THESIS

STUDIES TOWARDS THE BIOMIMETIC SYNTHESIS OF THE STEPHACIDIN FAMILY OF NATURAL PRODUCTS AND THE CONCISE AND VERSATILE SYNTHESIS OF *D,L*-BREVIANAMIDE B, C-12A-*EPI*-MALBRANCHEAMIDE AND STRUCTURALLY RELATED ANALOGS

Submitted by

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In partial fulfillment of the requirements

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MERIAH W. N. VALENTE ENTITLED "STUDIES TOWARDS THE BIOMIMETIC SYNTHESIS OF THE STEPHACIDIN FAMILY OF NATURAL PRODUCTS AND THE CONCISE AND VERSATILE SYNTHESIS OF *D,L*-BREVIANAMIDE B, C-12A-*EPI*-MALBRANCHEAMIDE AND STRUCTURALLY RELATED ANALOGS" BE ACCEPTED AS FULFILLING, IN PART, REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee of Graduate Work

Michio Kurosu, Department of Microbiology, Immunology and Pathology

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ABSTRACT OF THESIS

STUDIES TOWARDS THE BIOMIMETIC SYNTHESIS OF THE STEPHACIDIN FAMILY OF NATURAL PRODUCTS AND THE CONCISE AND VERSATILE SYNTHESIS OF *D,L*-BREVIANAMIDE B, C-12A-*EPI*-MALBRANCHEAMIDE AND STRUCTURALLY RELATED ANALOGS

The total synthesis of stephacidin A, avrainvillamide and stephacidin B was envisioned to proceed through a biomimetic intramolecular Diels-Alder cylcoaddition. The Diels-Alder precursor was thought to come from the coupling of (L)-prolinamide with a α-ketoacid indole, which would subsequently form the requisite azadiene. While the desired α-ketoacid indole was not stable enough to be synthetically formed, a *pseudo*-α-ketoacid was successfully designed. The coupling of this vinyl ether carboxylic acid indole moiety with (L)-prolinamide provides an interesting intramolecular Diels-Alder (IMDA) precursor, which might prove to be a useful synthetic prototype for the IMDA.

A similar coupling between a *pseudo*-α-ketoacid and (L)-prolinamide was applied to a different system of compounds, which led to a biomimetically-inspired intramolecular Diels-Alder reaction that diastereoselectively formed the characteristic bicyclo[2.2.2]diazaoctane core of the brevianamides. A concise

synthesis of *d,l*-brevianamide B was successfully designed through a Fischer indole reaction of the key tricyclic IMDA cycloadduct and phenyl hydrazine. This IMDA / Fischer indole route to form the indolic bicyclo[2.2.2]diazaoctane core has proven to be a versatile method to access C-12a-*epi*-malbrancheamide and structurally related analogs for biological activity studies.

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I thank my family (Mom and Dad, Amy, Sarah, and Jordan) for their continued support of my endeavors, and for always believing in me. I thank my

nieces and nephews (Hannah, Emma, Zachary, Lauren and Andrew) for bringing pure joy to my heart, and reminding me what is important in life: to never let go of the child within!

I really need to give thanks to Panino's, the little Italian establishment right next to the Chemistry building. It is here where good friends gather, eat good food, drink cheap beer, and can escape the hard life, even if we are only a couple hundred yards away from the building.

And lastly but not least, I owe everything to my best friend and husband, Joseph Valente, whom has made my life absolutely wonderful. We met here at CSU, and we will take all the great memories we've created here with us on our journey through life. I would never have made it without you… you make me a stronger person, a happier person…you make me complete! I love you so much.

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List of Abbreviations

AcOH	acetic acid
Boc	tert-butoxycarbonyl
BOPCl	bis(2-oxo-3-oxazolidyl)phosphinic chloride
Cbz	benzyloxycarbonyl
CD	circular dichroism
COSY	correlation spectroscopy
m-CPBA	meta-chloroperbenzoic acid
CSA	camphorsulfonic acid
1-D	1-dimensional
2-D	2-dimensional
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarization transfer
DIBAL-H	diisobutylaluminum hydride
DIPEA	N,N'-Diisopropylethylamine
DKP	diketopiperazine
DMAP	4-N,N'-dimethylaminopyridine
DMAPP	dimethylallyl pyrophosphate

DMF	N,N-dimethylformamide
DMS	dimethyl sulfide
DMSO	dimethyl sulfoxide
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
FAB+	Positive fast atom bombardment
HATU	2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyl uronium hexafluorophosphate
HETCOR	heteronuclear chemiccal shift correlation
HFIPA	hexafluoroisopropyl alcohol
HMBC	heteronuclear multiple bond coherence
HMDS	hexamethyldisilazane
HMQC	heteronuclear multiple quantum coherence
HRMS	high resolution mass spectroscopy
IMDA	intramolecular Diels-Alder
INEPT	insensitive nuclei enhanced by polarization transfer
IR	infrared spectroscopy
LDA	lithium diisopropylamine
LRMS	low resolution mass spectroscopy
2,6-lutidine	2,6-dimethylpyridine
Me	methyl
MeOH	methanol

MOM	methoxymethyl
NCS	N-chlorosuccinamide
NMR	nuclear magnetic resonance spectroscopy
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser enhancement spectroscopy
Ph	phenyl
PivOH	pivaloyl alcohol
PLP	Pyridoxal phosphate
PTLC	preparative thin layer chromatography
i-Pr	isopropyl
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
pyr	pyridine
RT	room temperature
TBAF	tetra-butylammonium flouride
TBAI	tetra-butylammonium iodide
TBDPSOTf	tert-butyldiphenylsilyl triflate
t-BuOH	tert-butanol
Tf	triflouromethanesulfonyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	N,N,N',N'-Tetramethylethylenediamine
TMS	trimethylsilyl

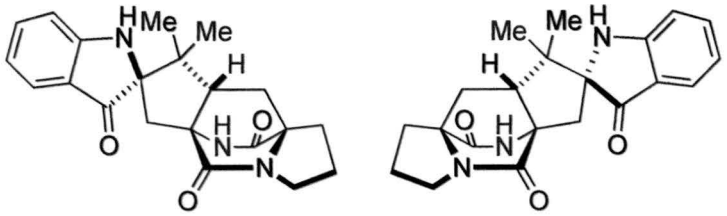
TOCSY	totally correlated spectroscopy
Ts	toluenesulfonyl

Chapter 1

Introduction

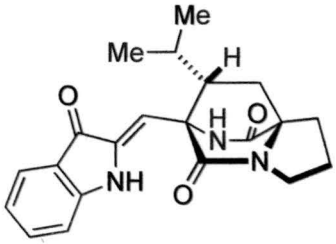
1.1 Isolation and Structural Determination

In 1969, Birch and co-workers reported the isolation of brevianamides A and B (**1** and **2**, Figure 1) from *Penicillium brevicompactum*.[1-3] This event marked the birth of a new family of prenylated indole alkaloids that contain a characteristic, but hitherto unknown bicyclo[2.2.2]diazaoctane core.[4] The brevianamides are made up of a tryptophan, proline and one isoprene unit. The structures of these mycotoxins were determined primarily on spectroscopic, degradative, and biogenetic evidence. Brevianamide B was shown to be a stereoisomer of brevianamide A through interconversion (Figure 2).[1] The relative and absolute stereochemistry of brevianamide A was determined by X-ray crystal structure of 5-bromobrevianamide A.[5] Brevianamides C and D (**3** and **4**) were also isolated from the same culture, yet they were found to be artifacts of isolation due to light irridation.[1]

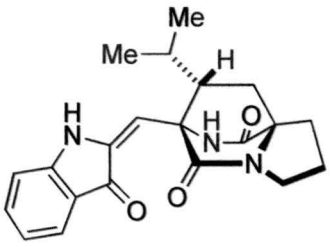


1, (+)-brevianamide A

2, (-)-brevianamide B

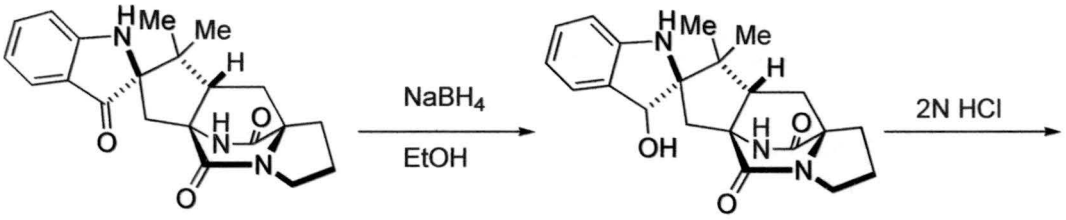


3, brevianamide C



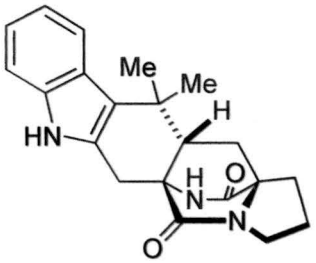
4, brevianamide D

Figure 1. Structures of the brevianamides.[1]

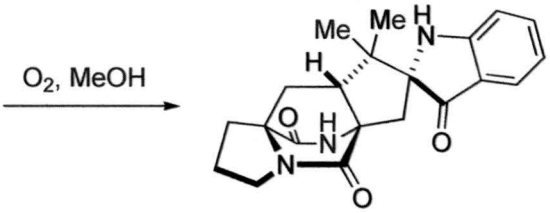


1, (+)- brevianamide A

5



6, deoxybrevianamide A

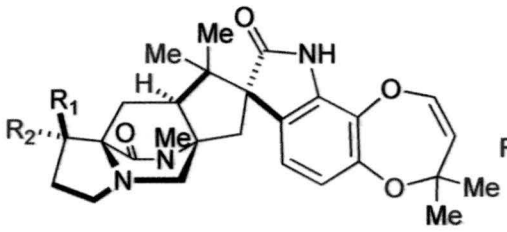


2, (-)-brevianamide B

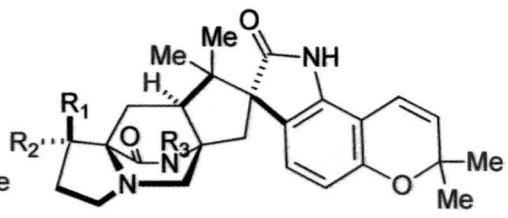
Scheme 1. Conversion of (+)-brevianamide A into (-)-brevianamide B.[1]

Over the next few decades, the family grew to include the paraherquamides (A-G, **7-13**), the asperparalines (**15-18**), VM55599 (**19**), the marcofortines (**20-22**), and sclerotiamide (**23**) from *Penicillium* and *Aspergillus* species (Figure 2).[6-9] While all of

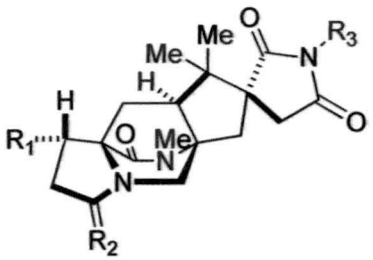
these compounds share the characteristic bicyclo[2.2.2]diazaoctane core of the brevianamides, they are structurally more complex and diverse, all sharing two isoprene units and a tryptophan (with the exception of the asperparalines, which contain a *spiro*-succinamide instead of the *spiro*-oxindole). Interestingly, the paraherquamides *spiro*-indole functionality is in the form of an oxindole ring, in contrast to the brevianamides indoxyl moiety. The paraherquamide compounds diverge from each other by either containing variously substituted proline derivatives (**7-19**) *vs.* a pipecolic acid unit in place of proline (**20-22**), or one *vs.* two oxygen substituents on the tryptophan moiety (**7-11** *vs.* **12-14**, or **20-21** *vs.* **22-23**). All of theses compounds except sclerotiamide (**23**) have the tertiary lactam reduced to the amine, and VM55599 (**19**) remains as the 2,3-disubstituted indole, rather than being oxidized to the *spiro*-oxindole. Importantly, all of these compounds in the paraherquamide family have a *syn* orientation (**25**) at the bicyclic core, in contrast to the *anti*-relationship **(24)** in the brevianamides (Figure 3). The *syn* and *anti* relationship is determined by the position of the C-H proton (or C-19, brevianamide numbering) at the bridgehead of the bicyclic core in relation to the bridging secondary lactam (Figure 3). [10]



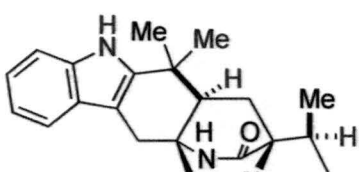
7, paraherquamide A, R_1 = OH, R_2 = Me
8, paraherquamide B, R_1 = H, R_2 = H
9, paraherquamide C, R_1 = R_2 = CH_2
10, paraherquamide D, R_1 = R_2 = OCH_2
11, paraherquamide E, R_1 = H, R_2 = Me



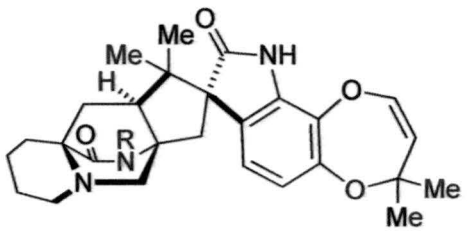
12, paraherquamide F, R_1=H, R_2=Me, R_3=Me
13, paraherquamide G, R_1=OH, R_2=Me, R_3=Me
14, VM55595, R_1=H, R_2=Me, R_3=H



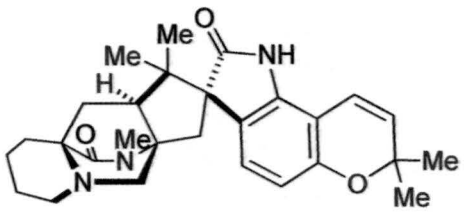
15, asperparaline A, R_1 = Me, R_2 = H_2, R_3 = Me
16, asperparaline B, R_1 = Me, R_2 = H_2, R_3 = H
17, asperparaline C, R_1 = H, R_2 = H_2, R_3 = Me
18, 16-keto-aspergillimide, R_1 = Me, R_2 = O, R_3 = Me



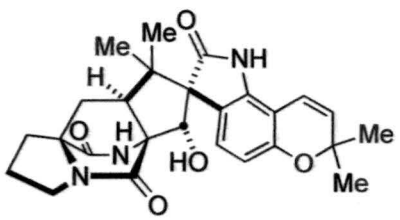
19, VM55599



20, marcfortine A, R = Me
21, marcfortine B, R = H

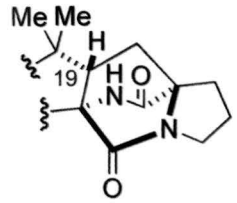


22, marcfortine C

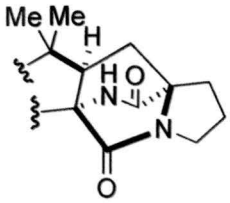


23, sclerotiamide

Figure 2. Structures of the paraherquamides and related alkaloids.[10]



anti-diastereomer of the brevianamides **24**



syn-diastereomer of the paraherquamides **25**

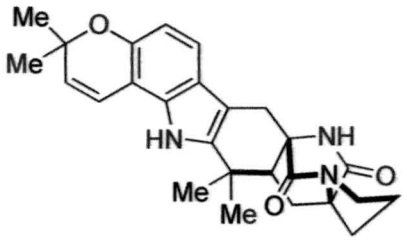
Figure 3. *Anti / syn* diastereomers at the bicyclo[2.2.2]diazaoctane core.

The brevianamide and paraherquamide family story has grown even more interesting by the recent isolation of the very peculiar derivatives stephacidin A and B (**26-27**), avrainvillamide (**28**), and aspergamide A and B (**29-30**, Figure 4). Researchers at Bristol-Myers Squibb isolated stephacidin A and B from the fungi *Aspergillus ochraceus* WC76466.[11] A similar fungal alkaloid, avrainvillamide (also named CJ-17665), was independently isolated from a different species of *Aspergillus*,[12] and later from the same.[13] The aspergamides were isolated from *Aspergillus ochraceus* as well.[14] The stephacidins are similar to paraherquamide F by sharing a chromene substituent on the tryptophan unit, yet are unique from most of the paraherquamides in that they contain a 2,3-disubstituted indole instead of the spiro-oxindole moiety, and the tertiary lactam remains intact. The very rare indole oxidation state of stephacidin B (**27**), avrainvillamide (**28**) and aspergamide A (**29**) is extremely intriguing. N-methoxyindoles are known to occur occasionally in nature, and there have been a few reported N-hydroxyindoles isolated.[15] However, stephacidin B, avrainvillamide and aspergamide A contain an indole nitrone moiety that has not been seen in any other natural products outside the *Aspergillus* species. Stephacidin B is believed to be the dimer of avrainvillamide, which itself is probably the oxidation product of stephacidin A.[11]

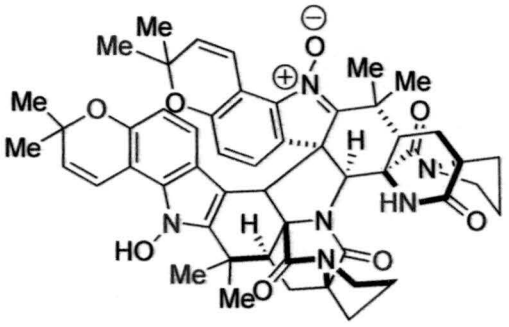
Aspergamide A and B seem to fit into this oxidation sequence from stephacidin A to avrainvillamide as well. Detailed 1D- and 2D-NMR spectral studies (DEPT, COSY, HETCOR, HMBC, HMQC, and NOE) led to elucidation of the molecular structure of the stephacidin A. Stephacidin B was more challenging to decipher, especially due to the difficulty in finding a suitable NMR solvent to eliminate severe signal broadening and overlapping. A solvent mixture of DMSO-d_6 and acetonitrile-d_3 finally gave well-resolved NMR spectra. While extensive 2D-NMR studies (COSY, HMBC, NOE and NOESY) led to the establishment of the structural fragments, the nature of the dimer linkage in stephacidin B remained unclear until a single-crystal X-ray structure was obtained. The dimer is formed from just 2 bonds, and the molecule has a butterfly-like structure. With 15 rings and 9 chiral centers, stephacidin B has been deemed to be one of the most structurally complex and novel alkaloids occurring in nature.[11] The structure of avrainvillamide was determined through 1-D and 2-D NMR studies (DEPT, COSY, INEPT, and NOEs). The presence of the N-oxide moiety was determined based on the downfield shifts of the nitrone carbon atom when in methanol solvent. This was explained by the formation of hydrogen bonding between the methanol solvent and the N-oxide moiety, in contrast to the spectral results when taken in chloroform.[13]

Earlier this year, the first chlorinated indole alkaloid belonging to this family was isolated form the ascomycete *Malbranchea aurantiaca*, and hence was named malbrancheamide (**31**, Figure 5).[16] Malbrancheamide is also the first of the family to be isolated outside of the *Penicillium* and *Aspergillus* species. Detailed 2D-NMR spectral analysis (COSY, HETCOR, HMBC, and NOESY) and X-ray analysis established that the compound had the same diastereomeric relationship as the stephacidins and the

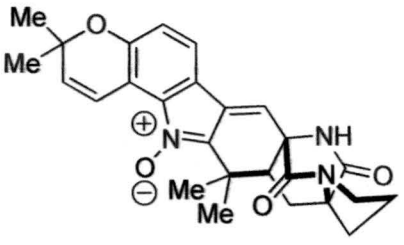
paraherquamides. While being the *syn*-diastereomer, malbrancheamide is the enanteomer of the stephacidins at the C-H bridgehead position. In addition, malbrancheamide's tertiary lactam is reduced, similar to the paraherquamides.



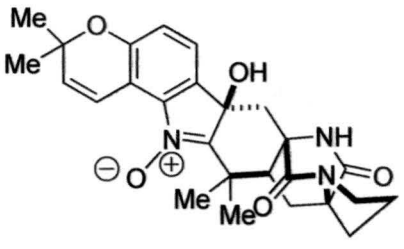
26, stephacidin A



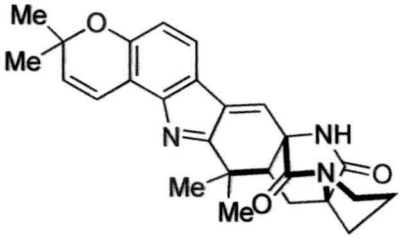
27, stephacidin B



28, avrainvillamide

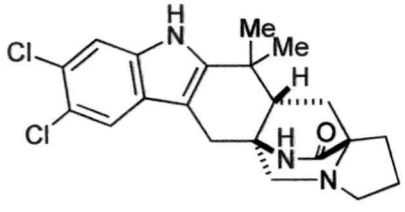


29, aspergamide A



30, aspergamide B

Figure 4. Structures of the stephacidins and related alkaloids.[11, 13]



31, malbrancheamide

Figure 5. Structure of malbrancheamide.[16]

1.2 Pharmacology

The brevianamides A and D have modest insecticidal activity, and recent studies have shown that some of the paraherquamides also display insecticidal activity.[10, 17] The paraherquamides have been largely examined for their potential in veterinary medicine. The paraherquamides have anthelmitic activity against several drug-resistant strains of nematodes, and have been suggested to possess a novel mechanism of action.[8, 9, 18-21]

The stephacidins demonstrated *in vitro* cytotoxicity against numerous human tumor cell lines (Table 1).[11] Stephacidin B (**27**) is much more potent and shows more selective antitumor activity than stephacidin A (**26**). The best selectivity was seen with prostate-dependent LNCaP cells, where **27** had an IC_{50} value of 0.06 μM. Significantly, it was proposed that these compounds have a novel mechanism of action, since the effects were not mediated by p53, mdr, bc12, tubulin or topoisomerase II. Stephacidin A was re-isolated last year from another group in a culture broth of *Aspergillus ochraceus* and tested for biological activity as an inhibitor of the mammalian mitochondrial respiratory chain.[22] Stephacidin A had only slight inhibitory potency against NADH oxidase (IC_{50} value of 34.6 μM), suggesting that mitochondrial chain inhibition is not part of the mechanism of action for this cytotoxic compound.

Avrainvillamide (**28**) was tested against multi-drug resistant (MDR) strains of Gram-positive bacteria. Vancomycin has been the typical drug used for treatment of MDR, but vancomycin-resistant Enterococci (VRE) and vancomycin-intermediate resistant *Staphylococcus aureus* (VISA) has caused the need to find a novel antibacterial drug with activity against MDR, VRE and VISA. Avrainvillamide was tested for activity against these strains, and compared to the antibiotics erythromycin, azithromycin and

vancomycin (Table 2). Avrainvillamide displayed good antibacterial activity against these MDR bacteria, yet had no antibacterial activity against *E. coli*. Avrainvillamide was also found to be cytotoxic to HeLa cells (cervical cancer cell lines), with an IC_{50} value of 1.1 μg/mL. [13]

cell line	histotype	characteristic	**26** (IC_{50})	**27** (IC_{50})
PC3	prostate	testosterone-independent	2.10	0.37
LNCaP	prostate	testosterone-sensitive	1.00	0.06
A2780	ovarian	parental	4.00	0.33
A2780/DDP	ovarian	mutp53/bcl2+	6.80	0.43
A2780/Tax	ovarian	taxol-resistant	3.60	0.26
HCT116	colon	parental	2.10	0.46
HCT116/mdr+	colon	overexpress mdr+	6.70	0.46
HCT116/topo	colon	resistant to etoposide	13.10	0.42
MCF-7	breast	estradiol-sensitive	4.20	0.27
SKBR3	breast	estradiol-independent	2.15	0.32
LX-1	lung	sensitive	4.22	0.38

Table 1. In vitro cytotoxicity of stephacidin A (**26**) and stephacidin B (**27**) (IC_{50} in μM).[11]

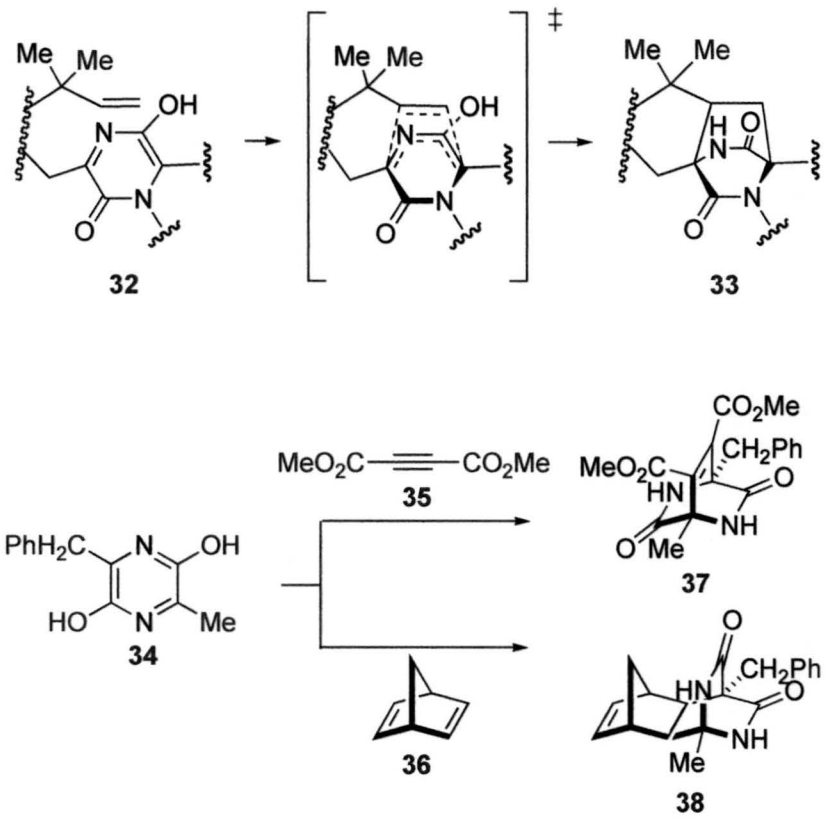
	MIC (μg/ml)			
Microorganism	CJ-17,665 (Avrainvillamide, **28**)	Erythromycin	Azithromycin	Vancomycin
Staphylococcus aureus 01A1105	12.5	>100	>100	1.56
Streptococcus pyogenes 02C1068	12.5	>100	>100	0.39
Enterococcus faecalis 03A1069	25	>100	>100	12.5
Esherichia coli 51A0266	>100	100	1.56	>100

Table 2. Antibacterial activities of avrainvillamide (CJ-17,665, **28**).[13]

Malbrancheamide (**31**) was shown to be a novel type of calmodulin (CaM)- inhibitor, where the compound competes with the formation of the CaM-PDE1 active complex. Malbrancheamide had a IC_{50} value of 3.65 μM, which is comparable to that of chlorpromazine (IC_{50} = 2.75 μM), a well-characterized CaM antagonist. [16] Calmodulin is a calcium-binding protein that can bind to and regulate a multitude of different protein targets, affecting many different cellular functions. Due to this multifunctional role, CaM inhibitors have been proposed as potential pharmaceutical targets for a wide range of uses, including MDR (multi-drug resistance) modifying agents and tumor metastasis inhibition.[23, 24] Therefore, the phytotoxic action of this new natural product could have pharmacological properties yet to be discovered.

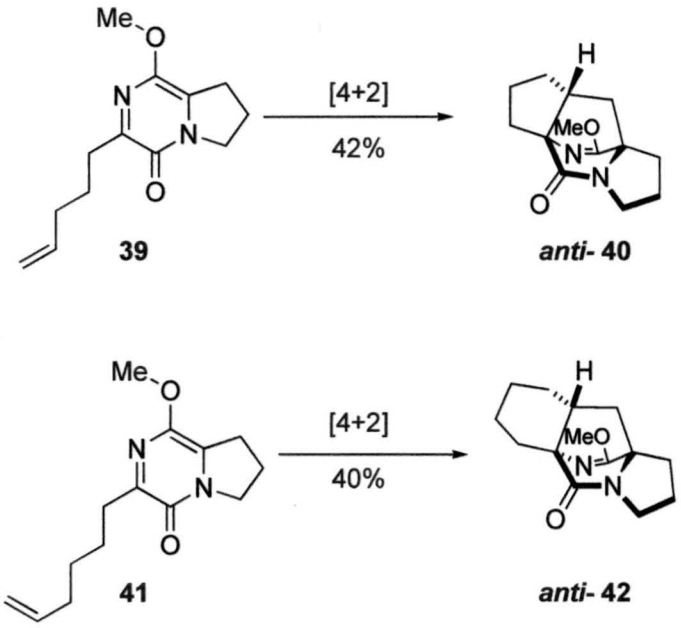
1.3 Biosynthetic origin of the bicyclo[2.2.2]diazaoctane core

Shortly after the brevianamides were isolated, Porter and Sammes proposed that the bicyclo[2.2.2]diazaoctane core could originate from a hetero Diels-Alder cycloaddition (**32-33**) (Scheme 2).[25] They showed this hypothesis to be possible by reacting the model dihydroxy pyrazine (**34**) with dimethyl acetylenedicarboxylate (**35**) and with norbornadiene (**36**), to provide the Diels-Alder cycloadducts **37** and **38**.



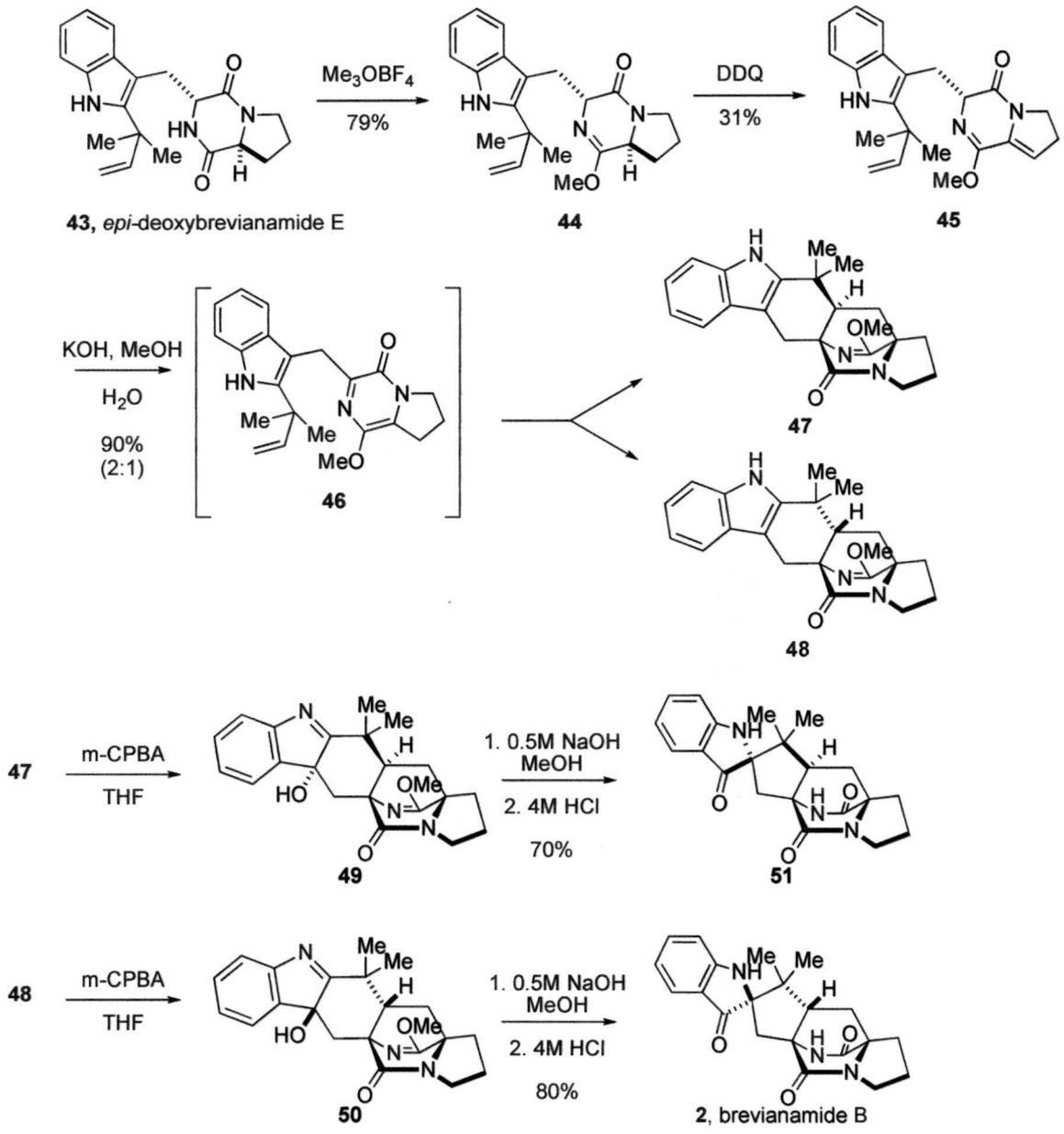
Scheme 2. Proposed biosynthesis of the brevianamides.[25]

Model systems using the diketopiperazine as the diene for the proposed [4+2] cycloaddition were explored to test this biosynthetic hypothesis (Scheme 3).[26] It was found that the intramolecular Diels-Alder reaction with either **39** or **41** proceeded spontaneously at room temperature to give exclusively the *anti* diastereomer products **40** and **42**. Interestingly, an intermolecular reaction with an analogous azadiene and either cyclopentene or cyclohexene could only be effected by harsh Lewis acidic conditions. These results suggested that the proposed Diels-Alder biosynthesis of the brevianamide and paraherquamide family was indeed feasible. While a specific enzyme might not be needed to catalyze the spontaneous reaction, it was proposed that an enzyme would be needed to control the relative *anti* or *syn* configuration of the natural products.



Scheme 3. Model studies examining the Diels-Alder biosynthetic hypothesis.[26]

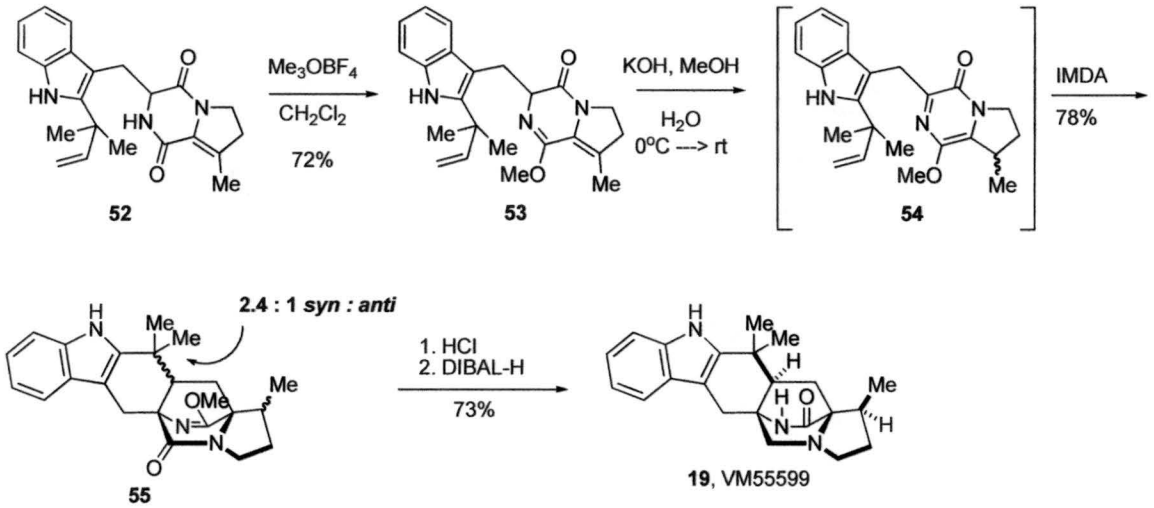
Williams and co-workers put this Diels-Alder biogenetic hypothesis to the test in their biomimetic synthesis of racemic brevianamide B (**2**, Scheme 4).[27, 28] The lactam ether **44** was formed from *epi*-deoxybrevianamide E **43** by treatment with trimethyloxonium tetrafluoroborate. Oxidation with DDQ gave the azadiene **45**, which spontaneously cyclized upon tautomerization in basic conditions to give a 2:1 mixture of cycloadducts **47** and **48**, favoring the unnatural *syn* diastereomer. Each cycloadduct was converted to the *spiro*-indoxyl moiety by diastereoselective oxidation, followed by base catalyzed pinacol-type rearrangements. Removal of the lactam ethers finally provided racemic brevianamide B (**2**) and C19-*epi*-brevianamide A (**51**). These results were the first to show that a biosynthetic Diels-Alder could form the brevianamide natural products. The synthetic observance of a 2:1 mixture of diastereomers suggests that nature may use protein organization to produce only one diastereomer in the natural products.



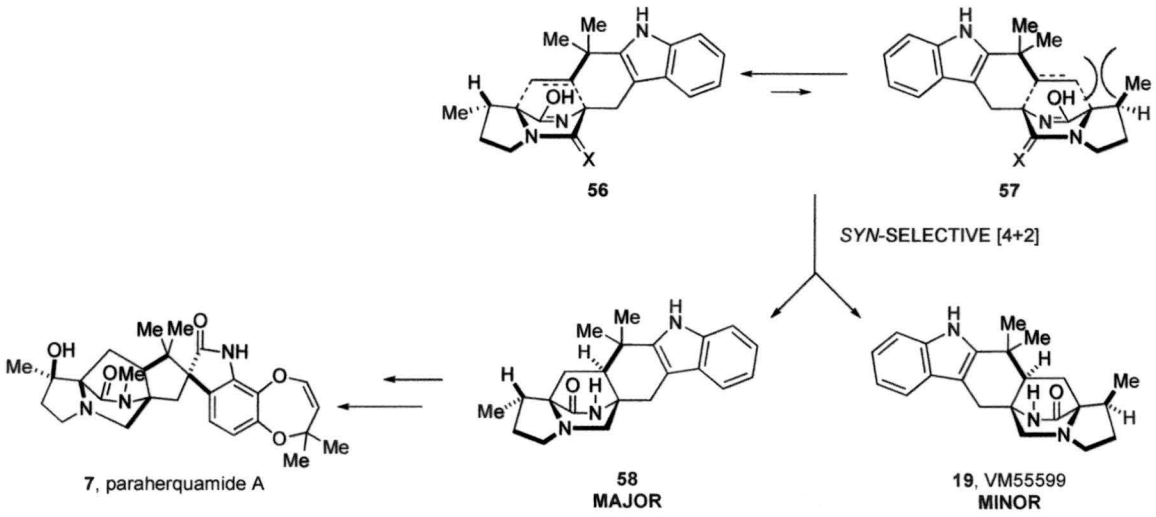
Scheme 4. Biomimetic synthesis of brevianamide B (**2**). [27, 28]

A biomimetic synthesis of VM55599 was also accomplished using the intramolecular Diels-Alder reaction (Scheme 5).[29] The azadiene **53** was obtained from lactam ether formation, and this compound also suffered spontaneous cycloaddition upon treatment with aqueous base to provide a mixture of all four possible racemic

cycloadducts (**55**). The *syn* diastereomers were preferred once again, in a 2.4 : 1 *syn : anti* ratio. The cycloadducts could be separated by PTLC, and their relative stereochemistry was assigned by NOE studies. With the correct diastereomer in hand, racemic VM55599 (**19**) was made after cleavage of the lactam ether and reduction of the tertiary amide. These synthetic results support the assumption that protein organization must have some control over the biosynthetic diastereoselective Diels-Alder reaction. Since VM55599 is a minor metabolite from the *Penicillium* species that produces paraherquamide A, and no metabolites with the *anti* configuration have been isolated, it appears that a *syn*-selective enzyme takes part in the paraherquamides biosynthesis (Scheme 6).



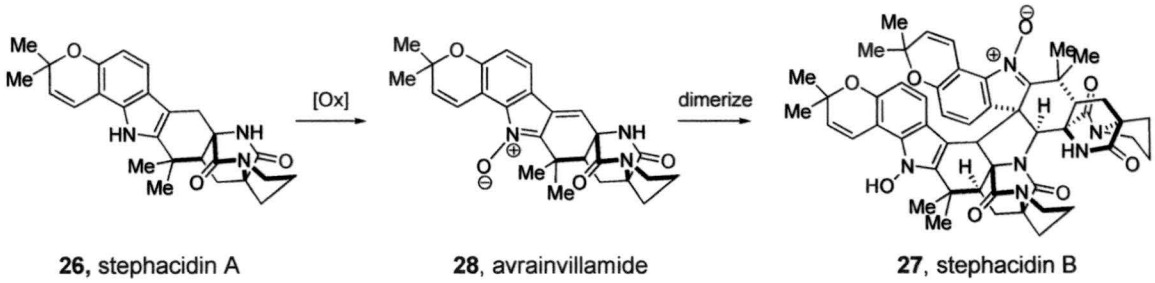
Scheme 5. The biomimetic synthesis of VM55599.[29]



Scheme 6. *Syn*-selectivity in biosynthesis of VM55599 and the paraherquamides.[29]

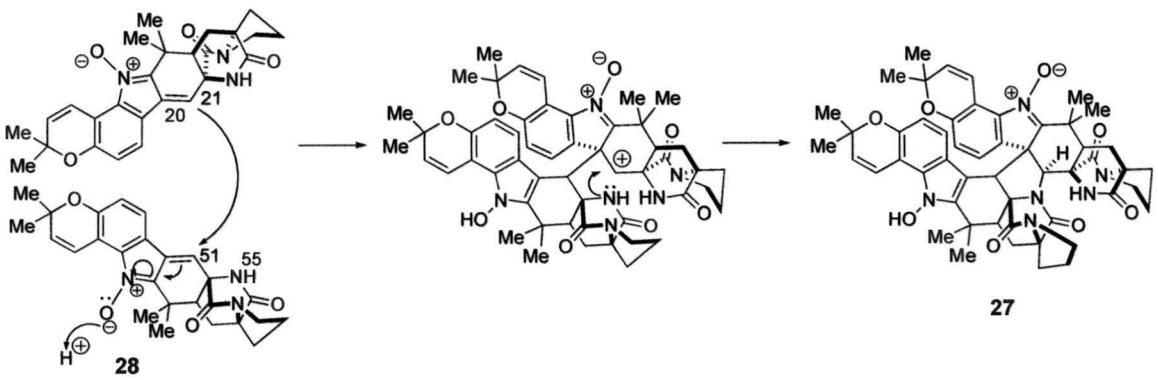
1.4 Biosynthetic pathway to stephacidin B

When stephacidin A and B were isolated, the isolation of avrainvillamide from the same *Aspergillus* species was already published.[13] Qian-Cutrone and co-workers immediately saw a connection between the molecules.[11] It appears that stephacidin B is the dimer of avrainvillamide, which itself is probably the oxidation product of stephacidin A (Scheme 7). Aspergamide A and B (**29** and **30**) seem to fit into this oxidation sequence from stephacidin A to avrainvillamide as well.

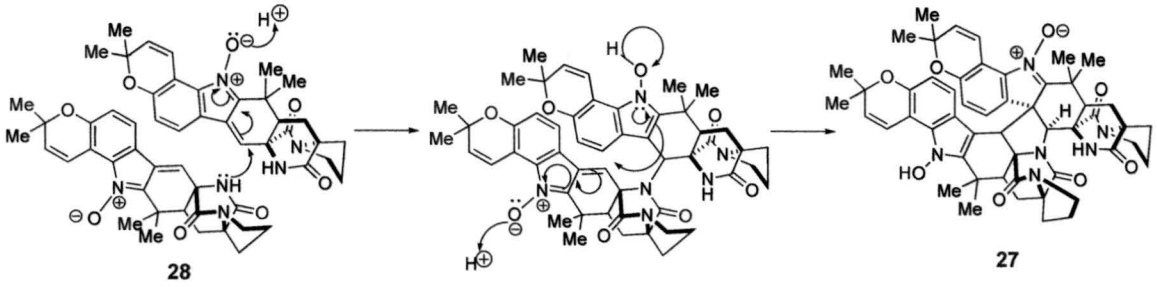


Scheme 7. Proposed relationship between the stephacidins and avrainvillamide.[11]

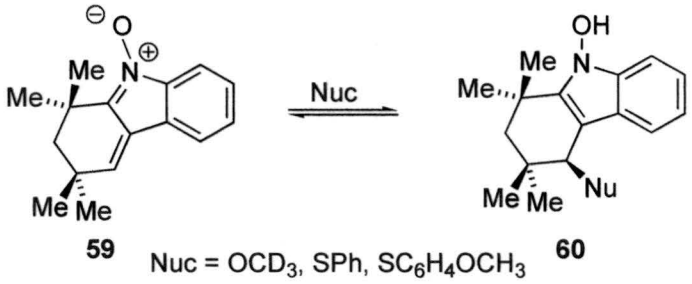
Two possible biosynthetic routes to stephacidin B from avrainvillamide have been proposed. Qian-Cutrone and co-workers proposed a nucleophilic attack on C51 by the C20-C21 olefin of another avrainvillamide unit, thus producing a secondary carbocation at C21 that would be attacked by the amide N55 to produce the dimer (Scheme 8).[11] Alternatively, Franz von Nussbaum proposed a novel Michael addition into the nitrone. Avoiding the creation of a secondary carbocation, he predicts that the amide N55 first nucleophilicly attacks the C21 nitrone Michael acceptor, thus affording the N-hydroxy-indole, which then reverses the nucleophilic attack to the nitrone Michael accepter of the other monomer (Scheme 9).[30] Myers and Herzon's model studies towards the synthesis of avrainvillamide showed that the 3-alkylidene-3H-indole 1-oxide functionality could function as novel Michael acceptor of oxygen and sulfur-based nucleophiles, yet not nitrogen (Scheme 10).[31] While the failure of nitrogen to nucleophilicly add to the unsaturated nitrone initially raised some speculation towards von Nussbaum's biosynthetic dimerization, Myers and Herzon's complete synthesis of avrainvillamide and stephacidin later showed that avrainvillamide's unique 3-alkylidene-3H-indole 1-oxide functionality does indeed act as a Michael acceptor (Scheme 11).[32]



Scheme 8. Qian-Cutrone's biosynthetic proposal on the formation of stephacidin B.[11]



Scheme 9. von Nussbaum's biosynthetic proposal on the formation of stephacidin B. [30]

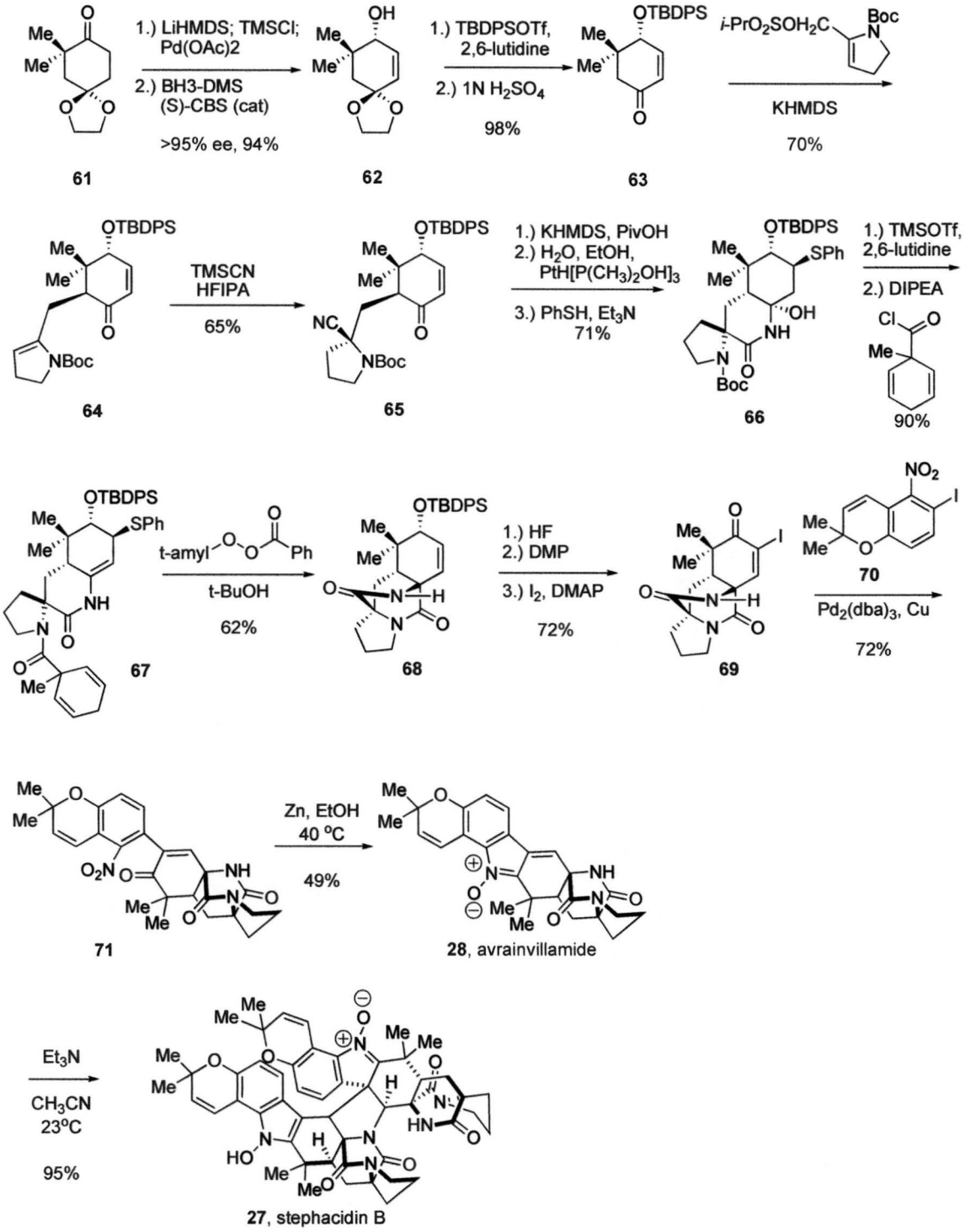


Scheme 10: 3-Alkylidene-3H-Indole 1-Oxide as a Novel Michael Acceptor. [31]

1.5 Past synthetic work on stephacidin A, avrainvillamide and stephacidin B

Myers and Herzon provided an elegant enantioselective synthesis of stephacidin B (**27**) through the synthesis of avrainvillamide (**28**, Scheme 11).[32] The stereochemistry that was set in **62** (>95% ee) from a Corey-Bakshi-Shabata (CBS) enantioselective reduction of the initial ketone **61** controlled the stereochemical outcome throughout the rest of the synthesis. At this point, the absolute stereochemistry of stephacidin A and B were not determined, so the authors randomly chose the (S)-CBS catalyst to illustrate their enantioselective route to stephacidin B. The silyl hydroxyl group directed diastereoselective alkylation of the enolate of **63**, providing **64** as a single diastereomer. Stecker-like addition of HCN gave **65** as the major diastereomer in 65% yield. Epimerization followed by nitrile conversion to the amide allowed cyclic hemiaminal

formation to give the tricyclic product **66**. Dehydration was accompanied with N-Boc deprotection, which after acylation led to the key acyl radical precursor **67**. By forming the acyl radical of **67**, the bicyclo[2.2.2]diazaoctane core was elegantly formed in **68** as a single diastereomer. The stage was then set for an Ullmann-like coupling of the vinyl iodide **69** and the aryl iodide **70**, to provide the nitroketone product **71.** Reductive cyclization then furnished avrainvillamide (**28**), which dimerized to stephacidin B (**27**) reversibly in the presence of excess triethylamine. Avrainvillamide was shown to be a novel Michael acceptor when brought up in a solution of methanol-d_4, where 1,5-addition was observed, as in their original model studies (Scheme 10).

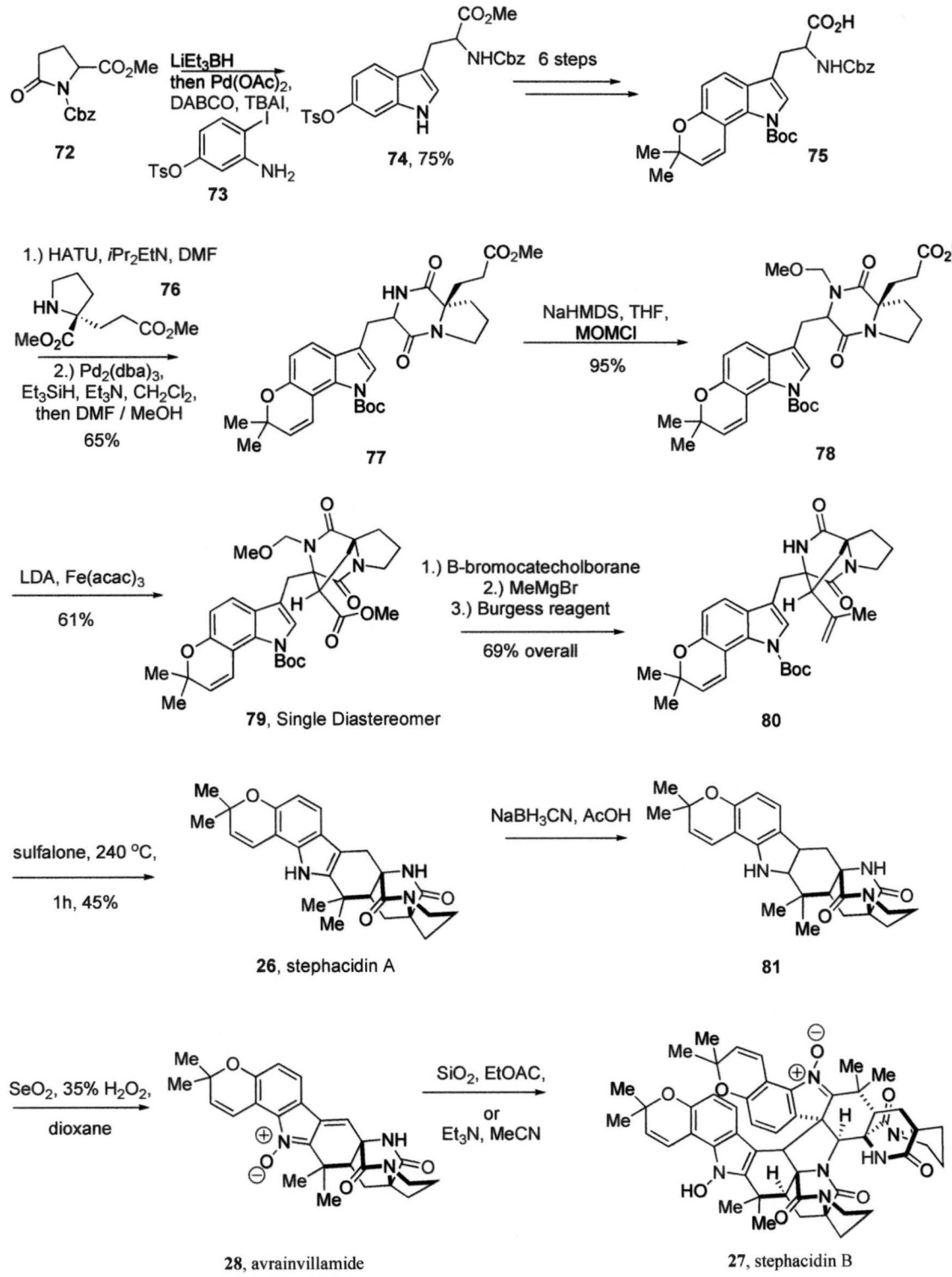


Scheme 11. Herzon and Myers' synthesis of avrainvillamide and stephacidin B. [32]

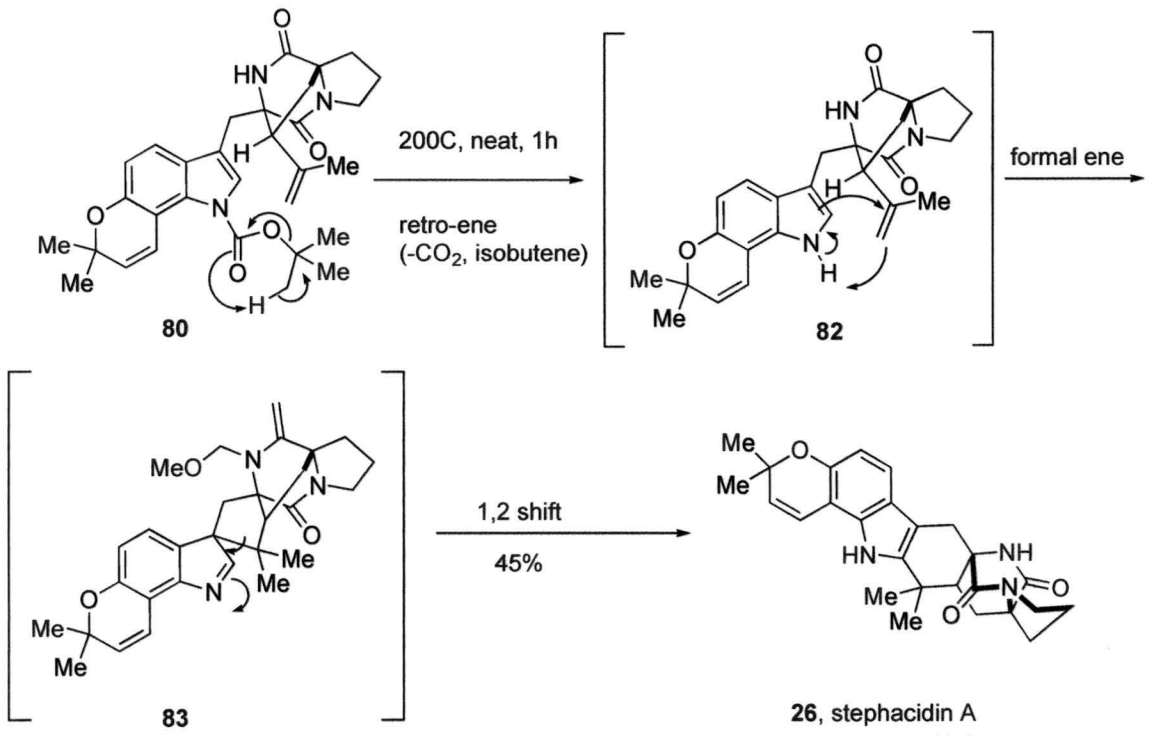
Baran and coworkers designed their synthesis of stephacidin B to proceed through the proposed biosynthetic pathway of oxidation of stephacidin A to avrainvillamide, and dimerization to stephacidin B (Scheme 12).[33, 34] Noting the difficulty in finding a practical and rapid synthesis of 6-hydroxytryptophan, the authors were finally able to obtain **74** in a fantastic yield after extensive optimization. With the indole made, subsequent chromene installation provided **75**. Peptide coupling of **75** with the proline derivative **76**, followed by a chemoselective cleavage of the N-Cbz group, allowed the cyclization to the diketopiperazine **77**. After MOM-protection, the bicyclic core was formed in a rare metal-mediated oxidative coupling of enolates. This remarkable reaction gave **79** as a single diastereomer. After removal of the MOM group with B-bromocatecholborane, reaction with MeMgBr provided a tertiary alcohol that was dehydrated with Burgess reagent to furnish **80**. The stage was now set for another remarkable reaction, where stephacidin A (**26**) was formed in one pot by simply heating **80** either neat or in sulfalone to 240°C (Scheme 13). The reaction is proposed to proceed by sequential thermolytic removal of the Boc group (by a retro-ene reaction) to give **82**, a formal ene reaction to give the *spiro*-cyclic intermediate **83**, which then finally undergoes a 1,2-shift to finish the cascade and provide stephacidin A (**26**). [33] Following the pioneering work of Somei in the synthesis of 1-hydroxyindoles (tautomers of indole nitrones),[15] Baran and coworkers reduced the indole of stephacidin A to the indoline **81**.[34] The indoline could then undergo a Somei oxidation using Na_2WO_4-$2H_2O$ to provide the α,β-unsaturated nitrone of avrainvillamide (**28**), however in the low yield of 20% where the product was mixed with inseparable impurities. Alternatively, it was found that SeO_2 and excess H_2O_2 would cleanly provide **26** in 27% yield along with 50%

recovered starting material. In accord with Herzon and Myers' synthesis of stephacidin B, Baran found that **28** would spontaneously dimerize to **27**, not only by treatment with base, but even with exposure to silica gel or simple evaporation from DMSO. Since the absolute stereochemistries of these compounds were unknown, and the original isolated material was no longer available, Baran collaborated with Professor Fenical to re-isolate avrainvillamide. By careful analysis of the extracts, they were pleased to find not only avrainvillamide (**28**), but also stephacidin A (**26**). Interestingly, there was no sign of stephacidin B (**27**). Comparison of CD spectra of the natural material to the synthetic material allowed them to synthetically assign the absolute configuration of all three natural products.

Due to the spontaneous dimerization and observed equilibration of both avrainvillamide and stephacidin B under mild conditions, speculation has been raised as to which compound is actually responsible for the observed biological activity.[32] Herzon and Myers suggested that the antitumor activity observed for stephacidin B may be attributable to avrainvillamide (**28**) formed from **27** *in vivo*. This assumption also leads to the possibility that stephacidin B could be an artifact of isolation.[35] Baran found that a solution of **28** in DMSO contained 20% of stephacidin B (**27**), yet when avrainvillamide was stored as a powder, the sample contained no evidence of **27**.[34] It is therefore feasible to speculate that stephacidin B is formed upon chemical solvent extraction of avrainvillamide from its natural source.



Scheme 12. Baran's enantioselective synthesis of stephacidin A, avrainvillamide, and stephacidin B.[33, 34]



Scheme 13. Indole annulation cascade in the synthesis of stephacidin A. [33, 34]

1.6 Research Objectives

The original intent of my studies was to explore the biomimetic synthesis of the stephacidins, avrainvillamide and paraherquamide F (Chapter 2). This work subsequently led to a biomimetically-inspired IMDA construction of racemic brevianamide B (Chapter 3). The chemistry that was developed for the synthesis of brevianamide B has proved to be a versatile route to synthesize *epi*-malbrancheamide and structurally related analogs (Chapter 4).

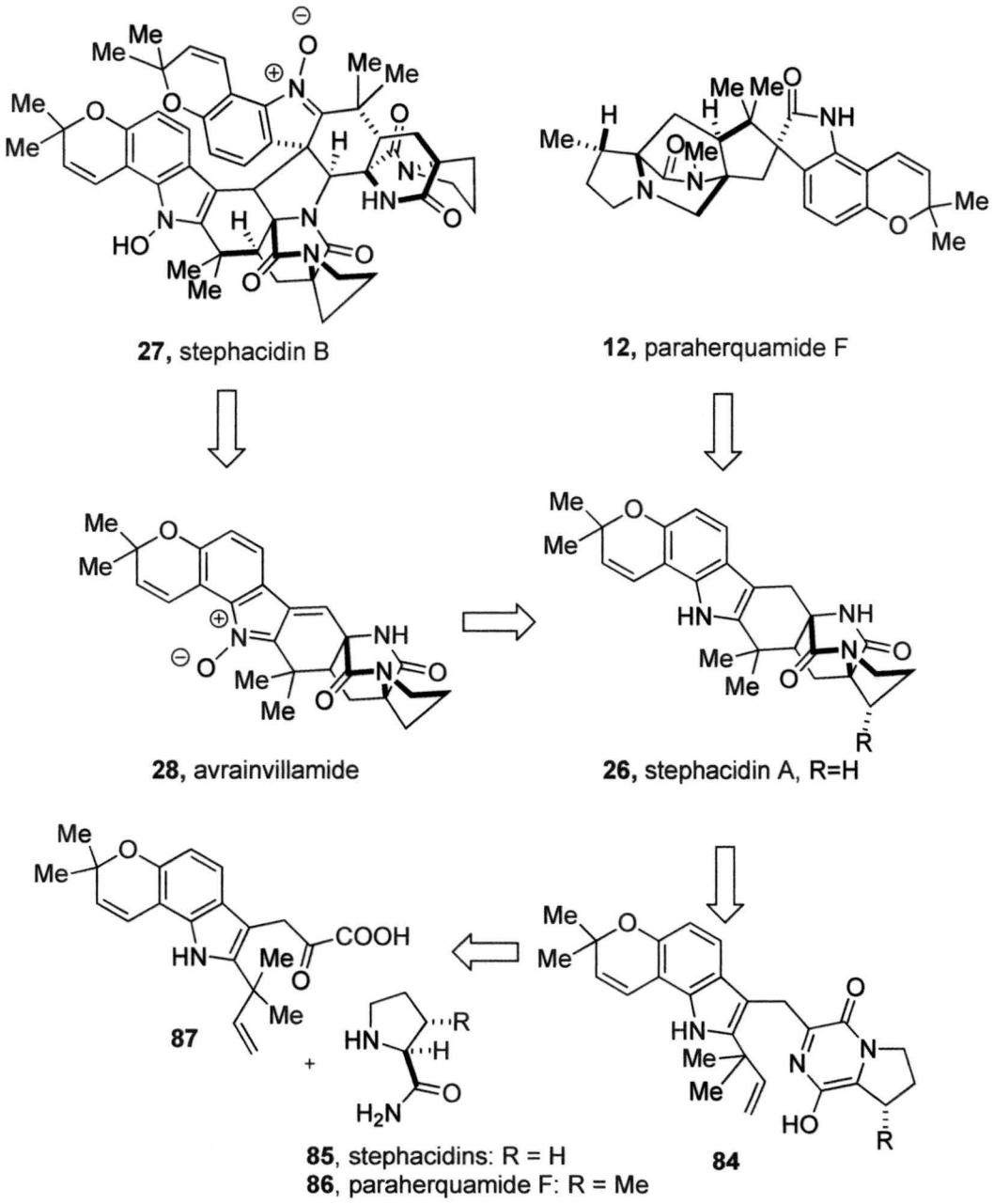
Chapter 2

Studies towards the synthesis of stephacidin A, avrainvillamide, stephacidin B, and paraherquamide F

2.1 Retrosynthetic Analysis

Stephacidin B has been considered the most complex prenylated alkaloid known with 15 rings and 9 chiral centers.[30] Due to its structural complexity and its potent *in vitro* cytotoxicity, we embarked on the total synthesis of stephacidin B. In addition, we hoped to learn more about the biosynthesis of this strangely oxidized indole by creating a biomimetic synthesis that would produce labeled intermediates for feeding experiments with *Aspergillus ochraceus*. A biomimetic synthesis producing stephacidin A, avrainvillamide and stephacidin B, would also be readily adaptable to prepare analogs for cytotoxicity studies. Our synthetic approach towards stephacidin A (**26**) was envisioned to proceed through a biomimetic Diels-Alder reaction of the azadiene **84** (Scheme 14). Condensation of the α-keto acid **87** with prolinamide **85**, followed by dehydration was expected to afford this Diels-Alder precursor **84**. Once stephacidin A had been made, direct indole oxidation would give rise to avrainvillamide (**28**), which in turn will be biomimetically dimerized to give stephacidin B (**27**). This route could be adapted towards the synthesis of paraherquamide F (**12**) by simply making the β-methyl

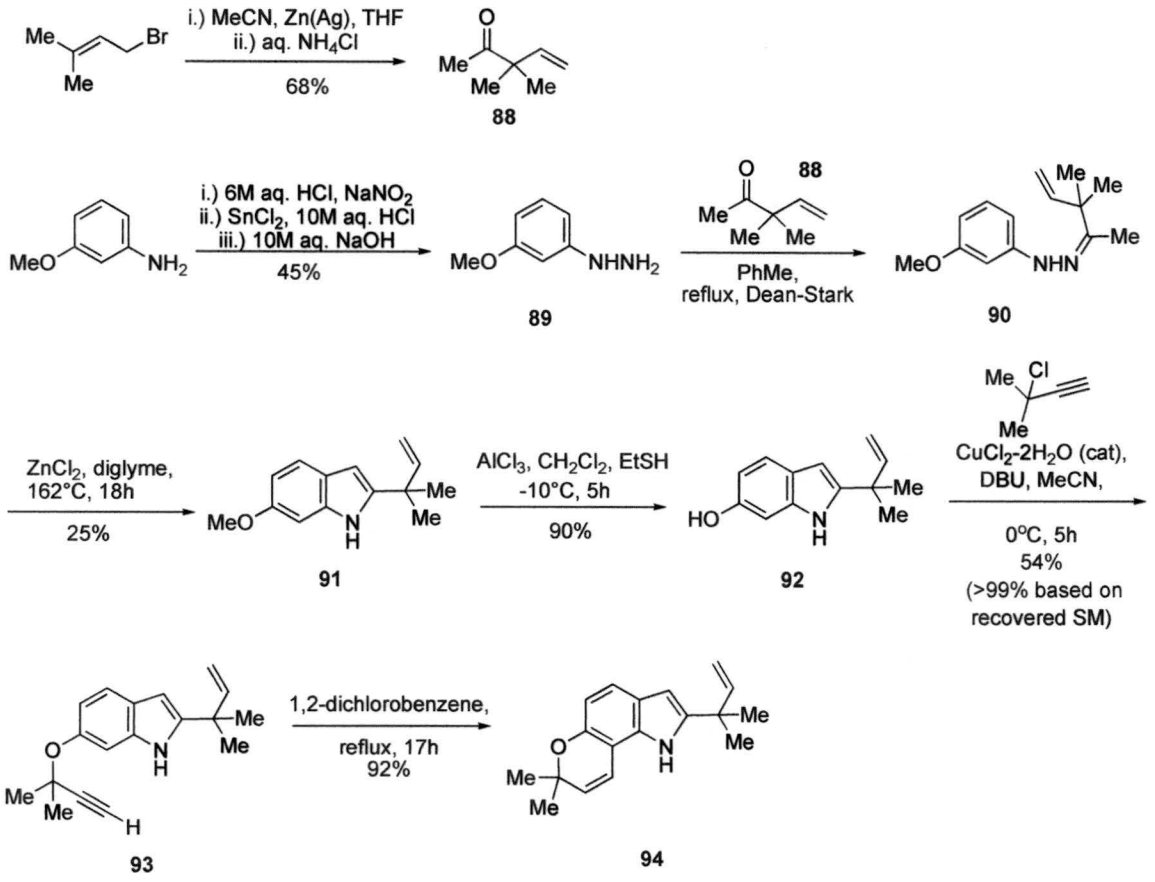
prolinamide derivative **86** (where R = Me), thereby providing an opportunity for the synthesis of four natural products.



Scheme 14. A biomimetic retrosynthetic analysis versatile for the synthesis of 4 natural products.

2.2 **Search for a more efficient route to the prenylated indole**

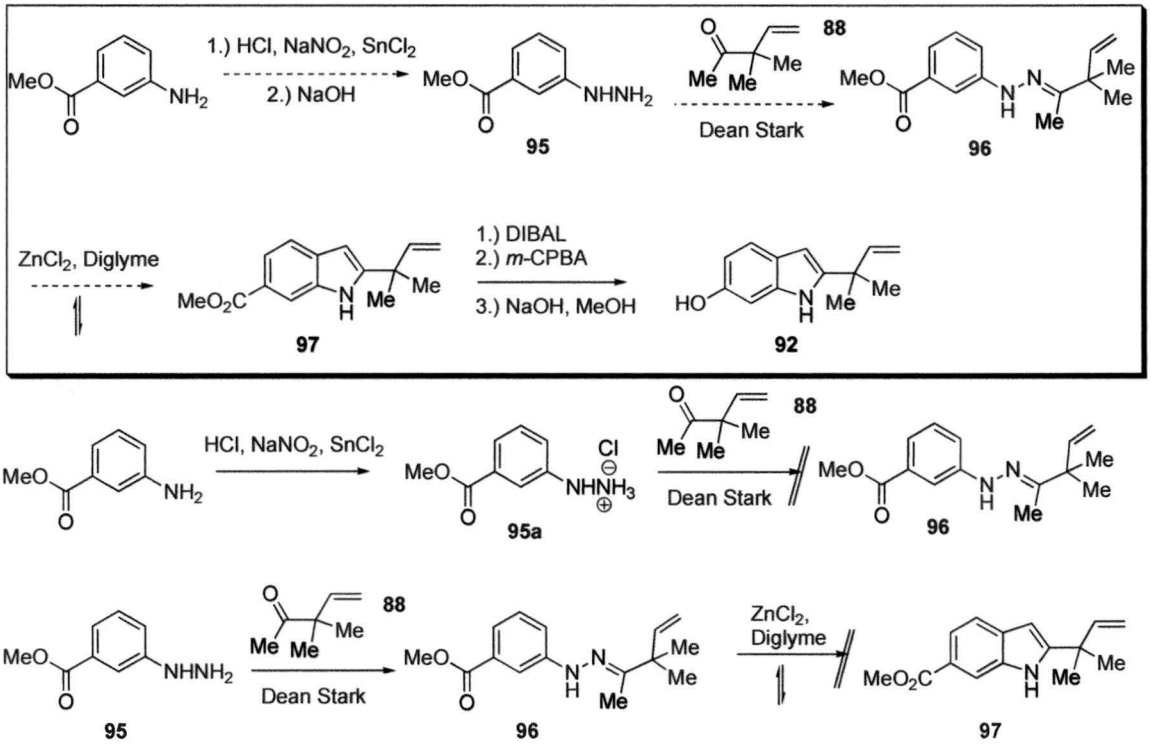
The prenylated pyrano-indole **94** has been previously described in our group's synthetic studies towards paraherquamide F (Scheme 15).[36] My designed biomimetic synthesis would require either the indoles **91** or **94**. This synthetic route proved to be quite challenging to reproduce. The bottleneck of this route is the Fischer indole reaction to produce indole **91**. The reaction gives two regioisomers, the desired 6-methoxyindole and the undesired 4-methoxyindole in a 3:1 ratio, respectively. The reported yield of **91** as 25% is a rather high estimate, and due to difficulties in purifying this compound, the yields usually were around 10-20%. Demethylation of the indole **91** gave the 6-hydroxyindole **92** in good yields, which then allowed for installation of the chromene piece by subsequent alkylation and a Claisen rearrangement. The total synthesis of **94** is achieved in 7 steps, with a yield no higher than 6%.



Scheme 15. Synthesis of the 1,7-dihydropyrano[2,3-*g*]indole ring system. [36]

Due to the low yields of the Fischer indole synthesis, I investigated other routes to obtain the desired indole. With the possibility in mind that the electron donating nature of the methoxyhydrazone was causing the observed low yields of the Fischer reaction, the electronics of the hydrazone were changed by replacing m-anisidine with electron withdrawing methyl-3-aminobenzoate (Scheme 16). If the methylester hydrazone **96** underwent the Fischer reaction in significantly higher yields, than the indole **97** could readily be converted to the hydroxyindole by a Baeyer-Villiger reaction of the aldehyde.[37] When the free hydrazine **95** was made from methyl-3-aminobenzoate, it polymerized upon standing, requiring that it be stored in the form of its HCl salt, **95a**.

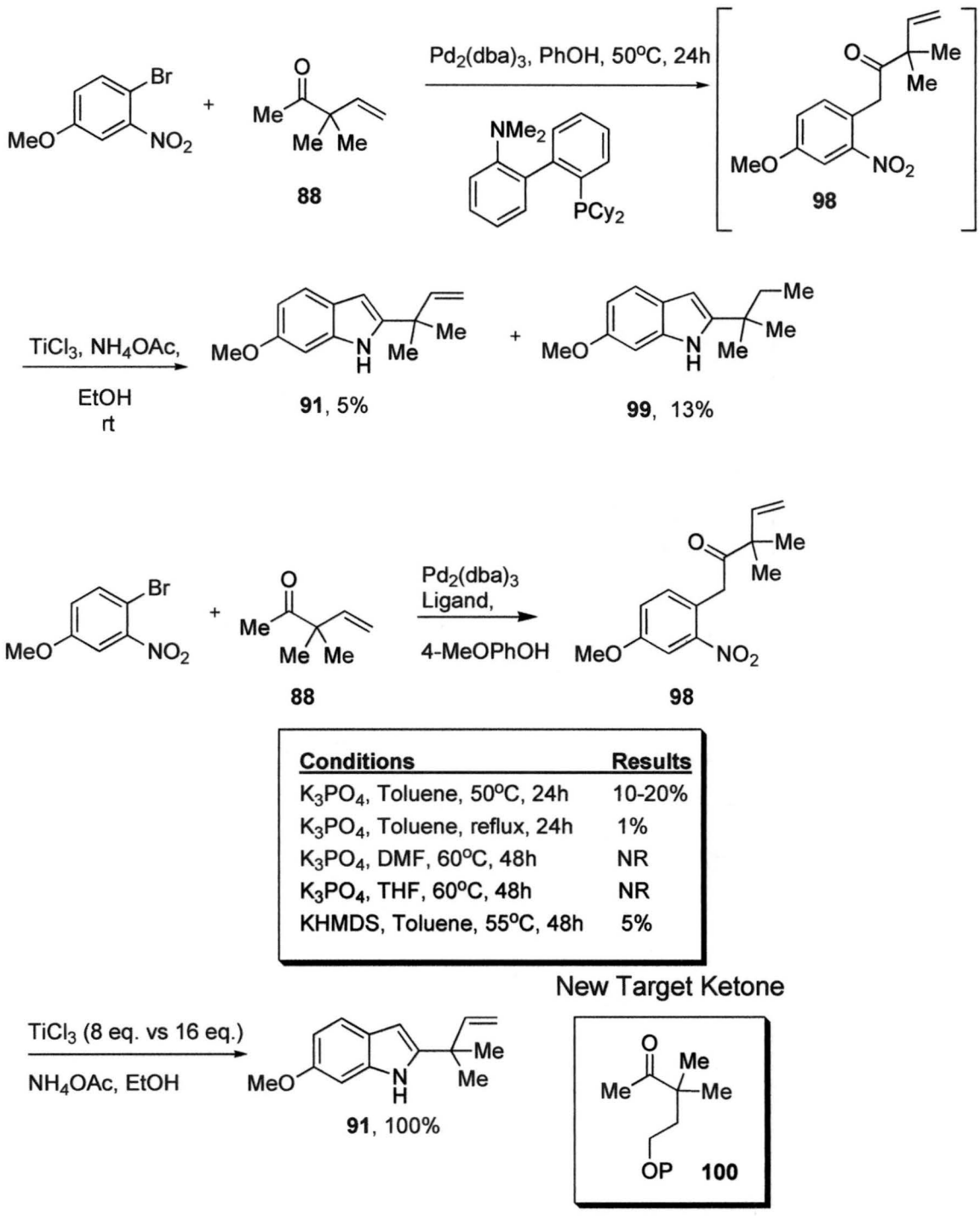
The hydrazine salt **95a** was obtained as stable white crystals, albeit in a low yield of 22%. However, the condensation of the hydrazine salt with the methyl prenyl ketone **88** failed to yield the desired hydrazone **96**. It was found that if the salt was neutralized and used immediately in the condensation reaction, the hydrazone **96** could be obtained. However, this hydrazone failed to undergo the Fischer reaction to give **97**.



Scheme 16. Attempts to create the methyl ester indole.

Buchwald's indole synthesis via the arylation of ketone enolates seemed to be an attractive alternative to design indole **91** using the ketone **88**.[38] The procedure for this reaction is one-pot, where the intermediate aryl ketone is not isolated and immediately subjected to reductive cyclization conditions to form an indole. Indole **91** was indeed formed upon initial investigation of this reaction, albeit in a very low yield of 5%. The

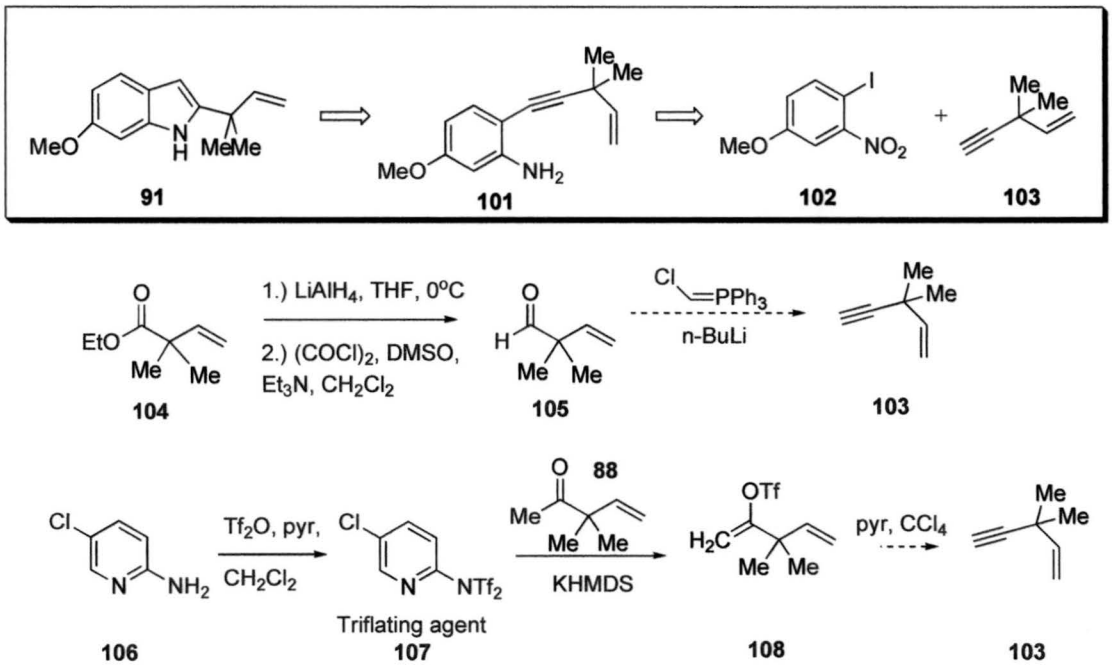
olefin-reduced indole **99** was isolated in slightly higher yields of 13%. Since it was unclear if the first step of Pd arylation was the cause for olefin reduction or if it was the second step of reductive cyclization, the intermediate **98** was isolated, and the reaction sequence divided into two steps. The first step appears to be the problem for the reaction, where a screening of various conditions revealed the best of yields at 20% with K_3PO_4 and toluene at 50°C. Since reductive cyclization occurred in quantitative yields, it appears that olefin is competing for coordination with the Pd, resulting in H insertion into the double bond to give the reduced indole **99** and minor yields of the desired indole. To overcome this, we would need a new ketone such as **100**, where the olefin is disguised as a protected hydroxyl group.



Scheme 17. Buchwald's arylation of ketone enolates. [38]

The Sonagashira coupling was also investigated as a route to the indole (Scheme 18).[39] It was envisioned that the alkyne **103** would couple with the aryl iodide **102**,

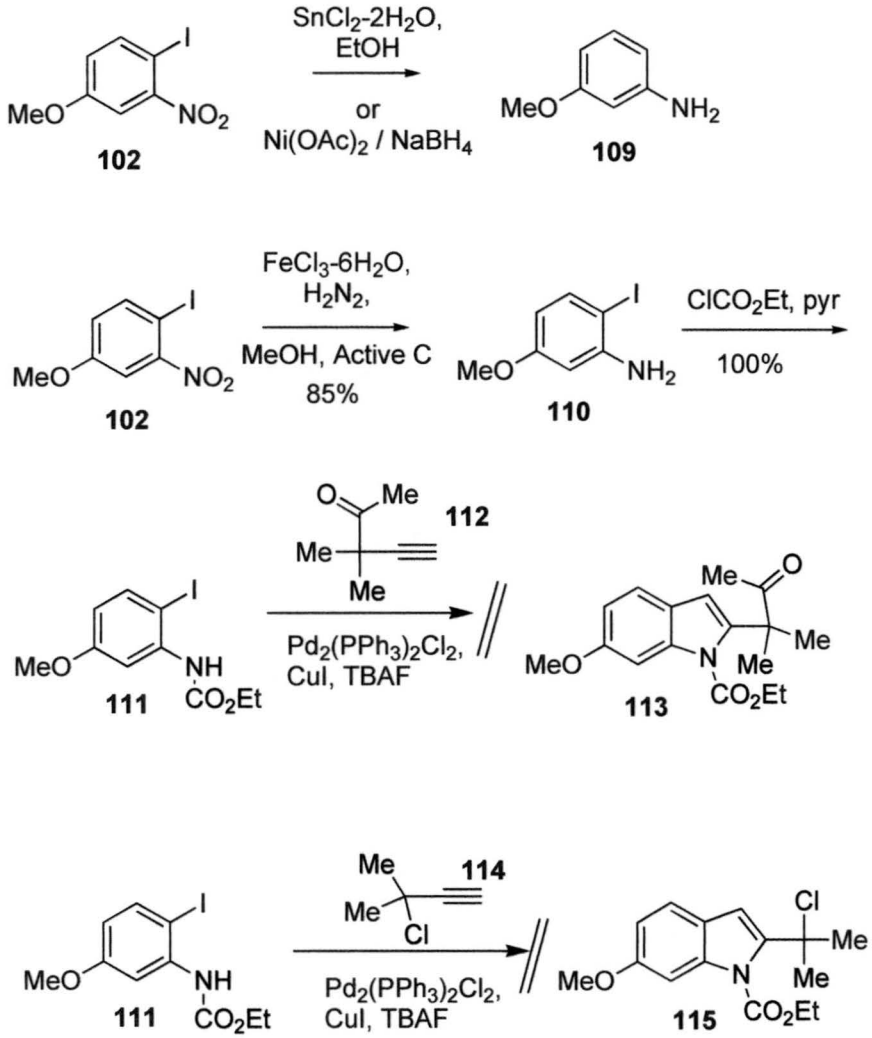
which would undergo cyclization to provide indole **91**. The desired alkyne **103** proved to be too volatile to synthesize. An attempted Corey-Fuchs homologation of the aldehyde **105** consumed all the starting materials, but left no products in hand.[40] The vinyl triflate **108** was prepared from the ketone **88** and the prepared triflating agent **107**.[41] It was similarly found that when eliminating conditions were applied to **108**, starting materials were consumed yet **103** was not obtained.[42]



Scheme 18. Initial investigation for the Sonagashira coupling of aryl iodide with prenylated alkyne. [39]

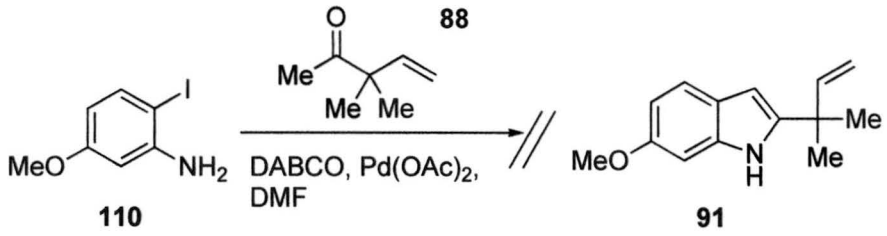
Alternatively, increasing the molecular weight of the alkyne gave a handle to this alkyne (Scheme 19). After the known alkyne **112** was prepared, the aryl iodide **102** needed to be transformed to carbamate **111**.[43] Standard conditions to reduce the nitro group to the analine resulted in de-iodination to **109**. Alternatively, it was found that $FeCl_3$ reduction in the presence of hydrazine and activated carbon gave the desired *o*-iodoanaline in good yields.[44] The 2-iodocarbanilate **111** was prepared in quantitative

yields by reacting the analine **110** with ethyl chloroformate.[45] To much dismay, the Sonagashira coupling of either the prepared alkyne **112** or the commercially available alkyne **114** failed to produce the indoles **113** or **115**, leaving only recoverable starting materials.[39]



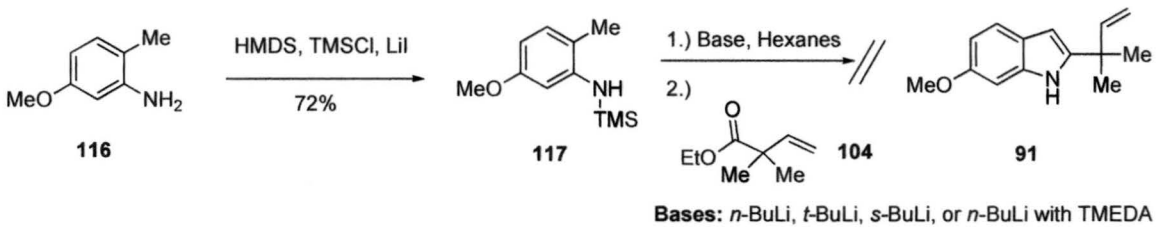
Scheme 19. Attempted Sonagashira coupling with known alkynes. [39]

The Pd catalyzed annulation of the analine **110** with the ketone **88** also failed to give the indole **91** (Scheme 20).[46] The enamine intermediate was never formed in this reaction. Again, only starting materials were recovered.



Scheme 20. An *o*-haloenamine route to the prenylated indole.[46]

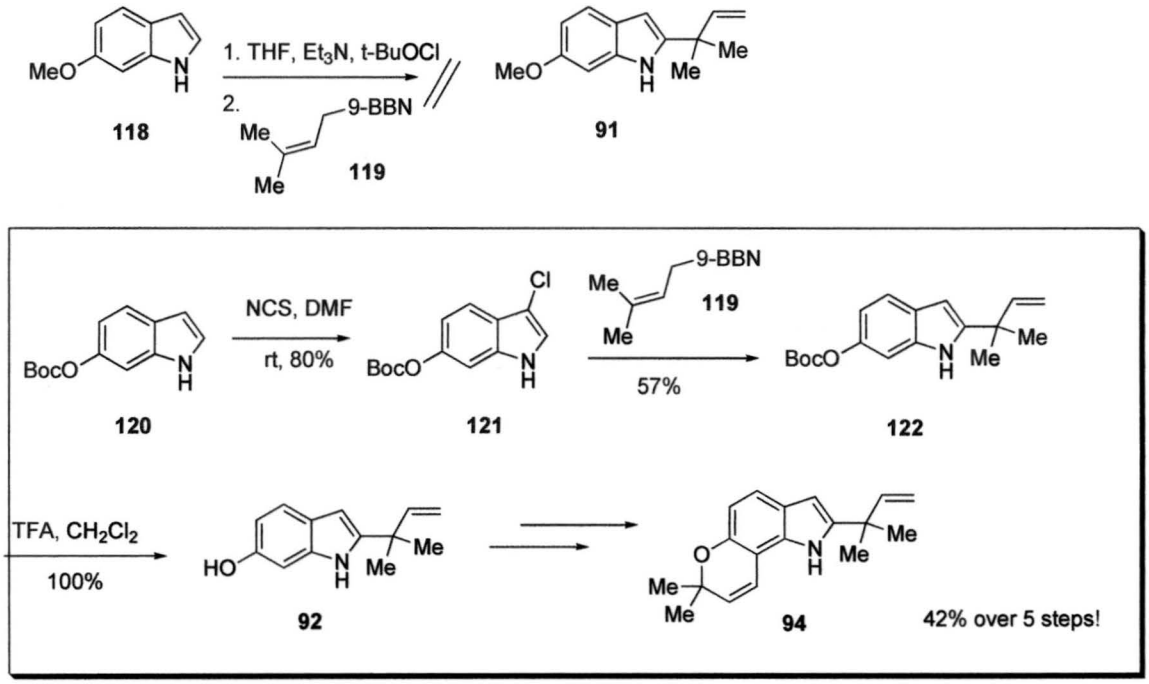
The Smith indole procedure was tried as well (Scheme 21).[47] Again, all attempts to obtain the desired indole failed. A variety of bases were used, along with the anion stabilizing additive TMEDA. In all cases, the toluidine anion would not be formed.



Scheme 21. The Smith indole procedure.[47]

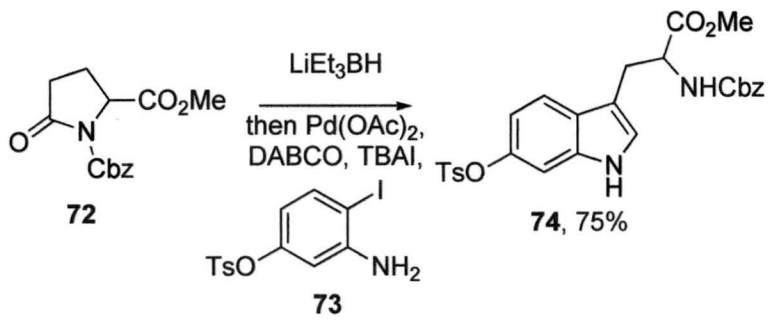
With all theses various indole procedures tried and failed, it started to become clear that the electronically donating nature of the methoxy group may be hindering these reactions. The electronic effects became more obvious after Danishefsky's reverse prenylation of the methoxyindole **118** was investigated, which again failed to give the indole **91**.[48] Collaboration with my co-workers led to discovery that if the 6-hydroxyindole was protected with an electron withdrawing functional group such as the Boc group in **120**, then reverse prenylation would give the indole **122**.[49] We found that Danishefsky's procedure does not work with 2,3-unsubstituted indoles, where 3-

prenylation is observed instead of 2-prenylation. Instead, it was found that a two-step procedure developed by Tatsuta and co-workers that first prepares the 3-chloroindole then reverse prenylates to the 2-postion was successfully applied to our system.[50] Our group had improved our past synthesis of **94** from a 7 step, 6% synthesis, to a more efficient 5 step, 42% route. [49]



Scheme 22. Reverse prenylation to give the desired indole. [49]

Soon after this discovery, Baran and co-workers published their results in the total synthesis of stephacidin A (Scheme 23).[33] They also noted the difficulties they had in producing a 6-hydroxytrptophan derivative. They found after much work and optimization, that indole **74** was obtainable in great yields when the electron withdrawing tosyl group protected the hydroxyl moiety. Interestingly, they used the same *o*-haloenamine route that had failed in my attempts with the electron donating substrate **110** in Scheme 20.

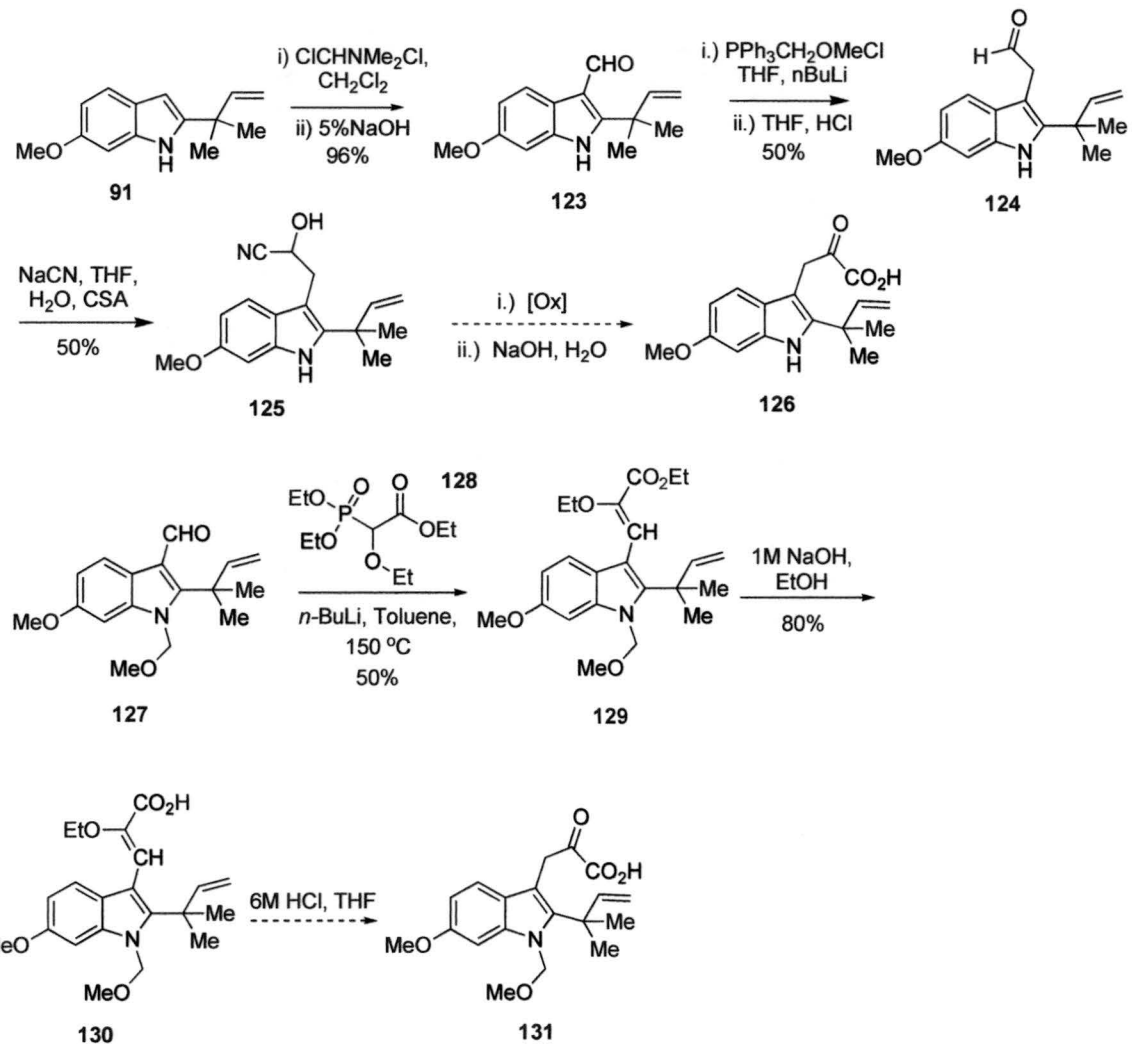


Scheme 23. Baran's synthesis of the 6-hydroxy-tryptophan derivative.[33]

2.3 Attempts to obtain the α-ketoacid

As the original retrosynthetic analysis depicted, the α-ketoacid **126** or **131** was desired for peptide coupling with prolinamide (Scheme 24). The indole **91** was converted to the aldehyde **123** in near quantitative yield by reaction with the Vilsmeier Reagent.[51] This aldehyde then undergoes a Wittig reaction with (methoxymethyl)triphenylphosphorane to yield the vinyl ether which is then converted to the aldehyde **124** by refluxing in THF and HCl.[52] The best yield obtained over this two-step sequence is 50%, along with recoverable starting material, due to the electron rich nature of the aldehyde **123**. The aldehyde **124** was then converted to the cyanohydrin **125** by reaction with NaCN and d-10-camphorsulfonic acid (CSA) in THF and water. This yield was optimized from 31% yield with KCN in AcOH/MeOH.[53] Despite the order of the next two reactions, oxidation to the acyl cyanide followed by hydrolysis of the cyano group to the carboxylic acid, or visa versa, decomposition occurred and the α-ketoacid indole **126** could not be obtained. Alternatively, MOM protection of indole **123** to give the more reactive aldehyde **127** allowed the Horner-Wadsworth-Emmons reaction

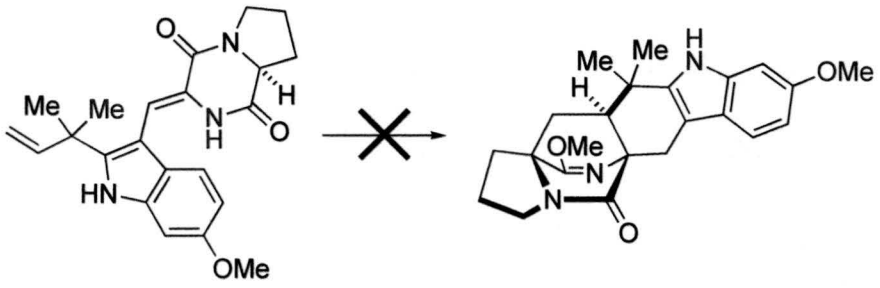
with the synthesized reagent **128** to give the vinyl ether **129** in 50% yield, leaving the remainder recoverable starting material.[54] When this reaction was done with the unprotected indole **123**, the yield was significantly lower (12%). Hydrolysis of the ester to the acid furnished the acid **130** in good yields, yet acidic transformation of the vinyl ether to the α-ketoacid **131** led to decomposition. The α-ketoacid appears to be unstable and unable to be synthesized.



Scheme 24. Attempts to synthesize the α-ketoacid.

2.4 A revised Diels-Alder precursor

Past experiments in our group have been unsuccessful in effecting the IMDA with 6-hydroxyindole derivatives in a similar fashion to the biomimetic brevianamide or VM55599 syntheses (Scheme 25). It was found through D_2O studies that **132** would not tautomerize to the azadiene needed for the intramolecular Diels-Alder reaction to take place (Unpublished results from Rhona Cox).

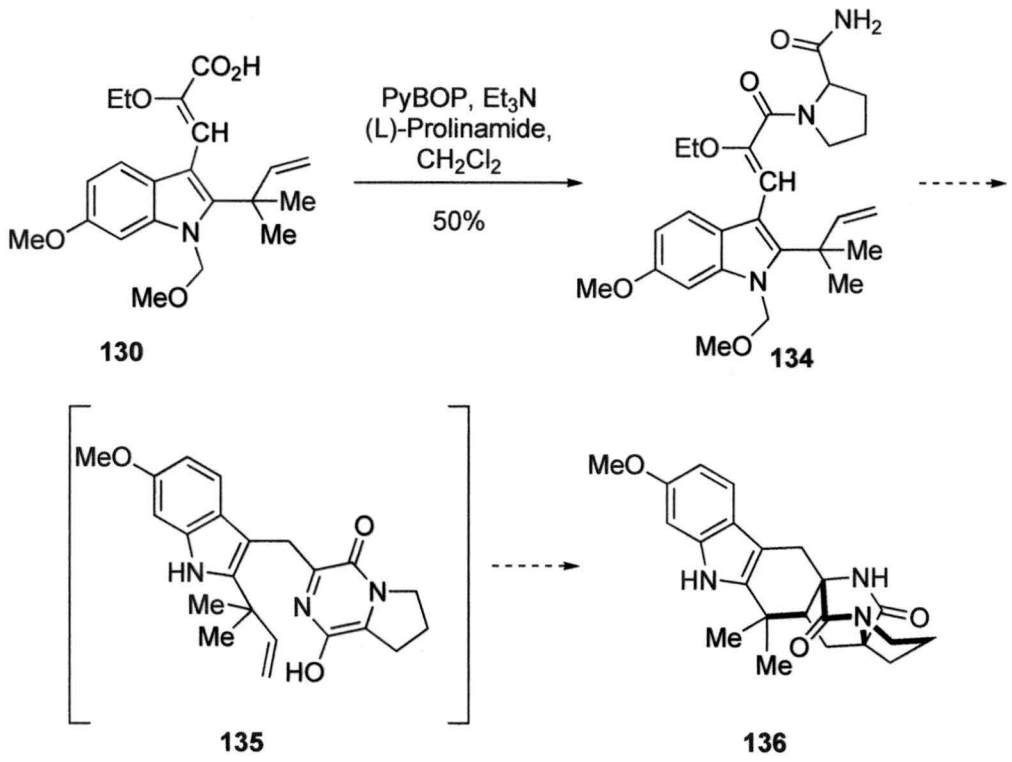


132 **133**

Scheme 25. Early experiments with 6-hydroxyindole Diels-Alder precursors.

After screening a few different coupling conditions, it was found that (L)-prolinamide would couple with the acid **130** in an optimized yield of 50% with PyBOP coupling agent (Scheme 26). The coupled product **134** provides an exciting new Diels-Alder precursor. It is envisioned that under one-pot acidic conditions, sequential N-MOM deprotection, vinyl-ether conversion into the ketone, condensation with prolinamide to form an internally unsaturated form of diketopiperazine, followed by enolization to provide the azadiene **135** would allow the Diels-Alder to occur to give **136**. This one-pot procedure may avoid the external olefin formation in **132**, which has been shown to be thermodynamically more favorable, and perhaps push the reaction in favor of an IMDA reaction. Unfortunately, due to the timing of this progress coming at a shift of research goals, I never was able to test different conditions to see if this Diels-Alder reaction would take place. Instead, my time was diverted to the synthesis of

brevianamide B, *epi*-malbrancheamide, and *epi*-stephacidin derivatives that will be discussed in the next two chapters.

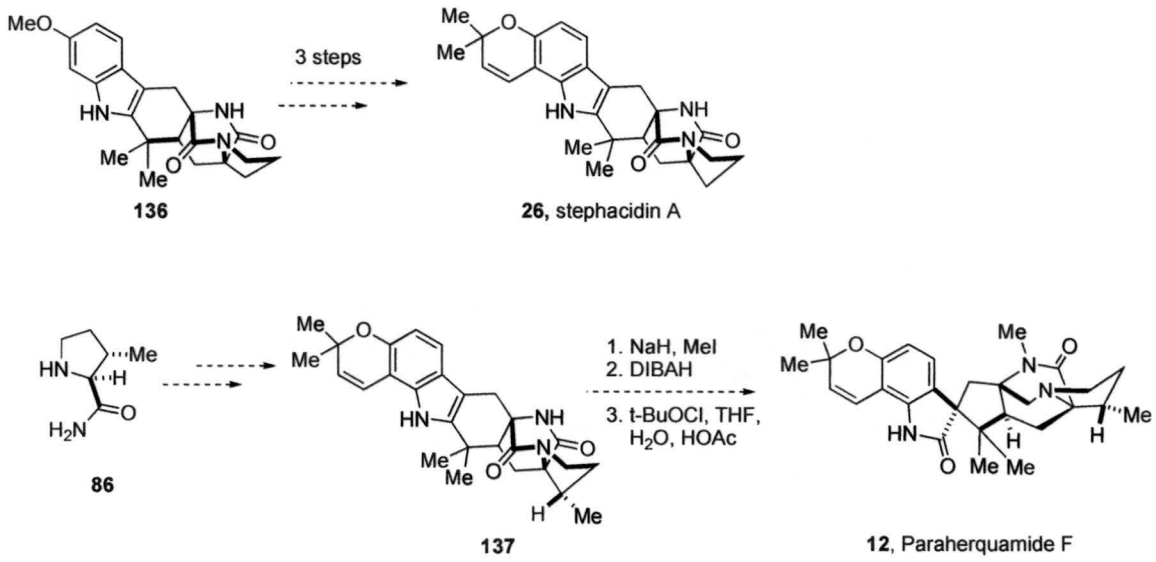


Scheme 26. An alternative approach to the Diels Alder reaction.

2.5 Future Investigations

If the Diels-Alder reaction would prove successful, then only 3 steps remain to convert the Diels-Alder product **136** into stephacidin A, simply by demethylation and installation of the pyran (Scheme 27). This synthesis would be convergent for the synthesis of paraherquamide F, by simple replacing the coupling of prolinamide with β-methyl prolinamide (**86**) in the reaction of **130** to **134** (Scheme 26). The same sequence of events already discussed would lead to the β-methyl proline derivative of stephacidin

A (**137**), which could be converted into the *spiro*-oxindole moiety of paraherquamide F by chemistry already developed in our group.[55]



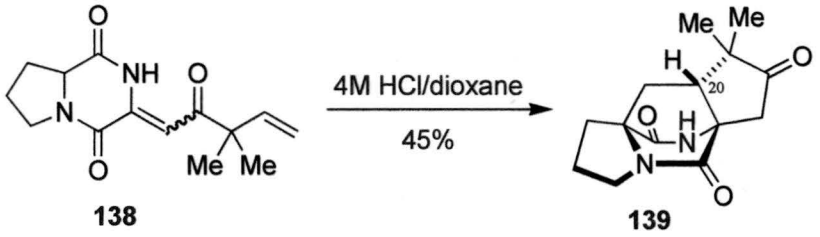
Scheme 27. Future work to complete the synthesis of stephacidin A and paraherquamide F.

Chapter 3

A concise synthesis of *d,l*-brevianamide B via a biomimetically-inspired IMDA construction

3.1 Past synthesis of the *anti-spiro-5* system of the bicyclo[2.2.2]diazaoctane core by the IMDA reaction

Our group recently reported an IMDA reaction of azadiene progenitor **138** that formed the *spiro*-fused 5-membered ring species **139** exclusively as the *anti*-diastereomer at C20 (Scheme 28).[56]



138 **139**

Scheme 28. Formation of the *anti-spiro*-5 system of the bicylo[2.2.2] by the Diels-Alder reaction. [56]

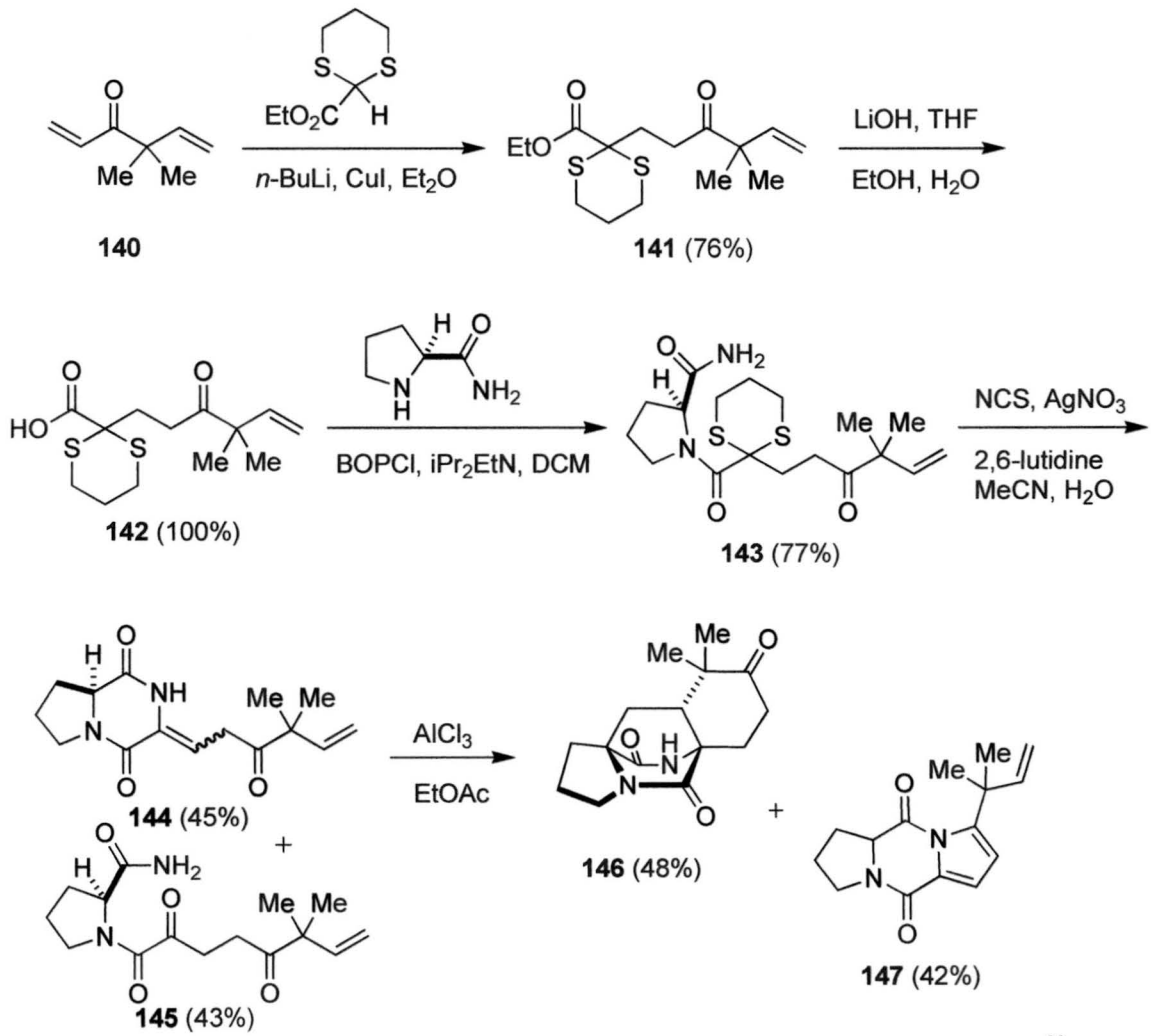
Somewhat conflicting theoretical calculations[57, 58] and experimental results[28, 29, 59] in various systems to be discussed below, motivated us to further explore this approach to construct a *spiro*-fused 6-membered ring system, and to evaluate the intrinsic facial bias of the IMDA reaction of these azadiene species. We had envisioned that by applying this

system to create a 6-membered ring, the Diels-Alder reaction may proceed *syn*-selectively, to give the bicyclic core related to the paraherquamides and stephacidins.

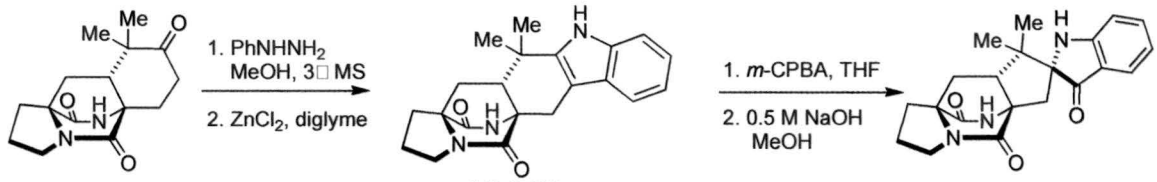
3.2 Synthesis of the *anti-spiro-6* system of the bicyclo[2.2.2]diazaoctane core by the IMDA reaction

As shown in Scheme 29, the synthesis commenced with the known ketone **140**,[60, 61] which was subjected to conjugate addition of ethyl 1,3-dithiane-2-carboxylate to provide the ester **141** in 76% yield.[62] Basic hydrolysis of the ester to the acid **142** was followed by optimized peptide coupling with (L)- prolinamide using BOPCl, to provide the protected peptide **143** in 77% yield. Oxidative deprotection of the dithiane **143** gave a mixture of diketopiperazine **144** (45%) and the uncyclized amide **145** (43%).[63] Noteworthy to mention is that the timing of the dithiane deprotection in the synthetic route was critical. Dithiane deprotection earlier in the synthesis failed to produce the presumable unstable α-keto acid or ester. Both the separable diketopiperazine **144** and the uncyclized amide **145** underwent the intramolecular Diels-Alder reaction when subjected to 3 equivalents of $AlCl_3$ in refluxing EtOAc for 24 hours, yielding the desired product **146** in 48% yield, along with the pyrrole by-product **147** in 42% yield. This Diels-Alder reaction gave a single diastereomer of **146** in the *anti*-configuration. The stereochemistry of this product could then be undoubtedly determined by converting it to *d,l*-brevianamide B (**2**, Scheme 30).[64, 65] The cyclic ketone **146** was converted into the corresponding phenyl hydrazone that without purification, was rearranged to the indole **148** by the Fischer indole reaction in an overall yield of 58%. This substance proved to be

identical to a species previously prepared in our laboratory.[27, 28, 64, 65] Using conditions previously deployed, the 2,3-disubstituted indole **148** was stereoselectively oxidized to the corresponding 3-hydroxyindolenine, which suffered pinacol-type rearrangement under basic conditions to provide racemic brevianamide B (**2**).[64, 65] Thus, brevianamide B was obtained in nine concise steps from the known ketone **140** (twelve steps from commercially available materials), and was identical by ^{1}H NMR, ^{13}C NMR, IR, and TLC to an authentic sample of brevianamide B obtained from *Penicillium brevicompactum*.[66]



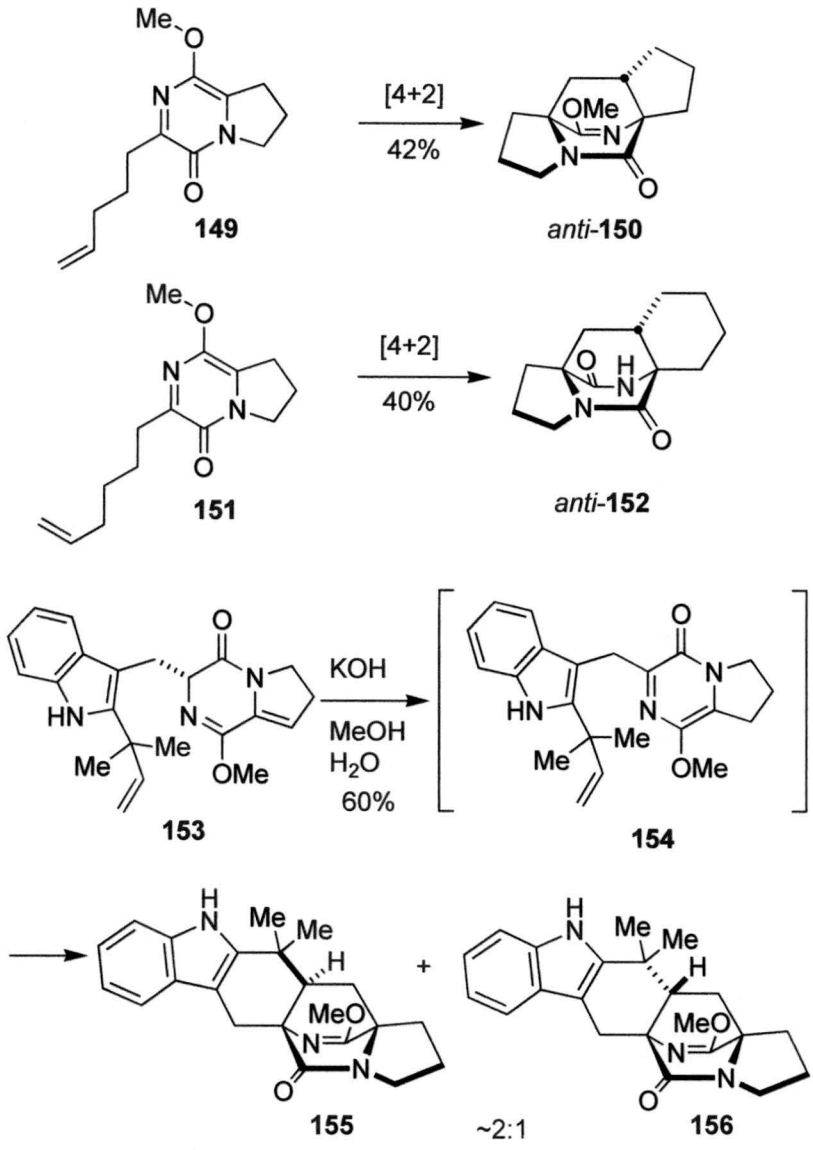
Scheme 29. IMDA reaction of **144/145** to provide a single diastereomer of **146**.[66]



146 148 (58%) 2, *d,l*-brevianamide B

Scheme 30. The total synthesis of brevianamide B. [66]

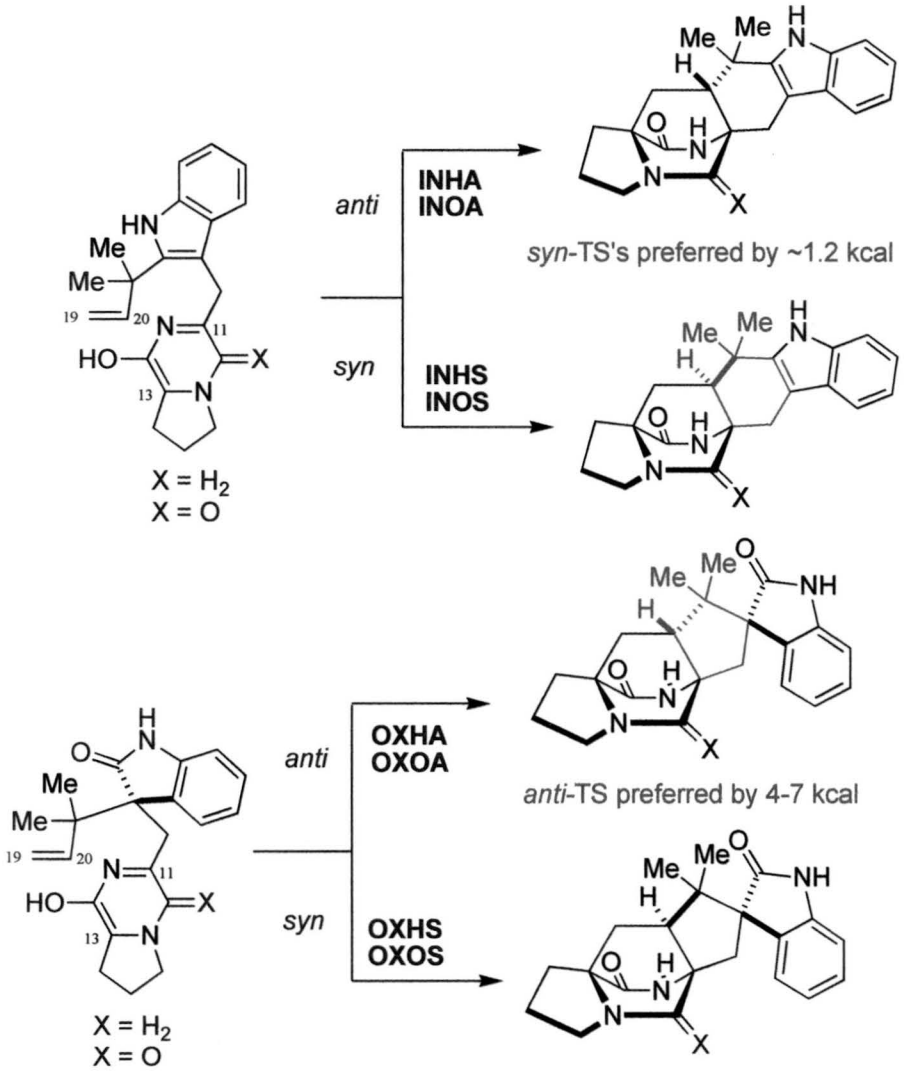
The *anti*-stereoselectivity of the IMDA reaction of **139** and **146** is consistent with the simple model systems **149** and **151** which produced the corresponding *spiro*-5 (**150**) and *spiro*-6 (**152**) cycloadducts, respectively, where in both instances a single diastereomer was formed with the *anti*-configuration at the C-20 position (brevianamide numbering, Scheme 31).[26] However, these systems are in stark contrast to the IMDA reaction we reported *via* azadiene species **153** that led (through the observable and isolable entity **154**) to the cycloadducts **155** and **156** where a ~2:1 *syn : anti* selectivity was observed.[27, 28] Comparison of **138** to **149** and **144** to **151** reveal that the *gem*-dimethyl group does not significantly affect the intrinsic *anti*-bias of these systems. Substrates **138** and **144**, which each contain the single trigonal carbon atom of the ketone function, retain the *anti*-selectivity bias in the IMDA reaction of the derived azadiene tautomers. Thus, the fused 2,3-disubstituted indole moiety in the tethering dienophile chain of **154**, which contains two trigonal atoms as opposed to the more saturated chain of atoms in **138**, **144**, **149** and **151**, reveals that the modest *syn*-bias of the former substrate is likely governed by the additional conformational rigidity imposed by the indole nucleus relative to the more saturated counterparts.



Scheme 31. Synthetic model systems. [26, 27, 28]

Ab initio calculations previously reported on the 2,3-disubstituted indole and oxindole substrates, predict a modest *syn*-selective bias for the 2,3-disubstituted indole species of ~1.2 kcal/mol furnishing the *spiro*-6 products, whereas the oxindole substrates that yield the *spiro*-5 products are substantially favored by 4~7 kcal/mol for the *anti*-stereochemistry (Scheme 32).[57, 58] Future work involving the preparation of additional substrates to examine other structural and electronic parameters of the azadiene, the

dienophile and the tethering chain of atoms, would help to build a deeper understanding of the subtle ground-state conformational and transition state energies that govern the important diastereoselectivity of these reactions which may have significant biogenetic implications.

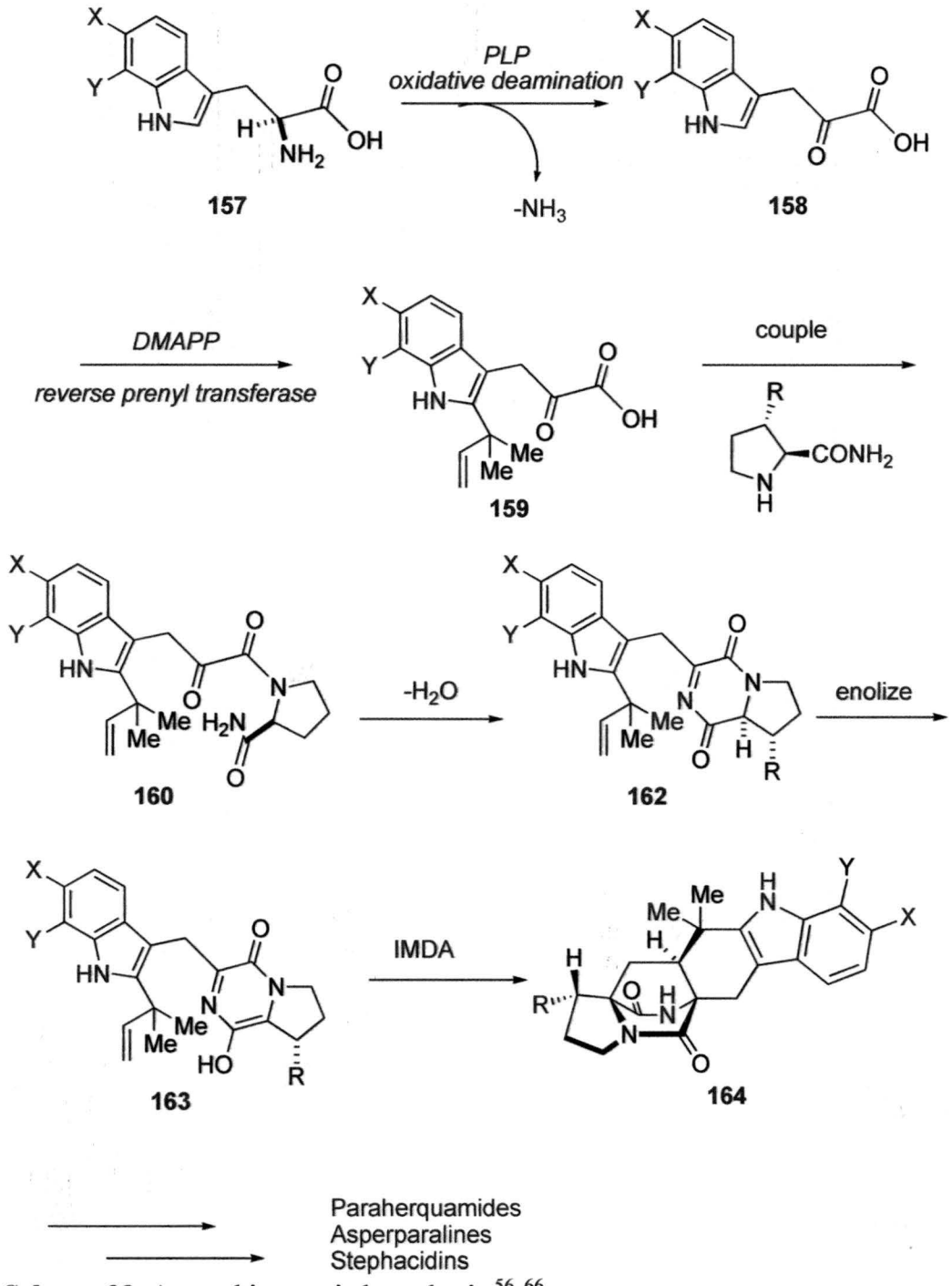


Scheme 32. *Ab initio* calculations on the *spiro*-5 and *spiro*-6 modes of IMDA cycloaddition.[58]

3.3 **Conclusions**

This concise and convergent synthesis of *d,l*-brevianamide B *via* an IMDA construction provides an alternative biogenetic proposal in the construction of the bicyclo[2.2.2] core of this unique family of alkaloids (Scheme 33).[56, 66] We have proposed that oxidative deamination of tryptophan and reverse prenylation would yield the α-keto acid **159**. Condensation of the appropriate proline amide derivative would result in a spontaneous cascade of cyclodehydration (**162**), tautomerization (**163**) and intramolecular [4+2] cycloaddition to afford the key hexacyclic substances **164** that is characteristic to each natural product in this family.

The tricyclic ketone substrate **146**, has proven to be a versatile intermediate from which a host of brevianamide, C20-*epi*-malbrancheamide and C20-*epi*-stephacidin analogs might be readily constructed using Fisher indole and related methodologies, which is discussed in the next chapter.



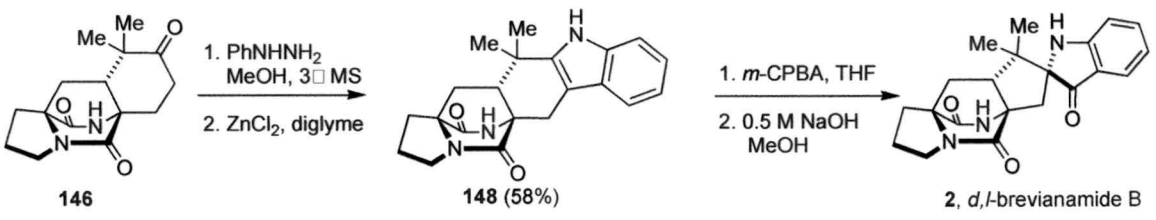
Scheme 33. A new biogenetic hypothesis.[56, 66]

Chapter 4

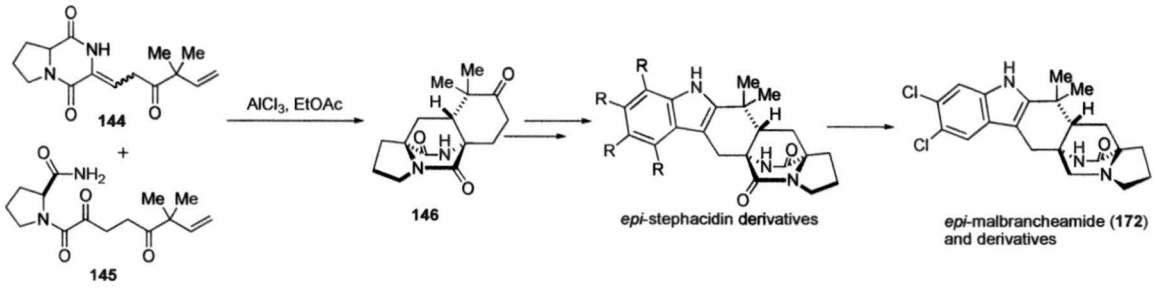
A concise and versatile synthesis of *epi*-malbrancheamide and structurally related analogs

4.1 Introduction

Recently, we have reported a concise synthesis of brevianamide B (**2**) through a key diastereoselective Diels-Alder reaction that establishes the characteristic bicyclo[2.2.2]diazaoctane core of **9** as the *anti*-diastereomer (Scheme 34).[66] This tricyclic ketone **146** was converted to *d,l*-brevianamide B through a Fischer indole reaction with phenyl hydrazine. The 2,3-disubstituted indole **148** has the same structural core as cytotoxic stephacidin A, yet is diastereomeric at the bicyclo[2.2.2]diazaoctane core. We have thus envisioned using this convergent route to access *epi*-malbrancheamide and *epi*-stephacidin-like derivatives that might prove to be possess biological activities (Scheme 35).



Scheme 34. The concise synthesis of brevianamide B (**2**). [66]



Scheme 35. Access to *epi*-stephacidin A derivatives, *epi*-malbrancheamide and derivatives.

4.2 Synthesis of *epi*-stephacidin A derivatives

The Fischer indole reaction was carried out with the hydrazones formed from the condensation of the ketone **146** and various commercially available substituted hydrazines (with the exception of 3-methoxyphenylhydrazine, which was made from 3-methoxyanaline)[67] (Table 3). All of these reactions were lower yielding than the reaction that formed **148** from **146** with phenyl hydrazine. From these various Fischer indole reactions, some interesting trends in substitution patterns were observed and are discussed below.

The methoxy-substituted hydrazines were chosen to make indole derivatives that resemble the oxygen substitution of stephacidin A. Indole **166** contains the corresponding 6-oxo substitution pattern of stephacidin, however it was much more difficult to obtain than indoles **165** and **168**. In general, it was observed that the electron donating groups in the *ortho* and *para* position gave higher yielding reactions than the *meta*-methoxyphenylhydrazone. Interestingly, when 3-methoxyphenylhydrazine was exchanged for the electron withdrawing 1-(3-triflouromethoxy)phenylhydrazine, the Fischer reaction failed altogether. The major difficulty in obtaining **166** was due to the difficulty in separating the two regioisomers formed in the reaction (**166** and **167**). The

same difficulty was observed in the isolation of indoles **170** and **171**. In both cases, the products were isolated only after tedious and repetitive preparative thin layer chromatography separations in very specific solvent elution conditions. While the 6-methoxyindole **166** could be obtained in pure form in only 7% yield, the regioisomer **167** was never isolated from the mixture.

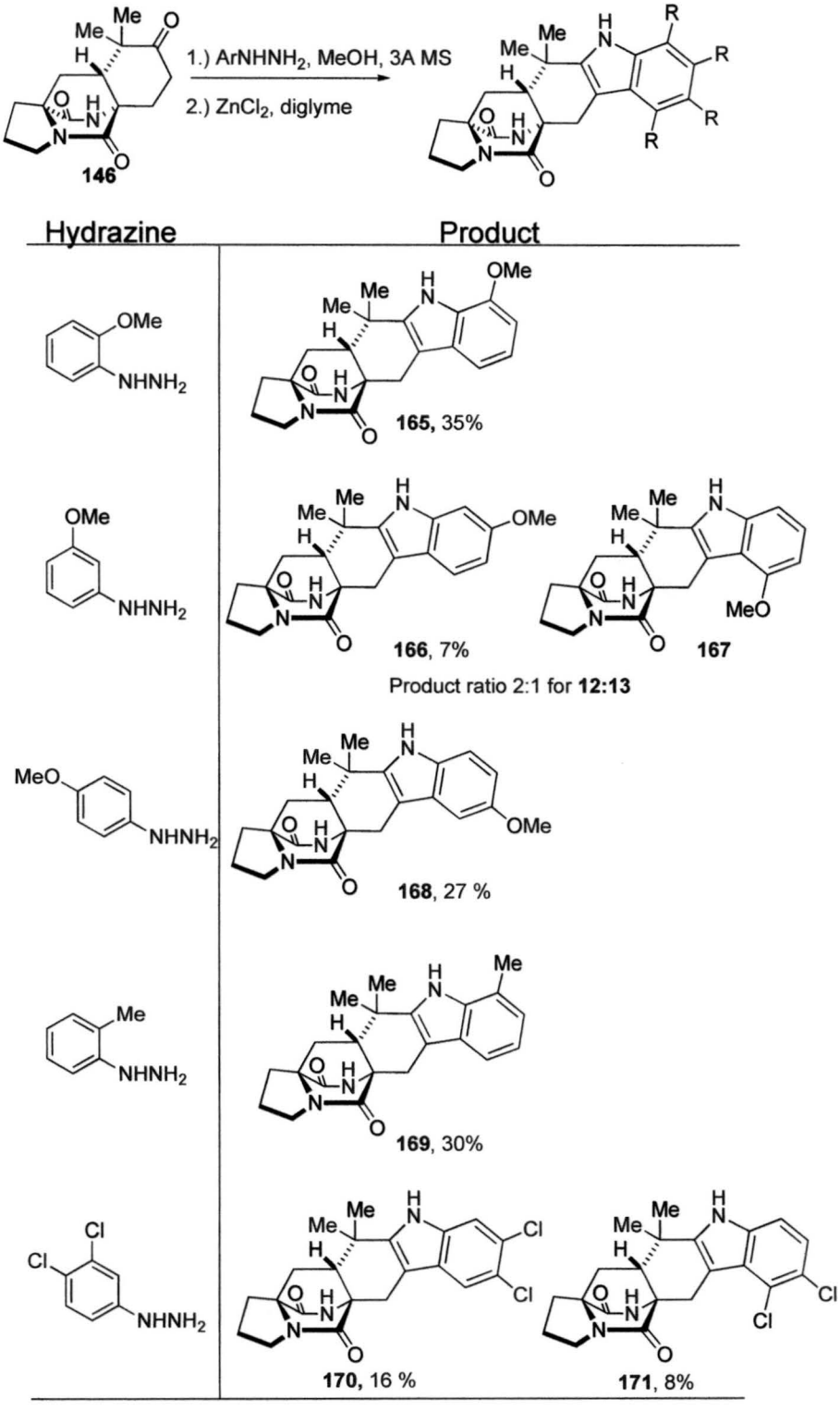
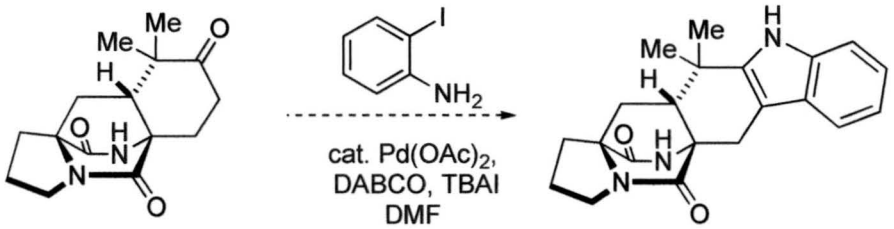


Table 3. Synthesis of *epi*-stephacidin A derivatives.[68]

The 1-(2-methylphenyl)hydrazine was chosen as the less polar alternative to the methoxy derivatives. The desired indole **169** was obtained in 30% yield. When 1-(2-chlorophenyl)hydrazine was used instead, the condensation with ketone **146** did not occur. The bulky chlorine on the hydrazine and the *geminal* dimethyl group *alpha* to the ketone presumably sterically hindered the condensation. In fact, the sterically encumbered ketone **146** had other limitations. Due to the initial shortcomings with the separation of the regioisomers formed from the Fischer reaction, the regioselective palladium-catalyzed annulation of *o*-iodoanaline and the ketone **146** was investigated (Scheme 36).[46] However, the sterically hindered ketone failed to react with the analine, despite the various conditions and modifications employed.



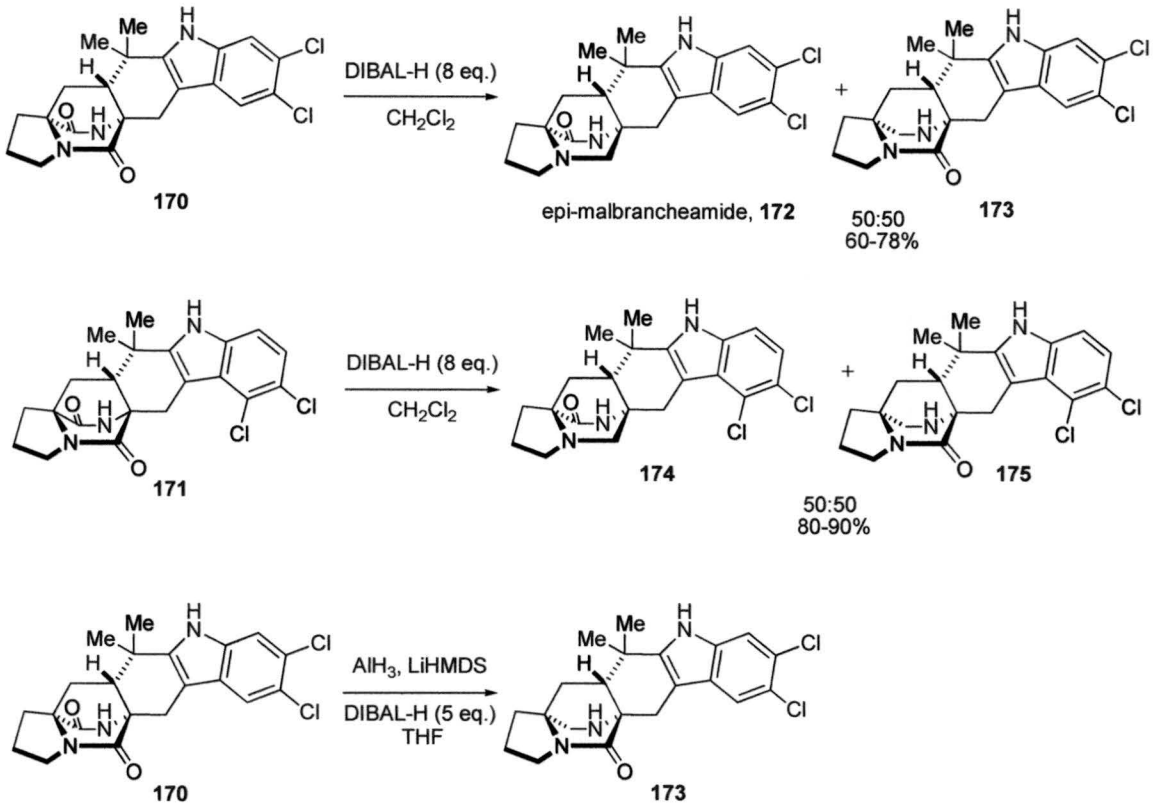
146 **148**

Scheme 36. Attempted Pd-catalyzed annulation.

4.3 Synthesis of *epi*-malbrancheamide and derivatives

Once the two dichloroindole regioisomers **170** and **171** were separated from their mixture, the final reduction of the tertiary lactam was needed to give C-19-*epi*-malbrancheamide (**172**) and its regioisomer derivatives. In the synthesis of paraherquamide A the tertiary lactam could be selectively reduced in the presence of the secondary lactam by treatment with excess DIBAL-H.[69] The reduction of either

regioisomer **170** or **171** with DIBAL-H gave a 50:50 mixture of both the tertiary reduced lactam (**172** and **174**) and the secondary reduced lactam (**173** and **175**) in good yields (Scheme 37). While the secondary reduced lactam derivatives are intriguing compounds for biological activity studies, we were interested to see if pre-complexation with $AlEt_3$ would change the selectivity of the reduction. In our laboratory's earlier synthesis of paraherquamide B, selective reduction of the tertiary lactam was achieved by pre-complexation of the secondary lactam with $AlEt_3$.[70] Interestingly, this reaction with the *anti*-diastereomer **170** led to the opposite selectivity observed with paraherquamide B, which has the same *syn* orientation as the stephacidins and malbrancheamide. In the present case, only the secondary lactam was reduced to give **173**, and no *epi*-malbrancheamide product (**172**) was observed. Examination of models of the two C-19 diastereomers reveals a plausible reason for the observed selectivities. The *syn*-diastereomer is twisted so that the *gem*-dimethyl groups effectively block one face of the tertiary amide, thus leaving the secondary amide more exposed for coordination with alane. In contrast, the *anti*-diastereomer's *gem*-dimethyl groups encumber the secondary amide, thereby leaving the tertiary amide free for complexation. These results could have biosynthetic implications. All of the natural products in this family with the tertiary reduced lactam have the *syn*-diastereomeric relationship. They include the paraherquamides (**7-14**), marcfortines (**20-22**), asperparalines (**15-18**), VM55599 (**19**), and malbrancheamide (**31**). Since secondary lactam reduction has never been observed in these natural products, it is feasible to believe that the more exposed secondary lactam is coordinated or anchored to the enzyme active site, thus allowing selective reduction of the tertiary lactam.



Scheme 37. Reduction of the lactam and synthesis of *epi*-malbrancheamide (**172**).[68]

4.4 Biological testing

Very recently, Baran published a full acount on his group's synthesis of stephacidin A, avrainvillamide and stephacidin B.[71] Herein they report the first analogues made of these natural products which were tested against human colon HCT-116 cell lines (Table 4).[71] The benzopyran ring appears to only have significant effects for cancer activity with stephacidin A (**26**), since the unsubstituted version **176** had no significant activity. However, in the higher oxidized analogs of **176**, activity appears to be restored (compounds **177, 178,** and **179**) where they even have better activity than stephacidin A. Interestingly, (±)-**179** has about half the activity as (+)-avrainvillamide, which probably is due to the fact that **179** is racemic. If this is the case, then only one enantiomorph is the active species. Baran proposed that the observed bioactivity of these compounds is attributable to the electrophilic α,β-unsaturated nitrone moiety of avrainvillamide. This functionality has already been shown to be an excellent Michael acceptor by Myers and Herzon.[32] Baran proposed that avrainvillamide may function by a selective protein alkylation event through a 1,5-addition.[71]

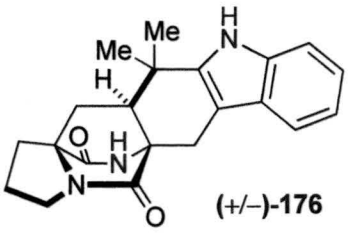
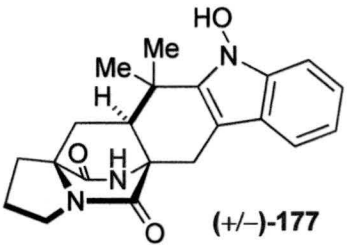
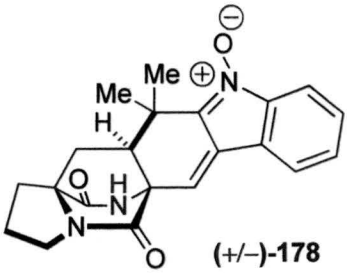
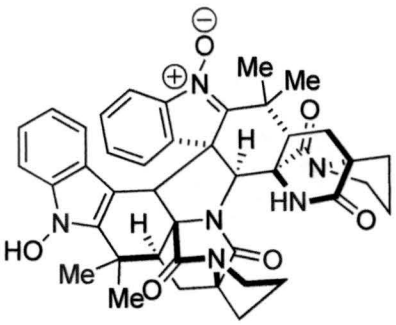
Substrate	Activity (μg/mL)
 **(+/–)-176**	no significant activity
 **(+/–)-177**	9.36
 **(+/–)-178**	5.47
 **(+/–)-179**	3.95
(+)-**26**: stephacidin A	10.4
(-)-**27**: stephacidin B	0.41
(+)-**28**: avrainvillamide	2.0

Table 4. Analogues made by Baran and co-workers (Activity = IC_{50} against human colon HCT-116 cell lines).

The analogs that we prepared are very interesting candidates for biological activity testing. By comparing our biological data to that of Barans', we may probe the significance of the substitution pattern on the indole ring as well as the stereochemistry around the bicyclic core. We sent the synthesized compounds to two different locations for biological testing. The *epi*-stephacidin derivatives (**165, 166, 167, 168, 169, 170** and **171**), and the *epi*-malbrancheamide derivatives (**172, 173, 174** and **175**), as well as synthetic brevianamide B (**2**) were sent to the University of Colorado at Denver and Health Science Center (UCDHSC) School of Pharmacy for testing against human prostate carcinoma PC-3 and 22rv 1 cells. *Epi*-malbrancheamide and derivatives (**172-175**) were sent to Rachel Mata's laboratory at the Universidad Nacional Autonoma de Mexico, to be tested with the same PDE1 assay that was used to examine malbrancheamide's competitive antagonistic effects of CaM.

By the time of this thesis submission, only the biological activity data from the prostate carcinoma cell lines were determined (Table 5). While the compounds have rather modest activity in general, some interesting results were obtained. The best activity was displayed from compound **165**. After 48 hours, **165** had more potency with PC-3 prostate carcinoma cell lines (IC_{50} of 5.91 μg/mL, Table 5) than stephacidin A had with HCT-116 colon carcinoma cell lines (IC_{50} of 10.4 μg/mL, Table 4). The 7-substitution pattern of this methoxy derivative appears to be essential, since **166** and **168** are significantly less active. The next best compound of these "stephacidin A" derivatives is the 7-methyl derivative **169**, which supports the hypothesis that 7-substitution on the indole ring provides the more potent of derivatives. Interestingly, **169** differs from **165** by displaying better selectivity for the 22rv 1 cells. Examination of the

"malbrancheamide" derivatives reveals *epi*-malbrancheamide (**172**) as the most active of the four compounds against PC-3 cell lines (IC_{50} of 18.95 μg/mL after 48 hours). It appears that the reduction of the tertiary amide of **170** to give **172** makes a more active species, while the reduction of the secondary amide to give **173** worsens the activity compared to **170**.

Compound	IC_{50} in ug/mL (12h)		IC_{50} in ug/mL (24h)		IC_{50} in ug/mL (48h)	
	PC-3 Cells	22rv1 Cells	PC-3 Cells	22rv1 Cells	PC-3 Cells	22rv1 Cells
(+/-)-165	25.46	N.D.	17.16	52.30	5.91	35.38
(+/-)-166	191.47	49.63	N.D.	93.58	67.52	75.58
(+/-)-168	N.D.	54.06	30.72	57.11	29.07	65.63
(+/-)-169	53.50	35.85	71.95	40.26	49.53	18.63
(+/-)-2: brevianamide B	N.D.	N.D.	165.26	1109.26	72.88	148.65
(+/-)-170	61.71	54.91	33.43	55.29	29.09	19.79
(+/-)-171	N.D.	38.81	90.50	49.58	77.87	28.42
(+/-)-172: *epi*-malbrancheamide	54.47	308.94	42.95	50.68	18.95	35.00
(+/-)-173	102.87	N.D.	N.D.	123.30	43.04	52.47
(+/-)-174	N.D.	N.D.	N.D.	N.D.	109.95	43.04
(+/-)-175	115.97	58.46	86.68	132.78	65.65	138.36

Table 5. Biological activity of the synthesized compounds against human prostate PC-3 and 22rv 1 cell lines.

While it is difficult to make any strong conclusions about the effect of the stereochemistry around the bicyclic core on the biological activity of these compounds, it is worthwhile to note that Baran's derivative of stephacidin A (**176**) had no reported significant activity, and our derivative **165**, showed very good activity towards PC-3 prostate carcinoma cell lines. From these results, it appears that the diastereomers that we have synthesized are potentially relevant pharmaceutical targets. Future work that should bear interesting results would be to oxidize the analogs **165-175** to the electrophilic α,β-unsaturated nitrone moiety of avrainvillamide, in order to test Baran and Myers assumptions that these compounds are the more active species.

4.5. Conclusions

We have demonstrated that the versatile ketone **146**, formed from a diastereoselective IMDA reaction, can be utilized to prepare *d,l*-brevianamide B, *epi*-malbrancheamide, and several malbrancheamide or stephacidin A analogs by deployment of the Fischer indole synthesis. The concise and convergent nature of the approach that we have employed provides ready access to these hexacyclic substances in a straightforward manner, which should allow a multitude of pharmaceutical targets to be easily prepared.

Chapter 5

Experimental

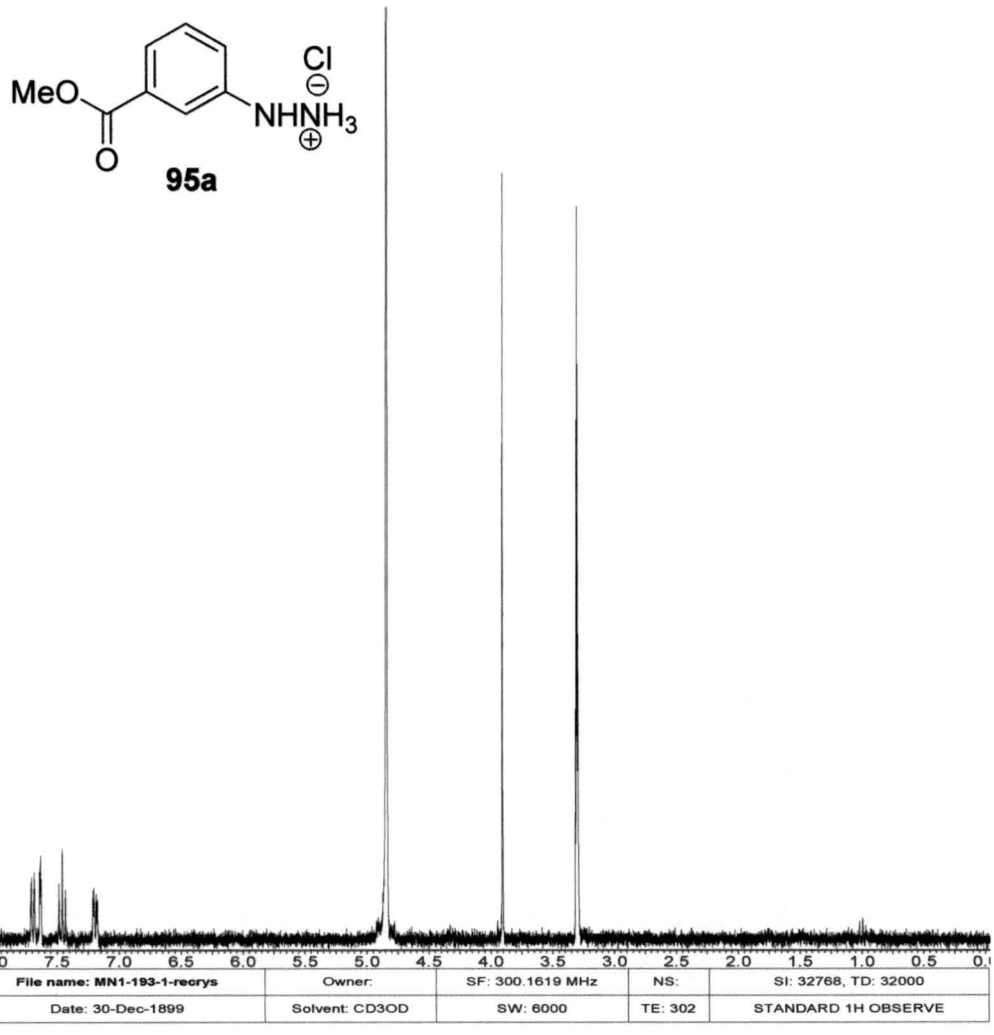
5.1 General synthetic considerations

Commercially available reagents were used as received without further purification. Thin layer chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm, or developed with either vanillan or potassium permanganate solutions by heating with a hot-air gun. Specified products were purified by flash column chromatography using silica gel 60 (230-400 mesh, Merck). IR absorptions on NaCl plates were run on a Perkin Elmer FT-IR 1600. ^{1}H NMR spectral data was obtained using Varian 300, 400 or 500 MHz instruments. ^{13}C NMR spectral data was obtained using a Varian 100 or 125 MHz spectrometer. Mass spectra were obtained at Colorado State University's Central Instrument Facility. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ 7.27 (^{1}H NMR) and δ 77.23 (^{13}C NMR). Chemical shifts are reported in ppm relative to CD_3OD at δ 3.31 (^{1}H NMR) and δ 49.15 (^{13}C NMR). For all NMR spectra, δ values are given in ppm and *J* values in Hz.

5.2 Chemical Synthesis Experimentals for Stephacidin Research (Chapter 2)

HCl salt of methyl 3-hydrazinylbenzoate (95a): Methylaminobezoate (1g, 6.62 mmol) was brought in solution with 2N HCl (13.5 mL) and cooled to -10°C. $NaNO_2$ (479.5 mg, 6.95 mmol) in 2 mL of H_2O was added drop wise over 30 minutes, and then stirred for a further 30 minutes at -10°C-0°C. $SnCl_2$-$2H_2O$ (3.73 g, 16.54 mmol) was dissolved in 15 mL of concentrated HCl and added drop wise over 30 minutes. After one hour of stirring at -10°C-0°C, the mixture was filtered and washed with n-butanol. Most of the n-butanol was distilled off, and the oily residue that remained was stirred into dichloromethane, which induced crystallization. The white crystals were collected by filtration and washed with dichloromethane to give **95a** (300 mg, 1.48 mmol) in a 22% yield. ^{1}H NMR (300 MHz, CD_3OD) 3.93 (3H, s), 7.22 (1H dd, J = 8.7, 2.4 Hz), 7.66 (1H, s), 7.72 (1H, dd, J = 8.1, 1.5 Hz). LRMS (FAB+): Calc. for $C_8H_{10}N_2O_2$: 166.07423. Found: 166.04 (M^+, 100 %), 167.04 (MH^+, 25 %).

Methyl 3-(2-(3,3-dimethylpent-4-en-2-ylidene)hydrazinyl)benzoate (96): 95a (25 mg, 0.123 mmol) was dissolved in 1M NaOH and extracted with ethyl acetate. The free hydrazine (10 mg, 0.0602 mmol) was then brought up in 5 mL of dry toluene and 3,3-dimethylpent-4-en-2-one (7 mg, 0.0602 mmol) was added. The mixture was fitted with a Dean-Stark trap and brought to a reflux for 2 hours. The solvent was removed *in vacou* and the crude oil was placed in a desiccator over P_2O_5 under high vacuum for 3 days.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NHNH3
95a
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SI: 32768, TD: 32000
Date: 30-Dec-1899
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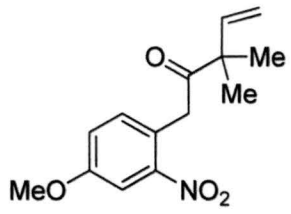
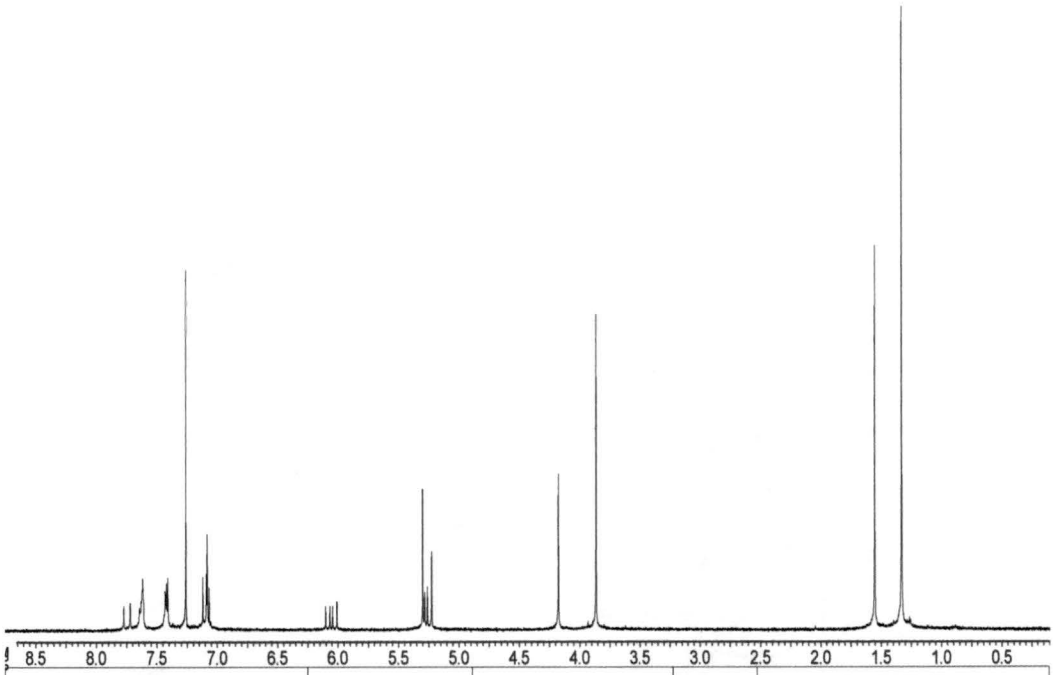
SW: 6000
TE: 302
STANDARD 1H OBSERVE

1-(4-methoxy-2-nitrophenyl)-3,3-dimethylpent-4-en-2-one (98): In a flame-dried round bottom flask fitted with a stir bar, 4-bromo-3-nitroanisole (500 mg, 2.15 mmol), 4-methoxyphenol (54 mg, 0.86 mmol), and ground K_3PO_4 (1.15 g, 5.38 mmol) were added and the flask was purged with argon and evacuated three times. The argon filled flask was transferred to a glove box with argon atmosphere, where $Pd_2(dba)_3$ (20 mg, 0.0215 mmol) and 2-dichlorohexyl-phosphino-2-dimethyl amino biphenyl (34 mg, 0.0863 mmol) were added. The flask was brought back to the bench and evacuated and purged with Ar three more times. Dry toluene (20 mL) was added, followed by 3,3-dimethylpent-4-en-2-one (535 mg, 4.74 mmol). The flask was fitted with a flame-dried condenser, and the mixture was brought to 50-60°C for 24 hours under Ar. After allowing the reaction to cool to room temperature, the mixture was extracted between ethyl acetate (15 mL) and H_2O (15 mL). The layers were separated and the organic layer was washed with brine (20 mL). After drying the organics over Na_2SO_4, the solution was concentrated *in vacou* to give the crude product as a brown oil. The product (R_f = 0.1) was purified by column chromatography using 3:2 CH_2Cl_2: Hexanes for elution to give 63 mg (0.24 mmol, 11% yield) of **98** as a orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.34 (6H, s), 3.87 (3H, s), 4.19 (2H, s), 5.24-5.31 (2H, m), 6.07 (1H dd, J = 17.1, 10.5 Hz), 7.09 (1H, m), 7.44 (1H, m), 7.63 (1H, s). HRMS (FAB+): Calc. for $C_{14}H_{17}NO_4$: 263.115758. Found: 264.123660 (MH^+), 263.115343 (M^+).



98



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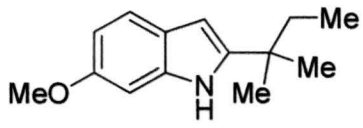
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

6-methoxy- 2-reverse prenyl indole (91) and 6-methoxy-2-tert-pentyl-1H-indole (99):

In a flame-dried round bottom flask fitted with a stir bar, 4-bromo-3-nitroanisole (2 g, 8.61 mmol), 4-methoxyphenol (216 mg, 1.72 mmol), and ground K_3PO_4 (4.57 g, 21.5 mmol) were added and the flask was purged with argon and evacuated three times. The argon filled flask was transferred to a glove box with argon atmosphere, where $Pd_2(dba)_3$ (79 mg, 0.086 mmol) and 2-dichlorohexyl-phosphino-2-dimethyl amino biphenyl (136 mg, 0.345 mmol) were added. The flask was brought back to the bench and evacuated and purged with Ar three more times. Dry toluene (20 mL) was added, followed by 3,3-dimethylpent-4-en-2-one (2.13 g, 18.96 mmol). The flask was fitted with a flame-dried condenser, and the mixture was brought to 50-60°C for 24 hours under Ar. After allowing the reaction to cool to room temperature, the mixture was extracted between ethyl acetate (15 mL) and H_2O (15 mL). The layers were separated and the organic layer was concentrated to a crude oil. The crude aryl ketone intermediate **98** was added to a flask containing 6.6 M aq. NH_4OAc (130 mL), $TiCl_3$ (142 mL of 20% in 3% HCl solution, 142 mmol), and EtOH (43 mL) under Ar. The reaction was stirred for 5 hours and then extracted with ethyl acetate (3x 100 mL). The combined extracts were washed with saturated $NaHCO_3$, followed by brine, and then dried over Na_2SO_4. The solvent was removed and the brown oil was purified by flash chromatography (3:2 CH_2Cl_2: Hexanes) resulting in a yield of 13% of indole **99** and a 5% yield of indole **91**.

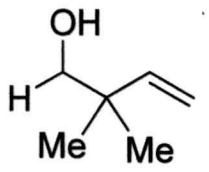
Indole **99**: ^{1}H NMR (300 MHz, $CDCl_3$) 0.79 (3H, t, J = 7.5 Hz) 1.34 (6H, s), 1.66 (2H, q, J = 15.0, 7.5 Hz), 3.87 (3H, s), 6.12 (1H, s), 6.75 (1H, d, J = 8.7 Hz), 6.85 (1H, s), 7.41 (1H, d, J = 8.7 Hz), 7.80 (1H, br s). LRMS (FAB+): Calc. for $C_{14}H_{19}NO$: 217.1467. Found: 217.1685 (M^+, 100 %), 218.1747 (MH^+, 35 %), 219.1783 (MH_2^+, 5 %).



99

2,2-Dimethyl-but-3-enal (105). $LiAlH_4$ (200 mg, 5.3 mmol) and ether (5 mL) were brought to 0°C and the ester **104** (500 mg, 3.5 mmol) was added drop wise. The reaction was stirred for 2 hours and carefully quenched with 0.5 mL water, 0.5 mL of 15 % NaOH, and 1.5 mL of water. The aq. layer was extracted with ether, and the combined organics were washed with saturated aq. NH_4Cl and brine. The solvent was dried with Na_2SO_4 and removed *in vacuo* to yield the alcohol (**105-Intermediate)** as a clear volatile oil in 57% yield (200 mg, 2.0 mmol). ^1H NMR (300 MHz, $CDCl_3$) 1.04 (6H, s) 3.35 (2H, s), 5.05-5.13 (2H, m), 5.79 (1H, dd, J = 17.4, 10.8 Hz).

Oxalyl chloride (9.3 g, 73.4 mmol) and CH_2Cl_2 (25 mL) were brought to -78°C. DMSO (7.6 g, 97.8 mmol) was added drop wise and stirred for 15 minutes. The alcohol (**105-Intermediate)** (2.45 g, 24.5 mmol) was added and the mixture was stirred for 30 minutes. Et_3N (17 mL, 122.3 mmol) was added and stirred for 20 minutes at -78°C, then warmed to room temperature and stirred for 3 hours. The reaction was quenched with water, and extracted with CH_2Cl_2. The combined organics were washed with saturated aq. NH_4Cl and brine. The solvent was dried with Na_2SO_4 and removed *in vacuo* to yield **105** (2.0 g, 20.4 mmol, 83 %) as a volatile oil. ^1H NMR (300 MHz, $CDCl_3$) 1.20 (6H, s) 5.12-5.25 (2H, m), 5.82 (1H, dd, J = 17.4, 10.8 Hz), 9.40 (1H, s).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H
Me
Me

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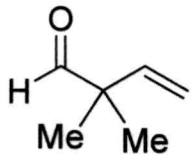
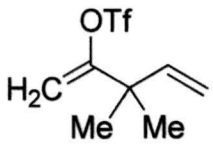
STANDARD 1H OBSERVE


O
H
Me
Me

105

10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0

3,3-dimethylpenta-1,4-dien-2-yl trifluoromethanesulfonate (108). A solution of the ketone (**88**, 230 mg, 2.05 mmol) in THF (25 mL) was brought to -78°C, when KHMDS (0.5 M in toluene, 3.08 mmol, 6.2 mL) was added. After the reaction stirred for 30 minutes, the triflating agent **107** (1.2 g, 3.08 mmol) in THF (10 mL) was added via cannula. The reaction was stirred for 4.5 hours at 0°C, after which time the solution was diluted with Et_2O (30 mL), washed with cold 10% NaOH (50 mL) and brine (50 mL). The organics were dried over Na_2SO_4 and concentrated *in vacuo* to yield 419 mg (1.71 mmol) of an orange oil (84%). ^1H NMR (300 MHz, $CDCl_3$) 1.27 (6H, s) 5.02-5.20 (4H, m), 5.84 (1H, dd, J = 17.4, 10.5 Hz).



108

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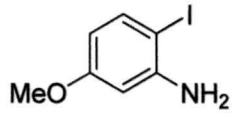
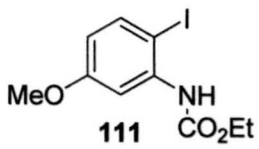
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

2-iodo-5-methoxybenzenamine (110): 4-iodo-3-nitroanisole (1g, 3.58 mmol), $FeCl_3$-$6H_2O$ (48 mg, 0.179 mmol), activated carbon (163 mg) and hydrazine monohydrate (551 mg, 11.0 mmol) were brought into solution with 50 mL of MeOH and brought to reflux for 4.5 hours. The reaction was cooled to RT and the catalysts were filtered off. The filtrate was concentrated to dryness and dissolved in dichloromethane. The organics were washed with water and brine, dried over Na_2SO_4 and concentrated *in vacou*. The product (754 mg, 3.03 mmol, 85% yield) is light sensitive and unstable at RT, so was stored covered in the freezer. ^1H NMR (300 MHz, $CDCl_3$) 3.76 (3H, s), 4.09 (2H, br s), 6.14 (1H, dd, J = 9.0, 3.3 Hz), 6.34 (1H, d, J = 3.0 Hz), 7.49 (1H, d, J = 9.0 Hz). LRMS (FAB+): Calc. for C_7H_8INO: 248.96506. Found: 248.99 (M^+, 100 %), 249.12 (MH^+, 6 %).



110

Ethyl 2-iodo-5-methoxyphenylcarbamate (111): 2-iodo-5-methoxyaniline (100 mg, 0.402 mmol) was brought into solution with pyridine (0.5 mL) and cooled to 0°C. Ethyl chloroformate (61 mg, 0.562 mmol) was added and the solution was stirred for 3.5 hours on the ice bath. The pyridine was removed *in vacou* and the residue was diluted with water and extracted with ether. The extracts were washed with 3N HCl, followed by 1N $NaHCO_3$ then brine. After being dried over Na_2SO_4, the solvent was removed *in vacou* to give **111** in 100% yield (137 mg, 0.4 mmol). ^1H NMR (300 MHz, $CDCl_3$) 1.36 (3H, t, J = 6.9 Hz), 3.82 (3H, s), 4.26 (2H, q, J = 14.1, 6.9 Hz), 6.44 (1H, dd, J = 11.7, 3.3 Hz), 6.96 (1H, br s), 7.59 (1H, d, J = 9.0 Hz), 7.81 (1H, d, J = 3.0 Hz). LRMS (FAB+): Calc. for $C_{10}H_{12}INO_3$: 320.98619. Found: 321.0777 (M^+, 100 %), 322.0827 (MH^+, 83 %).



111

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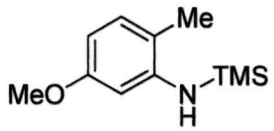
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

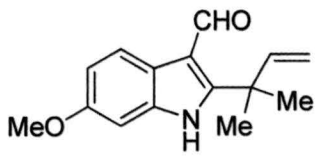
(5-Methoxy-2-methyl-phenyl)-trimethylsilanyl-amine (117). A mixture of 5-methoxy-2-methylaniline (2.0g, 14.46 mmol), HMDS (9.46 mL, 44.83 mmol), LiI (39 mg, 0.289 mmol), and TMSCl (0.110 mL, 0.868 mmol) was refluxed for 3.5 hours. A portion of cyclohexene oxide (0.295 mL, 2.89 mmol) was added and the reflux was continued for 15 minutes. Another portion of cyclohexene oxide (0.295 mL, 2.89 mmol) was added, and the reaction mixture was cooled. Short path distillation under normal atmosphere removed the excess HMDS, followed by distillation under high vacuum to yield the residual clear oil **117** (2.183 g, 10.38 mmol, 72%) that was stored under Ar. ^{1}H NMR (300 MHz, $CDCl_3$) 0.29 (9H, s), 2.09 (3H, s), 3.28 (1H, br s), 3.77 (3H, s), 6.24 (1H, dd, J = 8.1, 2.4 Hz), 6.34 (1H, d, J = 2.7 Hz), 6.96 (1H, d, J = 8.1 Hz).



117

File name: MN1-58	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

6-methoxy-2-(2-methylbut-3-en-2-yl)-1H-indole-3-carbaldehyde (123): To a cooled (0°C) solution of Vilsmeier reagent (87 mg, 0.68 mmol) in CH_2Cl_2 (4 mL) was added a solution of the indole **91** (140 mg, 0.65 mmol) in CH_2Cl_2 (6mL). The reaction mixture was heated to 40°C for 2 h, then EtOAc (10 mL) and a 10% aq. solution of NaOH (10 mL) and stirred for one hour at RT. The layers were separated and the aq. layer was extracted with EtOAc. The combined organics were washed with water and brine, and dried with Na_2SO_4. The solvent was removed *in vacou* and the crude material was purified by flash chromatography in 40% EtOAc : Hexanes to give the aldehyde (R_f = 0.2) as a yellow powder in 96% (152 mg, 0.62 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) 1.68 (6H, s), 3.85 (3H, s), 5.27-5.32 (2H, m), 6.23 (1H, dd, J = 17.4, 10.5 Hz), 6.87-6.94 (2H,m), 8.24 (1H, d, J = 8.7 Hz), 8.39 (1H, br s), 10.44 (1H, s). LRMS (FAB+): Calc. for $C_{15}H_{17}NO_2$: 243.12593. Found: 243.14 (M^+, 72 %), 244.14 (MH^+, 100 %).

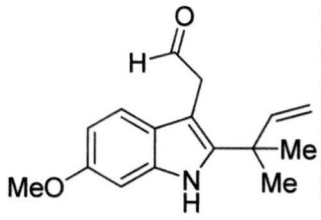


123

File name: MN1-109-cc15-22	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

2-(6-methoxy-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)acetaldehyde (124):

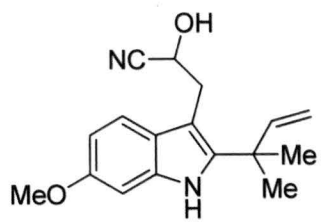
Ph_3PCH_2OMeCl (159 mg, 0.463 mmol) was brought to 0°C in 3 mL of dry THF and n-BuLi (0.3 mL of 1.6 M in Hexanes, 0.475 mmol) was added drop wise. The solution was stirred for 20 minutes and the aldehyde (**123**) (55 mg, 0.226 mmol) in 2 mL of THF was added. The reaction was stirred at 0°C for 30 minutes and then warmed to RT and stirred for 21 hours. The reaction was concentrated and the residue taken up in EtOAc. After being washed with water and brine and dried with $MgSO_4$, the concentrated residue was purified by flash chromatography in 40% EtOAc : Hexanes to give the vinyl ether intermediate. The intermediate was then brought to reflux in 5 mL of THF and 3 mL 1N HCl for 3.5 hours. After being cooled to room temperature, the mixture was partitioned between EtOAc and $NaHCO_3$. The extracts were washed with brine and dried over Na_2SO_4. After concentration, the residue was purified by flash chromatography in 40% EtOAc : Hexanes to give the homologated aldehyde (R_f = 0.4) as a yellow oil in 50% yield (29 mg, 0.11 mmol). ^1H NMR (300 MHz, $CDCl_3$) 1.52 (6H, s), 2.18 (2H, s), 3.85 (3H, s), 5.15-5.21 (2H, m), 6.09 (1H, dd, J = 16.8, 10.5 Hz), 6.78-6.86 (2H,m), 7.93 (1H, br s), 9.66 (1H, s). HRMS (FAB+): Calc. for $C_{16}H_{19}NO_2$: 257.14158. Found: 257.141399 (M^+).



124

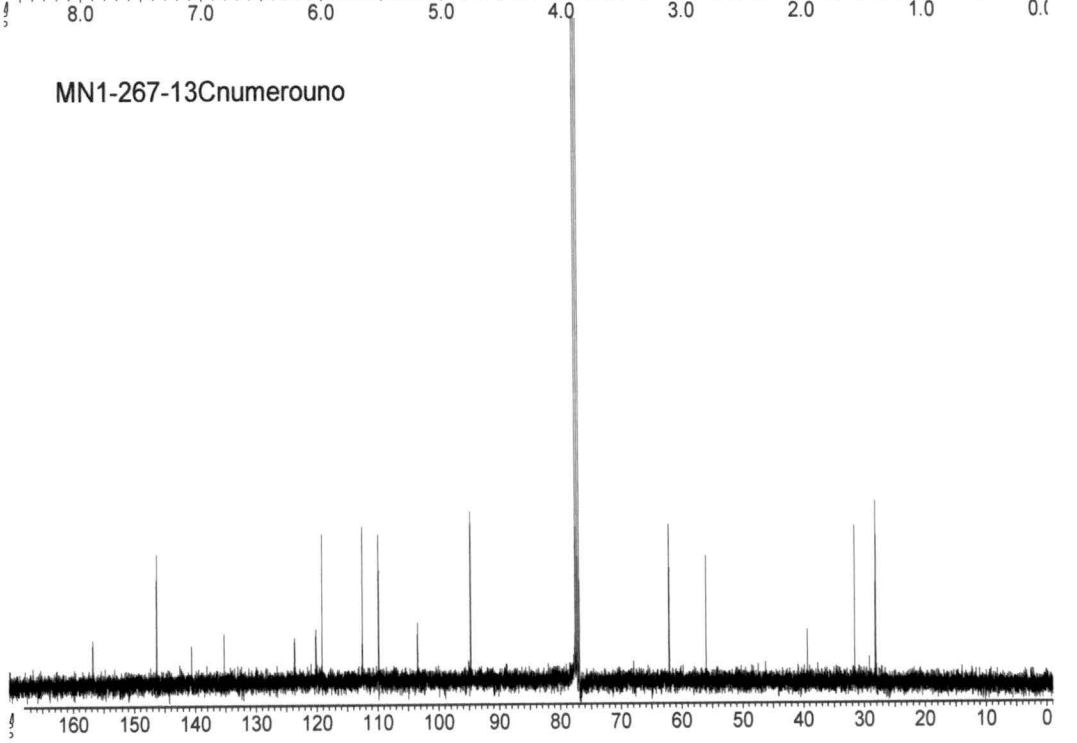
File name: MN1-228-cc3-6	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

2-hydroxy-3-(6-methoxy-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)propanenitrile (125): Aldehyde **124** (66 mg, 0.26 mmol) was dissolved in 4.5 mL THF and 1.2 mL of H_2O, NaCN (38 mg, 0.77 mmol) was added and the mixture was cooled to 0°C. CSA (66 mg, 0.28 mmol) was added. The reaction was stirred at 0°C for 2 hours and then quenched with saturated $NaHCO_3$ (5 mL). Extraction with EtOAc (10 mL) was followed by a wash of the organics with water and brine and drying over Na_2SO_4. After concentration, the residue was purified by flash chromatography in 40% EtOAc : Hexanes to give the cyanohydrin **125** (Rf = 0.6) as a yellow oil in 52% yield (39 mg, 0.14 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) 1.57 (6H, s), 3.44 (2H, d, J = 8.7 Hz), 3.85 (3H, s), 4.76 (1H, t, J = 7.8 Hz), 5.18-5.24 (2H, m), 6.15 (1H, dd, J = 17.7, 10.5 Hz), 6.79-6.84 (2H,m), 7.43 (1H, d, J = 8.4 Hz), 7.90 (1H, br s). ^{13}C NMR δ: 28.17 (2C), 31.58, 39.37, 55.97, 62.07, 94.72, 103.47, 109.83, 112.49, 119.10, 120.13, 123.66, 135.22, 140.58, 146.29, 156.73. HRMS (FAB+): Calc. for $C_{17}H_{20}N_2O_2$: 284.15248. Found: 284.151478 (M^+).

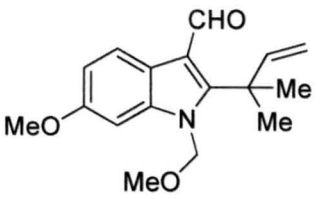


125

MN1-267-13Cnumerouno



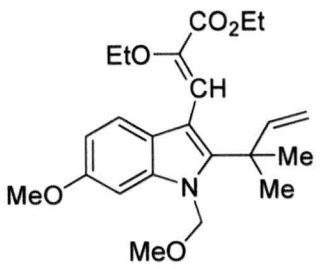
6-methoxy-1-(methoxymethyl)-2-(2-methylbut-3-en-2-yl)-1*H*-indole-3-carbaldehyde (127): To a stirred suspension of KH (35% in mineral oil; 124 mg, 1.1 mmol) in THF (1 mL) on an ice bath, was added a solution of the unprotected indole **123** (88 mg, 0.36 mmol) in THF (2 mL). The mixture was stirred at 0°C for 30 minutes before adding TMEDA (63 mg, 0.5 mmol), which was stirred for an additional 30 minutes at 0°C. MOMCl (87 mg, 1.1 mmol) was added and the mixture was warmed to RT and stirred for 18h. The mixture was quenched at 0°C with saturated aqueous $NaHCO_3$ solution (3 mL) and 5 mL of EtOAc was added. The layers were separated. The aqueous layer was extracted with EtOAc (3 x 5 mL), and the combined organic layers were washed with water, then brine, and dried with Na_2SO_4. The organics were concentrated *in vacou* and purified by flash chromatography (40% EtOAc / Hexanes) to yield the product ($R_f = 0.5$) as a yellow oil in 44% yield (45 mg, 0.16 mmol). ^1H NMR (300 MHz, $CDCl_3$) 1.76 (6H, s), 3.40 (3H, s), 3.89 (3H, s), 5.06-5.19 (2H, m), 6.29 (1H, dd, J = 17.4, 11.1 Hz), 6.88 (1H, s), 6.95 (1H, d, J = 12.0 Hz), 8.39 (1H, d, J = 9.0 Hz), 10.67 (1H, s).



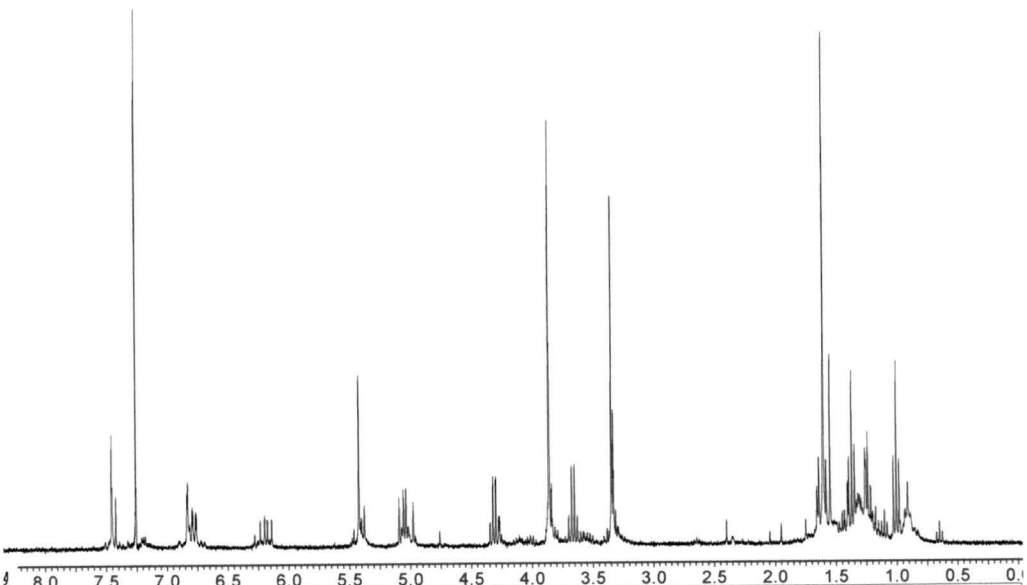
127

File name: MN1-335-cc9-16	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

(E/Z)-ethyl 2-ethoxy-3-(6-methoxy-1-(methoxymethyl)-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)acrylate (129): In a flame-dried sealed tube fitted with a septum, n-BuLi (0.36 mL, 1.6 M in Hexanes, 0.576 mmol) was added to an ice cold solution of the phosphanate **128** (155 mg, 0.576 mmol) in anhydrous toluene (3 mL). The solution was stirred for 30 mins at 0°C then allowed to warm to RT. The aldehyde **127** (138 mg, 0.48 mmol) in toluene (2 mL) was added and the resulting solution was sealed and brought to 150°C for 18 h. After cooling to RT, water was added to the solution, and the aqueous layer was extracted with ether (3x 5 mL). The combined extracts were washed with water, followed by brine, and then dried over Na_2SO_4. The solvent was removed and the brown oil was purified by flash chromatography (40% EtOAc / Hexanes) to yield the product as a brown oil in 50% yield (90 mg). ^{1}H NMR (300 MHz, $CDCl_3$) 1.02 (3H, t, J = 7.2 Hz), 1.38 (3H, t, J = 7.8 Hz), 1.66 (6H, s), 3.36 (3H, s), 3.68 (2H, q, J = 14.1, 6.9 Hz), 3.88 (3H, s), 4.32 (2H, q, J = 14.4, 7.2 Hz), 4.99-5.11 (2H, m), 5.44 (2H, s), 6.20 (1H, dd, J = 17.4, 10.5 Hz), 6.78-6.85 (2H, m), 7.45 (1H, d, J = 10.2 Hz). LRMS (FAB+): Calc. for $C_{23}H_{31}NO_5$: 401.22022. Found: 401.26 (M^+, 100 %), 402.26 (MH^+, 39%).

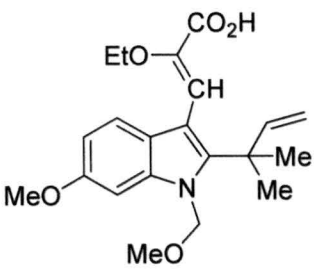


129



File name: MN1-345-cc1-3	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

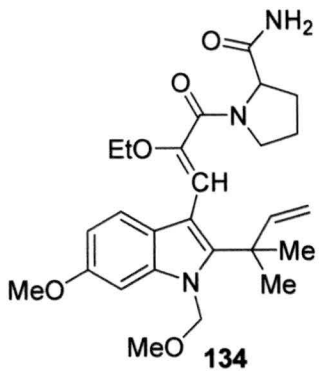
(E/Z)-2-ethoxy-3-(6-methoxy-1-(methoxymethyl)-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)acrylic acid (130): The ester **129** (9 mg, 0.022 mmol) was brought to solution with 1 mL EtOH (95%) and 1 mL of 1M NaOH, and brought to a reflux for 5 hours. After cooling to RT, the solution was acidified to pH 1 with 6 M HCl, and the aqueous solution was extracted with EtOAc (3 x 5 mL). The organics were washed with brine and dried over Na_2SO_4. The organics were concentrated *in vacou* to give the pure acid **130** in 84% yield (7 mg, 0.019 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) 1.05 (3H, t, J = 6.9 Hz), 1.76 (6H, s), 3.40 (3H, s), 3.68 (2H, q, J = 14.1, 6.9 Hz), 3.89 (3H, s), 5.06-5.19 (2H, m), 5.49 (2H, s), 6.28 (1H, dd, J = 17.4, 10.8 Hz), 6.81-6.95 (2H, m), 7.42 (1H, d, J = 9.0 Hz), 7.61 (1H, s), 8.39 (1H, d, J = 8.4 Hz), 10.66 (1H, s). LRMS (FAB+): Calc. for $C_{21}H_{27}NO_5$: 373.18892. Found: 373.18 (M^+, 100 %), 374.18 (MH^+, 33%).



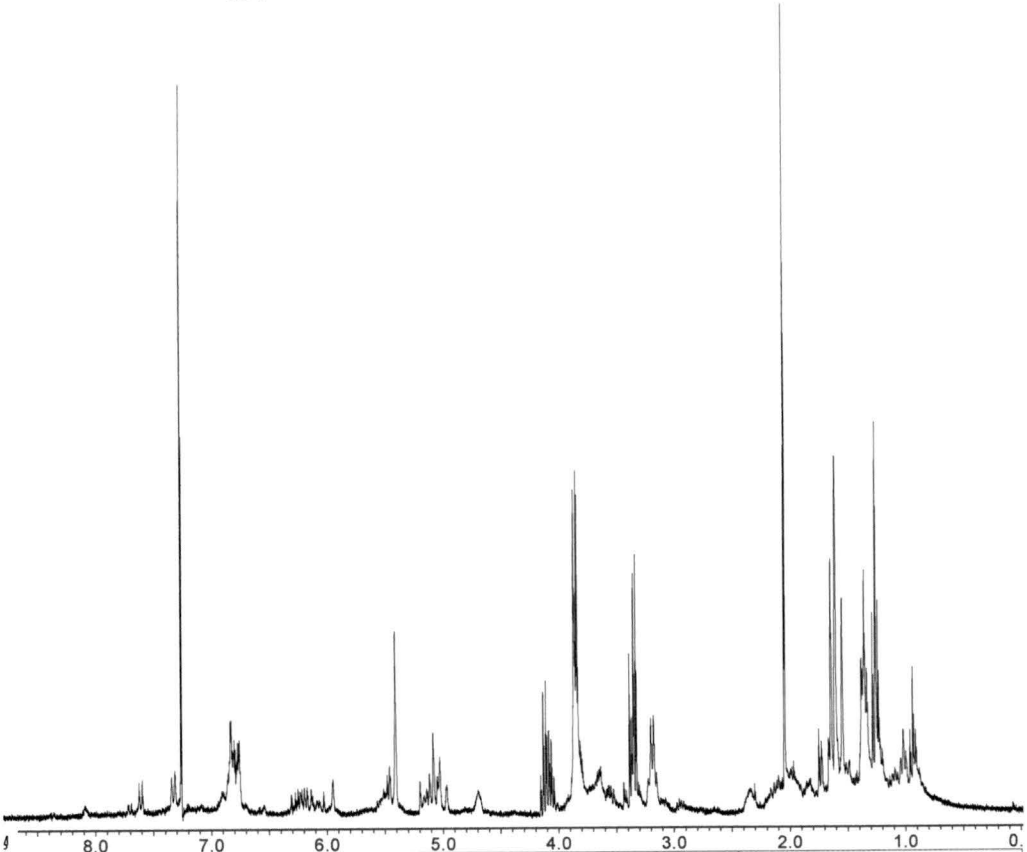
130

File name: MN2-25-cr	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

(E/Z)-1-(2-ethoxy-3-(6-methoxy-1-(methoxymethyl)-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)acryloyl)pyrrolidine-2-carboxamide (134): The acid **130** (52 mg, 0.14 mmol) was brought to solution with 2 mL of anhydrous DCM and Et_3N (58 μL, 0.41 mmol) and PyBOP (79 mg, 0.15 mmol) were added. The mixture was stirred for 10 minutes at RT before L-Prolinamide (17 mg, 0.15 mmol) was added. After stirring for 24 h at RT, the saturated aqueous NH_4Cl (2 mL) was added. After the layers were separated, the aqueous layer was extracted with DCM (2 x 5 mL). The combined organics were washed with brine, then dried over Na_2SO_4 and concentrated *in vacou* to give 33mg (50%) of a yellow oil. ^1H NMR (300 MHz, $CDCl_3$) is shown on the next page. The rotomers has left complete characterization too complicated. LRMS (FAB+): Calc. for $C_{26}H_{35}N_3O_5$: 469.25767. Found: 469.23 (M^+, 100 %), 470.23 (MH^+, 85%).



134

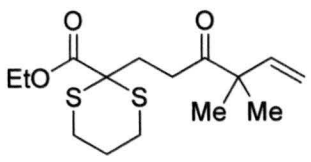


File name: MN2-31-cc40-end	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

5.3 Chemical Synthesis Experimentals for Brevianamide B Research (Chapter 3)

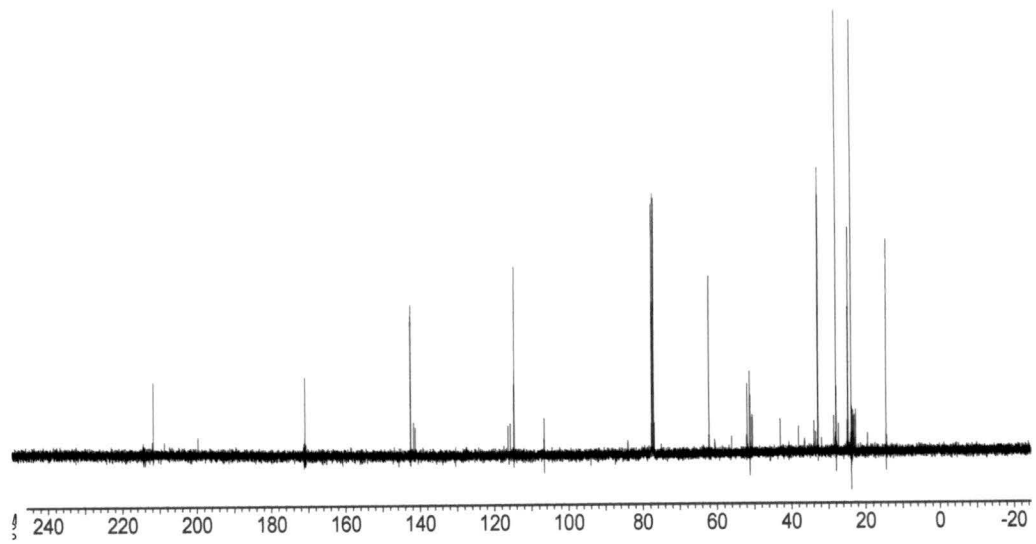
Ethyl 2-(4,4-dimethyl-3-oxohex-5-enyl)-1,3-dithiane-2-carboxylate (141). To a stirred, cooled (-60 °C) solution of ethyl 1,3-dithiane-2-carboxylate (13.3 mL, 84.2 mmol) in anhydrous Et_2O (100 mL), was added *n*-butyl lithium (52 mL, 84.2 mmol, 1.6 M solution in hexane) quickly. This mixture was stirred at 0 °C under argon for 45 min, cooled to -78 °C and then added *via* cannula to a cooled (-78 °C) suspension of CuI (8.55 g, 44.9 mmol) in Et_2O (200 mL). After stirring at this temperature for 30 min the white suspension was warmed to -15 °C for 5 min, cooled to -40 °C and stirred for 30 min, prior to the addition (*via* cannula) of a solution of ketone **140**[13] (3.48 g, 28.1 mmol) in anhydrous Et_2O (150 mL). The reaction was allowed to warm to room temperature slowly over 2.5 h. TLC (10:90 EtOAc / hexane) showed ethyl 1,3-dithiane-2-carboxylate (R_f 0.30) and dithiane **141** (R_f 0.19) potassium permanganate active. The reaction was quenched by the addition of saturated aqueous NH_4Cl (500 mL), stirred for 15 min and then filtered through Celite. The filter was washed with EtOAc (500 mL), the filtrate layers were separated and the aqueous phase extracted with EtOAc (3 × 500 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was purified by column chromatography on silica using a gradient of EtOAc / hexane (4:96 EtOAc / hexane to 10:90 EtOAc / hexane) as eluent. The mixed fractions were purified by column chromatography on silica using a gradient of EtOAc / hexane (5:95 EtOAc / hexane to 25:75 EtOAc / hexane) as eluent to give hexenyl dithiane **141** (6.63 g, 75%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.24 (6H, s), 1.32 (3H, t, J = 7.2 Hz), 1.82-1.94 (1H, m), 2.10-2.20 (1H, m), 2.23-2.31 (2H, m), 2.63-2.77 (4H, m), 3.25-

3.37 (2H, m), 4.25 (2H, q, J = 7.2 Hz), 5.14 (1H, d, J = 17.5 Hz), 5.15 (1H, d, J = 10.7 Hz), 5.92 (1H, dd, J = 17.5, 10.7 Hz). ^{13}C NMR δ: 14.1, 23.5 (2C), 24.4, 27.7 (2C), 32.6 (2C), 50.8, 51.5, 61.9, 114.4, 142.3, 170.7, 211.5. IR (NaCl): 3525, 2932, 1717, 1421, 1356, 1280, 1225, 1173, 1083, 1017, 917 cm^{-1}. HRMS (FAB+): Calc. for $C_{15}H_{25}O_3S_2$: 317.1245. Found: 317.1230 (MH^+).

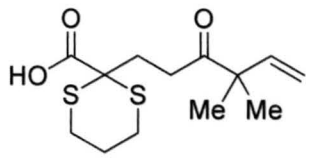


141

EsterC13

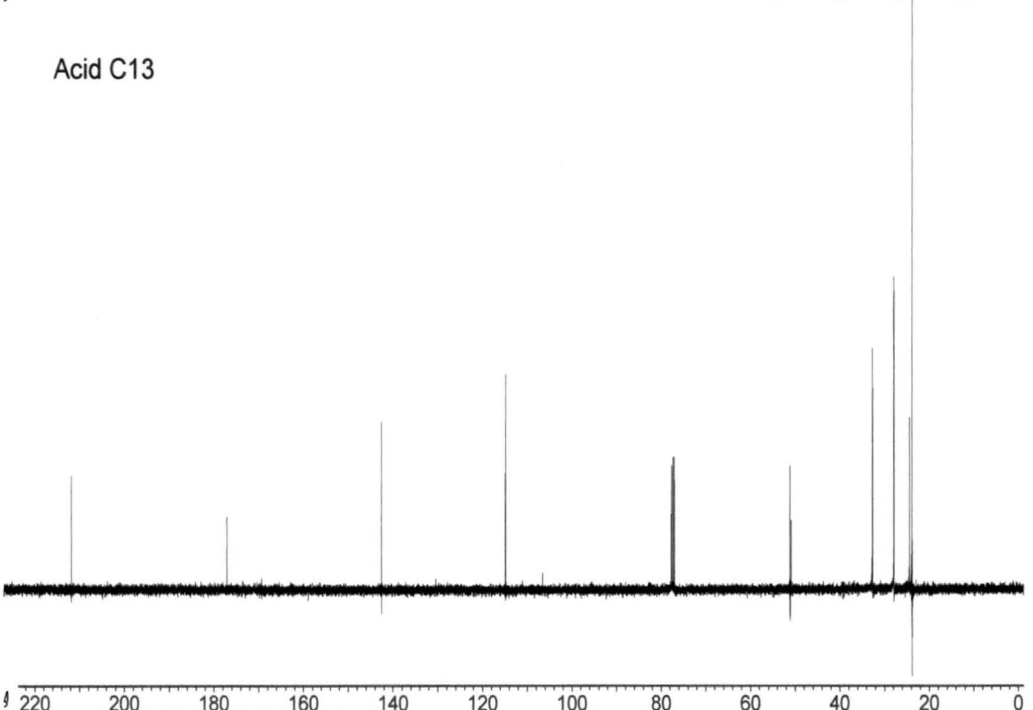


2-(4,4-Dimethyl-3-oxohex-5-enyl)-1,3-dithiane-2-carboxylic acid (142). To a stirred solution of dithiane ester **141** (3 g, 9.49 mmol) in THF (15 mL), ethanol (15 mL) and water (15 mL) was added lithium hydroxide (4.55 g, 190 mmol). The reaction mixture was heated under argon at 70 °C for 11 h and then the solvent was concentrated under reduced pressure. To the resultant aqueous mixture was added water (125 mL) and Et_2O (125 mL), the layers were separated and the aqueous further extracted with Et_2O (125 mL). EtOAc (250 mL) was added to the aqueous layer, which was then acidified to pH 1 with conc. HCl. The layers were separated and the aqueous phase was further extracted with EtOAc (3 × 250 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, then azeotroped with $CHCl_3$ to give the acid **7** (2.92 g, quantitative) as an off-white solid with a mp of 109-110 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.25 (6H, s), 1.78-1.95 (1H, m), 2.12-2.22 (1H, m), 2.28 (2H, br t, J = 7.9 Hz), 2.65-2.74 (2H, m), 2.79 (2H, br t, J = 7.9 Hz), 3.28-3.39 (2H, m), 5.16 (1H, d, J = 17.2 Hz), 5.17 (1H, d, J = 10.5 Hz), 5.93 (1H, dd, J = 17.2, 10.5 Hz). ^{13}C NMR: 23.5 (2C), 24.1, 27.6 (2C), 32.4, 32.5, 50.6, 50.8, 114.5, 142.2, 176.8, 211.5. IR (NaCl): 2971, 2929, 1703, 1414, 1364, 1254, 1081, 998, 919, 681 cm^{-1}. HRMS (FAB+): Calc. for $C_{13}H_{21}O_3S_2$: 289.0932. Found: 289.0928 (MH^+). Elemental analysis: Calculated: 54.13% C, 6.99% H, 22.23% S. Found: 54.07% C, 7.30% H, 21.80% S.



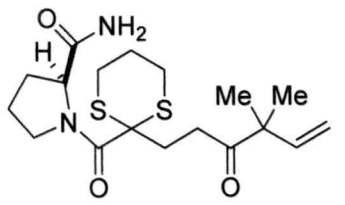
142 (100%)

Acid C13



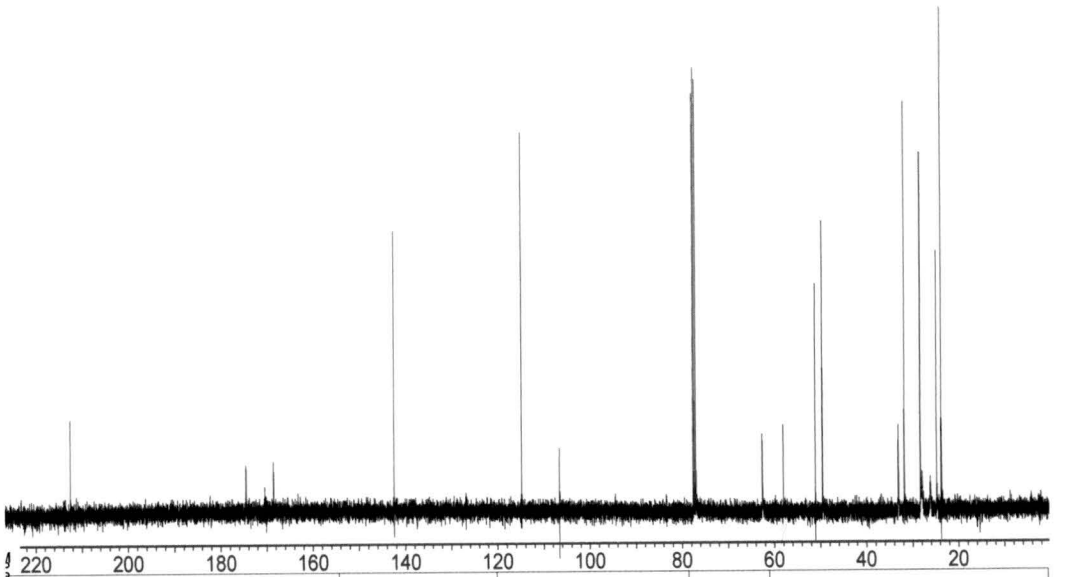
File name: MN1-Acid-H300	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

(*S*)-1-(2-(4,4-Dimethyl-3-oxohex-5-enyl)-1,3-dithiane-2-carbonyl)pyrrolidine-2-carboxamide (143). To a cold (0 °C) solution of acid **142** (1.6 g, 5.5 mmol) in anhydrous CH_2Cl_2 (20 mL) was added BOPCl (1.7 g, 6.6 mmol) and *i*-Pr_2EtN (1.16 mL, 6.6 mmol). The mixture was stirred for 10 min. before (L)-prolinamide (634 mg, 5.5 mmol) was added. The reaction was allowed to warm to RT and stirred for 48 h. After quenching the mixture with saturated aquous NH_4Cl, the layers were separated and the aqueous solution was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were washed with brine (50 mL) and dried over anhydrous Na_2SO_4, then evaporated under reduced pressure. TLC analysis in EtOAc showed the peptide **143** (R_f 0.20) as potassium permanganate active. This crude mixture was purified by column chromatography on silica using (EtOAc) as eluent to give peptide **143** (1.65 g, 77%) as a white solid with a mp of 111-112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (3H, s), 1.22 (3H, s), 1.86-2.08 (6H, m), 2.39 (2H, br t, J = 7.8 Hz), 2.64-2.85 (4H, m), 2.91-2.99 (1H, m), 3.13 (1H, br t, J = 11.1 Hz), 3.65-3.73 (1H, m), 4.17 (1H, br s), 4.52 (1H, br s), 5.12 (1H, d, J = 17.4 Hz), 5.12 (1H, d, J = 10.7 Hz), 5.66 (1H, br s), 5.91 (1H, dd, J = 17.4, 10.7 Hz), 6.34 (1H, br s). ^{13}C NMR δ: 23.5 (2C), 24.5, 25.9, 27.6, 28.0, 31.5, 32.8, 49.2, 50.8 (2C), 57.8, 62.3, 114.5, 142.2, 168.3, 174.2, 212.2. IR (NaCl): 3327, 2925, 2851, 1627, 1575, 1439, 1311, 1242, 1088, 643 cm^{-1}. HRMS (FAB+): Calc. for $C_{18}H_{29}N_2O_3S_2$: 385.1620. Found: 385.1629 (MH^+).



143

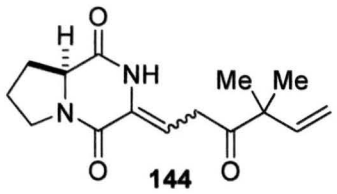
Amide 143



File name: MN1-Amide 143-H300	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

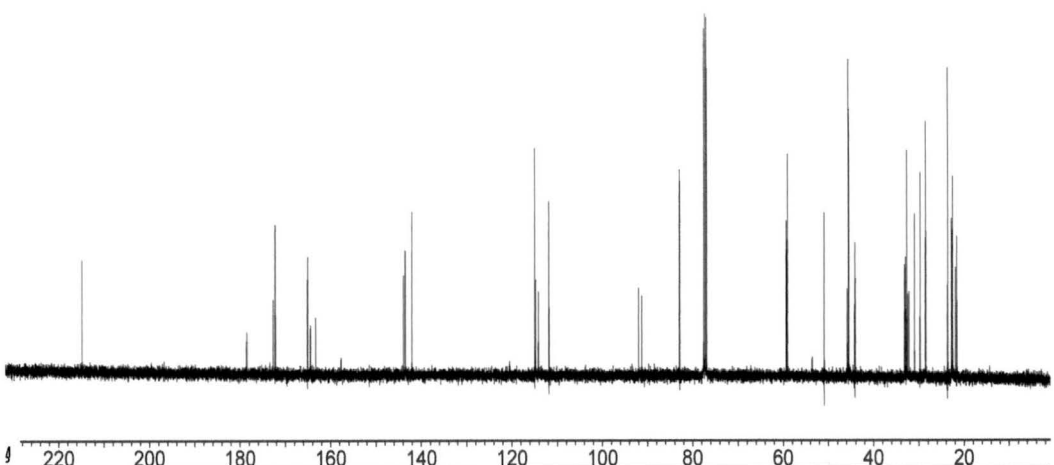
3-(4,4-Dimethyl-3-oxohex-5-enylidene)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (144) and (S)-1-(6,6-dimethyl-2,5-dioxooct-7-enoyl)pyrrolidine-2-carboxamide (145). To a stirred, cooled (0 °C) suspension of silver nitrate (1.08 g, 6.36 mmol) in 4:1 MeCN/water (25 mL) was added sequentially 2,6-lutidine (1.32 mL, 11.3 mmol), *N*-chlorosuccinimide (1.13 g, 8.49 mmol, recrystallised from AcOH), and a solution of dithiane **143** (543 mg, 1.41 mmol) in MeCN (3.5 mL) quickly. The reaction mixture was stirred at RT for 70 min., quenched by the addition of saturated aqueous NH_4Cl (10 mL), stirred for 15 min, and filtered through Celite. The filter was washed with Et_2O (100 mL), the filtrate layers were separated and the aqueous phase extracted with Et_2O (3 × 100 mL). The combined organic extracts were washed with 2% aqueous $Na_2S_2O_3$ (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was dissolved in the minimum amount of EtOAc, salts were removed by filtration, and the filtrate was purified by column chromatography on silica gel using (EtOAc) as eluent to give the diketopiperazine **144** (219 mg, 45%, 70:30 diastereomeric mixture of unassigned E/Z-geometry) as a white solid with a mp of 115-116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.08 (3H, s (isomer B)), 1.09 (3H, s (isomer B)), 1.21 (3H, s (isomer A)), 1.22 (3H, s (isomer A)), 1.89-2.38 (4H, m), 2.63 (1H, t, J = 6.7 Hz (isomer B)), 2.68 (1H, t, J = 6.7 Hz (isomer A)), 2.82 (1H, t, J = 6.7 Hz (isomer A)), 2.86 (1H, t, J = 6.7 Hz (isomer B)), 3.52-3.60 (2H, m), 4.27 (1H, dd, J = 9.7, 6.9 Hz (isomer B)), 4.34 (1H, dd, J = 9.5, 7.0 Hz (isomer A)), 5.05-5.15 (3H, m), 5.88 (1H, dd, J = 17.4, 10.4 Hz (isomer A)), 5.97 (1H, dd, J = 17.9, 10.6 Hz (isomer B)), 7.64 (1H, br s (isomer A)), 7.68 (1H, br s (isomer B)). ^{13}C NMR isomers A and B δ: 21.4, 21.7, 22.4, 22.5, 22.6, 23.5, 28.3, 28.3, 32.5, 32.7, 43.9, 44.0, 45.3, 45.6, 58.9, 59.0, 111.6, 113.9, 114.5, 114.7, 143.3,

143.7, 163.1, 164.9, 172.0, 172.0, 172.5, 178.3, 214.6 (2C). IR (NaCl): 3234, 2977, 1774, 1708, 1429, 1362, 1294, 1182, 1605, 919, 816, 639 cm^{-1}. HRMS (FAB+): Calc. for $C_{15}H_{21}N_2O_3$: 277.1552. Found: 277.1553 (MH^+), and amide **145** (43%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.22 (3H, s), 1.23 (3H, s), 1.92-2.14 (5H, m), 2.34-2.44 (1H, m), 2.66-2.72 (2H, m), 3.48-3.67 (2H, m), 4.28 (1H, dd, J = 9.0, 7.2 Hz), 4.83 (1H, br s), 5.15 (1H, d, J = 17.3 Hz), 5.16 (1H, d, J = 10.7 Hz), 5.91 (1H, dd, J = 17.3, 10.7 Hz), 6.76 (1H, br s). ^{13}C NMR δ: 22.7, 23.6 (2C), 28.6, 30.8, 33.3, 45.9, 50.7, 59.9, 114.6, 142.2, 166.9, 167.6, 212.1 (2C). IR (NaCl): 3338, 2974, 1696, 1636, 1445, 1297, 1178, 1074, 923 cm^{-1}. HRMS (FAB+): Calc. for $C_{15}H_{23}N_2O_4$: 295.1658. Found: 295.1662 (MH^+).

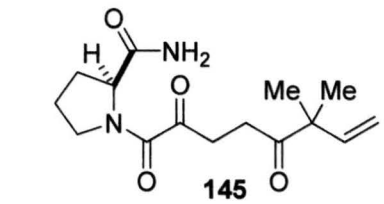


144

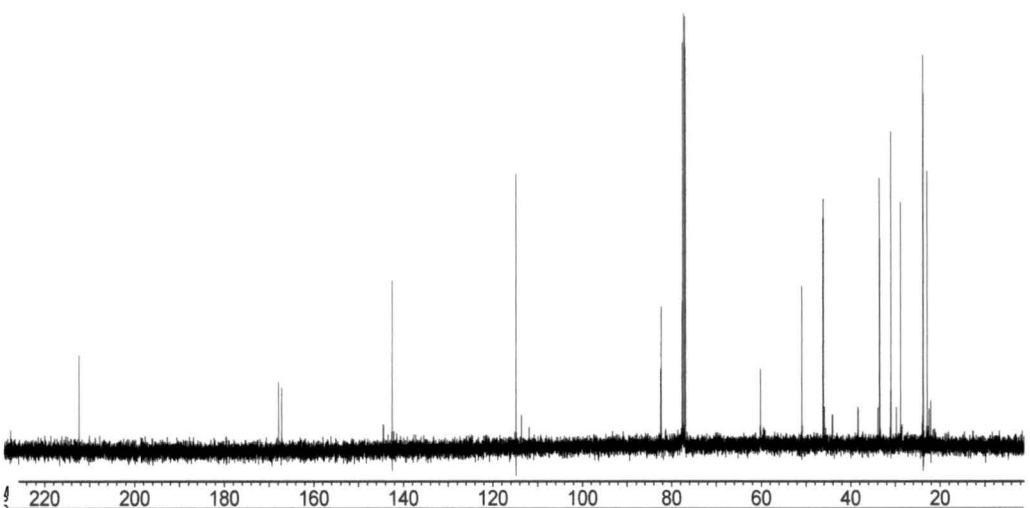
DKP C13



File name: MN1-DKP-H300	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE



Amide 145 C13

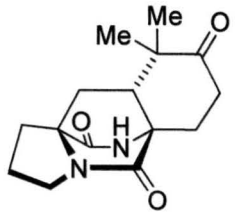


File name: MN1-Amide-145-H300	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

Tetracycle (146) from diketopiperazine (144). To a solution of the diketopiperazine **144** (110 mg, 398 μmol) in EtOAc (11 mL) was added aluminium trichloride (159 mg, 1.19 mmol). The solution was heated at reflux for 19 h, and then the solution was allowed to cool to room temperature. TLC analysis (EtOAc) showed the tetracycle **146** (R_f 0.18); UV and potassium permanganate active. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (10 mL), then filtered through Celite. The filter was washed with EtOAc (50 mL), the filtrate layers were separated and the aqueous phase extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was purified by column chromatography on silica gel using (EtOAc) as eluent to give the tetracycle **146** (48 mg, 44%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.97 (3H, s), 1.10 (3H, s), 1.76-2.07 (5H, m), 2.17 (1H, dd, J = 10.1, 5.9 Hz), 2.23 (1H, ddd, J = 14.9, 5.7, 4.4 Hz), 2.40 (1H, dt, J = 13.8, 4.4 Hz), 2.57 (1H, ddd, J = 14.9, 13.8, 5.7 Hz), 2.73 (1H, dd, J = 13.0, 5.9 Hz), 2.93 (1H, td, J = 13.8, 5.7 Hz), 3.45 (2H, t, J = 6.8 Hz), 8.78 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.5, 23.7, 24.5, 26.1, 28.8, 32.2, 32.8, 44.0, 47.1, 47.2, 59.9, 66.8, 168.9, 174.4, 213.1. IR (NaCl): 3230, 2926, 1692, 1411, 1297, 1073 cm^{-1}. HRMS (FAB+): Calc. for $C_{15}H_{21}N_2O_3$: 277.1552. Found: 277.1550 (MH^+).

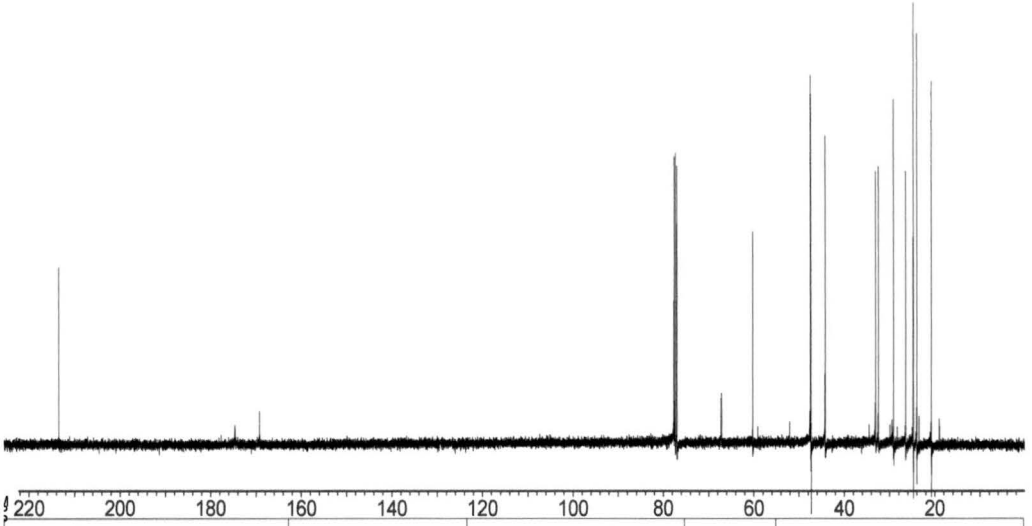
Tetracycle (146) from amide (145). To a solution of the amide **145** (20 mg, 67.7 μmol) in EtOAc (2 mL) was added aluminium trichloride (27 mg, 203 μmol). The solution was heated at 85 °C in a sealed reaction vessel for 19 h; TLC analysis (EtOAc) showed the tetracycle **146** (R_f 0.18); UV and potassium permanganate active. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (1 mL), EtOAc (10 mL) was added, the layers were separated and the aqueous phase extracted with EtOAc (3 × 10

mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was purified by PTLC on silica gel using (EtOAc) as eluent to give the tetracycle **146** (5.9 mg, 31%) as a colorless oil. See spectral data above.



146

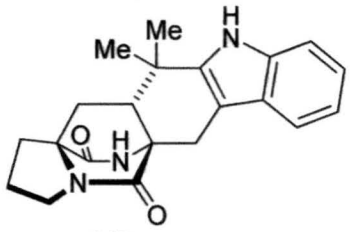
Ketone 146 C13



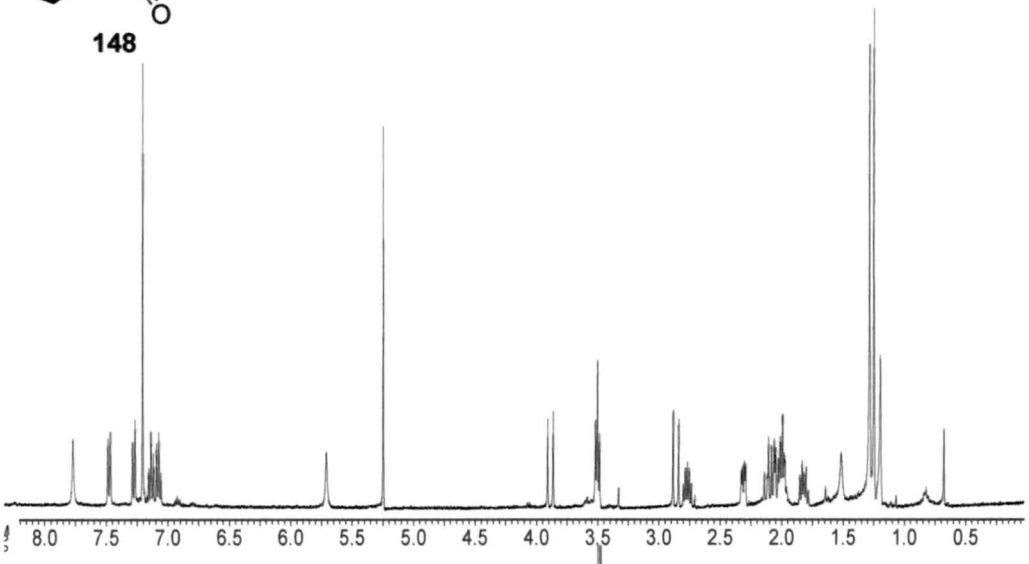
File name: Ketone 146 H	Owner:	SF: 400.1087 MHz	NS:	SI: 32768, TD: 29336
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6388	TE: 298	LAAII91B

Indole (148). To a solution of cyclic ketone **146** (100 mg, 360 μmol) in anhydrous methanol (3 mL), under argon, was added 3Å molecular sieves followed by phenylhydrazine (71 μL, 720 μmol). The mixture was heated at 90 °C in a sealed reaction vessel for 90 min, then it was allowed to cool to RT and the solvent was evaporated under reduced pressure to give the crude hydrazone that was taken on without further purification. The crude oil was dissolved in anhydrous 2-methoxyethyl ether (2 mL) under argon, and anhydrous zinc chloride (99 mg, 720 μmol) was added. The reaction mixture was heated at 172 °C in a sealed reaction vessel for 18 h; TLC analysis (EtOAc) showed the indole **148** (R_f 0.31); UV and potassium permanganate active. The reaction mixture was filtered through Celite, the filter was washed with toluene and the solvent was removed by short path distillation (5-mm Hg, 30-40 °C) to leave a crude residue that was brought up in EtOAc and washed with water. The aqueous phase was extracted with EtOAc (3 x 5 mL) and the combined organic extracts were washed with brine (10 mL) and dried over anhydrous Na_2SO_4. Concentration under reduced pressure gave the crude indole, which was purified by column chromatography on silica using (EtOAc) as eluent to give the indole **148** (73 mg) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.31 (3H, s), 1.35 (3H, s), 1.89 (1H, ddd, J = 13.4, 6.8, 6.8 Hz), 2.02-2.08 (2H, m), 2.10 (1H, dd, J = 13.4, 4.2 Hz), 2.18 (1H, dd, J = 13.4, 9.9 Hz), 2.38 (1H, dd, J = 9.9, 4.2 Hz), 2.83 (1H, ddd, J = 13.4, 6.8, 6.8 Hz), 2.93 (1H, d, J = 18.0 Hz), 3.57 (2H, t, J = 6.8 Hz), 3.95 (1H, d, J = 18.0 Hz), 5.78 (1H, br s), 7.12-7.23 (2H, m), 7.34 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz), 8.84 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.08, 24.68, 25.43, 29.60, 32.86, 34.72, 43.39, 45.96, 59.23, 61.82, 67.28, 103.82, 110.92, 118.55, 119.86, 122.28, 127.40, 136.64, 139.86, 169.29, 172.98. IR (NaCl): 3299, 2926, 2360, 1683, 1457, 1406,

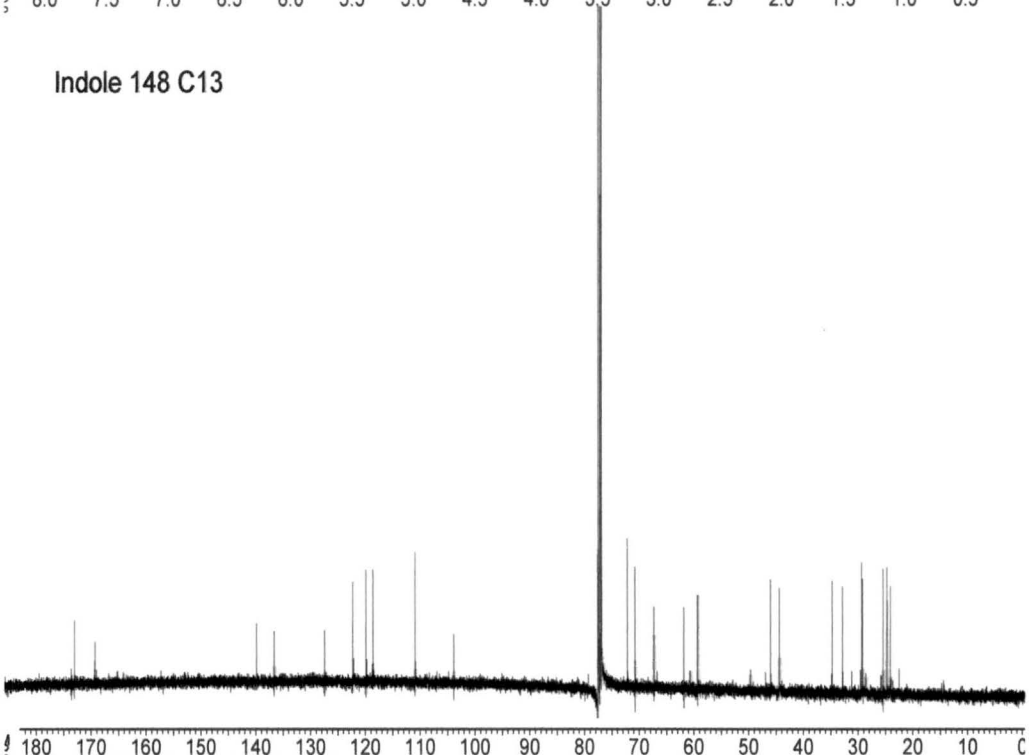
1294, 1140, 1014, 702 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{23}N_3O_2$: 350.17903. Found: 350.18588 (MH^+).



148

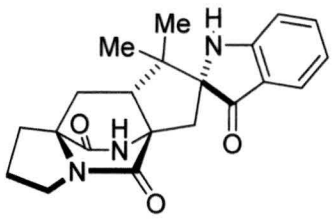


Indole 148 C13



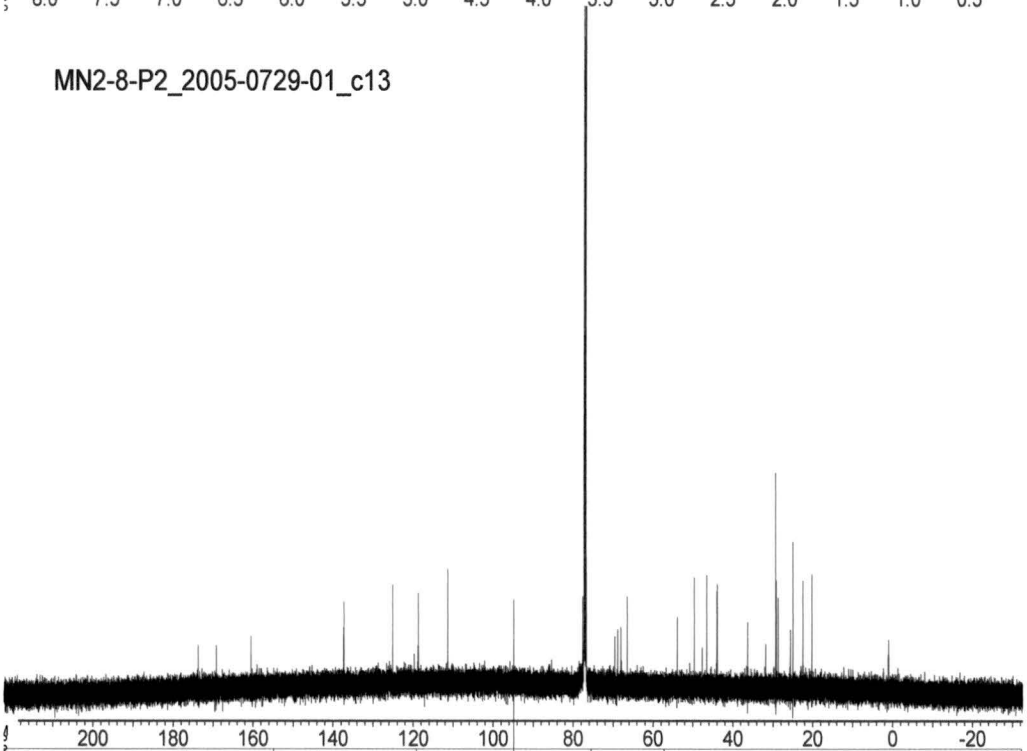
File name: Indole 148 H	Owner:	SF: 400.1081 MHz	NS:	SI: 32768, TD: 18374
Date: 30-Dec-1899	Solvent: CDCl3	SW: 4002	TE: 298	LAAII94A

***D,L*-Brevianamide B (2).** To a solution of indole **148** (25 mg, 70 μmol) in anhydrous THF (2.5 mL) under argon, was added dry 3-chloroperoxybenzoic acid (16 mg, 90 μmol). The reaction mixture was stirred at RT for 1.5 h and was quenched by the addition of dimethyl sulphide (3 drops). The mixture was stirred for 5 min and the solvent evaporated under reduced pressure to give the crude hydroxyindolenine, which was observed by TLC (EtOAc) with a R_f 0.15; UV and potassium permanganate active. To a stirred solution of the crude hydroxyindolenine in MeOH (2.5 mL) was added 0.5 M sodium hydroxide (5 mL). The bright yellow reaction mixture was stirred at room temperature for 18 h, and then heated to reflux for 2 h. The MeOH was removed under reduced pressure, the aqueous phase was neutralized to pH 6/7 with 1 M HCl, and finally extracted with CH_2Cl_2 (4 × 5 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a crude mixture that was purified by PTLC on silica gel using (10:90 MeOH/CH_2Cl_2) as eluent to give D,L-**2** (4 mg) as a yellow oil. This material was identical to natural brevianamide B by TLC (10:90 MeOH/CH_2Cl_2; R_f 0.35; fluorescent under UV), ^{1}H NMR, ^{13}C NMR, IR and HRMS. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.84 (3H, s), 1.13 (3H, s), 1.76-2.07 (5H, m), 2.71-2.78 (1H, m), 3.23-3.33 (1H, m), 3.25 (1H, d, J = 16 Hz), 3.49 (2H, t, J = 6.7 Hz), 5.10 (1H, br s), 6.51 (1H, br s), 6.79 (1H, t, J = 7.5 Hz), 6.84 (1H, d, J = 8), 7.42 (1H, t, J = 7.5 Hz), 7.55 (1H, d, J = 7.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 20.20, 22.45, 24.95, 28.60, 29.09, 36.20, 43.97, 46.46, 49.59, 66.38, 67.98, 77.57, 111.30, 118.70, 119.72, 125.04, 137.27, 160.46, 169.14, 173.73. IR (NaCl): 3583, 3259, 2971, 1690, 1618, 1469, 1392, 1326, 1197, 923, 731, 666 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{23}N_3O_3$: 366.17394. Found: 366.181498 (MH^+).

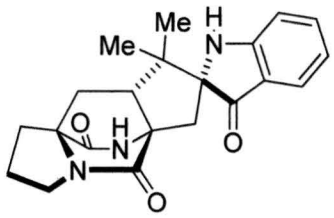


2, *d,l*-brevianamide B

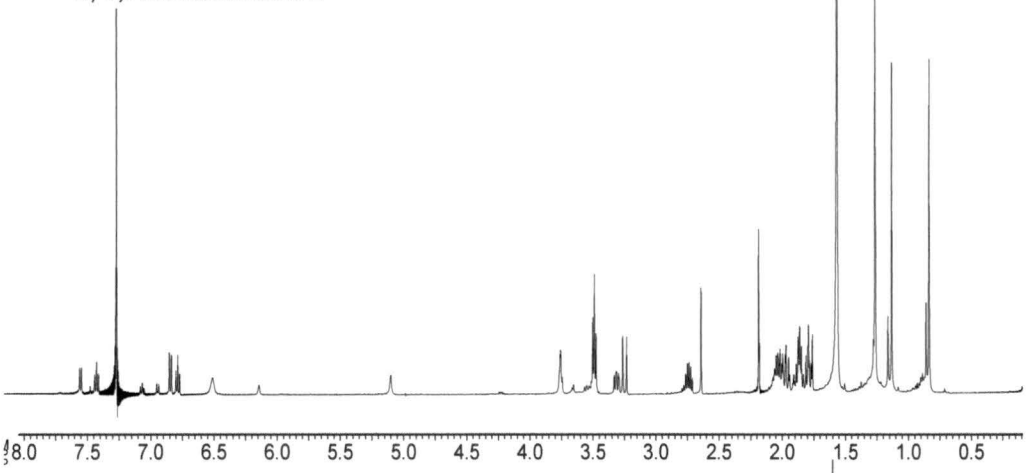
MN2-8-P2_2005-0729-01_c13



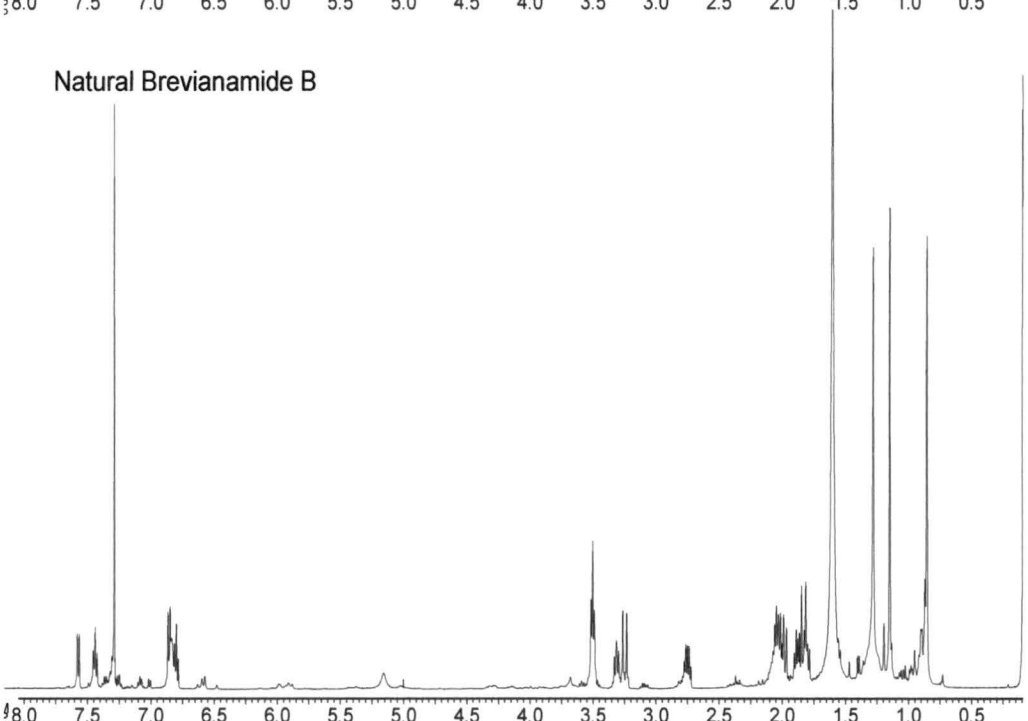
File name: MN2-10-P2-HNMR	Owner:	SF: 500.1751 MHz	NS:	SI: 32768, TD: 30272
Date: 30-Dec-1899	Solvent: CDCl3	SW: 8000	TE: 298	STANDARD PROTON PARAMETERS



2, *d,l*-brevianamide B



Natural Brevianamide B



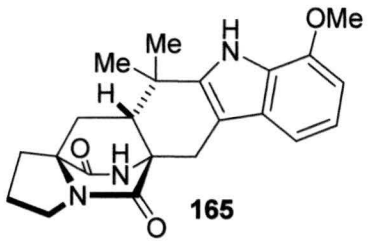
File name: MN2-10-P2-HNMR	Owner:	SF: 500.1751 MHz	NS:	SI: 32768, TD: 30272
Date: 30-Dec-1899	Solvent: CDCl3	SW: 8000	TE: 298	STANDARD PROTON PARAMETERS

5.4 Chemical Synthesis Experimentals for Malbrancheamide Research (Chapter 4)

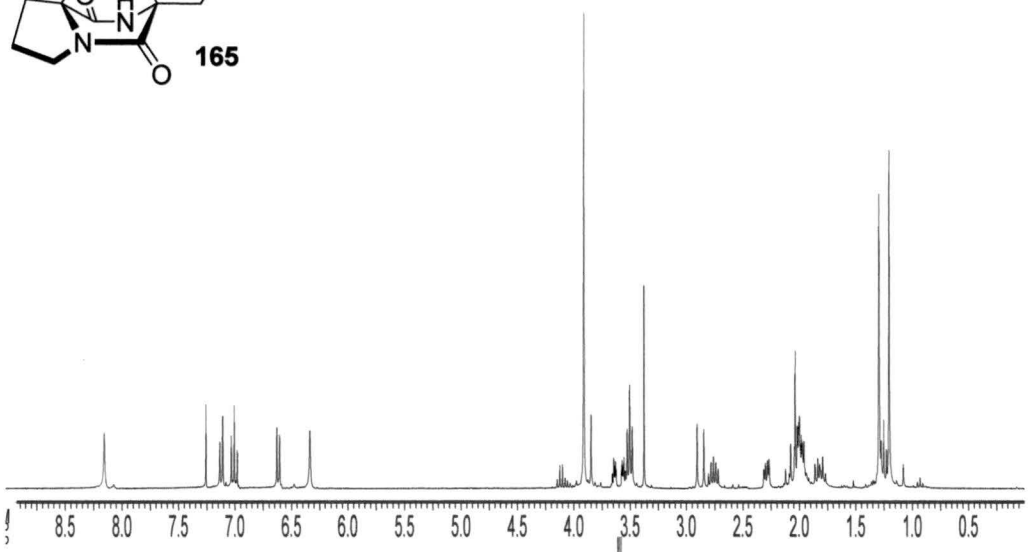
5.4.1 General Procedure for the synthesis of indoles **165-171** from ketone **146** with various commercial hydrazines:

To a solution of cyclic ketone **146** (1 mol equiv.) in anhydrous methanol (20 % vol/wt.), under argon, was added activated 3 Å molecular sieves (1 vol. equiv.) followed by phenylhydrazine (1.5 mol equiv.). The mixture was heated at 90 °C in a sealed reaction vessel for 18 h, then it was allowed to cool to room temperature and the solvent was evaporated under reduced pressure to give the crude hydrazone that was taken on without further purification. The crude oil was dissolved in anhydrous 2-methoxyethyl ether (20 % vol/wt.) under argon, and anhydrous zinc chloride (2 mol equiv.) was added. The reaction mixture was heated at 172 °C in a sealed reaction vessel for 24 h. The reaction mixture was filtered through Celite, the filter was washed with toluene and the solvent was removed by short path distillation to leave a crude residue that was brought up in EtOAc (10 mL) and washed with water (10 mL). The aqueous phase was extracted with EtOAc (3 x 5 mL) and the combined organics were washed with brine (10 mL) and dried over anhydrous Na_2SO_4. Concentration under reduced pressure gave the crude indole, which was purified by column chromatography on silica using (EtOAc) as eluent to give the indole. The regioisomers **166/167** and **170/171** were separated by preparative TLC, using 10:6:1 hexanes, ethyl acetate, and methanol. The plate was subjected to the chamber's solvent 3 times, allowing the plate to dry in between runs.

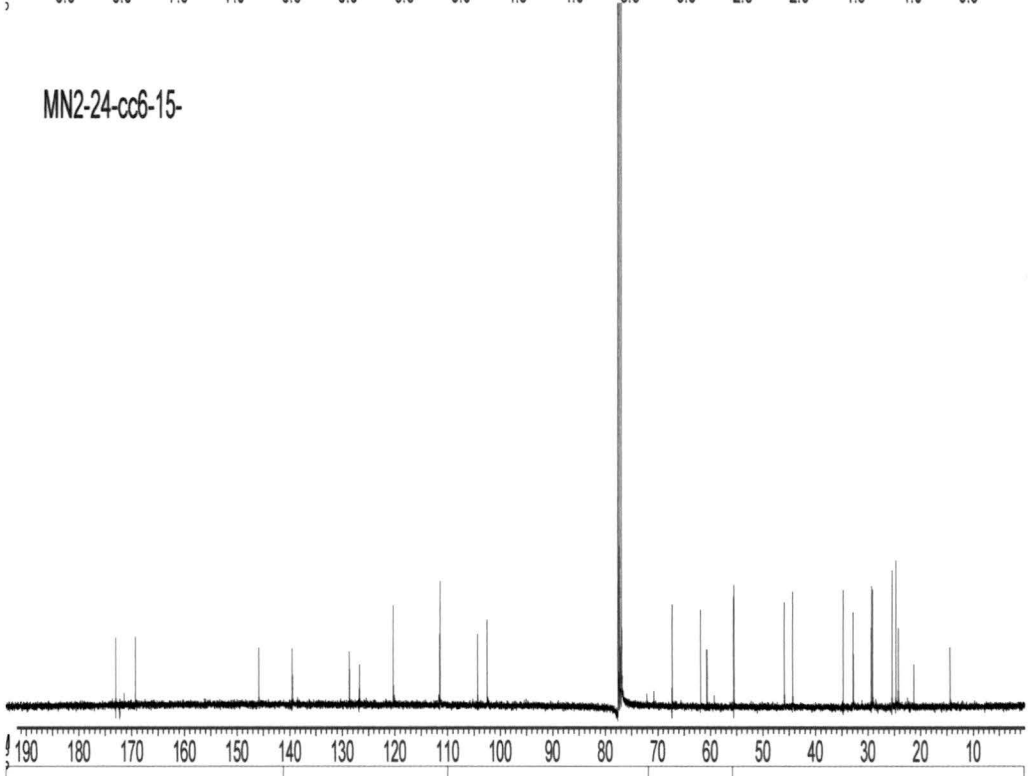
7-Methoxyindole (165). (from 54 mg of **146** and 50 mg of (2-methoxy-phenyl)-hydrazine: 35%, 26 mg, brown oil) ^{1}H NMR (300 MHz, $CDCl_3$) 1.21 (3H, s), 1.30 (3H, s), 1.89 (1H, ddd, J = 12.9, 7.5, 7.5 Hz), 1.96-2.13 (4H, m), 2.38 (1H, dd, J = 9.6, 4.2 Hz), 2.77 (1H, ddd, J = 12.6, 6.9, 6.0 Hz), 2.88 (1H, d, J = 18.0 Hz), 3.51 (2H, t, J = 6.9 Hz), 3.88 (1H, d, J = 18.9 Hz), 3.92 (3H, s), 6.34 (1H, br s), 6.62 (1H, d, J = 7.8 Hz), 7.01 (1H, t, J = 7.8 Hz), 7.12 (1H, d, J = 7.8 Hz), 8.16 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): 24.23, 24.68, 25.43, 29.15, 29.35, 32.84, 34.75, 44.37, 45.94, 55.52, 61.84, 67.26, 102.49, 104.29, 111.45, 120.32, 126.73, 128.62, 139.53, 145.89, 169.31, 173.02. IR (NaCl): 3583, 3244, 2957, 2359, 1683, 1576, 1397, 1305, 1256, 1075, 776, 729, 666 cm^{-1}. HRMS (FAB+): Calc. for $C_{22}H_{25}N_3O_3$ (MH^+): 380.19295. Found: 380.195818 (MH^+).



165

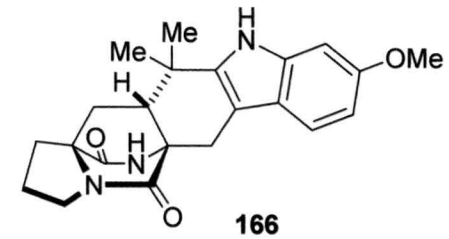


MN2-24-cc6-15-



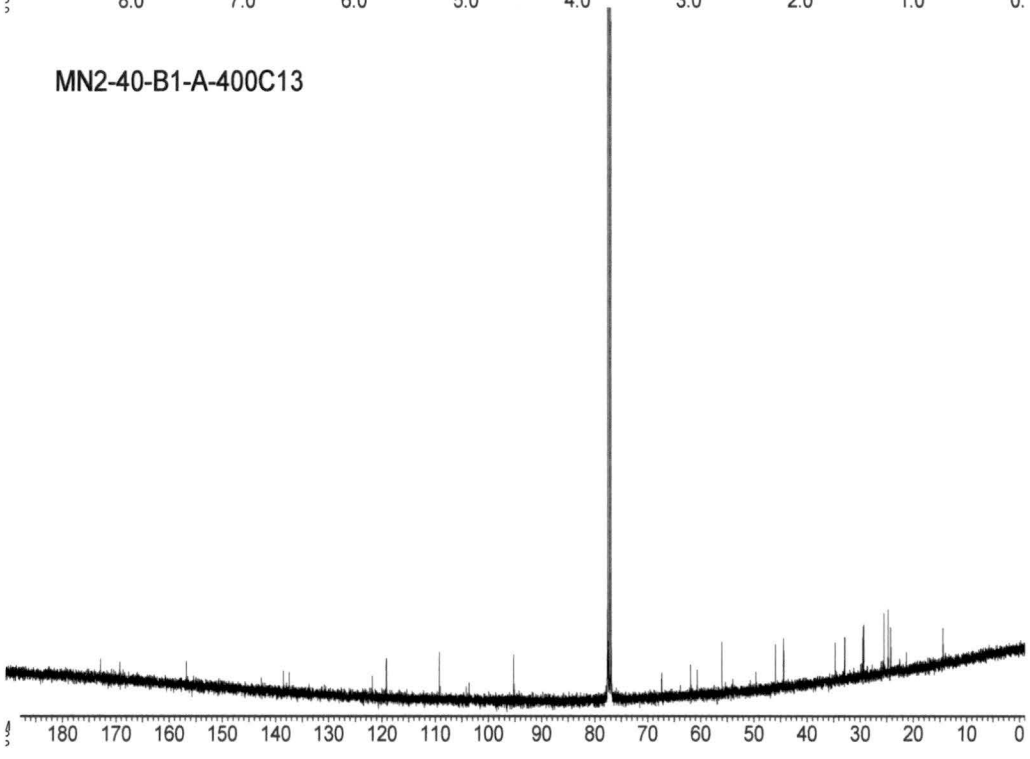
File name: MN2-24-cc6-15	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CDCl3	SW: 6000	TE: 302	STANDARD 1H OBSERVE

6-Methoxyindole (166). (from 141 mg of **146** and 106 mg of (3-methoxy-phenyl)-hydrazine: 7%, 14 mg, yellow oil) ^{1}H NMR (400 MHz, $CDCl_3$) 1.29 (3H, s), 1.32 (3H, s), 1.88 (1H, ddd, J = 12.4, 8.4, 8.0 Hz), 2.05-2.16 (4H, m), 2.35 (1H, dd, J = 9.6, 5.2 Hz), 2.83 (1H, ddd, J = 14.0, 7.2, 6.8 Hz), 2.88 (1H, d, J = 14.0 Hz), 3.56 (2H, t, J = 7.6 Hz), 3.85 (3H, s), 3.92 (1H, d, J = 9.2 Hz), 5.73 (1H, br s), 6.80 (1H, d, J = 8.8 Hz), 6.86 (1H, s), 7.39 (1H, d, J = 9.6 Hz), 7.72 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): 24.36, 24.85, 25.65, 29.61, 33.01, 34.85, 44.52, 46.03, 56.14, 60.75, 62.04, 67.45, 91.88, 94.79, 95.35, 100.07, 104.30, 109.34, 119.25, 121.91, 156.93, 173.01. IR (NaCl): 3583, 3284, 2957, 2360, 1685, 1457, 1404, 1258, 1158, 1107, 1031, 665 cm^{-1}. HRMS (FAB+): Calc. for $C_{22}H_{25}N_3O_3$ (MH^+): 380.19295. Found: 380.195779 (MH^+).

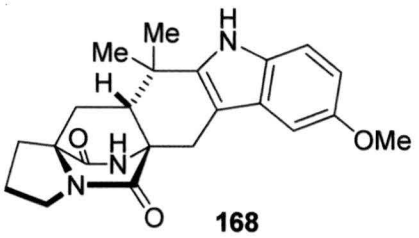


166

MN2-40-B1-A-400C13

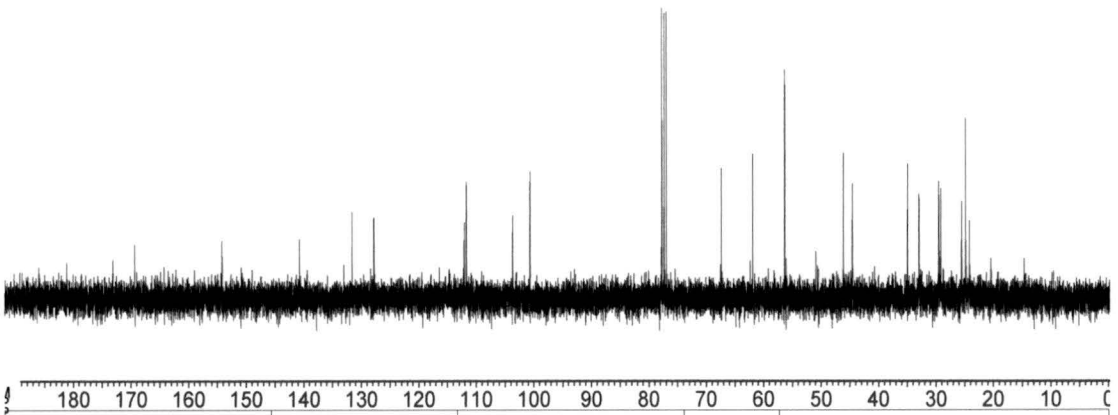


5-Methoxyindole (168). (from 70 mg of **146** and 88 mg of (4-methoxy-phenyl)-hydrazine: 27%, 26 mg, yellow oil) ^{1}H NMR (300 MHz, $CDCl_3$) 1.22 (3H, s), 1.28 (3H, s), 1.89 (1H, ddd, J = 13.2, 7.5, 5.7 Hz), 1.96-2.09 (4H, m), 2.28 (1H, dd, J = 9.6, 4.2 Hz), 2.76 (1H, ddd, J = 12.9, 7.2, 6.3 Hz), 2.85 (1H, d, J = 18.0 Hz), 3.50 (2H, t, J = 6.6 Hz), 3.77 (1H, d, J = 6.3 Hz), 3.83 (3H, s), 6.28 (1H, br s), 6.79 (1H, dd, J = 8.7, 2.7 Hz), 6.95 (1H, d, J = 2.4 Hz), 7.17 (1H, d, J = 8.7 Hz), 7.99 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): 24.13, 24.81, 25.48, 29.12, 29.46, 32.91, 34.91, 44.49, 46.02, 56.24, 61.86, 67.33, 100.65, 103.66, 111.65, 112.05, 127.76, 131.57, 132.34, 140.73, 154.18, 169.26. IR (NaCl): 3583, 3318, 2960, 2360, 1683, 1457, 1404, 1288, 1203, 1171, 1092, 1030, 734, 665 cm^{-1}. HRMS (FAB+): Calc. for $C_{22}H_{25}N_3O_3$ (MH^+): 380.19295. Found: 380.196995 (MH^+).



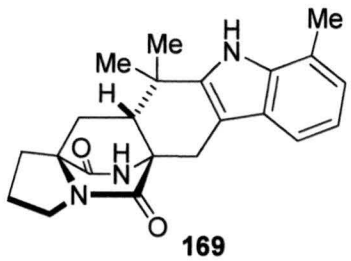
168

MN2-29-C1

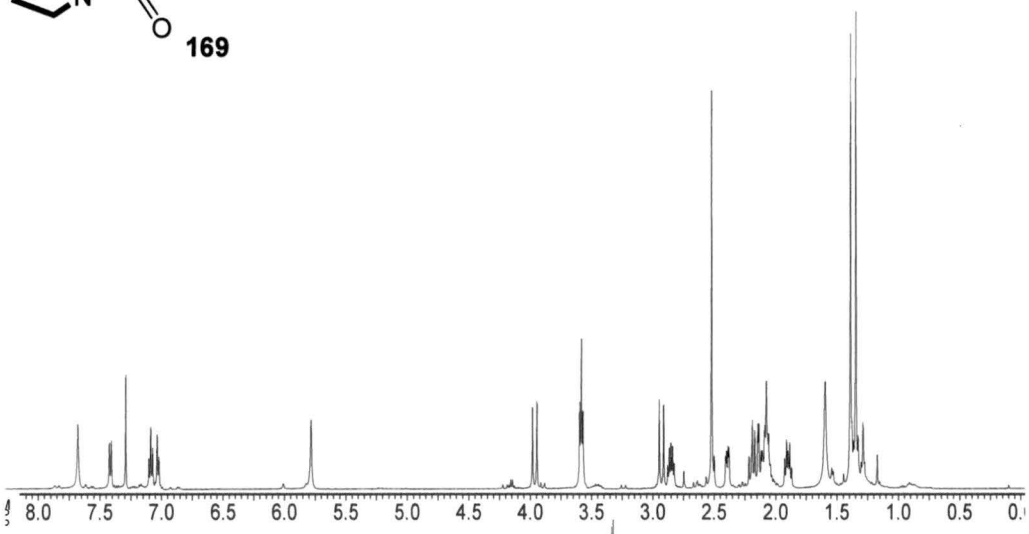


File name: MN2-29-B3	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 31994
Date: 30-Dec-1899	Solvent: CDCl3	SW: 5995	TE: 302	STANDARD 1H OBSERVE

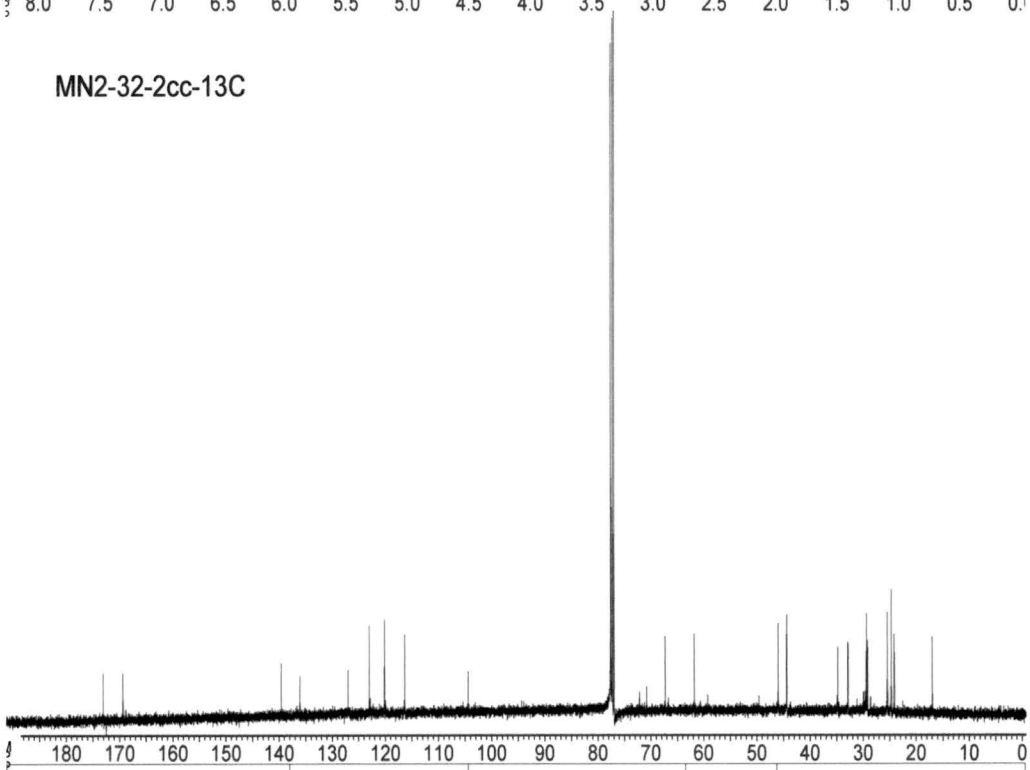
7-Methylindole (169). from 64 mg of **146** and 50 mg of (2-methyl-phenyl)-hydrazine: 30%, 25 mg, yellow oil) ^{1}H NMR (500 MHz, $CDCl_3$) 1.33 (3H, s), 1.37 (3H, s), 1.88 (1H, ddd, J = 12.5, 8.0, 7.0 Hz), 2.03-2.20(4H, m), 2.37 (1H, dd, J = 10.0, 4.0 Hz), 2.50 (3H, s), 2.83 (1H, ddd, J = 12.5, 6.5, 6.0 Hz), 2.91 (1H, d, J = 17.5 Hz), 3.56 (2H, t, J = 7.0 Hz), 3.94 (1H, d, J = 18.0 Hz), 5.77 (1H, br s), 7.02 (1H, d, J = 7.5 Hz), 7.08 (1H, t, J = 7.5 Hz), 7.40 (1H, d, J = 7.5 Hz), 7.66 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): 16.95, 24.12, 24.68, 25.44, 28.49, 29.36, 32.85, 34.75, 44.38, 45.99, 61.79, 67.26, 104.42, 116.29, 120.11, 122.98, 126.96, 136.08, 139.55, 169.32, 173.05. IR (NaCl): 3583, 3317, 2958, 1674, 1440, 1405, 1337, 1181, 1083, 775, 665 cm^{-1}. HRMS (FAB+): Calc. for $C_{22}H_{25}N_3O_2$ (MH^+): 364.19803. Found: 364.198917 (MH^+).



169

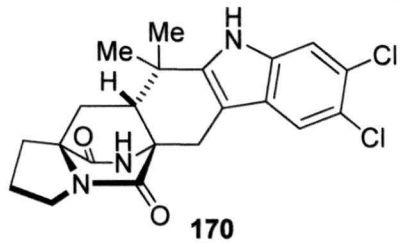


MN2-32-2cc-13C

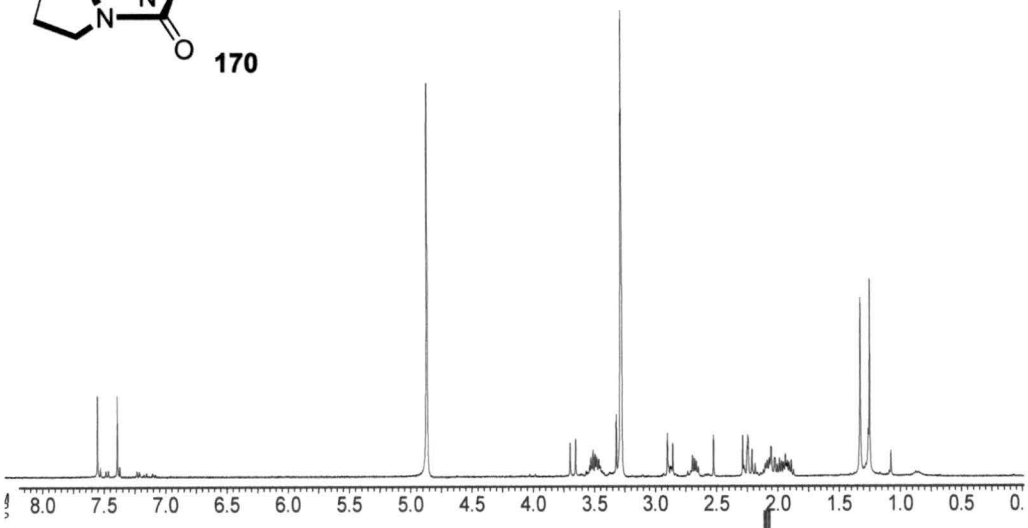


File name: MN2-32-H500	Owner:	SF: 500.1751 MHz	NS:	SI: 32768, TD: 30272
Date: 30-Dec-1899	Solvent: CDCl3	SW: 8000	TE: 298	Meriah/Williams

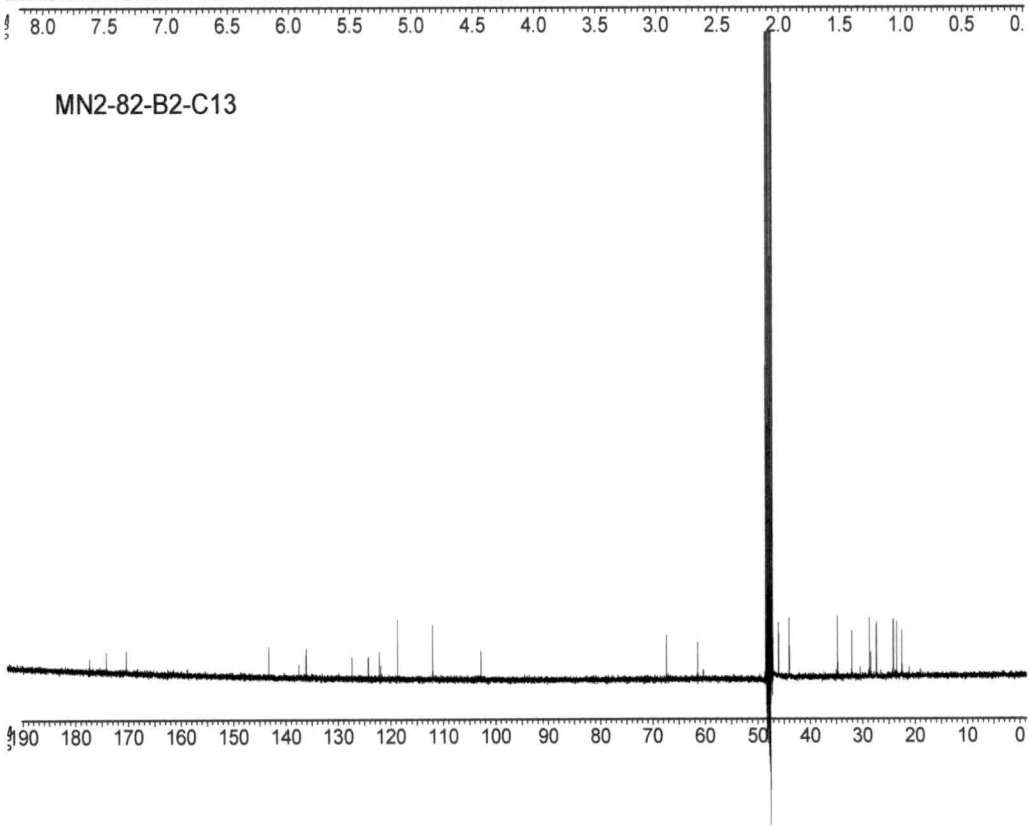
5,6-Dichloroindole (170). (from 70 mg of **146** and 81 mg of 3,4-dichlorophenylhydrazine hydrochloride: 16%, 17 mg, yellow oil) ^{1}H NMR (400 MHz, $CDCl_3$) 1.31 (3H, s), 1.34 (3H, s), 1.88 (1H, ddd, J = 15.6, 8.8, 6.8 Hz), 2.04-2.21 (4H, m), 2.45 (1H, dd, J = 9.6, 4.4 Hz), 2.80-2.86 (2H, m), 3.56 (2H, t, J = 7.2 Hz), 3.91 (1H, d, J = 18 Hz), 5.82 (1H, br s), 7.41 (1H, s), 7.58 (1H, s), 7.87 (1H, br s). ^{13}C NMR (100 MHz, CD_3OD): 23.89, 24.85, 25.51, 28.75, 30.05, 33.41, 36.13, 45.36, 47.35, 62.77, 68.77, 104.13, 113.35, 120.02, 123.48, 125.63, 128.72, 137.46, 144.57, 171.76, 175.53. IR (NaCl): 3583, 3272, 2923, 1669, 1404, 665 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{21}Cl_2N_3O_2$ (MH^+): 418.108908. Found: 418.106941 (MH^+).



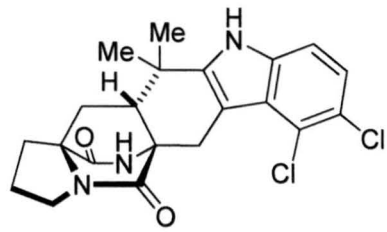
170



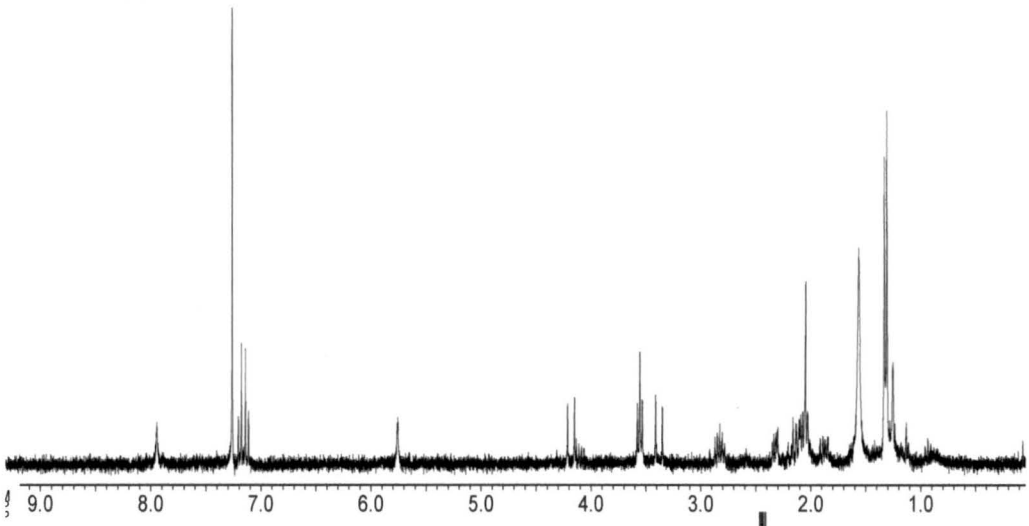
MN2-82-B2-C13



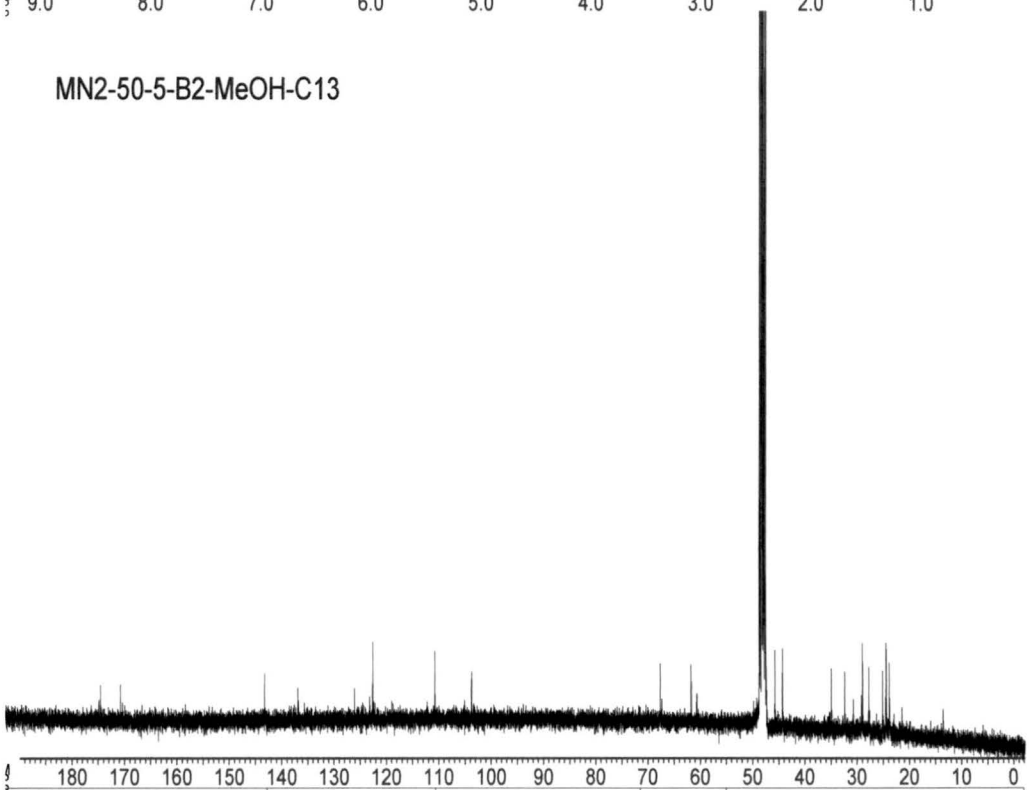
4,5-Dichloroindole (171). (from 70 mg of **146** and 81 mg of 3,4-dichlorophenylhydrazine hydrochloride: 8%, 8 mg, white residue) ^{1}H NMR (300 MHz, $CDCl_3$) 1.32 (3H, s), 1.34 (3H, s), 1.89 (1H, dd, J = 13.5, 7.2), 2.04-2.17 (4H, m), 2.34 (1H, dd, J = 10.2, 4.2 Hz), 2.84 (1H, ddd, J = 13.2, 6.9, 6.3), 3.39 (1H, d, J = 19.2 Hz), 3.56 (2H, t, J = 6.9 Hz), 4.19 (1H, d, J = 18.6 Hz), 5.77 (1H, br s), 7.17 (2H AB, J = 8.7 Hz, $\Delta\nu$ = 22.94 Hz), 7.96 (1H, br s). ^{13}C NMR (100 MHz, CD_3OD): 25.62, 26.22, 26.93, 29.57, 30.76, 34.16, 36.71, 46.07, 47.51, 63.52, 69.44, 105.52, 112.51, 120.73, 124.35, 127.83, 138.63, 145.06, 172.52, 176.29. IR (NaCl): 3583, 3273, 2923, 2360, 1674, 1429, 1314, 1249, 794, 665 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{21}Cl_2N_3O_2$ (MH^+): 418.108908. Found: 418.106941 (MH^+).



171



MN2-50-5-B2-MeOH-C13

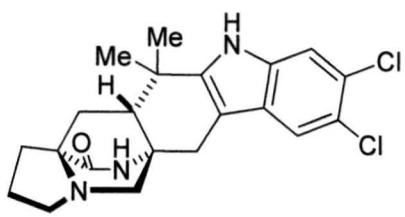


File name: MN2-78-7-B1	Owner:	SF: 300.1607 MHz	NS:	SI: 32768, TD: 31994
Date: 30-Dec-1899	Solvent: CDCl3	SW: 5995	TE: 302	STANDARD 1H OBSERVE

5.4.2 General procedure for the reduction of the tertiary and secondary lactams of **170** and **171**:

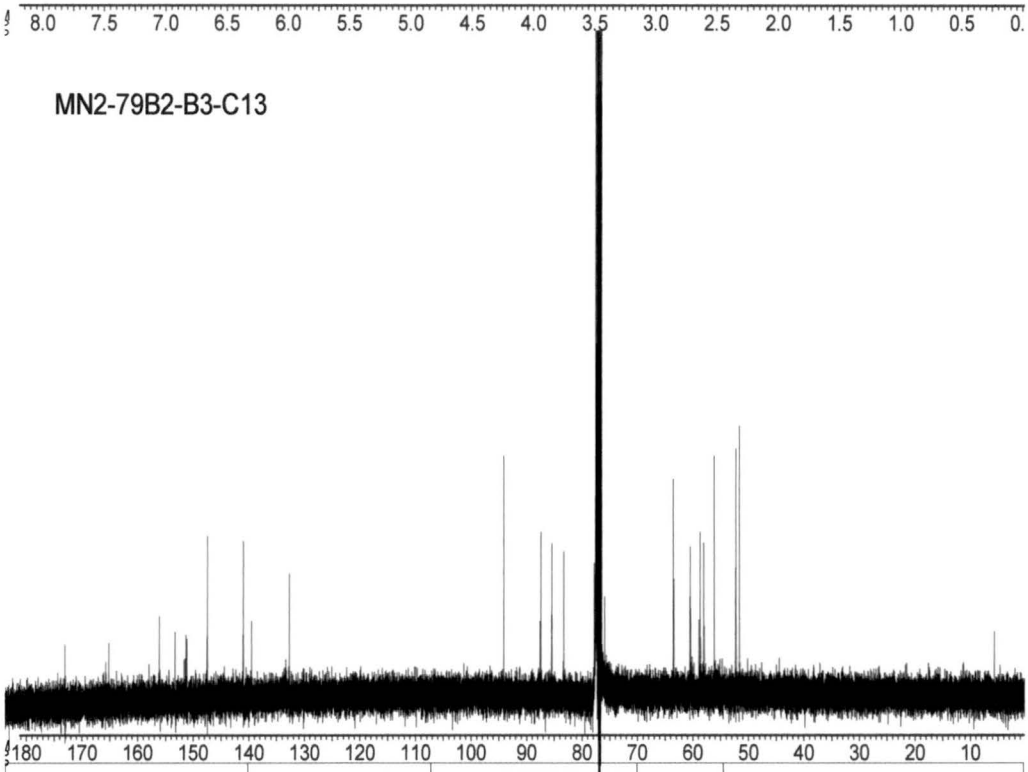
To a solution of the indole (1 equiv) in dichloromethane (10% vol/wt.) at 0°C, was added 5 equivalents of DIBAL-H (1M in dichloromethane). The solution was stirred at 0°C for one hour, then at room temperature for one hour. The solution was brought back to 0°C so that another portion of DIBAL-H (3.2 equiv.) could be added. The solution was again stirred at 0°C for 1 hour and at room temperature for 1 hour. After being brought back to 0°C, the reaction was quenched by the addition of saturated aq. NH_4Cl. The layers were separated, and the aqueous layer was extracted with dichloromethane (3 x 10 mL). The organic layer was washed with brine, dried with Na_2SO_4, and concentrated *in vacou* to give the crude product. The product was purified by preparative TLC, using 10:6:1 hexanes, ethyl acetate, and methanol. The plate was subjected to the chamber's solvent 3 times, allowing the plate to dry in between runs. The secondary reduced lactam product was obtained from the band with the lowest R_f, (0.2), and the tertiary reduced lactam product was obtained from the band with the higher R_f (0.5).

***Epi*-malbrancheamide (172).** (from 8 mg of **170**: 39%, 3 mg, colorless oil) ^{1}H NMR (500 MHz, CD_3OD) 1.33 (3H, s), 1.42 (3H, s), 1.44-1.48 (1H, m), 1.84-1.90 (2H, m), 1.93-2.03 (2H, m), 2.13-2.19 (2H, m), 2.26 (1H, dd, J = 10.0, 1.5), 2.50-2.55 (1H, m), 2.84 (2H, m), 3.04-3.08 (1H, m), 3.43 (1H, d, J = 10.5 Hz), 7.39 (1H, s), 7.48 (1H, s) ^{13}C NMR (125 MHz, CD_3OD): 23.62, 24.23, 28.17, 30.06, 30.67, 32.51, 35.56, 47.95, 55.36, 57.45, 59.40, 66.08, 104.70, 113.02, 119.48, 123.27, 125.25, 128.10, 137.16, 145.03, 176.47. IR (NaCl): 3583, 3313, 2922, 1653, 1471, 1260, 1260 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{23}Cl_2N_3O$ (MH^+): 404.129643. Found: 404.128497 (MH^+).

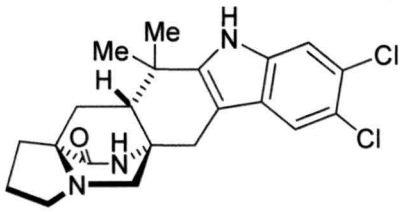


epi-malbrancheamide, **172**

MN2-79B2-B3-C13

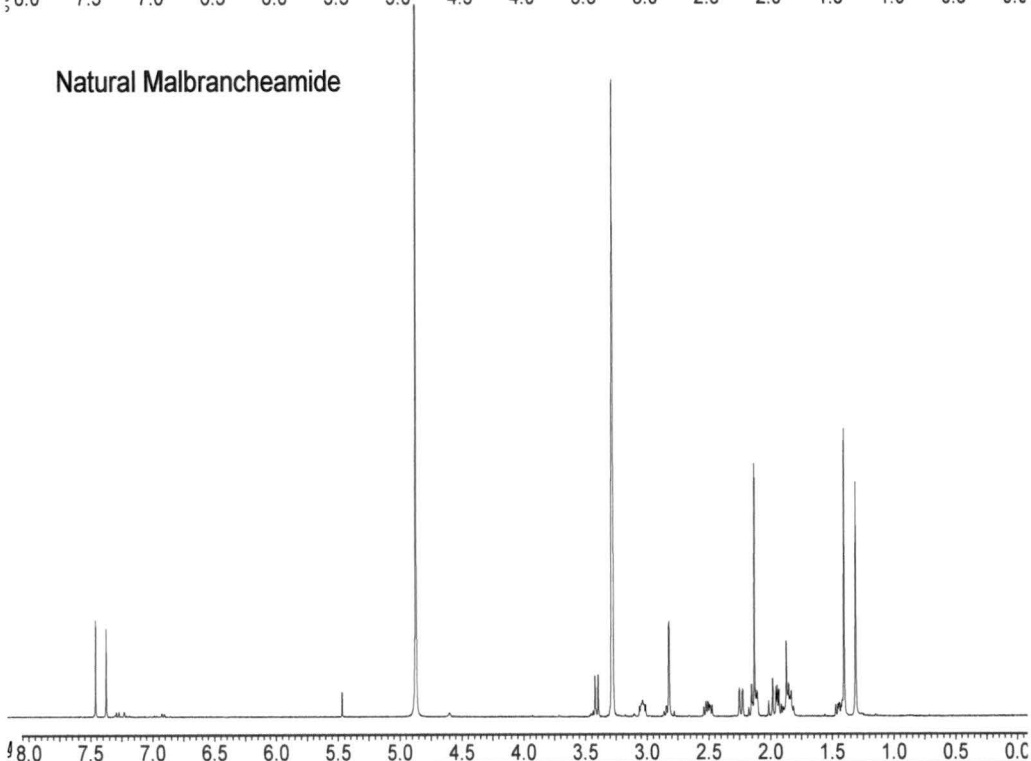


File name: MN2-85-B2	Owner:	SF: 400.1099 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CD3OD	SW: 6983	TE: 298	STANDARD 1H OBSERVE



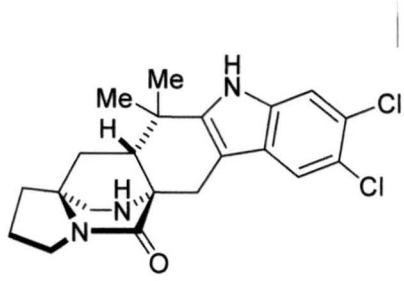
epi-malbrancheamide, **172**

Natural Malbrancheamide

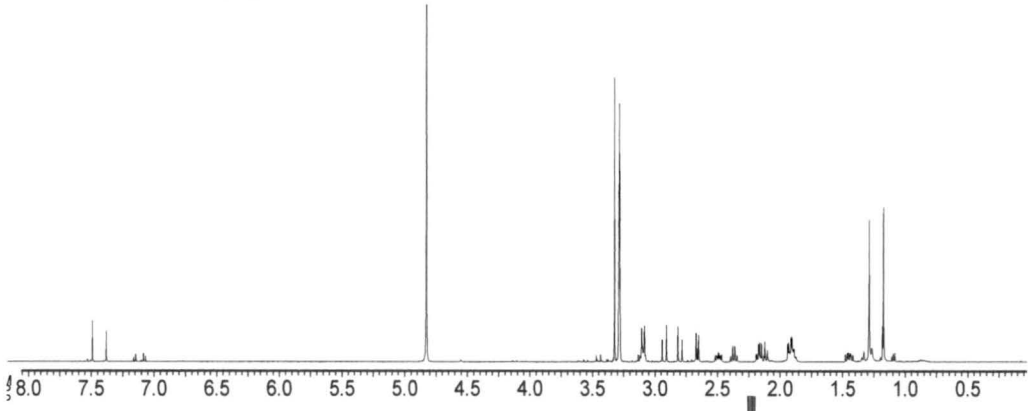


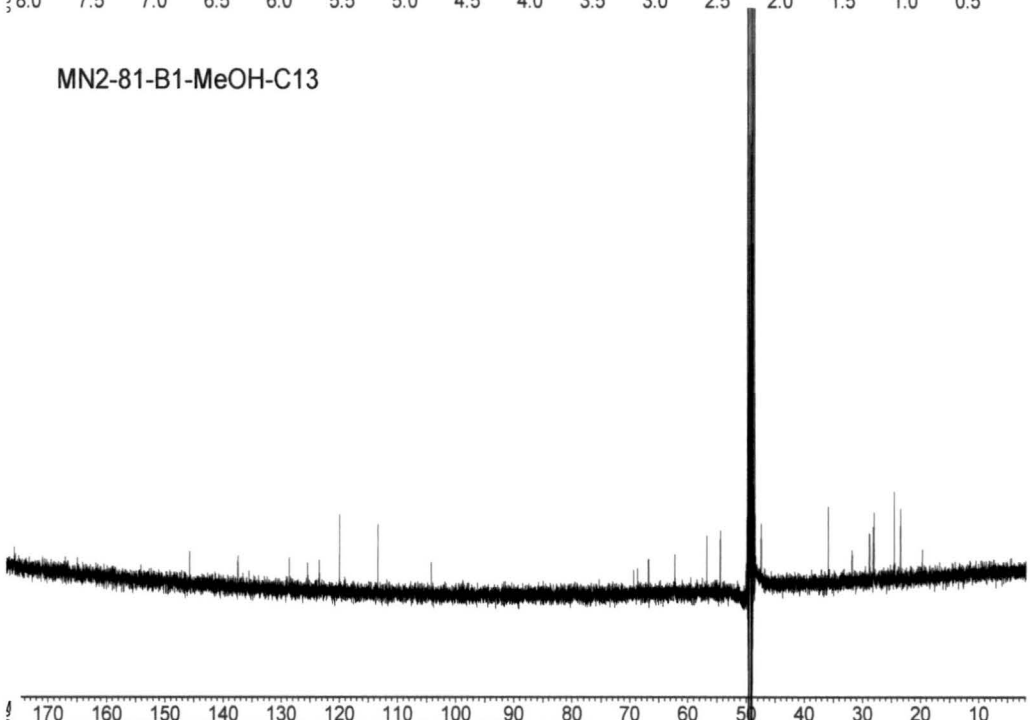
File name: MN2-79-B2-B3-H500	Owner:	SF: 500.1771 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: cd3od	SW: 8000	TE: 298	MN2-79-B1-B1

Indole (173). (from 8 mg of **170**: 39%, 3 mg colorless oil) ^{1}H NMR (500 MHz, CD_3OD) 1.17 (3H, s), 1.29 (3H, s), 1.42-1.48 (1H, m), 1.89-1.94 (3H, m), 2.10-2.19 (2H, m), 2.37 (1H, q, J = 17.0, 9.0), 2.47-2.52 (1H, m), 2.66 (1H, d, J = 10.5 Hz), 2.86 (2H AB, J = 8.7 Hz, $\Delta\nu$ = 60.99 Hz), 3.08-3.11 (2H, m), 7.38 (1H, s), 7.49 (1H, s). ^{13}C NMR (100 MHz, CD_3OD): 23.43, 24.53, 27.99, 28.15, 28.79, 31.76, 35.87, 47.40, 54.40, 56.73, 62.23, 66.78, 104.15, 113.30, 119.91, 123.37, 125.37, 128.56, 137.39, 145.70, 175.73. IR (NaCl): 3583, 2927, 1718, 1558, 1457, 1363, 1226, 665 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{23}Cl_2N_3O$ (MH^+): 404.129643. Found: 404.129725 (MH^+).



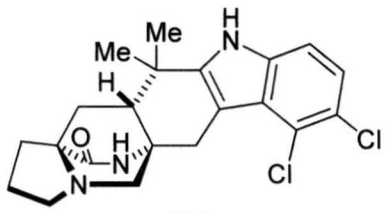
173





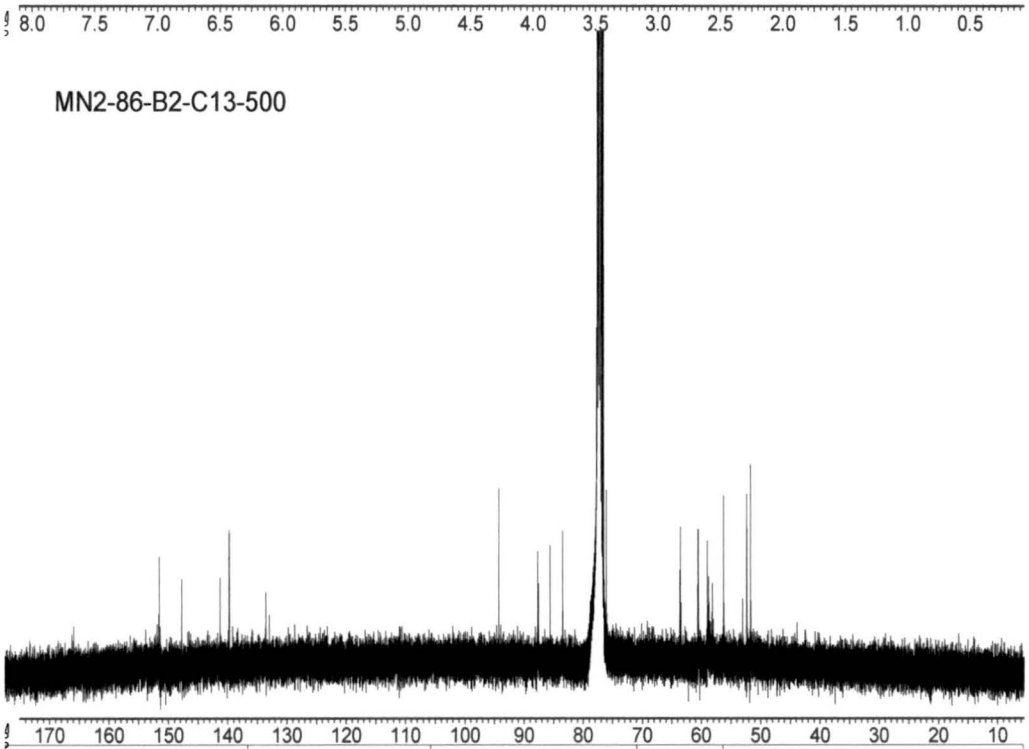
File name: MN2-79-B1-B1_h1-500	Owner:	SF: 500.1771 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: cd3od	SW: 8000	TE: 298	MN2-79-B1-B1

Indole (174). (from 6 mg of **171**: 35%, 2 mg, colorless oil) ^{1}H NMR (400 MHz, CD_3OD) 1.34 (3H, s), 1.45 (3H, s), 1.47-1.50 (1H, m), 1.85-1.94 (2H, m), 1.96-2.04 (2H, m), 2.12-2.19 (2H, m), 2.27 (1H, d, J = 10.0), 2.50-2.57 (1H, m), 2.85 (2H, m), 3.10 (1H, m), 3.49 (1H, d, J = 10.0 Hz), 7.15 (2H AB, J = 8.8 Hz, $\Delta\nu$ = 26.44 Hz). ^{13}C NMR (125 MHz, CD_3OD): 23.73, 24.40, 28.28, 30.18, 30.79, 32.60, 35.55, 48.10, 55.52, 57.61, 59.74, 66.25, 105.56, 111.73, 113.26, 119.73, 123.54, 137.45, 137.93, 145.22, 176.82. IR (NaCl): 3583, 3259, 2924, 2853, 2360, 1670, 1456, 1315 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{23}Cl_2N_3O$ (MH^+): 404.129643. Found: 404.128609 (MH^+).



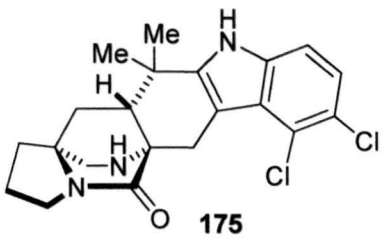
174

MN2-86-B2-C13-500

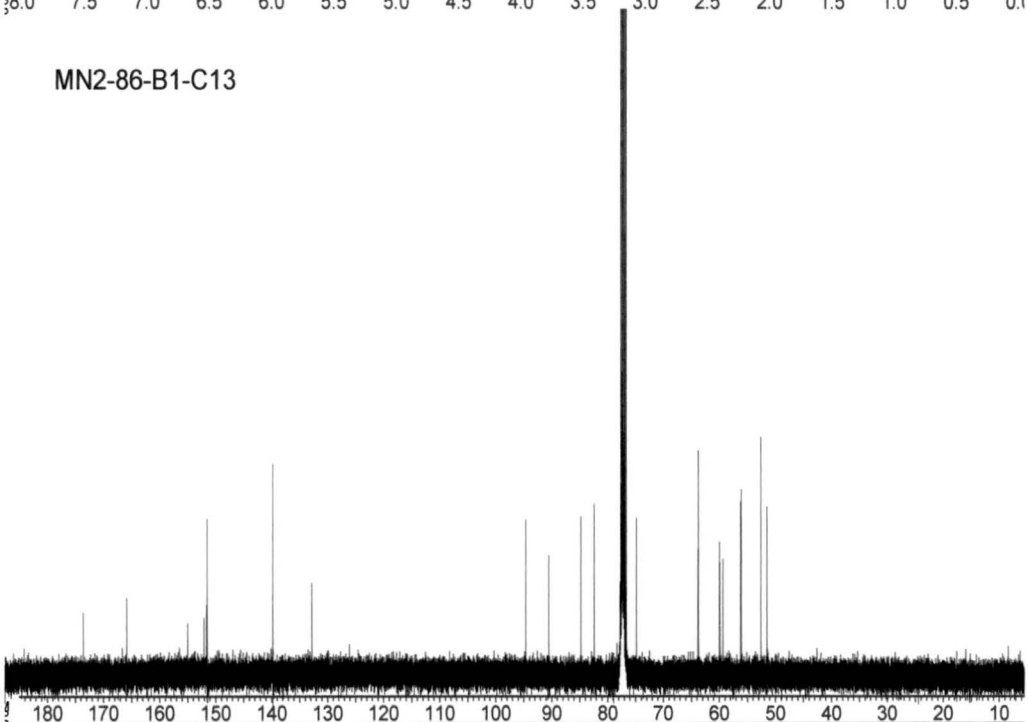


File name: MN2-86-B2	Owner:	SF: 400.1099 MHz	NS:	SI: 32768, TD: 32000
Date: 30-Dec-1899	Solvent: CD3OD	SW: 6983	TE: 298	STANDARD 1H OBSERVE

Indole (175). (from 6 mg of **171**: 53%, 3 mg colorless oil) ^{1}H NMR (400 MHz, CD_3OD) 1.18 (3H, s), 1.29 (3H, s), 1.41-1.48 (1H, m), 1.86-1.93 (3H, m), 1.99 (1H, d, J = 2.0 Hz), 2.09-2.18 (2H, m), 2.37 (1H, q, J = 17.2, 8.8 Hz), 2.46-2.52 (1H, m), 2.66 (1H, d, J = 10.4 Hz), 3.08-3.13 (3H, m), 3.45 (1H, d, J = 17.6 Hz), 7.11 (2H AB, J = 8.4 Hz, $\Delta\nu$ = 29.78 Hz). ^{13}C NMR (125 MHz, CD_3OD): 23.50, 24.61, 28.08, 28.21, 31.38, 31.96, 35.78, 46.87, 54.42, 56.82, 62.56, 66.64, 104.82, 111.77, 123.48, 123.60, 124.10, 126.98, 137.88, 145.56, 176.02. IR (NaCl): 3583, 3264, 2925, 2853, 1668, 1456, 1315, 1247, 665 cm^{-1}. HRMS (FAB+): Calc. for $C_{21}H_{23}Cl_2N_3O$ (MH^+): 404.129643. Found: 404.129383 (MH^+).



175



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Date: 30-Dec-1899	Solvent: CD3OD	SW: 6983	TE: 298	STANDARD 1H OBSERVE

5.4.3 Procedure to selectively reduce the secondary lactam of **170**:

To a solution of the indole **170** (4 mg, 0.0096 mmol) in THF (0.8 mL) at -78°C, was added LiHMDS (1M in THF, 0.019 mmol). After the solution was stirred for 30 minutes, Et_3Al (1M in hexanes, 0.020 mmol) was added and the solution was stirred at -78°C for 15 minutes. DIBAL-H (1M in toluene, 0.029 mmol) was added, and the mixture was stirred at -78°C for 10 minutes, then 0°C for 3 hours. The solution was allowed to warm to RT and was quenched with MeOH (0.1 mL) and taken up in EtOAc (10 mL). The organics were washed with saturated aqueous NH_4Cl, followed by brine, then dried over Na_2SO_4. The solvent was removed *in vacou*, and the crude product was purified by preparative TLC, using 10:6:1 hexanes, ethyl acetate, and methanol. The plate was subjected to the chamber's solvent 3 times, allowing the plate to dry in between runs. The secondary reduced lactam **173** product was obtained from the band with the R_f, (0.2) in 39% yield.

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Appendix 1. List of Publications

Adams, L. A.; Valente, M. W. N.; Williams, R. M., A concise synthesis of *d,l*-brevianamide B via a biomimetically-inspired IMDA construction. *Tetrahedron* **2006,** 62, (22), 5195-5200.

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