



The Richard A. Henson School of Science & Technology
Salisbury University, Salisbury, Maryland 21801

UNDERGRADUATE RESEARCH GRANT APPLICATION

THIS APPLICATION MUST BE TYPED!

NAME Kimberly Neutze

MAJOR CLASS: 1 1 = Senior, 2 = Junior, 3 = Sophomore, 4 = Freshman

TELEPHONE 410-219-3495

LOCAL ADDRESS 1710 Emerson Ave.

CITY/STATE/ZIP Salisbury, MD 21801

FACULTY SUPERVISOR Dr. Miguel Mitchell

PROJECT BEGINNING DATE Feb. 16, 2004 ENDING DATE May 2004

TOTAL FUNDS REQUESTED \$302.00 Is this your first Henson Grant Award? yes

SIGNATURE: Kimberly Neutze

DATE 2/23/04

In reviewing this proposal, I have examined both the text and the budget and, to the best of my knowledge, judge them both to be worthy of funding consideration and non-duplicative of other activities or supplies within my department. I agree to serve as Faculty Advisor on this project.

FACULTY SIGNATURE: Dr. Miguel O. Mitchell

DATE 2/23/04

Please attach the following to this cover sheet.

- 1) **Project Title**
- 2) **Abstract**
- 3) **Introduction and a clear problem statement**
- 4) **Methods and materials**
- 5) **Prioritized, itemized budget for your project. Include cost of supplies and materials, including catalog pages, and catalog item number. **Note: Materials for this project must be project specific supplies and materials and should not include major equipment items.***
- 6) **References**

Please return application in digital form (MS Word preferred) to:

hensonschool@salisbury.edu

Dawn Johnson, Henson School of Science and Technology

If you have questions, call 410 543-6425

Inhibition of the cyclopropane synthetase *pcaA* of *Mycobacterium tuberculosis* in order to interrupt the virulence of the bacteria

ABSTRACT

As new drug therapies for tuberculosis are surfacing there is significant evidence that they need to target mycolic acid biosynthesis. *Mycobacterium tuberculosis*'s ability to cord is directly related its virulence. There is a mycobacterial gene, *pcaA* that is responsible for cording and mycolic acid cyclopropane ring synthesis. When this gene is knocked out, the bacteria can not form cyclopropane rings, thus preventing them from cording and thus keeping them from killing the host. We plan to use this information to develop a three-step process to make new compounds that inhibit *pcaA* from causing cyclopropanation. This information can help future researchers understand the importance of cording to the disease and develop effective drug therapies to help those suffering with tuberculosis.

INTRODUCTION

Tuberculosis, which is caused by *Mycobacterium tuberculosis*, is the leading cause of death that results from infectious bacteria. An increasing number of tuberculosis cases are proving resistant to current drug therapies. In searching for new options to treat the bacteria the mycolic acid-rich cell envelope of *Mycobacterium tuberculosis* is being examined, since the unique chemicals that are found in this outer layer play a big part in determining the virulence of the disease.

The purpose of this study is to propose a synthetic route to a potential novel drug that would inhibit the bacteria, *Mycobacterium tuberculosis*, from cording by stopping cyclopropanation of the alpha mycolate mycolic acid. These modifications are due to cyclopropane synthases or synthetases. The one we are looking at is designated as *pcaA*. It is responsible for synthesizing the proximal cyclopropane ring of the alpha mycolate. The cyclopropane ring found on the proximal end of the alpha mycolate has been shown to be a main factor in the bacteria's ability to cord. Previous studies have shown a direct correlation in the strength of the bacteria's cording to its virulence against its host. In a study done with mice they inoculated mice with wild type version and a mutant inactive version of *pcaA*. The mice with the wild type *pcaA* all died, and there was bacterial cording. The mice with the mutant *pcaA* all lived, and there was no cording (Glickman 2000). The structure of *pcaA* is defined for us in a previous article so we knew we needed to design compounds that will be able to reach the binding site of the synthetase (Huang 2002). There our drug would interrupt the normal mechanism that produces a cyclopropane ring, stop cording, and allow the host to survive. In our study we will be attaching the adenosine with an amine to form a carbocation mimic that will fit into the binding site of the *pcaA*. Positioned in the active site, tyrosine-33 will bind to the positive charge on the nitrogen in our compounds. This tyrosine-33:positivelycharged nitrogen interaction will make a stable structure, in the same way that tyrosine-33 stabilizes the carbocation intermediate in the enzyme-catalyzed mechanism.

Our proposed three-step process begins with the preparation of the oxime ester itself. Using *Candida antarctica* lipase as an catalyst, acylation of the nucleoside, adenosine, to an oxime ester is the second step of the process (Moris 1993). Then through nucleophilic substitution, we will form our compounds (Vega 1999).

MATERIALS AND METHODS

Preparation of Oxime Ester:

This will be the first step in our three-step process. To make the ester, acetone oxime and chloroacetyl chloride will be combined under an inert atmosphere (Scheme). To complete the reaction diisopropylethylamine is added as well. This is to be stirred at ambient temperature for 2 h, until the white smoke disappears. After the reaction is complete, fractional distillation will be needed to separate the desired oxime ester from the other by-products of the reaction. ^{13}C -NMR and ^1H -NMR will be used to analyze and prove the structure of the ester to be correct before moving on to the next step.

Acylation of Adenosine with the Oxime Ester:

The procedure for the acylation was taken from a journal article. (Moris 1993) Following their method the adenosine will be dissolved in THF. To catalyze the acylation *Candida antartica* lipase will be added to the reaction flask. The oxime ester will then be added to the reaction flask. The whole mixture will be placed in an oil bath at elevated temperature (30-60°C) and allowed to mix for 24 hours or more. Several runs may be needed to find exact conditions as far as temperature and time of reaction in order to optimize the production of Compound 3 (Scheme). Flash chromatography will be used to isolate the pure product. ^{13}C -NMR and ^1H -NMR will be used to analyze the structure of Compound 3.

Nucleophilic Substitution of Compound 3 to form Compounds 1 and 2 (Scheme):

This will be the last step in the synthetic route. The procedure for the nucleophilic substitution is also taken from a journal article (Vega 1999). This reaction will be done with two different alkylating reagents, the pyridine and the standard amine. To get compound 1, a mixture of compound 3, pyridine and NaI will be microwaved for 35-40 minutes. The product will then be filtered and washed to clean and isolate compound 1 from other by-products that may form. ^{13}C -NMR and ^1H -NMR will be used to analyze the structure and confirm if the synthesis was a success. To get compound 2, we will use the same reaction procedure as compound 1, except the reacting mixture will be compound 3, the amine and NaI.

Virulence Data:

The completed compounds 1 and 2 will then be sent to the Southern Research Institute in Birmingham, Alabama. There they will determine if these compounds have any affect on killing TB cells. We will be collaborating with James C. Sacchettini in testing our compounds. This will determine whether the synthesis of these compounds will have the opportunity to create new drug therapies against tuberculosis.

LITERATURE CITED

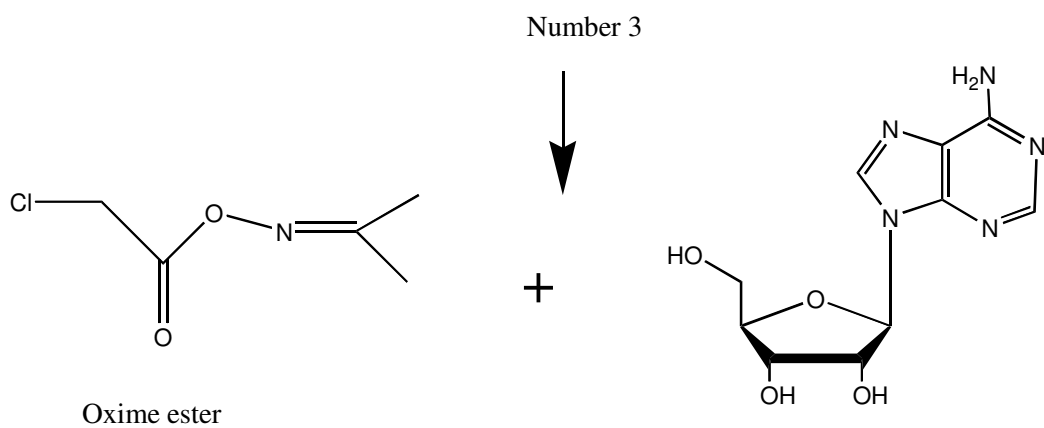
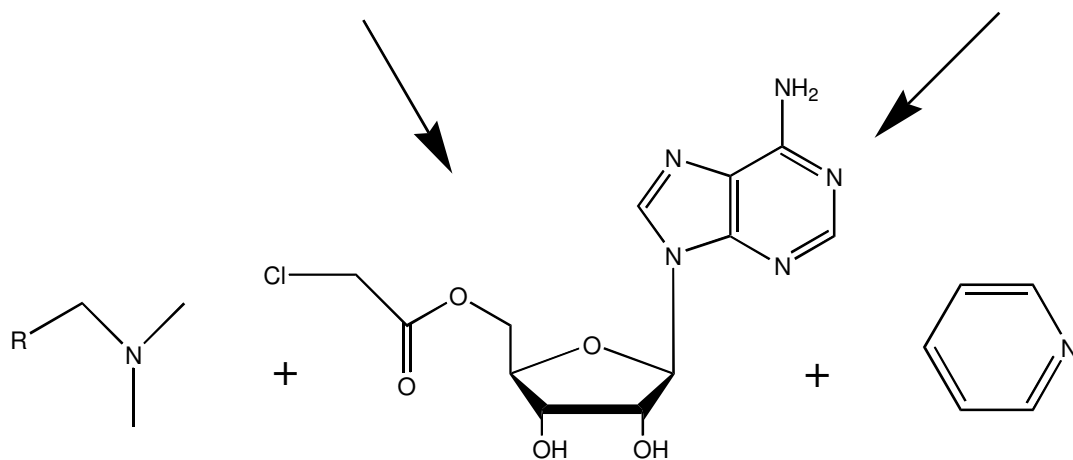
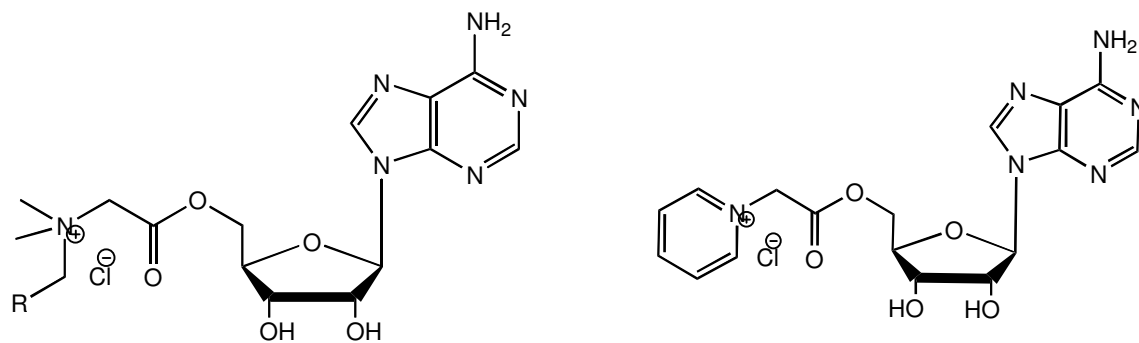
Glickman, M.S., Cox, J.S., Jacobs, W.R, Jr. (2000) A Novel Mycolic Acid Cyclopropane Synthetase Is Required for Cording, Persistence and Virulence of *Mycobacterium tuberculosis*. *Molecular Cell*. 5, 717-727.

Huang, C., Smith, C.V., Glickman, M.S., Jacobs, W.R, Jr., Sacchettini, J.C. *J. Biol. Chem.* **2002**, 277, 11559.

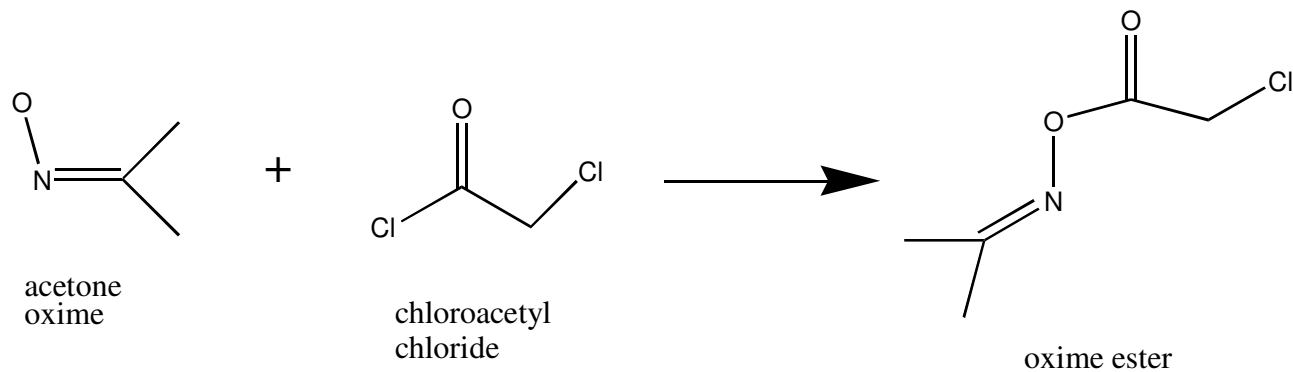
Moris, F., Gotor, V. *J. Org. Chem.* **1993**, 58, 653.

Vega, J.A, Vaquero, J.J., Alvarez-Builla, J.A., Ezquerro, J., Hamdouchi, C. *Tetrahedron Letters*. **1999**, 55, 2317.

- 4 -
Scheme



+ *C. antarctica* lipase



ITEMIZED BUDGET REQUEST

- For elemental analysis of my compounds
- From Desert Analytics in Tucson Arizona
- It is \$62.00 per run, there are going to be two runs at that price, plus an additional \$27.00 to analyze the final product.
- Minimum to run is \$151.00
- Total asking for is \$302.00 in case there is need for an additional elemental analysis or if I would need to duplicate the results



The Richard A. Henson School of Science & Technology
Salisbury University, Salisbury, Maryland 21801

UNDERGRADUATE RESEARCH GRANT APPLICATION

THIS APPLICATION MUST BE TYPED!

NAME Kimberly Neutze

MAJOR CLASS: 1 1 = Senior, 2 = Junior, 3 = Sophomore, 4 = Freshman

TELEPHONE 410-219-3495

LOCAL ADDRESS 1710 Emerson Ave.

CITY/STATE/ZIP Salisbury, MD 21801

FACULTY SUPERVISOR Dr. Miguel Mitchell

PROJECT BEGINNING DATE Feb. 16, 2004 ENDING DATE May 2004

TOTAL FUNDS REQUESTED \$302.00 Is this your first Henson Grant Award? yes

SIGNATURE: Kimberly Neutze

DATE 2/23/04

In reviewing this proposal, I have examined both the text and the budget and, to the best of my knowledge, judge them both to be worthy of funding consideration and non-duplicative of other activities or supplies within my department. I agree to serve as Faculty Advisor on this project.

FACULTY SIGNATURE: Dr. Miguel O. Mitchell

DATE 2/23/04

Please attach the following to this cover sheet.

- 1) **Project Title**
- 2) **Abstract**
- 3) **Introduction and a clear problem statement**
- 4) **Methods and materials**
- 5) **Prioritized, itemized budget for your project. Include cost of supplies and materials, including catalog pages, and catalog item number. **Note: Materials for this project must be project specific supplies and materials and should not include major equipment items.***
- 6) **References**

Please return application in digital form (MS Word preferred) to:

hensonschool@salisbury.edu

Dawn Johnson, Henson School of Science and Technology

If you have questions, call 410 543-6425

Inhibition of the cyclopropane synthetase *pcaA* of *Mycobacterium tuberculosis* in order to interrupt the virulence of the bacteria

ABSTRACT

As new drug therapies for tuberculosis are surfacing there is significant evidence that they need to target mycolic acid biosynthesis. *Mycobacterium tuberculosis*'s ability to cord is directly related its virulence. There is a mycobacterial gene, *pcaA* that is responsible for cording and mycolic acid cyclopropane ring synthesis. When this gene is knocked out, the bacteria can not form cyclopropane rings, thus preventing them from cording and thus keeping them from killing the host. We plan to use this information to develop a three-step process to make new compounds that inhibit *pcaA* from causing cyclopropanation. This information can help future researchers understand the importance of cording to the disease and develop effective drug therapies to help those suffering with tuberculosis.

INTRODUCTION

Tuberculosis, which is caused by *Mycobacterium tuberculosis*, is the leading cause of death that results from infectious bacteria. An increasing number of tuberculosis cases are proving resistant to current drug therapies. In searching for new options to treat the bacteria the mycolic acid-rich cell envelope of *Mycobacterium tuberculosis* is being examined, since the unique chemicals that are found in this outer layer play a big part in determining the virulence of the disease.

The purpose of this study is to propose a synthetic route to a potential novel drug that would inhibit the bacteria, *Mycobacterium tuberculosis*, from cording by stopping cyclopropanation of the alpha mycolate mycolic acid. These modifications are due to cyclopropane synthetases or synthetases. The one we are looking at is designated as *pcaA*. It is responsible for synthesizing the proximal cyclopropane ring of the alpha mycolate. The cyclopropane ring found on the proximal end of the alpha mycolate has been shown to be a main factor in the bacteria's ability to cord. Previous studies have shown a direct correlation in the strength of the bacteria's cording to its virulence against its host. In a study done with mice they inoculated mice with wild type version and a mutant inactive version of *pcaA*. The mice with the wild type *pcaA* all died, and there was bacterial cording. The mice with the mutant *pcaA* all lived, and there was no cording (Glickman 2000). The structure of *pcaA* is defined for us in a previous article so we knew we needed to design compounds that will be able to reach the binding site of the synthetase (Huang 2002). There our drug would interrupt the normal mechanism that produces a cyclopropane ring, stop cording, and allow the host to survive. In our study we will be attaching the adenosine with an amine to form a carbocation mimic that will fit into the binding site of the *pcaA*. Positioned in the active site, tyrosine-33 will bind to the positive charge on the nitrogen in our compounds. This tyrosine-33:positivelycharged nitrogen interaction will make a stable structure, in the same way that tyrosine-33 stabilizes the carbocation intermediate in the enzyme-catalyzed mechanism.

Our proposed three-step process begins with the preparation of the oxime ester itself. Using *Candida antarctica* lipase as an catalyst, acylation of the nucleoside, adenosine, to an oxime ester is the second step of the process (Moris 1993). Then through nucleophilic substitution, we will form our compounds (Vega 1999).

MATERIALS AND METHODS

Preparation of Oxime Ester:

This will be the first step in our three-step process. To make the ester, acetone oxime and chloroacetyl chloride will be combined under an inert atmosphere (Scheme). To complete the reaction diisopropylethylamine is added as well. This is to be stirred at ambient temperature for 2 h, until the white smoke disappears. After the reaction is complete, fractional distillation will be needed to separate the desired oxime ester from the other by-products of the reaction. ^{13}C -NMR and ^1H -NMR will be used to analyze and prove the structure of the ester to be correct before moving on to the next step.

Acylation of Adenosine with the Oxime Ester:

The procedure for the acylation was taken from a journal article. (Moris 1993) Following their method the adenosine will be dissolved in THF. To catalyze the acylation *Candida antarctica* lipase will be added to the reaction flask. The oxime ester will then be added to the reaction flask. The whole mixture will be placed in an oil bath at elevated temperature (30-60°C) and allowed to mix for 24 hours or more. Several runs may be needed to find exact conditions as far as temperature and time of reaction in order to optimize the production of Compound 3 (Scheme). Flash chromatography will be used to isolate the pure product. ^{13}C -NMR and ^1H -NMR will be used to analyze the structure of Compound 3.

Nucleophilic Substitution of Compound 3 to form Compounds 1 and 2 (Scheme):

This will be the last step in the synthetic route. The procedure for the nucleophilic substitution is also taken from a journal article (Vega 1999). This reaction will be done with two different alkylating reagents, the pyridine and the standard amine. To get compound 1, a mixture of compound 3, pyridine and NaI will be microwaved for 35-40 minutes. The product will then be filtered and washed to clean and isolate compound 1 from other by-products that may form. ^{13}C -NMR and ^1H -NMR will be used to analyze the structure and confirm if the synthesis was a success. To get compound 2, we will use the same reaction procedure as compound 1, except the reacting mixture will be compound 3, the amine and NaI.

Virulence Data:

The completed compounds 1 and 2 will then be sent to the Southern Research Institute in Birmingham, Alabama. There they will determine if these compounds have any effect on killing TB cells. We will be collaborating with James C. Sacchettini in testing our compounds. This will determine whether the synthesis of these compounds will have the opportunity to create new drug therapies against tuberculosis.

LITERATURE CITED

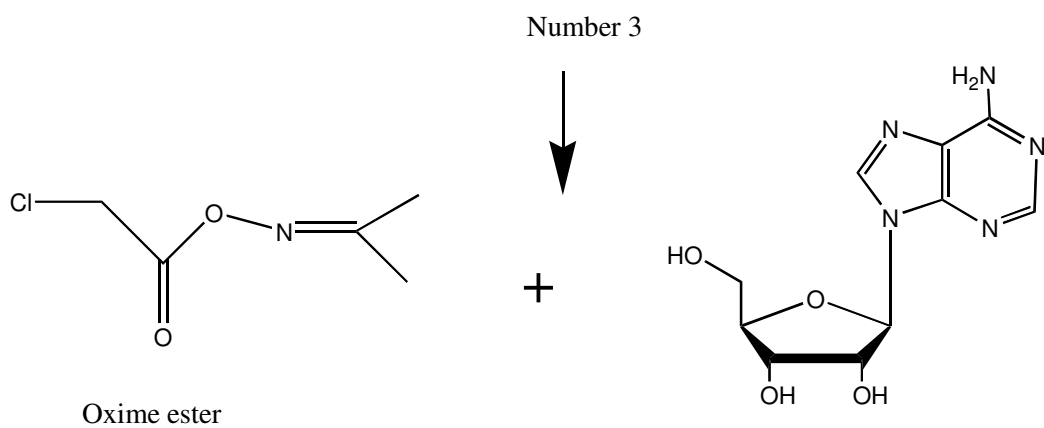
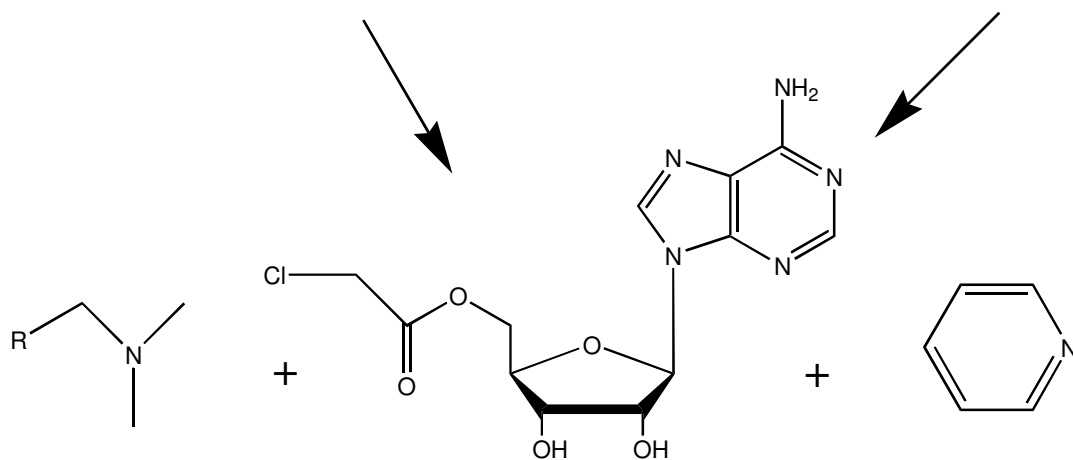
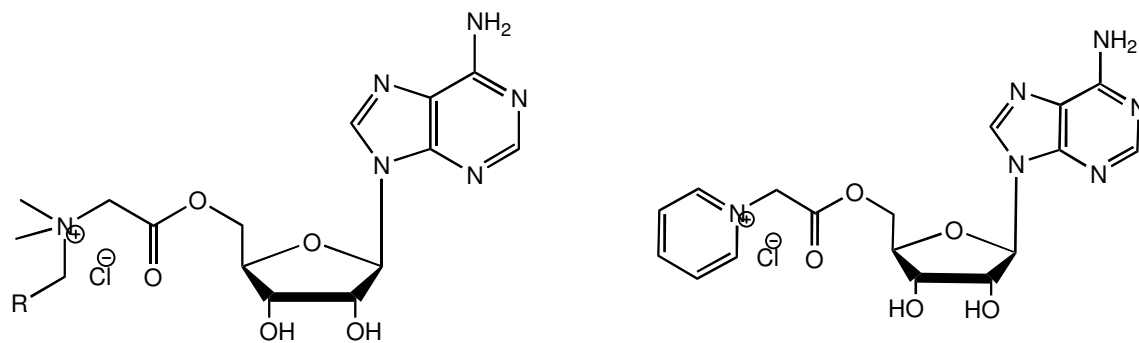
Glickman, M.S., Cox, J.S., Jacobs, W.R, Jr. (2000) A Novel Mycolic Acid Cyclopropane Synthetase Is Required for Cording, Persistence and Virulence of *Mycobacterium tuberculosis*. *Molecular Cell*. 5, 717-727.

Huang, C., Smith, C.V., Glickman, M.S., Jacobs, W.R, Jr., Sacchettini, J.C. *J. Biol. Chem.* **2002**, 277, 11559.

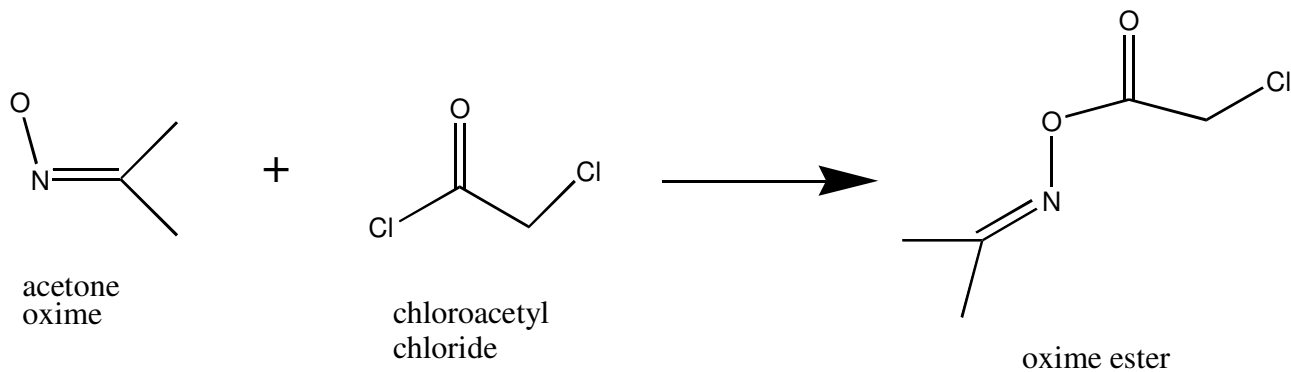
Moris, F., Gotor, V. *J. Org. Chem.* **1993**, 58, 653.

Vega, J.A, Vaquero, J.J., Alvarez-Builla, J.A., Ezquerra, J., Hamdouchi, C. *Tetrahedron Letters*. **1999**, 55, 2317.

- 4 -
Scheme



+ *C. antarctica* lipase



ITEMIZED BUDGET REQUEST

- For elemental analysis of my compounds
- From Desert Analytics in Tucson Arizona
- It is \$62.00 per run, there are going to be two runs at that price, plus an additional \$27.00 to analyze the final product.
- Minimum to run is \$151.00
- Total asking for is \$302.00 in case there is need for an additional elemental analysis or if I would need to duplicate the results



The Richard A. Henson School of Science & Technology
Salisbury University, Salisbury, Maryland 21801

UNDERGRADUATE RESEARCH GRANT APPLICATION

THIS APPLICATION MUST BE TYPED!

NAME Kimberly Neutze

MAJOR CLASS: 1 1 = Senior, 2 = Junior, 3 = Sophomore, 4 = Freshman

TELEPHONE 410-219-3495

LOCAL ADDRESS 1710 Emerson Ave.

CITY/STATE/ZIP Salisbury, MD 21801

FACULTY SUPERVISOR Dr. Miguel Mitchell

PROJECT BEGINNING DATE Feb. 16, 2004 ENDING DATE May 2004

TOTAL FUNDS REQUESTED \$302.00 Is this your first Henson Grant Award? yes

SIGNATURE: Kimberly Neutze

DATE 2/23/04

In reviewing this proposal, I have examined both the text and the budget and, to the best of my knowledge, judge them both to be worthy of funding consideration and non-duplicative of other activities or supplies within my department. I agree to serve as Faculty Advisor on this project.

FACULTY SIGNATURE: Dr. Miguel O. Mitchell

DATE 2/23/04

Please attach the following to this cover sheet.

- 1) **Project Title**
- 2) **Abstract**
- 3) **Introduction and a clear problem statement**
- 4) **Methods and materials**
- 5) **Prioritized, itemized budget for your project. Include cost of supplies and materials, including catalog pages, and catalog item number. **Note: Materials for this project must be project specific supplies and materials and should not include major equipment items.***
- 6) **References**

Please return application in digital form (MS Word preferred) to:

hensonschool@salisbury.edu

Dawn Johnson, Henson School of Science and Technology

If you have questions, call 410 543-6425

Inhibition of the cyclopropane synthetase *pcaA* of *Mycobacterium tuberculosis* in order to interrupt the virulence of the bacteria

ABSTRACT

As new drug therapies for tuberculosis are surfacing there is significant evidence that they need to target mycolic acid biosynthesis. *Mycobacterium tuberculosis*'s ability to cord is directly related its virulence. There is a mycobacterial gene, *pcaA* that is responsible for cording and mycolic acid cyclopropane ring synthesis. When this gene is knocked out, the bacteria can not form cyclopropane rings, thus preventing them from cording and thus keeping them from killing the host. We plan to use this information to develop a three-step process to make new compounds that inhibit *pcaA* from causing cyclopropanation. This information can help future researchers understand the importance of cording to the disease and develop effective drug therapies to help those suffering with tuberculosis.

INTRODUCTION

Tuberculosis, which is caused by *Mycobacterium tuberculosis*, is the leading cause of death that results from infectious bacteria. An increasing number of tuberculosis cases are proving resistant to current drug therapies. In searching for new options to treat the bacteria the mycolic acid-rich cell envelope of *Mycobacterium tuberculosis* is being examined, since the unique chemicals that are found in this outer layer play a big part in determining the virulence of the disease.

The purpose of this study is to propose a synthetic route to a potential novel drug that would inhibit the bacteria, *Mycobacterium tuberculosis*, from cording by stopping cyclopropanation of the alpha mycolate mycolic acid. These modifications are due to cyclopropane synthetases or synthetases. The one we are looking at is designated as *pcaA*. It is responsible for synthesizing the proximal cyclopropane ring of the alpha mycolate. The cyclopropane ring found on the proximal end of the alpha mycolate has been shown to be a main factor in the bacteria's ability to cord. Previous studies have shown a direct correlation in the strength of the bacteria's cording to its virulence against its host. In a study done with mice they inoculated mice with wild type version and a mutant inactive version of *pcaA*. The mice with the wild type *pcaA* all died, and there was bacterial cording. The mice with the mutant *pcaA* all lived, and there was no cording (Glickman 2000). The structure of *pcaA* is defined for us in a previous article so we knew we needed to design compounds that will be able to reach the binding site of the synthetase (Huang 2002). There our drug would interrupt the normal mechanism that produces a cyclopropane ring, stop cording, and allow the host to survive. In our study we will be attaching the adenosine with an amine to form a carbocation mimic that will fit into the binding site of the *pcaA*. Positioned in the active site, tyrosine-33 will bind to the positive charge on the nitrogen in our compounds. This tyrosine-33:positivelycharged nitrogen interaction will make a stable structure, in the same way that tyrosine-33 stabilizes the carbocation intermediate in the enzyme-catalyzed mechanism.

Our proposed three-step process begins with the preparation of the oxime ester itself. Using *Candida antarctica* lipase as an catalyst, acylation of the nucleoside, adenosine, to an oxime ester is the second step of the process (Moris 1993). Then through nucleophilic substitution, we will form our compounds (Vega 1999).

MATERIALS AND METHODS

Preparation of Oxime Ester:

This will be the first step in our three-step process. To make the ester, acetone oxime and chloroacetyl chloride will be combined under an inert atmosphere (Scheme). To complete the reaction diisopropylethylamine is added as well. This is to be stirred at ambient temperature for 2 h, until the white smoke disappears. After the reaction is complete, fractional distillation will be needed to separate the desired oxime ester from the other by-products of the reaction. ^{13}C -NMR and ^1H -NMR will be used to analyze and prove the structure of the ester to be correct before moving on to the next step.

Acylation of Adenosine with the Oxime Ester:

The procedure for the acylation was taken from a journal article. (Moris 1993) Following their method the adenosine will be dissolved in THF. To catalyze the acylation *Candida antarctica* lipase will be added to the reaction flask. The oxime ester will then be added to the reaction flask. The whole mixture will be placed in an oil bath at elevated temperature (30-60°C) and allowed to mix for 24 hours or more. Several runs may be needed to find exact conditions as far as temperature and time of reaction in order to optimize the production of Compound 3 (Scheme). Flash chromatography will be used to isolate the pure product. ^{13}C -NMR and ^1H -NMR will be used to analyze the structure of Compound 3.

Nucleophilic Substitution of Compound 3 to form Compounds 1 and 2 (Scheme):

This will be the last step in the synthetic route. The procedure for the nucleophilic substitution is also taken from a journal article (Vega 1999). This reaction will be done with two different alkylating reagents, the pyridine and the standard amine. To get compound 1, a mixture of compound 3, pyridine and NaI will be microwaved for 35-40 minutes. The product will then be filtered and washed to clean and isolate compound 1 from other by-products that may form. ^{13}C -NMR and ^1H -NMR will be used to analyze the structure and confirm if the synthesis was a success. To get compound 2, we will use the same reaction procedure as compound 1, except the reacting mixture will be compound 3, the amine and NaI.

Virulence Data:

The completed compounds 1 and 2 will then be sent to the Southern Research Institute in Birmingham, Alabama. There they will determine if these compounds have any effect on killing TB cells. We will be collaborating with James C. Sacchettini in testing our compounds. This will determine whether the synthesis of these compounds will have the opportunity to create new drug therapies against tuberculosis.

LITERATURE CITED

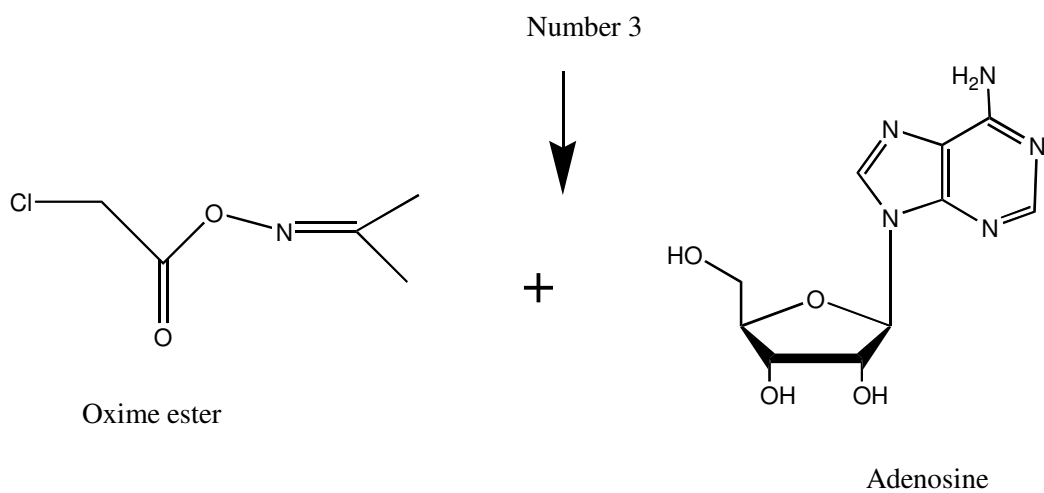
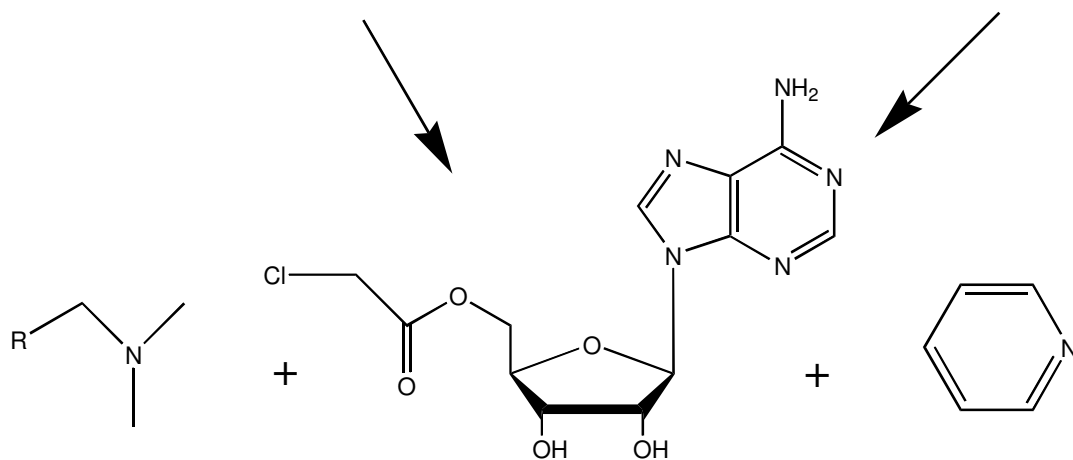
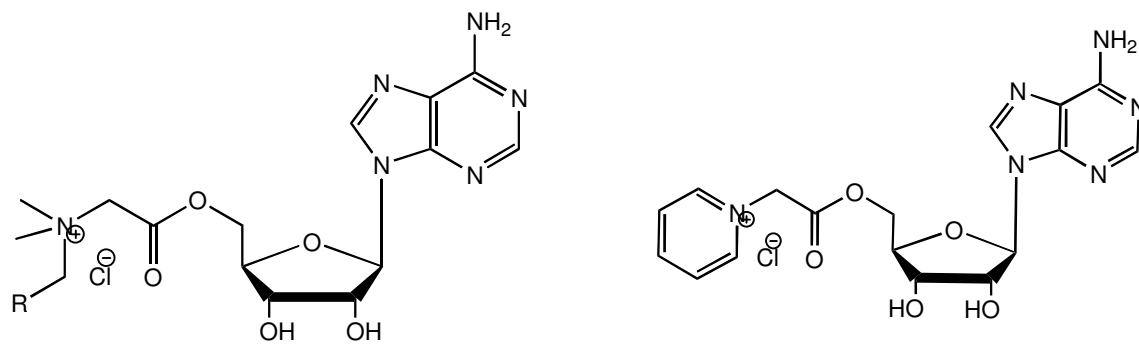
Glickman, M.S., Cox, J.S., Jacobs, W.R, Jr. (2000) A Novel Mycolic Acid Cyclopropane Synthetase Is Required for Cording, Persistence and Virulence of *Mycobacterium tuberculosis*. *Molecular Cell*. 5, 717-727.

Huang, C., Smith, C.V., Glickman, M.S., Jacobs, W.R, Jr., Sacchettini, J.C. *J. Biol. Chem.* **2002**, 277, 11559.

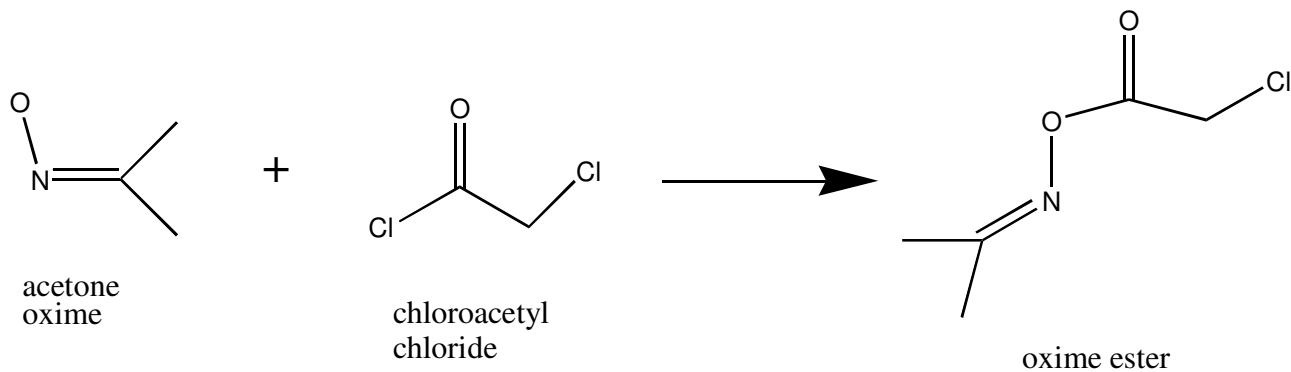
Moris, F., Gotor, V. *J. Org. Chem.* **1993**, 58, 653.

Vega, J.A, Vaquero, J.J., Alvarez-Builla, J.A., Ezquerra, J., Hamdouchi, C. *Tetrahedron Letters*. **1999**, 55, 2317.

- 4 -
Scheme



+ *C. antarctica* lipase



ITEMIZED BUDGET REQUEST

- For elemental analysis of my compounds
- From Desert Analytics in Tucson Arizona
- It is \$62.00 per run, there are going to be two runs at that price, plus an additional \$27.00 to analyze the final product.
- Minimum to run is \$151.00
- Total asking for is \$302.00 in case there is need for an additional elemental analysis or if I would need to duplicate the results