THESIS

PROGRESS TOWARDS PROPOSED BIOSYNTHETIC INTERMEDIATES OF STEPHACIDIN A

Submitted by

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For the degree of Master of Science

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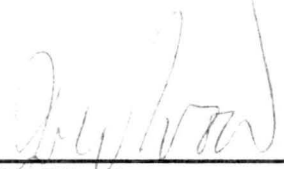
WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ANDREA GEISER ENTITLED PROGRESS TOWARDS PROPOSED BIOSYNTHETIC INTERMEDIATES OF STEPHACIDIN A BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate work



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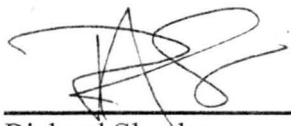

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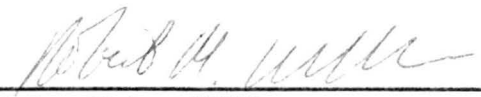

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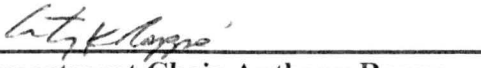

Department Chair Anthony Rappe

ABSTRACT OF THESIS

PROGRESS TOWARDS PROPOSED BIOSYNTHETIC INTERMEDIATES OF STEPHACIDIN A

Progress towards three potential biosynthetic intermediates of stephacidin A are presented. The first precursors has a 7-prenylated indole ring system, the second precursor has a 7-prenyl 6-hydroxy indole ring system, and the third precursor has a 6-hydroxy indole ring system.

The synthesis of the 7-prenyl indole precursor proved challenging. However, once addition of the reverse prenyl group had been achieved the synthesis proceeded without any further challenges. The remaining three steps of the synthesis should follow Williams group chemistry.

Once an efficient route to the starting material of the 7-prenyl-6-hydroxy and the 6-hydroxy precursors had been achieved, the synthesis progressed nicely. However, protecting group issues at the end of the 6-hydroxy precursor synthesis prevented the final product from being obtained. This also affected the efforts towards the 7-prenyl-6-hydroxy precursor indole. Once an adequate protecting group can be found, the syntheis of the 6-hydroxy precursor should follow group chemistry to completion.

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Chapter 1

Introduction

1.1 Isolation and Structural Determination

Birch and co-workers isolated brevianamides A, B, C, D and E (**1, 2, 3, 4, 5, 6**, Figure 1) between 1969 and 1973 from *Penicillium brevicompactum*.[1,2,3] The isolation of these metabolites began the ongoing study into prenylated indole alkaloids containing a bicyclo[2.2.2]diaazaoctane ring structure as their structural core. The structures of these new metabolites were determined by spectroscopic, degradative, and biogenetic evidence.[1] An X-ray crystal structure of 5-bromobrevianamide A provided the relative and absolute stereochemistry of brevianamide A.[4]

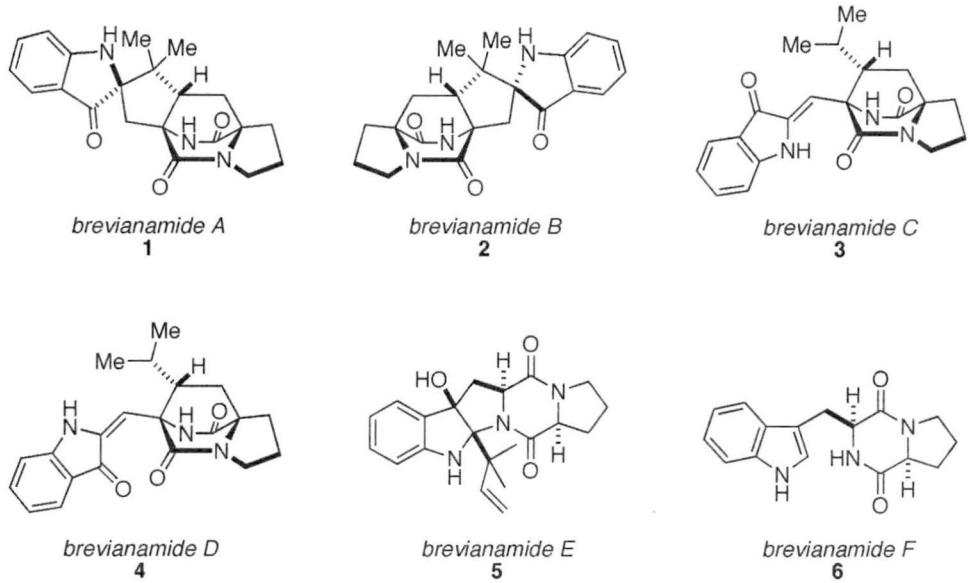


Figure 1: Brevianamides A-F.

Since 1973, the number of prenylated indole alkaloids have grown to over 40 secondary metabolites including the paraherquamides **7**, marcfortines **8**, notoamides **9,** avrainvillamide **(10)** and the stephacidins A **(11)** and B **(12)** (Figure 2).[5,6,7,8]

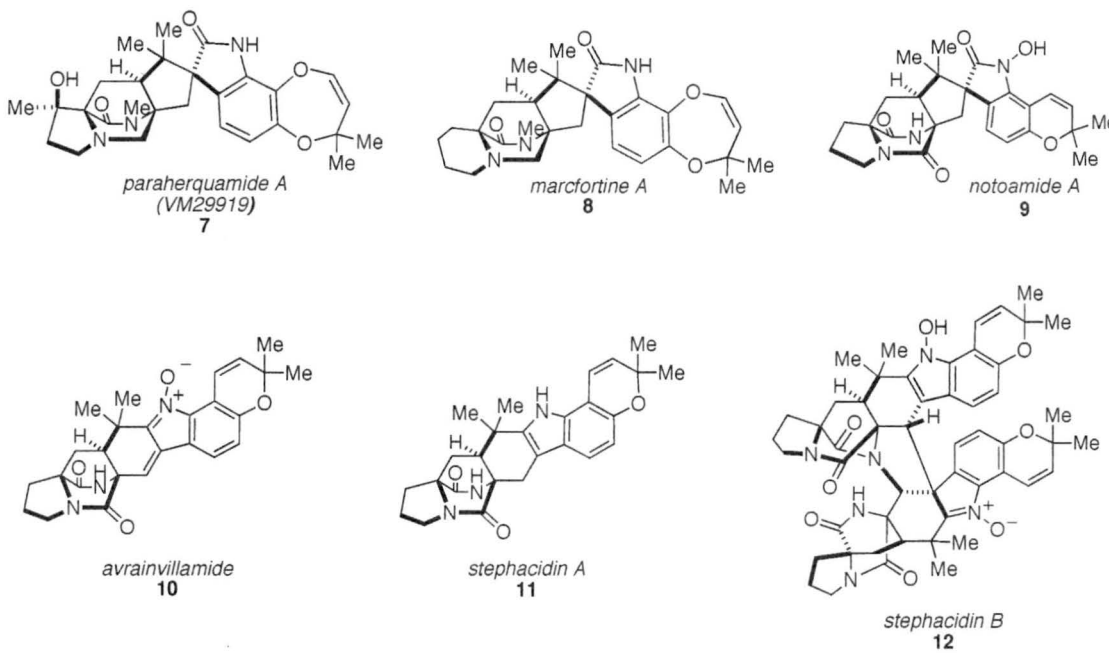
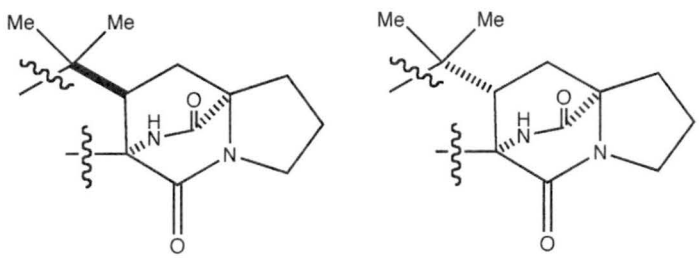


Figure 2: Various prenylated alkaloids.

These alkaloids are constructed from two isoprene units and a tryptophan piece.[1] They also contain unique functional groups that allow for the metabolites to be divided into various families. For example, the brevianamides consists of a diketopiperazine and a *spiro*-indoxyl ring system.[1] The paraherquamides **7** are also an important family of indole alkaloids and were considered the most structurally complex metabolites before the stephacidins were isolated.[5] The paraherquamides differ from the brevianamides in that their *spiro*-indole functionality is an oxindole ring instead of the brevianamides indoxyl moiety. It was also determined that the brevianamides have an *anti*-relationship **14** at the bicyclo core while the paraherquamides have a *syn*-relationship **13** (Figure 3).[9] The C-H proton at the bridgehead of the bicyclic core compared to the proline ring determines this *syn* v. *anti* relationship.



syn relationship of the paraherquamides **13**

anti relationship of the brevianamides **14**

Figure 3: *Anti* vs. *syn* relationship.

Another closely related family of metabolites are the marcfortines **8**.[6] Like the paraherquamides, the marcfortines have a monoketopiperazine and an oxygenated

dioxepin *spiro*-oxindole ring system. However, they differ from the paraherquamides in that they have a pyrrolidine ring system, while the marcfortines contain a piperidine.

A fourth family of metabolites are the notoamides **9**.[7] Like the previous three families the notoamides also contain a *spiro*-oxindole core. However, the notoamides are unique in that they have a pyran ring in place of the dioxepin ring found in the paraherquamides and the marcfortines.

In 2002 researchers at Bristol-Meyers Squibb isolated stephacidin A **(11)** and B **(12)** from the fungus *Aspergillus ochraceus* WC76466. The discovery of these two new metabolites only fueled the interest into prenylated indole alkaloids. The stephacidins, like the notoamides, contain an indole pyran ring system but are unique from the other families due to their 2,3-disubstituted indole, as opposed to the *spiro*-oxidole moiety. Stephacidin B shows greater structural complexity than stephacidin A. A rare indole oxidation state and an indole nitrone moiety that had not been found in natural products outside of these prenylated indole alkaloids was present in stephacidin B. The structure of stephacidin A was determined by 1D and 2D NMR (DEPT, COSY, HETCOR, HMBC, HMQC and NOE). Fragments of stephacidin B were structurally determined by 2D-NMR (COSY, HMBC, NOE and NOESY) however, the final structure was determined by an X-ray crystal structure.[8]

1.2 Pharmacology

These new metabolites have been isolated from various fungi including *Aspergillus* sp., *Malbranchea* sp., and *Penicillium* sp.. Several metabolites were found to exhibit various biological activities, with the brevianamides and paraherquamides

showing anti-insecticidal, anthelmintic and antinematodal properties.[9,10] The paraherquamides have shown activity against several drug-resistant strains of nematodes, which has led to an increasing interest for the use of the paraherquamides in veterinary medicine.[11]

The notoamides, isolated from *Aspergillus* sp., showed activity against HeLa (cervical cancer) and L1210 (lymphatic leukemia) cells.[12] The notoamides showed IC_{50} values in the range of 22-52 $\mu g\ mL^{-1}$.

Avrainvillamide **(10)** was found to exhibit antiproliferative effects in several human cancer cells. Myers and coworkers found that it works by binding to the nuclear phosphoprotein nucleophosimin at cystine-275. Nucleophosmin is thought to be an oncogenic protein that is overexpressed in tumors. Nucleophosmin is also known to regulate p53 a tumor suppressor. Restoration of p53 function could lead to tumor regression.[13]

Stephacidin A and B were found to be potent inhibitors to several human tumor cell lines. More specifically, the stephacidins showed *in vitro* cytotoxicity against prostate, ovarian, colon, breast and lung tumar cell lines (Table 1).

cell line	histotype	characteristic	Stephacidin A (IC_{50})	Stephacidin B (IC_{50})
PC3	prostate	testosterone-independent	2.1	0.37
LNCaP	prostate	testosterone-sensitive	1	0.06
A2780	ovarian	parental	4	0.33
A2780/DDP	ovarian	mutp53/bcl2+	6.8	0.43
A2780/Tax	ovarian	taxol-resistant	3.6	0.26
HCT116	colon	parental	2.1	0.46
HCT116/mdr+	colon	overexpress mdr+	6.7	0.46
HCT116/topo	colon	resistant to etoposide	13.1	0.42
MCF-7	breast	estradiol-sensitive	4.2	0.27
SKBR3	breast	estradiol-independent	2.15	0.32
LX-1	lung	sensitive	4.22	0.38

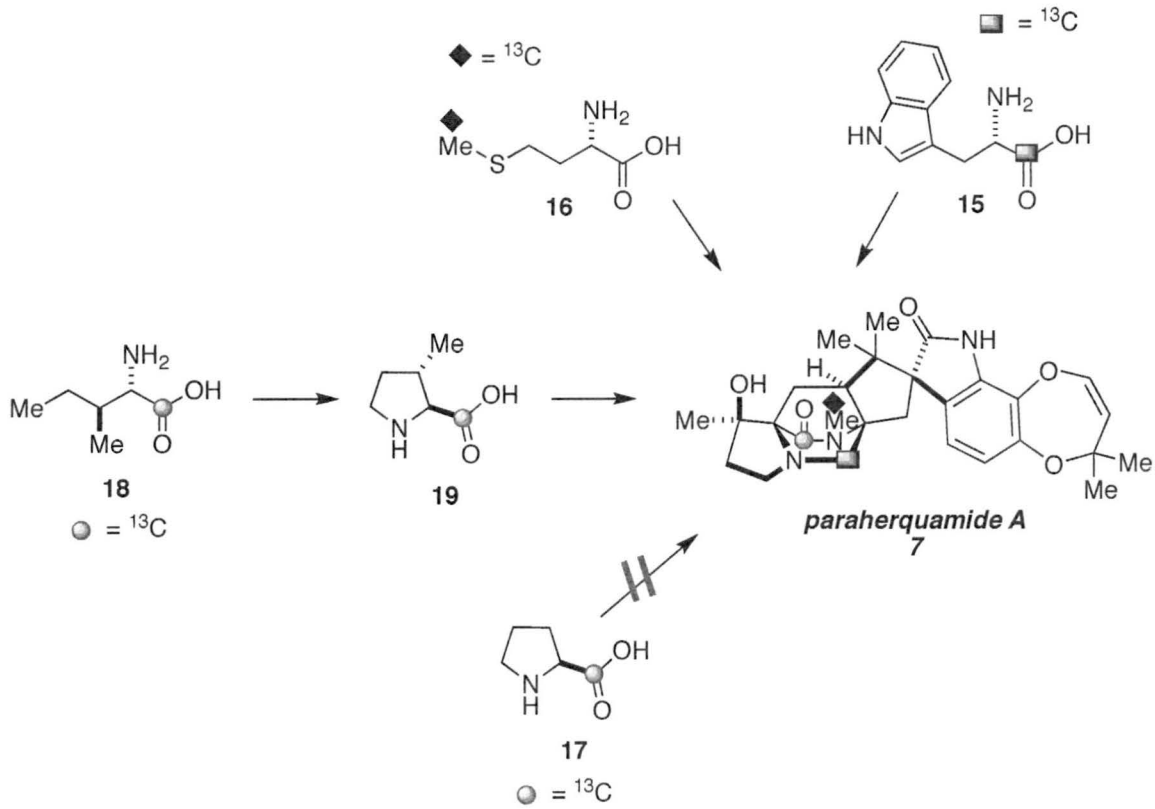
Table 1: Activity of stephacidin A and B

Stephacidin B **(12)** was found to be more potent and showed higher selectivity that stephacidin A **(11)**, especially against prostate dependent LNCaP cells. Stephacidin B **(12)** was shown to have a 5-30 fold higher activity than stephacidin A **(11)** with an IC_{50} value of 0.06 μM. Another finding that makes the stephacidins so interesting is that these metabolites appear to be inhibiting cell growth via a novel mechanism. The effects of the compounds are not mediated by known modes of action (p53, mdr or bcl2) and they are not tubulin or topoisomerase II-mediated.

1.3 Previous Biosynthetic Studies

Previous biosynthetic studies have led to a nearly completed biosynthetic picture with regards to how these metabolites are formed in nature. In order to determine the primary amino acid building blocks of paraherquamide A, several feeding experiments were preformed with *Penicillium fellutanum* (Scheme 1).[14] It was found that tryptophan **15**, methionine **16**, and isoleucine **18** incorporated into paraherquamide A **(7)**.

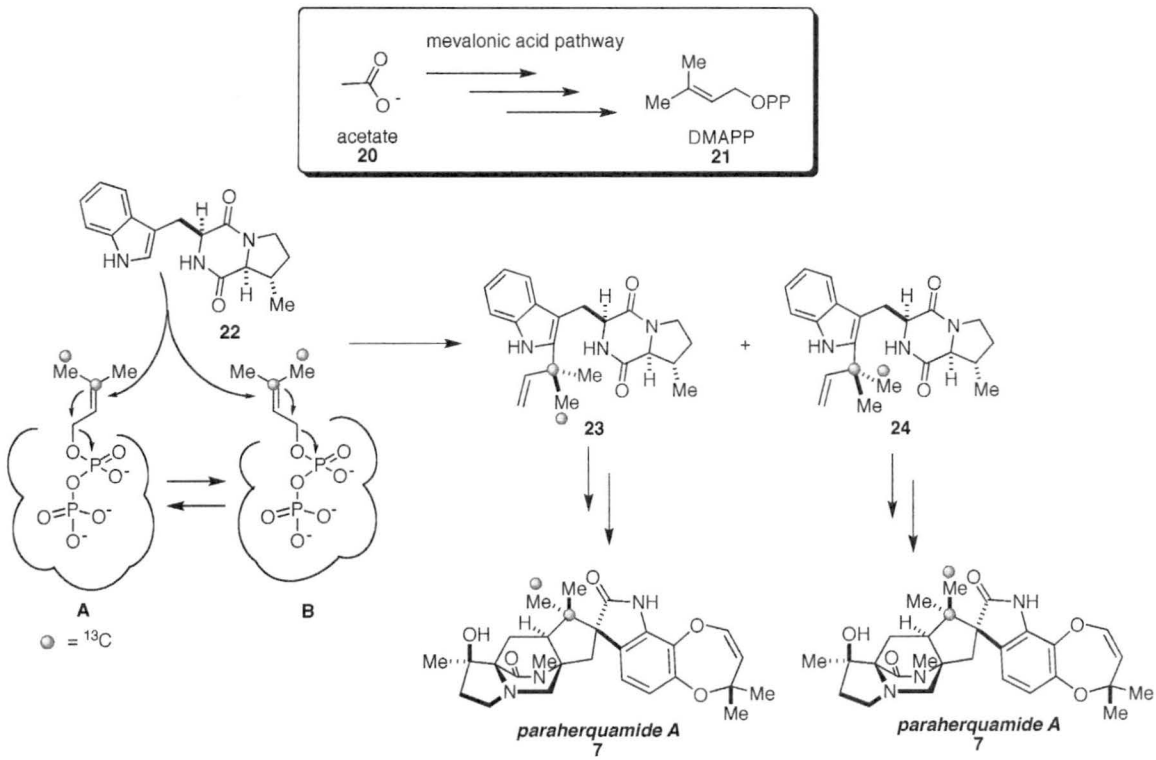
Tryptophan was found to incorporate into the oxindole ring at carbon 12, while the methionine incorporated at carbon 29. Proline **17**, interestingly, did not show any incorporation. This led to the conclusion that it is isoleucine and not proline serving as the precursor to the methyl hydroxy proline ring.



Scheme 1: Building blocks of paraherquamide A

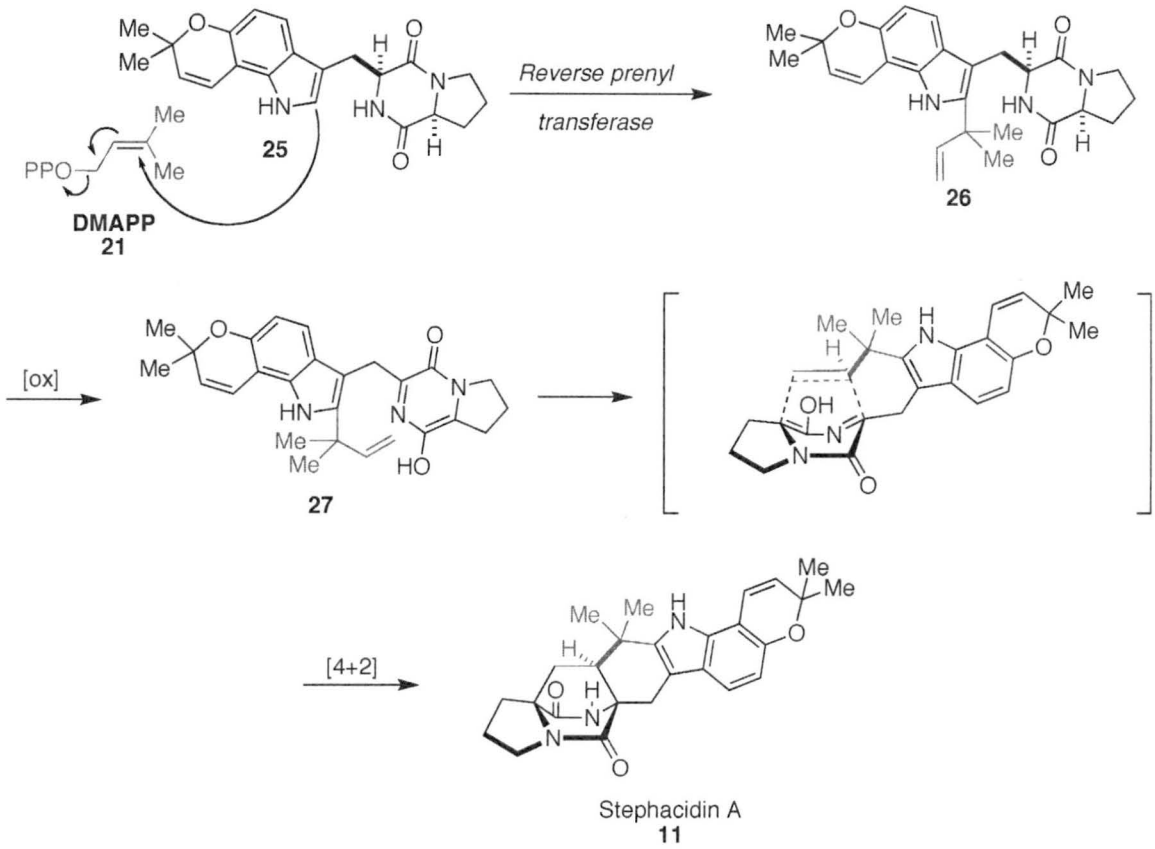
A second feeding experiment was completed on paraherquamide A **(7)** that established how the isoprene unit was incorporated into the molecule.[14] These studies showed that dimethylallypyrophosphate or DMAPP **21** is formed through the mevalonic acid pathway. Typically the DMAPP binds via a normal prenyl transferase. However, in this case, DMAPP appeared to be added as a reverse prenyl transferase (Scheme 2). The

binding of the DMAPP in this upside down manner allows for the indole **22** to attack the π bond of the DMAPP by an S_N2' mechanism. The pyrophosphate group is believed to be anchored in the enzyme active site while the hydrophobic isopropenyl moiety is presented in equilibrium between the two conformations **A** and **B**.



Scheme 2: Incorporation of the isoprene unit via an S_N2'.

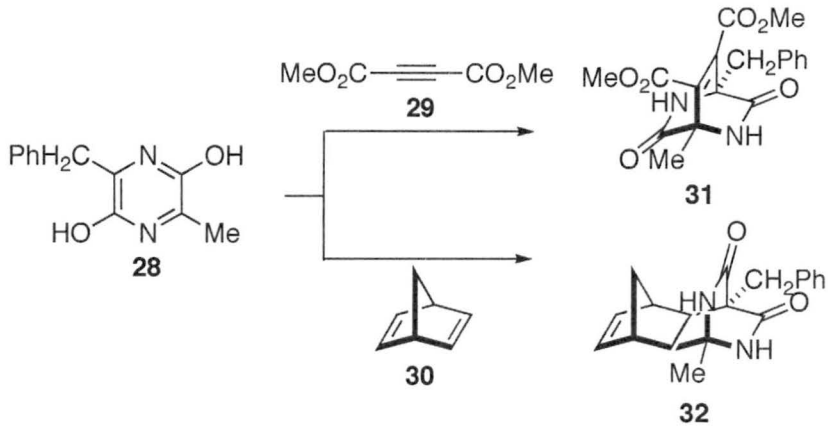
All of the metabolites have a common isoprene unit. It is believed that after the isoprene unit is incorporated by the reverse prenyl transferase the metabolite **26** can then be oxidized to the azadiene **27**, which can then be cyclized via an intramolecular Diels-Alder reaction to provide the metabolites such as stephacidin A **(11)** in this case (Scheme 3).[14]



Scheme 3: Proposed biosynthesis of secondary metabolites

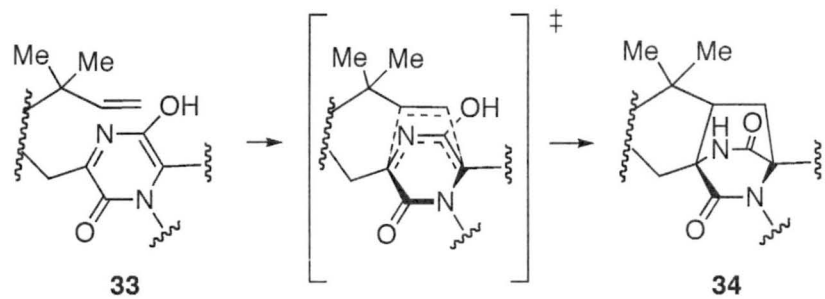
1.4 Biosynthesis of the Bicyclo[2.2.2]diazaoctane Core.

Porter and Sammes were the first to propose that a Diels-Alder reaction could form the bicyclo[2.2.2]diazaoctane core of the prenylated indole alkaloids.[15] They used a model system with dihydroxy pyrazine **28**, dimethyl acetylenedicarboxylate **29** and norbornadiene **30** to prove that this hypothesis was indeed plausible, thus providing the Diels-Alder products **31** and **32** (Scheme 4).



Scheme 4: Porter and Sammes proposed biosynthesis of the bicyclo core.

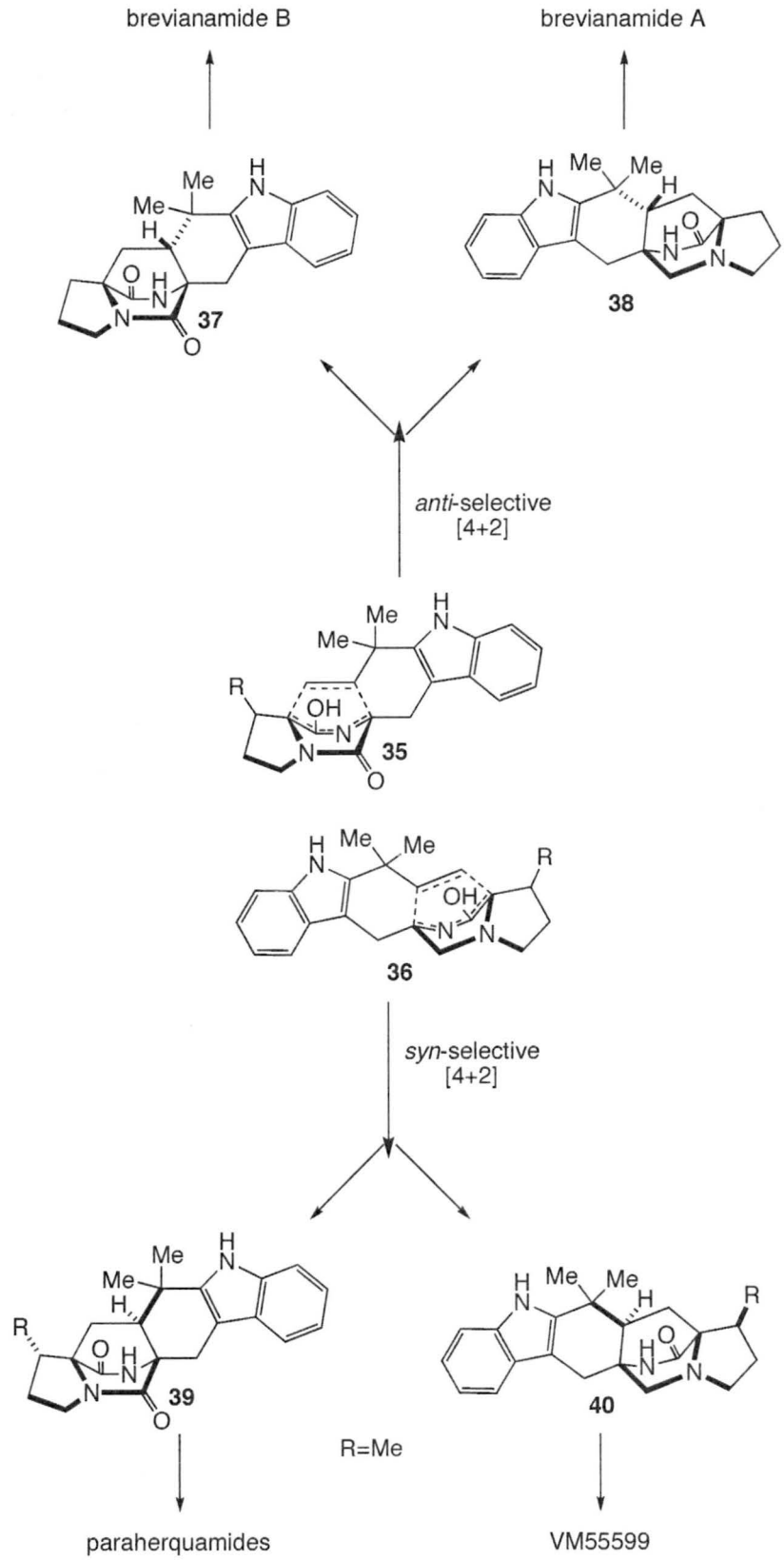
Since the Porter and Sammes proposal, the evidence supporting a Diels-Alder reaction has grown significantly. Although Diels-Alder reactions are utilized in the lab frequently, enzyme-catalyzed Diels-Alder reactions have not been proven in nature.[16] In a Diels-Alder reaction the transistion state is highly ordered and resembles the structure of the product **34** (Scheme 5).



Scheme 5: Diels-Alder mechanism.

In nature, an enzyme generally catalyzes reactions by stabilizing the structure and charge of the developing transition state. In these types of reactions the starting material and product will differ greatly from the transition state. Due to this difference, the product and starting material do not bind to the enzyme as tightly as the transition state, which

allows for turnover. In a Diels-Alder reaction, the enzyme that stabilizes the transition state could also bind to the product, which would prevent turnover. Thus, the question remains if a Diels-Alderase exists. There are currently no reports of an isolated Diels-Alderase from the various fungi that produce these metabolites. However, there are several other groups which also believe that there is a Diels-Alderase acting to biosynthesize other natural products, including Ichihara in the biosynthesis of solanapyrone, Oikawa in a macro phomate synthesis, and Vederase in a lovastatin nk synthesis.[15] Also, nature has provided some insight into the possibility of a Diels-Alderase within several known protein catalyzed [4+2] reactions in catalytic antibodies and in RNA catalysis. Another clue that an enzymatic Diels-Alder reaction may be occurring is that one could imagine an enzyme to provide either an *anti* or *syn* [4+2] reaction from one common intermediate (**32, 33** Scheme 6).[17]

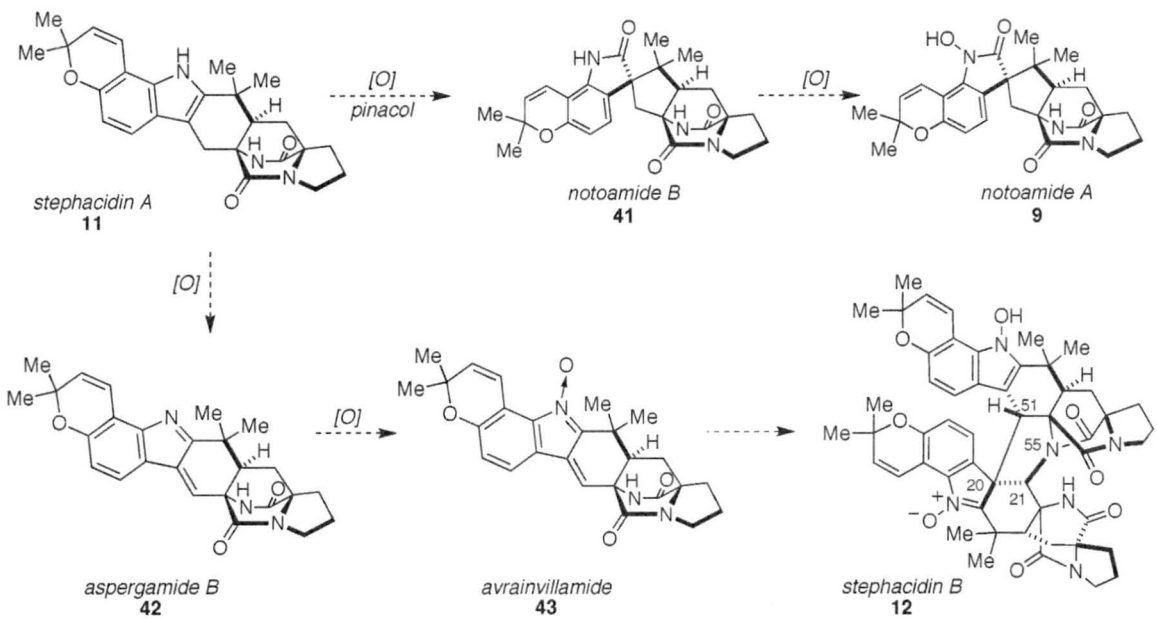


Scheme 6: *Anti* and *Syn* Diels-Alder.

If an *anti* [4+2] is occurring, then one could access the brevianamide family, and if a *syn*-selective [4+2] is occurring then one could access the paraherquamide family. In this case one could propose that different organisms could have enzymes that are selective towards either the *anti* or *syn* configurations and that the families could be accessed via the same advanced intermediate.

1.5 Previous Biosynthetic Work With the Stephacidin Family

Besides being biologically active and structurally unique, many different natural products appear to be biosynthetically derived from stephacidin A **(11)** (Scheme 7).[18]

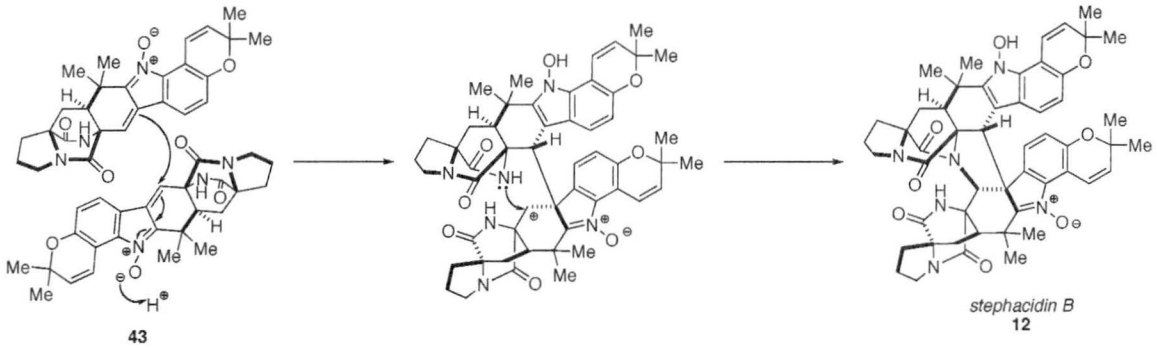


Scheme 7: Proposed biosynthesis of related alkaloids.

Oxidation of stephacidin A **(11)** followed by a Pinacol rearrangement generates the *spiro*-oxindole ring system found in notoamide A **(9)** and B **(41)**. Stephacidin A **(11)** could also participate in a different oxidation sequence where it could first undergo oxidation to

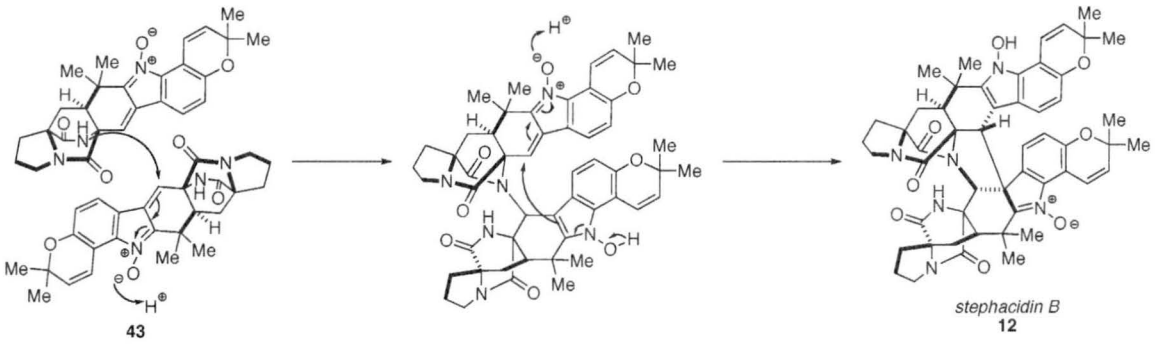
form aspergamide B **(42)**. Further oxidation of the vinyl imine of aspergamide B could yield the vinyl nitrone moiety of avrainvillamide **(43)**, which could then dimerize to produce the more active of the two stephacidins, stephacidin B **(12)**.

Two groups have proposed a mechanism for the dimerization of avrainvillamide into stephacidin B. The first was proposed by Qian-Cutrone and shows that a dimer could be formed from stephacidin A **(11)** via avrainvillamide **(43)**. Initiation from the protonation of the imine oxide in avrainvillamide **(43)** would generate a carbocation that could be attacked by the double bond of the other unit (Scheme 8).[10] The resulting cation could in turn be attacked by the amide to finish the dimerization.



Scheme 8: Qian-Cutron's Biosynthetic Proposal

Franz von Nussbaum proposed the second biosynthetic conversion of avrainvillamide **(43)** to stephacidin B **(12)** (Scheme 9).[19]



Scheme 9: von-Nussbaum's biosynthetic proposal.

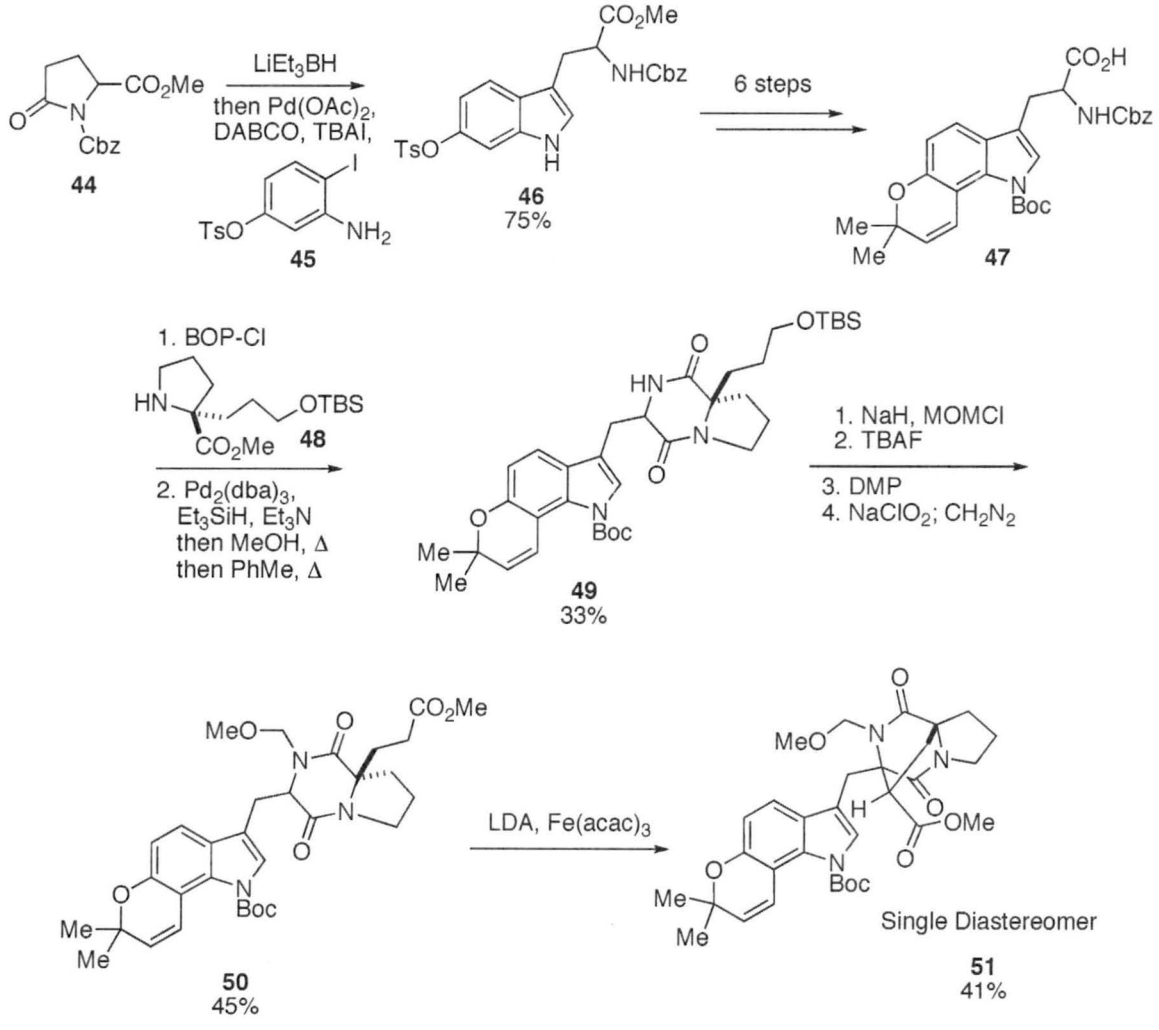
von Nussbaum's proposed route avoids the secondary carbocations and utilizes a novel Michael addition. This route is initiated by a nucleophilic attack of the amide at the nitrone Michael acceptor to install the C-N bond. The newly nucleophilic N-hydroxy indole system could then attack the double bond of the other unit to form the C-C and thus stephacidn B **(12)**.

1.6 Previous Syntheses of Stephacidin A

Two groups have previously synthesized stephacidin A **(11)**. Baran published the first synthesis of stephacidin A in 2005.[20] This was a 29 step route resulting in 0.49% overall yield. Baran (also in 2005) published a second, slightly improved, synthesis while converting stephacidin A to B.[21] The Williams group has since developed three other syntheses.[22,23] These include a 17 step asymmetric synthesis and two biominetic syntheses (a 17 and 14 step route), the best of which yielded an 11.1 % overall yield.

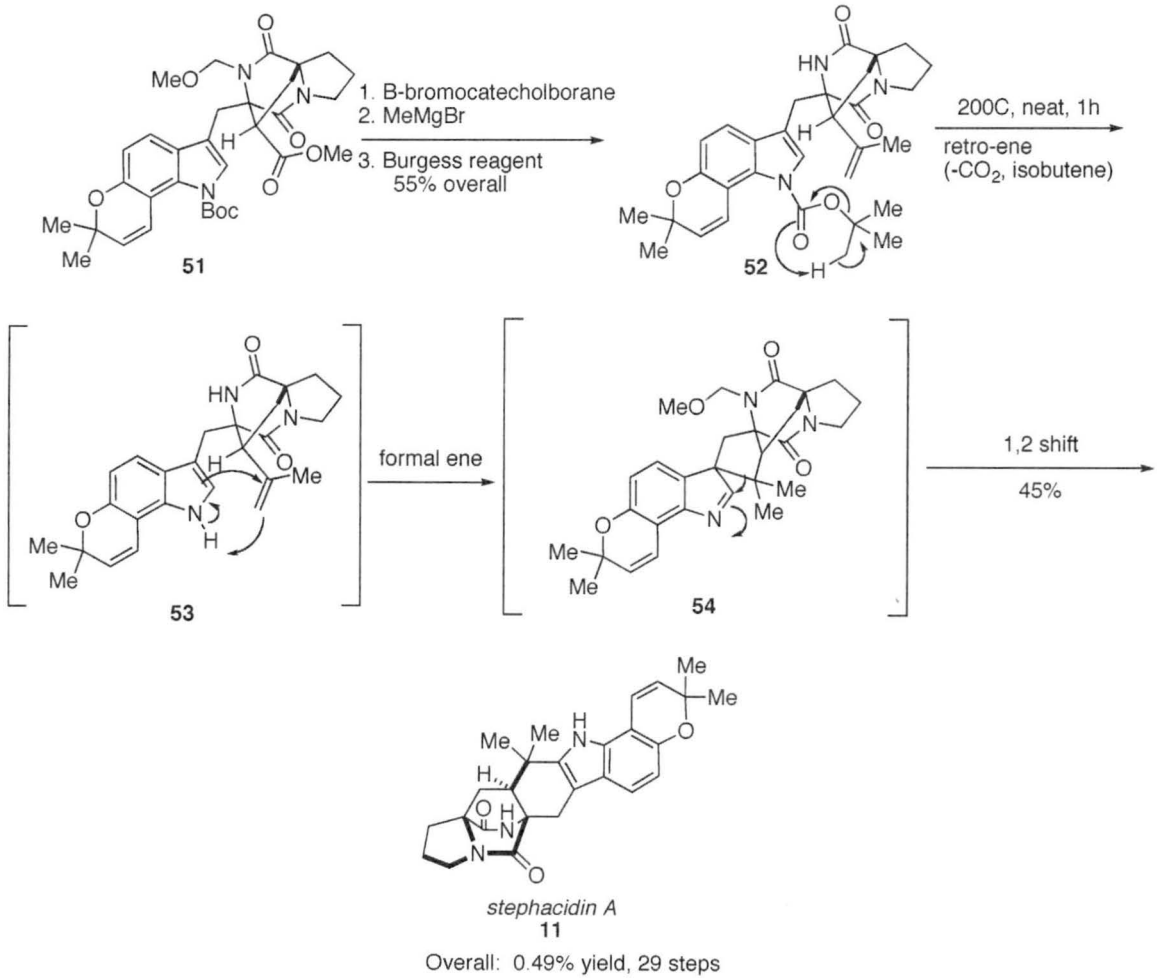
Baran's synthesis of stephacidin A began with commercially available pyroglutamate **44**. This was chemoselectively reduced with super hydride followed by a Pd-catalyzed coupling to **45** to yield the tryptophan **46**. Utilizing Williams group

chemistry, six more steps provided the acid **47**.[24] The acid underwent a peptide coupling with the proline derivative **48** and the Cbz group was removed, which allowed for cyclization to the diketopiperazine **49**. Protection of the DKP nitrogen with a MOM group was followed by TBAF mediated removal of the TBS group and oxidation to the ester **50**. Next, stereocontrolled oxidative coupling formed the C-C bond **(51)** (Scheme 10).[20]



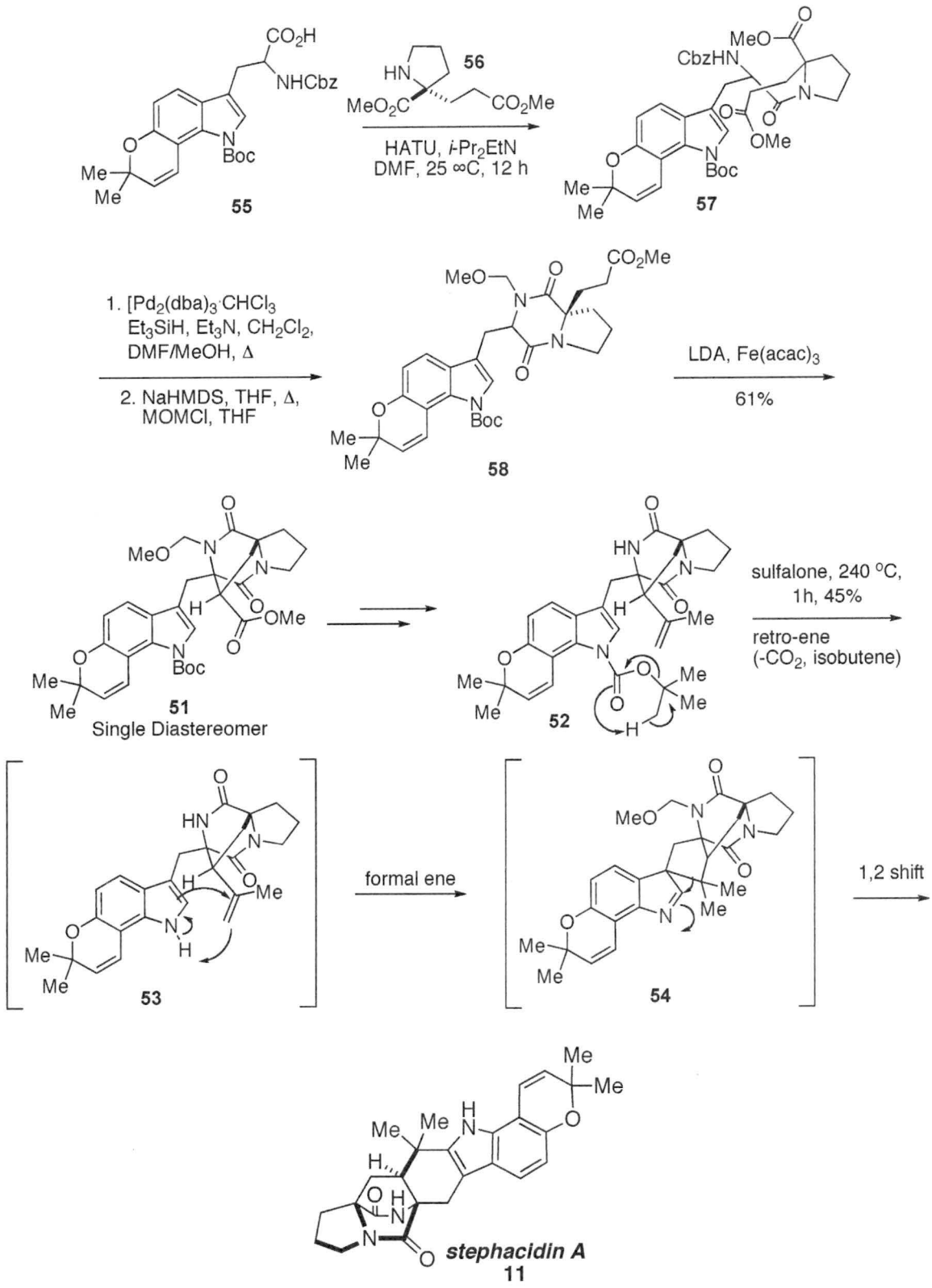
Scheme 10: Baran's enantioselective synthesis of stephacidin A.

From **51** removal of the MOM group and reaction with methyl magnesium bromide gave the tertiary alcohol, which was dehydrated with Burgess reagent to yield the olefin **52**. Ring closure, following the precedent from Williams' brevianamide B total synthesis, was achieved by heating the substrate neat leading to thermal removal of the Boc group **53,** followed by a formal ene reaction[25]. This led to the spirocyclic intermediate **54**. A 1,2 shift terminated the cascade reaction and afforded stephacidin A **(11)** (Scheme 11).



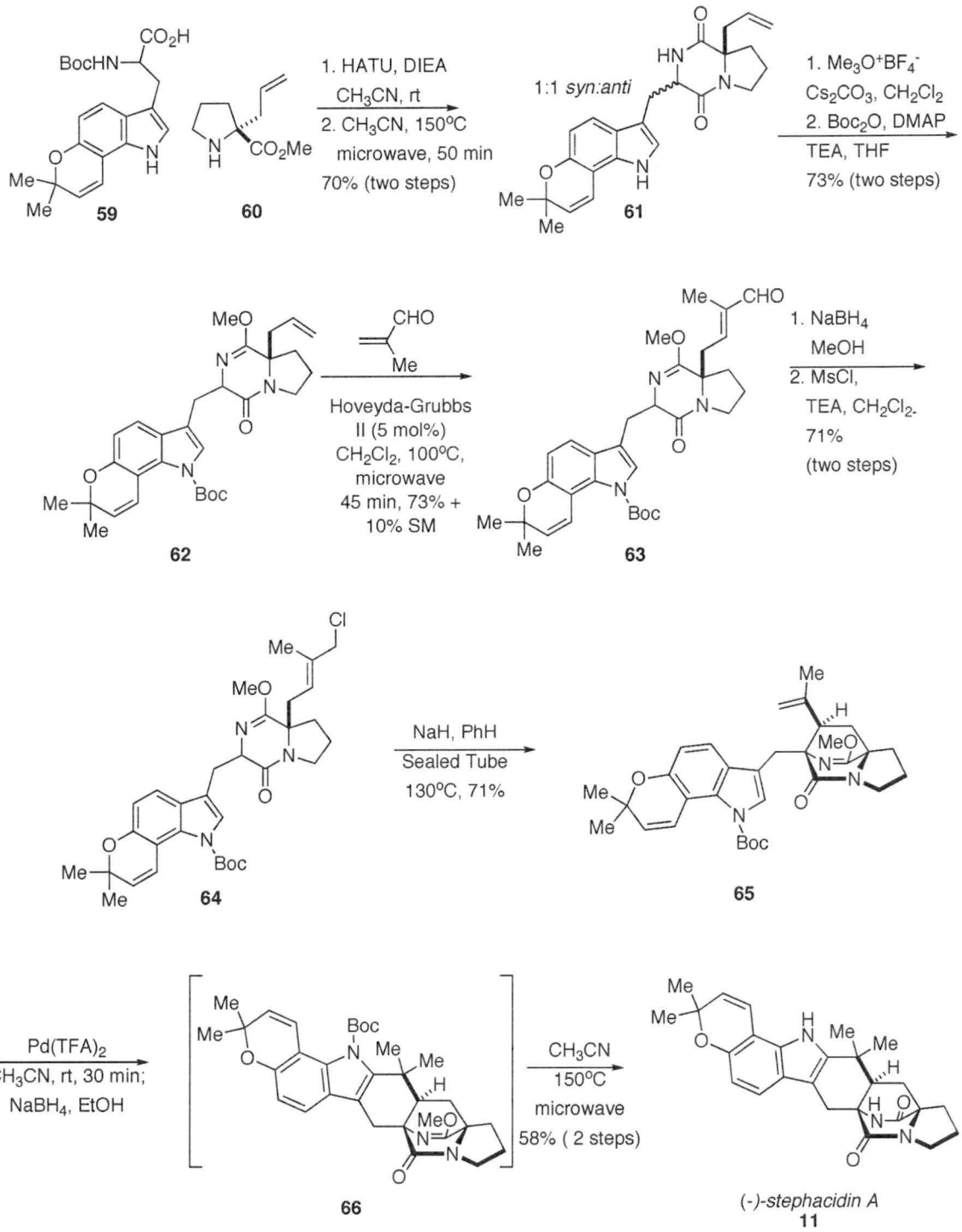
Scheme 11: Baran's ring closure and cyclization to stephacidin A.

Baran's second synthesis of stephacidin A is very similar to his previous route with three improvements.[21] First, changing the proline derivative **56** removed the need for a protection step and oxidation of the ester side chain. The yield of the enolate coupling reaction was also increased. The third change was an improvement of the thermal annulation by using sulfalone at a higher temperature, thus making the reaction more reproducible (Scheme 12). These changes resulted in a shortening of the synthesis by one step.



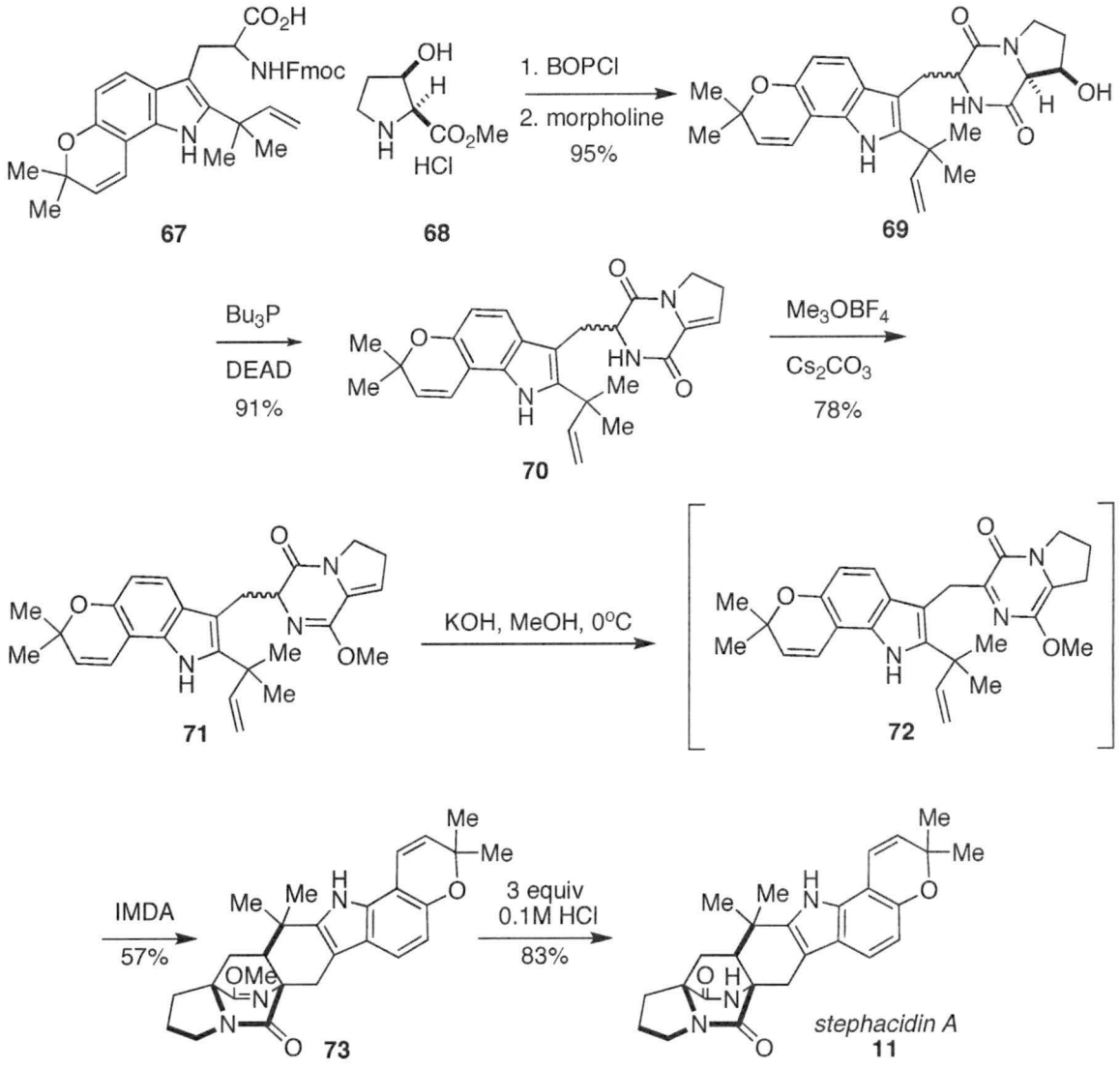
Scheme 12: Baran's second synthesis of stephacidn A.

The Williams group reported an asymmetric synthesis of stephacidin A **(11)** in 2007.[22] First the coupling of the allyl proline **60** to the tryptophan acid **59** was accomplished using HATU in the presence of diisopropylethylamine, followed by microwave heating to remove the Boc group afforded the DKP **61**. The secondary amine and the indole nitrogen were then protected to give **62**. Olefin cross metathesis gave the aldehyde **63**, which was subsequently reduced using sodium borohydride in methanol to give the allylic alcohol. Allyl chloride **64** was then formed from the allylic alcohol in good yield. Cyclization to the [2.2.2] bridged bicyclo core **65** was achieved using sodium hydride in benzene. Heating with palladium (II) in TFA provided the alkyl intermediate **66**. Finally, heating in acetonitrile led to Boc deprotection and afforded stephacidin A **(11)** over 17 steps in 5% overall yield (Scheme 13).



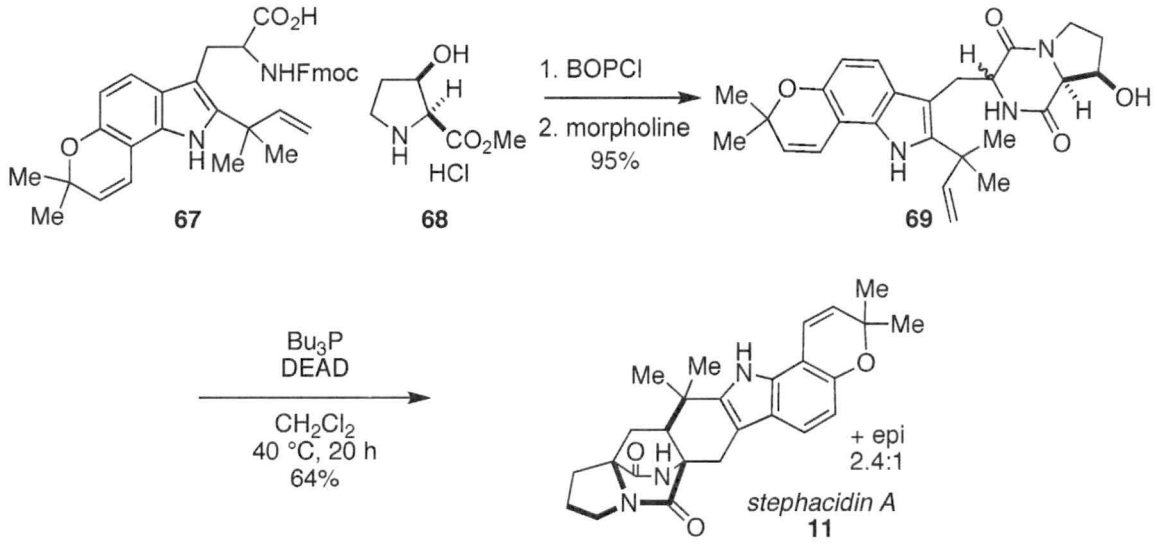
Scheme 13: Williams asymmetric synthesis of stephacidin A.

Williams' biomimetic synthesis of stephacidin A began with the coupling of the acid **67** with an L-proline derivative **68** in the presence of BOP chloride. Fmoc removal with morpholine and subsequent cyclization gave the DKP **69**. A Mitsunobu type elimination with tributyl phosphine and DEAD afforded the enamide **70**. Next, treatment with Meerwein's reagent and cesium carbonate afforded the lactim ether **71**. This was then treated with 20% potassium hydroxide in methanol to effect tautomerization to the intermediate azadiene **72**, which spontaneously suffered intramolecular Diels-Alder cycloaddition to provide the bicyclo core **73**. From there, treatment with HCl in THF affected cleavage of the lactim ether to afford stephacidin A (**11**) (Scheme 14).



Scheme 14: Williams' first biomimetic synthesis of stephacidin A.

The second biomimetic synthesis by the Williams group was a much-improved 14 step route (Scheme 15). Although the cycloaddition reactions of methoxy-protected azadienes had proven to be successful substrates for constructing the bicyclo core, a more concise route that removed the protecting group steps was desired. With Williams' third route it was found that subjecting the intermediate alcohol **69** to PBu_3 directly afforded stephacidin A **(11)**.



Scheme 15: Williams' second biomimetic synthesis of stephacidin A.

1.7 Research Objectives

Several efficient syntheses of stephacidin A **(11)** have previously been developed within the group. The remaining goal was to learn how this compound, specifically, the pyran ring is biosynthesized. Nature is unsurpassed in its ability to efficiently synthesize compounds. An understanding of the biosynthesis of these metabolites could lead to a more streamlined synthetic route in the lab. After several potential biosynthetic intermediates have been synthesized (chapter 2) the intermediates could then be ^{13}C labeled and feed to *Aspergilolus ochraceus* to check for incorporation of ^{13}C labels in stephacidin A (chapter 3).

Chapter 2

Progress Towards Proposed Biosynthetic Precursors Of Stephacidin A

2.1 Potential Biosynthetic Intermediates

Most of the biosynthetic work for these secondary metabolites have been determined by previous studies. However, the biosynthesis of the pyran ring system of the stephacidins represents one of the last missing pieces of this biosynthetic puzzle (Figure 2.1).

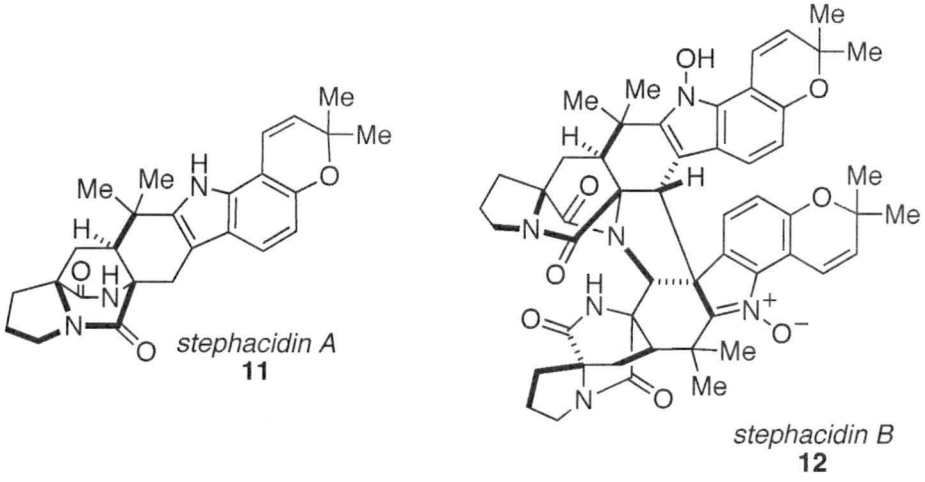
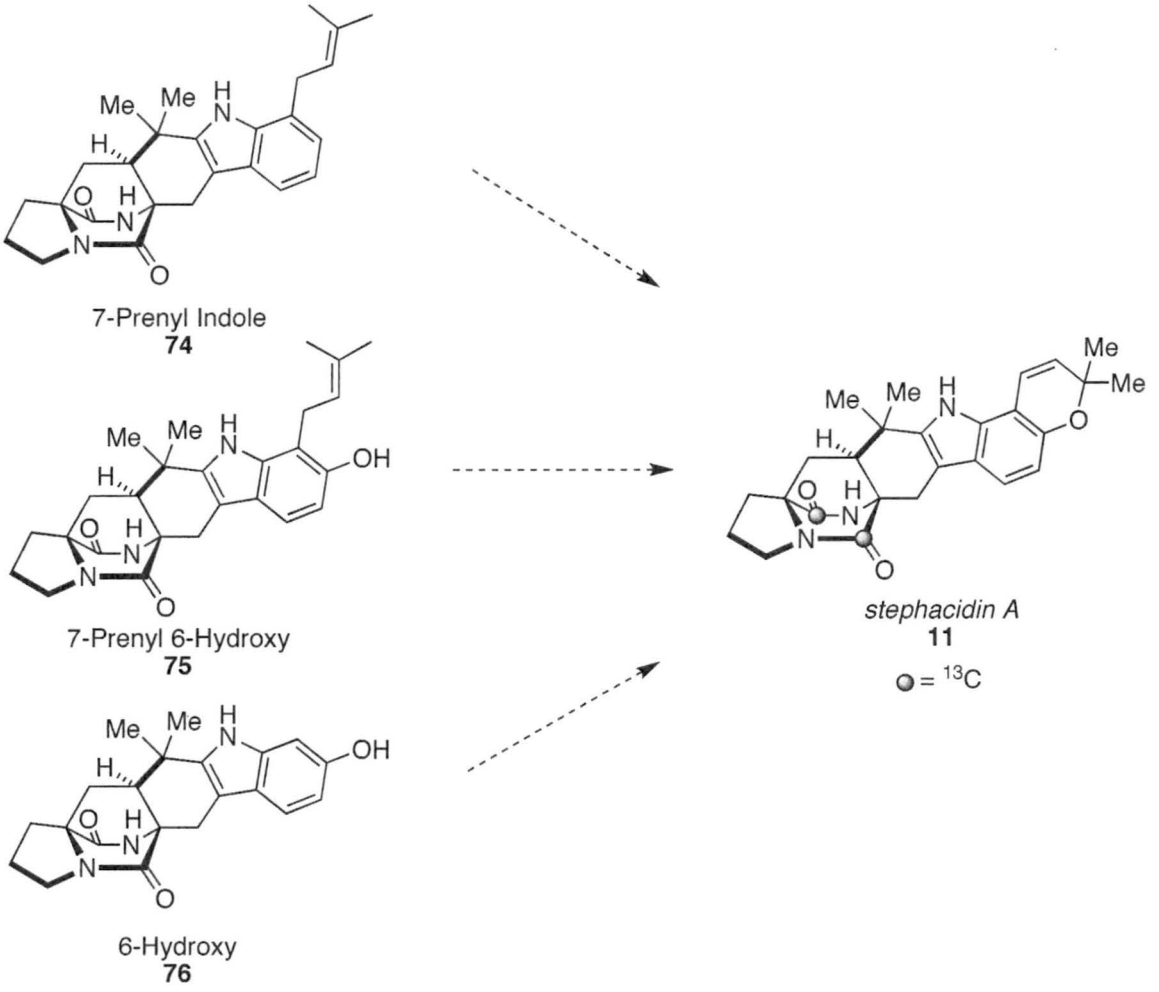


Figure 2.1: Stephacidin A and B.

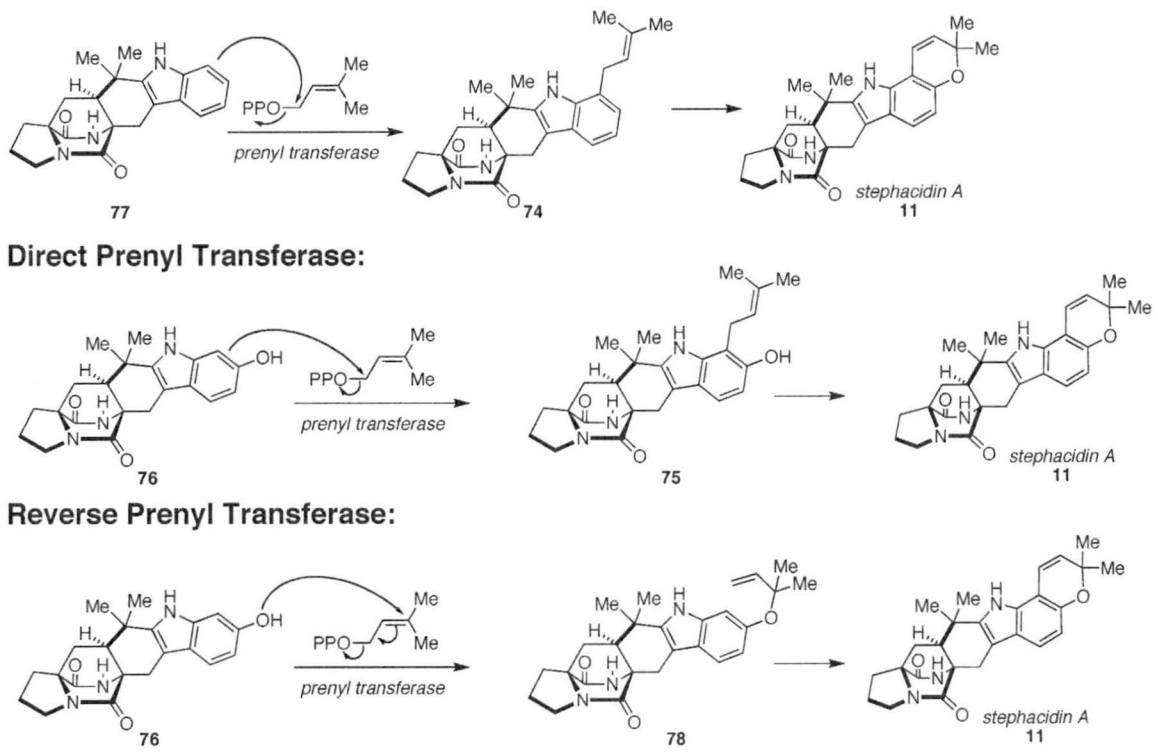
Our goal is to determine how the pyran ring is biosynthesized, and to establish the order in which these steps occur. We envision that the pyran ring can arise by one of three proposed routes (Scheme 16).



Scheme 16: Proposed biosynthetic precursors to stephacidin A.

The first of these precursors, **74**, has a 7-prenylated indole ring system. The second precursor has a 7-prenyl 6-hydroxy indole ring system **75**, and the third precursor has a 6-hydroxy indole ring system **76**. After these precursors have been synthesized they will be doubly ^{13}C labeled and fed to *Aspergillus ochraceus* to check for ^{13}C-incorporation into stephacidin A **(11)**.

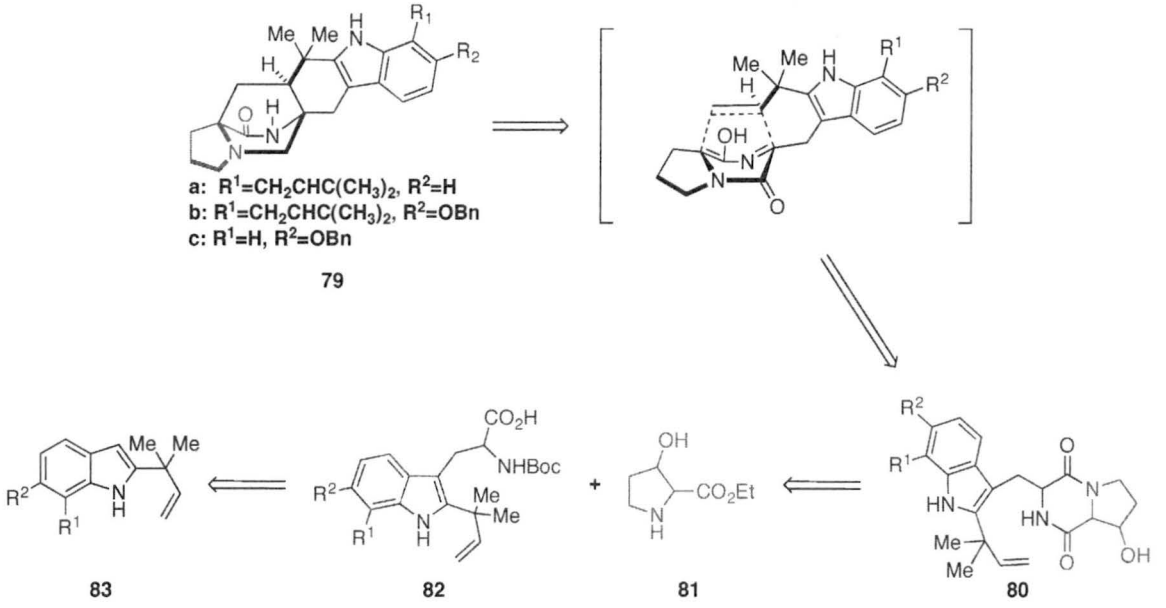
These precursors, **74**, **75**, and **76**, have been chosen as targets based on three hypotheses on whether the prenyl group at the 7-position of the indole is added via a direct or reverse prenyl transferase (Scheme 17). Beginning with pre-stephacidin **77** one could envision the prenyl group adding through a direct prenyl transferase resulting in the 7-prenyl precursor **74**. This substrate could then undergo oxidation and cyclize to give stephacidin A **(11)**. The second hypothesis also utilizes a direct prenyl transferase approach. In this case, the 6-hydroxy intermediate **76** could attack DMAPP to give the 7-prenyl 6-hydroxy intermediate **75**, which could then cyclize to stephacidin A **(11)**. The final hypothesis begins with the 6-hydroxy precursor **76**. This route utilizes a reverse prenyl transferase, which has also been observed in the addition of the isoprene unit in previous studies. The phenol **76** could attack the π bond of the DMAPP to give **78**, and then cyclize to afford stephacidin A **(11)**.



Scheme 17: Proposed pathways for insertion of the prenyl group.

2.2 Retrosynthetic Approach to Stephacidin A

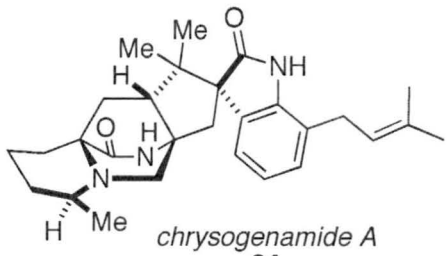
Our retrosynthesis for the three precursors, **74**, **75**, and **76**, followed Williams group chemistry as shown in Scheme 18.[26] Ring closure of the substrates can be achieved via an intramolecular Diels-Alder reaction of **80**. The Diels-Alder reaction will occur after dehydration and tautomerization of the diketopiperazine (DKP) **80**. The DKP portion of **80** would be provided by an amino acid coupling of the prenylated tryptophan derivative **82** and *cis*-3-hydroxy proline ethyl ester **81**.



Scheme 18: Retrosynthetic approach to the proposed precursors.

2.3 Progress Towards the 7-Prenylindole Precursor

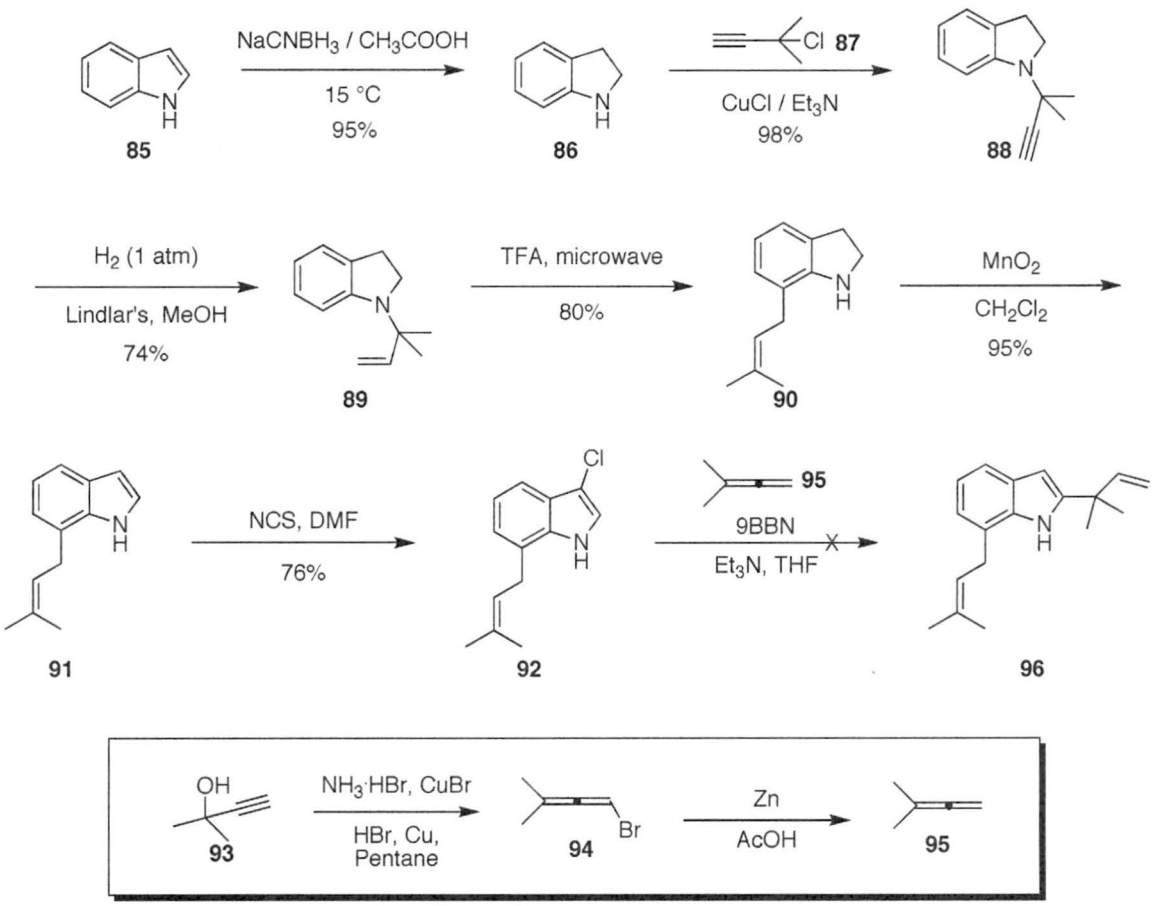
The recent discovery of a new metabolite in the marcfortine family, chrysogenamide A (**84**) strengthened the hypothesis that the 7-prenylindole precursor was a possible precursor (Figure 4).[27] This metabolite also has the 7-prenylatedindole ring system that is found in the 7-prenylindole precursor **74**. We envisioned that the 7-prenyl group in (**84**) could be arising through a similar biosynthetic pathway as that of stephacidin A (**11**).



chrysogenamide A
84

Figure 4: Chrysogenamide A.

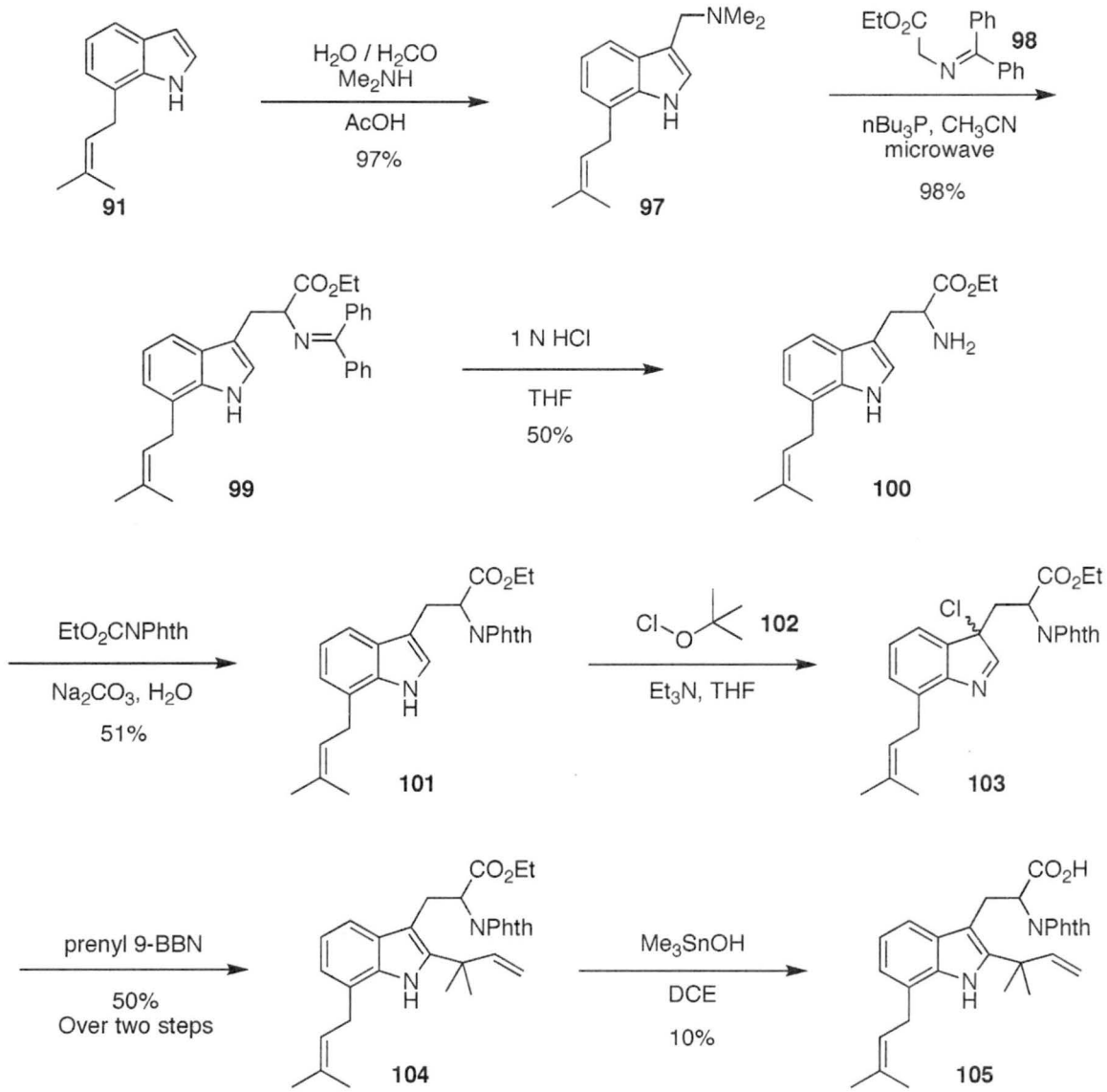
Progress towards the 7-prenyl precursor **74** began by following a known route to 7-prenylindole **91**.[28] Reduction of commercially available indole **85** with sodium cyanoborohydride to afford indoline **86** (Scheme 19) was followed by N-dimethylpropargylation with propargyl chloride **87**. Next, a hydrogenation of **88** with Lindlar's catalyst gave the alkene **89**, which underwent an acid promoted aza-claisen reaction to provide the 7-prenylated indoline **90**. Finally, an oxidation with manganese dioxide provided 7-prenylindole **91**, and treatment of **91** with NCS gave the 3-chloro derivative **92**. Allene **95** was formed by dehydrating and brominating the propargyl alcohol **93** followed by treatment with zinc and acetic acid. Attempts to form the reverse prenylated indole substrate **96** led to complicated mixtures and problematic purification.



Scheme 19: Attempted formation of the reverse prenylationed 7-prenyl indole.

It was then thought that by first installing the required amino acid portion the compound would become more polar leading to operationally simple separation from 9BBN, and indeed this proved to be the case (Scheme 20). To this end, treatment of **91** with formaldehyde and dimethylamine afforded the gramine **97** by a Mannich condensation reaction.[29] A microwave assisted Somei-Kametani coupling reaction between the gramine **97** and glycine benzophenone imine **98** provided imine **99**. Cleavage of the benzophenone imine afforded the amino ethyl ester **100** in good yield, which was subsequently reprotected and then chlorinated at C-3 to yield **103**. Finally,

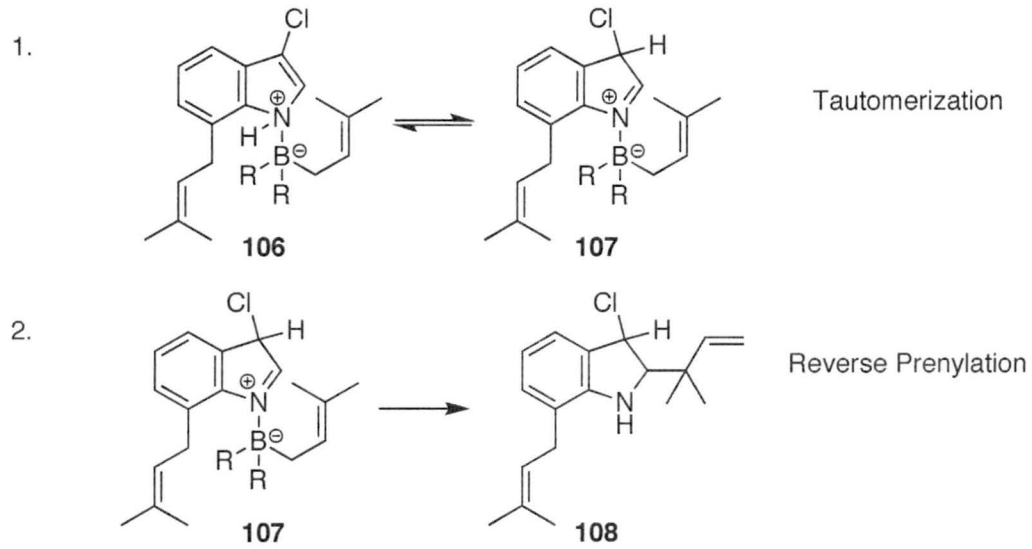
addition of the reverse prenyl group at C-2 provided **104**, and a saponification reaction with trimethyl tin hydroxide provided the acid **105**.



Scheme 20: Formation of 7-prenyl tryptophan derivative.

It was determined that purification of **104** was easier than that of **96** due to its less greasy nature. It is believed that formation of **104** was more straightforward than **106** because of the mechanism of the reaction. To reverse prenylate the indole **96** it must first undergo tautomerization and the 7-prenyl system may not allow this to occur. In the case

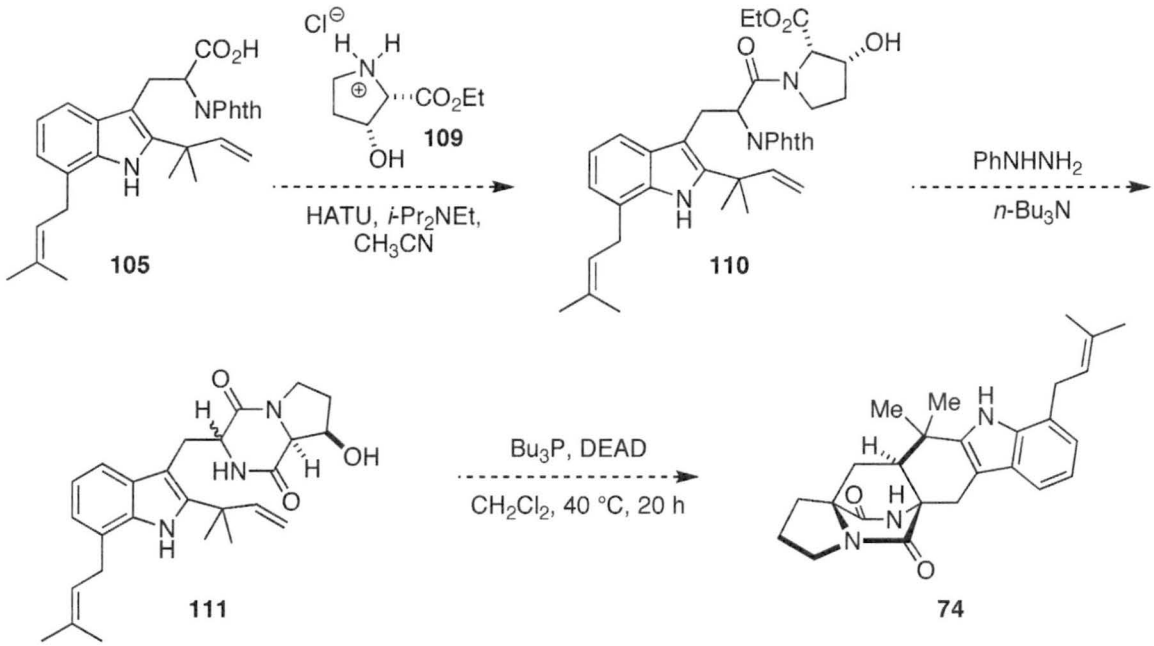
of **104** the imine is already in place by the chlorination of **101** and tautomerization is not required. After the imine is formed reverse prenylation succeeded (Scheme 21).



Scheme 21: Mechanism of reverse prenylation.

2.4 Future Work Towards the 7-Prenyl Precursor

From the acid **105** the 7-prenyl precursor **74** should be accessible in three more steps (Scheme 22). Amino acid coupling of the reverse prenylated tryptophan derivative **105** and the proline derivative **109** should provide the peptide **110**. Deprotection of the phthaloyl group and subsequent cyclization should provide the DKP **111**. Next, Mitsunobu conditions should allow for dehydration, which upon treatment with heat should lead to an intermolecular Diels-Alder reaction to provide the 7-prenyl precursor **74.** Since these steps have been utilized in the synthesis of stephacidin A, it is believed that they could also work for the very similar proposed precursor.

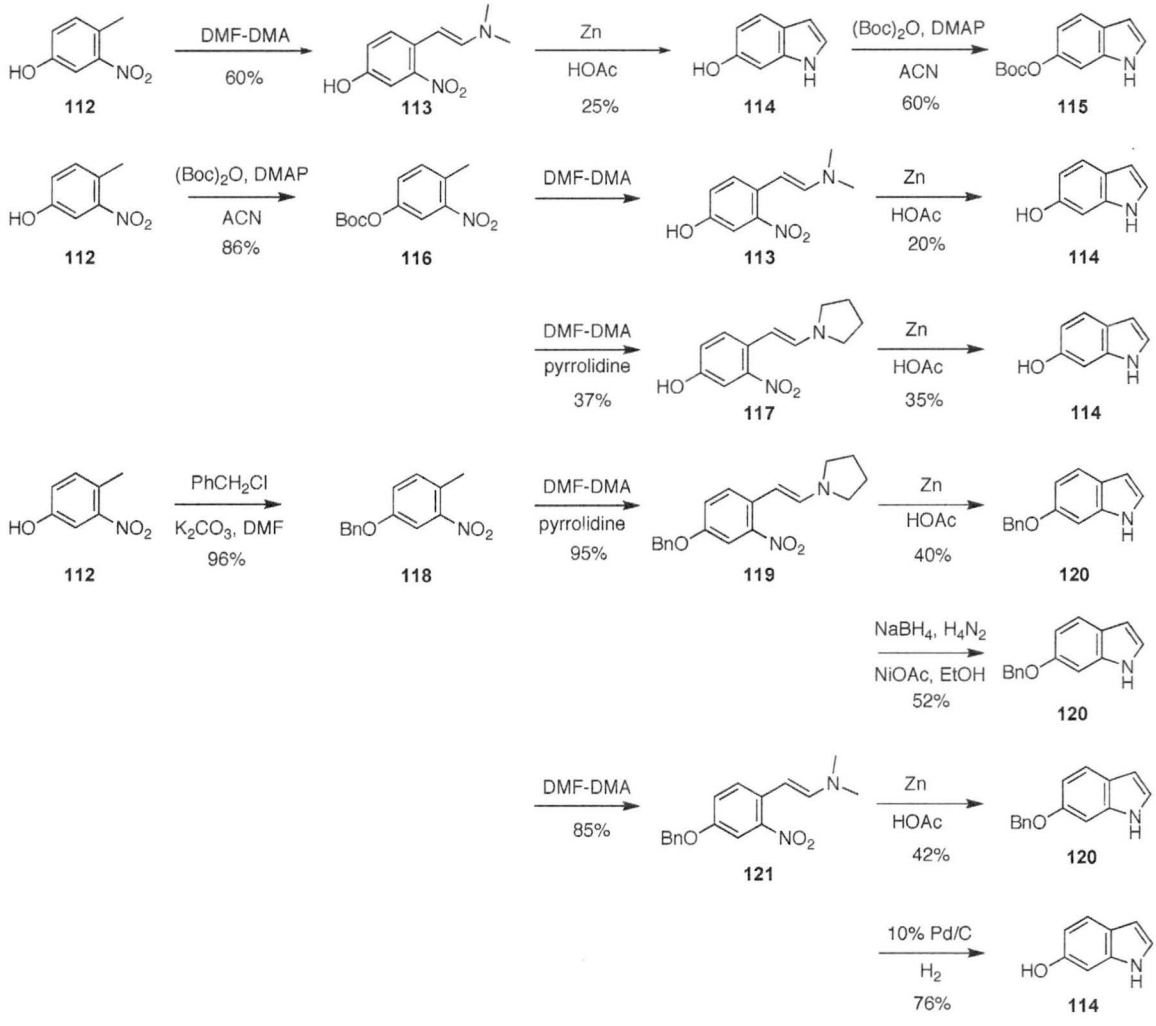


Scheme 22: Future work towards the 7-prenyl precursor.

2.5 Route to 6-Hydroxy Indole

An efficient synthesis of **114** proved to be difficult (Scheme 23). The literature precedent for the formation of these indoles follow the Batcho-Leimgruber method. Several different methods were attempted to form the 6-hydroxy indole. To start, both Boc and Bn protection of **112** were achieved, however, the Boc protecting group was found to be cleaved under the high temperature required for the formation of the enamine **113**, **117**, **119**, **121**. It was also found that a protecting group was not required, although the yield was considerably lower for each step. To achieve the best yield over the three steps, a Bn protecting group was utilized. Using the Bn protecting group, formation of either the pyrrolidine or dimethyl amine enamine **119, 121** resulted in a high yield. However, cyclization to the indole **120** proved troublesome. Several metal catalyst

including Raney Nickel and zinc were utilized and all resulted in poor yields of the indole **120**. Nickel acetate provided a moderate yield of the indole **120** but it was found that simultaneous cyclization and deprotection using 10% Pd/C resulted in a high and reproducible yield of **114**.

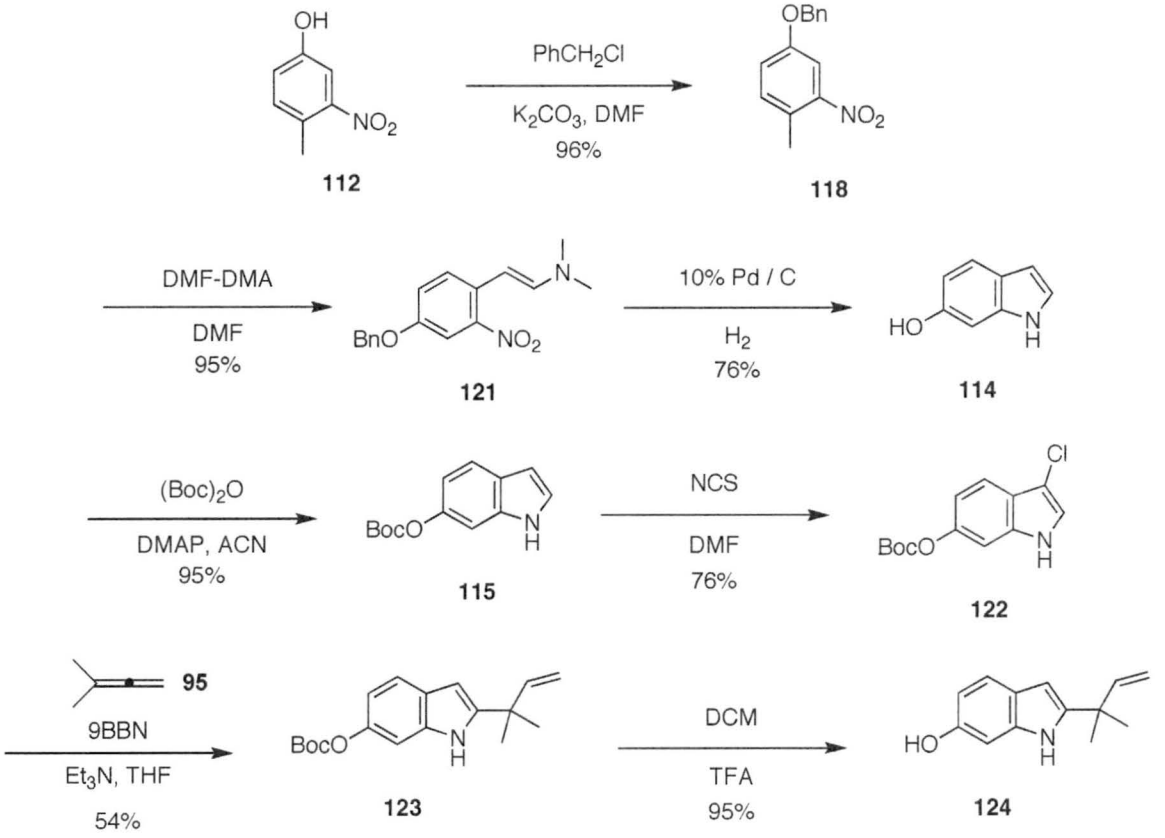


Scheme 23: Formation of 6-oxy indoles.

2.6 Progress Towards the 7-Prenyl 6-Hydroxy Precursor

The synthesis of the 7-prenyl 6-hydroxy precursor **75** has also been attempted (Scheme 24). Starting from commercially available 4-methyl-3-nitrophenol **112** 6-

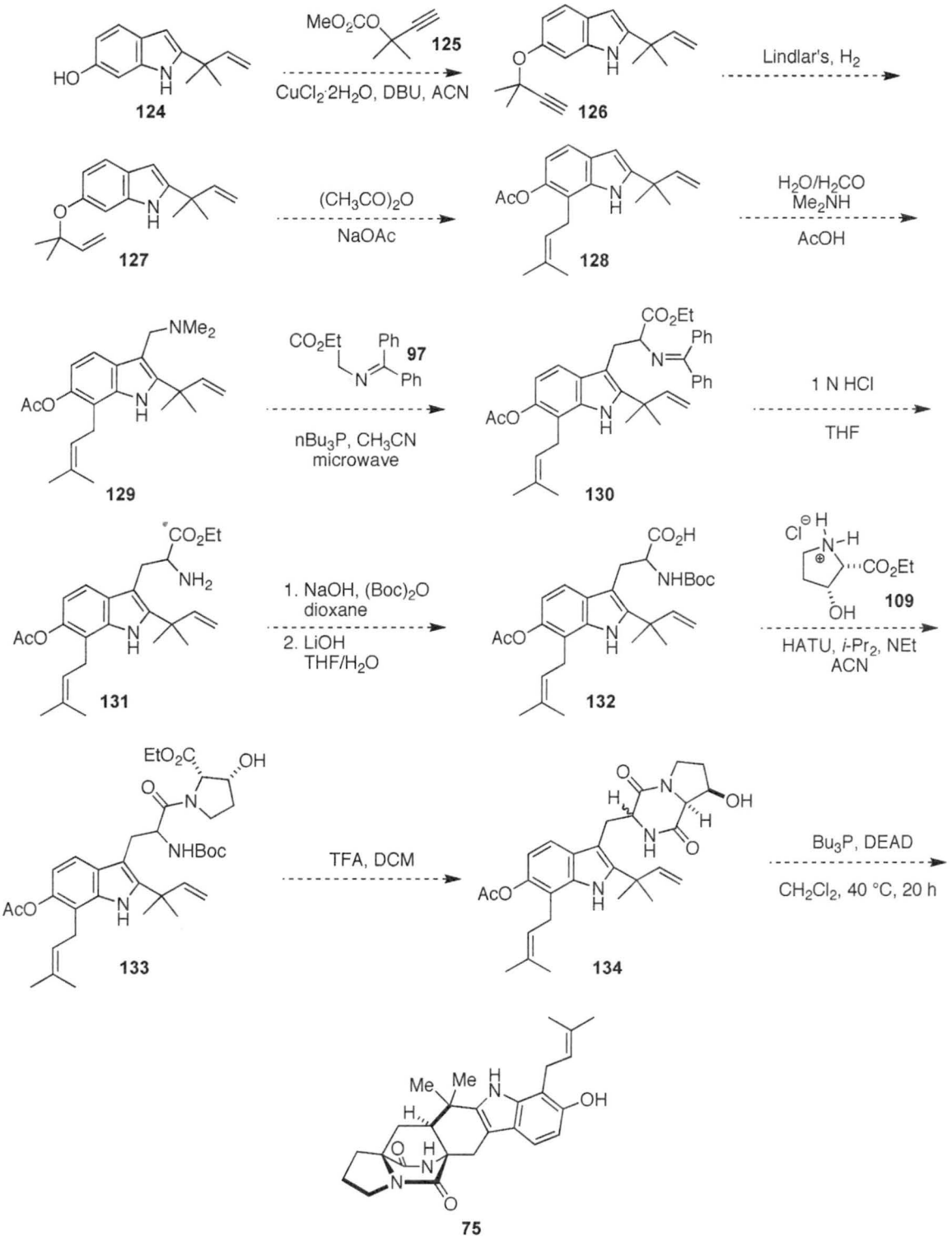
hydroxyindole **114** was prepared in good yield. Following benzyl protection of **112**, nitrotoluene **118** was converted to enamine **121** following Batcho-Leimgruber methods. Reduction with Pd/C led to cyclization and deprotection to give **114**. Following group chemistry the indole **114** was then Boc protected to provide **115** and then chlorinated at C-3 to afford **122**.[30] Next, the reverse prenyl group was added **123**, followed by deprotection to yield **124**.



Scheme 24: Synthesis of reverse prenylated 6-hydroxyindole

2.7 Future Work Towards the Synthesis Of the 7-Prenyl 6-Hydroxy Precursor

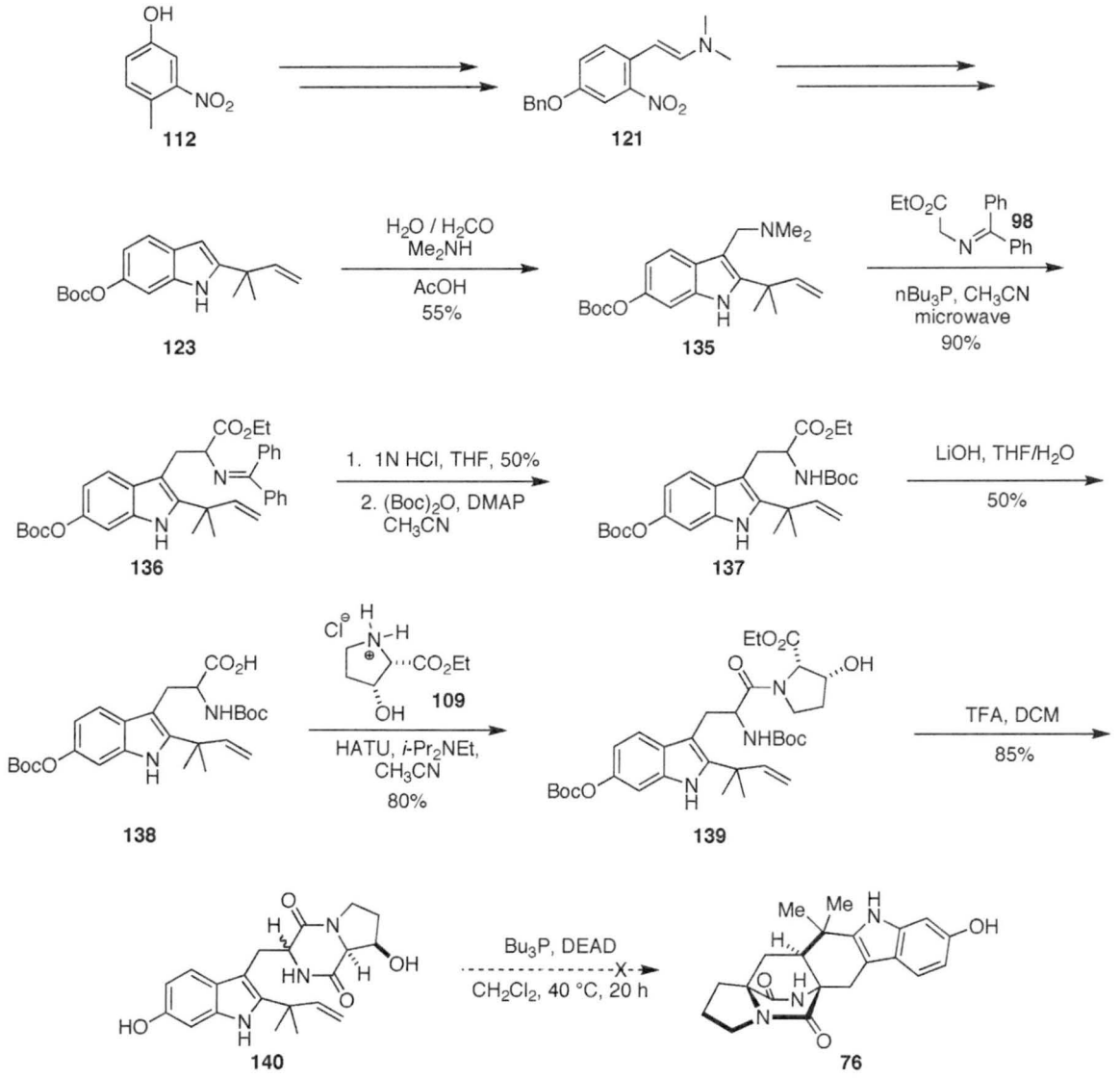
From **124** the desired 7-prenyl 6-hydroxy biosynthetic intermediate **75** can be obtained in 11 steps (Scheme 25). First the phenol will be transformed to the propargyl ether **126,** followed by reduction of the terminal alkyne to the alkene, thereby providing **127**. This will then be reacted with acetic anhydride and sodium acetate to afford the Claisen rearrangement product **128**.[31] Formation of the gramine **129** will be accomplished with a Mannich condensation reaction, followed by the Somei-Kametani coupling reaction to yield the imine **130**. Cleaveage of the benzophenone imine with HCl will afford the amino ethyl ester **131**, which can be protected with a Boc group. Saponification of the ethyl ester will provide the acid **132,** which will be coupled with the proline derivative **109** and then deprotection and subsequent cyclization will provide the diketopiperazine **134**. After undergoing an intramolecular Diels-Alder reaction, the desired 7-prenyl-6-hydroxy precursor **75** will be obtained.



Scheme 25: Future work towards 7-prenyl 6-hydroxy precursor.

2.8 Progress Towards the 6-Hydroxy Precursor

The 6-hydroxy precursor **76** can be accessed through an intermediate of the 7-prenyl-6-hydroxy precursor **75**. Beginning with 6-Boc protected hydroxy indole **123,** the gramine **135** was again formed by a Mannich condensation reaction (Scheme 26). Next, a Somei-Kametani coupling reaction with the glycine benzophenone imine **98** was completed to form the imine **136**, followed by cleavage of the benzophenone imine to provide the amino ethyl ester and protection with a Boc group to give **137**. Saponification of the ethyl ester **137** with LiOH provided the acid **138**. This was then coupled to the proline derivative **109** to give **139**. Next deprotection and subsequent cyclizaiton afforded the DKP **140**. At this point the Mitsunobu type reaction was attempted. Unfortunately, it seems that a side reaction occurred with the unprotected phenol and no product was obtained. Several possibilities exist for this side reaction, however, none have been confirmed. One possibility is that perhaps the Mitsunobu reaction is occurring at the phenol first which could potentially result in a polymer being formed. Unfortunately, time and material restraints did not allow for further studies.



Scheme 26: Progress towards the 6-hydroxy precursor.

2.9 Future Work Towards the Synthesis Of the 6-Hydroxy Precursor

There are several different possibilities for protection of the phenol **114**. Cbz protection at the early stage indole was attempted, however, this reaction also failed to produce the desired product. One could also imagine that protection after the formation of the DKP **140** could be achieved. The phenol is more acidic than the alcohol, which

should allow for protection with a benzyl group or some other protection group. under basic conditions.[32]

2.10 Conclusions

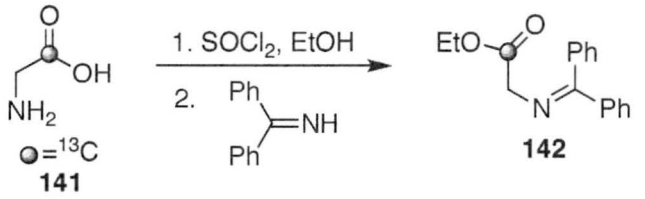
Extensive progress towards the synthesis of these three potential precursors **74**, **75**, and **76**, has been achieved. Future work to finish the 7-prenyl system **74** should follow Williams group chemistry since there are no functional groups off the indole to complicate reactions. The 7-prenyl-6-hydroxy **75** system will require the most attention, although it should also follow Williams group chemistry. The concern for this substrate is that there will also be a protecting group problem due to the 6-hydroxy group. Finally, the 6-hydroxy indole system **76** is near completion. A protecting group on the phenol should solve any issues with the Misunobu reaction and allow for completion of the substrate.

Chapter 3

Forward Direction: Feeding Experiments

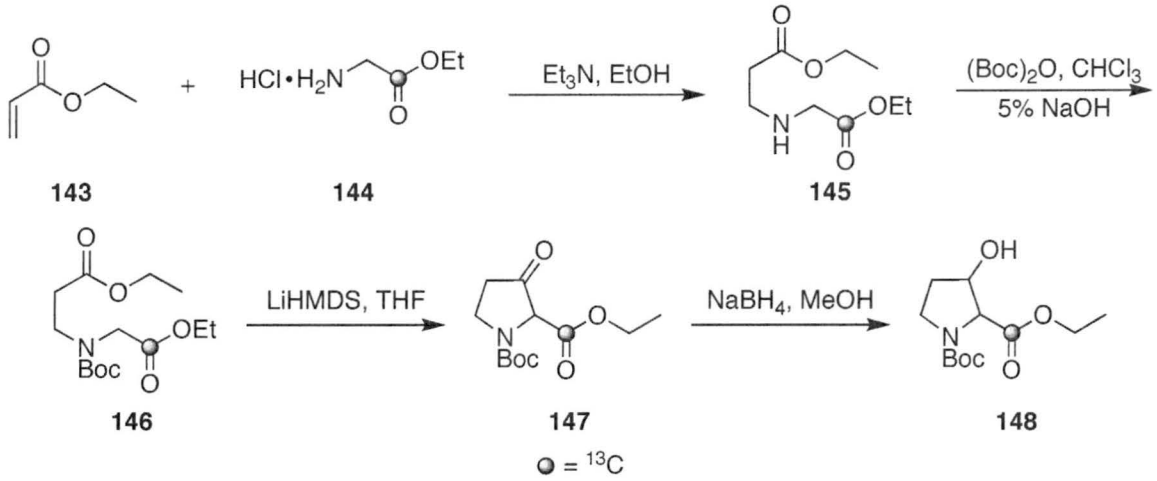
3.1 Feeding Experiments With the Proposed Precursors

With the possible biosynthetic intermediates of stephacidin A synthesized, various feeding experiments with *Aspergillus ochraceus* should confirm which, if any, of these substrates **74**, **75**, or **76** is the biosynthetic intermediate to stephacidin A. This will allow for the determination of how the pyran ring system is biosynthetically formed. After an efficient route to the precursors have been found, ^{13}C labeled building blocks will be used to synthesize doubly labeled advanced intermediates of each precursor. The first label will be provided by ^{13}C labeled glycine benzophenone imine **142**, which can be formed by following Williams group chemistry (Scheme 27).[33] Reacting ^{13}C labeled glycine **141** with thionyl chloride provided the glycine salt which upon treatment with benzophenone imine afforded ^{13}C labeled glycine benzophenone imine **142**.



Scheme 27: Synthesis of ^{13}C labeled glycine benzophenone imine.

The second ^{13}C label will be provided by *cis*-3-hydroxyproline ethyl ester **148** and will also follow Williams group chemistry (Scheme 28). [34] The proline derivative **148** was formed by first coupling ethyl acrylate **143** with ^{13}C labeled ethyl oxamate salt **144**. The resulting amino acid was then Boc protected to afford **146** and cyclized with hexamethylsilazane lithium salt to provide oxopyrrolidine **147**. **147** was then reduced to the ^{13}C labeled *cis*-3-hydroxyproline **148**.

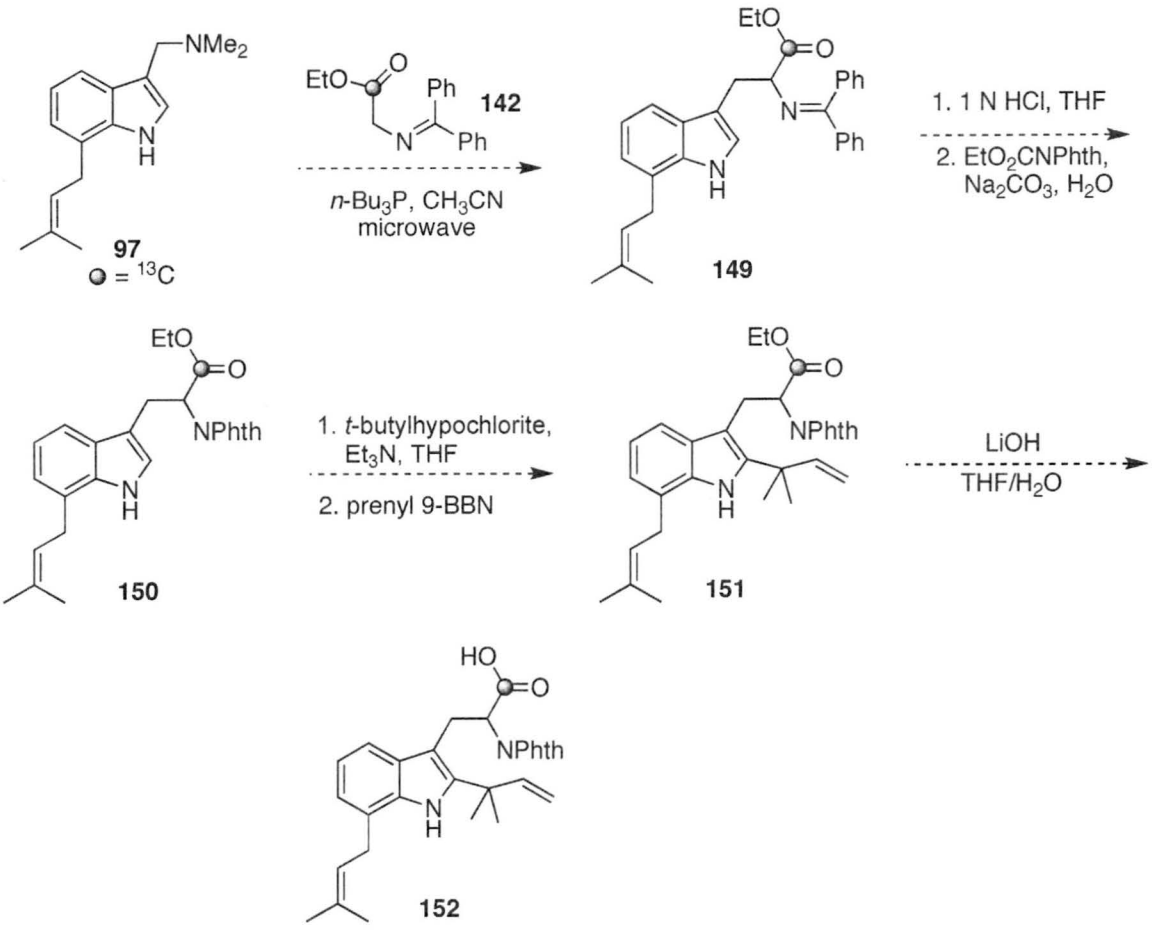


Scheme 28: Synthesis of the ^{13}C labeled *cis*-3-hydroxyproline ethyl ester.

3.2 Installation of the First ^{13}C Label on the Three Potential Precursors

The ^{13}C labeled glycine can be inserted through routes similar to those previously described in chapter 2. The 7-prenyl precursor **74** will have to receive its first ^{13}C label early on due to the installation of the amino acid portion of the compound before the reverse prenylation. To install the ^{13}C label, gramine **97** will be coupled with the ^{13}C labeled benzophenone glycine **142** through a Somei-Kametani coupling to provide imine **149** (Scheme 29). Cleavage of the benzophenone imine should afford the amino ethyl

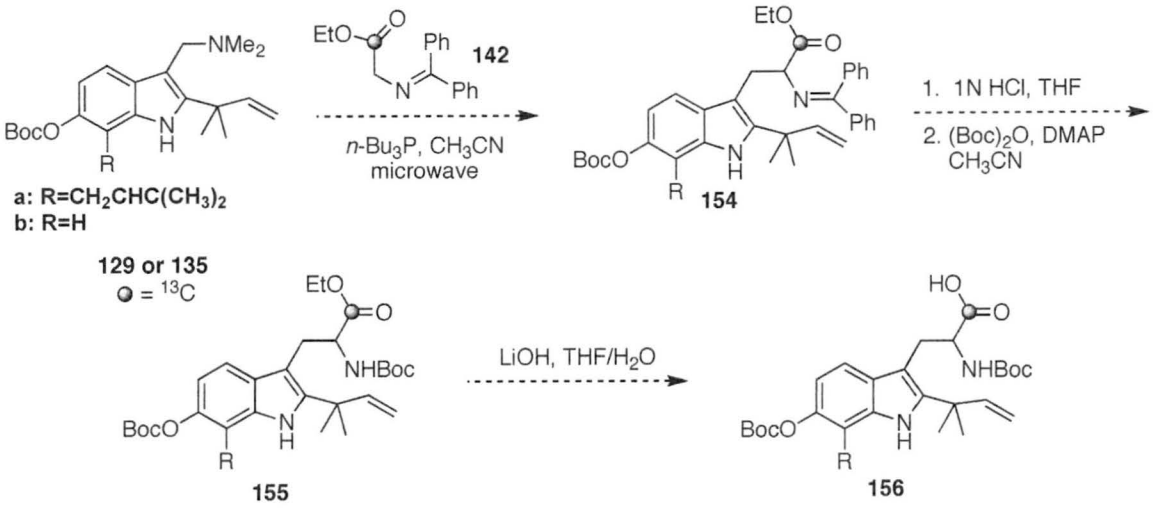
ester, which will be protected to provide **150**. From there the reverse prenyl group could be introduced to provide **151**, and saponification to acid **152** can be attempted.



Scheme 29: Synthesis of ^{13}C labeled 7-prenyl precursor.

The first ^{13}C label can be inserted later into the synthesis of the 7-prenyl-6-hydroxy **75** and 7-prenyl precursors **76**. Gramine **129** or **135** could undergo a Somei-Kametani coupling reaction with ^{13}C labeled glycine **142** to provide the imine **154** (Scheme 30). Cleavage of the benzophenone imine could then provide the amino ethyl

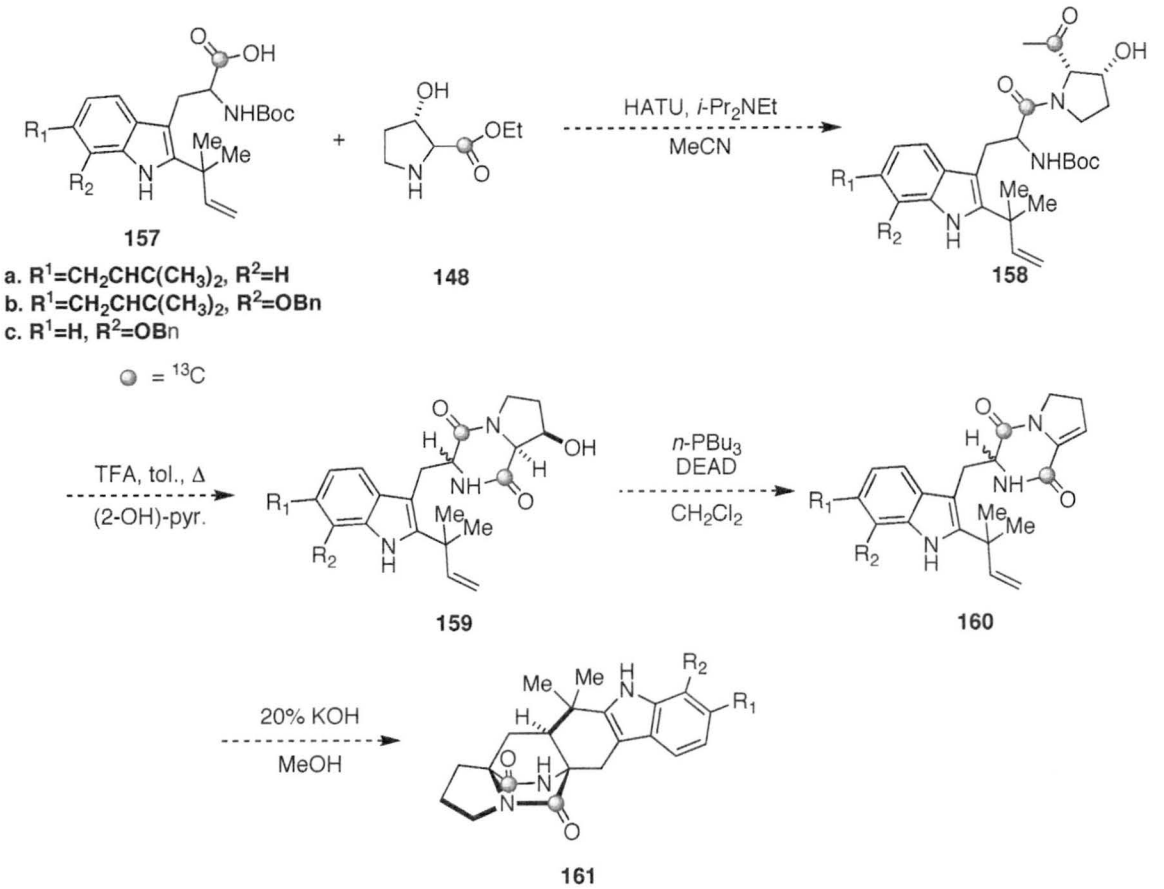
ester and protection with a Boc group could give **155**. Saponification of the ethyl ester **155** with LiOH should provide the acid **156**.



Scheme 30: Synthesis of ^{13}C labeled 7-prenylated acid.

3.3 Insertion of the Second ^{13}C Label

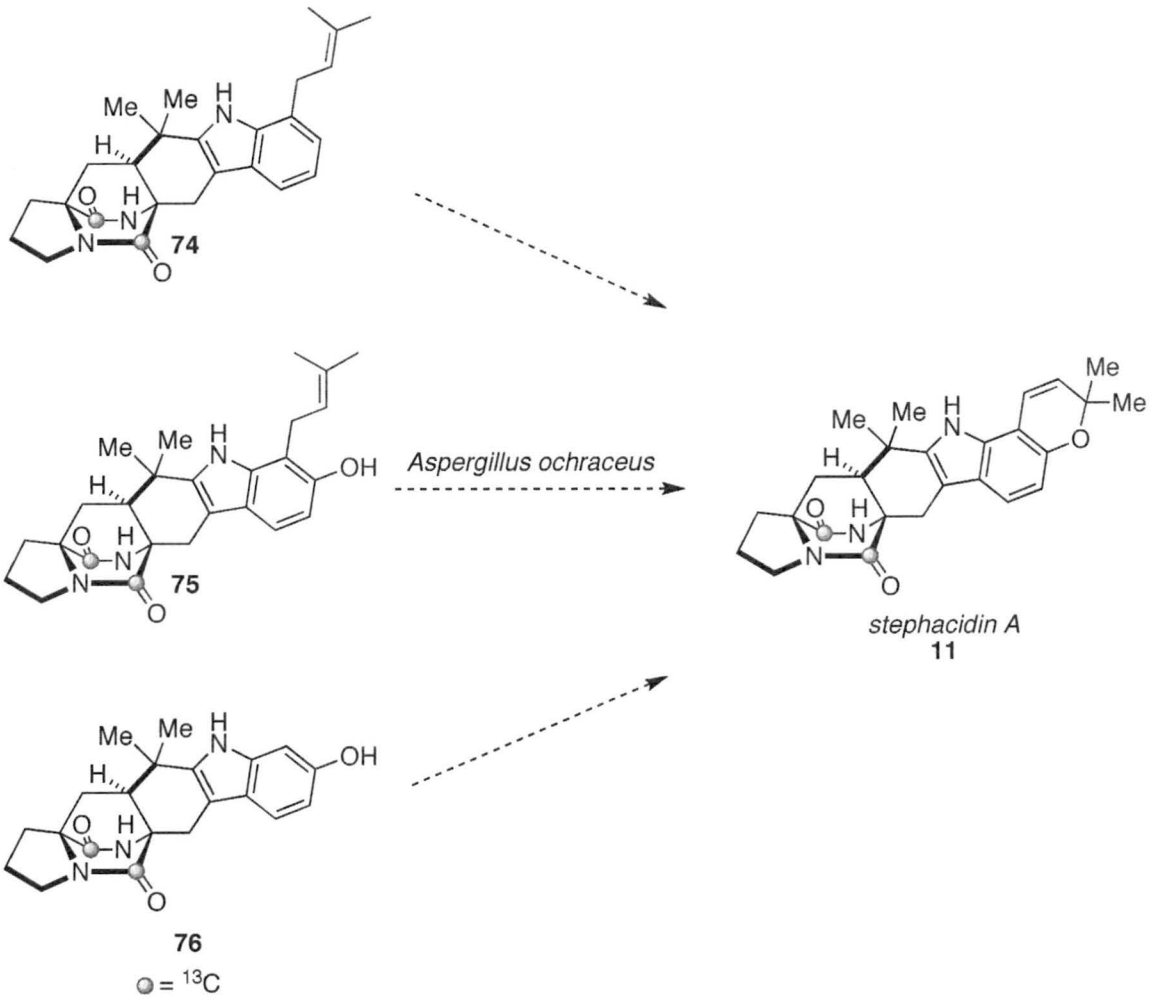
The precursors will be doubly ^{13}C labeled using a similar route previously described (Scheme 31).[23] Amino acid coupling of the ^{13}C labeled reverse prenylated tryptophan derivative **157** and ^{13}C labeled *cis*-3-hydroxyproline ethyl ester **148** should provide the peptide **158**. Treatment of the peptide with TFA should result in deprotection of the carbamate and subsequent heating of the unprotected amine could allow for cyclization to diketopiperazine **159**. Next, Mitsubobu conditions could allow for dehydration to enamide **160**, which after treatment with KOH can undergo intramolecular tautomerization and Diels-Alder to provide the cycloadduct **161**.



Scheme 31: ^{13}C labeling the precursors.

3.4 Planned Feeding Experiments of the Precursors to *Aspergillus Ochraceus*

Once all of the precursors have been synthesized and ^{13}C labeled as shown, various feeding experiments will be carried out with *Aspergillus ochraceus* in order to determine the biosynthesis of the pyran ring (Scheme 32).



Scheme 32: Feeding experiments with *Aspergillus ochraceus*

If any of the precursors are incorporated into stephacidin A the biosynthesis of the pyran ring system will be known.

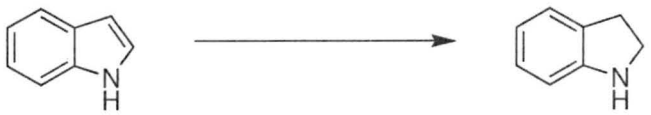
Chapter 4

Experimental

4.1 General Synthetic Conditions

Commercially available reagents were used without further purification. Thin layer chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm. Microwave reactions were conducted on a Discover monomode microwave apparatus monitored with the IR temperature monitoring feature of the Discover reactor. ^{1}H NMR spectral data was obtained using Varian 300, 400 or 500 MHz instruments. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ 7.27 (^{1}H NMR). For all NMR spectra, δ values are given in ppm and *J* values in Hz.

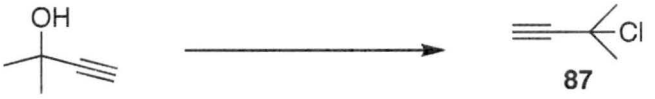
4.2 Experimentals for Chapter 2 Reactions



85 86

Indoline (86). To a stirred solution of indole **85** (10 g, 85.4 mmol) dissolved in glacial acetic acid (225 mL) was added sodium cyanoborohydride (16.5 g, 262.8 mmol) in one portion at room temperature. The reaction was allowed to stir for two hours. Water was then added to the mixture, which was then cooled in an ice bath. The mixture was then made basic using sodium hydroxide pellets. The mixture was then extracted with ether (4 x 1 L) and washed with water (2 x 1 L) and brine (2 x 1 L). The ether layers were combined and dried over anhydrous potassium carbonate and concentrated to dryness *in vacuo*. The crude material was then purified by flash chromatography (silica; 9:1 hexanes / ethyl acetate) affording **86** (yellow oil, 9.6 g, 94% yield from **85**).

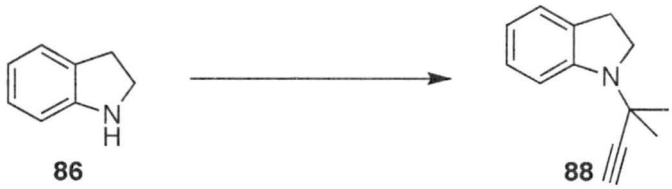
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched literature.



87

3-Methyl-1,2-butadiene (87). To a cold (0 °C), stirred solution of concentrated hydrochloric acid (2184 mL), 2-methyl-3-butyn-2-ol **(91)** (382.2 g, 4.55 mol) and hydroquinone (4.1 g, .037 mol) was added. Calcium chloride (507.8 g, 4.58 mol) was added in portions. After addition the ice bath was removed and the stirring was continued for 2 hours. The top layer was then separated and dried over sodium carbonate. The mixture was then distilled twice at 75 °C affording 3-methyl-1,2-butadiene **(84)** (97.8 g, 21% yield).

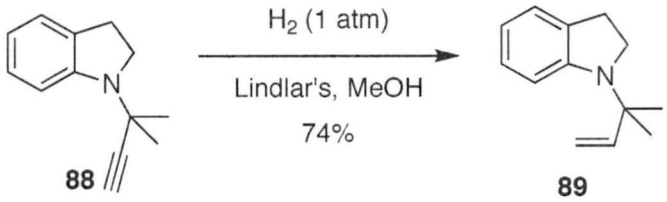
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



86 88

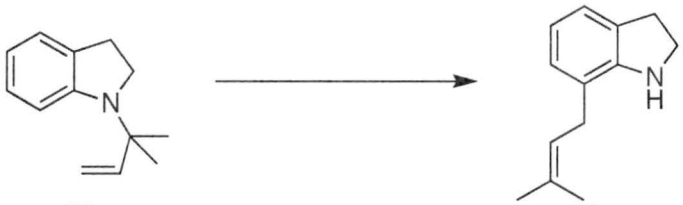
1-(2-Methylbut-3-yn-2-yl)indoline (88). To a 500 mL round bottom flask containing **86** (10 mL, .089 mol), **87** (12.22 mL), and CuCl (.884g, .00893 mol) in anhydrous THF (178 mL), under Ar, was added Et_3N (14.9 mL) dropwise at 0 °C. After the addition the reaction was stirred at room temperature overnight. The mixture was then filtered through a plug of silica and concentrated under vacuum. The crude material was then purified by column chromatograph (4:1 Hexanes / CH_2Cl_2) to afford **88** (16.06g, 98% yield from **86**).

^1H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



1-(2-Methylbut-3-en-2-yl)indoline (89). To a solution of alkyne **88** (11.3 g, .061 mol) in MeOH (180 mL) was added Lindlar catalyst (1.11g). The reaction mixture was then degassed and stirred under a H_2 atmosphere provided by a balloon and needle. The reaction was monitored by TLC. The mixture was then run through a plug of silica and concentrated. The mixture was then purified by column chromatography (95:5 Hexanes: Ethyl Acetate) to yield the product **89** (8.44 g, 74% yield from **88**) as a yellow oil.

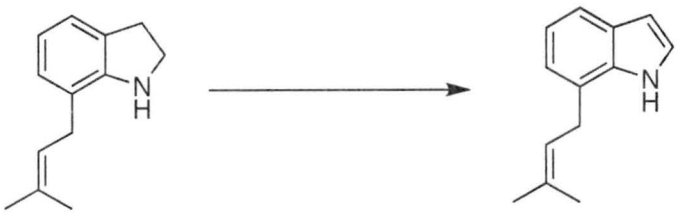
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



89 90

7-(3-Methylbut-2-enyl)indoline (90). To a solution of **89** (8.44 g, .045 mol) in toluene (13.5 mL) was added TFA (.451 mL). The reaction mixture was then sealed with a stir bar in a reaction vessel of a microwave apparatus. The mixture was then irradiated for 10 minutes at 150 °C. The mixture was then concentrated *in vacuo* and purified by column chromatography (95:5 Hexanes : Ethyl Acetate) to afford **90** (6.68 g, 79% yield from **89**) as a yellow oil.

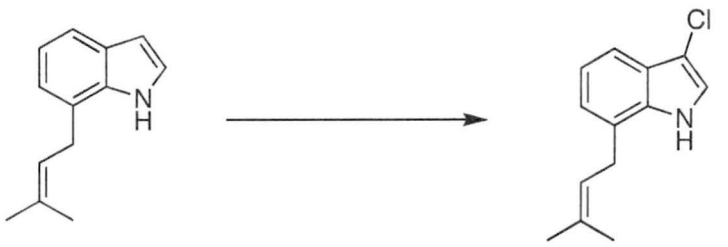
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



90 **91**

7-(3-Methylbut-2-enyl)-1*H*-indole (91). A stirred solution of **90** (10.5 g, .056 mol) and activated manganese (IV) oxide (13.57 g, mol) in dichloromethane (476 mL) was heated at reflux for 1.5 hours. After this time a second aliquot of activated manganese (IV) oxide (13.57g, mol) was added and the mixture was allowed to reflux an additional 1.5 hours. The mixture was then filtered through a plug of silica gel and concentrated under vacuum. The crude mixture was then purified by column chromatography (1% EtOAc in Hexanes) to afford **91** (6.75 g, 65% yield from **90**).

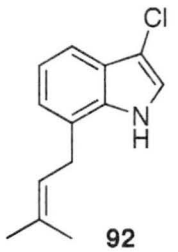
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



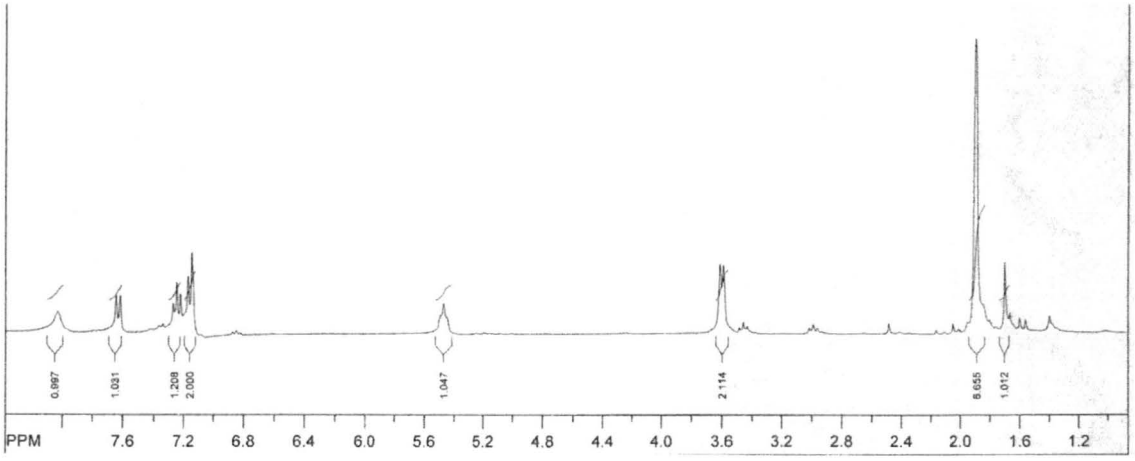
91 **92**

3-chloro-7-(3-methylbut-2-enyl)-1*H*-indole (92). 7-prenylindole **91** (.71 g, 3.83 mmol) was dissolved in DMF (19 mL). NCS (.512 g, 3.83 mol) was added and the reaction was allowed to stir for 4 hours. The reaction mixture was then washed with water and extracted with ether (3x 15 mL) and brine (2x 10 mL). The organic layers were then dried with Na_2SO_4 and concentrated. The crude material was then purified by column chromatography hexanes: ethyl acetate (95:5) to provide the chlorinated indole **92** as a brown oil (.74 g, 88% yield from **91**).

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 8.08 (1H, s), 7.69 (1H, d), 7.25 (1H, d), 7.17 (1H, d), 5.49 (1H, t), 3.6 (2H, d), 1.82 (6H, s).


Cl
N
H

92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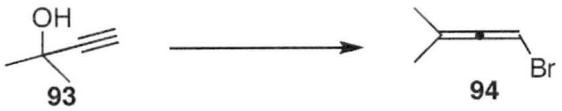
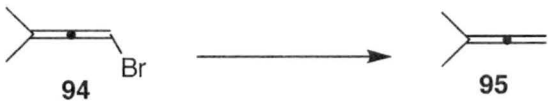
1.031
1.208
2.000
1.047
2.114
8.655
1.012
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2



3-methylbuta-1,2-diene (94). Ammonium bromide (2.49 g, .0254 mol), Copper(I) bromide (3.11 g, .022 mol), copper metal (158 mg, .025 mol) and 48% hydrobromic acid (12 mL, .105 mol) were added to a 50 mL round bottom flask. To the mixture 2-methyl-3-butyn-2ol **93** (6 mL, .062 mol) in pentane (15 mL) was added dropwise via an addition funnel over .5 h. The reaction was then heated to 30° C for 3 h. The biphasic material was then filtered through glass wool into a sepratory funnel and extracted with pentanes (2 x 15 mL), HBr (4 x 8 mL), water (2 x 10 mL), dried over $MgSO_4$, filtered through a plug of silica gel and concentrated in an iced roto vap bath to provide the brominated allene **92** as a clear solution (5.93g, 97% yield from **93**).

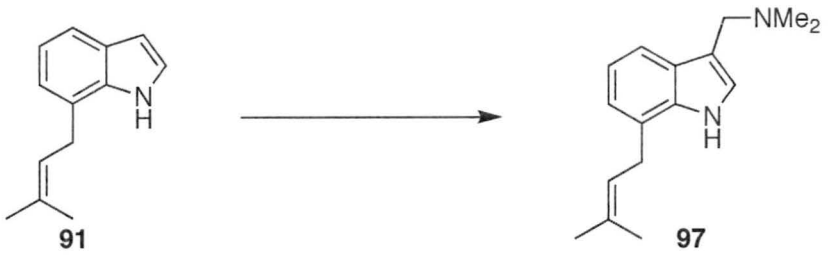
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



94 95

3-methylbuta-1,2-diene (95). **94** was then combined in a round bottom flask equipped with a vigor column and a simple distillation head with zinc dust (7.2 g, .111 mol) and acetic acid (46.3 mL, 2M). The mixture was heated to 85° C and the product was distilled off at atmospheric pressure to provide **95** as a clear oil (2.98g, 76% yield from **94**).

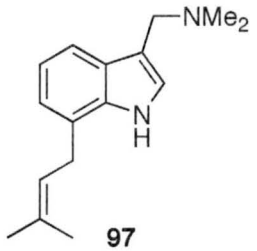
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



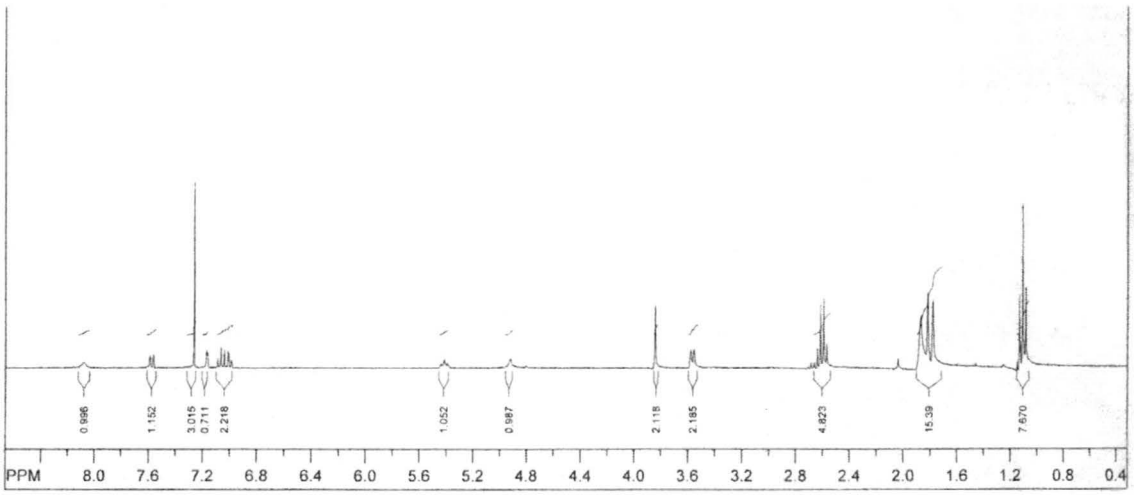
91 97

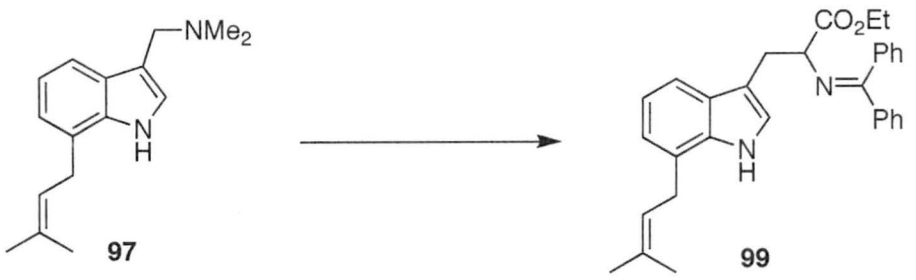
N, N-dimethyl-1-(7-(3-methylbut-2-enyl)-1H-indole-3-yl)methanamine (97). To a cooled flask containing glacial acetic acid (5.45 mL), dimethylamine (2.62 mL) was added. The mixture was then cooled in an ice bath. Formaldehyde (1.17 mL) was added and the above mixture was then added to room temperature indole **91** (4.84 g, 25.8 mmol) in one portion. The mixture was then allowed to stir under Ar overnight. The mixture was then basified with 2 N NaOH until the gramine precipitated. The solid was then filtered *in vacuo*, washed with water and allowed to dry to afford **97** (5.34 g, 85% yield from **91**).

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 8.09 (1H, s), 7.59 (1H, d), 7.18 (1H, s), 7.09 (2H, m), 5.41 (1H, t), 4.93 (1H, s), 3.82 (2H, s), 3.58 (2H, d), 1.81 (6H, s), 1.12 (6H, s).

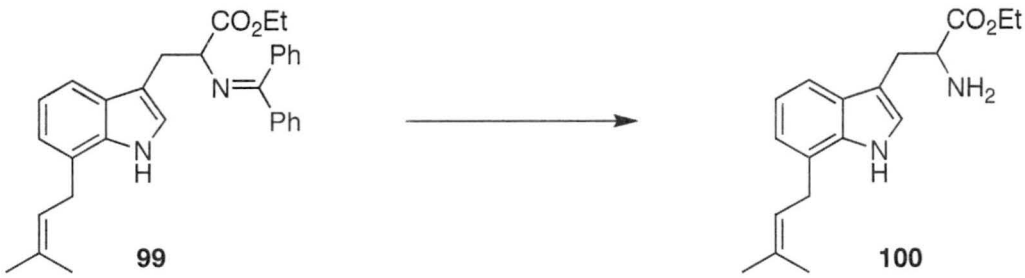

NMe2
N
H

97


0.998
1.152
3.015
0.711
2.218
1.052
0.987
2.118
2.185
4.823
15.39
7.670
PPM
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4

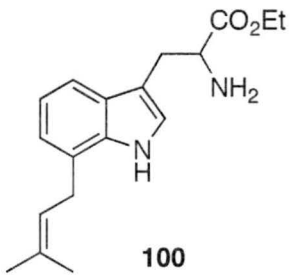


Ethyl 2-(diphenylmethyleneamino)-3-(7-(3-methylbut-2-enyl)-1H-indol-3-yl)propanoate (99). The gramine **97** (.479 g, 1.97 mmol) , N-(Diphenyl methylene) glycine ethyl ester **98** (.481 g, 1.79 mmol), Bu_3P (.177 mL), CH_3CN (8.99 mL) and a stir bar were sealed in a microwave reaction vessel. The mixture was irradiated at 130 °C for 30 minutes. The reaction mixture was then concentrated *in vacuo* and taken onto the next step without further purification.

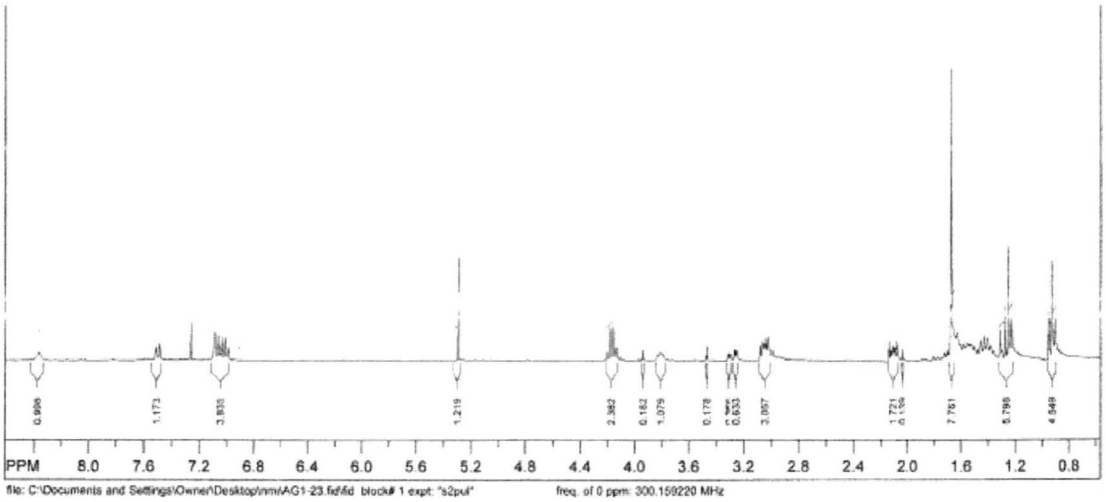


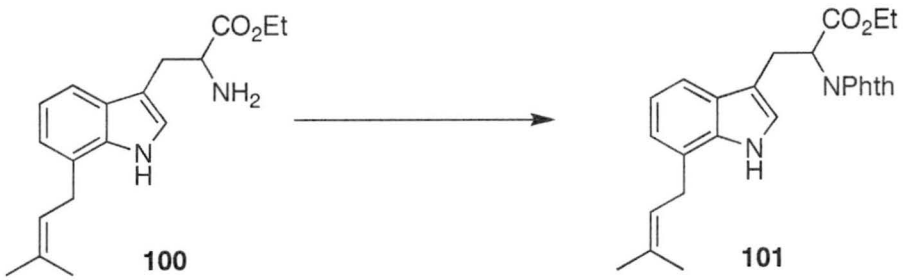
Ethyl 2-amino-3-(7-(3-methylbut-2-enyl)-1H-indol-3-yl)propanoate (100). To a stirred solution of the crude imine **99** in THF was added 1 N HCl. A color change indicated that the reaction was finished. The mixture was concentrated *in vacuo* and rediluted in $NaHCO_3$ until basic. The mixture was then extracted with dichloromethane and again concentrated *in vacuo*. The crude material was purified by column chromatography (95:5 DCM / MeOH) to give **100** (.63 g, 57% yield from **97**).

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 8.36 (1 H, s), 7.50 (1 H, d), 7.09 (1 H, s), 7.06 (1 H, d), 6.99 (1 H, s), 5.29 (1 H, s), 4.17 (2 H, m), 3.80 (1 H, t), 3.25 (2 H, d), 3.03 (2 H, m), 1.67 (6 H, s), 1.26 (3 H, t).


CO2Et
NH2
N
H

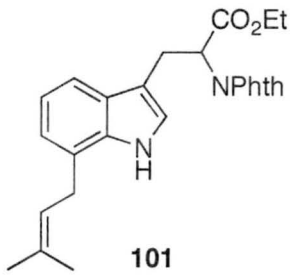
100


0.998
1.173
3.833
1.219
2.382
0.182
1.079
0.178
0.633
3.057
1.721
0.156
7.761
5.798
4.549
PPM
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
file: C:\Documents and Settings\Owner\Desktop\nmr\AG1-23.fid\fid block# 1 expt: 's2pul'
freq. of 0 ppm: 300.159220 MHz

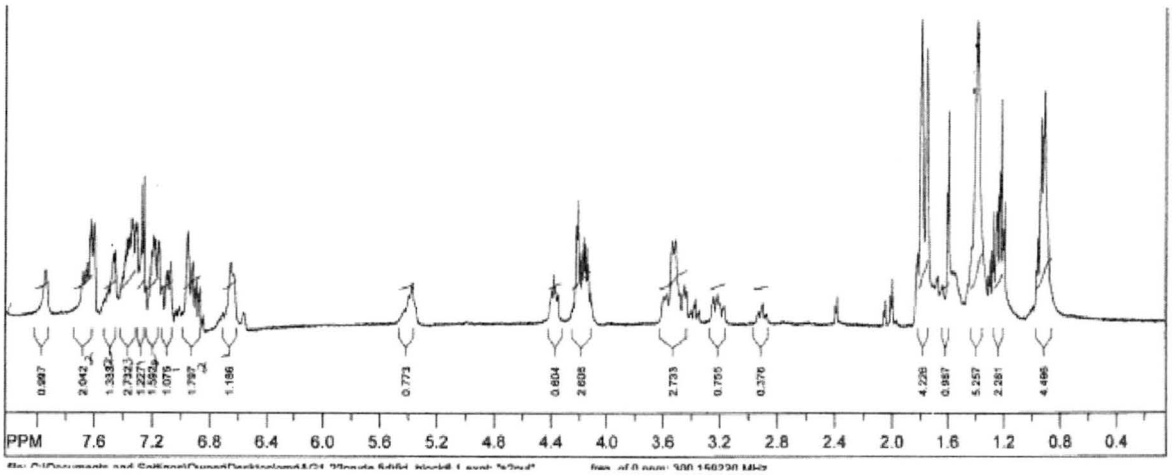


Phth Protected Ethyl 2-amino-3-(7-(3-methylbut-2-enyl)-1H-indol-3-yl)propanoate (101). The amine **100** (3g, 10 mmol) was stirred with N-carboethoxyphthalimide (2.19 g, 10 mmol), sodium carbonate (1.05 g, 10 mmol) in water for 6 hours at room temperature under Ar. The solution was then extracted with ether (2 x 25 mL) and then acidified to a pH of 2 with 10% $KHSO_4$. The mixture was then extracted with ethyl acetate (3 x 30 mL). The organic layers were then combined and washed with brine and dried with anhydrous Na_2SO_4. The mixture was concentrated *in vacuo*. The crude material was purified by column chromatography to give **101** (3.24 g, 75% yield from **100**).

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 8.17 (1 H, s), 7.77 (2 H, s), 7.67 (2 H, s), 7.48 (2 H, t), 7.02 (2 H, m), 5.26 (1 H, s), 4.24 (1 H, t), 4.13 (2 H, m), 3.74 & 2.98 (2 H, dd), 3.47 (2 H, d), 1.61 (6 H, s), 1.25 (3 H, t).


CO2Et
NPhth
N
H

101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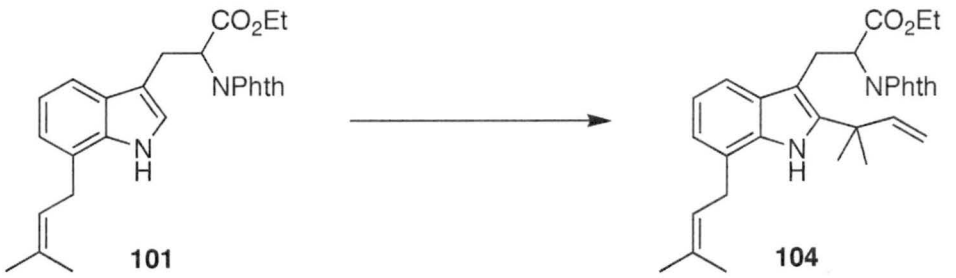
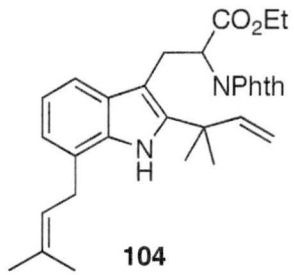
2.732
2.277
1.552
1.075
1.797
1.186
0.773
0.604
2.608
2.733
0.755
0.376
4.226
0.987
5.257
2.281
4.466
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4

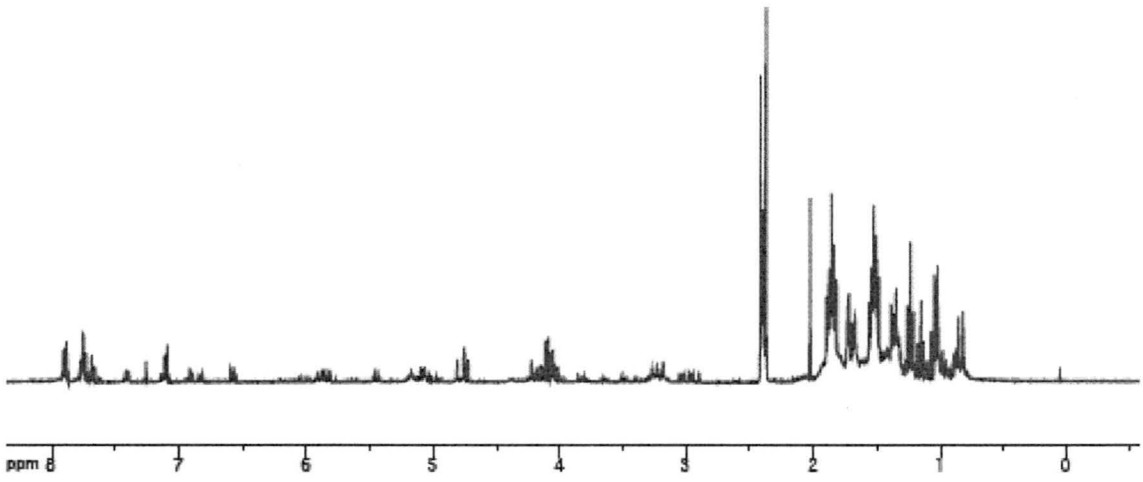


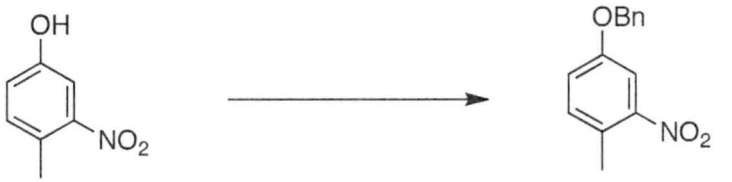
ethyl 2-(1,3-dioxoisoindolin-2-yl)-3-(7-(3-methylbut-2-enyl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propanoate (104). 9BBN (4.16 mL, .002 mol) was added to a 5 mL flask under Ar and chilled to 0° C. 3-methylbuta-1,2-diene (.341 mL, .0035 mol) was added and the mixture was allowed to slowly warm to room temperature and stir overnight. In a second round bottom flask, containing **100** (.3 g, .694 mmol) in Et_3N (.145 mL) was stirred and chilled to -78° C. To that flask was added freshly prepared t-butylhypochlorite (.1267 mL). The mixture was then stirred for half an hour at -78° C. The prenyl 9BBN was then added dropwise. The mixture was allowed to run for about 2 hours and followed by TLC. To work up the reaction was extracted with saturated K_2CO_3, and ethyl acetate. The organic layer was dried with $MgSO_4$ and concentrated. The mixture was purified by column chromatography (99:1, Hexanes : Ethyl acetate) to yield **104** (.11 g, 45% yield) as a yellow oil.

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 7.9 (1H, m), 7.75 (2H, m), 7.67 (2H, m), 7.14 (1H, t, J=6.6Hz), 7.09 (1H, d, J=7.5 Hz), 6.92 (1H, d, J=7.8Hz), 5.92 (1H, dd, J=2.4, 8.4 Hz), 5.85 (1H, dd, J=2.7, 8.1 Hz), 5.09 (2H, m), 4.82 (1H, t, J=9.6 Hz), 4.09 (2H, m), 3.59 (1H, d, J=9 Hz), 3.23 (2H, d, J=2.1 Hz), 3.22 (2H, d, J=2.1 Hz), 1.73 (2H, s), 1.69 (6H, s), 1.24 (3H, t, J=7.2 Hz).



104

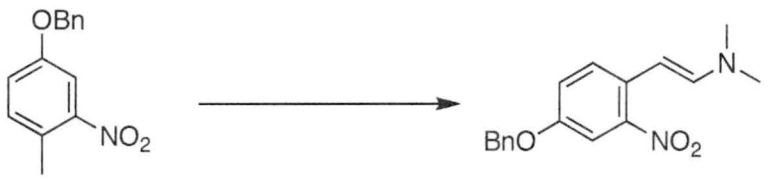




112 **118**

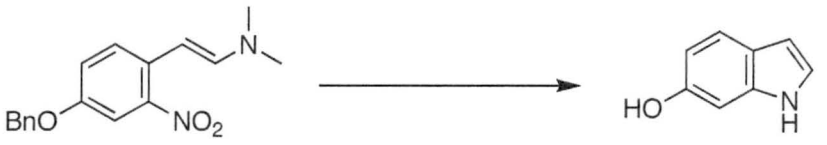
4-(benzyloxy)-1-methyl-2-nitrobenzene (118). To a stirred solution of 4-methyl-3-nitrophenol **112** (10g, 65.3 mmol) in DMF was added benzyl chloride (8.26 mL, 71.8 mmol) and potassium carbonate (9.03 g, 65.3 mmol). The reaction mixture was heated to 100°C for 4 hours. The mixture was then cooled to room temperate, poured into water and extracted with ethyl acetate. The organic layer was then washed with 1N NaOH and brine. The mixture was then dried over Na_2SO_4 and concentrated to afford a yellow flaky solid. The solid was recrystalized from ethyl acetate and hexanes to produce fine pale yellow crystals **118** (15.2 g, 96% from **112**).

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



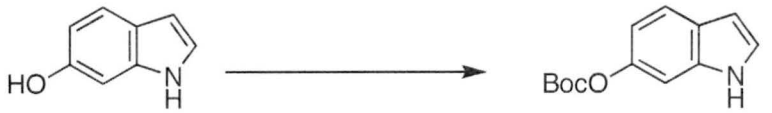
118 **121**

(*E*)-2-(4-(benzyloxy)-2-nitrophenyl)-*N*,*N*-dimethylethenamine (121). **118** (1 g, .0041 mol) in DMF-DMA (1.63 mL) were refluxed at 110° C for 6 hr. The mixture was allowed to cool to room temperature. Water was added and the mixture was then extracted with ether and water. The organic layers were combined and dried over $NaSO_4$ and concentrated to provide **121** as red oil. The material was taken on without further purification.



121 **114**

1*H*-indol-6-ol (114). **121** (10 g, .033 mol) was dissolved in ethanol (891 mL) and stirred with 10% Pd/C (1.79 g) in bomb under H_2 at 45 psi for 2 hours. The mixture was then filtered through a plug of silica gel, washed with absolute ethanol and concentrated. The mixture was then washed with anhydrous ether and concentrated to provide **114**. The material was taken on immediately with no further purification.



114 **115**

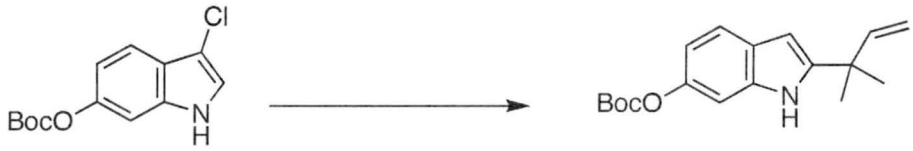
***tert*-butyl 1*H*-indol-6-yl carbonate (115).** **114** (4.46 g, .0335 mol) was stirred in ACN (134 mL) at 0° C. $(BOC)_2O$ (5.85 g, .026 mol), and DMAP (.041 g, .00034 mol) were added and the mixture was allowed to slowly warm to room temperature and stir overnight. The mixture was concentrated and purified via column chromatography (95:5, Hexanes : Ethyl Acetate) to provide **115** (7.4 g, 95% yield from **115**) as a white solid.

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.

115 **122**

***Tert*-butyl 3-chloro-1*H*-indole-6-yl carbonate (122).** **115** (1.96 g, 8.4 mmol) was stirred with NCS (1.12 g, 8.4 mmol) in DMF (42 mL) for 3-4 hours under Ar. The reaction mixture was then purified with column chromatography (95:5 Hex:EtOAc) to provide the chlorinated indole (1.97 g, 88% yield) as a brown solid.

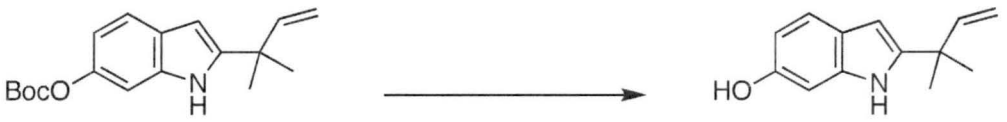
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



122 **123**

***tert*-butyl 2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-yl carbonate (123).** 9BBN (2.24 mL, 1.12 mmol) was added to a 5 mL flask under Ar and chilled to 0° C. 3-methylbuta-1,2-diene **95** (.184 mL, .1.867 mmol) was added and the mixture was allowed to slowly warm to room temperature and stir overnight. In a second round bottom flask, containing **122** was added THF (1.24 mL) and Et_3N (.169 mL) and the mixture was allowed to stir for 20 minutes. The mixture was then added in one portion to the prenyl-9BBN and allowed to stir for about 2 hours. The reaction was followed by TLC. The mixture was then concentrated and purified by column chromatography (95:5, hexanes : ethyl acetate) to yield **123** (.06 g, 54% yield from **122**) as a brown oil.

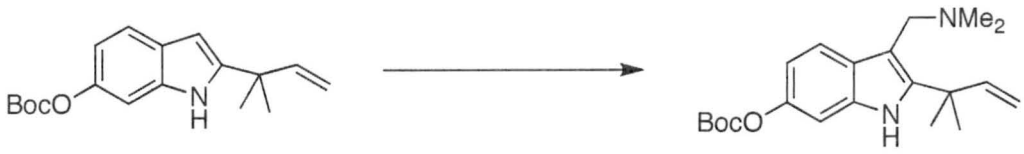
^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



123 **124**

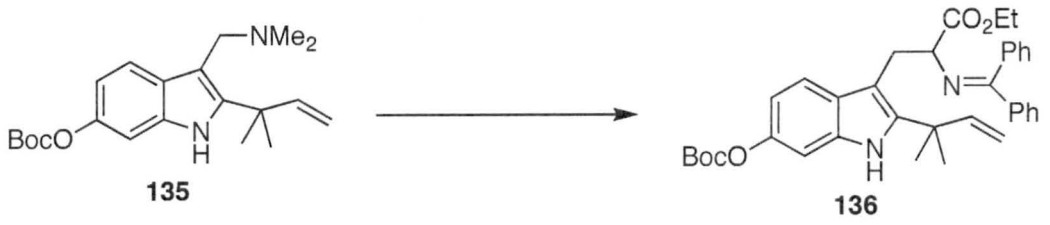
2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-ol (124). **123** (.426 g, .001 mol) was dissolved in DCM (10.65 mL) and chilled at 0° C. TFA (1.13 mL, .015 mol) was added dropwise and the reaction was followed by TLC. The mixture was chilled in an ice bath and sodium bicarbonate was added slowly until the solution turned basic. The mixture was then extracted with ethyl acetate, dried with $NaSO_4$ and concentrated. The mixture was then purified by column chromatography (95:5, hexanes : ethyl acetate) to yield **124** (.16 g, 95% yield) as a brown solid.

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: Matched Literature.



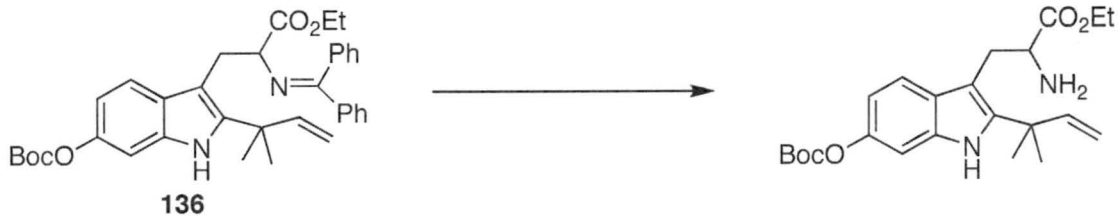
123 **135**

***tert*-butyl 3-((dimethylamino)methyl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-yl carbonate (135)**. Glacial acetic acid (.702 mL) and Me_2NH (.38 mL) were stirred at 2 °C in a round bottom flask. Formaldehyde (.145 mL) was added and the combined mixture was added in one portion to room temperature indole **123** (1 g, 3.5 mmol) and allowed to stir overnight. The mixture was then basified with 2 N NaOH and extracted with ethyl ether. The organic layers were dried with Na_2SO_4 and concentrated to provide **135** as a yellow oil which was taken on without further purification.



135 136

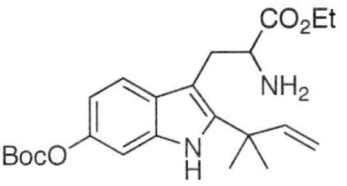
ethyl 3-(6-(*tert*-butoxycarbonyloxy)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)-2-(diphenylmethyleneamino)propanoate (136). In a microwave reaction vessel equipped with a stir bar gramine **135** (1.32 g, 3.68 mmol) was dissolved in ACN (16.75 mL). Ethyl 2-(diphenylmethyleneamino)acetate (.895 g, 3.35 mmol) and Bu_3P (.331 mL) were added. The mixture was irradiated at 130 °C for 30 minutes. The reaction mixture was then concentrated *in vacuo* to provide a red oil and taken onto the next step without further purification.

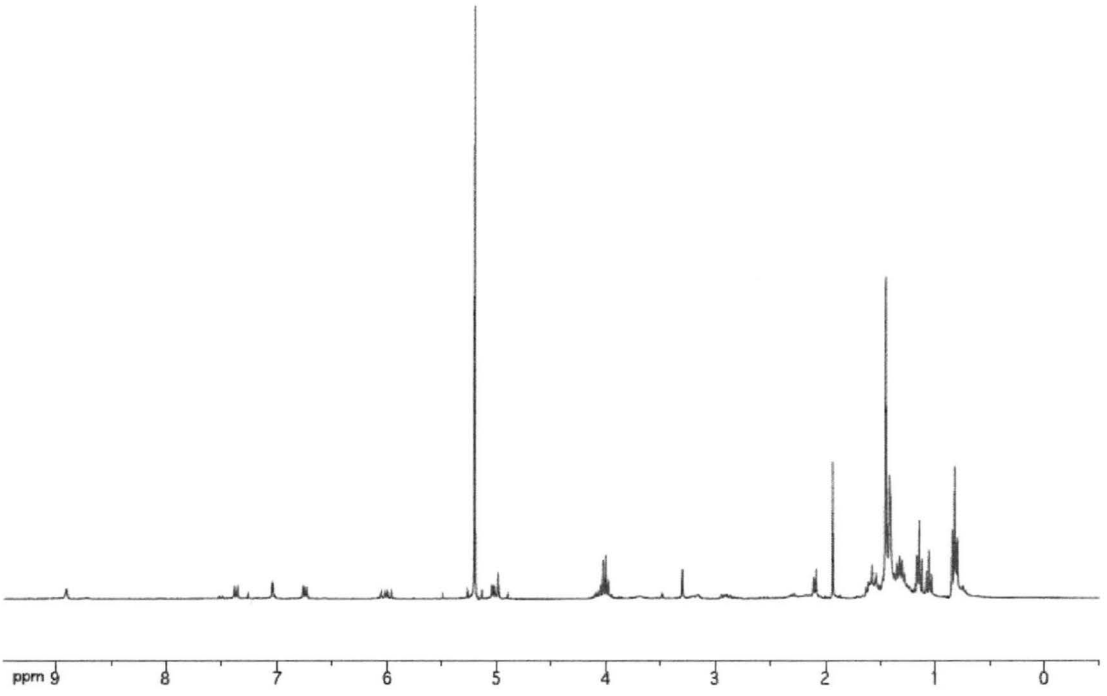


136

ethyl 2-amino-3-(6-(*tert*-butoxycarbonyloxy)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propanoate. The crude immine **136** (3.15 g, 5.42 mmol) was dissolved in THF (35.7 mL). 1 N HCl (11.9 mL) was added until the mixture was acidic and the color changed to red. The solution was concentrated to remove the THF. The mixture was then diluted with $NaHCO_3$ until basic and then extracted with DCM and concentrated. The material was purified by column chromatography 95:5 DCM:MeOH to provide the unprotected amine (2.05 g, 91 % yield from **136**) as a brown oil.

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 8.90 (1H, s), 7.38 (1H, d, J=8.7 Hz), 7.04 (1H, s), 6.73 (1H, d, J=.6 Hz), 5.99 (1H, m), 5.03 (2H, dd, J=10.8, 6.9 Hz), 4.00 (4H, m), 3.18 (2H, dd, J=3.9, 10.2 Hz), 1.93 (6H, s), 1.45 (9H, s), 1.17 (3H, t, J=7.2 Hz).


CO2Et
NH2
BocO
N
H


ppm 9
8
7
6
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4
3
2
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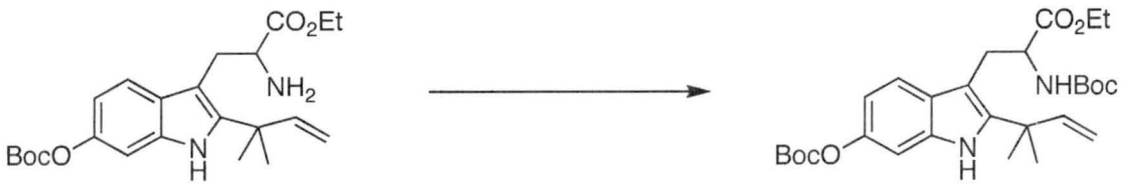
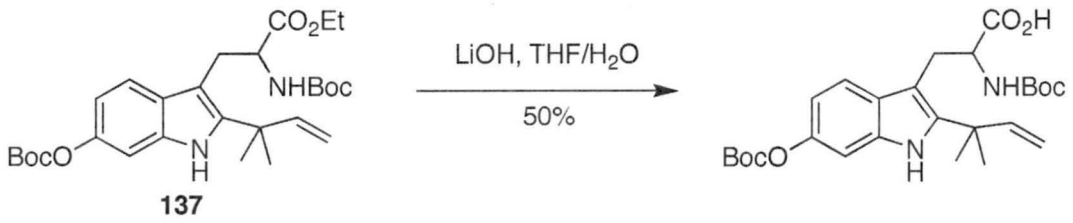


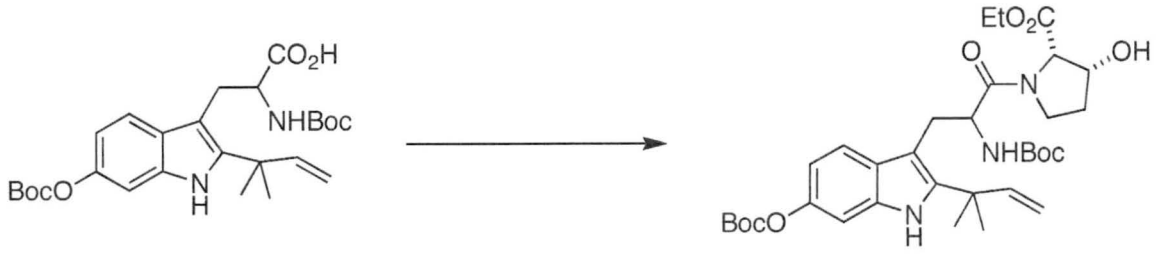
137

ethyl 2-(*tert*-butoxycarbonylamino)-3-(6-(*tert*-butoxycarbonyloxy)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propanoate (137). The amine (2.05 g, 4.925 mmol) was dissolved in dioxane (12 mL) and $(Boc)_2O$ (1.18 g, 5.41 mmol) was added. Aqueous NaOH was added and the reaction mixture was stirred at room temperature for 3 hours. The mixture was then concentrated to remove the dioxane and 10% $KHSO_4$ was added until the pH was approximately 2. The mixture was then extracted with ethyl acetate, washed with brine, dried with Na_2SO_4 and concentrated. The crude mixture was taken on without further purification.



137 **138**

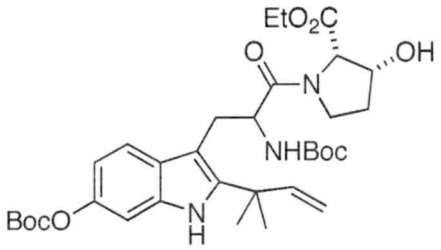
2-(*tert*-butoxycarbonylamino)-3-(6-(*tert*-butoxycarbonyloxy)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propanoic acid (138). **137** (1.46 g, 2.87 mmol) was dissolved in a 2:1 mixture of THF:H_2O (120 mL). LiOH (.338 g, 14.13 mmol) was added and the mixture was allowed to stir at room temperature 9 hours. The mixture was then concentrated to remove the THF and 10% $KHSO_4$ was added until the pH was approximately 2. The mixture was then extracted with EtOAc, dried over Na_2SO_4 and concentrated. The resulting brown solid was taken on without further purification.



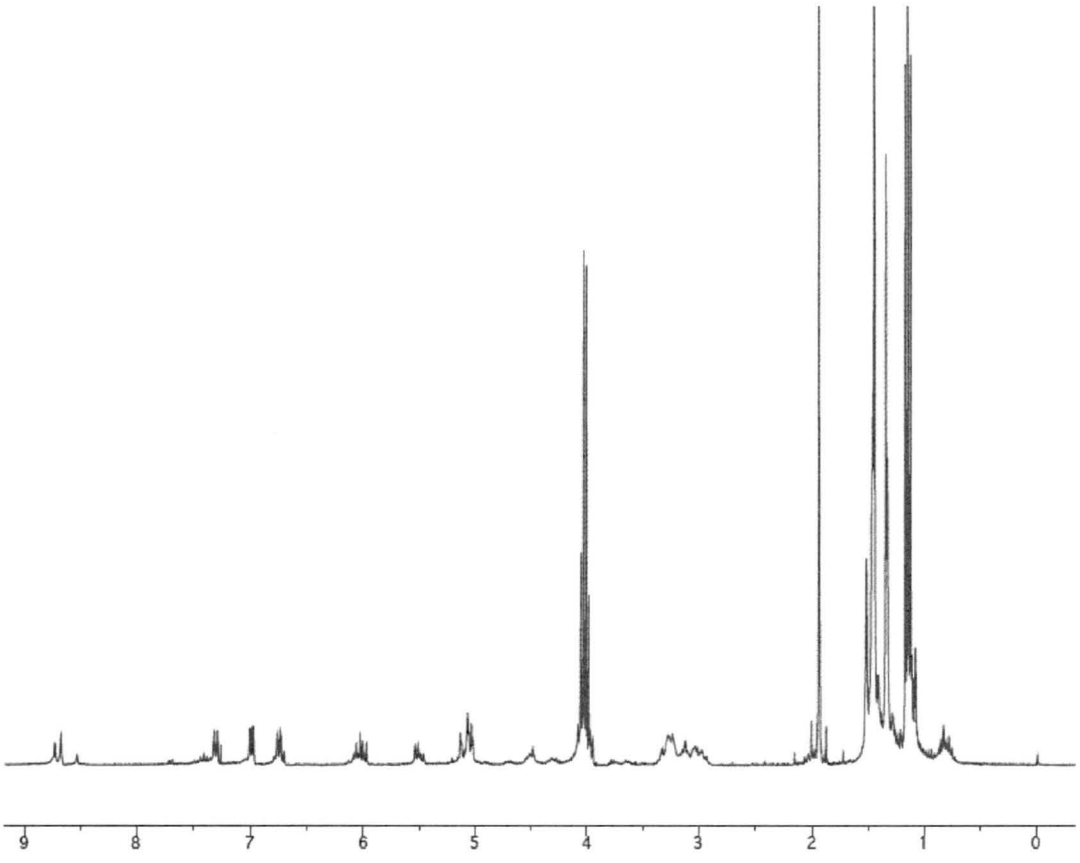
138 **139**

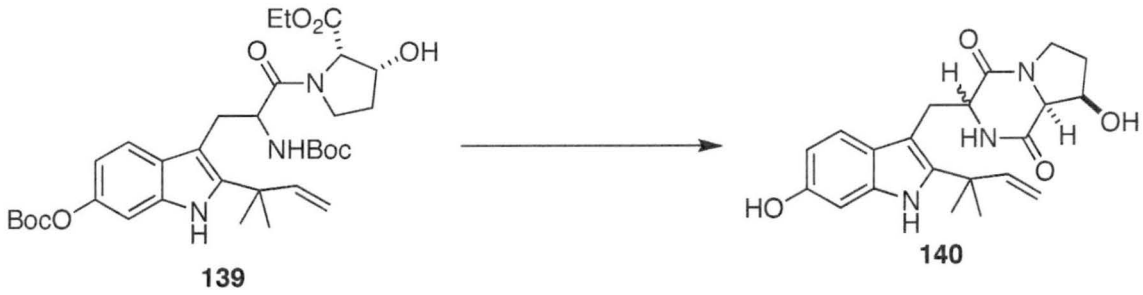
(2*S*,3*R*)-ethyl 1-(2-(*tert*-butoxycarbonylamino)-3-(6-(*tert*-butoxycarbonyloxy)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propanoyl)-3-hydroxypyrrolidine-2-carboxylate (139). 138 (.45 g, .92 mmol) was dissolved in ACN (8.77 mL). Sequentially HATU (.542 g, 1.38 mmol), *i*Pr_2Net (.642 mL, 3.68 mmol), (2*S*,3*R*)-2-(ethoxycarbonyl)-3-hydroxypyrrolidinium chloride (.359 g, 1.84 mmol) were added **109**. The reaction was allowed to stir for 3 hours at room temperature. 2 N HCl was added and the mixture was extracted with DCM, dried with Na_2SO_4 and concentrated. Purification was achieved by column chromatography 1:1 hexanes and ethyl acetate to afford **139** as a yellow foam (.43g, 48% yield from **138**).

^{1}H NMR (300MHz) ($CDCl_3$) δ TMS: 8.72 (1H, s), 8.531 (1H, s), 7.32 (1 H, d, J=8.7 Hz), 7.06 (1H, d, J=8.4 Hz), 6.73 (1H, s), 6.02 (1H, m), 5.13 (2H, t, J=9, 18 Hz), 4.48 (1H, m), 4.0 (4H, m), 3.32 (1H, s), 3.27 (1H, m), 3.18 (1H, m), 3.03 (1H, d), 2.97 (1H, d), 2.15 (1H, s), 2.00 (1H, s), 1.45 (6H, s), 1.34 (18H, s), 1.17 (3H, t, J=7.2 Hz).



139





139 **140**

***tert*-butyl 3-(((8*R*,8a*S*)-8-hydroxy-1,4-dioxooctahydropyrrolo[1,2-*a*]pyrazin-3-yl)methyl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-yl carbonate (140)**. TFA (1.46 mL) was added to a solution of **139** (.43 g, .683 mmol) in DCM (13.66 mL). The reaction was stirred for 3 hours and saturated $NaHCO_3$ was added and the mixture was extracted with ethyl acetate (3x 15 mL). The organic layers were combined and dried over Na_2SO_4 and then concentrated. The resulting brown oil was dissolved in toluene (13.6 mL) and 2-hydroxypyridine (.015 g) was added. The mixture was heated to reflux under Ar for 12 hours and then cooled to room temperature. The mixture was then concentrated and dissolved in DCM and washed with 1 N HCl, dried with Na_2SO_4 and concentrated to provide the DKP which was taken on without further purification.

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