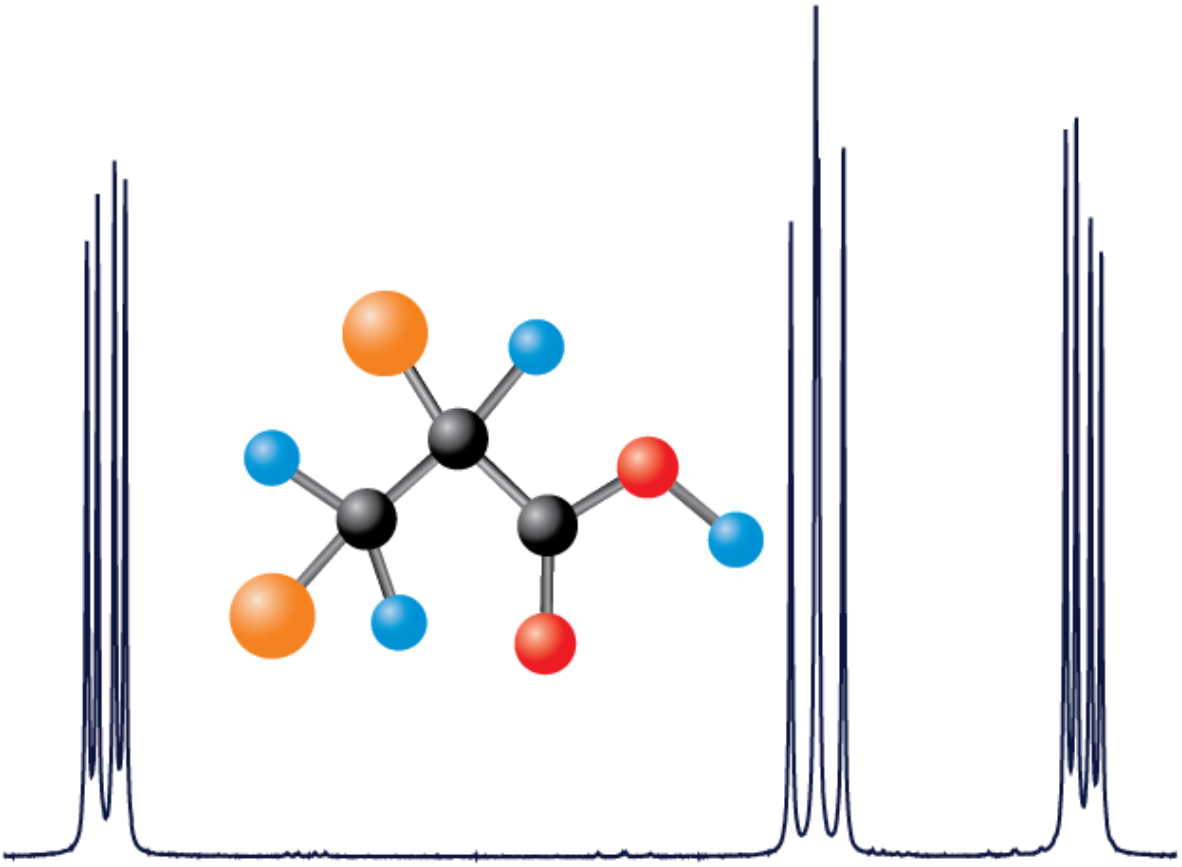


SMASH 2014

Conference Program

September 7th-10th, 2014
Atlanta, Georgia

SMASH 2014 NMR Conference

Dear SMASH 2014 Attendees,

We want to welcome you to the 2014 **S**mall **M**olecules **A**re **S**till **H**ot NMR conference in Atlanta, Georgia. The conference will be held this year at the Hyatt Regency Hotel in the downtown shopping district. Numerous restaurants and historical attractions are within walking distance of the conference site. The weather this time of year is typically warm and sunny with high temperatures of approximately 85-90 °F (30-32 °C) but be prepared for an occasional afternoon thunder shower.

This year's conference will kick off with a series of vendor user meetings sponsored by Advanced Chemistry Development, Inc. (ACD/Labs), Agilent Technologies, Bruker BioSpin, and Mestrelab Research. These symposia are always interesting and informative and we're sure that this year will be no exception.

While posters will be up the entire conference, those with even numbers will present on Monday and odd numbers on Tuesday. After dinner mixers on both evenings in the poster area will allow for discussions to continue.

The scientific program this year is packed with a plethora of leaders in small molecule NMR. We'll be hearing about new fields like hyperpolarization of liquids and solids in addition to new developments and applications of 'old' techniques like low-field NMR. There will be workshops on a variety of interesting topics like Pure Shift NMR, DFT calculations, Fast NMR techniques, and Process Monitoring by NMR. Other topics in the program include Stereochemical Analysis, Ligand Binding studies, Mixture Analysis, NMR of Inorganic Species, and the Structure Elucidation of Complex Small Molecules. Finally, we will have special session on NMR experiments outside of the normal spectroscopist's "toolbox". We hope that there will be something for everyone at this year's conference and as always we welcome your feedback on potential topics for future meetings.

One notable addition to this year's program is the inaugural Shoolery Award for Career Achievement in Small Molecule NMR spectroscopy. This year's recipient is William "Bill" Reynolds who will give an award presentation on Tuesday afternoon at the end of the day's scientific program.

On behalf of the Organizing Committee, we want to extend a warm welcome and thank you for attending SMASH 2014.

Sincerely,

Teo Parella and Thomas Williamson
Co-Chairs, SMASH 2014 NMR Conference

Conference Program

Sunday, September 7th

04:30 PM - 06:00 PM Registration
06:00 PM - 08:00 PM Dinner
08:00 PM - 11:00 PM Mixer

Monday, September 8th

07:30 AM - 08:45 AM Breakfast
08:45 AM - 09:00 AM Opening Remarks
09:00 AM - 10:30 AM **Hyperpolarization Techniques**
Chair: Jochem Struppe, *Bruker BioSpin, USA*

Overhauser Effects in Insulating Solids
Robert G. Griffin, *Massachusetts Institute of Technology, USA*
DNP-Enhanced NMR: Characterizing Pharmaceuticals at Natural Abundance
Cory Widdifield, *Université de Lyon, France*
SABRE Hyperpolarization and Quantitative Analysis of Complex Mixtures
Marco Tessari, *Radboud University, Netherlands*
Dissolution Dynamic Nuclear Polarization as a Tool to Characterize Small Molecules and Monitor Reactions
Christian Hilty, *Texas A&M University, USA*

10:30 AM - 11:00 AM Break
11:00 AM - 12:30 PM **Low Resolution/Low Field NMR**
Chair: Maria Victoria Silva Elipe, *Amgen, USA*

Applications of Variable Field NMR
Robert G. Bryant , *University of Virginia, USA*
Measurements of Hydrothermal Liquefaction (HTL) Combustion Products of Micro Blue-green Algae, Chlorella vulgaris by Low Resolution (and High Resolution) Benchtop NMR
Alan M. Kook, *NMR Service and Consulting, USA*
Survey of Low Field NMR Spectrometer Platforms for Successful Screening of Sexual Enhancement and Weight Loss Supplements for Adulteration with Drugs and Drug Analogs
John Edwards, *Process NMR Associates, LLC, USA*
Latest Developments and Applications for High Resolution Multinuclear Benchtop NMR Spectrometers (Upgraded Poster)
Bertram Manz, *Magritek Ltd, New Zealand*

12:30 PM - 01:30 PM Lunch, Free Time & Vendor Discussions
01:30 PM – 03:00 PM **Structure Elucidation of Complex Small Molecules (Natural Products, etc.)**
Chair: Dave Lankin, *University of Illinois at Chicago, USA*

Using Ultra-High Field NMR: The Structure Elucidation of Cyclic Lipopeptides
Jimmy Orjala, *University of Illinois at Chicago, USA*
NMR Characterization of Complex Natural Products: Opportunities and Challenges in Structure Elucidation
Kirk Gustafson, *NCI/NIH, USA*

Small Molecule Conformations in Solution Capture Drug-Protein Binding Poses
Jim Snyder, *Emory University, USA*
Determination of an Unprecedented Fused Pentacyclic Flavonoid Skeleton by Computer-Assisted Structure Elucidation of Black Chokeberry (Aronia melanocarpa) Fruit Juice Isolates (Upgraded Poster)
C. Benjamin Naman, *The Ohio State University, USA*

03:00 PM - 03:30 PM Break

03:30 PM - 05:30 PM **Workshop/Tutorial (concurrent)**

Pure Shift NMR
Coordinated by: Ralph Adams, *University of Manchester, UK*
Klaus Zangger, *University of Graz, Austria*
Jinfa Ying, *National Institutes of Health, USA*
Kinetics / Dynamics / Reaction Monitoring
Coordinated by: Jonas Buser, *Eli Lilly & Co., USA*
David Foley, *Pfizer, Inc., USA*

05:30 PM - 7:00 PM **Poster Session (even numbers)**
Co-chairs: Krish Krishnamurthy, *Agilent Technologies, USA*
Michael Hammer, *University of Regensburg, Germany*

07:00 PM - 11:00 PM Dinner/Social Hour

Tuesday, September 9th

07:30 AM - 08:45 AM Breakfast

09:00 AM - 10:30 AM **Metabolite ID and Other NMR Applications at Very Low Levels**
Chair: Melissa Lin, *Merck & Co. Inc., USA*

NMR Enabling Strategies and Platforms for Low Level Metabolite Characterizations
Janet Caceres Cortes, *Bristol-Myers Squibb Co., USA*
Exploring the Limits of Detection in 1D and 2D NMR for Metabolites Isolated from Biological Matrices
Greg Walker, *Pfizer, USA*
NMR and Natural Products Discovery at the Nanomole-scale
Ted Molinski, *University of California at San Diego, USA*
Effectiveness of CRAFT in Quantifying Serum Metabolites in the Presence of Spectral Distortions and Sharp/broad Peak Overlap (Upgraded Poster)
Erwin Garcia, *LipoScience Inc., USA*

10:30 AM - 11:00 AM Break

11:00 AM - 12:30 PM **Mixtures and Beyond**
Chair: Krish Krishnamurthy *Agilent Technologies, USA*

Online Reaction Monitoring for Biodiesel Production with Compact NMR Spectroscopy
Ernesto Danieli, *RWTH Aachen University, Germany*
High Resolution Multidimensional NMR Experiments
Wiktor Kozminski, *University of Warsaw, Poland*
Diffusion NMR and Applications to Small Molecules
William Price, *University of Western Sydney, Australia*
NMR for Quality Control of Injection Solution Mixtures: Comparison of Integration and CRAFT Analysis (Upgraded Poster)
Andrea Sefler, *Duke University, USA*

12:30 PM - 01:30 PM Lunch, Free Time & Vendor Discussions

01:30 PM - 03:00 PM **Applications of Inorganic / Organometallic Chemistry**
Chair: Roberto Gil, *Carnegie Mellon University, USA*

Parahydrogen Hyperpolarisation in NMR: Applications to Inorganic Chemistry
Meghan Halse, *University of York, UK*

A Step Closer Toward the Understanding of the Heart of the Reactivity for Coordination Complexes: When NMR and Quantum Mechanics Methods Squeeze Their Hands for Improving Metallo-Assisted Catalysis
Jonathan Farjon, *ICMMO - Université Paris-Sud, France*

Determining the Structure of Alkylaluminum Molecules Grafted on Silica Surface: A Combined Experimental Solid-State NMR / First-Principle Calculation Study
Pierre Florian, *CEMHTI-CNRS, France*

Mechanistic Studies of Transmetalation Intermediates in Copper-Catalyzed 1,4-Addition Reactions by Modified 1D $^{1}H^{31}P$-HMBCs (Upgraded Poster)
Carina Koch, *University of Regensburg, Germany*

03:00 PM - 03:30 PM Break

03:30 PM - 04:45 PM **Ligand Screening and Binding Studies**
Chair: Daneen Angwin, *SMASH NMR Conference, USA*

Discovery of a Novel Fragment Inhibitor of Acetyl CoA Carboxyltransferase
Parag Sahasrabudhe, *Pfizer, Inc., USA*

Taking a Gander and Kicking Some Tires: Setting Up an NMR Fragment Screen on a New Target
Ben Davis, *Vernalis, UK*

Integrating Physical Chemistry and Biophysics to Enhance Sensitivity of Based Fragment Screening
Elisabetta Chiarparin, *Astex Pharmaceuticals, USA*

04:45 PM - 05:15 PM **Presentation of the James N. Shoolery Award**
SMASH 2014 Recipient:

Teaching Young Dogs Old Tricks
William F. Reynolds, *University of Toronto, Canada*

05:15 PM - 07:00 PM **Poster Session (odd numbers)**
Co-chairs: Krish Krishnamurthy, *Agilent Technologies, USA*
Michael Hammer, *University of Regensburg, Germany*

07:00 PM - 11:00 PM Dinner/Awards/Social Hour

Wednesday, September 10th

07:30 AM - 08:45 AM Breakfast

09:00 AM - 10:30 AM **Venturing Outside the 'Standard' NMR Toolbox**
Chair: Craig Butts, *University of Bristol, UK*

Using Carbon Detected Experiments with Very High Resolution for More Complete Assignment of Fatty Acid Esters
Clark Ridge, *FDA, USA*

Non-Standard Ways of Monitoring Reaction Kinetics
Christina Thiele, *Technische Universität Darmstadt, Germany*

Revisiting Correlation Spectra in NMR Spectroscopy : Recent Developments in the Field of Spatial Frequency Encoding
Nicolas Giraud, *ICMMO - Université Paris-Sud, France*

Exploiting Natural Abundance ^{13}C-^{15}N Coupling as a Method for Identification of Nitrogen Heterocycles: Practical use of the HCNMBC Sequence (Upgraded Poster)
Steve Cheatham, *DuPont Crop Protection, USA*

10:30 AM - 11:00 AM Break

11:00 AM - 12:30 PM **Analysis of Chiral Molecules and Related NMR Methods**
Chair: Roberto Gil, *Carnegie Mellon University, USA*

Progress in the Determination of Configuration Using Anisotropic NMR Parameters
Burkhard Luy, *Karlsruher Institut für Technologie, Germany*

Searching for Chiral Derivatizing Agents (CDA) for the NMR Assignment of Absolute Configuration: A General Protocol
Ricardo Riguera, *CIQUS - Universidad de Santiago de Compostela, Spain*

New Methods for the Sign-Sensitive Determination of Homo- and Heteronuclear Coupling Constants
Josep Sauri, *SeRMN - Universitat Autonòma de Barcelona, Spain*

Sensitivity and Resolution Enhancement in EXSIDE and band Selective EXSIDE (Upgraded Poster)
Ikenna E. Ndukwe, *University of Bristol, UK*

12:30 PM - 01:30 PM Lunch, Free Time & Vendor Discussions

01:30 PM - 03:30 PM **Workshop/Tutorial (concurrent)**

Fast NMR Including NUS/Single Scan NMR/ASAP Methods
Coordinated by: Burkhard Luy, *Karlsruher Institut für Technologie, Germany*
Rainer Kerssebaum, *Bruker BioSpin*, *Germany*
Patrick Giraudeau, *Université de Nantes, France*

DFT Methods and Applications
Coordinated by: Dean Tantillo, *University of California at Davis, USA*
Alexei Buevich, *Merck & Co. Inc., USA*

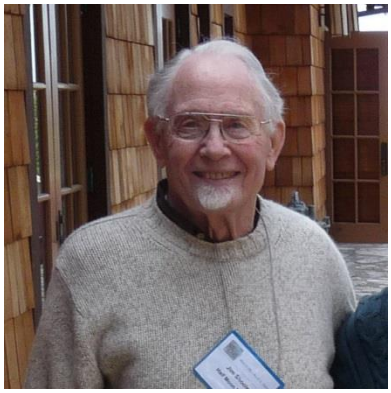
03:30 PM - 04:00 PM Break

04:00 PM - 06:00 PM **Structure Elucidation Boot Camp for Young Chemists**
Coordinated by: George Furst, *University of Pennsylvania, USA*
Brian Marquez, *Bruker BioSpin, USA*

06:00 PM - 06:30 PM Closing Remarks

The James Shoolery Award

This year SMASH established the James Shoolery Award as a grant, in honor of James N. Shoolery, to recognize the important contributions by an individual to the field of small molecule NMR spectroscopy.



In 1952, Jim Shoolery joined Varian Associates to set up an applications laboratory for NMR spectroscopy. His main initial goals were to develop applications of NMR in chemistry and to educate the wider chemistry community in the potential value of NMR spectroscopy in their research. In pursuit of these goals during the 1950's, he published a series of highly popular ads entitled "NMR at Work," initially in *Analytical Chemistry* and later on the back page of the *Journal of the American Chemical Society*. These illustrated a wide range of applications of NMR in chemistry and were based on work that he carried out in the applications lab. He also wrote a number of "Technical Information Bulletins" to help spectrometer owners in the operation of their instruments. Finally, he gave numerous lectures at conferences and research laboratories and at the annual NMR and EPR workshops that Varian Associates held in Palo Alto starting in 1958. In a 1993 article on the early history of NMR, he estimated that about 20,000 scientists had attended these different lectures by the end of the 1950's.

At the same time, Jim interacted with the R & D division of Varian on NMR instrument improvement, including the progression of ^{1}H operating frequency on Varian spectrometers from 30 to 40 to 60 and finally to 100 MHz by 1959. He was also involved in important technical improvements, including sample spinning, shim coils, spin decoupling, a flux stabilizer, and an electronic integrator. However, even with these improvements, the HR series of spectrometers were still extremely tricky to operate, requiring a significant amount of training, operating experience and patience. Jim realized that NMR spectroscopy would not reach its full potential as an analytical technique in chemistry until a spectrometer was developed that would be much easier to use, similar to the routine IR spectrometers that were already available from other manufacturers. Therefore, in 1957, Jim teamed with Emery Rogers of the marketing division of Varian to propose to the R & D division the development of a lower cost NMR spectrometer, which could use calibrated chart paper, which was rugged and reliable, and which could be run by graduate students and laboratory technicians with no training other than that provided by the spectrometer manual. He was heavily involved in this project, which resulted in 1961 in the introduction of the Varian A-60. This was a truly revolutionary development whose ease of operation triggered a dramatic increase in the use of NMR spectroscopy by chemists, in general, and by organic chemists, in particular. To illustrate its impact, the 1960 volume of the *Journal of Organic Chemistry* contained only one paper reporting the use of NMR while the 1967 volume included 220 papers, which used NMR data. In 2011, the seminal role of the A-60 in the development of NMR as a valuable analytical technique was recognized by the American Chemical Society as a National Historical Chemical Landmark in a ceremony at the Agilent facility in Santa Clara.

After the initial demonstration of FT NMR at Varian, Jim was involved in the development of the CFT-20 and FT-80 Varian spectrometers. These followed in the footsteps of the A-60 in being low cost and easy-to-use instruments for chemistry labs. In 1972, his book, "A Basic Guide to NMR," was published by Varian Associates and helped to educate many young chemists in the use of NMR. Later, with the development of multi-pulse sequences and 2D NMR, Jim was among the first to recognize the great value of these techniques for identifying unknown organic chemical structures, particularly in the natural products field. Jim, along with Steve Patt, developed the APT

sequence for spectral editing ^{13}C spectra of organic compounds and, through the 1980's, he collaborated with a number of natural products groups in establishing structures and assigning spectra of the compounds which they had isolated. He also, in 1984, published an important review article in the *Journal of Natural Products*, which clearly demonstrated the value of modern NMR techniques in the natural products field.

Scholarship Award Recipients

The following students received a scholarship to attend SMASH 2014

- **Jessica Bame**, University of Bristol, United Kingdom
- **Hanna Bartling**, University of Regensburg, Germany
- **Haoxi Ben**, National Renewable Energy Laboratory (NREL), United States
- **Andres Castillo**, Universidad Nacional de Colombia, Colombia
- **Franziska Fendt**, University of Regensburg, Germany
- **Manuela García**, Universidad Nacional de Cordoba, Argentina
- **Simon Glanzer**, University of Graz, Austria
- **Michael Hammer**, University of Regensburg, Germany
- **Erich Hellemann**, Carnegie Mellon University, United States
- **Carina Koch**, Universität Regensburg, Germany
- **C. Benjamin Naman**, The Ohio State University, United States
- **Ikenna Ndukwe**, University of Bristol, United Kingdom
- **Eliška Procházková**, Academy of Sciences of the Czech Republic, Czech Republic
- **Svetlana Pylaeva**, Saint-Petersburg State University, Russian Federation
- **Eduardo Sousa**, FIOCRUZ/UFRJ, Brazil
- **Godiraone Tatolo**, University of Bristol, United Kingdom
- **Mateusz Urbanczyk**, University of Warsaw, Poland
- **Christiane Wolff**, Technische Universität Darmstadt, Germany

Thanks to our scholarship sponsors for their generous support.

Acknowledgements

The SMASH 2014 Conference gratefully acknowledges the support provided by the following companies:

ACD/Labs
Agilent Technologies
BRUKER
Cambridge Isotope Laboratories, Inc.
CRYOMECH
WORLD LEADERS IN CRYOREFRIGERATION FOR MORE THAN 50 YEARS
HTS-110
ICON ISOTOPES
J S Research, Inc.
NMR Probes and Systems
JEOL
Serving Advanced Technology
Magnetic Resonance in Chemistry
KIMBLE CHASE
Life Science and Research Products
LEAP TECHNOLOGIES
magritek
www.magritek.com
MERCK
MESTRELAB RESEARCH
Chemistry Software Solutions
MODGRAPH
nanalysis
NEW ERA
The new standard in NMR Sampling
NMR CONCEPTS
OXFORD INSTRUMENTS
The Business of Science®
Pfizer
SIGMA-ALDRICH
ISOTEC™
Thermo SCIENTIFIC
Wilmad LabGlass

Executive Board and Planning Committee

Program Co-Chairs

Thomas Williamson
Merck & Co., Inc

Teodor Parella
SeRMN - Univ. Autònoma Barcelona

President

Brian Marquez
Bruker BioSpin Corp.

Outgoing President

Andreas Kaerner
Eli Lilly & Co.

Logistics Officer

Michael Kolpak
Janssen Pharmaceuticals

Sponsorship Officers

Margaret Levenberg
Stepan Company (Retired)

Craig Butts
University of Bristol

Treasurer & Registrar

Robert Espina
SMASH – Small Molecule NMR Conference

Secretary

Daneen Angwin
SMASH – Small Molecule NMR Conference

Poster Session Chairs

Krish Krishnamurthy
Agilent Technologies

Michael Hammer
University of Regensburg

Student Liaison

Maria Victoria Silva Elipe
Amgen, Inc.

Elizabeth McCord
E. I. du Pont de Nemours & Co.

Organizing Committee

Daneen Angwin
SMASH - Small Molecule NMR Conference

Craig Butts
University of Bristol

Ron Crouch
Agilent Technologies

Robert Espina
SMASH - Small Molecule NMR Conference

Juan Espinosa
Lilly

George Furst
University of Pennsylvania

Laurine Galya
Incyte Corporation

Roberto Gil
Carnegie Mellon University

Andreas Kaerner
Eli Lilly & Co.

Mike Kolpak
Jansenn Pharmaceuticals

Krish Krishnamurthy
Agilent Technologies

Dave Lankin
University of Illinois Chicago

Margaret Levenberg
Stepan Company (Retired)

Carla Marchioro
Research 4 Rent (R4R)

Brian Marquez
Bruker BioSpin Corp.

Gary Martin
Merck & Co., Inc

Eugene Mazzola
University of Maryland

Elizabeth McCord
E. I. du Pont de Nemours & Co.

Eric Munson
University of Kentucky

Maria Victoria Silva Elipe
Amgen

Christina Thiele
Technische Universitat Darmstadt

Martin Will
Sanofi-Aventis

Monday, September 8th
9:00 AM - 10:30 AM

Hyperpolarization Techniques

Chair: Jochem Struppe

Robert G. Griffin
Massachusetts Institute of Technology, USA

Cory Widdifield
Université de Lyon, France

Marco Tessari
Radboud University, Netherlands

Christian Hilty
Texas A&M University, USA

Return to Program Index

Overhauser Effects in Insulating Solids

R. G. Griffin

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

We report magic angle spinning (MAS), dynamic nuclear polarization (DNP) experiments at high magnetic fields (9.4 T, 14.1 T, and 18.8 T) using the narrow line polarizing agents BDPA dispersed in polystyrene, and sulfonated-BDPA and Trityl OX063 in glassy glycerol/water matrices. The ^{1}H DNP enhancement field profiles of the BDPA radicals exhibit a significant DNP Overhauser effect (OE) as well as a solid effect (SE) despite the fact that these samples are insulating solids, whereas trityl exhibits only a SE enhancement. In contrast to other DNP mechanisms such as the SE or CE, the experimental data suggest that the OE in non-conducting solids scales favorably with magnetic field, increasing in magnitude in going from 5 T, to 9.4 T and to 14.1 T. Simulations using a model two spin system consisting of an electron hyperfine coupled to a ^{1}H reproduce the essential features of the field profiles and indicate that the OE in these samples originates from the zero quantum (ZQ) cross relaxation induced by intramolecular delocalization of the unpaired electron and that the size of the hyperfine coupling is crucial to the magnitude of the enhancement. In ^{1}H-BDPA the OE is dominated by ZQ processes and is positive. In contrast, in ^{2}H-BDPA the double quantum (DQ) processes are dominant and yield a negative, albeit weak, enhancement as predicted by theory. Microwave field dependent studies show that the OE saturates at considerably lower power levels than the solid effect in the same samples. Our results provide new insights into the mechanism of the Overhauser effect, and also provide a new approach to perform DNP experiments at high magnetic fields.

Return to Session Index

DNP-Enhanced NMR: Applications in Characterizing Pharmaceuticals

Cory M. Widdifield, Aaron J. Rossini, Arthur Pinon, and Lyndon Emsley

Université de Lyon, Institut des Sciences Analytiques, (CNRS / ENS-Lyon / UCB Lyon 1), Centre de RMN à Très Hauts Champs, 69100 Villeurbanne, France.

Dynamic nuclear polarization (DNP)-enhanced NMR spectroscopy has recently been applied to a variety of chemically interesting systems, such as zeolites, metal-organic frameworks, and microcrystalline organics.[1] In this talk, we begin by clearly outlining the utility that DNP-enhanced NMR experiments has when attempting to interpret and ultimately assign the observed solid-state NMR spectra. This step is of great importance within NMR crystallography protocol development. Due to the substantial enhancements afforded by DNP (often one order of magnitude for samples of pharmaceutical relevance), we have been able to realize experimental observations that would have been prohibitive using traditional NMR methods. We underscore the benefits of these enhancements via several small molecules (gabapentin, thalidomide, and theophylline), all of which exhibit dauntingly large $^{1}H(T_1)$ values and therefore the acquisition of data via technically simple experiments, such as ^{1}H-^{13}C HETCOR, ^{1}H-^{15}N HETCOR, and ^{13}C-^{13}C INADEQUATE, is very inefficient, as they all rely upon initial polarization transfer from the ^{1}H spins. Subsequent discussions pertaining to the utility of the now fully assigned ^{13}C NMR spectra in determining the crystal structures of selected polymorphs are then presented. We also focus on the role that DNP-enhanced NMR spectroscopy can play in probing interactions between active pharmaceutical ingredients (APIs) and the excipient matrix in commercially available tableted drug formulations. Lastly, some preliminary discussion on the potential for the DNP-enhanced NMR spectra to establish rough guidelines of API domain sizes is provided.

1. Ni, Q. Z.; Daviso, E.; Can, T. V.; Markhasin, E.; Jawla, S. K.; Swager, T. M.; Temkin, R. J.; Herzfeld, J.; Griffin, R. G. *Acc. Chem. Res.*, **2013**, *46*, 1933.

Return to Session Index

SABRE Hyperpolarization and Quantitative Analysis of Complex Mixtures

Marco Tessari

Radboud University Nijmegen, Institute for Molecules and Materials, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

SABRE is a nuclear spin hyperpolarization technique based on the reversible association of a substrate molecule and parahydrogen (*p*-H_2) to a metal complex. A transient scalar coupling network within this complex allows the transfer of the spin-order from *p*-H_2 to the nuclear spin of the substrate molecules, resulting in NMR signals enhanced by one or two orders of magnitude. This sensitivity increase allowed the detection of NMR signals under 1 micromolar concentration in a single scan [1]. We have recently applied this technique to complex mixtures, showing that it is possible to quantify analytes at the low micromolar concentration.

1. Nan Eshuis , Niels Hermkens , Bram J. A. van Weerdenburg , Martin C. Feiters , Floris P. J. T. Rutjes , Sybren S. Wijmenga , and Marco Tessari "Toward Nanomolar Detection by NMR Through SABRE Hyperpolarization" *J. Am. Chem. Soc.*, 2014, **136**, 2695–2698.

Return to Session Index

Dissolution Dynamic Nuclear Polarization as a Tool to Characterize Small Molecules and Monitor Reactions

Christian Hilty, Guannan Zhang, Haifeng Zeng, Mukundan Ragavan, Youngbok Lee, Yaewon Kim, Hsueh-Ying Chen, Chia-Hsiu Chen, and Sean Bowen

Department of Chemistry, Texas A&M University, College Station, TX 77843, USA

Dissolution dynamic nuclear polarization (D-DNP) is an NMR sensitivity enhancement technique that can provide signal gains of several orders of magnitude in a single NMR scan[1]. Here, the potential is outlined for application of D-DNP to the characterization of small-molecule compounds, as well as chemical processes including reactions or binding events. In the D-DNP technique, the step of polarization in the solid state at low temperature, is separated from the NMR measurement in the liquid state. With the use of a rapid sample injector for the transfer of the dissolved, hyperpolarized sample between these two locations, signal loss due to spin relaxation is minimized. NMR spectra of most small molecules and a variety of NMR active nuclei can be detected either in a single scan, or a series of scans where the available spin polarization is distributed using multiple excitations with small-flip angle pulses. The latter option allows for the real-time monitoring of a reaction on the sub-second to second time scale after admixture of an initiating reagent immediately prior to NMR measurement. Most reactions that complete in a time frame of several seconds are amenable to study by D-DNP, including those catalyzed by organometallic catalysts or enzymes. Other NMR parameters such as spin relaxation can further be measured in single scan experiments and used for example to indicate binding to larger molecules, with applications in drug discovery. While the single-scan nature of D-DNP uniquely enables the study of non-equilibrium chemical processes, at the same time it prevents the application of many of the traditional NMR experiments used for the determination of molecular structure. In a final part, techniques for recovering spin correlation information from D-DNP samples are discussed, either in a single scan, or by splitting a hyperpolarized sample into multiple parts using a flow-NMR technique.

1. Ardenkjaer-Larsen, J.H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M.H.; Servin, R.; Thaning, M.; Golman, K. *Proc. Natl. Acad. Sci. U.S.A.*, **2003**, 100(18),10158.

Return to Session Index

Monday, September 8th
11:00 AM - 12:30 PM

Low Resolution/Low Field NMR

Chair: Maria Victoria Silva Elipe

Robert G. Bryant
University of Virginia, USA

Alan M. Kook
NMR Service and Consulting, USA

John Edwards
Process NMR Associates, LLC, USA

Bertram Manz*
Magritek Ltd, New Zealand

* Upgraded Poster

Return to Program Index

Applications of Variable Field NMR

Robert G. Bryant

Chemistry Department, University of Virginia, Charlottesville, VA, USA

Low field and variable field magnetic resonance provides a rich source of information for liquid as well as dynamically heterogeneous systems. Although spin-lattice relaxation may be coupled by intermolecular interactions, one may often select the observed molecule based on differences in diffusion constant or T2. Both transverse magnetization decay and spin-lattice relaxation carry fundamental information about the molecular dynamics in the system that may include chemical exchange rates, translational diffusion coefficients, rotational correlation times, the dimensionality of the space explored by the observed molecule, and the dynamical characteristics of molecules near interfaces. Fixed field magnetic resonance measurements have many applications ranging from standard high resolution spectral acquisition to down-the-hole NMR in the oil well. The magnetic field dependence of spin-lattice-relaxation-rate constants or the magnetic relaxation dispersion (MRD), provides a remarkably efficient approach to characterizing both large and small molecule dynamics. It is now remarkably easy to acquire magnetic resonance data in variable field NMR spectrometers that range in size from standard laboratory scale instruments to desk-top portable instruments (Stelar, Mede, Italy). This presentation will examine MRD applications to liquids, and semi-liquid or semi-solid systems.

Return to Session Index

Measurements of Hydrothermal Liquefaction (HTL) Combustion Products of Micro Blue-green Algae, Chlorella vulgaris by Low Resolution (and High Resolution) Benchtop NMR

Alan M. Kook[1], Rhykka Connelley[2], Stephanie Amack[2], and Patricia Phelps[3]

1. NMR Service + Consulting, Austin, TX
2. University of Texas Bioproducts and Bioenergy Program, Austin, TX
3. Dept. of Biotechnology, Austin Community College, Round Rock, TX

The last few decades have increasing shown the need for non-destructive, inexpensive, rapid, reproducible and repeatable analysis of microalgae lipids in vivo and in vitro. Low Resolution Benchtop NMR is the only technique that can meet these criteria without complex multivariate analysis software. By using deuterated solvents, the CPMG-T2 experiment and Laplacian transformation, we were able to reproducibly determine and identify 6 lipids of various chain lengths, unsaturation, and densities from 1% - 5% HTL reaction mixtures. High Resolution NMR at 60 MHz showed interesting evidence of oxidation and olefinic products

Return to Session Index

Survey of Low Field NMR Spectrometer Platforms for Successful Screening of Sexual Enhancement and Weight Loss Supplements for Adulteration with Drugs and Drug Analogs

John C Edwards[1], Kristie M Adams[2], and Anton Bzhelyansky[2]

1. Process NMR Associates, Danbury, CT
2. United States Pharmacopeial Convention, Rockville, MD

The adulteration of dietary supplements (or natural health products) with synthetic pharmaceuticals is an area of increasing concern, which presents substantial risk to public health. Widely available in retail and via the Internet, these products are often marketed as sexual enhancement, weight loss and/or bodybuilding supplements. Unlike prescription drugs, supplements do not require premarket approval by FDA before they are made available for public consumption. In fact, the agency can only take investigational action after the adulterated product has caused harm and the adverse event has been reported via MedWatch (FDA's online portal for *voluntary* reporting of adverse events associated with drugs, medical devices and dietary supplements).

Development of analytical tools for screening and identification of adulterated products in the marketplace represents a significant step forward in the fight against adulterated dietary supplements. Several organizations, including AOAC and USP, have undertaken initiatives to evaluate and recommend analytical methodologies for screening supplements for adulteration. HPLC and mass spectrometry have so far dominated the screening and quantitation studies published in the literature, with NMR spectroscopy often relegated to the status of structure elucidation tool. In this work, we investigate the ability of several-low field NMR spectrometric platforms to successfully identify and quantify the presence of adulterating drug substances in sexual enhancement and weight loss supplements purchased online and in US retail. ^{1}H qNMR of both types of samples was performed with 300 MHz NMR to confirm the presence of adulterants such as sildenafil, tadalafil, and their structural analogues (sexual enhancement supplements) and various synthetic stimulants (weight loss supplements). We have concluded that a simple sample preparation protocol combined with straightforward ^{1}H NMR spectroscopic analysis yields a rapid, robust and reliable screening test for adulterated supplements, presenting an attractive alternative to more labor-intensive, expensive and expertise-demanding techniques *du jour*.

Return to Session Index

Latest Developments and Applications for High Resolution Multinuclear Benchtop NMR Spectrometers

Federico Casanova[2], Andrew Coy[1], **Bertram Manz**[1], and Juan Perlo[2]

1. Magritek Ltd, 32 Salamanca Road, Wellington, New Zealand
2. Magritek GmbH, Pauwelsstr. 19 - D-52074, Aachen, Germany

Most high-resolution benchtop NMR spectrometers lack the ability to perform multidimensional and/or multinuclear NMR experiments. One of the main reasons for this is that the requirements on resolution, sensitivity and stability are much higher than for simple homonuclear 1D experiments. The benchtop system used here is based on a Halbach-array permanent magnet to provide sub Hertz resolution along with high sensitivity on regular 5mm diameter samples. An external lock system stabilises the magnetic field to enable long acquisition experiments. Additional stability is achieved by the in-built magnetic field shielding and temperature control, which make the system fairly insensitive to the local thermal and electromagnetic environment, enabling it to be operated on the bench in a chemistry lab or a production environment.

One of the advantages of measuring nuclei other than ^{1}H is the much wider chemical shift dispersion available. This intrinsically enables a larger number of NMR peaks to be resolved in the same spectral bandwidth. For lower magnetic field strengths this is especially useful, as it permits the elucidation of more complex molecules than proton NMR alone, where peak overlap and second order coupling tend to dominate the NMR spectra of molecules of even modest size.

For organic chemists ^{13}C NMR spectroscopy forms the backbone of routine molecular analysis. 1D ^{13}C methods such as DEPT, as well as 2D proton-carbon experiments, such as HETCOR, HSQC and HMBC, have been implemented. In this work we demonstrate multidimensional, multinuclear NMR spectra acquired on dilute, natural abundance samples with a benchtop NMR spectrometer.

Phosphorus is commonly found in many organic compounds, for example in biological membranes or DNA. The ^{31}P nucleus has a 100% natural isotopic abundance and a large chemical shift range, making it one of the most commonly used nuclei in biological NMR. We will show recent results of decoupled ^{31}P NMR spectra.

Benchtop NMR applications including reaction monitoring, QA/QC and complex mixture analysis have been investigated and demonstrate the power of NMR in providing quantitative, definitive and unique information about the sample under investigation.

Return to Session Index

Monday, September 8th
1:30 PM - 03:00 PM

Structure Elucidation of Complex Small Molecules (Natural Products, etc.)

Chair: Dave Lankin

Jimmy Orjala
University of Illinois at Chicago, USA

Kirk Gustafson
NCI/NIH, USA

Jim Snyder
Emory University, USA

C. Benjamin Naman*
The Ohio State University, USA

* Upgraded Poster

Return to Program Index

Using Ultra-High Field NMR: The Structure Elucidation of Cyclic Lipopeptides

Jimmy Orjala

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612

Cyclic lipopeptides are secondary metabolites produced by many different microorganisms, including cyanobacteria. Cyclic lipopeptides are biosynthesized by hybrid polyketide synthase (PKS)/non ribosomal peptide synthetases (NRPS). They often contain non-standard amino acids, such as D and L-, β-hydroxy -, and N- and/or O-methylated amino acids as well as other modified amino acids due to their NRPS origin. The lipid portion of the molecule is biosynthesized by PKS and is also often modified (i.e. branched, reduced, hydroxylated, halogenated, etc).

Together these modifications make the structure determination of cyclic lipopeptides challenging. At UIC we have access to ultra-high field NMR (Avance AV 900 spectrometer with CPTCI ATM 5 mm Z-gradient probe). The presentation will highlight our strategy using the exceptional resolving power of this spectrometer to assign the structures of cyclic lipopeptides. It will outline how we use the increased spectral dispersion, combined with the carbon sensitive cryoprobe (CPTCI), to obtain well-resolved direct detected ^{13}C/DEPTQ NMR spectra. Which, in combination with "band -selective" HMBC experiments of the carbonyl region greatly facilitate the structure determination of lipopeptides. Additional experiments and conditions used for structure determination of cyclic lipopeptides will also be discussed.

Return to Session Index

NMR Characterization of Complex Natural Products: Opportunities and Challenges in Structure Elucidation

Kirk R. Gustafson, Kentaro Takada, and Naoya Oku

Molecular Targets Laboratory, Center for Cancer Research,
National Cancer Institute-Frederick, MD, USA

NMR provides powerful structural elucidation tools that are particularly well suited for natural products studies. Comprehensive spectroscopic characterization of a native metabolite is often sufficient to fully assign a new structure. However assignment of the relative and absolute configuration of a molecule when there are multiple stereogenic centers often requires the development and application of additional experimental strategies. Many successful approaches in this regard rely on the formation of appropriate derivatives for more detailed NMR study. Since natural products are often obtained in very limited quantities, micro-scale chemical manipulations and the ability to analyze the structure of the resulting products is often key to the complete structural and configurational assignment of complex metabolites.

Return to Session Index

Small Molecule Conformations in Solution Capture Drug-Protein Binding Poses

James P. Snyder, Craig Grimmer, Bryan Cox, Brooke Katzmann, Pieter Burger, Gordon Wells, Qi Shi, Aaron Padwa, and Aiming Sun

Department of Chemistry, Emory University, Atlanta, GA, USA

The binding conformation of a drug-like molecule and its orientation in a macromolecular binding pocket constitutes the "binding pose" for a biological agent. X-ray crystallography comes closest to a full 3D description, while protein-ligand NMR contributes valuable but fragmentary information. Computer-guided ligand docking offers a palette of suggested poses based on application of one or more energy-parameterized scoring functions. We hypothesize that flexible small molecules in solution exist as an ensemble of conformations, one of which is either the bound conformer or a very close representation of it. A critical implication flows from this supposition. If the bioactive conformation can be detected by NMR in solution, its free energy is necessarily within a 2-3 kcal/mol window relative to the solution-derived global minimum. Identification of this form assures that the bound ligand is a low energy, unstrained structure that bypasses energy-inflated conformers which often arise by docking methodology. We propose complementary application of NMR-based conformer deconvolution in solution and ligand docking to avoid this conundrum and illustrate it with examples for soluble and membrane-embedded proteins with high resolution, low resolution and unknown 3D structures.

Return to Session Index

Determination of an Unprecedented Fused Pentacyclic Flavonoid Skeleton by Computer-Assisted Structure Elucidation of Black Chokeberry (*Aronia melanocarpa*) Fruit Juice Isolates

C. Benjamin Naman,[1] Jie Li,[1] Arvin Moser,[2] Jeffery M. Hendrycks,[2] Chunhua Yuan,[3] William J. Keller,[4] and A. Douglas Kinghorn[1]

1. Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, USA
2. Advanced Chemistry Development, Inc., Toronto, ON, Canada
3. Nuclear Magnetic Resonance Facility, Campus Chemical Instrument Center, The Ohio State University, Columbus, OH, USA
4. Nature's Sunshine Products, Inc., Spanish Fork, UT, USA

Black chokeberry [*Aronia melanocarpa* (Michx.) Elliott (Rosaceae)] has become a popular "superfruit" and botanical dietary supplement in the United States and some European countries.[1] The measured antioxidant potential and detailed phytochemical investigation of this plant material have been recently reported.[2,3] However, during the course of an earlier study on an extract of black chokeberry,[2] one compound was isolated that could not be structurally determined by conventional means. With the aid of computer-assisted structure elucidation (CASE) software, a feasible structure was generated that was not previously considered by manual data interpretation. At that time, the structure could not be confirmed due to diminished sample quantity and the need for additional experimentation. To validate the structure, a targeted isolation of the compound from the same plant source was undertaken that yielded, instead of the original compound of interest, a less complex analog. Analysis of the NMR spectra of this analog was more straightforward, and comparison with that of the previous molecule allowed for confirmation of the CASE-suggested structure. Presented are the structures for two new natural products of the flavonoid class that contain unprecedented pentacyclic cores, a description of the challenges overcome for their structure elucidation, and a plausible biosynthetic pathway.

1. Sidebottom, V. *Next-Generation Superfruits: Assessing the Potential of Emerging Ingredients Using Data from Patents, Clinical Trials, EFSA, and New Product Development*; Business Insights, Ltd.: London, UK, 2012.
2. Li, J.; Deng, Y.; Yuan, C.; Pan, L.; Chai, H.; Keller, W. J.; Kinghorn, A. D. *J. Agric. Food Chem.* **2012**, *60*, 11551–11559.
3. Taheri, R.; Connolly, B. A.; Brand, M. H.; Bolling, B. W. *J. Agric. Food Chem.* **2013**, *61*, 8581–8588.

Return to Session Index

Monday, September 8th
3:30 PM - 5:30 PM

Workshop/Tutorial (concurrent)

Pure Shift NMR

Ralph Adams*, University of Manchester, UK
Klaus Zangger, University of Graz, Austria
Jinfa Ying, NIH, USA

Kinetics / Dynamics / Reaction Monitoring

Jonas Buser*, Eli Lilly & Co., USA
David Foley, Pfizer, Inc. USA

* Coordinator

Return to Program Index

Poster Session

Monday, September 8th
5:30 PM - 7:00 PM

Co-Chairs: Krish Krishnamurthy and Michael Hammer

Return to Program Index

Tuesday, September 9th
9:00 AM - 10:30 AM

Metabolite ID and Other NMR Applications at Very Low Levels

Chair: Melissa Lin

Janet Caceres-Cortes
Bristol-Myers Squibb Co., USA

Greg Walker
Pfizer, Inc., USA

Ted Molinski
University of California at San Diego, USA

Erwin Garcia*
LipoScience Inc., USA

* Upgraded Poster

Return to Program Index

NMR Enabling Strategies and Platforms for Low Level Metabolite Characterizations

Janet Caceres-Cortes[1], Xiaohua Huang[2], Kim Johnson[2], Sarah Traeger[1], Haiying Zhang[1], Xiaohong Liu[3], Yue-Zhong Shu[1], Vikram Roongta[4], and Michael Reily[1]

1. Bristol-Myers Squibb Company, Princeton, NJ, USA
2. Bristol-Myers Squibb Company, Wallingford, CT, USA
3. Covance, Inc., Madison, WI, USA
4. NorthEast Bioanalytical Laboratories LLC, Hamden, CT, USA

NMR spectroscopy has evolved into an important technique in support of critical metabolite identification (ID) activities within the discovery and development workspace [1,2]. In Drug Discovery, NMR enables early identification of potential metabolic liabilities through soft spot analysis which helps drive structure–activity relationship (SAR) and improve the pharmacokinetic and safety profiles of newly synthesized drug candidates. At this stage, expediency is essential if it is to inform lead optimization. This has fostered a heavy reliance on LC-MS based approaches as the front-line analytical tool to analyze metabolites from in vitro studies in an efficient time frame. But, it can present challenges in precisely identifying the exact site and isomeric consequences of metabolic modifications. NMR spectroscopy is thus routinely applied to elucidate metabolite structures in those instances when ambiguities remain from LC-MS. For NMR studies at this stage, low quantity samples (1-15 µg) are routinely provided and the focus is not necessarily on completeness of assignment but rather on expediently identifying the site and type of modification. As a drug proceeds through the discovery - development continuum, the emphasis gradually shifts from soft spot analysis to metabolite profiling and understanding of the metabolites' contributions to on-target pharmacology and off-target receptor-mediated toxicology. Industry and regulatory focus on these activities underscore their importance and, therefore, considerably more time and resources are employed to exhaustively produce (25-100 µg per metabolite for NMR) and characterize the metabolic products of a drug.

In this presentation, we address two key challenges for metabolite identification by NMR within this drug discovery-development space [3]. It is well known that low amounts of isolated desired metabolites as well as contaminants arising from incubation matrices, coeluting metabolites and solvents are major impediments to NMR metabolite ID analyses. Strategies that address the challenges associated with isolation of sufficient amounts of metabolites and the generation of relatively pure samples for speedy NMR analysis will be discussed. Another challenge for metabolite ID is the issue of sensitivity. Because of the nature of this work, characterization must frequently be carried out on high nanogram-low microgram material. Whereas the most common NMR platform utilizes room temperature probes and sample volumes of 180 or 600 microliters, this technology is not well-suited for the analysis of metabolites in these low microgram quantities. This presentation provides selected examples of the benefit of utilizing nitrogen cryocooled probe technology, as well as reducing sample volumes to between 7 and 40 microliters in specially designed room temperature and helium cryogenic NMR probes.

1. Caceres-Cortes Janet, Reily Michael D., Bioanalysis, 2, 1263-1276, 2010.

2. Reily, Michael, D., Huang ,Stella, Caceres-Cortes, Janet, Johnson, Kim and Shu, Yue-Zhong, Encyclopedia of Drug Metabolism and Interactions, 5, 331-359, 2012.
3. Johnson, Kim, Liu Xiaohong, Huang Stella, Roongta, Vikram, Humphreys, Griffith, W. and Shu, Yue-Zhong, Analytical Methods, 2, 1542-1549, 2010.

Return to Session Index

Exploring the Limits of Detection in 1D and 2D NMR for Metabolites Isolated from Biological Matrices

Gregory S Walker[1], Raman Sharma[1], Shuai Wang[2]

1. Pfizer Global Research and Development, Phamacokintetic, Dynamics and Metabolism, Groton, CT 06340, USA
2. University of Illinois at Chicago, Department of Medicinal Chemistry and Pharmacognosy Chicago, IL 606012, USA

Over the last ten years the structural characterization of metabolites has had an increasing importance in both drug discovery and development. In drug development, MIST issues have driven this elevated scrutiny while in discovery benefits from improved SAR and pharmacokinetic parameters have raised the awareness of metabolites. In either case the definitive analytical techniques for structural characterization are high resolution mass spectrometry and NMR spectroscopy. Because of the relatively low abundance of metabolites the need for analytical sensitivity is critical. While mass spectrometry has historically been the much more sensitive technique, large strides in sensitivity have been achieved in NMR since the advent of cryo-probes. The limits of sensitivity in an NMR experiment can be defined strictly in terms of NMR signal to noise. This is frequently done using concentrated samples during the process of calibrating and establishing the performance of an instrument. Alternatively, sensitivity can be described in terms of limits of detection, which is more appropriate for drug metabolism studies. This has previously been performed with serial dilutions of pure standard compounds. While this work is very important in describing the ultimate sensitivity of an instrument, it does not account for the interferences by contaminating chemical entities from source material (e.g., microsomal preparations, cell lines and biological fluids) and the isolation process. In our laboratory we have executed a series of quantitative isolations of compounds with diverse structures to understand how isolation procedures as well as these background contaminants affect the limits of detection, relative to the ability to acquire interpretable 1D and 2D qualitative and quantitative NMR spectra.

Return to Session Index

NMR and Natural Products Discovery at the Nanomole-scale

Tadeusz F. Molinski

Department of Chemistry and Biochemistry and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California, USA

Technical advancements in NMR probe design, mass spectrometry, and chiroptical methods enable exploration of biodiversity and discovery of new marine natural products at extremely low abundances; sample amounts approaching ~1 nanomole. So-called 'nanomole-scale' natural products chemistry embraces integrated approaches for structure elucidation to achieve full characterization of new chemical entities from rare sources with available samples of only ~ 12–150 nmol (8 – 90 µg).[1-3].

More recent investigations have opened new dimensions; harnessing the innate biosynthetic capacity of marine sponges for synthesis of natural and non-natural alkaloids. In this talk, I will describe our recent results in this area, [4] and how these innovative approaches expand the scope of discovery of natural products from marine organisms.

1. Molinski, T. F. Curr. Opin. Drug Discov. Devel. 12, 197-206, 2009.
2. Molinski, T. F. Curr. Opin. Biotechnol. 21, 819-826, 2010.
3. Molinski, T. F. Nat. Prod. Rep. 27, 321-329, 2010.
4. (a) Stout, E. P.; Wang, Y.; Romo, D.; Molinski, T. F. Angew. Chem. Intl. Ed., 51, 4877-4881, 2011. (b) Stout, E. P.; Wang, Y.; Romo, D.; Molinski, T. F. J. Nat. Prod. 75, 527-530, 2012.

Return to Session Index

Effectiveness of CRAFT in Quantifying Serum Metabolites in the Presence of Spectral Distortions and Sharp/broad Peak Overlap

Erwin Garcia[1], Krish Krishnamurthy[2], Thomas O'Connell[3], Elias Jeyarajah[1]

1. LipoScience Inc., Raleigh, NC, USA
2. Agilent Technologies, Santa Clara, CA, USA
3. Duke University, Durham, NC, USA

The inherently quantitative nature of NMR lends itself to be used in analysis of complex mixtures including biological matrices such as in serum/plasma and urine. Extraction of quantitative information from NMR spectra is commonly performed by peak integration or deconvolution. The accuracy of the results from these methods can be compromised by imperfections in the peak shape and the baseline. While quantitation in the frequency–domain requires rigorously phased and baseline corrected spectra, time–domain Bayesian approaches derive quantitative information from the FID independent of the phase, and do not require baseline correction. We employed CRAFT (Complete Reduction to Amplitude Frequency Table) to perform time–domain analysis (a) on spectra unsuitable for phase adjustments (caused by misadjusted pre–acquisition delay), (b) on symmetrically broadened peaks (caused by suboptimal z3 shims), and (c) on spectra with misset transmitter offset. Furthermore, the quantitation of small metabolites by CRAFT analysis was performed on serum samples wherein, the sharp resonance lines arising from the metabolites of interest overlap with large, broad signals from macromolecules such as proteins and lipoproteins.

We report that, using CRAFT, the presence of spectral distortions arising from phase error, symmetric line broadening, and improperly placed transmitter offsets affect the average calculated concentrations < 6%, < 10% and < 3% respectively, relative to the reference spectra obtained under optimal conditions. The presence of broad resonances overlapping with the sharp peaks of interest affects the quantitation only slightly (% error < 5).

When CRAFT performance is evaluated for alanine, lactate and valine in dialyzed serum, in the presence of several suboptimal experimental settings and sharp/broad peaks overlap, the % CV and % error of the calculated concentrations are < 6 and < 2, respectively. These results demonstrate that with CRAFT analysis, accurate quantitative results can be obtained in complex mixtures without the need for advanced phasing and baseline correction algorithms. Further, it establishes the robustness of the Bayesian time–domain analysis against imperfect z3 shim and transmitter offset settings within tested limits. These features should prove to be highly advantageous in a high-throughput NMR environment where experimental conditions may vary over time, but it is important to maintain highly accurate quantitation.

Return to Session Index

Tuesday, September 9th
11:00 AM - 12:30 PM

Mixtures and Beyond

Chair: Krish Krishnamurthy

Ernesto Danieli
RWTH Aachen University, Germany

Wiktor Kozminski
University of Warsaw, Poland

William Price
University of Western Sydney, Australia

Andrea Sefler*
Duke University, USA

* Upgraded Poster

Return to Program Index

Online Reaction Monitoring for Biodiesel Production with Compact NMR Spectroscopy

Mario M. H. Killner[1], Yamila Garro Linck[2], **Ernesto Danieli**[2], Jarbas J. R. Rohwedder[1], and Bernhard Blümich[2]

1. Instituto de Química, Unicamp, Campinas, Brazil
2. Institut für Technische Chemie und Makromolekulare Chemie, RWTH Aachen University, Aachen, Germany

The use of biodiesel shows innumerous advantages compared to fossil fuels, since biodiesel is a biodegradable and non-toxic fuel. Nowadays, most of the biodiesel commercialized in the world is produced by the transesterification reaction of vegetable oils with methanol and basic catalysis. Understanding the reaction kinetics and controlling its optimum progress to improve the quality of the final product and to reduce production costs is of paramount importance. The present work explores compact ^{1}H NMR spectroscopy to follow the course of the transesterification reaction in real time. For this purpose the magnet is integrated into a flow setup which allows one to transport the neat solution from the reactor into the measurement zone and back again into the reactor. A multivariate calibration model applying Partial Least Squares (PLS) regression was built to analyze the measured data and to obtain information about the biodiesel conversion ratio with errors on the order of 1% of conversion. This information is used in combination with a Lorentzian deconvolution of the spectra to estimate the relative concentrations of methanol present in the ester-rich phase, in comparison with the one in the glycerol phase, the second medium involved in the reaction mixture. These results demonstrate that a compact NMR spectrometer can provide spectra with good quality and time resolution suitable for real time quality control of biodiesel production.

Return to Session Index

High Resolution Multidimensional NMR Experiments

Wiktor Koźmiński[1], Krzysztof Kazimierczuk[2], Maria Misiak[1], and Mateusz Urbańczyk[1]

1. Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Poland
2. Centre of New Technologies, University of Warsaw, Poland

A variety of different methods was proposed to overcome the sampling limitation in multidimensional NMR spectroscopy. They could be utilized in two different ways, either to shorten the experiment duration without loss of resolution, or to perform experiments that are not obtainable conventionally, i.e. with significantly improved resolution and/or of high dimensionality. Most often these methods are aplied in the studies of large molecules. However, in many cases spectra featuring extraordinary resolution may be very interesting in smal molecule experiments, as they reveal effects that are hidden, when spectral lines are broad, or enable resolving spectral ambiguities when peaks are overlapped. The examples of applications of nonconventional 2 and 3D spectra for smallmolecules include for example resonance assignement [1,2] and determination of couplings [3]. The sparse sampling techniques could be applied not only in frequency dimensions. Recently we have shown that sparse sampling can be extended to diffusion dimensions, and in this way acceleration of three-dimensional diffusion-ordered NMR spectroscopy could be achieved [4].

1. Misiak M., Koźmiński W., Magn. Res Chem., 45, 171-174 (2007).
2. Misiak M., Koźmiński W., Kwasiborska M., Wójcik J., Ciepichal E., Swiezewska E., Magn. Res. Chem., 47, 825–829 (2009).
3. Misiak M., Koźmiński W., Magn. Res. Chem., 47, 205-209 (2009).
4. Urbańczyk M., Koźmiński W., Kazimierczuk K., Angewandte Chemie Int. Ed. Engl, 53, 6464–6467 (2014).

Return to Session Index

Diffusion NMR and Applications to Small Molecules

William S. Price

Nanoscale Group, University of Western Sydney, Penrith, NSW, Australia

Nature generally uses the 'bottom up' approach in constructing materials. That is, small molecules are joined together to make larger ones. Many materials are also of a porous nature. Translational (or self-) diffusion is sensitive to the size and shape of the diffusing species and thus reports directly on whether the species is in some state of association or exchange (e.g., protein-protein self-association or drug-protein binding). Thus, diffusion is a natural probe for molecular dynamics and organisation at the nanoscale thereby providing information of fundamental chemical importance. If the timescale of the measurement is sufficiently long such that the mean square displacement of the diffusing molecules is sufficient for the molecules to interact with any boundaries (e.g., diffusion in a pore such as a vesicle or biological cell), then a diffusion measurement will provide information on the geometrical restrictions including the characteristic distances of the restriction. Further, it may also be possible to glean information on binding to and exchange through these boundaries.

For diffusion to be an effective probe it is important that the measurement does not perturb the system or the diffusive motion. Of the available methods for measuring diffusion, pulsed gradient spin-echo (PGSE) NMR diffusion measurements (also commonly referred to as DOSY, NMR diffusometry or q-space imaging) are now widely used due to their wide applicability, efficacy, resulting quantity and quality of information and non-invasive nature. Almost all modern NMR spectrometers are capable of at least some level of diffusion NMR measurements. Importantly, it is often possible to determine the diffusive behaviour of multiple species simultaneously.

Due to the rich information it provides coupled with the ease and non-invasive nature of the measurement, diffusion NMR has become a major characterisation tool with a diverse range of applications [1]. Recent methodological and theoretical improvements continue to expand the areas of application as well as providing increased precision and accuracy in established areas. This lecture will detail the theoretical underpinnings of diffusion NMR measurements and the type of modelling needed to analyse the resulting data. The talk will be illustrated with data selected from a number of experimental studies including supercooled water, solution structuring in aqueous alcohol systems, lithium salt - polymer electrolytes, drug binding and porous media.

1. Price, W.S. NMR Studies of Translational Motion: Principles and Applications, Cambridge University Press, Cambridge, 2009

Return to Session Index

NMR For Quality Control of Injection Solution Mixtures: Comparison of Integration and CRAFT Analysis

Andrea M. Sefler and Anthony Ribeiro

Duke NMR Center, Duke University, Durham, NC 27710, USA.

We present quantitative ^{1}H (NMR) studies on a formulated admixture of commercial injection solutions of ropivacaine, dexamethasone, epinephrine that is clinically administered to patients as a peripheral nerve block. Clinical practice often involves the mixing and/or dilution of packaged injection solutions from the manufacturer prior to administration to the patient for a variety of purposes. Despite this practice in the clinic, little information exists on the behavior or stability of the compounded injection solutions.

Our NMR study focuses on the stability of the admixtures as a function of light or dark storage conditions. Quantitative results have been generated using both traditional peak integration and the newly released CRAFT (Complete Reduction to Amplitude Frequency Table) algorithm to compare the results.

NMR is ideally suited for the task of assessing the quality control of such admixtures as it is highly sensitive to even minor structural changes, such as the addition of a sulfate group. Moreover, NMR is an inherently quantitative technique, even if the presence and/or identities of impurities are not expected or known *a priori*.

Return to Session Index

Tuesday, September 9th
1:30 PM - 03:00 PM

Applications of Inorganic / Organometallic Chemistry

Chair: Roberto Gil

Meghan Halse
University of York, UK

Jonathan Farjon
ICMMO - Université Paris-Sud, France

Pierre Florian
CEMHTI-CNRS, France

Carina Koch*
University of Regensburg, Germany

* Upgraded Poster

Return to Program Index

Parahydrogen Hyperpolarisation in NMR: Applications to Inorganic Chemistry

Meghan E. Halse, Barbara Procacci, Robin N. Perutz, and Simon B. Duckett

Department of Chemistry, University of York, York, UK

NMR spectroscopy using *para*hydrogen (*p*-H_2) induced polarisation (PHIP) is a powerful tool in inorganic and organometallic chemistry for the identification and study of intermediates in hydrogenation reactions.[1,2] The range of systems amenable to study by *p*-H_2 hyperpolarisation was recently extended to include non-hydrogenation reactions by the signal enhancement by reversible exchange (SABRE) method.[3] SABRE provides a mechanism for the transfer of hyperpolarisation from *p*-H_2-derived ^1H nuclei to non-hydrogenated substrates via catalysed reversible exchange reactions. In standard PHIP and SABRE NMR experiments, the hyperpolarisation is obtained by means of a thermally-initiated hydrogenation or reversible exchange reaction in the presence of *p*-H_2. The build-up of the net hyperpolarisation is asynchronous; therefore direct measurements of both the polarisation build-up and subsequent relaxation is a challenge.

In this talk, a new laser-synchronised *p*-H_2 approach [4] will be described and the utility of this method for observing *p*-H_2 polarisation transfer and monitoring chemical reactivity will be demonstrated. The technique uses a short laser pulse (10 ns) to reductively eliminate H_2 from a transition metal complex in an optically dilute solution under 3 atm of *p*-H_2. The subsequent oxidative addition of *p*-H_2 (μs) is monitored via NMR detection. High-resolution NMR spectra are acquired using laser-pump, NMR probe delays as short as 50 μs and the stability and reproducibility of the approach allows us to monitor changes in the spectra for increments in the pump-probe delay on the order of 10 μs. So far, we have applied the method to a range of complexes of the type $RuL_3L'H_2$ where L and L' are phosphine and arsine-based ligands or CO. Due to the large signal enhancements provided by the *p*-H_2 and the short delay times between laser initiation and NMR detection, this approach can be used to monitor chemical reactivity on microsecond timescales. In addition, the coherent nature of the *p*-H_2 oxidative addition makes this method an ideal tool for probing the transfer of *p*-H_2 hyperpolarisation from the *p*-H_2-derived hydride nuclei to other NMR active nuclei within the metal complex.

1. Bowers, C. R., Weitekamp, D. P., Phys. Rev. Lett., 57, 2645, 1986.
2. Duckett, S. B., Mewis, R. E., Acc. Chem. Res., 45, 1247, 2012.
3. Adams, R. W., Aguilar, J. A., Atkinson, K. D., Cowley, M.J., Elliott, P. I. P., Duckett, S. B., Green, G. G. R., Kahzal, I. G., Lopez-Serrano, J., Williamson, D.C., Science, 323, 1708, 2009.
4. Torres, O., Procacci, B., Halse, M. E., Adams, R. W., Blazina D., Duckett, S. B., Eguillor, B., Green, R. A., Perutz, R. N., Williamson, D. C., J. Am. Chem. Soc., 136(28), 10124–10131, 2014.

Return to Session Index

A Step Closer Toward the Understanding of the Heart of the Reactivity for Coordination Complexes: When NMR and Quantum Mechanics Methods Squeeze Their Hands for Improving Metallo-Assisted Catalysis

Jonathan Farjon[1], José Enrique Herbert Pucheta[1], Alexandre Requet[2], Flavien Bourdreux[2], Damien Prim[2], Anne Spasojevic[3], and Jean – Michel Gillet[3]

1. Equipe RMN en milieu Orienté, Institut de Chimie Moléculaire et des Matériaux d'Orsay (ICMMO) UMR CNRS 8182, Université Paris-Sud, 91405 Orsay Cedex, France.
2. Université de Versailles Saint-Quentin-en-Yvelines, Institut Lavoisier de Versailles UMR CNRS 8180, Versailles Cedex, France
3. Structures, Propriétés et Modélisation des Solides Ecole Centrale Paris, 92295 Châtenay - Malabry Cedex France

The economic and ecologic context requires the development of new more eco-compatible and cheaper chemical processes. Metallo-assisted catalysis appears to be a convenient domain in agreement with a lot of principles of a more virtuous chemistry [1]. Metallo-assisted catalysis processes are of main importance in modern syntheses since they permit to create bonds in mild conditions, maximizing the incorporation of reactants with an atom economy by using a small amount of catalyst to obtain the final product.

The installation of a pendant methylamine arm at pyridine rings generates an ideal 1,2-N,N-bidentate ligand and confers to the so-called pyridylmethylamine (pma) scaffold interesting complexation properties. Such motif can be encountered in numerous bioinspired Cu-, Fe-, Re- or Zn-based coordination compounds [2]. The combination of pma and transition metals revealed especially valuable as catalytic systems in synthetic transformations. A contribution of our research to this area on the synthesis, complexation and catalysis using pma-Pd was reported [3,4,5,6].

In order to improve the above mentioned processes to make them potentially industrialisable, it is necessary to understand the catalyst reactivity by deciphering mechanisms. Thus, it is essential to clarify the structure of target complexes and their intermediates. Modern Nuclear Magnetic Resonance (NMR) is one of the most utilized techniques to elucidate molecular structures but also to follow kinetics in solution. The combination of NMR with Quantum Mechanic (QM) calculations is the cornerstone able to access the conformations / configurations as well as dynamics of precatalyst and intermediates of metallic species involved in the processes of interest.

In a first step, five pma-ligands and their respective Pd complexes have been studied by liquid state NMR [7]. By comparing ^{1}H, ^{13}C and ^{15}N chemical shifts for each pma/pma-Pd couples, a general trend for the metallacycle atoms concerns variations of the electronic distribution at the pendant arm, especially at the nitrogen atom of the ligand. NMR allows a clear identification of electronic distribution and steric hindrance by comparing ligands to complexes chemical shifts at atomic resolution. The electronic contribution of each group on the pendant arm can be quantified by NMR. The comparison of five pma-

Pd complexes showed that Suzuki – Miyaura conversion and selectivity are strongly related to the substitution pattern at the pendant arm fitting with X Ray data [3,8]. In this field, new advances have been made for extending sites of chelation by using pyrimidylmelthylamine ligands [9].

Then, for clarifying the reactivity of a new Zn-pma family, we were interested in studying the structure of pma-Bz-ZnX2 complexes. For such complexes, four states have been detected by NMR/QM [10,11] and each exchange rate has been calculated by the full exchange matrix calculation from EXSY spectra. They are in agreement with QM energetic information. The structures optimized by DFT considering the solvent are in good agreement with the ones obtained by NMR. Then, we were able to predict chemical shifts, by using the DFT methods, as well as scalar couplings with the GIAO [12] technique, to complete the structural study.

For the first time, the structure of pma-Bz-ZnX2 family has been elucidated showing exchanges being the keys to monitor, in order to isolate a given conformer for getting high selectivity and great conversions. New advanced NMR techniques in combination with high level QM calculations open the avenue for most complicated multi-catalytic systems involving several metallic entities being the driving force for making valuable products of interest.

1. Anastas P., Eghbali N., Chem. Soc. Rev., 39, 301–312, 2010.
2. a) Suzuki K., Oldenburg P. D., Que Jr. L., Angew. Chem., Int. Ed., 47, 1887-1889, 2008; b) Hemmert C., Renz M., Gornitzka H., Meunier B., J. Chem. Soc., Dalton Trans., 3989-299, 1999.
3. Grach G., Pieters G., Dinut A., Terrasson V., Medimagh R., Bridoux A., Razafimahaleo V., Gaucher A., Marque S., Marrot J., Prim D., Gil R., Giner Planas J., Vinas C., Thomas I., Roblin J.-P., Troin Y., Organomet, 30, 4074-4086, 2011.
4. Terrasson V., Marque S., Scarpacci A., Prim D., Synthesis, 11, 1858-1862, 2006.
5. Puget B., Roblin J.-P, Prim D., Troin Y., Tetrahedron Lett., 49, 1706-1709, 2008.
6. Terrasson V., Giner Planas J., Vinas C., Teixidor F., Prim D.,. Light M. E, Hursthouse M. B., Organometallics, 29, 4130-4134, 2010.
7. Requet A., Colin O., Bourdreux F., Salim S.M., Marque S., Thomassigny C., Greck C., Farjon J., Prim D. Magn. Reson. Chem., 52, 273-278, 2014.
8. Terrasson V., Prim D., Marrot J., Eur. J. Inorg. Chem, , 2739-2745, 2008.
9. Candy M., Colin O., Requet A., Bourdreux F., Galmiche-Loire E., Gaucher A., Thomassigny C., Prim D., Leclerc E., Vranken E., Campagne J.M., Herbert Pucheta J.E., and Farjon J., submitted, 2014.
10. Diez V., Cuevas J. V., Garcia-Herbosa G., Aullon G., Charmant J. P. H., Carbayo A., and Muñoz A., Inorg. Chem., 46, 2, , 568-577, 2007.
11. Herbert Pucheta J.E., Recquet A., Bourdreux F., Gillet J.M., Spasojevic A., Prim D.and Farjon J., submitted, 2014.
12. Dithcfield R., Mol. Phys., 27, 789, 1974.

Return to Session Index

Determining the Structure of Alkylaluminum Molecules Grafted on Silica Surface: A Combined Experimental Solid-State NMR / First-Principle Calculation Study

Pierre Florian[1], Dominique Massiot[1], Christophe Copéret[2] and Philippe Sautet[3]

1. CNRS, CEMHTI UPR3079, Univ. Orléans, F-45071 Orléans, France
2. Department of Chemistry and Applied Biosciences, ETH Zürich, CH-8093 Zürich, Switzerland
3. University Lyon, ENS Lyon, Inst. Chim. Lyon, CNRS, F-69364 Lyon 07, France

Modification of a silica surface via grafting alkylaluminum molecules has been a long standing subject of interest in view of the preparation of solid activators for heterogeneous catalytic processes. Such systems are typically studied as a model for a supported methylaluminoxane and aluminum cocatalyst. However their chemistry involves complex surface reactions, in particular the cleavage of multiple Si–O–Si bridges by Al–C bonds. This makes the characterization of surface molecular species very difficult, as well as particularly challenging from a spectroscopic point of view.

While ^{27}Al solid-state NMR spectroscopy would be a method of choice, it has been difficult to apply because of large quadrupolar broadenings. Here, from a combined use of the highest stable field NMR instruments (17.6, 20.0, and 23.5 T) and ultrafast magic angle spinning (>60 kHz), high-quality spectra were obtained on triethylaluminum and triisobutylaluminum supported on silica, allowing NMR parameters to be extracted. Combined with first-principles calculations, these were assigned to actual structures of surface aluminum sites.[1,2]

This methodology enabled us to show that in the case of triethylaluminum the surface sites are mainly dinuclear Al species grafted on the silica surface via either two terminal or two bridging siloxy ligands (see figure below).[1] In stark contrast to those bis-grafted species that forms during Et_3Al silica grafting, reaction of triisobutylaluminum with silica yields three different Al sites: a quadruply grafted dimeric surface species and two incorporated $Al(O)_x$ species (x = 4 or 5).[2] Thus the isobutyl ligands, which render R_3Al monomeric, lead to greater reactivity towards the silica surface.

1. Kerber, R. N.; Kermagoret, A.; Callens, E.; Florian, P.; Massiot, D.; Lesage, A.; Coperet, C.; Delbecq, F.; Rozanska, X.; Sautet, P. J. Am. Chem. Soc. 2012, 134, 6767.
2. Kermagoret, A.; Kerber, R. N.; Conley, M. P.; Callens, E.; Florian, P.; Massiot, D.; Coperet, C.; Delbecq, F.; Rozanska, X.; Sautet, P. Dalton Transactions 2013, 42, 12681.

Return to Session Index

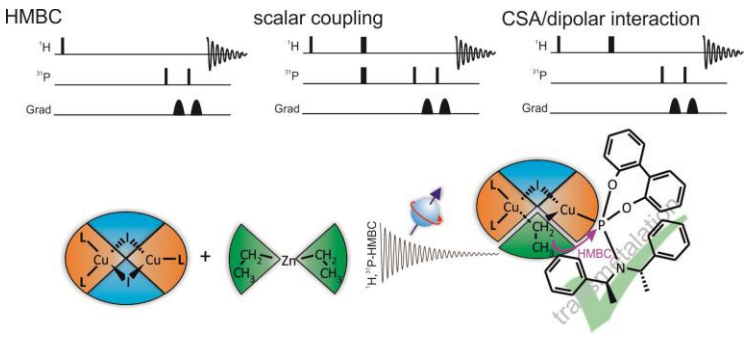
Mechanistic Studies of Transmetalation Intermediates in Copper-Catalyzed 1,4-Addition Reactions by Modified 1D $^{1}H^{31}P$-HMBCs

Carina Koch, Felicitas von Rekowski, and Ruth M. Gschwind

Department of Organic Chemistry, University of Regensburg, Germany

Asymmetric copper-catalyzed conjugate addition reactions are a very powerful and widely applied method for enantioselective carbon-carbon bond formation. By the use of a cheap catalytic system composed of Cu(I)-salts and chiral phosphoramidite ligands, it is possible to create new stereocentres with quantitative yields and *ee*-values up to 99 %.[1-4] Despite the great success of this class of reaction, a direct experimental proof of the widely accepted transmetalation intermediate[5] was missing until now, most likely because the magnetization transfer via copper of structures deviating from a highly symmetrical coordination is very challenging, due to the high quadrupole moment of $^{63/65}Cu$ and the high electric field gradient of these complexes. But the detection and characterization of intermediates in catalytic reactions is crucial for the understanding of the mechanisms and the rational optimization of reaction conditions. Therefore, we performed experiments, based on our experiences in the research field of the detection of H-bond networks,[6] where we used a special approach of modified 1D $^{1}H^{31}P$-HMBC spectra. With this special approach we were able to identify intermediates with alkyl groups directly bound to copper. Moreover, samples of enantiopure and enantiomeric mixtures of ligands for the structural characterization of the intermediates. With this special approach in hand and after screening different organometallic reagents (ZnR_2; R=Et, Me, Ph; MeLi) we were able to obtain the first direct experimental proof for the elusive transmetalation intermediate. Beside monomeric intermediates with one ligand, for the first time also a dimeric transmetalation intermediate with a mixed trigonal/tetrahedral coordination on the copper atoms is observed, which is comparable to the recently elucidated precatalytic complex of this reaction.[5,7,8]

Furthermore, we are investigating the appearing interactions between the phosphoramidite ligands and the organometallic reagents ($ZnMe_2$; MeLi).



1. Alexakis A. et al., Chem. Rev., 108, 2796, 2008.

2. T. Jeraphagnon et al., Chem. Soc. Rev., 38, 1039, 2009.
3. N. Krause et al, Synthesis, 2, 171, 2001.
4. B. L. Feringa et al., Angew. Chem. Int. Ed., 49, 2486, 2010.
5. R. M. Gschwind et al., Chem. Eur. J., 13, 6691, 2007.
6. R. M. Gschwind et al., J. Am. Chem. Soc., 130, 16846, 2008.
7. R. M. Gschwind et al., Angew. Chem. Int.Ed., 45, 6391, 2006.
8. R. M. Gschwind et al., J. Am. Chem. Soc., 130, 12310, 2008.

Return to Session Index

Tuesday, September 9th
3:30 PM – 4:45 PM

Ligand Screening and Binding Studies

Chair: Daneen Angwin

Parag Sahasrabudhe
Pfizer, Inc., USA

Ben Davis
Vernalis, UK

Elisabetta Chiarparin
Astex Pharmaceuticals, USA

Return to Program Index

Discovery of a Novel Fragment Inhibitor of Acetyl CoA Carboxyltransferase

Venkat Thanabal, Felix Vajdos, Kimberly Fennell, Marie Anderson, Kris Borzilleri, **Parag Sahasrabudhe**, and Jane Withka

Structural Biology and Biophysics, Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, USA

Fragment based screening methods for hit identification provides an opportunity to sample expanded chemical space than that covered by conventional library collections. Acetyl-CoA carboxylase (ACC), a >260KD multi-domain bifunctional enzyme, presents a challenge in the application of STD based screening method to identify selective binding fragment hits. We developed and implemented a novel NMR based method for fragment screening at high concentrations that uses ^{13}C-labeled substrate resulting in a simplified detection of enzymatic reaction by NMR. Previous reports describe the use of ^{19}F-NMR or proton NMR methods to monitor enzymatic reactions and apply them as functional assays to identify inhibitors of these enzymes. Use of isotope labeled substrate has the advantage to overcome the spectral overlap of signals in the proton NMR based functional methods. Conversion of acetyl-CoA into malonyl-CoA by the full length hACC1 enzyme was monitored by ^{13}C-edited proton NMR using ^{13}C-methyl labeled acetyl CoA (acetyl-1,2-$^{13}C_2$-CoA)substrate. This NMR functional assay was used for screening the proprietary fragment library against ACC in both forward and reverse second half-reactions of the enzymatic cycle. Fragment hits that inhibited the forward reaction of the full length protein were identified and validated against the Carboxyltransferase (CT) domain in a reverse enzymatic assay. NMR based STD binding studies were then performed with the CT domain to further validate these fragment hits and identify the binding pocket. From the list of all fragment hits, 33 were selected for soaking with CT domain crystals. Crystallization efforts using high concentration of fragment hits resulted in the crystal structure of one fragment with the CT domain.

Return to Session Index

Taking a Gander and Kicking the Tires: Setting up an NMR Fragment Screen on a New Target

Ben Davis

Vernalis R&D, Cambridge UK

In Fragment Based Screening (FBS), small low affinity ligands are identified as startpoints for lead discovery. Detecting these weak interactions can push screening technologies to their limits of sensitivity. It is therefore vitally important to have a high level of confidence in the initial fragment hits in order to minimise the risk of pursuing potentially misleading or costly artifacts.

I will discuss how we go about setting up an NMR fragment screen against a new target, assessing the target and using any available tool compounds to judge the suitability of the system for FBS by NMR. This will include the practicalities and requirements of an NMR screening campaign, along with some examples of the various issues which can arise with different proteins and ways of working around these problems.

I will also discuss ways in which we use the output of an FBS campaign, including target evaluation and integration of FBS with HTS as well as classical FBLD.

Return to Session Index

Integrating Physical Chemistry and Biophysics to Enhance Sensitivity of Fragment Based Screening

Elisabetta Chiarparin[1], Roberto Buratto[2], Daniele Mammoli[2], Glyn Williams[1], and Geoffrey Bodenhausen[2]

1. Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, UK
2. Ecole Polytechnique Fédérale de Lausanne 4 Avenue Forel, CH-1015 Lausanne, Switzerland

Over the last decade, Fragment Screening has emerged as a powerful and reliable source of drug-like leads and Fragment Based Drug Discovery (FBDD) has gained wide acceptance within the pharmaceutical industry, as evidenced by the number of fragments progressed into lead series and on to clinical candidates. Fragment hits are typically low affinity binders with Kd ranging from 100μM to 10mM or greater. As a consequence, very sensitive biophysical techniques such as X-ray crystallography, NMR spectroscopy, thermal unfolding and Surface Plasmon Resonance are applied to detect hits and measure their affinity.

NMR spectroscopy despite its low intrinsic sensitivity plays a major role in ensuring success of Fragment Screening campaigns. In this work, we will describe its use as physical chemistry technique to characterize fragment library solubility and aggregation properties in a quest to improve confidence on fragment hits from different biophysical techniques. Also, we will describe a new powerful NMR biophysical method (1,2), based on Long-Lived States (LLS) (3), for competitive binding experiments to measure reliable dissociation constants of fragments that bind weakly to the ATP binding domain of Heat Shock Protein 90 (Hsp90). This method extends the dynamic range of traditional 1H NMR ligand based-experiments, such as STD, WaterLogsy and T1rho for screening and determination of dissociation constants K_D beyond the current low millimolar range, using small concentrations of unlabeled proteins and reduced concentrations of fragments.

1. Salvi N., Buratto R., Bornet A., Ulzega S., Rebollo I.R., Angelini A., Heinis C., Bodenhausen G., *J Am Chem Soc*, **2012**. 134(27): p. 11076-11079.
2. Buratto R., Mammoli D., Chiarparin E., Williams G., and Bodenhausen G. Angewande Chemie, **2014** in press
3. Carravetta M., Levitt M.H., *J Am Chem So*c, **2004** 126(20):6228-6229.

Return to Session Index

Tuesday, September 9th
3:30 PM – 4:45 PM

Presentation of the

James N. Shoolery Award

SMASH 2014 Recipient:

William F. Reynolds
University of Toronto, Canada

Return to Program Index

William F. Reynolds

Bill Reynolds was born and raised in a mining town in Northern Manitoba where his father was one of North America's leading experts in the chemistry of gold recovery. This inspired Bill to pursue a BSc degree in Honors Chemistry at the University of Manitoba. He then obtained a PhD, specializing in NMR spectroscopy, with the late Professor Ted Schaefer in 1963. After spending two years at University College London as the Sir William Ramsey Fellow for Canada, he joined the Faculty of the Department of Chemistry, University of Toronto, where he has remained since, currently as Emeritus Professor.

Much of his early research at Toronto centered on using measurements of substituent-induced chemical shifts and long-range coupling constants to investigate transmission of electronic effects in aromatic derivatives, along with investigations of polymer structure by NMR. However, for the last 30+ years, he has focused on the use of 2D NMR to determine structures of natural products and the development of improved NMR methods for this purpose. A key early effort in this field was his pioneering work on the use of long-range ^{1}H-^{13}C shift correlation spectra which allowed total structure elucidation of unknown compounds by 2D NMR alone.

Another important contribution was his promotion of forward linear prediction as a time-saving method. His 210 publications in these areas involved about 150 scientists and students from Mexico and the Caribbean basin, including over 40 students from these areas who have visited his lab for periods of up to 4 months to carry out structure elucidations. He has also taught a number of short NMR courses in the latter locations and has also authored a number of reviews focusing on optimal use of NMR methods.

From 1993-2005, he served as an Editor for Magnetic Resonance in Chemistry. In 1998, he received the Gerhard Herzberg Award from the Spectroscopy Society of Canada and, in the same year, he was inducted as the first Canadian member of the Academia Mexicana de Ciencias, in recognition for his contributions to Mexican Science. He also received special awards in 1999 and 2010 for contributions to chemistry in the West Indies.

Return to Program Index

Teaching Young Dogs Old Tricks

William F. Reynolds

Department of Chemistry, University of Toronto, Toronto, Canada

This title is in response to the old joke that ‘You can’t teach old dogs new tricks’. Of course, the joke doesn’t really refer to dogs at all but rather to old timers like me, implying that we are too set in our ways to learn modern methods. At age 76, I am doing my best to show that this doesn’t apply to me, at least as far as NMR is concerned. Thus I decided to propose the opposite question- ‘can young dogs learn old tricks?’. Assuming that the answer is yes, I will first cover two NMR tricks which are both more than 50 years old but still useful. This will be followed by four examples where, under special circumstances, old methods may actually be better than new methods, including one case where, due to improvements in spectrometer hardware, I suspect that the old method may be generally better for routine use.

Return to Session Index

Wednesday, September 10th
9:00 AM - 10:30 AM

Venturing Outside the 'Standard' NMR Toolbox

Chair: Craig Butts

Clark Ridge
FDA, USA

Christina Thiele
Technische Universität Darmstadt, Germany

Nicolas Giraud
ICMMO - Université Paris-Sud, France

Steve Cheatham*
DuPont Crop Protection, USA

* Upgraded Poster

Return to Program Index

Using Carbon Detected Experiments with Very High Resolution for More Complete Assignment of Fatty Acid Esters

Clark D. Ridge, Shaun MacMahon, and Eugene Mazzola

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA

Updated ^{13}C detected 2D NMR experiments were used with a large number of points in the directly detected dimension to gain a more complete assignment of several fatty acid esters that are of interest as contaminants in processed edible oils. [1] Assigning carbon and proton NMR resonances of molecules containing long hydrocarbon chains has been known to be problematic due to extreme overlap in the proton spectrum. The carbon spectra are less crowded but are difficult to assign without sufficient data on correlations with protons and other carbons. The standard proton detected experiments for obtaining carbon-proton correlations (HSQC, HMBC, HMQC, etc.) all have limitations that make it difficult or impractical to get the higher resolution needed for assignment of such crowded spectra. Older carbon detected experiments, i.e. short and long range HETCOR, are not used as much because of their much lower sensitivity and the difficulty in handling the very large data sets the high resolution versions produce. However, these older experiments offer the possibility of much higher resolution then their proton detected counterparts and long-range couplings can be established through an updated FLOCK experiment. [2,3] Over the past 20 years carbon optimized probes have become more sensitive and computing power has increased making the processing of quite large data sets routine and therefore not an obstacle or time limiting step. Carbon detection becomes more practical with high sensitivity carbon-13 probes and in samples not limited in amount or solubility. While still challenging, the much higher resolution makes it possible to identify resonance that differ by only a few hertz in both the carbon and proton dimension.

1. MacMahon, S., Mazzola, E., Begley, T.H., Diachenko, G.W., J. Agric. Food Chem. 2013, 61 (20), pp 4737–4747
2. Reynolds, W.F., McLean, S., Perpick-Dumont, M., Enriquez, R.G., Magn. Reson. Chem., 1989, 27 (2), pp 162-169
3. Carpenter, K.A., Reynolds, W.F., Yang, J., Enriquez, R.G., Magn. Reson. Chem., 1992, 30 (13), pp S35-S41

Return to Session Index

Non-Standard Ways of Monitoring Reaction Kinetics

Christiane Wolff, Jonas Kind, and **Christina M. Thiele**

Clemens-Schöpf-Institut für Organische Chemie und Biochemie, Technische Universtität Darmstadt, Alarich-Weiss-Strasse 16, 64287 Darmstadt, Germany

NMR spectroscopy is a non-destructive and quantitative method for in situ monitoring of reactions while obtaining as much structural information as possible at the same time. Thus reaction kinetics can be observed in real-time.

In a kinetic analysis the first data points usually contain the highest information content. Thus it is essential to obtain these, which usually involves non-standard ways of initiating or monitoring the reaction.

One example discussed will be the study of a photochemical reaction. For this an irradiation source is essential.[1] So far there are several solutions for an illumination device inside the spectrometer, for example by modifying the probe itself or by using an optical fibre guiding the light.[2, 3] While several setups use laser as irradiation source, a new illumination setup was published last year using cheap and exchangeable LEDs and a sandblasted fibre tip.[4] Using this new setup we studied the photochemical equilibrium of a modified spiropyrane and merocyanine by irradiation of light with varying wavelengths. By changing the wavelength of the irradiating light the equilibrium composition of the sample can be easily manipulated and the kinetics of these composition shifts can be monitored.

1. G. Berkovic, V. Krongauz, V. Weiss, *Chem. Rev.* **2000**, *100*, 1741.
2. I. Kuprov, P. J. Hore, *J. Magn. Reson.* **2004**, *171*, 171.
3. T. Kühn, H. Schwalbe, *J. Am. Chem. Soc.* **2004**, *122*, 6169.
4. C. Feldmeier, H. Bartling, E. Riedle, R. M. Gschwind, *J. Magn. Reson.* **2013**, *232*, 39.

Acknowledgements: Funding by the ERC (starting grant no. 257041 to C.M.T.), LOEWE “Soft Control” and the Adolf-Messer-Foundation is gratefully acknowledged. Furthermore we want to thank the group of Ruth Gschwind for very helpful discussions and performing the experiments on the spyropyran/merocyanine system with us in Regensburg.

Return to Session Index

Revisiting Correlation Spectra in NMR Spectroscopy: Recent Developments in the Field of Spatial Frequency Encoding

Daisy Pitoux, Jonathan Farjon, Bertrand Plainchont, Jean-Michel Ouvrard, Denis Merlet, and **Nicolas Giraud**

Laboratoire de RMN en milieu orienté, ICMMO, UMR 8182 CNRS, Université Paris-Sud, Orsay, France

Most of the developments that have been made over the recent years in the field of so-called "Fast NMR" have focused on accelerating the acquisition of multidimensional data, sometimes with spectacular results. Despite the considerable progress accomplished in that field, the whole experimental and analytical process that leads to the extraction of proton-proton couplings remains however a hard and time-consuming task for chemists. One reason is the complexity and the amount of information that is made available in state-of-the- art experiments, even for small or medium sized molecules.

We will present our latest developments in the field of spatial frequency encoding, that allow us to control spin evolutions in a fully tailored manner, in localized regions of the sample, and to combine them into high resolution spectra whose analytical content is easily extractible.

In a first part, we will show that it is possible to combine pure shift and *J*-edited evolutions, by coding their evolution separately along different gradient axes, in order to acquire a general NMR experiment (PCR-COSY) that can give access, on a single spectrum, to a fully edited –and assignable- measurement of the whole proton coupling network. [1]

In a second part, we will discuss the advantages and limitations of implementing spatial frequency encoding techniques at very high field (1 GHz). To address this question, we have recently applied successfully a novel ∂-resolved TOCSY experiment to the analysis of a synthetic oligosaccharide whose standard TOCSY or COSY ^{1}H spectra usually show strongly overlapped regions. We will show that at very high field this sequence is robust and provides significant resolution enhancements. [2]

1. Giraud, N., Pitoux, D., Ouvrard, J.M. & Merlet, D. Chemistry –A European Journal, 19: 12221-12224, 2013
2. Pitoux, D., Farjon, J., Plainchont, B., Ouvrard, J.M., Bonnaffé, D. & Giraud, N. submitted, 2014

Acknowledgement: French Research Agency Grant (ANR-2011-JS08-009-01).

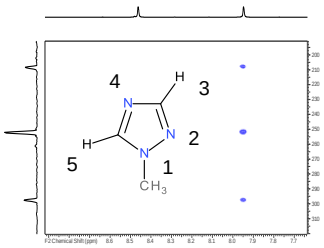
Return to Session Index

Exploiting Natural Abundance ^{13}C–^{15}N Coupling as a Method for Identification of Nitrogen Heterocycles: Practical Use of the HCNMBC Sequence

Eriks Kupce[1], Mike Kline[2], and **Steve Cheatham[2]**

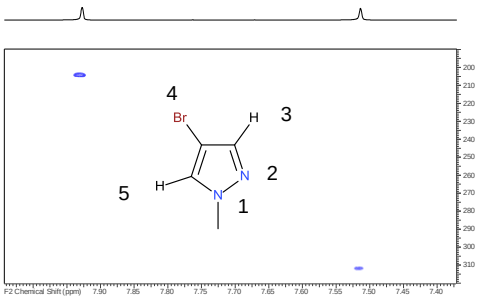
1. Bruker UK Limited, Banner Lane, Coventry, CV4 9GH, UK
2. DuPont Crop Protection, Stine Haskell Research Center, Newark, DE 19714, USA

Synthesis of heterocycles can often produce isomers that may be challenging to identify. Direct ^{1}H-^{15}N long range experiments are a invaluable tool for identifing isomers but the experiments are not without limitations. Recently, we have introduced a new experiment, HCNMBC[1], which permits ^{1}H-^{15}N correlation via the natural abundance ^{13}C-^{15}N coupling. In this paper we detail the results of the experiment on a number of classes of azoles including pyrazoles, imidazoles and triazoles. Sample quantities required are reasonable and on the same order as those needed for 1,1-adequate. The experiment produces data which can be highly complementary to direct ^{1}H-^{15}N HMBC type correlations in that it provides ^{15}N chemical shift data for nitrogens that may not show up in the HMBC. We demonstrate that this is particularly advantageous in the triazoles where ^{15}N chemical shift can be diagnostic of regiochemistry (Fig A).

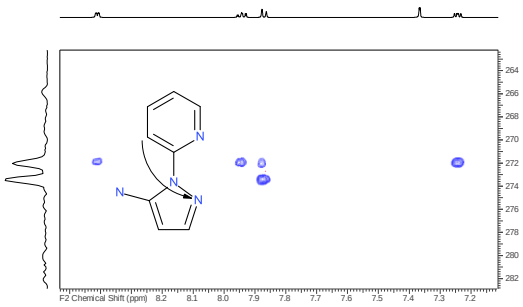


A

In addition, data indicate that the experimental parameters can be adjusted to produce defined results in certain heterocyclic systems emphasizing, for example, two-bond specific correlations in some instances (Fig. B) or, conversly, long range 4-bond correlations which occur through 3-bond ^{13}C-^{15}N coupling (Fig C).



B



C

It is therefore critical in setup and analysis of the data to have a general understanding of the magnitude of the carbon-nitrogen coupling constants within various heterocyclic types and to develop a database of this information. Preliminary results of our work in this area will also be presented.

1. Steve Cheatham, Peter Gierth, Wolfgang Bermel and Ēriks Kupče, HCNMBC – A pulse Sequence for H-(C)-N Multiple Bond Correlations at Natural Isotopic Abundance, *J. Magn. Reson. submitted, (2014).*

Return to Session Index

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

Analysis of Chiral Molecules and Related NMR Methods

Chair: Roberto Gil

Burkhard Luy
Karlsruher Institut für Technologie, Germany

Ricardo Riguera
CIQUS - Universidad de Santiago de Compostela, Spain

Josep Sauri
SeRMN - Universitat Autonòma de Barcelona, Spain

Ikenna E. Ndukwe*
University of Bristol, UK

* Upgraded Poster

Return to Program Index

Progress in the Determination of Configuration Using Anisotropic NMR Parameters

Burkhard Luy

Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Anisotropic NMR-parameters have been shown to be highly valuable in the determination of conformation, configuration, and even constitution of small molecules [1-3]. Although RDCs and RCSAs can be measured and interpreted straightforwardly for many (rigid) molecules, still many improvements concerning sample preparation, coupling extraction, and data interpretation of especially molecules with (limited) flexibility are highly desirable.

An update of several currently developed techniques will be given, including broadly applicable alignment media [4], pulse sequences on the way to semi-automated coupling determination [5-7] and data interpretation for relative configurational analysis using molecular dynamics with orienting constraints (MDOC). Finally, first attempts of a model for MD-based prediction of alignment in polymer gels will be presented that in principle might be useful for determining absolute configuration [8].

1. Gil R. R., Angew. Chem. Int. Ed. 50, 7222-7224, 2011.
2. Kummerlöwe G., Crone B., Kretschmer M., Kirsch S. F., Luy B., Angew. Chem. Int. Ed. 50, 2643-2645, 2011.
3. Kummerlöwe G., Luy B., Webb G., Annu. Rep. NMR Spectroscop. 68, 193-230, 2009.
4. Merle C., Kummerlöwe G., Halbach F., Lierse von Gostomski C., Höpfner J., Beskers T., Wilhelm M., Luy B., Angew. Chem. Int. Ed. 52, 10309-10312, 2013.
5. Ehni S., Luy B., J. Magn. Reson., in press, 2014.
6. Reinsperger T., Luy B., J. Magn. Reson. 239, 110-120, 2014.
7. Timari I., Kaltschnee L., Kolmer A., Adams R. W., Nilsson M., Thiele C. M., Morris G. A., Kover K. E., J. Magn. Reson. 239, 130-138, 2014.
8. Berger R., Courtieu J., Gil R. R., Griesinger C., Koeck M., Lesot P., Luy B., Merlet D., Navarro-Vazquez A., Reggelin M., Reinscheid U. M., Thiele C. M., Zweckstetter M., Angew. Chem. Int. Ed. 51, 8388-8391, 2012.

Return to Session Index

Searching for Chiral Derivatizing Agents (CDA) for the NMR Assignment of Absolute Configuration: A General Protocol

Ricardo Riguera

Department of Organic Chemistry and Centre for Research in Biological Chemistry and Molecular Materials, (CIQUS), University of Santiago de Compostela, Spain

The assignment of the absolute configuration of organic compounds by NMR is a rapid and inexpensive method useful for a variety of functional groups [1].

In the classical approach, the substrate is derivatized with the two enantiomers of a chiral derivatizing agent (CDA) and the absolute configuration is obtained by comparison of the ^{1}H-NMR spectra of the two resulting diastereomers. The most widely used CDAs are arylalkoxyacetic acids, and the nature of the aryl and alkoxy groups determines the usefulness and effectivity of each CDA for a safe assignment of a particular class of substrate.

In this presentation, a general protocol for the design of chiral derivatizing agents (CDAs) is described taken chiral thiols as example [2]. Theoretical calculations on modelled CDAs and NMR experimental data allow the prediction of the NMR shieldings and the optimisation of the CDA structure, leading to 2-naphtyl-*tert*-butoxyacetic acid (2-NTBA) as the most efficient auxiliary for the assignment of chiral thiols.

1. For reviews on this topic see:
    a. Seco, J. M.; Quiñoá E.; Riguera R. *Chem. Rev.* 2004, 104, 17-117;
    b. Seco, J. M.; Quiñoá, E.; Riguera, R. *Tetrahedron: Asymmetry* 2001, 12, 2915-2925; *Chem. Rev.* 2012, 112, 4603-4641
2. Porto, S.; Quiñoá E.; and Riguera R. *Tetrahedron*, 70, 3276-3283, 2014.

Return to Session Index

New Methods for the Sign-Sensitive Determination of Homo- and Heteronuclear Coupling Constants

Josep Saurí[1,2] and Teodor Parella[1]

1. Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona. Cerdanyola del Vallès, Barcelona, Catalunya, Spain
2. Merck Research Laboratories, NMR Structure Elucidation. Rahway, NJ, USA

Several new NMR methods for the sign-sensitive determination, as well as to the magnitude of homo- and heteronuclear coupling constants will be provided [1,2,3].

2D & 1D proton-selective HSQMBC-like experiments are proposed for the extraction of heteronuclear coupling constants either in low-natural or high-natural abundance heteronuclei. A special emphasis will be made on the easy and direct measurement of both the magnitude and the sign from the pure-phase multiplets obtained.

On the other hand, a spin-state-selective method that was born from the idea introduced in the P.E.HSQMBC experiment [4], will also be presented for the efficient measurement of individual $^{1}J(CH_A)$ and $^{1}J(CH_B)$ in diastereotopic CH_2 groups along the indirect dimension of a F1-coupled HSQC spectrum, as well as to the magnitude and the sign of the $^{2}J(H_AH_B)$ coupling constants from the straightforward analysis of a single four-component E.COSY cross-peak. The extraction of $^{1}J(CH)$ values for CH and CH_3 multiplicities are performed from the same spectrum. The success of the method will also be illustrated for the determination of residual dipolar $^{1}D(CH)$ and $^{2}D(HH)$ coupling constants in a small molecule weakly aligned in a PMMA swollen gel [5].

1. Saurí, Josep; Espinosa, Juan Félix; Parella, Teodor. Angew. Chem. Intl. Ed., 51, 3919-3922, 2012.
2. Saurí, Josep; Teodor. Magn. Reson. Chem., 50, 717-721, 2012.
3. Saurí, Josep; Nolis, Pau; Parella, Teodor. J. Magn. Reson., 236, 63-69, 2013.
4. Saurí, Josep; Castañar, Laura; Nolis, Pau; Virgili, Albert; Parella, Teodor. J. Magn. Reson., 224, 101-106, 2012.
5. Saurí, Josep; Castañar, Laura; Nolis, Pau; Virgili, Albert; Parella, Teodor. J. Magn. Reson., 242, 33-40, 2014.

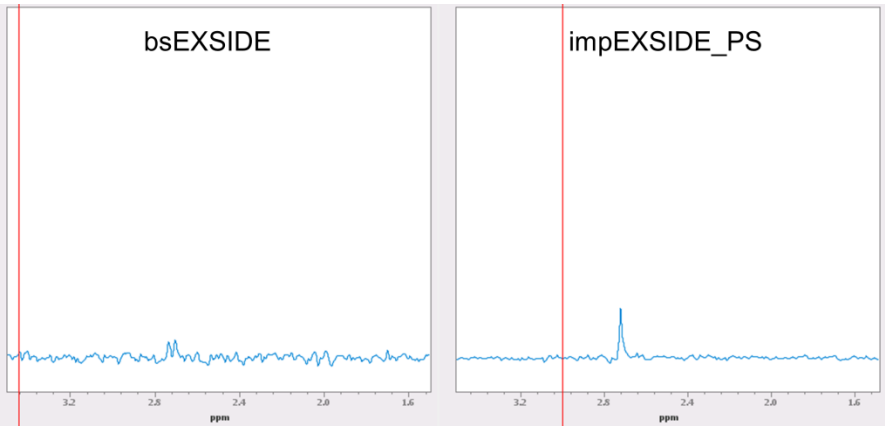
Return to Session Index

Sensitivity and Resolution Enhancement in EXSIDE and band selective EXSIDE

Ikenna E. Ndukwe, Godiraone Tatolo, and Craig P. Butts

University of Bristol, UK

The potential value of heteronuclear long range coupling constants ($^{n}J_{CH}$) for assigning stereochemical centres in small and complex molecules cannot be overemphasized. The literature is rife with NMR methods for measuring $^{n}J_{CH}$ (all of which have their limitations), but the EXSIDE[1] and ^{13}C band selective EXSIDE[2] methods afford easy means of obtaining $^{n}J_{CH}$, even for non-protonated centres, in addition to providing pure in-phase absorption lineshapes (devoid of distortion from or unmodulated by passive homonuclear coupling). However, the EXSIDE experiment requires a large number of t_1 increments in order to measure $^{n}J_{CH}$ in F1 (especially for small coupling constants). This leads to long acquisition times (hours) and a loss of sensitivity due to T_2 relaxation. The ^{13}C band selective EXSIDE method reduces the experimental time needed per $^{n}J_{CH}$ (minutes) but requires multiple experiments to be performed in order to reproduce the full spectra and also suffers from the same relaxation losses. Here we introduce three new pulse sequences based on the EXSIDE experiments. The first, **impEXSIDE,** employs the **IMPRESS** technique[3,4] (**imp**roved **re**solution using **s**ymmetrically **s**hifted pulses), which exploits Hadamard encoding in the F_1 dimension. The other two pulse sequences apply the band selective homonuclear decoupling (bash) **Pure Shift**[5] acquisition technique, and are called **EXSIDE_PS** and **bsEXSIDE_PS**. We see significant improvements in both signal to noise ratio and resolution in all of the these experiments over their parent versions, and their three-way combination **impEXSIDE_PS** could be a very powerful and enhanced tool for measuring $^{n}J_{CH}$ values.



1. Krishnamurthy, V. V., *Journal of Magnetic Resonance Series A* 1996, **121**, 33-41
2. Butts, C. P.; Heise, B.; Tatolo, G., *Organic Letters* **2012,** *14*, 3256-3259
3. Krishnamurthy, K. *Journal of Magnetic Resonance* 2001, **153**, 144 – 150
4. Krishnamurthy, K. *Journal of Magnetic Resonance* 2001, **153**, 124 – 132
5. Meyer, N. H. and Zangger, K. *Angewandte Chemie (International edition)* 2013, **52**, 7143 – 1746

Wednesday, September 10th
1:30 PM - 3:30 PM

Workshop/Tutorial (concurrent)

Fast NMR Including NUS/Single Scan NMR/ASAP Methods

Burkhard Luy*, Karlsruher Institut für Technologie
Rainer Kerssebaum, Bruker BioSpin
Patrick Giraudeau, Université de Nantes

DFT Methods and Applications

Dean Tantillo*, University of California at Davis
Alexei Buevich, Merck & Co. Inc.

* Coordinator

Return to Program Index

Wednesday, September 10th
4:00 PM - 6:00 PM

Structure Elucidation Boot Camp for Young Chemists

George Furst, University of Pennsylvania
Brian Marquez, Bruker BioSpin

Return to Program Index

Poster Session

Tuesday, September 9th
5:15 PM - 7:00 PM

Co-Chairs: Krish Krishnamurthy and Michael Hammer

Return to Program Index

Poster Index – Abstract Number

1	Emmanuel Hatzakis	Compositional Analysis of Encapsulated Fish Oil Supplements using Multinuclear and Multidimensional NMR Spectroscopy
2	Michael M Hammer	Unravelling Organocatalytic Reaction Mechanisms by NMR-Spectroscopy
3	Andrew Phillips	Using NMR to Gain Understanding of Dissolution Processes
4	Patrick Giraudeau	In situ EC-NMR by Real-time 1D and 2D Spectroscopy
5	Carina Koch	Mechanistic Studies of Transmetalation Intermediates in Copper-Catalyzed 1,4-Addition Reactions by Modified 1D $^{1}H^{31}P$-HMBCs
6	Christiane Wolff	Kinetic Study of Olefin Metathesis Reactions using a Benchtop NMR Spectrometer
7	Iain Day	Using Fluoroaromatics as Probes of Small Molecule Self-Aggregation
8	Steve Cheatham	Exploiting Natural Abundance ^{13}C-^{15}N Coupling as a Method for Identification of Nitrogen Heterocycles: Practical use of the HCNMBC Sequence
9	Franziska Fendt	^{29}Si and ^{119}Sn NMR Studies on $E4^{4-}$ Zintl Anions in Liquid Ammonia
10	Hanna Bartling	Merging NMR and UV/Vis for Mechanistic Studies on Photocatalytic Systems
11	Eduardo Sousa	Spectroscopic Characterization of Polimorphism in Efavirenz
12	Eliška Procházková	‘Planamerism’: A Novel Stereochemical Phenomenon?
13	Jessica Bame	Conformational Determination of Conformationally Biased Homologated Alkanes
14	Ikenna Ndukwe	Sensitivity and Resolution Enhancement in EXSIDE and Band Selective EXSIDE
15	Bertram Manz	Latest Developments and Applications for High Resolution Multinuclear Benchtop NMR Spectrometers
16	Simon Glanzer	Homonuclear J-Scaling During Acquisition
17	Jaroslav Havlicek	Characterization of Active Pharmaceutical Ingredients Using Solid-State NMR Spectroscopy
18	Godiraone Tatolo	Constant Time Gradient CRISIS HMBC (CTgc2HMBC) – Quantifying Improvements in SNR and Resolution
19	Mateusz Urbanczyk	Diffusion-ordered NMR with Joint Sparse Time-gradient Domain.
20	Haoxi Ben	Isotopic Tracing and In-situ NMR Monitoring – A Powerful Way to Investigate Mechanism of Upgrading Process
21	Michael Bernstein	Mixtures Analysis of Complex Samples
22	Regina Easley	Determination of Spectrophotometric pH Indicator Purity Using Quantitative ^{1}H NMR
23	Philip Keyes	Automated Structure Verification: High Profile Patent Issues Building a C.A.S.E. for Enhanced Compound Quality Assurance

24	Jochen Junker	Can NMR Correlations Determine Your Structure Unambiguously?
25	Andres Castillo	Online Analysis and Visualisation Tools for Chemical Information
26	Michael Bernstein	A Complete Wine Analysis Using Multiplets Detection
27	Michelle Markus	Full Automation of Routine NMR Aanalysis for Polymers, Using Assure-RMS
28	Michelle Markus	What is in Your Dishwashing Liquid? Developing Fully Automated Screening Methods for Commercial Products
29	Erwin García	Effectiveness of CRAFT in Quantifying Serum Metabolites in the Presence of Spectral Distortions and Sharp/Broad Peak Overlap
30	Andrea Sefler	NMR For Quality Control of Injection Solution Mixtures: Comparison of Integration and CRAFT Analysis
31	C. Benjamin Naman	Determination of an Unprecedented Fused Pentacyclic Flavonoid Skeleton by Computer-Assisted Structure Elucidation of Black Chokeberry (Aronia melanocarpa) Fruit Juice Isolates
32	Estelle Martineau	Non-linear Effects in Quantitative 2D NMR of Polysaccharides: Pitfalls and How to Avoid Them
33	Ard Kolkman	A Combined Approach: Highly Sensitive Pure Shift NMR and Structure Elucidation of ppm-impurities in Bulk Chemicals
34	Maria Victoria Silva Elipe	Application of ^{19}F Time-Domain NMR to Measure Content in FLuorine Containing Drug Products
35	Valdemar Lacerda Jr.	Unequivocal Structural Assignments of Three Cycloheptenoid Intermediates for Guaiane Sesquiterpenes: An Experimental and Theoretical Approach
36	Janaina Vilcachagua	Structural Assignment of Non-aromatic B-ring Flavanones from Piper Carniconnectivum C. DC by NMR, ECD and DFT Calculations
37	Radek Pohl	Tautomerism of Isocytosine - Low-temperature NMR Study
38	Iain Swan	Measuring and Mitigating Convection in NMR
39	Xiaoda Yuan	Characterizing Hydrogen Bonding Interactions in Amorphous Solid Dispersions Using Solid-State NMR Spectroscopy
40	Ion Ghiviriga	A Modified EXSIDE Experiment for the Measurement of ^{19}F-^{15}N Coupling Constants at Natural Abundance
41	Shi Bai	Multinuclear Solid-state NMR Studies of Form I of Atorvastatin Calcium
42	Alvaro Cunha Neto	Application of No-D NMR in Illicite Cocaine Analysis
43	Jan-Christoph Westermann	Free Ligand Conformational Populations in Solution – A Powerful Drug Discovery Tool
44	Donald Thomas	Oils of Provenance: Quantitative NMR Using Natural Abundance Deuterium
45	Alexei Buevich	NMR Structure Elucidation of Small Organic Molecules and Natural Products: Choosing ADEQUATE vs HMBC
46	Teodor Parella	Fast and Efficient Enantiodifferentiation Through Pure Shift NMR
47	Teodor Parella	Perfect-HSQC: Suppression of Phase and Amplitude J(HH) Modulations

48	Josep Saurí	Pure In-Phase Heteronuclear Correlation NMR Experiments
49	Josep Saurí	Simultaneous Measurement of the Magnitude and the Sign of Multiple Heteronuclear Coupling Constants in ^{19}F- or ^{31}P-Containing Molecules
50	Steve Coombes	Real Time On-Line Flow-NMR-MS For Reaction Monitoring
51	Rogelio Pereda	DFT ^{1}H-^{1}H Coupling Constants in the Configurational Reassignment of Synargentolide A
52	Dmitri Pichugin	Evaluating the Relative Merits of CRISIS-HSQC, Pure Shift-HSQC and ASAP-HMQC for Rapid Screening of Organic Compounds
53	Juan Carlos Cobas Gómez	Wide-band Relaxometry Analysis of Noisy Multiexponential Decay Data
54	Patrick Wheeler	Structure Revision of Acremolin: CASE Approach Based on 2D NMR vs. Total Synthesis
55	Igor Goljer	Utilizing Fluorine NMR in the Detection and Structural Elucidation of Metabolites
56	Thomas Williamson	LR-HSQMBC: A Highly Sensitive NMR Technique to Probe Very Long-Range Heteronuclear Coupling Pathways
57	Aleksej Krunić	Application of Ultra-high Field NMR Spectroscopy in Characterization of Natural Products
58	John Glushka	Software Tools for Simulating NOEs and J-Coupling from Long MD Trajectories of Flexible Molecules
59	Melissa Lin	Investigation of the Root Cause of Opalescence of an Injectable Drug Product by Diffusion Ordered Spectroscopy
60	You Zhuo	Monitoring In-Cell Metabolism with High Sensitivity Using DNP Polarized Substrates
61	Marek Kuzma	The MSG Mouse Model of Obesity Studied by NMR Metabonomics
62	Erich Hellemann	Pure Shift F1 Proton-Coupled J Scaled BIRD HSQC to Measure RDCs with Higher Accuracy and Sensitivity: Features and Limitations
63	Manuela E. García	Di(ethylene glycol) Methyl Ether Methacrylate (DEGMA) Gels Align Small Organic Molecules in Methanol
64	Till Kühn	From Automatic Structure Consistency Analysis to Structure Verification in NMR

Poster Index - Author

Shi Bai	41	Multinuclear Solid-state NMR Studies of Form I of Atorvastatin Calcium
Jessica Bame	13	Conformational Determination of Conformationally Biased Homologated Alkanes
Hanna Bartling	10	Merging NMR and UV/Vis for Mechanistic Studies on Photocatalytic Systems
Haoxi Ben	20	Isotopic Tracing and In-situ NMR Monitoring – A Powerful Way to Investigate Mechanism of Upgrading Process
Michael Bernstein	21	Mixtures Analysis of Complex Samples
Michael Bernstein	26	A Complete Wine Analysis Using Multiplets Detection
Alexei Buevich	45	NMR Structure Elucidation of Small Organic Molecules and Natural Products: Choosing ADEQUATE vs HMBC
Andres Castillo	25	Online Analysis and Visualisation Tools for Chemical Information
Steve Cheatham	8	Exploiting Natural Abundance ^{13}C-^{15}N Coupling as a Method for Identification of Nitrogen Heterocycles: Practical use of the HCNMBC Sequence
Juan Carlos Cobas Gómez	53	Wide-band Relaxometry Analysis of Noisy Multiexponential Decay Data
Steve Coombes	50	Real Time On-Line Flow-NMR-MS For Reaction Monitoring
Alvaro Cunha Neto	42	Application of No-D NMR in Illicite Cocaine Analysis
Iain Day	7	Using Fluoroaromatics as Probes of Small Molecule Self-Aggregation
Regina Easley	22	Determination of Spectrophotometric pH Indicator Purity Using Quantitative ^{1}H NMR
Franziska Fendt	9	^{29}Si and ^{119}Sn NMR Studies on $E4^{4-}$ Zintl Anions in Liquid Ammonia
Erwin García	29	Effectiveness of CRAFT in Quantifying Serum Metabolites in the Presence of Spectral Distortions and Sharp/Broad Peak Overlap
Manuela E. García	63	Di(ethylene glycol) Methyl Ether Methacrylate (DEGMA) Gels Align Small Organic Molecules in Methanol
Ion Ghiviriga	40	A Modified EXSIDE Experiment for the Measurement of ^{19}F-^{15}N Coupling Constants at Natural Abundance
Patrick Giraudeau	4	In situ EC-NMR by Real-time 1D and 2D Spectroscopy
Simon Glanzer	16	Homonuclear J-Scaling During Acquisition
John Glushka	58	Software Tools for Simulating NOEs and J-Coupling from Long MD Trajectories of Flexible Molecules
Igor Goljer	55	Utilizing Fluorine NMR in the Detection and Structural Elucidation of Metabolites
Michael M Hammer	2	Unravelling Organocatalytic Reaction Mechanisms by NMR-Spectroscopy

Emmanuel Hatzakis	1	Compositional Analysis of Encapsulated Fish Oil Supplements using Multinuclear and Multidimensional NMR Spectroscopy
Jaroslav Havlicek	17	Characterization of Active Pharmaceutical Ingredients Using Solid-State NMR Spectroscopy
Erich Hellemann	62	Pure Shift F1 Proton-Coupled J Scaled BIRD HSQC to Measure RDCs with Higher Accuracy and Sensitivity: Features and Limitations
Jochen Junker	24	Can NMR Correlations Determine Your Structure Unambiguously?
Philip Keyes	23	Automated Structure Verification: High Profile Patent Issues Building a C.A.S.E. for Enhanced Compound Quality Assurance
Carina Koch	5	Mechanistic Studies of Transmetalation Intermediates in Copper-Catalyzed 1,4-Addition Reactions by Modified 1D $^{1}H^{31}P$-HMBCs
Ard Kolkman	33	A Combined Approach: Highly Sensitive Pure Shift NMR and Structure Elucidation of ppm-impurities in Bulk Chemicals
Aleksej Krunić	57	Application of Ultra-high Field NMR Spectroscopy in Characterization of Natural Products
Till Kühn	64	From Automatic Structure Consistency Analysis to Structure Verification in NMR
Marek Kuzma	61	The MSG Mouse Model of Obesity Studied by NMR Metabonomics
Valdemar Lacerda Jr.	35	Unequivocal Structural Assignments of Three Cycloheptenoid Intermediates for Guaiane Sesquiterpenes: An Experimental and Theoretical Approach
Melissa Lin	59	Investigation of the Root Cause of Opalescence of an Injectable Drug Product by Diffusion Ordered Spectroscopy
Bertram Manz	15	Latest Developments and Applications for High Resolution Multinuclear Benchtop NMR Spectrometers
Michelle Markus	27	Full Automation of Routine NMR Aanalysis for Polymers, Using Assure-RMS
Michelle Markus	28	What is in Your Dishwashing Liquid? Developing Fully Automated Screening Methods for Commercial Products
Estelle Martineau	32	Non-linear Effects in Quantitative 2D NMR of Polysaccharides: Pitfalls and How to Avoid Them
C. Benjamin Naman	31	Determination of an Unprecedented Fused Pentacyclic Flavonoid Skeleton by Computer-Assisted Structure Elucidation of Black Chokeberry (Aronia melanocarpa) Fruit Juice Isolates
Ikenna Ndukwe	14	Sensitivity and Resolution Enhancement in EXSIDE and Band Selective EXSIDE
Teodor Parella	46	Fast and Efficient Enantiodifferentiation Through Pure Shift NMR
Teodor Parella	47	Perfect-HSQC: Suppression of Phase and Amplitude J(HH) Modulations
Rogelio Pereda	51	DFT ^{1}H-^{1}H Coupling Constants in the Configurational Reassignment of Synargentolide A
Andrew Phillips	3	Using NMR to Gain Understanding of Dissolution Processes
Dmitri Pichugin	52	Evaluating the Relative Merits of CRISIS-HSQC, Pure Shift-HSQC and ASAP-HMQC for Rapid Screening of Organic Compounds

Radek Pohl	37	Tautomerism of Isocytosine - Low-temperature NMR Study
Eliška Procházková	12	‘Planamerism’: A Novel Stereochemical Phenomenon?
Josep Saurí	48	Pure In-Phase Heteronuclear Correlation NMR Experiments
Josep Saurí	49	Simultaneous Measurement of the Magnitude and the Sign of Multiple Heteronuclear Coupling Constants in ^{19}F- or ^{31}P-Containing Molecules
Andrea Sefler	30	NMR For Quality Control of Injection Solution Mixtures: Comparison of Integration and CRAFT Analysis
Maria Victoria Silva Elipe	34	Application of ^{19}F Time-Domain NMR to Measure Content in FLuorine Containing Drug Products
Eduardo Sousa	11	Spectroscopic Characterization of Polimorphism in Efavirenz
Iain Swan	38	Measuring and Mitigating Convection in NMR
Godiraone Tatolo	18	Constant Time Gradient CRISIS HMBC (CTgc2HMBC) – Quantifying Improvements in SNR and Resolution
Donald Thomas	44	Oils of Provenance: Quantitative NMR Using Natural Abundance Deuterium
Mateusz Urbanczyk	19	Diffusion-ordered NMR with Joint Sparse Time-gradient Domain.
Janaina Vilcachagua	36	Structural Assignment of Non-aromatic B-ring Flavanones from Piper Carniconnectivum C. DC by NMR, ECD and DFT Calculations
Jan-Christoph Westermann	43	Free Ligand Conformational Populations in Solution – A Powerful Drug Discovery Tool
Patrick Wheeler	54	Structure Revision of Acremolin: CASE Approach Based on 2D NMR vs. Total Synthesis
Thomas Williamson	56	LR-HSQMBC: A Highly Sensitive NMR Technique to Probe Very Long-Range Heteronuclear Coupling Pathways
Christiane Wolff	6	Kinetic Study of Olefin Metathesis Reactions using a Benchtop NMR Spectrometer
Xiaoda Yuan	39	Characterizing Hydrogen Bonding Interactions in Amorphous Solid Dispersions Using Solid-State NMR Spectroscopy
You Zhuo	60	Monitoring In-Cell Metabolism with High Sensitivity Using DNP Polarized Substrates

1 Compositional Analysis of Encapsulated Fish Oil Supplements using Multinuclear and Multidimensional NMR Spectroscopy

Emmanuel Hatzakis[1], Maria Misiak[2], and Photis Dais[2]

1. Penn State University
2. University of Crete

Multinuclear (^{1}H, ^{13}C, ^{31}P) and multidimensional NMR spectroscopy was employed for the qualitative and quantitative analysis of marine oil supplements. A variety of selective and non-selective 2D NMR techniques facilitated the assignment of ^{1}H and ^{13}C NMR spectra The integration of the appropriate signals in the NMR spectra of the three nuclei allowed the determination of fish oil composition. NMR methodology requires minimal sample pretreatment compared to other analytical methods and offered a rapid and reproducible method for fish oil supplements evaluation. Results confirmed the presence of *trans* fatty acids in fish oil supplements and revealed for first time the presence of ω-1 acyl chains which exist in the form of free fatty acids as shown from our diffusion studies. Moreover, ^{31}P NMR spectroscopy after phosphitylation of the fish oil dietary supplement with the phosphorus reagent 2-chloro-4,4,5,5-tetrachloro-3,5-dioxaphospholane enabled the quantitative determination of mono- and diacylglycerols, free glycerol and fatty acids, as well as the precursor of the vitamin D3, 7-Dehydrocholesterol, the signal of which coincides with that of cholesterol in the spectrum.

1. Aursand, M.; Standal, I.; Axelson D. High-Resolution 13C Nuclear Magnetic Resonance Spectroscopy Pattern Recognition of Fish Oil Capsules. J. Agric. Food Chem. 2007, 55, 38-47.
2. Hatzakis, E.; Dagounakis, G.; Agiomirgianaki, A.; Dais, P. A Facile NMR Method for the Quantification of Total Free and Esterified Sterols in Virgin Olive Oil. Food Chemistry, 2009, 122, 346-352.

2 Unravelling Organocatalytic Reaction Mechanisms by NMR-Spectroscopy

Michael M. Hammer[1], Markus B. Schmid[2] and Ruth M. Gschwind[1]

1. University of Regensburg, Regensburg, Germany
2. Boehringer Ingelheim, Biberach, Germany

In this poster we present our efforts to shed light on organocatalytic reaction mechanisms by NMR spectroscopy. We focus on the field of aminocatalysis, with its three major pillars, the iminium, enamine and dienamine-catalysis.[1] In this class of catalysis an amine condeses with a carbonyl species to yield a reactive intermediate which itself can then react further to the desired products.

Our approach is to mainly investigate the key intermediates in all types of transformations by a combination of different NMR techniques.

We were able to stabilize the first enamine intermediate in proline catalysis and were able to perform extensive structural studies as well as reactivity studies with this key intermediate. By EXSY spectroscopy we deducted the interplay of all found intermediates and were able to construct a concise mechanism for the formation of enamine intermediates from their oxazolidinone partners.[2] We were also able to stabilize prolinol-derived enamines and determine reaction pathways with different outcomes by deuteration studies.[3] In a detailed structural study by NOE and selective NOE measurements we were able to unravel the most probable cause of stereodiscrimination in this class of enamine-catalysis.[4] The influence of cocatalysts on the structure of reaction intermediates was also addressed and we could identify a base-enamine complex which can offer the explanation for the observed reversed selectivity in base-cocatalysed cases.[5]

In the field of iminium catalysis we were able to stabilize the first non-conjugated iminium ion in aminocatalysis. We performed structural as well as reactivity studies and could deduce that the formation of iminium-ions is largely only dependent on the prevention of enamine or dienamine formation and presents as itself no stable intermediate as long as enamine or dienamine-formation is feasible.

We could stabilize a number of dienamine-intermediates in the rapidly developing field of dienamine-catalysis. Here an a,ß-unsaturated aldehyde is condensed with an aminocatalyst and yield a dien-structure which can further react with electrophiles in ?-position. We were able to determine the reaction barrier for dienamine formation by Arrhenius-analysis of react-NMR studies and were able to determine exchange processes within the dienamine-iminium network by VT-NMR studies. We were also able to structurally investigate the intermediates by NOE-spectroscopy and give conclusive hints towards the reason for stereodiscrimination in dienamine-catalysis.

1. Nielsen, M., Worgull, D., Zweifel T., Gschend, B., Bertelsen, S., Jørgensen, K. A., Chemical Communications, 47 (2), 632-649, 2011
2. Schmid, M. B., Zeitler, K., Gschwind, R. M., Angew. Chemie Int. Ed., 49, 4997–5003, 2010
3. Schmid, M. B., Zeitler, K., Gschwind, R. M., The Journal of Organic Chemistry, 76, 3005-3015, 2011

4. Schmid, M. B., Zeitler, K., Gschwind, R. M., Journal of the American Chemical Society, 2 (9), 1793-1803, 2011
5. Schmid, M. B., Zeitler, K., Gschwind, R. M., Chemistry - A European Journal, 18 (11), 3362-3370, 2011

3 Using NMR to Gain Understanding of Dissolution Processes

Steven R. Coombes, Leslie P. Hughes, **Andrew R. Phillips**, and Stephen A. C. Wren

AstraZeneca, Pharmaceutical Development, Macclesfield, Cheshire, United Kingdom.

Dissolution testing is a key aspect of the analysis of pharmaceutical products and in particular oral immediate release dosage forms such as tablets. Dissolution testing demonstrates that the active pharmaceutical ingredient (API) will be released from the tablet into solution and so will be available for absorption in the gastro-intestinal tract. It also helps to ensure the quality and consistency of established commercial pharmaceutical products.

We present the use of ^{1}H NMR as a new measurement approach for improved understanding of the dissolution of pharmaceutical tablets. [1] NMR has benefits over the conventional UV measurement approach in respect to much greater analyte selectivity and the ability to detect non-UV absorbing species such as sugars or polymers, commonly used excipients.

NMR data was obtained in two ways: using an in-line flow cell [2] and off-line sampling. From both sets of data we have determined the release profiles of multiple APIs and excipients present in the same tablet. All dissolution experiments were performed in a pharmacopoeial dissolution system with a standard protic buffer. Generally sufficient sensitivity can be obtained to determine the full release profile of even compounds present at relatively low amounts in the tablet. The in-line flow cell gives excellent quality NMR spectra having little impact on peak shape.

1. Analytical Chemistry, 86 (5), 2474 – 2480
2. Magn. Reson. Chem., 48 (7), 516 – 522

4 *In situ* EC-NMR by Real-Time 1D and 2D Spectroscopy

Renaud Boisseau[1], Ugo Bussy[1], Mohammed Boujtita[1] and **Patrick Giraudeau**[1,2]

1. Université de Nantes, CNRS, CEISAM UMR 6230, Nantes, France
2. Institut Universitaire de France (IUF), Paris, France

The *in situ* combination of electrochemistry and NMR (EC-NMR) is a rapid and essential way to elucidate in real time the molecular structures involved in electrochemical reactions. It is particularly useful to elucidate metabolic reaction pathways.[1] However, it is highly demanding in terms of experimental settings, due to the need to introduce electrodes inside the NMR tube and to implement adapted detection methods.

We recently designed a 3-electrode system made of carbon microfibers, carefully adapted to fit inside a 5-mm NMR tube and connected to a potentiostat, thus making it possible to monitor oxidation reactions in real time by liquid-state NMR experiments. We applied this approach to elucidate the oxidation pathway of phenacetin, a small molecule involved in the metabolism of paracetamol and also recognized as an emerging pollutant.[2] The reaction mechanisms were further elucidated by additional off-line quantitative NMR experiments, [3] revealing new insights in the field of drug oxidative metabolism.

While ^{1}H NMR is a useful tool for such real-time studies, it is hampered by significant line broadening brought by the electrochemical devices inside the sensitive volume. It results in strong peak overlap and partial loss of the multiplet structure. 2D NMR brings an attractive solution to this limitation, but its timescale is barely compatible with real-time EC-NMR. Fortunately, this drawback can be circumvented by fast 2D acquisition methods such as ultrafast (UF) 2D NMR.[4] We will present the first coupling of UF 2D spectroscopy with in situ EC-NMR, which enables the real-time acquisition of 2D COSY spectra in the course of an oxidation reaction. These results open promising perspectives towards a more general use of real-time EC-NMR.

1. H. Simon, D. Melles, S. Jacquoilleot, P. Sanderson, R. Zazzeroni, U. Karst, Anal. Chem. 84 (2012) 8777-8782.
2. U. Bussy, P. Giraudeau, V. Silvestre, T. Jaunet-LAhary, V. Ferchaud-Roucher, M. Krempf, S. Akoka, I. Tea, M. Boujtita, Anal. Bioanal. Chem. 405 (2013) 5817-5824
3. U. Bussy, P. Giraudeau, I. Tea, M. Boujtita, Talanta 116 (2013) 554-558
4. P. Giraudeau, L. Frydman, Annu. Rev. Anal. Chem. (2014), in press

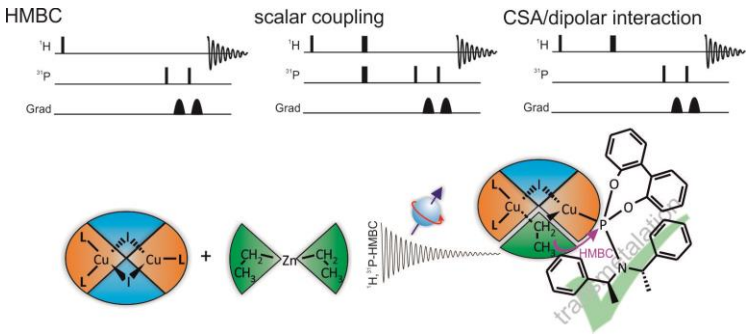
5 Mechanistic Studies of Transmetalation Intermediates in Copper-Catalyzed 1,4-Addition Reactions by Modified 1D $^{1}H^{31}P$-HMBCs

Carina Koch, Felicitas von Rekowski and Ruth M. Gschwind

Department of Organic Chemistry, University of Regensburg, Germany

Asymmetric copper-catalyzed conjugate addition reactions are a very powerful and widely applied method for enantioselective carbon-carbon bond formation. By the use of a cheap catalytic system composed of Cu(I)-salts and chiral phosphoramidite ligands, it is possible to create new stereocentres with quantitative yields and *ee*-values up to 99 %.[1-4] Despite the great success of this class of reaction, a direct experimental proof of the widely accepted transmetalation intermediate[5] was missing until now, most likely because the magnetization transfer via copper of structures deviating from a highly symmetrical coordination is very challenging, due to the high quadrupole moment of $^{63/65}Cu$ and the high electric field gradient of these complexes. But the detection and characterization of intermediates in catalytic reactions is crucial for the understanding of the mechanisms and the rational optimization of reaction conditions. Therefore, we performed experiments, based on our experiences in the research field of the detection of H-bond networks,[6] where we used a special approach of modified 1D $^{1}H^{31}P$-HMBC spectra. With this special approach we were able to identify intermediates with alkyl groups directly bound to copper. Moreover, samples of enantiopure and enantiomeric mixtures of ligands for the structural characterization of the intermediates. With this special approach in hand and after screening different organometallic reagents (ZnR_2; R=Et, Me, Ph; MeLi) we were able to obtain the first direct experimental proof for the elusive transmetalation intermediate. Beside monomeric intermediates with one ligand, for the first time also a dimeric transmetalation intermediate with a mixed trigonal/tetrahedral coordination on the copper atoms is observed, which is comparable to the recently elucidated precatalytic complex of this reaction.[5,7,8]

Furthermore, we are investigating the appearing interactions between the phosphoramidite ligands and the organometallic reagents ($ZnMe_2$; MeLi).



1. Alexakis A. et al., Chem. Rev., 108, 2796, 2008.
2. T. Jeraphagnon et al., Chem. Soc. Rev., 38, 1039, 2009.
3. N. Krause et al, Synthesis, 2, 171, 2001.
4. B. L. Feringa et al., Angew. Chem. Int. Ed., 49, 2486, 2010.
5. R. M. Gschwind et al., Chem. Eur. J., 13, 6691, 2007.
6. R. M. Gschwind et al., J. Am. Chem. Soc., 130, 16846, 2008.
7. R. M. Gschwind et al., Angew. Chem. Int.Ed., 45, 6391, 2006.
8. R. M. Gschwind et al., J. Am. Chem. Soc., 130, 12310, 2008.

6 Kinetic Study of Olefin Metathesis Reactions using a Benchtop NMR Spectrometer

Christiane Wolff[1], Ernesto Danieli[2], Bernhard Blümich[2], Federico Casanova[3], and Christina M. Thiele[1]

1. Clemens-Schöpf-Institute for Organic Chemistry and Biochemistry, TU Darmstadt, D-64287 Darmstadt, Germany
2. Institut für Technische Chemie und Makromolekulare Chemie, RWTH Aachen, D-52074 Aachen, Germany
3. Magritek GmbH, D-52074 Aachen, Germany

As the olefin metathesis is now an established tool for organic and polymer chemists [1-3], the need for a detailed understanding of its mechanism is inevitable for the development of new catalysts. While most of the investigations focus around the detection of the catalyst itself [4, 5], more recent work uses NMR spectroscopy to monitor the product formation [6]. By examining the conversion of the substrate all three key factors of the metathesis reaction (initiation, catalysis and decomposition) can be investigated simultaneously.

Using NMR spectroscopy as observing method the reaction can be monitored without disturbance over the whole time. Given that most of the reactions are run with a catalyst loading and activity so that the reaction time is about 30 minutes to 3 hours, the first few minutes contain most of the kinetic information. Because of an external injection and the experimental setup of high-field NMR spectrometer exactly this first minutes are lost or defective [6].

In the last years a benchtop NMR spectrometer based on permanent magnets was developed at RWTH Aachen University [7, 8]. Besides its economic advantages (lower acquisition and maintenance costs), this technology allows one to perform NMR spectroscopy measurements in an easy and fast way. By employing this kind of setup to our kinetic investigation, we were able to reduce the dead time by 50%. The new and more accurate data points from the first minutes can be used to support the kinetic studies done at a high-field NMR spectrometer.

1. Chauvin, Yves, Angew. Chem., 118(23), 3824, 2006.
2. Hoveyda, Amir H., Zhugralin, Adil R., Nature, 450(7167), 243, 2007.
3. Opper, Kathleen L., Wagener, Kenneth B., J. Polymer Sci. A, 49(4), 821, 2011.
4. Sanford, Melanie S., Love, Jennifer A., Grubbs, Robert H., J. Am. Chem. Soc., 123(27), 6543, 2001.
5. Thiel, Vasco, Hendann, Marina, Wannowius, Klaus-Jürgen, Plenio, Herbert, J. Am. Chem. Soc., 134(2), 1104, 2012.
6. Thiel, Vasco, Wannowius, Klaus-Jürgen, Wolff, Christiane, Thiele, Christina M., Plenio, Herbert, Chem. Eur. J., 19(48), 16403, 2013.
7. Danieli, Ernesto, Perlo, Juan, Blümich, Bernhard, Casanova, Federico, Angew. Chem., 122(24), 4227, 2010.
8. Küster, Simon K., Danieli, Ernesto, Blümich, Bernhard, Casanova, Federico, Phys. Chem. Chem. Phys., 13, 13172, 2011.

7 Using Fluoroaromatics as Probes of Small Molecule Self-Aggregation

Jonathan R. Katz[1], Roseanne Briffitt[1], Lucy J. Day[2], and **Iain J. Day[1]**

1. School of Life Sciences, University of Sussex, Brighton, UK
2. BHASVIC, 205 Dyke Road, Hove, East Sussex, UK

The self-association of small molecules has impact across a wide range of fields, from drug delivery and storage to liquid crystals and supramolecular chemistry. Small molecules are often used as probes of aggregation, for example the use of a thioflavin T fluorescence assay to monitor amyloid fibril formation, or are themselves incorporated into larger assemblies such as gas storage in metal-organic frameworks.

Recently, using the azo-dye sunset yellow FCF as a model system which shows concentration-dependent self-association [1], we have investigated the use of small fluoroaromatics as probes of this aggregation process [2]. We chose these molecules as they have similar structures to portions of sunset yellow and hence should interact with the assemblies without causing any significant disruption. The inclusion of a unique ^{19}F atom allows an NMR measurement which is background-free, i.e. there are no signals observed from the assemblies, just the probe molecules. The probe molecules chosen are the structure isomers of fluorophenol and two isomers of fluoronaphthoic acid.

We have utilised a combination of careful chemical shift and diffusion coefficient measurements to investigation the addition of the small molecule probes, initially at a low concentration of 1 mol%, to samples of sunset yellow [2,3]. Using a combination of chemical shift and diffusion coefficient measurements we can describe two binding modes for the probe: one to the ends of the sunset yellow stacks and a second for incorporation into the stackes [2,3].

In this poster we expand on this work to include the probe species at higher relative concentrations. It appears that the binding of fluorophenol to sunset yellow aggregates is largely independent of concentration, although minor variations are seen at very high relative concentrations. We also investigate the role of fluorine atom incorporation and isomerism on the self-assocation of the naphthoic acid probe species themselves.

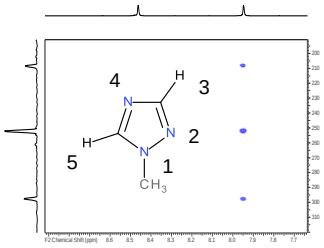
1. Renshaw, Matthew P.; Day, Iain J.; J. Phys. Chem. B. 114(31), 10032-10038, 2010
2. Katz, Jonthan R.; Day, Lucy J.; Day, Iain J.; J. Phys. Chem. B 117(39), 11793-11800, 2013
3. Katz, Jonathan R.; Day, Iain J.; Magn. Reson. Chem. In Press, 2014

8 Exploiting Natural Abundance ^{13}C–^{15}N Coupling as a Method for Identification of Nitrogen Heterocycles: Practical use of the HCNMBC Sequence

Eriks Kupce[1], Mike Kline[2], and **Steve Cheatham[2]**

1. Bruker UK Limited, Banner Lane, Coventry, CV4 9GH, UK
2. DuPont Crop Protection, Stine Haskell Research Center, Newark, DE 19714, USA

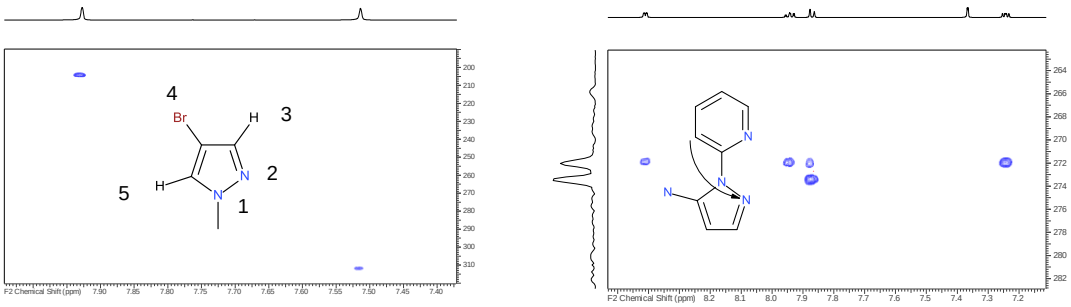
Synthesis of heterocycles can often produce isomers that may be challenging to identify. Direct ^{1}H-^{15}N long range experiments are a invaluable tool for identifing isomers but the experiments are not without limitations. Recently, we have introduced a new experiment, HCNMBC[1], which permits ^{1}H-^{15}N correlation via the natural abundance ^{13}C-^{15}N coupling. In this paper we detail the results of the experiment on a number of classes of azoles including pyrazoles, imidazoles and triazoles. Sample quantities required are reasonable and on the same order as those needed for 1,1-adequate. The experiment produces data which can be highly complementary to direct ^{1}H-^{15}N HMBC type correlations in that it provides ^{15}N chemical shift data for nitrogens that may not show up in the HMBC. We demonstrate that this is particularly advantageous in the triazoles where ^{15}N chemical shift can be diagnostic of regiochemistry (Fig A).



A

In addition, data indicate that the experimental parameters can be adjusted to produce defined results in certain heterocyclic systems emphasizing, for example, two-bond specific correlations in some instances (Fig. B) or, conversly, long range 4-bond correlations which occur through 3-bond ^{13}C-^{15}N coupling (Fig C).

It is therefore critical in setup and analysis of the data to have a general understanding of the magnitude of the carbon-nitrogen coupling constants within various heterocyclic types and to develop a database of this information. Preliminary results of our work in this area will also be presented.



B **C**

1. Steve Cheatham, Peter Gierth, Wolfgang Bermel and Ēriks Kupče, HCNMBC – A pulse Sequence for H-(C)-N Multiple Bond Correlations at Natural Isotopic Abundance, *J. Magn. Reson. submitted, (2014).*

9 ^{29}Si and ^{119}Sn NMR Studies on E4^{4-} Zintl Anions in Liquid Ammonia

Franziska Fendt[1], C. Koch[2], M. Neumeier[2], S. Gärtner[1], N. Korber[1], and R. M. Gschwind[2]

1. Institute of Inorganic Chemistry, University of Regensburg, Germany
2. Institute of Organic Chemistry, University of Regensburg, Germany

Soluble homoatomic, discrete compounds containing main group elements in negative oxidation states are widely limited to solid state materials, where an p-block element is reacted with an electropositive s-block metal. The resulting compounds are called Zintl phases. These intermetallic materials contain discrete anionic moieties and can be readily dissolved in appropriate solvents (en, NH_3) yielding naked cluster anions in solution. A second preparation method for these Zintl anions in solution is the direct reduction process of a p-block element by a electropositive metal in liquid ammonia. Both preparation methods have been known for a long time, but the underlying solvation, transformation and decomposition processes of group 14 compounds have not been investigated at all. In contrast, solutions of group 15 polyanions were studied intensely by Baudler *et al.* [1]. So, we focused on the dissolution of group 14 Zintl phases in liquid ammonia at low temperatures (bp(NH_3)=240.15K) monitored by one-dimensional solution NMR spectroscopy in order to get more insight. Recently, circumstantial evidence for solution chemistry of A_4E_4 (A=alkali metal, E=group 14 element) which contain tetrahedral E_4^{4-} anions in liquid ammonia gave rise for further systematic investigations [2]. For the preparation of the samples the extremely air and moisture sensitive precursor materials were placed in high pressure NMR tubes under inert atmosphere before condensing liquid ammonia. Melt off NMR tubes even allowed NMR measurements at room temperature and we were able to observe the dissolution of the highly charged tetrahedral Zintl clusters Si_4^{4-} and Sn_4^{4-} via ^{29}Si and ^{119}Sn NMR spectroscopy for the first time. To facilitate the NMR detection in solution, ^{29}Si isotope labeling was applied. We also could show that solvent protons are responsible for the oxidation of Sn_4^{4-} to Sn_9^{4-} by enhancing the S/N ratio using the ^{1}H PRESAT pulse sequence in order to suppress the solvent protons [3]. This allowed for the detection of low concentrations of amide NH_2^- beside NH_3. The distinctive ^{117}Sn satellite pattern was used for the assignment of the cluster sizes Sn_x based on previous ^{119}Sn NMR studies in en solutions [4].

The addition of chelating reagents like [2.2.2]-cryptand affected the predominant cluster sizes in solution significantly. The detection and characterization of these polyanions is crucial for the rational optimisation of reaction conditions and open up the opportunity of directed functionalization with (post-)transition metal complexes which up to now is based on empirical evidence of crystallized reaction products [5]. In this term we investigated the reactivity of Sn_4^{4-} towards mesitylcopper(I) and diphenylzinc by means of spectroscopic and crystallographic characterization of new coordination compounds of Sn_4^{4-} [6]. Obviously, the choice of synthetic route, additives, temperature and time range is determing for the stability of the E_4^{4-} species in solution. The common issue of all these variables is to reduce the proton mobility and slow down occuring oxidation reactions. Latest ^{87}Rb, ^{23}Na and ^{7}Li NMR investigations in liquid ammonia indicated that the role of the alkali cation is more significant as expected.

1. Baudler M., Glinka K., Chemical Reviews, 93, 1623-1667, 1993
2. Wiesler K., Brandl K., Fleischmann A., Korber N., Zeitschrift für Anorganische und Allgemeine Chemie, 635, 508-512, 2009
3. Neumeier M., Fendt F., Gärtner S., Koch C., Gärtner T., Korber N., Gschwind R. M., Angewandte Chemie, 125, 4579-4582, 2013
4. Haushalter R. C., Rudolph R. W., Journal of the American Chemical Society, 4629, 1978
5. Scharfe S., Kraus F., Stegmeier S., Schier A., Fässler T. F., Angewandte Chemie, 123, 3712-3754, 2011
6. Fendt F., Koch C., Gärtner S., Korber N., Dalton Transactions, 42, 15548-15550, 2013

10 Merging NMR and UV/Vis for Mechanistic Studies on Photocatalytic Systems

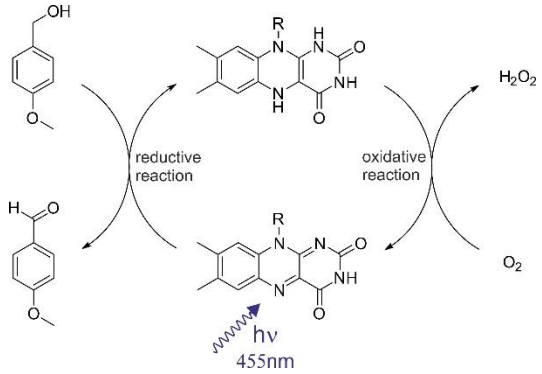
Hanna Bartling, Christian Feldmeier, and Ruth M. Gschwind

Institute of Organic Chemistry, University of Regensburg, Regensburg, Germany

Nowadays the sustainability and a "green" chemistry became of vast importance. This can be seen in the fast developing fields of photochemistry and photo- catalysis and the development and investigation of many new synthesis and synthetic applications is owed to that. However, the knowledge of the corresponding reaction mechanisms is really slight and mechanistic studies are scarce. But a profound knowledge of the mechanism is of high importance especially for the optimization of e.g. reaction conditions.

So for most comprehensive mechanistic studies of photocatalytic reactions the potentials of NMR and UV/Vis spectroscopy were merged. By this new approach we have the possibility to gain new insights in the reaction mechanism, we were not able to access before. UV/Vis spectroscopy can detect excited states and the initial reaction steps on an ultrafast time scale [1], while NMR spectroscopy can give information about structure, aggregation and solvent effects.

As a model system the photooxidation of *para*-methoxybenzyl alcohol to the corresponding aldehyde by riboflavin tetraacetate [2,3] was selected because of flavins optimal UV/Vis properties and the diamagnetic nature of starting material and products enabling both spectroscopies. The schematic mechanism is shown in the Figure below.



To enable the *in situ* detection of photoreactions and intermediates by NMR spectroscopy an LED based illumination device was developed, consisting of an optical fiber guiding the light of the LEDs into the NMR tube inside of the spectrometer [4]. This setup enables all the classical NMR methods for the detection and characterization of diamagnetic species, elusive intermediates and reaction profiles, but besides this even millisecond time resolved Photo-CIDNP spectroscopy (Photo-Chemically Induced Dynamic Nuclear Polarization) is possible [5,6].

For the NMR measurements the samples were illuminated with blue LEDs. By montoring the reaction profiles by a row of ^{1}H NMR spectra we found a strong solvent dependency of the reaction going from pure acetonitril to acetonitril/water mixtures of 1:1. The reaction turned out to be much more efficient in the mixture than in pure acetonitrile. Further the occurrence of a resting state in the solvents mixture, together with Photo-CIDNP effects and the UV/Vis measurements support our results of two different reaction mechanisms, switchable by the solvent.

1. Mergele U.; Wenninger M.; Kutta R.-J.; Lechner R.; König B.; Dick B.; Riedle E.; Phys. Chem. Chem. Phys. 13, 8869, 2011
2. Schmaderer H.; Hilgers P.; Lechner R.; König B.; Adv. Synth. Catal. 351, 163-174, 2009
3. Svoboda J.; Schmaderer H.; König B.; Chem. Eur. J. 14, 1854-1865, 2008
4. Feldmeier Ch.; Bartling H.; Riedle, E.; Gschwind R. M.; J. Magn. Reson. 232, 39-44, 2013
5. Kuprov I.; Hore P.J.; J. Mag. Res. 171, 171-175, 2004
6. Goez M., Annu. Rep. NMR Spec., 66. 77-147, 2009

11 Spectroscopic Characterization of Polimorphism in Efavirenz

Eduardo Gomes Rodrigues de Sousa[1,2], Erika Martins de Carvalho[1], Leandro B. Borré[2], and Rosane Aguiar da Silva San Gil[2]

1. Instituto de Tecnologia em Fármacos/Fiocruz, Rio de Janeiro, RJ, Brazil.
2. Universidade Federal do Rio de Janeiro, IQ, Rio de Janeiro, RJ, Brazil.

Despite efforts in the fight against the Human Immunodeficiency Virus (HIV) and the enormous advances in the therapy and prevention of this disease, the number of people living with the HIV virus continues to grow. Efavirenz (EFZ) is one of the most important drugs used in anti HIV cocktail distributed free by the *Sistema Único de Saúde (SUS)* - Unified Health System (UHS). The structure of EFZ presents different hydrogen bonding sites that increase the possibility to exhibit different crystal packing upon crystallization. Due to this fact, several authors have investigated the solid state structures of crystallized products of EFZ.

Today, about 90% of drugs administered in solid form, which implies the formulation and processing of the drug or active pharmaceutical ingredient (API) powder. Typically, the pharmaceutical development process conducted in powdered API most stable crystalline form, in order to ensure reproducible bioavailability of the product be subjected to various conditions in the real world. In the solid state, the compounds can exist in more than one polymorphic form. Such forms often have significant differences in solubility, bioavailability, processability and physical-chemical stability. Furthermore, the extreme conditions used for drug formulations may change with possible increase in interactions with excipients, or even affecting significantly on its stability [1].

Therefore, several spectroscopic and thermal techniques have been used in combination to characterize the drug in the solid state, identifying possible structural changes from the formulation and storage process. In this context, the technique of nuclear magnetic resonance (NMR) stands out especially among drug characterization techniques and more specifically, the solid state NMR because is a technique that can be used to study the crystallographic effects such as polymorphism, indicating the number of molecules in the unit cell, in the disorder of the system, the intermolecular and intramolecular hydrogen bonds, etc. In addition, unlike other techniques, solid state NMR can be applied to all physical forms of a solid (crystalline and amorphous), as well as the complexity of many different materials, such as pure API or solid dispersion, including the commercial formulations.

The aim of this study was to characterize the polymorphic forms of EFZ and to investigate the influence of different solvents in the recrystallization of EFZ form I, using spectroscopic methods, such as IR, NMR in solid and liquid state, and thermal analysis like DSC and TGA.

The API of micronized EFZ (EFZ/MIC), form I, was recrystallized using different organic solvents. For this purpose, approximately 1g of the API was dissolved in n-heptane (EFZ/HEP), acetonitrile (EFZ/ACN), methanol (EFZ/MeOH), tetrahydrofuran (EFZ/THF) and hexane (EFZ/HEX), under constant heating up to boiling [2]. After boiling, the solution was filtered and waited until complete precipitation of crystals. The samples were filtered under vacuum and stored in a desiccator.

I.R. analyzes were performed on equipment model Prestige 800 of Shimadzu FTIR in KBr pellets. The melting points were measured on a device BUCHI Melting Point B-545, with a heating rate of 10 °C/min. Thermogravimetric (TGA) and Differential scanning calorimetry (DSC) analysis were carried out in a NETZSCH STA 449 F3 Jupiter® thermal balance, by using 4.0-8.0 mg of samples with a 10 °C/min-1 heating rate. For TGA a nitrogen flow rate of 50 ml/min dry nitrogen and heating from 25 to 300 °C, whereas for DSC assays at 80 ml/min of dry nitrogen and heating from 25 till 210 ºC. The instrument was calibrated using indium reference material. The solution NMR spectra was obtained in a Bruker Avance 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) using $CDCl_3$ as solvent (zg30, d1 = 1s). ^{13}C CPMAS solid state NMR spectra were recorded on a Bruker Avance III 400 (9.4 T) spectrometer, operating at 100.64 MHz. The contact time was optimized to 10,000 μs. The recycle delay was 4s, and π/2 pulse length was 4 μs.

The experimental melting points were a little different than the others measured in the literature [2] (presenting ΔT(ºC) between 0.99 and 4.25. The preliminary DSC measurements showed, for the samples recrystallized with n-heptane (EFZ/HEP) and tetrahydrofuran (EFZ/THF), two endothermic peaks: one in the region of form I melting point, and another in a different region suggesting a different polymorphic form. Thermogravimetric analysis (TGA) showed the normal degradation of the material at 225ºC. The I.R. analysis showed differences in the region of 3100-3400 cm^{-1} characteristic of N-H, 1600-1800 cm^{-1} of carbonyl group and 1100-1250 cm^{-1} characteristic of CF_3 group. This suggests the existence of different intermolecular interactions. To confirm that the recrystallization process does not alter the structure of the molecule, ^{13}C NMR in $CDCl_3$ were obtained and showed the same chemical shifts (δ) for all samples when compared with the EFZ form I. However, the spectra of solid state NMR showed significant changes for the samples EFZ/HEP, EFZ/MeOH and EFZ/THF when compared with EFZ/MIC, form I. The solid state NMR can be used to verify and identify different polymorphic forms. For example, the signal at 95 ppm of the C-10 can be used to do this evaluation. In the spectrum of EFZ/MIC, this signal is a triplet suggesting that the chemical environment is different and that can be exists different conformations in the unit cell. This signal has the same behavior for the sample EFZ/MeOH. By the other hand, the signal of C-10 to the sample EFZ/HEP was a thin simplet and could be an unique conformation in the unit cell. Another interesting fact was the broadening of the signal of C-10 to the sample EFZ/THF that suggests a sample amorphization or various conformations in the unit cell.

With the preliminary results of solid state NMR it was possible to verify that there were different conformations at the same unit cell, the possible amorphization of the sample EFZ/THF and the existence of just one conformer in the unit cell to the sample EFZ/HEP.

1. Tishmack, Patrick A.; Bugay, David E.; Byrn, Stephen R. Journal of Pharmaceutical Sciences, 92(3), 441-474, 2002.
2. Chada, Renu; Arora, Poonam; Saini, Anupam; Jain, Dharamvir S. Journal of Pharmacy and Pharmaceutical Sciences, 15(2), 234-251, 2012.

12 ‘Planamerism’: A Novel Stereochemical Phenomenon?

Eliška Procházková[1,2], Lucie Čechova[1,3], Ivana Císařová[2], Zlatko Janeba[1] and Martin Dračínský[1]

1. Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
2. Charles University in Prague, Faculty of Science, Prague, Czech Republic
3. Institute of Chemical Technology, Faculty of Chemical Technology, Prague, Czech Republic

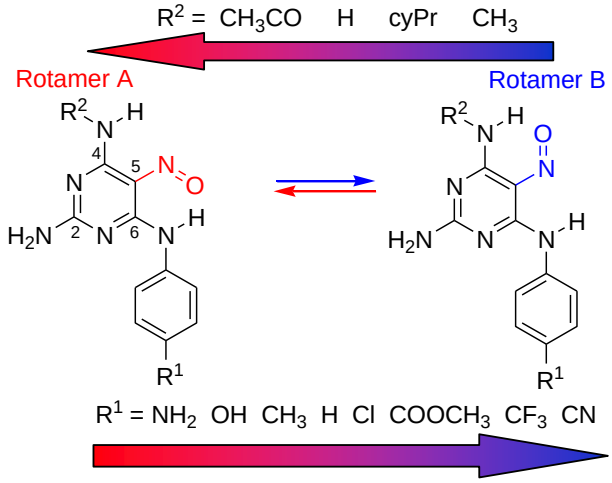


Figure 1. Substituent dependent switchable intramolecular hydrogen bonds

Recently, we have prepared a series of 32 polysubstituted 5-nitrosopyrimidine derivatives and we have studied a switchable intramolecular hydrogen bond using ^{1}H NMR spectroscopy [1]. The nitroso group can form hydrogen bonds with NH hydrogens in both neighboring positions 4 and 6, therefore, two orientations of the nitroso group are possible. It may lead to the two rotamers coexistence, see Fig. 1. They are observable in NMR spectra as two sets of signals. The rotamer ratio in equilibrium significantly depends on the nature of substituents and solvent and it can be finely tuned over a broad range of rotamer A (35 – 84 %), which can be well determined by ^{1}H NMR signals integration. Arisen hydrogen bonds are so strong that even at 140 °C no signal averaging in NH region was observed; the estimated rotation barrier is unusually high (> 20 kcal/mol). The orientation of nitroso group has a significant influence on the chemical shifts of the carbon atoms C4 and C6. For instance, when the nitroso oxygen atom is turned to the NH group at position 6 (rotamer A), the C-6 carbon atom is more shielded ($\Delta\delta$ higher than 10 ppm) than in rotamer B. The experimental data were confirmed by DFT calculations. Furthermore, we have also investigated the isotope exchange of hydrogen H-6 which participates on the hydrogen bonding in rotamer A in DMSO-d_6–CD_3OD mixture. As expected, the formation of hydrogen bond reduces the solvent accessibility to the hydrogen bond donors and the hydrogen-to-deuterium exchange is much slower than in the case of rotamer B.

Based on these findings, we decided to prepare a new series of substituted 5-nitrosopyrimidine derivatives with the aim to separate both rotamers from each other [2]. To our surprise, we were able to separate them by column chromatography even at room temperature and characterize them by common spectroscopic methods. We have also measured the kinetics of their interconversion using ^{1}H NMR spectroscopy in DMSO-d_6 solution. The NH hydrogen signals of the two rotamers are well separated and after their integration we determined the rotamer ratio in various times after dissolution. From these data, the rate constants and rotation barriers were determined.

We gently evaporated the solvents from separated rotamers of compound **1** (Fig. 2) and we measured solid-state ^{13}C NMR (CP-MAS) spectra. These data confirmed the rotamer purity (the minor rotamer NMR signals intensity was under the detection limit of solid-state NMR, ca. 5%). Interestingly, we were able to observe slow re-crystallization of amorphous rotamer B into the stable crystalline rotamer A in solid-state ^{13}C NMR spectra within several months. The separated rotamers have been also analyzed by solid-state IR spectroscopy and, like in the NMR spectra, significant differences between both rotameric forms have been observed. We also prepared single crystals of this compound suitable for X-ray diffraction experiments. After re-crystallization from different solvents we obtained different crystalline structures. From dry acetone we obtained pure rotamer A, but from an acetone-water mixture we obtained a monohydrate of this compound, where both rotamers were present (ca 80% of rotamer A). These experiments have unambiguously confirmed the structure of both rotamers.

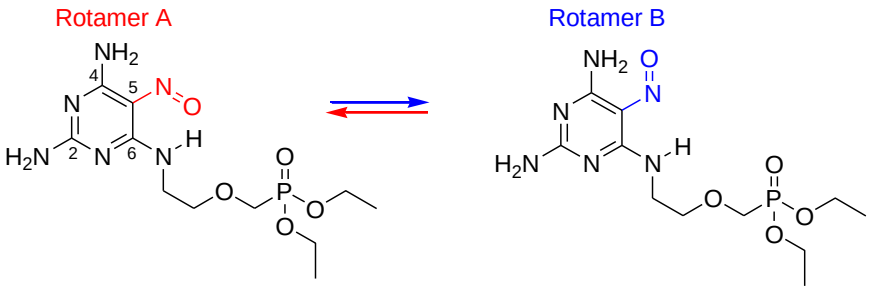


Figure 2. The two possible rotamers of compound **1**

Having the clear evidence of the structure of both separated rotamers and kinetics of their interconversion, we considered this behavior as a special case of atropisomerism. However, comparing atropisomers with our rotamers, we found significant differences between them. The most obvious one is their mode of stabilization. Contrary to atropisomers, which are stabilized by sterically hindered rotation, our rotamers are isolable thanks to the strong intramolecular hydrogen bonds. Because our rotamers are planar molecules, we term this phenomenon 'planamerism' (planar isomerism) and rotamers A and B 'planamers'. Planamers are stereoisomers with restricted rotation around a single bond where, due to strong intramolecular hydrogen bonding, the barrier to rotation is high enough to permit isolation of the isomeric species. We can speculate that planamers might play an important role in disciplines involving study of weak and reversible non-covalent interactions such as supramolecular assemblies or as potential biologically active compounds in development of novel drug-like molecules.

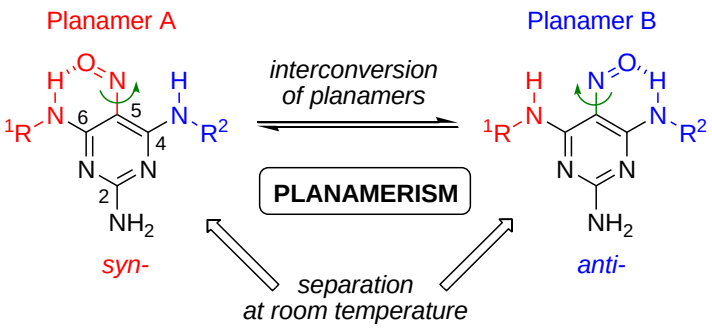


Figure 3. Planamerism = planar isomerism

1. Procházková E., Cechová L., Janeba Z., Dracínský M.: J. Org. Chem. 2013, 78, 10121.
2. Cechová L., Procházková E., Císarová I., Dracínský M., Janeba Z.: submitted.

13 Conformational Determination of Conformationally Biased Homologated Alkanes

Bame, J.R.; Burns, M.; Essafi, S.; Bull, S.P.; Butts, C.P.; Harvey, J.N.; Aggarwal, V.K.

School of Chemistry, University of Bristol, UK

NMR was used for the structural and conformational analysis of the alkane products ***1*** and ***2*** of a newly developed iterative homologation of boronic esters[1]. NMR was used to determine if the different diastereomers adopted predictable and controlled conformations as the all-syn alcohol, ***1,*** and syn-anti MoM ether, ***2***, were expected to take on a helical and linear configuration respectively. ^{1}H-^{1}H, and ^{1}H-^{13}C scalar couplings measurements provided a means to assess dihedral angles along the n-10 carbon chain to determine conformation. Interproton distances were determined via high accuracy NOE-distance analysis[2], further profiling the configuration of the n-10 carbon chain with sensitivity to low population conformers. The experimental scalar coupling constants and NOE distances were compared to population weighted averages of the computationally-determined parameters from conformer ensembles of the two compounds. The NMR results confirm that all-syn alcohol ***1*** takes on a helical conformation and syn-anti MoM ether ***2*** takes on a linear conformation in solution. This high accuracy approach to analysis of these remarkably conformationally-restricted systems highlights the power of this technique to probe molecular structure and dynamics in solution.

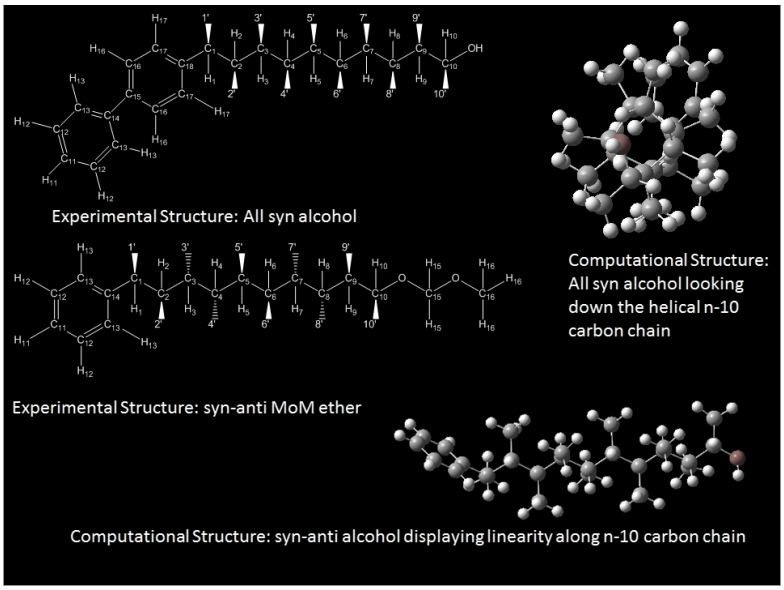


Figure 1: All syn alcohol and syn-anti MoM ether experimental and computational structures.

1. Burns, M.; Essafi, S.; Bame, J.R.; Bull, S.P.; Webster, M.P.; Balieu, S.; Dale, J.W.; Butts, C.P.; Harvey, J.N.; Aggarwal, V.K. High Precision Assembly Line Synthesis for Molecules with Tailored Shapes, 2014, Manuscript submitted

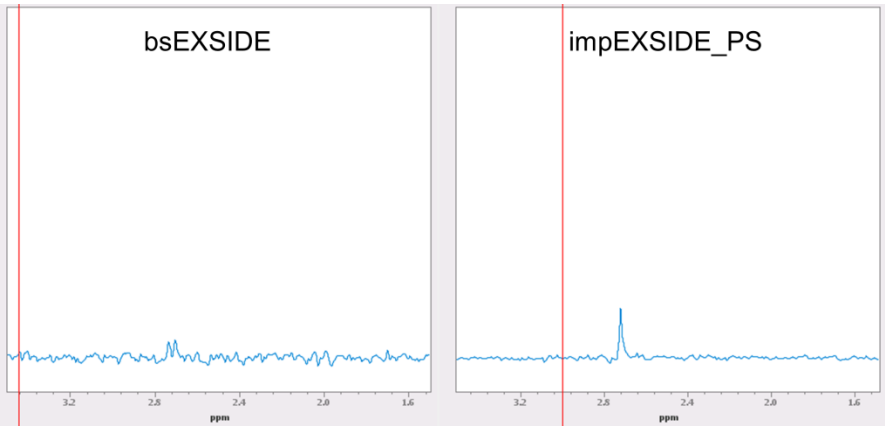
2. Butts, C.P.; Jones, C.R.; Towers, E.C.; Flynn, J.L.; Appleby, L.; Barron, N.J. Interproton distance determinations by NOE-surprising accuracy and precision in a rigid organic molecule Org. Biomol. Chem. 2011, 9, 177-184

14 Sensitivity and Resolution Enhancement in EXSIDE and Band Selective EXSIDE

Ikenna E. Ndukwe, Godiraone Tatolo, and Craig P. Butts

University of Bristol, UK

The potential value of heteronuclear long range coupling constants ($^{n}J_{CH}$) for assigning stereochemical centres in small and complex molecules cannot be overemphasized. The literature is rife with NMR methods for measuring $^{n}J_{CH}$ (all of which have their limitations), but the EXSIDE[1] and ^{13}C band selective EXSIDE[2] methods afford easy means of obtaining $^{n}J_{CH}$, even for non-protonated centres, in addition to providing pure in-phase absorption lineshapes (devoid of distortion from or unmodulated by passive homonuclear coupling). However, the EXSIDE experiment requires a large number of t_1 increments in order to measure $^{n}J_{CH}$ in F1 (especially for small coupling constants). This leads to long acquisition times (hours) and a loss of sensitivity due to T_2 relaxation. The ^{13}C band selective EXSIDE method reduces the experimental time needed per $^{n}J_{CH}$ (minutes) but requires multiple experiments to be performed in order to reproduce the full spectra and also suffers from the same relaxation losses. Here we introduce three new pulse sequences based on the EXSIDE experiments. The first, **impEXSIDE,** employs the **IMPRESS** technique[3,4] (**imp**roved **re**solution using **s**ymmetrically **s**hifted pulses), which exploits Hadamard encoding in the F_1 dimension. The other two pulse sequences apply the band selective homonuclear decoupling (bash) **Pure Shift**[5] acquisition technique, and are called **EXSIDE_PS** and **bsEXSIDE_PS**. We see significant improvements in both signal to noise ratio and resolution in all of the these experiments over their parent versions, and their three-way combination **impEXSIDE_PS** could be a very powerful and enhanced tool for measuring $^{n}J_{CH}$ values.



1. Krishnamurthy, V. V., Journal of Magnetic Resonance Series A 1996, 121, 33-41
2. Butts, C. P.; Heise, B.; Tatolo, G., Organic Letters 2012, 14, 3256-3259
3. Krishnamurthy, K. Journal of Magnetic Resonance 2001, 153, 144 – 150
4. Krishnamurthy, K. Journal of Magnetic Resonance 2001, 153, 124 – 132
5. Meyer, N. H. and Zangger, K. Angewandte Chemie (International edition) 2013, 52, 7143 – 1746

15 Latest Developments and Applications for High Resolution Multinuclear Benchtop NMR Spectrometers

Federico Casanova[1], Andrew Coy[2], **Bertram Manz**[2], and Juan Perlo[1]

1. Magritek Ltd, 32 Salamanca Road, Wellington, New Zealand
2. Magritek GmbH, Pauwelsstr. 19 - D-52074, Aachen, Germany

Most high-resolution benchtop NMR spectrometers lack the ability to perform multidimensional and/or multinuclear NMR experiments. One of the main reasons for this is that the requirements on resolution, sensitivity and stability are much higher than for simple homonuclear 1D experiments. The benchtop system used here is based on a Halbach-array permanent magnet to provide sub Hertz resolution along with high sensitivity on regular 5mm diameter samples. An external lock system stabilises the magnetic field to enable long acquisition experiments. Additional stability is achieved by the in-built magnetic field shielding and temperature control, which make the system fairly insensitive to the local thermal and electromagnetic environment, enabling it to be operated on the bench in a chemistry lab or a production environment.

One of the advantages of measuring nuclei other than ^{1}H is the much wider chemical shift dispersion available. This intrinsically enables a larger number of NMR peaks to be resolved in the same spectral bandwidth. For lower magnetic field strengths this is especially useful, as it permits the elucidation of more complex molecules than proton NMR alone, where peak overlap and second order coupling tend to dominate the NMR spectra of molecules of even modest size.

For organic chemists ^{13}C NMR spectroscopy forms the backbone of routine molecular analysis. 1D ^{13}C methods such as DEPT, as well as 2D proton-carbon experiments, such as HETCOR, HSQC and HMBC, have been implemented. In this work we demonstrate multidimensional, multinuclear NMR spectra acquired on dilute, natural abundance samples with a benchtop NMR spectrometer.

Phosphorus is commonly found in many organic compounds, for example in biological membranes or DNA. The ^{31}P nucleus has a 100% natural isotopic abundance and a large chemical shift range, making it one of the most commonly used nuclei in biological NMR. We will show recent results of decoupled ^{31}P NMR spectra.

Benchtop NMR applications including reaction monitoring, QA/QC and complex mixture analysis have been investigated and demonstrate the power of NMR in providing quantitative, definitive and unique information about the sample under investigation.

16 Homonuclear J-Scaling During Acquisition

Simon Glanzer and Klaus Zangger

Institute of Chemistry/Organic and Bioorganic Chemistry, University of Graz, Austria

NMR spectroscopy is one of the most frequently used techniques for the structural characterization of small to medium sized organic and biomolecules. Because of its widespread occurrence, high natural abundance and high sensitivity, ^{1}H nuclei are often used in this process. Structural information is obtained from the resonance frequencies and scalar coupling patterns. However, due to the limited chemical shift range of protons, the signals are often overlapped, rendering the extraction of structural information difficult or sometimes impossible. One way to increase the resolution of ^{1}H NMR spectra is the use of pure-shift methods [1-3], which yield singlet only spectra, reminiscent of ^{13}C NMR spectra. While, these experiments provide highest-resolution 1D proton NMR spectra, scalar coupling information, which is often key in analyzing chemical structures, is of course completely lost in such experiments. Several methods have been described in order to simplify spectra (increase their resolution) and still allow the extraction of scalar coupling information. Most commonly two-dimensional E-COSY type spectra are employed, yielding multiplets where active and passive couplings are distinguished or 2D J-resolved experiments, separating the chemical shift in the direct from J-coupling information in the indirect dimension. For complicated multiplets or larger molecules these spectra still often result in crowded multiplet patterns. The aim of this work is to provide a method which allows the extraction of homonuclear scalar coupling constants and chemical shifts at the same time with high resolution. While in some cases higher resolution is needed for chemical shifts, other situations require higher resolution in scalar coupling interactions. Therefore, it would be desirable to be able to continuously scale the ratio of chemical shift over scalar coupling constants, preferably in the detection dimension of the NMR spectrum.

Here we present a pulse-sequence[4], which allows the real-time (single scan) expansion or shrinking of homonuclear scalar-coupled multiplets by a user-defined factor, whereas the chemical shift values are left untouched. Two different pulse-sequences (one for down- and one for up scaling) are used. The down-scaling approach is based on a recently developed instant homonuclear decoupling technique. This method works with slice selective excitation, which can be achieved by selective pulses during a weak field gradien. The selective pulse hits all protons, but dependent on the position in the sample tube different signals are excited. By modifying this experiment, a total decoupling is replaced by a partial decoupling, which is dependent on a J-scaling factor λ. By this method overlapping peaks without any chance for detailed interpretation of regular 1D spectra can be separated without losing multiplicity information. On the other hand, in less crowded spectral regions up-scaling achieves a huge resolution improvement for scalar couplings. Furthermore, it has the potential to reveal splittings, that where are “buried” under the spectral line-width in regular spectra. For example by increasing the coupling constant up by a factor of 7 in n-propanol it was possible to observe and measure a splitting difference of 0.7 Hz. This was not possible in the regular NMR spectrum where the line width is on the order of 1.8 Hz. The up-scaling sequence does not depend on slice-selective excitation and has therefore a sensitivity comparable to regular NMR spectra.

1. Aguilar, Juan A, Faulkner, Stephen, Nilsson, Mathias, Morris, Gareth A, Angew. Chem. Int. Ed. Engl., 49(23), 3901–3903., 2010
2. Meyer, Helge N., Zangger, Klaus, Angew. Chem. Int. Ed. Engl., 52(28), 7143-7146, 2013
3. Dr. Foroozandeh, Mohammadali, Adams, Ralph W, Meharry, Nicola J, Jeannerat, Damien, Nilsson, Mathias, Morris, Gareth A, Angew. Chem. Int. Ed. Engl., 53(27), 6990-6992, 2014
4. Glanzer, Simon, Zangger, Klaus, in preparation, 2014

17 Characterization of Active Pharmaceutical Ingredients Using Solid-State NMR Spectroscopy

Jaroslav Havlicek[1] and Gerhard Althoff- Ospelt[2]

1. Laboratory of Solid Phase Analysis, Zentiva k.s., Prague, Czech Republic
2. Bruker BioSpin GmbH, Silberstreifen 76287 Rheinstetten, Germany

Polymorphs are chemically identical, but they have different chemical, physical and also spectroscopic properties.

Solid-state NMR spectroscopy is powerful method for the detection of polymorphs and polymorphic (crystalline) purity.

This advantage is excellently applied in API (active pharmaceutical ingredient) and also in the final solid dosage form.

^{13}C CP MAS is a routine technique to characterize pharmaceutical solids. For crystalline material ^{13}C CP MAS spectra are well resolved and spectra of different polymorphic forms often differ significantly. In the case of dosage forms, however, ^{13}C CP MAS spectra may suffer from overlapping signals of API and placebo and in case of low API concentration from the low intensity of the API lines. ^{19}F CP/MAS techniques may favorably be used in these cases. Usually ^{19}F is present in the API only, avoiding overlap of API and placebo lines. The ^{19}F CP/MAS spectra are very sensitive source of information about polymorphs and give us very clear and good results. This advantage is excellently applied in the final solid dosage form. How easily can ^{19}F CP/MAS spectra be used to determine the ratio crystalline and amorphous material is also shown in our study.

The aim of this presentation is to demonstrate how we can use different NMR techniques for characterization of polymorphs. This study shows the comparison of sensitivity and resolution of ^{13}C and ^{19}F CP MAS techniques for determination polymorphs and how to increase the detection of sensitivity of polymorphic purity.

18 Constant Time Gradient CRISIS HMBC (CTgc2HMBC) – Quantifying Improvements in SNR and Resolution

Godiraone Tatolo and Craig Butts

School of Chemistry, University of Bristol, UK

Since its introduction, the two-dimensional heteronuclear multiple bond correlation (HMBC) experiment has rapidly become the standard way of detecting the presence of long-range (two- and three bond), proton-carbon couplings in small- to medium- size molecules.[1,2] Though widely used, the HMBC experiment suffers from ^{1}H-^{1}H *J*-modulation during t_1, which lowers sensitivity (SNR) and resolution in F1 - limiting the amount of information which can be extracted from these spectra in some cases.[2]

In order to quantify improvements to the HMBC experiment we designed a constant time gradient HMBC experiment with adiabatic pulses to remove ^{1}H-^{1}H modulation. We compare its performance against comparable non-constant time experiments. This experiment termed CTgc2HMBC gave better peak separation, signal-to-noise ratio and resolution than normal HMBC experiment at both low and high F1 resolution.

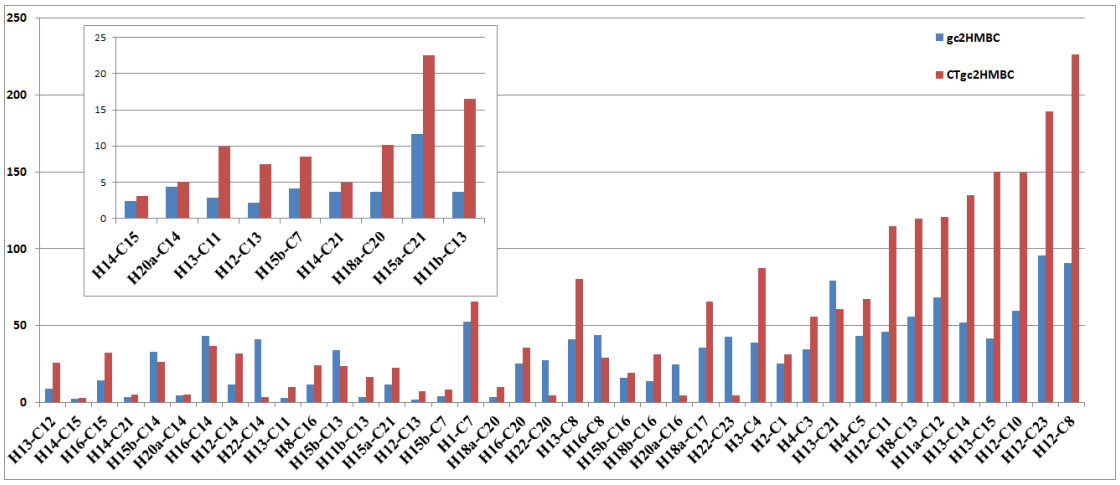


Figure 1: SNR for the weakest 20 gc2HMBC correlations (blue) for strychnine compared to their SNR in CTgc2HMBC (red)

1. Bax, A. Summers, M. Journal of the American Chemical Society, 108, 2093–2094, 1986 .
2. Furihata, K.; Seto, H. Tetrahedron Letters, 39, 7337–7340, 1998 and references therein.

19 Diffusion-ordered NMR with Joint Sparse Time-gradient Domain.

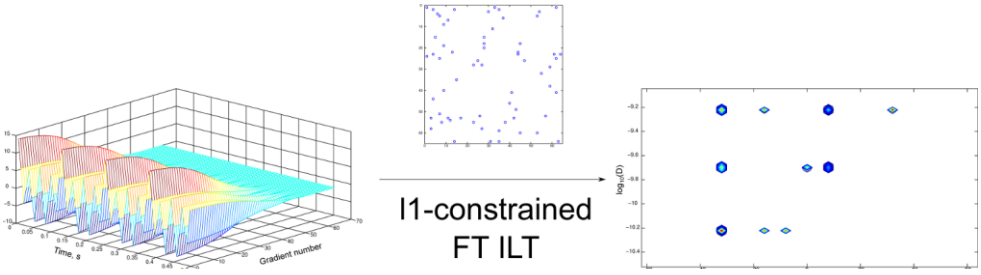
Mateusz Urbańczyk[1], Wiktor Koźmiński[1], and Krzysztof Kazimierczuk[2]

1. Faculty of Chemistry, Biological and Chemical Research Center, University of Warsaw, Warsaw, Poland
2. Centre of New Technologies, University of Warsaw,Warsaw Poland

Multidimensional diffusion-ordered NMR spectroscopy is a useful tool for analysis of chemical mixtures. However, the method is limited by a long experimental time.

In this study, we show how the limitation can be circumvented by an application of joint sparse sampling of both time and gradient domain (as shown in Drawing 1.)

Drawing 1: Idea of joint sparse sampling of time-gradient domain



The signal processing exploits the sparsity-enforcing properties of l_1-norm minimization.

The method joints two mathematical theories

- Compressed Sensing approach for Non-uniform sampling in frequency domain. Which is described by such functional:

$$\min_{\mathbf{S}} \ \|\mathbf{FS}-\mathbf{s}\|_{\ell_2}^2+\tau\|\mathbf{S}\|_{\ell_1}$$

  where **F** is Inverse Fourier Matrix, **S** is frequency domain, **s** is a measured signal, and τ keeps balance between two regularization terms.

- Tikhonov regularization for NMR diffusometry [2]:

$$\min_{\mathbf{\Psi}} \ \|\mathbf{LA}-\mathbf{\Psi}\|_{\ell_2}^2+\tau\|\mathbf{A}\|_{\ell_1}$$

  Where **L** is Laplace transform, **A** is a distribution of Diffusion coefficients and **Ψ** is a diffusion decay (signal).

This two signal processing approaches combined create such minimization functional:

$$\min_{\mathbf{Q}} \ \|\mathbf{PQ} - \mathbf{q}\|_{\ell_2}^2 + \tau\|\mathbf{Q}\|_{\ell_1}$$

Where **P** is defined as:

$$\mathbf{P} = \mathbf{F} \otimes \mathbf{L}$$

Q is reconstructed spectrum, and **q** is joint sparse sampled signal.

The approach was tested on 3D HSQC-DOSY spectra of two model mixtures.

For both experiments the use of sparse sampling shortened the experimental time and improved the quality of the reconstructed spectra compared to the classical approach[1].

1. Urbańczyk Mateusz., Koźmiński Wiktor, & Kazimierczuk Krzysztof. Angewandte Chemie (International Ed. in English), 53(25), 6464–7. 2014
2. Urbańczyk Mateusz, Bernin Diana, Koźmiński Wiktor, & Kazimierczuk Krzysztof (2013). Iterative Thresholding Algorithm for Multiexponential Decay applied to PGSE NMR data. Analytical Chemistry, 85(3), 1828–1833.

20 Isotopic Tracing and *In-situ* NMR Monitoring – A Powerful Way to Investigate Mechanism of Upgrading Process

Haoxi Ben[1], Glen A. Ferguson[1], Mark Jarvis[1*], Mary J. Biddy[1*], Gregg T. Beckham[1], Erica Gjersing[1], Mark Nimlos[1], Matt Sturgeon[1], Tom D. Foust[1], and Arthur Ragauskas[2]

1. National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401
2. Department of Chemical and Biomolecular Engineering, and Department of Forestry, Wildlife, and Fisheries, University of Tennessee, Knoxville, Tennessee 37996, USA.

Biofuels are increasingly being viewed as a promising alternative fuel resource due to the growing concerns about increasing global energy consumption and the effects of growing carbon dioxide emissions from fossil fuels. Among the various conversion technologies that have been investigated, the thermal decomposition processes (such as pyrolysis) has been reported as one of the most promising ways to produce biofuel precursors – pyrolysis oils. However, these pyrolysis oils always have higher oxygen content, molecular weight, viscosity and acidity than gasoline and diesel, and consequently they are characterized as having thermal instability, corrosiveness, poor volatility, low heating value, high coking tendency, cold flow problems and immiscible with petroleum fuels. Therefore, the upgrading processes including hydrogenation (HYD), hydrodeoxygenation (HDO) and selective ring opening (SRO) appear to be very necessary to convert biofuel precursors to drop in feed stocks that can be cost-effectively refined to renewable fuels.

One of the key challenges in studying the upgrading processes including HDO, HYD and SRO is to determine the mechanisms. Indeed, most research work in this area was trying to explore the reaction mechanisms by both experimental and computational methods. Our previous study developed the use of isotopic labeling to help solve this universal challenge. The HDO of lignin pyrolysis oil was examined employing deuterium gas to provide insights into the reaction mechanisms. A comparison of protio and deutero-labeled HDO products by Heteronuclear Single Quantum Coherence (HSQC) NMR provided information about which bonds were C-D labeled after the D_2-HDO process. In addition, ^2H NMR was also shown to be very effect technique to monitor the incorporation of deuterium into the HDO treated samples when employing D_2. However, such ex-situ investigation of reaction pathways could only characterize the final products. To further explore the reaction intermediates and possible mechanisms, in-situ monitoring of deuterium traced reactions appear to be very attractive. This study describes the development of an innovative in-situ NMR technique using high-pressure deuterium gas to monitoring the isotopic traced reaction. This method is an excellent tool for identifying the reaction intermediates to understand reaction mechanisms. Several model compounds (methylcyclopentane (MCP), cyclohenxane (CH), benzene, toluene and anisole) and commonly used noble metal catalysts (Ir, Pt, Pd and Ru) have been examined by this technique. For the study accomplished at 110 °C on Ir, Pt and Ru catalysts, the trend of H/D exchange rates were clearly open side > hindered side > methyl group, which may due to the presence of methyl group brings some barriers from the hindered side. However, very interestingly, Pd could perform almost equally efficient H/D exchanges for both sides, which may indicate that the "turnover" reactions could also

occur on Pd very easily. For both MCP and CH, Ru performed the most efficient H/D exchanges (C-H bond activation), and only Ru could accomplish the ring opening process (C-C bond activation) at 110 °C. Compared to the other two catalysts, the Ru/Al_2O_3 (1 wt%), appeared to have limited capabilities to upgrade all the model compounds. The in-situ monitoring of benzene indicated that there are no peaks could be assigned to two reported possible intermediates – cyclohexene and cyclohexadiene under the employed experimental condition for both Ir/Al_2O_3 (1 wt%) and Pt/Al_2O_3 (1 wt%). The reaction for toluene appeared to be more complicated than for benzene. Compare to the limited selectivity of H/D exchanges for Pt and Ru, the Ir favors to perform the H/D exchanges on the meta position of toluene. Compare to the other two catalysts, Pt appear to be the most active catalyst for hydrodeoxygenation reaction of anisole. A higher Ru loading and employing carbon as supporting material have been found could perform some advantages for the upgrading of anisole. Nevertheless, the innovative high pressure in-situ NMR monitoring technique used in this study proved to be a powerful method for both identifying the reaction intermediates and providing insights into the mechanisms. As far as we are aware, this is the first time in situ NMR monitoring was used to follow the reaction of lignin model compounds, over catalyst, at elevated pressures (deuterium gas).

21 Mixtures Analysis of Complex Samples

Mike Bernstein, Carlos Cobas, Santi Dominguez, Manuel Perez, and Agustín Barba

Mestrelab Research, Santiago de Compostela, Spain (www.mestrelab.com)

The quantitative determination of mixtures components is very suitable to NMR analysis. Building on the success of qNMR, mixtures analysis does, however, create quite significant new problems. These may be dependent on the sample, givien that these cover a huge range, with differing complexities.

We have briefly described an approach to the problem that can be applied when part or all of one multiplet can be clearly seen for each component [1,2]. Building on a quick and powerful Global Spectrum peak Deconvolution (GSD), the method has at its heart the capability for users to define their "solutions" with total flexibility. This approach can be easily applied to relatively simple cases such as edible oils, Aloe vera, and beer analysis. Red wine analysis is much more complex, but the technique has a validated application here as well, identifying >50 components. [3]

We have developed a workflow that is equally suitable to operation via the User Interface, in Batch Mode, or as a "listener", performing analyses as they become available.

We now have further developed this proven functionality with the addition of 2 significant new capabilities. A simple structure database can be used to store information on important compounds, metadata, experimental spectra, and representations of the spin systems. We then provide an interactive way to quantify a mixture component in a way that can use the simulated spectrum. In that way, multiplets are correctly calculated, and the data becomes field-strength independent.

Identification of multiplets can be performed using a novel method: the multiplet is treated as a shape for which mathematical polynomials are calculated to high orders. The experimental spectrum is searched for peak line combinations that best match the descriptors.

We will explain this new approach and show its utility with mixtures analysis and application to real analysis problems of varying complexity.

1. M Bernstein, C Cobas, S Dominguez, M Pérez, A Barba, and J Edwards, "Extending and Facilitating Simple Mixtures Analysis", ENC, 2014, Boston, USA
2. M Bernstein, C Cobas, M Pérez, S Domínguez, C Peng, and A Barba Sánchez, "A Versatile Software Tool for Mixtures Analysis", SMASH, 2013, Santiago de Compostela, Spain
3. Susanne Klein, Heidger Institute, personal communication.

22 Determination of Spectrophotometric pH Indicator Purity Using Quantitative ^{1}H NMR

Regina A. Easley, Katrice A. Lippa and Kenneth W. Pratt

Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD, USA

Approximately one third of the carbon dioxide released from fossil fuel combustion enters the ocean and causes decreases in both ocean pH (ocean acidification) and calcium carbonate saturation states [1]. Efforts to monitor these global changes in ocean chemistry require reliable measurements of the inorganic carbon system in seawater. The marine inorganic carbon system can be quantified by measuring two of the following parameters: pH, dissolved inorganic carbon, total alkalinity, and dissolved carbon dioxide. Highly precise pH measurements are achievable (0.001 pH units) by measuring the visible spectra of sulfonephthalein dyes in contrast to pH values obtained with glass electrodes (0.003 pH units). Previous studies indicate that batch-specific inconsistencies in indicator-based (*or* spectrophotometric) pH measurements (up to 0.1 pH units) are caused by dye impurities which also absorb in the visible range [2, 3]. A method to determine the purity of sulfonephthalein dyes using quantitative proton nuclear magnetic resonance (qHNMR) is presented. Analysis conditions (solvent, pH, and internal standard) were optimized to improve line shape and minimize the effect of exchangeable protons. Samples of meta-cresol purple and cresol red from several manufacturers were compared to dye samples purified with high pressure flash chromatography. The presented qHNMR method facilitates purity determinations for other sulfonephthalein dyes used for carbon system analyses: bromocresol green, bromocresol purple, phenol red and thymol blue.

1. Doney, S.C., et al., *Ocean acidification: The other CO_2 problem.* Annual Review of Marine Science, 2009. **1**(1): p. 169-192.
2. Liu, X., M.C. Patsavas, and R.H. Byrne, *Purification and characterization of meta-cresol purple for spectrophotometric seawater pH measurements.* Environmental Science & Technology, 2011. **45**(11): p. 4862-4868.
3. Patsavas, M.C., R.H. Byrne, and X. Liu, *Physical–chemical characterization of purified cresol red for spectrophotometric pH measurements in seawater.* Marine Chemistry, 2013. **155**: p. 158-164.

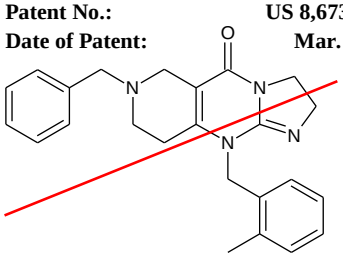
23 Automated Structure Verification: High Profile Patent Issues Building a C.A.S.E. for Enhanced Compound Quality Assurance

Philip Keyes, Joanne Rivera, and Vince Caruso

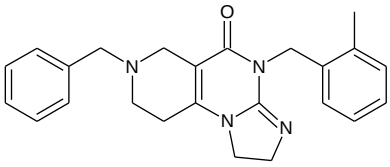
Lexicon Pharmaceuticals

Recent high profile cases of dug discovery and late stage pharmaceutical clinical candidate compound structure errors have shed light on a potentially risky situation. The current drama, as highlighted in the May 26 edition of the C&E News1, surrounding the elucidation of an active oncology agent by the Janda Group has brought into question the intellectual property protecting the claim of another group of scientists for the molecule known as TIC10 patented with an incorrect structure.2 This is the worst case scenario thus far.

TIC10 as submitted for patent
Patent No.: **US 8,673,923 B2**
Date of Patent: **Mar. 18 2014**



Actual structure of bioactive TIC-10 as elucidated
Angew Chem. Int. Ed. 2014, 53, 6628-6631
N. Jacob, J Lockner, V Kravchenko, K Janda



Were this the only such case of an important but miss assigned structure, one might point to an acute failure of scientific rigor, however this is only the most recent in a string of relatively similar events resulting in a great deal of concern for the pharmaceutical industry. Bosutinib (supplied by distributors) and other cases have also made the headlines due to the embarrassing discovery of structural inaccuracy. Whether the structural assignment error is the result of an errant starting material or a misunderstood or misinterpreted reaction, the outcome is the same: Lost productivity, misdirection of resources and far worse, the loss of the value of an entire project!

Emerging automated methods for challenging the validity of a structure, Automated Structure Verification (ASV), have been reported previously.3, 4, 5 Enhanced validation methods including multiple challenge structure generation, incorporation of multiple spectra and systematic tightening of constraints have been assessed to determine their usefulness in preventing such nightmare scenarios that have occupied the headlines of trade journal articles lately. The real question is not whether ASV works, but how well and additionally, how well it needs to work to protect against the risk of catastrophic loss of resources, productivity and even patent protection. Is ASV enough or do further computer assisted structure elucidation (CASE) algorithms need to be put to use to provide adequate reliability of validation results. Depending on a risk assessment a trade off exists between cost of implementation and loss of value from compound structural assignment errors that will be different for each organization.

I examine a major contributing source of errors in this battle: the necessary reliance on specialty and custom chemical manufacturers to supply chemical building blocks, fragment libraries and novel investigational compounds for screening. A growing body of evidence points to a systematic failure rate of materials supplied. In general, between 2% to 8% of milligram and gram scale quantities of specialty chemicals have been reported to be incorrect. ASV uses 1H and 13C NMR chemical shift prediction comparisons to experimental data and automated assignment of atoms to spectrum features, ASV aims to protect against the consequences of errors in supply chain ordered compounds and internal custom syntheses. ASV has the potential to identify nearly 70% - 80% of incorrect isomer structures that LCMS cannot. Recent high profile case studies, such as in C&E News, highlighting incorrectly synthesized Bosutinib sold by third party vendors6 for research benchmark purposes, demonstrate the vulnerability of our discovery programs to supply chain induced synthesis errors. ASV may be helpful in preventing future cases.

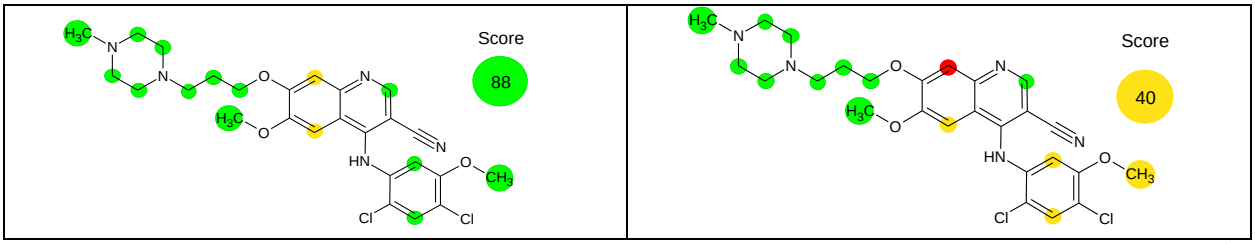


Figure 1: ASV Result for actual Bosutinib 1H + HSQC data against correct structure (score 88) and the ^{1}H + HSQC data of the isomer verified against the Bosutinib actual structure (score 40)

Vendors in the library and fragment screening industry also recognize the perils of producing erroneous hit results due to incorrectly identified or mislabeled compounds citing their own rates of failure for material they use and implementing quality control measures to reduce these issues.7 Additionally we have conducted and published our own internal examinations of commercial building blocks and have obtained similar results. 8, 9

Table 1: Structure Errors found in commercial compounds purchased for benchmark studies

All compounds (250 studied)	Incorrect Structure	Unacceptable purity	Registration Information Error	Total Error
Quantity	6	6	3	15
% Error	2.4 %	2.4 %	1.2%	6%

We have identified these errors as a significant risk in the synthesis process that contributes to submission_posters of incorrect structures. A growing work load of compound verification is helping to build a strong case for more routine implementation of quality control checks using ASV systems as they continue to mature in the future. Additional strides in the use of additional 1D and 2D-NMR experiments applied together with 1H and HSQC ASV currently being evaluated as an Automated Multi-Spectral Structure Verification (MSV) system will be presented. Ultimately one must ask: Is ASV enough?

1. Stu Borman , Tug of War over promising cancer drug candidate, C&E News May 26, 2014, p7
2. Nicholas T. Jacob et. Al., Angew. Chem. Int. Ed. 2014, 53, 6628-6631
3. Golotvin et al., Mag. Res. Chem. 2006, 44, 524-538
4. Keyes et al., Magn. Reson. Chem. 2009, 47, 38-52
5. Sergey Golotvin, Rostislav Pol, Ryan Sasaki, Asya Nikitina, Philip Keyes, Concurrent Combined Verification: Reducing False Positives in Automated NMR Structure Verification through the Evaluation of Multiple Challenge Control Structures, Mag. Res. Chem. 2012, 50, 429-35

6. Bethany Halford , Bosutinib Buyer Beware, C&E News May 21, 2012, p34
7. Darren Begley, Feb 04, 2013 "Grooming Your Fragment Library: Checking the quality of commercially-sourced fragments" http://www.embios.com/blog/bid/276487/Grooming-Your-Fragment-Library-Checki
8. Philip Keyes, Michael Messinger, Gonzalo Hernandez, *Automated Structure Verification by NMR, Part 1: Lead Optimization Support in Drug Discovery* Edit , American Laboratory, January 10, 2012
9. Philip Keyes, Michael Messinger, Gonzalo Hernandez, *Automated Structure Verification by NMR, Part 2: Return on Investment* Edit, American Laboratory, February 1, 2012 (March print edition)

24 Can NMR Correlations Determine Your Structure Unambiguously?

Cíntia Legramanti, Quézia Sant'Anna, and **Jochen Junker**

Fundação Oswaldo Cruz – CDTS, Rio de Janeiro, Brazil

The structure elucidation of an unknown organic compound remains a challenging task. Whilst NMR correlation data is relatively accessible, it's interpretation can still be hard. The difficulty of the structure elucidation problem depends more on the type of the investigated molecule than on its size. Saturated compounds can usually be assigned unambiguously using only COSY and ^{13}C-HMBC data, whereas condensed heterocycles are problematic due to their lack of protons that could show interatomic connectivities.

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. Since 2003 COCON has been available online as WEBCOCON at http://cocon.nmr.de . In 2005 WEBCOCON was presented as tool for the discussion of structural proposals, using theoretical correlation datasets that were generated based on the proposals. Now we present the results of a survey of publications from JNP in recent years, featuring new natural products whose structure has been determined solely by NMR. The published constitutions (at least one for each publication) have been submitted to a structure discussion with WEBCOCON.

As example, follows data for the publication year 2007: out of 198 publications, a total of 111 (~55%) had new compounds that verified straight forward, and 47 (~25%) had at least one compound with more then one possible structural assignment. A total of 40 (~20%) publications had compounds that could not be verified, because WEBCOCON did not produce any results. One part of the process of theoretical correlation data generation in WEBCOCON is the generation of carbon chemical shifts that are in agreement with COCON's chemical shift rules. Unfortunately the inversion of the chemical shift rules sometimes fails and the result does not fit. Currently this is being improved, and we hope to obtain better results in the future.

1. Lindel, T; Junker, J; Köck, M; Journal of Molecular Modeling, 3 (8), 364-368, 1997
2. Lindel, T; Junker, J; Köck, M; European Journal of Organic Chemistry, (3), 573-577, 1999

25 Online Analysis and Visualisation Tools for Chemical Information

Andrés M. Castillo[1,3], Luc Patiny[2], and Julien Wist[3]

1. Universidad Nacional de Colombia, Bogotá Colombia
2. Ecole Polytechnique Fédérale de Lausanne, Lausanne Switzerland
3. Universidad del Valle, Cali Colombia

Here we present a framework for the online analysis and visualization of chemoinformatic data allowing achieving complex tasks that involves several kind of experimental and theoretical. To illustrate the flexibility of this framework, a website was constructed that permits the automatic assignment of NMR spectra of small molecules.

Are you tired of firing up 5 different programs to analyze your data?

We present a set of tools for the analysis of chemical information intended to be used with a long range of experimental data like, images, spectra, molecules, and vectors in general, but also metadata associated with samples, such as geo-tagging.

The system is a JavaScript engine (Rhino) adapted to accept other java projects as plugins. The core logic is programmed within the plugins in pure java code, while the JavaScript interface ensures the communication between both elements. Therefore, any additional feature can be included as a new plugin and complex data mining and statistical analysis can thus be achieved within single JavaScript work-flow.

But nice results are not such unless you can display them in a fancy way. A plethora of tools are developed to deal with chemical information, nonetheless few offer a mature graphical interface. Here we propose a visualization layer to natively display chemical objects within a web browser. A collection of JavaScript objects have been defined for chemical data, such as: jcamp, molfile, mol2d, mol3d, chart, mf (molecular formula), etc. to facilitate the visualization of spectra, molecules and other chemical information.

As a case of study, this framework was used to tackle the NMR automatic assignment problem, which is complex enough to spotlight the power of our approach. First at all, we implemented a new algorithm for the automatic assignment of small molecules using 1D and 2D experiments (peak intensity and correlations). A ranking of all the possible assignments results according to a scoring function that captures how good the experimental data explains the predicted properties [1,2] of the molecule. A web page permits to explore interactively the results and once the data has been verified, a feedback can be stored with the correct assignment.

1. Christoph Steinbeck and Stefan Kuhn. NMRShiftDB - compound identification and structure elucidation support through a free community-built web database. Phytochemistry, 65(19):2711–7, October 2004.
2. Yuri Binev and João Aires-de Sousa. Structure-based predictions of 1H NMR chemical shifts using feed-forward neural networks. Journal of chemical information and computer sciences, 44(3):940–5, 2004.

26 A Complete Wine Analysis Using Multiplets Detection

Michael Bernstein[1], Agustín Barba[1], Susanne Klein[2], Andrea Dreiseitel[2], Daniel Heidger[2], and Volker Heidger[2]

1. Mestrelab Research S.L., Spain, www.mestrelab.com
2. Institut Heidger, Germany, www.institut-heidger.de

The correct and complete analysis of grape and wine metabolites is fundamental to product quality control and managed processes. qNMR has been shown to have huge benefit for foods analysis. In a related example, Peregrina and co-workers discussed its application to Rioja wine analysis [1], and the subject was reviewed by Hong [2].

We have designed and validated an NMR analysis of wine components using only signature peaks and multiplets. This software has general application, and is called Simple Mixture Analysis (SMA). [2, 3]

Here we demonstrate the program features that make this analysis possible and show results for a wine analysis. Quantification results were validated through certified compound additions. We use the program features that provide an enhanced, customized capability together with semi-automated work flow. These allow the trained user to quickly achieve an analysis that is checked and correct. Finally, analysis results are exported and imported to a corporate database.

A surprising level of analysis complexity can be achieved, with >50 metabolites reliably quantified from a single 600 MHz 1H NMR spectrum. The process is part of a streamlined analysis protocol that is performed on ca 100 samples per week.

1. E. Lopez-Rituerto, S. Cabredo, M. Lopez, A. Avenoza, J.H. Busto, J.M. Peregrina, “A thorough study on the use of quantitative 1H NMR in Rioja red wine fermentation processes”, J Agric Food Chem. 57 (2009) 2112–2118.
2. Y.-S. Hong, NMR-based metabolomics in wine science., Magn. Reson. Chem. 49 Suppl 1 (2011) S13–21.
3. M Bernstein, C Cobas, S Dominguez, M Pérez, A Barba, and J Edwards, “Extending and Facilitating Simple Mixtures Analysis”, ENC, 2014, Boston, USA
4. M Bernstein, C Cobas, M Pérez, S Domínguez, C Peng, and A Barba Sánchez, “A Versatile Software Tool for Mixtures Analysis”, SMASH, 2013, Santiago de Compostela, Spain

27 Full Automation of Routine NMR Aanalysis for Polymers, Using Assure-RMS

Michelle A. Markus[1], Jimmy Yuk[1], Christian Fischer[2], Kimberly L. Colson[1]

1. Bruker BioSpin Corporation, Billerica, MA, USA
2. Bruker BioSpin GmbH, Rheinstetten, Germany

NMR spectroscopy is a powerful tool for analyzing the composition of materials. Empirical methods have been developed to measure specific properties for different polymers. These methods typically involve region integration and calculations based on those integrals to yield a value. We have developed a tool, the Equation Builder, within the Assure-RMS software, for implementing these calculations. Assure-RMS is Bruker's software solution for NMR analysis with full automation, featuring data acquisition, processing, analysis, and report generation. Here we present several examples, using 1D ^{1}H and ^{13}C spectra to measure properties of polymers.

Poloxamers are copolymers of oxypropylene and oxyethylene. These polymers are amphiphilic and thus can be used as surfactants, improving the miscibility of materials of different hydrophobicity. They are used in various industries, including cosmetics and pharmaceuticals. Their properties depend on the percentage of oxyethylene in the copolymer. An NMR method to measure the percentage of oxyethylene has been developed [1], derived from the ratio of the areas of two regions in the 1D ^{1}H NMR spectrum. We will show how this method was implemented in Assure-RMS, using the Equation Builder.

Polyaromatic hydrocarbons (PAH) may be added to tire rubber to facilitate processing, but the amount of such oils in the finished product is limited by regulations due to their carcinogenicity and risk to the environment. There is a protocol measure the amount of bay region hydrogens in tire rubber by NMR [2]. We will show how this method was implemented in Assure-RMS, including the full workflow.

Propylene-1-Butene polymers are optically clear films and fine fibers having excellent low-temperature sealing properties and blocking resistance. An NMR method was developed to determine the composition of the polymers [3], important for monitoring different reaction conditions. This method is based on 1D ^{13}C spectroscopy and was implemented this method in Assure-RMS, as described here.

Poly(vinyl) acetate is a rubbery component of wood glue. It can be converted into **poly(vinyl) alcohol** by base hydrolysis. Poly(vinyl) alcohol is a soluble material used in paper making, textiles, and coatings. The conversion of the acetate into the alcohol can readily be monitored by 1D ^{1}H NMR. We compare the results using Assure-RMS with a manual calculation.

1. monograph, "Poloxamer", in the European Pharmacopeia 6.0
2. EU Directive ISO 21461 for Rubber Analysis
3. Yu-Dong Zhang, Macromolecules (2004) 37: 2471-2477

28 What is in Your Dishwashing Liquid? Developing Fully Automated Screening Methods for Commercial Products

Michelle A. Markus[1], Christian Fischer[2], Kimberly L. Colson[1]

1. Bruker BioSpin Corporation, Billerica, MA, USA
2. Bruker BioSpin GmbH, Rheinstetten, Germany

Manufacturers need tools for competitive intelligence, not to mention quality control of their own products. NMR spectroscopy is a powerful tool for identifying and quantifying components of complex mixtures, without fractionating the mixture. Simple sample preparation makes NMR an attractive tool for analysis. To increase the adoption of NMR, we are streamlining data acquisition and analysis by developing the Assure-RMS software. Here we show the application of NMR to a commercial product, hand dishwashing liquid.

Identification and quantification of components – For dishwashing liquid, we typically know the ingredients in advance. With this information, reference spectra of components can be acquired and stored in a reference spectral database (SBASE) for detergents. Analysis of test samples includes identification of components by matching against the SBASE and quantification with reference to an external standard [1].

Search for specific ingredients – Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol is a widely used antibacterial found in dishwashing liquid, hand soaps, and body washes. Although there are no proven adverse health effects, triclosan is a controversial ingredient. Triclosan is readily detected and quantified in the 1D ^{1}H NMR spectra of dish detergents.

Comparing formulations 1 - Hypoallergenic formulations of many detergents are currently being marketed to consumers who want products without unneccesary or irritating ingredients, with names including “pure essentials” and “pure+clear”. We can readily compare the reformulated product with the parent product using NMR – with some unexpected results.

Comparing formulations 2- With enough validated samples, we can build statistical models for a particular material. Here we show a preliminary model for Dawn dish liquid and demonstrate it groups another brand (Joy) made by the same company with Dawn but distinguishes a brand (Method) made by another company. Examination of the loadings plot suggests the differentiation is based on the surfactants.

All the analyses described above can be combined into one Assure-RMS method for complete automation. The information is obtained from a single 1D spectrum, acquired in less than 5 minutes.

1. Gerhard Wider, Lars Dreir (2006) “Measuring Protein Concentrations by NMR Spectroscopy” *J. Am. Chem. Soc.* **128**: 2571-2576.

29 Effectiveness of CRAFT in Quantifying Serum Metabolites in the Presence of Spectral Distortions and Sharp/Broad Peak Overlap

Erwin Garcia[1], Krish Krishnamurthy[2], Thomas O'Connell[3], and Elias Jeyarajah[1]

1. LipoScience Inc., Raleigh, NC, USA
2. Agilent Technologies, Santa Clara, CA, USA
3. Duke University, Durham, NC, USA

The inherently quantitative nature of NMR lends itself to be used in analysis of complex mixtures including biological matrices such as in serum/plasma and urine. Extraction of quantitative information from NMR spectra is commonly performed by peak integration or deconvolution. The accuracy of the results from these methods can be compromised by imperfections in the peak shape and the baseline. While quantitation in the frequency–domain requires rigorously phased and baseline corrected spectra, time–domain Bayesian approaches derive quantitative information from the FID independent of the phase, and do not require baseline correction. We employed CRAFT (Complete Reduction to Amplitude Frequency Table) to perform time–domain analysis (a) on spectra unsuitable for phase adjustments (caused by misadjusted pre–acquisition delay), (b) on symmetrically broadened peaks (caused by suboptimal z3 shims), and (c) on spectra with misset transmitter offset. Furthermore, the quantitation of small metabolites by CRAFT analysis was performed on serum samples wherein, the sharp resonance lines arising from the metabolites of interest overlap with large, broad signals from macromolecules such as proteins and lipoproteins.

We report that, using CRAFT, the presence of spectral distortions arising from phase error, symmetric line broadening, and improperly placed transmitter offsets affect the average calculated concentrations < 6%, < 10% and < 3% respectively, relative to the reference spectra obtained under optimal conditions. The presence of broad resonances overlapping with the sharp peaks of interest affects the quantitation only slightly (% error < 5).

When CRAFT performance is evaluated for alanine, lactate and valine in dialyzed serum, in the presence of several suboptimal experimental settings and sharp/broad peaks overlap, the % CV and % error of the calculated concentrations are < 6 and < 2, respectively. These results demonstrate that with CRAFT analysis, accurate quantitative results can be obtained in complex mixtures without the need for advanced phasing and baseline correction algorithms. Further, it establishes the robustness of the Bayesian time–domain analysis against imperfect z3 shim and transmitter offset settings within tested limits. These features should prove to be highly advantageous in a high-throughput NMR environment where experimental conditions may vary over time, but it is important to maintain highly accurate quantitation.

30 NMR For Quality Control of Injection Solution Mixtures: Comparison of Integration and CRAFT Analysis

Andrea M. Sefler and Anthony Ribeiro

Duke NMR Center, Duke University, Durham, NC 27710, USA.

We present quantitative ^{1}H (NMR) studies on a formulated admixture of commercial injection solutions of ropivacaine, dexamethasone, epinephrine that is clinically administered to patients as a peripheral nerve block. Clinical practice often involves the mixing and/or dilution of packaged injection solutions from the manufacturer prior to administration to the patient for a variety of purposes. Despite this practice in the clinic, little information exists on the behavior or stability of the compounded injection solutions.

Our NMR study focuses on the stability of the admixtures as a function of light or dark storage conditions. Quantitative results have been generated using both traditional peak integration and the newly released CRAFT (Complete Reduction to Amplitude Frequency Table) algorithm to compare the results.

NMR is ideally suited for the task of assessing the quality control of such admixtures as it is highly sensitive to even minor structural changes, such as the addition of a sulfate group. Moreover, NMR is an inherently quantitative technique, even if the presence and/or identities of impurities are not expected or known *a priori*.

31 Determination of an Unprecedented Fused Pentacyclic Flavonoid Skeleton by Computer-Assisted Structure Elucidation of Black Chokeberry (*Aronia melanocarpa*) Fruit Juice Isolates

C. Benjamin Naman,[1] Jie Li,[1] Arvin Moser,[2] Jeffery M. Hendrycks,[2] Chunhua Yuan,[3] William J. Keller,[4] and A. Douglas Kinghorn[1]

1. Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, United States
2. Advanced Chemistry Development, Inc., Toronto, ON, Canada
3. Nuclear Magnetic Resonance Facility, Campus Chemical Instrument Center The Ohio State University, Columbus, OH, USA
4. Nature's Sunshine Products, Inc., Spanish Fork, UT, USA

Black chokeberry [*Aronia melanocarpa* (Michx.) Elliott (Rosaceae)] has become a popular "superfruit" and botanical dietary supplement in the United States and some European countries.[1] The measured antioxidant potential and detailed phytochemical investigation of this plant material have been recently reported.[2,3] However, during the course of an earlier study on an extract of black chokeberry,[2] one compound was isolated that could not be structurally determined by conventional means. With the aid of computer-assisted structure elucidation (CASE) software, a feasible structure was generated that was not previously considered by manual data interpretation. At that time, the structure could not be confirmed due to diminished sample quantity and the need for additional experimentation. To validate the structure, a targeted isolation of the compound from the same plant source was undertaken that yielded, instead of the original compound of interest, a less complex analog. Analysis of the NMR spectra of this analog was more straightforward, and comparison with that of the previous molecule allowed for confirmation of the CASE-suggested structure. Presented are the structures for two new natural products of the flavonoid class that contain unprecedented pentacyclic cores, a description of the challenges overcome for their structure elucidation, and a plausible biosynthetic pathway.

1. Sidebottom, V. Next-Generation Superfruits: Assessing the Potential of Emerging Ingredients Using Data from Patents, Clinical Trials, EFSA, and New Product Development; Business Insights, Ltd.: London, UK, 2012.
2. Li, J.; Deng, Y.; Yuan, C.; Pan, L.; Chai, H.; Keller, W. J.; Kinghorn, A. D. J. Agric. Food Chem. 2012, 60, 11551–11559.
3. Taheri, R.; Connolly, B. A.; Brand, M. H.; Bolling, B. W. J. Agric. Food Chem. 2013, 61, 8581–8588.

32 Non-linear Effects in Quantitative 2D NMR of Polysaccharides: Pitfalls and How to Avoid Them

Estelle Martineau[1,2], Kamel El Khantache[1,2], Marion Pupier[3], Patricia Sepulcri[3], Serge Akoka[1], and Patrick Giraudeau[1,4]

1. EBSI Team, Chimie et Interdisciplinarité : Synthèse, Analyse, Modélisation (CEISAM), Université de Nantes, CNRS, UMR 6230, LUNAM Université
2. Spectromaîtrise, CAPACITÉS SAS, Nantes, France.
3. Sanofi-Pasteur, Analytical Research Department –Marcy l'étoile, France
4. Institut Universitaire de France, Paris, France

Quantitative 2D NMR is a powerful analytical tool which is widely used to determine the concentration of small molecules in complex samples [1]. Due to the site-specific response of the 2D NMR signal, the determination of absolute concentrations requires the use of a calibration or standard addition approach, where the analyte acts as its own reference [2,3]. Standard addition methods, where the targeted sample is gradually spiked with known amounts of the targeted analyte, are particularly well-suited for quantitative 2D NMR of small molecules. Here, we report unexpected results obtained when trying to apply such a quantitative 2D NMR approach to a high molecular weight polysaccharide. The standard addition method leads to a strong under-estimation of the target concentration, whatever the 2D NMR pulse sequence. It appears that this error is due to a non-linear behaviour of the 2D NMR signal as a function of concentration. This evolution can be correlated to a non-linear variation of the homonuclear Overhauser effect with the concentration, which can be attributed to spin-diffusion effects occurring in such high molecular weight molecules. Accurate quantitative results can still be obtained provided that an external calibration is performed with a wide range of concentrations surrounding the target value. This study opens the way to a number of studies where 2D NMR is needed for the quantitative analysis of macromolecules.

1. Giraudeau P., Magn. Reson. Chem., 52, 259-272, 2014
2. Giraudeau P., Tea I., Remaud G.S., Akoka S., J. Pharm. Biomed. Anal., 93, 3-16, 2014
3. Martineau E., Tea I., Akoka S., Giraudeau P., NMR Biomed., 25, 985-992, 2012

33 A Combined Approach: Highly Sensitive Pure Shift NMR and Structure Elucidation of ppm-impurities in Bulk Chemicals

Koen de Vries[1], Ard Kolkman[1], Marco Tessari[2], Sybren Wijmenga[2], and Maarten Honing[1]

1. DSM Resolve, Geleen, The Netherlands, koen.vries-de@dsm.com;
2. Radboud university Nijmegen, The Netherlands, Department of Biophysical Chemistry

For certain bulk chemicals (kton scale) the demands on quality are very high. Differences between on-spec and off-spec are in the low ppm-range.

The major challenge when dealing with ppm-level impurities in organic compounds, is how the impurities can be isolated for characterization. Unlike in the field of natural products characterization, isolation with SPE from an aqueous matrix is not an option. Better alternatives are Prep-GC or Prep- LC combined with the advantages of micro-cryoprobes and highly sensitive pure shift NMR.

A high sensitivity, pure shift NMR experiment for mass-limited samples (ppm-level) is presented to acquire simplified 1D-^{1}H NMR spectra where only chemical shift information is present. The experiment proves to be quantitative and can therefore be used to determine solutes concentrations. Because of its high sensitivity, our experiment provides a valuable support in the investigation of complex, low concentrated samples and mixtures.

34 Application of ^{19}F Time-Domain NMR to Measure Content in FLuorine Containing Drug Products

Maria Victoria Silva Elipe[1], Lan Li[1], Karthik Nagapudi[1], Alan Kook[2], Carlos Cobas[3], Isaac Iglesias[3],and Chen Peng[3]

1. Amgen, Inc., Thousand Oaks, CA 91320
2. NMR Service + Consulting, Austin, TX 78758
3. Mestrelab Research S.L., Santiago de Compostela, La Coruna 15706, Spain

It is necessary to show that the active content in the dosage form of drugs is within a certain narrow range of the label claim. In case of fluorinated drugs, the active content can be measured by solid state NMR because the excipients lack fluorine. To make NMR reachable to any laboratory, simple to use, and at low cost, measurement of ^{19}F nucleus using a 23 MHz (for ^{1}H) low resolution benchtop time-domain (TD) NMR was investigated. Three fluorinated drug products, cinacalcet, lansoprazole, and ciprofloxacin, were chosen for this study. The doses for these drug products range from 15 mg to 500 mg. The average drug content measured using ^{19}F TD-NMR compares well with the reported label claims for the three drugs tested. ^{19}F TD-NMR is a simple and non-destructive technique to measure drug content in tablets. In addition, the accessibility and simplicity of the technique makes it a good candidate for use as a process analytical technology (PAT) tool for development and manufacturing settings in the pharmaceutical industry. Besides, we have compared two software packages, to evaluate the results for quantitation studies and their flexibility for data analysis and reporting.

35 Unequivocal Structural Assignments of Three Cycloheptenoid Intermediates for Guaiane Sesquiterpenes: An Experimental and Theoretical Approach

Layla R. Barbosa,[1] Ygor W. Vieira,[2] **Valdemar Lacerda Jr.**,[1] Kleber T. de Oliveira,[2] Reginaldo B. dos Santos,[1] Sandro J. Greco,[1] Alvaro C. Neto,[1] Eustaquio V. R. de Castro[1] , and Timothy J. Brocksom[2]

1. Chemistry Departament, Federal University of Espirito Santo, Av. Fernando Ferrari 514, 29.075-910 – Vitória – ES, Brazil, Phone +55/27 4009 2908. Fax +55/27 4009 2826; *e-mail:valdemar.lacerda@ufes.br
2. Chemistry Departament, Federal University of São Carl os, 13.565-905, São Carlos – SP,Brazil, Phone +55/16 3351 8083

Within our endeavours to synthesize complex bioactive higher terpenoids, containing the seven-membered carbocyclic structural unit, we have developed several synthetic transformations starting from naturally occurring *para*-menthane monoterpenes. These transformations generally furnish cycloheptenones which are then alkylated at the alpha position, for subsequent cyclization chemistry [1,2].

The innumerable studies in the literature on the occurrence, isolation and structural determination of these natural products, has made them interesting objects of study in nuclear magnetic resonance (NMR) spectroscopy [3], which is a very important and well-established tool for the structural analysis of organic compounds [4-6].

We present here a complete NMR assignment for three synthetic cycloheptenone intermediates (compounds **2**, **3** and **4**, Fig. 1). These studies were performed using 1D and 2D NMR techniques and compared with the theoretical predictions of the chemical shifts and coupling constants using DFT calculations.

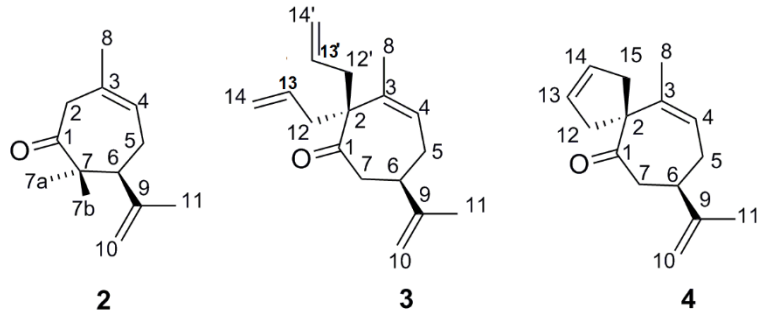


Figure 1. Cycloheptenones under study.

GIAO and CSGT models at DFT/B3LYP level of theory using the cc-pVTZ basis set was employed for calculations of ^{1}H and ^{13}C NMR chemical shifts (δ), using Gaussian03 program.

The experimental chemical shift data were correlated with the theoretical results. The B3LYP/cc-pVTZ model was used to calculate all of the properties (with and without solvent). From the correlation between the theoretical and experimental data, it was concluded that the model used was effective for calculating the shielding tensors for the compounds studied. Comparing the two calculation methods used, GIAO and CSGT, it was found that the mean deviation (MD), standard deviation (SD) and linear correlation factor (R) for all the calculations were generally better when using CSGT than GIAO. Moreover, calculations using CSGT were completed nearly three times faster than those using GIAO. Therefore, the CSGT method is very advantageous and more cost-efficient.

Acknowledgements

The authors thank CNPq, CAPES, FAPES and FAPESP for financial support and fellowships.

1. Brocksom, T. J.; Brocksom, U.; de Sousa, D. P.; Frederico, D. Tetrahedron-Asymmetr., 2005, 16, 3628-3632.
2. Brocksom, T. J.; Brocksom, U.; F.P. Barbosa, F. P. J. Braz. Chem. Soc., 2006, 17,792-796.
3. Breton, R. C.; Reynolds, W. F. Nat. Prod. Rep., 2013, 30, 501-524.
4. da Silva, R.; Pedersoli, S.; Lacerda Jr., V.; Donate, P. M.; Albuquerque, S.; Bastos, J. K.;Araújo, A. L. S. M.; Silva, M. L. A. Magn. Reson. Chem., 2005, 43, 966-969.
5. Lacerda Jr., V.; da Silva, G. V. J.; Constantino, M. G.; de Castro, E. V. R.; dos Santos, R. B.; Silva, R. C. Magn. Reson. Chem., 2008, 46, 268-273.
6. Salles, R. C.; Lacerda Jr., V.; Beatriz, A.; Ito, F. I.; dos Santos, R. B.; Greco, S. J.; de Castro, E. V. R.; de Lima, D. P. Magn. Reson. Chem., 2010, 48, 409-415.

36 Structural assignment of non-aromatic B-ring flavanones from *Piper carniconnectivum* C. DC by NMR, ECD and DFT calculations

Giovana C. Freitas[1], João M. Batista Jr.[2], Lydia F. Yamaguchi[1], **Janaina D. Vilcachagua**[1], Denize C. Favaro[1], Maysa Furlan[2], Elsie F. Guimarães[3], Christopher S. Jeffrey[4], and Massuo J. Kato[1]

1. Research Support Center in Molecular Diversity of Natural Products, Institute of Chemistry, University of São Paulo, SP, Brazil
2. NUBBE, institute of Chemistry, State University of São Paulo, UNIFESP, Araraquara, SP, Brazil
3. Research Institute Jardim Botânico from Rio de Janeiro, Rio de Janeiro, RJ, Brazil
4. Department of Chemistry, University of Nevada, Reno, USA

The species *Piper carniconnectivum* C. DC. (Piperaceae) is endemic to the Amazon region of Northern Brazil [1]. Previous phytochemical studies of its roots, resulted in the isolation of three cyclopentenediones, a prenylated coumarin, xanthyletin [2]; three C-methylated flavanones and a C-methylated chalcone [3].

Flavonoids are widespread class of secundary metabolites isolated from a multitude of angiosperms. Nevertheless, containing a partial or completely hydrated non-aromatic B-ring, called protoflavonoids, they have only been isolated from species within the Pteridophyte such as Thelypteridaceae [4,5] and Dryopteridaceae [6] and from one Magnoliophyte (*Ongokea gore*, Olacaceae) [7]. While flavonoids exhibit potent antioxidant activity, their citotoxic effects are typically negligible. In contrast, protoflavonoids, such as protoapigenone and protofarrerol, have promising cytotoxic effects mainly against breast, ovarian, lung, liver and prostate cancer cell lines [8,9,10]

The studies carried out on *P. carniconnectivum* in this work resulted in the isolation and characterization of two new flavanones that contain an unusual non-aromatic B-ring: 5-hydroxy-2-(1'-hydroxy-4'-oxo-cyclohex-2'-en1'-yl)-6,7-dimethoxy-2,3-dihydro-4*H*-chromen-4-one **(1)** and 5-hydroxy-2-(1',2'-dihydroxy-4'-oxo-cyclohexyl)-6,7-dimethoxy-2,3-dihydro-4*H*-chromen-4-one **(2)**.

The ^{1}H NMR spectra for both protoflavanones exhibited typical resonances of an oxygenated flavanone. However, despite characteristic signals of the A- and C-rings of a flavanone, typical proton resonances for the B-ring were absent. Instead, four alphatic hydrogen resonances, and two vinylic hydrogen resonances provided evidence of a cyclohexenone non-aromatic B-ring. The structural assignment of **1** and **2** were stablished via ^{13}C NMR spectra and COSY, NOESY, HSQC and HMBC correlation experiments to solve the relative configurations.

The absolute configuration of (+)-**1** was unanbiguously determined as 2*S*, 1'*R* by electronic circular dichroism (ECD) spectroscopy and comparison to simulated spectra that were calculated using TDDFT theory [11,12,13]. This metodology allowed the assignment of the absolute configuration of (+)-**2** also as 2*S*, 1'*R*, except for the stereogenic center at C-2', which was assigned as *R* because of the evidence drawn from high resolution NMR (800 MHz) experiments.

The analysis of long-range coupling in the high-resolution NMR was used to assign the configuration at C-2' [14,15]. The magnitude of vicinal escalar coupling constants $^{3}J_{HH}$ between H-2' and H-3'$_{ax}$ ($^{3}J_{H2'H3'ax}$=3.3 Hz) and H-3'$_{eq}$ ($^{3}J_{H2'H3'ax}$=2.7 Hz) of compound **2** suggested an equatorial orientation of H-2', which was futher confirmed by the strong NOE effect between H-2' (in 4.42 ppm) and both H-3' diastereotopic hydrogens H-3'$_{ax}$ (in 2.88 ppm) and H-3'$_{eq}$ (in 2.16 ppm). A long range H–C–C–C–H (*W*-type) coupling $^{4}J_{HH}$ of 2.1 Hz, observed between H-2' and H-6'eq (in 1.70 ppm), provided strong evidence of the equatorial orientation of H-2' and C-2' allowing definitive assignment of the C-2' absolute configuration as *R*.

1. Ribeiro, J.E.L.S., Hopkins, M.J.G., Vicentini, A., INPA-DFID, pp, 816, 1999.
2. Facundo, V.A., Sa, A.L., Silva, S.A.F., Morais, S.M., Matos, C.R.R., Braz-Filho, R., J. Braz. Chem. Soc., 15, 140, 2004.
3. Facundo, V.A., Braz-Filho, R., Biochem. Syst. Ecol., 32, 1215, 2004.
4. Lin, A.S., Chang, F.R., Wu, C.C., Liaw, C.C., Wu, Y.C., Planta Med., 71, 867, 2005.
5. Wada, H., Fujita, H., Murakami, T., Saiki, Y., Chen, C.M., Chem. Pharm. Bull., 35, 4757, 1987.
6. Noro, T., Fukushima, S., Saiki, Y., Ueno, A., Akahori, Y., Yakugaku Zasshi, 89, 851, 1969.
7. Jerz, G., Waibel, R., Achenbach, H., Phytochemistry, 66, 1698, 2005.
8. Chang, H.L., Wu, Y.C., Su, J.H, Yeh, Y.T., Yuan, S.S.F., J. Pharmacol. Exp. Ther., 325, 841, 2008.
9. Chang, H.L., Su, J.H., Yeh, Y.T., Lee, Y.C., Chen, H.M., Wu, Y.C., Yuan, S.S.F., Cancer Lett., 267, 85, 2008.
10. Wei, A., Zhou, D., Ruan, J., Cai, Y., Xiong, C., Wu, G., J. Ethnopharm., 139, 373, 2012.
11. Li, X.C., Ferreira, D., Ding, Y., Curr. Org. Chem., 14, 1678, 2010.
12. Batista Jr, J.M., Batista, A.N.L., Rinaldo, D., Vilegas, W., Ambrósio, D.L., Cicarelli, R.M.B., Bolzani, V.S., Kato, M.J., Nafie, L.A., López, S.N., Furlan, M., J. Nat. Prod., 74(1154), 1160, 2011.
13. Felippe, L.G., Batista Jr, J.M., Baldoqui, D.C., Nascimento, I.R., Kato, M.J., He, Y., Nafie, L.A., Furlan, M., Org. Biomol. Chem., 10, 4280, 2012.
14. Contreras, R.H., Peralta, J.E., Prog. Nucl. Magn. Reson. Spectrosc., 34, 21, 2001.
15. Krivdin, L.B., Contreras, R.H., Ann. Rep. NMR Spectrosc., 61, 133, 2007.

37 Tautomerism of Isocytosine - Low-temperature NMR Study

Radek Pohl and Martin Dračínský

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Isocytosine, a structural isomer of cytosine, can theoretically form 21 planar tautomers/rotamers [1]. However, experimental [2] and theoretical [3] studies have revealed that isocytosine exists in gas phase predominantly in the 2-amino-4-hydroxy form with smaller proportion of *3H*-2-amino-4-keto tautomer. On the other hand, approximetly 1:1 ration of *1H*-2-amino-4-keto and *3H*-2-amino-4-keto tautomers were observed in aqueous solution. These two tutomers crystallize in a 1:1 ratio and form a hydrogen-bonded dimer that can be observed by ^{13}C and ^{15}N CPMAS NMR [4].

In this poster, we present first direct observation of isocytosine tautomers in solution by low-temperature NMR using polar solvents such as CD_3OH, DMF-d_7, their mixture or DMSO-d_6/H_2O mixture. We have found that isocytosine exists in all tesetd solvents as a mixture of *1H*-2-amino-4-keto and *3H*-2-amino-4-keto tautomers with excess of *3H*-form as the most stable tautomer, which is in agreement with theoretical calculation of free energy. The particular tautomers were assigned using H,C-HMBC experiment and a comparison of experimental data with calculated NMR parameters.

Acknowledgements: The work is supported by the Czech Science Foundation (Grant No. 13-24880S).

1. Dračínský, Martin; Jansa, Petr; Chocholoušová, Jana; Vacek, Jaroslav; Kovačková Soňa; Holý, Antonín, Eur. J. Org. Chem. 777-785, 2011.
2. Vranken, Hertwig; Smets, Johan; Maes, Guido; Lapinski, Leszek; Nowak Maciej J.; Adamowicz, Ludwig, Spect. Acta 50A (5), 875-889, 1994.
3. Kwiatkowski, Józef S.; Leszczynski, Jerzy, Int. J. Quantum Chem. 61 (3), 453-465, 1997.
4. Dračínský, Martin; Jansa, Petr; Ahonen, Kari, Budešínský, Miloš, Eur. J. Org. Chem. 1544-1551, 2011.

38 Measuring and Mitigating Convection in NMR

Iain D. Swan[1], Matthew Reid[2], Peter W. A. Howe[2], Mathias Nilsson[1], and Gareth A. Morris[1]

1. School of Chemistry, University of Manchester, Manchester, UK
2. Syngenta, Jealott's Hill Research Centre, Bracknell, Berkshire, UK

Many modern liquid NMR experiments use pulsed magnetic field gradients (PFGs), which have the effect of spatially resolving spins. Unwanted convection occurs in an NMR sample more commonly than one might expect, meaning that spins experience unintended differences in PFG strength depending on the extent of their movement during an experiment. This has the effect of causing changes in phase, and ultimately signal loss in any experiment that uses PFGs.[1]

By manipulating the convection compensation element timing in a convection-compensated 2DJ-iDOSY pulse sequence[2], convection velocity profiles were determined[3] for $CDCl_3$ on three different spectrometers at 1°C temperature intervals between 5°C & 40°C in a variety of NMR tubes: thick wall, medium wall, and standard/thin wall 5 mm glass tubes, and a medium wall 5 mm sapphire tube; and on a single spectrometer, a thin wall 3 mm glass NMR tube of $CDCl_3$, and a thin wall 5 mm glass NMR tube containing D_2O were compared.

Convection was shown to occur both above and below the quiescent sample temperature (at which the variable temperature (VT) air is the same temperature as the ambient sample temperature). Rayleigh-Bénard convection has long been known to occur in an NMR sample when a threshold 'Rayleigh number' is reached, for a negative vertical temperature gradient along the axis of the NMR tube (i.e. heating the sample from below)[4], but does not lead to convection when cooling the sample (i.e. VT is set below the quiescent sample temperature). The driving force for convection here is a transverse temperature gradient, which has no threshold to be overcome.[5]

Convection was revealed to occur at any significant difference, in either direction, between VT air and quiescent sample temperature, due to transverse temperature gradients across the sample. Some practical methods of reducing convection speeds are demonstrated using NMR tubes with a more thermally conductive wall (e.g. sapphire), a smaller inner diameter, or a smaller outer diameter.

1. Jerschow A. and Müller N., J. Mag. Res., 125, 372, 1997
2. Nilsson M. and Morris G. A., J. Mag. Res., 177, 203, 2005
3. Loening N. M. and Keeler J., J. Mag. Res. 139, 334, 1999
4. Goux W. J., Verkruyse L. A. and Salter S. J., J. Mag. Res., 88, 609, 1990
5. Ostroumov G. A., Gosud. Izd. Techn-Teor. Lit., Svobodnaya convectzia v ousloviakh vnoutrennei zadachi, 1952

39 Characterizing Hydrogen Bonding Interactions in Amorphous Solid Dispersions Using Solid-State NMR Spectroscopy

Xiaoda Yuan and Eric J. Munson

University of Kentucky, Department of Pharmaceutical Sciences, Lexington, KY, USA

Formulation of solid dosage forms with an active pharmaceutical ingredient (API) in the amorphous form is a strategy used to enhance bioavailability of poorly water-soluble compounds, as the amorphous API typically has a higher apparent solubility and faster dissolution rate than its crystalline counterpart [1]. However, amorphous drugs possess higher free energies and have the risk of converting to the thermodynamically more stable crystalline form. To overcome physical instability, a polymer is mixed with the amorphous drug to form an amorphous solid dispersion. The formation of hydrogen bond between the drug and polymer is thought to play a significant role in reducing the crystallization of amorphous drugs [2].

FT-IR and Raman Spectroscopy have often been used to study hydrogen bonding in drug-polymer systems, but it is difficult to obtain quantitative data using these techniques. Here we describe an approach that combines single-site ^{13}C isotopic labeling and spectral subtraction to quantify the hydrogen bonding interactions of the amorphous drug indomethacin and polymer PVP in amorphous solid dispersions. The effect of moisture on hydrogen bonding was also investigated. The quantitative information obtained from this study was compared with results from MD simulations reported in the literature.

This work was funded by NSF I/UCRC Center for Pharmaceutical Development. EJM discloses that he is a partial owner of Kansas Analytical Services, a company that provides solid-state NMR services to the pharmaceutical industry. The results presented here are from academic work at University of Kentucky, and no data from Kansas Analytical Services are presented.

1. Hancock, Bruno C.; Zografi, George, Journal of Pharmaceutical Sciences, 86, (1), 1-12, 1997.
2. Taylor, Lynne S.; Zografi, George, Pharmaceutical Research, 14(12), 1691-1698, 1997.

40 A Modified EXSIDE Experiment for the Measurement of ^{19}F–^{15}N Coupling Constants at Natural Abundance

Ion Ghiviriga and William R. Dolbier

Chemistry Department, University of Florida, Gainesville, Florida

A modified ^{19}F-^{15}N EXIDE experiment [1] is proposed for the measurement of the ^{19}F-^{15}N couplings at natural abundance. The evolution of ^{1}H-^{15}N couplings in *f1* was suppressed by adding two composite 180 pulses, on the 3rd channel assigned to ^{1}H, in the middle of the *j*-scaling module, and in the midde of *t1*. ^{1}H waltz broadband decoupling was used during acquisition to collapse the multiplets due to ^{1}H-^{19}F couplings.

Compared to the alternative method of measuring the ^{19}F-^{15}N couplings as passive couplings in *f1* in a ^{1}H-^{15}N gHSQC spectrum, the proposed experiment has the benefits of *j*-scaling and of the assignment of the coupling constants when more than one fluorine atom is present. Application of the two methods is illustrated on a number of examples.

1. V.V. Krishnamurthy, Journal of Magnetic Resonance, Series A 121, 33–41, 1996.

41 Multinuclear Solid-state NMR Studies of Form I of Atorvastatin Calcium

Shi Bai

Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, USA

Atorvastatin calcium is the active pharmaceutical ingredient (API) of Lipitor. Solid-state ^{13}C**,** ^{19}F, and ^{15}N magic angle spinning NMR studies of Form I of atorvastatin calcium are present, including chemical shift tensors of all resolvable carbon sites and fluorine sites. The complete ^{13}C and ^{19}F chemical shift assignments are given based on an extensive analysis of ^{13}C-^{1}H HETCOR and ^{13}C-^{19}F HETCOR results. The solid-state NMR data indicate that the asymmetric unit of this polymorph contains two atorvastatin molecules. A possible structure of Form I of atorvastatin calcium (ATC-I), derived from solid-state NMR data and density functional theory calculations of various structures, is proposed for this important API.

42 Application of No-D NMR in Illicite Cocaine Analysis

Lucas de Almeida Gama[1], Wanderson Romão[2], Valdemar Lacerda Jr.[1], Bianca Bortolini Merlo[3], and **Alvaro Cunha Neto**[1]

1. Chemistry Departament, Federal University of Espirito Santo, Av. Fernando Ferrari 514, 29.075-910 – Vitória – ES, Brazil, Phone +55/27 4009 2908. Fax +55/27 4009 2826; *e-mail:valdemar.lacerda@ufes.br
2. Federal Institute of Espirito Santo, 29.106-010, Vila Velha – ES, Brazil, Phone +55/27 3149 0700
3. Laboratório de Química Legal, Superintendência de Polícia Técnico-Científica da Polícia Civil do Estado do Espírito Santo, 29.045-402, Vitória – ES, Brazil, Phone +55/27 3315 9154

Cocaine is the purification products of coca paste, extracted from leaves of the coca bush. Illicit cocaine is a common group of drugs spread in the world, moreover the presence of the adulterants as lidocaine, procaine, cafeine and phenacetine, and/or diluents (like suggar) is very usual in these cocaine seizured [1,2].

Cocaine abuse has increased dramatically during the past three decades, intensifying this way the interest in methods for detection and quantificcation of the parent drug. Cocaine and this adulterants/diluents are commonly identificated by gas chromatographic (GC) or gas chromatographic-mass spectrometric (GC-MS) analyses. The polar nature of the compounds make these analysis difficult and derivatization process is mandatory [3].

No-D NMR spectroscopy involves ^{1}H NMR spectra of samples dissolved in non-deuterium enriched solvents. No-D NMR is useful for *in situ* monitoring reaction, determining the concentration of many common reagents, especially for air-sensitive reagents. This methodology can be employed in routine analysis of mixtures, like illicit cocaine seizure [4,5].

In this work, the No-D NMR was employed to cocaine routine analysis, seized in Espirito Santo State, Brazil. The cocaine and adulterants (lidocaine, caffeine and phenacetin) were dissolved in commercial methanol (0.1 mol L^{-1}). The solution was applied in a 5mm NMR tube, with a coaxial insertion tube containing deuterated methanol (CD_3OD).

Figure 1 shows the cocaine (Figure 1a) and these adulterants in commercial methanol solutions.

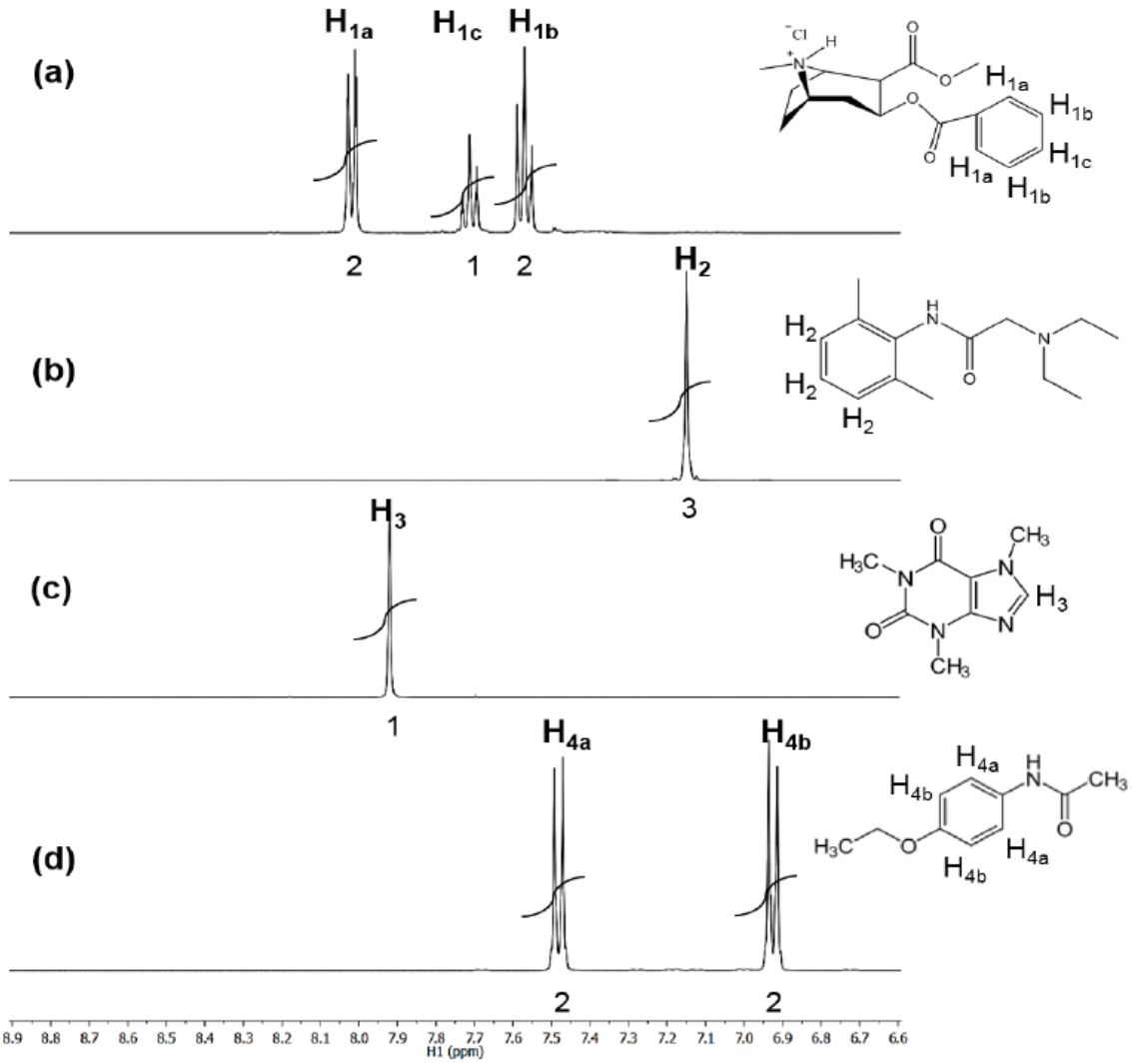


Figure 1. Expansion of aromatic region from ^{1}H NMR spectra for (a) cocaine, (b) lidocaine, (c) caffeine and (d) phenacetin.

The NMR analysis showed the adulterants distribution in the Espirito Santo State. An interesting result was observed in the samples with "no adulterants" (Figure 2). The major seizures without adulterants were observed in the North of the state, indicating a possible route of the international (cocaine paste) cocaine from the another states in the North of Espirito Santo State, south of the Bahia (BA) state and northeast of Minas Gerais (MG) state. The Metropolitan region of the state also presents a major distribution of adulterants, indicating a representative region in the illicit cocaine distribution inside the state.

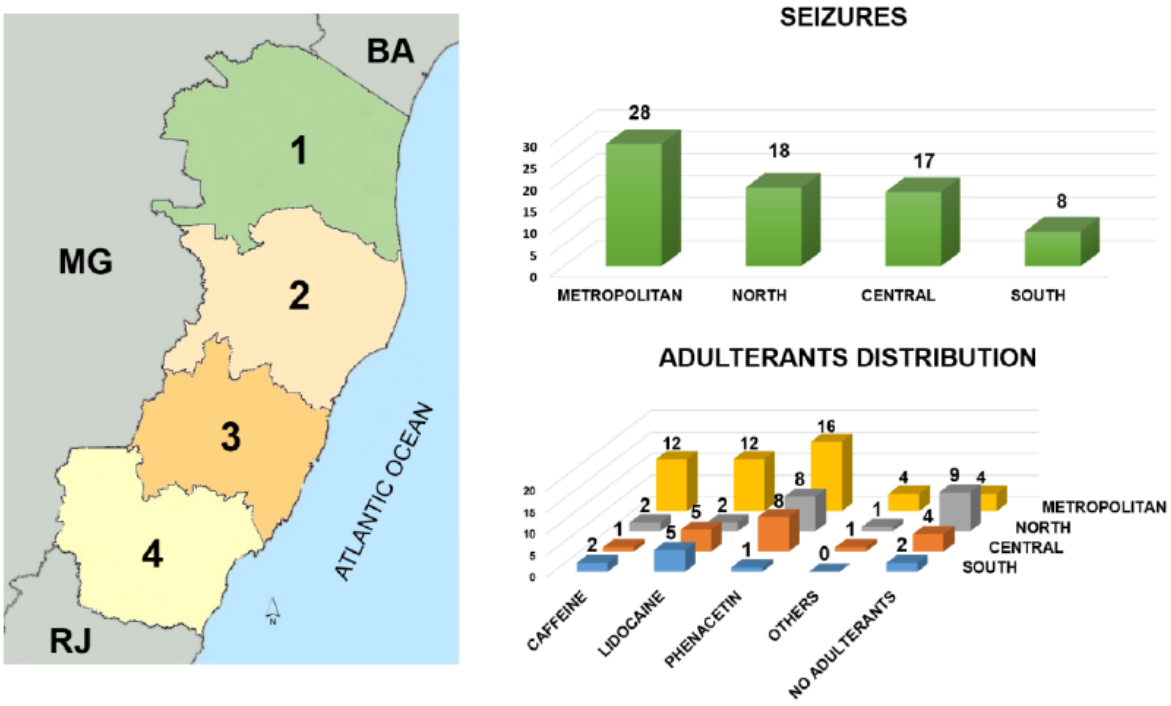


Figure 2. Espirito Santo State region (1- North, 2- Central, 3- Metropolitan and 4- South) and adulterants distribution.

Acknowledgements

The authors thank CNPq, CAPES, FAPES and FAPESP for financial support and fellowships.

1. B. Pagano, I. Lauri, S. De Tito, G. Persico, M.G. Chini, A. Malmendal, E. Novellino, A. Randazzo.. Forensic Science International, 2013, 231, 120-124.
2. N. Fucci. Forensic Science International., 2011, 209, e23-e25.
3. M. Farina, M. Yonamine, O.A. Silva. Forensic Science International, 2002, 127, 204-207.
4. T.R. Hoye, B.M. Eklov, M. Voloshim. Org. Lett., 2004, 6, 2567-2570.
5. T.R. Hoye, B.M. Eklov, T.D. Ryba, M. Voloshin, L.J, Yao. Org.Lett., 2004, 6, 953-956.

43 Free Ligand Conformational Populations in Solution – A Powerful Drug Discovery Tool

Jan-Christoph Westermann, Thorsten Nowak, Barrie Martin, and Charles D Blundell

C4X Discovery Ltd, Manchester, UK

The dynamic interchange of accessible low energy conformations for ligand molecules has traditionally been predicted computationally or extrapolated from small molecule crystal structure data. Here we present a new quantitative and precise NMR methodology that provides experimentally-derived detailed conformational information for free ligands in aqueous solution. These '4D-structures' give unprecedented insight in how to exploit and control ligand conformational preferences in drug design.

The detailed conformational information on ligand molecules contained in 4D-structures can be applied to all stages and settings of the drug discovery process. In Hit Identification, accurate 3D-pharmcophore information derived from reference compounds permits the rapid identification of chemical equity independently of other screening approaches (*e.g.*, VS, HTS). In Lead-Generation and Lead-Optimisation, 4D-structures can significantly impact on compound design separately or in conjunction with traditional structure-based drug design approaches. Specific examples for a range targets are presented, demonstrating how 4D-structures can be been used to great effect in drug discovery.

1. Blundell, C. D., Packer, M. J., & Almond, A., Bioorganic & Medicinal Chemistry, 21 (17), 4987–4976, 2013.

44 Oils of Provenance: Quantitative NMR Using Natural Abundance Deuterium

Donald S. Thomas, Adelle Amoore, Douglas G. Lawes, Aditya Rawal, Zhi Hao Chan, and James M. Hook

NMR Facility, Mark Wainwright Analytical Centre, The University of New South Wales, Sydeny NSW 2052 Australia

The isotopic distribution of deuterium within natural products, such as essential oils, can be used to determine their geographical origins. The same aim has been sought with both ^{1}H and ^{13}C NMR spectroscopy[1,2] and they have also been found effective for determining provenance. All of these NMR techniques have inherent advantages and disadvantages. In the case of ^{1}H, the over-crowding of large number of peaks can make analysis difficult, while ^{13}C, with a much larger range, is acquired with ^{1}H decoupling, and for quantitation, requires very long acquisitions for satisfactory outcomes.

Deuterium NMR may also suffer from these difficulties, but it has its own challenges.[3] The first, is obtaining sufficient signal to noise at natural abundance. To this end a pilot study into the distribution of deuterium within a variety of natural oils of commercial interest has been undertaken. Oils of Eucalyptus, tea tree, rosemary, lemon, lavender and peppermint have all been compared from a variety of sources to ascertain if a quantifiable difference in the D/H ratio can be observed, both within molecules and between sources.

Second, quantitative calibration of the observed signals must be achievable, and the choice of a standard for calibration is critical. Isotopically pure deuterated standards can be expensive, especially if used as an internal standard where it is added to each sample to be analysed. Vienna Standard Mean Ocean Water (VSMOW) is an internationally recognized standard for the isotopic distribution within water, and can now be used as an external standard using the ERETIC2 method for quantitative NMR (qNMR).

Practical applications of standards and acquisition protocols for the use of deuterium as a nucleus in quantitative NMR have been developed and are presented here.

1. Spraul, M. et al. NMR-Based Multi Parametric Quality Control of Fruit Juices: SGF Profiling. Nutrients 1, 148–155 (2009).
2. Caytan, E., et al. Precise and accurate quantitative C-13 NMR with reduced experimental time. Talanta 71, 1016–1021 (2007).
3. Lesot, P., et al. Natural abundance deuterium NMR spectroscopy. Developments and analytical applications in liquids, liquid crystals and solid phases. Prog. Nucl. Magn. Reson. Spectrosc. 55(2): 128-159 (2009).

45 NMR Structure Elucidation of Small Organic Molecules and Natural Products: Choosing ADEQUATE vs HMBC

Alexei V. Buevich[1], R. Thomas Williamson[2], and Gary E. Martin[2]

1. Merck Research Laboratories, Discovery and Preclinical Sciences, Process and Analytical Chemistry, NMR Structure Elucidation, Kenilworth, NJ 07033, USA
2. Merck Research Laboratories, Discovery and Preclinical Sciences, Process and Analytical Chemistry, NMR Structure Elucidation, Rahway, NJ 07065, USA

Long-range heteronuclear shift correlation methods have served as the cornerstone of modern structure elucidation protocols for several decades. The ^{1}H-^{13}C HMBC experiment provides a versatile and relatively sensitive means of establishing predominantly $^{3}J_{CH}$ connectivity with the occasional $^{2}J_{CH}$ or $^{4}J_{CH}$ correlation being observed. The two-bond and four-bond outliers must be identified specifically to avoid spectral and/or the structural misassignment. Despite the versatility and extensive applications of the HMBC experiment, it can fail to elucidate structures of molecules that are highly proton-deficient. In such cases, recourse to the ADEQUATE experiments should be considered. The current study was undertaken to facilitate a better understanding of situations where it might be beneficial to apply 1,1- or 1,n-ADEQUATE vs HMBC experiments to proton-rich and/or proton-deficient molecules. Strychnine (**1**) and cervinomycin A_2 (**2**) were employed as model compounds for each of these structural classes, respectively. DFT methods were employed to calculate the relevant heteronuclear proton-carbon $^{n}J_{CH}$ and homonuclear carbon-carbon $^{n}J_{CC}$ coupling constants for this study.

46 Fast and Efficient Enantiodifferentiation Through Pure Shift NMR

Laura Castañar[1], Míriam Pérez-Trujillo[1], Eva Monteagudo[1], Lars T. Kuhn[2], Pau Nolis[1], Albert Virgili[1], R. Thomas Williamson[3], and **Teodor Parella**[1]

1. Servei de Ressonància Magnètica Nuclear and Departament de Química, Universitat Autònoma de Barcelona, Bellaterra (Barcelona) Catalonia, Spain
2. DFG Research Center Molecular Physiology of the Brain (CMPB) & European Neuroscience Institute Göttingen (ENI-G), Göttingen, Germany.
3. Merck & Co. Inc, NMR Structure Elucidation, Rahway, NJ, USA.

The determination of enantiomeric purity can be accomplished by NMR spectroscopy using a great variety of auxiliary chiral sources. Of these, chiral solvating agents (CSAs), such as the so-called Pirkle alcohol (PA) or cyclodextrins (CDs), have been widely used. They do not typically introduce significant line-broadening, the sample is easily prepared and the analysis is quickly performed by observing chemical shift differences ($\Delta\Delta\delta$) between the resulting diasteromeric complexes in conventional ^{1}H NMR spectra. However, signal enantiodifferentiation using CSAs is not uniform for all protons and in many cases, low $\Delta\Delta\delta$ values and signal overlap caused by complex multiplets leads to the lack of spectral signal dispersion that precludes a straightforward analysis. Alternatively, enantiodifferentiation using ^{13}C NMR spectroscopy can be more advantageous because singlet signals are analyzed although its routine use is limited by its low sensitivity.[1]

We show how pure shift NMR can become a very efficient tool in enantiodifferentiation studies. First, a 1D Homodecoupled frequency-selective experiment is proposed for the fast ^{1}H chemical shift discrimination of overlapped signals.[2] Then, the concepts of spectral aliasing (SA) [3] and pure shift (PS) NMR [4] are combined into a novel 2D SAPS-HSQC experiment [5] which provides simultaneous ^{1}H and ^{13}C enantiodifferentiated data ($\Delta\Delta\delta(^{1}H)$ and $\Delta\Delta\delta(^{13}C)$) with high digital resolution and signal dispersion for both ^{1}H and ^{13}C dimensions. Its use increases significantly the probability to detect an enantiodifferentiated nucleus since more signals are observed (^{1}H and ^{13}C nuclei), overlapping problems of conventional 1D ^{1}H experiments are overcome, and poor enantiodifferentiation in 1D experiments can be now detected, allowing the study of cases abandoned in the past for reasons of poor enantioresolution and/or long experimental times.

1. Pérez-Trujillo, M.; Monteagudo E.; Parella T. Anal. Chem., 85, 10887-10894. 2013.
2. Castañar, L.; Pérez-Trujillo, M.; Nolis, P.; Monteagudo, E.; Virgili A.; Parella, T. ChemPhysChem, 15, 854-857, 2014.
3. Jeannerat, D. Magn. Reson. Chem., 41, 3-7, 2003.
4. Paudel, L.; Adams, R.W.; Kiraly, P.; Aguilar, J.A.; Foroozandeh, M.; Cliff, M.J.; Nilsson, M.; Sandor, P.; Waltho J.P; Morris, G.A. Angew. Chem. Int. Ed., 52, 11616-11619, 2013.
5. Pérez-Trujillo, M.; Castañar, L.; Monteagudo, E; Kuhn, L.T.; Nolis, P.; Virgili, A.; Williamson, R.T.; Parella T. Chem. Comm. 50, 10214-10217, 2014.

47 Perfect-HSQC: Suppression of Phase and Amplitude J(HH) Modulations

Laura Castañar[1], Eduard Sistaré[1], Albert Virgili[1], R. Thomas Williamson[2], and **Teodor Parella**[1]

1. Servei de Ressonància Magnètica Nuclear and Departament de Química, Universitat Autònoma de Barcelona, Bellaterra (Barcelona) Catalonia, Spain.
2. Merck & Co. Inc, NMR Structure Elucidation, Rahway, NJ, USA.

Many chemists use the HSQC experiment in a qualitative way to correlate the chemical shifts of carbon and attached protons. For these applications, the issues with phase distortion caused by J(HH) are for the most part invisible and therefore irrelevant. However, the amplitude and the phase of cross-peaks during the INEPT periods in conventional 2D HSQC experiments are modulated by both proton-proton, J(HH), and proton-carbon, ^{1}J(CH), couplings. These effects are present in the form of anti-phase components and become highly relevant when performing quantitative measurements in terms of J or intensity measurements because they do not cancel out.

On the other hand, it has been reported the concept of perfect echo which has been successfully implemented in a series of NMR applications to solve peak distortions caused by homonuclear J-coupling during echo periods.[1-3] Very recently, this concept has also been used in an effort to improve long-range heteronuclear transfers by means of a "perfect-echo INEPT" element.[4]

It is shown, experimentally and by simulation, that the typical J(HH) interferences present in conventional HSQC experiments can be efficiently suppressed in an improved perfect-HSQC pulse scheme, which replaces the classical INEPT ($\Delta/2$-$180_x(^1H,^{13}C)$-$\Delta/2$) by a perfect-echo INEPT module consisting of a double echo period ($\Delta/2$-$180_x(^1H)$-$\Delta/2$-$90_y(^1H)$-$\Delta/2$-$180_x(^1H,^{13}C)$-$\Delta/2$) in both defocusing/refocusing heteronuclear transfer periods. The resulting 2D perfect-HSQC spectra afford pure in-phase cross-peaks with respect to both ^{1}J(CH) and J(HH), irrespective to the experiment delay optimization. In addition, peak volumes are not influenced by J(HH), rendering practical applications such as phase correction, signal integration and multiplet analysis more appropriate.

1. Takegoshi, K.; Ogura, K.; Hikichi, K. J. Magn. Reson., 84, 611–615, 1989.
2. Torres, A. M.; Zheng, G.; Price, W. S. Magn. Reson Chem., 48, 129-133, 2010.
3. Aguilar, J. A.; Nilsson, M.; Bodenhausen, G.; Morris, G. A. Chem. Comm., 48, 811–813, 2012.
4. Baishya, B.; Khetrapal, C. L.; J. Magn. Reson., 242, 143-154, 2014.

48 Pure In-Phase Heteronuclear Correlation NMR Experiments

Laura Castañar[1], **Josep Saurí**[1,2], R. Thomas Williamson[2], Albert Virgili[1] and Teodor Parella[1]

1. Servei de Ressonància Magnètica Nuclear and Dept. de Química, Universitat Autònoma de Barcelona, Bellaterra (Barcelona) Catalonia, Spain.
2. Merck & Co. Inc, NMR Structure Elucidation, Rahway, NJ, USA.

The accurate quantification of long-range proton-carbon interactions from HMBC and HSQMBC spectra has been problematic due to the characteristic mixed phases of the resulting cross-peaks, which lead to difficult data analysis. The main source of problems arises from the fact that proton-proton coupling constants, J(HH), that have similar magnitudes to ^{n}J(CH) (typically ranging from 0-15 Hz), also evolve during the defocusing/refocusing periods. This unwanted modulation can introduce undesired effects in both the multiplet phase and the transfer efficiency.[1]

This work reports a simple and general broadband solution to obtain heteronuclear correlation spectra that yield truly pure absorption lineshapes and IP multiplet structures for all available cross-peaks with respect to both J(CH) and all passive J(HH) coupling constants along the detected dimension. The proposal is based on a conventional HSQC pulse train with an appended adiabatic z-filter [2] applied just before a refocusing gradient perfect-echo element and acquisition. Modification of HSQC and HSQMBC pulse sequences to include a PIP-module affords experiments that can be referred to as PIP-HSQC when the Δ delay is optimized as a function of ^{1}J(CH) or as PIP-HSQMBC when Δ is optimized as a function of ^{n}J(CH).

Experimental data will be provided showing how clean multiplet patterns obtained in PIP-HSQC and PIP-HSQMBC experiments [3] are suitable for a direct extraction of scalar and residual dipolar coupling constants in resolved signals, for a peak-fitting/matching process from a reference signal, and/or for the application of the IPAP technique in non-resolved multiplets.

1. Parella, T.; Espinosa, J.F. Prog. NMR Spect., 73, 17-55, 2013**.**
2. Thrippleton, M.J.; Keeler, J. Angew. Chem. Int. Ed., 42, 3938-3941, 2003.
3. Castañar, L.; Saurí, J.; Williamson, R.T.; Virgili, A.; Parella, T. Angew. Chem. Intl. Ed., 53, 8379-8382, 2014.

49 Simultaneous Measurement of the Magnitude and the Sign of Multiple Heteronuclear Coupling Constants in ^{19}F– or ^{31}P– Containing Molecules

Josep Saurí[1,2], Pau Nolis[1], and Teodor Parella[1]

1. Servei de Ressonància Magnètica Nuclear and Dept. de Química, Universitat Autònoma de Barcelona, Bellaterra (Barcelona) Catalonia, Spain.
2. Merck & Co. Inc, NMR Structure Elucidation, Rahway, NJ, USA.

The determination of both the magnitude and the sign of heteronuclear coupling constants is fundamental in the structural characterization of organic, organometallic and inorganic compounds. Recently, a family of proton-selective HSQMBC (selHSQMBC) experiments has been proposed as powerful NMR tools to obtain accurate long-range proton-carbon J coupling constants ($^{n}J(CH)$) [1-3]. The original selHSQMBC experiment allows the easy determination of the magnitude of $^{n}J(CH)$ by direct analysis of the relative displacement of the separate α/β multiplet components along the high-resolved detected F2 dimension [1]. Related selHSQMBC-COSY [2] and selHSQMBC-TOCSY [3] experiments allow extend the measurement to other protons belonging to the same spin system and, in addition, the information about the relative sign is also obtained by comparing the relative right/left or left/right sense of the signal displacement along.

In this work we present the successfully application of selHSQMBC experiments on fluoro- and phosphorus-containing small molecules. The presence of a high-abundant passive nucleus (such as Z=^{19}F or ^{31}P) allows the simultaneous determination of the magnitude and the sign of three different heteronuclear coupling constants from the analysis of each individual cross-peak observed in a ^{1}H-X selHSQMBC experiment. Whereas J(HZ) and J(XZ) coupling constants can be extracted from E.COSY multiplet patterns, J(XH) is independently determined from the complementary IPAP pattern generated along the detected F2 dimension. A related time-shared version is also proposed for the simultaneous measurement of J(CH) and J(NH) in nitrogen-containing compounds [5]. In this case, the presence of a passive spin also allows the additional measurement of J(CZ), J(NZ) and J(ZH) coupling constants. In addition, the incorporation of an extended TOCSY transfer allows the determination of a complete set of homonuclear and heteronuclear coupling constants for an entire 1H spin system. The use of non-uniform linear sampling (NUS) and/or the signal detection under homodecoupling conditions using the HOBS technique [4] will be also evaluated.

1. Gil, S.; Espinosa, J.F.; Parella, T. J. Magn. Reson. 213, 145-150, 2011.
2. Saurí, J.; Parella, T. Magn. Reson. Chem. 50, 717-721, 2012.
3. Saurí, J.; Espinosa, J.F.; Parella, T. Angew. Chem. Intl. Ed. 51, 3919-3922, 2012.
4. Castañar, L.; Saurí, J.; Nolis, P.; Virgili, A.; Parella, T., J. Magn. Reson., 238, 63-69, 2014.
5. Nolis, P.; Pérez-Trujillo, M.; Parella T. Magn. Reson. Chem., 44, 1031-1036, 2006.

50 Real Time On-Line Flow-NMR-MS For Reaction Monitoring

Anthony W.T. Bristow[1], **Steven R. Coombes**[1], Ana-Paola De Sousa[2], Michael I.T. Nunn[1], and Andrew D. Ray[1]

1. AstraZeneca, Pharmaceutical Development, Macclesfield, UK
2. Wadham College, University of Oxford, Oxford, UK

Here we describe the use of a fully hyphenated NMR-MS system for on-line reaction understanding and monitoring of batch processes. Combining the structurally rich information obtained from both techniques, along with the selectivity and sensitivity of MS and the quantitative nature of NMR, both on-line with no sub-sampling, enables a greater understanding of reaction mechanisms and profiles to be achieved.

The use of NMR Spectroscopy is widespread throughout pharmaceutical development providing a rich source of structural and quantitative information. NMR is commonly used for monitoring and understanding chemical reactions, however, this is normally achieved by sampling (and with the addition of deuterated solvents) to run in-process tests or checks (IPT's & IPC's) or in order to determine the end of reaction (EOR). We have a dedicated 500MHz NMR instrument for reaction monitoring using the NMR flow-tube system [1] with a broadband 5mm probe. The system allows for fully quantitative NMR data to be acquired on a sample pumped directly from the reaction vessel to enable better mechanistic understanding and also generate full kinetic profiles. The NMR experiments are routinely run unlocked (using protonated solvents so as not to introduce any isotope effects) and "on-flow" with linewidths comparable to standard tube based analysis (HHLW ~2Hz).

On-line monitoring of chemical reactions using MS can also provide data to (i) determine the fate of starting materials and reagents, (ii) confirm the presence of the desired product, (iii) identify intermediates and impurities, (iv) determine steady state conditions and (v) speed up process optimisation. Recent developments in portable mass spectrometers further enable this coupling, as they can be easily positioned with the reaction system to be studied. A major issue for this combination is the transfer of a sample that is representative of the reaction and also compatible with the mass spectrometer - this is particularly challenging as high concentrations of reagents and products are common in organic synthesis.

We have developed a system which pumps a reaction mixture directly from the vessel to the MS, then onto the NMR instrument before returning the reactor. Sample transfer to the MS is achieved using a mass rate attenuator (MRA) and a sampling make-up flow from a high pressure pump. This enables the appropriate sample dilution, transfer and preparation for electrospray ionisation (demonstrated recently by Bristow et al [2]). We will demonstrate the use of the system with case studies and also discuss quantitative versus qualitative analysis and observations regarding dynamic range and sensitivity capabilities.

The development and commercial manufacture of low field, portable small footprint NMR systems has the potential to make reaction monitoring by NMR a true Process Analytical Technology (PAT) tool. These systems can be taken directly to the chemist rather than the chemist bringing a sample to the NMR system, be it on the bench, large scale lab, pilot plant or manufacturing site. We have evaluated a 60MHz NMR system for monitoring reactions and report our findings.

1. Khajeh, Miriam, Bernstein Michael A and Morris, Gareth A, Magn. Reson. Chem., 48, 516, 2010.
2. Tony WT Bristow, Andrew D Ray, Anne O'Kearney-McMullan, Louise Lim, Bryan McCullough and Alessio Zammataro, J. Am. Soc. Mass Spectrom., in print – tbc

51 DFT ^{1}H-^{1}H Coupling Constants in the Configurational Reassignment of Synargentolide A

Francisco Juárez-González[1], G. Alejandra Suárez-Ortiz[1], Mabel Fragoso-Serrano[1], Carlos M. Cerda-García-Rojas[2] and **Rogelio Pereda-Miranda**[1]

1. Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City, 04510 DF, Mexico
2. Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, 07000 DF, Mexico

Using the DFT-NMR integrated approach as well as spectral simulation, the configurational reassignment of synargentolide A, a flexible and highly polyfunctionalized 6-heptenyl-5,6-dihydro-2*H*-pyran-2-one was accomplished. This class of biologically active plant derived metabolites are particularly abundant in the genus *Hyptis* of the mint family [1]. Their cytotoxicity against various tumor cell lines is related to the *α*,*β*-unsaturated *δ*-lactone system, which is a well-known Michael acceptor. These compounds are structurally related to pironetin, an anticancer acetogenin of microbial origin that selectively targets Lys352 of *α*-tubulin [2]. Determination of their stereochemical and conformational features has offered a substantial challenge due to their flexibility and the presence of multiple chiral centers [1]. Thus, after comparing DFT NMR calculations with experimental ^{1}H-^{1}H NMR coupling constants, the structural reassignment of synargentolide A was accomplished. Successful exploration of the full conformational space for the four possible diastereoisomers of spicigerolide A, all containing the (*S*)-configuration at C-6' (a unique biogenetic feature present in all natural 6-heptenyl-5,6-dihydro-2*H*-pyran-2-ones from the mint family), resulted in improving our DFT-NMR integrated protocol for the conformational and configurational analysis of this flexible molecule in gas phase. Calculated $^{3}J_{H,H}$ values established its configuration as 6*R*-[4'*S*,5'*S*,6'*S*-(triacetyloxy)-2*E*-heptenyl]-5,6dihydro-2*H*-pyran-2one, in contrast with the incorrect 6*R*,4'*R*,5'*R*,6'*R*-diastereoisomer previously proposed by synthesis [3]. Detailed values for ^{1}H chemical shifts and coupling constants revealed the noticeable spectral differences not previously estimated by any of the groups that claimed for the correct stereochemistry. Application of this approach increases the probability for successful enantiospecific total syntheses of flexible compounds with multiple chiral centers.

1. López-Vallejo, F., Fragoso-Serrano, M., Suárez-Ortiz, G.A., Hernández-Rojas, A.C., Cerda-García-Rojas, C.M., Pereda-Miranda. R. J. Org. Chem., 76, 6057–6066, 2011.
2. Bañuelos-Hernández, A.E., Mendoza-Espinoza, J.A., Pereda-Miranda, R., Cerda-García-Rojas, C.M. J. Org. Chem. 79, 3752–3764, 2014.
3. Sabitha, G., Gopal, P., Reddy, C.N.,. Yadav. J.S. Tetrahedron Lett. 50, 6298–6302, 2009.

52 Evaluating the Relative Merits of CRISIS-HSQC, Pure Shift-HSQC and ASAP-HMQC for Rapid Screening of Organic Compounds

Dmitri Pichugin, Darcy C. Burns, and William F. Reynolds

Department of Chemistry, University of Toronto

The relative advantages and disadvantages of the three named pulse sequences, used in conjunction with non-uniform sampling and forward linear prediction, are evaluated for their suitability for rapid sample screening. ASAP-HMQC is by far the fastest (minimum time ~ 30 seconds),

CRISIS-HSQC is intermediate (minimum time ~ 3 minutes) while Pure Shift-HSQC is slowest (minimum time ~ 6 minutes). However, ASAP-HMQC gives the poorest ^{13}C resolution and no-spectral editing, while Pure Shift-HSQC gives the best ^{1}H resolution and signal/noise. Non-uniform sampling and linear prediction can be used in conjunction with any of the sequences, the former to reduce the minimum experiment time and the latter to improve ^{13}C resolution. The relative merits of different methods of processing NUS spectra are also evaluated.

53 Wide-Band Relaxometry Analysis of Noisy Multiexponential Decay Data

Juan Carlos Cobas Gómez and Santiago F. López Ponte

Mestrelab Research SL, Santiago de Compostela, Spain

There is a plethora of physical phenomena such as nuclear science, chemical kinetics, fluorescence and NMR spectroscopy, to cite a few examples, where the observed signal can be represented by a sum of exponential decays.

The determination of the exponential decays and their amplitudes from a noisy signal is essentially an ill-posed inverse problem, meaning that several and very different solutions may fit the experimental data equally well. Often, regularization is incorporated to minimize the chances to be fitting to the noise and thus make the problem better conditioned. For instance, the classical approach to this problem involves adding in an adjustable smoothing parameter to the solution in order to favor the 'smoothest' solution that fits the data to within a certain error margin [1].

Many other different methods have been proposed for the analysis of these signals and the pros and cons of these methods are often the subject of intense debate [2]. In contrast to sinusoids, real-valued exponential decays do not form an orthogonal basis of functions on the real axis, thus making the estimation process extraordinarily sensitive to experimental noise and artifacts of the measured signal.

In this work we present a new approach to the inversion of exponential decays based on the addition of almost periodic, stochastical components in order to set the initial problem in the arena of the exponentially damped sinusoids. Then the problem is tackled by a Detection-Estimation Scheme for noisy signals based on a Matrix Pencil method [3], whose optimal order of calculation relies on the estimation of the number of components made upon a criterion derived from information theory [4].

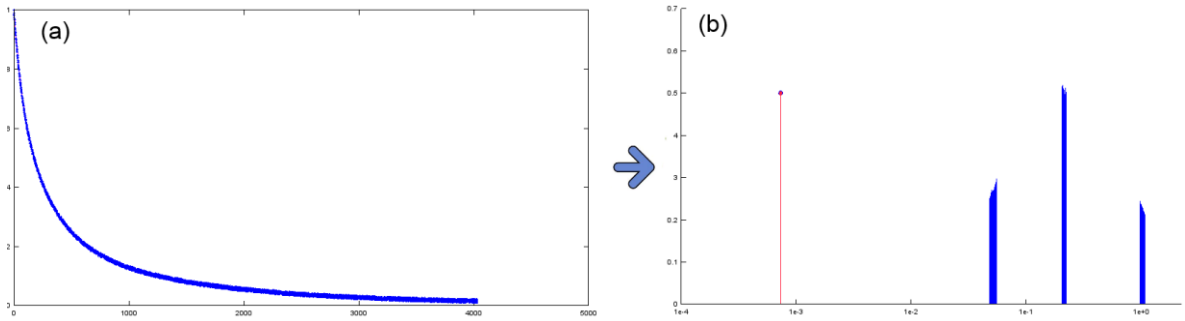


Figure: (a) Signal composed by the sum of 3 exponentials with amplitudes of 0.20, 0.50 and 0.25 and decays of 0.05, 0.20 and 0.90 respectively corrupted with synthetic noise (2% with respect to the signal maximum). (b) Resolved components by the algorithm proposed in this work. The red stick corresponds to the dwell time.

1. S.W. Provencher, "A constrained regularization method for inverting data represented by linear algebraic or integral equations". Comput. Phys. Commun. 27, 213 (1982)
2. Istratov and O. F. Vyvenko, "Exponential analysis in physical phenomena," Rev. Sci. Instrum., 70, 1233 (1999)

3. Y. Hua, T. K. Sarkar, “Matrix pencil method for estimating parameters of exponentially damped/undamped sinusoids in noise”, IEEE Trans. Signal Process. 39, 892 (1991)
4. Y. Y Lin, P. Hodgkinson, M. Ernst, A. Pines, “A Novel Detection–Estimation Scheme for Noisy NMR Signals: Applications to Delayed Acquisition Data”, J. Magn. Reson. 128, 30–41 (1997)

54 Structure Revision of Acremolin: CASE Approach Based on 2D NMR vs. Total Synthesis

Mikhail Elyashberg[1], Rostislav Pol[1], Sergey Golotvin[1], and **Patrick Wheeler**[2]

1. Advanced Chemistry Development, Moscow Department, Moscow, Russian Federation
2. Advanced Chemistry Development, Toronto, Canada

According to the review [1], around 1000 articles were published between 1990 and 2004 where originally determined structures were revised. The labor and other expenses used in structural mis-assignments and subsequent reassignments are thus roughly double the cost of getting the right answer at the start.

It has been shown [2,3] that application of modern Computer-Aided Structure Elucidation (CASE) systems [4] can usually help the chemist to avoid initial errors in structure identification. If nevertheless the researcher begins down the wrong track, the expert system can give a signal of caution. This is possible because molecular structure elucidation can be formally described as deducing all logical corollaries – without exclusions - from a system of statements ("axioms") which ultimately form a particular axiomatic theory related to a current spectrum-structural problem [4]. These corollaries constitute all conceivable structures that meet the initial system of axioms. When structure elucidation of a new chemical compound is performed with assistance of expert systems, all axioms are expressed explicitly. Therefore it becomes possible to investigate dependence of the solution of a structural problem on any change in the initial data.

However, in many publications, the structure revision is performed on the basis of total chemical synthesis. Often, to disprove a wrong structure and confirm a new structural hypothesis, both structures – original and revised, at the least – are synthesized by researchers. Computational experiments with the aid of expert system ACD/Structure Elucidator Suite lead to the following conclusions: if the 1D and 2D NMR data used by a researcher for inferring an original (wrong) structure were entered into the CASE system, then most frequently the correct (revised) structure would be assigned as the most probable, while the wrong one would be rejected by the program.

As an example, we discuss a recently performed acremolin structure revision by total synthesis (Januar and Molinsky [1]). The original (incorrect) structure was deduced by Julianti et al [2] from 1D and 2D NMR spectra. Januar and Molinski hypothesized the revised structure of acremolin and synthesized it in **five steps.** 1D and 2D NMR spectra of the product of synthesis coincided with those obtained for acremolin. A competing regioisomer structure whose possibility was also considered in [1] was rejected on the basis of ^{1}H-^{15}N HMBC spectrum.

We posed two questions. First, which structures would be delivered by CASE if it were used from the very beginning? The initial NMR data (^{1}H, ^{13}C and ^{1}H-^{13}C HMBC) from [6] were entered into the program and the problem was solved in fully automatic mode. The resulting structural file contained all three structures – original, revised and the competing regioisomer. ^{13}C chemical shift prediction

reliably selected the correct structure as the best and rejected both wrong ones – original and the competing regioisomer.

The second question was which structures can be generated from all *theoretically possible* ^{1}H-^{13}C HMBC correlations corresponding to the *original* structure? The program instantly (0.06 s) produced four structures and reliably ranked them in the following order: revised structure and tautomer, original structure, competing regioisomer. The erroneous hypothesis was immediately called into question, and that arduous total synthesis becomes unnecessary.

Though the classic dictum "synthesis is the ultimate proof of structure" remains valid, it should be enhanced: before starting total synthesis for structure revision it is very desirable to take into account results delivered by a CASE system.

1. Nicolaou K.C., Snyder S.A. *Angew. Chem. Int. Ed*., *44*, 1012-1044, 2005
2. Elyashberg M.E., Williams A.J., Blinov K.A. *Nat. Prod. Rep.*, 27(9), 1296-1328, 2010
3. Elyashberg M.E., Blinov K.A, Molodtsov S.G., Williams A.J. *J. Nat. Prod*., 76, 113−116, 2013
4. Elyashberg M.E., Williams A.J., Blinov K.A. *Contemporary Computer-Assisted*
5. *Approaches to Molecular Structure Elucidation*, Cambridge, RSC, **2012**, 482 P.
6. Januar L. A., Molinski T. F. *Org. Lett*. *15*, 2370–2373, 2013
7. Julianti E., Oh H., Lee H.-S., Oh D.-C., Oh K.-B. Shin J., *Tetrahedron Lett*., 53, 2885–2886, 2012

55 Utilizing Fluorine NMR in the Detection and Structural Elucidation of Metabolites

Igor Goljer, Amanda G. Culp, Steve Castellino, and Ernie M. Schubert

GlaxoSmithKline, DMPK, King of Prussia, Pennsylvania, USA

Many recently developed drugs and drug candidates contain fluorine for a variety of reasons: to improve potency, selectivity, tissue penetration and metabolic half life, to name a few. Fluorine NMR offers some unique properties (high sensitivity, large chemical shift range and negligible endogenous background) which can be used for screening, detection, and structural identification of drugs and metabolized products [1,2]. Recent advancements in cryoprobe technology enable the detection and quantitation of drug-related material at the sub-microgram level. The goal of this poster is to highlight some new and innovative strategies used to identify and structurally elucidate drug related material (DRM) in challenging samples from a clinical trial. The value of structural elucidation and quantitation of DRM is directly translated into the clarification of metabolic pathways and can be utilized in prediction of safety of drug candidates. Fluorine NMR was also a critical component in the structural elucidation of a product oxidative defluorination of the recently approved drug Dolutegravir [3].

1. Mutlib, A., et. al. Chem. Res. Toxicology 2012, 25, 572-583
2. Schubert, E., et. al., "Application of Alternative Analytical Methods to Yield Excretion Balance Information without the Use of a Radiolabel," Oral Presentation, Rozman Symposium, 2012
3. Castellino, S., et. al. Antimicrobial Agents and Chemotherapy, 2013, 57, p 3536-3546

56 LR-HSQMBC: A Highly Sensitive NMR Technique to Probe Very Long-Range Heteronuclear Coupling Pathways

R. Thomas Williamson[1], Alexei V. Buevich[2], Gary E. Martin[1], and Teodor Parella[3]

1. Merck Research Laboratories, Process & Analytical Chemistry, NMR Structure Elucidation, Rahway, NJ, 07065, USA
2. Merck Research Laboratories, Process & Analytical Chemistry, NMR Structure Elucidation, Kenilworth, NJ 07033, USA
3. Servei de Ressonància Magnètica Nuclear and Departament de Química, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain

HMBC is one of the most often used and vital NMR experiments for structure elucidation. We have developed a new, high sensitivity NMR pulse sequence that overcomes the typical $^{2}J_{CH}$, $^{3}J_{CH}$ limitation of HMBC by extending the visualization of long-range correlation to 4-, 5-, and even 6-bond long-range $^{n}J_{CH}$ heteronuclear couplings. This technique should prove to be an effective experiment to complement HMBC for probing the structures of proton-deficient molecules. The LR-HSQMBC NMR experiment can, in effect, extend the range of HMBC to provide data similar to that afforded by the 1,n-ADEQUATE experiment even in limited sample situations. This is accomplished by optimizing responses for very small $^{n}J_{CH}$ couplings as opposed to relying on the markedly less sensitive detection of long-range coupled ^{13}C-^{13}C homonuclear pairs at natural abundance. DFT calculations were employed to determine whether the very long-range correlations observed for cervinomycin A_2 were reasonable.

1. (a) Williamson, R. T.; Márquez, B. L.; Gerwick, W. H.; Kövér, K. E. Magn. Reson. Chem., 38, 265−273 (2000). (b) Williamson, R. T.; Boulanger, A.; Vulpanovici, A.; Roberts, M. A.; Gerwick, W. H. J. Org. Chem., 67, 7927−7936 (2002).
2. Omura, S.; Iwai, Y.; Hinotozawa, K.; Takahashi, Y.; Kato, J.; Nakagawa, A.; Hirano, A.; Shimizu, H.; Haneda, K. J. Antibiot. 35, 645−652 (1982).
3. Williamson, R. T.; Buevich, A. V.; Martin, G. E.; Parella, T. J. Org. Chem., 79, 3387-3394 (2014).
4. Williamson, R.T.; Buevich, A.V.; Martin, G.E., Tet. Lett., 55, 3365-3366 (2014).

57 Application of Ultra-High Field NMR Spectroscopy in Characterization of Natural Products

Aleksej M. Krunić and Jimmy Orjala

University of Illinois at Chicago, College of Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, 833 S. Wood St. Rm 539, Chicago IL, 60612, USA

Newly isolated natural products often represent analytical challenge due to the fact that they are more often than not isolated in sub-milligram quantities. Several NMR approaches can be applied with mass-limited samples. Cryogenic technology and high field magnets greatly enhance spectral resolution and sensitivity. Further gains can be achieved by restricting the sample volume or a combination with cryogenic approach (microcryoprobes). Alternatively, one can enhance the sensitivity by increasing the natural levels of spin active nuclei other than proton ($^{13}C/^{15}N$) relevant for structure characterization.

At our disposal, we have had the first Bruker 900 MHz US^2 spectrometer with carbon sensitive (TCI) 5mm cryoprobe, in the United States. We utilized a combination of restrictive volume and ultra-high field cryoplatform to achieve maximum signal-to-noise enhancements.

This methodology was applied to record a number of selective or hyphenated experiments, including 1,1-HSQC-ADEQUATE on a number of isolated secondary metabolites, in the most time-efficient manner. We will present our results in establishing connectivities and stereochemistries of Scytonemides A and B, Sanctolide A, Minutissamides E-L, and two new cyclic lipopeptides from UIC collection (strain ID 10045).

1. Krunic, A.; Vallat, A.; Mo, S; Lantvit, D. D.; Swanson, S. M.; Orjala, J.; Scytonemides A and B, cyclic peptides with 20S proteasome inhibitory activity from the cultured cyanobacterium Scytonema Hofmanii, J. Nat. Prod., 73(11), 1927-1932, 2010
2. Kang, H.-S.; Sturdy, M.; Krunic, A.; Kim, H.-J.; Swanson, S. M.; Orjala, J.; Minutissamides E – L, Antiproliferative Cyclic Lipodecapeptides from the Cultured Freshwater Cyanobacterium cf. Anabaena sp., Bioorg. Med. Chem., 20, 6134-6143, 2012
3. Kang, H.-S.; Krunic, A.; Orjala, J.; Sanctolide A, a 14-membered PK-NRP hybrid macrolide from the cultured cyanobacterium Oscillatoria sancta (SAG 74.79), Tetrahedron Lett., 53(28), 3563-3567, 2012

58 Software Tools for Simulating NOEs and J-Coupling from Long MD Trajectories of Flexible Molecules

Gordon Chalmers, Kari Pederson, **John Glushka**, James Prestegard, and Rob Woods

Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA

NOEs from cross-relaxation between proton pairs provide essential structural information on both large and small molecules. For rigid molecules, the analysis can be straightforward using the inverse sixth power and isolated spin-pair approximation. However for flexible molecules that have multiple conformational states, such as oligosaccharides, small peptides or intrinsically disordered proteins, simple averaging over conformational states may not provide structurally relevant models. Although formal treatments of this situation have been known for some time, it is difficult to apply these experimentally. One way is to make use of advances in computational power to generate long molecular dynamics trajectories. These calculated molecular motions can be used to generate correlation functions for dipolar interactions and then treat the system with a full relaxation matrix approach.

In general the correlation function has two $1/r^3$ terms that are typically combined into $1/r^6$ averaging, assuming motions are not correlated. It is not uncommon for small molecules to have internal motions such that the effective inter-nuclear distance is between the centers of motional range of the two nuclei. However, the steep $1/r^6$ averaging will heavily weight the positions of closest approach. Time points in an MD trajectory can be used to calculate the correlation function directly, if the trajectory is long enough to sample a uniform distribution of molecular orientations and conformations that would average the NMR data. For small molecules such as a disaccharide, these simulations would be on the order of hundreds of nanoseconds or millisecond range.

To make these calculations accessible and useful to the larger community, we are developing software tools that will be integrated into the existing GLYCAM website hosted at the CCRC. The current GLYCAM site is designed to assist in the analysis of carbohydrate conformations and interactions with proteins. It provides libraries of structures and tools for MD treatment of these molecules. The additional software tools, although primarily designed for use with the website libraries, will be sufficiently general to be applicable to other classes of molecules.

The current versions of the programs will be demonstrated using model data from sucrose and biologically interesting disaccharides derived from glycosaminoglycans (GAGs).

59 Investigation of the Root Cause of Opalescence of an Injectable Drug Product by Diffusion Ordered Spectroscopy

Melissa Lin[1], Alexei Buevich[1], Sachin Lohani[2], and R. Thomas Williamson[3]

1. Merck Research Laboratories, Process & Analytical Chemistry, Structure Elucidation Group, Kenilworth, NJ, USA
2. Merck Research Laboratories, Preclinical Development, Summit, NJ, USA
3. Merck Research Laboratories, Process & Analytical Chemistry, Structure Elucidation Group, Rahway, NJ, USA

Diffusion Ordered Spectroscopy (DOSY) is a NMR technique that distinguishes components in solution based on their translational diffusion coefficients. The technique can be used for analysis of mixtures, aggregation states, micelle formation, binding of small molecules to large molecules, etc. DOSY has found a wide range of applications in the pharmaceutical, food and agriculture industries and beyond. In this poster we describe the investigation of an opalescence observed in some of the injectable drug product formulations. Using ^{1}H NMR and DOSY measurements combined with dynamic light scattering (DLS) analysis, the root cause of the problem was discovered to be the result of incorporation of a small molecule additive to drug micelles. The incorporation of the small molecule induced formation of larger drug aggregates, which ultimately led to the apparent opalescence.

60 Monitoring In-Cell Metabolism With High Sensitivity Using DNP Polarized Substrates.

You Zhuo, Khan Hekmatyar, and James H. Prestegard

Complex Carbohydrate Research Center, University of Georgia, GA, USA

Dynamic Nuclear Polarization (DNP) provides dramatic improvements in NMR sensitivity for rapid real-time metabolism studies that can be pursued in living system. Using manual injection of polarized substrates into HEK-293 cells in suspension, we have demonstrated several conversions: pyruvate to lactate and alanine, and fructose to dihydroxyacetone phosphate and pyruvate using non-protonated ^{13}C sites, as well as glucose to phosphoglycerate using deuterated ^{13}C sites. The pyruvate conversion to other metabolites showed interesting differences in the presence of fructose or glucose. The influence of fructose uptake on pyruvate conversion to lactate and alanine was particularly apparent and may be linked to the regulatory effect of some fructose metabolic products on downstream pathways. The rapid metabolic conversions in some of these pathways would be difficult to observe without the high sensitivity advantages of the dissolution DNP technique. This method shows great promise for monitoring the trace amounts of metabolites on short time scales in both in-cell and in vivo applications.

61 The MSG Mouse Model of Obesity Studied by NMR Metabonomics

Marek Kuzma[1], Helena Pelantová[1], Jiří Anýž[2], Daniel Novák[2], Blanka Železná[3], Lenka Maletínská[3], Martin Haluzík[4]

1. Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic
2. Faculty of Electrical Engineering, Czech Technical University in Prague, Technicka 2, 166 27 Prague 6, Czech Republic
3. Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 166 10 Prague 6, Czech Republic
4. 3rd Medical Department, 1st Faculty of Medicine, Charles University and General Faculty Hospital in Prague, U nemocnice 1, 128 08 Praha 2, Czech Republic

Type 2 diabetes mellitus (T2DM) is one of the most prevalent metabolic diseases with serious chronic complications that markedly shorten patients´ lifespan and increase their mortality mostly due to cardiovascular complications. T2DM develops as a results of combination of insulin resistance and insufficient insulin secretion and it frequently clusters with other pathologies such as obesity, arterial hypertension, hyperglycemia and dyslipidemia commonly referred to as Metabolic syndrome[1].

The obesity development is dependent on genetic, environmental and lifestyle factors and is manifested also at the metabolic level by changes in the biochemical pathways. Several mouse models of obesity and diabetes are widely used. Our study was performed on model of chemically induced obesity which has been obtained by injection of monosodium glutamate (MSG) to new born mice. This treatment resulted in MSG obesity with dramatically increased body fat and development of insulin resistance [2].

Metabonomics represents a comprehensive method for metabolite assessment that involves measurement of overall metabolites of biological samples. Metabolic changes caused by insulin resistance resulting from obesity of the MSG mice were followed by 1H NMR of urine samples collected at 2, 6, and 9 months of age. The obtained data were worked-up by multivariate statistical analysis, which allowed us to follow changes in metabolic profiles and identify metabolites responsible for observed variations.

Primarily, it was evaluated optimized protocol for sample collection and data acquisition. It was compared acute morning urine excretion with 24 hour collection. Moreover, it was also compared tube diameter (3 and 5 mm) and pulse sequence used (1D NOESY, CPMG experiment and J-resolved).

The data analysis allowed us to identify more than 40 urine metabolites. PCA and PLS-DA shows metabolic changes during mice aging, which is mainly manifested by creatine, taurine, succinate, citrate, and 2-oxo-glutarate concentration changes. Moreover, it was also evaluated changes caused by obesity and T2DM. It was observed perturbation in the tryptophan-NAD+ pathway, which was demonstrated by increase of N-methylnicotinamide and its oxidation products N-methyl-2-pyridone-5-carboxamide and N-methyl-4-pyridone-3-carboxamide. On contrary, it was observed decreased concentration of nicotinamide-N-oxide and trigonelline.

Acknowledgements:

This work is supported by the Grant Agency of the Czech Republic (Grant no.13-14105S) and the project was conducted within the Prague Infrastructure for Structure Biology and Metabolomics, which was build up by Operational Program Prague – Competitiveness (Project No.: CZ.2.16/3.1.00/24023).

1 O'Rahilly, S. Brit Med J 1997, 314, 955.
2 R. Matyšková, L. Maletínská, J. Maixnerová, Z. Pirník, A. Kiss, B. Železná, Physiol. Res., 2008, 57, 727-734.

62 Pure Shift F1 Proton-Coupled J-Scaled BIRD HSQC to Measure RDCs with Higher Accuracy and Sensitivity: Features and Limitations

Erich Hellemann, Manuela E. García, Chakicherla Gayathri, and Roberto R. Gil

Carnegie Mellon University, Department of Chemistry, Pittsburgh, PA, USA

Due to their global orientation information content, residual dipolar couplings (RDCs) have shown significant impact on the constitutional, configurational and conformational structure determination of small organic compounds.[1,2] Among several alignment media compatible with organic solvents, we prefer to use compressed PMMA gels to partially align smalls organic molecules, as previously described by our group.[3]

One of the most common problems when measuring RDCs is the significant signal broadening in F2 of HSQC experiments due to proton-proton dipolar couplings. This is the major reason why we avoid the use of F2 ^{1}H-coupled HSQC experiments to measure one-bond ^{1}H,^{13}C RDCs ($^{1}D_{CH}$). Instead, in 2011, we have started to measure these RDCs with the F1 ^{1}H-coupled version of HSQC experiments.[4] We later found that the *J* Scaled BIRD version of this experiment generated RDCs with higher accuracy.[5] However, the proton-proton signal broadening is still present in the experiment. In order to circumvent this problem we implemented proton homonuclear broadband decoupling capabilities (pure shift, PS) to the *J* Scaled (JS) BIRD-HSQC to boost resolution in F2 and overall experimental sensitivity. Pure shift is achieved during the acquisition of a single FID, without any special data processing, using trains of BIRD-based homonuclear decoupling.[6]

We used strychnine (Figure 1) as model molecule and PMMA gels as alignment medium, to measure RDCs in order to demonstrate the advantages of implementing PS capabilities to the JS-BIRD-HSQC experiment.

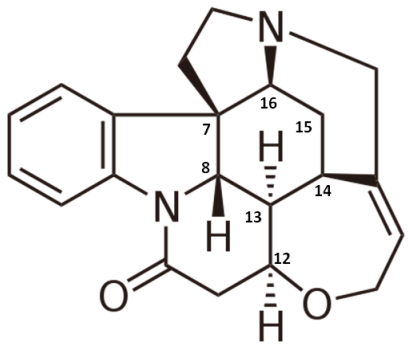


Figure 1. Strychnine structure

JS-BIRD-HSQC experiments with and without PS were collected on a sample of strychnine dissolved in $CDCl_3$, in both isotropic and anisotropic condition. The addition of PS has introduced better resolution in F2 and better signal to noise (S/N) ratio in most of the cross-peaks. The increase in S/N is shown in Figure 2 and the resolution improvement in F2 is clearly shown in Figure 3. It is important to note, as shown in the Figure 3, that signal multiplicities collapse efficiently in F2 and allow easy extraction of data in anisotropic media without multiplets overlapping. This would be particularly useful in complex molecules with many proton-carbon correlation peaks.

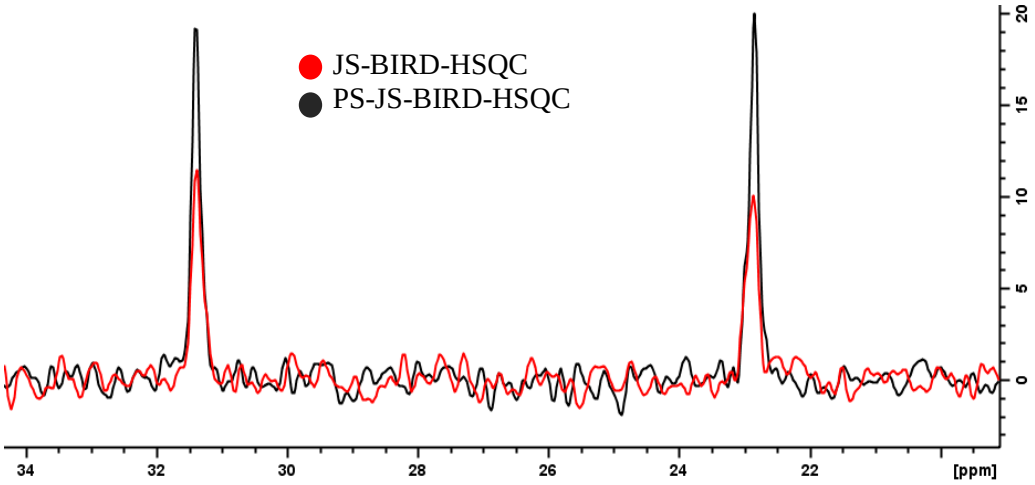


Figure 2. Comparison between CH_2 15 slice signal extracted of JS-BIRD-HSQC and PS-JS-BIRD-HSQC strychnine spectra.

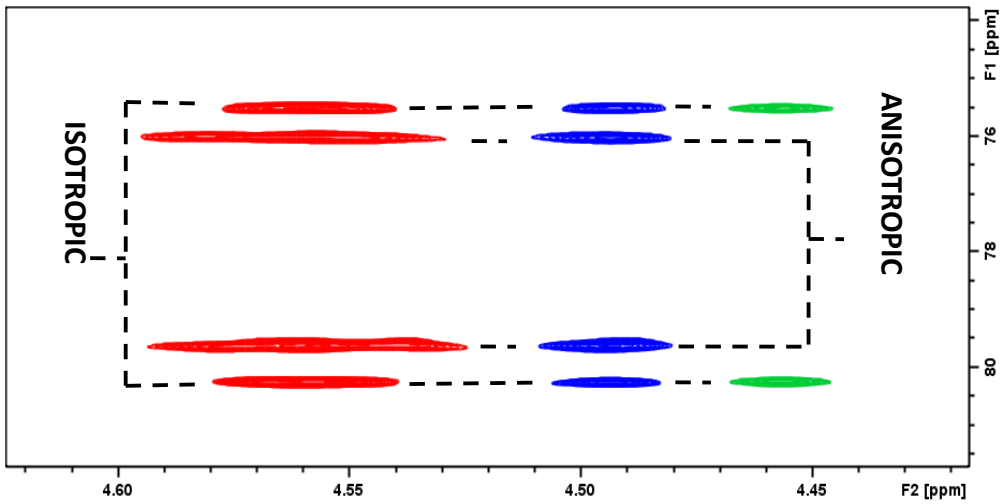


Figure 3. Overlapping of F1 proton-coupled J-scaled BIRD HSQC experiments in isotropic and anisotropic conditions. Comparison between CH 12 signal in anisotropic media of JS-BIRD-HSQC (red) and PS-JS-BIRD- HSQC (blue) and isotropic PS-JS- BIRD- HSQC (green) strychnine spectra.

Due to ring tension limitations, only eight geometrically possible 3D diastereomeric structures of strychnine were generated and energy minimized by DFT. SVD fitting of the RDC data to these eight structures permitted the selection of the correct configuration (7*R*,8*S*,12*S*,13*R*,14*R*,16*S*). RDC data collected with the PS experiments produced a slightly better quality factor (*Q*) for the correct structure (0.058 vs 0.069), while the Q factor for RDCs collected using F2 ^{1}H-coupled CLIP-HSQC was much higher (0.247). Comparison of the structural selection power of RDCs measured in F1 with and without PS capabilities, as well as RDCs measured in F2 with the CLIP-HSQC, are shown in Figure 4. Being configuration 6 the correct one, the graph shows the advantage of PS over non-PS JS-BIRD-HSQC experiments, and a significant improvement in the *Q* factor respect to RDCs collected in F2 using the CLIP-HSQC experiment.

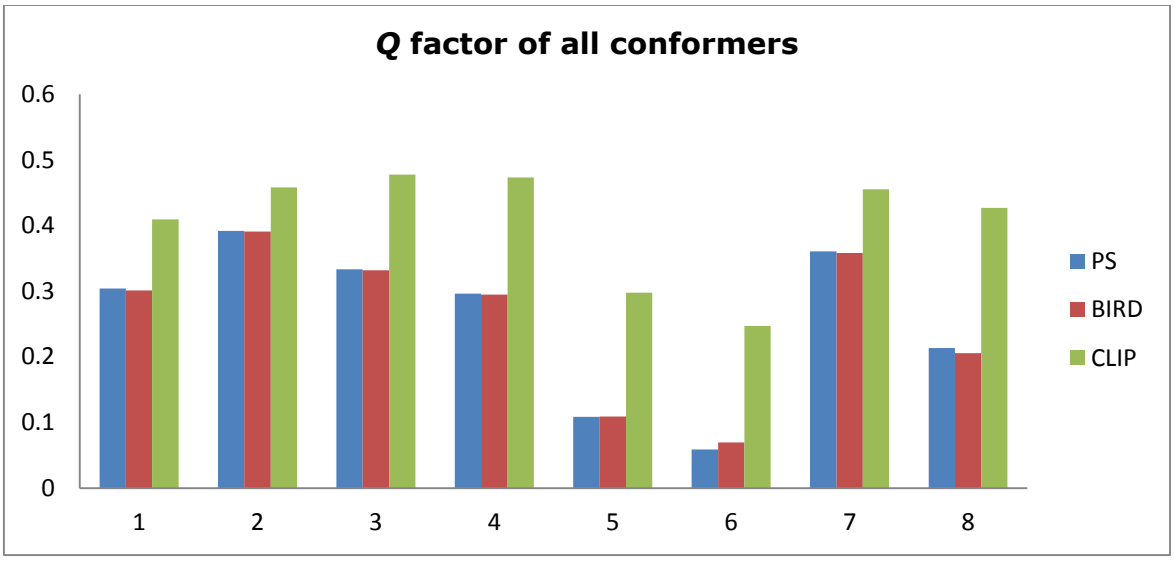


Figure 4: Comparison of *Q* factors obtained from the SVD fits of the experimental RDC data to each possible configuration of strychnine, with different HSQC coupled experiments PS-BIRD (blue), BIRD (red) and CLIP (green).

In a realistic view of the outcome of this work, considering its features and limitations, it is important to highlight the significant resolution introduced by the PS capabilities in many signals, as well as a significant increase in S/N of the cross-peaks. Unfortunately, not all of the signals are benefited by the addition of PS; *i.e.*, the BIRD decoupling cannot decoupled geminal protons, since both protons are attached to a ^{13}C carbon, because the filter decouples protons attached to ^{12}C from protons attached to ^{13}C. In addition, the BIRD filter is tuned to decouple protons with J values within a normal range of 0-18 Hz, while the alignment medium introduces total proton-proton signal-splittings ($^{n}T_{HH}=^{n}J_{HH}+^{n}D_{HH}$) that can be much larger than what the BIRD filter can remove. We particularly appreciate the increase in S/N since the amount of sample that can be loaded in the gel is limited.

1. Kummerlöwe, G.; Luy, B.; Graham, A. W. *Ann. Rep. NMR Spectrosc*. 68, 193-232, 2009.
2. Thiele, C. M. *Eur. J. Org. Chem*., 34, 5673-5685, 2008.
3. Gil, R.R. *Angew. Chem. Int. Ed.*, 50, 7222 – 7224, 2011.
4. Gayathri, C.; Tsarevsky, N.V.; Gil, R. R. *Chem. Eur. J.*, 16, 3622-3626, 2010.
5. Snider, J. D.; Troche-Pesqueira, E.; Woodruff, S.R.; Gayathri, C.; Tsarevsky, N.V.; Gil, R.R. *Magn. Res. Chem.* 50, S86-S91, 2012.
6. Lupulescu, A.; Olsen, G. L.; Frydman, L. *J. Magn. Res.*, 218, 141–146, 2012.

63 Di(ethylene glycol) Methyl Ether Methacrylate (DEGMA) Gels Align Small Organic Molecules in Methanol

Manuela E. García[1,2], Shannon R. Woodruff[3], Erich Hellemann[1], Nicolay V. Tsarevsky[3], and Roberto R. Gil[1]

1. Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA
2. Department of Organic Chemistry, National University of Cordoba, Medina Allende y Haya de La Torre, 5000, Argentina
3. Department of Chemistry, Dedman College, Southern Methodist University, Dallas, TX, USA

Residual dipolar couplings (RDCs) analysis has been successfully applied to the constitutional, configurational and conformational analysis of small organic molecules within the last 10 years. [1,2,3] This approach is particularly useful for structural problems, in which conventional NMR experiments such as NOE and ^3J coupling constant analysis fail to provide a unique solution.

The measurement of RDCs relies on the partial orientation of the molecule of interest, for which a so called alignment medium is necessary. While a series of adequate alignment media exists for aqueous solutions and apolar organic solvents, only few orienting media are reported for polar organic solvents, such as DMSO or methanol [4,5] and the compressed gels method developed in our group has been limited to the use of PMMA gels swollen in $CDCl_3$ and CD_2Cl_2.

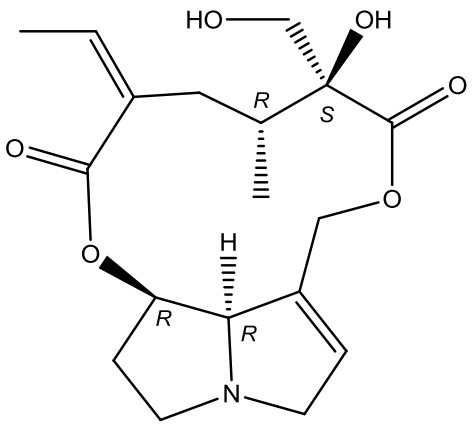
One of the objectives of this work was to develop an orienting gel that would exhibit good alignment properties in methanol-*d* (CD_3OD) since it is a solvent for sugars and peptides, which are an important class of molecules that are insoluble in other organic solvents.

Herein we propose the use of compressed DEGMA (di(ethylene glycol) methyl ether methacrylate) gels for partial orientation of molecules dissolved in methanol.

Some characteristics of these gels are their low cost and ease of preparation, their chemical structure without aromatic rings to minimize chemical shift perturbation, and flexibility to have reversible compression.

Briefly, gels rods were prepared in disposable NMR tubes by polymerizing di(ethylene glicol) monomethyl ether methacrylate (DEGMA) in the presence of various amounts of the cross-linker ethylene glycoldimethacrylate (EGDMA), initiated by V-70 (2,2´-azobis(2,4-dimethyl-4-methoxyvaleronitrile) at 50 °C, for 6 hrs. After confirming gelation, gels were let to dry. Then, gels were swollen in CH_3OH to wash the remaining monomer. Subsequent, they were let to dry again, cut in 2.5 cm rod and finally swollen in CD_3OD inside a 5mm NMR tube.

Retrorsine, a member of the natural pyrrolizidine alkaloids family was used to test the alignment capabilities of our gel. This molecule has four stereocenters, and its configuration is well established as *RRRS* (see below).



Retrorsine (3 mg) was dissolved in CD_3OD (200 µL), poured into the NMR tube containing the swollen gel, and the gel was gently compressed and decompressed several times (pumping action) with a Shigemi plunger. A 1D ^2H NMR spectrum was collected to measure the ^2H quadrupolar splitting of the solvent signal, in order the evaluate the degree of alignment. A small quadrupolar splitting value small values for the –OD signal (~7 Hz) with the gel fully compressed.

Pure-shift *J*-Scaled BIRD HSQC spectra (See Erich Hellemann *et al.* poster abstract for SMASH 2014) were acquired in both isotropic and anisotropic conditions. Due to the low viscosity of methanol and to the low degree of alignment, the resolution of both, isotropic and anisotropic spectra was very similar. $^1D_{CH}$ RDCs were extracted as the difference between the corresponding couplings measured. Fitting of the RDC data to all possible configurations/conformation combinations, alignment tensor determination, back computation of RDC values, and calculation of quality factors *Q* were performed using MSpin. The goodness of fit between experimental and back computed RDCs was expressed in terms of the Cornilescu quality factor *Q*. [6]

7. Gil, R. R. Angew. Chem. Int. Ed., 50, 7222-7224, 2011.
8. Thiele, C. M. *Eur. J. Org. Chem.*, 34, 5673-5685, 2008.
9. Kummerlöwe, G.; Luy, B.; Graham, A. W. *Ann. Rep. NMR Spectrosc.* 68, 193-232, 2009.
10. Freudenberger, J. C.; Knör, S.; Kobzar, K.; Heckmann, D.; Paululat, T.; Kessler H.; Luy, B., *Angew. Chem. Int. Ed.*, 44, 423-426, 2005.
11. Merle, C.; Kummerlöwe, G.; Freudenberger, J. C.; Halbach, F.; Stöwer, W.; Gostomski, C. L.; Höpfner, J.; Beskers, T.; Wilhelm, M.; Luy, B., *Angew. Chem. Int. Ed.*, 52, 10309 –10312, 2013.
12. Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A., *J. Am. Chem. Soc.* 120, 6836-6837, 1998.

64 From Automatic Structure Consistency Analysis to Structure Verification in NMR

Till Kühn[1], Sandra Groscurth[1], Björn Heitmann[1], Jochen Klages[1], Pavel Kessler[2], and Peter-René Steiner[2]

1. Bruker BioSpin AG, Fällanden, Switzerland
2. Bruker BioSpin GmbH, Rheinstetten, Germany

A molecular structure is considered to be in consistence with an NMR spectrum, if all peaks in the spectrum can be fully explained logically and by taking into account all available information with all spectroscopically relevant features of the molecule or with “artifacts” arising from solvent signals or impurities.

Therefore, for any structure verification assesment using NMR, typically the whole spectrum and the complete molecule are taken into account combined with information from sample workup and synthesis.

However, with a certain, real life spectral complexity, many alternative structures will also be consitent with the experimental NMR data. In this case, additional NMR data from different experiments will lead to higher certainty that the proposed structure is really the correct one. More and more alternative structures can be ruled out with more and more additional experimental information. The more experimental data is available, the more the analyst can be sure that the given structure is not only consistent with the data but it can even be considered as verified.

Here we show on several examples where these structural ambiguities arise and also how they can be resolved by the use of additional, orthogonal experimental NMR data. We also show an auomatic software approach which utilizes this additional information and can deliver a structural consitency statement with substantially higher confidence. We show automatic and semi-automatic applications of this software and present a statistical evaluaton of it's results compared to human expert analysis using the same amount of experimental information.