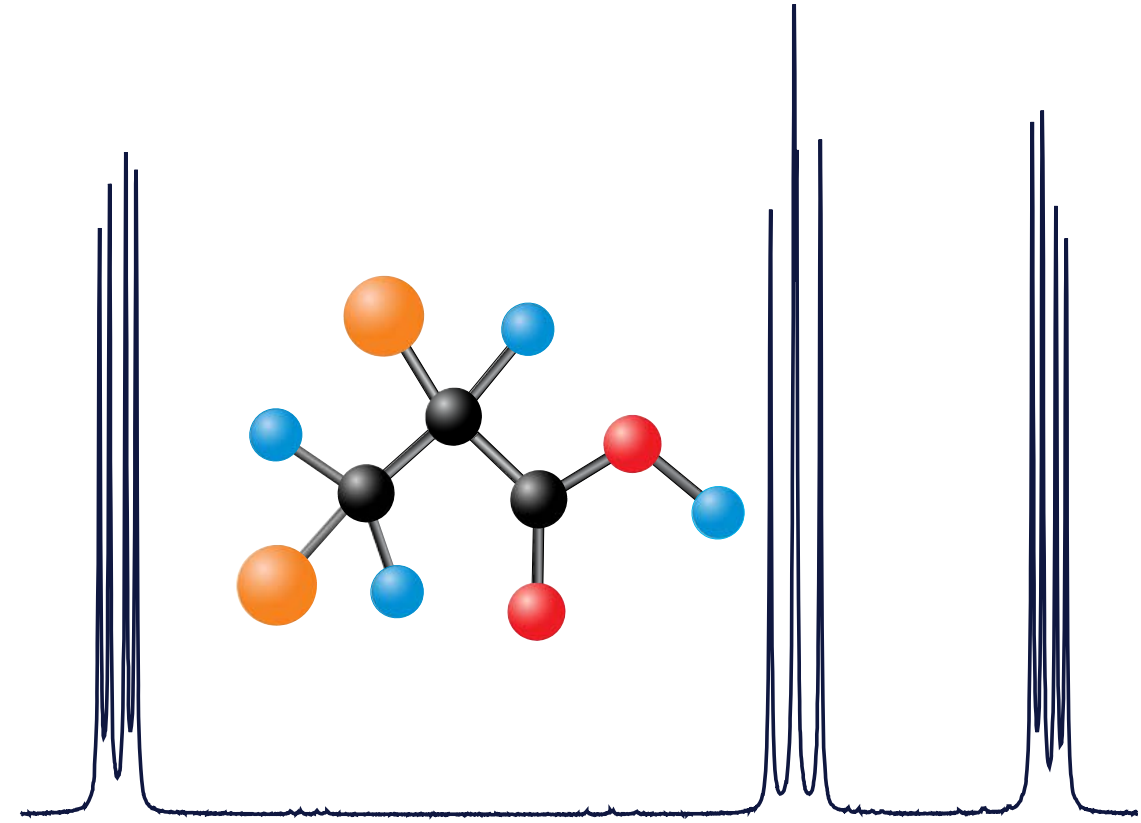

SMASH 2008

Conference Program

September 7th - 10th, 2008

Santa Fe, New Mexico

SMASH 2008 NMR Conference

Dear SMASH 2008 Attendees,

Welcome to Santa Fe, New Mexico and the 9th SMASH NMR Conference. This year's program encompasses many divergent areas of small-molecule research and includes the following sessions: New Experimental Techniques, Structure Elucidation of Natural Products and Organic Molecules, Metabolomics, NMR of Carbohydrates, Techniques and Characterization Methods of F-19 NMR, Computer Aided Structure Identification, NMR of Less Common Nuclei, and Student and Post-Doctoral Research.

In addition, there is an evening Poster Session with a buffet dinner and workshops on the following topics: Solid State NMR, Process and Reaction Monitoring, Structure Elucidation Pitfalls, and Cryoprobes.

Finally, we are honored to have one of the foremost experts in the application of NMR methods to the structural elucidation of natural products and organic molecules, Prof. William F. Reynolds, as our keynote speaker for the conference banquet.

On behalf of the entire SMASH Organizing Committee, we wish to thank you for your continued interest in, and support of, the SMASH NMR Conference. We hope that you enjoy the Conference and are able to sample the exquisite blend of old Spanish, Native American, and Anglo cultures of Santa Fe.

With warmest regards,

George Furst and Gene Mazzola
Co-Chairs, SMASH 2008 NMR Conference

SMASH 2008 NMR Conference
Program

Sunday September 7th

4:30 PM - 6:00 PM **Registration, La Fonda Hotel**
6:00 PM - 8:00 PM **Dinner, La Fonda Hotel**
8:00 PM -11:00 PM **Mixer, La Fonda Hotel**

Monday September 8th

8:15 AM - 8:30 AM **Opening Remarks**
8:30 AM - 10:00 AM **New Experimental NMR Techniques**
Chair: Krish Krishnamurthy, Lilly, USA

- **Covariance NMR Processing: Fundamentals, Applications, and Challenges**
  Bruce Hilton, Schering Plough, USA
- **NMR with Multiple Receivers**
  Ēriks Kupče, Varian, U.K.
- **Development of DNP Instrumentation for Small Molecule Applications**
  Steve Reynolds, Oxford Instruments Molecular Biotools Ltd, U.K.

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Structure Elucidation of Natural products and Organic Molecules**
Chair: Bill Reynolds, University of Toronto, Ca

- **Qualitative and Quantitative NMR Fingerprints of Small Natural Molecules**
  Guido F. Pauli, University of Illinois at Chicago, USA
- **The Virtue of the Impure: Chemical Biology of Natural Products Using Direct NMR**
  Frank C. Schroeder, Cornell University
- **The Utility of Coupled HSQC Spectra for Determining the Identities of Sugar Units in ^{1}H NMR - Spectrally Crowded Saponins**
  Eugene P. Mazzola, University of Maryland-FDA Joint Institute, USA

12:00 PM - 1:30 PM **Lunch**
1:30 PM - 3:00 PM **Metabolomics**
Chair: Greg Leo, J&J, USA

- **Metabolomics Methods Applied to Beverages: The Case of "Beer-omics"**
  Istvan Pelczer, Princeton University, USA
- **Advanced NMR-Based Metabolomics Methods for Improved Disease Biomarker Discovery**
  Daniel Raftery, Purdue University, USA
- **Boosting the Sensitivity of NMR by DNP for Small Molecule Applications**
  Ulrich Günther, University of Birmingham, U.K.

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

- **I. Solid State Workshop**
  Eric Munson, University of Kansas

- **II. Process and Reaction Monitoring**
  Brian Marquez, Pfizer, USA
  Andreas Kaerner, Eli Lilly, USA

5:00 PM - 6:00 PM **Free Time**
6:00 PM - 6:30 PM **Social Gathering**
6:30 PM - 9:00 PM **Dinner**
After Dinner Speaker: William Reynolds, University of Toronto
From the Frozen Wilds of Northern Manitoba to the Ancient Ruins of Mexico; A Forty Eight Year NMR Odyssey
9:00 PM - 11:00 PM **Mixer**

Tuesday September 9th

8:30 AM - 10:00 AM **NMR of Carbohydrates**
Chair: Daron I. Freedberg, F.D.A., USA

- **Conformational Studies of Oligosaccharides and their Analogues using NMR Spectroscopy**
  Goran Widmalm, Stockholm University, Sweden
- **The Stereochemical Dependence of Scalar ^{15}N-^{1}H Coupling Constants in Amino Sugars**
  Bruce Coxon, N.I.H., USA
- **Structures of Chondroitin Sulfate Oligomers from RDCs and CSA Offsets**
  James Prestegard, University of Georgia, USA

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Techniques and Characterization Methods in ^{19}F NMR**
Chair: Elizabeth McCord, Dupont, USA

- **BEBOP and BIBOP Pulses for Use in Analysis of Fluorinated Materials**
  Steve Cheatham, DuPont Crop Protection, USA
- **Practical Aspects of Selective ^{19}F Homonuclear Multidimensional NMR**
  Peter L. Rinaldi, University of Akron, USA
- **Probing the Interaction Between Fluorinated Compounds and Microorganisms Using ^{19}F NMR**
  Cormac D. Murphy, University College Dublin, Belfield, Ireland
- **Interest of ^{19}F NMR to Detect, Identify and Quantify New Metabolites of Fluorinated Antifungal and Anticancer Drugs in Humans**
  Myriam Malet-Martino, Université Paul Sabatier, France

12:00 PM - 1:30 PM **Lunch**
1:30 PM - 3:00 PM **Computer Aided Structure Identification**
Chair: Patrick Wheeler, ACD Labs, Canada

- **CASE Systems: A Review of the Last 40 Years**
  Mikhail Elyashberg, Russian Academy of Sciences, Russia
- **An In Silico Approach to Rational Antipsychotic Drug Design using Spectral Data Activity Relationship**
  Richard Beger, National Center for Toxicological Research
- **Computer Aided (Additivity) Chemical Shift Prediction in Multiply Halogenated Organics Using an Excel Visual Basic Program**
  Richard Newmark, Newark Consulting

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

- **I. Structural Elucidation Pitfalls**
  William Reynolds, University of Toronto, Canada
- **II. Cryoprobes**
  Kim Colson, Bruker-Biospin and Dave Russell, Varian .Inc.

5:00 PM - 6:00 PM **Free Time**
6:00 PM - 7:30 PM **Dinner**
8:00 PM - 10:00 PM **Poster Session & Mixer**
10:00 PM - 11:00 PM **Mixer**

Wednesday September 10th

8:30 AM - 10:00 AM **NMR of Less Common Nuclei**
Dave Lankin, University of Illinois at Chicago, USA

- **Phosphorous NMR Insights on Novel Inositol Sugars and Phosphoglycolipids from Gram-Negative and Gram-Positive Bacteria**
  Anthony Ribeiro, Duke University, USA
- **Current Topics in ^{29}Si NMR of Solutions**
  Jan Schraml, Institute of Chemical Process Fundamentals of the ASCR v.v.i.; Czech Republic
- **^{31}P NMR Discovers a Sex–Specific Metabolite**
  Rob Kleps, University of Illinois at Chicago

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Student and Post-Doctoral Session**
Chair: George Furst, University of Pennsylvania., USA

- **Multiple-Sample Probe for High-Throughput Solid-State NMR**
  Ben Nelson, University of Kansas
- **"Minimal Chemical Shift Maps" for Compound Matching and Identification**
  Xi Meng, University of California, Irvine
- **Xenon-129 NMR Biosensors for Cellular Imaging**
  P. Aru Hill, University of Pennsylvania, USA

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

SMASH 2008 NMR Conference
Acknowledgements

The SMASH 2008 Conference gratefully acknowledges the support provided by the following companies.

Advanced Chemistry Development, Inc., (ACD/Labs)
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Poster Session

Steve Cheatham
DuPont Crop Protection

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

New Experimental NMR Techniques
Chair: Krish Krishnamurthy

Speakers:

Bruce Hilton
Schering Plough, USA

Ēriks Kupče
Varian, U.K.

Steve Reynolds
Oxford Instruments Molecular Biotools Ltd, U.K.

Covariance NMR Processing: Fundamentals, Applications, and Challenges

Bruce D. Hilton[1], Gary E. Martin[1], Kirill A. Blinov[2], and Antony J. Williams[3]

1. Schering-Plough
2. Advanced Chemistry Development
3. Chem Zoo, Inc.

Covariance processing of NMR data, by definition, is a processing method. As such, a processing method cannot produce data that is not already implicit in the raw data acquired by the spectrometer. The value of covariance or any other processing method resides in the question of whether or not the method in question provides a faster and/or more convenient view of the experimental data. Several variants of covariance NMR processing have arisen over the past few years. Applications to homonuclear 2D NMR spectra have been prevalent. Applications to heteronuclear 2D NMR data have been termed indirect covariance processing when the method is applied to a single heteronuclear 2D NMR experiment. A newer variant of the processing algorithm allows a pair of 2D NMR spectra to be co-processed, a process that has been termed unsymmetrical indirect covariance processing. It is conceivable that unsymmetrical indirect covariance processing could invole the co-processing of a pair of homonuclear 2D experiments, but no examples have been reported. Applications have been reported of coprocessing homo- and heteronuclear 2D spectra or a pair of different heteronuclear 2D spectra. Examples of the fundamentals of covariance processing will be presented, as will examples of indirect and unsymmetrical indirect covariance processing.

NMR with Multiple Receivers

Ēriks Kupče[1] and Ray Freeman[2]

1. Varian Ltd., Oxford UK
2. Jesus College, Cambridge University, Cambridge

A recent innovation permits several quite distinct pulse sequences to be carried out in parallel, using separate receivers tuned to different nuclei, for example ^{31}P, ^{19}F, ^{15}N, ^{13}C, ^{2}H or protons [1,2]. Up to five nuclei can be detected simultaneously. We show that two or more multi-dimensional NMR correlation spectra can be recorded in a single shot, using a multi-coil radiofrequency probe designed for simultaneous parallel acquisition of signals from different nuclear species such as ^{1}H, ^{13}C and ^{15}N. Dubbed PANSY (Parallel Acquisition NMR Spectroscopy) this new technique shows promise for recording several multidimensional NMR spectra of different nuclear species in a very short time. For instance, two-dimensional NMR spectra correlating both ^{1}H and ^{19}F nuclei with either ^{13}C or ^{15}N, were recorded at the same time, using a broadband radio frequency probe feeding independent ^{1}H and ^{19}F receiver channels. This technique speeds up multidimensional NMR and is compatible with other fast-acquisition schemes. The method is illustrated with single-bond (HSQC) and multiple-bond (HMBC) experiments.

A different (but compatible) innovation in probe technology now makes it feasible to conduct a high-sensitivity INADEQUATE experiment using a new cryogenically-cooled probe optimized for ^{13}C. Combination of INADEQUATE with HSQC and HMBC offers an information-rich result in a single shot – carbon-carbon correlation, together with one-bond and long-range carbon-proton correlation. Few small molecules can resist such a high-calibre onslaught. Furthermore this double-barrelled attack lends itself to automation, so the busy spectroscopist need not be physically present at the kill. An extension of this "all-in-one" sequence with three parallel receivers permits the corresponding natural-abundance ^{15}N spectra to be included. As a 'proof of principle', experimental spectra are presented for some simple molecules.

1. Kupče Ē; Freeman R.; John BK. Parallel acquisition of two-dimensional NMR spectra of several nuclear species, J. Am. Chem. Soc. 2006; 128: 9606-9607.
2. Kupče Ē; Cheatham S.; Freeman R. Two-dimensional spectroscopy with parallel acquisition of ^{1}H–X and ^{19}F–X correlations, Magn. Reson. Chem. 2007; 45: 378–380.
3. Kupče Ē; Freeman R.; Molecular structure from a single NMR experiment, J. Am. Chem. Soc. 2008; in press.

Development of DNP Instrumentation for Small Molecule Applications

Steven Reynolds and Joost Lohman

Oxford Instruments Molecular Biotools Ltd., Tubney Woods, Abingdon, Oxon. UK

Dynamic Nuclear Polarisation (DNP) is a hyperpolarisation technique that offers an increase of over 10,000 times[1] in the signal-to-noise ratio (SNR) for many spin=1/2 NMR nuclei in solution-state NMR spectroscopy. The sample of interest is doped with a trityl radical and dissolved in a mixture of solvents that will form a glass when frozen. It is then exposed to a very low temperature (~1.4K) in the presence of a strong magnetic field (3.35T). Under these conditions, the unpaired electrons on the trityl radicals attain a high degree of Boltzmann polarisation(>90%). By applying microwave irradiation at the appropriate frequency (ca. 94GHz) polarisation is transferred to atomic nuclei. Once a sufficient level of hyperpolarisation has been reached, the sample is rapidly thawed using a dissolution solvent (e.g. water, methanol, toluene) and rapidly introduced into a high-resolution NMR spectrometer where the hyperpolarised spectrum is acquired.[2]

Understanding the polarisation mechanism in the solid state can provide insight into the optimum conditions for polarisation. Measurement of the polarisation build-up rate can also determine the most efficient polarisation time. Examples of polarised compounds for a number of different nuclei (^{31}P, ^{29}Si, ^{13}C, ^{15}N, ^{89}Y) are shown both in the solid and liquid state.

Also, discussed is the affect of T1 on the polarisation level observed in the liquid state and a method by which relaxation losses can be reduced.

1. Ardenkjaer-Larsen, J. H.; Fridlund, B.; Gram, A; Hannson, G.; Hansson, L.; Lerche, M.H.; Servin, R.; Thaning, M.; Golman, K. Proc. Nat. Acad. Sci. 2003, 100, 10158.
2. Wolber, J.; Ellner, F.; Fridlund, B.; Gram, A.; Johannesson, H.; Hansson, G.; Hansson, L.H.; Lerche, M.H.; Mansson, S.; Servin, R.; Thaning, M.; Golman, K.; Ardenkjaer-Larsen, J. H. Nucl. Inst. Meth. Phys. Res. A 2004, 526, 173.

Monday, September 8th
10:30 AM - 12:00 PM

Structure Elucidation of Natural products and Organic Molecules
Chair: Bill Reynolds

Speakers:

Guido F. Pauli
University of Illinois at Chicago, USA

Frank C. Schroeder
Cornell University, USA

Eugene P. Mazzola
University of Maryland-FDA Joint Institute, USA

Qualitative and Quantitative ^{1}H NMR Fingerprints of Small Natural Molecules

Guido F. Pauli

Department of Medicinal Chemistry and Pharmacognosy & PCRPS, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street,Chicago, Illinois 60612, USA

Small natural molecules often stand at the beginning of drug discovery chains [1], because they represent an (in)exhaustible global resource for evolutionary-shaped molecules of biological significance. As they are embedded into complex matrices, this appeal is paired with analytical challenges: "residual complexity" sets hurdles for structure determination and correlation of structure and bioactivity, because "pure" natural molecules may not necessarily be single chemical entities (SCEs). Because variation of structural parameters and deviation from SCE character have equal potential to cause variation in biological activity, the characterization of biologically active agents requires both qualitative as well as quantitative information, respectively. Its ability to deliver both simultaneously make ^{1}H NMR an invaluable tool for the comprehensive characterization of natural products. Examples of quantitative and qualitative ^{1}H NMR fingerprints are provided, illustrating this potential.

Quantitative ^{1}H NMR (qHNMR) has recently be recognized as highly applicable to natural products research [2]. Emphasizing the role of appropriate post-acquisition processing, qHNMR can be readily integrated into any NMR structure elucidation/dereplication workflow at almost no extra effort. Presented cases employ a routine ^{13}C GARP broadband decoupled qHNMR sequence that reduces spectroscopic complexity by removal of carbon satellites, and allows routine coverage of a dynamic range of 300:1 (0.3%) or better [3]. Qualitative ^{1}H NMR fingerprinting has become feasible only recently with the development of tools capable of spectral simulation and iteration of complex spin systems. Utilizing the PERCH environment, full spin analysis of small molecules such as the AChE inhibitor and drug lead for Alzheimer's disease, huperzine A [4], are feasible and at the verge of becoming routine tasks. Aided by ultra-high field measurements (900 MHz), the fingerprints of small molecules reveal a wealth of long-range couplings (^{4}J, ^{5}J, ^{6}J), in particular for constrained ring systems, and higher order effects, which altogether give rise to highly characteristic qualitative HNMR fingerprings.

Thus, the roles of qualitative ^{1}H NMR fingerprints and quantitation from a nuclear perspective cannot be underestimated for the development of small natural molecules.

1. Newman, D. J.; Cragg, G. M. J. Nat. Prod. 2007, 70, 461-477.
2. Pauli, G. F.; Jaki, B.; Lankin, D. J. Nat. Prod. 2005, 68, 133-149.
3. Pauli, G. F.; Jaki, B.; Lankin, D. J. Nat. Prod. 2007, 70, 589-595.
4. Niemitz, M.; Laatikainen, R.; Chen, S. N.; Kleps, R.; Kozikowski, A. P.; Pauli, G. F. Magn. Reson. Chem. 2007, 45, 878-882.

The Virtue of the Impure: Chemical Biology of Natural Products Using Direct NMR

Frank C. Schroeder

Boyce Thompson Institute, Cornell University, Ithaca, NY 14853

Differential Analyses of 2D NMR Spectra (DANS) constitutes a new approach to linking naturally occurring small molecules with their biological function. DANS offers considerable advantages for the detection of synergism as well as for the characterization of chemically labile signaling molecules. The approach is based on overlays of NMR-spectra of crude or largely unfractionated biological extracts, which can provide a comprehensive overview of a metabolome, often including detailed structural information for many of its components. The talk will give a brief history of the development of this method including several examples for early applications, including the discovery of sulfated nucleosides in spider venom and the identification of bacillaene, the long-sought product of the mixed non-ribosomal peptide/polyketide synthase complex pksX in *Bacillus subtilis*. Recently, we have employed DANS for the purpose of characterizing exocrine signals in the model organism *C. elegans* by linking genetic information to changes in the metabolite profiles of specific *C. elegans* mutants.

The Utility of Coupled-HSQC Spectra for Determining the Identities of Sugar Units in ^{1}H NMR-Spectrally Crowded Saponins

Eugene P. Mazzola[1], Ainsley Parkinson[2], Bruce Coxon[3], Edward J. Kennelly[2], and Daron I. Freedberg[4]

1. University of Maryland-FDA Joint Institute
2. Lehman College, CUNY
3. NIH, NICHD-LDMI
4. FDA, CBER

Saponins are glycosides of steroids, steroid alkaloids or triterpenes found in plants, especially in the plant skins where they form a waxy protective coating. The structures of three complex saponins, blighosides A-C from the tree Blighia sapida, were elucidated by one- and two-dimensional NMR and the H-1 and C-13 NMR spectra of these saponins assigned using only 1D and 2D NMR techniques. Blighoside A (MW 1086) has a tetrasaccharide linked to a triterpene aglycone, hederagenin. The four sugars in order of attachment at C-3 of hederagenin are arabinose, rhamnose, glucose, and another arabinose. Blighoside B (MW 1070) has the same tetrasaccharide linked to a different triterpene aglycone, oleanolic acid, which differs from hederagenin in that its C-23 is a methyl group rather than CH2OH [1]. Blighoside C (MW 1504) has a hexasaccharide linked to oleanolic acid. The six sugars are attached at C-3 of oleanolic acid in the following linear array: two xyloses, rhamnose, glucose, a second rhamnose, and another glucose. Additionally, the first glucose is acetylated at positions 3 and 6 and the terminal glucose at positions 4 and 6. High-resolution coupled-HSQC spectra, in which both the proton and carbon spectral widths were reduced to encompass just the respective carbohydrate regions, were especially useful for determining the identities of the monosaccharides in the three saponins. HMBC experiments enabled the linkages to be established between both the individual sugar units and C-3 of the two triterpenes. In all cases, complementary 3-bond connectivities were observed between the anomeric protons and carbinol carbons and the respective anomeric carbons and carbinol protons. In addition, equatorial methyl groups resulted in shielded ring protons [2,3] in both triterpenes and especially for the methine protons 3 and 5, with H-5 appearing very far upfield at 0.8 ppm in blighosides B and C. Both the H-1 and C-13 chemical shifts occur in relatively narrow spectral ranges, and the proton NMR spectra of all three saponins exhibit severe signal overlap. The coupled-HSQC spectra were of inestimable value in situations where protons on adjacent carbons had nearly coincident chemical shifts and the resulting strong H–H coupling made the measurement of vicinal couplings essentially impossible. Coupled-HSQC spectra elegantly obviated this problem because vicinal proton couplings are now determined from $^{1}H - {}^{13}C - {}^{12}C—{}^{1}H$ units, and the large one-bond C-H coupling effectively results in weak vicinal H–H coupling [4,5].

1. K. Hostettmann and A. Marston, Saponins. Cambridge University Press, Cambridge (1995).
2. E.L. Eliel, M.H. Gianni, T.H. Williams, and J.B. Stothers, Tet. Lett., 741 (1962).
3. H. Booth, Tetrahedron, 22, 615 (1966).
4. A.D. Cohen, N. Sheppard, and J.J. Turner, Proc. Chem. Soc., 188 (1958).
5. I. Leon, R.G. Enriquez, S. McLean, W.F. Reynolds, and M. Yu, Magn. Reson. Chem., 36, S111 (1998).

Monday, September 8th
1:30 PM - 3:00 PM

Metabolomics
Chair: Greg Leo

Speakers:

Istvan Pelczer
Princeton University, USA

Daniel Raftery
Purdue University, USA

Ulrich Günther
University of Birmingham, U.K.

Metabolomics Methods Applied to Beverages: The Case of "Beer-omics"

Istvan Pelczer and Ana C. De Roo

Department of Chemistry, Princeton University, Princeton, NJ 08544

The technical protocol of metabolomics, the analysis of small molecules in complex mixtures, such as biological fluids, tissues, cell extracts, etc., can equally be applied to any other mixture from industrial oil-well samples to food and beverages. Systematic analytical measurements followed by involved statistical analysis and additional component identification are the essential ingredients of such an effort.

We have been and are studying beer samples systematically -- beer is also result of a biological process, fermentation -- using NMR spectroscopy in collaboration with a local brewery, and call this project "beer-omics".

We compare and characterize various fresh brews of different types, as well as follow the brewing process over time both for diagnostic purposes and to understand better the associated biochemistry and chemistry. We developed a protocol to use the beer in its native condition without tampering with the sample in any way. Efficient water suppression is applied for the ^{1}H-NMR measurements in a cryoprobe-equipped 500 MHz instrument. We use PCA and additional supervised statistical methods to analyze the results. Component identification can be done either using the statistical methods themselves or applying dedicated software including spectral prediction tools.

A unique feature of our study is also using high-sensitivity ^{13}C-NMR aided by a dedicated, ^{13}C-detection optimized cryoprobe. The higher dispersion and relative simplicity of the ^{13}C spectra complement well the naturally higher sensitivity of the ^{1}H measurements. We apply both the same statistical and component level evaluation of the data, as well as careful curve fitting-based analysis of the sugar ingredients.

Advanced NMR-Based Metabolomics Methods for Improved Disease Biomarker Discovery

Daniel Raftery

Department of Chemistry, Purdue University, West Lafayette, Indiana

While the analysis of biofluid samples by 1D NMR and pattern recognition methods have proven effective at classifying populations such as "disease" and "healthy," these methods tend to focus on the metabolites with high concentration. New NMR approaches such as selective TOCSY experiments and chemical derivatization methods are being developed in our laboratory which focus on both major and minor components to provide an improved ability to discriminate similar samples [1,2]. Selective TOCSY can also be useful to validate putative biomarkers directly in the NMR spectrum, and may lead to some surprises when assessing "known" metabolite signals [3]. In addition, a new water suppression method we have developed that can improve baselines and sensitivities will be discussed [4]. We can also combine or correlate the results of NMR with several new and advanced MS methods to provide additional information for identifying putative biomarkers to distinguish diseased and healthy populations and to learn more about perturbed complex biological systems. The combination of NMR and MS improves our ability to classify cohorts and to identify additional potential biomarkers. Mapping the observed changes on to the metabolic pathways provides insight regarding the complex and correlated network of metabolic perturbations that occur in disease. Examples using both small animal and human studies will be discussed.

1. P. Sandusky and D. Raftery, Anal. Chem. 2005, 77, 7717-7723.
2. N. Shanaiah, M. A. Desilva, G. A. N. Gowda, M. A. Raftery, B. E. Hainline and D. Raftery, PNAS, 2007, 104, 11540–11544.
3. P. Sandusky and D. Raftery, in final preparation.
4. H. Mo and D. Raftery, J. Magn. Reson., 2008, 190, 1-6.

Boosting the Sensitivity of NMR by DNP for Small Molecule Applications

Martin Saunders, Ildefonso Montesinos, Christian Ludwig, and **Ulrich Günther**

HWB-NMR, CR UK Institute of Cancer Studies, University of Birmingham, Vincent Drive, Edgbaston, Birmingham B15 2TT

Dynamic Nuclear Polarisation (DNP) is used to transfer the high spin polarization of unpaired electrons to coupled nuclear spins. Stable radicals are added to a solution of the sample and irradiating with microwaves is applied for 1-3h at the EPR lines of the radical. In such experiments enhancements of >10,000 were achieved [1] after rapidly warming up polarized samples to approx. 300K where spectra are recorded after transfer to a high field magnet. This implementation of DNP requires efficient transfer of polarization from stable radicals to the samples which is facilitated by an optimal contact between the radical and the sample in a glass state formed at low temperature. The question is how to optimize polarization for small molecules, in particular for applications in organic chemistry, pharmaceutical chemistry and metabolomics.

We have therefore explored different strategies to optimize polarization for different substances, including typical metabolites and other small molecules. There are several determinants for efficient polarisation [2] including the time of the polarization, optimal contact between the radical and the sample [2]. Another contribution arises from methyl groups which seem to make a direct contribution to the overall polarization [3].

To improve the polarizability of small molecules we have explored different routes. One option is to introduce a long lived carbon by acetylation to enhance the life-time of other nuclei through a ^{13}CC-NOE. This allowed to record fast two-dimensional HMQC spectra during the life time of labeled carbonyl atom. Moreover, polarization transfer in the glass state can be further improved by optimal polarization matrix design. One option is to add co-polarization agents with long lived carbonyls to the glass state. This has substantially broadened the applicability of DNP to substances which are otherwise difficult to polarize, including citrate and glucose but also many small protein inhibitors. This new principle has also enabled metabolomics ^{13}C spectra or blood serum. Most interestingly the use of co-polarisation agents causes polarization arising from quantum rotor effects in methyl groups which can be exploited by choosing the correct microwave frequency [3].

In summary, the observed effects had significant impact for the application of DNP in metabolomics. Many molecules which could not be polarised yield a high polarization in the presence of co-polarisation agents and by using a different microwave frequency.

1. JH Ardenkjær-Larsen, B Fridlund, A Gram, G Hansson, L Hansson, MH Lerche, R Servin, M Thaning, and K Golman. Proc Nat Acad Sci USA, 100, 10158-10163 (2003).
2. Emwas A-H, Saunders M, Ludwig C, and Günther UL.. Determinants for Optimal Enhancement in ex situ DNP Experiments, Applied Magn Reson, in press.
3. Saunders MG, Ludwig C, and Günther UL.. Optimizing the Signal Enhancement In Cryogenic ex situ DNP-NMR, accepted in J Am Chem Soc.

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

NMR of Carbohydrates
Chair: Daron I. Freedberg

Speakers:

Goran Widmalm
Stockholm University, Sweden

Bruce Coxon
N.I.H., USA

James Prestegard
University of Georgia, USA

Conformational studies of oligosaccharides and their analogues using NMR spectroscopy

Göran Widmalm

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

Carbohydrates pose particular problems due to the narrow spectral range of the corresponding NMR resonances. A limited number of interactions between nuclei are usually present for analysis of their three-dimensional (3D) structure. Notably, in many cases only a single strong nuclear Overhauser effect is observed across each glycosidic linkage of an oligosaccharide. A complementary way to describe the conformational preference(s) at a glycosidic linkage is to utilize the hetero-nuclear three-bond coupling constants $^{3}J_{C,H}$, that subsequently can be interpreted by a Karplus-type relationship to give further information on the 3D structure. An additional source of information is attainable from NMR residual dipolar couplings (RDCs), which can be obtained when the molecule is dissolved in an orienting medium of low order. Importantly, high-resolution NMR spectroscopy techniques are then still possible to use [1].

Carbasugars are carbohydrate analogues in which the endocyclic ring oxygen has been replaced by a methylene group. Thus, the acetal linkage of the glycoside is then formally transformed into an ether for a carbasugar, and hence becomes hydrolytically stable [2]. In order to evaluate the conformation of carbaiduronic acid derivatives as hydrolytically stable mimics of iduronic acid, which may be of interest as inhibitors of carbohydrate-binding proteins, we have carried out a number of different NMR experiments such as J-HMBC [3], HSQC-HECADE [4], and an IPAP-hadamard-HSQC-TOCSY experiment with zero quantum suppression [5,6]. Using information from the NMR experiments we investigate conformation(s) of the analogues of iduronic acid.

In a previous study we determined the binding of a disaccharide to the protein WGA [7]. We here show that the conformational dynamics of the disaccharide can be unraveled using NMR spectroscopy experiments on the [6-^{13}C]-site-selectively labeled disaccharide.

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The Stereochemical Dependence of Scalar ^{15}N-^{1}H Coupling Constants in Amino Sugars

Bruce Coxon

National Institute of Child Health and Human Development,
National Institutes of Health, Bethesda, Maryland

The scalar ^{15}N-^{1}H NMR coupling constants of amino sugars over one to four bonds have been measured by three methods: (a) 1D ^{1}H NMR, (b) 1D and 2D ^{1}H-^{15}N heteronuclear, single quantum multiple bond correlation [1,2] (HSQMBC) using an HCN cryoprobe, and (c) 1D Carr-Purcell-Meiboom-Gill (CPMG) ^{1}H-^{15}N HSQMBC [3] using a normal broad band probe. Method (a) was applied to organic soluble, ^{15}N-labeled amino sugar derivatives synthesized by addition of phthalimide-^{15}N to carbohydrate epoxides [4-6], method (b) to similar, unlabeled derivatives, and method (c) to the biologically common glucosamine, mannosamine, and galactosamine (2-amino-2-deoxy sugars), as their pyranoid N-acetyl and hydrochloride derivatives in D2O and/or DMSO-d6 solutions. These amino sugars were usually present in the crystal as principally a single pyranose anomer, either α or β, which underwent inter-conversion to as many as four other forms on dissolution. The organic soluble group included a number of aminodeoxy-hexopyranose derivatives that were conformationally restricted to chair, boat, or skew forms by the attachment of anhydro or 4,6-O-benzylidene rings [4-7].

Dihedral angles φ_{HCCN} for the amino sugars were defined by molecular dynamics and mechanics calculations using either implicit solvent or explicit chloroform-d. Least squares fitting of the angles of the organic-soluble derivatives in the range φ_{HCCN} = 10°-172°, to the experimental $^{3}J_{HCCN}$ values allowed formulation [8] of a Karplus equation of the three-parameter type:

$$^{3}J_{HCCN} = 3.1 \cos2\varphi_{HCCN} - 0.6 \cos\varphi_{HCCN} + 0.4 \qquad (1)$$

The 4C1 chair conformations of the water-soluble amino sugars that were defined by their $^{3}J_{HCCH}$ values and molecular modeling provided a more restricted set of φ_{HCCN} angles (nominally 60° or 180°) than the conformationally limited, organic-soluble amino sugar derivatives. However, the $^{3}J_{HCCN}$ values measured for the water-soluble ones are in general agreement with equation (1). The results are potentially valuable for NMR characterization of amino sugar containing, bacterial polysaccharides of interest in vaccine development, and for structural analysis of aminoglycoside antibiotics.

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Structures of Chondroitin Sulfate Oligomers from RDCs and CSA Offsets

James H. Prestegard and Fei Yu

Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602

Chondroitin sulfate, like other members of the glycoaminoglycan family (heparin, heparan sulfate, keratin sulfate and dermatan sufate), is a linear polysaccharide composed of alternating amino sugar and uronic acid residues. It is widely expressed as a part of the proteoglycans found on mammalian cell surfaces. There it plays roles in adhesion and a variety of cell-cell signaling events. The origin of specificity in the molecular interactions underlying these events is a major unsolved issue. In part this is due to the extraordinary structural heterogeniety of these polymers. For chondroitin sulfate, which is built upon a simple alternation of N-acetylglactosamine and glucuronic acid residues, the heterogeniety arises entirely from differential sufation of the core residues. Even so, a simple hexasaccharide, carrying three sulfates on the most commonly used 4 and 6 sites of the N-acetyl galactosamine residues, can have more than 100 equal mass isoforms. NMR can, in principle, provide both primary structure and conformational characterization of such isoforms, and do so in the presence of proteins which preferentially bind the the isoforms.

The most effective NMR methods for primary structure analysis are based on two dimensional ^{1}H-^{13}C correlated spectra (HSQC, HMQC, HMBC). For conformational analysis NOEs can provide useful information, but as with other linear polymers, additional information relating relative geometries of remote regions is very important. This additional information can comes from residual dipolar couplings (RDCs) and, in some cases, chemical shift anisotropy (CSA) offsets.

When the analyis is carried out in the presence of receptor proteins it also becomes necessary to ^{13}C enrich some sites to facilitate acquisition of data at low protein and oligomer concentrations. Here we present an efficient method for introducing ^{13}C labeled acetates into native N-acetyl sites of chondroitin sulfate oligomers. RDC and CSA offset data from these sites are used to deduce preferred solution conformations as well as conformations when bound to a chemokine invovled in T-cell migration (CCL5). In addition, the CSA offsets, produced on partial orientation of oligomers are shown to greatly facilitate resolution of resonances for otherwise chemically degenerate sites.

Tuesday, September 9th
10:30 AM - 12:00 PM

Techniques and Characterization Methods in ^{19}F NMR

Chair: Elizabeth McCord

Speakers:

Steve Cheatham
DuPont Crop Protection, USA

Peter L. Rinaldi
University of Akron, USA

Cormac D. Murphy
University College Dublin, Belfield, Ireland

Myriam Malet-Martino
Université Paul Sabatier, France

BEBOP, BIBOP, and xy-BEBOP Pulses for Use in Analysis of Fluorinated Materials

Burkhard Luy[1] and **Steve Cheatham**[2]

1. Department of Chemistry, Technische Universität München, 85747 Garching, Germany
2. DuPont Crop Protection, Stine-Haskell Research Center, Newark, DE, 19714

Two-dimensional NMR on ^{19}F containing materials presents unique challenges because of the very wide chemical shift dispersion present in many fluorinated compounds. Recently, Optimal Control Theory has been used to develop broadband BEBOP and BIBOP pulses for excitation and inversion.1,2 The pulse families provide good phase behavior and have varying levels of B1-field compensation. However, pulses optimized for ^{19}F chemical shift ranges are exceedingly long and particularly excitation poses a significant problem. Therefore a new class of pulses has been developed, the so-called xy-BEBOP pulses capable of creating a 200 kHz excitation bandwidth at the cost of a defined phase behavior. The xy-BEBOP in combination with corresponding BIBOP inversion pulses enables the acquisition of various 2D NMR experiments on many fluorinated materials on spectrometers up to 1 GHz in magnitude mode. Here we demonstrate the use of the pulses for a series of 2D NMR experiments including ^{19}F-^{19}F and ^{19}F-^{13}C correlation experiments at 700 MHz. The advantages of the BEBOP and BIBOP pulses relative to other approaches to achieving wideband excitation in ^{19}F NMR will be discussed.

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Practical Aspects of Selective ^{19}F Homonuclear Multidimensional NMR

Peter L. Rinaldi[1] Silapong Baigern[1] Xiaohong Li[1] Sangrama Sahoo[2] and Elizabeth McCord[3]

1. Dept of Chemistry, The University of Akron, Akron, Ohio 44325-3601
2. Current address: Analytical & Engineering Sciences Division, Hercules Research Center, 500 Hercules Road, Wilmington, DE 19808-1599
3. Dupont Experimental Station, Bldg. 328, Rm 133, Route 141 and Powder Mill Rd, Wilmington, DE 19880-0328.

Structure studies of highly fluorinated materials offer some unique opportunities to use multidimensional NMR techniques. The high abundance and large chemical shift range make it possible to resolve resonances from many structure components. However, these properties, together with the large magnitude of $^{2}J_{FF}$ (ca. 200 Hz), and the prevalence of 4, 5, and even 6-bond ^{19}F homonuclear couplings present some interesting challenges when attempting to use variations of ^{19}F-^{19}F COSY experiments. In this presentation we will present some of our results and experiences in applying various versions of selective ^{19}F-^{19}F homonuclear correlation experiments. Results will be shown from model structures 2,2,3,4,4,4-hexafluorobutanol and low molecular weight oligomers (dimers and trimers) of poly(1,1,2,2,3,3,3-heptafluoro-propylene oxide).

Probing the Interaction Between Fluorinated Compounds and Microorganisms Using ^{19}F NMR

Cormac D. Murphy

UCD School of Biomolecular and Biomedical Science and Centre for Synthesis and Chemical Biology, Ardmore House, University College Dublin, Belfield, Dublin 4, Ireland

Microorganisms have an emerging role in the synthesis and degradation of organofluorine compounds, ever increasing numbers of which are employed as industrial, pharmaceutical and agrochemical reagents resulting in the accumulation of these compounds in the environment. Despite the potential of microorganisms for organofluorine production and remediation our understanding of microbial biotransformations of these compounds is at an early stage. A key technique in the study of microbial interactions with fluorinated compounds, either in whole cells or cell free systems, is ^{19}F NMR, which has the significant advantage of enabling analysis of fluorometabolites in culture supernatant or enzyme assay without purification or derivatisation. This technique has been highly instrumental in the recent discovery of the first fluorinase in the bacterium Streptomyces cattleya [1]. Furthermore, a considerable body of work has been conducted investigating the biodegradation of model fluorinated pollutants, such as fluorophenols and fluorobiphenyls, which has employed ^{19}F NMR to establish the biochemical pathways involved [2, 3]. In this paper I will explore the applications and limitations of this technique in microbial systems.

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Interest of ^{19}F NMR to Detect, Identify and Quantify New Metabolites of Fluorinated Antifungal and Anticancer Drugs in Humans

Myriam Malet-Martino

Groupe de RMN Biomédicale, Laboratoire SPCMIB, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse, France

^{19}F NMR spectroscopy provides a highly specific tool for the detection, identification and quantification of fluorine-containing drugs and their metabolites in intact human biofluids as well as fluorinated degradation compounds of fluorouracil in commercial vials. The value and difficulties encountered in investigations on drug metabolism will be first discussed. Then the metabolism of three fluoropyrimidines in clinical use, 5-fluorouracil, 5-fluorocytosine and capecitabine will be presented. Besides the parent drug and the already known fluorinated metabolites, twelve new metabolites were identified for the first time with ^{19}F NMR in human biofluids. Nine of them can only be observed with this technique: fluoride ion, N-carboxy-α-fluoro-β-alanine, α-fluoro-β-alanine conjugate with deoxycholic acid, 2-fluoro-3-hydroxypropanoic acid, fluoroacetic acid, O2-β-glucuronide of fluorocytosine, fluoroacetaldehyde hydrate and its adduct with urea, fluoromalonic acid semi-aldehyde adducts with urea. The contribution of ^{19}F NMR to the understanding of 5-fluorouracil cardiotoxicity will be highlighted.

Tuesday, September 9th
1:30 PM - 3:00 PM

Computer Aided Structure Identification
Chair: Patrick Wheeler

Speakers:

Mikhail Elyashberg
Russian Academy of Sciences, Russia

Richard Beger
National Center for Toxicological Research

Richard Newmark
Newark Consulting

CASE Systems: A Review of the Last 40 Years

Mikhail Elyashberg

Russian Academy of Sciences, Moscow, Russia

Research devoted to creating methods for Computer-Aided Structure Elucidation (CASE) began in the late 1960s by four independent groups of scientists in USA, Japan, and Russia. Their goal was to create artificial intelligence programs capable of modeling the thought processes of an NMR spectroscopist for the determination of the chemical structures of organic compounds using molecular spectra.

In the 1970s, researchers realized the general workflows required for an expert system. These systems were based mainly on 1D NMR spectra and on a set of "axioms" reflecting the relationship between spectrum and structure. The scheme included the selection of molecular fragments, subsequent structure generation from these fragments, and choosing the most probable structure based on the data provided. This structure was selected by comparing the best fit correlation between the candidate structure's experimental data vs. data generated from spectral predictions; an ideology which remains valid. These first generation systems provided invaluable knowledge to future CASE systems but were limited by their inability to identify molecules with more than 20 skeletal atoms. This limitation resulted in little progress in the 1980s.

The worldwide adoption of 2D NMR techniques breathed new life into this field in the 1990s, and second generation expert systems appeared. The most advanced 2D NMR systems used 1D and 2D NMR data along with corresponding sets of "axioms". The knowledge base consists of factual knowledge (databases containing structures and fragments with assigned NMR spectra) and axiomatic knowledge (rules of spectrum interpretation and spectrum-structure correlations). The validity of these systems was assessed by attempting to solve the structures of real-world complex natural products based on their 1D and 2D NMR data. Details on the performance of these systems will be outlined in this presentation.

In the early 2000s, the major challenges for CASE systems surrounded the size of output files, the speed of NMR prediction for verification of proposed structures, and the determination of relative stereochemistry. These challenges of file size and speed have been managed with the development of new methods to be discussed in this presentation. The challenge of determining the relative stereochemistry of rigid molecules having large numbers of stereocenters has been addressed with the use of NOESY/ROESY data and the development of a genetic algorithm.

The purpose of this presentation is to provide a detailed review on the progress of CASE over the last 40 years and an update on the most successful approaches and systems today. Based on the discoveries and developments over this 40 year quest, the presenter is deeply convinced that CASE expert systems can become a routine tool in NMR laboratories in the next 5–10 years.

An In Silico Approach to Rational Antipsychotic Drug Design using Spectral Data Activity Relationship

Neil Mitchell[1], Devin Howington[1], Dwight Miller[1], Bill Massey[1,2], Herbert Y. Meltzer[3], Dan Buzatu[4], Jon Wilkes[4], **Richard Beger**[4]

1. LITMUS Molecular Design, Ramona, CA 92065,
2. University of Arkansas for Medical Sciences, Little Rock, AR 72205,
3. Vanderbilt University, Nashville, TN 37212,
4. National Center for Toxicological Research, US FDA, Jefferson, AR 72079

Spectral data activity relationship (SDAR) modeling is a computational method that uses spectral characteristics of small molecules and relates them to their biological activity. SDAR modeling can be used to screen for molecules for a particular biological activity (e.g., efficacy, toxicity) and design novel drugs having a desired activity profile with a high degree of accuracy. The present purpose was to use SDAR modeling to create a series of atypical antipsychotic drug candidates predicted to have targeted characteristics that meet unmet medical treatment of schizophrenia. SDAR models were created for efficacy and human toxicity endpoints. The models were tested using a random leave-10%-out (q210%) cross validation and with external test sets. Highly accurate models were created for binding affinities to dopamine (D2) (q210% = 0.96) and 5-HT2A (q210% = 0.99) receptors. SDAR models were created for human agranulocytosis relative risk (q210% = 0.98) and hERG inhibition (q210% = 1.00). Data extracted from the models were used to create 125 in silico new atypical antipsychotic drug candidates. When screened against the efficacy SDAR models, 104 drug candidates were predicted to have antipsychotic activity, 21 of those 83 had the desired receptor binding affinities. After these drug candidates were predicted for toxicity, 12 were selected for synthesis and further validation. The first two compounds have been synthesized and confirmed in vitro and in vivo as exhibiting atypical antipsychotic behavior. Using SDAR models and rational drug design principles, the entire process took 6 months. The time and cost savings realized from SDAR modeling could have positive ramifications for the economics of the pharmaceutical industry.

Computer Aided (Additivity) Chemical Shift Prediction in Multiply Halogenated Organics Using an Excel Visual Basic Program

Richard A Newmark

Newmark Consulting (retired, 3M), St. Paul, Minnesota

Several methods have been reported for calculating aliphatic and olefinic ^{13}C NMR chemical shifts of substituted hydrocarbons. However, the simple additivity methods fail in the presence of multiply halogenated organic molecules. Dostovalova et al. (Org. Magn. Reson. 1982, 16, 251) proposed corrections to the calculated ^{13}C shifts as a function of the number of geminal and vicinal halogens and calculated the parameters for 48 brominated and/or chlorinated aliphatic hydrocarbons This concept has been extended to fluorine and halogenated olefins. A least squares fit of the observed chemical shifts of over 125 aliphatic and olefinic organic compounds containing multiple fluorine, bromine, or chlorine atoms were used to optimize the parameters. Calculations were performed using an Excel Visual Basic program.

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

NMR of Less Common Nuclei
Chair: Dave Lankin

Speakers:

Anthony Ribeiro
Duke University, USA

Jan Schraml
Institute of Chemical Process Fundamentals of the ASCR v.v.i.; Czech Republic

Rob Kleps
University of Illinois at Chicago

Phosphorous NMR Insights on Novel Inositol Sugars and Phosphoglycolipids from Gram-Negative and Gram-Positive Bacteria

Anthony Ribeiro

Duke NMR Center and Departments of Radiology and Biochemistry
Duke University Medical Center, Durham, North Carolina

^{31}P NMR is a critical tool allowing unique insights into three lipid-related areas:

1) Biochemical analysis of inositol phosphate kinases (IPKs). The water-soluble inositol phosphates (IP#s #=3-n) that result from IPK activity are secondary messenger molecules involved in cellular functions such as transcription, mRNA export, telomere length, chromatin remodeling and endocytosis. NMR, especially ^{31}P, plays a key role in identifying the phosphorylation level and structure of IPs and inositol pyrophosphates (PP-IPs) and verifying the key IPKs necessary for producing the diverse array of IP species [1,2].

2) Gram-negative bacterial pathogenesis. Gram-negative bacterial infections are associated with Lipid A (endotoxin), a water-insoluble glucosamine-based phosphoglycolipid (PGL) that makes up the outer monolayer of the outer membrane. Solution NMR studies of purified Lipid As encounter major challenges from lipid heterogeneity, chemical stability and molecular aggregation. NMR results are shown for Lipid A variants from diverse sources and pathogeneses such as *Escherichia coli* (sepsis or Gram-negative fever) [3], *Salmonella typhimurium* (Salmonella poisoning) [4], *Leptospira interrogans* (leptospirosis) [5] and *Franciscella novicida* (tularemia) [6,7].

3) Gram-positive bacterial pathogenesis. Gram-positive bacteria lack the outer membrane of Gram-negative bacteria but still produce PGLs. NMR results will be discussed for a novel water-insoluble PGL isolated from *Clostridium tetanii*, the pathogen involved in tetanus.

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Current Topics in ^{29}Si NMR of Solutions

Jan Schraml, Vratislav Blechta, Jan Sykora, and Milan Kurfurst

Institute of Chemical Process Fundamental of the ASCR, v. v. i.
Rozvojova 135, Prague 6, 165 02, Czech Republic

Components of organosilicon mixtures (be it mixtures of industrially important oligosiloxanes or silylated natural products) are separated and identified through LC-NMR with indirectly detected ^{29}Si NMR offering an additional advantage to the method. LC-NMR with reversed phase HPLC part surpasses in separation and resolution size exclusion chromatography as well as DOSY. The ^{29}Si NMR part offers the means of identification of each component.[1,2]

To enhance possibilities of oligosiloxane identification through ADEQUATE experiments, the structural dependence of ^{29}Si-O-^{29}Si couplings was established.[3]

For similar purpose of identification of silylated natural products ^{29}Si-^{13}C couplings over Si-O-C link were measured in various structural moieties, some signs of the coupling constants were determined [4] and the validity of the signs extended to other compounds as backed up by quantum chemical calculations. The found dependence of the geminal and vicinal ^{29}Si-^{13}C couplings on branching at the α-carbon calls for careful interpretation of 2D and 3D experiments involving a ^{29}Si-^{13}C polarization transfer.[5,6]

The financial support was provided by the Czech Science Foundation (grant no. 203/06/0738) and by the Grant Agency of AS CR (grant no. IAA 400720706).

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^{31}P NMR Discovers a Sex–Specific Metabolite

Robert A. Kleps, Rom N. Lipcius, and Thomas O. Henderson

Research Resources Center, University of Illinois at Chicago
Virginia Institute of Marine Sciences
Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago

Phosphorus, as phosphate and its esters and anhydrides, have many functions in biological systems: structural in bone and membranes, regulatory when phosphorylating small molecules and enzymes and providing pathways for energy metabolism and viability. Some phosphorus metabolites have an unknown function as in this presentation. What started as a simple P31 NMR survey of small phosphorus metabolites in blue crabs may become a new perspective on sexual dimorphism at the metabolite level.

Hormone level differences are generally accepted as the primary cause for sexual dimorphism in animal and human development. Levels of low molecular weight metabolites differ between men and women in circulating amino acids, lipids and carbohydrates and within brain tissue.

While investigating the metabolism of blue crab tissues using Phosphorus-31 Nuclear Magnetic Resonance, we discovered that only the male blue crab (Callinectes sapidus) contained a phosphorus compound with a chemical shift well separated from the expected phosphate compounds. Spectra obtained from male gills were readily differentiated from female gill spectra. Analysis from six years of data from male and female crabs documented that the sex-specificity of this metabolite (2-Aminoethyl phosphonic acid) was normal for this species. Analysis of a rare gynandromorph blue crab (literally and laterally, half male and half female) proved that this sex-specificity was an intrinsic biochemical process and was not caused by any variations in the diet or habitat of male versus female crabs.

The existence of a sex-specific metabolite is a previously unrecognized, but potentially significant biochemical phenomenon. An entire enzyme system has been synthesized and activated only in one sex. Unless blue crabs are a unique species, sex-specific metabolites are likely to be present in other animals. Would the presence or absence of a sex-specific metabolite affect an animal's development, anatomy and biochemistry?

I'm disappointed that this research has produced many more questions than answers, but I'm still fascinated when mysteries are found in the mundane.

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

Student and Post-Doctoral Session
Chair: George Furst

Speakers:

Ben Nelson
University of Kansas

Xi Meng
University of California, Irvine

P. Aru Hill
University of Pennsylvania, USA

Multiple-Sample Probe for High-Throughput Solid-State NMR Spectroscopy

B. Nelson[1], L. Schieber[1], D. Barich[1], J. Lubach[1], T. Offerdahl[1], D. Lewis[2], J. Heinrich[2], and E. Munson[1]

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

Solid-state NMR spectroscopy (SSNMR) is the most powerful technique for the analysis of pharmaceuticals and pharmaceutical formulations because of the vast quantity of molecular structure and dynamics information that can be obtained from NMR spectra. It is a non-invasive and non-destructive technique that can determine the physical state of the drug within a formulation matrix. It is selective in that the excipients in a drug formulation will often times have a different chemical shift than the API (active pharmaceutical ingredient). In addition, SSNMR can be used to quantitate solid forms within a pharmaceutical formulation. New drug compounds are often poorly crystalline or amorphous, and are present at low levels in a formulation. This leads to a greater number of transients needed to acquire an adequate signal to noise ratio, which could take hours to days to accomplish. To complete a series of formulations, which is often the case during drug development, would take much longer. Multiple sample solid-state NMR probes (MSS Probes) are being developed in order to combat these issues. The MSS Probes take advantage of relatively long T1 relaxation rates by switching between samples that are all spinning at the same time in the probe. One spectrometer, one magnet, and one set of amplifiers are used and there is the potential for through put increase of 2-20. Each separate circuit can be running a different experiment, be acquiring separate nuclei, and spinning at different rates.

"Minimal Chemical Shift Maps" for Compound Matching and Identification

Xi Meng, Bao D. Nguyen, A. J. Shaka

Chemistry Department, University of California, Irvine, CA 92697-2025

One way to unambiguously match and identify compounds is to obtain 2D heteronuclear correlation maps with the best possible resolution. Obtaining a single sharp peak for each and every C-H pair offers such resolution. To achieve this, proton-proton couplings must be eliminated or decoupled. Although a Constant Time HETCOR experiment has provided single peaks, the poor resolution along the proton dimension and missing peaks, caused by $^{n}J_{HH}$ antiphase coherence, limits its efficiency for accurate chemical shift maps [1]. We modified the pulse sequence and data analysis to obtain true high-resolution minimal chemical shift maps. The key is to use Filter Diagonalization Method (FDM) [2, 3]. Simultaneously, this strategy makes chemical shift database methods easier to construct and apply. Time permitting, we will also show how the basic idea can be extendended to more complex high-dimensional NMR experiments.

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Xenon-129 NMR Biosensors for Cellular Imaging

P. Aru Hill[1], Garry K. Seward[1], Julie A. Aaron[1], Najat Khan[1], Zhaohui Han[2], Jennifer M. Chambers[1], Tara Kaufman[1], Qian Wei[1], David W. Christianson[1], Nicholas N. Kuzma[2], and Ivan J. Dmochowski[1].

1. Department of Chemistry, University of Pennsylvania, 231 South 34th St., Philadelphia, PA 19104, USA.
2. Department of Biomedical Engineering, University of Rochester, Goergen Hall, Box 270168, Rochester, NY 14627, USA.

Xenon-129 biosensors offer an attractive addition to conventional MRI contrast agents. Conjugation of a xenon-binding molecule to a biological recognition moiety creates a biosensor that generates a new ^{129}Xe resonance upon binding to a target analyte. Given the chemical shift sensitivity and large NMR signal of hyperpolarized ^{129}Xe, the possibility exists of frequency-resolved imaging of multiple xenon-129 biosensor resonances associated with different biomarkers. For example, cryptophane biosensors have been targeted to human carbonic anhydrase (CA) by attaching para-substituted benzenesulfonamide. CA-biosensor association produced significant changes in the hyperpolarized ^{129}Xe NMR chemical shift, with $\Delta\delta$ (^{129}Xe) as large as 5.5 ppm. Binding affinities of up to 33,000 M-1 at 293 K have also been measured for water-soluble cryptophanes using both fluorescence quenching and ITC which is the highest known affinity for xenon of any synthetic or natural host molecule. These studies, and the insights gained from them, are guiding the rational design of new cryptophanes for detecting cancer biomarkers in living cells.

Tuesday, September 9th 8:00 PM - 10:00 PM

Poster Session

Chair: Steve Cheatham

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24. Determining Enantiomeric Excess by ^{13}C-NMR in Chiral Liquid Crystal Media: A Simple and Rapid Method with No Racemate Needed

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26. Determination of Herbal Drug Contents and Identification of Adulterants by 2D DOSY ^{1}H NMR

27. Monitoring of Grignard Reaction Intermediate by MICCS-NMR

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45. Automatic Chemical Shift Referencing of Homonuclear 2D NMR Spectra

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47. Bayesian Dosy: A New Approach to Diffusion Data Processing

48. HMBC (Heteronuclear Multiple-Bond Correlation) with Frequency-Swept Pulses

49. Analysis of Pretreated Corn Cob Extracts by Coupled HPLC/MS/SPE/NMR Technology

50. A Macro To Create All Compound Specific qNMR Macros

1 Automated Natural Product Extract Screening Using ^{1}H NMR

Kimberly L. Colson[1], Cherie A. Motti[2], Peter Neidig[3], Christian Fischer[3], Joshua M. Hicks[1] and Dianne Tapiolas[2]

1. Bruker BioSpin Corp., Billerica, MA, USA
2. Australian Institute for Marine Science, Townsville, Queensland, Australia
3. Bruker BioSpin GmbH, Rheinstetten, Germany

^{1}H NMR spectra of marine organic extracts contain a wealth of information on the compounds present and can help researchers identify extracts of interest. Targeting those extracts that promise to deliver natural products of a desired chemotype or from a particular biological pathway is of considerable interest to most natural product laboratories. In most such laboratories there is one or a few skilled individuals with a trained 'eye' towards identifying interesting extracts. Efficient dereplication of the extracts is essential if expensive isolation and structure elucidation resources are not wasted and that resources are focused primarily on the isolation of key compounds. Recent advancements in the analysis of complex mixtures in the field of metabolomics have driven extensive efforts to develop software, such as AMIX, and spectral databases capable of classifying spectra and detecting the presence of individual specific compounds within a mixture of hundreds of small molecules without requiring compound isolation. To this end, we applied these methods to marine organic extracts to establish techniques to assist the natural products researcher in rapidly classifying extracts of interest. Our results show that it is possible to rapidly screen and dereplicate marine extracts using ^{1}H NMR spectra. Automated identification of particular compounds present in the complex mixture is also possible.

2 Resolution of Diastereoisomers via NMR

S. Capacchi[1], M. Lipreri[1], A. Rizzi[2], C. Caló[2], S. Catinella[1], and T. Peveri[1]

1. Analytical Chemistry Department, Chiesi Farmaceutici s.p.a., Via S. Leonardo 96, 43100 Parma, ITALY.
2. Chemistry Unit - Chemical Synthesis Dept, Chiesi Farmaceutici s.p.a.,Via S. Leonardo 96, 43100 Parma, ITALY

Diastereoisomers CHF4226.01 and CHF4232.01, differing for a chiral centre, were investigated via NMR by means of 1D NMR and 2D NMR experiments. The aim of the work was to set up a new NMR method, fast and simple, that could easily be applied in every laboratory and allow the NMR resolution of the isomers. In order to hopefully form adducts showing bigger shift effects [1-7] than the ones found in the isomers alone, chiral reagent (S)-(-)-1-(2-napthyl)ethylamine (S-NEA) was added to each diastereoisomer. 1D and 2D (COSY, NOESY and ROESY) were then made on the adducts formed in the presence of S-NEA, and some important differences were pointed out helping us over recognizing one diastereoisomer from the other. Moreover computational studies, concerning conformational analysis and molecular dynamics, were also started and supported the NMR results.

Subsequently, we applied our procedure to practical problems, such as recognition and quantification of the two isomers in mixtures presenting varied ratios of CHF4226.01 and CHF4232.01 (50-50%, 90-10%, 95-5% and 99-1%). Another application was the NMR control on inhalation solution of CHF4226.01: following our procedure, it was confirmed that CHF4226.01 was still pure and no isomerisation to give CHF4232.01 occurred. In this way, we found an NMR method, simple and fast, able to recognize isomers and whose practical applications could lead to quick results for mixtures' and inhalation solutions' studies.

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3 Automated NMR Verification of Pharmaceutical Compounds with 2D HSQC Data in an Open Access Environment

Philip Keyes, Gonzalo Hernandez

Lexicon Pharmaceuticals

A novel approach to verify medicinal chemistry compounds during the registration process using open access NMR data has been implemented at Lexicon Pharmaceuticals. Chemists register their compounds in the corporate compound registration system resulting in automated database capture of analytical data. Upon passing prerequisite HPLC and LCMS analyses, automated combined verification of Proton and HSQC NMR data is triggered. A comparison of predicted to observed data, together with the generation of proposed assignments annotated on the spectra, is performed. The result ultimately reduces to a simple red, yellow, or green result. The automated workflow enhances efficiency and quality as it provides the spectroscopist with a virtual assistant to identify compounds more likely to need further examination. Examination of the process using commercial compounds with positive and negative controls (correct vs. incorrect structures) provided measurable feedback regarding the accuracy of the pass/fail results. The negative controls (similar imposter structures) were tested against the experimental data to measure false positive feedback. The similarity coefficient was calculated between the correct and incorrect structures to insure that the measurement of the systems accuracy was realistic. Results of over 750 compounds are described.

4 Solid State NMR of Curcumin and Derivatives

Sonia Recillas[1], Carolina Escobedo[1], Raul G. Enriquez [1],
Juan M. Fernandez-Gonzalez [1], Dino Gnecco[2], Benjamin Ortiz[1],
andWilliam F. Reynolds[1]

1. Instituto de Quimica, UNAM, México
2. Centro de Química, BUAP, México
3. Chemistry Department, University of Toronto, Canada.

Curcumin is a fascinating natural product which is currently receiving a great deal of attention for its therapeutic attributes including antitumoral activity, anti-inflammatory benefits, and general chemo-preventive agent against Alzheimer among others. The solid state NMR spectra of Curcumin and some metal derivatives as well as the preliminar results of cytotoxic studies in vitro are presented and discussed.

5 Miniaturised NMR Probe Based on a Microstrip Design

Hans Georg Krojanski, Jörg Lambert and Roland Hergenröder

ISAS – Institute for Analytical Sciences, P.O.Box 10 13 52, D-44013 Dortmund, Germany

Many potential NMR applications like membranes, thin layers, single cells and microfluidic chips come up with sample volumes in the low nanoliter range. Suitable NMR microprobes are usually based on capillary sample chambers with tightly wound helical coils wrapped around the capillary. The helical coil geometry imposes, however, restrictions on the sample shape and impedes measurements in microfluidic chips. Moreover, currently available microcoil designs have a poor radiofrequency field homogeneity, i.e. multiple quantum NMR techniques are difficult to implement. A novel NMR probe design, the slotted microstrip, not only addresses both shortcomings of conventional microcoils, but also allows sample volumes in the nanoliter range (and even smaller) to be measured. In microwave technology, microstrips are used as waveguides to route high frequency signals with low losses and well defined delays on printed circuit boards. The electric current generating the radiofrequency field is carried by a thin strip of copper, mounted on a PTFE board with a copper groundplane on its back. If a small slot (typically: 200 x 130 micrometer^2) is generated in the center of the microstrip, the radiofrequency field is confined in a small region of space and becomes very homogeneous, as simulations show. The sample is positioned in a capillary tube above the slot. Though the linewidths of the signals obtained with the microstrip probe are by factor of 5 higher than those of the commercial probe (the magnetic susceptibility of the components of the microstrip probe has not yet been matched), the sensitivity of the slotted microstrip probe is by a factor of approximately 70000 higher than that of the commercial probe. Results for hyphenation of the microstrip probe with capillary electrophoresis as well as a microfluidic chip based high throughput device are presented.

6 Current Topics in ^{29}Si NMR of Solutions

Jan Schram, Vratislav Blechta, Jan Sykora, and Milan Kurfurst

Institute of Chemical Process Fundamental of the ASCR, v. v. i. Rozvojova 135, Prague 6, 165 02, Czech Republic

Components of organosilicon mixtures (be it mixtures of industrially important oligosiloxanes or silylated natural products) are separated and identified through LC-NMR with indirectly detected ^{29}Si NMR offering an additional advantage to the method. LC-NMR with reversed phase HPLC part surpasses in separation and resolution size exclusion chromatography as well as DOSY. The ^{29}Si NMR part offers the means of identification of each component.[1,2]

To enhance possibilities of oligosiloxane identification through ADEQUATE experiments, the structural dependence of ^{29}Si-O-^{29}Si couplings was established.[3]

For similar purpose of identification of silylated natural products ^{29}Si-^{13}C couplings over Si-O-C link were measured in various structural moieties, some signs of the coupling constants were determined [4] and the validity of the signs extended to other compounds as backed up by quantum chemical calculations. The found dependence of the geminal and vicinal ^{29}Si-^{13}C couplings on branching at the α-carbon calls for careful interpretation of 2D and 3D experiments involving a ^{29}Si-^{13}C polarization transfer.[5,6]

The financial support was provided by the Czech Science Foundation (grant no. 203/06/0738) and by the Grant Agency of AS CR (grant no. IAA 400720706).

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7 Application of Band Selective Small Flip Angle COSY for the Analyses of ^{1}H NMR Spectra of Small Chiral Molecules

Uday Ramesh Prabhu and N. Suryaprakash

NMR Research Centre, Indian Institute of Science, Bangalore 560012, India

The NMR spectroscopic discrimination of optical enantiomers in the chiral liquid crystalline solvent is more often carried out using ^{2}H and ^{13}C detection. The employment of ^{1}H detection for such a purpose is severely hampered due to significant loss of resolution in addition to indistinguishable overlap of the spectra from the two enantiomers. The present study demonstrates that the well known band selected small flip angle homonuclear correlation experiment is a simple and robust method that provides unambiguous discrimination, incredible spectral resolution, reduced multiplicity of transitions, relative signs of the couplings and enormous saving of instrument time.

8 Indole Scaffold Anion Receptors: An NMR Study On Conformational Properties And Preorganization

Damjan Makuc and Janez Plavec

Slovenian NMR Centre, National Institute of Chemistry,
Hajdrihova 19, Ljubljana, Slovenia

Anion recognition and sensing can be effectively tuned by the choice of functional groups containing N–H hydrogen bond donors on an indole scaffold. The rapid growth in this area of supramolecular chemistry [1-5] has been stimulated by numerous roles that anions play in biology, medicine, catalysis and the environment. Receptors in question are based on the indole ring with a ureido as well as an amide moiety, which are further substituted with alkyl chain or aromatic ring. Indole based anion hosts undergo interactions with anions (e.g. halide, nitrate, acetate) through hydrogen bonding.

The purpose of our NMR study is to establish the potential conformational preorganization and conformational changes of indole anion hosts upon interactions with fluoride, chloride, bromide, nitrate and acetate anions. Our preliminary experiments showed that NMR parameters exhibit significant variations in different solvents. Dilution of the anion receptors suggested that they undergo considerable π-stacking interactions in non-polar solvents. Two indicative NMR parameters were monitored upon addition of anions. The perusal of chemical shift changes showed that some anions (F-, Cl-, AcO-) exhibit greater changes than others (Br-, NO3-) which indicates the role of size and shape of anions on the binding constant. Changes of NOE enhancements suggested that the preferred conformations in chloroform or acetone change significantly upon addition of anions. These results are consistent with our calculations at B3LYP/6-311+G(d,p) level in vacuo.

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We would like to thank Prof. Markus Albrecht and Triyanti from Institute of Organic Chemistry, RWTH Aachen for providing the anion receptors.

9 Investigation of the Structure and Source of Dimeric Impurities in AMG 517 Drug Substance

Maria Victoria Silva Elipe, Zhixin Jessica Tan, Michael Ronk, Tracy Bostick

Amgen, Inc., Thousand Oaks, CA, 91320, USA

In the initial scale-up batches of the experimental drug substance AMG 517, a pair of unexpected impurities was observed by HPLC. Analysis of data from initial LC-MS experiments indicated the presence of two dimer-like molecules. One impurity had an additional sulfur atom incorporated into its structure relative to the other impurity. Isolation of the impurities was performed, and further structural elucidation experiments were conducted with high resolution LC-MS and 2D NMR. The dimeric structures were confirmed, with one of the impurities having an unexpected C-S-C linkage. Based on the synthetic route of AMG 517, it was unlikely that these impurities were generated during the last two steps of the process. Stress studies on the enriched impurities were carried out to further confirm the existence of the C-S-C linkage in the benzothiazole portion of AMG 517. Further investigation revealed that these two dimeric impurities originated from existing impurities in the AMG 517 starting material, N-acetyl benzothiazole. The characterization of these two dimeric impurities allowed for better quality control of new batches of the N-acetyl benzothiazole starting material. As a result, subsequent batches of AMG 517 contained no reportable levels of these two impurities.

10 Vicinal $^3J_{FF}$ in a Perfluorinated Chain are Large but Opposite in Sign

Richard A. Newmark

Newmark Consulting, 810 Woodduck Drive; Woodbury MN 55125

It is generally assumed that $^3J_{FF}$ is small (under 1 Hz) in a CF_2CF_2 or CF_3CF_2 group in a perfluorinated chain, such as $C_7F_{15}CO_2H$. Vicinal $^3J_{FF}$ is very dependent on electronegativity [1] and ranges from +3.5 Hz in CF_3-CF_3 [2] to -24 Hz in CF_2X-CFH_2, for X = Br and I [3]. It is generally assumed that the electronegativity of CF_2 or CF3 groups is such that $^3J_{FF} \sim 0$. However, Harris and Woodman [3] in 1968 determined the vicinal CF_2CF_2 coupling constants $^3J_{MX}$ = +12.2 Hz and $^3J_{MX'}$ = -5.4 Hz in perfluorobutane, $CF_3CF_2CF_2CF_3$ by analyzing the naturally abundant ^{13}C isomer $CF_3{}^{13}CF_2CF_2CF_3$ that renders the two CF_2 groups chemically inequivalent. Harris and Sheppard [4] analyzed the ^{13}C isotopomers in CF_2BrCF_2Br and CF_2ClCF_2Cl. They found J_{MX} = 8.6 and $J_{MX'}$ = 4.5 in the dibromo compound and J_{MX} = 6.5 and $J_{MX'}$ = 1.0 in the dichlorocompound. Unlike the perfluorobutane example, both couplings are of the same sign, but this is consistent with the electronegativity of the vicinal J_{FF}. These result suggests that the near 0 values of $^3J_{FF}$ observed in CF_2CF_2 groups occurs by fortuitous cancellation of large gauche and trans $^3J_{FF}$ coupling constants of opposite signs. In most cases the AA′XX′ magnetic inequivalence of CF_2 groups is difficult to observe and analysis of this second order spin system requires observation of two very weak resonances. I have obtained ^{19}F NMR spectra at 3M of perfluorobutyric acid, $CF_3CF_2CF_2CO_2H$, and perfluorobutyl alcohol, $CF_3CF_2CF_2CH_2OH$. Analysis of the AA′XX′ CF_2CF_2 portion of the molecule indicates $J_{(AX)}$ = 4.6 and $J_{(AX')}$ = -4.1 in the acid and $J_{(AX)}$ = 8.9 and $J_{(AX')}$ = -4.1 in the alcohol. Details of the analysis will be presented including examples of highly resolved ^{19}F NMR spectra using 2D J-resolved spectroscopy.

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11 Structure-Activity Studies of Deoxycytidine Kinase with Novel 4'-azido-2'-deoxy-nucleosides Using NMR Analysis

Tatiana Maltseva[1], Elena Usova[2], Nils-Gunnar Johansson[1], Genadiy Kalayanov[1], Staffan Eriksson[2], Christian Sund[1], Anna Winqvist[1], Xiao-Xiong Zhou[1], David B. Smith3, Klaus Klumpp[3], Mark Smith[3], Hans Maag[3] and Joseph A. Martin[3]

1. Medivir AB, Huddinge, Sweden
2. Swedish University of Agricultural Sciences, Dept. Mol. Biosciences, The Biomedical Centre, Uppsala, Sweden
3. Roche Palo Alto LLC, 3431 Hillview Avenue, Palo Alto, CA 94304, USA

The study of the mechanism of the phosphorylation of the nucleoside by human recombinant deoxycytidine kinase (dCK) is a hot research area for the discovery of potential drug candidates. The aim of this study was to try to establish a relation between the conformation of the cytidine 4'-azido-2'-3' modified nucleosides in free form and bound with dCK, "near transition state", and the phosphorylation activity of the enzyme. The conformation of the bound state of these nucleosides has been defined by using transfer nOe experiments and J-coupling analysis. The main conclusions could be summarized as follows:

1. The phosphorylation activity of dCK does not correlate (and could not be predicted) in a direct manner based only on the conformation of the sugar moiety of the 4'-azido-2'-3' modified nucleosides in solution.
2. All studied nucleoside analogs, despite that they show different phosphorylation activity by dCK, form a weak binding complex with dCK. This unambiguously was confirmed by observing positive nOe belonging to the ligand-ligand interaction in 2D TRNOESY of the complex ligand-dCK versus the corresponding negative nOe of the free nucleosides.
3. The conformation of the sugar moiety of the 4'-azido-2'-or 3' modified nucleosides in the bound "near transition state" with dCK seems not to be locked by the enzyme and to be similar to the conformation observed in solution.
4. The "common wisdom" that a C3'-endo conformation enhances phosphorylation is not supported by the data obtained in this study.

12 ^{1}H NMR Study Of Northeast Brazilian Wines

Humberto G. Neto[1], João B.P. da Silva[1], Giuliano E. Pereira[2] and Fernando Hallwass[1]

1. Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil
2. EMBRAPA Uva e Vinho/Semi-Árido, Petrolina-PE-Brazil

The new Brazilian frontier in the vitiviniculture activity is located at Northeast region in Pernambuco State, closed to the equator line (8º latitude south). Comparing with other Brazilian vineyards, the grapes of this region possess peculiar characteristics, producing high quality wines called "wines of sun" (flavor, lights and ideal to be consumed at the first years after winemaking process). This characteristic is due to the singularity of the hot climate and soil of this region, being the second great producer of wine in Brazil. Additionally, in the last few years Northeast Brazilian wines have made their debut on both national and international markets.

The extensive European studies on wines have encouraged us to undertake a preliminary ^{1}H NMR study of Brazilian Wines [1]. Moreover, these studies have shown that regional and cultivars-based differences can be identified by NMR spectroscopy. ^{1}H NMR spectroscopy allows one to quantify, in a single analysis, the major sugars, organic and amino acids and phenolic compounds and thus to obtain the metabolic profiles in a complex mixture.

This study determined the metabolite profiles of Northeast Brazilian wines by ^{1}H NMR, associated to chemometric methods to analyze (dis)similarities from wines elaborated in 2005 and 2006 vintages. The wine samples analysed were Petit Verdot, Tempranillo, Castelão, Barbera and Periquita red wines; and Viognier and Chenin Blanc white wines.

Acknowledge: EMBRAPA (Brazilian Agricultural Research Corporation), CNPq (Brazilian National Council to Scientific and Technologic Development), FACEPE (Fundação de Amparo à Ciência e a Tecnologia do Estado de Pernambuco), ITEP (Technological Institute of Pernambuco) and UFPE (Federal University of Pernambuco).

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13 Characterization of Stannanes by ^{1}H, ^{13}C and ^{119}Sn NMR

Fernando Hallwass, Livia Cavalcanti and Ivani Malvestiti

Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil

The recent discussion concerning the synthesis of functionalized homoallylic alchools, promoted by metals, deals with the products stereo and regioseletivity. For instance, the allylic halide γ-substituted system, such as crotyl bromide, reacts with aldehyde producing α and γ compounds. Some articles in the literature describe the majority, or exclusively, formation of γ product [1]. However, the diastereoseletivity control depends upon the reaction conditions [2]. Probably, the mechanisms of these reactions involved the formation of organometallic species, and the investigation of organostannanes preformed are crucial to understand whose species take part in the addition reaction [3].

Most of the papers about organostannanes synthesis, as product or intermediate, used ^{1}H NMR to characterize the products. Additionally, the ^{119}Sn NMR is also in some extension apply. Nevertheless, our experience shown only ^{1}H NMR is not sufficient to characterize all structure formed in an organometallic reaction, because the overlap of signals are often observed when very similar structure are analyzed [4].

In this commucication we described an example where we have employed various NMR techniques, namely J-Resolved, ^{1}H-^{13}C HMQC and HMBC, and ^{1}H-^{119}Sn HMQC to elucidated all isomers produced in the reaction by crotyl bromide with tin in basic media. By ^{1}H NMR we could not assign the formed species. In contrast, by ^{119}Sn NMR we have observed five crotyl stannanes species. Besides, 2D experiments helped us to assign the NMR signals and through the correlations we have found out five different isomers. The addition of HBr drops at the NMR tube has been allowed to detect the formation of news species R_3SnBr and RSnBr (R=crotyl).

Acknowledge: CNPq (Brazilian National Council to Scientific and Technologic Development), FACEPE (Fundação de Amparo à Ciência e a Tecnologia do Estado de Pernambuco) and UFPE

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14 Interaction of a *S. enteritidis* O-antigen Octasaccharide with the Phage P22 TSP by NMR Spectroscopy, MD-Simulations and Docking Studies

Jens Landström and Göran Widmalm

Department of Organic Chemistry, Stockholm University, Stockholm, Sweden

The polysaccharide components of bacteria have important structural and functional roles. During infection bacteriophages use cell surface carbohydrates as receptors. These protein-ligand interactions are essential in the interplay between different organisms.

The Salmonella serogroups A, B and D1 have a trisaccharide element, Gal-Man-Rha in the O-antigen repeating unit in common and a fourth sugar, a 3,6-dideoxyhexose residue specific to the serogroup, namely, paratose, abequose or tyvelose, respectively. The antigenic determinants or epitopes recognized by antibodies are these 3,6-dideoxyhexoses.

We have investigated conformational preferences of an octasaccharide derived from the serogroup D1 LPS and its binding to the phage P22 tail spike protein (TSP).

The bound conformation of the octasaccharide was studied by transferred ^{1}H,^{1}H–NOESY (trNOESY) and Saturation Transfer Difference (STD) NMR. Docking studies have also been performed on this system with the Autodock program. The overall conformation of the octasaccharide in solution was investigated by 2D ^{1}H,^{1}H-T-ROESY experiments and showed a similar conformation to that of the bound molecule. Molecular Dynamics simulation has been performed on the octasaccharide, in a TIP3P water box, with NAMD, using a modified CHARMM type force field.

15 Conformational Analasys of some Carbasugars by NMR Spectroscopy

Elin Säwén, Mattias U. Roslund, Ian Cumpstey and Göran Widmalm

Department of Organic Chemistry
Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

Idose and iduronic acid are unusual among hexoses in that their pyranose forms do not exist exclusively in a single chair conformation but are rather flexible, having several low energy conformations. Iduronic acid is a component of various biologically active glycans, and the ring flexibility may be the key to the strong binding of these glycans to their receptors.

Carbasugars are carbohydrate analogues in which the endocyclic ring oxygen has been replaced by a methylene group. This means that the acetal linkage of a glycoside is formally transformed into an ether for a carbasugar, and hence becomes hydrolytically stable. Two different carbaiduronic acid derivatives were therefore synthesized.

To assign the ring conformations in solution of the carbaiduronic acid derivatives different NMR techniques as well as spin simulation was used to extract $^{3}J_{HH}$, $^{2}J_{CH}$ and $^{3}J_{CH}$ and proton – proton distances.

$^{3}J_{HH}$ coupling constants were extracted from 1D ^{1}H NMR spectra and refined by spin simulations with the PERCH NMR software. Heteronuclear long ranges ^{13}C–^{1}H coupling constants are other very important parameters in the determination of conformations. Three different 2D-NMR techniques have been used to investigate these coupling, the J-HMBC, HSQC-HECADE and IPAP-hadamard-HSQC-TOCSY experiment with zero quantum suppression. To determine proton – proton distances 1D-NOESY and 1D-TROESY experiments were used.

16 Optimization of a Large-scale Open-access N.M.R. Facility

Stephen Maginess, Dennis P. Anderson, Wei Wang, Kathleen A. Farley, Geeta Yalamanchi and George Perkins.

Pfizer Global Research & Development, Groton, CT. 06340
Exploratory Medical Sciences.

The operation of a large scale open-access NMR facility places large demands on an NMR administrator for upkeep of the instrumentation, training of users in the acquisition and processing of data, and the optimization of software. While instrument upkeep is necessarily handled on an instrument by instrument basis, efforts for user training and software optimization can be mitigated somewhat.

The time necessary to train hundreds of end users is significantly decreased by optimizing the user interface for data entry of sample information. Two versions of the MyNMR interface are shown here that provide several enhancements over the standard interfaces, including streamlining the data entry process in a "top down" manner, the use of LDAP lookup of user information without the need for separate database software, and the enforcement of time limitations according to local policies for instrument usage.

Many software files are generated or modified in order to optimize the NMR spectrometers for local needs. Therefore, a local application, known as VOICE (Vnmr Open-access Integrated Customization Environment), was developed to store all the customized files in a central location and quickly deploy changes when needed. When invoked, this application copies all files from a storage area within the local intranet to instrument workstations, determines if new files are present, and installs these files as necessary. This application ensures that a standardized operating environment is present on all systems, and greatly reduces the time needed for installations and updates.

17 Conformational Analysis of Cyclic Analogues of Inverse γ-Turns

Tim D. W. Claridge[1], Barbara Odell[1], Michael A. King[2], Morakot Kaewpet[1], Biswadip Banerji[1], and Christopher J. Schofield[1]

1. Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Oxford, UK.
2. CADD Group, UCB Celltech, Slough, UK.

Many pharmaceutical and biological probes may be viewed as conformationally constrained analogues of peptides and significant attention has been focused on β-turn mimetics. However, less work has been directed towards analogues of γ-turns which involve only three amino-acid residues. In γ-turns, the carbonyl oxygen of the first residue (i) may be hydrogen bonded to the amide NH of the third residue (i+2), giving rise to a seven-membered ring system.

γ-Turns occur in receptor~peptide ligand interactions, and in enzyme~substrate complexes. One example of the latter of interest to us occurs in the substrate conformation adopted by the complex formed between FIH, a dimeric asparaginyl hydroxylase, and its protein substrate, HIF-1α (hypoxia inducible factor-1α) [1]. The γ-turn positions the aparagine-803 β-methylene of HIF-1α adjacent to the active site iron of FIH in preparation for asparaginyl hydroxylation. FIH is one of the HIF-1α hydroxylases that act as oxygen sensors in humans; these enzymes are therapeutic targets with a view to inducing or suppressing the hypoxic response for beneficial effect.

In this work we report NMR and molecular modeling studies on conformationally restrained cyclic analogues of the γ-turn adopted by the substrate at the FIH active site, with a view toward development of enzyme inhibitors [2]. These studies reveal that the cyclic template can indeed stabilise a γ-turn conformation. Importantly, we find that the nature of the linker sequence (for example, E- versus Z-alkene) can significantly alter the observed conformations in solution.

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18 2,3'-Dihydroxy-2',5,6'-trichlorobibenzyl: Natural Product or Artifact?

William F Reynolds[1], Julia Frank[2] and Russel Ramsewak[2]

1. Dept. of Chemistry, University of Toronto, Toronto, Canada
2. Dept. of Chemistry, University of the West Indies, St. Augustine, Trinidad

The title compound was isolated from a shrub, *A. aripoense*, growing at an altitude of ca. 800 meters in the northern highlands of Trinidad. A combination of 1 and 2 dimensional NMR experiments indicated that it was a 2,3'-dihydroxybibenzyl with 3 additional unknown heteroatoms or groups attached at the 2',5 and 6' positions. High resolution mass spectrometry indicated that the three groups were all Cl atoms. Chlorinated compounds from plant sources are often regarded as artifacts somehow introduced in the isolation process. This compound seemed particularly suspicious since it has the same molecular formula as DDT. However, the skeletal structures are so different that any conversion seemed highly improbable. Also, chlorination would involve electrophilic substitution and there was no exposure to chlorine in an electrophilic form. Furthermore, chlorinated natural products from plant sources are more common than usually believed [1]. Also, dihydroxybibenzyls have been isolated from mosses, along with dimerized bibenzyls with attached chlorine atoms [2]. Since the plant was growing in a moss-rich rain forest, we strongly suspect that the compound is natural.

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19 Cap and CASE – Chasing Structures in the Drug Delivery Process

Ruth Boetzel[1], Anna Codina[2] & Katherine Mackay[1,2]

1. Pfizer Global R & D, Sandwich, Kent CT13 9NJ, UK
2. School of Engineering & Physical Sciences, Heriot-Watt University, Edinburgh, UK

The identification and characterisation of impurities and degradants is an essential step in delivering new drugs to the market safely. This often involves working with small and difficult to obtain amounts of materials, sometimes in the ug range, requiring careful consideration of acquisition conditions and equipment.

We have used a combination of MS, capillary (cap) NMR [1] for high mass sensitivity due to the incorporation of microcoils, and computer-assisted structure elucidation (CASE) [2] to identify families of structures of drug products containing only a limited number of protons. Cap NMR is ideal for this scenario because it allows the collection of all data sets in one go without having to change spectrometers or sample conditions. For CASE it is important to evaluate how to best use the software (Structure Elucidator [3]), depending on the amount of material available since the degree of interactivity and user input increases the less spectral information is available.

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20 Differentiation Between [1,2,4]triazolo[1,5-a]pyrimidine and [1,2,4]triazolo[4,3-a]pyrimidine Regioisomers by ^{1}H-^{15}N HMBC Experiments

A. Salgado, C. Varela and A. M. García

Department of Medicinal Chemistry, Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernández Almagro 3, 28029 Madrid, Spain

In our synthetic efforts to obtain fused heterocycles with high nitrogen contents, we came to the necessity to prepare triazolopyrimidine derivatives by condensation between 3-amino- or 3,5-diamino[1,2,4]triazole precursors with malonoaldehyde derivatives[1]. The compounds were expected to bear the [1,2,4]triazolo[1,5-a]pyrimidine or [1,2,4]triazolo[4,3-a]pyrimidine surrogate, although their complete structural characterization was difficult. We found that ^{1}H-^{15}N HMBC experiments could clearly and unambiguously differentiate between both regioisomers on the basis of their distinct ^{15}N chemical shifts[2]. The four ^{15}N signals of the [1,2,4]triazolo[1,5-a]pyrimidine scaffold fell into two main chemical shift areas (ca. 200 and 250 ppm), whereas the [1,2,4]triazolo[4,3-a]pyrimidine isomer gave signals distributed in three regions (ca. 175, 205 and 245 ppm). These results were fully corroborated by X-ray crystal analysis and 1D NOE experiments of representative examples.

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21 Enhanced Automated Structure Elucidation by Inclusion of 1,1-Adequate Data

Steve Cheatham[1], Mike Kline[1], Ryan Sasaki[2], Kirill Blinov[2]

1. DuPont Crop Protection, Stine-Haskell Research Center, Newark, DE 19714 USA
2. Advanced Chemistry Development, Inc., Toronto, ON M5C 1T4 Canada

Computer Assisted Structure Elucidation (CASE) has become much more common in recent years. Most algorithms make use of one dimensional ^{1}H NMR and ^{13}C NMR in conjunction with two dimensional ^{1}H-^{1}H and ^{1}H-^{13}C correlation experiments. Structure elucidation programs are subject to the same deficiencies in HMBC data as one encounters with manual interpretation, i.e., the inability to easily and unambiguously distinguish between two and three (and possibly four) bond correlations. The inclusion of 2-bond specific data would therefore greatly enhance automated structure elucidation.

Several pulse sequences have been proposed that can distinguish between 2 and 3-bond correlations. These include, but are not limited to, ^{2}J-^{3}J-HMBC, H2BC, and 1,1-adequate.[1,2,3,4] All of the sequences have limitations. In the case of H2BC and ^{2}J-^{3}J-HMBC, proton-proton coupling is required which limits their use in systems without extensive proton coupling networks.[1,2,3] The 1,1-adequate sequence has the significant advantage that it is more general but suffers from low sensitivity due to the need for ^{13}C-^{13}C double quantum excitation. However, the use of modern cryo-probes coupled with broadband adiabatic ^{13}C pulses has made the 1,1-adequate a much more routine experiment allowing data to be acquired with high resolution in two hours or less on a few milligrams of sample.[5,6] Here we show that the use of cryo-probe generated 1,1-adequate data in conjunction with HMBC dramatically improves CASE both in terms of speed and accuracy of structure generation.

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22 Shortcomings of INADEQUATE

Valerie Robertson[1], A. Konstantinov[2], Alex D. Bain [3] and Donald W. Hughes[3]

1. University of Guelph
2. Wellington Laboratories, Guelph Ontario
3. McMaster University

The INADEQUATE experiment is potentially a very powerful experiment, since it allows us to map out the carbon skeleton of an organic molecule without the need for attached protons. However, since it requires adjacent pairs of C-13's it has an appalling lack of sensitivity. Traditionally, almost a neat sample is required. However, modern cold probes, good consoles and high magnetic fields mean that smaller samples (<50 mg) can be analyzed.

This technology can also cause artifacts in the spectra, such as weak vs. strong correlations, and missing halves of a single correlation. A contributing cause of this is the fact that cold probes will only tolerate relatively weak rf power, and high magnetic fields mean large offsets from the carrier. The simple hard-pulse analysis of the experiment is no longer satisfactory.

In this poster we explore some typical artifacts which we encountered in a study of a polybrominated flame retardant. These artifacts are traced back to offset effects, flip–angle dependence and spin relaxation. A brief theoretical explanation is presented along with examples of the artifacts and possible solutions.

23 Design of a Universal Refocusing Pulse by Large-scale Nonlinear Optimization

Christopher K. Anand[1], Alex D. Bain[2], Andrew T. Curti[3], and Zhenghua Nie[4]

1. Computing and Software, McMaster University
2. Chemistry, McMaster University
3. Medical Biophysics, University of Western Ontario
4. Computational Engineering and Science, McMaster University

Modern NMR consoles give fantastic flexibility in the intensity and phases of shaped pulses. However, we need modern computer optimization methods to exploit this freedom and find the "best" pulses. For our purposes here, we restrict ourselves to non-selective pulses that can cover a much wider bandwidth than rectangular pulses. There are many such designs for inversion and excitation pulses, but relatively few for refocusing pulses. For excitation or inversion, the magnetization starts on the z axis, so an adiabatic pulse, for instance, will pull the magnetization into the required final state. Refocusing pulses may be more difficult, partly because the initial state of the magnetization is not defined, other than by being in the xy plane.

Large-scale open-source optimization methods are now available which allow us to optimize each intensity and phase in a 500 step shaped pulse. The designs include the effects of relaxation and of inhomogeneity of the rf field. One of the important choices in doing an optimization is the target function – what you are actually optimizing. In many published designs, it is the final value of the magnetization after the pulse. However, here we have chosen an objective function that forces the pulse to look like a rotation matrix. Since an infinitely hard pulse is equivalent to a rotation, this target function should make the pulse universal. A π pulse should not only refocus in the xy plane but also serve as an inversion pulse. Loss of universality has been thought of the price to be paid for broad bandwidth, but this appears not to be true.

In this poster we discuss the optimization method briefly and show both simulated and experimental results from using our optimized pulse in a CPMG T2 measurement.

24 Determining Enantiomeric Excess by ^{13}C-NMR in Chiral Liquid Crystal Media: A Simple and Rapid Method with No Racemate Needed

Vasilios M. Marathias[1], Peter Tate[1], Alvin C. Bach, II[2], Walt Massefski[1].

1. Wyeth Research, Chemical and Screening Sciences, Discovery Analytical Chemistry Cambridge, MA 02140
2. Wyeth Research, Chemical and Screening Sciences, Discovery Analytical Chemistry CN 8000 Princeton, NJ 08543

Described below is a NMR-based method used to measure enantiomeric excess (ee) of drug like molecules. The method utilizes chemical shift anisotropies observed when an orientation media composed of the chiral polypeptide liquid crystal, poly-γ-benzyl-L-glutamate, dissolved in organic solvents is used. This method is rapid and relatively simple. Only a one-dimensional carbon-13 NMR experiment on a 1 to 5 mg sample is required. When compared to ee measured by chiral SFC, the NMR method produced very comparable results but, most importantly, and unlike the chiral SFC methods, enantiomeric excess measured via NMR can be conducted directly on enandioselective reaction products when no racemate is available.

25 NMR Metabolomic Study of Biomarkers in the AppSwe Tg2576 Mouse Model of Alzheimer Disease

Julie Lalande[1], Stéphane Balayssac[1], Hélène Halley[2], Bernard Frances[2], Véronique Gilard[1], Jean-Michel Lassalle[2] and Myriam Malet-Martino[1]

1. Groupe de RMN Biomédicale, Laboratoire SPCMIB, UMR CNRS 5068, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex, France
2. Centre de Recherches sur la Cognition Animale, UMR 5169, Bât IVR3,118 route de Narbonne, 31062 Toulouse cedex, France

Alzheimer's disease (AD) is a complex illness, with no definitive biomarkers available that allow clinical diagnosis; this represents a major problem for the advance of efficient drug discovery programs. ^{1}H NMR-based metabolomics has been used to identify metabolic perturbations associated with a transgenic (AppSwe Tg2576) mouse model of AD. The metabolomic profiles of five brain regions (frontal cortex, entorhinal cortex, hippocampus, midbrain, cerebellum) were compared in control mice and in AD animals at two different disease stages (3 and 11 months).

After a biphasic extraction of the cerebral tissue, ^{1}H NMR spectroscopy analysis was performed on the aqueous fraction at pH 10. About 20 metabolites including N-acetyl-L-aspartate (NAA), glutamate, glutamine, taurine, glycine, glycerophospho-choline (GPC), creatine, γ-amino butyric acid (GABA), aspartic acid or lactate were identified and quantified. A principal component analysis was then performed with the KnowItAll® software (BioRad).

The five brain regions were discriminated in both control and transgenic animals at 3 and 11 months by taurine, creatine, GABA, GPC and NAA. Control and AD disease transgenic mice were then compared. At the anatomic level, hippocampus and midbrain regions were first affected at 3 months then entorhinal cortex at 11 months, which is coherent as these brain areas are spatially very close. Frontal cortex and cerebellum seem not to be affected. At the metabolome level, taurine and GABA had a similar behavior whatever the age of the transgenic mice. Taurine concentration increases with respect to control mice, whereas the amount of GABA decreases. On the other hand, lactate concentration drops in 3 month-old mice but increases in 11 month-old mice. Glutamine and glutamate are discriminating metabolites at 3 months. NAA concentration increases in the midbrain of transgenic 11 month-old mice. Other animals must be analyzed to confirm these encouraging preliminary data.

26 Determination of Herbal Drug Contents and Identification of Adulterants by 2D DOSY ^{1}H NMR

Stéphane Balayssac, Véronique Gilard, Myriam Malet-Martino and Robert Martino

Groupe de RMN Biomédicale, Laboratoire SPCMIB, UMR CNRS 5068
Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex, France

This study presents a new application of 2D DOSY ^{1}H NMR for the analysis of herbal drugs or dietary supplements. In the last few decades there has been an exponential growth in the field of herbal medicines. These products are regarded by many as being harmless because of their natural origin and helpful to the treatment of some chronic diseases and the maintenance of physical fitness. Nevertheless some manufacturers include synthetic drugs in the formulation of their products marketed as "herbal medicines" or "dietary supplements", in order to improve their effect. Particularly, in recent years, there have been several reports on adulteration of herbal drugs marketed for sexual dysfunction with synthetic phosphodiesterase-5 (PDE-5) inhibitors (sildenafil, tadalafil, vardenafil) or analogues in which minor modifications were brought to the molecular structure of PDE-5 inhibitors [1,2].

Seventeen herbal dietary supplements, coming from various coutries (China, Estonia, France, Germany, India, Sweden, Spain, Syria, Taiwan, USA) and marketed as natural products for the enhancement of sexual function, were analyzed by 2D Diffusion Ordered SpectroscopY (DOSY) ^{1}H NMR.

The method allows a global analysis of the samples with detection of both active and inactive ingredients present in the complex matrices. Eight formulations contain compounds related to the synthetic PDE-5 inhibitors. Sildenafil, tadalafil, vardenafil, hydroxyhomosildenafil, thiosildenafil, and the newly identified adulterant thiomethisosildenafil were detected. In addition to these actives, about 30 compounds or excipients were characterized in all formulations.

This study ends up by a three dimensional DOSY-COSY ^{1}H NMR experiment on an herbal formulation, which provides both virtual separation and structural information.

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27 Monitoring of Grignard Reaction Intermediate by MICCS-NMR

Satoshi Sakurai[1], Yutaka Takahashi[1], Hiroyuki Koshino[2], Hiroaki Utsumi[1] and Masamichi Nakakoshi[3]

1. Analytical Instrument Division, JEOL Ltd., Akishima
2. Advanced Technology Support Division, Advanced Science Institute, RIKEN, Wako
3. Instrumental Analysis Center, Yokohama Nat. Univ., Yokohama

The Grignard reaction is one of the most famous reactions, and various arguments are done about its reaction mechanism and the structure of the intermediate. However, analysis examples proving with spectral data almost never exist. The analyses of the reaction mechanism or the intermediate by the NMR have been performed by flow NMR mainly. However, in the case of conventional flow NMR methods, the quality of the data are not so good, because of decreasing of efficiency for mixing, and of reducing of resolution caused by turbulent flow. MICCS (MIcro Channeled Cell for Synthesis monitoring) -NMR resolves these problems by applying microtechnology, and it is the new technique that enabled real-time monitoring of the reaction by the NMR. [1][2]We have performed the structural analysis of intermediate of Grignard reaction for aim to resolving the reaction mechanism by MICCS-NMR. Because reaction velocity of Grignard reaction is too fast for analysis of reaction mechanism by standard NMR technique. In addition, there are many problems for flow conventional NMR, for example, the necessity of the anhydrous condition or generation of the methane gas, etc. However, by MICCS-NMR, we succeeded in getting signals of intermediate and in analyzing the structure of intermediate by utilizing ^{1}H-^{13}C correlation two dimensional NMR such as ^{1}H-^{13}C HMBC, and also ^{1}H-^{1}H COSY. In addition, we got the structure information of the intermediate by measuring of ESI-MS under the same reaction condition as MICCS-NMR at the same time.

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28 NMR Spectroscopic Evaluation of the Internal Environment of PLGA Microspheres

Kirby C. Wong-Moon, Xin Sun, Xichdao C. Nguyen, Bin P. Quan, Kai Shen, and Paul A. Burke

Department of Pharmaceutics, Amgen Inc., Thousand Oaks, CA 91320

The internal environment of poly(lactide-co-glycolide) (PLGA) microspheres was characterized using ^{31}P and ^{13}C solid-state and solution NMR spectroscopy. Physical and chemical states of encapsulated phosphate- and histidine-containing porogen excipients were evaluated using polymers with blocked (i.e., esterified) or unblocked (free acid) end groups. Spectroscopic and gravimetric results demonstrated the encapsulated porogen deliquesced upon hydration at 84% relative humidity to form a solution environment inside the microspheres. Dibasic phosphate porogen encapsulated in unblocked PLGA was partially titrated to the monobasic form, while in the same formulation ^{13}C NMR showed partial protonation of the histidine imidazole. Similarly, encapsulated monobasic phosphate was partially converted to phosphoric acid. Coencapsulation of monobasic and dibasic phosphate porogens resulted in a single peak on hydration, indicating chemical exchange between discrete excipient microphases. Exogenous buffer addition differentiated external from internal, non-titratable, excipient populations.

Microspheres containing dibasic phosphate porogen were hydrated with fetal calf serum, incubated at 37°C, and characterized by ^{31}P NMR through the polymer erosion phase. Within 48 hr, the ^{31}P chemical shift moved over 2 ppm upfield and the line width narrowed to <60 Hz; there was little additional change through day 14. This indicated complete conversion to the monobasic phosphate form throughout the polydisperse sample, and that pH remained below 4 but above the phosphoric acid pKa during matrix erosion.

29 Microprobes and Cryo-microprobes Applied to Structure Elucidation Problems in the Pharmaceutical Industry

Michael Ritzau, Lukas Oberer and Kayhan Gabriel Akyel

Novartis Institute for Biomedical Research, PO Box 4002 Basel, Switzerland

Structure elucidation of drug metabolites and impurities is mandatory for regulatory approval of each new drug. A complete determination of regio- and/or stereochemistry of such molecules by MS or MS/MS is often impossible. To avoid time-consuming synthesis of a number of possible isomers NMR is required. In the past, sample amounts of nano- to low micrograms were insufficient for most NMR experiments. Several years back the advent of cryo- and microprobes has improved matters by improving the filling factor or lowering thermal noise. Recently, combined micro-cryoprobes became available. We show our results with 1 to 1.7mm probe heads as well as our new 1.7mm cryoprobe.

30 Sensitive, Quantitative Carbon-13 NMR Spectra by Mechanical Sample Translation

Kevin J. Donovan, Mary Allen, Rachel W. Martin, A. J. Shaka

Chemistry Department, University of California, Irvine, CA 92697-2025

Collecting a truly quantitative carbon-13 spectrum requires a long relaxation delay between transients to allow the sample to return to nearly full equilibrium magnetization before the next read pulse is delivered [1]. Despite these drawbacks, a quantitative carbon-13 spectrum is useful in certain applications. These applications include metabolite quantification [2], food analysis [3-6], and petrochemical applications [7,8], to name a few. We have developed a technique to collect quantitative carbon-13 spectra without using a long relaxation delay, and instead changing the sample between scans. This technique uses an unusually long (1520 mm) NMR tube with a standard diameter (5 mm) that is moved incrementally between scans. By moving the tube after data acquisition is complete, the relaxing portion of the sample is removed from the coil and replaced by an equilibrated portion that has been sitting in the stray field, thus skipping the relaxation delay. In addition to carbon-13 quantification, this method was also found to produce an approximately 4-fold sensitivity enhancement. We demonstrate this technique on two small molecules (thymol and butylhydroxytoluene) to show that it is a useful platform for collecting quantitative spectra in situations with a large sample volume, required to fill the long tube.

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31 Indirect Covariance and 3D NMR: Assignment of Diastereotopic Protons

Martin Jaeger, Ruud Aspers, Elwin van der Cruijsen and Maarten Honing

Department of Medicinal Chemistry Oss
Schering-Plough Corporation
PO Box 20, NL-5340 BH Oss, The Netherlands

Structure elucidation and structure verification of small molecules are still major tasks in pharmaceutical analysis. As being part of today's standard procedures, such analyses are often subject to severe time constraints. Those restrictions comprise both experimental and interpretation time. While the latter can be significantly reduced by using suitable correlation spectroscopy, e.g in two dimensions, the experimental time increases with magnetization transfer steps that evoke the correlation signals.

NMR experiments that rely on complex but straight-forward pulse sequences and coherence pathways are well established and form the toolbox of every NMR laboratory. During the past 5 years, an alternative route has been discovered [1], explored [2,3] and made available [4]. This method was baptized Covariance Spectroscopy and is based on a mathematical transformation of two different types of spectra into one, containing the information of both. On top of it, the transformation correlates the formerly separate information.

Here, the assignment of the diasteretopic protons 16a and 16b of testosterone was investigated by applying experimental correlation spectroscopy, such as 3D HSQC-TOCSY and 3D HSQC-NOESY, and Indirect Covariance Spectroscopy to construct HSQC-COSY and HSQC-NOESY data sets. The pro's and con's of the experiments will be discussed with respect to routine implementation of such experiments into a standard industrial workflow. The possibility of the assignment of the 16a and 16b proton signals from testosterone with either method will be presented.

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32 Efforts at Providing High Content NMR Data to Our Client Chemists

David Whittern

Abbott Laboratories, Abbott Park, Illinois

The small molecule NMR lab has been using automation to collect and process NMR data, and then return the results to the submitting chemist for over 20 years. Over time, in response to an increasing emphisis on high-throughput synthetic efforts, we have been adding additional components to the automatic data processing in order to relieve some of the data analysis workload the chemists now face.

We have over 20 years worth of raw data in our archives available to our chemists within a couple minutes wait time via a variety of search methods using the lab's internal web pages. In addition to the raw data, we have been saving 1-D NMR data as Fourier transformed spectra in a J-camp format for over 8 years. These files are maintaned online for instant viewing.

Until recently, the J-camp file contained only the spectrum and the target structure. We are currently moving to Mnova as the platform for providing NMR spectrum viewing and data processing to the chemists' desktop computers. By adopting Mnova as the spectrum viewer/processor we can now incorporate additional components into the J-camp file. These include scaled intergrals, which have been already been cut into meaningful regions, multiplets analyzed with respect to number of protons and multiplicity, and chemical shift predictions from our in-house chemical shift predictor. In cases where a member of the NMR lab has made chemical shift assignments for a particular spectrum, the assignments can be included in the J-camp file as well. We feel that providing this addition information content with each spectrum rather than just more raw data, we are helping the chemists make better and faster descisions in their research efforts.

33 Quantification of NMR Spectra Obtained with New High-throughput Screening Method

Amy S Freund, Joshua Hicks, Michael Fey, Till Kuhn

Bruker Biospin Corp., Billeica, MA
Bruker Biospin Corp., Fallanden, Switzerland

The two rate limiting steps of compound library screening are throughput and concentration determination. Bruker Biospin has a variety of tools on the market to help address these issues raised by researchers tasked with analyzing or documenting these dipositories. At minimum, methods for compound library screening must be robust, fully automated, and encompass both data acquisition, processing and reporting. Our most recent method demonstrates significant improvements in performance and efficency as outlined below. Here we demostrate this method on the example of Library Screening, but it can be applied more generally to NMR applictions where large numbers of samples need to be analyzed.

Advances in Libraby Screening include the following key steps:

1. Fully automated sample preparation from well plate to NMR tube
    a. Addition of ~10% DMSO-d6
    b. Reliable and precise filling of tubes
    c. Working volume of 5-7uL with automated recovery
2. Fully automated acquisiton of spectra with protonated solvent
    a. Double solvent suppression with spoil gradients
    b. ^{13}C decoupling of DMSO satalites
    c. Flat baselines
    d. Integration within 100Hz
    e. Quantitation
3. Fully automated peak picking and integration with two concentration verification options
    a. Automated concentration determination
    b. Interactive interface for concentration determination
4. Total error rate under 10%

34 Marine Environmental Metabolomics

Daniel W. Bearden, Arezue F. B. Boroujerdi, Jerry L. Dallas and Tracey Schock

National Institute of Standards and Technology, Analytical Chemistry Division, Hollings Marine Laboratory, 331 Ft. Johnson Rd., Charleston, SC 29412

Changes in the marine environment due to pollution or urbanization often cause very subtle effects on organisms that participate in the marine ecosystem. Organisms are impacted in a way that may cause acute responses, like death, or through sub-lethal effects that may reduce the organism's ability to reproduce, avoid predation or flourish. These effects may cause unexpected and profound changes to the ecosystem balance, so sensitive tools are needed to carefully assess the response of multiple species to environmental insults.

Our laboratory is working towards using NMR-based metabolomics to understand the metabolomic response of organisms to relevant marine environmental stressors, and eventually to understand these responses on a population scale. Model organisms such as fish (*Fundulus heteroclitus* (mummichog)), crabs (*Callinectes sapidus* (Atlantic Blue Crab)) and bacteria (*Vibrio coralliilyticus*) and endangered/protected species like corals and dolphins are being investigated. In addition, efforts to improve data quality and improve comparability of interlaboratory results are progressing [1]. While NMR data is remarkably comparable and precise, the amount of variation from small numbers of biological samples causes problems that must be analyzed carefully and in full context.

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35 Validating the Quality of Large Collections of NMR Spectra Automatically

Sergey S. Golotvin[1], Eugene Vodopianov[1], Rostislav Pol[1], Mikhail Kvasha[1], Brent A. Lefebvre[1], Jim Brien[2], Chris Lein[2], and Tom Moertl[2]

1. Advanced Chemistry Development
2. Sigma-Aldrich Corp.

As NMR instrumentation continues to advance, more and more spectral information becomes available for researchers with a finite amount of time. A typical ^{1}H–^{13}C HSQC experiment for example, can now be performed in ten minutes or less, making it feasible to employ this spectrum for high-throughput evaluation of a proposed chemical structure. Although structure evaluation methods utilizing the combination of ^{1}H and ^{1}H–^{13}C HSQC data were shown to offer significantly higher accuracy [1,2], an evaluation based only on a 1D ^{1}H spectrum is still desirable in many instances.

In the work presented here, 15,000 ^{1}H NMR spectra from the Aldrich collection were evaluated in complete automation using a number of proton prediction methods [3]. The most accurate prediction method was able to confirm 88% of all structures as consistent, and flagged less than 5% as inconsistent. A manual examination of these flagged spectra did indeed reveal some amount of truly wrong structures as well as shortcomings in prediction, processing, and analysis steps. A discussion of factors limiting the verification performance is given.

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36 NMR Analysis of Unique Glycolipids Derived from Agricultural Byproducts

Gary D. Strahan, Aisha Abdul-Wakeel, Jonathan Zerkowski

USDA, ARS, Eastern Regional Research Center
Wyndmoor, Pennsylvania

Glycolipids possess immense structural variability and play essential roles in biological and artificial systems. Our work focuses on the 2D NMR structural characterization of unique, chemically-modified, glycolipids derived from the microbial fermentation of inexpensive agricultural wastes or byproducts. This report details the spectroscopic analysis of two such compounds, presenting evidence for the structure in terms of connectivity and resonance assignments. The first is an amino derivative of a rhamnolipid, which represents the first example of this kind of derivative for this class of biosurfactants. Such biosurfactants are potentially more environmentally-friendly than conventional synthetic detergents, and the amino functionality opens the door to new ways of modifying surfactancy or biocompatibility. The second is a sophorolipid that has been tethered to a triacylglycerol, and may serve as a model for a general method of anchoring saccharides to membranes. This compound was created by the copper-catalyzed Huisgen "click" reaction, which creates the possibility of tethering a wide array of "molecular cargo" to triacylglycerols for the purpose of attaching it to a membrane or other surface. We can, for example, attach sophorolipids, amino acids, and a second triacylglycerol unit to our functionalized lipids. The functionalized lipids might find utility as new classes of surfactants, transmembrane spanners, or possibly sites for interlayer molecular recognition.

37 Application of Restrictive Sample Volume and High Field Superconducting Magnet in Characterization of Natural Products

Aleksej Krunic, Jimmy Orjala and Shunyan Mo

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

Newly isolated natural products often represent analytical challenge due to the fact they are more often than not isolated in submiligram quantities. Our natural products discovery group commonly deals with NMR characterization of cyanobacterial isolates at low μmol levels, while certain peptidic samples encountered were between 0.01-0.4 μmol. Several different approaches can be used when dealing with mass-limited sample. Cryogenic technology and high field magnets greatly enhance spectral resolution and sensitivity. Further gains can be achieved by restricting the sample volume. At our disposal, we have had the first Bruker 900 MHz US2 spectrometer with carbon sensitive (TCI) 5mm cryoprobe, in the United States. Comparison was made between this instrument and a standard non-cryogenic 400 MHz instrument with broadband probe, and "standard cryoplatform" - 600 MHz spectrometer equipped with a TXI cryoprobe. Standard 3/5mm NMR tubes and the corresponding 3/5mm Shigemi NMR microcells were used. Sensitivity enhancements achieved with 3mm Shigemi micro cells at 900 MHz is a factor of 4 over standard 3mm tube at 600 MHz. This methodology was applied to obtain (Inverted Direct Response) IDR HSQC-TOCSY of 0.01 μmol sample in 24 hours, with S/N measured in F2 to be 10 for aliphatic protons and 47 for a methoxy group. Use of Shigemi 3 mm microcells was reported previously on Varian systems by Martin et al[1]. To our knowledge it represents first recorded usage of 3mm Shigemi microcells on Bruker spectrometers.

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38 Knowing More and More About Less and Less Until You Know Almost Everything About Almost Nothing: or Testing the Lower Limits of a CapNMR Probe on an Older Spectrometer

W F Reynolds[1], T Burrow[1], D McNally[1] and R G Enriquez[2]

1. Dept. of Chemistry, University of Toronto, Toronto, Canada
2. Instituto de Quimica, U.N.A.M., Mexico City, Mexico

We have investigated the minimum amount of sample of a typical natural product (300 molecular weight) needed to obtain a complete set of usable NMR spectra in 24 hours using a Protasis CapNMR probe on a 15 year old spectrometer. High quality ^{1}H COSY, T-ROESY, TOCSY and HSQC spectra and ^{13}C and HMBC spectra which were adequate for structure determination were obtained in 24 hours using 120 μg of kauadienoic acid in 20 μL of CDCL3 with robot-controlled injection. This amount could be significantly further reduced by a combination of direct sample injection, use of a state of the art spectrometer and elimination of the ^{13}C spectrum since the combination of HSQC and HMBC gave the same information as the ^{13}C spectrum.

39 Strategic Productivity: Impact of Microflow NMR on Key Applications in 2008

Dean L. Olson, Timothy L. Peck, and Robert A. Albrecht

Protasis/MRM Corporation, 101 Tomaras Avenue, Savoy, IL 61874 USA

Small molecule NMR research today faces formidable challenges. Greater demand for new drug leads, lower-level impurity characterization, higher cryogen and magnet expenses, lab space limitations, and stronger global competition all combine to apply new pressures on both pharmaceutical and academic NMR researchers and managers. An expectation of greater productivity has become the norm, which calls for fresh scientific approaches and advances in analytical instrumentation. Innovations must include cost reduction, less human intervention, and better equipment reliability to meet the demands of the modern laboratory workplace. A viable analytical instrument today must pay for itself, and in less time than before.

Due in part to significant up-front investment in both specialized equipment and technical staff, NMR is slower to adapt to changing needs when compared to benchtop analysis platforms such as mass spectrometry and liquid chromatography. A simple, yet effective way to address this shortcoming is to provide a more advanced workflow of samples to and from the spectrometer. Deliberate omission of the NMR tube in favor of maintaining samples in micro well plates and vials provides new ways for NMR to couple directly and non-disruptively to the overall sample workflow of the organization. Modern and efficient liquid handling robots combined with microfluidic advances now make this practical and economical. Microflow NMR links upstream processes such as reaction monitoring, synthesis, and mass-directed fractionation, with downstream activities that include library management, structure verification, and regulatory/IP protocols, all via a web-based, vendor-neutral desktop interface. Overall, the approach strengthens the role of NMR in the analytical laboratory, and carries both financial and scientific value for NMR as an indispensable and highly utilized institutional resource. This poster specifically describes unique accomplishments in 2008 of scientists in both academia and industry in 5 important focus areas: molecular library management, impurities, metabolomics, natural products, and open access. Groups are running up to hundreds of samples per week employing automation that monitors itself. Easily-configured software links together visualization, elucidation, verification, and statistical analysis packages via electronic reporting. Open-access and dedicated research platforms both benefit, resulting in a significantly improved role for NMR in service to the organization and individual researcher [1, 2, 3].

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40 Batch Data Analyses of NMR Spectra for Metabolic Profiling

Bruce A. Johnson[1], Ting-Chuan Wang[2] & Qiuwei Xu[2]

1. One Moon Scientific, Inc.
2. Merck & Co., Inc.

With increasing application of metabolomics to investigate the biochemical mechanisms of disease as well as toxicity, there come demanding requirements for consistent, efficient, robust and automatic data processing of NMR spectra to facilitate data mining.

Data analysis of NMR spectra for metabolic profiling includes trend based statistical differentiation, identification of chemicals in analyzed samples, quantification of detected chemicals, and statistical summary of quantitative changes of individual metabolites. Although each step or method is mature and commonly applied to many other applications, integration and flexibility of applying different methodologies in connection with individual spectra, peaks, and chemicals are challenging. In collaboration, we developed methodologies and implemented them in dataChord software from One Moon Scientific.

In the area of statistical differentiation we will illustrate and compare a variety of mathematical tools for analyzing collections of NMR spectra. These include Principle Component Analysis, Molecular Factor Analysis, and Non-Negative Matrix Factorization with Sparseness Constraints.

For metabolite identification, we collected reference spectra on a 700 MHz NMR spectrometer, processed the spectra to collect regions containing peaks of native line shapes and extracted peak related information (chemical shift, multiplicity, number of contributing protons), and stored them in a reference library associated with dataChord. The reference library can be searched by either chemical shift values or chemical names. In order to quickly scan through all spectra, we use an automated procedure to match peaks in sample spectra with reference spectra, translating NMR peaks to chemicals. We optimize the position of the reference peaks by minimizing the absolute value of the deviation between reference and sample spectra. Searching based on absolute value is more robust than using an rmsd based approach. In addition to traditional peak area based quantification, we also implemented linear deconvolution based metabolite quantification. For that purpose, each reference spectrum contained a quantity of DSS-d6, and the concentration of both DSS-d6 and reference are known. With known concentration of DSS-d6 in each sample, linear deconvolution can calculate chemical concentrations in samples readily. We fit spectra with a singular value decomposition. During fitting we can simultaneously include a polynomial baseline correction. We will present, in this poster, a general overview and comparison of integrating these different methodologies and illustrate their use in an application.

41 Exploring UCSC Marine Natural Products for Basic Research in Parasitic Diseases

Katharine R. Watts, Brent K. Rubio, Karen Tenney, Jennifer E. Compton, Kean-Hooi Ang, James H. McKerrow, Phillip Crews

University of California, Santa Cruz, CA
University of California, San Francisco, CA

Few formal programs in the US exist that are focused on targeting drug discovery approaches against what can be termed "neglected diseases." Among the most devastating of such diseases are African sleeping sickness (protozoan parasite: *Trypanosoma brucei*) and Chagas disease (protozoan parasite: *Trypanosoma cruzi*). The current situation for therapeutic treatments of these neglected diseases is bleak as the arsenal of available drugs is minimal and they often have adverse side effects. Chemotherapeutics derived from natural products such as suramin and ivermectin represent compounds discovered more than 30 years ago to treat Chagas disease. These early discoveries illustrate the value of using natural products as a discovery source. In an initial screen of 246 crude extracts from our laboratory, 11 distinct hits with growth inhibition activity of greater than 80% (at 0.5 μg/mL) against *T. brucei* were identified by the McKerrow laboratory at UCSF. Two of these samples are currently under investigation. The first project is a study of the marine sponge Diacarnus bismarkensis and the second project focuses on cultures of the deep water sediment-derived fungus *Aspergillus fumigatus*. These organisms have provided semi-pure fractions and pure compounds with IC50's $\leq$ 1μg/mL.

42 Isolation of a New Puupehenone Analog Using ELSD Directed Peak Library Generation

Sarah Robinson[1], Michelle Riener[1], Karen Tenney[1], Steven Loveridge[1], Frederick A. Valeriote[2], and Phillip Crews[2]

1. Dept. of Chemistry and Biochemistry and Inst. of Marine Sciences, University of California, Santa Cruz, CA 95064
2. Joshephine Ford Cancer Center, Ford Healthcare Systems, Detroit, MI 48202

In an effort to further isolate compounds with known protein and/or enzyme inhibition, specific assays including histone deacetylase inhibition and MCL-1 inhibition have been undertaken. Parallel testing between whole cell in vitro testing and protein specific testing will lead to information about both the protein target and the solid tumor selectivity of the puupehenone compounds isolated. New approaches to reach the active components in the extract include automated peak library generation with collection via ELSD led to the isolation of a new compound, 15-oxo-puupehenoic acid.

43 Identification of an Unknown Component Formed in Erythromycin Solution

Szabolcs Béni[1], Frederick Daidone[2], Erik Haghedooren[2], Larissa Van den Bossche[2], Murali Pendela[2], Erwin Adams[2], Jos Hoogmartens[2], and Béla Noszál[1]

1. Department of Pharmaceutical Chemistry, Semmelweis University, Budapest
2. Laboratorium voor Farmaceutische Analyse, Katholieke Universiteit Leuven

Erythromycin is a macrolide antibiotic that has an antimicrobial spectrum similar to or slightly wider than that of penicillin, and is often used for people that have an allergy to penicillins. Erythromycin is available in enteric-coated tablets, slow-release capsules, oral suspensions, ophthalmic solutions, ointments, gels, and injections. This macrocyclic compound contains a 14-membered lactone ring with ten asymmetric centers and two sugars: L-cladinose and D-desosamine, making this molecule a very complex structure drug.

It was observed that an unknown impurity (UNK) was formed in a commercial erythromycin A (EA) product. The stability of the freshly prepared solution was monitored over a period of 18 weeks, using four different chromatographic methods. The peaks in the different chromatograms were identified using 9 impurity standards except UNK.

For the structure identification of UNK, LC-MS experiments were conducted. The first order mass spectrum of erythromycin and UNK showed the same 734 m/z respectively, but it should be noted that in the ion trap UNK was behaving differently compared to EA and other related substances. Unfortunately, during the MS-MS experiments, the sensitivity was drastically decreased to instrumental noise level so, it was not possible to carry out detailed experiments. However, in the MS-MS spectrum, losses of the 158 Da (cladinose sugar) and 60 Da were observed. In the literature, a loss of 60 Da is not observed for EA and related substances with m/z 734. A loss of 60 Da is probably due to the loss of propanol instead of propionaldehyde at C-13 position. So, the UNK compound may correspond to pseudo erythromycin hemiketal (PsHK), one of the EA related substances.

To confirm it, LC-NMR experiments were carried out on a 600 MHz Varian spectrometer. The hypothesized PsHK was also prepared from EA according to the procedure mentioned in the literature. The complete ^{1}H and ^{13}C assignment of EA and PsHK were carried out in a separate offline experiment. The mobile phase was also transformed to a more NMR compatible one, and the obtained spectra confirmed the hemiketal structure of the unknown impurity.

This study was supported by Hungarian Research Fund OTKA-73804

44 NMR as a Quantitative Tool to Determine the Concentrations of Biologically Produced Metabolites: Implications in "Metabolites in Safety Testing" (MIST)

Robert Espina, Linning Yu, Jianyao Wang, Zeen Tong, Sarvesh Vashishtha, Rasmy Talaat, JoAnn Scatina and Abdul Mutlib

Biotransformation Division, Drug Safety and Metabolism, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426

NMR spectroscopy has traditionally been considered as an indispensable tool in elucidating structures of metabolites. However, with recent advancements in cryoprobe technologies, that have enhanced sensitivity quite considerably, NMR is increasingly being considered as a powerful quantitative tool. A specific quantitative application of NMR is in determining the concentrations of biologically isolated metabolites, which could potentially be used as "reference" standards for further quantitative work by LC/MS/MS. Traditionally, biologically isolated metabolites have not been considered as a way of generating "reference" standards for further quantitative work, relying on organic chemistry to produce the metabolites by synthetic methods. Due to the recent FDA guidance on safety testing of metabolites, one has to consider means of authenticating and quantitating biologically or non-biologically generated metabolites as early as possible in the drug discovery process. ^{1}H-NMR is being proposed as the method of choice as it is able to be used as both a qualitative as well as a quantitative tool; hence allowing structure determination, purity check and quantitative measurement of the isolated metabolite. In this poster, the application of NMR as a powerful and robust analytical technique in determining the concentrations of in vitro or in vivo isolated metabolites is discussed.

45 Automatic Chemical Shift Referencing of Homonuclear 2D NMR Spectra

Carlos Cobas

Mestrelab Research S.L. Xosé Pasín, 6, Santiago de Compostela, 15706, SPAIN

An accurate calibration of the chemical shift scales of NMR spectra is very important for both reproducibility and the correlation of the chemical shift with structural properties. Usually, this task is very easy in 1D NMR and in general, it is carried out graphically by simply selecting with the mouse cursor a reference signal (e.g. TMS or a residual solvent peak).

In the case of 2D NMR, the usual practice is to firstly measure a 1D spectrum, reference it, and then start the 2D experiment using the parameters of 1D spectrum. However, manual chemical shift referencing of 2D spectra after acquisition are usually required for the correction of any misalignment. The low digital resolution typically obtained in 2D NMR (nD in general) spectra makes this process more sensitive to the actual position of the reference peak selected for referencing.

In this work we present a robust algorithm for the automatic chemical shift referencing of 2D homonuclear experiments based on the Fast Fourier Cross-Correlation between a high resolution 1D spectrum and the internal projections of the 2D spectrum. The algorithm takes advantage of the higher digital resolution of the 1D spectrum (usually >20 times higher) to accurately reference the axis of the 2D spectrum. It's very fast, not iteractive and very insensitive to spectral distortions and artefacts.

46 Global Spectral Deconvolution (GSD) of 1D-NMR spectra

Carlos Cobas[1], Felipe Seoane[1],Stanislav Sýkora[2]

1. MESTRELAB RESEARCH, Santiago de Compostela, Spain, http://www.mestrelab.com
2. Extra Byte, Castano Primo (Mi), Italy, http://www.ebyte.it

A typical experimental NMR spectrum comprises a) spectral peaks arising from the transitions of the studied spin system(s), b) satellite peaks from the very same spin system(s) isotopomers, c) solvent peaks due to the employed solvent, d) impurity peaks due to undesired chemical components of the sample, e) folded-over peaks from outside the spectral window, f) baseline distortions due to the acquisition dead-time or undesirable broad solid-state signals, and g) spikes due to interfering RF signals from the instrument or from the broader environment. For both quantitative and qualitative analysis, it is desirable to decompose the spectrum into the individual components. In particular, one would like to reduce it automatically to an editable peaks list which matches all the recognizable peaks in the experimental spectrum, leaving out any baseline drift and noise. The peaks in such a peak list, each described in a parametric form, can be then subject to automatic and/or manual editing (filtering). For example, one can recognize automatically spikes (anomalously narrow peaks), solid impurities (very broad peaks), folded-over peaks (anomalous phase), and possibly even rotation sidebands and isotopomer satellites.

Several partial approaches to generate such peaks list have been developed in the past, including parametric Linear Prediction [1] and the Filter Diagonalization Method [2] (FDM). As opposed to those methods, based on the direct analysis of the FID, in this work we present a new approach which works directly on the frequency domain and makes use of novel methods for spectral resolution enhancement (Resolution Booster [3]) and automatic peak detection. This process can be carried out either by using a fast algorithm based on the knowledge of the first and second derivatives of the data and on limited, local fitting, or by employing a substantially slower massive fitting method to be used when highly accurate values are needed.

We will show several real-life examples of GSD as implemented in Mnova software [4]. Current limitations and restrictions, as well as the future potential and perspectives of this approach will be also presented.

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4. MestReNova (Mnova), version 5, Mestrelab Research S.L.

47 Bayesian Dosy: A New Approach to Diffusion Data Processing

Carlos Cobas[1], Stanislav Sýkora[2]

1. MESTRELAB RESEARCH, Santiago de Compostela, Spain, http://www.mestrelab.com
2. Extra Byte, Castano Primo (Mi), Italy, http://www.ebyte.it

In this work we present a newly developed Bayesian approach to the processing of NMR diffusion experiments which is computationally very efficient and physically eminently meaningful. It treats all data points in the same way, and gives very satisfactory and artifact-free results. Applied specifically to DOSY data sets, it leads to what we call the BDT algorithm (standing for Bayesian DOSY Transform). It is also an excellent example of the broad class of Bayesian approaches to the evaluation of NMR data.

The BDT algorithm[1] is the core of the BayDOSY evaluation and presentation method implemented in Mnova, which is now beta-tested in practice on real experimental data. Apart from BDT, BayDOSY includes also a novel algorithm for a substantial improvement of the resolution in the diffusion-constant dimension and a contextual improvement in the alignment of peaks belonging to the same molecular species. Ongoing work aims at Bayesian handling of overlapping spectral peaks belonging to different sample components.

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48 HMBC (Heteronuclear Multiple-Bond Correlation) with Frequency-Swept Pulses

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

GlaxoSmithKline, Inc., 5 Moore Drive, Research Triangle Park, NC

Frequency-swept pulses have been used to advantage to implement a number of useful NMR experiments, particularly HSQC (Heteronuclear Single-Quantum Correlation). The pulses offer nearly perfect inversion and refocusing over a range of power levels, resulting in robust experiments that require minimal calibration. Frequency-swept pulses also offer gains in sensitivity over hard-pulse experiments. The most important source of the improved sensitivity is "J-compensation," which takes advantage of the timing properties of swept pulses. Smaller gains in sensitivity are also possible, depending on the design of the swept-pulse experiment.

Frequency-swept pulses would appear to be of little value in the HMBC experiment. The HMBC pulse sequence uses far fewer inversion and refocusing pulses than HSQC. J compensation provides no gains in signal intensity because the multiple-bond coupling constants cover a range that does not correlate with chemical shifts. Nevertheless, we show that frequency-swept pulses do have desirable features that make them well suited to the HMBC experiment.

49 Analysis of Pretreated Corn Cob Extracts by Coupled HPLC/MS/SPE/NMR Technology

Laurie Howe[1], Steve Cheatham[2], and Keith Wing[1]

1. E.I. DuPont de Nemours and Company, Experimental Station, Wilmington, Delaware
2. E.I. DuPont de Nemours and Company, Stine-Haskell Research Center, Newark, Delaware

Analysis of complex mixtures to define the chemical composition is quite difficult largely due to limitations of various individual analytical techniques. The coupling of chromatographic analytical tools to provide separation, such as, gas chromatograpy(GC) and high pressure liquid chromatography (HPLC) and information rich detection, like mass spectrometry (MS) and nuclear magnetic resonance (NMR), has made significant advances in mixture analysis. HPLC/NMR, introduced commercially in the late 1990's, is one such technique that allows in-depth NMR characterization of individual components without performing laboratory isolation. While HPLC/NMR is quite powerful, many factors have an impact on the quality of the analysis. The difficulties encountered are mainly due to the inherent low sensitivity of NMR and the solvent gradients required to achieve HPLC separation. These issues often cause problems such as NMR spectra that are either poor or not observed. In addition, the technique requires the use of deuterated solvents which frequently results in shifting of HPLC peak retention time. This can render comparison with HPLC/MS, for example, impossible.

A relatively new technique, HPLC/MS/SPE/NMR successfully provides a solution to overcome these issues by combining MS and solid phase extraction (SPE) to the HPLC/NMR. This integrated system, located at the DuPont Stine Research Center, allows collection of both MS and NMR of individual HPLC peaks from a single injection of material. The addition of SPE technology, which traps individual components, eliminates the need for use of deuterated solvents in the mobile phase and problems associated with solvent gradients in the NMR probe. SPE also offers the opportunity to increase observed NMR sensitivity of low-level compounds through multiple trapping.

50 A Macro To Create All Compound Specific qNMR Macros

Patrick A. Hays

Senior Research Chemist, DEA Special Testing and Research Laboratory, Dulles, Virginia

Quantitative NMR macros have two general aspects: 1) they are compound specific (dealing with the unique properties of the compound being quantitated (such as name, molecular weight, integral and peak positions, and number of hydrogens for each integral); and 2) they contain generic, non-specific, instructions for processing and reporting the NMR data. I present here a design for one macro that creates a compound specific qNMR macro for any compound. This macro is easy to use and has been tested successfully on numerous compounds and reports not only integral, but peak height quantitative results. The examples are using Varian's Magical II programming language.