

THE NUCLEUS

April 2006

Vol. LXXXIV, No. 8

Monthly Meeting

*Esselen Award to
Prof. Richard D. DiMarchi*

Book Review

*The Physics of Superheroes
by Dennis Sardella*

Summerthing

2006 - Red Sox

Summer Research Scholar

"Efforts Towards the Structural Determination of LnmQ, a Novel Adenylation Domain"



ACS SHORT COURSE

Designed to improve the skills and marketability of practicing B.S., M.S., and Ph.D. chemists.
The NESACS Committee on Continuing Education is pleased to sponsor this new National ACS Two-Day Short Course,
at a registration fee about half of that charged at National ACS Meetings.

Introduction to Drug Metabolism: Role and Practice in Drug Discovery

This Short Course is designed for scientists and managers in the medicinal, analytical and drug metabolism fields of the chemical and pharmaceutical industries who want to learn about the applications of drug metabolism and registration.

Attendees should have a basic understanding of organic and analytical chemistry and of biochemistry.

DATES and TIME: **Thursday, May 18, 2006; 8:00 a.m. – 5:00 p.m.,** Room 333
and Friday, May 19, 2006; 8:30 a.m. – 3:00 p.m., Room 448

PLACE: Curry Student Center, Northeastern University, 360 Huntington Ave., Boston, MA

KEY TOPICS TO BE DISCUSSED:

Optimal ADME properties of a drug candidate;
Common biotransformation reactions and enzymes;
Optimization of lead compounds from the drug metabolism perspective;
Assessment and minimization of structural liabilities associated with drug metabolism;
Typical drug metabolism experiments for drug discovery and registration;
Regulatory consideration and PhRMA perspective on drug metabolism;
Application examples.

PROGRAM AGENDA:

Roles of physicochemical properties, disposition, trans-porters (e.g. Pgp, MRP2) and pharmacokinetics in drug discovery;	Determination of metabolic stability, soft-spot characterization, and metabolite identification;
Common metabolic reactions (types and mechanism);	Strategy and methodology for screening for and minimizing reactive metabolites;
Common metabolism enzymes (CYP, UGT, SULT);	Identification of metabolism enzymes: avoiding single enzyme-mediated pathways;
Typical chemical moieties that form reactive metabolites;	Assessment of CYP inhibition potential for lead selection.
Polymorphism and metabolizing enzymes;	
CYP inhibition and induction;	
<i>In vitro</i> metabolism and models and analytical tools;	

INSTRUCTORS: **Donglu Zhang** is a Senior Research Investigator at Bristol-Myers Squibb whose research interests include identification of metabolites and metabolism enzymes, investigative metabolism, LC/MS, bioactivation mechanism, microbial biotransformations and their application to drug discovery.

Mingshe Zhu is a Senior Research Investigator in the Biotransformation Group at Bristol-Myers Squibb whose principal responsibilities are drug metabolism support to discovery and development programs, development of LC/MS and other new analytical methodologies and investigation of drug metabolism issues.

PRE-REGISTRATION REQUIRED – Registration Fees:

ACS Members if **received** before May 3 \$500.00; if **received** after May 3 \$595.00

Non-ACS Members if **received** before May 3 \$600.00; if **received** after May 3 \$695.00

There will be a limited number of scholarships for unemployed ACS Members on a space-available basis.

Parking Fee: about \$14.00/day

University cafeterias will be available for lunches.

For further information contact: Marilou Cashman, NESACS Office, e-mail: mcash0953@aol.com
phone: (508) 653-6329

Short Course Registration form: Introduction to Drug Metabolism: Role and Practice in Drug Discovery. May 18-19, 2006

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Deadlines: Summer 2006 Issue: June 16, 2006

September 2006 Issue: July 14, 2006

THE NUCLEUS

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New England Association of Chemistry Teachers

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Bridgewater State College, Bridgewater, MA

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Keynote Speaker: Dr. John Warner, UMass Lowell

NEACT and NEACS Scholarships available

Program details will be posted at: www.neact.org

Registration information will be available soon.

Approximate cost for entire conference is about \$300 per participant.

Contact Kathy Siok, Registrar-Treasurer at [neactks\(at\)cox.net](mailto:neactks(at)cox.net)

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Speak Up & Speak Out

The Northeastern Section of the American Chemical Society (NESACS) is looking for good speakers. In a new effort to build awareness about the benefits of Chemistry in our schools, towns and other industries we are seeking willing and able speakers.

The James Flack Norris Speaker's Bureau of the NESACS is in the midst of a recruitment drive. Qualified volunteers will be asked to appear in front of audiences around the New England area and present a wide variety of subjects ranging from bioterrorism to bubble gum – whatever your area of

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Monthly Meeting

The 869th Meeting of the Northeastern Section of the American Chemical Society

Esselen Award Meeting

Thursday, April 6, 2006

Harvard University, Cambridge, MA

Harvard Faculty Club, 20 Quincy St.

5:30 pm Social Hour

6:30 pm Dinner

8:15 pm Award Meeting, Mallinckrodt Building, 12 Oxford St.

Pfizer Lecture Hall (MB23), ground floor.

Dr. Patricia Mabrouk, NESACS Chair, presiding

Welcome - Dr. William Klemperer, Chair, Esselen Award Committee

The Esselen Award - Dr. Myron S. Simon, Founding Member of the Esselen Award Committee

Introduction of the Award Recipient - Dr. David E. Clemmer, Indiana University

Presentation of the Award - Gustavus J. Esselen, III

"Chemical Biotechnology as a Means to Optimal Protein Therapeutics," Dr. Richard D. DiMarchi, Gill Chair of Biomolecular Sciences, Indiana University and Chairman, Ambrx, Inc.

Dinner reservations should be made no later than noon, Friday, March 31.

Please call or fax Marilou Cashman at (800) 872-2054 or e-mail at MCash0953(at)aol.com. Reservations not cancelled at least 24 hours in advance must be paid. Members, \$30.00; Non-members, \$35; Retirees, \$20; Students, \$10.

THE PUBLIC IS INVITED

Anyone who needs special services or transportation, please call Marilou Cashman a few days in advance so that suitable arrangements can be made.

Free Parking in the Broadway Street garage (3rd level or higher), enter from Cambridge St. via Felton St.

Next Meeting: The May Meeting is Education Night. The meeting will be held at Northeastern University on Thursday, May 11, 2006. The evening speaker will be Michael Gilbert of EIC Laboratories.

Abstract

The scientific work from this laboratory was central to the discovery and the commercial development of a number of prominent protein-based medicines, such as Humulin, Humalog Humatrope, rGlucagon, Xigris, and Forteo. Humalog represents the first biosynthetic hormone optimized by rDNA technology approved as a human medicine. It established the precedent that endogenous hormones were not optimized for use as drugs, and that through insightful structural modification a more efficacious and safer protein could be developed. The structural basis for the design of Humalog was derived from our previous work on the structure and function study of IGF-1.

The recent emergence of new technologies in protein biosynthesis is dramatically enlarging the structural space that can be utilized by protein medicinal chemists. This period in protein chemistry is quite analogous to the advent of rDNA-based synthesis when the first natural sequenced proteins were produced and the foundation for the delivery of optimized proteins was established. The integration of new synthetic tools with more conventional methodologies is dramatically enhancing the academic and commercial opportunities in protein chemistry. Our current scientific activities are focused on novel methods of drug delivery with

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human insulin). This designer insulin represents the first demonstration that structurally altered rDNA-derived biosynthetic proteins can improve pharmacological performance without increasing the risk of an abnormal immunological response. As scientist and administrator Dr. DiMarchi participated in the commercial development of Humulin, Humatrope, Xigris®, and Forteo®. The goal of his current research and commercial endeavors is to develop proteins with enhanced therapeutic properties through biochemical optimization with non-natural amino acids, an approach he has termed chemical-biotechnology ◇

Biography

Richard DiMarchi is the Linda & Jack Gill Chair in Biomolecular Sciences and Professor of Chemistry at Indiana University. He is a retired Group Vice President at Eli Lilly & Company where for more than two decades he provided leadership in biotechnology, endocrine research and product development. He currently serves as a co-founder and Board Chairman of Ambrx Inc. He previously served as a

board member to the biotechnology trade group BIO and the American Peptide Society, as well as such companies as Millennium Biotherapeutics and Inproteo. He currently serves as Board member to Isis Pharmaceuticals, and scientific advisor to Alba Inc., Epitome Biosciences, Kai Pharmaceuticals, Semafore Biotechnologies, SAM Ventures, and Twilight Ventures. Professor DiMarchi is readily recognized for discovery and development of rDNA-derived Humalog® (LisPro-

Lyman C. Newell Grants

The Northeastern Section of the American Chemical Society is again offering the Lyman C. Newell Grants for the NEACT 68th Annual Summer Conference on Green Chemistry at Bridgewater State College, in Bridgewater, MA, August 7-10, 2006.

(<http://www.neact.org/sumconf.htm>)

The Lyman C. Newell Grants commemorate a former chair of the Northeastern Section who was a distinguished chemist, teacher, and historian of chemistry. For many years Lyman Newell was chair of the Chemistry Department at Boston University. He served as the first president of NEACT from 1889 to 1900 and expressed a continuing interest in training chemistry students throughout his long career. His efforts are continued by grants that bear his name.

This year we will be awarding four grants. The total fees for Monday evening through Thursday morning, including registration, room and board, banquets and socials are expected to be from about \$300 to \$325. Each Newell Grant will be for \$225, paid to the NEACT Summer Conference Registrar/Treasurer.

While preference will be given to teachers who are new to teaching or returning to teaching, the awards are open to all secondary school teachers. Applicants need not be members of the Northeastern Section of the American Chemical Society or of NEACT. The application for the Newell Grants is available on the website of the Northeastern Section at <http://www.nesacs.org>. Applications for the grants are due by April 14, 2006, and all applicants will be notified of the results by e-mail on April 25, 2006. Mail your completed application to the following address.

Dr. Ruth Tanner
Telephone: (978) 934-3662
Education Committee Chair, NESACS

NERM 2006

34th NORTHEAST
REGIONAL ACS MEETING

October 5-7, 2006

Best Western Regency Hotel and
Conference Center, Binghamton, NY

Hosted by the Binghamton ACS Local
Section and sponsored by the Northeast
Region, Inc.

The Materials Research Society, the Watson School of Engineering and Applied Science, and the Integrated Electronics Engineering Center (IEEC) at Binghamton University join the ACS to present NERM 2006, "Emerging Technologies and the Chemical Sciences." The program of the meeting will highlight topics such as sensors and small scale systems integration, lithography, environmentally benign materials and processes, nanomaterials, and electronics packaging. In addition, there will be symposia in analytical, inorganic, organic, and physical chemistry, biochemistry, and chemical education, the display of undergraduate research posters, and an open public session on environmental issues important to the local region. Tobin Marks of Northwestern University will present a keynote lecture on his research in the area of molecular electronics and photonics.

Important dates:

May 1 – abstract submission and
advance registration begins

August 21 – abstract deadline

September 11 – advance registration
ends

October 5-7 – NERM 2006

See www.nerm2006.org for details. ◇

e-mail: Ruth.Tanner@uml.edu
University of Massachusetts-Lowell
Chemistry Department,
Olney Hall
1 University Avenue
Lowell, MA 01854
ATTN: Newell Grant Committee ◇

2nd Annual NESACS Golf Tournament

Please check our website
(www.nesacs.org) for details on the
Second Annual NESACS Golf Tourna-
ment. NESACS is looking for play-
ers, sponsors, and volunteers. ◇

Summerthing 2006 – Red Sox

The bearer of bad tidings is hardly ever cheered as a hero and so we come to you all with a heavy heart to report the change in the Red Sox group ticket sales distribution has devastated the Northeastern Section. The Red Sox have now limited groups to only 110 tickets per game for a maximum of three games. That's the bad news, but not the worst news.

The worst news turns out to be that our account executive who handled our reservations has left the Red Sox organization and, more importantly, did not pass along the list of organizations he handled. So when it came time to send out the new policy information and notification, no word was relayed to the Northeastern Section. E-mails we had sent in December in anticipation of group ticket availability went unanswered. Phone numbers did not seem to work. Finally, in January, we managed to get through to an answering machine and that's when we discovered that our executive was no longer with the Sox. We contacted someone who seemed to be in charge and was informed that all tickets were gone. We did manage to convince the powers that be, however, to give us fifty tickets to the May 14th game with Texas and we know that will not satisfy the voracious appetite of NESACS

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Book Review

The Physics of Superheroes, by James Kakalios

(Gotham Press, 2004) 292 pp., ISBN 1-592-40146-5; \$26.00 hardcover)

Reviewed by Dennis J. Sardella

Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, MA 02467

In an often-quoted essay, *New York Times* columnist Russell Baker wrote about the difference between being “serious” and being “solemn,” observing that “The transition from seriousness to solemnity occurs in adolescence, a period in which nature, for reasons of her own, plunges people into foolish frivolity. During this period the organism struggles to regain dignity by recovering childhood’s genius for seriousness. It is usually a hopeless cause.” The contrast between seriousness and solemnity can unfortu-

nately often be seen in introductory science courses, where serious subjects are presented in solemn ways, the result being that students who are not already committed to science end up being bored by them, see them as mere mathematical manipulations to be mastered, conclude that they lack “the science gene,” and vote with their feet.

In contrast, in the spirit of Heisenberg, who once observed that there are things that are so serious that one can only joke about them, James Kakalios has written *The Physics of Superheroes*, a book that tackles the task of presenting to non-scientists the basic ideas of physics – a serious subject if ever there was one – in a refreshing way that lacks all solemnity.

The book grew out of a freshman seminar entitled “All I Know About Physics I Learned From the Comics,”

in which Kakalios, a physics professor at the University of Minnesota reached back to his lifelong love of comic books, using the exploits of superheroes to illustrate the application (or, in some misapplication) of the principles of physics to such momentous questions as:

- Does the fact that Superman can leap tall buildings at a single bound tell us anything about the gravity on Krypton? (Newton’s first law)
- What really killed Spiderman’s girlfriend? (momentum)
- How much food does The Flash have to eat in order to run so fast? (conservation of energy)
- How can Electro run up the side of a steel-frame building? (Faraday’s laws)

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Abstract

Continued from page 5

particular emphasis in endocrinology. We are in the midst of exploring these chemical approaches in collaboration with academic, biotechnology, and pharmaceutical company collaborators.

◇


Summerthing – SOX

Continued from page 6

members. We pleaded, cajoled and begged, to no avail. We apologize profusely.

The tickets we have are right field grandstand seats with a base price of \$27 plus a \$6 contribution to the education fund plus \$2 for handling, postage and insurance for a total of \$35 per ticket. I checked the internet for ticket agency prices and discovered that they are charging \$60-80 for the same seats!!

To reserve your tickets (maximum 4) until the supply is exhausted, please contact Wally Gleekman at 617-527-1192 or [gleekman\(at\)msn.com](mailto:gleekman(at)msn.com). After you reserve your tickets you can mail a check made out to the Brookline Educators Association to Wally at 35 Rangely Road, West Newton, MA 02465-1218. ◇




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Book Review

Continued from page 7

- Why can't Superman change history by traveling into the past and altering events? (quantum mechanics and the many-world interpretation)

Kakalios writes with a clear style, incorporating plenty of humor, as illustrated both in the chapter headings (“Deconstructing Krypton,” “Does Size Matter?,” “The Central City Diet Plan. Conservation of Energy,” “Through A Wall Lightly. Tunneling Phenomena”), and in some of his numerous (but mostly serious) parenthetical remarks. Using examples taken from comics (of which he clearly has an encyclopedic knowledge) as a jumping-off point, he manages to cover all the basic topics in a physics survey course, including mechanics, thermodynamics, relativity and quantum mechanics. Aiming at non-scientists, he eschews mathematics almost entirely, relying instead on verbal exposition and the use of imaginative parallels that suggest he would be an enjoyable and effective classroom instructor.

In addition to principles of physics, *The Physics of Superheroes* conveys considerable information about the history and production of comic books. It also includes numerous illustrations from classic comics. Though it would have been more visually appealing had they been reproduced in color, I imagine this would have increased the price of the book considerably (though still keeping it far below the near-astronomical cost of the average contemporary science text).

I found no obvious mistakes and few typos (though manganese was accidentally transmuted typographically to magnesium at the top of page 275). One point that did seem potentially confusing, though, was the discussion of uncertainty, using as an example a hypothetical attempt to determine the frequency of a vibrating string by touching it gently and feeling its vibrations (p. 239). “... [O]nce we have touched the string, it will no longer be oscillating at the same frequency as before. It will either have stopped shaking altogether or be vibrating at a different frequency.”

Kakalios had already pointed out earlier that the frequency of a vibrating string, like that of a pendulum, depends only on its length. Thus, if one only touched the string lightly enough to feel it beating against one's finger (as opposed to pressing it firmly against a fret), one would logically expect its amplitude to be changed, but not its frequency (unless the touch creates a node, thereby producing an overtone or two frequencies beating against one another).

On p. 307, in his discussion of adamantium, the defect-free “covalently-bonded metal,” composing Wolverine's claws, Kakalios writes, “In order to break these bonds, one must remove the electrons from all of the bonds connecting an atom to all of its neighbors.” To a chemist's ear this sounds more like multiple ionization than bond cleavage, but is probably more a matter of unfortunate phrasing.

One amusing point: Kakalios' own alter ego. The photo on the back flyleaf of a smiling, benevolent teacher-author bursting with humor, contrasts strongly to the rather stern photo of the researcher in his University of Minnesota website, evoking an echo of the “serious-solemn” distinction!

For the reader who gets bitten by the physics bug after finishing *The Physics of Superheroes* and wants to scratch it, Kakalios includes plenty of suggestions for further reading in the back – mostly semi-popular books, biographies, histories, and occasional texts. This is still a good-sized jump (though not one of superhero dimensions), and, given people's propensity for brief reading, I would have liked him to have included some articles of the *Scientific American* genre, though this is at best a minor criticism.

Overall, Kakalios has done a great job of presenting the principles of physics to non-scientists in an effective and enjoyable way. By sensitizing them to look for the basic principles of physics at work in the everyday world of superheroes, Kakalios may succeed in getting them to apply a similar approach to their own daily experiences, thus helping them develop into

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Summer Research Scholar

Efforts Towards the Structural Determination of LnmQ, a Novel Adenylation Domain

William Hillmann and Steven D. Bruner
Boston College, Department of Chemistry

Introduction

A considerable number of the most potent anticancer and antibiotic natural products are produced by large, multi-functional proteins termed non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs). The modular structure of these megasynthetases suggests great potential for combinatorial manipulation of discreet modules to generate “unnatural” products using the existing enzymatic machinery. Before this may be attempted, a thorough understanding of the structure and function of individual domains must be obtained. In order to meet this end, it is necessary to solve the structure of NRPS/PKS domains and gain a mechanistic understanding of how these domains perform chemistry. Thus, the structure of LnmQ, a novel adenylation domain from the leinamycin biosynthetic pathway, will be determined by X-ray crystallography, and its structure refined through the use of synthetic inhibitors.

Leinamycin is an antitumor, antibiotic compound containing a novel 1,2-dithiolan-3-one 1-oxide heterocycle which has been shown to promote oxidative damage of DNA as well as alkylating DNA at the N7 position of guanine.¹ Leinamycin is a hybrid polyketide/non-ribosomal peptide natural product: its biosynthetic machinery of which contains several unique features.² One of the unusual aspects of the leinamycin synthetic machinery is that there are three stand-alone enzyme domains not covalently embedded in the PKS/NRPS hybrid machinery. Two of these domains are LnmQ, an adenylation domain, and LnmP, the peptidyl carrier protein (PCP) domain associated with LnmQ. This unique system presents many opportunities for studying NRPS machinery. Also, the stand-alone nature of these domains seems conducive to modular application of NRPSs to chemoenzymatic combinatorial synthesis of potentially bioactive compounds.

LnmQ is an adenylation domain which selectively adenylates D-alanine as opposed to the natural L isomer. The fact that LnmQ adenylates the unnatural D isomer of alanine is unique in NRPS systems. D-amino acids are not rare in non-ribosomal peptide natural products, but they are usually the result of epimerization domains associated with the NRPS machinery. This is not the case with LnmQ, which is highly selective for D-alanine. LnmQ is also an isolated adenylation domain, not followed by an NRPS condensation domain. Instead, the LnmQ/LnmP didomain is followed by two cyclization domains.³ This unusual composition may represent a new paradigm of adenylation domain function and chain elongation in NRPS systems. Following crystallization of LnmQ in its native form, the enzyme will be exposed to a specific synthetic inhibitor in co-crystallization experiments to obtain a clear picture of substrate recognition and catalysis.

Adenylation domains perform chemistry homologous to the aminoacyl tRNA synthetases of ribosomal peptide synthesis.⁴ Adenosine sulfamoyl amino acid derivatives have been used as inhibitors of these enzymes and used in co-crystallization experiments to gain mechanistic insights.⁵ Herein are reported the first efforts towards the synthesis of a D-alanine sulfamoyl adenosine inhibitor (1) of LnmQ.

Synthesis

The synthesis of the D-alanine sulfamoyl adenosine inhibitor begins with the protection of adenosine as the 2',3'-dimethyl acetal (see Scheme 1).⁶ A slurry of adenosine (2) in dry acetone is treated with excess amounts of 2,2-dimethoxypropane, as well as *p*-toluenesulfonic acid (TsOH). This affords 80% of the desired 1,2 acetonide-protected adenosine (3), with the main byproduct being the 1,3 acetonide. Following purification by silica gel flash chromatography, the acetonide-protected adenosine is treated with sodium hydride in 1,2-dimethoxyethane. The 5' hydroxyl group is deprotonated, resulting in the evolution of hydrogen gas, and the amine of the adenine base is transiently

Continued on page 10

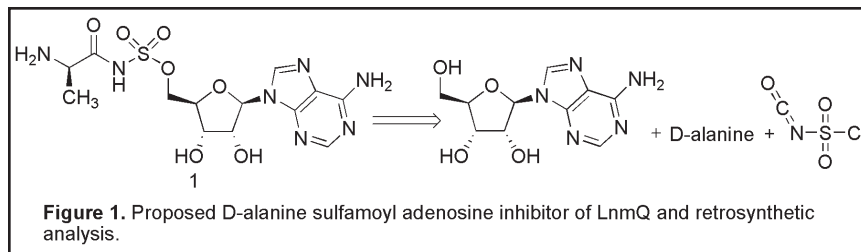
Book Review

Continued from page 8

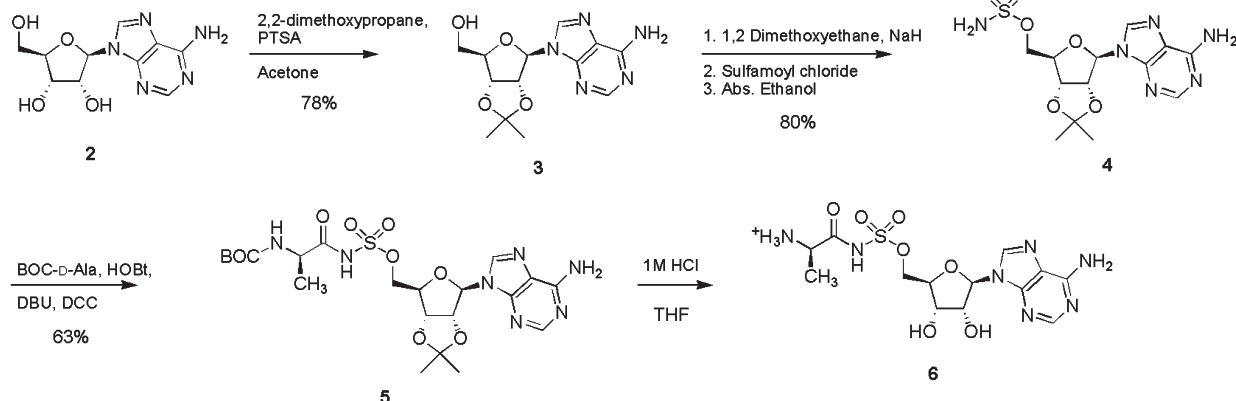
the kind of scientifically literate citizens our technological society needs. His book could also be profitably read by someone with only a modest, or a dimly-recalled, background in physics.

Personally, I thoroughly enjoyed reading *The Physics of Superheroes*, and I think I would have liked to be a fly on the wall in Kakalios' freshman seminar. Unfortunately, being a mere chemist of ordinary gifts, I have neither the requisite size nor sufficient adhesiveness in my hands and feet to accomplish this task. Maybe I should wander into the lab and wait for some transformative mishap to occur ...“◇

Continued from page 8



Scheme 1.



Summer Scholar

Continued from page 9

protected by the solvent. After stirring for several hours, freshly prepared sulfamoyl chloride⁷ is coupled to the 5' hydroxyl in 65-80% yield to give 4.

Boc-D-alanine is then coupled to the sulfamoyl moiety using a dicyclohexylcarbodiimide (DCC) coupling with 1-hydroxybenzotriazole (HOBT) to activate the amino acid and prevent racemization of the stereogenic center. This reaction proceeds with 60% yield to give Boc-D-alanine 5'-O-sulfamoyl adenosine (5).

Following the formation of the peptide bond, deprotection of the *t*-butyl carbamate and the acetonide protecting groups should be accomplished by treating the Boc-D-alanine 5'-O-sul-

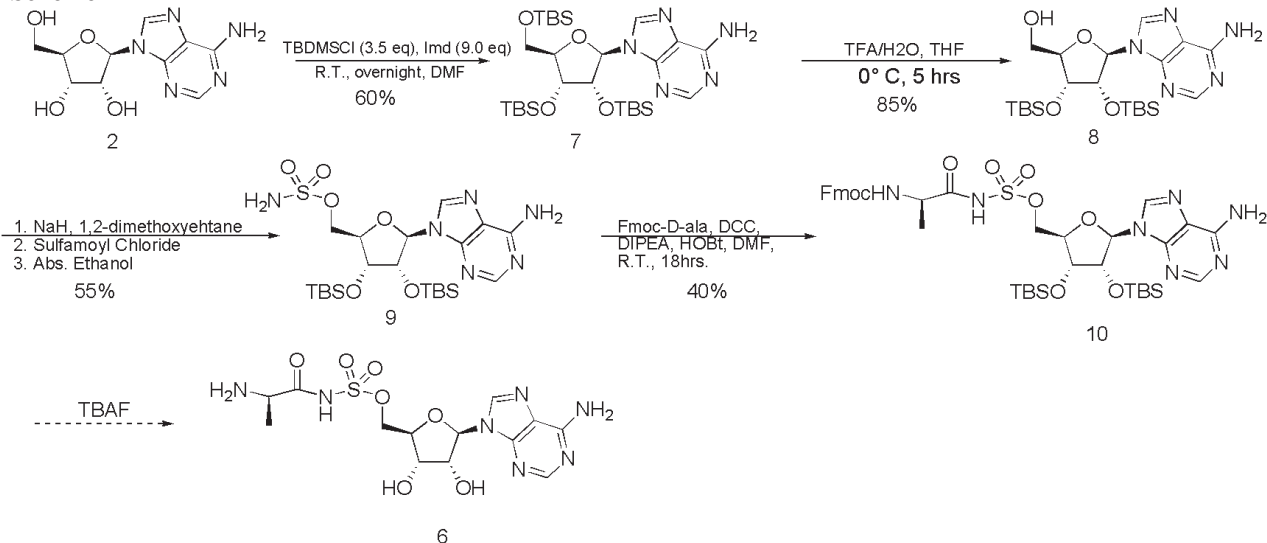
famoyl adenosine with acid. Several deprotection schemes were employed, using varying concentrations of trifluoroacetic acid (TFA) and hydrochloric acid, which generally resulted in decomposition of the starting material. It was found that the glycosidic bond was especially labile under the acidic conditions employed. Following many trials, it was found that treating the Boc-D-alanine 5'-O-sulfamoyl adenosine with 1 M HCl in 1:1 H₂O/THF resulted in the formation of product (6), as seen by mass spectrographic analysis (ESI, *m/z* of 418.1 corresponds to [M+H]⁺). Purification of the crude product has proved to be difficult, since the product is not amenable to silica gel chromatography. Currently, preparatory HPLC is being investigated as a method of purification, and

should yield the pure D-alanine 5'-O-sulfamoyl adenosine inhibitor 6.

Another deprotection scheme, one which avoids the acidic deprotection step, would solve many of the problems encountered with the synthesis. As such, another synthetic scheme was attempted (see Scheme 2).

In this scheme, the 2',3'-dimethyl acetal protection is replaced by silicon-based bis-TBDMS protection of adenosine.⁸ This initial protection installs TBDMS groups on all three hydroxyls of adenosine to give 7. Following selective deprotection of the 5' hydroxyl to give 8, the sulfamoyl coupling proceeds in the same manner as previously described to yield 9, as does the DCC coupling of D-alanine. However, the amino acid protection has been changed from a *t*-butyl carbamate

Scheme 2



group to an 9-fluorenylmethyl carbamate group (Fmoc), resulting in **10**. Following this coupling, deprotection of the TBDMS and the Fmoc protecting groups can be accomplished using tetrabutylammonium fluoride (TBAF) to give the final D-alanine 5' O-sulfamoyl adenosine inhibitor **6**.^{9,10} If TBAF does not remove the Fmoc group, it can be removed with base and then both TBDMS groups can be removed with TBAF. This synthesis has been completed through the peptide coupling in the yields shown in Scheme 2. Following deprotection, preparative reverse phase HPLC will be used to purify the final product.

Crystallography of LnmQ

Efforts were also made towards the crystallography of LnmQ. An *E. coli* expression vector (pET37b) containing the gene for LnmQ was obtained from Professor Ben Shen (U. Wisconsin, Madison). This expression vector contains an engineered hexahistidine tag on the *N*-terminus of the protein used to aid in purification of the protein. LnmQ was expressed in the BL21(DE3) strain of *E. coli*, obtained by cell lysis, and purified by successive chromatographic methods.

The first utilizes the hexa-histidine tag to bind to a nickel +2 resin. The histidines interact strongly with the nickel, allowing non tagged proteins to be removed by washing. The enzyme of interest may then be eluted by washing the resin with a buffer containing a high concentration of imidazole, which releases the hexa-histidine tag through competitive binding. Following nickel resin purification, the protein is purified, first using a strong anion exchange MonoQ column and finally using a gel filtration column. The purity of the enzyme is assessed by SDS-PAGE (see Figure 2A), and, following concentration to 10 mg/mL, the pure enzyme can be used in crystallography screens.

Initial crystal hits for LnmQ can be seen in Figure 2B. These crystals have a needle-like morphology and are of a relatively low quality. Many different conditions have been screened to try to optimize the morphology,

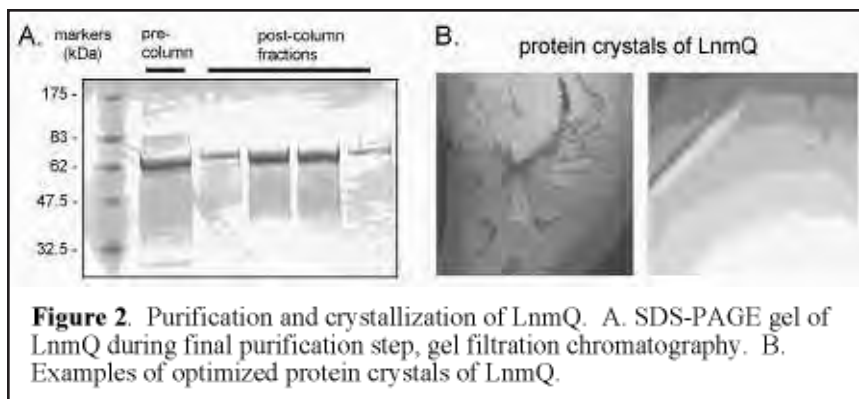
including commercially available additive screens; however, only needle-like, burst crystals are obtained. Further trials will be attempted, and it is hoped that, upon obtaining the D-alanine 5' O-sulfamoyl inhibitor, it can be used as an additive to aid in obtaining crystals with a good morphology. One of the major challenges of macromolecular crystallography is the disorder inherent in large molecules like enzymes. By adding the inhibitor which will bind in the active site, the structure of the enzyme will become more ordered and more amenable to crystal formation.

Conclusions and Future Directions

Advances have been made towards the synthesis of a D-alanine 5' O-sulfamoyl adenosine inhibitor of the NRPS adenylation domain LnmQ using two different synthetic schemes. Also, progress has been made towards obtaining high quality crystals of LnmQ, with hundreds of different conditions tested.

Future research entails the completion of both syntheses of the LnmQ inhibitor and the development of a suitable HPLC purification method. Following this, the inhibitor can be

Continued on page 12



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Continued from page 4

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Continued from page 11

used as an additive in obtaining high quality crystals of LnmQ, as well as in co-crystallization screens once a native LnmQ structure is obtained. After the structure of LnmQ is known, it can be used in co-crystallization experiments with other enzymes in the LnmQ biosynthetic pathway such as LnmP. These studies may provide insights into how NRPS systems are organized and how the different modular components interact to perform chemistry.

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December 2005 Meeting

Pictures Courtesy of Ms. Ying Wei



Edwin Villhauer speaking at the December Meeting



From left to right: Norton Peet; Liming Shao; Murali Ramachandra; G.Sridhar Prasad; Edwin Villhauer; Raj Rajur

January 2006 Meeting

Pictures Courtesy of Ms. Ying Wei



Prof. Gregory Petsko in discussion with Anthony Iavarone after presenting his seminar.



NESACS Chair-Elect, Mukund Chorghade, Prof. Gregory Petsko and Anthony Iavarone

February 2006 Meeting

Pictures Courtesy of Ms. Ying Wei



Michaeline Chen, Tim Frigo and Prof. Paula Hammond during the social hour at the February Meeting.



Patrick Gordon, NESACS Chair, Pam Mabrouk and Prof. Paula Hammond after the evening seminar in the Pfizer Lecture Hall at Harvard University.

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April 3

F. Dean Toste, (Univ. of California Berkeley) ;
John Hartwig (Yale Univ.) ; James Audia, (Eli
Lilly & Co.)
Eli Lilly Symposium.
Harvard Univ. Mallinckrodt: Pfizer Lecture Hall,
2:30 PM.
Maurice Brookhart (Univ. of North Carolina)
“Catalytic Transformations Based on C-H Bond
Activation Reactions”
Brandeis Univ. Gerstenzang 122, 3:45PM.

April 3-4

Michael A. Marletta (Univ. of California,
Berkeley)
TBA
MIT, Rm TBA, 9:00AM

April 5

Mahdi Abu-Omar (Purdue Univ.)
TBA
MIT Rm 6-120 4:00PM
Catherine Fenselau (Univ. Maryland, College
Park)
“Rapid Detection of Airborne Microorganisms
by Mass Spectrometry”
Northeastern Univ. 129 Hurtig Hall. 12 Noon

April 6

Klaus Schulten, (Univ. of Illinois Urbana-
Champaign)
“Towards Understanding Membrane Channels.”
(Woodward Lecture Series)
Harvard University, Mallinckrodt: Pfizer Lecture
Hall, 5:00PM

April 10

Melanie S. Sanford (Univ. of Michigan)
“Synthetic Applications and Mechanistic
Investigations Transition-Metal Catalyzed CH
Bond Functionalization.”
(Woodward Lecture Series.)
Harvard Univ. Mallinckrodt: Pfizer Lecture Hall,
4:15PM.

Anne Gershenson (Brandeis Univ.):
“Investigating Molecular Mousetraps: Single
Molecule Studies of Protease-Serpin
Complexes”
MIT: 56-114, 4:30PM

April 11

Krishna Kumar (Tufts Univ)
“A New Paradigm for Protein Design and
Molecular Engineering”
MIT. Room .TBD. All Day
Mukund Sibi, Ph.D. (North Dakota State Univ.)
TBA; Novartis Lecture Series, Part 4
Boston College. Merkert 130 4:00 PM

April 12

Professor Jeffrey Zaleski (Indiana Univ.)
TBA
MIT: 6-120, 4:00 PM
Eric Anslyn (Univ. Texas, Austin)
“Organic Chemistry Approaches to Single and
Multi-Analyte Sensing”
Northeastern Univ. 129 Hurtig Hall. 12 Noon

April 14

Karl Weiss Symposium on Membrane and
Membrane Systems.
Northeastern Univ. Curry Student Ctr, McLeod
Suites#318-322.
9:40AM-1:50PM.

April 18

James M. Cook (Univ. of Wisconsin,
Milwaukee)
“General Approach to the Synthesis of Ring-A
Alkoxy Substituted Indole Alkaloids via the
Asymmetric Pictet-Spengler Reaction”
Brandeis Univ. Gerstenzang 122 3:45PM.
Pamela Bjorkman (Cal Tech)
“ The Molecular Basis of Iron Overload in
Hereditary Hemochromatosis”
Univ. of New Hampshire, Iddles Auditorium,
Room L101, 11AM
Pamela Bjorkman (Cal Tech)
“What We can Learn from 3D Structures of
Proteins”
Univ. of New Hampshire Iddles Auditorium,
Room L101, 4 PM

April 19-20

Jackie Barton (California Institute of
Technology)
TBA; Arthur D. Little Lecture in Inorganic
Chemistry.
MIT, Rm: TBA, 4:00PM

April 24

Philip Baran, (The Scripps Research Institute);
Erik Sorensen (Princeton Univ.)
Pfizer Symposium.
Harvard. Mallinckrodt Pfizer Lecture Hall 2:30
PM.
Dr. Gerhard Hummer (NIH)
“Water, Proton, and Ion Transport: From
Nanotubes to Biomolecular Machines”
Brandeis Univ. Gerstenzang 122 Time: 3:45 PM.

April 25

Alanna Schepartz (Yale Univ.)
“Miniature and Non-Natural Proteins?”
MIT: 56-114, 4:00PM

April 26

John P Fackler (Texas A&M Univ.)
TBA
MIT. Location: TBA, 4:00PM

April 27

David Chandler, (Univ. of California, Berkeley)
TBA; G. B. Kistiakowsky Lecture,
Harvard Mallinckrodt: Pfizer Lecture Hall, ,
8:00PM.

April 27-28

Fred Wudl (Univ. of California Los Angeles)
TBA
MIT, Rm 6-120, 4:00PM

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