**Part I: Preliminary Information**

**Synthesis, Characterization, and Bioactivity of Vanadium Based Phosphonate Antibiotic Analogues**

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**Abstract:** The discovery of the first antibiotics in the early twentieth century was a breakthrough in biomedical science, within decades once deadly infections had become manageable. Since then the overuse of these treatments has led to an epidemic of microbial resistance, because of this there is a growing interest in developing new antibiotics that are capable of overcoming the modes of resistance of tolerant organisms.1 Interest in this area has led to research into the potential of organometallic molecules for their antibiotic properties, these molecules utilize metallic elements including palladium, platinum, rhodium, and others.2 Vanadium has been shown to have therapeutic properties in the form of oxovanadium complexes for the treatment of diabetes.3 Despite this, vanadium has been relatively underutilized in the area of antibiotics. This project focuses on the synthesis and evaluation of a vanadium based organometallic analogue of the known phosphonate antibiotic fosmidomycin, with an aim towards retaining its bioactivity, while overcoming known modes of pathogen resistance.

**Personal Statement:** Ever since I was young I have always been curious about how the world worked around me. One of my earliest memories of genuinely being excited about science was when I was a kid, my parents had gotten me a microscope for my birthday. As my Dad struggled to open the packaging on the kitchen table he slipped and cut himself with the knife. Before he could tell me to grab something for the blood, I was already picking up what I thought he needed. When I rushed back to the table my Dad reached for what I brought him, but instead of finding a cloth or a bandage like he expected he looked down to see me eagerly holding up a slide and a cover slip so he could donate a sample.

As I grew older and began taking challenging classes that curiosity never really diminished. As I progressed through adolescence my curiosity morphed it a fascination with research. Throughout high school and into college I found the classes I enjoyed the most involved challenging labs where I was given little direction and plenty of freedom to explore what interested me. During my freshman year at Elon the most memorable class I took was the general chemistry II SP lab with Dr. Matera. In her lab we were tasked with designing and preforming our own enzyme kinetic assay. The frustrating process of trial and error involved in executing one of these assays not only prepared me for more advanced labs at Elon, but also allowed me to develop valuable skills regarding designing and implementing my own experiment. In addition, one of the most valuable lessons I learned in classes like this was that nothing goes as planned and that failure was just a sign that I needed to approach the problem from a different direction.

This understanding helped me during my internship at Bausch & Lomb. I worked in the microbiology lab. One of my jobs was collaborating with an engineer to design and implement a digital imaging based colony counting system. I ran into problems almost immediately when trying to run the software I needed on my company issued laptop. I was faced with the possibility of scrapping the project all together; this seemed unacceptable to me. After days of following dead ends, I noticed in some of the back hallways of the R & D department there were scattered pieces of old computers. Knowing this was my last shot at being able to make any progress I grabbed the best pieces I could find until I had all the parts of what would hopefully be a functional computer. I brought all these up to the lab and after a few software installations my relatively ancient computer was doing the job that my brand-new laptop could not. By the end of the day I was producing useful data and finally making real progress toward the task at hand.

All of these experiences gave me the opportunity to succeed where I was right, to fail when I was wrong, and to approach problems like a scientist. As a biochemistry major I have always been excited to pursue a future in research. I have recently begun research under the guidance of my mentor Dr. Dabrowski and I am learning valuable laboratory techniques in the area of inorganic synthesis and characterization. I hope that the Lumen prize will help me to maintain this path by allowing me to pursue my research interest in the laboratory and at conferences.

**Part II: Project Description**

**Focus:**  The increasing prevalence of pathogen resistance against some of the most commonly used antibiotics has become an ever more pressing issue in recent years. The rate at which new resistant organisms are being discovered is particularly troubling. These discoveries have led to a greater interest into the development of novel antibiotics that are less prone to being rendered ineffective due to pathogen resistance.4 Recent research explores the possibility of using inorganic elements, such as palladium, platinum, and rhodium, to derive organometallic analogues of known antibiotics in order to accomplish this.2 This project builds on this endeavor and focuses on synthesizing an analogue of the antibiotic and antimicrobial drug fosmidomycin, illustrated in (figure 1A) along with the proposed analogue, an oxovanadium compound (figure 1B). Fosmidomycin has been shown to be effective against gram-negative and gram-positive bacteria, as well as eukaryotes such as *P.falciparum* which is responsible for malaria. It is because of this wide range of activity that an effective analogue of this molecule would be particularly valuable against the current trend of pathogen resistance.

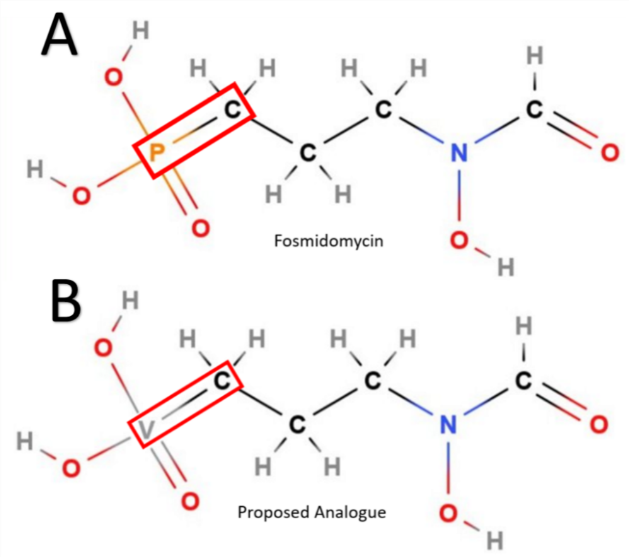
 In recent years there have been many advances in the understanding of phosphonate antibiotics, their modes of action, the mechanisms of pathogen resistance, as well as attempts to develop new organometallic antibiotics by incorporating metallic elements, *vide supra*, into natural models such as penicillin and platensimycin.5 Fosmidomycin functions by competitively inhibiting the reaction between a specific enzyme and substrate. In the case of these target organisms the system of interest involves the enzyme 1-deoxy-D-xylulose-5-phophate reductoisomerase (DXR) and the substrate deoxy-xylulose phosphate (DXP) in the MEP biosynthetic pathway found in a wide range of pathogenic organisms.6 This project will focus on a specific mode of resistance in which the enzyme and substrate of the biosynthetic pathway is upregulated so as to outcompete the antibiotic, fosmidomycin.7 Previous reports of oxovanadium complexes in the treatment of diabetes indicates these compounds have a greater affinity for the PTP1B protein, the key enzyme involved in the regulation of the insulin pathway in humans.8 Thus, a vanadium containing analogue of fosmidomycin may behave similarly with the DXR protein of the target pathogen to overcome the increased regulation of resistant organisms.9 The process of synthesizing, characterizing and testing the bioactivity of the analogue will provide insight into the effects of the modification of natural antibiotics through the introduction of inorganic atoms such as vanadium and allow a greater understanding of the viability of this process as a means to develop new organometallic antibiotics that replicate the bioactivity of the natural molecule, while overcoming modes of resistance known to be present in the target organism.

Figure 1. A. The antibiotic Fosmidomycin. B. The proposed vanadium based analogue. Boxed in red are the phosphorous-carbon (P-C) and the vanadium-carbon (V-C) bonds whose role in the bioactivity of the molecule will be investigated

**Scholarly Process:** The development of vanadium based antibiotics requires a diverse range of techniques with regards to the three main stages of the project: synthesis, chemical characterization, and antibiotic susceptibility testing.

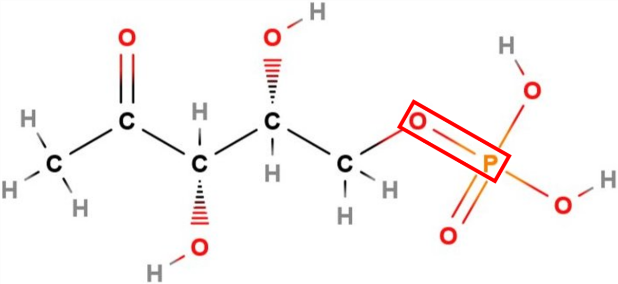
 The major structural component that allows fosmidomycin to function as an antibiotic is the relatively stable phosphorous-carbon bond (P-C fig. 1A); unlike the phosphorous-oxygen bond of the substrate DXP (P-O fig. 2), the enzyme DXR is unable to cleave this bond, effectively inhibiting the reaction. The first stage of the process will involve creating a vanadium-carbon bond (V-C, fig. 1B) in the organometallic analogue that mimics the phosphorous-carbon bond found in the antibiotic (P-C, fig. 1A). A combination of organic and inorganic synthesis techniques are required to produce the desired organometallic complex. The synthesis of the complex will consist of coupling the carbon fragment of fosmidomycin with vanadium through formation of a vanadium-carbon bond based on the techniques described by Preuss and Ogger.10 The carbon fragment itself will be created based on the procedure described in Sparr’s work on the synthesis of fosmidomycin.11

Figure 2. The substrate DXP. The labile Phosphorous-oxygen bond (P-O) is boxed in red.

The second stage involves spectroscopic analysis to validate the structure and evaluate bonding features between the vanadium and carbon in the analogue as compared to phosphorous and carbon in the original antibiotic. The initial part of this stage requires that the synthesized molecule be isolated and purified by thin layer chromatography before further tests are performed. Once the product has been purified it will be characterized using spectroscopic techniques including, infrared, UV-visible, nuclear magnetic resonance (NMR), as well as X-ray crystallography. There are relatively few known vanadium complexes with carbon-vanadium bonds, so a fundamental assessment of their properties and stability will be valuable before comparing the analogue to the phosphonate drug.10

After the desired compound has been synthesized and characterized it will have to be tested for antibiotic activity. I will compare the vanadium analogue, commercially available vanadium salts (e.g. VOCl3, VO(acac)2) the original phosphonate drug, fosmidomycin, and a control broad spectrum antibiotic (kanamycin) in a Gram negative (*E. coli*) and Gram positive (*B. subtilis*) model systems.12 Once these cultures are prepared, I will investigate the biological activity using biochemical and antibiological tests. These tests will include paper disc diffusion assays, turbidity assays, fluorescence assays, and dilution tests.13, 14 Since fosmidomycin is also an effective antimicrobial agent to treat malaria and an effective treatment against tuberculosis, another potential goal of the project will be to test the analogue with eukaryotic organisms such as *P. falciparum,* along with the bacteria responsible for tuberculosis, *M. tuberculosis* using the same biological tests. These studies will quantify the biological activity of the synthesized organometallic analogue and compare it to the model organic phosphate molecule.

In tandem, enzyme kinetic tests similar to those I performed in SP lab will be utilized to compare to the vanadium-based mimic molecule to the original fosmidomycin by analyzing the interactions between the antibiotic and the target enzyme, (DXR), utilizing UV/vis spectroscopy, as well as spectroscopic enzyme kinetic assays with natural substrate (DXP)15. This enzyme and the related substrate are both vital components of the MEP biosynthetic pathway that fosmidomycin inhibits as a means to limit pathogen viability.6 Consequently, these molecules are vital to evaluating the behavior of the vanadium analogue as compared to the original antibiotic, fosmidomycin.

**Proposed Products:** At the end of each semester of CHM 499 a formal, journal style, lab report will be written so that I can report my progress to my research mentor and receive feedback. Furthermore, I will attend multiple local, regional, and national conferences so that I may present my research to others in the field and receive feedback. These conferences include the Southeast Regional Meeting of the American Chemical Society (SERMACS) conference, the National American Chemical Society (ACS) conference, as well as Elon’s Spring Undergraduate Research Forum (SURF).

An initial goal will be to present a poster at SERMACS after the first two semesters of CHM 499. This will be followed by the presentation of a formal research report at the national ACS conference as well as Elon university’s SURF in the spring of my junior year. Additionally, when enough substantial results have been generated, a manuscript will be prepared for submission to the Journal of Inorganic Chemistry, the Journal of Biological Chemistry, or the Journal of Inorganic Biochemistry with the goal of publishing a formal scientific research paper on the project.

**Part III: Feasibility**

**Feasibility Statement:** Many of the resources needed for this project are not immediately available and will need to be purchased. These supplies include reagents for synthesis, purification, and characterization (e.g. NMR solvents) as well as access to UNC’s X-ray analysis lab for characterization intrumentation not available at Elon. The purchase of microbiological supplies, (i.e. agar, petri dishes, assay kits, ect.) as well as relevant organisms and enzymes will make examining the biological activity possible through antibiotic and enzyme kinetic tests.

A potential obstacle manifests from the structure of the phosphonate molecule, fosmidomycin itself. The phosphonate antibiotic family gains its antibiotic function via the relatively stable phosphorus-carbon bond (P-C, fig. 1A) as compared to the more labile phosphorus-oxygen bonds (P-O, fig. 2) of the substrate molecules they outcompete.7 While the synthesis of these phosphorus-carbon bonds are well established, the synthesis of this particular vanadium-carbon (V-C, fig. 1B) may prove to be much more difficult due to significantly less precedence in literature for its preparation.10 If attempts to synthesize these vanadium-carbon bonds proves to be too difficult a different route can be explored by focusing on replicating the phosphorus-oxygen bonds of the substrate with vanadium. The synthesis of vanadium-oxygen bonds is much more established and well understood and have been shown to exhibit biological activity (e.g., diabetes treatments).3, 10 Molecules with this variation on the vanadium bond can still be tested for antibiotic activity in the same way as the original intended molecule.

Furthermore, in regards to maintaining a range of viable options for this project, the molecule of focus for this project is fosmidomycin. This molecule was chosen because of its specific mechanism of action, and the modes of resistance developed by the targeted organisms. Fosmidomycin is one of a diverse family of phosphonate antibiotics, each with similar structures and different target organisms.7 If the structure of fosmidomycin proves too difficult to work with because of difficulty synthesizing the structure with vanadium, the project can simply be refocused on another member of the phosphonate family. While changing the project’s molecule of focus would not be ideal, the ultimate goal of assessing the concept’s viability for developing novel and effective antibiotics would still be viable.

If bacterial studies on the model systems (*E. coli* and *B. subtilis*) prove challenging additional organisms may be examined. Since fosmidomycin is also an effective treatment against tuberculosis as well as an antimicrobial agent to treat malaria, the analogue can be assessed with the two organisms (*P. falciparum* and the *M. tuberculosis* model organism *Mycobacterium smegmatis*) using the same biological tests. Access to the malaria organism *P. falciparum* is available via collaboration with Dr. Derbyshire of Duke University. She has worked extensively with *P. falciparum* and has agreed to allow me to utilize her lab, if necessary, in order to run the necessary biological tests.16 In regards to the model organism *M. smegmatis* must be used if this route were to be pursued because *M. Tuberculosis* is a highly infectious pathogenic organism that requires the use of a biosafety level three lab.17 A biosafety lab three is not available for use and it would be therefore be required to test the model organism instead. This however would not be an issue since *M. smegmatis* shares many of the same characteristics with the tuberculosis organism, except it is nonpathogenic.18 This would allow me to test it in one of the biosafety level one or two labs available at Elon.

The wide range of activity of fosmidomycin makes the flexibility in organisms of interest possible and allows me to refine the focus of the project as new information becomes available. This range of options allows for the project to be continually reassessed and altered in order to ensure its success.

**Budget:**

* **$2000.00:** Conference expenditures
* **$500.00:** Vanadium Reagents (VOCl3, VO(acac)2, VOSO4)

<http://www.sigmaaldrich.com/catalog/product/aldrich/200891?lang=en&region=US>

* **$1000.00:** Synthesis Reagents

<https://www.sigmaaldrich.com/united-states.html>

* **$250.00:** NMR Tubes (5): [http://www.sigmaaldrich.com/catalog/product/aldrich/z562262?lang=en&region= US](http://www.sigmaaldrich.com/catalog/product/aldrich/z562262?lang=en&region=%09US)
* **$400.00:** NMR Solvents

<http://shop.isotope.com/productdetails.aspx?itemno=DLM-51-PK>

* **$250.00:** Fosmidomycin (25mg)

<https://www.thermofisher.com/us/en/home/life-science/cell-culture/mammalian-cell-culture/antibiotics/fosmidomycin-sodium-salt-fr31564.html>

* **$700.00:** Deoxy-xylulose phosphate (10mg) <http://www.sigmaaldrich.com/catalog/product/sigma/13368?lang=en&region=US>
* **$1,500.00:** DXR recombinant protein (0.5mg)

<https://www.mybiosource.com/prods/Recombinant-Protein/1-deoxy-D-xylulose-5-phosphate-reductoisomerase-dxr/dxr/datasheet.php?products_id=1086729>

* **$350.00:** CyQUANT NF Cell proliferation assay kit

<https://www.thermofisher.com/us/en/home/brands/molecular-probes/key-molecular-probes-products/cyquant-cell-proliferation-assays.html>

* **$50.00:** Disk Assay Kit: Mueller Hinton Agar HDx, 15x150mm plate, R

<https://catalog.hardydiagnostics.com/cp_prod/product/h11-mueller-hinton-agar-large-for-kirby-bauer-disk-susceptibility-testing-large-15x150mm-plate-order-by-the-package-of-10-plates-by-hardy-diagnostics-media-prepared>

* **$2,000.00:** Microbiological supplies (Petri dishes, agar, growth media)
* **$2,000.00:** Consumables (Gloves, solvents, pipets, vials)
* **$1,500.00:** X-ray crystallography analysis (Lab usage fee at UNC)

<https://www.med.unc.edu/csb/mx/services-and-fees/fees>

* **$1,000.00:** Purification Supplies (Silica gels, TLC plates)
* **$100.00:** Organisms for biological tests (E. coli, B. subtilis)

[http://www.carolina.com/bacteria/escherichia-coli-microkwik-culture-vial/155065A.pr?question=#](http://www.carolina.com/bacteria/escherichia-coli-microkwik-culture-vial/155065A.pr?question=)

<http://www.carolina.com/bacteria/bacillus-stearothermophilus-living-tube/154910.pr?question=Bacillus+Subtilis>

* **$400.00:** Kanamycin (25g)

<http://www.sigmaaldrich.com/catalog/search?term=Kanamycin&interface=All&N=0+&mode=partialmax&lang=en&region=US&focus=product>

* **$1,000.00:** Turbidity assay supplies

**Total: $1500.00**

**Plan:**

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|  | Proposed Experiences | Proposed Product(s) |
| Summer 2017 | Non-Elon REU | None |
| Fall 2017 | Synthesis of vanadium antibiotic analogue  CHM499 | Prepare vanadium analogue  Formal research report  Southeast Regional Meeting of the American Chemical Society (Sermacs) conference |
| Winter 2018 | Synthesis and purification of vanadium antibiotic analogue  CHM499 | Finalize synthesis and purify substantive quantities for further tests  Formal research report |
| Spring 2018 | Characterization of synthesized molecule | Determination structure and bonding of synthesized analogue  National ACS conference  Formal research report  SURF presentation |
| Summer 2018 | Non-Elon REU | None |
| Fall 2018 | Disk diffusion and fluorescence assays with *E. coli* and *B. subtilis* | Determination of anti-bacterial properties of vanadium analogue  Formal research report  SERMACS conference |
| Winter 2019 | Dilution and turbidity assays | Determination of anti-bacterial properties of vanadium  Formal research report |
| Spring 2019 | Enzyme kinetics tests  Writing | Analysis of the molecule’s interaction with the DXR enzyme  Final research paper/draft of scientific paper to be published  SURF presentation |

**Citations**

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