SMASH 2003 NMR Conference

Verona, Italy

September 14th - 17th, 2003

SMASH 2003 NMR Conference

SMASH Attendees,

Welcome to Verona and the first European hosted SMASH conference. We have done our best to meet the suggestions and wishes of those who responded to the questionnaire after last years SMASH conference in Breckenridge, USA. The program focuses on the different aspects of small molecule research, including their interactions with larger molecules. From New Experiments, High Throughput Screening, Neutraceuticals and Tips & Techniques to Hyphenation, Sensitivity, Fast Sample Handling, Structure Elucidation and Conformational Analysis, the aim is to engage, communicate and educate. We are privileged to have one of the NMR communities Nobel Laureates, Richard Ernst, talk after the Conference banquet. Last year the Post-Graduate session was a great success and so we continue to encourage our young scientists by listening to them, present a broad series of cutting edge talks. The Workshops and Posters were popular with most and so we have included two sets of parallel workshops and an evening poster session with a buffet dinner.

On behalf of the whole SMASH Organizing Committee, we hope you enjoy the conference and thank you for your continued support and interest in SMASH.

Resonantly Yours,

Duncan Farrant & Carla Marchioro
Co-Chairs, SMASH 2003 NMR Conference

SMASH 2003 NMR Conference
Program

Sunday September 14th

5:00 PM - 6:00 PM **Registration**
6:00 PM - 8:00 PM **Dinner**
8:00 PM -11:00 PM **Mixer**

Monday September 15th

7:00 AM - 8:15 AM **Breakfast**
8:15 AM - 8:30 AM **Opening Remarks**
8:30 AM - 10:00 AM **New Experiments - James Keeler**

- *Real-time Chemical Shift Scaling: Low Field NMR Revisited*
  Gareth Morris, University of Manchester, UK
- *High-Resolution Solid-State NMR Methods For Visualising Drug Interactions With Transmembrane Receptors*
  David Middleton, UMIST, UK
- *NMR Sees The Mobile Side Of The Interaction: Protein Conformational Changes In Cell Adhesion And Drug Recognition*
  Stephan Grzesiek, University of Basel, Switzerland

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **HTS/Combinatorial - Marco Tatò**

- *Saturation Transfer Difference NMR To Characterize Ligand Binding To Protein Receptors*
  Bernd Meyer, Institut für Organische Chemie, Hamburg, Germany
- *NMR, CAMM And Crystallography For The Discovery And Validation Of High-Quality Leads*
  Wolfgang Jahnke, Novartis, Basel, Switzerland
- *HRMAS NMR A Tool To Monitor Chemical Transformation In Heterogeneous Systems*
  Pierre Rousselot-Pailley, University of Barcelona, Spain

12:00 PM - 1:30 PM **Lunch**
1:30 PM - 3:00 PM **Workshops (Concurrent)**

- I. *DOSY, Why, When And How?* - **Gareth Morris**
- II. *Choice Of Experiments To Answer The Question* - **Tim Claridge**

3:00 PM - 3:30 PM **Break**
3:30 AM - 5:00 PM **Neutraceuticals - Alberto Spisni**

- *Magnetic Resonance Imaging: Applications To Food -Composition, - Structure, -Storage, Processing, And -Cooking*
  Laurie Hall, University of Cambridge, UK
- *Curing Of Meat Products: ^{23}Na And ^{35}Cl NMR Studies*
  Jean-Pierre Renou, STIM, (INRA) Theix, France
- *^{1}H And ^{13}C High Resolution NMR Characterization Of Olive Oil*
  Annalaura Segre, CNR Rome, Italy

5:00 PM - 7:00 PM **Free Time**
7:00 PM - 7:15 PM **Bus To Palace Giusti**
7:15 PM - 8:00 PM **Garden Tour**
8:00 PM - 8:30 PM **Pre-Dinner Social Gathering**
8:30 PM - 11:00 PM **Social Dinner**
After Dinner Speaker: Richard Ernst
Double, Double Toil And Trouble, And My Numerous Pleasures In Science
11:30 PM **Bus Back To Hotel**

SMASH 2003 NMR Conference Program

Tuesday September 16th

7:00 AM - 8:30 AM **Breakfast**

8:30 AM - 10:00 AM **Tips & Techniques - Elisabetta Chiarparin**

- *Structure Elucidation Of Small Molecules Using Dipolar Couplings And Cross Correlated Relaxation Rates*
  Christian Griesinger, Max Planck Institut für Biophysikalische Chemie, Gottingen, Germany
- *The Automatic Confirmation Of Structure By ^{1}H Flow NMR*
  Lee Griffiths, AstraZeneca, Macclesfield, UK
- *Diffusion NMR. Not Just For Molecular Size*
  Michael Shapiro, Eli Lilly, Indianapolis, Indiana USA

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

10:30 AM - 12:00 PM **Hyphenated Techniques - John Shockcor**

- *600 MHz Cryoflowprobe - What Can And Cannot Be Done With This High Q Probe?*
  Michael Ritzau, Pfizer, Sandwich, UK
- *What's In A Hyphenated Name Anyway?*
  Mark O'Neil-Johnson, Sequoia Sciences, San Diego USA
- *The Use Of A Hyphenated Platform For Metabonomic Studies In Rat And Human Urine With NMR And A Hybrid Orthogonal Quadrupole Time Of Flight Mass Spectrometer*
  Jose Castro-Perez, Micromass, Manchester, UK

12:00 PM - 1:30 PM **Lunch**

1:30 PM - 3:00 PM **Sensitivity & Fast Sample Handling - Joyce James**

- *Developments In High-Throughput Capillary NMR With The VAST-HTSL Combination*
  Patrick Wheeler, Pfizer, San Diego, California, USA
- *High Resolution Capillary Tube NMR - A Miniaturized 5µl High-SensitivityTXI Probe For Mass-Limited Samples, Off-Line LC-NMR And HT-NMR*
  Götz Schlotterbeck, Hoffmann-La Roche, Basel, Switzerland
- *Cryoprobe High Throughput Problem Solving*
  Richard Upton, GSK, Stevenage, UK

3:00 PM - 3:30 PM **Break**

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

- I. Data Management And Automatic Archiving - **John Hollerton**
- II. Data Analysis And Chemometrics - **Hector Keun**

5:00 PM - 6:00 PM **Free Time**

6:00 PM - 6:30 PM **Pre-Dinner Social Gathering**

6:30 PM - 11:00 PM **Poster Session with Buffet Dinner followed by Mixer**

SMASH 2003 NMR Conference
Program

Wednesday September 17th

7:00 AM - 8:30 AM **Breakfast**

8:30 AM - 10:00 AM **Post-Graduate Session - Cynthia Larive**

- *Determination Of Rotamer-Specific Molecular Parameters By NMR Spectroscopy*
  Márta Kraszni, Semmelweis University, Budapest, Hungary
- *Identification Of Bioactive Compounds Employing cHPLC-NMR Coupling*
  Manfred Krucker, University of Tübingen, Tübingen, Germany
- *Multicoil NMR Probes For High-Throughput Analysis And Difference Spectroscopy*
  Megan A. Macnaughtan, Purdue University, West Lafayette, Indiana, USA
- *New Developments Of Metabonomics For Physiology*
  Marc Dumas, Imperial College, London, UK

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

10:30 AM - 12:00 PM **Structure Elucidation & Conformational Analysis - Adrian Davis**

- *Conformations And Bioactivities Of Proline-Rich Cyclic Peptides Isolated From The Fijian Marine Sponge Stylotella Aurantium*
  Marcel Jaspars, University of Aberdeen, UK
- *Understanding Chemical Interactions On Solid Supports*
  Nick Bampos, University of Cambridge, UK
- *Protonation, Tautomerism And Hydrogen Bonding Of Purine Analogues*
  Radek Marek, Masaryk University, Brno, Czech Republic

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

12:15 PM - **Box Lunch and Departure**

SMASH 2003 NMR Conference
Acknowledgements

The SMASH 2003 Conference gratefully acknowledges the support provided by the following companies.

Advanced Chemistry Development
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Poster Session

Gregory Nemeth

Monday, September 15th 8:30 AM - 10:00 AM

New Experiments

James Keeler, Session Chair

Speakers:

Gareth Morris, University of Manchester

David Middleton, UMIST

Stephan Grzesiek, University of Basel

Real-Time Chemical Shift Scaling: Low-Field NMR Revisited

Gareth A Morris[1], Neil P Jerome[1] and Lu-Yun Lian[2]

1. Department of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK

2. Department of Biomolecular Sciences, UMIST, P.O. Box 88, Manchester M60 1QD, UK

Few laboratories now have low field spectrometers. This is rarely a limitation, but causes problems for systems in intermediate exchange. Here linewidth is proportional to the square of the field strength, and lower field spectra can show both higher sensitivity and higher resolution. It would therefore be useful to be able to scale down the chemical shift in real time. The scaling of parameters such as chemical shift, scalar coupling and dipolar coupling has been used widely in solid-state NMR, but relatively little in high-resolution experiments. The sole reported application of real-time chemical shift scaling since its original proposal(1) has been for spatial localisation(2), ignoring the actual scaling properties.

A new approach to real-time delta-scaling(3) is proving highly effective. The use of a series of asymmetric spin echoes between successive data point samples during acquisition of a free induction decay allows the chemical shift to be scaled down uniformly by a user-defined factor. Appropriate phase cycling can compensate very effectively for <pi>-pulse imperfections, allowing high resolution to be retained.

Applications of the experiment to dynamic and coupled systems show clean control of strong coupling effects and straightforward manipulation of slow/intermediate/fast exchange regimes. Potential applications include improving the detection of exchanging signals, investigation of chemical exchange processes, distinguishing between homogeneous and inhomogeneous broadening, and analysis of spin systems. Spectra will be presented demonstrating the application of real-time delta-scaling to a range of systems and problems.

1. JD Ellett and JS Waugh, J. Chem. Phys. 1969, 51, 2851-8; JA DiVerdi and S Opella, J. Chem. Phys. 1982, 75, 5594-5.
2. M Nuss and ET Olejniczak, J. Magn. Reson. 1986, 69, 542-545.
3. GA Morris, NP Jerome and L-Y Lian, Angew. Chemie Int. Ed., 42, 823-825 (2003).

High-Resolution Solid-State NMR Methods For Visualising Drug Interactions With Transmembrane Receptors

David A. Middleton

Department of Biomolecular Sciences
University of Manchester Institute of Science and Technology
Manchester, U.K.

NMR spectroscopy is a valuable technique for characterising the interactions between small molecules and pharmaceutically interesting proteins in aqueous solution. Over half the targets of current drug therapies are transmembrane proteins, which must be embedded within an insoluble lipid bilayer to preserve their structure and function. Membrane protein-ligand interactions are difficult to characterise using solution NMR methods, particularly in the case of strongly binding ligands, because dipolar couplings and chemical shift anisotropy give rise to severe line broadening. This presentation will describe recent developments in multinuclear (^{13}C, ^{31}P, ^{19}F) solid-state cross-polarisation magic-angle spinning (CP-MAS) NMR for (i) screening small molecule interactions with transmembrane receptors, (ii) quantifying ligand binding affinities and (iii) solving the structures of ligands in their active sites. It will be shown how newly developed CP-MAS methods can eliminate interference from non-specific binding of hydrophobic ligands and determine the binding constants and dissociation rates of hydrophobic ligands. The talk will demonstrate how this combined information can contribute to drug discovery, with examples from recent work on ion pumps, bacterial transport proteins and G-protein coupled receptors.

NMR Sees The Mobile Side Of The Interaction: Protein Conformational Changes In Cell Adhesion And Drug Recognition

S. Grzesiek, M. Allan, M. Barfield, F. Cordier, J. Engel, D. Häussinger, P. Jensen, J. Kahmann, H.J. Sass, C. Thompson

Biozentrum, University of Basel, Switzerland
University of Arizona, Tucson, USA

High resolution NMR is a unique tool to study the interactions of biomolecules in solution. Traditionally, chemical shift mapping and NOEs are used to identify contact surfaces and to determine molecular geometries, but more recently weak alignment, anisotropic diffusion and scalar couplings across hydrogen bonds provide additional information. We will give examples of this technology applied to biomacromolecules involved in cellular adhesion, multidrug resistance and protein folding.

Monday, September 15th 10:30 AM - 12:00 PM

HTS/Combinatorial

Marco Tatò, Session Chair

Speakers:

Bernd Meyer, University of Hamburg

Wolfgang Jahnke, Novartis

~~Pierre Rousselot-Pailley, University of Barcelona~~

Saturation Transfer Difference NMR To Characterize Ligand Binding To Protein Receptors

Bernd Meyer, Jens Klein, Moriz Mayer, Robert Meinecke, Heiko Möller, Oliver Schuster and Jan Wülfken

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Martin Luther King Pl. 6, 20146 Hamburg, Germany
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Saturation transfer difference NMR spectroscopy is based on the long known principle that by saturating a protein one can transfer this saturation to the ligand if there is an exchange between ligand and protein. So far, the technique was largely used for extremely tight binding protein-ligand complexes and here especially for phosphorous containing ligands. In order to analyze the effect of saturation that is transferred from the protein to the ligand efficiently, we introduced the difference method by which we obtain a spectrum in which only signals remain in the spectrum that arise from molecules that have binding affinity. The technology is very robust and can be applied to any proteins larger than about 12kD. There is no limit to the size of the protein. In fact, we have been working with proteins up to 250kD. Using this method, it is easy to identify ligands from a mixture of compounds. The protein can be attached to a resin or can be anchored into the membrane of a liposome.

STD-NMR is very uncritical in terms of saturation excess of the ligand or amount of protein. In fact, we have been working with less than one nanomol (a few ug) of protein to analyze binding. Because the transfer of saturation is most efficient to ligand protons that are in direct contact with the protein, one can also determine the residues that are interacting directly with the protein. Furthermore, if the exchange between the ligand in its bound and free state is fast enough, one can even determine those individual protons that are making the direct contact even within an amino acid, a carbohydrate residue or an aromatic residue.

Because STD-NMR acts only as a filter, it can be interfaced with any pulse sequence. By competition titrations, it is possible to arrive at very precise binding constants for the ligands. On the ligand side, we have analyzed carbohydrates, peptides, glycopeptides and aromatic molecules. A quantitative analysis of binding curves as a function of the amount of the ligands can directly yield binding constants.
The maximum number of components in a library can be approximately 200. We have for example identified ligands to E-Selectin from a complex compound library obtained from combinatorial chemistry.

NMR, CAMM And Crystallography For The Discovery And Validation Of High-Quality Leads

Wolfgang Jahnke, P. Floersheim, B. Cutting, A. Strauss, G. Fendrich

Novartis Institute for Biomedical Research
CH-4002 Basel, Switzerland

Identification and characterization of molecular interactions has become a key area of NMR in drug discovery research. The ability of NMR to robustly detect even weak interactions is valuable in two respects: First, hits from biochemical high-throughput screening assays, which are often prone to artifacts and concomitant false positives, can be (in)validated by NMR, and their binding site, competition with a known ligand, and approximate dissociation constant can be determined. Second, NMR screening by itself can identify small fragments with weak but validated binding affinity. These fragments can then be optimized by similarity searches, NMR second-site screening, or virtual screening. Examples for both applications will be given.

HRMAS NMR A Tool To Study Chemical Transformations In Heterogeneous Systems

Rousselot-Pailley Pierre[2], Wieruszeski Jean-Michel[1] and Lippens Guy[1]

1. CNRS-University of Lille 2 UMR 8525, Pasteur Institute of Lille

1 rue du Professeur Calmette, 59019 Lille, France

2. University of Barcelona, Spain.

Since the last past decade HRMAS NMR has been recognized as a versatile and powerful tool to study compounds tethered to solid support. In our group we developed the technique to follow chemical transformations in heterogeneous systems. In the field of solid phase organic synthesis we used the technique to monitor and quantify a chemical transformation either on conventional resin or on lanterns.

We also studied the structuration of peptides tethered to water swollen solid support, in order to get a better understanding of some difficulties encountered during solid phase peptide synthesis.

In a last development we used HRMAS NMR to follow the metabolism of the pro-drug Ethionamide inside bacteria cells.

Those different study will be presented and discussed in this presentation

TALK CANCELLED

Monday, September 15th 3:30 PM - 5:00 PM

Neutraceuticals

Alberto Spisni, Session Chair

Speakers:

Laurie Hall, University of Cambridge

Jean-Pierre Renou, STIM, (INRA) Theix

Annalaura Segre, CNR Rome

Magnetic Resonance Imaging: Applications To Food -Composition, - Structure, -Storage, -Processing, And -Cooking

Laurie Hall and Kevin Nott

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The long title of this presentation reflects the enormous potential of Magnetic Resonance Imaging (MRI) for providing novel and/or important information about many aspects of "foods". The talk itself will use experimental data from HSLMC to illustrate each specific point as follows:

Food-Composition:

MRI can separately quantitate the spatial distribution of lipid and water in many foodstuffs (eg. Meat).

Food-Structure:
The above scans also map in two- or three -dimensions (2D, 3D) aspects of "structure", such as the separate physical compartments within either raw produce, or in prepared meals.

Food-Storage:
Many changes which occur during storage can be followed by measurements of the MRI parameters which depend on the molecular mobility of the water or lipid molecules. (e. ripening of fruit).

Food -Processing and -Cooking:
This section will demonstrate that MRI can provide unique information about both industrial and domestic aspects. For example, a 52 second duration MRI protocol can provide a 3D map of the temperature of a food, (which comprises 32 x 32 x 128 = 130,000 independent measurements) whilst it is being heated by immersion in hot -water or -air, by radiant heat or by microwaves. Similarly MRI can measure in 3D the flow of a liquid food during mixing, or extrusion.

Curing Of Meat Products: ^{23}Na And ^{35}Cl NMR Studies

J.P. Renou, L. Foucat, J.P. Donnat

STIM, INRA Theix
Saint-Genès Champanelle, 63122 France

Sodium chloride is widely used in the meat industry to improve such qualities of meat as water holding capacity. But its primary function is to preserve and to inhibit the microbial flora growth in meat products. However, it is well established that excess of salt consumption can induce cardiovascular disease. Thus, in the field of the public health, reduction of salt content in some brined products is suitable. This must be done without altering either organoleptic qualities or preservation properties. In this context, a better understanding of the role of Na^+ and Cl^- ions is necessary. In particular, it is essential to quantify and to characterise for each ion the "bound" and "free" fractions, and this in relation to the microbiological quality and the salt taste of the product.

^{23}Na and ^{35}Cl NMR spectroscopy offers a unique opportunity to access non-invasively the distribution and the state of Na^+ and Cl^- ions in tissues. To characterise the dynamics of these two quadrupolar nuclei (I=3/2), single quantum (SQ) and multiple quantum filter (MQF) acquisitions were used.

Applications on different brined meat products (pork, smoked salmon…) are reported. NMR results are discussed in terms of interactions of Na^+ and Cl^- ions with meat structure. ^{23}Na imaging was also used to study the distribution of brine in ham processed in various ways (tumbling, cooking).

^{1}H And ^{13}C High Resolution NMR Characterization Of Olive Oil

Annalaura Segre[1] and Luisa Mannina[1,2]

1. Ist.di Metodologie Chimiche CNR
00016 Monterotondo Staz., Roma, Italy
2. Università del Molise, Facoltà di Agraria
Dip. S.T.A.T., 86170 Isernia, Italy

^{1}H and ^{13}C NMR together with a multivariate statistical analysis are capable of characterizing olive oils according to their genuinity, origin and cultivar.

Authentication of olive oil. ^{1}H NMR can be used to analyse oils of different botanical origin such as hazelnut oil and seed oils. The adulteration of olive oil with soy, corn, rice or rapeseed oil is easily measured with most analytical techniques, not so in the case of adulteration with refined hazelnut oil. A full study performed within the European Project "MEDEO" allowed the evaluation of hazelnut oil present in adulterated olive oil samples. Linear Discriminant Analysis (LDA) was used to analyse NMR data. The determination of a possible fraud on olive oil down to limits where the addition of hazelnut oil becomes unprofitable is then possible, i.e. this method permits hazelnut oil addition to be detected at an extent of about 5%.

Geographical origin classification of Italian olive oils. ^{1}H NMR spectroscopy due to its intrinsic sensitivity allows to analyse minor components present in olive oil such as aldehydes, sterols, and terpenes. These minor components are very useful for the determination of the geographical origin of Italian olive oils; in fact, ^{1}H NMR is able to distinguish olive oils from all over Italy regardless of their cultivar. A proper choice of selected ^{1}H resonances allows the geographical selection. All data belonging to the same geographical area are grouped with errors less than 10%. A database was collected showing that the analysis works also for the geographical characterization of olive oils of different years. Therefore multivariate statistical analysis applied to ^{1}H NMR data may be a way to check the Protected Denomination of Origin (D.O.P.).

Varietal classification of olive oils. In order to group olive oils according to their own cultivars, ^{13}C high-field Nuclear Magnetic Resonance (NMR) and Gas Chromatography (GC) were used to analyze olive oils from the same Italian region) obtained from four monovarietal cultivars. ^{13}C NMR spectrum allows one to obtain information about glycerol tri-esters of olive oils. GC gives the complete fatty acid profile of olive oil samples. NMR or GC selected peaks submitted to a suitable statistical analysis allowed us to group olive oils according to their cultivars. Results obtained from ^{13}C NMR and GC techniques combined with the multivariate statistical procedure are in good agreement and prove the usefulness of fatty acids to group the monovarietal olive oils belonging to some particular cultivars.

Tuesday, September 16th 8:30 AM - 10:00 AM

Tips & Techniques

Elisabetta Chiarparin, Session Chair

Speakers:

Christian Griesinger, Max Planck Institut für Biophysikalische Chemie

Lee Griffiths, AstraZeneca

Michael Shapiro, Eli Lilly

Structure Elucidation Of Small Molecules Using Dipolar Couplings And Cross Correlated Relaxation Rates

Teresa Carlomagno, Jens Meiler, Marcel Blommers, Laurent Verdier, Peyman Sakhaii, Heike Neubauer, **Christian Griesinger**

Max Planck Institute for Biophysical Chemistry
Am Fassberg 11, 37077 Göttingen, Germany
&
Novartis, Basel, Switzerland

The measurement of dipolar couplings for water soluble (1) as well as insoluble organic compounds (2) shall be presented. Unique information on the structure and dynamics of these molecules can be derived.

In addition, the use of cross correlated relaxation to study structures of intermediately bound molecules will be described on the example of epothilone A bound to tubulin (3).

1. H. Neubauer, J. Meiler, W. Peti and C. Griesinger, Helv. Chim. Acta 84, 243-258 (2001)
2. L. Verdier, P. Sakhaii, M. Zweckstetter and C. Griesinger, J. Magn. Reson. in press
3. T. Carlomagno, I. C. Felli, M. Czech, R. Fischer, M. Sprinzl, C. Griesinger, J. Am. Chem. Soc. 121, 1945-1948 (1999); T. Carlomagno, M.J.J. Blommers, J. Meiler, W. Jahnke, T. Schupp, F. Petersen, D. Schinzer, K.-H. Altmann and C. Griesinger, Angewandte Chem. 115, 2615-2619 (2003); T. Carlomagno, M.J.J Blommers, C. Griesinger, Angewandte Chem. 115, 2619-2621 (2003)

The Automatic Confirmation Of Structure By ^{1}H Flow NMR

Lee Griffiths

AstraZeneca

Flow-NMR allows more rapid and convenient acquisition of NMR spectra. Its main application area has therefore been in multiple parallel synthesis or combinatorial chemistry. At the same time there is a significant need to automate the analysis of the resultant spectra. However, flow-NMR brings spectral imperfections, which compromise attempts to automate this analysis. The work described comprises experimental and computational expedients to accommodate the effects of residual solvent peaks, ^{13}C satellites, finite signal to noise, impurities, pre-saturation on integral calculations, the "silent" region and how multiplet areas can be scaled to numbers of protons in this environment.

Diffusion NMR. Not Just For Molecular Size

Michael Shapiro, Jiangli Yan, Allen D. Kline, Huaping Mo, Edward R. Zartler

Lilly Research Laboratories

In the pharmaceutical industry, diffusion based NMR techniques have been incorporated into a variety of methods, such as screening of chemical mixtures, determining the structures of bound ligands without physical separation, and measuring the diffusion coefficient of small metabolites in biofluids.

In our investigations of ligand-recpetor interactions,we have found that intermolecular NOE can build up during a long diffusion period and can seriously interfere with the diffusion measurement of ligands. The NOE creates a deviation from the linearity on the lnI-g2 plot used to determine the diffusion coefficient. The deviation varies from proton to proton because it is related to the intermolecular distance of ligand proton to the target surface. Therefore the deviation from linearity in the diffusion experiments can be used to create the epitope map of the ligand to the target.

Tuesday, September 16th 10:30 AM - 12:00 PM

Hyphenated Techniques

John Shockcor, Session Chair

Speakers:

Michael Ritzau, Pfizer

Mark O'Neil-Johnson, Sequoia Sciences

Jose Castro-Perez, Micromass

600 MHz Cryoflowprobe - What Can And Cannot Be Done With This High Q Probe?

Michael Ritzau

Pfizer Global R&D, Sandwich, UK

Recently the cryotechnology has been extended to flow- and LC-NMR probes. Having received such a probe in April 2003 we will present data that demonstrates not only its high S/N but more specifically covers the problem of solvent suppression in protonated solvents, where radiation damping becomes a problem on this type of probe. Examples will include parallel chemistry samples as well as metabolites and biofluids.

What's In A Hyphenated Name Anyway?

Mark O'Neil-Johnson

Sequoia Sciences, Inc.

HPLC hyphenation to an NMR measurement is assumed to be a well-established, mature technology. With the introduction of capillary HPLC, new opportunities were created to take the sample requirements down to the microgram scale. An entry point into the coil of an NMR was created with the coupling of capillary HPLC to the NMR which generated new opportunities as well as challenges from NMR sensitivity and HPLC isolation. Sequoia Sciences has created rugged HPLC isolation methodologies for its purified natural product chromatographic fractions and coupled this with a CapNMR probe, but not as a hyphenated technique. In our laboratory and as is the case with most real-life HPLC isolations, acceptable HPLC isolations require intelligent method development with analytical or semi-preparative columns. For full NMR data set acquisitions on compounds from challenging HPLC isolations at the microgram scale, the LC-NMR hyphenation technique was not a productive and viable option for our efforts. Data will be presented demonstrating routine acquisition of full data sets on 10-50 micrograms of material originating from tough HPLC isolations of natural products. Sequoia has created a new way for HPLC chemists and NMR spectroscopists to look at HPLC NMR without the hyphen.

The Use Of A Hyphenated Platform For Metabonomic Studies In Rat And Human Urine With NMR And A Hybrid Orthogonal Quadrupole Time Of Flight Mass Spectrometer

Jose Castro-Perez[1], Hilary Major[1], John P. Shockcor[2], Andrew Nichols[2], Henrik Antti[2]

1. Waters Corporation, MS Technology Center
Floats Road, Manchester,UK

2. Metabometrix , Prince Consort Road
South Kensington, London SW7 2BP, UK

Metabonomics is a systems approach for studying in vivo metabolic profiles and can provide information on disease state, toxicity and gene function. In metabonomics the effect of a pharmaceutical candidate on the whole animal or organism is investigated by studying the changes in metabolism over a time course following compound administration. LC-MS/MS and NMR spectroscopic analysis of biofluids, cells or tissues enables the generation of spectral profiles of a wide range of endogenous metabolites that reflect the metabolic status of an organism. The data generated in these studies are analyzed by statistical techniques such as principal component analysis (PCA) to highlight both subtle and gross systematic differences between the samples. The purpose of this study is to evaluate the use of Electrospray Mass Spectrometry in conjunction with NMR for Metabonomic studies followed by multivariate statistics and subsequent identification of biomarkers from the mass spectra and NMR. We will also present mass directed fractions containing bio-markers of interest which will be submitted to further MS and NMR analysis for identification purposes. The use of hyphenated techniques such as LC-MS-NMR together with multivariate statistical analysis was successfully applied in several studies including investigations of rat urine after dosing with a classical hepatotoxin. This data clearly shows that LC/MS together with NMR spectroscopy are very powerful tools for metabonomic applications in drug discovery and development.

Tuesday, September 16th 1:30 PM - 3:00 PM

Sensitivity & Fast Sample Handling

Joyce James, Session Chair

Speakers:

Patrick Wheeler, Pfizer

Götz Schlotterbeck, Hoffmann-La Roche

Richard Upton, GSK

Developments In High-Throughput Capillary NMR With The VAST-HTSL Combination

Patrick Wheeler[1], Cathy Moore[1], James Norcross[2], Dean Olson[2], Allan Pang[1]

1. Pfizer La Jolla Labs, San Diego, CA

2. MRM/Protasis, Savoy, IL, USA

The expectations for any technique advance ahead of the capabilities for that technique's routine implementation. The past year has seen a prime example of this phenomenon in the commercial introduction of gradient-equipped capillary NMR probes with matched sample loading apparatus. In principle, such a combination would be an excellent analytical adjunct to a combinatorial chemistry effort, with 5-10 fold reduction in required sample size at equivalent throughput rates. This presentation will detail the rigors of implementing use of a Protasis-MRM ICG probe into routine operation for high-throughput applications to support the flow-injection NMR analysis of combinatorial arrays. Particulars will include choice of capillary types and size, appropriate fittings, sample preparation, methods for handling extremely small volumes, and compromise choices between sensitivity and fast sample handling.

High Resolution Capillary Tube NMR - A Miniaturized 5µl High-SensitivityTXI Probe For Mass-Limited Samples, Off-Line LC-NMR And HT-NMR

Götz Schlotterbeck, Alfred Ross, Hans Senn

F. Hoffmann- La Roche, Pharmaceutical Research, Basel, Switzerland

Currently NMR measurements are performed in tubes of 5mm diameter and a detection volume of about 500µl. Miniaturizing the NMR measurement has many advantages the most important are: (i) the intrinsic higher sensitivity of the miniaturized detection coil leads to a decrease of the experiment time and thus potentially to a higher sample throughput of mass-limited samples and (ii) the use of costly deuterated solvents can significantly be reduced, and concomitantly the spectral artifacts from solvent impurities.

NMR measurements using a new 1mm TXI probe (Bruker) with an active sample volume of 2.5 microliter are reported. This is the first microliter NMR probe with optimized coil geometry for use with individual capillaries of outer diameter of 1 mm. The probe offers significant advantages over previous and other analytical-chemical NMR technologies: an increased mass sensitivity by a factor of 4 compared to the conventional setup and spectra with very low solvent background.

We demonstrate the potential of the probe for structure elucidation of mass- and volume-limited samples and micro-bore HPLC-NMR coupling on selected examples from pharmaceutical research. In addition applications with high sample throughput (HT-NMR) for quality control of large sample arrays are shown. This also includes the automatic sample preparation of 1mm capillaries.

Cryoprobe High Throughput Problem Solving

Richard J. Upton

GlaxoSmithKline R&D Stevenage, UK

As part of the Quality Assurance project on our compound screening collection it was necessary to run a subset of 20,000 x 1mg samples by NMR over one year. These samples had not given an appropriate response in the initial LCMS phase of the project for the expected structure, but could still be potentially pure and therefore valuable screening materials. A Bruker AV500 NMR with an inverse 3mm cryoprobe was utilised to provide the necessary sensitivity to run a full suite of problem solving experiments on every sample, with a 15 minute turnaround time. Experimental, logistical and practical problems involved in this task will be outlined, together with the associated software necessary for managing and interpreting the large quantity of data produced.

Wednesday, September 17th 8:30 AM - 10:00 AM

Post-Graduate Session

Cynthia Larive, Session Chair

Speakers:

Márta Kraszni, Semmelweis University

Manfred Krucker, University of Tübingen

Megan A. Macnaughtan, Purdue University

Marc Dumas, Imperial College

Determination Of Rotamer-Specific Molecular Parameters By NMR Spectroscopy

Márta Kraszni and Béla Noszál

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Bio- and drug molecules have long been hypothesized to take up different states of ionization and conformation in the various stages of their pharmacological journey. For example, membrane penetration and receptor binding take place preferably via the lipophilic and hydrophilic rotamers of the same molecular skeleton, respectively. Determination of the rotamer populations and related rotamer-specific parameters, such as basicity and lipophilicity is therefore not only a challenging theoretical task, but also a practical job to predict the viability of drugs in living systems.

We have therefore studied the stereochemical factors that influence two major physico-chemical properties: partition coefficient and basicity, both at the rotamer-specific levels. The first, experimental determinations of conformer-specific partition coefficients in octanol/water system were exemplified on amphetamine and clenbuterol, two small, flexible drugs possessing one major rotational axis. For the calculation of conformer-specific partition coefficients conformer populations were determined from vicinal ^{1}H-^{1}H NMR coupling constants of the ABX or ABC spin systems. The standard coupling values existing in distinct rotamers were calculated from the Altona-type Karplus equation[1]. Configuration- and conformation-dependent basicities were studied on two other small biomolecules: ephedrine and pseudo-ephedrine. These compounds are in diastereomeric relationship, and they differ significantly in the physico-chemical properties and also in the biological activity. We have quantitatively traced back some of the macroscopic differences into differences in population and basicity of the various rotamers. Determination of the rotamer populations was a complex task, since one single vicinal proton pair can be found at the ends of the rotational axis only. Therefore we introduced a set of model compounds and vicinal ^{1}H -^{13}C NMR coupling constants to solve the problem.

1. Altona, C.; Francke, R.; de Haan, R.; Ippel, J. H.; Daalmans, G. J.; Hoekzema,

A. J. A. W.; van Wijk, J. Magn. Reson. Chem. 1994, 32, 670-678.

Identification Of Bioactive Compounds Employing cHPLC-NMR Coupling

Manfred Krucker, Karsten Putzbach, Hongbin Xiao, and Klaus Albert

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To fulfil the need for fast and efficient identification of low concentration analytes from biological, environmental, and forensic samples, the hyphenation of capillary separation techniques to spectroscopic or spectrometric detection methods is getting of increased importance. Mostly used these days is the direct on-line coupling of high performance liquid chromatography (LC) to mass spectrometry (MS). Despite all the advantages of the LC-MS coupling shown in numerous applications, nuclear magnetic resonance spectroscopy (NMR) is considered to be one of the most powerful spectroscopic techniques for the unambiguous structural elucidation of unknown compounds. Unfortunately, the low sensitivity of the NMR detection still circumvents the widespread acceptance of this hyphenated technique. Major improvements increasing NMR sensitivity could be achieved utilising solenoidal capillary NMR probes and recent advances in microscale separations. Capillary NMR probes enable on-flow and stopped-flow NMR experiments in hyphenated systems, as well as flow injection measurements. Examples for all these different measurement types will be presented, in particular the continuous-flow cLC-^{1}H-NMR detection of tocopherols, stopped-flow experiments of carotenoid samples from gradient capillary LC separations, as well as stopped-flow experiments for the identification of iso-flavonoids from Radix Astralagi.

Multicoil NMR Probes For High-Throughput Analysis And Difference Spectroscopy

Megan Macnaughtan, Ting Hou, Jun Xu, Robert Santini, and Daniel Raftery

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West Lafayette, Indiana, USA,
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Recent advances in areas such as drug discovery and combinatorial chemistry require the development of new high-throughput analytical tools. In our laboratory, we have developed a new methodology, Multiplex NMR, which significantly increases the throughput of NMR measurements. The Multiplex NMR Probe currently can analyze four samples simultaneously using parallel, detection microcoils. Pulsed field gradients are used to distinguish signals from different samples, and rapid analysis of multiple samples can be achieved by selective excitation or chemical shift imaging methods. A completely automated, high-throughput system for injection and analysis of multiple samples has been achieved with the Multiplex NMR flow probe and a robotic liquid handler. Four samples can be simultaneously injected into the Multiplex Probe and then analyzed in succession using a selective excitation experiment giving analysis times as low as 34 s/sample for 1D ^{1}H NMR. A detailed description of the automated system, its applications, and throughput capabilities will be described.

In NMR, difference experiments are often used to eliminate background signals and reveal chemical interactions such as the binding of a ligand to a protein. Often two samples are analyzed separately and the resulting spectra are subtracted with software. We have developed the dual coil NMR Difference Probe to analyze two samples simultaneously and produce a single ^{1}H NMR difference spectrum. The difference spectrum is the result of a unique resonant circuit, which allows the two samples to be excited simultaneously by the same pulse sequence, while their signals are subtracted by destructive interference within the resonant circuit. Two dual-coil difference probes have been developed. A microcoil, flow-through difference probe is used to detect nanoliter to microliter, mass-limited samples under flowing or static conditions. A second NMR Difference Probe was constructed using saddle coils. The samples are placed in a modified 3 mm NMR tube, which can be spun. The saddle coil difference probe has a larger volume compared to the microcoil probe and is equipped with a pulsed field gradient for advanced NMR difference experiments. The resonant circuit, construction, and applications of the NMR Difference Probes will be described.

New Developments Of Metabonomics For Physiology

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The concept of "biological signature" is introduced in metabonomics by the study of chronic effects of anabolic steroids on metabolic homeostasis in the bovine model, by means of a simple urine analysis. This study hypothesizes that physiological disruptions induced by anabolics in cattle generate significant variations of the general metabolism, evidenced by parallel acquisition and treatment of a great number of variables. For this purpose, proton carbon HMBC nuclear magnetic resonance spectroscopy and metastable atom bombardment mass spectrometry have been validated as suitable variable generators. A variable-selection algorithm, extracting significantly affected non-redundant variables has been optimized for several pattern recognition devices, with a focus on Linear Discriminant Analysis. The global effect of anabolic steroids is delineated by decreasing variances in function of sex, treatment and their interaction, revealed by an apparent "pseudo-feminisation" of males and a "pseudo-masculinisation" of females. Specific effects of treatment in function of sex, resulting from treatment using testosterone in cows or trenbolone acetate and estradiol in steers, reveal responses that are related to the dosage and the duration of treatment. These effects can be diagnosed up to 90 days after implantation in the case of trenbolone acetate and estradiol administered to steers. Moreover, biological signatures obtained 90 days after implantation of steers seem to be similar, whatever the spectrometric variable generator used. Urinary metabolite assignments provide a metabolic and biochemical basis to the factorial analyses of anabolic steroid effects. Metabolites significantly affected, as described in this work, are related to nitrogen and energy metabolism, but also to osmolytic and electrolytic balance. Coordinated readjustments of these metabolites could outline a global modification of the functioning of the organism, in terms of adaptation for increased nitrogen retention.

Wednesday, September 17th 10:30 AM - 12:00 PM

Structure Elucidation & Conformational Analysis

Adrian Davis, Session Chair

Speakers:

Marcel Jaspars, University of Aberdeen

Nick Bampos, University of Cambridge

Radek Marek, Masaryk University

Conformations And Bioactivities Of Proline-Rich Cyclic Peptides Isolated From The Fijian Marine Sponge Stylotella Aurantium

Marcel Jaspars[1], Linda Morris[1], Bruce Milne[1], Jioji Tabudravu[1], Jantien Kettenes-van den Bosch[2]

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University of Aberdeen, Old Aberdeen, AB24 3UE, Scotland

2. Department of Biomolecular Mass Spectrometry
Utrecht Institute for Pharmaceutical Sciences, Utrecht University
Utrecht, The Netherlands

Proline-rich cyclic peptides have been isolated from diverse phyla. Bioactivity of cyclic peptides in Nature is improved over linear peptides due to their greater resistance to in vivo enzymatic degradation and increased bioavailability. Cyclisation also helps reduce conformational flexibility of the peptide backbone which enables systematic manipulation of the 3D conformation. The inclusion of several proline residues controls the conformation of the molecule in solution because of the restricted phi of proline. Sponges provide a large number of examples of proline-rich cyclic peptides: the hymenamides, stylopeptide, axinellins, axinastatins, and the phakellistatins.

During investigations of the Fijian marine sponge Stylotella aurantium we discovered several proline-rich cyclic hepta and octapeptides, the new wainunuamide and axinellin C and the known psuedoaxinellin and phakellistatin 2. The conformational preferences of axinellin C and two conformers of phakellistatin 2 were investigated using NOE-restrained molecular dynamics calculations. It was found that axinellin C adopts the same fold as phakellistatin 8 and the fungal natural product antamanide despite very divergent peptide sequences. Two different conformations of phakellistatin 2 were isolable by HPLC and were stable for long periods of time at room temperature. By NMR, one of these was identical to the previously isolated and subsequently synthesised phakellistatin 2. The second conformer was more compact and less polar, and is stabilised in part by a H-bond and a reduced solvent accessible surface compared to the more polar conformer. Molecular dynamics calculations have shown that there is a conformational lock preventing the more compact conformer from being converted into the more polar one. Assessment of bioactivity of the different conformers in different solvents suggests that the resulting conformational change may be responsible for loss of bioactivity.

Understanding Chemical Interactions On Solid Supports

Nick Bampos

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Characterisation of molecular recognition events have been extensively reported in both the solution and solid states. Most of our work centres on the binding interaction between organic guests and metal centred hosts and have been investigated primarily using ^{1}H NMR spectroscopy. In the solution state, the appearance of the spectrum is dominated by the strength of the binding interaction. By simply changing the temperature of the experiment, the binding interaction and resulting spectrum can be altered. Furthermore, by changing the metal used in a particular chemical system, the binding interaction can also be affected.

In this presentation we will outline the construction of simple rotaxanes using donor-acceptor and metal-ligand binding. Of particular interest is the transfer of the methodology from the solution state to the solution-solid interface by tethering components of the rotaxanes to solid supports (Argo-Gel™ beads).[1] Construction of the rotaxanes may then be dependent on where the chemistry is taking place (on the surface or within the bulk of the material). Using standard CPMG and suppression sequences, it is possible to obtain high quality spectra which shed light on molecular interactions using the solid supports,[2] and perhaps more importantly, give some insight into the morphology of the solid support.

1. K.D. Johnstone, N. Bampos, J.K.M. Sanders, M.J. Gunter A self-assembling polymer-bound rotaxane under thermodynamic control Chem. Commun. (12), 2003, 1396-1397.
2. Y.R. de Miguel, N. Bampos, K.M.N. de Silva, S.A. Richards, J.K.M. Sanders Gel phase MAS 1H NMR as a probe for supramolecular interactions at the solid-liquid interface Chem. Commun. (20), 1998, 2267-2268.

Protonation, Tautomerism, And Hydrogen Bonding Of Purine Analogues

Radek Marek

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Biogenetic purine bases play central roles in most biological processes. Structural modifications of the purine bases, nucleosides, and nucleotides have resulted in the discovery of thousands of biologically active compounds, including many clinically used drugs. Their biological effects range from antiviral and antineoplastic activities to antihypertensive properties.

The proper characterization of the tautomeric equilibria and protonation sites of the variously substituted purine derivatives under diverse conditions is of great importance and significance. Processes involving the change of the proton position play a direct role in the selectivity of recognition at the active sites of enzymes and alkylation reactions. These equilibria are governed by the electron distribution within the molecule and by the intermolecular effects such as solvation or crystal packing. ^{15}N NMR spectroscopy is a very sensitive probe for studying the electron distribution, tautomeric equilibria, and protonation processes [1].

The variously substituted purine derivatives were investigated by using inverse-detected ^{15}N NMR spectroscopy in the solution at a range of temperatures. Slow-spinning ^{15}N CP/MAS data of selected compounds were recorded in order to study intermolecular interactions in the solid-state and principal values of the ^{15}N chemical shift tensors [2,3]. DFT calculations of nitrogen chemical shifts were used for determining the solvation effects in the solution and bonding patterns in the solid state, assigning the nitrogen resonances observed in the solid-state spectra, and determining the orientation of the principal components of the chemical shift tensors. Structural arrangements have been correlated with the geometry obtained from single-crystal X-ray diffraction analysis.

Benefits of studying the structure by all these approaches will be discussed [4].

1. R. Marek, A. Lycka, Curr. Org. Chem., 2002, 6, 35-66.
2. D. Stueber, D.M. Grant, J. Am. Chem. Soc., 2002, 124, 10539-10552.
3. R. Marek, J. Brus, J. Toušek, L. Kovács, D. Hocková, Magn. Reson. Chem., 2002, 40, 353-360.
4. R. Marek, et al., unpublished results.

Tuesday, September 16th 6:30 PM - 9:30 PM

Poster Session

Sponsored by Varian Inc.

Gregory Nemeth, Session Chair

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55. Automated NMR Approaches In Pharmaceutical Discovery

1 Accurate Predictions Of ^{1}H Chemical Shifts Of Halogenated Hydrocarbons; Through Space Contributions

Mehdi Mobli[1], Raymond J. Abraham[1], Richard J. Smith[2], Ben Bardsley[2]

1. Chemistry Department. University of Liverpool

2. GlaxoSmithKline, Medicinal Research Centre, Stevenage

Our research group has developed a model, CHARGE, capable of accurately predicting 1H chemical shifts in a wide range of organic compounds.

The halogen substituent effect on ^{1}H chemical shifts has previously been calculated by CHARGE using electric field and steric effects as the only through space contributions. We found this model to be inadequate when applied to haloaromatics, where the halogen can in some cases be very close to the proton investigated.

We have found that the through space contribution of the substituent chemical shift of halogens can only be accounted for accurately if a combination of electric field, steric and magnetic anisotropy contributions are considered.

An anomalous effect on the meta proton chemical shifts was noted. This W effect was found to increase with the size of the halogen, being negligible for flourine. A gamma effect (three bond) from the ipso carbon in haloaromatic systems was derived to explain the effect. The large shielding of the meta proton was shown to be non-additive in ortho substituted halobenzenes, and implemented into the CHARGE scheme.

Here we also compare the calculated chemical shifts produced by CHARGE of 1-halo naphthalenes with those calculated by the quantum chemical GIAO method and chemical shift predictions by the database based ACD predictor.

2 Ring-Chain Tautomerism In 2-Substituted 1,2,3,4-Tetrahydroquinazolines: A ^{1}H, ^{13}C And ^{15}N NMR Study

Jari Sinkkonen[1], Kirill N. Zelenin[2], Abdul-Kadir A. Potapov[2], Igor V. Lagoda[2], Valeriy V. Alekseyev2 and Kalevi Pihlaja[1]

1. Structural Chemistry Group, Department of Chemistry
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2. Russian Military Medical Academy
Saint-Petersburg, Russia

In this work a set of 1,2,3,4-tetrahydroquinazoline derivatives were synthesized by the reaction of 2-aminomethylaniline with aldehydes and ketones and their ring-chain tautomerism studied by ^{1}H, ^{13}C and ^{15}N NMR spectroscopy. The ketone, as well as the alkyl aldehyde, derivatives were found to favor exclusively ring forms, whereas tautomeric equilibria were observed for aryl aldehyde derivatives. For para-phenyl substituted compounds, an excellent correlation was found between logK and the N=C nitrogen chemical shift, which shows that the electronic character of the aryl substituent is carried by the conjugation effect to the magnetic environment of the nitrogen atom.

3 A Precise And Generic ^{1}H NMR Method For The Rapid Quantitation Of New Synthetic Molecules

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NMR has never been extensively exploited as a quantitative tool although it is a unique quantitative detector because under appropriate conditions the response factor is independent of the molecular species and quantitation is therefore possible using a single reference compound. The main reasons are high cost of instrumentation, inherent low sensitivity and inadequate accuracy and precision of results. In this poster we demonstrate that with modern high field instruments most of these drawbacks are overcome.

We have validated for accuracy and precision a ^{1}H NMR quantitative method which requires a limited amount of sample (1-2 mg for MW = 500). Using a highly automated protocol - which comprises robotic solution preparation and spectra collection with an autosampler - accuracy and precision are around 2-3 %. In our labs ~ 400 samples/month are analyzed with this method and the turnaround time is 24 hours.
Accuracy and precision can be improved to better than 1% if two replicate solutions are manually prepared with a micropipette and analyzed. This high precision protocol is used - with benefits in terms of time - for reference materials where precision and accuracy must compare to the more customary HPLC methods followed by residue on ignition and thermo-gravimetric analysis.

One of the most challenging steps during the setup process was the selection of an appropriate reference standard. We tested several compounds reported in the literature but none of them performed satisfactorily. Among other commercially available derivatives we discovered that 1,4-bis(trimethylsilyl)benzene was the most suitable compound for our method.

4 High-Resolution Diffusion-Ordered Spectroscopy To Probe The Microenvironment Of Jandajel And Merrifield Resins[1]

Laura B. Pasternack, Celine Gambs, Tobin J. Dickerson, Suresh Mahajan, and Kim D. Janda

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The Scripps Research Institute
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Solid –phase organic synthesis (SPOS) has become a powerful methodology for the generation of compound libraries. The success SPOS is highly dependent on the accessibility of solvents, catalysts, and reagents to the interior of the resin. In order to explore the applicability of high-resolution ^{1}H DOSY (Diffusion Ordered Spectroscopy) NMR spectroscopy to the analysis of accessibility of reaction components, we undertook a comparative study of the diffusion coefficients of reaction components in two resins. These initial studies were conducted using Merrifield and JandaJel resins since these have different swelling properties and have significantly different kinetic behavior. Results show correlation between chemical reactivity, resin swelling, presence of intermolecular interactions and diffusion of solvents and reagents within the gel as determined by ^{1}H DOSY NMR Spectroscopy.

1. Gambs et al., J. Org. Chem. 2003, 68, 3673-3678.

5 Challenges In Determining Binding Specificity By trNOE During PFG-NMR Diffusion Measurements

Laura H. Lucas, Kristin E. Price, and Cynthia K. Larive

University of Kansas, Lawrence, KS USA

Ligand-protein binding interactions are of great significance in many receptor-mediated biological processes and in characterizing structure-activity relationships (SAR) during drug discovery. Pulsed-field gradient (PFG) NMR diffusion measurements have been utilized for many years to probe ligand-protein interactions, since the ligand diffusion coefficient decreases significantly when it binds to and takes on the motional properties of the protein. Recently, the parameters of simple diffusion experiments have been manipulated to detect transferred NOE (trNOE) that occurs via cross-relaxation during the experimental diffusion time and may provide insight into the bound ligand conformation. The trNOE manifests itself as a disruption in the exponential ligand signal decay as the gradient amplitude is increased, resulting in a range of diffusion coefficients for ligand function groups that may indicate their proximity to the protein at the binding site. Several ligands of varying affinity for human serum albumin (HSA) have been studied, and the results reveal that the ligand must bind strongly and specifically, be uninhibited by competing ligands, and maintain a relatively rigid conformation in the binding site to detect trNOE during PFG-NMR diffusion experiments.

6 A Study On The Nature And The Effect Of CyD Complexation Of 17-Stradiol

S. A. Fakhri, M. R. Rashidi, B. Shabani; A. Sadeghi

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Fac. of Chemistry, Tabriz University

Complexation nature of the two molecules is studied by ^{1}H-NMR, FT-IR, and ESI-MS. Analysis of the experimental data, evidenced that the inclusion complex of the two compounds contains two molecules of solubilizer (CyD) accomodating the two ends of the guest molecule via their wider sides. Optimized energy calculations (Hyperchem) also confirms this structure. The equilibrium constant of complexation at the phenolic end of the steroid (K_1) is considerably greater than the equilibrium constant of the complexation on the other end (K_2) according to our calculation.

7 Conformational Study Of Monosaccharide Analogues With NMR And Molecular Modeling

K. Martínez-Mayorga[1], J. L. Medina-Franco[1], Y. Bleriot[2], P. Sinäy[2], F. J. Cañada[1] and J. Jiménez-Barbero[1]

1. Centro de Investigaciones Biológicas, C.S.I.C., Madrid, Spain.

2. École Normale Supérieure, Paris, France.

The conformational analysis of three monosaccharide analogues with b-glycosidase inhibitory activity was conducted. NMR, at neutral and acidic pH, and NOE experiments were used. Also molecular mechanics, molecular dynamics and Monte Carlo calculations were employed. A very good agreement between experimental and calculated couplings and H-H distances was found. The three analogues exist in conformational equilibrium between two chair-like structures.

8 High Resolution NMR And Diffusion-Ordered Spectroscopy Of Port Wine

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Liquid foods such as port wine are highly complex mixtures of many types of metabolites. NMR spectroscopy has been proven to be a powerful technique to characterise intact food samples in a non-invasive way; many applications have been described for juices, oils, wines and beer. NMR spectroscopy clearly offers advantages such as non-invasiveness and the possibility of identifying many different compounds in one single analysis, which may take as little time as a few minutes; this avoids the use of traditional separation methods and discrete analysis for each family of compounds. The use of automation enables the analysis of a higher number of samples per unit of time. However, full use of NMR spectroscopy as a practical tool for rapid analysis of liquid foods requires previous detailed assignment of the signals of interest in the NMR spectra. Several assignment techniques/methods have been explored, trying to overcome problems such as signal overlap and signal loss- due to line broadening in larger compounds. For instance, a hyphenated NMR technique, LC-NMR/MS, has contributed to the identification of lower abundance metabolites resonating in strongly overlapped regions of the NMR spectra of beer, juices and wine. The NMR technique of Diffusion Ordered Spectroscopy (DOSY) may also be an important tool to differentiate between metabolites in food mixtures on the basis of their different hydrodynamic radii and, hence, diffusion rates. DOSY is a way to introduce diffusion coefficient as an extra dimension to the NMR spectrum. Diffusion coefficients are calculated from the decays of individual NMR signals in a series of spectra recorded with different pulsed field gradient strengths. The second dimension may be regarded as a pseudo-separation by apparent molecular size (i.e. hydrodynamic radius).

This work describes the use of high resolution 1D and 2D NMR and DOSY in the analysis of compositional differences of Port wines of different ages. Port wine is a well known Portuguese product with worldwide commercial importance, the composition and properties of which are strongly related to its origin and age. We show preliminary results of high resolution NMR (TOCSY, heteronuclear correlation) and of 2D and 3D DOSY analysis of Port wines of different ages. The assignment improvements achieved by DOSY relative to the results obtained by high resolution NMR alone are discussed.

9 Symmetry Breaking In A Per-6-Substituted-g-Cyclodextrin

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The per-6-(4-carboxyphenylthio)per-6-deoxy-γ-cyclodextrin Org 26142 is a reversal agent for the steroidal neuromuscular blocking drug, Rocuronium Bromide [1]. Org 26142 exhibits a broad spectrum at room temperature in D2O which is in contrast to the sharp and simple spectrum (C8 symmetry) for per-6-(3-carboxypropylthio)per-6-deoxy-γ-cyclodextrin, Org 25969, another potent reversal agent for Rocuronium Bromide. The ^{1}H spectrum of Org 26142 sharpens on heating to 353ºK and also on complexation with Rocuronium Bromide. The low temperature ^{1}H and ^{13}C spectra (273ºK) show distinct signals for each proton and each carbon of the glucosyl residues indicating that the minimum energy conformation of the cyclodextrin is asymmetric (C1). The asymmetry is attributed to either π-π stacking or self-complexation of one of the aromatic rings.

1. K. S. Cameron, J. K. Clark, A. Cooper, L. Fielding, R. Palin, S. J. Rutherford and M-Q. Zhang, Org. Letters, 4, 3403-3406 (2002)

10 Classification Of Protein Binding Sites Using 1D ^{1}H NMR And Statistical Methods

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A drawback of using 1D ^{1}H techniques for NMR detected screening is that they do not answer the key question: Are the interactions between the 'hits' and target specific or non-specific? Therefore false-positives can emerge from the screen unless displacement experiments are performed using ligands that are known to bind specifically to the active site.

Oida has shown that drugs binding to human serum albumin can cause changes in the 1D ^{1}H spectrum of the protein [1]. Simple spectral editing (difference spectroscopy) yields reproducible patterns that reflect the response of the protein to ligand binding. It was shown that these changes could be used to differentiate between drugs that bind to the ibuprofen and warfarin sites and also those that were bound non-specifically. We are investigating the possibility of using a similar approach in conjunction with statistical analysis to characterise binding sites.

1. T. Oida, J. Biochem. 100, 99-113 (1986)

11 qNMR Purity Analysis And Impurity Profiling Of Triterpenes

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Triterpenes (TTs) are natural products with a wide array of vital biological functions, such as the hormonal function of steroids, and pharmacological potential as new drugs, such as observed for plant TTs. Even in the age of 500+MHz gradient inverse probe NMR, their analysis remains a challenge due to the frequent lack of ^{1}H spectral dispersion of the tetra- and pentacyclic CH nucleus. Higher order spin systems, severe spectral overlap, and complex long-range coupling impede the elucidation of TT constitution and stereochemistry, and discourage the deconvolution of the information overload.

When performed appropriately, one piece of information that comes free with ^{1}H [q]NMR is sample purity. Considering their biological significance, and owing to the fact that analytical quality management is cumbersome for most TTs due to the lack of chromophores, their unfortunate [soapy] physicochemical properties, and their poor ability to ionize in MS, NMR has great potential. We are reporting the purity analysis and impurity profiling of 8 different batches of ursolic acid, which represents the prototype of a pentacyclic plant triterpene, with over 100 reported bioactivities. Although the purity information comes at the prize of deconvoluting crowded spectra and performing non-routine processing tasks, qNMR of TTs is rendered feasible and leads to a detailed quantitative and qualitative fingerprint of the materials prior to biological testing.

12 J Code Cracking In The Fingerprint Dereplication Of Small Molecules

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Dereplication of small molecules and identification of partial structures is a key task in drug discovery and metabolome research. Founded on three examples, this can be achieved using high-resolution ^{1}H NMR fingerprints in tandem with J code cracking. It can be demonstrated that already for molecules as trivial as carvone, menisdaurin, or even small prenyl C5 residues, the interpretation of the ^{1}H NMR spectral data is impossible without the application of off-line data-processing, optimized window functions, and spectral simulation in order to unravel the signal splitting pattern and the spin systems. Although carvone yields an apparently simple, well resolved ^{1}H NMR spectrum even at 300 MHz, interpretation is largely incomplete without understanding the multiple long-range J coupling, in which each proton is coupled to nearly every other proton. Considering that carvone represents a relatively simple monocyclic monoterpene, the true complexity of the spectra of unsaturated or higher oligocyclic terpenoids can only be (under)estimated. A second example for a simple molecule causing a highly complex spectrum is the aglycon portion of the cyanogenic glycoside menisdaurin. Spectral simulation was mandatory to elucidate a spin system, which revealed a previously unrecognized 6J bisallylic coupling. Thirdly, the detailed resolution of complicated spin systems also appears to be very valuable in the recognition of recurring structural elements like prenyl units. Once resolved, the characteristic fine-split septet of triplets of this hemiterpene is an almost predictive indicator of this residue.

13 Solution Structure And Orienting Interactions Of Small Peptides And Amides In An Aqueous Liquid Crystal

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The capped alanine dipeptide (AcAlaNHMe) is a benchmark during the development of in silico tools for protein structure determination. Previous experiments have failed to conclusively determine its solution conformation, however. We have extracted dipolar couplings for the dipeptide via LX-NMR, employing a liquid crystalline solution of cesium perfluoro-octanoic acid in water as orienting medium. Our data is consistent with the pII conformation encountered in unfolded proteins. We have performed similar experiments with the family of methyl-substituted formamides to describe the origin of the orientational anisotropy of small amides and peptides in the liquid crystal. We find that the orientation of these small compounds is surprisingly sensitive to the precise arrangement of methyl groups. We explain the orientational order in terms of competing hydrophobic and hydrophilic interactions with the mesogen and the surrounding solvent. These observations should prove of interest to investigators of molecular recognition, affinity, self-assembly, and related association phenomena in water.

14 Structure Elucidation Of A New Natural Tetrapeptide By NMR And Mass Spectrometry

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A new natural product, VIC-200031, was isolated(1) from fermentation of a Streptomyces sp.strain as a complex of four factors. VIC-200031 shows activity as inhibitor of bacterial protein synthesis in a cell-free system. The structures of the complex components have been elucidated by one and two-dimensional NMR techniques (^{1}H, ^{13}C, ^{13}C/DEPT, DQF-COSY, TOCSY, HMBC, HMQC, HMQC-TOCSY, NOESY, ROESY). The four complex constituents are structurally related; they are linear tetrapeptide containing unusual amino acids and seven stereocenters. High-resolution mass spectrometry (FT-ESI-MS, LC-MS, GC-MS) applied to the intact molecule and to the hydrolysis products supported the structure deduced from NMR data.

1. E.Selva, F.Marinelli, D.Losi, L.Cavaletti, A.Lazzarini, A.Marazzi Patent PCT WO 03/046192 (05.06.2003)

15 Structure Elucidation And Solution Conformation Of The Glycopeptide Antibiotic Enduracidin Determined By NMR And Molecular Dynamics

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Enduracidin as well as Ramoplanin belongs to the large family of the cyclic glycopeptide antibiotics, highly effective against Gram-positive bacteria. The primary and solution structure of ramoplanin is already well known, while the primary structure of enduracidins has been determined by a combination of chemical and NMR spectroscopic methods. Both antibiotics share a similar peptide core of 17 amino acids and differ mainly for the length of the acyl chain and the presence of two D-mannose moieties. Based on the high sequence-homology with ramoplanin, the solution structure of enduracidin is modeled as a cyclic peptide. The tertiary structure thus obtained is refined through Molecular Dynamics (MD) simulation, in which the interatomic NOE-derived distance restraints were imposed. MD simulations yielded a family of representative 3D structures (RMSD=0.89), which highlighted a backbone geometry similar to that of ramoplanin in its β-hairpin arrangement. In contrast, enduracidin displays a different arrangement of the side-chain and of the residues forming the hydrophobic core.

16 Probing The Solvation/Hydration Spheres And Ion-Pair Formation Of Anionic $PtCl_6^{2-}$ Complexes In Water-Methanol/Acetonitrile Solutions Using ^{195}Pt And ^{23}Na NMR

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The very high sensitivity of the chemical shifts of the ^{195}Pt nucleus to changes in the inner coordination sphere of Pt(II)/(IV) complexes has long been established. Less well known and understood is the sensitivity of the ^{195}Pt chemical shifts of inert platinum complexes to changes in solvent composition and temperature. We have found the ^{195}Pt chemical shifts of kinetically inert $PtCl_6^{2-}$ anions to depend strongly on mixed solvent compositions ranging from pure water through to pure methanol or acetonitrile at 303ºK. These ^{195}Pt shift trends have been interpreted in terms of changes in the outer, or second-sphere hydration/solvation shells of the complex anion. This interpretation is supported by ab initio and molecular dynamic computer simulations of the structure of the hydration/solvation spheres of $PtCl_6^{2-}$ complex anions, which predict relatively well structured hydration shells. Significantly in pure methanol and acetonitrile, substantial solvent-separated and/or contact ion pairs of type $\{Na^+[\ PtCl_6^{2-}]\}^+$ are favoured with increasing Na^+ ion concentration.

To our knowledge, this is the first significant experimental and computational verification of the existence of such ion-pairs in solution. By contrast in water, there is little tendency for such ion-pair formation at 303ºK. The corresponding ^{23}Na NMR chemical shift trends, although very much less sensitive than the ^{195}Pt shifts, mirror and support our conclusions.

17 Automated Structure Elucidation Of Organic Molecules From ^{13}C-NMR Spectra Using GA And ANN

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The routine work of structure elucidation of molecules produced by organic synthesis is one of the most important applications of NMR spectroscopy. For this purpose the ^{13}C NMR spectrum plays an important role. Since complex chemical information is encoded in the chemical shift, intensity and multiplicity, these data are suitable to be saved in databases and serve for further numerical analysis. A broad variance of excellent tools exist that assist the NMR spectroscopists during structure elucidation. However, a fully automated method for elucidating molecular structures from ^{13}C NMR data only has not been realized.

Even for relatively small molecular formulas a huge number of constitutions is theoretically possible. In order to approach an automated structure elucidation an intelligent structure generator needs to be implemented that uses the experimental 13C NMR spectrum as target function to restrict this huge constitutional space. In contrast to the existing programs MOLGEN (generates all possible constitutions), COCON (needs additional 2D NMR connectivity information) and SPECSOLV (database dependent) the approach presented here uses only the molecular formula and ^{13}C NMR chemical shift information and is independent from direct access to databases, since the database is only necessary for training the neural networks but not for predicting the ^{13}C NMR spectra. The exact molecular formula is often known from synthesis or can be experimentally determined by modern high resolution mass spectrometry. The generated structural space is dynamically determined during the optimization process by a genetic algorithm. The ^{13}C NMR spectra of generated molecules are calculated fast and precisely during the optimization process by artificial neural networks.

The genetic algorithm starts from a randomly generated set of m molecules for a defined molecular formula. This set of molecules undergoes iteratively the processes of selection, recombination and mutation in order to minimize the deviation of the experimental to the calculated ^{13}C NMR spectrum.

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18 New Aspects Of Automated Structure Elucidation Based On ^{13}C-NMR Spectroscopy

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Three new approaches for automated structure elucidations of organic molecules from NMR spectroscopic data were introduced recently. These approaches apply a neural network ^{13}C NMR chemical shift prediction method to rank the results of structure generators by the agreement of the predicted and experimental chemical shifts. These three existing implementations are compared using realistic molecules. The applicability and reliability of such approaches is addressed.

Artificial neural networks are capable of predicting the ^{13}C chemical shifts of organic molecules nearly as fast as incremental methods while maintaining the accuracy of database methods. We apply a neural network, to the screening of large sets of molecules obtained by structure generators in the process of automated structure elucidation. Specifically, we apply the network to sets of structures generated by MOLGEN for molecules of less than 13 non-hydrogen atoms. The computed ^{13}C NMR spectra are compared to the experimental spectrum; in all cases, the computed spectrum belonging to the example molecule yields a significantly smaller deviation to the experimental data than all other predicted spectra.

A genetic algorithm is implemented that uses molecular constitution structures as individuals. With this approach the structure of organic molecules can be optimized to fulfill experimentally obtained properties, if additionally a fast and accurate method for the prediction of the used physical or chemical features is available. ^{13}C NMR chemical shift, intensity and multiplicity information is readily available from ^{13}C NMR DEPT spectra. By means of artificial neural networks a fast and accurate method for calculating the ^{13}C NMR spectrum of the generated structures exists. The method is implemented and tested successfully at realistic example problems of organic molecules with up to 20 non-hydrogen atoms.

Systematical constitutional analyses of five model compounds were carried out using the NMR-based structure generator COCON. The resulting ensembles of up to 28,000 structural proposals were analyzed, filtered and validated using a substructure analysis and a fast method to calculate ^{13}C NMR chemical shifts as well as a combination of both approaches. Structure elucidation of organic compounds can be performed in a highly automated way by combining COCON with these new approaches

19 Novel Strategies For Drug Impurity NMR Analysis: A Real Case

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In pharmaceutical industry fast, cheap and reliable analytical methods for impurity analysis are needed. Traditionally drug impurities and degradation products are separated and quantified by chromatographic methods like HPLC. However, HPLC gives no structural information about impurities, and in addition, eluent optimization and standard calibration are time consuming. In this work, we present a strategy for drug impurity analysis as based on quantitative ^{1}H NMR (qHNMR).

A single ^{1}H NMR spectrum gives a full picture, including structural information, about impurities of a sample. Because calibration can be done using major resonances of the drug substance, no internal or external standards are needed and quantification of all the impurities can be performed with a single sample solution. This also simplifies the validation procedure. Our experiments show that with modern spectrometers the response factor is very close to 1.00 (intensity is directly proportional to the concentration of the proton) in the whole concentration range (0.05 to 100.00 %) used in this study. Our results also suggest that a typical ^{1}H NMR spectrum can be interpreted so that impurity concentrations well below 0.1 molar % can be determined with relative standard error below 10 %. It is also notable that qHNMR is more sensitive for small molecular weight impurities, in opposite to chromatographic methods. For example, the quantification of ethanol from a typical drug sample is possible well below 0.01 wt%.

A complete qHNMR analysis of a drug sample demands 1-2 hours, while the GC + HPLC analysis of the same sample may demand a period of days. The total spectrometer time demanded by one sample is 7-20 minutes; the analysis can be done in an ordinary personal computer. Our results mean that qHNMR rivals the chromatographic methods both in speed and in economy of chromatographic methods both in speed and in economy.

20 A First ^{13}C NMR Study Of South African Extra Virgin Olive Oils: Authentication And Major Component Analysis

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The South African olive oil industry is fairly young and small by international standards but nevertheless growing at a rapid rate. In the last few years high quality South African extra virgin olive oils have made their debut on both national and international markets. Unfortunately there is little legislation to date regulating the quality and analysis of olive oils in South Africa. As the demand and thus the cost of extra virgin olive oils increases, the incentive for marketing inferior or even adulterated oils does too. This indeed has already happened in the South African market, with the appearance recently of fake oils, marketed as imported extra virgin olive oil. While traditional methods of analysis and quality control for olive oils are well established, we believe there may be a need for a rapid method for assessing the authenticity of olive oils, particularly with reference to the quality and principle component analysis.

The extensive European studies on olive oils have encouraged us to undertake a preliminary ^{13}C NMR study of South African olive oils, which to our knowledge has not been previously attempted. Moreover these studies (particularly by Mannina et al. and Vlahov et al.) have shown that regional and cultivar-based differences in the olive oils can be identified by NMR spectroscopy. We have thus carried out major component analyses of a number of extra virgin South African olive oils using quantitative ^{13}C NMR methods with the intention of developing a "database" of South African olive oils on which to perform multivariate statistical analysis to assess regional and cultivar differences. The literature methods used for the quantitative ^{13}C NMR analyses were found to be time consuming and we have succeeded in reducing the analysis time to less than half an hour, without compromising the integrity of the data obtained.

21 The Composition of Some Liquid Foods By NMR And LC-NMR/MS

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This work describes the application of NMR spectroscopy and LC-NMR/MS to the direct analysis of beer, grape juice and a wine phenolic extract (1-3). These samples are good examples of complex food mixtures the composition of which may be tackled by high resolution NMR and hyphenated methods, potentially more rapid and informative than the chromatographic methods traditionally used.

Direct analysis of beer by LC-NMR/MS enabled the rapid (1-2 hours) identification of dextrins with degree of polymerization (DP) of up to nine monomers, degassing being the only sample treatment required. The presence of alpha(1? 6) branching points was easily indicated by NMR of each subfraction separated by LC, although difficulties arose for the unambiguous assignment of linear or branched forms of high DP dextrins, requiring further improvement of the methods used. In addition, LC-NMR/MS of beer was useful to confirm the identity of some aromatic compounds already assigned by NMR alone and to reveal new ones, such as the aromatic alcohols 2-phenylethanol, tyrosol and tryptophol. Hyphenated NMR was also found to aid significantly in the identification of aromatic compounds composing the grape juice and wine extract samples. Some examples are the identification of several cinnamic acids (e.g. p-coumaric, trans-coutaric and trans-caftaric) in grape juice, and the detection of phenolics such as catechin, epicatechin, trans-resveratrol, tyrosol and caffeic acid in the wine extract.

The results presented show that, in all cases studied, NMR and LC-NMR/MS offer great potential for the rapid and detailed characterisation of the composition of different liquid foods, knowledge which may aid in the development of novel quality control methods, as well as in the full evaluation of foods as important sources of natural compounds with varied possible uses.

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22 Comparison Of Cryo-, Nano-, And Conventional Probes For DOSY Experiments

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Diffusion-ordered NMR Spectroscopy (DOSY) is an attractive method for mixture analysis. However, DOSY analysis is difficult if the mixture is only available in limited quantities (i.e. drug metabolite mixtures) because a high signal-to-noise ratio is needed for good separation of the components. Since it is impractical to acquire data for a lengthy period of time, use of a cryogenically cooled probe (cryoprobe) or small sample volume magic-angle spinning probe (nanoprobe) is an alternative in this case. Unfortunately, these advanced probes are known to enhance other problems for DOSY acquisition, such as persistent eddy currents or excessive vibration. The purpose of this investigation was to determine if these probes, with their increased signal-to-noise and associated problems, could provide similar DOSY results as a conventional probe for sample-limited mixtures. DOSY results for three simple mixtures using several popular pulse sequences were obtained with cryo- and nanoprobes. The conditions necessary to obtain the best results with these probes will be presented in comparison to a conventional probe.

23 Steric Hindrance To The Solvation Of Melamines And Consequences For Noncovalent Synthesis

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Steric hindrance to solvation disfavors structures in which the melamine exposes to the solvent faces for whom binding to the ring nitrogen is hindered but not blocked. Steric hindrance to solvation lowers the barriers to rotation in solvents which bind the triazine nitrogens, therefore these solvents display the fastest rates for assembling/disassembling processes.

24 Evaluation Of A Capillary NMR Probe And Cap LC/NMR System

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Recent advances in NMR technology for the measurement of low-mass samples includes a flow probe with a very small active volume. This has the principal advantage of s/n that results from the excellent filling factor of the probe.

We report the results of an evaluation into the MRM micro-flow probe, in terms of both the absolute probe performance, and the performance when linked to a Waters Cap/LC system. We have paid particular attention to factors such as convenience and ease of use when tackling problems involving analytes at low mass (for example metabolites and drug substance impurities). Our investigation highlights potential advantages and disadvantages of the system when compared to alternative methods for obtaining spectral data on analytes at low mass.

25 Probing Ionic Association On Metal Oxide Clusters By Pulsed Field Gradient NMR: The Example Of Tin-12 Oxo-Clusters

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Pulsed field gradient ^{1}H NMR spectroscopy has been applied to investigate the electrostatic complexation behaviour of the tin-12-oxo cluster macrocation $\{(BuSn)_{12}O_{14}(OH)_6\}^{2+}$ with two different and smaller anions, p-toluene sulfonate (PTS^-) and diphenylphosphinate ($Ph_2PO_2^-$). By monitoring the translational diffusion coefficients of the various species involved, it is shown that complexation depends on the anion involved and on the solvent used. Moreover, the possibility to individually monitor the diffusion characteristics of multiple anionic and cationic species in mixtures, by virtue of resolved ^{1}H resonances available from each species, allows to evidence the occurrence of ion-exchange in such systems. Thus when $\{(BuSn)_{12}O_{14}(OH)_6\}(PTS)_2$ is mixed with two equivalents of $Ph_2PO_2NMe_4$, PTS^- is displaced by $PhPO_2^-$ highlighting the greater affinity of the organotin macrocation for the diphenylphosphinate. This example clearly illustrates the potential of pulsed field gradient ^{1}H NMR spectroscopy in inorganic/organometallic chemistry to address preferential ion pairing in multi ion systems at the level of each individually charged species.

26 Characterization And Determination Of Active Ingredients In Herbal And Dietary Supplements By NMR

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NMR methodologies are described for the determination and the characterization of active ingredients in important herbal and dietary supplements. Aristolochic acids are considered antineoplastic agents with acute nephrotoxicity. Nephropathy, the progressive form of renal fibrosis was developed in many individuals who take weight-reducing pills containing Chinese herbs. Melatonin (N-acetyl-5-methoxytryptamine), a hormone used as supplementary drug in the alleviation of jet-lag and other sleep disorders. S-Adenosyl-L-methionine, a significant biological agent in the human body, participates in detoxification reactions and in the manufacture of brain chemicals, antioxidants, joint tissue structures, and many other valuable components. The developed methodologies are able to differentiate between the toxic isomers of aristolochic acids, the biologically-active (S)-diastereoisomer and the biologically-inactive (R)-diastereoisomer of S-adenosyl-L-methionine, and melatonin and its precursor neurotransmitters tryptophan and tryptamine, assess chemical structure, and determine the quantity or relative ratio for each component. Results of the analysis of synthetic mixtures by the proposed methods demonstrated excellent agreements with the known values.

27 DFT Study Of ^{1}H-^{31}P Coupling Constants And Conformational Equilibria In Saturated Trans-Fused 1,3,2-Benzoxazaphosphorinane-2-oxides

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Recently it was shown that sufficient accuracy in the theoretical calculation of vicinal $^{1}H,^{1}H$ spin-spin coupling constants for studying conformational equilibria in saturated heterocyclic compounds can be reached by calculating only the Fermi contact contribution to the coupling.[1] Now, the same methodology was applied for calculating at the B3LYP/cc-pVTZ level of theory the ^{1}H-^{31}P coupling constants over three bonds or more in a set of various structurally well-defined organophosphorus compounds. The results were then used for calibrating selected couplings calculated for the conformations of two saturated trans-fused 1,3,2-benzoxazaphosphorinane derivatives. Finally, the conformational equilibria of the 1,3,2-benzoxazaphosphorinanes were analyzed by use of the calculated couplings and the results compared to the results of previous studies made by experimental NMR spectroscopic methods.[2,3]

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28 Solution Structure Of The U-S4U-U Oligonucleotide By 1D And 2D NMR

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Transfer RNAs (tRNAs) contain at least 92 posttranscriptional modified nucleosides affecting the chemistry, structure and function of these molecules (1). 4-thiouridine (S4U) is one of the naturally occurring modified uridines. It is found in tRNAs of bacteria, some viruses, and archaea at position 8 (2,3). In bacterial tRNAs one of the most frequent sequences around S4U is U-S4U-U (3). The goal of our project is the determination of the solution structure of the oligonucleotide U-S4U-U using NMR, molecular mechanics and dynamics. Unambiguous assignment of the 1H NMR signals was achieved by 2D DQFCOSY and ROESY at three different mixing times. 1H/31P correlation spectrum aided in the assignment of H5', H5" and H3' protons. Cross peaks intensity in ROESY spectra and coupling constants, that are obtained from the 1D and DQFCOSY, were used to generate the distance and dihedral angles constraints that will be used in the molecular mechanics/dynamics study of the trimer. Only H6/H2' intranucleotide correlation was observed in the 5'-U which indicates a predominant anti conformation around the glycosidic bond of that residue. However, for S4U and 3'-U residues both H6/H2' and H6/1' intranucleotide correlations were observed with the intensity of the H6/H1' correlation being less than H6/2'. That suggests the presence of both syn and anti conformations in these two residues with anti conformation being at higher percentage. A higher percentage of anti conformation in pyrimidines has been observed (4,5). The J1'2' and J3'4' coupling constants were used to calculate the ratio of C-2' endo/C-3' endo (S/N ratio) (5,6). Both 5' and 3' uridines had a lower S/N value relative to S4U which suggests that the C2'-endo conformation is more prevalent in S4U, an observation that was reported earlier (5). Internucleotides H6/H2' and H6/H1' dipolar correlations in the 5' to 3' direction indicate presence of weakly stacked, A-form structure (7).

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29 NMR Spectra Of Two Azo Dyes Derived From 5-Amino-1-naphthol. Hydrogen Bonding From Peri Position

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Two azo dyes were prepared by reaction of 4-nitrobenzenediazonium salt with 5-amino-1-naphthol both in acidic and alkaline media. The coupling product having 4-nitrophenylazo group in position 8 of naphthalene ring was prepared at low pH while proton in position 4 of naphthalene ring was substituted by 4-nitrophenylazo group at high pH. The ^{15}N selective labeled isotopomers were prepared, too. ^{1}H, ^{13}C and ^{15}N NMR spectra were measured. The effect of hydrogen bonding from peri position, especially on ^{15}N chemical shifts, will be discussed. The data will be compared with those obtained for analogous compounds which do not have amino or hydroxy group in peri position.

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30 2D Intermolecular Double Quantum Coherence Spectra. The Role Of Diffusion

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The role of diffusion in intermolecular multiple quantum coherence (i-MQC) has been examined by several authors, mostly from the point of view of non-linear echo amplitudes and their dependence upon various parameters 1,2. We will examine these effects by comparing simulated and experimental i-DQC 2D spectra. Various binary mixtures of molecules in solution with different diffusion coefficients and chemical shifts but no J-coupling are considered. The type of assumption concerning the leveling of the dipolar demagnetization field caused by diffusion during the observation period of the CRAZED sequence is shown to play a major role in the amplitude of the cross peaks of the 2D spectra.

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31 Automated Metabonomics Data Preparation

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Advanced Chemistry Development and GlaxoSmithKline

In the emerging field of Metabonomics, many processes for data preparation and analysis remain unstandardized. For this reason, the most efficient and reliable technique is yet to be found and agreed upon by the scientific community. What will be shown, is the advantage of a fully-automated data processing system and a comparison to the same data set prepared manually. The automated system will be shown to have two obvious advantages; the amount of time that needs to be devoted to working-up the data is reduced, but there is also an advantage in reducing user bias. As you will see, a significant improvement in quality of the processed data can be achieved when the processing is done automatically.

The proposed system starts with a comprehensive set of NMR processing tools. Robust auto-phasing and baseline correction that have been show previously, are key to the procedure. These routines are what makes the high-throughput nature of the process possible. Add to this the flexible and interactive bucketing procedure, as well as macros designed for data preparation and reduction, and the system is more complete than any other Metabonomics NMR data analysis package on the market. The process outlined here will be shown to be the most reliable and efficient method for preparing and analyzing NMR data for Metabonomics studies. By letting the statistics predict which mode of spectrum preparation is better, a purely unbiased evaluation of the two processing methods is presented.

32 ^{13}C NMR Chemical Shift Prediction – A Comparison Of Methods And A Case Study Analysis Of Taxol®

Brent Lefebvre and Antony Williams

Advanced Chemistry Development

The value and utility of software for the prediction of NMR spectra from a structural input cannot be doubted. In conjunction with the dramatic improvements in spectrometer technology has been an increased effort to put tools in the hands of both the professional spectroscopist and the organic chemist to speed the structure elucidation process. One has to be extremely cautious with the application of such tools however and be aware that prediction software commonly provides only confirmation that the structure is consistent since multiple molecular structures can generate extremely similar spectra and a good match between a predicted and an experimental spectrum is not verification of the structure.

Many approaches for the prediction of ^{13}C NMR spectra are available including incremental methods, neural nets, ab initio, and Hierarchically Ordered Spherical description of Environment (HOSE) code. Recently the application of these methods to the prediction of the ^{13}C spectrum of Taxol was reported (reference) with the conclusion that neural nets offer superior performance.

This work will examine in detail the advantages of a combined HOSE code and fragment-based approach in regards to ^{13}C NMR prediction. Comparisons with the previously reported work on TAXOL® allows a complete evaluation of the commercially available software products for ^{13}C NMR spectral prediction with our approach.

Through the study it will be shown that the use of a database or training set by these software products requires special attention to ensuring that the database is not only large enough to contain the needed information to properly predict the chemical shifts in question, but also that the accuracy of the information is much more important than the presence of it. An inaccurate database entry can severely impact the accuracy of a chemical shift prediction. As well, it will be shown that a continually evolving database is not necessarily needed for the prediction of shifts for a single compound, but during the course of day-to-day use, these software products can be expected to encounter many different structures; some of which may not closely resemble anything in the current database. In these cases, a database that allows easy incorporation of these novel structures for use in future predictions; lends itself well to accurate prediction on a reasonable time-scale.

33 Spectroscopic Validation Of Structures Assisted By Prediction And Auto-Assignment Algorithms-Verification Analysis Of High-Resolution ^{1}H And ^{13}C NMR Spectra

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Advanced Chemistry Development

The elucidation of chemical structures is distinctly different from the validation process. Validation of a chemical structure is an appropriate approach for walk-up NMR since in many cases the chemist has prior knowledge of the potential outcome of a reaction sequence. For the spectroscopist supporting product development, prior knowledge and experience in the spectroscopic examinations of related materials provides a foundation for aiding in the confirmation of chemical structure. Validation rather than elucidation is therefore a very common approach utilized in the NMR laboratory. The availability of NMR content databases, the assistance provided by NMR prediction algorithms and the utility of auto-assignment of spectra based on structural input can dramatically speed up the process of structure validation.

We will review our approach to automated verification analysis using a comparison of multiplets of experimental and calculated spectra and fully utilizing information about chemical shift, integral ratio and multiplicity. The process of NMR prediction makes these parameters readily available but in order to extract these parameters from experimental spectra an automated multiplet analysis algorithm has been developed.
The verification process depends on an auto-assignment algorithm allowing for variable weights for the NMR chemical shifts, the ratios of integrals and multiplicity contributions. The auto-assignment algorithm allows a whole range of analysis from comparison of experimental chemical shifts with predicted intervals to precisely matching all multiplet properties. The resulting analysis produces verification statistics including the Match Factor, RMS of Assignment and Structure Purity. The structure purity parameter accounts for the presence of multiple species in the sample. Multiple examples of the verification of ^{1}H and ^{13}C NMR spectra will be given.

34 All Good Things To Those Who Wait. The Application Of Automated Structure Elucidation Tools To Solve A Structure After 10 Years Of Human Effort!

Kirill Blinov, Mikhail E. Elyashberg, Antony Williams, and Gary Martin

Advanced Chemistry Development and Pfizer

The development of CASE applications, Computer Automated Structure Elucidation, for the elucidation of structures of organic compounds has been an ongoing effort for over 30 years. ACD/Structure Elucidator (StrucEluc) is a PC-based CASE system for elucidating structures of organic compounds from 1D and 2D NMR spectra is described. The system is based on a database of ^{13}C NMR spectra (about 150,000 structures), a library of molecular fragments and their ^{13}C NMR sub-spectra (about 500,000) and a library of spectrum-to-structure correlations for both NMR and IR spectra. We have applied StrucEluc to the structure elucidation of 60 recently isolated natural products. The molecules for study were chosen from recently published natural product structure determinations that contained listings of 2D NMR data. We analyzed molecules containing between 15 and 65 skeletal atoms and having molecular masses ranging from 200 to 900 a.m.u. We found that the correct structure was determined unambiguously for 58 of these molecules. The structures for 75% of the datasets were determined in less than one min.; 90% of the analyses required no more than 30 min on a 500 MHz PC computer. StrucEluc also was tested in a fragment mode for which fragments stored in a database and user-defined fragments were used for structure elucidation from 2D NMR data. It has been shown that the fragment approach markedly speeds up the generation of potential chemical structures and removed the need for user intervention in the task of problem solving. Commonly this frequently transformed very difficult if not impossible tasks for the expert system into nominal tasks. Strategies regarding the utilization of fragments under different situations were developed and will be described in this poster. The StrucEluc application has been applied to the structure elucidation of a series of challenging indoloquinoline alkaloid structures from the Cryptolepis series. The application of the system to elucidate a novel, proton-deficient alkaloid structure from this series is reported that was not satisfactorily resolved by a scientist periodically re-examining the 2D NMR data over a period of ten years. Synergistic interaction of the spectroscopist with the software package to guide the CASE process led to the generation of a manageable number of structural possibilities (12 structures) consistent with the available data that then allowed the final structure to be determined successfully.

35 ^{15}N Chemical Shift Prediction Databases, Algorithms, And Applications

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Advanced Chemistry Development and Pfizer

NMR chemical shift prediction algorithms and software have been available for many years and a number of commercial packages are available. Generally ^{13}C has been the preferred nucleus for the deployment of the algorithms though software is available which supports ^{1}H, ^{19}F, ^{29}Si, and ^{31}P prediction, all of which are sensitive nuclei. ^{15}N, by comparison, is an inherently insensitive, low natural abundance nuclide that has been under-utilized in structure characterization studies even though ^{15}N NMR parameters are sensitive indicators of both the structural environment and electronic arrangements in organic and bioorganic compounds. Direct detection of ^{15}N shifts is problematic due to the sensitivity issues but, despite the difficulties of recording long-range ^{1}H-^{15}N heteronuclear shift correlation data, there are now in excess of 100 studies reporting these data in the literature. Our recent work with automated structure elucidation shows that the availability of long-range ^{1}H-^{15}N data allowed the number of structures generated to be reduced dramatically so the collection of such data is of great value.

Recently we have developed algorithms to enable the prediction of both ^{15}N chemical shifts and heteronuclear coupling constants. These prediction algorithms were developed based on a training database of over 7500 structures and their associated NMR properties. This poster will review the issues with quality checking of the training set, the validation of the derived algorithms, and the application of predicted ^{15}N chemical shift values for setting up the appropriate acquisition windows for 2D NMR experiments and for structural validation.

36 The Measurement Of Enantiomeric Excess In Weakly Oriented Chiral Liquid Crystals Using Exchangeable Protons

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Within the pharmaceutical industry, measuring enantiomeric excess, or more particularly detecting small levels of the minor enantiomer is extremely important. This can be done in a number of ways using NMR, with perhaps the newest being a method in which the molecule in question is dissolved in a chiral liquid crystal phase. Resolution between enantiomers is often achieved through differences in residual dipolar coupling or chemical shift anisotropy. However the quadrupolar interaction of a deuterium atom is most sensitive to different enantiomeric forms. Therefore many techniques rely on selective deuteration – which is not usually practicable or natural abundance deuterium – which due to its extreme insensitivity cannot detect high enantiomeric excesses.

We therefore propose a method making use of exchangeable protons within molecules. By replacing these protons with deuterons, the quadrupolar couplings observed provide enantiotopic discrimination that is both simple to interpret and sensitive - to an extent that allows high enantiomeric excesses to be found.

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37 Structure Elucidation By NMR And Computer Assisted Structure Generation Of Bi- And Tri-Cyclic Products Derived From Electrocyclic Rearrangements In Biomimetic Synthesis

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Biomimetic synthesis of natural products is an active research area aimed not only at generating these products but also understanding the processes by which they may arise in nature. As part of their continuing efforts in the biomimetic synthesis of propionate derived natural products, the Baldwin group became interested in the crispatene family of compounds. Some of these are believed to be mild cytotoxic agents, although the full biological profile is still unknown. Subsequently, the bicyclic core of this family has been efficiently generated from all (E)-tetraene precursors in one step via photochemical cyclisation.

During these studies, however, the structural elucidation of products derived from palladium-catalysed, thermal and photochemical electrocyclic rearrangements of all-(E)-tetraenes and all-(E)-pentaenes presented a challenge because of three principle features: 1) the general lack of vicinal proton-proton couplings, 2) the ambiguity introduced by ^{2}JCH, ^{3}JCH, ^{4}JCH and even ^{5}JCH correlations in HMBC experiments of these extended conjugated systems, and 3) the potential variety and complexity of structures which may, in principle, result from the electrocyclisations.

This work describes an approach that combines, in particular, HMBC correlation data with computer assisted structure generation to produce candidate structures that are consistent with the input data. We employed the Logic for Structure Determination (LSD) program of Nuzillard and Massiot which employs ^{13}C, COSY, HMQC and HMBC data. The candidate structures are then crosschecked with other NMR data e.g. from NOE experiments, to ensure consistency and to provide stereochemical information on these rearrangement products.

38 LC-SPE-NMR: Separating The Chromatography From The NMR

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With the addition of a solid phase extraction unit for peak trapping, exciting new possibilities have become available to well established LC-NMR hyphenation. The obvious advantages of this new approach are:

- A sensitivity gain by a factor of 2 to 4, especially for broader peaks, due to concentration of the sample in a small volume (< 30 µl) and making use of a highly mass sensitive small scale probe.
- The dimensions of the chromatographic system can be freely chosen, thus the hyphenation of the widely used 4.6 mm chromatographic format to a 30 µl small scale NMR probe is possible.
- The signal to noise ratio can be further increased by multiple trapping of peaks from repeated sample injections.
- The chromatographic separation can now be performed using inexpensive non-deuterated solvents.
- Even NMR incompatible solvents systems can be used for chromatography.
- Only small amounts (approx. 240 µl) of deuterated solvents are required for the transfer.
- As no D_2O is used in the eluent, no H-D exchange occurs during the chromatographic process which results in the correct mass information.
- Exchangeable NMR signals can be observed by the appropriate choice of deuterated NMR solvent.

In this poster contribution, we will present an assessment of the potential of the new technique by giving a direct sensitivity comparison to conventional LC-NMR using identical real life samples from drug metabolite identification.

Advantages and disadvantages will be discussed and we will point out why this new way of doing LC-NMR has now taken an important place in our armoury of routine NMR techniques.

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39 Manipulating Small Volumes: Challenges For Capillary NMR

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The development of capillary NMR flow cells in recent years has been fueled in part by the need to analyze small amounts of sample. Cell capacities of ~5 µl and active volumes of 1-3 µl are typical, resulting in increased S/N compared to conventional probes with larger active volumes. These gains can only be realized if one can efficiently transfer the sample into volumes of >5 µl and thence into the probe. We have recently obtained a CapNMR probe from MRM, with an active volume of 1.5 ul in a flow cell with a volume of 5 µl. We will describe techniques for sample introduction and some of the performance measurements of this probe.

40 An Alternative Method For Enantiomeric Analysis Through ^{77}Se-NMR Using (+)-Methylephedrine As CSA

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Recently, we described a methodology to determine the enantiomeric purity of racemic selenium compounds using (+)- or (-)-methylbenzylamine (MBA) as a chiral solvating agent (CSA) through ^{77}Se NMR spectroscopy.(1) In the present work, aiming at achieving an even better separation of these anisochronous resonances, we chose (+)-methylephedrine (NME) as the CSA to be investigated. As such, we titrated samples of racemic selenides with the chiral base NME, followed by the acquisition of ^{77}Se NMR spectra. Signals separations up to 83 Hz, corresponding to the formation of two diastereomeric complexes for (+)-NME and CSA, were observed. These signals separations were relatively much larger than the previously observed ones (36 Hz)(1), allowing an even clearer diastereomeric signals resolution. This methodology can be done in situ in an NMR tube and avoids derivatization processes. Furthermore, the compound under investigation can be easily recovered.

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41 Applications Of Nano- And HR-MAS NMR In The Identification Of Resin Bound Small Molecules For Drug Discovery

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High resolution magic angle spinning (HR-MAS) NMR is today a superior method in the structural characterization of resin bound compounds. HR-MAS NMR is the only available NMR method for the study of these magnetic heterogeneous samples, and therefore NMR has become an important analytical tool in the area of library synthesis and drug discovery.

The small sample volume and high sensitivity of the probes have in addition contributed to resolve and/or reduce the problem with the inherent low sensitivity of NMR as compared to other analytical methods.

Examples of how HR-MAS NMR may be used for the structural characterization of resin bound small molecules will be presented. This includes a discussion of some specific experimental problems observed with the acquisition of given NMR experiments (e.g. TOCSY) when performed on single bead samples.

42 Binding Studies Of Small Molecules To Amyloid Peptide b-Ap(1-28) Using 1D NOESY Experiments

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Alzheimer's disease (AD) is the most common neurodegenerative disease. Neuronal cell death in AD is associated with the deposition of β-amyloid peptide (β-AP) in the extracellular part of the brain in the form of amyloid plaques. In order to identify small molecules that interact with β-AP and may potentially prevent the formation of amyloid plaques, we employed 1D gradient-enhanced NOESY experiments taking advantage of the fact that when a small molecule binds to a macromolecule its NOE signal changes from positive to negative. The use of 1D gradient-enhanced NOESY experiments reduces drastically the requirements in acquisition and storage capacity as well as data manipulation time compared to 2D techniques and, in addition, eliminates the subtraction artifacts present in conventional 1D NOE difference spectroscopy.

A series of small molecules, including thioflavin T, oleuropein, rifampicin, melatonin, and 4'-iododoxorubicin, have been investigated for binding to β-AP(1-28) in D2O and DMSO-d6 solutions with ligand/β-AP(1-28) concentration ratios of 5:1 and 15:1. Sucrose, that is known in the literature not to bind to β-AP, was used as the negative control in testing the method. The results of these studies will be presented.

43 Quality Control Of Substance Libraries By Micro NMR

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Over the past few years there has been a significantly growing demand for a more accurate characterization of the contents of lead discovery substance libraries of small molecules. In many cases mass spectrometry alone seems not to be sufficient. NMR would be an ideal addition to the MS data since intrinsically it yields the most structural information and it can also be used for quantification. But up to now NMR often was too time consuming, consumed too much sample or it was too elaborate to recover sample after the measurement.

Here we present a completely new and automated NMR setup to measure minute quantities of sample directly taken from a lead discovery library with outstanding sensitivity. This extremely time efficient method relies on the use of new spectrometer hardware which consumes only 5 microliters of the stock solution and therefore sample recovery is obsolete in most cases. Due to this new hardware, the NMR measurement itself is 4 times more sensitive than the same measurement on the same amount of sample measured on the conventional NMR hardware. Therefore the experiment can be carried out in 1/16th of the time needed before. Since only a very small volume is needed for the structure verification, the use of expensive deuterated solvent is reduced to a minimum or can be omitted completely.

In this poster we present examples of the new NMR setup for structure verification in parallel synthesis as well as the quality control in an existing library. We also present data of measurements, which focus on an assessment of substance concentration in products from parallel synthesis as well as in aging libraries.

44 Drug Trace Analysis By NMR Spectroscopy: A Comparative Study On Cryogenic And Micro-coil Probes

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NMR spectroscopy provides structure-rich and quantitative information in drug analysis. However, the inherent low sensitivity of NMR technique has curtailed the full utility of this powerful structure elucidation and quantitation tool in trace analysis. Improving NMR sensitivity in an effort to overcome NMR low detection barriers has therefore been a perpetual endeavor since the early days of the technology. Traditionally, increases in NMR field strength have been relied upon to produce incremental sensitivity gains at a disproportionally increased cost. Recent advances in NMR probe technology have dramatically changed the landscape of NMR spectroscopy. Cryogenic probes operating at very low temperature have yielded markedly improved sensitivity at a given field strength. Similarly, the newly emerging micro-coil probe technology has also demonstrated promises and potentials in NMR analysis of very little material. With the advent of the new techniques, most conventional NMR data are now obtainable for only micro-grams of samples within a short period of time. This presentation will discuss preliminary studies from a practical perspective using these two strains of NMR probe technology. The applications and implications of these technologies in drug R&D will be highlighted.

45 Small Molecule Concentrations By $_1$H NMR: Methods For Natural Product Standards And Hazardous Materials

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The production of Certified Reference Materials (CRM's) for use in phycotoxin monitoring requires several independent means of measuring concentrations of low MW compounds in solution. As phycotoxins are frequently available only in limited quantities and may be from uncharacterized sources, isolation to crystalline purity is usually impracticable. Furthermore, the compounds are frequently hygroscopic and in unknown salt forms, so weights provide only an upper limit of the amount present in typical samples. As the CRM's are intended for use with LC-MS equipment, deuterated solvents are to be avoided if H/D exchange with the reference compound would occur (e.g. with D2O, CD3OD).

Data will be presented to show that ^{1}H NMR can be used reliably to measure concentrations of small molecule solutions in sealed tubes without internal standards or concentric tubes, when care is taken to account for numerous factors influencing signal intensity. The methods apply to solutions in a variety of solvents, which need not be deuterated, over a large range of concentrations up to neat liquids. They are particularly useful for hazardous materials.

46 Characterization By NMR Of Two New Spirolides Isolated From Danish Strains Of The Toxigenic Dinoflagellate Alexandrium ostenfeldii

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Spirolides are a class of macrocyclic imines, first identified in digestive glands of scallops and mussels from the Atlantic Coast of Nova Scotia, Canada in the early 1990's. They are produced by the marine dinoflagellate Alexandrium ostenfeldii and cause "fast-acting" symptoms in the regulatory mouse bioassay for shellfish toxicity.

Cultures of recently-isolated Danish strains of A. ostenfeldii have been shown by LC-MS methods to contain some new spirolides. The use of NMR to characterize the two most abundant of these will be described, including evidence for a 5:6:6 trispiroketal ring system not previously observed in spirolides.

47 NMR Informatics – From Processing to Data Management to Prediction

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Within the field of NMR, spectroscopists have come to rely upon an assortment of software and database tools to perform tasks such as structure drawing, prediction, processing, data management. This poster introduces a system that combines tools within a fully integrated informatics environment to include spectral data and reliable prediction, tools for processing, database building, management, search, analysis, and reporting for HNMR, CNMR, and XNMR. It also demonstrates how working within a single interface can help scientists bring data together to manage workflow, improve productivity, and share information with others in the organization.

48 Structure Elucidation Of Monoalkylated Dihydroxycoumarins By NMR Spectroscopy

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We found that the Mitsunobu reaction allows a differential etherification of the phenolic groups in dihydroxycoumarins and we present here the first results obtained by sonochemical reaction of two commercially available differently substituted coumarins, namely the 6,7-dihydroxycoumarin (aesculetin)and 7,8-dihydroxycoumarin (daphnetin) with some biologically significant alcohols. The structural assignment for monoalkylated polyphenols (e.g. distinction between 6- or 7-, and 7- or 8-substituted coumarins) has always been problematic, as shown by the numerous corrections that from time to time have appeared in the literature. We will present a solution to the regiochemical problem via application of the homonuclear Overhauser effect.

49 ^{77}Se NMR Of Metabolites Using A 1 mm Probe – A New Hope

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A minute amount of a selenosugar (20 µg) was isolated from 15 L of urine. ^{1}H detetected HSQC (^{1}H -^{13}C), HMBC (^{1}H -^{13}C, ^{1}H -^{77}Se), COSY (^{1}H -^{1}H) and TOCSY (^{1}H -^{1}H) of an excellent quality were acquired from this small amount by means of a 1 mm probe. The spectra are compared to spectra from synthetic standards to prove the identity of the metabolite.

50 Automated Capillary Flow-Injection NMR

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As part of the program to develop NMR analytical methods for supporting high throughput chemistry, we report here the development and application of capillary based flow-injection NMR in an automated fashion.

Most flow-injection NMR applications utilize conventional flow probes with a flow cell volume no less than 60 µL (active volume 30 µL). On sites within GSK, Upper Merion/Upper Providence, PA and Research Triangle Park, NC, flow probes with 60 and 120 µL flow cells are used routinely. The drawbacks of these technologies are the amount of sample required and the solvent consumed.

These problems can be significantly alleviated with a Protasis/MRM Microflow CapNMR probe. The advantages of microcoil NMR is that the CapNMR probe has a very high mass sensitivity, and it consumes a very limited amount of deuterated solvents. This will enable us to use deuterated solvents exclusively, avoid using sophisticated solvent suppression schemes, and ultimately obtain better quality NMR data. All of these features encourage us to investigate ways to automate the data acquisition procedure using a CapNMR flow probe.

We have been able to integrate a high throughput sample loader (Protasis HTSL-1100) with a Gilson 215 liquid handler. The data of our pilot studies on a plate of 80 samples dissolved in DMSO-d6 are presented.

This exploration of automation using CapNMR flow probe at RTP certainly demonstrates a promising future of this technology in supporting high throughput chemistry applications.

51 Exploring Inclusion Complexation With Capillary Isotachophoresis With On-line Conductivity And Microcoil NMR Detection

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Microcoil probes can enhance the sensitivity of NMR experiments for small volume, mass limited samples. Solenoidal microcoil probes can also facilitate coupling of NMR wth on-line capillary separations, such as capillary isotachophoresis (cITP). In c-ITP, analytes are separated based on their electophoretic mobilities and simultaneously concentrated by one to two orders of magnitude. For example we have achieved greater than 200-fold concentration enhancements for solutions of the ß-blocker drugs atenolol and propranolol. The use of NMR to study the interactions of propranolol with ß-cyclodextrin in the c-ITP separation will be demonstrated. Propranolol is known to form an inclusion complex with ß-cyclodextrin, as confirmed by NMR chemical shift and line shape analysis. In our cITP experiments, addition of ß-cyclodextrin to the sample, leading and trailing electrolyte solutions at a constant concentration of 10 mM (the solubility limit in aqueous solution) lengthened the time required for the focused propranolol band to reach the NMR microcoil. In contrast, the experiment time for atenolol, which is not capable of forming an inclusion complex with ß-cyclodextrin, was unaffected. The NMR spectra collected on-line in real time during the separation show that the neutral ß-cyclodextrin is concentrated by c-ITP along with the positively charged propranolol as a result of the inclusion complexation equilibrium. A limitation of on-line, real time NMR detection of separations is the limited experiment time precluding signal averaging to improve signal-to-noise ratios or 2D experiments for structure elucidation. We have designed a contactless conductivity detector for use as an on-line detector in tandem with NMR detection. The conductivity detector facilitates trapping the focused analyte bands prior to NMR detection enabling signal averaging and 2D experimentation. This detector uses two silver electrodes separated by a gap and applied to the exterior of the separation capillary. An rf field applied to the electrodes measures the IR drop due to changes in solution conductance revealing the presence of a c-ITP focused band. The effect of parameters such as electrode diameter and length, size of the detection gap, and the distance between the conductivity detector and NMR microcoil are under investigation.

52 Screening Analyte-Cyclodextrin Binding By ^{1}H-^{13}C Chemical Shift Mapping: Applications To Chiral Chromatography

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Nuclear magnetic resonance (NMR) has recently been shown to be a useful tool in selecting cyclodextrins for use in chromatographic separations 1,2. These studies have utilized changes in diffusion rates or in the ^{1}H and ^{13}C chemical shifts upon complexation as indicators of binding. In this study, we propose the use of 2D heteronuclear single quantum coherence experiments (HSQC) to simultaneously observe both the ^{1}H and ^{13}C chemical shift changes. Given the added resolution of the 2D experiment, this new approach has the potential advantage that multiple cyclodextrins may be screened in a single experiment. Capillary electrophoresis (CE) will then be used to test if a chiral separation can be achieved based on the cyclodextrin modifier predicted by NMR. If successful, screening cyclodextrin binding by 2D NMR may be an efficient new technique for identifying the proper modifier to use for chiral separations.

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53 NMRQUANT - A Powerful Tool For The Quantitative Evaluation Of The NMR Spectra Of Mixtures

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Whereas there exist rigid prescriptions for quantitative data measurement and signal assignments, the evaluation of quantitative data consists of a more or less intensive application of the rule of three (depending on the particular analytical problem). At BASF we had developed a computerizable formalism based on several Visual Basic Applications of the spreadsheet program EXCEL. It decisively facilitates our daily routine work when evaluating quantitative NMR spectra. The evaluation of data is presented for an "easy" 3-component mixture and some "real-life" examples.

54 Capillary NMR: Capabilities And Applications Of The Microflow Approach

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Capillary-based microflow CapNMR™ probes are described which have a mass sensitivity comparable to cryoprobes, but with several distinct advantages. The probes have flow cell volumes of 5 µL, observe volumes of 1.5 µL and are equipped with either dual proton/carbon channels or proton/carbon/nitrogen with deuterium lock and z-gradient. The entire flow path is fused silica; inlet and outlet capillary inner diameter is 50 µm to minimize sample dispersion, making it well suited to mass- and volume-limited samples. An injected quantity of 1 nmol of sucrose (0.34 µg in 3 µL, 0.33 mM; MW = 342 g/mol) yields a 1D proton spectrum in 10 min on a spectrometer of 500 MHz or higher. Using a 3 µL injection of a natural product, muristerone A (75 µg, 50 mM, 150 nmol; MW = 497 g/mol), a gradient COSY spectrum was acquired in 7 min, a gradient HSQC in 2 hr, and a gradient HMBC in 13 hr. Examples of 1D, 2D and 3D NMR data will also be presented. Four basic modes of sample entry vary in degree of user intervention, speed, solvent consumption, and sample delivery efficiency. Manual (hand-held syringe), manual-assisted (employs a micropump), automated (uses a micropump and the Gilson 215 autosampler), and capillary HPLC modes of operation are illustrated with examples. The CapNMR™ probe is compatible with Bruker, Varian, and JEOL spectrometers.

55 Automated NMR Approaches In Pharmaceutical Discovery

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Wyeth Research

The modern drug discovery environment requires the preparation, measurement and data distribution of increasingly larger numbers of small molecule NMR samples. We describe here the design and utilization of a set of in-house and commercial hardware and software components which efficiently accomplish these tasks in an integrated, automated fashion.