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UNIVERSITY OF CALIFORNIA
RIVERSIDE

Synthesis of Indolylquinones as a Potential Therapy for
Diabetes and as Candidates for Neuroprotection

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Chemistry

by

Theresa Charbel Massoud

August 2010

Dissertation Committee:

Dr. Michael Pirrung, Chairperson

Dr. Michael Marsella

Dr. Pingyun Feng

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2010

The Dissertation of Theresa Charbel Massoud is approved:

Committee Chairperson

University of California, Riverside

Acknowledgments:

I am now considered a woman of science, but first and foremost I am a child of God. I thank God every single day for giving me faith, without which I would not have the determination to pursue my dreams. I would like to thank my parents for their encouraging words and unconditional love and support. They have taught me to become the person I am today. This Ph.D. is truly theirs. A sincere thank you goes to my brothers Joey and Elias, and to my sister Maria. I strive to be the best because they are my inspiration. They have provided me with a haven whenever I needed to escape the world of chemistry. I thank them for being my stress relief! A sincere thank you goes to the love of my life, Fouad. He has been my backbone since the day we met. I cannot express how far the words "Be Great" have taken me. Thank you for loving me.

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This dissertation is dedicated to my parents for their continuous support and overpowering love. Thank you for teaching me what it means to have faith. You are my heroes. To my brothers and sister, you three are my inspiration. Each of you has the potential to achieve anything you set your mind to. Thank you for being my siblings and my best friends. Finally, to the love of my life, Fouad, thank you for instilling greatness in me and for your belief in my potential. Your love kept me sane throughout my entire education.

ABSTRACT OF THE DISSERTATION

Synthesis of Indolylquinones as a Potential Therapy for
Diabetes and as Candidates for Neuroprotection

by

Theresa Charbel Massoud
Doctor of Philosophy, Graduate Program in Chemistry
University of California, Riverside, August 2010
Dr. Michael Pirrung, Chairperson

Asterriquinones display a wide range of biological activity. From antiretroviral and antitumor activity, to anti-diabetes properties and neuroprotection, asterriquinones and their analogs have quickly become a popular target in pharmaceutical research.

The asterriquinone demethylasterriquinone-B1 (DAQ-B1) exhibits insulin mimetic activity in two mouse models of diabetes. It directly activates the tyrosine kinase domain of the insulin receptor and, as a small molecule, is orally active. A substantial amount of SAR work in our and other laboratories identified the key pharmacophore, which includes the quinone. Despite promising early-stage animal studies with compounds of this class, concern about potential long-term toxicity linked to their quinone portion impeded further development. In order to surmount this obstacle, quinone replacements are sought in the synthesis of DAQ-B1 analogs. These replacements include cyclic ketones such as pyrones, pyridones, and cyclohexadienones containing a quarternary center at the 4-position. Two different methods are used in an attempt to synthesize the analogs: a Stille coupling between the desired indole and the

halogenated heterocyclic ketone, and a Claisen rearrangement of an (indole)-methyl-kojic acid ether.

Besides exhibiting insulin mimetic activity, DAQ-B1 demonstrates the ability to activate the nerve growth factor (NGF) receptor, which belongs to a class of neurotrophin proteins that support neuronal growth and survival. A fluorinated analog of DAQ-B1, **5E5**, activates the TrkA receptor and demonstrates NGF activator activity with 'approximately 200% the effect of a maximal dose of NGF.' A library of fluorinated monoindolylquinones, similar in structure to **5E5**, is synthesized from a two step procedure consisting of the acid-catalyzed coupling of fluorinated indoles to 2,5-dichloro-1,4-benzoquinone, followed by methanolysis.

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Chapter One: Introduction

1.1 Asterriquinones & Their Discovery

Natural products are a continuous and proven source of new lead compounds to drug discovery programs. Naturally occurring compounds are extracted from tissues of terrestrial plants, marine organisms, or microorganism fermentation broths. Asterriquinones are a large class of fungal natural products that were isolated from *Chaetomium*, *Aspergillus*, and *Pseudomassaria* species. The first asterriquinone reported, cochliodinol (Figure 1.1), was discovered by a Canadian group of scientists in 1968 from *Chaetomium* species and it was determined to have antibiotic properties.¹ Asterriquinones have been studied extensively by the Yamamoto group from Japan. The first compound they isolated was from the fungus *Aspergillus terreus* and was found to be 5-bis-[1'-(1'',1''-dimethyl-2''-propenyl)-indol-3'-yl]-3,6-dihydroxy-1,4-benzoquinone. Its structure was determined by spectroscopic data and the natural product was named “asterriquinone (ARQ)”, after *Aspergillus terreus* (Figure 1.1).²

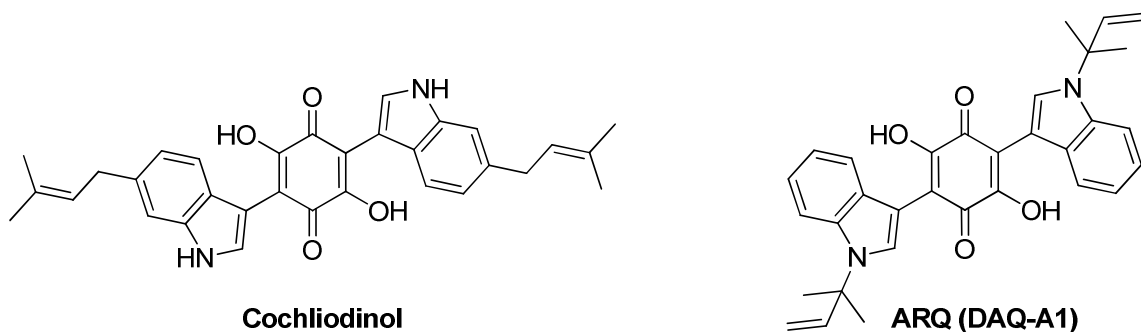


Figure 1.1: The first isolated asterriquinones, cochliodinol and DAQ-A1

ARQ would later come to be known as DAQ-A1 (demethylasterriquinone-A1). Purification of DAQ-A1 could not be accomplished using standard silica gel chromatography because the dihydroxybenzoquinone portion of this compound makes it unstable on silica. The Yamamoto group thus designed a new method of purification where the silica gel is precoated with oxalic acid. Use of oxalic acid-coated silica gel made isolation and full characterization of DAQ-A1 possible. Based on the chemical structure, this group postulated that DAQ-A1 was biosynthesized from tryptophan and an isopentenyl unit derived from mevalonic acid.

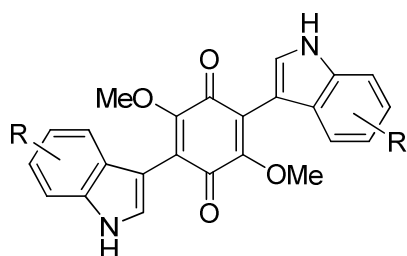


Table 1.1: Naturally existing asterriquinone compounds

Asterriquinone	Substitution		Asterriquinone	Substitution	
	R	R'		R	R'
AQ-A1	1-Isoprenyl	1-Isoprenyl	AQ-B3	1-Isoprenyl	H
AQ-A2	2-Isoprenyl-7-prenyl	7-Prenyl	AQ-B4	2-Isoprenyl	2-Isoprenyl
AQ-A3	1-Isoprenyl	2-Isoprenyl	AQ-C1	2-Isoprenyl	H
AQ-A4	2-Isoprenyl-7-prenyl	2-Isoprenyl	AQ-C2	7-Prenyl	H
AQ-B1	2-Isoprenyl	7-Prenyl	AQ-D	H	H
AQ-B2	2-Isoprenyl-7-prenyl	H			

Five years later, Yamamoto and co-workers isolated eleven new bisindolyl-dimethoxy-1,4-benzoquinones from *Aspergillus terreus*, and they proposed the general name “asterriquinone” for these types of compounds (Table 1.1). Verification of structure of these eleven compounds led to the conclusion that naturally occurring asterriquinones are composed of two indole units and a dimethoxy benzoquinone core. These newly discovered compounds are named as asterriquinone A1, A2, B1, B2 and so forth, which is a nomenclature based on indole structure.³

1.2 Asterriquinones as Potential Drug Therapies

Asterriquinones represent a class of ascomycete metabolic products whose significance stems from remarkable and useful pharmacological activities. These natural products and their analogs display a wide range of potential therapeutic properties. Some asterriquinones have shown antiretroviral (*e.g.*, against HIV) properties, while others have displayed antitumor activity against transplantable animal tumors. Certain asterriquinones have displayed anti-diabetes properties by mimicking insulin action. Recent studies have shown that asterriquinones can activate select Trk receptors and thus, could have potential utility in the treatment of neurodegenerative diseases. The ability of asterriquinones to either promote or prevent protein-protein interactions seems to be the source of their medicinal properties.

1.2.1 Asterriquinones as a Potential Cancer Therapy

As mentioned previously, one of the first asterriquinones isolated by the Yamamoto group is DAQ-A1 (Figure 1.1). In examining the biological activity of DAQ-A1, they found that it inhibited the growth of transplantable animal tumors *in vivo*.⁴ Mice bearing Ehrlich ascites carcinoma showed a dose-dependent increase in lifetime and a suppression of the growth of tumor mass when given DAQ-A1 intraperitoneally. Similar effectiveness was exhibited against Yoshida sarcoma, L-1210 mouse leukemia, and ascites hepatoma AH-13 in rats. Furthermore, DAQ-A1 was found to be relatively low in toxicity with an LD₅₀ of 400 mg/kg.

Although DAQ-A1 was shown to have antitumor activity in transplantable animal tumor models such as Ehrlich mouse carcinoma, P388 leukemia, and rat ascites hepatoma, its mechanism of action was not understood. The Kaji group from Japan wanted to determine whether: 1) the dihydroxybenzoquinone component was necessary for toxicity and 2) the mode of action was similar to that of other quinones. Therefore, they investigated the *in vitro* cytotoxic effects of DAQ-A1 on mouse leukemia P388 cells, and compared the mode of action with its dimethoxybenzoquinone analog, AQ-A1 (Table 1.1) and an anthracycline antitumor agent and antibiotic, adriamycin (ADR, Figure 1.2). They treated mouse leukemia P388 cells with each of the three compounds for 72 hours and obtained IC₅₀ values for DAQ-A1 and ADR of 1.28 μ M and 29 μ M, respectively.⁵ AQ-A1 did not show any cytotoxic effect, even at high doses. Quinone compounds, including ADR, are known to intercalate into genomic DNA and generate reactive oxygen species, producing DNA strand scission. DAQ-A1 formed DNA-

interstrand cross links (ISC) in P388 cells and was shown to induce cell cycle arrest and apoptosis. DNA fragmentation and apoptotic DNA degradation, however, were not observed in cells treated with AQ-A1. This suggests that the dihydroxybenzoquinone moiety of DAQ-A1 is essential to elicit cytotoxicity since AQ-A1, the dimethoxy analog of DAQ-A1 did not cause any cytotoxic action.

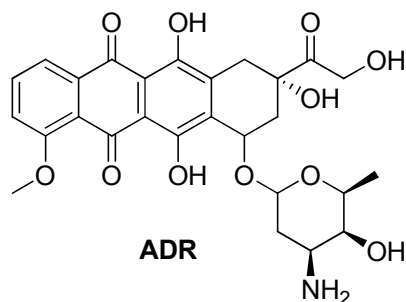


Figure 1.2: Adriamycin (ADR)

This group continued their investigation and determined that only one hydroxy group is necessary to elicit cytotoxicity.⁶ They did this by synthesizing several derivatives of DAQ-A1, where the dihydroxybenzoquinones were either monosubstituted or disubstituted with acetyl, methoxy, and/or amino groups; and their cytotoxicity towards P388 cells were examined (Table 1.2). The analogs bearing at least one hydroxy group displayed similar or more potent cytotoxic activity than DAQ-A1 against P388 cells. The analog ARQMeAc (Table 1.2, entry 5) does not possess a free hydroxy group anywhere in the molecule; however, it exhibited the best result with an IC_{50} of 0.56 μ M. This suggested that the acylated DAQ-A1 derivatives were converted into corresponding free hydroxy derivatives by hydrolysis of the ester group(s) in the cells.

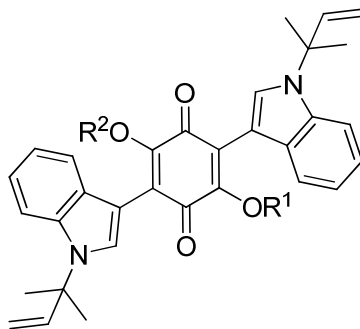


Table 1.2: ARQ derivatives used to study cytotoxicities

Entry	Compound	R ¹	R ²	IC ₅₀ (μM)
1	ARQ	OH	OH	1.28
2	ARQAc	OAc	OH	1.25
3	ARQDAc	OAc	OAc	1.07
4	ARQMe	OH	OMe	0.74
5	ARQMeAc	OAc	OMe	0.56
6	ARQDMe	OMe	OMe	>100
7	ARQA	NH ₂	OMe	0.75
8	ARQAMe	NH ₂	OMe	>100
9	ARQDA	NH ₂	NH ₂	>100

In 1994 the Kaji group isolated four new metabolites of *Aspergillus terreus* (Figure 1.3). The structures of these compounds were determined by chemical and spectral data. These four metabolites, along with DAQ-A1 (control), were tested against mouse leukemia P388 cells. DAQ-A1 inhibited cell growth with an IC₅₀ of 1.28 μM, and ARQ monoacetate, isoARQ, and neoARQ inhibited cell growth with a potency similar to that of DAQ-A1. Compound ARD, however did not affect the growth of mouse leukemia

cells, suggesting that the benzoquinone unit of these natural products is necessary for activity.⁷

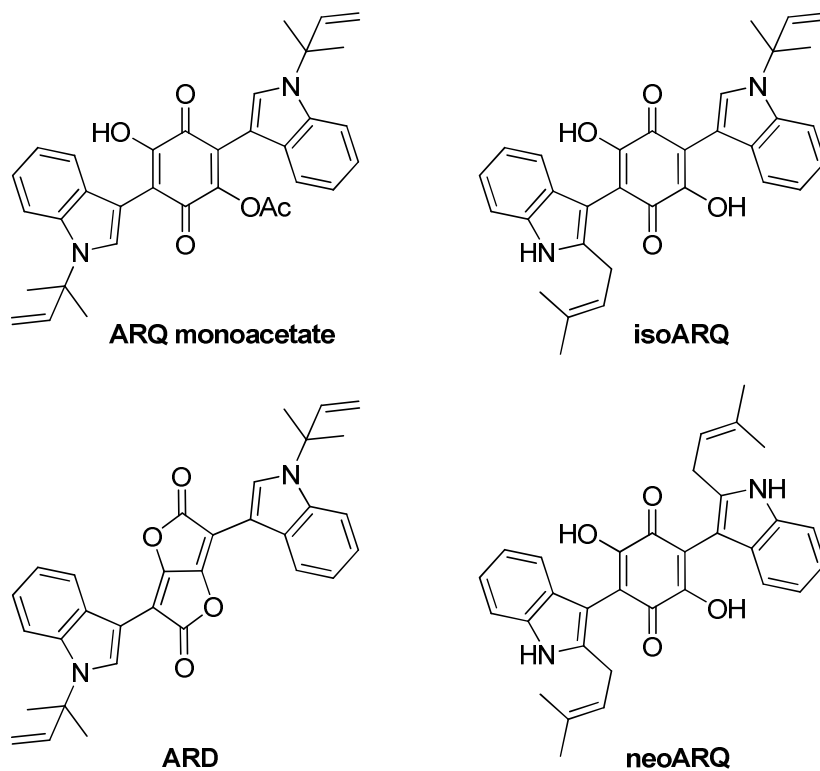


Figure 1.3: *Aspergillus terreus* metabolites tested for decrease in leukemia cell growth

1.2.2 *Asterriquinones as Inhibitors of HIV*

The step of reverse transcription from viral RNA to proviral DNA is unique for retrovirus infection to and multiplication in host cells. Thus, virus-associated reverse transcriptase is a target for effective antiretroviral agents. This is one of two key biological targets to which therapeutic drugs have been developed; the other being HIV-1 protease. This is verified by the fact that many reverse transcriptase inhibitors impede

human immunodeficiency virus (HIV) replication *in vitro* and *in vivo*.⁸ Many of these inhibitors that have been tested against HIV come from different sources such as a laboratory synthesis, or a natural product. The Ono group, from Japan tested a natural product, DAQ-A1, and its analogs (Figure 1.4) for inhibition of HIV-1 reverse transcriptase.⁹ Inhibition was examined in the presence of various concentrations of asterriquinones. Enzyme activity was found to be inhibited by approximately 90% in the presence of 5 μ M C1-1 or D-1 and by 75% in the presence of 5 μ M B1-4. The degree of inhibition was dose-dependent and nearly 100% inhibition was observed at 10 μ M C1-1 or D-1. DAQ-A1, the original asterriquinone, was less effective in inhibition than the other three compounds.

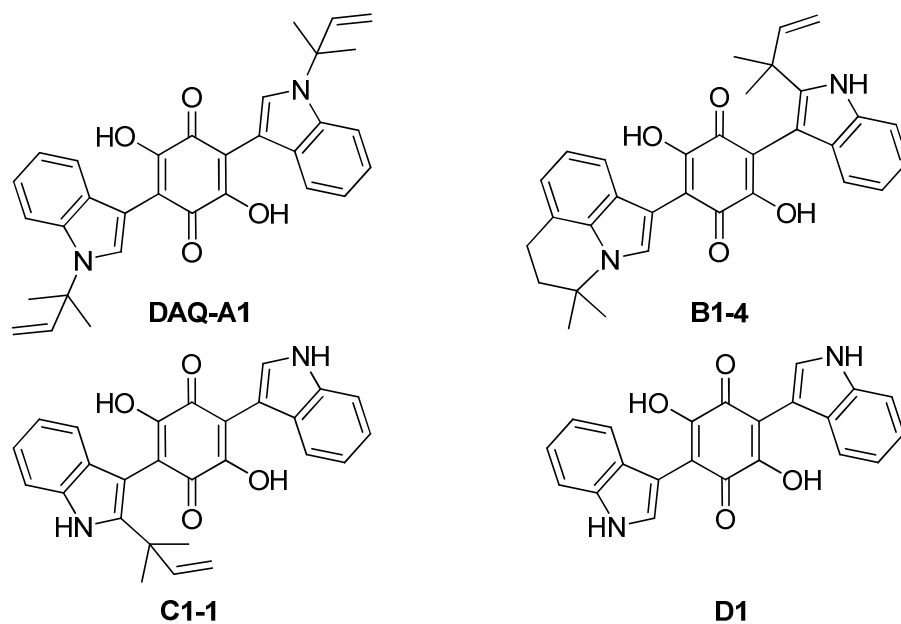


Figure 1.4: Asterriquinones examined for inhibition of HIV-reverse transcriptase activity

In addition to reverse transcriptase, HIV-1 encodes for an aspartic protease that is essential for maturation of infectious virus. A group of scientists from Switzerland pursued a drug discovery program aimed at identifying inhibitors of the HIV-1 protease. Their research led them to identify two novel asterriquinones, semicochliodinol A and semicochliodinol B (Figure 1.5) and two asterriquinones that have been isolated previously, asterriquinone-D1 (Figure 1.4) and cochliodinol (Figure 1.1), from the fungus *Chrysosporium merdarium*. These four compounds were tested for enzyme inhibitory activity.¹⁰ Asterriquinone-D1, semicochliodinol A, and cochliodinol inhibited HIV-1 protease with IC₅₀ values of 0.24, 0.37, and 0.18 μM, respectively. Semicochliodinol B displayed weaker inhibition properties, with an IC₅₀ value higher than 0.5 μM. After determination of activity against HIV-1 protease, all four compounds were tested for inhibition of epidermal growth factor receptor protein tyrosine kinase (EGF-R PTK), and displayed activity with IC₅₀ values ranging from 15-60 μM.¹⁰

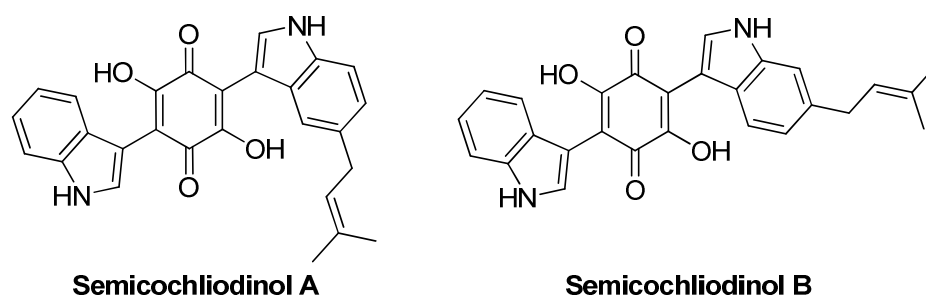


Figure 1.5: Asterriquinones examined for HIV-1 protease and EGF-R PTK inhibition

Currently, at least seven HIV-1 protease inhibitors are marketed as drugs. Due to the quick emergence of drug-resistant strains of HIV, use of HIV-1 protease inhibitors as a clinical monotherapy, in the absence of other antiviral agents, is contraindicated. Currently, a cocktail of antiviral, anti-fungal, and/or anti-bacterial drugs is used in the treatment for HIV-1. The discovery of novel HIV-1 protease inhibitors would considerably enhance the management of the disease. A Merck group screened numerous compounds for the inhibition of HIV-1 protease and discovered hinnuliquinone, also known as DAQ-B4 (Figure 1.6), a C_2 symmetric dimeric bis-indolylquinone from the fungal species *Nodulisporium hinnuleum*.¹¹ The first evaluation of DAQ-B4 in the HIV-1 protease screening assay revealed an IC_{50} value of 2 μ M. It was also tested against protease isolated from both wild-type virus and mutant HIV that is known to be resistant to commercially available HIV-1 protease inhibitors. Inhibition of wild-type and mutant protease occurred with IC_{50} values of 2.5 and 1.8 μ M, respectively.

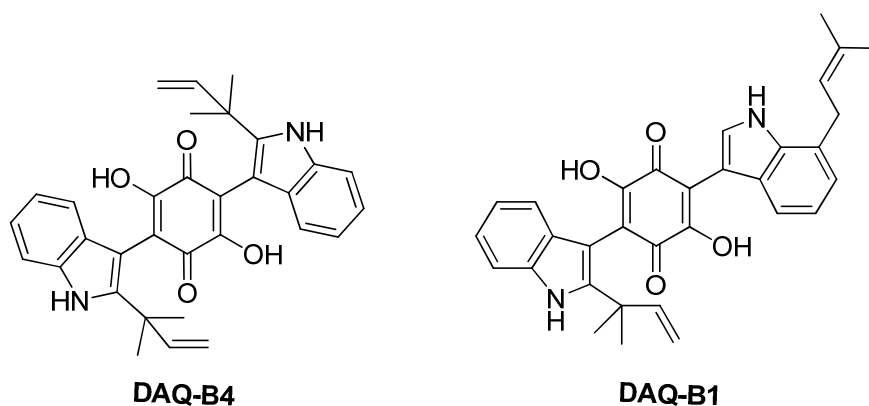


Figure 1.6: Structures of DAQ-B4 and DAQ-B1 (L-783,281)

In order to determine whether the inhibition was due to the reactive nature of the quinone moiety, other benzoquinones, including DAQ-B1 (Figure 1.6) were evaluated against wild-type HIV-1 protease. These compounds were found to be inactive, with DAQ-B1 having an IC_{50} value of $\sim 57 \mu\text{M}$, suggesting that the inhibitory potential of DAQ-B4 is due to structural features of the molecule other than the quinone.¹¹ Furthermore, DAQ-B4 was inactive in assays demonstrating DAQ-B1's ability to lower blood glucose (discussed later in this text), suggesting that the distinct and mutually exclusive activities of DAQ-B1 and DAQ-B4 is due to the C_2 symmetry present in DAQ-B4, but not in DAQ-B1.

1.2.3 Asterriquinones as a Potential Diabetes Therapy

In 1999 a group of scientists from Merck Research Laboratories discovered an asterriquinone in a screen for small molecule insulin receptor activators, and identified it as L-783,281, or demethylasterriquinone-B1 (DAQ-B1, Figure 1.6).¹² DAQ-B1 was isolated from the fungus *Pseudomassaria* and was found to induce 50% of the maximal effect of insulin on insulin receptor tyrosine kinase (IRTK) activity in Chinese hamster ovary cells over-expressing the human insulin receptor (CHO.IR) with a 3-6 μM IC_{50} . It induced phosphorylation of the insulin receptor β (in its tyrosine kinase domain), but not insulin-like growth factor receptor or epidermal growth factor receptor. Like insulin, activation of insulin receptor in β -cells by DAQ-B1 stimulates insulin gene transcription ($EC_{50} = 50 \text{ nM}$).¹³ However, it does not cause undesirable proliferation of vascular smooth muscle cells, whereas insulin does.¹⁴ Moreover, it was able to enhance the

insulin-stimulated IRTK activity at a lower concentration (0.6-2 μM) than the non-insulin-stimulated IRTK (3-6 μM); while DAQ-A1 (L-767,827), a structurally related analog (Figure 1.1) was ~ 100 times less active. These results indicate that DAQ-B1 is an insulin mimic; however, it does not compete with insulin since it was shown to bind to the intracellular domain of the receptor instead of binding to the extracellular domain, as growth factors do. Furthermore, this group demonstrated how DAQ-B1 was able to activate other components of the insulin signaling pathway, such as PI3 kinase, and Akt kinase in CHO.IR cells. Further testing demonstrated the ability of DAQ-B1 to cause dose-dependent glucose lowering in two mouse models of diabetes when administered orally. The initial studies on DAQ-B1 established novel insulin receptor activators that may lead to a potential new therapy for diabetes. However, improvements in potency and selectivity were necessary. Structure-activity relationship (SAR) studies of DAQ-B1 were conducted by the Merck group and they discovered that an *N*-methyl asterriquinone analog (Figure 1.7) was among the most potent and specific activator of human IRTK in intact mammalian cells of the series of compounds, with an IC_{50} of 0.3 μM .¹⁵

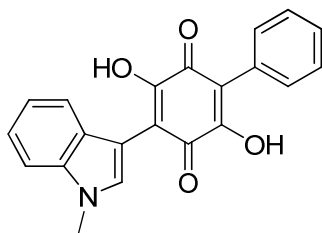


Figure 1.7: *N*-Methyl asterriquinone analog, an insulin mimic

Oral administration of this *N*-methyl asterriquinone analog to diabetic mice lowered blood glucose levels in a dose-dependent manner. Furthermore, it caused significant decrease in plasma insulin concentration, correction of mild hyperglycemia, and did not exhibit overt toxicity at pharmacological doses in the animal models.¹⁵

1.2.4 Asterriquinones as a Potential Therapy for Neurodegenerative Disorders

Neurotrophins play an essential role in the maintenance of neuronal populations of cells. Studies have shown that subcutaneous or intravenous administration of neurotrophins may be an effective treatment for peripheral neurodegenerative disorders. The receptors that bind neurotrophins are members of a family of highly similar transmembrane tyrosine kinases known as TrkA, TrkB, and TrkC. Each neurotrophin binds to a preferred receptor in the family: nerve growth factor (NGF) binds mainly TrkA, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) bind TrkB, and neurotrophin-3 (NT-3) binds TrkC. Neurotrophin binding to Trk receptors generates a cellular signaling cascade that produces neurotrophic effects such as neuronal survival and neurite growth. Preclinical and clinical data suggest that neurotrophins are a promising therapy for peripheral neuropathies¹⁶ and neurodegenerative diseases, such as Alzheimer's¹⁷ and Parkinson's disease.¹⁸ Neurotrophins, however, are not good drug candidates because of their poor pharmacokinetic behavior and bioavailability at the desired targets. Moreover, they are unable to cross the blood brain barrier. Much effort has been devoted to the search for nonpeptidyl small molecule neurotrophin mimics that

elicit the desired neuroregenerative responses of neurotrophins. As stated previously, DAQ-B1 activated the human insulin receptor at low micromolar concentrations. Given that Trk receptors belong to the same broad class of single transmembrane receptor tyrosine kinases, DAQ-B1 was evaluated for effects on this family of receptors, using CHO cell lines constructed to overexpress the different Trk receptor subtypes and primary cultures of cortical neurons.¹⁹ In the CHO-Trk cell-based assay, DAQ-B1 was able to activate all three Trk receptor subtypes, to different extents, at micromolar concentrations. In the same way DAQ-B1 was shown to act on the intracellular kinase domain of the insulin receptor, the Wilkie group was able to show that it also acted on the intracellular domain of Trk. Consequently, DAQ-B1 has a site of action different from the extracellular site of action for the neurotrophins. Further studies done by this group also suggested that DAQ-B1 was capable of activating Trk receptor dimerization and phosphorylation as well as downstream signaling pathways by interacting at a site within the cytoplasmic domain of the Trk receptor. One problem that arose in these investigations was that the cytotoxicity of DAQ-B1 prohibited the study of its effects in neuronal survival and neurite outgrowth assays. Although this compound itself is unsuitable as a drug candidate, it did activate three members of the Trk family of neurotrophin receptors, and it demonstrated an ability to promote receptor dimerization.

1.2.5 Other Therapeutic Uses for Asterriquinones

The discovery of DAQ-A1 and other biologically active asterriquinones encouraged scientists to search for other *Aspergillus* strains and test metabolites from those strains for

biological activity. In 1996 a group of scientists from San Francisco and Washington collaborated to isolate microorganisms from soil samples collected from Nevada and Mexico. This group was able to discover five asterriquinones from the fermentation broth of *Aspergillus*, *Humicola*, and *Botryotrichum* species (Figure 1.8).²⁰ The structures of these asterriquinones were determined by NMR and FAB-MS experiments. Once structure elucidation was complete, the effect of these compounds on proteolytic activity of several blood coagulation cascade enzymes was measured. Coagulation complexes have been implicated in conditions such as deep vein thrombosis, and coagulation inhibitors may be suitable as therapeutics. These five compounds were found to inhibit coagulation complexes with IC₅₀ values ranging from 6–28 μM. Furthermore, these compounds did not interfere with structurally related serine proteases such as thrombin and trypsin, making them useful as specific inhibitors.

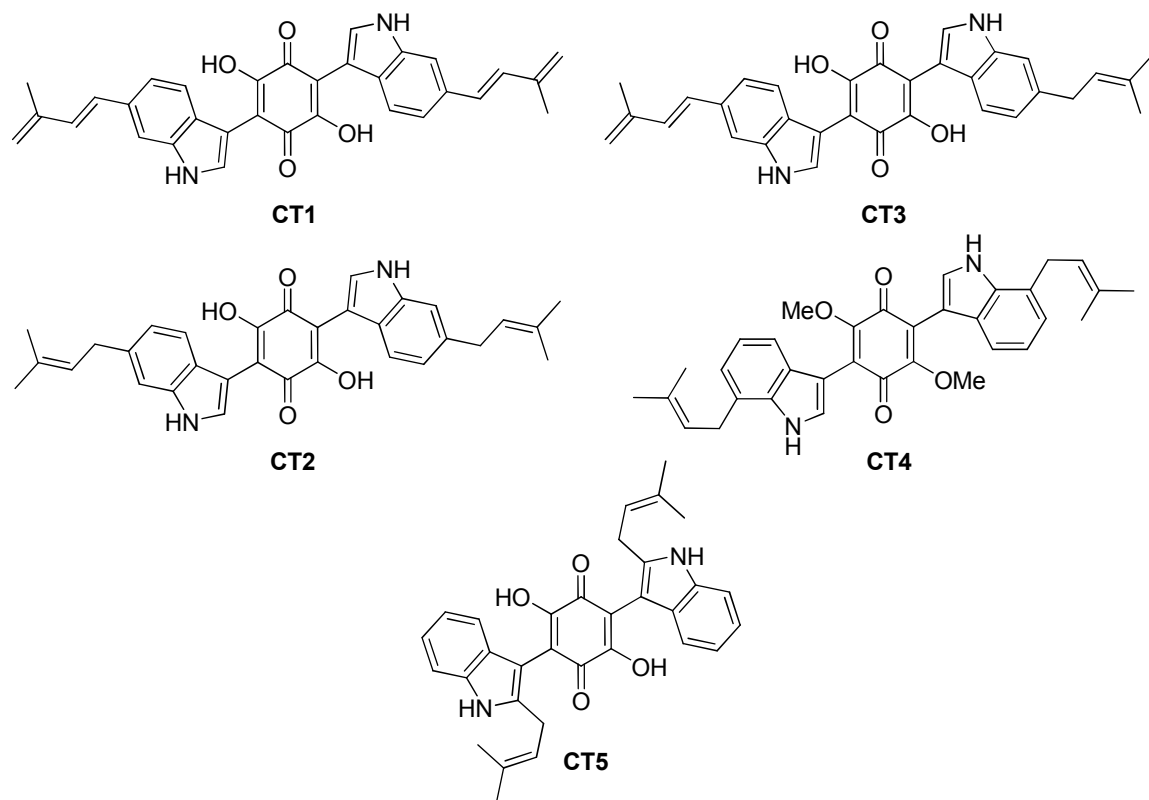


Figure 1.8: Asterriquinones examined for coagulation inhibition

1.3 Chemical Manipulations of Naturally Occurring Asterriquinones to Enhance Therapeutic Effects

1.3.1 Advances Made by the Yamamoto Group

As mentioned previously, after isolation of DAQ-A1, the Yamamoto group isolated eleven naturally occurring asterriquinones. In order to determine the structures of these natural products, they performed numerous chemical manipulations to obtain demethylated compounds.²¹ They discovered that hydroxy benzoquinone derivatives (demethylated asterriquinones) had more potent antitumor activity than the methoxy benzoquinones. Although this group was successful in forming the hydroxy analogs of the asterriquinones, they observed many side reactions with the methods they were using; including rearrangement, hydration, and cyclization of isoprenyl groups (Figure 1.9). These side reaction products were tested for antitumor activity, along with DAQ-A1. DAQ-A1 was very active against Ehrlich ascites carcinoma, and gave a dose-related prolongation of life span, while none of the side reaction products were effective, even at a 30 mg/kg dose.²² The Yamamoto group was able to show that the asterriquinones they isolated (Table 1.1) were effective in inhibiting the growth of several transplantable animal tumors. However, these compounds were found to exhibit unsatisfactory antitumor activities as compared with mitomycin C (Figure 1.10), an aziridine containing benzoquinone used as a chemotherapeutic. Thus, they proceeded to chemically modify the natural asterriquinones they isolated in hopes of increasing potency of these compounds.

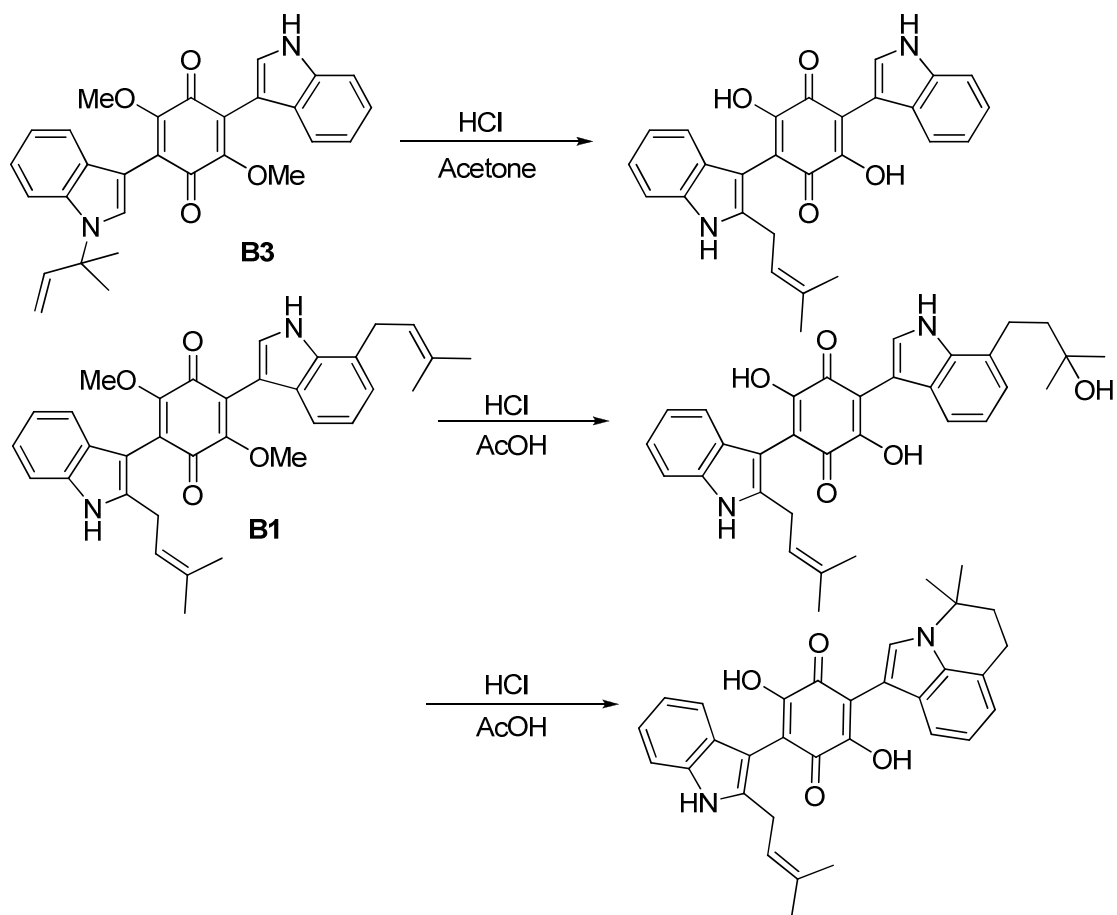


Figure 1.9: Side products from the hydrolysis of asterriquinones

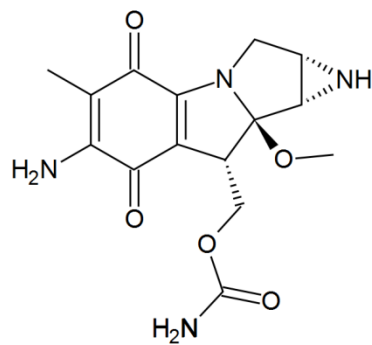


Figure 1.10: Mitomycin C

The first modifications consisted of replacing the functional groups at the 3 and 6 positions of the benzoquinone moiety. This was followed by alternating prenyl, isoprenyl, and allyl groups on the 1 and 2 positions of the indole rings to form seven new analogs. These chemical modifications are seen in Table 1.2.²³

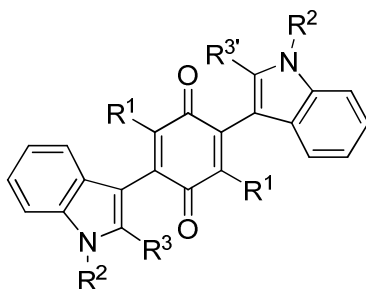



Table 1.3: Chemically modified asterriquinone compounds

Entry	Compound	R ¹	R ²	R ³	R ^{3'}
1	Amino-ARQ	NH ₂	Isoprenyl	H	H
2	Aziridinyl-ARQ		Isoprenyl	H	H
3	AQ C1	OMe	H	Isoprenyl	H
4	AQ C1-2	OH	Prenyl	H	Isoprenyl
5	AQ C1-3	OH	Allyl	H	Isoprenyl
6	AQ D	OMe	H	H	H
7	AQ D-2	OH	Prenyl	H	H

In order to test these compounds, male mice were either intraperitoneally or subcutaneously implanted with Ehrlich carcinoma cells. The effects of the asterriquinone derivatives on Ehrlich carcinoma in mice are shown in Table 1.4. Modification of the

DAQ-A1 to the amino (entry 2) or aziridinyl (entry 3) derivative failed to enhance antitumor potency, which is not a result they expected since synthesis of these analogs was based on the potent anticancer agent mitomycin C (aziridine containing benzoquinone). One of the asterriquinones that proved to be inactive was AQ-C1. In an attempt to augment antitumor potency, inactive AQ-C1 was modified to the *N*-prenyl derivative AQ-C1-2, which led to an increase in potency (entry 4). Modification of AQ-C1 to the *N*-allyl derivative AQ-C1-3 did not show any change (entry 5). AQ-D-2, produced by *N*-prenylation of AQ-D, was also ineffective. Thus, these results led them to the conclusion that in order to obtain a heightened potency of these asterriquinones, it is necessary to introduce hydrophilic groups, as well as demethylate the benzoquinone ring.

Table 1.4: Effect of the asterriquinone derivatives on Ehrlich carcinoma in mice

Entry	Compound	Dose (mg/kg/d)	MST ^a Treated	MST ^a Control	ILS ^b (%)	MTW ^c Treated	MTW ^c Control
1	DAQ-A1	30	31.5	14.0	125	0.55	2.05
2	Amino-ARQ	30	17.0	16.0	6	4.95	4.97
3	Aziridinyl-ARQ	10	15.0	18.0	-	2.99	3.65
4	AQ-C1-2	30	25.5	14.0	82	0.94	2.05
5	AQ-C1-3	30	14.0	14.0	0	1.43	2.05
6	AQ-D-2	30	17.0	14.0	21	1.36	2.05

^aMedian survival time. ^bIncrease of life span. ^cMean tumor weight (g)

1.3.2 Advances Made by the Pirrung Group

The wide range of biological activity exhibited by asterriquinones prompted our laboratory to develop novel synthetic methods for the preparation of their 3-indolylquinone precursors. Such methods are required to facilitate total syntheses of asterriquinones or to prepare reagents designed to probe the biological activity of the indolylquinones. As a starting point, our group examined anaerobic condensation reactions of 2-methylindole with a variety of 2,5-dihalogenated or 2,5-dioxygenated benzoquinones, promoted by stoichiometric hydrochloric acid (HCl) in tetrahydrofuran (THF). The resulting indolylhydroquinone product was oxidized with dichlorodicyanobenzoquinone (DDQ). The benzoquinone that gave the best result was found to be 2,5-dichloro-1,4-benzoquinone (**1**). This benzoquinone was then utilized in a reaction sequence using different indoles (**2(n)**) in a three step, one-pot protocol.²⁴ Standard reaction conditions included one equivalent (equiv) of **2(n)**, two equiv of **1**, and one equiv of concentrated HCl in THF. After stirring overnight at room temperature, one equiv of DDQ was added. Finally, the 3-indolylquinones were obtained after hydrolysis with 10% sodium hydroxide (NaOH) in methanol (MeOH) (Scheme 1.1).

Scheme 1.1: Synthesis of 3-indolyl-2,5-dihydroxybenzoquinones

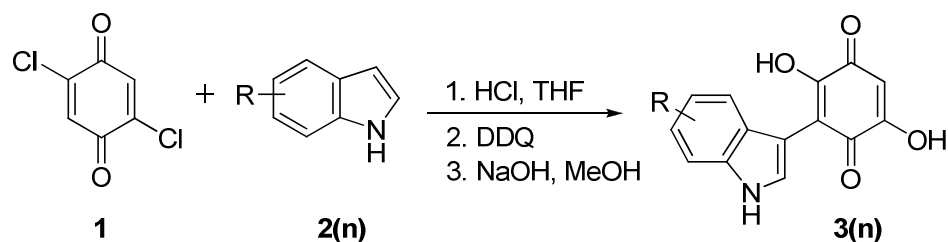


Table 1.5: Indoles used in Scheme 1.1

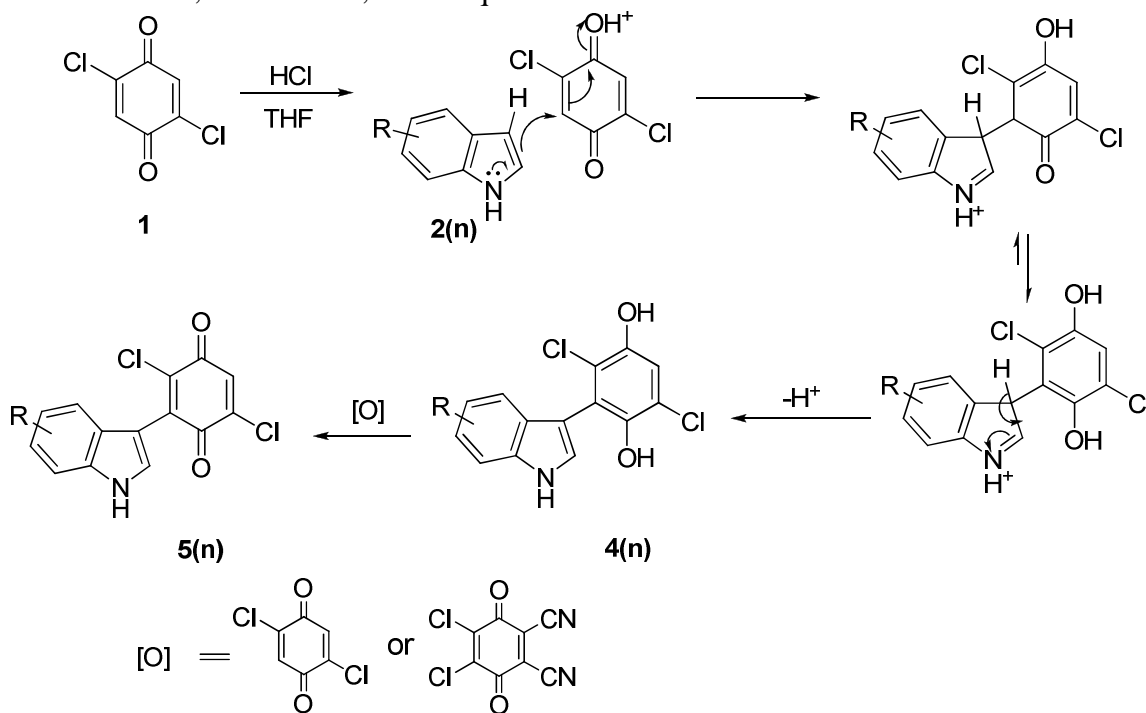
Entry	R	% Yield ^a	% Yield ^b
1	2-Methyl	97	62
2	2,5-Dimethyl	91	73
3	2-Methyl-5-methoxy	89	81
4	2-Methyl-5-chloro	75	87
5	2-Cyclopropyl	75	87
6	7-Prenyl	-	50 ^c
7	2-Phenyl	89	65
8	N-Methyl	87	62
9	2-Ethyl	95	77
10	2-Isopropyl	70	80
11	<i>tert</i> -Butyl	54	52
12	7-Methyl	81	66
13	H	70	59

^aAfter step 2. ^bAfter step 3. ^cYield for two steps.

Our group envisioned the mechanism of this reaction to commence with the addition of the nucleophilic carbon-3 of the indole to the unsubstituted position of the quinone, which was activated by protonation (Scheme 1.2). That addition product tautomerized to the hydroquinone **4n**. Generally, two equiv of quinone were used per equiv of indole to promote the oxidation from hydroquinone to benzoquinone. However,

the product in this case was a mixture of 3-indolylquinone (**5(n)**) and the corresponding hydroquinone (**4(n)**). To rectify this problem, DDQ was added to the mixture after the condensation reaction to yield the product in a single oxidation state.

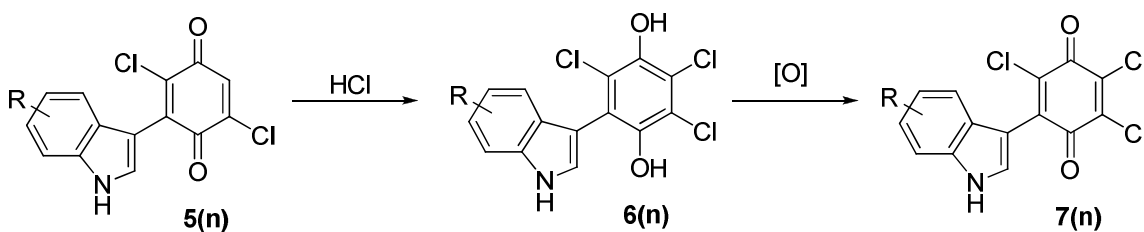
Scheme 1.2: Proposed mechanism of the acid-catalyzed condensation of an indole with 2,5-dichloro-1,4-benzoquinone



The reactivity of indoles in this condensation reaction is dependent on both steric and electronic factors. The most electron-rich indoles bearing a substituent at the 2-position were the most successful reactants; whereas indoles bearing electron-withdrawing groups do not react at all. Indoles that do not possess any substitution on the heterocycle gave a lower yield (Table 1.5, entry 13). This reaction is also limited by steric effects; indoles with a *tert*-alkyl substitution higher than 2-*tert*-butylindole (such as 2-

isoprenylindole) failed in this reaction. Furthermore, although this HCl-catalyzed process was an encouraging initial step toward general synthetic access to 3-indolylquinones, it was limited by side reactions involving HCl (Scheme 1.3). In some cases (Table 1.5, entries 3 and 4), the 3-indolylquinones reacted with HCl, leading to a trichlorohydroquinone (**6(n)**) that is further oxidized to give the side product, trichloro-3-indolylquinone (**7(n)**). The presence of chloride ion caused these unexpected problems thus, limiting this reaction. In addition, many desired 3-indolylquinones could not be synthesized using this procedure. Therefore, improvements needed to be made.

Scheme 1.3: The chlorination side reaction of the indole-benzoquinone condensation



These improvements were sought by examining other mineral acid promoters that would not offer nucleophilic counterions that could add to the product. A selection of acids was screened by the Pirrung group, and sulfuric acid (H_2SO_4) was found to be superior.²⁵ A large range of indoles were examined using these reaction conditions. In general, most indoles responded well to these conditions, producing 3-indolylquinones in good yield. However, these conditions were still not suitable for all indoles tested. The sensitivity of certain indoles to acid required the use of an even milder acid catalyst. Several organic acids, such as acetic acid (AcOH), *p*-toluenesulfonic acid, and

trifluoroacetic acid were screened. Refluxing AcOH was found to be very effective in promoting the addition reaction. In an effort to further improve this procedure, our lab also looked at the oxidation phase of the transformation. The potency of DDQ is unnecessary, as partial oxidation occurs with **1**. This led them to use silver carbonate on Celite, which can be removed by a simple filtration thus, making this transformation more convenient.²⁵ In general, our laboratory designed three different protocols for this addition reaction where, depending on the indole used, HCl, H₂SO₄, or AcOH was utilized as an acid catalyst. Using any of those protocols, alkylindoles gave good to excellent yields, halogenated indoles gave moderate yields, and indoles bearing electron withdrawing groups were unreactive. Moreover, having a substituent at the 4-position of the indole affects the reaction negatively due to the steric effects in such close proximity to the nucleophile.

Another study conducted by our group involved the production of dihydroxybenzoquinones from dichlorobenzoquinones. The transformation from dichloro- to dihydroxy- was done by the Harris group with potassium hydroxide (KOH) in MeOH.²⁶ These conditions are problematic for 3-indolyldichlorobenzoquinones because the unsubstituted quinone carbon can be readily attacked by hydroxide, leading to a hydroxylated **5(n)**. Investigations into hydrolysis experiments led to a protocol involving the addition of 10% NaOH to a warm solution of **5(n)** in MeOH. A challenging aspect of preparing these compounds is their instability to standard silica gel chromatography. The Harris group purified such compounds by reverse-phase HPLC. However, this limits the preparative scale on which such reactions can be carried out.

Thus, our group adopted the Yamamoto method for purification of these compounds, namely the use of oxalic acid-coated silica gel for column chromatography. Production of **3n** using these hydrolysis and purification methods proceeded in 52-90% isolated yields.²⁵

Approximately fifty 3-indolylquinones (**3(n)**) were synthesized by our group. These compounds were discovered to be a new class of Cdc25 phosphatase inhibitors.²⁷ Cdc25 phosphatases are regulatory proteins that modulate the activity of cyclin-dependant kinases (Cdks), which play a central role in controlling cell cycle progression. Three specific isoforms of Cdc25 exist in human cells, Cdc25A, Cdc25B, and Cdc25C. Cdc25A and Cdc25B have been consistently linked to a wide variety of cancers since they are important cell cycle regulators. For example, a high percentage of breast cancer patients displayed an overproduction of both Cdc25A and Cdc25B, where elevated expression levels are inversely associated with life expectancy. The Cdc25s have become appealing targets for drug development due to their important role in cell cycle regulation and their link to a wide variety of cancers. The 3-indolylquinones tested for inhibition were shown to bind reversibly to the active site with submicromolar potency. Structure-activity relationships displayed consistent trends that identified features required for inhibition of all of the Cdc25s. As inhibition did not appear to be time-dependent, our group proposed that these compounds do not oxidize the active site nor do they form covalent adducts with it. *In vitro* testing indicated that the 3-indolylquinones compete effectively with the protein substrate for Cdc25. *In vivo* testing led to a rapid death of the cells, without an increase in phosphorylation of the Cdks. Thus, the 3-indolylquinones

were viewed as useful lead compounds for drug discovery and further cell-based studies on the role of all three isoforms of Cdc25 in cell cycle control.

As mentioned previously, oral administration of DAQ-B1 caused lowering of glucose levels in diabetic mice without causing proliferation of vascular smooth muscle. This enabled DAQ-B1 to qualify as the first selective insulin receptor modulator (SIRM). In order to better understand some of its *in vivo* actions, the metabolic and transcriptional effects of DAQ-B1 were studied in cellular models by the Webster group, in collaboration with our laboratory.²⁸ Testing was conducted in rat fibroblasts that over-express human insulin receptor (IR). DAQ-B1 demonstrated activation of the extracellular-regulated kinase (ERK) similar to that achieved by insulin, but enhanced selective activation of the Akt kinase ($EC_{50} = 6 \mu\text{M}$). These features of DAQ-B1 indicate that it is a more selective agonist for the PI3 Kinase / Akt pathway than insulin. In adipocytes, DAQ-B1 promotes a dose-dependent increase in glucose transport. Furthermore, DAQ-B1 did not appear to directly compete with insulin since it did not inhibit any insulin-stimulated transport at the dose required for maximal effect. In addition, DAQ-B1 did not seem to have an inhibitory effect on signaling. In hepatoma cells, DAQ-B1 was found to up-regulate metabolic gene expression whereas insulin induced expression of cell-cycle genes. The ability of DAQ-B1 to enhance Akt phosphorylation relative to ERK and its failure to induce proliferative gene expression are what distinguish its SIRM properties.

Molecular recognition of the natural product, DAQ-B1 by its cellular receptor(s) has been studied by our laboratory using a methyl scan, which is a systematic approach

involving synthesis of a set of methylated derivatives and evaluation of their activity in cells.²⁹ In this process, twelve derivatives were synthesized where all aromatic positions not bearing a prenyl unit were substituted with methyl groups. If there was close contact between target proteins and a particular site, a methyl group at that site would sterically exclude binding. The sites where this chemical modification decreased activity are called "hot spots" and those sites where addition of a methyl group does not significantly affect activity are called "null spots". The positions of substitution for methyl scanning on DAQ-B1 are shown in Figure 1.11(A).

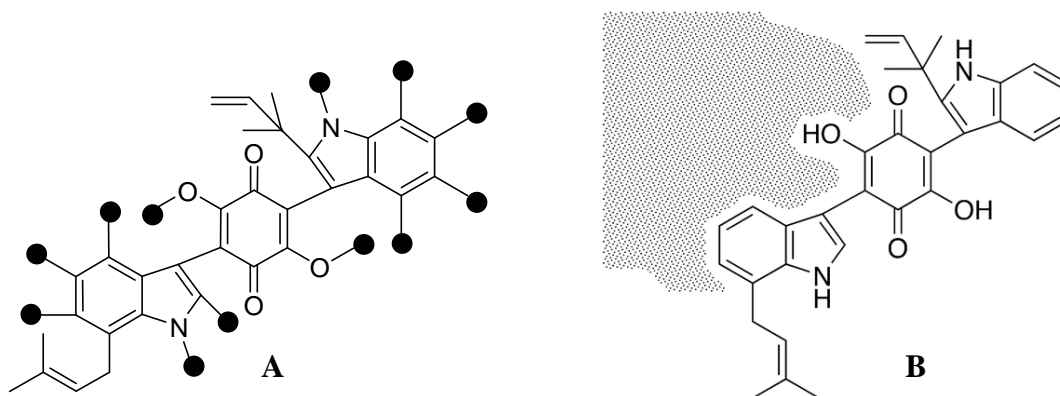


Figure 1.11: (A) Positions of substitution on DAQ-B1 for methyl scan. (B) Hot spots identified by methyl scan

Each of the twelve derivatives synthesized was examined for activation of human insulin receptor (hIR) tyrosine kinase autophosphorylation in a rat fibroblast cell line overexpressing the receptor (hIRcB).²⁸ Dose-response studies were performed and the percent activation by each compound at 30 μ M identified that the hot spots for protein-small molecule interaction are the 7-substituted indole ring and the hydroxy groups

(Figure 1.11(B)); the 2-isoprenylindole unit is of lesser importance. The methyl scan identified the part of DAQ-B1 that could potentially be responsible for activity, thus leading to the pharmacophore model, compound ZL-196 seen in Figure 1.12.

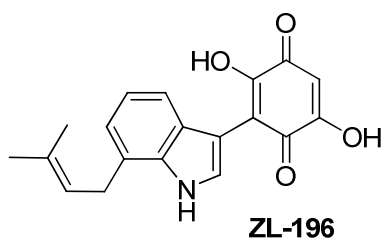


Figure 1.12: Compound ZL-196 – the pharmacophore unit of DAQ-B1

The asterriquinone compounds possess a unique ability to interact with a wide range of enzymes such as growth factor receptors and phosphatases; with a particular relevance to tyrosine kinase/protein phosphatase relationships. This characteristic suggests that they could be considered privileged structures. In order to evaluate the diverse biological activities and actions against various targets of asterriquinones, access to a wide variety of these compounds was necessary. The most efficient way to quickly produce a large library of compounds is through a combinatorial approach. The purpose of designing this bis-indolylquinone library was to discover novel DAQ-B1 analogs that could potentially display activity against the insulin receptor as well as potentially display biological activity against a large variety of targets. Development of synthetic approaches to asterriquinone derivatives has been made possible by the modular synthesis developed in our laboratory (discussed above). The indole building blocks were selected based on their commercial availability, given that they meet the coupling reactions' structural

criteria. A total of 35 indoles – 26 commercially available and nine prepared in our lab – and 21 indolylquinones were used in the acid-promoted coupling step.³⁰ These reactions were applied in parallel on an Argonaut Quest 210 synthesizer that accommodates 20 reaction vessels. Through solution-phase combinatorial synthesis, 424 diverse bis-indolylquinones were prepared from 21 indolylquinones and 35 indoles. The yields ranged from 6% to 100% and the purities of the products ranged from 50% to 100%. The compounds that were prepared in quantities of more than 10 mg and purities of greater than 80% were selected for hydrolysis.

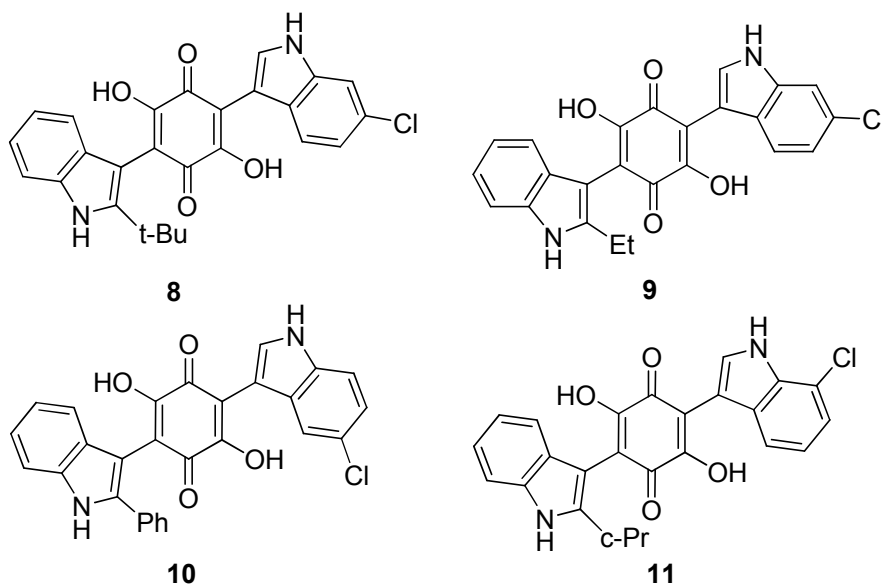


Figure 1.13: Most potent compounds from the parallel synthesis of indolylquinones

Of the 305 compounds selected, 269 (88%) yielded the desired hydrolysis product with yields ranging from 14% to 100% and an average purity of 70%.³⁰ Stock solutions of each of the 269 compounds were prepared and they were evaluated for their ability to

activate the hIR tyrosine kinase domain in an engineered CHO cell line. The most active compounds were found to be **8**, **9**, **10**, and **11**; with **8** being the most potent.³⁰

All members of this library were then screened for the toxicity towards CHO cells. Compounds bearing 7-*tert*-butylindoles were found to impact cell viability the most. Quantitative structure-activity relationship (QSAR) analysis of cell viability data for compound treatments at 100 μ M was conducted. Compounds with an aryl group at the indole 2-position demonstrated the least impact on cell viability. A data set was also provided from QSAR analysis for insulin receptor activation that could be readily compared to the cytotoxicity data set. This model demonstrated that indoles bearing a bulky substituent at the 2-position enhanced activity, as with structures **8-11**. The comparison of activity data for cytotoxicity versus that for insulin receptor activation demonstrated that there is no correlation between the two; cytotoxicity is not activity dependent. Predictors of toxicity derived from the QSAR study are different from predictors of insulin receptor activation.³⁰ Thus, it could be possible to develop compounds that could separate the two properties.

Continued qualitative inspection of the data obtained from the QSAR study suggested that substitution on the indole 6- or 7-position was associated with higher IR activation. Two compounds, ZL-196 (Figure 1.12), which was previously synthesized, and LD-17 (Figure 1.14) were chosen for further study, both of which possessed large, bulky substituents on the 7-position of their indole ring.³¹

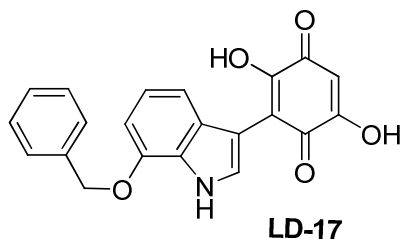


Figure 1.14: Structure of compound LD-17

Both compounds caused a dose-dependent increase in the phosphorylation of IR, Akt, and ERK and were able to stimulate downstream signaling as Akt and ERK phosphorylation was increased. At the cellular level, ZL-196, LD-17, and DAQ-B1 all dose-dependently increased glucose transport in adipocytes; and, both ZL-196 and LD-17 stimulated the production of glycogen and lipid from glucose to the same degree as insulin.³¹ When ZL-196 and LD-17 were administered as an oral suspension to both normal and diabetic mice, they displayed a hypoglycemic effect by lowering blood glucose levels acutely.

DAQ-B1 was initially shown to target the cytoplasmic tyrosine kinase domain of the β -chain of the insulin receptor. However, in order to develop analogs that selectively act on the insulin receptor and not other proteins, cellular targets other than the insulin receptor need to be identified. As mentioned previously, DAQ-B1 demonstrated the same glucose-lowering action as insulin, yet unlike insulin, DAQ-B1 does not promote the undesired proliferation of vascular smooth muscle cells of the endothelium. Our results discussed above suggested that this effect is based on the ability of DAQ-B1 to selectively stimulate the Akt branch of insulin signal transduction at the expense of the

ERK branch, whereas insulin stimulates both equally.²⁸ This could be because of the action of DAQ-B1 on other cellular protein targets. Our laboratory sought the identification of potential protein binding partners for DAQ-B1 using phage display cloning.³² This method consists of linking the small molecule to a solid phase to be used as an affinity matrix, then uses a library of proteins expressed on the surface of T7 phage to bind to it. In order to permit the immobilization of DAQ-B1 on an affinity matrix, a biotinylated form of the drug is used.³² Hence, a DAQ-B1 analog, derivatized with biotin at a position of the molecule that could be functionalized without altering its cell-based activity (as identified by methyl scanning) was synthesized. This compound, DAQ-biotin (Figure 1.15), was used as bait in phage display cloning.

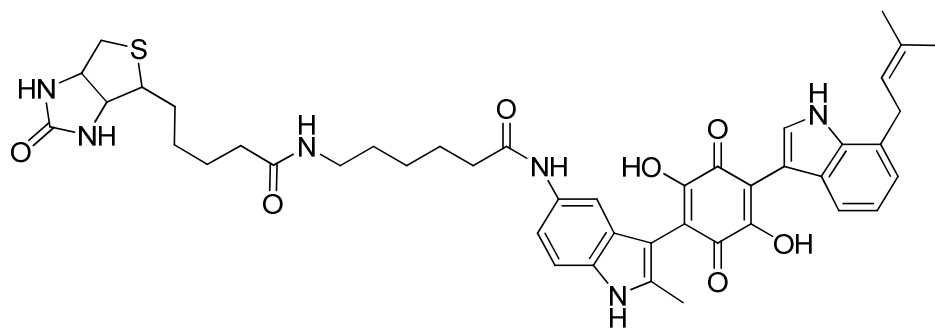


Figure 1.15: Bait molecule DAQ-biotin

The most interesting of the proteins identified was glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH is a recognized glycolytic enzyme, is involved in several nonglycolytic functions such as transcription and DNA repair, and binds strongly with the glucose transporter. Based on the work of Chang, who discovered a small

molecule that stimulates insulin signaling by acting on GAPDH³³, he reasoned that GAPDH, in its tetrameric form, is essential for promotion of cellular phosphatase activity that acts on phosphoinositides (such as phosphatidylinositol 3,4,5-trisphosphate). Thus, small-molecule binding caused impairment of GAPDH tetramerization, which maintained the phosphorylation state of phosphoinositides. This event was essential for insulin signaling to Akt. The ability of DAQ-B1 to selectively activate Akt could be ascribed to its capability of simultaneously activating the insulin receptor and inhibiting lipid phosphatase action upon phosphatidylinositol 3,4,5-trisphosphate. The Pirrung group utilized a technique known as surface plasmon resonance to verify binding of DAQ-B1 to GAPDH and measure its affinity.³² Results from this study demonstrated that binding of DAQ-B1 to GAPDH is not altered by the attachment of biotin, which had been predicted earlier by the methyl scan study. Consequently, an important discovery made here by our group was that human GAPDH is a cellular binding partner for DAQ-B1. Therefore, the cellular lipid phosphatase activity could be disrupted by binding of small molecules, such as DAQ-B1, to GAPDH. This could then potentiate insulin signaling via the PI3Kinase pathway, which is the branch of the insulin signaling pathway that promotes glucose transport. Another branch of the insulin signaling pathway, the MAPK branch, promotes proliferation of vascular smooth muscle cells. Thus, the claim that DAQ-B1 acts as a selective insulin receptor modulator can be verified by its ability to stimulate both the PI3Kinase and the MAPK pathways through human insulin receptor phosphorylation. Further verification is made by its ability to inhibit a lipid phosphatase activity, which enhances signaling in the PI3Kinase branch of intracellular insulin signal transduction.³²

As mentioned previously, DAQ-B1 demonstrated an ability to activate the NGF receptor. One problem, however, is that DAQ-B1 was toxic to neurons. Our laboratory, along with the Webster laboratory, hypothesized that compounds similar in structure to DAQ-B1 could potentially be used as oral NGF activators for neurotrophin therapy if they can activate signaling in the central nervous system.³⁴ Since these asterriquinone analogs target the kinase domain of TrkA, it may be possible to avoid the harmful effects caused by activation of the p75NTR receptor, which is an important advantage of these compounds. The p75NTR neurotrophin receptor is a member of the tumor necrosis factor (TNF) superfamily that lacks the tyrosine kinase domain and when activated, causes cell death rather than cell survival. Thus, DAQ-B1 was used as a lead compound and a library of closely related structures, synthesized using the combinatorial approach discussed above, were tested to identify nontoxic and specific NGF receptor activators that have neuroprotective properties.³⁴ A 334 compound library was screened for activation of TrkA using an ELISA assay that detects phosphorylated TrkA. Activation was observed by 91 compounds, which displayed activity of greater than 25% the effect of NGF. The parent compound DAQ-B1 activated TrkA to 61% of NGF. This data set was used to generate QSAR models for activation of TrkA, which suggested that substituents on carbons 2, 8, and 11 (Figure 1.16A) are crucial for the ability to activate TrkA. A library of 62 monoindolylquinone intermediates, the synthesis of which was previously discussed, was also tested against TrkA and many were found to activate it. QSAR was used to model the activity of the monoindolylquinones and the 4, 5, 6, and 7 (Figure

1.16B) substituents were found to be important for monoindolylquinone activation of TrkA.

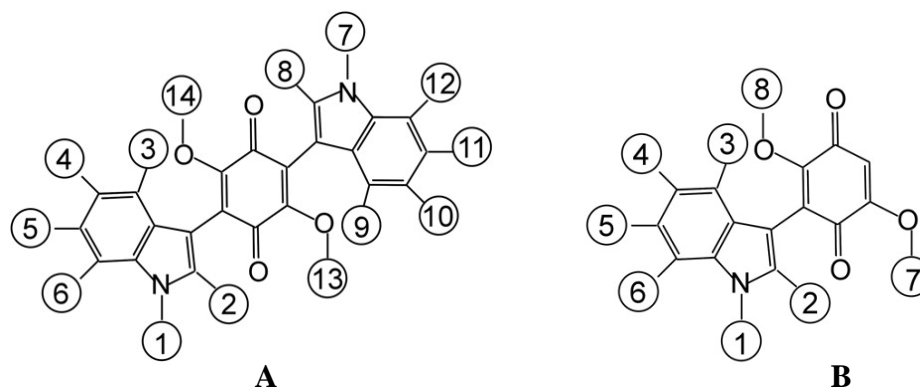


Figure 1.16: (A) Generic structure of the bisindolylquinone library. (B) Generic structure of the monoindolylquinone library

The toxicity of these compounds was measured against CHO-TrkA cells. A dose-dependent toxicity along with decreased cell viability was demonstrated by a large number of the compounds. This data was used to measure IC_{50} values, and the activation of TrkA did not correlate with IC_{50} for toxicity or cell viability at 100 μ M compound, suggesting that the toxicity is not dependent on activity. Out of 27 asterriquinones and six monoindolylquinones identified as Trk activators, two compounds, 1H5 and 5E5 (Figure 1.17) were selected for further study based on good NGF activator activity and low toxicity.

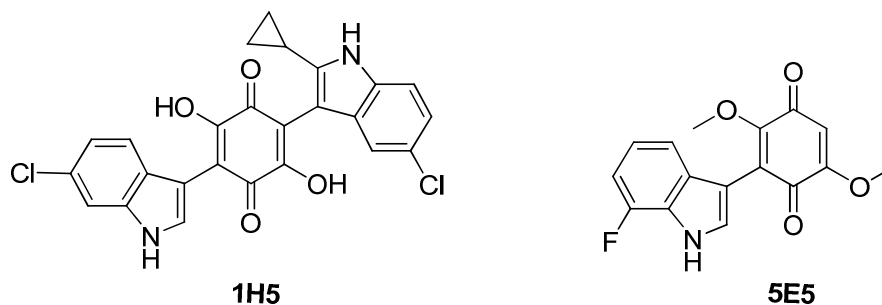


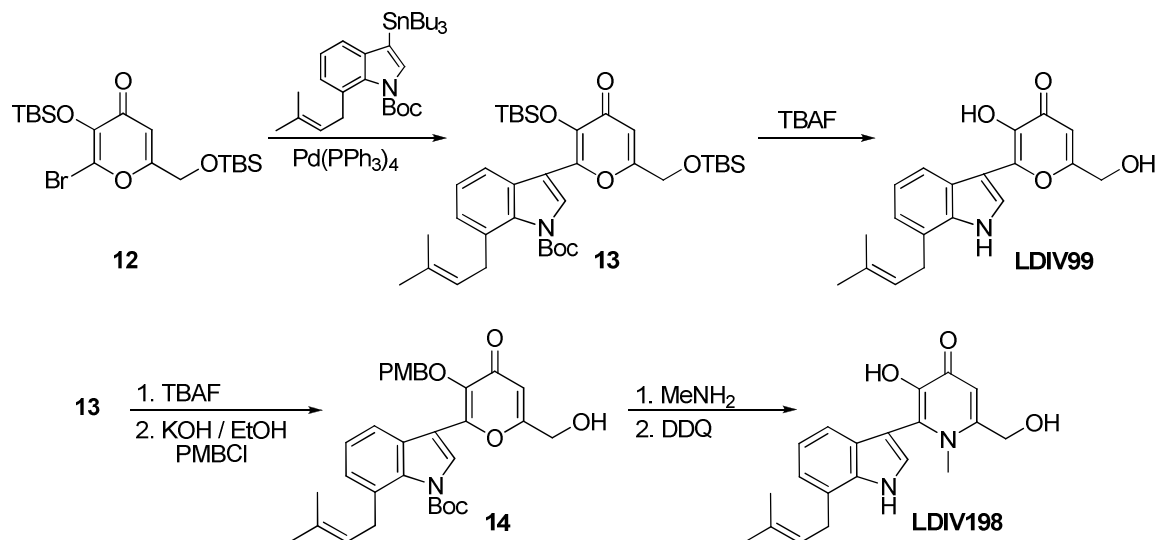
Figure 1.17: Structures of compounds 1H5 and 5E5

Compound 1H5 was chosen from the asterriquinone library and displayed approximately 50% the effect of NGF activator activity. Compound 5E5 was chosen from the monoindolylquinone library and had approximately 200% the effect of NGF.³⁴ The dose-dependent cytotoxicities of the two compounds were measured. The IC_{50} values for 1H5 and 5E5 were 30 μ M and 29 μ M, respectively; both of which were less toxic than DAQ-B1, with an IC_{50} of 5 μ M. The neurotrophic properties of 1H5 and 5E5 were tested using PC12 pheochromacytoma cells that undergo NGF-dependent neuronal differentiation, and require NGF for continued cell survival of the differentiated neurons. Compound 1H5 caused a dose-dependent increase in phosphorylation of TrkA, activated ERK and Akt downstream of the receptor, preserved the morphology of the cell bodies, and prevented apoptotic figures. Treatment with 5E5 caused phosphorylation of TrkA at a 1 μ M dose. Furthermore, TrkA and extracellular signal-regulated kinase were activated as strongly as a maximal dose of NGF (100 ng/ml) by a 3 to 10 μ M dose of 5E5. Neuronal differentiation of PC12 cells was promoted by a combination of a low dose of 5E5 (1 μ M) with a submaximal dose of NGF (10 ng/ml). A new class of TrkA activators has

thus emerged with the discovery of 1H5 and 5E5 that could potentially have an impact in neuroprotective therapy.

Despite all of the promising results mentioned regarding DAQ-B1 and other related asterriquinones, compounds of this family have not entered clinical trials. A major concern is the potential safety issues associated with the quinone substructure. The quinone-containing pharmaceuticals that are currently marketed are primarily used in acute therapies, such as anti-infectives and for cancer therapies. Quinone-containing drugs cannot be used to treat diseases like diabetes, which require chronic therapy. Our laboratory hypothesized that replacement of one of the quinone carbonyls with a heteroatom might alter toxicity while preserving activity. Two compounds that exchange the quinone of ZL-196 for a heteroatom, to form a pyrone or a pyridone, were synthesized by our laboratory (Scheme 1.4)³⁵ Bromopyrone **12** was coupled with a stannylated 7-prenylindole (discussed later) via Stille coupling to produce the protected intermediate **13**. The *O*-silyl and *N*-Boc protecting groups were removed with excess tetrabutylammonium fluoride (TBAF) to yield LDIV99. Formation of the pyridone began with the selective protection of the enol to produce *p*-methoxybenzyl-protected **14**. A pyrone-to-pyridone exchange and removal of the *N*-Boc group took place when **14** was treated with methylamine. Finally, deprotection of the PMB with DDQ produced LDIV198.

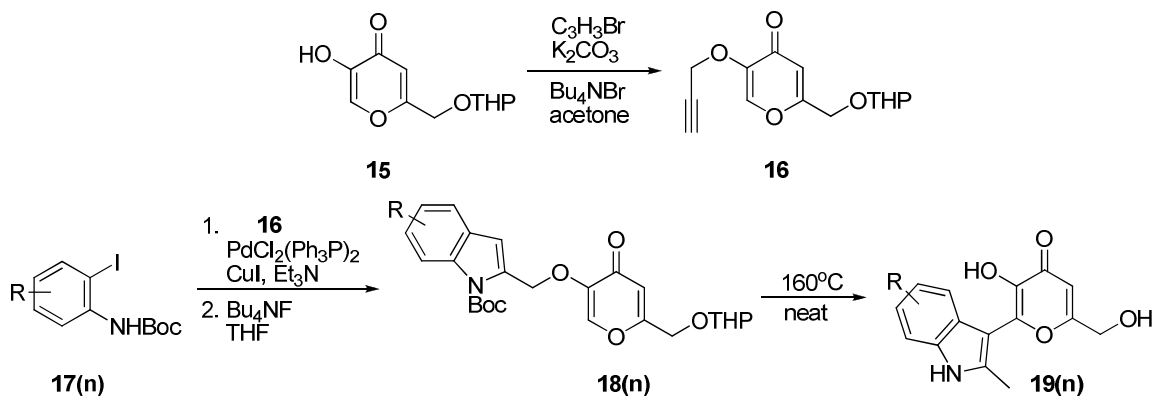
Scheme 1.4: Preparation of candidates for quinone replacements LDIV99 and LDIV198



These compounds were then examined for their ability to activate hIR in a cell-based receptor-phosphorylation assay. Activation of the IR by both these compounds was comparable to ZL-196, with an EC₅₀ of ~30 μM .

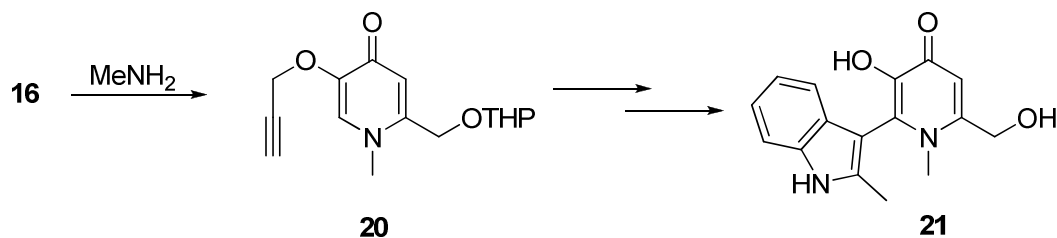
LDIV99 did not exhibit intrinsic safety issues when preliminary pharmacological screening was conducted. Therefore, this compound can serve as a lead structure and can be further modified, ultimately leading to an ideal biologically active drug candidate. The lack of versatility in the route shown in Scheme 1.4 did not allow for much structural variation of the indole portion. Consequently, our group reexamined this synthetic strategy and developed a synthetic route to indolyl-kojic acids based on the Claisen rearrangement of an (indole)methyl-kojic acid ether (Scheme 1.5).³⁶

Scheme 1.5: Synthesis of (indole)kojic acids via Claisen rearrangement



This route began with the alcohol protection of the kojic acid as the THP ether, followed by conversion to its propargyl derivative **16**. Propargyl ether **16** was coupled to various Boc-protected *o*-iodoanilines **17(n)**, via Sonogashira reaction. These anilines were obtained from commercial sources and protected using standard protocols. TBAF was added directly to the reaction mixture to produce intermediate **18(n)**, which was converted to the target compounds **19(n)** upon heating. The Claisen rearrangement can also be carried out for pyridone analogs (Scheme 1.6). First, the pyridone is formed by treating **16** with methylamine under slightly acidic conditions to convert it to pyridone **20**. Sonogashira coupling with 2-iodoaniline, followed by Claisen rearrangement (as before) yields indolyl-pyridone **21**. This process is important for varying the quinone replacements (such as kojic acid) as well as maintaining structural diversity of the indole portion in hopes of creating a library of asterriquinone analogs that could potentially function as insulin mimics.

Scheme 1.6: Synthesis of indolyl-pyridones via Claisen rearrangement



1.3.3 Other Advances Made in Asterriquinone Chemistry

As mentioned previously, the Kaji group demonstrated that dihydroxy groups on benzoquinone are not essential to elicit cytotoxicity in mouse leukemia P388 cells and that at least one hydroxy or acetoxy group is sufficient.⁵ This group wanted to further their investigations by constructing detailed structure activity relationships to determine whether compounds in which the 2,5-dihydroxybenzoquinone moiety was chemically modified would show a similar cytotoxicity to that of DAQ-A1, and whether the isoprenyl and/or indole ring of DAQ-A1 are necessary to its cytotoxicity. Thus, they synthesized 54 derivatives of DAQ-A1, with chemical modifications made to the 2, 3, 5, and 6 positions of the benzoquinone ring, and cytotoxic activity against mouse leukemia P388 cells was investigated.³⁷ Compounds containing one hydroxy group and one or two acetoxy groups demonstrated similar or more potent cytotoxic activity than the parent dihydroxy compounds. Moreover, the cytotoxic activity of acetoxy-methoxybenzoquinones ($IC_{50} = 0.56\text{--}1.18\ \mu\text{M}$) and diacetoxybenzoquinones ($IC_{50} = 1.07\text{--}1.80\ \mu\text{M}$) were higher than compounds having one or two hydroxy groups ($IC_{50} = 0.75\text{--}1.16\ \mu\text{M}$), suggesting that acetoxy or diacetoxy compounds could be more cell permeable due to

increased hydrophobicity. Next, they wanted to confirm the importance of the indole rings for cytotoxicity. Hence, they synthesized compounds where indoles had either been completely removed, or had been substituted for methyl groups. These compounds displayed very low cytotoxicities ($IC_{50} > 30 \mu M$), indicating that the indole ring is necessary for biological activity. Finally, they wanted to investigate the necessity of having a terminal double bond, such as that found in the isoprenyl groups of DAQ-A1, for cytotoxic activity. They synthesized asterriquinones without any terminal double bonds (Figure 1.18) and found that their cytotoxic activity was similar to that of DAQ-A1 and deduced that the presence of an isoprenyl group is not important for cytotoxic activity. They concluded that the contribution of at least one hydroxy or acetoxy group is more important to the cytotoxicity than that of the isoprenyl group.

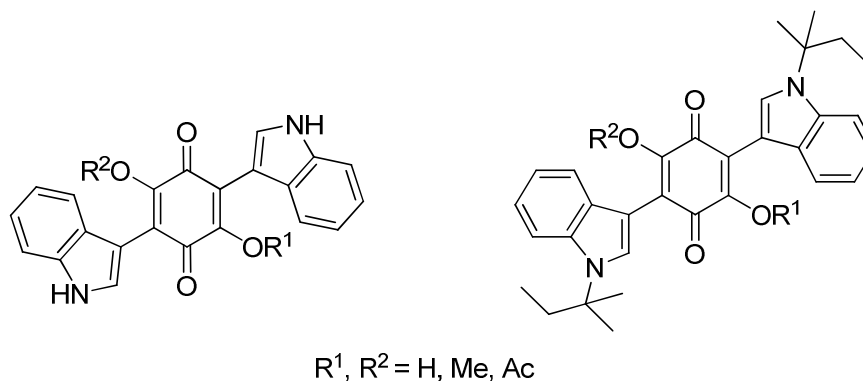


Figure 1.18: Asterriquinones with no terminal double bond

Further investigations by this group led them to discover that cytotoxic activity against mouse leukemia P388 cells increased with extension of the alkyl chain (R group) of DAQ-A1 monoalkyl ethers. The most potent of these DAQ-A1 monoalkyl ethers was

ARQHex (Figure 1.19). It had an IC_{50} value of $0.07 \mu\text{M}$ against P388 cells, which is about 18-fold more than DAQ-A1 ($IC_{50} = 1.28 \mu\text{M}$).³⁸

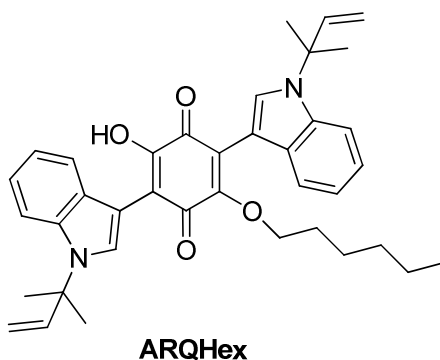
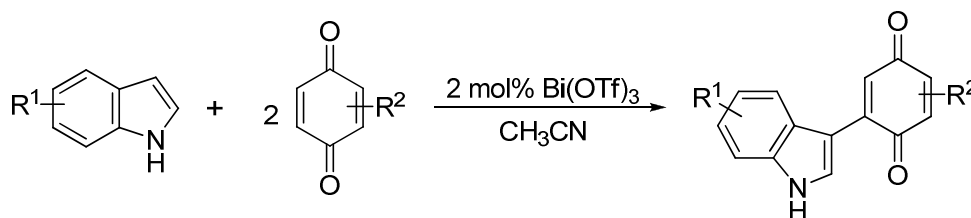


Figure 1.19: DAQ-A1 monoalkyl ether with the greatest potency

Early research conducted in the asterriquinone field consisted of isolation of asterriquinones and chemical manipulations of those natural products, along with testing for their respective biological activities. There was not much concentration on the synthesis of asterriquinones from simple starting material. In 2003 the Yadav group from India developed a protocol for the synthesis of 3-indolylbenzoquinones using catalytic amounts of bismuth(III) triflate ($\text{Bi}(\text{OTf})_3$) under mild conditions at room temperature (Scheme 1.7). A range of indoles and quinones were investigated, including monosubstituted quinones as well as 1,4-naphthoquinone. In all cases, the reactions proceeded rapidly under these mild conditions with high regioselectivity and the products obtained in yields ranging from 16-75%.³⁹ Regioselectivity played a role in the case of the monosubstituted quinones, with the indole adding to the quinones at the least hindered site. The authors reported that this method is applicable with electron-rich

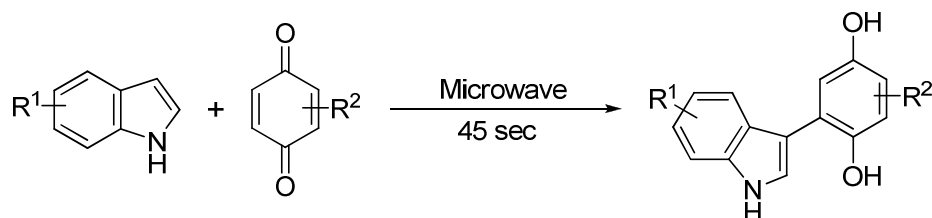
indoles as well as electron-deficient indoles. They tested ethyl indole-2-carboxylate ($R^1 = 2\text{-CO}_2\text{Et}$) and obtained the corresponding 3-indolylquinone in 78% yield. Most other methods to produce 3-indolylquinones with electron-deficient indoles fail.

Scheme 1.7: $\text{Bi}(\text{OTf})_3$ -catalyzed synthesis of 3-indolylquinones



This group continued their study of these reactions and in 2008 reported the synthesis of 3-indolylquinones by a microwave-assisted reaction under solvent-free conditions (Scheme 1.8).⁴⁰ Microwave irradiation is a process increasingly used to accelerate organic reactions. It has become a more common and powerful technique in organic synthesis. The main advantages of microwave chemistry over classical methods are reduced reaction times, improved yields, suppressed side reactions, decreased environmental pollution, minimized thermal decomposition, and safe operations.

Scheme 1.8: Microwave accelerated synthesis of 3-indolylquinones



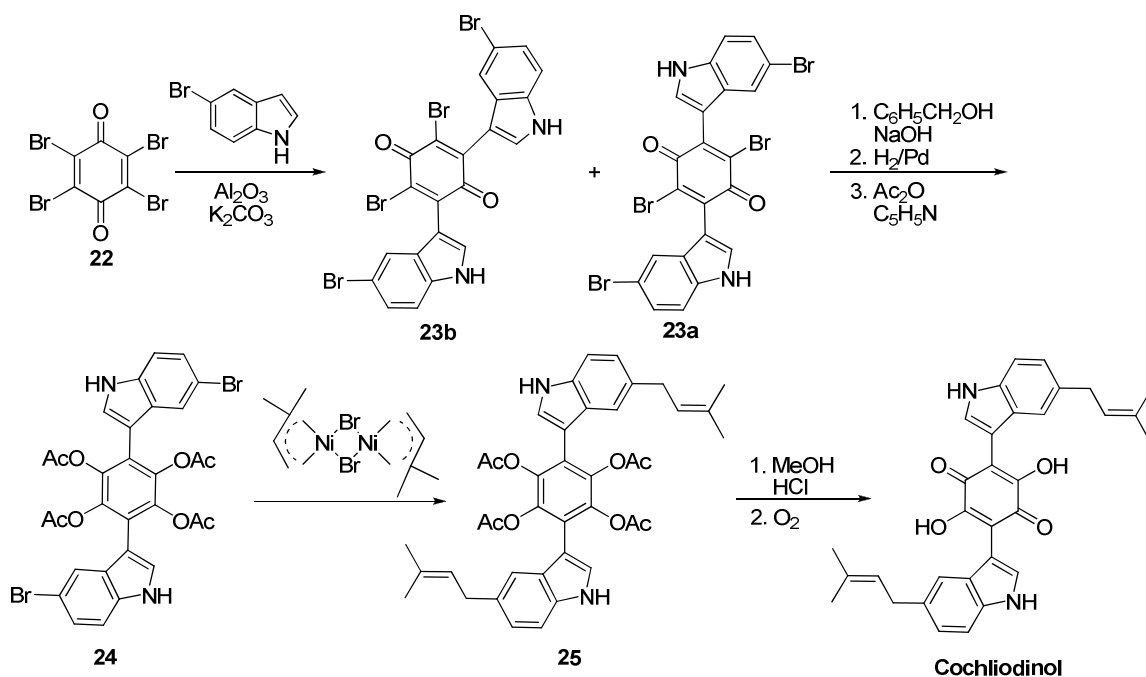
This also allows for the possibility to run reactions under solvent-free conditions. A variety of indoles, including electron-deficient indoles, reacted smoothly with substituted benzoquinones (including **1**) to produce the respective hydroquinones under excellent yields (78-94%).⁴⁰ The reactions progressed quickly in solvent-free conditions without catalyst interference. Two advantages of this method were that chlorinated side products usually observed under protic acid conditions (HCl) were not produced, and it is successful with electron deficient indoles.

1.4 Total Syntheses of Asterriquinones

1.4.1 Synthesis of Cochliodinol

The first total synthesis of an asterriquinone was directed toward the first isolated asterriquinone, cochliodinol, which is an antibacterial that was discovered in 1968. This synthesis used as a key step the alumina/potassium carbonate-promoted condensation of bromanil (**22**) with 2 equiv of 5-bromoindole to form a pair of regioisomers, **23a** and **23b** (Scheme 1.9).⁴¹ The para-regioisomer **23a** was isolated by chromatography, reduced to the hydroquinone, and protected by acetylation to give **24**. Prenyl groups were introduced to the 5-position of the indoles of compound **24** by nickel carbonyl-prenylbromide complex to produce intermediate **25**, which was oxidized to give the target molecule.

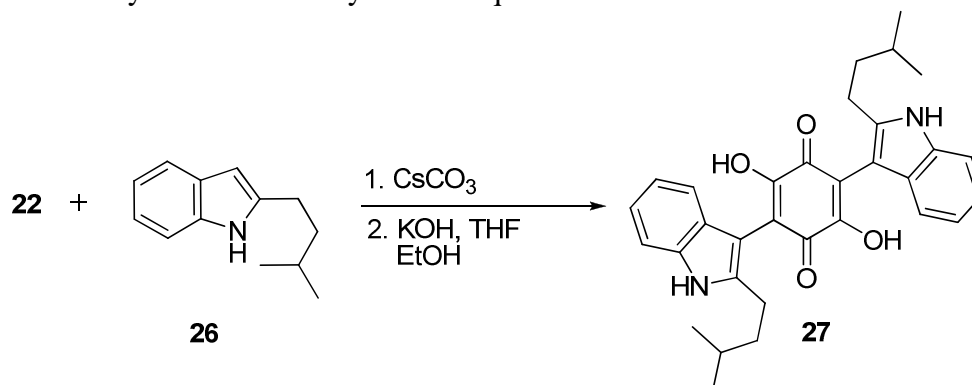
Scheme 1.9: Synthesis of cochliodinol



1.4.2 Synthesis of Tetrahydro Asterriquinone E

In 1999, the Harris group discovered that neoARQ (Figure 1.3), or what they called asterriquinone-E (AQ-E) was able to inhibit the binding of growth factor receptor-bound 2 (Grb2) adaptor protein to tyrosine-phosphorylated EGF receptor ($IC_{50} = 2.9 \mu\text{M}$). Many classes of cancerous tumors are associated with an interaction between adaptor proteins and protein tyrosine kinase molecules. AQ-E represents the first Grb2 inhibitor to directly inhibit this interaction. As of 1999, a general synthetic route to asterriquinones and their analogs had not been established, despite their therapeutic potential. Thus, an effort was undertaken by the Harris group to devise a general synthesis of asterriquinone analogs.²⁶ Their initial attempts were geared towards the production of AQ-E. However, they discovered it was easier to synthesize the tetrahydro derivative instead of the natural product, especially since it was presumed that the isoprenyl double bonds of AQ-E are prone to metabolism and they are irrelevant to biological activity. Their synthesis began by treating commercially available **22** with two equiv of 2-(3-methyl-*n*-butyl)indole (**26**) in the presence of cesium carbonate in acetonitrile. Aqueous KOH and EtOH were added directly to the crude mixture to form tetrahydroasterriquinone-E (tetrahydro-AQ-E) in a one pot procedure and an overall 31% yield (Scheme 1.10). Purification of this compound was carried out by high performance liquid chromatography (HPLC) since it was not stable on flash silica or neutral alumina. In the end, the olefinic portion of the indole moiety was not necessary for Grb2 inhibition since tetrahydro-AQ-E displayed biological activity.

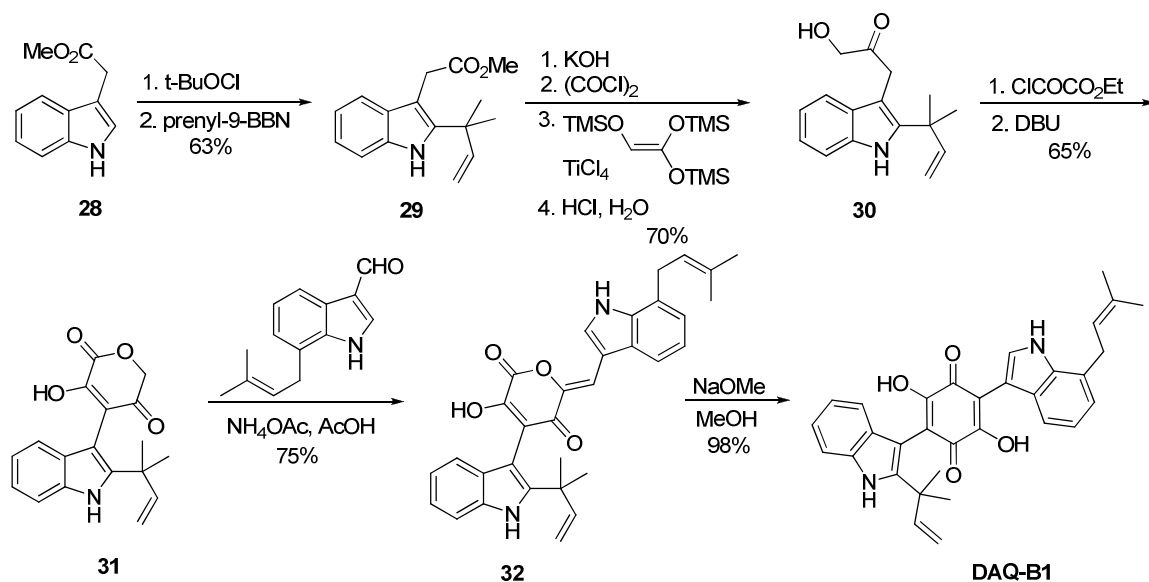
Scheme 1.10: Synthesis of tetrahydroasterriquinone E



1.4.2 Total Synthesis of DAQ-B1

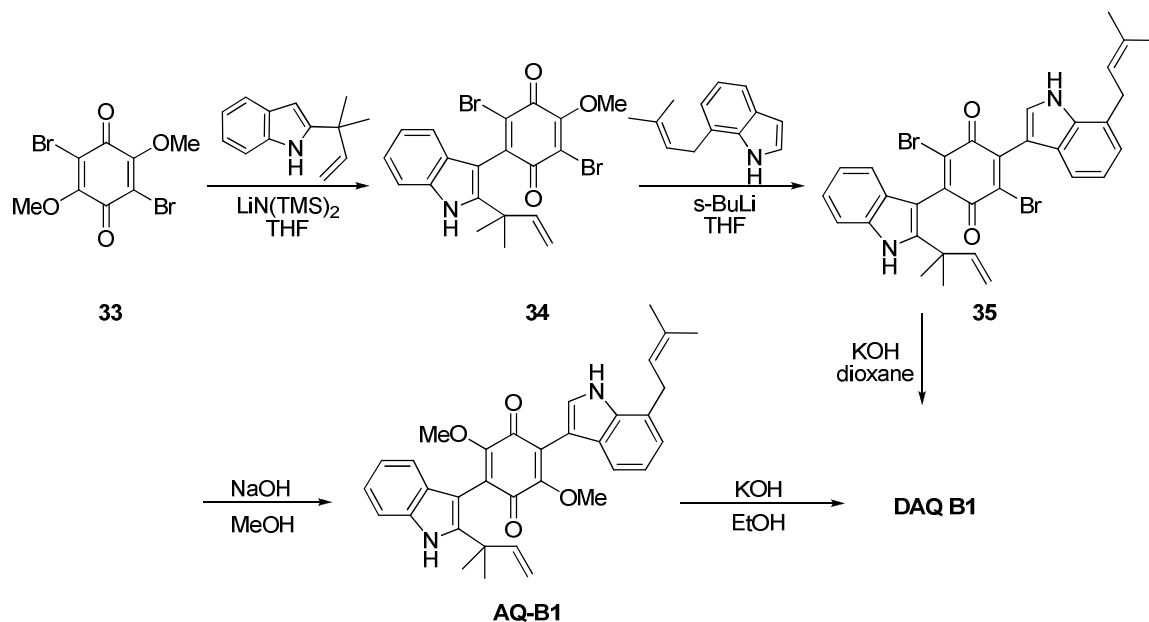
The first total synthesis of DAQ-B1 was achieved by a Merck group in 1999 (Scheme 1.11).⁴² Methyl indoleacetate (**28**) was treated with *tert*-butyl hypochlorite to generate the corresponding chloroindolenine, which was nucleophilically attacked by prenyl-9-BBN at the indole 2-position to produce **29**. Through a series of steps, **29** was transformed to the α -hydroxy ketone **30**, which was then treated with ethyl chlorooxalate and DBU to yield pyrandione **31**. Compounds **31** and 3-formyl-7-prenylindole were united in a Knövenagel condensation to give a bisindolyl pyrandione intermediate **32**, which underwent a base-catalyzed rearrangement reaction to give the natural product DAQ-B1 in a 10-step synthesis and a 20% overall yield.

Scheme 1.11: Merck total synthesis of DAQ-B1



Another synthesis of DAQ-B1 was reported by a group from Japan in 2001 (Scheme 1.12).⁴³ In this synthesis, 2-isoprenylindole was lithiated with $\text{LiN}(\text{TMS})_2$ to produce the indolyl anion, which reacted with dibromodimethoxyquinone **33** to give mono-substituted **34** in 61% yield. Another indole moiety, 7-prenylindole, was introduced onto **34** by using *s*-butyl lithium to give another indolyl anion, which reacted with **34** to produce disubstituted **35** in 72% yield. The resulting bisindolylquinone **35** was directly hydrolyzed to DAQ-B1 by KOH in dioxane, but only 30% yield was obtained. Consequently, they decided to transform **35** to asterriquinone-B1 (AQ-B1) by treatment with NaOH in MeOH at room temperature. This was then followed by hydrolysis of AQ-B1 with KOH in EtOH , to form DAQ-B1 in 58% yield from the two-step hydrolysis.

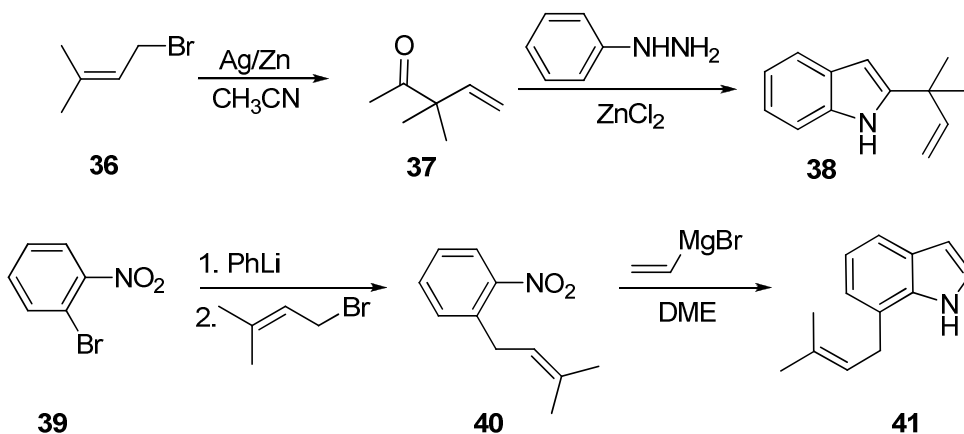
Scheme 1.12: Tatsuta group total synthesis of DAQ-B1



The Pirrung group attempted to synthesize DAQ-B1 in 2002. First however, their focus was on obtaining the two indoles required for the total synthesis (Scheme 1.13). 2-Isoprenylindole (**38**) was prepared from prenyl bromide (**36**) via the well known Fisher indole synthesis in two steps and 25% overall yield.⁴⁴ In this synthesis, **36** was treated with silver-zinc alloy in acetonitrile to yield methyl isoprenyl ketone (**37**). Reaction of **37** with phenyl hydrazine gave a hydrazone intermediate, which was treated with anhydrous zinc chloride to furnish **38** in multigram quantities. Synthesis of 7-prenylindole (**41**) was based on the Bartoli reaction.⁴⁵ 2-Bromonitrobenzene (**39**) was treated with phenyl lithium (PhLi) to form the nitrobenzene anion, which was treated with prenyl bromide to give prenyl nitrobenzene (**40**) quantitatively. Under traditional Bartoli reaction

conditions, the addition of vinyl magnesium bromide solution to **40** produces **41** in a total of two steps and ~50% yield.

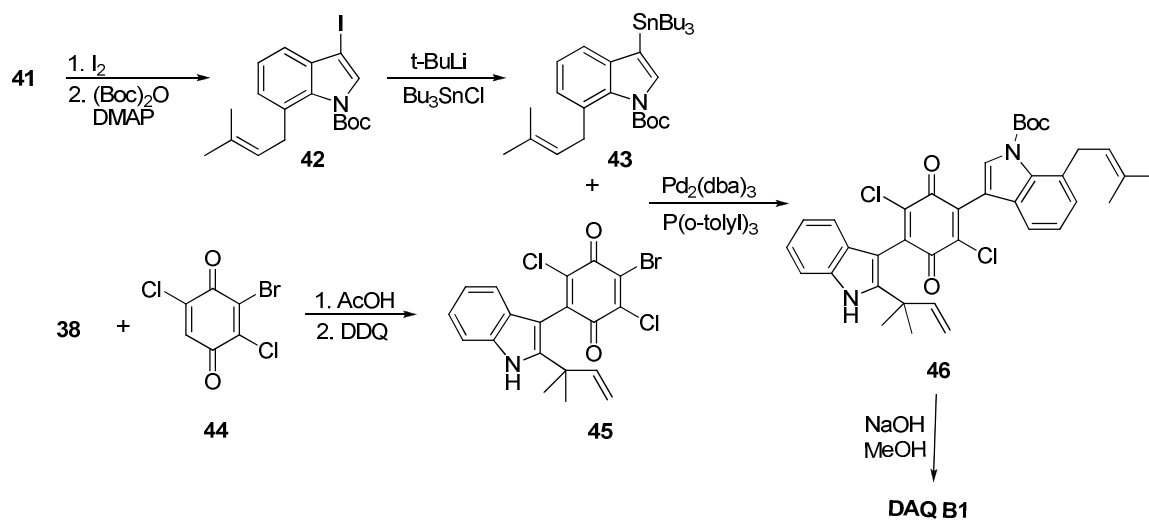
Scheme 1.13: Synthesis of 2-isoprenylindole and 7-prenylindole



The first generation total synthesis of DAQ-B1 developed by our group was based on the cesium carbonate-mediated coupling reaction between indole **38** and bromanil (**22**).⁴⁶ Although this synthesis was modular and time efficient, a lack of regiocontrol was observed when the second indole was introduced. Thus, in efforts to improve this synthesis, a second-generation route was sought. Our group reasoned that the para substitution of DAQ-B1 could be imposed with the use of 3-bromo-2,5-dichlorobenzoquinone (**44**). Once the initial addition of **38** to **44** takes place, introduction of the second indole could be accomplished through a bromide-selective Stille coupling reaction (Scheme 1.14). This synthesis began by selectively iodinating the 3-position of **41**, protecting the indole nitrogen with Boc, and metal-halogen exchange followed by stannylation with tributyltin chloride to form tin reagent **43** in a 55% overall yield. The

next step was the preparation of a starting material suitable for Stille coupling with **43**. Indole **38** was added to benzoquinone **44** in AcOH to form the corresponding hydroquinone, which was oxidized by DDQ to form **45** in 79% yield. Since the substitution pattern of **45** was built into the starting quinone, the difference in reactivities of vinyl bromides and vinyl chlorides in Stille coupling directed selective production of the *para*-bisindolylquinone regiochemistry. Traditional Stille coupling conditions (Pd(PPh₃)₄) were improved by using bulky phosphines, producing **46** in 76% yield. Hydrolysis of the vinyl halides and the Boc group using the 10% NaOH in MeOH procedure gave the natural product in 73% yield.⁴⁶

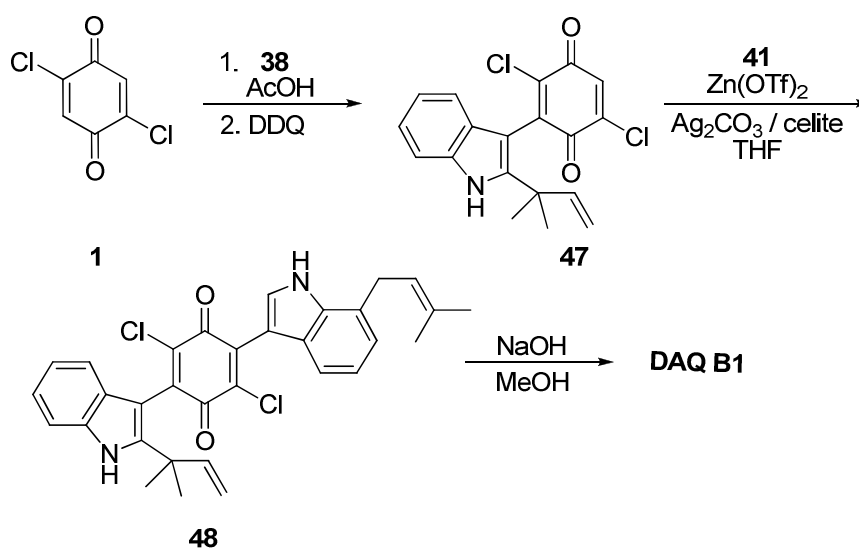
Scheme 1.14: Second generation synthesis of DAQ-B1 by the Pirrung group



As mentioned previously, our laboratory conducted a methyl scan experiment to identify the regions of DAQ-B1 that are necessary for biological activity. The design of this experiment required the synthesis of 12 derivatives substituted at all aromatic

carbons, oxygens, and nitrogens.²⁹ Although several syntheses of DAQ-B1 have been developed, including two in the Pirrung laboratory, none were adequate for conducting a methyl scan study. Hence, a new total synthesis was sought by our group that would add two different indole building blocks that have not previously been derivatized to a central quinone unit. Prior work done by our lab described an efficient method for the addition of a variety of indoles to the synthon **1**, through protic acid catalysis. Thus, only a method to add the second indole to the resulting monoindolylquinone was required. A desirable strategy was to add the second indole to a monoindolylquinone bearing a bulky 2-substituent. The reason for this addition sequence is to avoid double addition of indole onto the benzoquinone. Preparative experiments indicated that Brønsted acids are ineffective in promoting the addition of the second indole, but Lewis acids were successful.

Scheme 1.15: Superior method for the synthesis of DAQ-B1



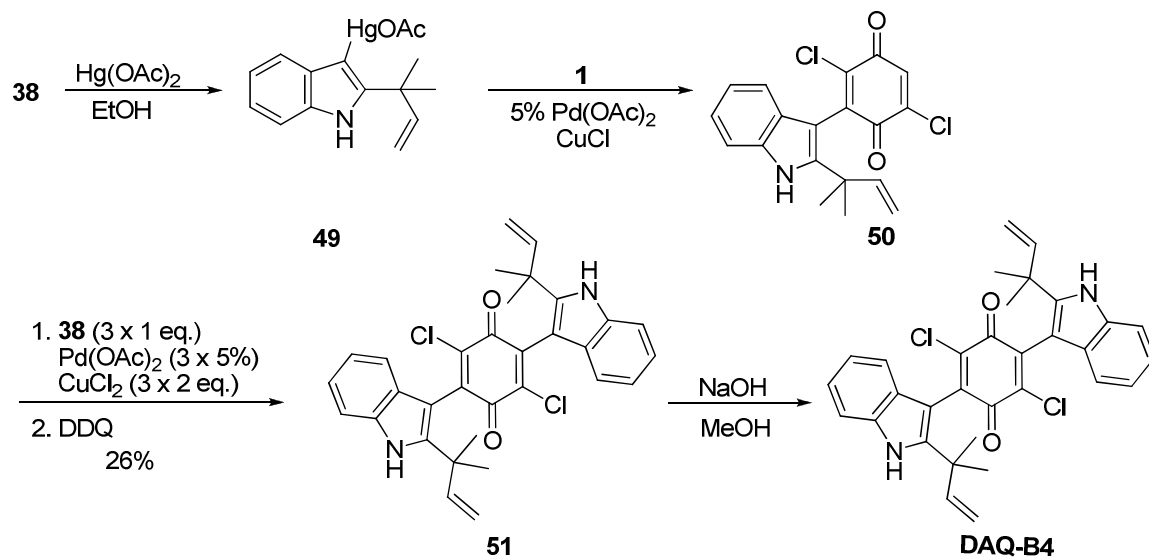
Zinc triflate ($\text{Zn}(\text{OTf})_2$) demonstrated promising catalytic capabilities, and further optimization led to THF as the best solvent.²⁹ During the preparation of monoindolylquinones, silver carbonate on Celite was found to be a convenient oxidant, and it worked similarly in the addition of the second indole. Through the use of this method, total synthesis of DAQ-B1 (Scheme 1.15) was completed in only three steps from the indoles, with full regiocontrol and an overall yield of 36%.²⁹

1.4.3 Total Synthesis of DAQ-B4

Demethylasterriquinone-B4 is another member of the large class of fungal natural products derived from *Aspergillus*, *Chaetomium*, and *Pseudomassaria* species. Total synthesis of DAQ-B4 was accomplished by our laboratory in 2005.⁴⁷ This synthesis was based on an adaptation of a method reported by Hegedus⁴⁸ for Heck reactions on indole 3-mercurials. The mercurial **49** was generated from treatment of **38** with mercuric acetate. Two equiv of **49** were added to **1**, in the presence of catalytic palladium acetate ($\text{Pd}(\text{OAc})_2$) and copper chloride (CuCl) as the reoxidant, to form monoindolylquinone **50** (Scheme 1.16). Compared to the acid-promoted methodology for the addition of indoles to dichlorobenzoquinones employed by our group, these reactions did not demonstrate much variation in efficiency, despite the bulk of the indole 2-substituent. Under similar conditions, a second indole mercurial was added to the monoindolylquinones, whose reactivity was expected to be similar to that of **1**, despite the presence of the indole. Molecular mechanics calculations previously conducted by our laboratory suggested that

the biaryl bond in these specific indolylquinones with four flanking substituents preferred a nonplanar conformation,²⁴ hence the predicted similar reactivities of **1** and **50**.

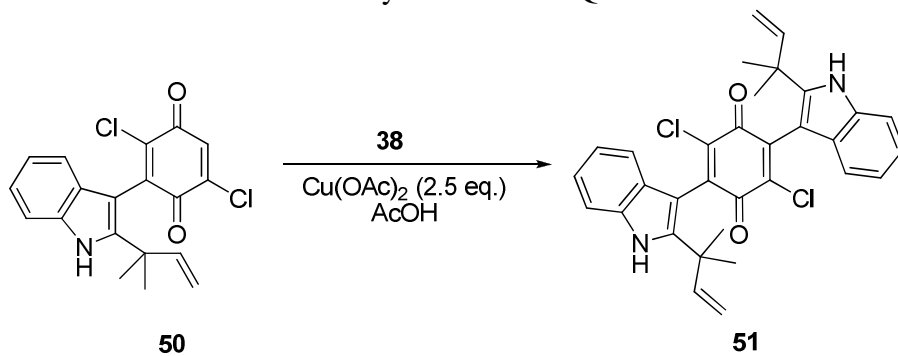
Scheme 1.16: Total synthesis of DAQ-B4



When less hindered mercurials were used (2-methylindole), the corresponding monoindolylquinone was formed in excellent yield (not shown).⁴⁷ However, the more hindered mercurials (**49**) required stringent conditions and gave a mixture of reduced product and starting material. The reduced compounds were oxidized by DDQ to form **51** in meager yield, which was converted to DAQ-B4 upon alkaline hydrolysis. In an effort to increase the environmental friendliness of this reaction sequence, the mercurial step was omitted (Scheme 1.17). In AcOH, the addition of indole **38** to **50** does not require a palladium catalyst, only copper acetate. In the absence of copper acetate, a large fraction of the product mixture is 2,5-dichlorohydroquinone, suggesting that the copper acetate

plays a dual role in promoting the conjugate addition by Lewis acidity and oxidizing the hydroquinone to the quinone.

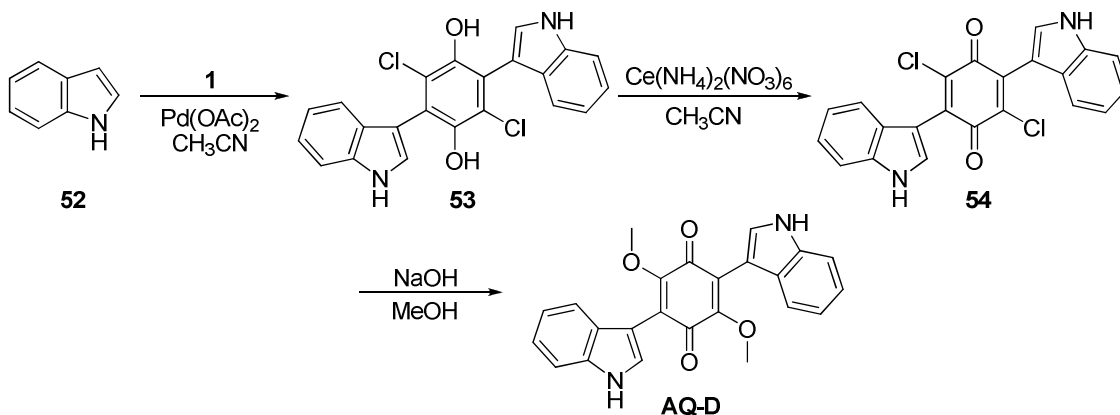
Scheme 1.17: Alternate conditions in the synthesis of DAQ-B4



1.4.4 Total Synthesis of AQ-D

Asterriquinone D (AQ-D) was shown to inhibit HIV-1 protease. Synthesis of this asterriquinone was reported by the Tanoue group (Scheme 1.18).⁴⁹

Scheme 1.18: Synthesis of asterriquinone D



Reaction of **1** with 3 equiv of indole (**52**) in the presence of Pd(OAc)₂ catalyst produces the bisindolylhydroquinone **53**. A side product of this reaction is the monoindolylhydroquinone. Oxidation of **53** with 3 equiv ceric ammonium nitrate (CAN) yielded quinone **57**, which was treated with NaOH in MeOH to provide AQ-D in 3 steps and 35% yield.⁴⁹

The focus of this dissertation is synthesis of DAQ-B1 analogs that can potentially mimic the anti-diabetic effects of insulin and play a role in neuroprotection as potential neurotrophin mimics.

Chapter Two: Synthesis of DAQ-B1 Analogs as Potential Insulin Mimics

2.1 Diabetes and its Modern Treatments

Diabetes is one of the most common diseases in the United States. It is a condition where the body either does not produce enough, or does not properly respond to insulin, leading to high levels of glucose in the blood. There are different types of diabetes recognized. The principal three are insulin dependent diabetes mellitus (IDDM), or type 1 diabetes; non-insulin dependent diabetes mellitus (NIDDM), or type 2 diabetes; and gestational diabetes. As of 2007, 23.6 million people in the United States, 7.8% of the population, have been diagnosed with diabetes.⁵⁰

Type I diabetes is usually diagnosed in children and young adults and constitutes about 5-10% of all diabetes cases. It is characterized by insulin deficiency due to loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas. This lack of insulin results in a buildup of glucose in the bloodstream. There is no known preventative measure against Type I diabetes. The only way patients can replace the insulin not produced by the pancreas is through insulin injections.

Type II diabetes is characterized by a combination of insulin resistance and relatively reduced insulin secretions. This is the most common form of diabetes and constitutes about 90-95% of the disease. Early diagnosis of type II diabetes makes it possible for patients to manage the disease without insulin injections. Mild cases can

control glucose level by maintaining a healthy weight through diet and exercise. Slightly more severe cases may require the use of an oral drug. There are several types of oral antidiabetic agents, classified according to their method of action. Sulfonylureas, which were the first widely used oral hypoglycemic compounds, and meglitinides stimulate more insulin secretion by the pancreatic beta cells. Another class of compounds known as “sensitizers” increase the sensitivity of target organs to insulin produced in the body. Drugs of this class include biguanides such as Metformin (Glucophage) and thiazolidinediones such as rosiglitazone (Avandia) and pioglitazone (Actose). The third class of drugs reduces gastrointestinal absorption of carbohydrates. They decrease the rate at which glucose is absorbed from the gastrointestinal tract by slowing down the digestion of starch in the small intestine, allowing glucose to enter the bloodstream more slowly. These compounds are known as α -glucosidase inhibitors. Examples of these drugs include miglitol (Glyset) and acarbose (Precose). Unfortunately, when some type II diabetes cases become too severe for oral treatment, insulin injections are required for these patients.

Gestational diabetes mellitus is another type of diabetes where glucose levels increase during a woman’s pregnancy. It resembles type II diabetes in many aspects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in 2%-5% of all pregnancies and may improve or disappear post-delivery.

As of 2007, 23.6 million people in the United States (7.8% of the population) have been diagnosed with diabetes. Over half of those people (12.2 million) are 60 years of age or older. Without proper treatment, diabetes runs the risk of leading to other

complications such as heart disease, stroke, high blood pressure, blindness, kidney disease, neuropathy, and amputation.⁵⁰

2.2 Insulin

2.2.1 Insulin Structure and Action

Insulin is a hormone produced by the beta cells of the islets of Langerhans, found in the pancreas (Figure 2.1). Beta cells are located adjacent to blood vessels and can easily respond to changes in blood glucose concentration by adjusting insulin production. This allows cells in the liver, muscles, and fat tissue to take up glucose from the blood and store excess glucose as glycogen in the liver and muscles. Thus, insulin level is a central metabolic control mechanism that has several anabolic effects throughout the body.

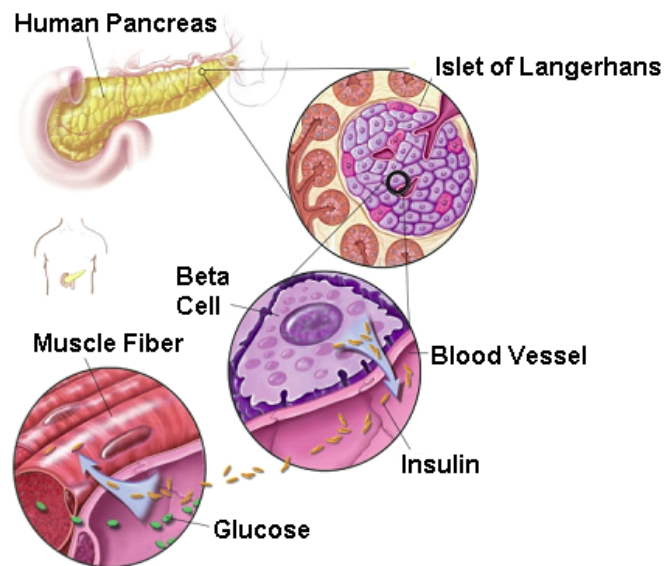


Figure 2.1: Insulin production in the human pancreas (© 2001 Terese Winslow, Lydia Kibiuk)⁵¹

The structure of insulin consists of 51 amino acids making up two polypeptide chains, the A chain and the B chain. The A chain is made up of 21 amino acids and contains a disulfide bond and the B chain is made up of 30 amino acids. The two chains are linked together by two disulfide bonds (Figure 2.2).

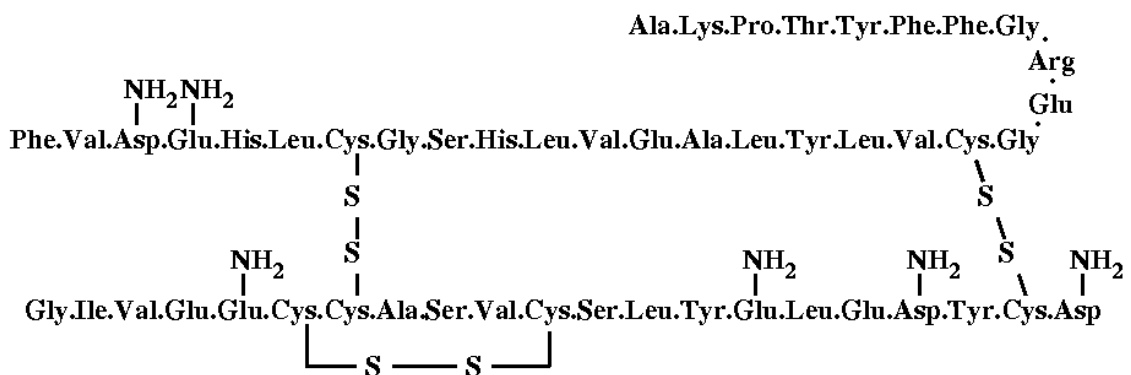


Figure 2.2: Primary structure of insulin⁵²

Insulin tends to dimerize in solution. At higher concentrations it polymerizes to form tetramers and hexamers (Figure 2.3). The amino acid sequence of insulin was characterized in 1951, 30 years after its discovery, by Frederick Sanger.⁵³ Insulin structure varies only slightly between species of animal. Porcine (pig) insulin is the closest to human, differing in only 1 amino acid residue. Similarly, bovine (cow) insulin differs from human insulin in only 3 amino acids. The first genetically-engineered, synthetic "human" insulin was produced in a laboratory in 1977.⁵⁴

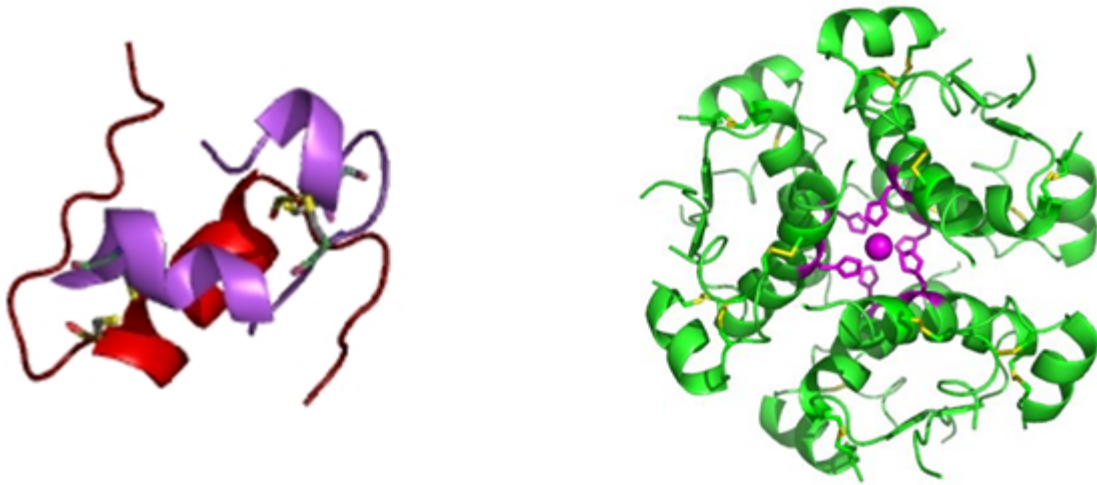


Figure 2.3: Structure of insulin monomer and hexamer (Protein Data Bank)⁵⁵

2.2.2 Insulin Action and Binding

As mentioned previously, insulin has many wide-ranging actions, as it plays a major role in carbohydrate, lipid, and protein metabolism. Insulin signals a state of energy abundance and activates glucose uptake, metabolism, and storage as glycogen in muscle and fat tissue. Furthermore, insulin also restrains processes that release stored energy such as lipolysis, ketogenesis, glycogenolysis, proteolysis, and gluconeogenesis.⁵⁶ The insulin signaling-pathway is a complex process that is under investigation to this day. This pathway is initiated by the liberation of glucose into the bloodstream from dietary carbohydrates such as starch or sucrose. Elevated concentrations of glucose in blood stimulate the release of insulin. Insulin then binds to the insulin receptor, which is embedded in the plasma membrane of the cell. The insulin receptor is a member of the tyrosine kinase family and is composed of two alpha subunits and two beta subunits, linked by a disulfide bond. Insulin binding takes place with the α -

subunit of the insulin receptor, causing a change in receptor conformation and inducing autophosphorylation of the β -subunit. The autophosphorylation of the β -subunit is believed to cause further conformation changes of the receptor, which will activate kinase activity of the receptor to phosphorylate other substrates.⁵⁷ This signal transduction stimulates the translocation of the glucose transporter GLUT-4 from intracellular sites to the membrane, where it will transport glucose from the bloodstream into the cell (Figure 1.4). A glucose transporter is required because the only mechanism by which cells can take up glucose is through some type of facilitated diffusion, hence the purpose of GLUT-4.

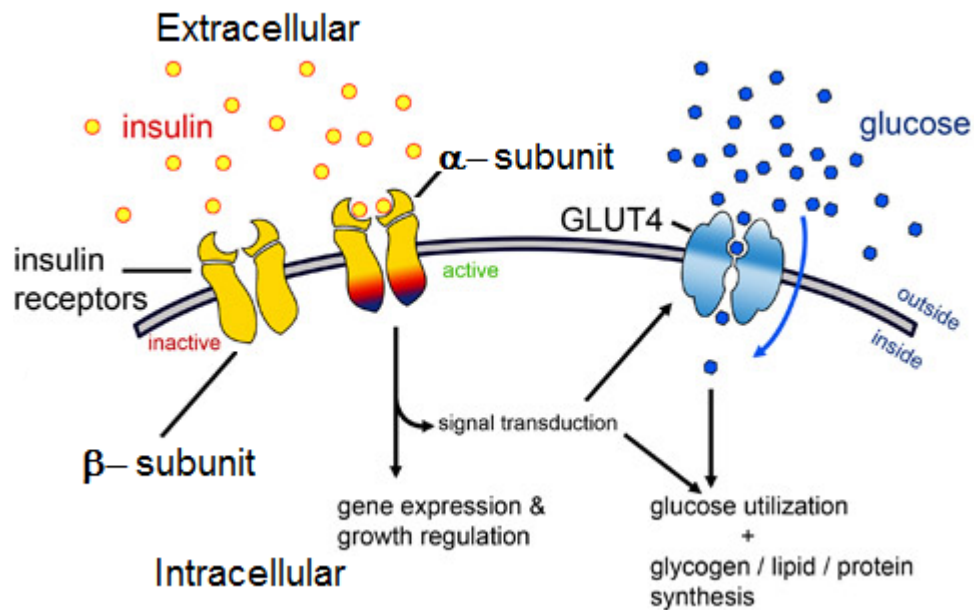


Figure 2.4: Simplified insulin binding pathway (© 2004 Beta Cell Biology Consortium)⁵⁸

It is important to note that the previously mentioned oral drugs used for the treatment of type 2 diabetes do not mimic this insulin signaling pathway. They only stimulate the

release of insulin or improve glucose utilization. Furthermore, they are completely ineffective in type 1 diabetes patients. This leads to the idea that the development of a small molecule that can mimic insulin activity by binding to the insulin receptor will greatly facilitate diabetes treatment, especially for type 1 diabetes patients.

2.3 Synthesis of DAQ-B1 Analogs via Stille Coupling

As mentioned in chapter one, compounds LDIV99 and LDIV198 displayed biological activities similar to ZL-196, an analog and pharmacophore model of DAQ-B1 that was shown to be an orally active insulin mimic. These two compounds are the result of substituting the carbonyl adjacent to the indole on ZL-196 with a heteroatom. The next phase of this project was to investigate the substitution of the second carbonyl (beta to the indole) with a heteroatom or a quaternary carbon (Figure 1.7). Before Stille coupling could take place; however, the first step would be the preparation of indoles.

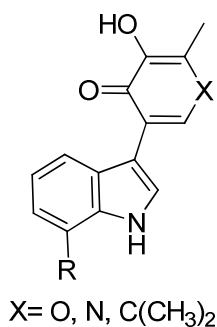
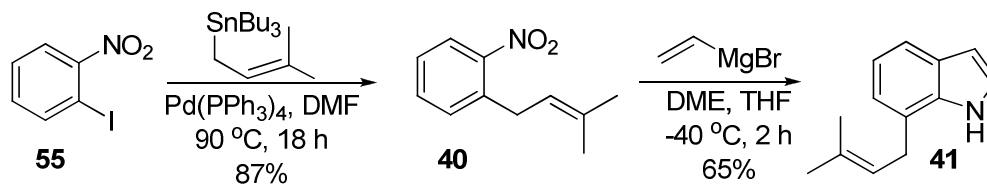


Figure 2.5: Position of substitution for DAQ-B1 analogs

2.3.1 Indoles

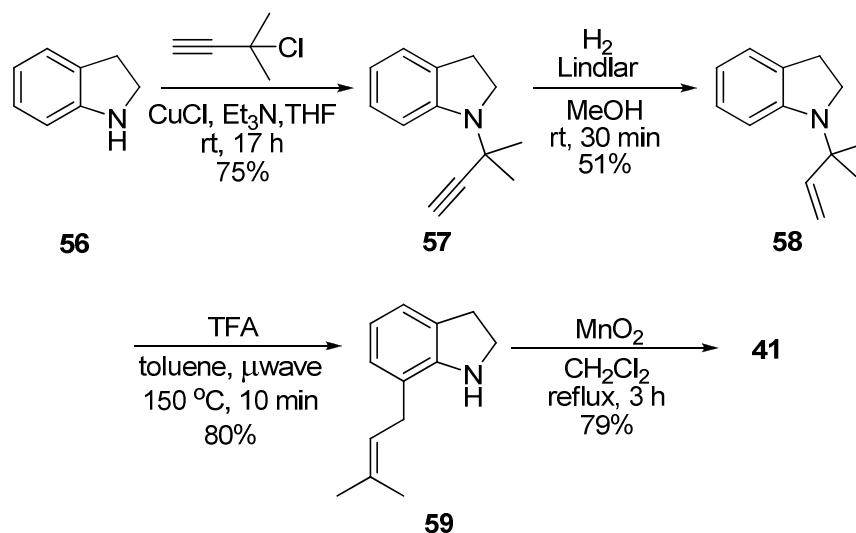
As mentioned in Chapter One, our laboratory developed an improved synthesis of 7-substituted indoles from bromonitrobenzene via metal-halogen exchange with PhLi, followed by alkylation, and finally Bartoli reaction conditions.⁴⁵ These reactions were used in the synthesis of 7-prenylindole (**41**). Repetition of this reaction sequence was attempted; however, formation of 2-prenylnitrobenzene was unsuccessful. Consequently, a different route was undertaken, where **41** was formed in two steps from two well known reactions, Stille coupling and Bartoli indole synthesis.⁵⁹ Commercially available 2-iodonitrobenzene (**55**) reacted with prenyltributyltin and the catalyst tetrakis(triphenylphosphine)-palladium(0) ($\text{Pd}(\text{PPh}_3)_4$) via Stille coupling to form 2-prenylnitrobenzene (**40**) in 87% yield. Other palladium catalysts such as tris(dibenzylideneacetone)dipalladium(0) ($\text{Pd}_2(\text{dba})_3$) with triphenylphosphine as ligand, were tried; however, $\text{Pd}(\text{PPh}_3)_4$ was found to be the best. Under traditional Bartoli reaction conditions, **40** is treated with vinyl magnesium bromide to furnish the desired indole **41** as a cream colored solid in 65% yield (Scheme 2.1). Although **41** has been synthesized by the Pirrung group numerous times, this was the first instance in which it was isolated as a solid material.

Scheme 2.1: Synthesis of 7-prenylindole via Stille coupling and the Bartoli reaction



While this 2-step method is relatively simple, it requires expensive reagents and catalysts. Since **41** is the common denominator in the syntheses of DAQ-B1 analogs, it is needed in ample supply. Thus, Dennis Xiong of our laboratory developed a synthesis of 7-prenylindole that required simple chemistry and on a gram quantity scale.⁶⁰ This synthesis began with indoline (**56**), which was *N*-dimethylpropargylated using 3,3-dimethylpropargyl chloride and CuCl in triethylamine (Et₃N) and THF to form **57** (Scheme 2.2). Semihydrogenation of **57** proceeded via hydrogen containing balloon and Lindlar's catalyst in MeOH, to yield the known dimethylallyl amine **58** in 20-30 min. Addition of the prenyl group ortho to the nitrogen was accomplished by heating **58** with trifluoroacetic acid (TFA) in a microwave, producing 7-prenylindoline (**59**) via the acid-promoted aza-Claisen rearrangement.

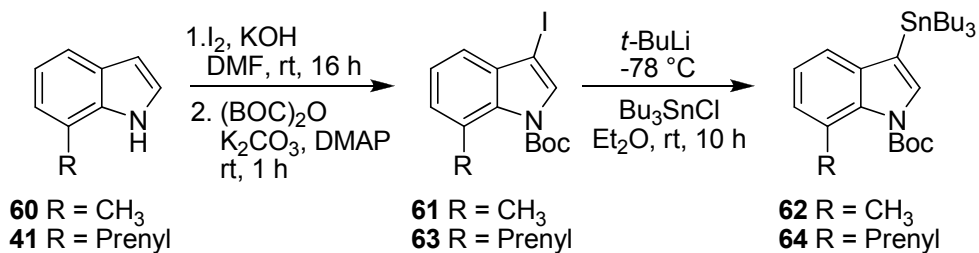
Scheme 2.2: Non-catalytic synthesis of 7-prenylindole



Oxidation of the indoline to the indole **41** was conducted with manganese dioxide (MnO_2) in DCM. Although use of this method can produce indoles in gram quantities for a lower price, it takes a longer time and has an overall lower yield than the procedure in Scheme 2.1.

In order to add indoles to any of the heterocyclic ketones, they needed to undergo a series of reactions to be prepared for Stille coupling. Since **41** is a precious compound, a model system was investigated using commercially available 7-methylindole (**60**). Selective iodination at the 3-position of **60** with iodine in DMF, followed by Boc protection of the indole nitrogen produces iodinated indole **61** in 78% yield. Compound **61** then undergoes metal-halogen exchange with *t*-BuLi, followed by trapping of the 3-indolyl lithium with tri-*n*-butyltin chloride (Bu_3SnCl) to form tin reagent **62** (Scheme 2.3) It is imperative that **61** undergoes stannylation immediately following its formation, due to its high instability. Furthermore, stannane **62** cannot be purified on silica gel since tin compounds undergo protodestannylation under acidic conditions. Thus, **62** was used in its crude form in the Stille coupling.

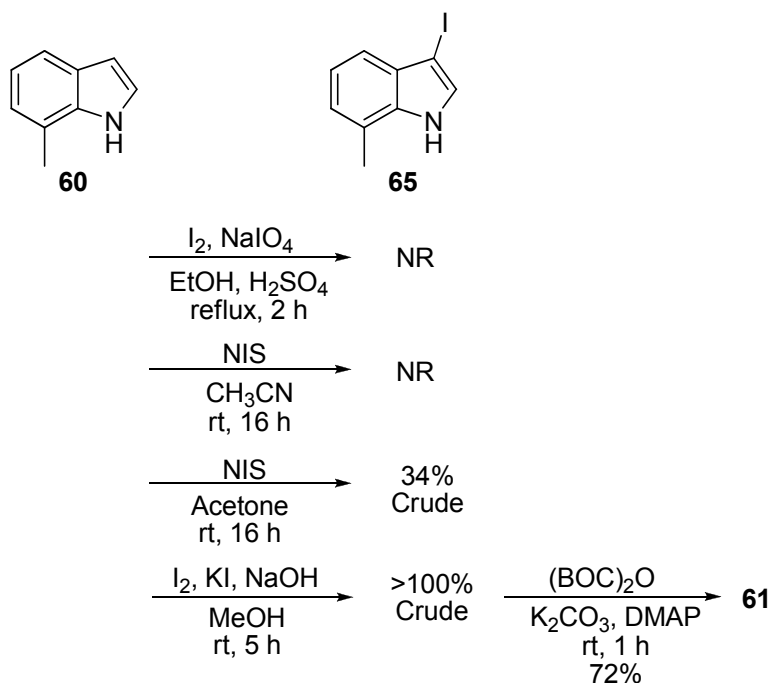
Scheme 2.3: Boc protection and stannylation of 7-methylindole and 7-prenylindole



Compound **64** was derived from the replication of a procedure used previously in our laboratory.⁴⁶ Initially, the results from this replication were disappointing. Therefore, troubleshooting began in order to identify the point at which the reaction was failing. It was assumed that the poor results were from the iodination step, and not so much the Boc protection. Consequently, much investigation went into the iodination of indoles (Scheme 2.4). The first procedure examined was based on a synthesis that was designed to iodinate the 5 position of ethyl indole 2-carboxylate with iodine, in the presence of sodium periodate (NaIO₄). However, what these researchers saw was iodination of the indole at the 3-position as well.⁶¹ Therefore, this synthesis proceeded by treatment of 7-methylindole with iodine in the presence of NaIO₄, EtOH, and H₂SO₄. Thin layer chromatography (TLC) indicated that all the starting material was consumed and a new fluorescent, violet spot appeared. The proton nuclear magnetic resonance (¹H NMR) spectrum of the final product, however, indicated that the compound had decomposed. The next iodination method utilized *N*-iodosuccinimide (NIS) in acetonitrile (CH₃CN). This procedure was previously used by the Townsend group to iodinate the 3-position of 2,5,6-trichloroindole.⁶² However, in the case of **60**, the reaction failed. Another group attempted a similar reaction using acetone instead of acetonitrile as solvent, and obtained their desired iodinated indole in 90% yield.⁶³ Switching solvents from acetonitrile to acetone proved to be successful, producing **65** in 34% recovered weight. Iodinated indoles are quite unstable and were used immediately without further purification. Such a low recovered weight, however, was unacceptable. Continued research led to an iodination method discovered in 1959, used to ultimately synthesize indoxyl acetate from

indole.⁶⁴ Implementing these same conditions, compound **60** was treated with an iodine-potassium iodide solution in MeOH, and indole **65** was obtained in 100% recovered weight. Boc-protection of crude **65** produced **61** in 72% yield (Scheme 2.4).

Scheme 2.4: Iodination of 7-methylindole, followed by Boc protection



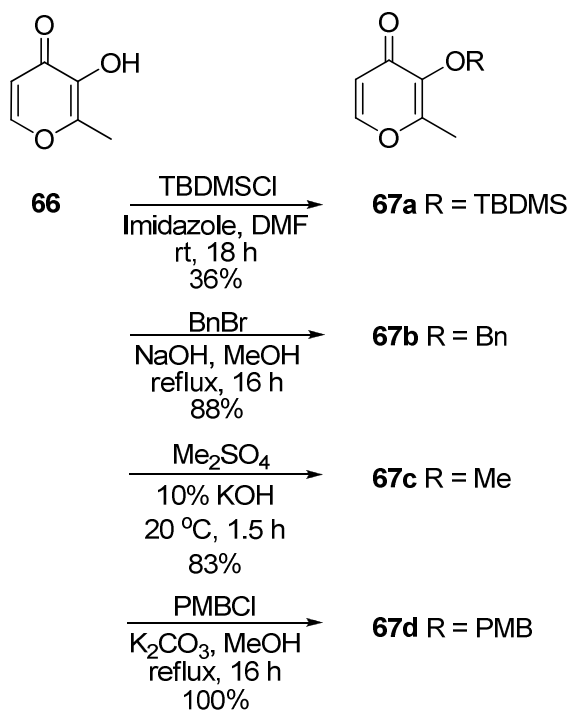
Although subjecting **60** to these iodinating conditions provided a successful outcome, the reaction failed with desired indole **41**. The second reaction that was successful in iodinating **60** was NIS in acetone. When indole **41** was subjected to the NIS iodinating conditions, the reaction failed in this instance as well. This led right back to the drawing board. Since the iodination method displayed in Scheme 2.3 was previously shown to be successful by our laboratory, it was reexamined with indole **60**. In this particular instance,

however, iodination reaction time was increased from the reported one hour to 16 hours, increasing the yield dramatically. When the conditions from Scheme 2.3 were applied to **41** with the increased reaction time, **63** was finally formed in 38% yield from the two steps.⁴⁶ Iodoindole **63** was immediately treated with *t*-BuLi, followed by Bu₃SnCl to form the desired stannane **64** in an overall 30% yield.

2.3.2 Pyrones

The first ZL-196 quinone replacement investigated was pyrone, where the carbonyl beta to indole was replaced with oxygen. The commercially available pyrone source closest in structure to the desired pyrone was maltol (**63**). Before maltol could be coupled to any indole, the alcohol needed to be protected. Multiple alcohol protection methods were investigated (Scheme 2.5). The first method consisted of using *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF. However, the TBDMS-ether **67a**⁶⁵ was formed in only 36% yield. In an attempt to improve the yield, benzyl protection was examined. Maltol was treated with benzyl bromide and NaOH in MeOH, to form benzyl-ether **67b**⁶⁶ in 88% yield. Although these are good results, deprotection of benzyl required the use of hydrogen (H₂) gas, which is inconvenient.

Scheme 2.5: Protection of the pyrone alcohol



This led to the use of the methylating agent, dimethylsulfate, which was combined with **66** in the presence of KOH as base, to form methylether **67c**⁶⁶ in 83% yield. Again, these results were good; however, a problem arises during deprotection. Removal of the methyl group and regeneration of the alcohol requires the use of boron tribromide (BBr₃), which is a fuming liquid that is inconvenient to use and did not work very well. Thus, investigation of protecting groups continued with the trial of *p*-methoxybenzyl chloride (PMBCl). Three different bases were examined for this procedure, KOH, NaOH, and potassium carbonate (K₂CO₃). Neither of the alkali bases were successful in deprotonation; however, K₂CO₃ worked marvelously, furnishing the PMB-ether **67d**⁶⁷ in quantitative yield.

Once the pyrone was protected, the next step was to iodinate at the alpha position. Several synthetic methods were attempted in order to obtain the α -iodopyrone; however, only one was partially successful. The first attempted synthesis of the α -iodopyrone followed a procedure that was used by the Ohshima group from Tokyo to iodinate the α -position of a cyclohexenone, in the enantioselective total synthesis of (-)-strychnine.⁶⁸ This method consisted of treating the alcohol-protected pyrone with iodine and 4-dimethylaminopyridine (DMAP) in dichloromethane (DCM). Although this method proved successful in the case of the cyclohexenone, only starting material was recovered (NR = no reaction) in the case of pyrone **67c** (Table 2.1, entry 1). Manipulation of these conditions, where DMAP was replaced by KOH and DCM was replaced by DMF, was examined. However, once again, only starting material was recovered (Table 2.1, entry 2). This led to the examination of a method reported in 1992 by Carl Johnson and his co-workers. In this study, they described the direct iodination at the α -position of cycloalkenones using iodine in a 1:1 mixture of pyridine and carbon tetrachloride (CCl₄).⁶⁹ Repetition of this method with pyrone **67c** produced the desired α -iodopyrone **68a** in only 4% yield, despite a three day reaction time (Table 2.1, entry 3). This prompted the addition of DMAP to the reaction, in hopes of increasing the yield. This addition however, only resulted in the recovery of starting material (Table 2.1, entry 4). Investigations continued with the discovery of a natural product synthesis reported in *Angew. Chem. Int. Ed.*,⁷⁰ where NIS was used in acetonitrile to iodinate the α -position of the diketone seen in Figure 2.6A. However, when the pyrone was subjected to these conditions, the reaction failed (Table 2.1, entry 5).

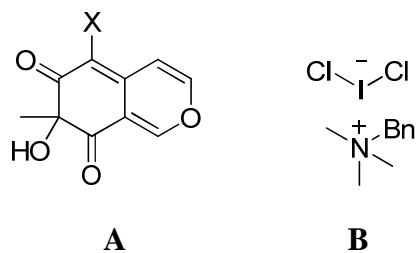


Figure 2.6: (A) Cyclic diketone, where X is the position of iodination. (B) BTMA-ICl₂

In 2002 a group from Tokyo reported the total synthesis of Garsubellin A, a macrocycle whose formation requires the α -iodination of an enone.⁷¹ They conducted that specific step using iodine in the presence of ceric ammonium nitrate (CAN), with acetonitrile as solvent. Repetition of their method with the alcohol-protected pyrone **67d** was attempted, but production of the desired iodo-product failed (Table 2.1, entry 6). One major problem was the low solubility of iodine. One of the final attempts at a synthetically pleasing procedure for the α -iodination of the pyrones came from a report by the Hayashi group from Tokyo.⁷² In this report, oxidative dimerization of epoxyquinols and epoxyquinones required the iodination of a cyclohexenone at the α -sp² carbon. This was done with iodine, [bistrifluoro(acetoxy)iodo] benzene (PhI(OCOCF₃)₂), and pyridine in DCM. Compound **67d** was subjected to these same conditions for 66 hours, and the iodinated pyrone **68b** was produced in 29% yield (Table 2.1, entry 7). In an effort to increase the yield, pyridine was replaced with DMAP; however, no product was observed with these changes (Table 2.1, entry 8). The search for the ideal conditions continued, leading to the discovery of the iodination of flavones, coumarin, and chromones (Figure 2.7). Iodination of these compounds utilizes LDA to lithiate the desired alpha position, followed by iodination with iodine.⁷³

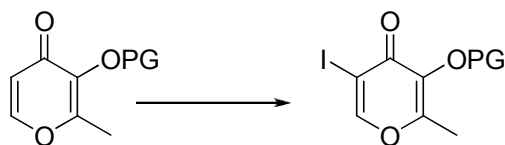


Table 2.1: Attempted methods of iodination for pyrones

Entry	PG ^a	Reagent	Solvent	Time (h)	Product	% Yield
1	Me	I ₂ /DMAP	DCM	15	-	SM ^b
2	Me	I ₂ /KOH	DMF	16	-	SM
3	Me	I ₂ /Py	CCl ₄	40	68a	4%
4	PMB	I ₂ /DMAP/Py	CCl ₄	16	-	SM
5	PMB	NIS	CH ₃ CN	17	-	- ^c
6	PMB	I ₂ /CAN	CH ₃ CN	15	-	-
7	PMB	I ₂ /PhI(OCOCF ₃) ₂	Py/DCM	66	68b	29%
8	PMB	I ₂ /PhI(OCOCF ₃) ₂	DMAP/DCM	52	-	-
9	PMB	I ₂ /LDA	THF	45 min	-	-
10	PMB	ICl/ZnCl ₂	AcOH	22	-	SM
11	PMB	BTMA-ICl ₂ / ZnCl ₂	AcOH	48	-	SM

^aProtecting group. ^bRecovered starting material. ^cNo reaction

Compound **67d** was subjected to these same conditions, yet, disappointingly, the reaction failed once again (Table 2.1, entry 9). Seeing as how the ¹H NMR spectrum indicated complete decomposition of starting material, perhaps these conditions were too severe. Thus far, all but one of the iodination methods have used molecular iodine as the source of iodine. Expanding research options led to a procedure that involved iodination of polymethylbenzenes with iodine monochloride (ICl) and zinc chloride (ZnCl₂) in AcOH

solution.⁷⁴ When **67d** was treated with these reagents, only recovered starting material was observed (Table 2.1, entry 10). Another source of iodine considered was benzyltrimethylammonium dichloroiodate (BTMA-ICl₂, Figure 2.6B). When used in the presence of ZnCl₂ in acetic acid, BTMA-ICl₂ was found to iodinate chroman compounds (Figure 2.7B) at aromatic positions.⁷⁵ Unfortunately, treatment of **67d** with this iodine source and ZnCl₂, only recovered starting material (Table 2.1, entry 11).

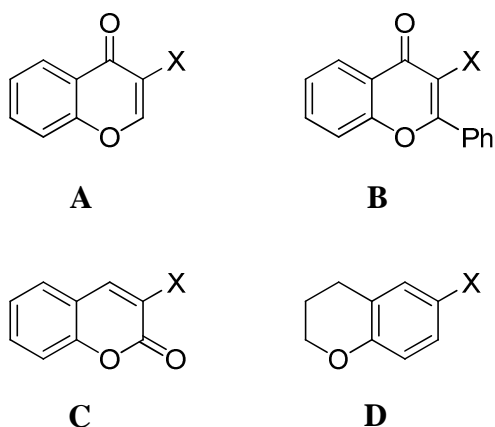


Figure 2.7: (A) Chromone. (B) Flavone. (C) Coumarin. (D) Chroman.
X indicates the position of iodination.

Consequently, the iodination method chosen for the remainder of the syntheses was the only one that produced a result, the method consisting of iodine in the presence of $\text{PhI}(\text{OCOCF}_3)_2$.

2.3.3 Pyridones

The second ZL-196 quinone replacement investigated was pyridone. There are not very many commercially available pyridones. Therefore, in order to attain pyridone analogs of DAQ-B1, the pyridones needed to be synthesized from alcohol-protected maltol. Synthesis began with TBDMS-protected pyrone **67a**, which was treated with a 40% solution of methylamine and refluxed in H₂O/HCl for 3.5 hours (Table 2.2, entry 1). Indication of the formation of a new compound on TLC prompted an extraction of the reaction mixture with ethyl acetate (EtOAc). It seemed however, that the product was hydrophilic and could not be extracted into an organic medium. In order to remove the water, the solution was distilled and a white solid, with a tint of orange color, resulted. The ¹H NMR spectrum of this material indicated the presence of the desired product, but it was not pure. Attempts were made at purification using silica gel chromatography; however, it seemed that the compound stuck to the silica and could not be retrieved. The next attempt at synthesizing a pyridone required the use of a 40% solution of aniline in H₂O/HCl, which was refluxed with compound **67a** for 20 hours (Table 2.2, entry 2). Once again, the hydrophilic product favored the aqueous layer. Upon removal of the water, a mixture of yellow solid and brown oil was produced. This mixture was cooled, in hopes of producing more precipitate, and the solid was filtered. A ¹H NMR spectrum of this solid was obtained and it indicated the presence of the desired pyridone. However, the mass spectrum (MS) showed complete compound decomposition. Formation of the N-phenyl pyridone from aniline was attempted once more; however, in this instance, benzyl protected maltol (**67b**) was used as the pyrone source (Table 2.2, entry 3).

Refluxing **67b** and 40% aniline in H₂O/HCl for 20 hours yielded a product that was not extractable by organic solvent. Consequently, the aqueous phase it was in was evaporated and a purification process on silica gel was attempted. In this case, a small amount of **69** was produced (3%); however, it required a very polar solvent to elute it, which affected the purity of the purified product. Although these pyridones were being formed, they could not be retrieved nor characterized because they were sticking to the silica gel during the purification process.

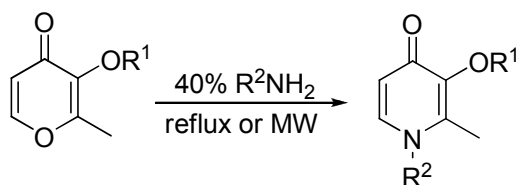


Table 2.2: Synthesis of Pyridones

Entry	R ¹	R ²	Time (h)	Solvent	Product	% Yield	Remarks
1		-CH ₃	3.5	H ₂ O/HCl	-	-	Pdt in aq layer
2			20	H ₂ O/HCl	-	NR ^a	-
3			20	H ₂ O/HCl	69	3%	Pdt in aq layer
4			24-48 2	EtOH	-	SM ^b	Reflux MW cond.
5			2	EtOH	-	SM	MW cond.
6			48 2	EtOH	70	13% 17%	Reflux MW cond.

^aNo reaction. ^bRecovered starting material.

Benzyl protected maltol (**67b**) was used in these pyridone forming reactions twice more. First, it was treated with 40% isopropylamine and refluxed in EtOH. After 24 hours, formation of product did not seem evident (Table 2.2, entry 4). As a matter of fact, the only compound recovered was **67b**. This procedure was run again for a longer reaction time of 48 hours. Yet again, however, only recovered **67b** was observed. Perhaps the reason these conversion reactions were failing is due to insufficient heat and pressure. This problem could be rectified by the use of a microwave reactor. Thus, pyrone **67b** and isopropylamine were combined in a sealed microwave vessel with no solvent, and placed in the microwave reactor at 120 °C and 150 watts (W). After a hold time of one hour, the reaction mixture was studied on the gas chromatograph (GC). The results from the GC indicated that not all the starting material had been consumed; thus, the mixture was left to react for one more hour. Upon completion of the reaction, the GC indicated complete consumption of starting material and the ¹H NMR spectrum suggested the potential formation of product (Table 2.2, entry 4). Attempts at purification were taken using silica gel chromatography; however, the ¹H NMR spectrum of the purified product did not show any sign of the pyridone. The next amine that was reacted with **67b** was cyclohexylamine. Both these compounds, along with EtOH were subjected to the microwave conditions described previously. After a two hour reaction however, only the starting pyrone was recovered (Table 2.2, entry 5). The next amine that was investigated possessed a property that the other amines did not have, ring strain. Methyl-protected maltol (**67c**) was treated with cyclopropylamine and refluxed in EtOH for 48 hours. After workup, the resulting viscous oil was purified using column chromatography on silica

gel, and the desired pyridone **70** was formed in 13% yield (Table 2.2, entry 6). ^1H NMR and MS both confirm the presence of the desired pyridone. The ^1H NMR however, shows the presence of impurities and the MS shows evidence of a mixture of two compounds. Consequently, the reaction was attempted once more, this time using microwave conditions. Pyrone **67b**, cyclopropylamine, and EtOH were combined in a microwave tube and the reaction was carried out in the microwave at 150 W and 80 °C. After a two hour hold time, the reaction mixture was tested for completion on the GC. The presence of starting material on the GC chromatogram prompted an extension of the reaction time to a total of 3 hours. This however, resulted in the complete decomposition of product. The reaction was attempted once more and stopped at the two hour mark. Workup, followed by purification provided the desired pyridone **70** in 17% of the theoretical weight (Table 2.2, entry 6). Once again, however, the ^1H NMR spectrum exhibited evidence of an impure compound.

The lack of success in the pyrone to pyridone conversion prompted further investigation into processes by which pyridones can be prepared. The main problem in the synthesis of pyridones is that they are hydrophilic compounds that are difficult to work with and purify. Thus, in an effort to facilitate the availability of these quinone replacements, commercially available pyridones were sought. The commercially available pyridone closest in structure to the desired target was 3-hydroxy-1,2-dimethylpyridone (**71**). In order to properly utilize this pyridone in Stille coupling reactions with indoles, the alcohol functionality needed to be protected first. Table 2.3 shows the many different attempts at protecting the alcohol of **71**. The first protecting

group examined was TBDMS (Table 2.3, entry 1). Pyridone **71** was treated with TBDMSCl and imidazole in DMF and the reaction was stirred at room temperature overnight. TBDMSCl did not seem to work as a protecting agent because only starting material was recovered. This led to the examination of the next protecting group, a benzyl group (Table 2.3, entry 2). Pyridone **71** and cesium carbonate (CsCO_3) were refluxed in DMF for 36 hours and the resulting reaction mixture displayed a promising outcome, as shown by the ^1H NMR spectrum. Spectral data of the purified sample however, did not directly indicate the presence of the desired product. A different combination of base (NaOH) and solvent (MeOH) was evaluated with this same protecting group, but that failed as well (Table 2.3, entry 2). When protecting pyrones such as maltol, PMB worked beautifully, producing PMB-protected maltol in quantitative yield. These results encouraged the use of PMB as a pyridone protecting group. Many different bases and solvents were examined in order to find the ideal reaction conditions with PMBCl (Table 2.3, entry 3). At first, sodium hydride (NaH) in DMSO was used, but these conditions were unsuccessful. Based on evidence from the ^1H NMR spectrum, it seemed that the 2-methyl group had undergone some sort of reaction because its corresponding signal did not appear. It is a possibility that NaH is too strong of a base, thus encouraging the use of a milder base, such as KOH. The use of KOH though, also resulted in a failed reaction; thus, prompting the use of K_2CO_3 as base in DMF. Refluxing **71** under those conditions for 16 hours produced trace amounts of PMB-protected pyridone **72**. Despite purification attempts, spectral data indicated impure **72**. In an effort to increase the yield, different solvents were examined for this reaction. Both acetone and acetonitrile were tested and

both solvents seemed to furnish the desired product, after a workup had been conducted. Purification of this slightly orange colored compound on silica gel was attempted. However, the compound seemed to stick to the column and isolation of a pure sample ultimately failed.

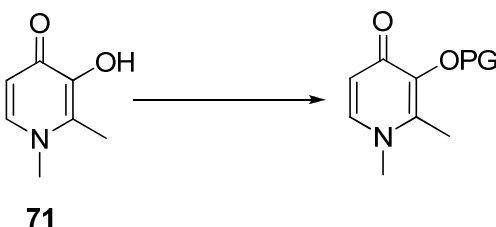


Table 2.3: Protection of the alcohol group on pyridone

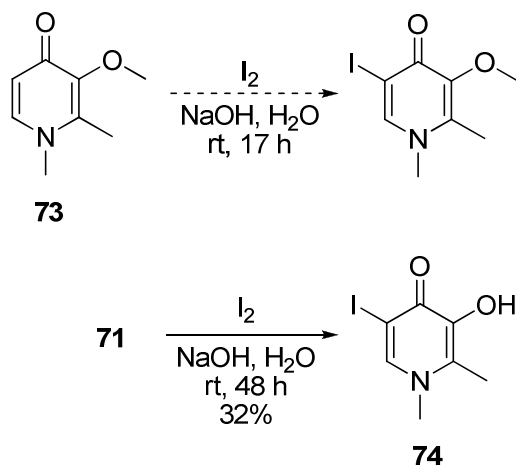
Entry	PG ^a	Reagent	Solvent	Temp. (°C)	Time (h)	Pdt.	% Yield
1		TBDMSCl/ Imidazole	DMF	rt	16	-	SM ^b
2		BnBr/CsCO ₃	DMF	Reflux	36	-	-
		BnBr/NaOH	MeOH	Reflux	48	-	-
3		PMBCl/NaH	DMSO			-	-
		PMBCl/KOH	DMSO			-	-
		PMBCl/K ₂ CO ₃	DMF	Reflux	16	72	4%
		PMBCl/K ₂ CO ₃	Acetone			-	-
		PMBCl/K ₂ CO ₃	CH ₃ CN			-	-
4		Me ₂ SO ₄ /KOH	H ₂ O	20	24	73	83%

^aProtecting group. ^bRecovered starting material.

Another protecting group that was successful in the case of the pyrones was methyl. Thus, the methylating agent dimethyl sulfate was used in the presence of an aqueous solution of KOH, and the mixture was stirred at just below room temperature (Table 2.3, entry 3). After 24 hours, an aqueous work up was undertaken, but this was challenging. The methyl-protected pyridone (**73**) was too hydrophilic to separate into the organic phase. The aqueous environment that held the desired product was removed and NMR data provided information to substantiate the correct structure. However, there were many impurities. Previous failed purification attempts with other pyridones sparked hesitation in using silica gel chromatography as a medium for purification. A second workup was attempted, where the crude product was dissolved in water and then extracted using 1:4 isopropanol:chloroform. Removal of this solvent mixture in vacuo provided the desired pyridone **73** in 83% yield.

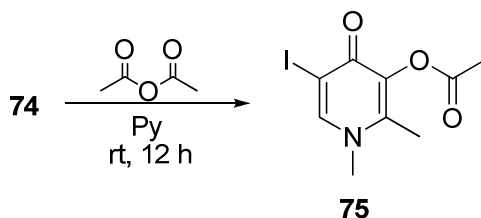
The next step in this series of reactions designed to prepare pyridones for Stille coupling, is the iodination at the α -position of the pyridone. Protected pyridone **73** was treated with iodine in an aqueous solution of NaOH and the reaction was stirred at room temperature for 17 hours, only to reveal recovered starting material (Scheme 2.6). These iodination conditions were used once more in an attempt to iodinate the non-protected pyridone starting material **71**. After a 48 hour reaction time, the ^1H NMR spectrum revealed what seemed to be recovered starting material. Upon work up however, the hydrophobic product separated from the hydrophilic starting material. Once again though, the previous failures in purification attempts sparked a hesitation to use silica gel for purification.

Scheme 2.6: Iodinations of pyridone



This problem could be by-passed by acetylating the pyridone, purifying the acetylated form, and finally hydrolyzing it back to the desired product. Iodinated pyridone **74** was treated with acetic anhydride in pyridine and the reaction was stirred for 12 hours (Scheme 2.7). Preliminary data suggested the presence of the acetylated product **75**; however, once again, another pyridone compound failed to cooperate on silica gel.

Scheme 2.7: Acetylation of N-methylpyridone

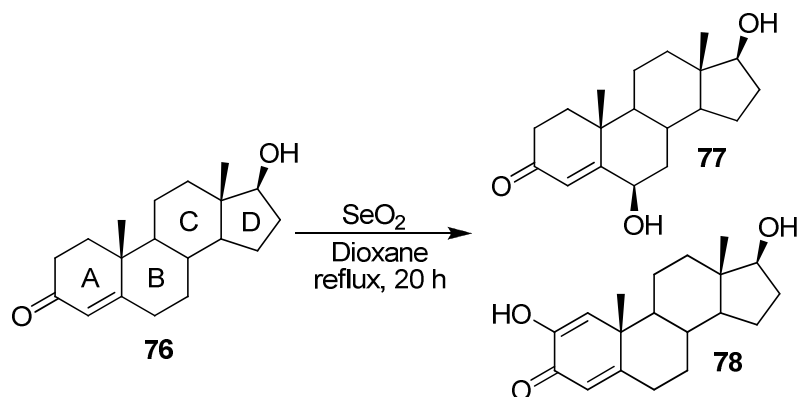


The pyridone compounds required to make up the core unit of the quinone-substituted DAQ-B1 analogs, proved to be problematic and synthesis of pyridone analogs could not be accomplished.

2.3.4 Cyclohexadienones

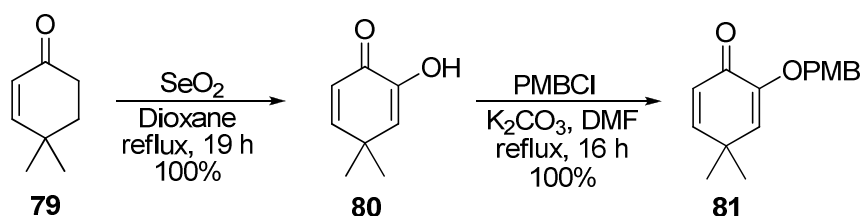
The final ZL-196 quinone replacement investigated was cyclohexadienone, where the carbonyl beta to the indole is substituted for a quaternary carbon. This quinone replacement has not been previously investigated by our laboratory. The core unit of the desired compound, 2-hydroxy-4,4-dimethylcyclohexa-2,5-dienone (**80**) is not commercially available; therefore, it had to be synthesized. In 2004 the Seger group from Austria attempted an allylic hydroxylation of the steroids progesterone and testosterone using selenium dioxide (SeO_2).⁷⁶ Oxidation of testosterone (**76**) yielded two major compounds, their desired product **77**, as well as 2-hydroxydienone **78** (Scheme 2.8).

Scheme 2.8: Hydroxylation of testosterone



These results are beneficial to quinone replacement research since the desired cyclohexadienone compound does not possess a steroidal moiety and contains only one possible position for oxidation. Therefore, oxidizing a precursor that resembles ring A in **76** using those same reaction conditions, may lead to a product similar to ring A in **78**. Fortunately, 4,4-dimethyl-2-cyclohexen-1-one (**79**) is commercially available and was the starting point for this synthesis (Scheme 2.9). Compound **79** was oxidized using SeO₂ in dioxane to form **80** in quantitative yield. Before coupling this cyclic ketone to the indole, the hydroxy group needed to be protected. Many different protecting groups were evaluated in the cases of the pyrones and pyridones; yet, only one protecting group demonstrated an ease in its addition as well as its cleavage, *p*-methoxybenzyl. Protection of the α -hydroxy group was accomplished by treating **80** with PMBCl and K₂CO₃ in DMF, to form the protected cyclohexadienone **81** in quantitative yield.

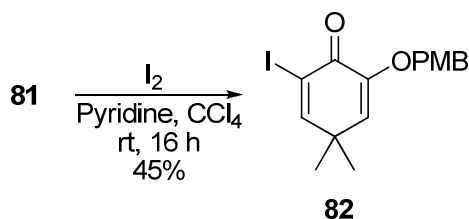
Scheme 2.9: Synthesis and protection of 4,4-dimethyl-2-hydroxycyclohexadienone



The next step in this sequence of reactions was the iodination of **81**, which is required in order to perform Stille coupling to an indole. In the cases of the pyrones and pyridones, the iodination proved to be quite a challenging task to achieve. In the case of the cyclohexadienone; however, it was less complicated. The iodination procedures for

the cyclohexadienone investigated were previously examined in the case of the pyrone. The protocol adopted consisted of the addition of iodine to **81** in a 1:1 solution of pyridine and CCl₄ to produce **82** in 45% yield (Scheme 2.10). The protocol involving the use of DMAP and DCM, instead of pyridine and CCl₄ was also attempted, but failed.

Scheme 2.10: Iodination of cyclohexadienone

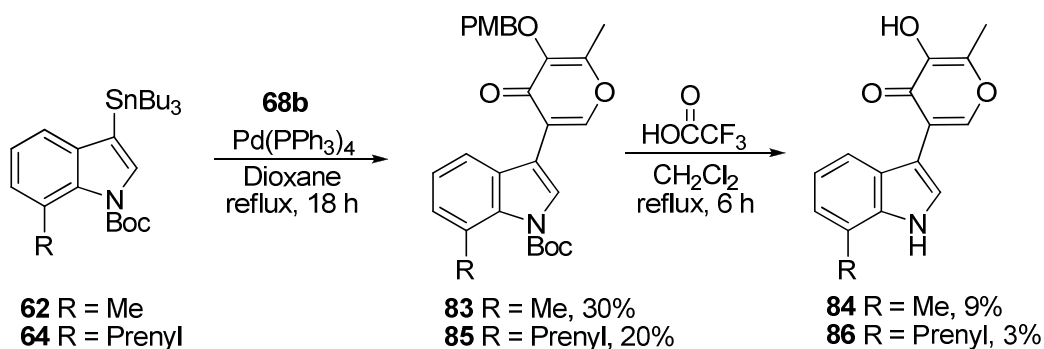


2.3.5 Stille Coupling of Indoles to Cyclic Ketones

As stated previously, 7-prenylindole is a precious compound. Therefore, once again, 7-methylindole served as a model system for the Stille coupling of indoles to pyrones. Stannylated methylindole **62** was added to PMB-protected iodomaltol **68b** in the presence of a palladium catalyst to form coupled product **83** in 30% yield (Scheme 2.11). Deprotection of the PMB and Boc groups with TFA in DCM produced **84** in a 9% yield. Such a low yield was the result of a challenging purification process. It appeared as if **84** stuck to the silica gel during flash column chromatography, and only a minute amount was retrieved. Despite the low quantity obtained, synthesis of the desired prenyl analog **86** was attempted. Stannylated 7-prenylindole was coupled to **68b** in the same manner as **65**, to form the protected product **85** in 20% yield. Deprotection with TFA produced

DAQ-B1 analog **86** in 3% yield (Scheme 2.11). Once again, this unsatisfactory yield was due to the highly polar nature of indolylpyrones, making them hard to purify. In addition to the low yield, a problem with the products from the Stille coupling reaction arose. Despite countless purification attempts, both **83** and **85** were impure.

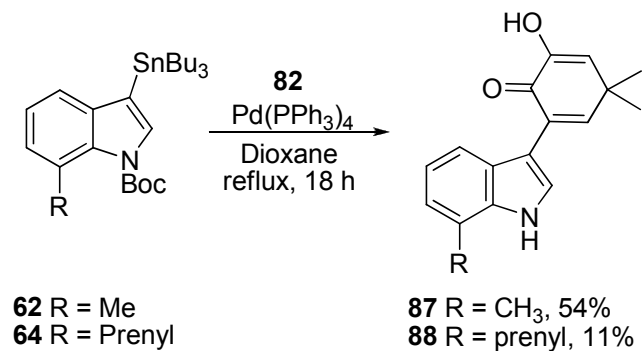
Scheme 2.11: Stille coupling of indoles to pyrone



The cyclohexadienone has proven to be easier to work with and involves more efficient reactions than the pyrone or pyridone. Such was the case when cyclohexadienone **82** was used in a Stille coupling to indoles **62** and **64** (Scheme 2.12). Stannylindole **62** was combined with cyclohexadienone **82** in the presence of Pd(PPh₃)₄ and refluxed in dioxane to produce the coupled product **87** in 54% yield. At some point during the course of the reaction, the PMB and Boc protecting groups must have been cleaved because their respective signals did not appear in the ¹H NMR spectrum. The next indole, stannylated 7-prenylindole (**64**) was coupled to **82** under the same conditions as **62** to produce the coupled product **88** in 11% yield. Once again, the ¹H NMR spectrum of the coupled product did not exhibit signals corresponding to the PMB and Boc

protecting groups. These substituents must have been cleaved during the course of the reaction.

Scheme 2.12: Stille coupling of indoles to cyclohexadienone



As can be seen from the many reactions discussed in section 2.3, the Stille coupling route to indolylquinone analogs was not versatile and posed many practical disadvantages, especially in the case of the pyridones.

2.4 Synthesis of DAQ-B1 Analogs via Claisen Rearrangement

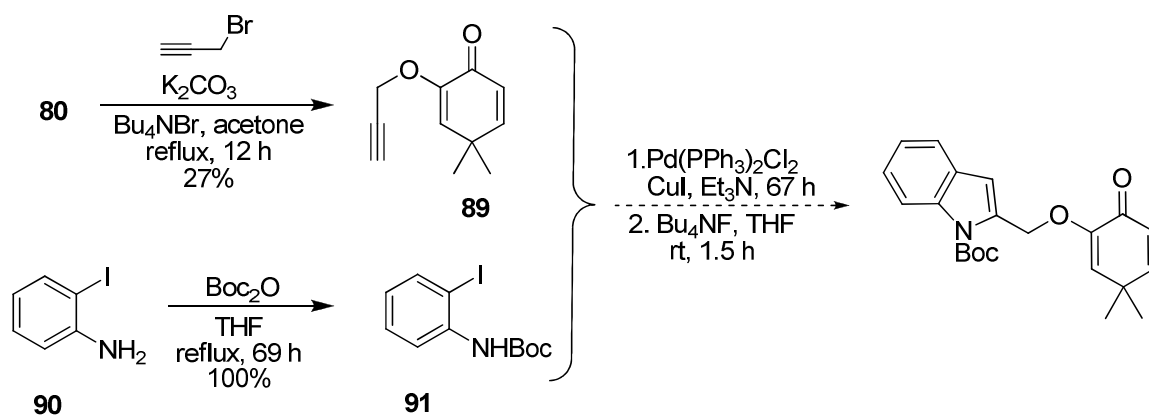
As mentioned in Chapter one, the Pirrung group reexamined the Stille coupling strategy and developed a synthetic route to indolyl-kojic acids based on the Claisen rearrangement of an (indole)-methyl-kojic acid ether.³⁶ This method was applied in the cases of the cyclic ketones described in Section 2.3, in hopes of producing the desired compounds in a simpler, more efficient method that avoids the use of organotin reagents.

2.4.1 (Indolyl)cyclohexadienones

The first cyclic ketone examined was cyclohexadienone **80**. In following the protocol set forth by the Pirrung group, the first step of this synthesis was the formation of propargyl ether **89** (Scheme 2.13). The starting cyclohexadienone **80** was refluxed with K₂CO₃ and TBAB in acetone to produce **89** in 27% yield. During the Claisen rearrangement, it is necessary for the indole nitrogen to be protected; otherwise, the excessive amounts of heat required for the rearrangement to take place causes the regeneration of **80** by solvolysis. Consequently, before propargyl ether **89** can be coupled to 2-iodoaniline (**90**), the amine needed to be protected. Initially, it was protected as the sulfonamide (tosyl group); however, removal of an N-sulfonamide could possibly be challenging. Therefore, the more easily removable Boc group was used to protect **90** instead. This was done using Boc anhydride in THF to form Boc-protected iodoaniline **91** in quantitative yield.⁷⁷ Coupling of aniline **91** to propargyl ether **89** should have produced the ((indolyl)methoxy)cyclohexadienone in a 2 step, one pot synthesis. However, despite

the many attempts and manipulations of the reaction conditions, this coupling reaction failed.

Scheme 2.13: Attempted synthesis of (indolyl)cyclohexadienone



2.4.2 (Indolyl)kojic Esters

Kojic acid (**92**) is a natural product used in cosmetics and as a preservative in foods⁷⁸, therefore making it a safe choice as a quinone replacement. The Pirrung group used kojic acid to form many (indolyl)kojic acids (**19(n)**) and (indolyl)pyridones (**21**) via the Claisen rearrangement.³⁶ The next phase of this insulin mimic library building process was the synthesis of (indolyl)kojic esters (Figure 2.8), where the alcohol portion of the kojic acid was transformed to an ester.

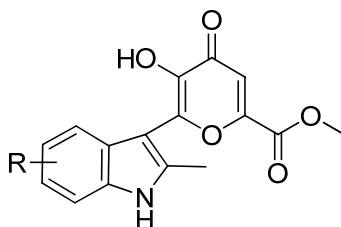
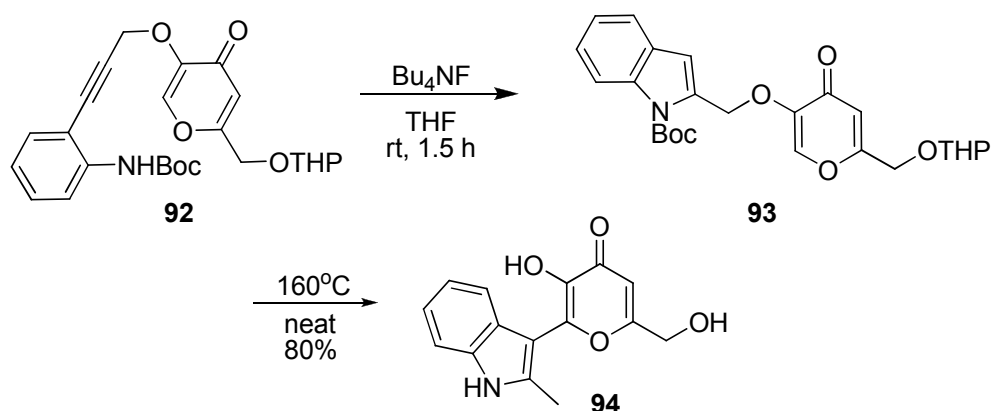


Figure 2.8: (Indolyl)kojic esters

Before this phase of the project can begin, the Claisen rearrangement experiments conducted by our laboratory needed to be reproduced to ensure success, especially since this protocol failed in the case of the cyclohexadienones. Synthesis of (2-methylindolyl)kojic acid (**94**) was carried out in the same manner as was previously seen in Scheme 1.5 in an overall 27% yield (Scheme 2.14). Certain manipulations of this scheme were undertaken in efforts to improve efficiency.

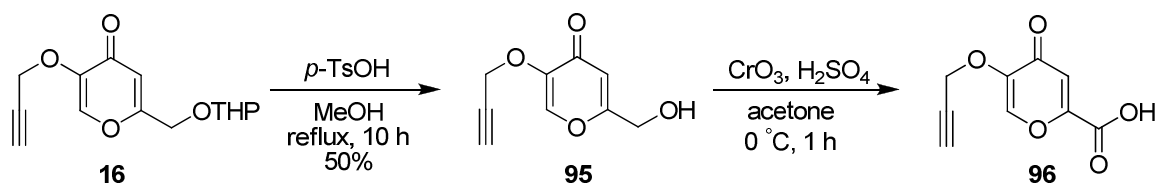
Scheme 2.14: Synthesis of (2-methylindolyl)kojic acid via Claisen rearrangement



For example, a one-pot synthesis of **94** from the propargyl ether **16** was attempted, without the isolation of intermediate **92**. Unfortunately, compound **93** was not produced under these conditions, thus affirming the necessity of isolating intermediate **92**.

Successful reproduction of the Claisen rearrangement to produce **94** allowed for the initiation of the next phase, which was the synthesis of (indolyl)kojic esters. In order to oxidize the alcohol portion of **16**, removal of the pyran protecting group was necessary to expose the alcohol. This was done by treating **16** with *p*-toluenesulfonic acid (*p*-TsOH) in methanol to yield the deprotected product **95** in 50% yield. Oxidation from alcohol to ester was a step-wise process that went through a carboxylic acid intermediate. Formation of the carboxylic acid was accomplished through a Jones oxidation to yield carboxylic acid **96** (Scheme 2.15).

Scheme 2.15: Deprotection and oxidation of propargylated kojic acid



The Jones oxidation is a process whereby primary or secondary alcohols are converted to carboxylic acids by using chromium trioxide (CrO_3) in H_2SO_4 (Jones reagent). One downfall of this reaction is the production of chromium (III) salts, which are green deposits that were very difficult to remove. Separation of the product from the chromium salts by column chromatography is improbable since **96** retains high polarity and would

stick to the silica gel. Consequently, other methods were considered, such as oxidation using pyridinium dichromate (PDC) in DMF. Compound **95** was subjected to these oxidizing conditions; however, failure to produce carboxylic acid **96** quickly terminated this attempt.

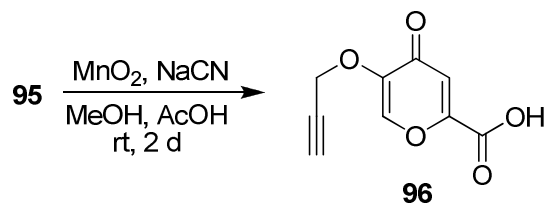
An interesting study conducted by the Taylor group from England demonstrated the ability of esters to be formed from activated alcohols using manganese(IV) dioxide (MnO_2) via a tandem oxidation process.⁷⁹ Benzyl alcohol was treated with MnO_2 and sodium cyanide (NaCN) in MeOH and glacial AcOH to form methyl benzoate in moderate yield (Scheme 2.16). They applied these conditions to numerous alcohols and the reaction was successful each time.

Scheme 2.16: Synthesis of methyl benzoate from benzyl alcohol



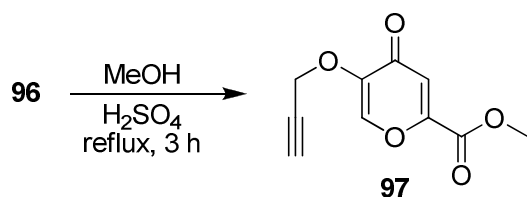
In an attempt to form the (indolyl)kojic ester directly from the alcohol, these oxidizing conditions were applied to compound **95** (Scheme 2.17). Despite a two day reaction time; however, these conditions were not sufficient to form the ester. Instead, carboxylic acid **96** was formed, as indicated by infrared (IR) and NMR spectroscopy.

Scheme 2.17: Alternative method to oxidize propargylated kojic acid



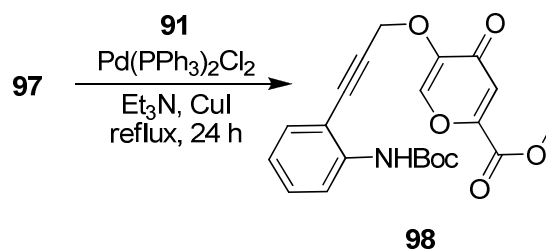
Despite the presence of chromium impurities, the Jones oxidation was determined to be the best method for producing this acid. In order to convert the acid into the ester, **96** was refluxed in MeOH and acetic acid to produce the esterified product **97** (Scheme 2.18).

Scheme 2.18: Synthesis of kojic ester



Kojic ester **97** was added to iodoaniline **91** via the same Sonogashira coupling conditions seen in Scheme 2.14. Spectral data of the crude mixture suggests the formation of the coupling product **98** (Scheme 2.19). Flash chromatography on silica gel however, failed to yield any product. This result indicated that compounds of this nature could possibly be too polar for silica gel purification. Cyclization attempts of crude **98** were taken; however, no product was seen.

Scheme 2.19: Sonogashira coupling of kojic ester with Boc-protected iodoaniline



2.4.3 (Indolyl)pyridone Esters

Unlike the maltol-derived pyridones discussed in section 2.3.3, the pyridones discussed in this section (Figure 2.9) are derived strictly from kojic acid. Many routes towards (indolyl)pyridone esters were examined. The first route carried out consisted of converting the propargylated pyrone ester **97** to the pyridone by treating **97** with a 40% aqueous solution of methylamine in EtOH.

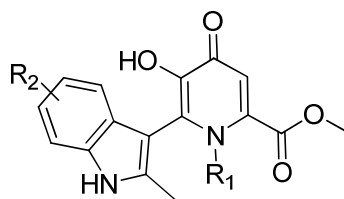
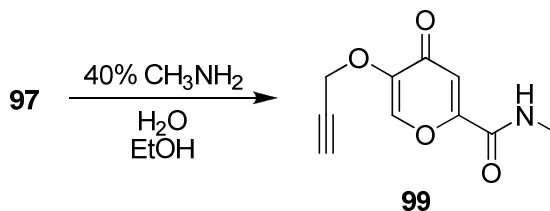


Figure 2.9: (Indolyl)pyridone esters

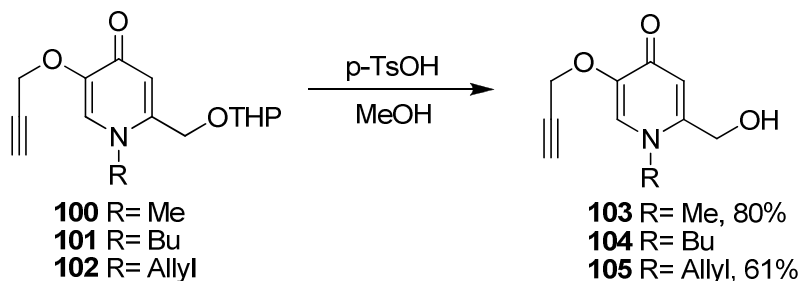
Unfortunately, the only product obtained from this process was amide **99** (Scheme 2.20). That amide product was resubmitted under the same reaction conditions, in hopes of forming the pyridone. Despite the continual addition of methylamine into the refluxing reaction mixture, only starting material could be recovered.

Scheme 2.20: Conversion of pyrone ester to pyrone amide



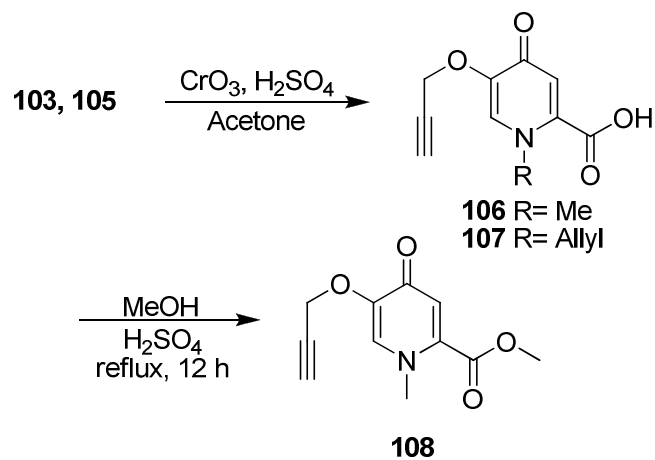
This pyridone-forming process needed to be re-evaluated. The problem in Scheme 2.20 was the presence of the ester in pyrone **97**. We hypothesized that amide formation is avoidable if the pyrone was converted to the pyridone prior to oxidation and esterification. A second route was examined where THP-protected pyridones **100**, **101**, and **102**⁸⁰ were individually deprotected using *p*-TsOH in MeOH. These pyridones possessed similarities to the maltol-derived pyridones described previously. Their high polarity and hydrophilicity were a disadvantage for purification since they had the tendency to stick to a flash column. Despite these difficulties, pyridones **103** and **105** were produced in 50% and 27% recovered weight, respectively. Pyridone **104** was harder to analyze because the amount of impurities associated with the formation of this analog was too vast to warrant isolation.

Scheme 2.21: THP deprotection of pyridone



The reason that such a small amount of product was formed in the cases of **103** and **105** was due to the large amounts of recovered starting material, 50% and 73%, respectively, despite the reaction times (up to 4 days for some cases). The recovered starting materials were subjected to the reaction conditions a second time to produce **103** and **105** in 80% and 61% yields, respectively (Scheme 2.21). Once structure determination of the isolated pyridones was complete (^1H NMR and LRMS), they were subjected to a Jones oxidation to yield carboxylic acids **108** and **109** (Scheme 2.22). Oxidation via Jones reagent (Cr^{+6}) produces chromium salts (Cr^{+3}), which were removed by filtration. Without further purification, acids **108** and **109** underwent the same esterification reaction seen in Scheme 2.18. The only methyl ester produced however, was *N*-methylpyridone **108**. The reaction failed in the case of *N*-allyl (**107**). Once again, extraction of the desired compound proved to be difficult since **108** was hydrophilic and favored the aqueous phase during work up, even when DCM:isopropanol was used as the organic extract. Due to the high polarity of these compounds, standard purification techniques were not operable. Therefore, characterizations of **108** were made on the crude product and a proper yield could not be determined.

Scheme 2.22: Synthesis of pyridone esters



Once synthesis of the pyridone was complete, the next step was to couple it to **91**. Despite numerous attempts, it was quite difficult to dissolve such a hydrophilic compound into an organic solvent suitable for Sonogashira coupling, such as THF. This made the moisture-sensitive Sonogashira reaction impossible.

2.5 Synthesis of the *Aspergillus* Natural Product Terrequinone A

2.5.1 Discovery of Terrequinone A

The genus *Aspergillus*, which contains many recognized species, has proven to be a rich source of bioactive metabolites. In 2004 the Gunatilaka group from the Southwest Center for Natural Products Research and Commercialization in Arizona discovered a novel metabolite from *Aspergillus* known as terrequinone A (Figure 2.10).⁸¹ Upon isolation and purification of the EtOAc extracts containing terrequinone A, spectral data was collected. Surprisingly, this data indicated that the central quinone moiety is monohydroxylated compared with all known asterriquinones, which contain dioxygenated quinone moieties. On the basis of the extensive spectral data collected, the structure of terrequinone A was elucidated as 2-hydroxy-6-(3-indolyl)-5-(3-methylbut-2-enyl)-3-(2-(2-methylbut-3-en-2-yl)-3-indolyl)cyclohexa-2,5-diene-1,4-dione.

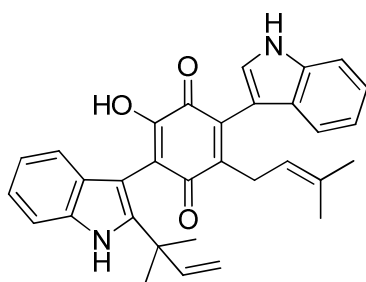


Figure 2.10: Terrequinone A

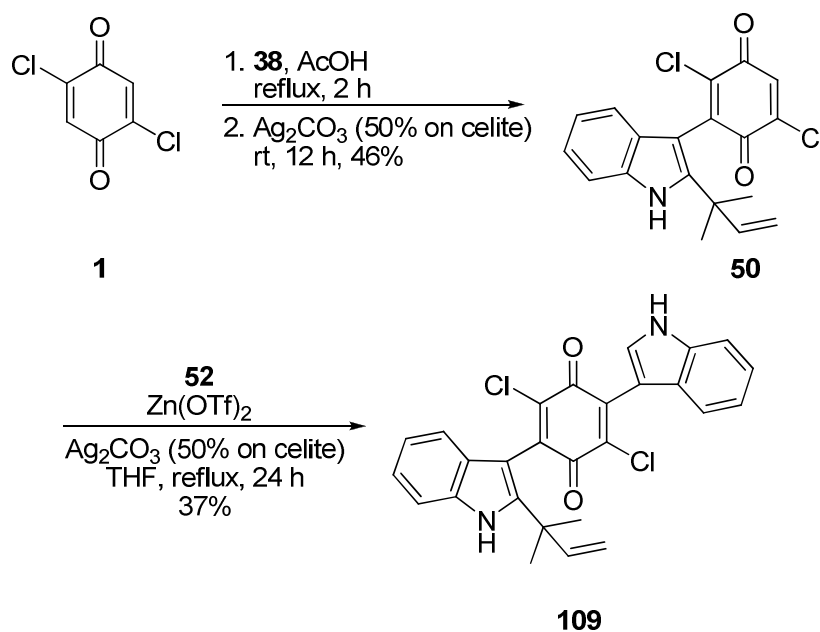
Once the structure was determined, they evaluated terrequinone A for *in vitro* cytotoxicity against a panel of four cancer cell lines (non-small cell lung carcinoma,

human breast carcinoma, human glioma, and human pancreatic cancer) and normal human primary fibroblast cells. It was found to be cytotoxic with an IC_{50} value ranging from 5.40 to 13.9 μ M.

2.5.2 Synthesis of Terrequinone A

Terrequinone A is an asterriquinone that possesses similarities to DAQ-B1. It contains many of the same substituents, located at different positions. DAQ-B1 was shown in Chapter 1 to have a strong impact on diabetes by activating the insulin receptor and consequently, displaying oral insulin mimetic activity in mice.

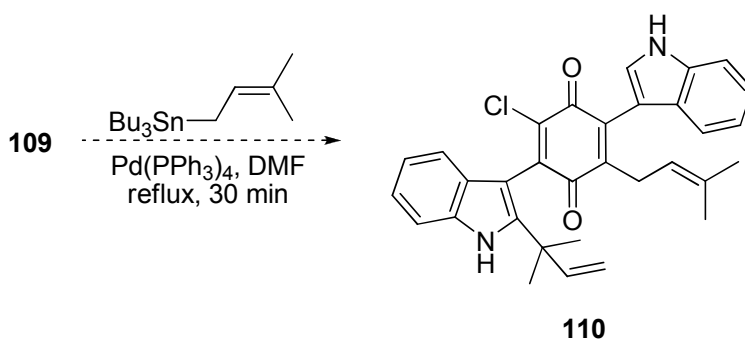
Scheme 2.23: Synthesis of terrequinone A intermediate



Since terrequinone A is very similar in structure to DAQ-B1, it could potentially possess similar anti-diabetes activity. Therefore, synthesis of this compound is crucial.

Synthesis of terrequinone A began with the addition of known indole **38** to commercially available **1**; producing the known coupled intermediate **50**⁴⁷ in 46% yield (Scheme 2.23).²⁵ Formation of the bisindolylquinone from **50** and **52** using the standard coupling methods designed by the Pirrung group²⁵ furnished the bis-coupled product **109** in 37% yield. The next step in the synthesis of this natural product was the addition of the prenyl group onto the 3-position of the benzoquinone moiety. The first attempt at this reaction consisted of a Stille coupling between **109** and prenyltributyltin, in the presence of Pd(PPh₃)₄ (Scheme 2.24). Despite numerous attempts at producing **110** using this standard method, after 30 minutes of refluxing in DMF, the starting material appeared to have decomposed.

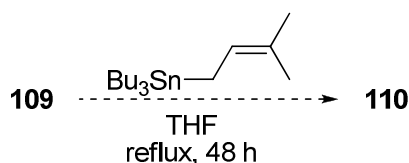
Scheme 2.24: First attempt at prenylation of benzoquinone



A second attempt to prepare **110** was undertaken, following a protocol by the Tam group.⁸² In order to prepare 2-allyl-1,4-naphthoquinones, they treated chlorinated

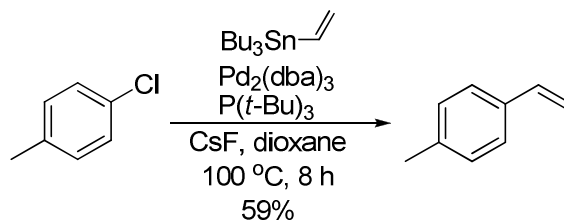
naphthoquinones with tetra-allyl tin in refluxing THF, without a catalyst. They produced their desired product in good yield. A parallel reaction was conducted with **109** and prenyltributyltin. Despite a 48 hour reaction time however, the product was not formed and some starting material was recovered (Scheme 2.25).

Scheme 2.25: Second attempt at prenylation of benzoquinone



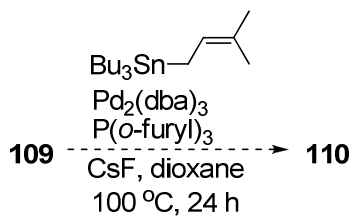
A final attempt at the formation of **110** was based on a synthesis by the Fu group from MIT.⁸³ They reported the cross-coupling of aryl chlorides with alkyl and aryl tin reagents, such as allyltributyltin, using $\text{Pd}_2(\text{dba})_3/\text{P}(\text{t-Bu})_3$ as catalyst (Scheme 2.26). Moreover, they discovered that fluoride sources can facilitate cross-coupling, with cesium fluoride (CsF) being the most efficient. This is because nucleophiles such as fluoride ion can increase reactivity of organotin species via hypercoordinate intermediates. This technique allowed them to effectively Stille couple unactivated aryl and vinyl chlorides and, cross-couple a wide variety of aryl bromides with a diverse range of organotin reagents.

Scheme 2.26: Stille coupling of aryl chloride to allyl tin



A third attempt at the synthesis of **110** was undertaken, with one small modification. The phosphorous portion of the catalyst, $\text{P}(t\text{-Bu})_3$ was replaced with tri-2-furylphosphine ($\text{P}(o\text{-furyl})_3$) simply for the reason of accessibility (Scheme 2.27). Prenyltributyltin was added to a solution of **111**, $\text{Pd}(\text{dba})_3$, $\text{P}(o\text{-furyl})_3$ and CsF in dioxane. Reaction at $100\text{ }^\circ\text{C}$ for 24 hours however, produced only a negligible amount of product. This coupling reaction will have to be addressed by future members of the Pirrung group.

Scheme 2.27: Third attempt at prenylation of benzoquinone



Chapter Three: Synthesis of DAQ-B1 Analogs as Neuroprotective Drugs

3.1 Alzheimer's Disease

Alzheimer's disease (AD) is an incurable, neurodegenerative, and terminal disease that signifies the most common form of dementia. The Alzheimer's Association has reported that over 5.3 million people in the US have AD and it is the sixth-leading cause of death.⁸⁴ It was first described by Alois Alzheimer, a German psychiatrist and neuropathologist in 1906.⁸⁵ The disease is generally diagnosed in people over 65 years of age and the most commonly recognized symptom is memory impairment.⁸⁶ As the disease advances, symptoms include confusion, irritability and aggression, mood swings, language breakdown, long-term memory loss, and the general withdrawal of patients as their senses decline.^{87,88} Gradually, bodily functions are lost, ultimately leading to death.⁸⁹

3.2 Traumatic Brain Injury

Damage to the brain caused by an external force to the head is known as traumatic brain injury (TBI). It is the leading cause of brain damage in children and young adults and the major cause of death among the Iraqi-war military. TBI is responsible for the temporary or permanent impairment of brain functions, and is classified based on the

severity (mild, moderate, or severe). In addition to the damage caused by the primary injury, secondary injury can occur gradually for days after the initial insult. Secondary injury could potentially be just as damaging as the primary injury because it is associated with a loss of neurons from the hippocampus, which could lead to deterioration of brain function. Although technological improvements in surgical procedures to treat the initial insult have been made, there are no current neuroprotection therapies that can halt damage caused by secondary injury.

Neurotrophins play an essential role in the maintenance of neuronal populations of cells by functioning to improve memory, neuronal growth, and development. In addition, there is good reason to believe that neurotrophins play a protective role in brain injury.⁹⁰ They do not however, make good drug candidate since they cannot cross the blood brain barrier and possess poor pharmacokinetic behavior and bioavailability at the desired targets. Therefore, the discovery of small molecule neurotrophin mimics could potentially play an important role in neuroprotection, provided that they elicit the desired neuroregenerative responses of neurotrophins.

3.3 Fluorinated Monoindolylquinones

As mentioned in Chapter 1, the Pirrung group, namely Zhitao Li and Liu Deng, prepared a library of dihydroxymonoindolylquinones²⁵ from the hydrolysis of their dichloro precursors. Upon screening, the fluorinated dimethoxy compound **5E5** was serendipitously discovered among this library to possess the best NGF activator activity. The Webster group screened this library of DAQ-B1 analogs to identify nontoxic and

specific NGF receptor activators that have neuroprotective properties. They discovered that the TrkA receptor was activated with a 30 μM dose of compound **5E5**, which also had the best NGF activator activity, with 'approximately 200% the effect of a maximal dose of NGF'.³⁴ The Webster group continued their studies in this area and predicted high activity in a number of indoles, some of which were fluorinated (Figure 3.1).

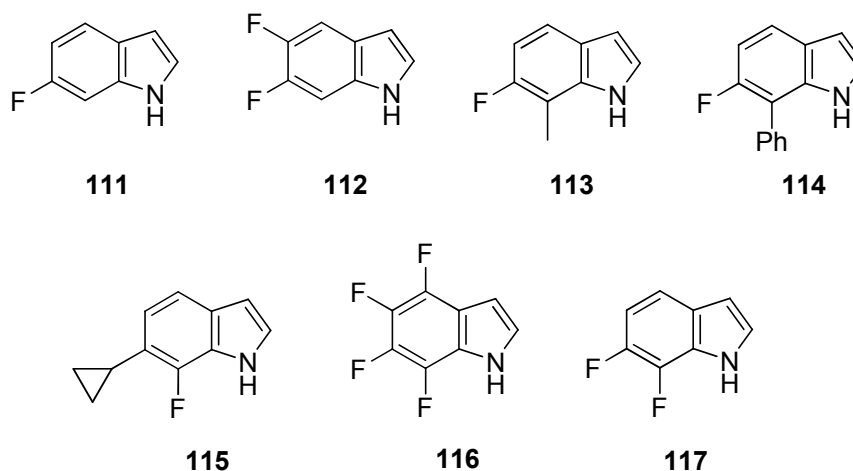


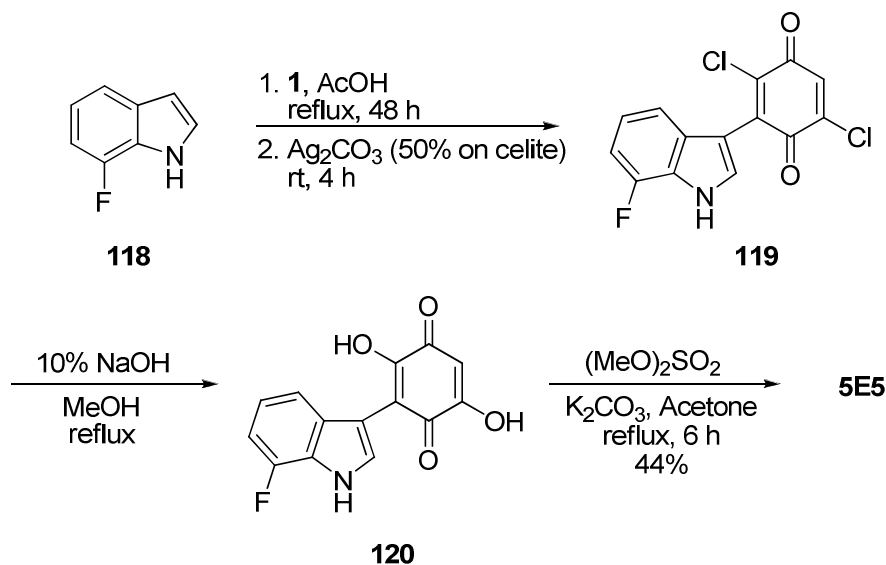
Figure 3.1: Fluorinated indoles predicted to possess high activity

The biological activity demonstrated by **5E5** prompted the synthesis of fluorinated monoindolylquinones using the indoles shown in Figure 3.1, in hopes that they would display similar results and can represent a new class of TrkA activators that could have potential utility in neuroprotection. However, before synthesis of these novel fluorinated compounds could take place, it was important to ensure the reproducibility of the synthesis of methoxylated monoindolylquinones such as **5E5**.

3.4 Synthesis of 5E5

The first synthesis of **5E5** was accomplished by Dennis Xiong from our laboratory. His procedure began with a two step, 1-pot anaerobic condensation of 7-fluoroindole (**118**) with 2,5-dichloro-1,4-benzoquinone (**1**) to form the coupled product **119**. Next, he treated this intermediate with a 10% solution of NaOH in MeOH to convert the dichloro moiety to dihydroxy product **120**. Methylation of **120** was accomplished by using dimethylsulfate to form **5E5** in 44% yield (Scheme 3.1). From this method, Dennis produced a total of 1 gram of **5E5**.

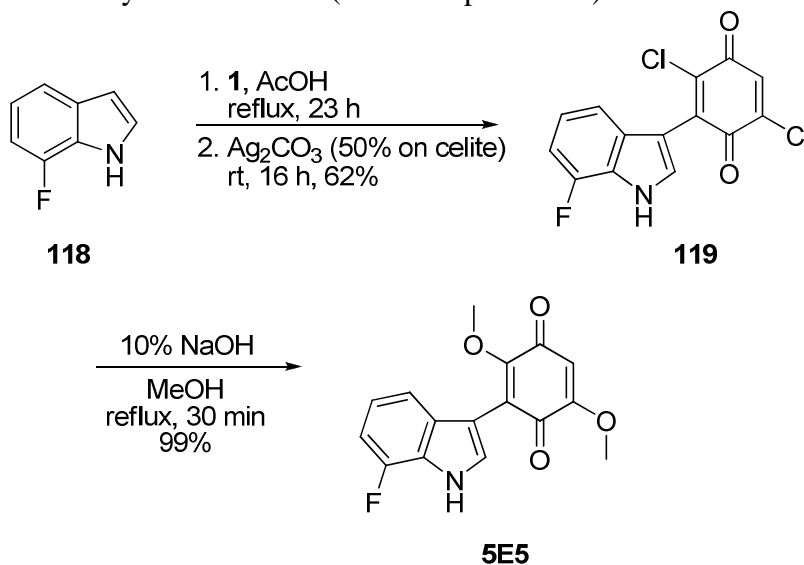
Scheme 3.1: First synthesis of **5E5** (Xiong procedure)



Careful manipulation of the NaOH addition process resulted in the formation of **5E5** immediately, eliminating the use of dimethylsulfate. The second synthesis of **5E5** was analogous to Dennis's procedure. First, indole **118** and **1** were refluxed in the

presence of AcOH catalyst for 23 hours. Next, silver carbonate (Ag_2CO_3) on celite was added to the stirring solution to oxidize the intermediate hydroquinone (not shown). Purification of the quinone product mixture furnished fluorinated intermediate **119** as a royal blue solid in 62% yield (Scheme 3.2). This procedure is similar to the acid-promoted synthesis of 3-indolylbenzoquinones demonstrated previously by the Pirrung group.²⁴

Scheme 3.2: Second synthesis of **5E5** (Massoud procedure)

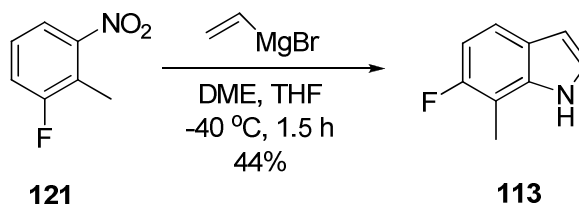


Intermediate **119** was then refluxed with a 10% solution of NaOH in MeOH for 30 minutes to produce **5E5** in 99% yield. In order to produce **5E5** directly from **119**, significant effort was focused on the process chemistry involved in the final methoxylation step, which will be discussed later in this text.

3.5 Synthesis of Fluorinated Indoles

The road to fluorinated monoindolylquinones began with synthesis of the fluorinated indoles **113-117**. These indoles were not available through a commercial source, whereas 6-fluorindole (**111**) and 5,6-difluoroindole (**112**) were both commercially available. 6-Fluoro-7-methylindole (**113**) was synthesized via the traditional Bartoli indole synthesis.⁵⁹ The starting material, nitrobenzene **121**, was treated with vinylmagnesium bromide and dimethoxyethane (DME) in THF for 1.5 hours to provide indole **113** in 44% yield (Scheme 3.3).

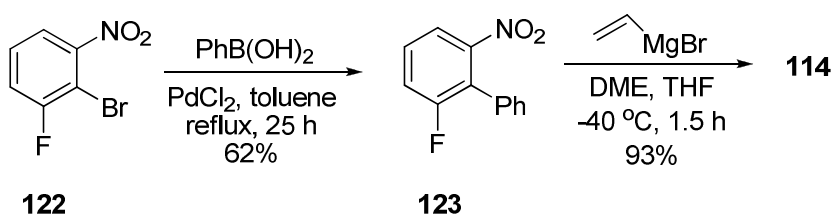
Scheme 3.3: Synthesis of 6-fluoro-7-methylindole



6-Fluoro-7-phenylindole (**114**) can be synthesized using Bartoli reaction conditions in the same manner as **113**. However, unlike **113**, the nitrobenzene precursor to indole **114** is not commercially available. Thus, nitrobenzene **123** was synthesized via a Suzuki coupling reaction between bromonitrobenzene **122** and phenylboronic acid in toluene, using palladium (II) chloride (PdCl₂) as catalyst. This Pd-catalyzed ligand-free Suzuki coupling was previously carried out by the Cheng group.⁹¹ They successfully cross-coupled a range of phenylboronic acids, possessing electron donating as well as electron withdrawing groups, with a variety of aryl bromides. Upon its preparation,

phenylnitrobenzene **123** was subjected to Bartoli reaction conditions to form the desired indole **114** in 93% yield (Scheme 3.4). A useful characteristic of most indoles is that their TLC spot stains bright burgundy when dipped in vanillin. This made tracking their production as product or their consumption as reactant by TLC straightforward.

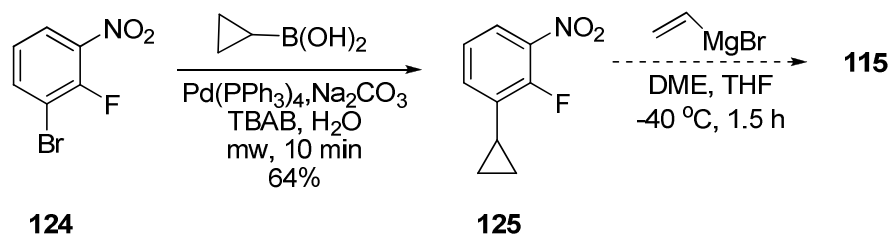
Scheme 3.4: Synthesis of 6-fluoro-7-phenylindole



In order to prepare 6-cyclopropyl-7-fluoroindole (**115**) via the Bartoli route, its precursor cyclopropylnitrobenzene **125** needed to be synthesized. In 2002 Nicholas Leadbeater reported a Suzuki cross-coupling of boronic acids with aryl bromides, chlorides, and iodides using a Pd catalyst in water. His procedure did not involve traditional heating, but rather used microwave irradiation instead.⁹² This methodology was applied in the synthesis of 3-cyclopropyl-2-fluoronitrobenzene (**125**). Commercially available 2-fluoro-3-bromonitrobenzene (**124**) underwent Suzuki coupling with cyclopropylboronic acid in the presence of Pd(PPh₃)₄ as catalyst, sodium carbonate (Na₂CO₃) as base, water as solvent, and tetrabutylammonium bromide (TBAB). The TBAB functioned as a phase-transfer catalyst for reactant mixing, which enhanced the rate of the coupling reaction.⁹² By using TBAB in the reaction, hydrodeboronation was

avoided and the yield of products increased. The reactants and catalyst were combined in a microwave vessel for 10 minutes and the Suzuki coupling product, cyclopropylnitrobenzene **125** was formed in 64% yield (Scheme 3.5). This intermediate was subsequently treated with vinylmagnesium bromide in an attempt to form indole **115**. However, the indole aromatic signals were not detected by ^1H NMR, indicating that the desired indole was not formed. Steric interactions play a major role in the success of the Bartoli indole synthesis. In order for the reaction to take place, the nitrobenzene precursor to the indole must bear a bulky substituent at the position ortho to the nitro group (2-position). In the case of nitrobenzene **125**, the substituent at the 2-position was a fluorine atom, which was too small to promote this reaction.

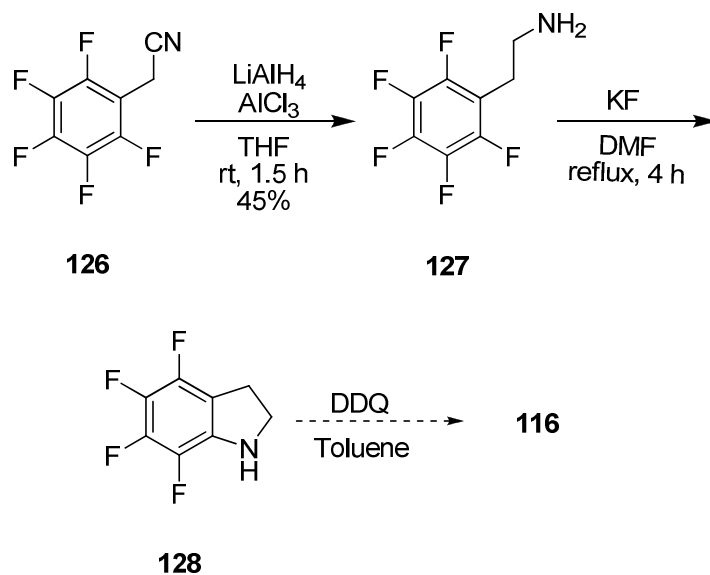
Scheme 3.5: Attempted synthesis of 6-cyclopropyl-7-fluoroindole



In 2002 the Filler group synthesized pentafluoroindole (**116**) from pentafluorophenylacetonitrile (**126**).⁹³ They began by reducing **126** with a mixed lithium aluminum hydride (LAH)/aluminum chloride (AlCl_3) reagent in ether to form an ethanamine **127**. Cyclization of this amine by a fluoride ion catalyst produced the indoline, which was oxidized to form **116**. They examined a number of oxidizing agents for the dehydrogenation of the indoline, including manganese dioxide (MnO_2). They found that

DDQ gave the best result.⁹³ When attempting to reproduce this work, there was difficulty in accessing the LAH/ AlCl_3 mixture in dry ether. Consequently, slight modifications were made to the procedure. Phenylacetonitrile **126** was treated with a commercially available LAH/ AlCl_3 mixture in THF to form the reduced product, **127**,⁹³ in 45% yield (Scheme 3.6). Indoline **128**⁹³ was then formed by cyclization of ethanamine **127** with potassium fluoride (KF). Purification of **128** proved to be difficult, as it decomposed on silica gel. Multiple purification attempts failed, including purification on alumina. As it turns out, the only known method of purification for **116** is steam distillation. Since this method is not practical, synthesis of **116** could not be completed.⁹³

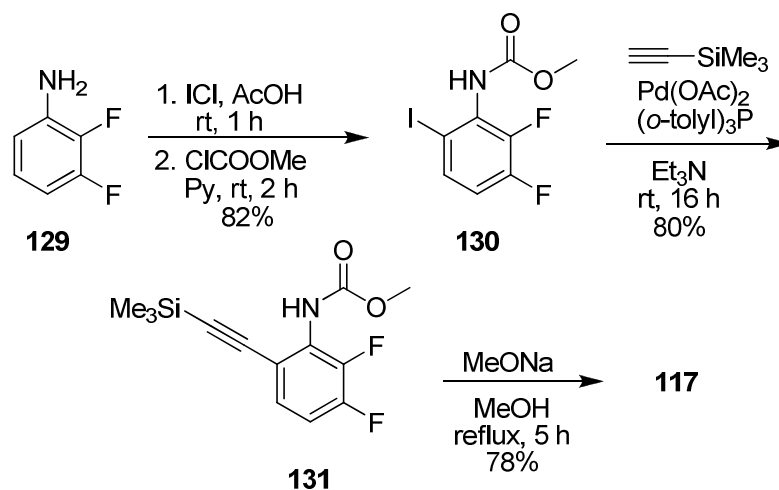
Scheme 3.6: Attempted synthesis of perfluoroindole



Synthesis of 6,7-difluoroindole (**117**) began with the iodination of commercially available 2,3-difluoroaniline (**129**) using iodine monochloride in acetic acid. Without

further purification, the amino group was protected by reaction with methyl chloroformate to give carbamate **130** in 82% yield. Sonogashira coupling of **130** with trimethylsilylacetylene, mediated by Pd(OAc)₂ and tris-*o*-tolylphosphine in Et₃N, produced acetylene **131** in 80% yield. Treatment of **131** with sodium methoxide (NaOMe) in MeOH completed the synthesis of **117** in 78% yield (Scheme 3.7). This synthesis is similar to work done by the Wang group at Bristol-Myers Squibb Pharmaceutical Research Institute; however, their target compound was 5,6-difluoroindole (**112**).⁹⁴ In the case of **117**, NaOMe in MeOH was used instead of NaOEt in EtOH because dry MeOH was more readily accessible.

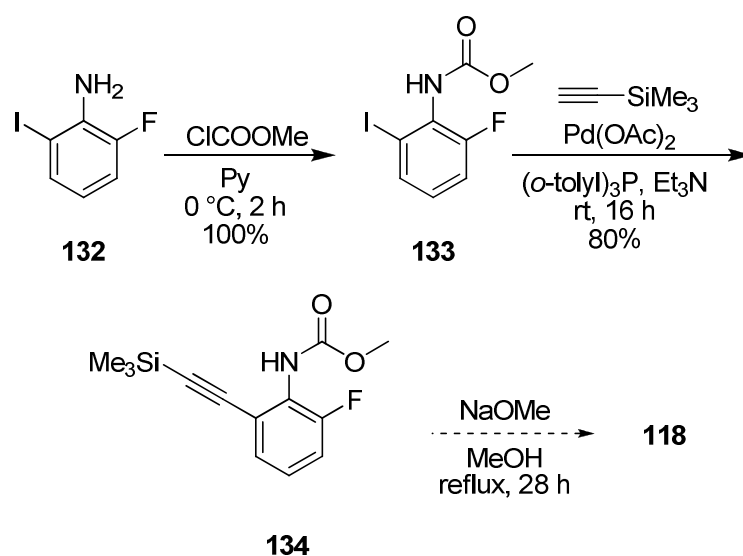
Scheme 3.7: Synthesis of 6,7-difluoroindole



Scheme 3.2 shows the synthesis of **5E5** from an initial acid-promoted coupling reaction between 7-fluoroindole (**118**) and **1**. Indole **118** can be obtained from a commercial source, however, with difficulty and at high cost. Successful production of

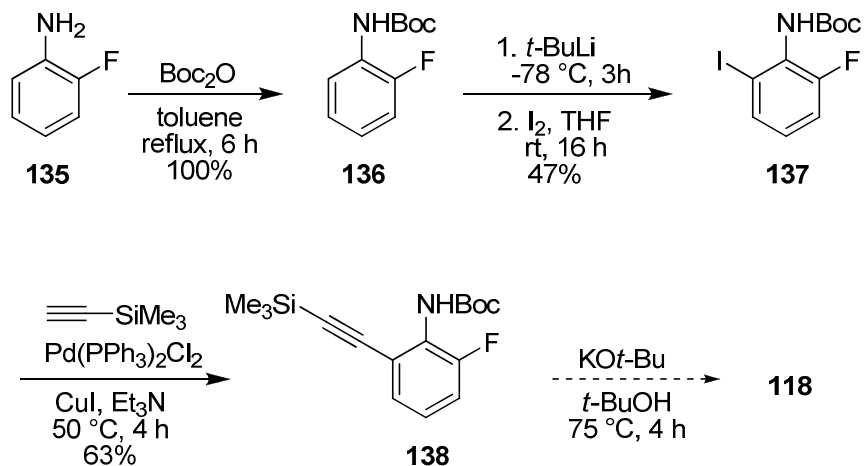
indole **117** prompted the synthesis of indole **118** (Scheme 3.8) via the route in Scheme 3.7. Commercially available 2-fluoro-6-iodoaniline (**132**) was protected as the carbamate using methyl chloroformate, producing **133** in quantitative yield. The carbamate underwent Sonogashira coupling with trimethylsilylacetylene to furnish **134** in 80% yield. The final cyclization step using NaOMe in MeOH proved to be difficult, as the ^1H NMR of the final product did not show evidence of the formation of **118**. Monitoring the progress of the cyclization reaction by TLC provided evidence that product was in fact forming (compared with a pure sample of **118**). This result suggests a loss of product during the purification step, which signifies a decomposition of indole **118** on silica gel. Continued failure in the purification of any 7-fluoroindole led to the hypothesis that indoles fluorinated at the 7-position, including **117**, decompose on silica gel upon purification, and can only be purified by steam distillation.

Scheme 3.8: First attempted synthesis of 7-fluoroindole



Failure to produce indole **118** via the method in Scheme 3.8 led to a second attempt at its formation that was initially investigated by the Schlosser group in 2006.⁹⁵ This synthesis began with BOC protection of 2-fluoroaniline (**135**) to produce **136** in quantitative yield (Scheme 3.9). Lithiation at the 6-position of **136**, followed by trapping of the intermediate with molecular iodine produced **137** in 47% yield. Iodo compound **137** was subjected to a Sonogashira coupling with trimethylsilylacetylene to form ethynylsilane **138** in 63% yield. Cyclization of **138** was attempted using potassium *tert*-butoxide (KO*t*-Bu) in *tert*-butanol (*t*-BuOH); however, according to chromatographic results, the desired indole **118** was not formed (compared with a pure sample of **118**). Failure to produce **118** from either of the two laboratory syntheses made it necessary to obtain it from a commercial source.

Scheme 3.9: Second attempt at the synthesis of 7-fluoroindole



3.6 Acid-Promoted Coupling of Fluorinated Indoles to 2,5-Dichloro-1,4-benzoquinone

Successful production of **5E5** (Scheme 3.2) allowed for the application of its synthesis towards the novel fluorinated indoles. As in the synthesis of **5E5**, the first step was an acid-promoted coupling of the two starting material to form the monoindolyl-dichloro intermediate. AcOH was chosen as the acid catalyst for this reaction based on experimentation conducted by the Pirrung group. They examined many acid catalysts, including mineral acids, and found that in most cases AcOH provided the best result.²⁴ Consequently, coupling of fluorinated indoles to **1** was performed using AcOH (Table 3.1).

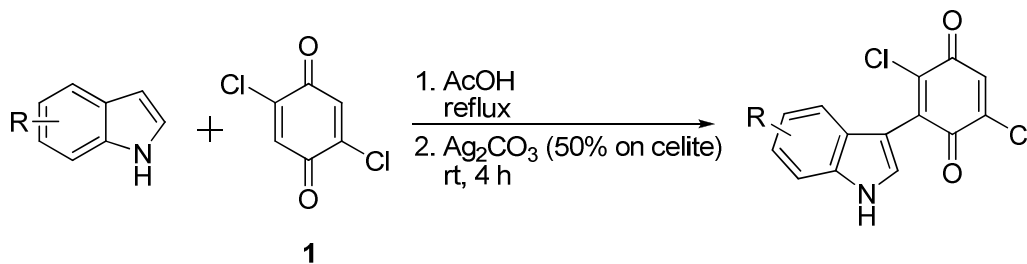


Table 3.1: Acid-promoted indole-benzoquinone coupling products

Entry	Indole	R	Reaction Time (h) ^a	Product	% Yield
1	111	6-Fluoro	23	139	40
2	112	5,6-Difluoro	24	140	25%
3	113	6-Fluoro-7-methyl	14	141	48%
4	114	6-Fluoro-7-phenyl	6	142	70%
5	117	6,7-Difluoro	48	-	-

^aFor coupling step 1

Synthesis of the fluorinated monoindolylquinone library continued with the addition of commercially available indole **111** to **1** in refluxing AcOH to yield the dichlorohydroquinone (not shown). Addition of Ag₂CO₃ oxidized the hydroquinone to the 6-fluoroindolylbenzoquinone intermediate **139**, a royal blue solid, in 40% yield (entry 1). Next, indole **112** was subjected to the same reaction conditions as **111** to yield the blue solid **140** in 25% yield (entry 2). Compounds **141** and **142** were also produced as blue solids in the same manner. However, coupling to produce **141** was completed in approximately half the time needed for **111** (entry 3), and **142** was formed in approximately one quarter of the time (entry 4). Furthermore, both dichlorobenzoquinones were produced with improved yields, 48% and 70%, respectively. The attempted addition of **117** to **1** (entry 5) proved complicated. Upon completion of this attempted coupling, the product formed did not possess the traditional dark blue/purple color of dichloromonoidolylquinones. Despite this inconsistency, purification of the resulting compound was attempted, but the unknown product decomposed on silica. While the structure of indole **117** was verified by ¹H NMR and MS, it did not stain red with vanillin on a TLC plate, as is characteristic of most indoles.

3.7 Synthesis of 5E5 Analogs

3.7.1 Optimization of Methanolysis Reaction

The last step in the synthesis of this library of fluorinated monoindolylquinones is the methanolysis of the dichlorobenzoquinone moiety, which is performed by the

addition of 10% NaOH to a refluxing solution of the dichloro intermediate in MeOH (Figure 3.2, A). Although this transformation appears to be simple, it is in fact highly problematic. As this protocol was originally used by many researchers as the hydrolysis step in the synthesis of asterriquinones and their analogs, addition of the 10% NaOH solution to the dichloro moiety could yield the undesired dihydroxy product (Figure 3.2, B).

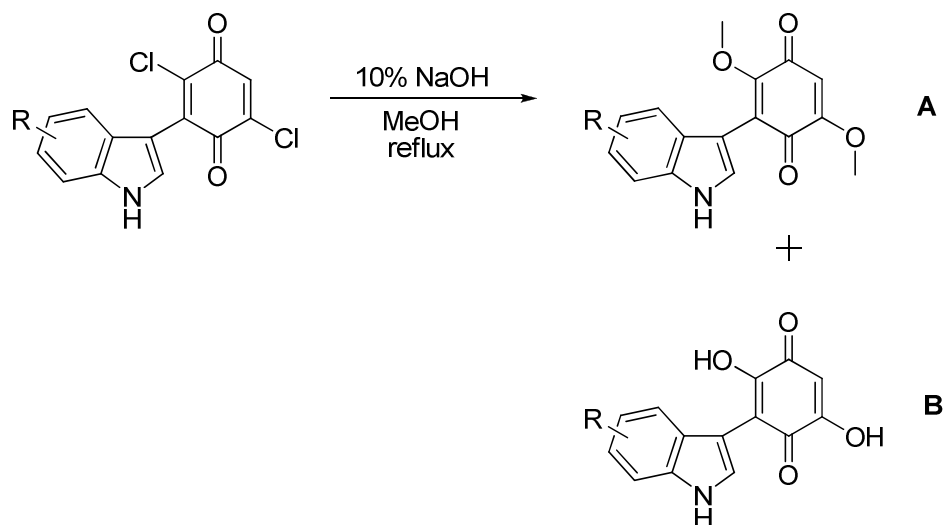
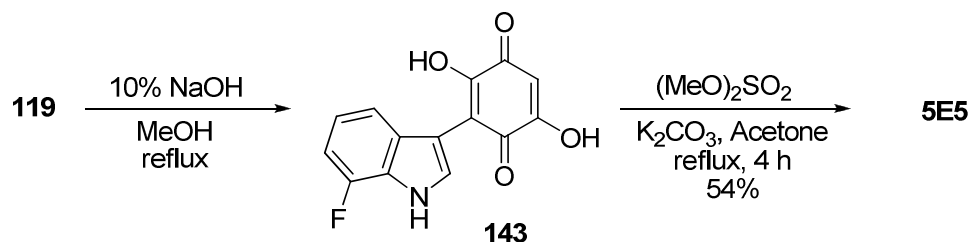


Figure 3.2: A) Formation of dimethoxy moiety B) Formation of dihydroxy moiety

The initial attempts I made to synthesize **5E5** from **119** were unsuccessful since the hydrolysis reaction prevailed over methanolysis, providing the dihydroxy compound **143** as a brown liquid (Scheme 3.10). In an effort to produce the desired compound, **143** underwent methylation by treating it with dimethylsulfate⁹⁶ to furnish **5E5** in 54% yield.

Scheme 3.10: Formation of **5E5** via hydrolysis and methylation



A second attempt at the formation of **5E5** via methanolysis was undertaken. This time, however, I added the NaOH solution at a slower rate. During the addition, the color of the solution changed from the royal blue of **119**, to a deep purple, and finally a dark red. The reaction was stopped at this point (before the solution turned brown). Analysis of this material revealed a mixture of **5E5** and **143**. Consequently, two different outcomes are a possibility from this same reaction, depending on the technique used in the NaOH addition process. It was important to establish the reaction pathway for this methanolysis step, in hopes of achieving complete conversion from the dichloro starting material to the dimethoxy product, without any formation of the dihydroxy product. Therefore, the reaction progress of the methanolysis of **119** was followed carefully by TLC in hopes of determining its reaction pathway. The first TLC (Figure 3.3A) demonstrates the royal blue color of pure starting material **119**. Chromatographic results were obtained after each addition of a precise volume of the NaOH solution. After the first addition of NaOH (20% of the total amount of NaOH), the starting material began to disappear (Figure 3.3B, spot 1), and a new product, red in color, began to appear (Figure 3.3B, spot 2).

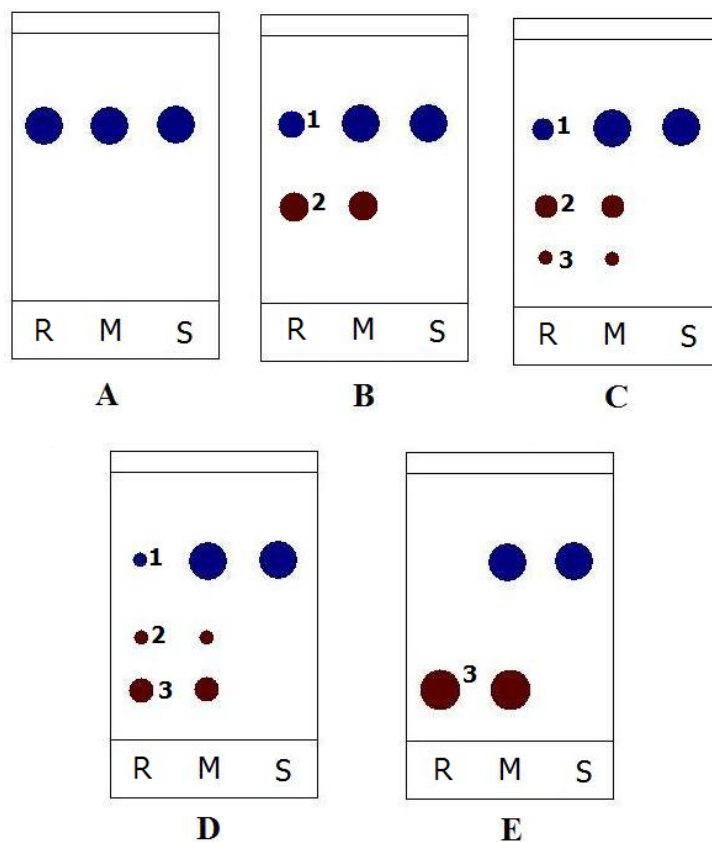


Figure 3.3: Progress of the **5E5** forming methanolysis reaction by TLC
Key: R= Product, M= Mixed spot, S = Starting material

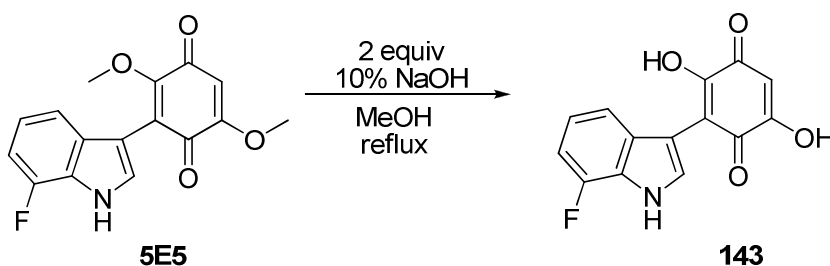
Continued addition of NaOH revealed a brand new spot on TLC, also red in color (Figure 3.3C, spot 3). Eventually, the recurrent, timely addition of NaOH transformed the compound representative of spot 2 into the compound representing spot 3. This can be seen by the gradual disappearance of spot 2 along with the gradual appearance and size increase of spot 3 (Figure 3.3D and E). The final crude, red material was purified and analysis of the compound indicative of spot 3 revealed it to be **5E5**. This diligent experimentation revealed that the addition of NaOH is a sensitive, time-dependent protocol that should be terminated once there is indication of complete conversion from

119 to **5E5** (Figure 3.3E). Furthermore, the reaction should not continue at reflux after the last drop of NaOH has been added. A work-up must take place immediately in order to avoid undesired hydrolysis.

3.7.2 Reaction Pathway of the Methanolysis Reaction

The protocol developed to monitor the reaction progress made it possible to elucidate the reaction pathway. Treatment of **5E5** with 2 equiv NaOH in MeOH instantaneously transformed the methoxy substituents to hydroxy groups (Scheme 3.11). Based on these results, a reaction pathway was envisioned in the following manner: dichloro (**119**) → dimethoxy (**5E5**) → dihydroxy (**143**).

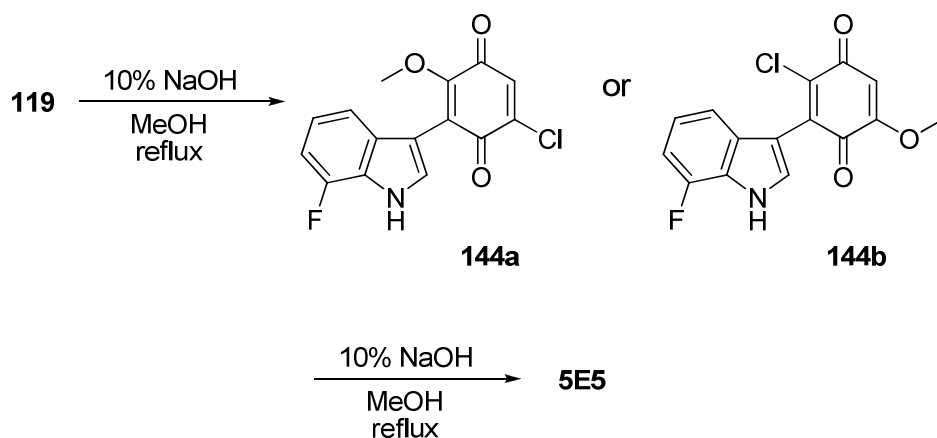
Scheme 3.11: Examination of the methanolysis reaction pathway; formation of the dihydroxy moiety from the dimethoxy moiety



The gradual transformation of spot 2 to spot 3 supports the idea that addition of methoxy or hydroxy to the 2 and 5 positions of the benzoquinone moiety is not simultaneous but rather stepwise. Based on that theory, the conversion of spot 2 to spot 3 in Figure 3.3 could be representative of the conversion of a monochloro-monomethoxy

intermediate to the dimethoxy product, **5E5** (Scheme 3.12). Although spot 2 was neither isolated nor characterized, it was postulated to be monochloro-monomethoxy intermediate **144a** or **144b**. After having perfected the method with which to synthesize **5E5**, a total of 1.2 g of **5E5** was produced in an overall 94 % yield from 7-fluoroindole.

Scheme 3.12: Step-wise conversion from the dichloro-precursor to **5E5**



3.7.3 Methanolysis of Fluorinated 3-Indolylquinones

The development of a successful protocol for the production of methoxy substituted indolylquinones provides a superior method to ensure the production of *methoxy* substituted analogs of **5E5** (Table 3.2). The first **5E5** analog precursor to undergo the novel methoxylation protocol was 6-fluoro intermediate **139** (entry 2). It was treated with the 10% NaOH solution for one hour to yield the desired analog **TM178** in 81% yield. In a similar fashion, **TM180** (entry 3) was synthesized from **140** in quantitative yield. In solution, both analogs **TM178** and **TM180** demonstrated the typical

dark red color of these indolylquinones. However, unlike **5E5**, these two analogs are gray solids in their pure forms. The 6-fluoro-7-methyl analog **141** took only 10 minutes for complete conversion to **TM183**. Unfortunately, the yield was low (20%).

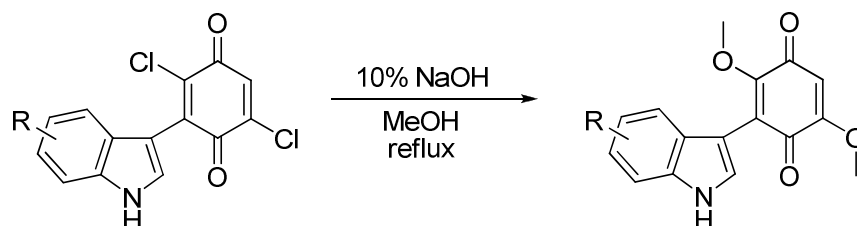


Table 3.2: Analogs of **5E5**

Entry	Dichloro-indolylquinone	R	Reaction Time (h) ^a	Product	% Yield
1	119	7-Fluoro	0.5	5E5	99%
2	139	6-Fluoro	1	TM178	81%
3	140	5,6-Difluoro	1	TM180	100%
4	141	6-Fluoro-7-methyl	10 min	TM183	20%
5	142	6-Fluoro-7-phenyl	0.5	TM186	99%

^aAmount of time measured from first drop of NaOH to last drop of NaOH, when reaction is terminated

The 6-fluoro-7-phenyl precursor **142** was completely transformed in 30 minutes to furnish dimethoxy analog **TM186** as a red solid in 99% yield. Both analogs **TM183** and **TM186** resembled the dark red **5E5** in solution as well as in pure form. All of the syntheses presented in Table 3.2 were monitored by TLC, which revealed that each reaction followed the same pathway indicated by Figure 3.3. This suggests formation of the desired analogs occurs in a step-wise fashion.

The compounds displayed in Table 3.2 are currently being screened for neuroprotection in Dr. Nicholas Webster's laboratory.

3.8 Conclusion

The prominent biological activity of **5E5** provides the motivation for this project to be carried on in the future. The library of **5E5** analogs can be extended to future targets, which include both fluorinated and non-fluorinated compounds, and can be screened for neuroprotective and diabetic activity. Furthermore, benzoquinone analogs that display activity can be re-synthesized with a quinone replacement such as pyrone or pyridone, which can also be tested for biological activity.

Chapter Four: Experimental

All reagents were purchased from commercial sources and used without further purification, unless otherwise stated. All reactions were carried out in oven-dried glassware under a nitrogen atmosphere using standard syringe and septum techniques unless otherwise stated. Organic extracts were dried over anhydrous magnesium sulfate (MgSO_4) or sodium sulfate (Na_2SO_4) and then filtered prior to removal of all volatiles under reduced pressure on a Büchi rotary evaporator. Chromatographic purification of products was accomplished using flash column chromatography on silica gels. Thin-layer chromatography (TLC) was carried out on aluminum sheets, Silica Gel 60 F₂₅₄ (Merck; layer thickness 0.25 mm). Visualization of the developed chromatogram was performed by UV light and/ or vanillin stains. GC analysis was carried out by using an Agilent Technologies 6890N with an HP-5 (30 m \times 0.32 mm) column and operating with an injector temperature of 325 °C and a detector temperature of 350 °C.

All melting points were measured on a Büchi Melting Point B-545 and are uncorrected.

¹H and ¹³C NMR spectra were recorded on Varian Inova 300 (300 MHz and 75 MHz respectively) or on Varian Inova 400 (400 MHz and 100 MHz respectively) as noted, are internally referenced to residual protio solvent signals unless otherwise stated, and are expressed in parts per million (ppm). Data for ¹H are reported as follows: chemical shift (δ ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m =

multiplet), and coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (ppm). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer using the ATR accessory and are reported in absorption frequency (cm^{-1}). Mass spectra were obtained from the University of California, Riverside Mass Spectral Facility or from the University of California, Irvine Mass Spectral Facility. Calculated accurate masses of parent compounds denote the mass of the ion (mass of electron is 0.00055Da).

General Procedure A — Bartoli Indole Synthesis:

A solution of vinylmagnesium bromide (12.9 mmol, 0.7 M solution in THF) and DME (1.3 mL) was cooled to $-40\text{ }^{\circ}\text{C}$ under nitrogen. The nitrobenzene (1.30 mmol) was dissolved in THF (1.3 mL) in a separate round bottom flask, and then added to the cooled vinylmagnesium bromide solution dropwise. The reaction mixture was stirred for 2.5 h at $-40\text{ }^{\circ}\text{C}$ until TLC indicated no remaining starting material. Upon quenching with ammonium chloride, the aqueous phase was extracted with ethyl acetate ($3 \times 10\text{ mL}$) and the collected organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography (1:9 hexanes:EtOAc) on silica furnished the target indole.

7-Prenylindole (41):

Synthesized according to General Procedure A from 2-prenylnitrobenzene (1.3 mmol). Cream solid (190 mg, 65%), $R_f = 0.50$, mp $43\text{-}44\text{ }^{\circ}\text{C}$. IR (neat) 3424, 3075, 2924, 1719,

1688, 1588, 1490, 1434, 1415, 1341, 1265, 1103, 1067 cm^{-1} . ^1H NMR (CDCl_3): δ 8.09 (NH, s, br), 7.51 (1H, d, $J = 7.7$), 7.15 (1H, t, $J = 2.8$), 7.08-6.98 (2H, m), 7.02-6.54 (1H, dd, $J = 3.2, 2.0$), 5.41 (1H, m), 3.56 (2H, d $J = 6.9$), 1.81 (3H, s), 1.78 (3H, d, $J = 1.0$). ^{13}C NMR (CDCl_3): δ 135.8, 132.9, 127.8, 124.1, 124.0, 122.3, 122.1, 120.3, 118.9, 102.9, 31.4, 26.2, 18.0. HRMS (EI): m/z calcd for $\text{C}_{13}\text{H}_{15}\text{NH}^+$ 186.1278, found 186.1282.

3-Iodo-7-methylindole (65):

Method A:

N-iodosuccinimide (190 mg, 0.82 mmol) was added to a solution of 7-methylindole (100 mg, 0.76 mmol) in acetone (13 mL) and the reaction was stirred at rt for 16 h. Acetone was removed *in vacuo* and the residue was redissolved in ether, washed sequentially with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (6×10 mL) and brine (10 mL), dried over MgSO_4 , and concentrated *in vacuo* to furnish a brown oil (0.07 g). This material was used immediately in its crude form due to its instability.

Method B:

A solution of iodine (0.39 g, 1.5 mmol) and potassium iodide (0.13 g, 0.76 mmol) in methanol (10 mL) was added all at once to a solution of 7-methylindole (1.0 g, 0.76 mmol) and sodium hydroxide (ground, 0.31 g, 0.76 mmol) in methanol (25 mL) and the reaction was stirred at rt for 5 h. Water was added and the precipitate was filtered, dissolved in dichloromethane (10 mL), and washed with water (10 mL). The final dichloromethane extracts were dried over Na_2SO_4 and concentrated *in vacuo* to produce a

brown oil (0.56 g). This material was used immediately in its crude form due to its instability.

5-Iodo-3-methoxy-2-methylpyran-4-one (68a):

A solution of iodine (2.2 g, 8.5 mmol) in carbon tetrachloride/pyridine (1:1, 15 mL) was added dropwise to a solution of 3-methoxy-2-methylpyran-4-one (0.564 g, 4.02 mmol) in carbon tetrachloride/pyridine (1:1, 15 mL) at 0 °C. The mixture was stirred for 40 h while the temperature was allowed to warm to rt. The mixture was diluted with ether (70 mL) and washed with water (2 × 30 mL), 1N HCl (2 × 30 mL), water (30 mL), and 10% aq. Na₂S₂O₃ (30 mL). The organic extracts were dried over MgSO₄ and concentrated *in vacuo* to yield a light orange oil (43 mg). This material was used without further purification.

5-Iodo-3-(4-methoxybenzyloxy)-2-methylpyran-4-one (68b):

[Bistrifluoro(acetoxy)iodo]benzene (0.78 g, 1.8 mmol) was added to solution of iodine (0.46 g, 1.8 mmol) and pyridine (0.32 mL, 4.0 mmol) in dichloromethane and the solution was stirred at rt for 15 min. 3-(4-methoxybenzyloxy)-2-methylpyran-4-one (0.45 g, 1.8 mmol) was added to the reaction mixture and the whole was stirred for 66 h in the dark and then quenched with satd. aqueous sodium bicarbonate (20 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL) and the combined extracts were washed with Na₂S₂O₃ (2 × 20 mL), brine (20 mL), and dried over Na₂SO₄. The crude material was concentrated *in vacuo* and purified by flash column chromatography (4:1

hexanes:EtOAc) on silica to furnish **68b** as a colorless oil (0.113 g, 17%). IR (neat) 3452, 2957, 2837, 1642, 1610, 1512, 1463, 1428, 1244, 1169, 1030 cm^{-1} . ^1H NMR (CDCl_3): δ 8.01 (1H, s), 7.29 (2H, m), 6.87 (2H, m), 5.07 (2H, s), 3.79 (3H, s), 2.09 (3H, s). ^{13}C NMR (CDCl_3): δ 175.1, 159.9, 159.7, 154.0, 143.5, 130.6 (2C), 129.0, 116.8, 113.7 (2C), 73.0, 55.0, 14.6. HRMS (EI): m/z calcd for $\text{C}_{14}\text{H}_{13}\text{O}_4\text{IH}^+$ 372.9934, found 372.9937.

3-(Benzyloxy)-2-methyl-1-phenylpyridin-4-one (69):

Aniline (1.10 mL, 12.1 mmol) was added dropwise to a solution of 3-(benzyloxy)-2-methylpyran-4-one (1.75 g, 8.09 mmol) and HCl (0.51 mL) in water (12.0 mL). The reaction mixture was heated at reflux for 20 h and washed successively with dichloromethane (6×20 mL). The aqueous layer was concentrated to reveal the crude product as a brown oil. Purification by flash column chromatography (1:4 hexanes:EtOAc) on silica furnished **69** as a brown oil (0.070 g). This compound could not be purified.

1-Cyclopropyl-3-methoxy-2-methylpyridin-4-one (70):

Reflux Conditions:

Cyclopropylamine (1.00 mL, 14.0 mmol) was added to a solution of 3-methoxy-2-methylpyran-4-one in ethanol and the mixture was heated at reflux for 48 h. Ethanol was removed under reduced pressure and the resulting residue was dissolved in water (6 mL). The aqueous layer was extracted with ethyl acetate (3×7 mL), dried over Na_2SO_4 , and

concentrated *in vacuo*. Purification by flash column chromatography on silica (4:1 hexanes:EtOAc) revealed a mixture of compounds, including **70** (85 mg). This compound could not be purified further.

Microwave Conditions:

A mixture of cyclopropylamine (1.70 mL, 23.9 mmol), 3-methoxy-2-methylpyran-4-one (0.335 g, 2.39 mmol), and ethanol (7.0 mL) was enclosed in a microwave reactor tube and subjected to microwave irradiation, operating at 150 W and 80 °C for 120 min using a CEM-Discover microwave reactor. After GC results indicated complete conversion, the reaction mixture was concentrated under reduced pressure and the resulting paste was dissolved in water (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), dried over Na₂SO₄, and concentrated *in vacuo*. Purification by flash column chromatography (3:7 hexanes:EtOAc) provided **70**, along with other impurities (0.11 g). This compound could not be purified further.

3-(4-Methoxybenzyloxy)-1,2-dimethylpyridin-4-one (72):

p-Methoxybenzyl chloride (0.63 mL, 4.7 mmol) was added to a solution of 3-hydroxy-1,2-dimethylpyridin-4-one (0.50 g, 3.6 mmol) and potassium carbonate (1.05 g, 7.5 mmol) in dry DMF (7.5 mL). The reaction mixture was heated at reflux for 16 h and concentrated under reduced pressure. The residue was dissolved in water (10 mL), extracted with ethyl acetate (3 × 10 mL), dried over Na₂SO₄, and concentrated *in vacuo*.

Purification by flash column chromatography (1:4 hexanes:EtOAc) yielded **72** as a brown oil (40 mg, 4%). This compound could not be purified further.

3-Methoxy-1,2-dimethylpyridin-4-one (73):

To a solution of 3-hydroxy-1,2-dimethylpyridin-4-one (1.0 g, 7.2 mmol) and potassium hydroxide (10% solution, 4.5 mL) was added dimethylsulfate (0.75 mL, 7.9 mmol) dropwise. The reaction mixture was stirred at 20 °C for 24 h and then extracted from the aqueous phase with dichloromethane (3 × 10 mL). The combined organic phase was washed with 5% sodium hydroxide (2 × 5 mL) and water (3 × 10 mL), and then concentrated *in vacuo*. The crude material was redissolved in water (20 mL) and washed with 1:4 isopropanol:chloroform (3 × 10 mL). The aqueous layer was concentrated to yield an orange paste (0.32 g, 83%). This compound was used without any further purification.

3-Hydroxy-5-iodo-1,2-dimethylpyridin-4-one (74):

Iodine (0.18 g, 0.72 mL) was added to a solution of 3-hydroxy-1,2-dimethylpyridin-4-one (0.10 g, 0.72 mL) and sodium hydroxide (0.03 g, 0.76 mmol) in water (4.0 mL). The reaction mixture was stirred at rt for 48 h and then concentrated under reduced pressure. The residue was dissolved in water (10 mL), extracted with ethyl acetate (3 × 10 mL), dried over Na₂SO₄, and concentrated *in vacuo* to produce a yellow oil (59.5 mg, 32%). This compound was used without any further purification.

2-hydroxy-4,4-dimethylcyclohexa-2,5-dienone (80):

Selenium dioxide (1.1 g, 9.7 mmol) was added to a solution of 2-bromonitrobenzene (1.0 g, 8.0 mmol) in dioxane (50 mL) and heated at reflux for 19 h. The yellow solution gradually turned reddish/burgundy. The reaction was cooled and a 5% KOH solution was added. The product was extracted with ethyl acetate (3 × 40 mL), washed with water until neutral pH, dried over Na₂SO₄, and concentrated *in vacuo*. Purification by flash column chromatography (4:1 hexanes:EtOAc) produced **80** as a yellow solid from acetone-hexane in quantitative yield (1.0 g), R_f = 0.53, mp 146.3-149.4 °C. IR (neat) 3250, 2966, 2864, 1640, 1621, 1588, 1468, 1344, 1226, 1121, 892, 827 cm⁻¹. ¹H NMR (CDCl₃): δ 6.93 (1H, dd, *J* = 9.9, 2.7), 6.84 (1H, d, *J* = 2.7), 6.27 (1H, d, *J* = 9.6), 1.58 (1H, s, br), 1.22 (6H, s). ¹³C NMR (CDCl₃): δ 183.6, 159.0, 153.1, 127.1, 125.2, 41.2, 26.8 (2C). HRMS (EI): *m/z* calcd for C₈H₁₀O₂H⁺ 139.0641, found 139.0642.

2-(4-Methoxybenzyloxy)-4,4-dimethylcyclohexa-2,5-dienone (81):

To a solution of **80** (1.0 g, 8.0 mmol) and potassium carbonate (2.31 g, 16.8 mmol) in DMF (17 mL) was added *p*-methoxybenzyl chloride (1.41 mL, 10.4 mmol). The reaction mixture was heated at reflux for 16 h and concentrated under reduced pressure. The residue was diluted with water (10 mL), extracted with ethyl acetate (3 × 10 mL), dried over Na₂SO₄, and concentrated *in vacuo*. Purification by flash column chromatography (9:1 hexanes:EtOAc) resulted in **81** as a yellow oil (2.03 g, 99%), R_f = 0.63. IR (neat) 2954, 2845, 1622, 1613, 1560, 1462, 1312, 1226, 1130, 1035 cm⁻¹. ¹H NMR (CDCl₃): δ 7.29 (2H, m), 6.86 (2H, dd, *J* = 8.9, 2.0), 6.81 (1H, d, *J* = 2.7), 6.24-6.21 (2H, m), 4.59

(2H, s), 3.78 (3H, s), 2.93 (3H, s), 2.85 (3H, s). ^{13}C NMR (CDCl_3): δ 179.8, 159.4, 153.2, 152.8, 130.2 (2C), 119.4, 125.9, 122.1, 113.5 (2C), 72.6, 54.7, 41.8, 25.3 (2C). HRMS (EI): m/z calcd for $\text{C}_{16}\text{H}_{18}\text{O}_3\text{H}^+$ 259.1249, found 259.1245.

2-Iodo-6-(4-methoxybenzyloxy)-4,4-dimethylcyclohexa-2,5-dienone (82):

Iodine (2.0 g, 8.1 mmol) dissolved in 1:1 carbon tetrachloride/pyridine (2 mL) was added dropwise to a solution of **81** (0.50 g, 1.9 mmol) in 1:1 carbon tetrachloride/pyridine (2 mL) at 0 °C and the reaction was stirred at rt for 16 h. The reaction mixture was diluted with ether (30 mL), washed successively with water (30 mL), 1N HCl (2×15 mL), water (30 mL) again, and 20% aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (15 mL). The organic phase was dried over MgSO_4 and concentrated *in vacuo*. Purification by flash column chromatography (2:1 hexanes:EtOAc) yielded **82** as an orange oil (0.34 g, 45% yield), $R_f = 0.50$. IR (neat) 2925, 2855, 1661, 1581, 1512, 1463, 1318, 1246, 1171, 1033, 987 cm^{-1} . ^1H NMR (CDCl_3): δ 7.65 (1H, s), 7.31 (2H, m), 6.92-6.87 (3H, m), 4.64 (2H, s), 3.82 (3H, s), 1.33 (3H, s), 1.30 (3H, s). ^{13}C NMR (CDCl_3): δ 174.0, 165.2 (2C), 159.5, 133.5, 130.6, 130.0 (2C), 113.8 (2C), 110.6, 96.8, 71.5, 55.7, 26.1 (2C). HRMS (EI): m/z calcd for $\text{C}_{16}\text{H}_{17}\text{O}_3\text{IH}^+$ 385.0216, found 385.0214.

General Procedure B — Stille Coupling of Indoles to Cyclic Ketones:

A mixture of stannylated indole (0.20 mmol), cyclic ketone (0.3 mmol), and $\text{Pd}(\text{PPh}_3)_4$ (0.01 mmol, 5 mol%) in previously degassed (10 min) dioxane (1.0 mL) was heated at reflux for 18 h. The reaction mixture was diluted with water (10 mL) and extracted with

dichloromethane (3×10 mL). The combined organic extracts were dried over MgSO_4 and concentrated *in vacuo*. Purification by flash column chromatography (9:1 hexanes: EtOAc) produced the desired coupled products.

2-hydroxy-4,4-dimethyl-6-(7-prenylindol-3-yl)cyclohexa-2,5-dienone (87):

Synthesized according to General Procedure B from *tert*-butyl-7-methyl-3-(tributylstannyl)-indole-1-carboxylate and **82** as a yellow oil (28.9 mg 54%). $R_f = 0.52$. IR (neat) 3403, 2928, 1749, 1456, 1370, 1326, 1251, 1221, 1153, 1046 cm^{-1} . ^1H NMR (CDCl_3): δ 7.44 (1H, d, $J = 3.8$), 7.31 (1H, dd, $J = 7.4, 1.2$), 7.09-7.01 (3H, m), 6.45 (1H, d, $J = 3.8$), 2.56 (3H, s), 1.56 (6H, s). ^{13}C NMR (CDCl_3): δ 182.0, 155.9, 144.6, 143.8, 135.2, 131.8, 127.5, 125.4, 122.0, 120.6, 120.2, 118.6, 105.4, 32.5, 29.7 (2C) 18.2. HRMS (EI): m/z calcd for $\text{C}_{17}\text{H}_{17}\text{NO}_2\text{H}^+$ 268.1253, found 268.1257.

2-Hydroxy-4,4-dimethyl-6-(7-prenylindol-3-yl)cyclohexa-2,5-dienone (88):

Synthesized according to General Procedure B from *tert*-butyl-7-prenyl-3-(tributylstannyl)-indole-1-carboxylate and **82** as a yellow oil (7.1 mg, 11%). $R_f = 0.8$. IR (neat) 3367, 2925, 2917, 1741, 1457, 1370, 1328, 1254, 1152, 1097, 1019 cm^{-1} . ^1H NMR (CDCl_3): δ 7.43 (1H, d, $J = 3.7$), 7.32 (1H, dd, $J = 6.8, 2.1$), 7.12-7.05 (3H, m), 6.46 (1H, d, $J = 3.8$), 5.18 (1H, m), 3.72 (2H, d, $J = 6.8$), 1.64 (3H, s), 1.55 (6H, s), 1.48 (3H, s). ^{13}C NMR (CDCl_3): δ 181.6, 156.6, 145.0, 144.1, 136.0, 133.0, 130.1, 127.7, 125.8, 124.3, 123.6, 122.6, 122.3, 119.1, 104.9, 32.6, 31.2, 29.7 (2C), 26.3, 18.2. HRMS (EI): m/z calcd for $\text{C}_{21}\text{H}_{23}\text{NO}_2\text{H}^+$ 322.1722, found 322.1723.

4,4-dimethyl-2-(prop-2-ynyloxy)cyclohexa-2,5-dienone (89):

Propargyl bromide (80% wt, 0.19 mL) was added to a solution of **80** (0.20 g, 1.5 mmol) in acetone (11 mL). Tetrabutylammonium bromide (0.05 g, 0.15 mmol), followed by potassium carbonate (0.24 g, 1.8 mmol) were then added and the reaction mixture was heated at reflux for 12 h. The mixture was concentrated under reduced pressure and the residue was dissolved in water (11 mL). The aqueous layer was extracted with ethyl acetate (3 × 11 mL) and the combined organic layers were washed with brine (11 mL), dried over MgSO₄ and concentrated *in vacuo*. Purification by flash column chromatography (4:1 hexanes:EtOAc) produced **89** as a colorless oil (70 mg, 27%), R_f = 0.53. IR (neat) 2962, 2925, 1646, 1466, 1376, 1298, 1265, 1121, 1028 cm⁻¹. ¹H NMR (CDCl₃): δ 6.91 (1H, dd, *J* = 6.9, 2.8), 6.86 (1H, d, *J* = 2.8), 6.24 (1H, d, *J* = 9.7), 3.37 (2H, d, *J* = 2.7), 2.19 (1H, t, *J* = 2.8), 1.30 (6H, s). ¹³C NMR (CDCl₃): δ 184.2, 158.7, 152.9, 126.4, 125.7, 76.1, 72.2, 59.0, 32.1, 29.9 (2C). HRMS (EI): *m/z* calcd for C₁₁H₁₂O₂H⁺ 177.0831, found 177.0833.

2-(Hydroxymethyl)-5-(prop-2-ynyloxy)-pyran-4-one (95):

To a solution of 5-(prop-2-ynyloxy)-2-((tetrahydro-2-pyran-2-yloxy)methyl)-pyran-4-one (0.300 g, 1.14 mmol) in methanol was added a few crystals of *p*-toluenesulfonic acid monohydrate. The reaction mixture was heated at reflux for 10 h and then concentrated under reduced pressure. The residue was diluted with ethyl acetate and washed with saturated sodium bicarbonate (2 × 20 mL). The organic phase was washed with brine, dried over Na₂SO₄, and concentrated *in vacuo*. Purification by flash column

chromatography (1:3 hexanes:EtOAc) on silica yielded **95** as beige crystals (98 mg, 50%) from hexanes, $R_f = 0.32$, mp 91.9-92.1 °C. IR (neat) 3281, 3089, 2130, 1647, 1607, 1578, 1436, 1333, 1255, 1209, 1083, 943, 699 cm^{-1} . ^1H NMR (CDCl_3) δ 7.81 (1H, s), 6.53 (1H, s), 4.77 (2H, d, $J = 2.41$), 4.51 (2H, d, $J = 6.04$), 2.66 (1H, m), 2.56 (1H, t, $J = 2.41$) ^{13}C NMR (CDCl_3) δ 175.2, 167.5, 146.0, 143.6, 112.8, 77.6, 77.3, 61.1, 58.2. HRMS (EI): m/z calcd for $\text{C}_9\text{H}_8\text{O}_4\text{H}^+$ 181.0501, found 181.0497.

4-Oxo-5-(prop-2-ynyloxy)-4-pyran-2-carboxylic acid (96):

Jones Reagent was prepared by adding a suspension of chromium trioxide (1.0 g, 0.1 mmol) in conc. sulfuric acid (1.0 mL) to water (3.0 mL) and stirring for 5 min.

To a solution of **95** (98 mg, 0.54 mmol) in acetone (10 mL) was added Jones reagent (0.5 mL) at 0 °C. The mixture was allowed to stir at rt for 1 h. Insoluble chromium salts were removed by filtration and the filtrate was concentrated *in vacuo* to yield **96** as a green solid. This compound was used without further purification.

Methyl 4-oxo-5-(prop-2-ynyloxy)-4-pyran-2-carboxylate (97):

Compound **96** (49 mg, 0.25 mmol) was treated with a solution of sulfuric acid (16 μL) in methanol (1.0 mL) and heated at reflux for 1 h. The solution was cooled to rt and concentrated *in vacuo*. The residue was dissolved in ether and washed with sodium bicarbonate and brine. The organic phase was dried over MgSO_4 and concentrated *in vacuo* to produce **97** as light yellow crystals (34 mg, 65%) from hexane-ether, $R_f = 0.38$, mp 127.9-128.6 °C. IR (neat) 3216, 3098, 2923, 2127, 1733, 1637, 1617, 1445, 1257,

1208, 1003, 960, 783 cm^{-1} . ^1H NMR (CDCl_3) δ 7.91 (1H, s), 7.18 (1H, s), 4.82 (2H, s), 3.97 (3H, s) 2.58 (1H, t, $J = 2.4$) ^{13}C NMR (CDCl_3) δ 174.4, 160.2, 151.9, 143.8, 119.4, 77.8, 77.4, 58.2, 53.8, 29.9. HRMS (EI): m/z calcd for $\text{C}_{10}\text{H}_8\text{O}_5\text{H}^+$ 209.0450, found 209.0448.

6-Fluoro-7-methylindole (113):

Synthesized according to General Procedure A from 1-fluoro-2-methyl-3-nitrobenzene. Recrystallized from hexanes-acetone as a white solid (85 mg, 44%), $R_f = 0.44$, mp 75.4-76.5 $^\circ\text{C}$. IR (neat) 3412, 2926, 1617, 1501, 1432, 1340, 1213, 1154, 1079, 1024 cm^{-1} . ^1H NMR (CDCl_3): δ 8.03 (NH, s, br), 7.39 (1H, dd, $J = 8.6, 5.1$), 7.18 (1H, t, $J = 2.9$), 6.87 (1H, dd, $J = 10.2, 8.7$), 6.52 (1H, dd, $J = 3.2, 2.2$), 2.39 (3H, d, $J = 1.5$). ^{13}C NMR (CDCl_3): δ 158.9-156.6 (d, $J = 235.2$), 136.0 (d, $J = 9.0$), 124.4, 123.9, 118.7 (d, $J = 10.1$), 109.1-108.8 (d, $J = 25.8$), 106.4 (d, $J = 22.1$), 103.4, 0.21. HRMS (EI): m/z calcd for $\text{C}_9\text{H}_8\text{NFH}^+$ 150.0714, found 150.0717.

6-Fluoro-7-phenylindole (114):

Synthesized according to General Procedure A from **122**. Recrystallized from hexanes-acetone as a white solid (0.26 g, 93%), $R_f = 0.47$, mp 76.8-78.0 $^\circ\text{C}$. IR (neat) 3427, 3037, 2919, 2850, 1961, 1848, 1605, 1485, 1425, 1405, 1332, 1218, 1177, 1083 cm^{-1} . ^1H NMR (CDCl_3): δ 8.24 (NH, s, br), 7.65-7.53 (5H, m), 7.48-7.43 (1H, m), 7.17 (1H, t, $J = 7.2$), 7.07-7.01 (1H, dd, $J = 10.6, 8.6$), 6.61 (1H, dd, $J = 3.2, 2.1$). ^{13}C NMR (CDCl_3): δ 157.3-154.9 (d, $J = 238.9$), 134.8 (d, $J = 7.7$), 133.0, 129.8 (2C), 129.3 (2C), 128.2, 124.9,

124.6, 120.7 (d, $J = 10.4$), 112.4 (d, $J = 20.5$), 109.4 (d, $J = 25.6$), 103.3. HRMS (EI): m/z calcd for $C_{14}H_{10}NFH^+$ 212.0876, found 212.0874.

6,7-Difluoroindole (117):

A mixture of carbamate methyl-2,3-difluoro-6-((trimethylsilyl)ethynyl)phenylcarbamate (0.50 g, 0.18 mmol) and sodium methoxide [prepared from sodium (0.20 mmol) and dry methanol (4 mL)] was heated at reflux for 5 h. After removal of the methanol, the residue was dissolved in water and the aqueous layer was extracted with chloroform (2×10 mL). The extracts were collected, dried over $MgSO_4$, and concentrated *in vacuo*. Purification by flash column chromatography (9:1 hexanes:EtOAc) yielded **117** as a yellow oil (0.022 g, 78%), $R_f = 0.5$. IR (neat) 3290, 2924, 2850, 1634, 1596, 1525, 1461, 1324, 1297, 1256, 1234, 1208, 1011 cm^{-1} . 1H NMR ($CDCl_3$) δ 7.81 (1H, t, $J = 7.9$), 7.15 (1H, t, $J = 7.9$), 7.17 (1H, s), 7.15 (1H, t, $J = 2.1$), 7.12 (1H, d, $J = 2.1$). LRMS (EI): m/z [$C_8H_6NF_2H$] $^+$ 154.0460.

General Procedure C — Coupling of Fluorinated Indoles to 2,5-Dichloro-1,4-benzoquinone:

A mixture of fluorinated indole (1.1 mmol), 2,5-dichloro-1,4-benzoquinone (0.40 g, 2.2 mmol) and acetic acid (3.3 mL) was stirred at 70 °C until the indole was completely consumed, as indicated by TLC (6:1 hexanes:EtOAc). After the reaction was cooled to room temperature, silver carbonate was added (50 wt % on celite, 0.31 g, 1.1 mmol) and the mixture was stirred an additional 4 h. The solution was filtered, the crude product was

concentrated *in vacuo*, and the residue was purified by flash column chromatography on silica (6:1 hexanes:EtOAc) to produce blue needles from toluene/hexane.

2,5-Dichloro-3-(7-fluoroindol-3-yl)-[1,4]-benzoquinone (119):

Synthesized according to General Procedure C from indole **118** (0.33 g, 95%), $R_f = 0.60$, mp 133.3-136.1 °C. IR (neat) 3466, 3354, 1672, 1655, 1557, 1501, 1478, 1415, 1232, 1186, 1112, 1032, 964, 888, 781, 727 cm^{-1} . ^1H NMR (CDCl_3): δ 8.86 (NH, s, br), 7.58 (1H, d, $J = 2.8$), 7.24 (1H, s), 7.18-7.09 (2H, m), 7.02-6.96 (1H, m). ^{13}C NMR (CDCl_3): δ 179.7, 179.5, 158.9, 150.9, 148.6, 144.4, 139.4-136.2 (d, $J = 317.6$), 134.9, 131.5, 124.8, 121.5, 120.7, 117.8, 107.8. HRMS (EI): m/z calcd for $\text{C}_{14}\text{H}_6\text{NO}_2\text{FCl}_2\text{H}^+$ 309.9832, found 309.9832.

2-Fluoro-6-nitrobiphenyl (123):

A solution of 2-bromo-1-fluoro-3-nitrobenzene (0.15 g, 0.68 mmol), phenylboronic acid (0.20 g, 1.7 mmol), and PdCl_2 (5 mol%, 0.03 mmol) in previously degassed (10 min) toluene (4.1 mL) was heated at reflux for 25 h and the reaction mixture was concentrated under reduced pressure. Purification by flash column chromatography (hexanes) on silica yielded **123** as white crystals (85 mg, 62%) from petroleum ether, $R_f = 0.41$, mp 66.3-67.1 °C. IR (neat) 3089, 2893, 1530, 1466, 1369, 1250, 1156, 1073 cm^{-1} . ^1H NMR (CDCl_3) δ 7.70 (1H, dt, $J = 7.9, 1.3$), 7.53, 7.38 (5H, m), 7.35-7.31, (2H, m). ^{13}C NMR (CDCl_3) δ 171.3-162.7 (d, $J = 865.3$), 161.2, 158.7, 130.3, 129.4 (d, $J = 8.9$), 129.0 (2C,

d, $J = 7.8$), 128.8 (2C), 125.0 (d, $J = 19.8$), 120.1 (d, $J = 23.6$), 119.8 (d, $J = 2.6$). HRMS (EI): m/z calcd for $C_{12}H_8NO_2FNa^+$ 240.0437, found 240.0446.

1-Cyclopropyl-2-fluoro-3-nitrobenzene (125):

A mixture of sodium bicarbonate (5 mg, 1.35 mmol), cyclopropylboronic acid (1.5 g, 0.45 mmol), 1-bromo-2-fluoro-3-nitrobenzene (0.10 g, 0.45 mmol), and tetrabutylammonium bromide (50 mg, 0.45 mmol) were degassed in a microwave reactor tube for 10 min. $Pd(PPh_3)_4$ (1 mol%, 0.0045 mmol) was added immediately and the reaction mixture was subjected to microwave irradiation, operating at 60 W and 130 °C for 10 min using a CEM-Discover microwave reactor. Upon completion, water (10 mL), followed by ether (10 mL) were added to the reaction mixture, and the aqueous layer was extracted with ether (4×10 mL). The combined ethereal extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography (6:1 hexanes:EtOAc) furnished **125** as a colorless oil (55 mg, 64%). $R_f = 0.55$. IR (neat) 3091, 2926, 1534, 1447, 1348, 1255, 1097 cm^{-1} . 1H NMR ($CDCl_3$): δ 8.12 (1H, dd, $J = 8.6, 1.8$), 7.95-7.87 (1H, m), 7.83-7.77 (1H, m), 2.23-2.14 (1H, m), 1.14-1.07 (2H, m), 0.81-0.75 (2H, m). ^{13}C NMR ($CDCl_3$): δ 156.0-153.3 (d, $J = 263.6$), 141.0, 131.5 (d, $J = 4.8$), 124.6, 123.9 (d, $J = 4.9$), 122.9, 29.9, 8.59 (2C).

Methyl 2,3-difluoro-6-iodophenylcarbamate (130):

A solution of iodine monochloride (0.66 g, 4.1 mmol) in acetic acid (3.9 mL) was added dropwise to a stirring solution of 2,3-difluoroaniline (0.39 mL, 3.9 mmol) in acetic acid

(9.7 mL) and the reaction mixture was left stirring for 1 h at rt. The acetic acid was removed and the resulting crude product was dissolved in dichloromethane under N₂. After the solution was cooled to 0 °C, pyridine (0.63 mL) and methyl chloroformate (0.48 mL, 6.2 mmol) were added dropwise and the solution was left stirring for 2 h at rt. Brine was added to the reaction mixture and the product was extracted with ethyl acetate (2 × 10 mL), dried over MgSO₄, and concentrated *in vacuo* to yield **130** as a white solid (1.0 g, 82%). This compound was used without further purification.

Methyl 2,3-difluoro-6-((trimethylsilyl)ethynyl)phenylcarbamate (131):

Trimethylsilylacetylene (0.33 mL, 2.4 mmol) was added to a stirring solution of carbamate **130** (0.50 g, 1.6 mmol), Pd(OAc)₂ (16.0 mg, 0.024 mmol), and (*o*-tolyl)₃P (0.97 g, 0.32 mmol) in triethylamine (3.7 mL) at rt. After stirring for 16 h, the reaction mixture was diluted with water and ether and then filtered through celite. The ethereal filtrate was collected, dried over MgSO₄, and concentrated *in vacuo*. Purification by flash column chromatography (6:1 hexanes:EtOAc) yielded **131** as a waxy, brown solid (0.36 g, 81%) from hexane-ether, R_f = 0.6, mp 63.8-65.0 °C. IR (neat) 3286, 2960, 2163, 1712, 1602, 1531, 1464, 1237, 1219, 1024, 973, 840, 821 cm⁻¹. ¹H NMR (CDCl₃): δ 7.85 (1H, t, *J* = 7.2), 7.19 (1H, m), 6.92 (1H, s, br), 3.81 (3H, s), 0.27 (9H, s). ¹³C NMR (CDCl₃): δ 153.4, 152.4-149.7 (dd, *J* = 252, 11.8), 141.8-139.3 (dd, *J* = 245, 14.8), 133.3, 128.8, 128.5, 114.1, 100.5, 96.6, 53.0, -0.047 (3C). HRMS (EI): *m/z* calcd for C₁₃H₁₅NO₂F₂SiH⁺ 284.0913, found 284.0900.

Methyl 2-fluoro-6-iodophenylcarbamate (133):

To a solution of 2-fluoro-6-iodoaniline (0.10 g, 0.42 mmol) in dichloromethane (1.4 mL) at 0 °C were added dropwise pyridine (0.07 mL, 0.84 mmol) and methyl chloroformate (0.05 mL, 0.68 mmol). The reaction mixture was stirred for 2 h at rt and diluted with dichloromethane. The organic layer was washed with water and brine, dried over MgSO₄, and concentrated *in vacuo*. The residue was crystallized from hexanes to yield **133** (0.12 g, 100%) as colorless crystals, $R_f = 0.48$, mp 101.1-101.5 °C. IR (neat) 3228, 3038, 2957, 1790, 1702, 1522, 1443, 1257, 1058, 826, 769 cm⁻¹. ¹H NMR (CDCl₃): δ 7.63 (1H, d, $J = 8.3$), 7.18 (1H, t, $J = 8.3$), 7.02-6.95 (1H, td, $J = 13.5, 4.1$), 3.79 (3H, s). ¹³C NMR (CDCl₃): δ 159-154 (d, $J = 366$), 156, 135 (d, $J = 3.3$), 130 (d, $J = 8.8$), 117, 116, 99, 53. HRMS (EI): m/z calcd for C₈H₇NO₂FIH⁺ 295.9544, found 295.9548.

Methyl 2-fluoro-6-((trimethylsilyl)ethynyl)phenylcarbamate (134):

Trimethylsilylacetylene (0.04 mL, 0.32 mmol) was added dropwise to a solution of **133** (0.06 g, 0.21 mmol), Pd(OAc)₂ (0.212 g, 0.003 mmol) and (*o*-tolyl)₃P (0.130 g, 0.004 mmol) in triethylamine (0.5 mL) and the reaction was allowed to stir at rt for 1 h. Water (5 mL) and ether (5 mL) were added to the reaction and the whole was filtered through celite, washed with brine, dried over MgSO₄, and concentrated *in vacuo*. Purification by flash column chromatography (5:1 hexanes:EtOAc) yielded **134** (44 mg, 79%) as a yellow oil, $R_f = 0.52$. ¹H NMR (CDCl₃): δ 7.25 (1H, m), 7.13 (2H, m), 6.39 (1H, s, br), 3.79 (3H, s), 0.27 (9H, s). ¹³C NMR (CDCl₃): δ 153.7-151.9 (d, $J = 287.8$), 152.0, 128.8

(d, $J = 9.5$), 128.6, 125.4 (d, $J = 2.8$), 120.9 (d, $J = 5.9$), 115.8, 115.0, 101.4, 52.9, -0.047 (3C). HRMS (EI): m/z calcd for $C_{13}H_{16}NO_2FSiH^+$ 266.0955, found 266.0939.

***tert*-Butyl-2-fluorophenylcarbamate (136):**

A solution of 2-fluoroaniline (0.17 mL, 1.8 mmol) and di-*tert*-butyl dicarbonate (0.59 g, 2.7 mmol) in anhydrous toluene (1.8 mL) was heated to reflux for 6 h. The mixture was concentrated *in vacuo* and the residue crystallized from hexanes to yield **136** as colorless needles (0.38 g, 100%), $R_f = 0.64$, mp 125-126 °C. IR (neat) 3458, 3382, 2981, 2934, 1743, 1721, 1469, 1367, 1274, 1251, 1151, 1113, 1002, 871, 761 cm^{-1} . 1H NMR ($CDCl_3$): δ 8.08 (1H, t, $J = 7.9$), 7.11-6.91(3H, m), 6.71 (1H, s, br), 1.54 (9H, s). ^{13}C NMR ($CDCl_3$): δ 153.9-150.7 (d, $J = 241.3$), 152.6, 127.1 (d, $J = 9.8$), 124.7 (d, $J = 2.4$), 123.1 (d, $J = 6.6$), 120.3, 114.9 (d, $J = 19.3$), 81.1, 28.5 (3C, t, $J = 2.7$). HRMS (EI): m/z calcd for $C_{11}H_{14}NO_2FH^+$ 212.1045, found 112.0554 ($[M-BOC]^+$).

***tert*-Butyl-2-fluoro-6-iodophenylcarbamate (137):**

tert-BuLi (0.6 mL, 1.7 M in pentane) was added to a solution of **136** (0.1 g, 0.5 mmol) in anhydrous THF (1.0 mL) at -78 °C and stirred for 3 h. After the solution was allowed to warm to rt, iodine (0.26 g, 1.0 mmol) was added and the solution was stirred for a few minutes. An aqueous solution of saturated $Na_2S_2O_3$ (20 mL) was added and the product was extracted with 3×10 mL ether, dried over $MgSO_4$, and concentrated *in vacuo*. This compound was used without any further purification.

***tert*-Butyl-2-fluoro-6-((trimethylsilyl)ethynyl)phenylcarbamate (138):**

A mixture of **136** (80 mg, 0.24 mmol), trimethylsilylacetylene (0.04 mL, 0.28 mmol), Pd(PPh₃)₂Cl₂ (16.6 mg, 0.024 mmol), CuI (4.50 mg, 0.024 mmol), and Et₃N (1.0 mL) was stirred at 50 °C for 4 h. The crude material was concentrated *in vacuo* and purified by flash column chromatography (3:1 hexanes:EtOAc) to yield **138** as colorless crystals (46 mg, 63%) from hexane, R_f = 0.68, mp 102.6-104.1 °C. ¹H NMR (CDCl₃): δ 7.70 (1H, t, *J* = 9.3), 7.45 (2H, m), 2.17 (9H), 0.01 (9H). ¹³C NMR (CDCl₃): δ 153.4, 152.5-149.1 (dd, *J* = 254, 11.2), 128.8 (d, *J* = 9.6), 128.5, 125.1 (d, *J* = 2.4), 121.7 (d, *J* = 6.3), 115.3, 114.6, 101.3, 96.8, 28.4 (3C), -0.047 (3C). HRMS (EI): *m/z* calcd for C₁₆H₂₂NO₂FSiH⁺ 308.1443, found 308.1440.

2,5-Dichloro-3-(6-fluoroindol-3-yl)-[1,4]-benzoquinone (139):

Synthesized according to General Procedure C from indole **111** (0.14 g, 40%), R_f = 0.60, mp 219.0-220.3 °C. IR (neat) 3389, 2925, 2851, 1676, 1654, 1623, 1561, 1453, 1267, 1233, 1141, 1011 cm⁻¹. ¹H NMR (CDCl₃): δ 8.69 (NH, s, br), 7.57 (1H, d, *J* = 2.7), 7.36-7.31 (1H, dd, *J* = 8.8, 5.2), 7.24 (1H, s), 7.18-7.14 (1H, m), 7.04-6.96 (1H, m). ¹³C NMR (CDCl₃): δ 178.0, 177.7, 161.9-158.7 (d, *J* = 240.6), 144.4, 138.3, 137.8, 135.9 (d, *J* = 12.4), 133.5, 130.0, 122.8 (d, *J* = 10.1), 121.9, 110.1 (d, *J* = 24.5), 107.8, 98.4, (d, *J* = 26.4). HRMS (EI): *m/z* calcd for C₁₄H₆NO₂FCl₂H⁺ 309.9837, found 309.9837.

2,5-dichloro-3-(5,6-difluoroindol-3-yl)-[1,4]-benzoquinone (140):

Synthesized according to General Procedure C from indole **112** (0.077 g, 24%), $R_f = 0.28$, mp 170.5-171.2 °C. IR (neat) 3403, 2923, 2854, 1675, 1552, 1472, 1337, 1275, 1186, 1151, 1111, 1073 cm^{-1} . ^1H NMR (CDCl_3): δ 8.77 (NH, s, br), 7.59 (1H, d, $J = 2.9$), 7.37 (1H, d, $J = 2.6$), 7.24 (1H, s), 7.23 (1H, s). ^{13}C NMR (CDCl_3): δ 177.8, 177.6, 150.9, 138.0 (d, $J = 21.2$), 133.4, 131.3, 130.7, 126.7, 125.3, 120.9, 118.7, 117.9, 108.9-106.7 (dd, $J = 208.4, 21.2$), 100.0-99.3 (dd, $J = 99.6, 22.2$). HRMS (EI): m/z calcd for $\text{C}_{14}\text{H}_5\text{NO}_2\text{F}_2\text{Cl}_2\text{H}^+$ 327.9738, found 327.9742.

2,5-Dichloro-3-(6-fluoro-7-methylindol-3-yl)-[1,4]-benzoquinone (141):

Synthesized according to General Procedure C from indole **113** (0.050 g, 48%), $R_f = 0.45$, mp 224.3-225.4 °C. IR (neat) 3340, 2922, 1676, 1641, 1553, 1502, 1436, 1262, 1219, 1191, 1163, 1118, 1077, 1036 cm^{-1} . ^1H NMR (CDCl_3): δ 8.63 (NH, s, br), 7.54 (1H, d, $J = 2.8$), 7.21 (1H, s), 7.17 (1H, s), 7.03 (1H, s), 2.42 (3H, s). ^{13}C NMR (CDCl_3): δ 179.2-178.7 (d, $J = 53.0$), 177.8 (d, $J = 28.0$), 157.5, 150.9, 145.3-144.3 (d, $J = 96.7$), 138.4-137.8 (d, $J = 59.8$), 133.4, 131.8-129.6 (d, $J = 223.3$), 131.1-129.0 (d, $J = 210.3$), 126.3, 123.0, 119.9 (d, $J = 10.0$), 118.5, 110.9, 29.9. HRMS (EI): m/z calcd for $\text{C}_{15}\text{H}_8\text{NO}_2\text{FCl}_2\text{NH}_4^+$ 341.0260, found 341.0258.

2,5-Dichloro-3-(6-fluoro-7-phenylindol-3-yl)-[1,4]-benzoquinone (142):

Synthesized according to General Procedure C from indole **114** (0.1 g, 70%), $R_f = 0.44$, mp 95.3-96.7 °C. IR (neat) 3362, 2923, 1675, 1654, 1562, 1509, 1428, 1263, 1212, 1110,

1079, 1010 cm^{-1} . ^1H NMR (CDCl_3): δ 8.83 (NH, s, br), 7.60-7.43 (5H, m), 7.33-7.27 (2H, m), 7.12-7.05 (2H, m). ^{13}C NMR (CDCl_3): δ 177.9, 177.7, 157.4-155.1 (d, $J = 241.5$), 144.3, 138.2, 137.8, 134.8 (d, $J = 8.3$), 133.5, 132.0, 130.2, 129.8 (2C), 129.4 (2C), 128.6, 121.8, 121.7, 113.2 (d, $J = 20.7$), 110.6 (d, $J = 29.9$), 107.5. HRMS (EI): m/z calcd for $\text{C}_{20}\text{H}_{10}\text{NO}_2\text{FCl}_2\text{Na}^+$ 408.0075, found 408.0077.

General Procedure D — Methanolysis of Fluorinated Dichloroindolylquinones:

An aqueous sodium hydroxide solution (10%, 0.2 mL) was added dropwise to a refluxing solution of dichlorobenzoquinone (varying amount) in methanol (5 mL). Progress of the reaction was monitored by TLC (TLC taken after every few drops of Sodium hydroxide added) and the reaction was complete once all the starting material was completely consumed. Appearance of the SECOND dark red spot on TLC indicates formation of product. The reaction mixture was diluted with water and acidified with 10% aqueous sulfuric to pH 2. The resulting mixture was extracted with ethyl acetate (2×15 mL), washed with brine, dried over Na_2SO_4 , and concentrated *in vacuo*. Purification by flash column chromatography on silica (7:3 hexanes:EtOAc), followed by recrystallization with hexanes produced the desired product.

2,5-Dimethoxy-3-(7-Fluoroindol-3-yl)-[1,4]-benzoquinone (5E5):

Synthesized according to General Procedure D from **119** (0.17 g, 0.54 mmol) as red crystals (0.16 g, 99%), $R_f = 0.3$, mp 187.8-188.6 $^\circ\text{C}$. IR (neat) 3376, 3145, 1638, 1597, 1526, 1445, 1318, 1294, 1231, 1195, 1092, 985 cm^{-1} . ^1H NMR (acetone- d_6): δ 9.00 (NH,

s, br), 7.61 (1H, d, $J = 1.4$), 7.24 (1H, d, $J = 8.2$), 7.04 (1H, dt, $J = 7.8, 4.8$), 6.95 (1H, m), 3.91 (3H, s), 3.85 (3H, s). ^{13}C NMR (acetone- d_6): δ 183.3, 181.3, 159.3, 154.7, 150.9-148.6 (d, $J = 242.8$), 129.0, 128.8, 124.3, 122.0, 119.9, 117.2, 106.4, 106.2, 105.8, 60.3, 56.3. HRMS (EI): m/z calcd for $\text{C}_{16}\text{H}_{12}\text{NO}_4\text{FH}^+$ 302.0829, found 302.0830.

3-(6-Fluoroindol-3-yl)-2,5-dimethoxy-[1,4]-benzoquinone (TM178):

Synthesized according to General Procedure D from **139** (0.14 g, 0.45 mmol) as a gray solid (0.11 g, 81%), $R_f = 0.33$, mp 224.1-225.2 °C. IR (neat) 3423, 3062, 2955, 2857, 2540, 1668, 1645, 1572, 1523, 1448, 1349, 1325, 1279, 1236, 1218, 1197, 1184, 1121, 1096, 1062, 1027 cm^{-1} . ^1H NMR (CDCl_3): δ 8.50 (NH, s, br), 7.49 (1H, d, $J = 2.0$), 7.43-7.38 (1H, dd, $J = 8.6, 5.3$), 7.10 (1H, d, $J = 9.2$), 6.93 (1H, t, $J = 9.0$), 5.91 (1H, s), 3.88 (3H, s), 3.80 (3H, s). ^{13}C NMR (acetone- d_6): δ 183.3, 181.6, 160.9, 159.2-158.6 (d, $J = 235.6$), 154.4, 136.2 (t, $J = 14.0$), 128.9 (d, $J = 16.4$), 123.8, 122.2 (d, $J = 9.8$), 108.0 (d, $J = 22.8$), 107.1, 105.2, 105.8, 97.6 (d, $J = 26.2$), 60.3, 56.2. HRMS (EI): m/z calcd for $\text{C}_{16}\text{H}_{12}\text{NO}_4\text{FH}^+$ 302.0829, found 302.0822.

3-(5,6-Difluoroindol-3-yl)-2,5-dimethoxy-[1,4]-benzoquinone (TM180):

Synthesized according to General Procedure D from **140** (78 mg, 0.24 mmol) as a gray solid (76 mg, 100%), $R_f = 0.35$, mp 214.8-215.7 °C. IR (neat) 3443, 3083, 3062, 2954, 1664, 1645, 1571, 1524, 1477, 1446, 1328, 1307, 1269, 1254, 1222, 1198, 1180, 1139, 1036 cm^{-1} . ^1H NMR (CDCl_3): δ 8.53 (NH, s, br), 7.53 (1H, d, $J = 2.7$), 7.28-7.16 (2H, m), 5.91 (1H, s), 3.88 (3H, s), 3.86 (3H, s). ^{13}C NMR (acetone- d_6): δ 183.2, 181.5, 159.1,

154.4, 148.8-146.4 (dd, $J = 239.1, 16.3$), 147.3-145.0 (dd, $J = 234.8, 14.9$), 131.3 (dd, $J = 15.3, 11.3$), 130.1 (d, $J = 16.4$), 122.7 (d, $J = 8.8$), 122.1, 107.9 (d, $J = 19.8$), 105.8, 105.4, 99.4 (dd, $J = 21.9, 4.5$), 60.4, 56.3. HRMS (EI): m/z calcd for $C_{16}H_{11}NO_4F_2H^+$ 320.0734, found 320.0725.

3-(6-Fluoro-7-methylindol-3-yl)-2,5-dimethoxy-[1,4]-benzoquinone (TM183):

Synthesized according to General Procedure D from **141** (50 mg, 0.15 mmol) as a red solid (9.5 mg, 20%), $R_f = 0.25$, mp 83-86 °C. IR (neat) 3347, 2927, 1722, 1639, 1588, 1445, 1322, 1219, 1199, 1022 cm^{-1} . 1H NMR ($CDCl_3$): δ 8.43 (NH, s, br), 7.49 (1H, d, $J = 2.6$), 6.95-6.88 (2H, t, $J = 9.4$), 5.91 (1H, s), 3.88 (3H, s), 3.80 (3H, s), 2.42 (3H, s). ^{13}C NMR (acetone- d_6): δ 183.4, 182.0, 158.9-156.6 (d, $J = 247.4$), 149.4 (d, $J = 13.3$), 146.0, 135.7, 130.9, 127.6, 122.5, 119.2 (d, $J = 10.0$), 109.4 (d, $J = 25.6$), 106.5 (d, $J = 22.1$), 105.7, 104.8, 60.8, 56.6, 29.6. HRMS (EI): m/z calcd for $C_{17}H_{14}NO_4FH^+$ 316.0985, found 316.0993.

3-(6-Fluoro-7-phenylindol-3-yl)-2,5-dimethoxy-[1,4]-benzoquinone (TM186):

Synthesized according to General Procedure D from **142** (96 mg, 0.25 mmol) as a red solid (93 mg, 99%), $R_f = 0.41$, mp 81.7-83.3 °C. IR (neat) 3356, 2925, 2854, 1729, 1642, 1588, 1432, 1211, 1096, 1058, 1029 cm^{-1} . 1H NMR ($CDCl_3$): δ 8.56 (NH, s, br), 7.62-7.51 (5H, m), 7.47 (1H, d, $J = 2.4$), 7.42-7.37 (1H, dd, $J = 9.0, 4.9$), 7.08-7.01 (1H, m), 5.92 (1H, s), 3.89 (3H, s), 3.87 (3H, s). ^{13}C NMR (acetone- d_6): δ 183.6, 182.1, 159.1, 157.8, 157.4-154.6 (d, $J = 286.5$), 132.4 (d, $J = 6.2$), 131.1, 129.8 (2C), 129.3 (2C),

129.0, 128.3 (d, $J = 10.2$), 123.2, 121.9 (d, $J = 8.0$), 121.6, 121.4 (d, $J = 10.9$), 110.0 (d, $J = 27.9$), 107.4, 105.9, 61.1, 56.8 HRMS (EI): m/z calcd for $C_{22}H_{16}NO_4FNa^+$ 400.0961, found 400.0951.

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