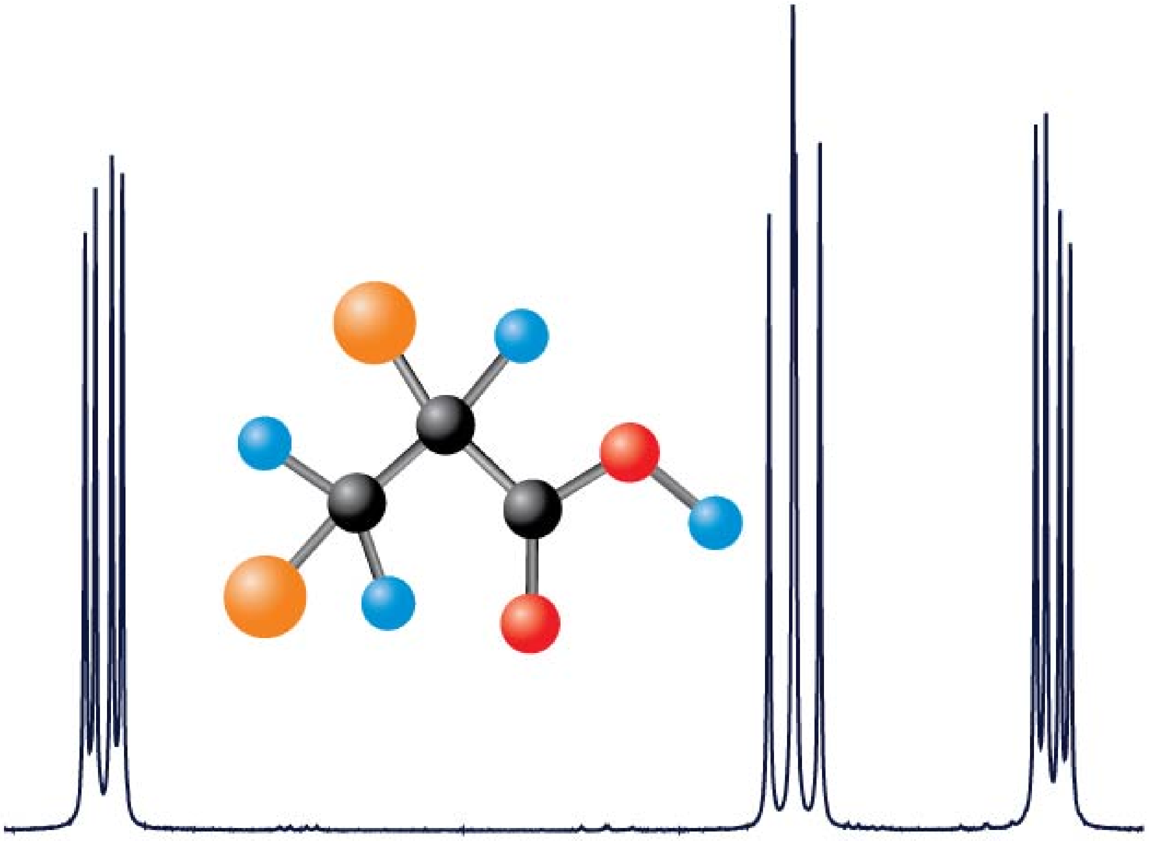


SMASH 2018

Conference Program

September 16th-19th 2018
Philadelphia, Pennsylvania

SMASH 2018 NMR Conference

Dear SMASH 2018 Attendees,

Welcome to the 2018 Small Molecules Are Still Hot conference in historical Philadelphia, Pennsylvania where the atmosphere that gave the United States its Constitution will provide us a great SMASH conference. We have left you a bit of time on Tuesday to explore this great city. We encourage you to try a cheesesteak topped with whiz, an ice cream topped with jimmies, or water ice at Rita's.

SMASH 2018 continues the tradition of combining first rate research from both the academic and industrial worlds into one conference. As in years past, there are no concurrent scientific presentations. We hope this allows you the flexibility to attend all the sessions and take every opportunity to have the free and open scientific discussions that have become a tradition at SMASH.

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 NMR analysis of chiral molecules, solid-state applications, low field NMR, and a session on natural products. Poster sessions are Monday evening and Tuesday evening, with workshops scheduled on Monday, Tuesday and Wednesday covering CASE, imaging, and chemometrics.

On Monday evening we are delighted to be presenting the James N. Shoolery Award for Career Achievement in Small Molecule NMR Spectroscopy to Dr. Krish Krishnamurthy. Krish's contributions to both our SMASH community and the NMR community in general are too numerous to list, and we hope you will join us in highlighting his 40-year career on Monday evening.

On behalf of the Organizing Committee, we want to extend a warm welcome and thank you for attending SMASH 2018.

Sincerely,
Amy Freund and Greg Walker
Co-Chairs, SMASH 2018 NMR Conference

SMASH 2018 Conference Program

Sunday, September 16th

16:00 - 17:00 Registration – Liberty Foyer
17:00 - 19:00 Mixer – Liberty Ballroom C & D
19:00 - 22:00 Dinner – Liberty Ballroom C & D

Monday, September 17th

08:50 - 09:00 Welcome, Announcements and Opening Remarks

09:00 - 10:30 **NMR Characterization of Natural Products**

Chair: Clark Ridge, *FDA*

New tools of structural elucidation by merging GIAO NMR calculations with statistical and artificial intelligence methods
Ariel Sarotti, *IQUIR (UNR-CONICET)*

The application of isotope labeling and NMR spectroscopy in biosynthetic, structural, and conformational studies
Jeffrey L. C. Wright, *UNC Wilmington*

NMR Supersequences for Small Molecule Analysis and Structure Elucidation
Tim Claridge, *University of Oxford*

Empirical equations for 3JCH prediction: introducing a general bond angle correction
Claire Dickson, *University of Bristol*

10:30 - 11:00 **Break**

11:00 - 12:30 **NMR in all stages of the Pharmaceutical Industry**

Chair: Kathleen Farley, *Pfizer*

High Confidence Protein-Ligand Complex Modeling by NMR-Guided Docking Enables Early Hit Optimization
Andrew Proudfoot, *Novartis Institute for BioMedical Research*

Application of Quantitative 1H-NMR Spectroscopy for the determination of silicone oil and degradation products thereof
Joan Malmstrøm, *Novo Nordisk A/S*

Optimization of ^{19}F Methodologies for Pharmaceutical Structure Elucidation
Dave Russell, *Genentech, Inc.*

Applications of quantitative NMR Spectroscopy in Pharmaceutical Development
Steve Coombes, *Astrazeneca*

12:30 - 14:00 **Lunch**

14:00 - 15:30 **Workshop I: CASE - Computer Aided Structural Elucidation**

Chair: Armando Navarro, *Federal de Pernambuco*

15:30 - 16:00 **Break**

16:00 – 18:00 **Poster session 1 - Evens**

Even numbered posters to be presented

18:00 - 19:30 **SHOOLERY AWARD LECTURE**
Krish Krishnamurthy, *Chempacker LLC*

19:30 – 22:00 **Dinner**

Tuesday, September 18th

09:00 - 10:30 **Industrial Applications of NMR**

Chair: John Edwards, *Process NMR*

NMR of Biomass Pyrolysis Products: Maximizing Derived Information

Gary Strahan, *USDA*

Characterization and Quantitation of Diiodosilane trace impurities by NMR Spectroscopy

Thomas M. Cameron, *Entegris*

Combined in situ NMR-UV/Vis-Illumination Spectroscopy: A Novel Mechanistic Approach in Photochemistry

Philipp Nitschke, *University of Regensburg*

Dynamic Nuclear Polarization Enables Fast Flow NMR Reaction and Process Monitoring

Raphael Kircher, *University of Kaiserslautern*

10:30 - 11:00 **Break**

11:00 - 12:30 **Workshop II: Imaging of Chemical Systems**

Chair: Melanie Britton, *University of Birmingham*

12:30 - 16:30 **Lunch followed by free afternoon**

16:30 - 18:00 **NMR analysis of Chiral Molecules**

Chair: Kirk Gustafson, *NCI*

Leveraging an expanded set of NMR parameters for more reliable structure elucidation

Thomas Williamson, *Merck*

Quantum Mechanical NMR Calculations as an Aid for Structure Elucidation of Organic Molecules

Alfonso Mangoni, *Università degli Studi di Napoli*

Co-Conformational Mobility of Amide-Based [2]Rotaxanes by Dynamic NMR Spectroscopy

Fátima Morales, *Universidad de Murcia*

Investigation of relative and absolute configuration of molecules using RDCs and RCSAs

Christian Griesinger, *Max Plank Institute*

18:00 – 20:00 **Poster session 2 - Odds**

Odd numbered posters to be presented

20:00 – 22:00 **Dinner**

Wednesday, September 19th

09:00 - 10:30 **Benchtop NMR in an Academic Setting**

Chair: Susie Riegel, *Nanalysis*

SIA-NMR: A novel hyphenated technique and it's application to the analysis of amphetamine derivatives

John Frost, *Thermo Fisher Scientific*

Improving Benchtop NMR Spectral Resolution for Simple Peptides by Ion Effects

Rio Febrian, *Saint Louis University*

A Multi-Outcome Experiment Involving the Oxidation of Alcohols in the Undergraduate Teaching Laboratories

Kasey Leigh Yearty, *The University of Georgia*

Studies of Phase Equilibria and Batch Distillation with Benchtop NMR Spectroscopy

Anne Friebel, *University of Kaiserslautern*

10:35 - 11:00 **Break**

11:00 - 12:30 **Small molecule solid-state NMR**

Chair: Heather Frericks Schmidt, *Pfizer*

Molecular Interaction and Miscibility in Pharmaceutical Products from Solid-State NMR

Yongchao Su, *Merck*

Identification and Quantification of Hydrates of a Drug Product using Solid-State NMR

Dewey Barich, *GSK*

NMR Crystallography Advancements for Exploring Polymorphism

Maria Baias, *New York University Abu Dhabi*

Structural studies on Cu(II) and Co(II) complexes and their free-ligands by Solid-State NMR, Solution-State NMR and X-Ray spectroscopy

Ayelen Florencia Crespi, *University of Buenos Aires*

12:30 - 14:00 **Lunch**

14:00 - 15:30 **Workshop III: Chemometrics and NMR Data**

Chair: Beata Mickiewicz, *University of Calgary*

15:30 - 16:00 **Break**

16:00 – 17:10 **Metabolomics: Making Metabolic Sense of Mixtures**

Chair: Aalim Weljie, *University of Pennsylvania*

New Approaches to In vivo Metabolomics

Art Edison, *University of Georgia*

Enriched Quantitative Chemicals to Support Metabolomics Application in Bio/Pharmaceutical R&D

Qiuwei Xu, *Merck*

Expanding the metabolomics toolkit with heteronuclear NMR

Fatema Bhinderwala, *University of Nebraska*

17:10 – 17:30 **Generation of High-quality NMR data using the NMReDATA format and top-resolution 2D NMR spectra of natural products**

Damien Jeannerat, *University of Geneva*

17:30 - 17:45 **Closing Remarks**

Thursday, September 20th

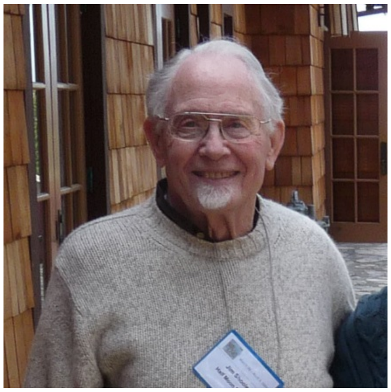
08:30 – 16:00 **Post-SMASH CRAFT Symposium**
Coordinator: Krish Krishnamurthy, *Chempacker LLC*

This is not a formal SMASH program event, but all interested parties are welcome to attend (no registration fee)

http://www.mrr.com/craft-symposium-at-smash-announcement/

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 2018 Scholarship Recipients

The following students received a scholarship to attend SMASH 2018

- **Nele Berg**, University of Regensburg, Germany
- **Fatema Bhinderwala**, University of Nebraska Lincoln, United States
- **Andrew Camp**, University of North Carolina at Chapel Hill, United States
- **Martina Čermáková**, Institute of Microbiology, Czech Academy of Sciences, Czech Republic
- **Ayelen Florencia Crespi**, Universidad de Buenos Aires, Argentina
- **Guilherme Dal Poggetto**, University of Manchester, United Kingdom
- **Emma Danelius**, Uppsala University, Sweden
- **Lydia Dewis**, University of Bristol, United Kingdom
- **Claire Dickson**, University of Bristol, United Kingdom
- **Anna Duell**, Portland State University, United States
- **Rio Febrian**, Saint Louis University, United States
- **Anne Friebel**, University of Kaiserslautern, Germany
- **Thomas Gloge**, Karlsruhe Institute of Technology, Germany
- **Ningyi Lyu**, Grinnell College, United States
- **Yevgen Matviychuk**, University of Canterbury, New Zealand
- **Fátima Morales**, Departamento de Química Orgánica/Universidad de Murcia/Spain, Spain
- **Philipp Nitschke**, University of Regensburg, Germany
- **Linqing Peng**, Grinnell College, United States
- **Dhanishta Poshetti**, Indian Institute of Science, Bangalore, India
- **Kerstin Rothermel**, University of Regensburg, Germany
- **Kanika Sarpal**, University of Kentucky College of Pharmacy, United States
- **Verena Streitferdt**, University of Regensburg, Germany
- **Kasey Leigh Yearty**, University of Georgia, United States
- **Siying Zhong**, University of Bristol, United Kingdom

Thanks to our scholarship sponsors for their generous support.

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* Emeritus

Monday, September 17th
09:00 AM - 10:30 AM

NMR Characterization of Natural Products

Chair: Clark Ridge

Speakers:

Ariel Sarotti
IQUIR (UNR-CONICET)

Jeffrey L. C. Wright
UNC Wilmington

Tim Claridge
University of Oxford

Claire Dickson
University of Bristol

New tools of structural elucidation by merging GIAO NMR calculations with statistical and artificial intelligence methods

Ariel M. Sarotti

Instituto de Química Rosario (IQUIR, CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas,
Universidad Nacional de Rosario, Suipacha 531, Rosario 2000, Argentina

Nuclear magnetic resonance (NMR) is undoubtedly the most important spectroscopic technique for the structural and stereochemical elucidation of natural products. However, despite the impressive advances that have been made in this field, it is striking the large number of structures erroneously assigned based on spectroscopic data [1].

Quantum chemical calculations of NMR shifts and coupling constants emerge as a powerful and simple option to shed light on structural or stereochemical issues of complex natural products. This approach has been extensively employed in recent years to facilitate the determination of the tridimensional structure of a wide variety of organic molecules [2].

Our research team has made important contributions in this field. On the one hand, we have developed an improved probability to determine the most probable structure of complex molecules when one set of experimental data is available (DP4+ method) [3a,b]. Alternatively, we have introduced a novel methodology for structural validation by merging NMR calculations with artificial neural network pattern recognition analysis (ANN-PRA method) [3c,d]. Finally, these computational tools were employed in the structural elucidation of several natural products [4]. In particular, in collaboration with Prof. Ronaldo Pilli (UNICAMP, Brazil), we have explored this alternative in the computer guided total synthesis of natural products [5].

1. Nicolaou, K. C.; Snyder, S. A. *Angew. Chem. Int. Ed.* **2005**, *44*, 1012-1044.
2. a) Grimblat, N.; Sarotti, A. M. *Chem. Eur. J.* **2016**, *22*, 12246-12261. b) Lodewyk, M. W.; Siebert, M. R.; Tantillo, D. J. *Chem. Rev.* **2012**, *112*, 1839-1862.
3. a) Grimblat, N.; Zanardi, M. M.; Sarotti, A. M. *J. Org. Chem.* **2015**, *80*, 12526-12534. b) Zanardi, M. M.; Suárez, A. G.; Sarotti, A. M. *J. Org. Chem.* **2017**, *82*, 1873-1879. c) Zanardi, M. M.; Sarotti, A. M. *J. Org. Chem.* **2015**, *80,* 9371-9378. d) Sarotti, A.M. *Org. Biomol. Chem.* **2013**, *11*, 4847-4859.
4. For example, see: a) Grimblat, N.; Kaufman, T. S.; Sarotti, A. M. *Org. Lett.* **2016**, *18*, 6420-6423. b) Li, C.-S.; Sarotti, A. M.; Huang, P.; Tang, U.; Hurdle, J; Kondratyuk, T.; Pezzuto, J.; Turkson, J.; Cao, S. *Scientific Reports – Nature*. **2017**, *7*, 10424, 1-10.
5. a) Novaes, L. F. T.; Sarotti, A. M.; Pilli, R. A. *J. Org. Chem.* **2015**, *80*, 12027-12037. b) Della-Felice, F.; Sarotti, A. M.; Pilli, R. A. *J. Org. Chem.* **2017**, *82*, 9191-9197. c) Della-Felice, F.; Pilli, R. A.; Sarotti, A. M. *J. Braz. Chem. Soc.* **2018**, *29*, 1041-1075.

The application of isotope labeling and NMR spectroscopy in biosynthetic, structural, and conformational studies

Jeffrey L C Wright[1]

1. UNC Wilmington Center for Marine Science, Wilmington NC

The use of stable isotopes has been an important tool in many studies employing NMR spectroscopy. To begin with the main focus was directed towards biosynthetic studies using ^{13}C-labeled precursors and this application completely transformed such biosynthetic studies by replacing the use of radioisotopes and tedious chemical degradations. Before the introduction of commercial ^{13}C NMR spectrometers, the incorporation of ^{13}C label could only be monitored through ^{13}C-^{1}H satellites. Later of course the introduction of ^{13}C spectrometers allowed us to observe every carbon in the molecule, such that today it is possible to get a complete biosynthetic picture in a single experiment. In addition we can determine whether labeled precursor units have been incorporated intact into the molecule of interest or have undergone some transformation beforehand. This is all vital information that aids any subsequent molecular or genetic studies of natural products.

Some examples of such experiments will be presented to illustrate the various applications and their outcomes. These include various polyketides such as the tropolone sepedonin, bikaverin, and the DSP toxins and spirolides.

Stable isotopes (for example ^{15}N) can also be used to establish or confirm the structure of a new alkaloid, and this will be illustrated in our studies of a fungal tetramic acid.

In a different context, ^{15}N can be used to probe the conformation of nitrogen-containing molecules such as peptides by measurement of the ^{13}C-^{15}N coupling values of the amide bonds, and this will be illustrated using a synthetically labeled peptide as well as a naturally occurring cyclic peptide.

Finally, by employing ^{13}C and D-labeled precursors, we have probed the stereochemistry of the polyketide process in different organisms, by a simple HSQC experiment. This will be illustrated with a marine polyketide.

NOAH: NMR Supersequences for Small Molecule Analysis and Structure Elucidation

Tim D W Claridge[1] and Ēriks Kupče[2]

1. Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK
2. Bruker UK Ltd., Banner Lane, Coventry, CV4 9GH, UK

Modern approaches to the structure characterization of small molecules by NMR spectroscopy largely follow well established protocols that are reliant on a core set of 2D correlation experiments such as HSQC, HMBC, COSY, NOESY, TOCSY, and similar [1]. Having established themselves as the primary techniques, much focus has now turned to developing experimental methods that allow the faster collection of these data sets. Herein, we exploit the concept of tailored polarization storage by recording multiple 2D data sets nested as individual modules within a single "supersequence" that requires only a single recovery delay, and so provides for reduced data collection times.

The proposed technique is outlined schematically in Figure 1 and combines several previously introduced approaches, as discussed in detail in our recent publication [2]. We term this concept NOAH (NMR by Ordered Acquisition using ^{1}H-detection). Herein we describe the general NOAH concept and its practical implementation, and present some recent sequences optmised for the characterisation of small molecules [3]. We illustrate the advantages and limitations of this approach and demonstrate the use of NOAH data in combination with computer-assisted strucuture elucidation (CASE) protocols.

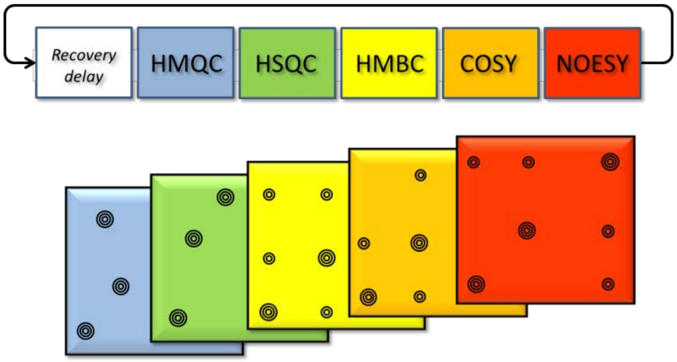


Figure 1. Schematic representation of the NOAH supersequences, here comprising a NOAH-5 experiment made up of five nested modules.

References:

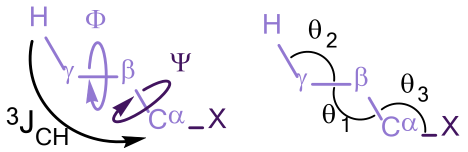
1. T. D. W. Claridge, High-Resolution NMR Techniques in Organic Chemistry, 3rd Edn, Elsevier, 2016.
2. Ē. Kupče and T. D. W. Claridge, *Angew. Chem. Int. Ed. Eng.*, 2017, 56, 11779-11783.
3. Ē. Kupče and T. D. W. Claridge, *Chem. Comm.* 2018, in press, DOI: 10.1039/C8CC03296C.

Empirical equations for ${}^3J_{CH}$ prediction: introducing a general bond angle correction

Claire L. Dickson[1], William Gerrard[1], Jacob Clements[1], Emily Johnston[1], Zoltan Takacs,[2] Charles D. Blundell[2], Martin J. Watson[2] and Craig P. Butts[1]

1. University of Bristol, Bristol, UK
2. C4X Discovery, Manchester, UK

Existing empirical equations for three-bond proton-carbon scalar couplings (${}^3J_{CH}$) either target specific coupling pathways, such as those common in carbohydrates [1], peptides/proteins [2] and nucleic acids [3], or more general equations accounting for β/γ [4] or α [5] substituents for H-C-C-C coupling pathways. To expand the breadth and improve the accuracy of empirical methods we have used density functional theory (DFT) to calculate ${}^3J_{CH}$ for >500 different molecular fragments while varying the dihedral angle(s) (Φ and/or Ψ). These DFT-calculated ${}^3J_{CH}$ were then used to identify and parameterize suitable equations relating ${}^3J_{CH}$ to the dihedral angles for each fragment.



A correction for the effects of bond angle ($\theta_{1\text{-}3}$) on ${}^3J_{CH}$ was generated by using DFT to calculate scalar couplings for over 1,000 organic molecules using 3D molecular structures selected from the Cambridge structural database (CSD). This resulted in a dataset of over 23,000 DFT-calculated ${}^3J_{CH}$ with a range of dihedral angles, bond angles, substituents and coupling pathways, which were used to fit the equation below:

$${}^3J_{CH} = {}^3J_{CH}\text{ (Fragment-based approach)} \times (B_1\Delta\theta_1 + B_2\Delta\theta_2 + B_3\Delta\theta_3 + C)$$

Where $\Delta\theta_n$ is the difference between the mean bond angle ($\bar{\theta}_n$) from the corresponding fragment and the bond angle from the molecule (θ_n): $\Delta\theta_n = \bar{\theta}_n - \theta_n$.

The performance of the empirical equations was tested against 102 experimentally measured ${}^3J_{CH}$ (as shown in the table below). This fragment-based approach achieved an accuracy of ~0.9 Hz with a >2800-fold time saving relative to performing full DFT calculations to determine ${}^3J_{CH}$.

Method	**DFT**	**Aydin *et al.* [6]**	**Palermo *et al.* [4]**	**This work.**
MAD / Hz	0.37	1.14	0.95	0.86
SD / Hz	0.44	1.38	1.06	1.04
Structural information required	Full Cartesian coordinates	Φ	Φ, β/γ substituent orientation and electronegativity	Φ, Ψ, $\theta_{1\text{-}3}$, coupling pathway α/β/γ substituent pattern

1. Coxon, B., Adv Carbohydr Chem Biochem, 62, 17-82, 2009.

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6. Aydin, R., Günther, H., Mag Res Chem, 28(5), 448-457, 1990.

Monday, September 17th
11:00 AM - 12:30 PM

NMR in all stages of the Pharmaceutical Industry

Chair: Kathleen Farley

Speakers:

Andrew Proudfoot
Novartis Institute for BioMedical Research

Joan Malmstrøm
Novo Nordisk A/S

Dave Russell
Genentech, Inc.

Steve Coombes
Astrazeneca

High Confidence Protein-Ligand Complex Modeling by NMR-Guided Docking Enables Early Hit Optimization

Andrew Proudfoot[1], Dirksen E. Bussiere[1], and Andreas Lingel[1, 2]

1. Novartis Institutes for BioMedical Research, Structural and Biophysical Chemistry, Global Discovery Chemistry, Emeryville, CA US
2. Novartis Institutes for BioMedical Research, Global Discovery Chemistry, Basel, Switzerland

In modern day drug discovery, the use of structure-based drug design has become a routine approach in the optimization of ligands that engage with a specific molecular target. For this approach to be successful, awareness is required of the geometries of the ligand and the protein, the types of interactions that are made, along with knowledge of the space available to expand the ligand and form additional interactions. This information can be determined using structural techniques such as NMR and EM, but such methodologies can be labor intensive and can only be used for a limited subset of targets. X-Ray crystallography is often the method of choice and can be used in a high throughput way to elucidate and characterize protein-ligand co-structures however, the success rate is often variable especially when working with weakly binding ligands or dynamic proteins. In these instances a lack of structural information will often result in identified hits not being pursued, which has an impact on the diversity of the chemical scaffolds that are explored for a given target. To overcome this impediment, we have developed a robust NMR restraint guided docking protocol to generate high quality models of protein-small molecule complexes [1]. Using the 120 kDa antibacterial target CoaD, a comprehensive set of experimentally determined intermolecular restraints are derived between selectively labeled methyl groups of the protein sidechains, and the unlabeled bound ligand. These restraints are then used to drive the docking process and determine the conformation of the ligand in the bound state. After benchmarking the process with several compounds for which X-Ray crystal structures were available, we show that the models produced for two independent crystallographically intractable fragments, with completely unknown binding modes, are of sufficient accuracy to drive a structure based drug campaign. The improved molecules are shown to interact with the target as predicted and represent newly established starting points for chemistry campaigns.

1. Proudfoot A, Bussiere D, Lingel A (2017) High Confidence Protein-Ligand Complex Modeling by NMR-Guided Docking Enables Early Hit Optimization. JACS 139, 17824-33

Application of Quantitative ^{1}H-NMR Spectroscopy for the determination of silicone oil and degradation products hereof

Joan Malmstrøm
CMC API Analytical Development, R&D, Novo Nordisk A/S, Denmark

During the past years there has been an increasing focus on the presence of silicone oil as a contaminant in pharmaceutical formulations kept in pre-filled syringes. As the pre-filled syringes are coated on the inner wall with silicone oil (PDMS) there is a potential risk of migration of silicone oil from the inner surface of the primary packing material into the aqueous solution. Several studies in the literature have demonstrated that presence of silicone oil as droplets in a high-concentrated protein formulation can cause unwanted protein aggregation. Hence, as the use of silicone coated primary packing material for protein formulations are increasing, the request for an easy and quantitative method for determination of silicone oil in pharmaceutical formulations is similar increasing. Several analytical techniques have been developed with the aim of detecting the presence of silicone oil. Most of these methods require either a derivatisation step or extraction steps followed by e.g. GC-MS or ICP-MS analysis. Appling these methods can cause a loss in detection of the hydrolytic degradation products of silicone oil, i.e. trimethylsilanol and dimethylsilandiol. Examination of an aqueous formulation obtained from a pre-filled syringe by ^{1}H-NMR spectroscopy using standard high field instruments equipped with cryoprobe provides data about the content and levels of silicone oil and the two silanols in levels above 0.1 µg/ml (LOQ). The ^{1}H-NMR method offers an easy and direct, quantitative measurement of samples intended for clinical use and samples kept at elevated temperature for a prolonged time-period (i.e. stability studies). In addition the results obtained by ^{1}H-NMR spectroscopy have been compared to results obtained by the orthogonal method MFI when measuring laboratory prepared samples with a well know amount of silicone oil coating the inner surface.

The method has been described in a standard operation procedure (SOP); making it useful to study other aspects were PDMS is of interest, i.e. depletion of anti-foam added during fermentation.

Optimization of ^{19}F Methodologies for Pharmaceutical Structure Elucidation

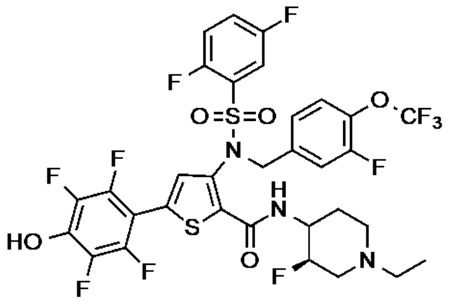
David Russell and Lydia Dewis

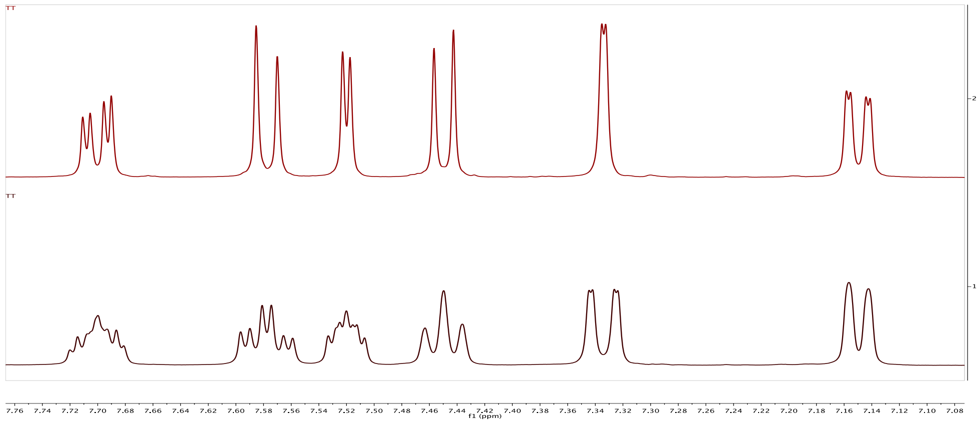
Genentech. Inc., South San Francisco, CA, US

The presence of fluorine in an active pharmaceutical ingredient (API) can impart important pharmacological attributes with regards to metabolism, stability, and selectivity. As such, nearly one-third of newly approved small molecule drugs contain at least one fluorine atom and the trend towards including more than one fluorine moiety per molecule is growing.

For pharmaceutical applications, the presence of a "new" NMR active nuclide with high receptivity and 100% natural abundance opens up many interesting avenues for obtaining structural data. The significant chemical shift window and large coupling constants associated with ^{19}F NMR requires very careful consideration of excitation, decoupling, and magnetization transfer schemes, however. As an example, are HF coupling pathways better elucidated using an HF-COSY approach, or does an indirect method like HETCOR provide more reliable data? And which nucleus should be used for the direct-observe dimension? In a similar vein, given that long-range FC coupling constants are significant over multiple bonds, what approaches work best to help simplify the wealth of information available using long-range experiments?

Results from our investigations using a three-channel HFX system will be presented.





Applications of Quantitative NMR Spectroscopy in Pharmaceutical Development

Steven R. Coombes[1], Ian C. Jones[1] and David T.E. Whittaker[2]

1. Pharmaceutical Technology and Development, AstraZeneca, Macclesfield, UK
2. Pharmaceutical Sciences, IMED Biotech Unit, AstraZeneca, Macclesfield, UK

The use of NMR spectroscopy is ubiquitous throughout discovery and development in the pharmaceutical industry as a primary tool for structure elucidation and identification. But perhaps the most useful application of NMR Spectroscopy is the inherently quantitiative structure-to-spectrum relationship, allowing for the unambigous assignment of a signal in the spectrum to a specific atom in the analyte molecule and the ability to determine the exact amount present – a capability almost entirely exclusive to the field of NMR. There is also the potential to utilise a variety of nuclei for quantitative analysis, with the possibility of using ^{1}H, ^{13}C, ^{19}F or ^{31}P NMR if acquired under appropriate conditions.

This phenomenen is well known and is commonly used for purity determinataion (assay by NMR), either with the use of internal or external reference standards, be they real or electronic, with accuracy and precision comparable to other analytical trechniques (e.g. HPLC-UV or LC-MS). One advantage of NMR spectroscopy is that no primary reference standard of a sample is required for quantification, as the quantitative NMR signal response is universal and not compound specific. This also allows for the direct (and quantitative) analysis of multicomponent systems, which would have different UV resposnes or MS ionisation potentials.

Here we will share a number of examples where quantitative NMR Spectroscopy has been used for more than routine assay in pharmaceutical development and demonstrate how it is also used for the analysis of a number commercial products (along with associated registered methods). In each case, NMR has been utilised because other quantitative analytical techniques were not suitable. Examples include the use of interleaved ^{1}H and $^{19}F/^{133}Cs$ NMR for reaction monitoring and process understanding, enabling the real-time tracking of multiple analytes within a complex mixture. Also, we have developed quantitiative selective excitation ^{1}H NMR methods using excitation sculpting for cleaning validation of non-chromorphoric analytes, allowing limit tests with sensitivity at single digit ppm levels.

We will describe a number of quantitative ^{13}C NMR methods used in the manufacture and release of the oncology drug Zoladex. These include a fully automated ^{13}C NMR method for ID and amino acid ratio of the deca-peptide goserelin (the active ingredient in Zoladex), along with quantitative ^{13}C NMR analysis for ID and determination of the lactide-glycolide ratio in the proprietary co-polymer used to formulate the Zoladex depot.

We also show how routine quantitative ^{1}H NMR spectroscopy has been utilised to perform 7 concurrent analyses in a single proton spectrum during the development of the antibiotic Ceftaroline. ^{1}H NMR is used in the assessment of suitability of material in the manufacture of Seroquel XR, by quantifying the ratios of hydropropoxy (HP) and methoxy (MeO) substituents on the cellulose backbone of the excipient Methocel. And finally, we show how quantitative NMR is being used by AstraZeneca in the global fight against counterfeit medicines.

Monday, September 17th
2:00 PM - 3:30 PM

Workshop

CASE - Computer Aided Structural Elucidation

Coordinated by:
Armando Navarro

CASE - Computer Aided Structural Elucidation

Coordinated by: Armando Navarro

Several commercial and open-source Computer-Assisted Structure Elucidation (CASE) software packages have evolved from the advances in NMR hardware, pulse sequences, computer hardware, and AI expert systems. CASE software is capable of successfully deducing the chemical constitution of complex natural and synthetic products with a minimum of human intervention. Learning to use a CASE software system for elucidating the structure of an unknown compound can be compared to learning to use a mathematical calculator for solving a math problem. Additionally, the application of CASE can strengthen a chemists' elucidation skills.

This workshop will discuss topics that cover:

1. Sample preparation and experiment choice amongst the multitude of options available in modern 1D and 2D NMR, such as ^{1}H-^{13}C and ^{1}H-^{15}N direct and long-range correlation experiments and 2D-ADEQUATE and INADEQUATE.
2. Selected examples comparing the different approaches for CASE and manual elucidation,
3. Recommendations for the interpretation of experimental NMR data and deducing possible fragments
4. Searching NMR databases for known fragments and applying filters to narrow down candidates,
5. The application of NMR chemical predictors, strategies for dealing with overlapping and/or absent signals in NMR spectra.
6. The impact of CASE algorithms within the generation workflow
7. Extension to the determination of three-dimensional structure (CASE-3D) using either empirical predictions, quantum mechanical calculations (DFT) and/or NOE, NOESY and RDC spectra. Discussion on Sustainability and Service Needs for NMR

Speakers:

Arvin Moser, Advanced Chemistry Development (ACD/Labs)

Pavel Kessler, Bruker BioSpin

Monday, September 17th
6:00 PM - 7:30 PM

Presentation of the James N. Shoolery Award

SMASH 2018 Recipient:

Krish Krishnamurthy
Chempacker LLC

Krish Krishnamurthy

Krish Krishnamurthy was born and raised in Chennai, South India. He received his B.Sc. in chemistry (1974) and M.Sc. in Organic Chemistry (1976) from the University of Madras, India, before moving to Kent State University, Ohio for his Ph.D. in synthetic organic chemistry. His passion for NMR spectroscopy was kindled during his tenure as the NMR research assistant at Kent and he decided to pursue his research career in that field. After his Ph.D., he did post-doctoral research with Prof. George A. Olah at University of Southern California focussing on NMR applications in the study and dynamics of carbocation intermediates. He followed that with a position as NMR specialist at University of California, Berkeley. During the next 25 years, his research career shifted back-and-forth between Varian Instruments and the pharmaceutical industry.

Fittingly, Krish was mentored by James Shoolery himself in the Varian NMR applications lab and this reinforced Krish's strong belief that state-of-the-art NMR methods should be made available for everyone. In this vein, he developed and administered the "Chempack" software package for Varian NMR users. In the spirit of Shoolery's vision, the package was built for "ease-of-use without compromise of quality" – revolutionizing the ease with which even very complex NMR experiments can be run by the chemist. He has continued to maintain this very popular package for the last 20+ years as a community service.

Krish also contributed to the early development of many pulse sequence elements that are incorporated into everyday experiments we would all recognise – most notably CRISIS pulses (Compensation of Refocusing Inefficiency with Synchronized Inversion Sweep), BASHD (F1 BAndSelective Homonuclear Decoupling), IMPRESS (a hybrid Hadamard-FT method for improved resolution in 2D), and STEP (Selective Tocsy-Editted Preparation). He also developed key long-range correlation experiments such as EXSIDE (J-scaling method for effective long-range coupling constant measurement) and, in collaboration with Gary Martin, a variety of accordion based HMBC type sequences. Working across more than just spectroscopy, Krish has also led a microimaging team and developed an in-vivo osteoarthritis biomarker method based on the use of residual chemical shift anisotropy of H2O in cartilage and time domain data processing. Krish's research now focuses on an unconventional data processing paradigm with the development of CRAFT, a Bayesian based approach to decimating time domain data (FID) into the tabular domain (Frequency-Amplitude-decay rate-phase) and its use in a variety of qNMR applications area.

Tuesday, September 18th
09:00 AM - 10:30 AM

Industrial Applications of NMR

Chair: John Edwards

Speakers:

Gary Strahan
USDA

Thomas M. Cameron
Entegris

Philipp Nitschke
University of Regensburg

Raphael Kircher
University of Kaiserslautern

NMR of Biomass Pyrolysis Products: Maximizing Derived Information

Gary Strahan and Charles A. Mullen

United States Department of Agriculture

Various standard and statistical data analytic methods, including principle component analysis (PCA) and partial least squares (PLS), have been applied to the NMR spectra of numerous fast pyrolysis oils in order to develop methods to more fully and rapidly characterize their chemical compositions. These bio-oils were generated from various energy crops and other agricultural feedstocks, such as woods, animal wastes and oil seed presscakes, using various treatment protocols and were compared to biodiesel, and fossil fuels (gasoline and diesel). In the first analytical approach, 1H and 13C NMR spectra were integrated over spectral regions to quantify classes of carbon and hydrogen atoms in each bio-oil sample, while DEPT spectra were used to determine proton multiplicity, providing detailed information on the types of molecules found in the bio-oils. The NMR spectra of the bio-oils varied greatly, and the percentage of carbons and protons at the downfield regions of the NMR spectra tracked with the energy content of the bio-oil as well as the feedstock. While this approach provided useful information on chemical composition, it can also be time-consuming and can oversimplify the spectral information, causing a loss of important details. Consequently, the 13C NMR spectra were re-interrogated using unsupervised principle component analysis (PCA) by binning their intensities in 2 ppm increments. Comparison of the PCA scores plots with their respective loadings plots enabled a determination of those chemical shifts, and hence the chemical functional groups, that were most important in discriminating amongst the clusters. The clustering of the biomass samples based on PCA results were shown to track with their biological origin and their energy contents. This approach was extended further by creating several partial least squares (PLS) models that correlated various properties and chemical composition measurements with the 1H and 13C NMR spectra of 73 different of pyrolysis bio-oil samples from various biomass sources (crude and intermediate products), finished oils and small molecule standards. Two models based exclusively on 13C-NMR data demonstrated the best over-all ability to predict these same characteristics for an unknown sample. Statistically relevant correlations were established between the binned 13C NMR spectra and the fractional mass contents of H, C, O, and N, as well as to their values for higher heating value (HHV), total acid number (TAN), and total concentration of phenol and cresols. The first two latent variables of the PLS models based on 13C spectra are qualitatively similar to the equivalent first two principle components reported previously, but their chemical properties and compositions can be now explicitly derived.

Characterization and Quantitation of Diiodosilane Trace Impurities by NMR Spectroscopy

Thomas M. Cameron, Thomas Baum, and David Kuiper

Entegris Danbury, Connecticut

A thorough understanding of the impurity profiles of fine chemicals destined for use in the semiconductor industry is critical. Trace impurities present in samples may drive performance yield issues, and some impurities are of concern from an EHS perspective. Chemicals used for the deposition of silicon-containing films have been an area of active development for many years. Diiodosilane is currently receiveing attention as a precursor for the low-temperature deposition of silicon-containing films. To date, we have identified at least six impurities that can be present in samples of diiodosilane at measurable levels. The impurity profile of diiodosilane as determined by various NMR spectroscopy techniques will be presented along with the identities of the impurities and their origins. In addition, we will discuss our preliminary findings on shelf-life studies as monitored by NMR spectroscopy. The use of NMR as a general characterization method and the comparison to a gas chromatography analysis will be addressed.

Combined in situ NMR-UV/Vis-Illumination Spectroscopy: A Novel Mechanistic Approach in Photochemistry

Philipp Nitschke[1], Andreas Seegerer[1], Ruth M. Gschwind[1]

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

Synthetic applications in photochemistry are booming. Despite great advances developing new reactions, details about the underlying mechanisms are often challenging to come by. Here, a fully automated *in situ* triple combination of NMR spectroscopy, UV/Vis spectroscopy and illumination to allow simultaneous and time resolved detection of paramagnetic and diamagnetic species is presented as a novel tool in photochemistry [1]. This optical fiber based setup enables the acquisition of combined UV/Vis and NMR spectra in photocatalysis under continuous or pulsed illumination. This is demonstrated on a consecutive photoinduced electron transfer (conPET) [2] process, which generates stable, paramagnetic radical anions which are usually inaccessible by conventional NMR spectroscopy. Further, the broad applicability of combined UVNMR spectroscopy for light induced processes is presented on a structural and quantitative analysis of a commercially available photoswitch [3] including rate modulation and stabilization of transient species by temperature variation. Besides the two examples, the flexible setup regarding NMR hardware, temperature and light sources is presented in detail.

1. Seegerer A., Nitschke P., Gschwind R. M., *Angew. Chem. Int. Ed.* **2018**(Early View).
2. Ghosh I., Ghosh T., Bardagi J. I., König B., *Science*, *346*, 725–728, **2014**.
3. J. Piard, *J. Chem. Educ.*, *91*, 2105–2111, **2014**..

Dynamic Nuclear Polarization Enables Fast Flow NMR Reaction and Process Monitoring

Raphael Kircher, Hans Hasse, and Kerstin Münnemann

Laboratory of Engineering Thermodynamics (LTD), University of Kaiserslautern, RLP, GER

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-invasive, quantitative and qualitative analysis method, established in a wide range of applications in medicine, physics, chemistry, and biology. NMR spectroscopy suffers from a lack of sensitivity due to the intrinsic low polarization of spins at ambient temperatures. To overcome this sensitivity issue many hyperpolarization techniques were developed in the last decades, e.g. Dynamic Nuclear Polarization (DNP). In the following, a mobile setup for continuous Overhauser DNP-enhanced NMR reaction and process monitoring is presented.

For Overhauser DNP, a transfer process of angular momentum between electron and nuclear spins, i.e. the use of stable radicals (electrons) is essential. After the hyperpolarization is accomplished the stable radicals are disturbing further applications or measurements of the sample, as they considerably lower the longitudinal relaxation time constant T_1 of the nuclei and thus the lifetime of the hyperpolarization. By separating the hyperpolarized nuclear spins from the electron spins, the decay of the hyperpolarization is related to the native T_1, and therefore the decay of the hyperolarization is decelerated. This is accomplished by immobilizing 4-amino-TEMPO on the organic support Sepharose [1,2].

NMR detection is performed with a mobile, medium-field NMR spectrometer (benchtop, 43.2 MHz proton frequency) due to many advantages of the device, e.g. low weight (55 kg) and adequate shim settings. Continuous hyperpolarization by Overhauser DNP enables NMR measurements in the fast flow regime of liquids at ambient temperatures. This is of high interest as it enables monitoring of reactions and processes that were previously inaccessible.

1. Ebert, Sandro; Amar, Andrea; Bauer, Christian; Kölzer, Michael; Blümler, Peter; Spiess, Hans W.; Hinerberger, Dariush; and Münnemann, Kerstin, Appl Magn Reson 43, 195-206, 2012
2. Lingwood, Mark D.; Siaw, Ting Ann; Sailasuta, Napapon; Ross, Brian D.; Bhattacharya, Pratip; and Han, Songi, J Magn Reson 205(2), 247-254, 2010

Tuesday, September 18th
11:00 AM - 12:30 PM

Workshop

Imaging of Chemical Systems

Coordinated by:
Melanie Britton

Imaging of Chemical Systems

Coordinated by: Melanie Britton

Nuclear Magnetic Resonance (NMR) spectroscopy is widely employed to determine molecular structure and dynamics by using the signals of NMR active nuclei contained within a molecule. When NMR measurements are performed in the presence of magnetic field gradients, the NMR signal becomes spatially-dependent, resulting in images (MRI), with a spatial resolution in the order of 10-100 µM, as well as measurements of molecular flow and diffusion. As such, NMR is uniquely able to provide an integrated, non-destructive view of the structure, dynamics, and function of molecular systems where spectroscopic information at the atomic level can be integrated with information at the mesoscopic and macroscopic length scales via NMR imaging and diffusion methods. The synergy between these two modalities enables MR techniques to probe the broadest range of systems, many of which not accessible via other analytical techniques. While MRI is a well-established analytical technique in biomedical research and clinical diagnosis, its ability to visualise the composition and behaviour of molecular materials is making it increasingly useful to study spatially-heterogeneous chemical systems in a diverse range of applications, including fast moving consumer goods (FMCG), pharma, manufacturing, materials science, reaction engineering, food technology, catalysis and energy storage. This workshop will explain the principles of MRI, including k-space, Fourier imaging, chemical shift imaging (CSI), pulsed gradient spin echo experiments and provide an overview of the different types of imaging sequences commonly used. The breadth of information available by MRI will be explored through examples from a diverse range of applications.

Tuesday, September 18th
04:30 PM - 06:00 PM

NMR analysis of Chiral Molecules

Chair: Kirk Gustafson

Speakers:

Thomas Williamson
Merck

Alfonso Mangoni
Università degli Studi di Napoli

Fátima Morales
Universidad de Murcia

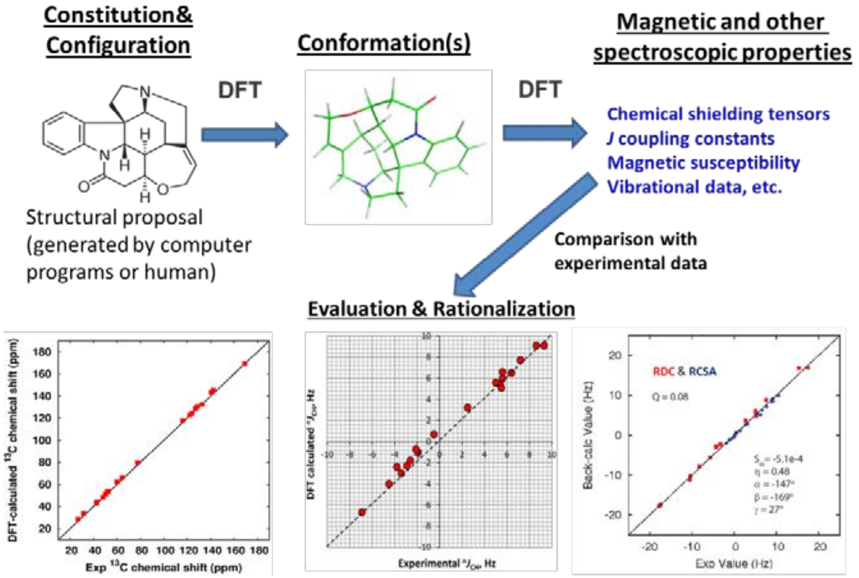
Christian Griesinger
Max Plank Institute

Leveraging an expanded set of NMR parameters for more reliable structure elucidation

Yizhou Liu[1], Alexei V. Buevich[2], Ryan Cohen[2], Gary E. Martin[2], **R. Thomas Williamson[2]**

[1]Pfizer Inc. Groton CT. [2]Merck Research Labs, Analytical Research & Development,
Structure Elucidation Group Merck & Co. Inc. Rahway and Kenilworth, NJ.

Theoretical calculations of molecular geometries and NMR parameters have advanced significantly over the past decade. These calculations provide a framework for the design of novel NMR experiments and revitalization of older experiments. Applications important within the pharmaceutical industry include calculations to support studies aimed at defining the constitution and configuration of small molecules, analysis of peptides in anisotropic media, conformational sampling, and spectral simulations for the analysis of crystal structures. Theoretical methods, most of which are based on QM DFT calculations, provide better strategies for choosing the optimal experiment for a specific task (e.g. using HMBC vs. ADEQUATE). DFT methods have also provided a foundational basis on which to enhance NMR tools for structural analysis. It is now common in our labs to simultaneously utilize two or three orthogonal methods (e.g. RDC/RCSA, chemical shifts, J-couplings, NOE/ROE, etc.) to afford robust structure confirmation. DFT methods are now highly efficient and these calculations can be carried out in parallel in a time frame comparable to that required for the collection of the experimental data.



1. Y. Liu, J. Saurí, E. Mevers, M. W. Peczuh, H. Heimstra, J. Clardy, G. E. Martin, R. T. Williamson, "Unequivocal determination of complex molecular structures using anisotropic NMR measurements", *Science*, **2017**, *356*, 43. dx.doi.org/10.1126/science.aam5349.

2. E. Troche-Pesqueira, C. Anklin, R. R. Gil, A. Navarro-Vazquez, "Computer-Assisted 3D Structure Elucidation of Natural Products using Residual Dipolar Couplings", *Angew. Chem. Int. Ed.* **2017***, 56,* 3660-3664.

Quantum Mechanical NMR Calculations as an Aid for Structure Elucidation of Organic Molecules

Alfonso Mangoni

Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Napoli, Italy

Structure elucidation has been, and still is, a central issue in the study of natural products. Even today, structure misassignment is not uncommon, in particular as stereochemistry is concerned. In addition, unassigned absolute or relative configurations are still very common.

Computational techniques may help to improve the quality of the structure elucidation work. The reliability of quantum mechanical prediction of NMR parameters (mainly chemical shifts, but also coupling constants) is steadily increasing, with a parallel decrease of the computational resources needed to perform the calculations. The predicted NMR parameters can be used not only to check regio- and stereochemical assignments made with other means, but also to make otherwise impossible structural assignments. On the other hand, quantum mechanical spectral simulation of non-first-order spin systems can be used to determine coupling constants between strongly coupled nuclei, which may be difficult or impossible to measure in other ways, but may be critical for stereochemical assignments.

Some real-life examples [1,2] of these techniques chosen from my own and others' research work will be presented.

1. Mangoni, A. Strategies for Structural Assignment of Marine Natural Products Through Advanced NMR-Based Techniques. In Fattorusso E., Gerwick W., Taglialatela-Scafati O. (eds), Handbook of Marine Natural Products, Springer Netherlands, Dordrecht, 2012, pp. 518–546.

2. Moosmann, P.; Ueoka, R.; Grauso, L.; Mangoni, A.; Morinaka, B. I.; Gugger, M.; Piel, J. Angew. Chemie Int. Ed. 56(18), 4987–4990, 2017

Co-Conformational Mobility of Amide-Based [2]Rotaxanes by Dynamic NMR Spectroscopy

Fátima Morales, Juan Carlos Martínez-Costa, Alberto Martínez-Cuezva, Mateo Alajarín and José Berná

Departamento de Química Orgánica, Facultad de Química, Campus de Excelencia Internacional Regional "Campus Mare Nostrum", Universidad de Murcia, Spain, **fatima.morales@um.es**

Research on the synthesis and applications of [2]rotaxanes, the most abundant category among the different types of mechanically-interlocked molecules (MIMs), has been a hot topic in many scientific areas in the last years [1,2]. These molecules are composed by a dumbbell-shaped component threaded through a ring with non-covalent bonding interactions, endowing the molecule with a unique kind of functionality due to the mechanical bond [3]. This particular linkage gives the feasibility for controlling their internal dynamic motions of rotation and translation (Figure 1).

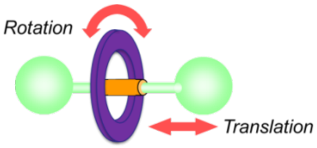


Figure 1.

Hydrogen bond interactions are known to assembly and stabile various supramolecular complexes, such as these [2]rotaxanes. Our research group is focused in the highly efficient-directed synthesis of amide based [2]rotaxanes [4], first discovered by Leigh and co-workers [5]. For this purpose, one the most important stages is the study of the affinity of the macrocycle for the binding sites embedded into the axis. In the Leigh-type rotaxanes, this affinity is related with rotational motion of the macrocycle around the axle [6].

Herein, we describe the synthesis of tetraamide-based [2]rotaxanes having different tetraalkyldicarboxamide-based templates, the dynamic NMR study of the rotational motion of their interlocked macrocycles, and the kinetics of the corresponding dethreading processes leading to the axis extrusion. These data will be compared with the corresponding analogues reported in literature.

Acknowledgments

This work was supported by the MINECO (CTQ2017-87231-P) with joint financing by FEDER Funds from the European Union, and Fundación Seneca-CARM (Project 19240/PI/14). F.M. also thanks the Fundación Seneca-CARM for her Saavedra Fajardo contract and funding (Contract No. 20025/SF/16).

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2. Alvarez-Lorenzo C, García-González CA, Concheiro A, J. Control. Release, 268, 269-281, 2017.
3. Barin G, Forgan RS, Stoddart JF, Proc. R. Soc. A., 468, 2849–2880, 2012.
4. Martinez-Cuezva A, Saura-Sanmartin A, Nicolas-Garcia T, Navarro C, Orenes RA, Alajarin M, Berna J, Chem. Sci., 8, 3775–3780, 2017.
5. Gatti FG, Leigh DA, Nepogodiev SA, Slawin AMZ, Teat SJ, Wong JKY, J. Am. Chem. Soc., 123, 5983, 2001.
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Unequivocal determination of 3D molecular structures using proton residual chemical shift anisotropy

N. Nath[1,3], Juan Carlos Fuentes[1], M. Reggelin[2], **C. Griesinger**[1]

[1] Dept. of NMR based structural biology, Max Planck Institute for Biophys. Chem., Göttingen, Germany
[2]Institute of Organic Chemistry, Technical university of Darmstadt, Darmstadt, Germany.
[3] present address: Department of Chemistry, Gauhati University, Guwahati 781 014, Assam, India.

Determination of 3D molecular structure remains a challenging task for natural products or organic compounds that are available in very minute amounts. While the constitution can be derived from proton/proton and proton/carbon correlations, *J* couplings and NOEs are used to determine the relative configuration oftentimes supported by RDCs between protons and carbons over one bond or carbon RCSAs (1-4). For compounds in the range of a few 10 µg, however, these RDC or carbon RCSAs are difficult to collect because of low sensitivity. Therefore, we introduce here the highly sensitive NMR observable ^{1}H RCSAs which, is similar to RDCs or ^{13}C RCSAs, provides spatial orientation of different structural moieties within a molecule. We report robust measurement of ^{1}H RCSAs by using constrained aligning gel or liquid crystal and their utilization in determining the complex 3D molecular structures of several molecules with varying complexities and rigidity of conformations. For amounts in the 10 µg deuterated alignment media are required to reduce the signals from the alignment medium. The determination of the so far unknown relative configuration of a natural compound will be demonstrated.

1) Hallwass, F.; Schmidt, M.; Sun, H.; Mazur, A.; Kummerlowe, G.; Luy, B.; Navarro-Vázquez, A.; Griesinger, C.; Reinscheid, U. M. *Angew. Chem., Int. Ed.* **2011**, *50*, 9487.
2) Y. Liu, J. Saurí, E. Mevers, M. W. Peczuh, H. Hiemstra, J. Clardy, G. E. Martin, R. T. Williamson, Science 2017, 356, eaam5349.
3) N. Nath,M. Schmidt,R. R. Gil,R. T. Williamson,G. E. Martin, A. Navarro-Vazquez, C. Griesinger, Y. Liu, *J. Am. Chem. Soc.***2016**, *138*, 548.
4) Leandro F. Gil-Silva, R. S.-F., Armando Navarro-Vázquez, and Roberto R. Gil. *Chemistry - A European Journal***22**, 472-476 (2016).

Wednesday, September 19th
09:00 AM - 10:30 AM

Benchtop NMR in an Academic Setting

Chair: Susie Riegel

Speakers:

John Frost
Thermo Fisher Scientific

Rio Febrian
Saint Louis University

Kasey Leigh Yearty
The University of Georgia

Anne Friebel
University of Kaiserslautern

SIA-NMR: A novel hyphenated technique and it’s application to the analysis of amphetamine derivatives

Timothy J. Trinklein[1], **John A. Frost**[2], Sandy M. Koresch[3], Joseph H. Aldstadt[1]

1. Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI, US
2. Thermo Fisher Scientific, Madison, WI, US
3. Wisconsin State Crime Laboratory Bureau, Milwaukee, WI, US

The growth of new psycoactive substances (NPS), including bath salts, and synthetic cathinones, have created a sproblem in forensic analysis. NPSs use a small structural modification of traditional psycoactive substances to circumvent laws and foil existing presumptive tests such as color, thin-layer chromatography, and infra-red spectroscopy. A fast, robust, and automated analysis method is needed to address this challenge. NMR is a powerful tool for identifying NPSs, but remains underutilized in this field because of the size and cost. Benchtop NMR’s lower size and cost make the technique more accessible, but the low fields used by these systems introduce their own limitations. We describe the development, optimization, and application of a novel hyphenated technique, Sequential Injection Analysis-Nuclear Magnetic Resonance (SIA-NMR). SIA-NMR combines the automated sample pretreatment capabilities of SIA, with the qualitative and quantitative power of NMR to create a high throughput, low-cost instrument with applications in a variety of areas including forensic science.

Improving Benchtop NMR Spectral Resolution for Simple Peptides by Ion Effects

Rio Febrian[1], William J. Ona[1], and Paul J. Bracher[1]

1. Department of Chemistry, Saint Louis University, St. Louis, MO 63103 USA

We have developed a method that improves low-field benchtop NMR resolution of simple peptides, such as glycylglycine (Gly-Gly), glycylglycylglycine (Gly-Gly-Gly), and alanylalanine (Ala-Ala), by the addition of salts. In benchtop NMR spectrometers such as the Nanalysis NMReady 60e, these molecules ordinarily give ^{1}H NMR spectra in which signals (i) are poorly resolved from each other and (ii) overlap with the signal for water. Our method involves adding an inorganic salt to aliquots of the sample prior to NMR analysis, which shifts signals in the subsequent ^{1}H NMR spectra such that many of the peaks will resolve.

In many cases, the improved resolution permits quantitative analysis of the individual signals. We demonstrate the utility of the method by using a Nanalysis instrument to measure the kinetics of hydrolysis of simple peptides. As the prevalence and accessibility of benchtop NMR instruments continues to grow in educational settings, we hope this method enables experiments that would otherwise have been impossible with a low-field magnet.

A Multi-Outcome Experiment Involving the Oxidation of Alcohols in the Undergraduate Teaching Laboratories

Kasey L. Yearty, Caroline E. Glessner, and Richard W. Morrison

Chemistry Department, The University of Georgia, Athens, GA, US

^{1}H NMR analysis is an important analytical technique presented in introductory organic chemistry courses. NMR instrument access is limited for undergraduate organic chemistry students due to the size of the instrument, price of NMR solvents, and the maintenance level required for instrument upkeep. The University of Georgia Chemistry Department currently employs benchtop ^{1}H NMR instruments in each of the undergraduate organic laboratories. These instruments can sit on a standard lab bench, can analyze samples without expensive NMR solvents, and are easily maintained. At the University of Georgia, undergraduate organic chemistry students traditionally perform a laboratory procedure for the oxidation of an unknown alcohol. This experiment was modified to utilize environmentally sustainable components, such as household bleach, as the oxidizing agent. Herein is described a multi-outcome experiment in which one unknown secondary alcohol (2-pentanol, 3-pentanol, or 3-methyl-2-butanol) is oxidized using bleach. Each student pair was assigned one unknown alcohol from a list of possible alcohols and analysis of their oxidation product was accomplished using benchtop ^{1}H NMR spectroscopy. Students interpreted their spectra and deduced the identity of the unknown alcohol they were assigned. This incorporation of modern analytical technology in the undergraduate teaching laboratories provides a tangible framework for student understanding of the utility of ^{1}H NMR spectroscopy.

Studies of Phase Equilibria and Batch Distillation with Benchtop NMR Spectroscopy

Anne Friebel, Kerstin Münnemann, Erik von Harbou, and Hans Hasse

Laboratory of Engineering Thermodynamics, University of Kaiserslautern, Kaiserslautern, Germany

Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for the investigation of complex liquid multicomponent systems [1-2]. It can resolve even chemically similar compounds and, in contrast to optical spectroscopy, no calibration is needed for determining the composition of the mixture. Furthermore, process can be investigated in-situ with NMR spectroscopy. Standard high-field NMR spectrometers require superconducting electromagnets that are cryogenically cooled. These instruments are expensive and sensitive and require a special laboratory infrastructure. Recently, compact benchtop NMR spectrometers have become available. They use permanent magnets with a medium field strength which still enables taking spectra that are well-suited for quantitative evaluation [3]. These benchtop NMR spectrometers are comparatively inexpensive, flexible, and robust, and have the potential to be used for the monitoring of industrial processes in the future.

In this work, two examples for the application of benchtop NMR spectrometers in thermodynamic studies are presented. The first application is the determination of liquid-liquid equilibria (LLE). Using benchtop NMR spectroscopy, the composition of both phases can be determined in a very simple manner by changing the position of the sample tube in the bore of the spectrometer. The results agree very well with literature LLE data for different ternary test systems. In a second application, the benchtop NMR spectrometer was connected to a laboratory batch distillation unit and was used for continuously monitoring the liquid phase composition. Different ternary mixtures were studied and the results were compared to results obtained from conventional sampling and GC analysis. Excellent agreement was found. The resulting residue curves describe the vapor-liquid equilibrium in the studied ternary system and are an interesting alternative to studies with conventional vapor-liquid equilibrium measurements.

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Wednesday, September 19th
11:00 AM – 12:30 PM

Small molecule solid-state NMR

Chair: Heather Frericks Schmidt

Speakers:

Yongchao Su
Merck

Dewey Barich
GSK

Maria Baias
New York University Abu Dhabi

Ayelen Florencia Crespi
University of Buenos Aires

Molecular Interaction and Miscibility in Pharmaceutical Products from Solid-State NMR

Yongchao Su, Xingyu Lu, Wei Xu, Matthew Lamm, Athanas Koynov, Chad D. Brown, R. Thomas Williamson, Gary E. Martin, David Hesk, Luke Schenck

MRL, Merck & Co., Inc., Kenilworth, New Jersey, 07033

The physicochemical properties, including thermodynamic and structural behaviors of active pharmaceutical ingredients (APIs) in solid dosages, can significantly manipulate phase stability, processibility and bioavailability. For example, physical stability is a key decision making factor for formulation excipients, processing and manufacturing parameters, and packaging and storage conditions. Macroscopic miscibility and microscopic interactions between API and excipients are mechanistic attributes responsible for various stability and instability events. The complexity of low drug loading, multi-component and often non-crystalline nature results in low intensity and poor temporal and spectral resolution for accessing these pharmaceutical properties. In recent years, solid-state NMR (ssNMR) has become an indispensable tool for pharmaceutical analysis due to its high resolution and quantitative capabilities in the characterization of molecular structures and dynamics. Particularly, it provides a unique advantage for investigating amorhpous systems.

Recent technical advances in ssNMR, including Dynamic Polymerization Enhancement (DNP) and ultra-fast magic angle spinning (UF-MAS), present high potential methodologies to overcome the current analytical challenges in solid state materials characterization and provide numerous avenues for investigating pharmaceuticals at a higher resolution and sensitivity. We will present a few pioneering development studies utilizing cutting-edge techniques in pharmaceutical sciences for structural investigation of small molecule drugs. For example, we have established a novel protocol for preparing DNP samples in an in-situ manner during spray drying and hot-melt extrusion, and achieved a 32-fold enhancement in signal to noise ratio which provides a reduction of ssNMR measurement time by ~1000 [1]. This approach enables efficient characterization of 1% drug-loading formulation and multidimensional ssNMR analysis of natural isotopic abundance pharmaceutical materials. In addition, we have published the first documented case utilizing ultrafast MAS at 60 kHz to investigate drug-polymer interactions [2]. We have also extended these investigations to amorphous solid dispersion of posaconazole and investigated site-specific molecular interactions and dynamics of these formulations. Interestingly, posaconazole exhibits a distinct interaction pattern and strength when formulated with different polymers (i.e., HPMCAS and HPMCP). These newtechniques allow us to comprehensively compare the structure of solid dispersion intermediates produced by different techniques including spray drying and hot-melt extrusion. Moreover, amorphous-amorphous phase separation, is a known issue for amorphous solid dispersion. Identification and quantification of phase-separated domains with sizes < 100 nm is technically challenging. We have successfully utilized relaxation ssNMR techniques to semi-quantitatively evaluate the API-polymer miscibility [3-4] These studies help to establish the correlation between molecular miscibility and various processing parameters and facilitate the optimization of process conditions.

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Identification and Quantification of Hydrates of a Drug Product using Solid-State NMR

Dewey H. Barich[1], Lyndsey Knight[2], Tran N. Pham[2], and Andy J. Edwards[2]

1. GlaxoSmithKline Global Spectroscopy, Collegeville, PA, US
2. GlaxoSmithKline Global Spectroscopy, Stevenage, UK

Drug substances can exist in many different forms, including polymorphs, solvates, and hydrates. These forms can exhibit different physical properties which can impact manufacturability, bioavailability, and stability. Identification and quantification of undesired forms is necessary. The authors here present a case in which the desired form is a hemihydrate that can convert to a monohydrate form. To assess degree of conversion, a solid state NMR technique was developed on drug substance. The technique was extended to drug product where the drug substance is only 50 mg of a 600 mg tablet.

NMR Crystallography Advancements for Exploring Polymorphism

Renny Mathew[1], Ivan Sergeyev[2], Melanie Rosay[2], Fabien Aussenac[3], Werner Maas[2], Chris J. Pickard[4], Bart Kahr[5], and **Maria Baias**[1]

1. Division of Science, New York University Abu Dhabi, Abu Dhabi, UAE
2. Bruker BioSpin, Billerica, Massachusetts, USA
3. Bruker BioSpin, Wissembourg, France
4. Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK
5. Chemistry Department, New York University, New York, USA

The development of NMR crystallography, a method that employs a combined experimental and computational approach for structural elucidation of powdered crystalline materials, opened new paths into exploring pharmaceutical polymorphism [1,2]. Solid-state NMR investigation of pharmaceutical drugs is faced with two main drawbacks: the low sensitivity of NMR experiments involving nuclei such as 13C, 15N at natural abundance and long 1H T1 relaxation times of many pharmaceuticals. The consequence of these cumulative effects is a very long experimental time for signal averaging required to obtain sufficiently high signal-to-noise ratio in two-dimensional NMR spectra, which are essential for the unambiguous chemical shift assignment of the investigated structure.

Our research is centered on further developing NMR crystallography by using new experimental and computational techniques that are more appropriate for studying complex molecular crystals. Experimentally we make use of the developments in Dynamic Nuclear Polarization (DNP)-enhanced solid-state NMR [3] to reduce the experimental time, allowing us to tackle more complex structures. Computationally we focus on tailoring the Ab Initio Random Structure Searching (AIRSS) [4] protocol to establish a DFT-based crystal structure prediction method for predicting new polymorphs of organic molecular crystals.

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3. Rosay, M.; Blank, M.; Engelke, F.; J. Magn. Reson. 264, 88-98, 2016
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Structural studies on Cu(II) and Co(II) complexes and their free-ligands by Solid-State NMR, Solution-State NMR and X-Ray spectroscopy

Ayelen F. Crespi [1], Yamila Garro-Link[3], Daniel Vega[4] and Juan M. Lázaro-Martínez [1,2].

1. Department of Organic Chemistry, UBA, FFyB, Buenos Aires, Argentina
2. IQUIMEFA-CONICET, Buenos Aires, Argentina
3. FaMAF/IFEG-CONICET, UNC, Córdoba, Argentina
4. CNEA, Buenos Aires, Argentina

Cu(II) and Co(II) ions are central atoms of complexes with heterocyclic alcohol and aldehydes that are relevant as catalysts, such as for degradation of organic pollutants present in effluents. Cu(II) complex with organic ligands are used to activate H_2O_2 that can produce $OH^{\bullet}$ via the Cu (II)/Cu (I) cycle involving different reaction pathways [1]. This is of particular interest because allows the replacement of traditional inorganic oxidants such as $K_2Cr_2O_7$ for benign ones as H_2O_2 and O_2. Taking this into account, we decided to use heterocyclic compounds containing the *gem*-diol functional group obtained from the hydration of carbonyl groups [2,3], to develop new coordination complexes. Most of the time, the stability of the functional group is not studied in the free ligand before the preparation of the metal complex. Understanding the chemistry and the coordination properties of *gem*-diols is decisive for the development of synthetic methods to obtain new organic ligands and new coordination complexes. Usually, solution-state NMR and X-ray spectroscopy are used to study the structure and to understand the chemistry of the metal complex. Although, obtaining single-crystals is not always possible.

For that reason, in this work, not only we develop new metal complexes of Cu(II) and Co(II) containing *gem*-diol and formyl groups in pyridine derivatives, but also, we propose a structural study combining solid-state and solution-state NMR together with single-crystal X-ray spectroscopy to characterize the Cu(II) and Co(II) complex and its free ligand. By using these NMR methodologies, we are able to approximate the functionalization present in various monomers used as the number of molecules per unit cell in cases where the samples do not yield single crystals.

Here, we used 3-formylpyridine, 4-formylpyridine and di-(2-pyridyl)ketone as ligands for the coordination of copper/cobalt ions. Previously, all the isomers were studied by solution-state and solid-state NMR using different strategies to favor the *gem*-diol or carbonyl moiety in order to try to isolate them, as they quickly revert to the aldehyde or ketone that originated them. The ^{1}H-NMR spectra in CD_3OD of di-(2-pyridyl)ketone shows that 85% of the ligand is in the carbonyl form and 15% is in the *gem*-diol form. Interestingly, by X-Ray diffraction studies we could see that in the coordination complex with Cu(II) which is prepared in methanol, the ligand is in the *gem*-diol form. Based on these results, we studied the di-(2-pyridyl)ketone in CD_3OD adding different amounts of copper ions by *ls*-NMR, and the relation between the *gem*-diol and the carbonyl group stays the same being majority product the ketone. Besides, we observed that the resolution of the ^{1}H-NMR spectra become poor and the line widths are larger as the copper concentration increases. The most affected signals are those that belong to the *gem*-diol. This is due to the paramagnetic effect of the copper that induces hyperfine shifts of NMR signals and shortening of nuclear longitudinal (T_1) and transverse (T_2) relaxation times. The closer the copper is to the protons, the greater the paramagnetic effect. Considering the X-ray structure, we observed that it coincides that the protons that are most affected are those involved in the copper complex. In this sense, the ketone form of the di-(2-pyridyl)ketone was present in its Cu-complex as a remainder uncoordinated compound according to the ^{13}C

CP-MAS and the solution-state NMR spectra. So, *ls*-NMR together with ss-NMR can be a very useful tool to approximate the structure of the complex in those cases where we do not have the single-crystal. Additionally, the ^{1}H-MAS and 2D ^{1}H-^{1}H SQ-DQ *ss*-NMR spectra (@60 kHz) can also bring structural information about the ligands surrounding the paramagnetic center.

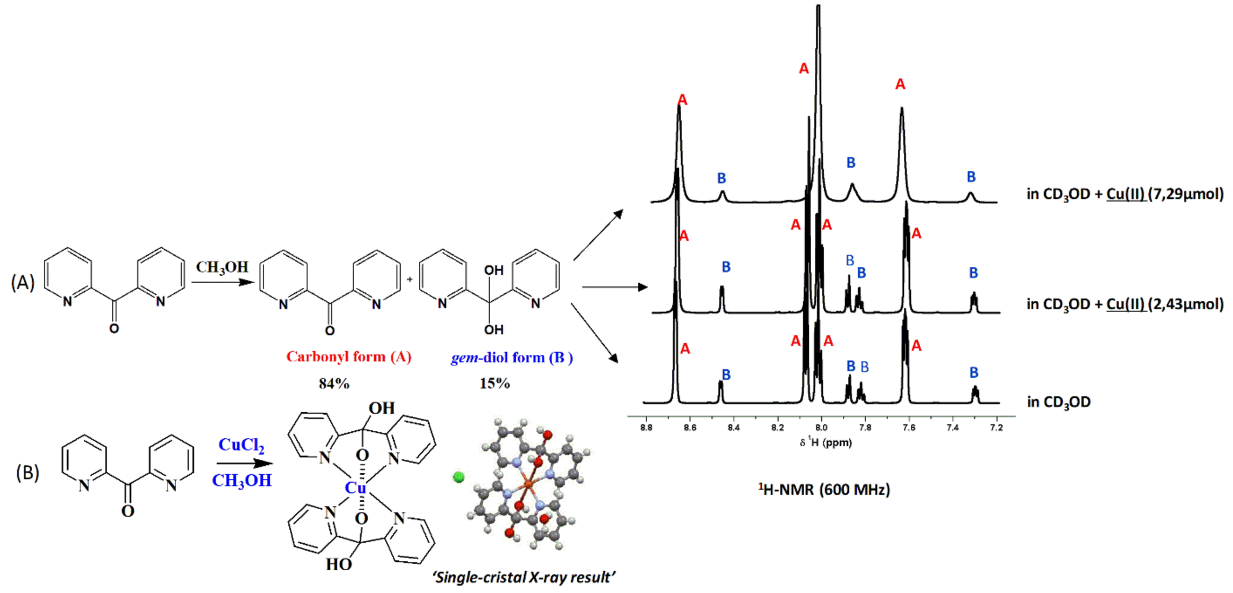

Figure 1. ^{1}H-NMR spectra in CD_3OD for di-(2-pyridyl)ketone (0.163 mmol) with 2.43 µmol of Cu (II), 7.29 µmol of Cu (II) and without Cu(II) (A). X-ray diffraction result for the Cu(II)-complex (B).

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Wednesday, September 19th
02:00 PM – 03:30 PM

Workshop

Chemometrics and NMR Data

Coordinator: Beata Mickiewicz

Chemometrics and NMR Data

Coordinated by: Beata Mickiewicz

Nowadays, NMR (Nuclear Magnetic Resonance) spectroscopy and MS (Mass Spectrometry) techniques are the most common platforms used in metabolomics studies. NMR spectroscopy benefits from being very specific, quantitative and highly reproducible. Despite lower sensitivity, a current NMR instrumentation allows to detect significant and unique changes in metabolic profiles between studied cohorts. The number of small-molecule metabolites detected in a biofluid, cell lysate, tissue or organ ranges from several dozen to several hundreds. Therefore, to analyze such big and very often heterogenic datasets specific statistical analysis tools need to be implemented. The most popular methods applied for metabolic data analysis are principal component analysis (PCA) and partial least squares (PLS) analysis. The PCA and PLS methods aim to differentiate between studied cohorts in highly complex data sets, despite a large background and the within class variability. During “The chemoMATRIX” workshop these multivariate analysis methods will be briefly described, along with a discussion of their application to metabolomics, with an emphasis on common errors and misconceptions. Additionally, participants will be introduced to the on-line available resources for the multivariate data analysis. Don’t wait, join the chemoMATRIX!

Wednesday, September 19th
04:00 PM - 05:10 PM

Metabolomics: Making Metabolic Sense of Mixtures

Chair: Aalim Weljie

Speakers:

Art Edison
University of Georgia

Qiuwei Xu
Merck

Fatema Bhinderwala
University of Nebraska

New Approaches to *in vivo* Metabolomics

Arthur S. Edison[1,2], Michael Judge[1], Yue Wu[1], Goncalo Gouveia[1], and Jonathan Arnold[2]

1. Complex Carbohydrate Research Center, University of Georgia, Athens, GA US
2. Genetics Department, University of Georgia, Athens, GA US

Neurospora crassa is a filamentous fungus with a circadian clock. Evidence from single-cell measurements show that cells can synchronize their oscillations, suggesting the possibility that small molecule(s) mediate the cell-to-cell communication [1]. We are attempting to measure the metabolome of *N. crassa* to identify candidates for circadian synchronization. Our initial experiments followed the traditional study design of making 3 replicates of large cultures that were synchronized at 4-hour intervals for a 48 hour period. This resulted in a rich, but noisy, dataset with the usual problems of alignment and normalization that are part of most NMR metabolomics experiments. These traditional results have been ambiguous because of high variance between replicates.

We recently began taking an alternate approach. Using a Bruker HR-MAS CMP probe operating at 600 MHz, we have been able to record the growth of a culture of *N. crassa* with 10 min resolution over several days. The resulting data are extremely rich in detail and require no alignment or normalization. We can obtain kinetic growth curves for individual resonances over the course of the experiment, and this rich dataset only requires a small 80 μL culture made with no sample preparation.

This experiment opens up several new opportunities but also involves some challenges. We will show the use of ^{13}C labeling to monitor the flux of metabolites over time, and we will describe approaches we are taking to model *N. crassa* metabolism using the detailed data from these experiments. The major challenge is oxygenation of the sample. *N. crassa* can grow under anaerobic (fermenting) conditions, but to link it with the bulk of the circadian data collected from this organism, we need to conduct the experiments under aerobic conditions. We will outline approaches we are taking to solve this problem. We have begun to apply these techniques to other organisms and mammalian cell cultures.

Acknowledgements: This work was supported by the NSF (MCB-17137460) and the Georgia Research Alliance. We thank all members of the UGA “clock” group for stimulating discussions. Jackie Walejko assisted in HR-MAS experiments.

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Enrich Quantitative Chemicals to Support Metabolomics Application in Bio/Pharmaceutical R&D

Qiuwei Xu

Merck Research Laboratories, West Point, PA, US

Metabolomics is gaining its acceptance and broad utilization in following changes of metabolomes or microbiomes [1-4]. Its most significant contribution is often its complementary role in working seamlessly with other tools such as genomics and proteomics to assist, for example, development of biomarkers or improvement of disease treatments [5-8].

As a powerful tool to metabolomics, NMR can readily quantify all detectable chemicals non-destructively with minimum sample preparation. Since proton is the most abundant and sensitive nucleus, an 1D proton NMR spectrum is naturally most efficient to profile metabolomes or microbiomes. To increase the number of chemicals that can be quantified, it is desirable to streamline automated acquisition of a large number of spectra with consistent quality, and develop intelligent software to identify and deconvolute individual chemicals in an efficient manner.

In this talk, I will summarize the operation of NMR metabolomics support to our drug development, and illustrate with examples of its application to the investigation of biochemical mechanisms of toxicities in preclinical animal models or *in vitro* cell models.

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Expanding the metabolomics toolkit with heteronuclear NMR

Fatema Bhinderwala[1,2] Thomas Smith[1] Samantha Lonergan[1] Chunyi Zhou[3] Martha Morton[1] Paul Fey[3] Robert Powers[1,2]

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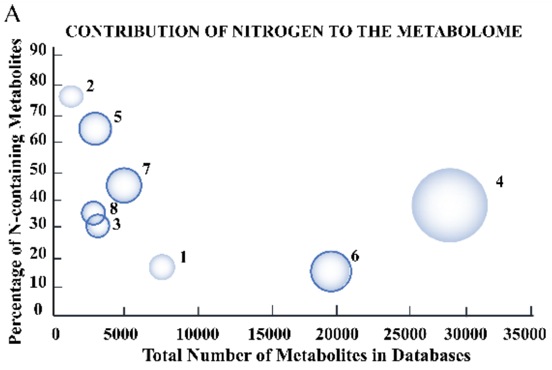
2. Nebraska Center for Integrated Biomolecular Communication
3. Center for Staphylococcal Research, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA 68198-5900

Isotopically labeling a metabolite and tracing its metabolic fate has provided invaluable insights about the role of metabolism in human diseases in addition to a variety of other issues. ^{13}C-labeled metabolite tracers or unlabeled ^{1}H-based NMR experiments are currently the most common application of NMR to metabolomics studies. Unfortunately, the coverage of the metabolome has been consequently limited to the most abundant carbon-containing metabolites.

Firstly, to expand the coverage of the metabolome and enhance the impact of metabolomics studies, we present a protocol for ^{15}N-labeled metabolite tracer experiments that may also be combined with routine ^{13}C-tracer experiments to simultaneously detect both ^{15}N- and ^{13}C-labeled metabolites in metabolic samples.[1].A database consisting of 2D ^{1}H-^{15}N HSQC natural abundance spectra of 54 nitrogen-containing metabolites are also presented to facilitate the assignment of ^{15}N-labeled metabolites. The methodology is demonstrated by labeling *Escherichia coli* and *Staphylococcus aureus* metabolomes with ^{15}N$_1$-ammonium chloride, ^{15}N$_4$-arginine, and ^{13}C$_3$-acetate. Efficient ^{15}N and ^{13}C metabolite labeling and identification was achieved utilizing standard cell culture and sample preparation protocols. Given that more than 30% of a metabolome **(Figure 1)** consists of nitrogen containing metabolites, we have demonstrated that a significant enhancement in the coverage of the metabolome may be achieved by utilizing ^{15}N-labeled metabolites with ^{15}N-based NMR experiments. Secondly, we extend this by implementing a ^{31}P-^{1}H HMBC approach to identifying phosphorus containing-metabolites present in cell lysates and biofluids. We are developing an optimized protocol to ease identification of nucleotide analogs using an optimized decoupled 2D^{1}H-^{13}C HMBC and 2D ^{1}H-^{31}P HMBC experiments.



B

No.	Databases	Total	N-Metabolites	% N-Metabolites
1	BMDB	7971	1284	16.1
2	BMRB	1323	967	73.1
3	ECMDB	2912	1000	34.3
4	foodDB	28800	10680	37.1
5	HMDB	3036	1899	62.6
6	MMCD	20236	3010	14.9
7	plantcyc	5142	2242	43.6
8	RikenPRIME	3230	971	30.0

Figure 1: (A) Bubble plot showing the contribution of nitrogen-containing metabolites to each of the metabolome databases. Bubbles are numbered and correspond to the rows in the table listed in B. (B) The number of nitrogen-containing metabolites in each of the databases

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Wednesday, September 19th
05:10 PM - 05:30 PM

Generation of High-quality NMR data using the NMReDATA format and top-resolution 2D NMR spectra of natural products

Damien Jeannerat
University of Geneva

Generation of High-quality NMR data using the NMReDATA format and top-resolution 2D NMR spectra of natural products

Damien Jeannerat

Chemistry Department, University of Geneva, Geneva, Switzerland

The NMR community is missing sources of NMR data for organic compounds in general and natural products in particular. An *Initiative* [1] gathering specialists of structure determination, cheminformatics, NMR software developments and journal editors recently introduced a new file format called “NMReDATA” [2] to report the NMR parameters extracted from “full NMR analysis”. A file contains the structure of the assigned molecule, the extracted chemical shifts, scalar couplings, and the lists of 2D correlations from COSY, HSQC, HMBC, (etc.) spectra. Associated with the NMR spectra, they constitute an “NMR record” which can be sent to journal to facilitate the reviewing and benefit the community with open user-friendly and computer-accessible data when articles are published. Many NMR software editors and providers of NMR databases and services made the commitment to introduce the NMReDATA format in their platforms.

The potential of the new format to change the NMR data landscape will be discussed. Besides the obvious benefit of providing a source of NMR data, the new format will open up opportunities to development tools to correct, improve, validate, certify these data. More specifically:

- An assignment could be automatically validated by checking the consistency of 2D correlations with the provided structure.
- The extracted NMR parameters (typically scalar coupling constants and chemical shifts) could be refined and complemented using spectral simulation including second-order effects, and could treat magnetic equivalence.
- The 3D structure could be determined or verified through DFT calculations or other predictor of chemical shifts, scalar couplings, etc.

We shall also present how the value of the NMReDATA can be significantly increased when including data from recent NMR spectra such as pure-shift, homonuclear 2D spectra recorded with homodecoupling in F1 [3] and other methods improving resolution in the carbon dimension of 2D heteronuclear spectra. [4]

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6	Guilherme Dal Poggetto	New Analysis Tool for 3D Diffusion NMR Experiments
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1

Development of a ^{13}C and ^{15}N Chemical Shift Prediction Procedure Using Density Functional Theory Calculations

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Density functional theory with the gauge invariant atomic orbital method (DFT-GIAO) has been utilized as a powerful and accurate tool in the prediction of NMR spectra for a diverse set of organic and bio-organic molecules.[1]

This study provides a systematic investigation of 13C[2] and 15N[3] chemical shift prediction employing DFT-GIAO methods.[4] Benchmarking analysis was carried out, incorporating several density functionals and basis sets commonly used for prediction of NMR chemical shifts, from which the B3LYP/cc-pVDZ level of theory was found to provide accurate results at low computational cost. Generalized linear scaling parameters for the conversion of isotropic shielding constants to calculated 13C and 15N chemical shifts were obtained from linear regression analyses based on calculations performed on structurally diverse organic molecules. Systematic errors were observed locally for key functional groups, and correction factors were determined. Statistical analyses performed on large data sets of 13C and 15N NMR chemical shifts enable an objective estimation of confidence level in proposed structure.

This computational process, associated correction factors and statistical parameters has shown sucessful applications in the structural assignment of natural products and therapeutically relevant compounds in cases where experimental data yielded inconclusive or ambiguous results. Overall, the use of B3LYP/cc-pVDZ with linear scaling and correction terms affords a powerful and efficient tool for structure elucidation.

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4

Assessment of the Performance of the DP4 Method and Subsequent Enhancements for Stereochemistry Problems

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The accurate and efficient determination of the relative stereoconfiguration of organic compounds is among the most challenging problems that NMR spectroscopists routinely encounter. There are several approaches to solve these problems, such as analysis of through-space correlations (i.e., NOEs) and scalar couplings. However, conclusions in certain cases can be ambiguous, especially for flexible structures. Over the past decade, density functional theory (DFT) calculations of chemical shifts have achieved a high level of accuracy, and consequently DFT calculations are often employed to bolster confidence in stereochemical assignments.

The DP4 method1, which combines DFT with Bayesian statistics, has been applied in the literature to determine relative stereochemistry of more than 200 rigid natural products. In 2015, Sarotti and coworkers2 improved on this method by employing a more accurate functional for NMR shieldings, DFT optimized geometries, and by accounting for systematic errors that correlated with orbital hybridization. Their modified DP4 method2 successfully predicted the stereochemistry of 42 out of 48 challenging structures: including flexible molecules and natural products with highly strained geometries.

We noted that further improvements could be garnered by using separate functionals for proton and carbon chemical shift predictions, including an implicit solvent correction to the optimized geometries, and more accurately determining the Boltzmann population of conformers using energies calculated from either a dispersion-corrected or a double hybrid functional. Finally, we evaluated the performance of all three DP4 methodologies for several challenging examples from the literature.

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5

Studies of Phase Equilibria and Batch Distillation with Benchtop NMR Spectroscopy

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Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for the investigation of complex liquid multicomponent systems [1-2]. It can resolve even chemically similar compounds and, in contrast to optical spectroscopy, no calibration is needed for determining the composition of the mixture. Furthermore, process can be investigated in-situ with NMR spectroscopy. Standard high-field NMR spectrometers require superconducting electromagnets that are cryogenically cooled. These instruments are expensive and sensitive and require a special laboratory infrastructure. Recently, compact benchtop NMR spectrometers have become available. They use permanent magnets with a medium field strength which still enables taking spectra that are well-suited for quantitative evaluation [3]. These benchtop NMR spectrometers are comparatively inexpensive, flexible, and robust, and have the potential to be used for the monitoring of industrial processes in the future.

In this work, two examples for the application of benchtop NMR spectrometers in thermodynamic studies are presented. The first application is the determination of liquid-liquid equilibria (LLE). Using benchtop NMR spectroscopy, the composition of both phases can be determined in a very simple manner by changing the position of the sample tube in the bore of the spectrometer. The results agree very well with literature LLE data for different ternary test systems. In a second application, the benchtop NMR spectrometer was connected to a laboratory batch distillation unit and was used for continuously monitoring the liquid phase composition. Different ternary mixtures were studied and the results were compared to results obtained from conventional sampling and GC analysis. Excellent agreement was found. The resulting residue curves describe the vapor-liquid equilibrium in the studied ternary system and are an interesting alternative to studies with conventional vapor-liquid equilibrium measure¬ments.Abstract

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6

New Analysis Tool for 3D Diffusion NMR Experiments

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In diffusion-ordered spectroscopy (DOSY),[1] one of the most commonly used NMR experiments in the analysis of mixtures, the amplitude (or integral) of each signal from an array of 1D spectra is fitted to an appropriate variant of the Stejskal-Tanner equation,[2] and displayed as a Gaussian peak in a second dimension which represents diffusion coefficient. DOSY is commonly used to identify signals from different components in a mixture. However, signal overlap can obscure diffusion information and complicate spectral interpretation. An elegant solution to minimise signal overlap in the spectral dimension is to add a diffusion dimension to a 2D (or higher dimensionality) NMR experiment, generating a 3D (or higher) DOSY spectrum.[3] This provides a major reduction in the probability of overlap, since cross-peaks are spread out over a plane rather than along a single axis. Here we introduce a new Matlab® based toolbox, for analysing 3D diffusion NMR data, which complements the recently introduced GNAT ("General NMR Analysis Toolbox",[4] the former DOSYToolbox[5]). Within this new environment it is possible for the first time to analyse 3D diffusion data (*e.g.* 3D DOSY-HSQC, as shown in the examples below) using both univariate and multivariate methods in a user-friendly graphical interface (GUI).

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7

A Multi-Outcome Experiment Involving the Oxidation of Alcohols in the Undergraduate Teaching Laboratories

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^{1}H NMR analysis is an important analytical technique presented in introductory organic chemistry courses. NMR instrument access is limited for undergraduate organic chemistry students due to the size of the instrument, price of NMR solvents, and the maintenance level required for instrument upkeep. The University of Georgia Chemistry Department currently employs benchtop ^{1}H NMR instruments in each of the undergraduate organic laboratories. These instruments can sit on a standard lab bench, can analyze samples without expensive NMR solvents, and are easily maintained. At the University of Georgia, undergraduate organic chemistry students traditionally perform a laboratory procedure for the oxidation of an unknown alcohol. This experiment was modified to utilize environmentally sustainable components, such as household bleach, as the oxidizing agent. Herein is described a multi-outcome experiment in which one unknown secondary alcohol (2-pentanol, 3-pentanol, or 3-methyl-2-butanol) is oxidized using bleach. Each student pair was assigned one unknown alcohol from a list of possible alcohols and analysis of their oxidation product was accomplished using benchtop ^{1}H NMR spectroscopy. Students interpreted their spectra and deduced the identity of the unknown alcohol they were assigned. This incorporation of modern analytical technology in the undergraduate teaching laboratories provides a tangible framework for student understanding of the utility of ^{1}H NMR spectroscopy.

8

NMR and DFT Analysis of Tailored Conformations for Flexible Small Molecules

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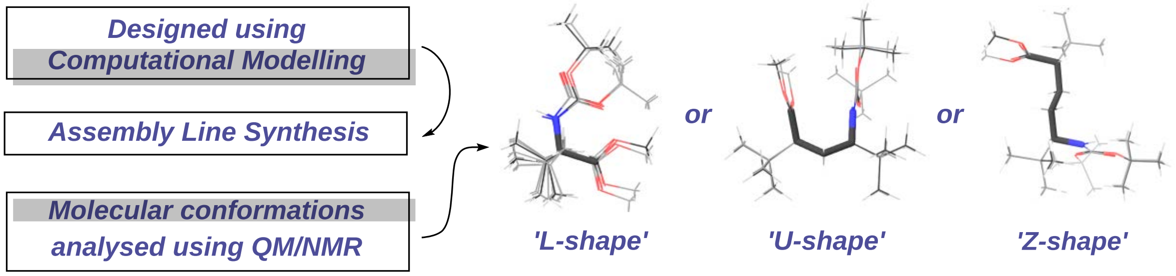
The conformational behaviour of small molecules influences their chemical properties and biological activities. However, the design of flexible, and yet conformationally controlled small molecules with optimal molecular function is challenging. It is hindered by the complexity of studying dynamic conformations in solution, our understanding of conformation design, and a paucity of stereoselective synthetic methods for highly flexible molecules.[1]

By exploiting the conformational control exerted by quaternary centres[2], a range of unnatural amino acids were designed and synthesised. Their solution state conformations were elucidated by a combination of Density Functional Theory calculations and quantitative analysis of $^{n}J_{HH}$, $^{n}J_{CH}$ and NOE derived interproton distances. The side chains are shown to take on defined orientations (0° - ‘U-shaped’; 120° - ‘L-shaped’, 180° - ‘Z’-shapeed) with controllable distances that could mimic peptide binding moieties.



The ability to predict and probe flexible molecules’ solution state behaviour using a combination of computational modelling and NMR spectroscopy, together with advanced synthetic methodology[3] should allow us to create molecules with bespoke conformations for drug design and materials applications.

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Solution Ensemble Analysis of Macrocycles

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Macrocycles are key drug leads for protein targets with large, flat and featureless binding sites, including protein-protein interfaces. Due to their conformational flexibility macrocycles typically exist as a mixture of interconverting geometries in solution, and hence cannot be represented by a single, averaged conformation. This flexibility is a result of continuously forming and breaking a number of weak intramolecular interactions. The yielded conformations in solution vastly impact the bioactivity, solubility and membrane permeability of the macrocycles. Therefore, describing their conformational ensembles, as well as the impact of conformation stabilizing weak interactions, is of fundamental importance, and the knowledge gained is directly applicable to medicinal chemistry.

In order to describe macrocycle structure and dynamics, time-averaged solution spectroscopic data has to be deconvoluted into the present conformations along with their respective probability. We have studied the solution ensembles of a series of macrocycles using the NAMFIS (NMR analysis of molecular flexibility in solution) algorithm. This combined computational and spectroscopic ensembles analysis deconvolutes time averaged NMR data by identifying the real conformations and assigning them with their molar fractions. Theoretical ensembles were predicted using Monte Carlo conformational searches with molecular mechanics minimization. The generated ensembles, typically containing 40-150 conformers, were then used together with experimental NOE-based distances and *J*-coupling-based dihedral angles to identify the molar fractions of the conformations present in solution.

We applied this technique to gain understanding of weak chemical interactions in a biologically relevant environment, by analyzing macrocyclic β-hairpin peptides. The stabilizing effect provided by an interstrand weak interaction, as compared to a reference peptide lacking this interaction, was quantified through ensemble analysis. We have shown that a single interstrand hydrogen [1,2,3] or halogen bond (Figure 1) [4], can significantly influence the folding, and increase the population of the folded conformation by up to 40%. The NMR results were corroborated by CD-spectroscopy and MD-calculations.

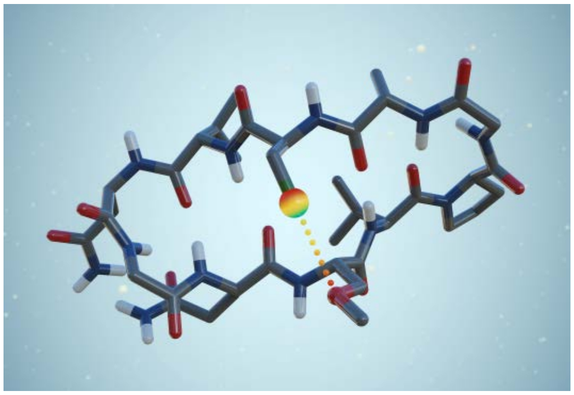

Figure 1. A macrocyclic β-hairpin peptide stabilized by a chlorine centered halogen bond.

Further, we have used the β-hairpin design for peptide inhibitors of the MDM2/p53 protein-protein interaction. Using ensemble analysis we have demonstrated that in contrast to common beliefs conformational flexibility is vitally important for the bioactivity of these macrocyclic inhibitors [5]. Similarly, we have identified the bioactive conformation of macrocyclic azapeptide inhibitors of the cluster of differentiation 36 (CD36) receptor, analyzed their dynamics and have shown that, in contrast to MDM2/p53 interaction inhibitors, rigidity promotes their bioactivity. Hence, for one target, rigidification into a presumptive bioactive conformation increased potency, whereas for the other it yielded decreased potency. Our results demonstrate that a balanced flexibility is important for the bioactivity of macrocyclic drugs.

Furthermore, we have correlated the cell permeability of orally available macrocyclic drugs to the their conformational ensembles in hydrophilic and hydrophobic environment, further demonstrating the huge impact of conformational dynamics and the importance of its proper description and understanding [6].

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Intramolecular Hydrogen Bonding Induced Stable Conformations in Benzoyl Phenyl Oxalamide Derivatives: Extensive NMR Studies and DFT Based Computation

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A number of benzoyl phenyl oxalamide derivatives have been synthesized and characterized by the extensive utility of one- and two-dimensional NMR experimental techniques. The manifestation of intramolecular hydrogen bonds in all the synthesized molecules, convincingly established using NMR studies, governs the stable conformations of the molecules. NMR experiments, viz., solvent dilution, temperature perturbation, $^{15}N{-}^{1}H$ HSQC and $^{19}F{-}^{1}H$ HOESY, have been exploited to derive unambiguous confirmation about the participation of organic fluorine in the hydrogen-bonding interaction. In the fluorine substituted molecules, the coupling between two NMR active nuclei mediated through hydrogen bonds, of significant strengths, have been detected. The measured chemical shift difference of an NH proton has been employed to calculate the energy of the HBs. NMR analysis revealed the electrostatic nature of the hydrogen bonds in all of the molecules. The NMR experimental findings have been validated using Density Functional Theory (DFT)-based Non Covalent Interactions (NCIs) and Quantum Theory of Atoms In Molecules (QTAIM) computations.

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Dynamic NMR Investigation of Asymmetric ^{195}Pt Satellites of Hydride in Stannylphosphine Complexes

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Platinum complexes with bidentate phosphine ligands $Ph_2P-(CH_2)_n-PPh_2$ exhibit varied regioselectivity and rates as catalysts for olefin hydroformylation and other processes [1-2]. While developing the chemistry of potential new catalysts, the platinum tin hydride complexes $L_2PtHSnPh_3$ (L_2=dppe, dppp) (Figure 1), we discovered that the hydride region of the proton NMR spectrum of $dpppPtHSnPh_3$ exhibits an apparent fluxional behavior in both THF-d_8 and C_6D_6. At room temperature in C_6D_6, a peculiar asymmetry in the appearance of the platinum satellites (Figure 2, ^{1}H NMR, 298K) piqued our interest, leading us to pursue variable temperature NMR experiments exploring this behavior. We observed changes in the asymmetry of these peaks in both THF-d_8 and C_6D_6 at various temperatures. Variable temperature NOESY and ROESY experiments suggest this process to be intramolecular and kinetic studies show that the rate of the exchange depends on the solvent as well as the concentration of $HSnPh_3$. We will report on our findings of the dynamic NMR studies of this surprisingly complex system, supplemented with density functional theory calculations (scalar relativistic PBE0/jcpl [3], Table 1) of the relevant Pt, Sn, H and P coupling constants.

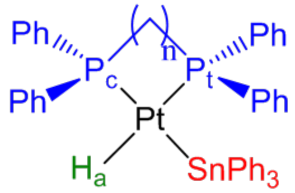


Figure 1
$dppePtHSnPh_3$ (n=2)
$dpppPtHSnPh_3$ (n=3)

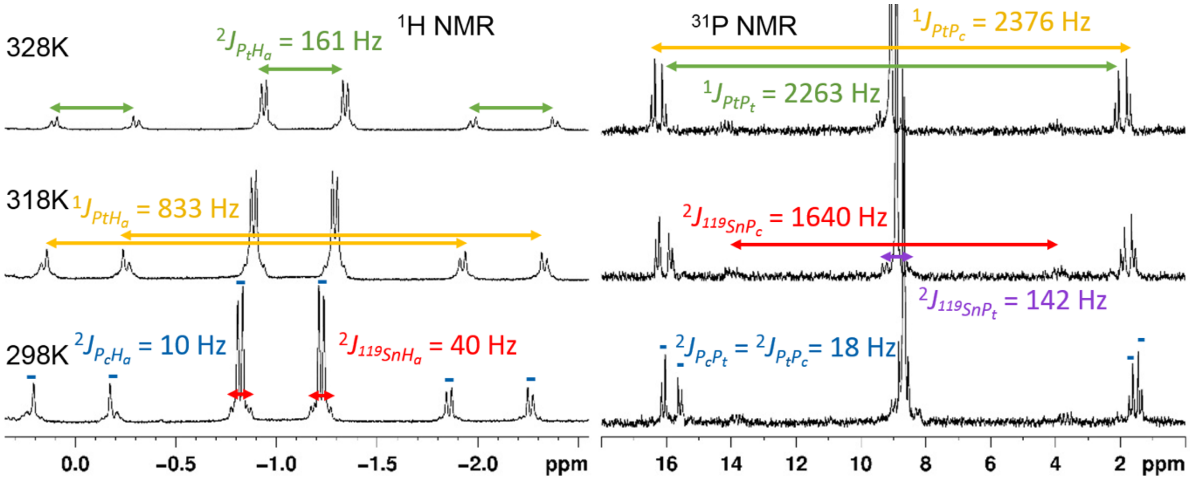
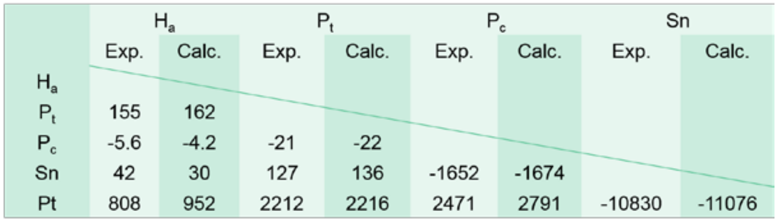


Figure 2 ^{1}H NMR (hydride region, left) and $^{31}P\{^1H\}$ NMR (right) of $dpppPtHSnPh_3$ in C_6D_6



	H_a		P_t		P_c		Sn	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
H_a								
P_t	155	162						
P_c	-5.6	-4.2	-21	-22				
Sn	42	30	127	136	-1652	-1674		
Pt	808	952	2212	2216	2471	2791	-10830	-11076

Table 1. Experimental (183 K) and DFT calculated coupling constants of $dpppPtHSnPh_3$ in THF-d_8 in Hz.

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12

The Shielding Effect of the Double Bond in 2-Norbornene and α- and β-Pinene

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The shielding effects of double bonds have been less well characterized than those of aromatic systems due in large part to the difficulty in observing this effect in isolation [1-3]. The effects are small and highly dependent on orientation and distance. Therefore, small rigid systems are needed with well-defined geometries. 2-Norbornene fits these criteria with a pair of bridge protons that provide one indication of deshielding (H-7syn) and also two pairs of methylene protons, with one pair aligned in the plane of the □-orbitals (H-5,6exo) and another well outside of the plane (H-5,6endo) [4-7]. NOE experiments were used to confirm the assignment and determine the relative proximities of these protons. Additionally, chemical shift and NOE comparisons were made for □- and □-pinene and their saturated counterparts, □- and □-pinane. Model compounds demonstrate that the shielding effect of double bonds can be described by shielding cones that corresponds to the magic-angle cone (55° 44') of solid-state NMR spectroscopy [7]. Shielding occurs inside the cones while deshielding occurs outside of the cones.

A series of "bis-norbornenes" in which one norbornene system has been fused to a second norbornene unit has also been investigated. One particular proton was especially interesting because it is located almost precisely on a horizontal line with respect to the 2,3-double bond and slightly less than 2 Å from it. A 1.49-ppm chemical shift difference between these geminal protons is one of the largest ever recorded for bridge protons in norbornyl systems and certainly demonstrates that double bonds are capable of producing large anisotropic effects when the molecular geometry is both rigid and optimum [8].

A second group of compounds in which double-bond shielding effects have been examined comprises isomeric α-pinene and β-pinene. Abraham et al. reported apparent shielding of the equatorial 9-methyl-groups in α- and β-pinene, which are located above and relatively near the 2,3-double bonds and were uncertain of the anisotropic properties of cyclobutyl groups [10]. Abstract

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Simple NMR Method for Assessing Aromatic Fluorination using Pure Shift NMR and CRAFT

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Aromatic fluorination is a critical step towards the development and manufacture of pharmaceuticals. Fluorination of drugs may protect them from metabolism and, as a result, often have elevated levels of activity and slower clearance [1]. However, reaction of fluorinating agents or elemental fluorine with an aromatic ring can yield a mixture of mono- and/or multifluorinated isomers at ortho, meta and para positions. The distribution of isomers in the mixture can be difficult to assess by standard ^{1}H NMR spectroscopy due to overlapped and crowded aromatic multiplets. Instead, components of the product mixture are separated and quantified by gas chromatography [2, 3]. We present here a simple NMR method to differentiate and quantify the products of aromatic fluorination using pure shift (PS) PSYCHE NMR [4] and CRAFT (Complete Reduction to Amplitude and Frequency Table) [5] analysis.

The method is illustrated using monofluorinated anilines, benzonitriles and benzotrifluorides. PS NMR is known to simplify ^{1}H spectra by removing the ^{1}H couplings with no effect on heteronuclear coupling and provides a simple way to measure heteronuclear coupling constants [6, 7]. The resulting PS spectra for 2F- isomers appear as one ^{1}H doublet (H3) with three-bond coupling ($^3J_{HF}$), two ^{1}H doublets (H4, H6) with four-bond coupling ($^4J_{HF}$) and a ^{1}H singlet (H5). This pattern codes for an ABCD-X spin system where X represents the ^{19}F spin. In contrast, PS spectra of 3F- compounds consist of two ^{1}H doublets (H2, H4) with three-bond coupling ($^3J_{HF}$), a ^{1}H doublet (H5) with four-bond coupling ($^4J_{HF}$) and a ^{1}H singlet (H6). This pattern codes for an ABCM-X spin system. Due to symmetry, the PS spectra of 4F- compounds consist of a 2-proton doublet (H3, H5) with three-bond coupling ($^3J_{HF}$) and a 2-proton doublet (H2, H6) with four-bond coupling ($^4J_{HF}$). This pattern codes for an A_2B_2-X (or AA'BB'-X) spin system. Mixtures of 2F-, 3F- and 4F-aniline yield overlapped and crowded multiplets in standard ^{1}H NMR spectra. Their PS spectra in contrast yield 2 unique singlets (2F- and 3F- isomers) and a doublet (4F- compound) that are well separated in shift from other doublet signals. Estimates of isomer distribution can hence be simply derived in the frequency domain by choosing a phase and a baseline and measuring peak heights or integrating peak areas for 2 singlets and a half-doublet in the PS NMR spectra. More accurate quantitation that is independent of phase or baseline choice can be achieved using CRAFT analysis to obtain signal amplitudes in the time domain [5]. The pure shift NMR data can be recorded in ~ 5 to 10 minutes with concentrations typical of organic chemistry reactions (~1 mM). This simple NMR procedure potentially allows rapid and direct assessment of aromatic ring fluorination for *in situ* reaction monitoring without separation of isomers. With rapid assessment of isomer distribution, reaction conditions can be quickly changed to tune for higher yields of the desired isomer.

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Combined in situ NMR-UV/Vis-Illumination Spectroscopy: A Novel Mechanistic Approach in Photochemistry

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Synthetic applications in photochemistry are booming. Despite great advances developing new reactions, details about the underlying mechanisms are often challenging to come by. Here, a fully automated *in situ* triple combination of NMR spectroscopy, UV/Vis spectroscopy and illumination to allow simultaneous and time resolved detection of paramagnetic and diamagnetic species is presented as a novel tool in photochemistry [1]. This optical fiber based setup enables the acquisition of combined UV/Vis and NMR spectra in photocatalysis under continuous or pulsed illumination. This is demonstrated on a consecutive photoinduced electron transfer (conPET) [2] process, which generates stable, paramagnetic radical anions which are usually inaccessible by conventional NMR spectroscopy. Further, the broad applicability of combined UVNMR spectroscopy for light induced processes is presented on a structural and quantitative analysis of a commercially available photoswitch [3] including rate modulation and stabilization of transient species by temperature variation. Besides the two examples, the flexible setup regarding NMR hardware, temperature and light sources is presented in detail.

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15

Improving INADEQUATE by F1 Spectral Aliasing using Prior Knowledge

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The INADEQUATE experiment determines the carbon-carbon backbone of a molecule directly. It is extremely helpful for the structure elucidation of small molecules, but is rarely used due to its inherently low sensitivity [1,2]. Modern hardware improvements such as increased B_0 fields and cold probes or microcoil probes can improve the sensitivity, but the INADEQUATE experiment remains underused.

The sensitivity of a 2D NMR experiment can be improved by focussing the acquisition on signal-containing regions of the spectrum, allowing more scans per increment to be acquired in a given amount of instrument time. A simple way is to reduce the number of increments in t_1 while reducing the F_1 spectral width to compensate, maintaining the digital resolution. Doing so causes aliasing, which can make some regions of the spectrum crowded and unassignable. However, if the 1D carbon spectrum is already known, then it is possible to estimate which spectral widths are likely to work well, remaining free of overlap. Jeannerat [3, 4] and Lescop *et al.* [5] have developed an approach for heteronuclear correlation experiments; here, we present an application to INADEQUATE:

We have developed a program which "guesses" a molecular structure based on the resonance positions in a 1D ^{13}C spectrum, and computes the INADEQUATE correlations at a given spectral width. From this, it determines the required digital resolution in F_1 and hence the optimum number of t_1 increments in order to fully resolve all F_1 correlations for that molecule at that spectral width. This procedure is then repeated for a large number of molecules across a range of spectral widths. The data is then analysed statistically to find the optimal spectral width for the given line list. An advantage of this method is that we can incorporate prior knowledge to improve the guessed structure (e.g. if some peaks are known to come from CH_3 groups then only a single correlation is permitted), which in turn improves the accuracy of the simulations.

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Structural studies on Cu(II) and Co(II) Complexes and Their Free-Ligands by Solid-State NMR, Solution-State NMR and X-Ray spectroscopy

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Cu(II) and Co(II) ions are central atoms of complexes with heterocyclic alcohol and aldehydes that are relevant as catalysts, such as for degradation of organic pollutants present in effluents. Cu(II) complex with organic ligands are used to activate H_2O_2 that can produce $OH^\bullet$ via the Cu (II)/Cu (I) cycle involving different reaction pathways [1]. This is of particular interest because allows the replacement of traditional inorganic oxidants such as $K_2Cr_2O_7$ for benign ones as H_2O_2 and O_2. Taking this into account, we decided to use heterocyclic compounds containing the *gem*-diol functional group obtained from the hydration of carbonyl groups [2,3], to develop new coordination complexes. Most of the time, the stability of the functional group is not studied in the free ligand before the preparation of the metal complex. Understanding the chemistry and the coordination properties of *gem*-diols is decisive for the development of synthetic methods to obtain new organic ligands and new coordination complexes. Usually, solution-state NMR and X-ray spectroscopy are used to study the structure and to understand the chemistry of the metal complex. Although, obtaining single-crystals is not always possible.

For that reason, in this work, not only we develop new metal complexes of Cu(II) and Co(II) containing *gem*-diol and formyl groups in pyridine derivatives, but also, we propose a structural study combining solid-state and solution-state NMR together with single-crystal X-ray spectroscopy to characterize the Cu(II) and Co(II) complex and its free ligand. By using these NMR methodologies, we are able to approximate the functionalization present in various monomers used as the number of molecules per unit cell in cases where the samples do not yield single crystals.

Here, we used 3-formylpyridine, 4-formylpyridine and di-(2-pyridyl)ketone as ligands for the coordination of copper/cobalt ions. Previously, all the isomers were studied by solution-state and solid-state NMR using different strategies to favor the *gem*-diol or carbonyl moiety in order to try to isolate them, as they quickly revert to the aldehyde or ketone that originated them. The ^{1}H-NMR spectra in CD_3OD of di-(2-pyridyl)ketone shows that 85% of the ligand is in the carbonyl form and 15% is in the *gem*-diol form. Interestingly, by X-Ray diffraction studies we could see that in the coordination complex with Cu(II) which is prepared in methanol, the ligand is in the *gem*-diol form. Based on these results, we studied the di-(2-pyridyl)ketone in CD_3OD adding different amounts of copper ions by *ls*-NMR, and the relation between the *gem*-diol and the carbonyl group stays the same being majority product the ketone. Besides, we observed that the resolution of the ^{1}H-NMR spectra become poor and the line widths are larger as the copper concentration increases. The most affected signals are those that belong to the *gem*-diol. This is due to the paramagnetic effect of the copper that induces hyperfine shifts of NMR signals and shortening of nuclear longitudinal (T_1) and transverse (T_2) relaxation times. The closer the copper is to the protons, the greater the paramagnetic effect. Considering the X-ray structure, we observed that it coincides that the protons that are most affected are those involved in the copper complex. In this sense, the ketone form of the di-(2-pyridyl)ketone was present in its Cu-complex as a remainder uncoordinated compound according to the ^{13}C CP-MAS and the solution-state NMR spectra. So, *ls*-NMR together with ss-NMR can be a very useful tool to approximate the structure of the complex in those cases where we do not have the single-crystal. Additionally, the ^{1}H-MAS and 2D ^{1}H-^{1}H SQ-DQ *ss*-NMR spectra (@60 kHz) can also bring structural information about the ligands surrounding the paramagnetic center.

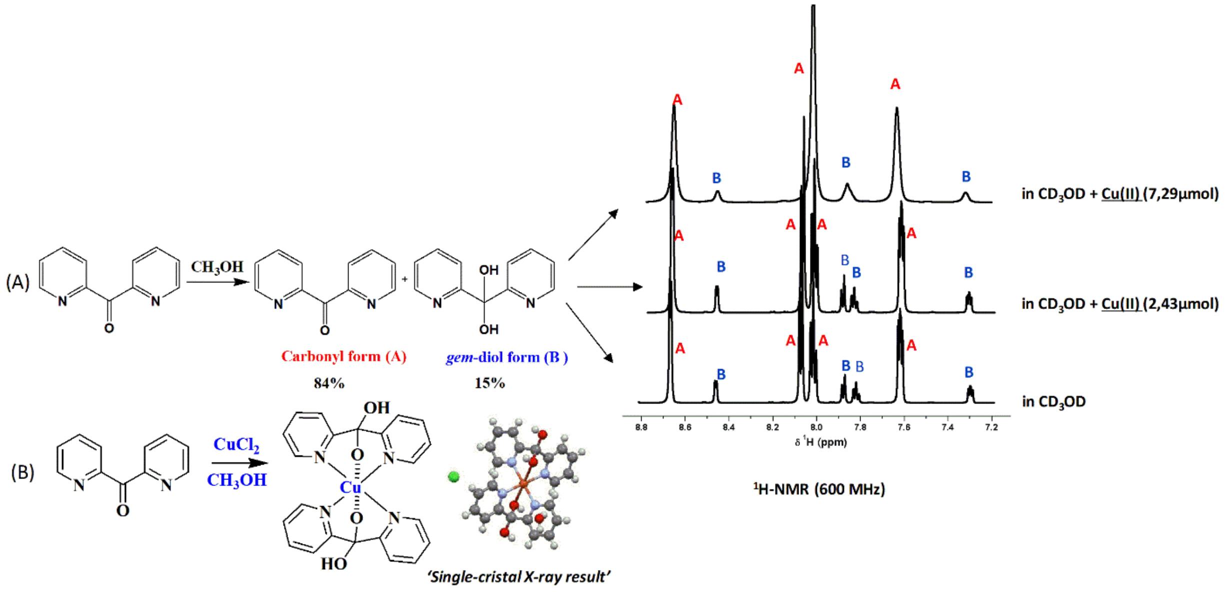

Figure 1. ^{1}H-NMR spectra in CD_3OD for di-(2-pyridyl)ketone (0.163 mmol) with 2.43 µmol of Cu (II), 7.29 µmol of Cu (II) and without Cu(II) (A). X-ray diffraction result for the Cu(II)-complex (B).

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The Quantitative Determination of H-F Internuclear Distance using ^{1}H-^{19}F 2D-HOESY

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It is well known that the conformation of a molecule is equally important as its consitution, with the conformation of a molecule often playing a crucial role in the biological and/or chemical activity of the molecule. The importance of conformation is particularly true when designing therapeutic molecules, with most drug molecules needing to access a certain conformation for binding to its receptor. If a large conformational reorganisation is required to access the bioactive conformation then weaker binding of the drug will result. However, deducing the conformational landscape of a molecule in solution is not a simple task. The principal method for investigating the conformation of a molecule in solution is to use NMR spectroscopy. NMR parameters such as HH and HC coupling constants can provide valuable information on the conformation of a molecule, due to the Karplus relationship that $^{3}J_{HH}$ and $^{3}J_{HC}$ values have with dihedral angle however, the quantitative determination of internuclear distances using NOE within the molecule provide the most useful information on deducing conformation.

A number of groups, including the Butts group[1], the Macura group[2] and the Krishnamurthy group[3] have refined the quantitative determination of interproton distances using PANIC and have shown that this methodology can be applied to increasingly flexible molecules with impressive accuracy. Fluorine is routinely incorportated into pharmaceutical molecules due to improved pharmacokinetic properties of fluorine containing drugs with nearly one-third of small molecule drugs containing at least one fluorine atom. Therefore, useful information about the conformation of many pharmaceutical molecules could be gained from internuclear HF distances. Previous work by Claridge demonstrated that 1D-$^{1}H^{19}F$ NOE could be used to accurately obtain quantitative internuclear distances.[4] This piece of work explores the use of 2D-$^{1}H^{19}F$ HOESY to obtain quantitative internuclear distances.

We aim to explore how we can use a 2D-$^{1}H^{19}F$ HOESY spectra to obtain HF internuclear distances to the same level of accuracy that has been observed when using NOE to extract interproton distances. To obtain quatntitative interproton distances to high levels of accuracy, PANIC is typically applied to correct for cross relaxation pathways. This project will explore how a PANIC type methodology can be applied to 2D-$^{1}H^{19}F$ HOESY to account for cross relaxation and accurately obtain quantitative HF internuclear distances.Abstract

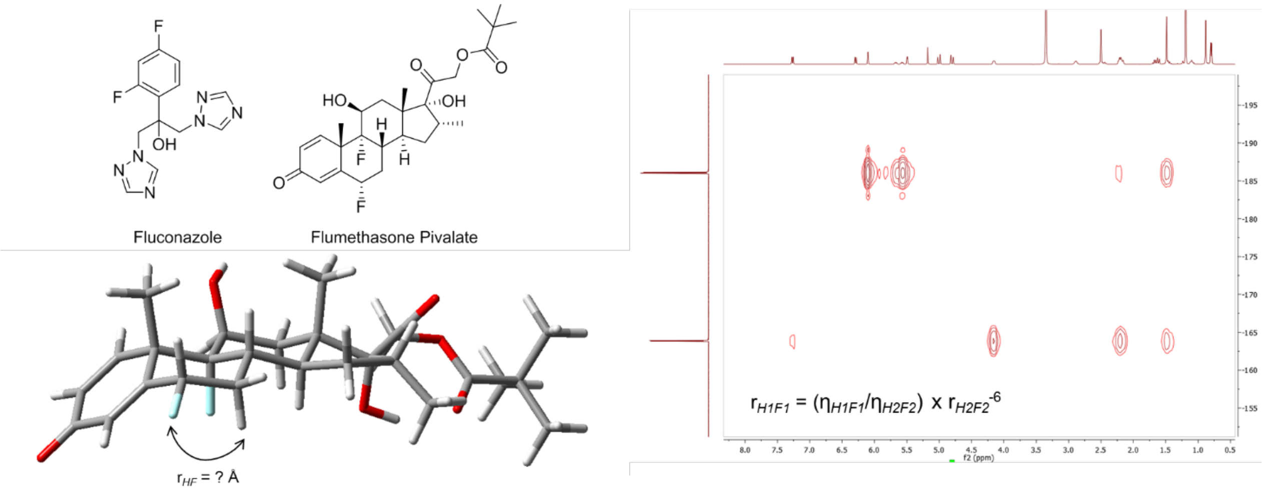

Figure 1. Two fluorinated drug molecules that we may employ in the investigation of using 2D-^{1}H^{19}F HOESY to obtain quantitative interproton distances.

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Combining NMR Spectroscopy, Computation and Synthesis to Design Conformationally Controlled Molecules

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Enforcing conformational control into flexible molecules is not a trivial challenge, however, through incorporating destabilising interactions into the unwanted conformations of a molecule it is possible to conformationally bias flexible molecules. A destabilising interaction that has been used previously to design molecules with tailored shapes is the *syn*-pentane interaction. The *syn*-pentane interaction will result in a roughly 3.5 kcal mol^{-1} energy penalty for a conformer, reducing its Boltzmann population considerably and thus conformationally biasing the molecule. The Aggarwal and Butts group at the university of Bristol employed this interaction to synthesise hydrocarbon molecules with tailored shapes. The molecules contained ten contigous stereochemically defined methyl groups, synthesised using Prof. Aggarwal's iterative assembly line synthesis, and depending on the relationship of the methyl groups the molecules could be conformationally biased into either a helical or linear conformation. The solution state conformation was confirmed using a combined NMR spectroscopy and computational approach in which DFT calculated interproton distances and scalar coupling constants were compared to experimental values.[1]

Recently, medicinal chemists have become more aware of the importance of molecular conformation on drug binding. If the unbound drug has to undergo significant conformational changes to access the bioactive conformation, a weaker binding affinity will result. If it is possible to conformationally preorganise a molecule so that it preferentially adopts the bioactive conformation then stronger binding should result. One field of therapeutics in which conformation is highly important is the inhibition of protein-protein interactions (PPIs) through the design of α-helix mimetics.[2] Typically α-helix mimetics are large molecules with many degrees of freedom however, they need to exhibit conformational control to ensure that they reproduce the key angular relationships and distances observed in the α-helix. We hope to explore whether the helical and linear scaffolds described above can be used as α-helix mimetics since it has been found that the methyl groups on one face of the scaffold reproduce the i, i +3/4 and the i + 7 positions of an α-helix. Through a combination of NMR spectroscopy, computation and synthesis we aim to design an inhibitor of the p53-Mdm2 PPI. We envisage carrying out an iterative lithiation-borylation sequence using the appropriate substituents at judicious points in the sequence to design a hydrocarbon framework that is capable of mimicking p53.[3] The solution state conformation of the molecule will be investigated using a combined NMR and computational approach, comparing experimental interproton distances derived from 1D-NOE and scalar coupling constants to those calculated at the DFT level of theory. EXSIDE and IPAP-HSQMBC will be used to extract ${}^{n}J_{CH}$ coupling constants.

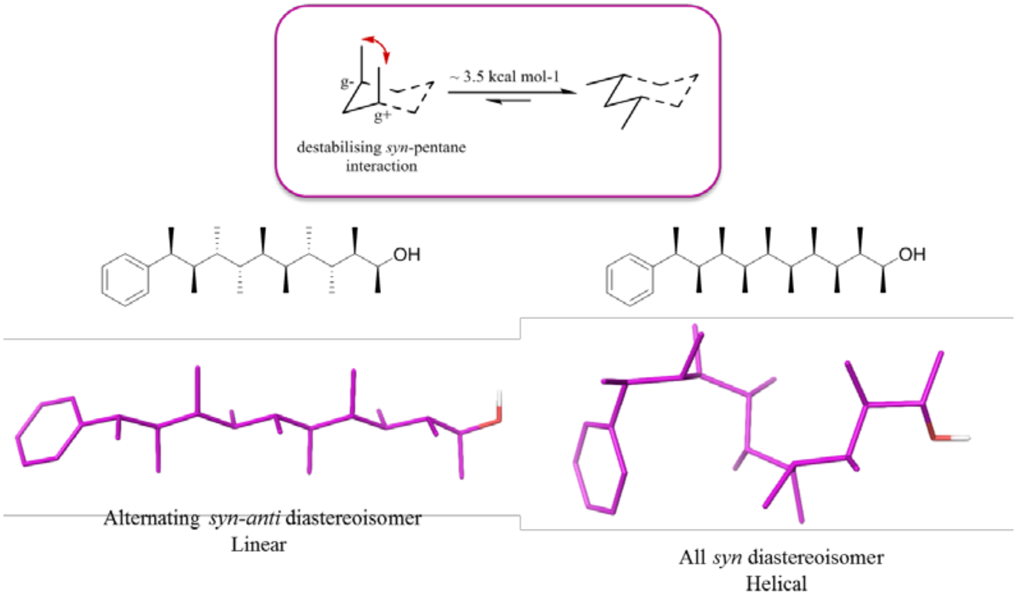

Figure 2. The helical and linear scaffolds investigated previously at the University of Bristol. The conformation of the molecules is controlled due to the avoidance of destabilizing syn-pentane interactions.

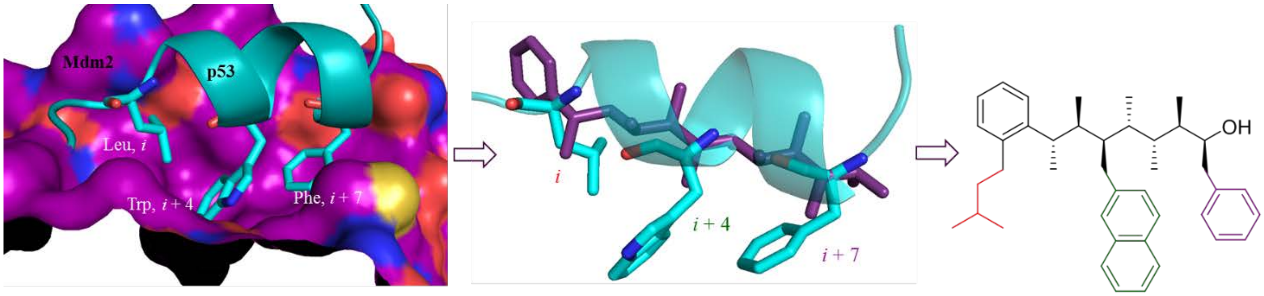

Figure 3. The design of an inhibitor of the p53-Mdm2 PPI using a combination of NMR spectroscopy, computation and synthesis.

We hope that this work will serve as a strong example of how NMR spectroscopy, when combined with computation, can be used to gain valuable information regarding the conformational landscape of flexible molecules and will encourage drug discovery chemists to consider using this approach to understand more about the unbound conformation of drug molecules. During this project we also want to explore the limits of the combined NMR and computational methodology used previously. It has been applied to increasingly flexible systems over recent years with great success, however we hope to explore whether this methodology can be used to investigate the conformation of a model peptide-drug conjuagte, in collaboration with AstraZeneca. Peptide-drug conjugates are an emerging class of therapeutics, however they often exhibit poor conformational control likely due to their size and flexiblity. We aim to use a variety of NMR methods, together with combined Molecular Mechanics/Quantum Mechanics calculations, to explore the conformation of a model peptide-drug conjugate. Once the conformation has been determined, we will investigate whether we can install conformational bias into the peptide-drug conjugate through the design of a linker, enforcing conformational control through the avoidance of *syn*-pentance interactions.

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Co-Conformational Mobility of Amide-Based [2]Rotaxanes by Dynamic NMR Spectroscopy

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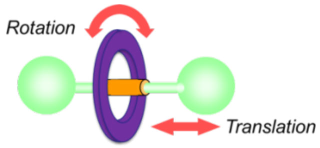
Research on the synthesis and applications of [2]rotaxanes, the most abundant category among the different types of mechanically-interlocked molecules (MIMs), has been a hot topic in many scientific areas in the last years [1,2]. These molecules are composed by a dumbbell-shaped component threaded through a ring with non-covalent bonding interactions, endowing the molecule with a unique kind of functionality due to the mechanical bond [3]. This particular linkage gives the feasibility for controlling their internal dynamic motions of rotation and translation (Figure).



Hydrogen bond interactions are known to assembly and stabile various supramolecular complexes, such as these [2]rotaxanes. Our research group is focused in the highly efficient-directed synthesis of amide based [2]rotaxanes [4], first discovered by Leigh and co-workers [5]. For this purpose, one the most important stages is the study of the affinity of the macrocycle for the binding sites embedded into the axis. In the Leigh-type rotaxanes, this affinity is related with rotational motion of the macrocycle around the axle [6].

Herein, we describe the synthesis of tetraamide-based [2]rotaxanes having different tetraalkyldicarboxamide-based templates, the dynamic NMR study of the rotational motion of their interlocked macrocycles, and the kinetics of the corresponding dethreading processes leading to the axis extrusion. These data will be compared with the corresponding analogues reported in literature.

Acknowledgments

This work was supported by the MINECO (CTQ2017-87231-P) with joint financing by FEDER Funds from the European Union, and Fundación Seneca-CARM (Project 19240/PI/14). F.M. also thanks the Fundación Seneca-CARM for her Saavedra Fajardo contract and funding (Contract No. 20025/SF/16).

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NMR-spectroscopic Investigations of Transition States in Brønsted Acid Catalysis

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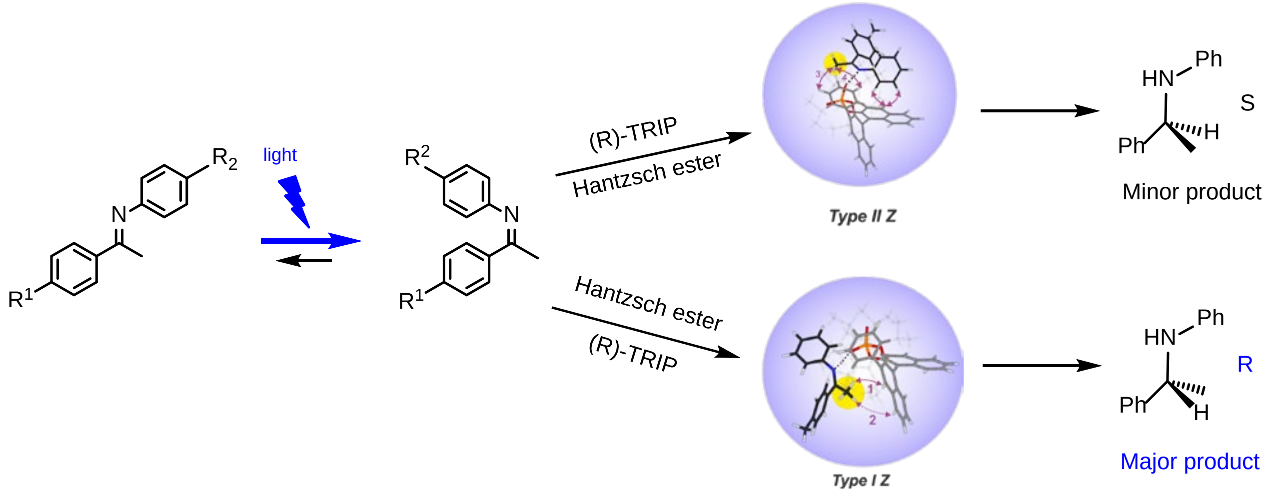
Recently, chiral Brønsted acids, like (R)-TRIP, have become more and more important as catalysts and have been used in several enantioselective transformations, like Mannich-type reactions [1] or asymmetric transfer hydrogenations of imines [2]. Most of the mechanistic studies in this field were done by theoretical calculations [3-7]. To the best of our knowelege, a direct experimental proof of the transition states was not possible. Our group developed a method, which uses photoisomerization of the imine double bond as mechanistic tool to decrypte transition states by light (DTS-hν) [8]. Because the isomerization is the rate-determining step we assumed that changes in rate and/or enantiomeric excess upon illumination create a characteristic fingerprint pattern, which is associated with a particular transition state. In our case we could show that photoisomerization of the imine double bond increases the yield of the amine. This result indicates that the Z-transition state, which is higher populated under irradiation, is the reactive one [8].



In all these reactions the activation by phosphoric acid catalysts takes place via hydrogen bonds. Hence, the characterization of the occuring hydrogen bond interactions [9] as well as structural investigations of the binary phosphoric acid-imine-complex [10] are indispensable to get more insight into the reaction mechanism. The NMR-spectroscopic investigations on hydrogen bonds of small molecules are very challenging because of the fast intermolecular exchange, which leads to averaged ^{1}H-signals of all hydrogen bond species. Very low temperature stabilizes the different hydrogen bond species. The slow hydrogen bond exchange regime is reached at 180K, which allowes the NMR-spectroscopic investigation of the occurring hydrogen bonds in small molecules.

Moreover, the proton transfer from the donor to the acceptor is an interesting process. The potential energy surface of the proton can be depicted as single-well or double-well potential. By replacing the hydrogen bond proton partially by a deuteron and measuring the H/D isotopic effect on the chemical shift, we could experimentally show that our phosphoric acid-imine-complexes are in agreement with an asymmetric single-well potential [9].

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11.

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Cross-Linked α,ω-Bisacrylated Poly(ethylene oxide) - An Enhanced Tunable Alignment Medium for the Measurement of Anisotropic NMR Parameters

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In high resolution NMR, residual anisotropic parameters like Residual Dipolar Couplings (RDCs), Residual Chemical Shift Anisotropies (RCSAs) and Residual Quadrupolar Couplings (RQCs) have proven to provide valuable information for the structure refinement of biomacromolecules [1] as well as for small organic molecules [2] and are still subject of ongoing research [3]. Homogeneous and appropriate weak partial alignment to avoid extensive line-broadening is needed, induced with so-called alignment media. Two types of alignment media, liquid crystalline phases and mechanically strained polymer gels, are commonly used to partially align solutes. However, common alignment media presently available are either specifically developed for application with small organic molecules or for big macromolecules. Furthermore, their use is restricted to certain solvents.

Earlier investigations with poly(ethylene oxide) hydrogels (PEO), that were covalently cross-linked by irradiation with β- or γ-rays, showed considerable swelling in wide variety of solvents [4] ranging from polar, like water, to apolar solvents, like toluene. We now have established an alternative with α,ω-bisacrylated PEO (PEO-DA), which can easily be cross-linked by free radical polymerization avoiding the use of radiation sources. Moreover the procedure offers control over the distribution of inter cross-link chain lengths, yielding quite homogeneous gels with narrow linewidths when low dispersity macromers are used as starting material. PEO-DA not only allows inducing scalable anisotropy both in compressing as well as in stretching devices, but additionally has been succesfully applied with pure solvents and mixtures. In accordance with parameter fine-tuning, cross-linked PEO-DA is suitable for the alignment of solutes reaching from small natural products up to proteins, making it a universal alignment medium. Here, we present the versatile medium based on covalently cross-linked poly(ethylene oxide) diacrylate and describe which considerations have to be taken into account for the measurement of residual anisotropic parameters in terms of synthesis and dimensioning the gel sticks on the basis of selected examples.

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NMR Mechanistic Studies of Visible Light Mediated Selective Single C(sp3)-F Bond Cleavage in Trifluoromethylarenes

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In our publication we report the first example of selective single C(sp^3)-F functionalization of trifluoromethylarenes via the combination of visible light catalysis and Lewis acid activation.[1] The conversion of easily accessible trifluoromethylarenes into aryl difluoromethyl compounds, which is of high interest in pharmaceutical chemistry, is atom- and step-economic. However, the single C(sp^3)-F bond cleavage of ArCF$_3$ is a challenge because of the inert chemical property of the C-F bond and the difficult selectivity control of mono-defluorination. The here developed method allows good chemo-selectivity control and shows a broad functional group tolerance. [1]

In order to get insight into the mechanism of the single C-F bond activation, we applied ^{1}H, ^{11}B, ^{19}F and 2D NMR spectroscopy. The reaction was moreover followed by direct illumination inside the NMR spectrometer according to Feldmeier *et al.*[2] As shown in the proposed catalytic cycle in Figure 1A the formation of the borenium cation TMP-Bpin$^+$ **4'** was identified by ^{11}B NMR via the comparison of the illuminated reaction mixture and the independently synthesized TMP-Bpin$^+$ B(C$_6$F$_5$)$_4^-$ **4** (Figure 1B).[3] As the addition of the *in-situ* generated borenium cationic species to the photo reduction system yielded the desired mono-fluorinated product, this species is suggested to be the key intermediate for the C-F bond cleavage acting as fluoride scavenger.

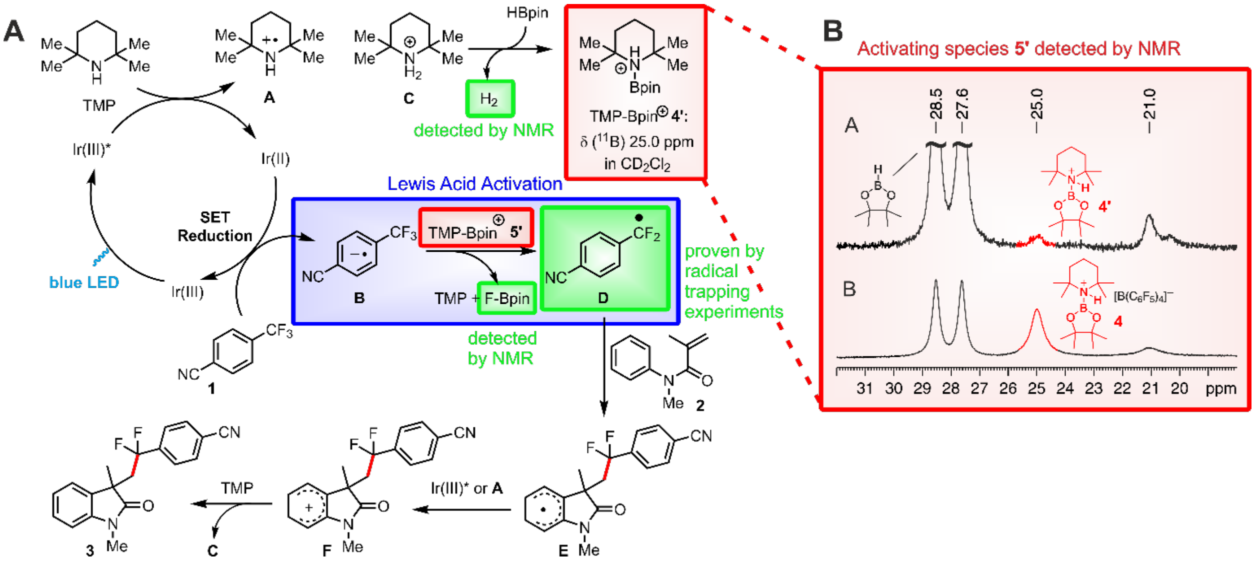

Figure 1: Proposed mechanistic cycle for the selective C-F bond activation combining photocatalysis and Lewis acid activation.[1]

Furthermore, we generally observed an interaction between TMP-Bpin$^+$ **4’** and the non-charged CF_3 substrate **1** via chemical shift change analysis of ^{1}H, ^{19}F and ^{11}B spectra and ^{1}H,^{1}H NOESY and ^{1}H,^{19}F HOESY experiments. As the reduced substrate **B** formed during reaction is characterized by a drastically higher hydrogen bond acceptor property a hydrogen bond mediated activation mechanism is therefore probable.[4,5]

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Empirical Equations for $^3J_{CH}$ Prediction: Introducing a General Bond Angle Correction.

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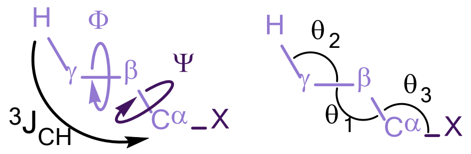
Existing empirical equations for three-bond proton-carbon scalar couplings ($^3J_{CH}$) either target specific coupling pathways, such as those common in carbohydrates [1], peptides/proteins [2] and nucleic acids [3], or more general equations accounting for β/γ [4] or α [5] substituents for H-C-C-C coupling pathways. To expand the breadth and improve the accuracy of empirical methods we have used density functional theory (DFT) to calculate $^3J_{CH}$ for >500 different molecular fragments while varying the dihedral angle(s) (Φ and/or Ψ). These DFT-calculated $^3J_{CH}$ were then used to identify and parameterize suitable equations relating $^3J_{CH}$ to the dihedral angles for each fragment.



A correction for the effects of bond angle ($\theta_{1\text{-}3}$) on $^3J_{CH}$ was generated by using DFT to calculate scalar couplings for over 1,000 organic molecules using 3D molecular structures selected from the Cambridge structural database (CSD). This resulted in a dataset of over 23,000 DFT-calculated $^3J_{CH}$ with a range of dihedral angles, bond angles, substituents and coupling pathways, which were used to fit the equation below:

$$^3J_{CH} = {}^3J_{CH}\text{ (Fragment-based approach)} \times (B_1\Delta\theta_1 + B_2\Delta\theta_2 + B_3\Delta\theta_3 + C)$$

Where $\Delta\theta_n$ is the difference between the mean bond angle ($\bar{\theta}_n$) from the corresponding fragment and the bond angle from the molecule (θ_n): $\Delta\theta_n = \bar{\theta}_n - \theta_n$.

The performance of the empirical equations was tested against 102 experimentally measured $^3J_{CH}$ (as shown in the table below). This fragment-based approach achieved an accuracy of ~0.9 Hz with a >2800-fold time saving relative to performing full DFT calculations to determine $^3J_{CH}$.

Method	**DFT**	**Aydin *et al.* [6]**	**Palermo *et al.* [4]**	**This work.**
MAD / Hz	0.37	1.14	0.95	0.86
SD / Hz	0.44	1.38	1.06	1.04
Structural information required	Full Cartesian coordinates	Φ	Φ, β/γ substituent orientation and electronegativity	Φ, Ψ, $\theta_{1\text{-}3}$, coupling pathway α/β/γ substituent pattern

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Rapid Assessment of the Coordination Modes of Hemilabile Pincer-Crown Ether Ligands by Solution NMR Methods

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Hemilabile ligands contain weak donors that are able to reversibly bind a metal center, often providing an optimal balance of stability and activity.[1] Thus, many exceptional catalysts incorporate hemilability into ligand frameworks. Despite the successes of hemilabile scaffolds in catalysis, characterization of active catalytic species in solution is difficult. Hemilabile ligands can shift between multiple coordination modes, complicating characterization of the coordination sphere in solution. In this poster, a protocol differentiating coordination mode of hemilabile ligands by solution-phase NMR is introduced. Our group has synthesized a series of iridium pincer-crown ether complexes with a hemilabile crown-ether macrocycle in pentadentate and tetradentate binding modes (with two or one ethers bound to Ir, respectively) and tridentate binding modes, providing a convenient framework to systematically explore the influence of coordination mode on chemical shift.[2]

COSY, ^{1}H-^{13}C HSQC-TOCSY, and ^{1}H-^{13}C HSQC-TOCSY were used to assign overlapping protons in the crown region for 5 model complexes; ^{1}H-^{1}H NOESY and crystallographic studies were subsequently used to confirm the 3D structure in solution. Comparison of 3D structure and chemical shift demonstrated that chemical shift was strongly influenced by proximity to ligands bound to the metal center. These strong ligand effects on chemical shift were attributed to ligand anisotropy. Comparison with an array of 8 additional structures characterized by ^{1}H, COSY, and ^{1}H-^{13}C HSQC experiments revealed that the chemical shift difference between diasterotopic protons ($\Delta\delta$) reports on the proximity of the macrocycle to the metal center, allowing differentiation between high- and low-coordinate ligand binding modes with few NMR experiments.[4] Characterization of coordination mode enabled by these few NMR experiments enable rapid and routine assessment of metal complexes *in situ*, with applications in monitoring catalytic intermediates and decomposition. Furthermore, the protocol can be coupled with non-uniform sampling and pure shift NMR to reduce the acquisition time and increase resolution.

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Assessment of Uncertainty in Quantification with Benchtop NMR using Bayesian Analysis

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Erik von Harbou[2,3], Kerstin Münnemann[2], and Daniel J. Holland[1]

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Benchtop instruments make chemometric analysis with NMR spectroscopy affordable, however, the lower field strength of permanent magnets results in reduced spectral resolution and overlap. Furthermore, in most common one-pulse proton NMR experiments, higher-order homogeneous coupling effects often severely distort the common multiplet patterns and alter peak shapes. Therefore, accurate quantitative analysis of benchtop data poses considerable challenges for the traditional peak integration approach. To overcome these obstacles, we use a model-based quantification to describe quantum interactions between coupled nuclei. Hence, one can define a model for the entire spectrum of each chemical specie parameterized by the set of its chemical shifts and coupling constants. Importantly, such quantum mechanical models are inherently field-invariant and can be easily transferred to any field strength using the same parameter values (see Fig. 1). The accuracy of the assumed model, and its parameters, eventually determine the uncertainty of estimated concentrations.

In this work, we investigate the accuracy of the model-based quantification specifically for the analysis of data obtained with a 43 MHz spectrometer. Our probabilistic signal model is formulated in terms of Bayesian statistics and provides a principled way to include any prior knowledge about the studied system (such as positions and widths of the peaks). Furthermore, unlike with least-squares fitting, the result of Bayesian estimation is not a single number but an entire distribution, which allows us to formally estimate the uncertainty of quantification, e.g. in terms of confidence intervals. We identify and study the main factors that contribute to the resulting uncertainty: peak overlap, deviation in chemical shifts, coupling constants, and peak widths, as well as the influence of noise and lineshape imperfection. Our quantification method is particularly well suited to noisy data and spectra with overlapping peaks.

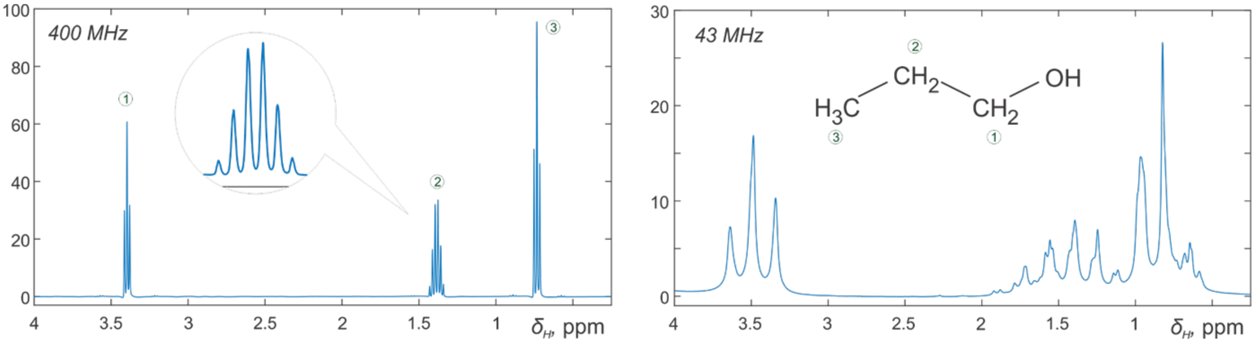

Figure 1. Spectra of 1-propanol in D_2O measured with superconducting and benchtop spectrometers. The appearance of the spectra in both cases, including severe higher-order distortions seen at 43 MHz, are determined by the same five parameters: three chemical shifts and two coupling constants.

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Sweet Vape May Not be Safe: Electronic Cigarette Degradation Profiles by ^{1}H NMR with and without Added Sucralose

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Sucralose is a synthetic sugar substitute commonly used in reduced calorie foods – and has been added to some electronic cigarette liquids (e-liquids) in an attempt to impart sweetness. Similar in structure to sucrose, sucralose differs in that it contains three chlorines and is perceived to be 400-700x sweeter (by weight) when consumed.[1] Sucralose is regarded as safe for consumption by the Food and Drug Administration as well as other sources,[2] but has not been approved for inhalation. Since commercial e-liquids currently do not list ingredients, it is difficult to assess how many consumers use sucralose-containing e-liquids. However, sucralose was present in some commercial e-liquids tested by our lab and is sold as an additive for consumers to add to any e-liquid. Fruit flavored e-liquids, which tend to be sweet and are more likely to contain sweeteners, are preferred by adolescents, but not by adults.[3]

^{1}H NMR has been used to study e-liquids as well as the degradation that can be produced when e-liquids are vaporized in e-cigarettes [4,5,6] since NMR is non-destructive and relative quantification is simple. The contents of an e-liquid/aerosol can be analyzed by NMR before and after being vaporized by an e-cigarette to detect the presence of new compounds (degradation products).

E-liquids are primarily composed of propylene glycol (PG) and glycerol (GL) in different ratios, and can contain smaller amounts of flavorants, sweeteners, and nicotine. Sucralose is one such additive that can be used in an attempt to sweeten e-liquids. In this work, the system was simplified to contain only PG, GL, and sucralose in order to more accurately determine the effect of sucralose on carrier solvent degradation during vaporization.

In order to evaluate coil diversity, three coils/tanks were tested using three different e-liquid compositions. Sucralose-free PG+GL, 0.05 mol% sucralose in PG+GL, and 0.10 mol% sucralose in PG+GL were tested in each coil/tank setup to determine the degree to which coil variability contributed to degradation profile changes. The concentrations of sucralose used were within the range of sucralose concentrations found in commercial e-liquids tested by our lab. Samples were collected using the standard CORESTA puffing method [7] as described previously, [8] and then tested by NMR.

It was found that 0.10 mol% sucralose significantly altered the degradation profile. The presence of aldehydes (propanal, acetaldehyde, glycolaldehyde, and acrolein), as well as propylene glycol and/or glycerol hemiacetals, were especially increased with the addition of 0.10 mol% sucralose. Changes in the production of other degradation products were also observed. A similar effect was seen for all three coils. Further studies are needed to determine the extent of the use of sucralose in commercial e-liquids by consumers, as well as to expand these studies to other e-cigarette devices.

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Metal-Free Route to Conjugated Enynes and Cyclic Nitronates: Mechanistic Investigations

Verena Streitferdt[1], Michael Haindl[1], Fabio Morana[1], Polyssena Renzi[1], Johnny Hioe[1], Felicitas von Rekowski[1], Alexander Zimmermann[1], Andreas Seegerer[1], Kirsten Zeitler[1] and Ruth M. Gschwind[1]

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Conjugated enynes can be subjected to different kinds of reactions e.g. ring-closing[1] and coupling reactions[2], or they can serve as transition metal ligands[3]. The moiety of conjugated enynes can be found in several natural products and pharmaceuticals.[4] Cyclic, five-membered nitronates are versatile building-blocks, which have been applied for the synthesis of biologically active compounds,[5] drug candidates[6] and natural products such as amino acids and amino sugars derivatives[7]. For the synthesis of cyclic nitronates, there already exist metal-free procedures.[8] In contrast, metal-free procedures to conjugated enynes are rarely found in literature.[9] Recent NMR investigations in our group on the organocatalytic nitroalkene dimerization with amines unveiled that this reaction can go beyond dimer formation.[10] Depending on the reaction conditions, different pathways can be followed leading to a metal-free conjugated enyne formation and/or to five-membered cyclic nitronates through a common central intermediate. *In situ* NMR reaction monitoring enabled the detection and characterization of key intermediates. In combination with quantum chemical calculations a mechanism for the formation of the cyclic nitronates and for the unusual build-up of the enyne triple bond out of a single bond could be proposed. In the reaction from nitroalkene dimers, product distribution (enyne versus nitronate) could be tuned according to the applied base and to the presence of acidic additives. Despite the promising aspects of the metal-free transformation to conjugated enynes, only moderate yields up to 55 % could be obtained because of polymerization, while cyclic nitronates were obtained in good yields (84%). Nevertheless, cheap and readily available nitroalkenes, amines (e.g. trimethylamine) and acids (e.g. benzoic acid) can be applied in the conjugated enyne formation under mild reaction conditions (room temperature).

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28

Dynamic Nuclear Polarization Enables Fast Flow NMR Reaction and Process Monitoring

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Nuclear Magnetic Resonance (NMR) spectroscopy is a non-invasive, quantitative and qualitative analysis method, established in a wide range of applications in medicine, physics, chemistry, and biology. NMR spectroscopy suffers from a lack of sensitivity due to the intrinsic low polarization of spins at ambient temperatures. To overcome this sensitivity issue many hyperpolarization techniques were developed in the last decades, e.g. Dynamic Nuclear Polarization (DNP). In the following, a mobile setup for continuous Overhauser DNP-enhanced NMR reaction and process monitoring is presented.

For Overhauser DNP, a transfer process of angular momentum between electron and nuclear spins, i.e. the use of stable radicals (electrons) is essential. After the hyperpolarization is accomplished the stable radicals are disturbing further applications or measurements of the sample, as they considerably lower the longitudinal relaxation time constant T_1 of the nuclei and thus the lifetime of the hyperpolarization. By separating the hyperpolarized nuclear spins from the electron spins, the decay of the hyperpolarization is related to the native T_1, and therefore the decay of the hyperolarization is decelerated. This is accomplished by immobilizing 4-amino-TEMPO on the organic support Sepharose [1,2].

NMR detection is performed with a mobile, medium-field NMR spectrometer (benchtop, 43.2 MHz proton frequency) due to many advantages of the device, e.g. low weight (55 kg) and adequate shim settings. Continuous hyperpolarization by Overhauser DNP enables NMR measurements in the fast flow regime of liquids at ambient temperatures. This is of high interest as it enables monitoring of reactions and processes that were previously inaccessible.

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NOAH: NMR Supersequences for Small Molecule Analysis and Structure Elucidation

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Modern approaches to the structure characterization of small molecules by NMR spectroscopy largely follow well established protocols that are reliant on a core set of 2D correlation experiments such as HSQC, HMBC, COSY, NOESY, TOCSY, and similar [1]. Having established themselves as the primary techniques, much focus has now turned to developing experimental methods that allow the faster collection of these data sets. Herein, we exploit the concept of tailored polarization storage by recording multiple 2D data sets nested as individual modules within a single "supersequence" that requires only a single recovery delay, and so provides for reduced data collection times.

The proposed technique is outlined schematically in Figure 1 and combines several previously introduced approaches, as discussed in detail in our recent publication [2]. We term this concept NOAH (NMR by Ordered Acquisition using ^{1}H-detection). Herein we describe the general NOAH concept and its practical implementation, and present some recent sequences optmised for the characterisation of small molecules [3]. We illustrate the advantages and limitations of this approach and demonstrate the use of NOAH data in combination with computer-assisted strucuture elucidation (CASE) protocols.

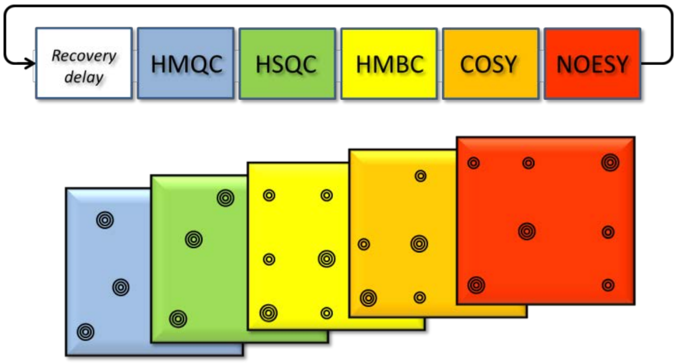

Figure 1. Schematic representation of the NOAH supersequences, here comprising a NOAH-5 experiment made up of five nested modules.

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Expanding the Metabolomics Toolkit with Heteronuclear NMR

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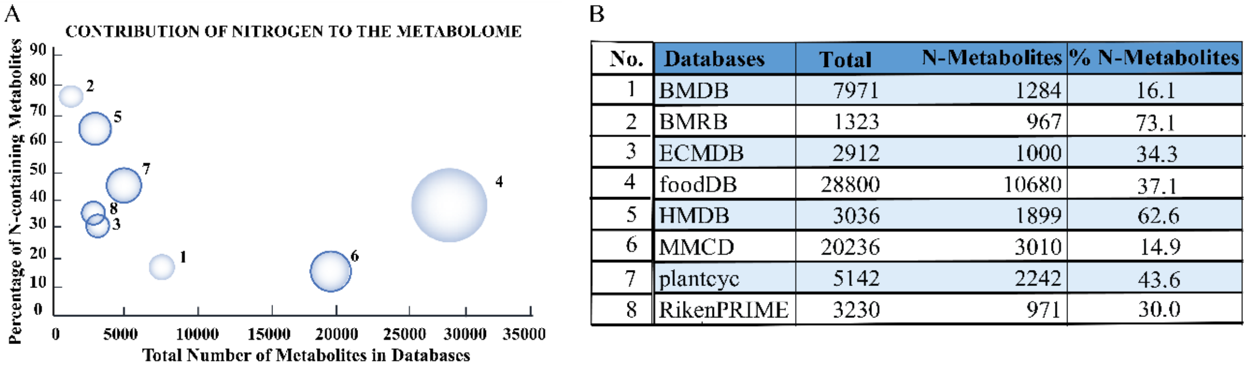
Isotopically labeling a metabolite and tracing its metabolic fate has provided invaluable insights about the role of metabolism in human diseases in addition to a variety of other issues. ^{13}C-labeled metabolite tracers or unlabeled ^{1}H-based NMR experiments are currently the most common application of NMR to metabolomics studies. Unfortunately, the coverage of the metabolome has been consequently limited to the most abundant carbon-containing metabolites.

Firstly, to expand the coverage of the metabolome and enhance the impact of metabolomics studies, we present a protocol for ^{15}N-labeled metabolite tracer experiments that may also be combined with routine ^{13}C-tracer experiments to simultaneously detect both ^{15}N- and ^{13}C-labeled metabolites in metabolic samples.[1].A database consisting of 2D ^{1}H-^{15}N HSQC natural abundance spectra of 54 nitrogen-containing metabolites are also presented to facilitate the assignment of ^{15}N-labeled metabolites. The methodology is demonstrated by labeling *Escherichia coli* and *Staphylococcus aureus* metabolomes with $^{15}N_1$-ammonium chloride, $^{15}N_4$-arginine, and $^{13}C_3$-acetate. Efficient ^{15}N and ^{13}C metabolite labeling and identification was achieved utilizing standard cell culture and sample preparation protocols. Given that more than 30% of a metabolome **(Figure 1)** consists of nitrogen containing metabolites, we have demonstrated that a significant enhancement in the coverage of the metabolome may be achieved by utilizing ^{15}N-labeled metabolites with ^{15}N-based NMR experiments. Secondly, we extend this by implementing a ^{31}P-^{1}H HMBC approach to identifying phosphorus containing-metabolites present in cell lysates and biofluids. We are developing an optimized protocol to ease identification of nucleotide analogs using an optimized decoupled 2D^{1}H-^{13}C HMBC and 2D ^{1}H-^{31}P HMBC experiments.



No.	Databases	Total	N-Metabolites	% N-Metabolites
1	BMDB	7971	1284	16.1
2	BMRB	1323	967	73.1
3	ECMDB	2912	1000	34.3
4	foodDB	28800	10680	37.1
5	HMDB	3036	1899	62.6
6	MMCD	20236	3010	14.9
7	plantcyc	5142	2242	43.6
8	RikenPRIME	3230	971	30.0

Figure 1: (A) Bubble plot showing the contribution of nitrogen-containing metabolites to each of the metabolome databases. Bubbles are numbered and correspond to the rows in the table listed in B. (B) The number of nitrogen-containing metabolites in each of the databases

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Optimization of ^{19}F Methodologies for Pharmaceutical Structure Elucidation

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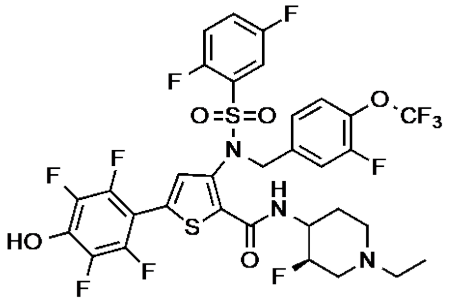
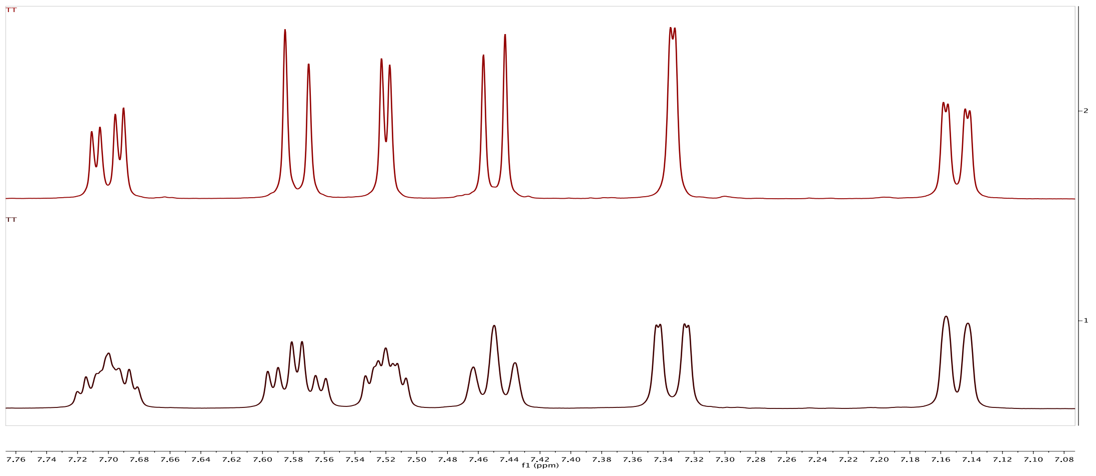
The presence of fluorine in an active pharmaceutical ingredient (API) can impart important pharmacological attributes with regards to metabolism, stability, and selectivity. As such, nearly one-third of newly approved small molecule drugs contain at least one fluorine atom and the trend towards including more than one fluorine moiety per molecule is growing.

For pharmaceutical applications, the presence of a “new” NMR active nuclide with high receptivity and 100% natural abundance opens up many interesting avenues for obtaining structural data. The significant chemical shift window and large coupling constants associated with ^{19}F NMR requires very careful consideration of excitation, decoupling, and magnetization transfer schemes, however. As an example, are HF coupling pathways better elucidated using an HF-COSY approach, or does an indirect method like HETCOR provide more reliable data? And which nucleus should be used for the direct-observe dimension? In a similar vein, given that long-range FC coupling constants are significant over multiple bonds, what approaches work best to help simplify the wealth of information available using long-range experiments?

Results from our investigations using a three-channel HFX system will be presented.





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Study of the Adsorption of Surfactants in Porous Media by NMR Relaxometry

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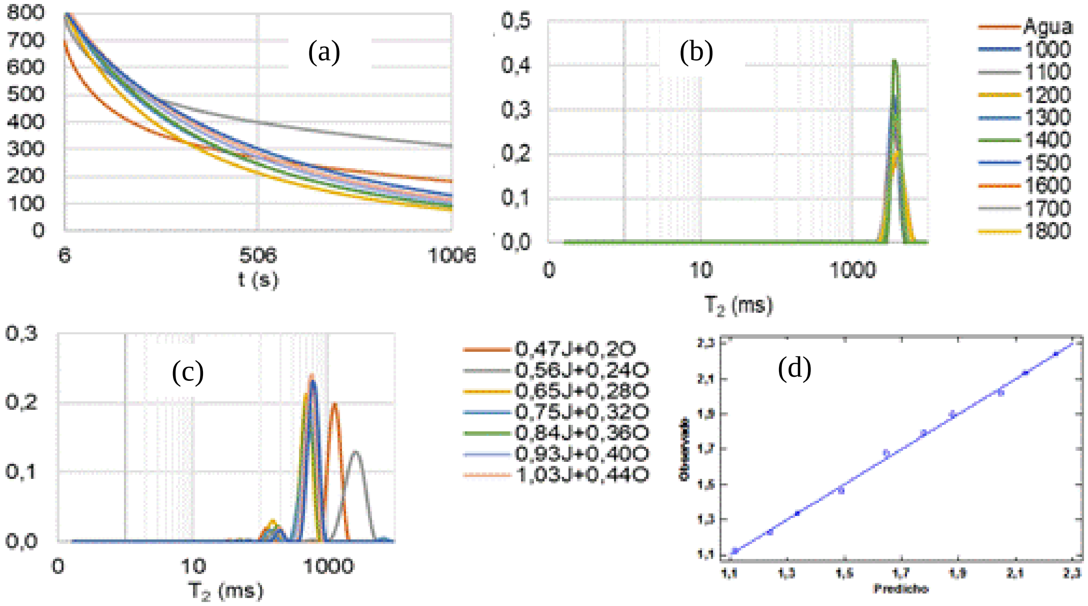
Phenomena studied by NMR techniques take place at different length scales, ranging from the molecular, through the colloidal to the macroscopic. In this paper, several topics of current importance for the recovery and production of crude oils are discussed. Proving that, increasingly, NMR is becoming an invaluable and versatile characterization tool in petroleum science, both in the laboratory and in the field.[1]

NMR low resolution (0,17T) was used to study the static adsorption of a mixture of anionic surfactants: J: extended of propoxy sulphate alcohol (APS) ($C_{12\text{-}13}(PO)_{13}SO_4^-$) and O: anionic double tail type of internal olefin sulfonate (IOS) type ($C_{15\text{-}18}SO_3^-$) in a porous media: mixture of Ottawa sand and kaolin (95:5 %w/w). The characteristics that are related to surfactant adsorption in tertiary oil recovery processes are the concentration and size of the injected pothole, for this the effect of consecutive additions of surfactants in the activation range (1000 to 1800 ppm, J+O) was studied using spin-spin relaxation of these surfactants in solution (figure b) and in the porous medium (figure c).

Models for the prediction of the concentration of surfactants adsorbed (J and O) were developed from the spin-spin T_2 relaxation times and distribution T_2 curves (figures a and c) and chemometric methods (Partial Least Squares). The best model obtained was with R^2= 0,995 and R^2 for validation (LOO) of 0,986. (figure d)



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33 Fermentation of Dietary Proteins in the Colon: NMR-based Metabolomic Study

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The nutrients, which escape digestion by host's digestive enzymes, may undergo fermentation by intestinal microbiota. These interactions between nutrients and microbiota have profound impact on the numerous physiological functions, thus influencing the host's health. Several of these microbial products have been already implicated in pathogenesis of severe human diseases, such as intestinal inflammation or cancer. Therefore, the presence of particular substrates in the diet is one of the most important factors that lead to these unfortunate consequences.

Recently, metabolomics has become a well-established tool to acquire the information on nutrients fermentation by analyzing the fermentation products excreted to feces. While carbohydrate fermentation has been widely studied, much less is known about protein fermentation and how these processes are influenced by the source of the protein.

In this study, we used NMR-based metabolomic approach to investigate the fecal metabolome in conventionally colonized BALB/c mice administered with high-protein (HP) diet of either plant or animal origin. First, we changed the diet in conventionally fed mice, thus creating four experimental groups: the groups fed with a diet containing normal amount of protein (about 17% crude protein) of either plant or animal origin and HP diets (about 50% crude protein) of either plant or animal origin. The feces samples were collected twice – before and two weeks after this diet switch. The fecal material was extracted using phosphate buffered saline (pH=7.4). The samples were analyzed on 600 MHz NMR spectrometer equipped with TCI cryoprobe using 1D-NOESY and CPMG spectra. Obtained spectral data were analyzed using combination of multivariate (principal component analysis) and univariate (nonparametric Wilcoxon-Mann-Whitney test) statistical procedures.

As expected, the introduction of new diets caused substantial changes in fecal metabolic profile. These changes were evaluated with respect to the protein content in the diet as well as the protein source (plant or animal). To our surprise, the amount of proteins did not have such a big effect on metabolic profile compared to the protein source. However, several differences between diets with high and normal protein content could have been observed. Interestingly, increase in dietary proteins led to a decrease in fecal acetate, regardless of the protein source. Among the mice fed with the plant protein-based diet, increase in crude protein correlated with an increase in phenolic compounds in feces, which have been previously reported as exclusive products of protein fermentation [1,2]. Furthermore, we found increased levels of citrate and branched-chain fatty acids (BCFAs) such as leucine and isoleucine, the latest two associated

with protein fermentation. Similarly, the high-protein diet of animal origin increased the levels of several BCFAs compared to respective control diet.
As mentioned above, the source of proteins had appreciable effect on mouse fecal metabolic profile. In fecal samples of mice fed the plant protein-based diet we recorded several additional signals in aromatic region, which corresponded to phenolic compounds and did not occur in samples obtained from animal protein-based diet-fed mice. Moreover, as compared to the animal-based diet, plant-based diet led to an increase in fecal levels of SCFAs (butyrate, acetate and isovalerate), glutamate, succinate and citrate. These observations indicate the substantial differences in the fermentation of plant and animal proteins present in the respective diets.

Taken together, our study describes the variability in protein fermentation process depending on the overall protein amount and source of proteins. Next, we will use this data to study the impact of these metabolic processes on the pathogenesis of intestinal inflammation and colon cancer.

Acknowledgements

This work is supported by the Czech Science Foundation (Grant No. 16-06326S) and the Operational Program Prague – Competitiveness (Project No.: CZ.2.16/3.1.00/24023). The authors gratefully acknowledge the project LO1509 of the Ministry of Education, Youth and Sports of the Czech Republic.

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SIA-NMR: A Novel Hyphenated Technique and it's Application to the Analysis of Amphetamine Derivatives

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The growth of new psycoactive substances (NPS), including bath salts, and synthetic cathinones, have created a sproblem in forensic analysis. NPSs use a small structural modification of traditional psycoactive substances to circumvent laws and foil existing presumptive tests such as color, thin-layer chromatography, and infra-red spectroscopy. A fast, robust, and automated analysis method is needed to address this challenge. NMR is a powerful tool for identifying NPSs, but remains underutilized in this field because of the size and cost. Benchtop NMR's lower size and cost make the technique more accessible, but the low fields used by these systems introduce their own limitations. We describe the development, optimization, and application of a novel hyphenated technique, Sequential Injection Analysis-Nuclear Magnetic Resonance (SIA-NMR). SIA-NMR combines the automated sample pretreatment capabilities of SIA, with the qualitative and quantitative power of NMR to create a high throughput, low-cost instrument with applications in a variety of areas including forensic science.

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Application of ^{19}F Solid-State NMR Spectroscopy for Detection of Low Levels of Crystalline Content in Amorphous Solid Dosage Forms

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There is a significant interest in quantification of low levels of crystalline content in amorphous formulations. The presence of crystalline phase in any amount has the potential to affect drug's efficacy. Traditional analytical tools such as differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) fail to detect crystalline content when present below 5% [1]. Solid-state nuclear magnetic resonance (SSNMR) spectroscopy is shown to be a useful probe to quantitate low levels of crystalline content in amorphous dosage forms [2]. Fluorine atom is present in a large range of pharmaceutical compounds and it makes an interesting NMR probe owing to its high gyromagnetic ratio and 100% isotopic abundance. In this contribution we describe the capability of ^{19}F SSNMR spectroscopy to study low levels (<1%) of crystalline content in amorphous solid dispersions (ASDs) of pharmaceutical fluorinated compounds. The dispersions along with pure active pharmaceutical ingredients (APIs) will be characterized exhaustively by SSNMR to get information on ^{19}F spectra. Small amounts of crystalline materials will be doped into the dispersions for probing through ^{19}F SSNMR spectroscopy. The proton relaxation time in the rotating frame ($^{1}HT_{1rho}$) filtering experiments will be done to filter out the amorphous signal and quantify the crystalline content. The study is currently in progress. Hence, the preliminary results acquired with triamcinolone acetonide are discussed here. The dispersion of triamcinolone acetonide was formulated with hypromellose acetate succinate (HPMCAS) at 20% w/w drug loading via spray drying. The dispersion was doped with low levels of crystalline API. The $^{1}HT_{1rho}$ filtering experiments revealed the presence of polymorph B and extracted the crystalline phase at less than 0.3%.

The preliminary results strongly support the suitability of ^{19}F SSNMR spectroscopy for characterizations of amorphous forms for crystalline content. Through these case studies, the intent is to highlight the versatility of ^{19}F SSNMR spectroscopy for complex dosage forms as ASDs.

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In-situ Solid MR Imaging of Tablet Dissolution

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Magnetic Resonance Imaging (MRI) allows intact pharmacuetical formulations to be investigated during static and dynamic events, such as dissolution. NMR imaging gives a three-dimensional spatially resolved visualization of the formulation. This visualization is accompanied by MR relaxation properties that can reveal the mobility of the formulation components. MR imaging of solid materials is well suited for looking at the fast relaxing components (i.e. excipients) in a solid pharmaceutical product. In this work solid-state MR relaxation and imaging were used to directly monitor the drug product, as opposed to the water component, during its dissolution to understand the changes that occur to the excipients both during and after water penetration into the tablet. The dissolution processes of two types of controlled release tablets in a USP4 flow cell device were monitored by ^{1}H NMR, both bulk FIDs and three dimensional imaging. Three different solid imaging methods, SPRITE[1], a modified Fast Spin Echo (FSE), and Pi-EPI[2], were employed. These sequences are designed for imaging solid and solid-like material with short T_2 or T_2* relaxation times. The image contrast was selected to highlight regions of high moisture contrast, such as the wetting front. The ability to image the *in situ* dissolution of controlled release tablets using the solids MR method is demonstrated. Dissolution events were correlated to the excipient hydration behavior through relaxation properities mesured on a bench-top TD-NMR.

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NMR Measurements and Molecular Simulation of Self-Diffusion Coefficients in Binary and Ternary Mixtures Containing Toluene, Acetone, Cyclohexane and Ethanol

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Recently, Guevara-Carrion et al. published a comprehensive molecular dynamics study on the thermodynamic properties of all binary mixtures comprising methanol, ethanol, acetone, benzene, cyclohexane, toluene and carbon tetrachloride, including self-diffusion [1]. For the mixtures acetone/cyclohexane, acetone/ethanol, acetone/toluene, cyclohexane/ethanol and toluene/ethanol no experimental self-diffusion data is yet available. Furthermore, there is no experimental data on the self diffusion in ternary mixtures of the studied components. Therefore, in the present work, the self-diffusion coefficients of aforementioned binary mixtures and ternary mixtures was measured by NMR spectroscopy. The employed pulse sequence is based on a stimulated echo and bipolar gradients. To ensure a high quality of the measurements, a preliminary gradient mapping was carried out. The results are compared to the aforementioned simulation data for binary mixtures and new meolcular simulation data for the ternary systems data, that were obtained in the present study. Good agreement is achieved for mixtures that do not contain ethanol. The deficiencies observed for mixtures that contain ethanol are attributed to deficiencies of the force field in describing the association of ethanol.

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Latest Development of Neat Certified Reference Material (CRM) and Novel CRM in Solution for Quantitative NMR (qNMR)

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Zearalenone is widely recognized as a hormone disruptor in animals and is under intense scrutiny as a probable endocrine-disrupting chemical in humans. To safeguard against zearalenone exposure in humans, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional maximum tolerable daily intake for zearalenone at 0.5 µg/kg body weight. Health Canada and the Canadian Food Inspection Agency have recommended zearalenone levels as low as 0.25 ppm in animal feed and monitor zearalenone contamination in food destined for human consumption.

To enforce regulatory limits, the accurate quantitation of zearalenone requires adherence to quality assurance guidelines, which recommend the use of certified reference materials (CRMs). Zearalenone CRMs on the market are few and are most commonly determined via the mass balance approach, whereby all potential impurities must be successfully identified and subtracted from the limit value. To avoid the limitations of identifying all exogenous species and maintain SI traceability, the National Research Council of Canada (NRC) used proton quantitative NMR (qNMR) to quantify mycotoxin standards by direct determination of zearalenone with dimethyl terephthalate as an SI-traceable internal standard. Complementary purity assignments of the bulk solid zearalenone material, destined for CRM development, were performed using standard ^{1}H-NMR and ^{1}H{^{13}C}-GARP decoupling pulse sequences. We show that both pulse sequences produce statistically similar results. However, elimination of ^{13}C-satellites from a ^{1}H{^{13}C}-GARP spectrum, enables easy identification of minor baseline impurities to facilitate accurate integration.

39 Probing the Acidity in FCC Catalysts by TMP/TPPO and Solid-State NMR

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Abstract: FCC catalysts responsible for the conversion of heavy fractions of crude oil into fuels (gasoline, naphtha, LPG), experience continuous loss of activity with the operating cycles due to coke deposition, Ni and V poisoning as well as zeolite dealumination, thus affecting the catalyst performance and economic activity of the FCC unit.[1] Solid-state NMR is a powerful spectroscopic technique known for the structural characterization and determination of the acidic properties that command the catalytic behavior.[2] TMP and TPPO were adsorbed on hydrothermally deactivated FCC catalysts and ^{31}P MAS and ^{1}H{^{31}P} HETCOR measurements were carried out on a Bruker Avance III WB 9.4T (400 MHz) spectrometer at temperature environment. ^{31}P-TMP-NMR spectra allowed to determine the interaction with the total Brönsted and Lewis acid sites because of the smaller kinetic diameter of the TMP that allowed the diffusion into the zeolite. By ^{31}P-TPPO-NMR was determined the Brönsted acid sites on the surface of the samples due to its larger molecular size.[3] The difference in the quantification of Brönsted sites with these two probe molecules resulted in the amount of internal sites in the material. It was revealed decreasing structural properties and change of the acidic strength of the Brönsted acid sites on FCC catalysts after hydrothermal deactivation and was correlated with the decrease in catalytic activity.

Acknowledgments: Supported by the National Research Program in Energy and Mining, Ecopetrol, Colciencias and Universidad Industrial de Santander through the 403+2013 contract and the Vice-Rectory of Research and Extension for 8821 Project.

Keywords: FCC catalyst, solid-state NMR, ^{31}P-TMP, ^{31}P-TPPO, deactivation.

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Improvement of NOE Distance Analysis

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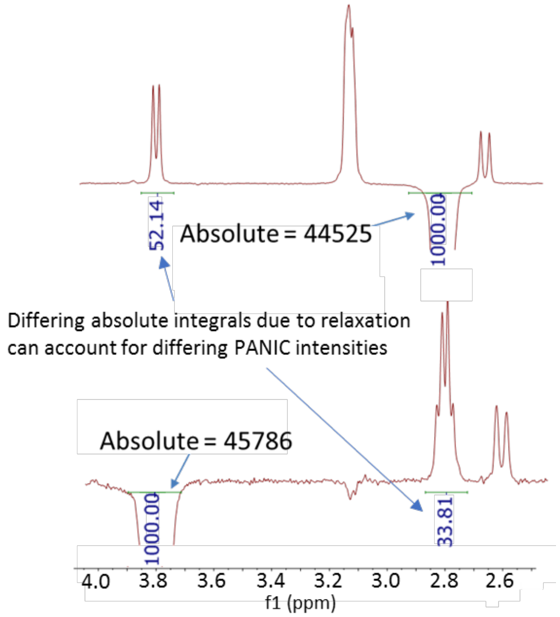
The transient NOE (Nuclear Overhauser Effect) is a valuable method of extracting distances from organic molecules and finds application to structural and conformational problems. Analysis often assumes a first order approximation of the build-up rate of enhancements. Unfortunately for most systems this approximation only holds true at the shortest mixing times, where S/N is poor.[1] The region where this approximation holds can be extended by PANIC (Peak Amplitude Normalization for Improved Cross Relaxation), where enhancements are normalized against irradiated/diagonal peak intensities – this accounts for external relaxation, the major cause of nonlinear build-up.[2] Previous work using PANIC in the Butts group has allowed calculation of internuclear distance to 3% deviation from computer models in ideal cases.[3,4]

However, PANIC is not perfect - for example it assumes that the rate of external relaxation on irradiated and enhanced nuclei is the same.[2] PANIC^2 is a novel modification which compares absolute integrals of irradiated peaks across multiple spectra and uses these to scale enhancements, accounting for differing external relaxation. PANIC^2 is also combined with PANIC+, a method developed in the Butts group by Dr Catherine Jones,[4] where enhanced peaks are normalized against all peaks present in a given 1D NOE spectrum, rather than solely against the irradiated peak. This corrects for differing amounts of cross relaxation between different irradiated peaks. These two corrections, requiring no additional experimental time, can substantially improve some NOE data - in the case of strychnine, deviation from computed distances is improved from 4.18% to 2.61%.



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High-Resolution and Multidimensional Solid-State NMR of Pharmaceutical Sciences

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The physicochemical properties, including thermodynamic and structural behaviors of active pharmaceutical ingredients (APIs) in solid dosages, can significantly impact phase stability, processability, and bioavailability. For example, physical stability is a key decision making factor for formulation excipients, processing and manufacturing parameters, and packaging and storage conditions. Macroscopic miscibility and microscopic interactions between drug and excipients are mechanistic attributes responsible for various stability and instability events. The complexity of low drug loading, multi-component and often non-crystalline nature results in low intensity and poor temporal and spectral resolution for accessing these pharmaceutical properties. In recent years, solid-state NMR has become an indispensable tool for pharmaceutical analysis due to its high resolution and quantitative capabilities in the characterization of molecular structures and dynamics. Particularly, it provides a unique advantage for investigating amorphous systems.

Recent technical advances in SSNMR, including ultra-fast magic angle spinning (UF-MAS), present high potential methodologies to overcome the current analytical challenges in solid state materials characterization and provide numerous avenues for investigating pharmaceuticals at a higher resolution and sensitivity. We will present a few pioneering development studies utilizing cutting-edge techniques in pharmaceutical sciences for structural investigation of small molecule drugs. For example, we have published the first documented case utilizing ultrafast MAS at 60 kHz to investigate drug-polymer interactions [1,2]; and developed the three-dimensional SSNMR analysis of natural isotopic abundance pharmaceutical materials [2]. We have also extended these investigations to the amorphous solid dispersion of posaconazole and investigated site-specific molecular interactions of these formulations. Interestingly, posaconazole exhibits a distinct interaction pattern when formulated with different polymers, i.e., HPMC-AS and HPMC-P. These new techniques allow us to comprehensively compare the structure of solid dispersion intermediates produced by different techniques. Moreover, phase separation is a known issue for amorphous solid dispersion. Identification and quantification of phase-separated domains with sizes < 100 nm is technically challenging. We have successfully utilized relaxation techniques to semi-quantitatively evaluate the drug-polymer miscibility [3,4]. These studies help to establish the correlation between molecular miscibility and various processing parameters and facilitate the optimization of process conditions. Most recently, we have developed the spin diffusion SSNMR method to quantify phase separation at an even higher resolution, i.e. a domain size of 50 nm, providing the highest resolution so far for detecting phase separation.

In summary, physical stability is one determining factor for optimizing formulations in drug development. Investigating molecular properties including drug-polymer miscibility and interaction carries significant scientific and business impacts. Our efforts push the boundaries of ssNMR for high-resolution investigations, enabling deep scientific understanding and confident controls of the thermodynamic and structural behavior and characteristics of drug products.

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Predicting NMR Parameters through Machine Learning

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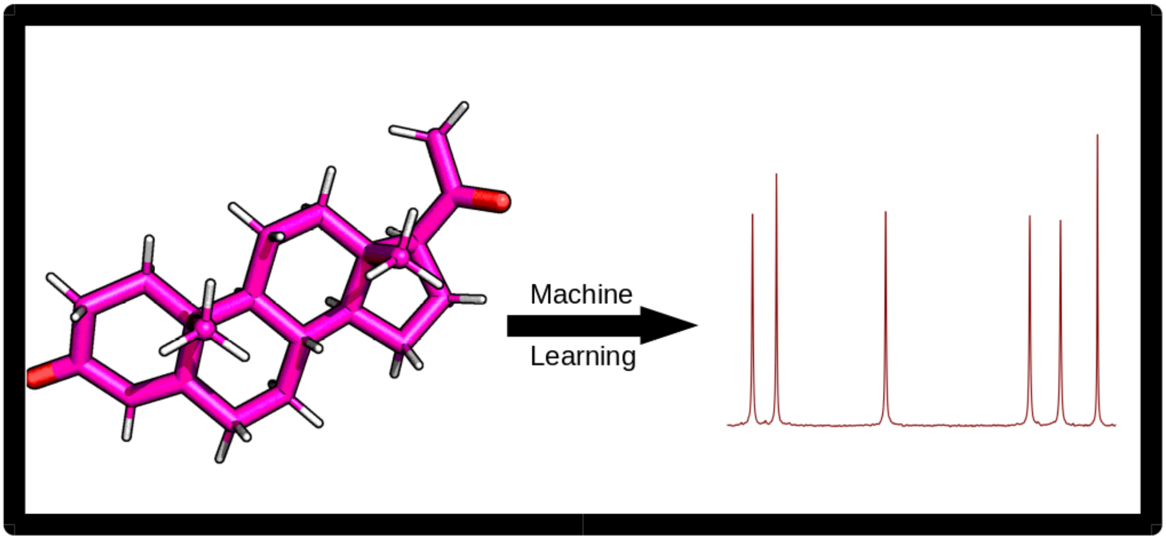
NMR parameters are widely used in the determination of 3D structure in molecules, especially in drug-discovery work-flows. Commonly these are calculated using DFT or other Quantum mechanical methods in replace of, or to complement, experimental measurements. Currently the computational power required for these calculations is prohibitive, especially for large sets of molecules. In this work, machine Learning techniques are applied to the prediction of NMR parameters (Scalar couplings, chemical shifts) with the use of a large dataset of small molecules (~1000 X-ray Crystal Structures) obtained from the Cambridge Structural Database. The aim of the project is to create machine learning solutions for the efficient and accurate prediction of NMR parameters in small molecules, matching the accuracy of DFT predicted values in a fraction of the time.

Currently the best prediction method tested is a Kernel Ridge Regression (KRR) algorithm utilising a Spectrum of London and Axillrod-Teller-Muto potential (SLATM) molecular representation. The accuracy of this method was tested through cross-validation on a dataset of 874 molecules (for chemical shift and scalar coupling). The prediction accuracy (mean absolute error in comparing out of sample machine learning prediction to DFT calculation) for carbon and proton chemical shift is 2.9ppm and 0.2ppm respectively. The one bond proton-carbon coupling prediction accuracy is 1.8Hz.

These results indicate that this method could be a viable replacement for DFT calculations in many workflows, especially with further development. To that end, more robust and effective training datasets are being produced, and new machine learning algorithms and molecular representations are being tested



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Generation of High-quality NMR data using the NMReDATA format and top-resolution 2D NMR spectra of natural products

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The NMR community is missing sources of NMR data for organic compounds in general and natural products in particular. An *Initiative* [1] gathering specialists of structure determination, cheminformatics, NMR software developments and journal editors recently introduced a new file format called "NMReDATA" [2] to report the NMR parameters extracted from "full NMR analysis". A file contains the structure of the assigned molecule, the extracted chemical shifts, scalar couplings, and the lists of 2D correlations from COSY, HSQC, HMBC, (etc.) spectra. Associated with the NMR spectra, they constitute an "NMR record" which can be sent to journal to facilitate the reviewing and benefit the community with open user-friendly and computer-accessible data when articles are published. Many NMR software editors and providers of NMR databases and services made the commitment to introduce the NMReDATA format in their platforms.

The potential of the new format to change the NMR data landscape will be discussed. Besides the obvious benefit of providing a source of NMR data, the new format will open up opportunities to development tools to correct, improve, validate, certify these data. More specifically:

-An assignment could be automatically validated by checking the consistency of 2D correlations with the provided structure.

-The extracted NMR parameters (typically scalar coupling constants and chemical shifts) could be refined and complemented using spectral simulation including second-order effects, and could treat magnetic equivalence.

-The 3D structure could be determined or verified through DFT calculations or other predictor of chemical shifts, scalar couplings, etc.

We shall also present how the value of the NMReDATA can be significantly increased when including data from recent NMR spectra such as pure-shift, homonuclear 2D spectra recorded with homodecoupling in F1 [3] and other methods improving resolution in the carbon dimension of 2D heteronuclear spectra. [4]

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New Insights in the Degradation Pathways of Deltamethrin

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The combination of ^{1}H-NMR and 2D-NMR experiments, along with GC-MS, has facilitated the determination of the stereochemical degradation pathways in a critically important pyrethroid for the first time. Pyrethroids are among the best insecticides for public health use and their biological activity is strongly related to the chiral and chemical composition [1].

Malaria, Zika virus, dengue fever and yellow fever are mosquito transmitted diseases for which vaccines either do not exist or are not widely available. In order to reduce their spread, the World Health Organisation recommends the use of insecticides as a key prevention measure [2-3]. However, pyrethroids suffer from stability issues - mainly pH related - which particularly affect the chiral centres [4-6]. For instance, deltamethrin (DMN, Figure 1), which is one of the most active type II pyrethroids, undergoes fast chemical and stereochemical degradation in alkaline environment [7]. The motivation behind this work is therefore to obtain better understanding of pyrethroids' degradation processes, so as to find ways to improve their stability which in turn should facilitate the development of more effective formulations. We show the *in-situ* identification of DMN degradation products originating in alkaline conditions and the potential pathways leading to their formation. Furthermore, the use of cyclodextrins (CDs, Figure 2) as possible DMN stabilisers was investigated.

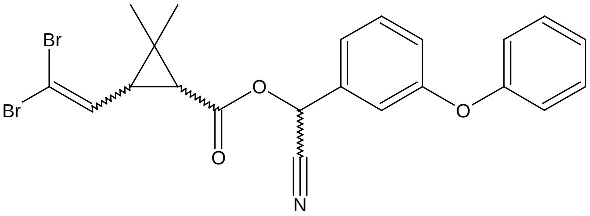

Figure 1- Deltamethrin (DMN) structure.

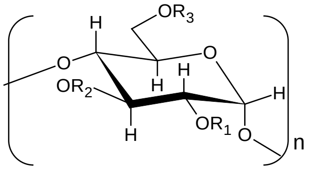

Figure 2- Cyclodextrins (CDs) basic structure.

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Counterintuitive Deshielding on ^{13}C Chemical Shift for the Trifluoromethyl Anion

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^{13}C NMR chemical shift ($\delta^{13}C$) trends are frequently explained through a direct association with electronic density surrounding the carbon nuclei. It is expected that a higher electron density produces a stronger nuclear magnetic shielding (σ), once the chemical shift is sensitive to the electronic environment experienced by the nucleus.[1] However, this association is not true for some systems [1,2] because the nuclear shielding depends actually on how the electronic system responds to the magnetic field.

The trifluoromethyl anion, CF_3^-, was recently characterized [3] by solution NMR spectroscopy and an unexpected $\delta^{13}C$ value of 175.0 ppm was observed. It would be expected a shielding effect for the carbanion in comparison to neutral (CHF_3) and carbocation (CF_3^+) derivatives, but $\delta^{13}C$ of 122.2 ppm [3] and 150.7 ppm [4] are reported, respectively. To understand why carbon nuclei of CF_3^- shows a deshielding effect and which shielding mechanisms are involved, we performed a deep investigation by density functional calculations and localized molecular orbital analyses.

The CHX_3 and their cation (CX_3^+) and anion (CX_3^-) derivatives with X = H or F were studied to evaluate the effect of fluorine atom on the $\sigma^{13}C$. The $\sigma^{13}C$ were calculated with the Amsterdam Density Functional suite (ADF 2017) using OPBE functional and QZ4P basis set. The $\sigma^{13}C$ tensor was decomposed into contributions of the diamagnetic, paramagnetic, and spin-orbit mechanisms and in terms of NBO orbitals generated by the NBO 6.0 program.

Different $\delta^{13}C$ trends were observed for the CHX_3 and derivatives when the X element is hydrogen or fluorine. While a deshielding behavior is observed for CF_3^- (178.70 ppm), CH_3^+ (393.55 ppm), and CF_3^+ (154.56 ppm), a shielding effect is estimated for CH_3^- (-43.41 ppm) in comparison to corresponding neutral CH_4 (-8.65 ppm) and CHF_3 (126.13 ppm) compounds. According to decomposition analyses of $\sigma^{13}C$ tensors, the magnetic interaction between carbon lone pair and antibonding $\sigma^*_{C\text{-}X}$ orbitals could cause a paramagnetic contribution on the carbon nuclei increasing the chemical shift for CF_3^- molecule.

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Quantitative ^{1}H-NMR for Certified Reference Material Value Assignment of the Mycotoxin Zearalenone.

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Zearalenone is widely recognized as a hormone disruptor in animals and is under intense scrutiny as a probable endocrine-disrupting chemical in humans. To safeguard against zearalenone exposure in humans, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional maximum tolerable daily intake for zearalenone at 0.5 µg/kg body weight. Health Canada and the Canadian Food Inspection Agency have recommended zearalenone levels as low as 0.25 ppm in animal feed and monitor zearalenone contamination in food destined for human consumption.

To enforce regulatory limits, the accurate quantitation of zearalenone requires adherence to quality assurance guidelines, which recommend the use of certified reference materials (CRMs). Zearalenone CRMs on the market are few and are most commonly determined via the mass balance approach, whereby all potential impurities must be successfully identified and subtracted from the limit value. To avoid the limitations of identifying all exogenous species and maintain SI traceability, the National Research Council of Canada (NRC) used proton quantitative NMR (qNMR) to quantify mycotoxin standards by direct determination of zearalenone with dimethyl terephthalate as an SI-traceable internal standard. Complementary purity assignments of the bulk solid zearalenone material, destined for CRM development, were performed using standard ^{1}H-NMR and ^{1}H{^{13}C}-GARP decoupling pulse sequences. We show that both pulse sequences produce statistically similar results. However, elimination of ^{13}C-satellites from a ^{1}H{^{13}C}-GARP spectrum, enables easy identification of minor baseline impurities to facilitate accurate integration.

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Applications of New 400 MHz HTS Power-Drive Magnet NMR Technology: Structure Elucidation and Reaction Monitoring

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NMR is a powerful technique for structure elucidation and reaction monitoring of organic molecules and significally important in the pharmaceutical industry supporting drug discovery and development processes. Typically, medium-high field NMR instruments are constrained to special laboratories due to facility and safety requirements, which make them less accessible to the work carried out in chemistry/analytical laboratories, and in manufacturing facilities. Recently, new developments using high-temperature-superconducting (HTS) magnets have provided a solution to locate NMR instruments in chemistry/analytical laboratories with minor needs from facilites and without cryogenic liquids. The elimination of cryogens eradicates the risks of quenches and safety concerns for the HTS NMR magnets and eliminates the needs of large cryogen dewars in the facility. Bringing the NMR to the chemistry/analytical laboratories increases the utilization of the technology to the same level of other instruments in those laboratories (e.g., mass spectrometries, HPLCs, infrared, etc.).

We have evaluated the performance of a medium-high field of 9.4 T (400 MHz for ^{1}H observation) HTS power-driven magnet technology connected to a standard console (Bruker Avance III HD NanoBay) and two probes with two shimstacks (BBFO with BOSS3 shims and QNP with BOSS1 shims) and compared with a conventional commercial NMR system (AVANCE III HD NMR console, 400 MHz Ascend magnet, and BBFO Smart probe) at the same field. From a practical perspective, we compared the performance on several compounds representing standard active pharmaceutical ingredients (API) in the pharmaceutical industry. In addition we have explored the understanding of reaction monitoring with NMR using the Bruker NMR flow tube for online reaction monitoring connected to the reactor vessel using only protonated solvents and at concentrations typical of reactions employed in manufacturing.

We are presenting here the scheme of the 400 MHz HTS power-driven magnet technology integrated in a standard chemistry laboratory, data comparison of the HTS magnet NMR system with a conventional NMR instrument towards the work of structure elucidation and comparing probes with different shimstacks to understand the differences in performance by 1D and 2D homonuclear and heteronuclear NMR experiments [1]. In addition, we are presenting our initial work on reaction monitoring for the synthess of cycloalkenes through the catalytic reaction of ring closure metathesis (RCM) using Grubbs catalysts. We will briefly discuss some pros and cons issues related to Suziki coupling reactions by NMR.

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G10 (a Polysiloxane Diamine) Imine Analysis by Solution State NMR

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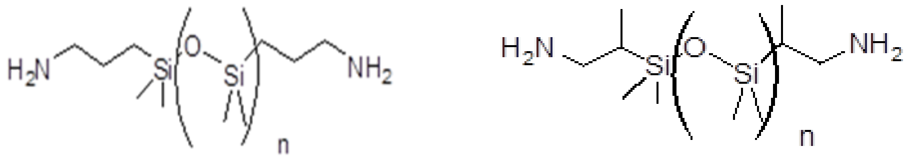
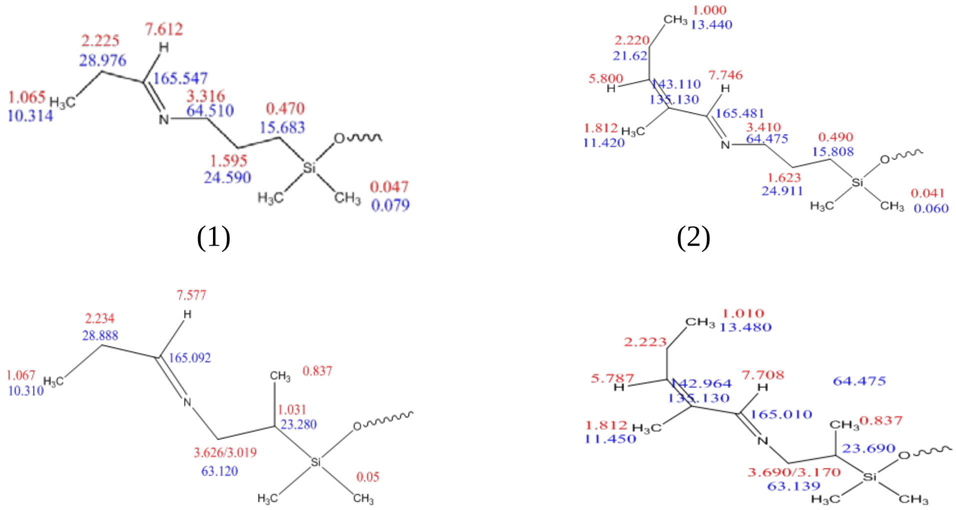
G10 is a raw material for the production of engineering thermoplastics with both high-heat resistance and flexibility properties. G10 has two isomers, gamma and beta, as displayed in Figure 1. These two isomers' structures were assigned with the combination of advanced 1D and 2D NMR spectroscopy (such as Pure shift 1D homonuclear decoupling, F1 band selective 2D HSQC and HMBC), and their relative ratio was determined by 1D ^{1}H NMR. Four kinds of imines (shown in Figure 2) in G10 derived from these two isomers and their reaction with two different aldehydes were identified and assigned with 2D ^{1}H and ^{13}C NMR correlation spectroscopies. The imine was found to be a part of the G10 molecule; therefore, small molecule methods of analysis such as GC or HPLC would not be effective in quantifying the contaminant.



(a) (b)

Figure 1. G10 has two isomers: (a) gamma isomer, and (b) beta isomer.



(1) (2)

(2) (4)

Figure 2: Four kinds of imines were identified and their structures were assigned. The 1H (in red) and 13C (in blue) chemical shifts are shown in the structures.

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Ion Difussion and Relaxometry NMR in Confined Mesoporous Materials

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NMR spectroscopy can explore the confinenment characteristics of ions on basis relaxometry properties of a particular nucleus sensible to the technique. T1 and T2 are strongly dependent on the physical and chemical conditions in the vicinity of the nucleus. In confined systems, inhomogenities resulting of the proximity of nucleus with the surface of material are mainly resposible for changes in T2 values. Then, for a particular nucleus such as $^7Li^+$ the T2 values can allow us to stablish correlations with confinement dimensions. In addition, the chemical composition can be an important factor when we consider the relaxation properties on different materials that are under observation.

In this work we explore the relaxometry behavior of $^7Li^+$ ion when it is confined in zeolites as Faujasite (pore 7.4Å, cavity 12Å), LTA (pore 4.2Å, cavity 11.4Å) and ZSM5 (pore 5.4-5.6Å). All the samples were obtained as acid solids. Through the analysis of XRF spectra, the SiO_2/Al_2O_3 ratio was measured and it was confirmed that Na ions were not present. Samples with different ion concentrations were prepared by saturation of 0.1g of solid material with 0.5 mL of Li^+ solutions at 1, 5, 10, 50 mM. Samples were packed in 4mm zirconia rotors and the ^7Li one pulse and proton-decoupled spectra were measure under MAS conditions in a Bruker Avance III (9.4T) NMR spectrometer. ^7Li NMR resonances broadened by quadrupolar interactions were observed. Under proton decouplig dipolar H-^7Li interactions could be suppresed and chemical shift anisotropy averaged by MAS.

For T1 relaxometry measurements the CPX T1 pulse sequence using Tochia method was employed, T2 experiments were performed using a CPMG pulse sequence. T1 and T2 values were measured from 298K to 358K every 10K. Due to acid protons in the zeolites can be easily exchanged by Li^+ ions, the local structure can alter the symmetry of ions, which in turn can show different characteristic relaxations. Quadrupolar constants and assymetry parameters were calculated from MQ-rotor synchronized experiment using a 2.5mm probe at 30kHz MAS. The chemical environment and relaxometry properties of Li ions in confined cavities as those shows by zeolites were probed. The effects of ion concentration and temperatures were analyzed.

Acknowledgments: Authors thanks to Vicerectoria de Investigaciones of the Universidad Industrial de Santander for NMR facilities at PTG-UIS, and the financial support through the Contract 403-2013 Ecopetrol-Colciencias-UIS.

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Theoretical Exploration of (Un)expectedly Small Aldehyde $^{3}J_{HH}$ Values: An NBO Perspective

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Aliphatic aldehyde vicinal proton-proton (R_2HCCHO, Fig.1) coupling constants have values around 1-3Hz [1]. These values are generally much smaller than those of corresponding alkenes ($R_2CHCHCR_2$, Fig.1), which are usually around 6-8Hz [2]. The fact that aldehyde $^{3}J_{HH}$ values are much smaller than alkene values is well known, but to our knowledge, an in-depth study about the underlying reasons for this small value has not been presented. Since an aldehyde differs from an alkene mainly by the oxygen atom that replaces the alkene $=CH_2$ group, we wished to understand how changes in the electronic structure due to the aldehyde oxygen atom give rise to such a small scalar coupling constant.

Acetaldehyde[1], $^{3}J_{HH}$=2.88Hz average 1-Propene[2], $^{3}J_{HH}$=6.40Hz average

Figure 1. Coupling pathway of acetaldehyde [1] and 1-propene [2].

In this study, we applied natural bond orbital (NBO) analyses to quantitatively explore the interactions between electronic groups in aldehydes, alkenes and thialdehydes. Donor-acceptor interaction analysis shows that expected donation between electron filled orbitals (*e.g.* O, S lone pair orbitals) and adjacent antibonding orbitals (*e.g.* C-H σ* and C-C σ* orbitals) is strong. As previously reported [3] and repeated in our study, acetaldehyde $O_{ald}(LP)\rightarrow C_{ald}\text{-}H_{ald}\ \sigma(BD)^*$ interaction has a donation energy of 28.63kJ/mol (Fig.2). However, Natural *J*-Coupling analysis (NJC) showed that these interactions do not directly contribute to the coupling constant.

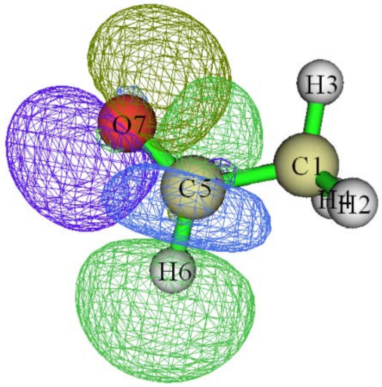

Figure 2. Surface plot of vicinal O Lone Pair (green and blue) and σ*C-H (olive green and dark blue) interaction. Isovalue 0.035.

Indirect contributions to the coupling constant of the aldehyde oxygen atom were therefore studied. Energetic analysis with NBO deletion (DEL) was applied to explore the change of geometry caused by the above mentioned LP→BD* interactions. Electron-withdrawing effect of aldehyde oxygen and the consequently deficient valance charge population were studied with Natural Population Analysis (NPA). Finally, the change of the *J*-coupling initiated by both the geometry changes and the reduced charge population in the coupling pathway was examined by Spin-Spin Coupling Constant (SSCC) calculation. .

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Top Resolution in 2D NMR Experiments Combining Chemical Shift Encoded Aliased Spectra and Spectral Reconstruction

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The resolution in the F1 dimension of 2D NMR spectra directly depends on the maximal t_1 evolution time $t_1max = N / 2SW$. A good choice of the number of time increment (*N*) and the spectral window (*SW*) is therefore crucial when aiming at obtaining spectra with highest possible information content. Spectral aliasing [1], obtained by reducing the F1 spectral window, can be used as an alternative to the brute force increase of the number of time increments to increase resolution by up to two orders of magnitudes. Because of aliasing, ambiguities in chemical shifts are introduced making it difficult to determine the true chemical shift of crude aliased spectra. But different methods have been reported to overcome the problems of chemical shift ambiguities [2,3].

We present here a general approach to resolve chemical shift ambiguities generated when recording experiments with spectral aliasing. Chemical shifts are encoded along the F1 dimension thanks to the inclusion of an additional t_1' evolution time block, for which quadrature discrimination is not applied, resulting in a split of the signals in the indirect dimension proportional to the chemical shift of the evolving nuclei. The incrementation of t_1' and t_1 being applied simultaneously does not increase the experimental time. A computer program processing the chemical-shift encoded spectrum is used to identify the splitting pattern generated by this additional evolution time block and to recalculate the true chemical shift for each pair of peaks. A full-width reconstructed spectrum version of the 2D experiments is therefore obtained by placing each of the reconstructed pairs of peaks at theirs true chemical shift in the indirect dimension.

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Metabolomics Analysis Reveals Altered BCAA Metabolism in Insomnia Patients

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Approximately 1/3 of a general population experiences symptom of insomnia. Clinically, insomnia is associated with physiological hyperarousal, but relationships with metabolism are unclear. The bidirectional relationship of insomnia with adverse medical outcomes is well known, however, the underlying mechanisms remain underexplored. Here we use comprehensive metabolomics to gauge the metabolic effect of insomnia in otherwise healthy individuals. NMR metabolic profiling of serum at high temporal resolution showed distinct changes in the metabolic profile in insomnia patients compared to age- and sex-matched good sleepers over two days. Insomnia subjects manifested clear metabolic desynchrony in terms of altered circadian oscillation of circulatory metabolites along with significantly different global metabolic profile from healthy controls. Nighttime metabolism of insomnia patients were dominated by altered branched chain amino acid catabolism and increased accumulation of glucose. These observations suggest that insomnia is associated with quantitative metabolic dysregulation and supports the hyperarousal hypothesis. These results were compared to a study of HIV patients with co-morbid depression under treatment for insomnia with comprehensive serum metabolomics analysis using NMR spectroscopy, polar LC-MS/MS and untargeted lipidomics. Improvement of the insomnia severity index (ISI) post treatment was found to strongly correlate with changes in BCAA and branched chain keto acid (BCKA) metabolites along with long chain acylcarnitines and several lipid features. These results suggest that BCAA catabolism is mechanistically linked with occurrences of insomnia irrespective of the cause. This result also connects epidemiological evidence that insomnia may lead to other metabolic disorders such as diabetes and metabolic syndrome.

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Adaptive 2D qNMR

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Over the past few years the application of 2-dimensional HSQC for quantification has grown in popularity [1]. The resolution afforded by two frequency axis massively improves resolution for both pure materials and especially mixtures. Great progress has been made with the development of pulse programs to overcome the inherent difficulties of achieving uniform excitation across all ^{1}H-^{13}C species. Such issues as variations of polarization transfer from different ^{1}H-^{13}C coupling constants with different atomic species and also the inherent limitation from wide excitation bandwidth have been addressed in many ways. What we will present is an automatically adaptive approach to the use of the HSQC experiment and a subsequent data analysis which can be easily done with any HSQC pulse program and any reasonably modern NMR console/probe combination. Furthermore, it will be shown that even relaxation delay choice within reasonable limits has almost no impact upon the quality and precision of the result. Finally the quantification result can be based on a few as a single ^{1}H-^{13}C correlation pair up to a maximum of the sum of all cross-peaks so the technique can have inherently very high signal to noise regardless of the NMR spectrometer frequency or probe.

This technique is simply to acquire 2D results with any HSQC pulse program and parameter choices on reference standards and simply summing up the amplitudes for all desired signals. Make careful note of exact acquisition parameters paying special attention to spectral regions and data acquisition times of both dimensions as these must be kept constant. It is very important to retain the repetition rate. It can be helpful to turn off such items as multiplicity editing. Because the response factors are automatically mapped out as a function of each resonance for each reference material, subsequent analysis of complex mixtures in solutions of similar dielectrics with the same pulse program and parameters can be reliably measured from a simple ratio of the sums of all resolved cross peak amplitudes against the same cross peak amplitudes in the reference standards. Results near 1 to 1.5% are routinely obtainable. The technique works equally well with linear or non-uniform sampled data.

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Implications of Single versus Multiple Quantum in HFX 2D Correlation Experiments

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Fluorine NMR is becoming more central in chemical analysis and poses challenges in 2D NMR studies using traditional applications such as HMBC and HSQC-based applications. A well-known complication arises simply because of the potentially wide bandwidth for ^{19}F chemical shifts which can easily approach 400 ppm. Methodologies to cover such bands are fairly mature and easily implemented on current state of the art NMR consoles though in the end the fundamental ^{19}F pulse efficiency factors significantly. A less obvious complication with decoupling ^{19}F in evolution while observing nuclei such as ^{1}H with correlation to ^{13}C (or ^{31}P or ^{15}N) is the issue of strong coupling with significant active and passive couplings arising from the ^{19}F species. With multiple quantum based experiments such as gHMBC and its many variants the simple expediency of decoupling evolution by a single ^{19}F pulse may or may not work as expected because the often wide bandwidth imposed by ^{19}F cannot be covered with a simple 180. A sufficiently short ^{19}F 180 degree pulse is not practical so tailored pulses must be used. As strong ^{19}F couplings evolve during application of the evolution and ^{19}F 180's multiple quantum may not be the best choice of the best results. The evolution 180 both refocuses chemical shift and swaps zero and double quantum and in the presence of strong coupling, a single tailored decoupling 180 may not be sufficient. Use of a single quantum based pulse program such as LRHSQMBC has no such issues and it is relatively simple to construct a suitable BIP pulse for the purpose of evolution ^{19}F decoupling. In this poster we will describe working alternatives to single pulse ^{19}F evolution decoupling and also explore MQ and SQ 2D experiment methods for HFX 2D chemical methods.

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Characterization of the Activated State of Oncogenic Human K-RAS by Two-Dimensional ^{1}H-^{15}N HSQC NMR for Use in Small Molecule Drug Discovery

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K-Ras, a key driver of oncogenesis, is a small GTPase protein that exists in two distinct states of inactive, GDP-bound, and an active, GTP-bound. Oncogenic mutations that impair the GTPase activity of K-Ras result in a hyperactivated state, consequently leading to uncontrolled cellular proliferation and tumorogenesis. Single amino acid mutations at positions 12, 13, and 61 in K-Ras are commonly found in various cancers, including lung, pancreatic, and colorectal adenocarcinomas, and have been a focus for therapeutic intervention in the past several decades.

Several regions within the G domain of K-Ras that are particularly important in the processes of nucleotide exchange and downstream signaling, include the P-loop (residues 10–17), Switch I (25–40), and Switch II (57–75) regions. For GTP-bound K-Ras, local polysterism and conformational exchange render residues in the aforementioned regions undetectable in the fingerprint region of the 2D ^{1}H-^{15}N HSQC spectrum under physiological conditions. In order to use NMR to characterize the binding mode and mechanism of action for GTP-selective K-Ras inhibitors currently in development, we optimized NMR experimental conditions for several prevalent K-Ras mutants, resulting in emergence of crosspeaks for the P-loop and Switch residues in the 2D ^{1}H-^{15}N HSQC spectrum. Here we present interim data showing the progress made in these efforts.

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Evaluation of Stereochemical Insights from VCD Data Alongside ^{13}C Chemical Shift, RDC and RCSA Measurements

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Understanding stereochemistry of an active pharmaceutical ingredient is critical to maintaining control during drug development. A common technique used to assess stereochemistry is X-Ray crystallography. While X-ray crystallography is often a straightforward and reliable method for stereochemistry assessment, the requirement of a single crystal of sufficient size and quality can slow the analysis of some molecules, or in some cases precludes it entirely.

Vibrational circular dichroism (VCD) is a spectroscopic techinque first developed in the 1970s and has become a routine and robust tool for determination of configuration.[1,2] Anisotropic nuclear magnetic resonance paramteres measured in weakly aligned samples has also proven to be an important tool to assess stereochemistry.[3,4] Specifically, residual dipolar couplings (RDCs) and residual chemical shift anisotropies (RCSAs) have been used for analysis of configuration. One advantage of VCD and NMR spectroscopy over X-ray analysis is the easy sampling on solutions without the need to grow a single crystal.

During our analysis, we evaluated between 2 – 4 diastereomers associated with three different compound classes. VCD, ^{13}C, RDC and RCSA measurements were acquired on each diastereomer. Concurrently, Gaussian DFT calculations were performed to predict the VCD spectrum and ^{13}C chemical shifts associated with the diastereomers. Additionally, Mspin was used to calculate scalar couplings for RDC and RCSA data fitting and interpretation. Data was evaluated to determine the alignment and discrimination of each technique towards one configuration. Results were variable depending on the rigidity, size and shape of the compound class that was evaluated.

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A New Method for the Reliable Detection of ^{13}C Multiplets of Fluorine Containing Compounds

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In modern organic and medicinal chemistry, fluorine is commonly used to enhance the chemical properties of molecules in many desirable ways: it may delay the metabolism of the molecule due to the increased stability of the C-F bond, reduce the toxicity of aromatic groups by forbidding the formation of poisonous peroxides during metabolism, or increase the bioavailability due to the higher lipophilicity of the C-F bond vs the C-H bond. As a result, it is estimated that more than 20% of commercial pharmaceutical APIs and 30% of agrochemicals contain at least one fluorine atom [1,2].

In contrast to these benefits, the ^{13}C NMR spectra of fluorinated organic compounds are highly susceptible to interpretation errors. This is because ^{13}C spectra are commonly recorded using only ^{1}H broadband decoupling and the ^{13}C-^{19}F couplings are still present. These coupling constants can be very large (up to 250 Hz or more), which may result in multiplets severely overlapping with other peaks in the spectrum. Additionaly, since ^{13}C spectra inherently have low S/N, it is not uncommon that the lower (outer) parts of a multiplet are below the noise level and not visible. To mitigate this, it is possible to record ^{13}C spectra broadband decoupled from both ^{1}H and ^{19}F but this requires specialized NMR probes and decoupling techniques. Moreover the very broad range of ^{19}F chemical shifts could pose a danger of damage to the probe due to the excessive power that would be required. Consequently, this approach is not considered practical for routine use.

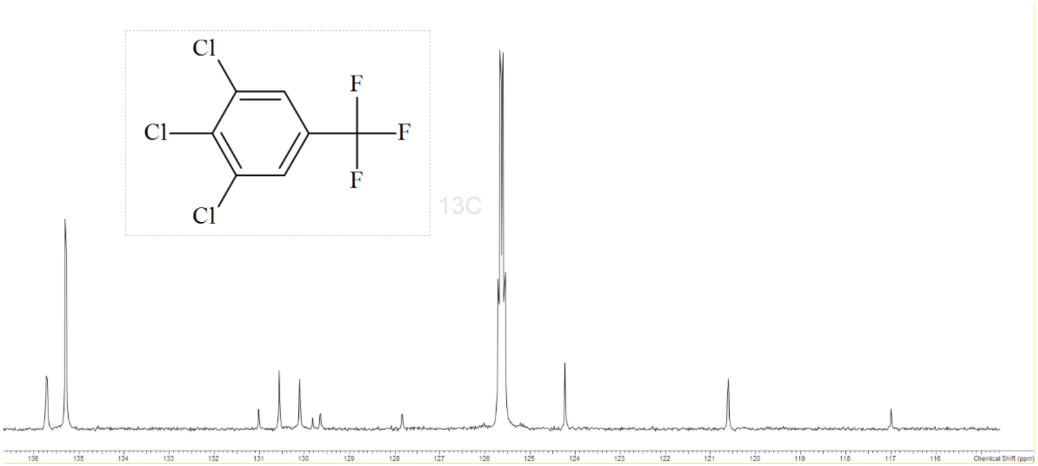

Figure 1: Expansion of the ^{1}H decoupled ^{13}C spectrum of 1,2,3-Trichloro-5-(trifluoromethyl)benzene, indicating the overlapping multiplets due to the ^{19}F-^{13}C couplings.

Here we present an analysis method that reliably peak-picks and identifies multiplets in the ^{13}C spectra of organic compounds. This technique is based on accurately predicting the ^{19}F coupled ^{13}C spectrum of the proposed compound. Following prediction, we examine the regions of the experimental spectrum where the ^{19}F coupled carbons are expected in order to identify multiplets by peak position and the agreement in the predicted and observed coupling constants. Provisions are taken if only part of a multiplet is observed. We show that regardless of whether the final results contain multiple, overlapping multiplets, the expected carbon resonances are reliably identified and assigned for each spectrum. Typical examples from common fluorine containing compounds are shown.

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Using Predicted ^{13}C NMR Spectra with Open Resources for Structure Dereplication of Natural Products

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For successful natural product-based drug discovery, it is critical to reliably separate and identify active components in natural product mixtures. Dereplication is the practice of screening active compounds early in the development process, to recognize and eliminate compounds that have been previously studied. This enables scientists to focus on testing truly 'unknown' compounds. For efficient dereplication, one must be able to easily identify characteristic spectral "fingerprints" of compounds in order to identify their structure and have access to databases containing known structures.

The ^{13}C NMR spectrum of a compound can be considered a fingerprint since it is virtually unaffected by conditions such as pH, concentration, and solvent effects. It is also largely magnetic field independent, since there are no couplings that could cause variations in stronger or weaker fields. As a result, it is very easy and accurate to predict. To identify experimental ^{13}C spectra, one can consider predicting the ^{13}C spectra of known chemical structures found in "open" chemistry databases (e.g. PubChem[1]) and seeing if there is a match. Predicted spectra benefit from being magnetic field independent, can be adjusted for solvents, and can be very accurate if the correct algorithms are used [2].

Isolated compounds in natural product research are typically low in quantity, thus it may not be experimentally possible to acquire a 1D-^{13}C spectrum. As a result, ^{13}C information is often obtained from indirect detection experiments like HSQC and HMBC. The proposed dereplication method can be adopted to work with this data, providing a very valuable resource to natural products chemists. This presentation explores the possibilities and limitations of this novel technique and applies it to natural products from the fungus *Ganoderma pfeifferi*, cyanobacteria strains from the genera *Nostoc* and *Cylindrospermum,* and actimomycetes of the genus *Streptomyces*.

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Structural Elucidation of Gramillin A and B, Novel Cyclic Lipopeptides Produced by Fusarium Graminearum

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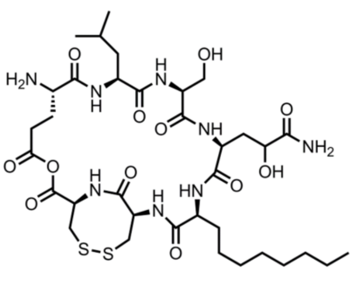
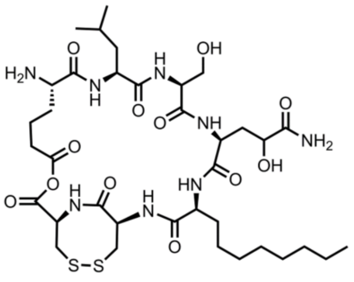
Fusarium graminearum is a broad host pathogen causing *Fusarium* Head Blight (FHB) in diverse cereal crops. The virulence and host range of *F. graminearum* is associated with its ability to secrete an arsenal of proteins and toxic secondary metabolites, including the regulated mycotoxin deoxynivalenol as a virulence factor. The *F. graminearum* genome harbors an array of unexplored biosynthetic gene clusters that are often co-induced with the *TRI* genes, including the nonribosomal peptide synthetase 8 (*NRPS8*) gene cluster. Here, two bicyclic lipopeptides, named gramillin A and B, are identified as the biosynthetic end products of *NRPS8*. Gramillin A and B have been shown to be phytotoxins, causing cell death in select plants including maize and Arabidopsis, but not in wheat, indicating that *F. graminearum* deploys the gramillins as a virulence agent in a host-specific adaptation.

The full structural elucidation was performed on a purified but inseparable mixture of unlabeled gramillin A and B and ^{15}N-labelled gramillins. ^{15}N enriched samples were produced by simply substituting nitrogen sources in *F. graminearum* liquid cultures with ^{15}N-ammonium chloride. A combination of high-resolution LC-MS, 1D and 2D NMR, including ^{1}H-^{15}N-^{13}C HNCO and HNCA on the isotopically enriched compounds revealed that the gramillins possess a fused bicyclic structure with ring closure of the main peptide macrocycle occurring via an anhydride bond. A disulfide bond between two adjacent cysteines forms the second smaller ring. Intra-residue connectivity was determined by COSY and TOCSY, the peptide sequence was solved by HNCA and absolute stereochemistry by chiral LC-MS.



Gramillin A
MF = $C_{35}H_{58}O_{12}N_8S_2$



Gramillin B
MF = $C_{36}H_{60}O_{12}N_8S_2$

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Applications of Quantitative NMR Spectroscopy in Pharmaceutical Development

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The use of NMR spectroscopy is ubiquitous throughout discovery and development in the pharmaceutical industry as a primary tool for structure elucidation and identification. But perhaps the most useful application of NMR Spectroscopy is the inherently quantitiative structure-to-spectrum relationship, allowing for the unambigous assignment of a signal in the spectrum to a specific atom in the analyte molecule and the ability to determine the exact amount present – a capability almost entirely exclusive to the field of NMR. There is also the potential to utilise a variety of nuclei for quantitative analysis, with the possibility of using ^{1}H, ^{13}C, ^{19}F or ^{31}P NMR if acquired under appropriate conditions.

This phenomenen is well known and is commonly used for purity determinataion (assay by NMR), either with the use of internal or external reference standards, be they real or electronic, with accuracy and precision comparable to other analytical trechniques (e.g. HPLC-UV or LC-MS). One advantage of NMR spectroscopy is that no primary reference standard of a sample is required for quantification, as the quantitative NMR signal response is universal and not compound specific. This also allows for the direct (and quantitative) analysis of multicomponent systems, which would have different UV resposnes or MS ionisation potentials.

Here we will share a number of examples where quantitative NMR Spectroscopy has been used for more than routine assay in pharmaceutical development and demonstrate how it is also used for the analysis of a number commercial products (along with associated registered methods). In each case, NMR has been utilised because other quantitative analytical techniques were not suitable. Examples include the use of interleaved ^{1}H and ^{19}F/^{133}Cs NMR for reaction monitoring and process understanding, enabling the real-time tracking of multiple analytes within a complex mixture. Also, we have developed quantitiative selective excitation ^{1}H NMR methods using excitation sculpting for cleaning validation of non-chromorphoric analytes, allowing limit tests with sensitivity at single digit ppm levels.

We will describe a number of quantitative ^{13}C NMR methods used in the manufacture and release of the oncology drug Zoladex. These include a fully automated ^{13}C NMR method for ID and amino acid ratio of the deca-peptide goserelin (the active ingredient in Zoladex), along with quantitative ^{13}C NMR analysis for ID and determination of the lactide-glycolide ratio in the proprietary co-polymer used to formulate the Zoladex depot.

We also show how routine quantitative ^{1}H NMR spectroscopy has been utilised to perform 7 concurrent analyses in a single proton spectrum during the development of the antibiotic Ceftaroline. ^{1}H NMR is used in the assessment of suitability of material in the manufacture of Seroquel XR, by quantifying the ratios of hydropropoxy (HP) and methoxy (MeO) substituents on the cellulose backbone of the excipient Methocel. And finally, we show how quantitative NMR is being used by AstraZeneca in the global fight against counterfeit medicines.

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NMR Study of Methyl Salicylate Hydrolysis in the Presence of Amino Acid

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Methyl salicylate (MeSA), the major flavor component in Wintergreen oil, has been extensively used in many consumer products, such as toothpaste, mouth rinse, and lotions. It has been known that MeSA can be easily hydrolyzed in aqueous solution, particularly in basic condition and in the presence of amino acids, yet the detailed mechanism of MeSA–amino acid interaction is not well understood.

In this work, a combination of ^{1}H NMR, UV-Vis, and GC-MS techniques was used to monitor the hydrolysis reaction of MeSA and to analyze the end products in the presence or absence of amino acids in alkaline solution. A composite pre-saturation pulse sequence was used to suppress the water peak in the ^{1}H NMR spectrum of the sample containing MeSA in the full presence of bulk water, allowing for detecting and quantifying the structural transition of MeSA during the hydrolysis at the MeSA concentration as low as 0.05 wt%. It was found that the amino acids examined, including glycine, lysine, and arginine, can accelerate the MeSA hydrolysis by an order of magnitude. By selectively protecting either the amine and/or carboxyl groups of amino acids, the hydrolysis rate of MeSA can be significantly reduced. This result suggests that both amine and carboxyl groups of amino acid are critical to MeSA hydrolysis, likely forming an intermediate complex with MeSA via hydrogen bonds, whereas hydroxyl ion acts as an effective nucleophile to attack the carboxyl group of MeSA in basic solution. Density functional theory (DFT) computations provide insight into the mechanism of MeSA–OH^- interaction. The potential prevention of MeSA hydrolysis by the encapsulation with cyclodextrin will also be discussed. The results of this study can be helpful to finding ways of stabilizing MeSA in the consumer products.

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Tautomeric Equilibrium in Lawsone and Curcuminoids: An NMR Study

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Quinones are a useful building block for the synthesis of heterocyclic compounds[1] *v. gr.* the synthesis of quinoxalines. The biological effects of naphthoquinone systems are quite varied [2] for example it is used for the synthesis of aminonaphthoquinone that posseses a great pharmacological potential as antibiotic and anticancer[3] or related compounds such as deoxylapachol that is reported as a cytotoxic component [4]. In addition have also been used for the synthesis of metal complexes[5]. Curcuminoids on the other hand have been reported to be useful agent for a wide variety of deseases including arthritis, Alzheimer and many other involving the disturbances that involve inflammation. [6]

Lawsone and some curcuminoids were studied in regard to their tautomerism using a simple approach involving deuterium exchange. The results allowed the observation of ^{13}C-D couplings in both cases.

Curcuminoid derivatives show the expected triplet at the methylene flanked by carbonyls and their C13 spectra allow estimation of the degree of deuterium exchange.

The tautomerism of lawsone allows to postulate an intermediary that explains the reactivity of this naphtoquinone that reacts as a 1,2 diketone with *o*-phenylenediamine. The mechanistic proposal is supported by deuterium exchange demonstrating the presence of α-diketone system in the equilibrium.

The chemical shifts for tautomers were assigned in liquids NMR and the exchange with D_2O provides the mechanism of scrambling (triplet in the ^{13}C NMR spectrum). The chemical shift assignments of both tautomers have been determined unambiguously using 1D and 2D experiments in several solvents at 600 MHz.

Two lawsone tautomers (α-diketone and α-hydroxyketone) can be clearly distinguished in solution (in several solvents) and their ^{1}H NMR spectra reveals two sets of multiplets with small overlap in the aromatic zone, while previous reports deal with asssignments for only one tautomer and only one set of ^{13}C signals is reported in DMSO [7].

The behavior of lawsone as well as the mechanism of tautomeric exchange (see diagram) is clearly observed through deuterium exchange that involves scrambling of deuterium at the –OH and vinylic sites. Both tautomers are observed while α-hydroxyketone form is predominant in solution. The exchange of deuterium in curcuminoids proceeds as expected and allows estimation of the degree of deuterium exchange.

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SSNMR as a Complementary Method for Studying Steroidal Supramolecular Gels

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Supramolecular gels formed by low molecular weight gelators have been under intensive research in the past decades [1-3]. The properties of these soft systems differ from those of pure solids or liquids leading to emergence of applications in numerous fields. Supramolecular gels manifest a network consisting of fibers or other nano- or microstructures, which immobilizes the bulk solvent by weak interactions.

Bile acids are end products of cholesterol metabolism formed in the hepatocytes and modified by the bacteria in the intestines. They have an essential role in digestion and absorption of lipids and lipid-soluble vitamins. In addition, they prevent cholesterol from precipitating in the gall bladder. Since the steroidal skeleton of the bile acids is curved and facially amphiphilic, and the hydroxyl groups and the carboxylic acid group enable tailoring of the water/lipid-solubility of the molecules [4], bile acids are ideal building blocks when designing low molecular weight gelators [5].

In continuation of our earlier work on the supramolecular gelation properties of bile acids [6], we present a detailed study on the self-assembly and gelation tendency for a series of bile acid alkyl amides [7]. Good quality single crystals of all nine amides were obtained and the subsequent single crystal X-ray studies revealed N···H, O···H, and H···H interactions to be the most important intermolecular interactions affecting organization of the molecules in the solid state. Of the 50 gel systems formed, 35 consisted of lithocholic acid derivatives and 15 of cholic acid derivatives, whereas deoxycholic acid derivatives did not form any gels. Even though the length and branching of the aliphatic side chain seemed to have clear effect on the gelation abilities of the compounds, the number of hydroxyl groups attached to the steroidal backbone played major role in the gelation. The ^{13}C CPMAS NMR spectra measured from both the single crystals and the corresponding wet gels were analogous, suggesting that the chemical environments and the intermolecular interactions found in the gel and in the crystalline state are similar.

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Mechanistic Study of a Re-Catalyzed Monoalkylation of Phenols

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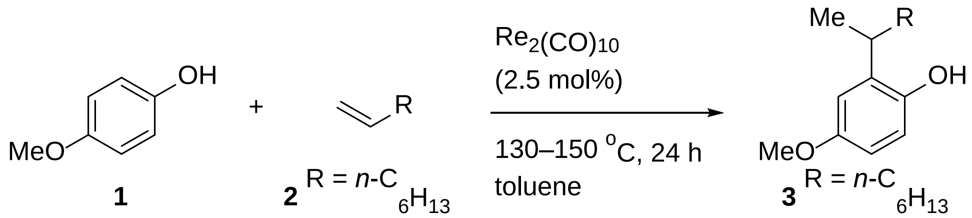
The Re-catalyzed alkylation of phenols with terminal alkenes was initially reported by Takai and coworkers.[1] This reaction provides exclusively the mono-alkylation product, is highly regioselective, and results in sole observation of the branched product. This result is in contrast to other methods of functionalizing phenols that afford mixtures of mono- and bis-alkylated products. We sought to gain understanding of the underlying reaction mechanism for this Re-catalyzed reaction in order to develop an efficient synthesis of ortho-monoalkylated phenols for the development of active pharmaceutical ingredients. Herein we report a detailed mechanism investigation of this reaction by NMR spectroscopy, isotoplic labelling studies, kinetic isotope effects and other kinetic studies, as well as characterization of several off-cycle rhenium species by NMR techniques. The results suggest that the reaction does not proceed via Friedel-Crafts type pathway, but instead proceeds via a reversible C–H activation, then a net hydroarylation process.



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