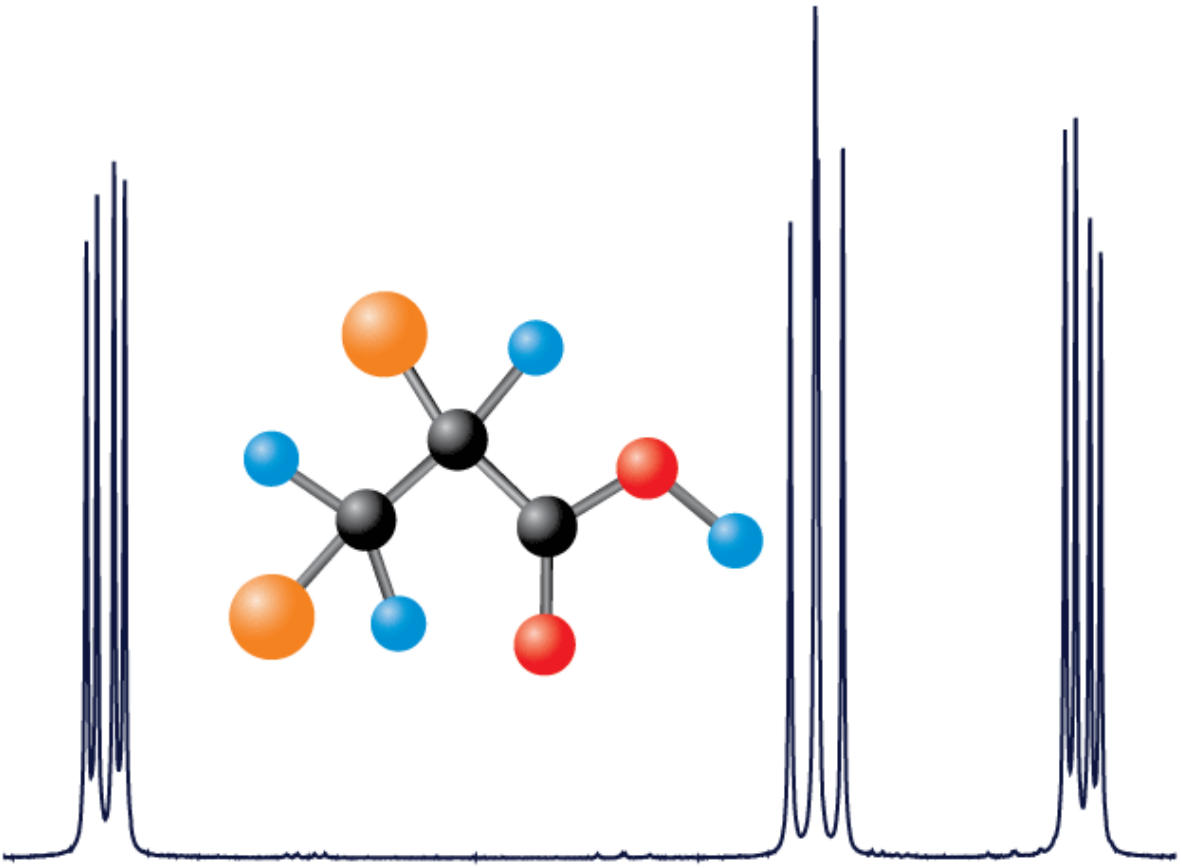

SMASH 2016

Conference Program

September 11th-14th, 2016
La Jolla, California

SMASH 2016 NMR Conference

Dear SMASH 2016 Attendees,

We welcome you to the 2016 Small Molecules Are Still Hot conference at The Hilton, Torrey Pines, in beautiful, La Jolla, California. La Jolla offers us warm sunshine and shimmering beaches plus ready access to the arts, fine dining, and world-famous museums.

SMASH 2016 continues the tradition of combining cutting edge research from both the academic and industrial worlds into one tightly woven international conference. As in years past, there are no concurrent scientific presentations so think of SMASH as your favorite restaurant where you get to enjoy every item on the menu.

The formal program for SMASH begins on Sunday evening with registration, mixer, and dinner. The oral sessions begin Monday morning and encompass a wide range of topics on current areas of research, including emerging methods, solid-state applications, numerical methods, low field NMR, and a session on antibody-drug conjugates. Poster sessions are Monday evening (even numbers) and Tuesday evening (odd numbers), with tutorial workshops scheduled on Monday and Wednesday covering validation of NMR systems, chiral determination by NMR, non-uniform sampling, and solvent suppression techniques.

On Monday evening we are delighted to be presenting the 3rd James N. Shoolery Award for Career Achievement in Small Molecule NMR Spectroscopy to Dr. Gary E. Martin of Merck. Gary's formidable CV exceeds 480 publications, and documents his distinguished record of pushing back boundaries with a richly interdisciplinary approach to small molecule NMR.

And of course, be sure to take advantage of the extensive opportunities to have the kinds of detailed discussions and interactions that have become a hallmark of SMASH. On behalf of the Organizing Committee, we want to extend a warm welcome and thank you for attending SMASH 2016.

Sincerely,

David Rovnyak and Dave Russell
Co-Chairs, SMASH 2016 NMR Conference

SMASH 2016 NMR Conference Program

Sunday, September 11th

04:00 PM - 06:00 PM Registration
06:00 PM - 07:00 PM Mixer
07:00 PM - 10:00 PM Dinner

Monday, September 12th

08:45 AM - 09:00 AM Opening Remarks
09:00 AM - 10:35 AM **How Low Can You Go - Pushing the Boundaries of Sensitivity and Low-Gamma**
Chair: Gary Martin, *Merck & Co. (US)*

Unambiguous Assignment of the Configuration of Oximes and Related Compounds using ^{1}H-^{15}N and ^{13}C-^{15}N Coupling Measurements at Natural Abundance
Steve Cheatham, *DuPont (US)*

Chasing Halogens and Embracing the 'Halogen Dance'. Band-Selective 2D NMR for Detecting Cl-^{13}C in Natural Products
Tadeusz (Ted) Molinski, *University of California – San Diego (US)*

Band-Selective 2d HSQMBC: A Universal Technique for Detection and Measurement of ^{35,37}Cl Isotope Effects for ^{13}C Nuclei (Upgraded Poster)
Josep Saurí, *Merck & Co. (US)*

Application of 1,1-ADEQUATE, HMBC, and Density Functional Theory to Determine Regioselectivity in the Halogenation of Pyridine N-Oxides
Tsang-Lin Hwang, *Amgen Inc. (US)*

10:35 AM - 11:00 AM Break
11:00 AM - 12:30 PM Workshop 1 (concurrent)

How to Validate an NMR in a Regulatory World
Coordinated by: Dave Detlefsen, *Novatia (US)*
Darryl Leblanc, *Avista Pharma Solution (US)*
Kim Colson, *Bruker BioSpin (US)*

Chiral Methods and Applications
Coordinated by: Tom Wenzel, *Bates College (US)*

12:30 PM - 02:00 PM Lunch & Vendor Discussions
02:00 PM - 03:35 PM **Work Smarter Not Harder - NMR Structure Elucidation and Emerging Methods**
Chair: Gary Sharman, *Eli Lilly (UK)*

Structure Elucidation of ^{13}C Labeled Small Molecules: Advantages and Challenges
Mikhail Reibarkh, *Merck & Co. (US)*

***Relativistic Force Field*: Structure Elucidation Made Easy with Fast and Accurate Computations of Nuclear Spin-Spin Coupling Constants**
Andrei Kutateladze, *University of Denver (US)*

PSYCHE Pure Shift NMR: Spectral Simplification and its Applications (Upgraded Poster)
Laura Castañar, *University of Manchester (UK)*

Determination of Configuration of Small Molecules from Residual Chemical Shift Anisotropy (RCSAs) at Microgram Levels
Nilamoni Nath, *Max Planck Institute for Biophysical Chemistry (DE)*

03:35 PM - 04:00 PM Break
04:00 PM - 06:00 PM **Poster Session 1 (even numbers presenting)**
Co-chairs: Krish Krishnamurthy, *Chempacker LLC (US)*
Clark Ridge, *U.S. Food and Drug Administration (US)*
06:00 PM - 07:00 PM **Presentation of the James N. Shoolery Award and Lecture**
SMASH 2016 Recipient: Gary Martin
07:00 PM - 10:00 PM Dinner/ Social Hour

Tuesday, September 13th

09:00 AM - 10:35 AM **Making it Crystal Clear - Solid-State NMR**
Chair: Joe Lubach, *Genentech (US)*

Sensitizing Solid State NMR for Characterization of Pure and Formulated Pharmaceuticals
Aaron Rossini, *Iowa State University, (US)*

Dynamic Nuclear Polymerization Enhanced Solid-State NMR Characterizations of Pharmaceutical Dispersions
Yongchao Su, *Merck & Co. (US)*

^{35}Cl Solid-State DNP NMR of Dosage Forms of Active Pharmaceutical Ingredients
Robert Schurko, *University of Windsor (CA)*

Using ^{1}H T_1 Relaxation Times for Measuring Particle Size, Purity, and Stability of Crystalline Organic Compounds
Eric Munson, *University of Kentucky (US)*

10:35 AM - 11:00 AM Break

11:00 AM - 12:35 PM **Carrying the Load - Small Molecule NMR of Antibody-Drug Conjugates**
Chair: Janet Caceres-Cortes *Bristol-Myers Squibb (US)*

1D ^{1}H NMR Spectroscopy for Characterization of Biologics
Leszek Poppe, *Amgen Inc. (US)*

Straightforward NMR Approach Toward Quantifying ADC Drug-Antibody Ratio
Kelly Sackett, *Pfizer, Inc. (US)*

The use of NMR Spectroscopy to Probe Macromolecule-Drug Conjugates
Mike Reily, *Bristol-Myers Squibb (US)*

12:35 PM - 02:00 PM Boxed Lunch (Available at 12:35 PM)

02:00 PM - 07:30 PM Free Time & Vendor Discussions

07:30 PM - 09:30 PM **Poster Session 2 with Desserts and Drinks (odd numbers presenting)**
Co-chairs: Roberto R. Gil, *Carnegie Mellon University (US)*
Armando Navarro, *Universidade Federal de Pernambuco (ES)*

Wednesday, September 14th

09:00 AM - 10:35 AM **Putting NMR on the Table - Low Fields and Benchtop NMR**
Chair: Mark Dixon, *Thermo Scientific (US)*

Thinking Outside the (Benchtop) Box: Pushing the Envelope in Low-Field NMR
Paul Bowyer, *Magritek Inc. (US)*

Widening the Scope: Heteronuclei for Simplification and Quantitation of Reaction Mixtures
Susanne Riegel, *Nanalysis Corp. (CA)*

Low Field NMR for Monitoring Reactions in Pharmaceutical Discovery Chemistry
Gary Sharman, *Eli Lilly (UK)*

Ultrafast NMR on a Benchtop Spectrometer: Reaction Monitoring and Rapid Screening
Boris Gouilleux, *University of Nantes (FR)*

10:35 AM - 11:00 AM Break

11:00 AM - 12:30 PM Workshop/Tutorial 2 (concurrent)

Non-Uniform Sampling and Why You Should Already Be Doing It
Coordinated by: Dave Rovnyak *Bucknell University (US)*
Frank Delaglio *NIST (US)*

Solvent Suppression, and All the Various Ways to Do It
Coordinated by: Ron Crouch *JEOL (US)*
Eric Johnson *Bruker BioSpin (US)*

12:30 PM - 02:20 PM Lunch, Free Time & Vendor Discussions

02:20 PM - 03:55 PM **Simple Molecules, Mixtures and Complex Data**
Chair: Robert Powers, *University of Nebraska at Lincoln (US)*

New Methods for NMR-Based, Hybrid, and Nanoparticle-Assisted Metabolomics
Rafael Brüschweiler, *Ohio State University (US)*
New Methods in Metabolomics and Applications to Colon Cancer
Daniel Raftery, *University of Washington (US)*
Quantitative Component Analysis of Solid Mixtures by Analyzing Time-Domain ^{1}H and ^{19}F T_1 Saturation Recovery Curves (Upgraded Poster)
Dirk Stueber, *Merck & Co. (US)*
Kinetic Analysis of the Metabolism of Food Protective Cultures by *in vitro* NMR and Chemometrics
Parvaneh Ebrahimi, *University of Copenhagen (DK)*

03:55 PM - 04:20 PM Break

04:20 PM - 05:55 PM **Getting More out of Less - Data Processing and Software**
Chair: Frank Delaglio, *NIST (US)*

Application of CRAFT (Complete Reduction to Amplitude Frequency Table) in Two-Dimensional NMR Data Processing
Krish Krishnamurthy, *Chempacker LLC (US)*
Practical Applications of Non-Uniform Sampling for 2D-NMR in the Pharmaceutical Industry
Dennis P. Anderson, *Pfizer Inc. (US)*
Burst Non-Uniform Sampling in NMR Experiments - EXACTly Why Would You Leave Holes in Your FID?
Craig Butts, *University of Bristol (UK)*
Application of NUS to Monitor *in vivo* Processes (Upgraded Poster)
Rupashree Dass, *University of Warsaw (PL)*

05:55 PM - 06:00 PM Closing Remarks

The James Shoolery Award

In 2014, 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.

SMASH 2016 Scholarship Recipients

The following students received a scholarship to attend SMASH 2016

- **Jessica Bame**, University of Bristol, United Kingdom
- **Laura Castañar Acedo**, University of Manchester, United Kingdom
- **Azzedine Abdelah Dabo**, University of Warwick, United Kingdom
- **Rupashree Dass**, University of Warsaw, Poland
- **Claire Dickson**, University of Bristol, United Kingdom
- **Julian Greindl**, Universität Regensburg, Germany
- **Florian Hastreiter**, Universität Regensburg, Germany
- **Lena Keller**, Scripps Institution of Oceanography, United States
- **Jerripothula K Lakshmi**, CSIR-Indian Institute of Chemical Technology, India
- **Guillermo Lucena**, University of Sussex, United Kingdom
- **Nilamoni Nath**, Max Planck Institute for Biophysical Chemistry, Germany
- **Ikenna Ndukwe**, University of Bristol, School of Chemistry, United Kingdom
- **Kerstin Rothemel**, University of Regensburg, Germany
- **Andreas Seegerer**, University of Regensburg, Germany
- **Eduardo Gomes Rodrigues de Sousa**, UFRJ / FIOCRUZ, Brazil
- **Lisabeth Wagner**, The University of Western Australia, Australia

Thanks to our sponsors for their generous support.

SMASH 2016 NMR Conference
Acknowledgements

The SMASH 2016 Conference gratefully acknowledges the support provided by the following companies:

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Maria Victoria Silva Elipe
Amgen Inc.

Christina Thiele
Technische Universität Darmstadt

Monday, September 12th
09:00 AM - 10:35 AM

How Low Can You Go - Pushing the Boundaries of Sensitivity and Low-Gamma

Chair: Gary Martin

Speakers:

Steve Cheatham
DuPont (US)

Tadeusz (Ted) Molinski
University of California (US)

Josep Saurí*
Merck & Co. (US)

Tsang-Lin Hwang
Amgen Inc. (US)

*Upgraded Poster

Unambiguous Assignment of the Configuration of Oximes and Related Compounds using ^{1}H-^{15}N and ^{13}C-^{15}N Coupling Measurements at Natural Abundance

Steve Cheatham, Dawn Pierce, and Mike Kline

DuPont Crop Protection, Newark, DE

One important task in supporting a Discovery chemistry effort is the determination of the correct configuration of regiosiomers.. Oximes, hydrazines and related compounds are common synthetic intermediates in heterocyclic synthesis which often exist as both E and Z isomers. Multiple methods have been described in the literature for determining configuration including the use of NOE[1] and examining chemical shift differences of both ^{15}N and ^{13}C.[2,3] While these methods are often successful they may be difficult or impossible to employ when only one isomer is present.

Early work utilizing ^{15}N and ^{13}C-^{15}N labeled materials indicated that both the ^{1}H-^{15}N and ^{13}C-^{15}N couplings were configuration dependent and that the values for the E and Z isomers were of sufficient difference to allow unambiguous assignment of configuration. [4,5,6,7] Unfortunately, because of the requirement at the time for isotopically labeled samples the early promise of this method could not be realized. Advances in spectrometer and probe technology now allow routine measurement of ^{1}H-^{15}N coupling to be accomplished using a variety of techniques.[8,9] Specifically we have found variants of the HSQMBC pulse sequence have proven especially useful.[10,11,12] In addition we recently introduced a new pulse sequence for measurement of ^{13}C-^{15}N coupling at natural abundance, the HCNMBC experiment.[13,14] Here we demonstrate that it is possible to definitively assign the configuration of a broad range of oximes, hydrazines and related derivatives utilizing ^{1}H-^{15}N and ^{13}C-^{15}N coupling constant measurements at natural abundance and propose a simple "decision tree" to aid in quickly choosing the correct experiment based on complexity of the ^{1}H spin system.

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Chasing Halogens and Embracing the 'Halogen Dance'. Band-selective 2D NMR for Detecting Cl-^{13}C in Natural Products

Tadeusz F. Molinski[1,2] and Xiao Wang[1]

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

Marine natural products, particularly those from red algae (Rhodophyta) are associated with molecular structures of high halogen content. For example, the formula of the halogenated terpene (C_{10}), halomon [1] – selected in the 1990s by the National Cancer Institute for preclinical trials as an antitumor agent – contains three Cl and two Br atoms. NMR-based structure elucidation of polyhalogenated molecules becomes more difficult as the number of halogens increases because ^{1}H and ^{13}C chemical shift predictions, based on empirical substiuent increment rules, or even DFT calculations, become less reliable due to confounding paramagnetic effects or poor prediction of spin-orbit contributions.

Here we present our recent work on development of novel 'nanomole-scale' NMR methods [2] for of complex halogenated compounds, peptides and polyketides from marine sponges and other invertebrates. The band-selective HSQC [3]– which allows ~ 1-5 ppb resolution in F1 – in combination with ^{35,37}Cl isotope shifts, is exploited for unambiguous assignment of chlorinated ^{13}C-^{1}H signals in rare natural products available only in microgram amounts. Recently, in collaboration with Martin and coworkers (Merck), the method has been extended to fully substituted Cl-^{13}C groups (bs-HSQMBC) [4].

Finally, we will describe the NMR- and MS-based structure elucidation of the first of a family of unusually large cysteine-rich peptides (4–7 kDa) from the marine sponge, *Spirastrella mollis* collected in the Bahamas – all at natural isotopic abundance – and made possible by use of microcryomicroprobe technology, in addition to deployment of 'pure shift' 2D NMR, MS-MS and degradative methods.

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Band-Selective 2D HSQMBC: A Universal Technique for Detection and Measurement of ^{35,37}Cl Isotope Effects for ^{13}C Nuclei

Josep Saurí[1], Mikhail Reibarkh[1], Ting Zhang[2], Ryan D. Cohen[1], Xiao Wang[3], Tadeusz F. Molinski[3,4], Gary E. Martin[1], and R. Thomas Williamson[1]

1. NMR Structure Elucidation, Process Research and Development, Merck Research Laboratories, Merck & Co., Inc., Rahway, NJ, USA.
2. Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co., Inc., NJ, USA
3. Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA, USA
4. Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA.

^{35,37}Cl Isotope effects on ^{13}C chemical shifts can be used to solve regiochemistry problems in the structure elucidation of compounds by NMR. Limited sample situations may preclude direct acquisition of ^{13}C spectra that can allow the observation of ^{35,37}Cl isotope effects, which are typically in the range of 4-14 ppb. [1-3] Very recently, Molinski and co-workers reported the utilization of a band-selective HSQC 2D NMR experiment to observe ^{35,37}Cl isotope effects to differentiate protonated chloro-bearing aliphatic carbons from protonated bromo-bearing aliphatic carbons in a series of marine natural products isolated from red algae. [4,5]

There have, however, never been any reports of the utilization of band-selective 2D NMR experiments for the observation of ^{35,37}Cl isotope effects for quaternary carbons, which are generally smaller than those observed for aliphatic carbons. Hence, we now wish to report the utilization of a modified, ^{13}C band-selective HSQMBC experiment that uses proton selective pulses during the INEPT transfer to avoid any modulation due to J_{HH}, to observe ^{35,37}Cl isotope effects for both protonated and non-protonated carbons. Several examples will be shown to illustrate the robustness, universality and superior performance of the method over bs-HSQC and 1D ^{13}C NMR.

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Application of 1,1-ADEQUATE, HMBC, and Density Functional Theory to Determine Regioselectivity in the Halogenation of Pyridine N-Oxides

Tsang-Lin Hwang, Michael D. Bartberger, and Ying Chen

Amgen Inc., Thousand Oaks, CA, USA

The application of 1,1-ADEQUATE [1-4] and HMBC [5] can be used to unequivocally elucidate the regioisomeric structures for the major and minor components in the product mixtures resulting from halogenation of 3,5-disubstituted pyridine derivatives. 1,1-ADEQUATE data provide specific 2-bond proton to carbon correlation information, and HMBC offers correlation patterns for the regioisomer structural determination and corresponding chemical shift assignments. The different regioselectivity with various substrates determined by NMR can be rationalized by density functional theory calculations [6]. Density functional theory calculations performed at the M06-2X/6-31+G(d,p) level demonstrate that the trend of regioselectivity can be predicted from the difference of the relative free energies of the intermediates, but not from the difference of the relative free energies of the final halogenation products [7]. The regioselectivity profile of a substrate of interest can then be accurately tuned in a prospective fashion via the choice of the ring substituent in order to produce the desired regioisomeric outcome.

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

Workshop 1 (concurrent)

How to Validate an NMR in a Regulatory World

Coordinated by:

Dave Detlefsen, Novatia
Darryl Leblanc, Avista Pharma Solutions
Kim Colson, Bruker BioSpin

Chiral Methods and Applications

Coordinated by:

Tom Wenzel, Bates College

Monday, September 12th
02:00 PM - 03:35 PM

Work Smarter Not Harder - NMR Structure Elucidation and Emerging Methods

Chair: Gary Sharman

Speakers:

Mikhail Reibarkh
Merck & Co. (US)

Andrei Kutateladze
University of Denver (US)

Laura Castañar*
University of Manchester (UK)

Nilamoni Nath
Max Planck Institute for Biophysical Chemistry (DE)

*Upgraded Poster

Structure Elucidation of ^{13}C Labeled Small Molecules: Advantages and Challenges

Mikhail Reibarkh[1], Thomas P. Wyche[2], Josep Saurí[1], Tim S. Bugni[2], Gary E. Martin[1], and R. Thomas Williamson[1]

1. Process Research and Development, NMR Structure Elucidation Group, Merck Research Labs, Rahway, NJ, USA
2. School of Pharmacy, University of Wisconsin-Madison, Madison, WI, USA

Utilization of ^{2}H, ^{13}C, and ^{15}N isotopically labeled proteins and peptides is now routine in biomolecular NMR investigations. The widespread availability of inexpensive, uniformly ^{13}C enriched glucose now makes it possible to isolate uniformly ^{13}C labeled natural products from microbial fermentation. We developed a streamlined approach for the rapid structural characterization of uniformly ^{13}C labeled small molecules and natural products [1]. The strategy utilizes ^{1}H-^{13}C CT-HSQC [2] for resonance assignments and ^{13}C-^{13}C COSY and COSYLR [3, 4] for establishing carbon skeleton of the molecule. We discuss the importance of utilizing experimental parameters optimized for small molecules to avoid the pitfalls of relying on parameters typically employed in biomolecular NMR studies [2].

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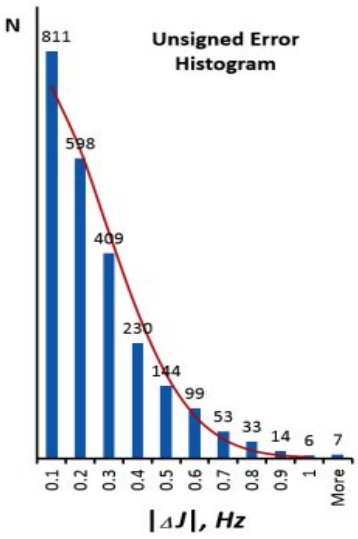
Relativistic Force Field: Structure Elucidation Made Easy with Fast and Accurate Computations of Nuclear Spin-Spin Coupling Constants

Andrei G. Kutateladze and Olga A. Mukhina

Department of Chemistry and Biochemistry, University of Denver, Denver, CO, USA

We have developed a fast method for computing accurate nuclear spin-spin coupling constants (SSCC), which is based on NBO (Natural Bond Orbital)-assisted scaling of DFT-computed Fermi contacts [1-3]. There has been growing consensus that the Fermi contact mechanism dominates nuclear spin scalar coupling. Other contributors, such as dia- and paramagnetic components of spin-orbit coupling are generally small in organic compounds, yet they require the lion share of computational time. Our approach is to augment the readily computed Fermi contacts with a parametric empirical *relativistic force field* (*rff*) designed to *emulate* these missing SOC contributions and improve the accuracy of SSCCs calculations, not unlike the ubiquitous *Force Fields* in molecular mechanics utilize sets of empirical parameters to emulate quantum mechanical effects and obtain accurate molecular structures.

The method is being refined and tested on complex organic molecules. The unsigned error histogram shows the test set as of April 2016, which contains >2400 experimental constants from prominent natural products with the cumulative rmsd of 0.28 Hz. (red line shows the ideal Gaussian distribution for σ=0.28). The new *rff DU8c* method [3] ("c" means that the parameterization is implemented not only for the proton-proton but also for the ^{13}C-^{1}H SSCCs) offers an equal or better accuracy of SSCCs computations in a small fraction of time compared with the existing state of the art methods (the acceleration for relatively large organic molecules like strychnine could be as dramatic as *several orders of magnitude*).



Current computations of ^{13}C NMR chemical shifts serve mostly the 'quality control' function in structure determination, whereas the computed SSCCs offer wealth of structural information to help guide and inform the process of stereochemical assignment.

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PSYCHE Pure Shift NMR: Spectral Simplification and its Applications

Laura Castañar, Mohammadali Foroozandeh, Guilherme Dal Poggetto, Mathias Nilsson, and Gareth A. Morris

School of Chemistry, University of Manchester, Manchester, UK

Resolution and sensitivity are essential for the analysis and interpretation of NMR spectra. In high-resolution ^{1}H NMR spectroscopy, because of the narrow range of chemical shifts and the many homonuclear couplings, multiplet overlap is very common and can severely complicate the analysis of spectra. Pure shift NMR techniques [1-3] have greatly improved signal resolution by removing homonuclear couplings, but at considerable cost in sensitivity. The most recent pure shift method, PSYCHE [4], although still costly in signal-to-noise ratio, typically offers almost an order of magnitude improvement over previous methods.

We present three new pure shift experiments, implementing PSYCHE in selective 1D TOCSY, Oneshot DOSY, and the recently published CLIP-COSY experiment [5]. The new selective 1D TOCSY-PSYCHE experiment is intended for the study of complex molecules and mixtures. The benefits of this method are shown in the analysis of a natural peppermint oil sample (*Mentha piperita*). Pure shift spectra showing only the connectivities of chosen signals (Figure 1) allow spectra of individual components in complex mixtures to be obtained.

The new F_1-PSYCHE-CLIP-COSY experiment is based on CLIP-COSY [5], using a PSYCHE element in the middle of the evolution time to obtain pure shift signals in the indirect dimension. Used in combination with covariance processing, the result is an ultra-high resolution phase-sensitive COSY spectrum with singlets in both dimensions.

Finally, the new Oneshot-PSYCHE DOSY experiment also facilitates the analysis of complex mixtures. Misleading peaks in the diffusion dimension due to signal overlap are minimized, and accurate high-resolution diffusion measurements are obtained.

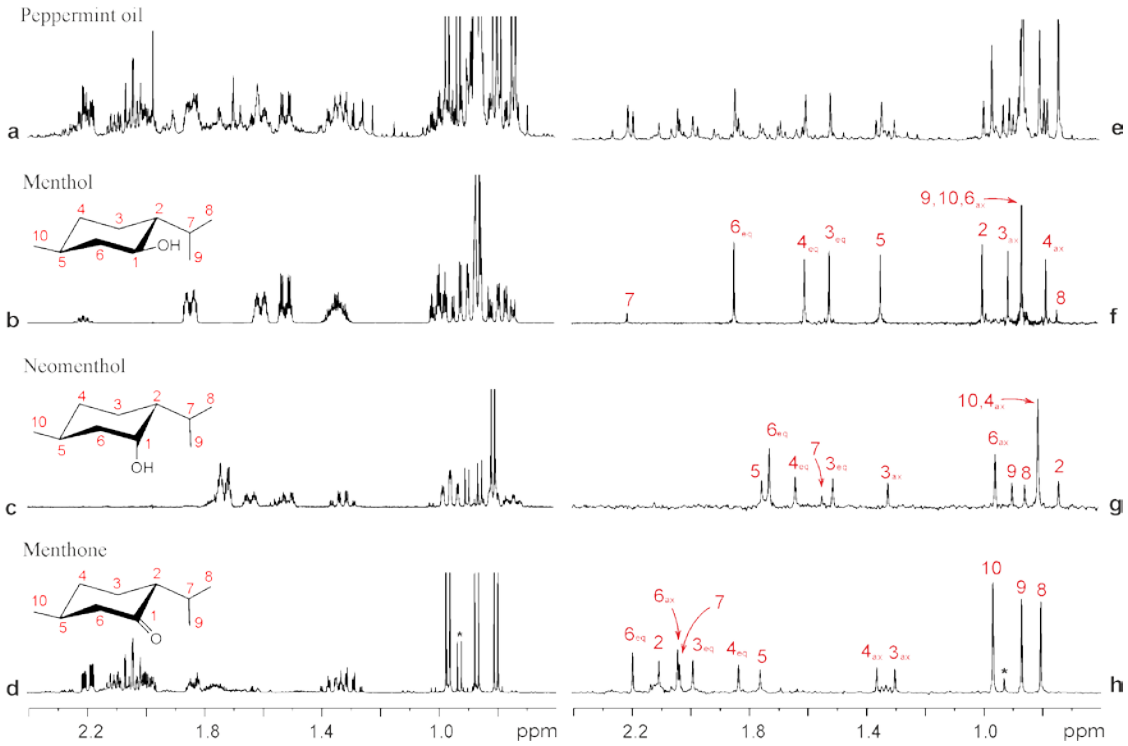

Figure 1. a) Conventional, b,c,d) selective 1D TOCSY, e) PSYCHE, and f,g,h) selective 1D TOCSY-PSYCHE ^{1}H spectra for peppermint oil 25% (v/v) in DMSO-d_6.

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Determination of Configuration of Small Molecules from Residual Chemical Shift Anisotropy (RCSAs) at Microgram Levels

Nilamoni Nath[1], Manuel Schmidt[1], Armando Navarro-Vázquez[2], Roberto R. Gil[3], and Christian Griesinger[1]

1. Department of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
2. Institute of Organic Chemistry and Institute for Biological Interfaces Karlsruhe, Institute of Technology (KIT), Karlsruhe, Germany
3. Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA

Wouldn't it be great to measure the chemical shifts of carbons and determine from these data the configuration of a natural or synthetic compound of which only several tens of micrograms are available? It has long been clear that this could be achieved if one could accurately measure residual chemical shift anisotropy (RCSA) [1,3], *e.g.* from the resonance positions under two alignment states. However, until now, it was difficult to differentiate the RCSA, *i.e.* the shift due to molecular alignment, from the isotropic NMR chemical shift change. The latter originates from a change in environment when switching from alignment state to another. Here we describe a technique to collect pure RCSAs which consists of compressing PMMA gel [4] dissolved in $CDCl_3$ inside a 5 mm NMR tube to obtain different alignments. Although, the sensitive volume is not changed, there is a change in gel-to-solvent ratio causing an isotropic contribution that can be removed post acquisition in a new and robust way. The main achievement obtained is the removal of obstacles for accurate measurement of RCSAs, making them available for routine applications. The simplicity of measuring just a simple ^{13}C 1D NMR experiment should make this method available for every chemist. The sensitivity of the method is very high and with 1.7 mm cryo-probes, this reduces the required amount of substance to 25 μg [5].

To demonstrate the robustness of the approach, RCSAs were measured on five different molecules some rigid, some flexible. We were able to determine the correct configuration in each case. To the best of our knowledge, this has never been achieved before. The most notable example is retrorsine in which flexibility aggravates the determination of the relative configuration. Furthermore, a residual dipolar coupling (RDC) alone were not able to assign the correct configuration since one of the key chiral carbons is quaternary and therefore does not exhibit an RDC. Using RCSAs, however, one can identify the correct configuration since RCSAs are observed regardless of the degree of a carbon's protonation. This attribute clearly highlights the power of RCSAs for molecules that contain chiral non-protonated carbons. We will show further progress of the method that has the potential to further reduce the required amount at least by a factor of 10.

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Monday, September 21st
7:30 PM - 8:30 PM

Presentation of the James N. Shoolery Award

SMASH 2016 Recipient:

Gary Martin
Merck & Co., US

Gary Martin

Dr. Gary Martin holds a B.S. in Pharmacy from the University of Pittsburgh and a Ph.D. degree in Medicinal Chemistry/Pharmaceutical Sciences from the University of Kentucky. He was a Professor of Medicinal Chemistry at the University of Houston from 1975–1989 and the director of the University of Houston NMR Facility between 1984-1989. He moved to the pharmaceutical industry in 1989 and worked at a number of pharmaceutical companies. He has published more than 275 papers, invited reviews, and chapters and is a frequently invited lecturer at national and international NMR meetings.Between 1989 and 1995 he worked at Burroughs Wellcome (later GlaxoSmithKline) and worked on the development of new one- and two-dimensional NMR experiments for the solution of complex structural and spectral assignment problems. He developed new methods for the acquisition of submicromole and sub-nanomole NMR data for molecular structure characterization, especially work involving inverse-detected heteronuclear shift correlation techniques. These efforts led to collaborative development with Nalorac Cryogenics Corp. to develop micro inverse detection probes which facilitated the acquisition of HMQC spectra on samples to the level of 0.05 µmole for small (200-500 Da) molecule NMR.

He moved to the Pharmacia corporation between 1996–2003 and ran the Rapid Structure Characterization Group. When Pharmacia was acquired by Pfizer, he served as the senior scientific consultant working on new methods development. He led the development of applications of unsymmetrical indirect covariance NMR, initially in an effort to eliminate artifacts and subsequently in the investigation of the mathematical combination of discretely-acquired 2D NMR data. The time savings for the latter was nearly a factor of 16 in time, with a 10-fold improvement in signal-to-noise ratios vs. directly acquiring an HSQC-TOCSY data set with the same sample. He conducted preliminary investigations into the utilization of indirect covariance NMR spectroscopy as an alternative means of evaluating NMR data for structure characterization and Computer-Assisted Structure Elucidation. He collaborated with a team of scientists at Advanced Chemistry Development, ACD/Labs, led by Antony John Williams, investigating the development of computational methods for automated structure verification and structure elucidation. He developed “accordion-optimized” long-range heteronuclear shift correlation methods to provide experimental access to small long-range heteronuclear couplings for the characterization of proton-“deficient” molecular structures, to experimentally access 4J heteronuclear couplings, to differentiate two-bond from three-bond long-range couplings to protonated carbons, to measure long-range heteronuclear couplings and to provide a reliable means of observing long-range proton-nitrogen correlations without concern for the variability of long-range proton nitrogen coupling constants.

He also collaborated on the development of a new generation of sub-micro inverse detection probes with Nalorac Cryogenics Corporation designed to allow heteronuclear shift correlation experiments to be performed at levels down to 0.01 µmole for small molecules. The collaboration extended to a new generation of cold metal (at temperatures of 8K) 3 mm micro inverse detection probes. In 2006 he joined Schering-Plough and was responsible for the chemical structure characterization of impurities and

degradants of candidate drug molecules in support of chemical process research. Schering Plough was acquired by Merck Research Laboratories in 2009. During his time at Merck he has continued to explore the limits of detection for low level samples by heteronuclear 2D NMR using newly developed 1.7 mm Micro CryoProbe™ technology. He has developed, in collaboration with ACD/Labs, and Bruker, unsymmetrical indirect covariance NMR spectroscopy, exploring the calculation of hyphenated heteronuclear 2D correlation spectra. He has also continued collaborative investigations in the area of Computer-Assisted Structure Elucidation (CASE) with ACD/Labs. He has also explored the use of unsymmetrical indirect covariance NMR processing methods to define ^{13}C-^{15}N and ^{13}C-^{13}C heteronuclear connectivity networks. He was awarded the 2016 EAS Award for Outstanding Achievements in NMR.

Tuesday, September 13th
09:00 AM - 10:35 AM

Making it Crystal Clear - Solid-State NMR

Chair: Joe Lubach

Speakers:

Aaron Rossini
Iowa State University, (US)

Yongchao Su
Merck & Co. (US)

Robert Schurko
University of Windsor (CA)

Eric Munson
University of Kentucky (US)

Sensitizing Solid-State NMR for Characterization of Pure and Formulated Pharmaceuticals

Aaron J. Rossini[1] and Lyndon Emsley[2]

1. Iowa State University, Department of Chemistry, Ames, IA, USA
2. École Polytechnique Fédérale de Lausanne, Institute of Chemical Sciences and Engineering, Lausanne, Switzerland

Solid-state NMR spectroscopy is routinely applied for the characterization of active pharmaceutical ingredients (APIs) in both pure and dosage forms. However, solid-state NMR experiments on many APIs suffer from poor sensitivity because many crystalline organic solids possess very long proton longitudinal relaxation times ($T_1(^1H)$ = 2-700 s). Furthermore, ^{13}C solid-state NMR experiments on dosage forms of APIs are challenging since the ^{13}C NMR signals from the API often overlaps with those from the excipient and the loading levels of the APIs are often very low (1-10 wt.%). In this contribution, I will describe how high field dynamic nuclear polarization (DNP) enhanced solid-state NMR[1] can be applied to "ordinary" micro-particulate organic solids (such as APIs) and how DNP can address the issues of long proton T_1, signal overlap and dilution in formulated APIs. In our "remote DNP" approach, finely ground micro-particulate solids are impregnated with a minimal amount of biradical solution.[2] The liquid for the impregnation step is chosen to be a non-solvent for the organic solids and coats the outside of the micro-particles. DNP enhanced proton polarization builds up at the surface of the particles and ^1H-^1H spin diffusion transports enhanced polarization into the interior of the particles.[2,3] With this procedure micro-particulate APIs can be remotely polarized by DNP, and sensitivity gains of one to two orders of magnitude can be obtained. This enables solid-state NMR experiments previously considered challenging or impossible to be rapidly performed. For example, with DNP it is now straightforward to perform natural abundance ^{13}C-^{13}C INADEQUATE,[2] ^1H-^{15}N HETCOR,[4] ^{14}N overtone,[5] ^{35}Cl, and natural abundance ^2H solid-state NMR experiments on pure APIs.[6] These techniques can then be adapted to formulated APIs to rapidly obtain high quality 1D and 2D natural abundance ^{13}C and ^{15}N solid-state NMR spectra. These spectra enable the API phae to be assessed and can probe for interactions between the API and excipients. Finally, analysis of the DNP enhancements and signal build-up rates enables the macroscopic size of the API particles to be measured, providing access to an important parameter that controls drug release.

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Dynamic Nuclear Polarization Enhanced Solid-State NMR Investigations of Pharmaceutical Formulations

Qing Zhe Ni[1], Fengyuan Yang[2], Thach V. Can[1], Ivan Sergeyev[3], Sudheer Jawla[4], Yongjun Li[2], Wei Xu[2], Anthony Leone[2], Robert G. Griffin[1], and **Yongchao Su**[2]

1. Department of Chemistry and Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA
2. Merck Research Laboratories, West Point, PA, USA
3. Plasma Science and Fusion Center, MIT, Cambridge, MA, USA
4. Bruker Incorporation, Billerica, MA, USA

Physical and chemical properties of active pharmaceutical intergradient (API) in pharmaceutical formations are core quality attributes potentially impacting stability and bioavailability. Therefore, solid phase characterizations become critical in the preformulaiton and formulation developments of drug substances and drug products. As a noninvasive and quantitative technique, solid-state NMR spectroscopy (ssNMR) has proven its indispensable role of investigating solid-state pharmaceutical systems. [1] Due to the low natural abundance of practical pharmaceuticals and low drug loading formulations, low sensitivity in ssNMR measurement has particularly become a technical challenge for pharmaceutical applications. Recent advances of Dynamic Nuclear Polarization (DNP) boosts ssNMR signals by factors of 102 to 103 [2-3] and have shown its great potential in pharmaceutical application. [4-6]

Our studies aim to utilize DNP-enhanced ssNMR in the characterization in practical development of pharmaceutical formulations. For this purpose, we show that various amorphous solid dispersions are successfully prepared by incorporating polarizing agents in the formulation processes of spray drying (SD) and hot-melt extrusion (HME). A few APIs and the excipient copovidone are utilized for binary solid dispersion to investigate the DNP enhancement. We first investigate the dependence of enhancement from the NMR intensity on the radical concentration. The spray dried API exhibits an optimum enhancement at a TOTAPOL radical concentration of 1% (w/w). API and polymer formulations with varying radical concentrations are also revealed similar optimal radical concentration. By deuterating the excipient, it gains an additional factor of 3 in enhancement. To further optimize the DNP sample of solid dosages, different polarizing agents at varying concentrations are compared. The optimal conditions exhibit an enhancement factor ~25, giving a reduction of NMR measurement time by 600. This largely boosted intensity facilitates the two dimensional homo- and heteronuclear correlation experiments, allowing the characterization of the solid dispersion sample in a much shorter measurement time. This also enables the investigation of the polymorph, phase conversions and API-excipient compatibility in low-drug loading formulations.

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^{35}Cl Solid-State DNP NMR of Dosage Forms of Active Pharmaceutical Ingredients

David A. Hirsh[1], Aaron J. Rossini[2], Lyndon Emsley[3], and **Robert W. Schurko**[1]

1. Department of Chemistry & Biochemistry, University of Windsor, Windsor, ON, Canada
2. Department of Chemistry, Iowa State University, Ames, IA, USA
3. Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Structural characterization of the bulk and dosage forms of active pharmaceutical ingredients (APIs) is of great importance to the pharmaceutical industry.[1] Most APIs have one or more distinct solid forms (*i.e.*, polymorphs, hydrates, solvates, salts, cocrystals, etc.) that can be crystalline or amorphous in nature. These different solid forms can have unique physicochemical properties, such as solubility, stability, or bioavailability; in addition, each solid form can represent unique intellectual property. Two of the most common methods for characterizing the bulk forms of solid APIs are X-ray diffraction and ^{13}C solid-state NMR (SSNMR) spectroscopy.[2] The former is restricted to the characterization of crystalline materials, and both are limited in terms of characterization of dosage forms, due to interfering signals arising from excipient molecules in tablets or capsules (*e.g.*, sugars, starches, polymers, and other binding ingredients and fillers).

More than 50% of solid APIs are produced as hydrochloride (HCl) salts; as such, our research group has proposed ^{35}Cl SSNMR spectroscopy as a means of structural characterization, polymorph differentiation, and impurity identification.[3,4] ^{35}Cl SSNMR spectra, which are dominated by the effects of the second-order quadrupolar interaction (SOQI) and chlorine chemical shift anisotropy (CSA), are extremely sensitive to the local hydrogen bonding environments about the chloride ions; hence, each powder pattern acts as a unique spectral fingerprint for each API phase. ^{35}Cl SSNMR may be of particular value for the study of dosage forms of HCl salts, since there are no interfering signals from the excipient. However, the weight percent of the API, and therefore the Cl, can be very low in many dosage forms, which necessitates very long experimental times using conventional NMR experiments.

Dynamic nuclear polarization (DNP) NMR[5,6] has emerged as a powerful method of characterizing all manners of solid materials.[7] The use of the surface-enhanced NMR spectroscopy (SENS) technique,[8] in which a radical polarization agent is impregnated into the solid material, has recently found use in ^{13}C and ^{15}N SSNMR studies of APIs.[9] DNP NMR is capable of providing signal enhancements of several orders of magnitude in comparison to conventional NMR experiments, greatly reducing experimental times, and permitting the study of dilute systems, including surfaces and nanoparticles.

I will present recent developments in the application of ^{35}Cl DNP SSNMR to the characterization of bulk and dosage forms of APIs that have been synthesized as HCl salts. ^{35}Cl DNP SSNMR ultra-wideline NMR spectra of static samples were acquired using DNP in combination with pulse sequences featuring frequency-swept pulses, which are utilized for broadband excitation, refocusing and polarization transfer.[10,11] I will introduce the spinning-on spinning-off (SOSO) technique, which increases DNP enhancement by spinning during the recycle delay and polarization period, and acquiring the spectra under

static conditions. The particular value of this technique for dosage forms with low-wt% API content will be demonstrated. Applications for polymorph identification, impurity screening, and quality assurance monitoring will also be discussed.

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Using ^{1}H T_1 Relaxation Times for Measuring Particle Size, Purity, and Stability of Crystalline Organic Compounds

Ashley Lay, Elodie Dempah, and **Eric J. Munson**

Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40502, USA

Relaxation times in the solid state are usually used to measure local mobility in a sample. However, we have found that ^{1}H T_1 relaxation times are sensitive to long-range effects for crystalline organic compounds having a substantially-long relaxation time (tens to thousands of seconds). For particle size, we have found that the rate of spin diffusion to the particle surface matches particle sizes up to ~100 nm, at which point the rate no longer obeys the relationship of particle size being proportional to time squared. Particle sizes from submicron to several hundred microns have been correlated back to relaxation times. NMR relaxation times correlate well to particle size, unless there are crystal defects present caused by milling or grinding the sample, which result in a shorter than predicted relaxation time for a given particle size. These crystal defects also are initiation sites for chemical reactions, and we have shown that shorter relaxation times correlate well with the reduced chemical stability of aspirin and gabapentin. Finally, increasing amounts of chemical impurities in a compound, such as other sugars in trehalose or salicylic acid in acetylsalicylic acid, have been correlated with shorter NMR relaxation times.

Eric Munson 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 his academic work at the University of Kentucky, and no data from Kansas Analytical Services are presented here. Funding was provided by the Center for Pharmaceutical Development, an NSF Industry-University Cooperative Research Center.

Tuesday, September 13th
11:00 AM - 12:35 PM

Carrying the Load - Small Molecule NMR of Antibody-Drug Conjugates

Chair: Janet Caceres-Cortes

Speakers:

Leszek Poppe
Amgen Inc. (US)

Kelly Sackett
Pfizer, Inc. (US)

Mike Reily
Bristol-Myers Squibb (US)

1D ^{1}H NMR Spectroscopy for Characterization of Biologics

Leszek Poppe

Amgen Inc., Thousand Oaks, CA, USA

There is an utmost need for technology that allows the thorough analytical characterization of therapeutic proteins in their formulation conditions. High-resolution NMR spectra are exquisitely sensitive to subtle differences in the structure of all orders, from primary all the way to quinary structural differences. Specifically, PROFILE-NMR allows the direct comparison of many of these structural properties between two different samples of the same target protein. Here we present an overview of applications of the PROFILE (Protein Fingerprint by Line Shape Enhancement) methodology to proteins of various molecular size, all the way up to individual monoclonal antibodies and antibody-drug conjugates.

Straightforward NMR Approach Toward Quantifying ADC Drug-Antibody Ratio

Kelly Sackett[1], Christopher Kohanski[1], Wes Swanson[2], Sen Zhang[2], and April Xu[2]

1. Pfizer, BTx R&D, Groton, CT, USA
2. Pfizer, BTx R&D, Pearl River, NY, USA

Degree of conjugation or drug:antibody ratio (DAR) in ADCs represents a critical quality attribute since it is correlated with pharmacokinetics. We introduce a straightforward approach to quantify average DAR by NMR. The proposed NMR approach relies on resolution of specific protein and drug resonances, and can complement orthogonal absorbance based approaches, particularly in cases where a suitable UV/Vis absorbing moiety is absent in the drug component. We expect the proposed NMR approach to perform approximately independent of ADC charge, and stability (i.e. protein aggregation with accelerated storage).

The use of NMR Spectroscopy to Probe Macromolecule-Drug Conjugates

Mike Reily

Bristol-Myers Squibb, Lawrenceville, NJ, USA

Highly selective targeted therapeutics have been in the crosshairs of drug hunters for over a century. Real world examples of such “magic bullets” are now available as antibody drug conjugates, or ADCs. The antibodies convey the target specificity and the attached conjugated drug, or payload, is designed to deliver the pharmacology exclusively at the target cell of interest. This new class of therapeutics requires new analytical approaches in order to adequately characterize them. NMR provides a powerful tool that can be applied to the gamut of ADC characterization, from directly evaluating the frequently highly toxic payload and payload-linkers molecule to studying the structure and dynamics of the complete macromolecular conjugates. The presentation will focus on the utility of NMR for payload quantification and characterization and for investigating dynamics within intact ADCs.

Wednesday, September 14th
09:00 AM - 10:35 AM

Putting NMR on the Table - Low fields and Benchtop NMR

Chair: Mark Dixon

Speakers:

Paul Bowyer
Magritek Inc. (US)

Susanne Riegel
Nanalysis Corp. (CA)

Gary Sharman
Eli Lilly (UK)

Boris Gouilleux
University of Nantes (FR)

Thinking Outside the (Benchtop) Box: Pushing the Envelope in Low-Field NMR

Paul Bowyer

Magritek Inc., San Diego, CA, USA

In recent years there has been rapidly growing interest in Benchtop NMR spectrometers. Benchtop systems are compact in size, and, because they utilise permanent rather than cryogen-thirsty superconducting magnets, they are less expensive to purchase, inexpensive to run, and require little or no system maintenance. Of course, the lower B_0 field strengths (less than 2 Tesla) used by Benchtop NMR spectrometers mean that these systems may be unable to provide the resolving power or sensitivity for some applications. However, these same limitations were also overcome in high field superconducting NMR systems as they measured larger and more complex biomolecules by drawing upon the rich vein of NMR methodological development that has taken place over the past six decades. Here we illustrate how a number of well-established and modern NMR techniques, such as Non Uniform sampling (NUS), Pureshift, and Inverse Detected 2D sequences from the high-field world can be implemented and used on a Benchtop NMR system to solve problems and application previously considered to be beyond the reach of these systems.

Widening the Scope: Heteronuclei for Simplification and Quantification of Reaction Mixtures

Juan F. Araneda and **Susanne D. Riegel**

Application Chemistry, Nanalysis Corp., Calgary, AB, Canada

Despite great potential, the substantial capital costs, significant operating expenditures and high degree of expertise required for use and maintenance of modern high-field NMR instrumentation have limited its use in industrial quantitative and routine research tasks.

The emergence of permanent magnet based benchtop NMR spectrometers promises a way to address affordability, maintenance and ease-of-use concerns. The well-known trade-offs in lower field NMR, however, are reduced chemical shift dispersion and sensitivity. The first concession is particularly acute in proton NMR, the most ubiquitous nucleus, given its narrow spectral width. In this presentation, we detail results of benchtop NMR applications involving heteronuclides, including ^{11}B, ^{19}F, and ^{31}P, where the larger chemical shift ranges permit novel sensing applications [1,2] well suited to automated, low-frequency NMR detection.

1. Zhao, Y.; Chen, L.; Swager, T. M. *Angew. Chem. Int. Ed.*, *55*, 917-921, **2016**.
2. Zhao; T. M. Swager, *J. Am. Chem. Soc.*, *137*, 3221, **2015**.

Low field NMR for Monitoring Reactions in Pharmaceutical Discovery Chemistry

Paul Tan, James Olerenshaw, and **Gary J Sharman**

Eli Lilly & Co., Erl Wood Manor, Windlesham, Surrey, UK

We present the results of an evaluation of a low field, benchtop NMR system for reaction monitoring. The work focusses on following or optimizing small scale chemical reactions within the context of discovery chemistry, which is perhaps not an area where most efforts on reaction monitoring are normally concentrated. We were particularly interested in testing whether 'real' reactions, carried out under unmodified conditions and concentrations, would be accessible to low field NMR analysis, or whether its inherent compromises of resolution and sensitivity would preclude its use. As such, a number of 'typical' reactions were chosen: all were studied under the conditions and concentrations normally used for the reaction. That is, they were not modified or optimized to make them more amenable to NMR investigation. The examples discussed include an imine formation, a room temperature heterogeneous Suzuki reaction, an alkylation reaction and two flow hydrogenation reactions, one where selectivity was required. The examples cover scenarios where kinetic information is obtained on flask type reactions done at a small scale (eg. 10 ml), and flow chemistry applications where an end point is assessed allowing rapid iteration of conditions. The work illustrates that although the quality of data compares poorly with that from a modern high field spectrometer, it can nonetheless be good enough to allow conclusions to be reached and decisions made based on the information.

Ultrafast NMR on a Benchtop Spectrometer: Reaction Monitoring and Rapid Screening

Boris Gouilleux[1], Benoît Charrier[1], Serge Akoka[1], François-Xavier Felpin[1,2], Mireia Rodriguez-Zubiri[1], and Patrick Giraudeau[1,2]

1. Université de Nantes, CNRS, CEISAM UMR 6230, Nantes, France
2. Institut Universitaire de France, 1 rue Descartes, 75005, Paris, France

High field (HF) spectrometers involve an expensive instrumentation, are located in dedicated laboratories and operated by specialized staffs. These drawbacks have hampered the use of HF NMR inside the synthetic laboratories and the production sites. A new generation of benchtop NMR spectrometers, compact and cryogen free, has emerged as a relevant alternative to extend the scope of NMR in harsh environments [1]. Obviously, the use of these permanent magnets involves a reduced frequency dispersion leading to crowded spectra with numerous overlapped resonances. 2D NMR is therefore an appealing solution, unfortunately incompatible with applications such as reaction monitoring due to its time scale and its incremental nature. In order to circumvent this drawback, we developed Ultrafast (UF) NMR on a benchtop spectrometer, yielding 2D specta within a single – or at most a few – scans [2]. These experiments were achieved on 43 MHz Spinsolve (Magritek) spectrometer including a gradient coil originally designed for diffusion experiments [3].

We will present two applications of UF NMR at 43 MHz for the online monitoring of organic reactions and for the rapid screening of food products. We first evaluated this approach on the real-time monitoring of a Heck-Matsuda reaction. This Pd-coupling reaction involves stringent conditions (i.e. the presence of metallic catalyst and gas bubbles inside the sensitive volume) and crowded ^{1}H spectra recorded in a non-deuterated solvent. This offers a challenging model to assess the robustness of the UF monitoring. Performed in an online fashion, the mixture is directly analyzed via a by-pass system connected to the benchtop spectrometer. The UF COSY spectra efficiently cope with the overlaps between the product and reactants by delivering well-resolved cross-peaks [4]. This on-line approach offers a better tunning range regarding the experimental conditions, e.g. temperature control and stirring. Another promising result relates to the rapid screening of edible oils. Recent work performed at 60 MHz enabled to detect cheap substitutes into an olive oil sample. However, the accuracy of this approach remains limited by the overlapped resonances from glycerides protons and those arising from the unsaturated chains. UF COSY overcomes this issue by providing well resolved cross peaks arising from the different protons of interest, whose integration provides an efficient and rapid screening tool [4]. Besides the development of UF NMR at 43 MHz, we will describe additional perspectives offered by the implementation of a robust and powerful gradient coil on benchtop devices, such as the implementation of modern solvent suppression methods, or even pure-shift NMR.

1. Danieli Ernesto, Perlo Juan, Blümich Bernhard and Casanova Federico, Angew. Chem. Int. Edit., 49, 4133-4135, 2010.
2. Frydman Lucio, Scherf Tali, Lupulescu Adonis, Proceedings of the National. Academy of Sciences, 99, 15858-15862, 2002

3. Gouilleux Boris, Charrier Benoît, Danieli Ernesto, Dumez Jean-Nicolas, Akoka Serge, Felpin François Xavier, Rodriguez-Zubiri Mireia and Giraudeau Patrick, Analyst, 140, 7854-7858, 2015.
4. Gouilleux Boris, Charrier Benoît, Akoka Serge, Felpin François Xavier, Rodriguez-Zubiri Mireia and Giraudeau Patrick, Trends Anal. Chem., 2016, in press, doi: 10.1016/j.trac.2016.01.014

Wednesday, September 14th
11:00 AM – 12:30 PM

Workshop/Tutorial 2 (concurrent)

Non-uniform Sampling and Why You Should Already Be Doing It

Coordinated by:

Dave Rovnyak, Bucknell University (US)
Frank Delaglio, NIST (US)

Solvent Suppression, and All the Various Ways to Do It

Coordinated by:

Ron Crouch, JEOL (US)
Eric Johnson, Bruker BioSpin (US)

Wednesday, September 14th
02:20 PM - 03:55 PM

Simple Molecules, Mixtures and Complex Data

Chair: Robert Powers

Speakers:

Rafael Brüschweiler
Ohio State University (US)

Daniel Raftery
University of Washington (US)

Dirk Stueber*
Merck & Co. (US)

Parvaneh Ebrahimi
University of Copenhagen (DK)

*Upgraded Poster

New Methods for NMR-Based, Hybrid, and Nanoparticle-Assisted Metabolomics

Rafael Brüschweiler

Professor and Ohio Research Scholar, Executive NMR Director of CCIC NMR
Departments of Chemistry & Biochemistry and Biological Chemistry & Pharmacology,
The Ohio State University, Columbus, OH, USA

Some of the key challenges in metabolomics concern the comprehensive, rapid and reliable identification of large numbers of metabolites in complex mixtures without the need for extensive purification. We will present new tools based on multidimensional NMR experiments that significantly help accomplish this task, some of which are being implemented in our COLMAR suite of web servers (http://spin.ccic.ohio-state.edu/index.php/colmar). It is demonstrated how the addition of certain additives, such as silica nanoparticles, to the NMR sample further simplifies this undertaking by either quenching the NMR signals of metabolites with certain physical-chemical properties or by eliminating proteins as will be demonstrated for human serum. The characterization of "unknown" metabolites is another key challenge for which an approach is presented that synergistically combines NMR with mass spectrometry in a novel way.

New Methods in Metabolomics and Applications to Colon Cancer

Daniel Raftery[1,2], G.A. Nagana Gowda[1], Lilahar Paudel[1], Danijel Djukovic[1], Haiwei Gu[1], and Min Zhang[3]

1. UW Medicine, University of Washington, Seattle, WA, USA
2. Fred Hutchinson Cancer Research Center, Seattle, WA, USA
3. Department of Statistics, Purdue University, West Lafayette, IN, USA

The high complexity inherent in biological samples provides a challenging analysis problem for the field of metabolomics, especially for NMR. Ideally, broad metabolome coverage provides the opportunity for deep insights into biological problems, while excellent quantitation allows high reproducibility, improved modeling capabilities, and an ability to compare across studies. However, these goals are difficult to achieve on a routine basis because the highly complex sample matrix often precludes reliable measurements of many metabolites and complicates quantitation efforts. As a result, researchers are increasingly using MS for metabolomics studies. However, NMR has an inherent advantage in quantitation. We have evaluated a simple protein precipitation procedure that allows the absolute quantitation of over 70 blood metabolites using a single standard compound [1]. These metabolites, including some at even sub-micromolar concentrations, span a broad range of classes and pathways, including organic and amino acids, as well as energy metabolites and co-enzymes (NAD+, NADH, NADP+, NADPH) and many others. This approach is even useful for calibrating quantitative MS measurements, and has led to some unusual findings about the stability of well-known metabolites such as glutamine [2] and others.

A second bottleneck in metabolomics is related to unknown identification. Currently much of the metabolome is unknown, and it is common for the results of global profiling to include 70% unknown species of features in the data sets. Ideally, improving unknown identification will involve the combination of NMR with MS, but the details of this marriage are complicated. We are developing two approaches to enhance the connection between NMR and MS for unknown identification. The first is the development of "smart tags," which are contain isotopically labeled nuclei that can be used to simplify NMR spectra and at the same time enhance MS data. The first of these is ^{15}N-labeled cholamine which allows for well resolved ^{1}H-^{15}N HSQC spectral after reacting with metabolites, and for improved MS data because of the inherent positive charge on the tag. Additional tags are in development to explore other parts of the metabolome. In a second approach, we have developed statistical methods that better connect the NMR and MS data which perform better than correlation in many instances.

Finally, to improve the biomarker discovery process, we are using an alternative statistical approach, seemingly unrelated regression (SUR), which was originally derived over 50 years ago in the context of econometrics. Here, we use SUR to model metabolite levels based on the (large) effects of various clinical and environmental factors [3]. The results have several important implications for biomarker efforts going forward. The application of the new methods for biomarker discovery for colon cancer will be discussed. Such efforts promise to broaden the capabilities of NMR based metabolomics.

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2. Nagana Gowda, G.A., Gowda, Y.N. and Raftery, D., Anal. Chem. 87, 3800 (2015).
3. Chen, C., Deng, L., Wei, S., et al., J. Proteome Res. 14, 2492 (2015).

Funding from the NIH (R01GM085291) is gratefully acknowledged.

Quantitative Component Analysis of Solid Mixtures by Analyzing Time-Domain ^{1}H and ^{19}F T_1 Saturation Recovery Curves

Dirk Stueber[1] and Stefan Jehle[2]

1. Materials & Molecular Characterization, Merck & Co., Inc., Rahway, NJ, USA
2. NMR Application, Bruker BioSpin, Fällanden, Switzerland

Active pharmaceutical ingredients (APIs) often exhibit extensive polymorphism and the tendency to form solvates and hydrates. In addition, the interaction of the desired API lead form with excipients in formulations during processing or during long-term storage may lead to form change and/or amorphization. Consequently, API and formulated materials studied in early drug development often contain complex mixtures composed of the desired API lead form in the presence of other polymorphs, solvates, amorphous material, and excipients. The ability to characterize and quantify relevant API forms in these complex mixtures in the presence of each other and excipients is crucial in the early development process because polymorphs often exhibit distinct physical properties that may alter the dissolution and bioperformance, processability, and/or chemical stability of formulated drug product [1].

Typical analytical tools to analyze API and formulated pharmaceutical materials include X-ray powder diffraction, optical and vibrational spectroscopy, and thermometric methods like differential scanning calorimetry (DSC) and thermogravimetry (TG) [2]. In recent years, high-field and high-resolution solid-state NMR has emerged as an invaluable tool for analyzing API and formulated pharmaceutical materials in the solid state [3]. Several ssNMR-based methods to quantify components in mixtures have been proposed and successfully applied. These methodologies include a number of chemometrics approaches, signal deconvolution, corrected signal integration, and relaxation-based methods. Among the chemometrics NMR tools, the direct exponential curve resolution algorithm (DECRA) has been applied most frequently on a variety of materials, including pharmaceuticals, polymers, and human brain MRI [4].

The method proposed here, QSRC, represents a new and very efficient approach for quantifying the components in solid mixtures. It utilizes ^{1}H and ^{19}F T_1 saturation recovery curves (SRCs) measured on a Bruker Minispec mq20 benchtop TD-NMR instrument [5]. For the analysis of a given mixture, the SRCs for the relevant pure components, as well as for the mixture itself, are measured. The relative amounts of the mixture components are obtained from a fit of the mixture SRC with a linear combination of weighted pure component SRCs.

1. Chemburkar, SR, Org Process Res Dev., 4, 413-417, 2000.
2. Brittain, HG, Physical Characterization of Pharmaceutical Solids. New York, NY: Marcel Dekker, Inc., 1995.
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4. Alam, TM, Ann Rep NMR Spect., 54, 41-80, 2004.
5. Schwartz, LJ, J Chem Ed., 65, 959-963, 1988.

Kinetic Analysis of the Metabolism of Food Protective Cultures by *in vitro* NMR and Chemometrics

Parvaneh Ebrahimi[1], Flemming H. Larsen[1], Henrik M. Jensen[2], Finn K. Vogensen[3], and Søren B. Engelsen[1]

1. Spectroscopy & Chemometrics, Department of Food Science, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark
2. DuPont Nutrition Biosciences ApS, Brabrand, Denmark
3. Food Microbiology, Department of Food Science, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark

Biopreservation is a safe and ecological approach to preserve food products, which has gained increased attention in recent years. Insight into the metabolism of food protective cultures can definitely lead to more efficient biopreservation systems. In this context, NMR spectroscopy can be particularly helpful for the kinetic analysis of the metabolism of protective cultures. NMR has a great potential for studying living organisms, owing to its non-destructive nature, i.e. it can be used for *in vivo* and *in vitro* measurements of biological processes, with no quenching of the metabolism required. In this work, this unique advantage of NMR spectroscopy was used to develop an efficient analytical protocol for the kinetic analysis of *in vitro* NMR measurements of Lactic acid bacteria (LAB) that play an important role in the food industry as protective cultures, and also as starter cultures to manufacture fermented food. The protocol includes guidelines for the different parts of the experiment, from sample preparation over data acquisition and preprocessing to the extraction of the metabolic kinetic profiles. The analytical protocol is applied to an experimental design with two LAB strains (*Lactobacillus rhamnosus* DSM 20021 and *Lactobacillus plantarum subsp. plantarum* DSM 20174), two initial pH levels (pHi 6.5 and 5.5), two levels of glucose concentration (2.5 and 0.25 g/l), and two batch fermentation replicates. Reference deconvolution [1, 2] was introduced as a very effective solution for enhancing the quality of *in vitro* NMR measurements of cells, and multivariate curve resolution alternating least squares (MCR-ALS) was used to extract the metabolic profiles from the processed time-series NMR data. The protocol allowed for detailed kinetic analysis of 11 major metabolites that are involved in the glycolysis, pyruvate catabolism, amino acid catabolism and cell energy metabolism of the bacteria. The developed analytical protocol, benefiting from the unique advantages of NMR spectroscopy, facilitates simple and easy investigation of different fermentation factors, such as new strains, cohabitations, new substrates, temperature, and pH, and thus has great potential for biopreservation studies, as well as studies that are related to the application of LAB for enhancing the sensory properties of food products [3].

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2. Ebrahimi, Parvaneh; Nilsson, Mathias; Morris, Gareth A.; Jensen, Henrik M.; Engelsen, Søren B., Journal of Chemometrics, 28(8), 656–662, 2014
3. Ebrahimi, Parvaneh; Larsen, Flemming H.; Jensen, Henrik M.; Vogensen, Finn K.; Engelsen, Søren B., Metabolomics, 12(4), 1-17, 2016

Wednesday, September 14th
04:20 PM - 05:55 PM

Getting More out of Less - Data Processing and Software

Chair: Frank Delaglio

Speakers:

Krish Krishnamurthy
Chempacker LLC (US)

Dennis P. Anderson
Pfizer Inc. (US)

Craig Butts
University of Bristol (UK)

Rupashree Dass*
University of Warsaw (PL)

*Upgraded Poster

Application of CRAFT (Complete Reduction to Amplitude Frequency Table) in Two-Dimensional NMR Data Processing

Krish Krishnamurthy

Chempacker LLC, San Jose, CA, USA

Two-dimensional data are typically truncated in both dimensions, but invariably and severely so in the indirect dimension. These truncated FIDs and/or interferograms are extensively zero-filled, and Fourier transformation of such zero-filled data is always preceded by a rapidly-decaying apodization function. Hence the frequency line width in the spectrum (at least parallel to the evolution dimension) is almost always dominated by the apodization function. Such apodization driven line broadening in the indirect (t_1) dimension leads to the lack of clear resolution of cross peaks in the 2D spectrum. Time-domain analysis (i.e. extraction of frequency, amplitudes, line width, and phase parameters directly from the FID, in this case via Bayesian modeling into a tabular format) of NMR data is another approach for spectral resonance characterization and quantification. The recently published CRAFT (Complete Reduction to Amplitude Frequency Table) technique converts the raw FID data (i.e., time domain data) into a table of frequencies, amplitudes, decay rate constants and phases. CRAFT analyses of time-domain data require minimal or no apodization prior to extraction of the four parameters. We used the CRAFT processing approach for the decimation of the interferograms and compared the results from a variety of two-dimensional spectra against conventional processing with and without linear prediction. The results show that use of the CRAFT technique to decimate the t_1 interferograms yields much narrower spectral line width of the resonances, circumventing the loss of resolution due to apodization.

Practical Applications of Non-Uniform Sampling for 2D-NMR in the Pharmaceutical Industry

Dennis P. Anderson

Pfizer, Inc., Groton, CT, USA

Not available at time of program release.

Burst NUS in NMR Experiments

Craig Butts[1], Ikenna Ndukwe[1], Krzysztof Kazimierczuk[2], Alexandra Shchukina[2], and Carlos Cobas[3]

1. University of Bristol, Bristol, UK
2. University of Warsaw, Warsaw, Poland
3. Mestrelab Research SL, Spain

Non-Uniform Sampling (NUS) is now a routine tool in multi-dimensional NMR spectroscopy. Reducing the number of increments in the indirect dimension(s) can enormously reduce experiment times while retaining high resolution in those dimensions.[1]

Herein, we propose the application of 'burst-sampling' in the direct dimension of an FID, which leaves gaps on the FID where were can incorporate RF pulses and delays to manipulate chemical shift and coupling evolution of selected spins. When combined with IST reconstruction[2] of the burst-sampled FID, this technique provides a powerful tool to:

(1) Reduce power demands/duty cycle of NMR sequences by >50%
(2) Reducing linewidths limitation of some 'pureshift' spectra by ~50%
(3) Acceleration of selected NMR experiments by >50%

We will illustrate (1) with EXtended ACquisition Time (EXACT) HSQC, where t2 acquisition times of >1.5 seconds are routinely possible. (2) is exemplified by EXACT modification of the Pureshift EXSIDE[3] where we can halve the linewidths of the resulting correlections in all cases examined (Figure 1). At the time of writing, we are still working on (3) and look forward to telling you about it!

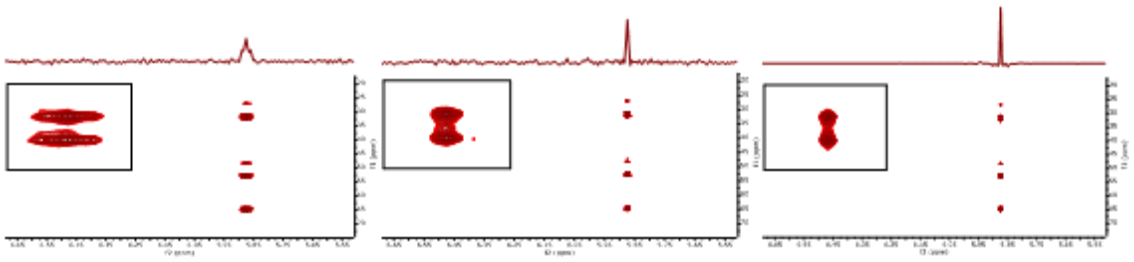

Figure 1: EXSIDE spectrum of H22 of strychnine (left, linewidth ~15.5Hz), corresponding Pureshift-EXSIDE (centre, linewidth ~4.5Hz, and EXACT-Pureshift EXSIDE (right, linewidth ~2.3Hz).

1. M. Mobli, M. Maciejewski, A. Schuyler, A. Stern, and J. C. Hoch, *Phys. Chem. Chem. Phys.*, **2012,** 14, 10835−10843
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Application of NUS to Monitor *in vivo* Processes

Rupashree Dass[1], Katarzyna Grudziąż[1], Takao Ishikawa[2] and Krzysztof Kazimierczuk[3]

1. Faculty of Chemistry, University of Warsaw, Warsaw
2. Department of Molecular Biology, Institute of Biochemistry, Faculty of Biology, University of Warsaw, Warsaw, Poland
3. Centre of New Technologies, University of Warsaw, Warsaw, Poland

Nuclear Magnetic Resonance (NMR) spectroscopy has the potential to non-invasively investigate chemical and biological processes. Conventionally, arrayed measurements are collected over equally spaced time intervals during an ongoing reaction. Multidimensional experiments provide additional benefits like increased resolution but are limited by the long acquisition times. It has been previously shown how Time-Resolved Non Uniform Sampling (TR-NUS) can be used as a method to monitor fast processes where time acts as the pseudo third dimension [1,2,3]. The idea is to sample the FID non-uniformly and in parallel to the process happening in the NMR tube. Further, subsets of such NUS dataset are created and reconstructed one by one using the Compressed sensing algorithm. The result is a stack of spectra showing the changes occurring during the reaction over time.

Previously spectral changes observed due to cell death [4] and specifically due to bacterial cell death by heat have been reported [5]. In this project, we have further extended the idea to monitor the biochemical processes which occur when bacteria interact with an antibacterial product. For our studies we have taken *Propionibacterium acnes*, which is a common cause for face acne and a commercially available face product. Since face products have a huge number of ingredients, multidimensional experiments become a necessity. Processes such as how the rate of cell death depends on the concentration of the product and the various biochemical changes that occur in the entire process over time have been studied. The effect of individual components of the product on the bacteria has also been taken into account for eg. the consumption of glucose , which is one of the ingredients in the face product.

TR-NUS provides one with the time resolution that makes such in-vivo studies feasible. We have acquired 2D TR-NUS zTOCSY, which shows the changes in peak intensities in a continuous “movie-like” manner. STOCSY [6] analysis of the 2D data also put some light on the metabolomic changes caused by the acid stress from the product. Sampling related artifacts, their effect on peak intensities and some methods to overcome this issue have also been discussed.

The aim of the project is to develop a method which can be used for the monitoring of complex biochemical reactions *in vivo* using time-resolved NMR spectroscopy. We believe such studies help to understand the mechanism of how pharmaceutical products work. Our main goal is to develop the method of TR-NUS by investigating some practical applications in the pharmaceutical industry.

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Poster Sessions

Monday, September 12th
04:00 PM - 06:00 PM
(even numbers presenting)

Co-Chairs: Krish Krishnamurthy and Clark Ridge

Tuesday, September 13th
7:30 PM - 9:30 PM
(odd numbers presenting)

Co-Chairs: Roberto R. Gil and Armando Navarro

Numerical Index to Posters

1	Lisabeth Wagner	Quantitative Discharge Water Analysis Using Mobile ^{1}H NMR
2	Maic Fredersdorf	Conformational Analysis of the Antibiotic Cyclodepsipeptide Methylgriselimycin with RDCs
3	Martin Dracinsky	"Through-Space" *J*-Coupling Between Hydrogen Nuclei
4	Azzedine Dabo	High Resolution NMR Based Approaches for Facilitating Chromatography Method Development
5	Dirk Stueber	Quantitative Component Analysis of Solid Mixtures by Analyzing Time-Domain ^{1}H and ^{19}F T_1 Saturation Recovery Curves
7	Eliška Procházková	Structural Insights Into the Enantioselectivity of an Organocatakyst for the Acylation of 1,2-diols from an NOE Investigation
8	Ralph Adams	Towards Optimal NMR Measurement of Carbon Kinetic Isotope Effects
9	Joan Malmstrøm	qNMR from In-process Support to Product on the Market – a Validation Perspective
10	Josep Saurí	Homodimericin-A: Elucidating the Structure of a Complex Natural Product with an Unprecedented Carbon Skeleton
11	Evan McCarney	The Application of Multinuclear Benchtop NMR Spectroscopy in the Analysis of Samples from Suspected Clandestine Methamphetamine Laboratories
12	Mari Victoria Silva Elipe	Reaction Monitoring by NMR in a Two-Chamber Glass System
13	Rupashree Dass	Application of NUS to Monitor in vivo Processes
14	Julian Greindl	Structural Preferences and Unexpected Mobility in Imine/Phosphoric Acid Complexes
15	Kerstin Rothermel	NMR-Spectroscopic Investigations on Hydrogen Bond Interactions between Chiral Phosphoric Acids and Imines
16	Andreas Seegerer	NMR Spectroscopic Investigations of Remote Stereocontrol in Dienamine Catalysis
17	Ikenna Ndukwe	EXACT NMR – Faster Pseudo-2D Acquisition and Safer ASAP-HSQC
18	Florian Hastreiter	NMR Spectroscopic Investigation of Silicon Zintl Anions
19	Jessica Bame	The Trouble with τ_c – How to Account for the Variance in Nuclear Motion in Small Molecules for NOE Distance Determination?
20	Jerripothula K Lakshmi	Determination of Relative Orientation of Triterpene Domain in Bis Triterpene-triazole Molecules, by Using RDC-enhanced NMR Spectroscopy
21	Alexei Buevich	Synergistic Combination of DFT Chemical Shift Predictions with CASE Algorithms: a New Powerful Approach for Structure Elucidation, Verification and Revision
22	Alexei Buevich	Mechanism of Atropisomerization of 8-membered Dibenzolactam: Experimental NMR and Theoretical DFT Study

23	Guillermo Lucena Alcalde	Extending the Application Range of Chromatographic NMR by Enantiomer Separation and Optimization of Diffusion Measurements Under MAS
24	Claire Dickson	$^{3}J_{CH}$ – Relating Scalar Coupling Constants to 3D Molecular Structure in Solution
25	Eduardo Sousa	Effects of Solvent, Concentration and Temperature on Development of a qNMR Assay Method for the Antiretroviral Drug Efavirenz
26	Lena Keller	NMR Characterization of a Complex Natural Product Macrolide
27	Radek Pohl	Tautomer Equlibrium Control Via Intramolecular Hydrogen Bonding
29	Laura Castañar Acedo	PSYCHE Pure Shift NMR: Spectral Simplification and its Applications
30	Josep Saurí	Band-Selective 2D HSQMBC: A Universal Technique for Detection and Measurement of ^{35,37}Cl Isotope Effects for ^{13}C Nuclei
31	Brendan Duggan	A Sensitivity Comparison of HMBC and LR-HSQMBC Experiments
32	Nathaniel Segraves	Exploring Cleanup Procedures for HPLC Contaminants to Aid Low Level NMR Sample Analysis
33	Arvin Moser	Advances in NMR Software and NMR Experiments: Three Novel Structures Solved with Computer-Assisted Structure Elucidation Enhanced with Modern NMR Experiments
34	Si Yan	Mechanistic Investigation of Membrane Residency of Membrane Protein Ligands
35	Thomas Wyche	Structure Elucidation by ^{13}C-^{13}C COSY NMR of Two Novel Peptides from a Mushroom-derived Streptomyces sp
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1 Quantitative Discharge Water Analysis Using Mobile ^{1}H NMR

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Quantitative measurement of oil contamination in produced water is required in the oil and gas industry at the ppm level prior to its discharge in order to meet ever more stringent environmental legislative requirements. An eligible method to accurately and reliably measure this oil contamination should be compact, robust and ideally account for both dispersed and dissolved oil components [1]. Here we present the use of mobile, comparatively low-field, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, in combination with solid phase extraction (SPE), to meet this metrology need.

The methodology developed exploits the use of a benchtop (1 T) Halbach magnet array with ^{1}H detection at 43.36 MHz [2]. This is sufficiently homogeneosu that it enables ready differentiation of the oil contaminant and a 'spiked solvent' NMR ^{1}H signal based on chemical shift resolution. The SPE apparatus is used to concentrate the oil contamination, which is then recovered by a solvent prior to NMR analysis. The solvent selected - 1% v/v chloroform in tetrachloroethylene – provides a reference ^{1}H signal from the chloroform with comparable signal intensity to the oil resonance resulting in the measurement being effectively self-calibrating. Compared to ultrasonic and optical commerical allternatives, this is a unique and highly desirable methodology feature. The measurement process has been successfully and widely demonstrated on water contaminated with both hexane and crude oil over the range 1-30 ppm. Excellent agreement was achieved with known solubility limits as well as alternative, established measurement methods, namely infrared analysis and gas chromatography. The measurement system is semi-automated, this will be detailed along with current work to deliver a fully automated system.

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2 Conformational Analysis of the Antibiotic Cyclodepsipeptide Methylgriselimycin with RDCs

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The discovery of streptomycin ushered in the era of tuberculosis therapy. Despite the subsequent development of a curative therapy for this disease tuberculosis remains a global problem and the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* has prioritized the need for new antibiotic drugs. An isolated minor component of griselimycin (GM) [1,2] from the strains *S. griseus* and *S. coelicus*, the derivative methylgriselimycin (MGM) and a chemically synthesized structural analogue cyclohexylgriselimycin (CGM) are showing both in vitro and in vivo activity against *Mycobacterium tuberculosis* and are therefore promising drug candidates.[3] GM, MGM and CGM are cyclic depsipeptides which are composed of ten amino acids. The structural difference between the three peptide derivatives is the presence of L-(R)-4-methylproline (MGM) or L-(R)-4-cyclohexylprolin (CGM) instead of L-proline (GM) at position 8 of the amino acid sequence. This small variation results in an increased metabolic stability of MGM and CGM in comparison to Griselimycin. To get a deeper insight into the structure-activity relationship we used nuclear-Overhauser-effect- (NOE) and residual-dipolar-couplings- (RDC) restraints to determine the conformation of the MGM macrocycle. In a first step we determined the conformations of MGM in $CDCl_3$ by using (NOE) distance measurements only. We obtained ten conformers by NOE-restrained-MD-simulation which were in good agreement with the NOE-data.[4] For the subsequent RDC-analysis we prepared anisotropic NMR-samples in a chemically cross-linked PDMS-gel in $CDCl_3$.[5] When fitting the RDC-restraints to the NOE-MD-derived structures we could not find a good agreement between the measured RDC-data and the proposed structures. In a subsequent simulated-annealing calculation with XPLOR-NIH [6,7] we used both NOE- and RDC-data as restraints. Here, we could identify a structural bundle of 20 low-energy structures which were in good agreement with NOE- and backbone RDC-restraints. After a structure refinement process by additionally using the proline side-chain RDCs we could identify two out of the 20 low-energy structures which are in good agreement with all experimental data.

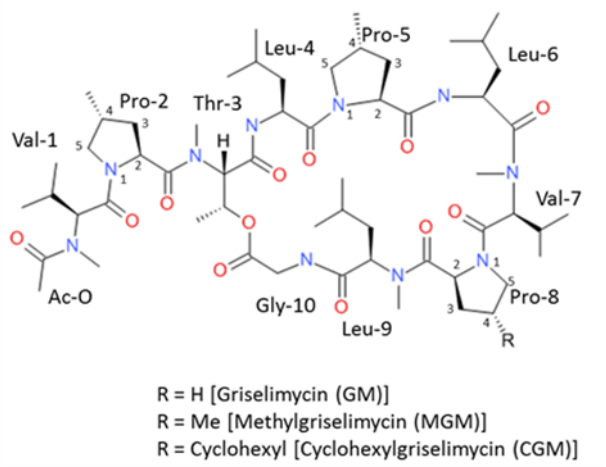

Figure 1: The structure of the cyclic depsipeptides GM, MGM and CGM.

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3 "Through-Space" J-Coupling Between Hydrogen Nuclei

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The J-coupling in NMR is conventionally associated with covalent bonds. So far, the non-covalent contribution, often called through-space coupling (TSC), was observed between haevier atoms bearing a lone electron pair or between atoms involved in hydrogen bonds. In this study, the TSC was detected and analyzed for more common ^{1}H-^{1}H nuclear pairs as well. In synthesized model molecules the hydrogen positions could be well controlled. We observed couplings between hydrogen nuclei close in space but formaly separated by many (up to 18) covalent bonds. The nature and magnitude of the phenomenon were also investigated by density functional computations. The calculated carbon and hydrogen coupling maps and perturbed electronic densities suggest that aromatic systems may strongly participate on the non-covalent contribution. Unlike for the covalent coupling usually governed by the Fermi contact, the through-space coupling is dominated by the diamagnetic term comprising interactions of nuclei with the electron orbital angular momentum. Moreover, the computations revealed a strong distance and conformational dependence of TSC [1]. This suggests that the through-space coupling can be explored in molecular structural studies in the same way as the covalent one, in particular for van der Waals complexes.

The detection of the through-space J-coupling raises questions about our understanding of chemical bonding [2]. Normally, the observation of indirect coupling is considered as a proof of a chemical bond. Chemical bonding between two hydrogen atoms has recently been proposed, for example, in dihydrogen bonds [3]. However, our analysis of the coupling pathway for the TSC between hydrogen nuclei does not support the idea of a chemical bond between the coupled hydrogen nuclei. Instead, an interaction of C–H bonding sigma electrons is observed.

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4 High Resolution NMR Based Approaches for Facilitating Chromatography Method Development

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Method development in reverse phase high pressure liquid chromatography (RP-HPLC) is both time and money consuming. Predictive modelling programs have been created for more efficient selection of the most optimal starting conditions for method development by the research and development team in Pfizer. Atomic resolution measurements of the interaction between analytes and stationary phases may facilitate further improvement to the predictive power of the program.

The aim of this project is to obtain a quantitative measure of the site-specific interaction between a series of analytes, mobile phases and five different reversed phases HPLC stationary phases.

In order to accomplish this, ^{1}H high resolution magic angle spinning (HR-MAS) nuclear magnetic resonance (NMR) spin-lattice (T_1) and spin-spin (T_2) relaxation measurements were carried out to probe site-specific interaction of a series of aromatic compounds (toluene, biphenyl, benzophenone, naphthalene, hydroxyzine dichloride and meclizine dichloride) and reverse phase HPLC stationary phases in two different mobile phases.

The NMR relaxation measurements provided insight about the nature of the site specific interaction between the compounds with different stationary phases. In addition, insights on the effect of different mobile phases were obtained.

Keywords: method development, reversed phase high pressure liquid chromatography; atomic resolution; spin-lattice relaxation; spin-spin relaxation; site-specific interaction; aromatic compounds; high resolution magic angle spinning NMR; Predictive modelling programs.

5 Quantitative Component Analysis of Solid Mixtures by Analyzing Time-Domain ^{1}H and ^{19}F T_1 Saturation Recovery Curves

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Active pharmaceutical ingredients (APIs) often exhibit extensive polymorphism and the tendency to form solvates and hydrates. In addition, the interaction of the desired API lead form with excipients in formulations during processing or during long-term storage may lead to form change and/or amorphization. Consequently, API and formulated materials studied in early drug development often contain complex mixtures composed of the desired API lead form in the presence of other polymorphs, solvates, amorphous material, and excipients. The ability to characterize and quantify relevant API forms in these complex mixtures in the presence of each other and excipients is crucial in the early development process because polymorphs often exhibit distinct physical properties that may alter the dissolution and bioperformance, processability, and/or chemical stability of formulated drug product [1].

Typical analytical tools to analyze API and formulated pharmaceutical materials include X-ray powder diffraction, optical and vibrational spectroscopy, and thermometric methods like differential scanning calorimetry (DSC) and thermogravimetry (TG) [2]. In recent years, high-field and high-resolution solid-state NMR has emerged as an invaluable tool for analyzing API and formulated pharmaceutical materials in the solid state [3]. Several ssNMR-based methods to quantify components in mixtures have been proposed and successfully applied. These methodologies include a number of chemometrics approaches, signal deconvolution, corrected signal integration, and relaxation-based methods. Among the chemometrics NMR tools, the direct exponential curve resolution algorithm (DECRA) has been applied most frequently on a variety of materials, including pharmaceuticals, polymers, and human brain MRI [4].

The method proposed here, QSRC, represents a new and very efficient approach for quantifying the components in solid mixtures. It utilizes ^{1}H and ^{19}F T_1 saturation recovery curves (SRCs) measured on a Bruker Minispec mq20 benchtop TD-NMR instrument [5]. For the analysis of a given mixture, the SRCs for the relevant pure components, as well as for the mixture itself, are measured. The relative amounts of the mixture components are obtained from a fit of the mixture SRC with a linear combination of weighted pure component SRCs.

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7 Structural Insights Into the Enantioselectivity of an Organocatakyst for the Acylation of 1,2-diols from an NOE Investigation

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The peptide-based organocatalyst **1** (Boc-L-(π-Me)-His-^{A}Gly-L-Cha-L-Phe-OMe) is highly effective in the enantioselective monoacylation of *trans*-cycloalkane-1,2-diols [1,2]. The selectivity observed was explained based on molecular dynamics as well as by DFT computations [3]. It has been proposed that the intermediate forms a pocket-shaped catalytic active site mimic. Only one enantiomer of *trans*-cycloalkane-1,2-diols fits into this pocket, where it is consequently acetylated. No experimental evidence confirming these computational results has been found up to now.

Here, we describe results from our NMR measurements of the organocatalyst in a mixture with *trans*-cyclohexane-1,2-diol (both enantiomers separately) studied by 1D and 2D NOE-based methods and T_1-relaxation time measurements. We noticed significant difference in chemical shift changes after the diol addition in ^{1}H as well as ^{13}C spectra. The chemical shift changes after the diol addition are bigger in the case of the R,R-diol. To determine conformational differences of tetrapeptide **1** in a mixture of the diols (both enantiomers), we selectively irradiated (1D NOE) well separated signals and measured a series of spectra differing in mixing time values. From these quantitative NOE data, using the PANIC approach [4], we extracted intramolecular distances. Additionally, we observed inter-molecular contacts between the R,R-diol and the imidazole part of the tetrapeptide. This is in contrast to the S,S-diol, for which these contacts are missing. This different bahevior is in good agreement with the proposed mechanism. We also obtained crosspeaks in 2D NOE spectra corresponding to intermolecular hydrophobic interactions between the R,R-diol and the cyclohexyl moiety. On the other hand, in the case of S,S-diol, these contacts are not observed which also suggests that the pocket-like shape of the tetrapeptide recognizes only the R,R-diol.

Finally, we measured T_1 relaxation times. We found significant differences in relaxation behavior between both diol enantiomers. In contrast to S,S-diol, the R,R-diol in a mixture with tetrapeptide behaves like a bigger molecule, which also supports the pocket-shaped intermediate theory. The results obtained increase an understanding of the origin of enantioselectivity of the newly prepared peptide-based organocatalyst and could be the basis for further design strategies.

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8 Towards Optimal NMR Measurement of Carbon Kinetic Isotope Effects

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A major established method for determining carbon kinetic isotope effects (KIEs) relies on measurement of multiple quantitative ^{13}C NMR spectra of the starting material and the recovered starting material, or products, of an incomplete competition reaction at natural abundance.[1] Relative integrals from the signals in these spectra can then be used to calculate the kinetic isotope effect for each carbon, and thus help identify the mechanism, or mechanisms, of reaction.

Even a 1% change in ^{13}C relative integral between starting material and recovered starting material corresponds to a significant KIE, so to avoid erroneous results measurements must be highly reproducible, relying on stable and robust NMR equipment and experiments. As a rule of thumb, quantitative ^{13}C experiments are typically recorded in sextuplicate, with a minimum signal-to-noise ratio of 300:1, to allow statistical analysis of results and increase the likelihood that results are significant.[2] Even at high concentrations, quantitative measurement of ^{13}C can take a very long time, sometimes days, and imperfect spectrometer stability can results in hours of wasted experiment time.

Two obvious ways to speed up NMR determination of carbon KIEs are to improve experiment robustness, to reduce reliance on replicate analysis; and to obtain ^{13}C relative abundances by an alternative, faster, method. Using recent advances in NMR methodology, including CHORUS [3] and pure shift HSQC [4], both strategies are investigated and the results of each are presented and discussed.

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9 qNMR from In-process Support to Product on the Market – a Validation Perspective

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Development of analytical methods with the acquired level of detection, specificity and robustness needed during a pharmaceutical projects lifetime (GxP environment) is a time requiring process. Especially during late phase in project development the demand for valid analytical methods increases. Traditionally, analytical methods like HPLC, GC and IR spectroscopy have been used. However, NMR is now becoming a significant player in the toolbox of available analytical methods. This is caused by the recent advances in the technical development of NMR instruments, such as acquisition electronics and probe design, which enables an improved detection limit (lower ppm range; i.e. 5-10 ppm) of components in liquid mixtures. A major obstacle with analytical methods in a GxP regulated environment is the validation of the instrument themselves and in this case the NMR related analytical methodology. The traditional terminology and standard approach for analytical method validations is not straightforwardly applied to the NMR environment. Some parameters like e.g. linearity are characteristic of the NMR instrument and only needs to be demonstrated upon installation and by regularly system suitability tests. We have applied quantitative NMR (qNMR) to several different aspects within analysis of small molecules. Examples of these applications will be presented along with the evaluation of chosen level of validation and parameters to validate. In addition, the results of a “quick and dirty” qNMR determination of low level impurities is compared to similar results obtained by a “ready-for market” validated UPLC method.

10 Homodimericin-A: Elucidating the Structure of a Complex Natural Product with an Unprecedented Carbon Skeleton

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Homodimericin-A ($C_{28}H_{26}O_8$) is a highly complex, proton-deficient natural product with an unprecedented carbon skeleton. The elucidation of the structure and the assignment of the relative configuration of the chiral centers will be described. An ensemble of routinely employed NMR experiments (COSY, ME-PS-HSQC, HMBC, and ROESY) was insufficient to unambiguously define the carbon skeleton of the molecule. Using the ensemble of basic 2D data as the input file, the Structure Elucidator CASE program was also unable to definitively establish the structure. The acquisition of a 60 Hz optimized 1,1-HD-ADEQUATE spectrum finally facilitated the elucidation of the complex, hexacyclic structure of homodimericin-A, which has eight chiral centers, five of which are quaternary carbons. The elucidation of the structure and the definition of the relative configuration of the chiral centers using a combination of ROESY, *J*-based configuration analysis (JBCA), residual dipolar couplings (RDCs) and residual chemical shift anisotropies (RCSAs) in conjunction with DFT calculations will be presented.

11 The Application of Multinuclear Benchtop NMR Spectroscopy in the Analysis of Samples from Suspected Clandestine Methamphetamine Laboratories

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Clandestine laboratory investigating chemists are tasked with using their knowledge of chemistry to answer basic questions about a suspected clandestine laboratory such as: What substance is being manufactured? How is it being manufactured? How much has or could be manufactured?

It has been recognized that NMR spectroscopy may be able to answer those questions, however the costs and maintenance of high-field NMR has limited its use in forensics laboratories. Recent advances in high resolution benchtop NMR spectroscopy overcome many of the limitations of high field NMR, becoming a more compact and affordable technique that is easy to use.

Studies have been carried out in order to evaluate the use of benchtop NMR in the analysis of samples from suspected clandestine laboratories, with a focus on precursors and phosphorus-containing compounds. The results of this study will be presented as a series of ^{1}H and ^{31}P NMR spectra used to identify the precursors, method of manufacture and isolation, and the potential yield for real suspected clandestine laboratories.

12 Reaction Monitoring by NMR in a Two-Chamber Glass System

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Traditionally, reaction monitoring has been carried in conventional NMR tubes (e.g., 5 mm) where the reactants are mixed in the appropriate solvent (e.g., deuterated) prior NMR acquisition. One of the drawbacks is the shimming process while the reaction is ongoing in the NMR tube because it delays the time to start acquiring data from the reaction. This problem is more acute when the reaction occurs at temperatures far from ambient due to equilibration time.

Mix et al. [1] developed a double-chamber NMR tube to decrease the shimming time after the reaction starts. Their system comprises the assembling of a 3-mm NMR tube with no close ends into a 5-mm tube to create two separate chambers. Silicon is used as sealant to seal the end of the 3-mm tube that seats at the bottom of the 5-mm NMR tube. To avoid potential mixing from the solutions in the two chambers, the system requires a series of parts purchased separately and mechanical work done at a workshop for assembling. Currently, the system is not commercially available. Another concern is the presence of sealant during the reaction for potential contamination and other effects.

Bosco's team at New Era Enterprises [2] designed a much simpler device, a sample reaction system with no sealant and fewer parts for assembling. The system is commercially available. Besides the easy assembling, the main difference from the system previously described is the Teflon tip in one end of the 3-mm tube that provides a perfect seal avoiding mixing of the solutions in the two separate chambers facilitating shimming prior mixing the solutions for the reaction to start.

We have tested the New Era system [2] with two reactions, a standard Fischer esterification at the temperature of the NMR probe near ambient, and a complex catalyzed cyclization at high temperature. In both cases, the Teflon tip sealed perfectly avoiding mixing prior lifting the inner 3-mm tube for mixing to follow the reaction by NMR. Even for reactions monitored online, having preliminary information of the kinetics and speciation of the reaction carried out in the NMR tube is valuable especially for complex reactions. In this poster, we will discuss the simplicity of using this device applied to reactions at different temperatures successfully.

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13 Application of NUS to Monitor *in-vivo* Processes

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Nuclear Magnetic Resonance (NMR) spectroscopy has the potential to non-invasively investigate chemical and biological processes. Conventionally, arrayed measurements are collected over equally spaced time intervals during an ongoing reaction. Multidimensional experiments provide additional benefits like increased resolution but are limited by the long acquisition times. It has been previously shown how Time-Resolved Non Uniform Sampling (TR-NUS) can be used as a method to monitor fast processes where time acts as the pseudo third dimension [1,2,3]. The idea is to sample the FID non-uniformly and in parallel to the process happening in the NMR tube. Further, subsets of such NUS dataset are created and reconstructed one by one using the Compressed sensing algorithm. The result is a stack of spectra showing the changes occurring during the reaction over time.

Previously spectral changes observed due to cell death [4] and specifically due to bacterial cell death by heat have been reported [5]. In this project, we have further extended the idea to monitor the biochemical processes which occur when bacteria interact with an antibacterial product. For our studies we have taken *Propionibacterium acnes*, which is a common cause for face acne and a commercially available face product. Since face products have a huge number of ingredients, multidimensional experiments become a necessity. Processes such as how the rate of cell death depends on the concentration of the product and the various biochemical changes that occur in the entire process over time have been studied. The effect of individual components of the product on the bacteria has also been taken into account for eg. the consumption of glucose , which is one of the ingredients in the face product.

TR-NUS provides one with the time resolution that makes such in-vivo studies feasible. We have acquired 2D TR-NUS zTOCSY, which shows the changes in peak intensities in a continuous “movie-like” manner. STOCSY [6] analysis of the 2D data also put some light on the metabolomic changes caused by the acid stress from the product. Sampling related artifacts, their effect on peak intensities and some methods to overcome this issue have also been discussed.

The aim of the project is to develop a method which can be used for the monitoring of complex biochemical reactions in vivo using time-resolved NMR spectroscopy. We believe such studies help to understand the mechanism of how pharmaceutical products work. Our main goal is to develop the method of TR-NUS by investigating some practical applications in the pharmaceutical industry.

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14 Structural Preferences and Unexpected Mobility in Imine/Phosphoric Acid Complexes

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Since the development of the first binol-derived phosphoric acids in 2004 by Akiyama and Terada,[1,2] Brønsted acid catalysts have emerged as a viable catalyst class for a variety of enantioselective reactions.[3-7] Due to their excellent yields and *ee* values, these catalysts are able to provide a complementary technique to transition metal catalyzed reactions. Despite the huge success of enantioselective Brønsted acid catalysis, experimental data about structures and activation modes of catalyst/substrate complexes in solution are very rare.

Here, for the first time, detailed insights into the structures of imine/Brønsted acid catalyst complexes are presented based on NMR data and underpinned by theoretical calculations. The chiral Brønsted acid catalyst *R*-TRIP (3,3′-Bis(2,4,6-triisopropylphenyl)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate) was investigated together with five aromatic imines. One dominant structure was observed for an *E*-imine/*R*-TRIP complex. In addition, an averaged set of signals from two *Z*-imine/*R*-TRIP complexes was obtained, which reveals a surprisingly high mobility at 180 K. Even at modulated electron density of the imine substituents similar structures were observed for all investigated imine/*R*-TRIP complexes. The observed dynamic movement of the *Z*-imine inside the complex is probably possible due to the compact structure of the imine, which reduces the kinetic barrier of the rearrangement. Initial theoretical investigation on the ternary complex suggested that this rearrangement process of the imines might be essential to form a transition state complex in the hydride transfer step.

The invariance of the structures across all imine substrates reflects the strength of this class of catalysts. Extended CH/π and π/π interactions between catalyst and substrate provide high stereoselectivities, whilst the strong hydrogen bond allows for a high substrate tolerance. For example, for phosphoramidites it is known that variations in the electron density of the substrate often have to be compensated by changes in the ligand structure.[8] The structural investigations presented here show that in Brønsted acid catalysis the strength of the hydrogen bond overwrites the variations in the CH/π and π/π interactions caused by different functional groups of the substrates. Thus, Brønsted acid catalysis seems to combine both high stereoinduction by excessive CH/π and π/π interactions and structural invariance caused by the hydrogen bond.

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15 NMR-Spectroscopic Investigations on Hydrogen Bond Interactions between Chiral Phosphoric Acids and Imines

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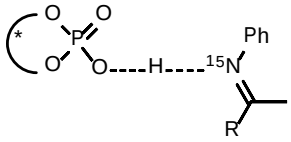
During recent years, chiral Brønsted acids have become more and more important as catalysts and therefore have been used in several enantioselective transformations, like Mannich-type reactions [1] or asymmetric transfer hydrogenations of imines [2]. In all these reactions the activation by phosphoric acid catalysts takes place via hydrogen bonds. To get a better insight into the Brønsted acid catalysis, the understanding of hydrogen bond interactions and proton transfer from the hydrogen donor to the acceptor are very important challenges. NMR-spectroscopic investigations on hydrogen bonds of small molecules are aggravated because of the fast intermolecular exchange, which leads to averaged ^{1}H-signals of all hydrogen bond species. In this case the stabilization of different hydrogen bond species is possible at very low temperatures, where the slow hydrogen bond exchange regime is reached. Thus, low-temperature NMR-spectroscopy at around 180 K is a suitable method to characterize the occurring hydrogen bonds of small molecules.



So far, our working group investigated the strong hydrogen bonds between phosphoric acid catalysts and different ^{15}N-labeld imines, as shown above [3]. For the characterization of the occurring hydrogen bonds the ^{1}H- and ^{15}N-chemical shifts as well as the coupling constants are adequate tools. Limbach *et al*. have shown in several publications how to calculate geometries of the hydrogen bonds between aldenamine Schiff bases and various carboxylic acids out of these parameters [4]. For example, it is possible to conclude the atomic distances r_{OH} and r_{HN} as well as the strength of the hydrogen bond out of the ^{1}H- and ^{15}N-chemical shifts [4]. While the coupling constants are informative about the bond angels within the hydrogen bond and the assignment of hydrogen donor and acceptor [4]. We investigated, if these correlations of chemical shift and coupling constants with the parameters of the hydrogen bond geometry are also applicable for our systems.

In order to characterize low-barrier hydrogen bonds, also the proton transfer from the donor to the acceptor is an interesting process. The barrier for the motion of the proton can be pictured as single-well or double-well potential. To investigate the potential energy surfaces in the liquid state once again low-temperature NMR-spectroscopy is a suitable method. One possibility is to replace the hydrogen bond proton partially by a deuteron and then the H/D isotopic effect on chemical shift is determined [5]. The isotopic effects facilitate the access to interesting properties of low-barrier hydrogen bonds, which also occur in our systems, consisting of ^{15}N-labeled imines and phosphoric acid catalysts.

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16 NMR Spectroscopic Investigations of Remote Stereocontrol in Dienamine Catalysis

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In the broad field of organocatalysis, catalysis with remote stereocontrol became very popular during the last years although it provides special challenges in design and comprehension. The dienamine catalysis, which is a prominent example, has emerged to one of the most promising catalytic models in the α- and γ-functionalization of unsaturated aldehydes using remote stereocontrol.[1,2] Depending of the electrophiles and catalysts used different reaction mechanisms and stereoinduction modes were proposed (e.g S_N-type, Diels-Alder or cycloaddition reactions).[3-6] However, independent of the proposed mechanisms, no correlations between the variable *Z/E*-ratios of the second double bond and the resulting *ee*-values were found ("*Z/E*-dilemma"). Futhermore, to the best of our knowledge, the exact reason for the excellent remote stereocontrol especially in S_N functionalization reactions in the γ-position and a quantitative analysis of the effect of the shielding of the catalyst were not available so far.

Therefore, the structure, thermodynamics and kinetics of the central dienamine intermediates were investigated by NMR and theoretical calculations. The NMR spectroscopic investigations showed a clear preference for an *E,s-trans,E* and *E-s-trans-Z* configuration of the diene subsystem as well as a *sc-exo* conformation of the exocyclic moiety and a *down* puckering of the pyrrolidine ring of the catalyst subsystem. Assuming the proposed effective shielding of one face [3,4], the high *ee*-value can be achieved if either one of the configuration isomers (*E-s*-trans,*E*/*Z*-dienamine) reacts significantly faster with the active electrophile than the other or the *Z/E*-ratio of the second double bond must reflect the final observed *ee*-value of the product. However, in our case the detected *Z/E* ratios does not correlate with the measured *ee*-values. Moreover, despite of similar *Z/E*-ratios across dienamines with different catalysts, the measured *ee*-values vary significantly. Based on experimental data and calculations, we could show an effective shielding of the diene system with different catalysts in case of large electrophiles, as Michler's hydrol blue. To investigate the conversion of the dienamines experimentally it was necessary to decelerate the electrophilic addition step by modulating the electrophile concentration using various Brønsted acids of different p*K*a values. The resulting NMR kinetic data showed a faster conversion of *Z*- than *E*-dienamines. Theoretical calculations confirmed these results by correlating the *ee*-values of three different catalysts with the kinetics of the electrophilic attack and display the importance of CH-π and stacking interactions in the transition states.

To summarize, for the first time a comprehensive understanding of the remote stereocontrol in a S_N reaction via dienamine catalysis is presented. In addition to the effectiveness of a partial shielding in the case of large catalysts and sterically demanding electrophiles, a kinetic preference for the formation as well as for the conversion of *Z*-dienamines could be shown. Due to these results, the "*Z/E*-dilemma" (i.e. no correlation

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17 EXACT NMR – Faster Pseudo-2D Acquisition and Safer ASAP-HSQC

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Limitations to the regular sampling of NMR signals at the Nyquist rate are mitigated by approaches involving non-uniform sampling (NUS) with subsequent algorithmic reconstruction of the un-acquired data points. Candès and Wakin in their paper on compressive sensing[1] expressed the idea that the "information rate of a continuous time signal may be much smaller than suggested by its bandwidth". In effect, under-sampling such signals at rates slower than the Nyquist rate often provides enough information to determine the frequencies within the signal. We have recently demonstrated this concept through EXACT (EXtended Acquisition Time) NMR.[2] Practical benefits to under-sampling data points in the direct dimension of some NMR experiments are hereby presented.

Faster – In pseudo-2D experiments, data points concatenated in the direct dimension are acquired over incremented time periods and one often wonders how many of these thousands of points acquired are actually needed. In this work, we reveal substantial time savings (more than 75%) employing EXACT NMR in the acquisition of pseudo-2D experiments such as PSYCHE[3] and ZS-1D[4, 5] without significant loss in spectral quality or resolution and most importantly with no signal intensity loss compared to the fully sampled spectrum.

Safer – The use of Homonuclear Hartmann-Hahn cross-polarisation sequence[6] to replace the long recovery delays (> 1s) of multidimensional NMR experiments in what is called 'acceleration by sharing adjacent polarization' (ASAP) increases, by orders of magnitude, the speed of acquisition to ~30s per scan. The practical use of the ASAP technique (first applied to HMQC[7] and subsequently HSQC[8]) is however, limited by sample heating and power handling concerns. We therefore propose a means of circumventing these challenges using EXACT NMR[2] and show acquisition of a full 2D spectra in ~ 6s using the proposed EXACT-ASAPHSQC pulse sequence (with multiplicity editing).

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18 NMR Spectroscopic Investigation of Silicon Zintl Anions

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Zintl Anions are intermetallic compounds of alkaline or earth alkaline metals and elements of group 13 to 16. These compounds form anionic cages (e.g. E_4^{4-}, E_5^{2-}, E_9^{n-}) and are interesting because of their properties as diamagnetic semiconductors. Although there is much known of Zintl anions in solid state, their behaviour in solution remains uncertain. Especially since their modification reactions take place in solution, studies on solution, transformation and degradation processes are necessary to predict and direct their synthetic pathways. [1,2]

In general, there are two ways to attain these polyanions, the direct reduction of the elements in liquid ammonia and the solid-state reaction of the elements at high temperature and then dissolving it in liquid ammonia. [3] In the case of silicon Zintl anions only the latter is possible. Therefore, $K_6Rb_6Si_{17}$ dissolved in liquid ammonia was chosen as a model system. The crystal structure of $K_6Rb_6Si_{17}$ shows it consists of two four-membered and one nine-membered cages. Liquid ammonia was chosen as solvent because of its stabilization of the highly charged anions, which are poorly soluble in other solvents, at rather mild conditions.

The only NMR active nucleus of Silicon is ^{29}Si, which is only a mediocre nucleus due to its low natural abundance (<5%) and also time-consuming because of its high longitudinal relaxation time (T_1 >5s).

For our first measurements a 20% ^{29}Si enriched sample in liqud ammonia at 233 K was used. But it resulted in no signal except the glass peak at around -100 ppm.

It was reported in the case of tin Zintl anions in liqud ammonia that cations can polarize the ammonia, which reduces Sn_4^{4-} to Sn_9^{4-} under formation of NH_2^-. [3] This can be surpressed by adding 2.2.2-cryptand (2:1), which binds the cations and separates them from ammonia. The sample of 20% ^{29}Si enriched $K_6Rb_6Si_{17}$ and cryptand gave one sharp singlet at -325 ppm, but with rather low intensity.

Therefore we synthesized a 100% ^{29}Si enriched $K_6Rb_6Si_{17}$ sample. Again without cryptand there was no signal detected. Only with cryptand several signals were obtained. An additional singlet at -360 ppm, a low intensity duplet at -358 ppm (J=27 Hz) and triplets at -352 (J=42 Hz) and -410 ppm (J=43 Hz), as well as a quartet at +345 ppm (J=42 Hz) have been observed.

So far we do not know which species these signals correspond to. We hope to gain insight by simulating theoretical spectra of different possible Zintl anions and compare them to the actual experimental data. This way it might be possible to understand the behaviour of silicon Zintl anions in solution.

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19 The Trouble with τ_c – How to Account for the Variance in Nuclear Motion in Small Molecules for NOE Distance Determination?

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Through the relationship of distance to observed NOE intensity, one can accurately estimate internuclear distances within a molecule, with average errors as low as 3.06% for rigid molecules such as strychnine.[1] This analysis assumes homogenous intramolecular tumbling (reported as the rotational correlation time, τ_c) however in reality each pair of nuclei have their own τ_c which should therefore cause an inherent error in quantitative NOE distance determination. This is supported by the increase in average NOE-distance errors for more flexible molecules such as 7.8% and 5.7% for conicasterol F and a C10 hydrocarbon chain adopting a helical conformation.[2-3] By correcting for any variation in effective τ_c for a given pair of nuclei (τ_{eff}), it is hoped that more flexible molecules will be able to provide NOE-distance data with accuracies approaching those obtained for rigid systems A number of literature methods to quantify τ_{eff} in small molecules by NMR have been examined and all fail to provide satisfactory results – indeed in most cases it is not possible to calculate a realistic τ_{eff} value for molecules near the extreme narrowing limit.[4-6]

Herein we propose that the temperature-dependence of NOE intensities can be used as a tool to estimate τ_{eff} for a given pair of protons. By measuring NOE at varying temperatures, it can be determined at what temperature no NOE intensity is observed ("zero-point" temperature).[4] If all NOE pairs have the same τ_c then all NOE correlations should have the same zero-point temperature. However variation of τ_{eff} across molecules will cause each NOE correlation to have a different zero point temperature and this is indeed observed experimentally (Figure 1). NOE correlations with higher τ_{eff} pass have higher zero-point temperatures than those with lower τ_{eff}. So by determining the point where each NOE correlation passes through zero, we can in principle scale each NOE correlation to one reference NOE zero point (Eq.2). This scaling has so far been applied to strychnine with a reduction in error in NOE distances determined from 2.23% (unscaled) to 1.45% (scaled), with more flexible rigid molecules being tested with this approach in future.

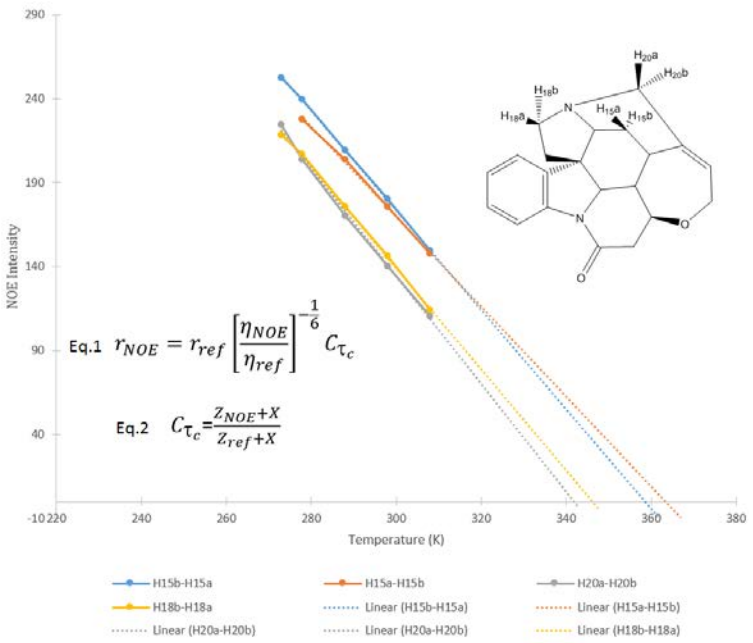

Figure 1. Temperature vs. NOE Intensity of Methylene Correlations in Strychnine. Linear trend lines show difference in zero point temperatures.

Equation 1. NOE distance determination where r is internuclear distance, η is NOE intensity and $C_{\tau c}$ as τ_c scaling factor

Equation 2. $C_{\tau c}$ is determined via ratio of zero point temperatures, Z, and undefined scaling factor, X.

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20 Determination of Relative Orientation of Triterpene Domain in Bis Triterpene-triazole Molecules, by Using RDC-enhanced NMR Spectroscopy

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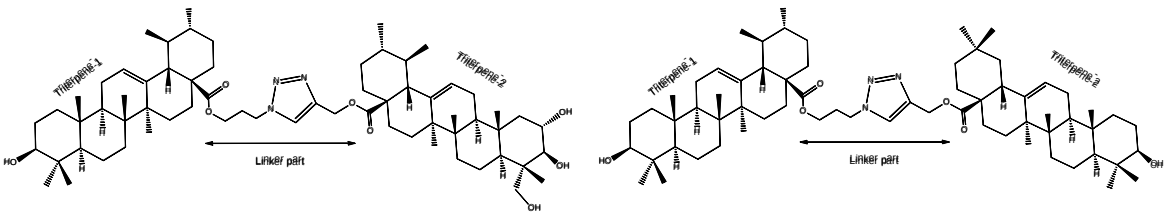
Structure based design and development of small organic molecules as therapeutics or ligands for target specific binding is an active area of research. Precise determination of configuration and conformation of these molecules are highly important for specific applications as well as for subsequent developments. Amongst these, two-domain molecules comprised of steroid [1] or tri-terpene units separated by flexible linkers are interesting due their potential therapeutic activity. However, their precise conformational determination is challenging due to absence of long-range NOEs and also due to the inherent large scale dynamics.

We will discuss the importance of RDC-enhanced (NOE+$^{1}J_{CH}$+$^{1}D_{CH}$) NMR in organic solvent media, to determine the conformation and relative domain orientation of triterpene units, that are isolated from the Diospyros melanoxylon and synthetically linked via highly potent pharmacophore linker (>5 Å). The results highlight that the conventional isotropic structural parameters viz., NOE-derived distances (<5 Å) and $^{2}J_{HH}$/$^{3}J_{HH}$ scalar couplings-derived dihedral angles, which are *local* in nature, alone cannot establish any meaningful conformation, On the other hand, by recording anisotropic parameters, one bond C-H RDCs for molecules incubated in stretched polymer/CDCl3 gel media, the analysis was unambiguous and has enabled to identify three different populations, with majority of them adopting extended/E-like relative orientation between the two-terpene units, whereas about 20% adopt U-shaped closed conformation. These distinct orientations of the two units are expected to have significant influence on therapeutic /inhibition activity. Interestingly single component tensor analysis was found adequate for RDC-enhanced structural refinement. Furthermore, the results were repeated for different starting structures and the results are found to be consistent.



a **b**

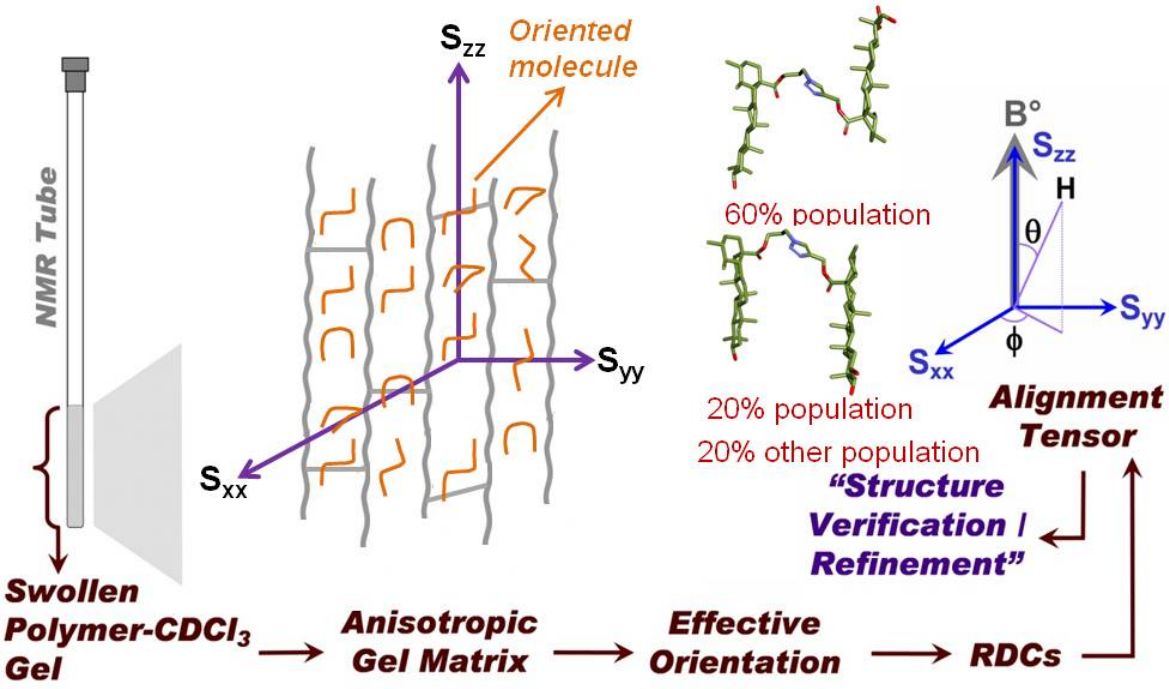


Figure1: Pictorial representation of how the orientation of molecules in anisotropic media can result in RDCs and thereby to structure verification or refinement.

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21 Synergistic Combination of DFT Chemical Shift Predictions with CASE Algorithms: a New Powerful Approach for Structure Elucidation, Verification and Revision

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Structure elucidation of new organic compounds is one of the most important challenges of organic chemistry. For this goal, CASE (Computer-Assisted Structure Elucidation) expert systems were developed. These systems are capable of generating a set of all possible structures which satisfy 2D NMR data and then selecting the most probable one on the basis of empirical NMR chemical shift prediction. However, in some cases, CASE systems failed to distinguish the correct structure when average deviations of chemical shifts were too large or when the correct structure was among several top-ranked structures with acceptable but very similar deviations. Herein, we show that the combination of CASE and density functional theory (DFT) methods of NMR data prediction allows determination of correct structures even in these challenging situations. This synergistic approach has been tested on three challenging structure elucidation problems of previously determined natural products: aquatolide,[1] coniothyrione[2] and epoxyroussoenone.[3] We believe that it can serve as an unbiased, reliable, and efficient structure elucidation method and can be applied to those difficult situations when molecular systems are chiral or conformationally flexible and/or when other experimental methods would be difficult or impossible to use.

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22 Mechanism of Atropisomerization of 8-membered Dibenzolactam: Experimental NMR and Theoretical DFT Study

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Biaryl moieties remain one of the most captivating structural motifs. They are frequently encountered in both pharmacologically active natural products and synthetic drugs. Axial chirality of biaryl systems has been widely employed in synthetic organic chemistry where they are used as chiral auxiliaries and catalysts to transfer chirality. When biaryls have bulky substituents in ortho positions they are prone to atropisomerism, a property of stereoisomerism defined by the high (>25 kcal/mol) barrier of rotation. Despite considerable experimental data on cyclic biaryl atropisomers, there is only very limited research published on a mechanism of their atropisomerization. Detailed experimental and theoretical quantum mechanical analysis of the atropisomerization mechanism of an 8-membered dibenzolactam[1] will be presented. Experimental Gibbs free activation energy, activation enthalpy, and activation entropy were established by temperature-dependent kinetic NMR experiments. The mechanism of atropisomer interconversion as a rotation of the eclipsed endocyclic coordinate in a clockwise or counterclockwise direction along the ring was proposed.

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23 Extending the Application Range of Chromatographic NMR by Enantiomer Separation and Optimization of Diffusion Measurements Under MAS

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University of Sussex

Analysis in chemistry has always been hindered by the presence of impurities in samples or mixtures that are difficult to separate. Nuclear magnetic resonance has proven to be one of the most powerful analysis techniques to enable the study of mixtures by pseudoseparation using molecular parameters such as diffusion coefficient [1]. However, when the sample to be studied is a mixture of enantiomers the best way to achieve this pseudoseparation is with the technique of matrix-assisted DOSY (MAD-DOSY) also known as chromatographic NMR. This technique is based on the addition of (chiral) sample modifier that will interact differently with the molecules varying and separating their diffusion coefficients or even changing slightly the chemical shifts [2, 3].

In this poster the analysis of a mixture of ethylenediamine cobalt complex enantiomers will be studied by NMR adding a chiral stationary phase such as Sephadex-G50 that will interact more strongly with one of the enantiomers.

One of the main issues when using DOSY is spectral overlapping that is the main cause of poor resolution. In addition to this problem a consequence of using stationary phases is the appearance of increased broadening of the signals due to differences in magnetic susceptibility. Thus, the objective is to extend the study of diffusion properties under HR-MAS conditions which can help to remove susceptibility effect but has complicating effects on the DOSY experiment.

In this poster experiments will be carried under MAS with a sample of water and tyrosine in order to find the most reliable way of studying diffusion under these conditions. Different aspects are investigated including different pulse sequences, parameters of the pulse sequence (diffusion delay or gradient strength), spinning rate and synchronization of the pulse sequence with the sample spinning. Also improvements in sample preparation as the addition of spacers in different locations to obtain the most accurate diffusion values [4].

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24 $^3J_{CH}$ – Relating Scalar Coupling Constants to 3D Molecular Structure in Solution

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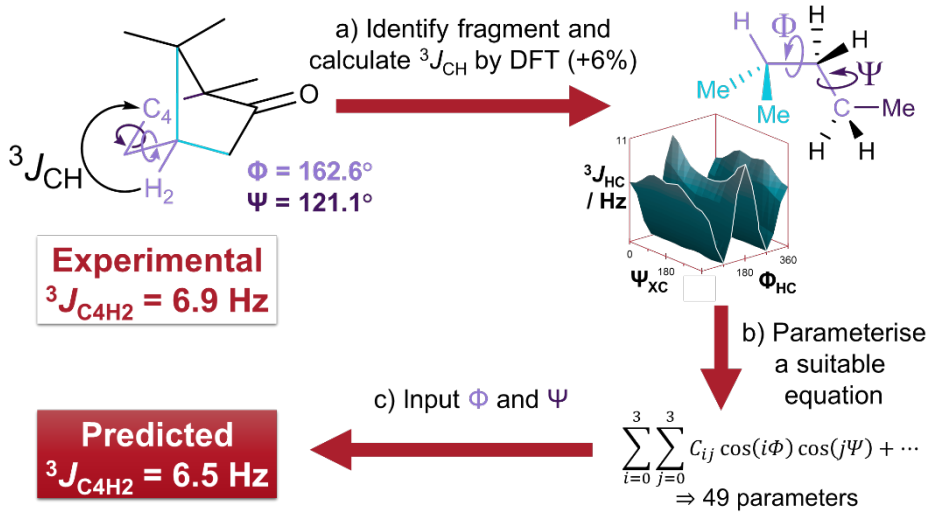
NMR spectroscopy has a wide application in the determination of three-dimensional molecular structure and the use of 3-bond scalar couplings is common in solution-state NMR. Scalar coupling between nuclei separated by three bonds can be described empirically by homonuclear (^{1}H-^{1}H) [1] and heteronuclear (^{1}H-^{13}C) [2,3] empirical equations relating the magnitude of the scalar coupling constant to the dihedral angle between the nuclei. Computational methods can also predict NMR properties such as chemical shift and scalar coupling constants for a given molecular structure [4].

Density functional theory (DFT) was used to examine the relationship between $^3J_{CH}$ and dihedral angles (Φ and Ψ) for a multitude of fragments. The effects of bond angle, substituent pattern and coupling pathway were examined. These $^3J_{CH}$ were used to identify and parameterise suitable equations relating $^3J_{CH}$ to the dihedral angles Φ and Ψ for different fragments. The performance of these equations was then tested using experimentally determined $^3J_{CH}$ as described below using $^3J_{C4H2}$ in camphor as an example.



Result: A significant improvement in the prediction of $^3J_{CH}$ in comparison to literature methods was found, with the standard deviation in the errors decreasing from ~1.5 Hz to less than 1.0 Hz.

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25 Effects of Solvent, Concentration and Temperature on Development of a qNMR Assay Method for the Antiretroviral Drug Efavirenz

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To apply the qNMR technique in quality control, satisfactorily, it is important to obtain spectra with high resolution, sensitivity and in the shortest possible time. These facts ensure that the technique could be rapid, precise, accurate and economically viable. The aim of this work was to study the solvent, concentration and temperature influence on proton chemical shifts in the development of a qNMR assay method for the antiretroviral drug Efavirenz (Figure 1).

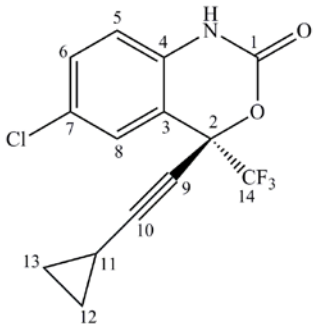

Figure 1. Chemical structure of Efavirenz.

The first step in developing a qNMR methodology (considering that the equipment suitability tests had been performed), is selecting the better ^{1}H NMR signal to be integrated and quantified [1]. Therefore, it was necessary to check the solvent, concentration and temperature influence on the proton chemical shifts. The variation of the solvent can lead to considerable changes in the chemical shift, signal dispersion and line shape [2]. The solvent effect study revealed that the aromatic H8 signal is a singlet when the solvent used was $CDCl_3$. In the case of the other solvents tested (acetone-d_6, methanol-d, dimethylsulfoxide-d_6 and acetonitrile-d_3), the H8 signal appeared as a doublet. In addition, by using DMSO, the signal of H8 has changed its position. All the other hydrogen signals presented considerable variations on its chemical shifts and in its multiplicity as result of the different solvents tested [3]. Then, ^{1}H NMR spectra were obtained at six different concentrations between 1.85 and 28.86 mg/mL in $CDCl_3$. H5, H6, H8, H11, H12 and H13 signals did not show significant chemical shift variation ($\Delta\delta$), while the H15 signal suffered deshielded as had been previously reported by Sousa and coworkers [3], in a lower concentration range (0.27 to 4.33 mg/mL). The influence of temperature on the chemical shift was also evaluated. The temperature range tested was from 25 ° C to 55 ° C. From the results, it was observed that the H15 signal suffered a change in the chemical shifts ($\Delta\delta$) at around 0.60 ppm for the shielded region of the spectrum, as a function of

increasing temperature. The other proton signals did not suffer modifications in its chemical shifts. Considering the results of the split signals in different solvents (singlet, in $CDCl_3$), the absence of chemical shift changes as a function of concentration and temperature increasing [3], the H8 signal was selected for the EFZ quantification method development by ^1H NMR.

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26 NMR Characterization of a Complex Natural Product Macrolide

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A complex polyhydroxy macrolide with a 40-membered ring was isolated from a tropical marine cyanobacterium collected from the Palmyra Atoll. Marine cyanobacteria (blue-green algae) have been shown to possess an enormous potential to produce structurally diverse natural products that exhibit a broad spectrum of potent biological activities, including cytotoxic, antifungal, antiparasitic, antiviral and antibacterial activities [1–3].

NMR-guided fractionation in combination with molecular networking and isolation via HPLC yielded 0.7 mg of the pure compound. The small amount as well as significantly overlapping proton and carbon signals complicated the NMR-based structure elucidation. Various NMR experiments and solvent systems were employed, including the LR-HSQMBC that allows the detection of long-range correlation data across 4-, 5-, and even 6-bond long-range $^{n}J_{CH}$ heteronuclear couplings [4]. This expanded NMR data set enabled the elucidation of the structure, which is characterized by several hydroxy groups as well as a *tert*-butyl group. Final results of bioactivity testings will be presented.

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27 Tautomer Equlibrium Control Via Intramolecular Hydrogen Bonding

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Prototropic tautomerism [1] of purine and pyrimidine nucleobases is of a great importance. It has implication in both reactivity (e.g. regioselectivity of nucleosidation) and ability to form hydrogen-bonding interaction (e.g. base pairing). Already in the second paper on the structure of DNA [2], Watson and Crick suggested that tautomerization of nucleobases may induce spontaneous mutation due to a presence of rare tautomeric forms. On the other hand, in RNA, the minor tautomeric forms have been proposed to enhance the structural and functional diversity of RNA enzymes and aptamers [3]. However, observations of rare nucleobase tautomers in the structures of DNA and RNA are uneasy from the physical chemistry point of view. Why the less populated high energy tautomer should be favored over the most stable tautomeric form? The answer is not simple at all, because there are many chemical and physical factors that can change tautomeric equilibria including pH, temperature, interaction with solvent or the presence of metals.

In this contribution, we would like to suggest another way of the minor tautomer selection based on the formation of a hydrogen-bonded complex. When a compound forms a hydrogen-bonded complex selectively with one tautomer, an addition of this compound may shift the tautomeric equilibrium significantly.

The proof of concept will be illustrated by shifting the tautomeric equilibrium of isocytosine observed by low temperature NMR. At SMASH 2014, we presented low temperature NMR study of isocytosine tautomerism in DMF-d_7 solution and we were puzzled by changes in tautomeric equlibrium when going down with the temperature. Recently, we were able to reach temperature as low as -120 °C with a mixture DMF-d_7 : dimethylether and proved the formation of the isocytosine dimer by NOE measurement. Therefore, tautomeric equlibrium is not simply the equilibrium between two possible tautomers but includes also the dimer (Fig. 1). This observation inspired us to shift the tautomeric equlibrium of isocytosine by addition of a compound that can form a system of hydrogen bonds with only one selected isocytosine tautomer. For example, 1-methylcytosine can from stable complex with the 2,3-I tautomer whereas the 1,2-I tautomer is preferentially selected by 2-amino-6-hydroxypyridine. We believe that such stabilization of individual tautomers via formation of stable complex may play the role in the observation of rare tautomers in the Nature.

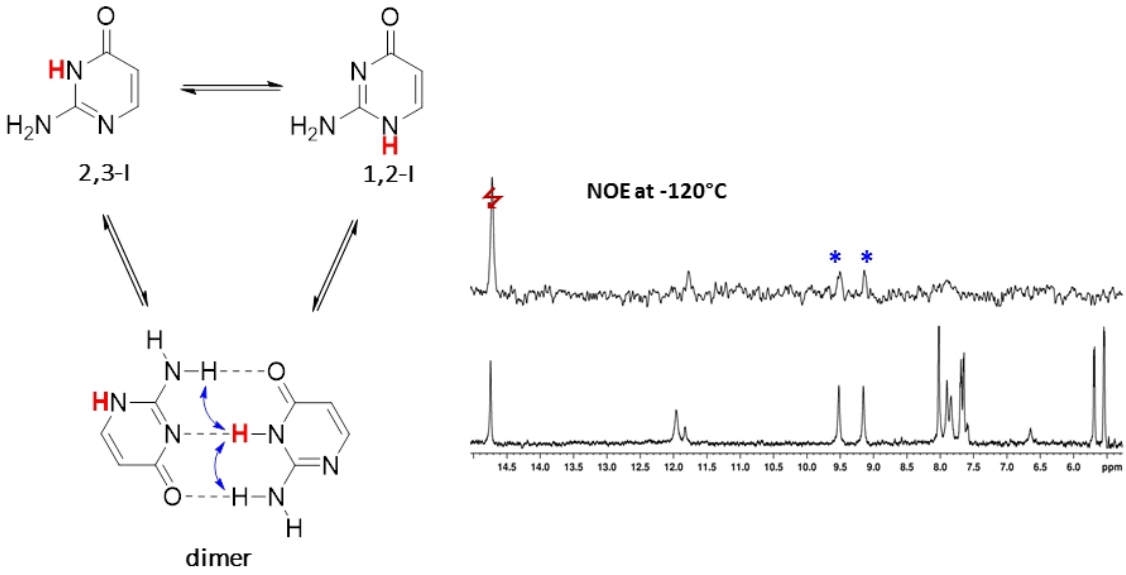

Figure 1. Proposed tautomeric equilibrium of isocytosine a NOE showing formation of the isocytosine dimer.

Acknowledgements: The work is supported by the Czech Science Foundation (Grant No. 15-11223S).

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29 PSYCHE Pure Shift NMR: Spectral Simplification and its Applications

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Resolution and sensitivity are essential for the analysis and interpretation of NMR spectra. In high-resolution ^{1}H NMR spectroscopy, because of the narrow range of chemical shifts and the many homonuclear couplings, multiplet overlap is very common and can severely complicate the analysis of spectra. Pure shift NMR techniques [1-3] have greatly improved signal resolution by removing homonuclear couplings, but at considerable cost in sensitivity. The most recent pure shift method, PSYCHE [4], although still costly in signal-to-noise ratio, typically offers almost an order of magnitude improvement over previous methods.

We present three new pure shift experiments, implementing PSYCHE in selective 1D TOCSY, Oneshot DOSY, and the recently published CLIP-COSY experiment [5]. The new selective 1D TOCSY-PSYCHE experiment is intended for the study of complex molecules and mixtures. The benefits of this method are shown in the analysis of a natural peppermint oil sample (*Mentha piperita*). Pure shift spectra showing only the connectivities of chosen signals (Figure 1) allow spectra of individual components in complex mixtures to be obtained.

The new F_1-PSYCHE-CLIP-COSY experiment is based on CLIP-COSY [5], using a PSYCHE element in the middle of the evolution time to obtain pure shift signals in the indirect dimension. Used in combination with covariance processing, the result is an ultra-high resolution phase-sensitive COSY spectrum with singlets in both dimensions.

Finally, the new Oneshot-PSYCHE DOSY experiment also facilitates the analysis of complex mixtures. Misleading peaks in the diffusion dimension due to signal overlap are minimized, and accurate high-resolution diffusion measurements are obtained.

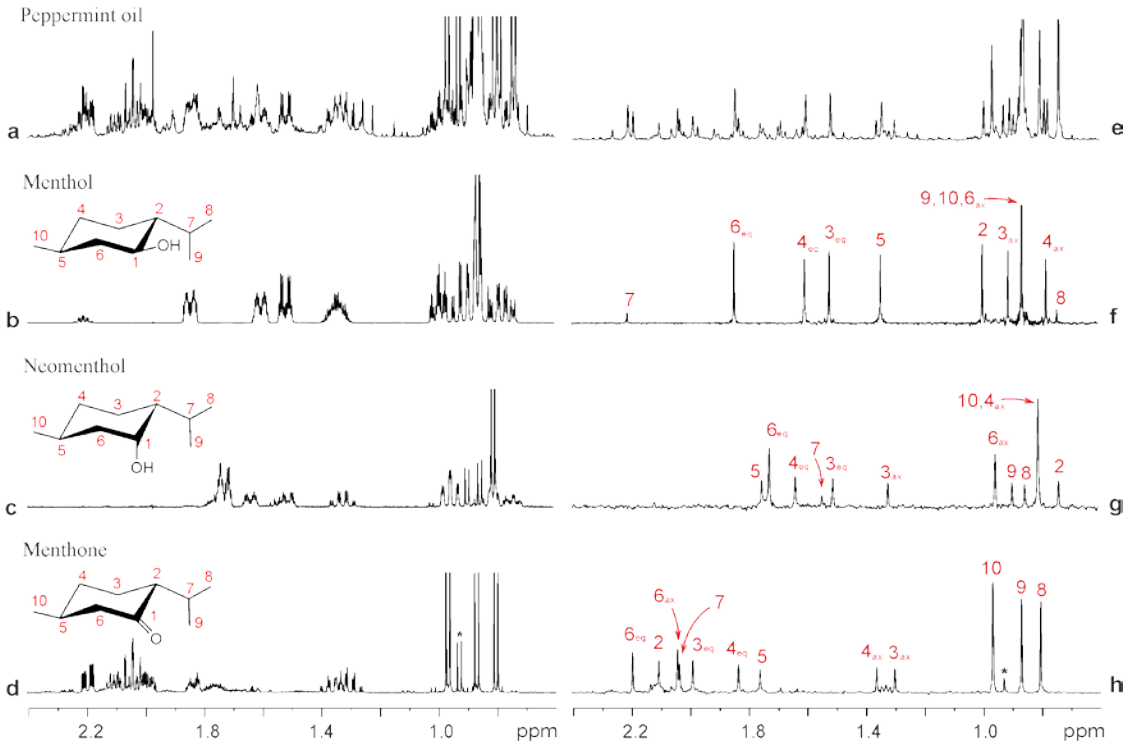

Figure 1. a) Conventional, b,c,d) selective 1D TOCSY, e) PSYCHE, and f,g,h) selective 1D TOCSY-PSYCHE ^{1}H spectra for peppermint oil 25% (v/v) in DMSO-d_6.

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30 Band-Selective 2D HSQMBC: A Universal Technique for Detection and Measurement of ^{35,37}Cl Isotope Effects for ^{13}C Nuclei

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^{35,37}Cl Isotope effects on ^{13}C chemical shifts can be used to solve regiochemistry problems in the structure elucidation of compounds by NMR. Limited sample situations may preclude direct acquisition of ^{13}C spectra that can allow the observation of ^{35,37}Cl isotope effects, which are typically in the range of 4-14 ppb. [1-3] Very recently, Molinski and co-workers reported the utilization of a band-selective HSQC 2D NMR experiment to observe ^{35,37}Cl isotope effects to differentiate protonated chloro-bearing aliphatic carbons from protonated bromo-bearing aliphatic carbons in a series of marine natural products isolated from red algae. [4,5]

There have, however, never been any reports of the utilization of band-selective 2D NMR experiments for the observation of ^{35,37}Cl isotope effects for quaternary carbons, which are generally smaller than those observed for aliphatic carbons. Hence, we now wish to report the utilization of a modified, ^{13}C band-selective HSQMBC experiment that uses proton selective pulses during the INEPT transfer to avoid any modulation due to J_{HH}, to observe ^{35,37}Cl isotope effects for both protonated and non-protonated carbons. Several examples will be shown to illustrate the robustness, universality and superior performance of the method over bs-HSQC and 1D ^{13}C NMR.

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31 A Sensitivity Comparison of HMBC and LR-HSQMBC Experiments

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Long range correlations are critical for the structure elucidation and characterization of small molecules. To observe long range correlations HMBC[1] experiments optimized for the detection of 8 Hz couplings are typically used. When searching for weak or long range correlations optimizing for 4 Hz couplings is a common practice. The HSQMBC[2] experiment can also be used to detect long range couplings and a recent variant, the LR-HSQMBC[3], is reported to extend the range over which couplings can be detected. Here we compare the sensitivity of the HMBC and LR-HSQMBC experiments.

HMBC and LR-HSQMBC spectra optimized for 8, 4 and 2 Hz couplings were recorded on RS2-153A (fragment library compound, MW 244.30) and cholesteryl-acetate (Bruker standard sample, MW 428.69). By measuring the signal to noise in ^{13}C columns extracted from the 2D spectra we found that in general the HMBC experiment was more sensitive than the LR-HSQMBC experiment, giving a greater chance of detecting the correlations when sample is limiting. Furthermore, we found that for small couplings (< 4 Hz) the 2 Hz optimized HMBC was more sensitive than the 4 or 8 Hz optimized spectra. In nearly all cases the 4 Hz HMBC was less sensitive than the 8 Hz experiment, making it a poor choice. In the LR-HSQMBC experiments a similar trend was observed with optimization for smaller couplings increasing sensitivity for small couplings. Some extremely long range correlations (5 or 6 bonds) were detected in the LR-HSQMBC spectra that were not present in the HMBCs.

When collecting data on a new compound we suggest first acquiring an 8 Hz HMBC, as it will provide most of the long range correlations. For detection of correlations involving small couplings a 2 Hz HMBC is most useful, while for extremely long range correlations the LR-HSQMBC may provide information that cannot be obtained from the other experiments.

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32 Exploring Cleanup Procedures for HPLC Contaminants to Aid Low Level NMR Sample Analysis

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Genentech

With ever increasingly sensitive NMR techniques, the need for sample are now in the mid microgram levels for complete structure analysis. This leads to new issues associated with contamination of the compounds coming from the purification. We evaluated numerous semi-preparative column types to develop NMR profiles of typical contaminants. This was followed by evaluating different cleanup steps including SPE, activated carbons, alumina, and solvent washing. The first three were evaluated with and without active drug to determine their tendencies to absorb the drug, drug and contaminant, or just contaminant.

33 Advances in NMR Software and NMR Experiments: Three Novel Structures Solved with Computer-Assisted Structure Elucidation Enhanced with Modern NMR Experiments

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5. Molecule Apps

Identifying and characterizing unknown compounds can be complex and time-consuming. To facilitate this process, we examine three examples where the practice of Computer-Assisted Structure Elucidation (CASE) was employed and we discuss how advanced NMR techniques improved the structure elucidation process. We compare the characterization times between ^{1}H-^{13}C HMBC and 1,1- and 1,n-HD-ADEQUATE and ^{1}H-^{13}C LR-HSQMBC data.

The first example covers the elucidation of an unexpected synthetic product, the rearrangement product that arose from the epoxidation of a polycyclic spiroketal. The molecular structure of the unknown was correctly proposed with CASE and standard NMR experiments, but unequivocally confirmed with a novel version of the 1,1-ADEQUATE NMR experiment.[1]

The next two examples are the elucidation of two novel natural products. The second is the example of cryptospirolepine, where the structure is of a particularly proton-deficient natural product. CASE produced millions of isomers after 420 hours of structure generation, however, the use of a 1,1-ADEQUATE experiment rendered the structure elucidation straightforward and reduced computational efforts to seconds.[2] The third is the example of a eudistidine alkaloid, in which CASE efforts were time-consuming; the application of LR-HSQMBC data was able to simplify the process and add assurance to the rectitude of the result.[3]

Computer-assisted structure elucidation can provide invaluable insight into a complex unknown. In addition, modern NMR experiments are vastly simplifying the process of structure elucidation.

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34 Mechanistic Investigation of Membrane Residency of Membrane Protein Ligands

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High-resolution magic angle spinning (HR-MAS) NMR is a powerful tool to provide atomistic information on structure, dynamics and interactions of molecules in insoluble system, such as membrane-related systems. Using this technique, we investigate the compound-membrane interaction for a few β2-agonists with distinct duration of action in model membrane bilayers. The HR-MAS NMR data is able to differentiate the compound insertion depth in membrane bilayer and the distribution profile of compound fragments. This study delineates the interactions between compounds molecular structure and their binding mode to membrane at atomic level, which is of great importance for the understanding of membrane-protein-targeted drugs with optimized duration.

35 Structure Elucidation by ^{13}C-^{13}C COSY NMR of Two Novel Peptides from a Mushroom-derived Streptomyces sp

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Natural products have historically been a rich source for therapeutics, but the structure elucidation of these complex chemical structures can often be time-consuming and inefficient. Isotopic labeling of natural products and NMR analysis, such as a ^{13}C-^{13}C COSY, can dramatically shorten the time for structure elucidation. Two novel peptides were discovered from a bacterial strain (*Streptomyces* sp.) isolated from a mushroom (*Hymenochaete rubiginosa*). The novel structures were determined by NMR and MS, including a ^{13}C-^{13}C COSY of isotopically enriched compounds, as well as the use of computer-assisted structure elucidation (CASE) software [1].

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36 Practical Considerations for Use of Either ^{1}H-^{15}N or ^{13}C-^{15}N Coupling Constant Measurements for Geometric Assignment

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In addition to correlation data, ^{1}H-^{15}N and ^{13}C-^{15}N coupling constants are a powerful tool for structure determination. The relationships between structure and coupling constants can be complex, however, and successful application requires selection of the appropriate experiment. The best experiment to utilize can be determined based on the complexity of ^{1}H-^{1}H homonuclear splitting, sample availability, and solubility. In this poster we detail a simple "decision tree" for structure determination using ^{1}H-^{15}N and ^{13}C-^{15}N coupling constants and provide typical sample conditions required to obtain the ^{1}H-^{15}N or ^{13}C-^{15}N coupling constants.

37 A novel method for distinguishing between Salts and Co-crystals using Solid State NMR Spectroscopy

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A potential drug candidate is usually developed as a salt or co-crystal in order to improve its physicochemical or biopharmaceutical properties. In the case of co-crystals, the molecular interaction between a compound and its conformer is nonionic, whereas the interactions in salts are ionic. According to the FDA guidance published in 2013, data supporting the classification as a co-crystal should be submitted for new drug applications and abbreviated new drug applications (NDAs and ANDAs) containing a co-crystal form [1]. New pharmaceutical co-crystals of known APIs are not considered as new APIs. This is in contrast to new salts of known APIs which are considered new APIs.

Although Raman, ATR-IR and solid state NMR spectroscopy can be used to define which type of interaction is occurring, it is impossible to identify a binding site using either Raman or ATR-IR spectroscopy. Only the chemical shift difference of ^{15}N-NMR and HETCOR of solid state NMR can specify the site [2, 3].

In order to prove this, four different complexes of Compound A were prepared using fumaric acid, succinic acid, citric acid and hydrochloric acid. Then, the chemical shift differences of ^{15}N-NMR and HETCOR of the four complexes were investigated in detail. It was found that the fumaric acid and the succinic acid complexes of Compound A were co-crystal compounds, and the citric acid and the hydrochloric acid complexes were salt compounds. The binding sites of four different complexes were accurately identified by using the combination of two methods. This novel combination approach can also be applied to amorphous compounds and compounds whose single crystals are not obtained.

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38 CRAFT 2D Aids the NMR Structural Identification of a Novel Diabetes Biomarker

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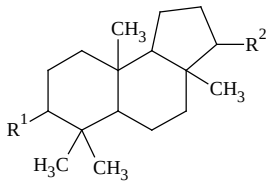
X12063 is a potential biomarker for type II diabetes that has been discovered in human plasma by Metabolon, Inc. Recently, a sufficient quantity (~50 ug) has been isolated and purified for structure elucidation by NMR using the standard suite of 2D homo- and heteronuclear sequences, e.g. TOCSY, HSQC, HMBC, and NOESY. A considerable number of overlapping methyl and methylene signals in a narrow region, however, complicate the identification of cross peaks in the 2D NMR data. Application of a 2D CRAFT (Complete Reduction to Amplitude Frequency Table)[1,2] algorithm was used to further analyze the data. CRAFT 2D uses a Bayesian analysis to reconstruct FID models of the f1 dimension. The Craft analysis creates a table of frequencies, amplitudes and decay constants for the fi dimension which can then be processed without apodization.



X12063

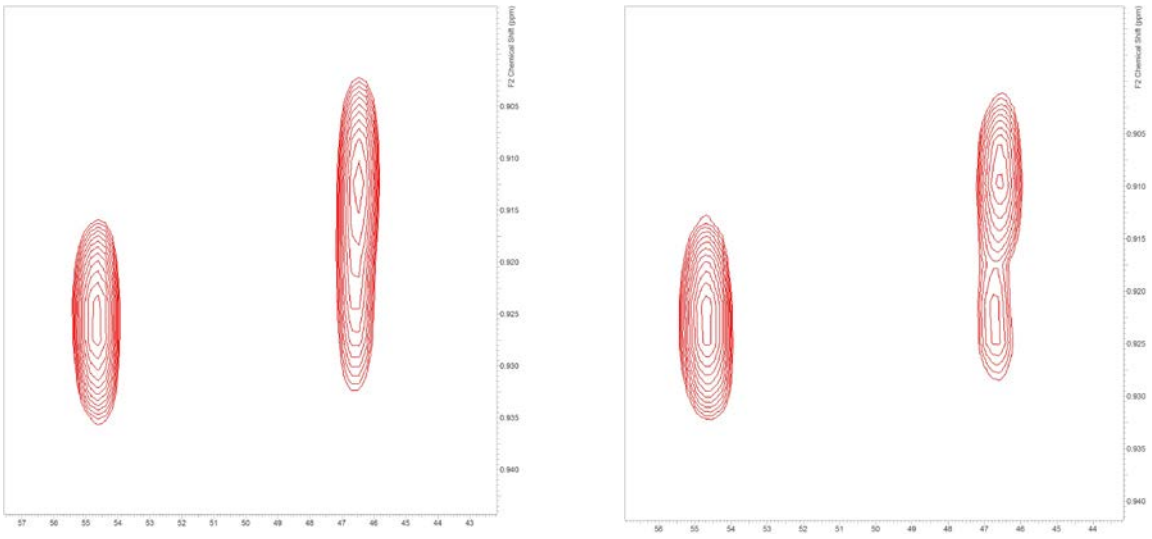


Figure 1. An example of improvement in the processed data. On the left is the 2D FT processed data and on the right is the CRAFTed data.

Truncation artifacts are circumvented by the use of the CRAFT analysis resulting in higher quality 2D spectra than 2d data processed by standard Fourier transformation.

This CRAFT reconstruction facilitates assignment ambiguities particularly for congested regions and greatly aided the structural identification of the biomarker.

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39 Extractive Ratio Analysis NMR Spectroscopy for Improved Unknown Metabolite Identification: in Crowded Specctra1D NMR to Robust Correlation Spectroscopy

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Resolving signals in crowded spectra is a bottleneck in metabolomics. In many biological systems, signals from compounds of different compound classes can overlap and dramatically increase the complexity of NMR spectra. Selective extraction of a compound class brings a level of simplification in the spectra. Extraction conditions and/or solvents can limit the compounds extracted and give rise to NMR spectra with exploitable varying peak intensities. Here we propose an extractive ratio analyisis NMR spectroscopy (E-RANSY) method to construct subspectra of individual metabolites from NMR spectra of the set of samples and demonstrate its application in the identification of human urine metabolites.

Ratios of peak intensities in NMR spectra are proportional to the relative numbers of magnetically active nuclei. Spectra of individual metabolites and/or biologically correlated metabolites can be obtained when peak ratios are divided by the standard deviation of the peak intensities over a set of NMR spectra, a method called ratio analysis nuclear magnetic resonance spectroscopy (RANSY)[1]. This method is useful in the selective identification of metabolites in biological fluids. However, in overcrowded NMR spectra such as that of urine, the RANSY method (and others) are severly challenged. In the proposed E-RANSY method, a set of samples are obtained by extraction using different extraction conditions, such as pH. RANSY is then applied to these extracted samples. This new method works well for the selective identification of acid metabolites in urine samples. We also propose a mathematical modification to the original RANSY approach to enhance strong correlations, which will also be described.

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40 Unequivocal Structural Assignments of a Macrolactone: An Experimental and Theoretical Approach

Layla R. Barbosa[1], João F. P. Bassane[1], Renato A. Lima[2], Fernanda B. Hurtado[2], Dionatas U. de O. Meneguetti[2], Valdir A. Facundo[2], Alvaro C. Neto[1], and **Valdemar Lacerda Jr.**[1]

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Medium-sized (7 to 11 members) and large-sized (12 or more members) cyclic compounds, particularly the lactones, are an important class of organic compounds, having a structural importance in the biological activity of a wide range of natural products [1,2]. An important factor in biological activity studies is the correct knowledge of their structures and many methods have been developed for this purpose.

Currently the Nuclear Magnetic Resonance (NMR) is an experimental technique widely used in structural determination of organic compounds [3]. However, the spectra of these compounds are not simple, there are overlapping ^{1}H and ^{13}C NMR signals and many couplings that hinder the unequivocal assignment of all ^{1}H and ^{13}C NMR signals and the determination of the relative stereochemistry.

The development of experimental NMR techniques has been accompanied by advances in computing and theoretical methods (especially the DFT methods), which permit shielding tensors and spin-spin coupling constants to be calculated with highly reliable results [4,5].

In this context, this work aims the complete NMR assignment of the macrolactone shown in Figure 1, using 1D and 2D NMR, and compare it with the theoretical predictions of the chemical shifts (δ) and coupling constants (*J*) using DFT calculations.

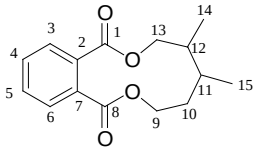

Figure 1. Macrolactone studied in the present work

In this study, we present the assignment of NMR data of 1D (^{1}H, $^{13}C\{^{1}H\}$, ^{13}C DEPT-135, NOEDIFF) and 2D (COSY, HMQC, HMBC and NOESY). The theoretical data obtained was used as support for the unequivocal assignment of the NMR signals of ^{1}H and ^{13}C and relative stereochemistry of the macrolactone. For that, the structures of *cis* and *trans* isomers were designed in the program GaussView 4 [6]. After that, optimization was carried out through GAUSSIAN 03 [7], using different methods. All calculations of shielding tensors NMR were made using the optimized structures at the B3LYP level of theory and with the basis set functions cc-pVTZ as input, through the GIAO. The calculated shielding tensors NMR were converted into chemical shifts (δ), considering the shielding tensors of ^{1}H and ^{13}C of TMS calculated at the same levels of the theory. The effect of solvent (chloroform) was also included in the calculations of

shielding tensors. For calculating the spin–spin coupling constants (*J*), the level of theory B3LYP was used with the GIAO method and the basis set functions cc-pVTZ and EPR-III.

The experimental ^{13}C and ^{1}H chemical shift (δ) data were correlated with the theoretical results for *cis* e *trans* macrolactone. Based on the data presented, a statistical analysis was performed, obtaining the standard deviations (SD) and mean deviation (MD). Graphs were plotted correlating the theoretical and experimental values, obtaining the linear correlation coefficient (R). The statistical analysis results are summarized in Table 1.

Table 1. Statistical analysis results for ^{13}C and ^{1}H chemical shift.

	^{13}C Chemical shift					**^{1}H Chemical shift**			
	Without Solvent		With Solvent			Without Solvent		With Solvent	
	cis	***trans***	***cis***	***trans***		***cis***	***Trans***	***cis***	***trans***
SD	5.03	4.38	5.19	5.91	SD	0.15	0.17	0.14	0.14
MD	7.10	7.93	7.93	7.94	MD	0.09	0.06	0.15	0.20
R	0.9922	0.9924	0.9927	0.9940	R	0.9973	0.9965	0.9986	0.9972

In Figure 2 it can be seen the difference (Δδ) between theoretical and experimental values for ^{13}C and ^{1}H chemical shift of *cis* and *trans* isomers without and with solvent effect.

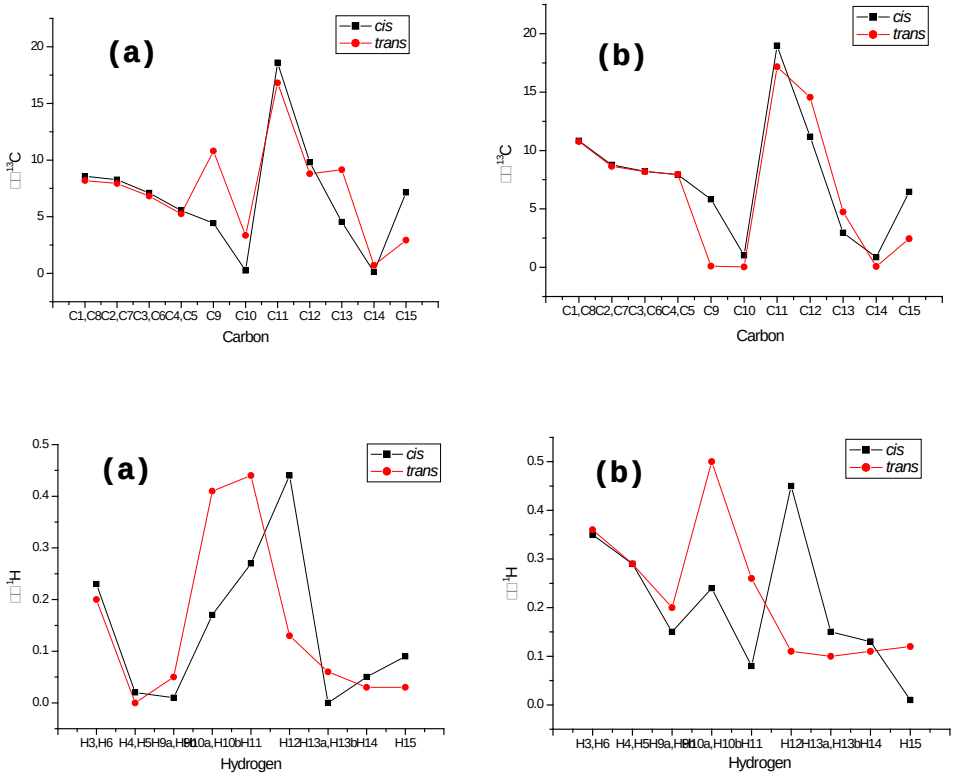

Figure 2. Comparison of Δδ values for the ^{13}C NMR and ^{1}H NMR data without (a) and with (b) solvent effect

Analyzing the table and graphs it can be noticed that for ^{13}C δ the *cis* macrolactone showed the best values for mean deviation, MD: 7.10 (without solvent effect) and for standard deviation, SD: 5.19 (with the solvent effect), being the other values closer for both isomers. For ^{1}H δ the results obtained were similar. The *cis* macrolactone showed the best values for calculations without solvent effect (SD=0.15 and R=0.99733) compared to the isomer *trans*. For calculations with the solvent effect the *cis* macrolactone also showed the best values for mean deviation, MD: 0.15 and R: 0.9986. However, the values for SD were identical for both isomers.

The statistical analysis results for coupling constants (*J*) are summarized in Table 2.

Table 2. Statistical analysis results for coupling constants (*J*) of H_{11} e H_{12}

Coupling Constants, *J*, H_{11} e H_{12}				
	cc-pvtz		EPR-III	
	cis	***trans***	***cis***	***trans***
SD	1.47	1.45	0.75	1.37
MD	0.61	2.61	1.79	2.20

With the basis set cc-pVTZ, the *cis* macrolactone showed best result for mean deviation,MD: 0.61 while SD values were very close. With the basis set EPR-III, the *cis* macrolactone showed better results compared to *trans* for SD and MD (0.75 and 1.79, respectively).

As conclusion, the unequivocal structural NMR ^{1}H and ^{13}C assignments of the macrolactone were performed using 1D and 2D experiments with the aid of molecular modeling (theoretical prediction of chemical shifts and coupling constants). Regarding the relative stereochemistry of the macrolactone, the results showed a slight indication for the isomer *cis.*

Acknowledgements: The authors thank to CNPq, CAPES and FAPES for financial support and fellowships.

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41 Improved INADEQUATE by F1 Spectral Aliasing and Non-Uniform Sampling

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Determining the carbon-carbon connectivities of an unknown molecule is a key step in elucidating its structure. The INADEQUATE experiment is specifically designed to reveal these correlations, however, it suffers from severe sensitivity problems [1, 2]. These problems are amplified in sample limited cases, such as those arising in natural product chemistry. Despite modern developments such as larger static magnetic fields and the advent of cold probe and microcoil technology, INADEQUATE is rarely used in routine structure elucidation.

The ability to accelerate the acquisition of an INADEQUATE experiment, or utilise spectrometer time to increase the signal-to-noise ratio via the accumulation of more scans per increment, would be a distinct advantage. Here we present studies decreasing the overall acquisition time by reducing the number of t_1 increments recorded. In order to obtain a sufficiently well digitised spectrum to enable the desired correlations to observed, aliasing in the indirect dimension is allowed to occur, with the degree of aliasing chosen so as to avoid any overlap in the indirect dimension. As the carbon-carbon correlations are not known *a priori*, a Monte Carlo method based on spectral aliasing optimisation algorithms by Jeannerat [3, 4] and Lescop *et al*. [5] has been developed allowing prediction of how much the spectral window can be narrowed without introducing ambiguity in the F_1 correlations.

We demonstrate this approach using a number of small molecules of differing degrees of structural complexity and also make comparisons with other acquisition-acceleration methods such as non-uniform sampling of the indirect dimension.

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42 Isolation and Structure Elucidation of SEG2011-1348, an Undesired Product Found in the Synthesis of Palbociclib

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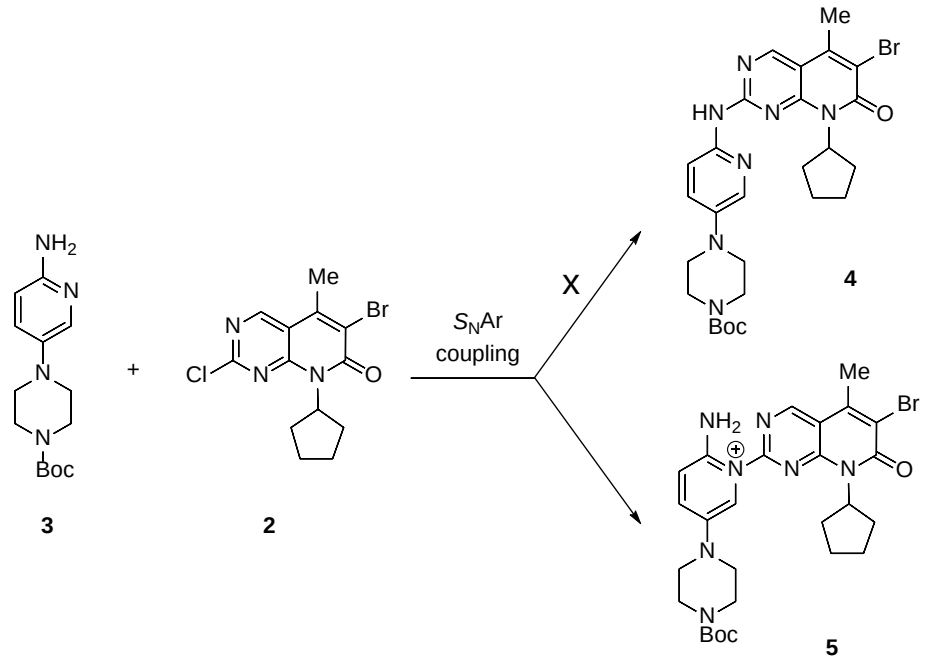
Palbociclib (**1**) is a highly selective, reversible inhibitor of CDK 4/6, intended to block tumor cell proliferation. It is currently approved by FDA for the treatment of postmenopausal women with estrogen receptor-positive, human epidermal growth factor receptor 2-negative (ER+/HER2-) advanced breast cancer. In the commercial manufacturing route for palbociclib, the first step involves an S_NAr coupling reaction between chloropyrimidine **2** and aminopyridine **3**. The biggest challenge for this coupling was the ambident reactivity of **3**, which resulted in an undesired regioisomer **5** as a dominant product in some cases. This poster is describing the isolation and structure elucidation of this undesired pyridinium salt **5** (SEG2011-1348).



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43 Small Molecule Accurate Recognition Technology (SMART): A Digital Frontier to Reshape Natural Products Research

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In most natural products research (NPR), the characterization of novel compounds as well as the dereplication of known compounds entails the collection and analysis of NMR spectra. This involves the running of 1D and 2D NMR spectroscopic experiments for the purpose of partial structure construction, assemblage and relative stereochemistry determination. As exciting advancements in the rapid genetic[1,2] and proteomic approaches[3] have made their way into NPR, conventional NMR practices have become one of several bottlenecks in the characterization and dereplication of new compounds. In regard to this challenge, we leveraged the advantages of Non Uniform Sampling Nuclear Magnetic Resonance (NUS NMR) and Artificial Intelligence (AI) to create Small Molecule Accurate Recognition Technology (SMART) as a tool to speed up marine natural products discovery. Fast NMR techniques like NUS NMR have the potential to further reduce detection limits while maintaining the same sampling time and quality.[4] Next, we applied over 4100 experimental Heteronuclear Single Quantum Correlation (HSQC) spectra for the AI training.[5] The outcome is that the AI algorithm provided us with structurally insightful embedding maps with nodes and clusters representing correlations of related families of natural products. By testing different HSQC spectra using this algorithm, we can greatly accelerate the rate of known compound identification as well as rapidly generating hypotheses about the relationship of new molecules to those used for the training - based entirely on their NMR properties.

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44 Cell Culture Media: NMR Approaches to Evaluating Quality and Nutrient Consumption

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Cell culture media is widely used to provide nutrients for cell growth in research, diagnostic and manufacturing applications. Use in research and manufacturing applications to produce secondary metabolites from microbes is long standing. Cell culture media use is increasing as a result of the growing focus on the development and production of biologics and biosimilars in the pharmaceutical industry. Typical cell culture media contain a mixture of defined nutrients that are expected to produce the desired product. The nutrients are often small molecule components that may be monitored using NMR spectroscopy utilizing a metabolomics approach.

In this poster we present techniques that we are developing to enhance the analysis of cell culture media. The ability for automated NMR analysis for quality control and monitoring post cell addition was evaluated. Solvent suppression and region selective 1D and 2D homonuclear and heteronuclear NMR techniques enhanced the monitoring of nutrients consumed and characterization of metabolites produced in the media. Drift compensation features of modern NMR systems provided the ability to obtain spectra of the pure media without requiring addition of a deuterium lock solvent, allowing for real time NMR monitoring of the media. These studies evaluate the ability to monitor fermentations in either (1) batch mode, sampling aliquots at time points and submitting them for analysis, or (2) continuous monitoring in flow mode (reaction monitor). NMR monitoring may allow optimization of real time conditions of the fermentation for maximum production of a desired product.

45 Pure Shift NMR with Water Suppression using CHEMPACK on Oligosaccharides

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The PureShift 1D (PS1D)[1,2] and Psyche[3] NMR experiments are useful to simplify complicated NMR spectra dominated by overlapping multiplets. Both produce a reconstructed homo-decoupled NMR spectrum of singlets at the true chemical shift of each multiplet. Here we use CHEMPACK modules to explore pure shift experiments with solvent suppression to simplify complex and overlapped ^{1}H NMR spectra of oligosaccharides. Solvent suppression can be a challenge for these types of experiments. We explore common modes of suppression (e.g. presaturation, PURGE, Wet, excitation sculpting) to find optimal methods for water suppression with homo-decoupling. We describe various artifacts encountered and explore their root causes. We also consider possible solutions and/or work-arounds to improve the data quality.

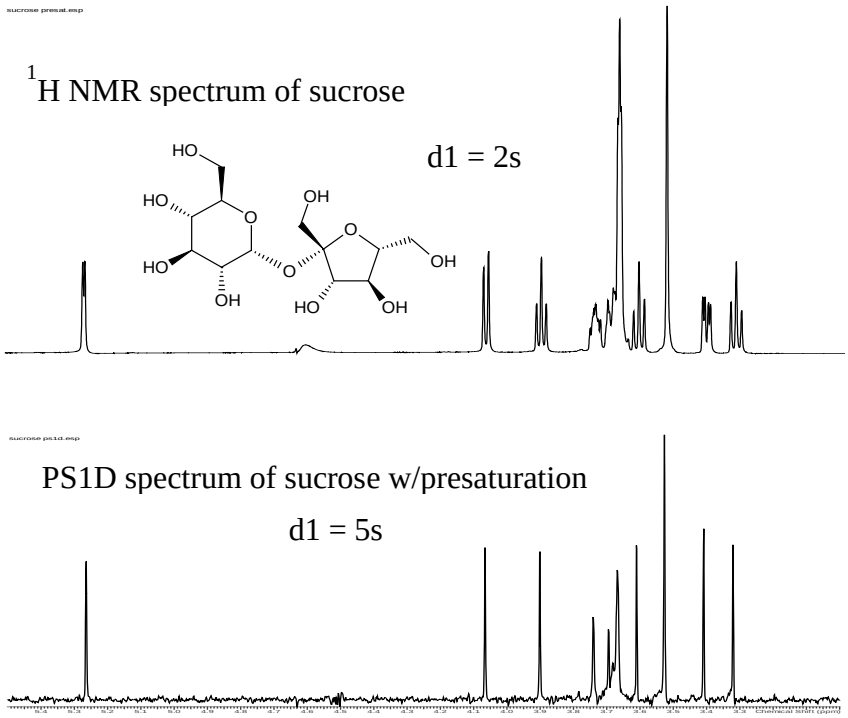

Figure 1. A comparison of 1D 1H and PS1D spectra of sucrose using presaturation to suppress the water signal.

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46 Detection of polymorphic purity by means of ^{13}C and ^{19}F ssNMR

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Polymorphs are chemically identical substances that differ in their structure, e. g. in their crystal forms. Often, these forms have significantly different chemical, physical, and also spectroscopic properties.

Solid-state NMR spectroscopy is one of the few techniques that allow unequivocal identification of polymorphs and also the detection of polymorphic (crystalline) purity.

It is advantageously applied both to the pure API and 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 excipient and in case of low API concentration from the low intensity of the API lines. ^{19}F CP/MAS and MAS techniques may favorably be used in these cases. Many active compounds contain ^{19}F nuclei, but usually ^{19}F is present in the API, only, avoiding overlap of API and excipient lines. Its high natural abundance and high Larmor frequency lead to strong signals even for low concentration samples.

The ^{19}F CP/MAS spectra are a very sensitive source of information about polymorphs and provide clear and unequivocal results. This is advantageously applied to the final solid dosage form. The ease of use of ^{19}F CP/MAS and MAS spectra to determine the ratio of crystalline to amorphous material is also shown in our study.

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

47 Optimal Flip Angle for Robust Quantitative NMR Measurement using a Fixed Pulse Width

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NMR quantification has been traditionally performed by using internal standards. Although methods using external reference in NMR quantification have been reported, one major obstacle in using external referencing method is the requirement of pulse width calibration for every sample. The calibration process is time-consuming and in some cases, impossible. We developed a quantitative NMR method using a fixed pulse width without sample-by-sample calibration based on the use of an optimal flip angle calibrated for an external standard so that the quantitative errors associated with the pulse width variations are minimized. The method can be implemented on most basic NMR spectrometers, and is robust and easily automated, making it applicable to a wide range of solution NMR samples in chemistry, biology and biomedical researches. The method may also find potential applications in quantitative solid-state NMR spectroscopy, MR Imaging and in vivo MR Spectroscopy.

48 Light-Assisted NMR for Photochemical Applications

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Nuclear Magnetic Resonance (NMR) has long been a major technique for both characterization of compounds and mechanistic studies. While ex-situ applications may provide considerable information on reaction mechanisms, they are often associated with loss of resolution in reaction time. Consequently, transcient intermediates might not be seen. In-situ investigations, on the other hand, have the advantage that timing of acquired NMR spectra can be tailored to the reaction conditions. Here, we present methods that combine NMR with various light sources and offer access to a wide range of light-enabled in-situ experiments.

The setup is based on light guided directly into 5mm NMR tubes placed in the magnet and probe using a fiber optic cable. The probe is tuned and the sample shimmed normally. All standard liquids NMR experiments can be performed in the presence of the fiber optic cable, with the light turned on or off. This in-situ approach eliminates dead-time issues and enables direct monitoring of reactions in real time. In our laboratories, high-power argon-ion and eximer lasers are utilized in this manner. Using appropriate photo-sensitizers, hyperpolarization can be achieved and used in, e.g., Photo-CIDNP (Chemically Induced Dynamic Polarization) for the investigation of, e.g., reaction mechanisms [1,2]. In a different application, large enhancements in signal — about 200 times for ^{13}C-^{1}H HSQC — can be produced in protein samples facilitating real-time investigation of protein and peptide folding [3].

Recently, the use of other light sources such as LED's or lamps simulating sunlight has been reported [4,5]. Here, we describe a simple, cost-effective setup using ultra high power (UHP)-LED systems to support not only photochemical research but also photochromic investigations. LED systems may be preferred over lasers when low power and continuous irradiation are needed. Our setup is portable and can easily be moved between spectrometers. Fiber patch cords are attached to the LED via a coupler adapter capable of delivering variable fiber optic output power of up to 500 mW at the tip of the fiber. Dedicated pulse sequences allow for precise LED control using spectrometer TTL ports.

This experimental setup has been applied by the Yoon group to study several types of photochemical reactions. For example, mechanistic studies of an iridium catalyzed [2+2] cycloaddition [6] were enabled by NMR-based rapid kinetic monitoring.

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49 ^{1}H NMR Analysis of Urinary Metabolome in Ischemia-Repurfusion Acute Kidney Injury

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Acute kidney injury (AKI) is a common and independent risk factor for chronic kidney disease (CKD). With cardiac surgeries becoming increasingly common, more people are at an increased risk for ischemia-reperfusion acute kidney injury (IR-AKI) as renal blood supply is temporarily cut off during surgery. Currently, there is no effective treatment of AKI due, in part, to the lack of reliable biomarkers of this disease. Urinary metabolite levels can potentially serve as disease biomarkers as they reflect metabolic status of the kidney.

In this study, IR-AKI was induced in 10 to 12 week-old male BALB/c mice by left renal pedicle clamping for 31 minutes followed by contralateral nephrectomy after 8 days. Urine samples were collected before the induction of IR-AKI (day 0) and on days 9, 14, 21 and 28 after the initial IR-AKI induction (1).

Proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy in combination with statistical methods specifically developed for NMR metabolomics (2) were employed to analyze concentrations and identities of small molecule metabolites in mouse urine. Concentrations of 9 urinary metabolites were found to be significantly different between the control and the IR-AKI groups, i.e. trigonelline, allantoin, taurine, trimethylamine, citrate, acetoacetate, acetate, urea, and 2-oxoglutarate. These metabolites have potential as biomarkers of AKI initiation and/or progression to CKD.

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50 New Approaches to in situ Analysis of Reactions by NMR

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The work presented will centre around our development of in situ NMR analysis techniques for reactions of interest to the synthetic chemist; these will be exemplified in the context of our recent investigations of reactions employing reagents based on boron and silicon.[1-3] The overall project that these developments have contributed to, aims to gain a better grasp of the fundamental physical and chemical processes that facilitate and govern these reactions, thereby allowing "informed control" of the processes, either directly, or indirectly via modulation of undesired side reactions. Mechanistic interrogation via analysis of reaction kinetics and other physical organic parameters is the key to this insight, and gaining sufficient quantity and quality of data is pivotal. The bulk of our data is obtained by UV, IR, MS, and most especially NMR, in concert with strategic isotopic labelling and computational analysis.

We will focus on the problems we faced when reactions became too fast, or too sensitive to be reproducible. This resulted in the conventional techniques for in situ analysis of reactions by IR and NMR either being of limited use in terms of reaction life-time, precision, or highly inefficient in terms of the physical handling aspects of accurate reaction assembly.

We thus began to explore new approaches and devices to give us traction in the analysis of these systems. These developments have allowed the design, assembly and interfacing of new devices with IR and NMR instruments. The presentation will reveal details of these new devices. We will show how they allow the exploration of synthetic organic reaction kinetics in situ, not just quickly and efficiently, but also in a way that the devices can be rapidly coupled / decoupled to standard IR and NMR instruments, thus requiring instrument access only when the device is being used.

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51 Application of a Computer-Assisted Structure Elucidation Program for the Structural Determination of a Difficult Natural Product

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Adansonia digitata commonly known as "Baobab" is found mainly in Africa.[1] The tree occurs naturally in dry areas, mainly in the Sahelian, Soudano-Sahelian, and Soudanian zones. The baobab is a multi-purpose tree with products having numerous food uses and medical properties. The fruits, seeds and leaves are all utilized and consumed daily by rural populations in Africa.[2,3] Baobab fruit pulp is been approved by statutory bodies for use in certain nutritional products. In 2008 the European Commission did recognize the dried fruit pulp of baobab as a novel food.[4]

A new terpenoid aldehyde, with an unusual fused 5/6-ring skeleton has been isolated. The complete proton and carbon assignments of this compound were determined by a series of 1D and 2D NMR experiments including ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H ROESY experiments. The structural elucidation proved more difficult than expected. After arriving at various combinations of fused 5/6-membered ring systems, all of which gave structures that were more or less consistent with the HMBC data and required a great deal of analysis time, it was decided to enter a set of experimental NMR data (^{1}H, ^{13}C, HSQC, and HMBC) into a Computer Assisted Structural Elucidation (CASE) program. Specifically, the ACD/Structure Elucidator program (v.14.04 Jul 30, 2015 running on a Windows 7 64 bit, RAM 8 GB, Dual Core 2.67 GHz computer platform) from Advanced Chemistry Development Inc.[4-6] The program generated eight candidate structures with the top ranked candidate being the final structure. Also, electronic structure calculations proved useful in supporting the experimentally determined conformation. This greatly simplified the analysis and gave confidence in the final structure.

Details of the structural elucidation will be presented with an overview of the particular information provided by the CASE program and electronic structure calculations and how they were used in the final determination.

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52 Solid Alkane Reverse Micelles (SARMs)- Confining Water in the Solid State

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Water is virtually insoluble in pure alkanes (~ 500 uM) yet, as was demonstrated several decades ago, the addition of small quantities of certain surfactants creates a ternary system where a significant amount of water can be taken up by the solution. These clear, macroscopically homogeneous solutions were shown to contain reverse micelles (RMs) - nanometer-sized pools of confined water, each surrounded by a monolayer of amphiphilic surfactant molecules immersed in a large volume of non-polar alkane. These RMs, made from an alkane that is liquid at room temperature, *i*-octane (C_8H_{18}), have been shown to possess unusual and interesting physical properties. Dynamical measurements intended to reveal details of internal structures, however, can be confounded by the overall micellar motions in solution.

Solid state RMs have been prepared previously by others and in our laboratory from liquid styrene that was then polymerized - purportedly with the micellar structures intact - into solid polystyrene. The solution dynamics are supposedly quenched yet fundamental questions arise as to the chemical environment in the aqueous pool during and after polymerization. The work presented here adopts a new, uniquely different yet related course - by utilizing various alkanes with greater molecular weight, for example icosane ($C_{20}H_{42}$), and with melting point higher than room temperature. Solid Alkane Reverse Micelles (SARMs) can be created with a chemically similar environment to liquid RMs by comparison to *i*-octane RMs and without the confounding solution dynamics presented by the polymerized RMs. Preliminary data on physical measurements and various spectroscopies will be presented.

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53 NMR-Based Cyanobacterial Natural Product Structure Elucidation Facilitated by MS/MS Molecular Networking

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Although the structure elucidation of natural products remains a complex and challenging research problem, advanced techniques such as LR-HSQMBC experiments, modern data analysis methods including computer-assisted structure elucidation, and other innovative approaches are changing the way that we go about determining unknowns.[1] Similarly, the dereplication of natural products in mixtures using NMR, LC-MS, and other methodologies has had a profound impact on the efficiency and pace of new natural product isolation and discovery.[2] In the present work, it is shown that the integration of LC-MS/MS based dereplication efforts using Global Natural Products Social molecular networking (GNPS [3]) can further facilitate natural product structure elucidation by generating testable hypotheses about the relationships between new unknowns and previously described molecules. In this study, cyanobacterial extracts from environmental collections were evaluated by LC-MS/MS and ^{1}H NMR for dereplication and sample prioritization. One sample from American Samoa was selected for further research due to the suggested presence of analogues of the cytotoxic natural product, dolastatin 10, which has been of pharmaceutical importance as a lead molecule that ultimately was developed into the antibody-drug conjugate berntuximab vedotin. By analyzing both MS/MS networking and NMR data, it was possible to rapidly identify a known compound without previous annotation in the searched library as being symplostatin 3 (= dolastatin 16), as well as facilitating the structure elucidation of other previously undescribed analogues to be presented.

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54 High-Performance quantitative NMR (HP-qNMR) – Expanding the Scope

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In recent years quantitative NMR (qNMR) spectroscopy has become one of the most important tools for the content determination of organic substances and the quantitative evaluation of impurities. Since the signal intensity is directly proportional to the number of protons contributing to the resonance, qNMR is considered as a relative primary method [1-3]. Quantitative NMR in combination with metrological weighing was optimized to show the power of the measurement method. It is possible to certify the purity of organic reference materials (expressed as mass fraction) with relative expanded measurement uncertainties down to 0.1 % for a 95 % confidence interval (k = 2) [4]. Following well-defined selection criteria, a set of 15 different chemical compounds was evaluated and certified to serve as internal references for ^{1}H-qNMR measurements.

An important requirement for the accurate accomplishment of qNMR spectroscopy is the presence of one well separated signal from each, analyte and reference, in the spectrum. The implementation of qNMR towards new application fields (e.g. metabolomics, biomarker discovery, studies on physiological pathways) brings along more complex molecules and systems, thus making the usage of ^{1}H-qNMR challenging. A smart workaround is given by the use of other NMR active nuclei, namely ^{31}P and ^{19}F. In 2014 Weber et al. described a new certification concept for ^{31}P qNMR certified reference materials (CRM) [5]. It has been successfully shown how traceability to the SI can be established by qNMR using different nuclei. The development of CRM for ^{19}F qNMR is ongoing work in R&D at Millipore Sigma. Detection sensitivity comparable with that of protons and a large chemical shift range (300ppm) underline the suitability of ^{19}F for qNMR [6].

We present the development of three classes of qNMR CRM, based on different NMR active nuclei, and the corresponding approaches to establish traceability to primary Standard Reference Material from the National Institute of Standards and Technology (NIST SRM) or to primary Certified Reference Material from the National Metrology Institute of Japan (NMIJ).

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55 Highly resolved pure shift NMR experiments for the analysis of complex mixtures of compounds

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The analysis of congested spectra of a mixture of structurally similar compounds poses a real challenge. Simplification and improved resolution of 1D and 2D proton spectra is highly desired to facilitate the spectral analysis hampered by severe signal overlap over narrow range of chemical shifts. The presence of the $J_{H,H}$ scalar coupling causing signal splitting greatly contributes to the complexity of the spectra. Broadband homonuclear decoupling leads to highly resolved pure shift proton spectra by a reduction of multiplets into singlets [1-3].

We present here a series of homonuclear 2D experiments (2D DIAG [1], CLIP-COSY [4] and TOCSY [5]) with a singlet structure in F1 and multiplets containing the *J*-coupling information in the F2 dimension. Several decoupling elements (nemoZS [1], PSYCHE [2] and BIRD [3]) were applied to achieve the decoupling in the indirect F1 dimension and compared. The greatly increased information content of the spectra, relative to standard ^{1}H 2D experiments such as DQF-COSY, will be demonstrated in the case of a mixture of carbohydrates.

The combination of the homonuclear decoupling and spectral aliasing leads to high-resolution DIAG spectra that can be acquired over a short experimental time (order of a few minutes).

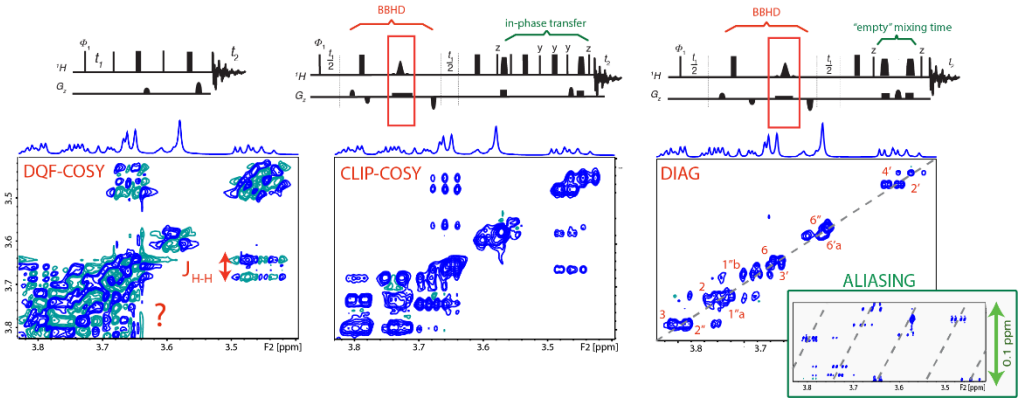

Figure 1. Step-wise simplification of the 2D spectra. Left to right: DQF-COSY, F1-decoupled CLIP-COSY, F1-decoupled DIAG of a sample of raffinose.

The high-resolution F1-decoupled homonuclear correlation experiments have the potential to find a wide range of applications in the field of small molecules and metabolomics.

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56 Precise Conformational Discrimination when ^{1}H-^{1}H NOESY & ^{3}J data are Sparse

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Accurately determined conformations of small organic molecules have a wide range of applications, not least in the pharmaceutical industry where the binding affinities of biological agents are highly dependent on molecular geometry. Although the use of NMR to accurately determine the range of conformations molecules adopt in solution (their 'dynamic 3D-structure') is becoming more routine [1], the success of this approach can be undermined by a sparsity of ^{1}H nuclei, and therefore a lack of measurable conformational restraints.

The fenamates are a group of small molecules (<300 Da) that have biological activity as non-steroidal anti-inflammatory drugs, and have been used to understand crystal nucleation, growth and polymorphism [2]. Although relatively ^{1}H-rich, their structure is such that that no conformation-dependent $^{3}J_{HH}$ and very few distance-defining ^{1}H-^{1}H NOE measurements can be made. Signal dispersion is also poor, deepening the challenge that these molecules present for accurate measurement of their solution conformational behaviour. Two very similar members of the group – mefenamic acid and flufenamic acid – were selected for NMR characterisation.

Despite the inherent difficulties that these molecules present to study, their solution conformational behaviours were accurately determined by the measurement, analysis and combination of NOE build-up rates, $^{3}J_{HC}$ coupling values, RDC data and parameters that indicate the presence of internal hydrogen bonds. These results reveal clearly measurable differences in the dynamic 3D-structures of the two molecules. In particular, we note that the use of any one of these data types alone would have failed to properly detail the conformational behaviour adopted by these molecules in solution and that the orthogonal data types synergistically combine to permit the detail of conformational behaviour to be precisely discriminated even when more routine experiments are inaccessible.

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57 Atrazine: Simple or Complex, or Both?

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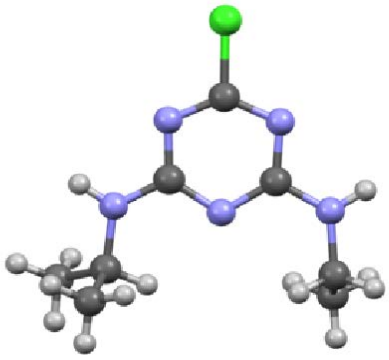
Atrazine, is a triazine herbicide and one of the most widely used agrichemicals in the world today for pre- and post-emergent weeds. In the course of more than fifty years of application during crop production, it has also gained notoriety as an endocrine disruptor especially in frogs, with a persistent environmental longevity, and, although banned in Europe, it is still heavily used in America and Australia.



In order to develop qNMR analytical methods for satisfying a Government regulation, we (re-)discovered the ^{1}H solution NMR of this simple organic molecule to be unexpectedly complex, with at least four different species in both polar and non-polar solvents. Exploration of its solution structure by 2-D EXSY and DOSY as well as by ^{13}C and ^{15}N NMR techniques unveiled some features of this complexity.

Removal of the influences from solvents, rather than simplifying, compounds the situation in the solid state, even after recrystallization. ^{13}C solid state NMR with CPMAS indicated structures which are still being untangled. Correlation spectroscopy with ^{1}H NMR ultra high speed MAS at 60 kHz, and ^{15}N solid state CPMAS NMR greatly enhanced with Dynamic Nuclear Polarisation (DNP), have both contributed key aspects to the investigation. Important insights have also come from single crystal X-ray diffraction which reveals the formation of dimers centred on -N…H-N triads, and their extension into thin ribbon-like structures.

The aim of this presentation is to showcase the insights into the structural complexity of this seemingly simple organic molecule, using solution and solid state NMR spectroscopy together with X-ray diffraction techniques.

58 Reconstruction of Two-Dimensional Spectra from One-Dimensional Projections—Examples from Solid State NMR.

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The use of Projection Reconstruction (PR) to obtain two dimensional (2D) spectra from one-dimensional (1D) data in the solid state is illustrated. The method exploits multiple 1D spectra obtained using magic angle spinning and off-magic angle spinning. The spectra recorded under the influence of scaled heteronuclear scalar and dipolar couplings in the presence of homonuclear dipolar decoupling sequences have been used to reconstruct J/D Resolved 2D-NMR spectra. The use of just two 1D spectra is observed sufficient to reconstruct a J-resolved 2D-spectrum while a Separated Local Field (SLF) 2D-NMR spectrum could be obtained from three 1D spectra. The experimental techniques for recording the 1D spectra and procedure of reconstruction are discussed and the reconstructed results are compared with 2D experiments recorded in traditional methods. The application of the technique has been made to small molecules in solid polycrystalline samples and partially oriented in magnetic field. Implementation of PR-NMR in solid state provides high-resolution spectra as well as leads to significant reduction in experimental time. The experiments are relatively simple and are devoid of several technical complications involved in performing the 2D experiments.

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59 NMR-Based Fragment Screen for Inhibitors of the Clostridium Difficile Binary Toxin, CDT

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Clostridium difficile infection (CDI) is one of the most common nosocomial infections in industrialized nations, often prevalent in hospital patients undergoing antibiotic therapy due to the disruption of normal colonic flora caused by antibacterial agents. C. difficile-associated disease (CDAD) includes antibiotic-associated diarrhea, colitis and pseudomembranous colitis (PMC) and in some cases, may progress to toxic megacolon, sepsis and death. Over the last decade, rates and severity of hospital infections in North America and Europe have increased dramatically and correlate with the emergence of a hypervirulent strain (NAP1) characterized by the production of a binary toxin, C. difficile transferase (CDT) (Gerding et al. 2014). Due to the opportunistic nature of C. difficile pathogenicity, treatment with broad-spectrum antibiotic therapies often fail to eliminate CDAD or lead to recurrent illness; thus, new methods of prophylactic or therapeutic treatment are needed.

Central to the mechanism of CDT virulence is the coordination of individual binary toxin components, CDTb (cell binding and translocation component) and CDTa (catalytic component) to form a functional toxin. From the perspective of CDTa, complex formation is mediated by catalysis-independent domain 1 (D1CDTa) (Gülke et al., 2001), making it an attractive target for pharmacological intervention. Therefore, we have applied protein-observed NMR-based compound fragment screening to the discovery and validation of chemical starting points for inhibition of CDTa complex formation. Mapping of ligand-induced chemical shift perturbations (CSP) of [1H-15N]-TROSY spectra revealed two sites of D1CDTa with high propensity for fragment binding and potential binary toxin inhibition.

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60 Investigating the Hydrate Forms and Functional Properties of Magnesium Stearate in Formulations using Solid-State NMR Spectroscopy

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Magnesium stearate (MgSt) is the most commonly-used pharmaceutical excipient in solid oral dosage forms. A fundamental challenge in using MgSt is that it is composed of naturally derived fatty acids salts, which create complexity in both its properties and its characterization. For example, MgSt can have up to five different crystalline forms which are difficult to identify because of the complex mixture of fatty acids. This batch-to-batch variability arises because MgSt is a mixture of stearate, palmitate and other acids in various ratios. We have found that solid-state NMR spectroscopy (SSNMR) is an extremely powerful technique for identifying the different hydrated forms of MgSt. We have used SSNMR to study both MgSt forms synthesized in our lab as well as samples obtained from commercial sources. We have found that many samples are composed of mixtures of different hydration states, which can affect the lubrication properties of the MgSt. Additionally, since we synthesize the MgSt, it is possible to make ^{13}C-labeled MgSt, which is extremely useful to study the MgSt in a formulated product, where it is typically present at only 0.5-2% by weight. Our fundamental goal is to relate the molecular properties of MgSt with functional properties such as dissolution and disproportionation.

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61 Automating the Evaluation of Screening Libraries by ^{1}H & ^{19}F NMR

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Ligand binding screening by NMR is becoming a popular approach in the pharmaceutical and biotechnology industries for the evaluation of the activity of specific compounds in fragment libraries, with the aim to guide drug design. Different pulse sequences have been developed (STD, T1ρ, WaterLOGSY, CPMG) for this screening method, and the evaluation of the results of such experiments, has been automated to remove the bottleneck of time consuming, labor intensive visual analysis.

However, robust hit finding and validation rely on the assumption that screening libraries are what they are supposed to be, i.e., that the identity and concentration of the screening compounds is as reported. A reliable NMR screening campaign, therefore, must have as one of its key elements identity and concentration checks. This check can be, once again, very time consuming, requiring the acquisition of ^{1}H and ^{19}F (the latter at different T2s) NMR data, and the visual inspection of the data to ascertain identity, ^{1}H concentration and ^{19}F T2 relative quantification.

In this poster, we present a novel, fully automated software method, which performs the following tasks:

- verification of the compound identity by ^{1}H NMR
- assignment of the compound ^{1}H atoms to the ^{1}H NMR signals
- measurement of the concentration of the sample by ^{1}H NMR
- automatic comparison of the ^{19}F integrals of spectra acquired at different T2s to ascertain the intensity loss expected of the ligand in CPMG experiments
- visualization, reporting and manipulation and editing of results.
- data storage in local, networked servers and databases

This automated software method combines advanced algorithmia for combining of different types of data (structure, ^{1}H NMR, ^{19}F NMR), data processing (automatic phase and baseline correction, blind regions), analysis (different integration approaches, concentration reference, methods for selection of suitable multiplets, automatic signal to atom assignment, structure verification, etc.), reporting and information storage for further study.

The result is a fast and reliable analysis of the screening library which allows the user to undertake NMR assays with confidence.

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62 StereoFitter. Mixing all NMR observables for Conformational deconvolution

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Conformational analysis of small molecules by NMR is a difficult subject since in most occasions NMR properties are observed as time-averaged quantities over the molecular trajectories. In a fair approximation these observables can be interpreted on the basis of a set of discrete conformations with different conformational weights. These weights should be determined from fitting to the available NMR observables such as for instance NOE determined distances or J-couplings.[1]

Here we present StereoFitter a new program for conformational deconvolution based on NMR observables. The program manages very different kind of properties such as q-NOE distances, J-couplings, chemical shifts as well as Residual Dipoalr Couplings. Additionally, to avoid overfitting, the program can select the simplest model that explains the experimental data, within a given level of uncertainty by using the Akaiko Information Criterion [2].

The program was succesfully applied to the conformational analysis of difficult targets as cinchonidine alkaloids and others.

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63 RDC/RCSA-Enhanced Conformational Analysis of Monocrotalin in Water and Organic Solvents

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The conformational space of monocrotalin has been investigated by means of RDCs and RCSAs[1] obtained in two different aligning media namely D_2O/cromoglycate [1] and PMMA/$CDCl_3$.[2] In combination with chemical shift ab initio predictions the data show a strong preference for a closed conformation with parallel carbonyl groups resembling the solid state X-ray structure. The possibility for the measurent of CSAs in the lyotropic cromoglycate phase has been also investigated and results contrasted with those obtained a recently published protocol. [3]

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64 SMP1, a Cystein-rich Peptide from Marine Sponge *Spirastrella mollis*

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SMP1 (Spirastrella mollis peptide 1) is a rare example of large cysteine-rich peptides isolated from marine sponges. 1.3 mg of SMP1 (MW=4896 Da, 42 residues, 8 cysteines) was obtained from *Spirastrella mollis* collected in the Bahamas. The structure elucidation of SMP1 by NMR techniques, including its sequence, disulfide bond connections, secondary and tertiary structure, is discussed. It is one of the largest peptide which structure is determined by NMR at the natural isotope abundance level without isotope labelling.

65 Complexing Behavior of Diacetylcurcumin and Perezone with Zn, Gd and Sm Seen by Liquid and Solid NMR.

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Curcumin and Perezone are two secondary metabolites with historical roots in their usage for culinary or therapeutic purposes. The first one has gained an oustanding actual relevance for its implications as generic antiinflammatory agent[1] and receives at present broad attention in biochemical and clinical studies while the latter,[2] is a naturally ocurring quinone discovered en 1852 although its chemical structure could be confirmed until 1965.

Curcumin is a phenolic heptanoid characterized by a beta-diketone chemical functionality and has attracted wide interest due to its complexing properties.[3] Perezone is the first secondary metabolite isolated in America where the quinone functionality contains a hydroxyl group which imparts also complexing properties of a different nature respect to those exhibited by the diacetylated derivative of curcumin.

In continuation of our chemical and pharmacological studies of both metabolites we have prepared their metal derivatives and their structures were submitted to high resolution NMR study as well as CPMAS determinations. Both types of chemical structures showed interactions that can be followed by spectral changes caused by the metal linkage at the site of complexation. The metals used in the present study were Zn, Sm and Gd and the spectral changes are discussed.

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66 Investigating Pharmaceutical Formulations Using Dynamic Nuclear Polarization CPMAS NMR

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Dynamic Nuclear Polarization (DNP) is an emerging technique for being able to investigate pharmaceutical systems. There are several advantages of the technique, including the ability to enhance signal intensity by two orders of magnitude or more, the capability of performing two-dimensional experiments, and the capability of detecting minor species present in samples. There are also several disadvantages, including the fact that the samples have to be cooled to near liquid nitrogen temperature, making them susceptible to potential polymorphic and other changes, non-uniform signal enhancement that can affect quantitation, and the need to incorporate a free radical into the sample. The relative merits of DNP will be discussed for several samples, including several APIs, excipients, and crystalline and amorphous formulations.

Eric Munson 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 his academic work at the University of Kentucky, and no data from Kansas Analytical Services are presented here. Funding was provided by the Center for Pharmaceutical Development, an NSF Industry-University Cooperative Research Center, and Iowa State University.

67 Homodecoupled *J*-resolved HSQC: Direct Determination of Accurate ^{1}H-^{13}C RDCs in a Single PMMA Compressed Gel Sample

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A robust *J*-resolved HSQC (HD-*J*-HSQC) experiment affording highly resolved $^{1}J_{CH}/^{1}T_{CH}$ splittings along the indirect dimension (F1) and homodecoupled ^{1}H signals in the detected dimension (F2) is proposed. The experiment enables the *in-situ* distinction of both isotropic and anisotropic components of molecules dissolved in compressible PMMA gels, allowing a rapid and reliable determination of accurate $^{1}D_{CH}$ for CH systems and sum $^{1}D_{CHa}+^{1}D_{CHb}$ for diastereotopic CH_2 protons from a single measurement. It will be shown that the combination of several resolution enhancement techniques introduces substantial improvements in spectral quality, sensitivity and both signal and digital resolution. This is a good example showing that RDCs of variable size and sign can be accurately determined from a single NMR spectrum, minimizing typical errors of measuring them from two different sample conditions. Experimental data will be used to evaluate the structural diastereoisomeric discrimination in two natural products: strychnine and ludartin.

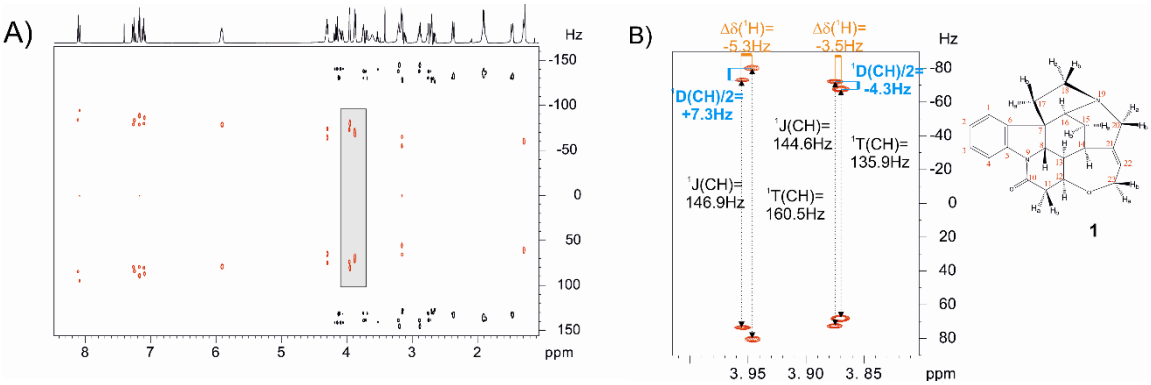

Figure 1. A) HD-*J*-HSQC spectrum of **1** in PMMA/$CDCl_3$ (33.4 Hz ^{2}H compression). B) Expanded region corresponding to the H-16 and H-8 protons showing how $^{1}J_{CH}$, $^{1}T_{CH}$ and $^{1}D_{CH}$ can be measured simultaneously with simplicity and high accuracy.

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68 Low Field NMR for Monitoring Reactions in Pharmaceutical Discovery Chemistry

Paul Tan, James Olerenshaw, and **Gary J Sharman**

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We present the results of an evaluation of a low field, benchtop NMR system for reaction monitoring. The work focusses on following or optimizing small scale chemical reactions within the context of discovery chemistry, which is perhaps not an area where most efforts on reaction monitoring are normally concentrated. We were particularly interested in testing whether 'real' reactions, carried out under unmodified conditions and concentrations, would be accessible to low field NMR analysis, or whether its inherent compromises of resolution and sensitivity would preclude its use. As such, a number of 'typical' reactions were chosen: all were studied under the conditions and concentrations normally used for the reaction. That is, they were not modified or optimized to make them more amenable to NMR investigation. The examples discussed include an imine formation, a room temperature heterogeneous Suzuki reaction, an alkylation reaction and two flow hydrogenation reactions, one where selectivity was required. The examples cover scenarios where kinetic information is obtained on flask type reactions done at a small scale (eg. 10 ml), and flow chemistry applications where an end point is assessed allowing rapid iteration of conditions. The work illustrates that although the quality of data compares poorly with that from a modern high field spectrometer, it can nonetheless be good enough to allow conclusions to be reached and decisions made based on the information.

69 *In-situ* Determination of $^1D_{CH}$ and $^2D_{HH}$ RDCs from a Single $^1J_{CH}$ / $^2J_{HH}$-Resolved NMR Measurement

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Residual dipolar coupling constants (RDCs) have becoming an alternative tool to classical isotropic NMR paramaters such as scalar coupling constants (*J*) or through-space NOE enhancements for determining the relative configuration and the 3D stereoview of small molecules in solution. RDCs provide structural information of non-local character, permitting the determination of the relative orientation of stereocenters regardless of the distance between them. This powerful advantage over conventional NMR parameters makes RDCs the tool of choice to lift structural ambiguities generated by using only scalar couplings and NOEs. We report here a fast RDC-assisted strategy involving the simultaneous determination of isotropic (scalar) and anisotropic (total) interactions in compressed PMMA gel samples. The concerted use of individual $^1D_{CH}$ for all CH_n multiplicities and $^2D_{HH}$ obtained directly from a single zero-quantum filtered $^1J_{CH}/^2J_{HH}$-resolved NMR spectrum offers an efficient discrimination between all eight possible diastereoisomeric structures of strychnine which contains six stereochemical centers. In addition, a new strategy to straightforwardly assign CH_2 diastereotopic protons that happens "on the fly" as the alignment tensor is calculated during the SVD fitting and the correct configuration is determined using the $^1D_{CH}$ from CH groups and the $^2D_{HH}$ from CH_2 groups was developed.

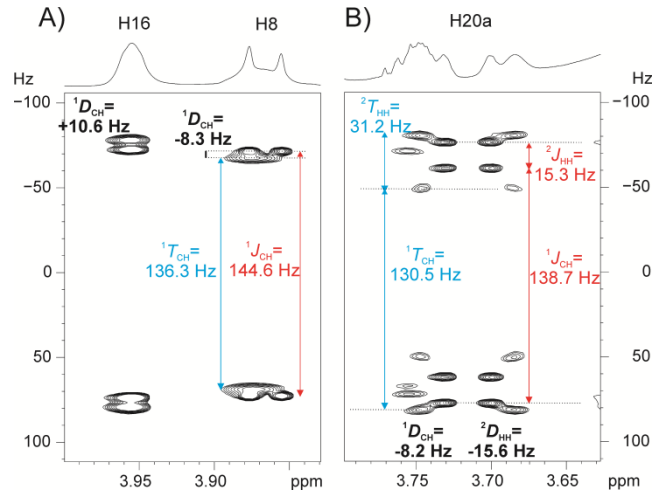

Figure 1. Coupling patterns for individual signals in the J_{CH}/J_{HH}-resolved spectrum of strychnine in a PMMA-$CDCl_3$ gel: (A) the C16-H16 and C8-H8 methine protons and B) the diastereotopic H20a proton belonging to the methylene C20 carbon. Signals corresponding to the anisotropic component can be distinguished from their broader line widths.

Acknowledgments: Financial support for this research provided by MINECO (project CTQ2015-64436-P) is gratefully acknowledged.

70 Pure Shift NMR Covariance

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The development of novel experimental strategies to significantly enhance signal resolution by broadband homodecoupling is a current topic of high interest in ^{1}H NMR spectroscopy.[1] The original Zangger-Sterk[2] experiment has been modified and improved in several ways. All of these novel building blocks have been implemented in a number of 1D and 2D homo- and heteronuclear pulse schemes to provide resolution-enhanced "pure" chemical shift ^{1}H NMR spectra, where signals appear collapsed to singlets. In contrast, covariance processing methods have been used to generate challenging NMR spectral representations. [3] We present here the first attempts towards a general solution to generate *Pure Shift NMR spectra by using Generalized Indirect Covariance* (psGIC).[4,5] The current strategy is based on the calculation of a new 2D psGIC spectrum from the combination of a parent homo- or heteronuclear spectrum and a reference 2D F1-homodecoupled ^{1}H-^{1}H correlation spectrum only showing diagonal cross-peaks (DIAG), which share a common ^{1}H frequency dimension. Using psGIC, the F1 dimension in the DIAG spectrum can be transferred to the F2 dimension of the parent spectrum, thus generating a new pure shift 2D spectrum, which includes collapse of geminal couplings that are not normally collapsed in current implementations of the psHSQC experiment. Examples are provided for a set of 2D NMR spectra of the alkaloid strychnine.

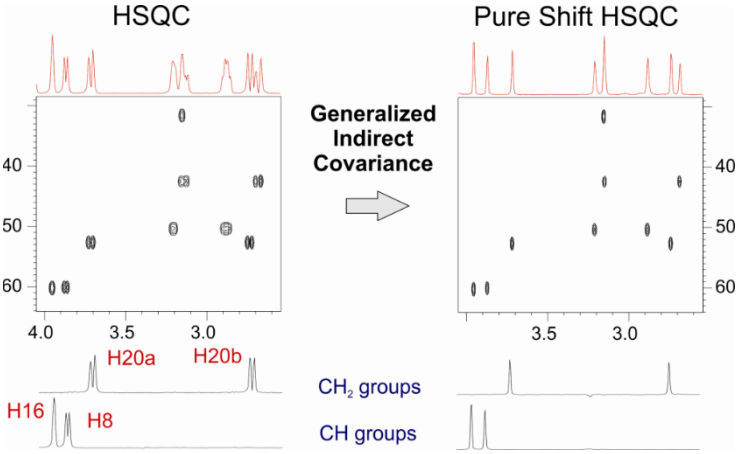

Figure 1. Generation of Pure Shift NMR spectra by using Generalized Indirect Covariance (psGIC).

Acknowledgments: Financial support for this research provided by MINECO (project CTQ2015-64436-P) is gratefully acknowledged. A.F. thanks CNPq- Brazil for a scholarship. We also thank the Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, for allocating instrument time to this project.

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71 Scalar Cross-Relaxation Observed in NOESY in NOESY Spectra of Thia- and Oxazolidines

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Apparently anomalous NOESY cross-peaks that cannot be explained by dipolar cross-relaxation or chemical exchange are described for disubstituted thiazolidines and oxazolidines.

Conventional analysis of the small molecule NOESY data would have assigned the cross-peaks as being indicative of a chemical exchange process occurring between correlated protons due to the cross-peak signs matching those of the diagonal-peaks. However, the pairs of nuclei displaying them cannot undergo exchange and the origin of these is in fact identified as *scalar cross-relaxation of the first kind (SCRFK)* [1].

This process arises from the slow, stochastic modulation of *J*-coupling between correlated protons and for the substituted pyrrolidine analogues described here, the origin of effect is ascribed to the on-off exchange of the amino proton that exhibits the scalar cross-relaxation cross-peaks. This process is shown to be highly sensitive to solution conditions (including acidity, temperature, and solute concentration) but which may occur at a rate suitable for SCRFK to be effective [2].

The scalar cross-relaxation process is likely to be effective in any system possessing slow (millisecond) modulation of proton-proton couplings and may well have been observed by others, possibly without realizing the true origin of the associated cross-peaks. Similar effects may also be anticipated in the related 1D NOESY experiments, where again the sign of the “NOE peaks” would suggest chemical exchange is occurring, despite scalar cross-relaxation being the true physical process in operation.

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72 Using ^{1}H T_1 Relaxation Times for Measuring Particle Size, Purity, and Stability of Crystalline Organic Compounds

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Relaxation times in the solid state are usually used to measure local mobility in a sample. However, we have found that ^{1}H T_1 relaxation times are sensitive to long-range effects for crystalline organic compounds having a substantially-long relaxation time (tens to thousands of seconds). For particle size, we have found that the rate of spin diffusion to the particle surface matches particle sizes up to ~100 nm, at which point the rate no longer obeys the relationship of particle size being proportional to time squared. Particle sizes from submicron to several hundred microns have been correlated back to relaxation times. NMR relaxation times correlate well to particle size, unless there are crystal defects present caused by milling or grinding the sample, which result in a shorter than predicted relaxation time for a given particle size. These crystal defects also are initiation sites for chemical reactions, and we have shown that shorter relaxation times correlate well with the reduced chemical stability of aspirin and gabapentin. Finally, increasing amounts of chemical impurities in a compound, such as other sugars in trehalose or salicylic acid in acetylsalicylic acid, have been correlated with shorter NMR relaxation times.

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73 Determination of Configuration of Small Molecules from Residual Chemical Shift Anisotropy (RCSAs) at Microgram Levels

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Wouldn't it be great to measure the chemical shifts of carbons and determine from these data the configuration of a natural or synthetic compound of which only several tens of micrograms are available? It has long been clear that this could be achieved if one could accurately measure residual chemical shift anisotropy (RCSA) [1,3], *e.g.* from the resonance positions under two alignment states. However, until now, it was difficult to differentiate the RCSA, *i.e.* the shift due to molecular alignment, from the isotropic NMR chemical shift change. The latter originates from a change in environment when switching from alignment state to another. Here we describe a technique to collect pure RCSAs which consists of compressing PMMA gel [4] dissolved in $CDCl_3$ inside a 5 mm NMR tube to obtain different alignments. Although, the sensitive volume is not changed, there is a change in gel-to-solvent ratio causing an isotropic contribution that can be removed post acquisition in a new and robust way. The main achievement obtained is the removal of obstacles for accurate measurement of RCSAs, making them available for routine applications. The simplicity of measuring just a simple ^{13}C 1D NMR experiment should make this method available for every chemist. The sensitivity of the method is very high and with 1.7 mm cryo-probes, this reduces the required amount of substance to 25 μg [5].

To demonstrate the robustness of the approach, RCSAs were measured on five different molecules some rigid, some flexible. We were able to determine the correct configuration in each case. To the best of our knowledge, this has never been achieved before. The most notable example is retrorsine in which flexibility aggravates the determination of the relative configuration. Furthermore, a residual dipolar coupling (RDC) alone were not able to assign the correct configuration since one of the key chiral carbons is quaternary and therefore does not exhibit an RDC. Using RCSAs, however, one can identify the correct configuration since RCSAs are observed regardless of the degree of a carbon's protonation. This attribute clearly highlights the power of RCSAs for molecules that contain chiral non-protonated carbons. We will show further progress of the method that has the potential to further reduce the required amount at least by a factor of 10.

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74 Long Range Residual Dipolar Couplings: A Tool for Determining the Configuration of Small Molecules

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Together with the NOE and the *J*-coupling, the one-bond Residual Dipolar Coupling (RDC) that reports on the three dimensional orientation of an internuclear vector in the moleculear frame plays an important role in the conformation and configuration analysis of small molecules in solution by NMR.[1-4] When the molecule has few C-H bonds or too many bonds are in parallel, the available RDCs may not suffice for obtaining the alignment tensor used for structure elucidation. Long range RDCs that connect nuclei over multiple bonds are normally not parallel to the single bonds and therefore complement the one-bond RDCs.[1,3] Herein we present a method for extracting the long range RDC of a chosen proton or group of protons to all remotely connected carbons, including non-protonated carbons, to complement the other NMR parameters.[2] Using alignment tensors fitted directly to the total long range couplings (T=J+D), an easy and straightforward analysis of both the long range and one-bond RDCs has been performed on the natural product strychnine using PMMA gel [4] as an alignment medium. The methodology for determining the alignment tensor by directly optimizing against absolute ${}^{n}T_{CH}$ values is a general one which has applications to all LR RDC experiment types. It has been demonstrated that LR RDC alone can discriminate between different configurations of strychnine in the absence of one-bond RDCs. Yet, utilization of different combinations of RDCs is beneficial, as the number of experimental parameters increases while the number of parameters to be fitted stays constant at five.

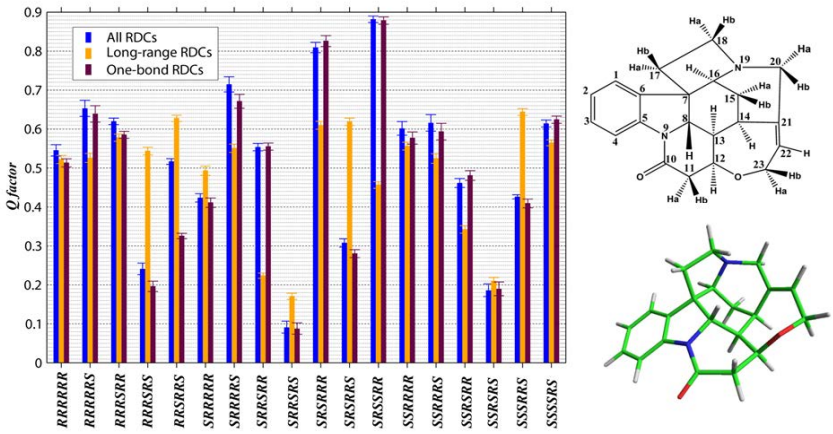

Figure 1. Comparison of quality factors (Q) obtained from the analysis of experimental RDC data to each viable diastereoisomers of strychnine. The lower the value of Q, the better the fit. Case 1 with only one-bond coupling (red bars); Case 2 with only long range couplings (yellow bars); Case 3 with the combination of one-bond and long range couplings (blue bars). Molecular and three dimensitional structure of strychnine were given on left side of figure.

The correct *SRRSRS* has the lowest *Q* factor of 0.17, while the next best configuration *SSRSRS* has a *Q* factor of 0.223 that is sufficiently higher, allowing the correct diastereomer to be isolated from long range couplings alone (Figure 1). The LR RDCs provide critical information for the structural analysis of the flexible retrorsine molecule, in which one-bond RDCs are not sufficient to isolate the correct configurations from the wrong configurations as one of the key chiral carbons is quaternary.

As the use of LR RDCs provides information complementary to the one-bond RDCs for determination of conformation and configuration, they can be used in all situations where the one-bond RDC is used, such as flexible molecules, and to new cases where one-bond RDCs are insufficient, such as molecules with an insufficient number of independent CH vectors.

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75 Development and Application of Homodecoupled 1,1- and 1,n-ADEQUATE to Solve Challenging Structure Elucidation Problems

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Routine small and medium-size molecules can generally be characterized by conventional NMR techniques such as 1D NMR and 2D NMR experiments including COSY, NOESY, HSQC and HMBC. However, when facing unknown natural products and/or complex small molecules, i.e. highly proton-deficient compounds, an assignment strategy augmented by NMR techniques to specifically establish carbon-carbon connectivities may be necessary to unambiguously address structural questions. 1,1- and 1,n-ADEQUATE are powerful and robust NMR experiments for this purpose that use modest sample quantities when small diameter cryogenic probe capabilities are available. However, these techniques still suffer from low sensitivity, especially in the case of 1,n-ADEQUATE.

Pure shift NMR methods have recently been the subject of intense research focus [1]. By collapsing homonuclear proton-proton couplings, resolution and experimental sensitivity both increase. We now wish to report the development of improved homodecoupled variants of 1,1- and 1,n-ADEQUATE (HD-ADEQUATE) and the utilization of these techniques in resolving complex structure elucidation problems [2,3]. Several examples will be shown.

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