DISSERTATION

SYNTHETIC AND BIOSYNTHETIC STUDIES OF THE BREVIANAMIDES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY KATHLEEN MARIE HALLIGAN ENTITLED SYNTHETIC AND BIOSYNTHETIC STUDIES OF THE BREVIANAMIDES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF DISSERTATION

SYNTHETIC AND BIOSYNTHETIC STUDIES OF THE BREVIANAMIDES

The preparation of a pinacol-type rearrangement precursor towards the total synthesis of target indoxyl **8** is presented. The route contains three key steps. These include a photooxidation reaction that installs a hydroxy group in the required stereospecific position, a pinacol-type rearrangement to provide the indoxyl core structure and a Strecker reaction to extend the carbon chain to the aminonitrile derivative. Though several other routes to hydroxyindolenines were pursued, this particular pathway shows the most promise for the completion of indoxyl **8** since the crucial pinacol rearrangement was successful. This route is also amenable to ^{13}C-labeled synthesis of indoxyl **8** and azadiene **29**.

The biomimetic synthesis of brevianamide B is described. Brevianamide B and epibrevianamide A were synthesized from deoxybrevianamide E in six steps. A Diels-Alder precursor was formed *via* oxidation of the diketopiperazine moiety and proline ring followed by rearrangement to the azadiene. A [4 + 2] cycloaddition reaction took place to provide two hexacyclic adducts. Oxidation at the 3-position of the indole supplied the hydroxyindolenines. A pinacol-type rearrangement of the respective hydroxy derivatives furnished brevianamide B and epibrevianamide A.

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Finally, I would like to thank Professor Robert M. Williams who provided financial resources necessary to pursue this synthesis. In addition, he provided an environment conducive toward intellectual discovery, personal growth and an eagerness for chemistry.

DEDICATION

For my parents,

John and Mary

and my brother and sisters,

Colleen, Maureen, and John

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ABBREVIATIONS

9-BBN	9-borobicyclo[3.3.1]nonane
Boc	*tert*-butoxycarbonyl
Cbz	benzyloxycarbonyl
m-CPBA	*meta*-chloroperbenzoic acid
Collidine	2,4,6-trimethylpyridine
CSA	camphorsulfonic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
Decomp	decomposition
DIBAH	diisobutylaluminum hydride
DMAP	4-*N,N*-dimethylaminopyridine
DMAPP	dimethylallyl pyrophosphate
DME	1,2-dimethoxyethane
DMF	*N,N*-dimethylformamide
DMS	dimethyl sulfide
DMSO	dimethyl sulfoxide
EtOH	ethanol
FMO	frontier molecular orbital theory
HMPA	hexamethylphosphoramide
HOAc	acetic acid
HOMO	highest occupied molecular orbital

KHMDS	potassium bis(trimethylsilyl)amide
LDA	lithium diisopropylamine
LUMO	lowest unoccupied molecular orbital
MeOH	methanol
Ms	methanesulfonyl (mesylate)
NaHMDS	sodium bis(trimethylsilyl)amide
PMB	*p*-methoxybenzyl
PPTS	pyridinium p-toluenesulfonate
PTLC	preparatory thin layer chromatography
Quant	quantitative
Red-Al	sodium bis(2-methoxyethoxy)aluminum hydride
RT	room temperature
Sm	starting material
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TEA	triethylamine
Tf	trifluoromethanesulfonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl

Chapter 1

Introduction and Biosynthetic Background

1.1 Significance and Implications

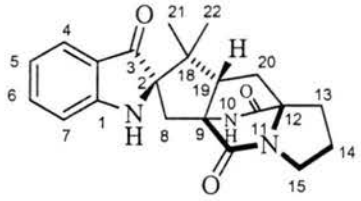
The ultimate goal of the Brevianamide / Paraherquamide project is to fully elucidate the biosynthetic pathway from primary amino acids and mevalonic acid-derived isoprene moieties to the polycyclic indole alkaloids comprising the paraherquamides and the brevianamides (**Figure 1**). The brevianamides have been shown to possess antifeedant and insecticidal effects[1] while the paraherquamides have potent antiparasitic properties.[2] It has been proposed that the core bicyclo [2.2.2] ring structure is formed *via* a biosynthetic [4 + 2] cycloaddition reaction. Synthesis and isotopic labeling of potential intermediates have been used to determine essential features of the biosynthetic pathways to these complex secondary metabolites.

Structural and stereochemical data strongly suggest a *metallo-enzyme-mediated* Diels-Alder reaction as the penultimate step in brevianamide biosynthesis.[3] If the postulated "Diels-Alderase" is isolated and characterized it will have considerable bearing not only in mechanistic enzymology, but also the potential design of an entire class of protein catalysts. The crucial element of this work is the synthesis of the putative indoxyl **8** (**Figure 1**), to assay for enzymatic activity in cell-free preparations. Synthesis of this key potential metabolite has been the primary research focus.

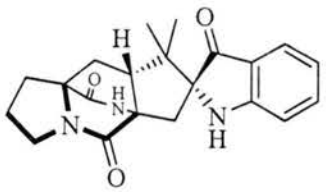
1.2 Value of Biosynthesis

Throughout history, mankind has used plant extracts both as remedies and toxins. In Native American medicine, podophyllins are used to cure warts and vinca alkaloids are used in the treatment of tumors and diabetes. Many extracts have been used as agents of death such as calabar beans and hemlock in addition to the South American curare arrow poisons.[4] Natural product research has become increasingly important in pharmaceutical drug design and discovery.

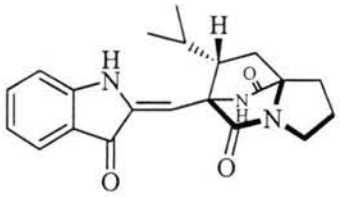
The study of biosynthetic pathways has led to highly advanced gene-targeted therapy and screening methods. These strategies are being utilized in the identification of new antibiotics, antitumor agents, and antiviral drugs. Our knowledge about enzymes of most biosynthetic pathways is limited. Their structure, mode of action, and ease of inhibition is not well understood. The continuing study of biological processes is thus, an important endeavor.



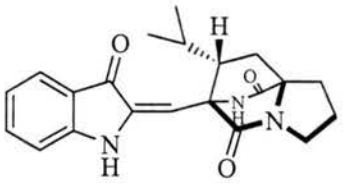
brevianamide A, **1**



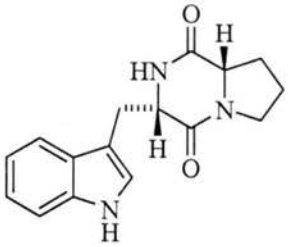
brevianamide B, **2**



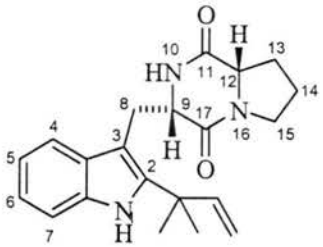
brevianamide C, **3**



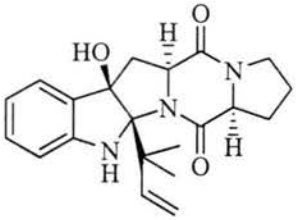
brevianamide D, **4**



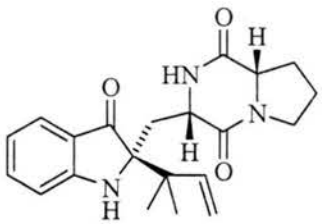
brevianamide F, **5**



deoxybrevianamide E, **6**



brevianamide E, **7**



target indoxyl **8**

Figure 1. The Brevianamides

1.3 Isolation of the Brevianamides

In 1969, Birch and Wright[5] isolated several neutral metabolites from the culture extract of *Penicillium brevicompactum*. They were named brevianamides A-E (**Figure 1**). Since then, the brevianamides have been isolated from several species of *Penicillium* molds and fungi associated with corn, rice and other stored grains (*Penicillium viridicatum, Penicillium ochraceum*).[6] The structural elucidations relied primarily on chemical degradation, spectroscopic data, and biogenetic theory due to low yields (0.1-10 mg/L) of the metabolites. A single crystal X-ray analysis of 5-bromo-brevianamide A determined the relative and absolute configuration of brevianamide A (**1**).[7] Brevianamide

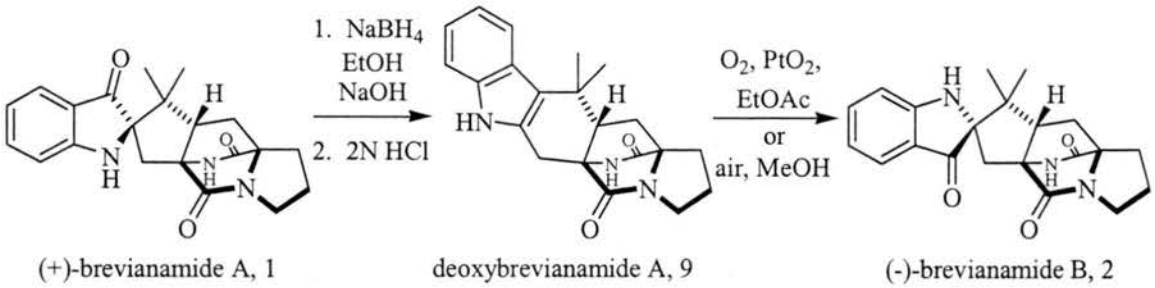
B (**2**) is epimeric to brevianamide A at the spiro-indoxyl carbon C_2. It is enantiomorphic with respect to the bicyclo [2.2.2] piperazinedione nucleus. Brevianamides C and D are thought to be artifacts since they can be obtained by irradiation of brevianamide A with white light. It was also shown that brevianamide F is consistent with *cyclo*-L-tryptophyl-L-proline.

1.4 Physical-Chemical and Structural Characteristics

Brevianamide A (**1**) is produced as the major metabolite of *P. brevicompactum* and has been extensively characterized.[5] It is a fluorescent yellow compound that can be crystallized as needles from chloroform. m.p. 190-220°C $[\alpha]_D^{25} = +413°$ (EtOH); ν_{max} 235, 256, 404 nm (28600, 7100, 3260 mμ); Found: C, 54.8; H, 5.0; N, 8.6. $C_{21}H_{23}N_3O_3$. $CHCl_3$ requires: C, 54.5; H, 5.0; N, 8.7; IR ν_{max} ($CHCl_3$) 3420, 3300, 1680-1715, 1625 cm^{-1}. ^1H NMR (100MHz) (d^5-pyridine)δ: 6.5-7.7 (4 H, AA'XX'); 8.4 (1 H, s); 4.8 (1 H, s); 3.3 (2 H, m2.94-3.18 (2 H, q, $J_{AA'} = 15$ Hz); 2.8 (2 H, m), 1.5-1.8 (5 H, m); 1.3 (3 H, s); 0.9 (3 H, s).

Brevianamide B (**2**) was isolated as the minor metabolite and is enantiomorphic to brevianamide A with respect to the bicyclo [2.2.2] ring system. Its structure was based on semi-synthetic conversion of brevianamide A (**Scheme 1**). The indoxyl carbonyl of brevianamide A was reduced with $NaBH_4$. Acid catalyzed retro-pinacol rearrangement afforded deoxybrevianamide A. Reoxidation to brevianamide B was accomplished by treatment with O_2 and PtO_2 in EtOAc or air and MeOH. It is a yellow crystalline solid that can be crystallized from MeOH or aqueous DMSO as small prisms, m.p. 324-328°C, ν_{max} 236, 254, 400 nm; ν_{max} 3240 (broad), 1695, 1670, 1618 cm-1 M^+ 365.1745 ($C_{21}H_{23}N_3O_3$ requires m/e 365.1739); m/e (relative intensity) 365 (100), 321 (15), 297

(22), 296 (60), 268 (9), 265 (7), 220 (11), 177 (8), 165 (18), 152 (11), 149 (12), 146 (11), 133 (14), 130 (21), 91 (11).



Scheme 1. Semi-Synthetic Conversion of Brevianamide A to Brevianamide B

Brevianamide C (**3**) formed an orange glass that could not be crystallized. It was the major component of the minor pigment fraction. It was present in the culture medium at a concentration of approximately 1 mg per liter. λ_{max} 234, 259, 277, 300, 450 nm; ν_{max} 3410, 3350, 1710, 1680, 1615 cm^{-1}; M^+ 365.1741 ($C_{21}H_{23}N_3O_3$ requires m/e 365.1739); m/e (relative intensity) 365 (13), 322 (15), 295 (100), 177 (5), 171 (4), 146 (3); ^1H NMR ($CDCl_3$) δ: 7.98 (s, 1H, NH), 6.8-7.7 (AA'XX', 4H, ArH), 7.34 (s, 1H, NH), 5.93 (s, 1H, CH), 3.48 (t, 2H, CH_2), 2.7-2.9 (m, 2H, CH_2), 1.7-2.5 (m, 5H), 0.84 (d, 6H, $CHMe_2$).

Brevianamide D (**4**) exists as a red glass and was present in the culture medium at a concentration of approximately 0.1mg per liter. λ_{max} 235, 264, 306, 470 nm; ν_{max} 3440, 3200, 1710 (sh), 1680, 1630, 1610 cm^{-1}; M^+ 365.1738 ($C_{21}H_{23}N_3O_3$ requires m/e 365.1739); m/e (relative intensity) 365 (25), 322 (14), 295(100), 239 (5), 177 (14), 171 (6), 146 (7), 133 (7). ^1H NMR ($CDCl_3$) δ: 10.40 (s, 1H, NH), 6.6-7.4 (m, 4H, ArH), 8.40 (s, 1H, NH), 5.90 (s, 1H, CH), 3.48 (t, 2H, CH_2), 2.7-2.9 (m, 2H, CH_2), 1.7-2.5 (m, 5H), 0.84 (q, 6H, $CHMe_2$).

Brevianamide F (**5**) was obtained from EtOH as a white crystalline solid, m.p. 173-175°C. It was present only in trace amounts in the culture medium. λ_{max} 277, 283, 292 nm; ν_{max}^{nujol} 3280, 1670, 1650 (weak), 1640 (weak) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.2-2.1 (m, 4H, methylene multiplet), 2.9-3.5 (4H, $=CCH_2$, NCH_2CH_2), 4.05 (t, 1H, NHCHCO, J = 7 c/s), 4.30 (t, 1H, NCHCO, J =6 c/s), 6.9-7.6 (m, 4H, ArH), 7.98 (d, 1H, indole NHCH=C, J = 2 c/s), 7.66 (s, 1H, NH), 10.8 b.s., 1H, indole NH); m/e (relative intensity) 283 (9), 154 (8), 130 (100), 83 (9). Found: C, 67.8, H, 6.1: N, 14.8. $C_{16}H_{17}N_3O_2$ requires C, 67.7: H, 5.9: N, 14.7%.

Brevianamide E (**7**) was obtained as a glassy solid after TLC in ether (R_f = 0.6). It was unstable to light while on a TLC plate and decomposed under basic conditions, giving a mixture of compounds; some of which possessed the indoxyl chromophore at 400 nm. $[\alpha]_D^{25}$ = -30° (EtOH); ν_{max} 239, 296 nm (7500, 2050 mμ); ν_{max} ($CHCl_3$) 3600, 3370, 1690, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.68-7.3 (m, 4 H, aromatic), 6.3 (s, 1 H, NH), 6.4 (X part of ABX system, 1 H, J_{AX} = 18.5 Hz, J_{BX} = 10.5 Hz, -CH=C$\underline{H}_2$), 5.1 (A part of ABX system, 1 H, J_{AX} = 18.5 Hz, J_{AB} = 1.5 Hz, CHC$\underline{H}_2$), 5.04 (B part of ABX system), 1 H, J_{BX} = 10.5 Hz, -CHC$\underline{H}_2$), 3.6-3.94 (m, 2 H, CH(CO)N-), 3.52 (t, 2 H, J = 7 Hz, -CH-N-), 2.86 (A part of ABX system, 1 H, J_{AB} = 13.0 Hz, J_{AX} = 11.0 Hz, $-CH_2$-), 2.61 (B part of ABX system, 1 H, J_{BX} = 8.0 Hz, J_{AB} = 13.0 Hz,–C$\underline{H}_2$-), 2.5 (s, 1 H, OH), 1.8-2.3 (m, 4 H, -CH-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-N-), 1.27 (s, 6 H, -$C(CH_3)_2$); M: 367.1899 ($C_{21}H_{25}N_3O_3$ requires: 367.1896).

1.5 Biological Activity

Brevianamides A and D were tested against the fall armyworm, *Spodoptera frugiperda*, for antifeedant activity, weight loss, and mortality. They were also screened for antifeedant activity against the tobacco budworm, *Heliothis virescens*. Brevianamides A and D were shown to be potent antifeedants against larvae of each species.[6]

1.6 Justification for Birch's Biosynthetic Proposal

Based on the metabolites isolated from cultures of *Penicillium brevicompactum*, Birch was able to speculate on possible biogenetic precursors.[5] Presence of an indole-derived unit pointed to the possibility of tryptophan as a biological precursor. The possible presence of a diketopiperazine moiety indicated that this might be linked with a second amino acid. Furthermore, the loss of C_5H_9 in the mass spectrum indicated the likely presence of a C_5-terpene unit. Feeding studies were conducted with ^{14}C-labeled tryptophan, ^{14}C-acetate, ^{14}C-mevalonolactone and L-[5-^{3}H]proline. Each showed significant incorporation into Brevianamide A. Mevalonic acid has been shown to be a virtually irreversible intermediate in terpenoid biosynthesis in molds,[8] and so its incorporation is therefore significant in terms of the presence of a terpene unit.

Based on Birch's proposed structure of Brevianamide A, Porter and Sammes postulated that the piperazinedione moiety is formed by a [4 + 2] cycloaddition reaction between the isopentenyl unit and the corresponding pyrazone (**Figure 2**).[8]

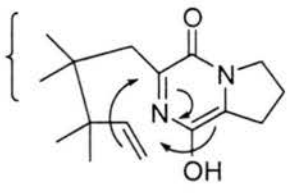
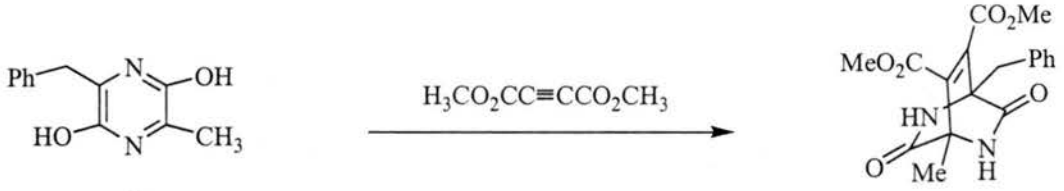


Figure 2. [4 + 2] Cycloaddition Reaction

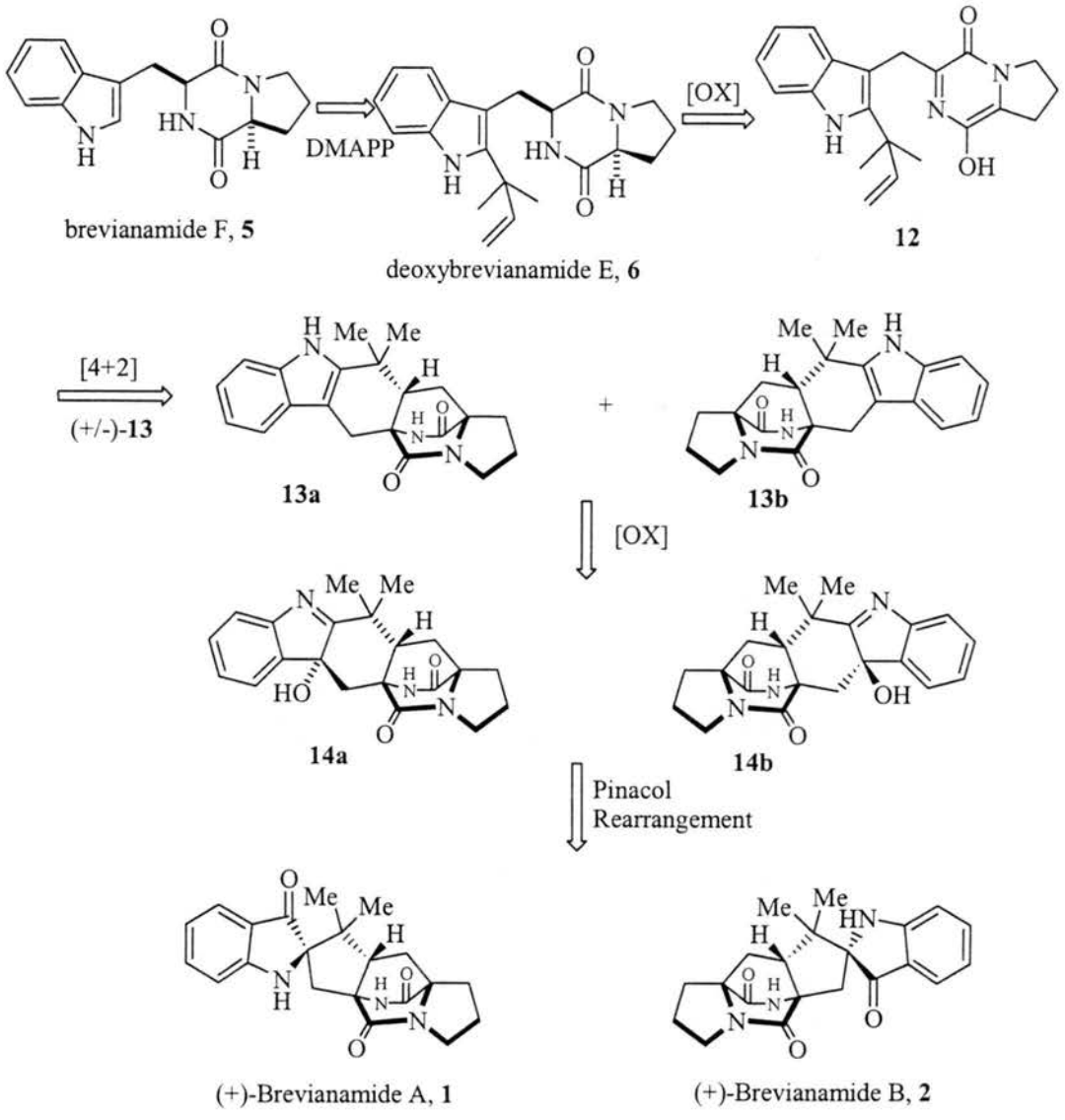
To test this hypothesis, pyrazine **10** was treated with dimethyl acetylenedicarboxylate in DMF at RT (**Scheme 2**). This resulted in the formation of bicyclic adduct **11**. Additional feeding studies showed that cyclo-L-[methylene-^{14}C]tryptophanyl-L-[5-^{3}H]proline (brevianamide F, **5**), was also incorporated intact into brevianamide A.[5]



10 11

Scheme 2. Porter and Sammes' Diels-Alder Test Reaction

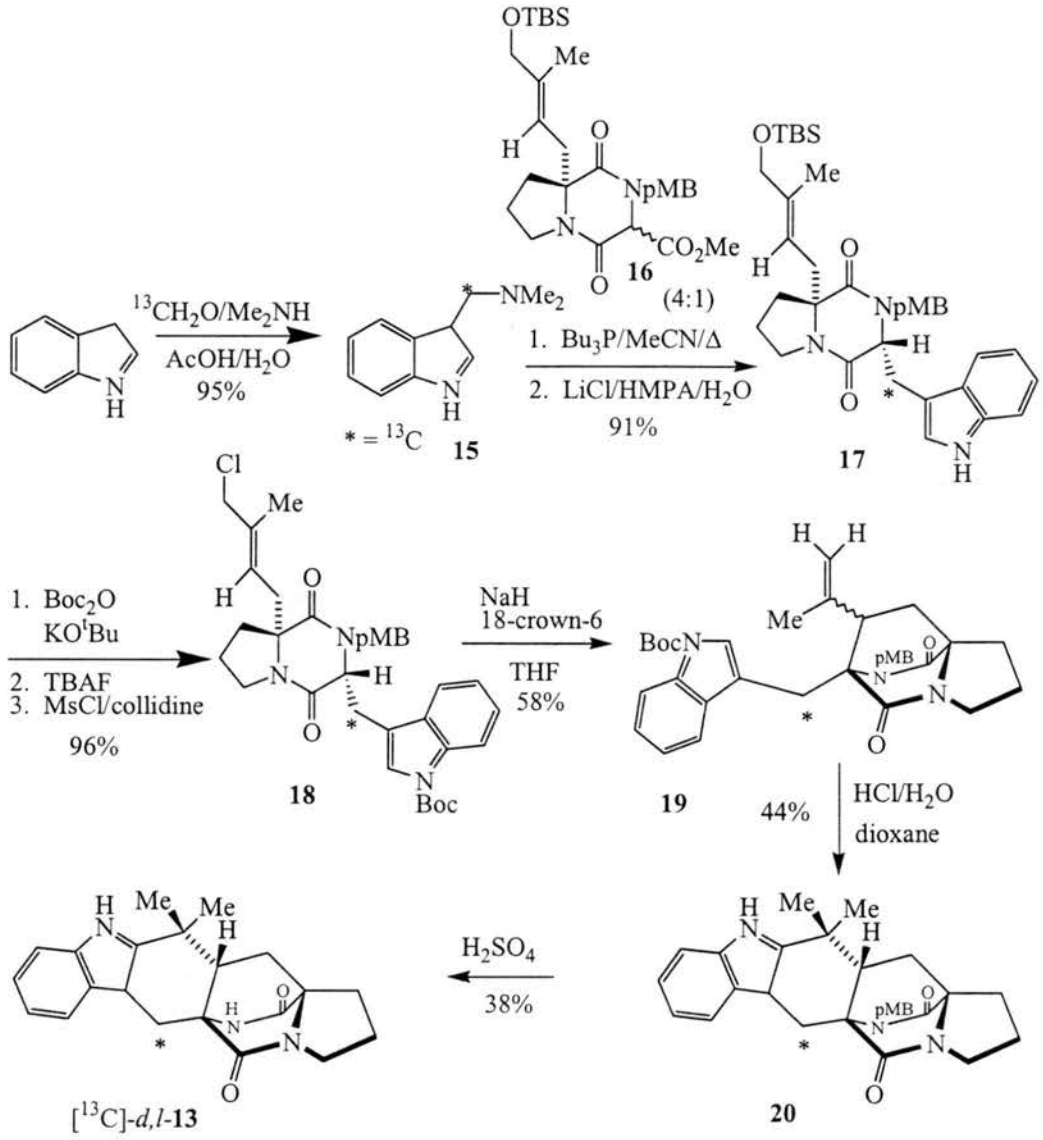
Therefore, according to biogenetic considerations, chemical degradation studies, labelled feeding studies, and the Diels-Alder studies by Porter and Sammes,[8] Birch proposed the following biosynthetic pathway (**Scheme 3**).[5]



Scheme 3. Biosynthetic Proposal

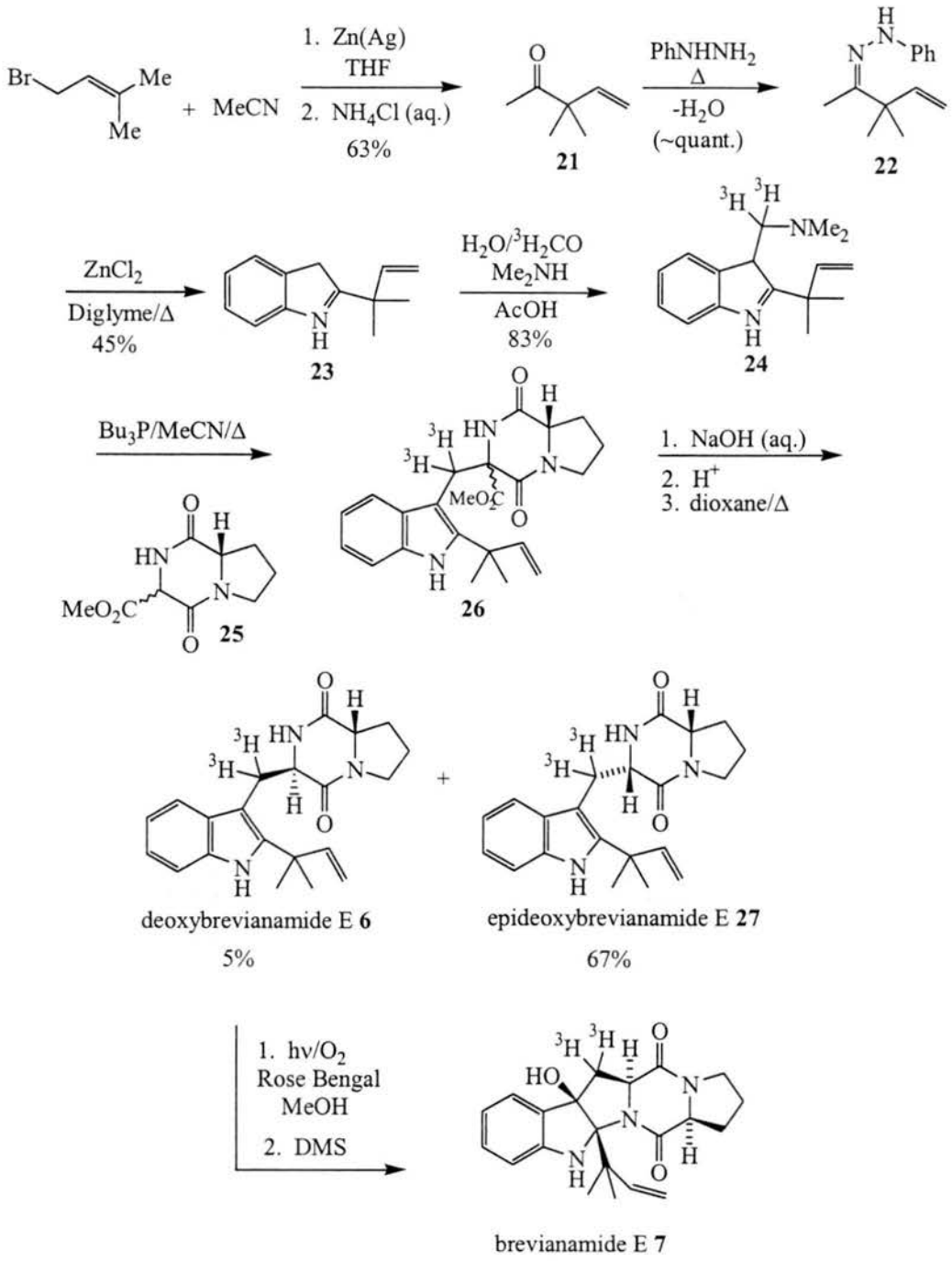
1.7 Williams' Group Experiments

Glinka and Kwast synthesized [^{13}C]-*d,l*-**13** (cyclic product), (**Scheme 4**) but found no significant incorporation of the label into brevianamide A or B.[9] This result led to the investigation of other possible biosynthetic pathways.



Scheme 4. ^{13}C-Labeled Synthesis of Cyclic Intermediate **13**

Deoxybrevianamide E (**6**) was also proposed as a biosynthetic intermediate so this substance was synthesized by Sanz-Cervera[9] with a tritium label at the trp-benzylic position, [8-^{3}H]-**6** (**Scheme 5**) using the methods of Kametani,[10] and Rousseau.[11] The label was introduced with tritiated formaldehyde during the Mannich reaction with 3,3-dimethylallylindole.



Scheme 5. ^{3}H-Labeled Synthesis of Deoxybrevianamide E **6**

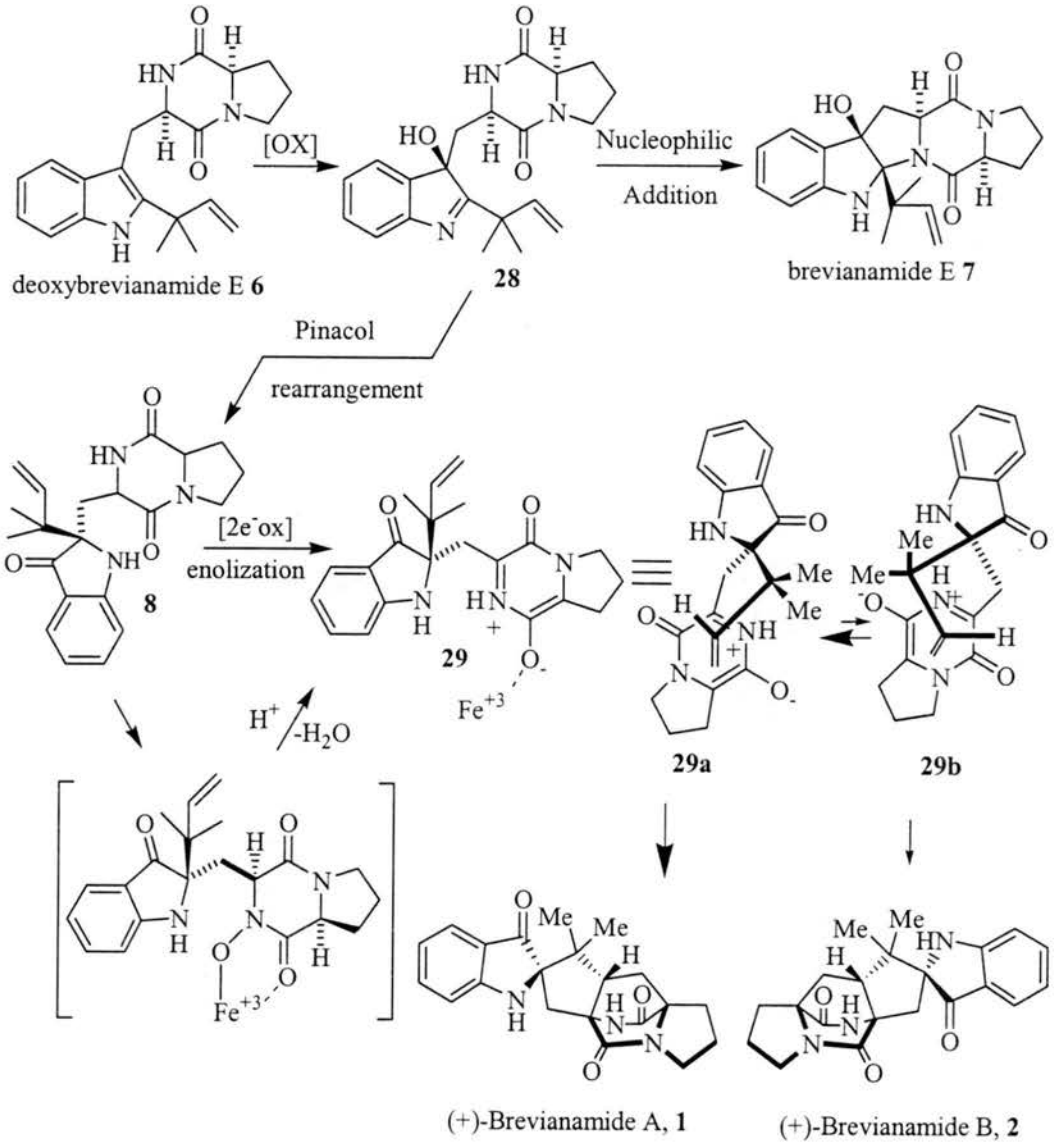
Deoxybrevianamide E, [8-^{3}H]-**6** (16.5mg with activity of 37.3 Ci/mol) was fed to cultures of *P. brevicompactum*. The results of the feeding study showed significant incorporation of the radiolabeled compound into both brevianamides A (**1**) (7.8% total

incorporation, 12.1% specific incorporation, 0.125 μCi, 6.12 μCi/mmol) and B (**2**) (0.9% total incorporation, 1.4% specific incorporation, 0.015 μCi, 10.8 μCi/mmol) as well as brevianamide E (**5**) (24.9% total incorporation, 38.5% specific incorporation, 0.40 μCi, 32.0 μCi/mmol).[9] This evidence strongly suggests that deoxybrevianamide E (**6**) is an intermediate in the brevianamide biosynthetic pathway.

Since brevianamide E (**7**) could also be a plausible biosynthetic intermediate *via* hydroxyindolenine **4** (**Scheme 6**),[9] this possibility was investigated. [5-^{3}H]-**7** was obtained from [5-^{3}H]-**6** *via* photooxidation followed by reduction of the resulting hydroperoxide with DMS (**Scheme 5**). Feeding experiments with [5-^{3}H]-**7**, however, gave brevianamides A (**1**) and brevianamide B (**2**) without significant incorporation. While some brevianamide E (**7**) may be formed by direct autooxidation of deoxybrevianamide E (**6**), compound **7** is not an artifact of the work-up or culture conditions, but rather a metabolite that represents a dead-end in this biosynthetic pathway.

1.8 Williams' Biosynthetic Proposal

In the brevianamide biosynthetic pathways proposed thus far, it has been assumed that oxidation of the indole unit to the indoxyl takes place at the very end of the sequence. However, the results of these feeding experiments led to the proposal of an alternate biosynthetic pathway (**Scheme 6**).



Scheme 6. Williams' Biosynthetic Pathway

1.9 Support for Williams' Pathway

This scheme accounts for the stereochemical outcome in the biosynthesis of brevianamides A and B. After the conversion of brevianamide F **5** into deoxybrevianamide E **6** by prenylation, an R-selective hydroxylation reaction occurs at the 3-position of indole **6** to afford 3-hydroxyindolenine **28**.[9] Nucleophilic addition of the dioxopiperazine secondary amide nitrogen to the imine bond of **28**, results in the

formation of brevianamide E **7**. However, a pinacol-type rearrangement of 3-hydroxyindolenine **28** sets the absolute stereochemistry at the indoxyl quaternary center as "R" to give **8**. Oxidation of dioxopiperazine **8** yields azadiene **29**. Lastly, it is possible that an intramolecular Diels-Alder cyclization from the major conformer **29a** leads to **1**, and from the minor conformer **29b** leads to **2**. Hence, this proposal supports the existence of the two enantiomorphic bicyclo [2.2.2] ring systems.

It is suggested that the preponderance of **29a** over **29b** could result from the relative activities of two different enzymes or the affinity of a single enzyme active site for the individual conformers. However, it is still puzzling why both brevianamides A and B are produced in the cultures. If precursor **29** was truly complexed in the enzyme active site, most likely the rotation between conformers **29a** and **29b** would not be allowed, therefore yielding just one of the brevianamides (**1** or **2**). It is possible that the supposed "Diels-Alderase" is just the oxidase that converts **8** into **29**. The hypothetical trifunctional enzyme (oxidase, dehydratase and Diels-Alderase) could be similar to the enzyme dehydroquinate synthase.[12] DHQ synthase appears to catalyze oxidation, elimination, ring-opening and aldolization reactions. It is now believed that DHQ synthase is only responsible for the oxidation and that the subsequent reactions occur spontaneously through substrate anchimeric assistance. Thus, it seems plausible that the brevianamide biogenesis could be involved in a similar circumstance.

Domingo and co-workers conducted *ab initio* calculations on the four possible transition-state structures of azadiene **29** (**Figure 3**).[13] Their purpose was to gain a better understanding of the intramolecular [4 +2] cycloaddition. The calculations were performed at the 3-21G and 6-31G* basis set levels. This Diels-Alder reaction appears to

be inverse electron demand. The calculations show that the HOMO of the dienophile (vinyl group) interacts with the LUMO of the diene (diketopiperazine). **Table 1** depicts the results obtained for the four possible transition-state structures. **Transition State 1** (**TS1**), has the lowest relative energy and leads to brevianamide A. **TS2** has a relative energy of 6.35 kcal/mol and leads to brevianamide B. This energy difference can be attributed to a favorable H-bond between the indoxyl NH and carbonyl oxygen of the diketopiperazine. **TS3** and **TS4** are considerably higher in energy (11.02 kcal/mol and 12.73 kcal/mol, respectively) and lead to the C-19 epimers of brevianamides A and B. These results are in accordance with the fact that neither C-19 epimer has been detected in the *Penicillium* cultures.

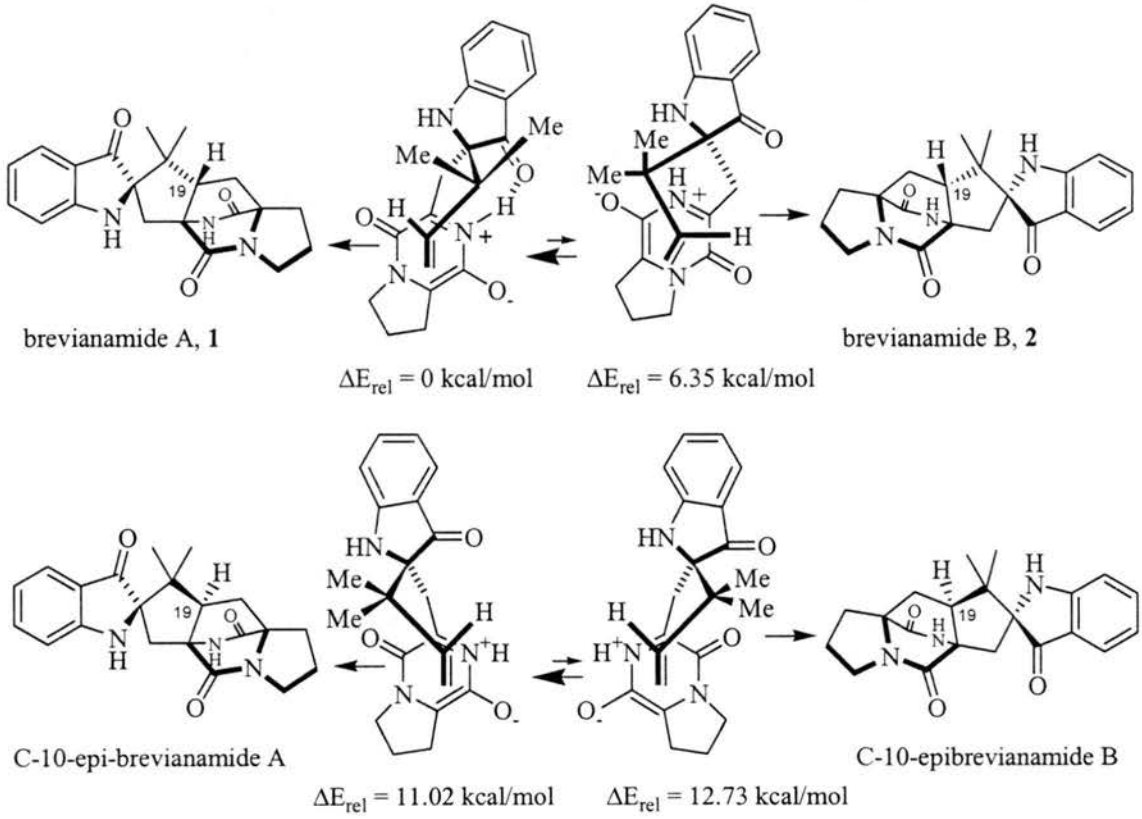


Figure 3. Transition State Structures for the Intramolecular [4 + 2] Cycloadditions

Table 1. Calculated Potential Energy Barriers and Relative Energies (kcal/mol) for **TS1**, **TS2**, **TS3**, and **TS4**

	3-21G/3-21G		6-31G*/3-21G	
	ΔE_a	ΔE_{rel}	ΔE_a	ΔE_{rel}
TS1	31.81	0.00	38.68	0.00
TS2	39.42	7.61	45.03	6.35
TS3	44.17	12.36	49.71	11.02
TS4	47.07	15.26	51.41	12.73

It has also been postulated that a non-heme metal such as Fe (III), could function as a chelator to the active site of the proposed oxidase. It could assist in the elimination of water to form azadiene **29**. In addition, the prolyl methine proton must also be removed at the active site of the enzyme. Therefore a metal-coordinated amide might also prove necessary to lower the activation barrier for the deprotonation step.[3]

1.10 Importance of a Diels-Alderase

The question as to whether or not the cycloaddition reaction is enzyme-catalyzed remains to be answered. There is little precedent for Diels-Alder reactions occurring in dioxopiperazines[5,8,14] and in these cases, highly electron-deficient dienophiles were used in the cycloaddition. To date, there is only one documented case[15] of a cell-free extract that catalyzes this valuable synthetic ring-forming reaction. Typically, enzymes catalyze reactions by stabilizing the structure and charge of the developing transition-state. For most reactions subject to this type of catalysis, both the starting substrate and the product differ significantly with respect to the transition-state. It is this primary difference that allows for turnover; ie., both the product and the starting substrate must bind to the enzyme less tightly than the transition state structure. The transition-state in the Diels-

Alder reaction is highly ordered and closely resembles the structure of the product (**Scheme 7**).

Scheme 7. Transition State Structure for the Diels-Alder Reaction

Therefore, an enzyme that was designed to stabilize the transition state structure for this reaction would be expected to be inhibited by the product (*via* tight binding) and thus catalysis would be prevented.[16,17] FMO theory would predict that for the energy levels of a relatively electron-rich diene (such as the present dioxopiperazine) to effectively interact with a dienophile, strong electron-withdrawing groups should be present on the dienophile (**Figure 4**). In the case of the brevianamides, the dienophile is an electron-neutral vinyl group. Accordingly, one would not expect this particular [4 + 2] cycloaddition reaction to be spontaneous.

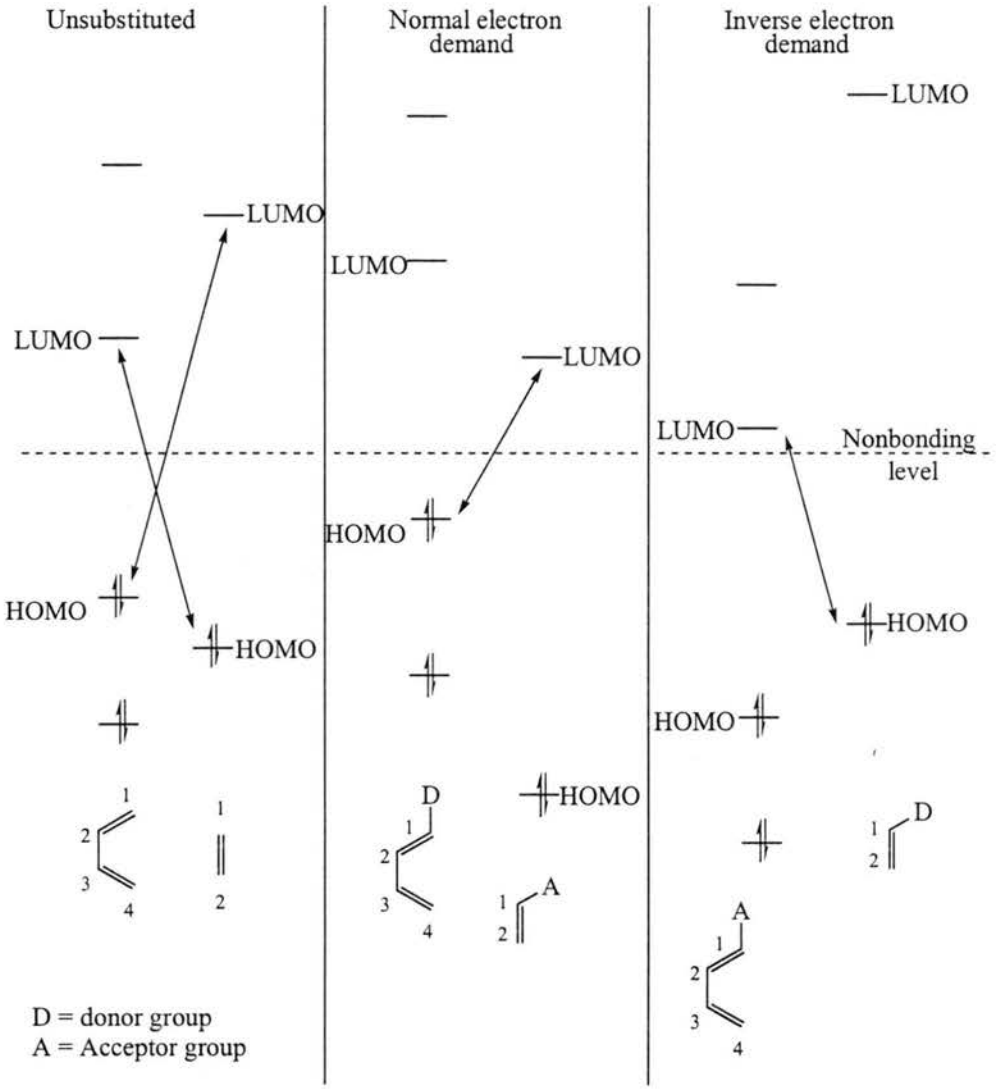


Figure 4. Frontier Orbital Interactions in the Diels-Alder Reaction

The only cases of protein-catalyzed Diels-Alder reactions are those of catalytic antibodies[17] and the recent cell-free example with solanopyrones (**Scheme 8**).[15] In the antibody cases, the initial Diels-Alder adducts were high energy substances that significantly change their structure or conformation after the initial cyclocondensation and were subsequently released from the antibody resulting in catalysis.

1.11 Biosynthetic Studies of Solanopyrones

There are a number of natural products whose structures may be derived from a biological Diels-Alder reaction.[16] These natural products have been isolated from various plants, marine animals and microorganisms. Structurally, these compounds can be classified as polyketides, isoprenoids, phenyl propanoids, alkaloids, and those of mixed biogenetic origin.

Until recently, there has been no concrete evidence for the existence of a Diels-Alderase. This lack of experimental data may be due to several factors. First, the Diels-Alder reaction is a symmetry-allowed thermal [4 + 2] cycloaddition that may be rare in biological processes. Second, identification of the precursor may prove difficult where many possibilities exist. Third, it may be synthetically challenging to obtain these precursors. Finally, one might find trouble incorporating such advanced intermediates into the organisms. There also exists the fact that several Diels-Alder type natural products are optically inactive and therefore, would be derived from non-enzymatic processes. In such cases, it seems conceivable that the cycloaddition is spontaneous or that an ionic process under physiological conditions is responsible for the reaction.[16m] Some of these examples include endiandric acid,[16g,18] lachananthocarpone,[16d] and yuehchukene (**Figure 5**).[19]

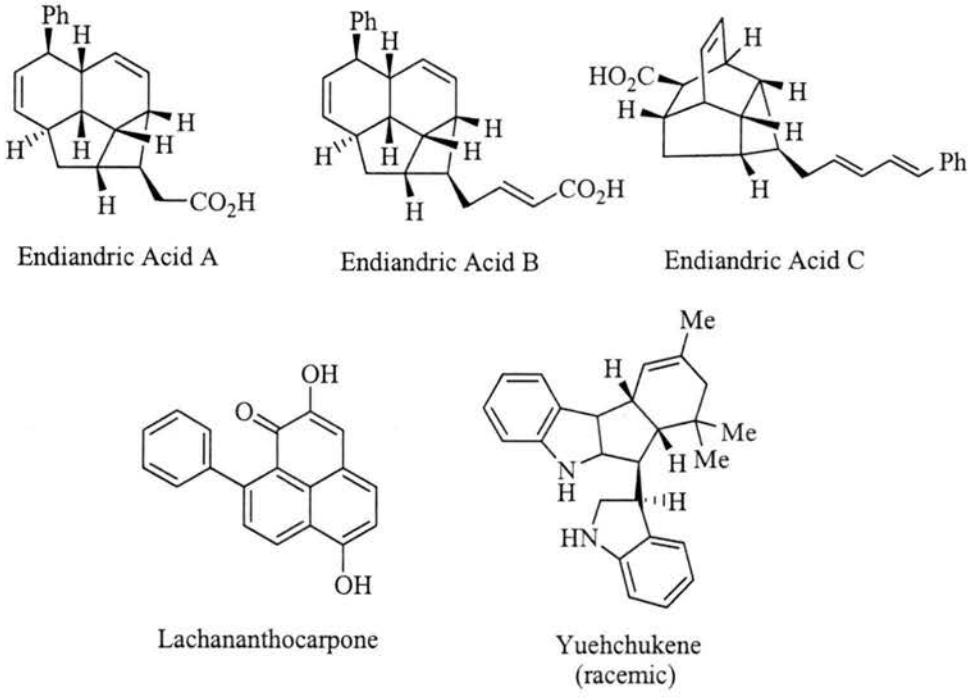
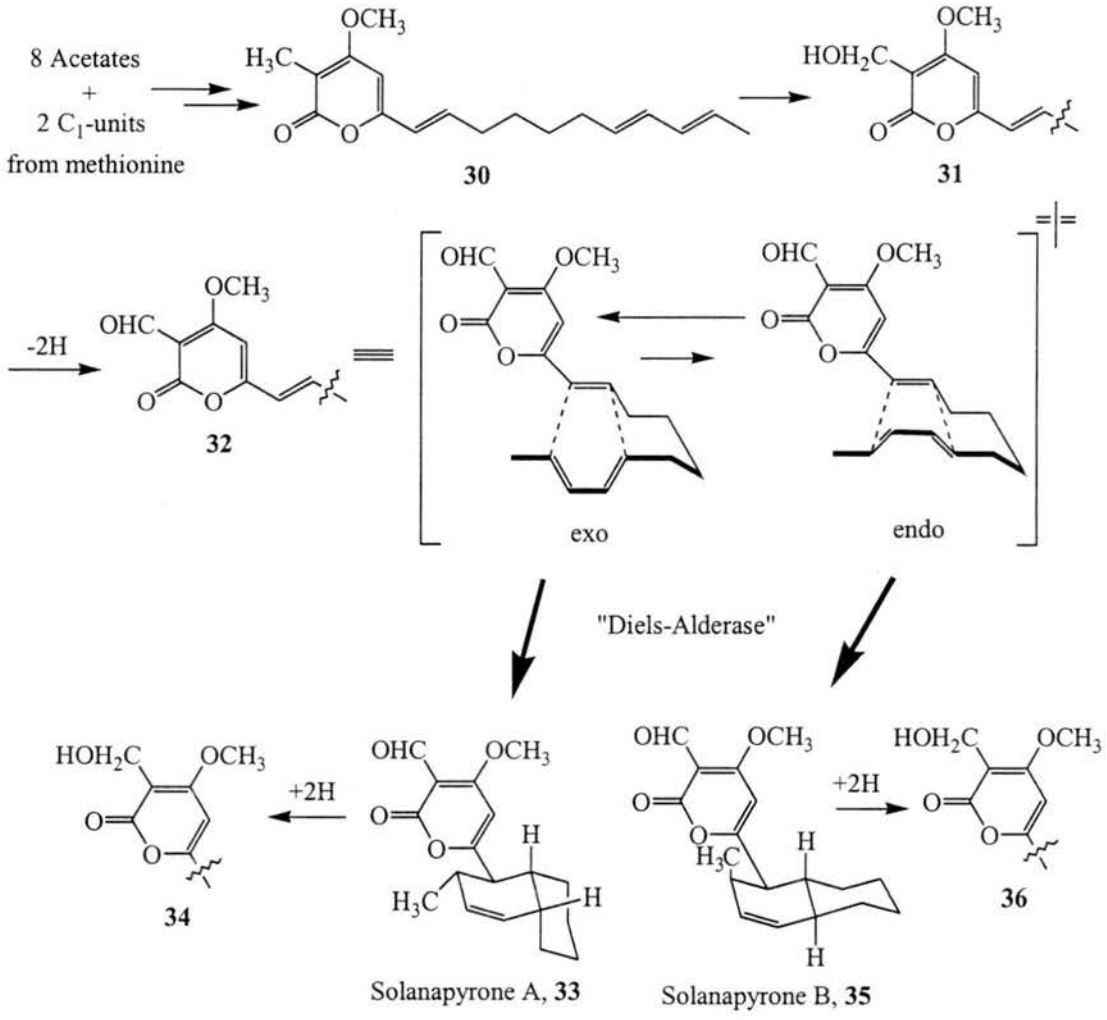


Figure 5. Non-Enzymatically Derived Diels-Alder Natural Products

In 1998, Ichihara and co-workers provided the first report of compelling evidence for the Diels-Alder enzyme in the biosynthesis of solanapyrones.[15e] The proposed biosynthetic pathway for the formation of solanapyrones is shown in **Scheme 8**.



Scheme 8. Biosynthetic Pathway of the Solanapyrones

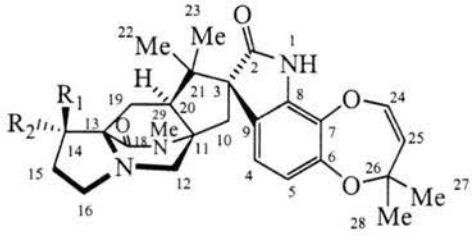
Total synthesis of prosolanapyrones I (**30**) and II (**31**) were achieved *via* the aldol reactions of pyrone and dienal fragments in 31% overall yield for **30** and 12% for **31**. To determine the diastereoselectivity and the inherent reactivity of prosolanapyrones, non-enzymatic Diels-Alder reactions were run under various conditions. It was found that the endo / exo selectivities for **30-32** were roughly equal in solvents of similar polarity whereas an increase in solvent polarity resulted in a slight increase in endo-selectivity. In the case of prosolanapyrone III (**32**), a substantial rate acceleration was observed in H_2O.

This is not surprising if one considers the impact of the hydrophobic effect[20] and the hydrogen bonding[21] between water and the carbonyl moiety of the dienophile. The highest observed *endo / exo* selectivity for non-enzymatic Diels-Alder reactions was that of **32** in H_2O for 3h (*endo / exo* = 23).

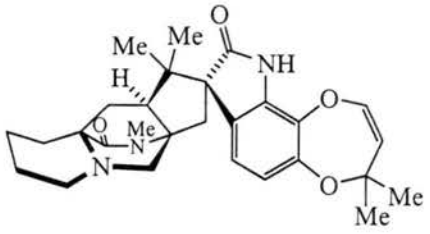
Next, a cell-free extract of the crude enzyme was used to convert prosolanapyrone III (**32**) to solanapyrone A (**33**) with high enantioselectivity (99%ee) and good exo-selectivity (6:1).[15d] Ichihara suggests that solanapyrone synthase is an oxidase that converts **31** into **32** and catalyzes the cycloaddition. Thus, the Diels-Alderase would be a bifunctional enzyme-catalyzing a two-step reaction. This implies that some oxidases or dehydrogenases could catalyze the Diels-Alder reaction if the proper substances were used.

1.12 Relatives of the Brevianamides

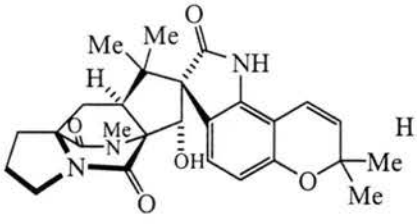
The paraherquamides,[2] marcfortines,[22] sclerotamides,[23] VM55599,[24] and asperparaline[25] are all structurally related to the brevianamide family due to their common bicyclo [2.2.2] ring system. They are indolic secondary mold metabolites isolated from numerous fungi (**Figure 6**).



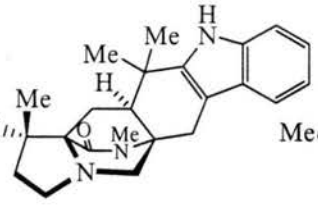
37, paraherquamide A, R_1 = OH, R_2 = Me
38, paraherquamide B, R_1 = H, R_2 =H



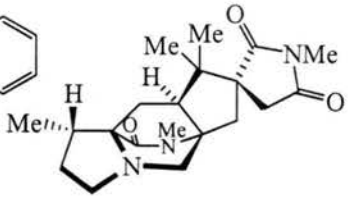
39, marcfortine A



40, sclerotamide



41, VM55599



42, asperparaline A

Figure 6. Structurally Related Natural Products

This group of natural products has proven curious due to their molecular complexity and interesting biogenesis. In addition, members of the paraherquamide family display potent anthelmintic and antinematodal activity. There exists a key stereochemical difference between the brevianamides and all of the aforementioned metabolites. The relative stereochemical relationship at C-19 position (brevianamide numbering) is *anti,* with respect to the proline ring in the brevianamdies, but *syn* for all of the paraherquamides and congeners. This phenomenon has been explained by invoking a facial divergence in the putative Diels-Alder cyclization that sets the relative stereochemical relationship at this stereogenic center.[9c] Furthermore, the brevianamides arise from oxidation of the indole at the 3-position, while the others are formed *via* oxidation of the indolic 2-position. Thus, brevianamides A and B are classified as

"indoxyls" whereas the paraherquamides, marcfortines and sclerotamides are categorized as "oxindoles."

1.13 Biosynthesis of Paraherquamides and Marcfortines

The paraherquamides are a group of fungal matabolites that have been isolated from various *Penicillium* species.[2] These alkaloids have been shown to possess potent anthelmintic and antinematodal activities. Williams and co-workers are currently investigating the biosynthetic origin of the core bicyclo [2.2.2] ring system that is common to these alkaloids.[9,26]

Feeding experiments with [1-^{13}C]-L-isoleucine, [1-^{13}C]-L-tryptophan and [*methyl*-^{13}C]-L-methionine to *P. fellutanum* showed significant incorporation into Paraherquamide A. The [1-^{13}C]-L-tryptophan was incorporated with the label at C-12 as predicted. The [*methyl*-^{13}C]-L-methionine was incorporated at C-29, the N-methyl position of the monoketopiperazine ring, contrary to the expected β-methylproline ring. Next, it was shown that [1-^{13}C]-L-isoleucine is also used to form the monoketopiperazine ring system with the label attached to C-18. In addition to these primary amino acid building blocks, [1-^{13}C]-β-methylproline has been fed to *P. fellutanum* and incorporation was observed at C-18 (**Figure 7**)[26c]. Furthermore, feeding studies with [U-$^{13}C_6$]-glucose and [$^{13}C_2$]-acetate confirmed that isoprene moieties are biosynthesized *via* the mevalonic acid pathway and not through the 1-deoxy-D-xylulose route.[26b]

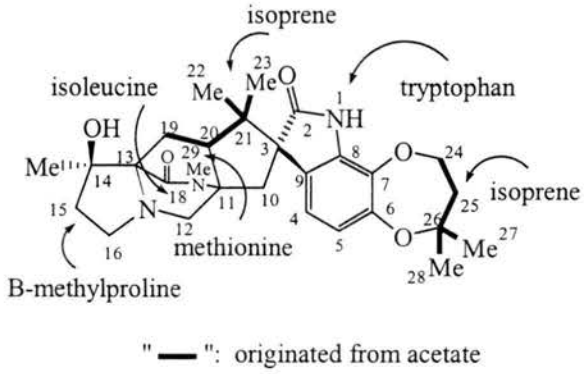


Figure 7. Biogenesis of Paraherquamide

In 1999, Williams and co-workers investigated the idea of "Reverse versus Normal Prenyl Transferases".[26b] Studies showed that the isoprene units of paraherquamide A are introduced in stereofacially distinct manners. According to previous reports, prenylation of the indole moiety in the biosynthesis of the brevianamides occurs in a similar fashion to that speculated for paraherquamide A.[9a] The prenyl transferase that installs this C_5 fragment must attract DMAPP to the 2-position of the indole in a π-facially indiscriminant manner. This result is significant because it represents the first case in which both a non face-selective and a face-selective addition to the trisubstituted olefinic portion of a DMAPP-derived moiety has occurred within the same molecule.

The marcfortine A biosynthesis has been studied by Kuo and co-workers[27] at Pharmacia & Upjohn Inc. This fungal metabolite was isolated from the cultures of *Penicillium roqueforti* by Polonsky et al. in 1980.[22] Biosynthetic feeding experiments with ^{13}C-labeled metabolites showed that marcfortine A is derived from methionine, tryptophan, lysine and two isoprenes (**Figure 8**). In a subsequent investigation, Kuo, *et al.* reported strong evidence suggesting that the marcfortine A-producing *Penicillium*

strain incorporates L-lysine to the pipecolate moiety through the α-keto-ε-amino caproic acid pathway (**Figure 9**-A) and not the L-α-aminoadipic acid semialdehyde route (**Figure 9**-B).

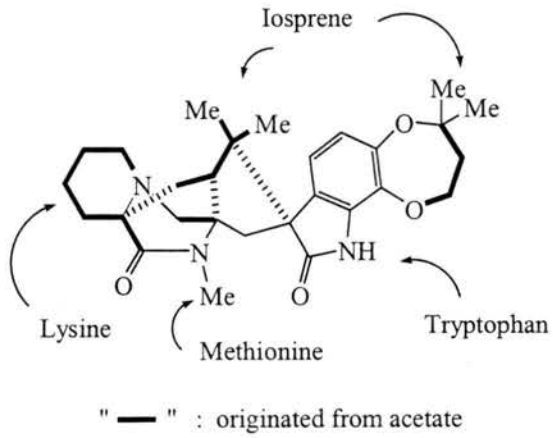


Figure 8. Biogenesis of Marcfortine A

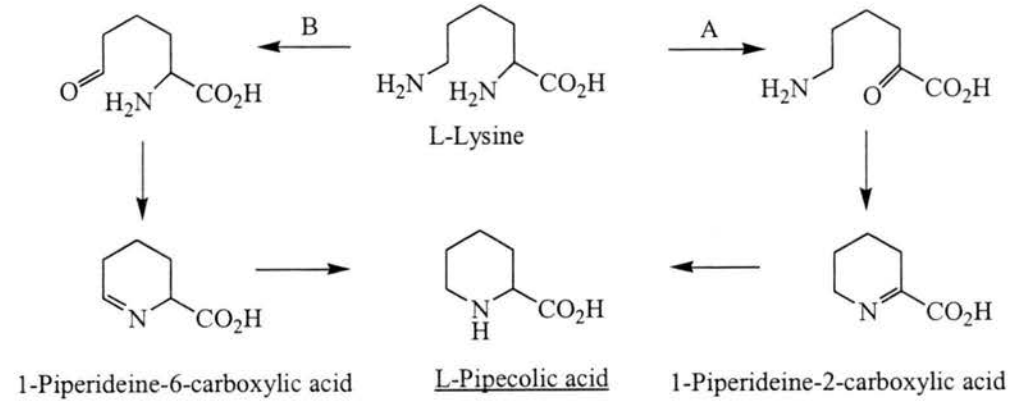


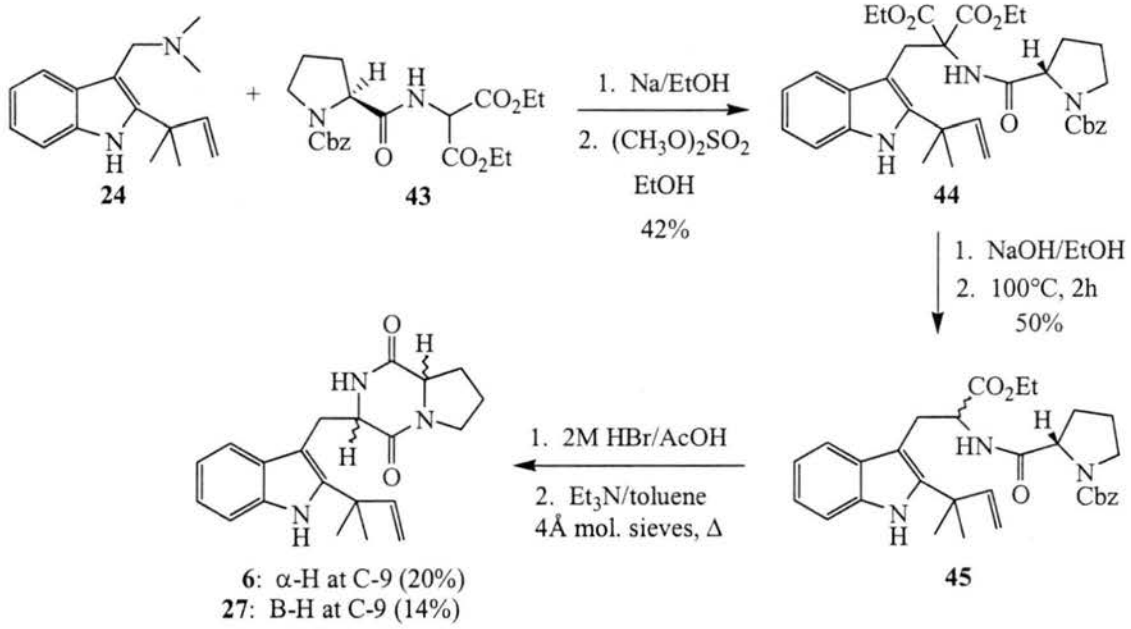
Figure 9. Two Possible Biosynthetic Pathways from Lysine to Pipecolic Acid

Chapter 2

Synthetic Studies Toward Brevianamides

2.1 Saxton's Deoxybrevianamide E Synthesis

Saxton and co-workers[28] chose to synthesize deoxybrevianamide E in order to confirm the brevianamide structures proposed by Birch.[5] Their synthesis began with the preparation of dipeptide **44** (**Scheme 9**).



Scheme 9. Saxton's Deoxybrevianamide E Synthesis

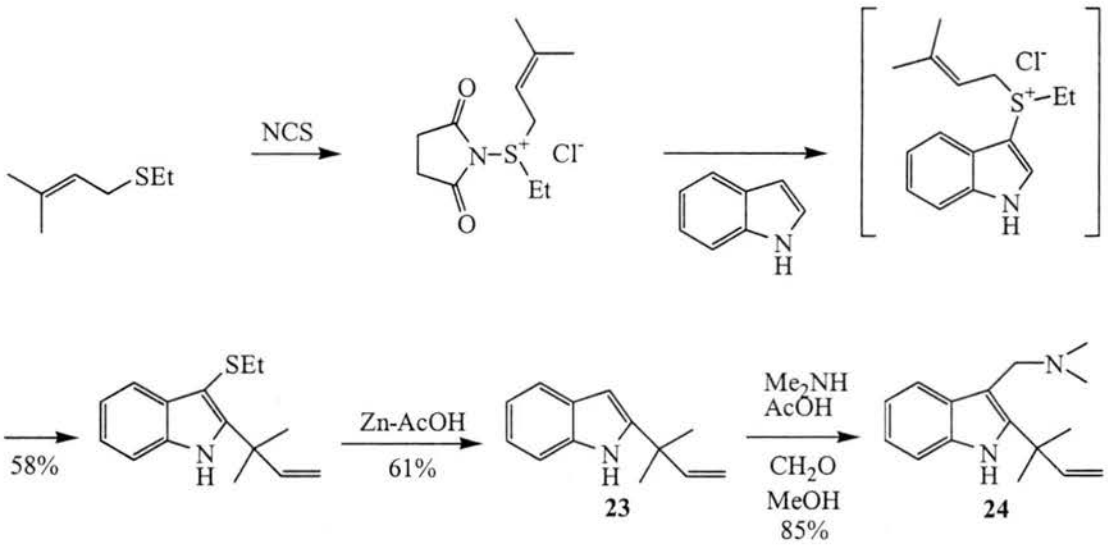
Benzyloxycarbonyl-L-proline and aminomalonic ester, were coupled with DCC to afford compound **43**. Condensation of the methosulfate of 2-(1,1-dimethylallyl)-indole

24[29] with the protected aminomalonate **43** afforded amide di-ester **44** in 42% yield. The diester **44**, underwent saponification followed by decarboxylation to give a mixture of the monoesters **45** in 50% yield. The Cbz group was removed by treatment with HBr in HOAc and the resulting free aminoester cyclized by refluxing in toluene with 4Å molecular sieves. After purification by silica gel chromatography, deoxybrevianamide E (**6**) was obtained as a colorless glass, together with some N-acetyl-2-(1,1-dimethylallyl)-tryptophan ethyl ester **27**.

2.2 Kametani's Deoxybrevianamide E and Brevianamide E Syntheses

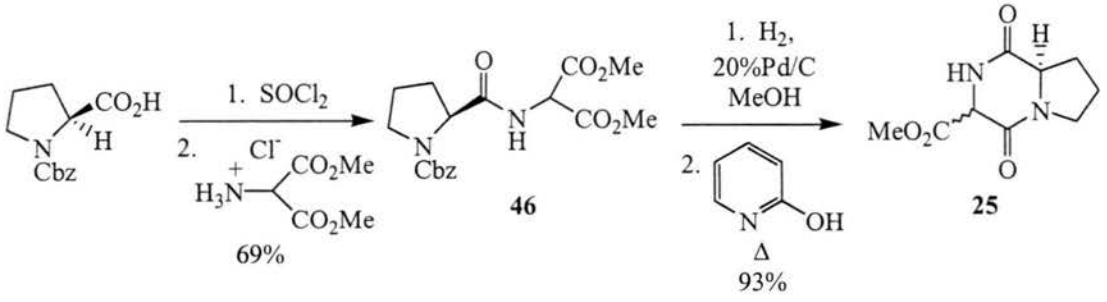
Kametani and co-workers published a chiral synthesis of brevianamide E and deoxybrevianamide E starting from L-proline to determine the relative stereochemistry and absolute configuration of brevianamide E.[10] This synthesis is attractive because it is convergent. In the final stages, gramine derivative **24** is coupled to diketopiperazine **25** in 74% yield.

Dimethylallyl indole **23** was prepared by the reaction of indole with succinimide-2-(3,3-dimethylallyl)ethylsulfonium chloride[30] followed by reductive desulfurization with zinc-acetic acid.[31] After subjection to Mannich conditions,[29] gramine derivative **24** was obtained in 85% yield (**Scheme 10**).



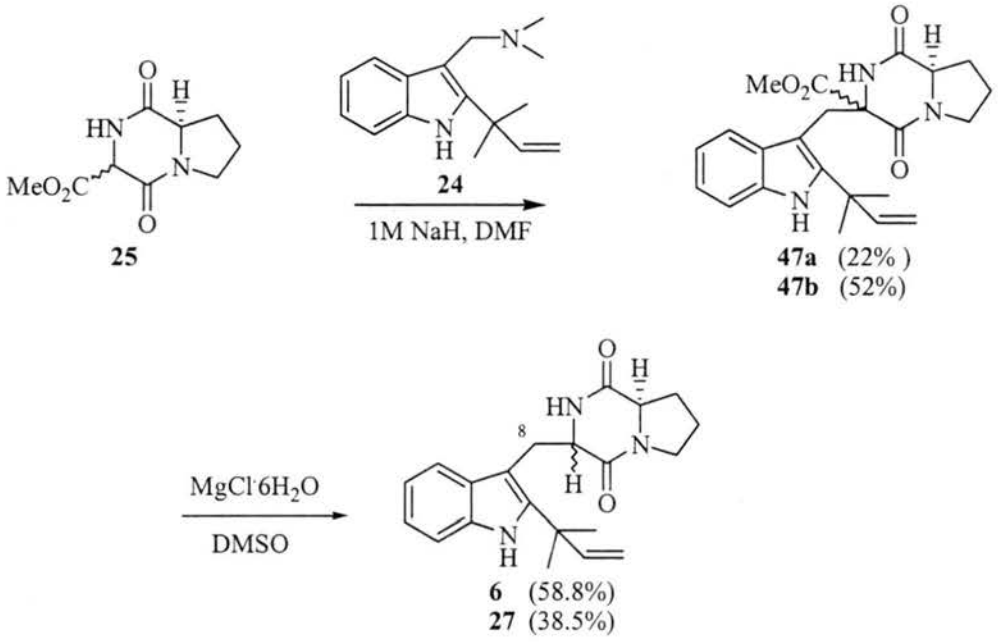
Scheme 10. Kametani's gramine (**24**) synthesis *via* Tomita's indole (**23**)

L-proline was used as the starting material for the requisite diketopiperazine **25**. Cbz-L-proline was converted to the acid chloride and then underwent a Schotten-Baumann reaction[31] with dimethyl aminomalonate to afford amide **46** in 69% yield. Hydrogenolysis of the Cbz group was accomplished using 20% Pd-C, H_2 in MeOH and the ensuing amine was cyclized to the diketopiperazine **25** (93% yield) after heating at 70°C for 1 hour in the presence of a catalytic amount of 2-hydroxypryidine (**Scheme 11**).



Scheme 11. Kametani's Synthesis of Diketopiperazine **25**

Condensation of **25** with gramine derivative **24** took place in the presence of 1 molar equivalent of NaH in DMF at 55-60°C to produce 2 epimers (**47a** in 22%, **47b** in 51.7%). Saponification and decarboxylation with $MgCl_2$ in DMSO, afforded deoxybrevianamide E **6** and epi-deoxybrevianamide E **27** in 58.8% (**6**) and 38.5% (**27**) yield, respectively (**Scheme 12**).



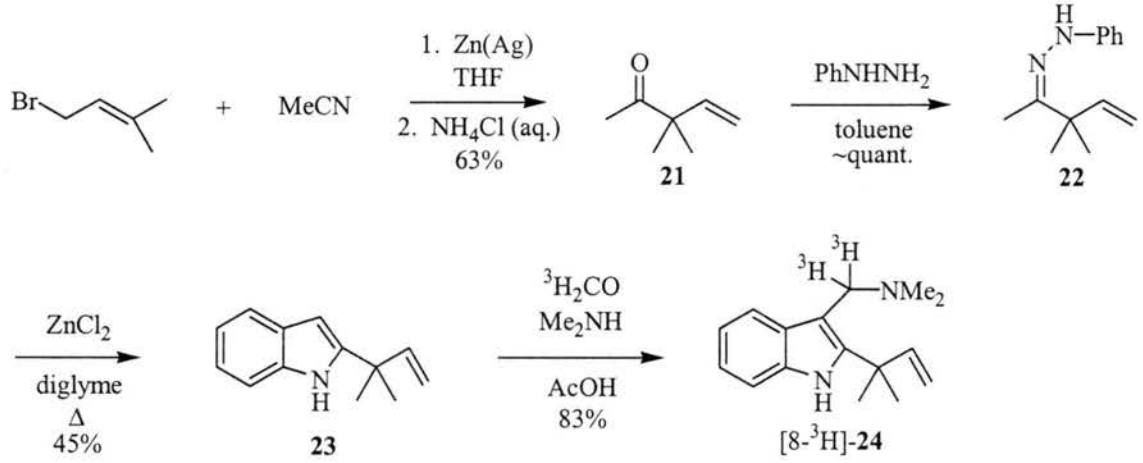
Scheme 12. Kametani's Synthesis of Deoxybrevianamide E (**6**)

2.3 Williams' Synthesis of Deoxybrevianamide E

Williams and co-workers synthesized deoxybrevianamide E for the purpose of ascertaining its intermediacy in the biosynthetic pathway of the brevianamides.[9a] Since [8-^{3}H] labeled deoxybrevianamide E was the species to be used in the feeding experiments, an efficient method for the introduction of the tritium label was sought.

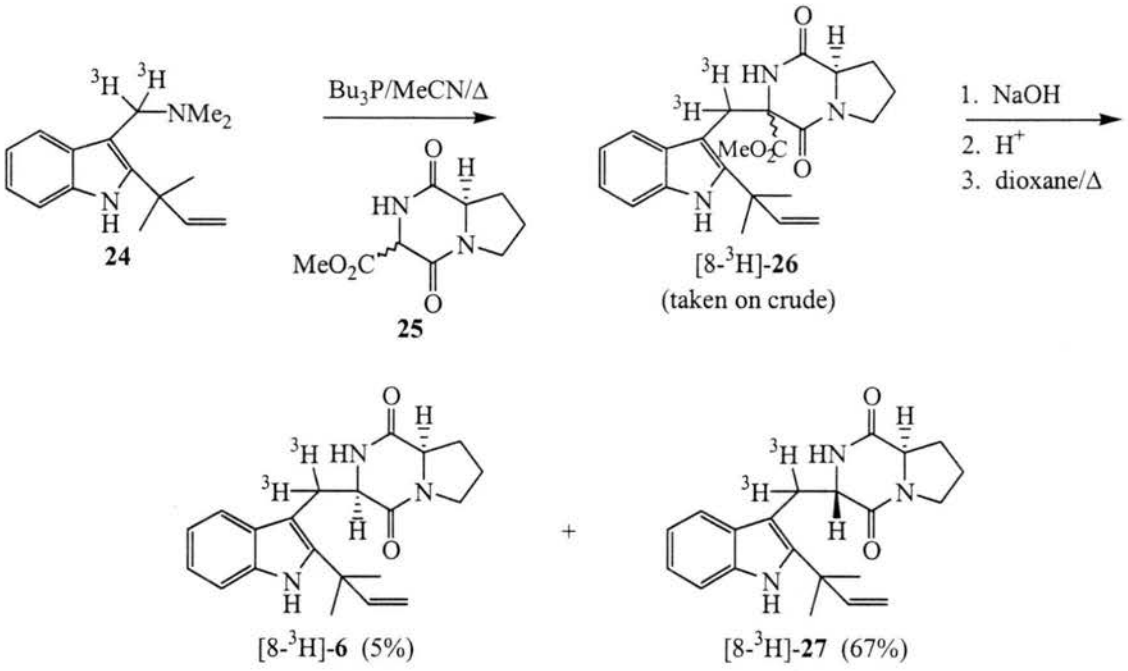
An improved synthesis for prenylated indole **23** was developed (**Scheme 13**). Isoprenyl bromide was treated with a solution of Zn(Ag) and CH_3CN in THF to give

ketone **21** in 63% yield according to the method of Rousseau and Conia.[11] Condensation of ketone **21** and phenylhydrazine furnished hydrazone **22** in quantitative yield. The Fischer indole synthesis[32] was used to produce the desired 2-(1,1-dimethylprop-2-enyl)indole **24** in 45% yield. Finally, gramine derivative **23** was prepared from indole **24** using Mannich conditions.[29]



Scheme 13. Williams' Synthesis of Gramine Derivative, [8-^{3}H]-**24**

Kametani's procedure was followed for the synthesis of diketopiperazine **25**.[10] Somei coupling[33] of **25** to gramine derivative **24** in the presence of Bu_3P in CH_3CN under reflux afforded **26** as a mixture of diastereomers (**Scheme 14**). Saponification and decarboxylation gave a mixture of deoxybrevianamide E **6** and its epimer which were purified by column chromatography resulting in a 5% yield of [8-^{3}H]-**6** and 67% yield of [8-^{3}H]-**27**.



Scheme 14. Williams' Synthesis of [8-^{3}H]-**6**, Deoxybrevianamide E

2.4 Kishi's Synthesis of Tetrahydroaustamide

Austamide (**56**) is a toxic metabolite isolated from *Aspergillus ustus*[34] whose structure is similar to brevianamide A **1**. Deoxybrevianamide E **6**, has been detected in cultures of *Aspergillus ustus* as well, which suggests that it could be an intermediate in the biosynthetic pathway of austamide **56.**

Kishi and Hutchinson published a stereospecific total synthesis of *d,l*-austamide in 1979.[35] One of the key intermediates in their synthesis is diketopiperazine ester **52**, whose structure closely resembles that of deoxybrevianamide E **6** (**Scheme 15**).

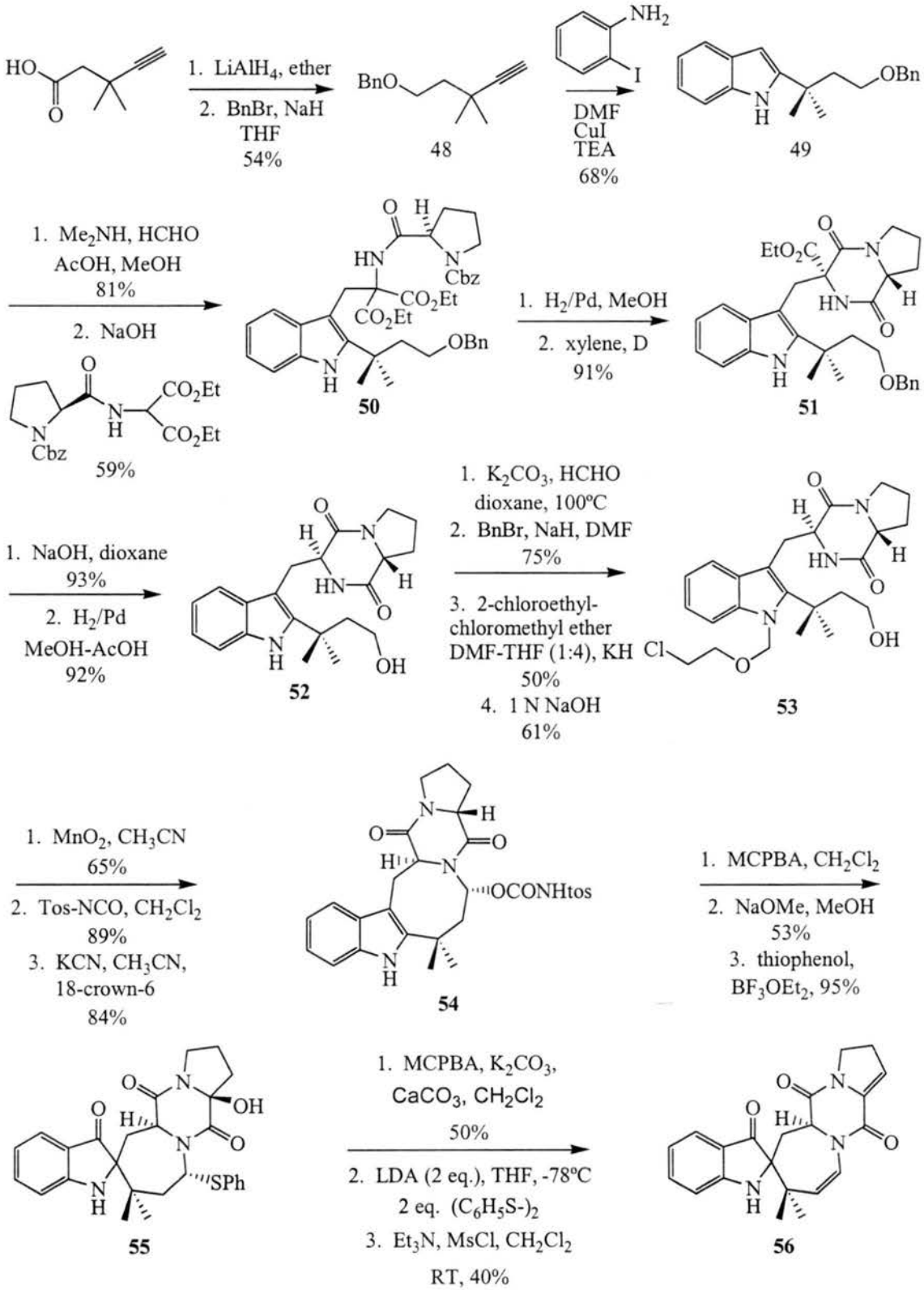
Reduction of 3,3-dimethyl-4-pentynoic acid using $LiAlH_4$ in ether[36] afforded the corresponding alcohol. The alcohol was protected as the benzyl ether by treatment with benzyl bromide and sodium hydride in THF to furnish 3,3-dimethyl-5-benzyloxy-1-

pentyne **48** in 54% overall yield.[37] Acetylene **48** was condensed with *o*-iodoaniline in DMF, TEA, and CuI at 145°C[41] to provide indole **49** in 68% yield. Indole **49** was subjected to Mannich conditions that resulted in an 81% yield of gramine derivative. Condensation with diethyl Cbz-L-prolylaminomalonate[29] in the presence of NaOH afforded aminomalonate derivative **50** in 59% yield.

Hydrogenolysis of the Cbz group was accomplished selectively using H_2/Pd in MeOH, resulting in the free amino comound. No cleavage of the benzyl ether was observed. The crude amine was cyclized to the diketopiperazine in refluxing xylene to give a 7:1 mixture of diastereomers. An overall yield of 91% was observed, with **51** as the major stereoisomer. Saponification of the ethyl ester was accomplished in 93% yield by treatment with NaOH in dioxane. The benzyl ether was cleaved by hydrogenolysis using H_2/Pd in MeOH-AcOH to give the resulting alcohol **52**.

Since the diketopiperazine nitrogen is more reactive than the indole nitrogen, it was necessary to protect it before the indole nitrogen. Alcohol **52** was treated with K_2CO_3 and HCHO in dioxane, then benzoylated to give the benzoate in 75% overall yield. The indole nitrogen was protected by treatment with 2-chloroethyl chloromethyl ether in DMF-THF with KH. The benzyl ester was hydrolyzed with 1 N NaOH to furnish indole-protected **53** in 61% yield. Oxidation of **53** with MnO_2 in CH_3CN (65% yield) was followed by tosyl protection of the carbamate (50% yield). Removal of the indole-protecting group was accomplished by treatment with KCN, 18-crown-6 and CH_3CN in 84% yield. The hydroxyindolenine was formed by treatment with MCPBA. A pinacol-type rearrangement was effected using NaOMe to give the indoxyl compound in 53% yield. Treatment with thiophenol and BF_3OEt_2 resulted in a 95% yield of indoxyl **55**.

The sulfide was oxidized with MCPBA then pyrolyzed *in situ*. Treatment with LDA resulted in dianion production. This was quenched by treatment with diphenylsulfide to give the tertiary alcohol. The alcohol was subjected to Et_3N and MsCl in CH_2Cl_2 to afford *d,l*-austamide (**56**) in 40% overall yield.



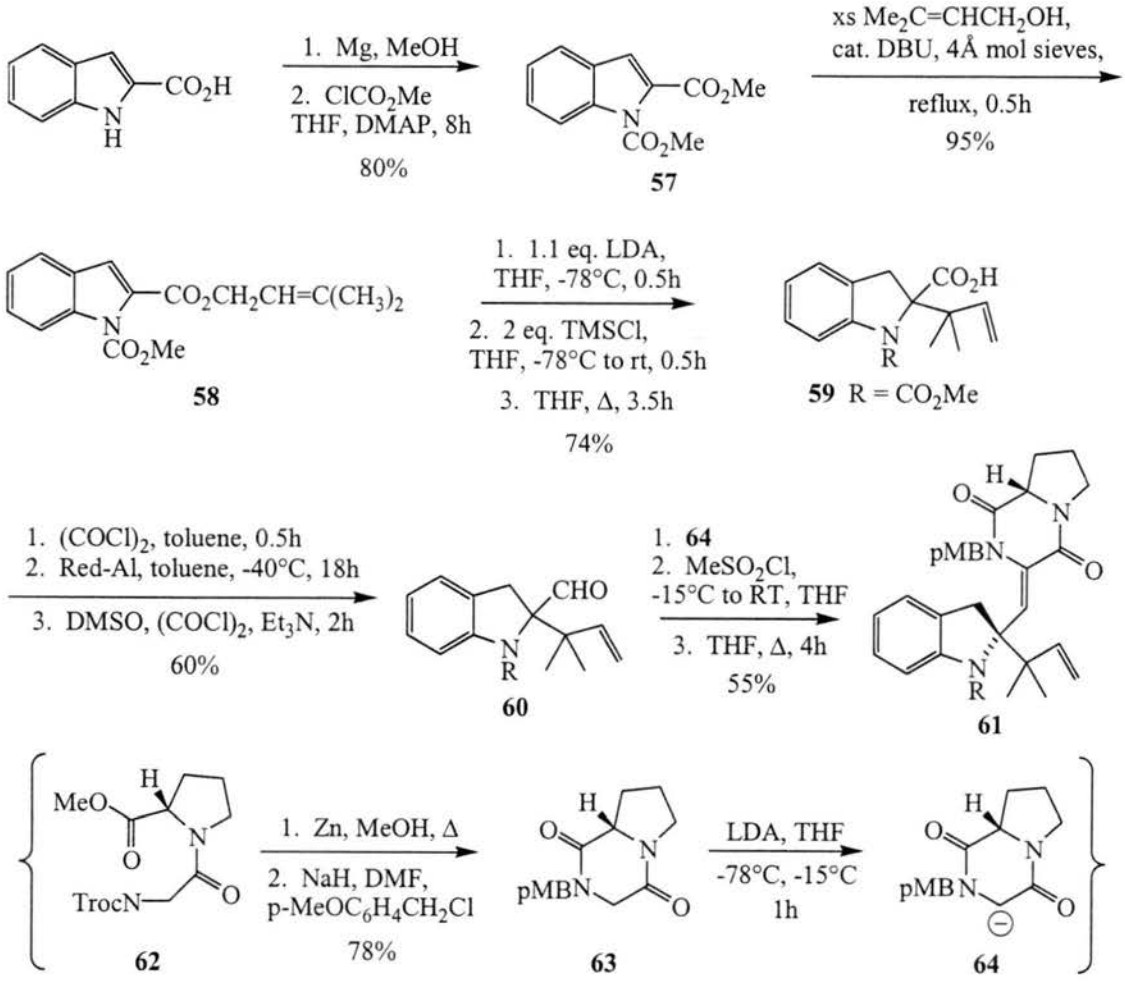
Scheme 15. Kishi's Synthesis of Austamide **56**

2.5 Dunkerton's Synthesis of Indole Derivative 61

Dunkerton and co-workers published the synthesis of a key intermediate (4-*p*-methoxybenzyl-5-(1'-carbomethoxy-2'-[(1'', 1''-dimethylallyl)-2', 3'-dihydroindole]methylidene)-1,2-L-pyrolidinopiperazine-3,6-dione (**61**) for a proposed synthesis of brevianamides A and B.[38] Starting with methyl indole-2-carboxylate[39], the indole was reduced with magnesium in MeOH and the nitrogen was protected as the methyl carbamate to afford **57** in 80% yield (**Scheme 16**). Transesterification in refluxing 3-methyl-2-buten-1-ol and catalytic DBU furnished **58** as a mixture of amide rotamers. Treatment of **58** with standard Ireland ester enolate Claisen rearrangement conditions[40] afforded indoline carboxylic acid **59** in 74% yield. Acid **59** was converted to its acid chloride, followed by *in situ* reduction using Red-Al, then Swern oxidation of the resulting alcohol furnished aldehyde **60** in 60% overall yield from **59**.

Preparation of the requisite diketopiperazine for coupling with aldehyde **60** was accomplished by cyclizing N-Troc-glycyl-L-proline methyl ester **62** with zinc in refluxing MeOH followed by amide nitrogen protection giving pMB-N-glycyl-L-proline anhydride **63** in 78% yield.

Generation of enolate **64** with LDA followed by addition of aldehyde **60** and mesylate elimination afforded the desired target **61** in 55% yield after chromatographic purification. Indole derivative **61** was formed as a mixture of epimers at the spiro center in approximately a 4:1 ratio. This ratio is based on 500 MHz ^{1}H NMR and 75.5 MHz ^{13}C NMR spectra. This suggests that partial kinetic resolution resulted during the aldol condensation.



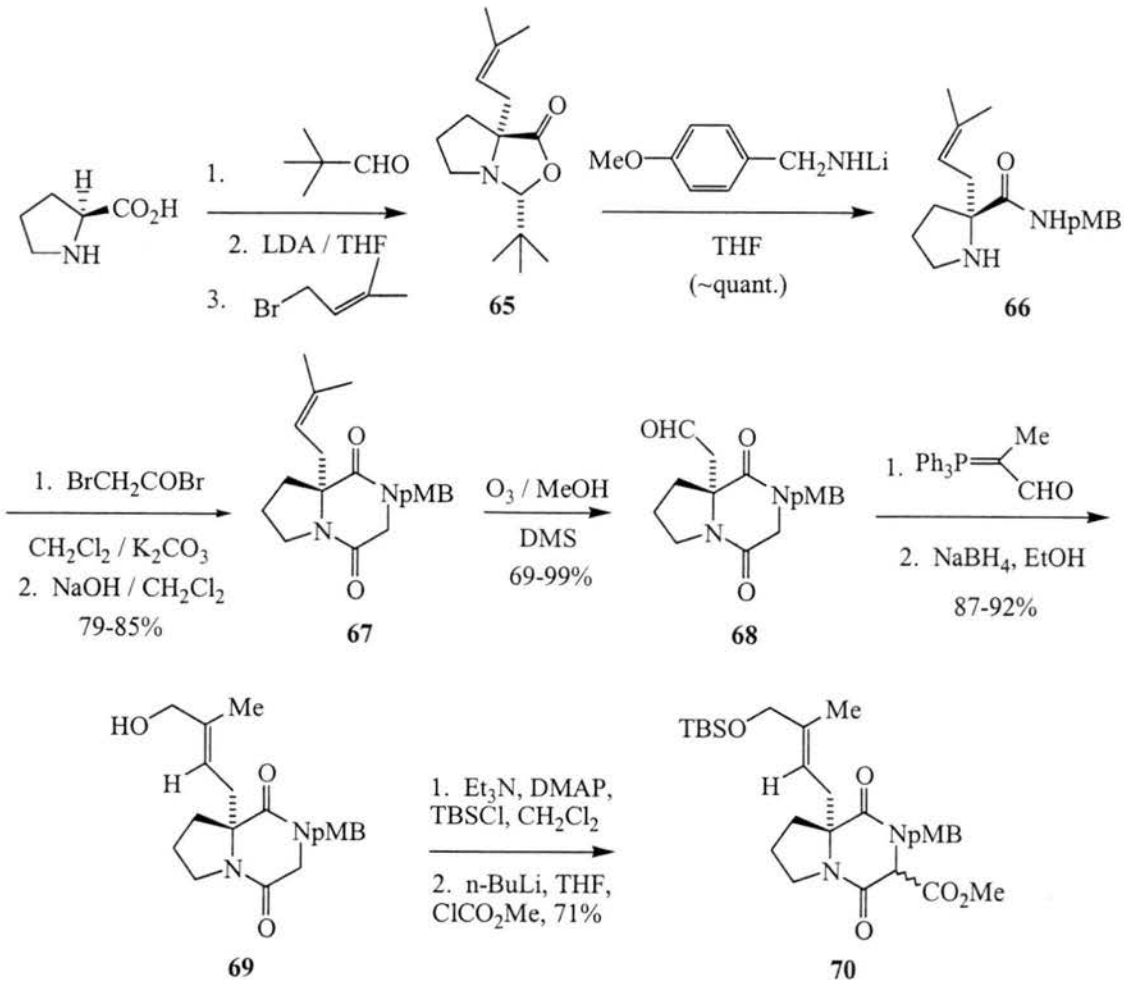
Scheme 16. Dunkerton's Synthesis of Indole Derivative **61**

Chapter 3

Synthetic Strategy for the Target Indoxyl

3.1 Brevianamide B Synthesis

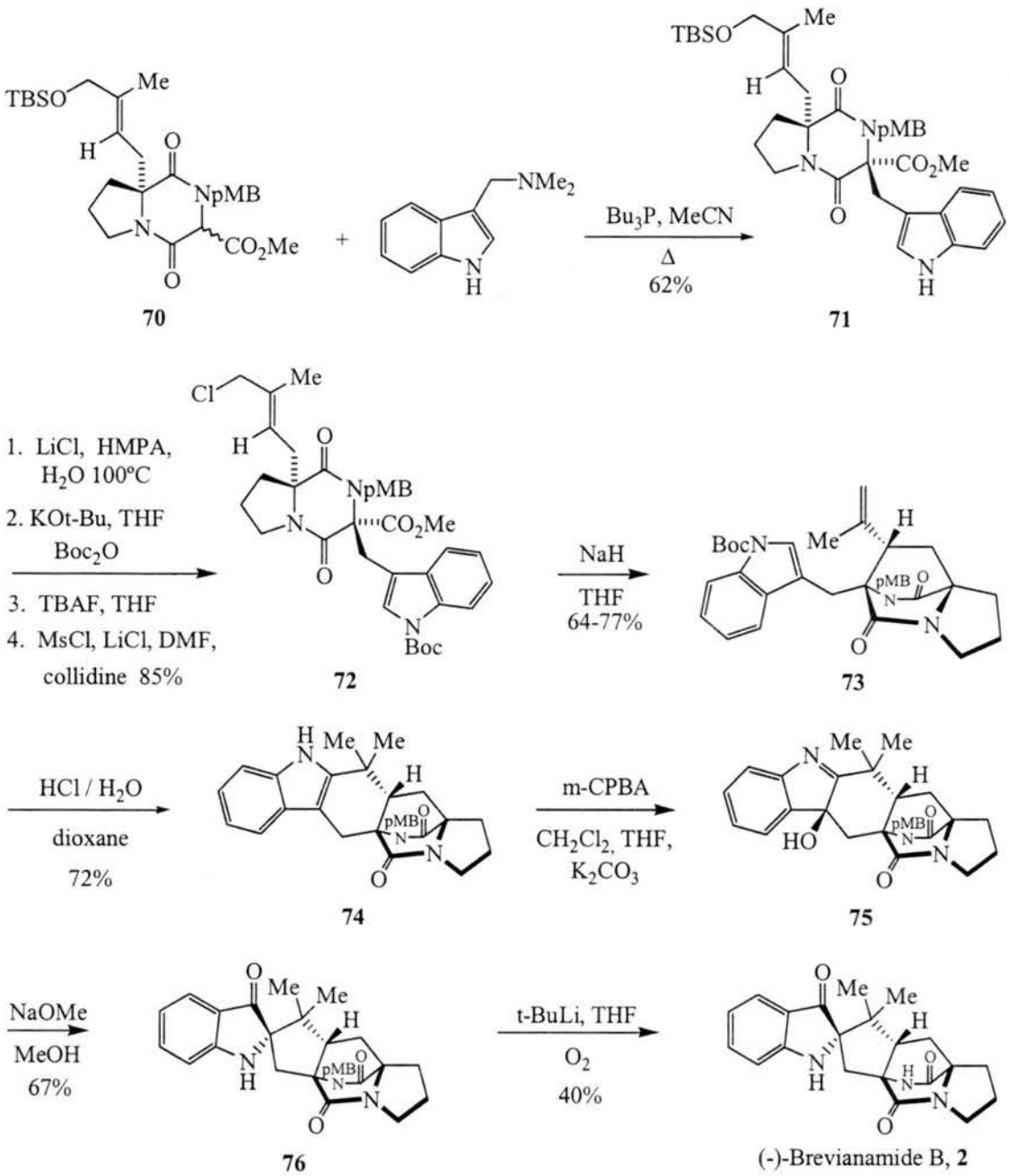
The synthetic strategy for the preparation of indoxyl **8** was based on the key pinacol rearrangement utilized in the total synthesis of brevianamide B (1989, Williams and co-workers).[41] An efficient and enantioselective synthesis of diketopiperazine **70** was achieved starting with L-proline (**Scheme 17**). Synthesis of the known pivaldehyde acetal[42] from L-proline followed by enolate alkylation with allyl bromide using Seebach's method afforded heterocycle **65**. Treatment of **65** with the lithium salt of *p*-methoxybenzylamine cleanly gave amide **66** in quantitative yield. Acylation of **66** with bromoacetyl bromide and K_2CO_3 in CH_2Cl_2 followed by ring closure (50% aqueous NaOH in CH_2Cl_2) provided the enantiomerically pure piperazinedione **67** (79-85% from **66**). Ozonolysis of diketopiperazine **67** in methanol followed by reductive workup, gave optically pure aldehyde **68**. Homologation with $Ph_3P{=}C(Me)CHO$ in 1,2-dichlorobenzene followed by reduction using $NaBH_4$ furnished allylic alcohol **69**. Protection of the allylic alcohol as the corresponding TBS ether followed by carbomethoxylation (*n*-BuLi/THF/$ClCO_2Me$) furnished a 4:1 diastereomeric mixture of ester **70**.



Scheme 17. Synthesis of Diketopiperazine **70**

Following the methodology devised by Kametani[10] and Somei[33] for the synthesis of brevianamide E, diketopiperazine **70** was coupled to gramine using Bu_3P in CH_3CN to give **71** as a single diastereomer (**Scheme 18**). Hydrolysis of ester **71** followed by decarbomethoxylation was achieved by treatment with LiCl in wet HMPA at 100°C. The indole was protected, desilylated, and then converted to chloride **72** (MsCl, LiCl, DMF, collidine) in 85% yield from **71**. Treatment of **72** with NaH and 18-crown-6 in THF provided a 64% yield of pentacyclic olefin **73** with a diastereomeric ratio of 4.9:1. Removal of the *tert*-butoxycarbonyl protecting group with HCl/H_2O in dioxane cleanly

afforded hexacyclic indole **74** in 58% yield. Oxidation of indole **74** with *m*-CPBA in CH_2Cl_2 furnished hydroxyindolenine **75** as a single isomer. A pinacol-type rearrangement was effected by treatment of **75** with NaOMe in MeOH to provide indoxyl **76**. The pMB deprotection of **76** proved difficult. Removal of this group was finally accomplished using the benzylic carbanion oxidation protocol (t-BuLi/THF/O_2), furnishing brevianamide B (**2**) in 40% yield.



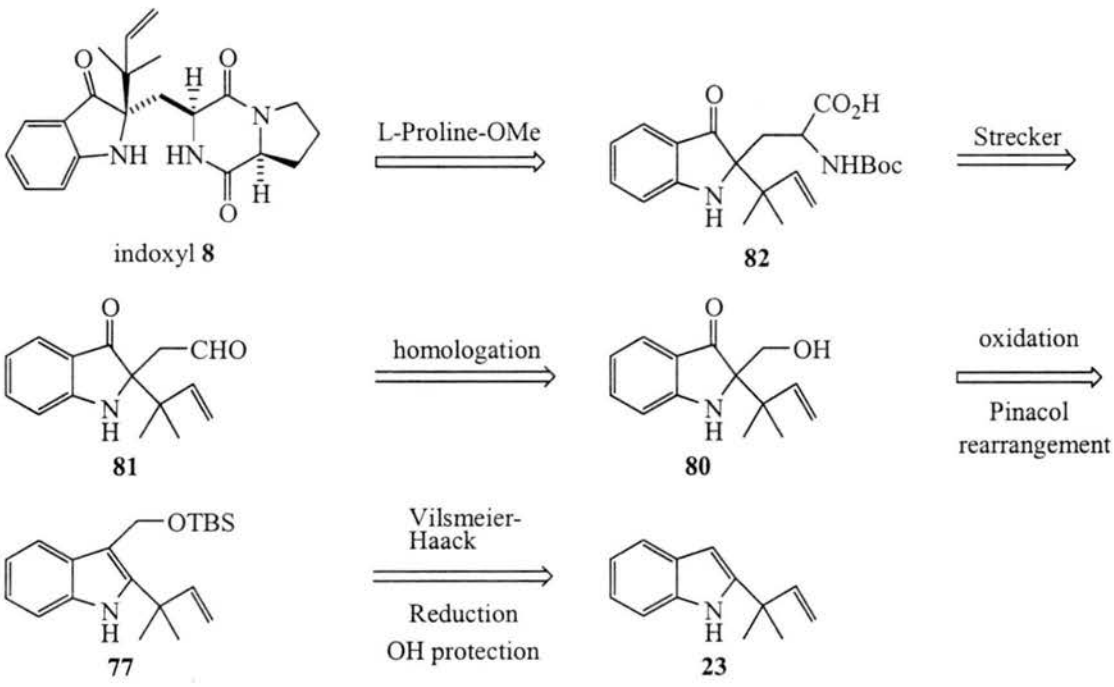
Scheme 18. Completion of Brevianamide B

3.2 Initial Indoxyl Route

The initial synthetic studies towards indoxyl **8** were conducted by Sanz-Cervera (unpublished results). The strategy incorporated methodology previously developed for

deoxybrevianamide E and brevianamide B within the Williams group. The retro-synthetic analysis is shown in **Scheme 19**.

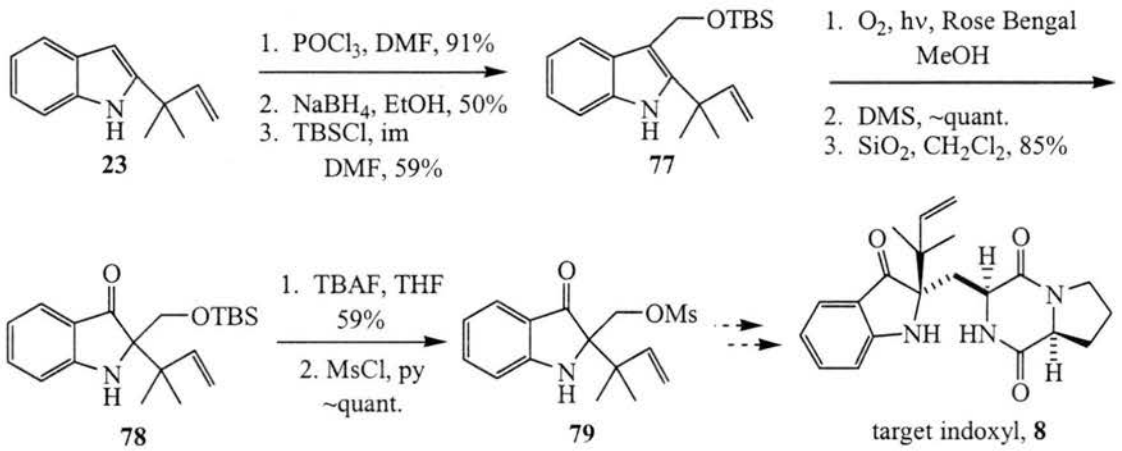
The final step in the indoxyl synthesis would be the coupling of L-proline-OMe to Boc-protected indoxyl **82** and cyclization to the diketopiperazine. Amino acid precursor **82** could come from a Strecker reaction with aldehyde **81**. A one carbon chain homologation of alcohol **80** would furnish **81**. Indoxyl **80** could be formed *via* photooxidation of **77** followed by a pinacol-type rearrangement to indoxyl **80**. The TBS protected alcohol could be derived from a Vilsmeier-Haack formylation of indole **23** followed by reduction to the alcohol. Indole **23** would be prepared according to the protocol used in the Williams deoxybrevianamide E **6** synthesis.[9a]



Scheme 19. Retro-Synthetic Analysis of Target Indoxyl **8**

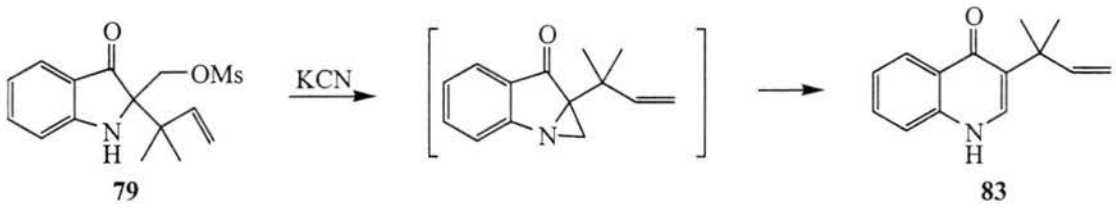
The synthetic route that was initially pursued is shown in **Scheme 20**. Prenylated indole **23** was subjected to Vilsmeier-Haack conditions[43] resulting in formylation at the 3-

position of the indole to give the aldehyde in 91% yield. Reduction of the aldehyde was accomplished by stirring with $NaBH_4$ in EtOH[44] resulting in a 50% yield of the corresponding alcohol after recrystallization. Protection of the alcohol as a silyl ether resulted in a 59% yield of TBS species **77**.[45] Next, photooxidation with Rose Bengal[46] followed by quenching of the ensuing hydroperoxide with dimethyl sulfide afforded the hydroxyindolenine in quantitative yield. The pinacol rearrangement was efficiently catalyzed by stirring with silica gel in CH_2Cl_2.[47] Removal of the silica gel using filtration followed by purification by PTLC furnished the fluorescent yellow indoxyl **78** in 85% yield. Cleavage of the TBS ether using TBAF afforded the corresponding alcohol in 59% yield. Treatment with methanesulfonyl chloride cleanly furnished mesylated indoxyl **79**.[48]



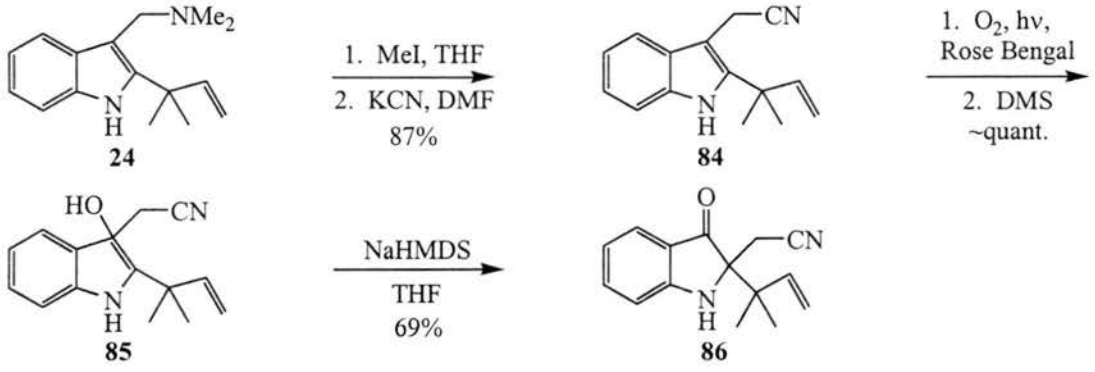
Scheme 20. Initial Synthetic Strategy for Indoxyl **8** (Sanz-Cervera)

I began work on the brevianamide project and the synthesis of target indoxyl **8**. In an attempt to homologate mesylate **79**, nucleophilic addition of KCN resulted in an undesired rearranged product **83** (**Scheme 21**).



Scheme 21. Undesired Rearrangement

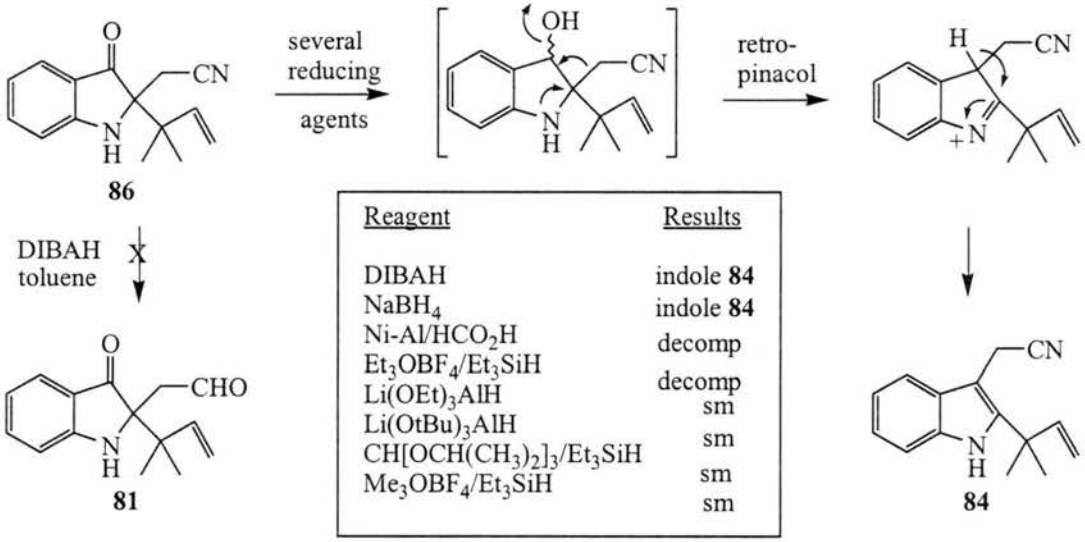
An alternate route to the homologated product was needed. Synthesis of nitrile indoxyl **86** was accomplished according to **Scheme 22**. Starting with gramine derivative **24**,[9a] attempts to directly displace the dimethylamine with NaCN, as well as trials using phase transfer conditions such as KCN, Bu_3P, and Bu_4NI in DMF, proved futile in the production of **84**. It was apparent that activation of the leaving group was necessary. Gramine derivative **24** was first converted to its quaternary ammonium salt using MeI. This substance was subsequently treated with KCN to afford nitrile **84**. Photooxidation with Rose Bengal cleanly furnished hydroxyindolenine **85** in quantitative yield. A few conditions such as DBU, SiO_2, and Al_2O_3 were tried before it was discovered that treatment with sodium bis(trimethylsilyl)amide in THF catalyzed the pinacol rearrangement to indoxyl **86**.



Scheme 22. Synthesis of nitrile **86**

The next goal was to convert indoxyl **86** to the Strecker precursor, aldehyde **81**. Treatment of nitrile indoxyl **86** with DIBAH led to reduction of the indoxyl carbonyl and

a subsequent retro-pinacol rearrangement, affording indole **84** (**Scheme 23**). Reduction with $NaBH_4$ also produced indole **84**. Milder conditions (Ni-Al/HCO_2H and Et_3OBF_4/Et_3SiH) were used in an attempt to avoid indoxyl carbonyl reduction, however only decomposition of the starting material was observed. The mild lithium alkoxyaluminum reagents did not have any affect on nitrile **86**. An attempt to activate the nitrile followed by reduction of the corresponding intermediate ($CH[OCH(CH_3)_2]_3/Et_3SiH$ and Me_3OBF_4/Et_3SiH) resulted only in recovery of starting material.



Scheme 23. Attempted Nitrile Reduction

3.3 Attempts to Block the Retro-Pinacol Rearrangement

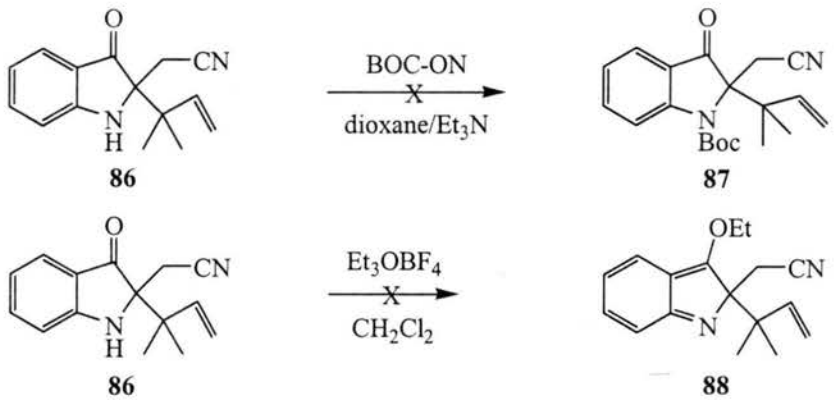
In order to block the retro-pinacol rearrangement, several attempts were made to protect the indoxyl carbonyl in **86** as the cyclic acetal (**Table 2**). Standard acetal forming conditions (ethylene glycol, Lewis acid) had no affect on indoxyl **86**. Treatment with more activated reagents (TMSOTf/$TMSOCH_2OTMS$ or ethylene chlorohydrin/$LiCO_3$ or ethylene chlorohydrin/K_2CO_3) afforded decomposition and/or recovery of starting material. It was discovered that due to its proximity to the aromatic ring, the carbonyl

carbon was not electrophilic enough to undergo nucleophilic attack required for ketal formation.

Table 2. Indoxyl Protection Conditions

Conditions	Results
ethylene glycol / TMSCl	sm
ethylene glycol / HOAc / BF_3-OEt_2	sm
ethylene glycol / benzene / PPTS	sm
TMSOTf / $TMSOCH_2OTMS$	sm
ethylene chlorohydrin	sm + decomp
ethylene chlorohydrin / K_2CO_3	sm + decomp

In a second effort to prevent the retro-pinacol reaction, a few attempts aimed at protecting the nitrogen failed to produce any desired compounds (**Scheme 24**). First, protection of the indoxyl nitrogen with a Boc group resulted in no desired product. In addition, attempts to form the ethyl enol ether with Et_3OBF_4 were also unsuccessful.

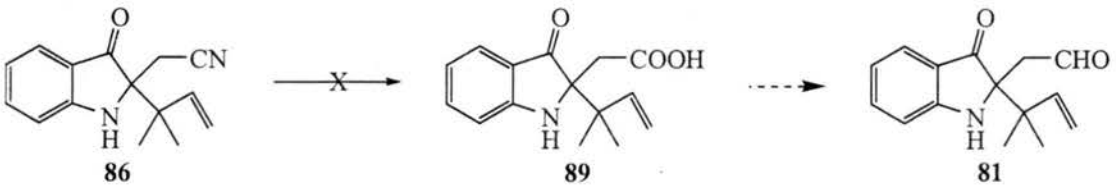


Scheme 24. Further Attempts to Block the Retro-Pinacol Rearrangement

3.4 Functional Group Transformations

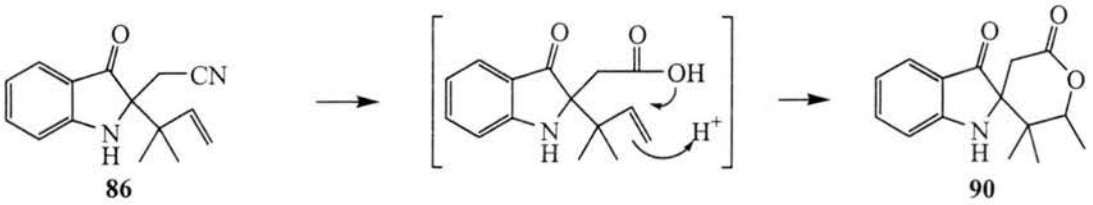
It was necessary to obtain the key intermediate aldehyde (**81**), possibly *via* a more mild reduction of an alternate functional group rather than the nitrile. Since the reduction of carboxylic acids can be accomplished using relatively mild boron reagents, synthesis

of carboxylic acid **89** was attempted. Under somewhat forceful conditions (diethylene glycol / NaOH as well as 95% EtOH / H_2SO_4), several products were produced, none of which corresponded to the desired carboxylic acid. Subjection of nitrile **86** to standard acidic hydrolysis conditions, resulted in lactone (**90**) formation, most likely *via* the mechanism shown in **Scheme 25**. Since lactone formation was catalyzed by acid, a few basic conditions were attempted. Unfortunately, only starting material and decomposition products were observed.



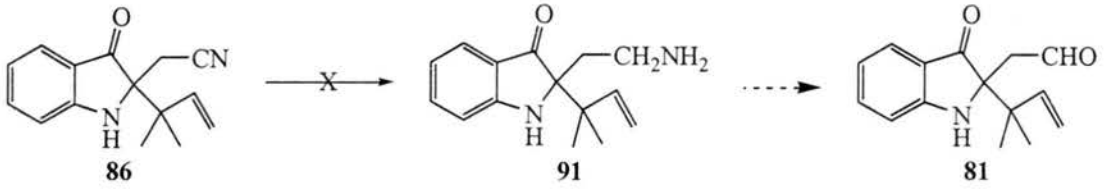
Reagent	Results
diethylene glycol/NaOH	several products
95% EtOH/H_2SO_4	several products
HCl/H_2O	lactone **90**
H_2SO_4/H_2O	lactone **90**
HCl/H_2O/HOAc	lactone **90**
KOH/ethylene glycol	sm + decomp
NaOH/MeOH	sm + decomp
KOH	sm + decomp

Lactone:



Scheme 25. Nitrile to Carboxylic Acid Transformation Attempts

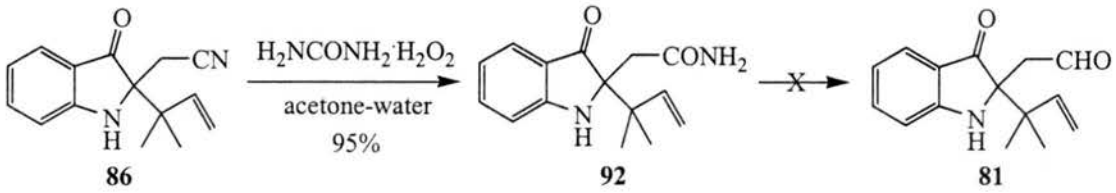
Primary amine **91** was sought as an intermediate to desired aldehyde **81**. Sodium trifluoroacetoxyborohydride had no effect on nitrile **86** (**Scheme 26**). Surprisingly, $LiAlH_4$ also resulted in recovery of starting material. This approach was not pursued further because successful conversion to the primary amide was achieved.



Reagent	Results
$NaBH_3(OCOCF_3)/THF$	sm (**86**)
$LiAlH_4/H_2SO_4/THF$	sm (**86**)

Scheme 26. Attempts to Reduce the Nitrile to the Amine

Treatment of nitrile **86** with urea-hydrogen peroxide complex[49] cleanly afforded the desired amide **92** in 95% yield (**Scheme 27**). This was encouraging since it is possible to reduce amides under milder conditions than DIBAH. With this substrate however, neither lithium alkoxy aluminum reagents nor triethylsilane were effective in the reduction to aldehyde **81**.

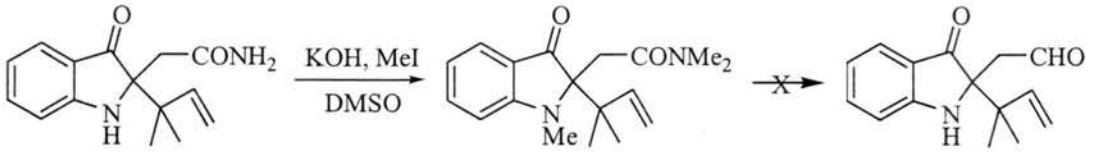


Reagent	Results
$Li(OEt)_3AlH$	sm (**92**)
CH_3OTf, $Li(OtBu)_3AlH$	sm (**92**)
$TFA/Et_3SiH/CH_2Cl_2$	sm (**92**)

Scheme 27. Nitrile to Amide Conversion, Then Attempts to the Aldehyde

It is likely that the reduction problems associated with the amide are due to the fact that it is a primary amide. Tertiary amides are usually more reactive. Primary amide **92** was alkylated using MeI, and KOH in DMSO (**Scheme 28**).[50] Alkylation of both the amide nitrogen and indole nitrogen was observed. It was discovered that the indole

nitrogen was most likely undergoing alkylation at an equal rate to the amide since 0.5 equivalents of MeI resulted in formation of compound **93**. Attempts to reduce tertiary amide **93** to aldehyde **81** were made using various lithium alkoxyaluminum hydride reagents to no avail. It was anticipated that removal of the indole methyl group was going to be very difficult, so this approach was abandoned.



92 **93** **81**

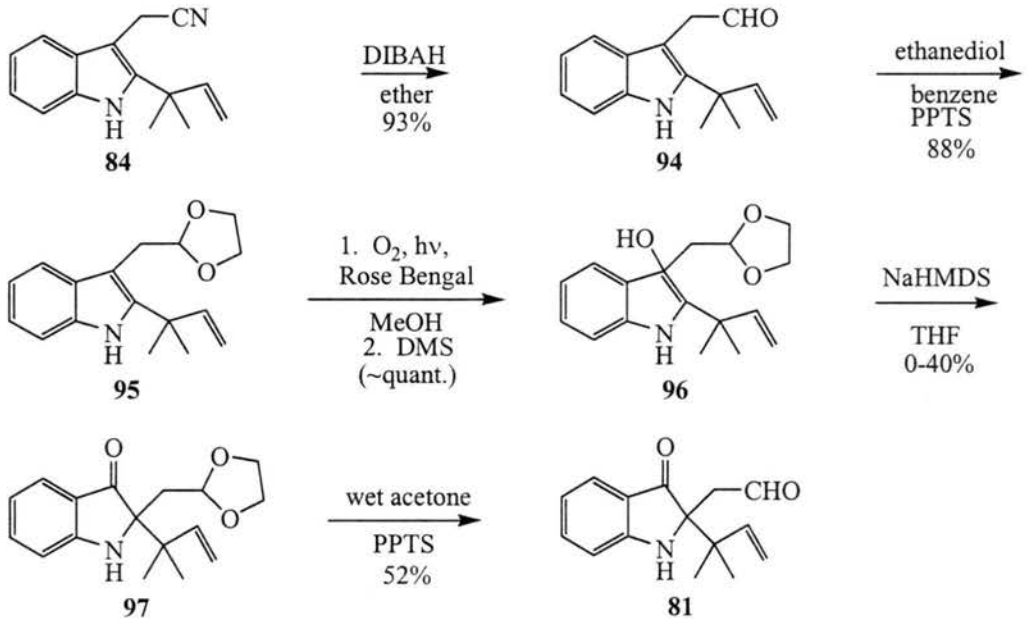
Reagent	Results
Red-Al/Ether	sm (**93**)
$Li(OEt)_3AlH$/Ether	sm (**93**)
$Li(OtBu)_3AlH$/Ether	sm (**93**)

Scheme 28. Conversion to the Tertiary Amide, Then Attempts to the Aldehyde

3.5 Pinacol Rearrangement of hydroxyindolenine 96

It was realized that the aldehyde functionality would have to be present before the pinacol rearrangement in order to avoid destruction of the indoxyl moiety or prenyl side chain. To this end, the nitrile was first reduced to aldehyde **94** (**Scheme 29**).[51] Next, acetal **95** was formed using ethylene glycol.[52] Treatment with O_2 and Rose Bengal in MeOH afforded hydroxyindolenine **96**.[46] The pinacol rearrangement was successful using a variety of reagents. Relatively neutral reagents such as silica gel and alumina catalyzed the rearrangement. Bases DBU and sodium bis(trimethylsilyl)amide are also capable of catalyzing indoxyl formation. The yields however, ranged between 0-40% with little consistency. Sometimes the oxindole is formed instead of indoxyl **97**. Moving

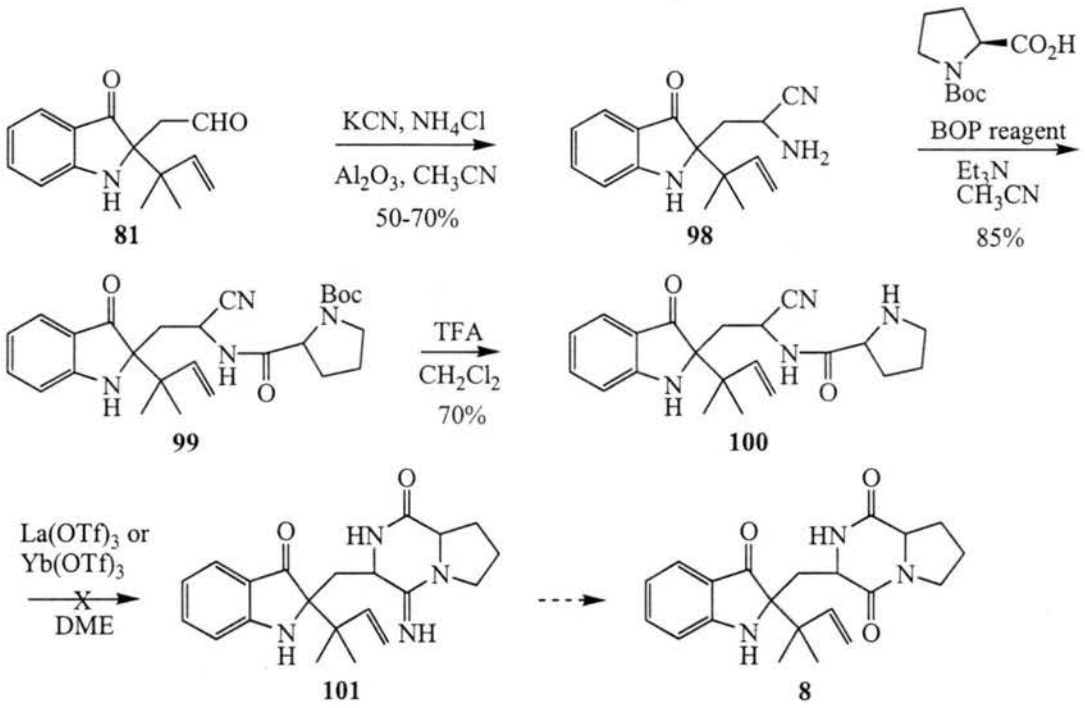
forward, acetal **97** was hydrolyzed in refluxing wet acetone and PPTS giving aldehyde **81** in 52% yield.[52]



Scheme 29. Synthesis of Key Aldehyde **81**

The next goal of this synthesis was to homologate the carbon chain and form the diketopiperazine. Aldehyde **81** was converted to aminonitrile **98** (50-70% yield), *via* Strecker conditions (**Scheme 30**).[53] It was envisioned that hydrolysis of nitrile **98** to the carboxylic acid followed by coupling to proline methylester would result in the necessary diketopiperazine precursor. Hydrolysis of the nitrile was attempted with several acids and bases, none of which gave the desired acid. To circumvent this problem, Boc-protected L-proline was coupled to the amino moiety of indoxyl **98** in 85% yield.[54] The Boc-protecting group was removed using TFA in CH_2Cl_2 to afford diketopiperazine precursor **100** in 70% yield.[55] The amidation/cyclization of **100** was not expected to occur spontaneously. According to literature precedent,[56] the nitrile needs to be more electrophilic for nucleophilic attack by an amine. It was therefore deemed necessary to

activate the nitrile for amidation. Lewis acids, aluminum amides or initial transformation into alkyl imidates have all been used to accomplish this. Cu(I)Cl[56] and lanthanide triflates[57] have also been successful in various intermolecular amidations. Attempts for amidation of nitrile **100** were unsuccessful and will be discussed in more detail below.



Scheme 30. Synthesis of Amidation Precursor **100**.

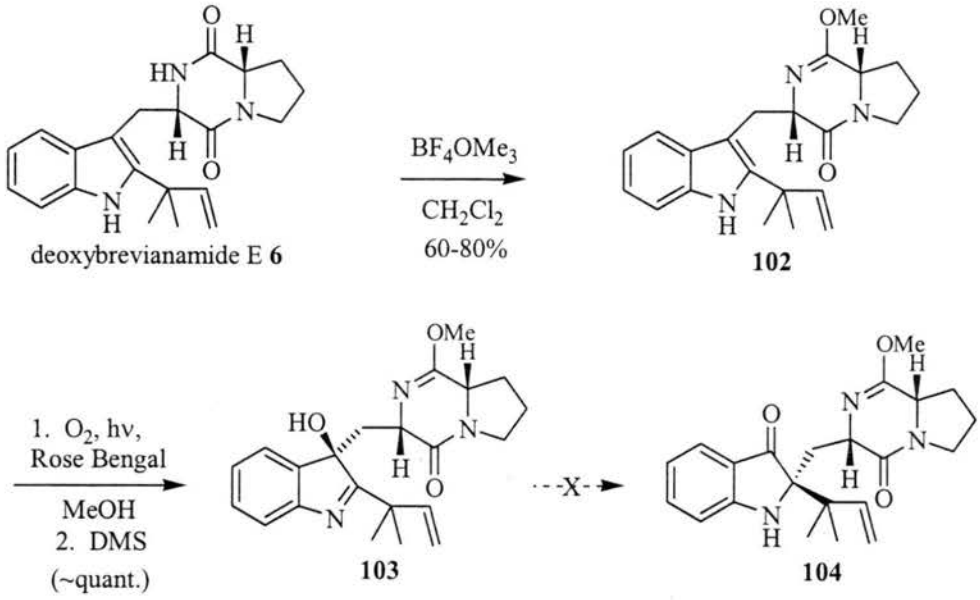
Several approaches to the amidation were pursued. For intermolecular systems, lanthanide triflates have been successful in activating the nitrile. In this case, $La(OTf)_3$ and $Yb(OTf)_3$ in DME were used, but only recovery of starting material was observed. In the literature methods, the nitriles were relatively simple in terms of sensitive functional groups. Most of the examples used acetonitrile or benzonitrile for the condensations.

Cu(I)Cl also has the potential of coordinating to the nitrile. However, this particular substrate (**100**) has several competing coordination sites. Experimentation with

the number of equivalents of Cu(I)Cl did not lead to cyclization of **100** to the desired diketopiperazine **101**. In combination with the plausible nitrile activating agents, bases such as NaH were used to deprotonate the proline nitrogen. It was expected that forming the anion would increase the likelihood of cyclization. It is possible that further exploration of this reaction could lead to diketopiperazine **101**.

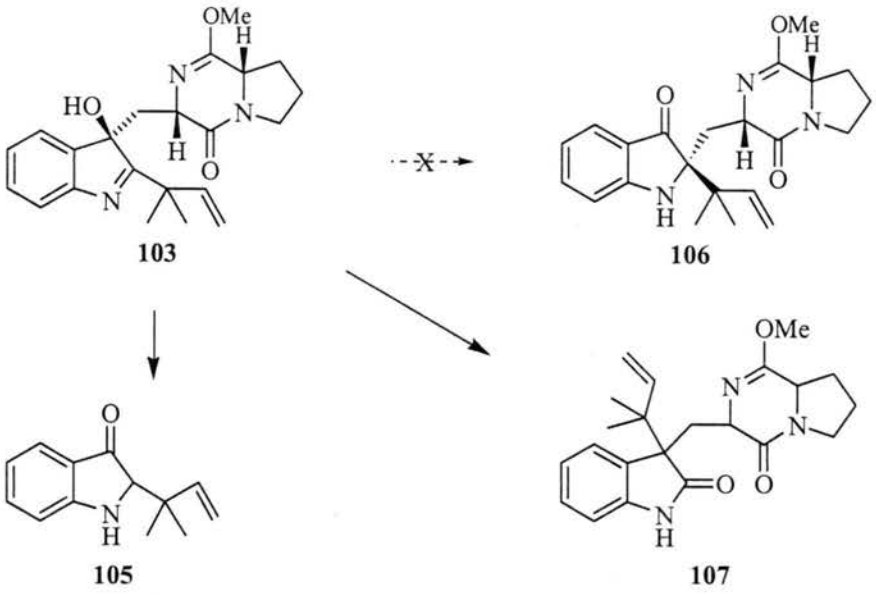
3.6 Biosynthetic Pathway Mimic

The next direction for the synthesis of target indoxyl **8** was based on the brevianamide biosynthetic proposal.[9] It is believed that deoxybrevianamide E undergoes hydroxylation followed by a spontaneous pinacol rearrangement to afford indoxyl **8**. It was necessary in the synthetic case to use the lactim ether of deoxybrevianamide E for this transformation since it is also possible to get nucleophilic addition of the diketopiperazine onto the 2-position of the indole, resulting in formation of brevianamide E (refer to **Scheme 6**). An approach was developed that incorporated this protecting group strategy. The lactim ether of deoxybrevianamide E had previously been synthesized in the Williams laboratory by treatment of **6** with BF_4OMe_3 in CH_2Cl_2 (**Scheme 31**). Hydroxyindolenine **103** was formed *via* photooxidation with Rose Bengal in MeOH.[46]



Scheme 31. Attempted Synthesis of Indoxyl **8** *via* Deoxybrevianamide E **6**

With the hydroxy compound in hand, next came the arduous task of rearrangement to the indoxyl. To this end, numerous conditions were tried (**Scheme 31**). Base catalyzed methods had been used successfully by several groups for this rearrangement.[35,41,58-60] Treatment of hydroxyindolenine **103** with NaOH, KOH, or NaH each furnished indoxyl **105**. Instead of deprotonating the hydroxy group, these bases were removing the tryptophan -hydrogen. **Scheme 33** shows the likely mechanism for production of indoxyl **105**. Indoxyl **105** has been detected by TLC and characterized by NMR. Bulkier bases such as potassium bis(trimethylsilyl)amide and DBU were tried as well in order to avoid deprotonating the tryptophan α-hydrogen. Since compound **103** is a tertiary alcohol, imidazole and 2,6-di-t-butylpyrine were used because they are more specific for this type of functionality. KOH in MeOH and K_2CO_3 in DMF led to formation of oxindole **107**. Instead of the diketopiperazine moiety migrating, the isoprenyl group moved to the 3-position of hydroxyindolenine **103** (**Scheme 34**).

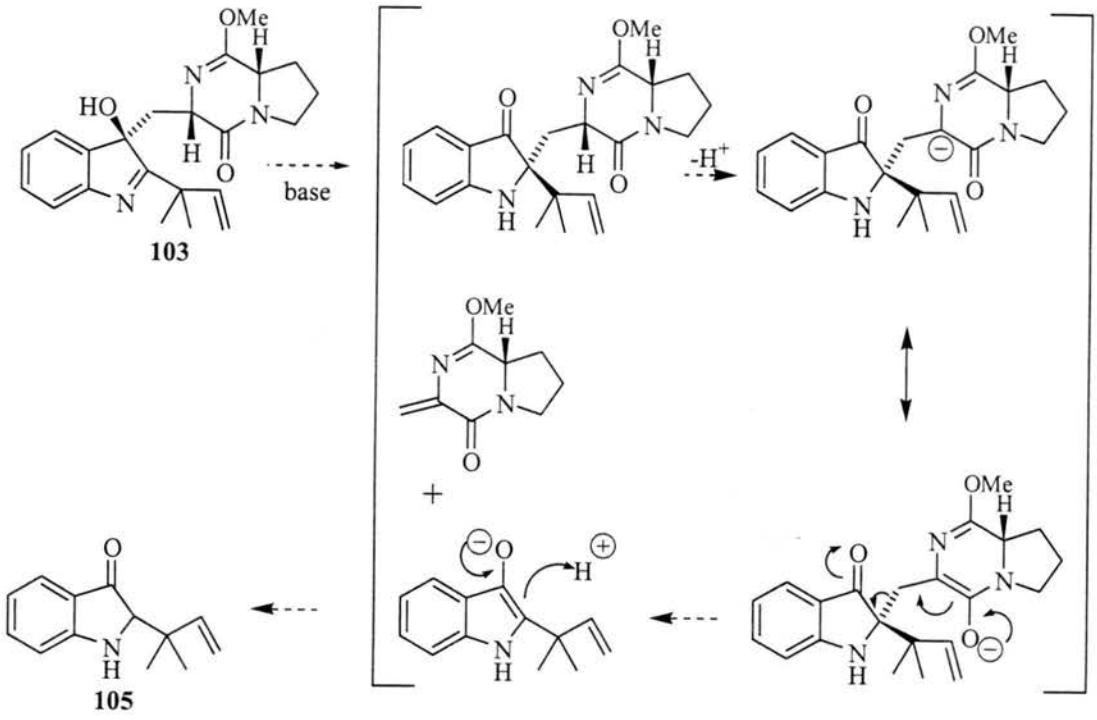


Conditions	Results
1M NaOH / MeOH	indoxyl **105** (30%)
KOH, PPTS / DMSO-MeOH	indoxyl **105** (85%)
NaOH / DMSO-MeOH	indoxyl **105** (85%)
NaH / toluene	indoxyl **105** (20%)
KHMDS, PPTS / CH_2Cl_2	sm + decomp
DBU / THF	sm + decomp
imidazole / DMF	sm + decomp
2,6-di-t-butylpyridine / DMF	sm + decomp
KH, 18-crown-6 / toluene	sm + decomp
KOH, PPTS / MeOH	oxindole **107** (90%)
K_2CO_3 / DMF	oxindole **107** (90%)
2M HCl / MeOH	sm + decomp
$BF_3{\cdot}OEt_2$ / $HC(OMe)_3$ / CH_2Cl_2	sm + decomp
Silica gel / CH_2Cl_2	sm + decomp
Al_2O_3 / toluene	sm + decomp

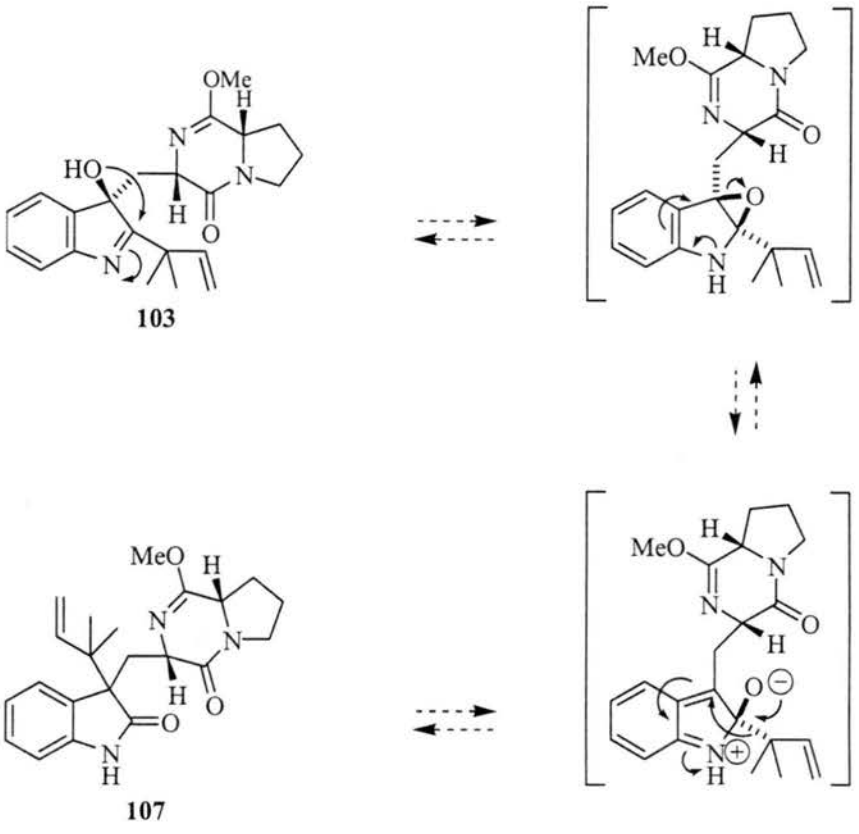
Scheme 32. Attempted Pinacol Rearrangement of Hydroxyindolenine **103**

Mineral acids and Lewis acids such as HCl and $BF_3{\cdot}OEt_2$ were used in an attempt to activate the imine towards indoxyl rearrangement, however, starting material and decomposition products were observed. Previously, treatment with slightly acidic or

neutral reagents (silica gel or Al_2O_3), was used successfully for the rearrangement of the hydroxyindolenine of TBS-protected alcohol **77** (**Scheme 20**). With hydroxyindolenine **103**, these conditions also led to decomposition and recovery of starting material.



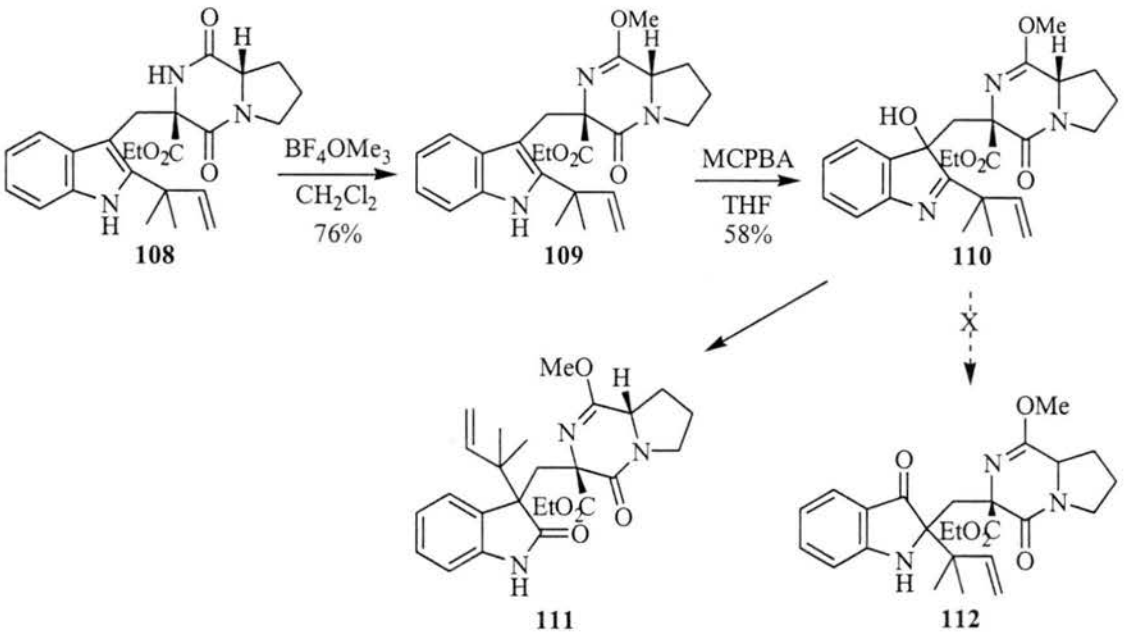
Scheme 33. Proposed Mechanism for Indoxyl **105** Formation



Scheme 34. Proposed Mechanism for Oxindole **107** Formation

The next course of action involved the synthesis of a hydroxyindolenine that had a functional group in place of the enolizable proton. Hydroxyindolenine **110**, which contains a carboethoxy group at the α-tryptophan position, was synthesized using methodology developed for [8-^{3}H]-**6**, deoxybrevianamide E (refer to **Schemes 13 and 14**).[9] Treatment of indole **108** with BF_4OMe_3 in CH_2Cl_2 furnished lactim ether **109** in 76% yield (**Scheme 35**). Oxidation of compound **109** with mCPBA in THF provided hydroxyindolenine **110** in 58% yield. Rearrangement attempts with DBU and NaOH produced the undesired oxindole **111**. Subjection of hydroxyindolenine **110** to CSA in EtOH led to decomposition products. These results indicate that the nature of the

migrating group is important. It is possible that the diketopiperazine unit is in a better position conformationally, to favor oxindole rather than indoxyl formation.



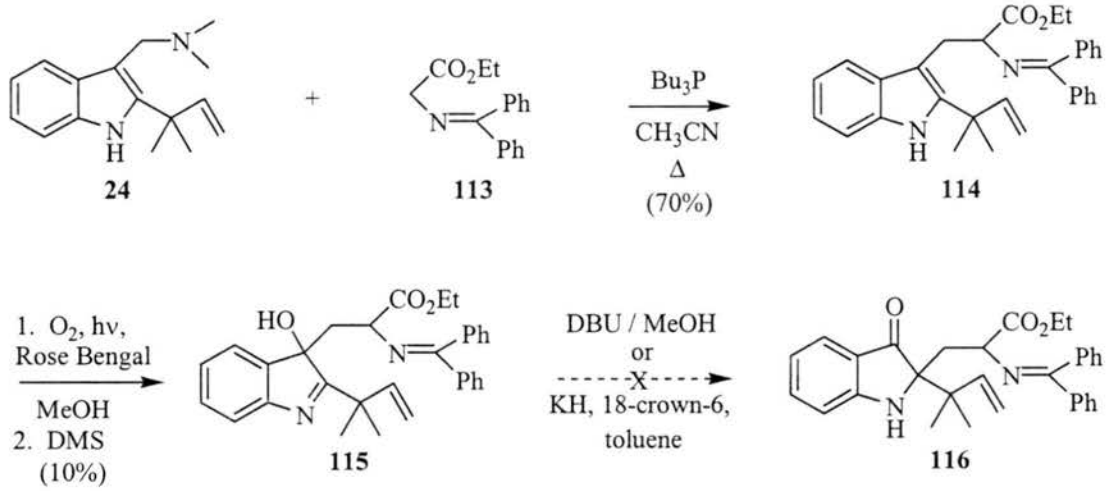
Conditions	Results
DBU / THF	oxindole **111** (50%)
1M NaOH / MeOH	oxindole **111** (70%)
CSA / EtOH	decomp

Scheme 35. Synthesis of Hydroxyindolenine **110**, Then Rearrangement Attempts

3.7 Synthesis of Glycine Derived Hydroxyindolenines

Since hydroxyindolenines containing the diketopiperazine unit failed to undergo the desired indoxyl rearrangement, a new approach was developed. The requirements for the migrating group were still unclear. In this strategy, cyclization of the diketopiperazine was left until after indoxyl formation. Hydroxyindolenines bearing a glycine unit with various protecting groups were synthesized.

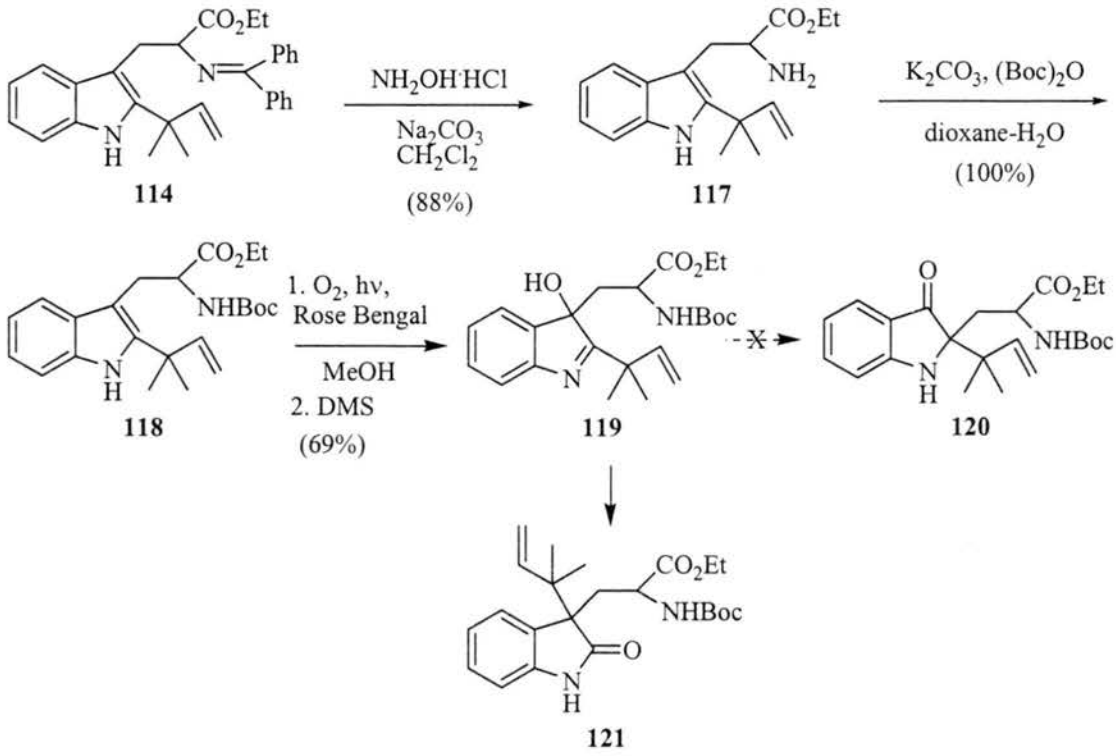
This approach was attractive from the standpoint that several glycine derived hydroxyindolenines were accessible *via* coupling of gramine derivative **24** to diphenylmethylene protected glycine ethyl ester **113** (**Scheme 36**).[33] The hydroxy group was introduced by photooxidation with O_2 and Rose Bengal in MeOH, giving hydroxyindolenine **115**.[46] The resulting product **115** was highly unstable to silica gel purification and could only be obtained in 10% yield. Pinacol-type rearrangements were attempted with DBU in MeOH and KH, 18-crown-6 ether in toluene, resulting in no desired products. Due to the instability of the diphenylmethylene amino protecting group, it was necessary to reprotect this functionality with a more durable group.



Scheme 36. Protected Glycine Route to Indoxyl

The t-butoxycarbonyl moiety was chosen as the new protecting group because these compounds can be purified by silica gel chromatography. Starting with indole **114**, removal of the diphenylmethylene group was accomplished by treatment with hydroxylamine and Na_2CO_3 in CH_2Cl_2, giving free amino compound **117** in 88% yield. The amine was reprotected with a Boc group in quantitative yield. Next, photooxidation

with O_2 and Rose Bengal in MeOH[46] was used to obtain hydroxyindolenine **119** in 69% yield (**Scheme 37**). Rearrangement of hydroxyindolenine **119** to the indoxyl was atttempted with a variety of reagents. Treatment of hydroxy compound **119** with CSA in EtOH had no effect at RT, however, upon gentle reflux, decomposition occurred. It was proposed that the prenyl group could be protonated under acidic conditions, leading to decomposition. Rearrangement was then attempted using basic conditions. There was concern as to whether or not the steric bulk of the base mattered in terms of deprotonating hydroxyindolenine **119** at the α-tryptophan carbon. To test this theory, both a sterically hindered base (KHMDS) and an unhindered one (K_2CO_3) were used for the rearrangement. Only starting material and decomposition were observed. A titanium Lewis acid and two chloroformates were used with the intention of activating the imine moiety in **119** for concomitant rearrangement, but these conditions led to formation of oxindole **121**. Reaction with DBU as well as HOAc also produced oxindole **121**. A higher temperature may favor rearrangement to the oxindole rather than the indoxyl. However, these results were seen in several solvents and varying temperatures.

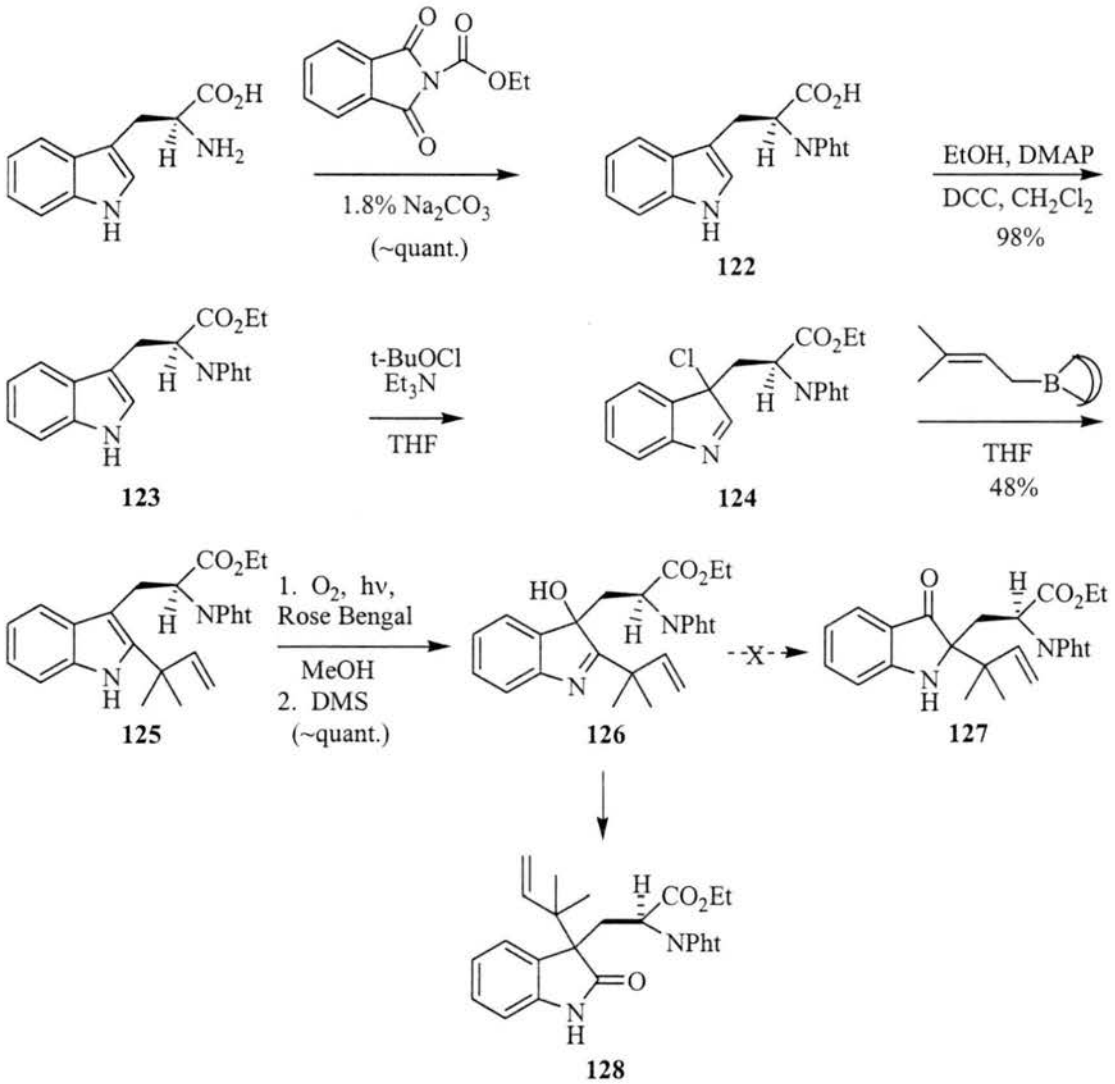


Conditions	Results
CSA / EtOH	sm + decomp
HOAc / MeOH	oxindole **121** (45%)
$Ti(IV)[CH_3)_2CHO]_4$ / CH_2Cl_2	oxindole **121** (20%)
$CH_3C(O)Cl$ / CH_2Cl_2	oxindole **121** (60%)
$ClC(O)OCH_3$ / CH_2Cl_2	oxindole **121** (60%)
DBU / THF	oxindole **121** (10%)
K-bisTMSamide / EtOH	sm + decomp
K_2CO_3 / EtOH	sm + decomp

Scheme 37. Synthesis of the Boc-Protected Hydroxyindolenine **117**

Since it was unclear whether or not the amino protecting group had any influence on the pinacol rearrangement, a hydroxyindolenine with a phthalimide group was synthesized. There was already an existing procedure to make the phthalimide protected indole from tryptophan,[61] so this opportunity was used to attempt an asymmetric synthesis of target indoxyl **8**. The amino group of L-tryptophan is first protected as

phthaloyl derivative **122** in quantitative yield (**Scheme 38**). Carboxylic acid **122** is esterified by treatment with EtOH, DMAP, and DCC in CH_2Cl_2. Subjecting **123** to t-BuOCl installed a chlorine atom at the 3-position of the indole. Addition of prenyl 9-BBN[61] to **124** gave indole **125** in 48% yield. Photooxidation with Rose Bengal followed by reductive work-up yielded the corresponding hydroxyindolenine **126**. When hydroxyindolenine **126** was subjected to Lewis acids ($ZnCl_2$, $TiCl_4$) as well as 1M NaOH, decomposition was observed. This was somewhat expected based on the results of Boc-protected hydroxyindolenine **119**. Treatment with CSA in EtOH led to formation of the undesired oxindole **127**. Activation of the imine in **126** was attempted using acetyl chloride, but only starting material and decomposition products were recovered. Diphenylmethylene, t-butoxycarbonyl, and now phthalimide-protected hydroxyindolenines have failed to undergo the desired indoxyl rearrangement. It is conceivable that since each of these hydroxyindolenines contains a protected amino acid unit in the migrating group, electronics might play a role in their potential to rearrangement.

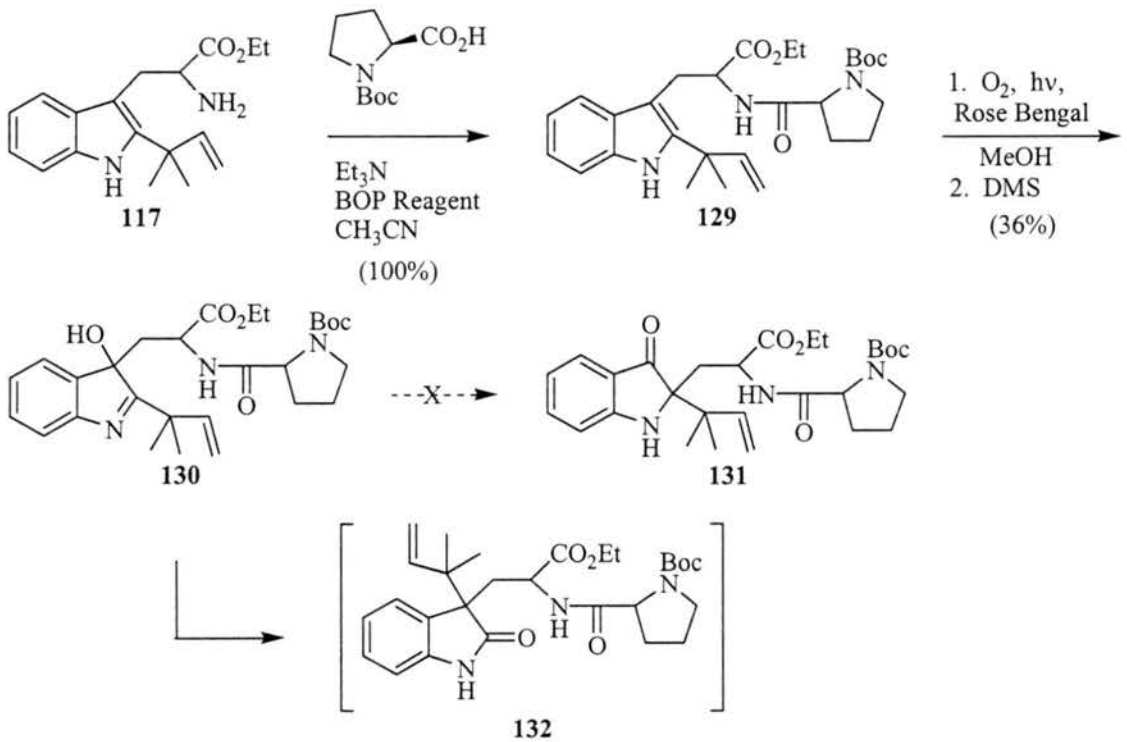


Conditions	Results
1M NaOH / MeOH	decomp
$ZnCl_2$ / Et_2O	decomp
$TiCl_4$ / Et_2O	decomp
CSA / EtOH	oxindole **128** (80%)
$ClC(O)OCH_3$ / CH_2Cl_2	sm + decomp

Scheme 38. Synthesis of Phthalimide Protected **126**

Hydroxyindolenine **130** became the next synthetic target. The electronics were slightly different with a proline coupled to the amine versus the other standard protecting

groups. This structure is very similar to the original hydroxyindolenine **103**, but the diketopiperazine cyclization is left until after the pinacol rearrangement. This compound does not have the same rigidity as **104** and thus could potentially adopt a conformation suitable for the rearrangement. The synthesis of **130** began with the deprotected indole **117**. Coupling of aminoester **117** to Boc-protected L-proline in dry CH_3CN furnished indole **129** in quantitative yield (**Scheme 39**).[54] Photooxidation of **129** using Rose Bengal followed by reductive work-up gave hydroxyindolenine **130** in 67% yield.[46] Attempts for the pinacol rearrangement of hydroxyindolenine **130** were made with an assortment of acids and bases. Oxindole formation was observed with both CSA and KHMDS. Treatment with various other mineral acids, Lewis acids, and bases resulted in decomposition.

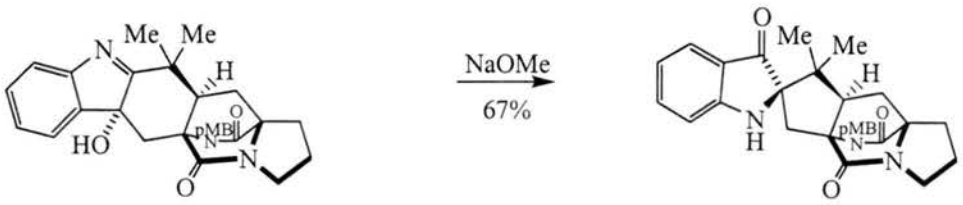


Scheme 39. Synthesis of the Proline-Coupled Hydroxyindolenine **130**

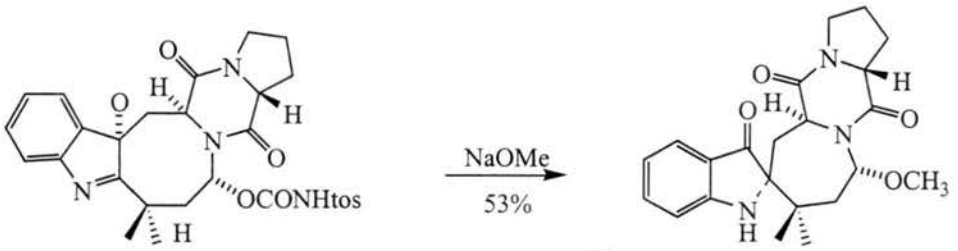
Conditions	Results
CSA / EtOH	sm + decomp + oxindole **119** (50%)
H_3PO_4 / EtOH	sm + decomp
Ti(IV) isopropoxide / CH_2Cl_2	sm + decomp
Al_2O_3	decomp
DBU / THF	decomp
KHMDS / EtOH	sm + decomp + oxindole **119** (20%)
2,6-lutidine / DMF	decomp
1M NaOH / MeOH	decomp

3.8 Indoxyl Versus Oxindole Formation

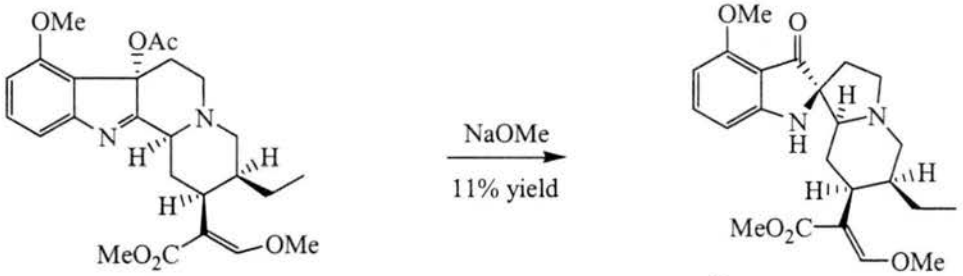
Pinacol-type rearrangements of hydroxyindolenines can lead to two different products: indoxyls and oxindoles. If the rearrangement involves a "ring-contraction," then the indoxyl is easily formed. Six-membered rings have been shown to undergo this contraction to yield the corresponding spiro compound most readily. Examples of these rearrangements are shown in **Figure 9**. The rearrangement to indoxyls can be catalyzed with bases such as NaOMe or mineral acids like H_3PO_4. Williams and co-workers treated hydroxyindolenine **75** with NaOMe, which resulted in a 67% yield of indoxyl **76**.[42] Kishi[35] and Takayama[58] also used NaOMe to accomplish this transformation. In the last example, Heathcock[59] and Borschberg[60] showed that pinacol-type rearrangement to form aristotelone, can take place under basic or acidic conditions.



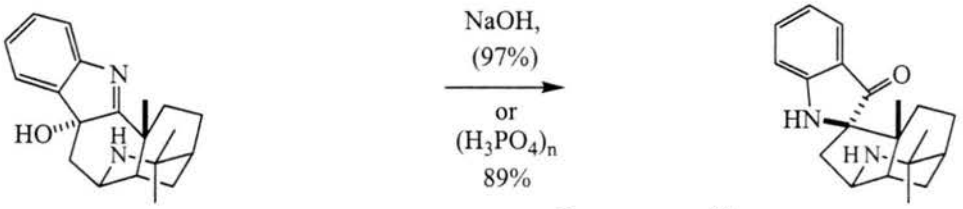
75 Brevianamide B -Williams[42] **76**



133 Austamide -Kishi[35] **134**



135 Mitragynine pseudoindoxyl -Takayama[58] **136**



137 Aristotelone -Heathcock[59], Borschberg[60] **138**

Figure 9. Successful Indoxyl Rearrangements

It is known that hydroxyindolenines can rearrange to oxindoles under Lewis acid catalysis.[60] It is also possible to convert indoxyls to oxindoles by heating. Oxindoles are more stable than indoxyls since they contain an amide bond rather than the β-keto amine functionality.

Borschberg and co-workers investigated the acid-catalyzed transformation of aristotelone **139**.[60] It was shown that treatment of **139** with a Lewis acid and heat leads to formation of oxindole **141** *via* the hydroxyindolenine (**Figure 10**). This was shown to be an irreversible process since **141** was recovered unchanged after exposure to $BF_3 \cdot Et_2O$ in CH_2Cl_2 at 95°C for 8 days.

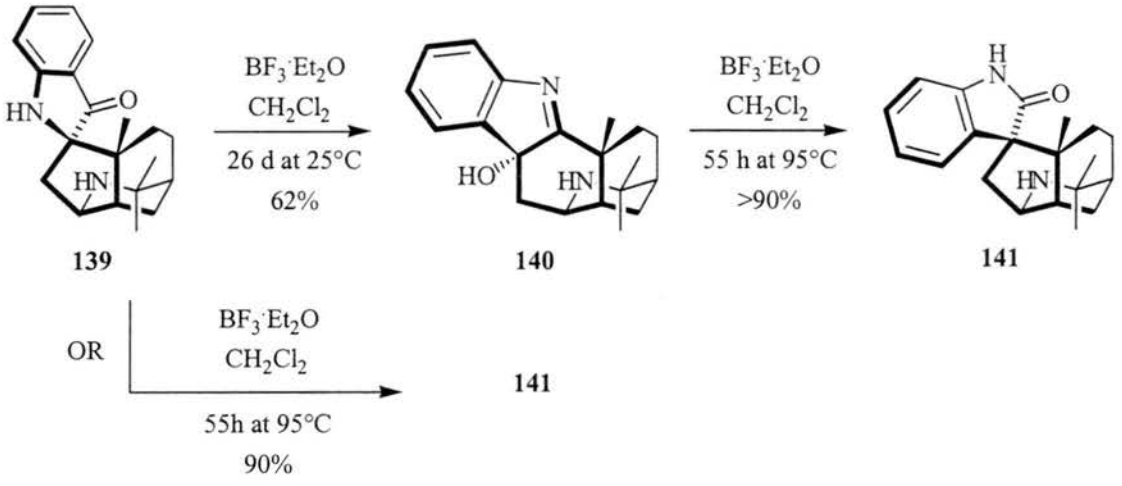


Figure 10. Indoxyl to Oxindole Conversion

The observed double 1,2-migration from carbon to carbon, accompanied by a concomitant 1,2-shift of a carbonyl group in the opposite direction, is depicted by an analogous transformation of 2,2-diphenyl-1,2-dihydro-3*H*-indol-3-one into 3,3-diphenyl-1,3-dihydro-2*H*-indol-2-one, reported by Witkop and Ek.[62] **Figure 11** shows the two potential pathways for the conversion of indoxyl **139** to oxindole **141**.

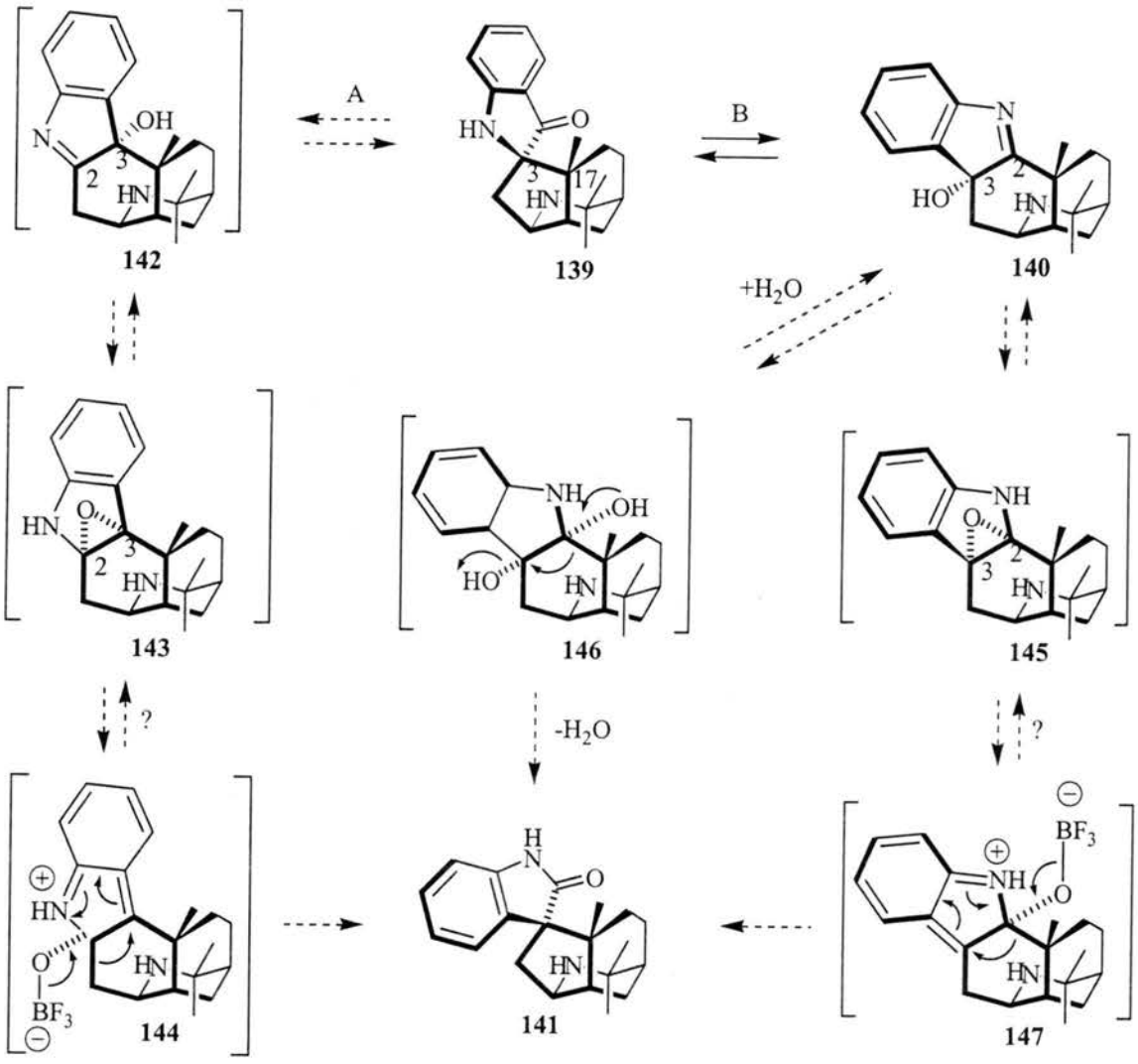


Figure 11. Rearrangement of Indoxyl **139**

Pathway **A** is electronically favored, but would lead to intermediate **142**, which is less stable than the alternative **140**.[63] Formation of epoxides **143** or **145** followed by oxirane ring-opening could give either the respective precursors **142** or **140** or the iminium salts **144** and **147**. Compounds **144** and **147** are then expected to rearrange to the observed final product **141**. Alternatively, hydrated hydroxyindolenines, such as *cis*-diol **146**, can be considered probable reactive intermediates.[63]

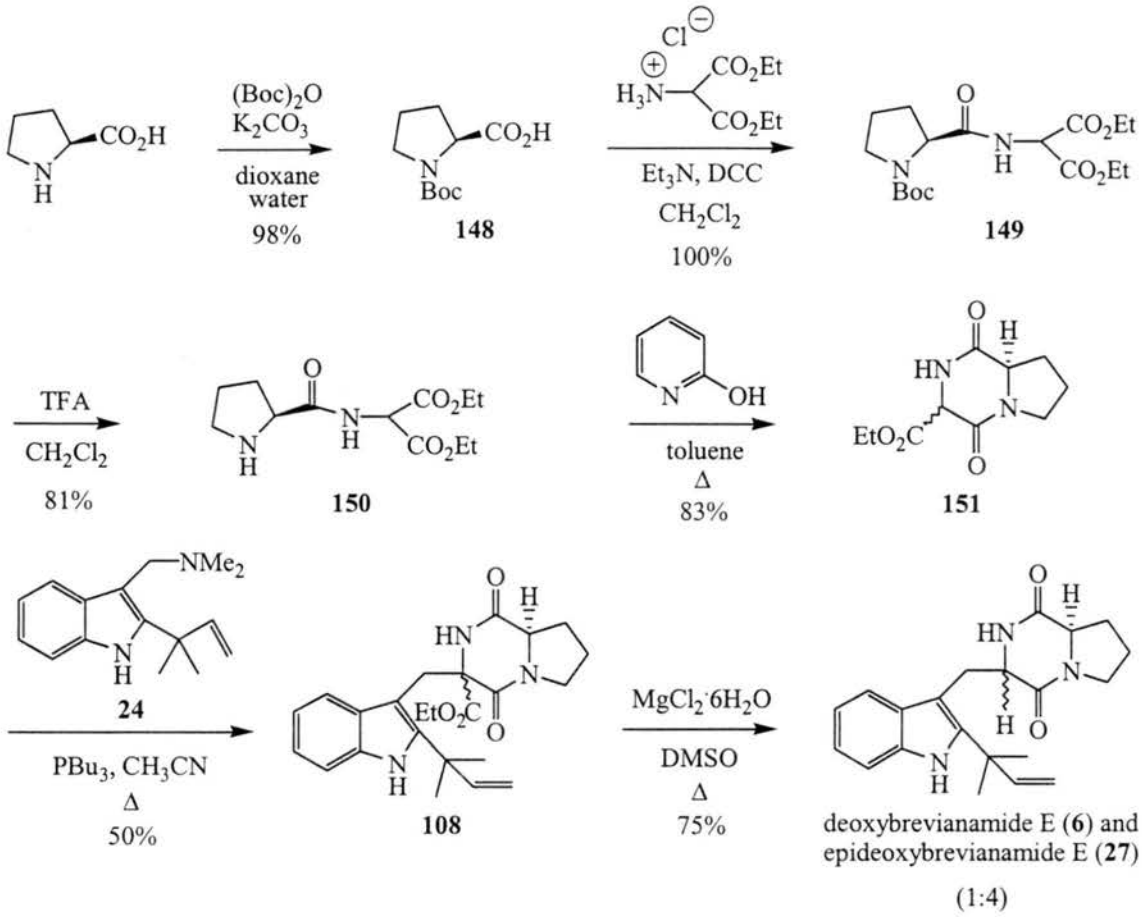
There are no literature examples of hydroxyindolenines rearranging to indoxyls that undergo an acyclic "R group" migration. Previously in the Williams group, however, Sanz-Cervera showed that TBS-protected hydroxyindolenine **77** rearranged to indoxyl **78** by treatment with silica gel and CH_2Cl_2 (**Scheme 20**) (unpublished results). This substrate could not be elaborated to target indoxyl **8** so a new indoxyl precursor was necessary. During the early indoxyl studies, nitrile hydroxyindolenine **85** rearranged to indoxyl **86** (**Scheme 22**). All attempts to convert indoxyl **86** to the key aldehyde **81**, failed. Finally, **81** was obtained *via* cyclic acetal hydroxyindolenine **96** (**Scheme 29**). Optimization of the rearrangement step is necessary for a viable synthesis. At this point, yields range from 0-40% for the pinacol-type rearrangement of **96**. Frequently, the oxindole is recovered as the sole product. As was shown by numerous attempts for rearrangement of hydroxyindolenines containing an amino acid derivative, this reaction does not produce the desired indoxyl. It is possible that the overall relative stability of the oxindole greatly hinders rearrangement of hydroxyindolenines containing an acyclic migrating group.

Chapter 4

Biomimetic Synthesis of Brevianamide B

In pursuit of identifying the biosynthetic pathway of the brevianamides, a biomimetic synthesis of brevianamide B (**2**), was accomplished.[64] Gathering information concerning the biogenesis of the core bicyclo [2.2.2] ring system, with respect to the possibility of an enzyme-catalyzed reaction, was the chief goal of this work. In particular, it was hoped that the stereoselectivity of the [4 + 2] cycloaddition of azadiene **154** could be controlled. If the Diels-Alder cyclization is influenced by solvent conditions, this would prove helpful toward understanding the biological system. The biomimetic synthesis began with deoxybrevianamide E (**6**), which was synthesized according to a slightly modified procedure originally reported by Kametani (**Scheme 40**).[10] Protection of L-proline with a Boc group resulted in 98% yield of amino acid **148**. Compound **148** was coupled to diethylaminomalonate, affording proline amide derivative **149** in quantitative yield. Removal of the Boc-protecting group was accomplished by treatment with TFA at 0°C in CH_2Cl_2. Aminomalonate **150** was cyclized in 2-hydroxypyridine and refluxing toluene to afford diketopiperazine **151** as a mixture of diastereomers (83% yield). Somei coupling of diketopiperazine **151** with gramine derivative **24** (refer to **Scheme 13**) in dry CH_3CN with Bu_3P furnished a mixture of monoesters **108** in 50% yield. Saponification of indole **108** followed by decarboxylation

furnished a 1:4 mixture of deoxybrevianamide E (**6**) and epideoxybrevianamide E (**27**) in 75% combined yield.

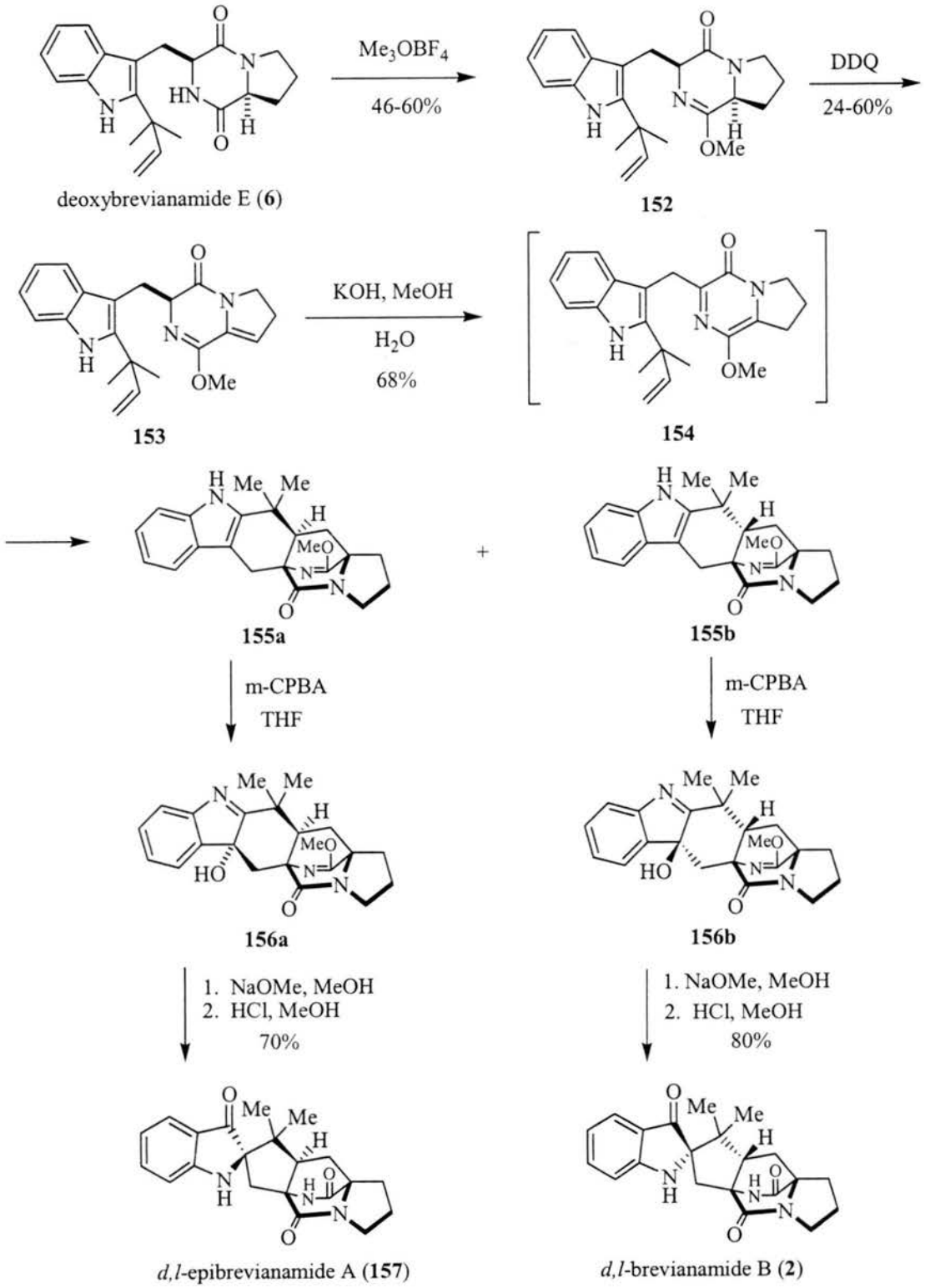


Scheme 40. Synthesis of Deoxybrevianmide E (**6**)

The biomimetic synthesis can be accomplished with either deoxybrevianamide E or epideoxybrevianamide E. Both methine protons in the diketopiperazine are removed in the formation of azadiene **154**. The biomimetic synthesis begins with treatment of **6** with Me_3OBF_4 in CH_2Cl_2 to afford lactim ether **152** (79% yield). Oxidation of **152** with DDQ provided unsaturated compound **153** in moderate yield (24-40%). Subjecting diene **153** to KOH in MeOH furnished the labile azadiene **154**. The azadiene intermediate could be isolated by silica gel chromatography and characterized spectroscopically.

Upon standing, however, **154** spontaneously cyclized to give a mixture of the cycloadducts **155a** and **155b** (2:1, 90% combined yield).

Hydroxylation of **155a** to C-19-*epi*-brevianamide A was accomplished by diastereoselective *m*-CPBA oxidation to hydroxyindolenine **156a** (~quant.). Next, a pinacol-type rearrangement of **156a** (0.5M NaOH, MeOH, RT, reflux) produced the corresponding *spiro*-indoxyl. Subsequent removal of the lactim ether with HCl gave *d,l*-C-19-*epi*-brevianamide A **157** in 70% overall yield from **156**. Epibrevianamide A (**157**) was previously synthesized by Kwast in the Williams group.[42] Compound **157** was identical to the authentic material except for being racemic. Transformation of the minor cycloadduct **155b** into *d,l*-brevianamide B (**2**) was achieved in similar fashion in 80% overall yield, thereby securing the relative stereochemistry of each respective cycloadduct **155a** and **155b**.



Scheme 41. Biomimetic Synthesis of Brevianamide B

This study supports the theory that the core bicyclo [2.2.2] ring system is very likely derived from an intramolecular Diels-Alder cyclization. It is interesting to note that the diastereofacial bias of the Diels-Alder cyclization of **154** is not strongly influenced by the choice of solvent. The ratio of **155a** : **155b** (~2:1) was the same in THF as well as aqueous MeOH. The C-19-*epi*- metabolites (corresponding to **155a** and epibrevianamide A) are not produced by *Penicillium brevicompactum* nor have there been documented cases on the isolation of similarly epimeric metabolites from the paraherquamide or sclerotamide families. Thus, it is plausible that an enzyme is involved in the organization of the transition state structures which accounts for the exclusive facial selectivity observed in their biosynthetic systems.

It would be interesting to continue studies on the Diels-Alder cyclization of diene **154**. One could test various Lewis acids to see if stereoselectivity is increased. It seems reasonable that Lewis acid coordination to the diketopiperazine carbonyl could potentially control whether or not the prenyl group dienophile approaches from the top or bottom face.

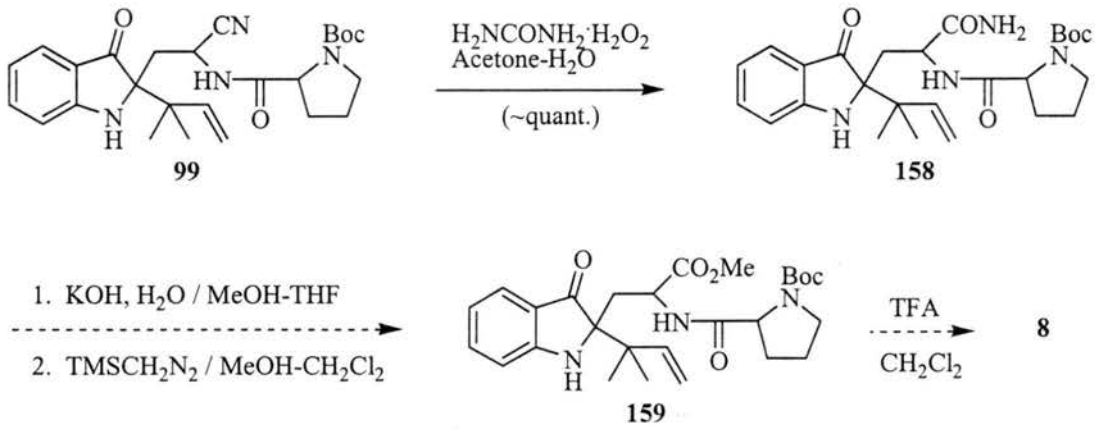
Chapter 5

Future Studies for the Brevianamide Project

5.1 Completion of Indoxyl 8

The most promising route for synthesis of indoxyl **8** is shown in **Scheme 42**. This strategy was attempted previously. However, difficulties in reaching the final target were encountered. The problem involved trying to cyclize the diketopiperazine through an amidation reaction. There have been recent examples in the literature that hydrolyze an amide under mild basic conditions.[65] If the carboxylic acid can be esterified *in situ* with $TMSCH_2N_2$, then removal of the Boc group should allow for spontaneous cyclization to form target indolxyl **8**.

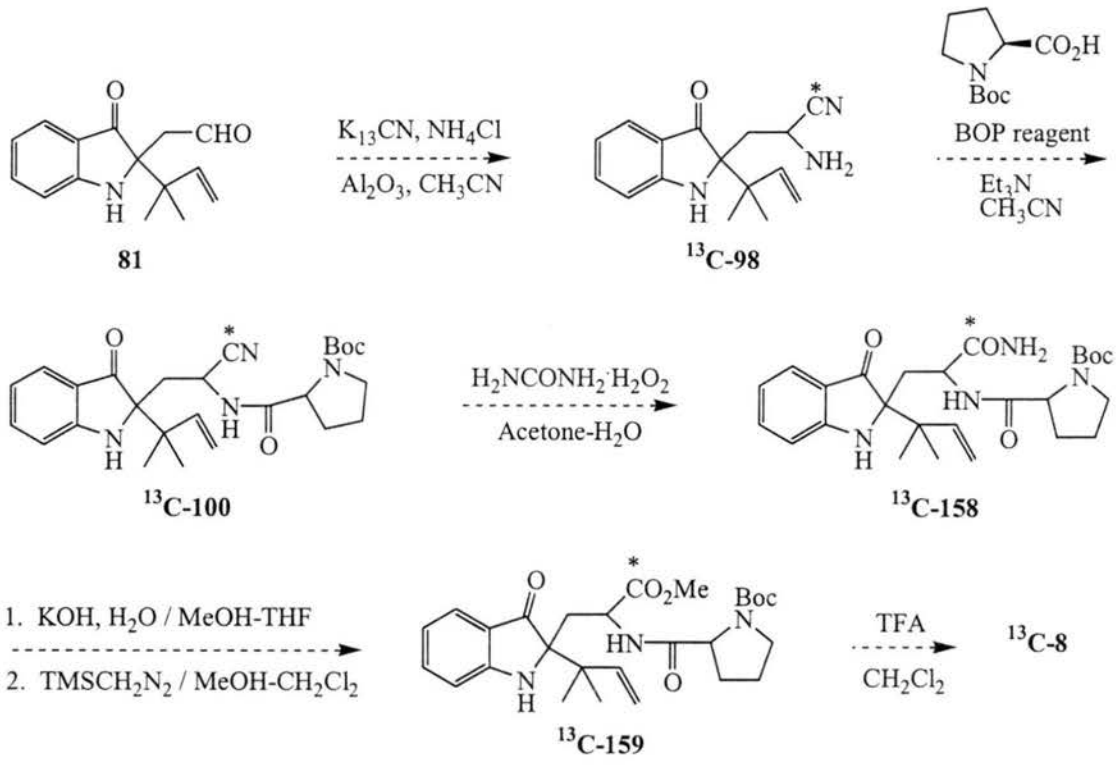
Nitrile **99** undergoes facile hydrolysis to the primary amide with urea-hydrogen peroxide complex in quantitative yield. Once amide **158** is obtained, treatment with KOH, H_2O in MeOH-THF followed by esterification *in situ* using $TMSCH_2N_2$ in MeOH-CH_2Cl_2 could provide **159**. Treatment of **159** with TFA should result in diketopiperazine cyclization, giving the desired indoxyl (**8**).



Scheme 42. Proposed Synthesis of Target Indoxyl **8**

5.2 ^{13}C-Labeled Synthesis of Indoxyl 8

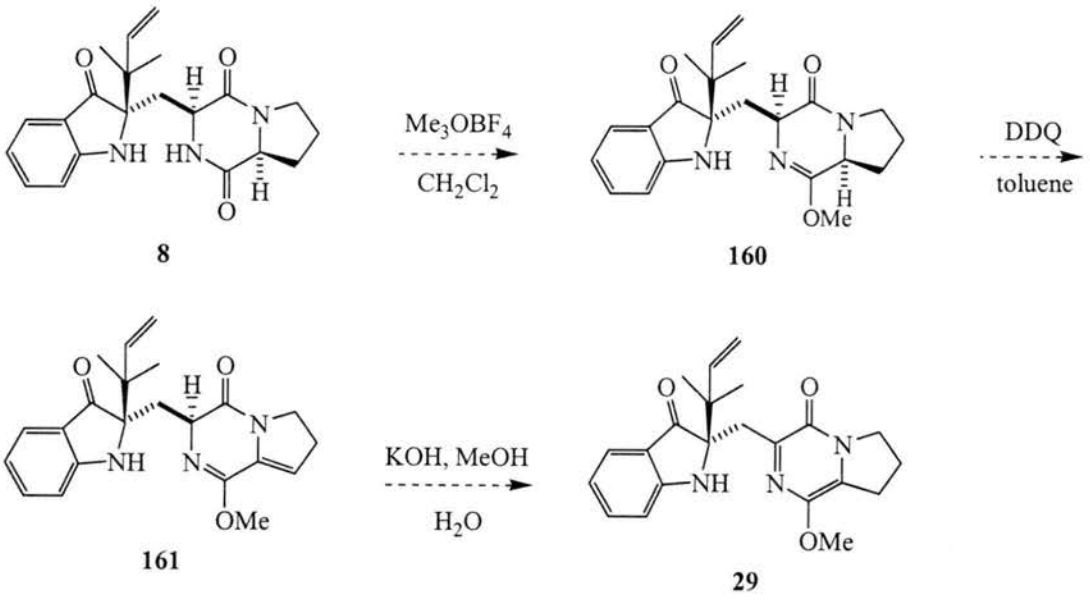
The strategy proposed in **Scheme 42** is amenable to a ^{13}C-labeled synthesis. During the Strecker reaction of aldehyde **81**, ^{13}C-labeled KCN could be used (**Scheme 43**). This route is attractive because the label could be introduced late in the synthesis. Feeding studies with labeled indoxyl **8** will be conducted in cultures of *Penicillium brevicompactum* to determine its possible intermediacy in the biological pathway.



Scheme 43. ^{13}C-Labeled Synthesis of Indoxyl **8**

5.3 Synthesis of Azadiene 29

The last intermediate in the proposed biosynthetic pathway is azadiene **29**. Its synthesis could follow the same protocol as in the brevianamide biomimetic synthesis (**Scheme 41**). Indoxyl **8** will be treated with Me_3OBF_4 in CH_2Cl_2 to form lactim ether **160** (**Scheme 44**). Oxidation with DDQ will lead to diene **161**. Finally, rearrangement of the unsaturated compound by treatment with KOH in MeOH, could produce azadiene **29**.



Scheme 44. Proposed Synthesis of Azadiene **29**

Once the synthesis of azadiene **29** is complete, a ^{13}C-labeled synthesis of **29** could be done in the same manner as indoxyl **8**. Feeding studies with *Penicillium brevicompactum* will be conducted to verify its intermediacy in the brevianamide biosynthetic pathway. The culture extracts from these feeding studies will be purified by Sephadex column chromatography in hopes of isolating the potential "Diels-Alderase."

References

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Experimental Section

6.1 General Procedures

Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. Diethyl ether and THF were distilled from sodium benzophenone keytl under a nitrogen atmosphere. Methylene chloride and triethylamine were distilled under a nitrogen atmosphere from calcium hydride. Dimethylformamide was dried over 4Å molecular sieves. The molecular sieves were activated by heating at 150°C at 1 mm Hg for 3 h in a vacuum oven.

All reactions involving hydroscopic substances were conducted with flame or oven dried glassware under an inert atmosphere (Ar) dried by passage of atmosphere gases through a column packed with $CaSO_4$.

Chromatographic separations were performed with EM Science TLC plates (silica gel 60, F254, 20 x 20 cm x 250 μm) or with EM Science 230-400 mesh silica gel under positive air pressure. Reactions and chromatographic fractions were monitored and analyzed with EM Science TLC plates. Visualization on TLC were achieved with ultraviolet light and heating of TLC plates submerged in a 5% solution of phosphomolybdic acid in 95% ethanol (pMB) or *p*-anisaldehyde in 95% ethanol or vanillin solution.

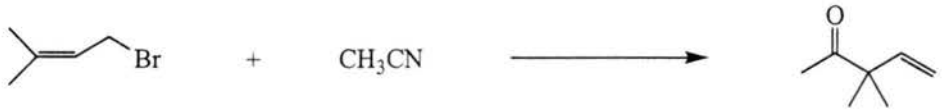
Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR neat and as thin films from methylene chloride and are reported as λ_{max} in wavenumbers (cm^{-1}). Mass spectra were obtained on a 1992 Fisions VG Autospec at the Chemistry Department at Colorado State University.

Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker AC-300, Varian 300 or 400 spectrometer. NMR chemical shifts are given in parts per million (ppm) relative to internal $CHCl_3$ or acetone. Proton (^1H) NMR are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), and coupling constant in hertz. When appropriate, the multiplicity of a signal is denoted as "br" to indicate that the signal was broad.

6.2 Preparation of Compounds, ^{1}H NMR and ^{13}C NMR Spectral Data

$$CH_3COO^-Ag^+ \quad + \quad Zn(powder) \longrightarrow Zn/Ag$$

Zn/Ag. The zinc dust was purified before use. Suspend 50 g Zn dust in 250 mL hot water, add 25 mL of 2M HCl, decant after 20 sec, wash twice with water, dry. The coupling agent was formed by adding silver acetate (50 mg, 0.30 mmol), and 50 g of purified zinc powder to 100 mL of refluxing glacial acetic acid. This was stirred for 30 sec and then quickly cooled in an ice bath. The Zn/Ag couple formed and was isolated by decantation, washed several times with anhydrous ether (6 x 50 mL), and dried under vacuum for 24 h. The yield was 50 g.

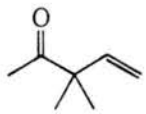


21

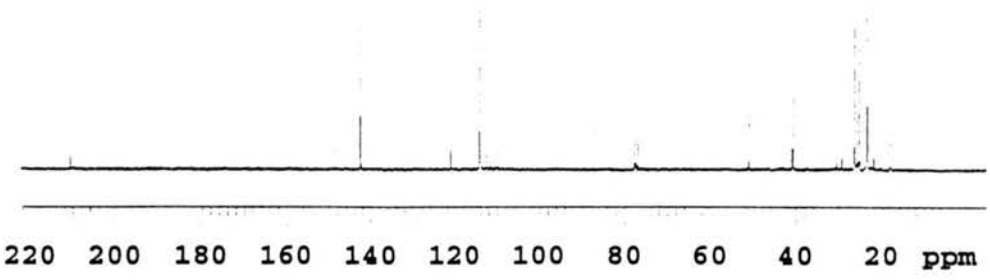
3,3-dimethyl-4-pentene-2-one (21). In a 250 mL 3-neck round bottom flask were introduced 13.1 mL CH_3CN (251 mmol, 0.75 eq.), 60 mL dry THF and 21.906 g of Zn/Ag coupling agent. While stirring vigorously at RT, 38.7 mL (335.8 mmol, 1.0 eq.) of 4-bromo-2-methyl-2-butene were added dropwise over 12 h. The mixture was stirred for an additional 24 h and then it was cooled to 0°C. 100 mL of an ice-cold saturated solution of NH_4Cl was added to the mixture and stirred for 10 min. Then it was transferred to a separatory funnel containing 150 mL of saturated NH_4Cl. The ketone was extracted with ether (4 x 75 mL), the organic extracts were pooled and dried over anhydrous $MgSO_4$.

The ether was removed by distillation at atmospheric pressure using a Vigreux column. The ether distilled at 34°C and the ketone distilled at 110-126°C. The ketone was obtained as a clear oil in 92.8% yield.

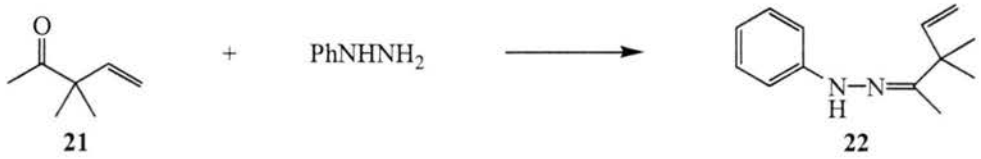
^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.2 (6 H, s), 2.1 (3 H, s), 5.06 (1 H, d, J = 10.5 Hz), 5.13 (1 H, d, J = 17.5 Hz), 5.9 (1 H, dd, J = 10.5 Hz, J = 17.5 Hz). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 23.1, 24.9, 26.1, 50.6, 113.7, 142.4, 209.8. IR (NaCl, neat): 3061, 2976, 2873, 1698, 1636, 1463, 1352 cm^{-1}. HRMS (FAB), calcd. For $C_7H_{12}O$ (MH^+): 113.0966. Found: 113.0963.



21



Compound **21**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

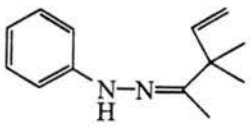


21 22

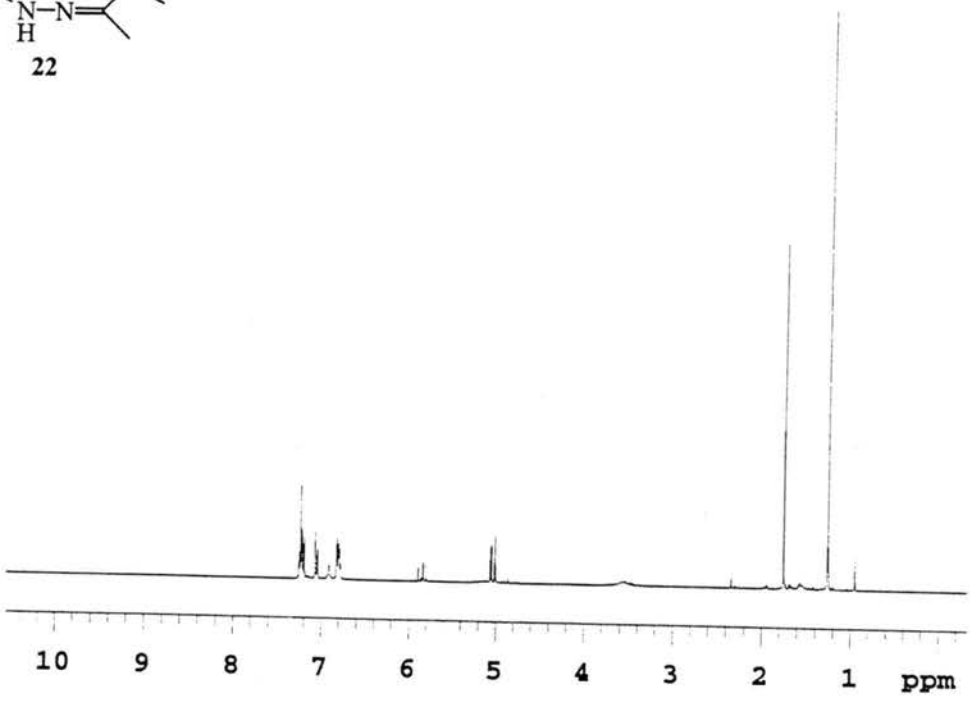
3,3-dimethyl-4-pentene-phenylhydrazone (22). 26.08 g of the ketone (232.8 mmol, 1.0 eq.), 117 mL of toluene, and 22.84 mL of phenyl hydrazine (232.8 mmol, 1.0 eq.) were put into a round bottom flask fitted with a Dean Stark distillation set. The mixture was refluxed for 30 min. while the water was removed. The toluene was removed *in vacuo*. The slight excess of phenyl hydrazine was removed under vacuum by gentle heating. The hydrazone was obtained as an orange oil in 82.6% yield (38.87 g).

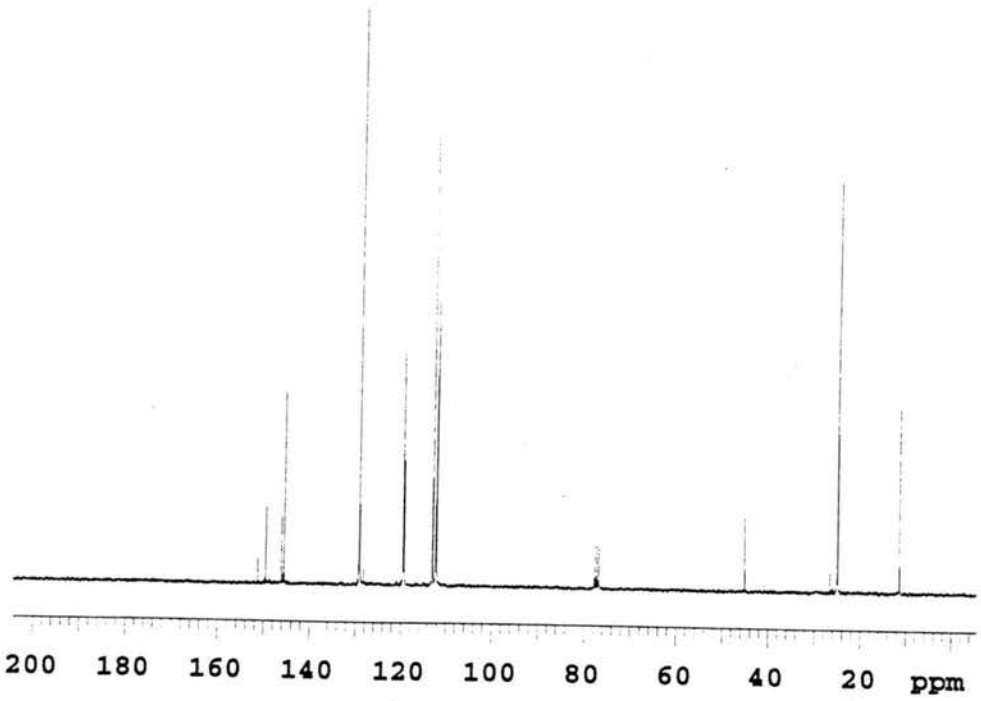
The hydrazone was dried thoroughly (overnight under vacuum over phosphorus pentoxide in a vacuum dessicator) before being used in the following reaction. Any water present will hydrate the double bond in the hydrazone.

^{1}H NMR (300 MHz) $CDCl_3$) δ TMS: 1.25 (6 H, s), 1.75 (3 H, s), 5.01 (1 H, d, J = 10.4 Hz), 5.05 (1 H, d, J = 17.7 Hz), 5.85 (1 H, dd, J = 10.4 Hz, J = 17.7 Hz), 6.78-7.25 (5 H, m), 6.91 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 11.0, 24.8, 44.8, 112.0, 112.1, 112.9, 119.3, 129.0, 145.5, 149.2. IR (NaCl, neat): 3352, 3052, 2970, 2928, 1607, 1503, 1246, 1099 cm^{-1}.



22





Compound **22**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

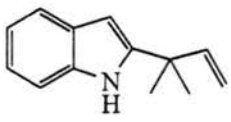
22 23

2-(1,1-dimethylallyl)indole (23). The $ZnCl_2$ was dried by heating in an erlenmeyer flask over a Bunsen burner until it became a solution. Heating was continued for a few minutes until all water was removed. It was quickly poured into an oven-dried mortar and ground with a pestal to a fine white powder. It was then immediately used for the reaction.

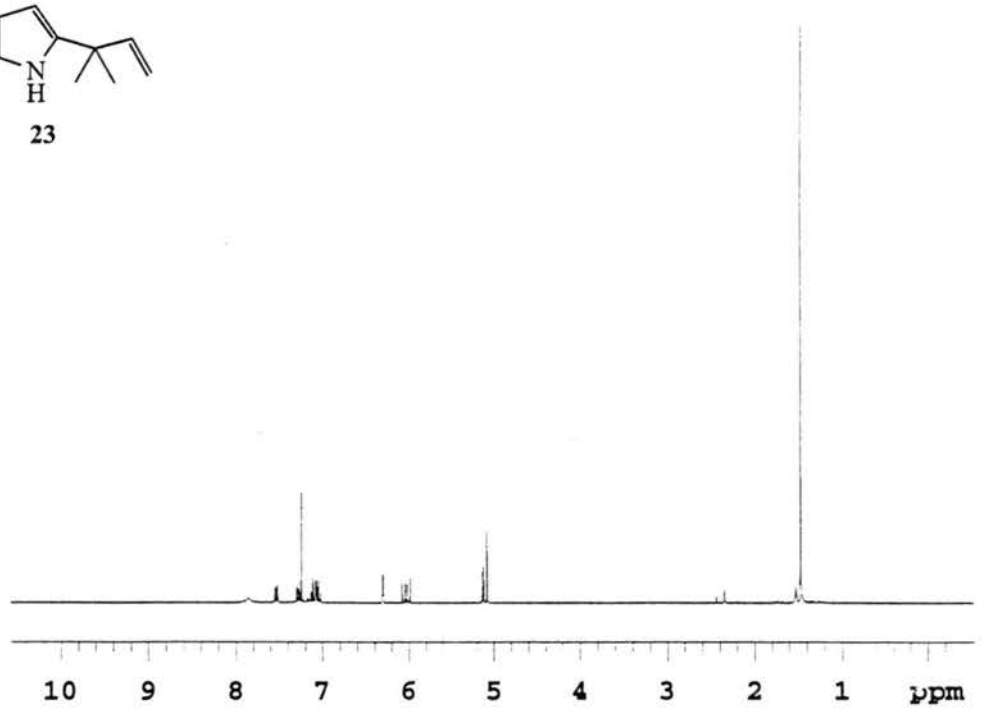
The crude hydrazone (38.87 g, 192 mmol, 1.0 eq.) was added to a hot solution (150°C) of 54.4 g of $ZnCl_2$ (400 mmol, 2.08 eq.) in 121 mL of dry diglyme. The reaction was refluxed for 9 h under argon.

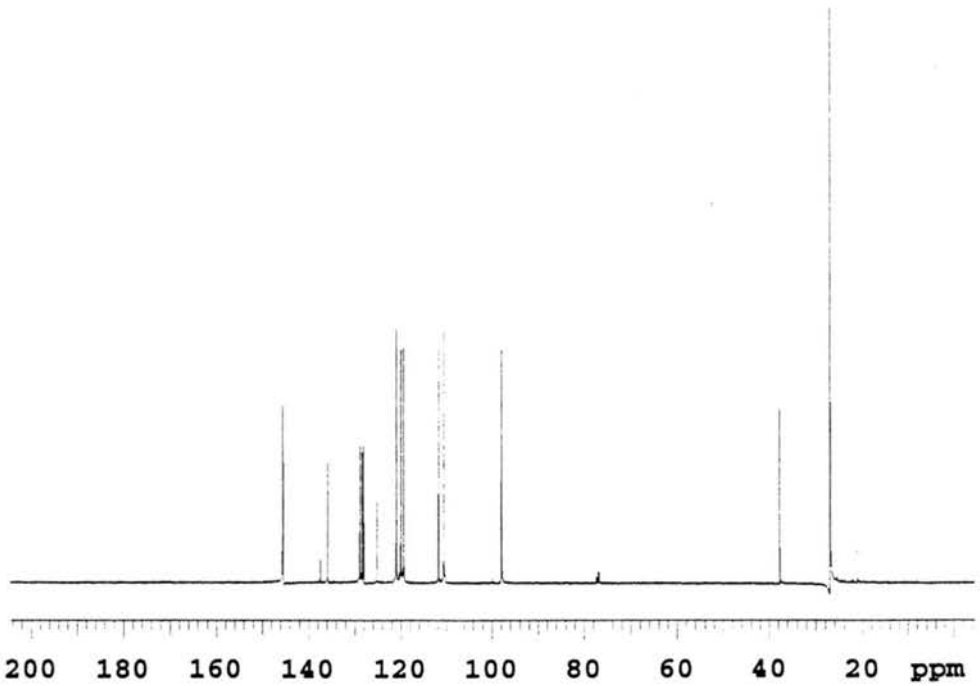
121 mL of toluene was added to the reaction mixture while it was still hot (otherwise the $ZnCl_2$ will crystallize). The mixture was stirred while it cooled to RT. The mixture was separated by CC using silica gel and toluene, giving 16.02 g of a yellow oil (45% yield). A small amount of MeOH can be used to load the crude mixture onto the column.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.47 (6 H, s), 5.09 (1 H, s), 5.14 (1 H, dd, J = 0.9 Hz, J = 5.4 Hz), 6.04 (1 H, dd, J = 10.5 Hz, J = 17.7 Hz), 6.32 (1 H, dd, J = 0.9 Hz, J = 2.1 Hz), 7.09 (2 H, dddd, J = 1.5 Hz, J = 7.2 Hz, J = 17.7Hz), 7.30 (1 H, d, J = 7.8 Hz), 7.55 (1 H, d, J = 7.5 Hz), 7.87 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 27.3, 98.1, 110.7, 112.0, 119.6, 120.2, 121.2, 145.9. IR (NaCl, neat): 3413, 2957, 1632, 1537, 1456, 1404, 1290 cm^{-1}. R_f = 0.67 (toluene, vanillin: dark blue).



23





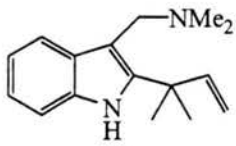
Compound **23**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

23 24

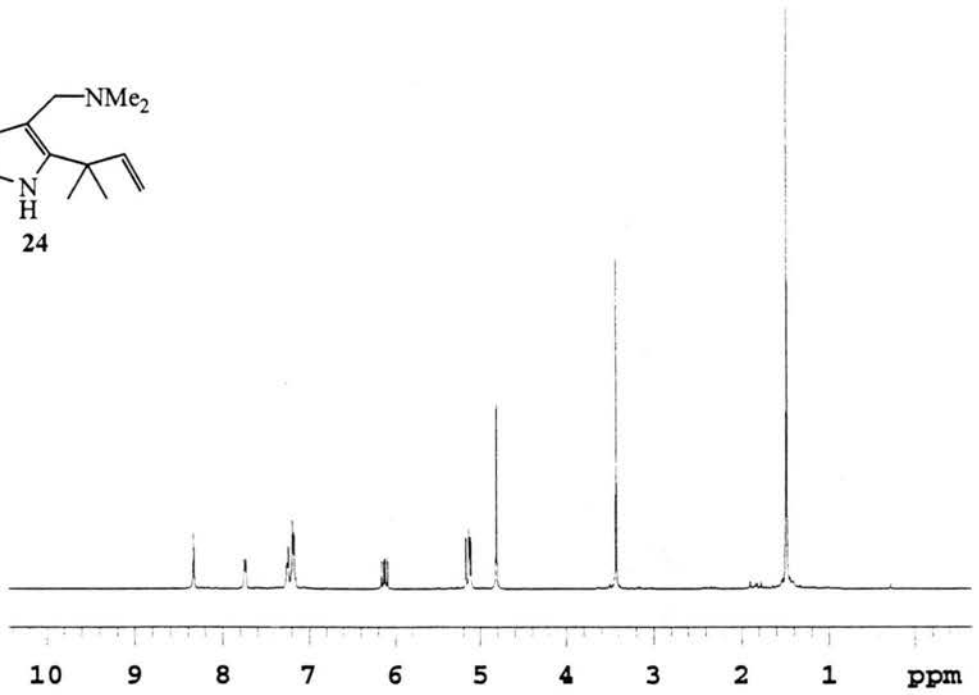
2-(1,1-dimethylprop-2-enyl)-3-(N,N-dimethylaminomethyl)indole (24). 8.79 g of a 40% aq. solution of Me_2NH (77.93 mmol, 0.9 eq.) was cooled to 2°C and 12.94 g of pre-cooled glacial AcOH (199.16 mmol, 2.3 eq.) was added. When the temperature cooled back down, 19.61 g of 38% formaldehyde (247.65 mmol, 2.86 eq.) were added. The mixture was stirred for a few minutes and then it was added to 16.02 g of the indole in a round bottom flask. Enough MeOH (52 mL) was added to make the solution homogeneous. The reaction was stirred for 18 h at RT.

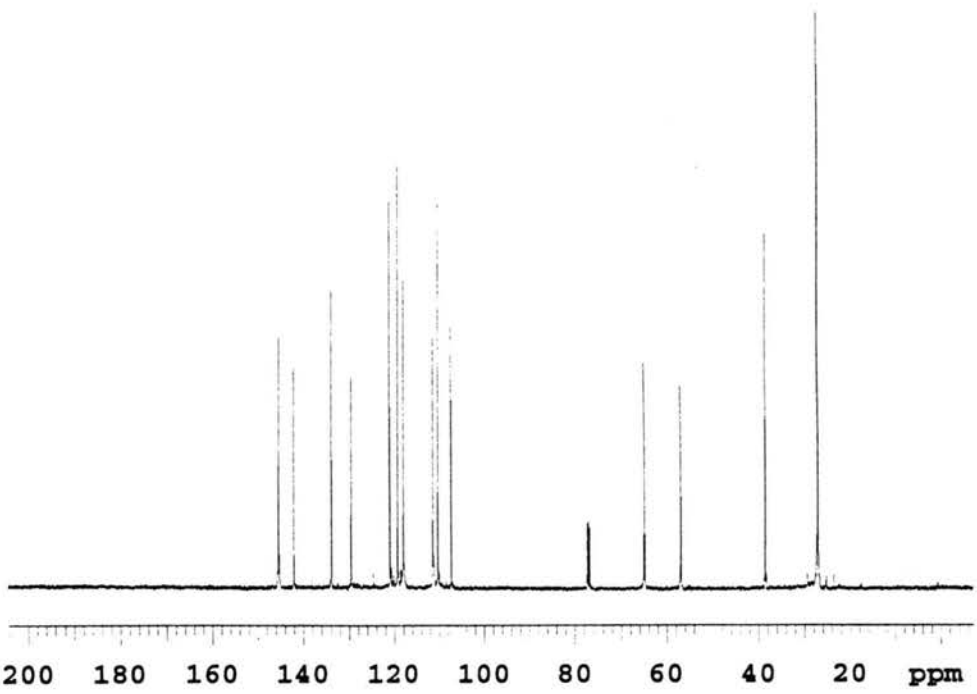
The reaction mixture was then partitioned between 187 mL ether and a solution of 19.6 g KOH in 220 mL water. The aq. phase was extracted once more with ether. The organic extracts were pooled and washed with brine. After drying over anhydrous Na_2SO_4, the ether was removed *in vacuo*, to give 20.53 g of the crude product (orange viscous oil, 98% yield). No purification was necessary.

^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.51 (6 H, s), 3.85 (2 H, s), 5.20 (1 H, d, J = 6.9 Hz), 5.25 (1 H, s), 6.09 (1 H, dd, J = 11.1 Hz, J = 17.1 Hz), 7.10-7.24 (2 H, m), 7.33 (1 H, d), 7.55 (1 H, d), 8.06 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 144.9, 141.1, 134.1, 128.5, 122.3, 120.4. IR (NaCl, CH_2Cl_2): 1008, 1072, 1261, 1425, 1460, 1478, 1542, 1613, 1631, 2754, 2801, 2931, 2954, 3036, 3330, 3448 cm^{-1}. R_f = 0.86 (10:1 CH_2Cl_2/MeOH, vanillin: brown)



24



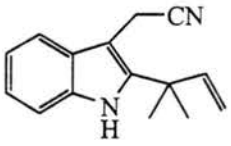


Compound **24**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

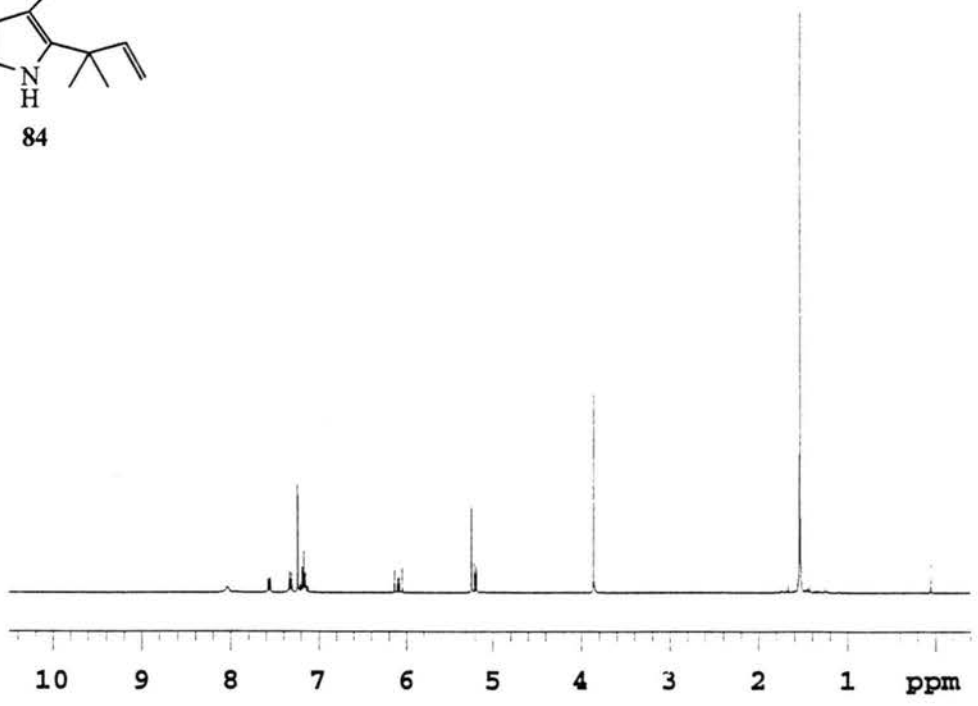
24 84

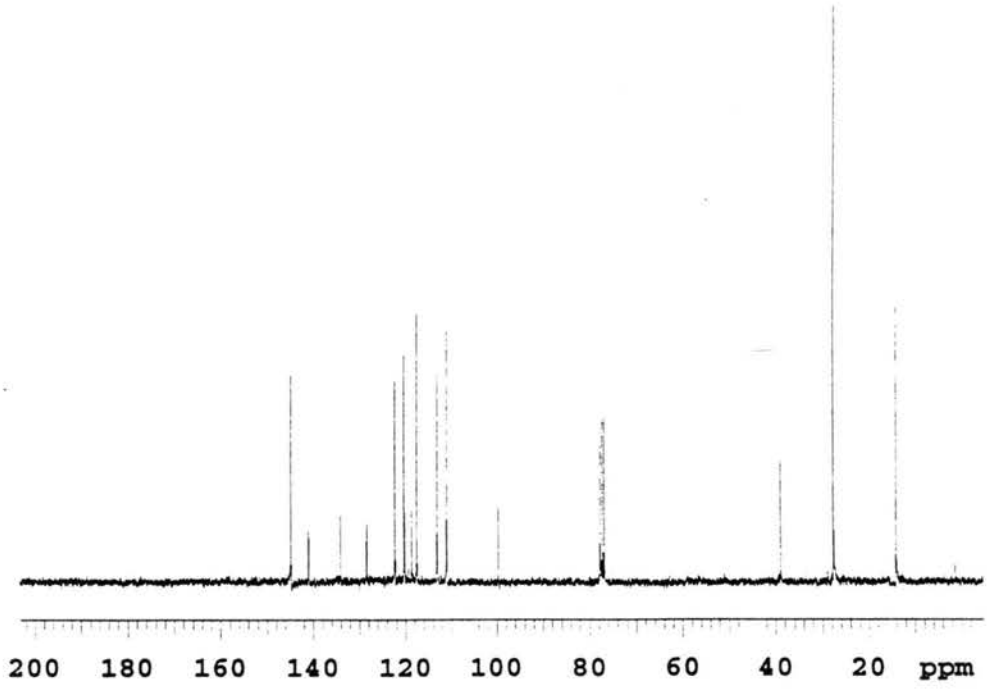
2-(1,1-dimethylprop-2-enyl)-3-(cyanomethyl)indole (84). To a stirred solution of **24** (55.4 mg, 0.2290 mmol, 1.0 eq) in THF (2.5 mL) under Argon at 0°C was added methyl iodide (160 mg, 0.07 mL, 1.1268 mmol, 5.0 eq) over 3 min. *via* syringe. After 1 h, THF was removed *in vacuo*. Potassium cyanide (15 mg, 0.229 mmol, 1.0 eq) and DMF (2 mL) were added and the resulting mixture was refluxed for 1 h. The reaction mixture was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The aqueous layer was extracted once more with CH_2Cl_2 (10 mL). The organic extracts were pooled and washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated. 44.5 mg (87%) of crude oil **84** was obtained. The crude material was purified by CC (silica; 100% CH_2Cl_2) giving indole **84** as a yellow powder.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.56 (6 H, s), 3.86 (2 H, s), 5.20 (1 H, d, J = 6.23 Hz), 5.25 (1 H, s), 6.09 (1 H, dd, J = 10.99 Hz, J = 16.85 Hz), 7.12-7.22 (2 H, m), 7.31-7.35 (1 H, m), 7.54-7.59 (1 H, m), 8.04 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 14.05, 27.63, 33.86, 99.63, 111.02, 113.10, 117.56, 118.65, 120.32, 122.28, 128.41, 134.05, 141.01, 144.84. IR (NaCl, CH_2Cl_2): 1264, 1421, 1618, 1636, 2247, 2305, 2975, 3054, 3380, 3469 cm^{-1}. R_f = 0.47 (100% CH_2Cl_2, vanillin: purple).

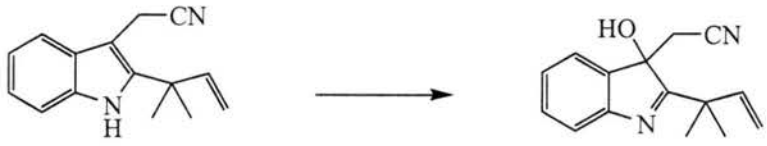


84





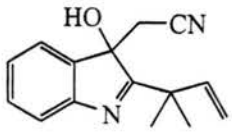
Compound **84**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



84 85

2-(1,1-dimethylprop-2-enyl)-3-cyano-3-hydroxyindolenine (85). To a stirred solution of methanol (15 mL) and Rose Bengal (0.10 g, 0.10 mmol, 0.3 eq.) was added nitrile **84** (220 mg, 0.9821 mmol, 1.0 eq). The mixture was irradiated with a 250 Watt Hg white spot lamp. O_2 was bubbled through the solution while stirring at 0°C. After 4.5 h, dimethyl sulfide (0.5 mL) was added and the mixture was placed in the cold room (3°C) to stir overnight. The reaction mixture was concentrated and filtered through an alumina column to remove the rose bengal. A yellow band was obtained in the first few fractions using CH_2Cl_2/MeOH 25:1 as eluent.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.20 (6 H, d, J = 7.5 Hz), 2.28 (1 H, d, J = 16.5 Hz), 3.01 (1 H, d, J = 16.2 Hz), 3.62 (1 H, s), 4.90 (1 H, d, J = 10.8 Hz), 4.95 (1 H, d, J = 17.7 Hz), 5.86 (1 H, dd, J = 10.2 Hz, J = 17.1 Hz), 6.89-6.96 (1 H, m), 7.08 (1 H, ddd, J = 1.2 Hz, J = 7.8 Hz, J = 9.0 Hz), 7.17-7.26 (2 H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 144, 142, 138, 136.5, 131, 127, 122.5, 121.4, 119.9, 114.3, 43.3, 40.9, 27.9, 27.4, 26.4. R_f = 0.36 (25:1 CH_2Cl_2/MeOH, PMA: light brown)



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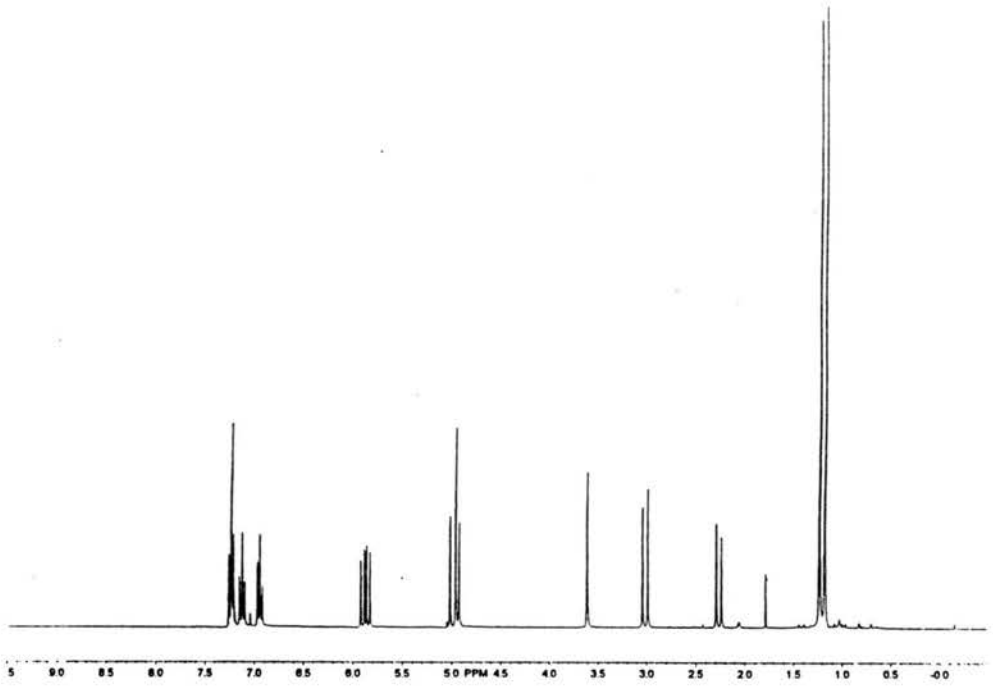
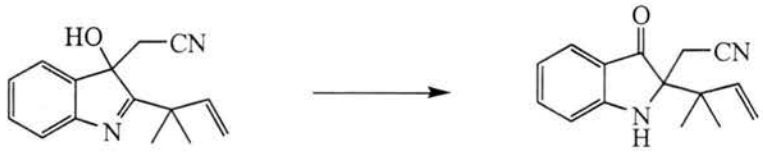


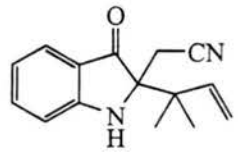
Compound **85**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



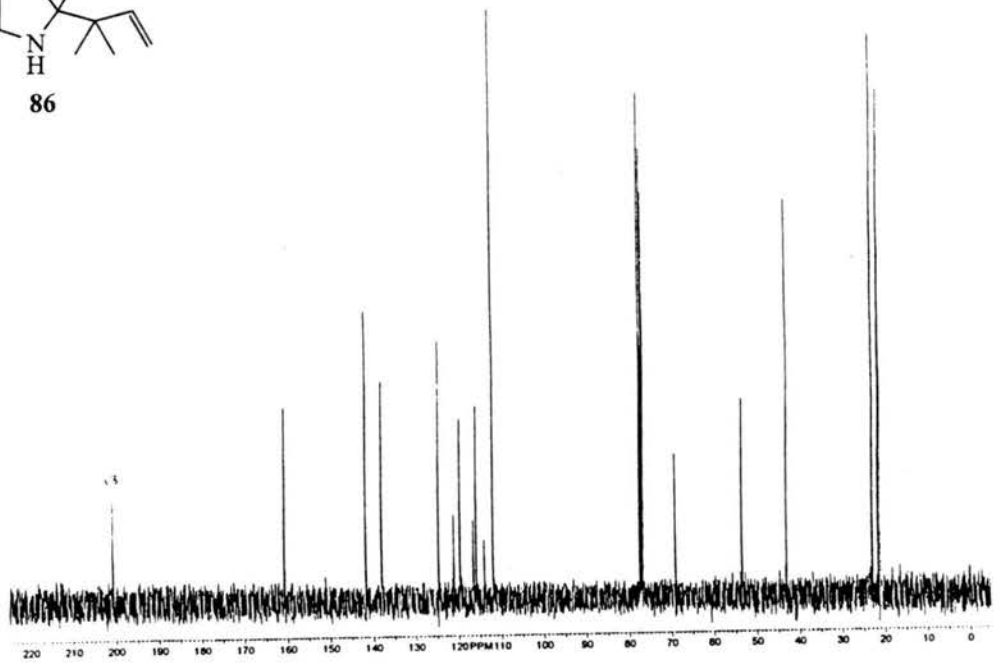
85 86

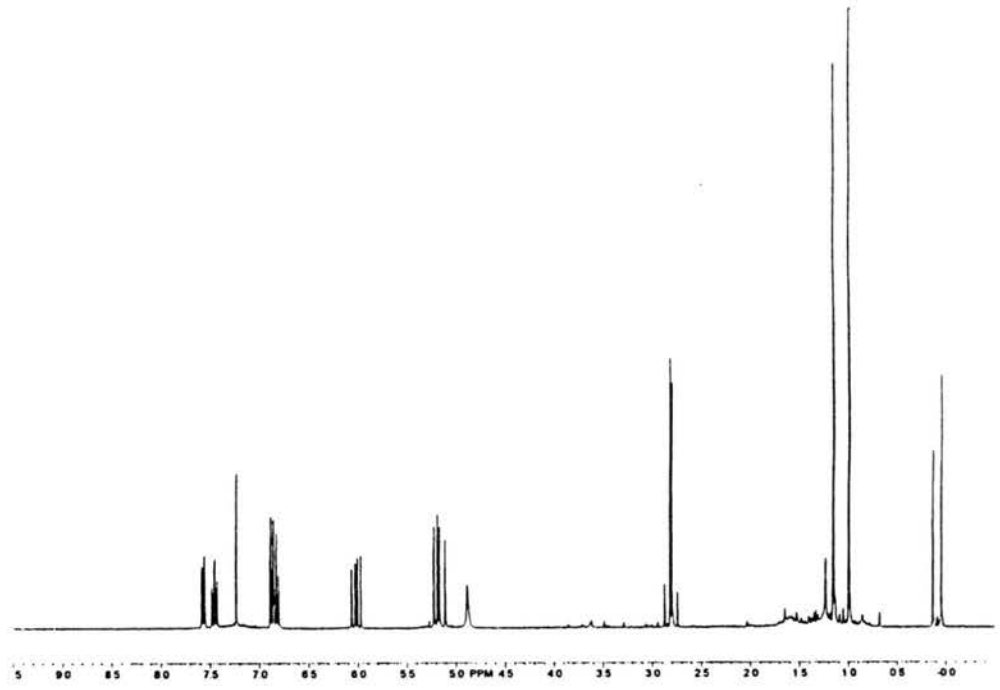
2-(1,1-dimethylprop-2-enyl)-2-(cyanomethyl)indoxyl (86) . Compound **85** (682.6 mg, 2.844 mmol, 1.0 eq) was stirred with sodium bis(trimethylsilyl)amide (144 μL, 0.711 mmol, 0.25 eq) in dry THF (25 mL) in an ice bath (0°C) under argon. After a few minutes, the reaction was moved to the cold room (3°C) to stir overnight (8 h). The color of the solution went from red to greeen-brown. The THF was removed *in vacuo*. The residue was taken up in ether and washed twice with water and once with brine. The organic layer was dried over Na_2SO_4 followed by solvent removal *in vacuo*. Crude NMR looked fine. A small amount of product was purified by PTLC using CH_2Cl_2/MeOH 25:1 for mass spectrometry. 470 mg (69%) of product was obtained.

^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.07 (6 H, d, J = 46.9 Hz), 2.81 (2 H, dd, J = 16.5 Hz, J = 22.2 Hz), 4.89 (1 H, s), 5.14 (1 H, d, J = 17.4 Hz), 5.21 (1 H, dd, J = 0.90 Hz, J = 10.8 Hz), 6.02 (1 H, dd, J = 10.8 Hz, J = 17.4 Hz), 6.80-6.90 (2 H, m), 7.42-7.48 (1 H, m), 7.55-7.60 (1 H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ: 200.9, 160.9, 141.9, 138.2, 124.9, 121.2, 119.8, 116.7, 116.0, 112.2, 43.2, 23.1, 21.6, 21.5. R_f = 0.52 (25:1 CH_2Cl_2/MeOH, fluorescent yellow)

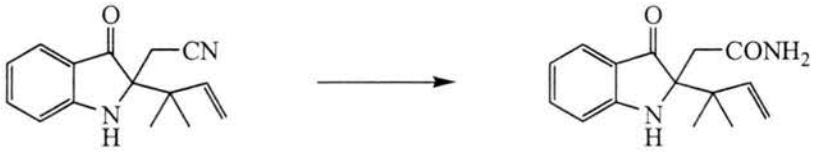


86





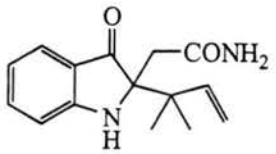
Compound **86**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



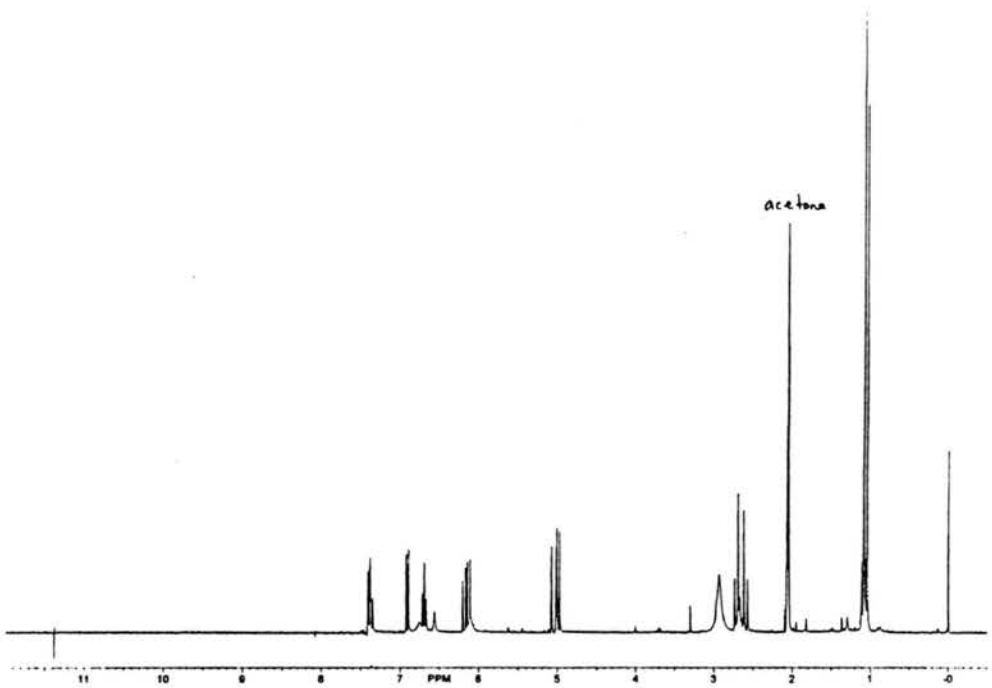
86 **92**

2-(1,1-dimethylprop-2-enyl)-2-(amidomethyl)indoxyl (92) . Compound **86** (8.6 mg, 0.0358 mmol, 1.0 eq) was refluxed 9 h in 0.2 mL acetone and 0.2 mL water with urea hydrogen peroxide addition compound (13.5 mg, 0.1432 mmol, 4.0 eq) and potassium carbonate (0.2 mg, 0.0011 mmol, 0.03 eq). The acetone was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with water (2 x 5 mL) and with brine (1 x 5 mL). The organic layer was dried over Na_2SO_4 followed by solvent removal *in vacuo*. Crude yield was 9.0 mg (98%) of a yellow powder.

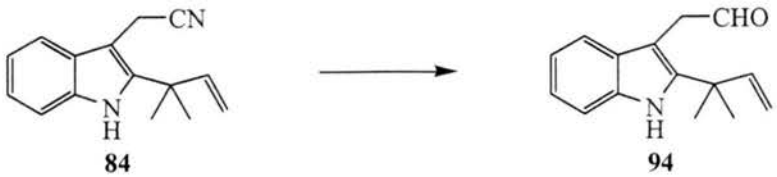
^1H NMR (300 MHz) ($CD_3C(O)CD_3$) δ TMS: 1.06 (6 H, d, J = 6.0 Hz), 2.65 (2 H, dd, J = 15.0 Hz, J = 36.0 Hz), 2.94 (2 H, s), 4.99 (1 H, dd, J = 3.0 Hz, J = 9.0 Hz), 5.05 (1 H, dd, J = 3.0 Hz, J = 16.5 Hz), 6.16 (1 H, dd, J = 12.0 Hz, J = 18.0 Hz), 6.57 (1 H, s), 6.69 (1 H, t, J = 7.5 Hz), 6.91 (1 H, d, J = 9.0 Hz), 7.33-7.43 (2 H, m). R_f = 0.14 (25:1 CH_2Cl_2/MeOH).



92



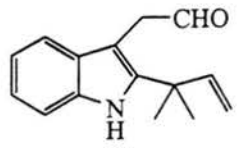
Compound **92**: 300 MHz ^{1}H NMR Spectrum in $CD_3C(O)CD_3$



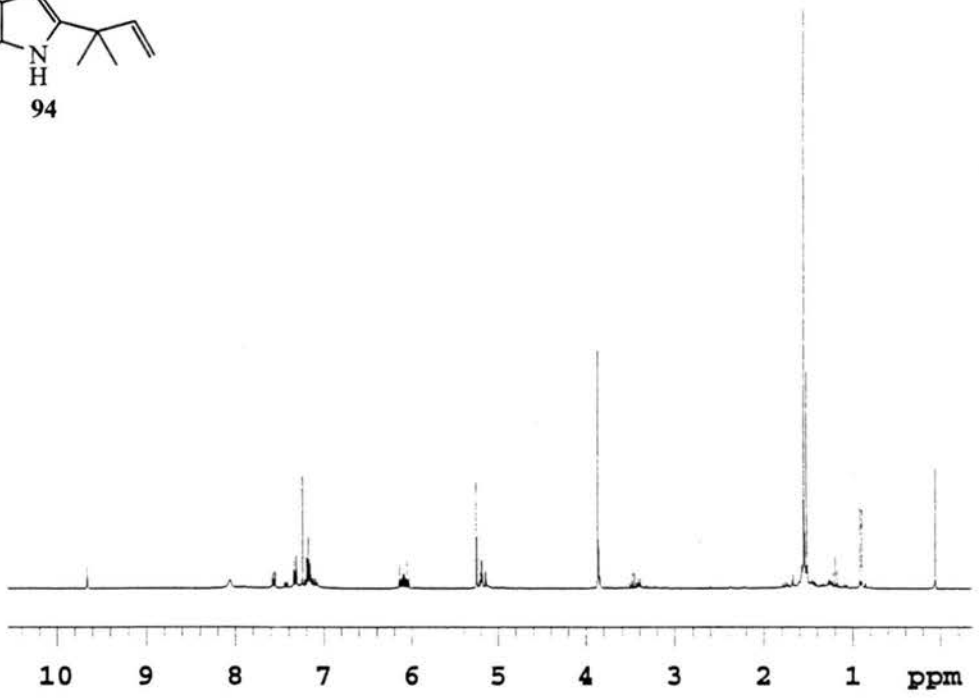
84 94

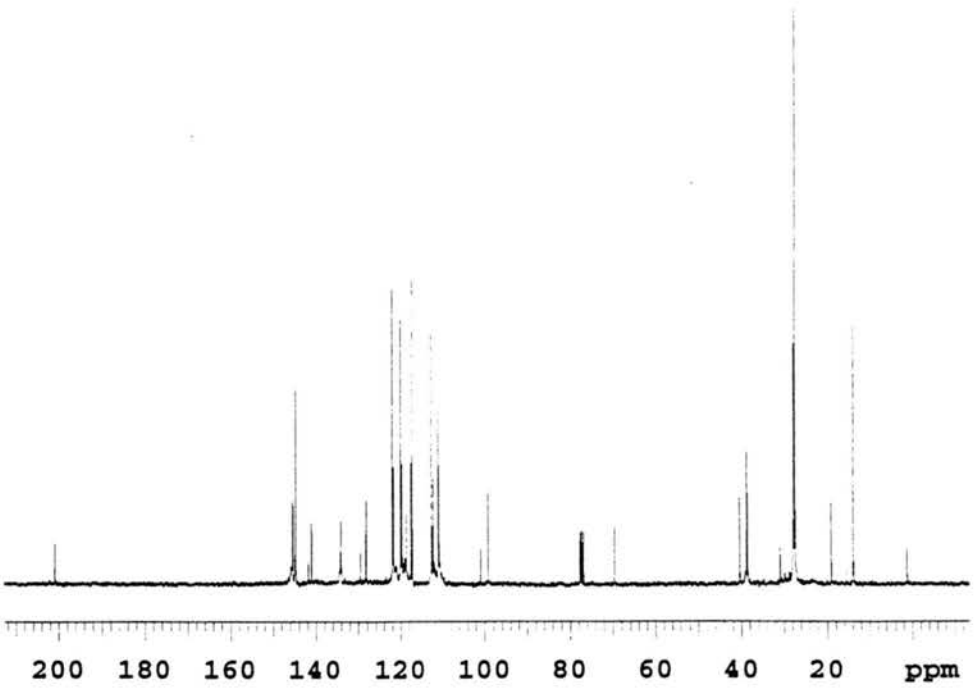
2-(1,1-dimethylprop-2-enyl)-3-(ethanal)indole (94). A solution of **84** (213 mg, 0.9509 mmol, 1.0 eq) in dry ether (5.3 ml) was stirred under argon at -78°C. A 1M solution of diisobutylaluminum hydride in hexane (1.04 mL, 5.1348 mmol, 5.4 eq) was added *via* syringe over 30 min. The reaction mixture continued to stir at -78°C for 2 h. It was quenched with 2.6 mL of 5% H_2SO_4. After stirring at room temperature for 1 h, the organic phase was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous Na_2SO_4 and concentrated to give 200 mg (93%) of an orange-yellow oil. This can be purified, if necessary, by flash CC using silica gel and 100% CH_2Cl_2. The aldehyde is eluted in the first few fractions

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.43 (6 H, s), 3.85 (2 H, s), 5.17-5.21 (2 H, m), 6.09 (1 H, dd, J = 1.09 Hz, J = 6.95 Hz), 7.05-7.20 (2 H, m), 7.30-7.60 (2 H, m), 8.06 (1 H, s), 9.66 (1 H, t, J = 2.57 Hz). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 13.91, 27.81, 38.82, 111.05, 112.78, 118.77, 119.75, 120.03, 122.01, 128.24, 134.09, 141.05, 144.78, 200.82. IR (NaCl, CH_2Cl_2): 1265, 1431, 1460, 1636, 1718, 2720, 2850, 2972, 3054, 3467 cm^{-1}. HRMS (FAB), calcd. $C_{15}H_{17}NO$ (MH^+): 227.1310. Found: 227.1307. R_f = 0.55 (100% CH_2Cl_2, vanillin: purple).

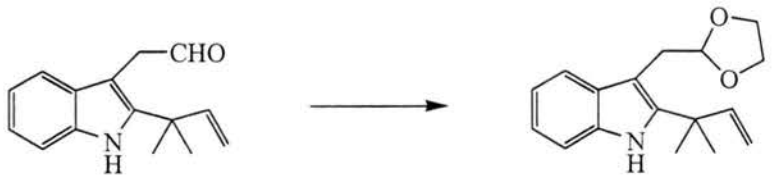


94





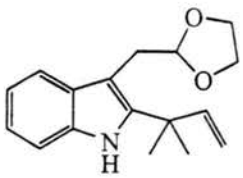
Compound **94**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



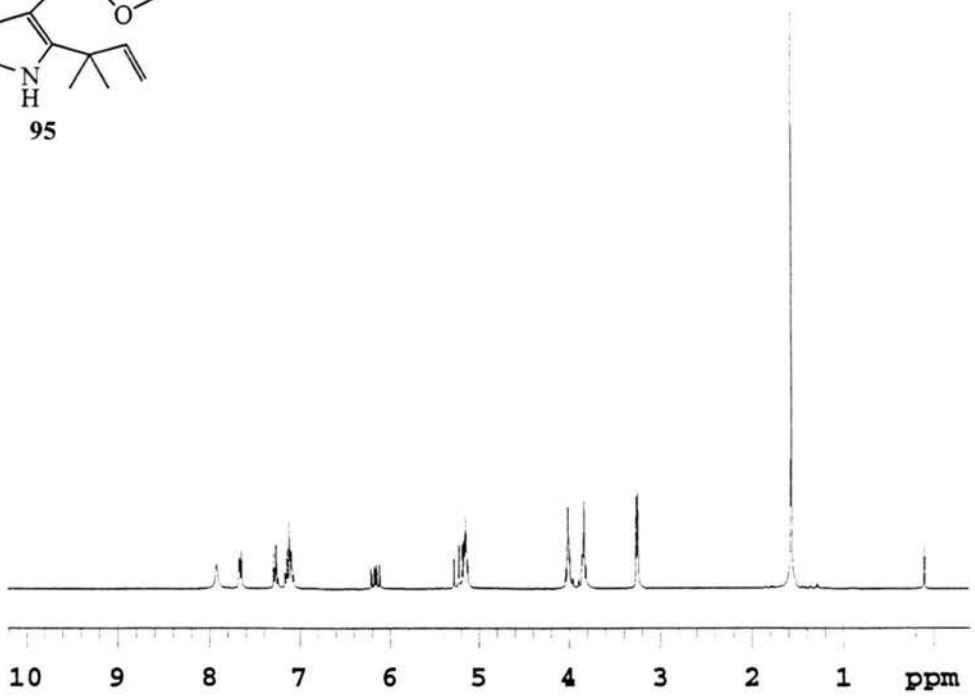
94 95

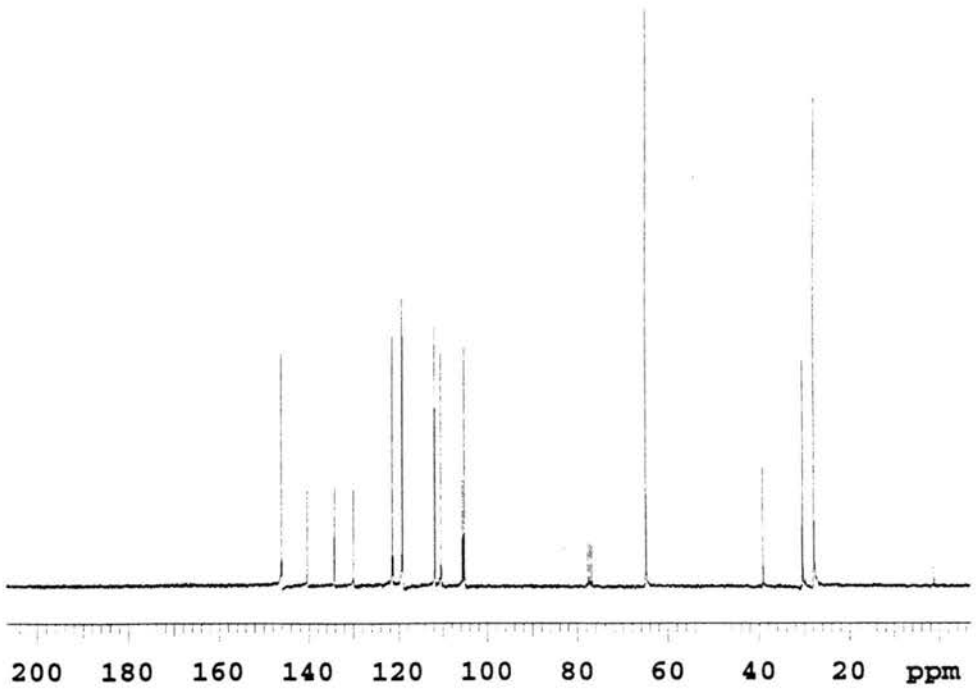
2-(1,1-dimethylprop-2-enyl)-3-(ethanal ethylene acetal)indole (95). To a solution of **94** (88 mg, 0.3877 mmol, 1.0 eq) in benzene (5 mL) was added ethylene glycol (75 μL, 1.3568 mmol, 3.5 eq) and pyridinium tosylate (19 mg, 0.0775 mmol, 0.2 eq). The mixture was refluxed with water separation *via* Dean Stark trap until starting material was consumed (3 h). Excess solvent was removed *in vacuo*. 20 mL ether was added and the mixture was washed with saturated sodium bicarbonate solution and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated to give 92.6 mg (88%) of acetal **95**.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.57 (6 H, s), 3.26 (2 H, dd, J = 2.20 Hz, J = 5.13 Hz), 3.80-4.08 (4 H, m), 5.10-5.30 (2 H, m), 6.16 (1 H, dddd, J = 1.83 Hz, J = 10.62 Hz, J = 12.45 Hz, J = 17.58Hz), 7.03-7.18 (2 H, m), 7.28 (1 H, d, J = 7.33 Hz), 7.65 (1 H, d, J = 6.96 Hz), 7.92 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 27.63, 30.32, 39.03, 64.60, 105.05, 105.50, 110.32, 111.73, 119.04, 119.11, 121.30, 129.93, 134.16, 140.30, 146.03. IR (NaCl, neat): 1134, 1265, 1430, 1636, 2250, 2343, 2360, 2885, 2972, 3054, 3455 cm^{-1}. HRMS (FAB), calcd. For $C_{17}H_{21}NO_2$ (MH^+): 271.1572. Found: 271.1567. R_f = 0.50 (100% CH_2Cl_2, Vanillin: purple).

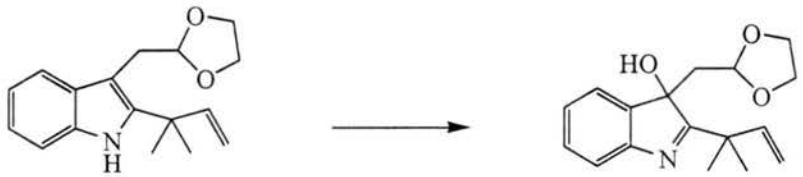


95





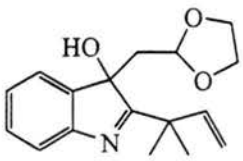
Compound **95**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



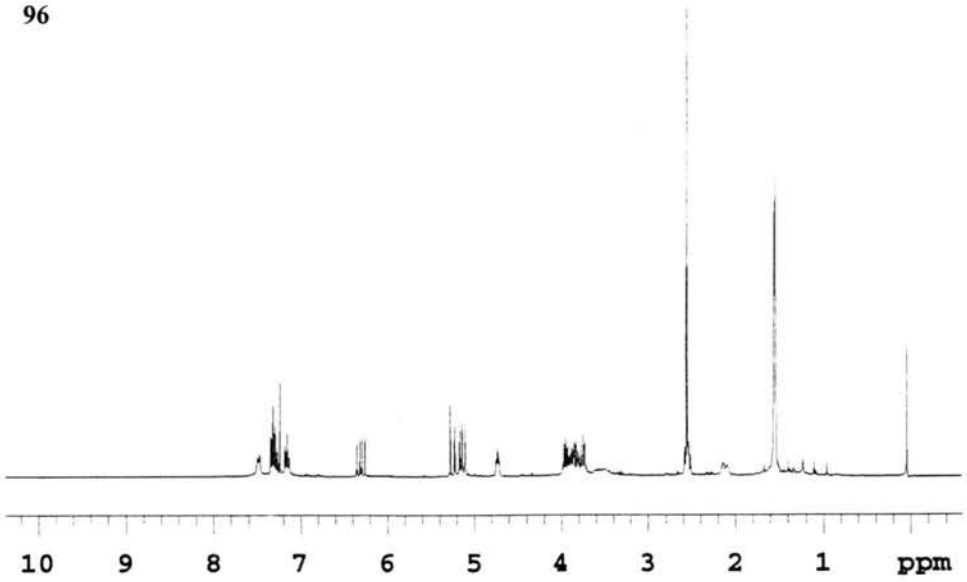
95 96

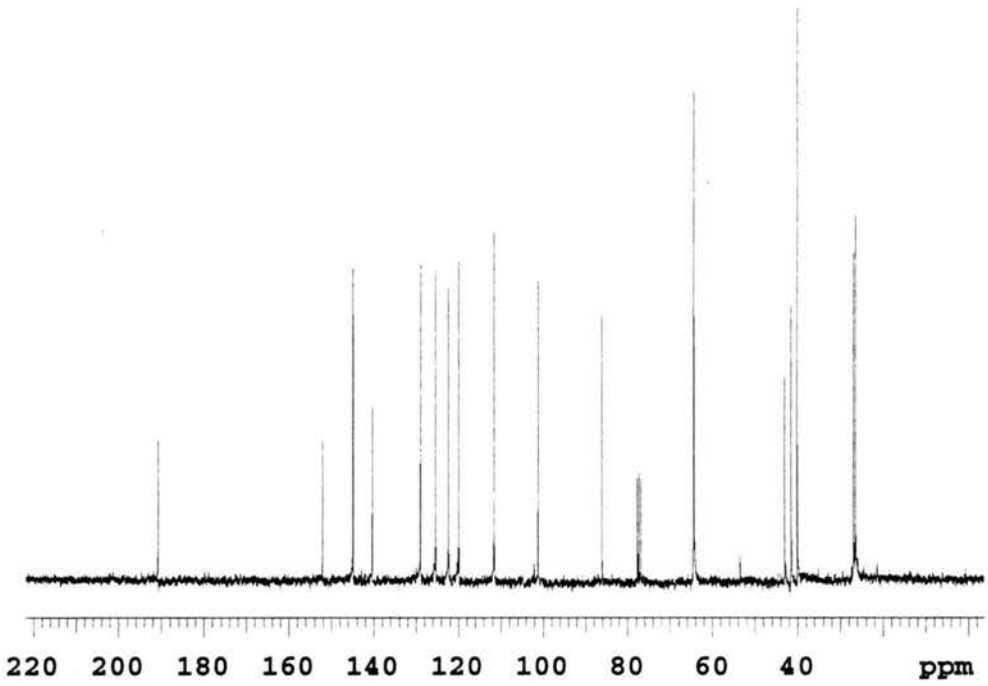
2-(1,1-dimethylprop-2-enyl)-3-ethanal ethyleneacetal-3-hydroxyindole (96). To a solution of **95** (32.1 mg, 0.1184 mmol, 1.0 eq) in 8.7 mL absolute MeOH was added Rose Bengal (0.04 g). The mixture was irradiated with a 250 Watt Hg white spot lamp in an ice bath (0°C) under O_2. After 3 h stirring, dimethyl sulfide (0.35 mL) was added to quench the reaction. It was stirred overnight in the cold room (3°C). The Rose Bengal was removed by filtration through an alumina column with 100% CH_2Cl_2 as solvent. A yellow oil resulted which was taken on to the next step without further purification.

^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.55 (6 H, s), 2.51-2.58 (2 H, m), 3.70-3.97 (4 H, m), 4.73 (1 H, t, J = 5.10 Hz), 5.12 (1 H, d, J = 10.50 Hz), 5.25 (1 H, d, J = 14.10 Hz), 6.31 (1 H, dd, J = 10.20 Hz, J = 17.1 Hz), 7.10-7.50 (4 H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 26.45, 26.92, 40.03, 41.44, 42.97, 64.30, 86.04, 101.11, 111.53, 119.88, 122.31, 125.32, 128.88, 140.26, 144.85, 152.04, 190.75.

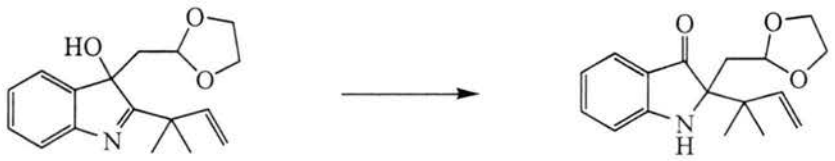


96





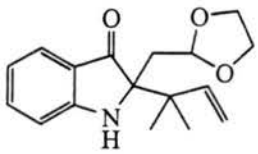
Compound **96**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



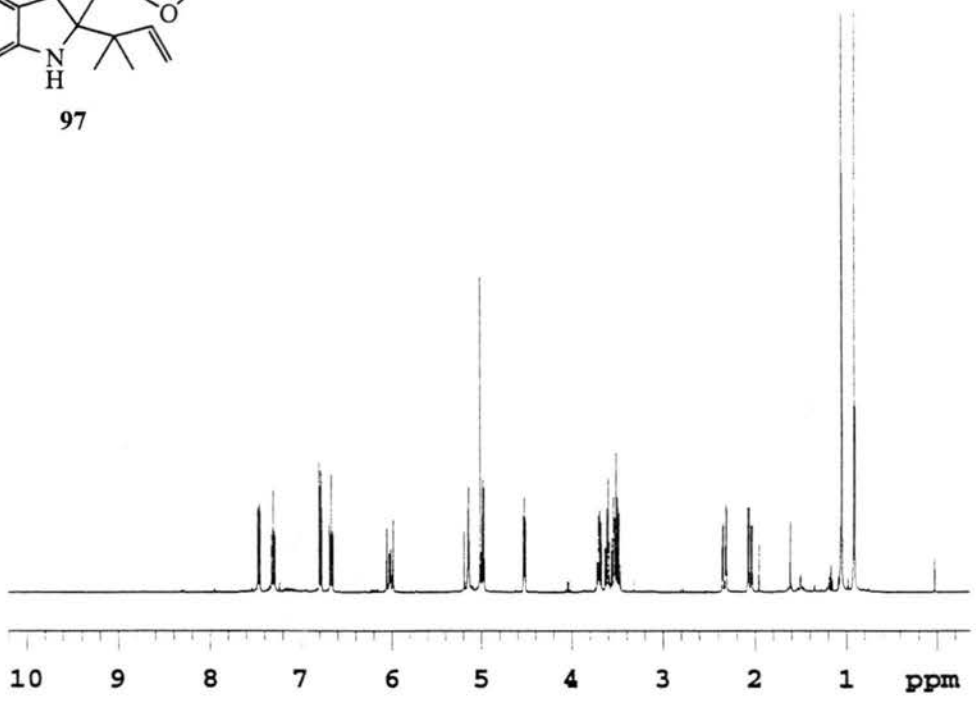
96 97

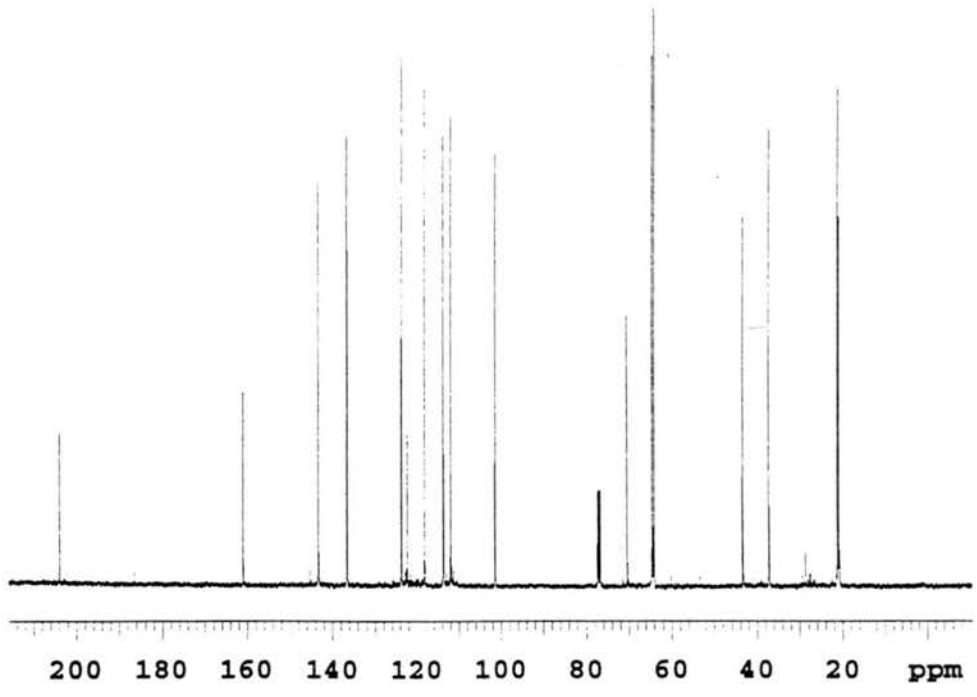
2-(1,1-dimethylprop-2-enyl)-2-(ethanal ethylene acetal)indoxyl (97). Hydroxyindolenine **96** (102 mg, 0.3554 mmol, 1.0 eq.) was stirred in 5 mL THF with sodium bis(trimethylsilyl)amide (18 μL, 0.0888 mmol, 0.25 eq) at 0°C. The temperature was allowed to warm to RT and the mixture stirred for 19 h. The crude product was concentrated and purified by CC using 4:1 hexane/ethyl acetate as eluent. Indoxyl **97** was obtained as a fluorescent yellow oil in 49% yield (50mg).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 0.99 (6 H, d, J = 56.03 Hz), 2.05 (1 H, dd, J = 5.49 Hz, J = 14.28 Hz), 2.33 (1 H, dd, J = 4.03 Hz, J = 14.65 Hz), 3.46-3.74 (4 H, m), 4.52 (1 H, dd, J = 4.03 Hz, J = 5.49 Hz), 4.98 (1 H, dd, J = 1.47 Hz, J = 6.60 Hz), 5.08 (1 H, s), 5.14 (1 H, s), 6.02 (1 H, ddd, J = 10.26 Hz, J = 11.72 Hz, J = 16.49 Hz), 6.67 (1 H, t, J = 7.32 Hz), 6.78 (1 H, d, J = 8.06 Hz), 7.28-7.34 (1 H, m), 7.46 (1 H, d, J = 7.69 Hz). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 20.83, 21.13, 37.10, 43.32, 64.03, 64.47, 70.36, 101.41, 111.89, 113.65, 118.13, 122.28, 123.68, 136.52, 143.26, 160.94, 203.87. IR (NaCl, neat): 1044, 1143, 1247, 1382, 1472, 1618, 1707, 2890, 2972, 3050, 3250 cm^{-1}. R_f = 0.67 (1:1 hexane/ethyl acetate, vanillin: brown).



97



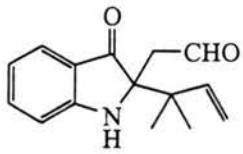


Compound **97**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

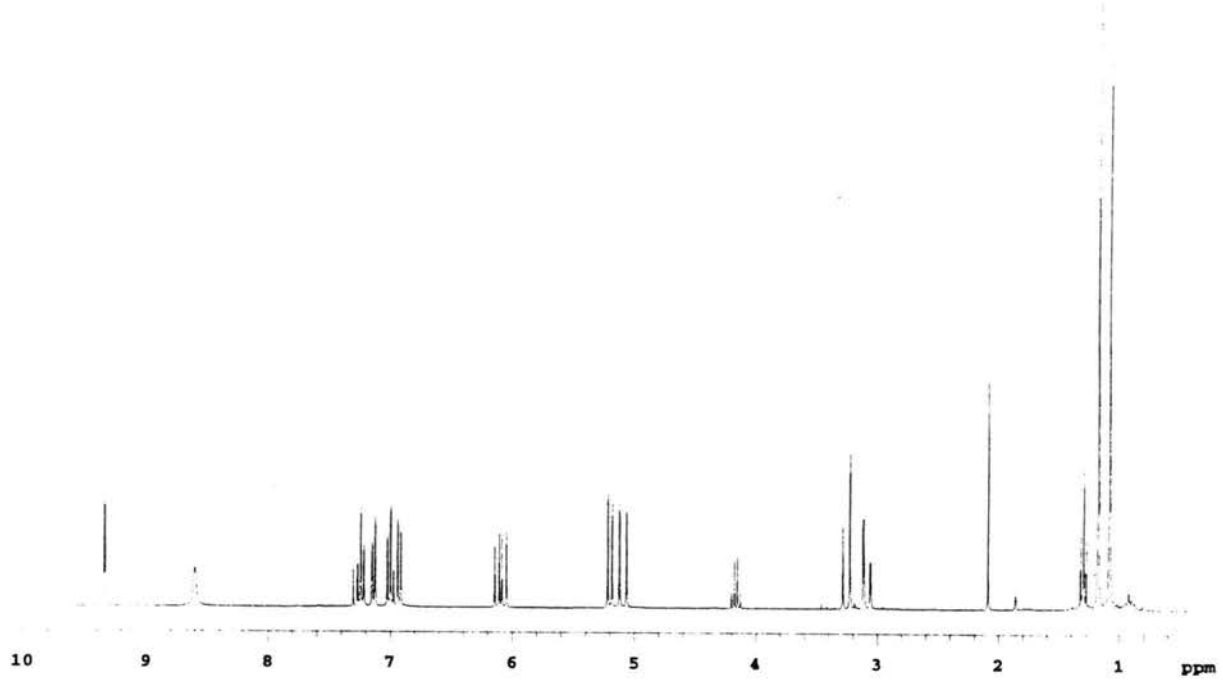
97 81

2-(1,1-dimethylprop-2-enyl)-2-ethanalindoxyl (81). To a solution of acetal **97** (230 mg, 0.801 mmol, 1.0 eq), in wet acetone (8 mL) was added pyridinium tosylate (100 mg, 0.400 mmol, 0.5 eq). This was refluxed for 12 h. Excess solvent was removed *in vacuo*. Ether was added (30 mL) and the mixture was washed with saturated sodium bicarbonate and brine. The organic phase was dried over anhydrous Na_2SO_4 and solvent evaporated to give the crude aldehyde **81**. This was purified by CC using silica gel and 1:1 hexane/ethyl acetate as eluent. 100 mg of **81** (52%) was obtained.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.13 (6 H, d, J = 27.83 Hz), 3.08 (1 H, dd, J = 2.56 Hz, J = 17.21 Hz), 3.25 (1 H, d, J = 17.21 Hz), 5.09 (1 H, dd, J = 1.10 Hz, J = 17.58 Hz), 5.20 (1 H, dd, J = 1.10 Hz, J = 10.99 Hz), 6.10 (1 H, dd, J = 10.62 Hz, J = 17.21 Hz), 6.93 (1 H, d, 7.69 Hz), 7.00 (1 H, ddd, J = 1.10 Hz, J = 7.69 Hz, J = 8.79 Hz), 7.14 (1 H, d, J = 6.22 Hz), 7.24 (1 H, ddd, J = 1.09 Hz, J = 7.69 Hz, J = 8.79 Hz), 8.60 (1 H, s), 9.34 (1 H, d, J = 2.20 Hz). R_f = 0.30 (1:1 hexane/ethyl acetate).



81

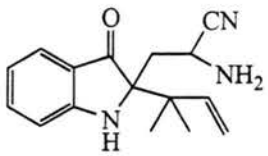


Compound **81**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

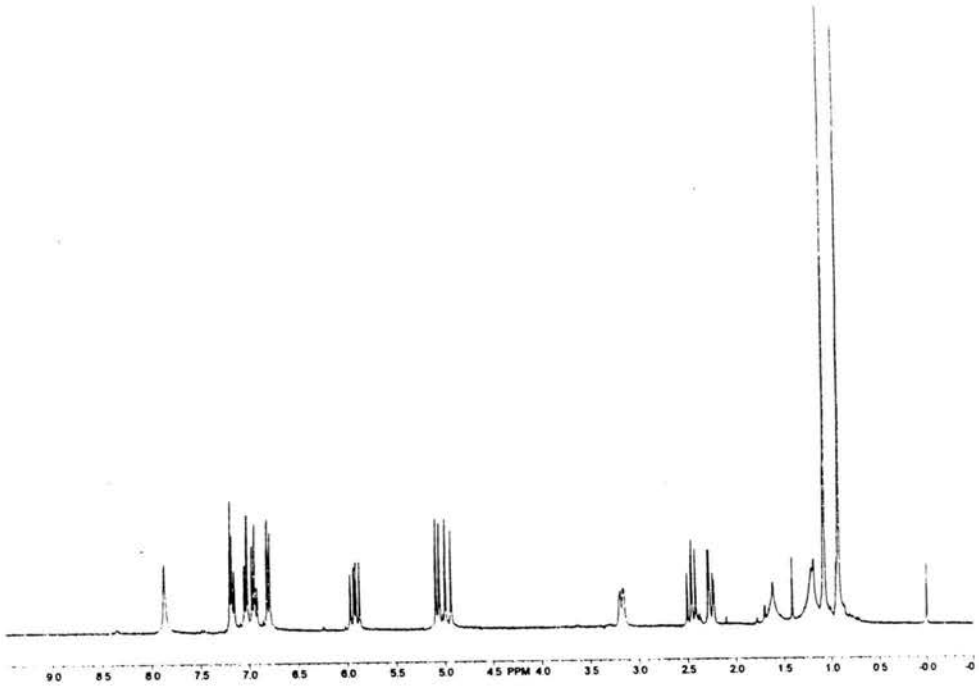
81 **98**

2-(1,1-dimethylprop-2-enyl)-2-(2-amino, 2-cyanoethyl)indoxyl (98). KCN (0.12 g, 1.85 mmol, 2.0 eq.), NH_4Cl (0.11 g, 2.04 mmol, 2.2 eq.), Al_2O_3 (0.10 g), indoxyl **81** (0.225 g, 0.93 mmol, 1.0 eq.) were sonicated at 50°C in dry CH_3CN (2 mL) for 24 h. The solids were filtered and the crude material purified by CC using 1:1 hexane/ethyl acetate as eluent. 40.5 mg (17%) aminonitrile was obtained.

^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 0.97 (3 H, s), 1.12 (3 H, s), 1.45 (2 H, s), 2.31 (1 H, dd, J = 3.90 Hz, J = 14.10 Hz), 2.51 (1 H, dd, J = 11.70 Hz, J = 14.10 Hz), 3.22 (1 H, dd, J = 3.90 Hz, J = 11.70 Hz), 5.01 (1 H, d, J = 17.4 Hz), 5.12 (1 H, d, J = 10.80 Hz), 5.97 (1 H, dd, J = 10.80 Hz, J = 17.40 Hz), 6.80-7.25 (4 H, m), 8.10 (1 H, s). IR (NaCl, CH_2Cl_2): 1265, 1422, 1719, 2305, 2987, 3054, 3400, 3650, 3700 cm^{-1}. R_f = 0.07 (1:1 hexane/ethyl acetate, vanillin: blue).



98

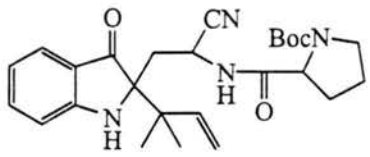


Compound **98**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

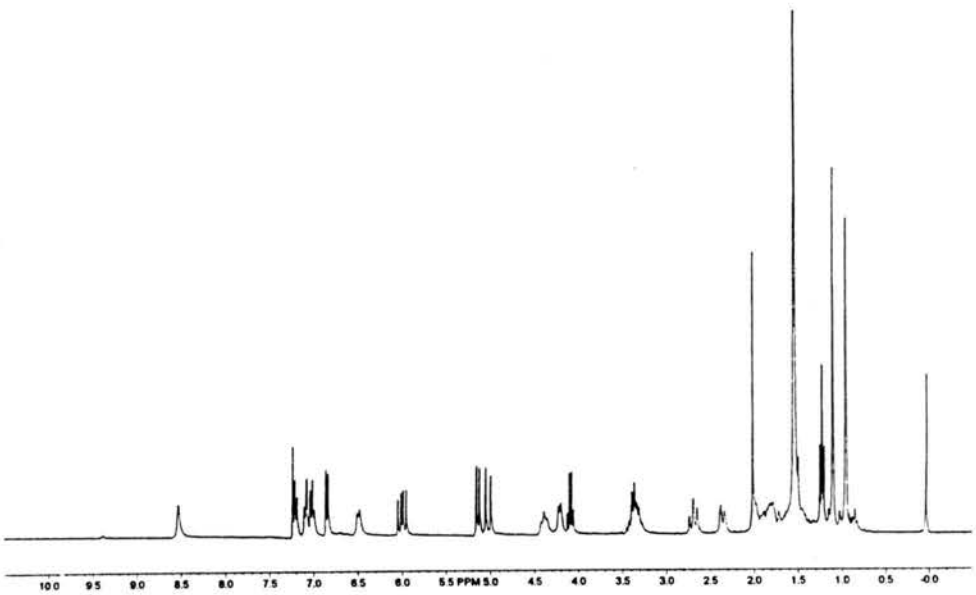
98 99

2-(1,1-dimethylprop-2-enyl)-2-(2-amido-Boc-proline, 2-cyanoethyl)indoxyl 99. Aminonitrile **98** (44 mg, 0.163 mmol, 1.0 eq.) Boc-proline (35 mg, 0.163 mmol, 1.0 eq.), BOP reagent (72 mg, 0.163 mmol, 1.0 eq.) and TEA (0.04 mL, 0.287 mmol, 1.76 eq.) were stirred at RT under argon in 2.4 mL CH_3CN. After 24h, the reaction mixture was poured into a separatory funnel with brine and extracted (3 x 15 mL) with ethyl acetate. The organic layers were pooled and washed with 2M HCl (aq.), 5% $NaHCO_3$ (aq.) and brine. It was dried over anhydrous Na_2SO_4 and the solvent removed *in vacuo*. After purification by PTLC using 10:1 CH_2Cl_2/MeOH as eluent, 32.2 mg of one diastereomer and 26.6 mg of another diestereomer were isolated for a combined yield of 85%.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 0.96 (3 H, d), 1.11 (3 H, s), 1.55 (9 H, s), 1.7-2.0 (4 H, m), 2.32 (1 H, dd, J = 2.4 Hz, J = 14.4 Hz), 2.65 (1 H, t), 3.2-3.4 (2 H, m), 4.17 (1 H, d), 4.3-4.4 (1 H, m), 5.03 (1 H, d, J = 17.40 Hz), 5.14 (1 H, d, J = 11.40 Hz), 5.96 (1 H, dd, J = 10.8 Hz, J = 17.4 Hz), 6.58 (1 H, d, J = 8.40 Hz), 6.80-7.30 (4 H, m), 8.73 (1 H, s). R_f = 0.69, 0.63 in 10:1 CH_2Cl_2/MeOH.



99

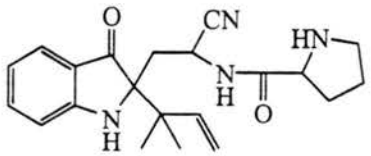


Compound **99**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

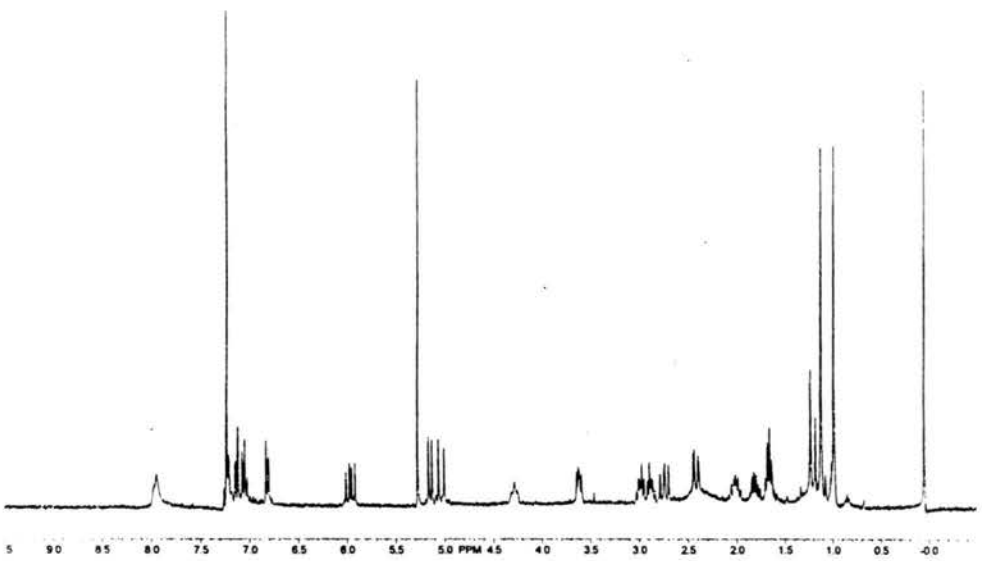
99 **100**

2-(1,1-dimethylprop-2-enyl)-2-(2-amido-proline, 2-cyanoethyl)indoxyl 100. Boc-proline nitrile **99** (26.6 mg, 0.057 mmol, 1.0 eq.) and TEA (44 mL, 0.57 mmol, 10 eq.) were stirred in 0.7 mL CH_2Cl_2 at RT under argon. After stirring 4 h, the reaction mixture was immediately purified by PTLC using 10:1 CH_2Cl_2/MeOH. 15 mg (72%) of **99** were obtained.

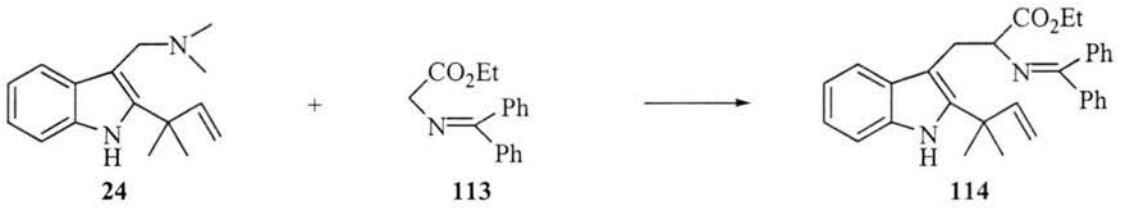
^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.00 (3 H, s), 1.13 (3 H, s), 1.60-2.10 (4 H, m), 2.42 (1 H, dd, J = 3.00 Hz, J = 14.40 Hz), 2.75 (2 H, dd, J = 11.10 Hz, J = 13.80 Hz), 2.82-3.05 (4 H, m), 3.58-3.67 (1 H, m), 4.51 (1 H, t), 4.72 (1 H, dd, J = 4.5 Hz, J = 9.0 Hz), 5.04 (1 H, d, J = 17.1 Hz), 5.18 (1 H, d, J = 11.4 Hz), 5.94 (1 H, dd, J = 11.1 Hz, J = 17.4 Hz), 6.81 (1 H, d, J = 7.8 Hz), 7.00-7.30 (3 H, m). R_f = 0.20 in 10:1 CH_2Cl_2/MeOH.



100



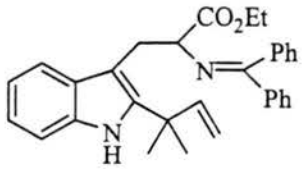
Compound **100**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



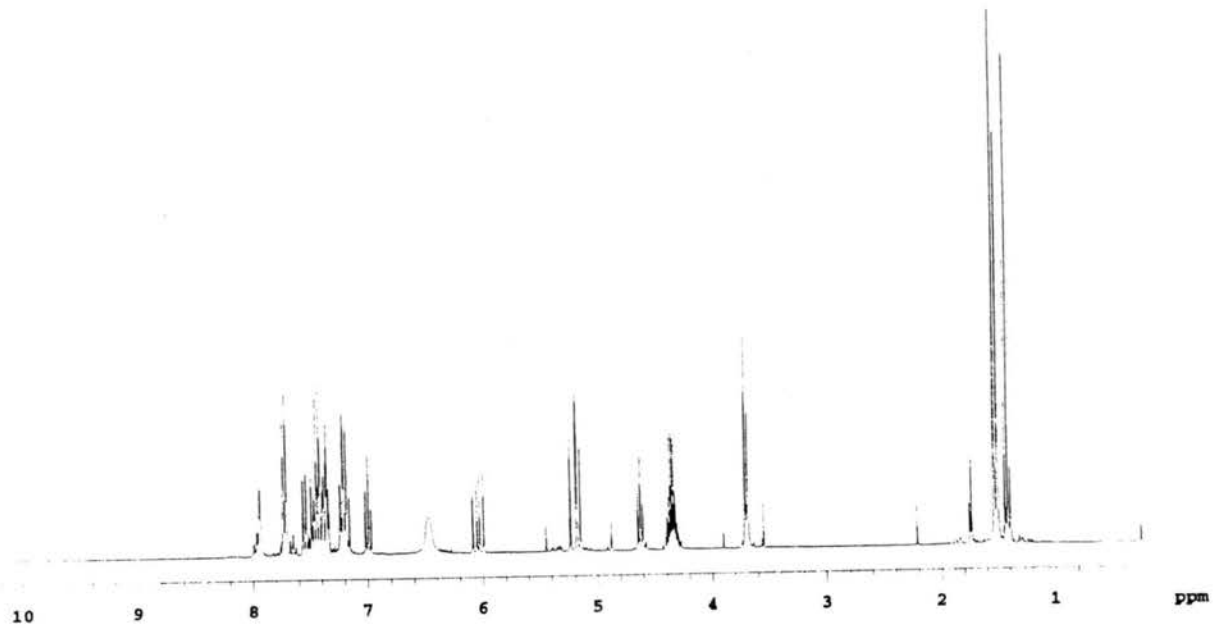
24 **113** **114**

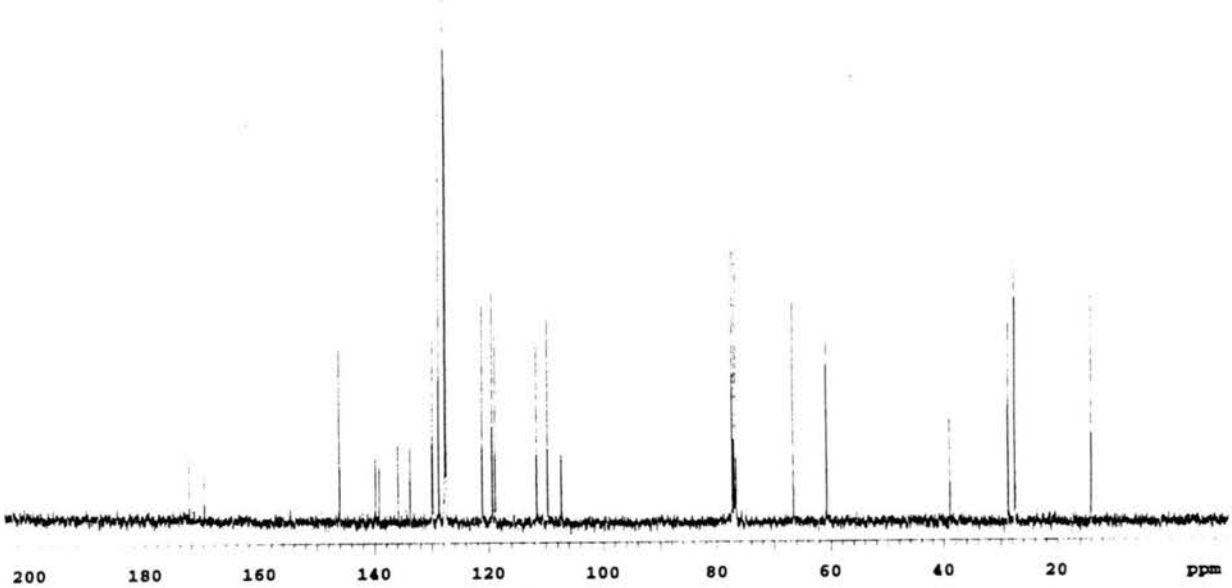
2-(1,1-dimethylallyl)-3-(ethyl-2-diphenylmethyleneamino) indolepropionate (104). To a stirred solution of gramine derivative **31** (100 mg, 0.4132 mmol, 1.0 eq.) and protected glycine **103** (110 mg, 0.4132 mmol, 1.0 eq.) in dry CH_3CN (3.0 mL) was added Bu_3P (0.10 mL, 0.4132 mmol, 1.0 eq.). The mixture was refluxed under argon for 19 h at which time the starting materials had disappeared. The solution was cooled to room temperature and the solvent removed *in vacuo*. The crude mixture was purified by column chromatography (silica: 25:1 - 10:1 hexane/ethyl acetate) affording indole **104** (off-white foam, 135 mg, 70% yield).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.32 (3 H, t, J = 7.2 Hz), 1.42 (6 H, d, J = 9.6 Hz), 3.62 (2 H, d, J = 6.3 Hz), 4.20-4.30 (2 H, m), 4.55 (1 H, t, J = 6.6 Hz), 5.09 (1 H, dd, J = 16.8 Hz, J = 0.9 Hz), 5.06 (1 H, dd, J = 9.6 Hz, J = 0.9 Hz), 5.94 (1 H, dd, J = 10.5 Hz, J = 17.4 Hz), 6.38 (1 H, bs), 6.83-7.88 (14 H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 14.17, 27.53, 27.59, 28.73, 39.01, 60.78, 66.63, 107.32, 109.72, 111.60, 118.91, 119.41, 121.16, 127.54, 127.68, 127.74, 128.81, 129.91, 130.01, 133.84, 135.92, 139.24, 139.86, 146.10, 169.77, 172.40. IR (NaCl, CH_2Cl_2): 3451, 3052, 2978, 1722, 1617, 1455, 1439, 1418, 1260, 1182 cm^{-1}. HRMS (FAB), calcd. for $C_{31}H_{32}N_2O_2$ (MH^+): 465.2542. Found: 465.2544. R_f = 0.49 Vanillin stain: purple (4:1 hexane/ethyl acetate).

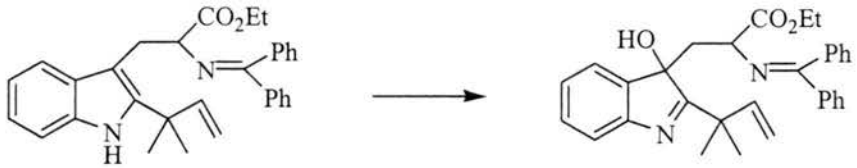


114





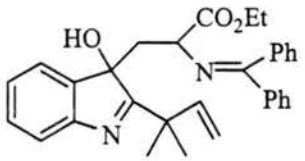
Compound **114**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



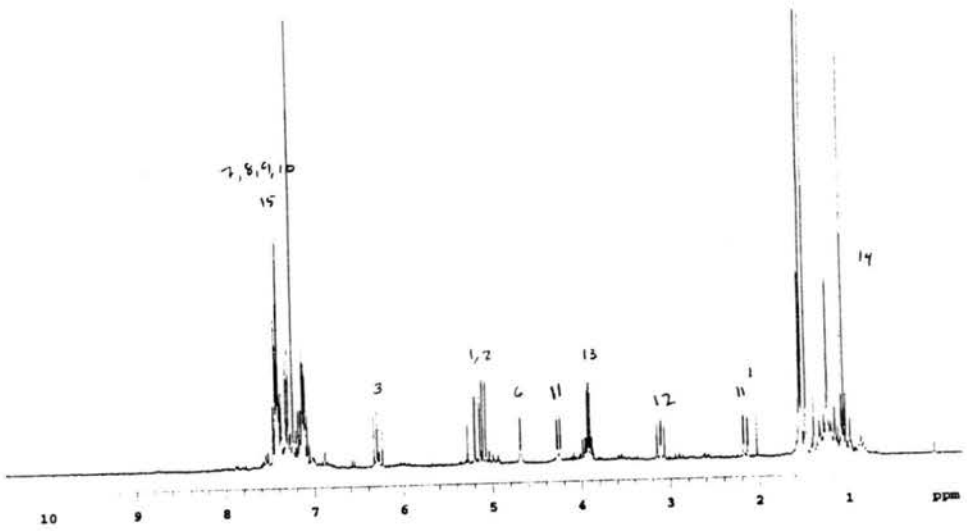
114 **115**

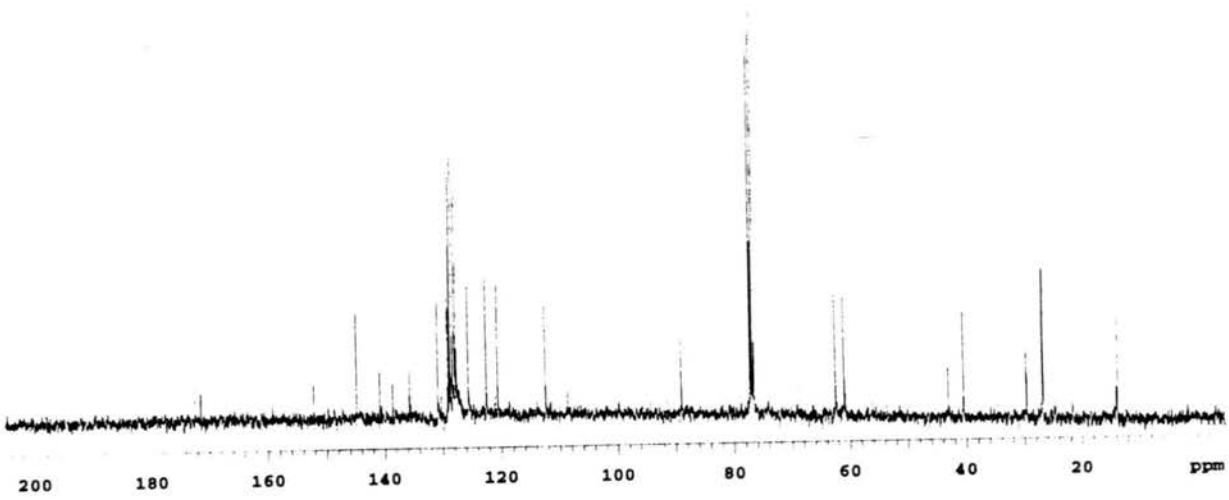
2-(1,1-dimethylallyl)-3-(ethyl-2-diphenylmethyleneamino) hydroxyindolenine propionate (115). The glycine indole **114** (1.0 g, 2.1552 mmol, 1.0 eq.) and Rose Bengal (0.30 g, 0.2956 mmol, 0.14 eq.) were stirred with dry MeOH (108 mL) at 0°C for 5 h. A lamp was used to irradiate the mixture and O_2 was bubbled through the solution. Once complete, DMS (3 mL) was added to quench the peroxide and stirred overnight. The solvent was removed *in vacuo*. The crude mixture was put through a short column of alumina (100% CH_2Cl_2 as eluent) to remove the Rose Bengal. It was further purified by column chromatography (silica: 10:1 - 4:1 hexane/ethyl acetate) to afford 100 mg of **115** (off-white foam, 10% yield).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.06 (3 H, t, J = 6.9 Hz), 1.50 (6 H, d, J = 13.8 Hz), 2.15 (1 H, dd, J = 2.7 Hz, J = 13.8 Hz), 3.11 (1 H, dd, J = 11.1 Hz, J = 14.1 Hz), 3.86-4.00 (2 H, m), 4.26 (1 H, dd, J = 2.7 Hz, J = 10.8 Hz), 4.69 (1 H, bs), 5.10 (1 H, dd, J = 1.2 Hz, J = 10.8 Hz), 5.17 (1 H, dd, J = 1.2 Hz, J = 17.1 Hz), 6.29 (1 H, dd, J = 10.5 Hz, J = 17.4 Hz), 7.04-7.56 (14 H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 13.93, 26.78, 26.89, 29.68, 40.51, 43.17, 61.08, 62.58, 89.09, 112.49, 120.64, 122.59, 125.66, 127.22, 127.72, 127.90, 128.04, 128.43, 128.83, 128.99, 129.22, 130.90, 135.77, 138.66, 140.89, 144.90, 152.28, 171.51, 172.58, 190.02. R_f = 0.32, 0.53 PMA stain: brown (4:1 hexane/ethyl acetate).

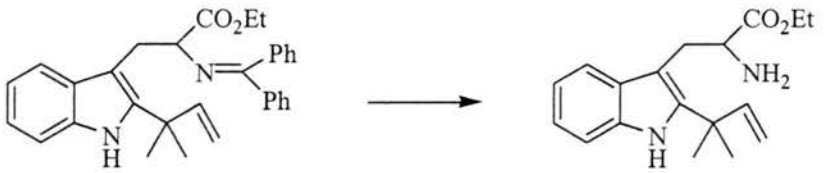


115





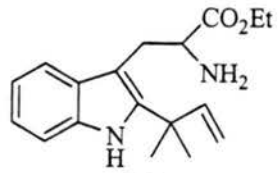
Compound **115**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



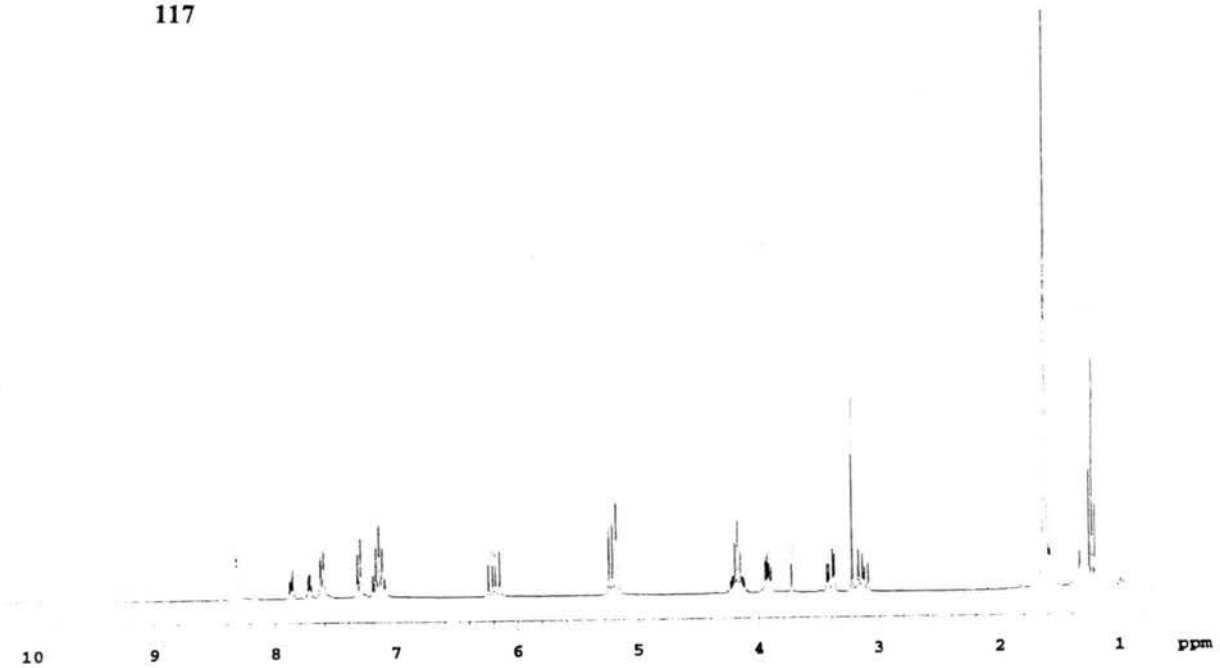
114 **117**

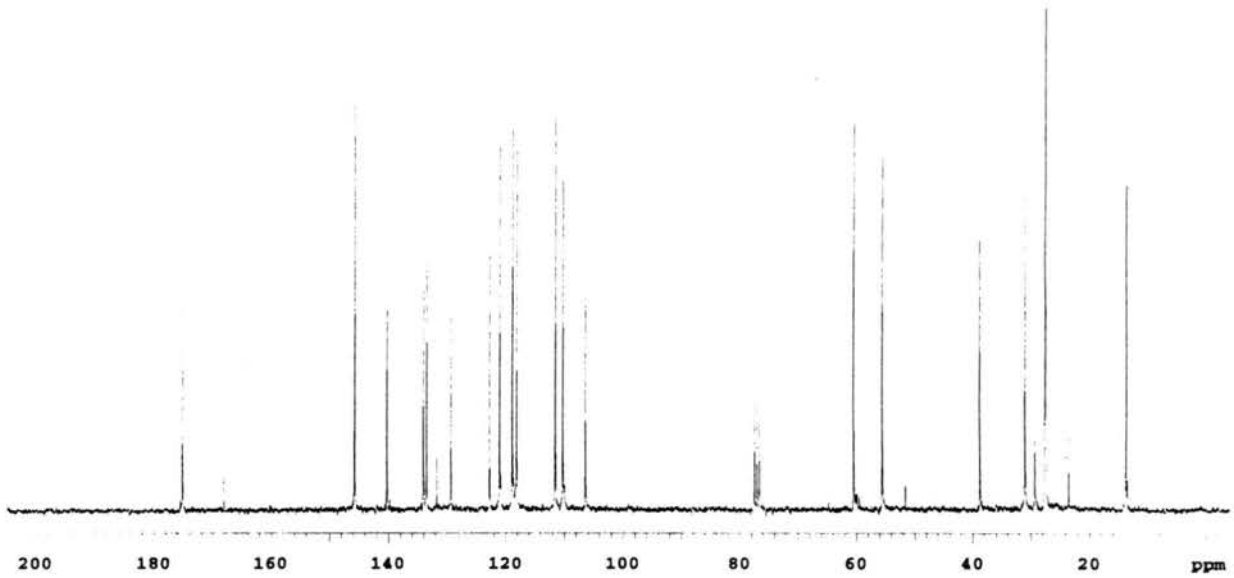
2-(1,1-dimethylallyl)-3-(ethyl-2-amino) indolepropionate (117). A solution of protected glycine indole **114** (304 mg, 0.6552 mmol, 1.0 eq.), Na_2CO_3 (493 mg, 4.6517 mmol, 7.1 eq.) and $NH_2OH{\cdot}HCl$ (346 mg, 4.9793 mmol, 7.6 eq.) in dry CH_2Cl_2 (2.7 mL) was stirred and refluxed gently for 15 h. The crude reaction was acidified to pH 3-4 with 10% $KHSO_4$ and then extracted with CH_2Cl_2 (3 x 50 mL) and washed with brine (50 mL). The organic layer was dried over Na_2SO_4 and the solvent evaporated. The mixture was purified by column chromatography (silica: 10:1-4:1-1:1 hexane/ethyl acetate) to give 172 mg of the tryptophan derivative **117** (88% yield).

^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.16 (3 H, t, J = 7.2 Hz), 1.56 (6 H, s), 1.58 (2 H, bs), 3.04 (1 H, dd, J = 9.6 Hz, J = 14.7 Hz), 3.32 (1 H, dd, J = 5.1 Hz, J = 12.6 Hz), 3.83 (1 H, dd, J = 5.1 Hz, J = 9.6 Hz), 4.02-4.20 (2 H, m), 5.14 (1 H, s), 5.18 (1 H, d, J = 5.7 Hz), 6.12 (1 H, dd, J = 11.1 Hz, J = 17.7 Hz), 7.02-7.14 (2 H, m), 7.26 (1 H, d, J = 6.3 Hz), 7.54 (1 H, d, J = 7.5 Hz), 7.92 (1 H, bs). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 15.09, 28.94, 32.39, 40.20, 57.00, 61.84, 107.82, 111.56, 112.91, 119.52, 120.26, 122.44, 124.14, 130.79, 135.45, 141.61, 147.17, 176.56. IR (NaCl, CH_2Cl_2): 909, 1006, 1027, 1106, 1193, 1265, 1382, 1438, 1460, 1718, 1771, 2873, 2932, 2973, 3054, 3400, 3454 cm^{-1}. HRMS (FAB), calcd. for $C_{18}H_{24}N_2O_2$ (MH^+): 301.1916. Found: 301.1916.

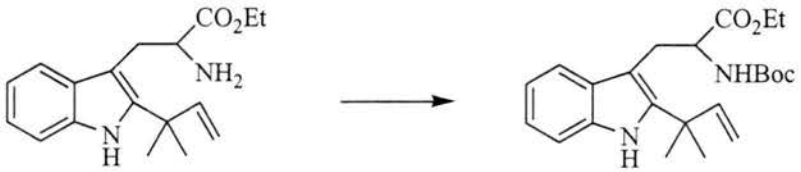


117





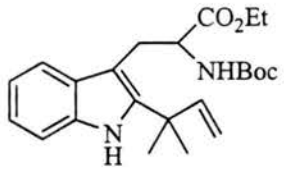
Compound **117**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



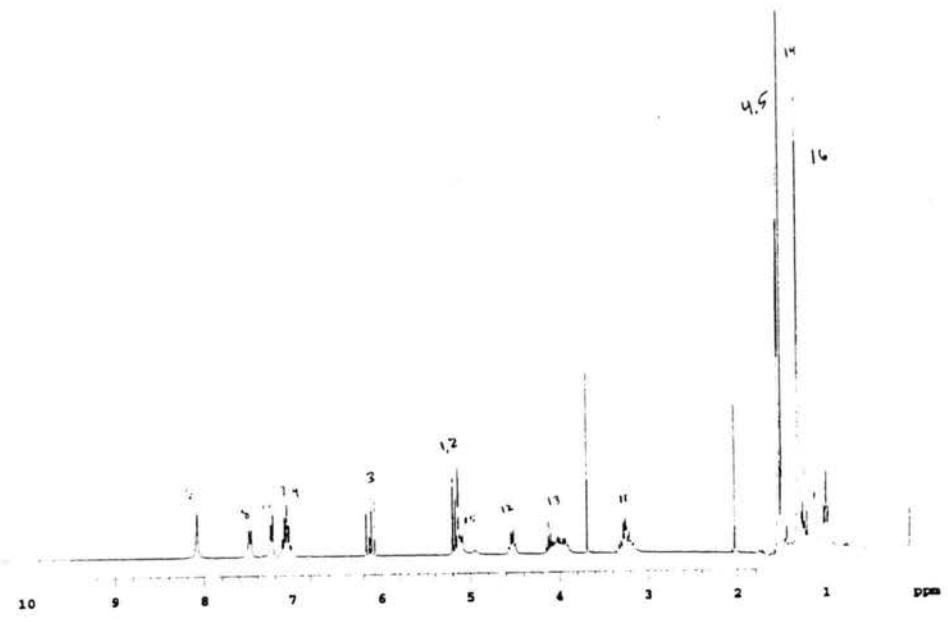
117 118

2-(1,1-dimethylallyl)-3-(ethyl-2-butoxycarbonylamino) indolepropionate (118). A solution of glycine indole **117** (120 mg, 0.400 mmol, 1.0 eq.), K_2CO_3 (55 mg, 0.400 mmol, 1.0 eq.) in dioxane (0.8 mL) and water (0.8 mL) was stirred and cooled in an ice-water bath. Di-tert-butyl-dicarbonate (96 mg, 0.440 mmol, 1.1 eq.) was added and the ice bath removed. It was stirred at room temperature for 12 h. The solution was concentrated *in vacuo*. It was cooled in an ice bath and a layer of EtOAc was added. The pH was adjusted to 2-3 with $KHSO_4$ (10% aq.). The aqueous phase was extracted with EtOAc (2 x 25 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated to give 160 mg **118** (100% yield).

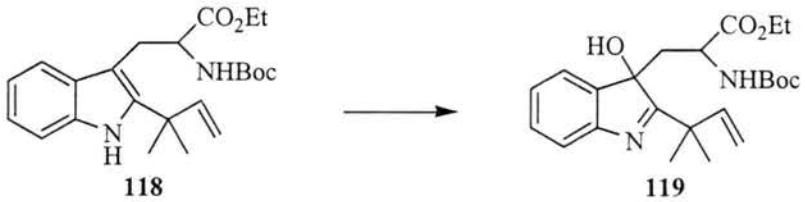
^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.32 (9 H, s), 1.51 (3 H, s), 1.54 (6 H, d J = 3.6 Hz), 3.16-3.34 (2 H, m), 3.90-4.16 (2 H, m), 4.48-4.58 (1 H, m), 5.16 (1 H, dd, J = 1.8 Hz, J = 9.9 Hz), 5.19 (1 H, dd, J = 1.2 Hz, J = 9.6 Hz), 6.13 (1 H, dd, J = 10.2 Hz, J = 17.1 Hz), 7.02-7.11 (2 H, m), 7.24 (1 H, d, J = 7.5 Hz), 7.48 (1 H, d J= 7.5 Hz), 8.08 (1 H, bs). R_f = 0.35 Vanillin stain: red-purple (4:1 hexane/ethyl acetate).



118

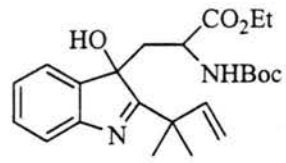


Compound **118**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

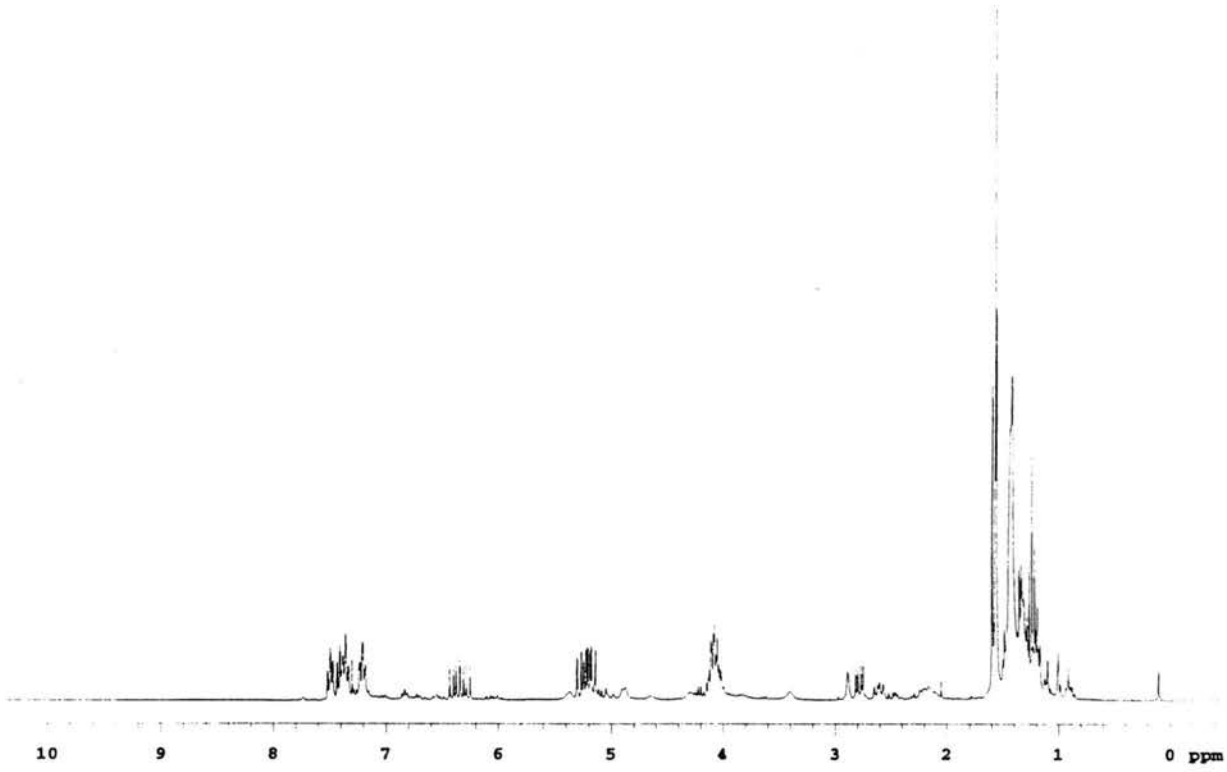


118 **119**

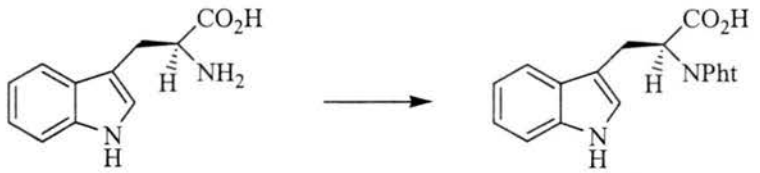
2-(1,1-dimethylallyl)-3-(ethyl-2-butoxycarbonylamino) hydroxyindolenine propionate (119). A solution of Boc-indole **118** (210 mg, 0.5250 mmol, 1.0 eq.) and Rose Bengal (0.20 g, 0.1970 mmol, 0.38 eq.) in dry MeOH (35 mL) stirred at 0°C for 3.5 h with a lamp to irradiate the reaction mixture. DMS (3.0 mL) was added to the solution and stirred overnight. The solvent was removed *in vacuo*. The Rose Bengal was removed by running the mixture through an alumina column (100% CH_2Cl_2). It was purified by column chromatography (silica: 10:1-4:1-1:1 hexane/ethyl acetate) to give 150 mg (68.8% yield) of hydroxyindolenine **119** as an off-white foam. ^{1}H NMR (300 MHz) ($CHCl_3$) δ TMS: 1.24 (3 H, t, J = 7.2 Hz), 1.42 (6 H, s), 1.57 (9 H, d, J = 3.0 Hz), 2.58 (1 H, dd, J = 11.4 Hz, J = 14.7 Hz), 2.80 (1 H, dd, J = 6.0 Hz, J = 14.4 Hz), 2.89 (1 H, s), 3.98-4.15 (2 H, m), 5.12-5.30 (2 H, m), 6.25-6.44 (1 H, m), 7.18-7.52 (4 H, m). R_f = 0.05, 0.12 Vanillin stain: red- purple (4:1 hexane/ethyl acetate).



119

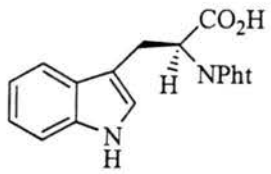


Compound **119**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

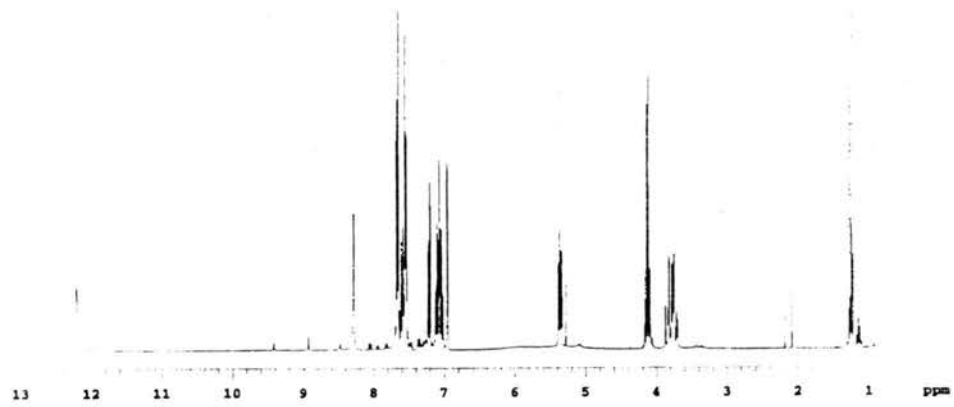


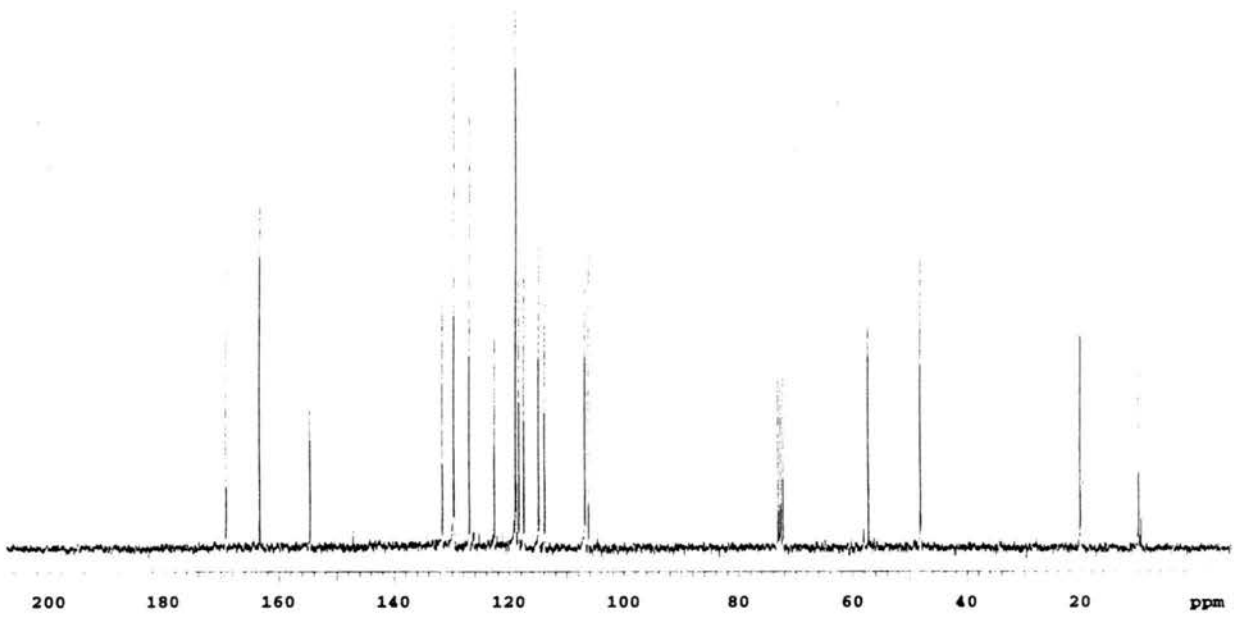
122

N-pthaloyl-tryptophan (122). A solution of L-tryptophan (15.0 g, 73.53 mmol, 1.0 eq.), N-carboethoxyphthalimide (16.1 g, 73.53 mmol, 1.0 eq.), and 1.8% (w/v) Na_2CO_3 (aq.) (430 mL) was stirred at room temperature for 10 h. The pH was adjusted to 2.0 with 10% $KHSO_4$ (aq.) and extracted with methylene chloride (3 x 300 mL). The organic layers were pooled together and washed with brine. It was dried over anhydrous Na_2SO_4. Concentration of the CH_2Cl_2 gave 24.50 g NPht-Trp **122** as a yellow solid (99.8% yield). ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 3.68-3.85 (3 H, m), 5.31 (1 H, dd, J = 5.1 Hz, J = 10.8Hz), 6.99-7.13 (4 H, m), 7.13-7.26 (1 H, m), 7.57-7.74 (4 H, m), 7.92 (1 H, bs), 12.25 (1 H, bs). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 14.25, 24.47, 52.47, 61.60, 110.50, 111.13, 118.19, 119.23, 121.80, 122.66, 123.22, 126.91, 131.26, 133.93, 135.92, 158.98, 167.67, 173.49. IR (NaCl, CH_2Cl_2): 1105, 1201, 1231, 1268, 1343, 1390, 1419, 1457, 1469, 1583, 1715, 1776, 2913, 2985, 3061, 3413, 3477 cm^{-1}. R_f = 0.55 PMA stain: blue (1:1 hexane/ethyl acetate).

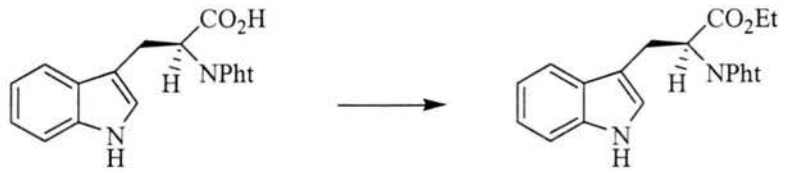


122





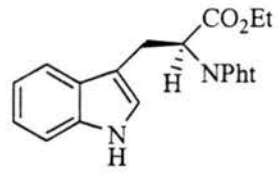
Compound **122**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



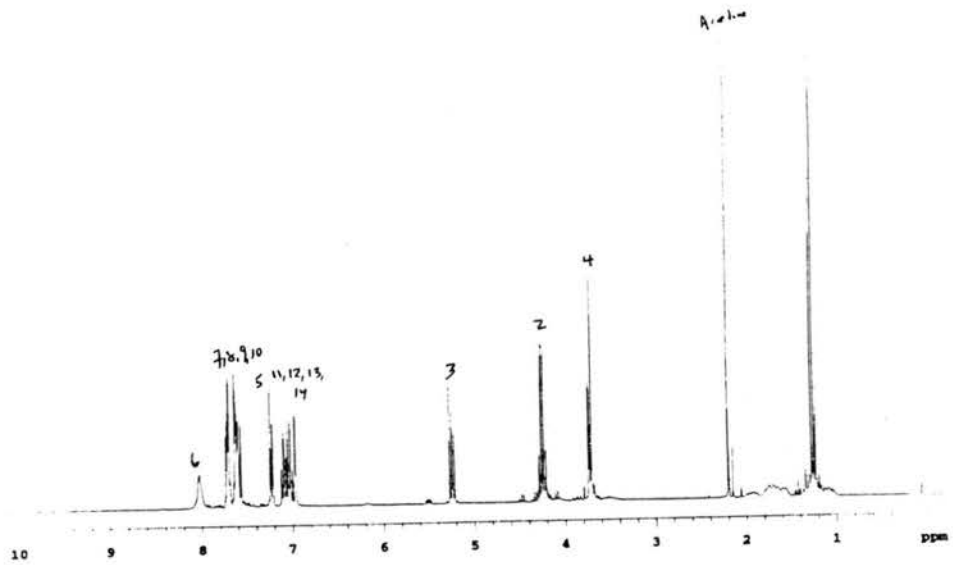
122 **123**

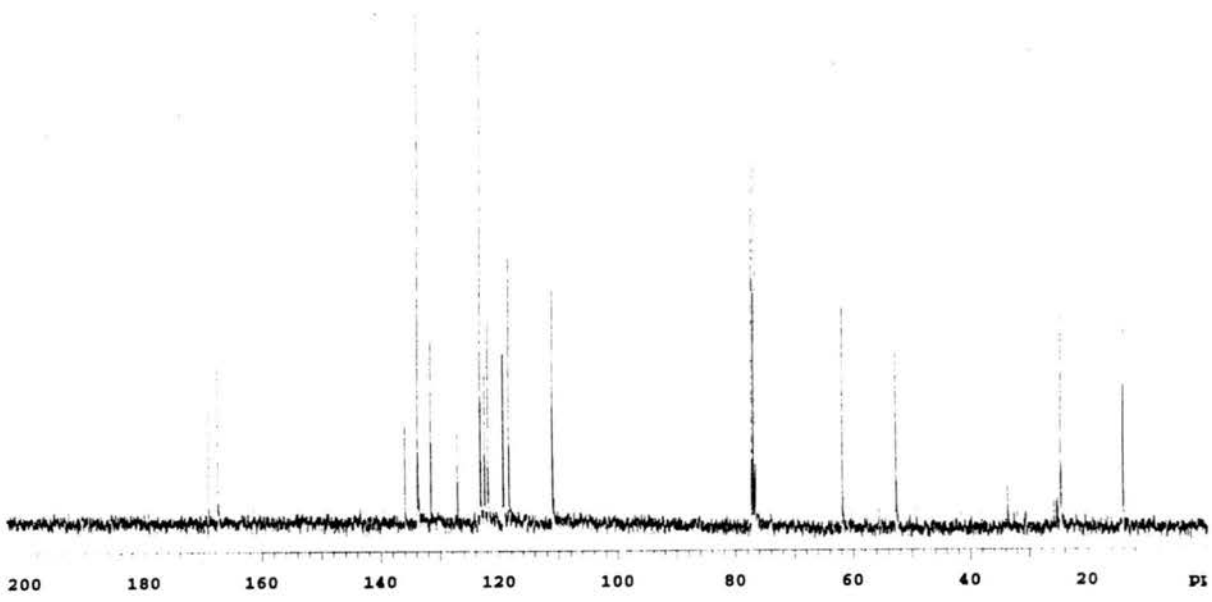
1H-Indole-3-propanoic acid, α-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-methyl ester (123). A solution of NPht-Trp **122** (15.0 g, 44.91 mmol, 1.0 eq.), N,N-dicyclohexylcarbodiimide (10.18 g, 49.40 mmol, 1.1 eq.), absolute ethanol (2.78 mL, 49.40 mmol, 1.1 eq.), and 4-pyrrolidinopyridine (0.55 g, 4.491 mmol, 0.1 eq.) in dry CH_2Cl_2 (150 mL) was stirred at room temperature for 12 h. The N,N-dicylohexylurea was filtered and the filtrate washed with water (3 x 200 mL), 5% acetic acid (3 x 200 mL), water (3 x 200 mL), and dried over Na_2SO_4. The solvent was evaporated to give 16.0 g of **123** as a yellow powder (98% yield).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.25 (3 H, t, J = 7.2 Hz), 3.71 (1 H, s), 3.74 (1 H, dd, J = 0.6 Hz, J = 3.3 Hz), 4.25 (2 H, ddd, J = 1.5 Hz, J = 6.9 Hz, J = 14.4 Hz), 5.22 (1 H, d, J = 6.9 Hz), 5.26 (1 H, d, J = 6.6 Hz), 6.93-7.26 (5 H, m), 7.55-7.76 (4 H, m), 8.03 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 14.08, 24.72, 33.72, 52.73, 61.90, 111.03, 111.06, 118.41, 119.37, 121.95, 122.51, 123.27, 127.11, 131.62, 133.92, 136.03, 167.57, 169.11. IR (NaCl, CH_2Cl_2): 1025, 1105, 1198, 1239, 1258, 1390, 1445, 1468, 1622, 1716, 1776, 1807, 2853, 2933, 2983, 3059, 3408 cm^{-1}. R_f = 0.11 (4:1 hexane/ethyl acetate).

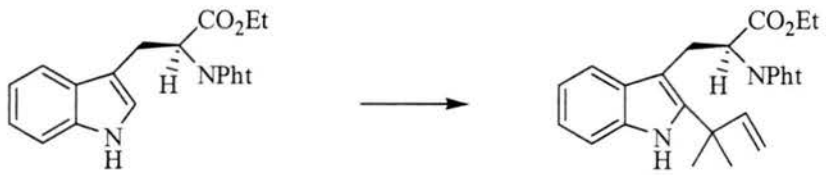


123





Compound **123**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

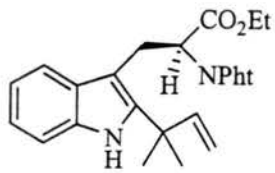


123 **125**

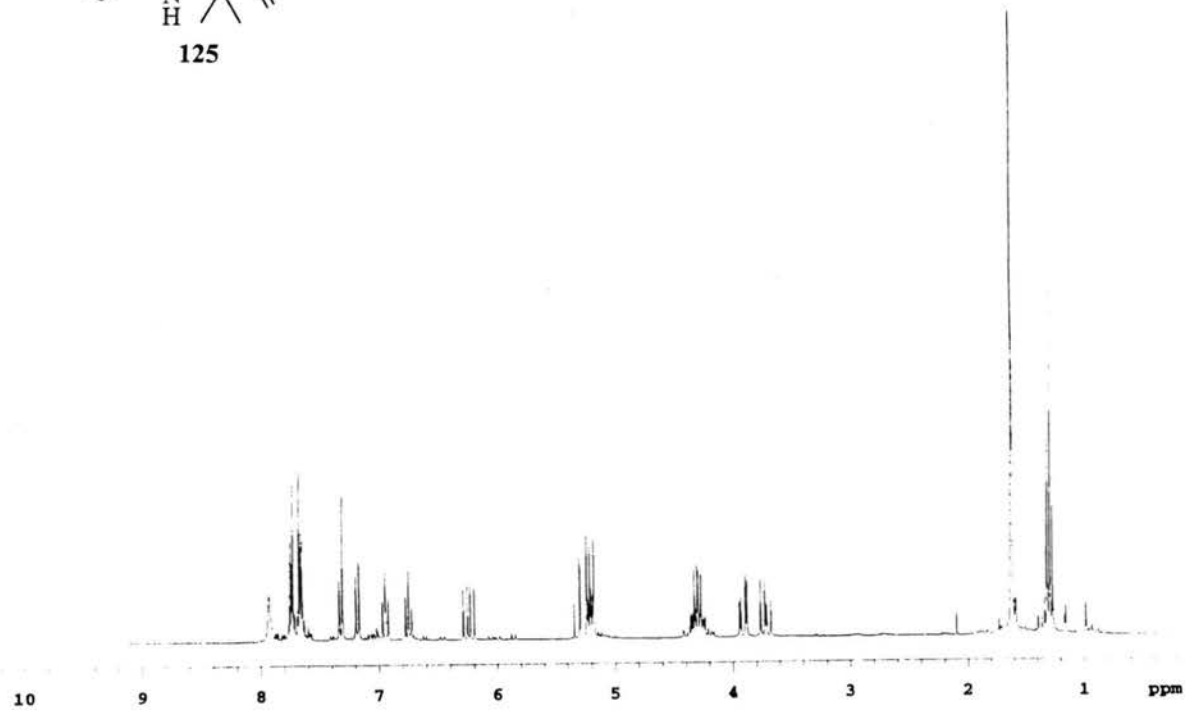
1H-Indole-3-propanoic acid, α-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-2-(1,1-dimethyl-2-propenyl)-methyl ester (125). To a stirred solution of NPht-Trp ester **123** (2.80 g, 8.86 mmol, 1.0 eq.), Et_3N (1.48 mL, 10.63 mmol, 1.2 eq.) in dry THF (22 mL) at -78°C was added freshly made t-butylhypochlorite (1.1 mL, 9.30 mmol, 1.05 eq.) This mixture continued to stir at -78°C for 2 h. Next, prenyl 9-BBN in THF (21.3 mL, 10.63 mmol, 1.2 eq) was added. The stirring continued for another 12 h slowly warming to room temperature. The mixture was concentrated and purified by column chromatography (silica: 10:1 - 4:1 - 3:1 hexane/ethyl acetate) to give 1.82 g **125** as a yellow powder (47.8% yield).

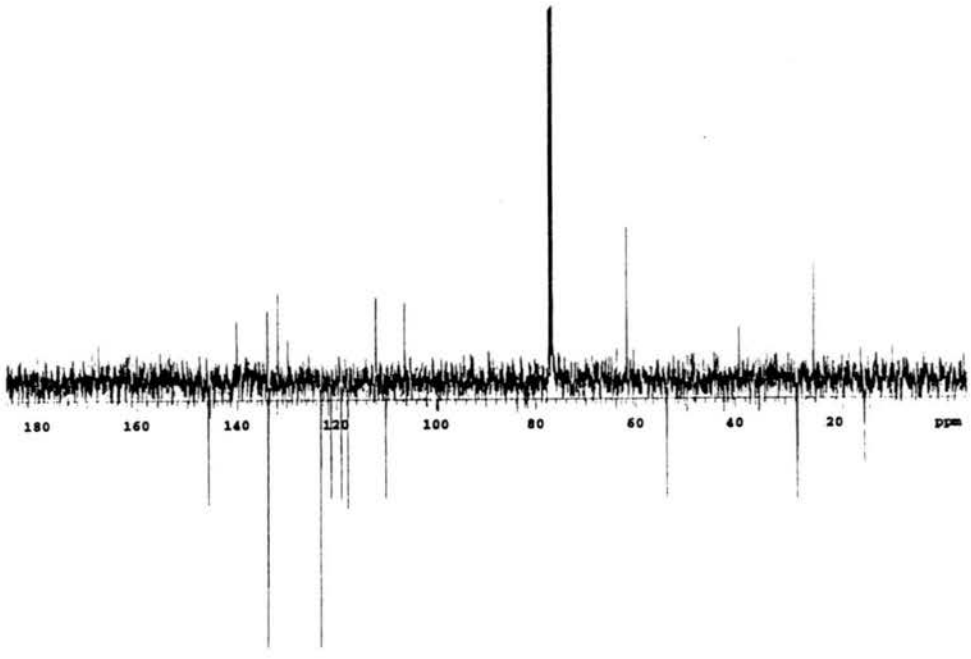
^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.23 (3 H, t, J = 6.9 Hz), 1.56 (6 H, d, J = 1.2 Hz), 3.65 (1 H, dd, J = 11.1 Hz, J = 15.0 Hz), 3.84 (1 H, dd, J = 3.6 Hz, J = 15.0 Hz), 4.23 (2 H, dddd, J = 3.9 Hz, J = 10.8 Hz, J = 14.1 Hz, J = 18.0 Hz), 5.13 (1 H, dd, J = 1.2 Hz, J = 10.8 Hz), 5.14 (1 H, dd, J = 3.6 Hz, J = 7.8 Hz), 5.20 (1 H, dd, J = 0.9 Hz, J = 17.4 Hz), 6.17 (1 H, dd, J = 10.2 Hz, J = 17.1 Hz), 6.68 (1 H, ddd, J = 0.9 Hz, J = 7.2 Hz, J = 8.1 Hz), 6.88 (1 H, ddd, J = 1.2 Hz, J = 6.9 Hz, J = 8.1 Hz), 7.11 (1 H, dd, J = 0.9 Hz, J = 8.1 Hz), 7.25 (1 H, d, J = 7.2 Hz), 7.57-7.69 (4 H, m), 7.85 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 14.09, 24.31, 27.45, 27.63, 39.16, 53.68, 61.78, 106.35, 110.18, 112.09, 117.74, 119.08, 121.10, 123.15, 129.76, 131.89, 133.78, 140.06, 145.80, 167.70, 169.02. IR (NaCl, CH_2Cl_2): 1030, 1106, 1202, 1265, 1337, 1390, 1422, 1469, 1716, 1740, 1776,

2934, 2985, 3054, 3472 cm^{-1}. R_f = 0.48 Vanillin stain: red- purple (4:1 hexane/ethyl acetate).

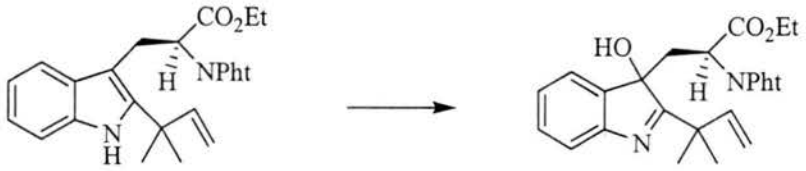


125





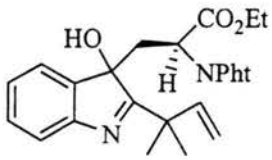
Compound **125**: 300 MHz ^{1}H NMR and APT NMR Spectrums in $CDCl_3$



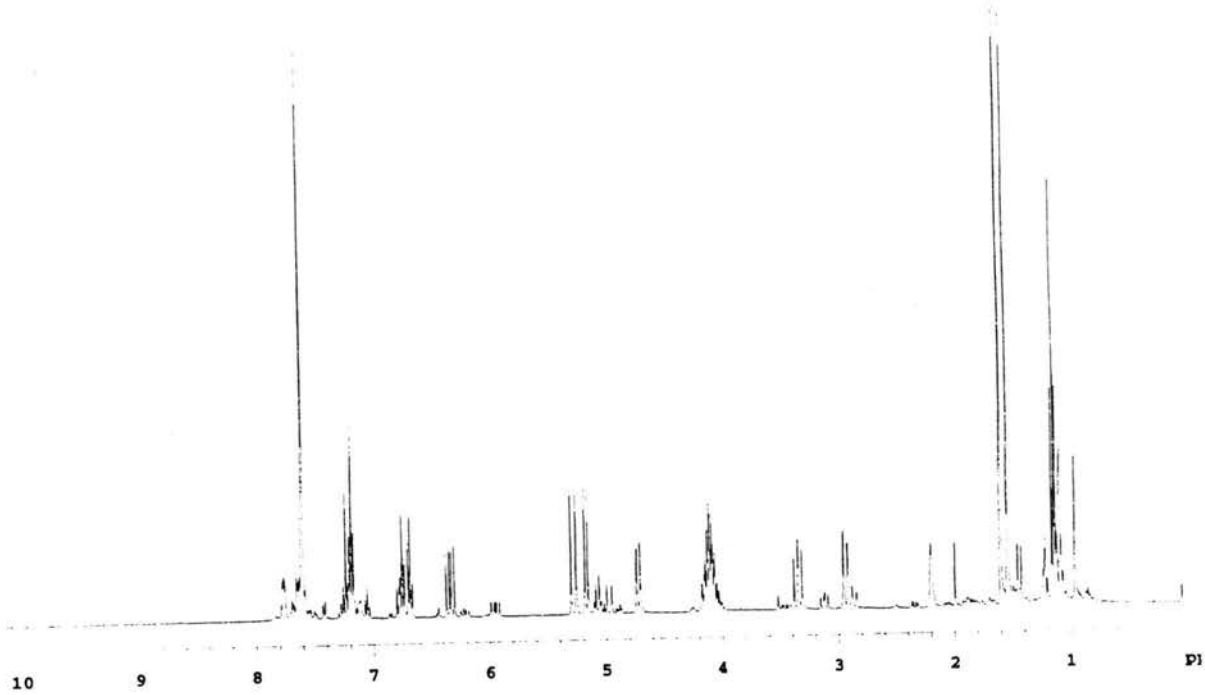
125 126

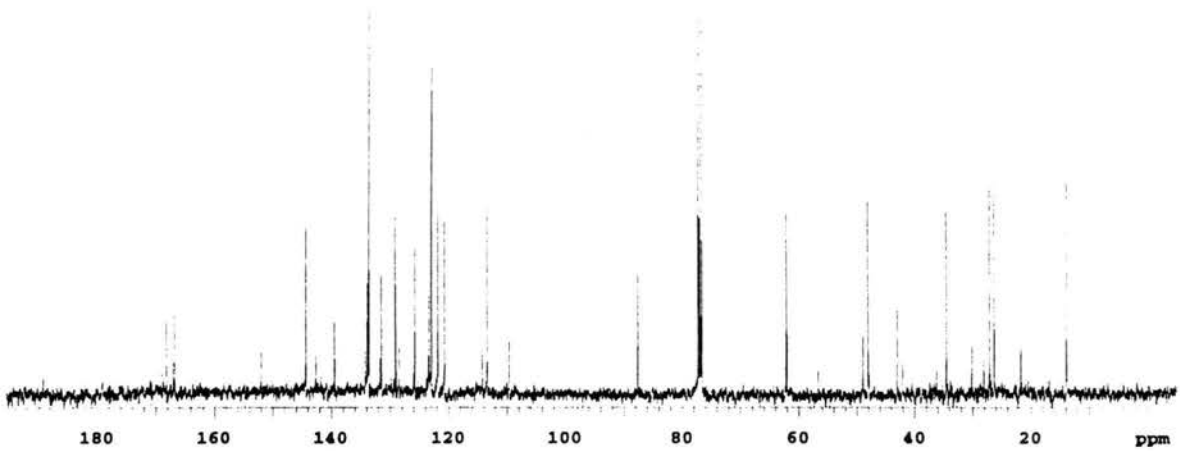
1H-Indole-3-propanoic acid, α-(1,3-dihydro-1,3-dioxo-2H-hydroxyindolenine-2-yl)-2-(1,1-dimethyl-2-propenyl)-methyl ester (126). A solution of Pht-indole **125** (770 mg, 1.7907 mmol, 1.0 eq.) and Rose Bengal (0.30 g, 0.2956 mmol, 0.17 eq.) in dry MeOH (200 mL) stirred at 0°C for 3.5 h with a lamp to irradiate the reaction mixture. DMS (3.0 mL) was added to the solution and stirred overnight. The solvent was removed *in vacuo*. The Rose Bengal was removed by running the mixture through an alumina column (100% CH_2Cl_2). It was purified by column chromatography (silica: 10:1-4:1-1:1 hexane/ethyl acetate) to give 400 mg (50.1% yield) of hydroxyindolenine **126** as an off-white foam.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.14 (3 H, t, J = 5.7 Hz), 1.56 (6 H, d, J = 17.7 Hz), 2.20 (1 H, bs), 2.93 (1 H, dd, J = 0.9 Hz, J = 10.8 Hz), 3.35 (1 H, t, J = 10.2 Hz), 4.00-4.20 (2 H, m), 4.72 (1 H, dd, J = 1.2 Hz, J = 9.3 Hz), 5.16 (1 H, d, J = 8.1 Hz), 5.27 (1 H, d, J = 11.1 Hz), 6.33 (1 H, dd, J = 8.1 Hz, J = 12.9 Hz), 6.66-6.80 (2 H, m), 7.15-7.21 (2 H, m), 7.38-7.80 (4 H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 13.96, 26.40, 27.21, 34.62, 43.10, 48.15, 62.17, 87.71, 113.43, 120.76, 121.93, 123.01, 123.41, 125.85, 129.16, 131.55, 133.66, 133.92, 139.51, 144.38, 152.01, 166.85, 167.11, 168.25, 189.18. IR (NaCl, CH_2Cl_2): 1265, 1389, 1421, 1468, 1552, 1597, 1718, 1777, 2986, 3054, 3420, 3582 cm^{-1}. LRMS (MH^+): 447.2

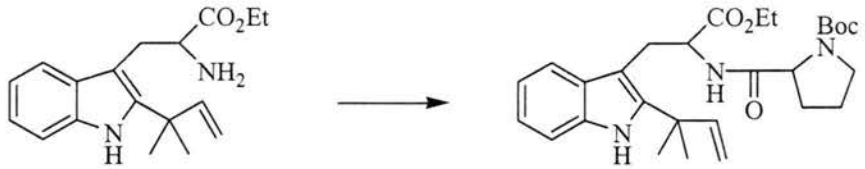


126





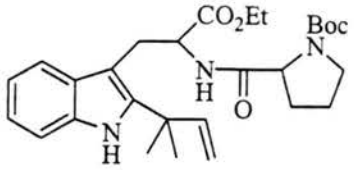
Compound **126**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



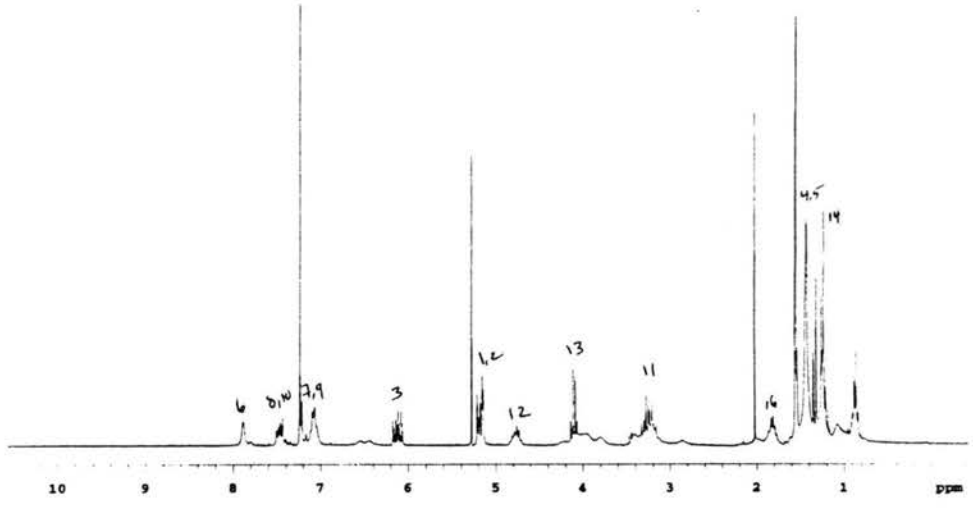
117 129

2-(1,1-dimethylallyl)-3-(ethyl-2-L-prolylamino) indolepropionate (129). A solution of indole **117** (500 mg, 1.6667 mmol, 1.0 eq.) Boc-Proline (358 mg, 1.6667 mmol, 1.0 eq.) Bop-Reagent (737 mg, 1.6667 mmol, 1.0 eq.), Et_3N (0.46 mL, 3.3333 mmol, 2.0 eq.) in dry CH_3CN (24 mL) was stirred under argon at room temperature for 16 h. The reaction mixture was poured into a separatory funnel containing brine (75 mL) and EtOAc (75 mL). The organic layer was washed with 2M HCl (50 mL), saturated bicarbonate solution (50 mL), and brine (50 mL). It was dried over Na_2SO_4 and concentrated. The crude material was purified by column chromatography (silica: 4:1-1:1 hexane/ethyl acetate). 830 mg of an off-white foam **129** were obtained (100 % yield).

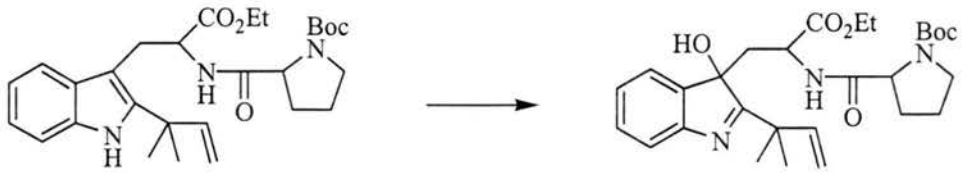
^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.31 (3 H, t), 1.50 (6 H, s), 1.62 (9 H, s), 1.82-1.94 (1 H, m), 3.16-3.56 (2 H, m), 3.80-4.24 (2 H, m), 4.74-4.92 (1 H, m), 5.18-5.30 (2 H, m), 6.12-6.26 (1 H, m), 7.06-7.60 (4 H, m), 7.95 (1 H, s). R_f = 0.11 Vanillin stain: red-purple (4:1 hexane / ethyl acetate).



129



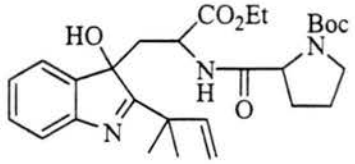
Compound **129**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



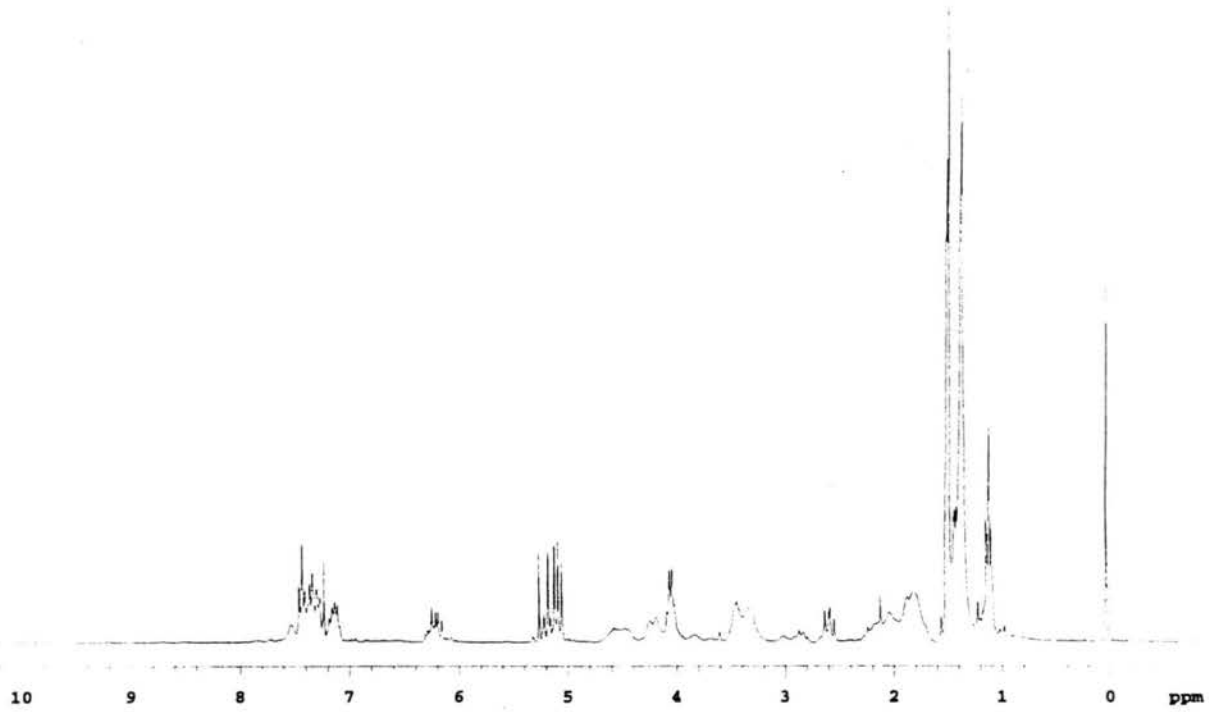
129 **130**

2-(1,1-dimethylallyl)-3-(ethyl-2-L-prolylamino) hydroxyindolenine propionate (130). A solution of Boc-Pro-indole **129** (440 mg, 0.8853 mmol, 1.0 eq.) and Rose Bengal (0.25 mg, 0.2463 mmol, 0.28 eq.) in dry MeOH (150 mL) stirred at 0°C for 2 h with a lamp to irradiate the reaction mixture. DMS (3 mL) was added to the solution and stirred overnight. The solvent was removed *in vacuo*. The Rose Bengal was removed by running the mixture through an alumina column (100% CH_2Cl_2). It was purified by column chromatography (silica: 10:1-4:1-1:1 hexane/ethyl acetate) to give 165 mg (36% yield) of hydroxyindolenine **130** as a pinkish-off-white foam.

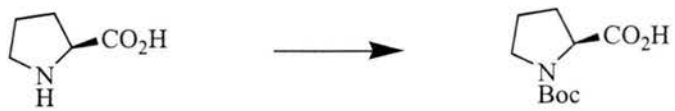
^{1}H NMR (300 MHz) ($CHCl_3$) δ TMS: 1.35 (9 H, s), 1.46-1.50 (9 H, m), 1.68-2.24 (4 H, m), 2.56 (1 H, dd, J = 11.4 Hz, J = 14.7 Hz), 2.80 (1 H, dd, J = 9.0 Hz, J = 14.4 Hz), 3.10-3.50 (3 H, m), 3.70-4.30 (4 H, m), 4.40-4.70 (1 H, m), 5.10-5.30 (2 H, m), 6.14-6.32 (1 H, m), 7.01-7.50 (4 H, m). R_f = 0.27 Vanillin stain: light purple (1:1 hexane/ethyl acetate).



130



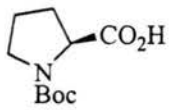
Compound **130**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



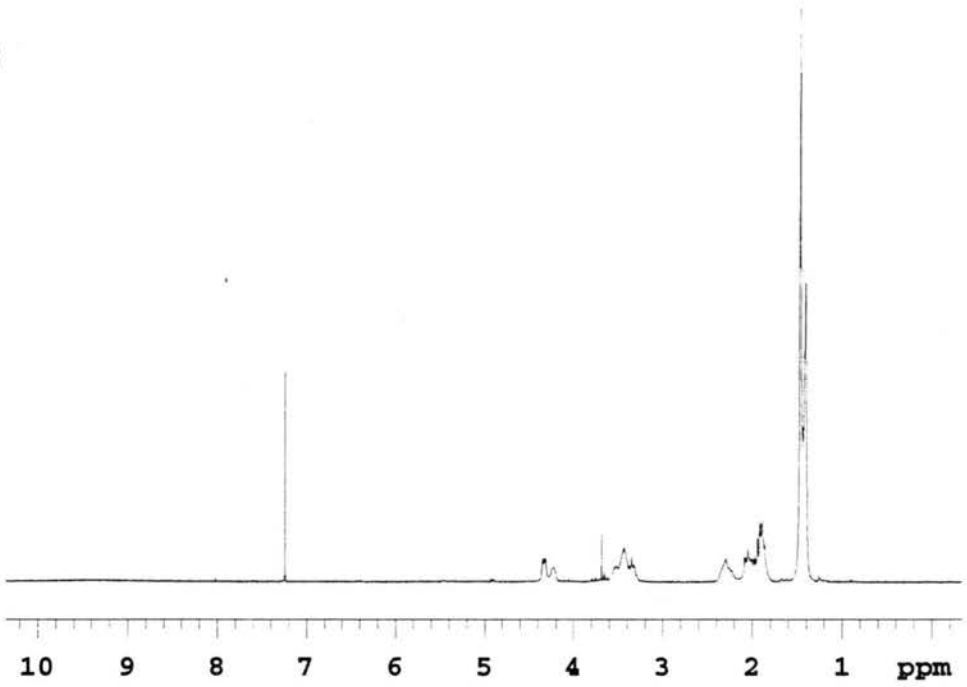
148

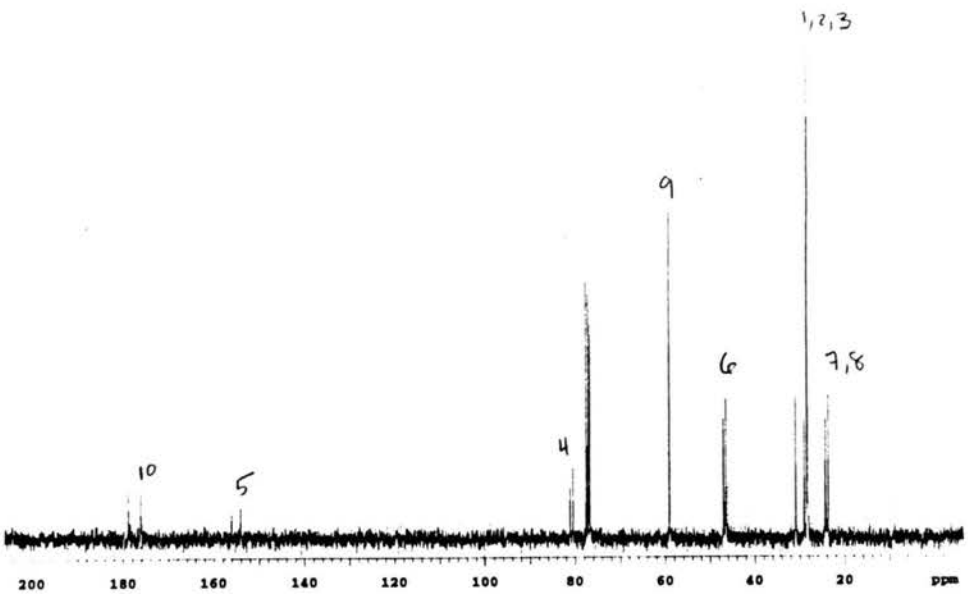
Butoxycarbonyl-L-Proline (148). A solution of L-proline (20.0 g, 174 mmol, 1.0 eq), 350 mL dioxane, 350 mL water and K_2CO_3 (24 g, 174 mmol, 1.0 eq) was stirred at 0°C in an ice bath. Once cool, di-t-butyldicarbonate (41.7 g, 191 mmol, 1.1 eq) was added. The reaction was stirred at room temperature for 12 h. The solution was concentrated *in vacuo* to about 200 mL. It was cooled in an ice bath and 100 mL ethyl acetate was added. The pH was adjusted to 2-3 with 10% $KHSO_4$ (aq). The aqueous phase was extracted with ethyl acetate (2 x 150 mL) and dried over anhydrous Na_2SO_4. Solvent was evaporated to give 36.48 g (97.5%) of a white powder.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 4.3-4.4 (1 H, m), 3.3-3.55 (2 H, m), 1.85-2.50 (4 H, m), 1.45 (9 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 23.80, 24.47, 28.54, 46.50, 59.13, 80.51, 156.09, 178.89. IR (NaCl, CH_2Cl_2): 3550-2800, 2964, 1746, 1650, 1407 cm^{-1}. R_f = 0.51 (10:1 CH_2Cl_2/MeOH)



148



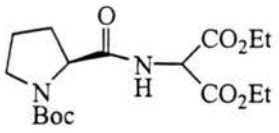


Compound **148**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

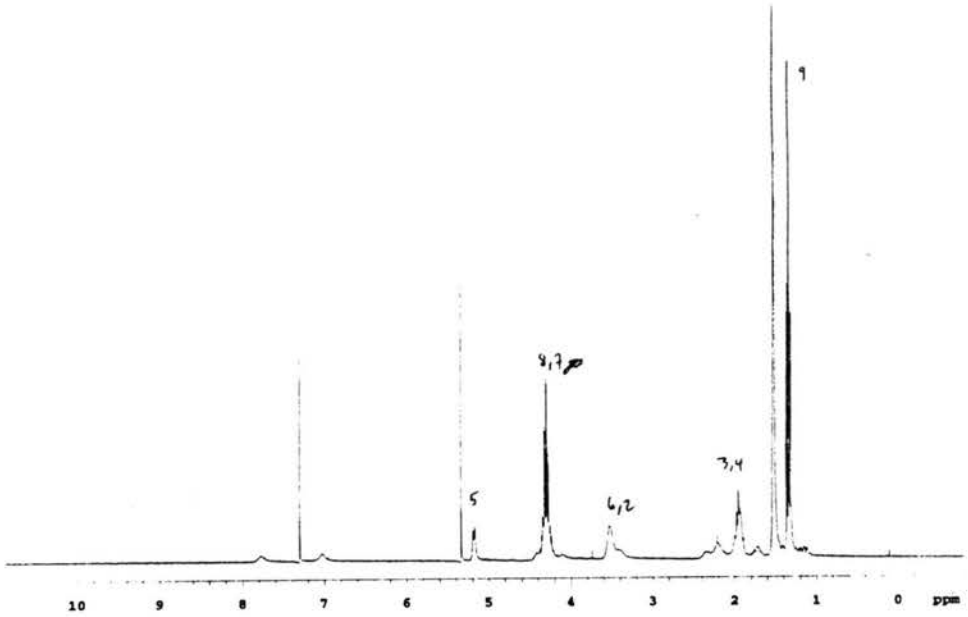
148 149

Butoxycarbonyl-L-prolylaminomalonate (149). Diethylaminomalonate hydrochloride (35.8 g, 169 mmol, 1.0 eq), Et_3N (23.6 mL, 169 mmol, 1.0 eq) , and dry CH_2Cl_2 were stirred at room temperature for 10 minutes. The mixture was then cooled to 0°C in an ice bath and Boc-proline (36.4g, 169 mmol, 1.0 eq) was added and stirred for 10 minutes. DCC (34.9 g, 169 mmol, 1.0 eq) was added and the mixture stirred an additional 10 minutes at 0°C. The reaction continued stirring at room temperature for 45 minutes. During this time, the solution turned milky white, indicating the conversion of DCC to DCU. The urea derivative was filtered with a buchner funnel and washed with CH_2Cl_2. The crude mixture was washed with 500 mL 1M aq. HCl followed by 500 mL 1M aq. K_2CO_3 and then brine. It was dried over anhydrous $MgSO_4$. The solvent was evaporated to give 71.8 g (100%) of a yellow oil.

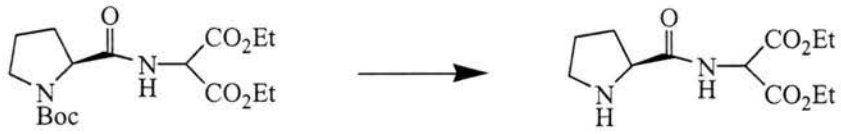
^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 5.18 (1 H, d, J = 6.3 Hz), 4.21-4.38 (5 H, m), 3.35-3.51 (3 H, m), 1.89-1.99 (4 H, m), 1.52 (9 H, s), 1.34 (3 H, t, J = 7.2 Hz), 1.33 (3 H, t, J = 7.2 Hz). R_f = 0.65 (10:1 CH_2Cl_2/MeOH).



149



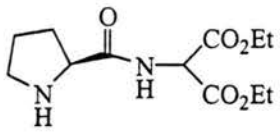
Compound **149**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



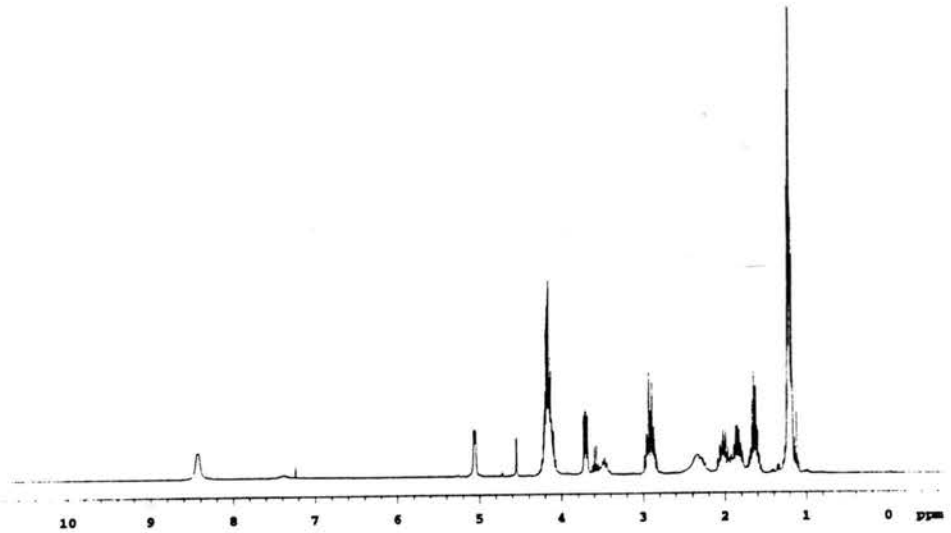
149 150

L-prolylaminomalonate (150). Boc-aminomalonate (25.96 g, 61.23 mmol, 1.0 eq) were dissolved in 246 mL dry CH_2Cl_2. This solution was cooled to 0°C in an ice bath. While stirring, TFA (46.7 mL, 610 mmol, 10 eq) were added over 5 minutes. The mixture stirred for 6 h at 0°C. The reaction was made basic with the slow addition of 100 mL 2M aq. NaOH while stirring at 0°C. The aminomalonate was extracted CH_2Cl_2 (3 x 500 mL) and washed with brine. It was dried over anhydrous $MgSO_4$ and the solvent evaporated to give 13.51 g (81%) of a yellow oil.

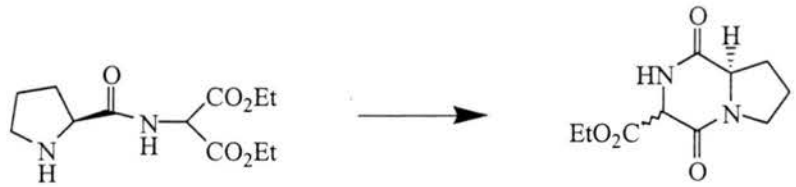
^{1}H NMR (300 MHz) ($CDCl_3$), δ TMS: 8.47 (1 H, d, J = 6.3 Hz), 5.11 (1 H, d, J = 7.8 Hz), 4.10-4.28 (2 H, m), 3.76 (1 H, dd, J = 8.7 Hz, J = 4.8 Hz), 2.88-3.03 (2 H, m), 2.01-2.14 (1 H, m) , 1.84-1.95 (1 H, m), 1.60-1.78 (2 H, m), 1.24 (6 H, ddd, J = 14.4 Hz, J = 7.2 Hz, J = 3.0 Hz). R_f= 0.35 (10:1 CH_2Cl_2/MeOH).



150



Compound **150**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

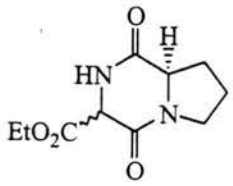


150 **151**

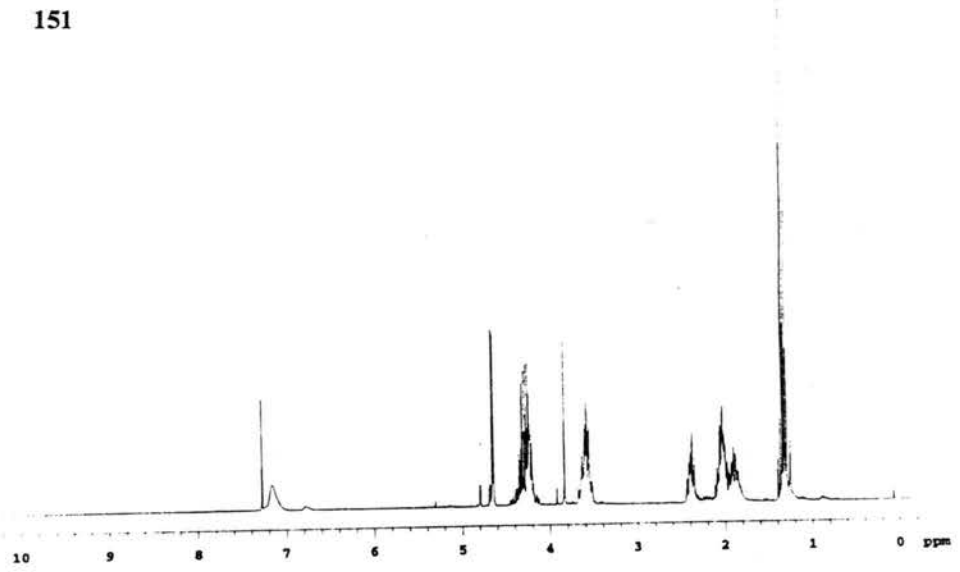
Pyrrolo[1,2-a]pyrazine-3-carboxylic acid, octahydro-1,4-dioxo, ethyl ester (151). 1.148 g of crude amine **150** (4.22 mmol, 1.0 eq.) was dissolved in 5 mL of dry toluene. 41 mg of 2-hydroxypyridine (0.43 mmol, 1.10 eq.) were added. The mixture was refluxed for 3 h.

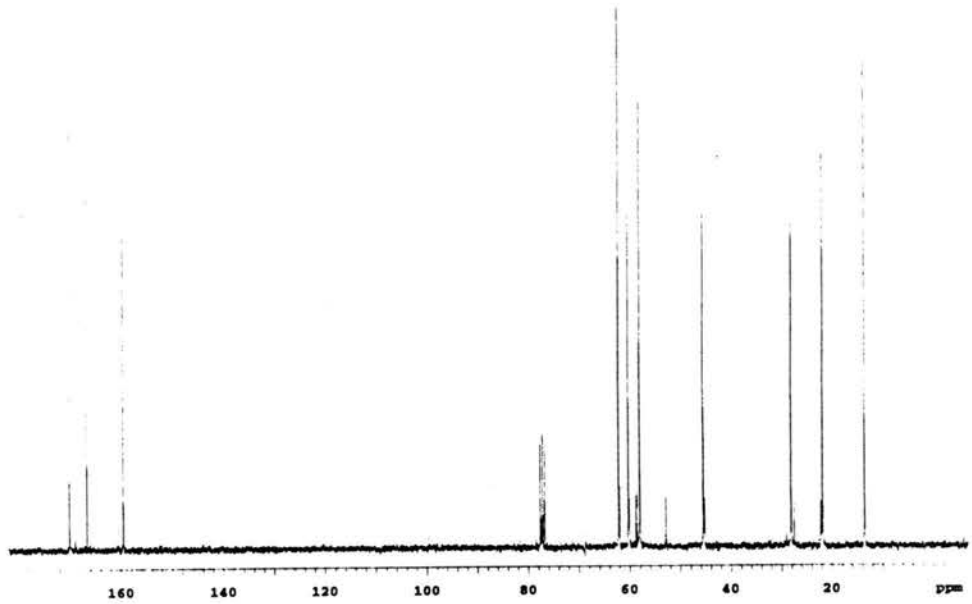
The solvent was removed *in vacuo*, and the residue was purified using CC with CH_2Cl_2/MeOH (50:1 to 25:1 to 10:1) as eluent. 0.9665 g of diketopiperazine were obtained (100%).

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.32 (3 H, t, J = 6.90 Hz), 1.80-2.42 (4 H, m), 3.50-3.70 (2 H, m), 4.20-4.42 (3 H, m), 4.65 (1 H, d, J = 4.50 Hz), 7.16 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 13.56, 21.89, 28.10, 45.49, 58.09, 60.34, 62.25, 159.61, 166.68, 170.16. IR (NaCl, CH_2Cl_2): 3244, 3056, 2985, 2887, 1743, 1678, 1443, 1265, 1194, 1023 cm^{-1}. HRMS (FAB), calcd. for $C_{10}H_{14}N_2O_4$ (MH^+): 227.1032. Found: 227.1029.

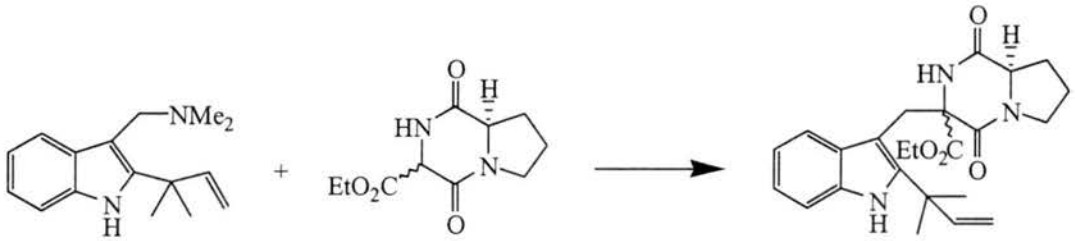


151





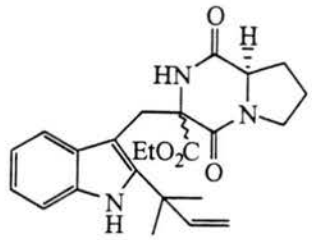
Compound **151**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



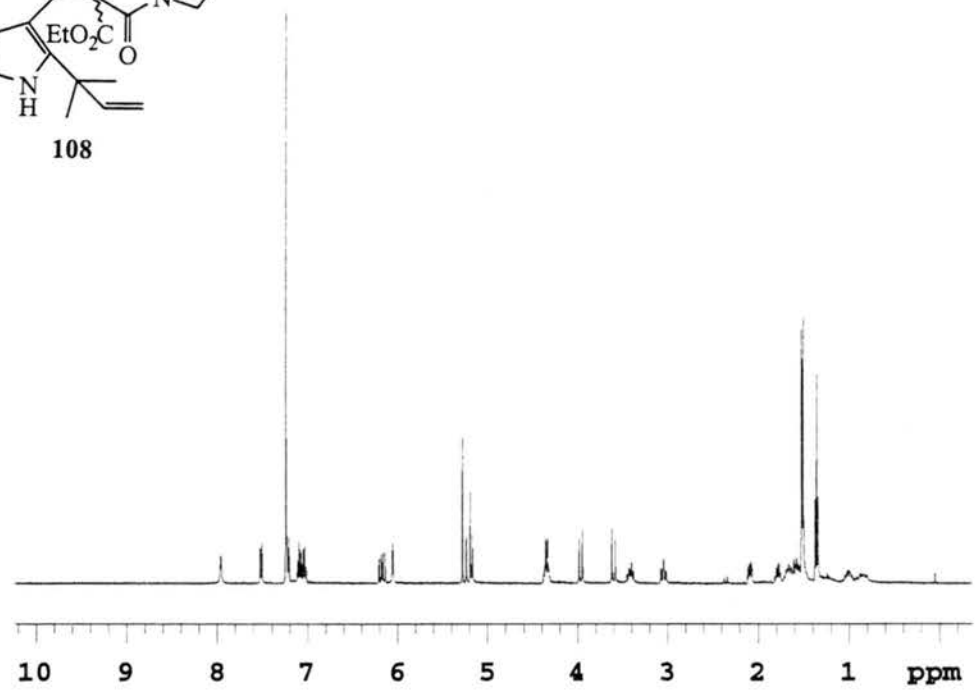
ethyl 3-[2-(1,1-dimethylallyl)indol-3-ylmethyl]-1,4-dioxoperhydropyrrolo[1,2-b]pyrazine-3-carboxylate (108). Under inert atmospere, 2.26 g of the indole **24** (9.159 mmol, 1.0 eq.) and 2.07 g diketopiperazine **151** (9.159 mmol, 1.0 eq.) were dissolved in 76 mL of dry CH_3CN. Then 1.14 mL Bu_3P (4.5796 mmol, 0.50 eq.) was added. The mixture was refluxed for 4 h.

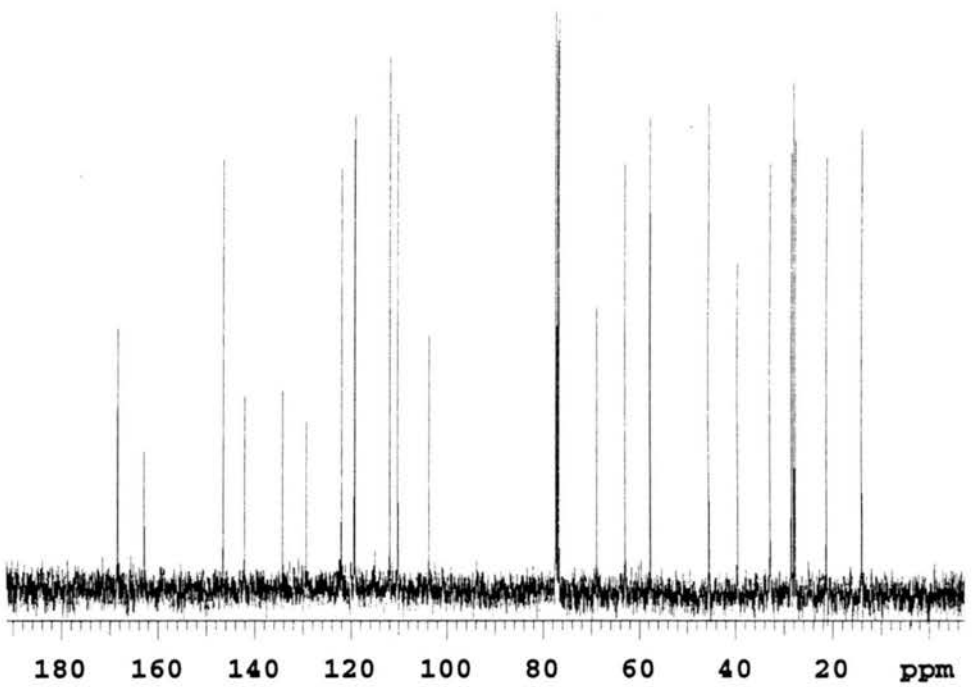
The solvent was removed *in vacuo*, to give the crude product. The mixture was purified by CC using 100:1 CH_2Cl_2/MeOH as eluent. 603.8 mg of one diastereomer and 1.13 g of the other diastereomer were obtained for a combined yield of 44.7%.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.35 (3 H, ddd, J = 1.50 Hz, J = 5.70 Hz, J = 10.80 Hz), 1.51 (3 H, d, J = 4.80 Hz), 3.02-3.10 (1 H, m), 3.38-3.46 (1 H, m), 3.59 (1 H, d, J = 11.7 Hz), 3.96 (1 H, d, J = 11.4 Hz), 4.00-4.40 (2 H, m), 5.20 (1 H, d, J = 21.3 Hz), 5.24 (1 H, d, J = 27.3 Hz), 6.05 (1 H, s), 6.17 (1 H, dddd, J = 1.20 Hz, J = 7.80 Hz, J = 9.0 Hz, J = 13.2 Hz), 7.01-7.11 (2 H, m), 7.21-7.25 (1 H, m), 7.51 (1 H, d, 6.0 Hz), 7.96 (1 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$): 13.97, 21.26, 27.64, 28.01, 28.47, 32.85, 39.53, 45.52, 57.67, 62.92, 68.82, 103.74, 110.21, 110.87, 119.07, 119.26, 121.92, 129.23, 134.18, 142.09, 168.29, 168.30, 168.45. IR (NaCl, CH_2Cl_2): 1265, 1422, 1665, 1689, 1748, 2986, 3054, 3351, 3471 cm^{-1}.

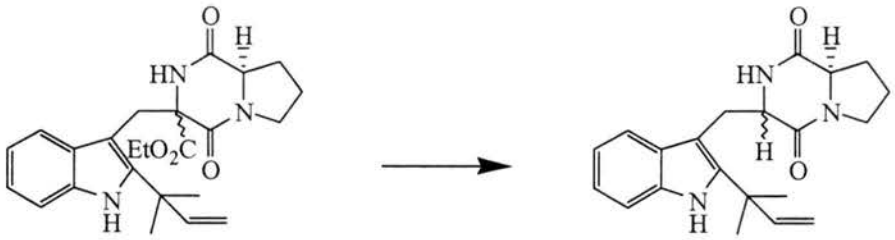


108





Compound **108**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$

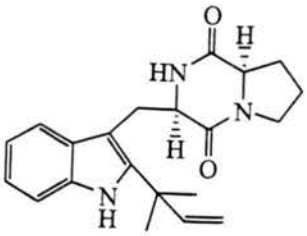


108 **6,27**

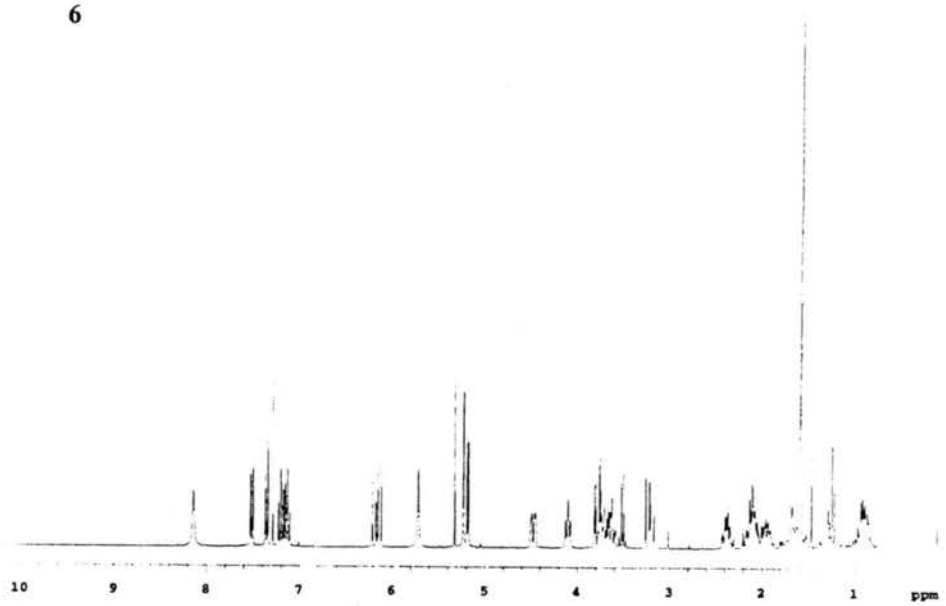
Deoxybrevianamide E and Epideoybrevianamide E (6, 27). In a 25 mL round bottom flask, 950 mg of indole **108** (2.245 mmol, 1.0 eq.) were dissolved in 3.61 mL of DMSO and 0.50 g of $MgCl_2 6H_2O$ (2.463 mmol, 1.1 eq.) were added. The reaction was refluxed for 2.5 h. Then the solvent was removed *in vacuo*. A few mL of CH_2Cl_2 were added. The mixture was stirred and filtered to remove the $MgCl_2 6H_2O$. After removing the solvent *in vacuo*, the crude mixture was purified by CC using 50:1 CH_2Cl_2/Ether as eluent. 133 mg of deoxybrevianamide E and 270 mg of epideoxybrevianamide E were obtained for a combined yield of 51%.

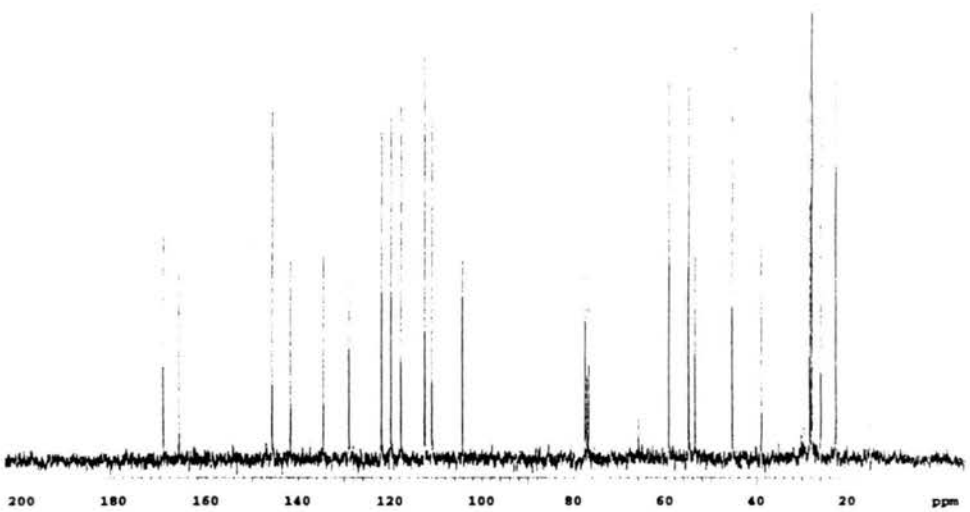
(Deoxybrevianamide E). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.23 (6 H, s), 1.85-2.4 (4 H, m), 3.16 (1 H, dd, J = 3.6 Hz, J = 15.3 Hz), 3.64 (1 H, dd, J = 3.9 Hz, J = 12.2 Hz), 5.12 (1 H, d, J = 16.8 Hz), 5.14 (1 H, d, J = 10.4 Hz), 6.11 (1 H, dd, J = 10.4 Hz, J = 17.6 Hz), 7.05-7.47 (4 H, m), 5.67 (1 H, s), 8.10 (1 H, s).

(Epideoxybrevianamide). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.50 (6 H, d), 1.35-2.0 (4 H, m), 3.24-3.32 (2 H, m), 3.44 (2 H, s), 4.20-4.32 (1 H, m), 5.12 (1 H, d, J = 10.5 Hz), 5.15 (1 H, d, J = 16.5 Hz), 6.10 (1 H, dd, J = 10.5 Hz, J = 17.5 Hz), 7.05-7.57 (4 H, m), 5.80 (1 H, s), 8.10 (1 H, s).

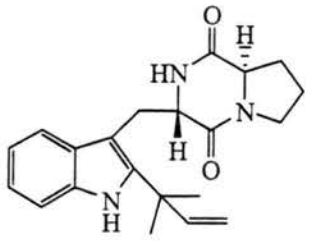


6

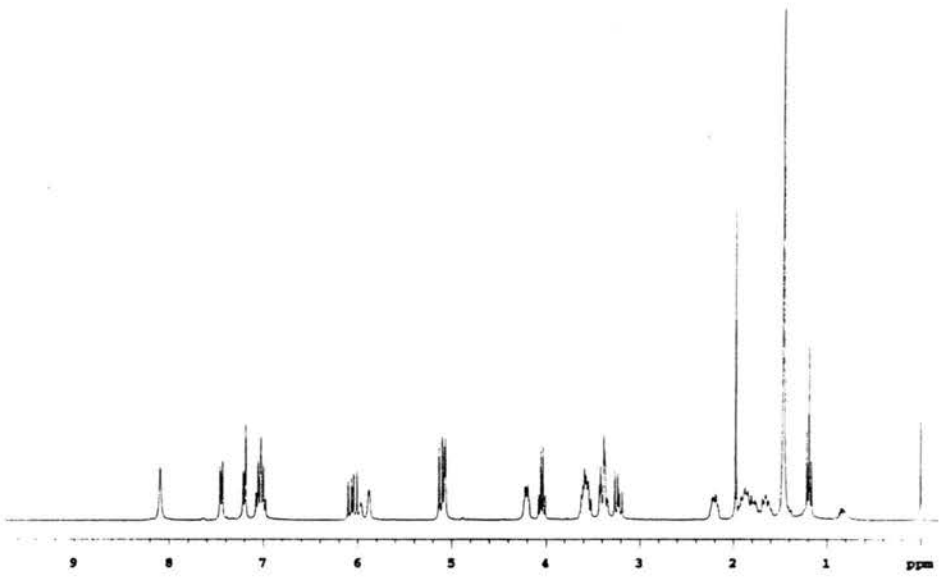




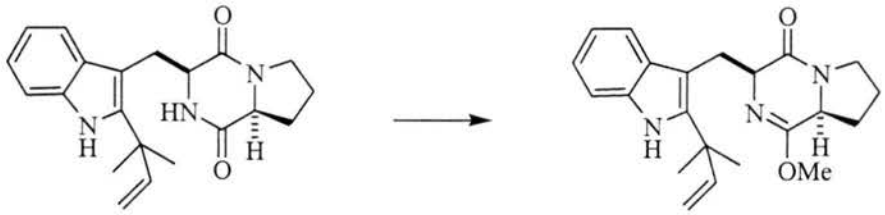
Compound **6**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



27



Compound **27** 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

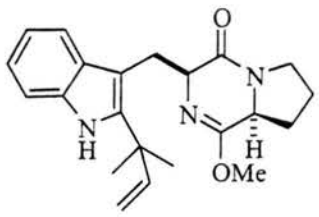


6 152

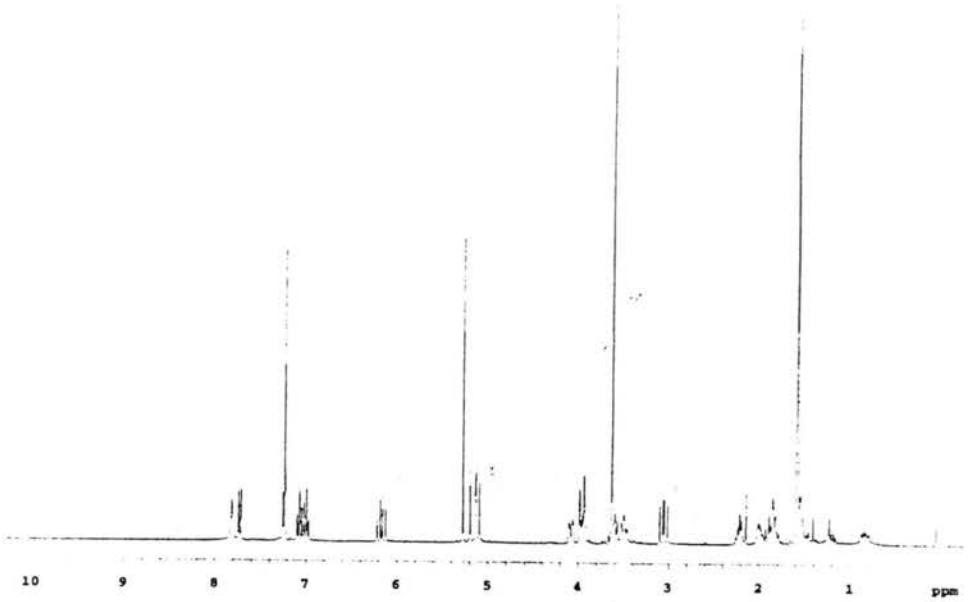
3-[[2-(1,1-dimethyl-2-propenyl)-1H-indol-3-yl]methyl]6,7,8,8a-tetrahydro-1-methoxy-pyrrolo[1,2-a]pyrazine-4(3H)-one (152). Deoxybrevianamide E (3.41 g, 9.71 mmol, 1.0 eq.) was dissolved in dry CH_2Cl_2 (88 mL). It was stirred at 0°C under argon. After 10 min, BF_4OMe_3 (4.31 g, 29.15 mmol, 3.0 eq.) was added. The mixture was stirred in the cold room (3°C) with an argon balloon and drying tube for 16 h. The crude material was partitioned between CH_2Cl_2 and $NaHCO_3$ (aq.). It was extracted with CH_2Cl_2 (3 x 100 mL), and washed with brine. The organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (silica: 2:1 CH_2Cl_2/ether) to afford 2.8 g (79%) of lactim ether **152** as a yellow foam.

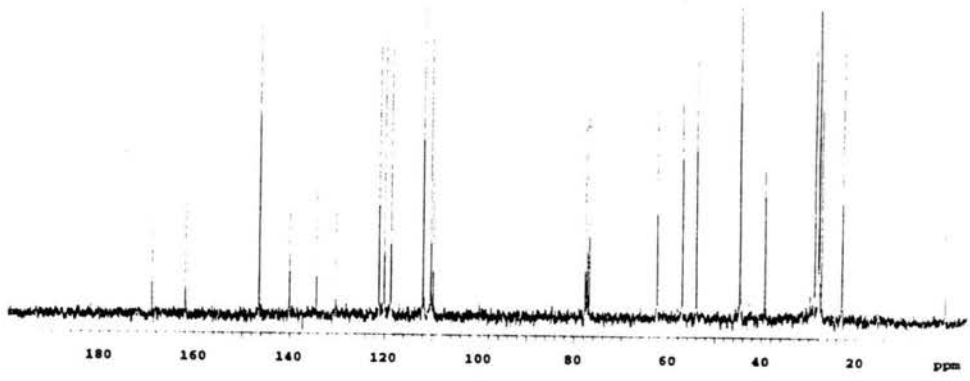
^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.96 (1 H, brs), 7.53 (1 H, dd, J = 7.3 Hz, J = 1.2 Hz), 7.19 (1 H, ddd, J = 7.2 Hz, J = 0.7 Hz, J = 1.3 Hz), 7.02 (2 H, m), 6.15 (1 H, dd, J = 17.5 Hz, J = 10.6 Hz), 5.13 (1 H, dd, J = 17.5 Hz, J = 1.0 Hz), 5.08 (1 H, dd, J = 10.6 Hz, 1.0 Hz), 4.53 (1 H, ddd, J = 5.7 Hz, J = 5.0 Hz, J = 1.6 Hz), 3.65 (3 H, s), 3.53 (1 H, m), 3.50 (1 H, dd, J = 14.7 Hz, J = 5.0 Hz), 3.33 (1 H, dd, J = 14.3 Hz, J = 6.4 Hz), 3.18 (2 H, m), 2.02 (1 H, m), 1.84 (1 H, m), 1.41-1.63 (2 H, m), 1.54 (3 H, s), 1.53 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 169.44, 161.54, 146.38, 140.40, 134.23, 129.92, 106.59, 121.29, 119.34, 118.68, 109.97, 111.55, 63.82, 56.18, 53.11, 44.33, 39.49, 29.29, 29.14, 27.83 (2 C), 21.81. IR (NaCl, neat): 3320, 3078, 3053, 2970, 1681, 1644, 1462,

1431,1322, 1257, 1027, 999, 917, 744 cm^{-1}. HRMS (FAB), calcd. for $C_{22}H_{27}N_3O_2$ (MH^+): 366.2182. Found: 366.2168.

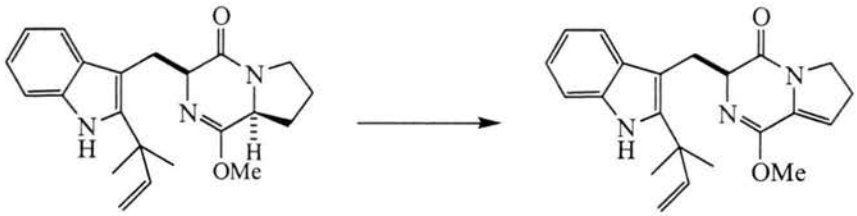


152





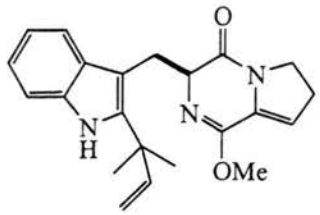
Compound **152**: 300 MHz ^{1}H NMR and 75 MHz ^{13}C NMR Spectrums in $CDCl_3$



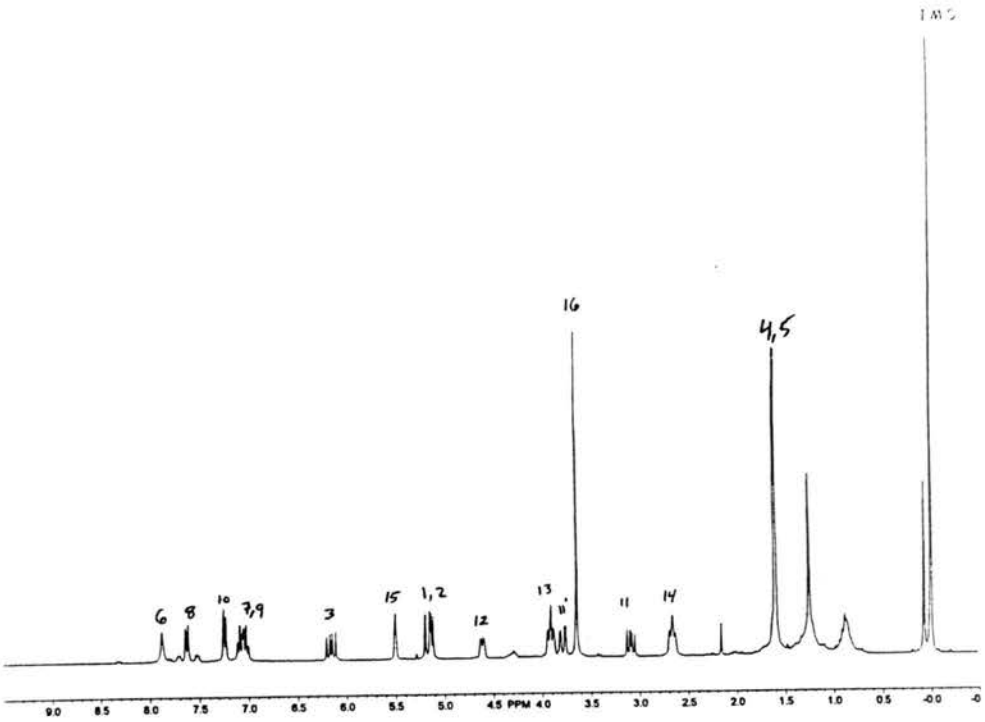
152 **153**

3-[[2-(1,1-dimethyl-2-propenyl)-1H-indol-3-yl]methyl]6,7-dihydro-1-methoxy-pyrrolo[1,2-a]pyrazine-4(3H)-one (153). Lactim ether **152** (128 mg, 0.3507 mmol) was dissolved in dry toluene (9.6 mL). The reaction was stirred under argon at -78°C. Once cooled, DDQ (95 mg, 0.4208 mmol) was dissolved in toluene (3.2 mL) and added *via* syringe to the mixture. The mixture stirred for 34 h and went from -78°C to room temperature. It was refluxed for 8 h. The crude reaction was filtered through alumina (50:1 CH_2Cl_2/MeOH). Purification by preparative TLC (25:1 CH_2Cl_2 / EtOAc) provided 22 mg (31%) of the oxidized product (**153**) as a yellow foam.

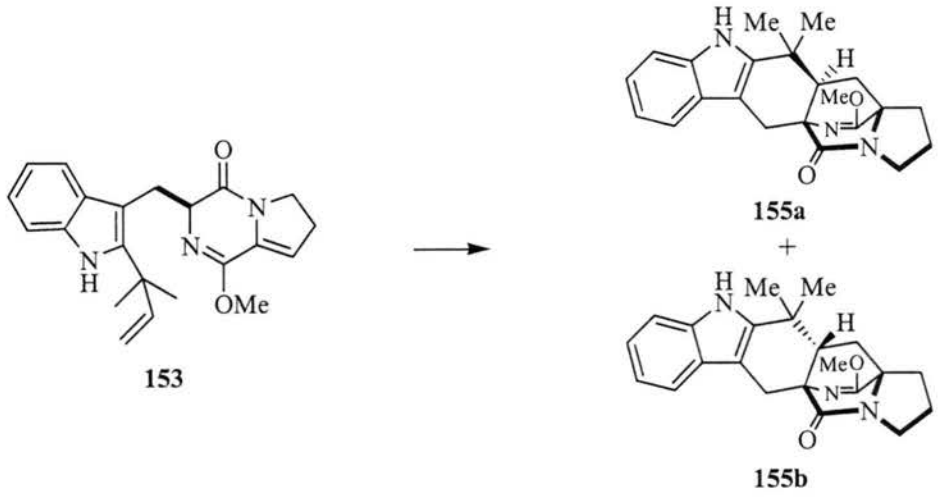
^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.85 (1 H, brs), 7.62 (1 H, d, J = 8 Hz), 7.22 (1 H, d, J = 8 Hz), 7.05 (2 H, m), 6.12 (1 H, dd, J = 17.0 Hz, J = 10.0 Hz), 5.50 (1 H, dd, J = 3.3 Hz, J = 3.3 Hz), 5.16 (1 H, dd, J = 17.0 Hz, J = 1.0 Hz), 5.12 (1 H, dd, J = 10.0 Hz, 1.0 Hz), 4.61 (1 H, br, dd, J = 3.5 Hz, J = 9.5 Hz), 3.85-3.95 (2 H, m), 3.77 (1 H, dd, J = 14.0 Hz, J = 3.5 Hz), 3.64 (3 H, s), 3.08 (1 H, dd, J = 9.5 Hz, J = 14.5 Hz), 2.63 (2 H, m), 1.59 (3 H, s), 1.58 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 172.0, 166.9, 151.3, 146.1, 140.1, 134.1, 130.3, 121.2, 119.7, 118.7, 111.9, 110.5, 110.0, 108.1, 64.5, 52.9, 44.3, 39.3, 31.4, 29.6, 27.7, 25.9;IR (NaCl, neat): 3337, 3078, 3043, 2960, 2913, 2878, 2854, 1680, 1644, 1633, 1627, 1622, 1454, 1335, 1245, 1049, 914, 744 cm^{-1}. HRMS (FAB), calcd. for $C_{22}H_{25}N_3O_2$ (MH^+): 364.2025. Found: 364.2023.



153



Compound **153**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



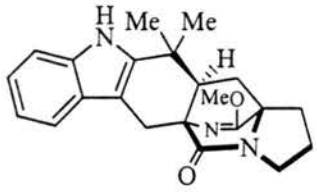
153

2,3,11,12,12a,13-hexahydro-14-methoxy-12-12-dimethyl-(5aR,12aS,13aR)-5H,6H-5a,13a-(Nitrilometheno)-1H-indolizino[7,6-b]carbazol-5-one (155a, 155b). The azadiene lactim ether **153** (22 mg, 0.0606 mmol) was dissolved in dry THF (0.6 mL). DBU (4.6 μL, 0.0303 mmol) was added to the mixture *via* syringe. It was stirred at room temperature under argon for 18 h. The crude mixture was partitioned between CH_2Cl_2 and $NaHCO_3$ (aq.). It was extracted with CH_2Cl_2 (3 x 10 mL) and washed with brine. It was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by preparative TLC (25:1 CH_2Cl_2/MeOH) yielded 6.2 mg and 8.2 mg of **155a** and **155b** respectively (combined yield of 65%).

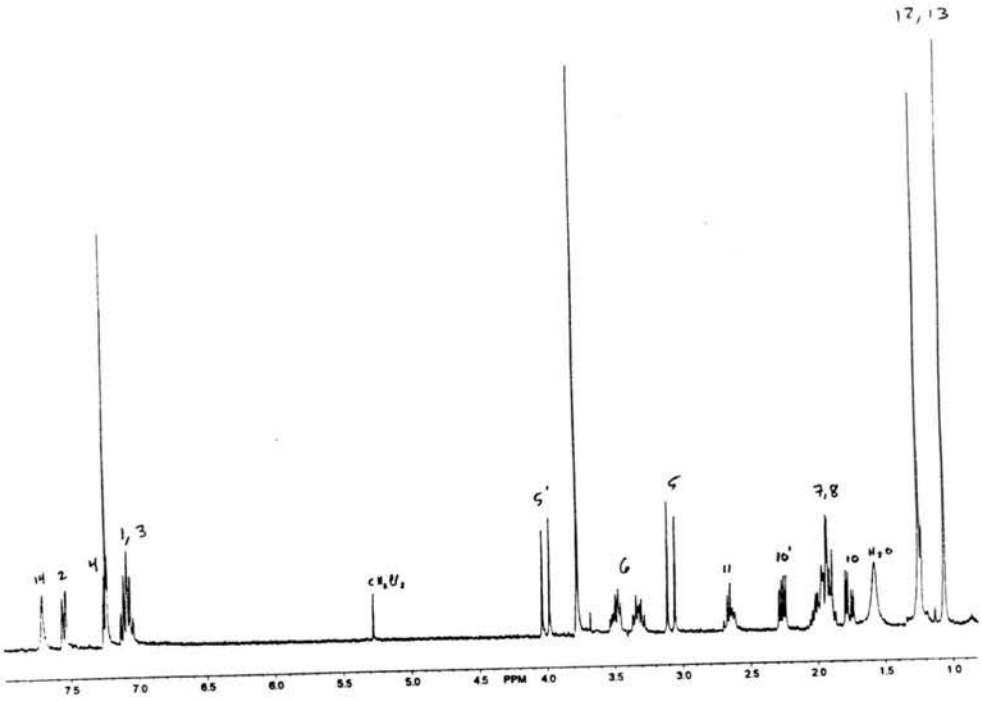
Major cycloaddition product (**155a**). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.76 (1 H, brs), 7.56 (1 H, d, J = 7.0 Hz), 7.25 (1 H, d, J = 7.0 Hz), 7.09 (2 H, m), 4.01 (1 H, d, J = 16.0 Hz), 3.79 (3 H, s), 3.50 (1 H, ddd, J = 5.5 Hz, J = 6.3 Hz, J = 11.8 Hz), 3.33 (1 H, ddd, J = 7.1 Hz, J = 7.1 Hz, J = 11.3 Hz), 3.10 (1 H, d, J = 16.0 Hz), 2.66 (1 H, m), 2.27 1 H, dd, J = 5.0, 10.2 Hz), 1.88-2.04 (4 H, m), 1.78 (1 H, dd, J = 5.0 Hz, J = 12.6 Hz), 1.26 (3 H, s), 1.07 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 172.6, 171.6, 136.4, 133.4, 127.5,

121.4, 119.1, 118.8, 110.3, 107.0, 66.3, 64.2, 54.5, 47.3, 43.4, 35.1, 32.2, 29.3, 28.6, 27.8, 24.8, 22.8. IR (NaCl, neat): 3306, 3118, 3046, 2925, 1662, 1445, 1353, 1313, 1262, 1190, 1144, 1108, 1005, 990, 742, 703 cm^{-1}. HRMS (FAB), calcd. for $C_{22}H_{25}N_3O_2$ (MH^+): 363.1947. Found: 363.1940.

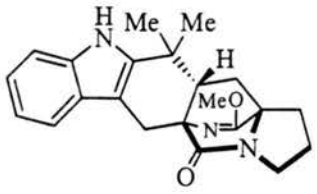
Minor cycloaddition product (**155b**). ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.69 (1 H, brs), 7.59 (1 H, d, J = 7.0, Hz), 7.27 (1 H, d, J = 7.0 Hz), 7.10 (2 H, m), 3.91 (1 H, d, J = 17.0 Hz), 3.69 (3 H, s), 3.48 (2 H, m), 3.30 (1 H, d, J = 17.0 Hz), 2.66 (1 H, m), 2.36 (1 H, dd, J = 4.0 Hz, J = 9.5 Hz), 1.88-2-08 (4 H, m), 1.82 (1 H, dd, J = 4.0 Hz, J = 13.0 Hz), 1.25 (3 H, s), 1.15 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 172.3, 170.5, 139.7, 136.3, 128.0, 121.5, 119.2, 118.9, 110.3, 106.3, 67.3, 64.2, 54.3, 45.8, 43.6, 34.9, 33.6, 29.2, 28.5, 26.1, 25.3, 24.7. IR (NaCl, neat): 3306, 3046, 2952, 1662, 1456, 1428, 1351, 1310, 1256, 1200, 1108, 1005, 990, 741, 697 cm^{-1}. HRMS (FAB), calcd. for $C_{22}H_{25}N_3O_2$ (MH^+): 363.1947. Found: 363.1942.



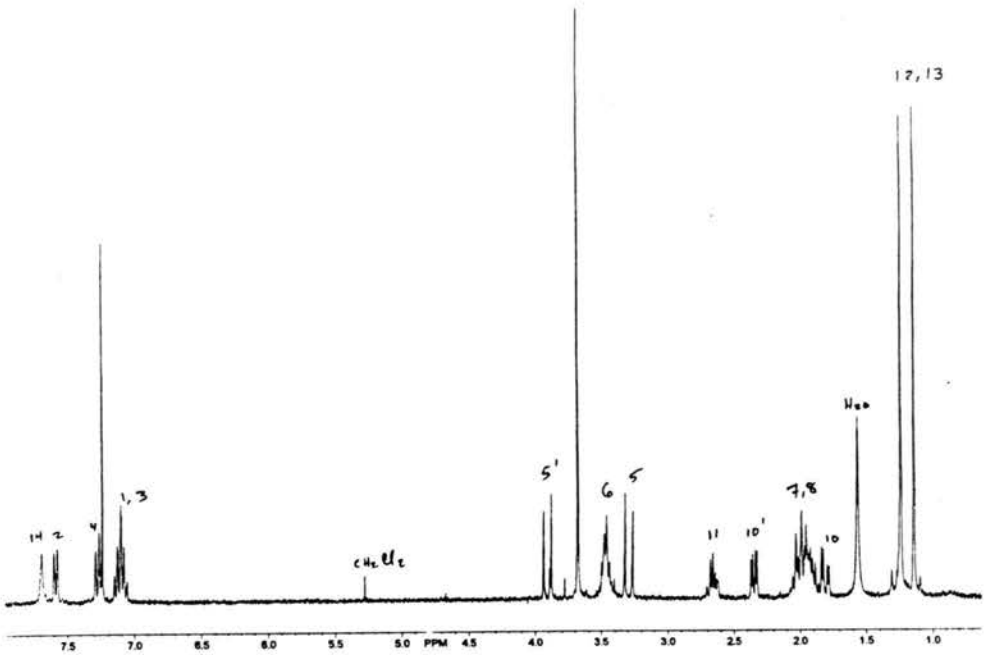
155a



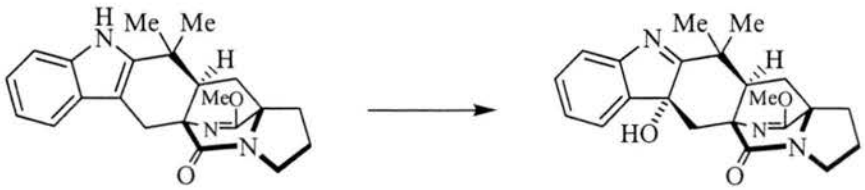
Compound **155a**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



155b



Compound **155b**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

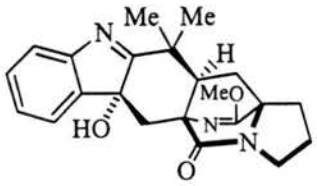


155a **156a**

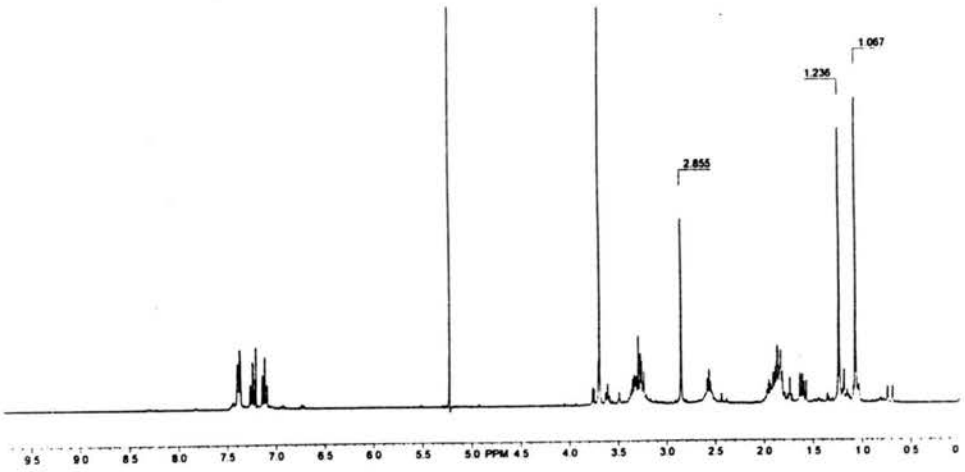
2,3,6a,12,12a,13-hexahydro-6a-hydroxy-14-methoxy-12,12-dimethyl-(5aR, 6aS, 12aS, 13aR)-5H, 6H-5a, 13a-(nitilomethane)-1H-indolizino[7,6-b]carbazol-5-ones. Indole (**155a**) (6.2 mg, 0.0176 mmol, 1.0 eq.) was dissolved in dry THF (0.85 mL). *m*-CPBA (4 mg, 0.0230 mmol, 1.3 eq.) was added and the mixture stirred at room temperature under argon. After 12 h, the crude reaction was quenched with one drop of DMS. The hydroxyindolenines **156a** and **156b** were not purified, but taken on crude.

3-Hydroxyindolenine (156a). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.48 (1 H, ddd, J = 7.0 Hz, J = 1.5 Hz, J = 0.5 Hz), 7.44 (1 H, ddd, J = 8.0 Hz, J = 1.5 Hz, J = 0.5 Hz), 7.29 (1 H, ddd, J = 8.0 Hz, J = 8.0 Hz, J = 1.5 Hz), 7.18 (1 H, ddd, J = 8.0 Hz, J = 7.0 Hz, J = 1.5 Hz), 3.66 (3 H, s), 3.2-3.5 (2 H, m), 2.60 (1 H, m), 1.99 (1 H, dd, J = 13.0 Hz), 1.93 (1 H, d, J = 13.0 Hz), 1.92-2.17 (5 H, m), 1.60 (1 H, dd, J = 7.0 Hz, J = 12.5 Hz), 1.30 (3 H, s), 1.18 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 190.68, 173.21, 170.55, 153.08, 140.44, 129.24, 126.11, 122.29, 120.22, 82.62, 65.91, 65.47, 54.25, 43.76, 43.69, 39.37, 37.66, 32.04, 28.70, 27.96, 24.63, 23.49. IR (NaCl, neat): 3222, 3055, 2948, 2872, 1682, 1668, 1652, 1636, 1581, 1456, 1418, 1359, 1206, 1108, 755, 734 cm^{-1}.

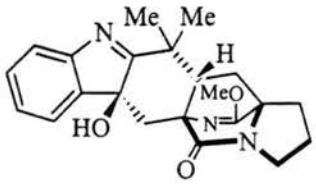
3-Hydroxyindolenine (156b). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.36-7.41 (2 H, m), 7.24 (1 H, ddd, J = 7.5 Hz, J = 7.0 Hz, J = 1.5 Hz), 7.18 (1 H, ddd, J = 7.5 Hz, J = 7.0 Hz, J = 0.5 Hz), 3.69 (3 H, s), 3.23-3.36 (2 H, m), 2.57 (1 H, m), 1.92-2.17 (5 H, m), 1.99 (1 H, dd, J = 13.0 Hz), 1.93 (1 H, d, J = 13.0 Hz), 1.60 (1 H, dd, J = 7.0 Hz, J = 12.5 Hz), 1.24 (3 H, s), 1.07 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_2$) δ: 190.81, 171.57, 170.69, 153.33, 140.52, 129.44, 126.05, 122.15, 120.42, 83.86, 65.81, 64.32, 54.62, 46.05, 43.61, 38.76, 36.14, 31.86, 29.29, 28.75, 24.66, 21.33. IR (NaCl, neat): 3324, 3052, 2950, 2876, 1668, 1652, 1634, 1580, 1428, 1361, 1337, 1264, 1227, 1195, 1180, 1145, 1115, 1088, 1007, 988, 762, 735 cm^{-1}.



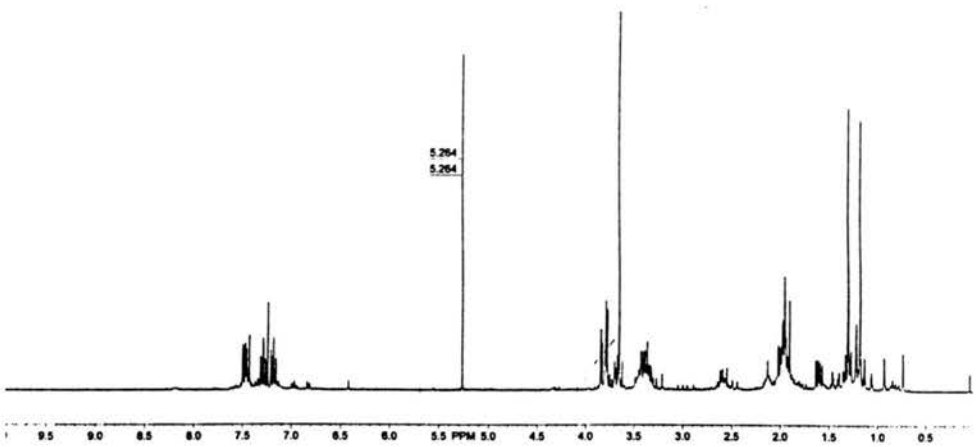
156a



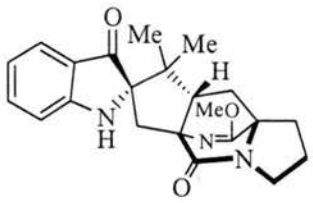
Compound **156a**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



156b

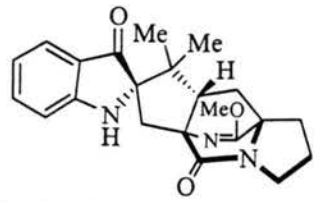


Compound **156b**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

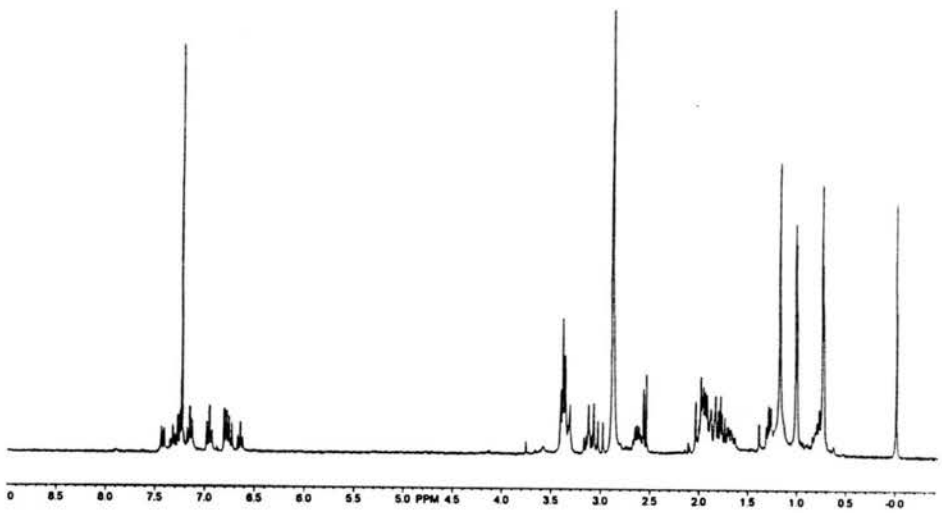


lactim ether of brevianamide B

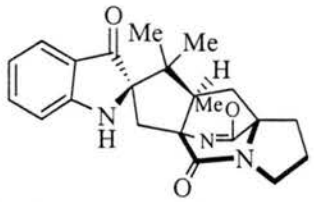
Spiro[2H-indole-2,7'(8'H)-[5H,6H-5a,9a](nitrilometheno)[1H]cyclopent[f]indolizine]-3,5'(1H)-dione,2',3',8'a,9'-tetrahydro-10'-methoxy-8',8'-dimethyl-,(5'aα,7'α,8'aα,9'aα). . ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.51 (ddd, J = 7.8, 1.2, 0.6 Hz, 1H), 7.37 (ddd, J = 8.4, 7.2, 1.5 Hz, 1H), 6.69-6.78 (m, 2H), 4.95 (brs, 1H), 3.79 (s, 3H), 3.37 (dd, J = 6.6, 6.6 Hz, 2H), 3.20 (d, J = 14.5 Hz, 1H), 3.15 (dd, J = 10.0, 6.6 Hz, 1H), 2.51-2.61 (m, 1H), 2.28 (d, J = 14.5 Hz, 1H), 1.78-2.05 (m, 4H), 1.48 (dd, J = 12.5, 7.0 Hz, 1H), 0.95 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 204.63, 171.60, 171.30, 160.21, 136.89, 124.81, 119.96, 118.14, 111.16, 78.12, 72.74, 66.93, 54.34, 51.11, 46.40, 43.17, 39.08, 30.16, 28.85, 25.00, 23.27, 21.36. IR (NaCl, neat): 3322, 3024, 2946, 2873, 1694, 1682, 1668, 1652, 1621, 1470, 1416, 1360, 1324, 1259, 1199, 990, 924, 752, 730 cm^{-1}. HRMS (FAB), calcd. For $C_{22}H_{25}N_3O_3$ (MH^+): 380.1974. Found: 380.1982.



lactim ether of brevianamide B

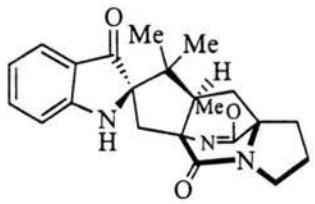


Lactim ether of brevianamide B: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

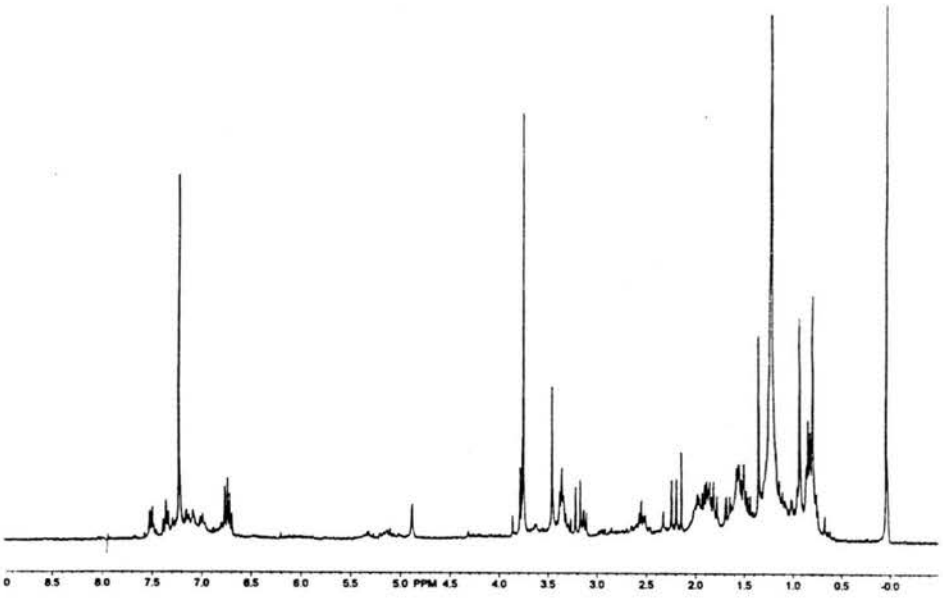


lactim ether of epibrevianamide A

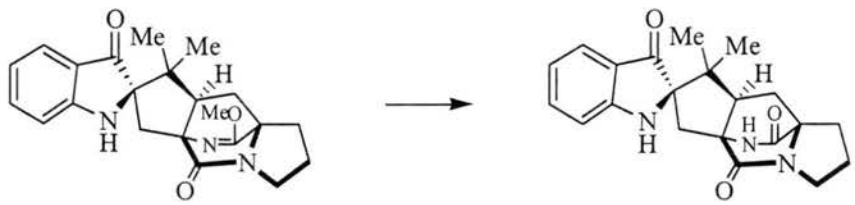
Spiro[2H-indole-2,7'(8'H)-[5H,6H-5a,9a](nitrilometheno)[1H]cyclopent[f]indolizine]-3,5'(1H)-dione,2',3',8'a,9'-tetrahydro-10'-methoxy-8',8'-dimethyl-,(5'aα,7'α,8'aα,9'aα).. ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.50 (1 H, d, J = 7.5 Hz), 7.37 (1 H, ddd, J = 8.0 Hz, J = 7.5 Hz, J = 1.0 Hz), 6.75 (1 H, d, J = 8.0 Hz), 6.70 (1 H, dd, J = 7.5 Hz, J = 7.5 Hz), 5.43 (1 H, brs), 3.66 (3 H, s), 3.28-3.48 (2 H, m), 2.91 (1 H, d, J = 14.0 Hz), 2.61 (1 H, dd, J = 11.5 Hz, J = 6.0 Hz), 2.57 (1 H, dd, J = 10.0 Hz, J = 7.0 Hz), 2.49 (1 H, d, J = 14.0 Hz), 1.82-2.09 (3 H, m), 1.81 (1 H, dd, J = 12.5 Hz, J = 10.0 Hz), 1.54 (1 H, dd, J = 12.5 Hz, J = 7.0 Hz), 0.87 (3 H, s), 0.86 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 204.88, 172.18, 172.05, 160.59, 137.04, 124.62, 119.47, 117.78, 111.32, 77.15, 72.91, 66.06, 54.71, 54.45, 44.93, 43.16, 39.67, 31.19, 29.33, 24.98, 24.92, 20.97.IR (NaCl, neat): 3353, 3030, 2942, 2871, 1694, 1660, 1613, 1488, 1470, 1428, 1320, 1295, 1179, 920, 768 cm^{-1}. HRMS (FAB), calcd for $C_{22}H_{25}N_3O_3$ (MH^+): 380.1974. Found: 380.1980.



lactim ether of epibrevianamide A

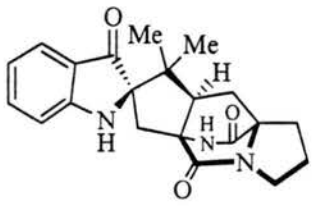


Lactim ether of epibrevianamide A: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

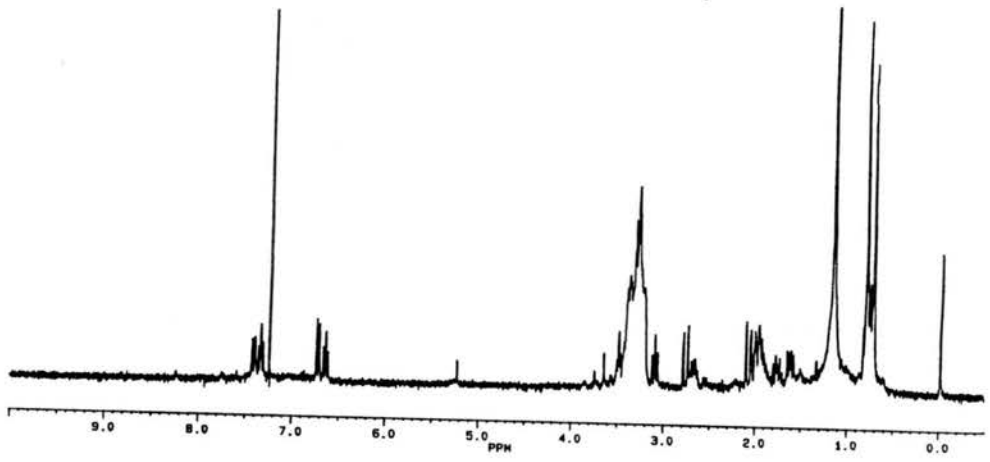


d,l-epibrevianamide A, **157**

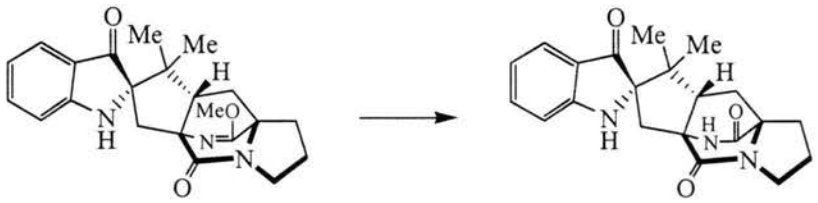
***d,l*-19-Epibrevianamide A.** ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.50 (1 H, d, J = 7.5 Hz), 7.37 (1 H, ddd, J = 8.0 Hz, J = 7.5 Hz, J = 1.0 Hz), 6.75 (1 H, d, J = 8.0 Hz), 6.63 (1 H, dd, J = 8.0 Hz, J = 7.5 Hz), 3.30-3.50 (2 H, m), 3.09 (1 H, dd, J = 10.0 Hz, J = 8.0 Hz), 2.76 (1 H, d, J = 14.5 Hz), 2.67 (1 H, ddd, J = 12.5 Hz, J = 7.0 Hz, J = 5.2 Hz), 2.08 (1 H, d, J = 14.5 Hz), 2.00 (1 H, dd, J = 14.0 Hz, J = 10.0 Hz), 1.85-2.00 (2 H, m), 1.77 (1 H, dd, J = 13.0 Hz, J = 8.0 Hz), 1.54 (1 H, dd, J = 13.0 Hz, J = 8.0 Hz), 0.81 (3 H, s), 0.73 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 206.10, 173.95, 169.92, 161.22, 137.69, 124.50, 118.53, 117.92, 111.27, 76.57, 68.83, 65.27, 55.80, 45.28, 43.86, 34.85, 30.42, 29.53, 24.58, 23.02, 19.18. IR (NaCl, neat): 3282, 3077, 2925, 2851, 1682, 1615, 1467, 1395, 1323, 1251, 1189, 1149, 1097, 913, 754, 728 cm^{-1}. HRMS (FAB), calcd for $C_{21}H_{23}N_3O_3$ (MH^+): 366.1818. Found: 366.1818.



d,l-epibrevianamide A, **157**



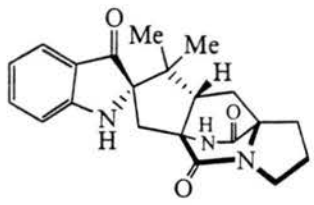
Compound **157**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$



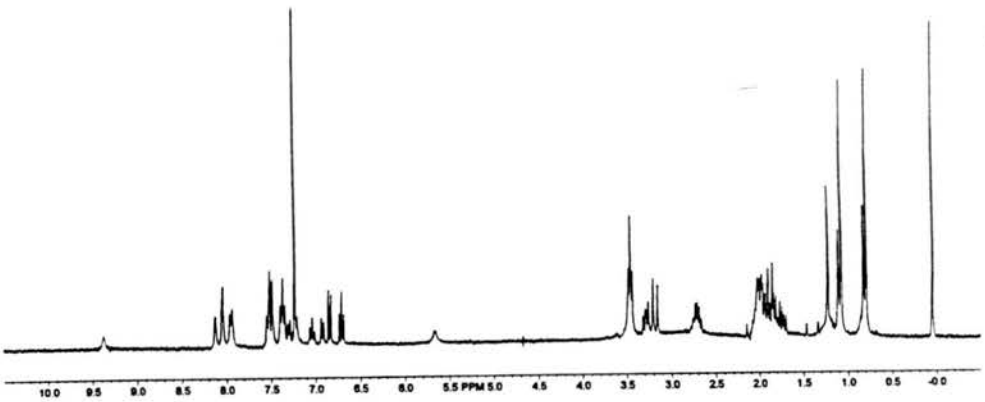
brevianamide B (**2**)

d,l-Brevianamide B (2). The residues (6 mg, and 8 mg, respectively) were each dissolved in 1M NaOMe / MeOH (3.8 mL), refluxed for 1 h and cooled to room temperature. 2.5 mL 3N HCl were added and stirred for 30 min. Most of the MeOH was removed *in vacuo*. The crude reaction was poured into water, extracted with CH_2Cl_2 (3 x 5 mL) dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to furnish **157**and ***d,l-2***.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.67 (1 H, brs), 7.50 (1 H, d, J = 7.5 Hz), 7.37 (1 H, br dd, J = 8.0 Hz, J = 7.5 Hz), 6.75 (1 H, d, J = 8.0 Hz), 6.63 (1 H, dd, J = 7.5 Hz, J = 7.5 Hz), 3.37-3.47 (2 H, m), 3.20 (1 H, dd, J = 10.0 Hz, J = 8.0 Hz), 3.09 (1 H, d, J = 15.0 Hz), 3.09 (1 H, d, J = 15.0 Hz), 2.67 (1 H, ddd, J = 12.3 Hz, J = 6.5 Hz, J = 6.5 Hz), 1.90-2.02 (3 H, m), 1.82 (1 H, dd, J = 13.0 Hz, J = 7.0 Hz), 1.72 (1 H, dd, J = 13.0 Hz, J = 8.0 Hz), 0.81 (3 H, s), 0.73 (3 H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: 205.72, 173.42, 169.92, 161.12, 137.22, 123.89, 118.20, 117.39, 110.77, 77.29, 68.57, 65.87, 49.33, 45.94, 43.30, 33.64, 29.06, 28.29, 24.24, 21.50, 19.11. IR (NaCl, neat): 3279, 3070, 2927, 1683, 1616, 1490, 1466, 1389, 1324, 1304, 1258, 1139, 1119, 1098, 1031, 980, 924, 751, 694 cm^{-1}. HRMS (FAB), calcd for $C_{21}H_{23}N_3O_3$ (MH^+): 366.1818. Found: 366.1820.



brevianamide B (**2**)



Compound **2**: 300 MHz ^{1}H NMR Spectrum in $CDCl_3$

Appendix 1

Publications

1. *Biomimetic Diels-alder Cyclization for the Construction of the Brevianamide, Paraherquamide, Sclerotamide, Asperparaline and VM55599 Ring Systems* Williams, R. M.; Sanz-Cervera, J. F.; Sancenón, F.; Marco, J. A.; Halligan, K. M. *Bioorganic and Medicinal Chemistry* **1998**, *6*, 1233-1241.
2. *Biomimetic Diels-alder Cyclization for the Construction of the Brevianamide, Paraherquamide, Sclerotamide, Asperparaline and VM55599 Ring Systems* Williams, R. M.; Sanz-Cervera, J. F.; Sancenón, F.; Marco, J. A.; Halligan, K. M. *J. Am. Chem. Soc.* **1998**, *120*, 1090-1091.

Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1233–1241

BIOORGANIC & MEDICINAL CHEMISTRY

Biomimetic Diels–Alder Cyclizations for the Construction of the Brevianamide, Paraherquamide, Sclerotamide, Asperparaline and VM55599 Ring Systems

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Abstract—A potentially bio-mimetic Diels–Alder cyclization to construct the bicyclo[2.2.2] ring system common to the paraherquamides, marcfortines, sclerotamides, brevianamides, VM55599, and asperparaline is reported. *Epi*-deoxybrevianamide E (**22**) is converted into the corresponding lactim ether (**23**) and then oxidized with DDQ to provide an azadiene (**24**) which is tautomerized in the presence of base to azadiene **25** which, spontaneously cyclizes to give a 2:1 mixture of cycloadducts **26** and **27**. These cycloadducts are each in turn, converted into D,L-C-19-*epi*-brevianamide A (**20**) and D,L-brevianamide B (**6**). The stereochemical implications of the [4 + 2] cycloaddition is discussed in the context of a working hypothesis on the biosynthesis of this family, particularly VM55599.

Introduction

The paraherquamides (**1**, **2**),[1] marcfortines (**3**),[2] sclerotamides (**4**),[3] brevianamides (**5**, **6**),[4] VM55599 (**7**),[5] and most recently, asperparaline (aspergillimide, **8**)[6] are indolic secondary mold metabolites isolated from various fungi (Fig. 1). This family has attracted considerable attention due to their molecular complexity, intriguing biogenesis[7] and some members, most notably the paraherquamides, display potent anti-parasitic activity.[8] These substances are the result of a mixed biogenesis, being derived from isoprene units oxidatively woven into an ral metabolite). Significantly, the enantiomorphic bicyclo[2.2.2] ring system that has been proposed to arise via the [4 + 2] cycloaddition of the isoprene moiety across the oxidized α-carbons of the amino acid units.[9] A striking stereochemical difference between the brevianamides and all of the other members of this family, is the relative stereochemical relationship at the tertiary carbon at C-19 (brevianamide numbering) which is *anti*-[10] in the brevianamides and *syn*- for all of the others.

Previous work on the biosynthesis of these substances, invoked a facial divergence in the putative Diels–Alder cyclization which sets the relative stereochemical relationship at this stereogenic center.[11] In addition, the brevianamides are the result of oxidation at the indolic 3-position while, the paraherquamides, marcfortines and sclerotamides are the consequence of indole oxidation at the 2-position (indole numbering).

One of the original proposals on the biosynthesis of the brevianamides, postulated the conversion of brevianamide F (**9**) into deoxybrevianamide E (**10**), followed by a 2-electron oxidation and enolization to afford the azadiene **11** (Fig. 2).[7d] Intramolecular Diels–Alder cycloaddition would then furnish *racemic* **12** which, was proposed to be converted into the two optically pure diastereomers **13** and **14** by a pro-(*R*)-selective indole oxidase. Final spiro-rearrangement culminates in brevianamide A (**5**, the major natural metabolite) and brevianamide B (**6**, the minor natural metabolite). Significantly, the enantiomorphic bicyclo[2.2.2] ring systems extant in **5** and **6** were, in principle, accommodated by this scheme. However, subsequent total synthesis of

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0968-0896/98/$—see front matter
PII: S0968-0896(98)00102-3

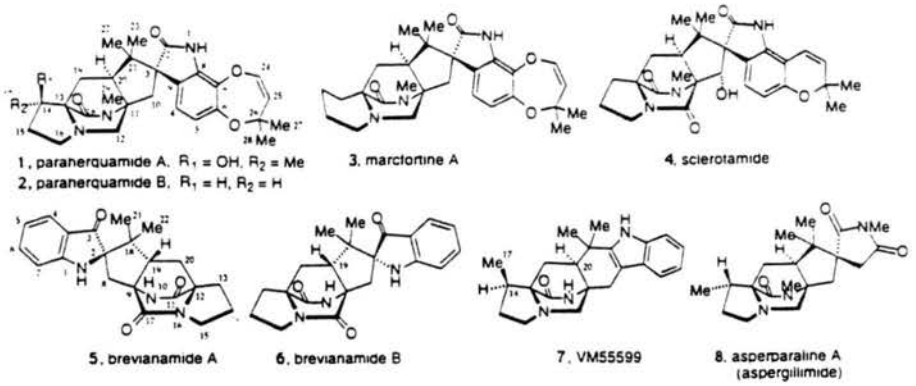

Figure 1.

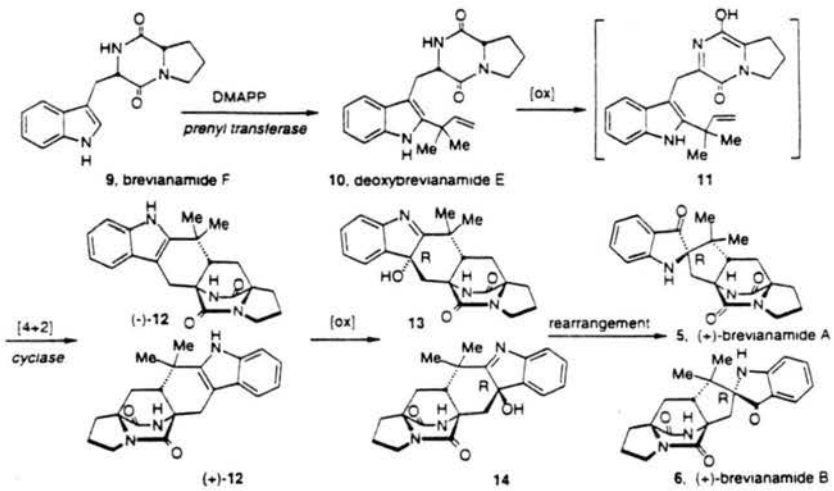

Figure 2. Early biosynthetic proposal for brevianamides A and B.

^{13}C-labeled, racemic **12** and feeding experiments with the producing organism *Penicillium brevicompactum*, failed to provide experimental evidence for the intermediacy of and/or the production of structures **12** in *Penicillium brevicompactum*.[7d]

Subsequent work from this laboratory, based primarily on the metabolite co-occurence of brevianamide E (**16**), gave rise to an alternate biogenetic proposal shown in Fig. 3.[7c,7d] Tritium-labeled deoxybrevianamide E (**10**) was synthesized and found to incorporate efficiently into brevianamide A (**5**), brevianamide B (**6**) and brevianamide E (**16**) in *Penicillium brevicompactum*.[7d] When tritium-labeled brevianamide E (**16**) was fed to *Penicillium brevicompactum*, no incorporation was observed into either **5** or **6** indicating that, the conversion of **10** into **16** is an irreversible, dead-end shunt pathway. Thus, our working hypothesis envisions an (*R*)-selective indole oxidase that converts **10** into the hydroxyindolenine **15** which can suffer either of two fates: (1) irreversible nucleophilic ring closure to **16** or; (2) spiro-rearrangement to the (*R*)-indoxyl **17**. Subsequent 2-electron oxidation and enolization provides azadiene **19** that can suffer intramolecular [4+2] cycloaddition to directly furnish brevianamide A (**5**) and brevianamide B (**6**). Whether this reaction occurs spontaneously or involves the agency of the enzyme active site to organize and select from the four possible diastereomeric transition states, is not known and constitutes the focus of our program.

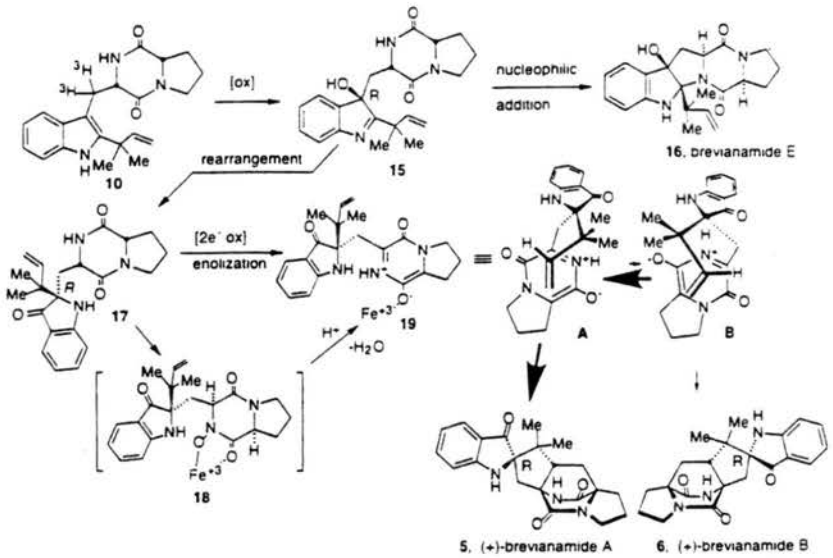

Figure 3.

Recent theoretical work on an indoxyl-based Diels–Alder cyclization pathway, supported the observed isomer production of the brevianamides in *Penicillium brevicompactum* which produces brevianamide A as the major metabolite and brevianamide B as the minor metabolite (Fig. 4).[12] Of interest in the biosynthetic process, is the fact that cycloaddition appears to occur primarily from transition state/conformer **A**, with a small amount occurring from transition state/conformer **B** with no detectable metabolite production (of **20**/**21**) from either transition state/conformer **A′** and/or transition state/conformer **B′**.

The relatively recent isolation of VM55599 (**7**) forces somewhat of a resurrection of the original biosynthetic [4 + 2] cycloaddition pathway (Fig. 2) that we have already probed in the brevianamide pathway via substance **12**. In particular, the existence of **7** begs the question of the timing of the indole and amino acid oxidation reactions in each biosynthetic system.

Our laboratory has been concerned with elucidating the biosynthetic mechanism of formation of the unique bicyclo[2.2.2] ring system, particularly with respect to the question of possible enzymatic catalysis of this reaction. In this paper, we provide a full account of a possibly biomimetic intramolecular Diels–Alder cyclization reaction that constructs the core framework of this class of fungal metabolites. The stereochemical implications of our findings bear on the possible biosynthetic construction of this ring system.

Results and Discussion

The starting material for this investigation, 9-*epi*-deoxybrevianamide E (**22**, Fig. 5) was synthesized according to a slightly modified procedure originally reported by Kametani.[13] Conversion of this substance to the lactim ether (**23**) was accomplished with Me_3OBF_4 in CH_2Cl_2 (79% yield). Next, oxidation of **23** with DDQ gave the unsaturated substance **24** in moderate yield (24–40%). Treatment of **24** with aqueous methanolic KOH at room temperature, cleanly produced the labile azadiene **25** which could be isolated by silica gel chromatography and characterized spectroscopically. Upon standing however, **25** spontaneously cyclized to give a mixture of the cycloadducts **26** and **27** (2:1, 90% combined yield). The structures of the cyclization products were secured through analysis of their respective NMR spectra as well as by chemical correlation to known substances as described below. Alternatively, azadiene **24** could be tautomerized and cyclized to **26** (37%) and **27** (28%) by treatment with DBU in THF at room temperature.

Conversion of **26** to C-19-*epi*-brevianamide A (**20**), a non-natural isomer of brevianamide A previously synthesized in this laboratory,[14] was accomplished by diastereoselective *m*-CPBA oxidation to the corresponding hydroxyindolenine (**28**, ~quant.) and pinacol-type rearrangement (0.5 M NaOH, MeOH, rt, reflux) to the corresponding *spiro*-indoxyl; subsequent removal of the lactim ether with HCl afforded D,L-**20** in 70% overall yield from **26**.

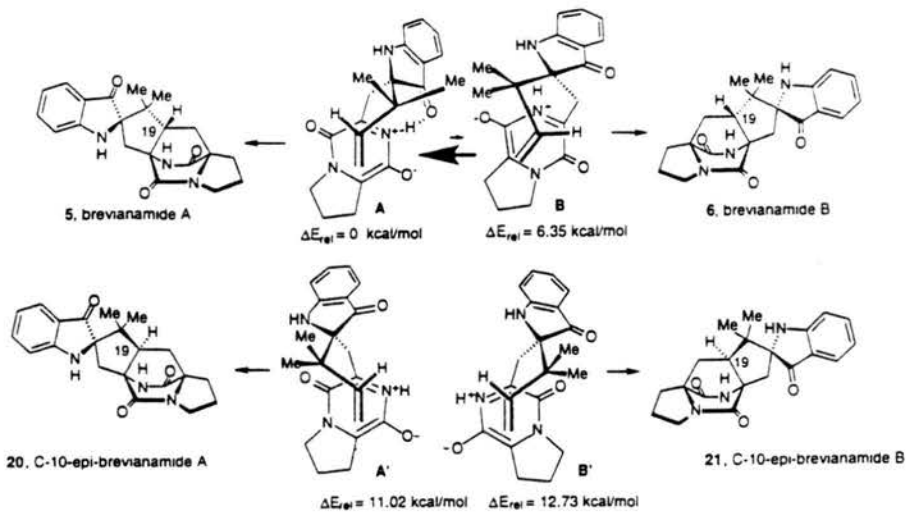

Figure 4. Calculated relative energies for transition state structures for intramolecular [4 + 2] cycloadditions.[12]

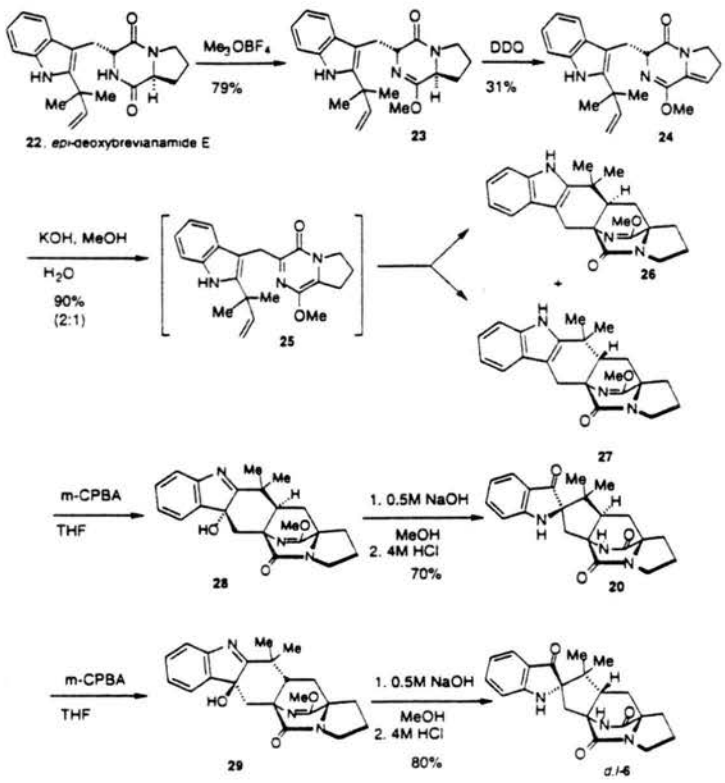

Figure 5.

Initially, the labile ring-opened aminoester (**30**, Fig. 6) was produced from this sequence but could be cyclized in hot toluene containing 2-hydroxypyridine furnishing D,L-**20**. Careful control of the reaction time and temperature obviated the formation of **30**. Curiously, we have never observed a related ring-opened amino ester derived from **29** (see below). The C-19-*epi*-brevianamide A (**20**), was identical to the authentic material[14] in every respect (except for being racemic).

Conversion of the minor cycloaddition product **27** to D,L-brevianamide B (**6**) was accomplished in like manner in 80% overall yield from **27** securing the relative stereochemistry of each respective cycloadduct **26** and **27**. This represents an alternative (albeit racemic) total synthesis of brevianamide B (six steps from **22**). In addition, the construction of **26** constitutes a model study for the synthesis of VM55599.

A significant implication of these observations concerns the biogenesis and stereochemistry of the related metabolite VM55599 (**7**) isolated from the paraherquamide-producing mold *Penicillium* sp. IMI332995. Since Paraherquamide A and VM55599 both possess the bicyclo[2.2.2] monoketopiperazine ring system, it seems plausible that these substances arise via a related or, more provocatively, a common [4 + 2] cycloaddition intermediate. The relative stereochemistry of VM55599 was originally assigned by extensive ^{1}H NMR NOE studies where the methyl group in the β-methylproline moiety was assigned as being *syn*- to the bridging isoprene unit.[5] Thus, if a similar Diels–Alder cyclization, whether it be un-catalyzed or enzyme-catalyzed, is operating in the biosynthetic construction of these metabolites, the isoprene unit must approach the azadiene from the same face as the methyl group in the proline ring (**31b**, Fig. 7), whereas in paraherquamide A, which has been shown by this laboratory[15] to be derived from L-isoleucine, Diels–Alder cyclization must occur from the face *opposite* to the methyl group (**31a**).

The absolute stereochemical implications of the *relationship* of VM55599 to the biosynthesis of the paraherquamides has been previously discussed[15] and is an issue that is currently under study. It is important to note that, the absolute stereochemistry of VM55599 has not been determined and constitutes an important, missing piece of this puzzle. Efforts are underway to determine the intrinsic facial bias of related Diels–Alder cyclizations on β-methylproline-containing substrates (i.e., **31**) to address these and related issues.

Conclusion

This study provides experimental validity to the hypothesis that, the core bicyclo[2.2.2] ring system

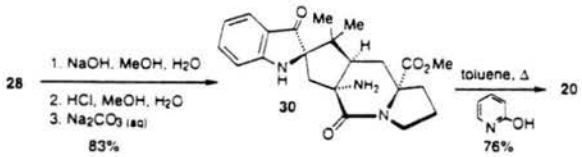

Figure 6.

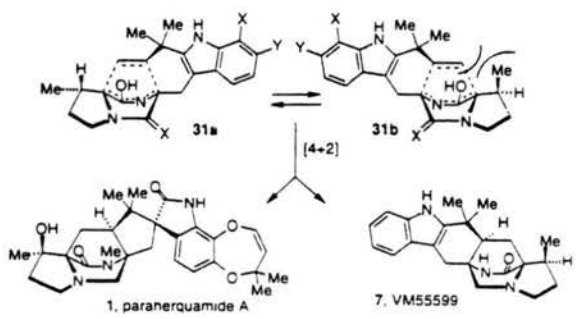

Figure 7.

common to this family of secondary metabolites very likely arises by an intramolecular Diels–Alder cyclization from a pre-formed dioxopiperazine[16] that subsequently undergoes oxidation to an azadiene species. The diastereofacial bias of the Diels–Alder cyclization of **25** is significant in that, the diastereoselectivity is not strongly affected by solvent. A comparable ratio of **26**:**27** was observed in THF as in aqueous methanol (~1.3–2:1).[17] We have also shown that, C-19- *epi*-metabolites (corresponding to **26** and **20**) are not produced by *Penicillium brevicompactum* and there have been no reports on the isolation of similarly epimeric metabolites from paraherquamide-, sclerotamide- or asperparaline-producing organisms. *Thus, in each biosynthetic system, there appears to be complete facial exclusivity with respect to the isoprene unit in the construction of the bicyclo[2.2.2] ring nucleus.* Since the laboratory cycloaddition reported here does not show a strong bias for either the *syn*- or the *anti*-relationship, it would appear plausible that there is some organization of the transition state structures in the putative biosynthetic cycloadditions. Uncertainties as to the oxidation state of the indole moiety as being either oxindole (for the paraherquamides, marcfortine and sclerotamide), or indoxyl (for the brevianamides) as opposed to the non-oxidized indole (for VM55599) at the [4 + 2] cyclization phase as well as the question of possible protein organization of the transition state structures still exists and are the subject of ongoing investigations in these laboratories.

Experimental

Lactim ether of deoxybrevianamide E (23)

A solution of *epi*-deoxybrevianamide E (**22**) (3.41 g, 9.71 mmol) in dry CH_2Cl_2 (88 mL) was stirred at 0 °C under argon. After 10 min, BF_4OMe_3 (4.31 g, 29.15 mmol) was added. The mixture was stirred in the cold room (3 °C) with an argon balloon and drying tube for 16 h. The crude material was partitioned between CH_2Cl_2 and $NaHCO_3$ (aq). It was extracted with CH_2Cl_2 (3×100 mL) and washed with brine. The organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (silica, 2:1 CH_2Cl_2/ether) to afford 2.8 g (79%) of lactim ether (**23**) as a yellow foam. IR (neat, cm^{-1}) 3320, 3078, 3053, 2970, 1681, 1644, 1462, 1431, 1322, 1257, 1027, 999, 917, 744; ^1H NMR (300 MHz, $CDCl_3$) δ 7.96 (brs, 1H), 7.53 (dd, J = 7.3, 1.2 Hz, 1H), 7.19 (ddd, J = 7.2, 0.7, 1.3 Hz, 1H), 7.02 (m, 2H), 6.15 (dd, J = 17.5, 10.6 Hz, 1H), 5.13 (dd, J = 17.5, 1.0 Hz, 1H), 5.08 (dd, J = 10.6, 1.0 Hz, 1H), 4.53 (ddd, J = 5.7, 5.0, 1.6 Hz, 1H), 3.65 (s, 3H), 3.53 (m, 1H), 3.50 (dd, J = 14.7, 5.0 Hz, 1H), 3.33 (dd, J = 14.3, 6.4 Hz, 1H), 3.18 (m, 2H), 2.02 (m, 1H), 1.84 (m, 1H), 1.41–1.63 (m, 2H), 1.54 (s, 3H), 1.53 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) (169.44, 161.54, 146.38, 140.40, 134.23, 129.92, 106.59, 121.29, 119.34, 118.68, 109.97, 111.55, 63.82, 56.18, 53.11, 44.33, 39.49, 29.29, 29.14, 27.83 (2 C), 21.81; MS m/z (M^+) calcd 366.2182, obsd 366.2168.

Azadiene (24)

Lactim ether (**23**) (128 mg, 0.3507 mmol) was dissolved in dry toluene (9.6 mL) and stirred under argon at −78 °C. Once cooled, DDQ (95 mg, 0.4208 mmol) was dissolved in toluene (3.2 mL) and added via syringe to the mixture. The mixture stirred for 34 h and was allowed to warm from −78 °C to room temperature. The mixture was then brought to reflux temperature for 8 h. The crude reaction was filtered through alumina (CH_2Cl_2/MeOH, 50:1). Purification by preparative TLC (CH_2Cl_2/EtOAc, 25:1) provided 22 mg (31%) of the oxidized product (**24**) as a yellow foam. IR (neat, cm^{-1}) 3337, 3078, 3043, 2960, 2913, 2878, 2854, 1680, 1644, 1633, 1627, 1622, 1454, 1335, 1245, 1049, 914, 744; ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (brs, 1H), 7.62 (d, J = 8 Hz, 1H), 7.22 (d, J = 8 Hz, 1H), 7.05 (m, 2H), 6.12 (dd, J = 17.0, 10.0 Hz, 1H), 5.50 (dd, J = 3.3, 3.3 Hz, 1H), 5.16 (dd, J = 17.0, 1.0 Hz, 1H), 5.12 (dd, J = 10.0, 1.0 Hz, 1H), 4.61 (br dd, J = 3.5, 9.5 Hz, 1H), 3.85–3.95 (m, 2H), 3.77 (dd, J = 14.0, 3.5 Hz, 1H), 3.64 (s, 3H), 3.08 (dd, J = 9.5, 14.5 Hz, 1H), 2.63 (m, 2H), 1.59 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 166.9, 151.3, 146.1, 140.1, 134.1, 130.3, 121.2, 119.7, 118.7, 111.9, 110.5, 110.0, 108.1, 64.5, 52.9, 44.3, 39.3, 31.4, 29.6, 27.7, 25.9; MS m/z (M^+) calcd 364.2025, obsd 364.2023.

Cycloaddition of 24 (formation of 26 and 27) with DBU

To a stirred solution of azadiene (**24**) (22 mg, 0.0606 mmol) in dry THF (0.6 mL) was added DBU (4.6 mL, 0.0303 mmol) via syringe. The mixture was stirred at room temperature under argon for 18 h. The crude mixture was partitioned between CH_2Cl_2 and $NaHCO_3$ (aq), extracted with CH_2Cl_2 (3×10 mL) and washed with brine. The organic extracts were combined and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by preparative TLC (silica gel, CH_2Cl_2/MeOH, 25:1) afforded **26** (8.2 mg, 37%) and **27** (6.2 mg, 28%) as yellow foams.

Cycloaddition of 24 (formation of 26 and 27) with KOH

Azadiene (**24**) (10 mg, 0.0275 mmol) was stirred in MeOH (2 mL) and 20% KOH (aq) (0.5 mL) under argon at 0 °C in an ice bath. The ice bath was allowed to warm to room temperature and after 4 h, the pH of the crude mixture was adjusted to 7 with phosphate buffer

The mixture was extracted with CH_2Cl_2 (3×10 mL) and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by preparative TLC (silica gel, CH_2Cl_2/MeOH, 25:1) afforded **26** (6.0 mg, 60%) and **27** (3.0 mg, 30%) as yellow foams.

Major cycloaddition product (26)

IR (neat, cm^{-1}) 3306, 3118, 3046, 2925, 1662, 1445, 1353, 1313, 1262, 1190, 1144, 1108, 1005, 990, 742, 703; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (brs, 1H), 7.56 (d, J = 7.0 Hz, 1H), 7.25 (d, J = 7.0 Hz, 1H), 7.09 (m, 2H), 4.01 (d, J = 16.0 Hz, 1H), 3.79 (s, 3H), 3.50 (ddd, J = 5.5, 6.3, 11.8 Hz, 1H), 3.33 (ddd, J = 7.1, 7.1, 11.3 Hz, 1H), 3.10 (d, J = 16.0 Hz, 1H), 2.66 (m, 1H), 2.27 (dd, J = 5.0, 10.2 Hz, 1H), 1.88–2.04 (m, 4H), 1.78 (dd, J = 5.0, 12.6 Hz, 1H), 1.26 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 171.6, 136.4, 133.4, 127.5, 121.4, 119.1, 118.8, 110.3, 107.0, 66.3, 64.2, 54.5, 47.3, 43.4, 35.1, 32.2, 29.3, 28.6, 27.8, 24.8, 22.8; MS m/z (M^-) calcd 363.1947, obsd 363.1940.

Minor cycloaddition product (27)

IR (neat, cm^{-1}) 3306, 3046, 2952, 1662, 1456, 1428, 1351, 1310, 1256, 1200, 1108, 1005, 990, 741, 697; ^1H NMR (300 MHz, $CDCl_3$) δ 7.69 (brs, 1H), 7.59 (d, J = 7.0, Hz, 1H), 7.27 (d, J = 7.0 Hz, 1H), 7.10 (m, 2H), 3.91 (d, J = 17.0 Hz, 1H), 3.69 (s, 3H), 3.48 (m, 2H), 3.30 (d, J = 17.0 Hz, 1H), 2.66 (m, 1H), 2.36 (dd, J = 4.0, 9.5 Hz, 1H), 1.88–2–08 (m, 4H), 1.82 (dd, J = 4.0, 13.0 Hz, 1H), 1.25 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) ppm 172.3, 170.5, 139.7, 136.3, 128.0, 121.5, 119.2, 118.9, 110.3, 106.3, 67.3, 64.2, 54.3, 45.8, 43.6, 34.9, 33.6, 29.2, 28.5, 26.1, 25.3, 24.7; MS m/z (M^-) calcd 363.1947, obsd 363.1942.

3-Hydroxyindolenine (28)

Compound (**26**) (38 mg, 0.1079 mmol) was dissolved in dry THF (5 mL). *m*-CPBA (24.5 mg, 0.14 mmol) was added and the mixture was stirred at room temperature under argon. After 12 h, the crude reaction was quenched with one drop of DMS. The hydroxyindolenine **28** was not purified, but taken on crude for the next step. IR (neat, cm^{-1}) 3222, 3055, 2948, 2872, 1682, 1668, 1652, 1636, 1581, 1456, 1418, 1359, 1206, 1108, 755, 734; ^1H NMR (300 MHz, $CDCl_3$) δ 7.48 (ddd, J = 7.0, 1.5, 0.5 Hz, 1H), 7.44 (ddd, J = 8.0, 1.5, 0.5 Hz, 1H), 7.29 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H), 7.18 (ddd, J = 8.0, 7.0, 1.5 Hz, 1H), 3.66 (s, 3H), 3.2–3.5 (m, 2H), 2.60 (m, 1H), 1.99 (dd, J = 13.0 Hz, 1H), 1.93 (d, J = 13.0 Hz, 1H), 1.92–2.17 (m, 5H), 1.60 (dd, J = 7.0, 12.5 Hz, 1H), 1.30 (s, 3H), 1.18 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.68, 173.21, 170.55, 153.08, 140.44, 129.24, 126.11, 122.29, 120.22, 82.62, 65.91, 65.47, 54.25, 43.76, 43.69, 39.37, 37.66, 32.04, 28.70, 27.96, 24.63, 23.49.

3-Hydroxyindolenine (29)

Indole (**27**) (20 mg, 0.0568 mmol) was dissolved in dry THF (2.7 mL). *m*-CPBA (12.9 mg, 0.0742 mmol) was added and the mixture was stirred at room temperature under argon. After 12 h, the crude reaction was quenched with one drop of DMS. The hydroxyindolenine **29** was not purified, but taken on crude for the next step. IR (neat, cm^{-1}) 3324, 3052, 2950, 2876, 1668, 1652, 1634, 1580, 1428, 1361, 1337, 1264, 1227, 1195, 1180, 1145, 1115, 1088, 1007, 988, 762, 735; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36–7.41 (m, 2H), 7.24 (ddd, J = 7.5, 7.0, 1.5 Hz, 1H), 7.18 (ddd, J = 7.5, 7.0, 0.5 Hz, 1H), 3.69 (s, 3H), 3.23–3.36 (m, 2H), 2.57 (m, 1H), 1.92–2.17 (m, 5H), 1.99 (dd, J = 13.0 Hz, 1H), 1.93 (d, J = 13.0 Hz, 1H), 1.60 (dd, J = 7.0, 12.5 Hz, 1H), 1.24 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.81, 171.57, 170.69, 153.33, 140.52, 129.44, 126.05, 122.15, 120.42, 83.86, 65.81, 64.32, 54.62, 46.05, 43.61, 38.76, 36.14, 31.86, 29.29, 28.75, 24.66, 21.33.

D,L-19-*epi*-Brevianamide A (20)

Crude compound **28**, prepared as described above (10 mg, 0.0264 mmol) was dissolved in 0.5 M NaOH (2 mL) and MeOH (1 mL) and stirred at room temperature for 12 h then refluxed for 2 h. After cooling to room temperature, most of the MeOH was removed in vacuo. At this point, the lactim ether of **20** could be isolated and purified, but was in practice taken on crude (purified by TLC, silica gel, CH_2Cl_2/ether, 5:1). Data for the lactim ether: IR (neat, cm^{-1}) 3353, 3030, 2942, 2871, 1694, 1660, 1613, 1488, 1470, 1428, 1320, 1295, 1179, 920, 768; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 7.5 Hz, 1H), 7.37 (ddd, J = 8.0, 7.5, 1.0 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.70 (dd, J = 7.5, 7.5 Hz, 1H), 5.43 (brs, 1H), 3.66 (s, 3H), 3.28–3.48 (m, 2H), 2.91 (d, J = 14.0 Hz, 1H), 2.61 (dd, J = 11.5, 6.0 Hz, 1H), 2.57 (dd, J = 10.0, 7.0 Hz, 1H), 2.49 (d, J = 14.0 Hz, 1H), 1.82–2.09 (m, 3H), 1.81 (dd, J = 12.5, 10.0 Hz, 1H), 1.54 (dd, J = 12.5, 7.0 Hz, 1H), 0.87 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.88, 172.18, 172.05, 160.59, 137.04, 124.62, 119.47, 117.78, 111.32, 77.15, 72.91, 66.06, 54.71, 54.45, 44.93, 43.16, 39.67, 31.19, 29.33, 24.98, 24.92, 20.97; MS m/z (M^-) calcd 380.1974, obsd 380.1980. The crude mixture was treated with 4 M HCl until pH 4 and then 2 M NaOH was added to adjust the pH to 7. The mixture was extracted with CH_2Cl_2 (3×5 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 25:1) furnished **20** (6.7 mg, 70%) as a fluorescent yellow oil. IR

(neat, cm^{-1}) 3282, 3077, 2925, 2851, 1682, 1615, 1467, 1395, 1323, 1251, 1189, 1149, 1097, 913, 754, 728; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 7.5 Hz, 1H), 7.37 (ddd, J = 8.0, 7.5, 1.0 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.63 (dd, J = 8.0, 7.5 Hz, 1H), 3.30–3.50 (m, 2H), 3.09 (dd, J = 10.0, 8.0 Hz, 1H), 2.76 (d, J = 14.5 Hz, 1H), 2.67 (ddd, J = 12.5, 7.0, 5.2 Hz, 1H), 2.08 (d, J = 14.5 Hz, 1H), 2.00 (dd, J = 14.0, 10.0 Hz, 1H), 1.85–2.00 (m, 2H), 1.77 (dd, J = 13.0, 8.0 Hz, 1H), 1.54 (dd, J = 13.0, 8.0 Hz, 1H), 0.81 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 206.10, 173.95, 169.92, 161.22, 137.69, 124.50, 118.53, 117.92, 111.27, 76.57, 68.83, 65.27, 55.80, 45.28, 43.86, 34.85, 30.42, 29.53, 24.58, 23.02, 19.18; MS m/z (M^+) calcd 366.1818, obsd 366.1818.

D,L-Brevianamide B (6)

Crude compound **29**, prepared as described above (10 mg, 0.0264 mmol) was dissolved in 0.5 M NaOH (2 mL) and MeOH (1 mL) and stirred at room temperature for 12 h then refluxed for 2 h. After cooling to room temperature, most of the MeOH was removed in vacuo. At this point, the lactim ether of **6** could be isolated and purified, but was in practice taken on crude (purified by TLC, silica gel CH_2Cl_2/MeOH, 15:1, silica gel then CH_2Cl_2/EtOAc, 3:1). Data for the lactim ether: IR (neat, cm^{-1}) 3322, 3024, 2946, 2873, 1694, 1682, 1668, 1652, 1621, 1470, 1416, 1360, 1324, 1259, 1199, 990, 924, 752, 730; ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 (ddd, J = 7.8, 1.2, 0.6 Hz, 1H), 7.37 (ddd, J = 8.4, 7.2, 1.5 Hz, 1H), 6.69–6.78 (m, 2H), 4.95 (brs, 1H), 3.79 (s, 3H), 3.37 (dd, J = 6.6, 6.6 Hz, 2H), 3.20 (d, J = 14.5 Hz, 1H), 3.15 (dd, J = 10.0, 6.6 Hz, 1H), 2.51–2.61 (m, 1H), 2.28 (d, J = 14.5 Hz, 1H), 1.78–2.05 (m, 4H), 1.48 (dd, J = 12.5, 7.0 Hz, 1H), 0.95 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.63, 171.60, 171.30, 160.21, 136.89, 124.81, 119.96, 118.14, 111.16, 78.12, 72.74, 66.93, 54.34, 51.11, 46.40, 43.17, 39.08, 30.16, 28.85, 25.00, 23.27, 21.36; MS m/z (M^+) calcd 380.1974, obsd 380.1982. The crude mixture containing the lactim ether of brevianamide B was treated with 4 M HCl until pH 4 and then 2 M NaOH was added to adjust the pH to 7. The mixture was extracted with CH_2Cl_2 (3×5 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 25:1) furnished D,L-**6** (7.7 mg, 80%) as a fluorescent yellow oil. This material was identical to natural brevianamide B by TLC, and ^1H NMR. IR (neat, cm^{-1}) 3279, 3070, 2927, 1683, 1616, 1490, 1466, 1389, 1324, 1304, 1258, 1139, 1119, 1098, 1031, 980, 924, 751, 694; ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (brs, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.37 (br dd, J = 8.0, 7.5 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.63 (dd, J = 7.5, 7.5 Hz, 1H), 3.37–3.47 (m, 2H), 3.20 (dd, J = 10.0, 8.0 Hz, 1H), 3.09 (d, J = 15.0 Hz, 1H), 3.09 (d, J = 15.0 Hz, 1H), 2.67 (ddd, J = 12.3, 6.5, 6.5 Hz, 1H), 1.90–2.02 (m, 3H), 1.82 (dd, J = 13.0, 7.0 Hz, 1H), 1.72 (dd, J = 13.0, 8.0 Hz, 1H), 0.81 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.72, 173.42, 169.92, 161.12, 137.22, 123.89, 118.20, 117.39, 110.77, 77.29, 68.57, 65.87, 49.33, 45.94, 43.30, 33.64, 29.06, 28.29, 24.24, 21.50, 19.11; MS m/z (M^+) calcd 366.1818, obsd 366.1820.

Acknowledgements

This paper is dedicated to Professor Stuart Schreiber in recognition of being awarded the Tetrahedron Prize. This work was supported by the NIH (Grant CA 70375) and the DGICYT of Spain (Proyecto PB95-1089). Mass spectra were obtained on instruments supported by the NIH Shared Instrumentation Grant GM49631 and by the Servicio de Espectroscopia de la Universidad de Valencia.

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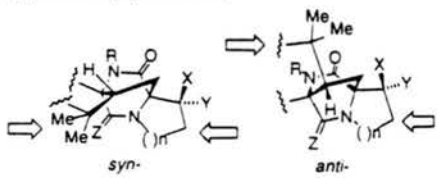
10. The *syn*-/*anti*-relationship refers to the relative stereochemistry between the C-19 stereogenic center (brevianamide numbering) and the cyclic amino acid residue (proline, β-methylproline, or pipecolic acid):



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Biomimetic Diels−Alder Cyclizations for the Construction of the Brevianamide, Paraherquamide Sclerotamide, and VM55599 Ring Systems

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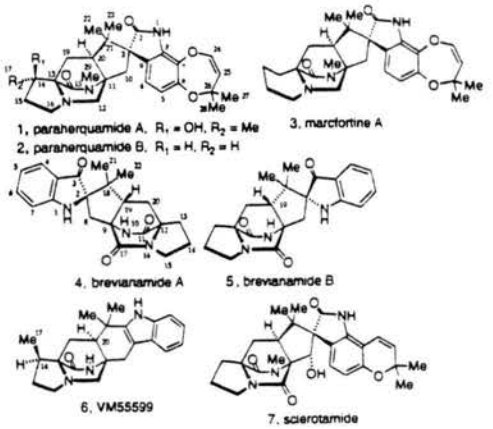
The paraherquamides,[1] brevianamides,[2] marcfortines,[3] VM55599,[4] and most recently, the sclerotamides[5] are indolic secondary metabolites isolated from various fungi. These substances have attracted considerable attention due to their molecular complexity and intriguing biogenesis,[6] and some members, most notably the paraherquamides, display potent anti-parasitic activity.[7] These alkaloids share the unusual bicyclo[2.2.2] ring system that has been proposed to arise via the [4+2] cycloaddition of the isoprene moiety across the α-carbons of the amino acid units.[8] A striking stereochemical difference between the brevianamides and all of the other members of this family is the relative stereochemical relationship at the tertiary carbon at C-19 (brevianamide numbering), which is *anti*[9] in the brevianamides and *syn* for all of the others. Previous work on the biosynthesis of these substances invoked a facial divergence in the Diels−Alder cyclization, which sets the relative stereochemical relationship at this stereogenic center.[10] In addition, recent theoretical work on an indoxyl-based Diels−Alder cyclization pathway supported the observed isomer production of the brevianamides in *Penicillium brevicompactum* which produces brevianamide A as the major metabolite and brevianamide B as the minor metabolite.[11] As part of a program directed primarily at elucidating the biosynthetic mechanism of formation of the unique bicyclo[2.2.2] ring system, particularly with respect to the question of possible enzymatic catalysis of this reaction, we report here a possibly biomimetic intramolecular Diels−Alder cyclization reaction that constructs the core framework of this class of alkaloids.



9-*epi*-Deoxybrevianamide E (**8**, Scheme 1) was synthesized according to the procedure of Kametani.[12] Conversion of this substance to the lactim ether (**9**) was accomplished with Me_3OBF_4 in CH_2Cl_2 in the presence of Na_2CO_3. Next, oxidation with DDQ gave the unsaturated substance **10**. Treatment of **10** with aqueous methanolic KOH at room temperature cleanly produced the labile azadiene **11**, which cyclized to give a mixture of **12** and **13** (2:1, 68% combined yield). The structures of the cyclization products were secured through analysis of their respective NMR spectra as well as by chemical correlation to known substances. Conversion of **12** to C-19-*epi*-brevianamide A (**16**), a nonnatural isomer of brevianamide A previously synthesized in this laboratory,[13] was accomplished by diastereoselective m-CPBA oxidation to the corresponding hydroxyindolenine (**14**, ca. quantitatively) and pinacol-type rearrangement (1 M NaOMe, MeOH, reflux) to the corresponding *spiro*-indoxyl; subsequent removal of the lactim ether with HCl afforded the corresponding ring-opened amino ester,[14] which was cyclized in hot toluene containing 2-hydroxypyridine furnishing *d,l*-**16** in 46% overall yield from **12**. This material was identical with the authentic material in every respect (except for being racemic). Conversion of **13** to *d,l*-brevianamide B was accomplished in like manner in 65% overall yield from **13** securing the relative

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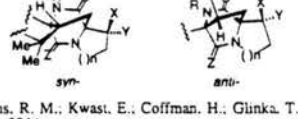
(9) The *syn*/*anti* relationship refers to the relative stereochemistry between the C-19 stereogenic center (brevianamide numbering) and the cyclic amino acid residue (proline, β-methylproline, or pipecolic acid):



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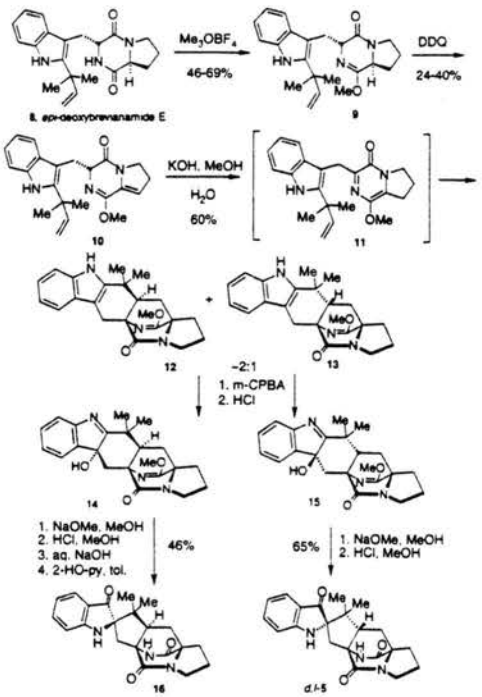
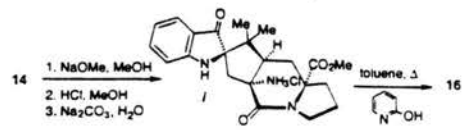
Scheme 1



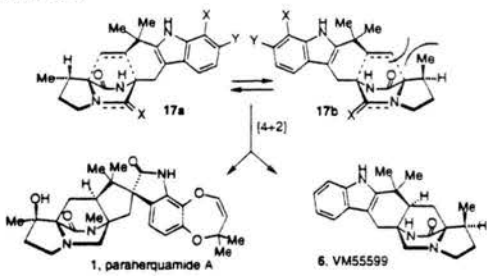
stereochemistry of each respective isomer **12** and **13**. However, in this case the cleavage of the lactim ether with HCl in aqueous methanol led directly to brevianamide B without any intermediate ring-opened amino ester detectable.

A significant implication of these observations concerns the biogenesis and stereochemistry of the related metabolite VM 55599 (**6**) isolated from the paraherquamide-producing mold *Penicillium* sp. IMI332995. Since paraherquamide A and VM 55599 both possess the bicyclo[2.2.2] monoketopiperazine ring system, it seems plausible that these substances arise via a related or common [4+2] cycloaddition. The relative stereochemistry of VM55599 was assigned by extensive ^{1}H NMR nOe studies where the methyl group in the β-methylproline moiety was assigned as being *syn* to the bridging isoprene unit.[4] Thus, if a similar Diels–Alder cyclization, whether it be un-catalyzed or enzyme-catalyzed, is operating in the biosynthetic construction of these metabolites, the isoprene unit must approach the azadiene

(14) Cleavage of the lactim ether derived from **14** with HCl produced the ring-opened amino-ester *i* that can be isolated by PTLC. Cyclization of this substance with 2-hydroxypyridine in hot toluene provided **16**.



Scheme 2



from the *same* face as the methyl group in the proline ring (**17b**, Scheme 2), whereas in paraherquamide A, which has been shown by this laboratory[15] to be derived from L-isoleucine, Diels–Alder cyclization must occur from the face *opposite* to the methyl group (**17a**, Scheme 2). The absolute stereochemical implications of the *relationship* of VM55599 to the biosynthesis of the paraherquamides have been previously discussed[15] and are an issue that is currently under study. Efforts are underway to determine the intrinsic facial bias of related Diels–Alder cyclizations on β-methylproline-containing substrates to address these issues.

This study demonstrates that the core bicyclo[2.2.2] ring system common to this family very likely arises by an intramolecular Diels–Alder cyclization from a preformed dioxopiperazine[16] that subsequently undergoes oxidation to an azadiene species. It is significant that the diastereofacial bias of the Diels–Alder cyclization of **11** is not strongly affected by solvent. The same ratio of **12**:**13** (~2:1) was obtained in THF as in aqueous methanol. We have also shown that C-19-*epi*- metabolites (corresponding to **14** and **16**) are not produced by *Penicillium brevicompactum* and there have been no reports on the isolation of similarly epimeric metabolites from paraherquamide- or sclerotamide-producing organisms. Thus, in each biosynthetic system, there appears to be complete facial exclusivity in the construction of the bicyclo[2.2.2] ring nucleus; such is not the case for the laboratory cycloaddition reported here. Uncertainties as to the oxidation state of the indole moiety as being either oxindole (for the paraherquamides, marcfortine and sclerotamide) or indoxyl (for the brevianamides) as opposed to the non-oxidized indole (for VM55599) at the [4+2] cyclization phase as well as the question of possible protein organization of the transition state structures still exists and are the subject of intense investigation in these laboratories.

Acknowledgment. Dedicated to Professor Yoshito Kishi on the occasion of his 60th birthday. This work was supported by the NIH (Grant CA 70375) and the DGICYT of Spain (Proyecto PB95-1089). Mass spectra were obtained on instruments supported by the NIH Shared Instrumentation Grant GM49631 and by the Servicio de Espectroscopia de la Universidad de Valencia.

Supporting Information Available: Spectral data for all new compounds employed in this study (11 pages). See any current masthead page for ordering and Internet access instructions.

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Appendix 2

Research Proposal

Research Proposal

Kathleen Halligan

September 13, 1999

Total Synthesis and Biological Studies of a New Class of Antimalarial Agents

Title: Total Synthesis and Biological Studies of a New Class of Antimalarial Agents

Abstract

Malaria is an acute infectious disease affecting the tropics of Asia, Africa and Latin America. Resistance to current antimalarial agents has now spread and the development of new drugs with specific activity is required. The goal of this program is to develop new antimalarial drugs based on the lead structure **1** (**Scheme 1**). Diterpene **1**, ((1(14)-E, 3S*, 4R*, 7S*, 8S*, 11R*, 12R*, 13R*)-7 isocyanoneoamphilecta-1-(14), 15-diene), isolated from the sponge *Cymblastela hooperi*, represents the first and only member of a new class of tricyclic marine-derived compounds known as neoamphilectane derivatives. Compound **1** has shown significant biological activity *in vitro* against two clones of the malaria parasite *Plasmodium falciparum* with selectivity that rivals currently used antimalarial drugs. A synthetic strategy for neoamphilectane **1** and several analogues is proposed. Bioassays will be performed to determine their antiplasmodial activity.

Specific Aims

Diterpene 1. This structurally unique molecule will be synthesized according to the retrosynthetic analysis outlined in **Scheme 1**. The core neoamphilectane ring structure will be formed *via* a transannular intramolecular Diels-Alder (IMDA) cyclization, followed by a Heck reaction. A judicious selection of reagents will be used to exert stereocontrol and install appropriate functional groups.

Analogues of 1. The current synthetic strategy will serve to investigate the synthesis of neoamphilectane analogues with potentially greater antiplasmodial activity and better selectivity. Attention will be focused on modification of the isocyanide functionality, isoprenyl moiety, and demethylation at C_3 and C_{11}.

Bioactivity Studies. The analogues of **1** will be bioassayed against the four species of the protozoan *Plasmodium: P. falciparum, P. vivax, P. malariae, and P. ovale.* A selectivity index will also be constructed in order to assess whether the observed antiplasmodial activity was a specific or general toxic effect. The currently used antimalarial agents: chloroquine, quinine, mefloquine, and artemisinin will be used as standards.

Background and Significance

Malaria is caused by protozoa of the genus *Plasmodium: P. falciparum, P. vivax, P. malariae, and P. ovale* which are transmitted to humans by mosquitoes.[1] Infection by *P. falciparum* is the most severe form of malaria. When an infected female Anopheles mosquito bites, it injects *Plasmodium* organisms into the wound. These migrate to the liver, where they reproduce, invade red blood cells, and spread throughout the circulatory system. The infected person becomes a carrier of malaria, infecting any mosquito that bites him, thus perpetuating the cycle of transmission.

Malaria is endemic to the tropics of Asia, Africa, and Latin America. An estimated 1.5 billion people live in these regions and roughly 1.5 million people die annually from this

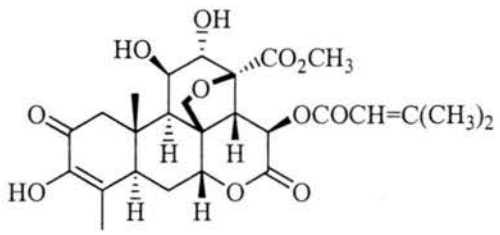
disease.[2] To control the disease, chloroquine has been the drug of choice in Africa, but resistance to this compound has now spread to all major malaria endemic regions. In Southeast Asia, malaria cases no longer respond to mefloquine and a decreased sensitivity to quinine has been reported.[3]

The goal of antimalarial drug development is to discover and characterize drug targets that are unique to the malaria parasite. Emphasis is being placed on testing compounds for inhibition of specific targets and for activity on the intact parasite.[3]

Current natural product isolation and synthetic studies encompass a wide range of classes of compounds. For instance, in 1972, endoperoxide research began with the isolation of 1,2,4-trioxane artemisinin (qinghaosu) as the key ingredient in a tea of *Artemisia annua* leaves used for thousands of years in China and Southeast Asia to treat fever.[4,5] Hierridin B and two other phenols have recently been isolated from a marine cyanobacterium and show antiplasmodial activity towards *Plasmodium falciparum*.[6] The West African vine *Triphyophyllum peltatum* contains a number of antimalarial naphthylisoquinoline alkaloids. Dioncophylline C has shown the greatest antimalarial activity of this group. The synthesis of Dioncophylline C was accomplished by Bringmann in 1998.[7] Three lignans and one flavan were isolated from the extract of *Terminalia bellerica* fruit rind and have shown significant antimalarial activity.[8]

The bitter degraded triterpenoid quassinoids, (e.g. brusatol **21**, **Figure 1**), have been identified as the active constituents of plants from the Simbaroubaceae which are used to treat malaria, leukemia, amoebiasis and other illnesses. It has been shown that quassinoids are potent inhibitors of protein synthesis. Although the mode of action is not fully known, it has been established that the presence of a diosphenol group or an

unsaturated ketol in ring A, an oxygen methylene group in ring C, and an ester moiety at C_{15} or C_6 have substantial effects on antoplasmodial activity.[9] Semisynthetic derivatives and bicyclic analogues of Quassin have been prepared.



21

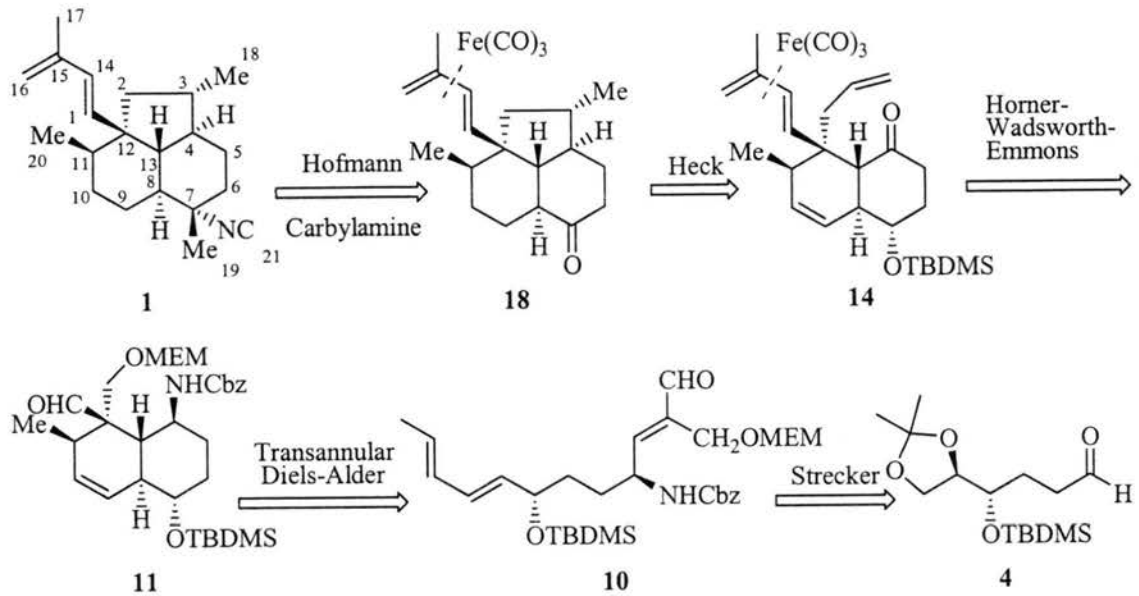
Figure 1: Antimalarial quassinoid, brusatol, **21**.

The marine sponge *Cymbastela hooperi* has proven to be a rich source of novel potent antimalarial diterpene isocyanates, isothiocyanates, and isonitriles. König and co-workers[2,10] have identified fifteen diterpenes of which only one compound, **1**, incorporates the neoamphilectane ring structure. To date, this is the only known member of this new class of compounds; its synthesis has not been reported. Diterpene **1** was tested against mammalian KB cells (IC_{50} 19100 ng/ml), and two clones of *P. Falciparum* (clone D6: IC_{50} 90.0 ng/mL, clone W2: IC_{50} 29.7 ng/mL). Its *in vitro* antiplasmodial activity and selectivity rival the current antimalarial treatments. Synthetic studies on amphilectanes have been reported in the literature,[11a-e] none of which seemed promising for the construction of neoamphilectane target **1**. The synthetic challenges present in the neoamphilectane core are the novel 5-membered carbocycle and the C_{12} tetrasubstituted center. It is hoped that modification of the isoprene moiety, demethylation, and functional group transformation will lead to the total synthesis of more potent antimalarial neoamphilectane analogues.

Experimental Design and Methods

Retrosynthetic analysis of neoamphilectane 1

Scheme 1



1 **18** **14**

11 **10** **4**

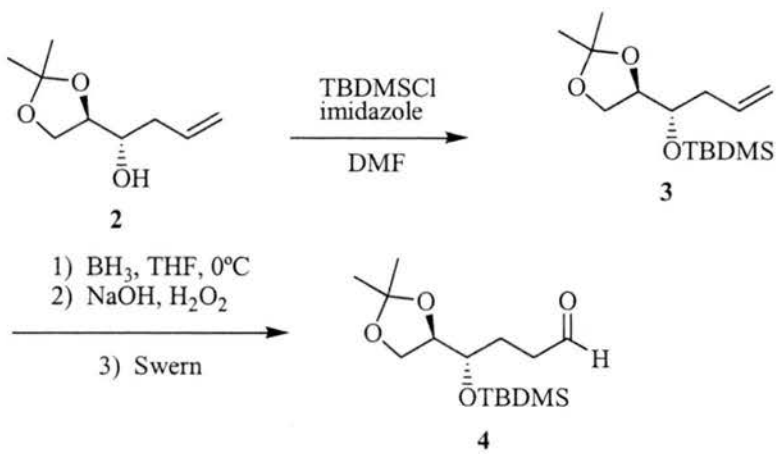
The sensitive isocyanide moiety will be installed late in the synthesis using the Hofmann Carbylamine reaction after amination of ketone **18**. Disconnection at C_3-C_4 allows for final ring closure of the neoamphilectane core *via* an intramolecular Heck reaction of the enol triflate of ketone **14**. The cis-isoprenyl group of **14** will arise from a stereocontrolled Horner-Wadsworth-Emmons reaction of aldehyde **11**. Disconnection at C_{11}-C_{12} will allow for the key intramolecular transannular Diels-Alder cyclization of triene **10** where the OH protecting group at C_7 has been shown to control diastereoselectivity of this intramolecular cyclization.[12a,b] Triene **10** can be derived from aldehyde **4** *via* a Strecker reaction, followed by three sequential Wittig-type olefinations.

Aldehyde **4** will be prepared by the asymmetric reaction of D-glyceraldehyde acetonide and tartrate allylboronate.[13,14a,b]

Synthesis of aldehyde 4

The proposed synthesis of neoamphilectane **1** begins with the construction of aldehyde **4** (**Scheme 2**). Allylic alcohol **2** can be furnished in 86-91% yield with an 96% de according to Roush and co-workers.[13,14a,b] Protection of the alcohol as the TBDMS ether will provide the cyclic acetal **3**. The alkene is then subjected to hydroboration-oxidation[13] conditions followed by a Swern[15] oxidation to provide aldehyde **4**.

Scheme 2



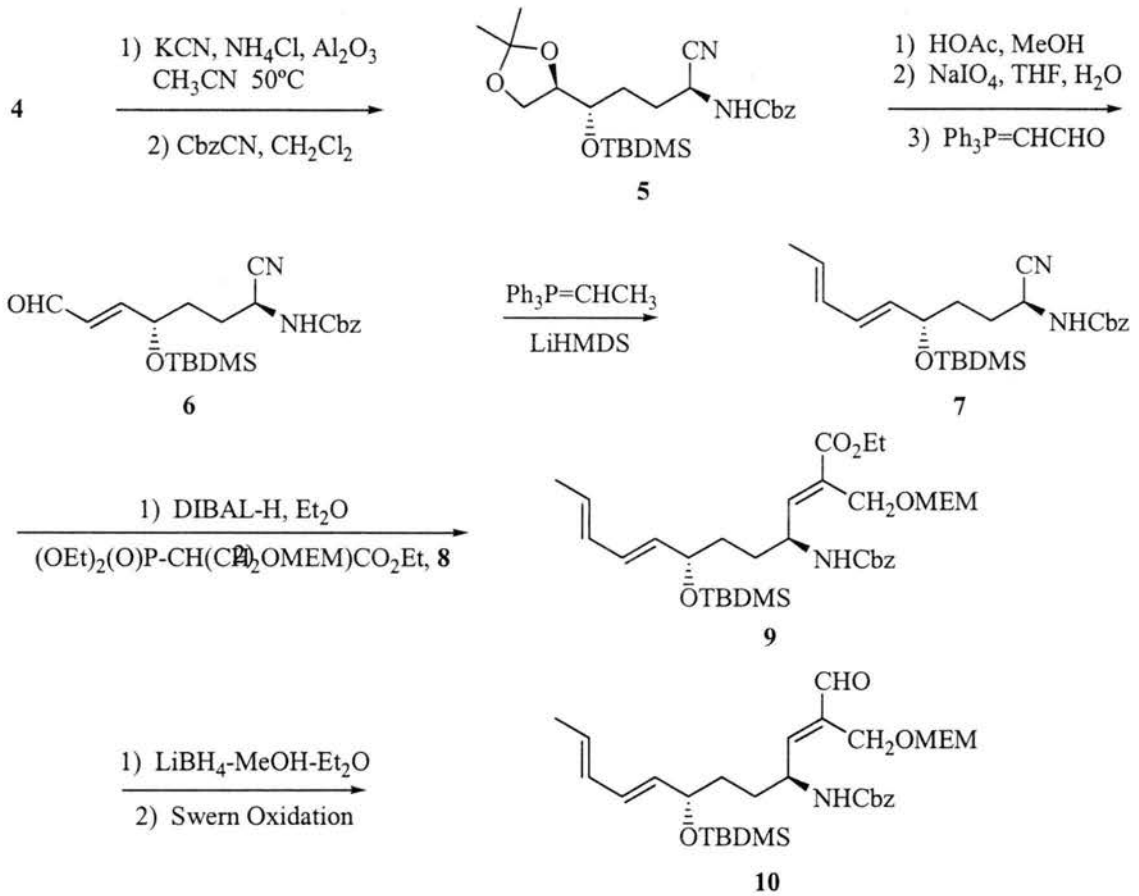
2 **3** **4**

Synthesis of triene 8

The aminonitrile functionality will be introduced using the following Strecker conditions: KCN, NH_4Cl, Al_2O_3 in CH_3CN (**Scheme 3**).[16] The desired amine diastereomer will be taken on and protected with CbzCN in CH_2Cl_2 to furnish compound **5**.[17] Removal of the cyclic acetal followed by periodate cleavage of the diol intermediate will provide the corresponding aldehyde.[13] Condensation with $Ph_3P{=}CHCHO$ followed by a second Wittig olefination using $PhP{=}CHCH_3$ and a lithium salt would provide the requisite trans-

diene **7**.[18a,b] Reduction of the nitrile with an excess of DIBAL-H in ether at –78°C, will give the corresponding aldehyde. Mild work-up conditions (e.g. citric acid)[19] will be used to prevent cleavage of the TBDMS ether. A Horner-Wadsworth-Emmons[20a-d] reaction with phosphonate ester **8**, should afford triene **9**. Ester **8** could be made by starting with commercially availabe $(EtO)_2P(O)CH_2CO_2Et$. Reaction with MEMCl and base should yield the desired compound **8**.[21]

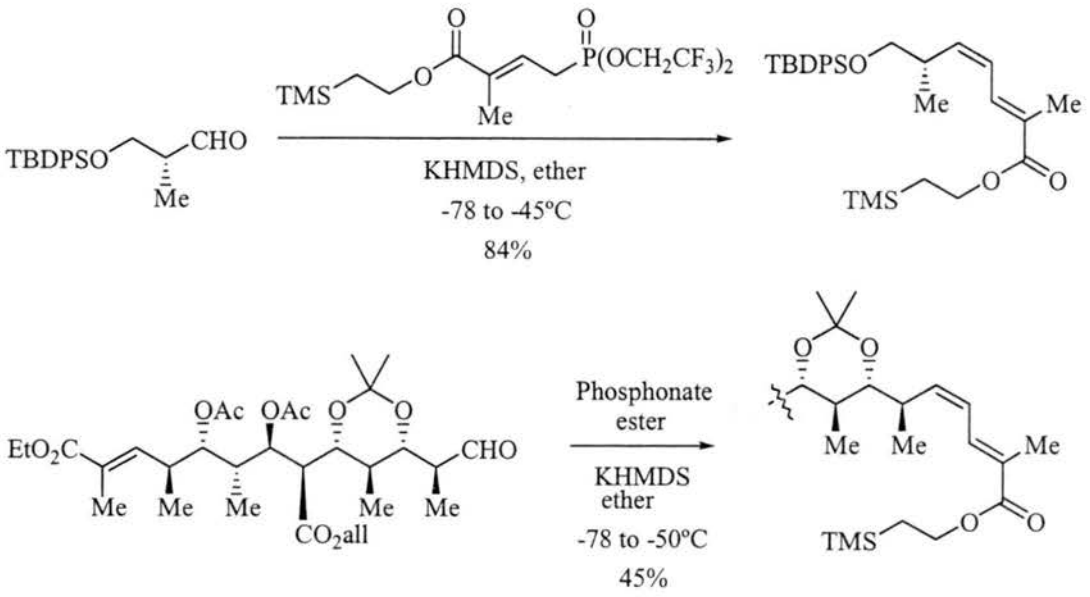
Scheme 3



Roush and Ando have achieved successful olefinations using bulky and complex phosphonate reagents.[20a,b] In the synthesis of the (E,Z)-Dienoate precursor of (+)-Damavaricin D, Roush and co-workers condensed the phosphonate ester and aldehyde with potassium hexamethyldisilazide in ether to furnish the diene in 84% yield. (**Scheme**

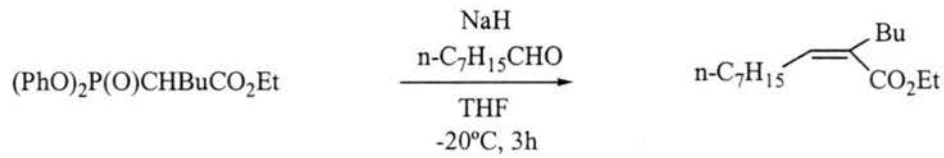
4; E,Z:E,E = 4.7:1) With a more complex aldehyde, the diene was furnished in 45% yield (**Scheme 4**; E,Z:E,E = 4:1).

Scheme 4



Ando and co-workers have utilized phosphonate esters for olefinations that contain alkyl groups at the α-carbon that are larger than the typical "H or Me" that is used.[20b] An example of this reaction in which butyl is the α-alkyl group is shown in **Scheme 5**. The resultant olefin was produced in 88% yield and 82:18 Z:E ratio.

Scheme 5



Reduction of the ester moiety of **9** could be achieved by using $LiBH_4$-MeOH-Et_2O.[22a,b] The resulting alcohol could be oxidized back up to the aldehyde under Swern conditions to afford triene **10**.[15]

Intramolecular Diels-Alder Cyclization of Triene ***8***

In order to control the trans-ring-fused stereochemistry and induce asymmetry in the cycloadduct, a transannular IMDA reaction will be employed. Marshall[12a,b] has demonstrated that Lewis acid catalyzed cycloadditions of the corresponding trienals (**Figure 2**) occur efficiently at low temperatures with great selectivity for the trans-fused product.

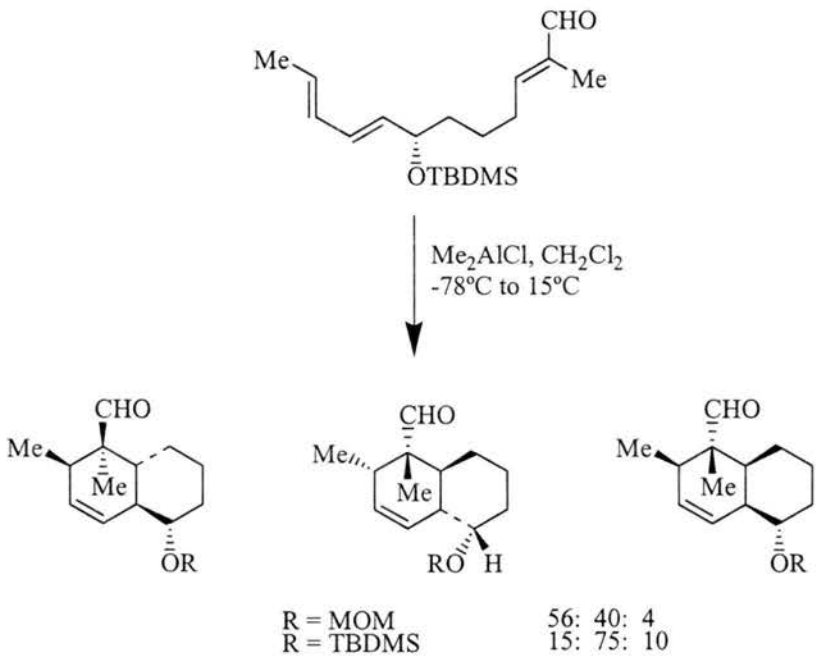


Figure 2. Lewis acid catalyzed IMDA

It has been observed that an allylic alkoxyl protecting group bears significant influence on the diastereoselectivity of the Diels-Alder reaction. When the allylic alcohol is protected as a TBDMS ether (**Scheme 6**), the stereochemical outcome matches that required for neoamphilectane **1**.[12a,b] The intramolecular Diels-Alder precursor **10**, can adopt two favorable transition state conformations (**Figure 3**). The dienophile may

approach the diene from the top or bottom face. Both transition states are likely since the TBDMS ether and the benzyl carbamate occupy an equatorial position, thus avoiding 1,3-diaxial interactions. A 50:50 mixture of diastereomers is expected from the cycloaddition of triene **10**. Compound **11** will be used for the synthesis of the target neoamphilectane, **1**. Based on the metabolites isolated by König in *Cymbastela hooperi*[2,10] it was observed that stereochemistry at C_{11} and C_{12} varied from one compound to the next. So, diastereomers of **11** should provide access to several other neoamphilectanes. Due to the complexity of triene **9**, in particular the two additional basic moieties (NHCbz, CH_2OMEM), it might become necessary to explore other Lewis Acid conditions for the Diels-Alder cyclization.

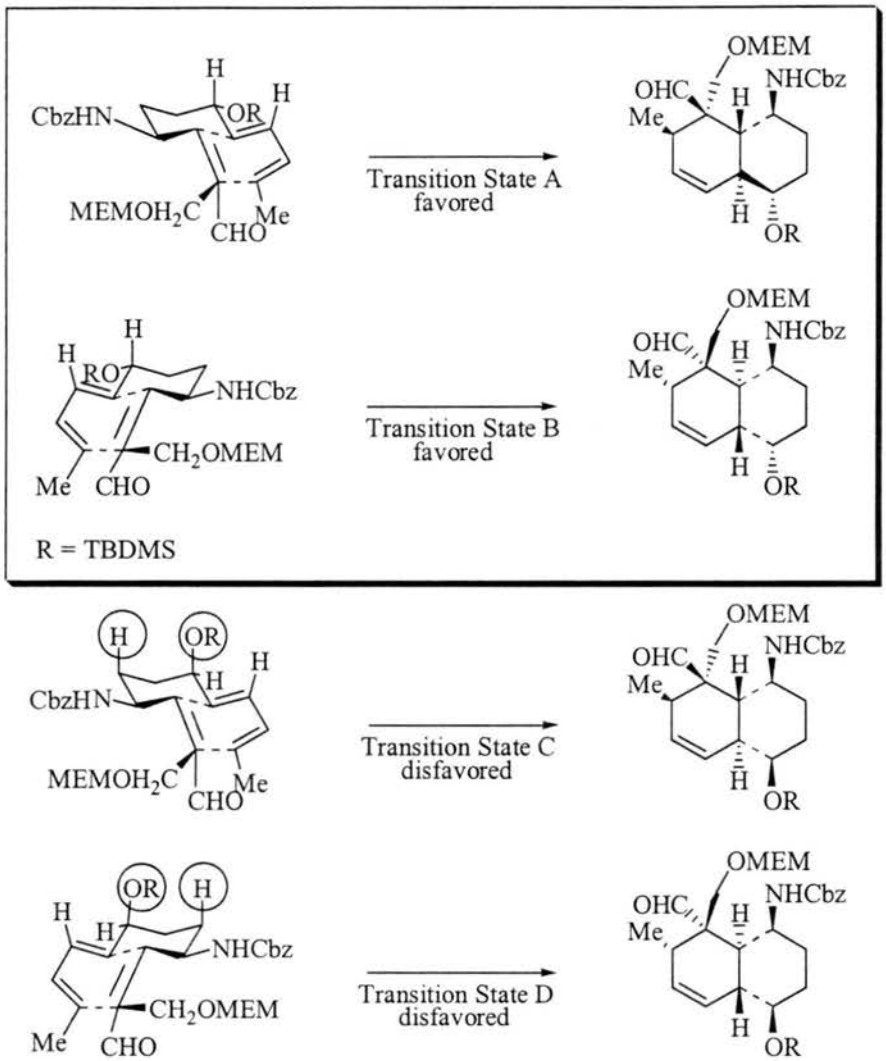
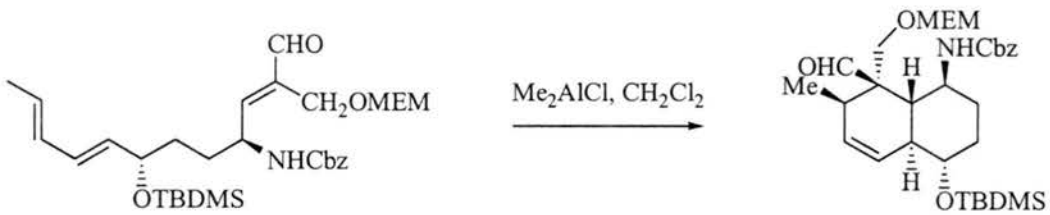


Figure 3: Transition state structures for the intramolecular Diels-Alder cyclization

Scheme 6

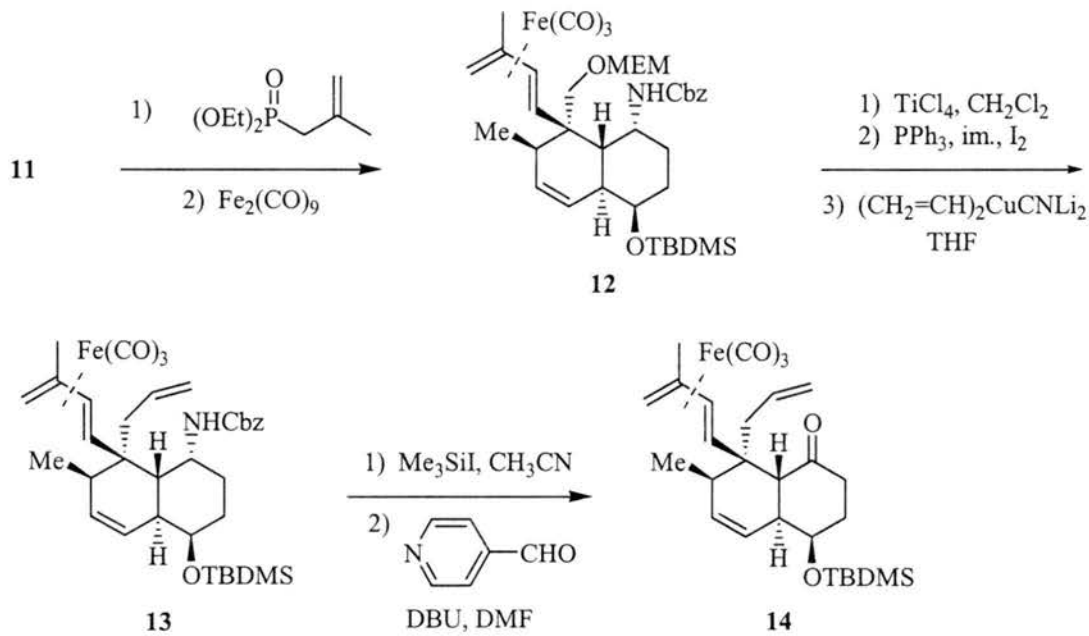


10 **11**

Synthesis of the Heck precursor ***12***

At this point, a Horner-Wadsworth-Emmons[20a-d] reaction (**Scheme 7**), could be used to introduce the isoprenyl chain. Though aldehyde **11** is sterically hindered, there are a number of successful cases involving hindered aldehydes reacting with Wittig or Horner-Wadsworth-Emmons reagents.[23a,b,c] If aldehyde **11** is too hindered to accept the required isoprenyl-forming phosphonate ester, diene **12** could be achieved in two sequential Wittig reactions. The diene should be capable of rotating to the cisoid conformation followed by protection with $Fe_2(CO)_9$[24] to give metallo adduct **12**.

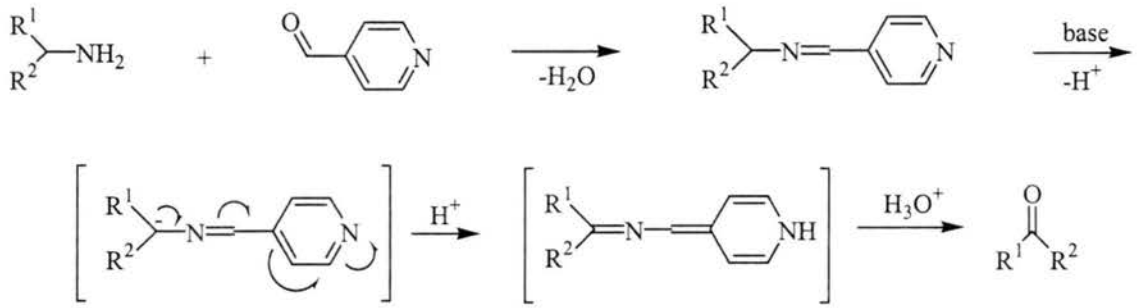
Scheme 7

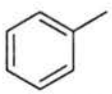
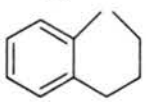
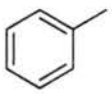
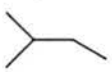


The MEM protecting group can be removed by stirring with $TiCl_4$ in CH_2Cl_2 at 0°C.[21] The ensuing alcohol will be converted to the iodo-derivative by treatment with Ph_3P, imidazole, and iodine.[12b] Though substitution reactions with neopentyl systems have proven difficult in the past, Masada and co-workers have achieved moderate yields

in the synthesis of neopentyl 2,4,6-tri-tert-butylphenyl ether and related hindered ethers.[25] Addition of an organocuprate to the iodo-compound will provide the vinyl group of trans-decalin derivative **13**.[26] After careful removal of the Cbz group using TMSI,[27a,b] the primary amine will be oxidatively deaminated to ketone **14** when subjected to pyridine-4-carboxaldehyde and DBU in DMF.[28] It is anticipated that the bulky base, DBU, will give predominantly the kinetic enolate, thereby avoiding racemazation at C_{13}. Hydrazone formation takes place first and then after electron movement, acidic hydrolysis affords the carbonyl. A few examples from Ohta and co-workers are shown in **Scheme 8**.[28]

Scheme 8

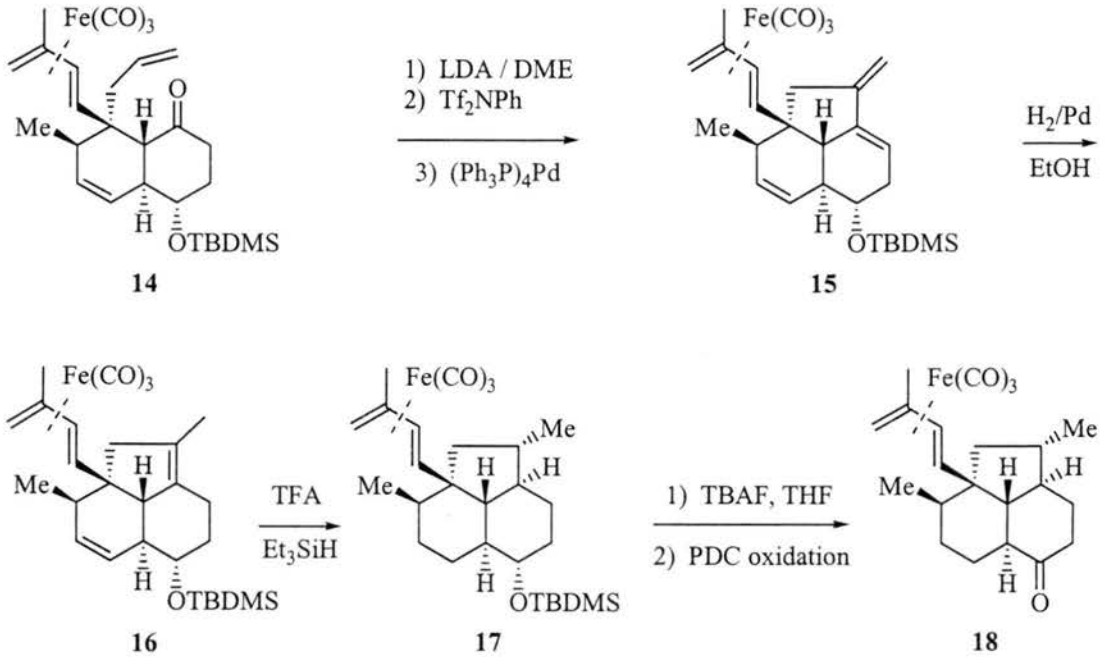


R_1	R_2	yield [%]
	H	100
	H	100
	CH_3	84
	COOH	85

*Construction of 6,6,5 portion of the neoamphilectane core (**18**)*

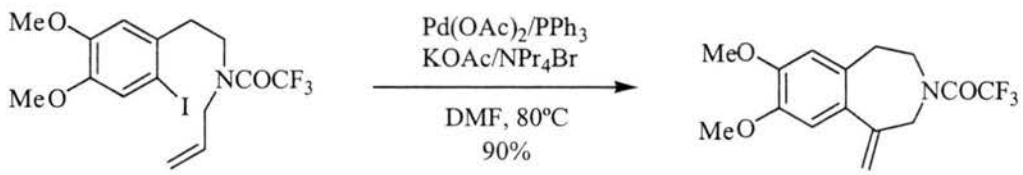
The enol triflate[29] of ketone **14** will undergo a palladium catalyzed intramolecular Heck reaction (**Scheme 9**), to give the 5-*exo* cyclization product **15**.[11e]

Scheme 9



Precedent for the 5-*exo* compound has been observed by several groups.[30a-d] Kinetically, this product is favored. Tietze and co-workers[30d] demonstrate this outcome in the synthesis of 2,3,4,5,-tetrahydro-1H-3-benzazepines (**Scheme 10**).

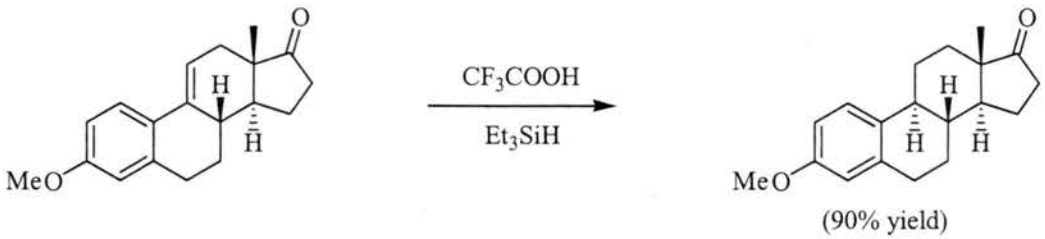
Scheme 10



Danishefsky has conducted palladium catalyzed 5-*exo* cyclizations enroute to taxol.[30c] Diene **15** will be reduced to olefin **16** using conditions that proved successful for Shibasaki and co-workers in the synthesis of carbacyclin.[31] Further reduction of the olefin with TFA and Et_3SiH should provide **17**. The stereochemical outcome shown in **17** is anticipated due to the potential directing affect of the iron tricarbonyl protected diene.

Posner and co-workers demonstrated successful reduction of a cyclic olefin (**Scheme 11**) using this procedure.[32]

Scheme 11



Deprotection of the TBDMS ether[33] followed by PDC oxidation,[34] would provide ketone **18**. Donaldson and co-workers[34] have shown that the iron tricarbonyl protecting group is stable to PDC oxidation (**Figure 4**).

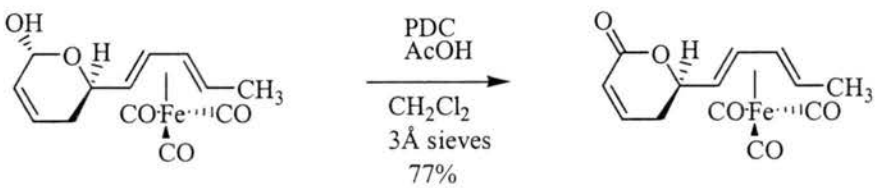


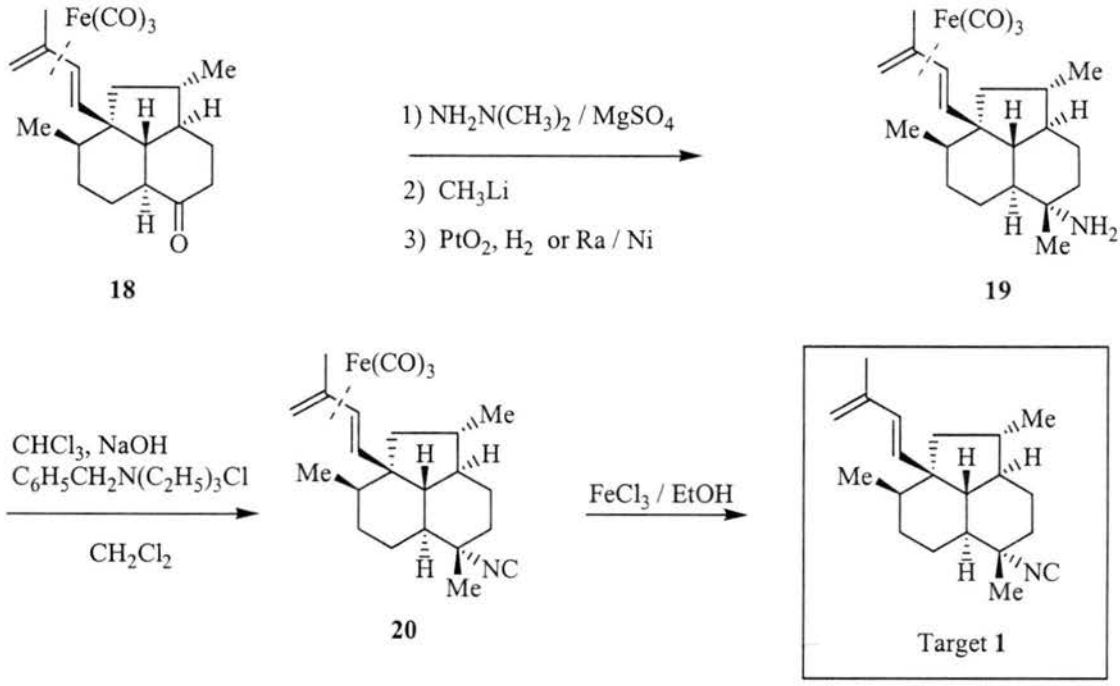
Figure 4: PDC Oxidation by Donaldson[34]

One potential problem with the use of base in the reactions from compound **13** to **15** is the loss of stereochemistry at C_{13}. Bulky bases and lower temperatures should help keep this to a minimum.

Completion of target diterpene 1

Organolithium addition to the hydrazone of ketone **18** followed by hydrogenation should provide **19** as a mixture of diastereomers (**Scheme 12**).[35a-c] Treatment of the newly formed primary amine with dichlorocarbene and a phase transfer catalyst (benzyltriethylammonium chloride) will provide the isonitrile moiety via the Hofmann Carbylamine reaction.[36] Finally, removal of the $Fe(CO)_3$ diene-protecting group[24] using $FeCl_3$ will result in the completion of the antimalarial neoamphilectane compound **1**.

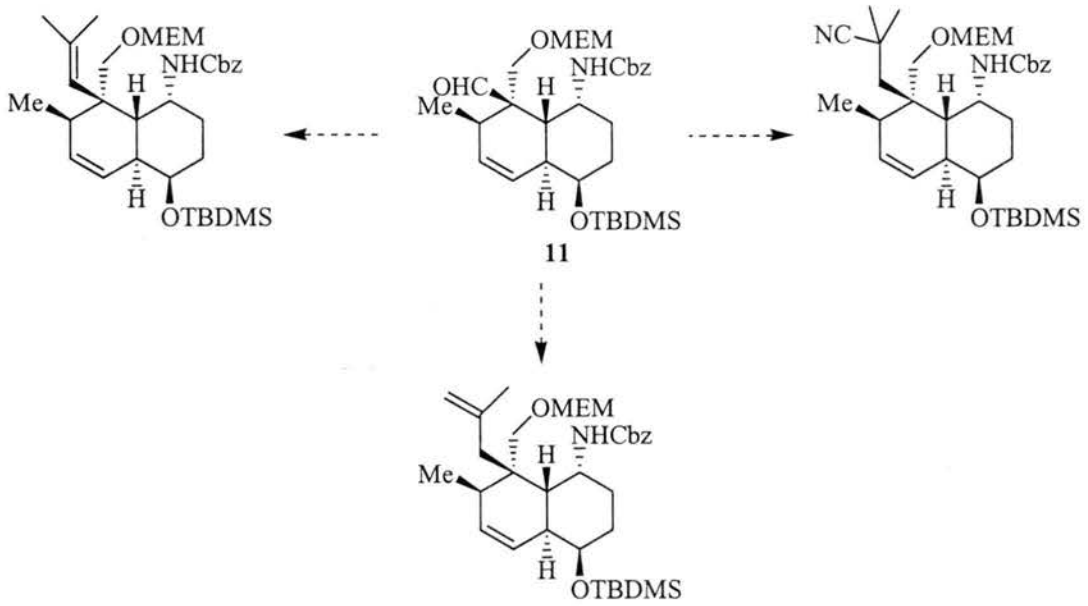
Scheme 12



Analogues of 1

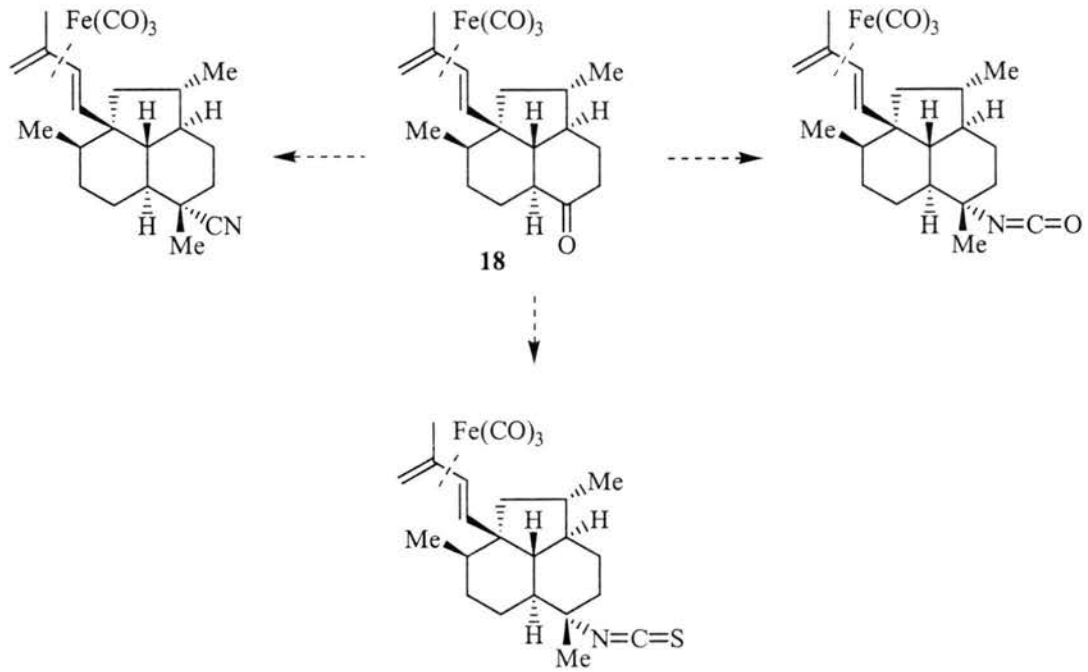
Within the amphilectane family of antimalarial compounds, functionality at C_1, C_3, C_7, and C_{11} plays an important role in antiplasmodial activity and selectivity. The current synthetic strategy allows for modification of the isoprenyl group at C_1. Aldehyde **11** will provide access to additional isoprenyl derivatives (**Scheme 13**).

Scheme 13



Ketone **18** will serve as a template for the application of various nitrile, isocyanate, and isothiocyanate manipulations (**Scheme 14**).

Scheme 14



Biological Studies

The analogues of **1** will be tested for biological activity *in vitro* against two clones of the malaria parasite *Plasmodium falciparum* according to the procedures used by König and co-workers.[10] Additional examination of the compounds against the mammalian KB cell line will enable experimental Selectivity Index (SI) calculations, in order to assess whether the observed antiplasmodial activity is a specific or general toxic effect.

Alternative Routes

In performing a total synthesis of a natural product containing a new ring system, there will undoubtedly be unforeseen complications that will require further attention. Here are some possible solutions for steps that are particularly challenging.

The transannular intramolecular Diels-Alder reaction looks promising, but it has not been proven with the exact functionality present in triene **10**. If poor diastereoselection in the cycloaddition is observed, the possibility of different protecting groups for the hydroxyl and amino moieties will be examined.

Another problematic step may be the intramolecular Heck reaction of the triflate of ketone **14**. It is conceivable that the olefin of the product could isomerize to the C_2-C_3 position. If this problem arises, there are several asymmetric hydrogenation[24] methods to ensure the correct stereochemistry of the C_{18} methyl group. As an alternative to the Heck reaction, the C_3-C_4 bond could also be formed *via* a McMurry coupling from the appropriate diketone.[36]

It should be pointed out that there exist a number of other examples in the literature for the preparation of transannular IMDA adducts.[12,37a-e] The Deslongchamps[38] Diels-Alder strategy using temporary tethers is one possible method to pursue if problems are encountered in the current synthesis.

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