The synthesis of new biotin derivatives and their bioactivity

Latisha Chanette Johnson

Louisiana State University and Agricultural and Mechanical College

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THE SYNTHESIS OF NEW BIOTIN DERIVATIVES AND THEIR BIOACTIVITY

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The Department of Chemistry

Latisha C. Johnson
B. S. Southern University, 1999
December, 2002
This thesis is dedicated to my parents and family who has been my support system. To my paternal grandparents, Faye Johnson and the late Samuel “Tiny” Johnson, thank you for setting the foundation.
Acknowledgements

I would first like to acknowledge my advisor, Dr. Robert Strongin, for giving me the opportunity to do meaningful research. Thank you for your guidance and encouragement. I am grateful that you took the time to understand me as a chemist as well as a person. I would also like to thank the entire Strongin research group for their support. I would especially like to express my appreciation to Nadia St. Luce and Rolanda Johnson for being my cheerleaders, my counselors, and my friends.

Secondly, I would like to recognize all my collaborators: Keith Levert, Dr. Grover Waldrop, Dr. Anne Grove, Steven Wilkinson, Dr. Jackie Stephens, Dr. Isiah Warner, and Dr. Lui Marzilli. My story would not be complete without you. I would also like to acknowledge the following staff: Dr. Dale Treleavan, Dr. Frank Zhou, and Dr. Tanaji Telele. I am grateful to the Petroleum Research Fund of the American Chemical Society and the National Institute of Health for funding my research.

Lastly, I would like to thank Dr. Robert Gooden, my undergraduate advisor and lifelong mentor, for pushing me to achieve my goals and for reassuring me when I felt they were out of reach. To Mrs. Kris Purdy, my high school chemistry teacher, thank you for presenting chemistry as a challenging yet exciting science. My story began with you.
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<tr>
<td>CoA</td>
<td>coenzyme A</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine 5’-triphosphate</td>
</tr>
<tr>
<td>Pi</td>
<td>phosphate</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>1’-N</td>
<td>one prime nitrogen</td>
</tr>
<tr>
<td>$\varepsilon$-NH$_3^+$</td>
<td>epsilon amine</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine 5’-monophosphate</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine 5’-diphosphate</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylamino pyridine</td>
</tr>
<tr>
<td>HOBT</td>
<td>1-hydroxybenzotriazole</td>
</tr>
<tr>
<td>DMAP</td>
<td>dimethylaminopyridine</td>
</tr>
<tr>
<td>Et$_3$N</td>
<td>triethylamine</td>
</tr>
<tr>
<td>P(OEt)$_3$</td>
<td>triethylphosphite</td>
</tr>
<tr>
<td>TMSBr</td>
<td>trimethylsilyl bromide</td>
</tr>
<tr>
<td>LiOH</td>
<td>lithium hydroxide</td>
</tr>
<tr>
<td>DMF</td>
<td>N-N-dimethylformamide</td>
</tr>
<tr>
<td>Pd/C</td>
<td>palladium/carbon</td>
</tr>
<tr>
<td>THF</td>
<td>terahydrofuran</td>
</tr>
<tr>
<td>H$_2$</td>
<td>hydrogen</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
</tbody>
</table>
h  hours
Km  Michaelis-Menlen constant
MM  milimolar
CHCl₃  chloroform
¹H NMR  Hydrogen Nuclear Magnetic Resonance
HPLC  high performance liquid chromatography
M  molar
HCl  hydrogen chloride
UV  ultraviolet
NaHCO₃  sodium bicarbonate
DMSO  dimethyl sulfoxide
Asp  aspartate
Thr  threonine
Gly  glysine
β  beta
Ile  isoleucine
Ki  equilibrium constant for inhibitor binding
µM  micromolar
FDA  Food and Drug Administration
C  Celsius
mL  mililiter
EtOAc  ethyl acetate
CH₂Cl₂  dichloromethane
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>KBr</td>
<td>potassium bromide</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>magnesium sulfate</td>
</tr>
<tr>
<td>CaH₂</td>
<td>calcium hydride</td>
</tr>
<tr>
<td>(EtO)₂PCl</td>
<td>ethylchlorosulfonate</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
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**Abstract**

Biotin is an essential growth factor found in all living cells. It functions as a cofactor for a group of coenzymes that catalyze transcarboxylation, decarboxylation, and carboxylation reactions. Acetyl CoA carboxylase catalyzes the first committed and regulated step in fatty acid synthesis in which malonyl CoA is the product. The biotin-dependent enzyme is found in all animals, plants, and bacteria. We studied the kinetic and structural aspects of acetyl CoA carboxylase, and in 1999 we reported the bioactivity and total synthesis of a unique first generation biotin-derived inhibitor of acetyl CoA carboxylase, 1(BP1). Kinetic studies on the activity of *E. coli* biotin carboxylase yielded an inhibition constant of 8.4±1 mM. Since that time, we have improved the yield and purification of 1. Currently, we are designing multsubstrate analogs which involve the attachment of adenosine moieties at the 1-N of biotin. We reason that these compounds will have a greater affinity for acetyl CoA carboxylase. In addition, we are synthesizing biotin derivatives for their study in three main areas: (1) understanding the mechanism of biotin carboxylase and acetyl CoA carboxylase for the development of new therapeutic agents, (2) screening for ribozyme activity to support the “RNA World” hypothesis, and (3) investigation of chiral cationic micelles for analytical separations.
Chapter One
Introduction

1.1 Biotin-dependent Carboxylases

Biotin, or vitamin H, is an essential component of all living cells. It was first isolated as a growth factor for yeast in 1936 in its methyl ester form. The proposed structure for (+)-biotin (Figure 1) was confirmed by the first total synthesis by Merck. Structurally, the molecule consists of a bicyclic ring system whose mirror symmetry is broken by a valeric acid side chain. The top ring of the bicyclic system is a ureido ring, and the bottom ring, containing sulfur, is a tetrahydrothiophene (or thiophane) ring. Biotin further possesses three contiguous stereocenters on the thiophane ring in the all-cis configuration.

Figure 1: Structure of (+)-biotin

The natural sources of biotin are the pancreas, liver, kidney, yeast, and egg yolk. Low levels of biotin in poultry and red meat cause a series of symptoms. These deficiencies are corrected by using biotin as a feed additive. Biotin functions as a cofactor in carboxylation, decarboxylation, and transcarboxylation reactions related to biochemical processes such as glucogenesis and fatty acid synthesis. Because of its
fundamental and commercial importance biotin has attracted the attention of both academic and industrial biologists and chemists.

Acetyl CoA carboxylase (ACC) carries out the first committed and regulated step in fatty acid biosynthesis. The biotin-containing enzyme catalyzes the ATP-dependent formation of malonyl CoA from acetyl CoA and bicarbonate. Acetyl CoA carboxylase is found in all animals, plants, and bacteria. Biotin-dependent enzymes, such as ACC, in general have three domains: the biotin carboxyl carrier protein, biotin carboxylase, and carboxytransferase (Figure 2). Depending on the organism, these components may occur as separate subunits. For example, pyruvate carboxylase has the biotinyl and transcarboxylase domains on one subunit and the biotin carboxylase on another, whereas in the carboxylases from some yeast, insects, vertebrates, and bacteria, such as *Escherichia coli*, all three components are found on one polypeptide chain.

**Figure 2**: The three domains of biotin-dependent enzymes.
Biotin-dependent carboxylases function by means of a two-step reaction sequence shown in Scheme 1. Biotin is covalently attached to the carboxyl carrier protein via an amide bond between the valeric acid side chain of biotin and the ε group of a lysine residue. In the first half-reaction, Knowles describes the carboxylation of biotin whereby there is an ATP-driven attachment of carbon dioxide from bicarbonate at the 1’-N position of the biotin cyclic urea ring through the catalysis of biotin carboxylase. A carboxyphosphate intermediate is involved, and 1’-N-carboxybiotin is generated stepwise. In the second half reaction, the activity of carboxytransferase allows for the transfer of carbon dioxide from carboxybiotin to the acceptor, acetyl CoA, forming the product of fatty acid synthesis, malonyl CoA.

(1) Enzyme-biotin + Mg^{2+} - ATP + HCO_3^- \rightarrow \text{Enzyme-biotin-}CO_2^- + Mg^{2+} - ADP + Pi

(2) Enzyme-biotin-\text{CO}_2^- + \text{acceptor} \rightarrow \text{acceptor-}CO_2^- + \text{Enzyme-biotin}

**Scheme 1:** The two-step reaction used by all biotin-dependent enzymes.

Since Knowles’s review in 1989, there have been several reviews on biotin-dependent enzymes which describe the progress towards elucidating their mechanism of action. For several years, Strongin and Waldrop have collaboratively studied the structural and mechanistic aspects of the biotin carboxylase subunit of acetyl CoA carboxylase. In 1999, they reported the total synthesis and bioactivity of the first biotin-derived inhibitor of biotin carboxylase, compound 1, or BP1 (Figure 3). This thesis focuses on the efforts currently being made to improve the yield and purification of the
BP1 analog as well as the synthesis of new biotin derivatives for their study in three main areas: (1) understanding the mechanism of biotin carboxylase for the development of new therapeutic agents, (2) screening for ribozyme activity to support the “RNA World” hypothesis, and (3) investigation of the first chiral cationic micelles.

**Figure 3:** Compound 1 embodies a stable analog of the naturally occurring carboxyphosphate intermediate involved in biotin-mediated CO$_2$.

### 1.2 Background

A number of proposed mechanisms for the carboxylation of biotin in reaction 1 (Scheme 3) have been considered. These included the initial formation of a phosphobiotin intermediate which then reacts in a concerted reaction with bicarbonate to form carboxybiotin and Pi (Scheme 2a). Another mechanism involved formation of carboxyphosphate. Biotin is then directly carboxylated via a nucleophilic attack of the 1-$N$ of biotin on the carboxyl carbon. Carboxyphosphate can also decarboxylate to form the strong electrophile CO$_2$, which is then the carboxylating species (Scheme 2b). Knowles$^6$ concluded that the most likely route to carboxybiotin was via a carboxyphosphate intermediate from ATP and bicarbonate. To date, however, there has not been a direct demonstration of the existence of carboxyphosphate.$^{13,14}$
Scheme 2: Mechanisms a and b are proposed mechanisms for the carboxylation of the 1’-N of biotin to form carboxybiotin. Mechanism c suggests that a cysteine-lysine base pair form the base and conjugate acid involved in the enolation of biotin.

The favored route to carboxylation of biotin from carboxyphosphate involves initial proton abstraction from the 1’-N of biotin by an enzymic base, resulting in the formation of the enolate which can attack the carboxylating species. Attwood and coworkers\(^9\) proposed that a cysteine-lysine ion pair are involved in the conversion of biotin from the ureido to eneamine form (Scheme 2c). The lysine would deprotonate cysteine to form a thiolate anion, which would remove the 1’-N proton from biotin. The positively charged ε-NH\(_3^+\) of lysine stabilizes the enolate oxygen of biotin. This would result in the enolate of biotin containing a very nucleophilic 1’-N, ready to attack the
carboxylating species. On the contrary, a recent theoretical study by Grant\textsuperscript{15} has raised the possibility of there being concerted carboxylation and deprotonation of biotin in reaction 1 (Scheme 1). He also proposed a mechanistic role for sulfur on the thiophane ring of biotin. Grant has calculated that a twisted conformation of the bicyclic ring structure can lead to orbital overlap between the sulfur and the ureido group, increasing the nucleophilic character of the 1’-N on biotin.

Although many questions remain to be answered, many advances have been made relating to the study of the catalytic mechanism of biotin carboxylase as a result of the determination of the three–dimensional structure of biotin carboxylase by X-ray crystallography.\textsuperscript{15} High-resolution crystallographic structures of the biotin carboxylase subunit of acetyl CoA carboxylase co-crystallized with substrates would be an important breakthrough.

It is known from recent studies reported by Blanchard and Waldrop that biotin has a low affinity (Km =134 mM) for the enzyme acetyl CoA carboxylase in contrast to the Km values of 0.7 mM and 0.05 mM for bicarbonate and ATP, respectively.\textsuperscript{16} Therefore, one possible way to overcome low affinity of biotin for the enzyme is to synthesize a multisubstrate analog linking biotin to tighter binding substrates like bicarbonate and ATP as shown in Figure 4. The design and synthesis of 1 was the direct result of this reasoning.
The activity of biotin carboxylase was measured in the absence and presence of increasing amounts of 1. As the concentration of the analog increased the initial velocity of biotin carboxylase from *E. coli* decreased. Fitting the data to the equation for linear competitive inhibition by linear regression analysis yielded a slope inhibition constant of 8.4±1mM. While this is a modest degree of inhibition, placing a phosphonoacetic acid moiety at the 1-*N* of biotin allows BP1 to bind 10 times more tightly to the enzyme than free biotin.

The new biotin derivatives we are currently synthesizing include multisubstrate inhibitors (Figure 5) that will incorporate AMP, ADP, and ATP, respectively. The
inhibition of these compounds will be compared to that of the patented BP1 analog. We hypothesize that these derivatives will have a significantly lower inhibition constant than the first-generation analog.
Chapter Two
Carboxyphosphatase Reaction Intermediate Analog

2.1 Introduction

There has been growing interest in our reaction intermediate analog BP1 since its total synthesis and inhibitory effect on biotin carboxylase were reported in 1999.\textsuperscript{10,11} In several collaborative efforts, we have studied BP1 as both an antiobesity and anti-HIV therapeutic agent. In addition, compound 1 has been utilized as a substrate in the screening of ribozyme activity to support the “RNA World” hypothesis.

The total synthesis of 1, (Scheme 3) described previously by Amspacher,\textsuperscript{10} begins with the protection of (+)-biotin as the corresponding ester in 95% yield via reaction with benzyl alcohol, DCC, and catalytic amounts of HOBT and DMAP. Biotin benzyl ester 2 is reacted with chloroacetyl chloride to furnish compound 3 in 98% yield. Compound 3 then undergoes an Arbuzov reaction with P(OEt)\textsubscript{3} at 100°C to obtain phosphonate ester 4 in 88% yield. Hydrolysis of 4, promoted by TMSBr, provides the corresponding phosphonic acid in 72% yield. Subsequent saponification of 5 with LiOH affords target compound 1 in 59% yield and an overall 22% yield.

The synthesis of BP1 is straightforward and the yields of all intermediates are excellent. The saponification step, however, is not as efficient when compared to the preceding four steps in the synthesis. I thus optimized the reaction conditions as well as the purification methods to improve the yield of the saponification reaction before submitting the BP1 analog to our collaborators for utilization in biological assays.
Scheme 3: The preparation of 1 from (+)-biotin.

2.2 Improved Yield of Carboxyphosphate Reaction Intermediate Analog

We first attempted the cleavage of the benzyl ester on phosphonic acid 5 by more classical synthetic organic means, such as hydrogenation. Although it is a known fact that sulfur poisons palladium catalysts, successful hydrogenation of sulfur-containing compounds has been previously reported.\textsuperscript{17} Compound 5 was reacted with 10% Pd/C in THF under H\textsubscript{2} (50 psi) overnight; the target compound was not obtained. Further work may be necessary. We meantime concluded reaction with LiOH followed by immediate neutralization and lyophilization of the reaction mixture were the optimum conditions.
The carboxphosphate reaction intermediate analog had only been synthesized previously on milligram scale. In my efforts to scale up the saponification step, the pH sensitivity of BP1 as well as the polarity of starting material and final product became challenging issues. The time required for the conversion of compound 5 to 1 is approximately 2 hrs ± 20 minutes. I discovered this 20-minute window made a significant difference in the yield of BP1 obtained. Due to the polarity of the reactant and desired product, the exact time at which the reaction was complete was difficult to determine by thin layer chromatography (TLC). Stopping the reaction prematurely resulted in a polar mixture of starting material and product, which was seemingly impossible to separate. Even with polar solvent systems, such as 9:1 CHCl₃: MeOH, 5% v/v Et₃N, the retention times of the two compounds were indistinguishable. Removal of the phosphonic acid side chain at the C-N bond to afford biotin results when the reaction is allowed to exceed the time required for maximum conversion of phosphonic acid 5 to BP1.

I resolved these synthetic issues by carefully monitoring the saponification reaction by ¹H NMR. A small aliquot was taken from the reaction flask every 20 minutes and evaporated. The concentrate was dissolved in D₂O, and a ¹H NMR was generated on a high-resolution spectrometer. I monitored the change in the chemical shift of the valeric acid α protons and stopped the reaction when the integral ratio of product to starting material was greatest. Because the reaction proceeds towards the production of biotin as long as the reaction mixture is basic, the solution was immediately neutralized then lyophilized. In previous instances, the water was removed by evaporation in vacuo at high temperatures, contributing to partial decomposition of the desired product. By
making these minor yet necessary changes, I increased the yield of BP1 from 59% to 68%.

2.3 HPLC Isolation of Carboxyphosphate Reaction Intermediate Analog

Target compound BP1 was formerly purified by recrystallization, which often affords a diminished yield of the desired compound due to the high temperatures employed. Because BP1 is unstable at elevated temperatures, an alternative purification method was needed. We anticipated isolation by HPLC coupled with the modified saponification experimental procedure would further increase the yield of reaction intermediate analog 1.

The complexes of biotin with streptavidin and its structural homologue avidin are known to be the strongest ligand-protein complexes, with measured binding constants of $1.7 \times 10^5$ and $2.5 \times 10^{13}$, respectively. The high affinity binding of these two systems has found many applications in affinity chromatography, and it is most commonly the technique of choice for the purification of biotin derivatives. Contrarily, we decided to try another HPLC method for the isolation of compound 1-reverse phase chromatography. As simple as the concept seemed, to our knowledge, isolation of biotin or biotinylated compounds had not been achieved without the employment of an affinity column.

With the aid of my colleague Jorge Escobedo, reverse phase analysis of BP1, was performed with H$_2$O and MeOH. Composition of the eluent was isocratically changed from 100% H$_2$O for 10 minutes to 100% MeOH over a 30-minute time period. The UV detector was set at 205 nm and 240 nm. The chromatogram generated (Figure 6) three peaks of interest, with retention times of 9.17, 11.83, and 41.0 minutes, respectively.
These fractions were immediately lyophilized upon collection. The fraction at 11.83 minutes was confirmed by $^1$H NMR to be biotin. Therefore, we believed some degree of decomposition occurred. Interestingly, the sample from which the fractions were isolated was allowed to sit at basic pH overnight before being injected. A proton NMR of the BP1 standard from which the sample was prepared, however, contained all significant peaks, specifically the phosphonic acid α protons at $\approx$2.5 ppm. In addition, the $^{31}$P NMR showed one single peak at approximately 15 ppm. This result supported our original hypothesis that the basicity of the solution causes removal of the phosphonic acid side chain. The NMR spectrum of the eluent at 9.17 minutes was also identified as a decomposed BP1 product, but to a lesser degree than 11.83 minute eluent. In contrast to spectrum of the fraction identified prove to biotin; the α protons were observed, but they were broad and diminished in size and integral ratio. The peak at 41.0 minutes was not the desired product or any significant by-product, so no further characterization was performed.

Figure 6: Chromatogram of BP1 aqueous solution (pH 8) allowed to sit overnight.
A series of experiments were performed to obtain conclusive evidence of whether the pH was affecting the separation of BP1 or merely the appearance of the $^1$H NMR spectrum generated. Three BP1 aqueous solutions of varying pH’s were prepared. The initial pH of each solution was approximately 9. Using a 1.0M solution of HCl, the pH was adjusted to 5.5, 6.0, and neutral pH, respectively. The samples were injected immediately upon preparation. Using the same gradient conditions described above, the best resolved chromatogram was obtained from analysis of the pH 6 solution. This chromatogram is shown in Figure 7. For this particular case, the peaks of interest were at 9.0, 10.5, and 16.8 minutes. The 9.0 minute peak was determined to be insignificant, or a “ghost peak”. The NMR spectrum of the 10.5 minute fraction beared the closest resemblance to that of BP1, with the exception of the two characteristic $\alpha$ protons. The spectrum of the 16.8 minute peak overlayed with that of biotin with the exception of the amide protons, which have a chemical shift of approximately 6.5 ppm. Thus I could not yet conclude that BP1 was successfully isolated by HPLC.

![Figure 7: Chromatogram of BP1 aqueous solution (pH 6).]
A $^1$H NMR of the standard BP1 solution (pH 8) was taken. The alpha protons were visible at the correct chemical shift and integrated properly. The pH of the same sample was adjusted to 6; then another spectrum was generated. The two peaks of interest were no longer present. In fact, the spectrum overlayed with that of the 10.5 minute fraction isolated from the pH 6.0 solution (Figure 7). The sample pH was then elevated back to $\approx$8.5, using saturated NaHCO$_3$. A final NMR was obtained, which was analogous to the spectra of the BP1 standard. Hence, isolation of BP1 by reverse phase HPLC was accomplished. I concluded that the absence or presence of the $\alpha$-protons in the NMR spectrum is the result of an ionization effect.
Chapter Three
Bioactivity

3.1 Effect of CABI on Acetyl CoA Carboxylase Activity

Obesity is characterized by an increase in the number and size of adipocytes. During adipogenesis, the activities of several lipogenic enzymes such as acetyl CoA carboxylase, fatty acid synthase, and ATP citrate lyase are increased. The elevated regulation of these enzymes suggests they could be targets for anti-obesity agents. It has been demonstrated recently that mice treated with inhibitors of fatty acid synthase resulted in decreased food intake and weight loss. The hypothesis that acetyl-CoA carboxylase could be a target for anti-obesity agents was strengthened by a recent study demonstrating that mice lacking the gene coding for the mitochondrial isoform of acetyl-CoA carboxylase lost weight despite eating more food.

A bisubstrate analog inhibitor of carboxyltransferase 6, the component of acetyl CoA carboxylase, which transfers carbon dioxide from biotin to acetyl CoA to form malonyl CoA, was synthesized by our collaborators Keith Levert and Grover Waldrop of Biological Sciences at Louisiana State University. Synthesis of this compound involved attack of the primary sulfur of coenzyme A to displace the chlorine on compound 3. Since human acetyl CoA carboxylase is now a target for anti-obesity drugs, the question arose as to whether the bisubstrate analog could inhibit mammalian acetyl CoA carboxylase and in turn reduce lipid accumulation. Unfortunately, the bisubstrate analog lacks the hydrophobic character needed to permeate a cell membrane. However, the chloroacetylated biotin derivative (CABI), or compound 3 is sufficiently hydrophobic to diffuse across the cell membrane. Although the mechanism is not yet fully understood, it is believed that CABI is able to permeate the cell membrane via passive diffusion,
owing to its hydrophobicity. Once inside the cell, 3 reacts with endogenous coenzyme A to form the bisubstrate analog, compound 6 (Figure 8), and thus inhibits acetyl CoA carboxylase.

![Compound 6](image)

**Figure 8:** Bisubstrate analog inhibitor of carboxyltransferase.

To aid in elucidating the mechanism by which CABI reacts with coenzyme A to inhibit acetyl CoA carboxylase activity, I have synthesized an adduct of CABI (Figure 9). Compound 7 is obtained in 75% yield via reacting biotin (0.25g, 0.75 mmol, 1 equiv), Et$_3$N (0.32 mL, 2.24 mmol, 3 equiv), and acetyl chloride (0.081, 1.1 mmol, 1.5 equiv) under the same conditions employed to produce chloroacetylated compound 3.\textsuperscript{10} It will be used to determine if displacement of the chlorine by the primary sulfur of coenzyme A is the key mechanistic step.
Our collaborators also performed a series of inhibition experiments to provide support for the CABI-CoA hypothesis. To test the ability of CABI to reduce acetyl CoA carboxylase activity, Levert and Waldrop treated 3T3-L1 preadipocytes with a 10 µM solution of CABI in DMSO solution or a DMSO control for 4 hours. The whole cell extracts were prepared and immediately used to measure acetyl CoA carboxylase activity by analytical reverse phase HPLC. Treatment of preadipocytes with CABI resulted in a 79% reduction of enzyme activity. As shown in Figure 10a, the specific activity for adipocytes treated with DMSO for 4 hours was 1.40 nmol malonyl CoA/min/mg protein. The activity significantly decreased to 0.30 nmol malonyl CoA/min/mg protein when 3T3-L1 cells were treated with the 10 µM CABI of CABI in DMSO for the same period of time.

Professor Jackie Stephens, also of the department of Biological Sciences at Louisiana State University, analyzed the same cytosolic extracts for the expression of acetyl CoA carboxylase protein levels using streptavidin HRP (Figure 10b). These results clearly demonstrate that the reduced activity of acetyl CoA carboxylase is not due to altered expression levels of the enzyme. In other words, CABI treatment of 3T3-L1 preadipocytes results in decreased acetyl CoA carboxylase activity, but not protein levels, in isolated cell extracts.
Figure 10: (A) The specific activity of preadipocytes treated with DMSO (control) or a 10 µM solution of CABI in DMSO. (B) CABI shows no effect on protein levels of preadipocytes as measured by streptavidin HRP.

The suggestion that CABI-CoA is formed from the reaction of CABI with endogenous coenzyme A was further strengthened by the fact that CABI had no effect on acetyl CoA activity in isolated cellular extracts. The bisubstrate analog 6, was indeed able to inhibit acetyl CoA carboxylase activity when added to isolated cellular extracts in
vitro. The inhibition constant of CABI-CoA for bacterial acetyl CoA carboxylase was determined to be 23 µM. Detection of CABI-CoA in extracts treated with CABI would provide conclusive evidence for the mechanism proposed. Unfortunately, Levert’s attempts to isolate the bisubstrate analog have been unsuccessful using HPLC absorption optics. Waldrop and coworkers have begun studies to detect compound 6 by more sensitive methods.

3.2 Effect of CABI on Adipogenesis and Lipid Content

In addition to demonstrating that treatment of 3T3-L1 cells with CABI inhibits the activity of acetyl CoA carboxylase, our collaborators examined the effects of CABI on the adipogenesis of these cells. Adipogenesis is coincident with expression of several key transcription factors. To assess the adipocyte differentiation expression, Stephens analyzed PPARγ (Peroxisome Proliferator Activated Receptors), STAT1, and STAT5A (Signal Transducers and Activators of Transcription) by Western Blot Analysis. All three markers decreased in cells treated with 17µM and 8µM solutions of CABI in DMSO (Figure 11). Untreated and control cultures showed no decrease in these markers.

STAT3 is a protein whose expression is not substantially regulated during differentiation. As shown in Figure 11, the level of STAT3 was not affected by CABI at any concentration. This observation was significant, because it suggests that CABI is not acting as a non-specific alkylating agent. Moreover, the fact that the level of acetyl CoA carboxylase did not decrease with CABI treatment further indicates that CABI is not exerting a general toxic effect.
Figure 11: CABI blocks the induction of adipogenic transcription factors in a dose dependent manner. Whole extracts were prepared one week after induction of differentiation in the presence of various doses of CABI, as indicated at the top of the figure. For each condition, the cells were cultured in the presence or absence of CABI or DMSO, and the cells were treated every 24 hours. One hundred µg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western Blot Analysis. The molecular mass of each protein is indicated to the left of the blot in kilodaltons.

Exposure of differentiating adipocytes to 17µM or 8µM solutions of CABI in DMSO also blocked induction of lipid accumulation in a dose-dependent manner as measured by Oil red O staining (Figure 12). It should be noted that the inhibition of CABI was reversible. If the addition of compound 3 was not repeated every 24 hours, the cells began to differentiate.
Figure 12: CABI inhibits lipid accumulation in differentiating 3T3-L1 cells. Oil red O staining was performed on 3T3-L1 cells one week following the induction of differentiation. Cells were induced to differentiate at 2 days post-confluence and CABI was replaced with DMEM. The cells were treated every 24 hours with a fresh solution of CABI or DMSO. Each plate was treated with the following concentrations of CABI: (A) 0 µM, DMSO control; (B) 4 µM; (C) 8 µM; and (D) 17µM.

These results presented by Levert, Waldrop, and Stephens\textsuperscript{28} are the first demonstration of a link between a pharmacological modulation of cytosolic acetyl CoA carboxylase and inhibition of adipogenesis. These studies support the gene knockout experiments in mice, which indicated that acetyl CoA carboxylase is a very promising target for antiobesity agents.\textsuperscript{26} The ability of CABI to prevent weight gain in mice fed high-fat diets is being investigated by biologists at Pennington Biomedical Research Center. To aid in these experiments, I synthesized 40 grams of compound 3. Currently, we are searching for alternative solvents and trying to synthesize a more soluble adduct.
for these animal studies. It is our desire to examine the effects of CABI on other types of cells in which acetyl CoA carboxylase is up-regulated, such as in breast cancer cells.29

3.3 The “RNA World” Hypothesis

For centuries, scientists have been wrestling with the challenge of formulating a plausible scenario for the origin and early evolution of life. How did a self-replicating assembly of molecules emerge on early earth and give rise to cellular life? One hypothesis is that early life was based on RNA.30, 31 That is, the first biocatalysts on Earth were not made of enzymes but were made of RNA or a very similar polymer.

The appeal of the “RNA World” hypothesis is that catalytic RNA molecules, which could have served as their own genes, would have been much simpler to duplicate than proteins. According to this theory, RNA promoted reactions required for life with the help of metals, pyridines, amino acids, and other small-molecule cofactors. As metabolism became more complex, RNA developed the ability to synthesize coded polypeptides that served as more sophisticated cofactors. DNA later replaced RNA as the genetic polymer, and protein replaced RNA as the prominent biocatalyst.

In a collaborative effort with Professor Anne Grove and Steven Wilkinson of Biological Sciences at Louisiana State University, we are currently exploring another aspect of the “RNA World” hypothesis- CO2 fixation. If the early atmosphere was abundant in carbon dioxide, as many biologists believe, then an RNA molecule(s) that can catalyze the carboxylation of biotin, the carrier of CO2, would have been essential for the existence of early life. Typical metabolic reactions involve small molecules that are not attached to RNA. Demonstrating that RNA can promote interesting reactions using a tethered substrate is difficult yet important in addressing the question of whether RNA
can catalyze a reaction involving small compounds. The ability to explore the repertoire of RNA catalysis dramatically improved with the development of randomization, selection, and amplification methods.

Ribozymes with new or enhanced activities can now be isolated from large libraries of ribozyme variants, and entirely new ribozymes can be isolated from large pools of random-sequence molecules.

Figure 13: SELEX process in which BP1 was used for the selection of RNA molecules that demonstrate the greatest potential to catalyze the carboxylation of biotin.
Compound 1, which mimics the carboxyphosphate intermediate in the carboxylation of biotin, is currently being used as a substrate to allow for the selection of RNA aptamers that likely possess the desired catalytic activity. I synthesized 500 mg of BP1 using the optimized synthetic and purification conditions previously described. The final product was submitted to our collaborators to be utilized to generate ribozymes that catalyze the carboxylation of biotin.

In a selection-amplification process known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment), a library of RNA aptamers with affinity for BP1 was isolated (Figure 13). First, BP1 was coupled to an aganose column in aqueous solution. During pre-selection, those RNA molecules possessing modest affinity for the tethered substrate were eluted from the column. Next, the RNA aptamers that bound tightest to the BP1-aganose column were amplified. This selection-amplification process was repeated until sequences with the desired activity dominated the pool. The ultimate goal is to screen these RNA sequences for their catalytic capabilities. Grove and Wilkinson are diligently working to accomplish this task. If we are successful in discovering a ribozyme(s) that can catalyze the carboxylation of biotin, we will have extended the known catalytic capabilities of RNA to include CO₂ fixation.

3.4 Potential of BP1 As A Potent HIV Protease Inhibitor

Human immunodeficiency virus (HIV) is the causative agent of AIDS (Acquired Immunodeficiency Syndrome). It encodes an aspartic acid protease, HIV PR, whose function is essential for proper viron assembly and maturation. Inactivation of HIV protease by either mutation or chemical inhibition leads to the production of immature, non-infectious viral particles. Thus inhibition of HIV PR is recognized as an important
therapeutic strategy for treatment of AIDS. Structural biochemical methods, nuclear magnetic resonance, and computational biochemistry are recent additions to the plethora of approaches used in the rational drug design and discovery process. High-resolution crystal structures of HIV protease and HIV PR/inhibitor complexes have stimulated the synthesis of potent protease inhibitors.

Figure 14: (a) Molecular model of BP1 (green) bound to the active site of HIV protease. (b) Position of BP1 in the flaps of the aspartic protease and its relation to Asp25/25' and Ile50/50' (Fig. con'd.)
The experimental crystal structure of recombinant native HIV PR was first reported by scientists from Merck. While studies initially disagreed about certain details of the structure, they all confirmed that HIV protease is a homodimer and that the active site closely resembles that of other known aspartic proteases. Figure 14a shows BP1 bound to the active site of the aspartic acid protease. Generation of this molecular model inspired us to explore the inhibitory effect of compound 1 on HIV PR.

Preliminary experiments were performed by Dr. Lui Marzilli, Chairman of the Department of Chemistry at Louisiana State University. He reported minimal inactivation of HIV PR by compound 1. Nevertheless, the reaction intermediate analog possesses several functional features that have been proven essential for the design of
effective protease inhibitors: a cyclic urea moiety, symmetry or pseudosymmetry, and a biotin scaffold. Hence, we believe, BP1 remains a strong anti-HIV drug candidate.

The two halves of HIV protease are topologically related by an approximate intramolecular C$_2$-axis. Each monomer consists of 99 amino acid residues. The active site triad (Asp25, Thr26, Gly27) is located in a loop whose structure is stabilized by a network of hydrogen bonds. The flap, which consists of residues 45-55, is a β hairpin that covers the catalytic site and participates in the binding of inhibitors and substrates. Crystal structures indicate that interactions of molecules with Asp25/25’ and Ile50/50’ are particularly critical for high affinity and specificity for the viral protease.

![Figure 15: DMP323, a potent symmetrical HIV protease inhibitor developed by DuPont Merck.](image)

In 1993, the DuPont Merck group succeeded in the development of a potent nonpeptide orally bioavailable HIV protease inhibitor, DMP323$^{43}$ (Figure 15). Design of this subnanomolar inhibitor was based on careful inspection of the high-resolution of X-ray structures available for HIV protease-inhibitor complexes.$^{44}$ In all of these complexes, the backbone amide hydrogens of Ile 50 and 50’ in the flaps of the enzyme are hydrogen bonded to the carbonyl oxygen of the inhibitors through a tetra-coordinated H$_2$O molecule.$^{44}$ This H$_2$O molecule is considered to play a critical role in closing the
“flaps” over the protease inhibitor and its presence has not been observed in mammalian aspartic proteases. The rationale underlying the development of DMP323 was to displace the H$_2$O molecule by the cyclic urea carbonyl oxygen. Indeed the conserved H$_2$O molecule was not seen in the HIV protease-DMP323 crystal structure. The structure of the protease/DMP323 complex reveals many stabilizing protein-ligand interactions. It shows that the carbonyl oxygens of the catalytic aspartic residues, Asp 25,25’, and the diol oxygens of the inhibitor are positioned to form a network of hydrogen bonds.

A magnified representation of the BP1-HIV PR complex (Figure 14b) shows the sites of interaction between compound 1 and the flap residues of the aspartic protease. Although energy minimization studies have not been performed, I believe that the cyclic urea oxygen of BP1 is similarly positioned to perform the important role of accepting two hydrogen bonds from the Ile residues. In fact, I propose that the modest inhibition reported by Marzilli is most attributed to this interaction. Understanding the relationship of BP1 to Asp 25 and 25’, which is less apparent from Figure 14b, is very likely the key to improving activity.

Initially, I concluded that the lack of activity of BP1 was directly related to the geometry of the compound. However, recent studies revealed that the symmetry of the HIV protease dimer has guided the design of twofold and pseudosymmetric compounds. Kempf et al demonstrated that high oral bioavailability can be achieved with pseudosymmetric molecules. One such compound, A-7703 (Figure 16) possessed sufficient aqueous solubility for intravenous administration and was examined in clinical trials (unpublished results). In the search for related inhibitors with improved bioavailability, A-80987 was produced. It retained submicromolar antiviral activity.
More importantly, this potent inhibition was accompanied by significant oral bioavailability in three animal species and in humans. Intense study of a series of A-80987 analogues has now yielded valuable insight into the relationship of chemical structure to antiviral activity, aqueous solubility, and hepatic metabolism. Application of these insights to compound design culminated in the discovery of ABT-538 (Figure 16), an HIV protease inhibitor with enhanced antiviral activity and exceptional oral pharmacokinetics.

\[ \text{Figure 16: Structures of pseudosymmetric bioavailable HIV protease inhibitors.} \]

The X-ray structure of the streptavidin-biotin complex revealed the importance of the urea oxygen of (+)-biotin as a superior hydrogen-bond acceptor. The excellent
hydrogen bond accepting ability of urea was the reason Han and coworkers incorporated this functional group into their cyclic urea series of HIV PR inhibitors.\textsuperscript{43, 49} It later occurred to them that (+)-biotin, composed of two \textit{cis}-fused five-membered rings with the important urea group, could be used as a scaffold for synthesizing anti-HIV compounds. Modeling suggested that the (+)-biotin derivatives can fit into the binding site of the protease, as demonstrated by the BP1-HIV PR complex. (Figure 14a). In addition, the sulfur atom in (+)-biotin may form a weak hydrogen bond with the aspartic acid residues (25 and 25\textsuperscript{\textprime}).\textsuperscript{50} Han recently reported preliminary results of the synthesis and biological activities of biotin-derived HIV PR inhibitors.\textsuperscript{51} They succeeded in developing a new class of compounds with good transitions from $K_i$ to antiviral IC\textsubscript{90} values. The most potent inhibitor of this series, shown in Figure 21, has $K_i$ of 0.5 $\mu$M and an IC\textsubscript{90} of 7 $\mu$M.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{structure.png}
\caption{Structure of the potent active HIV protease inhibitor from of a new class of biotin-derived compounds.}
\end{figure}

In recent years, scientists have reported the design and synthesis of high-affinity bioavailable inhibitors that lack a C\textsubscript{2} axis as well as potent anti-HIV compounds that possess a biotin scaffold. Hence, I conclude that the modest activity exhibited by our
pseudosymmetric biotin-derived analog (1) is a matter of size rather than geometry or functionality. I am convinced that extended substitution of the valeric acid and phosphonic acid side chains of BP1 will position the carbonyl oxygens closer to those of the flap residues Asp 25 and 25’. As a result, a stronger network of hydrogen bonds will be formed, which is essential to the stabilization of the inhibitor/protease complex.
4.1 Introduction

The separation of enantiomeric mixtures into individual optical isomers is one of the most challenging issues in analytical chemistry. Development of new methodologies for improved chiral separation is recognized as an important achievement by researchers in many areas of science, particularly, the pharmaceutical and agricultural industries. In the former case, many drugs are chiral and therefore the design of and synthesis of these compounds often involve a racemic mixture rather than a pure form of the drug. The problem is that one form of a racemic mixture may have a medicinal application while the other enantiomer may be very toxic, as in the case of thalidomide.\textsuperscript{52} These observations have resulted in the recent release of new FDA guidelines regulating the marketing of chiral drugs. Such regulation has influenced the potential growth in the chirotechnology industry, specifically in the area of chiral drug separations.\textsuperscript{53}

Early attempts to separate racemic mixtures into individual enantiomers employed naturally occurring chiral materials, such as wool\textsuperscript{54,55} and cellulose or other polysaccharides,\textsuperscript{56} in conjunction with standard separation methods. These early strategies of the 1920’s provided some degree of success for separation of racemic mixtures. However, isolations employing naturally occurring compounds are often complicated by their poor mechanical and chromatographic behavior.

Recent studies have demonstrated the use of chiral micelles as a viable alternative for achieving enantioselective separations. These studies have been performed primarily by use of capillary electrophoresis,\textsuperscript{57,58} more specifically micellar electrokinetic
chromatography (MEKC). In chiral recognition by this method, the micelles themselves can become the major site of interaction, or they can incorporate various chiral selectors which are added to the separation medium. Even these systems are limited in their applications. One major problem encountered with the use of normal micelles in MEKC is that the interactions of many analytes with these micelles are often too strong to allow adequate discrimination. Warner and coworkers previously hypothesized that a polymeric surfactant will have stronger chiral recognition properties than conventional (non-polymerized) chiral micelles.

Professor Isiah Warner has dedicated the past five years of his career to offering unique possibilities for chiral separations. During this time, the Warner research group has made significant advances and contributions to the scientific literature in this area of analytical chemistry. Currently, the specific aim of his research is to examine the structural parameters that may contribute to the enhanced chiral recognition using polymeric surfactants rather than micelles. The Warner and Strongin research groups will collaboratively assess the effect of polar head group type on the utility of the polymeric surfactant as a stationary phase in MEKC.

![Figure 18: Structure of biotin-derived chiral cationic monomer.](image)
The type of head group can provide drastic differences in the properties of the polymeric surfactant and thus in the elution window in the MEKC process. To date, Warner et al. have only explored chiral head groups which are derivatives of amino acids. Previous experiments have shown that these anionic surfactants have particular appreciation for separation of cationic and neutral analytes. However, there has been some difficulty with separation of anionic analytes. It is believed that this is related to charge repulsion between the analyte and the highly anionic chiral polymeric surfactant. Therefore, a logical extension of the current research is to evaluate cationic polymeric surfactants. To this end, we describe the synthesis of compound 8, a new chiral cationic biotion-derived monomer (Figure 18).

4.2 Synthesis of Biotin-Derived Chiral Cationic Monomer

The synthesis of 8 (Scheme 4) begins with the transformation of (+)-biotin to the corresponding undecylenyl ester in 52% via stirring DMF (40 mL), biotin (1.0 g, 4.1 mmol, 1 equiv), undecylenyl alcohol (1.02 mL, 5.11 mmol, 1.25 equiv), DMAP (0.05 g, 0.41 mmol, 0.1 equiv), HOBT (0.06 g, 0.41 mmol, 0.1 equiv), and DCC (0.93 g, 4.5 mmol, 1.1 equiv) at rt for 12 h. Chloroacetylated compound 9 was obtained by two different synthetic methods. The first procedure is based on Amspacher’s published procedure for highly regioselective acylation of biotin esters. Chloride 10 is afforded in 59% yield by careful dropwise addition of Et₃N (0.69 mL, 4.9 mmol, 3 equiv) and chloroacetylchloride (0.39 mL, 2.5 mmol, 1.5 equiv) in three portions (0.5 equiv every 12 h) to a solution of biotin undecylenyl ester (0.65 g, 1.6 mmol, 1 equiv) in CH₂Cl₂ at −78 °C; then the solution is warmed to rt. The alternative procedure was derived by Tanaji Talele, a post doc at Louisiana State University. This method involves stirring of ester 9
(0.85 g, 2.14 mmol, 1 equiv) in chloroacetylchloride (8.5 mL, neat) at 65 °C for 3 h. Compound 10 is obtained in 24% yield. This is a 50% decrease in the yield afforded by the original method. Although we have not yet optimized these conditions, the advantage of the new method is a rapid conversion time of several hours as opposed to 2 days. Subsequently, allowing 10 (0.45g, 0.95 mmol, 1 equiv) to stir in pyridine (5 mL, neat) at rt for 12 hr results in a 41% yield (13% overall yield) of the biotin-derived chiral cationic monomer.

Scheme 4: Preparation of chiral cationic monomer from (+)-biotin.
Chapter Five
Conclusion

5.1 Future Work

5.1.1 A New Synthetic Route to Carboxyphosphate Reaction Intermediate 1

In our efforts to improve the yield of the conversion of 5 to 1 (Scheme 3), it occurred to us that an alternative route to target compound 1 may be via a synthetic pathway which eliminates the saponification step completely. (Scheme 5). This method would reduce the number of steps required for the total transformation of (+)-biotin to BP1 from four to six. Chloroacetylated compound 11 has been synthesized and characterized by $^1$H NMR and mass spectrometry. (+)-Biotin (1.00 g, 0.409 mmol, 1 equiv) and chloroacetylchloride (10 mL, neat) were stirred at 65 °C for 5 hr to obtain 11 in 14% yield. The formation of phosphonate ester 12 has been confirmed by $^1$H NMR as well, however, further purification is required. Currently, the specific aim of our research is to produce BP1 directly from the hydrolysis of unprotected compound 12. Upon achieving this goal, the next task will be to fully characterize all intermediates and increase the efficiency of the modified synthesis.

5.1.2 Multisubstrate and Transition State Analogs

The current work in this area focuses on attachment of adenosine moieties to the 1’-N of (+)-biotin to form the structure(s) shown in Figure 5. Two strategies are currently being employed. The first method involves coupling of CABI (3) to the thio-derivatives AMP, ADP, ATP to form the corresponding multisubstrate analog (Scheme 6). The second procedure entails two reactions. Compound 3 first undergoes an Arbuzov reaction to form an ethylchlorophosphonate adduct. This product is then reacted with the adenosine salt to form the desired multisubstrate analog (Scheme 7). Further work
on these schemes is required; therefore, yields have not been reported. We are researching the most efficient synthetic and analytical conditions that will provide us with these compounds in greatest yield.

Scheme 5: Alternative route to compound 1 from (+)-biotin.

Scheme 6: Proposed reaction scheme for the attachment of adenosine moieties to the 1-N of biotin.
Scheme 7: Proposed synthesis of multisubstrate analogs which involves the preparation of adenosine salts.

Work in progress also includes the design and synthesis of transition state analogs to complement our reaction intermediate analog work (Figure 19). The development of these compounds may be applicable to our collaborative research project with Professor Anne Grove to support the “RNA World” hypothesis. Isolation of RNA aptamers based on their ability to bind transition-state analogs is a current approach to the conversion of ribozymes to forms that use free small-molecule substrates.\textsuperscript{62,63}

Figure 19: Structures of proposed transition state analogs.
The development of analogs 19a and 19c is premature. The reaction conditions by which these compound will be synthesized are still being investigated. In contrast, an efficient route to transition state analog 19b has been derived (Scheme 8). The $^1$H NMR spectrum of the crude reaction mixture indicated that the desired compound was produced. Because the compound was not pure, the percent yield was not determined. In addition, mass spectrometry generated a peak at 443.2 (calculated 442.55). A mass peak of 459.4 was recorded as well. The calculated mass of the oxidized by-product is 458.6 g/mole. The ratio of this peak to the former was approximately 9:1. Future work includes optimizing reaction conditions that will decrease the amount of side product formed for sulfonate transition-state analog 19b. We will also investigate the best analytical method for isolating the two products.

**Scheme 8:** Preparation of transition state analog 19b from (+)-biotin.

5.2 Conclusion

In conclusion we have demonstrated the potential of reaction intermediate analog 1 and and other biotin derivatives in several areas. We have synthesized and studied BP1 and analogs for inhibition of HIV protease and acetyl CoA carboxylase as well as began the screening for ribozyme activity. We have synthesized CABI for in vitro adipogenesis and lipid content studies. These results have led to scale up of the CABI synthesis for antitobesity studies in mice. The synthesis of multsubstrate and transistion state analogs is
ongoing. These compounds could serve as leads to a series of more potent inhibitors, based on biotin carboxylase’s role in fatty acid synthesis. The transition state analogs may be especially useful in the selection-amplification process employed in ribozyme screening. We have also synthesized a chiral cationic monomer that will be utilized to provide alternative methods for analytical chiral separations.
Chapter Six
Experimental

6.1 Materials and Methods

Biotin was purchased from Bioworld. All other reagents were purchased from Sigma or Aldrich. All nonaqueous reactions were carried out under N\textsubscript{2} or Ar in flame dried glassware. CH\textsubscript{2}Cl\textsubscript{2} and TMSBr were distilled over CaH\textsubscript{2}. Anhydrous DMF and pyridine were purchased from Aldrich. Analytical thin-layer chromatography (TLC) was performed using general purpose 60-Å silical gel on glass (Aldrich). TLC plates were visualized with aqueous KMnO\textsubscript{4} or I\textsubscript{2}. Chromatography columns were packed with Kieselgel 60-Å silica gel 230-400 mesh (Sorbent Technologies). Proton (\textsuperscript{1}H) and carbon (\textsuperscript{13}C) NMR spectra were generated on a Bruker ARX400, 400 MHz and a Bruker ARX250, 250 MHz spectrometer. IR spectra were recorded on a Nicolet 320 FT-IR spectrometer. High-resolution mass spectrometry was provided by the Louisiana State University Mass Spectrometry Facility; spectra were recorded on a Finnigan MAT 900 with a PATRIC detector.

6.2 Experimental Procedures

6.2.1 Purification of 5-(6,8-diaza-7-oxo-6-(2-phosphonoacetyl)-3-thiabicyclo[3.3.0]oct-2-yl) pentanoic acid 1

For reverse phase HPLC analysis a C-18 column (Dynamax 60 Å, C18 83-221-C) was used as a stationary phase. The mobile phase was changed from 100% H\textsubscript{2}O to 100% MeOH over a 30 minute time period. Flow rate was 5 mL/min. MeOH was removed in \textit{vacuo} and the remaining filtrate was lyophilized.
### 6.2.2 Benzyl 5-[(3aS,4S,6aR)-1-acetyl-3-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoate 7

Benzyl ester 2 (0.25 g, 0.75 mmol) and Et$_3$N (0.32 mL, 2.24 mmol) are added to 10 mL of CH$_2$Cl$_2$. Acetylchloride (0.081 mL, 1.1 mmol) is added in three portions. The mixture is stirred and brought to –78 °C; then acetylchloride (0.027 mL, 0.37 mmol) is added. The mixture is stirred for 12 hours and allowed to warm to room temperature. This series of steps is repeated 2 more times. The reaction mixture is filtered and the filtrate is evaporated in vacuo. Then the resultant thick brownish oil is dissolved in CH$_2$Cl$_2$, absorbed on to silica, and purified by column chromatography. A solution of 4% MeOH, 48% hexane, and 48% EtOAc is used as the mobile phase to afford a yellowish oil (0.21 g, 75%). $^{1}$H NMR (DMSO-$d_6$) $\delta$ 7.90 (s, 1H), 7.35 (s, 5H), 5.08 (s, 2H), 4.75 (m, 1H), 4.12 (m, 1H), 3.29 (m, 1H), 2.95-2.85 (m, 2H), 2.49 (t, $J = 7.2$ Hz, 2H), 2.34 (s, 3H), 1.72-1.25 (m, 6H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 173.58, 170.22, 157.04, 137.14, 129.30, 128.85, 66.20, 61.85, 57.95, 55.58, 34.48, 34.11, 28.84, 25.26, 24.32; IR (thin film) 3252, 3131, 2936, 1684, 1375, 1251, 755, 699, 632; HRMS: found 377.40, calculated 376.47.

### 6.2.3 Undec-10-enyl 5-[(3aS, 4S, 6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoate 9

(+)-Biotin (1.00 g, 4.09 mmol), undecylenyl alcohol (1.02 mL, 5.11 mmol), DMAP (0.05g, 0.409 mmol), and HOBT (0.055 g, 0.409 mmol) are added to a 40 mL of DMF in a 250 mL 3-neck round bottom flask. The mixture is mildly heated while stirring until a homogeneous solution of reagents is achieved. DCC (0.93 g, 4.5 mmol) is added; then the reaction mixture is allowed to stir at room temperature for 12 hours. The reaction mixture is filtered and the filtrate was concentrated in vacuo. The resultant solid
is dissolved in CH$_2$Cl$_2$ and extracted three times with H$_2$O. The organic layer is dried over MgSO$_4$ and filtered. The organic filtrate is concentrated and the pale yellow solid is purified by flash chromatography with 5% MeOH, 95% EtOAc to obtain a white solid 9 in 52% yield. $^1$H NMR (DMSO-$d_6$) $\delta$ 6.42 (s, 1H), 6.35 (s, 1H), 5.74 (m, 1H), 5.02-4.91 (m, 2H), 4.35 (m, 1H), 4.11 (m, 1H), 3.98 (t, $J$ = 7.3 Hz, 2H), 2.01 (m 2H), 2.87-2.51 (m, 2H), 1.51-1.25 (m, 20H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 173.75, 163.56, 139.70, 115.52, 76.09, 64.54, 61.90, 60.04, 56.23, 34.05, 29.75, 29.48, 29.35, 29.13, 28.99, 28.88, 26.24, 25.43; IR (KBr) 3257, 2918, 2815, 1710, 1731, 1473, 1422, 1267, 1178, 901, 731, 658; HRMS: found 397.32, calculated 396.59

**Figure 20:** $^1$H NMR spectrum of 7.
Figure 21: Carbon 13 spectrum of 7.

Figure 22: $^1$H NMR spectrum of 9.
Figure 23: COSY NMR spectrum of 9.
6.2.4 Undec-10-enyl 5-[3aS,4S,6aR]-1-(chloroacetyl)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoate 10

Undecylenyl ester 9 (0.65g, 1.64 mmol) and Et₃N (0.69 mL, 4.92 mmol) are added to 20 mL of CH₂Cl₂. Chloroacetylchloride (0.39 mL, 2.46 mmol) is added in three portions. The reaction mixture is stirred at –78 °C and chloroacetylchloride (0.065 mL, 0.82 mmol) is added to the flask. The solution is allowed to stir at room temperature for 12 hours. This sequence of steps is repeated two more times. The crude reaction mixture was evaporated in vacuo and the resultant light brown oil is dissolved in CH₂Cl₂ and purified by flash chromatography with 4% MeOH, 48% EtOAc, 48% hexane. A yellow oil is furnished in 59% yield. Via an alternative method, compound 9 (0.85 g, 2.14 mmol) is added to chloroacetylchloride (0.85 mL, neat). The reaction mixture is allowed to stir at 65 °C for 3 hours. The solution is allowed to warm to room temperature then
diluted with CH₂Cl₂. While stirring, H₂O was carefully added over 3 minutes. Then the organic phase is washed twice more with H₂O and once with concentrated NaHCO₃. The CH₂Cl₂ layer is dried over MgSO₄ then filtered. The filtrate is evaporated in vacuo. The resultant yellow oil is dissolved in 4% MeOH 48% hexane 48% EtOAc and purified by column chromatography with the same solvent system. A yellow solid is afforded in 24% yield. $^1$H NMR (DMSO-$d_6$) δ 8.09 (s, 1H), 5.77 (m, 1H), 5.01-4.90 (m, 2H), 4.79 (m, 3H), 4.18 (m, 1H), 3.98 (t, $J = 6.5$ Hz, 2H), 3.13 (m, 1H), 3.03-2.87 (m, 2H), 2.28 (t, $J = 7.3$ Hz, 2H), 2.01 (m, 2H), 1.68-1.24 (m, 20H); $^{13}$C NMR (DMSO-$d_6$) δ 173.72, 166.32, 156.65, 139.69, 115.53, 64.56, 62.21, 58.87, 55.46, 44.84, 34.05, 29.76, 29.65, 29.49, 29.36, 29.13, 29.01, 28.85, 26.24, 25.31; IR (KBr) 3346, 2927, 2855, 1740, 1700, 1394, 1234, 1100, 795; HRMS: found 473.32, calculated 473.07.

**Figure 25:** $^1$H NMR spectrum of 10.
Figure 26: Carbon 13 NMR spectrum of 10.

Figure 27: $^1$H NMR spectrum of 8.
Chloracetylated compound 10 (0.45 g, 0.95 mmol) is allowed to stir in pyridine (5 mL, neat) at room temperature for 12 hours. The reaction mixture is concentrated in vacuo. The resultant yellow oil was dissolved in CH$_2$Cl$_2$ and purified by column chromatography in 10% MeOH 90% CH$_2$Cl$_2$ to afford a yellow solid in 41% yield. $^1$H NMR (DMSO-$d_6$) $\delta$ 9.0 (m, 2H), 8.75 (m, 1H), 8.43 (s, 1H), 8.31 (m, 2H), 6.0 (m, 2H), 5.80 (m, 1H), 5.01-4.83 (m, 2H), 4.78 (m, 1H), 4.30 (m, 1H), 3.99 (t, $J = 6.5$ Hz, 2H), 3.15 (m, 1H), 2.98-2.87 (m, 2H), 2.30 (t, $J = 7.3$ Hz, 2H), 1.95 (m, 2H), 1.64-1.25 (m, 20H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 173.73, 165.93, 156.74, 147.40, 139.68, 128.30, 115.52, 76.12, 64.56, 62.00, 59.48, 55.45, 34.05, 29.74, 29.64, 29.49, 29.36, 29.13, 29.00, 26.24, 25.31; IR (KBr) 3206, 3078, 2922, 2854, 1743, 175, 1653, 1634, 1327, 1254, 1187, 851, 768, 690; HRMS: found 516.41, calculated 516.40.
6.2.6 5-[3aS,4S,6aR]-1-(chloroacetyl)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid 11

(+)-Biotin (1.00 g, 0.409 mmol) was added to chloroacetylchloride (10 mL, neat) in a 50 mL round bottom flask. The reaction mixture was allowed to stir at 65 °C for 5 hours. The chloroacetylchloride was removed by vacuum distillation. The resultant milky suspension was dissolved in CH$_2$Cl$_2$ and washed three times with H$_2$O and once with saturated NaHCO$_3$. The organic layer was dried over MgSO$_4$. The solid was purified by column chromatography using 10% MeOH 90% EtOAc mobile phase to obtain a white solid in 15% yield. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.10 (s, 1H), 4.79 (m, 3H), 4.31 (m, 1H), 3.32 (m, 1H), 3.04-2.79 (m, 2H), 2.18 (t, $J = 7.3$ Hz, 2H), 1.78-1.23 (m, 6H); HRMS: found 321.3, calculated 320.79

Figure 29: $^1$H NMR spectrum of 11.
References


Vita

Latisha C. Johnson was born on July 30, 1977, in Shreveport, Louisiana, to the parents of Rev. Edward H. Johnson and Sandra C. Johnson. She is the second oldest of four children. After attending magnet schools from kindergarten through twelfth grade, Latisha graduated from Caddo Parish Magnet High School in 1995. It was there at Caddo Magnet High that she developed an interest in chemistry and realized she wanted to establish a career in this field. She received a Bachelor of Science degree in chemistry from Southern University in Baton Rouge, Louisiana in May of 1999. In the fall of the same year, she continued her education at Louisiana State University where she received a Master of Science degree in the Department of Chemistry in December of 2002. Ms. Johnson hopes to apply the experience and knowledge she has attained to research and development in the pharmaceutical industry.