DESIGN AND SYNTHESIS OF MEDIUM-RING LACTAM LIBRARIES
INSPIRED BY THE ANTITUMOR AGENT OCTALACTIN A

A THESIS IN
Chemistry

Presented for the Faculty of the University
of Missouri-Kansas City in partial fulfillment of
the requirements for the degree

MASTER OF SCIENCE

by

GE GAO

B.S., China Pharmaceutical University, China, 2005

Kansas City, Missouri

2009
ABSTRACT

With the aid of novel synthetic methodologies and new analytical techniques, studies on natural products opened a new era of pharmaceutical science. In many cases, these candidates are derivatives of the active compound of the natural product synthesis.

Octalactin A, a novel eight-membered lactone-containing natural product with potent cytotoxic properties, was isolated from marine bacterium, \textit{Pacifigorgia sp.}, which lives in association with a type of octacoral. Its isolation and structure determination were reported by Fenical and Clardy in 1991.\textsuperscript{1} In 1994, the first total synthesis of octalactin A was achieved by Buszek.\textsuperscript{2} The Buszek laboratory published another approach to octalactin A via the ring closing metathesis (RCM) method in 2002\textsuperscript{3} and continued the research on the novel medium-ring libraries. The research presented in this thesis is focused on the synthesis of a speculative eight-membered lactam library inspired by octalactin A.
This abstract of 140 words is approved as to form and content.

Keith R. Buszek, Ph.D.
Associate Professor of Chemistry
College of Arts and Sciences
The undersigned, appointed by the Dean of the College of Arts and Sciences, have examined a thesis titled "Design and Synthesis of Medium-Ring Lactam Libraries Inspired by the Antitumor Agent Octalactin A" presented by Ge Gao, candidate for the Master of Science degree, and hereby certify that in their opinion it is worthy of acceptance.

Keith R. Buszek, Ph.D.
Department of Chemistry
06/05/09

Kathleen V. Kilway, Ph.D.
Department of Chemistry
08/17/09

Zhonghua Peng, Ph.D.
Department of Chemistry
08/17/09
# Table of Contents

ABSTRACT .................................................................................................................. ii

LIST OF SCHEMES .................................................................................................... vi

LIST OF ILLUSTRATIONS ......................................................................................... vii

ACKNOWLEDGMENTS .............................................................................................. viii

CHAPTER

1. INTRODUCTION ....................................................................................................... 1

   1.1 Introduction ........................................................................................................ 1

   1.2 Background ......................................................................................................... 4

       1.2.1 Structures of the Octalactins .................................................................. 4

       1.2.2 First Total Synthesis of the Octalactin A ............................................. 5

       1.2.3 New Method of the Octalactin A Synthesis.......................................... 8

2. RESULTS AND DISCUSSION .................................................................................. 11

   2.1 Synthesis of the Scaffold for the Library ....................................................... 11

   2.2 Derivation of the Amine .................................................................................. 14

3. CONCLUSION .......................................................................................................... 18

4. EXPERIMENTAL SECTION .................................................................................. 20

REFERENCES .............................................................................................................. 29

VITA ............................................................................................................................... 32
# SCHEMES

<table>
<thead>
<tr>
<th>Scheme Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation of the Left-hand Segment of the Octalactins</td>
<td>5</td>
</tr>
<tr>
<td>Preparation of the Lactone</td>
<td>6</td>
</tr>
<tr>
<td>Preparation of the Vinyl Iodide</td>
<td>7</td>
</tr>
<tr>
<td>Preparation of Octalactins A and B</td>
<td>7</td>
</tr>
<tr>
<td>Preparation of Carboxylic Acid</td>
<td>8</td>
</tr>
<tr>
<td>Synthesis of Octalactin A through Ring Closing Metathesis</td>
<td>9</td>
</tr>
<tr>
<td>Retrosynthetic Analysis of the Scaffold</td>
<td>12</td>
</tr>
<tr>
<td>Scaffold Synthesis via RCM</td>
<td>13</td>
</tr>
<tr>
<td>Tertiary Amine Derivatives</td>
<td>15</td>
</tr>
<tr>
<td>Amide Derivatives</td>
<td>15</td>
</tr>
<tr>
<td>Sulfonamide Derivatives</td>
<td>16</td>
</tr>
</tbody>
</table>
ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Octalactin A, 1 and Octalactin B, 2</td>
<td>4</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

I would like to express my gratitude to my advisor, Professor Keith R. Buszek, for his dedicated support, patience, and continuous encouragement. He inspired me and led my discovery in the research field. This thesis could not have been written without his advice.

My thanks also go to the members of my committee, Professors Kathleen V. Kilway and Zhonghua Peng, for watching over my progress and for valuable support to my future career.

I extend many thanks to all my colleagues and friends, especially Dr. Neil Brown for his consistent enormous support, motivation, and time spent in helping me. Also, I need to thank Dr. Baohan Xie, Dr. Sampathkumar Ellapan, and Mr. Diheng Luo for their cooperation and help.

I would like to thank all the academic and technical staff in the Department of Chemistry at University of Missouri-Kansas City. They always have been cooperative and helpful.

Last, but not least, I would like to show my great appreciation to my parents, Huakang Gao and Manjing Zheng, for their support and dedication.
CHAPTER 1
INTRODUCTION

1.1 Introduction

As early as four thousand years ago, people in ancient cultures such as China, Egypt, and India started using "herbal medicines" as therapies for illness and disease. For most of this period, these herbal medicines were assumed to possess mystical powers of healing. Although herbal medicines are typically not widely used today by people in the West, many populations in the world still do use such entities in their daily lives. By the middle twentieth century, a revolutionary idea postulated that the individual chemical components in herbal extracts and other remedies interacted with biological molecules, such as proteins and nucleic acids, in order to exert their medicinal effect. As this theory was tested and developed, a new era of pharmaceutical science and medicinal chemistry began to emerge. As chemical structures of newly discovered bioactive compounds were elucidated, synthetic chemists were able to prepare analogues in order to enhance activity while simultaneously minimizing side effects.

Prior to 1980, most of the new drug candidates came from natural product sources. From 1981 to 2006, 63% of the 974 small-molecule new chemical entities (NCEs) approved by the FDA, mainly in the areas of anticancer and anti-infective agents, were derived from or inspired by natural products. With the introduction of high-throughput screening (HTS) and the concurrent development of combinatorial chemistry as a
potential new avenue for drug discovery, medicinally active compounds resulting from chemical methodology began to replace natural product leads.

Combinatorial chemistry is an approach that is designed for the solid-phase or parallel solution phase synthesis of large numbers of new and diverse chemical entities, usually by combining a small number of readily available reagents into all combinations using a small set of reactions. It permits the synthesis in a manner that has the potential to minimize labor and maximize the structural diversity of the resulting libraries. These libraries can then be screened against hundreds of biological assays to identify potential drug candidates. Merrifield disclosed the first solid-phase synthesis of peptides in 1963, but he did not ever make libraries with this technique. In 1984, Geysen explored this technique further by creating different peptides on separate supports. Researchers usually aim to create focused or speculative libraries. Focused libraries are designed to enhance a particular biological response, whereas speculative libraries are purposely designed to elicit many different activities. In either type of library, a compound that possesses a provocative profile can be used to create a library of libraries in order to identify a lead candidate.

Combinatorial chemistry was not successfully utilized by the pharmaceutical industry until 1990. The numerically large, quality libraries with a practical scale of synthesis can be produced as the synthesis technology has advanced. New instrumentation, robotics, and extensive use of computers have efficiently controlled the
preparation and analysis of the great number of resulting data. Since the elucidation of
the chemical formula is a significant issue in order to avoid double hits, new techniques
and methods have also been applied in this field, thus accelerating and expediting the
process of drug discovery.\textsuperscript{14}

However, from these efforts only one drug, a multikinase inhibitor, Sorafenib,
emerged and was approved for clinical use by FDA for the treatment of kidney and liver
cancer.\textsuperscript{7} The promise of combinatorial chemistry appeared so limited while it has been
employed as an essential part of drug discovery for many years. One possible reason is
that the compounds that arise from library efforts typically lacked the stereochemical and
structural diversity found in natural products. Furthermore, natural products generally
have more complex chemical space coverage, which is the space extended by all possible
stoichiometric combinations of atomic nuclei, electrons, and topologies in molecules and
compounds.\textsuperscript{15} A more recent trend has been directed toward the synthesis of
diversity-oriented libraries that enhances chemical space coverage.\textsuperscript{16}

At the same time, natural products tend to have higher number of stereogenic centers,
greater level of non-aromatic unsaturation, and a lower number of aromatic moieties.\textsuperscript{17}
Last but not least, natural products are typically rich in heteroatoms like oxygen and
nitrogen, which are greatly found in human bodies as well. Above all, natural products
have rekindled the inspiration of templates for new generation of small molecule
chemical libraries.
1.2 Background

1.2.1 Structures of Octalactins

Since the majority of living organisms (e.g., terrestrial- and marine-derived plants and animals, bacteria, and fungi) has not ever been tested for biological activity, natural products hold enormous potential as pharmaceutical leads and as templates for drug development. For example, the search and identification of new natural products derived from marine organisms remain a very active area of research.

Octalactins A and B were isolated from the marine bacterium Streptomyces sp. in 1991 by Fenical and Clardy. Octalactin A (1) exhibits the potent cytotoxic activity against several tumor cell lines. By contrast, octalactin B (2), which differs only in having an enone as opposed to an epoxy ketone, is completely inactive in these same assays. The striking structural feature of the octalactins consists of the saturated eight-membered lactone framework, which is rarely found in naturally occurring compounds. Medium-sized rings in general and eight-membered rings in particular, are considered the most challenging to synthesize chemically and bio-synthetically.

![Figure 1. Octalactin A (1) and Octalactin B (2)](image)

The construction of such rings is considered most challenging and difficult due to
both entropic and enthalpic factors. As a result, it was widely accepted that saturated monocyclic eight-membered lactones in particular are difficult or impossible to be prepared directly from their corresponding hydroxy carboxylic acids. As a consequence, enormous efforts have been directed toward developing alternative strategies to address this challenge, on which the disconnection of acyl-oxygen bond seems like the simplest retrosynthetic approach.

1.2.2 First Total Synthesis of Octalactin A

Fenical and Clardy have provided the connectivity and relative stereochemical assignments of the octalactins. Buszek found that the synthesis of the eight-membered rings is greatly facilitated by the presence of substituent in the tether connecting the reactive ends of the seco acid. The absolute configurations of octalactin A and B were established in 1994 through the total synthesis of the natural (-)-octalactins by Buszek.

Methyl (S)-3-hydroxy-2-methylpropionate (3) was used as a starting material to prepare the left-hand segment (7) of the octalactins (Scheme 1). para-Methoxybenzyl (PMB) ether was used to protect the secondary alcohol.

![Scheme 1. Preparation of the Left-hand Segment of the Octalactins](Image)
The other enantiomer of Roche's ester, ethyl (R)-3-hydroxy-2-methylpropionate (ent-3), was used as a right-hand three-carbon unit. In the catalyst presence of a Ni(II)/Cr(II), the left-hand segment 7 was reacted with aldehyde 8 to give an inseparable alcohol mixture 9. Then, 9 was converted to the unsaturated seco acid 11, which could form the desired unsaturated eight-membered lactone through lactonization using the Corey-Nicolaou S-pyridyl ester method. However, the Buszek group used saturated hydroxyl carboxylic acid to prepare the trisubstituted eight-membered ring, avoiding the double bond in lactone, which was difficult to carry out reduction. Then, the key lactonization proceeded to afford the desired eight-membered lactone 12. Furthermore, aldehyde 13, a main skeleton of the octalactins, was accomplished in good yield by the desilylation of 12 and successive oxidation (Scheme 2).

Scheme 2. Preparation of Lactone 13

The side chain was synthesized as follows: the desired vinyl iodide 17, which is
common to all of our octalactins syntheses, was obtained from a sequence of reactions on L-valine 14.

\[
\begin{align*}
14 & \xrightarrow{1) \text{NaNO}_2, \text{HCl}} \xrightarrow{2) \text{LiAlH}_4} 15 \\
& \xrightarrow{3) \text{KOH, H}_2\text{O}} 16 \\
16 & \xrightarrow{1) \text{TMSC}≡\text{CLi}} \xrightarrow{2) \text{TBSCI, imidazole}} 17
\end{align*}
\]

Scheme 3. Preparation of the Vinyl Iodide

A separable mixture of diastereomers 18 was acquired by the Ni(II)/Cr(II)-mediated Kishi-Nozaki coupling of the aldehyde 13 with the vinyl iodide 17.\(^{26,27}\) The synthetic (-)-octalactin B (2) was provided in good yield from the oxidation and desilylation on mixture 18. Finally, the synthetic (-)-octalactin A (1) was furnished by epoxidation, oxidation, and deprotection\(^{29}\)(Scheme 4).

\[
\begin{align*}
17 & \xrightarrow{1) \text{TBHP, VO(\text{acac})}_2} \xrightarrow{2) \text{DMP}} \xrightarrow{3) \text{HF}} \xrightarrow{4) \text{DDQ, H}_2\text{O}} 18 \\
18 & \xrightarrow{1) \text{DMP}} \xrightarrow{2) \text{aq. HF}} \xrightarrow{3) \text{DDQ, H}_2\text{O}} 1
\end{align*}
\]

Scheme 4. Preparation of Octalactins A and B

Though this is the first total synthesis approach of octalactin A, the procedure is long and not necessarily open to library expansion.
1.2.3 New Method of Octalactin A Synthesis

In addition to the approach from saturated seco acid, the Buszek group alternatively synthesized octalactins via the formation of the eight-membered lactone moiety using ring-closing olefin metathesis (RCM).³

The desired carboxylic acid 23 was first synthesized starting from methyl (S)-3-hydroxy-2-methylpropionate (3) (Scheme 5).

![Scheme 5. Preparation of Carboxylic Acid](image)

The synthesis of the other alcohol part was similar starting from methyl (R)-3-hydroxy-2-methylpropionate (ent-13).²⁵ Through the intermediate primary alcohol ent-19, the Buszek group prepared the secondary allylic alcohols ent-20 in the same method described in former section. The component parts 23 and ent-20 were coupled to yield the precursor diene ester 24, which was used for the vital ring closure. The desired eight-membered lactone 25 was acquired in 86% yield, while the diene exclusively went through RCM in the presence of the Grubbs' Ru catalyst.³⁰ Via ring closing metathesis, generating the medium-sized ring effectively from simple precursors is a very notable
Lipinski’s rules\textsuperscript{31} have been investigated for decades to determine and develop better lead structure in drug discovery. It is a guiding principle to evaluate if a certain chemical compound has drug-like properties in the human body. Lipinski’s rule of five states that an orally active drug should follow at least three of the criteria including a molecular weight under 500 daltons, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and an octanol-water partition coefficient log $P$ of less than 5. Octalactins, as pharmacologically active compounds, inspired us to set up and optimize libraries based on criteria above.
Small molecule compound libraries based on medium-sized rings are unfamiliar because of the uncommon of medium ring natural products themselves and the difficulties to synthesize them. It can be seen from scheme 6 above, that the total synthesis of the octalactins is either a facile direct lactonization from the saturated seco acid or works via a ring-closing metathesis to afford oxocenes from the diene ester. Our original plan was to utilize either approach to construct the eight-membered lactone core, then adapt the synthetic route in Scheme 3 to generate vinyl iodides with other amino acids. However, both approaches to the core are still too long and not generally amenable for use in library synthesis. Accordingly, it was decided to retain the key structural element, namely, the eight-membered ring, and build a library based on this unprecedented scaffold.
CHAPTER 2
RESULTS AND DISCUSSION

2.1 Synthesis of the Scaffold for the Library

For the construction of library, Lipinski’s rules is also a guiding principle to incorporate drug-like features into the proposed library members in order to improve the chances of finding bioactive molecules during the screening phase of the program. The replacement of the oxygen atom with a nitrogen in the eight-membered ring would not cause violation to Lipinski’s rules. Since we need to increase flexibility to introduce additional functionality, which could be easily modified based on new library template, a closely related eight-membered amino acid-derived lactam scaffold, rather than the lactone one, appeared promising as a flexible chemotype.

With Lipinski’s rules in mind, we employed a convergent-divergent strategy of constructing lactam rather than lactone libraries via RCM. In the convergent phase, diversity is bestowed upon the template by three simple, commercially available building blocks including protected L-amino acids, benzaldehydes, and benzyl amines. In the divergent phase, we diversified the secondary amine in parallel fashion after the desired frameworks were available, producing compound libraries of amines, amides, sulfonamides, carbamates, and ureas (Scheme 7).
Scheme 7. Retrosynthetic analysis of the scaffold

The synthesis of the scaffold began with the tert-butyl ester salts (41-43) of the amino acids glycine, alanine, and phenylalanine. They underwent allylation by reacting the ester salts with CH$_2$=CHCH$_2$Br, NaHCO$_3$, and LiI$^{33}$ followed by Fmoc protection to give the esters 44-46 in 53-81% yield for two steps. Instead of using conventional ester hydrolysis strategy to unmask the carboxylic acid, which would result in significant racemization with the two chiral amino acids, we used the Et$_3$SiH in TFA/CH$_2$Cl$_2$ (1:1)$^{34}$ producing the desired acids 47-49 (Scheme 8).
Scheme 8. Scaffold Synthesis via RCM

A single racemate is utilized as the coupling partner instead of stereoisomer, in order to maximize the efficiency of synthesis and following biological screening on available compounds. After a mixture of the same amount of para-methoxybenzaldehyde (39) and para-fluorobenzylamine (40) was stirred in ether with the presence of anhydrous magnesium sulfate, vinyl Grignard reagent was added with BF₃•OEt₂ catalyst to produce the secondary allylic amine (37). The yield was 82% on a 10-gram scale.

Through the acyl chloride, a resin-bound triphenylphosphine could help accomplish the amidation to produce the requisite diene amides (50-52) in 64-87% yield in the most
effective way.\textsuperscript{36} When the amides were available, the key ring-closing metathesis reaction was carried out. No reaction was observed at room temperature or elevated temperatures up to 100 °C, even though RCM with esters in previous octalactins works at 40 °C.\textsuperscript{3}

A favorable result was observed in the acquisition of the 1:1 ratio diastereomeric (or racemic in the case of glycine) lactams 53-55. This was affected by heating diene amides 50-52 in refluxing toluene with either the Grubbs' first or second generation catalyst for 5 h. Neither diastereomer preference during the cyclization nor double bond migration was observed.

In order to optimize the yield, a continuous purge of nitrogen or argon gas was applied to get rid of the ethylene, driving the reversible reaction to completion. Furthermore, a 57-75% yield in a one to two gram scale, a significantly improved outcome,\textsuperscript{37} can be achieved if P(CH\textsubscript{2}OH)\textsubscript{3} as a ruthenium scavenger at the end of the reaction is utilized.

Finally, quantitative production of the secondary amine 56{1-3} can be attained by deprotection of the Fmoc group, which is carried out with TBAF in THF/MeOH (1:1).\textsuperscript{38}

2.2 Derivation of the Amine

Derivitization of the amine was performed by using the available key scaffolds with sufficient supply. Functional groups were selected based on their occurrence rates in pharmacophores, and this thesis includes the derivitization of tertiary amines (Scheme 9),
amides (Scheme 10), and sulfonamides (Scheme 11). We can even develop further diversity of the final structures by incorporating additional rings, functional groups, and stereocenters in these units.

Scheme 9. Tertiary Amine Derivatives

Scheme 10. Amide Derivatives
Resin-bound scavengers (e.g., trisamine) were employed to assist with isolation, and all of the compounds were further purified via chromatography for characterization and annotation purposes. Their identity and purity were confirmed by $^1$H NMR spectroscopy and high resolution mass spectrometry. The unoptimized yields for the total library ranged from 27-79%.

From the very beginning, all of these library members, as well as intermediate compounds, were synthesized by project members of the Buszek Group, including Dr. Neil Brown, Dr. Baohan Xie, Machiko Minatoya, Diheng Luo, and myself. Among glycine, alanine, and phenylalanine, I synthesized the latter two scaffolds 56{2} and 56{3} from starting materials 42 and 43, respectively. Then I finished synthesizing the sub-libraries from tertiary amine derivitization of alanine scaffold 58{2}{1-10}, amide
derivitization of alanine as well as phenylalanine scaffold $60{2-3}{1-10}$, and sulfonamide derivitization of alanine scaffold $62{2}{1-10}$. The NMR spectra for all these compounds can be found in our previously published paper.\textsuperscript{32} The other compounds were prepared by the colleagues in our group, and their hard work is appreciated in setting up this magnificent molecular library.

All these compounds have been submitted to the NIH Molecular Libraries Screening Centers Network (MLSCN) for evaluation with a broad range of assays.\textsuperscript{32} Also, biological screen with murine L1210 lymphocytic leukemia cell lines has shown inhibition of some randomly selected compounds from our library. Although their activities are lower than those of many common anticancer drugs, these validated compounds still confirm the success of our medium-ring library, especially considering the fact that there are no similar octalactin analogues to these compounds in the entire PubChem Compound Database.
The remarkable chemical diversity encompassed by natural products continues to be important to drug discovery. As exemplified by the recent review of Newman and Cragg, there is no doubt that numerous novel bioactive chemotypes awaiting discovery from both terrestrial and marine sources.

Efforts to expand the impact of natural chemical diversity on the drug discovery process depend on two main chemistry-driven paths. One seeks to simplify crude mixtures, as well as enhance the impact of minor components in assays, through the creation of fractionated natural-product libraries. Crucial breakthroughs in separation and structure determination technologies have made screening mixtures of structurally complex molecules easier.

The other approach uses the power of combinatorial synthesis to amplify the structural context in which the unique features of natural products are expressed. Those compounds that are obtained through diverted total synthesis, i.e., through methodology, were redirected from the original (and realized) goal of total synthesis, to encompass otherwise unavailable congeners.

The total synthesis of octalactins and the following design of medium-ring lactam libraries inspired by these lactones gave a good example for the confluence of these technologies mentioned above. It proved that in the pursuit for new drugs, exciting
molecules can be achieved by exploiting the amazing chemical diversity of natural products. As an eight-membered medium-sized ring, octalactins were extracted from a marine bacterium and considered not possible to synthesize from their corresponding seco acids until the Buszek group successfully achieved the total synthesis in 1994. As the absolute configurations of the octalactins A and B had been established at the same time, more synthesis strategies were supplied and the Buszek group alternatively reported another approach using the ring-closing olefin metathesis in 2002.3

Inspired by this RCM method, we developed a convergent-divergent approach to design and synthesize a novel medium ring library based on amino acid-derived lactam scaffold. This library possesses a diversity of topology, stereochemistry, and functionality. We can expect that progress in synthetic studies of medium-sized ring compounds will contribute to the fruitful achievements of active drugs in the future.
CHAPTER 4

EXPERIMENTAL SECTION

4.1 General Details

$^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectroscopy were performed in CDCl$_3$ unless otherwise noted with reference to residual solvent at $\delta$ 7.26 ppm and 77.0 ppm, respectively. Unless otherwise noted, all commercially obtained starting materials were used as received. Dichloromethane and toluene were distilled from calcium hydride under nitrogen prior to use. THF and diethyl ether were distilled from sodium benzophenone ketyl under nitrogen prior to use. Ring-closing metathesis reactions were performed under constant nitrogen sparging to remove ethylene formed during the reaction.

4.2 Representative procedures

General procedure for two-step allylation/Fmoc protection of amino-esters; (S)-$^t$er-$^{\text{t}}$-butyl-2-(((9$^T$-fluoren-9-yl)methoxy)carbonyl(allyl)amino)-3-phenylpropanoate (46): In a dry 250 mL round-bottom flask was added 4.12 g (16 mmol) of H-Phe-OtBu•HCl, followed by 5.40 g (64.3 mmol) sodium bicarbonate and 106 mg (0.79 mmol, 5 mol%) of lithium iodide in the dark. To this was added 60 mL of acetonitrile, and the suspension was heated to reflux with stirring. To the suspension was then added 1.44 mL (16.8 mmol) of allyl bromide, and the reflux was continued for 20 h, after which time the mixture was cooled to room temperature. To the cooled mixture was then added
a solution of 6.18 g (23.9 mmol) Fmoc-Cl in 20 mL of acetonitrile. The mixture was then stirred for 1 h. The mixture was then poured into 350 mL of diethyl ether and the resulting solution was washed three times with 100 mL of 1 M HCl, once with 100 mL of distilled water and once with 50 mL of brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to give the crude product as viscous oil. The crude material was then purified via column chromatography on silica gel using 20% ethyl acetate in hexanes as eluent to give 6.27 g (81%) of the title compound as a viscous oil.

(S)-tert-Butyl-2-(((9-fluoren-9-yl)methoxy)carbonyl(allyl)amino)propanoate (45): The above general procedure was applied to 1.45 g (8.0 mmol) H-Ala-OtBu•HCl to give 2.45 g (75%) of the title compound as a viscous oil.

tert-Butyl-2-(((9-fluoren-9-yl)methoxy)carbonyl(allyl)amino)ethanoate (44): The above general procedure was applied to 5.0 g (29.8 mmol) H-Gly-OtBu•HCl to give 6.21 g (53%) of the title compound as a viscous oil.

General procedure for removal of tert-Bu ester protecting group:

(S)-2-(((9-fluoren-9-yl)methoxy)carbonyl(allyl)amino)-3-phenylproanoic acid (49): In a 250 mL round-bottom flask was dissolved 4.68 g (9.7 mmol) 46 in 75 mL of dichloromethane. To the stirring solution was added 3.55 mL (22.2 mmol) of triethylsilane. To the solution was then added 30.0 mL of trifluoroactetic acid dropwise over 30 min. The solution was then stirred at room temperature for 2 h, after which time
the solution was concentrated under reduced pressure and immediately purified via column chromatography on silica gel using 5% methanol in dichloromethane as the eluent to give 4.10 g (99%) of the title compound as a viscous oil.

**(S)-(((9//-Fluoren-9-yl)methoxy)carbonyl(allyl)amino)propanoic acid (48):** The above general procedure was applied to 1.94 g (4.76 mmol) 45 to give 1.49 g (89%) of the title compound as a viscous oil.

**tert-Butyl-2-(((9//-fluoren-9-yl)methoxy)carbonyl(allyl)amino)ethanoic acid (47):** The above general procedure was applied to 6.21 g (15.8 mmol) 44 to give 2.51 g (47%) of the title compound as a viscous oil.

**N-(4-Fluorobenzyl)-1-(4-methoxyphenyl)prop-2-en-1-amine (37):** In a 500 mL round-bottom flask was added 4.85 mL (40 mmol) of para-anisaldehyde. This was dissolved in 150 mL of anhydrous diethyl ether with stirring. To this solution was added 6.04 mL (50 mmol) of 4-fluorobenzylamine dropwise over 2 min. To the stirring mixture was added 10 g anhydrous magnesium sulfate, and the mixture was stirred for 24 h at room temperature under nitrogen. At this time, the Schiff base solution was filtered through a sintered glass funnel into a 1000 mL flask, and the residue was rinsed with 3 x 30 mL of anhydrous diethyl ether. The solution was then concentrated at the rotary evaporator until a colorless oil of ca. 10 mL remained. The volatiles were then pumped off under high vacuum for 6 h. The flask was then sealed with a septum cap and purged with nitrogen. The crude Schiff base was then dissolved in 300 mL of anhydrous
tetrahydrofuran and cooled to -78 °C with a dry-ice acetone bath. To the cold solution was added 28.38 g of boron trifluoride diethyl etherate dropwise over 5 min. The solution was stirred for 2 min and then 120 mL (120 mmol) of a 1.0 M solution of vinylmagnesium bromide in tetrahydrofuran was added dropwise over 10 min. The mixture was allowed to warm to room temperature over a period of 6 h at which point TLC indicated complete reaction. The solution was then cooled to 0 °C and quenched with dilute sodium bicarbonate solution (150 mL of a 5 % aqueous solution) dropwise over 5 min. The biphasic mixture was transferred to a separatory funnel and diluted with 300 mL of diethyl ether, and the phases were separated. The organic layer was washed with 3 x 200 mL of 10 % aqueous sodium bicarbonate, then the desired product was extracted with 3 x 200 mL of 1.0 M HCl. The organic layer was tested for the presence of secondary amine via TLC and then discarded. The acidic layer was adjusted to pH 8 with solid sodium bicarbonate, and the resulting basic suspension was extracted with 3 x 200 mL of dichloromethane, dried over sodium sulfate, filtered, and washed with 3 x 20 mL of dichloromethane. This solution was then concentrated under reduced pressure to yield 8.91 g (82%) of the title compound as a pale yellow oil.

General procedure for two-step amide formation: (9H-Fluoren-9-yl)methyl allyl((2S)-1-((4-fluorobenzyl)(1-(4-methoxyphenyl)allyl)amino)-1-oxo-3-phenylprop-2-yl)carbamate (52): In a flame-dried 250 mL three-necked round-bottom flask under nitrogen was added 6.60 g (10.5 mmol) resin-bound triphenylphosphine
(polystyrene, 2% DVB). To this was added 100 mL of dry dichloromethane and the suspension was stirred at room temperature. To the suspension was then added a solution of 1.4962 g (3.50 mmol) of carboxylic acid 49 in 20 mL of dry dichloromethane, followed by dropwise addition of a solution of 606.4 mg (4.20 mmol) trichloroacetonitrile in 5 mL of dry dichloromethane over 2 min. The resulting suspension was stirred at room temperature for 4 h, after which time the suspension was filtered through a medium porosity glass sintered funnel into a 500 mL dry round-bottom flask containing a solution of 1.93 g (7.10 mmol) 5 and 1.83 mL (10.5 mmol) of diisopropylethylamine in 20 mL dry dichloromethane under nitrogen. The resin was washed three times with 20 mL of dry dichloromethane into the 500 mL flask. The resulting solution in the 500 mL flask was stirred at room temperature for 20 min, then poured into a 1.0 L separatory funnel and was washed with 3 x 150 mL of 1 M HCl and 1 x 75 mL of brine. The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude material was then purified via column chromatography on silica gel using 25% ethyl acetate in hexanes as eluent to give 2.07 g (87%) of the title compound as an off-white foam.

\[(9H-\text{Fluoren-9-yl})\text{methylallyl(}2S\text{-1-((4-fluorobenzyl)(1-(4-methoxyphenyl)allyl)\ amino)-1-oxopropan-2-yl) carbamate (51):}\] The above general procedure was applied to 1.49 g (4.24 mmol) 48 to give 2.18 g (87%) of the title compound as an off-white foam.
(9H-Fluoren-9-yl)methylallyl((2S)-1-((4-fluorobenzyl)(1-(4-methoxyphenyl)allyl)amino)-2-oxoethyl)carbamate (50): The above general procedure was applied to 1.0411 g (3.2 mmol) to give 3.57 g (64%) of the title compound as an off-white foam.

General procedure for ring-closing metathesis: (2S,Z)-(9H-Fluoren-9-yl)methyl 2-benzyl-4-(4-fluorobenzyl)-5-(4-methoxyphenyl)-3-oxo-2,3,4,5-tetrahydro-1,4-diaza cine-1(8H)-carboxylate (55): In a dry 1.0 L round-bottom flask under nitrogen was dissolved 2.07 g (3.04 mmol) of 52 in 700 mL of dry toluene. This solution was heated to reflux with constant nitrogen sparging and stirring. To the refluxing solution was added a solution of 135 mg (0.15 mmol, 5 mol%) Grubbs' 2nd Generation Catalyst in 5 mL dry toluene. The red-brown solution was stirred at reflux with nitrogen sparging for 5 h, during which time three portions of 60 mg (3 x 0.071 mmol) catalyst was added after each of the first 3 h. The brown solution was then cooled to room temperature. To the cooled solution was added a solution of 5.00 g (40.3 mmol) tris(hydroxymethyl)phosphine in 50 mL of methanol, followed by 0.3 mL of triethylamine. The solution was then stirred at room temperature overnight. The yellow solution was then poured into a 2.0 L round-bottom flask and diluted with 700 mL of distilled water. The resulting emulsion was rapidly stirred at room temperature for 30 min, after which time, the mixture was poured into a 2.0 L separatory funnel. The phases were separated, and the organic layer was washed with 200 mL of 1:1 distilled water:brine. The organic layer was then dried over anhydrous magnesium sulfate, filtered, and
concentrated under reduced pressure. The crude material was then purified via column chromatography on silica gel using a gradient of ethyl acetate in hexanes (10% to 35%) to give 1.49 g (75%) of the title compound as a white foam.

**(2S,Z)-(9H-Fluoren-9-yl)methyl-4-(4-fluorobenzyl)-5-(4-methoxyphenyl)-2-meth-yl-3-oxo-2,3,4,5-tetrahydro-1,4-diazocine1(8H)-carboxylate (54):** The above general procedure was applied to 1.30 g (2.15 mmol) of 51 to give 1.24 g (70%) of the title compound as an off-white foam.

**(Z)-(9H-Fluoren-9-yl)methyl-4-(4-fluorobenzyl)-5-(4-methoxyphenyl)-3-oxo-2,3,4,5-tetrahydro-1,4-diazocine1(8H)-carboxylate (53):** The above general procedure was applied to 2.11 g (3.65 mmol) of 50 to give 1.14 g (57%) of the title compound as a white foam.

General procedure for removal of Fmoc protecting group; 56 {1-3}: Into a 250 mL round-bottom flask was added a solution of the Fmoc protected amine (53-55) in THF (0.027 M). To this was added a 1.0 M solution of TBAF in THF (2 equivalents). The solution was then stirred for 3 min (complete by TLC) after which time an equal volume of methanol (with respect to THF) was added. The color changed from yellow to colorless. The solution was then stirred for 20 min, then diluted with a three-fold excess of diethyl ether (with respect to combined volume of THF and methanol). The solution was extracted with 3 portions of 1 M HCl (equal volume with respect to ether). The acidic layer was then neutralized with solid sodium bicarbonate, and the resulting
aqueous mixture was extracted with three portions of dichloromethane (equal volume with respect to aqueous layer). The dichloromethane layer was then dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the crude secondary amine, which was used without purification.

General procedure for tertiary amine derivitization; 58{1-3}{1-10}: Into a 20 mL vial was added a solution of the secondary amine 56{1-3} (0.054 mmol) in 1 mL of dichloromethane. To this was added 5 eq. of the aldehyde 57{1-10}. The mixture was stirred for 5 min, then 5 eq. of sodium triacetoxyborohydride (58 mg) were added. The mixture was stirred for 1 h, then subjected to a basic aqueous work-up. The crude tertiary amines were then purified via column chromatography in parallel.

General procedure for amide derivitization for 60{1-3}{1-10}: Into a 20 mL vial was added a solution of the secondary amine 56{1-3} in 1 mL of dichloromethane. To this was added 5 eq of DIEA, followed by 5 eq. of the acyl chloride 59{1-10}. The mixture was stirred for 1 h at room temperature, after which time 500 mg (10 eq.) of PS-trisamine (polystyrene, 1% DVB) was added, followed by 1 mL of dichloromethane. The suspension was stirred for 1 h at room temperature, after which time, the suspension was filtered and the resin washed with 3 x 2 mL of dichloromethane. The crude solution was concentrated under reduced pressure, and the crude products were purified via column chromatography in parallel.

General procedure for sulfonamide derivitization for 62{1-3}{1-10}: Into a 20 mL
vial was added a solution of the secondary amine 56{1-3} in 1 mL of dichloromethane. To this was added 5 eq of DIEA, followed by 5 eq. of the sulfonyl chloride 61{1-10}. The mixture was stirred for 1 h at room temperature, after which time 500 mg (10 eq.) of PS-trisamine (polystyrene, 1% DVB) was added, followed by 1 mL of dichloromethane. The suspension was stirred for 1 h at room temperature, after which time, the suspension was filtered and the resin washed with 3 x 2 mL of dichloromethane. The crude solution was concentrated under reduced pressure, and the crude products were purified via column chromatography in parallel.
REFERENCE


VITA

Ge Gao was born on October 13, 1982 in Huaibei, Anhui, China. He obtained his bachelor’s degree in pharmaceutical science from China Pharmaceutical University in 2005. Ge Gao decided to continue his graduation education at UMKC and started in chemistry in August of 2005. He had a Graduate Teaching Assistant appointment for two semesters followed by a Graduate Research Assistant appointment by Dr. Buszek from June, 2006. After one year’s graduate research assistant work under Dr. Buszek, he went back to teaching assistant position for another four semesters. During his study in UMKC, Ge Gao also served as a vice president of the UMKC Chinese Student and Scholar Association for three years.