2011 – Bloomsburg University
2009 – Wilkes University
2007 – Bucknell University
2005 – Bucknell University
2004 – Bucknell University

2014 Conference on Research and Education in Nuclear Magnetic Resonance and Mass Spectroscopy

Department of Chemistry
Wilkes University
Wilkes-Barre, PA 18766

28 May 2014

Program Schedule

8:45 – 9:30	**Registration and Refreshments** (Breiseth Lobby)
9:30 – 9:45	**Welcome and Introduction** Donald Mencer, Chemistry Department, Wilkes University
9:45 – 11:25	**Morning Session** (Breiseth 107)
11:25 – 11:35	**Conference Photo**
11:35 – 1:00	**Lunch, Poster Session**
1:00 – 2:15	**Afternoon Session** (Breiseth 107)
2:15 – 2:30	**Break**
2:30 – 3:45	**Afternoon Session**, continued
3:45 – 4:00	**Break**
4:00 – 5:00	**Plenary Lecture** Gætano T. Montelione Jerome and Lorraine Aresty Chair & Distinguished Professor of Molecular Biology and Biochemistry, Center for Advanced Biotechnology and Medicine, and Robert Wood Johnson Medical School Member, Cancer Institute of New Jersey, Rutgers, The State University of New Jersey
5:00 – 5:10	**Closing Comments** Donald Mencer
5:30 – 7:00	**Dinner for presenters, organizers, and helpers**

Presentation Schedule

Morning Session (Breiseth 107)

9:45 – 10:35	**Mark Tapsak**, Bloomsburg University *The Use of Diffusion Ordered Spectroscopy (DOSY) on Polymers*
10:10 – 10:35	**Geneive Henry**, Susquehanna University *NMR Structure Elucidation of Spirocyclic Acylphloroglucinol Derivatives from Hypericum pyramidatum*
10:35 – 11:20	**Emmanuel Hatzakis**, Penn State University *Studying Parallel-Stranded G-Quadruplex Structures of promoter DNA using NMR Spectroscopy*

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List of Authors

11:00 – 11:25 **Debashish Sahu**, Penn State University
Deciphering Structural Features of Intrinsically Disordered Proteins using Carbon-detect NMR

Afternoon Session (Breiseth 107)

1:00 – 1:25 **Michæl J. Shifflet**, Johnson & Johnson Consumer Products Company
Use of Gas Chromatography-Mass Spectroscopy to Perform Extractables and Leachables Studies for Use in Undergraduate Research Programs

1:25 – 1:50 **James Swan**, Bucknell University
Deprotonated Dipeptides in Electrospray Ionization Mass Spectrometry: Teaching Applications and Research Studies

1:50 – 2:15 **Rob McDonough**, Advion, Inc.
Bringing Mass Spectrometry to a Variety of Chromatographic Techniques

Break

2:30 – 2:55 **Toni Trumbo-Bell**, Bloomsburg University
Quantitative Analysis of Mixtures from Alcoholic Fermentation and Distillation by NMR

2:55 – 3:20 **C. Anderson Evans**, Drew University
Use of NMR in a Research Program Teaching Drug Discovery Techniques in an Undergraduate Environment

3:20 – 3:45 **Scott McCallum**, Rensselær Polytechnic Institute
Enabling NMR to Investigate Heparin Structural Biology

Break

Plenary Lecture

4:00 – 5:00 **Gætano T. Montelione**, Cancer Institute of New Jersey, Rutgers University
Combining Solution NMR and X-ray Crystallography for Analysis of Structure-Function Relationships in Proteins and Protein-Protein Complexes

Presentation Abstracts

Presentation I

The Use of Diffusion Ordered Spectroscopy (DOSY) on Polymers

Tapsak, M.
Department of Chemistry, Bloomsburg University, Bloomsburg, PA, 17815

Unique challenges exist for the characterization of synthetic polymers due to their inherent polydispersity. The standard tool used for this analysis is size exclusion chromatography (SEC). This powerful tool is used to simultaneously determine parameters such as the number average molecular weight (M_n), the weight average molecular weight (M_w) and the molecular weight distribution (polydispersity) of polymers. Like one may expect, however, there are certain limitations to this well accepted technique. Recently, diffusion-ordered NMR spectroscopy (DOSY) has been applied to polymer systems in an attempt to overcome some limitations of SEC. This talk will begin with a general overview of the application of DOSY to polymeric samples. It will conclude with specific examples of how DOSY has been used by the presenter to address specific analytical problems for copolymer materials.

Presentation II

NMR Structure Elucidation of Spirocyclic Acylphloroglucinol Derivatives from *Hypericum pyramidatum*

Henry, G.
Department of Chemistry, Susquehanna University, Sellinsgrove, PA 17870

The *Hypericum* genus is widely known as a rich source of biologically active polycyclic polyprenylated acylphloroglucinol derivatives (PPAP's). The PPAP class of natural products is structurally diverse, and consists of compounds containing fused and/or bridged polycyclic cores. *Hypericum ascyron* subspecies *pyramidatum* (*H. pyramidatum*) is one of nineteen *Hypericum* species growing in Pennsylvania. Prior phytochemical study of a different *H. ascyron* subspecies from China yielded a series of PPAP's, containing an unusual 6/6/5 spirocyclic skeleton. The hexanes extract of the leaves of *H. pyramidatum* was studied to compare its chemical constituents to those of the China subspecies. Four new spirocyclic PPAP's, pyramidatones A-D (**1**–**3**, **5**), together with a known spirocyclic PPAP, chipericumin C (**4**), were isolated from the extract. The structures of compounds **1**–**5** were elucidated on the basis of 1D- and 2D-nuclear magnetic resonance (NMR) spectroscopic data. The compounds isolated from *H. pyramidatum* contain the same 6/6/5 spirocyclic skeleton as the PPAP's from the China *ascyron* species.

Poster 6

The Recovery and Characterization of Type II Polyketides from Soil Metagenomes

Low-Beinart, L.; Iqbal, H.; Brady, S.; Jain, S.*
Bard College Chemistry Department, Annandale-On-Hudson, NY 12504

Antibiotic-resistance has emerged as one of the major public health problems of the 21st Century. The rate at which novel antibiotics are discovered has declined for over thirty years, while simultaneously the number of antibiotic resistant strains of harmful bacteria has risen. Natural products or synthetic variations of such account for about two-thirds of all FDA approved drugs. However, the standard drug discovery platform — growing and extracting liquid bacterial cultures — has become ineffective as 99% of microbes cannot be grown in the laboratory. A novel culture-free technique allows access by heterologous expression to the biosynthetic pathways of these unculturable microbes by extracting DNA directly from the environment. In the current work, three heterologously expressed bacterial type II polyketide pathways were examined for the production of new molecules. Extraction of liquid cultures and analysis by LCMS revealed the presence of clone- specific metabolites in all three pathways. Purification by HPLC and silica flash chromatography were attempted with varying degrees of success, and the product of one incomplete pathway was successfully characterized by 1-D and 2-D NMR.

Poster 4

NMR Studies on the Conformational Changes in the Poliovirus RNA Dependent RNA Polymerase

Yang, X.; Liu, X.; Musser, D.M.; Boehr, D.D.*
Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

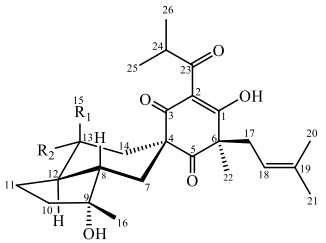
The fidelity of the viral RNA-dependent RNA polymerase (RdRp) is closely related to the stable heritage of the genome and the evolution of virus. Previously we used solution ^{13}C NMR spectroscopy to demonstrate that the closed conformation of the motif-D active-site loop in poliovirus RdRp could be achieved only upon binding the correct nucleotide. Here, we have employed ^{31}P NMR spectroscopy to directly monitor conformational changes of the triphosphate of the incoming nucleotide itself. The results show that the triphosphates of correct and incorrect nucleotide are held in different conformations in the wild-type enzyme. However, in some fidelity variants, the motif-D loop appeared to close while the triphosphate of correct nucleotide displayed the same conformation as that for incorrect nucleotide. We conclude that the triphosphate conformation change is a fidelity checkpoint, separate from the structural rearrangement of the motif-D loop.

Poster 5

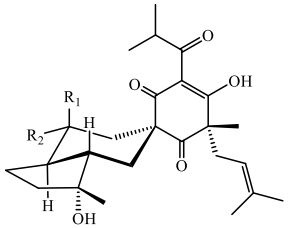
Synthesis and Characterization of Fluorinated Unnatural Amino Acids

Zack, L.; Dudeck, B.; Henkels, C.*
Department of Chemistry, Wilkes University, Wilkes-Barre, PA 18766

In vivo unnatural amino acid (UAA) incorporation is a powerful tool to study or modify proteins as one can increase the number of possible chemical probes for structure-function studies through the expansion of the genetic code to include a 21st amino acid. The designed UAA can introduce novel chemical functionalities not found within the canonical 20 amino acids with the fidelity and the specificity of site-directed mutagenesis. In this study, we synthesized UAAs that provided a unique ^{19}F probe. ^{19}F nuclear magnetic resonance (NMR) has many exclusive and intriguing advantages in studying protein structure, dynamics, kinetics, and notably, the reduction of NMR spectra complexity. Additionally, ^{19}F is a naturally abundant isotope of fluorine, is NMR-active, has a large chemical shift range, and high sensitivity. The fluorinated UAAs para-triflouromethyl- phenylalanine (ptfmF), para-methyl-meta-flouro- phenylalanine (pmmfF), para-trifluoromethyl-meta- fluoro-phenylalanine (ptfmmfF) were successfully synthesized and purified to homogeneity. The three UAAs were verified by using ^{1}H-NMR, ^{13}C NMR, and Mass Spectroscopy.



Pyramidatone A **1**: $R_1 = CH_3$, $R_2 = OH$
Pyramidatone B **2**: $R_1 = CH_3$, $R_2 = OOH$
Pyramidatone C **3**: $R_1 = OOH$, $R_2 = CH_3$



Chipericumin C **4**: $R_1 = CH_3$, $R_2 = OH$
Pyramidatone D **5**: $R_1 = CH_3$, $R_2 = OOH$

Presentation III

Studying Parallel-Stranded G-Quadruplex Structures of promoter DNA using NMR Spectroscopy

Hatzakis, E.
Department of Chemistry, Pennsylvania State University, University Park, PA 16802

DNA G-quadruplexes make up a family of secondary DNA structures that consist of stacked G-tetrads connected by Hoogsteen hydrogen bonds and stabilized by monovalent cations such as potassium and sodium (Fig 1). Intramolecular G-quadruplexes have been found in a number of G-rich regions with biological significance, such as human telomeres and oncogene promoters. We determined the NMR solution structure of the 1:2:1 parallel-stranded loop isomer, formed in Myc1234, the region containing the four consecutive 5′ runs of guanines of c-MYC promoter NHE III1. This major loop isomer, although sharing the same folding structure, appears to be markedly less stable than the major loop isomer formed in the single-stranded c-MYC NHE III1 oligonucleotide, the Myc2345 G-quadruplex. Our NMR structures indicated that the different thermostabilities of the two 1:2:1 parallel c-MYC G-quadruplexes are likely caused by the different base

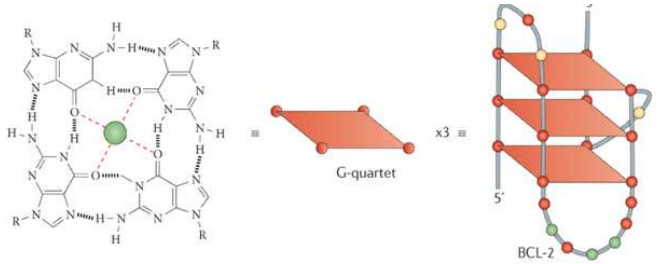


Fig 1. G-quadruplexes consist of stacked G-tetrads connected by Hoogsteen hydrogen bonds and stabilized by monovalent cations.

conformations of the single nucleotide loops. We also performed a systematic thermodynamic analysis of modified c-MYC NHE III1 sequences, which provided quantitative measure of the contributions of various loop sequences to the thermostabilities of parallel-stranded G-quadruplexes. This information is important for understanding the equilibrium of promoter G-quadruplex loop isomers and for their drug targeting.

Presentation IV

Deciphering Structural Features of Intrinsically Disordered Proteins using Carbon-detect NMR

Sahu, D.
Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

The ubiquitous use of intrinsically disordered proteins (IDPs) in various signaling and transcription pathways has created an extraordinary need for their structural characterization to better understand their respective mechanisms of action. With the invention of cryo-probes and novel ^{13}C direct-detect NMR experiments, it is now feasible to obtain structural parameters on IDPs which can be observed as ^{15}N,^{13}C CON spectrum. Here we present a wide variety of NMR based tools to easily assign chemical shifts of IDPs that correlate ^{15}N chemical shifts of adjacent residues as well as amino-acid filtered CAS experiments. In addition to H_N start experiments that are most suitable under low pH conditions, we have also developed H_α start experiments that aid in the assignments on samples under pH > 7.0 conditions. Adding to these experiments, we have also developed suite of CON based carbon-detect experiments to accurately measure $^1J_{HN}$, $^1J_{C'C}$, $^1J_{NC'}$, $^1J_{H_\alpha C_\alpha}$, $^2J_{HNC'}$ and $^3J_{HNH_\alpha}$, which is used to further obtain residual dipolar couplings, giving vital information on the structures of IDPs.

Presentation V

Use of Gas Chromatography-Mass Spectroscopy to Perform Extractables and Leachables Studies for Use in Undergraduate Research Programs

Shifflet, M.J.
Johnson & Johnson Consumer Products Company, Lititz PA

Gas chromatography-mass spectrometry plays a large role in solving many analytical challenges. One of those challenges is Extractables and Leachables. Packaging of foods, drugs and consumer products bring those products in contact with packaging components. The packaging components need to maintain the integrity of the product and not add substances to the packaged item. GC-MS can be used to test packaging materials to determine if the packaging material may add substances to the packaged item.

final week, students incubate their lidocaine analog with rat liver microsomes and collect samples at various time points. Students analyze the samples using LC-MS-MS to find the concentrations of their derivative at each time point, allowing them to them to determine the rate of metabolism of the derivative.

Poster 2

NMR and MS investigations of the sodium borohydride reduction of benzil

Lloyd, C.T.; Shoffler, C.A.; Denny, R.R.; Nguyen, T.V.; Peelen, T.J.*
Lebanon Valley College, Department of Chemistry, Annville, PA 17003

We have developed a simple set of experiments for the undergraduate organic chemistry laboratory course that explore the mechanism of the reduction of benzil by sodium borohydride. The reduction of benzil by sodium borohydride is a common undergraduate laboratory experiment that results in high selectivity for the *meso*-hydrobenzoin diastereomer. We have designed a set of experiments that can be performed in the context of the undergraduate laboratory course that probe the mechanism of benzil reduction, most notably using a mixture of $NaBH_4$:$NaBD_4$ to determine how the first carbonyl reduction is connected to the second carbonyl reduction. The experimental data allow the students to make conclusions about the mechanism of the reaction and expose students to the mechanism of borohydride reductions, the use of isotope labeling experiments in interrogating mechanistic problems, and stereochemical models for carbonyl reactions.

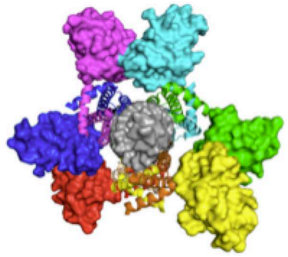
Poster 3

Diastereomeric Interactions of Chiral Alcohols

Maple, M.; Stoltzfus, A.; Denny, R.R.; Peelen, T.J.*
Lebanon Valley College, Department of Chemistry, Annville, PA 17003

Chiral alcohols and amines have been previously shown to be responsible for enantiomeric enrichment of optically active compounds during routine purification. These self-disproportionation processes have been attributed to differing diastereomeric interactions among enantiomers capable of hydrogen bonding. In our work, we aim to characterize the nature of diastereomeric hydrogen-bonded aggregates by formation of stable, covalently-bound acetals. NMR serves as an effective tool for distinguishing and quantifying these diastereomeric interactions. Furthermore, we will report on initial efforts to exploit these diastereomeric interactions as stereocontrol elements in kinetic resolutions.

sample production and protein complexes to the broader biological ntegrated technology platform is organized through web-based data information community. The focus of our work is on developing methods which can be broadly used by the biology research community. Examples utilizing these methods include specific studies of viral protein–human host protein complexes associated with influenza virus infection, and protein-protein complexes associated with cancer biology. Interfaces in these complexes are potential therapeutic targets for development of new drugs. The unique role of protein NMR in these projects will be highlighted.



Hybrid model of the complex formed between the influenza A non-structural protein 1 (NS1) and double-stranded RNA. (Aramini et al. **J. Biol. Chem.** 2011, 286: 26050)

Poster Abstracts

Poster 1

A project-based laboratory using LC-MS that introduces students to drug discovery

Peelen, T.J.;* Kontra, J. M.; Parks, B. W.
Lebanon Valley College, Department of Chemistry, Annville, PA 17003

A multiple week project for the undergraduate organic laboratory has been developed that introduces students to the drug discovery process and the critical role played therein by liquid chromatography–mass spectrometry (LC-MS). The project combines parallel synthesis of a library of molecules with the power of LC-MS to quantitatively analyze samples from complex, biological matrices.

The goal of the project is to compare the metabolic stability of a library of drug-like molecules. In the first week, students adapt the known synthesis of the anesthetic drug, lidocaine, to prepare a library of analogs. In the second week, students perform experiments using the mass spectrometer to develop MS detection methods tailored to the analysis of their lidocaine analog and then prepare a standard curve that allows them to quantify the concentration of their derivative relative to an internal standard. In the

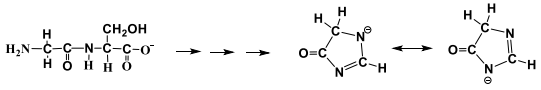
This presentation will demonstrate how GC-MS can be used to demonstrate that substances are not being leached into products from packaging materials. These studies are generically titled extractable and leachables studies and they can be completed using a single quadrupole or ion trap unit mass analyzers that are common to most laboratories.

Presentation VI

Deprotonated Dipeptides in Electrospray Ionization Mass Spectrometry: Teaching Applications and Research Studies

Swan, J.
Chemistry Department, Bucknell University, Lewisberg, PA 17837

A brief introduction to Electrospray Ionization Mass Spectrometry will be followed by a discussion of how ESI-MS is used in a course currently offered as part of the Cell Biology/Biochemistry major at Bucknell. Recent findings on fragmentation mechanisms of dipeptides containing Serine will also be presented. Computational work and Oxygen-18 labeling studies will be used to support a proposed mechanism of formation of an unusual fragment in the collision induced dissociation of the dipeptide Gly-Ser.



Presentation VII

Bringing Mass Spectrometry to a Variety of Chromatographic Techniques

McDonough, R.
Advion Biosciences, Inc.

Advion BioSciences, Inc. was founded in 1993 based on the novel techniques developed within the Cornell University laboratory of Dr. Jack Henion, a leading researcher in the field of Liquid Chromatography/Mass Spectrometry (LC/MS). Now Advion, Inc., its mission: To excel as innovators of purpose-built products that improve workflows for the life science industry. The expression CMS (Compact Mass Spectrometer) provides essential information quickly and improves the chemist's workflow. Modern organic labs have become automated, yet real-time mass assays remain just out of reach ??' the expression CMS solves this at an affordable price with kits available to interface it to many other techniques (Flash, SFC, any HPLC) and it can even perform direct MS measurements of TLC spots. With over 20 years of MS and chemistry expertise, the collaborative efforts produced a MS for chemists.

Presentation VIII

Quantitative Analysis of Mixtures from Alcoholic Fermentation and Distillation by NMR

Trumbo-Bell, T.

Department of Chemistry, Bloomsburg University, Bloomsburg, PA, 17815

Alcoholic fermentation of sugars in fruit and grains by yeast has been employed by countless cultures around the world. The products form an important part of modern cultures and, as such, is an important industry about which science students should learn. In this work, students in Biochemistry 2 used common baker's yeast (*S. cerevisiae*) to ferment a selection of sugars: galactose, sucrose, and fructose. The crude extracts were purified by simple distillation. The simple distillates were purified by fractional distillation. Samples were taken at each point for analysis by densitometry and quantitative NMR.

Presentation IX

Use of NMR in a Research Program Teaching Drug Discovery Techniques in an Undergraduate Environment

Evans, C.A.

RISE Department, Drew University, Madison NJ, 07940

Small molecule drug discovery in a given therapeutic area involves the synthesis and structural determination of a number of compounds related to a starting biologically active entity. Biological screening and the identified structures of these compounds allow the creation of a Structure-Activity Relationship (SAR) of this region of "structure space". A well-formed SAR provides a picture of the active binding site in terms of the chemical groups most responsible for eliciting the biological response.

Unquestionably the central part of developing a good SAR is the accuracy of the molecular structures being tested in the assay. Mass Spectrometry and Nuclear Magnetic Resonance are the most important tools used to determine or verify structures produced in the synthesis of the drug candidate molecules. In this talk I will describe the drug discovery approach used as a platform for student instruction in drug discovery. I will also provide examples of the use of NMR in structure determination for this program at Drew.

The presence of the RISE department, apparently unique to Drew University, greatly enhances our ability to provide a high level of supervision of undergraduate research projects. The University supports the department by providing office and laboratory research space for all department Fellows. The active members of the department are all retired industrial scientists who volunteer their time to individually mentor undergraduate research projects. A number of these projects will be discussed in this presentation.

Presentation X

Enabling NMR to Investigate Heparin Structural Biology

Masuko, S.[1]; Liu, J.[2]; Linhard, R.J.[1]; McCallum, S.A.[3]

1. *Departments of Chemistry and Chemical Biology, Rensselær Polytechnic Institute*
2. *University of North Carolina, Chapel Hill, North Carolina, 27599*
3. *Department of Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselær Polytechnic Institute, Troy, New York, 12180*

Despite the importance of heparin in the most fundamental processes in human health and cancer biology, our understanding of the structural basis of heparin function is poor largely due to challenges in obtaining structural data at atomic resolution. To overcome these challenges, novel heparin-based probes are developed by our group that enable the structural interrogation of heparin-protein complexes at atomic resolution by modern solution and solid-state NMR in addition to pulsed EPR based approaches. More specifically, results for the preparation and utilization of both site specific and uniformly $^{13}C/^{15}N$-enriched heparin and site-directed spin labeled heparin-based probes are presented. In addition, NMR-based binding studies between a defined spin-labeled heparin oligosaccharide and VEGF are reported and clearly demonstrate how simple experiments can be used to rapidly map the boundaries and polarity of the heparin-binding site of VEGF with precision. Results from these studies provide insight into the structural determinates of heparin recognition and the structural basis of heparin in the cooperative assembly and activation of the KDR-VEGF signaling complex.

Plenary Speaker

Presentation XI

Combining Solution NMR and X-ray Crystallography for Analysis of Structure-Function Relationships in Protein-Protein Complexes

Montelione, G.T.

Center for Advanced Biotechnology and Medicine and Robert Wood Johnson Medical School, Rutgers University

As structural biology attempts to tackle more and more challenging problems, opportunities have evolved for the development of hybrid methods for structure-function analysis. In our research team, we combine high throughput protein expression technologies, bioinformatics for construct design, sequence-based genomics data, NMR spectroscopy, X-ray crystallography, and small angle X-ray scattering (SAXS) to address challenges in obtaining samples and structures of biomedically-important protein-protein complexes. This imanagement systems, providing data on protein