SOAR Research Proposal – Summer 2019

Interstrand Cross-linking of DNA by Novel Rhodium Complexes

Faculty: Shari U. Dunham, Ph.D., Associate Professor of Chemistry
Student: Megan Konrath (GPA 3.75)
Project Start Date: June 3, 2019
Length of Project: 10 weeks

Description of the project
Interstrand Cross-linking of DNA by Novel Rhodium Complexes

DNA damaging agents come in all shapes and sizes. Some are small inorganic compounds like cisplatin that contain less than a dozen atoms, others are organic compounds like those in mustard gases that contain about 20-30 atoms, while yet others are large organic natural products like bleomycin that are comprised of almost 200 atoms. The DNA damaging ability of these three compounds, as well as many other DNA damaging agents, is exploited in therapies to kill cancer cells. Research in our laboratory focuses on small inorganic complexes that contain two atoms of the metallic element rhodium at their core, and investigates their ability to damage double-stranded DNA. Our ultimate goal is to screen for DNA damage to identify potential new rhodium-based anticancer agents.

Not all DNA damage is created equal. Some agents cause breaks in the DNA strands while others bind directly to the DNA and "stick" there. Previous work in our laboratory has indicated that the rhodium compounds we study "stick" to DNA by forming covalent adducts[1] instead of causing strand breaks. These rhodium compounds can covalently bind to just one strand of the DNA double helix or, in some cases, they can "reach out" and bind to both strands of the same DNA helix causing what we refer to as an interstrand cross-link. Interstrand cross-links are particularly toxic forms of DNA damage, as few of the DNA repair mechanisms in cells can correctly repair these adducts.

In the past, we have measured the amount of interstrand cross-linked damage sites on a DNA duplex after treatment with a limited number of rhodium compounds[1] but by using a radioactive DNA probe. In the summer of 2016, with former student Swathi Kanakamedala ('17), we started to adapt these kinds of experiments to the non-radioactive DNA probes and laboratory resources we have available here at Moravian College. Our goal was to use non-radioactive methods to quantitate the amount of DNA interstrand cross-links formed by a variety of novel dirhodium compounds synthesized in the laboratory of Dr. Stephen Dunham.[2]

Swathi started these interstrand cross-linking experiments with the same large (>100 bp) double-stranded DNA probe we had previously used, but without a radioactive label. By the end of Swathi's honors project, we had designed a 39-bp DNA probe that was easier and cheaper to obtain

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[1] S.U. Dunham, A.E. Burr, S. Mikulski, H.T. Chifotides and K.R. Dunbar, "Covalent Binding and Interstrand Cross-Linking of Duplex DNA by Dirhodium(II,II) Carboxylate Compounds", *Biochemistry* 44(3), pp 996-1003 (2005).
[2] S.U. Dunham, T.S. Remaley, B.S. Moore, D.L. Evans and S.U. Dunham, "Isolation, Characterization, and DNA Binding Kinetics of Three Dirhodium(II,II) Carboxyamidate Complexes: $Rh_2(\mu\text{-}L)(HNOCCF_3)_3$ where L= $[OOCCH_3]^-$, $[OOCCF_3]^-$, $[HNOCCF_3]^-$", *Inorganic Chemistry*, **50**, pp 3458-3463 (2011).

and imaged well with a non-radioactive fluorescent stain that was applied after gel electrophoresis.[3] This work was continued by Ariana Caiati ('19) starting in the winter of 2018 and, unfortunately, she determined that the fluorescent stain does not image rhodium-modified DNA very well. As a result, we permanently attached a different fluorescent beacon directly to the 39-bp DNA probe, such that the DNA is highly fluorescent before any reaction with metal compound. Recently, Ariana has used denaturing gel electrophoresis to successfully separate and quantitate interstrand cross-links formed by control platinum compounds on this fluorescent 39-bp DNA probe, using only small amounts of the probe and the imaging facilities here at Moravian College. Before the end of this spring semester (2019), we anticipate that Ariana will have used this same method to analyze the DNA interactions of the two rhodium compounds that were reported in our original radioactive experiments.[1]

This summer, with Megan Konrath ('20), we plan to use Ariana's DNA probe and electrophoresis method to survey, quantitate, and summarize the interstrand cross-linking efficiency of a number of novel rhodium compounds that have been synthesized here at Moravian College. For some of these rhodium compounds, we have recently collected cytotoxicity data (Jared Miller '19) that indicates how well they can kill human cancer cells (in an ongoing collaboration with Dr. Anastasia Thévenin). Megan's goals for the summer are (1) to react the fluorescent 39-bp DNA probe with a variety of novel rhodium compounds, (2) to screen these reactions for formation of interstrand cross-links, and (3) to determine if there is a correlation between the nature of the 4 bridging groups around the rhodium core and the DNA interstrand crosslinking efficiency. We have also discussed an extension of this project with Megan for next year ('19-'20) and hope to include some cell culture training with Dr. Thévenin before the end of the summer. Ultimately, our research goal is to determine the extent of DNA interstrand cross-links formed by each rhodium compound in the series and to use this information, along with DNA-binding rates, DNA unwinding angles, and cell toxicity studies, to identify the most likely potential candidates for new chemotherapy treatments.

Roles and Responsibilities

- The project director (Shari Dunham) will be available regularly to train Megan to prepare and quantitate stock solutions of the DNA probe and the metal compounds, to form and purify metal-modified DNA samples, to pour denaturing polyacrylamide gels and perform gel electrophoresis of metal-modified DNA samples, to image these gels using the BioRad imager and ImageQuant software, and to appropriately disposal of all materials used in the laboratory. The project director will also oversee Megan's data analysis in Excel and visual representations of gel results in Microsoft Word or PowerPoint.
- The project director will work with Megan to make plans to identify appropriate experiments and appropriate contact faculty for those occasions when the project director will be out of town.
- In addition to attending and occasionally presenting at weekly SOAR program meetings, Megan will prepare and present at approximately biweekly meetings (~15-30 min) with the project director.
- Megan will maintain a research laboratory notebook that will include regular and complete entries and have an updated table of contents at the beginning. Entries will be dated, clearly written and organized, and made at least daily with details of ideas for experiments,

[3] S. Kanakamedala, "Interstrand Crosslinking of Duplex DNA by Dirhodium Compounds", Moravian College, Honors Thesis in Biochemistry, May 2017.

planning of experiments, clear reference to location and organization of electronic data for each experiment, and a summary of results from each experiment. The project director will look at Megan's notebook periodically and provide informal feedback throughout the summer. The notebook will be submitted to the project director upon completion of the Summer Research.

- Throughout the summer, Megan will prepare a summary figure for each set of experiments (with a detailed figure caption!) to clearly illustrate the results. These summary figures will be submitted electronically to the project director before completion of the Summer Research.
- Megan will be encouraged to present the results of her summer work in either a poster or talk format at the Landmark Conference for Science Research near the end of the summer (location and date TBD). If Megan does not continue this research into the 2019-2020 academic year, she will be required to submit a draft of a research report on her work before the first day of classes in the fall. If Megan does continue this research in some form during the 2019-2020 academic year, a final report/poster will instead be due by the end of the experience and in time for the Annual Student Scholarship and Creative Endeavors Day in spring of 2020.

Project Timetable

- ☐ Weeks 1-2: Safety training, lab orientation, pipettor calibration check, buffer preparation, preparing solutions of rhodium compounds, measuring Rh concentrations by GFAAS, preparing DNA solutions/dilutions, measuring DNA concentrations and purity by UV-Vis, checking size and purity of DNA samples by gel electrophoresis (preparing, running, staining, imaging, preparing a gel results Figure).
- ☐ Week 3: Anneal more DNA duplex for metal modification reactions. Carry out reactions between one new rhodium compound and the DNA duplex at different concentrations of rhodium compound. Continue training on the Atomic Absorption Spectrometer (AAS) to quantitate the total amount of rhodium that has bound to a DNA sample.
- ☐ Week 4: Prepare and run a denaturing polyacrylamide gel of the metal-DNA reactions from Week 3 to assess interstrand cross-link formation. Be trained on how to use the ImageQuant software to quantitate the amount of DNA cross-linking in the sample. Prepare an annotated gel Figure to illustrate how interstrand crosslink formation changes with the concentration of rhodium compound. Research and perform calculations that indicate the percent of adducts that are interstrand cross-links for this rhodium compound.
- ☐ Weeks 5-6: Carry out reactions between another rhodium compound DNA duplex at different concentrations of rhodium compound. Use the AAS to measure the amount of rhodium that has bound to DNA (with the goal of becoming more independent on the instrument). Prepare and run a denaturing polyacrylamide gel of these new metal-DNA reactions to assess interstrand cross-link formation.
- ☐ Weeks 7-8: Meet with the project director to review the results so far. Choose at least two more novel rhodium compounds and react them with the DNA duplex at a variety of rhodium compound concentrations. Prepare and run a denaturing polyacrylamide gel of these metal-DNA reactions to assess interstrand cross-link formation. Prepare an annotated gel Figure to illustrate how interstrand crosslink formation changes with the concentration of rhodium compound.
- ☐ Week 9: Cell culture training with Dr. Anastasia Thévenin in preparation for cytotoxicity and cell permeability experiments (with human cancer cells).

- ☐ Week 10: Perform any necessary replicate measurements, complete final edits on annotated gel figures for presentation, prepare a summary table of comparison results, complete lab clean up and appropriate cataloging of samples and reactions.

Summary of benefits

Student engagement in discipline-appropriate scholarly research. Megan will be engaged in biochemistry laboratory research that includes reading and decoding primary references for laboratory methods, planning and performing experiments that require the biochemistry lab techniques and several new instrumental methods, and collecting/analyzing/organizing significant amounts of electronic data. The project director will help Megan present her results in visual representations that are clear and well-captioned. Hopefully, Megan will be working on this project in the presence of other peer researchers, and that research environment is an important experience that is the most representative of a medical research environment and is difficult to simulate during the semester when few students work on independent study projects on different days. The project director will also work with Megan to prepare a scientific poster or talk for presentation of this work at a local and/or a national conference: examples of such conferences are the American Chemical Society meeting in April 2020 (Lehigh Valley Chapter), the National Conference on Undergraduate Research in April 2020, and the more selective Gordon Research Conference on Metals in Medicine which meets biannually and is scheduled to meet in the Summer of 2020 (this conference only recently started accepting undergraduate attendees).

Impact on faculty, campus community, and discipline. For many of the rhodium complexes, our previous work has already determined DNA-binding rates and DNA-unwinding angles. Megan's contribution will benefit the Dunham laboratories by adding a measure of DNA interstrand crosslink formation to the list of characteristics of these potential antitumor agents. When combined with data on cytotoxicity (a project in collaboration with Dr. Anastasia Thevenin in the Department of Biological Sciences), these contributions will allow us to start to predict (and design!) the most important changes to bridging groups in the rhodium compounds that lead to potential for treating cancer. Megan will be encouraged to present her work at local and national meetings and she will be required to present her results during the Annual Student Scholarship and Creative Endeavors Day in spring of 2020.

Proposed Expenses (beyond stipends)

$250 Custom oligonucleotides, 0.2 μmole scale. Additional samples of each of the purified DNA strands (39 bases) may be needed for the reactions this summer.

Urea, TRIS, Boric acid, EDTA (I need to check our stocks of these!)

(I have enough acrylamide)

$150 Spin filters (3kD, 1.5 mL, to purify metal-modified DNA for gel electrophoresis)

$400 Total laboratory expense request

Interstrand Cross-linking of DNA by Novel Rhodium Complexes

Project Title: Interstrand Cross-linking of DNA by Novel Rhodium Complexes
Student: Megan Konrath
Major: Biochemistry
Projected Graduation Date: May 2020
Faculty Mentor: Dr. Shari U. Dunham, Associate Professor of Chemistry
On-Campus Housing Requested: Yes

Participation Rationale and Expected Outcomes:

I am enthusiastic about participating in a SOAR project in which I will be working with novel rhodium complexes to determine how efficiently they target DNA and to what extent the complexes form interstrand cross-links in DNA. When I came to Moravian in Fall of 2016, I already knew what I wanted to commit the rest of my life to: treating children with cancer. In 2007, I was diagnosed with Acute Lymphoblastic Leukemia. With this one diagnosis, I embarked on a lifelong journey and, throughout that journey, met many people who have irrevocably changed my life. Unfortunately, some of the friends I found during my treatment (specifically fellow patients) passed away from metastasized tumors. They have inspired me to want to perform research to test possible cancer-reducing agents and drugs, in hopes of one day saving others. Participating in the SOAR project with Dr. Shari U. Dunham would provide a very hands-on experience in precisely this field, as well as a sense of independence that I could not receive in a lab for a regular one-credit course at Moravian College.

This experience will expand my thinking skills and research ability, and it will help to enhance my laboratory skills. I could possibly use the techniques and learned skills in the future at Medical School or in my line of work as a pediatric oncologist. I expect this research will lead to a better understanding of a way to treat cancer with novel rhodium complex drugs. I also expect it will give myself and future researchers a better understanding as to how the novel rhodium complexes affect and target DNA. As a future medical professional, this experience will allow me to understand the mode of action of drugs and gain an appreciation for the synthesis side of medicine, as I will be the person prescribing the drugs. Ultimately, our goal is to determine the extent of DNA interstrand cross-links formed by each rhodium complex and to use this information to identify the novel rhodium complexes that have the greatest potential to become new chemotherapy treatments. Hopefully I can continue an extension of this work on cells under the advisement of Dr. Anastasia Thévenin and Dr. Shari U. Dunham in the 2019-2020 academic year.