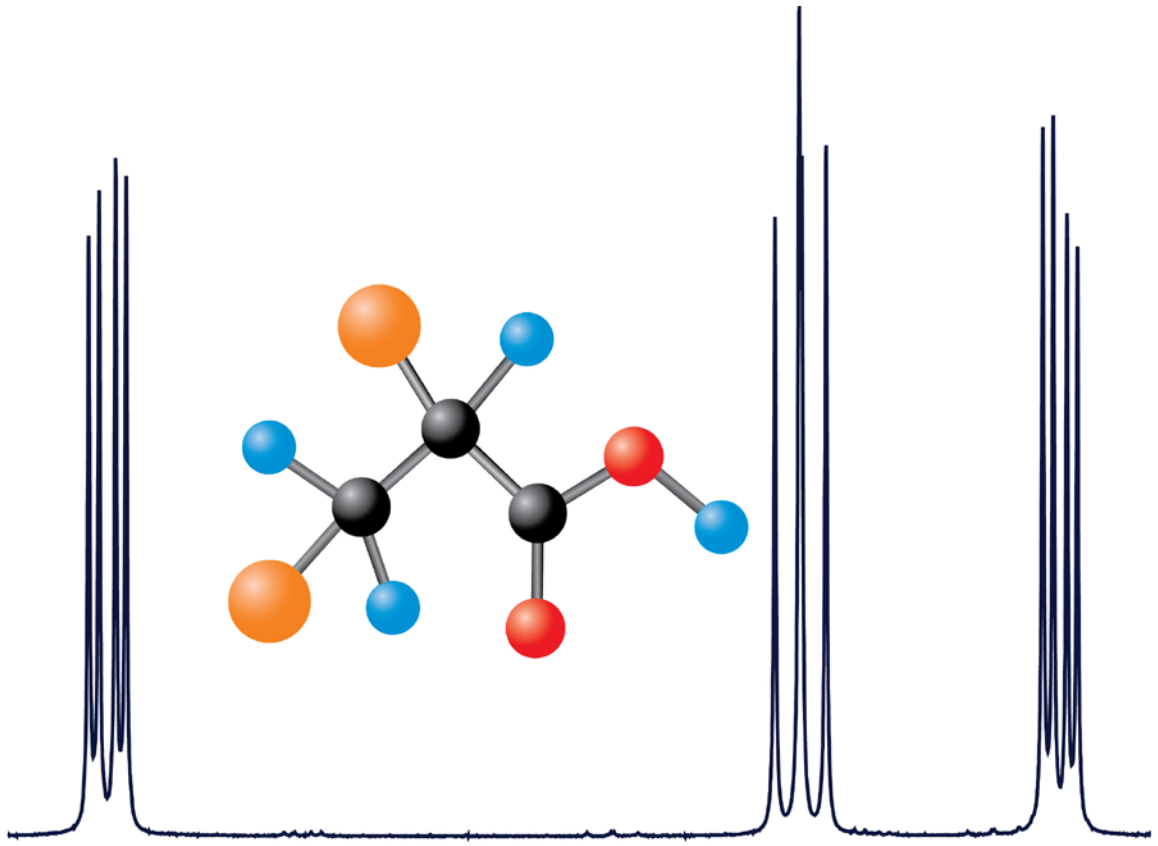

SMASH 2011

Conference Program

September 18th-21st, 2011

Chamonix, France

SMASH 2011 NMR Conference

Dear SMASH 2011 Attendees,

Welcome to SMASH 2011, held in the beautiful French Alps in the shadow of breath-taking Mont Blanc. The charming ski and mountaineering resort town of Chamonix is our venue once again, where we take inspiration from the majesty of the surroundings whilst profiting from the close proximity of conference activities and attendees.

We are making a significant effort this year to bring a balance to the programme so that the talks and posters are of equal interest to industrial and academic spectroscopists. We have also made every effort to appeal to researchers at every stage in his or her career, and to this end we have been very fortunate to receive excellent sponsorship that allows us to assist a record 17 early-career NMR spectroscopists to attend and present their work at SMASH. Reinforcing this outreach, we have set aside time for more talks promoted from excellent posters. Vendors continue to give us strong support and add to the proceedings through their Users' Meetings and booths.

The success of SMASH lies equally in the attendees and speakers and we believe that 2011 will follow in what is becoming a well-established tradition of excellence. This year we have seven oral sessions entitled "*Optimizing NMR in the Modern Laboratory*", "*Student/PostDoc Session*", "*Critical Applications of NMR in Pharmaceutical Development*", "*Further Progress in Solid State NMR*", "*Advances in Determining Configuration/Conformation*", "*Enhancements for Physical Organic Chemistry*", "and "*NMR Solutions to Combating Counterfeits and Achieving Quality*." There will also be two workshops and two tutorials: *"University vs Industry: Challenges for Doing Innovative Science"* and *"Automated Structure Verification"* are workshops that will focus around general discussion. "*Quantification in NMR without Using Internal References*" and "*Optimization of Small Molecule NMR Experiments*" are more lecture in nature and geared toward spectroscopists who are interested in learning more about these techniques or perhaps just want a refresher on the topic. We have more than 100 posters spread over two sessions which, together with the strong response we received for poster contributors to present their work as an oral presentation, we are confident will provide an excellent and varied scientific programme.

It is also our pleasure to welcome Professor Ray Freeman from Jesus College, Cambridge University who has kindly agreed to do us the honour of presenting the after dinner talk entitled "Heroes, Mentors, Colleagues and Accomplices". Many will know Ray both for his outstanding contribution to small molecule NMR and his captivating and witty talks.

On behalf of the entire SMASH Organising Committee we thank you for your continuing interest and support of the SMASH NMR Conference and hope that you will enjoy your stay in Chamonix.

Sincerely,

Christina Thiele and Michael Bernstein
Co-Chairs, SMASH 2011 NMR Conference

SMASH 2011 NMR Conference Program

Sunday, September 18th

5:00 PM - 6:00 PM **Registration, Le Majestic Centre de Congrès**

6:00 PM - 8:00 PM **Dinner, Le Vista Restaurant (Alpina Hotel)**

8:00 PM -11:00 PM **Mixer, Le Vista Restaurant (Alpina Hotel)**

Monday, September 19th

8:45 AM - 9:00 AM **Opening Remarks**

9:00 AM - 10:30 AM **Optimizing NMR in the Modern Laboratory**

Chair: Gary Sharman, Eli Lilly and Company, UK

High-Throughput Screening with ^{19}F NMR Spectroscopy

Claudio Dalvit, Italian Institute of Technology, Italy

Speeding Up Common NMR Experiments - SMART NMR

Philippe Pelupessy, ENS, France

Multiscan Single Shot 2D NMR: a New Tool to Optimize Fast Quantitative Analysis

Patrick Giraudeau, University of Nantes, France - Upgraded Poster

Automatic Generation of Negative Control Structures for Automatic Structure Verification Systems

Gonzalo Hernández, Vis Magnetica, Uruguay - Upgraded Poster

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

11:00 AM - 12:30 PM **Poster Session I (even numbered posters)**

Chair: Philippe Lesot, Université Paris-Sud, France

12:30 PM - 2:30 PM **Lunch, La Caleche Restaurant, Free Time & Vendor Discussions**

2:30 PM - 4:00 PM Student and Post-Doctoral Session

Chair: Philippe Lesot, Universite Paris-Sud, France

Spectral Aliasing and Homonuclear Decoupling in F1: Two Strategies to Increase the Resolution in 2D NMR Spectra

Mohammadali Foroozandeh, Université de Genève, Switzerland

^{1}H NMR Profiling for Authentication of Pomegranate Juice

Daniel Orr, University of California, Riverside, USA

RDC-based Determination of the Relative Configuration of the Fungicidal Cyclopentenone Hygrophorone A

Volker Schmidts, Technische Universität Darmstadt, Germany - Upgraded Poster

Detection of ^{1}H-^{1}H Proximities in Small Organic Solids through Imperfect Homonuclear Dipolar Decoupling

Giulia Mollica, Universities of Aix-Marseille, France - Upgraded Poster

4:00 PM - 4:30 PM **Break**

4:30 PM - 6:00 PM **Workshop: University vs. Industry: Challenges for Doing Innovative Science**

Facilitators: Gareth Morris, University of Manchester, UK; Mike Bernstein, MestreLabs, UK and Brian Marquez, Pfizer, USA

Tutorial: Quantification in NMR without Using Internal References

Facilitator: Richard Upton, GSK, UK

6:00 PM - 6:30 PM **Free Time**

6:30 PM - 11:00 PM **Conference Dinner, La Baita Restaurant (Hotel Prieuré)**
After Dinner Speaker: Professor Ray Freeman, University of Cambridge, UK
Heroes, Mentors, Colleagues and Accomplices

Tuesday, September 20th

9:00 AM - 10:30 AM Critical Applications of NMR in Pharmaceutical Development
Chair: Adrian Davis, Pfizer, UK

NMR Techniques and Applications in the Drug Development Phases (from drug substances to drug product to market)
Carla Marchioro, Aptuit Inc., Italy

What Lies Beneath the Surface? Impacting Drug Product Formulation Through NMR Imaging
Daneen T. Angwin Hadden, Eli Lilly and Company, USA

NMR as a Technique for Quality Control: Pharmacopoeial Tests Analyzing Intact Samples
Paulo Dani, Merck, Netherlands

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

11:00 AM - 12:30 PM **Workshop: Automated Structure Verification**
Facilitators: Gary Sharman, Eli Lilly and Company, UK; John Hollerton, GSK, UK

Tutorial: Optimization of Small Molecule NMR Experiments
Facilitators: Rainer Kerssebaum, Bruker, Germany; Eriks Kupče, Agilent, UK

12:30 PM - 2:30 PM **Box Lunch, Free Time & Vendor Discussions**

2:30 PM - 8:30 PM **Free Time**
Continuation of Automated Structure Verification Workshop
Note: There is no Conference Dinner Planned
Attendees are encouraged to try out the numerous local restaurants

8:30 PM - 11:00 PM **Poster Session II (odd numbered posters) and Mixer**
Chair: Philippe Lesot, Université Paris-Sud, France

Wednesday, September 21st

9 :00 AM - 10:30 AM Further Progress in Solid State NMR
Chair: Gerd Buntkowsky, Technische Universitat Darmstadt, Germany

Solid-State NMR of Small Molecules in Small Rotors
Marek Pruski, Ames Laboratory, USA

Energy Storage and Conversion: Using Local Structural Probes to Understand and Optimise Function of Battery and Fuel Cell Materials
Clare Grey, University of Cambridge, UK

Solid State ^{19}F NMR Spectroscopy Sensitive Tool for Detection of Polymorphic Purity
Jaroslav Havlicek, Zentiva a.s., Czech Republic - Upgraded Poster

^{1}H MAS Line Widths of Small Organic Guest Molecules Confined in Porous Silicas
Gábor Szalontai, University of Pannónia, Hungary - Upgraded Poster

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

11:00 AM - 12:30 PM Advances in Determining Configuration/Conformation

Chair: Christina Thiele, Technische Universitat Darmstadt, Germany

NMR for Small Molecule Configuration and Drug Validation

Christian Griesinger, MPI Biophys. Chem., Germany

^{13}C-NMR as a General Tool for the Assignment of Absolute Configuration

Ricardo Riguera, Universidade de Santiago de Compostela, Spain

Revisiting the (D/H) Isotopic Fractionation Analysis of (Un)saturated Fatty acids and Triglycerides: What NAD 2D NMR Spectroscopy in Aligned Media Can Do for You?

Philippe Lesot, Université de Paris-Sud, France - Upgraded Poster

Precise Measurement of Heteronuclear Coupling Constants from ^{1}H Selective HSQMBC Experiments

Teodor Parella, Universitat Autònoma de Barcelona, Spain - Upgraded Poster

12:30 PM - 2:30 PM **Lunch, La Caleche Restaurant, Free Time & Vendor Discussions**

2:30 PM - 4:00 PM Enhancements for Physical Organic Chemistry

Chair: Craig Butts, University of Bristol, UK

Quantifying Conformational Control

Jonathan Clayden, University of Manchester, UK

Atropisomerism: NMR Investigations and Optimization

Richard Lewis, AstraZeneca, Sweden

Dynamics and Structure of Organolithiums by Modern NMR Spectroscopy

Ann-Christin Pöppler, Georg August Universität Göttingen, Germany - Upgraded Poster

Trilinear Analysis: NMR Reaction Monitoring for Overlapped Spectra

Mathias Nilsson, University of Manchester, UK - Upgraded Poster

4:00 PM - 4:30 PM **Break**

4:30 PM - 6:00 PM NMR Solutions to Combating Counterfeits and Achieving Quality

Chair: Carla Marchioro, Aptuit Inc., Italy

Applications of Low Field NMR in Quality Control: Established Methods and Developments

Gisela Guthausen, Karlsruher Institut für Technologie, Germany

Mobile NMR of Art and Cultural Heritage

Bernhard Blümich, RWTH Aachen University, Germany

Isotopic NMR Spectrometry as an Efficient Tool to Fight Against Counterfeiting: High Accuracy Requirement and Sensitivity Improvement

Gérald S. Remaud, University of Nantes, France - Upgraded Poster

2D DOSY ^{1}H NMR Analyses for the Characterization of Fake Drugs

Myriam Malet-Martino, University of Paul Sabatier, France - Upgraded Poster

6:00 PM **Closing Remarks**

SMASH 2011 Scholarship Recipients

The following students received a scholarship to attend SMASH 2011

- Manuel Alvaro Berbis
- Adolfo Botana
- Luis Calle
- Elodie Dempah
- Raluca M. Fratila
- Victoria Gomez
- Agnes Haber
- Michael Haindl
- Michael Hammer
- Peter Kiraly
- Andreas Kolmer
- Damjan Makuc
- Silvia Mari
- Jens Pilger
- Ann-Christin Pöppler
- Sarah Pyszczynski
- Bruno Vitorge

Thanks to our scholarship sponsors for their generous support.

SMASH 2011 NMR Conference Acknowledgements

SMASH gratefully acknowledges the support provided by the following companies.

act-GmbH / Magritek
Advanced Chemistry Development, Inc., (ACD/Labs)
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Armar Chemicals
Bruker
Chenomx, Inc.
CortecNet
Euriso-Top
GDCh
GPE Limited
Isotec/Sigma Aldrich
JEOL
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SMASH 2011 NMR Conference Committee

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Poster Session

Philippe Lesot
Universite Paris-Sud, France

Monday, September 19th
9:00 AM - 10:30 AM

Optimizing NMR in the Modern Laboratory
Chair: Gary Sharman

Speakers:

Claudio Dalvit
Italian Institute of Technology, Italy

Philippe Pelupessy
ENS, France

Patrick Giraudeau
University of Nantes, France

Gonzalo Hernández
Vis Magnetica, Uruguay

High-Throughput Screening with ^{19}F NMR Spectroscopy

Claudio Dalvit

Italian Institute of Technology, Department of Drug Discovery & Development, Genova, Italy

NMR Spectroscopy is now well recognized for its important role in the fragment-based drug discovery projects. A plethora of experiments exploiting different observable NMR parameters has been proposed for this purpose. Although NMR has an intrinsic low sensitivity, thus allowing only a limited throughput, it has one of the highest relative sensitivity to protein binding. This is due to the large dynamic range, defined as the difference of the NMR measured response in the free and protein-bound states. 19F R2 filter NMR experiments are among the most sensitive techniques for binding detection and can identify binders where other NMR experiments and other biophysical techniques fail. In this approach, a library of fluorinated fragments with different Local Environment of Fluorine (LEF)[1] is first screened. The identified binders are then used as starting chemical scaffolds for medicinal chemistry activities or as spy molecules for subsequent screening efforts with FAXS (Fluorine chemical shift Anisotropy and eXchange for Screening) and for measuring with accuracy the dissociation binding constants of the molecules binding to the receptor. The possibility of screening large chemical mixtures[1] along with sensitivity improvements deriving from recent technological and pulse sequence[2] developments allow an increase in the screening throughput making 19F NMR spectroscopy an attractive and versatile technique for screening large compound collections.

1. A. Vulpetti, U. Hommel, G. Landrum, R. Lewis and C. Dalvit J. Am. Chem. Soc. 131, 12949-12959, (2009)
2. C. Dalvit, A.D. Gossert, J. Coutant and M. Piotto Magn. Reson. Chem. 49, 199-202, (2011)

Speeding Up Common NMR Experiments - SMART NMR

Bruno Vitorge, Geoffrey Bodenhausen and Philippe Pelupessy

École Normale Supérieure - Paris

Due to technological advances, NMR is becoming an increasingly sensitive technique. This can be used either to perform measurements on more dilute samples or to reduce the experimental time. The latter can be achieved by minimizing the recovery delay between consecutive increments of a multidimensional experiment. However, signal leakage from previous increments may lead to considerable artifacts when this delay is smaller than the relaxation times of the different coherences. We present a method to effectively suppress the interference of longitudinal and transverse magnetization components from one scan to another. The use of SMAll Recovery Times (SMART) permits a reduction of the experimental duration by more than an order of magnitude.

Multi-scan Single Shot 2D NMR: a New Tool to Optimize Fast Quantitative Analysis

Meerakhan Pathan, Serge Akoka, Illa Tea, Benoit Charrier and Patrick Giraudeau

CEISAM UMR 6230, University of Nantes, Nantes, France

2D NMR is a powerful tool for quantitative analysis of complex mixtures. The main drawback affecting 2D NMR experiments is their experimental duration, due to the time incrementation necessary to sample the indirect dimension. Moreover, when 2D NMR is used for quantitative measurements, a calibration procedure is required, thus increasing further the overall experiment time.

This time limitation can be circumvented by the so-called Ultrafast 2D NMR methodology [1], allowing the acquisition of a 2D spectrum in a single scan, and thus in a fraction of a second. Recently, we have considerably improved the analytical performances of ultrafast 2D experiments, in terms of resolution, lineshape, sensitivity and spectral width [2,3]. However, the molecular concentrations available in complex biological mixtures are often not sufficient to obtain, in a single scan, the sensitivity required for a precise quantification and detection. Still, ultrafast signals can be accumulated in order to increase the sensitivity of these experiments while preserving reasonable experiment durations. But little is known about the sensitivity of ultrafast experiments versus conventional 2D NMR. A fair and relevant comparison has to consider the SNR ratio per unit of time, in order to determine the optimum choice: for a given experiment time (e.g. 1 h), should we run a conventional 2D experiment or is it preferrable to accumulate ultrafast acquisitions for 1 h?

To answer this question, we performed a systematic comparison between accumulated, optimized ultrafast experiments and conventional acquisitions, for different conditions and pulse sequences. Assuming that the sensitivity of 2D experiments is limited by the dimension where the SNR has the lowest value, it was found that the sensitivity per unit of time is much higher in ultrafast NMR (e.g. by a factor of 5 for COSY). This result is mainly attributed to the absence of t1 noise in ultrafast experiments and highlights the interest of accumulating ultrafast signals instead of conventional ones. This new multi-scan single shot approach offers a very high flexibility and can be easily implemented on standard spectrometers. Analytical aspects of this promising quantitative methodology and applications to quantitative analysis of metabolic samples [4] will be presented.

1. L. Frydman, T. Scherf, A. Lupulescu, Prod. Natl. Acad. Sci. USA, 99 (2002) 15858-15862.
2. P. Giraudeau, S. Akoka, J. Magn. Reson., 205 (2010) 171-176.
3. P. Giraudeau, S. Akoka, Magn. Reson. Chem., 49 (2011) 307-313
4. P. Giraudeau, S. Massou, Y. Robin, E. Cahoreau, J.-C. Portais, S. Akoka, Anal. Chem., 83 (2011), 3112-3119.

Automatic Generation of Negative Control Structures for Automatic Structure Verification Systems

Gonzalo Hernández

Vis Magnetica, Montevideo, Uruguay

The generation of positive and negative controls is a fundamental part of good experimental design. Getting a positive outcome on a test performed over a subject known to give a positive result, reassures the scientist the test is working properly. As important, if not more, is to test over subjects known to give negative results. Getting a negative outcome when expected validates the test and increases the results confidence when applied to unknowns.

Automated Structure Verification (ASV) is no different than any other scientific test. Positive as well as negative controls should be frequently tested to optimize performance and to obtain a measure of robustness and confidence in the results.

In this poster I will show how to automatically generate relevant negative control structures for any type of NMR data. Furthermore, I will argue that ASV systems fall in the category of binary classifiers, and that their performance can be measured by a host of metrics, already in use in the fields of statistical classification and signal detection theory.

Monday, September 19th
2:30 PM - 4:00 PM

Student and Post-Doctoral Session
Chair: Philippe Lesot

Speakers:

Mohammadali Foroozandeh
Université de Genève, Switzerland

Daniel Orr
University of California, Riverside, USA

Volker Schmidts
Technische Universität Darmstadt, Germany

Giulia Mollica
Universities of Aix-Marseille, France

Spectral Aliasing and Homonuclear Decoupling in F1: Two Strategies to Increase the Resolution in 2D NMR Spectra

Mohammadali Foroozandeh1, Rupali Shivapurkar1, Patrick Giraudeau2, Damien Jeannerat1

1. Departement de chimie organique, Université de Genève, Genève, Suisse
2. CEISAM UMR CNRS 6230, Université de Nantes, Nantes, France

Sensitivity and low resolution in F1 are two long-standing problems especially in 1H-13C heteronuclear spectra where the carbon window is typically 250 kHz broad. Among the numerous techniques aiming at increasing the resolution in F1, one of the most simple consists in the optimization of the sampling of the F1 dimension by spectral aliasing [1,2]. The problem is that high resolution makes little sense if scalar couplings disperse the signals over a large number of weak transitions. It is therefore preferable to be able to decouple all scalar interactions [3] except when their measurement is needed.

Heteronuclear decoupling techniques have been available for quite some time, but eliminating homonuclear interactions is a much more difficult challenge. The first broadband homodecoupling based on spatial encoding was introduced by Zanger and Sterk for 1D proton spectra and extended to COSY and DOSY experiments by James Keeler and Gareth Morris respectively. In order to address the specific problem of 13C-enriched small molecules, we introduced a generally applicable broadband 13C-homodecoupled HSQC experiment (BBHD-HSQC) [4] which effectively eliminates 13C-13C couplings in fully 13C labeled molecules. It was successfully applied to enriched cholesterol. Solutions to overcome the intrinsically low sensitivity of spatially encoded sequences will be discussed.

1. Jeannerat, D., Rapid multidimensional NMR: high resolution by spectral aliasing, Encyclopedia of Magnetic Resonance, Wiley
2. Foroozandeh, M., Jeannerat, D., ChemPhysChem, 11, 12, 2503-2505, 2010
3. Shaka, A.J., Keeler, J., Frenkiel, T., Freeman, R., J. Magn. Reson., 52, 335-338, 1983
4. Foroozandeh, M., Giraudeau, P., Jeannerat, D., ChemPhysChem, in press, 2011

^{1}H NMR Profiling for Authentication of Pomegranate Juice

Daniel J. Orr, Sumukh M. Sathnur and Cynthia K. Larive

Chemistry Department, University of California Riverside, CA, USA

Exploding demand for pomegranate juice provides economic motivation to adulterate juice products. Methods of adulteration are diverse thus analytical methods are needed that can detect and quantify many components simultaneously and provide a more complete description of pomegranate juice composition. A 1H NMR approach to profiling the major components of pomegranate juice, screening for adulterated samples, and identification of specific markers of adulteration are described. A total of 41 authentic juice samples from many countries and different pomegranate cultivars were examined. The natural variation in the composition of authentic pomegranate sources is compared to that of 30 commercial samples of unknown quality for authenticity testing. Analysis of the 1H NMR spectra allows the identification of sugars and a variety of organic and amino acids in pomegranate juice. Sugar composition is consistent across most pomegranate cultivars as previously described [1]. Commercial samples with relative glucose, fructose or sucrose content more than two standard of deviation from the mean of pomegranate sample may be adulterated. In this study, 13 of 30 commercial samples analyzed have suspect quality based on relative sugar composition. Organic acid content varies among authentic pomegranate samples such that adulterated samples cannot be confidently identified based on the concentration of any one organic acid. Analysis of individual organic acids was avoided by calculating the ratio between each pair of identified components. The ratios from individual samples were then compared to the average for authentic pomegranate samples using z-scores to account for the magnitude natural variance in each ratio. To provide rapid interpretation z-scores are visualized in matrices and heat mapped. If the concentration of an individual component is well beyond the natural variance high z-scores result in paring with all other components. Samples with multiple high scoring components are flagged as potentially adulterated. Elevated succinic, malic, lactic or citramalic acid and abnormal sugar composition are the most common reason that commercial samples failed the z-score test. The utility of this method for rapid screening was demonstrated by using blends of pomegranate with common adulterating juices. Spectra acquired in 10 minutes allow identification of pomegranate juice containing apple and pear juice at 10% and white grape juice at 20%(v/v).

1. Zhang, Yanjun et. al, J. Agric. Food Chem., 57, 2550-2557, 2009

RDC-based Determination of the Relative Configuration of the Fungicidal Cyclopentenone Hygrophorone A

Volker Schmidts1, Maic Fredersdorf1, Tilo Lubken2, Andrea Porzel2, Ludger Wessjohann2 and Christina M. Thiele1

1. Clemens-Schopf-Institute, Technische Universität Darmstadt, Darmstadt, Germany
2. Leibnitz-Institute for Plant Biochemistry, Halle/Saale, Germany

After their isolation from fungi of the Hygrophorus family, the Hygrophorones and their acetylated derivates have been subject of intense study due to their structural similarity with the antibiotic pentenomycin and antifungal activity. [1] The relative configuration of the sp3 carbons in the five-membered ring (C4 and C5) was established by comparison of 3J and 4J coupling constants with the known (epi-)pentenomycin structure and NOESY measurements. However the relative configuration of the exocyclic C6 carbon remained unknown and also the proposed 4,5-trans configuration has not yet been determined unambiguously.

We used RDCs [2] to determine the relative configuration of all three stereogenic centers at once. By aligning about 2 mg of Hygrophorone A in a liquid crystalline phase of high-molecular-weight PBLG in CD2Cl2 [3] we were able to measure eight one-bond and long-range C-H RDCs. We studied possible conformational flexibility in the five-membered ring and along the C5-C6 bond by conventional force-field and DFT methods. Fitting these calculated structure models with our RDC module in the software hotFCHT, [4] we found only a single relative configuration reproducing the experimental data.

1. Lubken, Tilo, Schmidt, Jurgen, Porzel, Andrea, Arnold, Norbert, Wessjohann, Ludger, Phytochem., 65, 1061-1071, 2004.
2. Reviews: Thiele, Christina M., Conc. Magn. Reson. A, 30A, 65-80, 2007. Thiele, Christina M., Eur. J. Org. Chem., 2008, 5673-5685, 2008.
3. Marx, Andreas, Schmidts, Volker, Thiele, Christina M., Magn. Reson. Chem., 47, 734-740, 2009.
4. Berger, Robert, Fischer, C., Klessinger, Martin, J. Chem. Phys. A, 102, 7151-7167, 1998.

Detection of ^{1}H-^{1}H Proximities in Small Organic Solids through Imperfect Homonuclear Dipolar Decoupling

1. Laboratoire Chimie Provence, Universities of Aix-Marseille I, II Giulia Mollica,1 Pierre Thureau,1 Fabio Ziarelli,2 Stephane Viel1 and Andre Thevand1

1. Laboratoire Chimie Provence, Universities of Aix-Marseille I, II et III, CNRS, UMR 6264, 13397 Marseille, France
2. Federation des Sciences Chimiques de Marseille, CNRS-FR1739, Spectropole, 13397 Marseille, France

Despite protons are the nuclei of choice for NMR studies due to their high natural abundance and magnetogyric ratio, strong homonuclear dipolar couplings have for long time limited the access to high resolution 1H spectra in solid samples. Recent advances in hardware and pulse sequence development have allowed to partially overcome the problem, opening the access to the detection of 1H-1H correlations in solids. Notably, beyond Double-Quantum (DQ) experiments, the most successful experimental methods are based on NOESY-type spin diffusion pulse schemes, in which 1H-1H proximities can be estimated following the transfer of z-magnetization between dipolar-coupled nuclei [1]. In these experiments, two periods of single-quantum (SQ) coherence evolution (usually in the presence of homonuclear-decoupling schemes or fast MAS conditions) are separated by a mixing time during which spin diffusion occurs [2,3]. We discuss here the possibility of exploiting imperfections in homonuclear amplitude-modulated decoupling schemes as an alternative approach to obtain information on 1H-1H proximities in small organic solids through the observation of SQ-SQ correlations among protons. In particular, the method proposed combines windowed-PMLG homonuclear decoupling [4] and DQ filtering under moderate MAS conditions to generate the weak recoupling conditions [5]. The possibility of extending these principles to the selective measurement of 1H-1H distances is also discussed.

1. Brown, S.P. Progr. Nucl. Magn. Reson. Spectrosc. 50, 199-251, 2007.
2. Elena, B., de Paepe, G., Emsley, L. Chem. Phys. Lett. 398, 532-538, 2004.
3. Elena, B., Emsley, L. J. Am. Chem. Soc. 127, 9140-9146, 2005.
4. Vinogradov, E., Madhu, P.K., Vega, S. Chem. Phys. Lett. 354, 193-202, 2002.
5. Thureau, P., Sauerwein, A. C., Concistre, M., and Levitt, M. H. Phys. Chem.Chem. Phys. 13, 93-96, 2011.

Monday, September 19th
4:30 PM - 6:00 PM

Workshop: University vs. Industry: Challenges for Doing Innovative Science

Gareth Morris, University of Manchester, UK; Mike Bernstein, MestreLabs, UK and Brian Marquez, Pfizer, USA

Tutorial: Quantification in NMR without Using Internal References

Richard Upton, GSK, UK

University vs. Industry: Challenges for Doing Innovative Science

Michael A Bernstein
Brian L Marquez
Gareth A Morris

MestreLabs UK
Pfizer Inc, Groton, CT 06340 USA
School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK

This workshop will discuss the challenges of research in industry and academia at a time when both environments are undergoing rapid change. Particular attention will be paid to the joys and sorrows of collaborative research at the university-industry interface. The popular images of the absent-minded academic in his or her ivory tower and the white-coated technocrat in the industrial laboratory have lost any relevance they might once have had: high quality research knows no boundaries. The challenges are, to a greater or lesser extent, the same for all researchers: generating new ideas; marshalling resources, particularly funding; finding, and keeping, collaborators; solving problems, whether intellectual, technical or managerial; meeting regulatory requirements; managing uncertainty and risk; and publishing and/or exploiting results. Nevertheless, differences in research culture, expectations, mechanisms and constraints between industrial and university research can often get in the way of effective collaboration, and experiences with and strategies for coping with such issues will be discussed.

Quantification in NMR Without Using Internal References

Richard J. Upton[1] and Gregory S. Walker[2]

[1] GSK, Stevenage, UK.
[2] Pfizer Inc., Groton, USA.

Quantification in NMR has generally been dominated by the internal standard method, despite early demonstrations of the accuracy and ease of external standard referencing methods [1]. Greater use of external reference quantification was encouraged by the ERETIC (Electronic Reference to Access in-Vivo Concentrations) technique [2,3], which enabled a more flexible approach to the subject. Recently, publications[4,5] highlighting the utility of the principle of reciprocity in compensating for the NMR response of variable load samples and it's simple dependence on other parameters have stimulated an interest in creating more straightforward protocols and automation for external referencing[6,7]. Vendors have now incorporated these protocols into their software. In this session quantification in general is discussed in combination with an evaluation of the external referencing versus internal referencing. All topics will be open for delegate input including the lack of commercially available, sealed, known concentration standards that are individually able to test a wide range of spectrometer performance for small molecules and also suitable for use in external quantification methods.

[1] D.P. Hollis, Anal. Chem., 35, p1682 (1963).
[2] L. Barantin, A. Le Pape and S. Akoka, Magn. Reson. Med., 38, p179 (1997).
[3] S. Akoka, B. Laurent and M. Trierweiler, M. Anal. Chem., 71, p2554 (1999).
[4] I.W. Burton, M. A. Quilliam and J. A. Walter, Anal. Chem., 77, p3123 (2005).
[5] W. Gerhard and L. Dreier, J. Am. Chem. Soc., 128, p2571 (2006).
[6] R.D. Farrant, J.C. Hollerton, S.M. Lynn, S. Provera, P.J. Sidebottom, and R.J. Upton, Magnetic Resonance in Chemistry, 48, p753 (2010).
[7] G.S. Walker, T.F. Ryder, R. Sharma, E.B. Smith and A. Freund, Drug Metabolism and Disposition, 39, p433 (2011).

Tuesday, September 20th
9:00 AM - 10:30 AM

Critical Applications of NMR in Pharmaceutical Development

Chair: Adrian Davis

Speakers:

Carla Marchioro
Aptuit Inc., Italy

Daneen T. Angwin Hadden
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Merck, Netherlands

NMR Techniques and Applications in the Drug Development Phases (from Drug Substances to Drug Product to Market)

Carla Marchioro, Silvia Davalli, Stefano Provera

Aptuit Medicines Research Center, Verona, Italy

The lecture will cover the different contributions of the NMR Spectroscopy techniques in the Drug Development phases covering chemical synthesis, compound characterization, and form and formulation selections.

Focus will be given to the combined use of techniques to result in a complete profile of the drug substance with data on structure, solid form, and related stability impacting on the formulation of the drug product. Quantitation aspects in the different phases will be also discussed.

In addition, studies on the aggregation behaviour of a new drug by means of NMR spectroscopy and surface tension measurements will be covered highlighting experimental aspects in the critical micelle concentration (CMC) determination and influencing parameters. Finally, possible options for an appropriate, reliable and comprehensive impact from the various structural techniques on the drug discovery and development process will be discussed.

What Lies Beneath the Surface? Impacting Drug Product Formulation Through NMR Imaging

Daneen Angwin Hadden and Andreas Kaerner

Lilly Research Laboratories, Analytical Sciences R&D,
Eli Lilly and Company, USA

Nuclear Magnetic Resonance (NMR) provides many different types of information during the drug discovery/development process. How about taking solution NMR another step further through the use of NMR imaging? Both physical and chemical information can be obtained utilizing this technique in situ. Results ranging in type from qualitative to quantitative can bring insight to release mechanisms that influence decisions regarding drug product formulation. A qualitative example is demonstrated in images illustrating different disintegration behaviors between samples stored under varying conditions. Quantitative information such as gel layer thickness and dry core area measurements over time can aid formulation decision when certain drug product performance characteristics are desired. Multiple dosage forms, immediate to controlled release, are amenable to these types of investigations. Studies performed on low and high field MR imaging systems will be presented illustrating how NMR imaging data complements all other analytical data by understanding what is really going on beneath the surface in situ.

NMR as a Technique for Quality Control: Pharmacopoeial Tests Analyzing Intact Samples

Paulo Dani and Edwin R. Kellenbach

Merck, Oss, the Netherlands

NMR qualitative and quantitative data are often collected from intact samples in an easy, reproducible, precise, and accurate manner. Automation and user-friendly software makes the utilization of the technique by non-experts common practice. Technological developments are continuously increasing NMR sensitivity, speed and scope, while the equipment is fairly robust, lasting for decades.

All these aspects would place NMR as an important technique not only in research divisions in the pharmaceutical world but also in quality control environments. However, NMR methods in pharmacopeias are scarce and use only the very basic features from the technique. Why is that?

This presentation touches these aspects and shows examples on how NMR is currently adopted in pharmacopeias. It discusses the role of NMR in the quality control of batches of heparin sodium, in which qualitative and quantitative NMR is used to ensure material identity and purity. As another example an identity test for peptides is discussed, in which NMR is applied as an alternative to the classical analysis of amino acids.

Tuesday, September 20th
11:00 AM - 12:30 PM

Workshop: Automated Structure Verification

Gary Sharman, Eli Lilly and Company, UK; John Hollerton, GSK, UK

Tutorial: Optimization of Small Molecule NMR Experiments

Rainer Kerssebaum, Bruker, Germany; Eriks Kupče, Agilent, UK

Automated Structure Verification

Gary Sharman & John Hollerton

Eli Lilly and Company, UK
GlaxoSmithKline, UK

Software for Automated Structure Verification (ASV) by NMR is being developed by a number of organisations around the world. There are even commercial offerings available to use today. This workshop looks at the challenges associated with ASV and will allow a full discussion with the vendors of this software. We will look at issues such as the acceptable levels of false negative and false positive results for different uses of ASV and what outcomes people expect from such software.

This is a unique opportunity to discuss this challenging topic with most of the experts in one place. Expect a lively debate!

Tutorial: Optimization of Small Molecule NMR Experiments

Rainer Kerssebaum

Bruker, Germany

The idea of this tutorial is to discuss the "implementation of modern NMR experiments". We will highlight typical NMR experiments (use of adiabatic pulses) with Agilent/Bruker spectrometers, give an introduction to newer methods like fast methods ("ultra-fast" single scan experiments, sparse sampling) and talk about less typical experiments like ADEQUATE. There will be time to ask questions and any question about other topics is wellcome.

Fast Techniques and Multiple Receiver Experiments in VNMRJ

Eriks Kupče

Agilent Technologies, 6 Mead Road, Yarnton, Oxford, OX5 1QU, UK

The latest NMR consoles that are equipped with multiple receivers allow design of principally new NMR experiments. The PANACEA pulse sequence [1-3] that is designed to unambiguously determine structure of organic molecules in a single measurement is just one example of such developments that employ the new technology. On the other hand, the continuing development of the compressive sensing techniques (also known as “sparse sampling”), such as optimized aliasing, Hadamard spectroscopy, random sampling, and projection-reconstruction NMR provide the means for speeding up such measurements. We shall discuss the software tools for implementing the new techniques and the graphical user interface in VNMRJ for setting up these experiments and for processing non-conventional data sets recorded using the methods outlined above.

References:

1. E. Kupce, R. Freeman, Molecular Structure from a Single NMR Experiment, J. Am. Chem. Soc. 130 (2008) 10788-10792.

2. E. Kupce, R. Freeman, High-resolution NMR Correlation Experiments in a Single Measurement (HR-PANACEA), Magn. Reson. Chem. 48 (2010) 333-336.

3. E. Kupce, R. Freeman, Molecular Structure from a Single NMR Sequence (fast PANACEA) J. Magn. Reson. 206 (2010) 147-153.

4. E. Kupce, R. Freeman, Parallel Receivers and Sparse Sampling in Multidimensional NMR, J. Magn. Reson. (2011) in press.

Wednesday, September 21st
9:00 AM - 10:30 AM

Further Progress in Solid State NMR
Chair: Gerd Buntkowsky

Speakers:

Marek Pruski
Ames Laboratory, USA

Clare Grey
University of Cambridge, UK

Jaroslav Havlicek
Zentiva a.s., Czech Republic

Gábor Szalontai
University of Pannónia, Hungary

Solid-State NMR of Small Molecules in Small Rotors

Marek Pruski

U.S. DOE Ames Laboratory and Department of Chemistry, Iowa State University, Ames, Iowa, 50011, USA

We will review the applications of several advanced techniques in solid-state nuclear magnetic resonance spectroscopy to the structural studies of systems comprising small interacting molecules. These latest capabilities were made possible by combining fast MAS (at >> 40 kHz)[1] with several multiple RF pulse sequences for two-dimensional correlation measurements. Non-incremental sensitivity gains were achieved in heteronuclear correlation (HETCOR) spectroscopy through the detection of high-gamma (1H) rather than low-gamma (e.g., 13C, 15N) nuclei, the use of optimized 1H homonuclear decoupling under fast MAS, or by multi-echo refocusing of magnetization during data acquisition.[2-8] These techniques can yield through-space and through-bond 2D HETCOR spectra of systems that were previously inaccessible to multidimensional inspection due to low concentration of NMR-active nuclei. The intermolecular interactions can be probed under fast MAS by 1H-1H homonuclear correlation NMR, e.g., double quantum (DQ) MAS or NOESY.

Examples of the studied materials include mesoporous silica nanoparticles (MSN) functionalized with various types of organic groups, where solid-state NMR allows definitive characterization of the structure and absolute/relative concentration of moieties inside the mesopores, their spatial distribution and orientation with respect to the surface, as well as dynamic behavior. The reaction products and intermediates, and catalysts' stability under the reaction conditions can also be studied. In particular, the basic understanding of the dynamics of molecules on the solid-liquid interface, provided by solid-state NMR characterization and theory, served as a predictive tool in the design of a new catalyst for the esterification reaction.[9] In other studies, homo- and heteronuclear correlation methods provided valuable insights into the arrangement of surfactants in MSN materials synthesized from a mixture of cetyltrimethylammonium bromide (CTAB) and cetylpyridinium bromide (CPB),[10] and into the host-guest interactions in the matrix of a metal-free corrole.[11]

References

[1.] A. Samoson, in: Encyclopedia of Nuclear Magnetic Resonance, D.M. Grant, R.K. Harris (Eds.), John Wiley & Sons, Chichester, U.K., 2002; Vol. 9, p. 59.
[2.] Ishii, Y.; Tycko, R.; J. Magn. Reson. 2000, 142, 199.
[3.] Wiench, J.W.; Bronnimann, C.E.; Lin, V.S.-Y.; Pruski, M., J. Am. Chem. Soc. 2007, 129, 12076.
[4.] Mao, K.; Wiench, J.W.; Lin V.S.-Y.; Pruski, M., J. Magn. Reson. 2009, 196, 92-95.
[5.] Elena, B.; Lesage, A.; Steuernagel, S.; Bockman, A.; Emsley, L., J. Am. Chem. Soc. 2005, 127, 17296.
[6.] Mao, K.; Pruski, M., J. Magn. Reson. 2009, 201, 165.
[7.] Mao, K.; Pruski, M., J. Magn. Reson. 2010, 203, 144.
[8.] Leskes, M.; Madhu, P. K.; Vega, S., J. Magn. Reson. 2009, 199, 208.
[9.] Mao, K.; Kobayashi, T.; Wiench, J.W.; Chen, H.-T.; Tsai, C.-H.; Lin, V.S.-Y.; Pruski, M., J. Am. Chem. Soc. 2010, 132, 12452.
[10.] Kobayashi, T.; Mao, K.; Wang, S.-G.; Lin V.S.-Y.; Pruski M., Solid State NMR, 2011, 39, 65-71.
[11.] Mao, K.; Nowak-Krol, A.; Kobayashi, T.; Gryko, D.T.; Pruski, M.; Potrzebowski, M., in preparation.

Energy Storage and Conversion: Using Local Structural Probes to Understand and Optimise Function of Battery and Fuel Cell Materials

Clare P. Grey

Chemistry Department, Cambridge University

The application of new Nuclear Magnetic Resonance (NMR) approaches to correlate structure and dynamics with function in materials lithium-ion batteries and solid oxide fuel cells will be described. A particular focus is the development of methodology to allow these systems to be investigated in-situ, i.e., under realistic operating conditions. This allows processes to be captured, which are very difficult to detect directly by ex-situ methods. For example, we can detect side reactions involving the electrolyte and the electrode materials, and processes that occur during extremely fast charging and discharging. The approach will be demonstrated for the anode materials silicon and lithium. Lithium-ion batteries (LIBs) containing silicon have been the subject of much recent investigation, because of the extremely large gravimetric and volumetric capacity of this anode material. This material undergoes a crystalline-to-amorphous phase transition on electrochemical Li insertion into crystalline Si, during the first discharge, hindering attempts to link structure in these systems with electrochemical performance. We apply a combination of static, in-situ and magic angle sample spinning, ex-situ 7Li and 29Si nuclear magnetic resonance and pair distribution function analysis studies to investigate the changes in local structure that occur in the actual working LIB. The first discharge occurs via the formation of isolated Si ions and smaller Si-Si clusters embedded in a Li-ion matrix; the latter are broken apart at the end of the discharge forming isolated Si ions. In a second example, we illustrate the use of NMR to investigate the nature of the defects in materials that have been proposed for use as electrolytes that operate via either oxygen-ion or protonic conduction in solid oxide fuel cells. For example, $BaZrO_3$ or $BaSnO_3$ can be doped with Y^{3+} to create oxygen vacancies. These vacancies can be filled with H_2O, the water molecules dissociating to form mobile ions that contribute to the long-range ionic transport in these systems. NMR experiments are used to examine the local structure, the locations of the vacancies and how this affects protonic/oxygen ion motion in these systems.

Solid-state ^{19}F NMR Spectroscopy Sensitive Tool for Detection of Polymorphic Purity

Jaroslav Havlicek1, Gerhard Althoff-Ospelt2

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

Polymorphs are chemically identical, but they have different chemical, physical and also spectroscopic properties. Solid-state NMR spectroscopy is one of the few techniques that allow unequivocal identification of polymorphs and also the detection polymorphic (crystalline) purity. This advantage is excellently applied in API and also in the final solid dosage form. 13C CP MAS is a routine technique to characterize pharmaceutical solids. For crystalline material 13C CP MAS spectra are well resolved and spectra of different polymorphic forms often differ significantly. In the case of dosage forms, however, 13C CP MAS spectra may suffer from overlapping signals of API and placebo and in case of low API concentration from the low intensity of the API lines. 19F CP/MAS and MAS techniques may favorably be used in these cases. Usually 19F is present in the API only, avoiding overlap of API and placebo lines. Its high natural abundance and high Larmor frequency lead to strong signals even for low concentration samples.

This study shows the comparison of sensitivity 19F MAS techniques for determination polymorphs with 13C CP MAS and XRPD and how to increase the sensitivity of detection of polymorphic purity.

^{1}H MAS Line widths of Small Organic Guest Molecules Confined in Porous Silicas

Gábor Szalontai

1. University of Pannónia, NMR laboratory, Veszprem, Hungary

It is known that 1H MAS line widths are dominated by the homonuclear dipolar interactions. In the last decade several ingenious multipulse sequences have been proposed to overcome this problem [1], for the same purpose others proposed the decrease of proton spin density by deuteration of the sample molecules [2]. Under fast or very-fast MAS conditions line widths of about 0.5 ppm can be achieved nowadays.

We report now on results based on simple spatial separations of the interacting proton spins. It has been noticed recently that small organic molecules (solids at room temperature) show liquid-like behavior if confined in silica pores [3]. The origin of this high molecular mobility (that scale down the dipolar couplings) is not well understood as yet. The first reports hinted that a sharp drop of the phase-transition temperature (the so called Gibbs-Thomson effect) might be credited for the phenomenone, however our observations do not support this view.

We have managed to confine small organic molecules (camphor, menthol, HMB, HMTA, terphenyl, anthacene, etc.) in gradually increasing regular (MCM-41, SBA-15) and irregular (silica aerogels) pores and observed steadily decreasing proton line widths. 2H MAS spectra confirmed the molecular mobility as main reason. Comparison of the static spectra of the confined and free molecules show an impressive resolution improvement (a factor of 5 to 10 was observed in each case looked at so far). When combined with fast MAS rotation line widths of 30-40 Hz could be readily achieved.

Effects of loading, silica pore size and possible applications will also be discussed.

1. E. Vinogradov. P.H. Madhu, S. Vega, New Techniques in Solid-State NMR, vol.246, Springer Verlag, Berlin, 2005, p.33.
2. Q. Wang, E. Jordan, D.F. Shantz, J.Chem.Phys.C. 18151, 113, 2009.
3. T. Azais, C. Tourné-Péteilh, F. Aussenac, N. Baccile, C. Coelho, J.M. Devoisselle, F. Babonneau, Chem.Mater. 6382, 18, 2006.

Wednesday, September 21st
11:00 AM - 12:30 PM

Advances in Determining Configuration/Conformation
Chair: Christina Thiele

Speakers:

Christian Griesinger
MPI Biophys. Chem., Germany

Ricardo Riguera
Universidade de Santiago de Compostela, Spain

Philippe Lesot
Université de Paris-Sud, France

Teodor Parella
Universitat Autònoma de Barcelona, Spain

NMR for Small Molecule Configuration and Drug Validation

Han Sun1, Manuel Schmidt1, Fernando Hallwass1, Edward d'Auvergne1, Emily Whitson2, Chris M. Ireland2, Armando Navarro-Vazquez3, J. Orts1,4, A. Mazur1, J. Pilger1, T. Carlomagno1,4, Uwe M. Reinscheid1, and C. Griesinger1,5

1Department for NMR-based Structure. Biology, Max-Planck Institute for Biophysical Chemistry, Goettingen, Germany; 2Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah, USA; 3Organic Chemistry Department, Universidade de Vigo, Vigo, Spain; 4EMBL, Heidelberg, Germany
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NMR spectroscopy is especially suited for the investigation of structurally heterogeneous systems. In the presentation, several avenues towards the characterization of such disordered molecules will be given. First, the combination of NMR spectroscopy employing anisotropic parameters such as residual dipolar couplings and residual chemical shift anisotropies will be demonstrated in combination with chiroptical methods to provide absolute configurations of non-crystallizable molecules whose structures can only be described by ensembles (1). In a second part of the talk, the application of NMR methods for the characterization of complex structures between small molecules and their target proteins will be presented. Some insight for challenging molecules such as microtubule binders will be discussed and comparisons with scattering techniques will be made (2).

(1) H. Sun, U. M. Reinscheid,E.L. Whitson, C. Ireland, A. Navarro-Vázquez and C. Griesinger: The challenge of large scale motion for RDC analysis of configuration: the case of fibrosterol sulfate A, J. Am. Chem. Soc.in press, 17, 1811-17 (2011); H. Sun, E. J. d'Auvergne, U. M. Reinscheid, L. C. Dias, C. Kleber Z. Andrade, R. Oliveira Rocha and C. Griesinger: Bijvoet in Solution Reveals Unexpected Stereoselectivity in a Michael Addition, Chem. Eur. J. 17, 1811-17 (2011); U. M. Reinscheid, M. Koeck, C. Cychon, V. Schmidts, C. M. Thiele and C. Griesinger: The Absolute Configuration of Dibromopalau'amine Eur. J. Org. Chem. 36, 6900-6903 (2010); Schuetz, A., Murakami, T., Takada, N., Junker, J., Hashimoto, M., Griesinger, C: Rdc-enhanced NMR Spectroscopy in Structure Elucidation of the Natural Product Sucroneolambertellin. Angew. Chem. Int. Ed. 47, 1-4. (2008); Schuetz, A., Junker, J., Leonov, A., Lange, O., Molinski, T. F., Griesinger, C. (2007): Stereochemistry of Sagittamide A from Residual Dipolar Coupling enhanced NMR. J. Am. Chem. Soc. 129 (49), 15114-15115; Haberz, P., Farjon, J., Griesinger, C. (2005) The First DMSO Compatible Orientating Medium: Towards the Investigation of the Stereochemistry of Natural Products, Angew. Chem. 117, 431-433(2005), Angew. Chem. Int. Ed. 44, 427-429

(2) Bartoschek, S., Klabunde, T., Defossa, E., Dietrich, V., Stengelin, S., Griesinger, C., Carlomagno,T., Focken, I., and K.U. Wendt: Drug Design for G-Protein-Coupled Receptors by a Ligand-Based NMR Method. Angewandte Chemie International, 49, 8, 1426-1429, (2010); Orts, J., Tuma, J., Reese, M., Grimm, S K., Monecke, P., Bartoschek, S., Schiffer, A., Wendt, K. U., Griesinger, C., Carlomagno, T.: Crystallography-independent determination of ligand binding modes, Angew. Chem. Int. Ed. 47, 7736-40 (2008); Reese ,M., Sanchez-Pedregal, V. M., Kubicek, K., Meiler, J., Blommers, M. J. J. Griesinger, C., Carlomagno, T. (2007). Structural Basis of the Activity of the Microtubules Stabilizing Agent Epothilone studied by NMR Spectroscopy in Solution, Angew. Chem. Int. Ed. 46, 1864-8; Sánchez-Pedregal,V, M., Reese, M., Meiler, J., Blommers, M. J. J., Griesinger, C., Carlomagno, T. (2005) The INPHARMA Method: Protein Mediated Interligand NOEs for Pharmacophore Mapping, Angew. Chem. 117, 2-4 Angew. Chem. Int. Ed. 44,2-4

^{13}C-NMR as a General Tool for the Assignment of Absolute Configuration

Ricardo Riguera

Department of Organic Chemistry and CIQUS, Universidad de Santiago de Compostela, SPAIN

In the last few years, the use of 1H-NMR for the assignment of the absolute configuration of a variety of organic compounds has been established as a reliable tool. In this presentation I will show that 13C-NMR can also be used for the assignment of absolute configuration of chiral alcohols, amines, thiols, carboxylic acids, cyanohydrins, sec,sec-diols and sec,sec-aminoalcohols. This is carried out in exactly the same way as for 1H -NMR, by comparison of the 13C-NMR spectra of the derivatives obtained with the R and the S enantiomers of selected auxiliaries. The auxiliaries and the graphical models showing the correlation sterochemistry- $\Delta\delta$RS signs, are the same employed for the 1H-NMR approach, therefore, there is no need for additional derivatives to be prepared. Also, 13C-NMR can be used jointly with or instead of the 1H-NMR, increasing the number of signals used for the assignment (13C plus 1H) but also allowing the absolute configuration of fully deuterated or of no proton containing compounds to be determined.

Experimental data on more than 70 compounds of varied structure and functional groups and theoretical calculations demonstrate the general character of this methodology that is founded on the aromatic shielding effect produced by the auxiliary reagent on the 13C-NMR chemical shifts.

Revisiting the (D/H) Isotopic Fractionation Analysis of (Un)saturated Fatty Acids and Triglycerides: What NAD 2D NMR Spectroscopy in Aligned Media Can Do for You?

Philippe Lesot

RMN en milieu Oriente, ICMMO UMR 8182, Université de Paris-Sud, 91405 Orsay cedex, France

Natural abundance deuterium 2D NMR (NAD 2D-NMR) in weakly ordering, chiral liquid crystals (CLC), constituted by olutions of polypeptide (PBLG, PCBLL) dissolved in organic solvents is a powerful alternative to the isotropic SNIF-NMR method for studying the natural isotopic (D/H) distribution of biointerest molecules such as fatty acid methyl esters (FAMEs) [1-5]. The "CLC method" is superior to SNIF-NMR for two reasons: i) the presence of deuterium quadrupolar splittings on NAD spectra considerably enhances the spectral separation of almost all deuterium inequivalent sites in the molecule; ii) the chirality of the CLC allows the determination of the (D/H) ratios on the enantiotopic sites of prochiral molecules (spectral discrimination of corresponding pairs of site-specific enantio-isotopomers). The combination of both advantages can provide new detailed data to biochemists for the understanding of stereoselectivity of enzymatic mechanisms, or the determination of hydrogen sources [3].

In this work, we report the influence of organic co-solvent used (CHCl3, DMF, TMU, Pyridine) on the number and the quality of spectral enantiodiscriminations. The best NMR results were obtained using high polarity organic co-solvents [4,5]. To illustrate the efficiency of this approach, a particular attention is focussed on the analysis of three important, unsaturated FAMEs (sequentially biosynthetised in plants): the methyl oleate, the methyl linoleate and the methyl α-eleostearate [2,4,5]. For methyl linoleate, we show that it is possible using the system 'PBLG/Pyr' as CLC to determine the site-specific natural enantio-isotopomeric excesses at each methylene group [4]. In the saturated series, we present the current experimental NMR results obtained for three saturated FAMEs: the methyl myristate, the methyl palmitate and the methyl stearate. Finally, the first NAD NMR results concerning the analysis of (identical) short-chain triglycerides will be briefly reported.

The assignment of quadrupolar splittings observed on NAD 2D spectra in oriented solvents for these two classes of FAMEs was confirmed by numerical simulations including: i) Monte-Carlo sampling of the conformational distribution of these flexible solutes; ii) calculation of the orientational distribution of conformers, based on their excluded-volume interactions with the polymer [6]. Experimental results and theoretical predictions are compared and discussed.

References:

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(1) Lesot, P.; Courtieu, J., Prog. in NMR Spectrosc., 2009, 55, 128-159.
(2) Lesot, P.; Baillif, V.; Billault, I., Anal. Chem., 2008, 80, 2963-2972.
(3) Baillif, V.; Robins, R.J.; Billault, I.; Lesot, P., J. Biol. Chem., 2009, 284, 10783-10792.
(4) Serhan, Z.; Martel, L.; Billault, I.; Lesot, P., Chem. Commun., 2010, 46, 6599-6511.
(5) Lesot, P.; Serhan, Z.; Billault, I., Anal. Bioanal. Chem., 2011, 399, 1187-1200.
(6) Serhan, Z.; Lesot, P.; Billault, I.; Borgogno, A.; Ferrarini, A., Chem. Phys. Lipids, 2011, submitted.

Precise Measurement of Heteronuclear Coupling Constants from ^{1}H Selective HSQMBC Experiments

Teodor Parella1, Sergi Gil1 and Juan Felix Espinosa2

1. Servei de RMN, Universitat Autònoma de Barcelona, Bellaterra, Barcelona
2. Centro de Investigación Lilly S.A., Alcobendas, Madrid, Spain

1D and 2D 1H-selective HSQMBC experiments are proposed for the direct and accurate measurement of long-range H-C coupling constants in small molecules without need for an individualized and time-consuming post-processing fitting procedure.

The use of long-range proton-carbon coupling constants (nJCH, n>1) is a very good complement to proton-proton coupling constants (JHH) and/or NOE data for the structural and conformational analysis of natural-abundance molecules [1]. It is known that these small nJCH coupling constants (ca. 0-10 Hz) present strong dependences with respect to coupling pathways, patterns substitutions and structural constrains such as dihedral angles. However, the lack of extensive experimental data and trustworthy structural correlations often prevent its successful application to resolve routine problems [2]. Long-range optimized experiments (such as HMBC [3] and HSQMBC [4]) are highly suitable when quaternary carbons are involved. The value of nJCH is usually extracted from an individualized and time-consuming post-processing fitting procedure of the resulting anti-phase coupling pattern. Unfortunately, undesired mixed-phase multiplet distortions originated by the additional JHH-coupling evolution during the long evolution INEPT-type introduce a common source of inaccuracy.

In this work, improved 1H-selective versions of the HSQMBC experiment will be proposed for the straightforward, direct and accurate measurement of nJCH without the need of the classical fitting procedure. It will be shown that specific nJCH values can be accurately extracted from pure in-phase multiplets. Otherwise, IPAP technique is also implemented in versions of the HSQMBC. In this way, the relative displacement of separate alpha- and beta-HSQMBC-cross-peaks that result of the time-domain addition/subtraction procedure of complementary. In-phase (IP) and Anti-phase (AP) HSQMBC data provides a simple way to extract the nJCH value.

1. Bifulco, G., Dambruoso, P., Gomez-Paloma, P. et al, Chem. Rev, 107(9), 3744-3779, 2007.
2. Parella, T, 2D Methods for the Measurement of Long-Range H-C Coupling Constants in Multidimensional NMR methods for the Solution State, Morris, G.A., Emsley, J.W., John Wiley & Sons LTD, UK, Chap. 23, 305-314, 2010.
3. Eden, R.A.E., Keeler, J, J. Magn. Reson. 181(1), 89-97, 2006.
4. Williamson, R.T., Marquez, B.J., Gerwick, W.H., Kover, K.E., Magn. Reson. Chem., 38(4), 265-273, 2000.

Wednesday, September 21st
2:30 PM - 4:00 PM

Enhancements for Physical Organic Chemistry
Chair: Craig Butts

Speakers:

Jonathan Clayden
University of Manchester, UK

Richard Lewis
AstraZeneca, Sweden

Ann-Christin Pöppler
Georg August Universität Göttingen, Germany

Mathias Nilsson
University of Manchester, UK

Quantifying Conformational Control

Jonathan Clayden

University of Manchester

We showed some years ago that a combination of steric and dipole effects allows the orientation of functional groups to be controlled, and we have exploited these effects in the stereocontrolled synthesis of a series of new families of atropisomers, most recently the biaryl sulfones. By identifying structures with well-defined conformational preferences - for example poly aromatic amides in which the amides adopt an all-anti conformation and achiral peptide analogues with a strong helical preference - we can synthesise molecules which offer the possibility of communication of stereochemical information over distances exceeding 2.5 nm, the typical thickness of a cell membrane. We report simple NMR methods for analysing ratios of helical conformers even at fast exchange, and for quantifying relative and absolute local helical preferences, by comparing the anisochronicity of diastereotopic signals in both 1H and 13C spectra.

Atropisomerism: NMR Investigations and Optimisation

Richard J Lewis

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431 83 Mölndal, Sweden

Atropisomers are stereoisomers resulting from hindered rotation about a single bond which can be physically isolated due to a high thermodynamic barrier to rotation. When atropisomerism occurs in pharmaceutical compounds, this can be an issue for drug development (1) as the separable isomers are enantiomers (or diastereoisomers in cases where an additional chiral centre is present) and therefore are likely to exhibit different pharmacological and metabolic activities. When the half life for interconversion of the atropisomers falls in the range hours to months, it becomes nearly impossible to develop such a compound as the isomer delivered to the patient cannot be controlled effectively.

This talk will describe a project (2) in which an initial lead compound could be separated into atropisomers by HPLC and was therefore unsuitable for further development. Unfortunately, the functional groups causing the atropisomerism were found to be vital to retain activity. Variable temperature NMR was found to be an excellent method for screening project compounds with an aim to reducing the half life for interconversion to an acceptable level.

The compounds have also proved useful to probe the effect of conformation on chemical shift, and implications for chemical shift prediction will also be discussed.

1. The Challenge of Atropisomerism in Drug Discovery Jonathan Clayden, Wesley J. Moran, Paul J. Edwards, and Steven R. LaPlante Angew. Chem. Int. Ed. 2009, 48, 6398-6401
2. Optimization of Monocarboxylate transporter 1 blockers through analysis and modulation of atropisomers interconversion properties. Simon D Guile et al, J. Med. Chem. 2007, 50, 254-263

Dynamics and Structure of Organolithiums by Modern NMR Spectroscopy

Ann-Christin Pöppler, Michael John and Dietmar Stalke

Georg-August-Universität Göttingen, Institut fur Anorganische Chemie Göttingen, Germany

Due to their versatile application in synthetic chemistry, organometallic compounds have been widely investigated concerning their aggregation behavior or coordination sphere [1]. In order to gain deeper understanding of their reactivity detailed analyses both in liquid and in solid state are required. By a combination of NMR techniques like DOSY, HOESY and NOESY we investigated the solution dynamics of the interconversion of the two mixed organolithiums [Me2NPhLi]4-[tBuLi]4 that were observed in the crystal (Figure 1). So far, only one other crystal structure of a [tBuLi]4 co-crystallized organolithium compound has been structurally characterized [2].

Despite the wealth of information provided by conventional NMR methods, there are a lot of procedures chemists do not understand completely, in particular selectivities of catalytic reactions. Thiele et al. could demonstrate an alternative approach to gain structural information on catalytic reaction pathways by measuring RDCs (Residual Dipolar Couplings) [3]. RDCs can only be measured if the molecules - that normally rotate freely in solution - are aligned with respect to the applied magnetic field. The common alignment techniques have been used mainly for proteins in aqueous solutions, and only few media are compatible with organic solvents [4,5]. Apart from Thieles work on a reactive Pd intermediate no organometallic compound has so far been analyzed by this technique.

In this work, we employed polystyrene sticks swollen in organic solvents in an inert atmosphere. Due to the high reactivity of organolithiums the polymer has to be of high quality - as evidenced by 1H CPMG, 2H NMR - and free of remaining monomer, side products or radical starter. Here we show first results on the highly reactive organolithium compounds nBuLi and 2-Thienyllithium•PMDTA.

References:

1. e. g. Tatić, Tanja, Hermann, Stefanie, John, Michael, Loquet, Antoine, Lange, Adam, Stalke, Dietmar, Angew. Chem. Int. Ed. 2011, online, DOI: 10.1002/anie. 201102068.
2. Bojer, Daniel, Kamps, Ina, Tian, Xin, Hepp, Alexander, Pape, Tania, Frohlich, Roland, Mitzel, Norbert W., Angew. Chem. Int. Ed., 46, 4176-4179, 2007.
3. Bottcher, Benjamin, Schmidts, Volker, Raskatov, Jevgenij A., Thiele, Christina M., Angew. Chem. Int. Ed., 49, 205-209, 2010.
4. Review: Thiele, Christina, M. Conc. Magn. Res., 30A, 65-80, 2007.
5. Luy, Burkhard, Kobzar, Kyryl, Kessler, Horst, Angew. Chem. Int. Ed., 43, 1092-1094, 2004.

Trilinear Analysis: NMR Reaction Monitoring for Overlapped Spectra

Mathias Nilsson and Gareth A. Morris

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NMR spectroscopy can in principle allow every species involved in a chemical reaction to be monitored simultaneously, providing both real-time quantitation and information on chemical structure. Typically this is done by acquiring 1H spectra at regular intervals and monitoring the integrals of diagnostic peaks, but when signals overlap it is often difficult interpret the data obtained. In principle, multivariate statistical methods can be applied here to separate the component spectra. However, this typically results in rotational ambiguity, where a wide range of candidate component spectra fit the experimental data equally well. Multilinear analysis (where the data vary independently in more than two dimensions) offers a way around this problem, allowing experimental data to be decomposed into physically realistic component spectra where multilinear data can be obtained experimentally.

One way to obtain trilinear NMR data for the course of a chemical reaction is to acquire successive DOSY [1.2] (Diffusion-Ordered SpectroscopY) datasets during the reaction. Each individual DOSY dataset records how the NMR spectrum varies with pulsed field gradient strength at a given time. Provided that each species has a different diffusion coefficient and a different timecourse, the dataset is trilinear and can be decomposed (without prior knowledge of the spectra, reaction kinetics or diffusion behaviour) using the PARAFAC (PARAllel FACtor Analysis) algorithm to yield the spectrum, concentration time course, and diffusional attenuation for each component of the reaction separately.

1Nilsson M, Khajeh M, Botana A, Bernstein MA, Morris GA. Chem. Commun. 2009, 1252-1254.
2Khajeh M, Botana A, Bernstein MA, Nilsson M, Morris GA. Anal. Chem. 2010, 82, 2102-2108.

Wednesday, September 21st
4:30 PM - 6:00 PM

NMR Solutions to Combating Counterfeits and Achieving Quality

Chair: Carla Marchioro

Speakers:

Gisela Guthausen
Karlsruher Institut für Technologie, Germany

Bernhard Blumich
RWTH Aachen University, Germany

Gerald S. Remaud
University of Nantes, France

Myriam Malet-Martino
University of Paul Sabatier, France

Applications of Low Field NMR in Quality Control: Established Methods and Developments

Gisela Guthausen, Franz Dalitz, Richard Bernewitz, Maria Vargas

Institut für Mechanische Verfahrenstechnik und Mechanik, SRG10-2, KIT, Karlsruhe, Germany

Nuclear magnetic resonance (NMR) is known to be a versatile analytical method. Apart from the well-known spectroscopy for studies of molecular structure and dynamics, spatial resolution, diffusion and relaxation properties can be exploited to answer specific questions in diverse scientific and industrial sectors. Nevertheless, NMR is considered as an expensive and complicated technique with only limited use regarding reaction and process monitoring as well as quality control.

In the last decade, the development of analytical on-line techniques has made considerable progress. Also the value of NMR for process development and quality control was recognized increasingly. NMR is very attractive due to its non-invasiveness and the possibility to obtain both qualitative and quantitative information. Especially at low magnetic fields, progress in instrument and magnetic field design has been made which shows the potential of NMR also in the above mentioned fields of interests. Whereas most conventional NMR experiments aim at obtaining qualitative or structural information, additional requirements have to be fulfilled in order to acquire reliable data well suited for quantification. Amongst these are reproducibility, short- and long-term stability, which will be discussed. Additionally, examples will be shown of well established applications of low-field NMR in industrial environment which are mainly based on relaxation rate differences. Actually, forthcoming applications aim mainly at spectral resolution. First examples in the context of process and reaction monitoring will be presented.

Mobile NMR of Art and Cultural Heritage

Bernhard Blümich

Institute of Technical and Macromolecular Chemistry,
RWTH Aachen University

The miniaturization of NMR equipment has led to a variety of mobile NMR devices for relaxometry, imaging, and spectroscopy with open and with closed magnets. Open magnets are used to investigate large objects non-destructively in the stray field the magnet. Apart from well-logging devices, one of the first small stray-field relaxometers for materials analysis is the NMR-MOUSE®. It has found interesting applications in studies of the objects of cultural heritage like paper, wood, bones, master paintings, stone, and mortar. Undocumented previous restoration measures can be identified, the mortar layers supporting frescoes classified, and the age of paintings be estimated following a universal aging curve of paint over more than six decades.

References:

B. Bluemich, S. Anferova, S. Sharma, A. L. Segre, C. Federici, Degradation of historical paper: nondestructive analysis by the NMR-MOUSE, J. Magn. Reson. 161 (2003) 204 - 209

F. Casanova, J. Perlo, B. Bluemich, Depth Profiling by Single-Sided NMR, in: S. Stapf, S. Han, eds., NMR in Chemical Engineering, Wiley-VCH, Weinheim, 2006, pp. 107-123

F. Ruehli, T. Boeni, J. Perlo, F. Casanova, M. Baias, E. Egarter, B. Bluemich, Noninvasive spatial tissue discrimination in ancient mummies and bones by in situ portable nuclear magnetic resonance, J. Cult. Heritage 8 (2007) 257-263

F. Presciutti, J. Perlo, F. Casanova, S. Gloeggler, C. Miliani, B. Bluemich, B.G. Brunetti, A. Sgamellotti, Non-invasive NMR profiling of painting layers, Appl. Phys. Lett. 93 (2008) 033505

B. Bluemich, J. Perlo, F. Casanova, Mobile Single-Sided NMR, Progress in Nuclear Magnetic Resonance Spectroscopy 52 (2008) 197-269

B. Bluemich, F. Casanova, J. Perlo, F. Presciutti, C. Anselmi, B. Doherty, Noninvasive Testing of Art and Cultural Heritage by Mobile NMR, Acc. Chem. Res. 6 (2010) 761 - 770

B. Bluemich, A. Haber, F. Casanova, E. del Federico, V. Boardman, G. Wahl, A. Stilliano, L. Isolani, Noninvasive depth profiling of walls by portable nuclear magnetic resonance, Anal. Bioanal. Chem. 397 (2010) 3117 - 3125

Isotopic NMR Spectrometry as an Efficient Tool to Fight Against Counterfeiting: High Accuracy Requirement and Sensitivity Improvement

Gérald S. REMAUD, Virginie SILVESTRE and Serge AKOKA

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Isotope ratio measurements used in forensic chemistry have mainly obtained by mass spectrometry (IRMS). However, this technique provides only the average contribution of a given element, hiding the internal isotopic distribution. Further valuable information for source attribution and for the authentication of a given molecule can be obtained by using isotopic NMR spectrometry, which provides intra-molecular isotope distributions. For the last 25 years, 2H NMR has been used to measure (2H/1H) ratios in many molecules and matrices and has effectively been applied in such areas as authentication, metabolism, and counterfeiting [1]. Very recently, isotopic 13C NMR has been validated [2]. While it is challenging due to the high level of accuracy required (better than 1&#65533;), this is a new and very promising approach to fight against counterfeiting in its broadest sense [3,4]. A new breakthrough has been achieved by using polarization transfer (INEPT in particular). A major modification of the sequence was the introduction of adiabatic schemes for the 180 deg pulse for inversion and refocusing on both channels 1H and 13C. Using this modified pulse sequence a huge reduction of the duration of the NMR analysis is achieved because (i) the polarization transfer leads to a sensitivity gain of around a factor 4 (γ1H/γ13C) and (ii) the repetition rate between transients is dictated by 1H T1 relaxation times that are much shorter than 13C T1 times [5]. Our latest work on authentication and on the pedigree of active molecules will be illustrated.

References:

1. Martin G.J., Martin M.L., Remaud G.S. SNIF-NMR part 3: From mechanistic affiliation to origin inference. Modern Magnetic Resonance, 1647-1658, (2006) Webb GA editor, Springer Berlin.
2. Caytan E., Botosoa E.P., Silvestre V., Robins R.J., Akoka S., Remaud G.S. Anal. Chem. 79, 8266-8269, 2007.
3. Silvestre V., Maroga Mboula V., Jouitteau C., Akoka S., Robins R.J., Remaud G.S. J. Pharm. Biomed. Anal. 50, 336-341, 2009.
4. Thomas F., Randet C., Gilbert A., Silvestre V., Jamin E., Akoka S., Remaud G., Segebarth N., Guillou C. J. Agric. Food Chem. 58, 11580-11585, 2010.
5. Thibaudeau C., Remaud G., Silvestre V., Akoka S. Anal. Chem. 82, 5582-5590, 2010.

2D DOSY ^{1}H NMR Analyses for the Characterization of Fake Drugs

Myriam Malet-Martino, Veronique Gilard, Stephane Balayssac and Robert Martino

Groupe de RMN Biomedicale, Laboratoire SPCMIB, University of Paul Sabatier, Toulouse, France

The chemical analysis of suspected fake drugs is a crucial step for differentiating counterfeit versus genuine drugs. Most analytical methods proposed for the detection of counterfeit pharmaceutical formulations focus on the active pharmaceutical ingredient (API) expected in the formulation and do not provide any other information on the pharmaceutical formulation. The detection of counterfeiting requires methods orthogonal to the conventional separation techniques described in the pharmacopoeias and pharmaceutical guidelines.

2D Diffusion Ordered SpectroscopY (DOSY) 1H NMR [1] is a very efficient tool for analyzing drug formulations. DOSY NMR experiments provide the spectral signature of a drug formulation with identification of the various ingredients (API, excipients, adulterants). The main strength of the method lies in its holistic nature, which enables the drug formulation to be considered as a whole.

DOSY NMR was applied to the analysis of various formulations of conventional drugs (fluoxetine, sildenafil, tadalafil, artesunate) [2-5] as well as herbal drugs marketed as natural slimming products or for sexual dysfunction [6,7]. The presentation will give an overview of the application of DOSY NMR in the field of drug analysis. It will outline the benefits and limits of DOSY NMR as a screening method for detecting and analyzing fake drugs [8].

1. Morris, K.F., Johnson, C.S., J. Am. Chem. Soc., 114, 3139-3141, 1992
2. Trefi, S., Gilard, V., Balayssac, S., Malet-Martino, M., Martino, R., J. Pharm. Biomed. Anal., 46, 707-722, 2008
3. Trefi, S., Gilard, V., Balayssac, S., Malet-Martino, M., Martino, R., Magn. Res. Chem., 47, S163-S173, 2009
4. Trefi, S., Routaboul, C., Hamieh, S., Gilard, V., Malet-Martino, M., Martino, R., J. Pharm. Biomed. Anal., 47, 103-113, 2008
5. Nyadong, L., Harris, G., Balayssac, S., Galhena, A., Malet-Martino, M., Martino, R., Parry, R., Wang, M., Fernandez, F., Gilard, V., Anal. Chem., 81, 4803-4812, 2009
6. Vaysse, J., Balayssac, S., Gilard, V., Desoubzdanne, D., Malet-Martino, M., Martino, R., Food Addit. Contam. Part A, 27, 903-916, 2010
7. Balayssac, S., Trefi, S., Gilard, V., Malet-Martino, M., Martino, R., Delsuc, M.A., J. Pharm. Biomed. Anal., 50, 602-612, 2009
8. Holzgrabe, U., Malet-Martino, M., J. Pharm. Biomed. Anal., 55, 679-687, 2011

Monday, September 19th
11:00 AM - 12:30 PM

Tuesday, September 20th
8:30 PM - 11:00 PM

Poster Sessions

Chair:
Philippe Lesot

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99.		Evaluation Automatic Structure Verification Tools
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101.		Automated Analysis of NMR Data in the Context of Fragment Screening

1 Study of Pollutant Interactions with Clay

Adolfo Botana, Ronald Soong, Jasmine Wang, and Andre J. Simpson

Environmental NMR Centre and Department of Chemistry,
University of Toronto, Toronto, Ontario, Canada

An understanding of interactions between man-made substances and clay is essential for the study of numerous systems. Clay is an ubiquitous material used as liner material in containment of waste, drugs and agrochemical agents, and present in soils and sediments, where it retains and releases metal nutrients [1]. In this project, it is shown that it is possible to study weak interactions of pollutants with clay through the use intermolecular NOE effects in experiments like CLEANEX [2].

Clays have the capability of adsorbing water in several layers that interact with exchangeable cations, platelet edge hydroxyl groups, surface oxygen atoms and with each other. Some pollutants bind strongly to clays and, therefore, irradiation of the water signal causes an NOE effect on the bound pollutants. However, when pollutants interact weakly there is not necessarily a significant NOE effect, as many pollutants fall in the intermediate motional regime. It is then necessary to study these effects by other means. CLEANEX is a 1D ROESY-type sequence that allows the study of chemical exchange and intermolecular NOEs by selectively irradiating the water resonance and suppressing TOCSY-type transfer and intramolecular NOE effects (preventing the appearance of additional resonances where water signal overlaps with other peaks, as would often be the case in soils spectra). Exchangeable protons show up as positive peaks, and those species that interact with the hydration water of the clay would present negative peaks. Therefore, an analysis of the build-up rate can aid estimating the degree of interaction between pollutants and clay. This is illustrated for the interactions of different pesticides, such as diflufenzopyr and acephate, with montmorillonite, as an initial step for the study of soil remediation.

References:

1. Hensen, E. J. M. and Smit, B., The Journal of Physical Chemistry B, 106 (49), 12664-12667, 2002
2. Hwang, T.-L., Mori, S., Shaka, A. J., and van Zijl, P. C. M., Journal of the American Chemical Society, 119, 6203-6204, 1997

2 The Use of 3H NMR to Verify Structure and Labeling position(s) of 3H Labeled Compounds

Alexandra Bernlind1, Jonas Malmquist2, Johan Sandell2 and Peter Strom2

1. Medicinal Chemistry, CNSP iMed Science Sodertalje
2. Isotope Chemistry, Screening and Profiling Global DMPK IM
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When performing in vivo studies with tritium labelled compounds, it is important to consider the metabolic fate of the molecule in order to avoid loss of label. Consequently, knowing the exact position of the label can be crucial in order to avoid a compromised study.

NMR (Nuclear Magnetic Resonance) is a very powerful tool when it comes to verifying chemical structures. Tritium NMR can be used to verify the position of the tritium atom(s), as well as the degree of isotope incorporation in each position. 3H NMR can also give information about other, sometimes unexpected, positions that have been radio labelled.

This poster will present our 3H NMR work flow as well as a few examples where 3H NMR has been used in structure verification of compound(s), as well as in the determination of labelling position(s).

3 PARAFAC and J-Resolved Spectra for Metabolic Profiling

Ali Yilmaz, Nils T. Nyberg, and Jerzy W. Jaroszewski

Faculty of Pharmaceutical Sciences, University of Copenhagen

Metabolic profiling of natural products is used to map correlated concentration variances of known and unknown secondary metabolites in extracts. Two-dimensional J-resolved NMR-spectra are used in this context to resolve overlapping signals by separating the effect of J-coupling from the effect of chemical shifts. Often one-dimensional projections of these data are used as input for standard multivariate statistical methods and only the intensity variances along the chemical shift axis are taken into account. On this poster we describe the use of parallel factor analysis (PARAFAC) as a tool to pre-process a set of two-dimensional J-resolved spectra with the aim of keeping the J-coupling information intact. A set of saffron samples, directly extracted with methanol-d4, were used as a model system to evaluate the feasibility and merits of the method.

4 Dynamics and Structure of Organolithiums by Modern NMR Spectroscopy

Ann-Christin Pöppler, Michael John and Dietmar Stalke

Georg-August-Universitat Gottingen, Institut fur Anorganische Chemie Gottingen, Germany

Due to their versatile application in synthetic chemistry, organometallic compounds have been widely investigated concerning their aggregation behavior or coordination sphere [1]. In order to gain deeper understanding of their reactivity detailed analyses both in liquid and in solid state are required. By a combination of NMR techniques like DOSY, HOESY and NOESY we investigated the solution dynamics of the interconversion of the two mixed organolithiums [Me2NPhLi]4-[tBuLi]4 that were observed in the crystal (Figure 1). So far, only one other crystal structure of a [tBuLi]4 co-crystallized organolithium compound has been structurally characterized [2].

Despite the wealth of information provided by conventional NMR methods, there are a lot of procedures chemists do not understand completely, in particular selectivities of catalytic reactions. Thiele et al. could demonstrate an alternative approach to gain structural information on catalytic reaction pathways by measuring RDCs (Residual Dipolar Couplings) [3]. RDCs can only be measured if the molecules - that normally rotate freely in solution - are aligned with respect to the applied magnetic field. The common alignment techniques have been used mainly for proteins in aqueous solutions, and only few media are compatible with organic solvents [4,5]. Apart from Thieles work on a reactive Pd intermediate no organometallic compound has so far been analyzed by this technique.

In this work, we employed polystyrene sticks swollen in organic solvents in an inert atmosphere. Due to the high reactivity of organolithiums the polymer has to be of high quality - as evidenced by 1H CPMG, 2H NMR - and free of remaining monomer, side products or radical starter. Here we show first results on the highly reactive organolithium compounds nBuLi and 2-Thienyllithium•PMDTA.

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2. Bojer, Daniel, Kamps, Ina, Tian, Xin, Hepp, Alexander, Pape, Tania, Frohlich, Roland, Mitzel, Norbert W., Angew. Chem. Int. Ed., 46, 4176-4179, 2007.
3. Bottcher, Benjamin, Schmidts, Volker, Raskatov, Jevgenij A., Thiele, Christina M., Angew. Chem. Int. Ed., 49, 205-209, 2010.
4. Review: Thiele, Christina, M. Conc. Magn. Res., 30A, 65-80, 2007.
5. Luy, Burkhard, Kobzar, Kyryl, Kessler, Horst, Angew. Chem. Int. Ed., 43, 1092-1094, 2004.

5 Metabolomic Profiling of Serum Samples by 1H NMR Spectroscopy as a Novel Diagnostic Approach for Septic Shock

Beata Mickiewicz1, Gavin E. Duggan1, Josee Wong2, Paul Kubes3 and Hans J. Vogel1

1. Bio-NMR-Center, University of Calgary, Calgary, Alberta, Canada
2. CCEPTR, University of Calgary, Calgary, Alberta, Canada
3. Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada

Septic shock as a result of sepsis is a leading cause of mortality in hospital intensive care units (ICUs). Despite the ongoing efforts of doctors and intensive care staff, active treatment and huge financial costs, the death rate from septic shock remains high; around 50% by some estimates. Therefore early prognosis of sepsis and proper intervention are very important aspects of increasing the survival rate of hospital patients. This study is focused on improvement and development of septic shock diagnosis using NMR-based metabolomic approach. 39 Serum samples were collected from septic shock patients and 20 from ICU patients not suspected of an infection (ICU controls). 1H NMR spectra of studied samples were analyzed and quantified using a targeted profiling methodology and the concentrations of 60 metabolites were identified in all 59 specimens. To detect specific patterns in changes of metabolites' concentrations and distinguish the difference between septic shock and ICU control samples we applied MultiVariate Data Analysis (MVDA). The supervised Orthogonal Partial Least Square (OPLS) modeling method which was used in this study presented a clear separation between defined classes (septic shock and ICU control) with high validation metrics (R2 = 0.72, Q = 0.64). Based on obtained concentrations, two predictive OPLS models were created using a small and large training set respectively. In both cases the accuracy demonstrates a good predictive ability of the designed model to predict septic shock. These results indicate that model created such as this may be a promising tool for the prediction of septic shock disease. Additionally, 31 metabolites detected in serum samples had significant influence on the separation between septic shock samples and controls. These metabolites could be interpreted as a pattern of human metabolic response to septic shock and they might be postulated as biomarkers for prognostic diagnosis of septic shock in the ICUs.

Acknowledgements: Funding was provided by the Alberta Sepsis Network (ASN), which in turn is funded by Alberta Innovates Health Sciences. HJV and PK hold Scientist awards from the Alberta Heritage Foundation for Medical Research. CCEPTR receives funding from ASN and from the Canada Foundation for Innovation.

6 High-Performance Quantitative 1H-NMR (HP-qNMR(R)) yields a New Generation of Organic Certified Reference Materials

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Quantification of organic substances is usually performed using chromatographic techniques such as HPLC or GC. Over the last years quantitative NMR (qNMR) has evolved not only in pharmaceutical industry but also in many other fields [1]. In the R&D laboratories of Sigma-Aldrich Buchs (Switzerland) a new Bruker Avance III 600 MHz NMR spectrometer was installed for high resolution qNMR measurements of organic substances, and the lab was fully accredited under both ISO/IEC 17025 and also ISO Guide 34 for the certification of organic reference materials using 1H-qNMR. The most outstanding attribute of qNMR is that it is a relative primary method. The signal intensity is in direct proportionality with the number of protons contributing to the resonance [2]. Hereby the structures of the chemical substances are fully irrelevant. The signal intensities of the sample of interest and a reference substance can be directly compared. Therefore, a direct traceability to internationally accepted reference standards (e.g. NIST SRM) can be achieved [3], which is usually not possible with chromatographic techniques. The signal ratio of two different protons can be measured with tremendous precision and the integration of the signals in most cases dominates the measurement uncertainty. Therefore, the high trueness and the low uncertainty makes it possible to generate certified reference materials (CRMs) for use as qNMR standards. Buoyancy correction was implemented into the content calculation and the final certificate contains a proper uncertainty calculation [4], comprehensive documentation and storage information based on stability studies.

[1] Weber M, Hellriegel C, Zertifizierte Standards fur die qNMR, GIT Labor-Fachzeitschrift 2010, 07, 5272.
[2] Malz F, Jancke H, Validation of quantitative, J Pharm Biomed Anal. (2005), 38(5):813-233.
[3] Eurachem/CITAG Guide, 1-37, 1st Ed. (2003), Traceability in chemical measurement.
[4] Eurachem/CITAG Guide, 1-120, 2nd Ed. (2000), Quantifying uncertainty in analytical measurement.

7 Saturation Transfer Difference NMR Experiments of Membrane Proteins in Living Cells under HR-MAS Conditions: The Interaction of the SGLT1 Cotransporter with its Ligands

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In the last decade, Saturation Transfer Difference (STD)-NMR spectroscopy has been extensively exploited to study receptor-ligand interactions [1]. This technique has also been used to study molecular recognition events involving membrane receptors and their ligands, by working on samples which contained platelets or whole cells, and exploiting liquid state NMR [2,3].
This strategy allowed, at least in some particular cases, to overcome the inherent problems associated with the extreme difficulty of isolating and maintaining certain membrane receptors in solution with the correct folding and the proper functionality. Nevertheless, the authors employed platelets and cells, that live in suspension, particularly in the bloodstream, and that present a low tendency to aggregate and precipitate.
In contrast, cells derived from solid tissues show a strong tendency to aggregate and precipitate very slowly which, in most cases, prevents the application of STD-NMR experiments. In this context, we aimed to develop a robust NMR methodology to study the interaction of ligands with membrane proteins, employing samples which contain whole and vital cells, as we considered important to have access to a method not affected by restrictions related to the nature of the tissue of origin. Therefore, the use of high resolution magic angle spinning (HR-MAS) NMR techniques has been explored to exploit the rotation at a relative high speed as a tool to maintain cells into the sample active window.
To verify the feasibility of this approach, we selected a model system composed by the hSGLT1 cotransporter interacting with two of its known ligands. hSGLT1 is a Na+/glucose co-transporter membrane protein that uses the energy from a downhill sodium gradient to transport glucose across the apical membrane against an uphill glucose gradient. The glycoside phlorizin and naphthyl-beta-D-galactoside are both competitive inhibitors of hSGLT1, but presenting affinities for the receptor that differ for more than three orders of magnitude [4].
The phlorizin/hSGLT1 and naphthyl-beta-D-galactoside/hSGLT1 interactions have been verified and characterized by STDD experiments acquired on samples containing the ligands and a cell line over-expressing the transporter or a cell line expressing a basal level of the protein [5]. Data obtained will be presented in this communication.

1. Biet T., Peters T., Angew. Chem. Int. Ed., 40, 4189-4192, 2001.
2. Claasen B., Axmann M., Meinecke R., Meyer B., J. Am. Chem. Soc., 127, 916-919, 2005.
3. Mari S., Serrano-Gomez D., Canada F. J., Corbi A. L., Jimenez-Barbero J., Angew. Chem. Int. Ed,. 44, 296-298, 2005.
4. Hirayama B. A., Diez-Sampedro A., Wright E. M., Br. J. Pharmacol., 134, 484-495, 2001.
5. Airoldi C., Giovannardi S., La Ferla B., Jimenez-Barbero J., Nicotra F., submitted.

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8 Conformational Preferences and Interactions of Functionalized Indoles as Anion Receptors

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The conformational preorganization and anion-induced conformational changes of indole-based receptors have been studied by a combination of heteronuclear NMR spectroscopy and quantum mechanical calculations. In the first group of receptors, a single indole scaffold has been functionalized with an amide group at C2 and a variety of amide, urea and thiourea moieties at C7 [1-3]. NOE enhancements showed that anti-anti conformation across C2-C2(alpha) and C7-N7(alpha) bonds is preferred in an acetone-d6 solution in the absence of anions. Upon anion binding to receptors, syn-syn conformation becomes predominant. Anion-receptor interactions were evaluated through 1H and 15N chemical shift changes. The second group of receptors exhibited an extra indole group, which resulted in diindolyl(thio)ureas [4]. NOE experiments showed that the anti-anti conformer along the C7-N7(alpha) bonds was favored in DMSO-d6 solution in the absence of anions. Anion-induced 1H and 15N chemical shift changes suggested weak binding of chloride anions and negligible conformational changes. Strong deshielding of the ureido protons and moderate deshielding of the indole NH has been observed upon the addition of acetate, benzoate, bicarbonate and dihydrogen phosphate, which indicates that the predominant hydrogen bond interactions occurred at urea donor groups. Binding of oxoanions caused remarkable conformational changes along the C7-N7(alpha) bonds and the syn-syn conformer was preferred for anion-receptor complexes. The conformational changes in functionalized indoles and diindolyl(thio)ureas upon anion binding are in good agreement with the energy preferences established by ab initio calculations.

References

1. Makuc, D.; Lenarcic, M.; Bates, G. W.; Gale, P. A.; Plavec, J. Org. Biomol. Chem., 7, 3505-3511, 2009.
2. Makuc, D.; Triyanti; Albrecht, M.; Plavec, J.; Rissanen, K.; Valkonen, A.; Schalley, C. A. Eur. J. Org. Chem., 2009, 4854-4866, 2009.
3. Makuc, D.; Albrecht, M.; Plavec, Supramol. Chem., 22, 603-611, 2010.
4. Makuc, D.; Hiscock, J. R.; Light, M. E.; Gale, P. A.; Plavec, J. article submitted.

9 1H NMR Profiling for Authentication of Pomegranate Juice

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Exploding demand for pomegranate juice provides economic motivation to adulterate juice products. Methods of adulteration are diverse thus analytical methods are needed that can detect and quantify many components simultaneously and provide a more complete description of pomegranate juice composition. A 1H NMR approach to profiling the major components of pomegranate juice, screening for adulterated samples, and identification of specific markers of adulteration are described. A total of 41 authentic juice samples from many countries and different pomegranate cultivars were examined. The natural variation in the composition of authentic pomegranate sources is compared to that of 30 commercial samples of unknown quality for authenticity testing. Analysis of the 1H NMR spectra allows the identification of sugars and a variety of organic and amino acids in pomegranate juice. Sugar composition is consistent across most pomegranate cultivars as previously described [1]. Commercial samples with relative glucose, fructose or sucrose content more than two standard of deviation from the mean of pomegranate sample may be adulterated. In this study, 13 of 30 commercial samples analyzed have suspect quality based on relative sugar composition. Organic acid content varies among authentic pomegranate samples such that adulterated samples cannot be confidently identified based on the concentration of any one organic acid. Analysis of individual organic acids was avoided by calculating the ratio between each pair of identified components. The ratios from individual samples were then compared to the average for authentic pomegranate samples using z-scores to account for the magnitude natural variance in each ratio. To provide rapid interpretation z-scores are visualized in matrices and heat mapped. If the concentration of an individual component is well beyond the natural variance high z-scores result in paring with all other components. Samples with multiple high scoring components are flagged as potentially adulterated. Elevated succinic, malic, lactic or citramalic acid and abnormal sugar composition are the most common reason that commercial samples failed the z-score test. The utility of this method for rapid screening was demonstrated by using blends of pomegranate with common adulterating juices. Spectra acquired in 10 minutes allow identification of pomegranate juice containing apple and pear juice at 10% and white grape juice at 20%(v/v).

1. Zhang, Yanjun et. al, J. Agric. Food Chem., 57, 2550-2557, 2009

10 Dual Flow Cell NMR Probe Installation and Use

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The first production model microscale Protasis CapNMR Dual Sample Probe (DSP) was installed at our Groton campus. Each independent detection element of the two-cell probe achieved pulse width, resolution, and sensitivity standards similar to a single-cell probe of similar design. The degree of electrical isolation of each cell limits the amount of cross-over signal between cells such that simultaneous high resolution experiments can be run in both cells simultaneously. Both elements are co-shimmed to maintain performance in each channel, while shim sets for multiple solvent combinations can be created and used as needed.

Samples are run through the DSP probe in a high throughput manner. As chemists order NMR analyses of purified compounds through automated screening software, the site's compound management group delivers 0.9 micromoles of plate-based compounds for data acquisition. After protonated solvent is replaced with deuterated solvent, the DPC probe is used to acquire 1-D proton and HSQC data for every sample. Sensitivity of the probe is sufficient to acquire data for one 96-well plate per day.

11 NMR Characterization of Glycofused Tricycles as New Abeta Peptide Ligands for the Diagnosis and Therapy of Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder that affects over 30 million individuals worldwide. [1] A central pathological feature of AD is the accumulation of misfolded Abeta peptides in the form of oligomers and amyloid fibrils in the brain. Many small molecules that are able to bind Abeta peptides and inhibit their aggregation are already known; most of them are natural compounds bearing aromatic moieties. Among them tetracycline is particularly attractive for the development of new Abeta ligands. It is known that tetracycline displays anti-amyloidogenic activity against many amyloidogenic proteins both in vivo and in vitro, [2] and we verified its ability to bind Abeta1-40 and Abeta1-42 oligomers by NMR experiments. [3] Anyway, tetracycline presents some drawbacks, as it suffers from chemical instability, low water solubility and possesses, in this contest, undesired anti-bacterial activity. [4] In order to overcome these limitations, and to increase the diversity and derivatisation potential, we developed the synthesis of ductile tricyclic scaffolds presenting the structural requirements allowing the interaction tetracycline-Abeta peptides.[5]

Glycofused aromatic tricyclic compounds with improved chemical stability and water solubility have been generated, the properties of hydrophilicity/hydrophobicity of which may be easily modulated adding proper substituents on the hydroxyl groups. The ability of these compounds to bind Abeta oligomers was verified by STD-NMR and trNOESY experiments. All these compounds were able to bind Abeta peptides but their affinities were modulated by the functional groups present at the aromatic ring.

We demonstrated with molecular dynamic studies that all these molecules have the same 3D-structure and conformation, so the diverse affinity may be only due to the different polarity determined by the aromatic substituents. No influence on the binding was observed for the sugar moiety, which in fact displays only a minor involvement in the interaction with amyloid peptides.

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1. Forloni G. et al., FEBS Lett., 487, 404-407, 2001.
2. a) Ono K. et al. FASEB J., 17, 803-809, 2003,; b) Aitken J.Fet al. Biochem J., 374, 779-84, 2003.
3. Airoldi C. et al. Org. Biomol. Chem., 9, 463, 2011.
4. Howlett D.R. et al. Biochem. J., 343, 419, 1999.
5. Patent RM2011A000264.

12 NMR of Self-Assembled Bifunctional Organocatalysts

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Organocatalysis plays an increasingly important role among synthetic methodologies. The continuous search for new and efficient enantioselective organocatalysts is one of the great challenges in the advancement of modern organic synthesis. Among the many applications reported in the past years bifunctional amin-thiourea catalysts represent a proven and successful family of catalysts in the field. Inspired by biological models mechanistic studies describe their modes of action in the various enantioselective C-C forming reactions. The key to their function is the simultaneous presence of both hydrogen-bond donor and acceptor sites and the ability to reversibly control their non-covalent interactions.

Our research aims the understanding of the nature and thermodynamics of these non-covalent interactions using the methodologies offered by low-temperature, high-field nuclear magnetic resonance (NMR) spectroscopy. In the past years we recognized that self-association is one of the consequences of bifunctionality that leads to the self-assembly of the catalyst via intermolecular hydrogen bonding [1]. Experimental results suggest that in apolar-aprotic solvents self-assemblies are still abundant in the reaction mixtures. Today we raise ourselves the question whether the geometrical preference of the catalyst self-assembly provides a natural pool for specific proton exchange processes contributing to the process "catalyst self-activation". Knowing the three dimensional solution structure of the catalyst is a necessary step in answering this question. Here, we present some of our lates results showing that self-assemblies produce odd, "unnatural" conformers that may be regarded as catalytically active states next to the catalytically inactive, intramolecularly hydrogen bonded monomeric species. In this respect self-assemblies of the catalysts are important in creating certain geometries and lowering the activation energy of conformational transitions [2]. Since catalysis is inherently a dynamic process we hope that our structural investigations contribute to the better understanding of the question: self-association - a friend or foe in bifunctional organocatalysis?

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References:

1. Tarkanyi, G.; Kiraly,P.; Varga, Sz.; Vakulya, B.; Soos, T. Chem. Eur. J. 14, 6078 - 6086 (2008)
2. Kiraly, P.; Soos, T.; Varga, Sz.; Vakulya, B.; Tarkanyi, G. Magn. Reson. Chem. 48, 13-19, (2010)

13 Automatic Generation of Negative Control Structures for Automatic Structure Verification Systems

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The generation of positive and negative controls is a fundamental part of good experimental design. Getting a positive outcome on a test performed over a subject known to give a positive result, reassures the scientist the test is working properly. As important, if not more, is to test over subjects known to give negative results. Getting a negative outcome when expected validates the test and increases the result's confidence when applied to unknowns.

Automated Structure Verification (ASV) is no different than any other scientific test. Positive as well as negative controls should be frequently tested to optimize performance and to obtain a measure of robustness and confidence in the results.

In this poster I will show how to automatically generate relevant negative control structures for any type of NMR data. Furthermore, I will argue that ASV systems fall in the category of binary classifiers, and that their performance can be measured by a host of metrics, already in use in the fields of statistical classification and signal detection theory.

14 Crystal Structure Determination of Powdered Pharmaceuticals using a Combined Solid State Characterization Approach

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A single crystal structure of a new pharmaceutical candidate can provide vast amounts of information to aid in its development. In addition to reporting on chemical structure and crystal interactions, the crystal structure can be used by computational predictive tools to indentify the most stable polymorph, understand the solid form landscape, and predict physical properites in manufacturing. In cases where a suitabile single crystal is not available for structure determination by single crystal x-ray diffraction alternative methods must be explored. We present the crystal structure of a pharmaceutical powder solved using a combination of solid state characterization methods. The crystal structure of a powdered sample was determined from powder x-ray diffraction (PXRD) structure solution methods and verified with solid state NMR, CASTEP NMR predicted chemical shifts and vibrational spectroscopy. The sensitivity of each characterization technique was evaluated with a blind study of three structure solutions, having varying Rwp values with the PXRD pattern, and the final refined structure. Our results show that Infrared and Raman spectra, and calculated isotropic chemical shifts are senstive to hydrogen bonding differences, selecting the correct structure solution. The solid state NMR isotropic chemical shifts were also sensitive to hydrogen bonding differences in addition to crystal packing, correctly selecting the final refined structure.

15 BSS Estimation of Components in DOSY

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1HNMR analysis of complex mixtures has to deal with the overlap of signals, a problem that increases with the number and complexity of the spectral sources, or there similarity. One way to improve the spectral resolution is the use of DOSY NMR, which provides a means to separate the different components in a mixture based on the difference between their diffusion coefficients.[1] The basic processing of DOSY data, based on ILT or on simple fitting, provides limited in resolution which requires the use of statistical tools to analyze these complex data.[2,3,4]

Among the possible mathematical tools that have not been tested for DOSY processing, we explored the Blind Source Separation (BSS),which allows estimating the spectra of N unknown sources (the spectra of pure components) from a series of P mixed spectra (observations) without a parametric modeling of the sources. [5]

To seek this spectral separation, we explore two different approaches of BSS which rely on different prior assumption:

* Independent Component Analysis (ICA-JADE), which is based on the assumption that the sources are statistically independent [6,7]
* Factoring in non-negative matrices (NMF), a specific model imposing constraints of non negativity and sparseness of the sources [8]

References:

1. JohnsonJr C. S, Progress in Nuclear Magnetic Resonance Spectroscopy,34, 203-256, 1999.
2. Antalek, B. Concepts in Magnetic Resonance,14 (4), 225-258,2002.
3. Stilbs, P.Journal of Magnetic Resonance, 135 (1), 236-241,1998.
4. (a) Khajeh, M.; Botana, A.; Bernstein, M. A.; Nilsson, M.; Morris, G. A, Analytical Chemistry,82 (5), 2102-2108, 2010;
(b) Nilsson, M.; Morris, G. A., Analytical Chemistry,80 (10), 3777-3782,2008.
5. Cardoso Jean-Francois.Proceedings of the IEEE, vol. 9, no 10, pp. 2009-2025, oct. 1998.
6. James V. Stone. TRENDS in Cognitive Sciences Vol.6 No.2 February 2002.
7. Aapo Hyvarinen and Erkki Oja.Neural Networks, 13(4-5):411-430,2000.
8. Michael W. Berry and all. Computational Statistics & Data Analysis 155-173, 2007.

16 Solid-state 19F NMR Spectroscopy Sensitive Tool for Detection of Polymorphic Purity

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Polymorphs are chemically identical, but they have different chemical, physical and also spectroscopic properties. Solid-state NMR spectroscopy is one of the few techniques that allow unequivocal identification of polymorphs and also the detection polymorphic (crystalline) purity. This advantage is excellently applied in API and also in the final solid dosage form. 13C CP MAS is a routine technique to characterize pharmaceutical solids. For crystalline material 13C CP MAS spectra are well resolved and spectra of different polymorphic forms often differ significantly. In the case of dosage forms, however, 13C CP MAS spectra may suffer from overlapping signals of API and placebo and in case of low API concentration from the low intensity of the API lines. 19F CP/MAS and MAS techniques may favorably be used in these cases. Usually 19F is present in the API only, avoiding overlap of API and placebo lines. Its high natural abundance and high Larmor frequency lead to strong signals even for low concentration samples.

This study shows the comparison of sensitivity 19F MAS techniques for determination polymorphs with 13C CP MAS and XRPD and how to increase the sensitivity of detection of polymorphic purity.

17 NMR-based Pharmacophore Mapping and Binding Mode Determination

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In pharmaceutical industries, many target proteins are not tractable by NMR because they are too big or they are not amenable for labelling with NMR enabling stable isotopes. For such cases, we have developed INPHARMA[1], Interligand NOEs for PHarmacophore MApping, which requires two ligands that bind competitively and sequentially to a protein. INPHARMA peaks emerge from the magnetization transfer from the protons of one ligand to the protons of the other ligand via the protein protons, which can be observed in a NOESY experiment. We have shown that with these experimental restraints, docking modes of protein-ligand complexes can be cross validated which leads in many cases to an unambiguous determination of protein-ligand complex structures. For protein kinase A (PKA) we could reproduce, by docking and INPHARMA restraint cross validation, complex structures known from X-ray crystallography[2].

Now we present an improvement of the method by including Saturation Transfer Difference (STD)[3] restraints on top of the INPHARMA restraints. We show on the example of four PKA-ligand complexes that cross validation of docking results against INPHARMA-STD restraints leads to the same binding modes as X-ray crystallography.

For the diabetes related drug target GPR40, which is a membrane protein and for which only homology models exist, INPHARMA-STD reproduces previous INPHARMA derived relative binding modes of two ligands[4] and provides in addition absolute binding modes of the ligands in the GPR40 homology model.

Finally we demonstrate how STD data can be used as a final selection criterion for ambiguous binding modes on the example of epothilone A (epoA), a natural product used as an anticancer agent that targets the taxane-binding site of b-tubulin. An electron crystallography (EC) derived binding mode of epothilone to Zn-stabilized tubulin sheets is known[5], but is in severe disagreement with NMR data in solution[6]. STD data of the tubulin-epoA complex were measured and back-calculated for the NMR and the EC structure. Correlation clearly favours the NMR solution structure.

1. Sanchez-Pedregal, V., M.; Reese, M.; Meiler, J.; Blommers, M. J. J.; Griesinger, C.; Carlomagno, T., Angew. Chem. Int. Ed., 44, 4172-4175, 2005.
2. Orts, J.; Tuma, J.; Reese, M.; Grimm, S. K.; Monecke, P.; Bartoschek,S.; Schiffer, A.; Wendt, K. U.; Griesinger, C.; Carlomagno, T., Angew. Chem. Int. Ed., 47, 7736-40, 2008.
3. Mayer, M.; Meyer, B., Angew. Chem. Int. Ed., 38, 1784-1788, 1999.
4. Bartoschek, S.; Klabunde, T.; Defossa, E.; Dietrich, V.; Stengelin, S.; Griesinger, C.; Carlomagno, T.; Focken, I.; Wendt, K. U., Angew. Chem. Int. Ed., 122, 1468-1471, 2010.
5. Nettles, J. H.; Li, H.; Cornett, B.; Krahn, J. M.; Snyder, J. P.; Downing, K. H., Science, 305, 866-869, 2004.
6. Reese, M.; Sanchez-Pedregal, V. M.; Kubicek, K.; Meiler, J.; Blommers, M. J. J.; Griesinger, C.; Carlomagno, T., Angew. Chem. Int. Ed., 46, 1864-1868, 2007.

18 In Situ Monitoring of Biodiesel Oxidation within an NMR Spectrometer

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Biodiesel, consisting of fatty acid alkyl esters (FAAEs) derived from biological sources, is becoming increasingly important as a renewable fuel. Knowledge of how it degrades via oxidation at high temperature is of particular importance, since this is a major factor limiting its use.1 Various mechanisms have been proposed, and NMR spectroscopy is one of several analytical techniques that can be used to monitor the degradation, usually by the periodic sampling of a bench experiment.1

Using NMR spectroscopy to monitor reactions of liquids with gases in situ can be problematic, mainly because of limited gas solubility. We found that FAAEs will not oxidise significantly under standard NMR conditions, i.e. in a Young's tap NMR tube at 383 K under an atmosphere of air. We therefore constructed a simple NMR tube which facilitates the ready monitoring of liquid-phase reactions with gases. The design is related to that reported by previous workers,2,3 and is based on a standard 5 mm NMR tube containing a concentric inner tube reaching to the bottom through which gas can be bubbled. The inlet tube is connected via high temperature silicone tubing to a peristaltic pump, whilst a second exit tube prevents excessive pressure build-up. The bubbling is controlled and timed to periodically turn off, allowing the liquid to settle, during which time NMR spectra can be acquired.

Using this approach, we have monitored the oxidation of various FAAE samples at 383 K. Air was bubbled through the samples at a rate of 1 ml/min over a period of 24-72 hours, and 1H NMR spectra were recorded every 10 minutes. The rates of oxidation of different FAAEs were vastly different: complete oxidation was observed for some samples (e.g. highly unsaturated samples such as linolenic acid methyl ester) within 24 hours, whereas no significant reaction was seen over 72 hours for algal oil-derived biodiesel. Further, using this technique, the intermediates in the reactions (aldehydes, formates etc.) have been monitored and quantified over time, and in separate NMR experiments we have also detected new intermediates in the oxidation pathway. This method is therefore a cheap and simple way to study the rate and mechanism of biodiesel degradation and other gas-liquid reactions.

1. Bannister, CD, Chuck, CJ, Bounds, M and Hawley JG, Proc. IMechE Part D, 225, 99, 2011
2. Baumann, W, Mansel, S, Heller D and Borns, S, Mag. Reson. Chem., 35, 701, 1997.
3. Bowers CR and Weitekamp DP, J. Am. Chem. Soc., 109, 5541, 1987

19 Stereochemistry of 2-fluoro-ribose Derivatives: NMR, Molecular Modeling and Outcome

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In ongoing studies on the synthesis of the Hepatitis C Virus (HCV) NS5B RNA polymerase inhibitor RG7128 the stereochemistry of the intermediate 3,5-dibenzoyl-1-bromo-2-deoxy-2-fluoro-2-C-methyl-D-ribose was to be assigned by NMR.[1] 1H, 1H-NOESY-NMR as well as 1H, 19F- HOESY-NMR spectra did not provide unambiguous results. Therefore molecular modeling techniques [PERCH MMS and Quantum Mechanic calculations using Jaguar][2,3] were applied to several related molecules and expected NMR coupling constants and NOE constraints were calculated. Based on the X-ray diffraction data of a deprotected intermediate related to its 3J-1H-19F-coupling constants in NMR, the stereochemistry assignment of the particular representatives of this structural class could be done. Moreover the quality of both molecular modeling approaches was assessed by means of these examples.

1. Sofia, M., J. Furman, P. A., Symonds, W. T., RSC Drug Discovery Series, 4, 238-266, (2011).
2. MMS, Perch Solutions (www.perchsolutions.com).
3. Jaguar, v.7.7, Schroedinger, LLC, New York, NY, 2010 (http://www.schrodinger.com)

20 Characterization of Sulfur Compounds in Heavy Crude Oil Fractions

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Characterization and quantification of sulfur compounds within crude oils and their corresponding heavy fractions such as resins, vacuum residues, asphaltenes, and others, is a task of paramount importance for quality assessment of secondary petroleum products. Many heavy crude oils present higher amounts of sulfur of up to 10 per cent in weight. The main problem with heavy fractions is the impossibility to use conventional analytical procedures for the characterization of these heavy sulfur compounds as them are present mainly as thiophenic species and as sulfides in a few cases.

In a previous work [1], we report the chemical derivatization of sulfur compounds provided by a Light Cycle Oil from a Colombian crude oil. The characterization was tentatively for 30 compounds, and handicapped due to the low magnetic field of the 13C-NMR experiments. Here we use chemical derivatization using isotopic enriched compounds and high magnetic field to perform the 13C-NMR experiments. In addition to heteronuclear correlation, H-C, a new tool to obtain 2D maps which can be used as finger prints for the sulfur compounds present in fractions of heavy crude oils was introduced. The characterization of derivatized molecules was ambiguous since no reference compounds were accessible. Thus, we use the alternative approach of running a series of quantum chemical computations to calculate the chemical shifts of over 120 possible sulphur compounds present in heavy crude oil fractions.

Initially, the sample was prepared by suspension in dry C2H4Cl2 as solvent, and further derivatized with CH3I (13C, 99%) to produce S-13CH3 sulphur compounds stabilized with tetrafluoroborate anion. The salts were separated from the non reactive compounds and washed out using a non polar solvent. In the second step NMR spectroscopy was performed using CD3CN as solvent; 1H and 13C 1D NMR spectra were acquired at 700 and 175 MHz, respectively. One bond 1H-13C heteronuclear 2D correlation experiments were used to obtain the finger prints of the samples.

Using theoretical quantum chemical computations, the absolute chemical shifts were calculated for a set of 120 sulfur compounds at a density functional theory level of theory using the GIAO method for the tensors calculation. Here, we used different basis sets to describe the atomic orbitals. For carbon, and hydrogen a 6-311G(2d,2p) was used, and a 6-311G(3df,2dp) for sulfur. Calculated chemical shifts were referenced to tetramethyl silane, TMS. A set of 20 sulfur compounds were used as reference and their chemical shifts used to establish a correlation between theoretical and experimental data.

Using this methodology we had identified more than hundred sulfur compounds in heavy crude oils fractions, in asphaltenes samples particularly. Additional work is required because we need to dentify more complex thiopenic compounds present in asphaltenes, which require additional quantum chemical computations.

1. J.C. Poveda, D. Molina, C. Baldrich, Fuel 83 (2004) 337-342

21 Through Space 5JFH Spin-spin Coupling Constants in N,N-dimethylfluoroacetamides

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Among the main parameters of NMR, the nuclear spin-spin coupling constants (SSCCs) are of substantial interest due to its application on several stereochemical aspects. This interest leads to a breakthrough in experimental techniques to detect SSCCs and theoretical approaches to calculate these spectroscopy parameters. The SSCCs measured in an isotropic phase by high resolution NMR spectroscopy were rationalized by Ramsey using a nonrelativistic formulation as originating in four different terms: Fermi contact (FC), spin-dipolar (SD), paramagnetic spin-orbit (PSO) and diamagnetic spin-orbit (DSO). In the past recent, mechanism transmission of the FC through the electronic structure has been discussed[1] and it is known now that electron delocalization interactions are the main contribution for transmitting the FC term for long-range SSCCs. Recently, our research group reported a practical approach for identifying coupling pathways for the FC term of SSCCs based on an analysis of the spatial distribution of canonical molecular orbitals (CMOs). The Fermi hole is propagated through the whole region spanned by each CMO, and each CMO is studied in terms of natural bond orbitals (NBO). This approach is dubbed FCCP-CMO, Fermi contact coupling pathways in terms of canonical molecular orbitals [2]. In this work, we reassesed some experimental couplings constants on the N,N-dimethylfluoroacetamides and verified the mechanisms involved on its transmission by use of the new and efficient FCCP-CMO methodology. During the studies of conformational analysis of this compounds, was observed the long-range coupling 5JFH between the fluorine atom and the hydrogen atoms of N-methyl fragment [3]. Now we report the studies of these acetamides from experimental and theoretical point of view. The NMR spectras were obtained and compared to theoretical data to show the pathway transmission of 5JFH SSCCs. The structures were optimized at the BHandH/epr-iii level using the Gaussian03 program. 5JFH couplings and all four terms (FC, SD, PSO and DSC) were calculated using epr-iii basis set. The CMO information was obtained as CMOs expanded in NBOs basis. The dates showed that the coupling transmission have an important component occurring through space (TS). The TS transmission of FC term occurs by the overlap of the lone pair of fluorine atom and bond C-H orbital. The FCCP-CMO approach allows the graphic visualization as a picture showing TS contribution for 5JFH.

1. Castillo, N, Matta, CF, Boyd, RJ. J. Chem. Inf. Model. 45, 354, 2005.
2. Contreras, RH, Gptelli, G, Ducati, LC, Barbosa, TM, Tormena, FC. J. Phys. Chem. A 114, 1044, 2010.
3. Tormena, FC, Rittner, R, Abraham, RJ, Basso, EA, Pontes, RM. J. Chem. Soc. Perk. Trans 2, 2054, 2000.

22 From Retrospective Assessment to Prospective Decisions in Natural Product Isolation

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Repetitive isolation of known or even readily available natural products is one of the main factors limiting productivity of natural products research. Thus, a cornerstone of lead discovery programs based on natural products is dereplication, which is primarily based on HPLC-MS and HPLC-DAD with database support [1,2]. With advances in cryogenically cooled NMR probes and miniaturization it is now possible to acquire 2D NMR data of metabolites in the ng-range [3]. This makes the structurally very informative NMR technique feasible for dereplication purposes when hyphenated to HPLC and a solid phase extraction interface for post column up-concentrating of metabolites (HPLC-SPE-NMR). In this work, we describe a model study of Carthamus oxycantha employing the hyphenated NMR technique, HPLC-DAD-MS-SPE-NMR, to obtain structural information of metabolites prior to the laborious preparative isolation, thereby rendering prospective decision-making possible. Through comparison to a traditional isolation approach we show the strength of miniaturization and automation by isolating and acquiring 1H NMR data of individual metabolites of the crude plant extract overnight. Based on these initial data, interesting metabolites were selected for further 2D NMR analysis and complete structure elucidation in the hyphenation mode. Finally, metabolites selected on the latter data were subjected to targeted preparative-scale isolation. This approach enabled us to rationalize isolation procedure and focus isolation efforts on a series of unusual spiro[4.5]decane derivatives, in contrast to the traditional approach, when the value of metabolites is assessed retrospectively after investment in laborious, non-focused isolation efforts.

References :

1. Fredenhagen, Andreas; Derrien, Caroline; Gassmann, Ernst, J. Nat. Prod., 68, 385-391, 2005
2. Hansen, Michael E.; Smedsgaard, Jorn; Larsen, Thomas O., Anal. Chem., 77(21), 6805-6817, 2005
3. Hilton, Bruce D.; Martin, Gary E., J. Nat. Prod., 73, 1465-1469, 2010

23 Qualitative Source Identification and Quantitative Chlorogenic Acid Analysis for Multilab Method Validation of Blueberry Leaf extract by NMR

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The ability to quickly assay natural product extracts for new compounds or higher levels of known compounds, monitor ripeness, or confirm the natural source or origin can enhance the selection of natural products for ensuring efficacy, safety, origin, and quality. Similarly, the ability to perform these assays reproducibly on different instruments and at multiple locations frees users from time consuming sample validation on multiple instruments independently. NMR is well documented as an analytical technique which provides structurally definitive and quantitative data simultaneously in a single NMR experiment. NMR high reproducibility across platforms allows qualitative assessment (chemometric modeling) of highly complex samples such as botanical extracts, which enables data comparison at different sites.

Concepts and results of a multi-site reproducibility study are presented, which aims to identify and quantify compounds directly from the spectra of raw blueberry leaf extract and qualitatively identify the natural source of the raw plant material. Data were acquired at 12 different locations in North America on various generations of NMR spectrometers using a proton (1H) experiment which takes less than 10 minutes. Evidence for high reproducibility and the ability to acquire all data, both extracts and system suitability tests, under automation underscore NMR significant capacity to serve as an R & D screening tool and as a quality control tool operating under GLP guidelines.

24 Dynamic Nuclear Polarization and Scalar Coupling Measurement with a Squid-Based Micro-Tesla NMR System

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Micro-Tesla nuclear magnetic resonance (NMR) technique is a challenging application based on superconducting quantum interference device (SQUID) technology. The high sensitivity of the SQUID magnetometer enables to measure weak magnetic resonance free induction decay signals even for the low Larmor frequency at μT static field level. Measuring the NMR signals at such a low field gives many benefits like development of an open-type low-cost surgery monitoring magnetic resonance imaging (MRI) system, high-contrast cancer detector, J-coupling chemical analyzer, metal-penetration explosive material detector, and etc. For those applications, we need to generate a strong enough polarization. Generally, pre-polarization by using a strong electromagnet has widely been adopted. But, it causes troublesome electromagnetic interference to the sensitive SQUID sensor. One substantial way to increase the polarization is to utilize the dynamic nuclear polarization (DNP). This approach can enhance the nuclear polarization by using cross-relaxation from saturated the electron spin. In this presentation, we introduce experimental details for DNP of ULF MRI and demonstrate the first enhanced NMR signal in 120-MHz electron spin resonance and ~2 μT detection field and measurement of heteronuclear J-coupling between 1H and 31P of trimethylphosphate.

25 Synthesis, Biological Evaluation and Structural Characterization of Novel Glycopeptide Analogues of Nociceptin N/OFQ

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Nociceptin (N/OFQ) peptide is the endogenous ligand for NOP receptor, whose distribution is mainly in the central nervous system and in the spinal cord. It is implied, as its name indicates, in process of nociception, but there are several controversies about its effects. For instance, the route of administration determines opposite effects. After i.c.v. administration of nociceptin the analgesic effects of morphine and other opioids are diminished, whereas this peptide produces analgesia after intrathecal administration potentiating morphine analgesia.

In our laboratories some glycopeptides analogues of nociceptin were synthesized and analyzed in order to understand the function-structure correlation. The function was studied by biological activity in competition binding assays with zebrafish animal mode, previously tested for this nociceptin system, and the structural analysis were performed by NMR and circular dichroism. The distinct glycosilations determine different changes of the structure of the peptide (N/OFQ), which can be coupled to the measured inhibition constants. One of the glycopeptides with O-glycosilation at position 10 ([Ser10-O-alpha-D-GalNAc]-N/OFQ) showed stronger inhibition constant than native nociceptin, and the improvement of biological activity was explained by a modification of its structure.

26 Planar Microcoils for the Swift Optimization of Continuous-flow Processes

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Despites the diverse utility of NMR, it ranks as one of the least sensitive tools in the analytical arsenal. Advances in superconducting magnets, electronics, and probe design have greatly increased sensitivity, resolution, and ease of NMR spectroscopy. The signal-to-noise ratio (SNR) is increased by the use of reduced diameter coils since the coil efficiency, characterized as B1/i, is inversely proportional to the diameter of the coil. Thus, the concept of microcoil appeared.

Here we present the use of planar spiral microcoils integrated in a glass microfluidic chip (NMR-chip) [1]. The volume underneath the coil (detection volume) is 6 nL, allowing the detection of nanomol/picomol absolute amount of material. We have designed a setup for the rapid optimization of microwave assisted chemical processes since in most cases when performing microwave reactions, the analysis of the reaction progress takes longer time than the reaction itself. The two major components of the setup, the microwave flow cell and the NMR-chip are connected by means of capillaries enabling the on-line monitoring of the reaction progress. The setup has been designed and optimized to have a very small reaction volume (1,6 microliters) and total volume (around 5 microliters) for an optimization of a chemical process with low cost and in short time. This is the first time than a microwave reactor has been hyphenated to an NMR-probe.
The NMR-chip, as a consequence of having a smaller active volume than the reaction volume, provides several data points just from a single constant-flow experiment what accelerates the optimization process. It allowed an optimization of a Diels-Alder reaction with low cost and in short times [2]. We are currently focused on extending the scope of the system to the preparation of a small library of heterocycles compounds with significant biological activity as well as on the scale-up of the setup for the synthesis of the compound of interest.

1. Gomez, M. V; Reinhoudt D N; Velders A H, Small 4, 1293-1295, 2009.
2. Gomez, M. V; Verputten, H H J; Diaz-Ortiz, A; Moreno, A; de la Hoz, A; Velders A H, Chem. Commun. 46, 4514-4516, 2010.

27 Tools for Determination of Absolute Configuration in Small Chiral Molecules.

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Most compounds generated in the pharmaceutical industry today are chiral. They are often syntesized as racemate and then purified and separated into its different isomers by chromatography. The compounds can have one, two or even more chiral centres. It is essential to determine the absolute configuration of all centres before a compound can be submitted as a candidate drug. The by far mostly used method for such a determination is X-ray crystallography. This method implies good quality crystals of the compound, which sometimes can be very tedious and labour intense to get. It is therefore of great importance to have a wider set of tools for these determinations. Here, we present some typical cases for small molecule drug research and the determination of the chiral centres by NMR spectroscopy and vibrational circular dichroism (VCD).

28 Trilinear Analysis: NMR Reaction Monitoring for Overlapped Spectra

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NMR spectroscopy can in principle allow every species involved in a chemical reaction to be monitored simultaneously, providing both real-time quantitation and information on chemical structure. Typically this is done by acquiring 1H spectra at regular intervals and monitoring the integrals of diagnostic peaks, but when signals overlap it is often difficult interpret the data obtained. In principle, multivariate statistical methods can be applied here to separate the component spectra. However, this typically results in rotational ambiguity, where a wide range of candidate component spectra fit the experimental data equally well. Multilinear analysis (where the data vary independently in more than two dimensions) offers a way around this problem, allowing experimental data to be decomposed into physically realistic component spectra where multilinear data can be obtained experimentally. One way to obtain trilinear NMR data for the course of a chemical reaction is to acquire successive DOSY [1.2] (Diffusion-Ordered SpectroscopY) datasets during the reaction. Each individual DOSY dataset records how the NMR spectrum varies with pulsed field gradient strength at a given time. Provided that each species has a different diffusion coefficient and a different timecourse, the dataset is trilinear and can be decomposed (without prior knowledge of the spectra, reaction kinetics or diffusion behaviour) using the PARAFAC (PARAllel FACtor Analysis) algorithm to yield the spectrum, concentration time course, and diffusional attenuation for each component of the reaction separately.

1Nilsson M, Khajeh M, Botana A, Bernstein MA, Morris GA. Chem. Commun. 2009, 1252-1254.
2Khajeh M, Botana A, Bernstein MA, Nilsson M, Morris GA. Anal. Chem. 2010, 82, 2102-2108.

29 Enhanced Arrayed NMR Data Analysis Through New Automatic and Computer Assisted Alignment Algorithms and Global Spectral Deconvolution

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Many NMR experiments are acquired in the so-called "arrayed mode" which consists of a back-to-back series of FID recorded one after the other by varying a single parameter [1]. Such spectra include T1/T2 relaxation, Pulse Field Gradient and kinetics experiments. In particular, monitoring reaction progression in real time using NMR is gaining in popularity in industry, for example, in process development, but also in academia. However, these experiments present a number of challenges in the process of extracting the relevant NMR descriptors (i.e. resonance heights or integrals) across the different spectra. Firstly, during the course of a reaction, it is very common that the chemical shifts of one or several signals move as a result of, for example, the change in pH, concentration or temperature. On the other hand, peaks of different chemical species in the mixture might overlap to the point where the extraction of the integrals or heights of an individual component without the interference of the other species might become extremelly difficult. Finally, although automatic phase correction algorithms are usually very efficient, there are cases in which tiny phase errors are still present, hampering the correct quantification of the NMR signals.

In this work, we present an integrated solution specifically designed for the analysis of NMR arrayed experiments and which addresses the aforementioned problems. It includes an automatic alignment algorithm as well as a new graphical selection feature intended for the extraction of NMR descriptors in spectra facing large signal frequency drifts even in cases in which the chemical shift ordering changes during the experiment. Application of Global Spectral Deconvolution (GSD) [2] to alleviate the problems of phase correction and peak overlap will also be illustrated

1. Neil E. Jacobsen, NMR Spectroscopy Explained, Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology, John Wiley and Sons, 2007.

2. Carlos Cobas, Felipe Seoane, Stanislav Sykora, SMASH 2008 Conference, Santa Fe, New Mexico (USA), September 7-10, 2008

30 Detection of Slow Processes using Slowly Relaxing Nuclei

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NMR spectroscopy provides a range of useful methods for the investigation of dynamic processes at various timescales in solution. Among these, 2D exchange spectroscopy (EXSY) is of outstanding importance as it allows to study processes that are too slow to affect the lineshapes and under equilibrium conditions [1]. The method is limited by longitudinal relaxation of the nucleus of interest.

In recent years, several attempts have been made to extend the lifetime of nuclear magnetization using so-called singlet states [2-4]. Another approach would be the use of low-gamma nuclei with inherently long T1-times such as 109Ag or 183W. Here we present an example where 109Ag longitudinal magnetization was used to study the temperature-dependent dynamic equilibrium between two silver-NHC (NHC = N-heterocyclic carbene) complexes.

The challenge is the low inherent sensitivity of low-gamma nuclei which makes use of polarization transfer methods mandatory. We have designed an X-relayed HMBC experiment (where X is the nucleus bonded to 109Ag, usually 13C or 31P) that under certain conditions is superior to the conventional 1H,109Ag-HMBC detection scheme. Exchange was studied using a pulse scheme derived from the original heteronuclear exchange experiment reported by the Kay group [5].

References:

1. Jeener, J., Meier, B.H., Bachmann, P., Ernst, R.R., J. Chem. Phys., 71, 4546-4553, 1979.
2. Carravetta, M., Johannessen, O.G., Levitt, M.H., Phys. Rev. Lett., 92, 153003, 2004.
3. Cavadini, S., Dittmer, J., Antonijevic, S., Bodenhausen, G., J. Am. Chem. Soc., 127, 15744-15748, 2005.
4. Sarkar, R., Vasos, P., Bodenhausen, G., J. Am. Chem. Soc., 129, 328-334, 2007.
5. Farrow, N.A., Zhang, O., Forman-Kay, J.D., Kay, L.E., J. Biomol. NMR, 4, 727-734, 1994.

31 Rapid Enantiodifferentiation of Ibuprofen and its Major Metabolites in Human Urine using 1H NMR Spectroscopy and a Chiral Solvating Agent Without Prior Chromatography or Derivatisation: Towards Chiral Metabolic Profiling

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Differences in molecular chirality remain an important issue in drug metabolism and pharmacokinetics for the pharmaceutical industry and regulatory authorities. NMR spectroscopy is a powerful tool for the study of biofluids for the monitoring of endogenous metabolic effects of drug toxicity effects and disease[1]. It is, as well, a valuable tool for the de novo identification of both endogenous and xenobiotic metabolites. However, when dealing with stereo-recognition of chiral metabolites in biofluids, mainly chromatographic and mass spectrometric techniques have been utilised, usually preceded by a derivatisation and/or purification of the metabolites of interest. We present a straightforward method for the rapid differentiation and identification of chiral drug enantiomers directly in human urine without a previous physical separation and/or derivatisation. Using the well-known anti-inflammatory pharmaceutical ibuprofen as an example we demonstrate that the enantiomers of the parent and the diastereoisomers of its three main urinary metabolites (the glucuronides of ibuprofen and its carboxylate and hydroxyl derivatives) can be resolved directly in the biofluid by 1H NMR spectroscopy and by the direct addition of an appropriate chiral solvating agent (CSA) prior to analysis. This approach is simple, rapid, robust, and involves minimal sample manipulation. It avoids labour-intensive steps such as purification and/or derivatisation of the chiral compounds, all of which can reduce the opportunity for resolution. Moreover, the versatility of NMR spectroscopy allows the simultaneous characterisation of drug metabolites, both expected and unexpected. In addition, the method allows the enatio-differentiation of endogenous chiral metabolites and this has implications for improved understanding of metabolic pathways, both mammalian and microbial. From these initial findings we believe that more extensive and detailed chiral metabolic profiling could be possible using CSA-NMR spectroscopy than has been previously reported. Such an approach paves the way forward for more routine chiral metabolic profiling using NMR spectroscopy in the context of drug metabolism and metabolomics studies.

1. Nicholson, J. K.; Connelly, J.; Lindon, J. C.; Holmes, E., Nat. Rev. Drug Discov., 1(2), 153-161, 2002.

32 2D DOSY 1H NMR Analyses for the Characterization of Fake Drugs

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The chemical analysis of suspected fake drugs is a crucial step for differentiating counterfeit versus genuine drugs. Most analytical methods proposed for the detection of counterfeit pharmaceutical formulations focus on the active pharmaceutical ingredient (API) expected in the formulation and do not provide any other information on the pharmaceutical formulation. The detection of counterfeiting requires methods orthogonal to the conventional separation techniques described in the pharmacopoeias and pharmaceutical guidelines.

2D Diffusion Ordered SpectroscopY (DOSY) 1H NMR [1] is a very efficient tool for analyzing drug formulations. DOSY NMR experiments provide the spectral signature of a drug formulation with identification of the various ingredients (API, excipients, adulterants). The main strength of the method lies in its holistic nature, which enables the drug formulation to be considered as a whole.

DOSY NMR was applied to the analysis of various formulations of conventional drugs (fluoxetine, sildenafil, tadalafil, artesunate) [2-5] as well as herbal drugs marketed as natural slimming products or for sexual dysfunction [6,7]. The presentation will give an overview of the application of DOSY NMR in the field of drug analysis. It will outline the benefits and limits of DOSY NMR as a screening method for detecting and analyzing fake drugs [8].

1. Morris, K.F., Johnson, C.S., J. Am. Chem. Soc., 114, 3139-3141, 1992
2. Trefi, S., Gilard, V., Balayssac, S., Malet-Martino, M., Martino, R., J. Pharm. Biomed. Anal., 46, 707-722, 2008
3. Trefi, S., Gilard, V., Balayssac, S., Malet-Martino, M., Martino, R., Magn. Res. Chem., 47, S163-S173, 2009
4. Trefi, S., Routaboul, C., Hamieh, S., Gilard, V., Malet-Martino, M., Martino, R., J. Pharm. Biomed. Anal., 47, 103-113, 2008
5. Nyadong, L., Harris, G., Balayssac, S., Galhena, A., Malet-Martino, M., Martino, R., Parry, R., Wang, M., Fernandez, F., Gilard, V., Anal. Chem., 81, 4803-4812, 2009
6. Vaysse, J., Balayssac, S., Gilard, V., Desoubzdanne, D., Malet-Martino, M., Martino, R., Food Addit. Contam. Part A, 27, 903-916, 2010
7. Balayssac, S., Trefi, S., Gilard, V., Malet-Martino, M., Martino, R., Delsuc, M.A., J. Pharm. Biomed. Anal., 50, 602-612, 2009
8. Holzgrabe, U., Malet-Martino, M., J. Pharm. Biomed. Anal., 55, 679-687, 2011

33 ERETIC: A NMR Quantitative Tool for the Pharmaceutical Industry

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NMR spectroscopy is a powerful tool for the quantification of molecules in solution, since the area under a given resonance signal is proportional to the number of moles of nuclei responsible for that signal. On this principle, the use of a known concentration of an internal reference allows the direct quantification of the molecule of interest by calculating areas ratio of a specific resonance of the standard and of the molecule to quantify. This method is accurate and efficient but has some drawbacks. For example, the internal standard must be chemically inert towards the sample, soluble in the NMR solvent, compatible with the temperature, the pH and stable during the analysis. Moreover, it must not overlap resonances of the sample and must have preferentially a small relaxation time.

The ERETIC (Electronic Reference To access In vivo Concentrations) method described by the team of Akoka in 1995 was a major technological solution as instead of using an internal chemical reference, a radio frequency (RF) electronic reference is used for the quantification. The ERETIC signal is an exponential decay generated on a free channel of the spectrometer and sent to the unused coil of the probe or directly routed from the transmitter to the receiver. It is then combined with the free induction decay (fid) and after Fourier transform, a spectrum of the sample with the additional ERETIC peak is obtained. The initial calibration of the ERETIC signal against a standard of known concentration allows the use of this peak for molecule quantification. Using this adjustable ERETIC signal, an internal chemical reference is not needed anymore which eliminates the previously mentioned drawbacks.

We will demonstrate and illustrate here that this ERETIC quantitative tool has a wide range of pharmaceutical applications, especially for vaccines characterization and control. It can be applied on the quantification of small molecules used, for example, as raw material to the quantification of large biomolecules, such as the bacterial polysaccharides used as active substances of some vaccines.

1. Akoka S, Barantin L and Trierweiler M. Analytical Chemistry, 7, 2554-57, 1999
2. Barantin L, Le Pape A, and Akoka S. Magnetic Resonance in Medicine, 38, 179-82, 1997
3. Barantin L, Akoka S and Le Pape A. French Patent CNRS no.95 07651, June 26, 1995

34 Does 10B Have Advantages over 11B NMR Spectroscopy?

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Boron NMR can be an essential part of the structural studies of boron containing small organic molecules. However, rapid quadrupolar relaxation and the appearance of strong background signals may hamper the detection of broad resonances. Either the subtraction of the background spectrum or the utilization of multipulse sequences (DEPTH, RIDE) leads to unacceptable spectral quality owing to incomplete suppression and/or complete sensitivity loss induced by rapid quadrupolar relaxation. This precludes the routine 11B NMR investigations of the asymmetric boron environments. We have recently observed that 10B NMR spectra of tetrazolo[5,1][1,2]azaborinin derivatives in solution do not show measurable background signals contrary to the 11B NMR spectra [1]. It has turned my attention to the reconsideration of the generally accepted view that 11B is more suitable for solution NMR investigations than 10B. The signal-to-background ratio is determined by the proportion of the signal-to-noise ratio of the sample solution (real signal) and the signal-to-noise ratio of solid-state materials (background signal of the probe itself and the NMR tube). Solid-state 10B and 11B NMR spectrum simulations were carried out by using SIMPSON [2] to investigate the effect of Qcc on the signal intensity. It can be shown that the difference of the signal-to-noise ratio between 11B and 10B is increasing with Qcc. However, the boron environment has an opposite effect in solution, because the line broadening induced by the quadrupolar relaxation is more effective for 11B as compared to 10B [3]. Therefore, the strong background signals of 11B NMR are generally not observed in 10B NMR. The application of 10B NMR re-enables the detection of high quality, background-free boron NMR spectra without the need of special probe design and quartz tubes. This gives new perspectives for the structural studies of boron containing compounds, in particular for asymmetric boranes. One can push the advantage either to observe the extremely broad resonances of previously uncharacterized boranes or to lower the concentration of the sample in order to reduce solute-solute interactions. The growing field of metal-free borane/amin catalyst systems (frustrated Lewis pairs) is an example for this [4]. This work was supported by the GVOP-3.2.1.-2004-04-0210/3.0 grant.

1. Takacs, D.; Kiraly, P.; Nagy, I.; Bombicz, P.; Egyed, O.; Riedl, Z.; Hajos, G. J. Organomet. Chem., 695, 2673-2678, (2010).
2. Bak, M.; Rasmussen, J. T.; Nielsen, N. C. J. Magn. Reson., 147, 296-330, (2000).
3. Akitt, J. W. J. Magn. Res., 3, 411-414, (1970).
4. Eros, G.; Mehdi, H.; Papai, I.; Rokob, T.A.; Kiraly, P.; Tarkanyi, G.; Sojas T. Angew. Chem. Int. Ed., 49, 6559-6563, (2010).

35 High-Throughput Flow NMR Automation

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NMR-based metabolomics has been used to identify markers of chemical exposure and toxicity. Prior to this work, NMR-based metabolomics was not practical for high-throughput screening and prioritization of chemicals or for development of biomarkers because of limitations with spectral acquisition. Historically, NMR spectroscopy is not highly automated. Samples were placed in the magnet manually. Recently, there has been an escalating need to increase throughput of NMR analysis in modern NMR laboratories. Robot-type sample automation was developed to place NMR tubes into the magnet. However, because of issues with sample tubes broken or missed, this type of NMR automation is not suitable for studies requiring analysis of a large number of samples. Additional disadvantages of the robot automation include (a) difficulty in maintaining samples at low temperature when waiting in queue; (b) the extensive amount of time required for washing reusable NMR tubes; and (c) production of a large amount of organic solvent waste. Flow or direct-injection (DI) NMR automation has been recognized to have the potential to increase throughput because of its 96-well plate format. However, flow NMR analysis has not been widely used because of a few unsolved technical challenges such as sample carryover (cross contamination), sample diffusion at the front end of the sample, transfer line blockage, and air bubbles entrapped in the flow cell. Several variants of flow NMR such as flow injection analysis (FIA) and microflow NMR have been proposed to address one or more of the issues, but not all together.

It is known that the sample carryover problem can be reduced by repeated washing the tubing and flow cell. This wash process is not ideal because it requires more deuterium solvent, which increases operation cost on per sample basis. During conventional flow NMR analysis, wash solvent is retracted from the flow cell as waste through the injection line. This setup inevitably intensifies the carryover contamination problem because the previous sample at the front end will contaminate the line after the wash solvent passes from the flow cell back to waste. We have developed a high-throughput flow NMR method that overcomes all the above-mentioned problems with the conventional flow NMR automation methods. This new method simultaneously eliminates sample carryover and diffusion by using the minimum volume of wash solvent and is free of air bubbles in the flow cell; also, samples can be kept at low temperature when queuing and can be retrieved at the end of the NMR experiments. During the automations of thousands of samples, the new flow NMR automation technique has been proven to be reliable, efficient and free of carryover contamination and sample diffusion.

36 Second Generation Microfluidic Chips for Nanoliter NMR Spectroscopy

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The amplitude of the NMR signal is maximized when the filling factor is optimal; therefore, an intense effort has been devoted over the past decade to the development of microcoil NMR probes for the analysis of mass-limited and volume-limited samples [1]. We previously reported the study of supramolecular interactions using 1H and 19F NMR spectroscopy in a microfluidic chip equipped with a planar transceiver microcoil [2,3]. Recently, the on-line monitoring of a microwave-assisted cycloaddition was also described as a proof-of-concept for the hyphenation of small-volume NMR spectroscopy to other techniques [4].

Here we present the design of a second generation of microfluidic chips for nanoliter NMR spectroscopy, as well as the corresponding chip holder [5]. The new setup allows a precise positioning of the microprobe inside the bore of the magnet, and enables the use of plug-and-play electrical connections on one side, and microfluidic connections on the other side. A sample cooling/heating channel was also included for variable temperature experiments. The possibility of detecting different nuclei combined with the advantages of working on-flow opens the path for many lab-on-a chip applications, including real time monitoring of chemical reactions using sub-microliter volumes of reagents.

References:

1. Fratila, R. M., Velders, A. H., Ann. Rev. Anal. Chem., 4, 227-249, 2011.
2. Gomez, M. V., Reinhoudt, D. N., Velders, A. H., Proceedings of Micro Total Analysis Systems, Vol. 1 (Eds.: J.-L. Viovy, P. Tabeling, S. Descroix, L. Malaquin), Paris, 506-508, 2007.
3. Gomez, M. V., Reinhoudt, D. N., Velders, A. H., Small, 4, 1293-1295, 2008.
4. Gomez, M. V., Verputten, H., Diaz-Ortiz, A., Moreno, A., de la Hoz, A., Velders, A. H., Chem. Commun., 46, 4515-4516, 2010.
5. Fratila, R.M., Gomez, M.V., Sykora, S., Velders, A.H., submitted.

37 NMR and Quantum Theory of Methyl Esters Alpha-Amino Acids Conformational Analysis

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Glycine and alanine have been extensively studied by both theoretical and experimental approachs [1,2], mostly in the gas phase. Moreover, amino acids exhibit the zwitterionic form, which is prohibitive for NMR studies in organic solvents [1]. They present high melting points, low vapor pressures and thermally unstable. An alternative is the study of their methyl esters, not explored yet.

Thus, it is reported here the study of valine, phenylalanine and tyrosine methyl esters conformational behavior in several solvents by NMR and infrared spectroscopies. Theoretical calculations at the B3LYP/aug-cc-pVDZ level for all possible conformers were also performed. The hydrogen atoms vicinal coupling constants were also calculated for each conformer in solution using the IEFPCM model at the B3LYP/aug-cc-pVDZ and B3LYP/EPR-III levels for N and O atoms and for H and C atoms, respectively, in excellent agreement with experimental values. These couplings give their conformational preferences. All these information provided by NMR can not be obtained by any other spectroscopic technique. Their conformer populations were also obtained by infrared C=O stretching band deconvolution and confirmed those results. Theoretical calculations indicated that each of the aminoesters occur as 16 minima in the PES. However, a selection of the lower energie (Erel.<1 kcal mol-1) conformers leads to six conformers to Val-OCH3, nine for Phe-OCH3 and seven for Tyr-OCH3. Taking into account the (N-C-C=O) dihedral angle, their geometries for the most stable conformer are cis for Val-OCH3 (as 57 %) and gauche for Phe-OCH3 (as 44 %) and for Tyr-OCH3 (as 43 %). Their geometries for the  [nN-N-C-C(O)] dihedral angle are trans for the three aminoesters. The remaining dihedral angle [(H-CH-C-H] presented variable values for the three compounds. It is remarkable that these compounds present the same geometry for the main dihedral angle [(N-C-C=O)] of carbon skeleton.

These similarities reinforce the results obtained by the Quantum Theory of Atoms in Molecules (QTAIM) and Natural Bond Orbital (NBO) calculations. They showed that steric and hyperconjugative interactions are the main effects responsible for the stabilities of the main conformers. These results show how powerful is the NMR spectroscopy for the study of -aminoesters conformational equilibria, giving results in excellent agreement with IR data and theoretical calculations. FAPESP.

1.Alonso, J. L., Pena, I., Lopez, J. C., Vaquero, V., Angew. Chem. 121, 6257, 2009.
2.Ke, H.-W., Rao, L. I., Xu, X., Yan, Y.-J., J. Theor. Comput. Chem. 7, 889, 2008.

38 13C, 2H Enrichment of Cholesterol and Ergosterol for NMR Applications

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Cholesterol is one of the most important constituents of biological membranes and is implicated in the development of cardiovascular diseases. The structure of many complexes and structures involving cholesterol and ergosterol and their roles in the formation of membrane microdomains are still unknown. We introduced an efficient biosynthesis and purification of isotipically enriched cholesterol using a strain of Saccharomyces cervisiae capable of synthesizing sterols with a yield of c.a. 1 mg of cholesterol per gram of glucose.1

We produced cholesterol and ergosterol with diverse levels of 13C and/or 2H enrichment and will present relevant NMR spectra.

1. R. Shivapurkar, C. M. Souza, D. Jeannerat, H. Riezman. Journal of Lipid Research, 52, 1062-1065, 2011.

39 An Approach for the Thorough Evaluation of Automatic Structure Verification Software Systems

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Over the last few years automatic evaluation of NMR data has become a main driver for the pharmaceutical industry, given the desire within the pharmaceutical industry to analyze large data sets in the context of high throughput analytics and contract research. When assessing the adequacy of such systems and choosing between them, the development of a thorough evaluation protocol is fundamental to a successful conclusion aligned with user objectives. A well designed evaluation can not only provide prospective users with information as to the general capabilities of an automated structure evaluation system and its potential for application in their specific environment but also contribute an in depth understanding of discriminating power, strengths and weaknesses of the different stages of the process, limitations generated by the quality of data and possibilities and potential for user assisted training of the algorithms.

In this poster, we present an approach to the development of such a protocol. A variety of issues, such as relevant size and quality of data set, curation, generation of negative control structures [1], structure and spectra variety, correct assessment of false positive and false negative results are all analyzed. The selection of different types of data to provide granularity which allows an understanding of the different stages of the process which can be responsible for different results is also addressed by this approach.

The implementation of this approach is illustrated with its practical application in the in-house evaluation of the Mnova Automatic Structure Verification system and a blueprint for such tests or evaluations described.

1. MolSimNMR: A Molecular Similarity Method Predictive of NMR Data Similarity, Gonzalo Hernandez, Giovanni Cianchetta, Phil Keyes, Poster at ENC 2011 Conference, Asilomar, CA.

40 Dramatically Reducing False Positives in Automated Structure Verification by NMR

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Automated structure verification using 1H NMR data or a combination of 1H and HSQC NMR data is gradually gaining more interest as a routine software application for quality evaluation of large compound libraries produced by synthetic chemistry. The goal of this software application is to identify a manageable subset of compounds and data that require human expertise and review. In practice, the software will flag structure and data combinations that exhibit some inconsistency and automatically validate those that appear consistent. One drawback of this approach is that no automated software system can guarantee that all passed structures are indeed correct structures with correct structural assignments. The major reason for this is that approaches using only 1H or even 1H and HSQC spectra do not always provide enough information to properly distinguish between similar structures. As a result, current implementations of automated structure verification systems allow, in principle, false positive results.

Presented in this work is a method that greatly reduces the probability of an automated validation system to pass incorrect structures (i.e. false positives). The essence of the approach is to validate the proposed structure as well as several similar compounds against the same experimental NMR data. The software then evaluates all structures under tighter conditions to determine whether multiple structures can be clearly distinguished from the data. This novel method was applied to automatically validate a series of spectra for non-proprietary compounds from several sources. Presented and discussed is the impact of this approach on false positive and false negative results.

41 NMR as a Versatile Tool for the Quantitative Analysis of Active Pharmaceutical Ingredients

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NMR spectroscopy is a well-known and widely used analytical technique for the identification of the molecular structure of a compound. Moreover, it is a very powerful technique for the quantification of compounds, either pure, or in a complex mixture, without the need of a structurally related internal standard. At the DSM Biotechnology Center in Delft, NMR spectroscopy is routinely used for the identification and quantification of active pharmaceutical intermediates in several stages of process development ranging from high-throughput screening for micro organisms that produce the desired drug to pharmaceutical formulations containing the purified end product.

The power of quantitative NMR in drug analysis is outlined using the development of beta-lactam antibiotics such as penicillins and cephalosporins as an example. By means of metabolic pathway engineering in a medium throughput screening setup, the biosynthetic route to these products was optimized to obtain a micro organism with high production levels. By being able of measuring one sample per minute, flow-injection NMR allowed time-efficient support to this screening and provided a ranking of the micro organisms according to productivity.

Subsequently, conventional NMR is used to identify and quantify the product during fermentation, isolation and purification and in the final product. Since side products, starting materials and other components originating from the fermentation broth can be simultaneously identified and quantified, the information thus obtained about the metabolism of the micro organism and the process itself can be used for further optimization.

42 2D TR-NOESY Experiments Interrogate and Rank Ligand Receptor Interactions in Human Cancer Cells

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Integrins, the major class of heterodimeric transmembrane glycoprotein receptors, and the membrane-spanning surface protein aminopeptidase N (CD13) play a pivotal role in tumour growth and metastatic spread, as they are two of the major membrane bound receptors highly expressed on the surface of tumour cells during angiogenesis, therefore gaining increasing importance as drug targets in antiangiogenic cancer therapy. Using 2D-TR-NOE experiments we investigated the binding of this small library of cyclopeptides onto 2 human cancer cell lines differently expressing αvβ3 and CD13, including a melanoma (MSR3: αvβ3+CD13- cells) and a non-small lung carcinoma (MR300:αvβ3+CD13+) cell line, which display different phenotypes for CD13 and integrins [2]. Only very small amount of receptors are needed to prove binding (in the picomolar range), as it is sufficient that the receptor is detectable by FACS analysis. The method allows to prove recognition specificity using different cell lines, with different receptors, which can be also silenced with siRNA techniques. Most importantly, non specific binding can be straightforwardly established by competitive binding with stronger ligands.
Finally, we have characterized peptide conformations and αvβ3-binding ability by a combination of NMR and computational methods, including docking and molecular dynamics simulations. The conformational properties of the cyclopeptides were first analysed by classical solution NMR methods, and then refined by MetaDynamics (MetaD) simulations using as collective variable the glycine phi/psi dihedral angle. The MetaD refinement allowed to identify the most populated conformers in solution, which were next docked onto the αvβ3 crystallographic structure using HADDOCK-2.0. Both NMR and metadynamics results show that acetyl-CisoDGRC has a reduced conformational flexibility compared to CisoDGRC. In addition, this peptide has the correct bioactive conformation to dock inside the αvβ3 binding pocket, thus reducing the unfavourable entropic binding contributions observed in the flexible non-acetylated CisoDGRC macrocycle [2].

1. Mari S. et al. Angew. Chemie Int. Ed., 49, 1071-1074 (2010).
2. Spitaleri A. et al.Angew. Chem. Int. Ed., 50, 1832-1836 (2011).

43 Quantitative Aspects of Solid-State NMR: Quantitation of Polymorphic and Solvate Impurities Using MAS 19F and 2H NMR

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Understanding and control of solid state form is vital to the development of drug products in the pharmaceutical industry. In this context, it is imperative that the nature of solid state impurities such as undesired polymorphs and solvates are characterized and ultimately controlled. Solid state NMR (ssNMR) is not only sensitive to changes in crystal packing (i.e. can differentiate polymorphs), but is also inherently quantitative. The ssNMR spectra of these solid state impurities need to be understood to allow quantitative methods to be developed and potentially validated.

This poster describes the use of solid state 19F NMR to characterize and quantify a polymorphic impurity in the active pharmaceutical ingredient (API); a discussion of method validation is also presented.

In a second example, solid state 2H NMR was used to characterize a labelled solvate and discriminate free vs. bound solvent. In tandem with solution state 1H NMR, it was possible to quantify the solvate as a solid state impurity in API.

44 Fully Automated Metabolomics Analysis using Flow-NMR/LC-MS: From Sample to Spectrum

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Metabolomics has proven to be a well suited approach to study the metabolism of biological systems. In particular, nutritional metabolomics investigates the metabolic effects of nutrition and lifestyle. The relatively low amplitude of nutrition-induced metabolic effects, together with the high intrinsic inter-individual variability of complex mammalian systems require the analysis of a large number of samples in order to generate statistically robust metabolic information. In addition, the introduction of new automatic sample preparation devices and the progress achieved to couple the two most used and complementary techniques, namely nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), guaranty both robustness and in-depth analysis of the metabolome. In fact, the combination of NMR and LC-MS allows the acquisition of metabolic profiles of high biochemical detail, necessary not only for the statistical differentiation of samples but also for chemical identity.

Here, we report an application of a fully automated set-up of flow-NMR/LC-MS for the metabolomics analysis of human urine samples. Sample preparation, NMR on-flow sample delivery and acquisition and LC-MS acquisition are processed in parallel, under the control of a sample manager software and database. This system processes samples at comparable technical errors to manual handling, reducing human intervention and increasing the daily throughput and the efficiency in book-keeping.

45 The Rapid Identification of Bioactive Compounds from Korean Natural Products by LC-NMR

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The hyphenation of HPLC (high performance liquid chromatography) with NMR (LC-NMR) has recently become a valuable tool in analyzing complex mixtures such as natural products and pharmaceutical metabolites. This technique combines the separation power of HPLC with structural information provided by NMR for each component after online separation and offers the potential for the target components to be identified rapidly without the need for additional purification [1, 2]. We have applied this powerful tool to directly identify the bioactive constituents from three Korean natural products (Petasites japonicus, Angelica dahurica, Aceriphyllum rossii)

P. japonicus; Six compounds were successfully separated and identified as 5-caffeoylquinic acid (5-CQA), fukinolic acid (FA), 3,5-di-O-caffeoylquinic acid (3,5-DCQA), quercetin-3-O-(6"-acetyl)-b-glucopyranoside (QAG), 4,5-di-O-caffeoylquinic acid (4,5-DCQA) and kaempferol-3-O-(6"-acetyl)-b-glucopyranoside (KAG) by LC-NMR and LC-MS. The relative antioxidant capacities of the compounds were evaluated by an HPLC system with post-column on-line antioxidant detection based on 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging. Among these compounds, those containing a caffeoyl moiety (5-CQA, FA, 3,5- and 4,5-DCQA) showed relatively strong radical scavenging capacity, with 3,5-DCQA having the greatest radical scavenging capacity inleaf (23.09% of total antioxidant capacity) and root (26.47%) extracts.

A. dahurica; Five furanocoumarins were separated and identified as byakangelicol, oxypeucedanin, imperatorin, phellopterin and isoimperatorin by LC-NMR/MS. In search of the anti-inflammatory constituents, the HPLC-based activity profiling approach was used to investigate the extract's NO inhibitory activity. The results showed that the anti-inflammatory activities could be linked to imperatorin and phellopterin.

A. rossii; We evaluated the estrogenic activities in MCF-7 cells. In search of the estrogenic constituents, bioactivity-guided isolation using solvent partition and column chromatography was carried out. Two compounds from the active fraction were identified as Gallic acid ethyl ester and quercetin by LC-NMR/MS.

1. Andrade FDP, Santos LC, Datchler M, Albert K, Vilegas W., J Chromatogr A, 953, 287-291, 2002
2. WilsonID., J Chromatogr A, 892, 315-327, 2000

46 Precise Measurement of Heteronuclear Coupling Constants from 1H Selective HSQMBC Experiments

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1D and 2D 1H-selective HSQMBC experiments are proposed for the direct and accurate measurement of long-range H-C coupling constants in small molecules without need for an individualized and time-consuming post-processing fitting procedure.

The use of long-range proton-carbon coupling constants (nJCH, n>1) is a very good complement to proton-proton coupling constants (JHH) and/or NOE data for the structural and conformational analysis of natural-abundance molecules [1]. It is known that these small nJCH coupling constants (ca. 0-10 Hz) present strong dependences with respect to coupling pathways, patterns substitutions and structural constrains such as dihedral angles. However, the lack of extensive experimental data and trustworthy structural correlations often prevent its successful application to resolve routine problems [2]. Long-range optimized experiments (such as HMBC [3] and HSQMBC [4]) are highly suitable when quaternary carbons are involved. The value of nJCH is usually extracted from an individualized and time-consuming post-processing fitting procedure of the resulting anti-phase coupling pattern. Unfortunately, undesired mixed-phase multiplet distortions originated by the additional JHH-coupling evolution during the long evolution INEPT-type introduce a common source of inaccuracy.

In this work, improved 1H-selective versions of the HSQMBC experiment will be proposed for the straightforward, direct and accurate measurement of nJCH without the need of the classical fitting procedure. It will be shown that specific nJCH values can be accurately extracted from pure in-phase multiplets. Otherwise, IPAP technique is also implemented in versions of the HSQMBC. In this way, the relative displacement of separate alpha- and beta-HSQMBC-cross-peaks that result of the time-domain addition/subtraction procedure of complementary. In-phase (IP) and Anti-phase (AP) HSQMBC data provides a simple way to extract the nJCH value.

1. Bifulco, G., Dambruoso, P., Gomez-Paloma, P. et al, Chem. Rev, 107(9), 3744-3779, 2007.
2. Parella, T, 2D Methods for the Measurement of Long-Range H-C Coupling Constants in Multidimensional NMR methods for the Solution State, Morris, G.A., Emsley, J.W., John Wiley & Sons LTD, UK, Chap. 23, 305-314, 2010.
3. Eden, R.A.E., Keeler, J, J. Magn. Reson. 181(1), 89-97, 2006.
4. Williamson, R.T., Marquez, B.J., Gerwick, W.H., Kover, K.E., Magn. Reson. Chem., 38(4), 265-273, 2000.

47 1D HOESY Sequences for 19F-1H Heteronuclear nOe Experiments

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The past decade has witnessed an increasing contribution of fluorine chemistry to the field of pharmaceutical and agrochemical research with up to one quarter of drugs containing at least one fluorine atom. The stereospecific incorporation of fluorine into organic compounds represents an expansive field of research at the interface of bioorganic and medicinal chemistry, and chemical biology. As a consequence, methods for the structure elucidation of fluorinated compounds are becoming increasingly important. One approach to this is to employ the nuclear Overhauser effect (nOe) to establish close internuclear relationships between spins and in the context of fluorinated compounds the 19F-1H heternuclear nOe has considerable potential. Nevertheless, despite recent spectrometer hardware developments, many 19F-1H nOe experiments reported in the literature still utilise the traditional 2D HOESY experiment to observe these interactions, despite this being far from the optimum approach in many cases. Thus, with only a single 19F centre in any compound a classical 19F detected 2D approach leads to poor 1H resolution in the 'indirect' dimension, with only the single 19F resonance observed. For the 'inverse' 2D approach employing 1H observation, the indirect frequency dimension contains only a single fluorine resonance, and there is no need to record a full 2D data set. An optimum approach in such cases may be to employ a 1H observe heteronuclear 1D nOe experiment which is time efficient and provides high proton resolution. Herein we describe robust and clean 1D 19F-1H HOESY experiments optimised for small molecules that are suitable for qualitative analysis and for quantitative internuclear distance estimates.

48 Determining Stereochemistry and Conformational Analysis Using Residual Dipolar Couplings and Molecular Modeling

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One of the main complications in the structure determination of nonrigid molecules in solution by NMR spectroscopy is interconversion of conformers taking place fast on the NMR time scale that averages NMR parameters (3J coupling constants, NOEs and residual dipolar couplings) which are essential for the determination of the 3D structure of organic and biomolecular compounds. Recently it has been shown that residual dipolar couplings (RDCs) can be used to determine 3D structure of molecules even in the presence of limited conformational motion [1,2].

Herein we want to present an example of molecule (2-(5,7-dimethyl-1,8-naphthyridin-2-yl)-3-isobutylhexahydro-1H-pyrrolo[1,2-c]imidazol-1-one), comprising of three moieties with different degree of conformational flexibility, whose stereochemistry and conformational dynamics was assessed using combined experimental and computational approach.

References:

1. Thiele, C. M.; Schmidts, V.; Boettcher, B.; Louzao, I.; Berger, R.; Maliniak, A.; Stevensson, B. Angew. Chem. Int. Ed., 48, 6708-6712, 2009, and references therein.
2. Stoll, R. S.; Peters, M. V.; Kuhn, A.; Heiles, S.; Goddard, R.; Buehl, M.; Thiele C. M.; Hecht, S. J. Am. Chem. Soc., 131, 357-367, 2009.

49 RDC-based Determination of the Relative Configuration of the Fungicidal Cyclopentenone Hygrophorone A

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After their isolation from fungi of the Hygrophorus family, the Hygrophorones and their acetylated derivates have been subject of intense study due to their structural similarity with the antibiotic pentenomycin and antifungal activity. [1] The relative configuration of the sp3 carbons in the five-membered ring (C4 and C5) was established by comparison of 3J and 4J coupling constants with the known (epi-)pentenomycin structure and NOESY measurements. However the relative configuration of the exocyclic C6 carbon remained unknown and also the proposed 4,5-trans configuration has not yet been determined unambiguously.

We used RDCs [2] to determine the relative configuration of all three stereogenic centers at once. By aligning about 2 mg of Hygrophorone A in a liquid crystalline phase of high-molecular-weight PBLG in CD2Cl2 [3] we were able to measure eight one-bond and long-range C-H RDCs. We studied possible conformational flexibility in the five-membered ring and along the C5-C6 bond by conventional force-field and DFT methods. Fitting these calculated structure models with our RDC module in the software hotFCHT, [4] we found only a single relative configuration reproducing the experimental data.

1. Lubken, Tilo, Schmidt, Jurgen, Porzel, Andrea, Arnold, Norbert, Wessjohann, Ludger, Phytochem., 65, 1061-1071, 2004.
2. Reviews: Thiele, Christina M., Conc. Magn. Reson. A, 30A, 65-80, 2007. Thiele, Christina M., Eur. J. Org. Chem., 2008, 5673-5685, 2008.
3. Marx, Andreas, Schmidts, Volker, Thiele, Christina M., Magn. Reson. Chem., 47, 734-740, 2009.
4. Berger, Robert, Fischer, C., Klessinger, Martin, J. Chem. Phys. A, 102, 7151-7167, 1998.

50 Validation of 1mm CMCQ HT-NM, A Novel Pharmaceutical Repository Compound Quality Screening Technology

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The goal of this study is to validate a high-throughput and fully automated quantitative NMR (qNMR) technique, 1mm HT (high throughput)-NMR with CMCQ (Complete Molecular Confident Quantification). The method has been developed and implemented to assess CM (Compound Management). Although accuracy and sensitivity of qNMR have been well established, performance of qNMR on compounds stored in non-deuterated DMSO under high throughput operation was not thoroughly investigated before. In order to validate this new qNMR tool, we conducted various analytical studies. Particularly, we designed and developed an orthogonal HPLC method, which examined sample concentration changes within the sample preparation and data collection steps, and compared the results with the HT-NMR results repository compound quality since 2009. The results show clearly that 1mm HT-NMR meets criteria for determining concentration of deck compounds. Our study also confirms that CM standard protocols of distributing BMS compounds are reliable and accurate.

51 NMR Structure Characterization of Lasso Peptides

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Lasso peptides are ribosomally assembled natural products of bacterial origin. Their sequence contains 16-21 amino acids, whereby the N-terminal starts with Gly or Cys, an Asp or Glu occupies position 8 or 9, and the amino group of Gly1/Cys1 and the carboxyl side chain of Asp/Glu at position 8/9 form an isopeptide bond and thus an intralmolecular macrolactame ring with 8/9 residues. Through the macrocyclic macrolactame ring the rest linear exocycli C-terminal part of the sequence is threaded. Residues with sterically demanding side chains (Arg, Phe, Trp, and Tyr) trap the threaded tail within the macrocyclic ring and thus stabilize the lasso fold. The currently known lasso peptides are RP 71955, RES-701-1, siamycin I, siamycin II, microcin J25 (MccJ25), lariatin, capistruin, and BI-32169 [1-8]. We identified capistruin by genome mining approach and determined the structure of capistruin and BI-32169 by NMR spectroscopy. The unique structural characteristics of lasso peptides make them as potential candidates for biomedical applications and drug design. Recently, MccJ25 was used as structure scaffold and the tripeptide integrin binding motif RGD has been successfully grafted onto it [9]. Furthermore, through genome mining approaches more and more lasso peptides are going to be discovered.

Here we present a summary of the spectroscopic studies on lasso peptides. Detailed procedures for a full signal assignment, the identification of crucial long-range NOE contacts, the diverse behavior of the NH towards temperature and H/D exchange, and the stability against protein denaturing reagents will be discussed. The structure details of the RGD grafted MccJ25 will also be presented.

1. D. Frechet et al. Biochemistry, 1994, 33, 42-50.
2. R. Katahira et al. Bioorg. Med. Chem. 1995, 3, 1273-1280.
3. R. Katahira et al. Bioorg. Med. Chem. 1996, 4, 121-129.
4. K. L. Constantine et al. J. Biomol. NMR 1995, 5, 271-286.
5. K. J. Rosengren et al. J. Am. Chem. Soc. 2003, 125,12464-12474.
6. M. Iwatsuki et al. J. Am. Chem. Soc. 2006, 128, 7486-7491.
7. T. A. Knappe et al. J. Am. Chem. Soc. 2008, 130, 11446-11454.
8. T. A. Knappe et al. FEBS Lett. 2010, 584, 785-789.
9. T. A. Knappe et al. Angew. Chem. 2011, accepted.

52 Localized Single Scan 2D NMR

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In order to sample an arbitrary space, at least two different approaches are possible. The first one involve a unique reporter giving its state at different time, a series of time dependent experiments is then needed to characterized the full space. A faster solution would be to excite simultaneously several reporters with different evolution rates. Then the full space can be characterized in one experiment. The classical way to record 2D Nuclear Magnetic Resonance (NMR) experiments is described by the first solution whereas the single scan 2D experiment proposed by Lucio Frydman[1] is based on the second one. Actually, in the single scan scheme, the excitation of the spins is achieved by simultaneous use of radio frequency (RF) and field gradient (GF) pulses distributing their time evolution in function of their position in the sample. Thus the multiple reporters are created. This excitation period can be followed by any classical mixing scheme. Therefore, a single scan version of TOtal Correlated SpectroscopY (TOCSY) can be obtained by the concatenation of the excitation scheme with a sequence of RF pulses as MLEV, or DIPSI[1].

Recently Pelupessy et al.[2], describe a single scan version of the Spin Echo Correlated Spectroscopy (SECSY[3]). In this case the difference of the precession frequencies of two coupled spins is recorded canceling all the effect of the magnetic field inhomogeneities. Herein, we present a new version of this experiment which includes a Double Quantum Filter (DQF), leading to much less disturbances of the diagonal peaks.

The detection scheme should be designed to readout the state of the reporters. This is achieved by using a series of alternated GF pulse during the acquisition, as is it done in the Echo Planar Imaging (EPI) technique. In the Pelupessy et al's case it the echo arise for the difference of the chemical shift between two coupled spins.

Walter Kockenberger and co-wokers have demonstrated the feasibility of the localized single scan experiment[4], in the context of Dynamic Nuclear Polarization (DNP).

Herein, we propose to concatenate a dedicated volume selection sequence as the STEAM (Stimulated Echo Acquisition Mode), or the PRESS (Point Resolved Spectroscopy), with single scan experiment type. As a proof of concept we have done a Localized single scan TOCSY, and based on Pelupessy et al's sequence a localized single scan (DQF) experiment insensible to the inhomogeneities of the Bo magnetic field, that mean no shimming is required.

1. Frydman, Lucio, Scherf, Tali, Lupulescu, Adonis, PNAS, 99(25), 15858-15862, 2002.
2. Pelupessy, Philippe, Rennella, Enrico,Bodenhausen, Geoffrey, Science,324,1693-1697, 2009.
3. Nagayama, K., Wuthrich, Kurt, Ernst, R Richard, Biochem. Biophys. Res. Commun., 90, 305-311, 1979.
4. Panek, Rafal, Granwehr, Josef,Leggett, James Kockenberger, Walter, PCCP ,12, 5771-5778, 2010.

53 A Pulse Sequence for Optimal Signal-to-Noise Over Wide Spectral Ranges: A Long-Range 1H-15N Correlation Experiment Using Frequency-swept Radiofrequency Pulses

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The Heteronuclear Multiple-Bond Correlation (HMBC) experiment is a valuable tool for elucidating the structures of small molecules. A number of groups have refined the HMBC pulse sequence to suppress unwanted signals and improve the intensities of observed correlations. Even the best published sequences are subject to some signal losses, especially when the spectral width in the indirect dimension is large. Although improvements in probe performance can reduce the signal losses, a better option is to improve the pulse sequence. This poster presents a modified HMBC sequence that offers full signal intensity over very wide spectral widths.

54 Quality Assessment of Pharma Industry Compound Solutions by Automated Quantitative NMR and LC-MS

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Like in any pharma company, Actelion's medicinal chemists synthesize thousands of low molecular weight compounds within an iterative drug optimization process. These innovative compounds are characterized by molecular biologists and biochemists in relation to the chosen molecular drug targets.
In order to reduce the ratio of false positive screening results already very early in the drug discovery process, Actelion's compound management team has implemented a process to validate the quality of the compounds distributed to the biological screening. This quality assurance process is based on novel, automated NMR techniques in combination with optimized LC-MS conditions to confirm the sample identity, purity, and compound concentration along with the water content of the H-DMSO screening solutions. NMR has traditionally been the preferred tool for sample quality control with the highest information content. The NMR spectrum provides the analyst with information on the structural integrity of the compound. But NMR also provides inherent information on the concentration of the compound in solution and it supplies additional information on the purity, orthogonal to other methods such as optical HPLC detectors. In the same way, NMR can also be used to determine the water content on a sample in a solvent, which serves as an important information for a quality factor in pharma industry compound management facilities.
NMR has not been used until very recently as the standard quality assurance tool for all these analytical tasks since the data analysis had to be done from scratch and completely manually on each dataset. NMR also suffered for a long time of the prejudice of being very insensitive, and thus, being slow and requiring large sample amounts which are preferably dissolved in deuterated solvents. Here we present Actelion's NMR setup and which may easily be adapted to any other routine NMR setup. This setup is able to deal with microliters of solvents, works in full automation and delivers data within few minutes. We also present Bruker's latest developments to support the spectroscopists with the analytical tasks associated with this increased amount of data. We will show latest results of an automation tool for the full automatic NMR setup, acquisition and analysis with respect to the determination of concentration- and water content determination of large batches of samples. We will show details on the workflow implementation and outline the analytical algorithm. We present the NMR application in an industry environment where it is seamlessly combined with information derived from different analytical tools such as LC-MS data-analysis, for maximum confidence on the resulting quality factors.

55 1H MAS Line widths of Small Organic Guest Molecules Confined in Porous Silicas

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It is known that 1H MAS line widths are dominated by the homonuclear dipolar interactions. In the last decade several ingenious multipulse sequences have been proposed to overcome this problem [1], for the same purpose others proposed the decrease of proton spin density by deuteration of the sample molecules [2]. Under fast or very-fast MAS conditions line widths of about 0.5 ppm can be achieved nowadays.

We report now on results based on simple spatial separations of the interacting proton spins. It has been noticed recently that small organic molecules (solids at room temperature) show liquid-like behavior if confined in silica pores [3]. The origin of this high molecular mobility (that scale down the dipolar couplings) is not well understood as yet. The first reports hinted that a sharp drop of the phase-transition temperature (the so called Gibbs-Thomson effect) might be credited for the phenomenone, however our observations do not support this view.

We have managed to confine small organic molecules (camphor, menthol, HMB, HMTA, terphenyl, anthacene, etc.) in gradually increasing regular (MCM-41, SBA-15) and irregular (silica aerogels) pores and observed steadily decreasing proton line widths. 2H MAS spectra confirmed the molecular mobility as main reason. Comparison of the static spectra of the confined and free molecules show an impressive resolution improvement (a factor of 5 to 10 was observed in each case looked at so far). When combined with fast MAS rotation line widths of 30-40 Hz could be readily achieved.

Effects of loading, silica pore size and possible applications will also be discussed.

1. E. Vinogradov. P.H. Madhu, S. Vega, New Techniques in Solid-State NMR, vol.246, Springer Verlag, Berlin, 2005, p.33.
2. Q. Wang, E. Jordan, D.F. Shantz, J.Chem.Phys.C. 18151, 113, 2009.
3. T. Azais, C. Tourné-Péteilh, F. Aussenac, N. Baccile, C. Coelho, J.M. Devoisselle, F. Babonneau, Chem.Mater. 6382, 18, 2006.

56 An Expert System for Automatic Classification of NMR Peaks Using a Fuzzy Scoring Logic

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Over the last few years automatic evaluation of NMR data has undergone a profound renaisssance, particularly driven by the strong motivation from the pharmaceutical industry where the need for high throughput and reliable analysis of large data sets is of paramount importance. In that context, a number of procedures of varied sophistication have been developed to extract relevant information from the NMR spectra, ranging from traditional peak picking algorithms to more advanced evaluation methods such as FDM [1], Bayesian [2] and GSD [3].

However, regardless of the peak evaluation technique, automation is usually hampered by the presence of solvent and impurity resonances, and the process can be made even more difficult when experimental acquisition conditions are less than perfect. Identification of these peaks is a task an experienced chemist is very familiar with, but extremely difficult for a computer program, since the exact location and multiplicty of those peaks can vary significantly depending on experimental conditions. Additionally, these peaks can overlap with compound resonances, making some simple strategies based on the definition of &#65533;solvent&#65533; regions ineffective.

In this work we present an expert system for the automatic classification of NMR peaks that addresses these problems by means of a fuzzy logic scoring system applied to a list of peaks derived using the GSD algorithm. Solvent peaks and known impurity compounds in a spectrum are effectively identified even in cases where those resonances overlap with compound multiplets. The system presented here takes into account both the uncertainty in exact peak position as well as incompleteness of information (e.g. insufficient resolution).

Reducing an NMR spectrum in the way described is an important prerequisite for effective Automatic Structure Verification (ASV) and Elucidation (ASE).

1. V.A. Mandelshtam, H. S. Taylor, A. J. Shaka, J. Magn. Reson. 133, 304-312 (1998)

2. Denis V. Rubtsov, Claire Waterman, Richard A. Currie, Catherine Waterfield, Jose; Domingo Salazar, Jayne Wright, and Julian L. Griffin, Anal. Chem., 2010, 82 (11), 4479-4485

3. Carlos Cobas, Felipe Seoane, Stanislav Sykora, SMASH 2008 Conference, Santa Fe, New Mexico (USA), September 7-10, 2008

57 Speed, Selectivity and Structures: NMR Adding Value to Reaction Monitoring

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NMR is commonly used as a tool for analysing isolated reaction products. However, many of the strengths of NMR also make it highly desirable as a real time method for obtaining kinetic and structural information during a chemical reaction. These include selectivity for certain nuclei, inherently quantitative peaks, high resolution, ability to determine structures, fast acquision times, flexibility in the setup and the ability to run different experiments in a sequence. This poster will show some of the specific advantages of applying NMR to reaction monitoring over techniques such as HPLC and NIR. The examples will demonstrate that this approach can add value by providing structural and kinetic information on fast reactions and selectively observing multiple species involved in more complex sequential reactions.

58 NMR Characterization of Osteogenic Growth Peptide Interaction with alpha2-Macroglobulin

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The Osteogenic Growth peptide (OGP) is a highly conserved, naturally occurring tetradecapeptide that exerts regulatory effects on bone and bone marrow and shows mitogenic effects on fibroblasts. It is physiologically present in the blood circulation, mainly in the form of an OGP-OGP binding protein (OGPBP) complex. These complexes maintain large reservoirs of inactive OGP protected from proteolytic degradation, and provide a mechanism for the control of peptide availability to its target cells. Since α2-Macroglobulin is a plasma OGPBP that appears to be an important modulator of the OGP action, we are currently exploiting high resolution NMR techniques in order to investigate the formation of OGP alpha2-Macroglobulin complex at molecular level. In particular, STD, waterlogsy, 1H-13C-HSQC and NOESY experiments has been employed in order to characterize the OGP epitope mapping, that is the aminoacid residues of the peptide directly involved in the binding, at atomic level and to obtain information about the OGP conformation in the OGP α2-Macroglobulin complex. Experimental results obtained will be presented in this communication. Structural data obtained will be exploited for the rational design of OGP mimetics that will be employed for biomaterial decoration.

The research leading to these results has received funding from the CARIPLO Foundation (CARIPLO 2008-3175: Development of NMR techniques for tissue engineering studies)

59 GSD (Global Spectrum Deconvolution) in Metabolomics: Absolute Quantification and GSD Binning

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NMR spectroscopy is an important tool in many metabolomic applications. Its potential capability to handle complex mixtures of metabolites makes it a prime choice in both identification and quantification of the multitude of different species constituting unprocessed biological mixtures. For this reasons, and because the actual nature of all the metabolites is rarely known in advance, metabolomics often uses alternative statistical evaluation methods, such as multivariate factor analysis [1], which sidestep the need for a complete interpretation of the spectra and a full solution of the inverse problem, while still permitting the correlation of the spectra with specific biological aspects. However, such approaches require integration over predefined intervals (bins) and a meaningful integration of such intricate and artifact-burdened spectra may often be just as arduous as peaks fitting. Recently, a new algorithm called GSD (Global Spectrum Deconvolution) has been developed [2] and made available in the Mnova software package of Mestrelab. GSD is capable of identifying even poorly resolved spectral peaks and of fitting all recognizable peaks in even a very complex 1D spectrum in a surprisingly short time (typically a dozen seconds for up to 1000 peaks). Here we present the first controlled tests of the applicability of GSD to the multivariate analysis of complex metabolite mixtures such as cell culture media containing serum and mouse urine. GSD allows the user to generate synthetic spectra that can be used in the subsequent steps of multivariate statistical analysis. In particular, we compare the quality of PCA plots obtained by means of conventional binning of experimental spectra (NOESYPR, CPMG and projection obtained from J-resolved spectra) with PCA plots obtained by binning the synthetic spectra as well as direct GSD-binning based just on the GSD peaks table. In addition, we have taken advantage of GSD to implement under Mnova the quantitative referencing strategy known as PULCON [3]. By combining the GSD algorithm with a PULCON script we are able to deconvolute overlapped regions and perform absolute quantification even of metabolites whose peaks are buried under these areas. In order to verify the quality of the quantification, we have acquired spectra of a commercial cell culture medium which is a complex but well known mixture of more than 20 compounds. We then compare the metabolite concentrations obtained from experimental spectra by means of the GSD - PULCON algorithm with those declared by the medium vendor.

1. HG.J. Issaq et al. J. Sep.Sci., 32, 2183-2199 (2009)
2. C. Cobas et al. Poster at 50th ENC
3. G. Wider, et al. JACS 128, 2571-2576 (2006)

60 Direct C-13 NMR Detection in HPLC Hyphenation Mode: Analysis of Ganoderma Lucidum Terpenoids

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Hyphenated NMR spectroscopy has been demonstrated as a powerful analytical tool in determination of small molecules chemical structure [1]. Studies have shown the possibility of structural evaluation based on 1H-detected experiments in hyphenation mode. However, direct 13C NMR detection is a challenge due to natural paucity of 13C nuclei and its lower gyromagnetic ratio. Therefore virtually all hyphenated NMR studies reported thus far use 1H-detected experiments.

In the present work, we demonstrate the possibility of direct 13C LC-SPE-NMR spectroscopy using three terpenoids from Ganoderma luciduma, a macro fungus with remarkable value in Chinese traditional medicine [2], as model compounds. These terpenoids are known to maintain a well-conserved carbon skeleton. Differences are mostly pronounced by the degree of oxidation and placement of double bonds. Therefore, 13C chemical shifts are particularly useful in their structure elucidation, whereas use of 1H-detected 2D experiments is hampered by the necessity of assigning often unresolved and overlapping peaks of diastereotopic methylene protons of the triterpenoid core.

Optimal chromatographic loading and post column concentration of the compounds followed by elution with appropriate deuterated solvent enabled the first LC-NMR experiments with direct 13C detection using 600 MHz Avance III system equipped a cryogenically cooled gradient inverse triple-resonance 1.7 mm TCI probe head. In addition to standard Fourier transformation with the application of Traficante window function, spectral calculation are also performed using the maximum entropy method, MaxEnt [3], for improved signal to noise ratios.

References:

1. Kesting, J. R.; Johansen, K. T.; Jaroszewski, J. W. In Biomolecular NMR Spectroscopy, Dingley, A. J., Pascal, S. M., Eds., IOS Press, Amsterdam; pp. 413-434, 2011.
2. Paterson R. R. M. Phytochemistry, 67, 1985-2001, 2006.
3. Gull, S. F. & Daniell, G. J. Nature, 272, 686-690, 1978.

61 Solid-State NMR Studies of Trehalose

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Trehalose, a disaccharide composed of two α-glucose units, has applications as an excipient in pharmaceutical formulations, as a therapeutic, and as an additive in the food and cosmetic industries [1]. Despite significant interest in the stabilizing properties of trehalose, as well as its frequent use as a model compound to study the amorphous state, confusion still surrounds the relationships among the solid forms. We believe that 13C solid-state NMR (SSNMR) has the potential to address many of the questions about trehalose that more common solid-state characterization techniques have left unanswered. In addition to the documented effects of particle size on the dehydration of trehalose dihydrate [2], studies in our lab have shown that source variability and lot-to-lot variability of trehalose dihydrate also significantly influence which anhydrous forms are produced upon dehydration. We have used 13C SSNMR to identify the anhydrous forms of trehalose that are present in complex mixtures resulting from isothermal dehydrations. Unidentified peaks present in spectra of samples that were dehydrated at 75 degree C suggest that a new form of trehalose may have been generated. Both preparation method and aging have been shown to affect the tendency of amorphous trehalose to crystallize to the anhydrate [3,4]. Amorphous trehalose prepared by dehydration at 125 degree C has a greater tendency to crystallize than amorphous trehalose prepared by lyophilization. Following aging at 100 degree C for 24 hours, both amorphous samples have an increased tendency to crystallize. 1H T1 values for amorphous trehalose samples are typically ~5 s (lyophilized) or ~8 s (dehydrated). Following aging, the 1H T1 of the lyophilized sample increased to ~8 s, but the 1H T1 of the dehydrated sample remained the same. Samples of the dihydrate from different sources were dehydrated at 75 degree C and also display different tendencies to crystallize. Spectral subtractions and deconvolutions of these SSNMR spectra may reveal subtle differences that will help explain why certain samples crystallize more readily than others. SSNMR studies of trehalose have the potential to provide information on how all forms of trehalose, both crystalline and amorphous, are related. It is uniquely suited to study complex mixtures of anhydrous forms and provide detailed information on amorphous materials that may contain short-range order. Increased use of SSNMR to study trehalose will likely answer current and future questions about this complicated system.

1. Ohtake, Satoshi, Wang, Y. John, J. Pharm. Sci., 100(6), 2020-2053, 2011.
2. Taylor, Lynne S., York, Peter, J. Pharm. Sci. 87(3), 347-355, 1998.
3. Surana, R., Pyne, A., Suryanarayanan, R., Pharm. Res., 21(7), 1167-1176, 2004.
4. Surana, R., Pyne, A., Suryanarayanan, R., Pharm. Res., 21(5), 867-874, 2004.

62 Validating the ChemSpider Open Spectral Database using NMR Verification Algorithms

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ChemSpider is a free access online database of over 26 million chemical compounds sourced from over 400 different sources including government laboratories, chemical vendors, public resources and publications. ChemSpider allows its users to deposit data including structures, properties, links to external resources and various forms of spectral data. ChemSpider has aggregated over 3000 high quality NMR spectra and continues to expand as the community deposits additional data. The majority of spectral data is licensed as Open Data allowing it to be downloaded and reused. The validation of the data can be performed by members of the community but an automated validation of the data was undertaken using ACD/Labs software using NMR prediction and verification routines. The dataset is a real world dataset containing the contributions of a number of laboratories around the world supplying data of varying quality including S/N issues, misreferencing, impurities etc. This work will report on the batch analysis of the ChemSpider spectral data including the identification of multiple errors in the spectra.

63 Particle Size Measurement of Lipoprotein Fractions using Diffusion-Ordered NMR Spectroscopy

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The sizes of certain type of lipoprotein particles have been associated with an increased risk of cardiovascular disease (CVD). However, there is currently no gold standard technique for the determination of this parameter. Here, we propose an analytical method to measure lipoprotein particles sizes using diffusion-ordered NMR spectroscopy (DOSY). The method was tested on six lipoprotein fractions, VLDL, IDL, LDL1, LDL2, HDL2, and HDL3, which were obtained by sequential ultracentrifugation from four hyperlipoproteinemic patients. We performed a pulsed-field gradient experiment on each fraction to obtain a mean diffusion coefficient, and then determined the apparent hydrodynamic radius using the Stokes-Einstein equation. To validate the hydrodynamic radii obtained, the particle size distribution of these lipoprotein fractions was also measured using transmission electron microscopy (TEM). The standard errors of duplicate measurements of diffusion coefficient ranged from 0.5% to 1.3%, confirming the repeatability of the technique. The coefficient of determination between the hydrodynamic radii and the TEM-derived mean particle size was r2=0.96 and the agreement between the two techniques was 99%. DOSY experiments have proved to be accurate and reliable for estimating lipoprotein particle sizes and could serve as a valuable tool to improve CVD risk assessment.

64 Isotopic NMR Spectrometry as an Efficient Tool to Fight Against Counterfeiting: High Accuracy Requirement and Sensitivity Improvement

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Isotope ratio measurements used in forensic chemistry have mainly obtained by mass spectrometry (IRMS). However, this technique provides only the average contribution of a given element, hiding the internal isotopic distribution. Further valuable information for source attribution and for the authentication of a given molecule can be obtained by using isotopic NMR spectrometry, which provides intra-molecular isotope distributions. For the last 25 years, 2H NMR has been used to measure (2H/1H) ratios in many molecules and matrices and has effectively been applied in such areas as authentication, metabolism, and counterfeiting [1]. Very recently, isotopic 13C NMR has been validated [2]. While it is challenging due to the high level of accuracy required (better than 1&#65533;), this is a new and very promising approach to fight against counterfeiting in its broadest sense [3,4]. A new breakthrough has been achieved by using polarization transfer (INEPT in particular). A major modification of the sequence was the introduction of adiabatic schemes for the 180 deg pulse for inversion and refocusing on both channels 1H and 13C. Using this modified pulse sequence a huge reduction of the duration of the NMR analysis is achieved because (i) the polarization transfer leads to a sensitivity gain of around a factor 4 (γ1H/γ13C) and (ii) the repetition rate between transients is dictated by 1H T1 relaxation times that are much shorter than 13C T1 times [5]. Our latest work on authentication and on the pedigree of active molecules will be illustrated.

References:

1. Martin G.J., Martin M.L., Remaud G.S. SNIF-NMR part 3: From mechanistic affiliation to origin inference. Modern Magnetic Resonance, 1647-1658, (2006)Webb GA editor, Springer Berlin.
2. Caytan E., Botosoa E.P., Silvestre V., Robins R.J., Akoka S., Remaud G.S. Anal. Chem. 79, 8266-8269, 2007.
3. Silvestre V., Maroga Mboula V., Jouitteau C., Akoka S., Robins R.J., Remaud G.S. J. Pharm. Biomed. Anal. 50, 336-341, 2009.
4. Thomas F., Randet C., Gilbert A., Silvestre V., Jamin E., Akoka S., Remaud G., Segebarth N., Guillou C. J. Agric. Food Chem. 58, 11580-11585, 2010.
5. Thibaudeau C., Remaud G., Silvestre V., Akoka S. Anal. Chem. 82, 5582-5590, 2010.

65 Prediction of Chiral Sulfoxides and N-oxides Configuration by GIAO DFT Calculations

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Current achievements in calculation of NMR parameters (nuclear shieldings and spin-spin couplings) offer possibility to differentiate between all possible diastereoisomers when calculated data are compared with experimental [1]. Relative configuration can be in some cases derived directly from the NMR measurement using NOE and spin-spin coupling constants but in the case when these indicia are not easily available or missing then the calculation/experiment comparison approach might be the method of chioce. This is the case of the configuration determination of asymmetric S and N center in chiral sulfoxides and N-oxides.

We have already shown that selection of appropriate geometry optimization and NMR parameters calculation method is necessary for unambiguous results [2,3,4]. In this poster we would like to present application of the calculation/experiment comparison approach on real molecules containing chiral S and N centers. Examples of such compounds are sulfoxides derived from nonbornane derivatives with annealed tetrahydrothiophene ring and N-oxides of selected tropane derivatives.

The calculation/experiment comparison procedure includes optimization of the geometry of studied compound with respect to all possible conformers and then calculation of NMR parameters by selected method. If several possible conformers have to be considered, NMR parameters have to be weighted by corresponding distribution of the conformers. We have used B3LYP and OPBE functionals for geometry optimization and NMR parameters calculation, respectively.

The experimental NMR data were obtained by in situ oxidation of parent sulfides or amines with MCPBA in CDCl3 solutions in NMR tube. All 1H and 13C resonances were assigned using standard 1D and 2D (COSY, C,H-HSQC, C,H-HMBC and ROESY) methods. In case of amine N-oxides, the product of in situ oxidation is a protonized N-oxide that has to be taken into account when calculating NMR parameters.

References:

1. Smith S., Goodman J.: J. Am. Chem. Soc. 132(37), 12946-12959, 2010
2. Dracinsky M., Pohl R., Slavětinska L., Budesinsky M.: Mag. Reson. Chem. 48(9), 718-726, 2010
3. Pohl R., Dracinsky M., Slavětinska L., Budesinsky M.: Mag. Reson. Chem. 49(6), 320-327, 2011
4. Dracinsky M., Pohl R., Slavětinska L., Janků J., Budesinsky M.: Tetrahedron:Asymmetry, 22(3), 356-366, 2011

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66 Identification of Black Raspberry Polyphenols by High Field NMR and HPLC-ESI-MS/MS

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Polyphenolic-rich black raspberry fruit extracts have shown chemoprotective activity against oral, esophageal, colonic and rectal forms of aerodigestive cancers. However, correlating individual components with the total activity of berry extracts requires the identification of these constituents on the molecular level. Statistically based models that relate 1H nuclear magnetic resonance (NMR) spectra with biological activity against proliferation of HT-29 colon cancer cells permitted quantitative determination of relationships between activity and the NMR resonances. Comparisons of these resonances with spectra of HPLC fractions permitted the identification of biologically active components. A number of HPLC fractions were subjected to structure determination by 1D and 2D NMR and HPLC-ESI-MS-MS analyses. These studies confirmed that anthocyanin polyphenols, namely cyanidin 3-rutionside, cyanidin 3-xylosylrutinoside, cyanidin 3-glucoside, and cyanidin 3-sambubioside were important antiproliferative constituents of the extracts. A variety of non-anthocyanin phenolic compounds, including: hydroxybenzoic acids, hydroxycinnamic acids, quercetin, myricetin, ellagic acid, and their glycosylated and methoxylated derivatives were identified in the HPLC fractions. Our metabolomic analysis showed that some of these components also contributed to the activity of the fruit extract. Thus, our work demonstrates that statistical analysis can be combined with NMR and mass spectral techniques to analyze highly variable fruit extracts and provide valuable information when assessing their medicinal benefits.

67 Multi-scan Single Shot 2D NMR: a New Tool to Optimize Fast Quantitative Analysis

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2D NMR is a powerful tool for quantitative analysis of complex mixtures. The main drawback affecting 2D NMR experiments is their experimental duration, due to the time incrementation necessary to sample the indirect dimension. Moreover, when 2D NMR is used for quantitative measurements, a calibration procedure is required, thus increasing further the overall experiment time. This time limitation can be circumvented by the so-called Ultrafast 2D NMR methodology [1], allowing the acquisition of a 2D spectrum in a single scan, and thus in a fraction of a second. Recently, we have considerably improved the analytical performances of ultrafast 2D experiments, in terms of resolution, lineshape, sensitivity and spectral width [2,3]. However, the molecular concentrations available in complex biological mixtures are often not sufficient to obtain, in a single scan, the sensitivity required for a precise quantification and detection. Still, ultrafast signals can be accumulated in order to increase the sensitivity of these experiments while preserving reasonable experiment durations. But little is known about the sensitivity of ultrafast experiments versus conventional 2D NMR. A fair and relevant comparison has to consider the SNR ratio per unit of time, in order to determine the optimum choice: for a given experiment time (e.g. 1 h), should we run a conventional 2D experiment or is it preferrable to accumulate ultrafast acquisitions for 1 h?

To answer this question, we performed a systematic comparison between accumulated, optimized ultrafast experiments and conventional acquisitions, for different conditions and pulse sequences. Assuming that the sensitivity of 2D experiments is limited by the dimension where the SNR has the lowest value, it was found that the sensitivity per unit of time is much higher in ultrafast NMR (e.g. by a factor of 5 for COSY). This result is mainly attributed to the absence of t1 noise in ultrafast experiments and highlights the interest of accumulating ultrafast signals instead of conventional ones. This new multi-scan single shot approach offers a very high flexibility and can be easily implemented on standard spectrometers. Analytical aspects of this promising quantitative methodology and applications to quantitative analysis of metabolic samples [4] will be presented.

1. L. Frydman, T. Scherf, A. Lupulescu, Prod. Natl. Acad. Sci. USA, 99 (2002) 15858-15862.
2. P. Giraudeau, S. Akoka, J. Magn. Reson., 205 (2010) 171-176.
3. P. Giraudeau, S. Akoka, Magn. Reson. Chem., 49 (2011) 307-313
4. P. Giraudeau, S. Massou, Y. Robin, E. Cahoreau, J.-C. Portais, S. Akoka, Anal. Chem., 83 (2011), 3112-3119.

68 Alternative Approaches to Hydrogen Atom Location in the Solid State

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Different strategies for determining the position of hydrogen atoms in the crystalline solid state have been investigated. A comparison was made of molecular structures determined using both X-ray and neutron diffraction data, and these were used to calculate solid state NMR parameters to compare with experimental values. To achieve these aims, high quality X-ray diffraction data were collected. Various techniques were applied to determine the hydrogen atomic parameters which were compared to those determined from neutron diffraction.

Hydrogen bond vectors showed excellent correlation. However, hydrogen bond lengths determined by X-ray diffraction data were shorter than those from neutron diffraction [1]. A systematic relationship was found between the distances determined by both methods and that a linear function can be used to adjust X-ray bond lengths to neutron distances. These corrected structures were successfully used in density function theory (DFT) calculations to give an improved prediction of the experimental NMR spectra. Geometry optimizations [2] were carried out using DFT energy minimizations. Optimizations of; all atoms and only hydrogen atoms (keeping the heavy atoms fixed) were carried out, using the X-ray and neutron structures as starting models, and the resulting structures were used as the basis for NMR predictions [3]. Allowing only the hydrogen atoms to relax gave the best fit to the experimental NMR. The results show that optimizing hydrogen positions from X-ray structures using DFT methods gives the best model to predict experimental NMR spectra, however using a simple (and far less computationally expensive) correction to X-ray structure hydrogen positions allows NMR spectra prediction that on average are as good as prediction from a neutron diffraction study.

1. Taylor R, Kennard O, Acta Cryst. B39, 133-138, 1983.
2. Yates JR, Pickard CJ, NMR Crystallography, Chapter 3, John Wiley & Sons Ltd: Chichester, 29, 2009.
3. Harris RK, Hodgkinson P, Pickard CJ, Yates JR, Zorin V, Mag. Res. Chem., 45, S174-186, 2007.

69 Spectral Aliasing and Homonuclear Decoupling in F1: Two Strategies to Increase the Resolution in 2D NMR Spectra

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Sensitivity and low resolution in F1 are two long-standing problems especially in 1H-13C heteronuclear spectra where the carbon window is typically 250 kHz broad. Among the numerous techniques aiming at increasing the resolution in F1, one of the most simple consists in the optimization of the sampling of the F1 dimension by spectral aliasing [1,2]. The problem is that high resolution makes little sense if scalar couplings disperse the signals over a large number of weak transitions. It is therefore preferable to be able to decouple all scalar interactions [3] except when their measurement is needed.

Heteronuclear decoupling techniques have been available for quite some time, but eliminating homonuclear interactions is a much more difficult challenge. The first broadband homodecoupling based on spatial encoding was introduced by Zanger and Sterk for 1D proton spectra and extended to COSY and DOSY experiments by James Keeler and Gareth Morris respectively. In order to address the specific problem of 13C-enriched small molecules, we introduced a generally applicable broadband 13C-homodecoupled HSQC experiment (BBHD-HSQC) [4] which effectively eliminates 13C-13C couplings in fully 13C labeled molecules. It was successfully applied to enriched cholesterol. Solutions to overcome the intrinsically low sensitivity of spatially encoded sequences will be discussed.

1. Jeannerat, D., Rapid multidimensional NMR: high resolution by spectral aliasing, Encyclopedia of Magnetic Resonance, Wiley
2. Foroozandeh, M., Jeannerat, D., ChemPhysChem, 11, 12, 2503-2505, 2010
3. Shaka, A.J., Keeler, J., Frenkiel, T., Freeman, R., J. Magn. Reson., 52, 335-338, 1983
4. Foroozandeh, M., Giraudeau, P., Jeannerat, D., ChemPhysChem, in press, 2011

70 Conformational Preferences of Prolinol (Ether) Enamines

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Enamine key intermediates [1] in organocatalysis, derived from aldehydes and Jorgensen/Hayashi-type prolinol [2] or prolinol ether catalysts, [3,4] were investigated conformationally [5] in different solvents by means of NMR spectroscopy in order to provide an experimental basis for a better understanding of the origin of stereoselectivity. Owing to the bulkiness of the pyrrolidine-substituent, the enamines of diarylprolinol (ether) catalysts exist exclusively in the s-trans conformation, while a prolinol enamine is shown for the first time to partially populate the s-cis conformation in solution. In all enamines studied and in contrast to the free catalysts, the pyrrolidine ring is found to adopt the down conformation. In the case of diarylprolinol ether enamines, exclusively the sc-exo conformation around the exocyclic Cα-Cε bond is observed, which is stabilized by CH/π interactions. In contrast, diarylprolinol enamines adopt the sc-endo conformation allowing for an OH...N hydrogen bonding and a CHπ interaction. A rapid conformational screening method for these conformational enamine features was developed and applied to show their generality for various catalyst, aldehyde, and solvent combinations. Thus, by revealing unexpectedly pronounced conformational preferences of prolinol and prolinol ether enamines in solution, our study provides for the first time an experimental basis for discussing the previously controversial questions of s-cis/s-trans and sc-endo/sc-exo conformation of prolinol and prolinol ether enamines. [5,6] The presented conformational preferences are in agreement with the experimental results from synthetic organic chemistry. [3-7] Our results are therefore expected to have a significant impact for future theoretical calculations and synthetic optimizations of asymmetric prolinol (ether) enamine catalysis.

References:

1. Schmid M. B., Zeitler K., Gschwind R. M., Angew. Chem. Int. Ed., 49, 4997-5003, 2010
2. Lattanzi A., Chem. Commun., 1452-1463, 2009
3. Marigo M., Wabnitz T. C., Fielenbach D., Jorgensen K. A., Angew. Chem. Int. Ed., 44, 794-797, 2005
4. Hayashi Y., Gotoh H., Hayashi T., Shoji M., Angew. Chem. Int. Ed., 44, 4212-4215, 2005
5. Schmid M. B., Zeitler K., Gschwind R. M., Chem. Sci, accepted, 2011
6. Groselj U., Seebach D., Badine D. M., Schweizer W. B., Beck A. K., Krossing I., Klose P., Hayashi Y., Uchimaru T., Helv. Chim. Acta, 92, 1225-1259, 2009
7. Mager I., Zeitler K., Org. Lett., 12, 1480-1483, 2010

71 Formation and Stability of Prolinol (Ether) Enamines

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Enamine key intermediates in organocatalysis, derived from aldehydes and Joergensen-Hayashi-type prolinol or prolinol ether catalysts, [1-5] were generated in different solvents and investigated by NMR spectroscopy. Depending on the catalyst structure, trends for their formation and amounts are elucidated. For prolinol catalysts, the first enamine detection in situ is presented and the rapid cyclization of the enamine to the oxazolidine ("parasitic equilibrium") is monitored. [6] In the case of diphenylprolinol, this equilibrium is fully shifted to the kinetic endo-oxazolidine ("dead end") by the two geminal phenyl rings most probably because of the Thorpe-Ingold-effect. [7-8] With bulkier and electron-withdrawing aryl rings, however, the enamine is stabilized relative to the oxazolidine allowing for the parallel detection of the enamine and the oxazolidine. In the case of prolinol ethers, the enamine amounts decrease with increasing sizes of the aryl meta-substituents and the O-protecting group. In addition, for small aldehyde alkyl chains, Z-configured enamines are observed for the first time in solution. Prolinol silyl ether enamines are evidenced to undergo slow desilylation [9-11] and subsequent rapid oxazolidine formation in DMSO. For unfortunate combinations of aldehydes, catalysts, solvents, and additives, the enamine formation is drastically decelerated, but can be screened for by a rapid and facile NMR approach. Altogether, especially by clarifying the delicate balances of catalyst selectivity and reactivity, our NMR spectroscopic findings can be expected to substantially aid synthetically working organic chemists in the optimization of organocatalytic reaction conditions and of prolinol (ether) substitution patterns for enamine catalysis.

1. Chi Y., Gellman S. H., Org. Lett. 2005, 7, 4253-4256.
2. Marigo M., Wabnitz T. C., Fielenbach D., Joergensen K. A. , Angew. Chem. Int. Ed. 2005, 44, 794-797.
3. Hayashi Y.,Gotoh H.,Hayashi T.,Shoji M., Angew. Chem. Int. Ed. 2005, 44, 4212-4215.
4. Palomo C.,Mielgo A., Angew. Chem. Int. Ed. 2006, 45, 7876-7880.
5. Mielgo A.,Palomo C., Chem. As. J. 2008, 3, 922-948.
6. Schmid M. B. ,Zeitler K., Gschwind R. M., J. Am. Chem. Soc. 2011, 133, 7065 - 7074
7. Beesley R. M., Ingold C. K., Thorpe J. F., J. Chem. Soc., Trans 1915, 1080 - 1106.
8. Bachrach S. M., J. Org. Chem. 2008, 73, 2466 - 2468.
9. Groselj U., Seebach D., Badine D. M., Schweizer W. B., Beck A. K., Krossing I., Klose P., Hayashi Y., Uchimaru T., Helv. Chim. Acta 2009, 92, 1225 - 1259.
10. Varela M. C., Dixon S. M., Lam K. S., Schore N. E., Tetrahedron 2008, 64, 10087 - 10090.
11. Alza E., Pericas M. A., Adv. Synth. Catal. 2009, 351, 3051 - 3056.

72 Complete NMR Spectral Analysis - The Golden Standard of Structure Verification?

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NMR spectroscopy provides valuable information for the verification of molecular structures. NMR parameters (chemical shifts and coupling constants) extracted from most easily available 1D-1H spectra already provide comprehensive structural information at least for proton rich structures.

However, spectral overlap and higher order effects often make it difficult to extract these NMR parameters. NMR spectral analysis based on a quantum mechanical optimization of NMR parameters to match the actual ones in the experimental data is a classic approach to handle this problem and used to be “the golden standard of NMR spectral analysis”. We now present a highly automated procedure extracting the actual NMR spectral parameters from 1D-1H spectra even with overlapping signals and higher order effects optionally also utilizing HSQC-information. By evaluating the similarity of these actual spectral parameters to the predicted ones the procedure can make a safe assessment on the consistency between structural and spectral data. We present the workflow and examples illustrating the power of this approach.

73 Calculations of Solid-State NMR Parameters of Isocytosine and Sesquiterpene Lactones

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Isocytosine crystallizes as a 1:1 ratio of two tautomers in a manner similar to that of the guanine and cytosine pairs in DNA. The experimental solid-state NMR chemical shifts of crystalline neutral 15N labeled isocytosine were compared with those calculated by three different methods: (1) calculations on isolated molecules, (2) calculations on isocytosine clusters of various sizes, and (3) infinite crystal calculations, that is, the gauge including projector-augmented wave (GIPAW [1]) method. The data obtained with the GIPAW method were in best agreement with the experimental data. [2] The GIPAW method was also used for calculations of solid-state 13C chemical shifts of a series of sesquiterpene lactones. Two polymorphs of helenalin and aromaticin were studied. In the asymmetric unit cells of geigerinin and badkhysin, two geometrically different molecules are present. The experimental differences in 13C chemical shifts between the polymorphs and between the geometrically different molecules were reproduced very well with the GIPAW calculations.

References:
1. Pickard, C. J.; Mauri, F. Phys. Rev. B, 6324, 245101, 2001.
2. Dracinsky, M.; Jansa, P.; Ahonen, K.; Budesinsky, M. Eur. J. Org. Chem., 1544-1551, 2011.

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74 NMR Fragment Screening Against Human Galectin-7

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Fragment-Based Drug Discovery (FBDD) is a powerful strategy to lead identification and optimization. It represents an efficient alternative to high-throughput screening, based on the fact that a small set of chemically diverse molecules of low molecular weight, called fragments, covers a wider portion of the chemical diversity space than a much larger collection of heavier, drug-sized compounds. [1,2]

In an FBDD project, a small library of fragments, typically ranging from a few hundreds to a few thousands, is screened against a target through a variety of biophysical and/or biochemical techniques, yielding a moderate rate of weak-interacting fragment hits.

NMR represents the dominant biophysical technique for fragment screening, given its reliability and versatility. Ligand-detected methods, such as saturation transfer difference (STD), allow inferring very weak interactions between fragment hits and the target, whereas protein-detected methods, such as heteronuclear single quantum coherence (HSQC) provide information on the target binding epitopes.

Galectin-7, also referred to as the p53-induced gene 1 product, is a pro-apoptotic lectin which has been correlated with cancer cell malignancy in a number of cancer models, including thymic lymphoma and mammary carcinomas. [3]

Here, we report an NMR-driven fragment screening process against human galectin-7 combining STD, 1H-15N HSQC and ligand-competition experiments, as well as molecular modeling.

1. Erlanson D., McDowell RS. and O'Brien T., J Med Chem., 47, 3463-3482 (2004)
2. Hajduk PJ. and Greer J., Nat Rev Drug Discov., 6, 211-219 (2007)
3. Saussez S., and Kiss R., Cell Mol Life Sci., 6, 686-697 (2006)

75 Evaluation of Automated Structure Verification for Industrial Purposes

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Automated structure verification is considered as a tool to reduce unnecessary NMR expert activity in analytical core service groups. Open access users should be supported by trustful software for identifying correct, doubtful or incorrect structures. A larger number of compounds from different sources are investigated and the scores manually revised by our NMR experts. The results and suggestions will be reported to the provider, for achieving a higher reliability of the software. Avoidance of false-positive ratings is in the focus of the study. The objective of this evaluation is to adapt programs for the needs in a pharmaceutical company.

76 A Novel Design for High Pressure NMR Compatible with Commercial NMR Probes

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This research covers the design and performance of a high pressure NMR sample cell that is compatible with standard, commercially available liquids NMR probes. The sample cell reproducibly holds pressures over 3 kbar and is therefore recommended for use at up to 1 kbar for normal operations. The 3 mm internal diameter of the sample cell provides 50% of the sample volume of standard, commercial glass 5 mm NMR tubes and four times the sample volume of other closed end NMR cells published in the literature capable of comparable operating pressures. Finite element analyses plus extensive experimental burst pressure measurements were used to demonstrate that this design is able to withstand elevated pressure across a wide temperature range (-50oC to 100oC) for prolonged periods with no evidence of material fatigue. In addition, it is compatible with a wide array of standard NMR solvents.

77 Elucidating "Undecipherable" Chemical Structures Using Computer Assisted Structure Elucidation Approaches

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In parallel with the development of new 2D-NMR techniques, the last two decades have seen the development of new approaches for Computer-Assisted Structure Elucidation (CASE). Starting from a number of pioneering works published in the late 1960s, researchers created several generations of CASE expert systems. Nowadays expert systems are powerful analytical tools capable of assisting in the elucidation of very complex molecular structures. These systems adequately mimic the systematic reasoning of spectroscopists but markedly outperform the human expert in logical-combinatorial reasoning. Experience accumulated in the application of expert systems show that CASE methods can dramatically accelerate the procedure of structure elucidation, provide improved reliability of results and, consequently, can save researchers significant amounts of time.

This work will investigate two recent examples from the literature that were deemed by the authors as impossible or too difficult to elucidate using traditional methods of 1D and 2D NMR spectra structural interpretation. The application of Computer Assisted Structure Elucidation on these examples will be explored and the CASE results will be presented and explained.

It was demonstrated that the application of a CASE approach allowed solving both problems quickly and reliably. We conclude that a modern CASE expert system should be considered as an integral part of a spectroscopist's armory for quick and reliable structure elucidation. It is now impossible to evaluate the capabilities of NMR experimental techniques in isolation from powerful mathematical aids developed for 2D NMR data analysis. We believe that in future CASE software will become a common tool for NMR spectroscopists to apply, much like the software that is today an integral part of X-ray crystallography.

78 Detection of Adulterated Natural Product Extracts Containing Sildenafil (Viagra) Derivatives

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Adulteration of natural products with sildenafil (Viagra), sulfoaildenafil, tadalafil, vardenafil or other derivatives is common. To elude detection, adulterators regularly change the derivative supplied in the natural product sample in hopes that the presence of such adulterants go undetected due to the use of analytical methods using targeted analysis. Regenerect, which targets an audience for erectile dysfunction, was voluntarily recalled in April 2011 as a result of a FDA lab analysis detecting Sulfoaildenafil in two lots (100521 and 112850). In this work, we evaluated Regenerect samples, both from a recalled lot and unrecalled lot, using 1H NMR spectroscopy. Lot to lot comparisons, detection and quantification of sulfoaildenafil and component reconstruction was undertaken. Methods for automated detection of sildenafil derivatives through substructure analysis were appraised. Evaluation by NIR and IR was also performed.

79 Particle Size Evaluation and Physical State Characterization of Pharmaceutical Systems by Solid-state NMR

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The purpose of this study was to use solid-state nuclear magnetic resonance (SSNMR) to evaluate the particle size range present in a sample as well as its physical state (amorphous or crystalline).

Samples of various particle sizes were prepared using sieving, spray drying and precipitation. As-received salicylic acid was sieved to separate particles based on their size. Various concentrations of salicylic acid in methanol (0.5-5%) were spray dried to generate particles of different sizes. Niflumic acid was dissolved in methanol and precipitated surfactant-containing water under sonication and subsequently freeze-dried to generate particles in the nanometer size range. Dicumarol was cryoground and ball milled for 8 and 10 min respectively. Differences in thermal behavior between the as-received and processed materials were assessed by differential scanning calorimetry. SSNMR proton spin lattice relaxation (1H T1) times were measured and SEM images of the materials were obtained.

The 13C SSNMR spectra of the samples showed that all the processed samples were still crystalline. The 1H T1 values of the sieved salicylic acid material ranged from 3300s for the smallest fraction to 6000s for the largest fraction, with a linear trend in relaxation times versus particle size. The spray-dried samples were best described with a two-component 1H T1 fit, with a long 1H T1 value on the order of hundreds of seconds and a short 1H T1 value on the order of tens of seconds. The precipitated niflumic acid also had two 1H T1 values differing by an order of magnitude. The cryoground and ball-milled dicumarol samples had 1H T1 values two orders of magnitude shorter than the as-received material.

1H T1 relaxation time values decreased with decreasing particle size for salicylic acid, niflumic acid and dicumarol. The relaxation times measured were determined to correlate with the particle sizes present in the sample. The particle size-relaxation time correlation allows the use of SSNMR as a powerful characterization technique of both the physical state and the particle size of the API. This is particularly appealing since SSNMR can be used to study the API within a formulation.

80 Cytotoxic Constituents of Exophiala Xenobiotica, Fungus Isolated from Deep-see Hydrothermal Vents

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Deep-sea hydrothermal vents have scarcely been investigated for biologically active natural products.[1] Nevertheless, the diversity and also density of deep-sea vent organisms suggest that they should be considered as new source for natural products discovery.[2] Therefore, to maximize the diversity of natural product chemotypes isolated in our laboratory, we have collected and cultured fungi from deep-sea hydrothermal vents. Among the isolated organisms, the fungus Exophiala xenobiotica was identified, whose presence in the deep-sea environment has been reported previously.[3] Fungi from the genus Exophiala are best known for causing cutaneous and subcutaneous infections in humans.[4] Despite their pathogenic character, other representatives of the genus Exophiala have already been investigated for the production of novel secondary metabolites.[5-8] This has lead to the discovery of new benzodiazepine alkaloids, a chromone dimer, indole alkaloids, tritepenes and sterols.[5-8]

The Exophiala xenobiotica cultured in our laboratory, was isolated from a microbial mat sample collected near Gollum Vent in the caldera of Axial Volcano (1,541 m depth, Juan de Fuca Ridge, Pacific Ocean). The organism was grown in six different media to determine the optimal fermentation conditions for secondary metabolite production. After four weeks, the fungal mycelium and culture filtrate from each medium were extracted separately and tested at 30 μg/mL for cytotoxicity to neuro 2a mouse blastoma and NCI-H460 lung cancer cells. Only an extract of the culture filtrate from the fungus grown in FR-23 media (high hydrocarbon content) showed activity, causing 52% lethality to neuro 2a cells and 71% lethality to NCI-H460 cells. Bioassay-guided fractionation of the extract of a 2 L culture yielded 0.3 mg of a new sulfur-containing chromone, the structure elucidation of which was made challenging by a low proton-carbon ratio. Nevertheless, assignment of the structure was greatly facilitated by experiments acquired on a 13C cryogenic probe (at 700 MHz). NMR techniques including quantitative 1H NMR, 2D HSQC and diffusion NMR experiments using both standard BBO 5 mm and capillary NMR flow probes are being compared for use in determining optimal growth conditions and incubation times for the production of this and related minor chromone metabolites. Future work includes cultivation of the fungus on 13C-labeled substrates to afford 13C-labeled chromones for which 13C-13C COSY data may be obtained to confirm/assign minor congeners.

1. Pettit, R. K., Mar. Biotechnol. 13, 1-11, 2011
2. Thornburg, Ch. C., Zabriskie, M., McPhail, K.L, J. Nat. Prod. 73, 489-499, 2010
3. Singh, P., Raghukumar, Ch., Verma, P., Schouche, Y., Fungal Diversity, 40, 89-102, 2010
4. Calvo, E.; Pastor, J.; Guarro, J. J. Antimicrob. Chemoter. 65, 1455-1459, 2010
5. Li, F., Li, K., Li, X., Wang, B., Chinese Journal of Oceanology and Limnology. 29, 63-67, 2011
6. Zhang, D., Yang, X. et al., J. Antibiot. 61, 40-42, 2008 7. Hussain, H., Krohn, K., Draeger, S., Schulz, B., Heterocycles. 74, 331-337, 2007
8. Kindler, B. L. J., Kraemer, H-J., et al., Eur. J. Org. Chem. 2084-2090, 2010

81 Curcumin and Diacetylcurcumin: Theoretical NMR Chemical Shifts and its Correlation with Liquid and Crystalline Solid State NMR

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Curcumin is a fascinating molecule with a variety of biological effects and great potential for pharmacological applications. At the molecular level, it has several single bonds and shows various degrees of rotational freedom, active in the liquid phase but restricted either at low temperatures or in the solid state.

In the present work, we carried out the solid state CP-MAS NMR spectra of curcumin and diacetilcurcumin (DAC). The latter exhibits two isomorphs which could be analyzed separately by NMR and x-ray crystallography.

Furthermore, we analyzed the rotational barrier among several possible geometrical arrangements of curcumin, and their relative populations have been calculated using the Boltzmann approach. Therefore, using minimum energy geometries we have calculated the chemical shift tensors using the GIAO aproximation at DFT level of theory. Results were compared with experimental liquid state data measured in different solvents.

In the solid phase, (DAC) showed different unit cells being analyzed as P21 and P21/n asymmetric units, with two and four molecules per asymetric unit, respectively. For comparative purposes chemical shifts tensors were calculated using geometries from x-ray data for single molecules in the two unit cells. Here, we considered it convenient to analysis the effect of interactions among molecules in the assymetric unit. Two and four molecules in the P21 and P21/n cells were also used to calculate the shift tensors. These results were compared with the solid state CP-MAS NMR spectra of samples.

Also, the interactions between molecules in these phases were analyzed using Natural Bonding Orbital analysis derived from the calculation of chemical shifts, so the possible contributions attributed to magnetic effects and electronic perturbations were analyzed to allow the order assignment of specific molecular orbitals. The CP-MAS NMR spectra and the x-ray structure of each DAC isomorph are discussed.

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82 NMR Studies on a D-Glucosamine-Based Macrocycle

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Macrocycles are important class of compounds due to biological activities [1]. This work reports the preparation of a 12-membered macrocycle by Bu3SnH/AIBN-mediated radical carbocyclization [2] of a suitable N-2-iodobenzoyl-D-glucosamine intermediate [3]. The main signals in the H-1 NMR spectrum that were critical to the establishment of the cyclization were two double doublets at 3.24 ppm and 3.57 ppm and a centre overlapped double doublet at 5.35 ppm composing an ABX spin system. Moreover, the C-13 NMR spectra showed two signals at 34.7 ppm and 47.9 ppm that were promptly assigned. So far, the regioselectivity was still unresolved. The values of these chemical shifts as the so- le explanation for discriminate between the two possible cyclisation pathways i.e. 12-exo-trig and 13-endo-trig that will lead to different macrocycles would not be an unequivocal statement. HMBC experiment was fundamental for this establishment. In the HMBC we found typical correlations that certify the linkage between the ortho carbon of the benzamidic aromatic ring to the alpha-carbonyl olefinic carbon at the cinnamoyl moiety. Other main correlations confirm the 12-exo-trig cyclisation process. Furthermore, a benzylic stereogenic center that was created could generate two diastereomers. Nevertheless, radical reactions are highly stereoselectives [4] and because of the purity of the compound and the high quality of the NMR spectra, it can be expected only one diastereomer. The facial diastereoselectivity should be inspected by nOe's experiments. The macrocycle in DMSO-d6 was submitted to NOESY and ROESY experiments at 400 MHz. The NOESY experiment showed negative nOe's for the bulky molecule and positive nOe's for the more mobile benzyl group. These results were not conclusive about the facial diastereoselectivity because the lack of nOe's does not mean that the nuclei are far apart. The same result was observed in CDCl3. Fortunately we measured qualitative nOe's at another different NMR field. So, DMSO-d6 and CDCl3 solutions of the macrocyclic compound were evaluated by NOESY at 600 MHz. The experiment with the CDCl3 solution is not conclusive. On the other hand, the NOESY experiment performed on the DMSO-d6 sample with 400 ms for mixing time, showed a very discrete nOe between the benzylic hydrogen and H-2 from the sugar moiety, attesting for the abso- lute configuration at the benzylic carbon. For this molecule all these dependences were verified, it means, the very mobile system in CDCl3 with positive nOe's, the highly viscous DMSO-d6 solutions with negative nOe's and the field strength dependence, 9.4 T or 14.1 T.

1. Kohli R. M.; Walsh C. T. Chem. Comm., 3, 297, 2003
2. Beckwith A. L. J.; Bowry V. W.; Bowman W. R.; Mann E.; Parr, J.; Storey J. M. D. Angewandte Chemie, International Edition, 43(1), 95, 2004
3. Pires M. C. Dissertacao de Mestrado, Faculdade de Farmacia, UFMG, 2009
4. Srikanth G. S. C.; Castle, S. L. Tetrahedron, 61(44), 10377, 2005 FAPEMIG, CAPES, PRPq-UFMG, LAREMAR, BRUKER-BIOSPIN

83 Structural Studies of Small Cationic Antimicrobial Peptides by NMR and MD: a Refined Pharmacophore Model Able to Predict Activity vs. Staphylococcus Aureus

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Synthetic antimicrobial peptides (SAMPs) with increased stability against proteolytic degradation and good efficacy and selectivity for methicillin-resistant staphylococci over human erythrocytes have been developed over the last decade1,2. The pharmacophore of this class of molecules is intimately related to its general mode of action. This relationship has been investigated by a by liquid NMR and computer simulations both in pure solvent and in small unilamellar vesicles (SUVs)3. The role of the three dimensional structure in general, and the role of the positively charged arginines in particular, in solution as well as inside the lipid membrane and its effect on the solvatization energy of the SAMPs have been studied, resulting in a new refined pharmacophore able to successfully predict the efficacy of other antibiotics targeting the bacterial membrane, even ones not belonging to the peptide class.

References:

1. Strom, M. B.; Haug, B. E.; Skar, M. L.; Stensen, W.; Stiberg, T.; Svendsen, J. S. The pharmacophore of short cationic antibacterial peptides. J Med Chem 2003, 46, 1567-70.
2. Haug, B. E.; Strom, M. B.; Svendsen, J. S. The medicinal chemistry of short lactoferricin-based antibacterial peptides. Curr Med Chem 2007, 14, 1-18.
3. Isaksson, J.; Brandsdal, B. O.; Engqvist, M.; Flaten, G. E.; Svendsen, J. S. M.; Stensen, W. A Synthetic Antimicrobial Peptidomimetic (LTX 109): Stereochemical Impact on Membrane Disruption. Journal of Medicinal Chemistry Article ASAP, DOI: 10.1021/jm200450h.

84 ALPESTRE Processing of 2D J-resolved NMR Spectra

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The standard way of acquiring and processing 2D homonuclear J-resolved NMR data produces magnitude mode spectra [1,2]. Solutions have recently been proposed to improve the resolution of these spectra through the obtention of pure absorption peaks [3]. Such a goal can also be reached by processing usual spin echo data sets with the ALPESTRE (A Linear Prediction Estimation of Signal Time REversal) algorithm [4]. ALPESTRE reconstructs the experimentally inaccessible data that corresponds to negative t1 values by linear prediction. The Fourier transformation of the hybrid (acquired + reconstructed) data set produces the expected result.

The first implementation of ALPESTRE relied on GIFA, the precursor of the NMRnotebook (NMRtec, Illkirch-Graffenstaden, France) NMR processing software. The linear prediction routine was later recoded in Octave (a free Matlab clone) language. More recently, a Python script was added to glue the Octave code with the GUI of the Bruker TopSpin software, thus making it easy to use ALPESTRE (www.univ-reims.fr/LSD/JmnSoft/Alpestre).

A new resolution enhancement step was introduced before the final Fourier Transformation in dimension 1. A family of time-domain filter functions was designed in order to reduce the spectral line width along F1. The severity of the filter can be controlled by two parameters.

The ALPESTRE transformation procedure interestingly reveals the relative signs of the peaks in a 2D J-resolved spectrum. Assuming that "regular" peaks have a positive sign, a part of the strong coupling artifacts show up with a negative sign. The observation of negative peaks helps to identify the columns of the 2D spectrum that result from strong coupling. This is demonstrated on the ABC spin system of serine [5], an example for which experimental and simulated data are compared.

1. Aue W.P., Karhan J., Ernst R.R., J. Chem. Phys., 64, 4226-4227, 1976
2. Bax A., Freeman R., Morris G.A., J. Magn. Reson. 43, 333-338, 1981
3. Pell A.J., Keeler J., J. Magn. Reson., 189, 293-299, 2007
4. Nuzillard J.-M., J. Magn. Reson. A, 118, 132-135, 1996
5. Wider G., Baumann R., Nagayama K., Ernst R.R., Wuthrich K., J. Magn. Reson., 42, 73-87, 1981

85 Optimized Process for Validation of NMR Fragment Library

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Over the 15 past years, fragment-based drug discovery (FBDD) has become a well established component of new drug discovery process in pharmaceutical industries [1]. Among the different techniques, NMR has proved to be a method of choice [2]; since the introduction of NMR as screening technique pioneered by Abbott [3], several NMR techniques have emerged. The ligand-based approaches which do not require labeled material have gained increasing attention, and among them STD [4] and WaterLogsy [5] have become very popular and widely used. The major advantage of these techniques over other screening approaches is their high sensitivity and robustness to the detection of low affinity binders, classically observed with fragments.

As binding evidence is brought by direct observation of ligand signals, ligand-detected NMR screening is more stringent than other techniques regarding fragments. Success of NMR screens strongly depends on the quality of the library. NMR validation of fragments and cocktails is essential besides thoughtful design of the library [6]. In this context, additionally to integrity and purity check, and assessment of aqueous solubility, correct NMR behavior of fragments in the screening conditions has to be assessed, especially with respect to aggregation.

In this poster, we present an optimized protocol for validation of NMR fragment library. We have developed an NMR approach where all the chemical, physicochemical, and NMR criteria are checked in a single experiment. This centralized procedure allows for simplified workflow, along with a saving of sample. The experimental protocol will be described and illustrated by several examples.

1. Hajduk P.J and Greer J, Nature Rev. 6, 211-219, 2007.
2. Dalvit C, DDT 14, 1051-1057, 2009.
3. Shuker S.B, Hajduk P.J, Meadows R.P, and Fesik S.W, Science 274, 1531-1534, 1996.
4. Meyer B and Peters T, Angew Chem int Ed 42, 864-890, 2003.
5. Dalvit C, Fogliatto G, Stewart A, Veronesi M and Stockman B, J Biomol NMR 21, 349-359, 2001.
6. Lepre C, DDT 6, 133-140, 2001.

86 Probing Small Molecule Aggregation using NMR Spectroscopy

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The (self) association of small molecules in solution has implications across a wide variety of disciplines, from influencing optical properties in liquid crystal science to complications of non-specific binding in ligand screening experiments [1,2,3]. Investigating this aggregation is therefore vital in understanding the solution behavior of a range of important small molecules.

Recently, we have used pulsed field gradient NMR spectroscopy to probe the aggregation states of the azo-dye Sunset Yellow [4]. These results show significantly larger assemblies compared to those observed by optical and x-ray scattering techniques.

We present here some preliminary results from a number of new approaches being developed to investigate small molecule aggregation phenomena. The use of size-exclusion chromatographic stationary phases within the NMR tube [5] acts to perturb the equilibrium between the various aggregate states and hence provides information on sizes of assemblies and the thermodynamics of the self association, akin to equilibrium sedimentation experiments. Small molecule probes with reporter nuclei such as 19F or 31P are being utilized as minimally perturbing "spies" to circumvent problems of spectral complexity upon aggregation, allowing detailed analysis of diffusion and relaxation behavior as probes of assembly.

References:
1. Selkoe, D. J.; Nature, 426, 900-904, 2003
2. Whitesides, G. M.; Mathias, J. P.; Seto, C. T.; Science, 254, 1312-1319, 1991
3. Lydon, J.; Curr. Opin. Colloid. In. Sci. 8, 480-490, 2004
4. Renshaw, Matthew P.; Day, Iain J.; J. Phys. Chem. B 114(31), 10032-10038, 2010
5. Joyce, Rebecca E.; Day, Iain J.; J. Phys. Chem. B, In preparation

87 The Utility of Non-Uniform Sampling in 2D-NMR Analysis of Small Molecules

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Structural characterization of drug compounds via two-dimensional (2D) NMR has become a standard in the pharmaceutical industry. However, because of the number of data points required in the f1 dimension, these experiments often have long acquisition times, and are limited in sensitivity and/or resolution. Reducing the required number of f1 data points by using a non-uniform sampling (NUS) approach coupled with multi-dimensional decomposition (MDD) has been used with protein and peptide samples to enhance resolution and decrease the acquisition time of multidimensional experiments. In 2D and 3D experiments, this model can result in a 75% reduction in acquisition time. Much of these gains can also be realized in the analysis of small molecules. The reduction in acquisition time by using NUS-MDD can be utilized to promote higher sample throughput (through a reduction in total acquisition time), increased sensitivity (by using more pulses per f1 data point) or increasing resolution by adding more data points in the f1 dimension. These three approaches have been evaluated with several structurally diverse small molecules using common homonuclear and heteronuclear experiments. Complete 1H-1H COSY, 1H-13C HSQC and 1H-13C HMBC data sets can be acquired, using 50 mM (1-5 mg) samples, in under twenty minutes of acquisition time. When NUS-MDD data sets are compared with data sets acquired using traditional methods, comparable integration and sensitivity is achieved in 50% less acquisition time. Spectra generated using non-uniform sampling (25% of a standard acquisition) methods with 1024 experiments in the f1 dimension were comparable to spectra, acquired similarly, using traditional methods. While this level of resolution in the indirect dimension is not always needed, the enhanced resolution greatly aids in the interpretation of spectra with overlapping resonances. The one obvious detriment of using the NUS-MDD approach is computational time. Using current computers, processing times for traditionally acquired 2D spectra are almost instantaneous while processing time for NUS acquired 2D spectra can take from five to twenty minutes.

88 Detection of 1H-1H Proximities in Small Organic Solids through Imperfect Homonuclear Dipolar Decoupling

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Despite protons are the nuclei of choice for NMR studies due to their high natural abundance and magnetogyric ratio, strong homonuclear dipolar couplings have for long time limited the access to high resolution 1H spectra in solid samples. Recent advances in hardware and pulse sequence development have allowed to partially overcome the problem, opening the access to the detection of 1H-1H correlations in solids. Notably, beyond Double-Quantum (DQ) experiments, the most successful experimental methods are based on NOESY-type spin diffusion pulse schemes, in which 1H-1H proximities can be estimated following the transfer of z-magnetization between dipolar-coupled nuclei [1]. In these experiments, two periods of single-quantum (SQ) coherence evolution (usually in the presence of homonuclear-decoupling schemes or fast MAS conditions) are separated by a mixing time during which spin diffusion occurs [2,3]. We discuss here the possibility of exploiting imperfections in homonuclear amplitude-modulated decoupling schemes as an alternative approach to obtain information on 1H-1H proximities in small organic solids through the observation of SQ-SQ correlations among protons. In particular, the method proposed combines windowed-PMLG homonuclear decoupling [4] and DQ filtering under moderate MAS conditions to generate the weak recoupling conditions [5]. The possibility of extending these principles to the selective measurement of 1H-1H distances is also discussed.

1. Brown, S.P. Progr. Nucl. Magn. Reson. Spectrosc. 50, 199-251, 2007.
2. Elena, B., de Paepe, G., Emsley, L. Chem. Phys. Lett. 398, 532-538, 2004.
3. Elena, B., Emsley, L. J. Am. Chem. Soc. 127, 9140-9146, 2005.
4. Vinogradov, E., Madhu, P.K., Vega, S. Chem. Phys. Lett. 354, 193-202, 2002.
5. Thureau, P., Sauerwein, A. C., Concistre, M., and Levitt, M. H. Phys. Chem.Chem. Phys. 13, 93-96, 2011.

89 qNMR in Applied Foods Research

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Quantitative 1H NMR (qNMR) is highly suitable for the simultaneous detection and quantification of organic acids, sugars, amino acids and nucleotides in foods and natural products. Due the diversity and complexity of these biological systems, however, various critical aspects have to be considered and optimized before the qNMR application can actually be integrated in a routine workflow. The examination of a wide range of fruits, herbs and vegetables, led to a value-added (interactive) qNMR approach with the potential for generic use in applied foods research. The proposed protocol involves a series of optimized conditions for the 1H qNMR experiment, a validation strategy, a comprehensive deconvolution routine (using Chenomx and Perch), custom-made data exchange scripts and a laboratory information management system (LIMS). To demonstrate the entire qNMR workflow, bell pepper and tomatoes were selected as working examples. In the aqueous extracts of these natural products we were able to quantify a series of >15 compounds in the range 0.1 to 200 mg/g by using a 1D 1H experiment with low-power water suppression. These compounds represent a major fraction of all observable 1H resonances in the NMR spectrum, and matched well with the absolute quantitative results obtained from established analytical methods. Based on the currently achieved performance, accuracy, measurement precession and convenience we concluded that qNMR is a rational, versatile and robust alternative for currently applied compositional analysis of foods and natural products.

90 NMR Studies of Derivatives of Furanoditerpene Isolated from Pterodon Polygalaeflorus Benth

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The genus Pterodon (Leguminosae) comprises five species widely distributed over central Brazil, among which Pterodon polygalaeflorus Benth, popularly known as Sucupira-branca and Faveiro [1]. Alcoholic infusions from the fruits of this vegetal are used in folk medicine to treat as antirheumatic, anti-inflammatory (sore throat), and pain killer preparations [1]. From hexane extract of the fruits of this vegetal it was isolated the 6alpha,7beta-dihydroxyvouacapan-17beta-oic acid - ADV (1), a furanoditerpene that presents multiple biological activities including analgesic, antifungal, anti-inflammatory, antinociceptive, cancer cells antiproliferative, larvicidal, photosystem II inhibitory, photosynthesis uncoupler, and plant growth regulatory properties [2].

To obtain information about the chemical structure-biological activity relationship, and to search for more potent compounds, several amino, ester, keto, Mannich-base, and delta-lactone derivatives of ADV have been synthesized, among which its lactones: 6alpha-hydroxyvouacapan-7alpha,17beta-lactone - HVL (2), 6alpha-acetoxyvouacapan-7alpha,17beta-lactone - AVL (3), and 6-oxovouacapan-7alpha,17beta-lactone - POL (4) [4,8,9]. The amino, hydroxyl and carbonyl groups are able to receive hydrogen bonds, while the NH and OH can also donate hydrogen to hydrogen bonds. To map the receptor structure, the ADV OH group at C-6 was replaced by a carboxyl (3) and a carbonyl (4) group, while the H-16 at the furane ring was replaced by N-alkyl-substituted groups. This work reports the NMR study of some ADV derivatives relative to the conformational study of the three lactones 2 to 4 by NMR and X-ray. The syntheses of these compounds have already been reported [2]. It will be present the NMR conformational analysis of some ADV derivatives. The 1H and 13C NMR spectra, as well as the 1H,1H-COSY (1JH,H and 3JH,H), 1H,1H-NOESY (JH,H long distance), 1H,13C-HMBC (nJH,C, n=1, 2, 3, and 4), 1H,13C-HMQC (1JH,C), and 1H,13C-HSQC (1JH,C) experiments were recorded at 400 MHz, using CDCl3, DMSO-d6, or Py-d5 as NMR solvent. The chemical shifts (δ) were registered in ppm relative to TMS.

1. Correa, M. P. MA-IBDF, Imprensa Nacional:Rio de Janeiro, 1978, p. 153
2. Euzebio, F. P., Santos, F. J. L., Pilo-Veloso, D., Alcantara, A.F.C., Ruiz, A. L. T. G., Carvalho, J. E., Foglio, M.A., Ferreira-Alves, D. L., Fatima, A. Bioorg. Med. Chem. 18, 8172, 2010.
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91 Stereochemical Dependence of 3JCH Coupling Constant in 2-Substituted 4-t-Butyl-Cyclohexanone

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Indirect spin-spin coupling constants (SSCCs) 3JCH are of importance in many areas of structural chemistry, like for studying constitutions, configurations and conformations in organic and bioorganic molecules, mainly when other parameters such as 3JHH SSCCs and NOE experiments are inconclusive [1, 2]. Both types of coupling are transmitted mainly by Fermi contact (FC) and, seen to be similarly affected by structural factors (dihedral and bonding angles, for example). It is known [1] that in 4-t-butylcyclohexanone 3JC6H2eq coupling across the carbonyl group have an unusually small value (3JC6H2eq = 3.1 Hz) corresponding to a C6-C1-C2-H2eq coupling pathway with a 173.9 deg dihedral angle. This contrasts notably with 3JC1H3eq = 8.4 Hz for a 176.9 deg C1-C2-C3-H3eq dihedral angle measured in the same compound [3]. In this work, we focus our attention on measuring and rationalizing the difference in 3JC2H6eq SSCCs for cis- and trans-2-X-4-t-butyl-cyclohexanones (X= H, Me, F, Cl and Br). 3JCH couplings were measured using the HSQC-TOCSY-IPAP [4] pulse sequence and hyperconjugative interactions were evaluated using the Natural Bond Orbital (NBO) analysis. There is an excellent agreement between theoretical and experimental 3JC2H6eq SSCCs, and there were observed similar values for the C2-C1-C6-H6eq dihedral angle for both cis and trans isomers while the respective 3JC2H6eq SSCCs for cis-2-X-4-t-butylcyclohexanones are twice as large as those for the respective trans isomers, except for 2-Me-4-t-butylcyclohexanone which presented similar values of 3JC2H6eq for both isomers.

Some calculations changing the C2-C1-C6 angle (θ) in 5 deg steps from 90 deg ≤ θ ≤ 130 deg and re-optimizing geometries for each step, were performed to study the influence of θ in the 3JC2H6eq, SSCCs were calculated for these new geometries and the results obtained for the different cis- and trans-2-X-4-t-butylcyclohexanones show an inverse relationship between internal angle (θ) and 3JC2H6eq values (increasing bond angle it was observed a decrease in the coupling constant). It has been observed a linear correlation between sum of occupancy for the bonding and antibonding orbitals involving in the coupling pathway and theoretical values of 3JC2H6eq coupling. These results together with the dependence of 3JC2H6eq with the internal angle, allow us to conclude that there is a direct dependence of the stereochemistry of the carbon C2 and the value of 3JC2H6eq.

1. Parella,T., Sanchez-Ferrando, F., Virgili, A., Magn. Reson. Chem., 32, 657-664, 1994.
2. Santos, F. P., Tormena, C. F., Contreras, R. H., Rittner, R., Magalhes, A. Magn. Reson. Chem. 46, 107-109, 2008.
3. Contreras, R. H., Peralta, J. E., Prog. NMR Spectrosc. 37, 321-425, 2000.
4. L. Lerner, A. Bax, J. Magn. Reson. 69, 375-380, 1986. FAPESP, CNPq and IQ-UNICAMP

92 Identification of adulterants in an antihypertensive Chinese herbal medicine by LC-HRMS and LC-MS-SPE/NMR

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Based on anecdotal evidence of anti-hypertensive effect of Gold Nine Soft Capsules, an in vivo study of this complex Chinese "herbal-based" medicine was initiated. Dosage of the content of Gold Nine capsules in spontaneous hypertensive rats showed a remarkably good effect. This led to further investigation of the components of the preparation and eventual identification of three known anti-hypertensive drugs; amlodipine, indapamide and valsartan, which were not declared on the label. Compounds were rapidly identified using LC-HRMS and LC-MS-SPE/NMR, quantified by HPLC, and the in vivo activity of a combination of commercially purchased standards was shown to be equivalent to that of the capsule content. Adulteration of herbal remedies and dietary supplements with synthetic drugs is an increasing problem that may lead to serious adverse effects. LC-MS-SPE/NMR as a method for the rapid identification of such adulterants is highlighted in this case study [1].

1. Journal of Pharmaceutical and Biomedical Analysis 2010, 51, 705-711.

93 NASCA-HMBC and DENA-HMBC, Two Approaches to Obtain High-resolution Multiple-bound Correlations Spectra

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The low resolution of standard 2D HMBC spectra makes it often difficult to assign close pairs of signals to individual carbons. This is especially problematic when determining or confirming the structures of natural products such as biflavonoids isolated from plant extracts because carbons tend to appear as clusters of signals. The NASCA-HMBC (Non-ambiguous Assignment by Superposition of Coupled Aliased HMBC) combines a pair of aliased HMBC spectra to provide one order of magnitude increase in the resolution and unambiguous chemical shifts. Alternatively, a single spectrum can provide the same information after insertion of the DENA (Differential Evolution for Non-ambiguous Aliasing) sequence element1 into classical HMBC sequences. The DENA element consist in bringing together two magnetization pathways having experienced slightly different 13C evolutions so that they can encode the chemical shift information lost because of aliasing. Application to agathisflavone, a biflavonoid found in Ouratea gilgiana resulted in spectra with a sufficient resolution to make signal assignment absolutely straightforward.

1. M. Foroozandeh and D. Jeannerat, ChemPhysChem, 11, 2503-2505, 2010.
2. G. Bayiha Ba Njock et al, Phytochem. Anal, in press, 2011.

94 NMR Tools for Protein-Ligand Interaction Studies Under Non-Homogeneous Conditions: The Example of Lectin-Carbohydrate Recognition

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NMR techniques allow to obtain structural information useful for the comprehension of biological processes and nowadays high-resolution magic-angle-spinning (HR-MAS) NMR spectroscopy is a well established tool for the study of heterogeneous system, especially semisolid materials such as lipid membranes, drug delivery system, cell suspensions, biopsy samples, molecules adsorbed on a solid support, and resin-bound molecules. Here we present the generation of a feasible model-system used to explore the possibility to reveal interactions between a soluble ligand and another molecular entity linked to a solid support. In particular we based our studies on the carbohydrate recognition process. We obtained a pseudo-receptor, that mimics lectin binding site, coupling a Tryptophan residue to a Sepharose resin and we characterize its interaction with different monosaccharides. The results support the theoretical model according to which lectins bind carbohydrates exploiting the CH-π interactions that occur in their active site. Moreover the experimental approach here described can be generally applied when the interacting species do not have the same solubility properties in physiological conditions.

The research leading to these results has received funding from the CARIPLO Foundation (CARIPLO 2008-3175: Development of NMR techniques for tissue engineering studies)

95 New Developments of LSD, a Structure Elucidation Software

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The aim of the LSD program [1] is to find all possible molecular structures of an organic compound that are compatible with its spectroscopic data. Structure building relies on connectivity data found in 2D NMR spectra, without any reference to a chemical shift database [2]. The measurement protocol that is required by LSD includes the recording of 1D 1H and 13C as well as 2D COSY, HSQC and HMBC spectra. The status of each atom must be defined. It includes the atom symbol, the hybridization state and the number of attached hydrogen atoms. This part of the data set is most often easily deduced by the user from elementary chemical shift knowledge. The status of the heteroatoms is deduced by the user from the molecular formula. Carbon-carbon bonds are inferred from COSY and HSQC data while HMBC and HSQC data provide connectivity relationships through one or two bonds for non-hydrogen atoms. The constraints imposed by atom status and 2D NMR data may be enforced by other atom neighborhood relationships.

A recent development of LSD is the possibility of defining ambiguous COSY correlations between atom groups with nearly identical chemical shifts. Another novelty is the possibility of defining the interval of coupling path length that is associated to HMBC or COSY correlations. The coupling path length of a strong intensity correlation can thus be forced to never be strictly greater than three (3J 13C-1H or 3J 1H-1H). This would save time and reduce the number of solutions if very long range correlations are allowed. Other recent implementations extend the diversity of molecules the program could manage. New status of atoms are taken into account such as charged atoms or sp hybridized atoms. These new functionalities have permitted the structure elucidation of compounds by means of the LSD program.

1. http://helios.univ-reims.fr/Labos/UPRESA6013/LSD
2. Nuzillard Jean-Marc, Chinese Journal of Chemistry, 21, 1263-1267, 2003

96 NMR Studies of Organometallic Chalcogenides and Similar Compounds

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So-called N,C,N chelating ligands (NCN = -(2,6-di(Me2NCH2)2C6H3)) stabilize low valence metals (Sb, Bi, Se, Te, Sn) bound in position 1. These compounds can react with many reactants to give rather unusual structures [1-4]. X-ray data proved the new structures undoubtedly.

Multinuclear NMR was used to study the constitutions in solution. The 1H, 13C, 31P, 77Se, 125Te and 119Sn NMR spectra were used for such a purpose. In some cases, the different constitution or dynamic behaviour was observed comparing the situation in the solid state and in solution.

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1. Svoboda T., Dostal L., Jambor R., Ruzicka A., Jirasko R., Lycka A., Inorg. Chem., 50, 6411-6413, 2011
2. Bouska M., Dostal L., de Proft F., Ruzicka A., Lycka A., Jambor R., Chem. Eur. J., 17, 455-459. 2011
3. Mairychova B., Dostal L., Ruzicka A., Fulem M., Ruzicka K., Lycka A., Jambor R., submitted
4. Svoboda T., Jambor R., Ruzicka A., Jirasko R., Lycka A., Dostal L., submitted.

97 Towards a Better Understanding of the Selectivity of an Organocatalyst

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The tetrapeptide Boc-L-(-Me)-His-AGly-L-Cha-L-Phe-OMe (AGly represents γ-aminoadamantanecarboxylic acid) is used as an organocatalyst for the enantioselective monoacylation of trans-cyclohexane-1,2-diol, yielding exceptionally high selectivities [1]. The reaction course is assumed as follows: First, the tetrapeptide is acylated at the nitrogen of the methylsubstituated histidine. This intermediate then transfers the acyl group selectively to one alcoholic group of one enantiomer of the trans-cyclohexane-1,2-diol. The structure of the assumed intermediate should therefore be crucial to understand the selectivity of this reaction.

Quantum chemical calculations have been used by SCHREINER ET AL. [1] to examine the structure of the tetrapeptide and of the acylated intermediate in solution. These calculations led to an environment at the catalytic active site in the intermediate which would explain the selectivity of the reaction by suggesting a hydrogen-bridge from a carbonyl group to one of the alcoholic groups of the trans-cyclohexane-1,2-diol and by a hydrophobic interaction of two cyclohexyl rings, resulting in the monoacylation of only one enantiomer. With three conformers of quite similar structure within a range of 8 kJ/mol, the calculations suggest conformational flexibility for the intermediate. The calculations also suggest conformational flexibility for the tetrapeptide in solution with ten conformers showing a large variety of structures within a range of 8 kJ/mol [1].

So far no experimental evidence towards the existence of the proposed intermediate nor towards the solution structure of the tetrapeptide itself is available. Thus we started to investigate the solution structure of the tetrapeptide by NMR spectroscopy. It is difficult to determine the structure with routine procedures if conformational flexibility for the organocatalyst is present, as was suggested by the calculations. We started using a combination of NOE (Nuclear Overhauser Effect) [2] data and RDCs (Residual Dipolar Couplings) [3] of the tetrapeptide in the liquid crystal phase of the homopolypeptide poly-gamma-benzyl-D-glutamate [4] to study the structure of the tetrapeptide in solution. The presentation will show the results of this study.

1. Muller, Christian E., Wanka, Lukas, Jewell, Kevin, Schreiner, Peter R., Angew. Chem., 120, 6275-6278, 2008
2. Neuhaus, David, Williamson, Michael P., The Nuclear Overhauser Effect in Structural and Conformational Analysis, 2. Edition, Wiley, Chichester, 2000
3. Reviews: Thiele, Christina M., Concepts Magn. Reson., 30A, 65-80, 2007, Thiele, Christina M., Eur. J. Org. Chem., 2008, 5673-5685, 2008
4. Marx, Andreas, Thiele, Christina M., Chem. Eur. J., 15, 254-260, 2009

98 Quantum Mechanical Calculations of NMR Parameters: Applications to Structure Elucidation Problems in Drug Discovery

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Structure elucidation by NMR is essentially the process which aims to reconstruct experimental NMR data by a theoretical model of a molecular structure and a set of NMR parameters associated with that structure. One way to simplify that process is to rely on experimental data of known compounds and statistical analysis of that information. This approach has been successfully applied to NMR chemical shifts. However, it has limited applicability when it comes to the analysis of spin-spin interactions, such as J-couplings, a major parameter used in stereochemical analysis. In this case, quantum mechanical calculations of J-couplings appear to be an invaluable source of independent and unbiased structural information. For the past 5 years we have been testing this approach with regard to structure elucidation problems in drug discovery. A major contribution of quantum mechanical calculations was observed for long-range heteronuclear (HC, HN, CF, FF) spin-spin coupling predictions. These calculations were often the only source of quantitative structural information, particularly for molecules with a limited number of homonuclear couplings. Moreover, quantum mechanical calculations of carbon and nitrogen chemical shifts were overall in better agreement with experimental data as compared to those between statistically estimated chemical shifts (ACD, ChemDraw) and experimental data.

99 Evaluation Automatic Structure Verification Tools

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Several automatic structure verification tools were tested out. They differ from highly qualitative verification to more badge and automation oriented. Also a quantification package is tested with structure verification as a side result.

Mainly their capabilities to give an automatic verification support in a medicinal chemistry environment is evaluated. Although numbers will be reported, it was not really the idea to go for big numbers. Neither was the intention to limit to percentages only. A more in dept evaluation of the advantages but also problems of the different packages will be discussed. Small sets of different kind of structures are evaluated: fragments, standard size medicinal chemistry compounds and a set of synthesized compounds proven to be wrong by NMR experts. In the last case both the structure proposed by the chemist as the elucidated structure are given to the software for verification. If possible in the software, both proton and 2D results are compared.
Improvements and possible applications are suggested.

100 Objects of Cultural Heritage Analyzed by the NMR-MOUSE

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The stratigraphies of decorated walls in ancient Herculaneum, Italy, were analyzed by single-sided 1H NMR using a large version of the NMR-MOUSE® with a maximum penetration depth of 25 mm [1]. It was used to map proton density profiles at different positions of the mosaic of Neptune and Amphitrite showing considerable differences between different tesserae and the mortar bed at different times of the year. The recorded profiles revealed undocumented conservation treatment of the tesseare and different moisture content in the supporting mortar. Also a strong dependence of the proton signal with the measurement position was observed. In the House of the Black Room, different mortar layers were observed on painted walls as well as different proton content in different areas due to different moisture levels and different conservation treatments. The three walls that were analyzed had parts where an organic fixative was applied, parts where it was removed and parts where new inorganic conservation treatments, ammonium oxalate and barium hydroxide, were applied. The proton density profiles of the differently treated areas indicated that one method leads to higher moisture content than the other and in this way the efficacy of each of the conservation treatments was estimated. For the first time depth profiles of untreated wall paintings from different times were recorded in a recently excavated room at the Villa of the Papyri showing two different types of mortar layer structures which identify two different techniques of preparing the walls for painting [2]. Furthermore laboratory studies were conducted on mock-up fresco and secco samples to determine the moisture transport using diffusion measurements at different depths for different conservations treatments. The conservation treatments were the same as the ones used in the Black Room. These studies demonstrate that NMR is a suitable tool to analyze and characterize the layer structures of different wall paintings and mosaics. By analyzing the proton content in terms of proton density and mobility, the NMR-MOUSE® provides a method for distinguishing layer structures and properties in a non-destructive way. Measurements taken in the same point but at different time periods demonstrate the accuracy and reproducibility of the measurements.

References:

1. Eidmann G, Savelsberg R, Blümler P, Blümich B (1996) J Magn Reson A 122:104–109
2. Haber A., Blümich B., Souvorova D., Del Federico E. (2011) Anal. Bioanal. Chem. DOI: 10.1007/s00216-011-5180-3

101 Automated Analysis of NMR Data in the Context of Fragment Screening

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NMR experiments have been widely used to screen libraries of small molecules in the search of compounds which bind weakly to target proteins [1]. Our analysis has been focused in the interpretation of 1D experiments, such as STD, T1ρ and WaterLOGSY.

The visual inspection of hundreds, sometimes thousands, of experimental results is tedious, prone to operator errors and may be the cause of delays in medicinal chemistry projects. In order to automate the analysis of such datasets several obstacles must be overcome, to mention a few:

- Alignment of all spectra must be as accurate as possible to compensate for changes in experimental conditions, mainly pH and temperature
- Deconvolution of all acquired spectra to allow efficient peak peaking and spectra manipulation
- Evaluate the presence in solution of expected small molecules
- Match library peaks with STD or T1ρ peaks, and compare intensity changes to identify primary hits from STD or T1ρ spectra

In this poster we detail a combination of approaches which address these four main points, as well as other practical aspects of the analysis.

1. Shuker, Suzanne B.; Hajduk, Philip. J.; Meadows, Robert P.; Fesik, Stephen W., Science, 274, 1531–1534, 1996