DISSERTATION

STUDIES ON THE BIOSYNTHESIS OF THE STEPHACIDINS AND NOTOAMIDES. TOTAL SYNTHESIS OF NOTOAMIDE S AND NOTOAMIDE T. AND PROGRESS TOWARD THE SYNTHESIS OF CHRYSOGENAMIDE A.

Submitted by

Timothy Joseph McAfoos

Department of Chemistry

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Doctoral Committee:

Advisor: Robert M. Williams

Alan J. Kennan
John L. Wood
Richard G. Finke
Dean C. Crick

ABSTRACT

STUDIES ON THE BIOSYNTHESIS OF THE STEPHACIDINS AND NOTOAMIDES. TOTAL SYNTHESIS OF NOTOAMIDE S AND NOTOAMIDE T. AND PROGRESS TOWARD THE SYNTHESIS OF CHRYSOGENAMIDE A.

Herein I discuss my efforts toward the elucidation of the biosynthesis of the stephacidins and notoamide family of natural products. Notoamide S has been suggested to be the final common precursor between two different fungal strains, *Aspergillus* sp. and *Aspergillus versicolor*, before diverging to form enantiomerically opposite natural products (+) and (–)-stephacidin A and (+) and (–)-notoamide B. The synthesis of notoamide S comes from coupling *N*-Fmoc proline with a 6-hydroxy-7-prenyl-2-reverse prenyl tryptophan derivative synthesized through a late stage Claisen rearrangement. The oxidation of notoamide S affords an achiral azadiene that leads to an intramolecular Diels-Alder providing a new product, notoamide T, containing the bicyclo[2.2.2]diazaoctane ring system with the 6-hydroxy-7-prenyl indole ring of notoamide S. The synthesis of notoamide T

is accomplished through a radical addition to the pyran ring of stephacidin A followed by an elimination ring opening event to provide the 6-hydroxy-7-prenyl indole.

Chrysogenamide A is the newest member of the marcfortine family of natural products. Herein I discuss the synthesis of 7-prenyl-2-reverse prenyl indole through a thio-Claisen reaction and subsequent Lewis acid mediated sulfide removal. Coupling of a pipecolic acid derivative with the 7-prenyl-2-reverse prenyl tryptophan leads to the dipeptide containing all of the carbons needed in chrysogenamide A. I propose that chrysogenamide A can be synthesized through an unprecedented intramolecular Diels-Alder reaction of a monoketopiperazine by a condensation/tautomerization event leading to the appropriate azadiene for the intramolecular Diels-Alder reaction. A final oxidation of the intramolecular Diels-Alder product would lead to chrysogenamide A and what could be a newly proposed biosynthesis of a monoketopiperazine.

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My family for being there. Mom and dad you have been a great inspiration for me, and great role models. I am proud to be the person I am

today, and I owe it all to you. You did a great job at raising Allen and me and I can't thank you enough. I know I haven't always been the best kid, but you were always willing to give me a second chance and let me learn from my mistakes. I still can't believe you took bets on how long I was going to last at wrestling; I proved you both wrong. I really want to thank the biggest inspiration in my life, my brother Allen. I always look up to you and I always will. You have always been there for me and it was you who got me back on track. I am so proud to be your brother, and I miss hanging out with you all the time. You have a great family, Kim is the best, and she knows how to put you in your place and keep you in line. Your daughter is so beautiful, you did a great job, and you and Kim will be great parents. Thanks for everything you have done for me and thanks for always being there for me.

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realize how great you are, and how much you mean to me. I love you and I want to thank you for being you.

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In the words of a BMX company that a good friend rides for:

"Never give up, and never give in!"

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LIST OF ABBREVIATIONS

9-BBN	9-Borabicyclo[3.3.1]nonane
Ac_2O	Acetic anhydride
AcOH	Acetic acid
AIBN	Azobis(isobutyronitrile)
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
$(Boc)_2O$	Di-*tert*-butyl dicarbonate
BOP	Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
Bu	Butyl
n-BuLi	Butyllithium
s-BuLi	*sec*-Butyllithium
t-BuLi	*tert*-Butyllithium
t-BuOK	Potassium *tert*-butoxide
Cbz	Benzyloxycarbonyl
CSA	Camphorsolfonic acid

COSY	Correlation spectroscopy
DA	Diels-Alder
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DEPT	Distortionless enhancement by polarization transfer
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethyl azocarboxylate
DIBAl-H	Diisobutylaluminum hydride
DIPEA	Diisopropylethylamine
DKP	Diketopiperazine
DMAP	4-(Dimethylamino)pyridine
DMAPP	Dimethylallyl pyrophosphate
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide
DMPU	*N,N*'-Dimethylpropyleneurea
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDCI	*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide

Et	Ethyl
EtOAc	Ethyl acetate
Et_2O	Diethyl ether
EtOH	Ethanol
eq	equivalents
Fmoc	Fluorenylmethyloxycarbonyl
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HMBC	Heteronuclear multiple bond correlation
HMPA	Hexamethylphosphoramide
HMQC	Heteronuclear multiple quantum correlation
IMDA	Intramolecular Diels-Alder
imid.	Imidazole
KHMDS	Potassium (bis)trimethylsilyl amide
K-Naphth.	Potassium naphthalenide
LDA	Lithium diisopropylamine
LiHMDS	Lithium (bis)trimethylsilyl amide
2,6-lutidine	2,6-Dimethylpyridine
*m*CPBA	*meta*-Chloroperbenzoic acid
Me	Methyl
MeI	Methyl iodide
MeOH	Methanol

MOM	Methoxymethyl
Ms	Methanesulfonyl (mesylate)
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
μW	Microwave
NaHMDS	Sodium (bis)trimethylsilyl amide
NCS	*N*-Chlorosuccinamide
NBS	*N*-Bromosuccinamide
NMM	*N*-Methyl morpholine
NOSEY	Nuclear Overhauser effect spectroscopy
PCC	Pyridinium chlorochromate
Ph	Phenyl
PHPLC	Preparative high pressure liquid chromatography
PMB	*para*-Methoxybenzyl
PPTS	Pyridinium p-toluenesolfonate
PTLC	Preparative thin layer chromatography
i-Pr	Isopropyl
Py. or Pyr	Pyridine
SEM	2-(Trimethylsilyl)ethoxymethyl
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDPS	*tert*-Butyldiphenlsilyl

TBS	*tert*-Butyldimetylsilyl
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
Tf	Trifluoromethanesulfonate
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMEDA	*N,N,N',N'*-Tetramethylethylenediamine
TMS	Trimethylsilyl
pTSA	*para*-Toluenesulfonic acid

Chapter 1: Biosynthetic Studies of Stephacidin A

1.1 Introduction

1.1.1 Isolation

1.1.1a Isolation of (+)-Stephacidin A

Isolated by Qian-Cutron and coworkers, (+)-stephacidin A (**1**) was extracted from the fungus *Aspergillus ochraceus* WC76466, which was collected from a soil sample from Sirsaganj, Uttar Pradesh, India and grown on Nunc fermentation plates.[3] The plates were extracted with MeOH and concentrated under reduced pressure to give an aqueous solution, which was partitioned against ethyl acetate. The ethyl acetate fraction was subjected to Sephadex LH-20 gel filtration chromatography, followed by further purification by reverse-phase HPLC. Three major products were isolated from the extracts: (+)-stephacidin A (**1**), (–)-stephacidin B (**2**), and (+)-avrainvillamide (**3**) (Figure 1).[3]

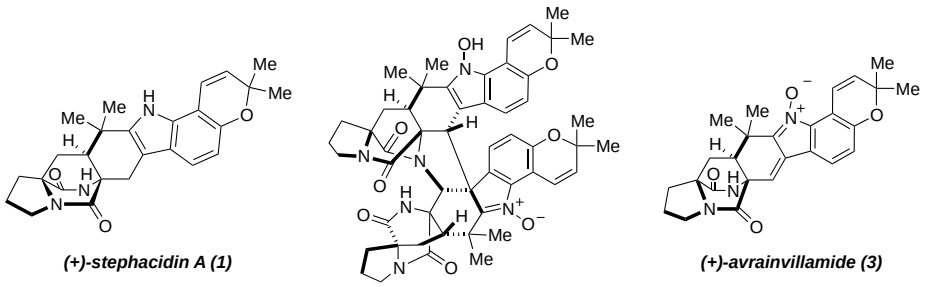


Figure 1: (+)-Stephacidin A, (–)-stephacidin B, and (+)-avrainvillamide.

Tsukamoto and coworkers isolated a second sample of (+)-stephacidin A collected off the Noto Peninsula in the Sea of Japan. This sample was taken from the fungus *Aspergillus sp*., which was separated from the mussel *Mytilus edulis*.[4] After fermentation on agar plates and extraction with ethanol, the fungus was found to produce four new metabolites along with three previously identified metabolites. Concentration of the extracts under reduced pressure was followed by extraction of the aqueous residue with ethyl acetate. That residue was further partitioned between MeOH/H_2O (9:1), then *n*-butanol to give two separate fractions in which the cytotoxic activity was found. The two fractions were combined and chromatographed through reverse-phase chromatography on an octadecasilane column with aqueous methanol as the eluent. The collected fractions were further purified by HPLC to afford four new compounds: notoamide A (**4**), B (**5**), C (**6**), and D (**7**) as well as sclerotiamide (**9**), deoxybrevianamide E (**8**) and (+)-stephacidin A (**1**) (Figure 2).[4]

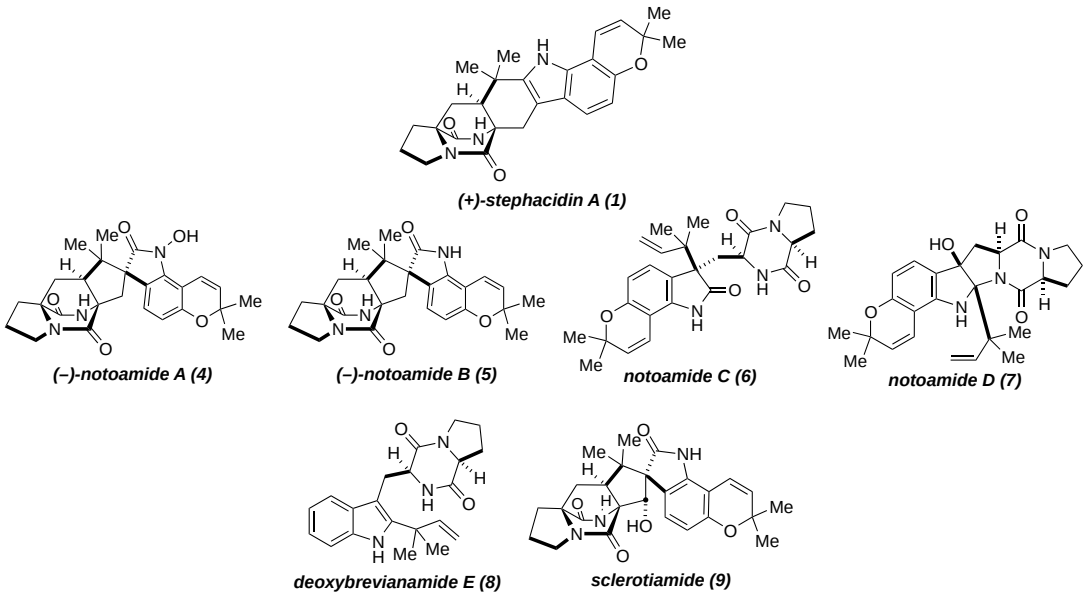


Figure 2: Notoamide A-D, sclerotiamide, deoxybrevianamide E, and (+)-stephacidin A.

1.1.1b Isolation of (–)-Stephacidin A

Collected by Gloer and coworkers from a Hawaiian forest, *Aspergillus versicolor* was obtained from a basidioma of *Gandoderma australe*.[20] Isolation of the metabolites began by culturing *Aspergillus versicolor* through solid-substrate fermentation on rice for 30 days at 25ºC before being extracted with ethyl acetate. The fractions were combined and concentrated under reduced pressure to give crude extract, which was further partitioned between acetonitrile and hexane. The acetonitrile layer was purified by Sephadex LH-20 chromatography. The final isolation of the metabolites was accomplished by reverse-phase HPLC to provide (–)-stephacidin A (**10**), (+)-notoamide B (**11**), and (+)-versicolamide B (**12**) (Figure 3).[20]

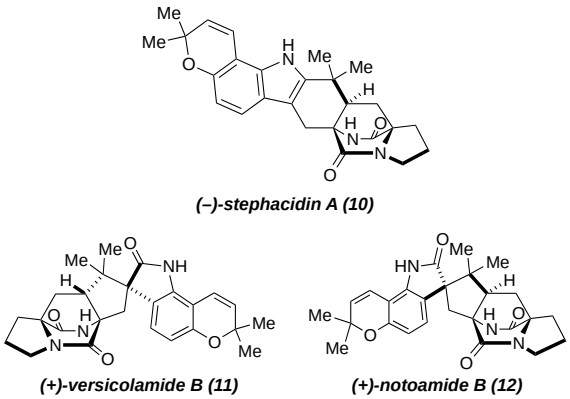


Figure 3. (–)-stephacidin A, (+)-notoamide B and (+)-versicolamide B.

1.1.2 Previous Work

The paraherquamide[1], brevianamide[2], stephacidin[3], and notoamide[4] families of prenylated indoles are of great interest to chemists and biologists because of their structural complexity and biological properties. In particular, the members of these families containing the unique bicyclo[2.2.2]diazaoctane ring system have drawn the most attention. Our group has been very interested in these bicyclo[2.2.2]diazaoctane ring-containing natural products for quite some time. With the synthesis of paraherquamides A[18] and B[5], pre-paraherquamide[6], VM55599[6], and brevianamide B[18] previously completed by our group, we envisioned the stephacidins and notoamides as applicable targets for our previously developed chemistry (Figure 3a).

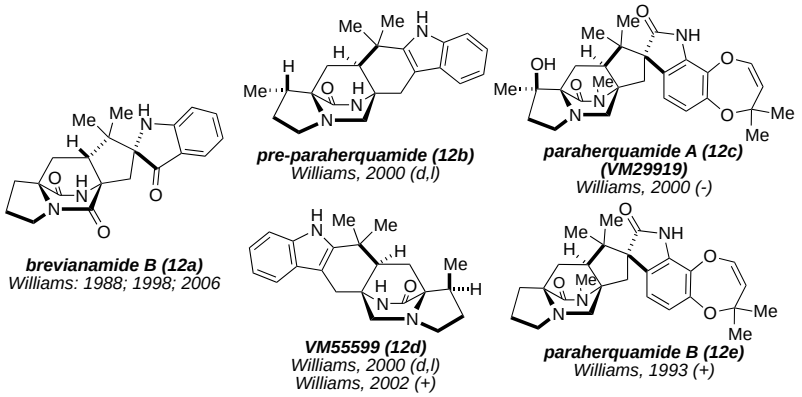
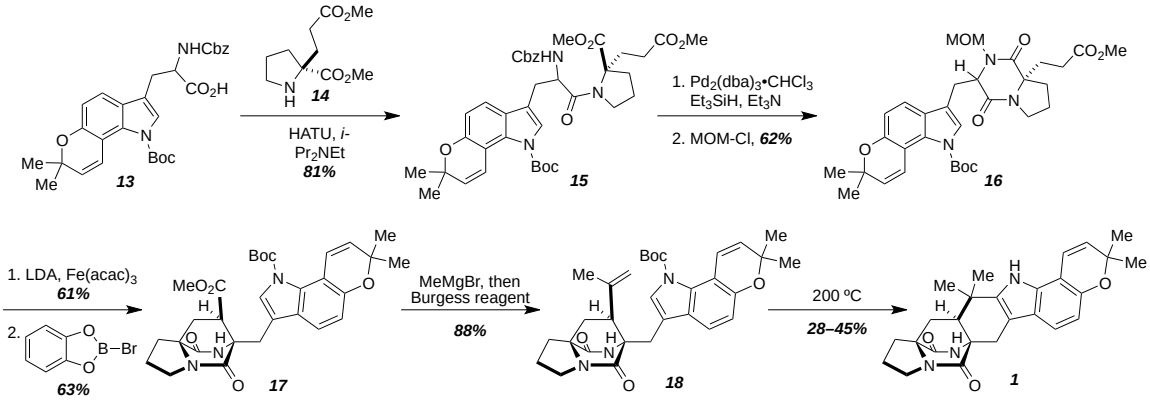


Figure 3a: Williams' completed syntheses of bicyclo[2.2.2]diazaoctane containing natural products.

The first synthesis of stephacidin A was completed by Baran and coworkers in 2006 through the use of an oxidative enolate coupling reaction to gain access to the bicyclo[2.2.2]diazaoctane core.[7] Starting from an advanced tryptophan core system (**13**), coupling to a functionalized proline (**14**) gave dipeptide **15**. Deprotection of the Cbz group followed by cyclization gave the desired diketopiperazine core system (**16**), which was protected as the MOM ether needed for the key oxidative coupling reaction. Treatment of **16** with LDA in the presence of $Fe(acac)_3$ afforded the desired bicyclo[2.2.2]diazaoctane core (**17**). Bis-methylation with methyl Grignard followed by addition of Burgess reagent gave the primary 1,1 alkene (**18**), which, when heated at 200ºC, afforded stephacidin A.

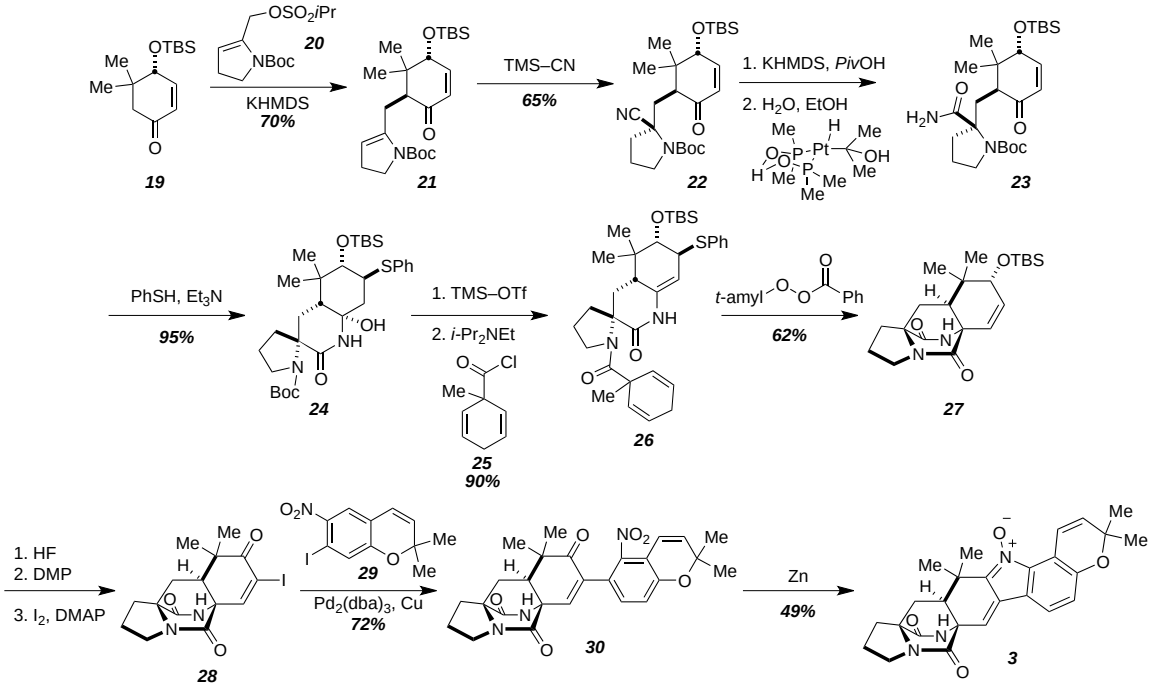


Scheme 1: Baran's total synthesis of stephacidin A.

Myers and coworkers also completed the synthesis of a related natural product, avrainvillamide (**3**), en route to stephacidin B (**2**) in 2005. Containing the same bicyclo[2.2.2]diazaoctane core system as stephacidin A, avrainvillamide was synthesized through a key aminoacyl radical cyclization to form the desired bicyclo core (Scheme 2).[8]

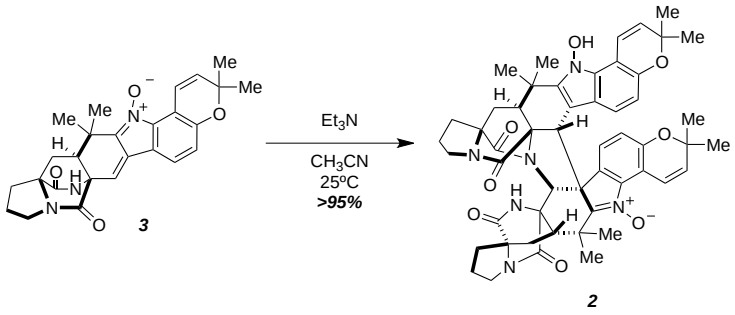
Their synthesis started with the alkylation of ketone **19** with proline derivative **20** in the presence of KHMDS to give the *trans*-coupled product (**21**) as a single diastereomer. Treatment of **21** with TMSCN gave the Strecker-like addition of hydrogen cyanide to afford nitrile **22**. Epimerization of **22** followed by treatment with the platinum catalyst developed by Ghaffar and Parkins[9] gave primary amide **23**. Treatment of amide **23** with thiophenol in the presence of TEA led to sulfide addition, along with cyclization to hemiaminal **24**. Removal of the Boc group as well as dehydration of the hemiaminal led to the desired enamide, wherein acylation of the pyrrolidinyl amino group with the 1-methyl-2,5-cyclohexadiene-1-carbonyl chloride (**25**) provided the desired radical cyclization substrate (**26**). Heating **26** in the

presence of tert-*amyl* peroxybenzoate initiated the cascading radical cyclization to give the bridged diketopiperazine core (**27**) in a 62% yield. Deprotection of the silyl enol ether followed by oxidation of the resultant alcohol gave the desired α,β-unsaturated ketone, which was transformed to the α-iodoenone (**28**) with iodine and DMAP. Coupling of **28** with nitroarene **29** under Ullmann-like conditions[10] (10% $Pd_2(dba)_3$, copper powder) gave coupled product **30** in a 72% yield. Reduction of the coupled nitroarene with activated zinc powder lead the formation of avrainvillamide as a yellow solid in a 49% yield. The synthesis of avrainvillamide (**3**) was completed in 17 steps with a 4.2% overall yield (Scheme 2).



Scheme 2: Myers synthesis of avrainvillamide.

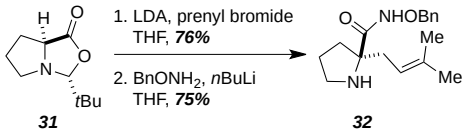
Treatment of avrainvillamide (**3**) with TEA in acetonitrile at room temperature ultimately led to the dimerization of the natural product to form stephacidin B (**2**) in greater than 95% yield (Scheme 3).



Scheme 3: Conversion of avrainvillamide to stephacidin B.

A second family of natural products related to the stephacidin family is the malbrancheamides. The malbrancheamides contain the same bicyclo[2.2.2]diazaoctane ring system of the stephacidin family[3], but lack the pyranyl indole ring. In 2009 Simpkins and coworkers completed the total synthesis *ent*-malbrancheamide B through a double cyclization of hydroxyldiketopiperazine compound via an α-amido *N*-acyliminium species.[11] Simpkins' synthesis of *ent*-malbrancheamide B started from *tert*-butyl 6-chloro-3-formyl-1*H*-indole-1-carboxylate (**33**) and hydroxamic acid derivative **34**. For the synthesis of the hydroxamic acid moiety, Simpkins and co-workers used the method developed by Seebach[12] for the "self-reproduction of chirality" in the installation of the dimethylallyl group onto a protected proline. The Seebach acetal (**31**) was treated with LDA, followed by addition of dimethylallyl bromide giving the desired prenylated proline acetal. Treatment

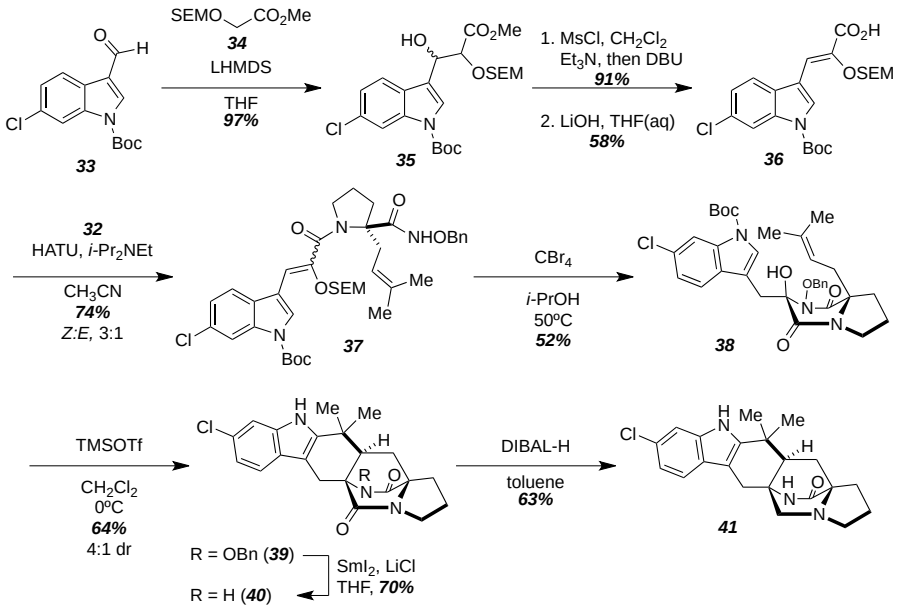
with *O*-benzylhydroxylamine gave the proline-derived hydroxamic acid (**32**) needed for coupling (Scheme 4).



31 ***32***

Scheme 4: Synthesis of hydroxamic acid derivative.

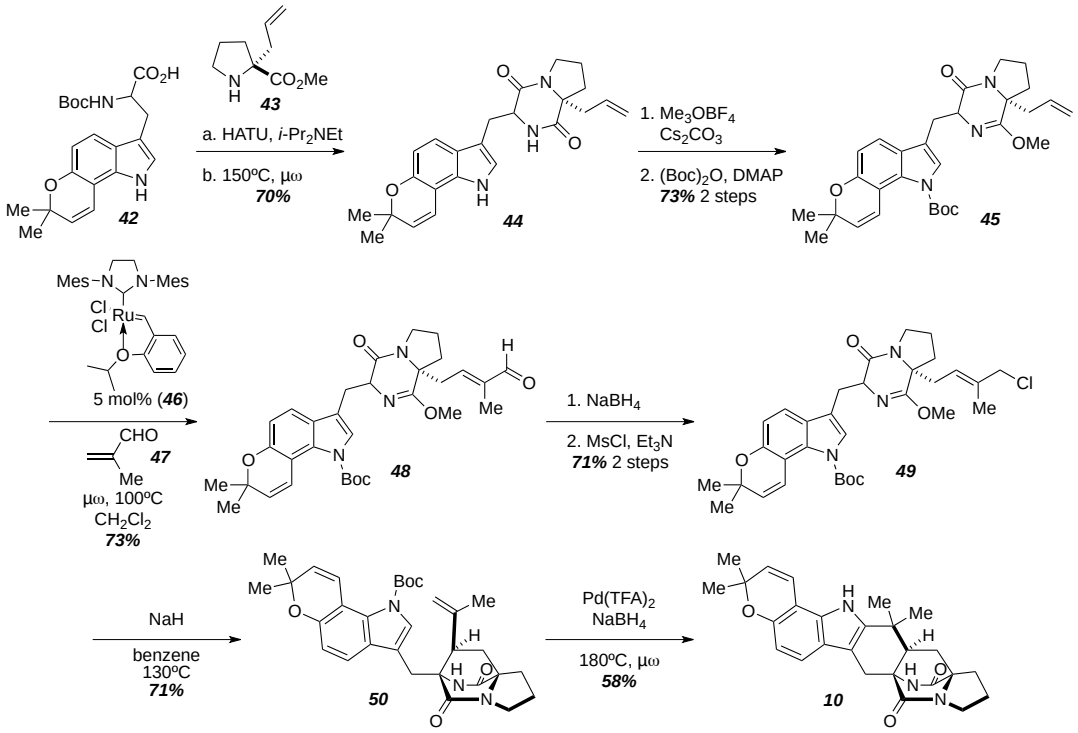
For the indole-3-pyruvic acid portion, an aldol condensation of ester **34** with aldehyde **33** gave alcohol **35**. Mesylation followed by elimination of the alcohol gave the unsaturated ester, as a 3:1 *Z/E* mixture, which was then hydrolyzed to the corresponding acid (**36**). Coupling of the proline amide derivative (**32**) to masked indole pyruvic acid **36** was completed with HATU in the presence of *i*-Pr_2NEt in a 74% yield and with retention of the 3:1 *Z/E* mixture. With the use of unconventional conditions, the SEM protecting group was removed to afford the cyclized diketopiperazine **38** in modest yield. With the key substrate in hand, exposure of **38** to TMSOTf at 0ºC gave the bicyclo[2.2.2]diazaoctane core (**39**) in a 4:1 diastereomeric ratio and a 64% yield. Samarium diiodide-mediated removal of the OBn group gave the desired secondary amide (**40**) in a 70% yield. DIBAl-H reduction of the tertiary amide completed the synthesis of *ent*-malbrancheamide B (**41**) in 10 linear steps starting from commercially available 6-chloroindole (Scheme 5).



Scheme 5: Simpkins' synthesis of *ent*-malbrancheamide B.

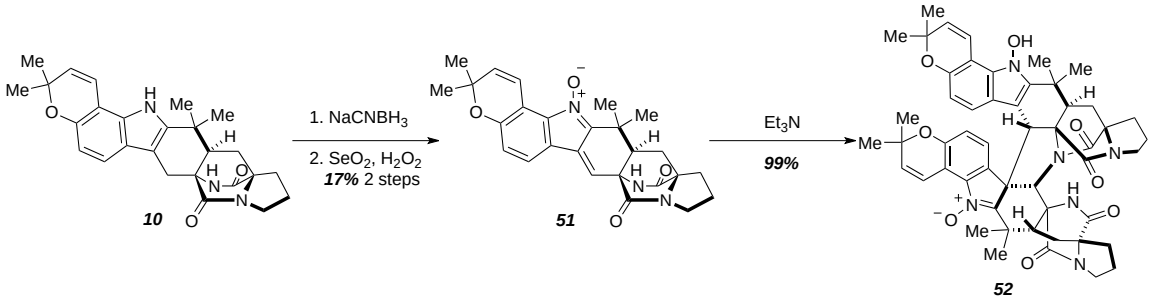
Our group also contributed to the synthesis of (–)-stephacidin A, (–)-avrainvillamide (**51**), and (+)-stephacidin B (**52**) through an S_N2' cyclization to form the bicyclo[2.2.2]diazaoctane ring system.[13] Coupling of tryptophan derivative **42** with functionalized proline derivative **43** in the presence of HATU and *i*-Pr_2NEt provided the corresponding *N*-Boc protected dipeptide. Thermal removal of the Boc group under microwave conditions afforded cyclized diketopiperazine **44** in a 70% yield. Formation of the lactim ether with methyl Meerwein salt, followed by carbamate protection of the indole nitrogen gave DKP ether **45**. Treatment of **45** with Hoveyda-Grubbs catalyst (**46**) transposed the primary olefin to the α,β-unsaturated aldehyde **48**. To set up for the key S_N2' reaction, **48** was reduced with $NaBH_4$ followed by mesylation and chlorination to give the corresponding allylic chloride **49**. With the allylic chloride in hand, **49** was treated with NaH and heated in benzene to give the

desired bicyclo[2.2.2]diazaoctane ring system (**50**) in a 71% yield. The *syn*-selectivity of the intramolecular S_N2' cyclization was believed to be due to a "closed" transition state via a tight contact-ion pair. The heptacycle was completed through a palladium mediated cyclization followed by thermal deprotection of the Boc group to provide **10** in 58% yield (Scheme 6).



Scheme 6: Williams' S_N2' synthesis of stephacidin A.

(–)-Stephacidin A was transformed to (–)-avrainvillamide (**51**) through sodium cyanoborohydride reduction followed by selenium dioxide oxidation, which when treated with TEA gave (+)-stephacidin B (**52**).



Scheme 7: Conversion of stephacidin A to stephacidin B through avrainvillamide.

A variety of conditions, from radical to metal mediated, were used in the formation of the bicyclo[2.2.2]diazaoctane ring system. However, in all of the cases described above, the bicyclo[2.2.2]diazaoctane ring system was formed prior to connecting the indole ring system. Though each synthesis was unique and complex, our interest lay more in the biosynthesis of these families and the origin of the bicyclo[2.2.2]diazaoctane ring system.

1.1.3 Biosynthesis

First isolated by Birch, Wright, and Russel in the late 1960's and early 1970's from *Penicillium brevicompactum,* brevianamide A (**62**), along with minor metabolite brevianamide B, were the first of the bicyclo[2.2.2]diazaoctane ring containing natural products to appear in the literature.[14] Sammes proposed that these metabolites could arise through an intramolecular hetero-Diels-Alder of an olefin with a pre-formed azadiene of the DKP ring (Figure 4).[15]

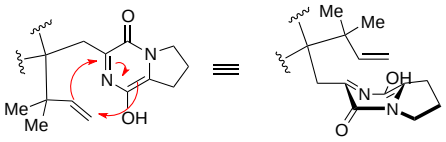
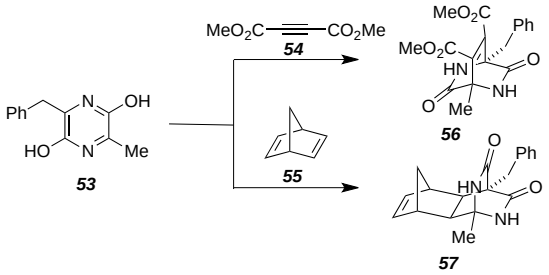


Figure 4: Azadiene formation for the intramolecular Diels-Alder reaction

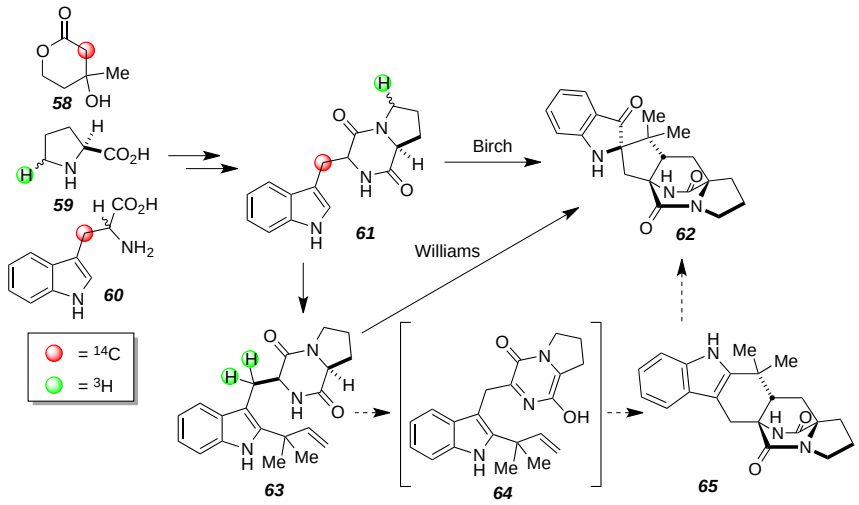
To test this hypothesis, Sammes performed a series of experiments on simple model systems to see whether the Diels-Alder reaction was plausible. Treatment of dihydroxypiperazine **53** with either dimethyl acetylenedicarboxylate (**54**) or norbornadiene (**55**) indeed led to the desired bicyclo[2.2.2]diazaoctane ring system (**56** or **57**, respectively) (Scheme 8). Though both examples were model systems, it created a great deal of interest in the possible intramolecular Diels-Alder reaction.



Scheme 8: Sammes' model system for the Diels-Alder reaction.

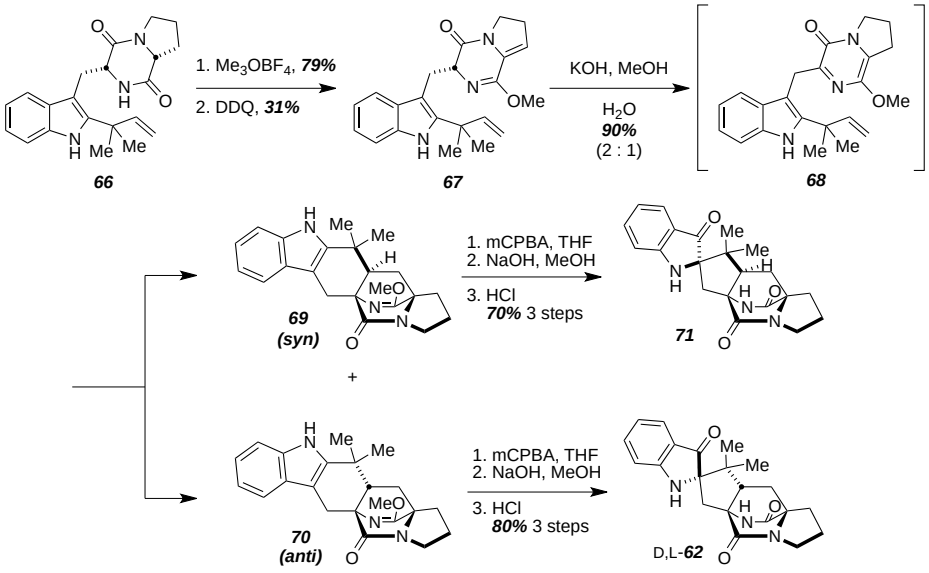
Through a series of radiolabeling and feeding experiments, Birch and coworkers showed that tryptophan (**60**), proline (**59**), and mevalonic acid (**58**) were biosynthetic precursors to **62**.[16] Further investigation led to the discovery that isotopically labeled brevianamide F (**61**) also showed significant incorporation into brevianamide A (**62**). With the results of the radiolabeling experiments, it was postulated that **61** undergoes a reverse prenylation reaction at the C2 position of indole to give deoxybrevianamide E

(**63**). It was then hypothesized that **63** is the substrate that participates in the IMDA, which can give rise to **62** following an oxidation (Scheme 9).



Scheme 9: Incorporation studies by Birch and Williams in brevianamide A.

Our group also took part in labeling studies of the biosynthesis of **62**, helping to provide strong evidence that **63** is the biosynthetic precursor to **62**.[17] We propose that **63** can undergo a net two-electron oxidation leading to azadiene **64**, which can cyclize to give **65**. Oxidation of **65** followed by a pinacol-type rearrangement would lead to **62**. To explore this proposed biosynthesis, our group synthesized **62** through the use of a key IMDA reaction to form the bicyclo[2.2.2]diazaoctane ring system.[18] Starting from epi-deoxybrevianamide E (**66**), treatment with trimethyloxonium tetrafluoroborate (Me_3OBF_4) afforded the desired lactim ether in 79% yield. Oxidation of the proline portion with DDQ gave conjugated diene **67**. Treatment of **67** with KOH in MeOH and H_2O gave a mixtue of *syn* and *anti*-cycloadducts in a 2:1 ratio and 90% yield (Scheme 10).[??]

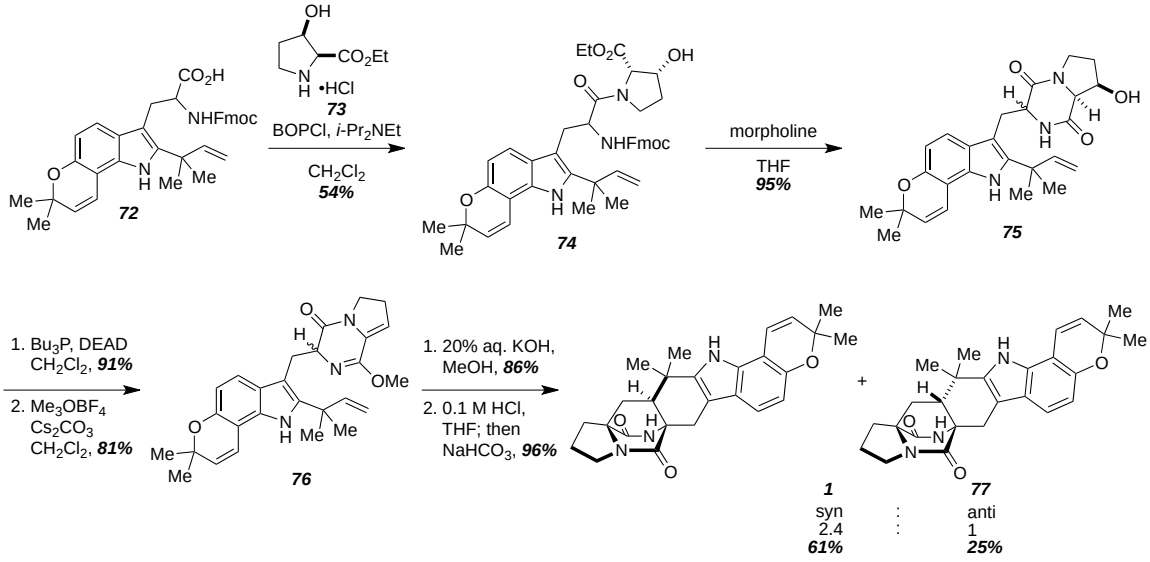


Scheme 10: Synthesis of **62** through an IMDA.

One of the major observations made in both the synthetic and biosynthetic systems was the outcome of the *syn* and *anti* ratio of the IMDA. Through a series of ab initio calculations it was found that the most favorable product of the Diels-Alder reaction should be the *anti* stereochemistry, relative to the secondary amide.[19] However, in the synthesis of **62**, it was found that the IMDA afforded a 2:1 ratio favoring the *syn* adduct, with respect to the secondary amide. One aspect that was initially overlooked was the substrate that was participating in the IMDA. Initial calculations showed that the favored transition state for the indoxyl cycloaddition was for the *anti* product[19] (which was later discovered to be correct) but the cycloaddition for the synthesis of **62** began from the indole.[18] This is an important factor for the synthesis of the bicyclo[2.2.2]diazaoctane containing natural products made through an IMDA. The *syn* relationship can be favored if the cycloaddition is performed on the

indole moiety, whereas the *anti* relationship can be favored if the cycloaddtion is performed on the oxindole or indoxyl moiety.

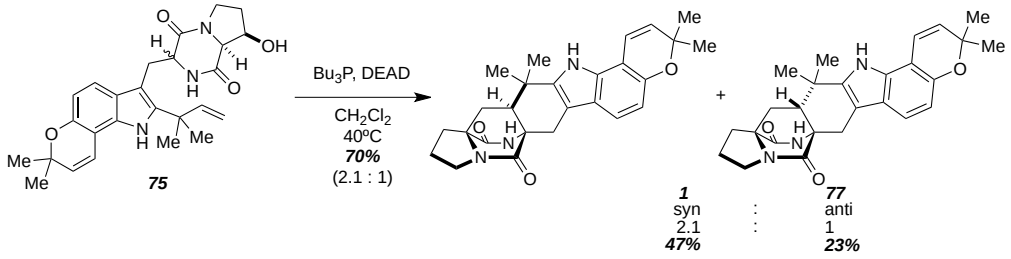
Synthetically, our group has shown that the IMDA is an efficient and probable biosynthetic pathway leading to metabolites containing the bicyclo[2.2.2]diazaoctane ring system. With the core of stephacidin A containing the same bicyclo[2.2.2]diazaoctane system, we became interested in its biosynthesis. Previously, we have shown that stephacidins A and B can be synthesized through an S_N2' methodology, but now we also wanted to prove that it could come from an IMDA reaction in what might be its biosynthetic pathway.



Scheme 11: Williams' biomimetic synthesis of stephacidin A.

The biomimetic synthesis of stephacidin A started by coupling previously reported **72**, with cis-hydroxy proline **73** in the presence of BOPCl and *i*-Pr_2NEt to give amide **74** in 54% yield (Scheme 11).[20] Removal of the Fmoc group with morpholine afforded the primary amine, which would

spontaneously cyclize to give diketopiperazine **75**. Mitsunobu-type elimination[21] of the alcohol followed by lactim ether formation with Me_3OBF_4 and Cs_2CO_3 provided azadiene **76** in good yield. Treatment of **76** with aqueous KOH in MeOH provided the IMDA product, which, upon hydrolysis of the lactim ether with aqueous HCl and subsequent basification with $NaHCO_3$ provided stephacidin A (**1**) (*syn*, 58%) and its diastereomer **77** (*anti*, 24%) in an 82% yield for the two steps.[20] The synthesis of stephacidin A was further improved with a one pot IMDA during the Mitsunobu-type elimination.[22] Treatment of **75** with 5 equivalents of both Bu_3P and DEAD followed by heating to 40ºC afforded stephacidin A and its epimer **77** in a 70% combined yield and a 2.1:1 ratio favoring the *syn* stereochemistry (Scheme 12).



Scheme 12: One pot elimination, tautomerization and cyclization to give **1**.

Our group has shown that synthetic stephacidin A can come from an IMDA reaction of the corresponding azadiene to afford stephacidin A and its epimer in 2.1:1 ratio and 70% yield as mentioned above. The existence of both enantiomers of stephacidin A in separate fungi led to an important biosynthetic question. When do these two separate fungi deviate to produce enantiomerically opposite metabolites, and what is the final common intermediate between the two fungi?

1.1.4 Genome-based Study of the Biosynthesis of Stephacidin A

Since the isolation of (+)-stephacidin A (**1**) in 2002 by Bristol-Myers Squibb from *Aspergillus ochraceus*, the biosynthetic pathway for the stephacidin family of alkaloids has been of particular interest to our laboratories.[3] (+)-Stephacidin A was also subsequently identified as a co-metabolite with several new prenylated indole alkaloids, the notoamides, by Tsukamoto and coworkers from a marine derived *Aspergillus* sp. Our interest in the biosynthesis of the stephacidin and notoamide families was heightened when the corresponding enantiomer, (–)-stephacidin A (**10**), was isolated from a terrestrial fungi, *Aspergillus versicolor* that was found growing in a Hawaiian forest (Figure 5).[4]

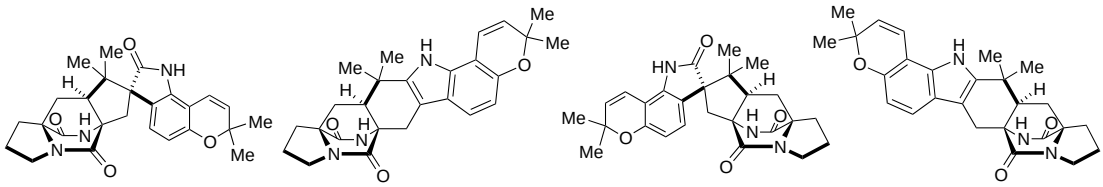
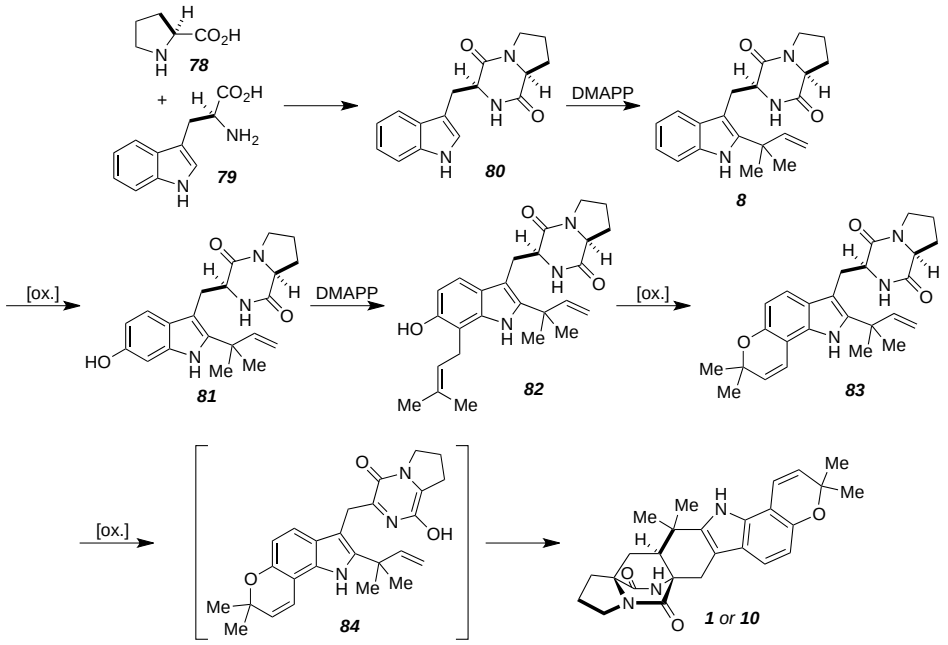


Figure 5: (+) And (–)-stephacidin A and (+) and (–)-notoamide B.

Previously, our group has shown that stephacidin A can arise synthetically from an IMDA reaction to form the bicyclo[2.2.2]diazaoctane ring system.[20] Biosynthetically, one can envision stephacidin A coming from two separate amino acid residues, tryptophan **79** and proline **78**. These two amino acids come together to form the secondary motabolite brevianamide F (**80**). Reverse prenylation of **80** at the C2 position of the indole afforded

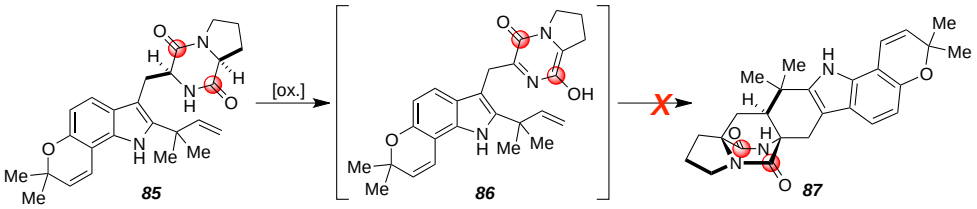
deoxybrevianamide E (**8**). Our first proposed biosynthesis started from **8** undergoing an oxidation of the indole ring at the C6 position to give 6-hydroxy-deoxybreveanamide E (**81**). **81** would then be prenylated at the C7 position of the indole affording **82**, followed by an oxidation, cyclization and elimination of the C7 prenyl group to give notoamide E (**83**). Notoamide E would then undergo a net two electron oxidation to form the azadiene which would cyclize to give (+) or (–)-stephacidin A (Scheme 13).



Scheme 13: Proposed biosynthesis of stephacidin A.

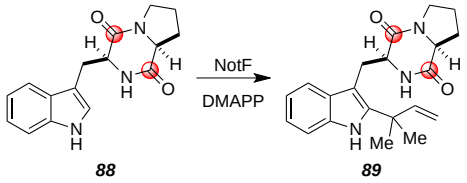
However, through precursor feeding and incorporation experiments of doubly ^{13}C-labeled **85**, it was discovered that **85** does not incorporate into **87** (Scheme 14).[23] This interesting discovery made us question the next steps in the synthesis of stephacidin A. We believe that our assumptions up to this point were correct based on incorporation studies of related molecules such as breveanamides A and B.[17] It was at this time we undertook a collaborative

study on the biosynthesis of stephacidin A with the Sherman group at the University of Michigan.



Scheme 14: Incorporation study of **85** into **87**.

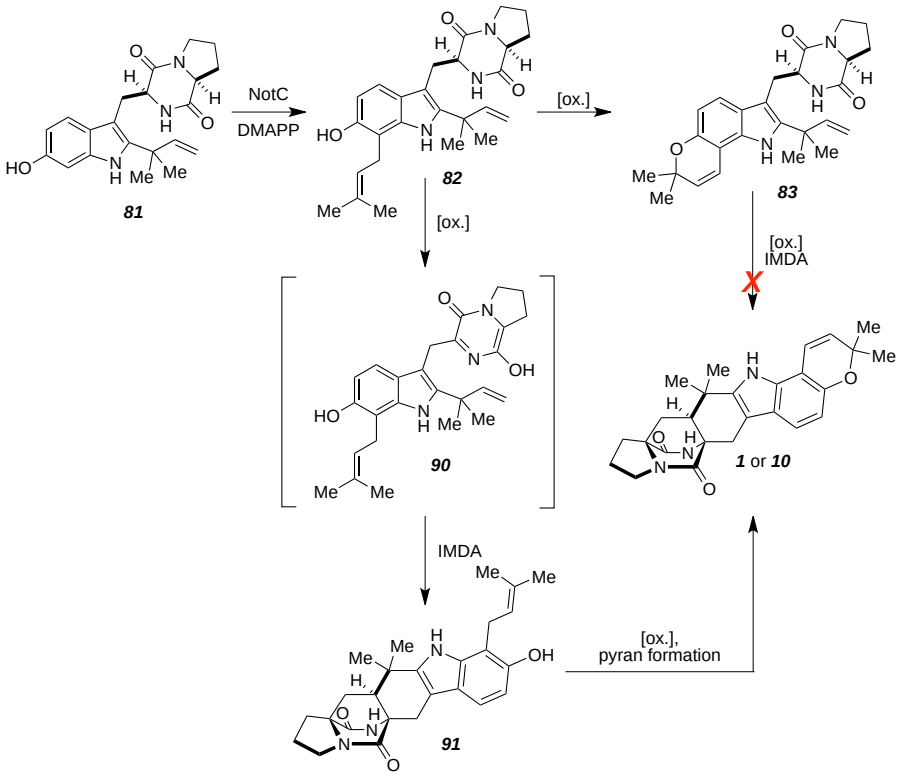
Our collaboration took us in to a much more modern approach in the discovery of the biosynthesis of the stephacidins. Gene sequencing of the stephacidin and notoamide families through gene mining and biochemical analysis led to identification of the first gene cluster involved in the biosynthesis.[24] Through isolation and expression of individual enzymes, the identification of two central pathway enzymes catalyzing both a normal and reverse prenyltransfer reactions was described in detail.[24] The first of these enzymes was the reverse prenyltransferase, NotF, which installs the reverse prenyl group at the C2 position of the indole of **80**. Subjecting the NotF to doubly ^{13}C-labled **88** afforded **89** (Scheme 15).



Scheme 15: Reaction of NotF with ^{13}C labeled **88**.

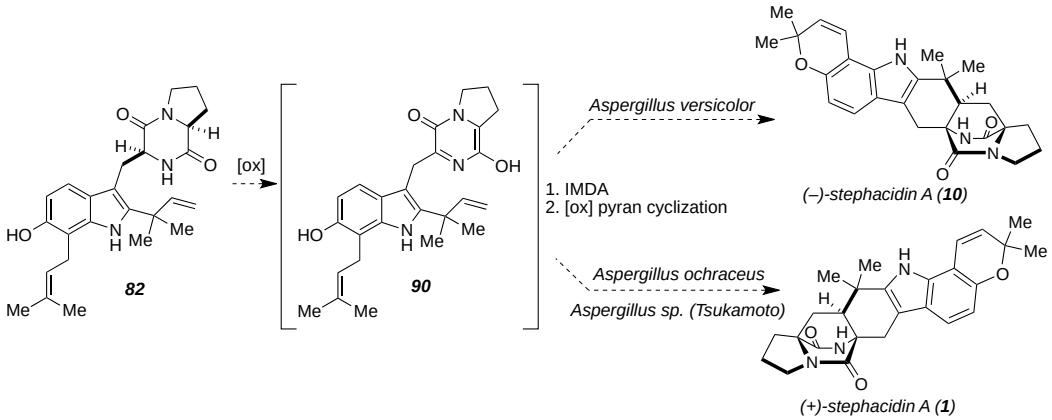
This intriguing result helps to prove what was already expected in the biosynthesis of stephacidin A; from **80**, the first step is a reverse prenylation

reaction to give **8**. The substrate selectivity was also tested in order to see whether this enzyme was selective to one particular substrate or whether, when exposed to any similar type of substrate it would perform its designated task. Subjecting NotF to a variety of substrates showed it to be selective for **80** alone, making it not only site selective but substrate selective as well. Next was the identification of the normal prenyltransferase NotC. Without knowing the exact timing of the normal prenylation, a variety of substrates were subjected to the enzyme to see whether there was any type of selectivity. Interestingly, only one of the substrates, **81**, was found to have any activity with the enzyme NotC. However, the product of the prenylation reaction, notoamide S (**82**), has not been identified as a metabolite in previous isolations. To confirm the structure of the newly isolated product, a synthetic sample needed to be synthesized. Through our synthetic efforts it was confirmed that the product of NotC was notoamide S (**82**).[25] This led to a change in our original proposed biosynthetic route (Scheme 16).



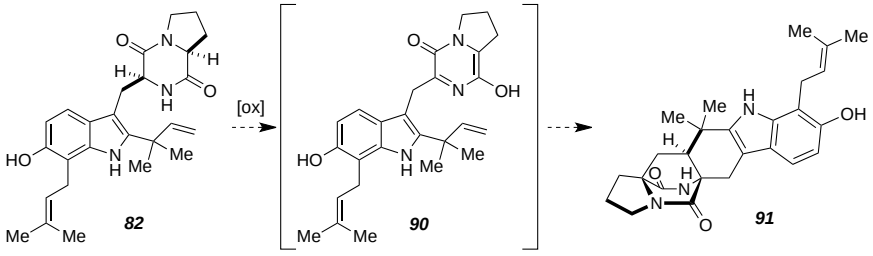
Scheme 16: New proposed biosynthetic pathway to stephacidin A.

From previous incorporation studies, it was shown that **83** did not incorporate into stephacidin A.[23] Knowing that **83** did not lead to stephacidin A, and with the newly isolated metabolite from the NotC enzyme, we now believe that stephacidin A could arise from notoamide S. It is also believed that notoamide S may be the common intermediate leading to either enantiomer of stephacidin A (Scheme 17).



Scheme 17: The proposed final steps in the synthesis of **1** or **10**.

With our newly proposed biosynthetic pathway, we believe that it is notoamide S that undergoes the two-electron oxidation to form azadiene **90** for the IMDA. If this proposal is correct then there is another new metabolite that should be formed before stephacidin A and contains the bicyclo[2.2.2]diazaoctane ring system. We chose to call this proposed metabolite notoamide T (**91**). We believe that notoamide S is oxidized to form azadiene **90**, which undergoes an IMDA reaction to give notoamide T. To test this hypothesis, we needed to synthesize a sample of notoamide T (**91**) for comparison (Scheme 18).



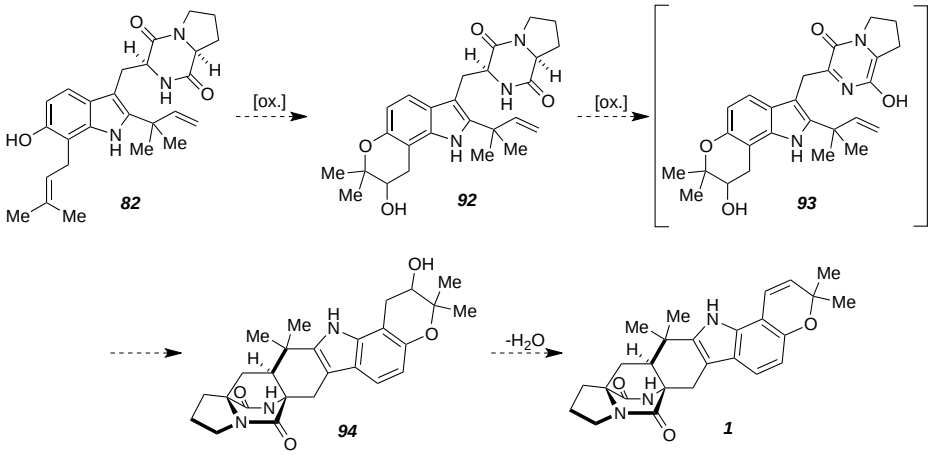
Scheme 18: IMDA of notoamide S to form notoamide T.

With the completion of notoamide T, our current efforts are focused on searching for, and identifying it as a metabolite of the fungus, thereby leading

us one step closer to the elucidation of the biosynthesis of stephacidin A. For further corroboration to our proposed biosynthetic synthesis of stephacidin A, we have synthesized doubly ^{13}C- and ^{15}N-labeled notoamide S for feeding incorporation studies in order to confirm its role in stephacidin A's biosynthesis. One of the major hopes of the incorporation study is to not only identify the incorporation of notoamide S into stephacidin A, but also to identify notoamide T and its role in the biosynthesis.

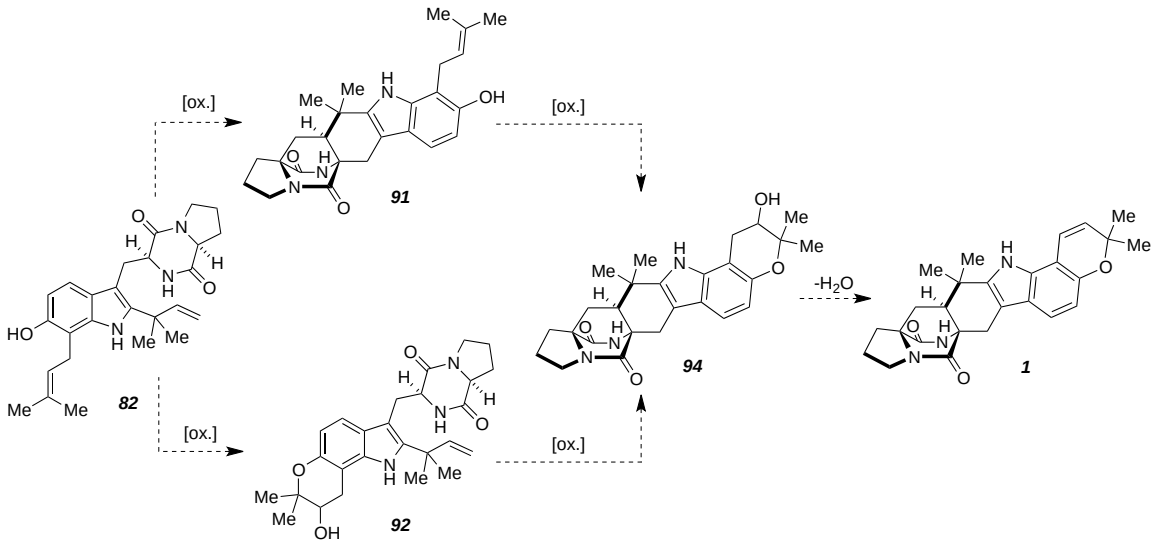
Current work in our lab to understand the biosynthesis of stephacidin A is conducted through the collaboration with the Sherman group at the University of Michigan and their genome based studies. The Sherman group has identified notoamide S as a metabolite along the pathway to stephacidin A, and we believe that it is the key intermediate at which the pathways diverge in different fungi to give the two enantiomers. Our logic, combined with the knowledge of previous incorporation studies, lead us to believe that notoamide T is the product of the IMDA leading to stephacidin A. With the synthesis of notoamide T, we can begin to search for it as a possible metabolite leading to stephacidin A. However, one concern is that the existence of notoamide T may be fleeting, making it nearly impossible to detect through current techniques. If this is the case, further genetic studies must be completed to either prove or disprove our hypothesis. Incorporation studies of notoamide T must also be completed to see whether it is a possible precursor to stephacidin A. If it is not, then there are very few options left on

the biosynthetic path to stephacidin A. Current efforts in the Sherman group are directed towards the identification of the oxidase responsible for the two-electron oxidation leading to the azadiene for the IMDA. As notoamide S has all of the appropriate carbons intact, the final steps to stephacidin A are oxidation to form the azadiene, and oxidation of the prenyl group followed by pyran ring formation. This does leave one final intermediate that could possibly be the Diels–Alder precursor (Scheme 19).



Scheme 19: Another possible biosynthetic pathway to stephacidin A.

The oxidation of the prenyl group followed by immediate cyclization to form β-hydroxy cyclic ether **92**, which could undergo an IMDA to give **94**, is another possibility. There is also the chance that notoamide S is oxidized and cyclized to form notoamide T, which would in turn be oxidized at the prenyl group and cyclized to form **94** – the same β-hydroxy cyclic ether. This would make **94** a common intermediate between the two pathways and the possible final metabolite before stephacidin A (Scheme 20).



Scheme 20: The proposed pathway leading to common intermediate **94**.

Through our collaborative efforts, we have discovered what might be the final common precursor before the formation of the achiral diene leading to the two separate enantiomers (+)-stephacidin A and (–)-stephacidin A. This would indicate that both fungi may have the same genes to produce notoamide S, at which time the two fungi diverge to form separate enantiomers. We have synthesized notoamide S and notoamide T for comparison with the work being done by our collaborators in the Sherman group. We have confirmed the structure of the metabolite notoamide S (**82**) through the synthesis of a synthetic sample,[25] and, with a synthetic sample prepared, are now pursuing the possible existence of notoamide T as a metabolite. Notoamide S has also been synthesized with double ^{13}C and ^{15}N labels for feeding incorporation studies stephacidin A. The biosynthetic path by which stephacidin A is synthesized is being whittled down to a very few select options, and with our synthetic efforts and our collaborators' genome

based studies,[24] we believe that the biosynthesis of stephacidin A will soon be determined.

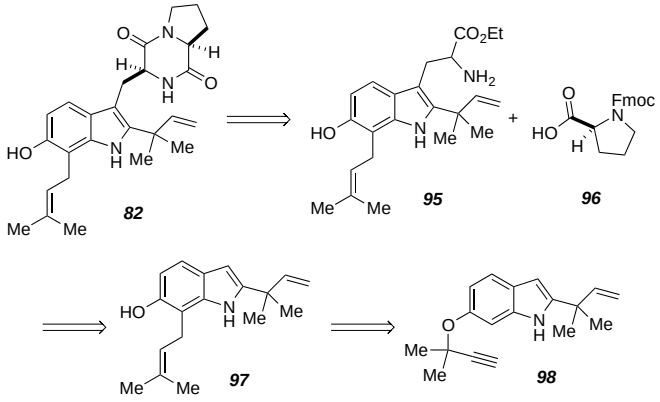
1.1.5 Synthetic Goals

Our goal for this project is the identification of each step along the biosynthetic path leading to the stephacidin and notoamide family of natural products. Through our collaborative efforts, we have identified a possible new metabolite, which we called notoamide S. Synthetically, we want to develop a short, high yielding route to notoamide S that would allow us to use ^{13}C-labels for incorporation studies into the synthesis of stephacidin A. Also, along the path of our newly proposed biosynthesis is what we believe to be a possible new metabolite, notoamide T. The synthesis of notoamide T would help our efforts toward the complete picture in the biosynthesis by providing an authentic sample to our collaborators for comparison in their work on the isolation of possible metabolites along the pathway. The synthesis of notoamides S and T would provide us with both a better understanding and a synthetic route to possible compounds on the pathway to the stephacidin and notoamide family of natural products.

1.2 Notoamide S

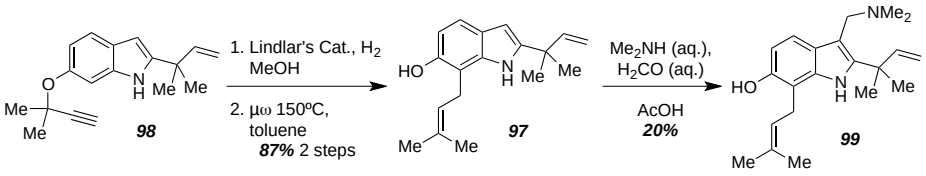
1.2.1 Synthesis of Notoamide S

We envisioned that, notoamide S (**82**) could come from the coupling of commercially available *N*-Fmoc L-proline (**96**) with the 6-hydroxy-7-prenyl-2-reverse prenyl tryptophan derivative **95**. This tryptophan derivative can be accessed from the corresponding 6-hydroxy-7-prenyl-2-reverse prenyl indole (**97**), which can be synthesized from the known indole **98** (Scheme 21).



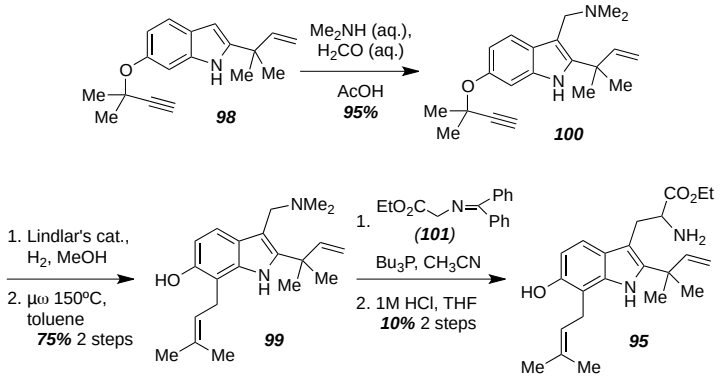
Scheme 21: Retrosynthetic analysis of notoamide S.

The assembly of notoamide S began from the previously reported indole **98**.[26] The treatment of **98** with Lindlar's catalyst under an atmosphere of hydrogen resulted in partial reduction of the alkyne (Scheme 22). The reverse prenyl ether was heated under microwave conditions in toluene to give the desired 6-hydroxy-7-prenyl-2-reverse prenyl indole **97**. Indole **97** was treated with dimethyl amine and aqueous formaldehyde in acetic acid to give the desired gramine **99**, albeit in only 20% yield.



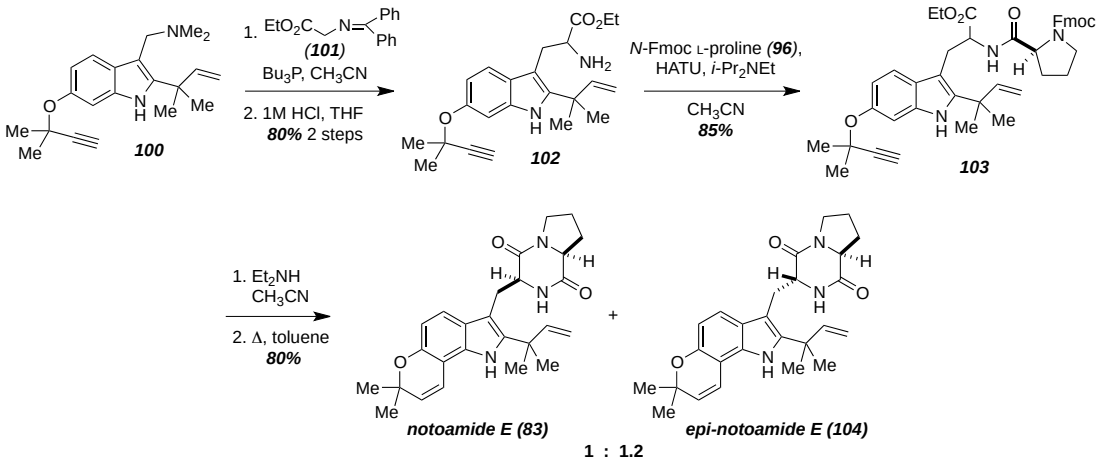
Scheme 22: Synthesis of gramine **99**.

With the poor yield in the synthesis of **99**, it was decided to reverse the order of steps to see whether the desired gramine could be synthesized in a higher yield. Formation of gramine **100** from **98** was followed by reduction and subsequent Claisen rearrangement[27] to give the desired gramine (**99**) in a 71% overall yield (Scheme 23). Somei-Kametani[28] coupling of **99** with *N*-(diphenylmethylene)glycine ethyl ester (**101**) in the presence of tributylphosphine, followed by deprotection of the incipient benzophenone imine gave only a 10% yield of the desired tryptophan derivative **95**. It is believed that the free phenol is problematic in this reaction due to the acidity of the phenolic proton; therefore a different approach was adopted to avoid this problem.



Scheme 23: Synthesis of tryptophan derivative **95**.

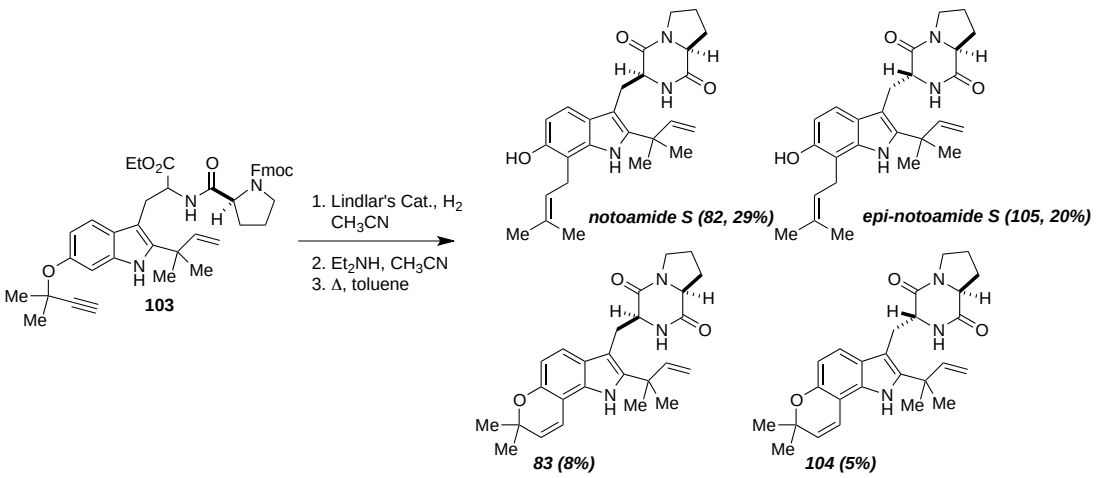
With **100** being readily available on multi-gram scale, we decided to explore the use of the propargyl group as a temporary protecting group for the phenol by performing the reduction and Claisen rearrangement[27] at a later stage (Scheme 24). Under Somei-Kametani[28] conditions **100** can be converted to the desired tryptophan derivative, which upon removal of the benzophenone imine with aqueous acid gave the free amine **102**. Coupling of **102** with *N*-Fmoc L-proline (**96**) in the presence of HATU and *i*-Pr_2NEt gave coupled product **103** in 85% yield. However, after removal of the Fmoc group of **103**, the cyclization to the diketopiperazine was also accompanied by undesired pyran formation to give the natural product notoamide E (**83**) and *epi*-notoamide E (**104**) (Scheme 24).



Scheme 24: Unanticipated formation of notoamide E and *epi*-notoamide E.

Although unanticipated, the adventitious synthesis of notoamide E is three steps shorter than our previously reported route starting from the same indole substrate **98**. To prevent the formation of the pyran ring it was decided to reduce the alkyne to the alkene prior to the formation of the

dioxopiperazine, thus allowing for the possibility of performing the desired Claisen and diketopiperazine formation in one operation. Reduction of **103** with Lindlar's catalyst gave the desired alkene in quantitative yield. With the full skeleton in hand all that remained was cleavage of the Fmoc group from the proline residue followed by cyclization to the dioxopiperazine ring (Scheme 25). As shown previously with the formation of the pyran ring, it was believed that the Claisen rearrangement[27] of the reverse prenyl ether would take place during the cyclization of the dioxopiperazine. Removal of the Fmoc group proceeded smoothly in the presence of diethylamine, and upon refluxing in toluene, the desired compound notoamide S (**82**) was isolated in a modest yield (18%); however, three other products were also found to be present in the mixture. These include: *epi*-notoamide S (**105**), notoamide E (**83**), and *epi*-notoamide E (**104**). Further investigation revealed that the formation of the pyran ring was occurring during the Somei-Kametani[28] reaction, and these inseparable by-products were invariably carried through the synthesis. Suppression of this undesired side reaction can be achieved by lowering the temperature of the Somei-Kametani[28] reaction below reflux. Under these conditions, notoamide S (**82**) was obtained in 29% isolated yield along with **105** (20%), **83** (8%) and **104** (5%). The synthetic notoamide S exactly matched the natural metabolite obtained from the marine-derived *Aspergillus* sp. (Tsukamoto) by ^{1}H NMR and retention time on LC.



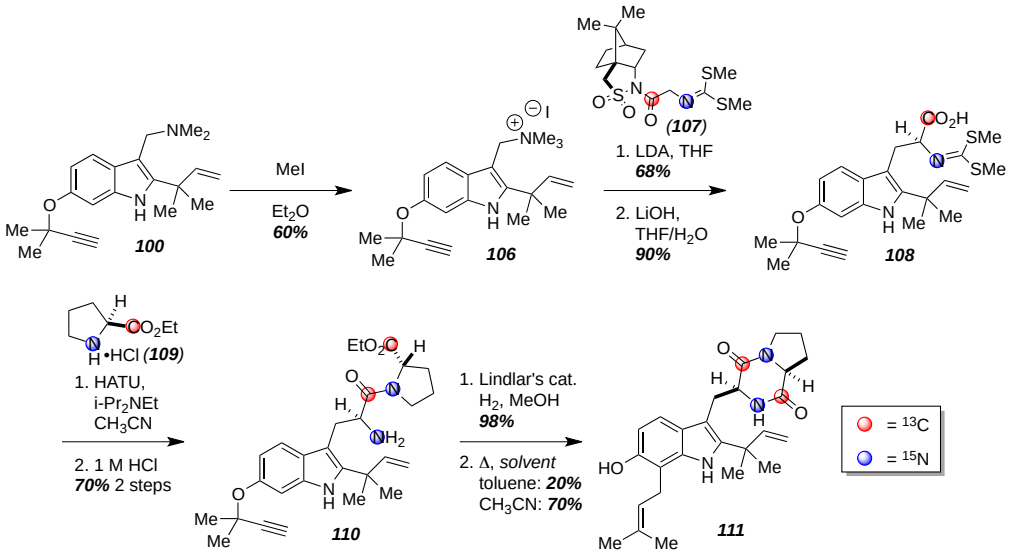
Scheme 25: Synthesis of notoamide S from the propargyl dipeptide **103**.

With the synthesis of notoamide S we were able to match our synthetic sample with what was found to be the product of the NotC enzyme. At this point, our next step was to use the synthetic route developed for notoamide S to synthesize doubly ^{13}C-labeled notoamide S for feeding incorporation experiments. If our speculations are correct, we should see the incorporation of the labeled notoamide S into the synthesis of stephacidin A.

1.2.2 Synthesis of Doubly ^{13}C, ^{15}N Labeled Notoamide S

For incorporation studies into the biosynthesis of stephacidin A we required a labeled sample of notoamide S. The synthesis of doubly ^{13}C-labeled notoamide S started from the previously described gramine **100**. One of the major changes in the synthesis of the labeled **111** from the non-labeled notoamide S was the use of a chiral auxiliary to set the tryptophan portion of the molecule, in order to maximize the yield of pure material. Using the Oppolzer auxiliary, derived from (+)-CSA, the glycine imine **107** can be

synthesized in three steps in good yield.[29] With the problematic Somei-Kametani[28] reaction leading to the undesired pyran ring formation we chose to use an alternative two step sequence. Treatment of MeI with gramine **100** in ether afforded ammonium salt **106**, which could be displaced with the enolate of glycine imine sultam **107**. Treatment of the resultant sultam with LiOH in THF and H_2O afforded acid **108** with recovery of the auxiliary. Coupling of **108** with ^{13}C, ^{15}N labeled L-proline ethyl ester in the presence of HATU and *i*-Pr_2NEt provided the dipeptide in moderate yield. Hydrolysis of the imine with 1 M HCl provided free amine **110**, which was treated with Lindlar's catalyst under an atmosphere of hydrogen to give the alkene in high yield. The dipeptide was then heated in toluene to cyclize to the diketopiperazine, as well as undergoing a Claisen reaction to give the 6-hydroxy-7-prenyl indole portion. Unfortunately, the yield of this reaction was very low due to the insolubility of the dipeptide in toluene. By switching solvents from toluene to acetonitrile, the yield of **111** was increased dramatically from 20% to 70% (Scheme 26).



Scheme 26: The synthesis of doubly ^{13}C-, ^{15}N-labeled **111**.

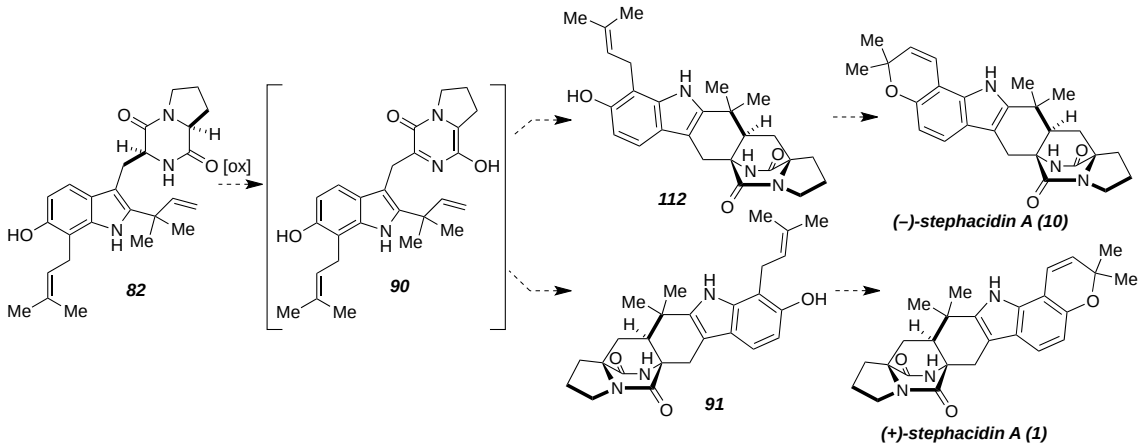
The synthesis of doubly ^{13}C-, ^{15}N-labeled **111** allowed us to conduct feeding experiments to see if **111** does incorporate into stephacidin A. This will help confirm that, not only is notoamide S a metabolite of the fungus but that it is also part of the biosynthetic pathway leading to stephacidin A. At this point, we began to think about the next step in the synthesis. As we had time while labeled **111** was being used in feeding studies, we chose to look at what would happen if notoamide S were to be oxidized and cyclized to give a new bicyclo[2.2.2]diazaoctane containing metabolite.

1.3 Notoamide T

1.3.1 Synthesis of Notoamide T

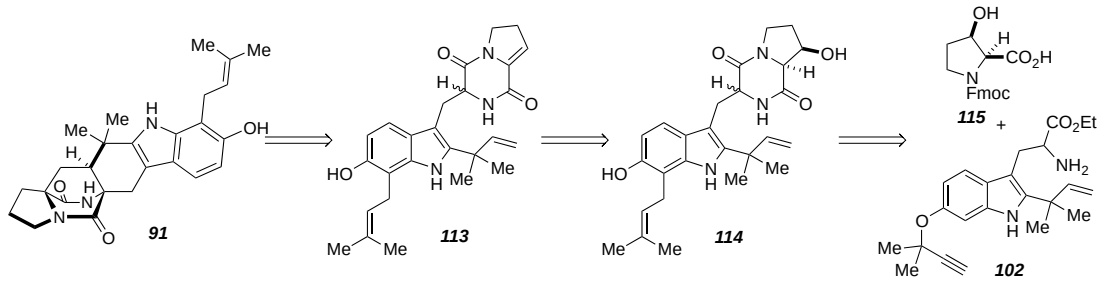
In our new biosynthetic proposal, we believe that notoamide S is the key intermediate before the IMDA. If notoamide S (**82**) undergoes a two-electron oxidation to form the azadiene, it could cyclize to form a new

metabolite, which we will call notoamide T. **112** would contain the bicyclo[2.2.2]diazaoctane ring system, but lack the pyran ring of stephacidin A. Instead, it would contain the 6-hydroxy-7-prenyl indole portion much like notoamide S (Scheme 27).



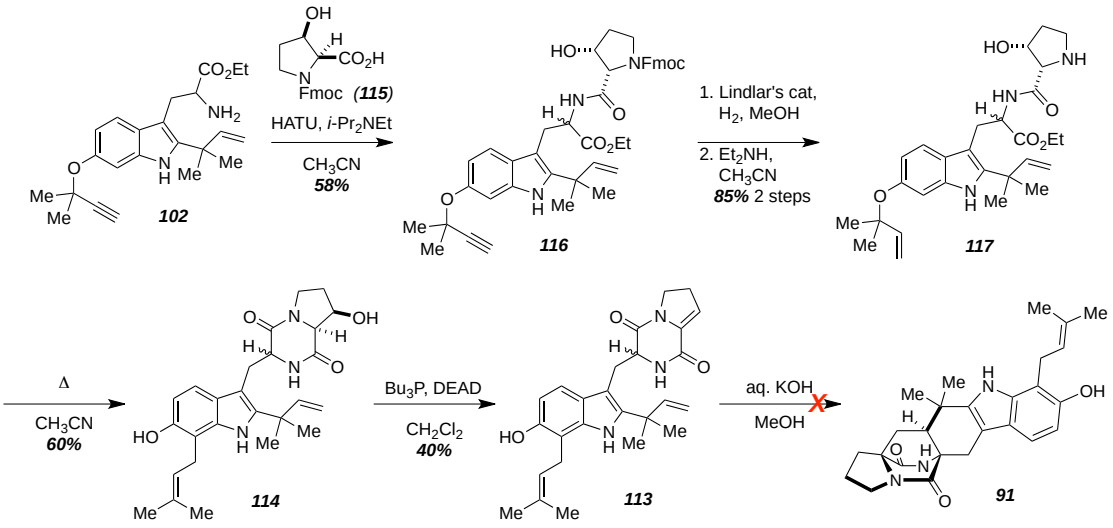
Scheme 27: Our proposed biosythesis of **1** from **82**.

We envisioned the synthesis of notoamide T starting from eneamide **113**, which could come from an elimination of the alcohol of **114**. Diketopiperazine **114** could come from the coupling of tryptophan derivative **102** with cis-β-hydroxy L-proline derivative **115**. This route should afford notoamide T in a rapid and scaleable manner, and allowing for the possibility of incorporating ^{13}C labels for further feeding experiments.



Scheme 28: Retrosynthesis of notoamide T.

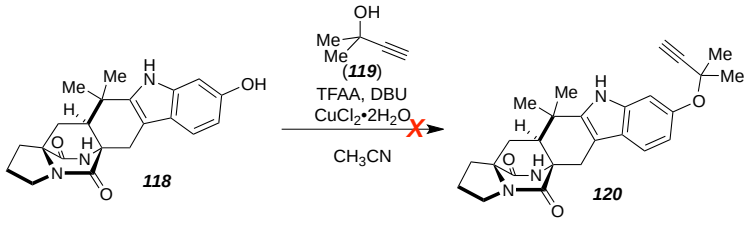
The synthesis of notoamide T was to follow the chemistry that our group has developed for the construction of the bicyclo[2.2.2]diazaoctane ring system.[20] Starting from the previously described tryptophan derivative **102**, coupling of cis-β-hydroxy-N-Fmoc-L-proline in the presence of HATU and *i*-Pr_2NEt provided amide **116** in moderate yield. Reduction of the alkyne with Lindlar's catalyst under an atmosphere of H_2, followed by removal of the Fmoc group with diethylamine gave alkene **117**. Heating in acetonitrile provided the diketopiperazine as well as the Claisen rearrangement of the reverse prenyl ether to give **114**. The next step was the elimination of the alcohol by treatment with Bu_3P and DEAD to give eneamide **113** in low yield. The enamide was treated with aqueous KOH in MeOH for the key IMDA; however, no product was found from the reaction (Scheme 29).



Scheme 29: Attempted synthesis of notoamide T through an IMDA.

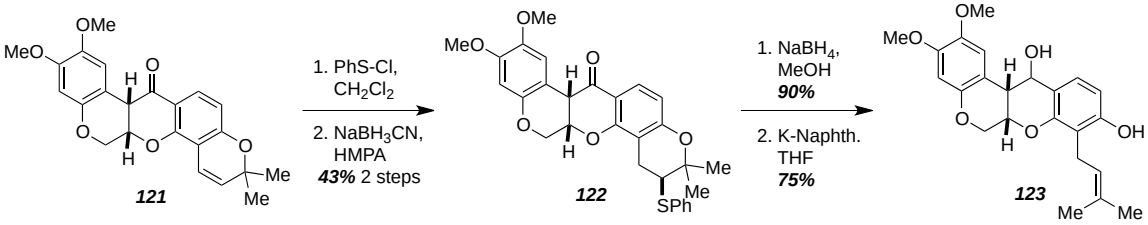
With the unfortunate results of the IMDA of eneamide **113** we decided to try a late stage propargylation followed by reduction and Claisen

rearrangement to gain access to notoamide T. Treatment of phenol **118**, synthesized by another group member, with $CuCl_2 \bullet 2H_2O$ and DBU followed by addition of a preformed solution of trifloropropargyl carbonate failed to give **120** (Scheme 30). One of the major problems with this route is that the propargylation requires a very precise amount of $CuCl_2 \bullet 2H_2O$, and on small scale this is extremely difficult to accomplish.



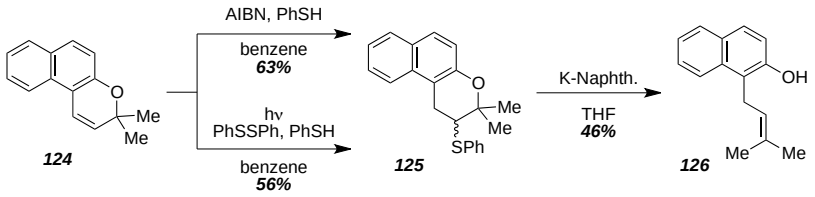
Scheme 30: Attempted late stage propargylation of phenol **118**.

Difficulties with the synthesis of led us to think about alternative ways to access notoamide T. One of the proposed methods was the use of stephacidin A as a precursor for notoamide T. We were curious to see whether there was a possibility to open the pyran ring to afford notoamide T. In work by Whiting and coworkers, it was shown that the opening of a pyran ring can be accomplished through a metal mediated elimination of a phenylsulfide to give the corresponding hydroxy prenyl compound (Scheme 31).[30]



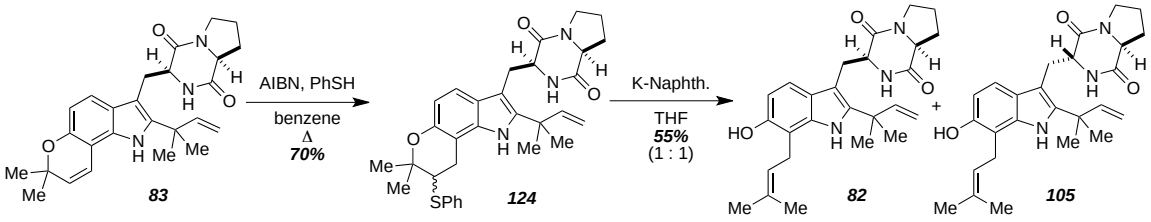
Scheme 31: Whiting's pyran ring opening through sulfide elimination.

Whiting also showed that a ring opening can be done in the same manor by installing the phenylsulfide through a radical reaction.[31] Treatment of **124** with AIBN and PhSH lead to the anti-Markovnikov addition of the phenylsufide radical to give **125**. This intermediate can be opened through a metal mediated elimination to give phenol **126** (Scheme 32).



Scheme 32: Anti-Markovnikov addition of phenylsulfide radical to a pyran ring.

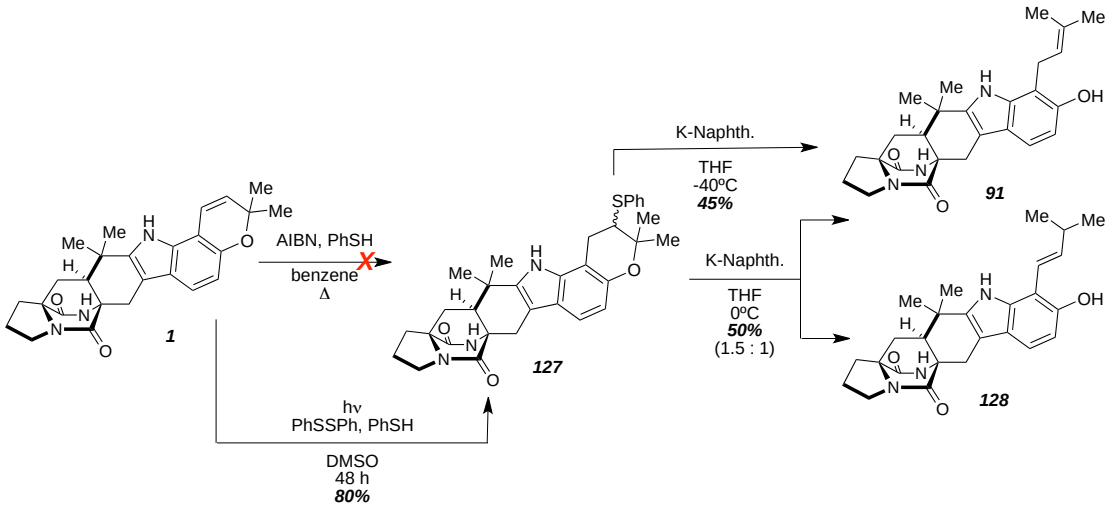
With Whiting's examples, we were hopeful that we could use stephacidin A as a precursor for the synthesis of notoamide T, but to explore the utility of this sequence we chose to start with the ring opening of notoamide E to notoamide S. Treatment of **83** with AIBN and PhSH in refluxing benzene provided an inseparable mixture of diastereomers of the anti-Markovnikov addition of PhSH to afford sulfide **124**. Treatment of sulfide **124** with potassium naphthalenide at room temperature gave the desired notoamide S along with *epi*-notoamide S in a 1:1 mixture. The strong basicity of the potassium naphthalenide caused an epimerization to the more stable trans-diketopiperazine of *epi*-notoamide S (Scheme 33).



Scheme 33: Ring opening of notoamide E.

Converting notoamide E to notoamide S gave us hope that we would be able to do the same for stephacidin A. Treatment of stephacidin A with AIBN and PhSH in refluxing benzene failed to produce the desired sulfide due to the insolubility of stephacidin A in benzene. Whiting also found that changing the conditions of the sulfide addition from an initiator-based radical formation to light – initiated radical formation was also effective for the installation of the sulfide, and this could be done in DMSO; one of the few solvents in which stephacidin A is soluble.[31] Irradiating a solution of stephacidin A, diphenyl disulfide and PhSH in DMSO gave the desired sulfide in a modest yield. The next step in the synthesis to gain access to notoamide T (**91**) is the ring opening of the cyclic ether with potassium naphthalenide. Our first attempt at the ring opening was with potassium naphthalenide at 0ºC, but an inseparable mixture of two products was obtained. The first of the two products was the desired notoamide T, but the second of the two products contained the ring-opened ether with the trisubstituted olefin isomerized to the benzylic position to form styrene **128**. Countless efforts to separate the two regioisomers all failed. However, changing the conditions of

the ring opening from 0ºC to -40ºC gave notoamide T in a modest 45% yield (Scheme 34).



Scheme 34: Synthesis of notoamide T through ring opening of the pyran.

Through the use of a ring opening technique, we have shown that we can gain access to a possible new metabolite, notoamide T. Our conventional methods for the synthesis of notoamide T did not provide the desired product; however, current efforts are being made to redesign our original route to provide notoamide T in a higher yield. The main purpose of the initial synthesis of notoamide T was to generate an authentic sample of the compound with which our collaborators could begin their search for the metabolite and possibly identify it as a key in the biosynthetic pathway.

1.4 Conclusions

Through the use of modern genomic techniques and the use of isotopically labeled incorporation studies, we believe that we have come a few

steps closer to understanding the biosynthetic pathway of the stephacidin and notoamide family of natural products. With the assistance of our collaborators at the University of Michigan, we have identified a new metabolite on the pathway to the synthesis of stephacidin A.[??] In the genetic aspect of the biosynthesis, the Sherman group at the University of Michigan has identified the enzymes involved in both the reverse prenylation (NotF), and normal prenylation (NotC), through gene sequencing and gene mining. The Sherman group has also identified the relationship between the two separate fungi. Recent results have shown that *Aspergillus sp*. and *Aspergillus versicolor* have a 70.8% nucleotide identity (Figure 5a), leading us to believe that the two fungi proceed through the same pathway until the point of divergence where enantiomerically opposite natural products are produced.

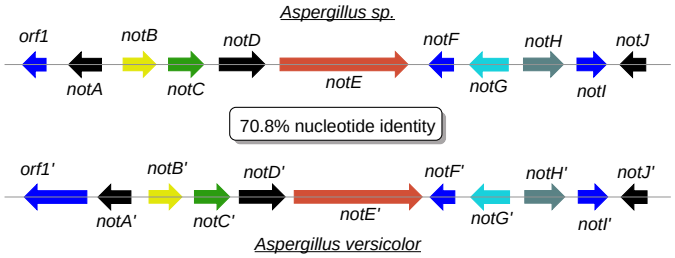
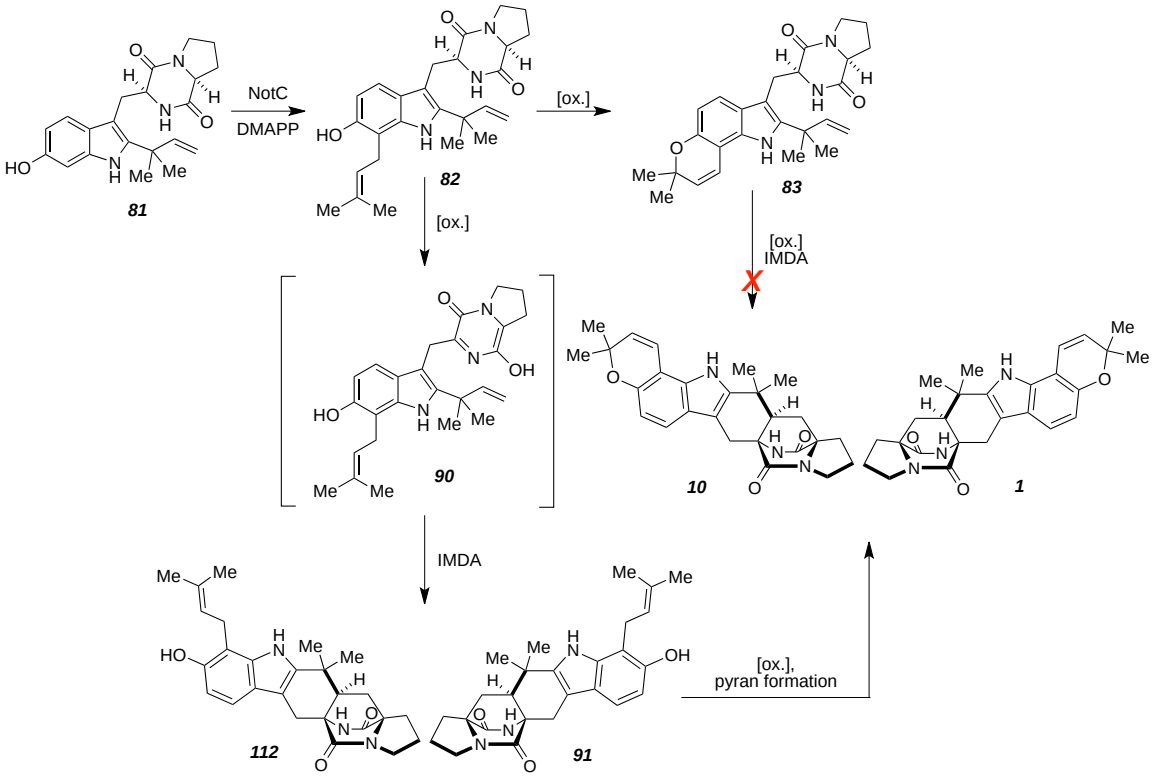


Figure 5a: The genetic relationship between *Aspergillus sp*. and *Aspergillus versicolor*.

With the assistance of the Sherman group, we believe that we are getting closer to identifying each step in the biosynthesis of the stephacidin and notoamide families of natural products. We have completed the synthesis of notoamide S to confirm by analytical techniques the structure of the newly isolated product of the NotC enzyme.[??] The synthesis of notoamide

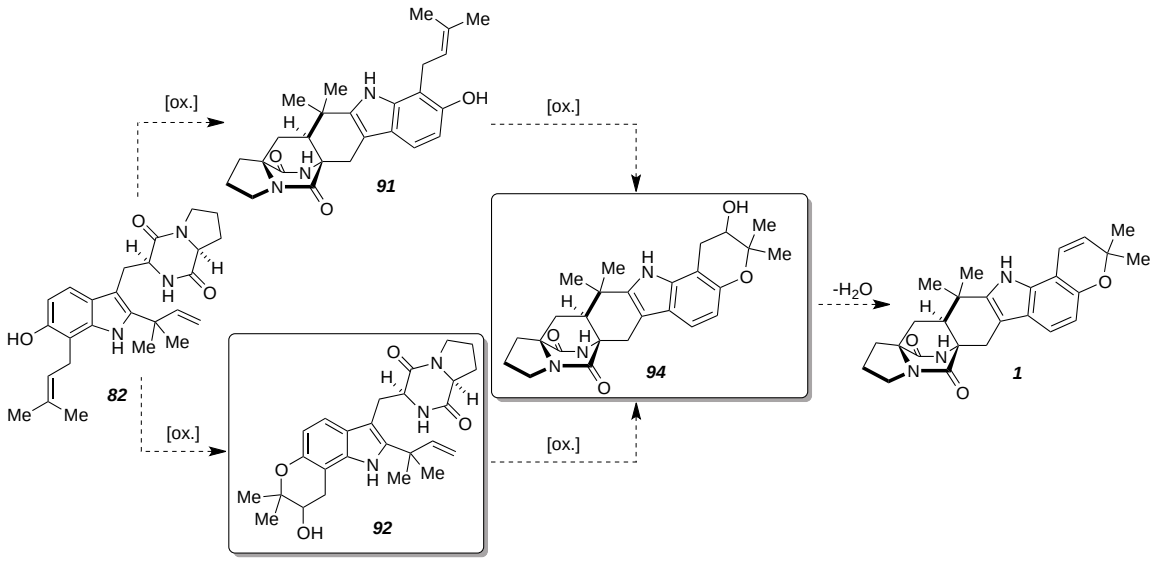
S has allowed us to incorporate ^{13}C- and ^{15}N-labels into the natural product for use in incorporation studies to help confirm notoamide S as a metabolite on the path to stephacidin A. With the knowledge of previous incorporation studies[23] and the discovery of notoamide S[24], we believe that our new biosynthetic proposal is closing in on the biosynthesis of stephacidin A and the possible discovery of a Diels-Alderase enzyme (Scheme 35).



Scheme 35: Our current biosynthetic proposal for the synthesis of **1**.

In our current proposal, we believe that the product of the IMDA contains the same substitution as notoamide S. Through our synthetic efforts we have synthesized an authentic sample of what we call notoamide T. Our conventional group chemistry failed to give us access to the desired 6-hydroxy-7-prenyl bicyclo[2.2.2]diazaoctane containing product; however, we

gained access to notoamide T through unconventional methods by a key ring opening of the pyran system of stephacidin A. With the completion of notoamide T, very few intermediates remain that could be potential precursors on the path to stephacidin. Two possible intermediates still remain unsynthesized (Scheme 36).



Scheme 36: Possible remaining metabolites leading to stephacidin A.

The first of the possible metabolites that could possibly remain on the path to stephacidin A would be **92**; the result of the oxidation of the prenyl group of notoamide S followed by cyclization of the phenol. At this point the next metabolite yet to be synthesized is IMDA adduct **94**. Both of these possible metabolites should be considered on the path to stephacidin A, especially **94** as it is not only the product of the oxidation of notoamide T, but it could also be the product of the IMDA of **92**. Knowing that these two metabolites could still be potential precursors is a major step in understanding the biosynthesis of the stephacidin and notoamide family of natural products.

With the help of our collaborators we hope to resolve this puzzle on a molecular level as well as genetic level. Through the genome based studies of the Sherman group alongside our incorporation studies, we believe that we can fully characterize the biosynthesis of (+) and (–)-stephacidin A as well as the origin of their divergence within the two fungi.

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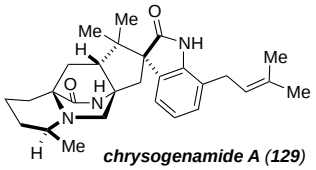
Chapter 2: Progress Toward the Synthesis of Chrysogenamide A

2.1 Introduction

2.1.1 Isolation

Chrysogenamide A was isolated by Zhu and coworkers in 2008 from *Penicillium chrysogenum* No. 005 in northwest China from the root of a parasitic plant *Cistanche deserticola*.[1] The stem of the *C. deserticola* plant has been used for the treatment of kidney deficiency and neurasthenia in ancient Chinese medicine. *C. deserticola* phenylthanoid glycosides have been reported to display free radical scavenging activity, and have also been used in the treatment and prevention of cranial nerve diseases. While searching for new neuroprotective compounds from endophytic fungi in *C. deserticola,* three active strains were screened using SH-SY5Y cells (human neuroblastoma cells). The culture broth of fungus No. 005 (authenticated as *Penicillium chrysogenum)* showed significant neurocyte protection effects against oxidative stress-induced cell death in SH-SY5Y cells. Chrysogenamide A (**129**) was found to be the active constituent of this strain.



chrysogenamide A (129)

Figure 6: Structure of chrysogenamide A.

Fungus No. 005, was grown under static conditions at 24ºC in 35 1000-mL flasks in a medium (300 mL/flask) composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \bullet 7H_2O$ (0.3 g/L), corn steep Liquor (1.0 g/L) and yeast extract (3.0 g/L) and seawater at a pH of 7.0. After 30 days the fermentation broth (10.5 L) was extracted three times with EtOAc and concentrated under reduced pressure to give the crude extract (9.5 g). The crude extract was chromatographed on a silica gel column where 15 fractions were collected by eluting in a step gradient of petroleum ether and acetone. The fourth fraction collected was further purified by reverse phase column chromatography giving 9 additional fractions. Extensive PHPLC separation of fractions 4-9 in 80% MeOH at 4.0 mL/min. gave chrysogenamide A (4.0 mg) as a pale yellow solid.

2.1.2 Characterization

Chrysogenamide A was characterized by high resolution mass spectrometry, NMR (^1H, ^{13}C, NOESY, HMBC, HMQC, DEPT, COSY), and IR. The molecular formula was determined by HRESI-MS -observed $[M+H]^+$ at *m/z* 448.2951, calcd $[M+H]^+$ $C_{28}H_{38}N_3O_2$, 448.2964. ^1H-NMR spectra revealed

three methyl groups, as well as three ortho coupled aromatic signals, indicating a 1,2,3-trisubstituted benzene ring. An olefinic proton coupled with methylene protons, along with two vinylic methyls, indicated the presence of a prenyl group in **129**. The ^{13}C-NMR and DEPT spectra showed 28 signals and confirmed the presence of a prenyl group as well as a trisubstituted benzene ring. Two carbonyls, four quaternary carbons, two methines and six methylenes were also observed in the ^{13}C-NMR spectra.

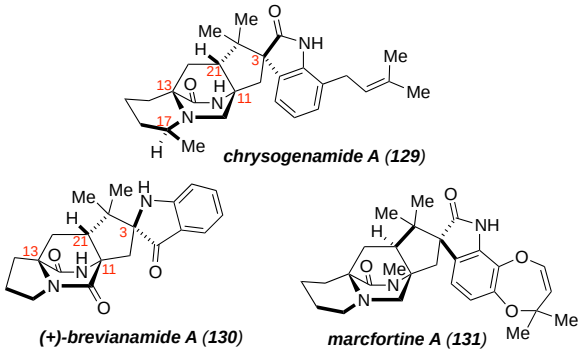


Figure 7: Relationship of **129** to brevianamide A and marcfortine A.

Chrysogenamide A showed a close relation to the marcfortine group of fungal metabolites.[2] Based on further evaluation of the spectral data and subsequent ^{1}H-^{1}H COSY and HMBC results, 6 out of 12 unsaturations were accounted for, from which a heptacyclic structure could be inferred. A similar relation to marcfortine A[2] was assessed due to further HMBC correlation, differences in the benzene side chain with a prenyl group rather than a dioxapine ring, and a methyl group instead of hydrogens at the C17 of the pipecolic acid portion. NOSEY data were used to elucidate the relative stereochemistry of **129**, which was found to be in agreement with the relative

stereochemistry of marcfortine A (**131**).[2] The Cotton effect at 200-250 nm from an *n*-π^* transition of the monoketopiperazine amide bonds was indicitive of a bicyclo[2.2.2]diazaoctane core system. The CD spectrum of chrysogenamide A correlated to the relevant regions of (+)-brevianamide A (**130**), thereby permitting the absolute stereochemistry to be assigned.[3] The absolute stereochemistry of **129** was therefore assigned as 3*S*, 11*S*, 13*S*, 17*S*, 21*R*, and the compound was given the name chrysogenamide A (**129**).

2.1.3 Pharmacological Properties

MTT assays on neurocytes were evaluated using oxidative stress-induced cell death in SH-SY5Y cells for a preliminary evaluation of the protective effect of chrysogenamide A.[1] Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical donor, the free radical scavenging activity of **129** was compared to that of vitamin C (IC_{50}: 29.0 μM) and showed reduced protective character (IC_{50}: >100 μM) at concentrations up to 100 μM. At concentrations of $1x10^{-4}$ μM, cell viability in the presence of hydrogen peroxide was improved; 59.6% of cells survived in comparison to only 43% of the control group. Chrysogenamide A therefore displayed protective effect against oxidative stress-induced cell death, though not through its antioxidant activity.

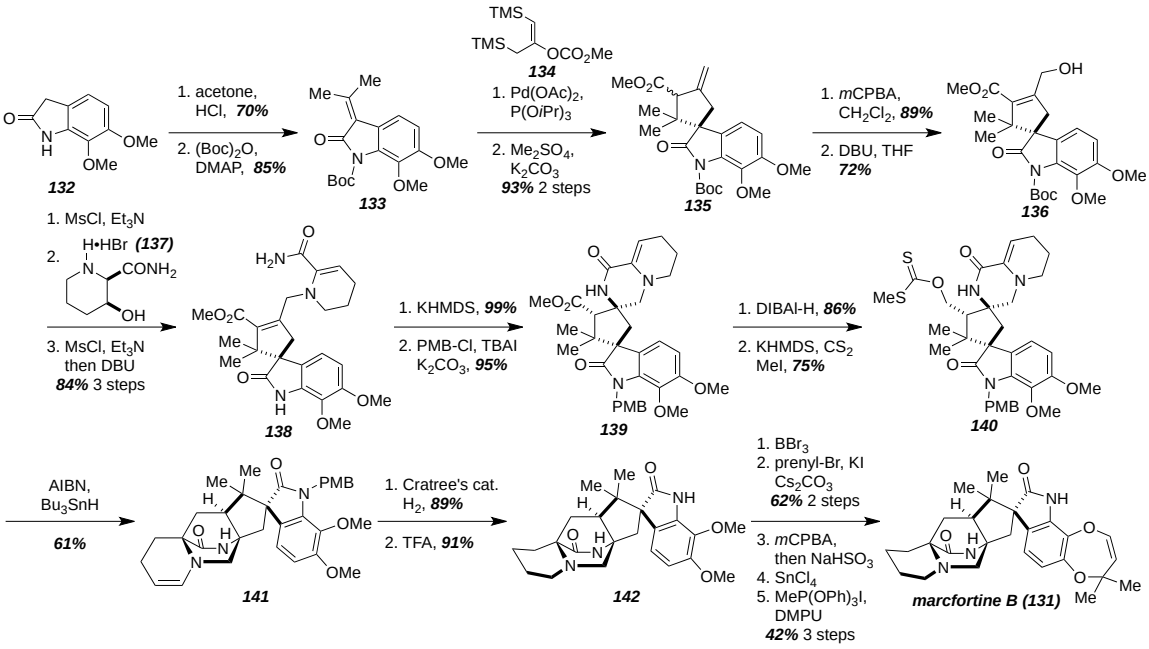
2.1.4 Background

Chrysogenamide A (**129**) is believed to be a member of the marcfortine family of prenylated indoles due to its bicyclo[2.2.2]diazaoctane ring system.[2] However, **129** contains the relative structural framework of an intermediate of the cintrinadins A and B, suggesting that chrysogenamide A could be a biosynthetic intermediate in the cintrinadin pathway.[4]

Structurally, chrysogenamide A is unique in that its pipecolic acid is methylated at C17, and it contains a C7 prenyl group on the spiro-oxindole. This stands in contrast to the dioxepin ring of marcfortine A and the pyran ring of marcfortine C. Related C7 prenylated molecules have been shown to exhibit potent antiparasitic activity,[5] inhibition of human tumor cells,[6] and cytotoxicity.[7]

2.1.4a Trost's Synthesis of (±)-Marcfortine B

Marcfortine B was first synthesized by Trost and co-workers in 2007, through a key palladium catalyzed carboxylative trimethylenemethane (TMM) [3+2]-cycloaddition reaction to form the spiro-oxindole cylcopentene core.[8] The bicyclo[2.2.2]diazaoctane ring system was expected to form through alkylation of 2-hydroxypipecolic amide, intramolecular Michael-addition,[9] and radical ring closure. Further manipulations of the core would give (±)-marcfortine B (Scheme 37).[8]



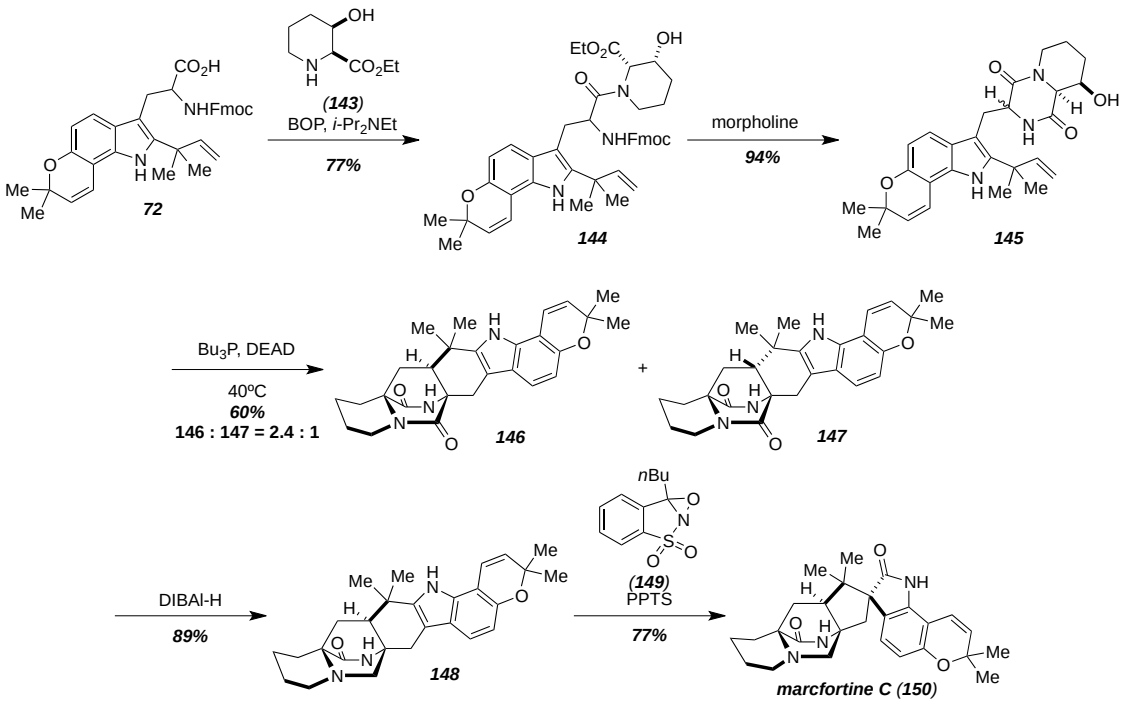
Scheme 37: Trost's synthesis of marcfortine B.

Starting from known oxindole **132**, treatment with acetone in the prescence of HCl gave the α,β-unstaturated oxindole, which, when treated with Boc anhydride and DMAP, gave **133**. The key TMM step proceeded nicely through the use of palladium acetate, triisopropyl phosphine ligand, and TMM donor **134** gave **135** in a 93% yield after methylation of the resultant carboxylic acid. Epoxidation of the exomethylene with *m*CPBA, followed by ring opening of the epoxide with DBU, gave primary alcohol **136**. Mesylation and displacement of the primary alcohol followed by elimination of the secondary alcohol in the piperdine ring gave **138**; the desired substrate for the Michael addition[9] cyclization. Treatment of **138** with KHMDS smoothly afforded the Michael addition[9] product as a single diastereomer, which was protected as the PMB oxindole in a 95% yield over two steps. Reduction of ester **139**, followed by alkylation with carbon disulfide and methylation to the

xanthante ester, gave the radical cyclization precursor for the key closure of the bicyclo[2.2.2]diazaoctane core. Treatment of xanthate ester **140** with superstoichiometric AIBN and catalytic Bu_3SnH initiated the radical cyclization to the bicyclo[2.2.2]diazaoctane core. In this step, the nitrogen centered radical of the AIBN participates in a 1,4-hydrogen abstraction to the alkyl radical, which undergoes fragmentation to give alkene **141**. Reduction of the resultant olefin with Crabtree's catalyst in the prescence of H_2, followed by deprotection of the PMB group, gave **142** in 81% yield over two steps. Cleavage of the methoxy ethers with boron tribromide to give the catechol, followed by prenylation gave the desired prenyl ether. Marcfortine B was completed in three more steps: epoxidation of the prenyl ether with *m*CPBA, an exclusive endo cyclization with tin tetrachloride to give the seven membered ring, followed by elimination of the secondary alcohol. The target was achieved in 22 linear steps from oxindole **132,** with an overall 3% yield.[8] The Trost group showed the utility of the developed TMM chemistry to form the spirocyclic tricycle, in which the configuration of the spiro center was used to relay the remaining stereochemistry. Formation of the strained bicyclo[2.2.2]diazaoctane core was achieved through a radical-mediated cyclization in a moderate yield to give the core of marcfortine B (Scheme 37).[8]

2.1.4b Williams' Synthesis of Marcfortine C

Our group has also participated in the synthesis of the marcfortine family of natural products with the total synthesis of marcfortine C.[10] Our approach to marcfortine C involved a biosynthetic approach, relying on a tryptophan derivative and a hydroxylated pipecolic acid to form a diketopiperazine. With the use of the previously described intramolecular hetero-Diels-Alder[11] reaction to form the bicyclo[2.2.2]diazaoctane ring system, the core structure of marcfortine C could be accessed (Scheme 38).



Scheme 38: Williams' synthesis of marcfortine C.

The synthesis of marcfortine C started from the previously described tryptophan derivative **72**.[12] Coupling with cis-hydroxypipecolic ester **143** gave the Fmoc protected dipeptide **144**, which was deprotected and cyclized to give diketopiperazine **145** in 72% yield over two steps. With the key

precursor in hand for the intramolecular hetero-Diels-Alder[11] reaction, **145** was treated with tributyl phosphine and DEAD at 40ºC to facilitate both the Mitsonobu elimination[13] and tautomerization to the corresponding azadiene for the Diels-Alder reaction, giving a 1 : 2.4 mixture of *anti* (**147**) to *syn* (**146**) diastereomers. Reduction of tertiary amide **146** with DIBAl-H, followed by an oxidative Pinacol-like[14] rearrangement with Davis' oxaziradine[15] (**149**) in the presence of PPTS, gave marcfortine C (**150**). Completion of the synthesis took five steps from the previously reported tryptophan derivative, giving marcfortine C in 30% overall yield (Scheme 38).[10] This synthesis successfully capitalized on a proposed biosynthetic route in which an intramolecular hetero-Diels Alder[11] reaction was used to form the bicycle[2.2.2]diazaoctane core.

2.2 Synthetic Goals and Approach to Chrysogenamide A

2.2.1 Synthetic Goals

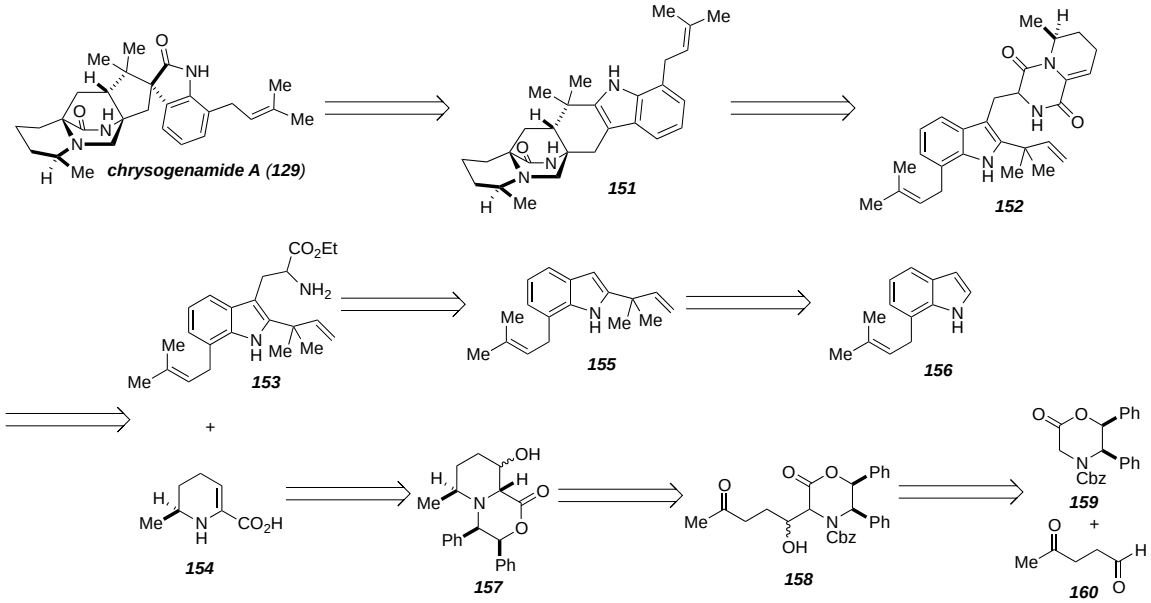
Our synthetic goal for the synthesis of chrysogenamide A was to develop a simple, high yielding route that would enable the incorporation of labeled substrates, possibly leading to the elucidation of the compound's biosynthesis. The synthetic tasks were constructing two separate portions of the molecule for use in the well established IMDA[11] reaction to form the bicyclo[2.2.2]diazaoctane ring system. The first synthetic challenge was to synthesize a tryptophan portion containing a 7-prenyl and 2-reverse prenyl

indole moiety; this tryptophan derivative would act as a possible biomimetic intermediate. The second goal was to construct an α-methyl pipecolic acid derivative that could be coupled to the desired tryptophan, again in a process possibly mimicking the actual biosynthesis. With the two pieces joined, our group's well established IMDA[11] chemistry could be used to complete the synthesis of chrysogenamide A.

2.2.2 Retrosynthesis of Chrysogenamide A

Retrosynthetically, we envisioned chrysogenamide A arising from an oxidative rearrangement of the corresponding indole containing bicyclo[2.2.2]diazaoctane system, followed by DIBAl-H reduction of the tertiary amide to afford **151**. Amine **151** could come from a tautomerization event leading to an IMDA reaction of unsaturated DKP **152**. The Diels-Alder precursor would be the product of the coupling and cyclization of two amino acid derivatives to give the corresponding DKP. Two amino acid derivatives would be needed to form the DKP: a tryptophan derivative (**153**) and pipecolic acid derivative **154**. The tryptophan derivative could come from a Mannich[16] reaction to form the gramine, followed by a Somei-Kametani[17] coupling to afford tryptophan **153**. The indole portion of **153** would come from the reverse prenylation of a previously reported 7-prenylindole (**156**).[18] We envisioned the pipecolic acid portion of the molecule coming from an aldol addition of Williams' lactone **159** to aldehyde **160** to give **158**.[19] Cleavage of

the lactone template and reductive amination would give the desired pipecolic acid piece (Scheme 39).



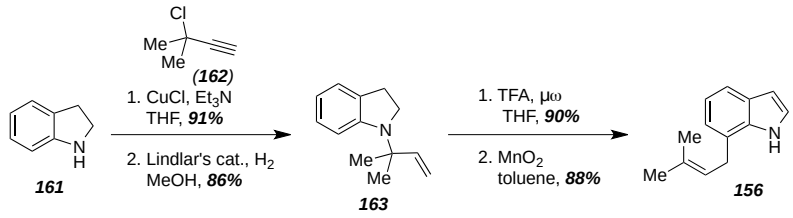
Scheme 39: Retrosynthesis of chrysogenamide A.

2.3 Tryptophan Portion

2.3.1 Prenylation and Reverse Prenylation

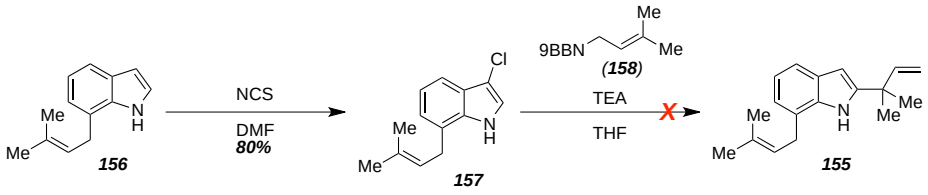
Our initial synthetic efforts were aimed at the synthesis of the desired tryptophan derivative. Previously, it has been shown that 7-prenylindole **156** could be synthesized in high yields through a scalable four step process.[18] Propargylation of indoline with chloride **162** in the presence of catalytic CuCl and TEA gave *N*-propargyl indoline in 91% yield. Reduction of the alkyne to the alkene with Lindlar's catalyst under an atmosphere of H_2, followed by a microwave assisted aza-Claisen[20] rearrangement, gave 7-prenyl indoline.

Oxidation of the indoline with MnO_2 gave the desired indole in a 62% overall yield for the four steps (Scheme 40).



Scheme 40: Synthesis of 7-prenyl indole.

With the 7-prenyl indole in hand, the next task was to install the desired C2 reverse prenyl group following the protocol developed by Danishefsky and coworkers.[21] Employing NCS rather than *t*-BuOCl (as in the Danishefsky method) prevented any possible chlorination of the tri-substituted olefin. Chlorination of indole **156** with NCS gave the desired C3 chloroindole **157** in 80% yield. Subjecting of indole **157** to prenyl-9BBN with TEA did not give the desired product; rather, this reaction led to the recovery of starting material (Scheme 41).



Scheme 41: Attempted reverse prenylation of 7-prenyl chloroindole.

This unfortunate result led us to believe that the prenyl group at the C7 position of the indole may block the boron from adopting the correct conformation, preventing the reverse prenylation at the indole's C2 position. The steric interaction between the prenyl group of the indole with the

cyclooctane ligand of the boron may prevent the overlap needed for reverse prenylation.

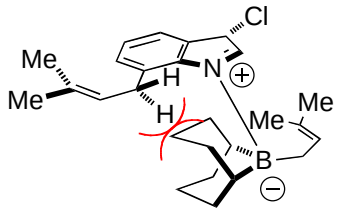


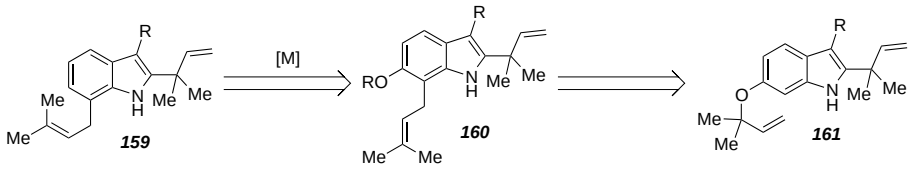
Figure 8: Steric interaction of the C7 prenyl group with 9BBN.

Previously unpublished work from our group showed that the C7 *O*-Boc chloroindole was also unreactive to the prenylation conditions described above. This led us to believe that any bulky substituent at the indole C7 position would prohibit the use of the previously described prenylation conditions. One solution to this problem may be the use of smaller ligands bound to boron as these may allow for sufficient rotation of the boron to achieve the needed overlap for the reverse prenylation event. However, these experiments were never explored as we found a different method to solve the problem.

2.3.2 Aryl Triflate Reduction

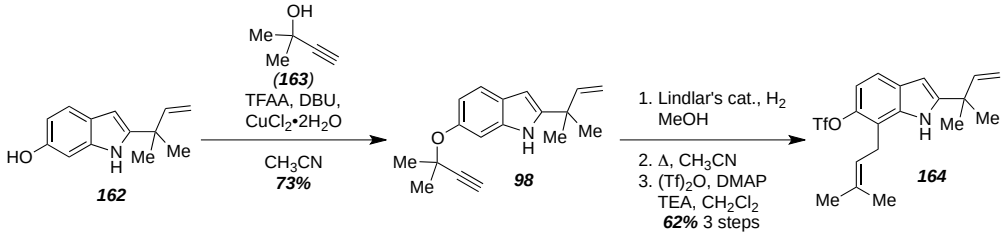
Our next attempt to access the desired indole was through a previously described Claisen[22] rearrangement. In our synthesis of notoamide S, we showed that propargyl ether **103** underwent a Claisen[22] rearrangement to give the desired 6-hydroxy-7-prenyl indole. Following reduction of the alkyne, we were ultimately able to synthesize notoamide S.[23] We hoped to use this same

reaction to access a 6-hydroxy-7-prenyl indole, which could undergo a metal-mediated reduction to afford the desired 7-prenyl indole.



Scheme 42: Metal mediated deoxygenation of the 6-hydroxy group.

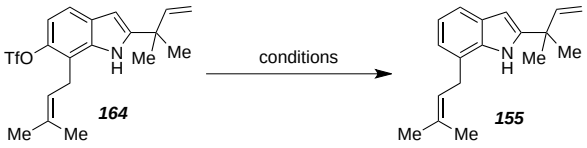
The synthesis of our phenolic intermediate started from 6-hydroxy-2-reverse prenyl indole **162**. Propargylation of indole **162** with alcohol **163** in the presence of TFAA, DBU and $CuCl_2 \bullet 2H_2O$ gave **98** in a 73% yield. Reduction of the alkyne with Lindlar's catalyst under an atmosphere of H_2 gave the corresponding alkene, which when heated in CH_3CN underwent a Claisen[22] rearrangement to afford 6-hydroxy-7-prenyl indole in near quantitative yield. Triflation of the phenol with triflic anhydride in the presence of TEA and catalytic DMAP gave aryl triflate **164** in a modest 65% yield (Scheme 43).



Scheme 43: Synthesis of aryl triflate **164** from phenol **162**.

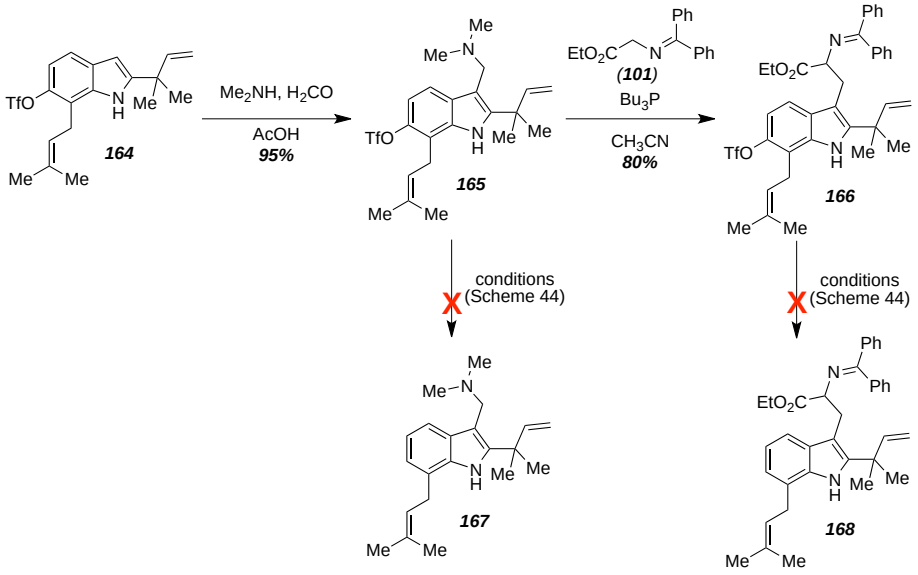
Our next step to gain access to the indole core of chrysagenamide A was to reduce the aryl triflate using metal mediated transfer hydrogenation in order to avoid reduction of the prenyl groups. Various aryl triflate reduction

methods failed to afford the desired reduced indole system, resulting instead in the recovery of starting material. More highly functionalized aryl triflates were also subjected to reduction conditions; these substrates unfortunately gave the same result (Scheme 44).



Catalyst	Hydrogen Source	Solvent	Ligand	Base
Pd / C	Et_3SiH	MeOH	–	–
Pd / C	CO_2H_2	toluene	–	–
$Pd(OAc)_2$	Et_3SiH	THF	Ph_3P	*i*-Pr_2NEt
$Pd(OAc)_2$	CO_2H_2	DMF	Ph_3P	*i*-Pr_2NEt
$Pd(OAc)_2$	CO_2H_2	THF	dppe	K_2CO_3
$Pd(PPh_3)_4$	Bu_3SnH	THF	–	–
Lindlar's cat.	H_2	MeOH	–	–

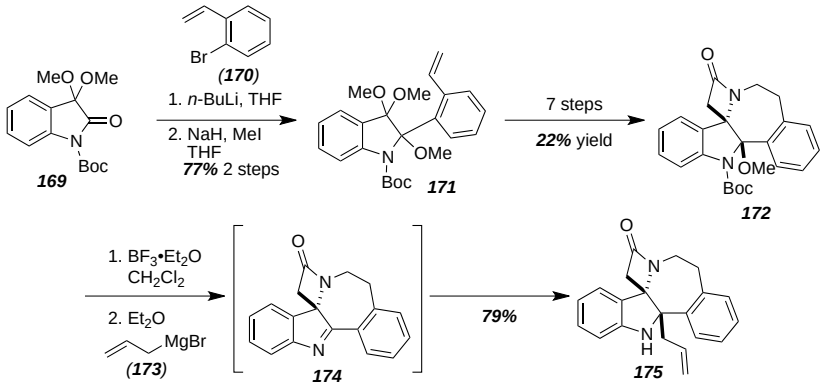
Scheme 44: Conditions used for reduction of aryl triflate.



Scheme 45: Reduction of gramine and tryptophan derivatives.

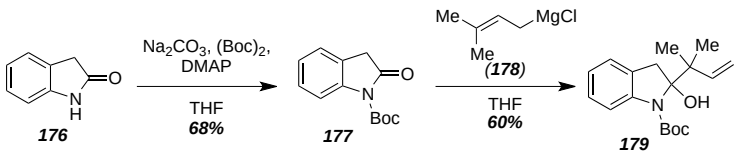
2.3.3 Oxindole Formation

In the preliminary studies towards the syntheses of chartellamide A and B by Weinreb and coworkers, an aryl lithium moiety was added to the amide of a Boc-protected oxindole species to give the corresponding hemi-aminal (**171**).[24] This hemi-aminal was then methylated and eliminated under acidic conditions to give imine **174**, which was alkylated with allyl Grignard to provide their key substrate.



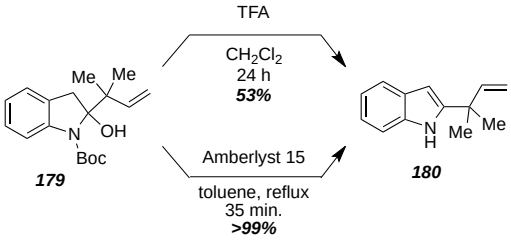
Scheme 46: Weinreb's preliminary studies toward chartellamide A and B.

We saw Weinreb's Grignard addition methodology[24] as a possible means to gain access to C2 substituted indoles. To test this hypothesis, a simple model system was used, starting with the protection of the oxindole nitrogen as the *t*-butyl carbamate to give *N*-Boc oxindole **177**. Treatment of **177** with freshly prepared prenyl Grignard gave the corresponding hemiaminal (**179**) in 60% yield, with the deprotected oxindole comprising the balance of the material.



Scheme 47: Grignard addition to *N*-Boc oxindole.

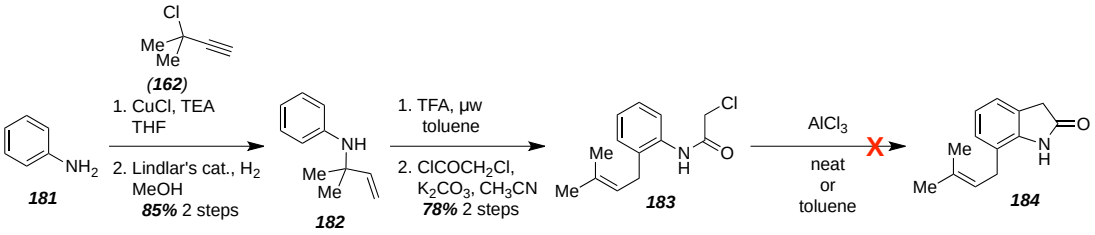
Treating hemiaminal **179** with excess TFA in CH_2Cl_2 lead to indole **180** in moderate yield after a 24 hour period. Due to the sluggish nature and moderate yield of the TFA deprotection and subsequent dehydration, an alternative method was needed (Scheme 48). Refluxing hemi-aminal **179** in toluene in the presence of Amberlyst 15 acidic resin gave the desired indole **180** cleanly in 35 minutes and near quantitative yield (Scheme 48).



Scheme 48: Deprotection and dehydration of hemi-aminal **179**.

With the deprotection and dehydration of the hemi-aminal resolved, the next step was to add a prenyl group at the seven position of the oxindole starting material. Intial attempts focused on an intramolecular Friedel-Crafts reaction developed by Beer.[25] Propargylation of aniline with chloride **162** in the presence of CuCl and TEA gave the desired derivative **182** in 95% yield. Reduction of the alkyne followed by an acid mediated aza-Claisen[20] rearrangement gave 7-prenyl aniline. Treatment with chloroacetyl chloride and potassium carbonate in acetonitrile, gave α-chloroamide **183**, which we

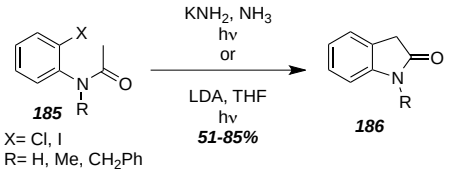
expected to cyclize to the desired oxindole when treated with $AlCl_3$. However, when **183** was heated with neat $AlCl_3$, the desired oxindole was not seen. The same decomposition was observed when **183** was refluxed with $AlCl_3$ in toluene, with the major product showing loss of the prenyl group (Scheme 49).



Scheme 49: Attempted Beer type oxindole synthesis.

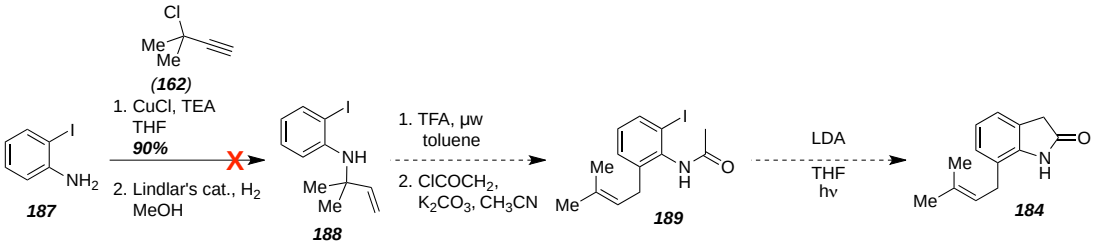
With the product of the Friedel-Crafts not being observed, and the high reactivity of the prenyl group now known, this route to a 7-prenyl oxindole was abandoned. Although $AlCl_3$ was the only Lewis acid tried, one possible solution to this problem may be the use of a different Lewis acid.

In another attempt to access the desired 7-prenyl oxindole, a radical method was chosen as the target reaction. Wolfe and coworkers have shown that when *N*-acyl-2-iodo- or 2-chloroaniline are treated with excess base (in this case LDA) and irradiated with a 240nm light, the desired oxindole can be obtained in high yield (Scheme 50).[26] This transformation can also be accomplished by irradiating *N*-acyl 2-iodo or 2-chloroaniline in potassium amide and ammonia, but the yields were found to be lower.



Scheme 50: Wolfe's oxindole formation through an $S_{RN}1$ reaction.

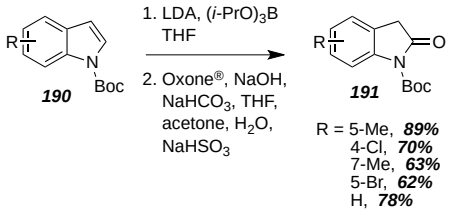
Starting with 2-iodoaniline, propargylation of the amine with chloride **162** in the presence of CuCl and TEA gave propargyl aniline. Reduction of the alkyne, however, was problematic. Treatment of propargyl aniline with Lindlar's catalyst under an atmosphere of H_2 returned only starting material with none of the desired alkene being formed (Scheme 51). Higher pressures of H_2 were not attempted for fear of over reduction of the alkyne.



Scheme 51: Attempted cyclization through an $S_{RN}1$ reaction.

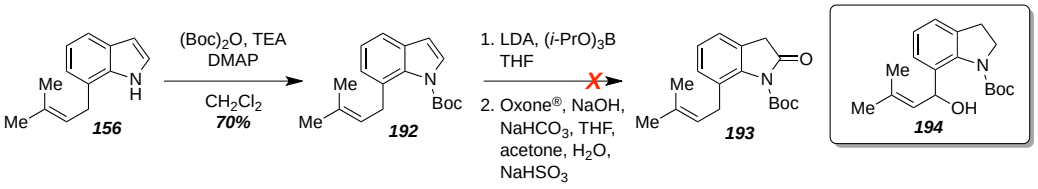
With these disappointing results for the direct formation of a 7-prenyl oxindole, an alternative route was sought for the oxidation of 7-prenyl indole to its corresponding oxindole. Previously, it was shown we could access 7-prenyl indole in a scalable and high-yielding four step sequence (Scheme 40).[18] With the rapid availability of **156**, a variety of oxidation conditions could be screened for the synthesis of 7-prenyl oxindole. The first of the oxidation conditions chosen were developed by Payack and Vazquez.[27] Treatment of *N*-Boc indole with LDA, followed by addition of triisopropylborate, gave the

corresponding 2-(indolyl) borate, which was used without further purification in the second step. The borate can then be hydrolyzed to the boronic acid and oxidized to the oxindole with Oxone® in a one-pot procedure (Scheme 52). We anticipated success, since one of the examples shown was a 7-methyl indole being oxidized to 7-methyl oxindole in moderate yield.



Scheme 52: Payack and Vazquez oxindole formation.

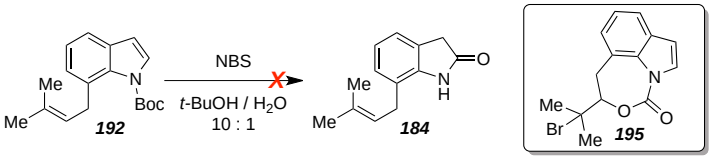
Boc protection of **156** proceded smoothly with the use of Boc anhydride, TEA, and DMAP to give *N*-Boc indole **192** in high yield. Treatment of **192** with LDA and $(i\text{-PrO})_3B$, followed by oxidation, gave one product in high yield. Characterization revealed that the oxidation took place at the allylic carbon of the prenyl group (**194**) rather than giving desired oxindole **193** (Scheme 53).



Scheme 53: Oxidation of the prenyl group of **192**.

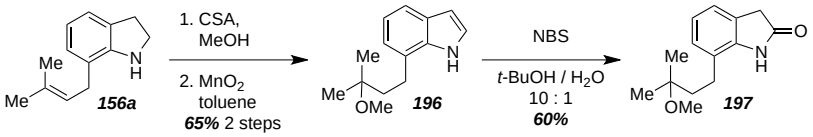
With the unexpected oxidation of the allylic-benzylic carbon of the prenyl group, a different oxidation technique was sought. Funk and coworkers had shown that treatment of indole with two equivalents of NBS in

the presence of a 10:1 mixture of *t*-BuOH and H_2O gave the desired 3-bromo oxindole in high yield.[28] It was thought that reducing the number of equivalents of NBS would provide the desired oxindole without over-bromination. However, treatment of **192** with NBS in a mixture of *t*-BuOH and H_2O did not provide the desired oxindole, but instead gave **195** through reaction with the prenyl group (Scheme 54).



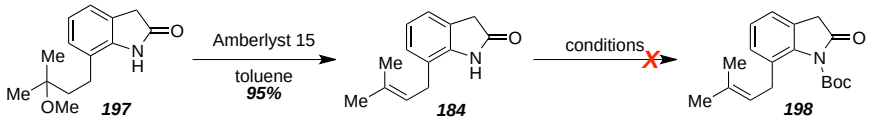
Scheme 54: NBS mediated oxindole formation.

From this reaction it was seen that the olefin of the prenyl group is very reactive to a variety of oxidative conditions and must therefore be masked to prevent formation of these undesired side products. It was determined that formation of the methyl ether would be the best way to overcome this problem. Protection of the trisubstituted olefin was accomplished by refluxing **156a** in MeOH in the presence of CSA, followed by refluxing with MnO_2 in toluene to provide **196** in decent yield. With the reactivity of the prenyl group moderated, the oxidative conditions of NBS, *t*-BuOH and H_2O were revisited to see whether the desired oxindole could be formed. Indeed, the protection of the olefin of the prenyl group allowed for the oxidation of indole **196** to the desired oxindole (**197**) (Scheme 55).



Scheme 55: Protection of the prenyl olefin and oxidation to the oxindole.

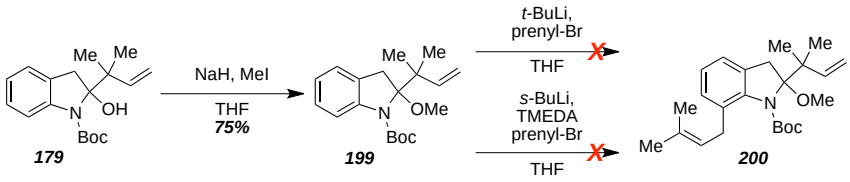
The next step in the approach to a 7-prenyl-2-reverse prenyl indole was to Boc protect oxindole **197** in preparation for the Grignard addition. After screening a variety of conditions, Boc protection of oxindole **197** was found to be unsuccessful. It is possible that placing the Boc group adjacent to the methyl ether (**196**) is too sterically demanding. In order to rectify this problem, it was decided to remove the methyl ether, regenerating the olefin. Treatment of protected oxindole **197** with Amberlyst 15 acidic resin in refluxing toluene eliminated the methyl ether to give oxindole **184**. However, protection of oxindole **184** failed to give the desired oxindole for the Grignard addition (Scheme 56).



Scheme 56: Synthesis of 7-prenyl oxindole.

Beak and coworkers have shown that when using Boc protected indolines, one can lithiate ortho to the carbamate and trap the lithiated species with a variety of electrophiles.[29] Using this same type of ortho-lithiation method, **199** was protected as the methyl ether, then treated with *t*-BuLi and prenyl bromide as the electophile. However, the 7-prenylated

product was not formed. Changing to *s*-BuLi in the presence of TMEDA still failed to produce the desired product (Scheme 57).



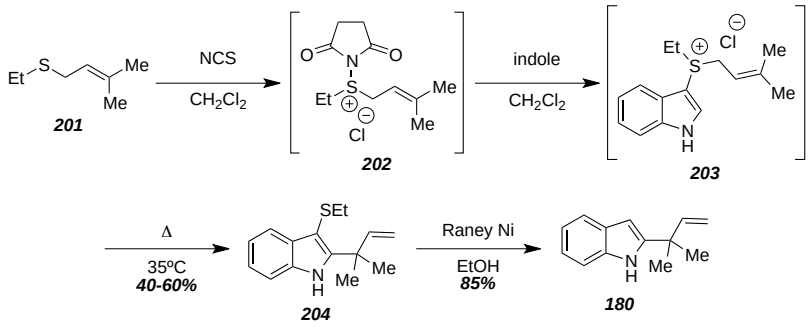
Scheme 57: Attempted ortho-lithiation to form 7-prenyl indoline.

The synthesis of an oxindole system containing a bulky prenyl group at the C7 position with an equally bulky carbamate protecting group on the nitrogen proved more difficult than expected. With sterics apparently playing a large role, a different route was to be pursued in which steric interaction of the C7 prenyl group could be avoided. This method does show promise for the synthesis of C2 substituted indoles through the corresponding oxindole precursor.

2.3.4 Thio-Claisen Reaction

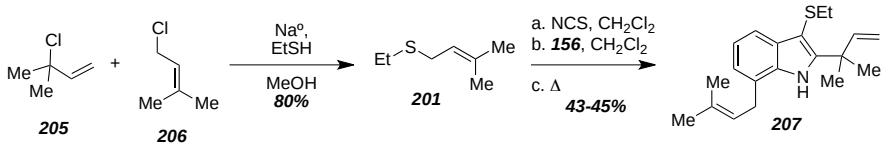
With attempts at synthesizing indole **155** through a Grignard addition to a protected oxindole having failed, a common trend of unfavorable steric interactions was recognized. The bulk of the C7 prenyl group, in conjunction with its moderately reactive olefin, makes it a significant obstacle in the synthesis of **155**. In 1976, Tomita and coworkers showed that C2 reverse prenyl indole can be synthesized through the use of a thio-Claisen[22] reaction.[30] Treatment of ethyl prenyl sulfide (**201**) with NCS affords the alkylprenylsulfonium chloride **202**, which when treated with indole gives the

indole-3-sulfonium chloride **203**. Warming the reaction to 35ºC causes the indole-3-sulfonium chloride to undergo a thio-Claisen[22] reaction to give C2 reverse prenyl vinyl sulfide **204**. Reduction of the C3 vinyl sulfide with Raney Nickel or Zn–acetic acid gave the desired C2 reverse prenyl indole **180** (Scheme 58).



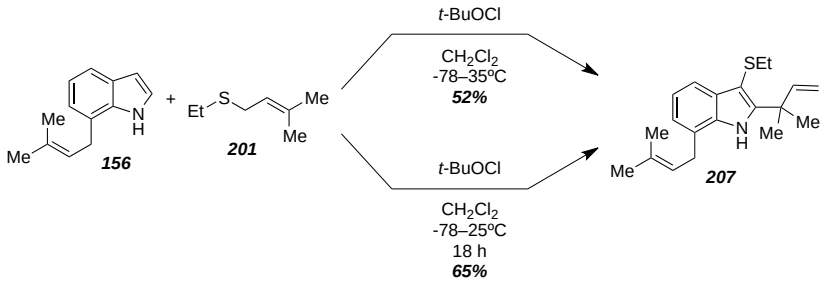
Scheme 58: Tomita and coworkers synthesis of reverse prenyl indole.

This type of thio-Claisen reaction deals exclusively with the C2 and C3 position of indole, far removed from the C7 position. It was therefore hoped that this method would alleviate this steric problem.[30] With indole **156** in hand, the first step for the thio-Claisen[22] reaction was the synthesis of ethyl prenyl sulfide **201**. A freshly prepared solution of sodium methoxide was treated with ethane thiol and mixture of prenyl (**205**) and reverse prenyl (**206**) chlorides to afford the desired sulfide (**201**) in 80% yield. Treatment of **201** with NCS at -30ºC, followed by the addition of indole **156,** gave a golden-colored solution. Upon heating to 35ºC over the course of an hour, the golden solution became dark brown in color. Evaporation of solvent, followed by flash chromotagraphy, gave the desired indole sulfide **207** in 43% yield.



Scheme 59: Synthesis of C7 prenyl C2 reverse prenyl indole sulfide.

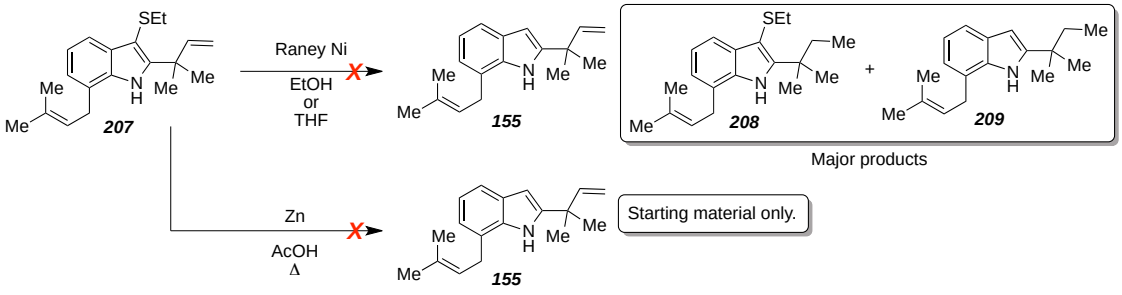
Attempts to improve the yield by varying the conditions of the reactions failed to produce yields higher than 45%. In looking at other thio-Claisen reactions, Walsh and coworkers used *t*-BuOCl instead of NCS to initiate a thio-Claisen reaction in high yields.[31] They also utilized a one pot method as opposed to Tomita's pre-formation of the sulfonium chloride followed by addition of the indole to afford the sulfide.[30,31] Utilizing Walsh's one-pot *t*-BuOCl method, a solution of ethyl prenyl sulfide (**201**) and **156** in CH_2Cl_2 was treated with *t*-BuOCl at -78ºC.[31] Upon warming to room temperature, the reaction mixture turned dark brown in color, and the consumption of starting material was monitored by TLC. The reaction mixture was then heated to 35ºC over the course of one hour and allowed to remain at that temperature for another hour. Evaporation of the solvent, followed by flash chromotagraphy, afforded indole sulfide **207** in 52% yield (Scheme 60). Varying the conditions of the reaction, it was found that slowly warming to room temperature and allowing to stir for 18 hours gave an improved 65% yield. This reaction could also be run on a 10 gram scale without significant loss in yield.



Scheme 60: Improved synthesis of indole sufide **207**.

2.3.5 Sulfide Reduction

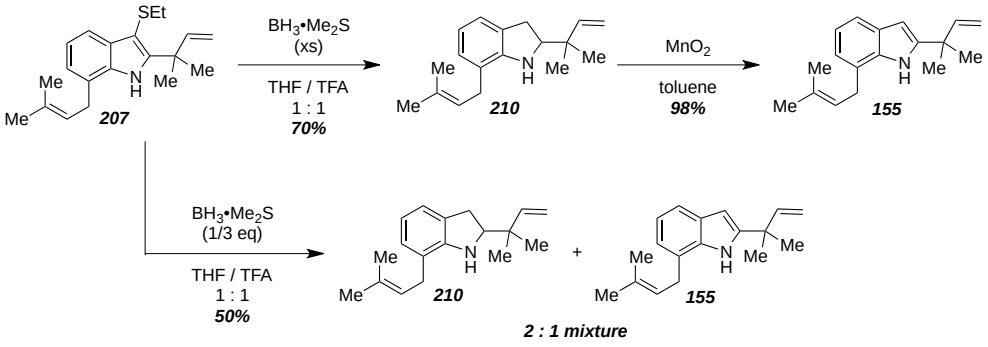
Removal of the sulfide group was the next challenge in the synthesis of indole **155**. Tomita and coworkers had shown that treatment of indole **204** with Raney nickel in EtOH gave the desired indole in an 85% yield (Scheme 58).[] However, when treating indole sulfide **207** with Raney nickel in EtOH the desired product was not obtained; instead, a variety of products varying from reduction of the primary olefin to reduction of the sulfide and primary olefin were isolated (Scheme 61). Changing solvents from EtOH to THF had no effect on the outcome of the reduction, and varying grades of Raney nickel again resulted in failure to form the product.



Scheme 61: Major biproducts of the Raney nickel reduction.

Tomita also showed that treatment of indole sulfide **204** with zinc in acetic acid at 60ºC for 10 hours gave the desired indole in a moderate 61% yield.[31] Treatment of indole **207** with zinc in acetic acid at 60ºC for 24 hours failed to give **155** (Scheme 61). Freshly washed zinc powder and zinc granules were tested, however, but both failed to give the desired product.

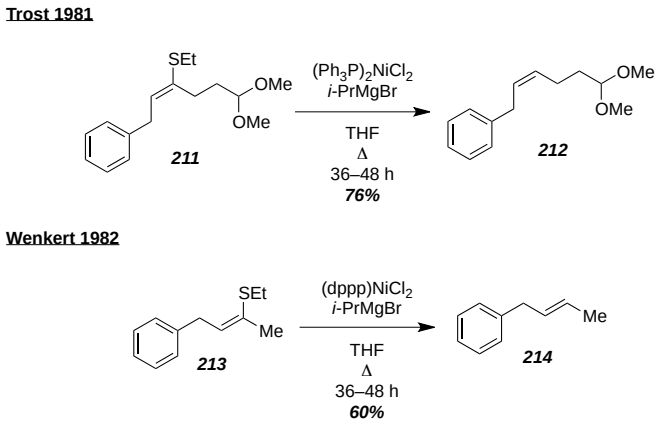
Our next approach to removal of the sulfur group was through the reduction of the indole to the indoline, followed by elimination of the sulfide to give the corresponding indole. Treatment of indole **207** with excess BH_3•Me_2S in a 1:1 mixture of THF/TFA gave the desired indoline in 70% yield. To our delight the use of excess BH_3•Me_2S not only facilitated the reduction of the indole but also caused the elimination and reduction to indoline **210**. The indoline was then oxidized to the indole by refluxing in toluene in the presence of MnO_2 (Scheme 62).



Scheme 62: BH_3•Me_2S reduction of sulfide **207**.

All attempts to use only one equivalent of hydride failed to give indole **155** exclusively, instead giving a 2:1 mixture of indoline **210** and indole **155** in 50% yield (Scheme 62). This two-step procedure provided the desired indole

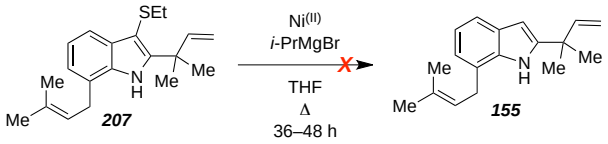
155 in a 68% overall yield. However, with the undesirable use of excess borane and a large excess of TFA for the first step, as well as nine equivalents of MnO_2 for the oxidation, more efficient conditions were sought for removal of the sulfide. In 1981, Trost and coworkers showed that treatment of vinyl phenyl sulfides with 2-propylmagnesium bromide and catalytic $(Ph_3P)_2NiCl_2$ at reflux for 36–48 hours affords reduction of the sulfide with retention of the olefin geometry.[32] Wenkert and coworkers later elaborated this methodology by changing from an aryl sulfide to alkyl sulfide under the same conditions, and found that the desired olefin could be synthesized (Scheme 63).[33]



Scheme 63: Trost's and Wenkert's reduction of vinyl sulfides.

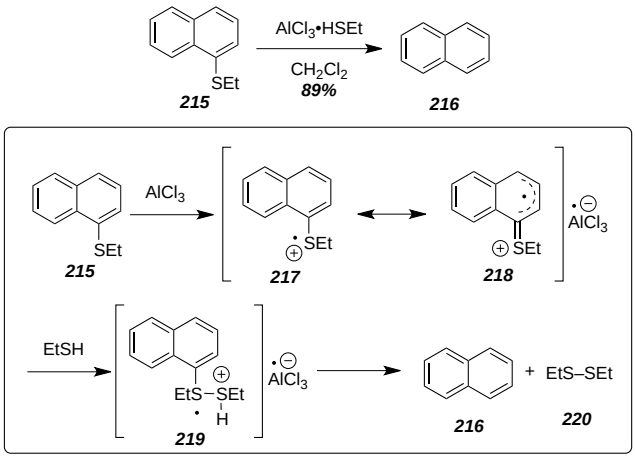
The methodology developed by Trost and Wenkert was applied to the reduction of the sulfide of indole **207**.[32,33] Isopropylmagnesium bromide was added to a solution of indole **207** and the appropriate $Ni^{(II)}$ catalyst in THF, which was then heated to reflux. After 48 hours the reaction was stopped; decomposition of the starting material was observed. Both $Ni^{(II)}$ catalysts

were tried, and both led to decomposition of the starting material (Scheme 64).



Scheme 64: Attempted reduction of the vinyl sulfide with a $Ni^{(II)}$ catalyst.

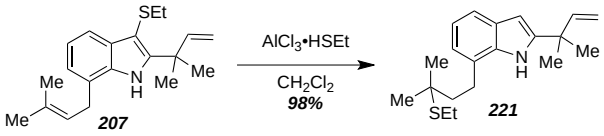
Our next attempt at sulfide reduction focused on Lewis acid mediated disulfide elimination. Fujita and coworkers have shown that aryl sulfides can be reduced in the presence of $AlCl_3$ and excess of EtSH at room temperature.[34] Treatment of 1-naphthyl ethyl sulfide with 1.5 equivalents of $AlCl_3$ and 5 equivalents of EtSH at room temperature for 5.5 hours afforded naphthalene in 89% yield (Scheme 65). It is proposed that the reduction of the sulfide takes place through a disulfide formation with $AlCl_3$ as a one-electron oxidant.[34]



Scheme 65: Reduction of an aryl sulfide through a disulfide mechanism.

One concern with this approach was the use of a stronge Lewis acid like $AlCl_3$ in the presence of the prenyl group. It has been shown previously

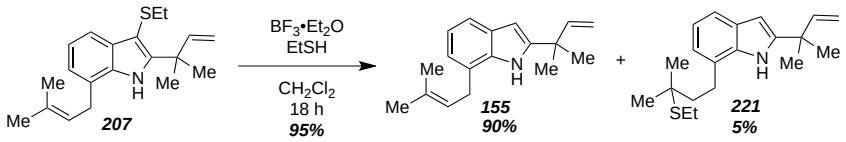
that strong acids reacted with the C7 prenyl group to give a variety of undesired side products. The possibility that the $AlCl_3$ would react exclusively as a one electron oxidant dictated that the sulfide reduction be faster than any interactions with the prenyl group. Treatment of $AlCl_3$ in CH_2Cl_2 with EtSH to form an $AlCl_3$•HSEt complex afforded the reduced indole in almost quantitative yield, unfortunately with the addition of EtSH to the prenyl group to give **221** (Scheme 66).



Scheme 66: Reduction of the **207** with addition of EtSH to the prenyl group.

Switching from the Lewis acid $AlCl_3$ to a Bronsted acid such as H_2SO_4 in the presence of EtSH also gave the reduced product, but again with the addition of EtSH to the prenyl group to afford **221**. In both cases, the acid used was strong; we therefore shifted our focus to the possibility of using a weaker Lewis acid to promote the reaction without interfering with the prenyl group. Moving from $AlCl_3$ to the relatively less acidic BF_3•Et_2O was the first attempt at reducing the sulfide without affecting the prenyl group. Following the same procedure as before, BF_3•Et_2O was treated with EtSH, followed by indole **207**. After 18 hours, the reaction had gone to completion. The major product of the reaction was the desired indole **155** in 90% yield, with approximately a 5% yield of the EtSH addition product **221** (Scheme 67). The only major drawback to switching from $AlCl_3$ to BF_3•Et_2O was the reaction

time need to consume the starting material: from 5 hours with $AlCl_3$ to 18 hours with BF_3•Et_2O.



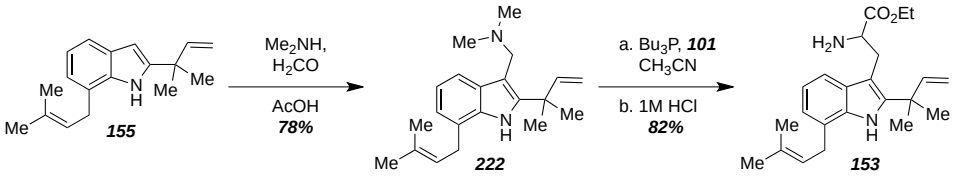
Scheme 67: Sulfide reduction with the use of BF_3•Et_2O and EtSH.

The desired indole **155** for the tryptophan portion of chrysogenamide A could then be accessed in two steps from the previously reported 7-prenylindole **156** in a scalable 58% yield. The key thio-Claisen[22] reaction allows formation of the indole portion with the 7-prenyl group already attached, obviating a late stage installation of the 7-prenyl moiety. However, the two-step reduction/oxidation used for removing the sulfide provides indole **155** in conjunction with the large excess of TFA, BH_3•Me_2S and MnO_2 made this route unappealing on larger scale. Through screening of a variety of reduction condition, the BF_3•Et_2O mediated disulfide formation proved to be not only scalable, but high yielding.

2.3.6 Tryptophan Synthesis

The synthesis of the tryptophan portion of **129** follows the same steps described in the previous chapter. With a scalable route to the 7-prenyl-2-reverse prenyl indole **155** now developed, the first step in the tryptophan synthesis was a Mannich[16] reaction of indole **155** with the imine of dimethyl amine and formaldehyde to give gramine **222**. Gramine **222** was then

subjected to Somei-Kametani[17] conditions to form the protected tryptophan in high yield. Hydrolysis of the benzophenone with 1 M HCl gave the desired free amine of the newly formed tryptophan derivative **153** (Scheme 68). This scaleable and high yielding route allowed for any protecting group manipulations that might be needed later in the synthesis.

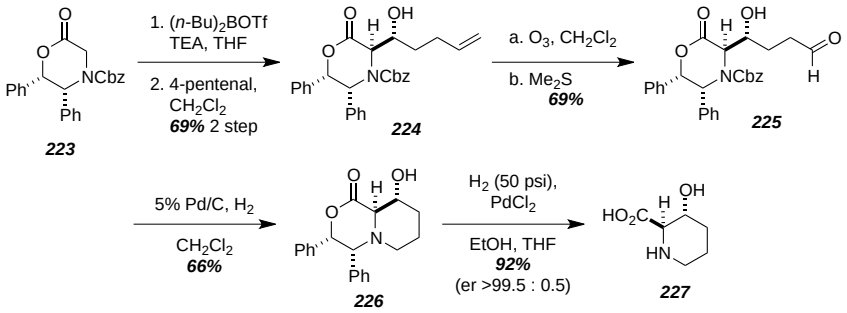


Scheme 68: Synthesis of the tryptophan derivative **153**.

2.4 Pipecolic Acid

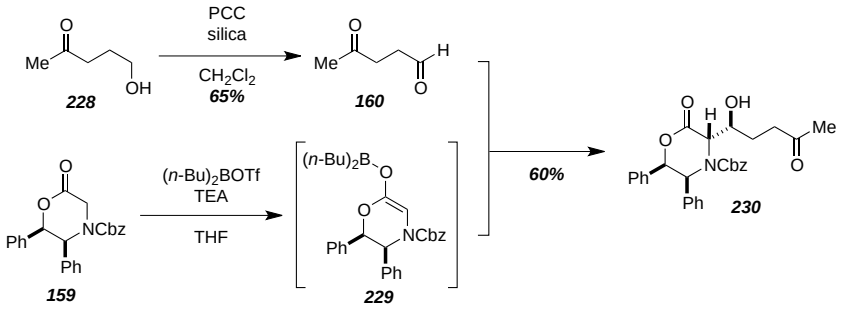
2.4.1 Lactone Approach

The pipecolic acid portion of chrysogenamide A was the next task at hand. Our first approach was inspired by previous group work in the synthesis of tetrazomine.[19,35] Commercially available lactone **223** was converted into the corresponding boron enolate with di-*n*-butyl boron triflate.[19] Aldol condensation of the boron enolate with 4-pentenal gave the anti-β-hydroxy aldol product **224** in 69% yield. Ozonolysis of the primary alkene under reductive conditions afforded aldehyde **225**, which underwent reductive amination during catalytic hydrogenation of the *N*-Cbz group to afford **226**. Finally, removal of the chiral template through catalytic hydrogenation with $PdCl_2$ gave β-hydroxy pipecolic acid **227** in 92% yield, and greater than 99 enantiomeric ratio (Scheme 69).[19]



Scheme 69: Williams' synthesis of β-hydroxy pipecolic acid **227**.

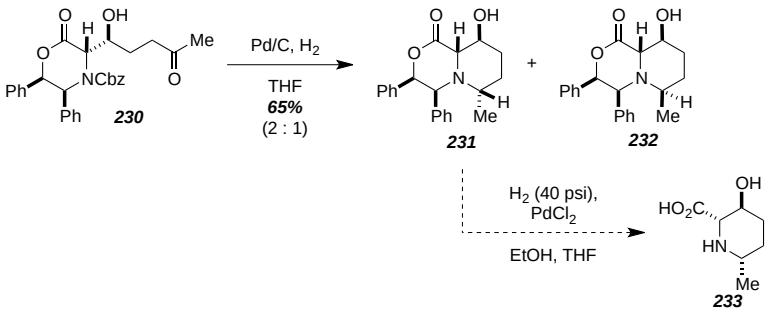
With the chemistry for the pipecolic acid well established, we decided to use this same approach for **154**. Staring from commercially available 5-hydroxy-2-pentanone, oxidation of the primary alcohol with PCC in the presence of silica gel gave aldehyde **160**. Boron enolate formation of lactone **159** with di-*n*-butyl boron triflate and TEA, followed by aldol addition to aldehyde **160,** gave β-hydroxy lactone **230** in 60% yield (Scheme 70).



Scheme 70: Synthesis of β-hydroxy lactone **270**.

At this point, we envisioned a reductive amination of the secondary amine onto the ketone in a facially selective manner influenced by the bis-phenyl group of the template. Treatment of lactone **230** with catalytic palladium on carbon under an atmosphere of H_2 gave the desired bicyclic

lactone. Unfortunately, there was little facial selectivity during the reductive amination step (Scheme 71). A crystal structure of the major diastereomer was obtained, which displayed the relative stereochemistry shown in **231** (Figure 9).



Scheme 71: Synthesis of the bicyclic lactone and removal of the template.

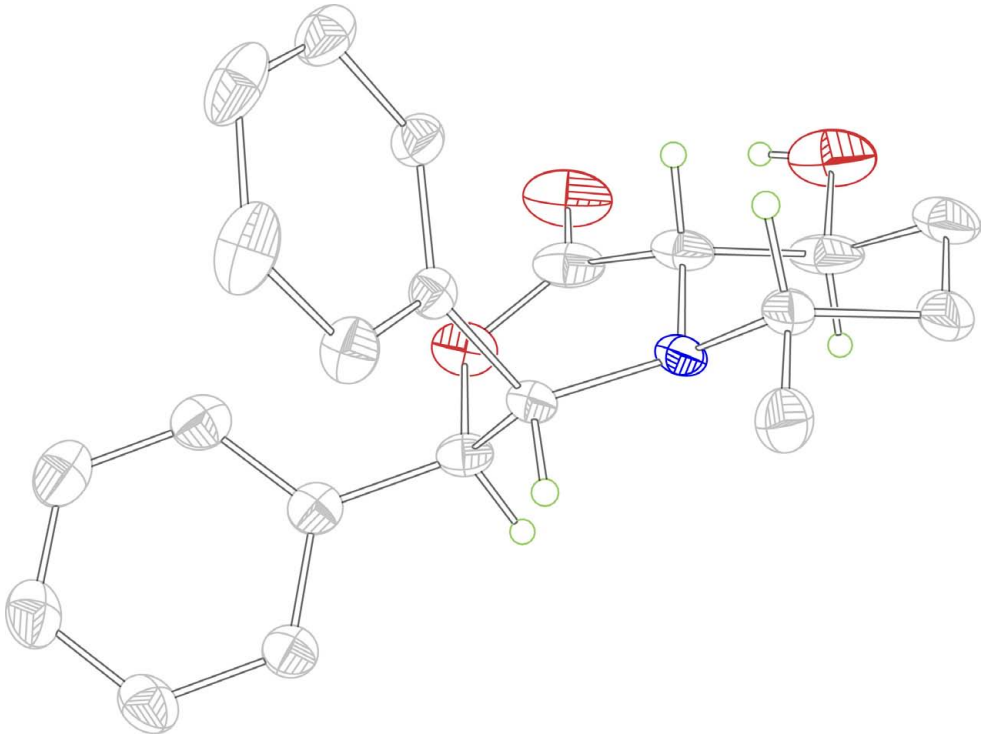
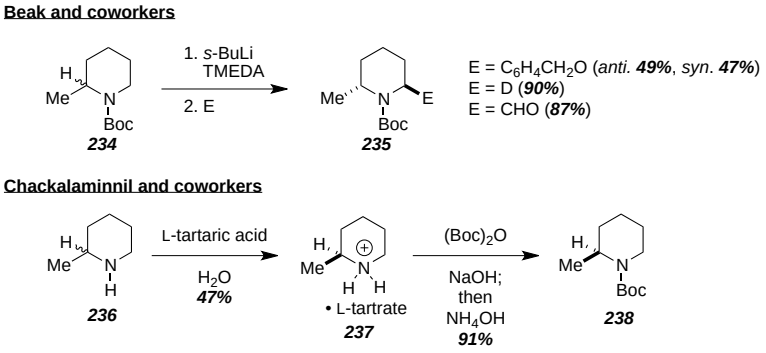


Figure 9: Crystal structure of the major diastereomer **231**.

Under an atmosphere of H_2 at 40 psi, the lactone template was removed with catalytic $PdCl_2$ to afford α-methyl-β-hydroxy pipecolic acid **233**.

With only a 2:1 selectivity from the reductive amination, the total yield for the four step sequence was a disappointing 13.5%. Although the synthesis of pipecolic acid **233** could be completed through this sequence the low yields and poor diastereoselectivity led us to pursue a more efficient route.

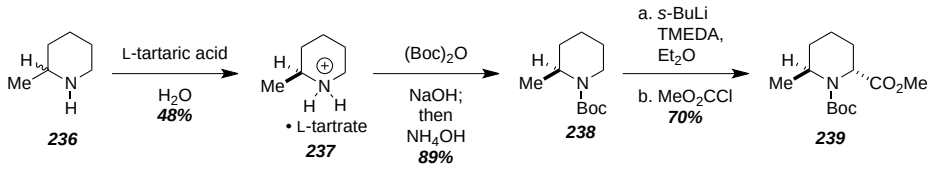
By this point, we had concluded that the stereochemistry of the α-methyl group was the most important aspect of **154**. Beak and coworkers have shown that treatment of *N*-Boc-2-methyl piperidine **234** with *s*-BuLi in the presence of TMEDA affords the ortho-lithiated species, which can be trapped with a variety of electrophiles (Scheme 72).[36] Chackalamannil and coworkers have shown that the resolution of (±)-2-methyl piperidine **236** with L-tartaric acid gives (*S*)-2-methyl piperidine tartaric acid salt in good yield.[37] Boc protection of the resultant tartaric acid salt with $(Boc)_2O$ in aqueous NaOH afforded the (*S*)-N-Boc-2-methyl piperidine (**238**) needed for the preparation of pipecolic acid **154** (Scheme 72).



Scheme 72: Alkylation and resolution of 2-methyl piperidine.

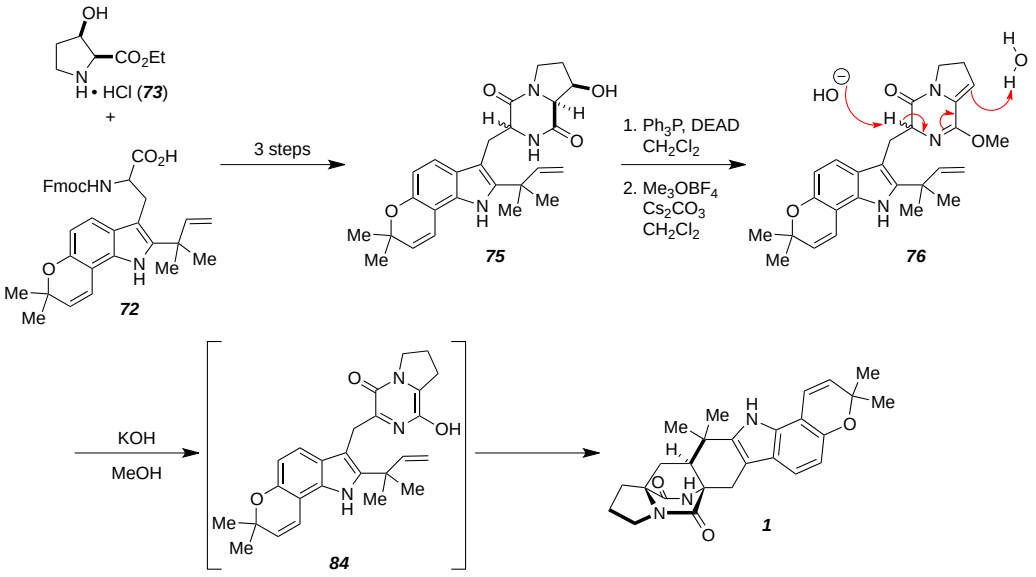
Combining Chackalamannil's[37] resolution of (±)-2-methyl piperidine with Beak's ortholithiation method, we believed that **154** could be synthesized.

This proved to be successful; (*S*)-*N*-Boc-2-methyl piperidine was obtained in 41% overall yield on 200 gram scale. Treatment of a solution of **238** and TMEDA with *s*-BuLi, according to Beak's method[36] gave the desired ortholithiated intermediate, which, when reacted with methylchloroformate gave the desired (*S*)-*N*-Boc-2-methyl pipecolic methyl ester **239** (Scheme 73).



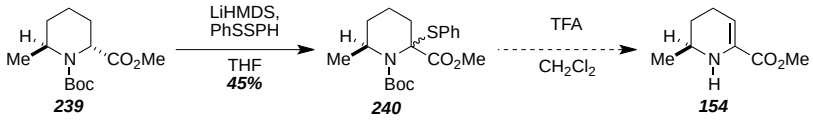
Scheme 73: Synthesis of (*S*)-*N*-Boc-2-methyl piperidine methyl ester.

For the IMDA[11] to work properly, a degree of unsaturation is required in the pipecolic acid portion. In the previous synthesis of stephacidin A,[38] a hydroxy proline moiety was coupled to a tryptophan derivative, which later underwent elimination to afford enamide **76**. Tautomerization of the enamide in a solution of MeOH and KOH provided azadiene **84**, which underwent an IMDA to give stephacidin A (Scheme 74).[38]



Scheme 74: Tautomerization, Diels-Alder for the synthesis of stephacidin A.

To install a functional group that could be used for the formation of the desired enamide, we turned to the use of diphenyl disulfide.[39] Deprotonation of **239** with LiHMDS, followed by quenching with diphenyl disulfide, gave the desired α-phenylsulfide **240**. This reaction could also be accomplished with the use of pheylselenium chloride; however, we chose to use the much less toxic diphenyl disulfide. We envisioned that removal of the Boc group would cause the elimination of the phenylsulfide through formation of the imine, which would tautomerize to give enamine **154** (Scheme 75). Enamine **154** could then be coupled and cyclized to give DKP **152**.



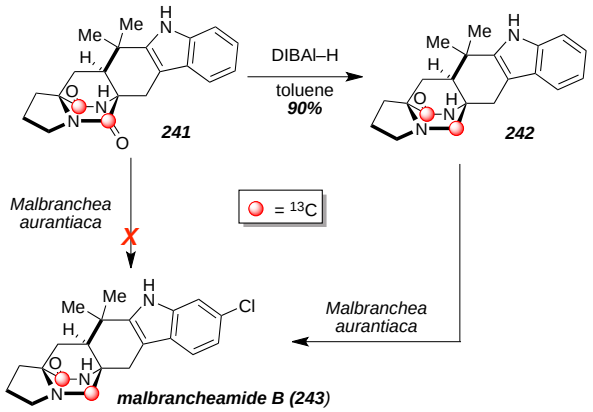
Scheme 75: Proposed synthesis of enamide **154**.

The synthesis of enamine **154** was only continued to the installation of the phenylsulfide group before another idea was explored. We are confident that enamine **154** can be synthesized through the remaining proposed step, which has been taken into consideration as an alternative plan.

2.5 Proposed Biosynthesis

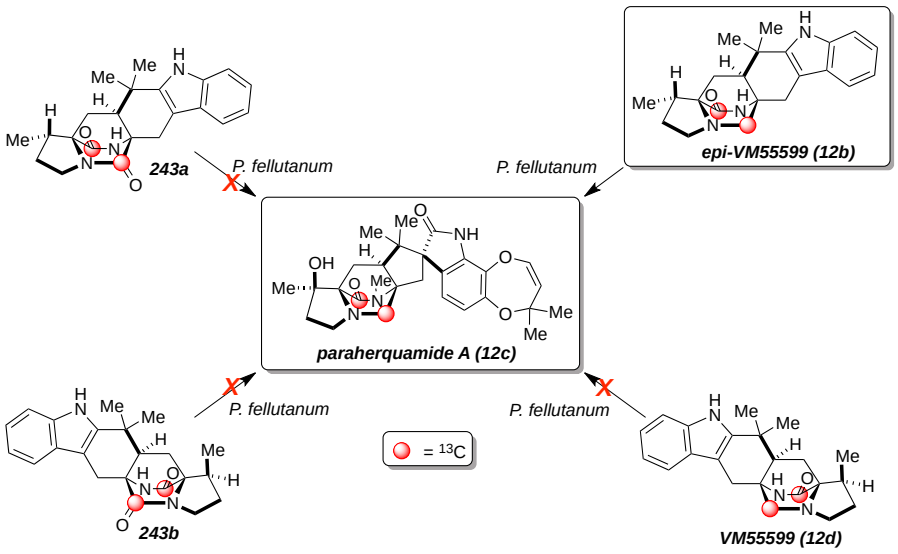
2.5.1 Previous Work

Previously in our group, we completed the synthesis of malbrancheamide B (**243**) using an approach centered around an IMDA.[40] This work was prompted by a desire to interrogate the biosynthetic pathway leading to malbrancheamide B due to its structural differences in comparison to related natural products.[38,40] Specifically, while malbrancheamide B lacks the tertiary amide functionality and exists as a monoketopiperazine, realated products – e.g. stephacidin A – exist as the diketopiperazine. This in itself is neither new nor novel, as there are many monoketopiperazine natural products, including the malbrancheamides, marcfortines, and paraherquamides. However, during a series of feeding studies focusing on the halogenation of malbrancheamide B, an interesting discovery was made. When the producing fungus was fed doubly ^{13}C labeled diketopiperazine derivative **241**, no incorporation into malbrancheamide B was found.[40] When the fungus was fed doubly ^{13}C labeled monoketopiperazine derivative **242**, it was found to incorporate into **243** (Scheme 76).



Scheme 76: Incorporation studies for the biosynthesis of malbrancheamide B.

This was not the first incident of a diketopiperazine not being incorporated into its monoketopiperazine counterpart. Previously, former group member Dr. Emily Stocking interrogated the biosynthesis of the catechol-derived dioxepin ring formation of paraherquamide A (**12c**) through labled incorporation studies.[41] During her studies a variety of labeled precursors were synthesized and fed to *Penicillium fellutanum* (ATCC:20841) to examine the timing of the formation of the dioxepin ring. Feeding of doubly ^{13}C labled **243a**, **243b**, **12b**, and **12d** to *P. fellutanum* proved that only **12b** showed significant incorporation into paraherquamide A (Scheme 76a).



Scheme 76a: ^{13}C labled incorporation studies for the biosynthesis of paraherquamide A.

This again demonstrating the possiblility that the bicyclo[2.2.2]diazaoctane ring-containing monoketopiperazine natural products may be the products of a completely different biosynthetic pathway than the diketopiperazine-containing natural products. More interesting results leading to a possible new biosynthetic propsal were realized when feeding *P. fellutanum* a second set of ^{13}C labled precursors **243c**, **243d**, **243e**, **243f**, and **243g**.[] Results of the feeding incorporation studies showed that none of these potential precursors were incorporated intact, and only **243f** and **243g** showed any incorporation into paraherquamide A. However, only the C18 of **243f** and **243g** showed incorporation (0.44 and 0.92%, repectively) with no incorporation at C12. This suggests that **243f** and **243g** are catabolized, and that 3(*S*)-methyl-L-proline is re-incorporated, but the reverse prenylated tryptophan moiety is not.[]

[]

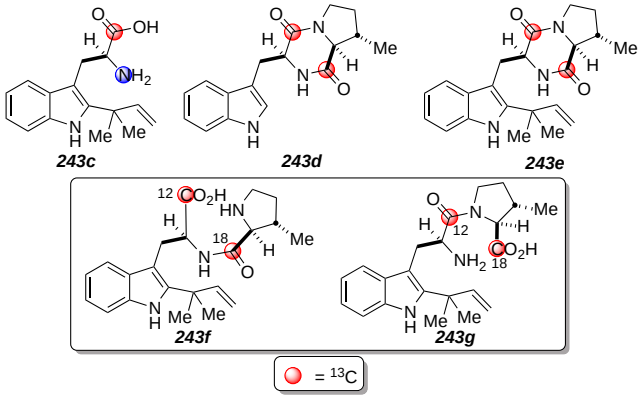
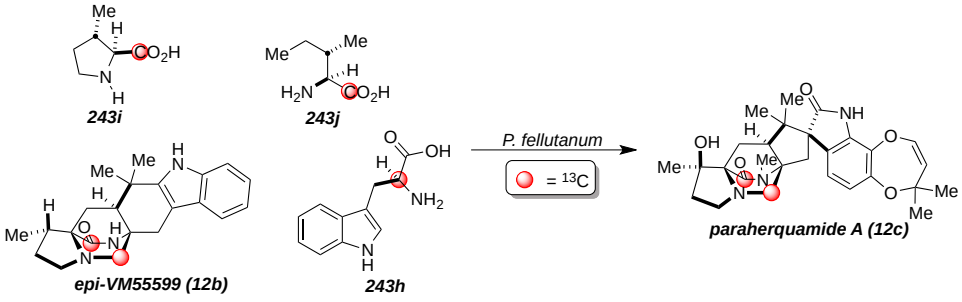


Figure 9a: ^{13}C labeled metabolites fed to *Penicillum fellutanum* for paraherquamide A studies.

It has been shown through feeding incorporation studies that a few substrates do incorporate into paraherquamide A (Scheme 76b). These substrates are generally simple amino acids: L-tryptophan, 3(*S*)-methyl-L-proline and L-isoluecine, with the most complex metabolite being monoketopiperazine **12b** (Scheme 76b).[?] This leads to questions concerning the timeline of the biosynthetic pathway: at what point is the carbonyl of the tryptophan moiety reduced, and when is the bicyclo[2.2.2]diazaoctane ring formed?



Scheme 76b: ^{13}C metabolites that incorporated into paraherquamide A.

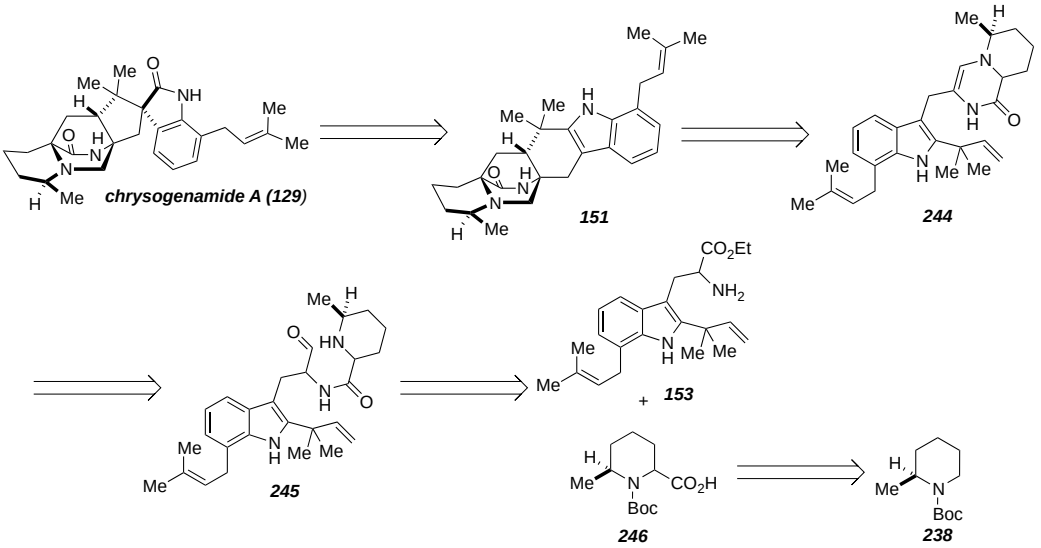
Regarding the first question, is it possible that there is a net two-electron reduction leading to the formation of the azadiene for the IMDA? This proposal would stand in contrast to the oxidation pathway followed in the cases of the stephacidins and notoamides. Alternatively, it is possible that the monoketopiperazine based bicyclo[2.2.2]diazaoctane containing natural products could proceed through a completely different mechanism. We believe that the monoketopiperazine of natural products such as the paraherquamides, malbrancheamides, and marcfortines is produced early in their biosynthesis through the possible reduction of tryptophan.

This led us to explore the possibility that the reduction of the tertiary amide may take place before the IMDA reaction. It may also be possible that the same reduction may lead to formation of the azadiene needed to facilitate the IMDA. With chrysogenamide A being a monoketopiperazine derivative, we wanted to explore the hypothesis that the reduction of the tertiary amide, or a mimic of this reduction, could lead to bicyclo[2.2.2]diazaoctane ring system.

2.5.2 Amended Retrosynthesis

This new hypothesis in mind, we revisited our synthetic route to incorporate this key reaction. As before, chrysogenamide A could arise from an oxidative rearrangement of its indole precursor. The bicyclo[2.2.2]diazaoctane ring system of **129** could come from an IMDA

reaction of enamine **244**. Condensation of the secondary amine of the pipecolic acid onto the aldehyde of tryptophan **245** would afford enamine **244**. The tryptophan derivative **153** could be synthesized as previously discussed. The pipecolic acid could also be formed through chemistry discussed earlier, but without the installation of the phenylsulfide (Scheme 77).

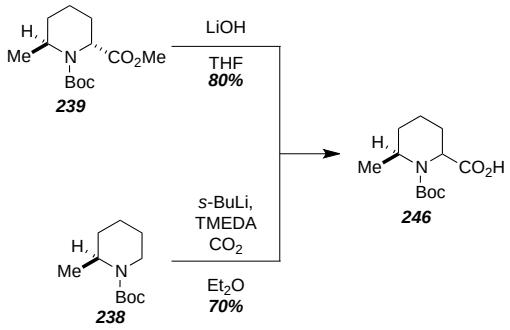


Scheme 77: Amended retrosynthesis for chrysogenamide A.

2.5.3 Revised Pipecolic Acid

As mentioned above, pipecolic acid **246** was to be synthesized through the use of the previously discussed chemistry. Our first synthesis of **246** was achieved through hydrolysis of methyl ester **239** with LiOH in THF. This proceeded nicely to give the desired acid in 80% yield (Scheme 78). Upon reexamination however, we decided to access **246** directly from the previously synthesized **238**. Treatment of a solution of **238** and TMEDA with

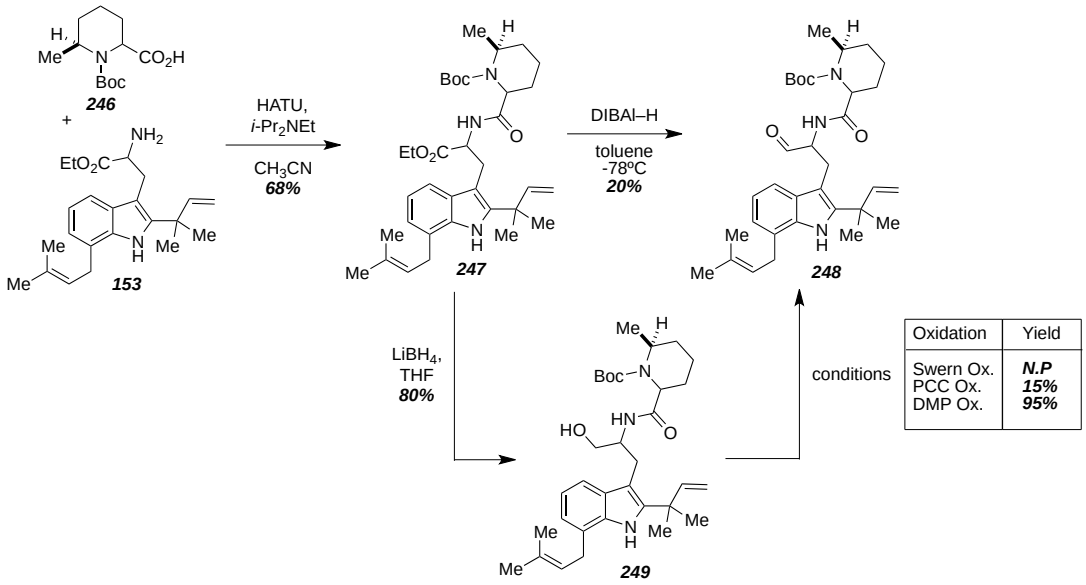
s-BuLi afforded the desired lithiated species, which, when reacted with CO_2 gas and aqueous acid, gave pipecolic acid **246** in 70% yield (Scheme 78).[42]



Scheme 78: Synthesis of pipecolic acid **246**.

2.5.4 Progress Towards the Synthesis of Chrysogenamide A

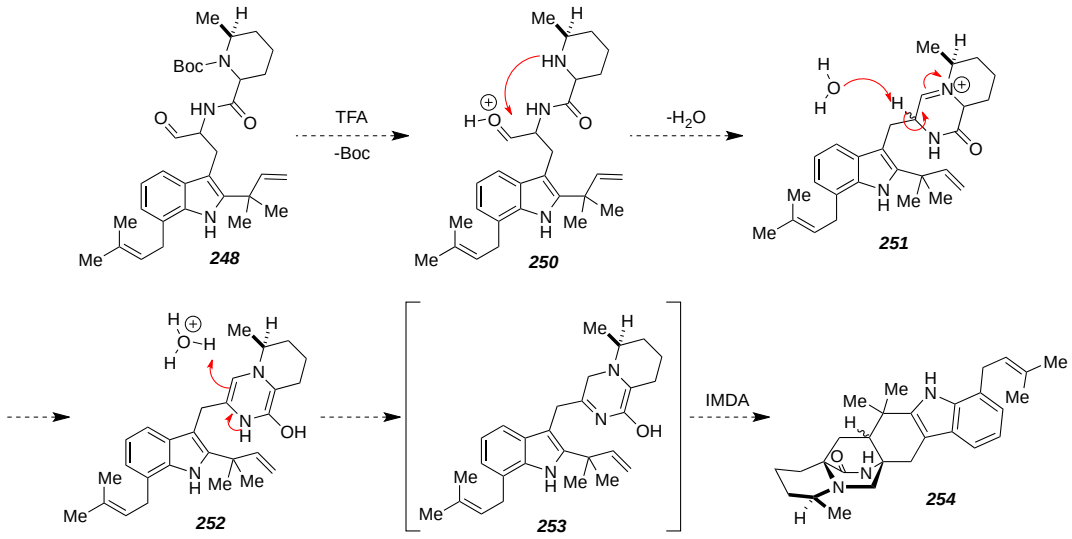
With both amino acid derivatives in hand, the next step toward the synthesis of **129** was to couple pipecolic acid **246** with tryptophan **153**. Treatment of a solution of acid **246** and amine **153** with HATU and *i*-Pr_2NEt at room temperature gave the desired coupled product in 68% yield. Reduction of the ester to aldehyde **248** with DIBAl–H at -78ºC afforded the product in a low 20% yield. This poor result was circumvented through reduction of ester **247** to its corresponding alcohol with $LiBH_4$ in THF to give a separable 1:1 mixture of two diastereomers in 80% combined yield. The first attempt at oxidizing the alcohol to the desired aldehyde through Swern oxidation[43] resulted in a complex mixture of products, with none of the desired aldehyde obtained (Scheme 79).



Scheme 79: Coupling and formation of aldehyde **248**.

This led us to try oxidation of **249** with PCC and silica gel in CH_2Cl_2. After stirring for five hours, only 15% of the aldehyde was obtained, despite complete consumption of the starting material. Finally, DMP[44] was employed treating a solution of **249** in wet CH_2Cl_2, which afforded the desired aldehyde as a 1:1 mixture of diastereomers in 95% yield.

Our next step in the syntesis of **129** was deprotection of the Boc group, followed by condensation onto the aldehyde. We were interested to see whether a tautomerization would occur during the deprotection/condensation step to give rise to the azadiene needed for the IMDA. We proposed that this might be possible under strongly acidic conditions (Scheme 80).



Scheme 80: Proposed condensation/tautamerization for the IMDA.

Treatment of **248** with TFA in THF for 24 hours gave a mixture of products, but one had the distinct NMR peaks of the IMDA[11] cycloadduct. Scaling the reaction up and subjecting **248** to the same conditions still failed to produce more than a 2% yield of cycloadduct, with insufficient product obtained to complete all of the NMR studies needed for the determination of the relative stereochemistry.

One of the drawbacks to this type of IMDA reaction is the selectivity of both the diene and dieneophile. Taking this fact into account, there are four possible products from the IMDA[11] (Figure 10).

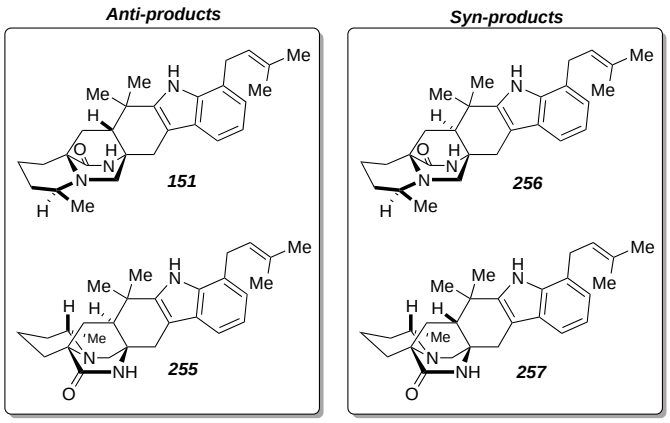
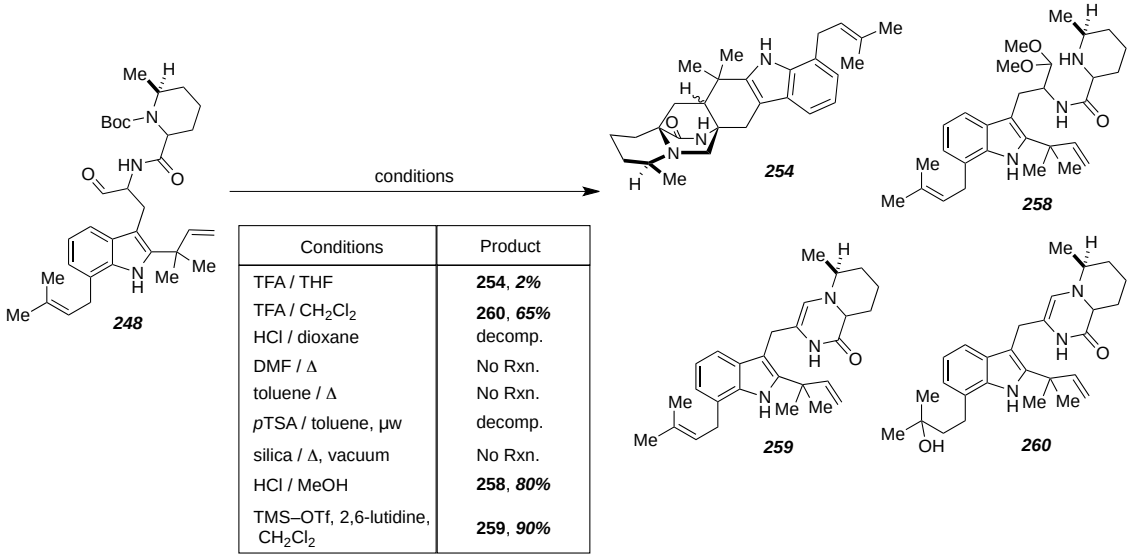


Figure 10: Possible IMDA products.

To improve the overall yield of the cyclization, a variety of conditions were screened. Our first attempt involved switching from TFA in THF to HCl in dioxane, but none of the desired product was formed.[45] Switching solvents from THF to CH_2Cl_2 with TFA as the acid source yielded a new product which was eventually found to be formed from the addition of water to the 7-prenyl group of the indole along with cyclization to enamine **260**. Attempts at thermal deprotection of the Boc group led to recovered starting material. Treatment of **248** with PTSA in toluene under microwave conditions gave complete decomposition of the starting material (Scheme 81).[46] Absorption of aldehyde **248** onto silica gel, followed by heating to 180ºC under vacuum, failed to produce any cycloadduct, again resulting in recovery of starting material.[47] Exposure of **248** to HCl in MeOH led to deprotection of the Boc group and formation of the dimethoxy acetal of aldehyde **258**. Finally, treatment of aldehyde **248** with TMS–OTf in the presence of 2,6-lutidine led to

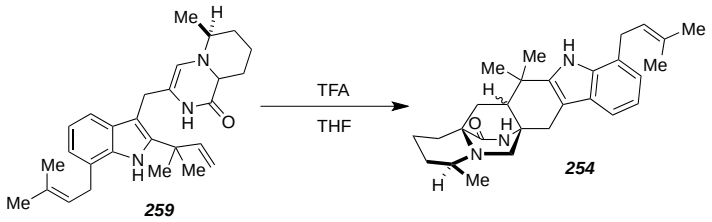
the exclusive formation of cyclized enamine **259** upon workup with NH_4Cl (Scheme 81).[48]



Conditions	Product
TFA / THF	**254**, ***2%***
TFA / CH_2Cl_2	**260**, ***65%***
HCl / dioxane	decomp.
DMF / Δ	No Rxn.
toluene / Δ	No Rxn.
*p*TSA / toluene, μw	decomp.
silica / Δ, vacuum	No Rxn.
HCl / MeOH	**258**, ***80%***
TMS–OTf, 2,6-lutidine, CH_2Cl_2	**259**, ***90%***

Scheme 81: Reaction conditions and products of *N*-Boc deprotection.

With only the TFA in THF conditions succeeding to give the cycloadduct in one step, we decided to examine the two-step cyclization of aldehyde **248**. Deprotection of the Boc group with TMS–OTf and 2,6-lutidine gave the crude enamine in seemingly high yield; however, the product decomposed during flash chromotagraphy and only 40% was recovered. It was also noted that the clean enamine began to decompose in $CDCl_3$ when taking the NMR; the substrate was therefore used without purification in the step that followed the deprotection. Subjecting **259** to one equivalent of TFA in THF gave a single product resembling the cycloadduct of an IMDA in a low 20% yield (Scheme 82).



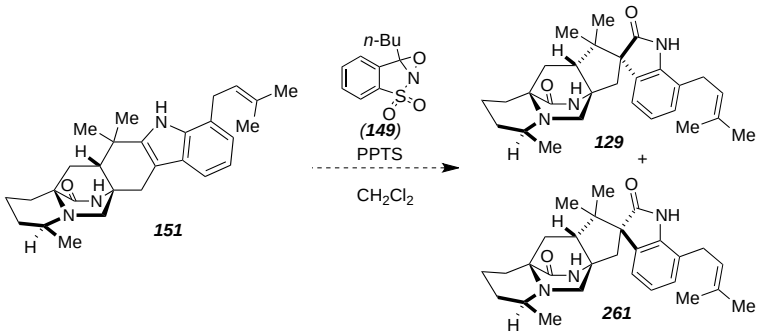
Scheme 82: Cyclization of enamine **259** to IMDA product **254**.

Currently, full characterization of the proposed cycloadduct, along with structural determination, is underway in hopes of confirming our hypothesis for the plausible IMDA of a monoketopiperazine. We are also exploring the use of AcCl to afford cyclization to the bicyclo[2.2.2]diazaoctane ring system; however, this reaction takes 14 days to complete and is currently underway.[49]

2.6 Future Work

2.6.1 Oxidative Rearrangement

The end game for the chrysogenamide A project has two possibilities. The first, assuming the product of the previously described IMDA can be detected, is to attempt the oxidative rearrangement of indole **151** to **129** and its diastereomer (**261**). The oxidative rearrangement can take place by first treating the cycloadduct with PPTS to prevent any *N*-oxide formation of the tertiary amine, followed by the addition of Davis' oxiziradine[15] (Scheme 83), as was shown in the synthesis of marcfortine C.[10]

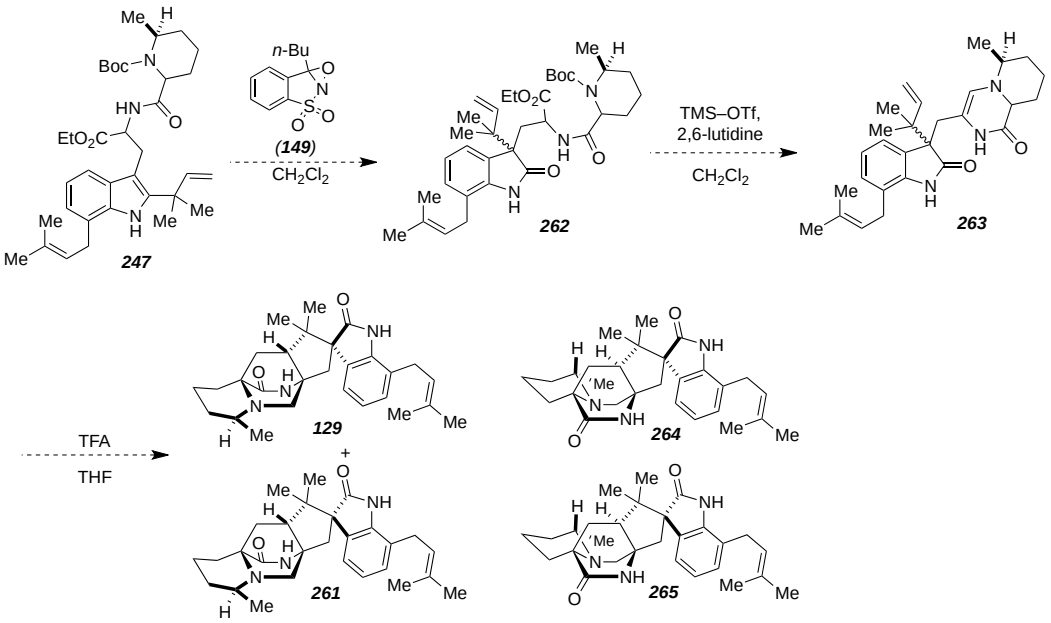


Scheme 83: Oxidative rearrangement of indole **151** to chrysogenamide A.

This should provide chrysogenamide A and its C3 diastereomer in a 1:1 ratio if results follow those of the marcfortine C synthesis.[10] This route is fully dependent on the IMDA of the indole in the previous step. If the correct precursor can be synthesized, this final step should pose no problem in the synthesis of chrysogenamide A.

2.6.2 IMDA of the Oxindole

The second plausible concluding strategy for the synthesis of chrysogenamide A is to perform the oxidative rearrangement before the IMDA. It has been shown that the IMDA on the oxindole moiety provides the *anti*-cycloadduct exclusively. Alternatively, when cyclized on the indole there is usually a greater than 2:1 ratio favoring the *syn*-cycloadduct. We envision the possibility of performing the oxidative rearrangement following the coupling of the pipecolic acid with **247**. This would allow for the IMDA to take place on the oxindole substrate and possibly give the *anti*-cycloadduct exclusively (Scheme 84).[50]



Scheme 84: Early stage oxidative rearrangement to the IMDA.

In the case of the early stage oxidative rearrangement, there would be four possible products in the end, assuming that the spirooxindole diastereomers could not be separated along the way. If the two diastereomers are separable, there would be two possible products at the end of the IMDA. This is a more appealing route to chrysogenamide A due to the possibility of being able to separate the two spirooxindoles before the key IMDA. However, if they are not separable, there is the possibility of not being able to separate the four products by normal purification methods.[51]

2.6.3 Acid vs Base

The final consideration in the synthesis of chrysogenamide A lies in the conditions used for the final IMDA reaction. In every case discussed above, the IMDA was proposed through an acid-catalyzed reaction. However, in the

majority of the sytheses of related natural products, base was used to facilitate the tautomerization event that led to the IMDA. For this reason, it may be in our best interest to treat the enamine that is formed from the deprotection/cyclization of the secondary amine with a solution of KOH in MeOH as shown in previous syntheses.

2.7 Conclusions

In our progress toward the synthesis of chrysogenamide A, we have shown that typical prenylation conditions were not suitable for our 7-prenyl indole **156**. Because of this, we were forced to use unconventional methods to gain access to the desired indole **155**. Though the thio-Claisen reaction of Tamita and coworkers worked well for our substrate,[30] the reported reduction of the sulfide failed. Adapting the work of Fujita and coworkers,[34] we utilized a weaker Lewis acid to facilitate the reduction of sulfide **207**, thereby affording indole **155** in high yield. We showed that indole **155** can be converted to tryptophan **153** in moderate yield to give one half of chrysogenamide A. The synthesis of the pipecolic acid portion of **129** was made through a resolution, as reported by Chackalamannil and coworkers,[37] followed by an ortholithiation of (*S*)-*N*-Boc-2-methyl piperidine and quenching with CO_2 and aqueous acid to give **246**. We also believe that the origin of the bicyclo[2.2.2]diazaoctane ring system in the monoketopiperazine could arise from the condensation of the secondary amine, or possible reduction of the amide, to form an enamine.

This enamine could undergo a tautomerization to give the azadiene for the IMDA. We envision that through the correct conditions, the IMDA should take place and give rise to the synthetic target: chrysogenamide A.

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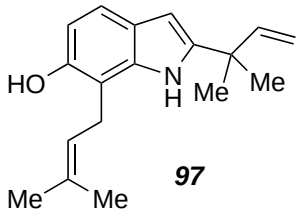
Chapter 3: Experimentals

3.1 General Considerations

Unless otherwise noted, all materials were obtained from commercial sources and used without purification. All reactions were carried out under anhydrous conditions, unless otherwise specified, and performed under positive pressure of argon using flame-dried glassware. Dichloromethane, acetonitrile, toluene, and tetrahydrofuran were degassed with argon and dried through a solvent purification system (J.C. Meyer of Glass Contour). Flash chromatography was performed on standard grade silica gel (230 x 400 mesh) from Sorbent Technologies with the indicated solvent. Microwave reactions were run using a CEM Discover set to a constant temperature and allowing for variable power. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 300 and 400 MHz spectrometers as indicated. Chemical shifts are reported in parts per million downfield from tetramethylsilane. Peak multiplicities are denoted s (singlet), bs (broad singlet), d (doublet), t (triplet), m (multiplet) or by a combination of these *e.g*. dd (doublet of doublets). Infrared spectra were recorded on a Bruker Tensor 27 IR spectometer on

NaCl plates. Mass spectra were obtained at the Colorado State University CIF on a Fisons VG Autospec.

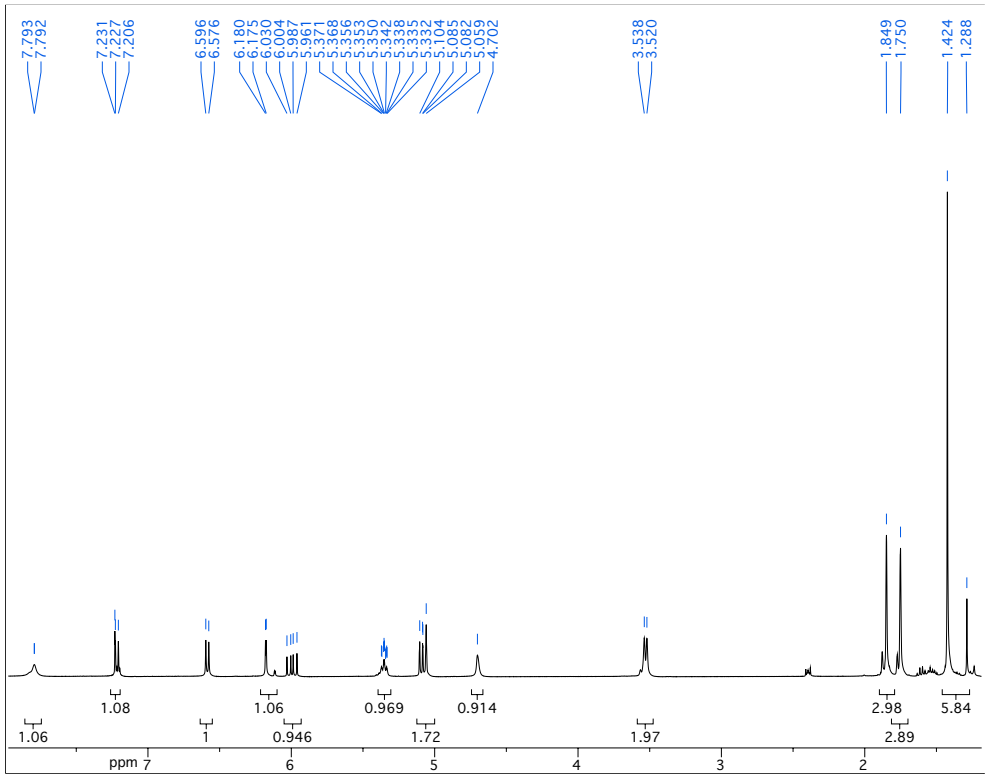
3.2 Notoamide S and Notoamide T



97

7-(3-Methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-ol (97). To **98** (100 mg, 0.37 mmol, 1 eq) in MeOH (3 mL) was added Lindlar's catalyst (10 mg, 10 wt %) at rt followed by purging with a balloon of hydrogen gas. The reaction was vigorously stirred for 30 min. The reaction was monitored by NMR for loss of alkyne proton. The reaction was then filtered through a plug of Celite and concentrated under reduced pressure to give a crude yellow oil. The resultant crude yellow oil was then taken up in toluene (3 mL) and the reaction was heated to 150ºC in a microwave reactor for 30 min. The reaction was then concentrated under reduced pressure to give the crude **97** as a brown oil. The residue was purified by flash chromatograph (AcOEt/hexane, 1:20) to give indole **97** (87 mg, 87%) as an off white solid. R_f = 0.38 (AcOEt/hexane, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) d 7.79 (bs, 1 H), 7.20 (d, *J* = 8.3 Hz, 1 H), 6.57 (d, *J* = 8.3 Hz, 2 H), 6.18 (s, 1 H), 6.03 (dd, *J* = 17.3, 10.5 Hz, 1 H), 5.37 (t, *J* = 7.25 Hz, 1 H), 5.10 (d, *J* =17.3 Hz, 1 H), 5.08 (d, *J* = 10.5 Hz, 1 H), 4.70 (s, 1 H), 3.55 (d, *J* = 7.25 Hz, 2 H), 1.84 (s, 3 H), 1.75 (s, 3 H),

1.42 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 148.7, 146.5, 144.5, 136.4, 134.0, 123.1, 122.3, 118.4, 112.0, 110.1, 108.1, 97.99, 38.3, 27.5, 25.9, 24.5, 18.1; IR (n_{max}) 3447, 1621, 1103, 913, 852 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{18}H_{23}NO$ (M + H) 270.1852, found 270.1851.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5.350
5.342
5.338
5.335
5.332
5.104
5.085
5.059
4.702
3.538
3.520
1.849
1.750
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1.288
1.08
1.06
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0.914
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1.06
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1.97
2.89
ppm 7
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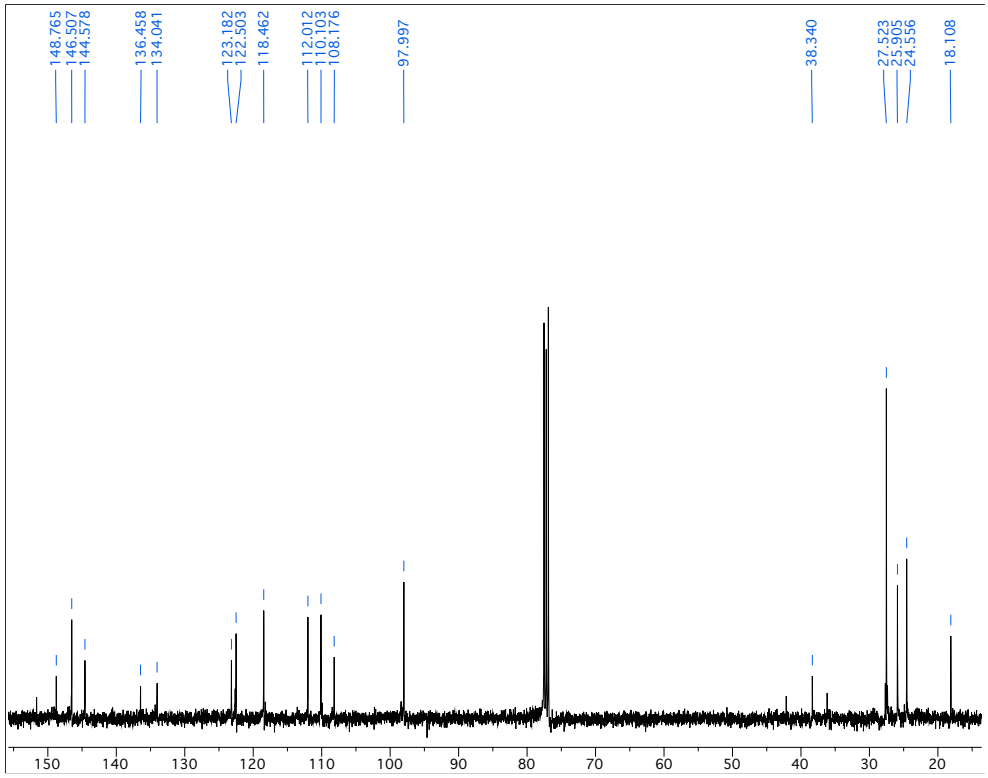
5
4
3
2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90
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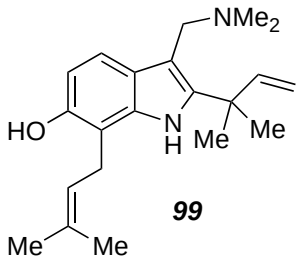
60
50
40
30
20

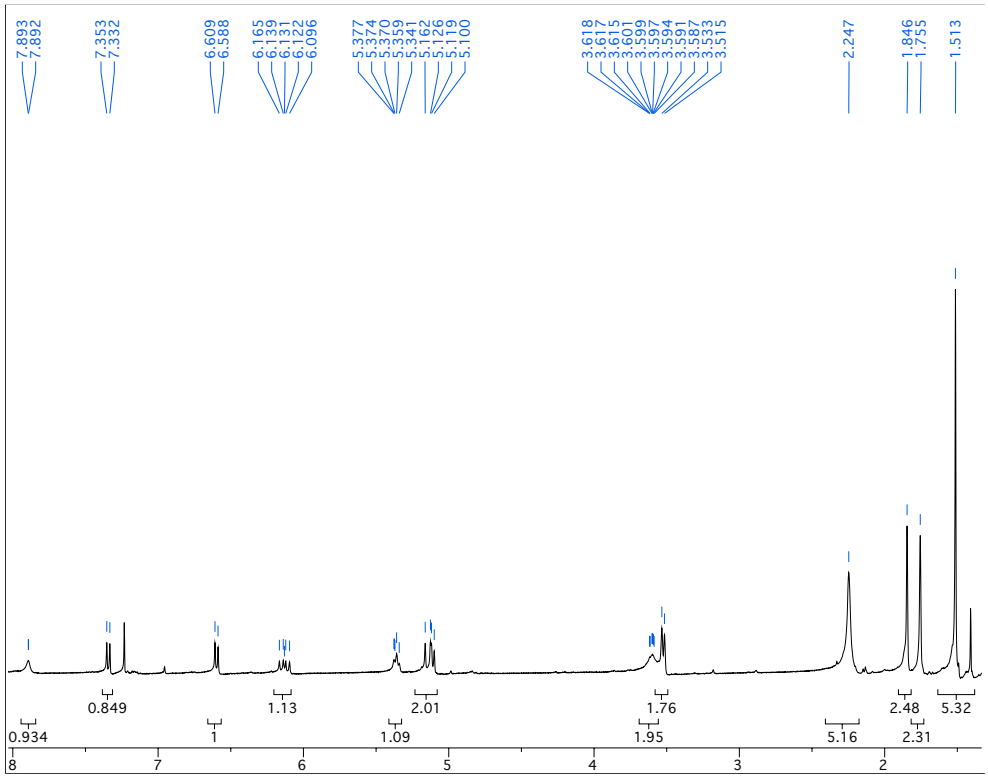


99

3-((Dimethylamino)methyl)-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-ol (99) from 97. To AcOH (500 mL) was added at rt, Me_2NH (6 mL, 0.074 mmol, 1 eq, 40% aqueous solution) followed by H_2CO (8 mL, 0.074 mmol, 1 eq, 37% aqueous solution) and stirred for 30 min. To this was added at rt a solution of **97** (20 mg, 0.074 mmol, 1 eq) in AcOH (500 mL) and stirred for 4 h. The reaction was basified with 2M NaOH to a pH of 10–12 and extracted with Et_2O (3 x 10 mL). The organic layers were combined and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure, to give **99** (4 mg, 20%) as a yellow oil.

(99) from 100. To **16** (100 mg, 0.30 mmol, 1 eq) in MeOH (3 mL) was added at rt Lindlar's catalyst (10 mg, 10 wt %) followed by purging with a balloon of hydrogen gas. The reaction was vigorously stirred for 30 min. The reaction was monitored by NMR for loss of the alkyne proton. The reaction was then filtered through a plug of Celite and concentrated under reduced pressure. The resultant yellow oil was then taken up in toluene (3 mL) and subjected to microwave conditions of 150ºC for 30 min. The reaction was then concentrated under reduced pressure to give crude **15** (75 mg, 75%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) d 7.89 (bs, 1 H), 7.35 (d, *J* = 8.4 Hz, 1

H), 6.60 (d, *J* = 8.4 Hz, 1 H), 6.16 (dd, *J* = 17.5, 10.6 Hz, 1 H), 5.35 (t, *J* = 7.3, 1 H), 5.16 (d, *J* = 17.3, 1 H), 5.12 (d, *J* = 10.6, 1 H), 3.59 (bs, 1 H), 3.53 (d, *J* = 7.3 Hz, 2 H), 2.24 (s, 6 H), 1.84 (s, 3 H), 1.75 (s, 3 H), 1.51 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 148.8, 146.5, 134.4, 133.8, 122.6, 117.4, 111.8, 109.9, 107.8, 54.0, 45.3, 39.5, 30.5, 29.8, 27.3, 25.9, 25.7, 24.4, 18.1; IR (n_{max}) 3457, 1623, 1241, 1011, 915, 842 cm^{-1}; HRMS (ESI/APCI+), calcd for $C_{19}H_{24}NO$ (M – $N(CH_3)_2$) 282.1852, found 282.1860.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5.162
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5.119
5.100
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3.617
3.615
3.601
3.599
3.597
3.594
3.591
3.587
3.533
3.515
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1.846
1.755
1.513
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1.13
2.01
1.76
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3
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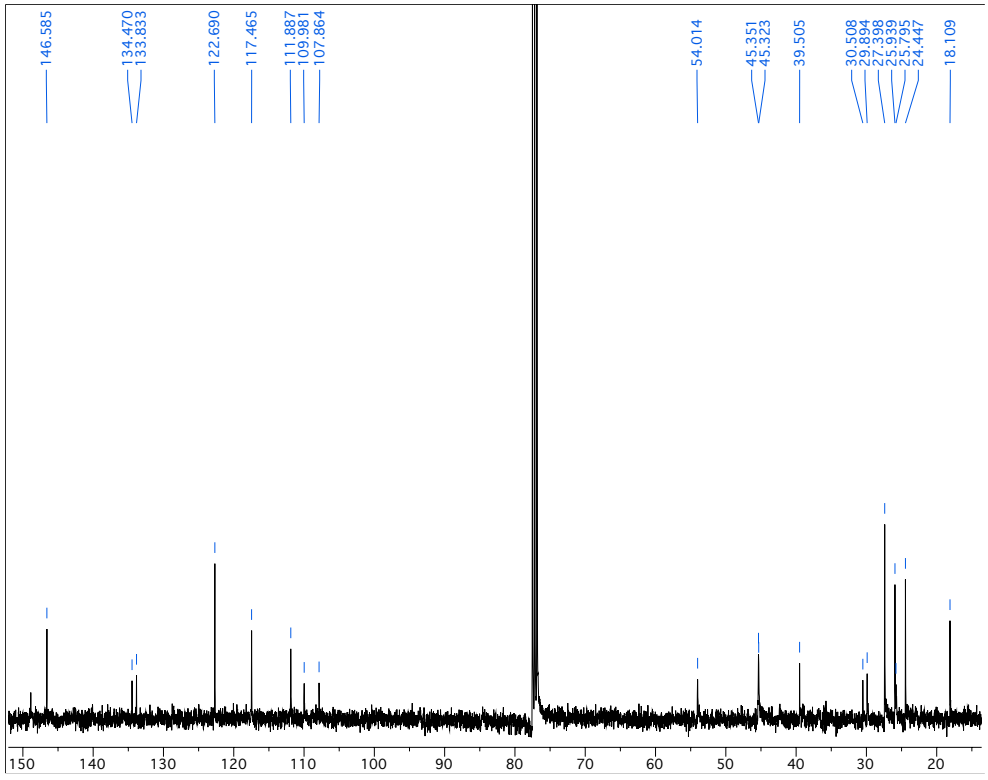
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50
40
30
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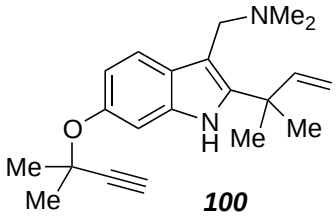
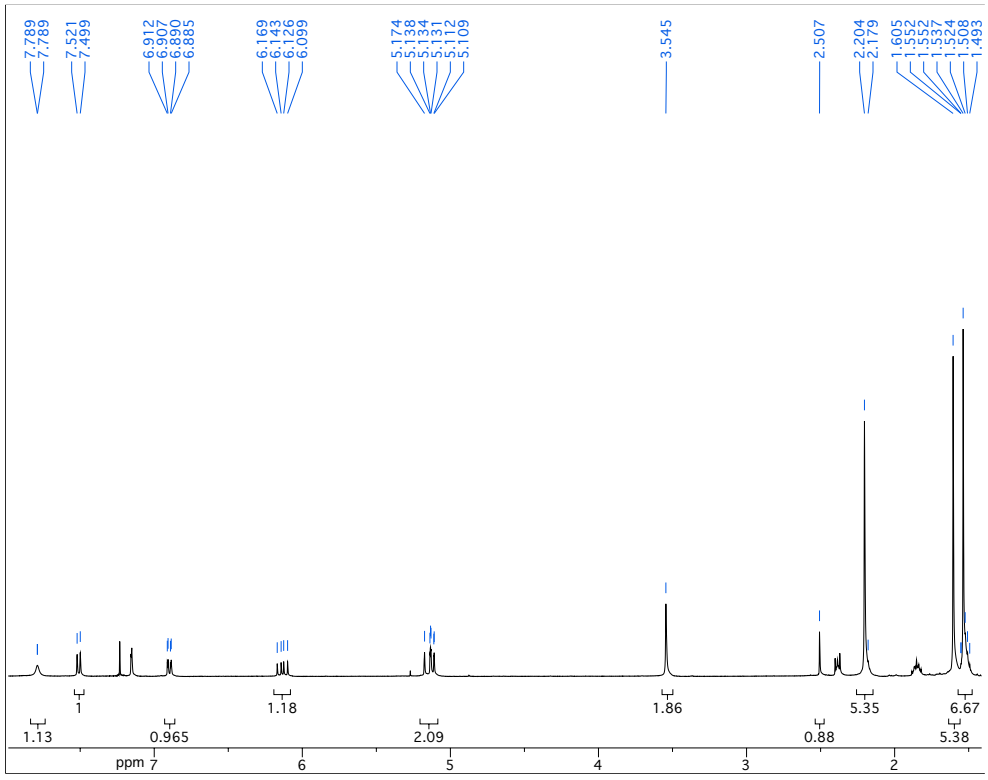


100

***N*,*N*-Dimethyl-1-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)methan- amine (100).** To AcOH (1.5 mL) was added at rt Me_2NH (320 mL, 2.84 mmol, 1.05 eq, 40% aqueous solution) followed by H_2CO (230 mL, 2.84 mmol, 1.05 eq, 37% aqueous solution) and stirred for 30 min. To this was added at rt a solution of **98** (724 mg, 2.70 mmol, 1 eq) in AcOH (1.5 mL) and stirred for 4 h. The reaction was basified with 2M NaOH to a pH of 10–12 and washed with Et_2O (3 x 10 mL). The organic layers were combined and dried over anhydrous $MgSO_4$, and concentrated under reduced pressure, to give **100** (835 mg, 95%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) d 7.78 (bs, 1 H), 7.52 (d, *J* = 8.5 Hz, 1 H), 7.15 (d, *J* = 1.9 Hz, 1 H), 6.90 (dd, *J* = 8.5, 1.9 Hz, 1 H), 6.16 (dd, *J* = 17.5, 10.5, 1 H), 5.17 (d, *J* = 17.5, 1 H), 5.13 (d, *J* = 10.5, 1 H), 3.54 (s, 2 H), 2.50 (s, 1 H), 2.20 (s, 6 H), 1.60 (s, 6 H), 1.53 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 146.4, 140.8, 133.9, 127.0, 119.0, 115.9, 112.0, 103.9, 73.3, 73.0, 54.2, 45.5, 42.1, 39.4, 29.8, 27.3, 25.8; IR (n_{max}) 3301, 1622, 1463, 1231, 1134, 1042, 1012, 968, 914 cm^{-1}; HRMS (ESI/APCI+), calcd for $C_{19}H_{22}NO$ (M – $N(CH_3)_2$) 280.1696, found 280.1695.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1.537
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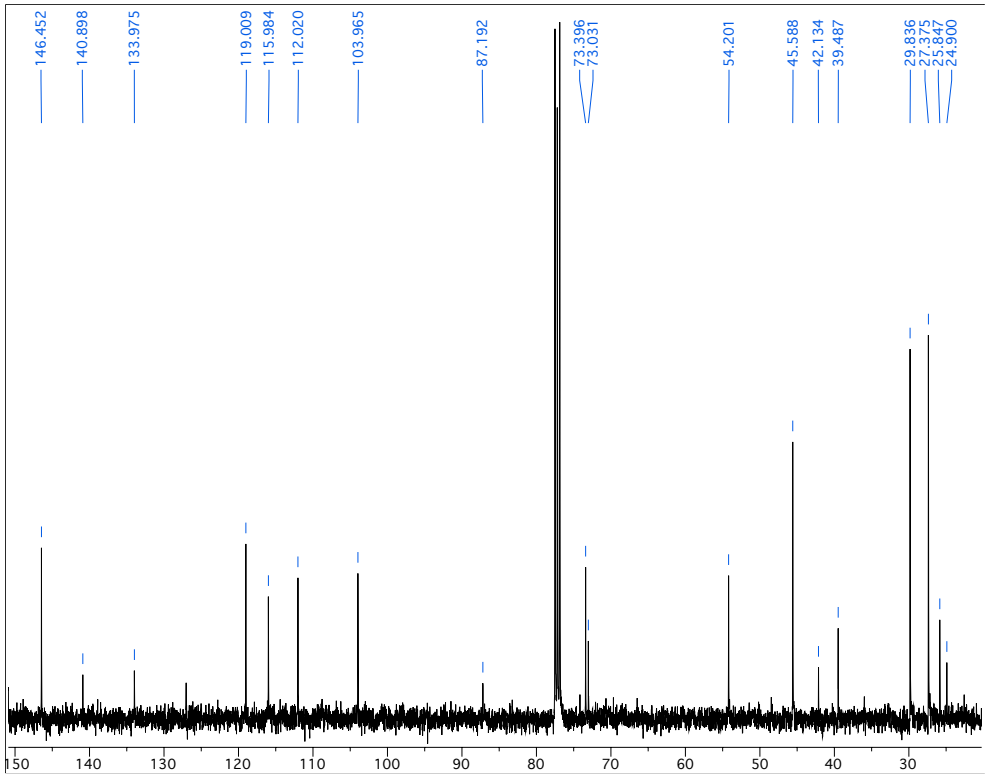
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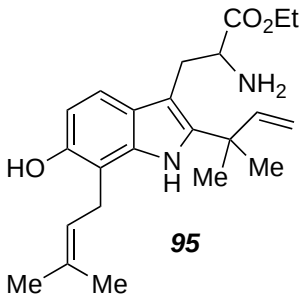
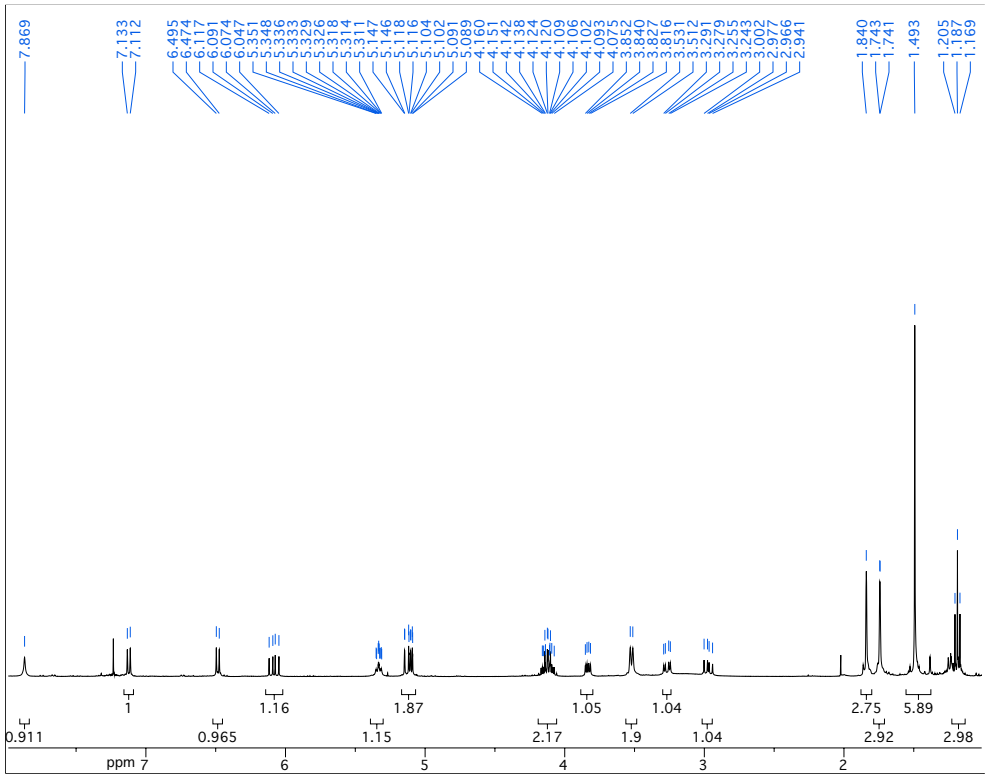


95

Ethyl-2-amino-3-(6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)pro-panoate (95). To a solution of **99** (61 mg, 0.18 mmol, 1 eq) and *N*-(diphenylmethylene)glycine ethyl ester (52 mg, 0.19 mmol, 1.05 eq) in acetonitrile (2 mL) was added at rt Bu_3P (10 mL, 0.074 mmol, 0.4 eq). The reaction was heated under reflux for 24 h. The reaction was cooled to rt and concentrate under reduced pressure. The residue was taken up in THF (3 mL) and 1 M HCl (2 mL) was added and the reaction was stirred at rt for 3 h. The solution was basified with sat. aq. $NaHCO_3$ and washed with AcOEt (3 x 10 mL). The organics were combined and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The yellow residue was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0:1–1:20) to give **95** (7 mg, 10%) as a yellow oil. R_f = 0.36 ($MeOH/CH_2Cl_2$, 1:20); ^1H NMR (400 MHz, $CDCl_3$) d 7.86 (bs, 1 H), 7.13 (d, J = 8.4 Hz, 1 H), 6.49 (d, J = 8.4 Hz, 1 H), 6.11 (dd, J = 17.5, 10.5 Hz, 1 H), 5.33 (t, J = 7.3 Hz, 1 H), 5.14 (dd, J = 17.4, 11.8 Hz, 2 H), 4.16-4.07 (m, 2 H), 3.85-3.81 (m, 1 H), 3.53 (d, J = 7.3 Hz, 2 H), 3.29 (dd, J = 14.4, 4.7 Hz, 2 H), 3.00 (dd, J = 14.4, 4.5 Hz, 2 H), 1.84 (s, 3 H), 1.74 (s, 3 H), 1.49 (s, 6 H), 1.18 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 175.4, 149.2, 146.4, 139.0, 134.9, 133.4, 124.2, 122.9,

116.8, 111.9, 110.1, 108.3, 106.7, 61.1, 55.8, 39.3, 31.2, 28.0, 27.9, 25.9, 24.3, 18.1, 14.2; IR (n_{max}) 3449, 1730, 1621, 1444, 1100, 917, 799 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{23}H_{32}N_2O_3$ (M + H) 385.2486, found 385.2483.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4.106
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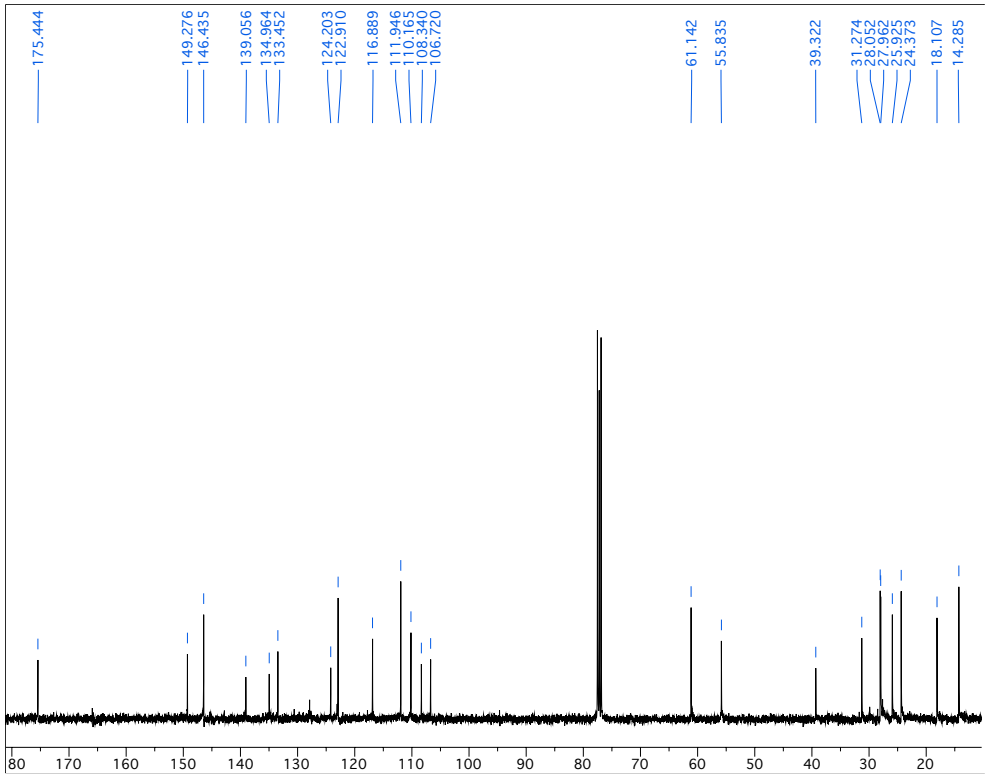
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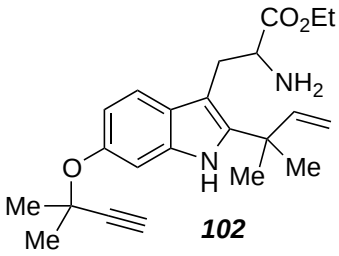
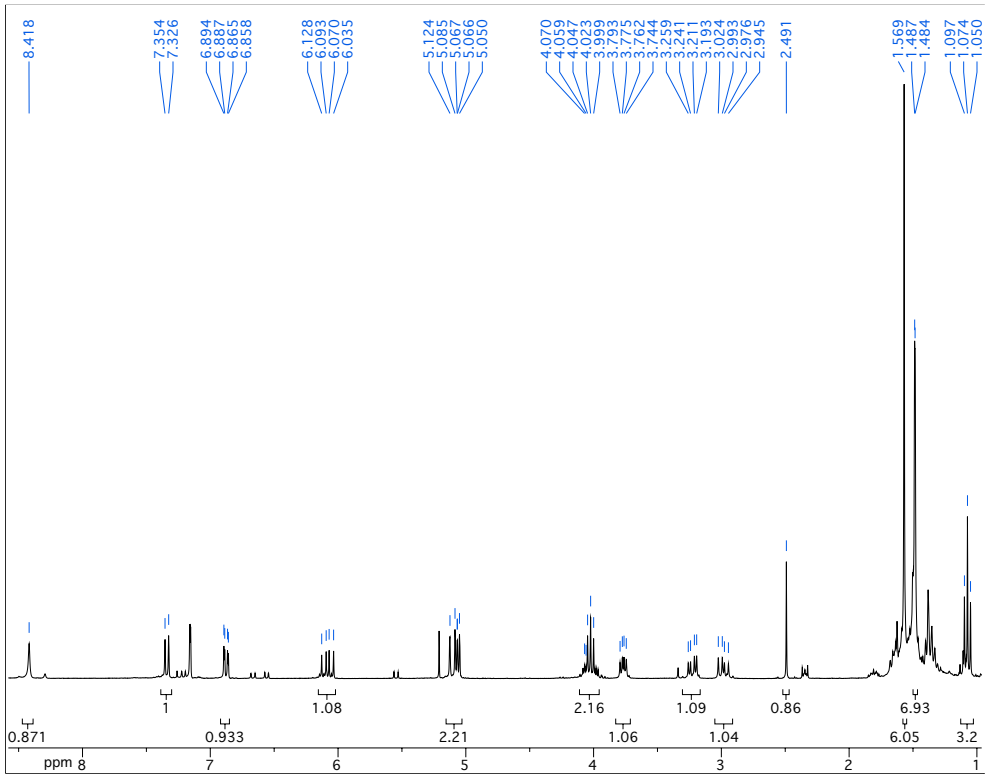


102

Ethyl-2-amino-3-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)propane- ate (102). To a solution of **100** (180 mg, 0.52 mmol, 1 eq) and *N*-(diphenylmethylene)glycine ethyl ester (155 mg, 0.57 mmol, 1.1 eq) in acetonitrile (5 mL) was added at rt Bu_3P (29 mL, 0.21 mmol, 0.4 eq). The reaction was taken to 70ºC and stirred for 24 h. Cool to rt and concentrate under reduced pressure. The residue was then taken up in THF (5 mL) at rt was added 1M HCl (1 mL) and stirred at rt for 1 h. The solution was basified with 2M NaOH and washed with Et_2O (3 x 10 mL). The combined organic layers were washed with Brine (15 mL), dried of over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give crude **102** as a yellow oil. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0:100–1:20) to give **102** (160 mg, 80%) as a light yellow foam. R_f = 0.36 ($MeOH/CH_2Cl_2$, 1:20); ^1H NMR (300 MHz, $CDCl_3$) d 8.41 (bs, 1 H), 7.35 (d, *J* = 8.5 Hz, 1 H), 7.16 (s, 1 H), 6.89 (d, *J* = 8.5 Hz, 1 H), 6.12 (dd, *J* = 17.5, 10.5 Hz, 1 H), 5.12 (d, *J* = 17.5 Hz, 1 H), 5.08 (d, *J* = 10.5 Hz, 1 H), 4.08-3.96 (m, 2 H), 3.79 (q, *J* = 5.3 Hz, 1 H), 3.25 (dd, *J* = 14.4, 5.3 Hz, 1 H), 3.02 (dd, *J* = 14.3, 9.3 Hz, 1 H), 2.49 (s, 1 H), 1.56 (s, 6 H), 1.48 (s, 6 H), 1.07 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) d 175.8, 151.1, 146.2, 140.2, 134.4, 118.5, 116.1, 112.2, 104.3, 87.0, 73.5, 73.1, 61.0, 56.1, 39.3,

31.5, 29.8, 28.0, 24.6, 24.4, 23.9; IR (n_{max}) 3267, 1733, 1622, 1464, 1136, 969 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{23}H_{31}N_2O_3$ (M + H) 383.2329, found 383.2328.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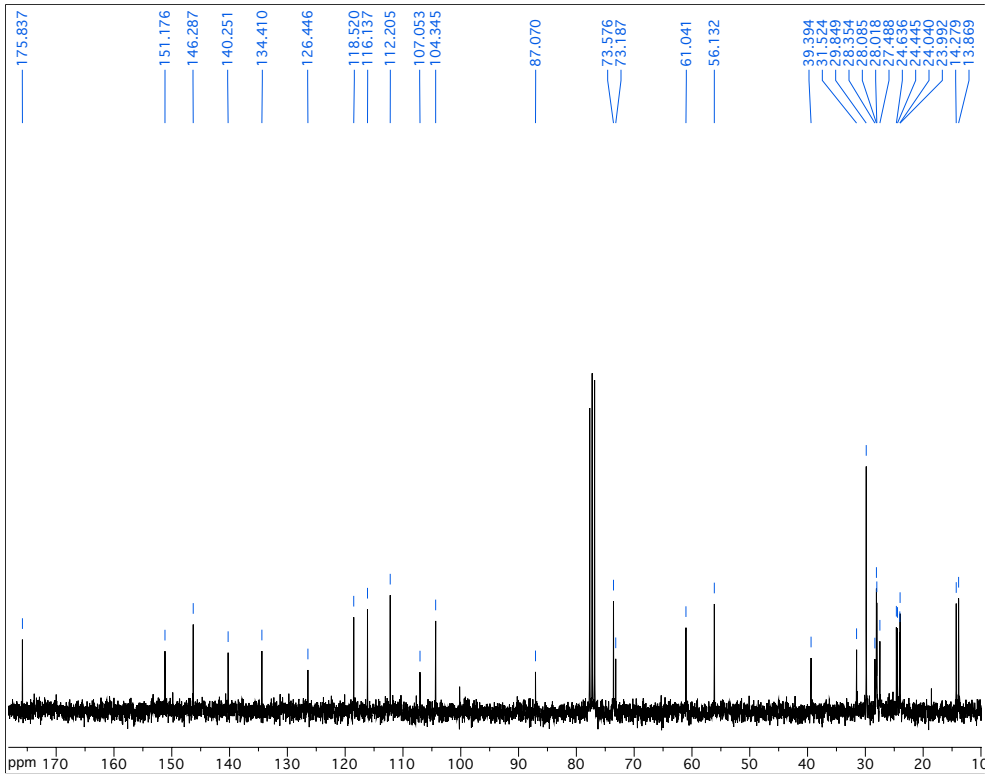
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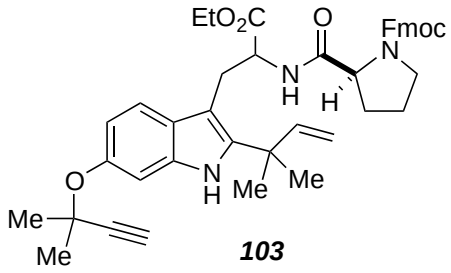
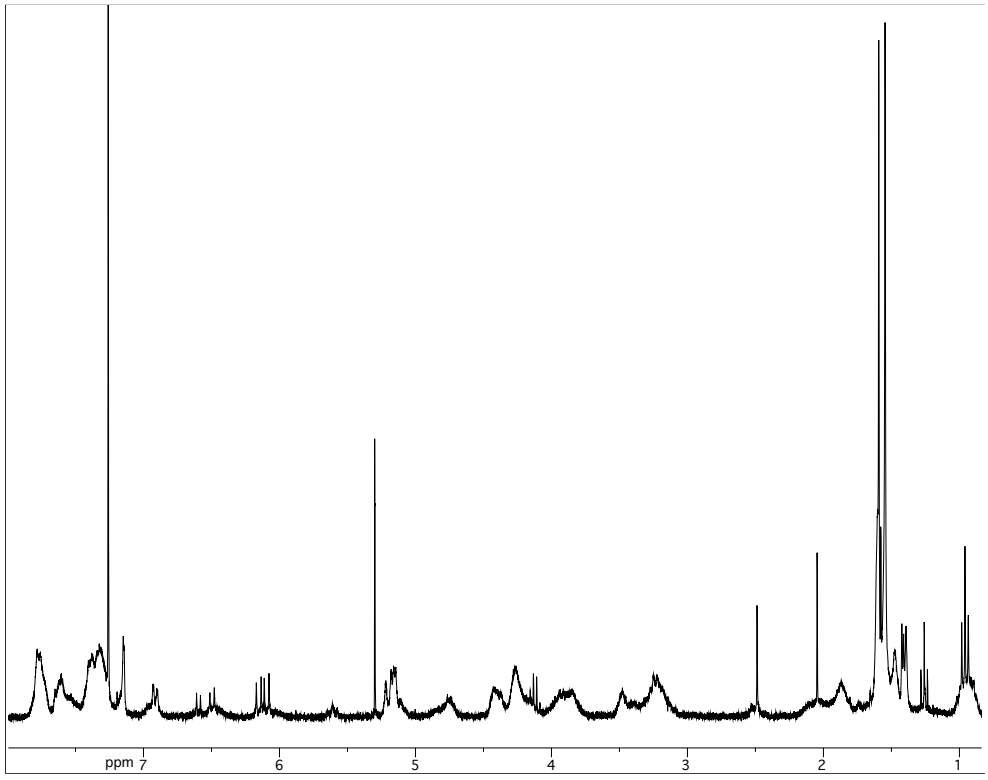


103

(2*S*)-(9*H*-Fluoren-9-yl)methyl-2-((1-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)butan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (103). To a solution of **102** (50 mg, 0.130 mmol, 1 eq) and *N*-(9-fluorenylmethoxycarbonyl)-L-proline (46 mg, 0.137 mmol, 1.05 eq) in acetonitrile (1.5 mL) at 0ºC was added HATU (62 mg, 0.163 mmol, 1.25 eq) followed by *i*-Pr_2NEt (47 mL, 0.287 mmol, 2.2 eq). The reaction was allowed to warm to rt and was stirred for 12 h. The solution was concentrated under reduced pressure and the residue was purified by flash chromatography (AcOEt/hexane, 1:5–9:20) to give dipeptide **103** (77 mg, 85%) as a white foam. R_f = 0.35 (AcOEt/hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotomers, diastereomers) d 7.79-7.77 (comp, 3 H), 7.61-7.58 (comp, 2 H), 7.37-7.26 (comp, 5 H), 7.14 (bs, 1), 6.93-6.89 (comp, 1 H), 6.17-6.07 (comp, 1 H), 5.22-5.14 (comp, 2 H), 4.79-4.70 (comp, 1 H), 4.45-4.33 (comp, 2 H), 4.29-4.15 (comp, 3 H), 4.00-3.77 (comp, 3 H), 3.51-3.33 (comp, 2 H), 3.29-3.08 (comp, 3 H), 2.48 (bs, 1 H) 1.99-1.93 (comp, 2 H), 1.92-1.80 (comp, 2 H), 1.59 (bs, 6 H), 1.54 (bs, 6H), 1.01-0.89 (comp, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$, mixture of diastereomers) d 172.6, 151.1, 151.0, 145.9, 144.2, 144.1, 141.4, 134.3, 127.9, 127.3, 125.4, 120.1, 112.4, 104.5, 87.1, 87.0, 73.6, 73.1, 67.8, 61.4, 60.6, 53.8, 47.3, 39.3, 29.8, 27.7, 21.2, 14.4, 13.9, 13.8; IR (n_{max}) 3346, 1684, 1126, 968, 739 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{43}H_{47}N_3NaO_6$ (M + Na) 724.3357, found 724.3355.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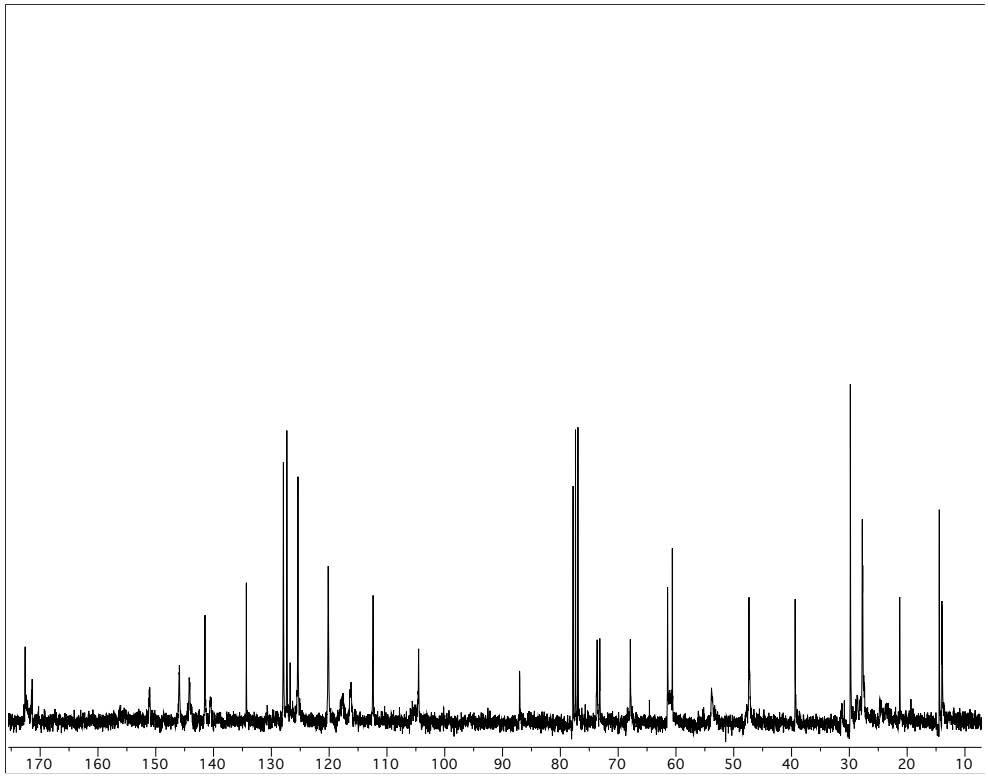
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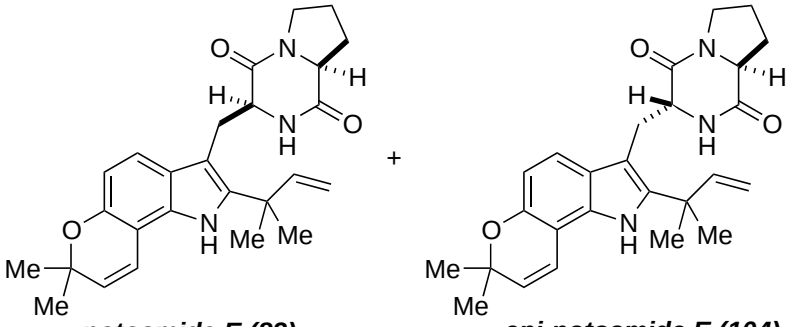
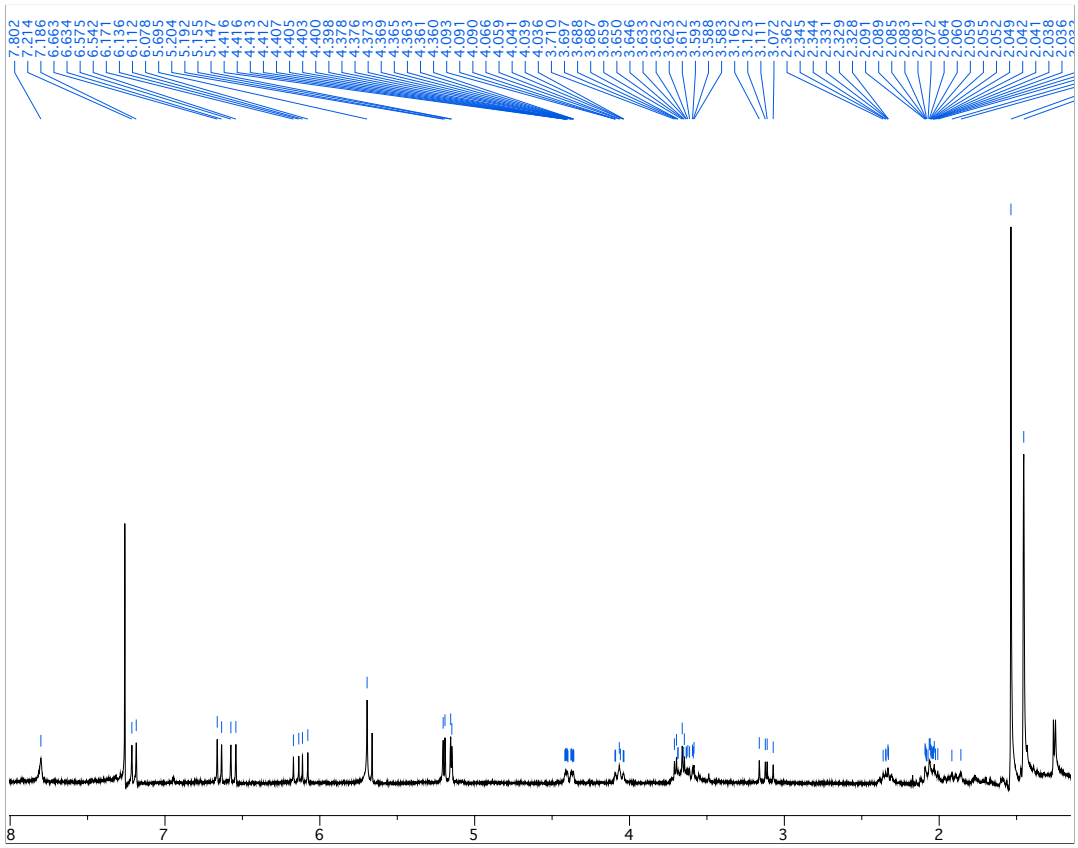


notoamide E (83) *epi-notoamide E (104)*

(3*S*,8a*S*)-3-((7,7-Dimethyl-2-(2-methylbut-3-en-2-yl)-1,7-dihydropyrano[2,3-g]indol-3-yl)methyl)hex-ahydropyrrolo[1,2-a]pyrazine-1,4-dione (83) and (3*R*,8a*S*)-3-((7,7-Dimethyl-2-(2-methylbut-3-en-2-yl)-1,7-dihydropyrano[2,3-g]indol-3-yl)methyl)- hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (104). To **103** (71 mg, 0.10 mmol, 1 eq) in acetonitrile (1.5 mL) at rt was added Et_2NH (700 mL) and the reaction was stirred for 1 h at rt. The reaction was concentrated under reduced pressure to give an orange/brown residue. The residue was dissolved in toluene (3 mL) and heated under reflux for 18 h. The solution was then concentrated under reduced pressure and the residue was purified by flash chromatography (MeOH/CH_2Cl_2, 1:20) to give **83** (15 mg, 36%) & **104** (19 mg, 44%). (**9**) R_f = 0.41 (MeOH/CH_2Cl_2, 1:20); ^{1}H NMR (300 MHz, $CDCl_3$) d 7.91 (s, 1 H), 7.21 (d, *J* = 8.5 Hz, 1 H), 6.65 (d, *J* = 8.5 Hz, 1 H), 6.58 (d, *J* = 9.7 Hz, 1 H), 6.13 (dd, *J* = 17.5, 10.4 Hz, 1 H), 5.70 (bs, 1 H), 5.67 (d, *J* = 9.7 Hz, 1 H), 5.21-5.10 (m, 2 H), 4.44-4.33 (m, 1 H), 4.12-4.00 (m, 1 H), 3.75-3.51 (m, 3 H), 3.12 (dd, *J* = 15.3, 11.7 Hz, 1 H), 2.41-2.27 (m, 1 H), 2.21-1.81 (m, 3 H), 1.54 (s, 6 H), 1.46 (s, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) d 169.5, 166.0, 149.1, 146.0,

140.1, 131.0, 130.2, 123.9, 118.2, 117.0, 112.8, 111.1, 105.2, 105.1, 76.0, 59.4, 55.1, 45.6, 39.2, 28.6, 28.1, 27.6, 27.5, 26.1, 22.8; HRMS (FAB) calcd for $C_{26}H_{32}N_3O_3$ [M + H] 434.2444, found 434.2422.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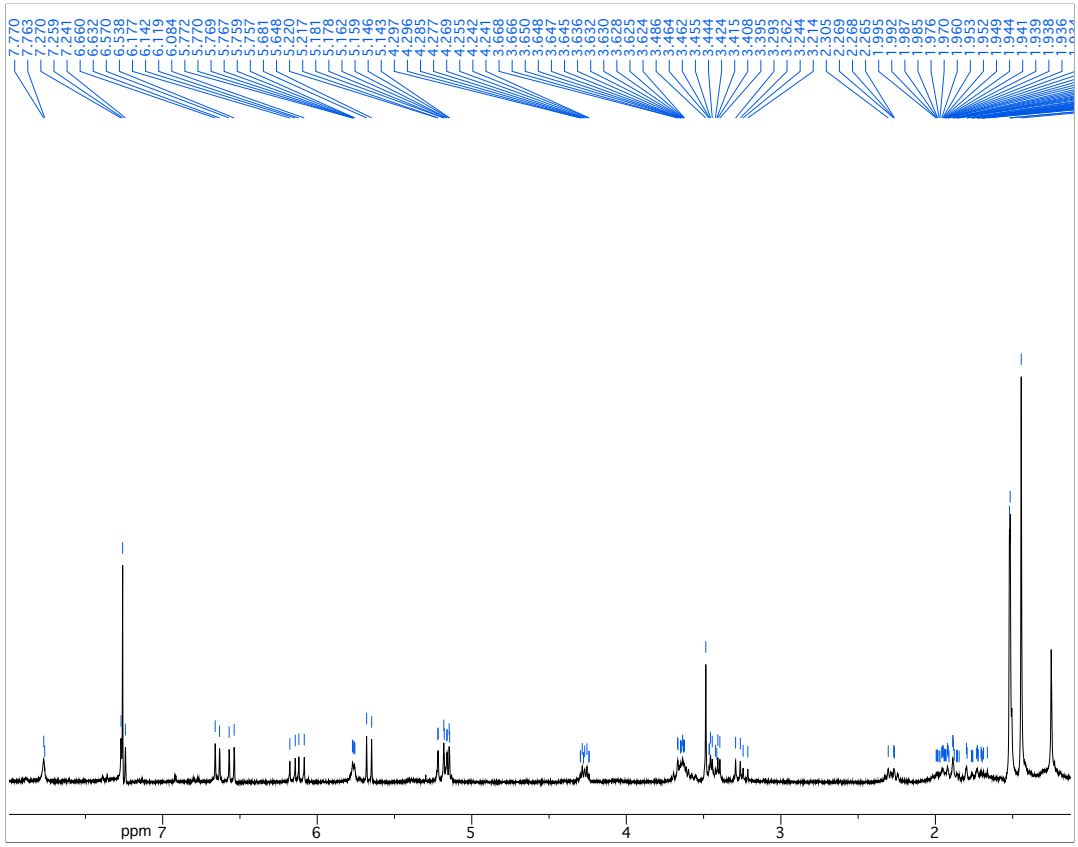
2

(**104**) R_f = 0.29 (MeOH/ CH_2Cl_2, 1:20); ^{1}H NMR (300 MHz, $CDCl_3$) d 7.87 (bs, 1 H), 7.25 (d, *J* = 8.4 Hz, 1 H), 6.64 (d, *J* = 8.4 Hz, 1 H), 6.58 (d, *J* = 9.7 Hz, 1 H), 6.13 (dd, *J* = 17.5, 10.6 Hz, 1 H), 5.84 (d, *J* = 3.7 Hz, 1 H), 5.66 (d, *J* = 9.9, 1 H), 5.19 (d, *J* = 11.7 Hz, 1 H), 5.15 (d, *J* = 4.4 Hz, 1 H), 4.31-4.22 (m, 1 H), 3.76-3.55 (m, 2 H), 3.52-3.35 (m, 2 H), 3.26 (dd, *J* = 14.6, 9.0 Hz, 1 H), 2.37-2.21 (m, 1 H), 2.05-1.62 (m, 4 H), 1.52 (s, 6 H), 1.45 (s, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) d 168.8, 166.0, 148.9, 146.4, 140.2, 130.9, 130.0, 123.7, 118.9, 117.0, 112.0, 110.8, 105.7, 104.8, 75.9, 58.7, 58.4, 45.7, 39.3, 30.0, 29.2, 28.2, 28.0, 27.5, 22.1; HRMS (FAB) calcd for $C_{26}H_{32}N_3O_3$ [M + H] 434.2444, found 434.2422.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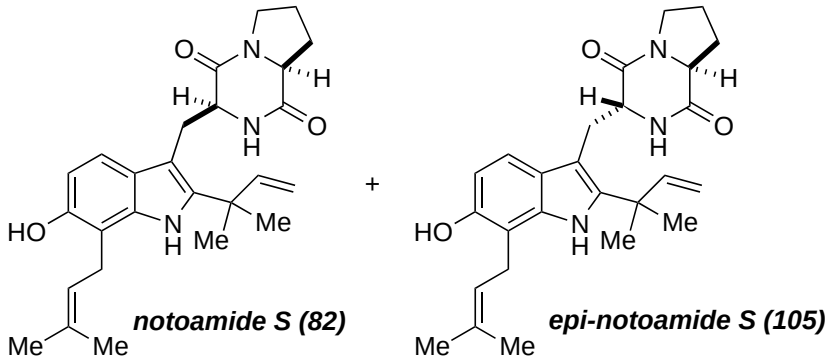
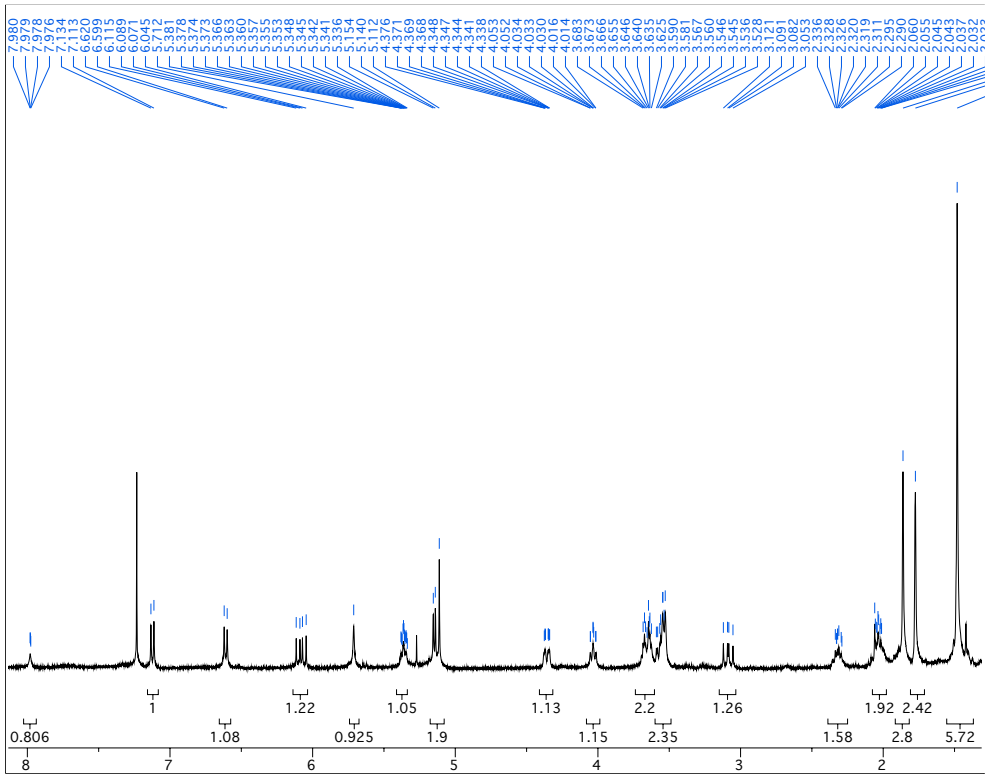
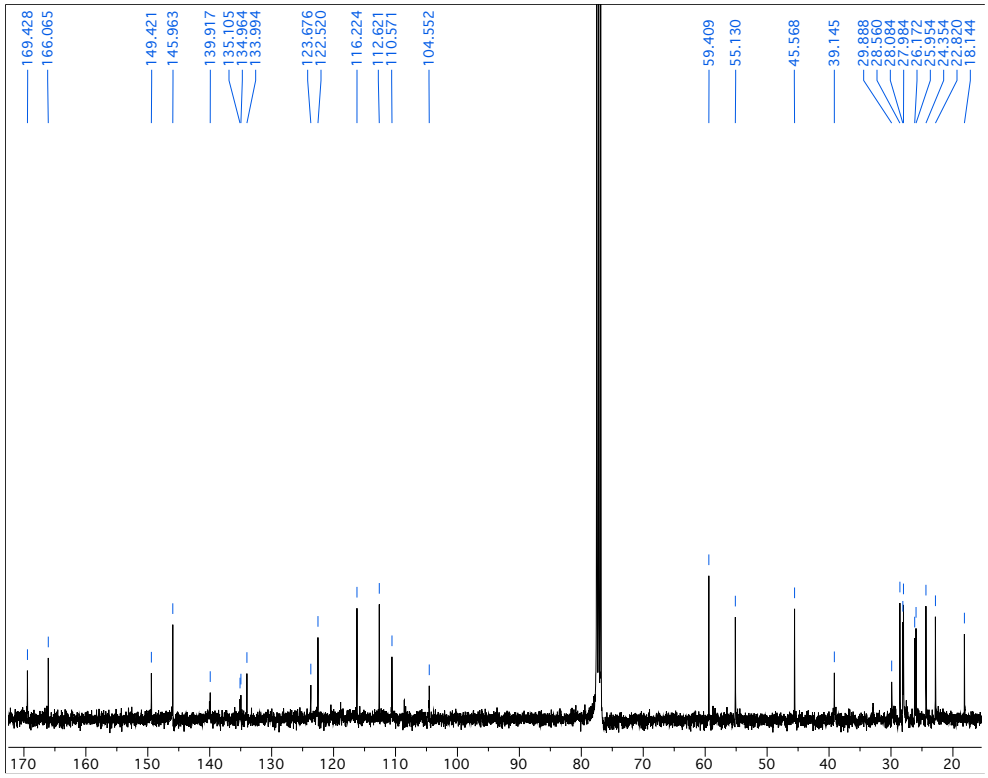


notoamide S (82) ***epi-notoamide S (105)***

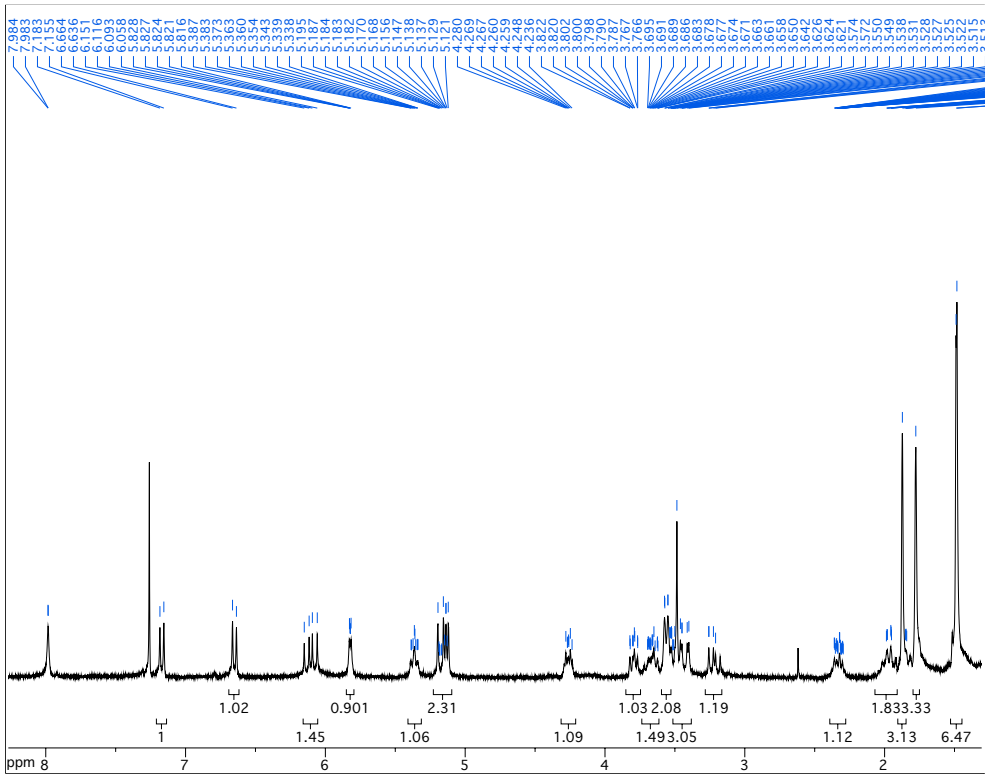
(3*S*,8a*S*)-3-((6-Hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)- hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (82) (Notoamide S) and **(3*R*,8a*S*)-3-((6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (105).** To **103** (286 mg, 0.41 mmol, 1 eq) in MeOH (4 mL) was added at rt Lindlar's catalyst (28 mg, 10 wt %) followed by purging with a balloon of hydrogen. The reaction was vigorously stirred for 30 min. The reaction was monitored by NMR for loss of the alkyne proton. The reaction was then filtered through a plug of Celite and concentrated under reduced pressure to give the alkene as yellow foam which was directly taken on to the next step without further purification. Et_2NH (2 mL) was added to a solution of the crude alkene in acetonitrile (3 mL) and the reaction was stirred for 1 h at rt. The reaction was concentrated under reduced pressure to give a yellow oil. The residue was dissolved in toluene (5 mL) and heated under reflux for 18 h. The reaction was then concentrated under reduced pressure and purified by flash chromatography ($MeOH/CH_2Cl_2$, 1:20) to give **82** (51 mg, 29%), as a yellow oil. R_f = 0.67 ($MeOH/CH_2Cl_2$, 1:10); ^1H NMR (300 MHz,

$CDCl_3$) d 8.01 (bs, 1 H), 7.15 (d, *J* = 8.5 Hz, 1 H), 6.65 (d, J = 8.5 Hz, 1 H), 6.15 (dd, *J* = 17.5, 10.3 Hz, 1 H), 5.75 (bs, 1 H), 5.38 (t, *J* = 7.2 Hz, 1 H), 5.18 (d, *J* = 5.35 Hz, 1 H), 5.13 (s, 1 H), 4.41 (dd, *J* = 10.7, 2.5 Hz, 1 H), 4.08 (t, *J* = 7.3 Hz, 1 H), 3.71-3.61 (m, 2 H), 3.57 (d, *J* = 7.2 Hz, 2 H), 3.15 (dd, *J* = 15.3, 11.6 Hz, 1 H), 2.39-2.31 (m, 1 H), 2.12-2.00 (m, 2 H), 1.88 (s, 3 H), 1.79 (s, 3 H), 1.50 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 169.4, 166.0, 149.4, 145.9, 139.9, 134.9, 133.9, 123.6, 122.5, 116.2, 112.6, 110.5, 104.5, 59.4, 55.1, 45.5, 39.1, 29.8, 28.5, 28.0, 27.9, 25.9, 24.3, 22.8, 18.1; IR (n_{max}) 3362, 1665, 1438, 919, 800 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{26}H_{32}DN_3O_3$ [M + Na] 459.2477, found 459.2475.





(**105**) (35 mg, 20%) R_f = 0.53 (MeOH/CH_2Cl_2, 1:10); ^{1}H NMR (400 MHz, $CDCl_3$) d 7.94 (bs, 1 H), 7.15 (d, *J* = 8.4 Hz, 1 H), 6.63 (d, *J* = 8.4 Hz, 1 H), 6.11 (dd, *J* = 17.4, 10.3 Hz, 1 H), 5.83 (bs, 1 H), 5.76 (bs, 1 H), 5.32 (t, *J* = 7.4 Hz, 1 H), 5.15 (dd, *J* = 17.4, 10.3 Hz, 2 H), 4.24-4.21 (m, 2 H), 3.78-3.74 (m, 1 H), 3.68-3.61 (m, 1 H), 3.53 (d, *J* = 7.4 Hz, 2 H), 3.48-3.37 (m, 3 H), 3.22 (dd, *J* = 14.4, 10 Hz, 1 H), 2.32-2.27 (m, 1 H), 1.99-1.86 (m, 2 H), 1.84 (s, 3 H), 1.74 (s, 3 H), 1.45 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 168.7, 166.0, 149.4, 146.3, 139.9, 135.0, 133.6, 123.3, 122.7, 116.7, 111.8, 110.3, 108.3, 105.0, 58.9, 58.4, 45.7, 39.2, 30.1, 29.2, 28.0, 27.8, 25.9, 24.3, 22.1, 18.1; IR (n_{max}) 3336, 1655, 1448, 918, 802 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{26}H_{33}N_3NaO_3$ [M + Na] 458.2414, found 458,2413.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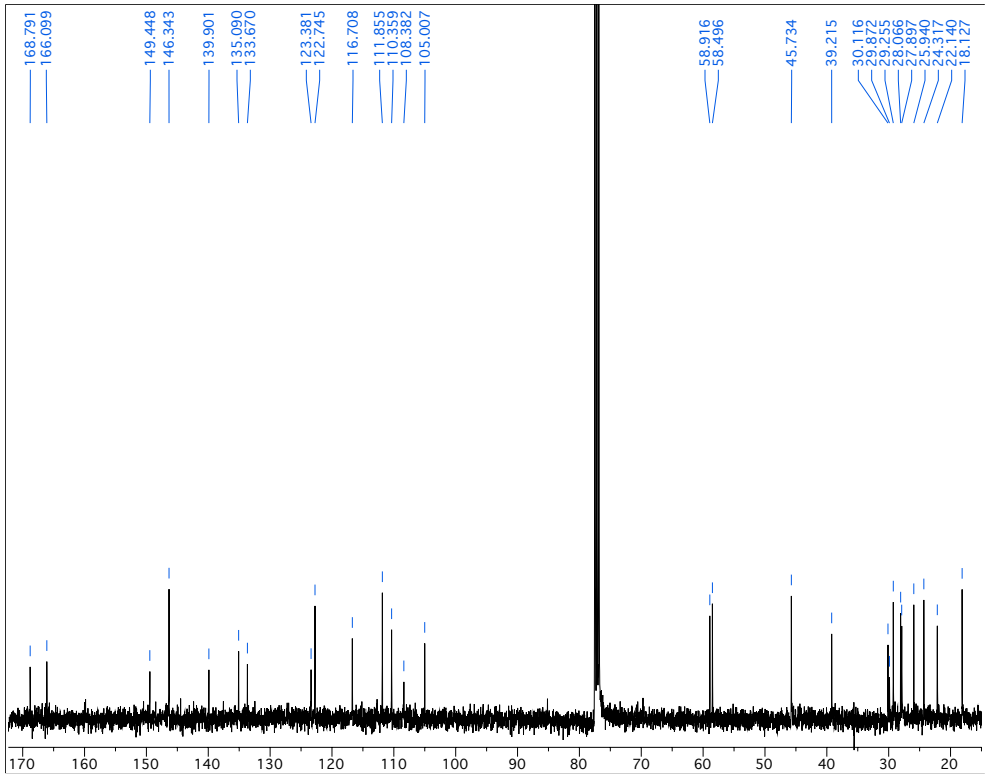
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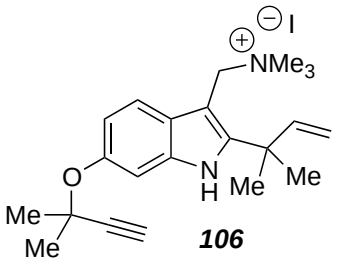
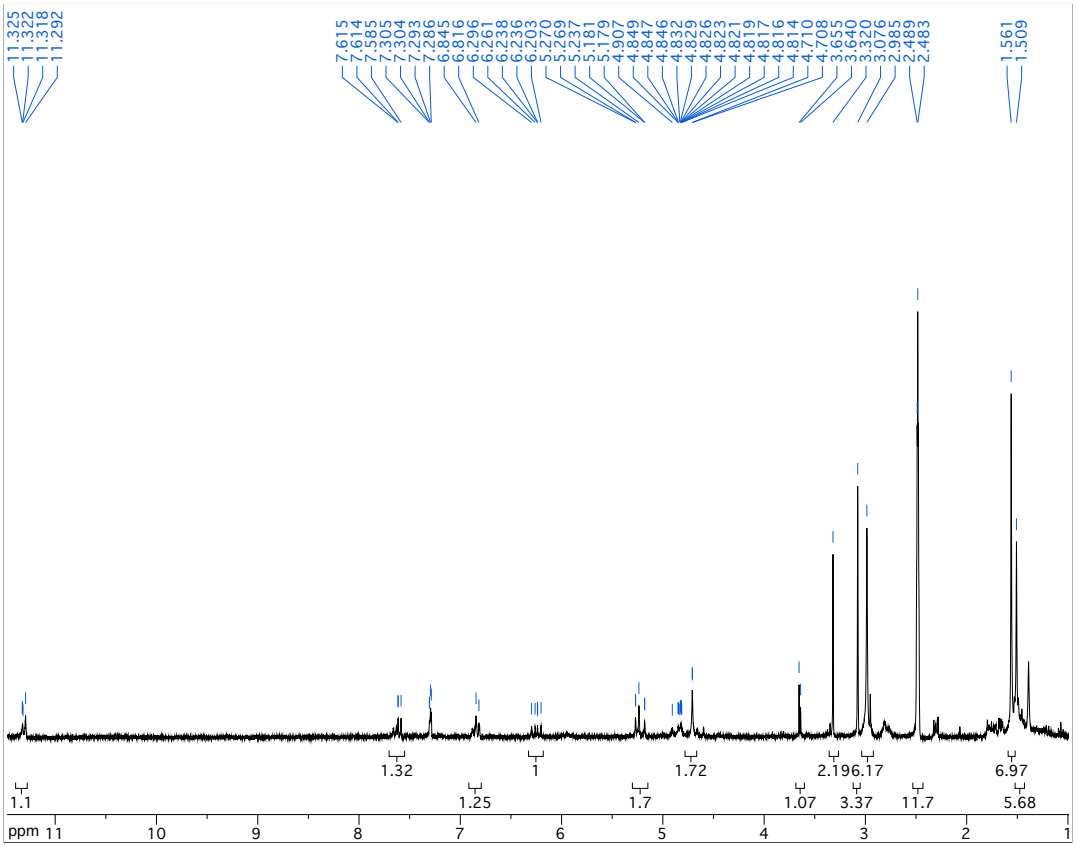


106

***N*,*N*,*N*-trimethyl-1-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)methanaminium iodide (106).** To a solution of MeI (9.5 mL, 147.93 mmol, 40 eq) was added a solution of **100** (1.2 g, 3.69 mmol, 1 eq) in Et_2O (48 mL) over a period of 2 h. The reaction continued to stir for an additional 3 h at which time it was concentrated under reduced pressure. The crude yellow white solid was used in the proceeding step as a mixture of iodide salt and dimmer. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.32 (bs, 1 H), 7.58 (d, *J* = 8.8 Hz, 1 H), 7.29 (s, 1 H), 6.84 (t, *J* = 8.8 Hz, 1 H), 6.20 (dd, *J* = 10.4, 17.4 Hz, 1 H), 5.18 (dd, *J* = 10.4, 17.4 Hz, 2 H), 4.70 (s, 2 H), 3.64 (s, 1 H), 3.32 (s, 2 H), 3.07 (s, 3 H), 2.98 (s, 6 H), 1.56 (s, 6 H), 1.50 (s, 6 H).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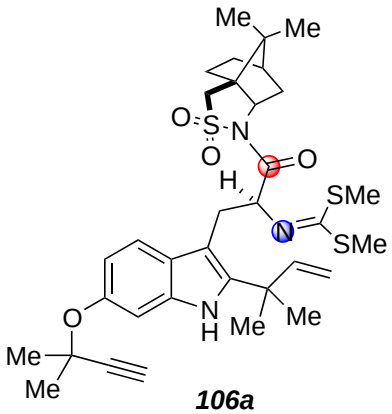
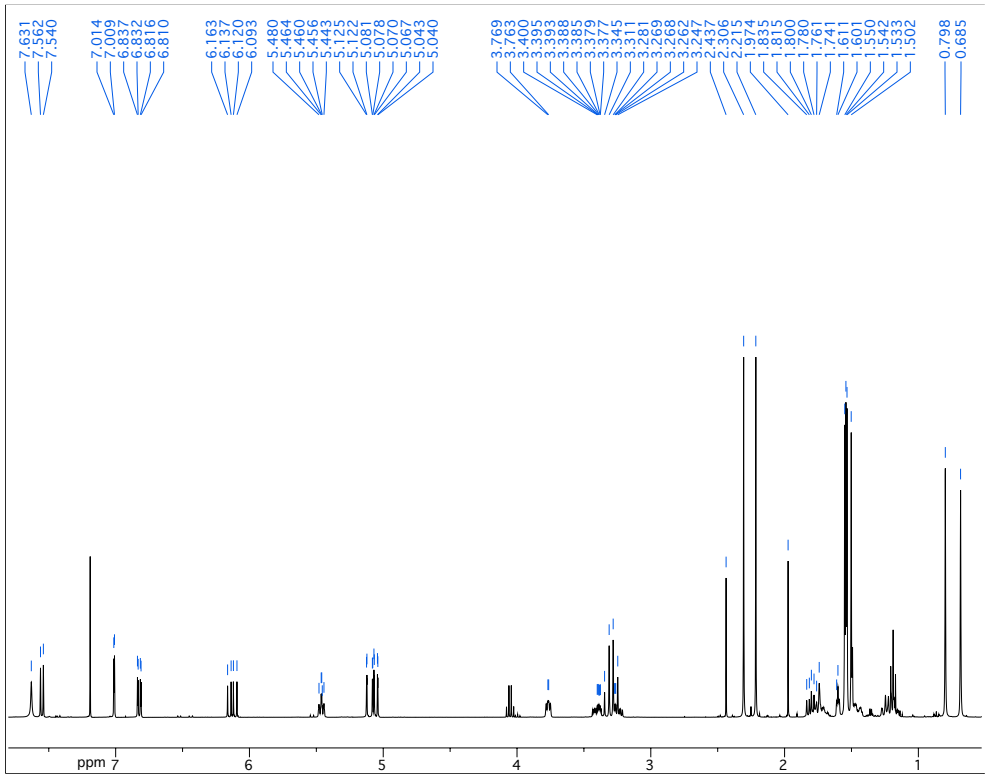


106a

dimethyl ((2*S*)-1-((3a*S*,6*R*)-8,8-dimethyl-2,2-dioxidohexahydro-1*H*-3a,6-methanobenzo[*c*]isothiazol-1-yl)-3-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)-1-oxopropan-2-yl)carbonimidodithioate (106a). To a solution of **107** (1.1 g, 2.90 mmol, 1.1 eq) in THF (10 mL) was added LDA (310 mg, 2.90 mmol, 1.1 eq) at -78ºC. The reaction was allowed to stir at -78ºC for 1 h. In a separate flask a solution of **106** (1.23 g, 2.63 mmol, 1 eq) in THF (8 mL) was cooled to -78ºC, at which time the solution of **107** was canulated at -78ºC. The mixture was allowed to warm to room temperature where in stirred for 5 h. Sat. NH_4Cl (20 mL) was added and the mixture was partitioned in to CH_2Cl_2 (30 mL). The organic layer was washed with H_2O (3 x 15 mL), brine (15 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded the crude residue. Purification by flash chromatography (EtOAc / hex., 1 : 5) to provide **106a** (738 mg, 68%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (bs, 1 H), 7.60 (d, *J* = 8.6 Hz, 1 H), 7.07 (d, *J* = 2.1 Hz, 1 H), 6.87 (dd, *J* = 2.1, 8.6 Hz, 1 H), 6.14 (dd, *J* = 10.1, 17.0 Hz, 1 H), 5.51 (t, *J* = 7.3 Hz, 1 H),

5.13 (dd, *J* = 10.1, 17.0 Hz, 2 H), 4.67 (m, 1 H), 4.03 (m, 1 H), 3.85-3.80 (m, 1 H), 3.46 (m, 1 H), 3.35 (d, *J* = 7.3 Hz, 2 H), 3.28-3.26 (m, 1 H), 3.10 (d, *J* = 2.9 Hz, 1 H), 2.55 (s, 1 H), 2.50 (s, 1 H), 2.37 (s, 3 H), 2.28 (s, 3 H), 1.93-1.80 (m, 6 H), 1.61 (s, 3 H), 1.60 (s, 6 H), 1.56 (s, 3 H), 1.12 (s, 3H), 0.93 (s, 3 H), 0.86 (s, 3 H), 0.74 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 150.6, 146.5, 140.3, 134.1, 126.4, 119.8, 115.6, 111.4, 105.4, 103.5, 87.0, 73.0, 72.8, 66.9, 66.4, 65.2, 53.1, 48.1, 47.4, 44.6, 39.5, 38.1, 32.8, 29.8, 29.6, 29.5, 27.6, 27.5, 26.3, 20.3, 19.7, 15.7, 14.8, 14.7, 14.1; HRMS (ESI/APCI+) calcd for $C_{33}(^{13}C)H_{46}N_2(^{15}N)O_4S_2$ [M + H] 658.2649, found 658.2655.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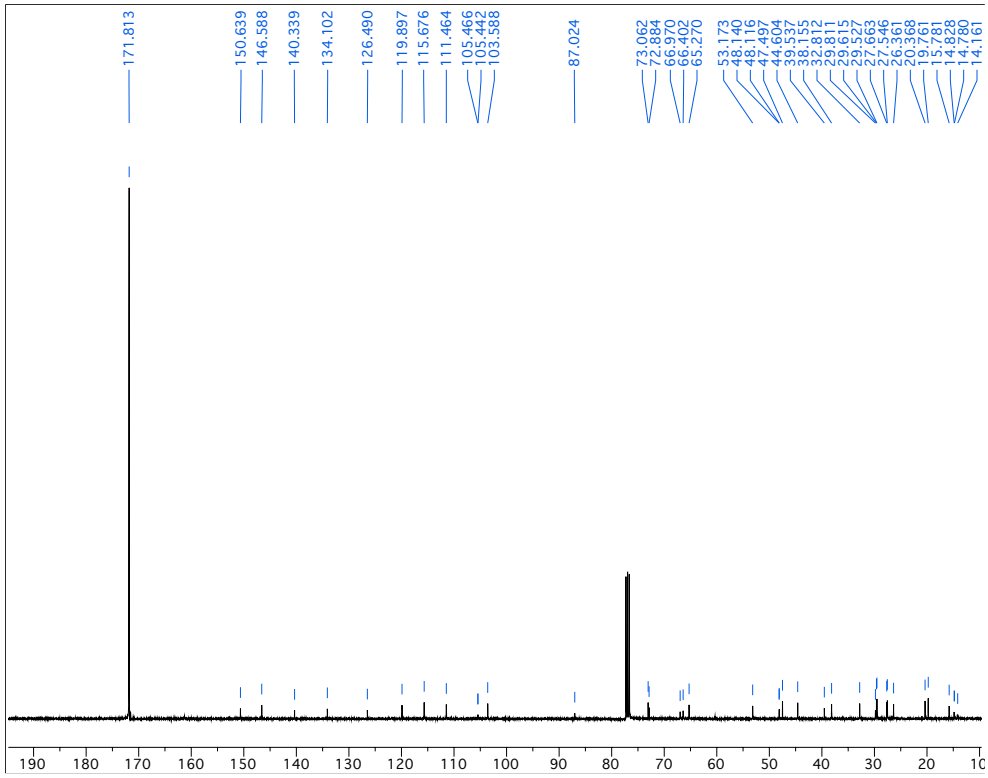
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20.368
19.761
15.781
14.828
14.780
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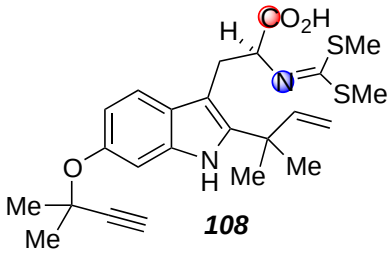
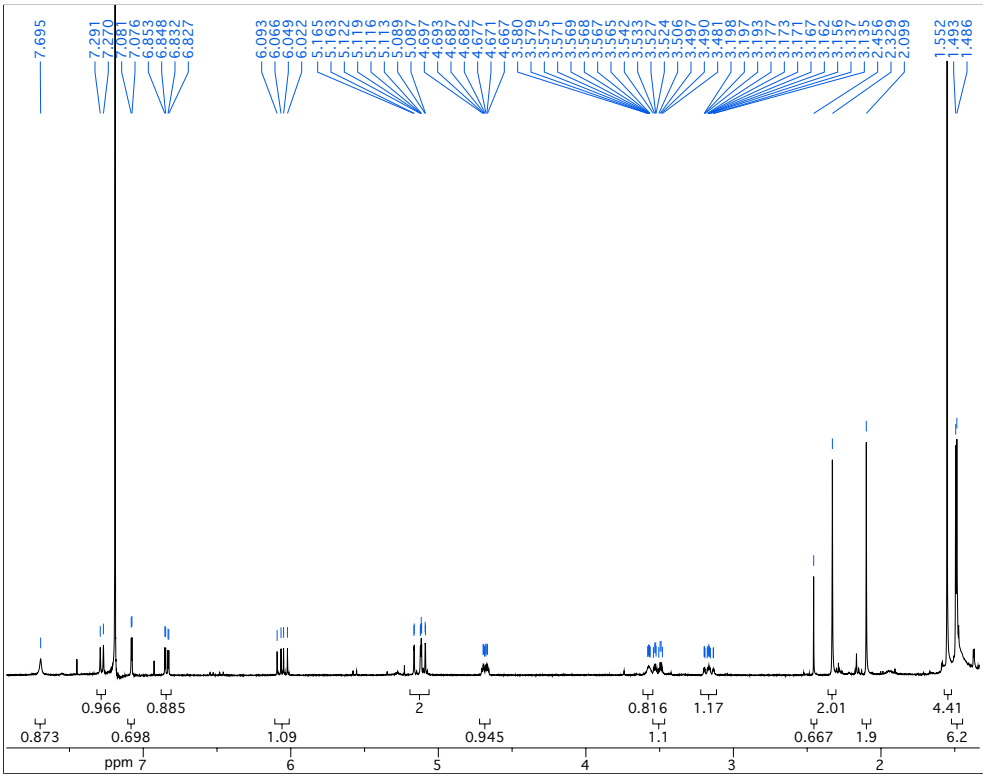


108

(*S*)-2-((bis(methylthio)methylene)amino)-3-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)propanoic acid (108). To a solution of **106a** (1.2 g, 1.82 mmol, 1 eq) in THF (12 mL) and H_2O (6 mL) at 0ºC was added solid LiOH (218 mg, 9.11 mmol, 5 eq). The reaction mixture was warmed to room temperature where it remained stirring for 24 h. The THF was removed under reduced pressure and the remaining aqueous solution was acidified with 1 M HCl (5 mL) to a pH of 2. The solution was extracted with CH_2Cl_2 (3 x 10 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided crude **108**, which was purified by flash chromatography (MeOH / CH_2Cl_2, 1 : 30) to afford **108** (752 mg, 90%) as a white solid. The sultam can also be recovered during flash chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (bs, 1 H), 7.27 (d, *J* = 8.5 Hz, 1 H), 7.07 (d, *J* = 2.0 Hz, 1 H), 6.83 (dd, *J* = 2.0, 8.5 Hz, 1 H), 6.02 (dd, *J* = 10.6, 17.5 Hz, 1 H), 5.08 (dd, *J* = 10.6, 17.5 Hz, 2 H), 4.67 (m, 1 H), 3.56 (m, 1 H), 3.49 (dq, *J* = 3.6, 14.6 Hz, 1 H), 3.17 (m, 1 H), 2.45 (s, 1 H), 2.32 (s, 3 H), 2.09 (s, 3 H), 1.55 (s, 6 H), 1.49 (s, 3 H), 1.48 (s, 3 H).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3.137
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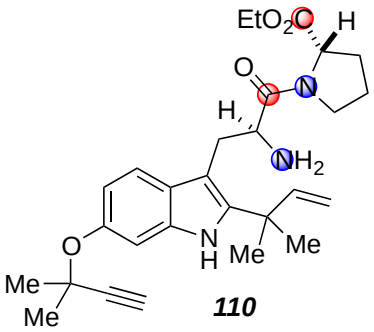
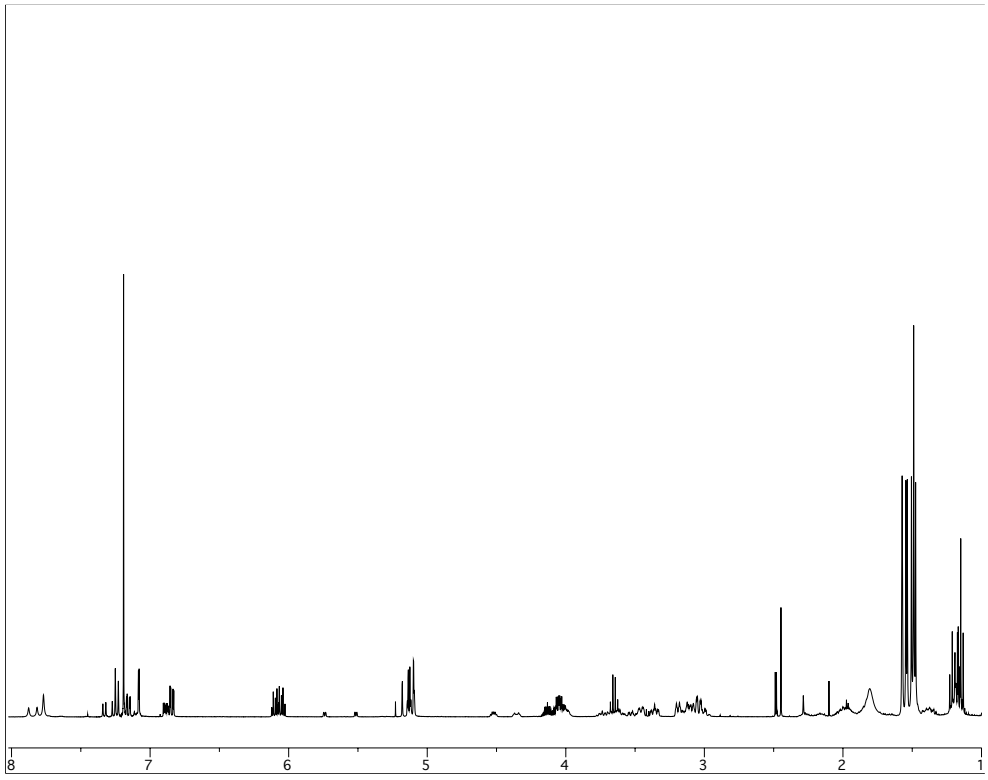


110

(*S*)-ethyl 1-((*S*)-2-amino-3-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)propanoyl)pyrrolidine-2-carboxylate (110). To a solution of **108** (300 mg, 0.65 mmol, 1 eq) and **109** (125 mg, 0.68 mmol, 1.05 eq) in CH_3CN (10 mL) was added consecutively HATU (310 mg, 0.81 mmol, 1.25 eq) and *i*-Pr_2NEt (240 µL, 1.43 mmol, 2.2 eq) at 0ºC. The reaction was warmed to room temperature and allowed to stir for 24 h. 1 M HCl (5 mL) was added to the reaction mixture and stirring continued for 3 h. The mixture was concentrated under reduced pressure and purified by flash chromatography (MeOH / CH_2Cl_2, 1 : 20) to provide **110** (218 mg, 70%) as a white solid. NMR is a mixture of four products: free amine and DKP of the alkyne, as well as free amine and DKP of the pyran ring. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33-8.31 (comp., 2 H), 8.10-8.05 (comp., 1 H), 7.25-7.23 (comp., 2 H), 6.93-6.91 (comp., 1 H), 6.18-6.08 (comp., 1 H), 5.28-5.17 (comp., 2 H), 4.34-4.28 (comp. 1 H), 4.17-4.00 (comp., 2 H), 3.77-3.70 (comp., 1 H), 3.47-3.38 (comp., 2 H), 3.14-3.09 (comp., 2 H), 2.55 (bs, 1 H), 1.54 (bs, 12 H); HRMS (ESI/APCI+) calcd for $C_{26}(^{13}C)_2H_{38}N(^{15}N)_2O_4$ [M + H] 484.2865, found 484.2877.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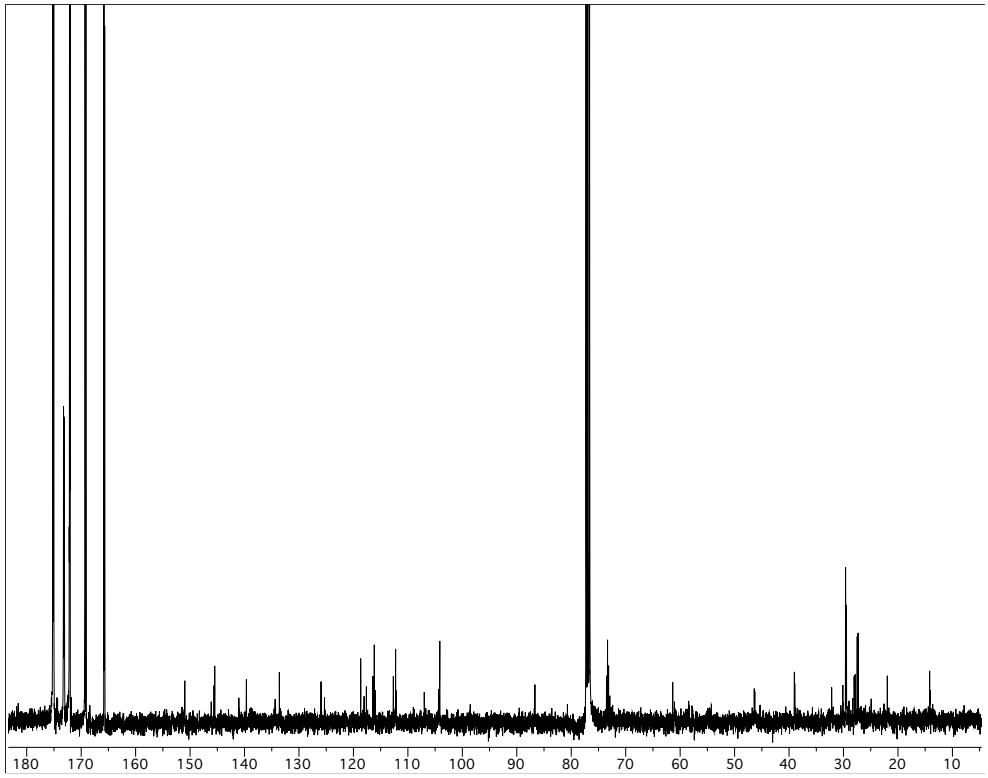
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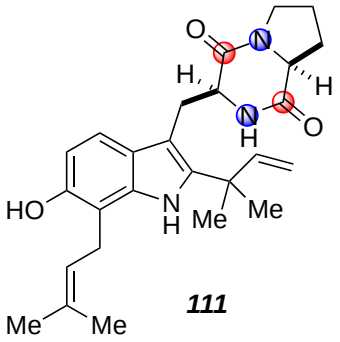
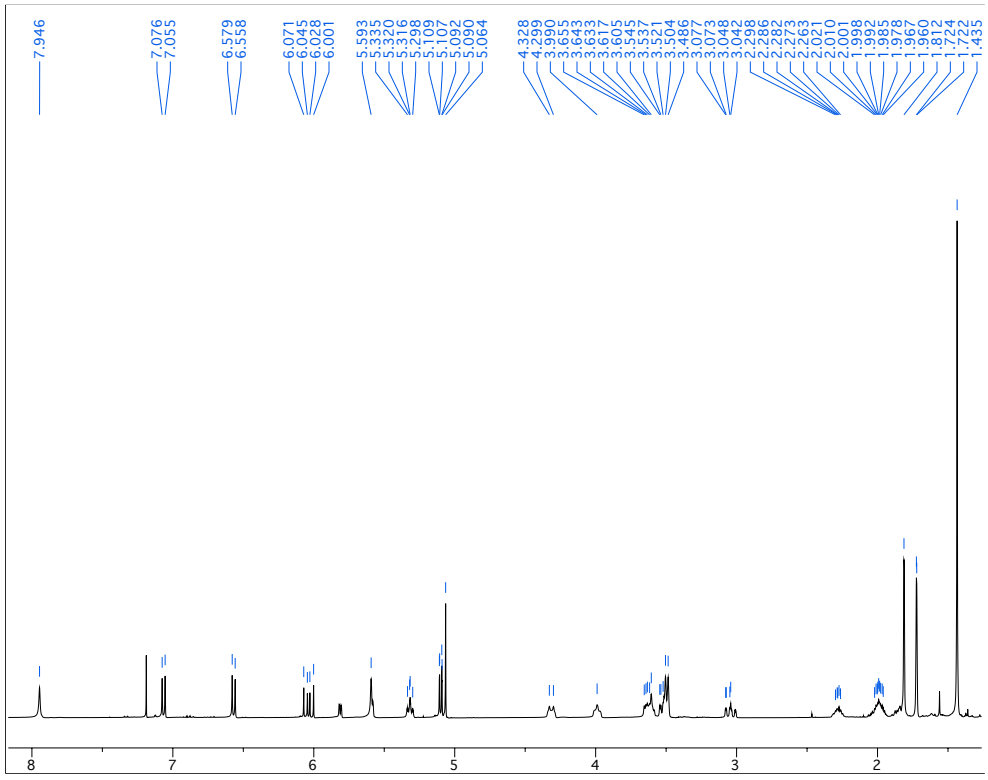


111

(3*S*,8a*S*)-3-((6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (111). To a solution of **110** (183 mg, 0.37 mmol, 1 eq) in MeOH (5 mL) was added Lindlar's catalyst (18 mg, 10 weight %) followed by a balloon of H_2. The reaction was vigorously stirred for 1 h. Filtration through a pad of Celite followed by concentration under reduced pressure provided the crude alkene. The alkene was diluted with CH_3CN (5 mL) and heated to reflux for 18 h. Concentration under reduced pressure and purification by flash chromatography (MeOH / CH_2Cl_2, 1 : 20) provided **111** (112 mg, 68%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (bs, 1 H), 7.11 (d, *J* = 8.3 Hz, 1 H), 6.62 (d, *J* = 8.3 Hz, 1 H), 6.05 (dd, *J* = 10.5, 17.6 Hz, 1 H), 5.38 (t, *J* = 7.4 Hz, 1 H), 5.12 (dd, *J* = 10.5, 17.6 Hz, 2 H), 4.40-4.35 (m, 1 H), 4.09-4.03 (m, 1 H), 3.73-3.61 (m, 2 H), 3.55 (d, *J* = 7.4 Hz, 2 H), 3.10 (t, *J* = 12.8 Hz, 1 H), 2.36-2.32 (m, 1 H), 2.06-2.02 (m, 2 H), 1.88 (s, 3 H), 1.78 (s, 3 H), 1.50 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 169.1, 165.9, 165.7, 149.3, 145.7, 139.7, 134.9, 133.4, 123.3, 122.4, 115.9, 112.3, 110.3, 108.4, 104.2, 45.3,

45.2, 38.9, 28.3, 27.8, 27.7, 25.9, 25.7, 24.0, 22.5, 17.9; HRMS (ESI/APCI+) calcd for $C_{24}(^{13}C)_2H_{33}NaN(^{15}N)_2O_3$ [M + Na] 462.2422, found 462.2428.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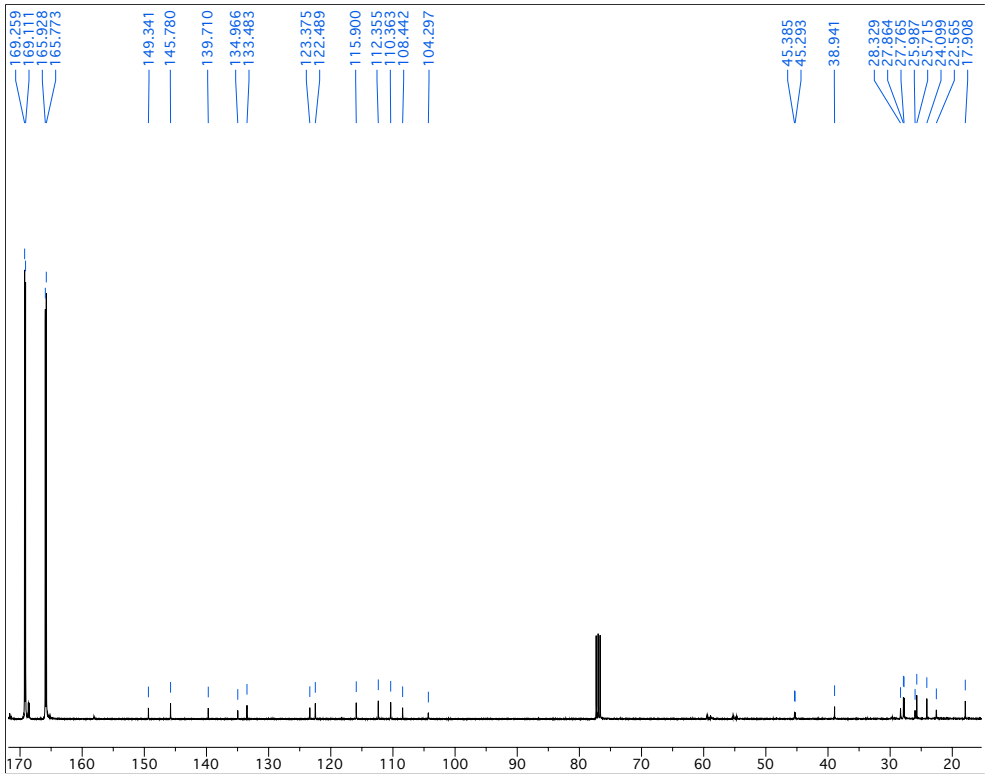
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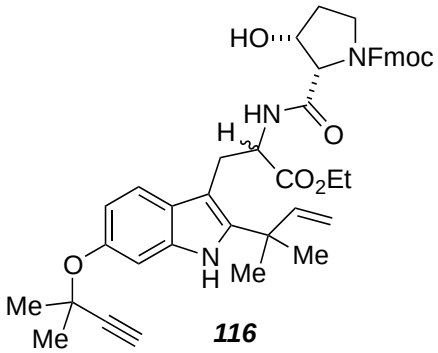
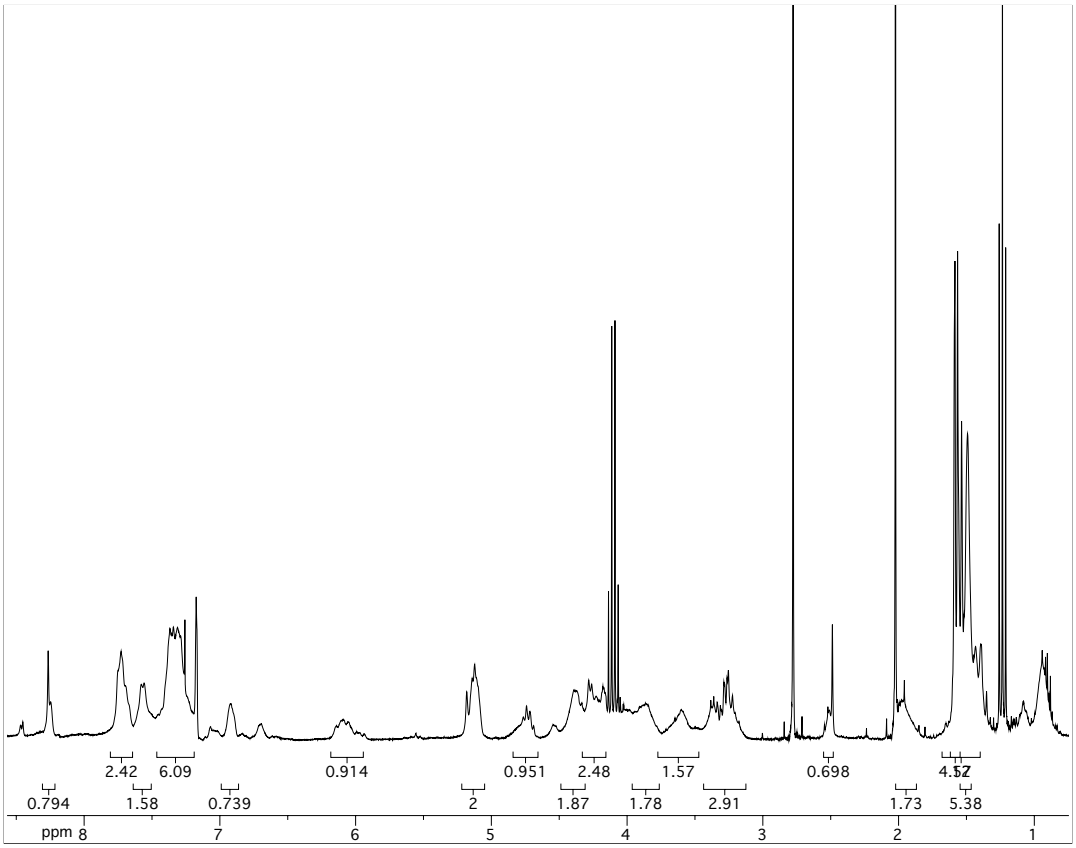


116

(2*S*,3*R*)-(9*H*-fluoren-9-yl)methyl 2-((1-ethoxy-3-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-3-hydroxypyrrolidine-1-carboxylate (116). To a solution of **102** (1.0 g, 2.61 mmol, 1 eq) and **115** (970 mg, 2.74 mmol, 1.05 eq) in CH_3CN (25 mL) was added consecutively HATU (1.25 g, 3.26 mmol, 1.25 eq) and *i*-Pr_2NEt (940 µL, 5.75 mmol, 2.2 eq) at 0ºC. The reaction was warmed to room temperature and allowed to stir for 24 h. The mixture was concentrated under reduced pressure and purified by flash chromatography (EtOAc / hex., 1 : 5–2 : 5) to provide **116** (1.08 g, 58%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (bs, 1H), 7.74-7.62 (comp., 3 H), 7.57-7.55 (comp, 1 H), 7.36-7.17 (comp., 6 H), 6.92-6.89 (comp., 1 H), 6.14-5.93 (comp., 1 H), 5.18-5.09 (comp., 2 H), 4.82-4.68 (comp., 1 H), 4.39-4.31 (comp., 1 H), 4.28-4.16 (comp. 3 H), 3.91-3.84 (comp., 2 H), 3.64-3.56 (comp., 2 H), 3.38-3.20 (comp., 3 H), 2.48 (s, 1 H), 2.01-1.94 (comp., 1 H), 1.53-1.35 (comp., 12 H).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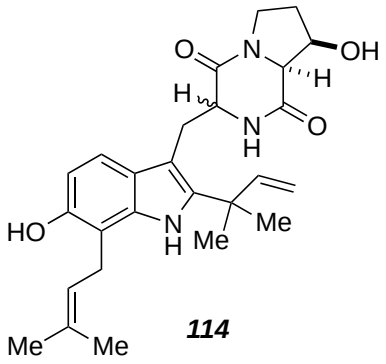
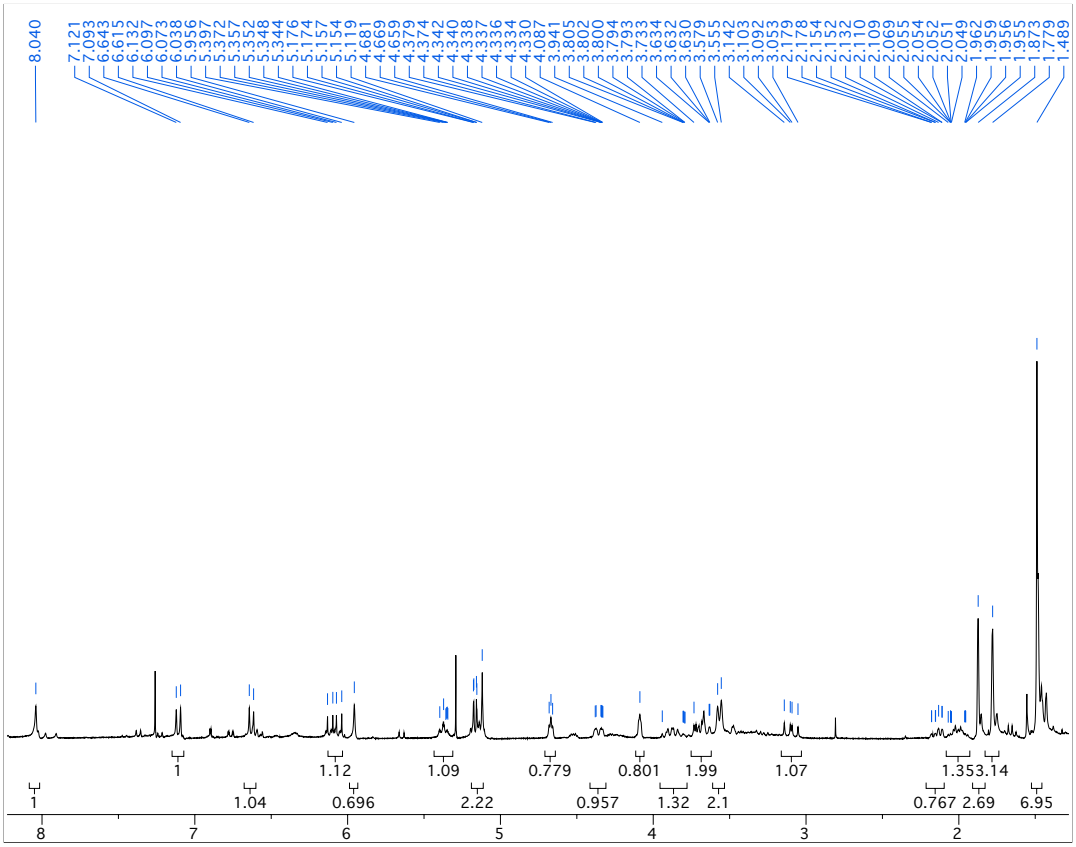


114

(8*R*,8a*S*)-8-hydroxy-3-((6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (114). To a solution of **116** (322 mg, 0.44 mmol, 1 eq) in MeOH (4.5 mL) was added Lindlar's catalyst (96 mg, 30 weight %) followed by a balloon of H_2. The reaction was stirred vigorously for 1 h. The mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude material was diluted with CH_3CN (5 mL) and Et_2NH (2 mL) was added. The reaction mixture continued to stir for 5 h. at room temperature. The mixture was concentrated under reduced pressure and placed under vacuum for 18 h. CH_3CN (5 mL) was added to the residue and heated to reflux for 18 h. The reaction was then concentrated under reduced pressure and purified by flash chromatography (MeOH / CH_2Cl_2, 1 : 20) to afford **114** (102 mg, 51%) as a yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (bs, 1 H), 7.09 (d, *J* = 8.4 Hz, 1 H), 6.61 (d, *J* = 8.4 Hz, 1 H), 6.03 (dd, *J* = 10.4, 17.5 Hz, 1 H), 5.95 (bs, 1 H), 5.35 (t, *J* = 7.2 Hz, 1 H), 5.11 (dd, *J* = 10.4, 17.5 Hz, 2 H), 4.66 (t, *J* = 3.5 Hz, 1 H), 4.37-4.33 (m, 1 H), 4.08 (bs, 1 H), 3.94-3.79 (m, 1 H), 3.73-3.63 (m, 2 H), 3.55 (d, *J* = 7.3 Hz, 2 H), 3.05 (dd,

J = 11.7, 15.2 Hz, 1 H), 2.17-2.10 (m, 1 H), 2.06-1.95 (m, 2 H), 1.87 (s, 3 H), 1.77 (s, 3 H), 1.48 (s, 6 H).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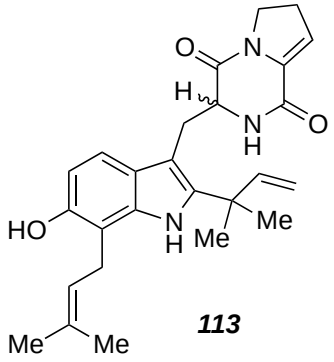
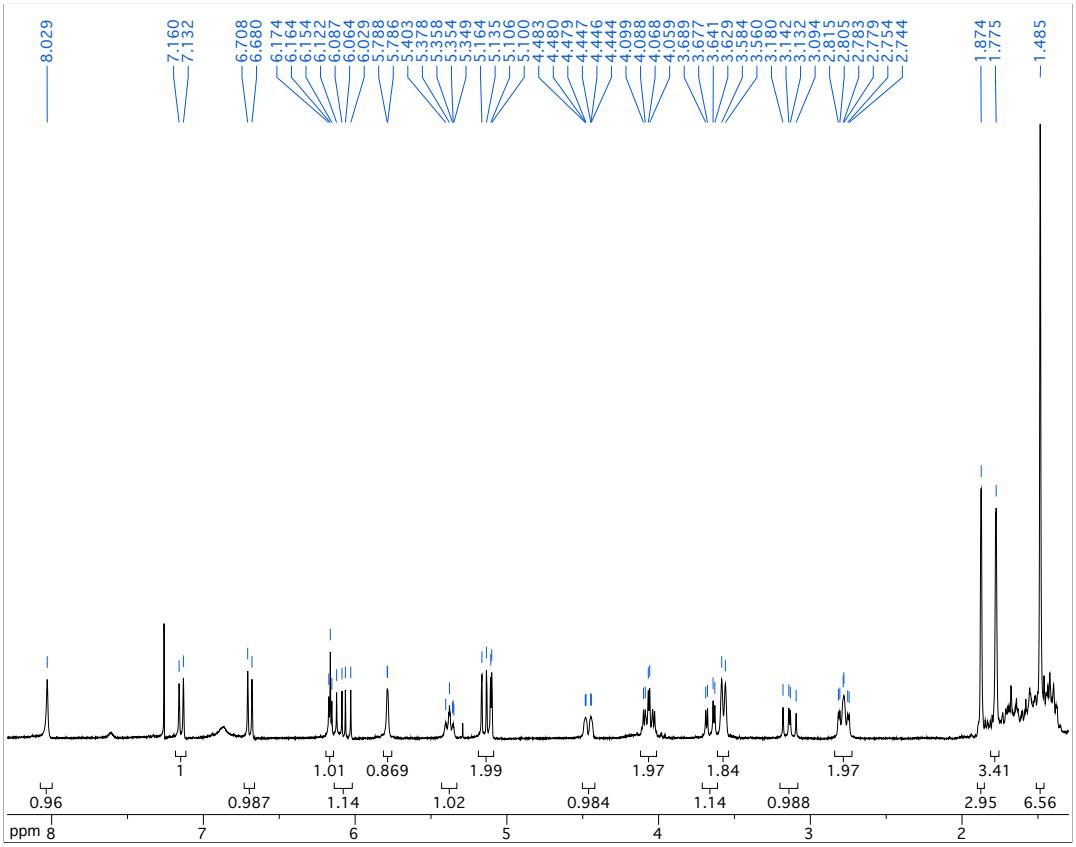
3
2



113

3-((6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)-2,3,6,7-tetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (113). To a solution of DEAD (100 µL, 0.22 mmol, 2 eq) in CH_2Cl_2 (2 mL) at 0ºC was added Bu_3P (55 µL, 0.22 mmol, 2 eq). The reaction was allowed to stir at 0ºC for 15 min. at which time a solution of **114** (50 mg, 0.11 mmol, 1 eq) in CH_2Cl_2 (1 mL) was added. The reaction was stirred at room temperature for 3 h., and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (EtOAc / hex., 1 : 1) to give **113** (19 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (bs, 1 H), 7.13 (d, *J* = 8.5 Hz, 1 H), 6.68 (d, *J* = 8.5 Hz, 1 H), 6.16 (t, *J* = 3.0 Hz, 1 H), 6.02 (dd, *J* = 10.5, 17.4 Hz, 1 H), 5.78 (bs, 1 H), 5.37 (t, *J* = 7.2 Hz, 1 H), 5.10 (dd, *J* = 10.5, 17.4 Hz, 2 H), 4.44 (bd, *J* = 10.4 Hz, 1 H), 4.05 (td, *J* = 3.5, 9.4 Hz, 2 H), 3.62 (dd, *J* = 3.5, 14.5 Hz, 1 H), 3.56 (d, *J* = 7.2 Hz, 2 H), 3.09 (dd, *J* = 11.4, 14.5 Hz, 1 H), 2.77 (m, 2 H), 1.87 (s, 3 H), 1.77 (s, 3H), 1.48 (s, 6 H).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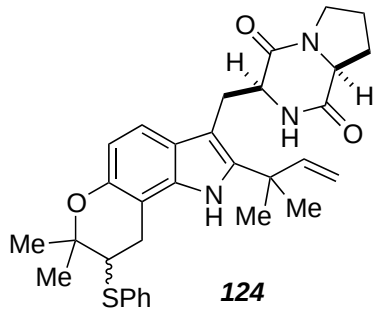
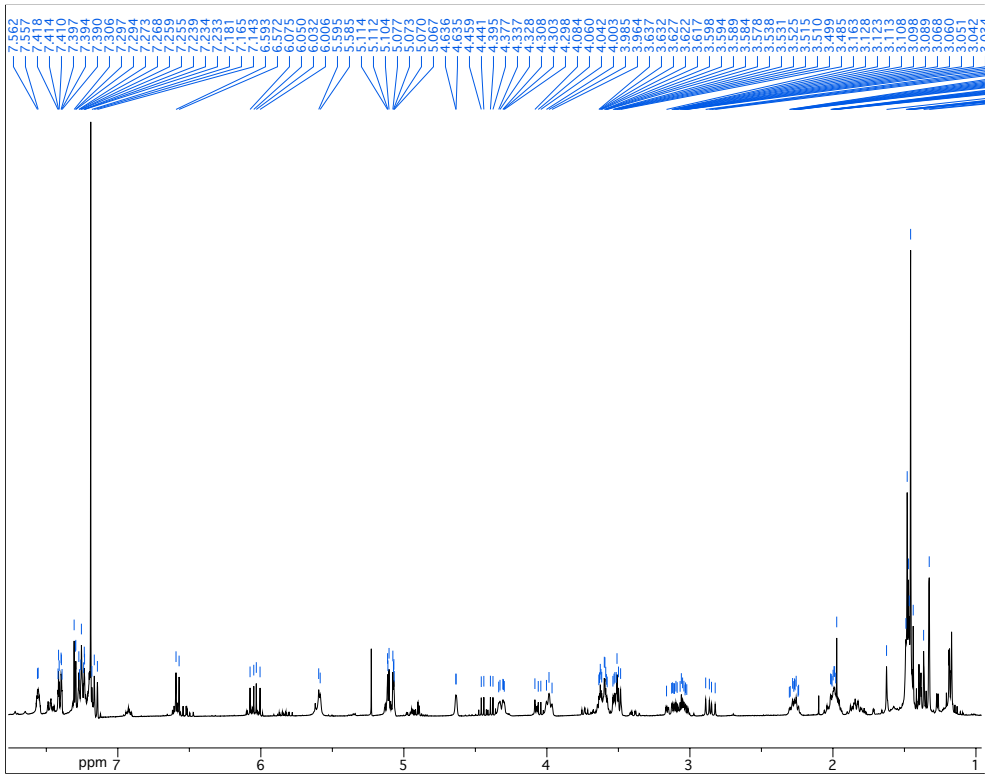


124

(3*S*,8a*S*)-3-((7,7-dimethyl-2-(2-methylbut-3-en-2-yl)-8-(phenylthio)-1,7,8,9-tetrahydropyrano[2,3-*g*]indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (124). To a solution of **83** (50 mg, 0.11 mmol, 1 eq) in benzene (1 mL) was added PhSH (26 µL, 0.25 mmol, 2.2 eq) and AIBN (1 mg, 0.0046 mmol, 0.04 eq) and heated to reflux for 24 h. The mixture was diluted with EtOAc (5 mL) and the organic layer washed with 2 M NaOH (3 x 5 mL), H_2O (3 x 5 mL), brine (10 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provide the crude residue which was purified by flash chromatography (EtOAc / hex., 1 : 1–4 : 5) to afford **124** (43 mg, 70%),as a mixture of diastereomers, as a yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ complex mixture; ^{13}C NMR (100 MHz, $CDCl_3$) δ complex mixture; HRMS (ESI/APCI+) calcd for $C_{32}H_{37}N_3O_3S$ [M + H] 544.2628, found 544.2629.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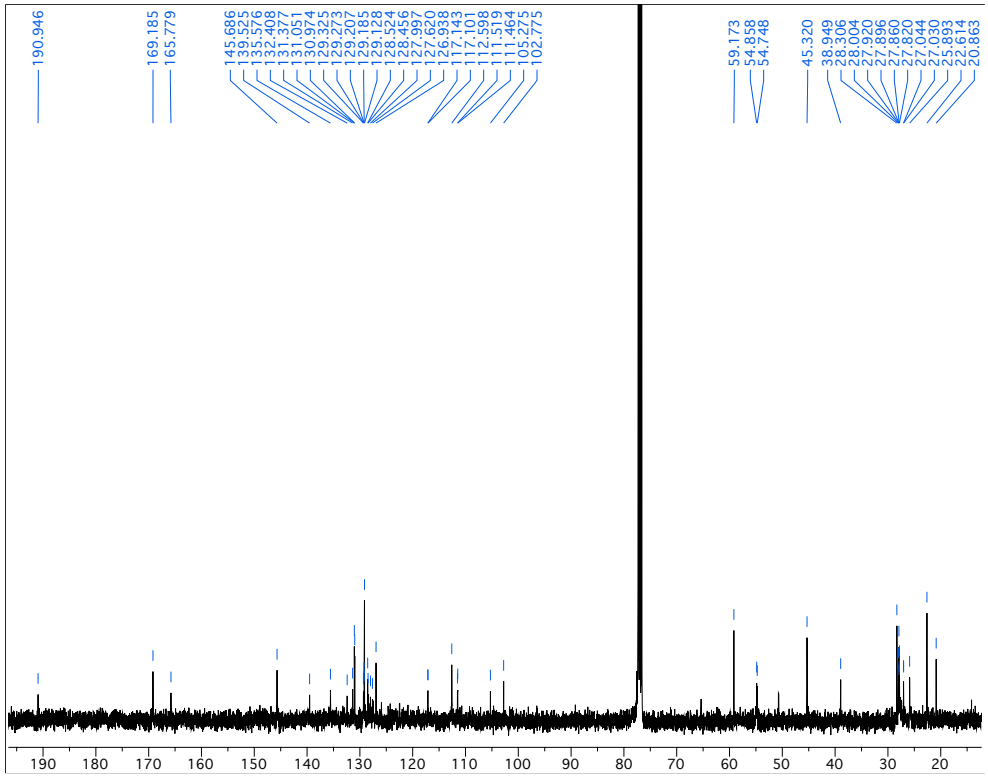
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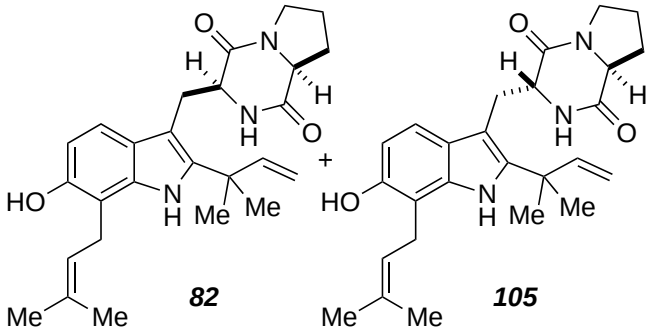
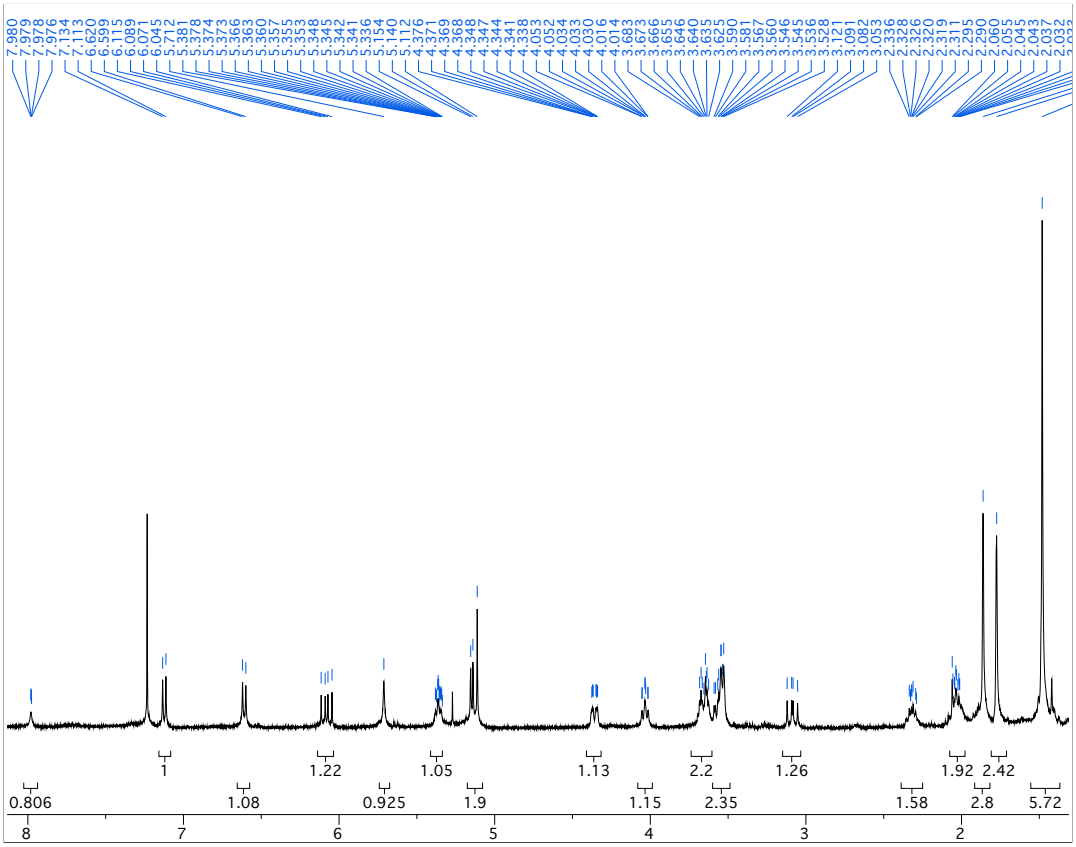
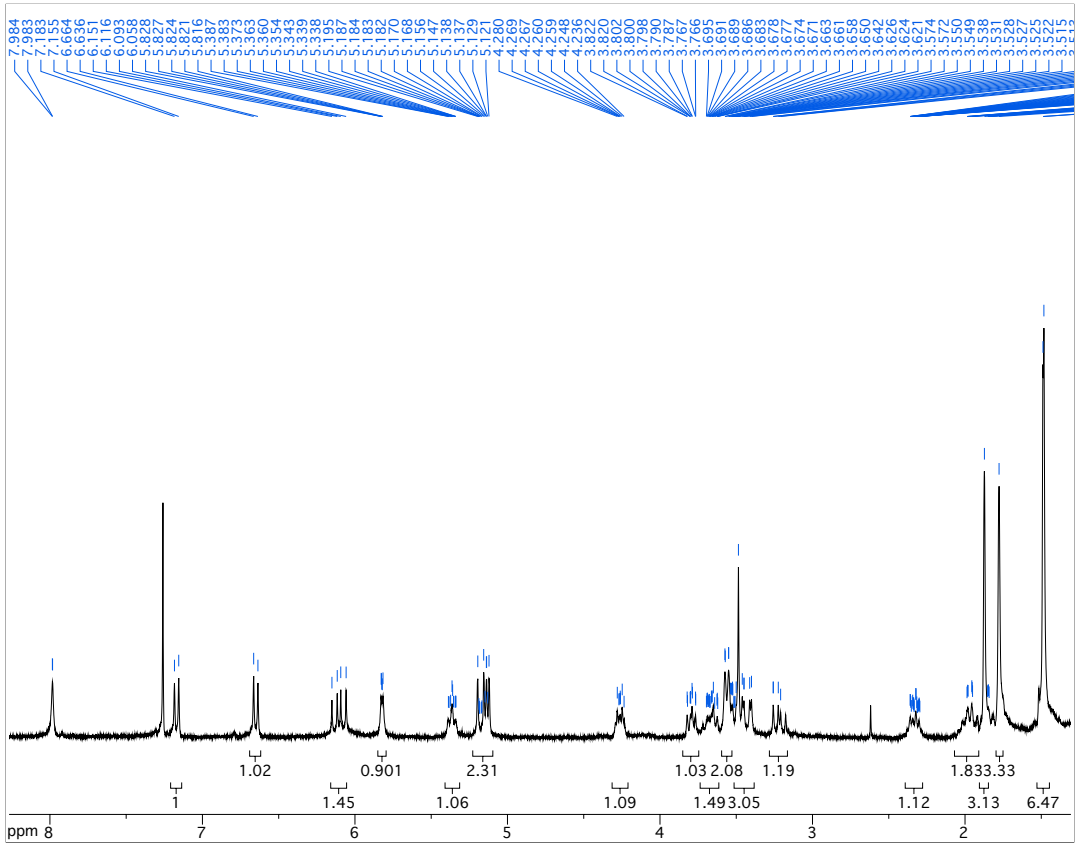


(3*S*,8a*S*)-3-((6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (82) and **(3*R*,8a*S*)-3-((6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (105).** To a solution of **124** (26 mg, 0.047 mmol, 1 eq) in THF (1 mL) at -40ºC was added a solution of K-Naphth. until the reaction mixture remained green in color for 10 min. H_2O (5 mL) was added slowly at -40ºC and the mixture was allowed to warm to room temperature. The mixture was diluted with EtOAc (5 mL) and separated. The organic layer was washed with H_2O (10 mL), brine (10 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded the crude material which was purified by flash chromatography (MeOH / CH_2Cl_2, 1 : 50) to afford **82** (6 mg) and **105** (5 mg) in a 55% yield as a 1 : 1 mixture. R_f = 0.67 (MeOH/CH_2Cl_2, 1:10); ^{1}H NMR (300 MHz, $CDCl_3$) d 8.01 (bs, 1 H), 7.15 (d, *J* = 8.5 Hz, 1 H), 6.65 (d, J = 8.5 Hz, 1 H), 6.15 (dd, *J* = 17.5, 10.3 Hz, 1 H), 5.75 (bs, 1 H), 5.38 (t, *J* = 7.2 Hz, 1 H), 5.18 (d, *J* = 5.35 Hz, 1 H), 5.13 (s, 1 H), 4.41 (dd, *J* = 10.7, 2.5 Hz, 1 H), 4.08 (t, *J* = 7.3 Hz, 1 H), 3.71-3.61 (m, 2 H), 3.57 (d, *J* = 7.2 Hz, 2 H), 3.15 (dd, *J* = 15.3, 11.6 Hz, 1 H), 2.39-2.31 (m, 1 H), 2.12-2.00 (m, 2 H), 1.88 (s, 3

H), 1.79 (s, 3 H), 1.50 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 169.4, 166.0, 149.4, 145.9, 139.9, 134.9, 133.9, 123.6, 122.5, 116.2, 112.6, 110.5, 104.5, 59.4, 55.1, 45.5, 39.1, 29.8, 28.5, 28.0, 27.9, 25.9, 24.3, 22.8, 18.1; IR (n_{max}) 3362, 1665, 1438, 919, 800 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{26}H_{32}DN_3O_3$ [M + Na] 459.2477, found 459.2475.



(**105**) (35 mg, 20%) R_f = 0.53 (MeOH/CH_2Cl_2, 1:10); ^{1}H NMR (400 MHz, $CDCl_3$) d 7.94 (bs, 1 H), 7.15 (d, *J* = 8.4 Hz, 1 H), 6.63 (d, *J* = 8.4 Hz, 1 H), 6.11 (dd, *J* = 17.4, 10.3 Hz, 1 H), 5.83 (bs, 1 H), 5.76 (bs, 1 H), 5.32 (t, *J* = 7.4 Hz, 1 H), 5.15 (dd, *J* = 17.4, 10.3 Hz, 2 H), 4.24-4.21 (m, 2 H), 3.78-3.74 (m, 1 H), 3.68-3.61 (m, 1 H), 3.53 (d, *J* = 7.4 Hz, 2 H), 3.48-3.37 (m, 3 H), 3.22 (dd, *J* = 14.4, 10 Hz, 1 H), 2.32-2.27 (m, 1 H), 1.99-1.86 (m, 2 H), 1.84 (s, 3 H), 1.74 (s, 3 H), 1.45 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 168.7, 166.0, 149.4, 146.3, 139.9, 135.0, 133.6, 123.3, 122.7, 116.7, 111.8, 110.3, 108.3, 105.0, 58.9, 58.4, 45.7, 39.2, 30.1, 29.2, 28.0, 27.8, 25.9, 24.3, 22.1, 18.1; IR (n_{max}) 3336, 1655, 1448, 918, 802 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{26}H_{33}N_3NaO_3$ [M + Na] 458.2414, found 458,2413.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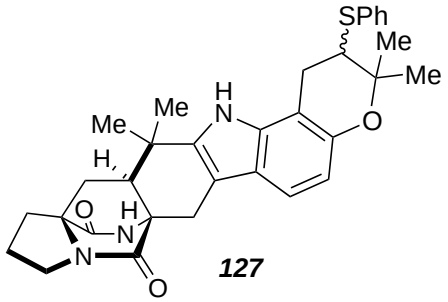
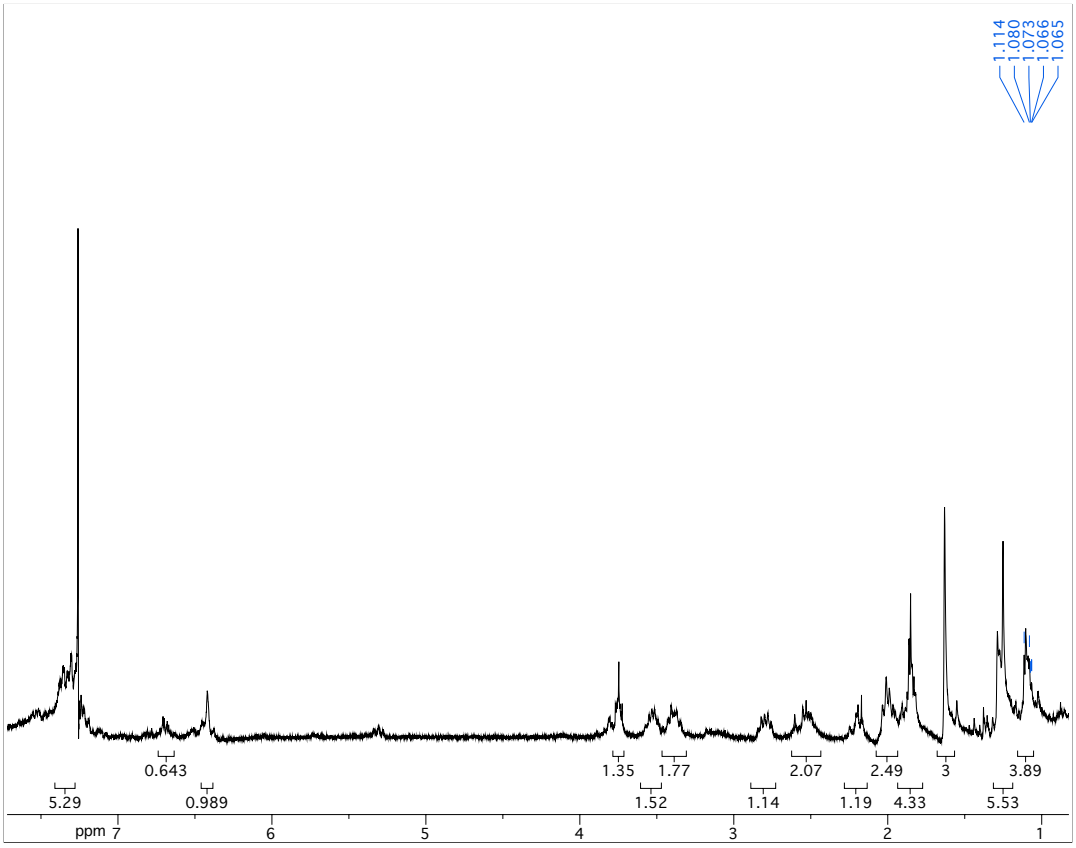


127

(7a*S*,12a*S*,13a*S*)-3,3,14,14-tetramethyl-2-(phenylthio)-2,3,7,10,11,12,13,13a,14,15-decahydro-7a,12a-(epiminomethano)indolizino[6,7-*h*]pyrano[3,2-*a*]carbazole-8,16(1*H*)-dione (127). To a solution of **1** (100 mg, 0.23 mmol, 1 eq) in DMSO (4 mL) was added PhSH (380 µL, 3.70 mmol, 16 eq) and PhS-SPh (101 mg, 0.46 mmol, 2 eq). The solution was degassed via freeze pump thaw method. The reaction was irradiated with hν (400W medium pressure Hg arch lamp) for 48 h. The reaction mixture was diluted with Et_2O (10 mL) and washed with 2 M NaOH (3 x 10 mL), H_2O (3 x 10 mL), brine (10 mL) and dried over $MgSO_4$. Filtration and concentration under reduced pressure provided the crude residue which was purified by flash chromatography (MeOH / $CHCl_2$, 0 : 1–1 : 65) to provide **127** (100 mg, 80%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.27 (comp., 7 H), 6.70-6.67 (comp., 1 H), 6.41 (bs, 1 H), 3.76-3.72 (comp., 1 H), 3.57-3.48 (comp., 1 H), 3.43-3.33 (comp., 1 H), 2.82-2.75 (comp., 1 H), 2.60-2.46 (comp., 2 H), 2.25-2.16 (comp., 1 H), 2.03-1.94 (comp., 2 H), 1.90-1.80 (comp., 4 H), 1.63 (bs, 3 H), 1.28-1.21 (comp., 6 H), 1.11-1.06 (comp., 3 h).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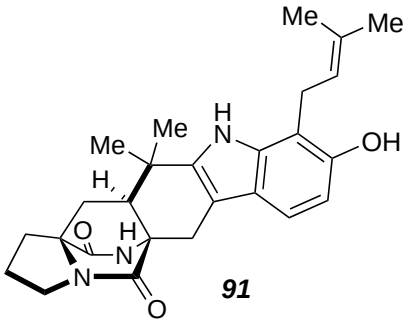
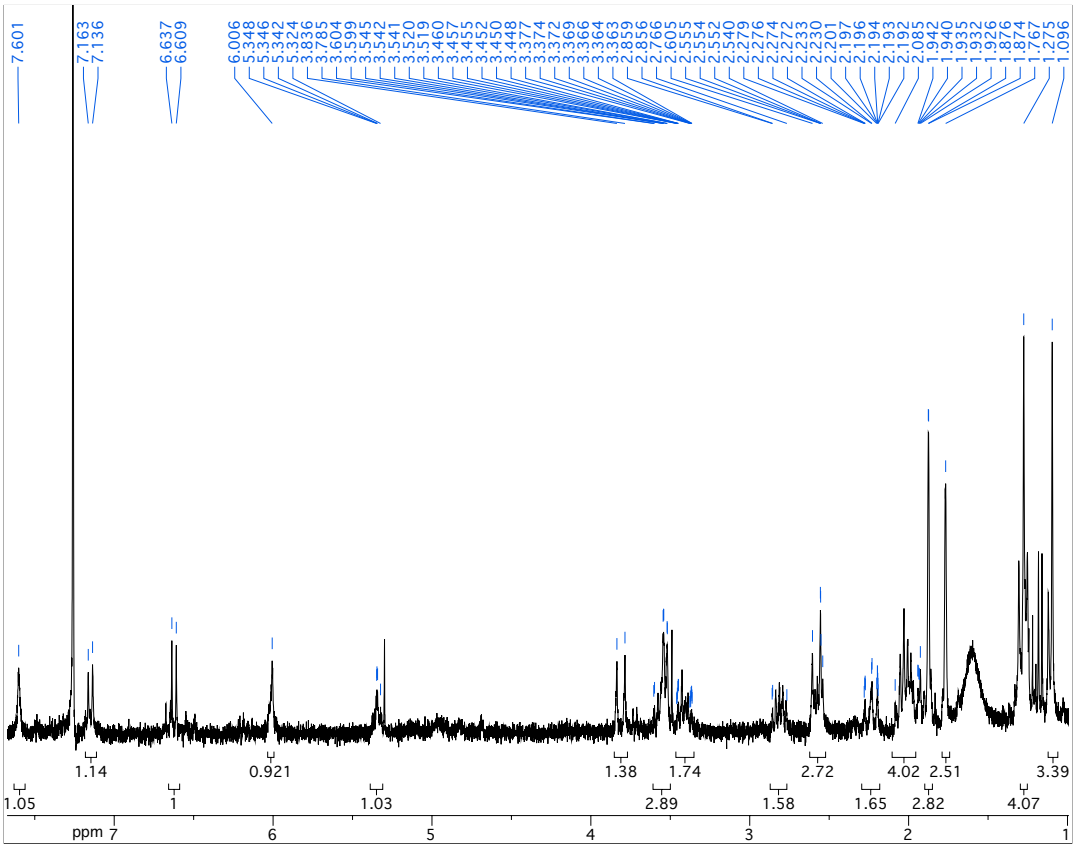


91

(5a*S*,12a*S*,13a*S*)-9-hydroxy-12,12-dimethyl-10-(3-methylbut-2-en-1-yl)-2,3,11,12,12a,13-hexahydro-1*H*-5a,13a-(epiminomethano)indolizino[7,6-*b*]carbazole-5,14(6*H*)-dione (91). To solution of **127** (100 mg, 0.18 mmol, 1 eq) in THF (2 mL) as -40ºC was added a solution of K-Naphth. until a green color remained for 10 min. H_2O (5 mL) was added slowly at -40ºC and the mixture was allowed to warm to room temperature. The mixture was diluted with EtOAc (5 mL) and separated. The organic layer was washed with H_2O (10 mL), brine (10 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded the crude material which was purified by flash chromatography (MeOH / CH_2Cl_2, 1 : 50) to afford **91** (29 mg, 40%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (bs, 1 H), 7.13 (d, *J* = 8.1 Hz, 1 H), 6.60 (d, *J* = 8.1 Hz, 1 H), 6.00 (bs, 1 H), 5.34 (t, *J* = 7.5 Hz, 1 H), 3.78 (d, *J* = 15.3 Hz, 1 H), 3.60-3.51 (m, 3 H), 3.46-3.36 (m, 2 H), 2.85-2.76 (m, 1 H), 2.60-2.54 (m, 3 H), 2.27-2.19 (m, 1 H), 2.08-1.92 (m, 4 H), 1.87 (s, 3 H), 1.76 (s, 3 h), 1.27 (s, 3 H), 1.09 (s, 3 H). HRMS (ESI/APCI+) calcd for $C_{26}H_{31}N_3O_3$ [M + Na] 456.2258, found 456.2255.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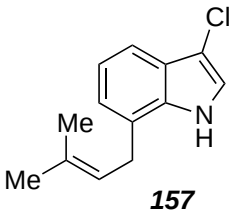
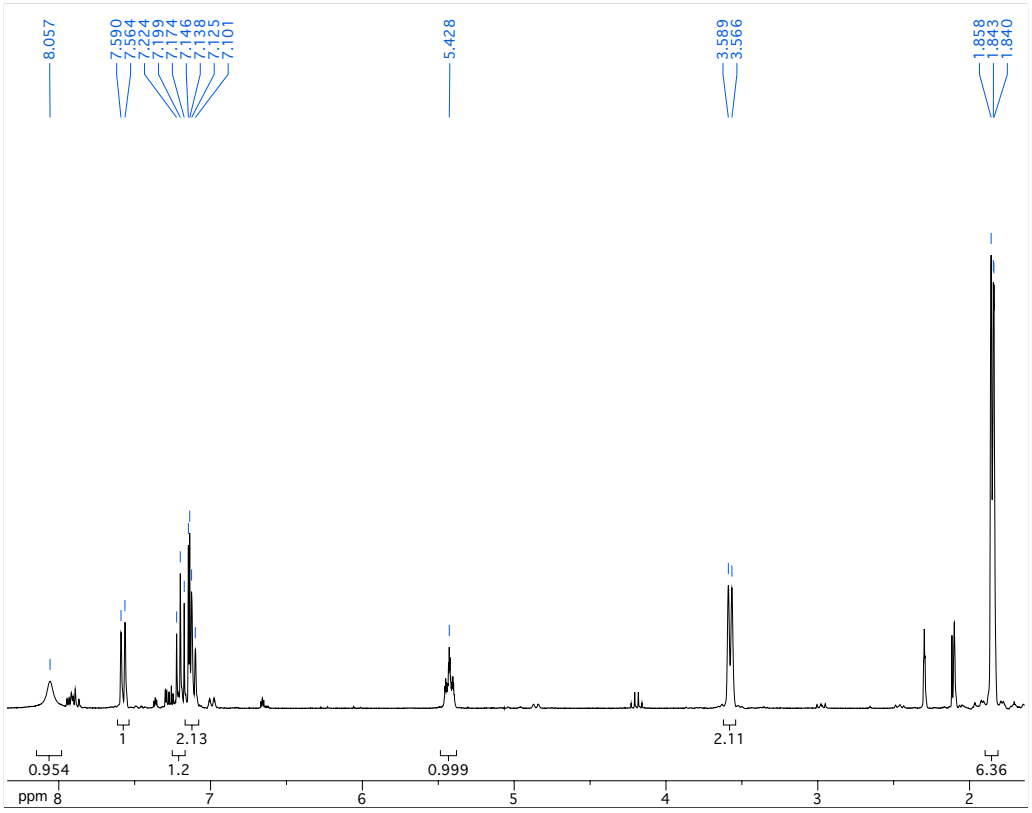


157

3-chloro-7-(3-methylbut-2-en-1-yl)-1*H*-indole (157). To a solution of indole **156** (100 mg, 0.53 mmol, 1 eq) in DMF at 25ºC was added NCS (65 mg, 0.48 mmol, 0.9 eq) in one portion. The reaction was stirred for 4 h at 25ºC, at which time brine (20 mL) was added and the solution was partitioned between Et_2O (20 mL) and H_2O (20 mL). The aqueous layer was extracted with Et_2O (3 x 10 mL). All organic layers were combined and extracted with H_2O (3 x 20 mL) and brine (2 x 10 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc / hex., 1:10) to give **157** as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (bs, 1 H), 7.56 (d, *J* = 7.8 Hz, 1 H), 7.19 (t, *J* = 7.3 Hz, 1 H), 7.13 (s, 1 H), 7.10 (d, *J* = 7.3 Hz, 1 H), 5.42 (t, *J* = 7.1 Hz, 1 H), 3.56 (d, *J* = 7.1 Hz, 2 H), 1.85 (s, 3 H), 1.84 (s, 3 H).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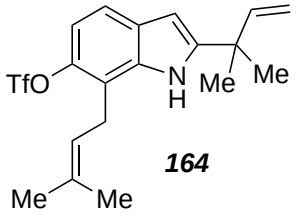
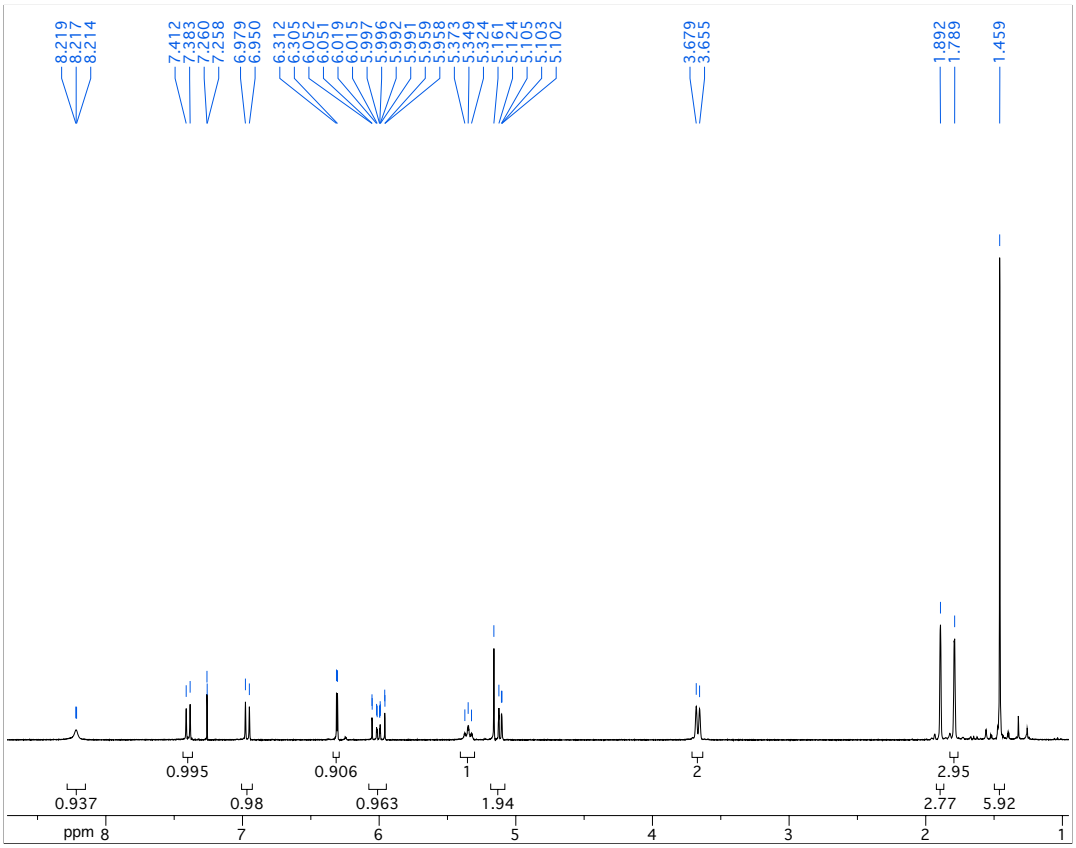


164

7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-yl trifluoromethanesulfonate (164). To a vigorously stirred solution of **98** (737 mg, 2.75 mmol, 1 eq) in MeOH (3 mL) was added Lindlar's catalyst (36 mg, 1.37 mmol, 0.5 eq) followed by a balloon of H_2. After stirring for 30 min. the reaction was filtered through a pad of Celite and concentrated under reduced pressure. The crude oil was taken up in CH_3CN (4 mL) and heated to reflux for 6 h. Removal of the CH_3CN under reduced pressure gave a yellowish oil, which was passed through a plug of silica eluting with 5% EtOAc / hex and concentrated under reduced pressure. Treatment of the yellow oil in CH_2Cl_2 (5 mL) consecutively with DMAP (458 mg, 3.75 mmol, 1.5 eq), TEA (2.45 mL, 17.50 mmol, 7 eq), and $(Tf)_2O$ (0.845 mL, 5.00 mmol, 2 eq) at 0ºC. Stir at 0ºC for 20 min. at which time sat. $NaHCO_3$ (5 mL) is added. Dilute with CH_2Cl_2 (10 mL) and separate. The organic layer is washed with sat. $NaHCO_3$ (10 mL), brine (10 mL) and dried over Na_2SO_4. The solution is filtered and concentrated under reduced pressure to give a yellow oil. Crude **164** is purified by flash chromatography (EtOAc / hex., 1 : 20) to give **164** (618 mg, 56%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (bs, 1 H), 7.38 (d, *J* = 8.7 Hz, 1 H), 6.95 (d, *J* = 8.7 Hz, 1 H), 6.30 (d, *J* = 2.2 Hz, 1 H), 5.99

(dd, *J* = 10.1, 5.6 Hz, 1 H), 5.34 (t, *J* = 7.3 Hz, 1 H), 5.12 (dd, *J* = 10.1, 5.6 Hz, 2 H), 3.65 (d, *J* = 7.3 Hz, 2 H), 1.89 (s, 3 H), 1.78 (s, 3 H), 1.45 (s, 6 H).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7.258
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6.019
6.015
5.997
5.996
5.992
5.991
5.959
5.958
5.373
5.349
5.324
5.161
5.124
5.105
5.103
5.102
3.679
3.655
1.892
1.789
1.459
0.937
0.995
0.98
0.906
0.963
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2.95
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ppm
8
7
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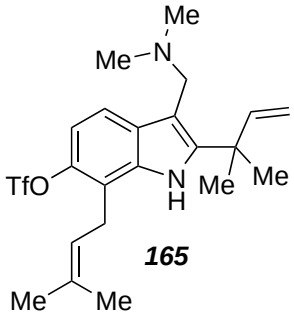
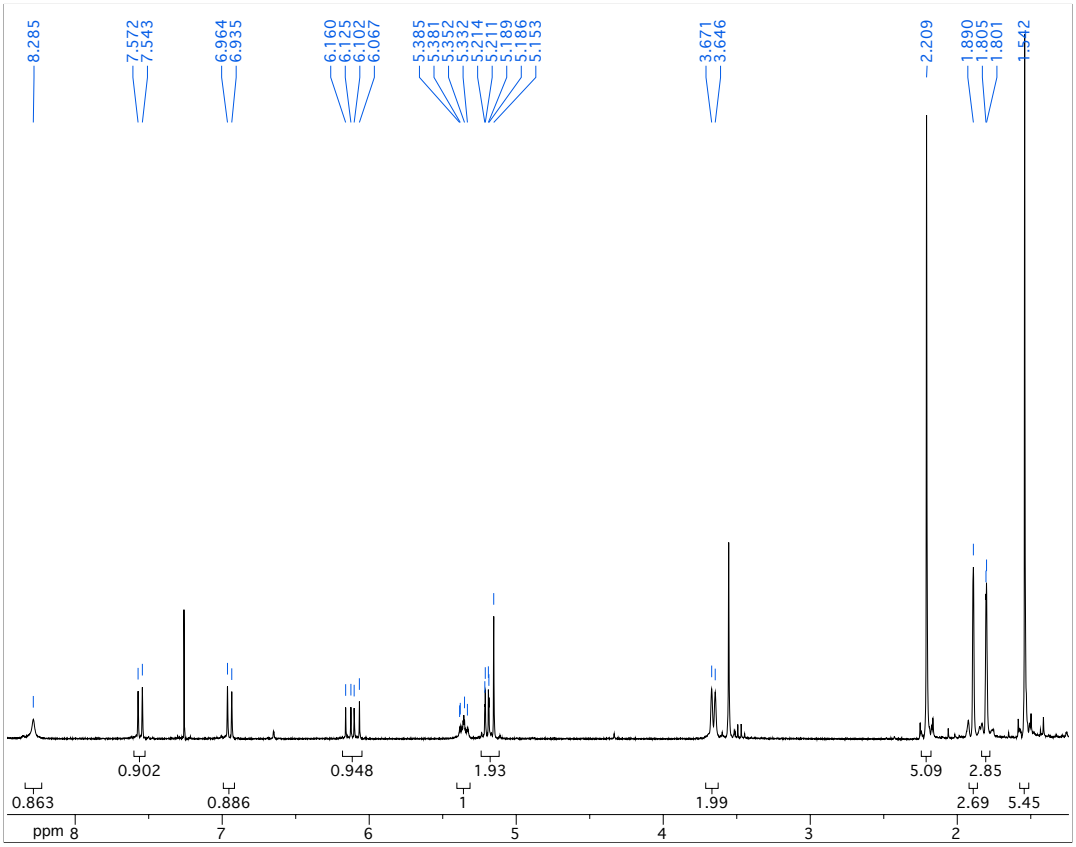
5
4
3
2
1



165

3-((dimethylamino)methyl)-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-yl trifluoromethanesulfonate (165). To a solution of Me_2NH (15 µL, 0.13 mmol, 1.1 eq, 40% aqueous) in AcOH (400 µL) was added H_2CO (11 µL, 0.13 mmol, 1.1 eq, 37% aqueous) at 25ºC. The reaction mixture was stirred for 30 min. before being canulaed to a solution of **164** (50 mg, 0.12 mmol, 1 eq) in AcOH (100 µL). After 8 h. the reaction was basified with 2 M NaOH (5 mL) and extracted with Et_2O (5 x 10 mL). The organics were combined and dried with $MgSO_4$, filtered and concentrated under reduced pressure to give a yellow oil, which was taken on crude to the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (bs, 1 H), 7.54 (d, *J* = 8.7 Hz, 1 H), 6.93 (d, *J* = 8.7 Hz, 1 H), 6.06 (dd, *J* = 10.1, 6.3 Hz, 1 H), 5.35 (t, *J* = 7.3 Hz, 1 H), 5.15 (dd, *J* = 10.1, 6.3 Hz, 2 H), 3.64 (d, *J* = 7.3 Hz, 2 H), 2.20 (s, 6 H), 1.89 (s, 3 H), 1.80 (s, 3 H), 1.54 (s, 6 H).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5.381
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5.214
5.211
5.189
5.186
5.153
3.671
3.646
2.209
1.890
1.805
1.801
1.542
0.902
0.948
1.93
5.09
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0.886
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3
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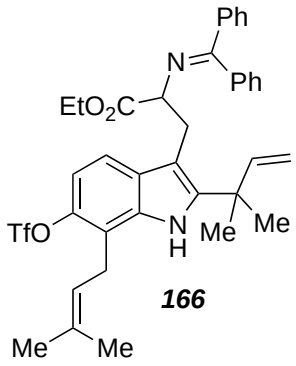
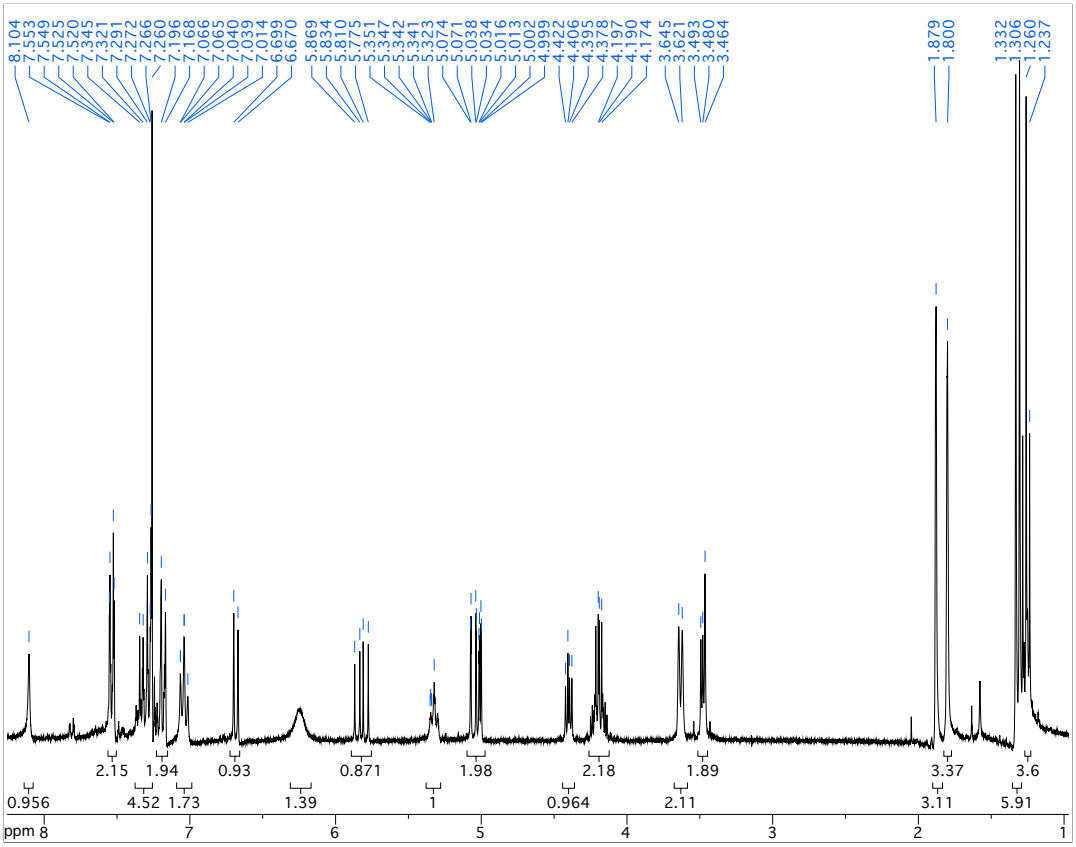


166

ethyl 2-((diphenylmethylene)amino)-3-(7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-6-(((trifluoromethyl)sulfonyl)oxy)-1*H*-indol-3-yl)propanoate (166). To a solution of **165** (46 mg, 0.10 mmol, 1 eq) in CH_3CN (2 mL) was added **101** (27 mg, 0.10 mmol, 1 eq) and Bu_3P (12 µL, 0.050 mmol, 0.5 eq). The reaction mixture was heated to reflux for 18 h., upon cooling to room temperature the reaction was concentrated under reduced pressure. The crude mixture was purified by flash chromatography (EtOAc / hex., 1 : 20–1 : 10) to give **166** (54 mg, 80%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (bs, 1 H), 7.52 (d, *J* = 8.5 Hz, 2 H), 7.34-7.26 (m, 5 H), 7.16 (d, *J* = 8.5 Hz, 2 H), 7.01 (t, *J* = 7.4 Hz, 2 H), 6.67 (d, *J* = 8.7 Hz, 1 H), 5.77 (dd, *J* = 10.5, 17.5, 1 H), 5.32 (t, *J* = 5.3 Hz, 1 H), 4.99 (dd, *J* = 10.5, 17.5 Hz, 2 H), 4.37 (dd, *J* = 4.8, 8.2 Hz, 1 H), 4.19-4.17 (m, 2 H), 3.62 (d, *J* = 5.3 Hz, 2 H), 3.46 (dd, *J* = 4.8, 8.2 Hz, 2), 1.87 (s, 3 H), 1.80 (s, 3 H), 1.33 (s, 3 H), 1.30 (s, 3 H), 1.26 (t, *J* = 7.2 Hz, 3 H).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7.321
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7.066
7.065
7.040
7.039
7.014
6.699
6.670
5.869
5.834
5.810
5.775
5.351
5.347
5.342
5.341
5.323
5.074
5.071
5.038
5.034
5.016
5.013
5.002
4.999
4.422
4.406
4.395
4.378
4.197
4.190
4.174
3.645
3.621
3.493
3.480
3.464
1.879
1.800
1.332
1.306
1.260
1.237
2.15
1.94
0.93
0.871
1.98
2.18
1.89
3.37
3.6
0.956
4.52
1.73
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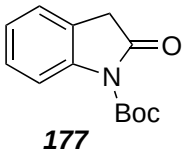
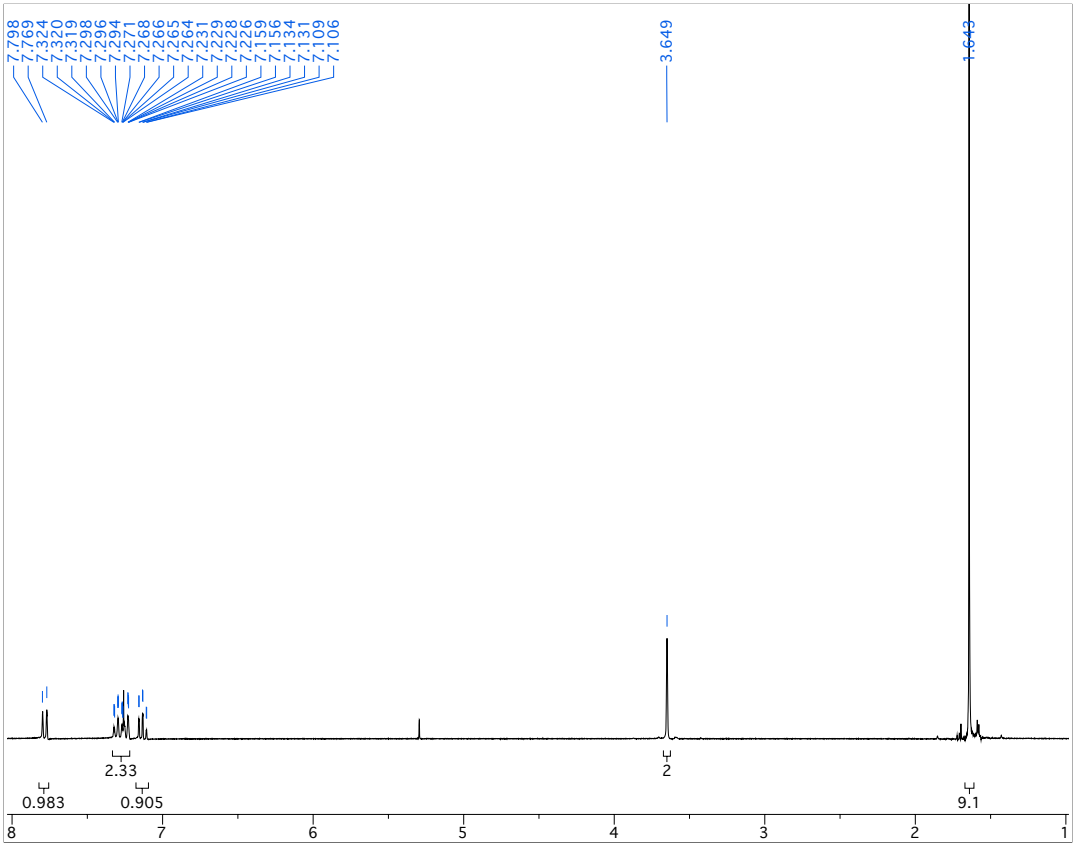
5
4
3
2
1



177

***tert*-butyl 2-oxoindoline-1-carboxylate (177).** To a solution of **177** (2 g, 15.03 mmol, 1 eq) in THF (70 mL) was added Na_2CO_3 (8 g, 75.15 mmol, 5 eq) followed by $(Boc)_2O$ (8.2 g, 37.57 mmol, 2.5 eq). The reaction was stirred at 25ºC for 24 h. The mixture was then filtered through a medium porosity sintered glass frit and evaporated under reduced pressure. The crude mixture was purified by flash chromatography (EtOAc / hex., 1 : 20–3 : 20) to give **177** (2.3 g, 68%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.7 Hz, 1 H), 7.32-7.22 (m, 2 H), 7.10 (t, *J* = 7.5 Hz, 1 H), 3.64 (s, 2 H), 1.64 (s, 9 H).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7.231
7.229
7.228
7.226
7.159
7.156
7.134
7.131
7.109
7.106
3.649
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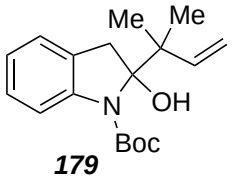
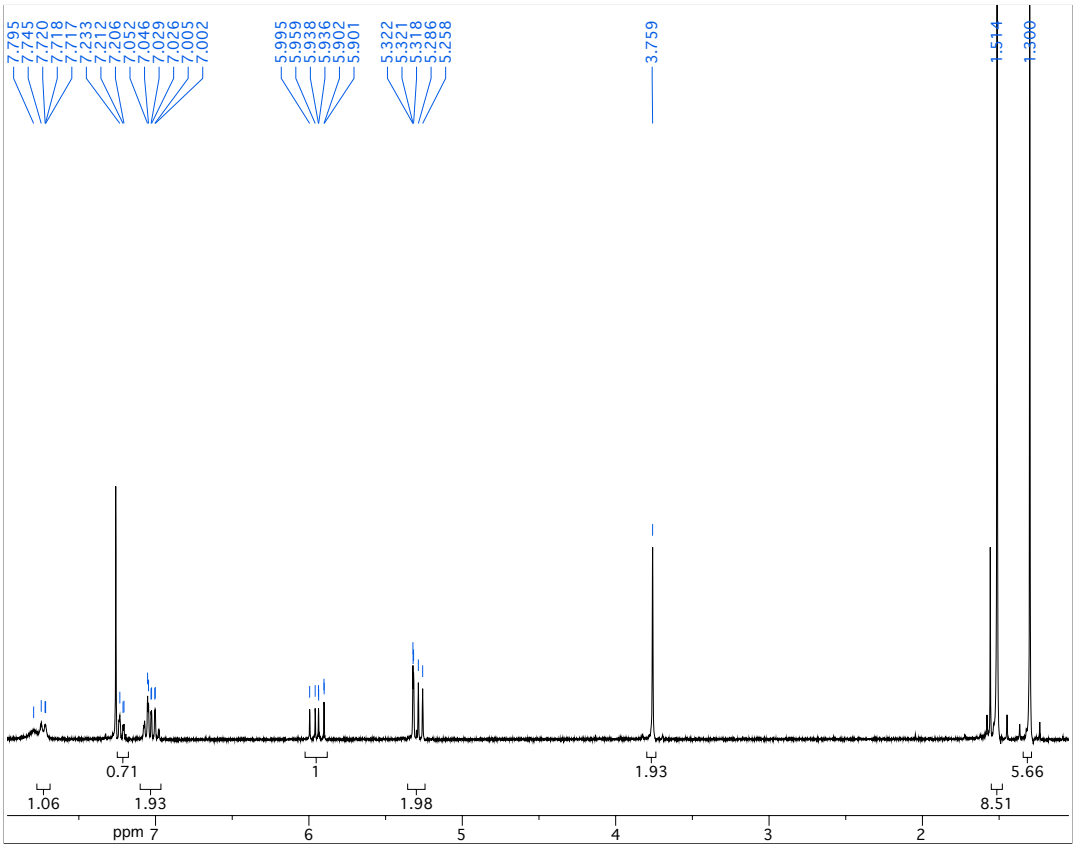
2.33
2
0.983
0.905
9.1
8
7
6
5
4
3
2
1



179

***tert*-butyl 2-hydroxy-2-(2-methylbut-3-en-2-yl)indoline-1-carboxylate (179).** To magnesium turnings (281 mg, 11.27 mmol, 5.4 eq) in THF (2 mL) was added a solution of prenyl chloride (270 mg, 2.57 mmol, 1.2 eq) in THF (6 mL) over a period of 2 h. The reaction mixture continued to stir for 1 h. at 25ºC. In a separate round bottom, **177** (500 mg, 2.14 mmol, 1 eq) in THF (4 mL) was cooled to -78ºC at which time the freshly prepared Grignard was added dropwise over a period of 1 h. Stirring continued at -78ºC for an additional 1 h, upon which sat. NH_4Cl (5 mL) was added at -78ºC and allowed to warm to room temperature. The reaction mixture was diluted with EtOAc (20 mL) and extracted. The organic lay was then washed with H_2O (15 mL) and brine (15 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded crude **179**, which was purified by flash chromatography (EtOAc / hex., 1 : 20) to give pure **179** (390 mg, 60%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (bs, 1 H), 7.74 (d, *J* = 7.7 Hz, 1 H), 7.20 (m, 1 H), 7.07-7.00 (m, 2 H), 5.90 (dd, *J* = 10.8, 17.3 Hz, 1 H), 5.25 (dd, *J* = 10.8, 17.3 Hz, 2 H), 3.75 (s, 2 H), 1.51 (s, 9 H), 1.30 (s, 6 H).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5.995
5.959
5.938
5.936
5.902
5.901
5.322
5.321
5.318
5.286
5.258
3.759
1.514
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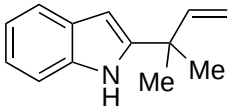
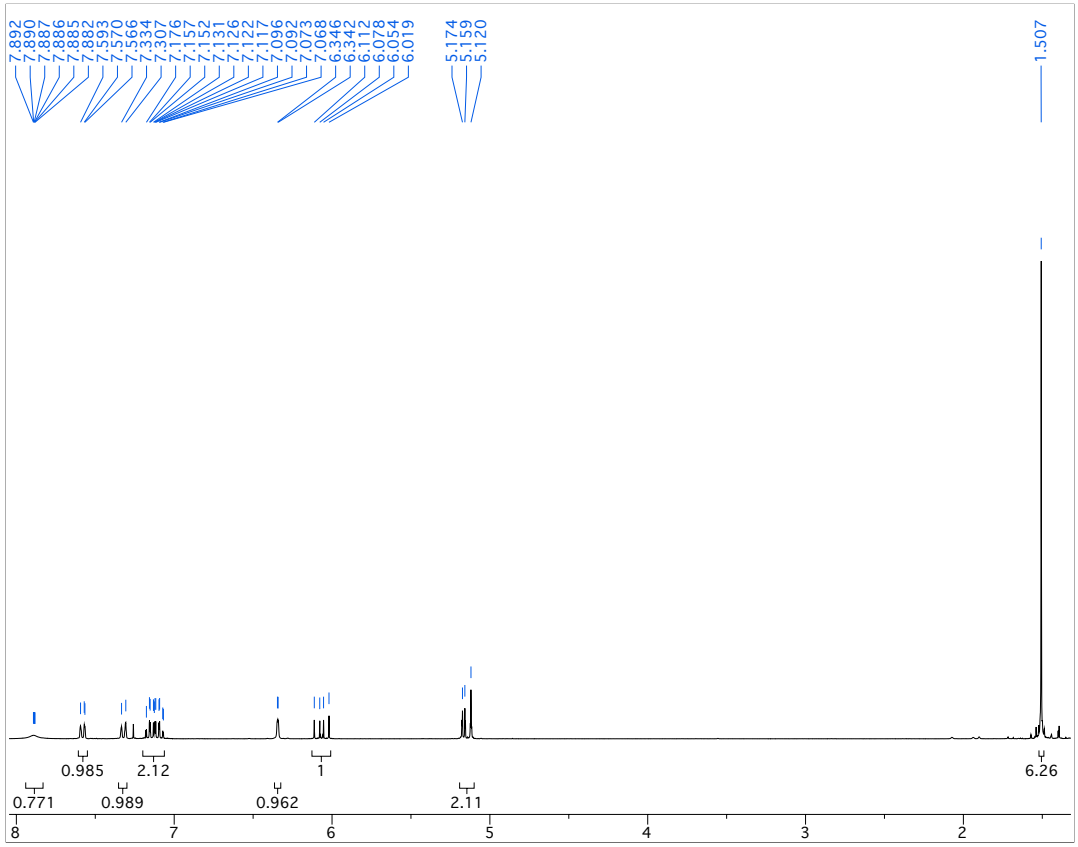
2



180

2-(2-methylbut-3-en-2-yl)-1*H*-indole (180). [A] To a solution of **179** (305 mg, 1.00 mmol, 1 eq) in CH_2Cl_2 was added TFA (235 µL, 3.01 mmol, 3 eq) at 25ºC. The reaction mixture was stirred for 3 h, at which time it was concentrated under reduced pressure. To the crude mixture was added sat. $NaHCO_3$ (5 mL) followed by EtOAc (10 mL) and extracted. The organic layer was washed with H_2O (2 x 5 mL), brine (5 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded crude **180**. Purification by flash chromatograph (EtOAc / hex., 3 : 20) provided **180** (98 mg, 58%) as a clear oil.

[B] To a solution of 179 (200 mg, 0.65 mmol, 1 eq) in toluene (10 mL) was added Amberlyst 15 resin (400 mg). The reaction mixture was heated to reflux for 35 min. and cooled to room temperature. The mixture was filtered and concentrated under reduced pressure to give crude 180. Purification by flash chromatograph (EtOAc / hex., 3 : 20) provided **180** (121 mg, >99%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (bs, 1 H), 7.56 (d, *J* = 8.3 Hz, 1 H), 7.30 (d, *J* = 8.1 Hz, 1 H), 7.17-7.06 (m, 2 H), 6.34 (s, 1 H), 6.01 (dd, *J* = 10.3, 17.6 Hz, 1 H), 5.12 (dd, *J* = 10.3, 17.6 Hz, 2 H), 1.50 (s, 6 H).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7.068
6.346
6.342
6.112
6.078
6.054
6.019
5.174
5.159
5.120
1.507
0.771
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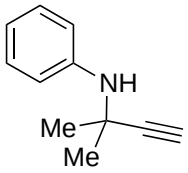
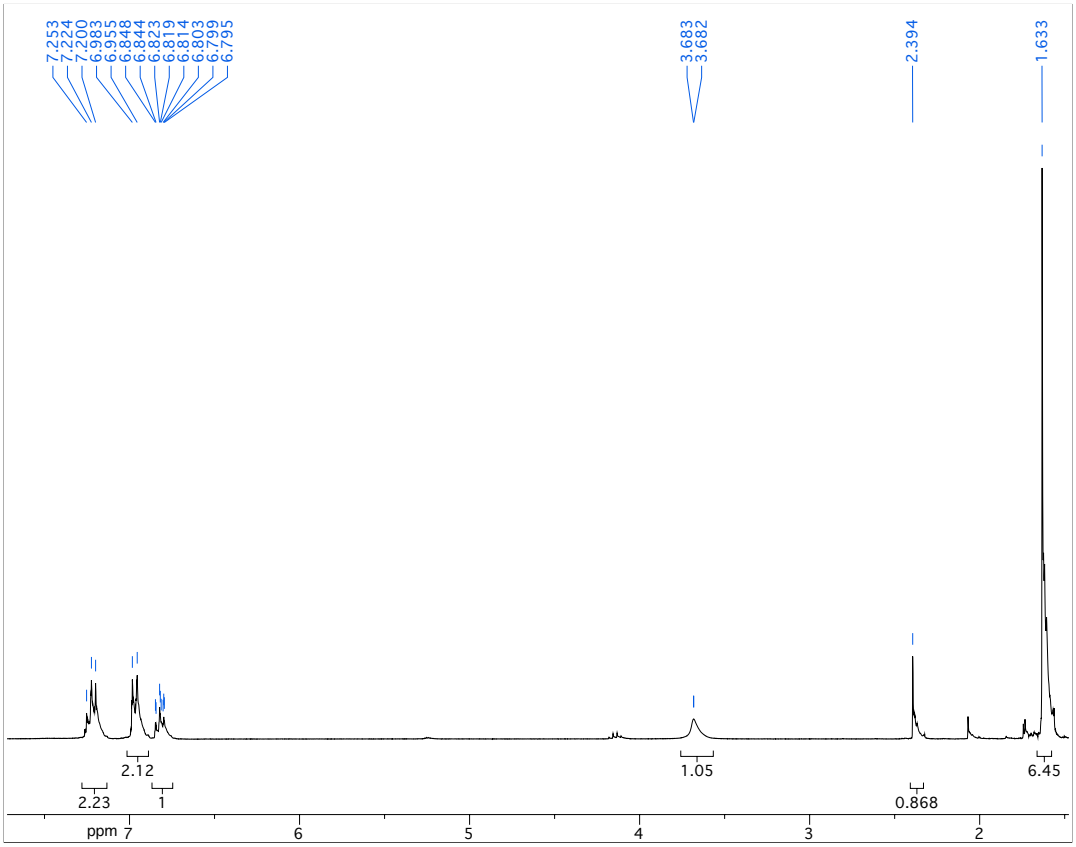


181a

***N*-(2-methylbut-3-yn-2-yl)aniline (181a).** To a solution of aniline (5 g, 53.70 mmol, 1 eq) in THF (250 mL) was added CuCl (531 mg, 5.37 mmol, 10 mol%) at 25ºC and allowed to stir for 5 min. **162** (7.24 mL, 64.44 mmol, 1.2 eq) was added to the mixture over a period of 5 min., and allowed to stir for an additional 10 min. The reaction mixture was cooled to 0ºC at which time TEA (9.02 mL, 64.44 mmol, 1.2 eq) was added over a period of 5 min. Warming to 25ºC, the reaction mixture was allowed to stir for 18 h. The mixture was filtered through a small plug of silica gel and concentrated under reduced pressure. Purification by flash chromatography (EtOAc / hex., 1 : 10) provided **181a** (7.6 g, 90%) as a yellow oil that solidified upon freezing. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (t, *J* = 8.6 Hz, 2 H), 6.95 (d, *J* = 8.6 Hz, 2 H), 6.82 (t, *J* = 7.3 Hz, 1 H), 3.68 (bs, 1 H), 2.39 (s, 1 H), 1.63 (s, 6 H).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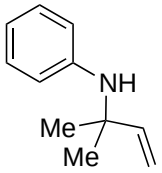
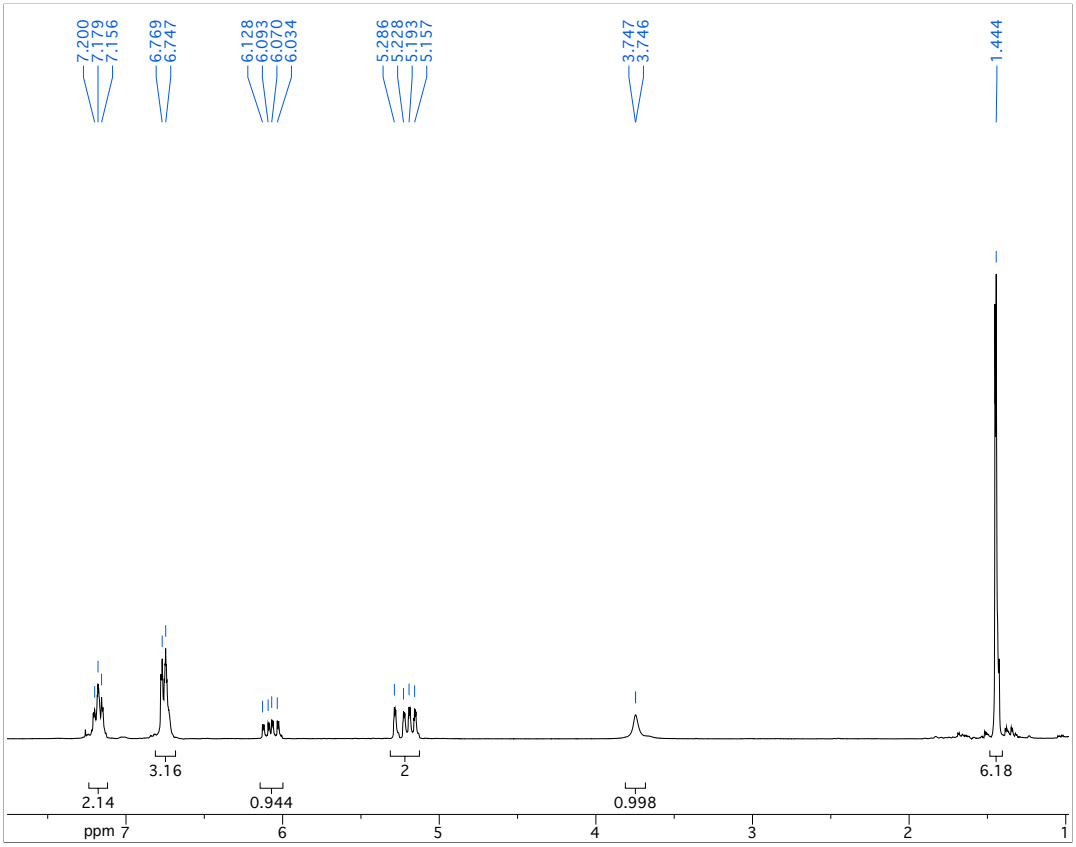


182

***N*-(2-methylbut-3-en-2-yl)aniline (182).** To a solution of 181a (1 g, 6.28 mmol, 1 eq) in MeOH was added Lindlar's catalyst (100 mg, 10 weight %) followed by a balloon of H_2. The reaction was stirred at 25ºC for 30 min., at which time it was filtered through a pad of Celite and concentrated under reduced pressure to give 182 (961 mg, 95%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (t, *J* = 6.5 Hz, 2 H), 6.74 (d, *J* = 6.5 Hz, 3 H), 6.03 (dd, *J* = 10.7, 17.5 Hz, 1 H), 5.15 (dd, *J* = 10.7, 17.5 Hz, 2 H), 3.74 (bs, 1 H), 1.44 (s, 6 H).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3.747
3.746
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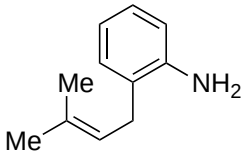
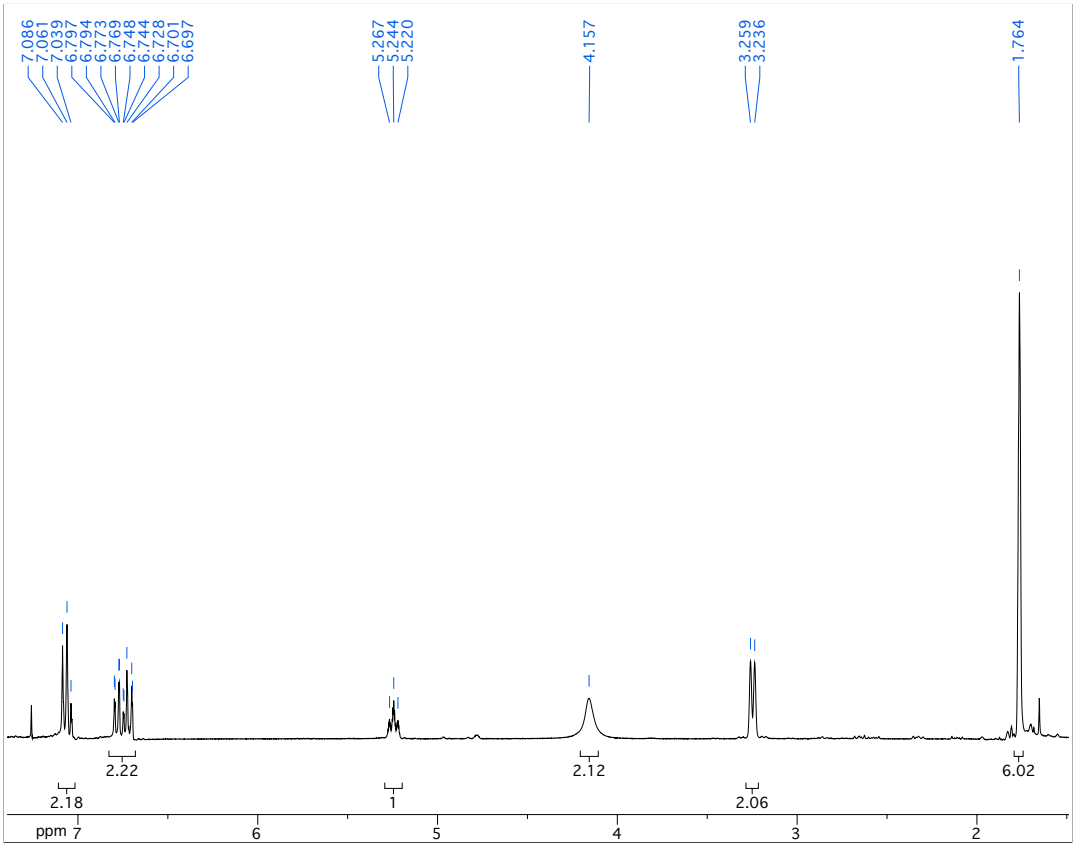


182a

2-(3-methylbut-2-en-1-yl)aniline (182a). To a solution of **182** (100 mg, 0.62 mmol, 1 eq) in toluene (5 mL) was added TFA (50 µL, 0.62 mmol, 1 eq) and heated under microwave condition of 150ºC for 10 min. Concentration of the reaction mixture under reduced pressure followed by addition of sat. $NaHCO_3$ (3 mL) and extraction with EtOAc (3 x 5 mL). The organic layer was washed with H_2O (3 x 5 mL), brine (2 x 5 mL) and dried with Na_2SO_4. Filtration and concentration under reduced pressure gave crude product **182a**. Purification by flash chromatography (EtOAc / hex., 1 : 10) afforded **182a** (90 mg, 90%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (t, *J* = 7.5 Hz, 2 H), 6.79-6.69 (m, 2 H), 5.24 (t, *J* = 7 Hz, 1 H), 4.15 (bs, 2 H), 3.23 (d, *J* = 7 Hz, 2 H) 1.76 (s, 6 H).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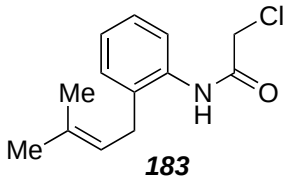
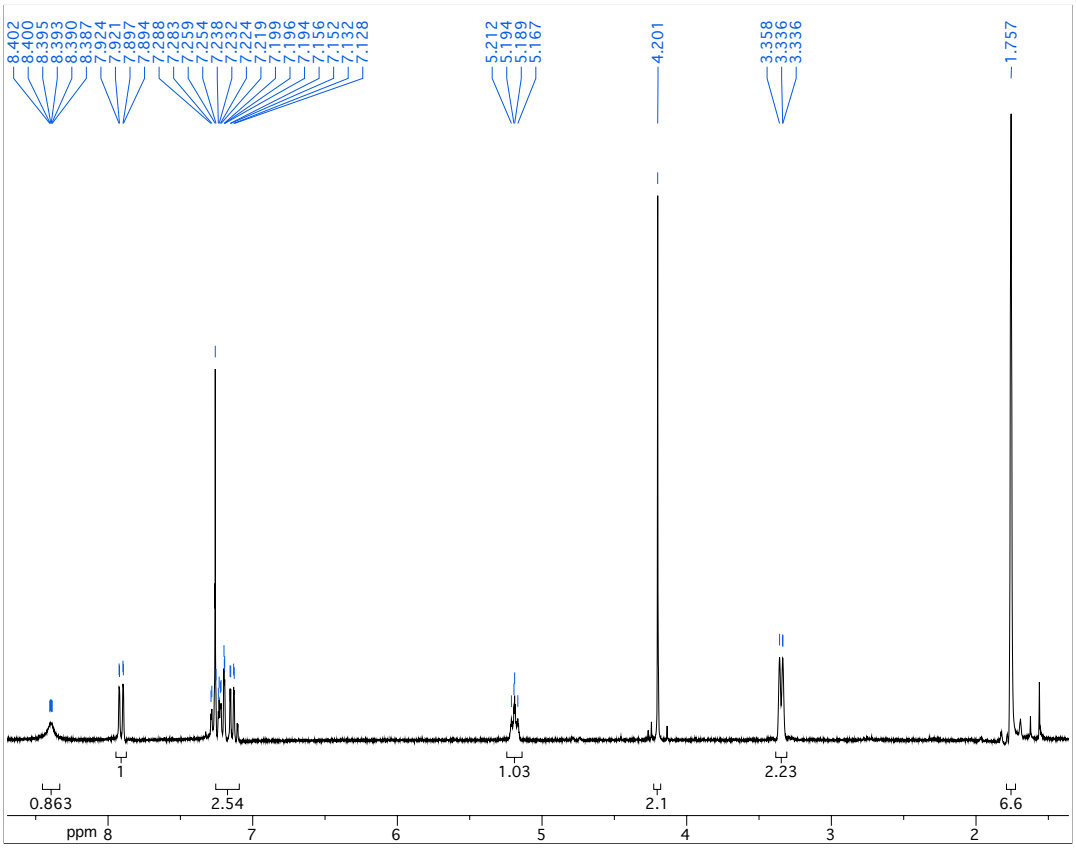


183

2-chloro-*N*-(2-(3-methylbut-2-en-1-yl)phenyl)acetamide (183). To a solution of **182a** (970 mg, 6.01 mmol, 1 eq) and K_2CO_3 (795 mg, 6.01 mmol, 1 eq) in CH_3CN (50 mL) was added dropwise $ClCOCH_2Cl$ (622 mL, 7.82 mmol, 1.3 eq) at 0ºC. The reaction was stirred at 0ºC for 1 h., at which time it was allowed to warm to room temperature where it stirred for 18 h. The reaction mixture was filtered and diluted with CH_2Cl_2 (100 mL) and washed with 1 M HCl (3 x 50 mL), H_2O (3 x 50 mL), brine (50 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided **183** (1.2 g, 87%) as a white solid. No purification was required. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (bs, 1 H), 7.89 (d, *J* = 8.0 Hz, 1 H), 7.28-7.12 (m, 3 H), 5.18 (t, *J* = 6.7 Hz, 1 H), 4.20 (s, 2 H), 3.33 (d, *J* = 6.7 Hz, 2 H), 1.75 (s, 6 H).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7.894
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7.254
7.238
7.232
7.224
7.214
7.199
7.196
7.194
7.156
7.152
7.132
7.128
5.212
5.194
5.189
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ppm
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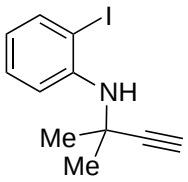
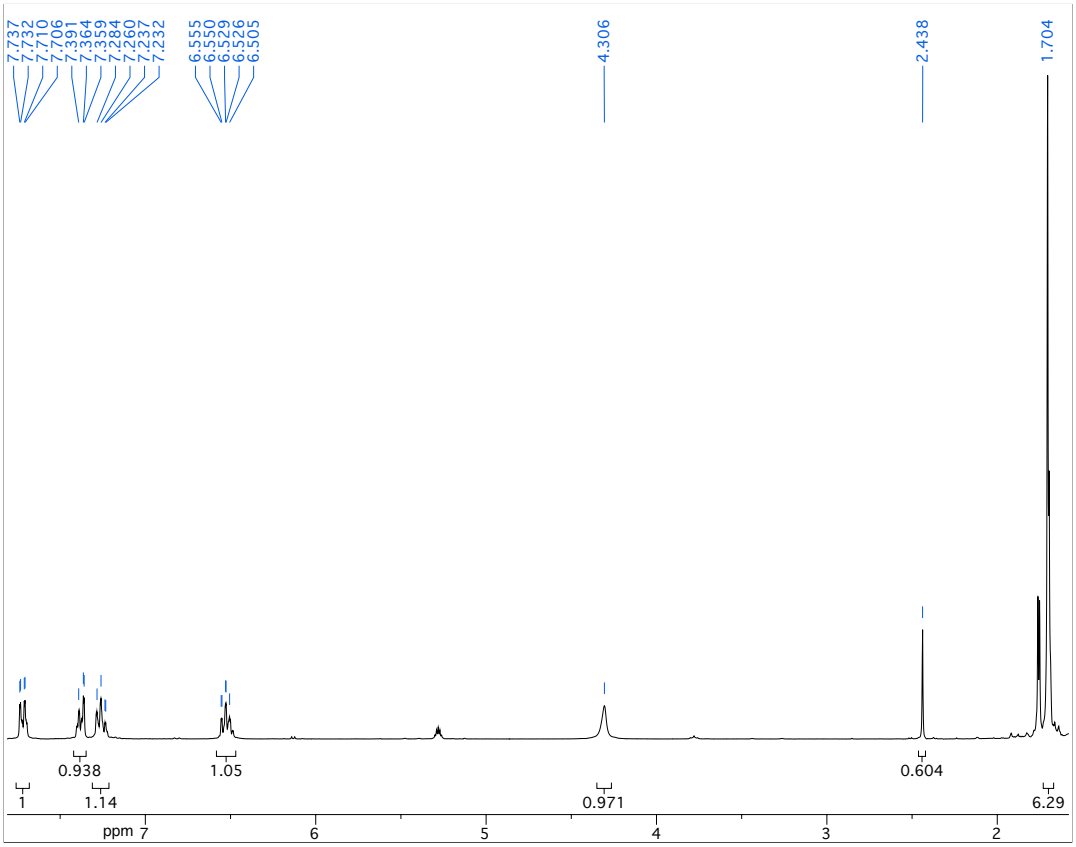
4
3
2



187a

2-iodo-*N*-(2-methylbut-3-yn-2-yl)aniline (187a). To a solution of 2-iodoaniline (200 mg, 0.91 mmol, 1 eq) in THF (9 mL) was added CuCl (18 mg, 0.1826 mmol, 20 mol%) at 25ºC and allowed to stir for 5 min. **162** (125 µL, 1.09 mmol, 1.2 eq) was added to the mixture over a period of 5 min., and allowed to stir for an additional 10 min. The reaction mixture was cooled to 0ºC at which time TEA (110 µL, 1.09 mmol, 1.2 eq) was added over a period of 5 min. Warming to 25ºC, the reaction mixture was allowed to stir for 18 h. The mixture was filtered through a small plug of silica gel and concentrated under reduced pressure. Purification by flash chromatography (EtOAc / hex., 1 : 10) provided **187a** (229 mg, 88%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.9 Hz, 1 H), 7.35 (d, *J* = 9.6 Hz, 1 H), 7.26 (t, *J* = 7 Hz, 1 H), 6.52 (t, *J* = 7 Hz, 1 H), 4.30 (bs, 1 H), 2.43 (s, 1 H), 1.70 (s, 6 H).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7.359
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7.260
7.237
7.232
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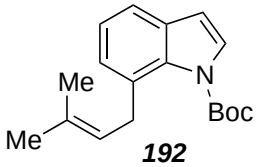
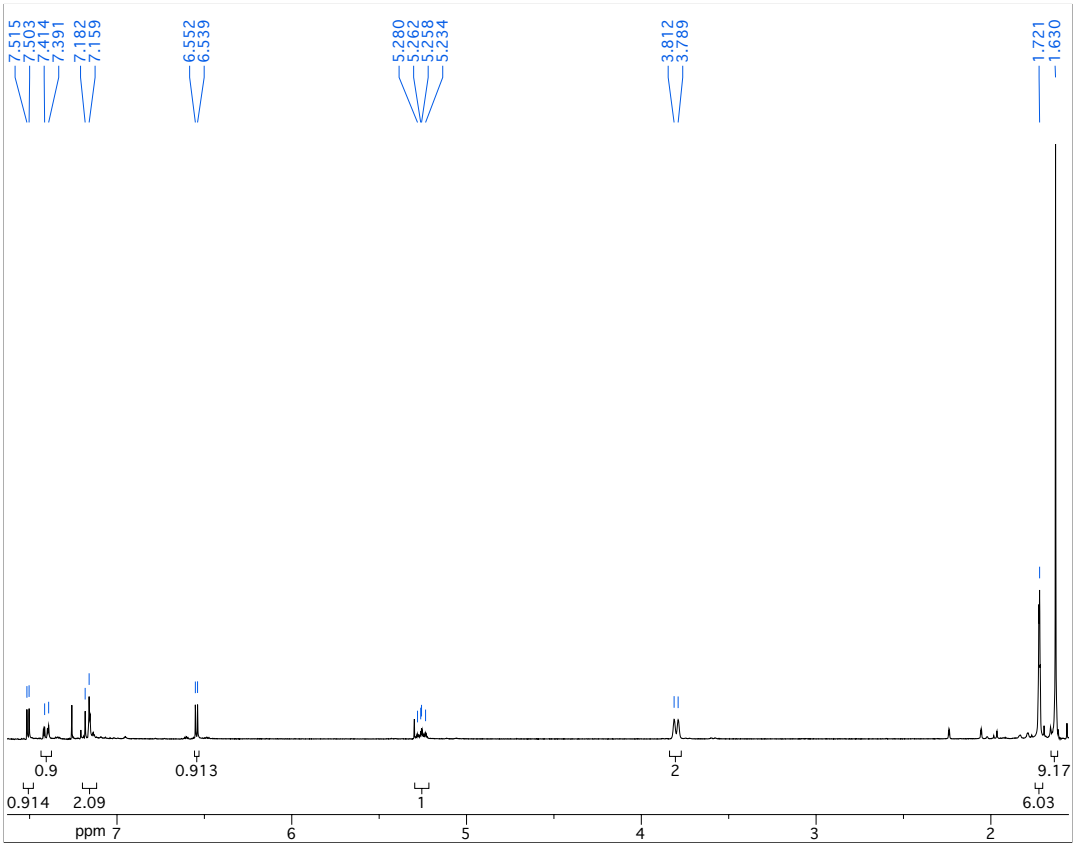
6.550
6.529
6.526
6.505
4.306
2.438
1.704
0.938
1.05
0.604
1
1.14
0.971
6.29
ppm 7
6
5
4
3
2



192

***tert*-butyl 7-(3-methylbut-2-en-1-yl)-1*H*-indole-1-carboxylate (192).** To a solution of **156** (500 mg, 2.69 mmol, 1 eq) in CH_2Cl_2 (25 mL) was added consecutively $(Boc)_2O$ (884 mg, 4.04 mmol, 1.5 eq), DMAP (65 mg, 0.53 mmol, 0.2 eq) and TEA (455 µL, 3.23 mmol, 1.2 eq) and allowed to stir for 18 h. 1 M HCl (20 mL) was added to the reaction mixture followed by dilution with CH_2Cl_2 (25 mL) and extracted. The organic layer was washed with H_2O (3 x 25 mL), brine (2 x 25 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided crude **192**, which upon purification by flash chromatography (EtOAc / hex., 1 : 20) provided **192** (538 mg, 70%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, *J* = 3.8 Hz, 1 H), 7.39 (d, *J* = 3.6 Hz, 1 H), 7.15 (m, 2 H), 6.53 (d, *J* = 3.8 Hz, 1 H), 5.25 (t, *J* = 6.8 Hz, 1 H), 3.78 (d, *J* = 6.8 Hz, 2 H), 1.72 (s, 6 H), 1.63 (s, 9 H).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5.258
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3.789
1.721
1.630
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0.914
2.09
1
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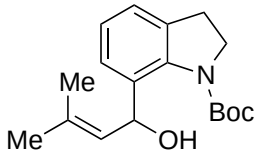
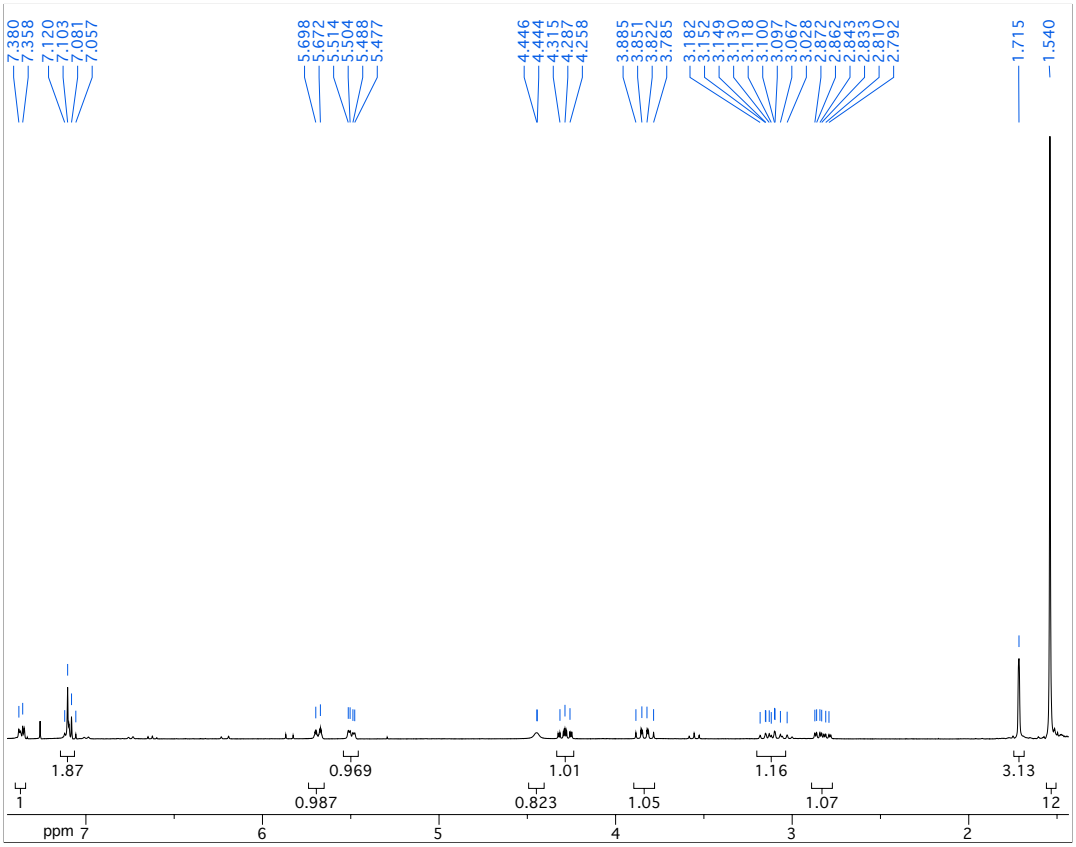
ppm
7
6
5
4
3
2



194

***tert*-butyl 7-(1-hydroxy-3-methylbut-2-en-1-yl)indoline-1-carboxylate (194)**. To a solution of **192** (500 mg, 1.75 mmol, 1 eq) in THF (2 mL) was added $(i\text{-PrO})_3B$ (610 µL, 2.62 mmol, 1.5 eq) and cooled to 0ºC. Freshly prepared LDA (245 mg, 2.27 mmol, 1.3 eq) was added dropwise over a period of 1 h. The reaction mixture was stirred at 0ºC for an additional 30 min., at which time 1 M HCl (3.6 mL) was added to the mixture and allowed to stir for 5 min. The water was removed and the organic layer was washed with H_2O (4 mL). Removal of the H_2O followed by dilution of the organic layer with acetone (4 mL) and H_2O (4 mL). Solid NaOH (105 mg, 2.62 mmol, 1.5 eq) and $NaHCO_3$ (1.2 g, 14.01 mmol, 8 eq) were added to the mixture and cooled to 0ºC, and Oxone™ (1.06 g, 1.73 mmol, 0.99 eq) was added in one protion. The reaction mixture was allowed to stir at 0ºC for 30 min., upon which time $Na_2S_2O_3$ (5 mL) was added. The mixture was partitioned in EtOAc and the organic layer was dried with Na_2SO_4. Filtration and concentration under reduced pressure provide crude **194**. Purification by flash chromatography (EtOAc / hex., 1 : 10) provided **194** (422mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 6.5 Hz, 1 H) 7.12-7.05 (m, 2 H), 5.67 (d, J = 7.9 Hz, 1 H), 5.47 (d, J = 7.9 Hz, 1 H), 4.44 (bs, 1 H), 4.28 (t, J = 8.5 Hz, 1 H), 3.78 (dd,

J = 10.1, 8.7 Hz, 1 H), 3.18-3.02 (m, 1 H), 2.79 (dd, *J* = 10.1, 8.7 Hz, 1 H), 1.71 (s, 3 H), 1.54 (s, 12 H).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4.315
4.287
4.258
3.885
3.851
3.822
3.785
3.182
3.152
3.149
3.130
3.118
3.100
3.097
3.067
3.028
2.872
2.862
2.843
2.833
2.810
2.792
1.715
1.540
1.87
0.969
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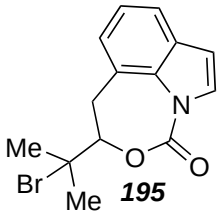
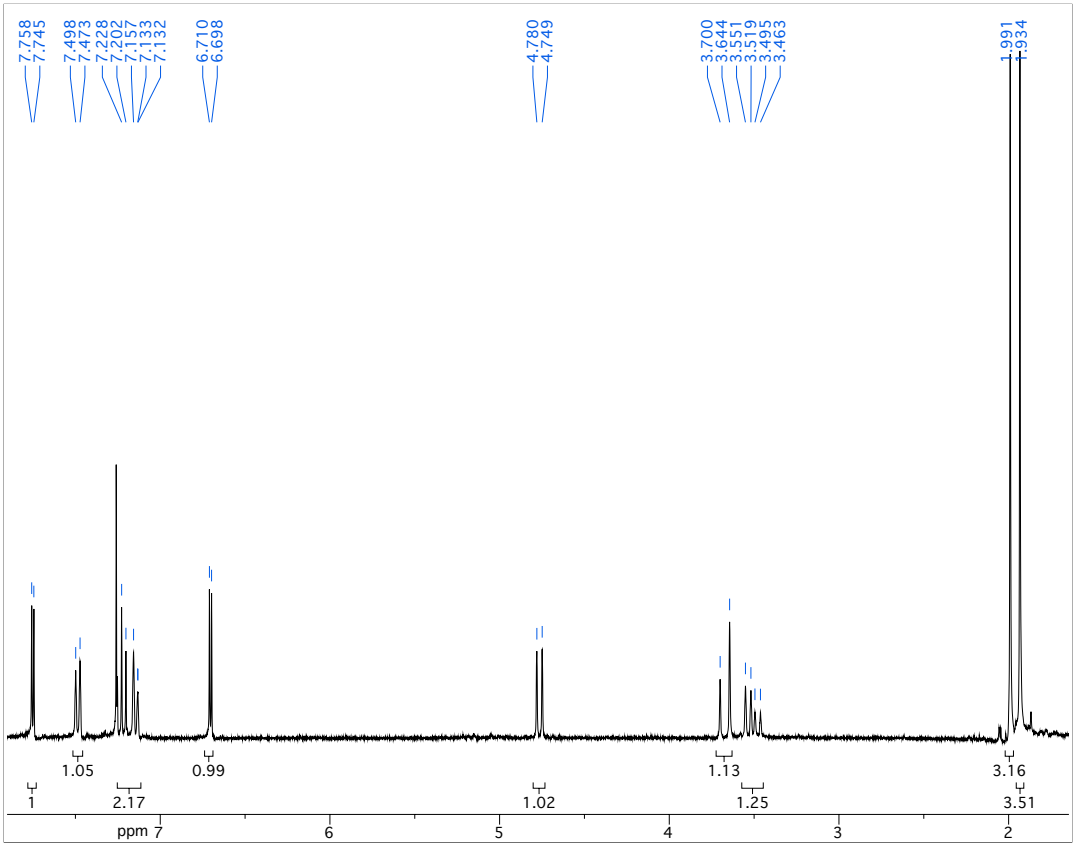


195

2-(2-bromopropan-2-yl)-1*H*-[1,3]oxazepino[5,4,3-*hi*]indol-4(2*H*)-one (195). To a solution of **156** (40 mg, 0.21 mmol, 1 eq) in *t*-BuOH (1.15 mL) and H_2O (350 µL) was added solid NBS (42 mg, 0.23 mmol, 1.1 eq) in one portion. The reaction mixture was allowed to stir at 25ºC for 8 h., at which time sat. $Na_2S_2O_3$ (4 mL) was added. The mixture was diluted with EtOAc (10 mL) and separated. The organic layer was washed with 1 M HCl (3 x 5 mL), H_2O (3 x 5 mL), brine and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided crude product. Purification by flash chromatography (EtOAc / hex., 1 : 20) afforded **195** (22 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 3.8 Hz, 1 H), 7.47 (d, *J* = 7.7 Hz, 1 H), 7.22 (t, *J* = 7.7 Hz, 1 H), 7.13 (d, *J* = 7.4 Hz, 1 H), 6.69 (d, *J* = 3.8 Hz, 1 H), 4.74 (d, *J* = 9.4 Hz, 1 H), 3.64 (d, *J* = 16.8 Hz, 1 H), 3.46 (dd, *J* = 9.4, 16.8 Hz, 1 H), 1.99 (s, 3 H), 1.93 (s, 3 H).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3.700
3.644
3.551
3.519
3.495
3.463
1.991
1.934
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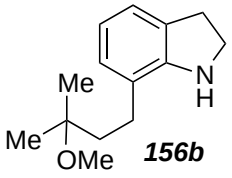
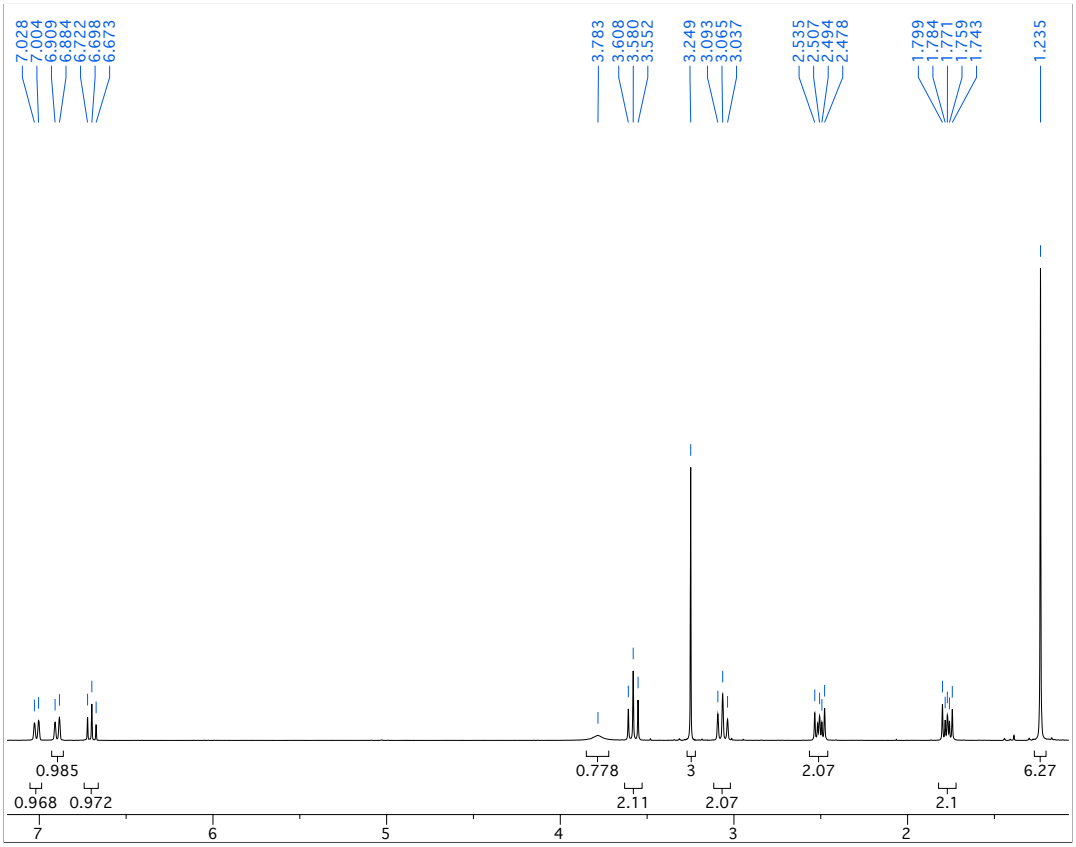
2



156b

7-(3-methoxy-3-methylbutyl)indoline (156b). To a solution of **156a** (2.48 g, 13.24 mmol, 1 eq) in MeOH (75 mL) was added CSA (15 g, 66.21 mmol, 5 eq) and heated to reflux for 48 h. The reaction was cooled to room temperature and sat. $NaHCO_3$ (30 mL) was added. The mixture was extracted with EtOAc (3 x 25 mL), the organic layers were combined and washed with H_2O (2 x 20 mL), brine (20 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided crude **156b**. Purification by flash chromatography (EtOAc / hex., 1 : 10–1 : 5) afforded pure **156b** (1.86 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (d, *J* = 7.2 Hz, 1 H), 6.88 (d, *J* = 7.6 Hz, 1 H), 6.69 (t, *J* = 7.6 Hz, 1H), 3.78 (bs, 1 H), 3.58 (t, *J* = 8.4 Hz, 2 H), 3.24 (s, 3 H), 3.06 (t, *J* = 8.4 Hz, 2 H), 2.50 (m, 2 H), 1.77 (m, 2 H), 1.23 (s, 6 H).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1.771
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1.743
1.235
0.985
0.968
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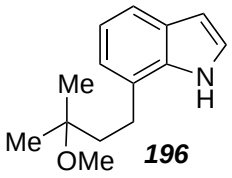
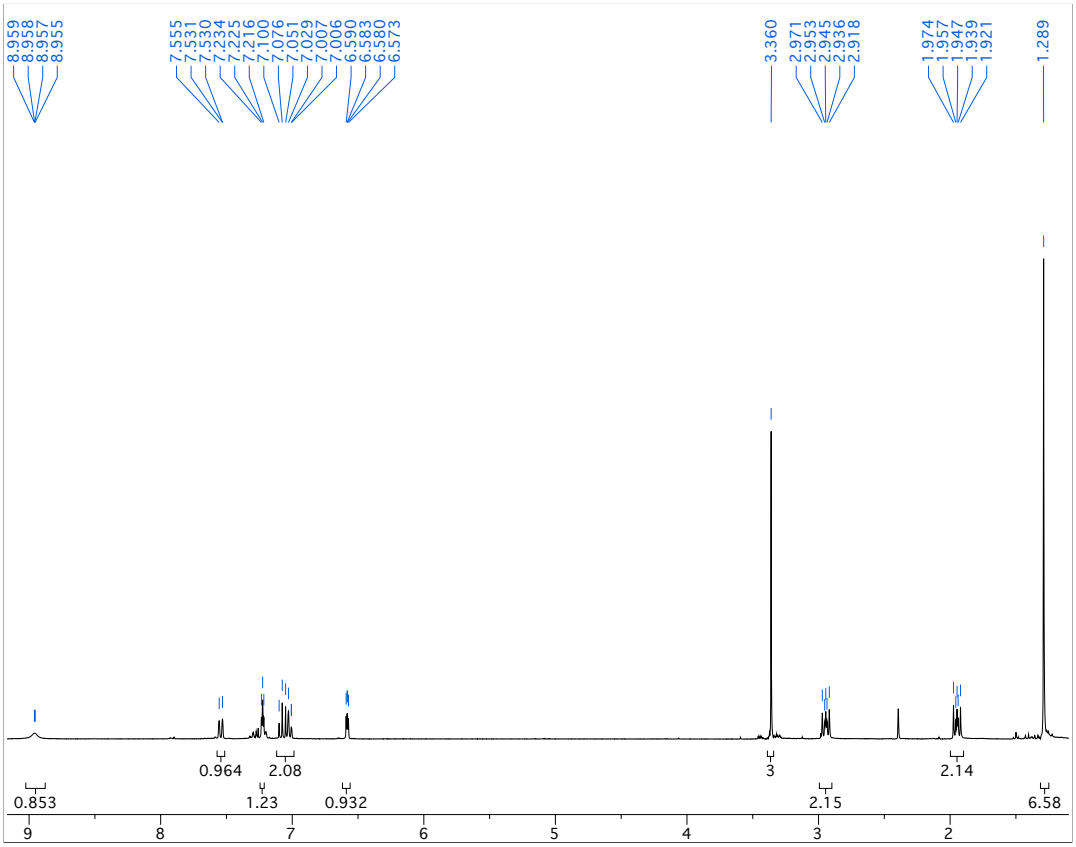


196

7-(3-methoxy-3-methylbutyl)-1*H*-indole (196). To a solution of **156b** (2.8 g, 12.76 mmol, 1 eq) in toluene (130 mL) was added MnO_2 (11 g, 127.66 mmol, 10 eq). The reaction mixture was heated to reflux for 18 h., upon which it was filtered through a pad of Celite and concentrated under reduced pressure. Purification by flash chromatography (EtOAc / hex., 1 : 5) provided **196** (1.37 g, 50%) as a brown orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (bs, 1 H), 7.53 (d, *J* = 7.3 Hz, 1 H), 7.22 (t, *J* = 2.8 Hz, 1 H), 7.10-7.00 (m, 2 H), 6.58 (t, *J* = 2.8 Hz, 1 H), 3.36 (s, 3 H), 2.94 (m, 2 H), 1.94 (m, 2 H), 1.28 (s, 6 H).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2.945
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2.918
1.974
1.957
1.947
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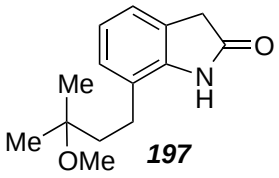
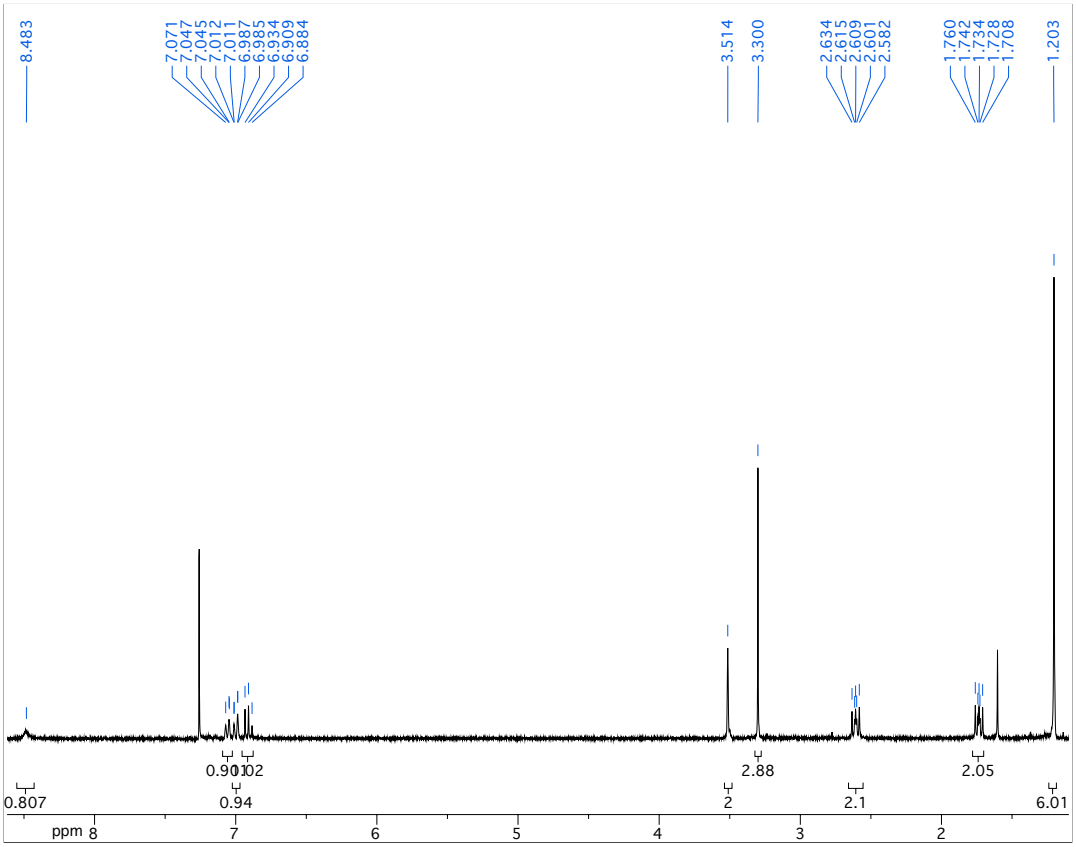


197

7-(3-methoxy-3-methylbutyl)indolin-2-one (197). To a solution of **196** (151 mg, 0.69 mmol, 1 eq) in *t*-BuOH (6.37 mL) and H_2O (630 µL) was added solid NBS (148 mg, 0.83 mmol, 1.2 eq) in one portion. The reaction was covered in foil and allowed to stir for 18 h. After the allotted time $Na_2S_2O_3$ (5 mL) was added and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with H_2O (3 x 10 mL), brine (20 mL) and dried over Na_2SO_4. Filtration and concetration under reduced pressure provided crude **197**. Purification by flash chromatography (EtOAc / hex., 1 : 5–9 : 20) to afford **197** (97 mg, 60%) as a yellow orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (bs, 1 H), 7.04 (d, *J* = 7.1 Hz, 1 H), 6.98 (d, *J* = 7.6 Hz, 1 H), 6.90 (t, *J* = 7.6 Hz, 1 H), 3.51 (s, 2 H), 3.30 (s, 3 H), 2.60 (m, 2 H), 1.73 (m, 2 H), 1.20 (s, 6 H).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2.609
2.601
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1.734
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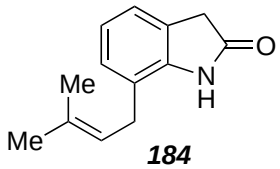
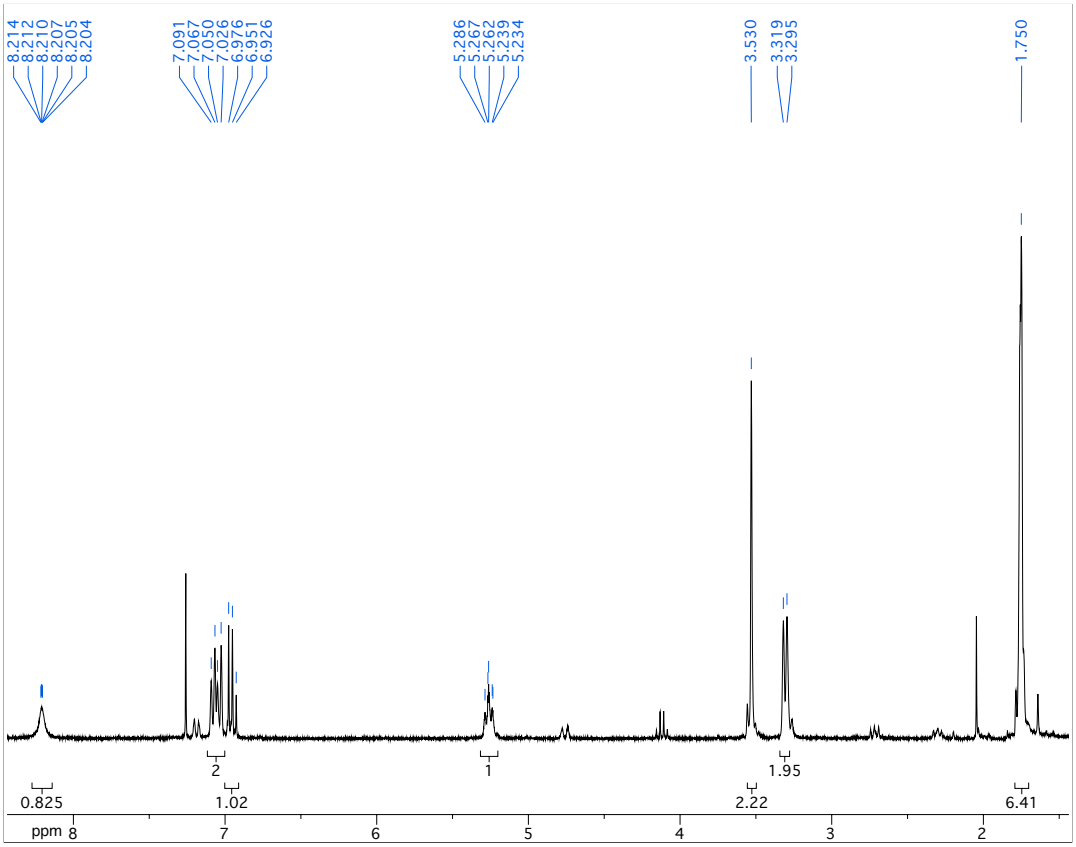


184

7-(3-methylbut-2-en-1-yl)indolin-2-one (184). To a solution of **197** (50 mg, 0.21 mmol, 1 eq) in toluene (2 mL) was added Amberlyst 15 (50 mg, 1 weight eq) and refluxed for 3 h. The mixture was cooled to room temperature, filtered and concentrated under reduced pressure to afford **184** (41 mg, 95%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (bs, 1 H), 7.05 (d, *J* = 12.4 Hz, 1 H), 7.02 (d, *J* = 12.4 Hz, 1 H), 6.95 (t, *J* = 7.5 Hz, 1 H), 5.26 (t, *J* = 7.1 Hz, 1 H), 3.53 (s, 2 H), 3.29 (d, *J* = 7.1 Hz, 2 H), 1.75 (s, 6 H).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5.286
5.267
5.262
5.239
5.234
3.530
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3.295
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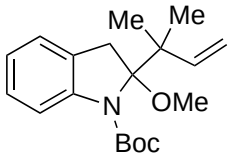
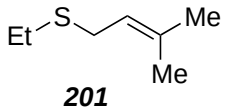


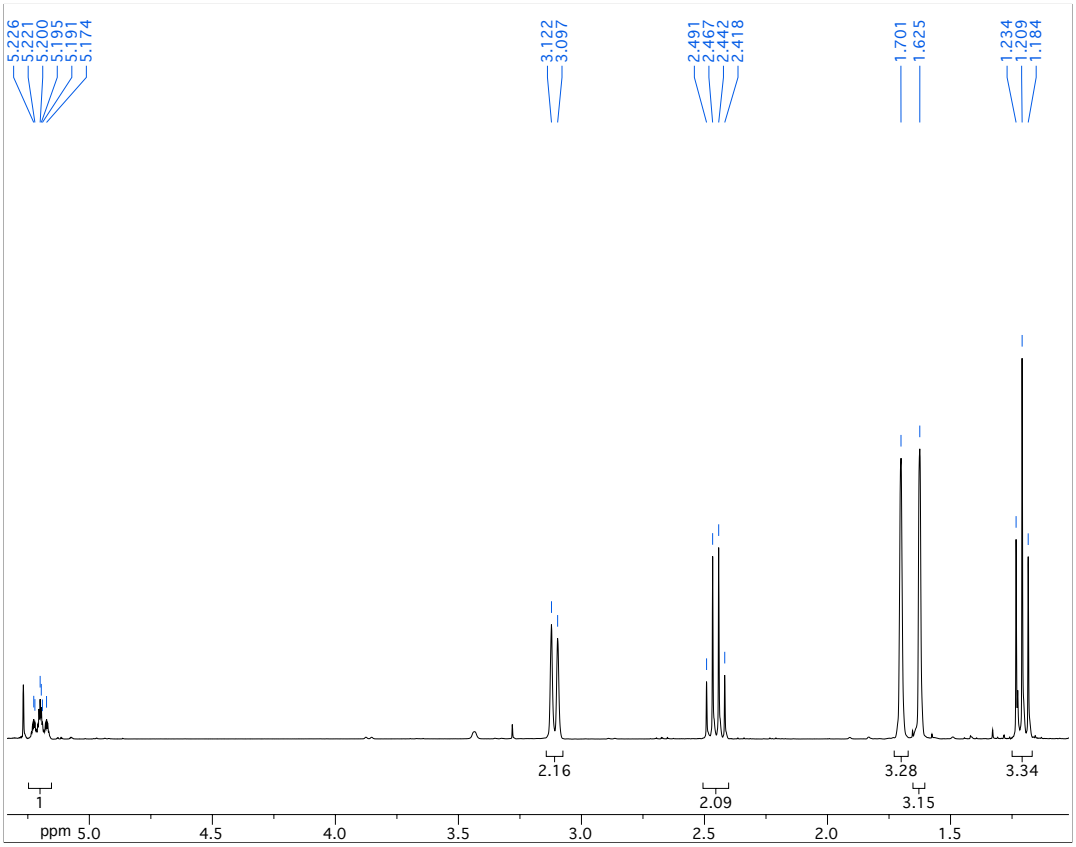
199

***tert*-butyl 2-methoxy-2-(2-methylbut-3-en-2-yl)indoline-1-carboxylate (199)**. To a solution of NaH (4 mg, 0.16 mmol, 1 eq, 60%) in THF (2 mL) was added **179** (50 mg, 0.16 mmol, 1 eq) in THF (1 mL) at 0ºC. The reaction was warmed to room temperature and allowed to stir for 10 min. MeI (10 mL, 0.17 mmol, 1.05 eq) was added to the reaction mixture and allowed to stir for 18 h. Sat. NH_4Cl (3 mL) was added and the mixture was extracted with Et_2O (2 x 5 mL). The organics were washed with H_2O (2 x 5 mL), brine (5 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided crude **199**. Purification by flash chromatography (EtOAc / hex., 1 : 5) afforded **199** (69 mg, 75%) as a white solid.



201

ethyl(3-methylbut-2-en-1-yl)sulfane (201). Na^{o} (1.96 g, 85.34 mmol, 1.05 eq) was added slowly to MeOH (40 mL) at 0^{o}C and allowed to stir until all Na^{o} was dissolved. EtSH (6.1 mL, 82.90 mmol, 1.02 eq) was added to the solution drop wise over a period of 2 h. A mixture of **205** and **206** (8.5 g, 81.27 mmol, 1 eq) was added to the reaction mixture at a drop wise pace, upon addition the reaction was allowed to stir for 30 min. The mixture was poured into a separation funnel containing H_2O (50 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The organic layers were combined and concentrated under reduced pressure to afford **201** (8.4 g, 80%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.20 (t, *J* = 7.5 Hz, 1 H), 3.09 (d, *J* = 7.5 Hz, 2 H), 2.44 (q, *J* = 7.4 Hz, 2 H), 1.70 (s, 3 H), 1.62 (s, 3 H), 1.20 (t, *J* = 7.3 Hz, 3 H).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3.097
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1.209
1.184
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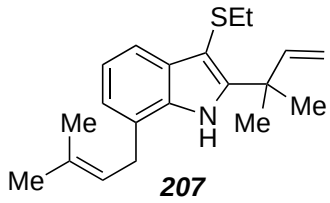
3.5
3.0
2.5
2.0
1.5

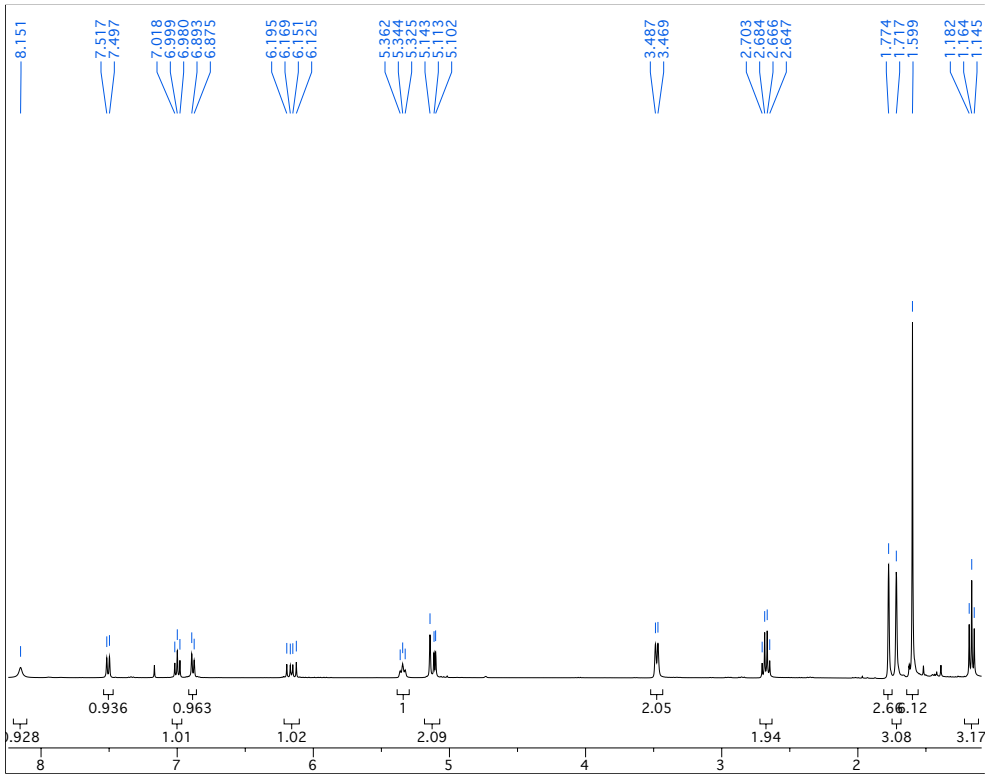


207

3-(ethylthio)-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indole (207). [A] To a solution of **201** (281 mg, 1.61 mmol, 1.5 eq) in CH_2Cl_2 (10 mL) at -30ºC was added NCS (216 mg, 1.61 mmol, 1.5 eq), and allowed to stir at -30ºC for 45 min. **156** (200 mg, 1.07 mmol, 1 eq) in CH_2Cl_2 (1 mL) was then added to the reaction mixture at -30ºC and stirring continued for an additional 30 min. The mixture was then warmed to 30ºC over a period of 1 h., where it remained for an additional 2 h. After cooling to room temperature, H_2O (5 mL) was added and the two phases separated. The organic layer was washed with 1 M HCl (2 x 10 mL), H_2O (3 x 10 mL), brine (10 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided crude **207**. Purification by flash chromatography (EtOAc / hex., 1 : 20) afforded **207** (145 mg, 43%) as a yellow oil.

[B] To a solution of **156** (100 mg, 0.53 mmol, 1 eq) and **201** (102 mg, 0.58 mmol, 1.1 eq) in CH_2Cl_2 (5 mL) at -78ºC was added *t*-BuOCl (66 µL, 0.58 mmol, 1.1 eq). The reaction mixture was allowed to stir at -78ºC for 1 h., at which point it was allowed to warm to room temperature where it remained for 18 h. H_2O (5 mL) was added and the two phases separated. The organic layer was washed with 1 M HCl (2 x 10 mL), H_2O (3 x 10 mL), brine (10 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure

provided crude **207**. Purification by flash chromatography (EtOAc / hex., 1 : 20) afforded **207** (109 mg, 65%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (bs, 1 H), 7.49 (d, *J* = 7.9 Hz, 1 H), 6.99 (t, *J* = 7.4 Hz, 1 H), 6.87 (d, *J* = 7.4 Hz, 1 H), 6.12 (dd, *J* = 10.3, 17.7 Hz, 1 H), 5.34 (t, *J* = 7.2 Hz, 1 H), 6.12 (dd, *J* = 10.3, 17.7 Hz, 2 H), 3.46 (d, *J* = 7.2 Hz, 2 H), 2.66 (q, *J* = 7.5 Hz, 2 H), 1.77 (s, 3 H), 1.71 (s, 3 H), 1.59 (s, 6 H), 1.16 (t, *J* = 7.5 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.5, 145.5, 133.1, 132.9, 132.1, 123.3, 122.7, 121.6, 120.2, 117.0, 112.8, 101.1, 39.5, 31.1, 30.7, 27.2, 25.6, 17.8, 14.9. HRMS (ESI_APCI) calcd for $C_{20}H_{27}NS$ [M + H] 314.1937, found 314.1944.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5.344
5.325
5.143
5.113
5.102
3.487
3.469
2.703
2.684
2.666
2.647
1.774
1.717
1.599
1.182
1.164
1.145
0.936
0.963
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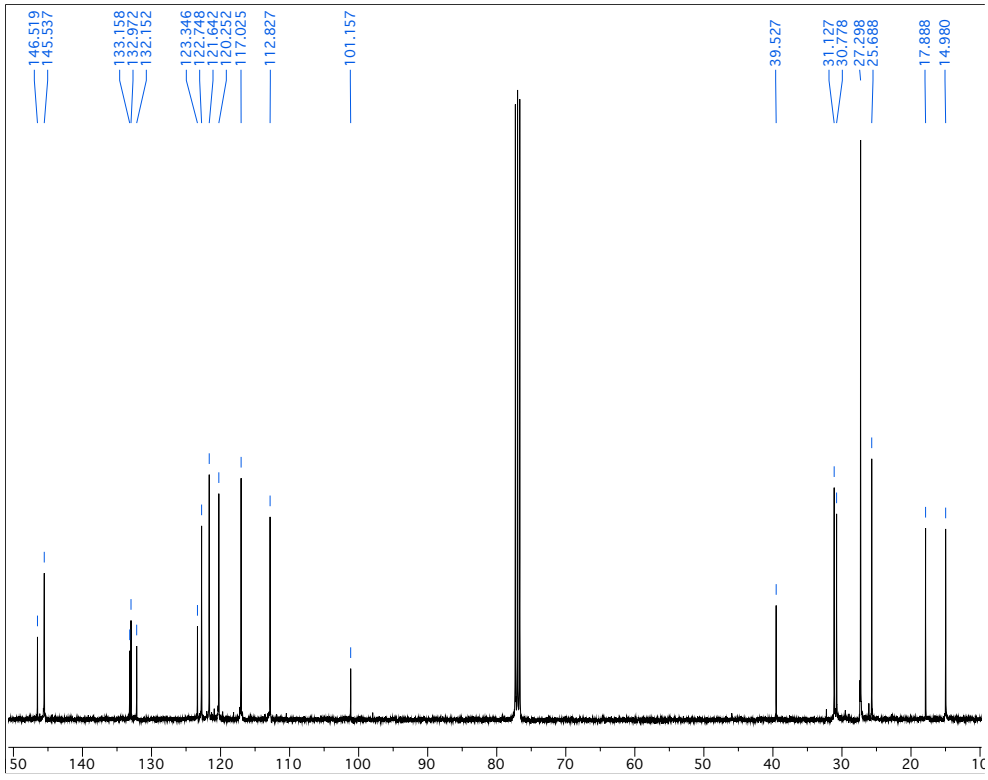
6.12
0.928
1.01
1.02
2.09
1.94
3.08
3.17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132.152
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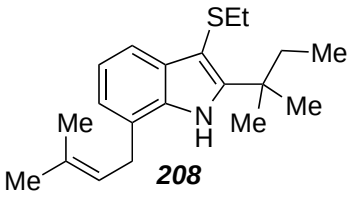
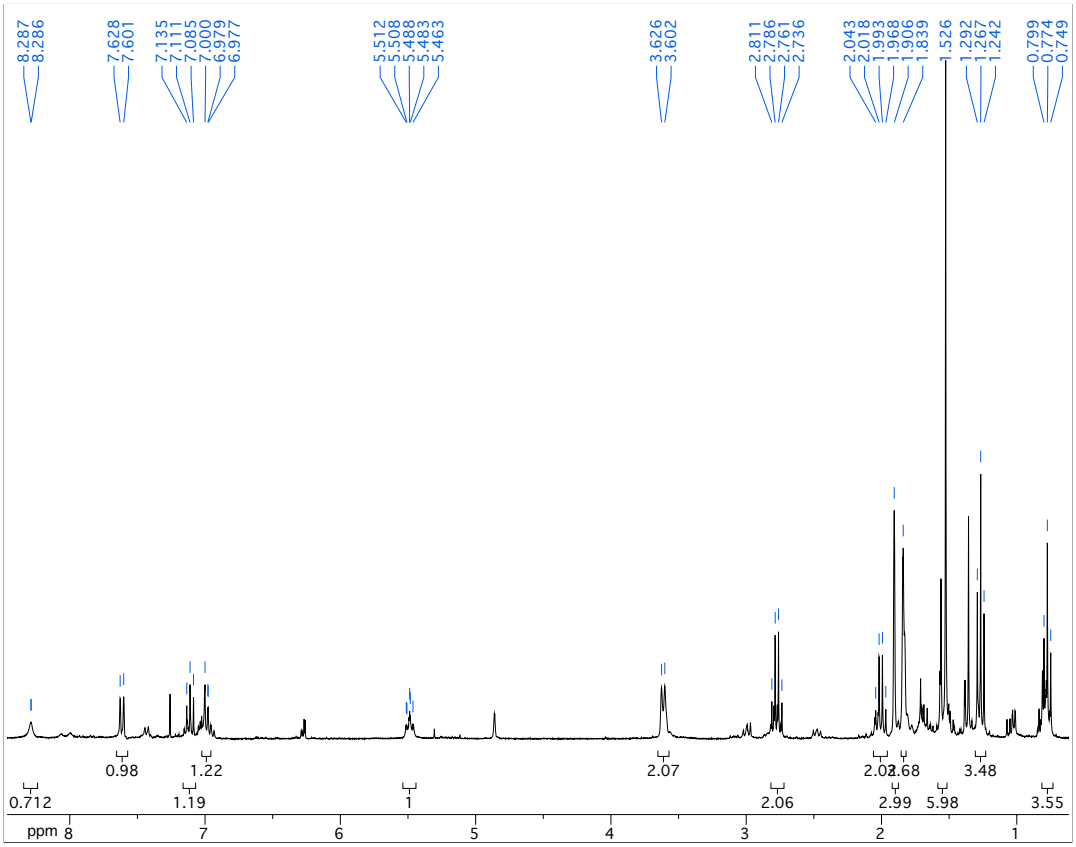
31.127
30.778
27.298
25.688
17.888
14.980



208

3-(ethylthio)-7-(3-methylbut-2-en-1-yl)-2-(*tert*-pentyl)-1*H*-indole (208). To a solution of **207** (50 mg, 0.15 mmol, 1 eq) in EtOH (1.5 mL) was added Raney Ni^{o} (50 mg, 1 weight eq) at room temperature. The solution was allowed to stir for 30 min., upon which it was filtered through a pad of Celite and concentrated to give **208** (42 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (bs, 1 H), 7.60 (d, *J* = 7.8 Hz, 1 H), 7.11 (t, *J* = 7.3 Hz, 1 H), 7.00 (d, *J* = 7.3 Hz, 1 H), 5.48 (t, *J* = 7.3 Hz, 1 H), 3.60 (d, *J* = 7.3 Hz, 2 H), 2.76 (q, *J* = 7.5 Hz, 2 H), 1.96 (q, *J* = 7.5 Hz, 2 H), 1.90 (s, 3 H), 1.83 (s, 3 H), 1.52 (s, 6 H), 1.26 (t, *J* = 7.5 Hz, 3 H), 0.77 (t, *J* = 7.5 Hz, 3 H).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7.085
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5.488
5.483
5.463
3.626
3.602
2.811
2.786
2.761
2.736
2.043
2.018
1.993
1.968
1.906
1.839
1.526
1.292
1.267
1.242
0.799
0.774
0.749
0.98
1.22
2.07
2.02
2.68
3.48
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2.99
5.98
3.55
ppm
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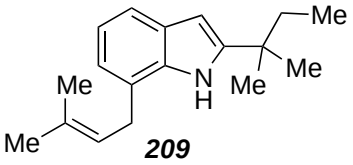
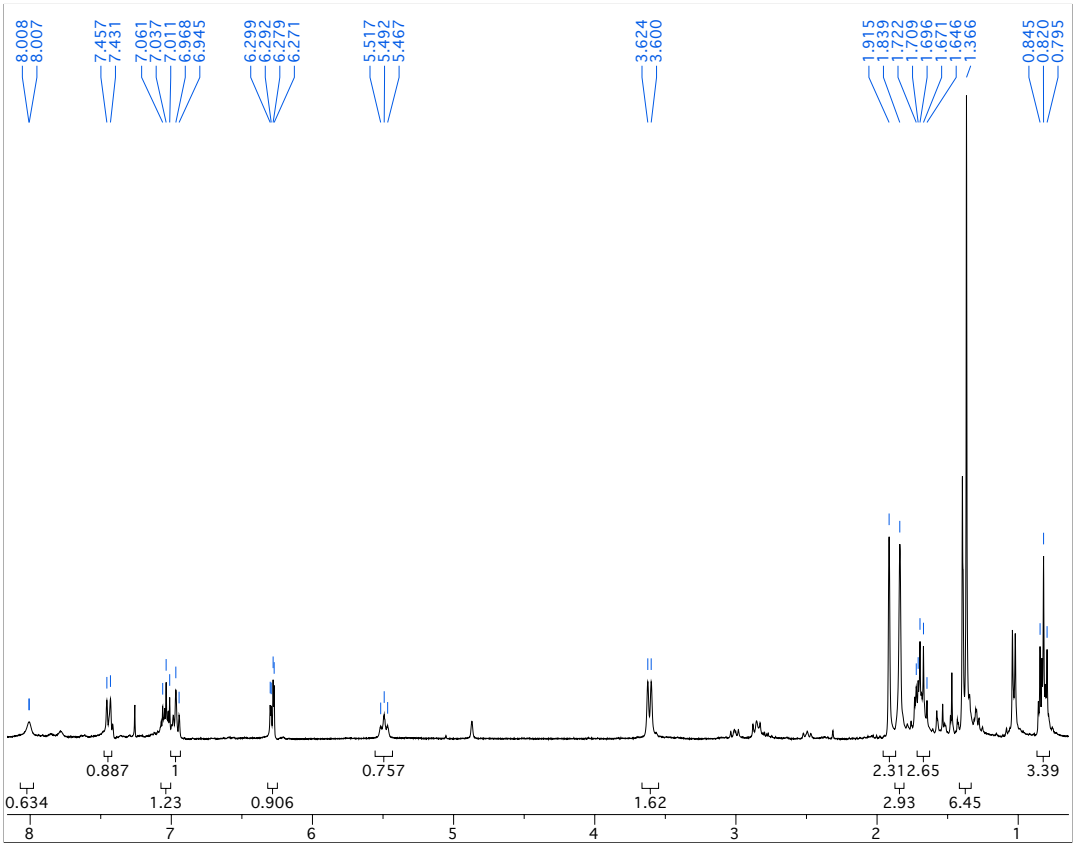
3
2
1



209

7-(3-methylbut-2-en-1-yl)-2-(*tert*-pentyl)-1*H*-indole (209). To a solution of **207** (50 mg, 0.15 mmol, 1 eq) in THF (2 mL) was added Raney Niº (50 mg, 1 weight eq) at room temperature. The solution was allowed to stir for 45 min., upon which it was filtered through a pad of Celite and concentrated to give **208** (37 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (bs, 1 H), 7.43 (d, *J* = 7.6 Hz, 1 H), 7.03 (t, *J* = 7.6 Hz, 1 H), 6.94 (d, *J* = 7.0 Hz, 1 H), 6.27 (dd, *J* = 2.1, 6.1 Hz, 1 H), 5.49 (t, *J* = 7.4 Hz, 1 H), 3.60 (d, *J* = 7.4 Hz, 2 H), 1.91 (s, 3 H), 1.83 (s, 3 H), 1.67 (q, *J* = 7.5 Hz, 2 H), 1.36 (s, 6 H), 0.82 (t, *J* = 7.5 Hz, 3 H).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5.517
5.492
5.467
3.624
3.600
1.915
1.839
1.722
1.709
1.696
1.671
1.646
1.366
0.845
0.820
0.795
0.887
1
0.757
2.31
2.65
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1.23
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1.62
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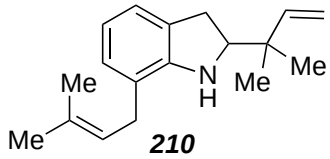
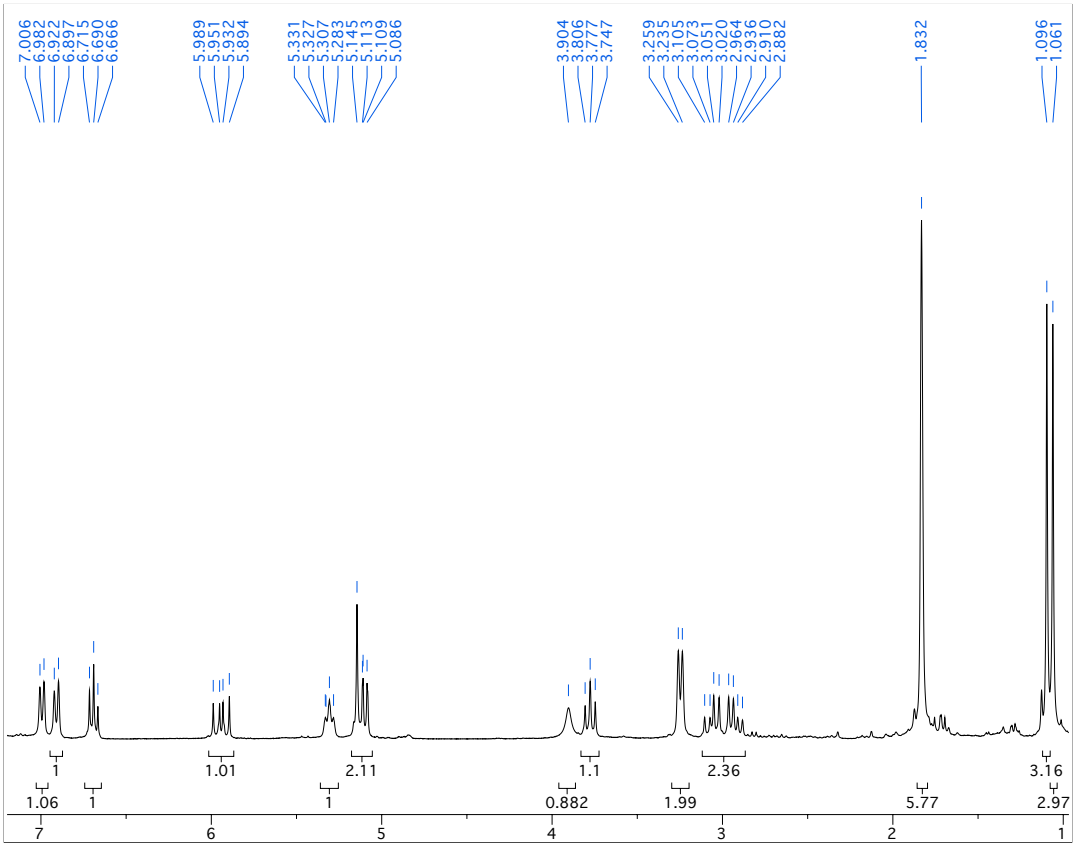
5
4
3
2
1



210

7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)indoline (210). To a solution of **207** (951 mg, 3.03 mmol, 1 eq) in THF (15 mL) and TFA (15 mL) at 0ºC was added drop wise BH_3•Me_2S (910 µL, 9.10 mmol, 3 eq). The reaction mixture was stirred at 0ºC for 30 min., at which time H_2O (25 mL) was added. The mixture concentrated under reduced pressure and azeotroped with toluene (3 x 40 mL). The residue was diluted with EtOAc (25 mL) and washed with 1 M NaOH (3 x 10 mL), H_2O (3 x 10 mL), brine (20 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided crude **210** as a brown oil. Purification by flash chromatography (EtOAc / hex., 1 : 10–1 : 5) afforded **210** (542 mg, 70%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (d, *J* = 7.1 Hz, 1 H), 6.89 (d, *J* = 7.4 Hz, 1 H), 6.69 (t, *J* = 7.4 Hz, 1 H), 5.89 (dd, *J* = 11.2, 17.1 Hz, 1 H), 5.30 (t, *J* = 7.2 Hz, 1 H), 5.08 (dd, *J* = 11.2, 17.1 Hz, 2 H), 3.90 (bs, 1 H), 3.77 (t, *J* = 8.9 Hz, 1 H), 3.23 (d, *J* = 7.2 Hz, 2 H), 3.10-2.88 (m, 2 H), 1.83 (s, 6 H), 1.09 (s, 3 H), 1.06 (s, 3 H).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5.283
5.145
5.113
5.109
5.086
3.904
3.806
3.777
3.747
3.259
3.235
3.105
3.073
3.051
3.020
2.964
2.936
2.910
2.882
1.832
1.096
1.061
1
1.01
2.11
1.1
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3.16
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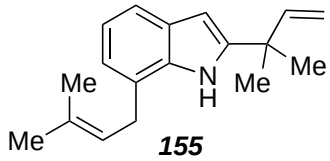
2
1

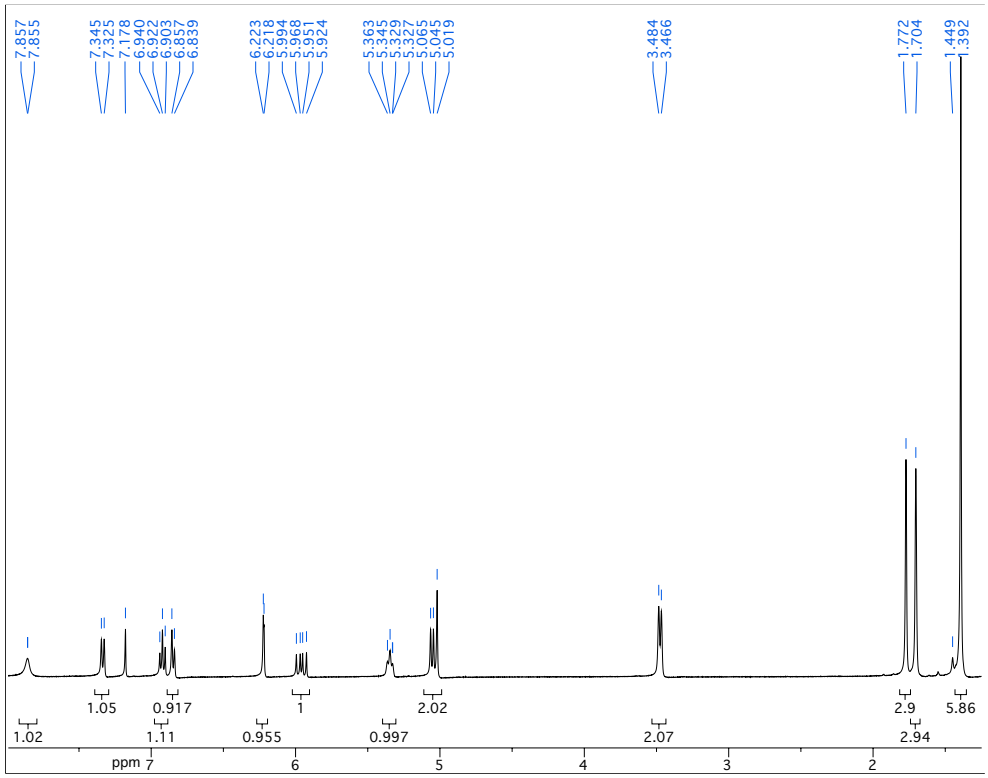


155

7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indole (155). [A] To a solution of **210** (150 mg, 0.58 mmol, 1 eq) in toluene (5 mL) was added MnO_2 (460 mg, 5.28 mmol, 9 eq) at room temperature. The reaction mixture was heated to reflux for a period of 18 h. Upon cooling the mixture was filtered through a pad of Celite and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc / hex., 1 : 5) to provide **155** (145 mg, 98%) as a yellow oil.

[B] To a solution of BF_3•Et_2O (185 µL, 1.47 mmol, 1 eq) in CH_2Cl_2 (15 mL) at 0ºC was added EtSH (545 µL, 7.35 mmol, 5 eq). The reaction stirred for 5 min. at 0ºC at which time a solution of **207** (461 mg, 1.47 mmol, 1 eq) in CH_2Cl_2 (3 mL) was added to the mixture. Upon warming to room temperature the mixture continued to stir for 18 h. The mixture was then poured in to a cold solution of sat. $NaHCO_3$ (15 mL) and allowed to stir for 15 min. The solution was diluted with CH_2Cl_2 (20 mL) and separated. The organic layer was washed with 1 M NaOH (3 x 10 mL), H_2O (3 x 10 mL), brine (20 mL) and dried over Na_2SO_4. Filtration through a pad of Celite and concentration under reduced pressure provided crude **155**. Purification by flash chromatography (CH_2Cl_2 / hex., 1 : 50–3 : 100) provided **155** (353 mg, 95%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (bs, 1 H), 7.32 (d, *J* = 7.7 Hz, 1 H), 6.92 (t, *J* =

7.3 Hz, 1 H), 6.83 (d, *J* = 7.3 Hz, 1 H), 6.21 (s, 1 H), 5.92 (dd, *J* = 10.7, 17.3 Hz, 1 H), 5.34 (t, *J* = 7.3 Hz, 1 H), 5.01 (dd, *J* = 10.7, 17.3 Hz, 2 H), 3.46 (d, *J* = 7.3 Hz, 2 H), 1.77 (s, 3 H), 1.70 (s, 3 H), 1.39 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.1, 145.1, 135.1, 132.6, 128.4, 123.0, 122.7, 120.9, 119.6, 118.1, 111.8, 97.9, 38.1, 31.1, 27.3, 25.6, 17.8. HRMS (ESI_APCI) calcd for $C_{18}H_{23}N$ [M + H] 254.1903, found 254.1907.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5.329
5.327
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3.484
3.466
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1.704
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1.392
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2.02
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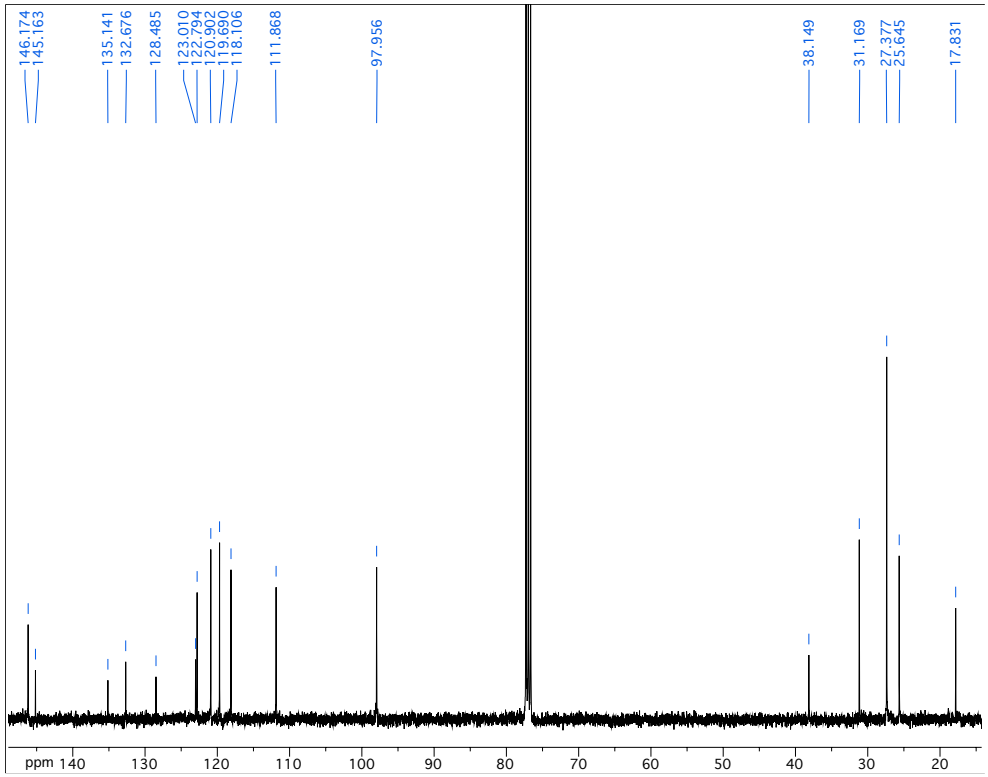
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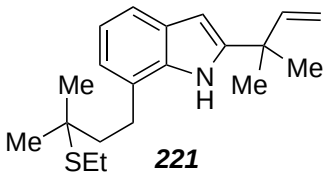
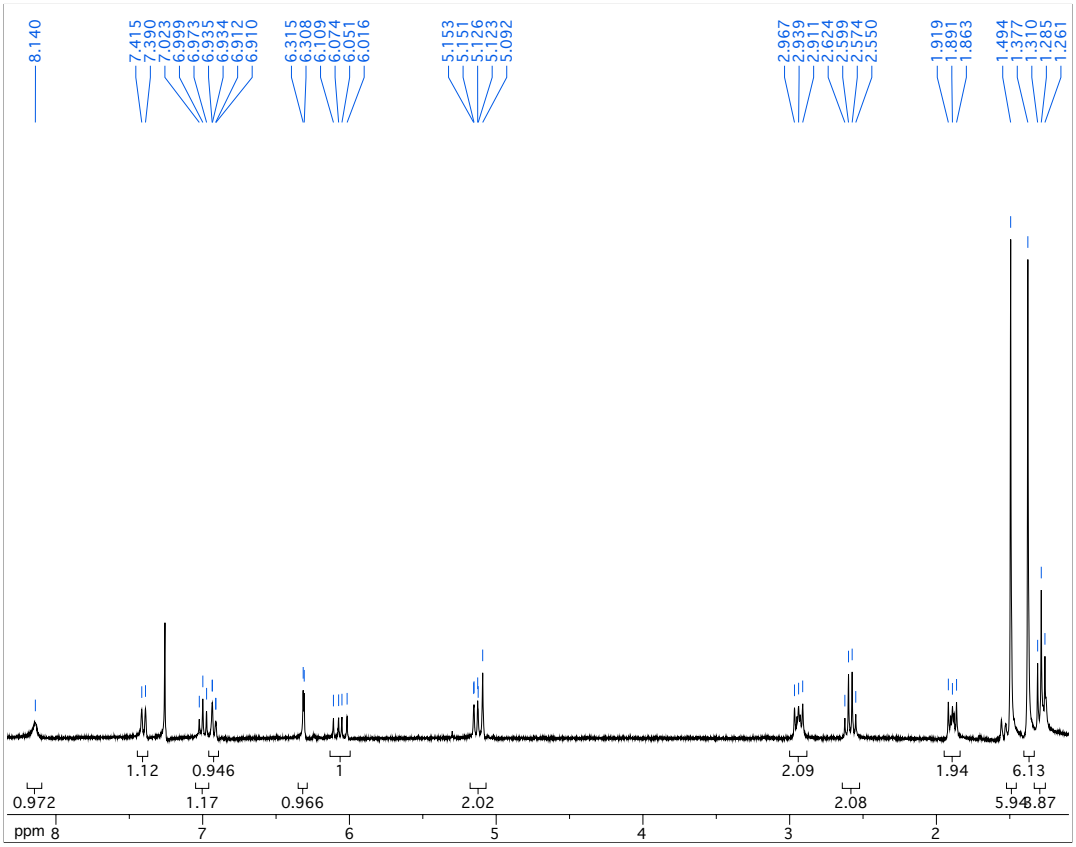
30
20



221

7-(3-(ethylthio)-3-methylbutyl)-2-(2-methylbut-3-en-2-yl)-1*H*-indole (221). To a solution of $AlCl_3$ (12 mL, 0.095 mmol, 1.5 eq) in CH_2Cl_2 (200 µL) at 0ºC was added EtSH (25 µL, 0.31 mmol, 5 eq). The reaction stirred for 5 min. at 0ºC at which time a solution of **207** (20 mg, 0.063 mmol, 1 eq) in CH_2Cl_2 (100 µL) was added to the mixture. Stirring continued at 0ºC for 5 h. The mixture was then poured in to a cold solution of sat. $NaHCO_3$ (5 mL) and allowed to stir for 15 min. The solution was diluted with CH_2Cl_2 (10 mL) and separated. The organic layer was washed with 1 M NaOH (3 x 5 mL), H_2O (3 x 5 mL), brine (10 mL) and dried over Na_2SO_4. Filtration through a pad of Celite and concentration under reduced pressure provided the crude residue. Purification by flash chromatography (CH_2Cl_2 / hex., 1 : 50–3 : 100) provided **221** (19 mg, 98%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (bs, 1 H), 7.39 (d, *J* = 7.6 Hz, 1 H), 6.96 (t, *J* = 7.2 Hz, 1 H), 6.91 (d, *J* = 7.2 Hz, 1 H), 6.31 (s, 1 H), 6.01 (dd, *J* = 10.6, 17.4 Hz, 1 H), 5.09 (dd, *J* = 10.6, 17.4 Hz, 2 H), 2.91 (m, 2 H), 2.57 (q, *J* = 7.5 Hz, 2 H), 1.86 (m, 2 H), 1.49 (s, 6 H), 1.37 (s, 6 H), 1.28 (t, *J* = 7.5 Hz, 3 H).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6.016
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5.126
5.123
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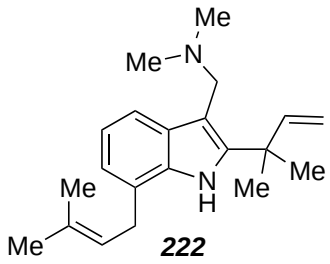
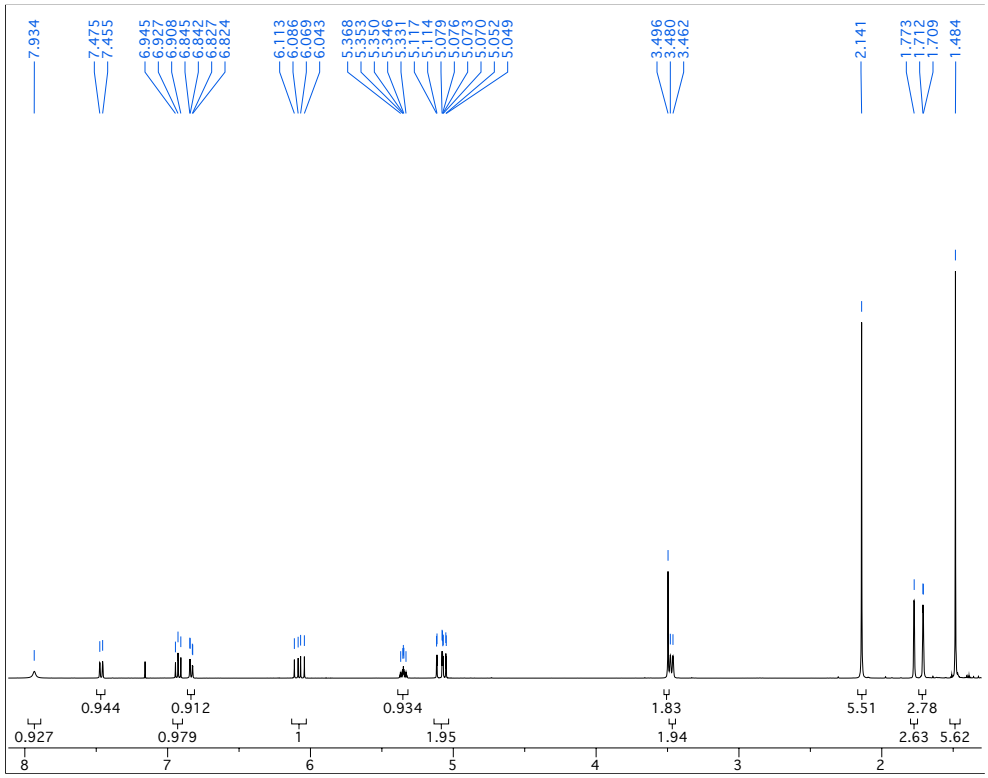


222

***N*,*N*-dimethyl-1-(7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methanamine (222).** To a solution of Me_2NH (81 µL, 0.64 mmol, 1.05 eq, 40% aqueous) in AcOH (1 mL) was added H_2CO (48 µL, 0.64 mmol, 1.05 eq, 37% aqueous) at room temperature. The mixture stirred at room temperature for 30 min. at which time it was added to a solution of **155** (155 mg, 0.61 mmol, 1 eq) in AcOH (1 mL). The mixture continued to stir for 8 h., upon which it was basified to a pH of 10 with 2 M NaOH (3 mL). The solution was extracted with Et_2O (3 x 10 mL), and the organic layers dried with $MgSO_4$. Filtration and concentration provided **222** (148 mg, 78%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (bs, 1 H), 7.57 (d, *J* = 7.8 Hz, 1 H), 7.05 (t, *J* = 7.3 Hz, 1 H), 6.94 (d, *J* = 7.3 Hz, 1 H), 6.15 (dd, *J* = 10.6, 17.5 Hz, 1 H), 5.47 (t, *J* = 7.3 Hz, 1 H), 5.17 (dd, *J* = 10.6, 17.5 Hz, 2 H), 3.63 (s, 2 H), 3.58 (d, *J* = 7.3 Hz, 2 H), 2.27 (s, 6 H), 1.89 (s, 3 H), 1.83 (s, 3 H), 1.60 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.3, 140.6, 133.1, 132.6, 130.4, 122.9, 122.7, 120.8, 119.3, 117.0, 111.6, 108.8, 54.0, 45.3, 39.3, 31.2, 27.0, 25.6, 17.8. HRMS (ESI/APCI+), calcd for $C_{19}H_{23}N$ (M – $N(CH_3)_2$) 266.1903, found 266.1912.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6.043
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5.353
5.350
5.346
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5.114
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5.070
5.052
5.049
3.496
3.480
3.462
2.141
1.773
1.712
1.709
1.484
0.944
0.912
0.934
1.83
5.51
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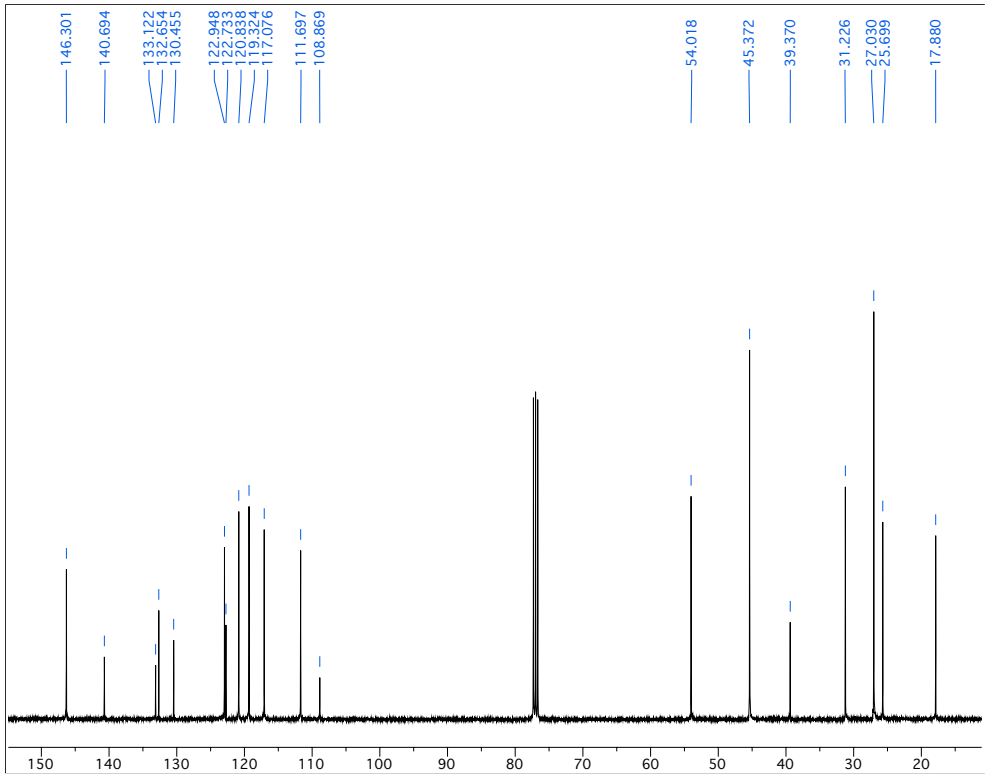
3
2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45.372
39.370
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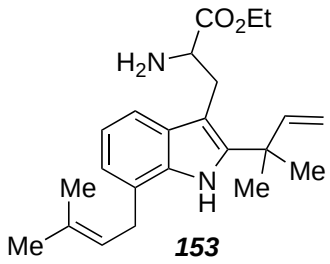
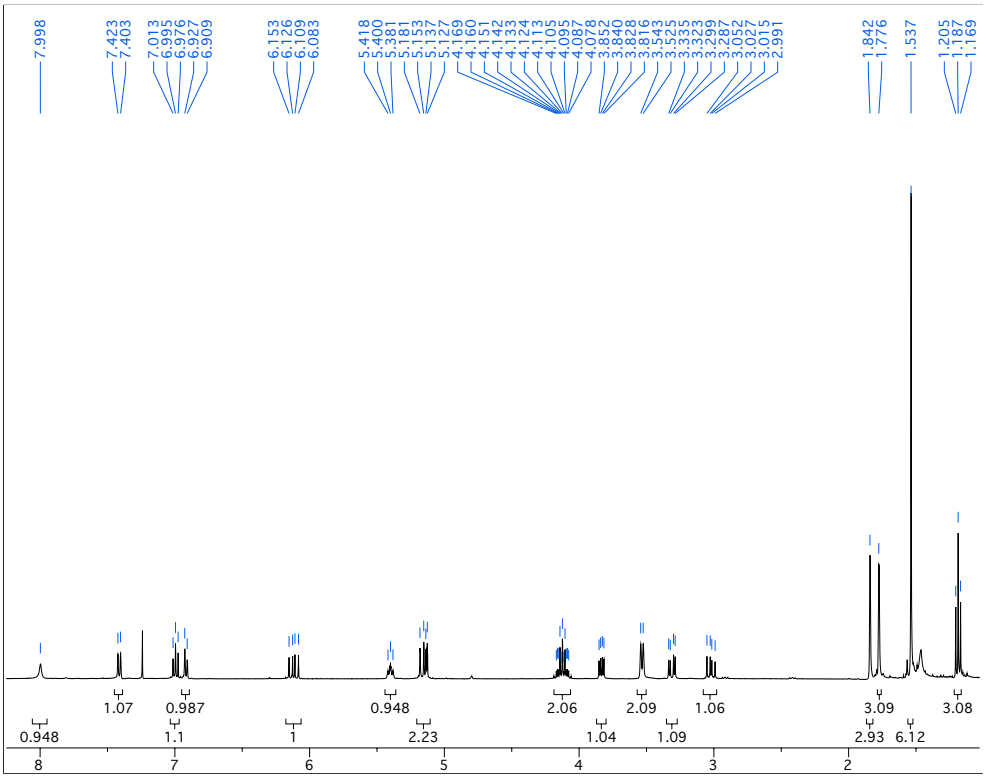


153

ethyl 2-amino-3-(7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propanoate (153). To a solution of **222** (92 mg, 0.29 mmol, 1 eq) in CH_3CN (3 mL) was added **101** (87 mg, 0.32 mmol, 1.1 eq) and Bu_3P (16 µL, 0.11 mmol, 0.4 eq). The reaction mixture was taken to reflux where it remained for 24 h. Upon cooling to room temperature 1 M HCl (2 mL) was added and allowed to stir for 3 h. The crude mixture was concentrated under reduced pressure and purified by flash chromatography (MeOH / CH_2Cl_2, 1 : 20) to provide **153** (89 mg, 82%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (bs, 1 H), 7.40 (d, *J* = 7.8 Hz, 1 H), 6.99 (t, *J* = 7.8 Hz, 1 H), 6.90 (d, *J* = 7.1 Hz, 1 H), 6.08 (dd, *J* = 10.6, 17.5 Hz, 1 H), 5.40 (t, *J* = 7.3 Hz, 1 H), 5.12 (dd, *J* = 10.6, 17.5 Hz, 2 H), 4.16-4.07 (m, 2 H), 3.81 (dd, *J* = 4.8, 9.8 Hz, 1 H), 3.52 (d, *J* = 7.3 Hz, 2 H), 3.28 (dd, *J* = 4.8, 9.8 Hz, 1 H), 2.99 (dd, *J* = 4.8, 9.8 Hz, 1 H), 1.84 (s, 3 H), 1.77 (s, 3 H), 1.53 (s, 6 H), 1.18 (t, *J* = 7.1 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 146.0, 139.9, 133.4, 132.7, 129.7, 122.9, 122.8, 121.2, 119.5, 116.6, 111.8, 107.1, 60.7, 55.9, 39.1, 31.2, 31.1, 27.8, 27.7, 25.6, 17.8, 14.0. HRMS (ESI_APCI) calcd for $C_{23}H_{32}N_2O_2$ [M + H] 369.2537, found 369.2542.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5.400
5.381
5.181
5.153
5.137
5.127
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4.160
4.151
4.142
4.133
4.124
4.113
4.105
4.095
4.087
4.078
3.852
3.840
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3.816
3.543
3.525
3.335
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3.287
3.052
3.027
3.015
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1.842
1.776
1.537
1.205
1.187
1.169
1.07
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0.948
2.06
2.09
1.06
3.09
3.08
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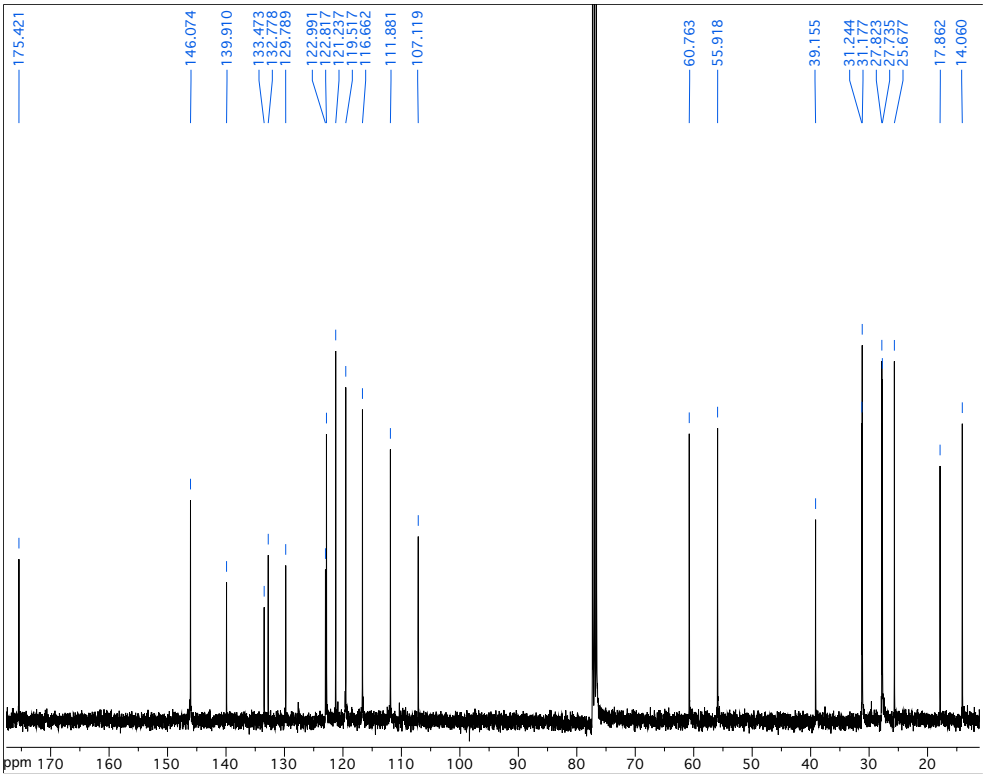
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60.763
55.918
39.155
31.244
31.177
27.823
27.735
25.677
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30
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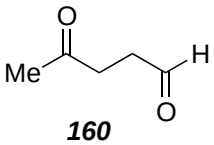
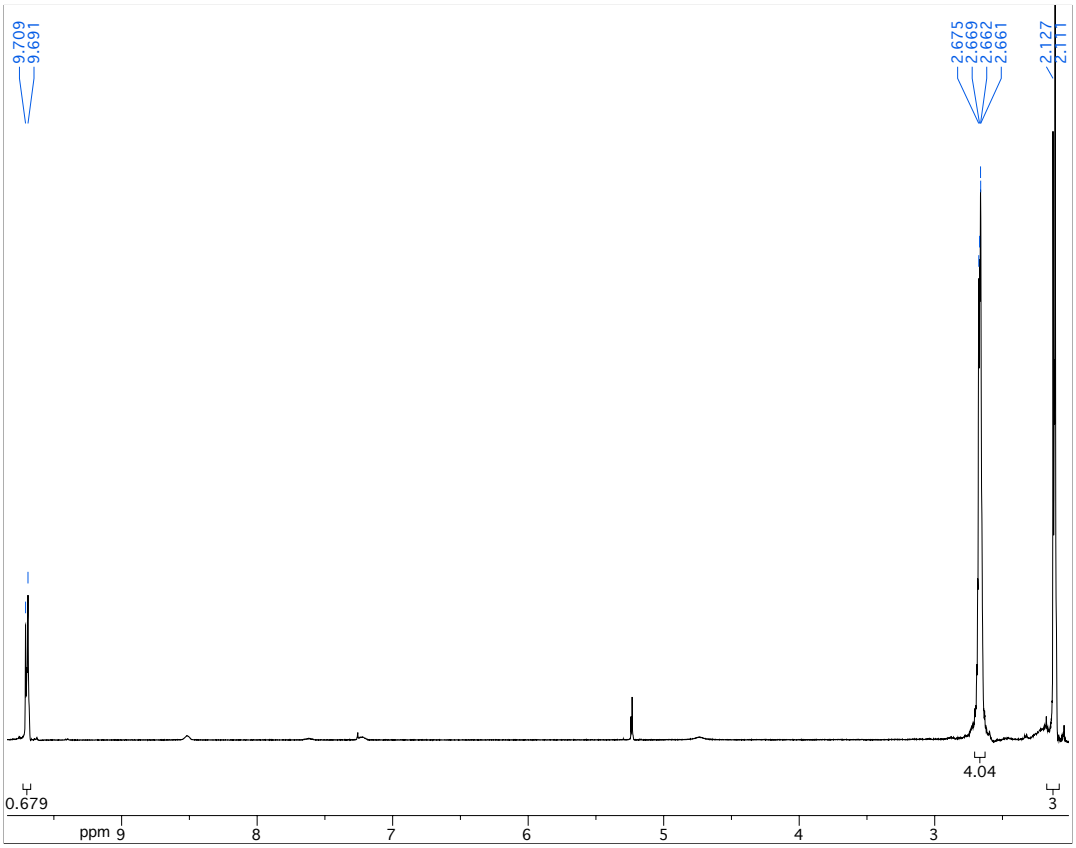


160

4-oxopentanal (160). To a solution of PCC (16.2 g, 73.43 mmol, 1.5 eq) and silica gel (25 g) in CH_2Cl_2 (150 mL) was added **228** (5 g, 48.95 mmol, 1 eq) drop wise. The solution was allowed to stir for 16 h. at which time it was filtered through a plug of silica gel and concentrated under reduced pressure. The crude aldehyde was purified by flash chromatography (EtOAc / hex., 1 : 5–2 : 5) to give **160** (3.81 g, 65%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (s, 1 H), 2.66 (s, 4 H), 2.11 (s, 3 H).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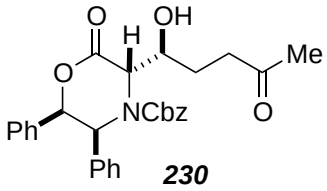
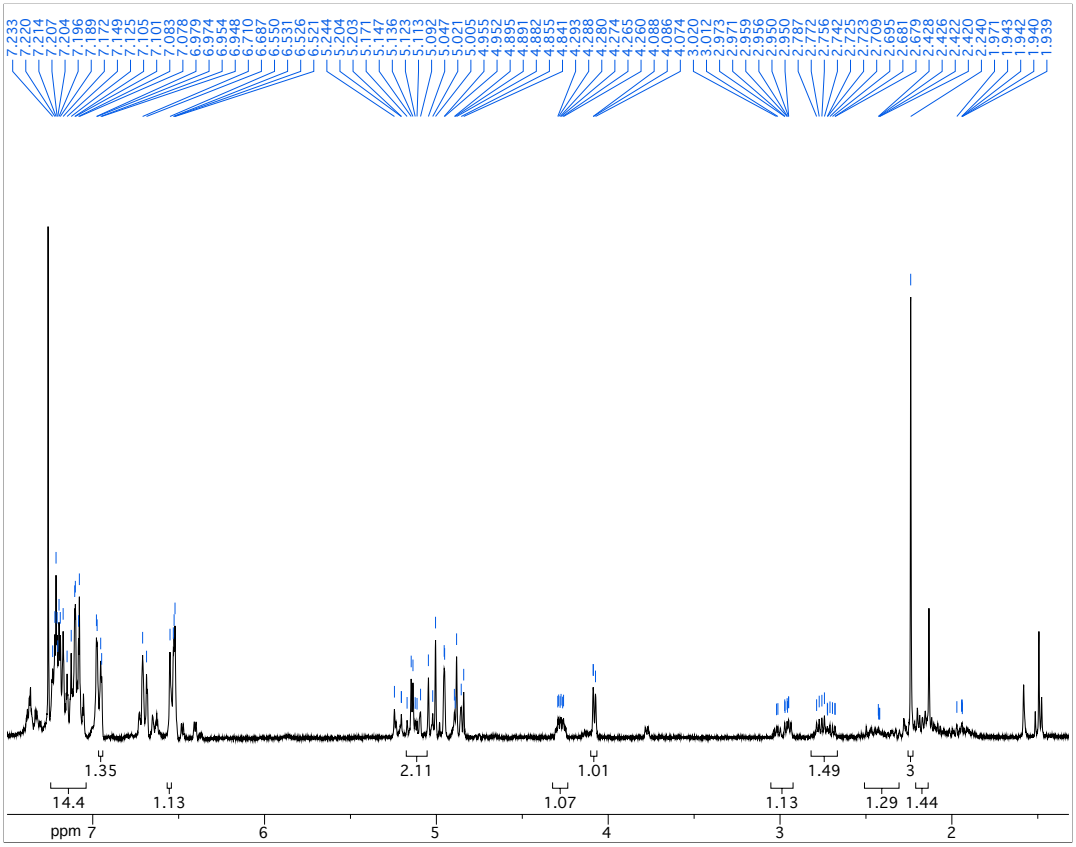


230

(3*S*,5*S*,6*R*)-benzyl 3-(1-hydroxy-4-oxopentyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (230). To a solution of **159** (1.34 g, 3.45 mmol, 1 eq) in CH_2Cl_2 at -5ºC was added drop wise *n*-Bu_2BOTf (6.9 mL, 6.90 mmol, 2 eq) followed by drop wise addition of TEA (1.45 mL, 10.36 mmol, 3 eq). The reaction mixture was stirred for 1 h. at -5ºC. Cooling the reaction mixture to -78ºC prepared for drop wise addition of **160** (415 mg, 4.14 mmol, 1.2 eq) in CH_2Cl_2 (5 mL). The mixture stirred at -78ºC for 1 h. At -78ºC pH 7 phosphate buffer (25 mL) was added and the reaction was slowly warmed to room temperature. The aqueous layer was separated and extracted with EtOAc (3 x 15 mL). The organics were combined and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded the crude mixture. Purification by flash chromatography (EtOAc / hex., 1 : 5–2 : 5) provided **230** (1.0 g, 60%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23-7.07 (m, 14 H), 6.94 (d, *J* = 9.1 Hz, 1 H), 6.52 (d, *J* = 9.1 Hz, 1 H), 5.09 (m, 2 H), 4.26 (m, 1 H), 4.07 (d, *J* = 3.7 Hz, 1 H), 3.02-2.95 (m, 1 H), 2.78-2.67 (m, 1 H), 2.42 (m, 1 H), 2.24 (s, 3 H), 1.94 (m, 1 H).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ppm
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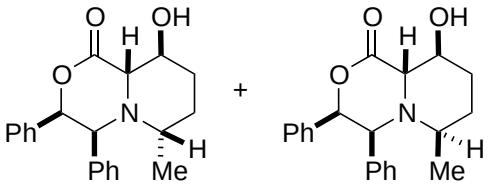
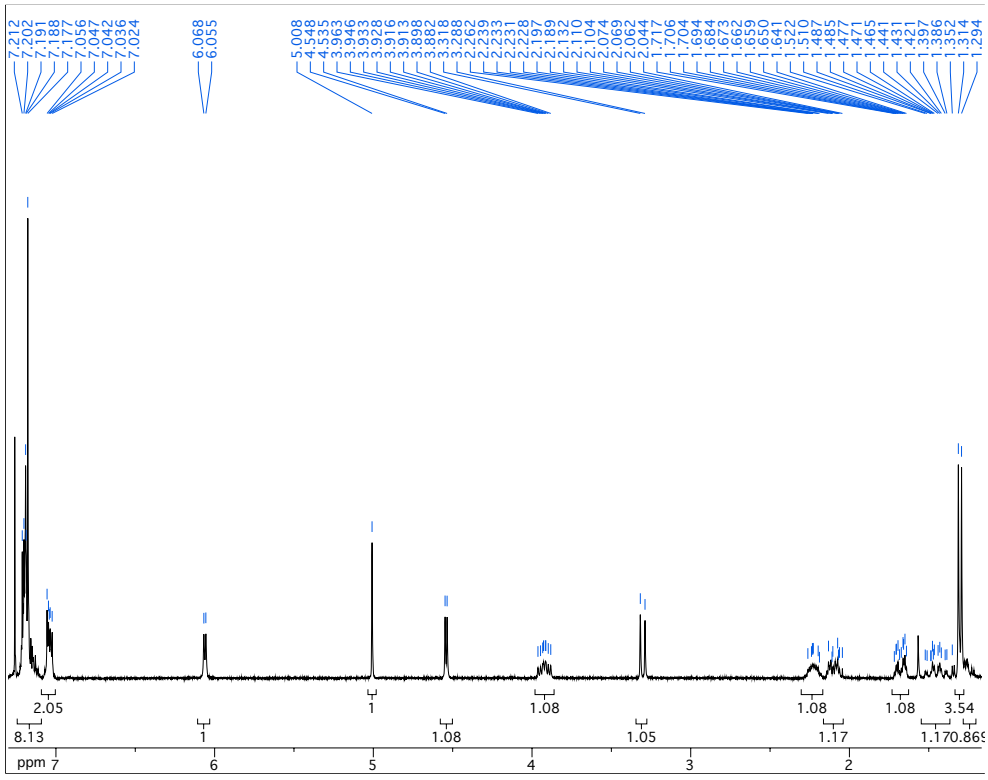
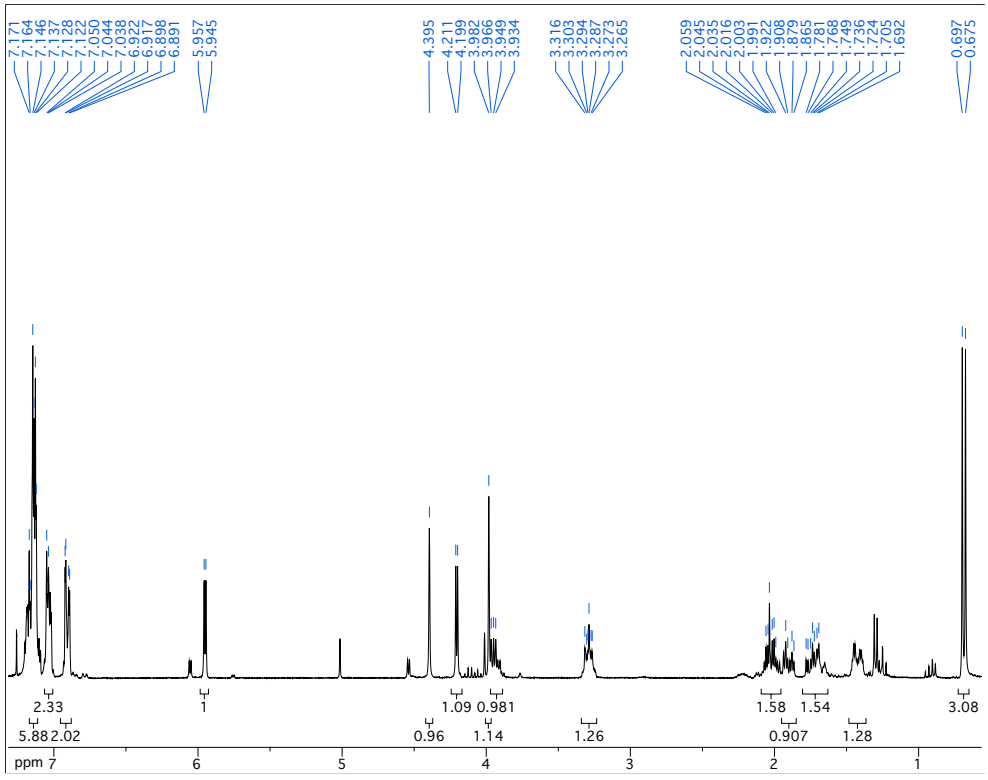
2

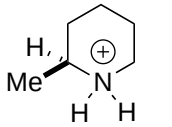


231 ***232***

(3*R*,4*S*,6*S*,9*S*,9a*S*)-9-hydroxy-6-methyl-3,4-diphenylhexahydropyrido[2,1-*c*][1,4]oxazin-1(6*H*)-one (231) and **(3*R*,4*S*,6*R*,9*S*,9a*S*)-9-hydroxy-6-methyl-3,4-diphenylhexahydropyrido[2,1-*c*][1,4]oxazin-1(6*H*)-one (232).** To a solution of **230** (4.3 g, 8.81 mmol, 1 eq) in THF (90 mL) was added 10% Pd/C (430 mg, 10 weight %) followed by a balloon of H_2. The reaction was stirred at room temperature for 18 h. The H_2 balloon was removed and the reaction mixture was purged with argon for 5 min., after which time it was filtered through a pad of Celite. Concentration under reduced pressure afforded the crude mixture which was purified by flash chromatography (EtOAc / hex., 1 : 10–3 : 20) to provide **231** (1.28 g, 43%) and **232** (650 mg, 22%) as white solids. **(231)** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21-7.17 (m, 8 H), 7.05-7.02 (m, 2 H), 6.05 (d, *J* = 3.8 Hz, 1 H), 5.00 (s, 1 H), 4.53 (d, *J* = 3.8 Hz, 1 H), 3.96-3.88 (m, 1 H), 3.28 (d, *J* = 8.8 Hz, 1 H), 2.26-2.13 (m, 1 H), 2.11-2.04 (m, 1 H), 1.71-1.64 (m, 1 H), 1.51-1.38 (m, 1 H), 1.29 (d, *J* = 6.0 Hz, 3 H), 1.27-1.25 (m, 1 H). **(232)** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17-7.12 (m, 6 H), 7.05-7.03 (m, 2 H), 6.92-6.89 (m, 2 H), 5.94 (d, *J* = 3.7 Hz, 1 H), 4.39 (s, 1 H), 4.19 (d, *J* = 3.7 Hz, 1 H), 3.98 (s, 1 H), 3.96-3.93 (m, 1 H), 3.31-3.26 (m, 1 H), 2.05-1.99 (m, 1 H), 1.92-1.86 (m, 1H), 1.78-1.69 (m, 1 H), 1.45-1.39 (m, 1 H), 0.67 (d, *J* = 6.7 Hz, 3 H).



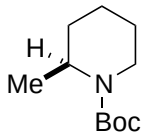




• L-tartrate

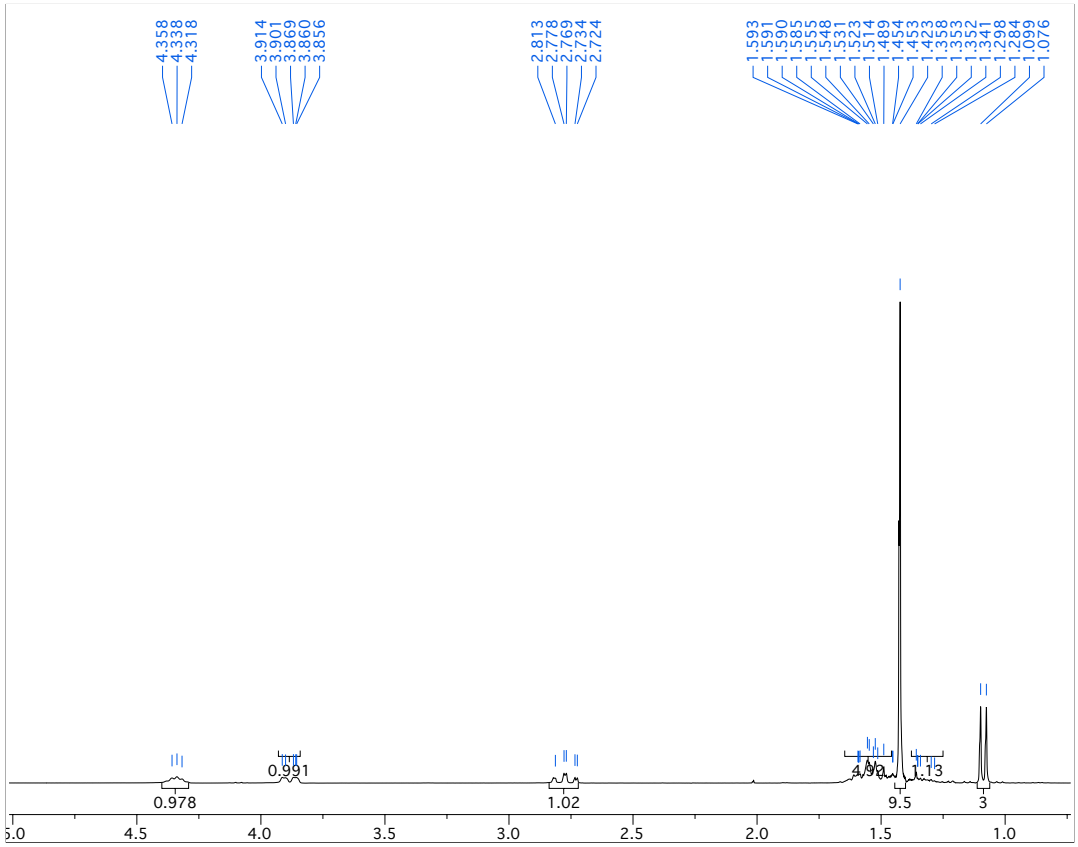
237

(*S*)-2-methylpiperidine-L-tartrate (237). To a solution of L-tartaric acid (200 g, 1.30 mmol, 1 eq) in H_2O (133 mL), cooled in an ice bath, was added (±)-2-methylpiperidine (156g, 1.30 mmol, 1 eq) in portions, keeping the temperature below 30ºC. The solution was concentrated under reduced pressure to give a thick syrup. The residue was dissolved in H_2O (135 mL) heating with a heat gun to dissolve and then cooled in an ice bath. The solid was collected by filtration and washed with cold H_2O (100 mL). The precipitate was dissolved in H_2O (80 mL) and cooled in an ice bath. The solid was collected by filtration and recrystallized from H_2O (30 mL). The solid was collected and the final recrystallization was carried out in H_2O (16 mL). The solid was collected by filtration and dried under reduced pressure to afford **237** (200 g, 48%) as a white crystalline solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.59 (m, 1 H), 3.52 (m, 1 H), 3.30 (d, *J* = 7.9 Hz, 1 H), 3.15 (m, 1 H), 2.90 (t, *J* = 7.9 Hz, 1 H), 1.88-1.75 (m, 3 H), 1.70-1.32 (m, 3 H), 1.19 (d, *J* = 6.3 Hz, 3 H).



238

(*S*)-*tert*-butyl 2-methylpiperidine-1-carboxylate (238). To a solution of **237** (13 g, 45.56 mmol, 1 eq) in aqueous NaOH (40 mL, 10%) at 0ºC was added a solution of $(Boc)_2O$ (57.7 g, 264.29 mmol, 5.8 eq) in THF (35 mL). The reaction mixture was warmed to room temperature where it stirred for 18 h. Sat. NH_4OH (400 mL, 30%) was added to the reaction mixture slowly, and continued to stir for 2 h. The mixture was diluted with EtOAc (500 mL) and separated. The aqueous phase was extracted with EtOAc (2 x 100 mL) and the organics were combined and washed with 1 M HCl (200 mL), H_2O (2 x 200 mL), brine (200 mL) and dried over $MgSO_4$. Filtration and concentration under reduced pressure afforded the crude residue. Purification by flash chromatography (EtOAc / hex., 7 : 100) provided **238** (8.2 g, 89%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.35-4.31 (m, 1 H), 3.85 (dd, *J* = 4.0, 9.5 Hz, 1 H), 2.76 (td, *J* = 2.9, 10.4 Hz, 1 H), 1.59-1.45 (m, 5 H), 1.42 (s, 9 H), 1.35-1.28 (m, 1 H), 1.07 (d, *J* = 7.0 Hz, 3 H).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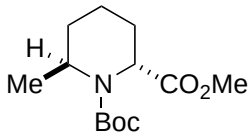
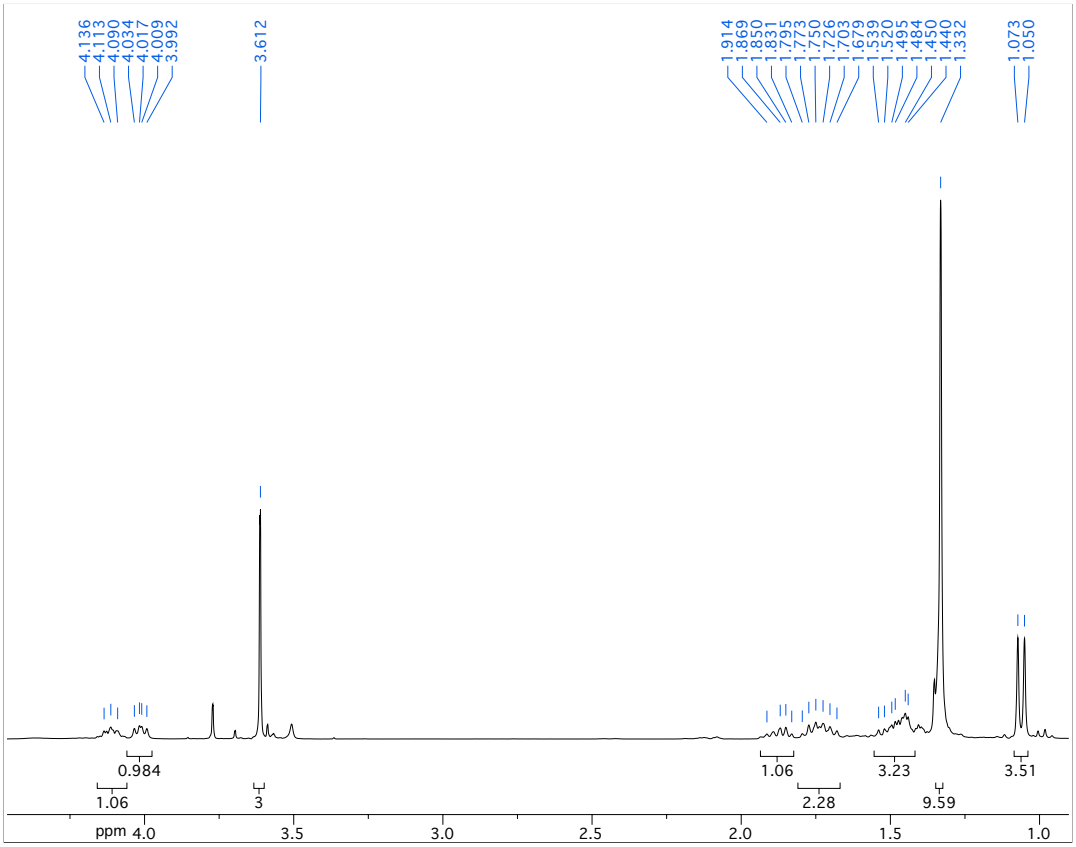


239

(2*R*,6*S*)-1-*tert*-butyl 2-methyl 6-methylpiperidine-1,2-dicarboxylate (239). To a solution of **238** (1 g, 5.01 mmol, 1 eq) and TMEDA (822 µL, 5.51 mmol, 1.1 eq) in Et_2O (50 mL) at -65ºC was added drop wise *s*-BuLi (3.95 mL, 5.51 mmol, 1.1 eq). The reaction was warmed to -20ºC where it remained for 30 min. The mixture was then cooled to -78ºC and CH_3O_2CCl (970 µL, 12.54 mmol, 2.5 eq) was added drop wise. The reaction mixture was warmed to room temperature where it remained for 3 h. The mixture was partitioned between H_2O (20 mL) and Et_2O (20 mL). The aqueous layer was extracted with Et_2O (3 x 10 mL). The organics were combined and washed with brine (2 x 20 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided the crude mixture. Purification by flash chromatography (EtOAc / hex., 1 : 50–1 : 10) afforded **239** (900 mg, 69%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13-4.09 (m, 1 H), 4.03-3.99 (m, 1 H), 3.61 (s, 3 H), 1.91-1.83 (m, 1 H), 1.79-1.67 (m, 2 H), 1.53-1.44 (m, 3 H), 1.33 (s, 9 H), 1.05 (d, *J* = 6.7 Hz, 3 H).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1.850
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1.795
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1.679
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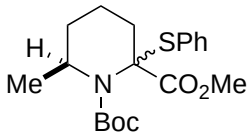
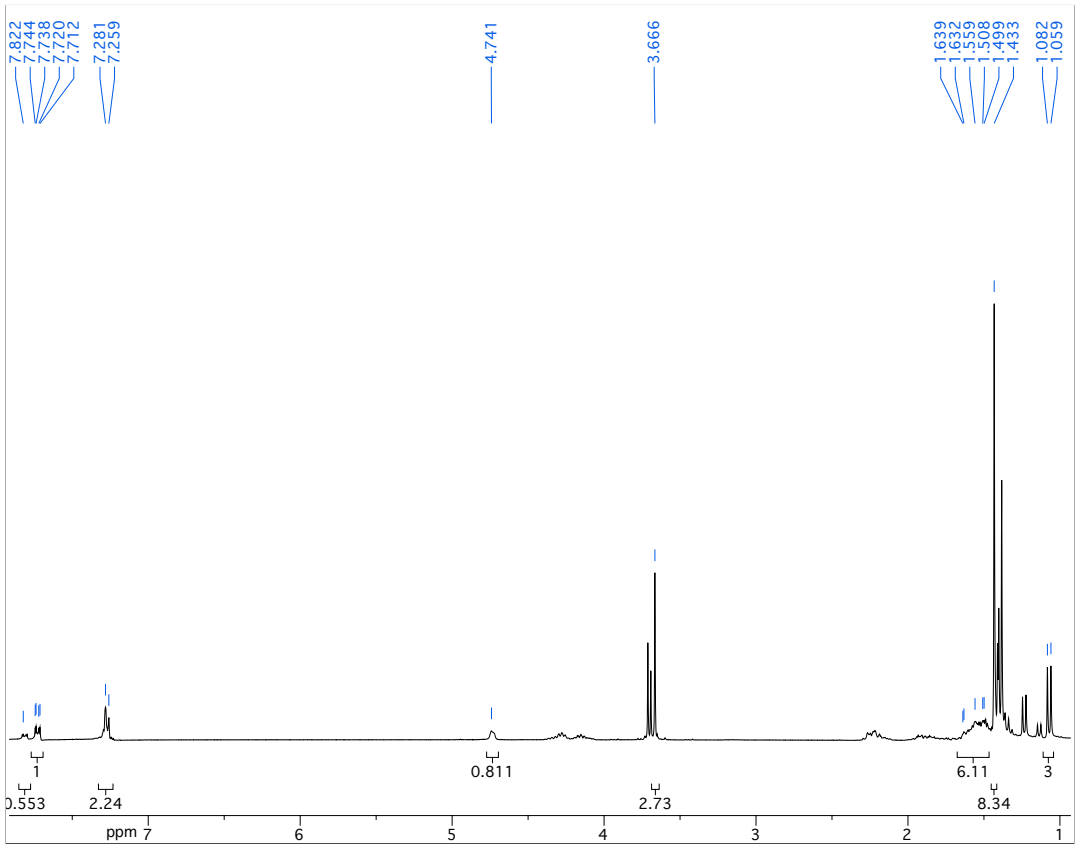


240

(6*S*)-1-*tert*-butyl 2-methyl 6-methyl-2-(phenylthio)piperidine-1,2-dicarboxylate (240). To a solution of **239** (200 mg, 0.77 mmol, 1 eq) in THF (7 mL) at -78ºC was added LiHMDS (893 µL, 0.89 mmol, 1.15 eq). The reaction mixture was stirred at -78ºC for 30 min. A solution of PhS-SPh (186 mg, 0.85 mmol, 1.1 eq) in THF (2 mL) was added to the reaction mixture at -78ºC. The reaction continued stirring at -78ºC for 10 min., at which time it was allowed to warm to room temperature. The mixture was concentrated under reduced pressure and purified by flash chromatography (EtOAc / hex., 1 : 20) to provide **240** (127 mg, 45%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (m, 1 H), 7.74-7.71 (m, 2 H), 4.74 (m, 1 H), 3.66 (s, 3 H), 1.63-1.49 (m, 6 H), 1.43 (s, 9 H), 1.05 (d, *J* = 6.9 Hz, 3 H).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1.082
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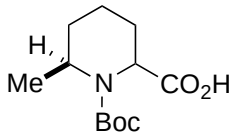
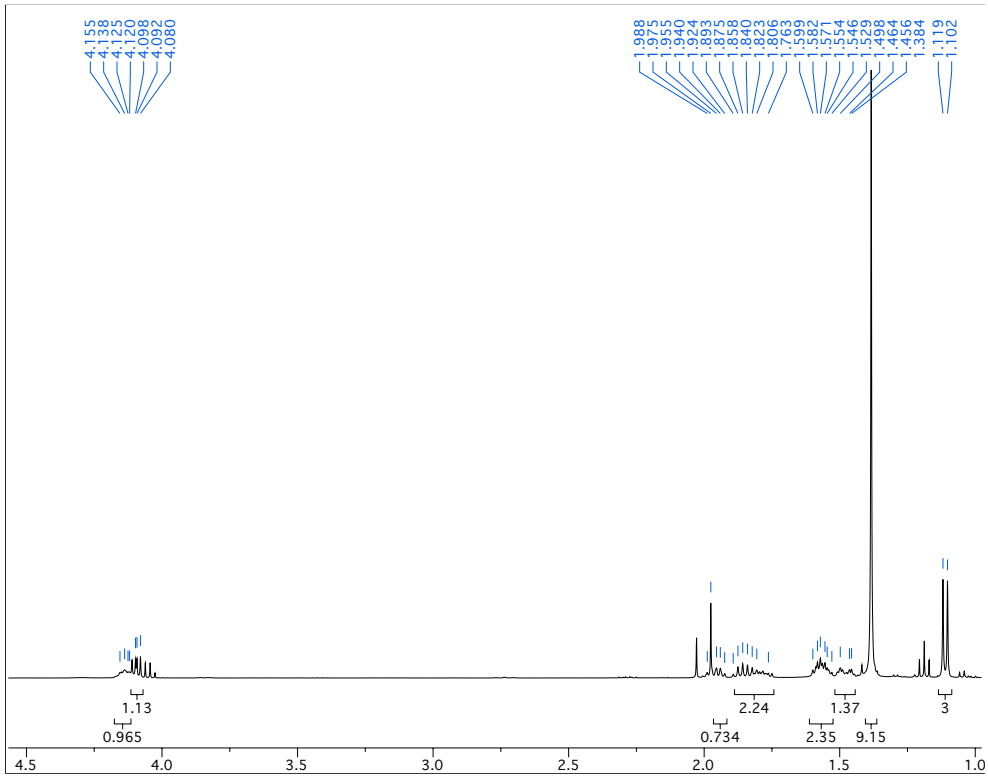


246

(6*S*)-1-(*tert*-butoxycarbonyl)-6-methylpiperidine-2-carboxylic acid (246). [A] To a solution of **239** (500 mg, 1.94 mmol, 1 eq) in THF (10 mL) was added an aqueous 1 M LiOH (10 mL) at 0ºC. The reaction mixture was warmed to room temperature and allowed to stir for 5 h. The mixture was acidified with 3 M HCl (5 mL) and extracted with CH_2Cl_2 (15 mL). The organic layer was extracted with 2 M NaOH (3 x 20 mL). The combined aqueous layers were acidified with 3 M HCl (100 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided **246** (378 mg, 80%) as a clear oil.

[B] To a solution of **238** (1 g, 5.01 mmol, 1 eq) and TMEDA (822 µL, 5.51 mmol, 1.1 eq) in Et_2O (15 mL) at -78ºC was added drop wise *s*-BuLi (4.3 mL, 6.02 mmol, 1.2 eq). The reaction was stirred at -78ºC for 6 h., at which time a stream of CO_2 was bubbled into the reaction mixture for 1 h at -78ºC. The reaction was stirred for an additional 1 h. at -78ºC. 1 M HCl (20 mL) was added and the reaction was allowed to warm to room temperature. The mixture was separated and the aqueous layer was extracted with Et_2O (3 X 10 mL). The organics were combined and basified with 2 M NaOH (30 mL). The organics were removed and the aqueous layer was acidified with 3 M HCl

(45 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded **246** (854 mg, 70%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15-4.12 (m, 1 H), 4.09-4.08 (m, 1 H), 1.98-1.92 (m, 1 H), 1.89-1.76 (m, 2 H), 1.59-1.52 (m, 2 H), 1.49-1.45 (m, 1 H), 1.38 (s, 9 H), 1.10 (d, *J* = 6.6 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7, 177.0, 80.75, 53.8, 47.4, 28.2, 27.7, 25.6, 18.7, 15.0.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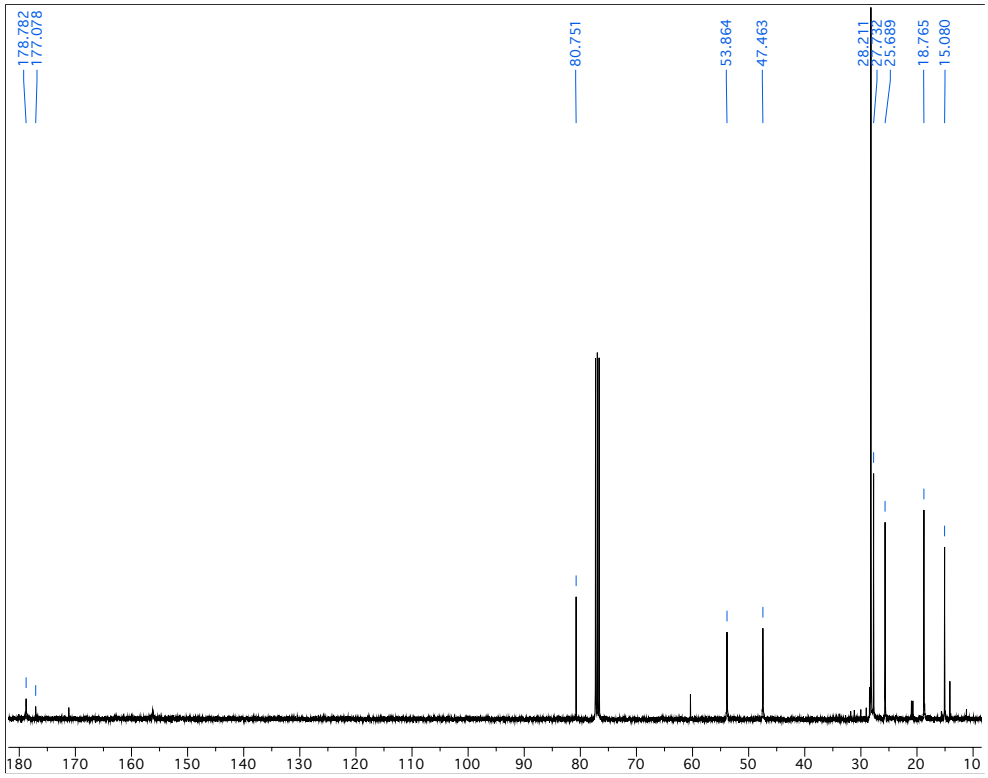
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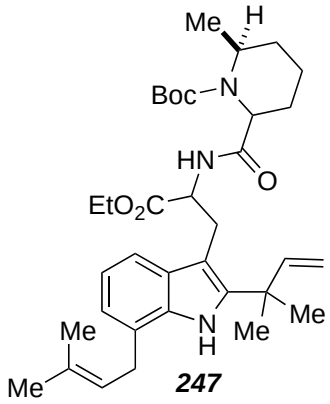
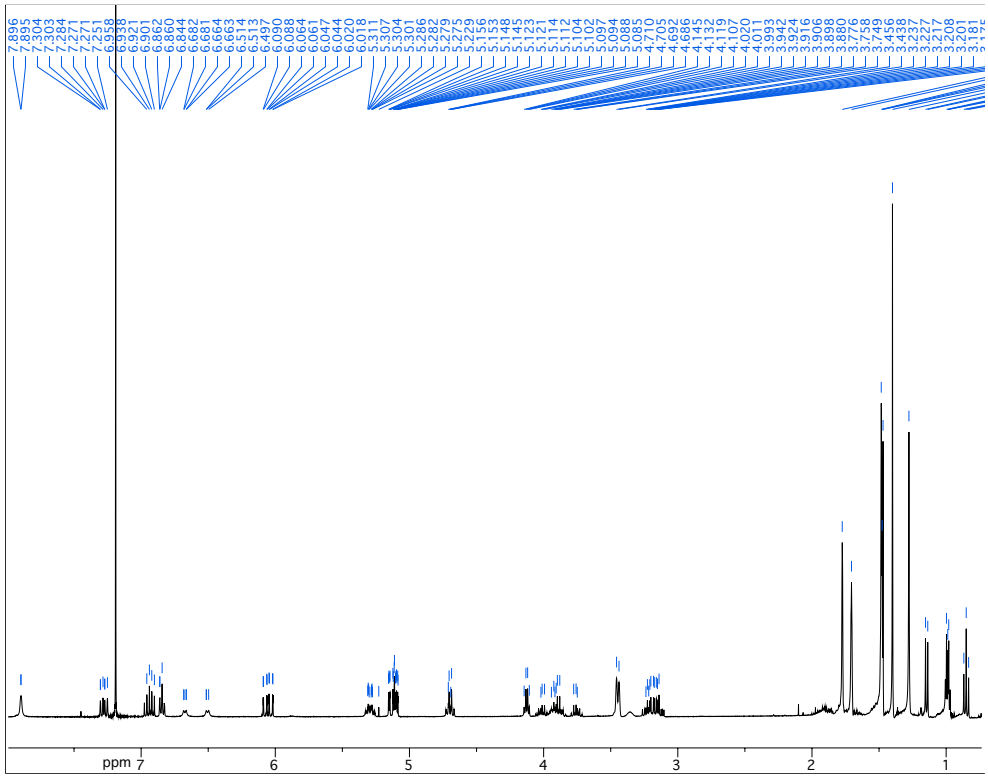
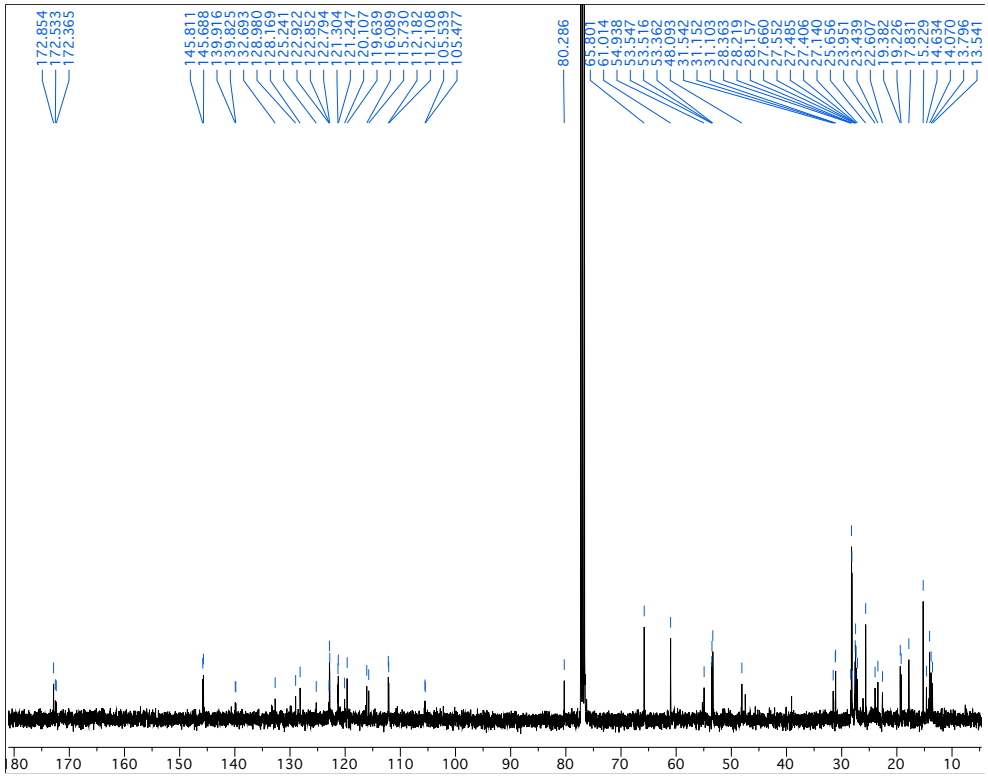
10

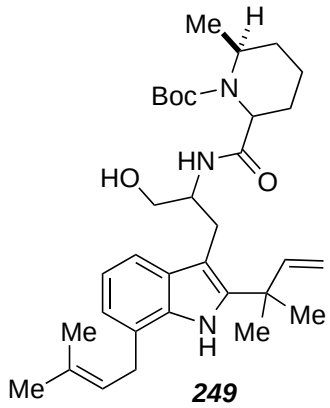


247

(6*S*)-*tert*-butyl 2-((1-ethoxy-3-(7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-6-methylpiperidine-1-carboxylate (247). To a solution of **153** (100 mg, 0.27 mmol, 1 eq) and **246** (70 mg, 0.28 mmol, 1.05 eq) in CH_3CN (3 mL) was added consecutively HATU (130 mg, 0.33 mmol, 1.25 eq) and *i*-Pr_2NEt (100 mL, 0.59 mmol, 2.2 eq) at room temperature. The reaction mixture was allowed to stir for 18 h., at which time it was concentrated under reduced pressure. Purification by flash chromatography (EtOAc / hex., 1 : 10–1 : 5) provided **247** (109 mg, 68%) as a white foam. Mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (bs, 1 H), 7.34 (t, *J* = 8.3 Hz, 1 H), 7.05-6.96 (m, 1 H), 6.91 (t, *J* = 6.3 Hz, 1 H) 6.59 (dd, *J* = 6.7, 52 Hz, 1 H), 6.07 (dd, *J* = 10.6, 17.5 Hz, 1 H), 5.34 (t, *J* = 7.3 Hz, 1 H), 5.15 (dd, *J* = 10.6, 17.5 Hz, 2 H), 4.21-4.16 (m, 1 H), 4.12-4.07 (m, 1 H), 3.99-3.91 (m, 1 H), 3.50 (d, *J* = 7.3 Hz, 2 H), 3.31-3.15 (m, 2 H), 1.84 (s, 3 H), 1.76 (s, 3 H), 1.54 (s, 6 H), 1.46 (s, 5 H), 1.33 (s, 4 H), 1.19 (d, *J* = 6.6 Hz, 3 H), 1.05 (t, *J* = 6.5 Hz, 3 H). HRMS (ESI/APCI) calcd for $C_{35}H_{51}N_3O_5$ [M + H] 594.3901, found 594.3910.



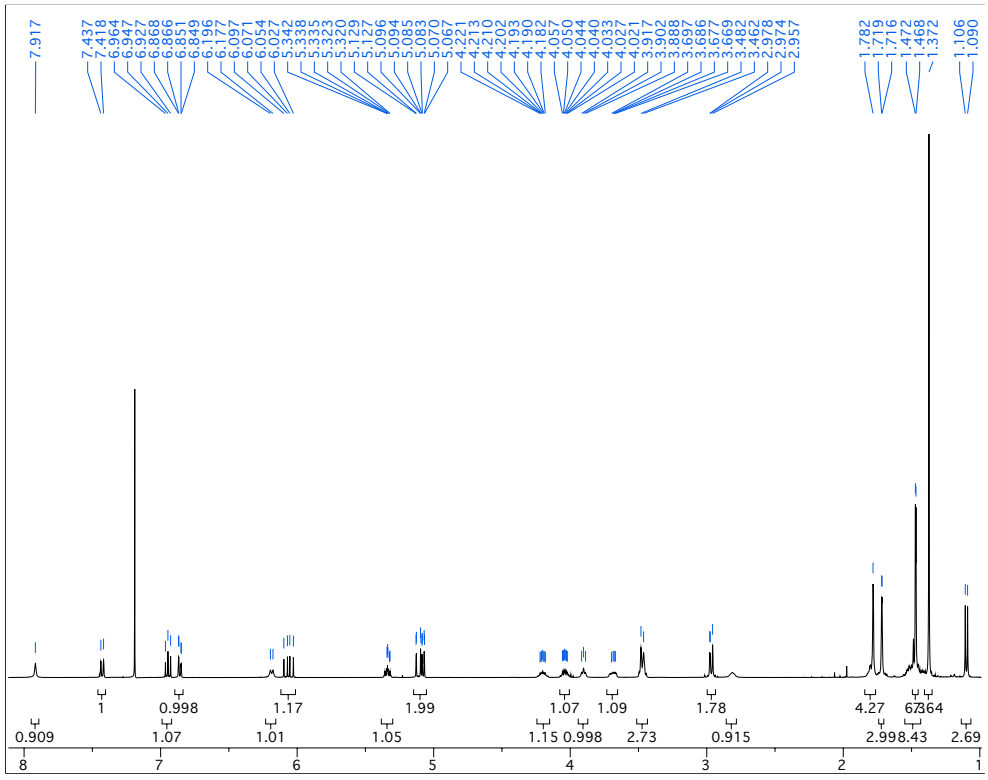




249

(6*S*)-*tert*-butyl 2-((1-hydroxy-3-(7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propan-2-yl)carbamoyl)-6-methylpiperidine-1-carboxylate (249). To a solution of **247** (200 mg, 0.33 mmol, 1 eq) in THF (3 mL) was added solid $LiBH_4$ (18 mg, 0.84 mmol, 2.5 eq). The reaction mixture was stirred at room temperature for 18 h. Sat. NH_4Cl (5 mL) was added and the mixture was partitioned into EtOAc (10 mL). The organic layer was separated and washed with 1 M NaOH (15 mL), H_2O (3 x 10 mL), brine (10 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure provided the crude mixture. Purification by flash chromatography (EtOAc / hex., 3 : 10–1 : 1) afforded **249** (148 mg, 80%) as a white foam. (**249a**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (bs, 1 H), 7.41 (d, *J* = 7.8 Hz, 1 H), 6.92 (t, *J* = 7.2 Hz, 1 H), 6.85 (d, *J* = 7.2 Hz, 1 H), 6.17 (bd, *J* = 7.6 Hz, 1 H), 6.02 (dd, *J* = 10.6, 16.6 Hz, 1 H), 5.33 (t, *J* = 7.4 Hz, 1 H), 5.06 (dd, *J* = 10.6, 16.6 Hz, 2 H), 4.20 (m, 1 H), 4.04 (m, 1 H), 3.90 (t, *J* = 5.8 Hz, 1 H), 3.67 (m, 1 H), 3.48 (d, *J* = 7.8 Hz, 3 H), 2.97 (d, *J* = 8.5 Hz, 2 H), 2.81 (bs, 1 H), 1.78 (s, 3 H), 1.71 (s, 3 H), 1.47 (s, 6 H), 1.37 (s, 9 H), 1.09 (d, *J* = 6.7 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) d 191.0, 173.2, 146.1, 139.6, 133.3, 132.7, 129.9,

122.9, 122.8, 121.2, 119.6, 116.4, 111.8, 107.0, 80.5, 63.7, 55.4, 53.0, 48.2, 39.1, 31.1, 28.2, 27.6, 27.5, 27.4, 26.4, 25.6, 25.0, 18.7, 17.8, 15.2. HRMS (ESI/APCI+) calcd for $C_{33}H_{49}N_3O$ [M + H] 552.3796, found 552.3802.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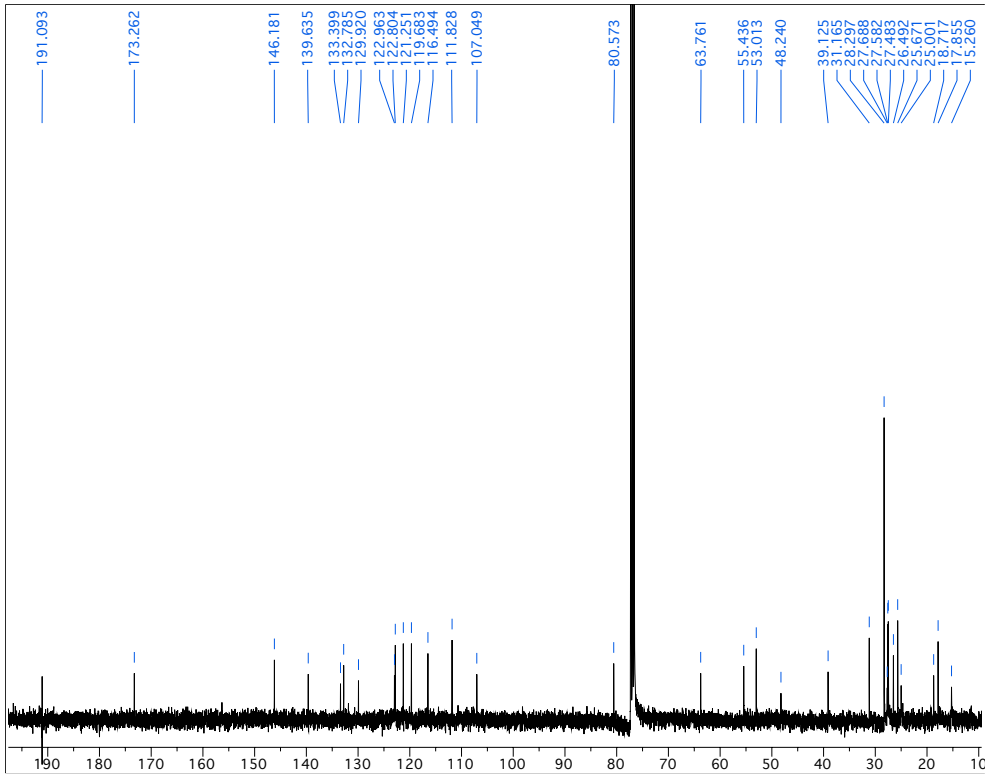
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90
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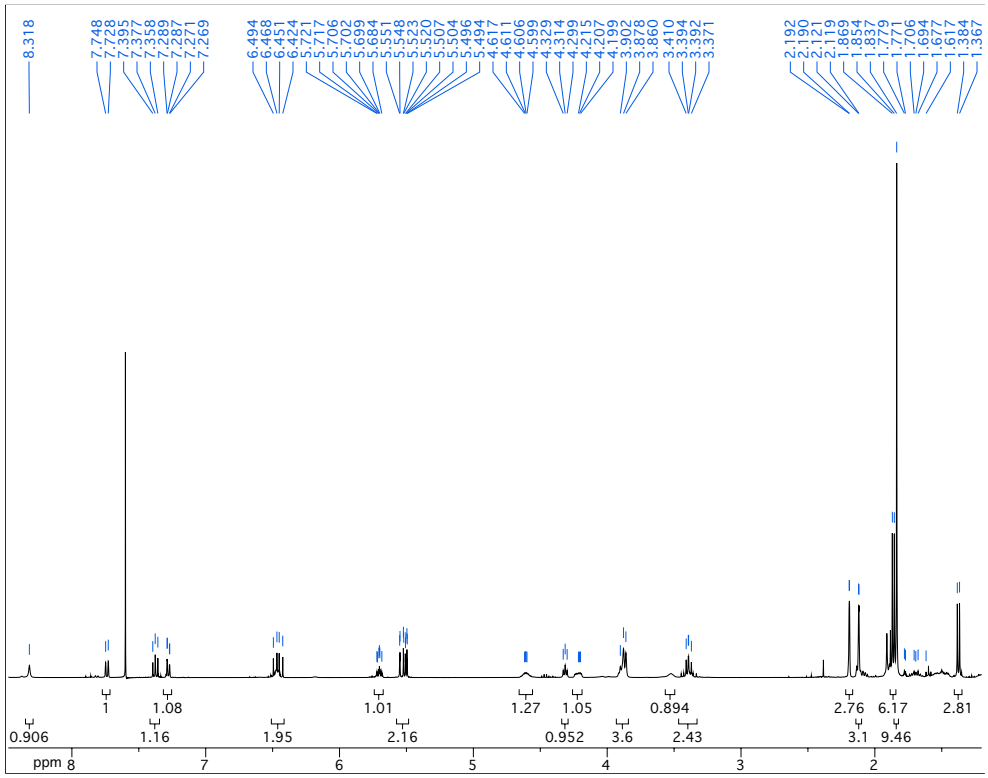
70
60
50
40
30
20
10

(**249b**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (bs, 1 H), 7.72 (d, *J* = 7.9 Hz, 1 H), 7.37 (t, *J* = 7.2 Hz, 1 H), 7.26 (d, *J* = 7.2 Hz, 1 H), 6.42 (dd, *J* = 10.6, 17.4 Hz, 2 H), 5.70 (t, *J* = 7.2 Hz, 1 H), 5.49 (dd, *J* = 10.6, 17.4 Hz, 2 H), 4.60 (m, 1 H), 4.31 (t, *J* = 5.8 Hz, 1 H), 4.20 (m, 1 H), 3.90 (bs, 1 H), 3.86 (d, *J* = 7.2 Hz, 2 H), 3.52 (bs, 1 H), 3.39 (m, 2 H), 2.19 (s, 3 H), 2.11 (s, 3 H), 1.86 (s, 3 H), 1.85 (s, 3 H), 1.83 (s, 9 H), 1.67 (m, 2 H), 1.36 (d, *J* = 6.6 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 173.9, 145.8, 139.8, 133.3, 132.7, 130.0, 123.0, 122.7, 121.3, 120.0, 115.8, 112.0, 106.6, 80.6, 55.7, 53.1, 47.5, 39.0, 31.1, 28.3, 27.6, 27.4, 26.8, 25.6, 25.5, 24.9, 18.7, 17.8, 14.3. HRMS (ESI/APCI+) calcd for $C_{33}H_{49}N_3O$ [M + H] 552.3796, found 552.3802.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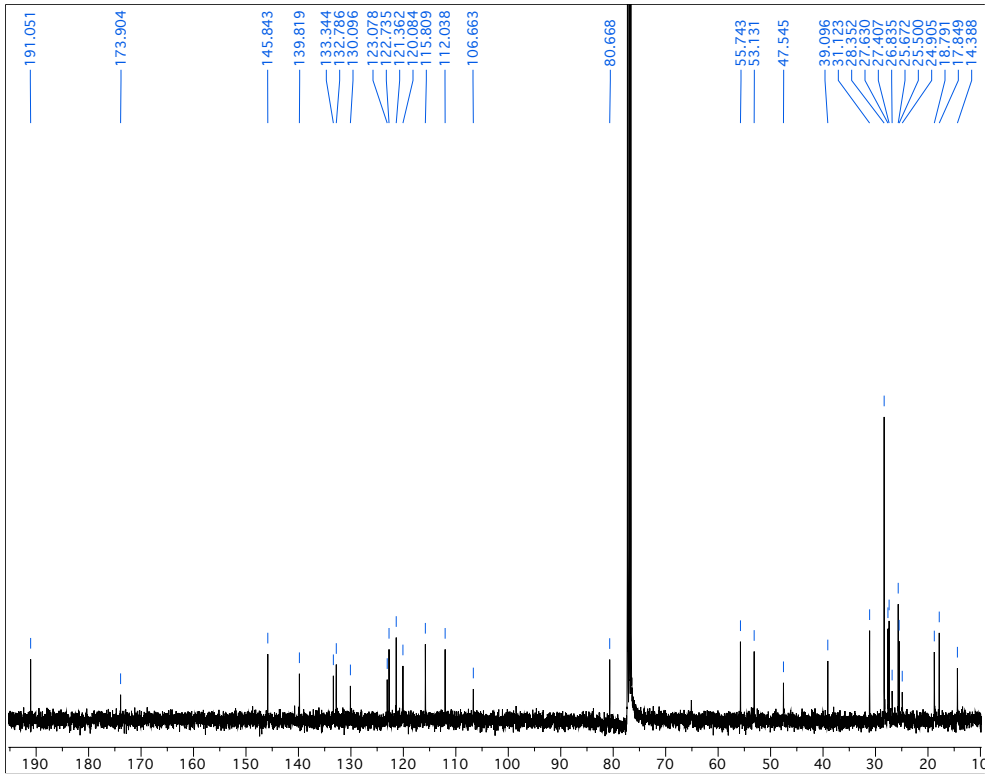
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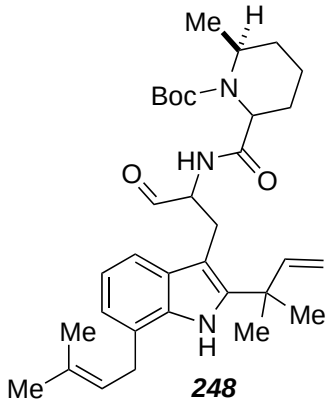
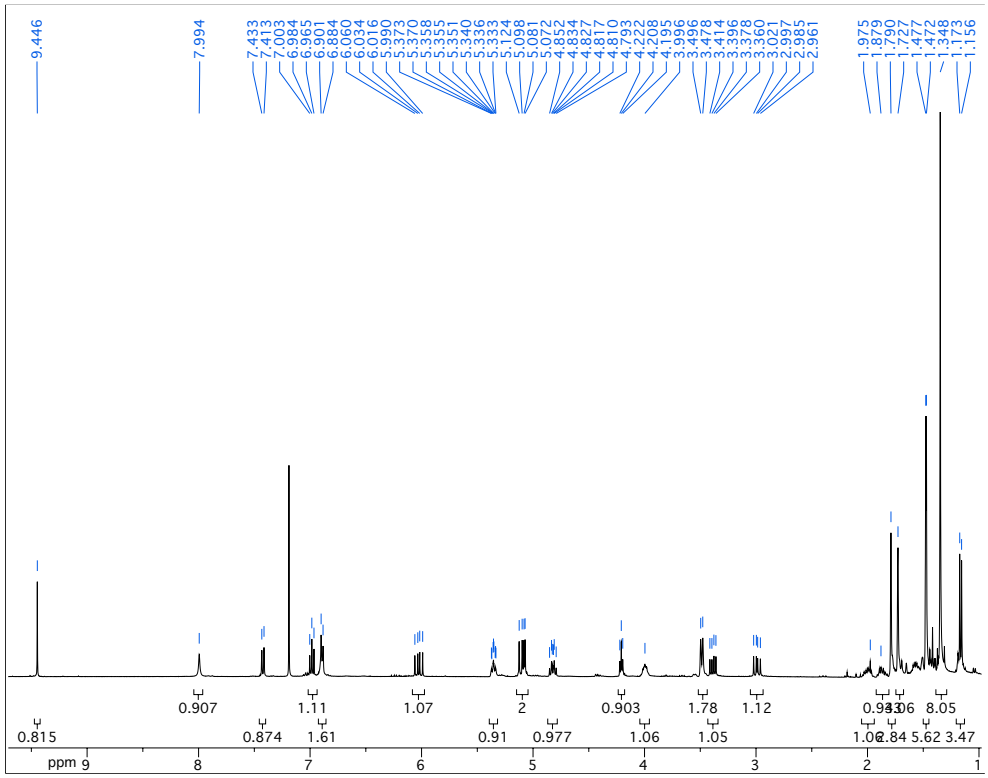


248

(2*S*)-*tert*-butyl 2-methyl-6-((1-(7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)-3-oxopropan-2-yl)carbamoyl)piperidine-1-carboxylate (248). To a solution of **249** (74 mg, 0.13 mmol, 1 eq) in wet CH_2Cl_2 (4 mL) was added solid DMP (63 mg, 0.14 mmol, 1.1 eq) at room temperature. The reaction mixture was stirred at room temperature for 30 min., at which time it was concentrated under reduced pressure. The residue was diluted with Et_2O (5 mL) and washed with a 1 : 1 mixture of sat. $Na_2S_2O_3$ (3 mL) and sat. $NaHCO_3$ (3 mL), H_2O (3 x 5 mL), brine (10 mL) and dried with Na_2SO_4. Filtration and concentration under reduced pressure provided the crude residue. Purification by flash chromatography (EtOAc / hex., 1 : 10–3 : 10) to provide **248** (70 mg, 95%) as a seperable mixture of diastereomers (**249a, 249b**). (**249a**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 (s, 1 H), 7.99 (bs, 1 H), 7.41 (d, *J* = 7.8 Hz, 1 H), 6.98 (t, *J* = 7.4 Hz, 1 H), 6.88 (d, *J* = 6.9 Hz, 1 H), 5.99 (dd, *J* = 10.6, 17.4 Hz, 1 H), 5.35 (t, *J* = 7.4 Hz, 1 H), 5.08 (dd, *J* = 10.6, 17.4 Hz, 2 H), 4.81 (m, 1 H), 4.20 (t, *J* = 5.3 Hz, 1 H), 3.99 (m, 1 H), 3.47 (d, *J* = 7.4 Hz, 2 H), 3.36 (dd, *J* = 7.3, 14.4 Hz, 1 H), 2.96 (dd, *J* = 9.8, 14.4 Hz, 1 H), 1.97 (m, 1 H), 1.87 (m, 1 H), 1.79 (s, 3 H), 1.72 (s, 3 H), 1.47

(d, *J* = 2.3 Hz, 6 H), 1.34 (s, 9 H), 1.15 (d, *J* = 6.7 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.8, 191.0, 173.2, 145.5, 139.9, 133.4, 132.9, 129.4, 123.1, 122.7, 121.5, 120.1, 116.4, 112.4, 104.8, 80.4, 58.8, 55.1, 48.0, 38.9, 31.1, 28.2, 27.6, 27.4, 27.1, 26.7, 25.6, 24.0, 19.3, 17.8, 14.5. HRMS (ESI/APCI+) calcd for $C_{33}H_{47}N_3O4$ [M + H] 550.3639, found 550.3638.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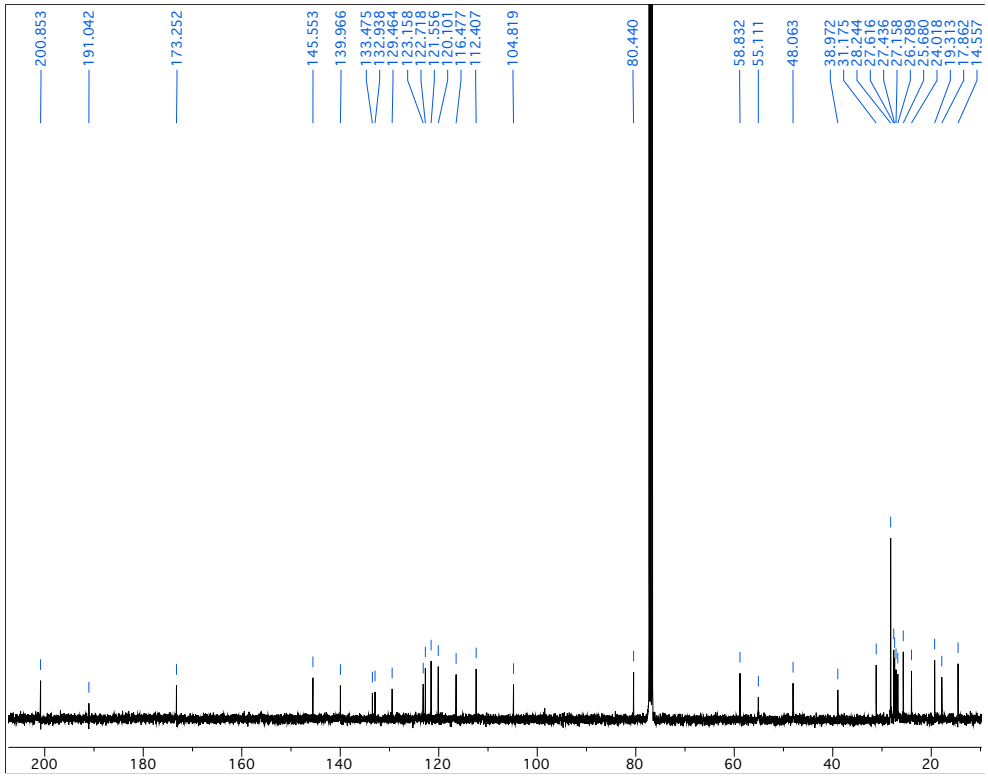
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112.407
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80.440
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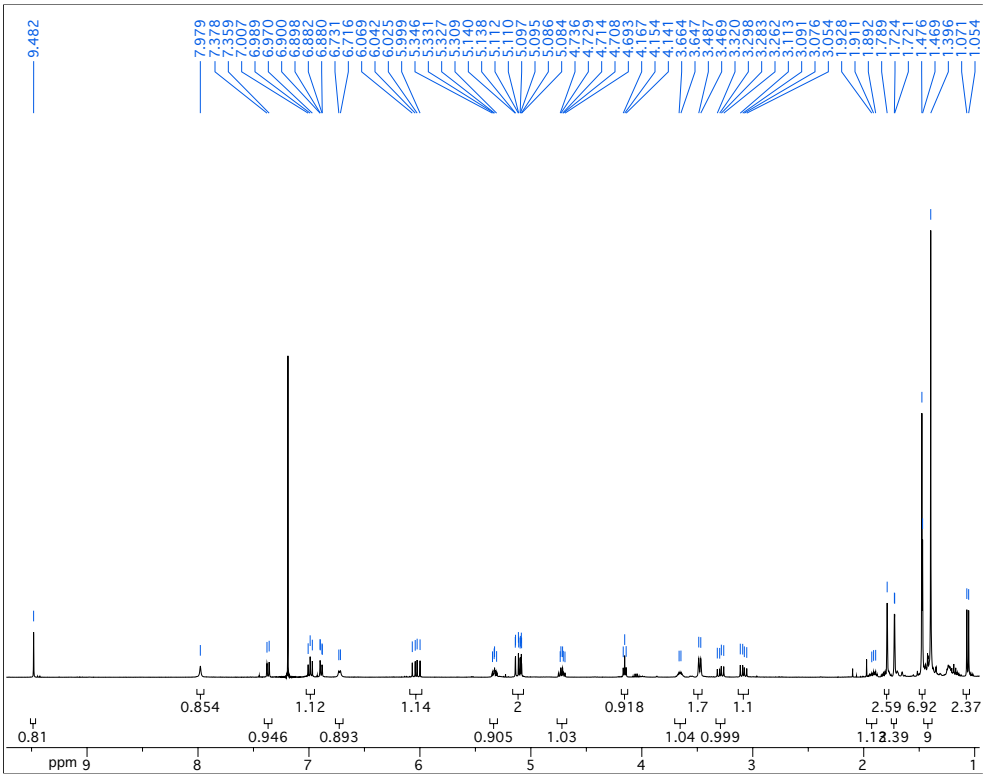
31.175
28.244
27.616
27.436
27.158
26.789
25.680
24.018
19.313
17.862
14.557
200
180
160
140
120
100
80
60
40
20

(**248b**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.48 (s, 1 H), 7.97 (bs, 1 H), 7.35 (d, *J* = 7.8 Hz, 1 H), 6.90 (t, *J* = 7.8 Hz, 1 H), 6.88 (d, *J* = 7.1 Hz, 1 H), 6.71 (bd, *J* = 5.8 Hz, 1 H), 5.99 (dd, *J* = 10.5, 17.4 Hz, 1 H), 5.32 (t, *J* = 7.4 Hz, 1 H), 5.08 (dd, *J* = 10.5, 17.4 Hz, 2 H), 4.71 (m, 1 H), 4.15 (t, *J* = 5.2 Hz, 1 H), 3.66 (m, 1 H), 3.46 (d, *J* = 7.4 Hz, 2 H), 3.26 (dd, *J* = 8.6, 14.6 Hz, 1 H), 3.05 (dd, *J* = 8.6, 14.6 Hz, 1 H), 1.91 (m, 1 H), 1.78 (s, 3 H), 1.72 (s, 3 H), 1.46 (s, 6 H), 1.39 (s, 9 H), 1.05 (d, *J* = 6.7 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.6, 191.0, 173.5, 145.5, 140.0, 133.4, 132.9, 129.6, 123.1, 122.6, 121.5, 120.2, 116.1, 112.4, 104.8, 80.4, 59.0, 55.0, 47.7, 39.0, 31.1, 28.2, 27.5, 26.6, 25.6, 24.0, 19.1, 17.8, 14.2. HRMS (ESI/APCI+) calcd for $C_{33}H_{47}N_3O4$ [M + H] 550.3639, found 550.3644.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1.1
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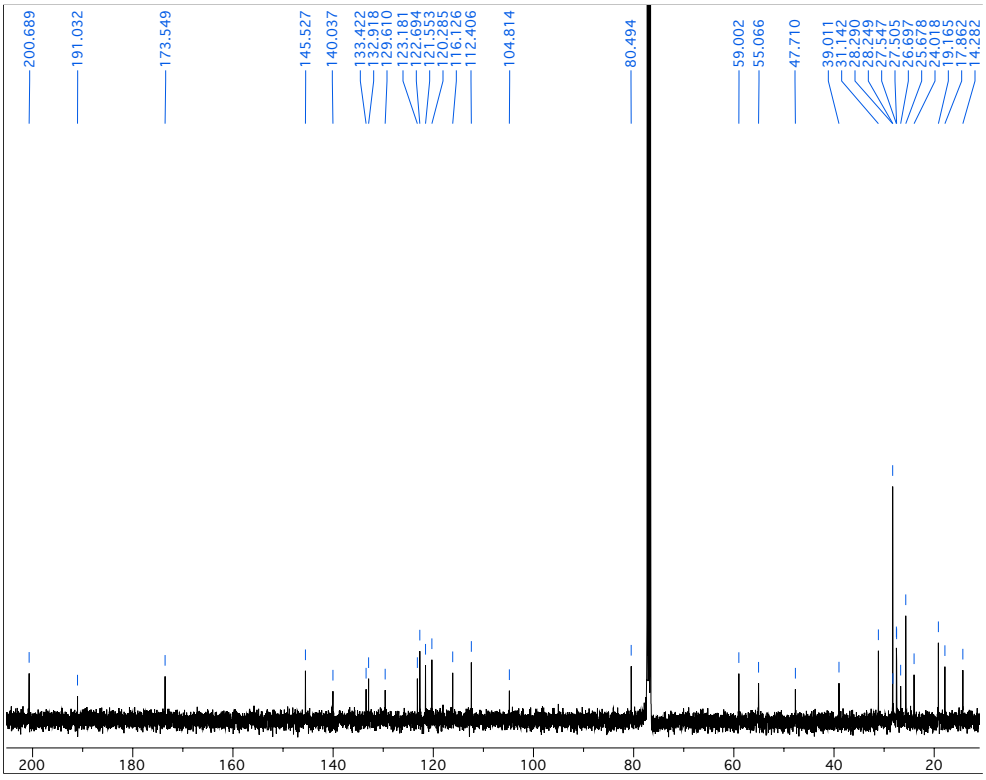
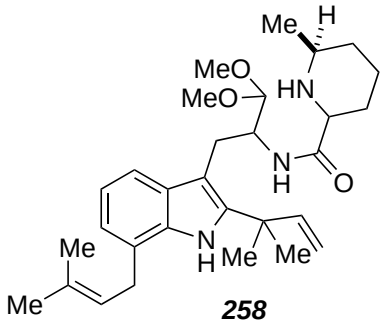
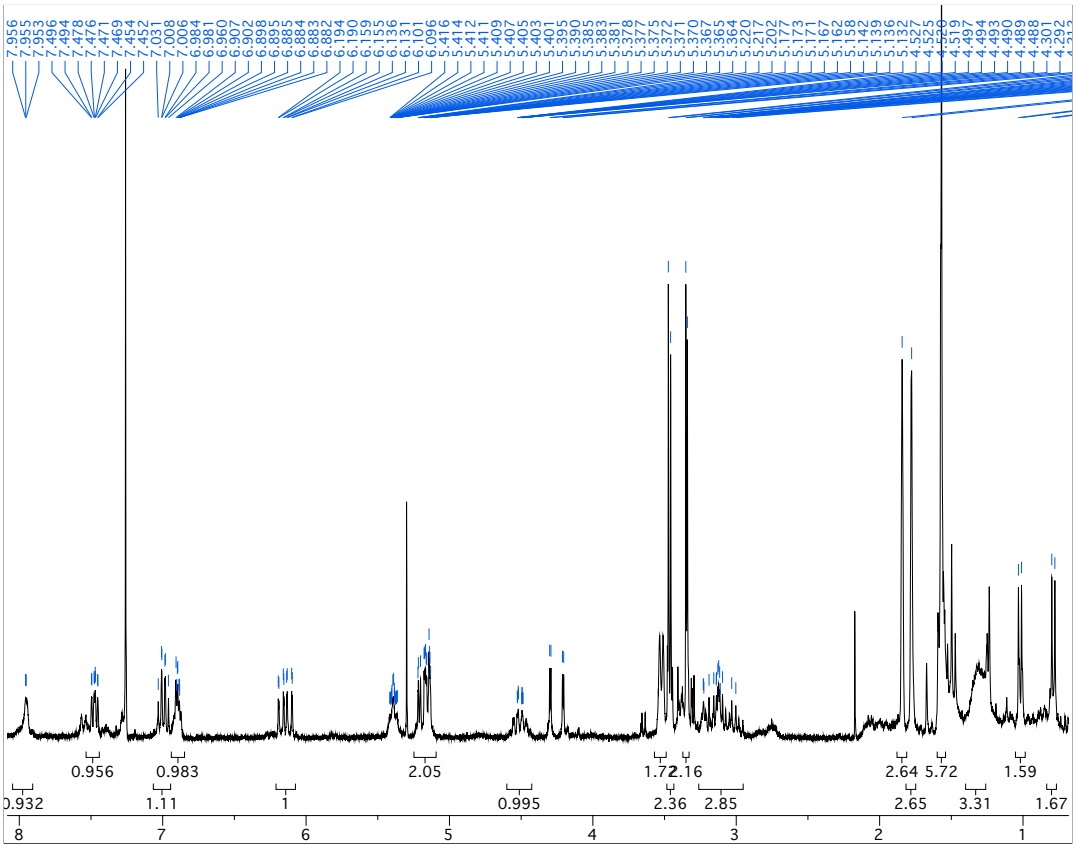




258

(6*S*)-*N*-(1,1-dimethoxy-3-(7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)propan-2-yl)-6-methylpiperidine-2-carboxamide (258). To a solution of **248** (11 mg, 0.02 mmol, 1 eq) in MeOH (200 µL) was added 1 M HCl in MeOH (40 µL, 0.04 mmol, 2 eq). The reaction was heated to reflux for 6 h. Concentration under reduced pressure afforded crude residue. Purification by preprative chromatography (EtOAc / hex., 1 : 1) to afford **258**

(7 mg, 80%) as a mixture of diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (bs, 1 H), 7.49-7.45 (m, 1 H), 7.03-6.96 (m, 1 H), 6.90-6.88 (m, 1 H), 6.09 (dd, *J* = 10.6, 17.1 Hz, 1 H), 5.41-5.36 (m, 1 H), 5.22-5.13 (m, 2 H), 4.52-4.48 (m, 1 H), 3.51 (d, *J* = 7.3 Hz, 2 H), 3.45 (s, 3 H), 3.34 (s, 3 H), 3.23-3.03 (m, 3 H), 1.84 (s, 3 H), 1.77 (s, 3 H), 1.56 (s, 6 H), 1.35-1.28 (m, 3 H), 1.01 (d, *J* = 6.5 Hz, 1.5 H), 0.77 (d, *J* = 6.5 Hz, 1.5 H).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4.292
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2
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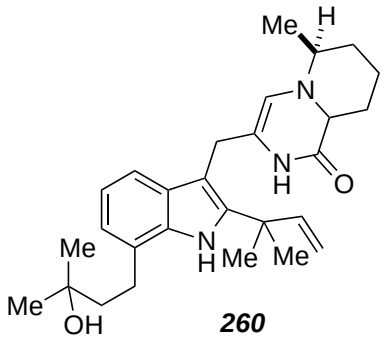
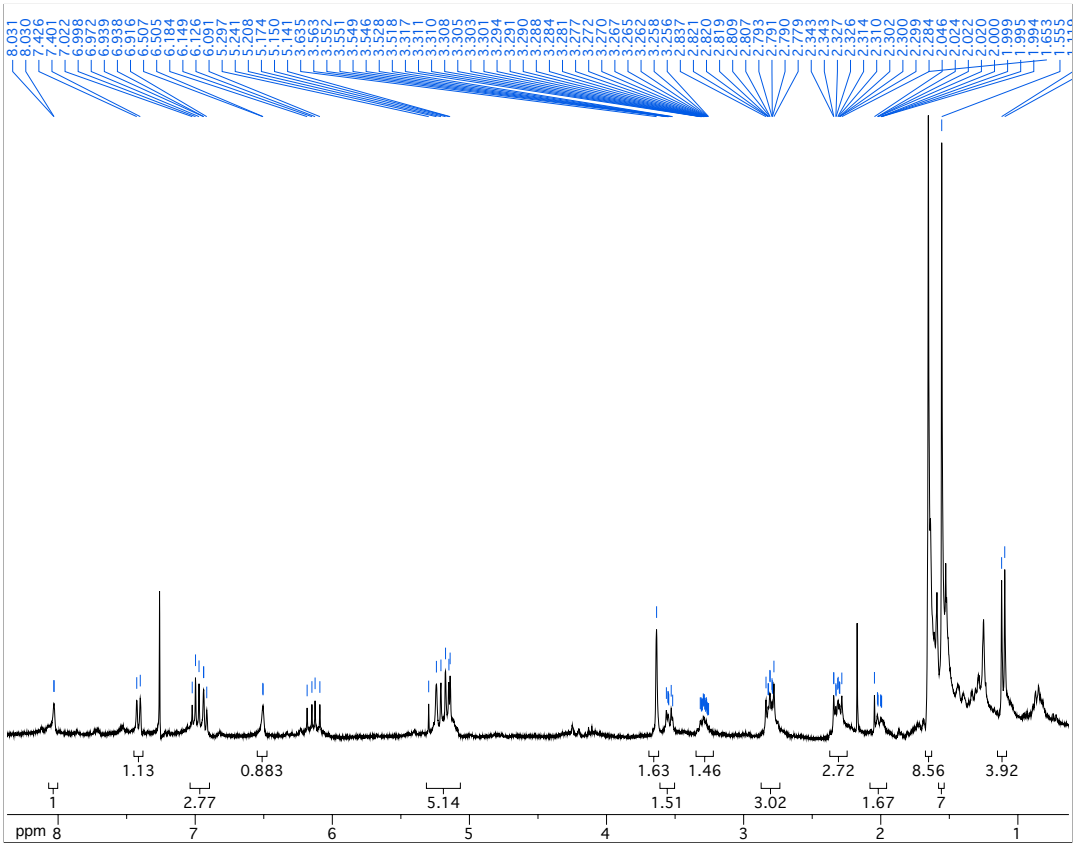


260

(6*S*)-3-((7-(3-hydroxy-3-methylbutyl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)-6-methyl-2,6,7,8,9,9a-hexahydro-1*H*-pyrido[1,2-*a*]pyrazin-1-one (260). To a solution of **248** (11 mg, 0.02 mmol, 1 eq) in CH_2Cl_2 (200 µL) was added TFA (100 µL). The reaction was allowed to stir at room temperature for 3 h. Concentration under reduced pressure afforded the crude imine. Purification by preprative chromatograph (EtOAc / hex., 2 : 5) afforded **260** (5 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (bs, 1 H), 7.40 (d, *J* = 7.8 Hz, 1 H), 7.02-6.91 (m, 3 H), 6.50 (bs, 1 H), 6.09 (dd, *J* = 10.6, 17.1 Hz, 1 H), 5.24-5.10 (m, 4 H), 3.63 (s, 2 H), 3.56-3.52 (m, 1 H), 3.31-3.25 (m, 1 H), 2.83-2.75 (m, 3 H), 2.34-2.28 (m, 2 H), 2.04-1.99 (m, 1 H), 1.65 (s, 6 H), 1.55 (s, 6 H), 1.09 (d, *J* = 6.8 Hz, 3 H).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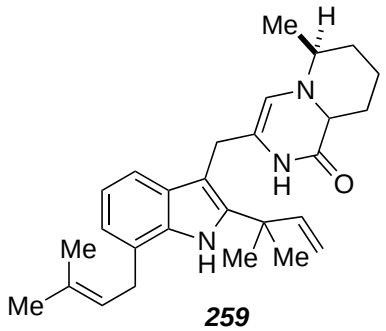
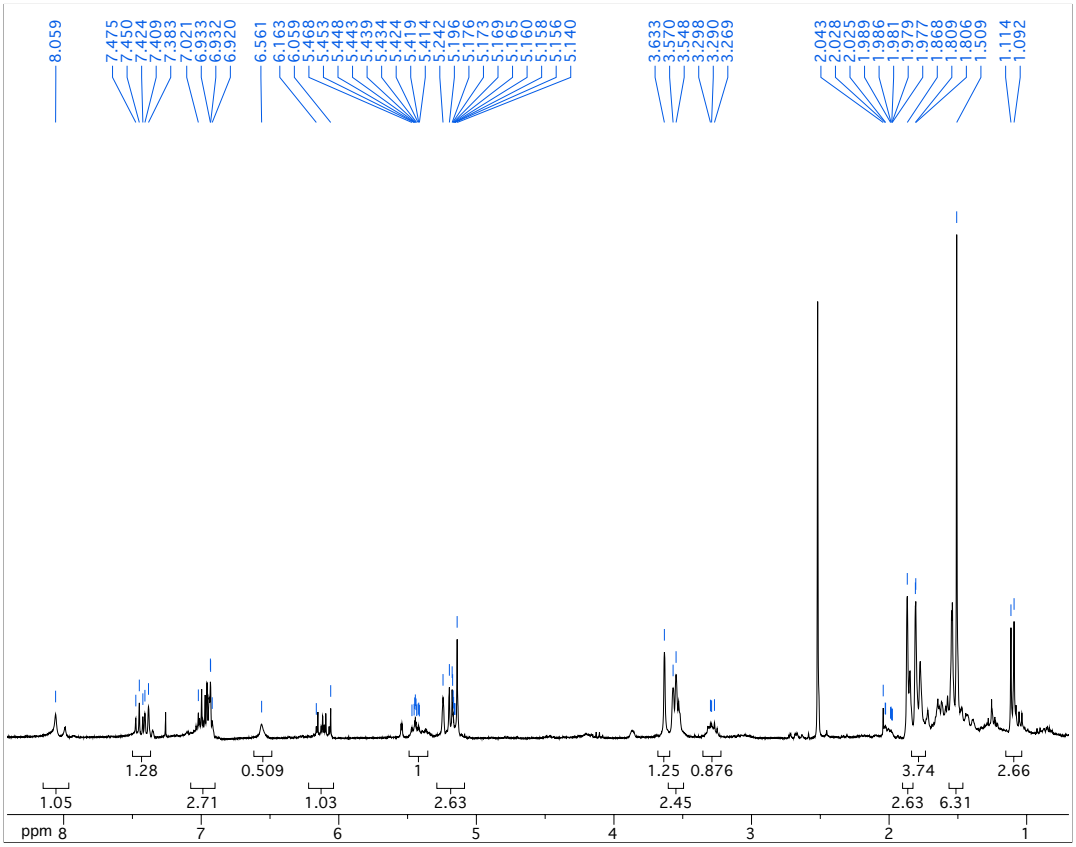


259

(6*S*)-6-methyl-3-((7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)-2,6,7,8,9,9a-hexahydro-1*H*-pyrido[1,2-*a*]pyrazin-1-one (259). To a solution of **248** (11 mg, 0.02 mmol, 1 eq) and 2,6-lutidine (6 µL, 0.05 mmol, 2.5 eq) in CH_2Cl_2 (500 µL) at 0ºC was added TMS-OTf (15 µL, 0.08 mmol, 4 eq). The reaction mixture was allowed to stir at 0ºC for 15 min. before removing the ice bath and stirring at room temperature for 95 min. The reaction was cooled to 0ºC at which time sat. NH_4Cl (3 mL) was added. The reaction mixture was extracted with EtOAc (3 x 5 mL). The organics were combined and washed with sat. $NaHCO_3$ (2 x 5 mL), brine (2 x 5 mL) and dried over Na_2SO_4. Filtration and concentration under reduced pressure afforded crude **259**. The crude product was used in further reactions. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (bs, 1 H), 7.47-7.38 (m, 1 H), 7.02-6.92 (m, 2 H), 6.56 (bs, 1 H), 6.16-6.05 (m, 1 H), 5.46-5.41 (m, 1 H), 5.24-5.15 (m, 2 H), 5.14 (s, 1 H), 3.63 (s, 2 H), 3.54 (d, *J* = 7.3 Hz, 2 H), 3.29-3.26 (m, 1 H), 2.04-1.97 (m, 1 H), 1.86 (s, 3 H), 1.80 (s, 3 H), 1.50 (s, 6 H), 1.09 (d, *J* = 6.8 Hz, 3 H).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8
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5
4
3
2
1

Appendix 1:

Publications

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Received, 15th April, 2010, Accepted, 14th May, 2010, Published online, 17th May, 2010
DOI: 10.3987/COM-10-S(E)19

STUDIES ON THE BIOSYNTHESIS OF THE STEPHACIDINS AND NOTOAMIDES. TOTAL SYNTHESIS OF NOTOAMIDE S

Timothy J. McAfoos,[a] Shengying Li,[c] Sachiko Tsukamoto,[d] David H. Sherman,[c] and Robert M. Williams[*a,b]

[a]Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, USA. [b]University of Colorado Cancer Center, Aurora, Colorado 80045, USA. [c]Life Sciences Institute and Departments of Medicinal Chemistry, Microbiology & Immunology, and Chemistry, University of Michigan, Ann Arbor, Michigan 48109, USA. [d]Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan

Abstract – Notoamide S has been suggested to be the final common precursor between two different *Aspergillus* sp. fungal strains before diverging to form enantiomerically opposite natural products (+)- and (–)-stephacidin A and (+)- and (-)-notoamide B. The synthesis of notoamide S comes from the coupling of *N*-Fmoc proline with a 6-hydroxy-7-prenyl-2-reverse prenyl tryptophan derivative that was synthesized via a late stage Claisen rearrangement from a 6-propargyl-2-reverse prenylated indole.

INTRODUCTION

The paraherquamide,[1] brevianamide,[2] stephacidin,[3] and notoamide[4] families of prenylated indoles are of great interest to chemists and biologists because of their structural complexity and their biological properties; in particular, the members of these families containing the unique bicyclo[2.2.2]diazaoctane ring system have drawn the most attention. Since the isolation of (+)-stephacidin A (**2**) in 2002 by Bristol-Myers Squibb from *Aspergillus ochraceus*,[3] the biosynthetic pathway of the stephacidin family of alkaloids has been of particular interest to our laboratories.[5] (+)-Stephacidin A was also subsequently identified as a co-metabolite with several new prenylated indole alkaloids, the notoamides, by Tsukamoto and co-workers from a marine derived *Aspergillus* sp.[4] Our interest in the biosynthesis of the stephacidin and notoamide families was heightened when the corresponding enantiomer, (–)-stephacidin A (**4**), was isolated from a terrestrial fungi, *Aspergillus versicolor* that was found growing in a Hawaiian forest (Figure 1).[6]

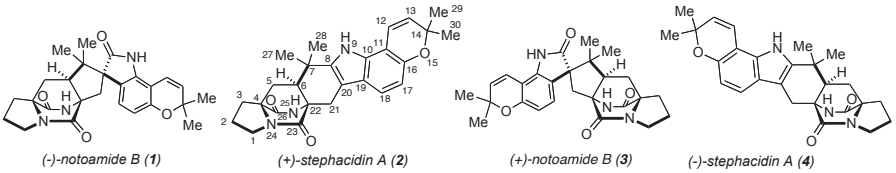
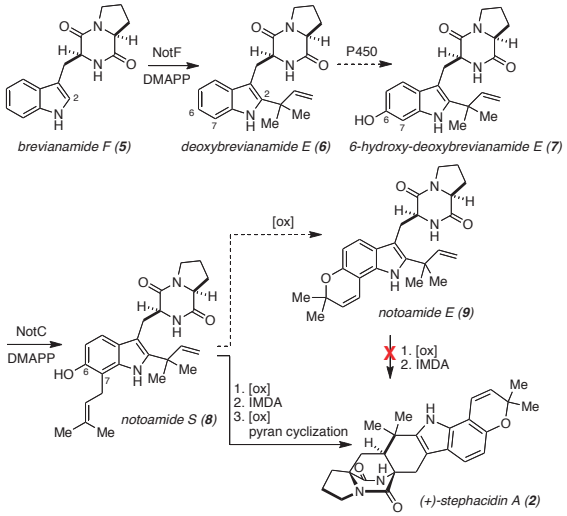


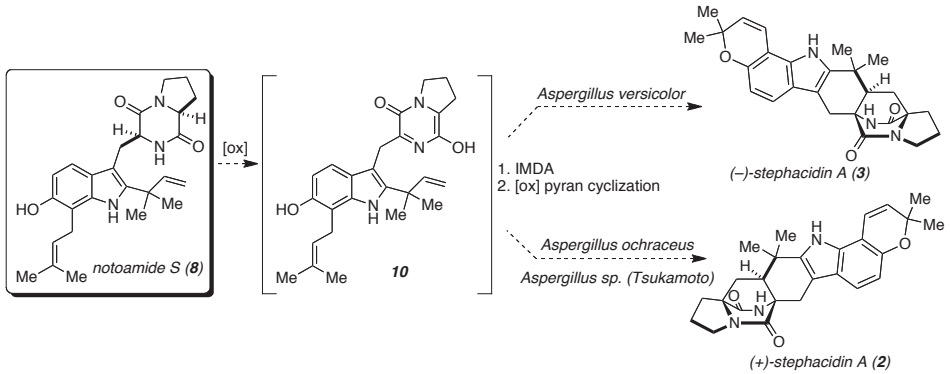
Figure 1. (+) And (–)-stephacidin A and (+) and (–)-notoamide B.

This most intriguing discovery lead to the as of yet unanswered biosynthetic question, at what point does the biosynthetic pathway of these fungi diverge to produce these two enantomerically distinct secondary metabolites? This assumes of course, that the biosynthesis of stephacidin in each respective species of the *Aspergillus* sp. are related through a common intermediate. Previous work by Sammes[7] and Birch,[8] as well as from our laboratories,[9] has shown that the bicyclo[2.2.2]diazaoctane ring system found in many of these natural products likely arises *via* a biosynthetic intramolecular Diels-Alder of an azadiene. One postulate concerning the enantiomeric selectivity seen in each fungus is that the facial selectivity during the key intramolecular Diels-Alder is respectively, enantioselective in the two fungi.[10] If the intramolecular Diels-Alder is the point of stereochemical divergence, then both fungi could potentially produce the same achiral azadiene intermediate, which in turn is believed to be produced via a common biosynthetic pathway from a mutual precursor. During our genome-based characterization experiments of the marine-derived *Aspergillus sp*. aimed at elucidation of each step of the biosynthesis, it was shown that brevianamide F (**5**) undergoes a reverse prenylation at the C2 position of the indole to give deoxybrevianamide E (**6**) by a reverse prenyl transferase NotF (Scheme 1).[11]



Scheme 1. Proposed biosynthesis of stephacidin A.

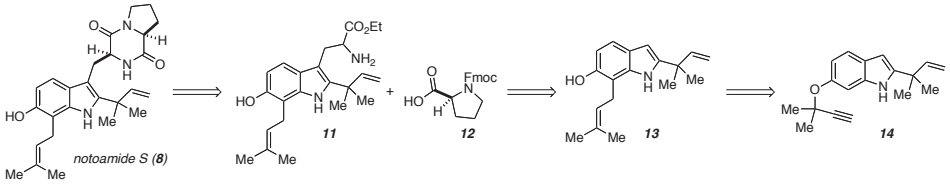
After oxidation of deoxybrevianamide E at C6, it was found that a prenylation takes place at C7 via the prenyl transferase NotC giving a previously undiscovered metabolite **8**, which we have named notoamide S.[11] We identified notoamide S (**8**) by ^{1}H and ^{13}C NMR as a metabolite constituted of two isoprene units, tryptophan and proline residues but lacking the bicyclo[2.2.2]diazaoctane ring system and pyranyl indole ring, as the possible precursor to the achiral azadiene.[11] Previously, we learned that notoamide E (**9**) is not a biosynthetic precursor to stephacidin A (**2**) through labeled precursor incorporation experiments, mandating that the putative intramolecular Diels-Alder preceeds the formation of the pyran ring. This narrowed the realm of possible precursors from **7** to stephacidin, suggesting that notoamide S is potentially the penultimate metabolite leading to the achiral azadiene, which can be transformed to either (+) or (–)-stephacidin A (Scheme 2).[12] In order to confirm the structure of natural **8**, an authentic sample was required; herein we detail the total synthesis of notoamide S (**8**).



Scheme 2. Notoamide S and the final achiral precursor (**10**) leading to (+)- or (–)-stephacidin A.

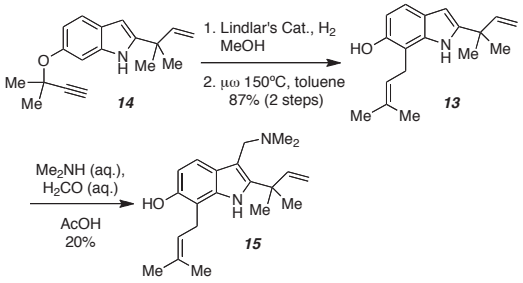
RESULTS AND DISCUSSION

We envisioned that, notoamide S (**8**) could come from the coupling of commercially available *N*-Fmoc L-proline (**12**) with the 6-hydroxy-7-prenyl-2-reverseprenyl tryptophan derivative (**11**). This tryptophan derivative can be derived from the corresponding 6-hydroxy-7-prenyl-2-reverseprenyl indole (**13**), which can be synthesized from the known indole **14**[13] (Scheme 3).



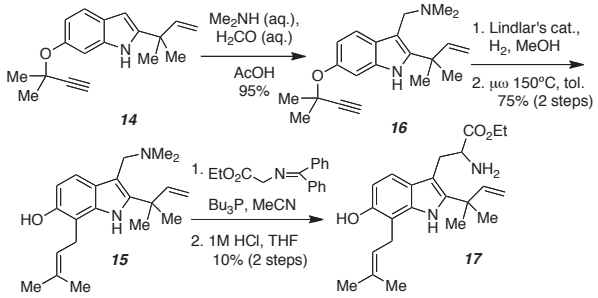
Scheme 3. Retrosynthetic analysis of notoamide S.

The assembly of notoamide S began from the previously reported indole **14**.[13] The treatment of **14** with Lindlar's catalyst under an atmosphere of hydrogen resulted in partial reduction of the alkyne (Scheme 4). The propargylic ether was heated under microwave conditions in toluene to give the desired 6-hydroxy-7-prenyl-2-reverseprenyl indole **13**. Indole **13** was treated with dimethyl amine and aqueous formaldehyde in acetic acid to give the desired gramine **15**, however in only 20% yield.



Scheme 4. Synthesis of gramine **15**.

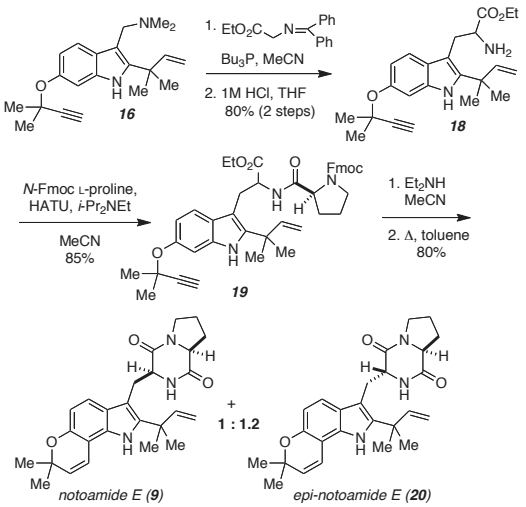
With the poor yield of the **15** it was decided to reverse the order of steps to see if the desired gramine could be synthesized in a higher yield. Formation of gramine **16** from **14** was followed by reduction and subsequent Claisen rearrangement to give the desired gramine **15** in a 71% overall yield (Scheme 5). Somei[14]-Kametani,[15] coupling of **15** with *N*-(diphenylmethylene)glycine ethyl ester in the presence of tributylphosphine followed by deprotection of the incipient benzophenone imine gave only a 10% yield of the desired tryptophan derivative **17**. It is believed that the free phenol is problematic in this reaction due to the acidity of the phenolic proton, therefore a different approach was adopted to avoid this problem.



Scheme 5. Synthesis of tryptophan derivative **17**.

With **14** being readily available on multi-gram scale, we decided to explore the use of the propargyl group as a temporary protecting group for the phenol by performing the reduction and Claisen rearrangement at

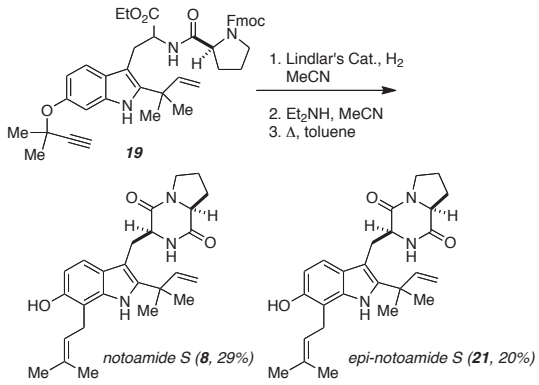
a later stage (Scheme 6). Under Somei-Kametani[14,15] conditions **16** can be converted to the desired tryptophan derivative which upon removal of the benzophenone imine with aqueous acid gave the free amine **18**. Coupling of **18** with *N*-Fmoc L-proline (**12**) in the presence of 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethyl amine gave the coupled product **19** in 85% yield. However, after removal of the Fmoc group in **19**, the cyclization to the diketopiperazine was also accompanied by undesired pyran formation to give the natural product notoamide E (**9**) and *epi*-notoamide E (**20**) (Scheme 6).



Scheme 6. Unanticipated formation of notoamide E and *epi*-notoamide E.

Although unanticipated, the adventitious synthesis of notoamide E is three steps shorter than our previously reported route starting from the same indole substrate **14**.[16] To obviate the formation of the pyran ring it was decided to reduce the alkyne to the alkene prior to the formation of the dioxopiperazine, thus allowing for the possibility of performing the desired Claisen and dioxopiperazine formation in one operation. Reduction of **19** with Lindlar's catalyst gave the desired alkene in quantitative yield. With the full skeleton in hand all that remained was cleavage of the Fmoc group of the proline residue followed by cyclization to the dioxopiperazine ring (Scheme 7). As shown previously with the formation of the pyran ring, it was believed that the Claisen rearrangement of the reverse prenyl ether would take place during the cyclization of the dioxopiperazine. Removal of the Fmoc group proceeded smoothly in the presence of diethylamine, and upon refluxing in toluene the desired compound **8** (notoamide S) was isolated in a modest yield (18%); however, three other products were also found to be present in the mixture. These include: *epi*-notoamide S (**21**) followed by notoamide E and *epi*-notoamide E. Further investigation

revealed that the formation of the pyran ring was occurring during the Somei-Kametani[14,15] reaction, and these inseparable by-products were invariably carried through the synthesis. Suppression of this undesired side reaction can be achieved by lowering the temperature of the Somei-Kametani[14,15] reaction below reflux. Under these conditions, notoamide S was obtained in 29% isolated yield along with **21** (20%), **9** (8%) and **20** (5%). The synthetic notoamide S exactly matched the natural metabolite obtained from the marine-derived *Aspergillus* sp. (Tsukamoto) by ^{1}H nmr and retention time on LC.[11]



Scheme 7. Synthesis of notoamide S from the propargyl dipeptide **19**.

CONCLUSION

The synthesis of notoamide S (**8**) was accomplished in seven steps from known indole **14** in 19% overall yield. Synthetic access to this potentially pivotal intermediate, will allow us to further interrogate the steps from notoamide S to the distinct natural enantiomers of stephacidin A and notoamide B. These studies are in progress and will be reported in due course.

EXPERIMENTAL

General. Unless otherwise noted, all materials were obtained from commercial sources and used without purification. All reactions were carried out under anhydrous conditions, unless otherwise specified, and performed under positive pressure of argon using flame-dried glassware. Dichloromethane, acetonitrile, toluene, and tetrahydrofuran were degassed with argon and dried through a solvent purification system (J. C. Meyer of Glass Contour). Flash chromatography was performed on standard grade silica gel (230 x 400 mesh) from Sorbent Technologies with the indicated solvent. Microwave reactions were run using a CEM Discover set to a constant temperature and allowing for variable power. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 300 and 400 MHz spectrometers as indicated. Chemical shifts are reported in parts per million downfield from tetramethylsilane. Peak multiplicities are denoted s (singlet),

bs (broad singlet), d (doublet), t (triplet), m (multiplet) or by a combination of these *e.g*. dd (doublet of doublets). Infrared spectra were recorded on a Bruker Tensor 27 IR spectometer on NaCl plates. Mass spectra were obtained at the Colorado State University CIF on a Fisons VG Autospec.

7-(3-Methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-ol (13). To **14** (100 mg, 0.37 mmol, 1 eq) in MeOH (3 mL) was added Lindlar's catalyst (10 mg, 10 wt %) at rt followed by purging with a balloon of hydrogen gas. The reaction was vigorously stirred for 30 min. The reaction was monitored by NMR for loss of alkyne proton. The reaction was then filtered through a plug of Celite and concentrated under reduced pressure to give a crude yellow oil. The resultant crude yellow oil was then taken up in toluene (3 mL) and the reaction was heated to 150 ºC in a microwave reactor for 30 min. The reaction was then concentrated under reduced pressure to give the crude **13** as a brown oil. The residue was purified by flash chromatograph (AcOEt/hexane, 1:20) to give indole **13** (87 mg, 87%) as an off white solid. R_f = 0.38 (AcOEt/hexane, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (bs, 1 H), 7.20 (d, *J* = 8.3 Hz, 1 H), 6.57 (d, *J* = 8.3 Hz, 2 H), 6.18 (s, 1 H), 6.03 (dd, *J* = 17.3, 10.5 Hz, 1 H), 5.37 (t, *J* = 7.25 Hz, 1 H), 5.10 (d, *J* =17.3 Hz, 1 H), 5.08 (d, *J* = 10.5 Hz, 1 H), 4.70 (s, 1 H), 3.55 (d, *J* = 7.25 Hz, 2 H), 1.84 (s, 3 H), 1.75 (s, 3 H), 1.42 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 146.5, 144.5, 136.4, 134.0, 123.1, 122.3, 118.4, 112.0, 110.1, 108.1, 97.99, 38.3, 27.5, 25.9, 24.5, 18.1; IR (ν_{max}) 3447, 1621, 1103, 913, 852 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{18}H_{23}NO$ (M + H) 270.1852, found 270.1851.

3-((Dimethylamino)methyl)-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-ol (15) from 13. To AcOH (500 μL) was added at rt, Me_2NH (6 μL, 0.074 mmol, 1 eq, 40% aqueous solution) followed by H_2CO (8 μL, 0.074 mmol, 1 eq, 37% aqueous solution) and stirred for 30 min. To this was added at rt a solution of **13** (20 mg, 0.074 mmol, 1 eq) in AcOH (500 μL) and stirred for 4 h. The reaction was basified with 2M NaOH to a pH of 10–12 and extracted with Et_2O (3 x 10 mL). The organic layers were combined and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure, to give **15** (4 mg, 20%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (bs, 1 H), 7.35 (d, *J* = 8.4 Hz, 1 H), 6.60 (d, *J* = 8.4 Hz, 1 H), 6.16 (dd, *J* = 17.5, 10.6 Hz, 1 H), 5.35 (t, *J* = 7.3, 1 H), 5.16 (d, *J* = 17.3, 1 H), 5.12 (d, *J* = 10.6, 1 H), 3.59 (bs, 1 H), 3.53 (d, *J* = 7.3 Hz, 2 H), 2.24 (s, 6 H), 1.84 (s, 3 H), 1.75 (s, 3 H), 1.51 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.8, 146.5, 134.4, 133.8, 122.6, 117.4, 111.8, 109.9, 107.8, 54.0, 45.3, 39.5, 30.5, 29.8, 27.3, 25.9, 25.7, 24.4, 18.1; IR (ν_{max}) 3457, 1623, 1241, 1011, 915, 842 cm^{-1}; HRMS (ESI/APCI+), calcd for $C_{19}H_{24}NO$ (M – $N(CH_3)_2$) 282.1852, found 282.1860.

***N*,*N*-Dimethyl-1-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)methanamine (16).** To AcOH (1.5 mL) was added at rt Me_2NH (320 μL, 2.84 mmol, 1.05 eq, 40% aqueous

solution) followed by H_2CO (230 μL, 2.84 mmol, 1.05 eq, 37% aqueous solution) and stirred for 30 min. To this was added at rt a solution of **14** (724 mg, 2.70 mmol, 1 eq) in AcOH (1.5 mL) and stirred for 4 h. The reaction was basified with 2M NaOH to a pH of 10–12 and washed with Et_2O (3 x 10 mL). The organic layers were combined and dried over anhydrous $MgSO_4$, and concentrated under reduced pressure, to give **16** (835 mg, 95%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (bs, 1 H), 7.52 (d, *J* = 8.5 Hz, 1 H), 7.15 (d, *J* = 1.9 Hz, 1 H), 6.90 (dd, *J* = 8.5, 1.9 Hz, 1 H), 6.16 (dd, *J* = 17.5, 10.5, 1 H), 5.17 (d, *J* = 17.5, 1 H), 5.13 (d, *J* = 10.5, 1 H), 3.54 (s, 2 H), 2.50 (s, 1 H), 2.20 (s, 6 H), 1.60 (s, 6 H), 1.53 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.4, 140.8, 133.9, 127.0, 119.0, 115.9, 112.0, 103.9, 73.3, 73.0, 54.2, 45.5, 42.1, 39.4, 29.8, 27.3, 25.8; IR (ν_{max}) 3301, 1622, 1463, 1231, 1134, 1042, 1012, 968, 914 cm^{-1}; HRMS (ESI/APCI+), calcd for $C_{19}H_{22}NO$ (M – $N(CH_3)_2$) 280.1696, found 280.1695.

3-((Dimethylamino)methyl)-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-6-ol (15). To **16** (100 mg, 0.30 mmol, 1 eq) in MeOH (3 mL) was added at rt Lindlar's catalyst (10 mg, 10 wt %) followed by purging with a balloon of hydrogen gas. The reaction was vigorously stirred for 30 min. The reaction was monitored by NMR for loss of the alkyne proton. The reaction was then filtered through a plug of Celite and concentrated under reduced pressure. The resultant yellow oil was then taken up in toluene (3 mL) and subjected to microwave conditions of 150 ºC for 30 min. The reaction was then concentrated under reduced pressure to give crude **15** (75 mg, 75%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (bs, 1 H), 7.35 (d, *J* = 8.4 Hz, 1 H), 6.60 (d, *J* = 8.4 Hz, 1 H), 6.16 (dd, *J* = 17.5, 10.6 Hz, 1 H), 5.35 (t, *J* = 7.3, 1 H), 5.16 (d, *J* = 17.3, 1 H), 5.12 (d, *J* = 10.6, 1 H), 3.59 (bs, 1 H), 3.53 (d, *J* = 7.3 Hz, 2 H), 2.24 (s, 6 H), 1.84 (s, 3 H), 1.75 (s, 3 H), 1.51 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.8, 146.5, 134.4, 133.8, 122.6, 117.4, 111.8, 109.9, 107.8, 54.0, 45.3, 39.5, 30.5, 29.8, 27.3, 25.9, 25.7, 24.4, 18.1; IR (ν_{max}) 3457, 1623, 1241, 1011, 915, 842 cm^{-1}; HRMS (ESI/APCI+), calcd for $C_{19}H_{24}NO$ (M – $N(CH_3)_2$) 282.1852, found 282.1860.

Ethyl 2-amino-3-(6-hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)-propanoate (17). To a solution of **15** (61 mg, 0.18 mmol, 1 eq) and *N*-(diphenylmethylene)glycine ethyl ester (52 mg, 0.19 mmol, 1.05 eq) in acetonitrile (2 mL) was added at rt Bu_3P (10 μL, 0.074 mmol, 0.4 eq). The reaction was heated under reflux for 24 h. The reaction was cooled to rt and concentrate under reduced pressure. The residue was taken up in THF (3 mL) and 1 M HCl (2 mL) was added and the reaction was stirred at rt for 3 h. The solution was basified with sat. aq. $NaHCO_3$ and washed with AcOEt (3 x 10 mL). The organics were combined and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The yellow residue was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0:1–1:20) to give **17** (7 mg, 10%) as a yellow oil. R_f = 0.36 ($MeOH/CH_2Cl_2$, 1:20); ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.86 (bs, 1 H), 7.13 (d, J = 8.4 Hz, 1 H), 6.49 (d, J = 8.4 Hz, 1 H), 6.11 (dd, J = 17.5, 10.5 Hz, 1 H), 5.33 (t, J = 7.3 Hz, 1 H), 5.14 (dd, J = 17.4, 11.8 Hz, 2 H), 4.16-4.07 (m, 2 H), 3.85-3.81 (m, 1 H), 3.53 (d, J = 7.3 Hz, 2 H), 3.29 (dd, J = 14.4, 4.7 Hz, 2 H), 3.00 (dd, J = 14.4, 4.5 Hz, 2 H), 1.84 (s, 3 H), 1.74 (s, 3 H), 1.49 (s, 6 H), 1.18 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 149.2, 146.4, 139.0, 134.9, 133.4, 124.2, 122.9, 116.8, 111.9, 110.1, 108.3, 106.7, 61.1, 55.8, 39.3, 31.2, 28.0, 27.9, 25.9, 24.3, 18.1, 14.2; IR (ν_{max}) 3449, 1730, 1621, 1444, 1100, 917, 799 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{23}H_{32}N_2O_3$ (M + H) 385.2486, found 385.2483.

Ethyl 2-amino-3-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)propanoate (18). To a solution of **16** (180 mg, 0.52 mmol, 1 eq) and *N*-(diphenylmethylene)glycine ethyl ester (155 mg, 0.57 mmol, 1.1 eq) in acetonitrile (5 mL) was added at rt Bu_3P (29 μL, 0.21 mmol, 0.4 eq). The reaction was taken to 70ºC and stirred for 24 h. Cool to rt and concentrate under reduced pressure. The residue was then taken up in THF (5 mL) at rt was added 1M HCl (1 mL) and stirred at rt for 1 h. The solution was basified with 2M NaOH and washed with Et_2O (3 x 10 mL). The combined organic layers were washed with Brine (15 mL), dried of over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give crude **18** as a yellow oil. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$, 0:100–1:20) to give **18** (160 mg, 80%) as a light yellow foam. R_f = 0.36 ($MeOH/CH_2Cl_2$, 1:20); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (bs, 1 H), 7.35 (d, *J* = 8.5 Hz, 1 H), 7.16 (s, 1 H), 6.89 (d, *J* = 8.5 Hz, 1 H), 6.12 (dd, *J* = 17.5, 10.5 Hz, 1 H), 5.12 (d, *J* = 17.5 Hz, 1 H), 5.08 (d, *J* = 10.5 Hz, 1 H), 4.08-3.96 (m, 2 H), 3.79 (q, *J* = 5.3 Hz, 1 H), 3.25 (dd, *J* = 14.4, 5.3 Hz, 1 H), 3.02 (dd, *J* = 14.3, 9.3 Hz, 1 H), 2.49 (s, 1 H), 1.56 (s, 6 H), 1.48 (s, 6 H), 1.07 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 151.1, 146.2, 140.2, 134.4, 118.5, 116.1, 112.2, 104.3, 87.0, 73.5, 73.1, 61.0, 56.1, 39.3, 31.5, 29.8, 28.0, 24.6, 24.4, 23.9; IR (ν_{max}) 3267, 1733, 1622, 1464, 1136, 969 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{23}H_{31}N_2O_3$ (M + H) 383.2329, found 383.2328.

Ethyl (2*S*)-(9*H*-Fluoren-9-yl)methyl-2-((1-(2-(2-methylbut-3-en-2-yl)-6-((2-methylbut-3-yn-2-yl)oxy)-1*H*-indol-3-yl)butan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (19). To a solution of **18** (50 mg, 0.130 mmol, 1 eq) and *N*-(9-fluorenylmethoxycarbonyl)-L-proline (46 mg, 0.137 mmol, 1.05 eq) in acetonitrile (1.5 mL) at 0 ºC was added HATU (62 mg, 0.163 mmol, 1.25 eq) followed by *i*-Pr_2NEt (47 μL, 0.287 mmol, 2.2 eq). The reaction was allowed to warm to rt and was stirred for 12 h. The solution was concentrated under reduced pressure and the residue was purified by flash chromatography (AcOEt/hexane, 1:5–9:20) to give dipeptide **19** (77 mg, 85%) as a white foam. R_f = 0.35 (AcOEt/hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotomers, diastereomers) δ 7.79-7.77 (comp, 3 H), 7.61-7.58 (comp, 2 H), 7.37-7.26 (comp, 5 H), 7.14 (bs, 1), 6.93-6.89 (comp, 1 H), 6.17-6.07 (comp, 1 H),

5.22-5.14 (comp, 2 H), 4.79-4.70 (comp, 1 H), 4.45-4.33 (comp, 2 H), 4.29-4.15 (comp, 3 H), 4.00-3.77 (comp, 3 H), 3.51-3.33 (comp, 2 H), 3.29-3.08 (comp, 3 H), 2.48 (bs, 1 H) 1.99-1.93 (comp, 2 H), 1.92-1.80 (comp, 2 H), 1.59 (bs, 6 H), 1.54 (bs, 6H), 1.01-0.89 (comp, 3 H).
^{13}C NMR (100 MHz, $CDCl_3$, mixture of diastereomers) δ 172.6, 151.1, 151.0, 145.9, 144.2, 144.1, 141.4, 134.3, 127.9, 127.3, 125.4, 120.1, 112.4, 104.5, 87.1, 87.0, 73.6, 73.1, 67.8, 61.4, 60.6, 53.8, 47.3, 39.3, 29.8, 27.7, 21.2, 14.4, 13.9, 13.8; IR (ν_{max}) 3346, 1684, 1126, 968, 739 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{43}H_{47}N_3NaO_6$ (M + Na) 724.3357, found 724.3355.

(3*S*,8a*S*)-3-((7,7-Dimethyl-2-(2-methylbut-3-en-2-yl)-1,7-dihydropyrano[2,3-*g*]indol-3-yl)methyl)-hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (9) and (3*R*,8a*S*)-3-((7,7-Dimethyl-2-(2-methylbut-3-en-2-yl)-1,7-dihydropyrano[2,3-*g*]indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (20). To **19** (71 mg, 0.10 mmol, 1 eq) in acetonitrile (1.5 mL) at rt was added Et_2NH (700 μL) and the reaction was stirred for 1 h at rt. The reaction was concentrated under reduced pressure to give an orange/brown residue. The residue was dissolved in toluene (3 mL) and heated under reflux for 18 h. The solution was then concentrated under reduced pressure and the residue was purified by flash chromatography (MeOH/CH_2Cl_2, 1:20) to give **9** (15 mg, 36%) & **20** (19 mg, 44%). (**9**) R_f = 0.41 (MeOH/CH_2Cl_2, 1:20); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1 H), 7.21 (d, *J* = 8.5 Hz, 1 H), 6.65 (d, *J* = 8.5 Hz, 1 H), 6.58 (d, *J* = 9.7 Hz, 1 H), 6.13 (dd, *J* = 17.5, 10.4 Hz, 1 H), 5.70 (bs, 1 H), 5.67 (d, *J* = 9.7 Hz, 1 H), 5.21-5.10 (m, 2 H), 4.44-4.33 (m, 1 H), 4.12-4.00 (m, 1 H), 3.75-3.51 (m, 3 H), 3.12 (dd, *J* = 15.3, 11.7 Hz, 1 H), 2.41-2.27 (m, 1 H), 2.21-1.81 (m, 3 H), 1.54 (s, 6 H), 1.46 (s, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.5, 166.0, 149.1, 146.0, 140.1, 131.0, 130.2, 123.9, 118.2, 117.0, 112.8, 111.1, 105.2, 105.1, 76.0, 59.4, 55.1, 45.6, 39.2, 28.6, 28.1, 27.6, 27.5, 26.1, 22.8; HRMS (FAB) calcd for $C_{26}H_{32}N_3O_3$ [M + H] 434.2444, found 434.2422. (**20**) R_f = 0.29 (MeOH/CH_2Cl_2, 1:20); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (bs, 1 H), 7.25 (d, *J* = 8.4 Hz, 1 H), 6.64 (d, *J* = 8.4 Hz, 1 H), 6.58 (d, *J* = 9.7 Hz, 1 H), 6.13 (dd, *J* = 17.5, 10.6 Hz, 1 H), 5.84 (d, *J* = 3.7 Hz, 1 H), 5.66 (d, *J* = 9.9, 1 H), 5.19 (d, *J* = 11.7 Hz, 1 H), 5.15 (d, *J* = 4.4 Hz, 1 H), 4.31-4.22 (m, 1 H), 3.76-3.55 (m, 2 H), 3.52-3.35 (m, 2 H), 3.26 (dd, *J* = 14.6, 9.0 Hz, 1 H), 2.37-2.21 (m, 1 H), 2.05-1.62 (m, 4 H), 1.52 (s, 6 H), 1.45 (s, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.8, 166.0, 148.9, 146.4, 140.2, 130.9, 130.0, 123.7, 118.9, 117.0, 112.0, 110.8, 105.7, 104.8, 75.9, 58.7, 58.4, 45.7, 39.3, 30.0, 29.2, 28.2, 28.0, 27.5, 22.1; HRMS (FAB) calcd for $C_{26}H_{32}N_3O_3$ [M + H] 434.2444, found 434.2422.

(3*S*,8a*S*)-3-((6-Hydroxy-7-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-1*H*-indol-3-yl)methyl)-hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (8) (Notoamide S). To **19** (286 mg, 0.41 mmol, 1 eq) in MeOH (4 mL) was added at rt Lindlar's catalyst (28 mg, 10 wt %) followed by purging with a balloon of hydrogen. The reaction was vigorously stirred for 30 min. The reaction was monitored by NMR for loss

of the alkyne proton. The reaction was then filtered through a plug of Celite and concentrated under reduced pressure to give the alkene as yellow foam which was directly taken on to the next step without further purification. Et_2NH (2 mL) was added to a solution of the crude alkene in acetonitrile (3 mL) and the reaction was stirred for 1 h at rt. The reaction was concentrated under reduced pressure to give a yellow oil. The residue was dissolved in toluene (5 mL) and heated under reflux for 18 h. The reaction was then concentrated under reduced pressure and purified by flash chromatography ($MeOH/CH_2Cl_2$, 1:20) to give **8** (51 mg, 29%), as a yellow oil. R_f = 0.67 ($MeOH/CH_2Cl_2$, 1:10); ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (bs, 1 H), 7.15 (d, *J* = 8.5 Hz, 1 H), 6.65 (d, J = 8.5 Hz, 1 H), 6.15 (dd, *J* = 17.5, 10.3 Hz, 1 H), 5.75 (bs, 1 H), 5.38 (t, *J* = 7.2 Hz, 1 H), 5.18 (d, *J* = 5.35 Hz, 1 H), 5.13 (s, 1 H), 4.41 (dd, *J* = 10.7, 2.5 Hz, 1 H), 4.08 (t, *J* = 7.3 Hz, 1 H), 3.71-3.61 (m, 2 H), 3.57 (d, *J* = 7.2 Hz, 2 H), 3.15 (dd, *J* = 15.3, 11.6 Hz, 1 H), 2.39-2.31 (m, 1 H), 2.12-2.00 (m, 2 H), 1.88 (s, 3 H), 1.79 (s, 3 H), 1.50 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 166.0, 149.4, 145.9, 139.9, 134.9, 133.9, 123.6, 122.5, 116.2, 112.6, 110.5, 104.5, 59.4, 55.1, 45.5, 39.1, 29.8, 28.5, 28.0, 27.9, 25.9, 24.3, 22.8, 18.1; IR (ν_{max}) 3362, 1665, 1438, 919, 800 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{26}H_{32}DN_3O_3$ [M + Na] 459.2477, found 459.2475. (**21**) (35 mg, 20%) R_f = 0.53 ($MeOH/CH_2Cl_2$, 1:10); ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (bs, 1 H), 7.15 (d, *J* = 8.4 Hz, 1 H), 6.63 (d, *J* = 8.4 Hz, 1 H), 6.11 (dd, *J* = 17.4, 10.3 Hz, 1 H), 5.83 (bs, 1 H), 5.76 (bs, 1 H), 5.32 (t, *J* = 7.4 Hz, 1 H), 5.15 (dd, *J* = 17.4, 10.3 Hz, 2 H), 4.24-4.21 (m, 2 H), 3.78-3.74 (m, 1 H), 3.68-3.61 (m, 1 H), 3.53 (d, *J* = 7.4 Hz, 2 H), 3.48-3.37 (m, 3 H), 3.22 (dd, *J* = 14.4, 10 Hz, 1 H), 2.32-2.27 (m, 1 H), 1.99-1.86 (m, 2 H), 1.84 (s, 3 H), 1.74 (s, 3 H), 1.45 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 166.0, 149.4, 146.3, 139.9, 135.0, 133.6, 123.3, 122.7, 116.7, 111.8, 110.3, 108.3, 105.0, 58.9, 58.4, 45.7, 39.2, 30.1, 29.2, 28.0, 27.8, 25.9, 24.3, 22.1, 18.1; IR (ν_{max}) 3336, 1655, 1448, 918, 802 cm^{-1}; HRMS (ESI/APCI+) calcd for $C_{26}H_{33}N_3NaO_3$ [M + Na] 458.2414, found 458,2413.

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ARTICLES

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Genome-Based Characterization of Two Prenylation Steps in the Assembly of the Stephacidin and Notoamide Anticancer Agents in a Marine-Derived *Aspergillus* sp.

Yousong Ding,[†] Jeffrey R. de Wet,[‡] James Cavalcoli,[‡] Shengying Li,[†] Thomas J. Greshock,[§] Kenneth A. Miller,[§] Jennifer M. Finefield,[§] James D. Sunderhaus,[§] Timothy J. McAfoos,[§] Sachiko Tsukamoto,[||] Robert M. Williams,*[,§] and David H. Sherman*[,†]

Life Sciences Institute and Departments of Medicinal Chemistry, Microbiology & Immunology, and Chemistry, University of Michigan, Ann Arbor, Michigan 48109, Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan 48109, Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, USA and University of Colorado Cancer Center, Aurora, Colorado 80045, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan

Received June 6, 2010; E-mail: davidhs@umich.edu; rmw@lamar.colostate.edu

Abstract: Stephacidin and notoamide natural products belong to a group of prenylated indole alkaloids containing a core bicyclo[2.2.2]diazaoctane ring system. These bioactive fungal secondary metabolites have a range of unusual structural and stereochemical features but their biosynthesis has remained uncharacterized. Herein, we report the first biosynthetic gene cluster for this class of fungal alkaloids based on whole genome sequencing of a marine-derived *Aspergillus* sp. Two central pathway enzymes catalyzing both normal and reverse prenyltransfer reactions were characterized in detail. Our results establish the early steps for creation of the prenylated indole alkaloid structure and suggest a scheme for the biosynthesis of stephacidin and notoamide metabolites. The work provides the first genetic and biochemical insights for understanding the structural diversity of this important family of fungal alkaloids.

Introduction

Structurally complex fungal-derived natural products account for a significant number of clinical therapeutics for treatment of human and animal diseases.[1] Due to emerging appreciation for the high level of biodiversity within this group of eukaryotes, an increasing number of natural products have been isolated from fungal sources and screened for bioactive secondary metabolites.[2] Recently, a family of fungal-derived prenylated alkaloids has attracted increasing interest for its remarkably diverse bioactivities including insecticidal, antitumor, anthelmintic, calmodulin inhibitory, and antibacterial properties, and intriguing structural features. These natural products are comprised of L-tryptophan, a second cyclic amino acid residue, and one or two isoprene units (Scheme 1).[3] The isolation and characterization of two key biosynthetic intermediates, preparaherquamide (**1**) and premalbrancheamide (**2**), in the biosynthesis of the paraherquamides (**3**) and malbrancheamides (**4**),[4,5] respectively, suggest that two amino acid residues are condensed to generate the *cyclo*-L-tryptophan-L-proline analog **5** or **6**. The tryptophanyl subunit of the dipeptide is subsequently prenylated in a reverse manner to generate compound **7** or **8** (Scheme 1). The bicyclo[2.2.2]diazaoctane core in **1** and **2** possibly arises from an intramolecular Diels−Alder (IMDA) reaction after oxidizing **7** or **8** to form a putative pyrazine-derived azadienophile. However, the detailed understanding of assembly and modification of these biosynthetic building blocks remain highly obscure.

Recently, a group of new prenylated indole alkaloids, the notoamides (A−E, **9**−**13**), were isolated from a marine-derived *Aspergillus* sp. (Figure 1a).[6,7] Interestingly, stephacidin A (**14**) and deoxybrevianamide E (**15**) were purified from the same fungal strain, indicating the possible role of **15** as a common biosynthetic intermediate.[7] In 2006, a bimodular nonribosomal peptide synthetase (NRPS) gene (*ftmA*) was mined from an *A. fumigatus* genome sequence, and its heterologous expression led to accumulation of the *cyclo*-L-tryptophan-L-proline product

[†] Life Sciences Institute and Departments of Medicinal Chemistry, Microbiology & Immunology, and Chemistry, University of Michigan.
[‡] Center for Computational Medicine and Bioinformatics, University of Michigan.
[§] Colorado State University.
[||] Kumamoto University.

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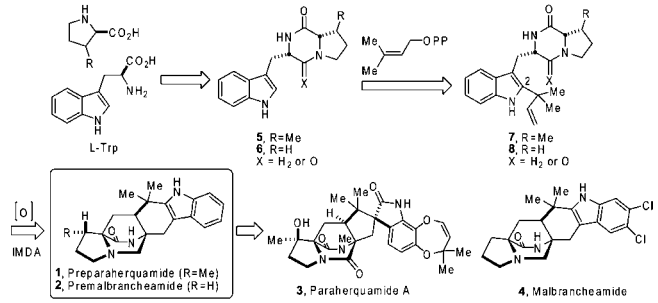
Scheme 1. Biosynthetic Subunits and Putative Route to Paraherquamide (**3**) and Malbrancheamide (**4**)[a]



[a] Molecules in the boxes have been validated as the building blocks or biosynthetic intermediates based on precursor incorporation studies.

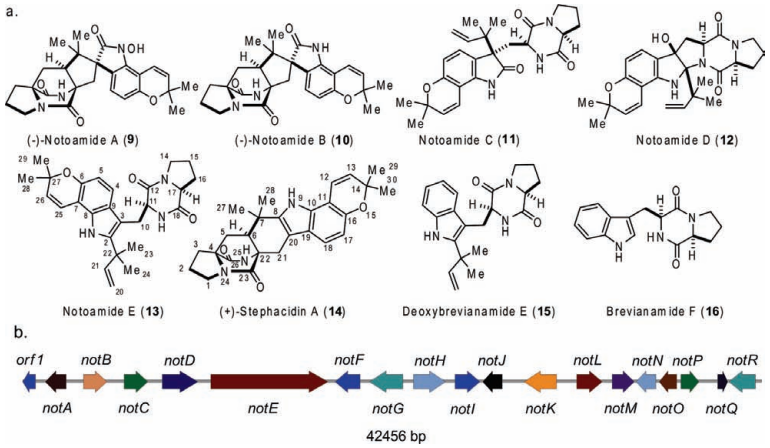


Figure 1. Genetic studies of fungal alkaloids produced in the marine-derived fungus *Aspergillus* sp. (a) Selected fungal alkaloids isolated from the marine-derived *Aspergillus* sp. Compound **16** was not reported in the fungal strain but was expected as the direct precursor of compound **15**. (b) The notoamide (*not*) biosynthetic gene cluster derived from complete sequencing and bioinformatic mining of *Aspergillus* sp. MF297-2 genome.

brevianamide F (**16**).[8] We reasoned that an NRPS with a function coincident with FtmA would be expected for the notoamide biosynthetic pathway, where **16** in this marine-derived *Aspergillus* sp. is elaborated in an alternative manner compared to *A. fumigatus*[9] that mediates biosynthesis of one ergot alkaloid, fumitremorgin. Herein, we report the identification of the first gene cluster for the biosynthesis of the stephacidin and notoamide family of prenylated alkaloids based on genome mining and biochemical analysis. These studies include a detailed characterization of the elusive deoxybrevianamide E synthase (e.g., reverse prenyl-transferase) as well as a second normal prenyltransferase that provide new insights into the assembly of the structurally and biologically diverse class of bicyclo[2.2.2]diazaoctane-derived natural products.

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Results

Localization and Analysis of the Notoamide (*Not*) Gene Cluster from a Marine-Derived *Aspergillus* sp. through Genome Mining. The genome of the stephacidin- and notoamide-producing marine-derived *Aspergillus* sp. MF297−2 was sequenced to ~15 times coverage of the average published *Aspergillus* genome size (32.5 Mb) using Roche 454FLX technology (unpublished data). An open reading frame (*orf*) named *notE* (Figure 1b) was identified from the genome sequence using *ftmA* to probe for homologous genes.[8] NotE is a presumed bimodular NRPS (Table 1) with adenylation (A)-thiolation (T)-condensation (C)-A-T-C domain organization and shares 47% amino acid sequence identity with FtmA (Table 1). In addition to *notE*, eighteen other genes were identified in a 42456-bp region of the chromosome encompassed by four overlapping genome assembly nodes (Figure 1b). At the left end of the gene cluster, the product of *orf1* was predicted to be the *N*-terminus of a capsule polysaccharide biosynthesis protein

Table 1. Features of the *Not* Gene Products

Protein	Size bp/aa	Exon	Function	Relative (identity/similarity [%])	Accession number
Orf1	731/224	1−113, 173−731	partial polysaccharide synthase	capsule polysaccharide biosynthesis protein from *Aspergillus fumigatus* (43/63)	XP_748327
NotA	1199/339	1293−1568, 1643−1971, 2023−2179, 2234−2491	negative regulator	NmrA family protein from *Ajellomyces capsulatus* (45/64)	EEH03447
NotB	1344/401	3486−4079, 4141−4487, 4568−4829	FAD binding domain protein	FAD binding domain protein from *A. clavatus* (44/63)	XP_001268514
NotC	1350/427	5819−6996, 7066−7168	prenyl-transferase	FtmH from *A. fumigatus* (50/66)	BAH24002
NotD	2025/621	8012−8294, 8389−8927, 8996−10036	oxidoreductase	oxidoreductase from *Microsporum canis* (40/59)	EEQ33235
NotE	6723/2241	10787−17509	NRPS	FtmA from *A. fumigatus* (47/67)	XP_747187
NotF	1431/453	17924−18053, 18126−19354	prenyl-transferase	tryptophan dimethylallyltransferase from *Coccidioides posadasii* (40/62)	EER24759
NotG	1901/544	19899−20086, 20171−20272, 20337−20635, 20689−20810, 20879−21799	P450	cytochrome P450 from *A. fumigatus* (62/75)	XP_747185
NotH	1836/502	22422−22668, 22734−22822, 22897−22996, 23060−23128, 23187−23765, 23836−24257	P450	cytochrome P450 from *Neosartorya fischeri* (47/65)	XP_001261652
NotI	1423/434	24803−24962, 25021−25939, 26003−26225	FAD binding domain protein	FAD binding domain protein from *A. clavatus* (44/63)	XP_001268514
NotJ	1113/371	26390−27502	unknown	hypothetical protein from *Salinispora arenicola* (52/65)	YP_001537335
NotK	1851/564	28771−29141, 29196−29569, 29620−30389, 30445−30621	efflux pump	MFS transporter from *N. fischeri* (87/93)	XP_001265322
NotL	1455/484	31789−33243	transcriptional activator	C6 zinc finger domain protein from *N. fischeri* (53/62)	XP_001265321
NotM	1266/402	33816−34597 34654−35080	unknown	hypothetical protein from *Talaromyces stipitatus* (74/82)	XP_002482929
NotN	1126/340	35192−35244, 35299−35895, 35948−36317	dehydrogenase	alcohol dehydrogenase from *Penicillium marneffei* (60/76)	XP_002147947
NotO	993/331	36520−37512	short-chain dehydrogenases/ reductase	hypothetical protein from *Nectria hematococca* (66/80)	EEU36425
NotP	1020/322	37770−37930, 37985−38789	unknown	metallo-β-lactamase domain protein from *T. stipitatus* (80/88)	XP_002482927
NotQ	569/152	39871−40059, 40120−40316, 40370−40439	unknown	hypothetical protein from *T. stipitatus* (88/94)	XP_002482928
NotR	1517/461	40514−41140, 41212−41727, 41791−42030	transcriptional coactivator	hypothetical protein from *P. marneffei* (45/61)	XP_002144868

involved in a primary metabolic pathway. At the right end of the gene cluster, a protein encoded by *notR* showed 38% sequence identity to the AflJ aflatoxin pathway transcriptional coactivator.[10] Bioinformatic analysis indicated that NotB and NotI show high similarity to FAD-dependent monooxygenases while NotD is a presumed flavin-dependent oxidoreductase. NotG and NotH show high sequence similarity to fungal CYP450s, both of which might be involved in the formation of the isoprene-derived pyran ring (Scheme 2). Furthermore, NotN and NotO are predicted to function as a dehydrogenase and a short-chain dehydrogenase/reductase, respectively. The *notK* gene encodes a putative efflux pump, which might specify excretion of alkaloid products from the cell. As with NotR, NotL shares high protein sequence similarity to AflR while NotA is a predicted biosynthetic pathway transcriptional repressor.[11] These regulators offer opportunities to understand the notoamide pathway gene expression, and the potential to manipulate fungal alkaloid production in this unique marine-derived *Aspergillus* sp.[12] NotC and NotF, two predicted aromatic prenyltransferases, presumably catalyze the two key prenylation reactions including a first reverse prenyltransfer step leading to **15**. NotC shows a 50% sequence identity to FtmH (also called FtmPT2) in *A. fumigatus* while NotF shows the highest identity (40%) to a putative dimethylallyl tryptophan synthase (EER24759) in *Coccidioides posadasii*.[13] However, the putative functions of products encoded by *notJ*, *notM*, *notP*, and *notQ* remain unknown based on bioinformatics analysis.

Determination of NotF as the Deoxybrevianamide E Synthase. We first examined the role of NotF in notoamide biosynthesis. Its cDNA was prepared by removing the 72-bp intron using an overlapping PCR strategy (Supplementary Table 1). The recombinant enzyme was purified with Ni-NTA resin to about 90% purity, and its native protein status was determined as an oligomer with an observed molecular weight of 292 kDa

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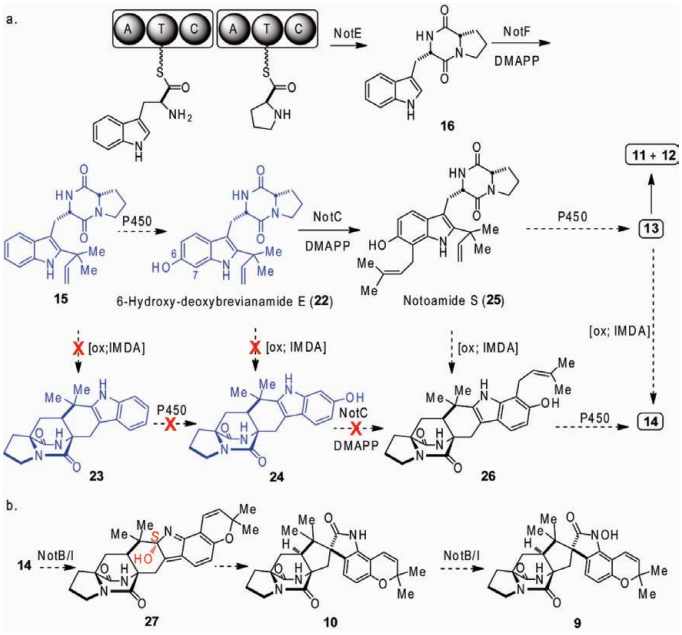
Scheme 2. The Putative Biosynthetic Pathway for Stephacidin A (**14**) and the Notoamide Natural Products[a]



[a] (a) The early stages in the stephacidin and notaomide biosynthesis. **25** serves as the common precursor to **13** and **14**. **13** is then converted into **11** and **12**. Substrates used in NotC studies were labeled in blue. (b) Notoamide A (**9**) an dB (**10**) are possibly derived from **14**. The solid arrows represent reactions that have been confirmed with bioinformatic analysis, biochemical analysis, or precursor incorporation experiments, while the dashed arrows indicate proposed biosynthetic steps. The red symbol X indicates the reaction is not supported by the current study.

(53.6 kDa as the calculated monomeric size) by gel filtration (Supplementary Figure 1).

Next, the function of NotF was tested with doubly ^{13}C-labeled brevianamide F (**17**, Supplementary Figure 2). The product **18** exhibited the same retention time (17.38 min) as authentic **15** but its $[M+H]^+$ ion was 354.19, bearing the expected 2.00-Da shift from $[\mathbf{15}+H]^+$ (352.19) (Figure 2a). In MS^2 analysis, **18** was fragmented in the same manner as **15** and the *m*/*z* differences (1 or 2 Da) of three major fragments (*m*/*z* values at 199.14, 286.17, and 298.14 for **18**) in two MS^2 spectra originated from the two ^{13}C atoms in **17** (Supplementary Figure 3). These results demonstrate that NotF is the deoxybrevianamide E (**15**) synthase and catalyzes the key reverse prenylation at C-2 of the indole ring leading to the bicyclo[2.2.2]diazaoctane core during biosynthesis of many fungal alkaloids within this family. In contrast, **16** in *A. fumigatus* is ultimately converted to fumitremorgin following normal prenylation at C-2 by FtmB.[9]

As a next step, the substrate selectivity of NotF was investigated with L-Trp, **17**, *cyclo*-(L-Phe-L-Pro) **19**, *cyclo*-(L-Trp-L-Trp) **20**, and *cyclo*-(L-Trp-L-Tyr) **21** (Supplementary Figure 2). No prenylated products for any of these unnatural substrates were detected by LC-MS analysis. This result provides strong evidence for the early timing and high selectivity of the NotF-catalyzed reaction in the alkaloid biosynthetic pathway (Scheme 2a). Moreover, the structural similarities among **16**, **19**, **20**, and **21** suggested that both amino acid residues in **16** are critical for selective interactions between the substrate and the NotF reverse prenyltransferase.

Determination of NotC as the 6-Hydroxy-7-prenyl-deoxybrevianamide E Synthase. The role of NotC, the second predicted prenyltransferase from the marine-derived *Aspergillus* sp. MF297−2, was also investigated. Its cDNA was similarly generated by an overlapping PCR strategy and was expressed in *E. coli* (Supplementary Table 1). The recombinant protein was purified with a single Ni-NTA affinity column, and its native protein status was determined as a monomer with an observed molecular weight of 61 kDa (51.1 kDa as the calculated monomeric size) by gel filtration (Supplementary Figure 1).

Stephacidin A (**14**) is a central advanced intermediate featuring a pyran ring but lacking a spiroxindole implicating the mode of assembly and timing of the bicyclo[2.2.2]diazaoctane family of fungal alkaloids.[7,14] We propose that **14** is produced from deoxybrevianamide E (**15**) in a series of reactions, including hydroxylation at C-6 (following **13** numbering system), followed by normal prenylation at C-7, oxidation of the dioxopiperazine ring, IMDA and ring closure to the pyran (Scheme 2a). This hypothesis and the order of these reactions were examined by the determination of NotC activity with four

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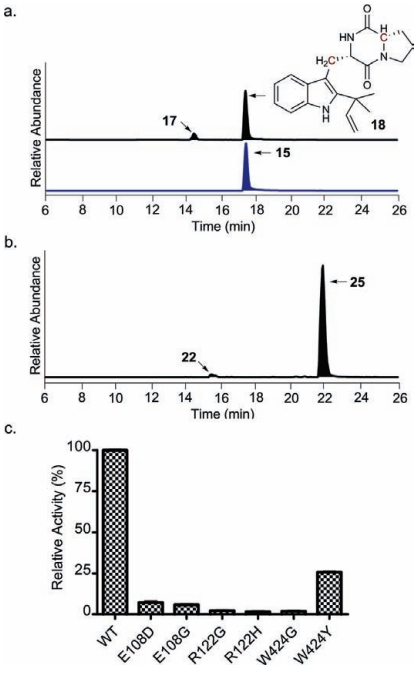

Figure 2. Determination of NotF and NotC prenyltransferase activities. (a) Identification of NotF product (**18**) by LC-MS/MS analysis as described in Methods. (b) Identification of NotC reaction product (**25**) by LC-MS/MS analysis as described in Methods. The product (**25**) was further characterized by ^{1}H and ^{13}C NMR analysis. (c) Investigation of key residues in the reaction of the reverse prenyltransferase NotF by site-directed mutagenesis. Data shown are means ± s.d. from two independent experiments.

structurally related putative substrates, **15**, **22**, **23**, and **24** (Scheme 2a). Compounds **22** and **24** were synthesized according to Supplementary Schemes 1 and Scheme 2, respectively. NotC showed high selectivity toward 6-hydroxy-deoxybrevianamide E (**22**) with three additional substrates failing to be converted to products by the enzyme (Figure 2b). The *m*/*z* value of the product was 436.17, the same as that of the singly prenylated **22** (calculated MW of 435.25), in MS analysis. Also, three major fragment ions in the MS^2 spectrum of the enzyme product exhibited *m*/*z* values at 280.20, 368.17, and 380.22 (Supplementary Figure 4). The difference between these and the major ions in MS^2 spectrum of **15** is 84-Da, which is consistent with the MW sum of one oxygen atom and one isoprene unit linked to the indole ring of **15**. We also chemically synthesized notoamide S (6-hydroxy-7-prenyl-deoxybrevianamide E, **25**)[15] as the authentic standard and compared the authentic substance with the NotC product by LC and ^{1}H NMR analyses. Both compounds exhibit the same LC retention time (Supplementary Figure 5) and displayed identical ^{1}H NMR spectra (Supplementary Figure 6). The NotC product was further confirmed to be notoamide S (**25**)[15] by comparison with **13** (Supplementary Table 2). Chemical shifts of ^{1}H and ^{13}C NMR spectra between the product and **13** were essentially identical with the exception of C-25 and C-27.[6] The significant differences at these two positions reflect the double bond position in the attached isoprene unit and also indicate that the pyran is not formed in **25**. These results demonstrated that NotC catalyzes a normal prenyltransfer reaction at C-7 of the indole aromatic ring system in **22**. Moreover, analysis of NotC substrate selectivity suggests that the biosynthetic pathway of **13** and **14** might not proceed through substrates **23** or **24** (Scheme 2a). Instead, **15** is first hydroxylated at C-6 of the indole ring, and the product **22** is subsequently prenylated at C-7 by NotC to generate **25**, a biosynthetic precursor of both **13** and **14**. Accordingly, notoamide J was isolated from the culture of this marine-derive fungus, and contains only one C-6 (following **13** numbering system) hydroxy group.[16] The precise path from **25** to **14** requires further investigation, although we have demonstrated that stephacidin A (**14**) does not arise directly from notoamide E (**13**), suggesting that generation of the pyran follows formation of the bicyclo[2.2.2]diazaoctane core[6].

Biochemical Characterization of NotF and NotC. Both NotF and NotC tolerated a broad range of temperature (4 to 42 °C for NotF while 16 to 42 °C for NotC) and pH (6.0 to 9.0) (Supplementary Figure 7). Enzyme activity was independent of divalent cation, although addition of 5 mM Mg^{2+}, Ca^{2+} or Mn^{2+} slightly enhanced catalysis (about 100−120%) (Supplementary Figure 8). Significant reduction of enzyme activity (2% − 35%) was observed with Cu^{2+}, Zn^{2+}, Fe^{2+}, or Sn^{2+} (5 mM). Unlike previous observations with a normal aromatic prenyltransferase (MaPT)[17] and CloQ,[18] EDTA caused only minor effects on NotF and NotC activity (remaining 90−95%), possibly indicating that the active-site pocket of both prenyltransferases might be less exposed to solvent. However, the exact mechanism for nonchelating inhibition of this group of metal-independent enzymes by EDTA remains unclear.

The reactions of both NotF and NotC followed Michaelis−Menten kinetics (Supplementary Figure 9). The K_m and V_{max} values for doubly ^{13}C-labeled brevianamide F (**17**) in the NotF reactions were 4.33 ± 0.43 μM and 0.89 ± 0.02 μM/min, respectively, giving a maximal turnover value of 19.1 ± 0.4 min^{-1}. Similarly, the enzyme K_m and V_{max} values for dimethylallyl diphosphate (DMAPP) were 1.31 ± 0.22 μM and 1.18 ± 0.03 μM/min, respectively. Its maximal turnover value (25.3 ± 0.6 min^{-1}) and enzyme catalytic efficiency value (19.31 μM$^{-1}\cdot$min^{-1}) were slightly higher than those of **17** (k_{cat}/K_m = 4.41 μM$^{-1}\cdot$min^{-1}). Compared to FtmB using **16** as a substrate in a normal prenylation step (K_m = 55 μM, k_{cat}/K_m = 6.08 μM$^{-1}\cdot$min^{-1}), NotF showed more restricted substrate selectivity and higher substrate binding affinity with a similar enzyme catalytic efficiency.[19] In addition, we also determined the kinetic parameters for the NotC normal prenyltransferase. The K_m and V_{max} values for **22** in the NotC reactions were 2.64 ± 0.33 μM and 1.30 ± 0.04 μM/min, respectively, while for DMAPP these values were determined to be 1.89 ± 0.20 μM and 1.45 ± 0.03 μM/min, respectively. Similar to NotF, the maximal turnover

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value (67.4 ± 1.4 min^{-1}) and enzyme catalytic efficiency value (35.66 $\mu M^{-1}\cdot min^{-1}$) of NotC were also slightly higher than those of **22** (60.5 ± 1.9 min^{-1}, 22.92 $\mu M^{-1}\cdot min^{-1}$, respectively).

In this study, three key amino acid residues located in the predicted NotF reaction pocket were mutated to probe the reaction mechanism of this reverse aromatic prenyltransferase (Supplementary Figure 10). R108 was predicted to be a substitute for the divalent metal ion and to interact with the DMAPP pyrophosphate group. Generation of the R108H and R108G mutants (Supplementary Figure 1a) resulted in proteins with less than 2% catalytic activity, confirming the vital role of R108 for effective catalytic function (Figure 2c). Another highly conserved key residue for substrate binding in NotF is E108, which may form a H-bond with N−H in the **16** indole ring system.[20] Both E108D and E108G mutants lost at least 92% of their activity (Figure 2c). This result suggests that a specific side chain length in this acidic amino acid residue is important for H-bond formation. Recently, the crystal structure of FgaPT2, a normal fungal aromatic prenyltransferase, revealed the presence of a defined network consisting of five Tyr residues to prevent the DMAPP-derived carbocation from reacting adventitiously with nucleophiles.[20] All of these residues are conserved in NotF except Y413, which is replaced with W424 (Supplementary Figure 10). Although W424Y still retained about 25% of its activity for production of **18**, the corresponding W424G lesion resulted in >98% loss of catalytic activity, validating the importance of this analogous aromatic network in the notoamide reverse prenyltransferase reaction (Figure 2c).

Proposed Biosynthetic Pathway for the Stephacidin and Notoamide Biosynthesis in Marine-Derived *Aspergillus* sp. MF297-2. Based on the biochemical characterization of two prenyltransferases and the predicted biochemical function of related gene products in the isolated gene cluster, a putative notoamide biosynthetic pathway is proposed (Scheme 2). Briefly, we presume that **16** is produced from L-Trp and L-Pro by the NotE NRPS and is subsequently reverse prenylated at C-2 by NotF to produce **15**. In the next step, the indole ring is hydroxylated at C-6, which is likely catalyzed by one of the two *not* pathway-encoded P450s. NotC is then responsible for normal prenylation at C-7 position of **22** to produce **25**. From this intermediate, notoamide E (**13**) is generated following ring closure to the pyran, a process possibly controlled by the second P450, and then may be converted into notoamide C (**11**) and notoamide D (**12**).[6] The direct connection from **13** to **14** was not observed in double ^{13}C-labeled precursor incorporation experiments, whereby **13** was previously envisioned to be converted by an oxidase to the pyrazine-derived dienophile followed by IMDA to directly produce stephacidin A (**14**) (Scheme 2a).[6] The enzyme(s) that promote bicyclo[2.2.2]diazaoctane formation remain unknown. Recently, a flavin-dependent oxidase in solanapyrone biosynthesis was shown to catalyze both oxidation and subsequent Diels−Alder cycloaddition reactions,[21] indicating that the predicted oxidoreductases (e.g., NotB, NotD, and NotI) are possible candidates for catalyzing the IMDA reaction in the notoamide biosynthesis. Alternative to the pathway through **13**, **14** may be produced from **25** after the stepwise oxidation, IMDA, and ring closure (Scheme 2a). We propose that **14** is regiospecifically hydroxylated by a monooxygenase, possibly NotB or NotI, to give **27** for subsequent pinacol-like rearrangement to produce notoamide B (**10**). Such an intriguing rearrangement reaction has not been previously observed in a natural product biosynthetic pathway, and a putative mechanism is proposed (Supplementary Figure 11). A rare *N*-hydroxylation reaction is required to generate the final notoamide A product (**9**) (Scheme 2b). The biosynthetic scheme proposed here provides an initial understanding of the assembly and modification of biosynthetic building blocks for this important group of bioactive prenylated fungal alkaloids.

Discussion

The advent of next-generation sequencing has provided tremendous opportunities to identify novel natural products and their biosynthetic pathways through genome mining of bacterial, fungal,[22] and plant[23] genomes. This approach has proved increasingly important in current natural product biosynthesis studies with the availability of increasing numbers of microbial genome sequences. For example, many orphan biosynthetic gene clusters were identified from the genome sequence of well-studied *Streptomyces coelicolor* A3(2), and novel natural products such as 2-alkyl-4-hydroxymethylfuran-3-carboxylic acids were uncovered with the guidance of genome mining.[24,25] In this study, we employed an allied approach to study the biosynthesis of prenylated indole alkaloids and also to initiate an understanding of fungal genetic evolution and adaptation to different environmental niches. The entire genome of the notoamide-producing marine-derived *Aspergillus* sp. was sequenced at ~15X coverage, enabling the *not* gene cluster identification through sequence file database comparison to *ftmA* (Figure 1). With this gene cluster it is now possible to pursue studies toward a complete understanding of the assembly, tailoring, and regulation of this family of bioactive fungal alkaloids and to further develop them as medicinal agents.

Fungal aromatic prenyltransferases have attracted increasing interest because of their important roles in the biosynthesis of natural products and potential applications in drug development. Currently, more than 100 putative indole prenyltransferases have been revealed by BLAST searches in the public genome database.[26] Biochemical investigations of over 10 recombinant enzymes in this group finds that catalytic functions are independent of divalent metal ions.[26,27] In these reactions, an isoprene unit can be transferred onto different positions of the indole ring system.[27] Moreover, in a normal prenylation reaction, DMAPP alkylates an aromatic substrate through its C1′ atom via an S_N2 displacement, while the C3′ position is involved in the reverse prenyltransfer reaction via an S_N2' displacement. Remarkably, these enzymes can utilize a series of structurally similar analogs as their aromatic substrates.[17,27,28] In contrast to other characterized enzymes in this group, NotF (reverse) and NotC (normal) prenyltransferases showed highly restricted substrate specificities. NotF specifically prenylates **16** (at C-2),

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confirming its role as the elusive deoxybrevianamide E synthase, while only **22** is utilized by NotC (C-7 alkylation) in the biosynthesis of the stephacidin and notoamides (Figure 2). The mechanism of the reverse prenyltransferase was also probed by site-directed mutagenesis to understand the reaction of this group of enzymes.[17,20] Future structural studies of NotF and the comparison to FgaPT2[20] are expected to contribute further information about the regio- and stereospecificity of the reverse and normal prenyltransfer reactions. We expect this analysis will likely illuminate the lack of facial selectivity previously observed for the reverse prenylation step[29] and facilitate expansion of the enzyme substrate range and efficiency. More importantly, the combined studies of NotF and NotC provide direct evidence to establish a biosynthetic scheme for this family of bioactive prenylated fungal alkaloids. Finally, the high *in vitro* catalytic efficiencies of recombinant NotF and NotC suggest their potential value as biocatalysts for chemoenzymatic production of bioactive fungal alkaloid analogs in drug development.

Identification and characterization of the notoamide gene cluster also provides the initial basis to understand the formation of three pairs of antipodal natural products derived from a marine-derived and a terrestrial *Aspergillus* sp.[30,31] In the marine-derived fungal strain, (−)-notoamide B (**10**), (−)-versicolamide B (**38**), and (+)-stephacidin A (**14**) are produced,[7,30] while their antipodal counterparts, **39**, **40**, and **41**, respectively, are isolated from the terrestrial *A. versicolor* NRRL 25660 strain (Supplementary Figure 12).[31] Based on the putative notoamide biosynthetic pathway, we propose that formation of **14** and **41** might be controlled by the IMDA reaction. Subsequently, **10** and **39** are possibly derived from **14** and **41**, respectively, in these two distinct fungal strains. It remains unclear whether generation of (−)- and (+)-versicolamide B (**38** and **40**) occurs in the pathway through stephacidin A due to their opposite stereogenic centers at C-6 (Supplementary Figure 12).[30] Instead, **13** might be converted into **11**, subsequently producing **38** in the following IMDA reaction in the marine-derived fungus.[30] The detailed biochemical characterization of biosynthetic enzymes from both fungal strains is in progress and will shed more light on the biosynthesis of these unique antipodal natural products.

Identification of biocatalysts from fungal alkaloid biosynthetic pathways may also enable production of natural products and their analogs through heterologous expression and metabolic engineering.[32−34] It is estimated that >99% of microorganisms in the environment fail to grow in the laboratory, and the potential to find pharmaceutically important natural products from fungal sources remains vastly underexplored. Introducing natural product gene clusters into more technically and industrially amenable microorganisms such as *E. coli* and yeast represents an attractive way to obtain suitable quantities of natural products and to identity novel leads in drug discovery and development programs.[34] Moreover, a microorganism can be further optimized for the efficient production of a target metabolite using traditional mutation and selection methods, as well as new tools from systems biology and synthetic biology.[35] Identification of the notoamide gene cluster provides such an opportunity to produce bioactive fungal alkaloids and analogs thereof through pathway engineering and heterologous expression.

Methods

Materials and Strains. Authentic deoxybrevianamide E (**15**), doubly ^{13}C-labeled brevianamide F (**17**), and keto-premalbrancheamide (**23**) were synthesized following previously published procedures.[4,36] Standard methods for DNA isolation and manipulation were performed as described by Sambrook et al.[37] Genomic DNA from *Aspergillus* MF297-2 was isolated with a MasterPure Yeast DNA Purification kit (Epicenter Biotechnologies) as described in the manual. The GenBank accession numbers for *notC*, *notF*, and the complete assembled *not* gene cluster are GU564534, GU564535, and HM622670, respectively. *E. coli* DH5α was used for cloning and plasmid harvesting while *E. coli* BL21 CodonPlus-(DE3)-RIPL was used for protein overexpression.

Expression and Purification of NotC and NotF. Details about the preparation of *notC* and *notF* cDNAs and of *notF* mutant DNAs are included in the Supporting Information and Supplementary Table 1. The expressed enzymes were purified with a single Ni-NTA column (Supporting Information). As determined by SDS-PAGE analysis, the purity of proteins was more than 90%. The native status of proteins was determined by gel filtration (Supplementary Figure 1).

Determination of Enzyme Activities. Compounds **22** and **24** were chemically synthesized to examine NotC activity (Supporting Information). The 100-μL reaction mixture contained 0.5 μg of NotF, its mutants, or NotC; 5 mM $MgCl_2$; 0.1 mM **17** (NotF or its mutants) or **22** (NotC); and 0.15 mM DMAPP in the reaction buffer (50 mM Tris-Cl, pH 7.5, 10% glycerol, and 3 mM β-mercaptoethanol). The reaction was initiated by adding enzyme after prewarming the other components at room temperature for 1 min. After mixing well and briefly centrifuging, the reactions were further incubated at room temperature for 45−60 min and stopped with 10 μL of 1.5 M trichloroacetic acid. The mixtures were mixed and centrifuged at 13 000*g* for 5 min. An aliquot of the 100-μL solution was subjected to HPLC coupled with an XBridge C18 column (5 μm, 4.6 mm × 250 mm), at a wavelength of 222 nm. Solvent B (acetonitrile in 0.1% TFA) was increased from 30% to 40% for 5 min and then increased to 80% over 20 min for the detection of products. LC-MS^2 analysis was performed by using a ThermoFinnigan LTQ linear ion-trap instrument equipped with an electrospray source and a Surveyor HPLC system at room temperature. Separations were performed with an XBridge C18 (3.5 μm, 2.1 mm × 150 mm) column at a flow rate of 200 μL/min with solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). Solvent B was kept at 2% in solvent A for 4 min and then was gradually increased to 90% over 16 min. After being washed with 90% solvent B for 2 min, the column was further re-equilibrated with 2% solvent B for 10 min. The spectra were recorded in positive ion mode. Product **25** was further characterized with ^{1}H and ^{13}C NMR analysis (Supporting Information).

Kinetics Analysis. The 100-μL reaction mixture contained 0.25 μg of NotF or 0.11 μg of NotC and 5 mM $MgCl_2$ in the reaction buffer. Details about the experiment procedures were included in the Supporting Information. All experiments were performed in duplicate. The data were fit to the Michaelis−Menten equation in Prism 4.0 (GraphPad Software).

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Supporting Information Available: Remaining methods, including genome sequencing and assembly, assay for enzyme metal independence, detailed synthetic procedures of **22** and **24**, determination of enzyme optimal conditions, characterization of **25**, antipodal fungal prenylated alkaloids, and the complete list of authors in ref 25. This material is available free of charge via the Internet at http://pubs.acs.org.

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Appendix 2:

Research Proposal

Abstract

Alchivemycin A (**1**) is a newly isolated metabolite from *Streptomyces sp.* containing an unprecedented 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring system (Figure 1).[1] This novel polycyclic polyketide also shows potent antimicrobial activity against *Micrococcus luteus* and inhibitory effects on tumor cell invation. Due to its interesting stucture and its potent activeity, alchivemycin A is an ideal synthetic target to explore the formation of the unprecedented 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring system.

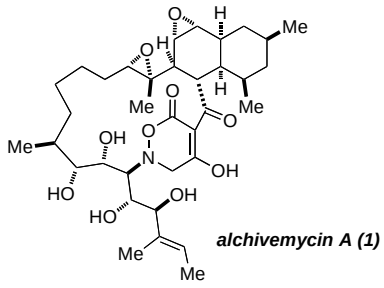


Figure 1: Alchivemycin A.

The synthesis of **1** in envisioned to come from three separate pieces with a late stage installation of the unprecedented ring system (Figure 2). The cis-decalin ring system can come from a transannular Diels-Alder (TADA) reaction to give the resultant lactone. The hydroxyl amine containing side chain can be formed from the condensation of a hydroxyl amine derivative onto an acetinide protected aldehyde, derived from galactose, followed by a Mannich addition of a terminal alkyne to the nitrone. The formation of the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring could arise through a possible biosynthetic pathway. Coupling of the hydroxyl amine side chain with an acid derivative of the cis-decaline system followed by Dieckmann-type condensation[25] would give the desired 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring system. Macrocyclization could be afforded through a Horner-Wadsworth-Emmons reaction[21,22] to give the desired E-olefin. Through a

series of deprotection and epoxidations, it is envisioned that the synthesis of alchivemycin A could be accessed.

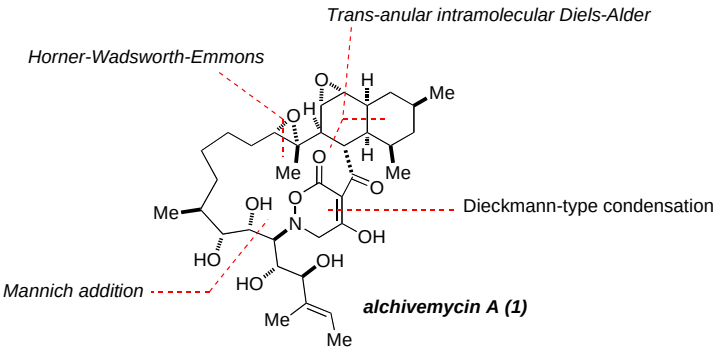
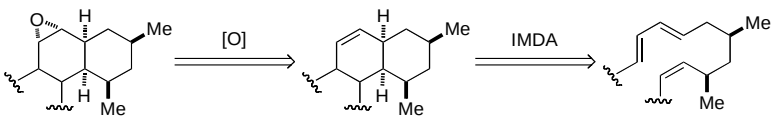


Figure 2: Key disconnection for the synthesis of alchivemycin A.

One of the major concerns in the synthesis of alchivemycin A is the stability of this unprecedented ring system. Though it resides in the natural product the question remains as to what type of conditions this ring system can withstand. A second issue is that of the selectivity of the TADA reaction. Though this reaction has been performed to synthesize cis-decalin systems, the cis-methyl groups that are meta to each other in the cyclohexane ring my play a large role in the relative stereochemistry of the TADA. Keeping these potential problems in mind the synthesis of **1** can be divided into two parallel pieces, which can be brought together through what is thought to be a possible biosynthetic pathway.

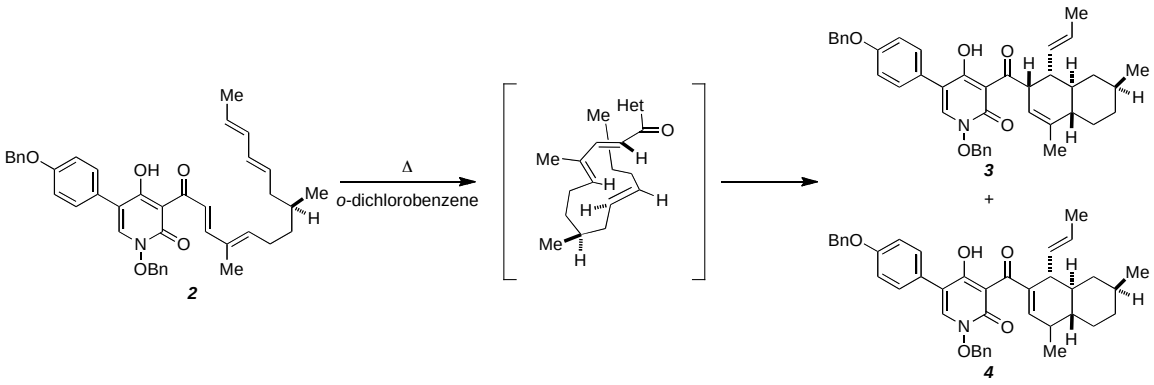
Background

A major key interest in **1** is the cis-decalin ring system. Though this system is not new, the epoxide containing system resembled the product of a Diels-Alder reaction (Scheme 1).



Scheme 1: Intramolecular Diels-Alder reacion to form the cis-decalin of **1**.

Intamolecular Diels-Alder (IMDA) reactions have been used in the synthesis of a multitude of natural products such as (±)-ilicicolin H,[2,3] (+)-phomopsidin,[4,5] and (–)-oblongolide[6] to name a few. In each case the stereochemical outcome of the IMDA varied between a cis-decalin and trans-decalin system. In the earliest case of these three examples Williams and coworkers used a IMDA reaction for the synthesis of (±)-ilicicolin H giving a trans-decalin system.[2,3] The key compound **2** when heated in *o*-dichlorobenzene at reflux for 5 minutes gave exclusively the exo-bridged Diels-Alder adducts (Scheme 2).

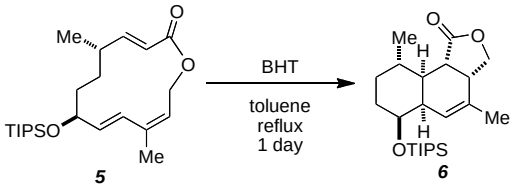


Scheme 2: Williams TADA to gain access to the core of ilicicolin H.

Although the product of the IMDA for ilicicolin H was a trans-decaline system, it gave exclusive formation of a chair conformer in the cyclohexane (B) ring with the C12 methyl substituent in an equatorial position. This important factor gives some insight into the geometry and transition state needed for the IMDA for alchivemycin A. One other factor that may need to be explored for the synthesis of alchivemycin A is the geometry of the olefins involved in the IMDA.

In the synthesis of (+)-phomopsidin, Nakada and coworkers used a TADA as the key step for the formation of a cis-decaline ring system to gain access to the molecules core.[4,5] One of the major differences between the IMDA of Nakada and Williams is the formation of a macrolactone for

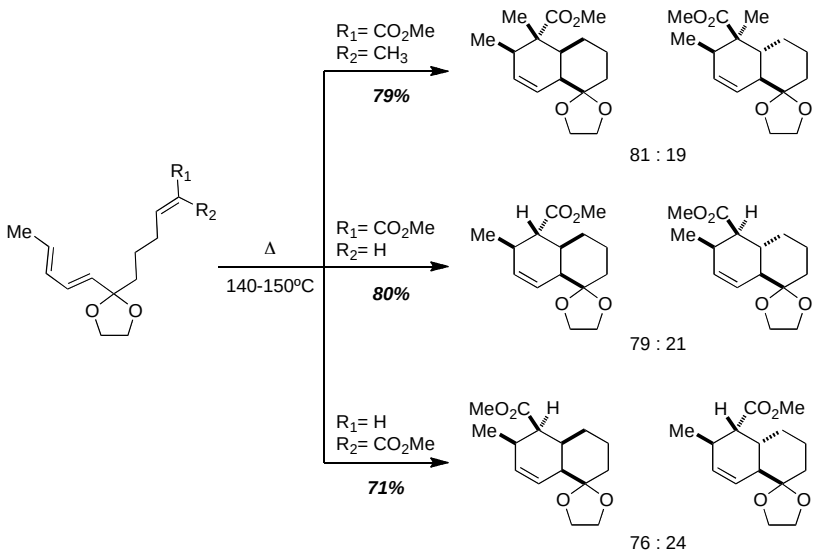
transannular Diels-Alder versus an untethered chain of an IMDA. Macrocyclic lactone **5** was used in the synthesis of phomopsidin to entropically activate and diastereoselectively control the TADA (Scheme 3).



5 ***6***

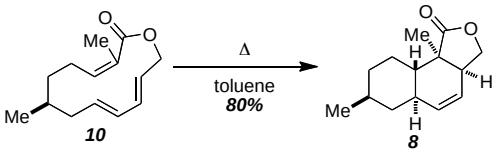
Scheme 3: TADA of a (*E,Z*)-diene to give the cis-decalin product.

One major reason to use **5** was because of the poor reactivity of (*E,Z*)-dienes in Diels-Alder reactions predominantly due to their energetically unfavored *s*-cis conformation in the transition state. By restricting the rotation of the diene with the use of a macrocyclic lactone, activation of the (*E,Z*)-olefins can be achieved for an intramolecular Diels-Alder reaction. It should also be noted that a chair conformer of the cyclohexan (A) ring with the methyl and OTIPS groups being in the equatorial position was achieved. The cis-decaline system of phomopsidin was achieved through the use of a *(E,Z*)-diene, but this isn't always the case it has been shown by Roush and Hall that many (*E,E*)-dienes give cis-decaline systems.[7] Roush and Hall explore a varity of different IMDA systems and the decaline products obtained relative to their triene system (Scheme 4). The outcome of Roush and Hall's work explained that in a large number of cases the cis-decaline ring system in favored, however factors of substitution and reaction conditions play a major factor in observed product.



Scheme 4: Roush and Hall's observation into the IMDA of different trienes.

The synthesis of (–)-oblongolide by Shing and Yang took the previous information pioneered by Roush and Hall and used it to construct the core of **8** by varying the condition of the IMDA to construct the desired trans-decaline core.[6] Shing and Yang pursued the synthesis of oblongolide through the use of a IMDA knowing that in the key cyclization step the cis-decaline system would be the favored product as previously discussed by Roush and Hall. However, by varying the reaction temperature and reaction time of the IMDA Shing and Yang found that the trans-decaline could be achieved exclusively. Through the work of Roush and Hall, it was known that lower temperatures, in the range of 100-150ºC, would lead to a cis-decaline product, so in a first attempt Shing and Yang performed the IMDA at 210ºC for 56 hours. The products obtained were a 2 : 1 mixture of trans and cis-decaline systems, with the trans being the major. Extending the length of the reaction to 72 hours lead to only trans product in a 55% yield showing that changing the reaction condition and time can vary the products. Shing and Yang also looked at the use of a TADA for the formation of 8 and found that by making the macrolactone the TADA proceeded at a much lower temperature and a higher yield (Scheme 5).



Scheme 5: TADA of **10** to give exclusively trans-decaline **8**.

The use of a TADA for the formation of the cis-decaline core of alchivemycin A has great potential. However, with the knowledge of the previously described Diels-Alder reactions a variety of different reaction conditions and olefin geometries may need to be explored. Through modeling of the macrolactone system there seems to be a favored conformation that could lead to the desired cis-decaline system. A synthesis that would allow access to each of the desired macrolactones (**11**, **12**, **13, 14**; Figure 3) would be optimal for both exploratory and synthetic analog purposes.

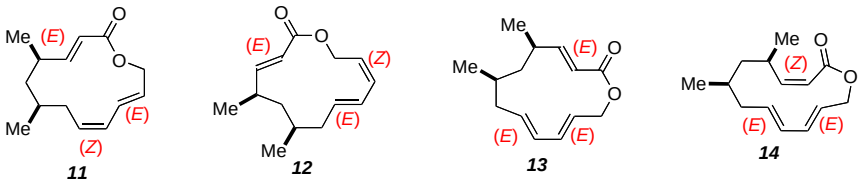


Figure 3: Four different combinations of macrolactones for a TADA.

The second most intriguing aspect of alchivemycin A is the unprecedented 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring. It is believed that the biosynthesis of this ring system proceeds through a pathway similar to that of tetramic acid. The biosynthesis of tetramic acid is by condensation of an α-amino acid with a growing polyketide chain followed by a Dieckmann condensation and release of an enzyme complex.[8] One could envision, *N*-hydroxyglycine, which could be derived from the *N*-hydroxylation of glycine,[9,10] incorporated on to the tail of a polyketide chain, followed by a Dieckmann-type cyclization to provide the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring (Figure 4).

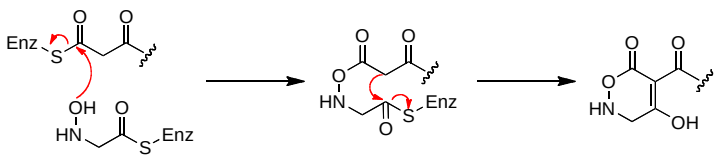


Figure 4: Proposed biogenesis of the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring.

Synthetically the use of the same type of connections for the synthesis of the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring can be used. Coupling of a N-hydroxy glycine with an acid derivative of the cis-decaline system, followed by a Dieckmann-type condensation should afford the desired 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring. This sequence of steps may also be used as the final steps in the formation of the macrocyclic core of alchivemycin A.

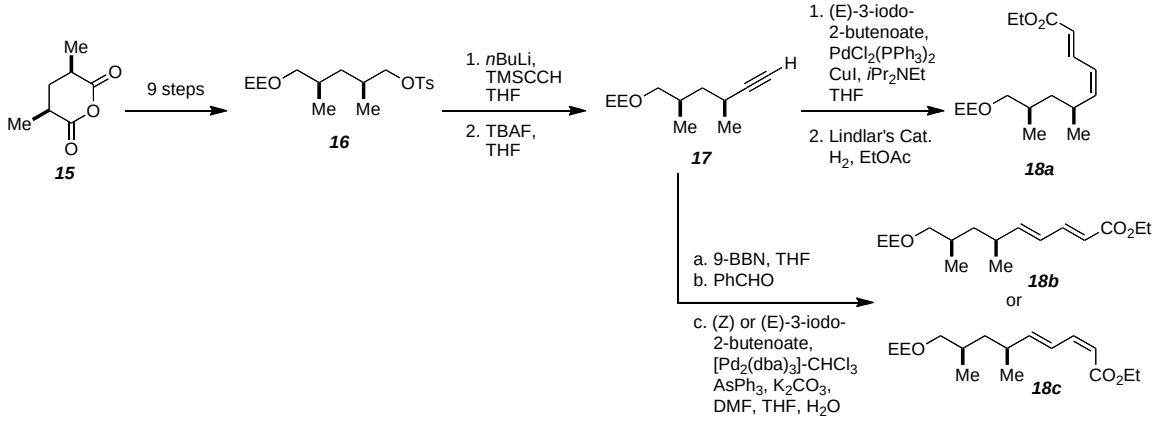
These two aspects of alchivemycin A combined with its potent antimicrobial activity and inhibitory effects on tumor cell invasion have made this molecule an interesting synthetic target. The use of a TADA to synthesize the cis-decaline ring, though not novel, does provide room for the construction of stereochemical analogs that can be used for further testing. Construction of the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring through a proposed biosynthetic pathway also leads to the intrigue of unprecedented ring system. These two aspects along with current synthetic strategies should allow access to the total synthesis of alchivemycin A and any possible analogs.

Synthetic Design

The synthesis of alchivemycin A can be broken down into three major parts. The first of these three parts being the cis-decaline system, which can arise through a TADA reaction. Second, a long sidechain containing the protected diols along with an *N*-hydroxy glycine for the synthesis of the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring. The final piece of alchivemycin A will

be the construction of the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring through a proposed biosynthetic pathway where in the coupling of the *N*-hydroxy glycine derivative with the acid of the decaline system followed by a Dieckmann-type condensation to give the desired ring system.

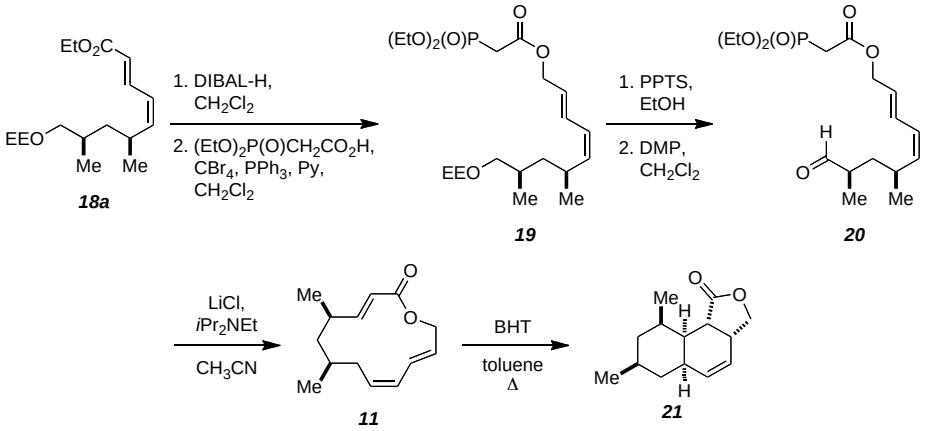
The cis-decalin system can start from known anydride **15** and through 9 steps be desymmetrize to protected alcohol **16** through the procedure of Lautens and coworkers.[11] Displacement of the tosylate of **16** with TMS-acetalene followed by TMS deprodection will give **17.** A Sonanashira coupling[12] with ethyl (*E*)-3-iodo-2-butenoate will give the corresponding ene-yne system, which reduction of the alkyne with Lindlar's catalyst under an atmosphere of H_2 would give the desired (*Z,E*)-diene **18a** for the TADA. Alternatively, **17** could be reacted with excess 9BBN-H and treated with benzaldehyde to convert the byproduct, 1,1-bisboryl adduct,[13] to the trans-alkenylborane, with can be use in a Suzuki–Miyara coulpling[14] with either (*E*) or (*Z*)-3-iodo-2-butenoate to give the corresponding diene **18b**, **18c** systems (Scheme 6).



Scheme 6: Synthesis of the three different diene systems.

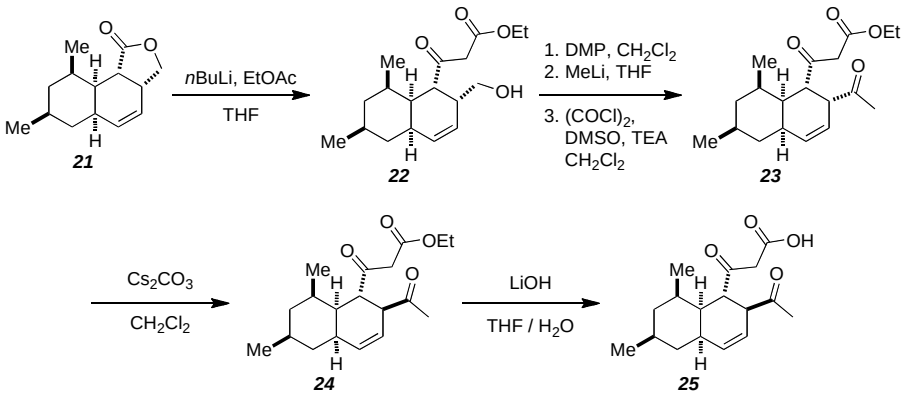
Either 18s could be reduced to the alcohol with DIBAl-H, followed by condensation with diethyl phosphonacetic acid to give **19**. Deprotection of the ethoxyethyl group with PPTS would give the resultant alcohol which can be oxidized using Dess–Martin periodinane[15] to furnish **20**. Using the conditions

developed by Nakada and coworkers, in the synthesis of (+)-phomopsidin,[5] an intramolecular Horner–Wadsworth–Emmons would provide the desired macrolactone **11** needed for the TADA.[4,5] Finally, heating of lactone **11** in toluene with catalytic BHT should provide cis-decaline **21** (Scheme 7).



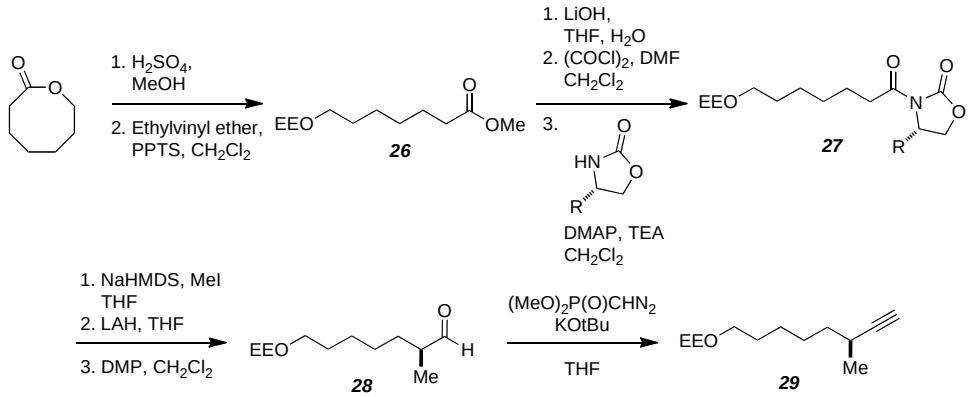
Scheme 7: Synthesis of the lacton **21** through a TADA.

Lactone **21** can be opened to the corresponding β-ketoester **22** with *n*-BuLi and ethyl acetate. Oxidation of the primary alcohol with Dess–Martin periodinane[15] followed by addition of methyl lithium and a second oxidation to give give **23**, which can be epimerized to give **24**. Hydrolysis of the β-ketoester with LiOH to the β-ketoacid **25** would complete the northern portion of alchivemycin A (Scheme 8).



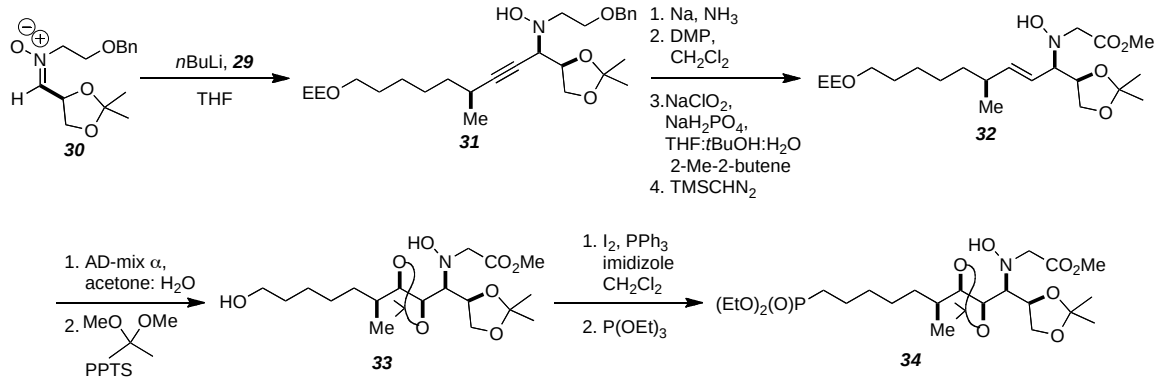
Scheme 8: Synthesis of the northern portion of alchivemycin A.

The synthesis of the sidechain needed for **1** can start from ring opening of ζ-lactone with H_2SO_4 in MeOH, followed by protection of the primary alcohol as ethoxyethyl ether **26**. Hydrolysis **26** to the corresponding acid and subsequent formation of the acid chloride followed by addition of Evans auxiliary[16] ((*S*) stereochemistry) will afford **27**. Methylation of **27** with *n*-BuLi and methyl iodide followed by cleavage of the auxiliary with LAH will give the primary alcohol which can be oxidized to aldehyde **28** with Dess–Martin periodinane[15]. Aldehyde **28** can be converted to the primary alkyne **29** with Seyferth–Gilbert's reagent[17,18] to give a portion of the sidechain needed for **1**.



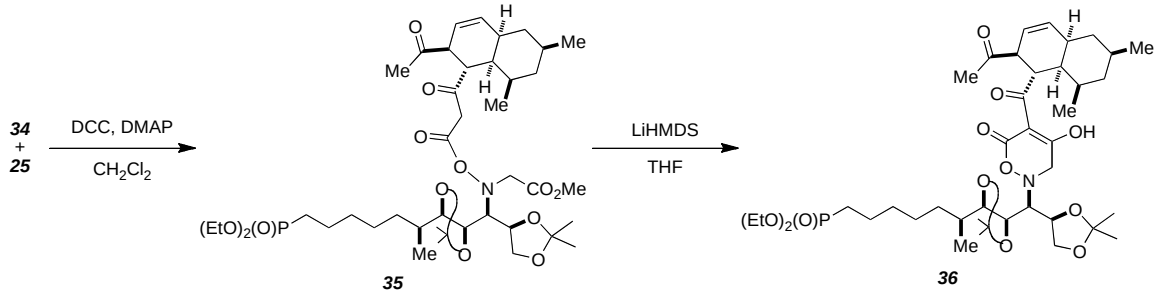
Scheme 9: Synthesis of alkyne needed for the sidechain of **1**.

Treatment of alkyne **29** with *n*-BuLi followed addition of nitrone **30** will give the major portion of the sidechain of **1**.[19] Reduction of **31,** with Na and NH_3, to the trans-alkene and deprotection of the benzylether followed by oxidation to the carboxyl acid and methylation will afford methyl ester **32**. **33** will be accessed by an asymmetric Sharpless dihydroxilation[20] to give the resultant diol, followed by protection of the diol as an acetanide and deprotection of the ethoxyether. Formation of the Horner–Wadsworth–Emmons[21,22] reagent of **33** through a two step process of halogenation and displacement of the primary alcohol followed by and Arbuzov reaction[23,24] to afford phosphonate **34** (Scheme 10).



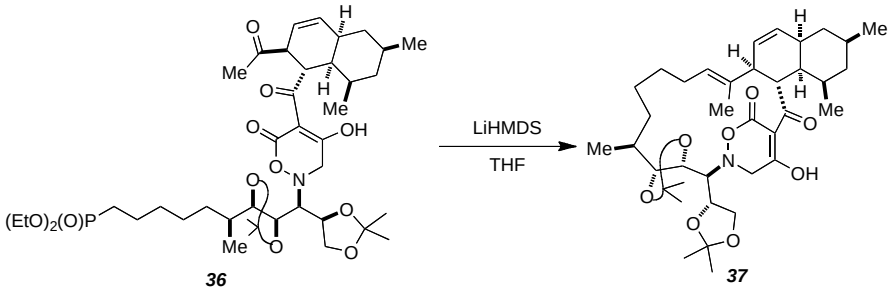
Scheme 10: Synthesis of the sidechain for **1**.

With the two pieces in hand, coupling of sidechain **34** with acid **25** in the presence of DCC will give **35**. Employing an intramolecular Dieckmann cyclization[25] will provide the desired 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring of **36**, in what is thought to be a biosynthetic manor[1] (Scheme 11).



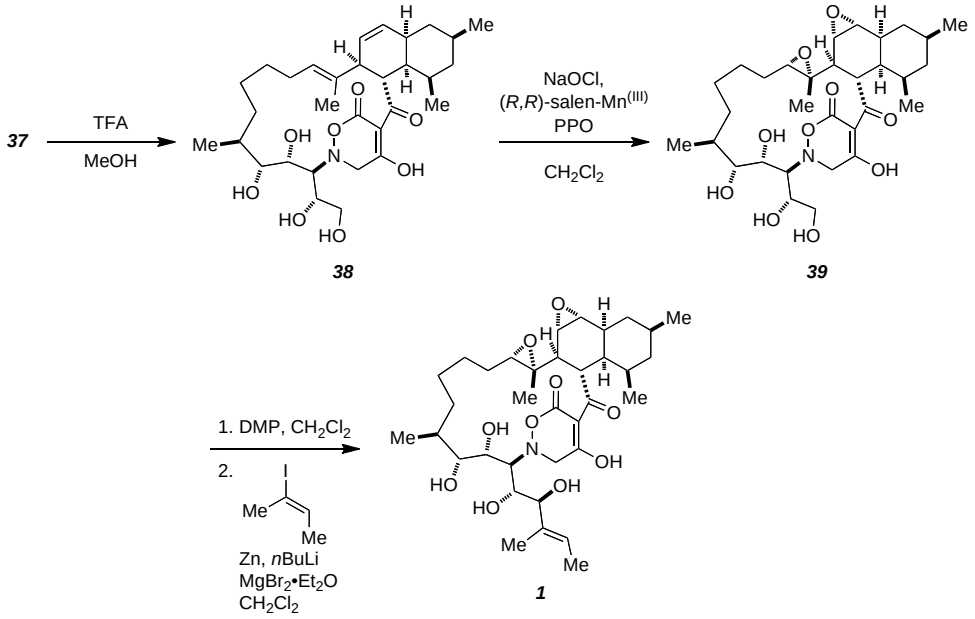
Scheme 11: Coupling of **34** and **25** along with Dieckmann cyclization.

At this point, the macrocyclization can be completed by an intramolecular Horner–Wadsworth–Emmons[21,22] reaction to give the desired (*E*) olefin of **37** (Scheme 12). There is a possibility that the macrocyclization may also occur during the previous step with the Dieckmann cyclization[25] if enough base is used. This possible one pot procedure can be explored.



Scheme 12: Macrocyclization of **36** to give the core of **1**.

Deprotection of the acetinides with TFA in MeOH would give both sets of diols, **38**. Epoxidation of the di- and tri-substituted olefins can be set through a Jacobsen–Katsuki[26,27] asymmetric epoxidation to give **39**. Alternatively, other epoxidation condition, such as the Shi epoxidation[28], may be explored for an asymmetric epoxidation of the two olefins. Oxidation of the primary alcohol of **39** with Dess–Martin periodinane[15] followed by a zinc mediated addition of 2-iodo-2-butene with $MgBr_2 \bullet Et_2O$, for a keelation controlled addition of the resultant zinc reagent, will stereoselectively set the final alcohol to give **1** (Scheme 12).[29]



Scheme 12: Completion of **1** from **37**.

The synthesis of alchivemycin A can be completed with the use of modern techniques through two key intermediates. With precedence on the use of TADA to form cis-decaline ring systems, this is a very viable and versatile approach for the cis-decaline system of **1**. There may be some exploratory work required for the correct olefin geometries to obtain the correct cis-decaline system but these substraits can easily be made in parallel. The formation of the 2H-tetrahydro-4,6-dioxo-1,2-oxazine ring through a proposed biosynthetic pathway also makes this synthesis of **1** an appealing target. These two major features along with the potent antimicrobial activity against *Micrococcus luteus* and inhibitory effects on tumor cell invasion make the synthesis of alchivemycin A (**1**) an interesting and useful synthetic target.

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