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NESACS

THE NUCLEUS

March 2006 *Vol. LXXXIV, No. 7*

Monthly Meeting

Richards Medal Award to Prof. Richard R. Shrock

Historical Notes

Henry Brown, M.D. and Nathan N. Crounse, Ph.D.

Northeastern Section ACS Scholars

Summer Research Scholar

"Novel Fluorescent Nitric Oxide Sensors"

Historical Notes

Henry Brown, M.D.
(1920-2005)

Henry Brown trained as a physician, but his longtime interest in biochemistry brought him to membership in the Northeastern Section, where he found a congenial home, and more challenging and stimulating meetings than among his sur gical brethren. He and his wife, Julia, were usually to be found at our monthly meetings, and his questions to the speaker frequently started a new and profitable line of thought. He had been elected Director-at-Large of this Section just four months before his death. We shall all miss his warmth and courtesy at meetings of the Board of Directors.

Dr. Brown was born in Erie, PA , where he attended high school, going on to the University of Michigan for undergraduate work, the University of Pennsylvania Medical School for his medical degree, and internship at St Vincent's Hospital in Erie. He married Julia Reagle, his life partner for over 60 years. During World War II he served in the Navy as Medical Officer on the USS Independence.

After the war he returned to Erie and worked as a general practitioner for five years, then, in a change of career, studied biochemistry in Cambridge, England, in the laboratory of Dr. Frederick Sanger. Following this, he took a surgery residency at the University of Wisconsin until the Navy called him back during the Korean War. His move to Boston to study hand surgery under Dr. Flynn brought him into the Harvard medical services at Boston City, New England Deaconess and Brigham and Women's Hospitals. He was a faculty member at Harvard Medical School and also Chief of Hand Surgery at the Manchester, NH, VA Hospital until his late seventies.

Research was an important part of his life. Summer weeks and sabbaticals were spent in Paris, Buenos Aires, and

continued on page 13

Contents

THE NUCLEUS

The Nucleus is distributed to the members of the Northeastern Section of the American Chemical Society, to the secretaries of the Local Sections, and to editors of all local A.C.S. Section publications. Forms close for advertising on the 1st of the month of the preceding issue. Text must be received by the editor six weeks before the date of issue.

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continued on page 13

Golf Tournament - Osmium Level Donor
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Candidates Wanted

Petition candidates for the 2006 NESACS election are welcomed.

The positions to be filled in the 2006 election are Chair-Elect, Treasurer, Auditor, Trustee, Nominating Committee, Norris Award Committee, Councilor / Alternate Councilor, Director-at-Large.

Petitions must be received no later than March 19, 2006.

Please contact Amy Tapper, chair of the Nominating Committee, at amy.tapper(at)peptimmune.com. ◇

Monthly Meeting

The 868th Meeting of the Northeastern Section of the American Chemical Society

2006 ACS Richards Medal Award Meeting

Thursday – March 9, 2006

Harvard Faculty Club, 20 Quincy St., Cambridge, MA.

5:30 pm Social Hour

6:30 pm Dinner

8:15 pm **Richards Award Ceremony**
Mallinckrodt Building, Pfizer Lecture Hall - MB23,
12 Oxford Street, Cambridge, MA.
Introduction by Professor Daniel Nocera
Prof. Richard R. Schrock, Frederick G. Keyes Professor of Chemistry, Massachusetts Institute of Technology, *"Catalytic Reduction of Dinitrogen to Ammonia at Room Temperature and One Atmosphere."*

Dinner reservations should be made no later than noon, Thursday, March 2, 2006. Please call or fax Marilou Cashman at 800-872-2054 or e-mail at Mcash0953(at)aol.com. Please specify vegetarian. Reservations not cancelled at least 24 hours in advance must be paid. Members,$28; Non-members, $30; Retirees, $15; 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 St. Garage (3rd level or higher), enter from Cambridge Street via Felton, St.

Next Meeting: April 6, 2006, Esselen Award Meeting, Harvard Faculty Club. Reception and dinner 5:30 pm. Award Meeting: 8:15 pm, Pfizer Hall, Mallinckrodt Chemistry Building, 12 Oxford Street, Cambridge. Dr. Richard D. DiMarchi, Linda & Jack Gill Chair in Biomolecular Sciences and Professor of Chemistry, Indiana University: "Chemical Biotechnology as a Means to Optimal Protein Therapeutics."

Biography

Richard R. Schrock received his Ph. D. in inorganic chemistry from Harvard in 1971. He spent one year as an NSF postdoctoral fellow at Cambridge University, followed by three years at the Central Research and Development Department of E. I. DuPont de Nemours and Company. In 1975 he moved to M.I.T. In 1980 he became full professor, and in 1989 he was named the Frederick G. Keyes Professor of Chemistry. His interests include the inorganic and organometallic chemistry of high oxidation state, early metal complexes (especially those that contain an alkylidene or alkylidyne ligand), catalysis and mechanisms, the chemistry of high oxidation state dinitrogen complexes and catalytic dinitrogen reduction, and the controlled polymerization of olefins and acetylenes. From the ACS he has received the ACS Award in Organometallic Chemistry (1985), the Harrison Howe Award (Rochester section, 1990), the ACS Award in Inorganic Chemistry (1996), an ACS Cope Scholar Award (2001), and the F. Albert Cotton Award in Synthetic Inorganic Chemistry (2006). From the German Chemical Society he has received the August Wilhelm von Hofmann Medal (with R. H. Grubbs, 2005), and from the Royal Society of Chemistry the Sir Geoffrey Wilkinson Medal (2002) and the Sir Edward Frankland Prize (2004). He is a member of the National Academy of Sciences. In 2005 he shared the Nobel Prize in Chemistry with R. H. Grubbs and Y. Chauvin. ◇

Martin Freier

We have the very sad task of informing you that our outstanding contributer, Martin Freier, passed away suddenly in early January.

A remembrance will appear in a future issue.

The NUCLEUS Staff

Abstract

We have been able to reduce dinitrogen selectively and catalytically to ammonia at 1 atm and room temperature with protons and electrons. The reduction takes place at a single molybdenum center that is sterically protected against bimetallic decomposition reactions with *meta*-terphenyl-substituted triamidoamine ligands such as $[(HIPTNCH_2CH_2)_3N]^{3-}$, where HIPT is hexaisopropyl-*meta*-terphenyl. The proton source is $(\{2,6\text{-lutidinium}\}\{BAr'_4\}$; $Ar' = 3,5\text{-}(CF_3)_2C_6H_3)$ and the reductant is decamethyl chromocene. The reducing equivalents make either ammonia (~60% from dinitrogen) or dihydrogen. All evidence suggests that N_2 is being reduced at a single Mo center in which the oxidation state of the metal varies between Mo(III) and Mo(VI). Similar $[(HIPTNCH_2CH_2)_3N]^{3-}$ complexes of tungsten, chromium, vanadium, and iron fail to yield any catalytic turnover of dinitrogen to ammonia. ◇

Call for Papers

The 8th Annual Northeast Student Chemistry Research Conference

Saturday, April 22, 2006, 9am – 4pm

Massachusetts Institute of Technology

Undergraduate, graduate, and post-doctoral students in all areas of chemistry welcome

CASH AWARDS for best presentations

Abstract Deadlines:

Oral presentations: **April 7, 2006**

Poster presentations: **April 14, 2006**

For details, directions, registration, and abstract submission, please visit the Northeastern Section Younger Chemists Committee website: www.nsycc.org ◇

Third Annual Northeastern

Student Chemistry Career Fair

Friday, April 21, 2006

Brookline Holiday Inn
1200 Beacon Street, Brookline, MA

Schedule:

(Whitney Hall A) 3pm-7pm
ACS Career Services workshops on resume writing and interviewing skills

(Whitney Hall B) 3pm-7pm
Job Fair – Meet with representatives from companies in the Northeast about employment opportunities

Have your resume reviewed by ACS Careers services

FREE REGISTRATION (Returnable Deposit):

Please register *at the Northeastern Section Younger Chemists Committee website:* **www.nsycc.org**

Participating companies *to be listed.* ◇

Call for Applications

The James Flack Norris and Theodore William Richards Undergraduate Summer Research Scholarships

The Northeastern Section of the American Chemical Society (NESACS) established the James Flack Norris and Theodore William Richards Undergraduate Scholarships to honor the memories of Professors Norris and Richards by promoting research interactions between undergraduate students and faculty.

Research awards of $3250 will be given for the summer of 2006. The student stipend is $2750 for a minimum commitment of ten weeks of full-time research work. The remaining $500 of the award can be spent on supplies, travel, and other items relevant to the student project.

Institutions whose student/faculty team receives a Norris/Richards Undergraduate Summer Research Scholarship are expected to contribute toward the support of the faculty members and to waive any student fees for summer research. Academic credit may be granted to the students at the discretion of the institution. Award winners are required to submit a report (~5-7 double-spaced pages including figures, tables, and bibliography) of their summer projects to the NESACS Education Committee by November 3, 2006 for publication in *The Nucleus*. They are also required to participate in the Northeast Student Chemistry Research Conference (NSCRC) in April 2007.

Eligibility: Applications will be accepted from student/faculty teams at colleges and universities within the Northeastern Section. The undergraduate student must be a chemistry, biochemistry, chemical engineering, or molecular biology major in good standing, and have completed at least two full years of college-level chemistry by summer, 2006.

Continued on page 13

The Analytical Laboratory Managers Association

Announcement of the first New England Local Chapter Meeting

Proposed Date: Tuesday, April 4, 2006
9:30 A.M. – Noon
Cabot Corporation
Billerica, MA

We are pleased to announce the first meeting of the New England chapter of the local Analytical Laboratory Manager's Association. If you are not familiar with this organization, ALMA is an organization that benefits managers of analytical chemistry laboratories in industry, government, and universities all over the world. ALMA's unique strength lies in providing a forum for analytical managers to interact with managers who have similar problems and who are willing to share solutions and ideas that have worked in their laboratories. Participation in ALMA helps analytical managers develop better management skills and practices. You can find more information about ALMA by visiting the ALMA website at http://www.labmanagers.org.

Presently, there are 17 official ALMA members from CT, MA and NH but you do not need to be a member of the national chapter to join the meeting. As managers of analytical labs, we are faced with lots of challenges, lots of opportunities and ever shrinking resources. If you would like to find a local network of professionals facing the same challenges as you then the ALMA local chapter meetings are an excellent opportunity to network, share and learn first-hand about best practices. There are several local chapters already established around the country and we hope that this chapter with all the outstanding laboratories in the area has the potential to be a dynamic and exciting group.

For our first meeting, the following topics are being considered.

- RoHS (Regulation of Hazardous Substances)
- Turn-around time
- Capital
- Out-sourcing

The proposal would be to have a breakfast meeting at Cabot Corp. followed by a round-table discussion of one or two of those topics and then leave sufficient time to allow us to brainstorm for future meetings and the formation of the chapter. Please send us your suggestion for future topics.

Feel free to extend this invitation to fellow analytical lab managers in your company and neighboring companies who may not know about ALMA or our local chapter. Please ***confirm your interest in attending*** by sending an e–mail to Larry or Lynne. Additional details and driving directions will follow. We look forward to meeting with you in this networking and learning opportunity for analytical laboratory managers.

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Summer Research Scholar

Novel Fluorescent Nitric Oxide Sensors

Jacqueline Tio, Mihee Lim, and Stephen J. Lippard
Massachusetts Institute of Technology, Department of Chemistry

Abstract

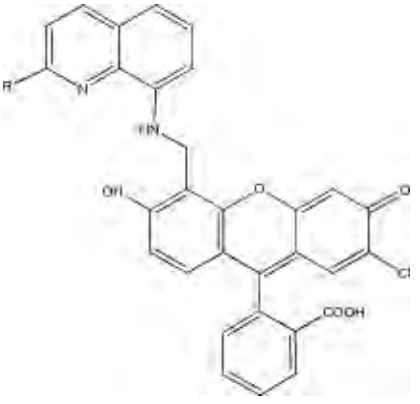
Nitric oxide (NO), widely known as a toxic environmental pollutant, has also been increasingly recognized as an important player in biological systems and been implicated in research concerning cancer therapy, neurobiology, and liver disease. Peroxynitrite ($ONOO^-$), a closely related reactive nitrogen oxides species, has also been implicated in the onset of neurodegenerative disorders. The ability to track NO and $ONOO^-$ would thus provide a conduit to a vast amount of information concerning their roles in biological systems. This project has identified several viable fluorescence-based sensors to detect NO and $ONOO^-$. One series of metal-ligand sensors composed of Cu^{2+} complexed with aminoquinoline-fluorescein-based ligands was studied as an extension of earlier studies using the Fl-AQR series of ligands to detect NO at 25°C. In this study, conducted at 37°C and in aqueous environments, the Fl-AQ-CH_3 ligand showed the most significant detection of NO, and the QZ1 and Fl-AQ-CO_2CH_3 ligands showed the most significant detection of $ONOO^-$. Up to six-fold fluorescence intensity changes of these ligands were observed within 2 minutes of NO injection. This study therefore indicates the viability of pursuing future research with these ligands to detect NO produced *in vivo* rather than NO introduced through gas injection.

Introduction

Nitric oxide (NO), widely known as a toxic environmental pollutant, has also been increasingly recognized as an important player in biological systems. In addition to being known as a vasodilator, it has also been implicated in research concerning cancer therapy, neurobiology, and liver disease.[1-5] Despite the many different roles that nitric oxide has been found to take, it is quite evident that nitric oxide is an important biological signaling agent whose movement and activity impact a wide range of scientific disciplines.

The ability to track nitric oxide would thus provide a conduit to a vast amount of information concerning its role in biological systems. However, past methods to detect NO face several limitations, such as the use of indirect chemical intermediates, irreversibility of the detection method, and the inability to detect NO *in vivo*. Using fluorescence-based sensors to detect nitric oxide would be particularly advantageous because it would allow for direct detection of NO, the use of a reversible sensor, the acquisition of both temporal and spatial information, and compatibility with biological systems.[6] Furthermore, these fluorescence-based sensors may also be able to detect other reactive nitrogen oxide species, such as peroxynitrite ($ONOO^-$), that have also been implicated in the occurrence of neurodegenerative disorders.

This project has explored several different candidates for fluorescence-based sensors composed of various metal-binding and fluorescence components. One series of metal-ligand sensors, known as the Fl-AQR series, was studied and is shown in Figure 1.



QZ1 Family Ligands	
QZ1	R = H
Fl-AQ-CH_2OH	R = CH_2OH
Fl-AQ-CO_2CH_3	R = CO_2CH_3
Fl-Q-CH_3	R = CH_3

Fig 1. Fl-AQR series of ligands.

The Fl-AQR series of ligands is based on an aminoquinoline-fluorescein structure that allows for the formation of a Cu^{2+} metal complex and for the detection of NO. Fluorescein-based sensors are particularly advantageous because fluorescein absorbs at longer wavelengths than other fluorophores, such as dansyl chloride and coumarin -343. As such, excitation of fluorescein to detect NO would not excite proteins and molecules present *in vivo* that respond to ultraviolet radiation. These sensors showed promising results in the detection of both NO and $ONOO^-$ in aqueous environments at 25°C and 37°C. They have inspired the synthesis of 2-[2-chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid, shown in Scheme 1, to further elucidate the mechanism of NO detection by the Fl-AQR series. To make this compound, the synthesis of an asymmetric fluorescein-aldehyde derivative, 7'-chloro-4'-fluoresceincarboxaldehyde, was also undertaken and is shown in Scheme 2.

The successful detection of NO and $ONOO^-$ in aqueous environments has laid the groundwork for further research

to be conducted in biological systems. In mammalian systems, NO is synthesized by nitric oxide synthase (NOS), of which there exists three forms: neuronal NO (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). Future research concerning the Fl-AQR series of fluorescence-based sensors, therefore, can gear studies toward the detection of NO produced in mammalian endothelial cells. This may, for the first time, produce sensors that can detect NO produced *in vivo* rather than NO introduced through gas injection using fluorescence-based sensors.

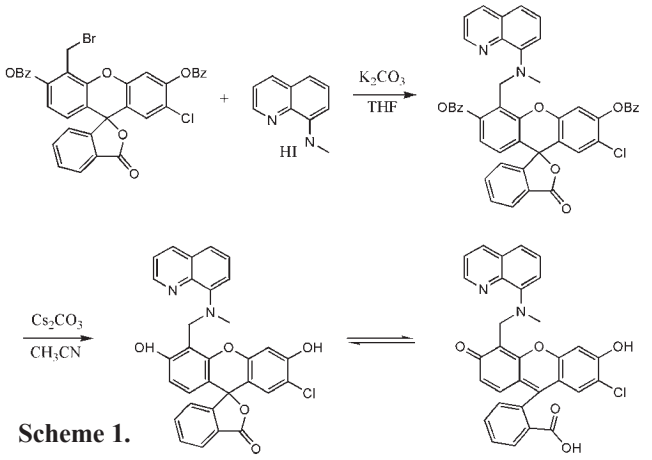
Experimental Synthesis

Preparation of Fl-AQR Series

The Fl-AQR ligands were previously synthesized and acquired from Mi Hee Lim.[7]

Synthesis of 2-[2-Chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid.

The synthesis of the methylated derivative of the Fl-AQR ligands, 2-[2-chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid, is shown in Scheme 1.



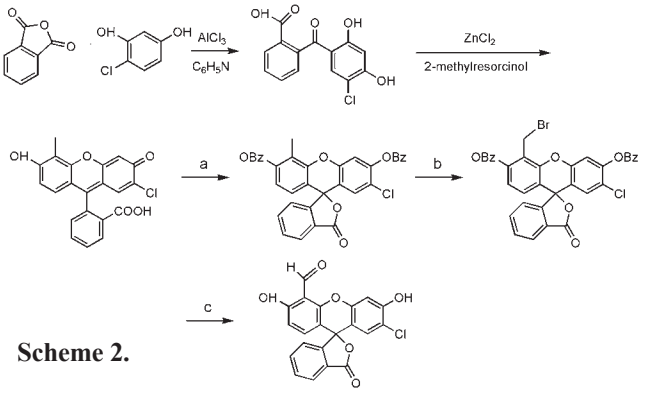
Scheme 1.

2-[2-chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid. 7'-Chloro-4'bromomethylfluorescein dibenzoate (0.10 g, 0.15 mmol) was combined with n-methyl-8-quinolinamine monohydriodide (0.04 g, 0.15 mmol) in 3 ml of tetrahydrofuran in the presence of potassium carbonate (0.10 g, 0.74 mmol). The bright orange solution, with traces of hot pink solid, was stirred and allowed to reflux overnight at 70-80°C. The product was purified by column chromatography on silica gel (2:1 hexanes/ethyl acetate) and deprotected by adding cesium carbonate in 5 ml of acetonitrile. The orange solution was refluxed overnight under nitrogen at 90°C. The product was purified, using a reverse phase chromatography in methanol, to yield a crusty red solid (50 mg, 100% yield), and ^{1}H-NMR was analyzed in $CDCl_3$, CD_3OD, DMF/CD_3OD, DMSO-d_6/NaOD, and C_5D_5N; however, there were no consistently significant ^{1}H-NMR chemical shifts. Calcd for MH^+ 536; Found 537.1.

Synthesis of Precursors

Synthesis of 7'-Chloro-4'-fluoresceincarboxaldehyde

The synthesis of 7'-chloro-4'-fluoresceincarboxaldehyde was adapted from Nolan et al. and Burdette et al. and is shown in Scheme 2.[8,9]



Scheme 2.

(a) Benzoic anhydride, pyridine, 150°C
(b) 1,3-Dibromo-5,5-dimethylhydantoin, VAZO 88, HOAc, chlorobenzene, 40°C
(c) NaHCO3, DMSO, 150°C

2'Carboxy-5-chloro-2,4-dihydroxybenzophenone. Phthalic anhydride (9 g, 61 mmol) and 4-chlororesorcinol (8.5 g, 59 mmol) were combined in 150 ml nitrobenzene to form a tea brown solution that was stirred rigorously and chilled to 0°C in an ice bath under nitrogen. Then aluminum chloride (18.4 g, 138 mmol) was added in three parts over an hour to form a jet black solution. After warming to room temperature and stirring overnight, the solution was poured into 1 L of 1M hydrochloric acid and 300 mL hexanes (85% n-hexane), whereupon a tan precipitate was formed. After stirring for an hour, a light brown solid was collected and air dried. The solid was then recrystallized with boiling methanol and boiling milli-Q water to form very light brown crystals (1.8 g, 11% yield). ^{1}H-NMR (CD_3OD, 300 MHz) ‰ 6.47 (1 H, s), 6.94 (1 H, s), 7.38 (1 H, dd), 7.65 (1 H, td), 7.73 (1 H, td), 8.11 (1 H, dd).

7'chloro-4'methylfluorescein. 2'Carboxy-5-chloro-2,4-dihydroxybenzophenone (3.9 g, 13 mmol) and 2-methylresorcinol (1.6 g) were combined and heated until melted at 300°C, whereupon zinc chloride (1.8g) was added to form a dark red liquid that was allowed to boil for 30 minutes. The solution was cooled to room temperature and pulverized to form a dark red crystalline powder that was poured into 200 ml boiling 1M HCl. The mixture was stirred for 30 minutes, then cooled in an ice bath. A dark orange solid (also some 4',5'-dimethylfluorescein present) was collected with a frit and dried under vacuum (5.0 g, 99% yield). ^{1}H-NMR ($CDCl_3$/CD_3OD) ‰ 2.36 (2 H, s), 2.58 (1 H, s), 6.68 (2 H, m), 6.81 (1 H, s), 7.00 (1 H, s). 7.10-7.39 (2 H, m), 7.60-7.85 (2 H, m) 8.17 (1 H, d).

7'Chloro-4'methylfluorescein Dibenzoate. 7'chloro-4'methylfluorescein (4.8 g, 13 mmol) and benzoic anhy-

Continued on page 10

Summer Scholar

Continued from page 9

dride (13.1g) were combined in 75 ml anhydrous pyridine and refluxed at 150-160°C for 2.5 hours, whereupon the dark red solution was allowed to cool. The cooled solution was then added to 300 mL milli-Q water and stirred for 30 minutes, whereupon the solution was filtered to isolate an orange powder. Water was changed three times and solid collected before allowing the solution to stir overnight. Suspecting more reactants present than product, the orange powder collected (6.0 g) was refluxed again under nitrogen in pyridine for 5 hours. The solution was allowed to cool and poured into 300 mL milli-Q water whereupon water was changed several times over 5 days, each time collecting an orange powder with a glass frit. The orange powder was dried under vacuum, dissolved in a minimal amount of toluene, and washed with ethanol. The solid was washed once more with toluene and ethanol and dried under vacuum to afford a light orange powder (4.0 g, 55% yield) ^{1}H-NMR ($CDCl_3$, 300 MHz) ‰ 2.37 (3 H, s), 6.76 (1 H, d), 6.95 (1 H, d), 7.45-7.60 (4 H, m), 7.60-7.80 (4 H, m), 8.05 (1 H, 2) 8.20-8.25 (4 H, m).

7'-Chloro-4'bromomethylfluorescein Dibenzoate. 7'Chloro-4'methylfluorescein dibenzoate (3.7 g, 6.2 mmol) and 1,3-dibromo-5,5-dimethylhydantoin (1.6g, 5.6 mmol) and 1,1'-azobis(cyclohexanecarbonitrile) (0.17 g, 0.7 mmol) were combined in a flask whereupon 230 ml of chlorobenzene, that was run through an aluminum oxide plug, was added and allowed to stir. Glacial acetic acid (100 ml of glacial acetic acid was added and the solution was heated to 60°C and allowed to stir for five days. The brown solution was extracted with 2 x 200 mL of a warm sodium bicarbonate solution and 2 x 200 mL warm milli-Q water. The tea brown organic layer was filtered through a celite pad, and magnesium sulfate was added to dry the brown solution. The solution was then filtered through a celite pad, and solvent was removed under vacuum in a heat bath to yield a dark, reddish-orange brown oil. The oil was washed three times by dissolving in a minimal amount of toluene and adding ethanol until a solid powder was formed. A pink-peach powder was collected and dried under vacuum (2.7 g, 70% yield). ^{1}H-NMR ($CDCl_3$, 300 MHz) ‰ 4.80 (2 H, s), 6.90 (1 H, d), 7.00 (1 H, s), 7.08 (1 H, d), 7.20 (1 H, d), 7.50 (1 H, s), 7.53-7.60 (4 H, m), 7.65-7.81 (4 H, m), 8.05 (1 H, d), 8.10-8.15 (4 H, m).

7'-Chloro-4'-fluoresceincarboxaldehyde. 7'-Chloro-4'bromomethylfluorescein dibenzoate (1.0 g, 1.4 mmol) and sodium bicarbonate (1.1 g, 13 mmol) were combined in 75 ml of anhydrous methyl sufoxide, stirred rigorously, and heated to 150°C. After three hours, the dark red solution was allowed to cool to 67°C and then poured into 100 ml of 4M hydrochloric acid. The mixture formed a muddy orange precipitate and was allowed to stir overnight. The mixture was then filtered and washed with water to collect a crude orange solid. Column chromatography with silica gel (33:1 chloroform:methanol) was used to isolate a light orange powder (0.2 g, 37% yield). ^{1}H-NMR ($CDCl_3$/CD_3OD, 300 MHz) ‰ 6.56 (1 H, s), 6.60 (1 H, d), 6.65 (1 H, s), 6.82 (1 H, d), 6.88 (1 H, s), 7.13 (1 H, d), 7.60-7.73 (2 H, m), 8.00 (1 H, d), 10.59, (1 H, s).

NO Experimentation

A 500 ml buffer solution of pH 7was made by adding deionized water to 7.56 g (50 mM) of 1,4-piperazinebis(ethanesulfonic acid) and 3.73 g (100 mM) of potassium chloride (KCl). Concentrated sodium hydroxide (NaOH) was added to adjust buffer solutions. The solution was purged under nitrogen for 2 hours before being placed in the glovebox for subsequent use. A 10 mL solution of Cu^{2+} (~1mM) was made from deionized water and $CuCl_2 \bullet H_2O$.

Fluorimeter samples were prepared in 3 ml quartz cuvettes to form 1 µM solutions of the ligand complex. Samples containing metal centers maintained a 1:1 ratio of fluorophore:Cu^{2+} in pH 7 buffer solutions before the addition of NO.

Nitric oxide was obtained as in earlier studies.[10,11] To avoid reactions with O_2, addition of NO (100 microliters, 1 atm) to ligand or metal-ligand solutions by syringe occurred in an anaerobic atmosphere. Fluorescence emission spectra were obtained from a Hitachi F-3010 spectrophotometer at 25.0 ± 0.2°C. Samples were excited at 500 nm, and fluorescence emission spectra were recorded at 37°C.

$ONOO^-$ Experimentation

Fresh solutions of 1 µM peroxynitrite were prepared from diluting 75 ml of 13 µM peroxynitrite with 925 ml of 0.3 M sodium hydroxide. Fluorimeter samples were prepared in 3 ml quartz cuvettes to form 1 µM solutions of the ligand complex in the presence of either 1 or 10 µM PN .

Fluorescence emission spectra were obtained from a Hitachi F-3010 spectrophotometer at 25.0 ± 0.2°C. Samples were excited at 500 nm, and fluorescence emission spectra were recorded at 37°C.

Results and Discussion

NO Reactivity of Fluorescein-Ligands with Cu^{2+} ion

In previous studies the detection of NO at 25 °C proved the viability of using Fl-AQR as fluorescence-based NO sensors.[12] In this study, the viability of using these ligands to detect NO at a more biologically relevant temperature of 37°C was confirmed. The fluorescence spectra of each ligand-metal complex in the NO experiments compared the fluorescence of the ligand, the ligand-metal complex, the ligand-metal complex in the presence of NO, and, as a control, the ligand in the presence of NO. Each NO experiment was repeated three times. When the Fl-AQR ligands complex with Cu^{2+}, the Cu^{2+} metal is paramagnetic and is able to quench the fluorescence of the fluorophore, which was, in this case, fluorescein. In the presence of NO, however, Cu^{2+} is reduced to Cu^+, a diamagnetic metal which is unable to quench the fluorescence of the fluorophore. Thus, fluorescence should decrease in the presence of Cu^{2+} and increase

upon the addition of NO. These trends were confirmed in the study of the Fl-AQ-CH_2OH and Fl-AQ-CO_2CH_3 ligands at 37°C. A summary of the fluorescence increase seen from these ligands previously obtained at 25 °C, as compared to that seen at 37°C, is shown in Table 1. The fluorescence spectra for NO detection with these ligands at 37°C are shown in Figures 2 and 3. These trends were each confirmed by results obtained from two additional rounds of experimentation. NO experimentation with Fl-AQ-CH_3 had already been conducted.[12]

Ligand-Cu2+ Complex (1 µM)	NO (1.36 mM), 25° C		NO (1.36 mM), 37°C	
	Fluorescence increase	Time (min)	Fluorescence increase	Time (min)
QZ1	22.0	20		
AQ-CH2OH	3.4	15	3.5	15
AQ-CO2CH3	1.6	60	2.4	60
AQ-CH3	10.5	6	6.5	2

Table 1. Fluorescence increase of Fl-AQR series of ligands at 25ºC compared to data at 37ºC

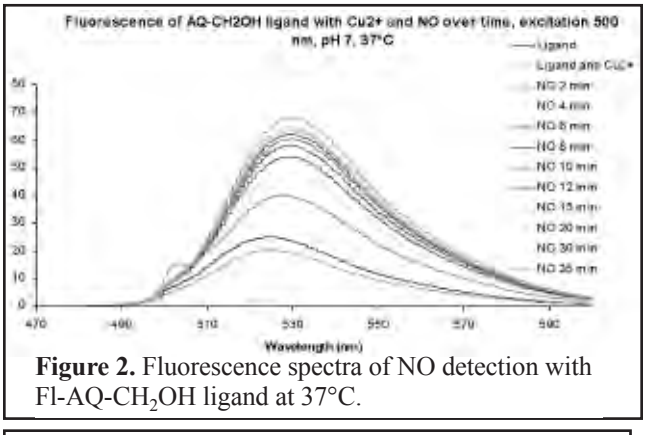


Figure 2. Fluorescence spectra of NO detection with Fl-AQ-CH_2OH ligand at 37°C.

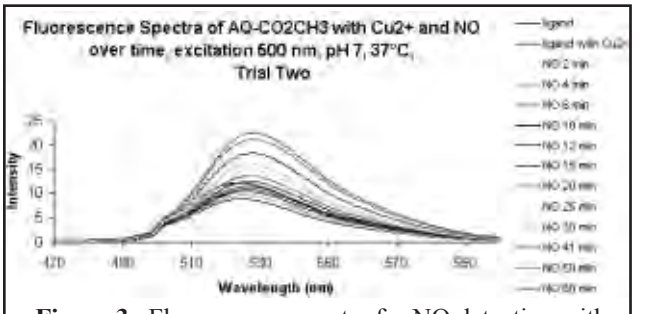


Figure 3. Fluorescence spectra for NO detection with Fl-AQ-CO_2CH_3 at 37°C

These results showed that the most promising ligand in the detection of NO at 37°C, or at body temperature, was Fl-AQ-CH_3. Not only was the fluorescence of the ligand quenched in the presence of Cu^{2+}, but the presence of NO yielded a 6-fold increase in fluorescence seen in just 2 minutes.[12] Nitrosylation of the secondary amine is suspected to be the mechanism of interaction between the NO and the fluorescence-based sensor. More specifically, the hydrogen of the secondary amine bridging the n-methyl-8-quinolinamine group and the fluorescein group is suspected to interact with NO, thus causing the rapid increase in fluorescence by the binding of NO. To test this hypothesis, the synthesis of 2-[2-chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid, a similar ligand with a methyl group instead of the hydrogen on the bridging amine, was attempted. The synthesis of a pure compound has been difficult to characterize, but if synthesized successfully, an NO experiment can be run to confirm whether or not the aforementioned mechanism is correct. Since the hydrogen that is suspected to interact with NO would be replaced by a methyl group, no NO detection should be observed.

Peroxynitrite ($ONOO^-$) Reactivity of Fluorescein-Ligands with Cu^{2+} Ion

The fluorescence spectra of each ligand-metal complex in the $ONOO^-$ experiments compared the fluorescence of the ligand with the ligand in the presence of 1 or 10 µM PN in pH 7 buffer. Results for Fl-AQ-CH_3 had already been collected before.[12] The fluorescence spectra from experiments testing QZ1, Fl-AQ-CH_2OH, and Fl-AQ-CO_2CH_3 indicated the significant changes in the fluorescence of the ligands in the presence of $ONOO^-$. A summary of the fluorescence increase seen by these ligands in the presence of peroxynitrite at 37°C is shown in Table 2. The corresponding fluorescence spectra are shown in Figures 4-6.

Ligand	Fluorescence Peak Relative Intensities, initial changes					
	pH 7, initial changes			NaOH, maximum change		
	Ligand	1 µM PN	10 µM PN	Ligand	1 µM PN	10 µM PN
QZ1	1	1.9	4.9	1	1.2	2.1
Fl-AQ-CH2OH	1	1.4	1.8	1	0.99	1.2
Fl-AQ-CO2CH3	1	1.9	5.4	1	0.96	1.4
Fl-AQ-CH3	1	1.2	5.0	1	1.2	5.9

Table 2. Fluorescence increase of Fl-AQR series of ligands in the presence of peroxynitrite.

To ensure that at pH 7, $ONOO^-$ was forming NO through the mechanism shown below:

$ONOO^-$ $\quad ONOOH \quad \bullet NO_2 + \bullet OH$

a set of control experiments in 0.3M NaOH was conducted. In data not shown, the NaOH experiments suggested that, whereas there was some residual amount of NO coming from $ONOO^-$, the majority of the changes in fluorescence observed occurred at pH 7, in which reactive nitrogen oxide species $\bullet NO_2$ and $\bullet OH$ were most likely the key players.

These $ONOO^-$ detection results showed the most promising sensor to be the QZ1 and Fl-AQ-CO_2CH_3 ligands. Both showed an initial two-fold increase in fluorescence intensity upon the addition of $ONOO^-$ at 1 µM concentrations, thus indicating the possibility for reactive nitrogen

Continued on page 12

Summer Scholar

Continued from page 11

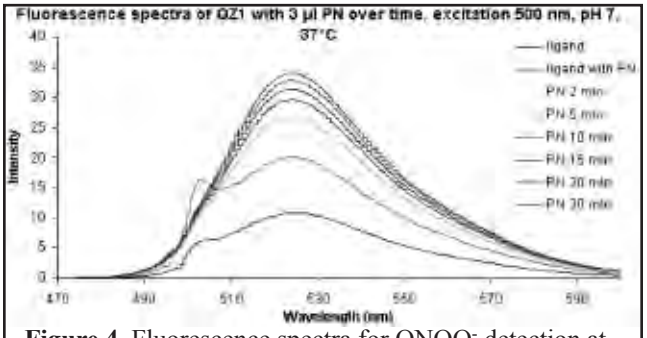


Figure 4. Fluorescence spectra for $ONOO^-$ detection at 1 µM with the QZ1 ligand at 37°C

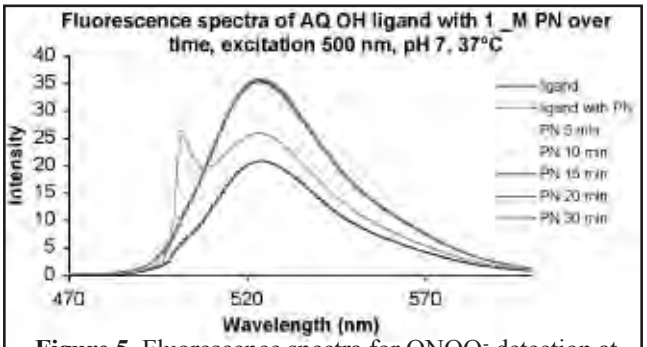


Figure 5. Fluorescence spectra for $ONOO^-$ detection at 1 µM with the Fl-AQ-CH_2OH ligand at 37°C.

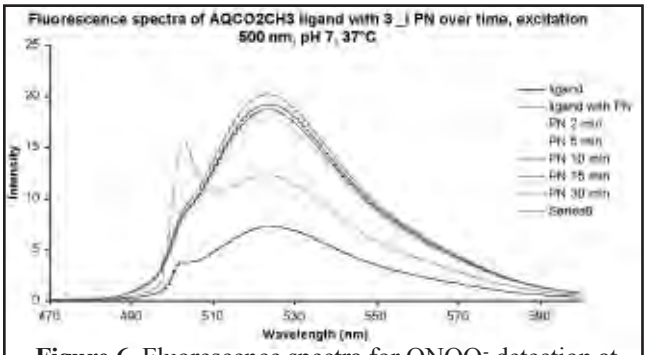


Figure 6. Fluorescence spectra for $ONOO^-$ detection at 1 µM with the Fl-AQ-CO_2CH_3 ligand at 37°C.

oxide species detection on a significantly fast timescale.

Synthesis of 2-[2-chlor o-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid

The synthesis of 2-[2-chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid had been previously attempted.[13] In an effort to progress further in this endeavor, the synthesis of 2-[2-chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid was undertaken for potential use in identification of the NO binding mechanism that was occurring with the Fl-AQR series of ligands. The steps taken in this study are shown in Scheme 1.

The mechanism tested was based on the SN2 substitution of the bromine in 7'-chloro-4'bromomethylfluorescein dibenzoate with the amine group of the n-methyl-8-quinolinamine monohydriodide in a solution of tetrahydrofuran and in the presence of potassium carbonate. The product was then deprotected with 3 equivalents of cesium carbonate in a solution of acetonitrile that was refluxed overnight. This immediately formed an orange solution, from which suspected product was isolated as a crusty red solid in ~100% yield. Although the ESI-MS data confirmed the MH^+ expected for the deprotected product, strong and consistent chemical shift patterns using ^{1}H-NMR proved extremely difficult to obtain in a number of solvents tested. The product itself dissolved well in methanol; however, the resulting NMR data, with the most peaks seen using CD_3OD as a solvent, were not clean enough to verify that the desired product had, indeed, been isolated from its impurities. Therefore, further NO experiments with this compound could not be conducted to verify whether or not the mechanism of NO detection involved the interaction between NO and the hydrogen of the secondary amine bridging the n-methyl-8-quinolinamine group and the fluorescein group in the Fl-AQR series of ligands.

Conclusions

The detection of NO by the Fl-AQ-CH_3 ligand has been shown to be viable in both an aqueous environment and at 37°C. The detection of $ONOO^-$ with the QZ1 and Fl-AQ-CO_2CH_3 ligands has also shown to be viable in aqueous environments at 37°C. Although the incomplete synthesis of 2-[2-chloro-6-hydroxy-3-oxo-5-(methylquinolin-8-ylaminomethyl)-3H-xanthen-9-yl]-benzoic acid has not allowed for NO experimentation that can elucidate the mechanism of NO detection, future synthesis routes may prove to be more successful. The results of this experiment, however, indicate a promising future for further research focusing on the detection of biologically produced NO or $ONOO^-$ with the Fl-AQ-CH_3 ligand and $ONOO^-$ detection with the QZ1 and Fl-AQ-CO_2CH_3 ligands.

References

1. Furchgott, R. F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1870-1880.
2. Ignarro, L. J. *Angew. Chem., Int. Ed.* **1999**, *30*, 1882-1892.
3. Lala, P. K. *Cancer Metastasis Rev.* **1998**, *17*, 1-6.
4. Prast, H.; Philippu, A. *Prog. Neurobiol.* **2001**, *64*, 51-68.
5. Shah, V.; Kamath, P. S. *Liver Transplantation* **2003**, *9*, 1-11.
6. Hilderbrand, S. A.; Lim, M. H., Lippard, S. J. "Fluorescence-based Nitric Oxide Detection."
7. Lim, M. H.; Lippard, S. J., unpublished results.
8. Nolan, E.; Burdette, S. C.; Harvey, J. H.; Hilderbrand, S. A.; and Lippard, S. J. *Inorganic Chemistry.* **2004**, *43*, 2624-2635.
9. Burdette, S.C.; Frederickson, C. J.; Bu, Weiming, and Lippard, S. J. *J. Amer. Chem. Soc.* **2003**, *125*, 1778-1787.
10. Hildebrand, S. A.; Lim, M. H.; Lippard, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 4972-4978.
11. Lorkoviç, I. M.; Ford, P. C. *Inorg. Chem.* **2000**, *39*, 632-633.
12. Lim, M. H.; Lippard, S. J., unpublished results.
13. Nolan, E.; Lippard, S. J., unpublished results. ◇

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Massachusetts Institute of Technology

Continued from page 4

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Historical Notes

Henry Brown, M.D.

Continued from page 2

Cambridge, England, researching the biochemistry of wound healing and the anatomy of cranial nerves. A colleague describes those studies as leading to important publications. He spent four summers in Liberia doing reconstructive surgery for leprosy patients. He published three books and 90 peer-reviewed articles. He even found time to run twenty-five Boston Marathons.

Besides his wife, Julia, Henry leaves his five children, Dana, Theodore, Rodney, Jennifer and Gregory, thirteen grandchildren, one great grandchild, and two sisters. He also leaves a big hole in the hearts of the many people he helped with advice, information and support. M.S.Simon

Nathan N. Crounse, Ph.D. (1917-2005)

Dr. Nathan N. Crounse died in Myrtle Beach on January 30, 2005, at the age of 87. During the last years of his life he lived in Belmont, MA, and was a member of the Northeastern Section.

He was born in Omaha, Nebraska, attended grammar school and Omaha Central High School. He received his bachelor degree in chemistry in 1938 from Iowa State University and his doctorate in organic chemistry from the University of Iowa in 1942. In 1939, he married Margaret Burkey, also an organic chemistry graduate student at Iowa, whom he met and courted in the laboratory.

Dr. Crounse began his career at Hilton-Davis Chemical Company in Cincinnati, but resigned in 1947 to do antimalarial research at Christ Hospital Institute for Medical Research. In 1951 he returned to Hilton Davis, by then part of Sterling Drug, and in 1963 became Director of Chemical Research at Hilton- Davis.

He published extensively, contributed a chapter to Organic Reactions, and, was inventor or co-inventor of eighty US patents. He was the inventor of the red pigment used on the cover of Time magazine, as well as whiteners in laundry detergents and the production process used in the manufacture of NCR carbonless paper.

He chaired the Cincinnati Section of ACS in 1954, coached a Knothole League baseball team, and became an avid golfer. He is survived by his wife Margaret, two daughters, Cynthia Smith of Belmont, MA and Elizabeth Grippe of White Plains, NY, six grandchildren and three great-grandchildren.

MSS ◇

Call for Applications

Continued from page 6

Application: Application forms are available on the NESACS web site at http://www.nesacs.org. Completed applications are to be submitted no later than April 7, 2006, to the Chair of the Selection Committee:

Professor Edwin Jahngen
University of Massachusetts Lowell
Chemistry Department, Room 520,
Olney Hall
1 University Avenue
Lowell, MA 01854-5047

Notification: Applicants will be notified of the results by e-mail on April 26, 2006 with written confirmation to follow. ◇

NESACS Board Update

A number of changes have occurred in the NESACS board. These changes were approved at the January board meeting. David Warr will fill the alternate councilor position vacated when Michael Singer was elected councilor. 2004 Chair Jean Fuller-Stanley has resigned from her position as councilor to be replaced by alternate councilor, Pamela Nagafuji. Susan Chiri-Buta has resigned from her alternate councilor position. The two vacant alternate councilor positions will filled by Mary Mahaney and Mark Froimowitz. These changes are all noted on page 3. In addition, Dr. Harry Mandeville will fill the Director-at-Large position vacated by the death of Dr. Henry Brown ◇

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Calendar

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Note also the Chemistry Department web pages for travel directions and updates.
These include:
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http://www.unh.edu/chemistry/seminars.html

March 1
Dr. Bryant C. Nelson (NIST, Gaithersburg, MD)
"Folate Deficiency - An Emerging Cardiovascular Disease Risk Factor"
Northeastern Univ., 129 Hurtig Hall,
12 noon

March 2
Prof. Rick Geier (Colgate University)
"Synthesis of Porphyrinic Macrocycles with Altered Core Structures"
Brandeis University, Gerstenzang 122
3:30 pm

March 6
Prof. Oliver Steinbock (Florida State University)
"Self-organization in Nonequilibrium Systems"
Brandeis University, Gerstenzang 122
3:30 p.m.

March 7
Russell Petter, Ph.D., Biogen Idec
"Discovery and Development of a New Adenosine A1 Receptor Antagonist"
Organic Chemistry Seminar
Boston College, Merkert 130, 4:00 PM

March 13
Prof. Soumya Ray (Harvard Medical School)
Title: TBA
Brandeis University, Gerstenzang 122
3:30 p.m.

March 14
Gary Weisman, Ph.D., University of New Hampshire
"Cross-Bridged Tetraamines: Synthesis, Coordination Chemistry, Biomedical Utility, and Conformational Contortions"
Organic Chemistry Seminar
Boston College, Merkert 130, 4:00 PM

March 15
Dr. Timothy Logan (Florida State University, Tallahassee FL)
"Mechanisms of Metal Activation in Diphtheria Toxin Repressor"
Northeastern Univ., 129 Hurtig Hall,
12 noon

March 16
Glenn D. Prestwich, Ph.D., University of Utah
"Affinity Probes for Visualizing and Manipulating Phosphoinositides and Phospholipids in Cells"
Organic Chemistry Seminar
Boston College, Merkert 130, 4:00 PM

March 20
Prof. Arthur Johnson (Texas A&M)
"Protein Trafficking at the ER Membrane"
Brandeis University, Gerstenzang 122
3:30 p.m.

March 21
David Sherman, Ph.D., University of Michigan
"Chemical Diversity and Enzymatic Versatility of Microbial Natural Product Systems"
Chemical Biology Seminar
Boston College, Merkert 130, 4:00 PM

March 22
Dr. Plamen Atanassov (University of New Mexico, Albuquerque NM)
"Electrochemical Biosensors Based on Direct Electron Transfer"
Northeastern Univ., 129 Hurtig Hall,
12 noon

March 27
Prof. Kenneth Showalter (West Virginia University)
"Collective Behavior in Addressable Excitable Media"
Brandeis University, Gerstenzang 122
3:30 p.m.

March 29
Dr. Vivek Murthi (Case Western Reserve University, Cleveland OH)
Dept. of Chemistry & Chemical Biology Graduate Student Research Award Winner
"Alkaline Fuel Cells: Current Challenges and Potential Routes for Future Technological Viability"
Northeastern Univ., 129 Hurtig Hall,
12 noon

Notices for the Nucleus Calendar FOR MARCH 2006 AND THE FOLLOWING MONTHS should be sent to:
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