CROSS-COUPLING REACTIONS:

SYNTHESIS AND PHOTOACTIVITY

OF

ENEDIYNES

A Thesis

Presented to

The Faculty of Graduate Studies

Of

Lakehead University

By

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In partial fulfillment of requirements

For the degree of

Masters of Science

January 6th, 2015

ABSTRACT

CROSS-COUPLING REACTIONS:
SYNTHESIS AND PHOTOACTIVITY
OF
ENEDIYNES

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The syntheses of a number of photoactive enediynes using the Sonogashira Coupling reaction are described. Alkynes with different characteristics were coupled to 2-bromoiodobenzene to yield the desired drugs. The enediyne's functionality and their ability to undergo the Bergman Cyclization reaction is of interest given that a variety of naturally occurring and few synthetically produced compounds containing this unit demonstrate the ability to cleave DNA. The compounds were tested against human alveolar type 11-epithelial A549 cancer cells and the non-cancer cell line H9c2 and it was found that in the presence of light, they showed an enhanced ability to increase cell death. The enediyne with electron withdrawing functionality resulted in the greatest increase in cell death.

In addition, an independent study on the direct arylation of heteroaromatic arenes with benzene was undertaken. In this study, substituted furans were treated under palladium-catalyzed conditions in the presence of benzene to synthesize biaryl compounds from unactivated starting materials. The presence of the electron with drawing aldehyde resulted in best yield of cross-coupling product.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my parents, sister and boyfriend for their love and support through my 6 year journey at Lakehead University. I would like to thank my friend Sean Cuninghame for always coming to my rescue whenever I needed help, especially for the formatting of this thesis. I would like to thank Dr. Justin Jiang and Dr. Craig MacKinnon for allowing me to work in their labs in previous years, which has broadened my chemistry horizons. I would like to thank Brad Miller, Jarret Sylvester and most importantly Debbie Puumala for their endless assistance over the years with anything I needed. Debbie- the chemistry department would be completely lost without you! I would like to thank Pam Tallon for providing me with an orientation at the Northern Ontario School of Medicine, Dr. Suntres for his knowledge and help with my cell testing, and Heidi Forsyth for training and helping me for countless hours at the Med School testing my compounds. I would like to thank Ain Raitsakas for running my ICP and GCMS samples, Mike Sorokopud for training and constantly coming to my rescue on the NMR, and lastly Greg Kepka for running my thousands of GCMS and LCMS samples. I would have been lost without you guys. I would like to thank Dr. Justin Jiang and Dr. Zach Suntres again for being on my committee for the past two years. Finally and most importantly, I would like to thank Dr. Christine Gottardo for absolutely everything she has done for me these past 6 years. During my undergrad I was very close to giving up and you helped me believe in myself and pushed me to make it through and now I am a Master of Chemistry. I have worked for you for many years now and it has been an unbelievable experience and I would work for you forever if I could. Your lab has been my second home and I will miss you and the lab dearly. You have been an absolute amazing supervisor and mentor and you really couldn't have done more for me.

Your understanding and support has carried me through some rough times and words cannot describe how grateful I am to have been one of your minions. Writing these acknowledgements has been the toughest part of my thesis because I cannot say enough about how extraordinary you are! You will never understand how thankful I am to have worked under you and will never forget what you have done for me.

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LIST OF ABBREVIATIONS

Å	Angstrom
BC	Bergman cyclization
cd	carbon distance
$CDCl_3$	deuterated chloroform
Δ	heat
Da	dalton
DMSO	dimethylsulfoxide
DMAP	dimethylaminopyridine
DNA	deoxyribonucleic acid
eq.	equivalent
Et_3N	triethylamine
FID	flame ionization detector
FTIR	Fourier transform infrared spectroscopy
FDA	food and drug administration
GCMS	gas chromatography mass spectrometry
GLC	gas-liquid chromatography
hν	light
IR	infared
Λ_{max}	lambda maximum
MS	mass spectrum
$MgSO_4$	magnesium sulphate

MTT	method of transcriptional and translational assay
MPLC	medium pressure liquid chromatography
m/z	mass to charge ratio
NMR	nuclear magnetic resonance
$NaHCO_3$	sodium bicarbonate
NH_4Cl	ammonium chloride
PDT	photodynamic theory
ppm	parts per million
PPh_3	triphenylphosphine
R_F	retardation factor
RT	retention time
THF	tetrahydrofuran
TEMPO	2,2,6,6-tetramethylpiperidine 1-oxyl
TLC	thin layer chromatography
TMS	trimethylsilane
UV	ultraviolet

CHAPTER ONE: A SYNTHETIC AND MECHANISTIC REVIEW OF ENEDIYNE CHEMISTRY

1.1 Introduction

Enediyne chemistry has become of more interest to researchers ever since these compounds were isolated from bacteria in the 1960s. This interest is due to their potential as anticancer agents. Their three unique characteristics grant enediynes the capability to impart biological activity and have displayed great cancer fighting characteristics. The behaviour of these compounds can be controlled *in vivo* by synthetically modifying the different functionality found around the enediyne core.[1] Currently, scientists have been experimenting with diverse synthetic analogs of enediynes so that their mode of action can be better understood and therefore demonstrate better control of activity. Although the exploration continues, few enediynes have been FDA approved for use as anticancer agents.

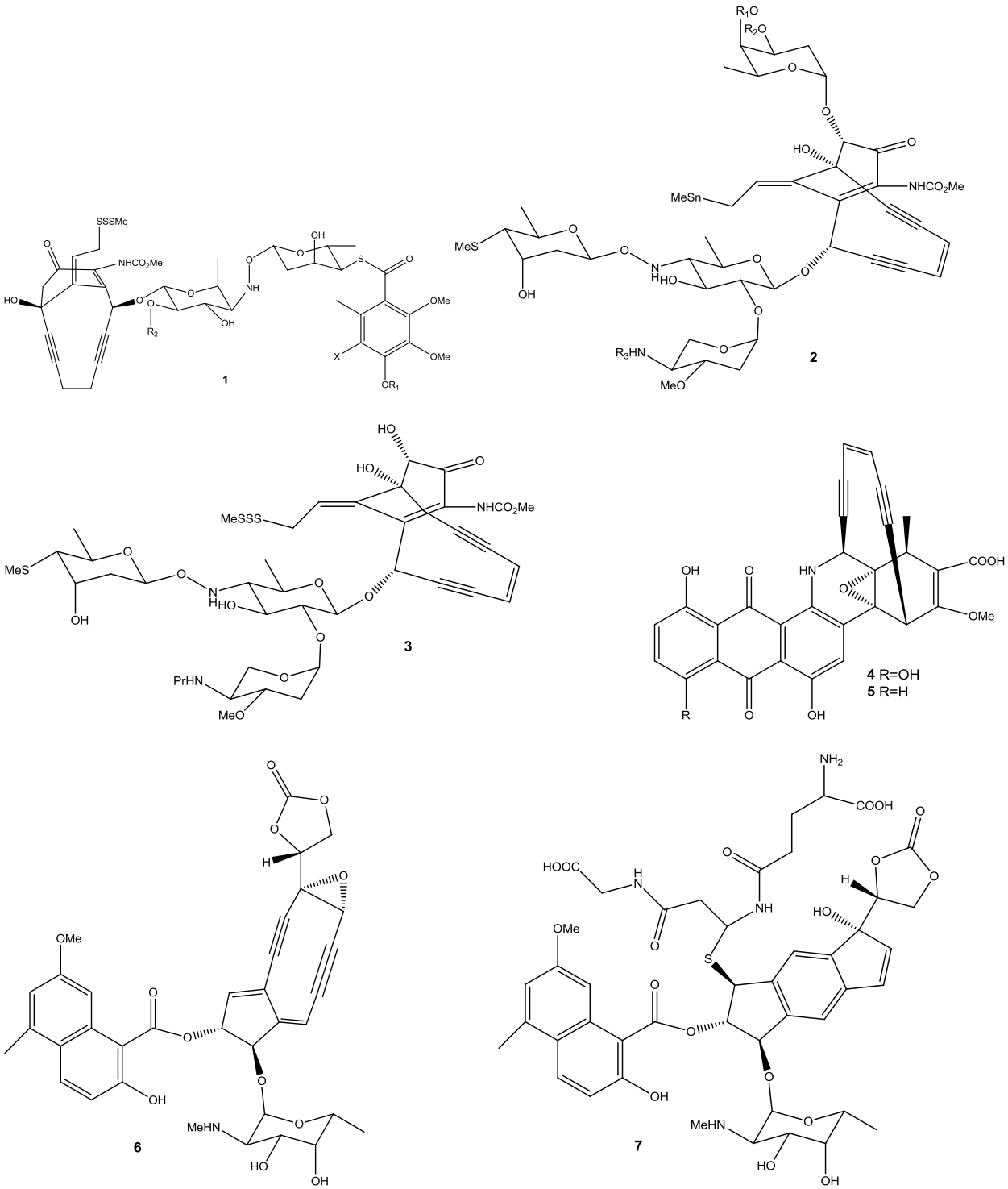
Herein, previously synthesized anticancer agents will be discussed, along with the synthetic approach and cycloaromatization reactions demonstrated for these compounds. A closer look at enediynes that are thermally stable under biological conditions and still exhibit anticancer activity will also be reviewed.

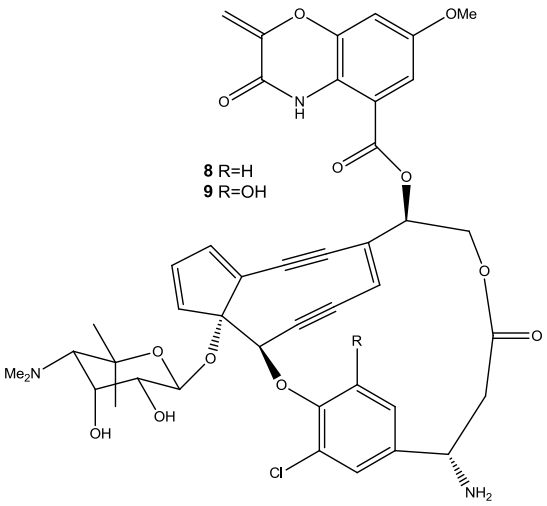
1.2 Enediyne Anticancer Agents

Enediynes are a bacterial natural product that consists of aromatic rings and triple bonds, separated by a double bond. They are unique due to the three different sections in their structure that impart biological activity. An enediyne's functionality, delivery system and triggering system can all be modified synthetically to control the behavior of the enediyne *in vivo* to make

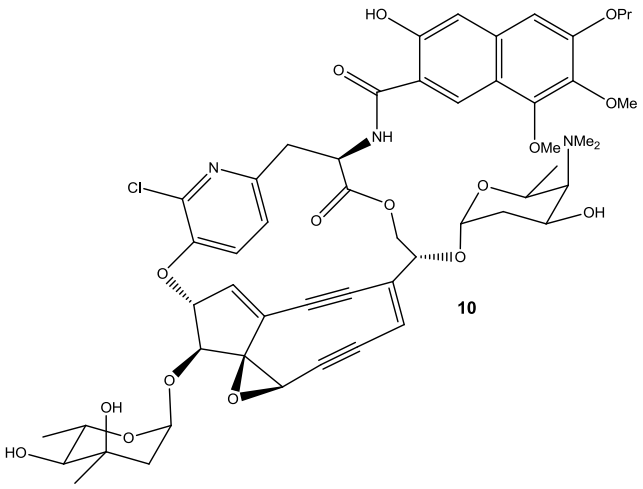
it a more selective molecule.[1] They were originally isolated from bacteria, various marine and terrestrial plant sources as early as the 1960's, and in 1972, Bergman reported that (Z)-enediynes prepared synthetically could undergo thermal cyclization *in vitro*.[2] However, it wasn't until the 1980s that their anticancer activity was discovered. It was found that their functionality allows the enediynes to cyclize, producing a biradical intermediate which imparts biological activity.[2] These anti-tumour agents interested researchers from a number of disciplines and brought more attention to the Bergman cyclization.[3] Since then, as many as eleven families of natural enediynes have been reported.[4] These families are Calicheamicin (**1, R** group depends on member of family)[5], Esperamicins (**2** and **3**)[6], Dynemicins (**4** and **5**)[7], Neocarzinostatin chromophores (**6** and **7**-post activated)[8], Lidamicins C-1027 (**8** and **9**)[9], Kedaricidin (**10**)[10], N1999-A2 (**11**)[11], Maduropeptin (**12**)[12], Namenamicin (**13**)[13], Shishijimicins (**14-16**)[14], and most recently, Uncialamycin (**17**)[15] (Figure 1). Presently, there has yet to be discovered an anti-tumour agent that is more potent than the class of enediyne anticancer agents.

Figure 1: Enediyne Anticancer Agents





8 R=H
9 R=OH



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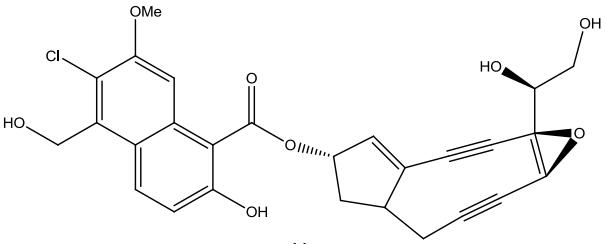

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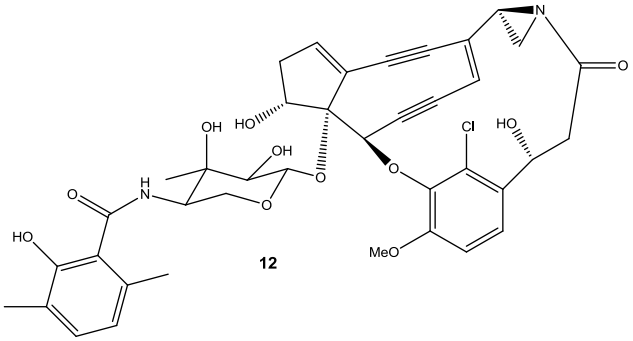

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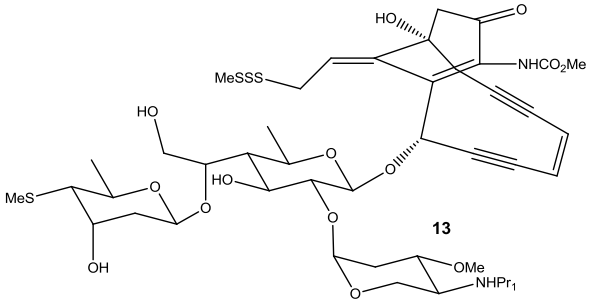

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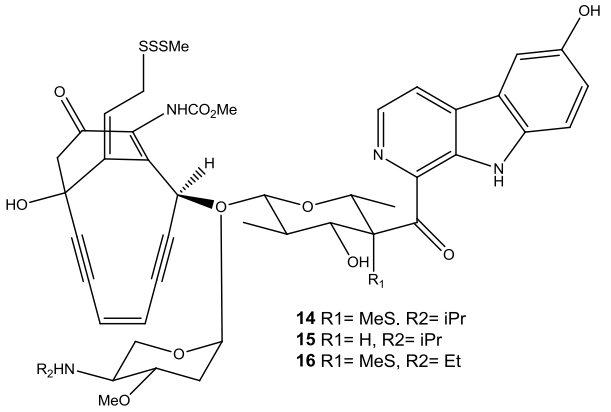
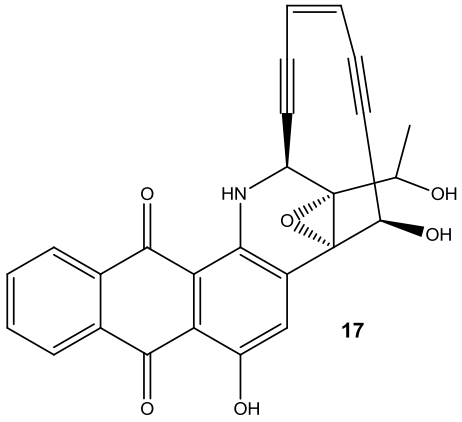


14 R1= MeS. R2= iPr
15 R1= H, R2= iPr
16 R1= MeS, R2= Et



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1.2.1 Mode of Action

Due to the potent activity, molecular framework and their specific mode of action, these compounds can generate cell death in the desired regions. This ability to trigger cell death is due to the cytotoxicity of the highly unsaturated hex-3-ene-1,5-diyne unit that can undergo the Bergman cyclization reaction, which in turn generates a benzene-1,4-diradical.[2] This biradical species can then proceed to abstract hydrogen atoms from the sugar-phosphate backbone of double stranded DNA.[2] The biradical species formed from the cyclization of the (Z)-hex-3-ene-1,5-diyne reacts further to produce DNA strand cleavage, resulting in cell death. The final product of the cyclization reaction performed is an aromatic ring.[4] The mechanism of the Bergman cyclization reaction will be discussed further in Section 1.3.

There can be many different conditions and structural components arranged which result in better control and efficiency of this mode of action. Different chemical properties such as electronic, geometric, steric factors, planarity, oxidation states and transition states can affect the favourability of the cyclization reaction.[4] When designing enediynes, all of these characteristics must be taken into consideration. In order for the abstraction of hydrogen atoms to take place, the chelating unit must be able to insert into the targeted DNA sequence. Therefore, the features of the molecule must be designed carefully so that the cyclization biradical intermediate is formed in the desired region and can access the DNA. Another important factor in this process is the triggering device, which establishes the time and location of the cyclization. If complications with the trigger are observed, the desired outcome will not be obtained due to the fact that the opposite ends of the enediyne are kept far enough apart so contact, and therefore, cyclization, is impossible. Improvements on the triggering mechanism have been reported and will be discussed in Section 1.4.

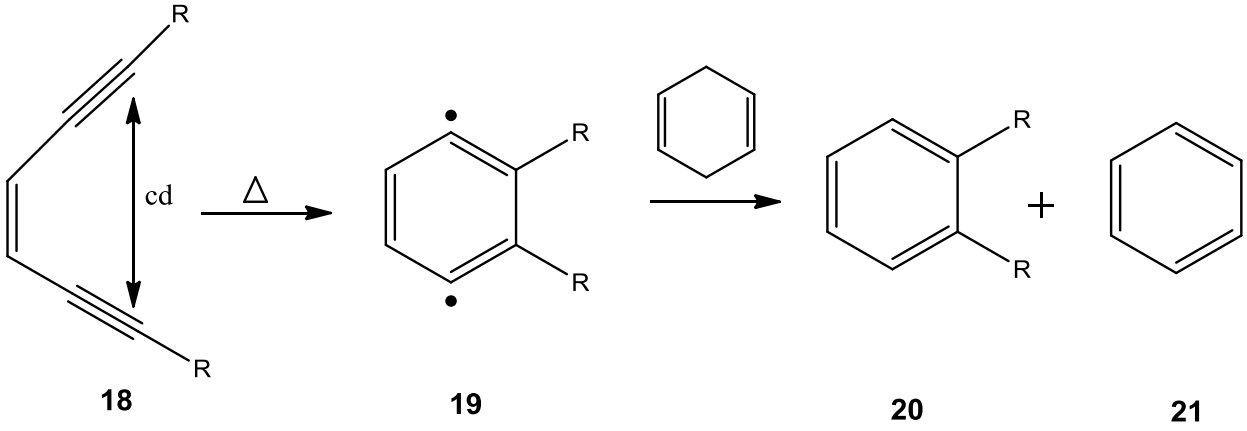
1.3 Cycloaromatization

As previously discussed, the anti-tumour activity demonstrated from the enediynes reviewed would not be observed if the cyclization reaction did not take place. For this reason, an understanding of the mechanism is crucial for the production of these anticancer agents. Although there are many cycloaromatization pathways presented in chemistry, the Bergman Cyclization reaction will be thoroughly discussed as most of the enediynes function through this mechanism.

1.3.1 The Bergman Cyclization Reaction

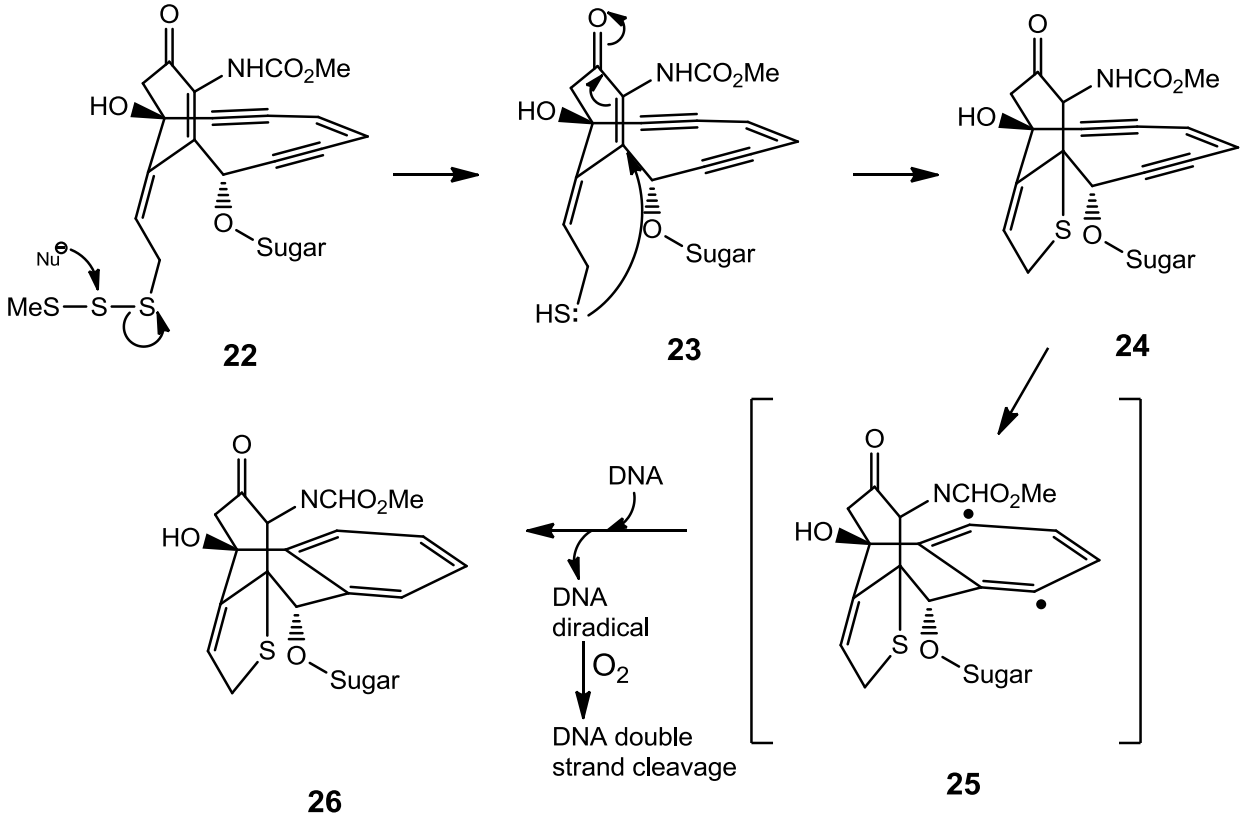
As mentioned earlier, the Bergman cyclization reaction was first reported in 1972 for Z-enediynes. After being triggered with heat, the formation of a biradical intermediate was proposed. This intermediate species would then abstract protons from a hydrogen donor, resulting in the final aromatic product (Scheme 1).[4] Unfortunately, the mechanism for this reaction was poorly understood due to lack of evidence to support the biradical intermediate's presence. However, radical trapping experiments have been subsequently run with the use of TEMPO to prove that the biradical intermediate does exist.[16] Further research provided evidence of biradical intermediates in enediynes showing that the intermediates could undergo subsequent chemistry characteristic of radical reactions.

Scheme 1



Herein, using Calicheamicin as an example, a proposed mechanism will be discussed for better understanding of the cyclization. Calicheamicin contains two distinct structural regions that contribute to biological activity. The largest region, which is the sugar residue, serves to deliver the molecule to its target and will bind tightly in the minor groove of the DNA.[17] The second region, referred to as the aglycon, acts as the "warhead" and the trisulfide group serves as the trigger. Once the molecule is in the vicinity of the DNA, cleavage may take place due to a nucleophile attacking the central sulfur atom, which causes the formation of a thiolate. This thiolate attacks the unsaturated ketone which converts the trigonal bridgehead to a tetrahedral center. This causes an increase in the strain energy of the 10 membered ring, which is relieved by the Bergman cyclization reaction. This produces the highly reactive benzenoid diradical, which can then go on to abstract protons from the DNA backbone, causing cell death (Scheme 2).[17]

Scheme 2



1.3.2 Triggering Mechanisms

In regards to the natural enediynes' mode of action, a certain triggering mechanism is in place to guarantee control of the cyclization process. Until the trigger is in position, the opposite ends of the enediyne are kept apart so an interaction cannot proceed. Once the trigger is activated or removed, the ends can be brought together so cyclization can take place. Both the critical distance (c-d) and strain factor act synergistically to drive the process to form the biradical species. To have better control of the reaction, knowing the critical distance between the two ends is essential. Nicolaou and his research team studied simple monocyclic enediynes without limited strain and tethered the ends with different hydrocarbon chain lengths to successfully

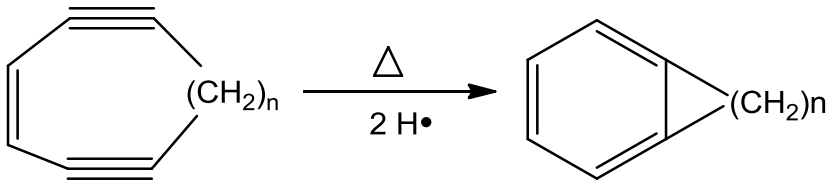
determine this distance.[18] The use of chains with different lengths allowed the distances between the terminal carbon of the two alkynes (cd) to be changed. They then went on to perform numerous cyclization reactions to determine the distances required at room temperature to allow for cyclization. They discovered that the distances must be in the range of 3.20-3.31Å in order for the cyclization to take place at room temperature. If the critical distances are longer than this range, the reaction cannot proceed without the use of heat or other methods of increasing the reaction energy.

This information proved to be quite interesting to other scientists and the experimentation continued. Another group of researchers, Schreiner and his team, analyzed similar compounds to those investigated by Nicolaou[18] and reported a different outcome (**28**a-f) (Scheme 3).[19] They found that there is no predictive relationship between the alkyne carbon distance and the cyclization activation energy. However, they found that similar monocyclic systems were able to cyclize at room temperature within a longer cd range. The only compounds that would cyclize, **28**c(n=3) and **28d**(n=4), had critical distances calculated to be 3.40Å and 3.90Å.

Studying the substituent effects on the terminal alkynyl carbons provides evidence that the cd distances are a critical factor in the enediynes reactivity due to the Bergman cyclization being an endothermic process. Grissom and her research team discovered that the activation energy can be raised from 25.1 kcal/mol to 28.1 kcal/mol by simply adding an alkyl group onto a terminal carbon of the alkyne.[20] It was also reported that the energy can be raised to a further 34.0 kcal/mol with the addition of a second alkyl group. If the substituents on the ends are any larger, thereby increasing the cd distances, steric interactions would be detected. In turn, the ends would be pushed farther apart, making interaction impossible. This research demonstrates the

importance of the cd distances being within this certain range in order for the reaction to proceed.

Scheme 3



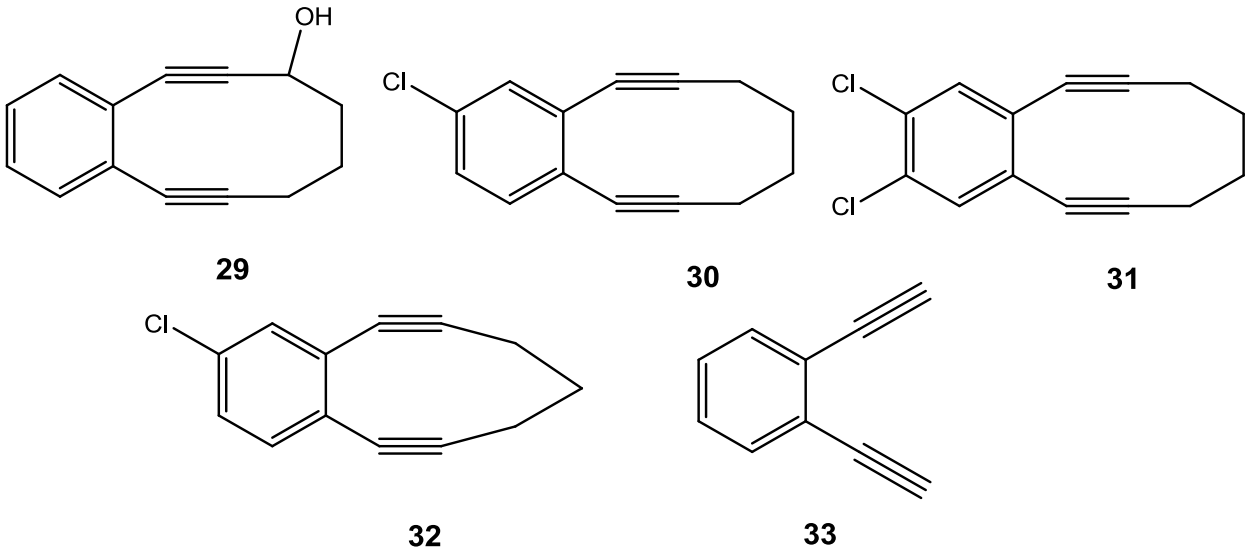
27a-f
(n=1-6)

28a-f

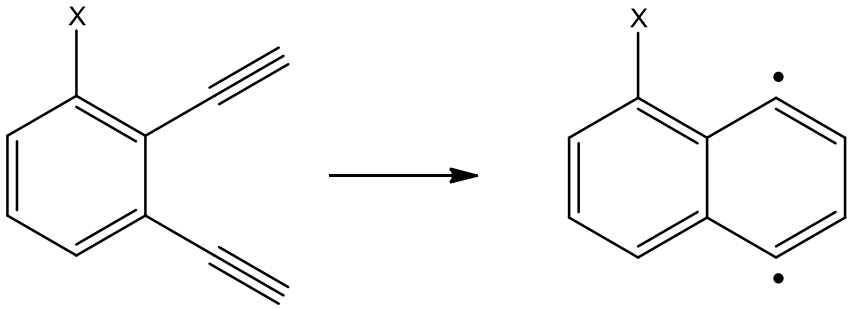
Although these distances play an important role in the cyclization process, other substituents have proven to contribute to the reactivity. An increase in rate has been observed when electron withdrawing substituents are located adjacent to the alkynyl carbon (**29**). [21] Other researchers found that vinyl substituents can increase the activation energy due to the electron withdrawing group's ability to lower the rate of reaction (**30,31,32**).[22,23] More recently, Albugin et al.[24,25] reported that benzannulated enediynes (**33**) impact the kinetics of the reaction significantly when the *ortho* effect is observed (Scheme 4) (X= OMe, NH_3^+, NO_2, CF_3). Through the interaction of the in-plane acetylenic orbitals and the *ortho*-substituents, the cyclization can be controlled. Hydrogen bonding, hyperconjugation and electron transfer can be observed to stabilize the interaction or steric interactions can be implied to destabilize the enediyne. In conclusion, the cycloaromatization reaction has presented researchers with numerous opportunities for developing strategies to make enediynes more selective molecules. Herein, a review of synthetically designed enediynes that have been structured to enhance Bergman

cyclization reactivity, and decrease toxicity while using favorable triggering mechanisms, will be presented.

Figure 2: Various Substituted Enediynes



Scheme 4



1.3.3 Internal and External triggering in *vivo*

One alternate solution used to develop target-specific chemotherapeutic agents, is the use of pH and photo-irradiation cyclization as the triggering method. Kar et al report that greater selectivity against tumour cells is expected with pH triggering due to the tumour cells having an acidic pH, which can be lowered by the use of hyperglycaemic agents.[26] Several enediynes have been synthesized and tested amongst cell lines although no specific advantage seems noticeable under alkaline conditions. Under mildly basic conditions, the cyclization reaction has taken place for select enediynes and has displayed therapeutically useful IC_{50} values against cancer cells. However, lack of selectivity is displayed and therefore high toxicity results which is unfavourable.

An internal triggering approach has also been discovered by Jones and his team.[29] They designed prodrugs that demonstrate selectivity towards certain characteristics found in tumour cells such as bulges in nucleic acid targets. They reported that the molecules undergo a conformational change when interactions occur with these bulges. This brings the ends of the enediyne to the desired critical distance so that cyclization may occur.

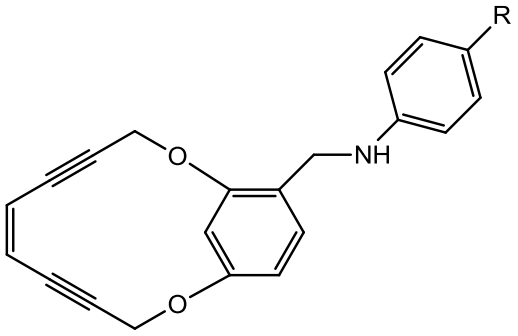
Another approach described in the literature is a light-mediated procedure known as photodynamic therapy (PDT).[27] With the use of an anti-tumour drug and light at a certain wavelength from sources such as a laser, this method has become useful for terminating tumour cells selectively. Although it displays promising results, PDT is not sufficient for large tumours as the light cannot penetrate through the tissue. Therefore, longer wavelengths must be used.

Another external method has also been approached by Grissom et al, where they experimented with thermal cyclization.[30] Here, the conformational change is observed when sufficient energy is applied. However, in order for cyclization to occur in acyclic compounds,

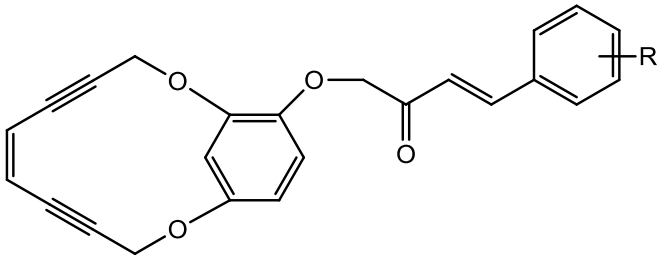
high temperatures are needed *in vitro*. Yet cyclic systems can cyclize at lower temperatures to relieve ring strain due to their shorter bond critical distances.[28]

Most recently, a group of researchers designed 13-membered cyclic enediynes which displayed marginal to good inhibition for select cell lines (Figure 3). [28] An external method of activation was used by applying light in the desired region. In order for this method to be successful, the enediynes designed had to remain stable until activation at the specific wavelength. In turn, they had to ensure the wavelength of light did not damage the cells independently. This method proved advantageous as a photo-irradiation trigger can display selective treatment to particular regions. However, as mentioned earlier, not all tumours can be reached by the photo-irradiation light.

Figure 3: Sharma's Synthetically Designed13-Membered Cyclic Enediynes



36a R=Cl
36b R=Br
36c R=H
36d R=F

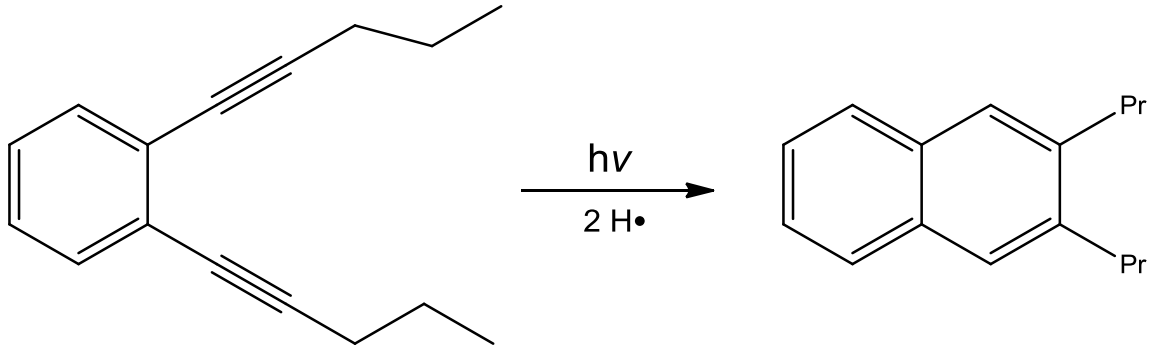


37a R= 4-H
37b R=4-CH_3
37c R= 3-CH_3
37d R=2-CH_3
37e R= 4-Cl
37f R= 3-Cl
37g R= 2-Cl
37h R=3-F
37i R=4-iso-Pr
37j R= 4-C_2H_5
37k R= 1-Naphthyl
37l R= 3-NO_2
37m R= 4-Br
37n R= 3-Br
37o R=2-Br

1.3.4 Photochemical Triggering

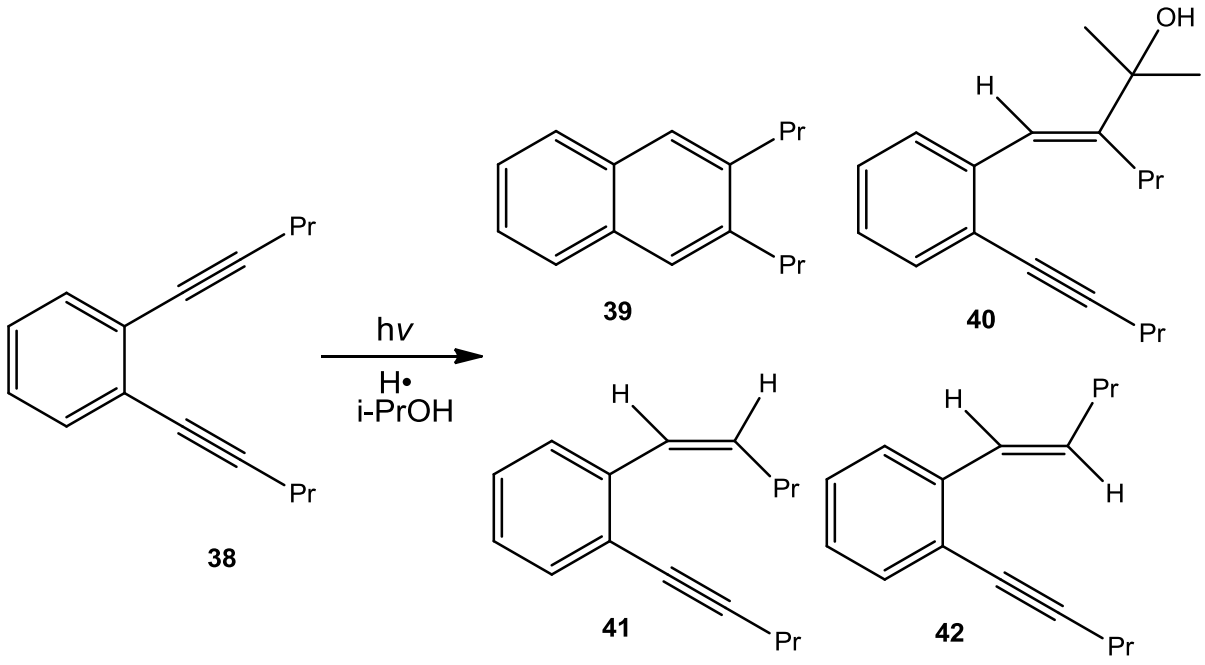
Although the triggering mechanisms discussed have shown promising results, the idea of using photochemical triggering has become the most interesting approach yet. In 1994, Evenzahav and Turro reported that compound **38** underwent a photochemical Bergman cyclization by irradiation in different solvents to produce **39** (Scheme 5).[31] After further research, the same group reported that **38** also produced four different products upon photochemical activity (Scheme 6).[32] They also reported that compound **43** underwent cyclization to produce the compound **44** (Scheme 7).

Scheme 5



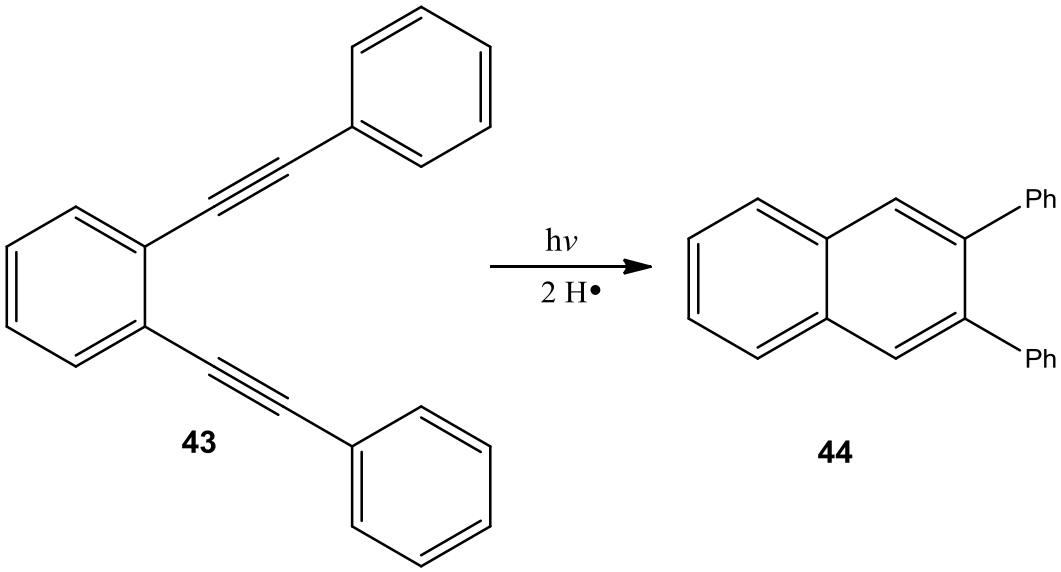
38 **39**

Scheme 6



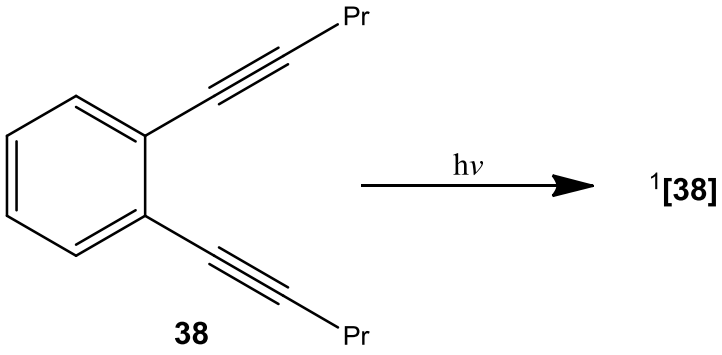
A mechanism for this photochemical Bergman cyclization has been proposed by this group (Scheme 8). Based on the theory of the quantum yield of fluorescence which depends on the rigidity of the molecule, they proposed that the cyclization begins in the singlet excited state and that the photoreduction occurs in the triplet excited state.[33] When the enediynes are less rigid, chances of deactivation, such as intersystem crossing, are higher which could be problematic. However, the aromatic benzene rings shown in **43** make the compound more rigid and less susceptible to intersystem crossing. This explains why **43** only forms one cyclization process, since photoreduction occurs from the triplet state. The opposite is displayed for compound **38**, as it is less rigid, granting further production of intersystem crossing (Scheme 9).

Scheme 7



43 **44**

Scheme 8



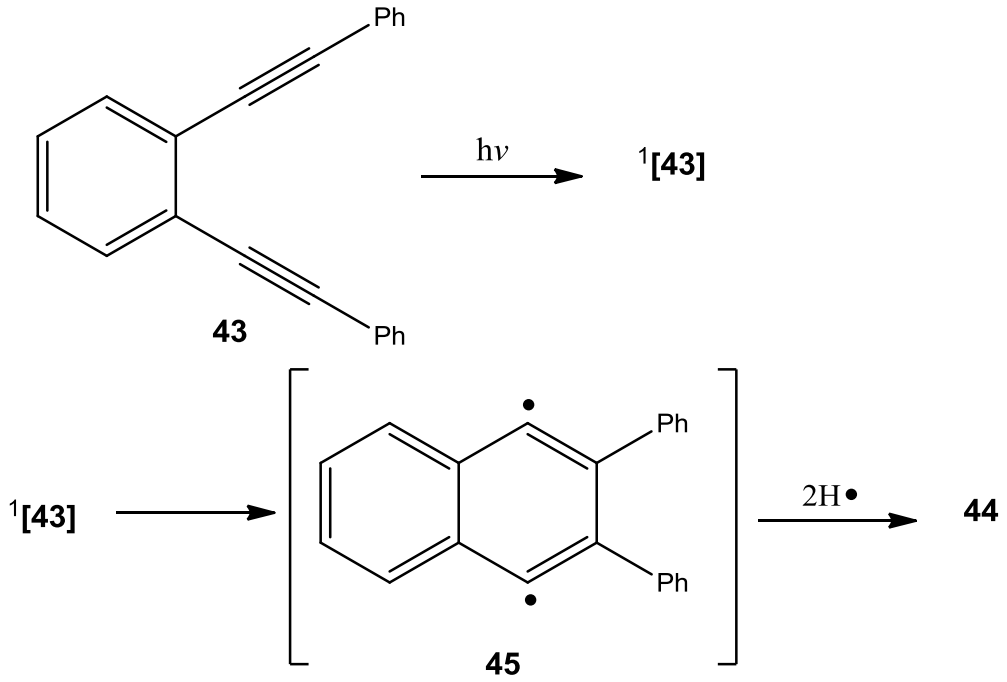
38

^{1}**[38]** $\xrightarrow{2\ H\bullet}$ **39**

^{1}**[38]** $\xrightarrow{2\ H\bullet}$ ^{3}**[38]**

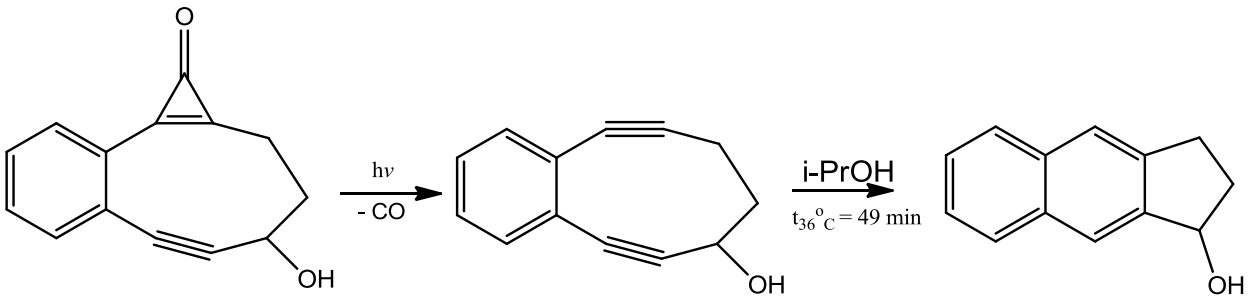
^{3}**[38]** $\xrightarrow{2\ H\bullet}$ **40 + 41 + 42**

Scheme 9



More recently, Polukhtine and his team synthesized enediynes that improved yields of cyclized products by adjusting the electronic properties of substituents.[34] They also used different modes of excitation energy transfer hoping to alleviate toxicity and allow spatial and temporal control of the reactivity. They found that when tested in tumour cells, **46** did not produce DNA damage whatsoever (Scheme 10). Alternately, **47** is generated in *situ* and survives until encountering DNA. It then goes on to induce single to double stranded cleavage of dsDNA with moderate efficiency.

Scheme 10



46 **47** **48**

1.4 Carbon-Carbon Cross Coupling Reactions

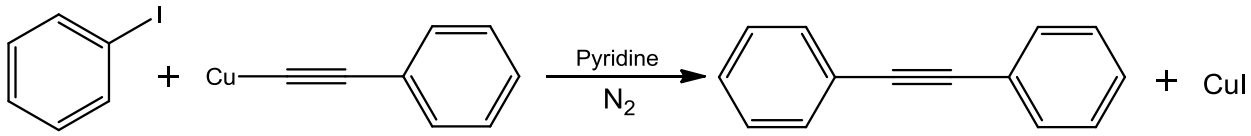
In order to design anti-tumour agents, a synthetic approach must be considered. One of the most common methods used to produce enediynes is through carbon-carbon cross coupling reactions. The reactions proceed with the use of a metal catalyst and vinyl or aryl halides coupling with acetylenes. Since the 1960s, synthetic chemists have been experimenting with different cross coupling methodology and are still to this day working to improve reaction conditions.

1.4.1 Stephens-Castro Coupling

One of the earliest cross coupling reactions reported which experimented with alkynes and aryl halides was by Stephens and Castro.[35] In 1963, they discovered that compound **51** can be formed by reacting an aryl iodide with a cuprous acetylide in refluxing pyridine (Scheme 11). Heterocyclic compounds were formed when the aryl iodide contained an *ortho* nucleophilic substituent due to a subsequent cyclization reaction. The order of reactivity observed for the aryl halides was found to be I>Br>Cl with F showing no reactivity at all. It must also be noted that the reactions progressed with more success if the electron withdrawing groups or poorly

donating groups are attached in the *para* position. The order of reactivity for these substituents is as follows: p-NO_2 > p-H > p-CH_3O. However, they did recognize difficulty with cuprous acetylide production and it also displayed much instability. Nonetheless, this research paved the way for different types of coupling reactions to be explored.

Scheme 11

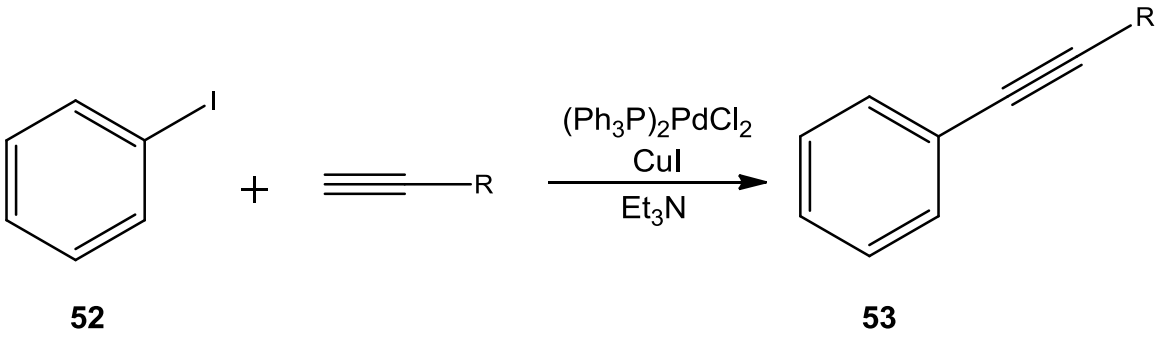


49 **50** **51**

1.4.2 Sonogashira Coupling

The Stephens-Castro reaction struck the interest of chemists Sonogashira and Hagihara, who then reported success in the coupling reaction by adding a catalytic amount of copper (I) iodide.[36] This copper catalyst displayed a better rate of formation for alkynylation at room temperature. The original Sonogashira reaction conditions involve $PdCl_2$ (the main catalyst), PPh_3(ligand), CuI(cocatalyst) and triethylamine (other amine solvents can be used) (Scheme 12). The $PdCl_2$ and PPh_3 ligate together which changes the electronic and steric properties of the catalyst. The role of the amine is to neutralize the reaction by removing any acidic materials while also acting as a solvent. The participation of CuI co-catalyst is not completely understood, however the acceleration of the reaction is observed when it is included in the synthesis.[37, 38]

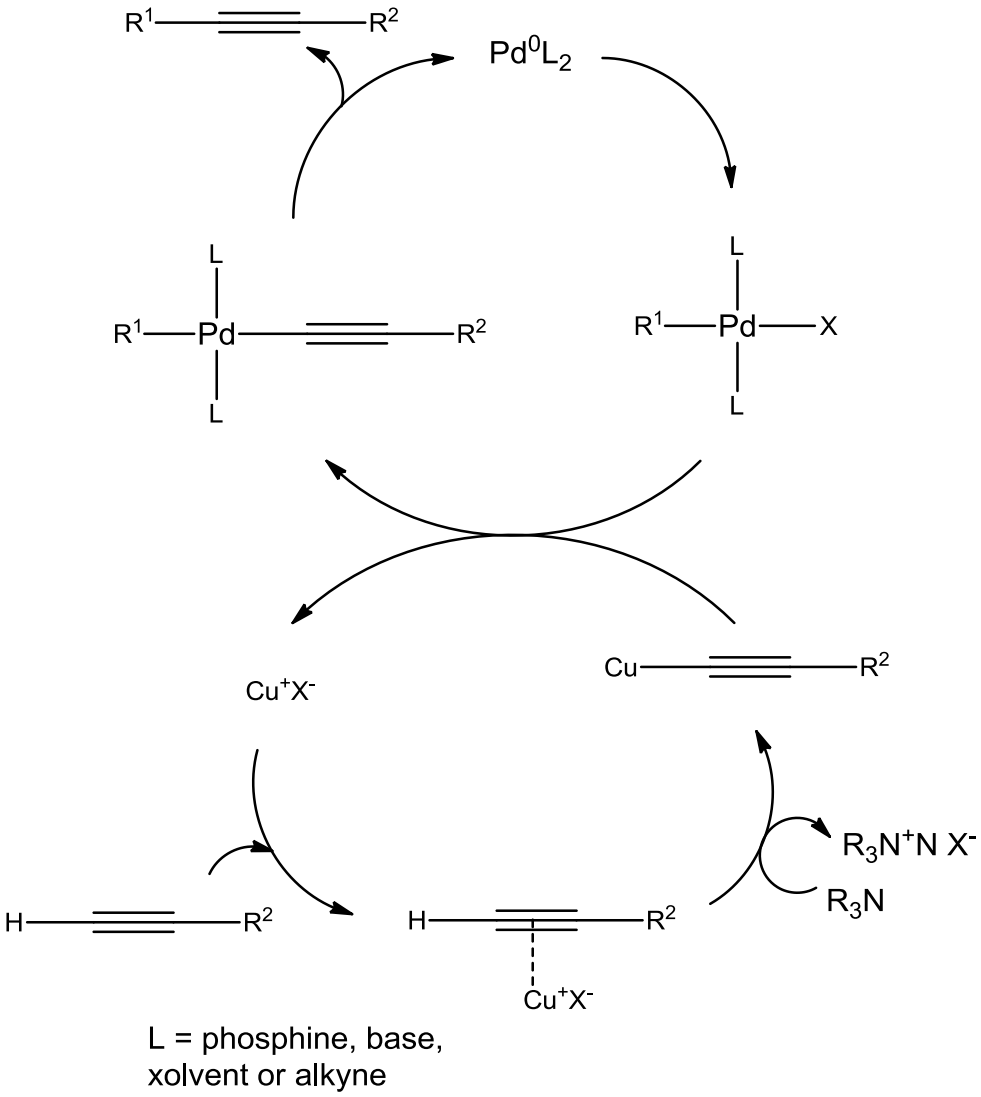
Scheme 12



52 **53**

The exact mechanism for the Sonogashira reaction is actually unknown, but a proposed mechanism has been reported (Scheme 13).[39] It is believed to take place in two independent catalytic cycles, the first being the palladium cycle. In the first cycle, a fast oxidative addition is observed when the R1-X (R1=aryl, heteroaromatic, vinyl; X=I, Br, Cl, OTf) complex enters the palladium cycle. The R1-X complex interacts with the active catalyst which is generated from the original palladium complex, a 14-electron Pd^0L_2 species results from a reduction of the different palladium (II) species. As the reaction proceeds, the intermediate interacts with the copper co-catalyst in the second cycle. The rate determining transmetalation reaction taking place in the copper cycle generates an alkyne complex, which undergoes a trans/cis isomerization and reductive elimination to produce the final coupled alkyne. The complete regeneration of the catalyst is also obtained. The second cycle, the copper cycle, is even more poorly understood. In theory, it is claimed that the base abstracts a proton from the alkyne forming a copper acetylide in the presence of a copper (I) salt.

Scheme 13



Since this discovery, there have been some improvements in the Sonogashira coupling with regards to the catalyst. The most common catalysts used to date are $Pd(PPh_3)_4$ and $PdCl_2(PPh_3)_2$. Although the mechanism is not completely understood, the reaction has been successful using both Pd^0 and Pd^{+2} catalysts. However, this reaction proceeds under much

milder and easier conditions than the Stephens-Castro reaction; it still requires purification as side reactions often take place, generating homocoupled products.

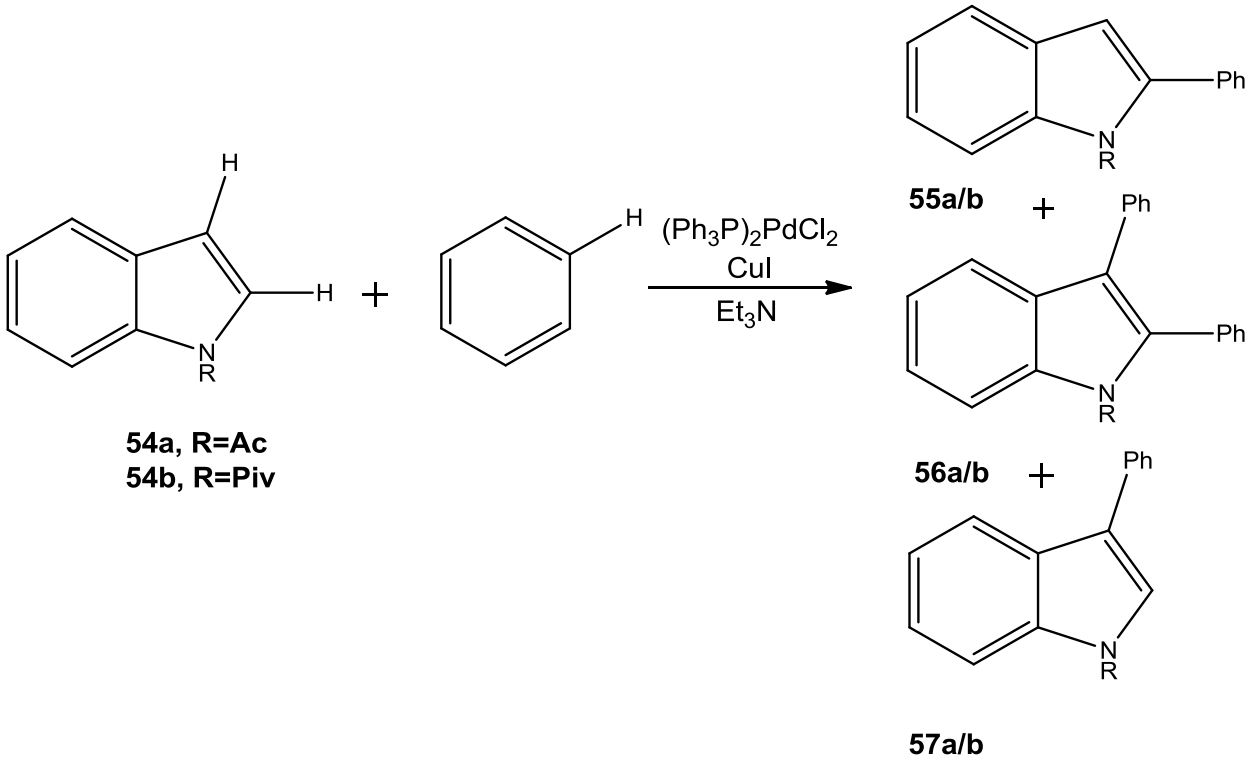
1.4.3 Cross-coupling of Unactivated arenes

Although Sonogashira coupling has proved to be a successful pathway to form carbon-carbon bonds, other synthetic approaches using unactivated arenes have demonstrated more ideal conditions. The costly preactivated starting materials, added reactions, undesirable side/by products, generated waste and purification can be decreased by inducing cross-coupling of unactivated arenes. This direct activation of a C-H bond permits the direct arylation forming a C-C bond from two unactivated arenes. In 2007, David R. Stuart and Keith Fagnou[43] reported their methodology requiring a specifically chosen catalyst capable of reacting with one arene in the first step of the catalytic cycle to avoid production of homo-coupling and eliminate consumption of the starting material and production of unwanted by-products. The catalyst would then invert its selectivity in the second step of the cycle and react with the second arene. This inversion in selectivity and reactivity is the most crucial and difficult challenge in cross-coupling reactions.

Stuart and Fagnou describe their achievement with direct arylation using benzene and an electron rich indole or pyrrole. Palladium (II) trifluroacetate was the chosen catalyst accompanied by the oxidant copper (II) acetate. Not only did the reaction provide high yields of coupled product, high regioselectivity was observed for the C-3 arylated product when this specific oxidant was used (Scheme 14). They also found that when they implemented the oxidant silver acetate instead, direct C-2 arylation of the indole was observed.[44] The mechanism for C-H cross-coupling reactions is not fully understood or yet determined due to many reasons. 3-Nitropyridine and cesium pivalate, additives in the reaction, are said to stabilize palladium (0)

before it is oxidized back to palladium (II), but this is still not fully conclusive. The sensitivity of C3/C2 selectivity for arylation has been compared by using varying amounts of catalysts and has been proven that 2 mol% of palladium catalyst offers high desirable isomeric ratios that depend on the oxidant used in the reaction. When the amount of catalyst is increased, the selectivity is decreased even though the reaction is proven to be very successful. Also, even though there is much reactivity observed for indoles with electron-donating groups, no clear trends have been observed with respect to benzene or other heteroaromatic compounds.[43]

Scheme 14



In conclusion, cross-coupling synthesis has progressed immensely over the past few decades and has led to numerous discoveries. In regards to enedyiene chemistry, new antitumor agents have been generated, along with different methods for the synthesis of these compounds. Even though this experimentation has been investigated for the past 30 years, only a few enediynes have been FDA approved and are used to treat a few diseases such as myeloid leukemia.[40,41] In this thesis, Sonogashira coupling and photochemical activation of the Bergman cyclization reaction will be used to synthesize aromatic enediynes and subsequently test their biological activity. In Chapter Two, the synthetic methodology of these compounds will be discussed along with the reasoning behind each procedure. Chapter Three will consist of an interpretation of the biological activity displayed by these enediynes, once they have been tested between certain cell lines. Chapter Four will describe a separate study of direct arylation of heteroaromatic arenes with benzene.

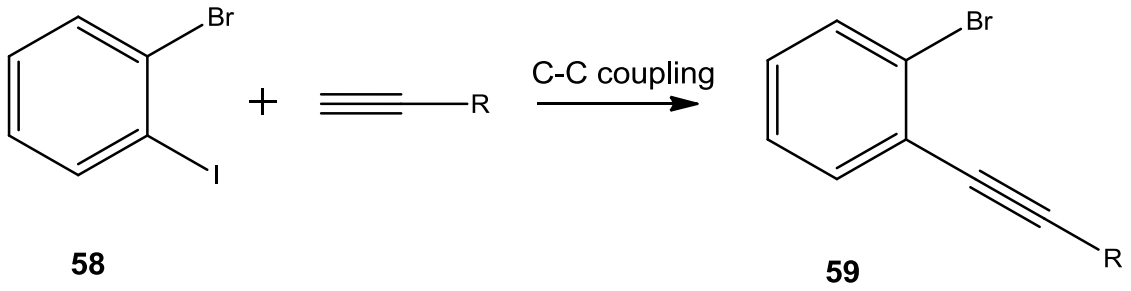
CHAPTER TWO: A STUDY OF ENEDIYNE SYNTHESIS

2.1 Introduction

In Chapter One, a review of enediyne chemistry was presented along with the synthetic methodology used for their syntheses, and a discussion of different approaches to triggering the Bergman cyclization reaction. It has been noted that past methodology which relied on the non-specific enzymatic triggering mechanism of the cyclization reaction is lacking in a number of ways and there has been greater success implementing photochemical triggering via UV light. Herein, methodology towards the synthesis of enediynes designed to undergo the Bergman cyclization reaction photochemically *in vivo* will be discussed, along with the rationale behind the production of each compound.

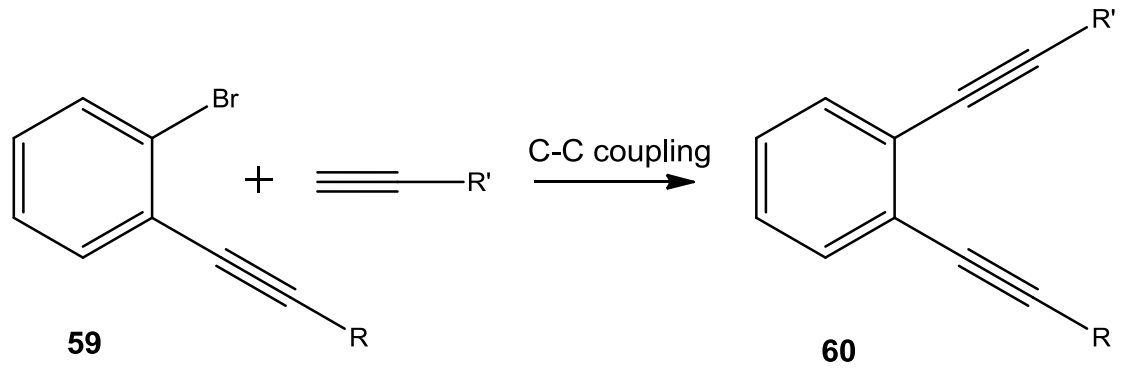
In this chapter, the initial stages of various methodologies to synthesize enediynes are presented. As mentioned previously, in order to undergo the Bergman cyclization reaction each compound must consist of two alkyne substituents. Therefore, starting the Sonogashira coupling reaction with an arene that consists of two heteroatoms will make this possible. The enediynes can then be designed specifically to include favourable substituents that would be promising candidates for cyclization. The first step of this methodology incorporates a Sonogashira coupling reaction between an alkyne and 2-bromoiodobenzene (Scheme 15).

Scheme 15



Due to the presence of the iodide heteroatom, the reactivity at C-1 is much higher than the reactivity at C-2. As a result of the reactivity differences between iodides and bromides, the reaction can be stopped after one coupling has taken place, compound **59** can then be isolated and undergo another Sonogashira reaction with a second alkyne (Scheme 16). Due to each coupling reaction taking place individually, different alkynes containing different substituents can be coupled to test the reactivity of the final enediyne.

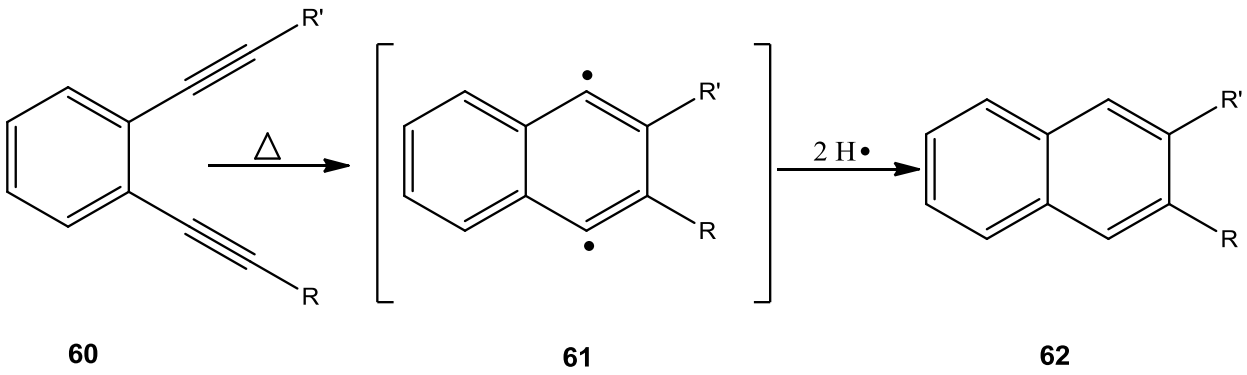
Scheme 16



After a second Sonogashira coupling reaction has been completed, the 1,5-diyne-3-ene desired structure is produced (**60**). It can then be tested under the photochemical Bergman

cyclization reaction conditions in hopes that the biradical intermediate will be generated, and therefore result in the desired cell death (Scheme 17) *in vivo*. Due to the fact that the substituents can be chosen in the initial stages of the Sonogashira coupling reactions, the reactivity of the enediynes can be manipulated so that the best outcome can be observed, that is cell death under photochemical conditions only. As previously mentioned, the Bergman Cyclization can take place under various conditions due to its ability to cyclize under thermal and photochemical reaction pathways. Therefore, the R and R' substituents on the second benzene ring of the final product are determined by the substitution displayed on the enediyne, provided by the two alkynes.

Scheme 17



60 **61** **62**

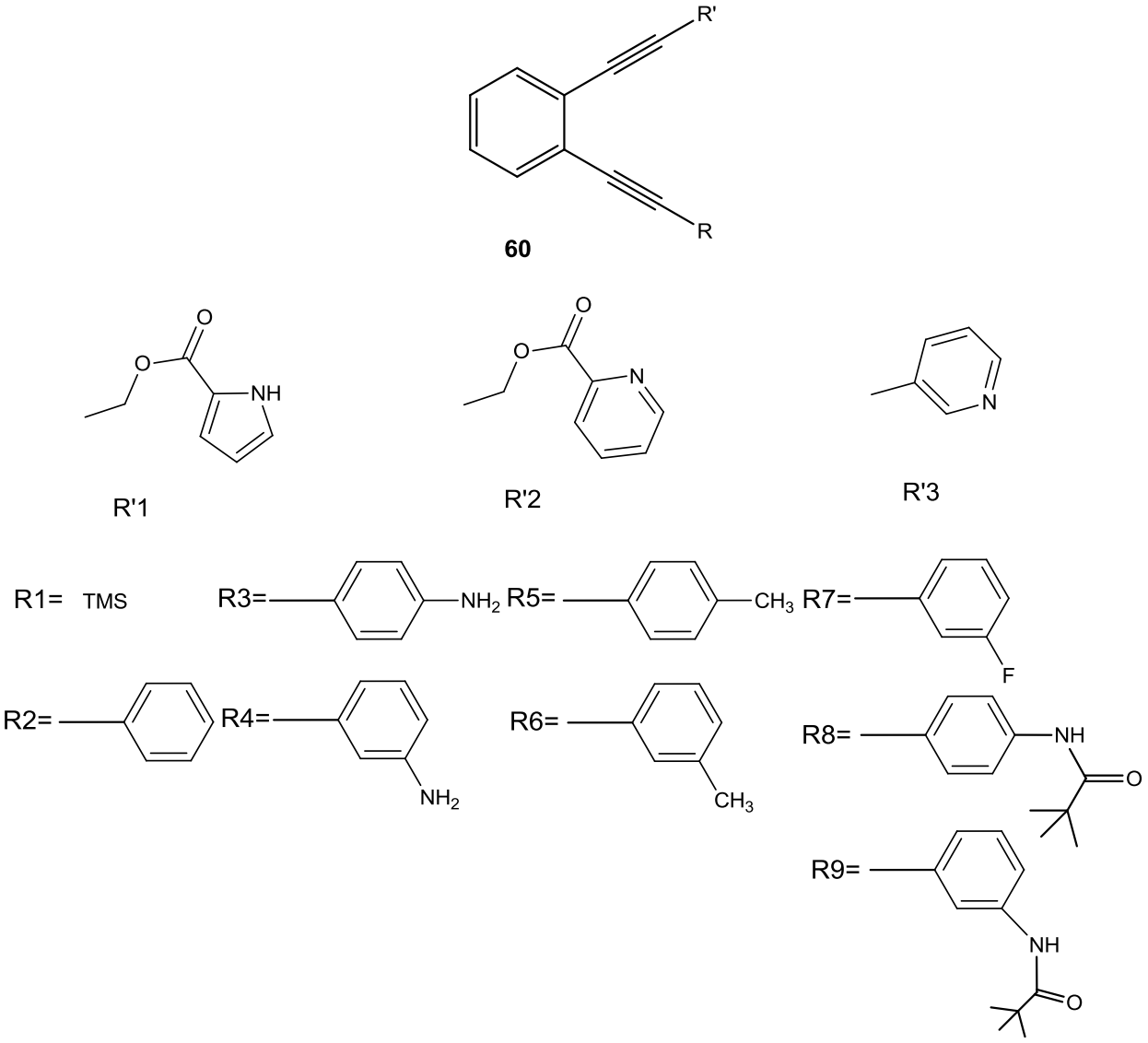
Herein, a synthetic strategy will be discussed that illustrates the production of enediynes that will be able to undergo Sonogashira coupling and photochemical Bergman cyclization *in vivo*. These enediynes have been specifically designed to be thermally stable and biocompatible in stable cells, along with having the ability to form the biradical intermediate only after application of UV light. The functionalities of the substituents have been selected to display different characteristics such as electron withdrawing and donating abilities, so that different

reactivities can be observed. The viability of select cell lines, in the presence of these synthetically designed enediynes, after being triggered selectively, will be discussed in Chapter Three.

2.2 Designing Enediynes

The research goal for this project is to design enediynes that can be synthesized via Sonogashira coupling and can cyclize photochemically through the Bergman cyclization reaction. It is believed that the molecules synthesized in this thesis will have specific features that give them the desired reactivity to increase cell death (Figure 4). They have been designed to adsorb light at wavelengths that should cause less damage directly on the cells and only increase cell death in selective locations due to their strong chromophoric characteristics. They have molecular features such as pyrrole rings that allow importation into the cells, which can be found in hemoglobin, bioavailable molecules that have the capability to easily go into cells.

Figure 4: Enediynes Designed to Increase Cell Death



It has been decided that the first alkyne to undergo the Sonogashira coupling reaction and perhaps the easiest, are the R substituents. Due to the fact that most of these enediynes contain a benzene substituent, they can be easily modified. By changing between electron-donating and electron-withdrawing substituents it is hoped that a trend will emerge that leads to enhanced reactivity and decreased cytotoxicity to healthy cells. Some substituents chosen, such as **R3** and **R4**, have the ability to hydrogen bond with the DNA backbone and could help aid in cellular

interactions. Other substituents chosen have lone pairs of electrons that should display a longer wavelength of absorption, thereby lowering the wavelength of light energy required.

As for the second Sonogashira coupling reaction, the three alkynes chosen were (2-propynol) pyrrole-2-carboxylate (**R'1**), 2-propynol pyridine-3-carboxylate (**R'2**), and 3-ethynylpyridine (**R'3**). Each alkyne contains nitrogen in the different sized ring systems, since there has been great success reported in the literature with nitrogen containing compounds. Two of the compounds contain an ester linkage that should aid in DNA cleavage and breakup the direct chromophore of the two rings. In turn, they can absorb light at a longer wavelength and require less energy for cyclization, which would be less harmful to the cells. The pyridine containing alkyne lacks this ester linkage, and therefore should display a long chromophore. Herein, the compounds that were synthesized via this methodology will be presented and discussed. There will also be a discussion of molecules that we could not successfully prepare.

To rationalize the hypothesis that these specifically designed enediynes will undergo the Bergman cyclization reaction, the theoretical cd distances were calculated for all compounds. These results can be observed in Table 1. It can be noted that the distances fall outside the critical ranges determined by Nicolaou and Schreiner by approximately 0.1Å. However, as mentioned previously, if the separation of the ends is outside their ranges, the reaction must be driven by the addition of energy, thermal or photochemical. Therefore, it seems plausible based on the results in Table 1 that the enediynes should cyclize when irradiated by UV light.

Table 1: Theoretical Calculations for the cd Distance[a]

R	R'1	R'2	R'3
R1	4.060	4.069	4.117
R2	4.071	4.077	4.046
R3	4.074	4.071	4.049
R4	4.082	4.060	4.062
R5	4.071	4.061	4.049
R6	4.077	4.076	4.046
R7	4.070	4.069	4.056
R8	4.095	4.049	4.054
R9	4.025	3.936	4.094

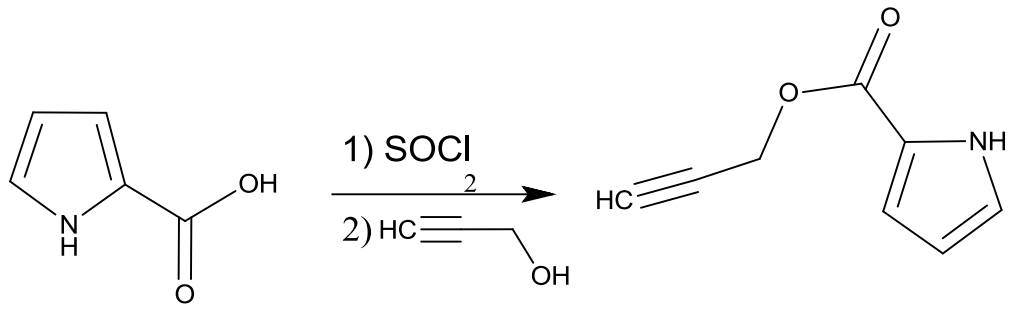
[a]: Calculations were done using MM2 minimization with Chem Draw

2.3 Synthetic Studies on Enediynes

2.3.1 Preparations for Sonogashira Coupling

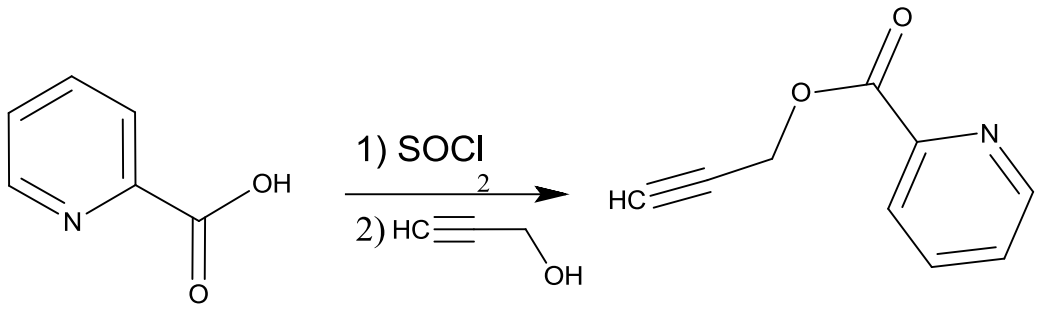
Before the Sonogashira coupling reactions could begin for the monocoupled and dicoupled products, certain starting materials needed to be synthesized. Fortunately, 3-ethynylpyridine was commercially available from Sigma-Aldrich, but **64** and **66** needed to be synthesized. These syntheses were done by performing a simple esterification reaction on pyrrole-2-carboxylate acid and nicotinic acid with propargyl alcohol (Scheme 18 and Scheme 19).

Scheme 18



63 **64**

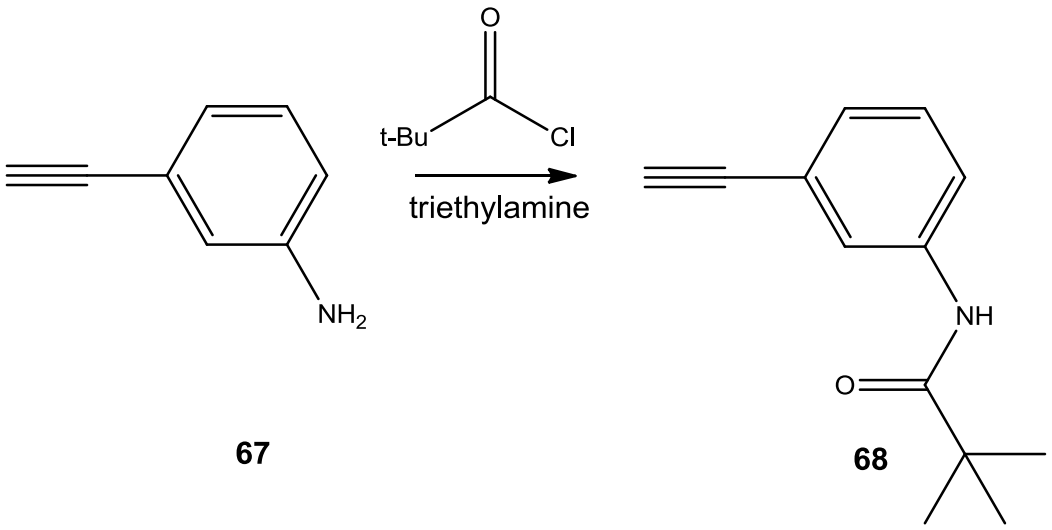
Scheme 19



65 **66**

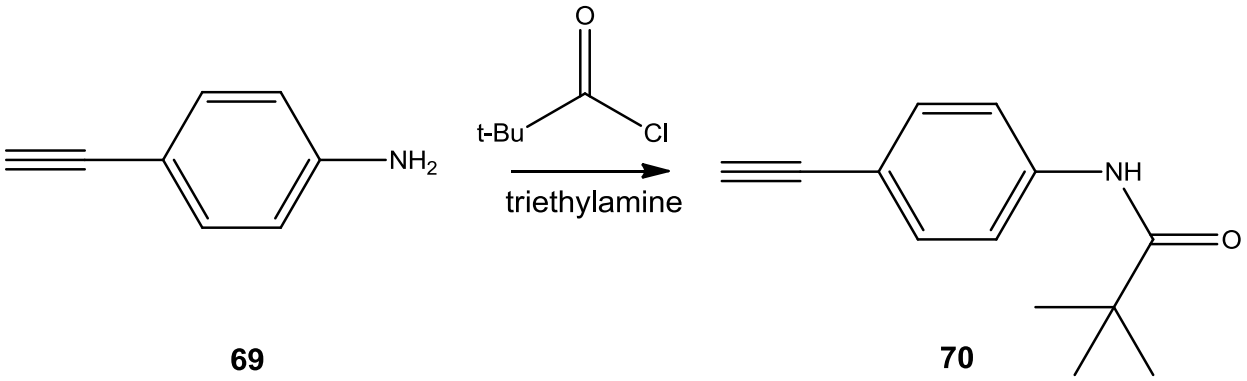
Substituents **R8** and **R9** were synthesized by adding a protecting group on the nitrogen through a pivaloylation reaction of 4-ethynylaniline and 3-ethynylaniline (Scheme 20 and Scheme 21). This reaction was performed using methodology presented by Stuart and Fagnou.[44] The exact reaction conditions for these reactions can be found in Chapter Five in the experimental section.

Scheme 20



67 **68**

Scheme 21



69 **70**

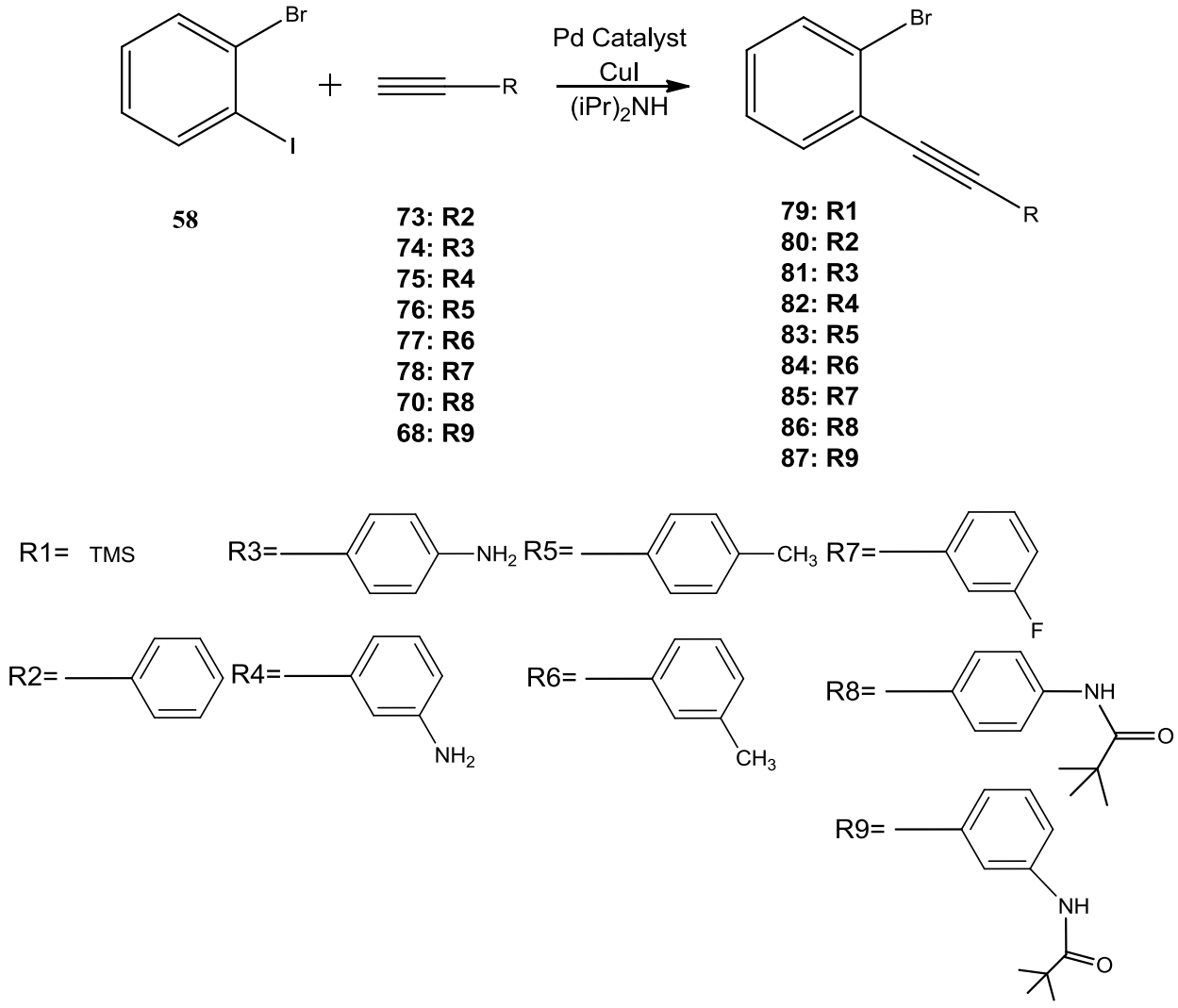
2.3.2 Formation of Monocoupled Products

In order to begin the Sonogashira methodology, the most effective catalyst must be chosen in order to maximize the alkyne coupling results. Based on successful previous work in the Gottardo lab for the preparation of enediynes, different Pd-catalysts, *tetrakis*(triphenylphosphine) palladium (0) [$Pd(PPh_3)_4$] (**71**) and palladium (II) acetate [$Pd(OAc)_2$] (**72**) were selected.[66] Both catalysts were chosen to determine if one would produce

higher yields than the other, as one is Pd^0 and the other Pd^{II}. It was anticipated that the reactions would be successful using the $Pd(OAc)_2$ catalyst, so that the unwanted triphenylphospine and triphenylphosphine oxide side products which are generated when using the Pd 0 catalyst could be eliminated, as they are tedious to separate from the desired product. The amine chosen was triethylamine since it was used most frequently in the literature. CuI was used as a co-catalyst and the reactions were run for 5 hours at 110^0C based on classic Sonogashira reaction conditions.

The first stage of the Sonogashira coupling reaction is to yield a product with one alkyne in the C-2 position (Scheme 22). Compound **79**, (2-bromophenylethynyl)trimethylsilane was purchased from Sigma-Aldrich, so the first step of the Sonogashira coupling was unnecessary.

Scheme 22



From previous work in the Gottardo lab, it was known that compound **80** can be successfully synthesized. It was then hypothesized that the rest of the monocoupled products could easily be formed through the same procedure. As previously mentioned, all of compounds **80-87** were formed with both catalysts for yield comparisons and the results are tabulated in Table 1. Although it seems that the $Pd(PPh_3)_4$ catalyst produced higher yields for most compounds, $Pd(OAc)_2$ was used to make more starting materials. This was decided due to the fact that the only major by-products formed by these reactions were triphenylphosphine and

triphenylphosphine oxide, which could be eliminated by simply using the palladium (II) acetate catalyst. However, both reaction pathways led to the final product after purification through column chromatography, which removed any unwanted compounds. Once the products were purified, gas chromatography-mass spectrometry (GCMS) analyses were run to make sure the separation was successful and the desired product was obtained.

Table 2: Percent Yields of Monocoupled Products

Catalyst	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**87**
$Pd(PPh_3)_4$ (**63**)	56%	43%	66%	75%	57%	56%	12%	14.5%
$Pd(OAc)_2$ (**64**)	84%	20%	30%	32%	54%	11%	ND	ND

After all nine monocoupled products were synthesized; it was possible to begin the second stage of the Sonogashira coupling reaction. The formation of the enediyne proved to be the most challenging and time consuming step in all of the enediyne synthetic process.

2.3.3 Formation of Enediynes

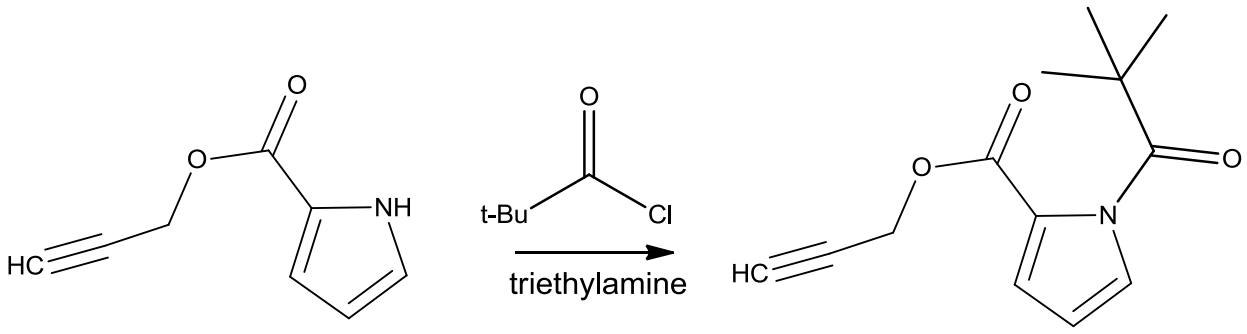
After the monocoupling products were produced, the methodology for coupling the second alkyne needed to be determined (Scheme 16). Considering the monocoupled product was produced quite easily with reasonable yields in most instances, using the same methodology seemed acceptable. The reactions were originally run at 110^{o}C for five hours using the same amounts of materials as described in Chapter Five. However, these reaction conditions did not

prove to be successful. Herein, the modifications tried on each reaction to produce the desired enediyne will be discussed along with the results obtained from each synthetic approach.

2.3.3.1 Formation of Enediynes with Ester Linkages

In previous years in the Gottardo lab, compounds **79** and **80** were reacted with **64** for approximately 24 hours and produced the desired enediynes using the same conditions as previously mentioned. However, when the reactions were tried for the work presented in this thesis, these results could not be produced. All compounds, **79-87,** were tried with **64** using different amounts of both catalysts and CuI, different temperatures ranging from 0^{o}C-140^{o}C and different lengths of reactions including running up to 72 hours. Each reaction seemed to only produce the unwanted by-products from the $Pd(PPh_3)_4$ when used, and unreacted monocoupled starting material. In most cases, (2-propynol) pyrrole-2-carboxylate could not be found as unreacted starting material in the final product. After numerous failed attempts, a different method to achieve the second coupling was attempted. It was thought that the N-H group on the pyrrole was interfering in the coupling process via hydrogen bonding and that the reaction might be more successful with a protecting group. A pivaloylation reaction was run on **64**, (Scheme 23) using the same conditions that had allowed us to successfully produce **68** and **70**.

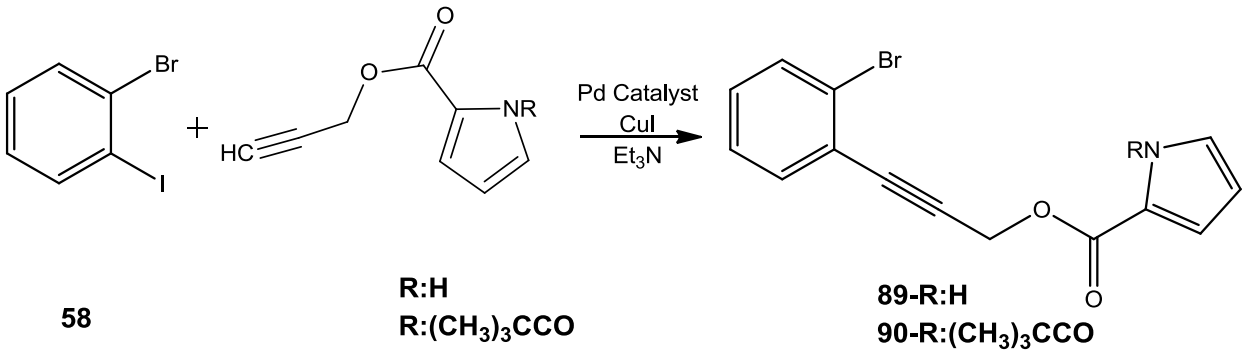
Scheme 23



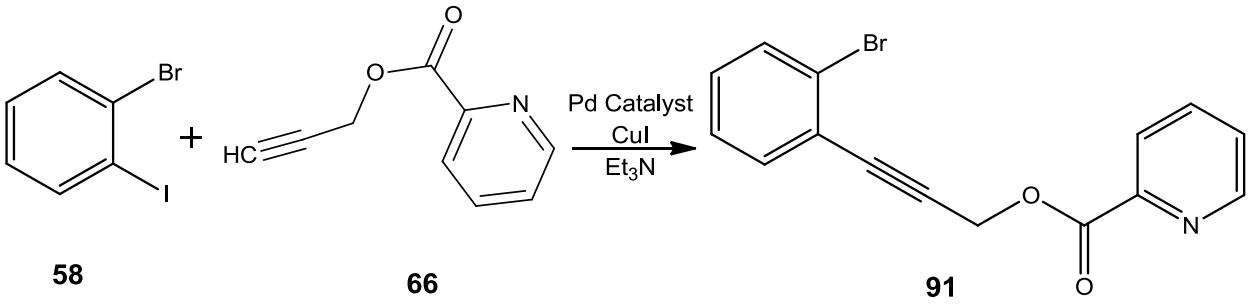
64 **88**

Reactions were run again with all nine monocoupled products with **88** using the different reaction conditions described previously, and the enediynes still would not form. Another option to be rid of the interfering hydrogen on **64** was to try coupling the monocoupled products with 2-propynol pyridine-3-carboxylate, **66**. Compounds **79-87** were reacted with **66** using the multiple conditions tried with **64** and yet again, no dicoupled products were observed and another approach needed to be taken into consideration. It was surmised that the aromatic groups on **64** and **66** were too bulky to be attached second, so coupling them first would eliminate this issue. Compounds **64, 66** and **88** were all reacted with 2-bromoiodobenzene using the same reaction conditions for the monocoupled products, except they were run for 48 hours (**89**) and 72 hours (**90**, **91**) (Scheme 24 and 25).

Scheme 24



Scheme 25



Compound **91** seemed to form in just trace amount and no further attempts to characterize this compound were made, even after several experiments. Perhaps this compound could be produced if the reaction was run longer, but this theory could not be tested due to time constraints. The preparations of compounds **89** and **90** were successful and they were reacted with alkynes **79-87** in an attempt to obtain the desired enediyne product. Again, all reactions were run at different temperatures with different catalyst amounts and monitored over a 72 hour period to check the progression of the reactions. The reactions did not proceed efficiently and no product was formed. It was difficult to determine why the reactions were not successful but a few ideas have been considered. Due to these reactions being run at different times of the year, ambient humidity in the laboratory could be affecting the reaction conditions. Perhaps there was too much steric strain occurring in the ring system due to these alkyne groups being too bulky

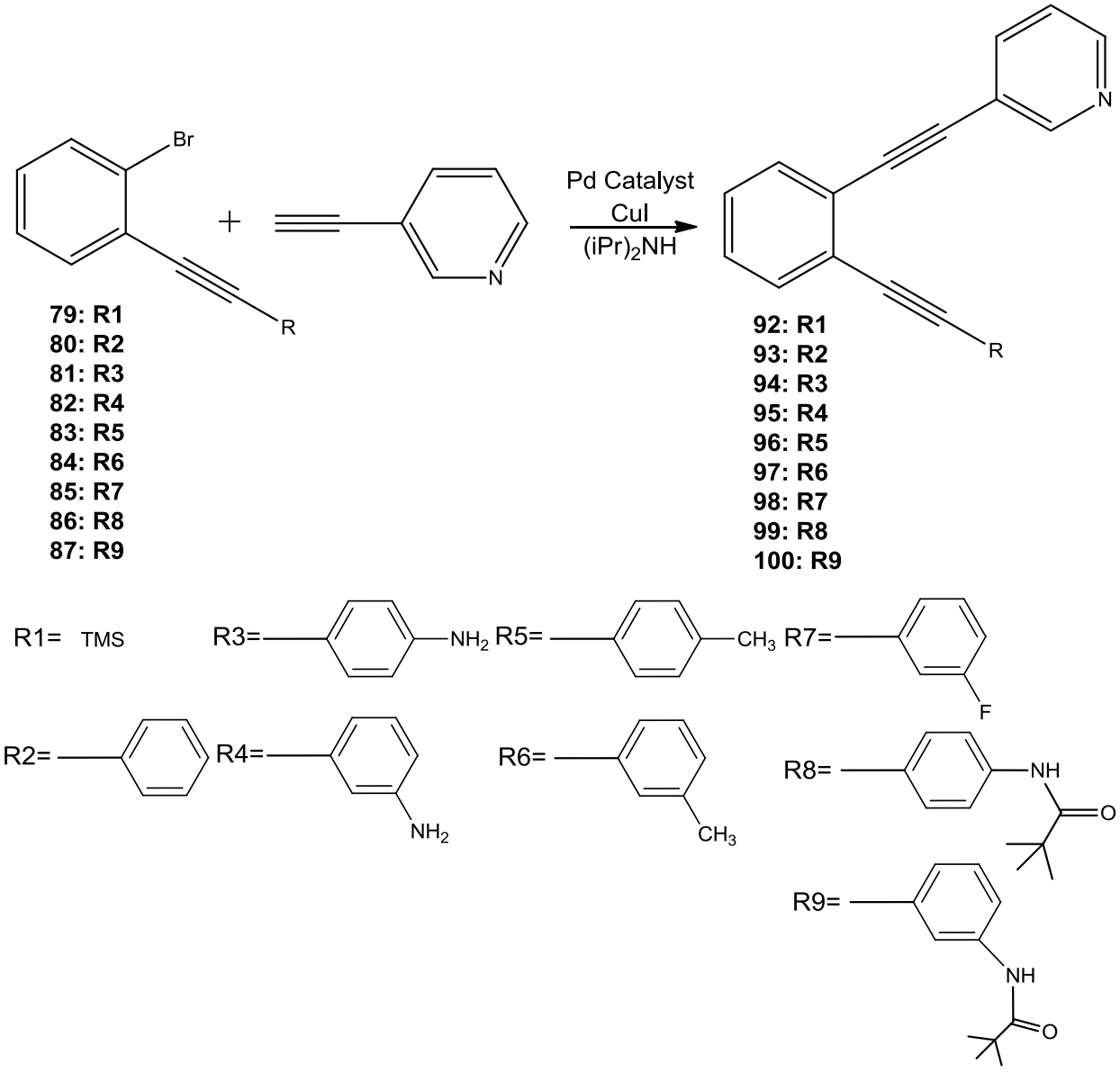
and these circumstances prevented activation. Also, there were some difficulties with the production of the $Pd(PPh_3)_4$ catalyst. At first, it seemed to oxidize and change colour over a few days which resulted in questions around its reactivity in the reactions. Although, once the procedure was perfected and the right colour of the catalyst was obtained, the desired products were still not formed.

2.3.3.2 Formation of Enediynes with 3-ethynylpyrridine

Fortunately, a more productive outcome was observed when reacting the monocoupled products with 3-ethynylpyridine. The reactions produced the desired enediynes rather simply after a few modifications of the original conditions were applied. The reactions were first tried for 5 hours at 110°C with 2% of the palladium catalysts. After GCMS analysis, it was found that minimal product was formed using $Pd(PPh_3)_4$, and the starting materials did not seem to react with the $Pd(OAc)_2$ catalyst. Due to these results, the reactions were run for 24 and 48 hours with $Pd(PPh_3)_4$ at 130°C until it was observed that 72 hours optimized production of product. However, the yields determined are still quite low. Our first successful reaction utilizing this methodology was done with 3-ethynylpyridine and (2-bromophenylethynyl)trimethylsilane, resulting in **92** (Scheme 26). Compound **92** can be separated from the reaction mixture via column chromatography very easily but in poor yields (11%). This was found acceptable as very little was needed for the cell testing. After changing the amount of catalyst to 4% and the temperature back down to 110°C, the other 8 enediynes were synthesized. Compound **93** was produced using 1-bromo-2-(2-phenylethynyl) benzene as the first alkyne, and it proved to be very easily isolated with a 13% yield. It was predicted that these two enediynes should show

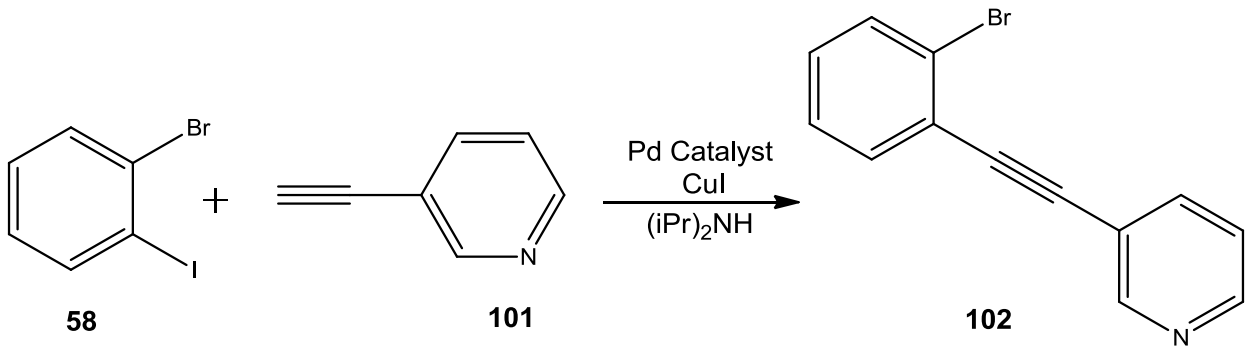
biological activity as similar molecules containing a phenyl substituent in place of the pyridine substituted had shown activity in experiments run previously in the Gottardo lab.

Scheme 26



Compounds **96**, **97** and **98** were also successfully synthesized and purified albeit in low yields of 12.5%, 12% and 25%, respectively. However, at first the same outcome did not seem to be observed for compounds **94** and **95**. These reactions were run with the exact same conditions, but the GCMS showed only trace product being formed with no reason to purify further. After many different tries changing the amount of catalyst, temperature and time of reaction, the enediynes still did not seem to form. It was hypothesized that as for **64**, the hydrogens on the NH_2 group were interfering with the reaction and were preventing the formation of the enediyne. In order to prevent this from happening, 3-ethynylpyridine was tried as the first alkyne to be coupled using the same conditions for the monocoupling reactions (Scheme 27).

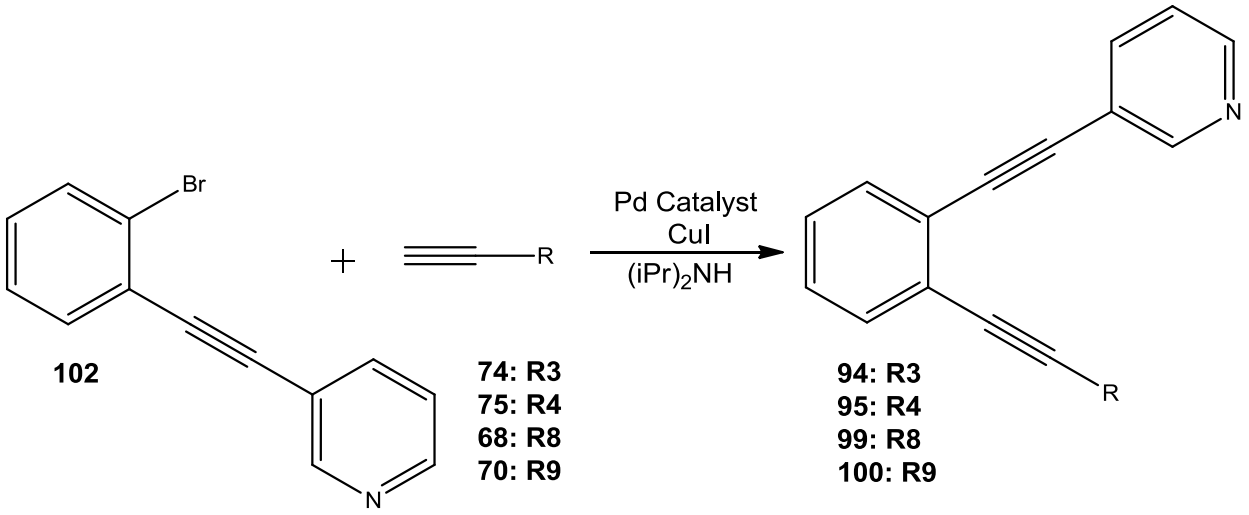
Scheme 27



58 **101** **102**

Compound **102** was synthesized easily when run for a 72 hour period, purified and yielded 13% of product. It was then reacted with 3-ethynylaniline (**75**) and 4-ethynylaniline (**74**) under various reaction conditions, but once again, minimal product seemed to form (Scheme 28).

Scheme 28



As with **64**, a pivaloylation reaction was run on both 3-ethynylaniline (Scheme 20) and 4-ethynylaniline (Scheme 21) to produce compounds **68** and **70** with 14.5% and 12% yields. These compounds were reacted with **102** via Scheme 28 and via Scheme 26 and yet again, the end result did not seem promising. This seemed very peculiar. All of the crude products formed from the different reaction schemes (Scheme 26 and 28) of each compound (**94, 95, 99, 100**) were characterized via NMR spectroscopy and IR spectroscopy. The data collected from the NMR and IR spectra are consistent with the formation of the desired products. What if the product was successfully synthesized through these reactions but the product was too sticky and could not be detected via GCMS? A direct injection ION TRAP was run on each compound and proved that the enediyne was in fact produced and in reasonable amounts. These compounds were then purified by column chromatography and produced the following yields: 12% (**94**), 14% (**95**), 4% (**99**), 9% (**100**). Compounds **99** and **100** were not deprotected so that they could also be tested for biological activity against the cells. Although only 9 of the 27 proposed enediynes were formed in this thesis, the question of whether or not these enediynes would undergo the Bergman

cyclization reaction and produce cell death in the desired regions was still investigated. These procedures and results are presented in Chapter Three.

2.4 Future Work

It has been demonstrated that the Sonogashira coupling reaction can be applied effectively to the synthesis of both homo- and heterodisubstitued enediynes. Additional work must be performed in order to extend the range of enediynes which can be produced. Other substituents should be synthesized to study reactivity *in vivo*. Due to successful previous work in the Gottardo lab, other reaction conditions could be tried to produce enediynes with **64, 66,** and **88**. Other commercially available alkynes could also be studied, with similar characteristics and functionality.

The thermal cyclization of these compounds could also be investigated. Due to their calculated c-d values, they should be able to undergo the Bergman cyclization *in vitro*, which would have been explored if more time was provided.

CHAPTER THREE: A STUDY OF THE BIOLOGICAL ACTIVITY OF PHOTOACTIVATED ENEDIYNES

3.1 Introduction

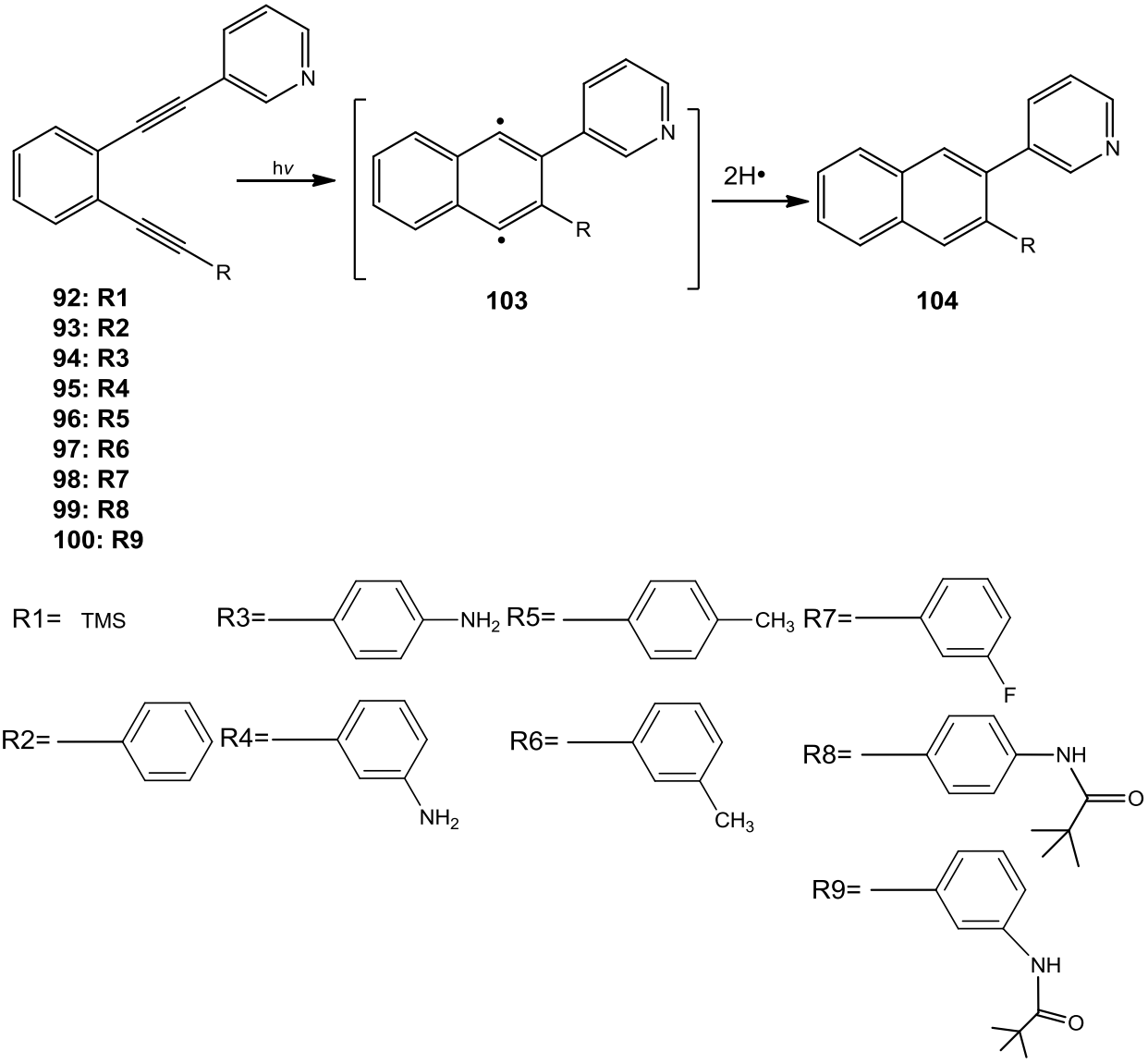
In Chapter One, a review of enediyne chemistry was presented along with synthetic methodology used for the synthesis of these compounds. As mentioned previously, enediynes are of interest due to their specific functionality that allows them to impart biological activity. This functionality is known to cyclize via a Bergman biradical intermediate which is then capable of cleaving DNA.[2] *In vivo,* it has been shown that this cyclization is triggered enzymatically, which results in reduced selectivity and increases cytotoxicity.[3] Numerous attempts have been reported in the literature to control the cyclization of enediynes through the use of photo-irradiation triggering of the enediynes.[26] However, after 30 years of investigating photoactivated enediynes, to date there are still only few drugs which have been approved by the FDA.

Since the cytotoxicity of enediynes is not limited to tumour cells or diseased cells, synthetic analogues have been proposed. The design of enediyne analogues requires that the pro-drug nature of the molecule be maintained to ensure that the "triggering" can occur selectively in the desired cells. These triggers can be internal or in this case, external to the cell. An external method of activation to cyclization, thermal cyclization, relies upon sufficient energy being applied to promote the conformational change. Due to cyclic systems having the advantage of shorter bond critical distances (Figure 1), cyclization can occur more readily at lower temperatures to relieve ring strain.[28] External triggers such as light can also be applied.[34] However, it is extremely important to ensure that the light sensitive molecules are stable *in vivo* until activation by a specific wavelength and that the wavelength of activation is not in itself

damaging to the cells. Triggering via photo-irradiation is advantageous as it can be selectively applied to specific treatment areas, although this could be limiting as not all tumours can be reached by photo-irradiation.

The approach presented in this thesis relies on the synthesis of enediynes, which are thermally stable under biological conditions, that cyclize to generate the reactive biradical after the application of light (Scheme 29). Due to enediynes having three different sections, optimal functionality, a delivering and triggering system, these compounds can be modified synthetically to control the behavior of the drug *in vivo* to make it a more selective molecule. Chapter Two presents the enediynes that have been synthesized and designed to have sufficient biocompatibility to enter the cells but also be stable within the cell environment until activation. Herein, the anti-cancer activity of these compounds tested on two different cell lines will be presented and discussed.

Scheme 29



3.2 Cell Culture and Testing

The nine enediyne compounds synthesized in this thesis were tested on two types of cells, cancerous and non-cancerous cell lines. They were first tested against human alveolar type II-like epithelial A549 cancer cells. These cells are squamous in nature and are responsible for the diffusion of certain substances such as water and electrolytes, across the alveoli of the human

lungs. They also have the ability to synthesize lecithin and contain a high percentage of desaturated fatty acids, which are utilized by the cytidine-diphospho-choline pathway and are important for the maintenance of membrane phospholipids in cells.[67] This cell line was chosen due to its cancerous characteristic and its ability to absorb light at the desired wavelength. The other cell line chosen was the non-cancer H9c2 cardiomyocytes. This myoblast cell line was derived from embryonic rat heart[68] and was chosen so that a comparison of non-cancerous and cancerous cell lines could be established. Both cells were readily available and proved to be good candidates with enediyne testing in the Gottardo lab previously.

The experiments were performed using serum-free media. Before the testing could take place, each cell line needed to be thawed, grown and split so that the required amount of cells could be obtained. Once each line was fully grown and prepared, they were seeded into sterile flat-bottom 96-well plates at 10 000 cells/well and grown to 80% confluence overnight. In the meantime, the enediynes were added to the serum-free media so that it could dissolve and be taken up by the cells. However, this was not the case. The enediynes would not dissolve directly into the media. Previously in the Gottardo lab, the enediynes were dissolved in a 1% DMSO/ethanol solution and then added to the media, but when this was tried they did not stay dissolved in the media. Adding up to 1mL of DMSO was tried and still the drug candidate came out of solution. This was very problematic as if the cells did not dissolve in the media, they could not be tested. After much research, it was decided that Tween 80 may be the best solution to this solubility issue.[69] Each enediyne was mixed with 200 μL of Tween 80 and after sitting overnight and stirring with a sterile micro spatula and vortex, the compounds fully dissolved in the media.

After 24 hours of incubation of the cells, the media was removed and replaced with control, control and Tween 80, or the enediyne containing media. Each enediyne was tested at

two different concentrations (7 and 15 μM final concentration) with three wells per concentration in two plates. After 3 hours and 50 minutes of pre-incubation of the A549 or H9c2 cells with enediynes, one plate containing the drug were activated with a 10 minute or 6.5 minute exposure to 302 nm UV light, and were then incubated for a further 16 hours. The length of irradiation was chosen to ensure that the cells did not overheat. It was found by previous monitoring of temperature that longer time frames resulted in temperature increase that could be harmful to the cells. Control cells with and without Tween 80 were also exposed or left in the incubator with the other non-exposed plate. Each enediyne was characterized via UV spectrometry (Chapter Five), and it was found that the absorbance's ranged from 230-328nm. Due to this observation, treating the cells at 302 nm seemed appropriate.

After the 16 hour incubation period was complete, the culture media (150μL) was replaced with fresh media containing 10% yellow MTT (Thiazolyl Blue Tetrazolium Bromide) reagent and then incubated for an additional four hours. The culture media was then aspirated and 50 μL of dimethylsulfoxide was added per well to solubilize the formazan crystals, and the absorbance of each well was measured.

3.3 Anti-cancer Activity Results

The absorbances were measured spectrophotometrically at a wavelength of 490 nm (650 nm background correction wavelength), 8 watts, 115 V, ~60 Hz and 0.7 Amps using a PowerWave XS Microplate Spectrophotometer (BioTek, Winooski, VT, USA). Cell death of challenged cells was assessed relative to control cells. The results for each compound **92-100** (Scheme 26) are reported in Table 3 and 4. The original report of absorbance readings from the BioTek microplate reader can be found in Appendix I and Appendix II.

Table 3: Cell Death of human alveolar type II-like epithelial A549 cells in the Presence of Enediyne

Compound	Concentration (μm)	% Cell Death	
		No UV Exposure	UV Exposure
92	7	83.10	81.31
	15	70.77	77.24
93	7	81.78	74.80
	15	61.44	78.46
94	7	84.33	82.53
	15	78.80	79.27
95	7	83.9	81.71
	15	77.54	70.74
96	7	85.20	86.18
	15	90.25	86.58
97	7	86.44	78.86
	15	72.88	77.65
98	7	85.20	92.28
	15	89.00	89.80
99	7	92.80	89.03
	15	91.53	89.03
100	7	90.68	87.40
	15	87.70	79.67
Control	0		~0

Table 4: Cell Death of H9c2 cells in the Presence of Enediyne

Compound	Concentration (μm)	% Cell Death	
		No UV Exposure	UV Exposure
92	7	90.77	76.89
	15	68.12	72.86
93	7	82.23	75.88
	15	79.62	70.61
94	7	79.45	75.13
	15	58.54	51.51
95	7	87.28	75.38
	15	82.06	68.60
96	7	81.19	76.38
	15	82.41	75.13
97	7	84.67	72.86
	15	84.67	76.64
98	7	85.37	73.37
	15	83.28	73.37
99	7	92.16	84.44
	15	ND	ND
100	7	87.29	81.41
	15	86.59	79.65
Control	0		30.66

3.4 Cytotoxic Properties of Activated Enediynes- Cell Death

As previously mentioned, the compounds tested were designed specifically to have the ability to undergo the Bergman cyclization reaction with the addition of light, and increase cell death due to their desired reactivity characteristics. The enediynes have electron-donating and electron-withdrawing substituents that should enhance reactivity and decrease cytotoxicity and others have the ability to hydrogen bond with the DNA backbone to aid in cellular interactions. Compounds with substituents with lone pairs of electrons should also technically display a longer wavelength of absorption and lower the wavelength of light energy required. Each of these compounds were tested at two different concentrations to see if the effect of the drug would increase or increase cell death at higher or lower concentrations. Each enediyne was tested in three wells per concentration with UV or without so that an average could be taken for better results. Due to previous trial and error experimentation in the Gottardo lab, the cells were irradiated at 10min (A549) and 6.5min (H9c2). They found that when the H9c2 cells were irradiated for longer than 6.5 minutes, cell death was increased significantly. However, if time was permitted, other times would have been tried and altered to optimize results for these specific drugs.

Control groups consisted of the cells challenged alone or with UV light alone. They were also tested this way with Tween 80, but were not included in Table 3 & 4 as the results seemed to suggest complete cell death, which should not be the case. Exposure of A549 cells to UV light resulted in slightly less than 0% cell death. This also seemed strange as the value should not be less than 0%, so experimental error has been observed here as well. When compounds **92-100** were tested with and without ultraviolet light, cell death was greatly increased compared to the control. However, the standard values (no UV exposure) are very similar which raises the

question of how effective the drug was. Also, unfavorable patterns have been observed for most compounds. For compounds **92-94, 97,** the cell death decreased after the application of light for 7μM, which should not be. This was also found for both concentrations for compounds **95, 99-100**. In some cases, cell death was found to be greater for 7μM as opposed to 15μM, which is very unusual as the high concentration should be displaying a higher increase in cell death. However, compound **92** enhanced the cytotoxicity to 77.24% (15μM), **93** to 78.46% (15μM), **94** to 79.27% (15μM), **96** to 86.18% (7μM), **97** to 77.65% (15μM), **98** to 92.28% (7μM) and 89.80% (15μM). This suggests that these compounds do have the ability to increase cell death under photochemical conditions. However, a DNA extraction was not completed and it is unknown of whether or not the drug killed the cells or if other factors affected the results.

The exposure protocol of the H9c2 cardiomyocytes to the di-alkynes and/or UV light was the same as the one used for the experiment with the A549 cells, except for the length of time irradiated. The exposure of H9c2 cells to UV light resulted in 30.66% cell death. However, a lot of the same patterns observed for A549 cells were observed here as Tween 80 was also used. All compounds except for **92**(15μM) displayed a smaller amount of cell death when UV light was applied when compared to the standard UV exposure values which should again not be the case. Only compound **92**(15μM) displayed an enhancement of cytotoxicity to 72.86%. Compound **99** could not be run at 15μM as there was not enough of the compound available to be tested.

The standard no UV exposure values observed should be approximately 0% since the drug should be able to survive in the cells until the application of light. This determines that either the enediyne is killing the cells or the Tween 80 is. Based on previous results obtained in the Gottardo lab, in which enediynes only increased cell death by less than 85% it can be extrapolated that the enediyne should not be harming the cells in this way; it appears that there

may have been problems with the Tween 80. The Tween could be potentially blocking the light, making the Bergman cyclization reaction impossible to proceed. It could be that the Tween is toxic to the cells or perhaps it is destroying the drug before addition to the cells. A bad batch of Tween 80 could have also been used, which may have been harmful to the cells. In no way should the cells be growing after the application of light, which was displayed in most cases and displayed great experimental error.

3.5 Future Work

In theory, the drugs designed in this thesis should display marginal to good inhibition when tested between the cell lines used. However, this was not necessarily the result obtained from this research. Additional work must be performed in order to optimize these results. Most importantly, improving the solubility of the compounds to ensure testing without Tween would be desirable. Attempts should be made to see if the compounds could be dissolved in less than 200μL of Tween 80 to see if the amount of Tween 80 accelerated cell death. Other methods of dissolving the compounds should also be tried, such as other solvents or surfactants. Changes in the enediyne structure synthetically could also be taken into consideration to make them more soluble and stable for the cells. A further decrease of energy of light needed by the extending chromophore would be ideal. Also, the amount of time needed for irradiation could be optimized by increasing or decreasing the time through trial and error. Perhaps testing other potential cell lines for activity would be better for this type of drugs and other choices in media could improve solubility issues.

CHAPTER FOUR: A STUDY OF DIRECT ARYLATION OF HETEROAROMATIC ARENES WITH BENZENE

4.1 Introduction

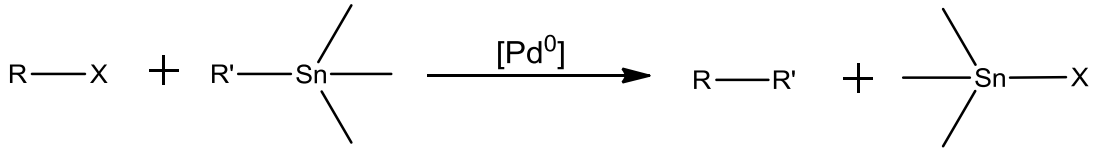
There are numerous reports in the literature that describe methods for the synthesis of biaryl compounds using favourable cross-coupling reaction conditions. The scientific and commercial value of biaryl molecules is demonstrated through their ability to be building blocks in liquid crystals, electron transport devices, medicinal compounds and in light emitting diodes. These types of compounds are also found in natural products such as alkaloids and are also present in commercial dyes. Applications of these compounds towards organic semi-conductors and conductors rely on the physical properties of polyaromatic compounds. [48] As a result, the formation of biaryl compounds has been closely studied over the past 30 years due to the many applications these molecules can be part of, ranging from pharmaceutical to materials chemistry. Many different methods for biaryl compound synthesis have been established using combinations of preactivated, preactivated and unactivated, and just unactivated starting materials. In these reactions, the aromatic compounds usually require different arene coupling patterns which are activated towards reaction with the help of a palladium catalyst and a copper oxidant. The most widely used syntheses of cross-coupling reactions are the Suzuki and the Stille reaction both of which couple aryl organometallics and aryl halides.[43]

4.1.2 Direct Arylation of Preactivated Arenes

The Stille reaction involves the coupling of organostannanes with organic electrophiles (Scheme 30).[49] The work of coupling organostannanes was originally discovered in 1976-1977

but became a standard method in 1978 with the work of Stille and his research team. This reaction seemed quite effective due to its tolerance towards most functional groups resulting in the transformations of highly functionalized molecules. Numerous ring systems bearing sensitive functional groups have been successfully constructed with the application of the Stille reaction. Although the Stille reaction has established itself as one of the most general and most selective palladium-catalyzed cross-coupling reactions, the synthesis has many disadvantages which result in this reaction not being optimal. The transmetalation step is a complex reaction and can operate with different Pd (II) species in solution. Depending upon the catalyst, reagents and the solvent, the species can change uncontrollably. The toxicity and low polarity of the tin compounds also make them unfavourable starting materials. The solvent and additives can also influence the nucleophilicity of the tin reagent. Recent studies carried out on these reactions used electrophiles that were unreactive with conventional ligands. These reactions used complexes with sterically demanding ligands as catalysts.[49] A different view of transmetalation to palladium has emerged using organotin compounds and other nucleophilic organometallic compounds. These observations have suggested that by using other organometallic reagents with less electropositive metals, similar dual pathways may be found in these reactions under the appropriate conditions. The Suzuki coupling is an example of a cross-coupling reaction that has successfully used less electropositive metals.[50]

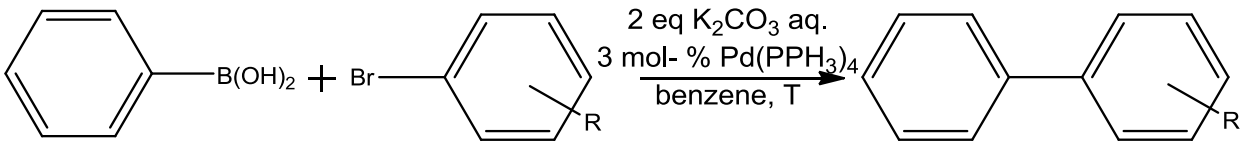
Scheme 30



105 **106** **107** **108**

The 2010 Nobel Prize winner A. Suzuki worked with palladium-catalyzed cross-coupling reactions between different types of organoboron compounds and various organic electrophiles which included triflates and halides in the presence of a base (Scheme 31). There are many advantages to this type of cross-coupling which include mild reaction conditions, high product yields and high regio- and stereoselectivity, good water stability, readily available reactants, tolerance of a broad range of functional groups and the usage of a small amount of catalyst.[50] Also, the reaction is non-toxic, environmentally friendly and can be completed in a one-pot synthesis. In 1981, the first method to cross-couple aryl boranes with haloarenes was reported which proceeded even under heterogeneous conditions. Numerous modifications have been made to the reaction conditions since 1981 such as using CsF or Bu_4NF as bases. Originally, phosphine based palladium catalysts were used due to their stability under prolonged heating conditions until it was discovered that using palladium catalysts without a phosphine ligand such as $Pd(OAc)_2$, also achieved high coupling reaction rates.[50] The Pd(0) catalyst that is used in these basic conditions is generated *in situ* from either the $Pd(OAc)_2$ or $PdCl_2$.[51] Although the Suzuki reaction is environmentally friendly, constant advances in Suzuki coupling have been made throughout the years to obtain even greener reaction conditions and improve the yields.

Scheme 31



108 **109** **110**

In 2008, Evangelos Aktoudianakis and his research team at the University of Toronto designed a Suzuki reaction with "greenness" to it. This method of cross-coupling uses water as the sole solvent in the reaction and an inexpensive palladium on carbon active catalyst. These reaction conditions also do not follow the purification protocol as seen in previous Suzuki reactions, other than the recrystallization of the solid product. This research team worked with phenylboronic acid and 4-iodophenol to produce 4-phenylphenol. These reaction conditions are quite favourable due to the use of water because it is a non-flammable solvent that has no toxicity issues and is the most cost efficient solvent on an industrial scale.[51]

The Suzuki reaction has been one of the most popular research investigations in academic institutions over the past few years. However, there have been many synthetic problems and disadvantages with the need for preactivation of starting materials. Due to using preactivated arenes, several synthetic steps are required which can generate waste from reagents and can become quite costly. Also, the solvents and the purification protocol used in these reactions can produce undesired by-products such as homo-coupling.[48] Therefore; direct C-H arylation of unactivated arenes with aryl halides is an attractive approach for biaryl bond formation.

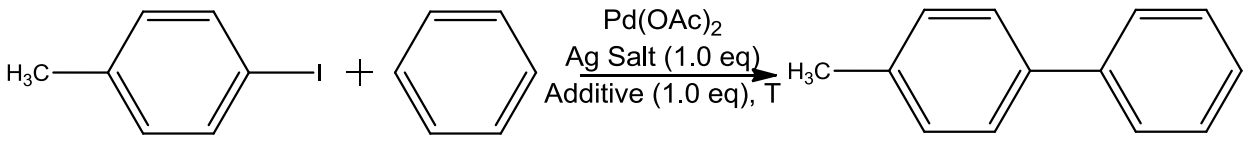
4.1.3 Direct Arylation of Preactivated and Unactivated Arenes

The most dominant class of C-H bond arylation reactions involves the use of a directing group to direct a transition metal into a C-H bond or to promote transition metal oxidative addition of a C-X bond (X=halogen). There are also many examples of single-activated cross-coupling reactions, in which the proposed mechanisms of the reaction rely on the catalyst to insert into the activated aryl group first, usually an aryl halide. Once the Ar-M-X species has been formed, the unactivated insertion will occur. Numerous reactions have been reported

illustrating the success of this type of methodology working with indoles[53,54,55], thiophenes and furans[56] with aryl halides. Researchers have reported cross-coupling reactions with a directing group such as the intermolecular reactions of o-bromophenols[57] or the use of a removable 2-pyridylsulfinyl group.[58] It has been reported that use of the N-directing group of benzo[*h*]quinoline allowed better understanding of the selectivity of the reaction and the reductive elimination that produces the C-C bond formation.[59] A different group of researchers also reported successful directing ability of o-phenylcarbamates which selectively produce mono- and diarylated products.[60]

In 2011, Wenkun Hong and his research team worked to develop a new method to improve the efficiency and generality of this reaction. They worked with preactivated and unactivated arenes to produce a Pd-catalyzed direct arylation without the use of directing groups. This reaction is environmentally friendly and produces high-yields. The substrates used were benzene and 4-methyl iodobenzene with the catalytic assistance of $Pd(OAc)_2$ and benzene as the solvent. Salts were also added to the reaction to be used as oxidizing agents, such as $AgNO_3$, AgOAc, AgO_2CCF_3, and $Cu(OAc)_2$. They reported that the use of additives was less effective and produced no yield. To eliminate homo-coupling of biphenyl, the reaction conditions were modified to 10 mol % $Pd(OAc)_s$ and 1.0 equivalent $AgNO_3$ at 110^{o}C (Scheme 32).[52] In the presence of multiple potentially reactive groups at higher temperatures, this methodology shows great functional group tolerance and high selectivity.

Scheme 32



108 **109** **110**

Although these synthetic approaches are successful, they unfortunately still use one preactivated starting material which can be avoided via other synthetic approaches. The costly preactivated starting materials, added reactions, undesirable side and or by products, and generated waste from reagents, solvents and purification will be decreased by inducing cross-coupling of unactivated arenes.

4.1.4 Direct Arylation of Unactivated Arenes

In order to successfully form a C-C bond in the absence of a directing group, the ability to control the reactivity of each species is crucial. The reactivity of the two unactivated starting materials and the mechanism of this reaction must be understood in order to result in the desired products. Selectivity of heteroarenes with different electron densities that produce different C-H bond strengths, and reacting simple arenes with different substituents have been reported.[60,61] In 2007, Fagnou et al. reported success in coupling unactivated indole and pyrrole to benzene with the use of a palladium catalyst [$Pd(TFA)_2$] and silver/copper oxidants.[43,44] 3-Nitropyridine, and cesium pivalate were also used as additives in the reactions. They discovered that when copper (II) acetate and the additives were included in the reaction, arylation was directed into the C-3 position producing no homo-coupling and relatively high yields. This demonstrates complete

inversion in catalyst selectively occurring at the crucial arene metallation step of the catalytic cycle under mild conditions. Remarkably, it was also found that when implementing the oxidant silver (I) acetate with the additives, coupling to the C-2 position was observed with higher regioselectivity. Unfortunately, undesirable benzene homo-coupling was observed but was only considered as a minimal loss. The reactions were run with different amounts of palladium catalyst and were most successful with using only 2 mol%. Different amounts of catalyst were tested and it has been reported that when the amount of catalyst is increased, the selectivity is decreased even though the reaction is proven to be very successful. Even though there is much reactivity observed for indoles and pyrroles with electron-donating groups, no clear trends have been observed with respect to benzene. Due to many successes reported in the literature using unactivated arenes, further investigation will be discussed on furan coupling to benzene in this thesis, along with the reactivity and selectivity of these compounds.

4.2 Synthetic Studies on Unsubstituted and Substituted Furans

The reaction conditions for the furan reactions with benzene were designed to match Fagnou's previously reported synthesis with few modifications. Pivalic acid was chosen as an additive due to Fagnou's reported results in 2006, where they discovered that PivOH (Pivalic acid) works as a cocatalyst providing a key component anion in the C-H bond cleaving. This anion lowers the dissociation energy of the C-H thereby operating as a catalytic proton transporter from benzene to a base.[62] Benzoquinone was used as an additive instead of 3-nitropyridine based on results from Sanford who reported success with using a substoichiometric quantity of 1,4-benzoquinone in order to promote the oxidative coupling reaction.[63] Due to benzoquinone's unique structure, it has the ability to influence regioselectivity with certain

arenes by acting as a ligand during the selectivity-determining step.[64] This conclusion has depended on the production of a C-H activated palladium benzoquinone intermediate which then initiates a second C-H activation of a substituted benzene. Herein, similar energy demands have been observed for this reaction sequence.

Due to cross-coupling reaction mechanisms not being fully understood, Palladium II and Palladium 0 catalysts were chosen in this thesis to see if there is any correlation observed to the coupling product and the catalyst. Two of the three palladium catalysts chosen for these reactions are $Pd(TFA)_2$ and $Pd(OAc)_2$ which provide both monomeric and dimeric Pd^{II} species. The third catalyst chosen, $Pd(PPh_3)_4$, is a Pd^0 bearing a phosphine instead of an oxygen ligand. The reactions were run using the same oxidants, silver and copper (II) acetate, as Fagnou reported successful C2/C3 selectivity with in 2007. Four unactivated aromatics were investigated: Furan, 2-Furaldehyde, 2-methylfuran and 2,3-benzofuran. These compounds were chosen based on their different electron withdrawing and electron donating characteristics. Herein, we report the reactivity and selectivity observed for these compounds when different substituents, additives, catalysts and oxidants are implemented in various combinations.

4.3 Results

The reaction conditions chosen for each arene and the observed outcome are presented in Table 5. Although the reported yields for each reaction are quite low, there are some interesting patterns detected with regards to reactivity and selectivity.

Scheme 33

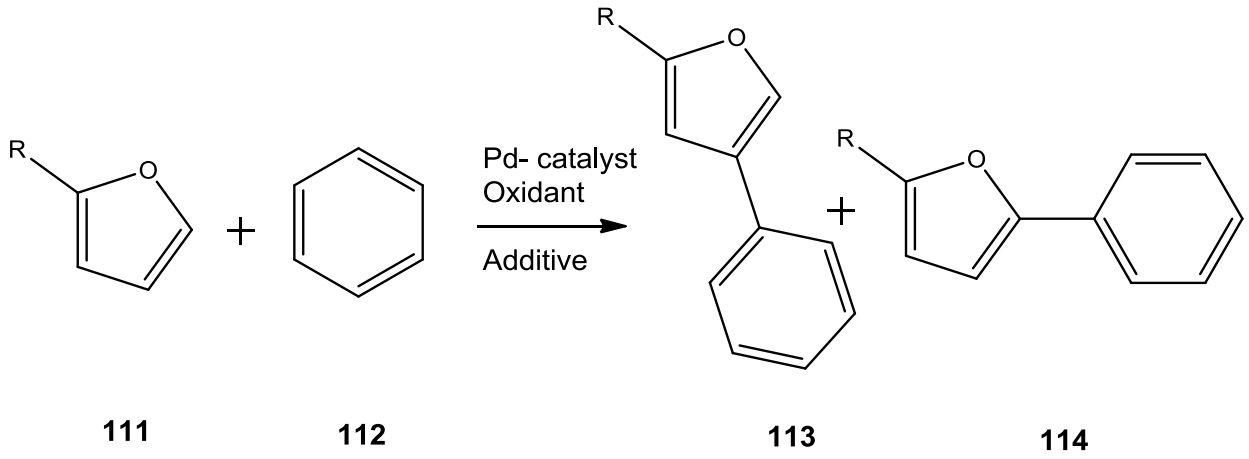


Table 5: Carbon-carbon Bond forming results for O-Containing Heteroatoms

Entry	**R**	**Pd-catalyst**	**Oxidant**	**Additive**	**Yield % (113:114)[a]**	**Conditions[b]**
1	H	$Pd(OAc)_2$	AgOAc	None	<1% (14:86)	A
2	H	$Pd(OAc)_2$	$Cu(OAc)_2$	None	<1% (44:56)	A
3	H	$Pd(OAc)_2$	AgOAc	Benzoquinone	NR	B
4	H	$Pd(OAc)_2$	$Cu(OAc)_2$	Benzoquinone	NR	B
5	H	$Pd(TFA)_2$	AgOAc	None	<1% (1<100)	A
6	H	$Pd(TFA)_2$	$Cu(OAc)_2$	None	NR	A

7	H	$Pd(TFA)_2$	AgOAc	Benzoquinone	<1% (30:70)	B
8	H	$Pd(TFA)_2$	$Cu(OAc)_2$	Benzoquinone	NR	B
9	H	$Pd(PPh_3)_4$	AgOAc	None	1.4% (53:47)	A
10	H	$Pd(PPh_3)_4$	$Cu(OAc)_2$	None	<1% (80:20)	A
11	H	$Pd(PPh_3)_4$	AgOAc	Benzoquinone	<1% (41:59)	B
12	H	$Pd(PPh_3)_4$	$Cu(OAc)_2$	Benzoquinone	<1% (67:33)	B
13	CH_3	$Pd(OAc)_2$	AgOAc	None	5.1% (100>1)	A
14	CH_3	$Pd(OAc)_2$	$Cu(OAc)_2$	None	1% (100>1)	A
15	CH_3	$Pd(OAc)_2$	AgOAc	Benzoquinone	1% (100>1)	B
16	CH_3	$Pd(OAc)_2$	$Cu(OAc)_2$	Benzoquinone	NR	B
17	CH_3	$Pd(TFA)_2$	AgOAc	None	1%	A
18	CH_3	$Pd(TFA)_2$	$Cu(OAc)_2$	None	1%	A
19	CH_3	$Pd(TFA)_2$	AgOAc	Benzoquinone	1.4%	B
20	CH_3	$Pd(TFA)_2$	$Cu(OAc)_2$	Benzoquinone	NR	B
21	CH_3	$Pd(PPh_3)_4$	AgOAc	None	3.6%	A

22	CH_3	$Pd(PPh_3)_4$	$Cu(OAc)_2$	None	1%	A
23	CH_3	$Pd(PPh_3)_4$	AgOAc	Benzoquinone	<1%	B
24	CH_3	$Pd(PPh_3)_4$	$Cu(OAc)_2$	Benzoquinone	<1%	B
25	CHO	$Pd(OAc)_2$	AgOAc	None	5.8% (100>1)	A
26	CHO	$Pd(OAc)_2$	$Cu(OAc)_2$	None	<1% (73:27)	A
27	CHO	$Pd(OAc)_2$	AgOAc	Benzoquinone	30.8% (100>1)	B
28	CHO	$Pd(OAc)_2$	$Cu(OAc)_2$	Benzoquinone	7.5% (100>1)	B
29	CHO	$Pd(TFA)_2$	AgOAc	None	21.8% (79:21)	A
30	CHO	$Pd(TFA)_2$	$Cu(OAc)_2$	None	1.4% (84:16)	A
31	CHO	$Pd(TFA)_2$	AgOAc	Benzoquinone	22.9% (98:2)	B
32	CHO	$Pd(TFA)_2$	$Cu(OAc)_2$	Benzoquinone	6.1% (100>1)	B
33	CHO	$Pd(PPh_3)_4$	AgOAc	None	9.4% (93:7)	A
34	CHO	$Pd(PPh_3)_4$	$Cu(OAc)_2$	None	<1% (100>1)	A

35	CHO	$Pd(PPh_3)_4$	AgOAc	Benzoquinone	33.3% (97:3)	B
36	CHO	$Pd(PPh_3)_4$	$Cu(OAc)_2$	Benzoquinone	9.5% (100>1)	B
37	CHO	$Pd(OAc)_2$	AgOAc	Pyridine	<1%	C
38	CHO	$Pd(OAc)_2$	$Cu(OAc)_2$	Pyridine	5.9%	C
39	CHO	Pd(OAc)2	AgOAc	None	5.8%	A + PivOH (6 eq)
40	CHO	Pd(OAc)2	AgOAc	Benzoquinone	19% (1:15)	D
41	CHO	Pd(OAc)2	$Cu(OAc)_2$	Benzoquinone	17%	E
42	CHO	$Pd(PPh_3)_4$	AgOAc	Benzoquinone	12%	F
43	CHO	Pd(OAc)2	AgOAc	Benzoquinone	17%	F
44	$-(CH=CH)_2-$	$Pd(OAc)_2$	AgOAc	None	<1%	A
45	$-(CH=CH)_2-$	$Pd(OAc)_2$	$Cu(OAc)_2$	None	<1%	A
46	$-(CH=CH)_2-$	$Pd(OAc)_2$	AgOAc	Benzoquinone	<1%	B
47	$-(CH=CH)_2-$	$Pd(OAc)_2$	$Cu(OAc)_2$	Benzoquinone	1.4%	B
48	$-(CH=CH)_2-$	$Pd(TFA)_2$	AgOAc	None	2.5%	A
49	$-(CH=CH)_2-$	$Pd(TFA)_2$	$Cu(OAc)_2$	None	<1%	A
50	$-(CH=CH)_2-$	$Pd(TFA)_2$	AgOAc	Benzoquinone	1.3%	B
51	$-(CH=CH)_2-$	$Pd(TFA)_2$	$Cu(OAc)_2$	Benzoquinone	<1%	B
52	$-(CH=CH)_2-$	$Pd(PPh_3)_4$	AgOAc	None	2.0%	A

53	-(CH=CH)$_2$-	Pd(PPh$_3$)$_4$	Cu(OAc)$_2$	None	<1%	A
54	-(CH=CH)$_2$-	Pd(PPh$_3$)$_4$	AgOAc	Benzoquinone	1.0%	B
55	-(CH=CH)$_2$-	Pd(PPh$_3$)$_4$	Cu(OAc)$_2$	Benzoquinone	<1%	B
56	-(CH=CH)$_2$-	Pd(TFA)$_2$	AgOAc	Pyridine	<1%	C
57	-(CH=CH)$_2$-	Pd(TFA)$_2$	Cu(OAc)$_2$	Pyridine	1.1%	C

[a] Where no ratio of **113:114** is given, only compound **113** was detected by GC; [b] Conditions : **A**. Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110 ^{o}C for five hours; **B**. Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol), benzoquinone (2.0 mmol, 216 mg), pivaloyl acid (6.0 mmol, 613 mg) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110 ^{o}C for five hours; **C**. . Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol), 4-methyl-3-nitropyridine (2.0 mmol, 216 mg), cesium pivalate (mmol, mg), pivaloyl acid (6.0 mmol, 613 mg) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110 ^{o}C for five hours; **D**. Heteroaromatic compound (1.0 mmol), oxidant (6.0 mmol), benzoquinone (2.0 mmol, 216 mg), pivaloyl acid (6.0 mmol, 613 mg) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110 ^{o}C for five hours; **E**. Heteroaromatic compound (1.0 mmol), oxidant (1.5 mmol), benzoquinone (2.0 mmol, 216 mg), pivaloyl acid (6.0 mmol, 613 mg) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110 ^{o}C for five hours; **F.** Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol), benzoquinone (2.0 mmol, 216 mg), pivaloyl acid (6.0 mmol, 613 mg) and Pd-catalyst (0.05

mmol) were combined in a quartz tube containing dry benzene (2.7 mL), sealed and irradiated via UV light for five hours.

4.4 Discussion

Although similar synthetic conditions were followed, much lower yields were observed for furan and benzene coupling compared to Fagnou's research. For the unsubstituted furan reactions (**Entries 1-12**), less than a 5% yield was observed even when the additives were included. It can also be seen that in the presence of benzoquinone, the reactivity is decreased, opposite to Sanford's observations. For all of the reactions with a regioselectivity of greater than 2:1, 3-phenylfuran was produced with the AgOAc oxidant while the 2-phenylfuran was produced with the $Cu(OAc)_2$ oxidant, which is the opposite of Fagnou's reported selectivity patterns. However, there is no observation of dimerization production of bifuran or biphenyl and there is no evidence of diphenyl substitution occurring on the furan.

As for the substituted furan compounds, low yields were also observed with or without the use of additives. The overall reactivity seems to decrease when the electron-donating 2-methylfuran (**Entries 13-24**) is reacted with the copper oxidant and benzoquinone promoter. There seems to be no production of the 4-phenylsubstitution and the product of 3-phenylsubstitution is formed with both silver and copper oxidant with less than 5% yield. With the 2,3-benzofuran (**Entries 44-57**), the Cu^{2+} adversely affects the reactivity and little is gained by the presence of a promoter. Again, no production of the 4-phenylsubstitution is observed, and the 3-phenylsubstitution is produced with no more than 3% yield unlike previously reported literature.[65] The 2-furaldehyde reactions (**Entries 25-43**) were the only ones that showed reaction progress. Up to a 33% yield was observed for the reaction containing the copper oxidant,

benzoquinone and the $Pd(PPh_3)_4$ catalyst. It is also observed that again, the copper oxidant seemed to decrease the reactivity. 2-Furaldehyde was the only substituted furan that showed evidence of 2- regioisomers. As displayed in Table 5, it can be observed that the C-3 substitution is favoured in the presence of the copper oxidant but it is also favoured in the presence of AgOAc. Reactions were run with 2,3-benzofuran and 2-furaldehyde using nitropyridine instead of benzoqunione to see if Fagnou's exact conditions would produce more success (**Entries 37,38,56,57**). Unfortunately, the same observations were made for these compounds as when they were run with benzoquinone.

Two separate reactions were run for 2-furaldehyde changing the amount of the oxidant used to see if the same results would be observed. The reactions were run with $Pd(OAc)_2$, PivOH, benzoquinone and either half or double the amount of the oxidant. Both of these reactions were run to determine if the amount of the cation, copper versus silver, (6.0 mmol vs. 1.5 mmol) is the aspect of the oxidant change that governs changes in the overall reactivity or the C2/C3 selectivity. The reaction run with 6.0 mmol of AgOAc (**Entry 40**) seemed to result in products at the C-3 position and minimal amounts of C-2 products with a 19% yield and the reaction run with 1.5 mmol of $Cu(OAc)_2$ (**Entry 41**) produced only the C-3 coupled product with a 17% yield. Also, the two reactions which produced the best yield were run again, using the same reaction conditions except they were reacted via UV light. 2-Furaldehyde was chosen to run with benzoquinone, silver acetate and with $Pd(PPh_3)_4$ (**Entry 42**) and $Pd(OAc)_2$ (**Entry 43**). **Entry 42** produced the C-3 product along with trace amounts of the C-2 coupled product (12% yield), whereas **Entry 43** only formed the C-3 product with a 17% yield.

Overall good selectivity was seen for the 2-furaldehyde reactions, but not for the other substituted and unsubstituted furans. In most cases only one product was formed with a greater

than 3:1 ratio. In light of the poor yields observed in the absence of a promoter or with the use of benzoquinone promoter, other reactivity patterns were observed opposite to those reported in the literature. In order to determine if the reactivity demonstrated by indole and pyrrole was intrinsically tied to the N-containing promoter; a few reactions were run with 4-methyl-3-nitropyridine instead of benzoquinone. As mentioned earlier, the yields did not improve nor did any ratios of product change. On the other hand, it was noticed that the copper oxidant was more successful in the coupling reactions than the silver oxidant. Recently, successful arylation has been reported without the use of promoters although PivOH additive is included. These reaction conditions were also applied as well as no PivOH, as shown in Table 5. However, no further improvement on the outcome of these reactions was observed. When the oxidant amount was increased to 6.0 mmol (**Entry 40**), yields were decreased by at least half compared to the original reaction conditions (**Entry 27**) and both compounds were produced. On the other hand, when the oxidant was decreased to 1.5 mmol (**Entry 41**), yields improved compared to the original reaction (**Entry 28**) and still only one product was formed. Both of these observations lead to suspicion of whether or not the amount of oxidant affects the yields and C2/C3 selectivity. It is hard to conclude if this is the case as only two reactions were run, thus future experimentation could lead to explanation of the relationship between reactivity/selectivity and oxidant. It is also interesting to note that **Entries 42** and **43** were successfully synthesized via UV light. Although yields were much lower than the original protocol (**Entries 35, 27**), the same product was obtained in each case. Similarly, this creates vast synthetic opportunity for direct arylation on these compounds.

Even though the yields were quite low, some product was formed for each starting heteroaromatic species. This proves that C-H activation arising from a palladium intermediate

did occur and could perhaps be optimized by altering some conditions. The fact that the palladium catalyst is only being used at a 5% mole factor and the reactions are run for only five hours could potentially prevent a successful turnover. In an attempt to optimize these yields, some reactions were run for 24 hours at 110^{o}C (Table 5), and some improvements have been recognized. The electron- withdrawing furaldehyde arenes yield rose to 35% from 30%, while the rest of the compounds did not show much improvement. However, these reactions did maintain their high level of selectivity and no increase of homo-coupling products were detected.

4.4 Future Work

As mentioned previously, the fact that some product was obtained for each starting heteroaromatic implies that C-H activation did occur and therefore demonstrates that these reactions could be optimized in the future. The reaction conditions can be altered to optimize yields and selectivity. Reactions could be run at higher temperatures for longer periods of time, and the amount of palladium catalyst can be adjusted. The amount of oxidant used can also be increased or decreased based on the results found in this thesis when using either half or double the original amount. Since the most promising results were shown with an electron withdrawing reactant, different compounds could be tested using the same conditions that also have this characteristic. Lastly, reactions could be run with each heteroaromatic arene under UV light as somewhat successful results were found with the two reactions run on 2-furaldehyde.

CHAPTER FIVE: EXPERIMENTAL

5.1 Experimental

5.1.1 General Experimental Technique, Instrumentation and Materials

Analytical gas chromatography (GC) was performed on a Hewlett Packard 5890 equipped with a flame ionization detector (FID) using a 30 m by 0.25 mm DB-5HT capillary column of (5% phenyl)methylpolysiloxane. The carrier gas was helium or nitrogen with a flow rate of 2.0 mL/min and a column head pressure of 21 psi. The temperature program used was the following: initial temperature = 50^{o}C, initial time = 3 minutes, rate = 8^{o}C/minute, final temperature = 280^{o}C, final time = 2 minutes. Gas chromatography-mass spectrometry (GCMS) was performed on a Varian 460GC-300 MS/MS chromatogram equipped with a 30 m by 0.25 m Varian FActorFour VF-5 ms Capillary Column. The carrier gas used was helium with a linear velocity of 25.5 cm/s at 80^{o}C and a column head pressure of 11.5 psi. The temperature program used was the following:

Table 6: Temperature Program for Varian 460GC-300MS/MS

	Temperature (^{o}C)	Rate (^{o}C/min)	Hold (min)	Total (min)
1	45		2	2
2	155	8	0	15.75
3	275	50	11	29.15

The gas chromatograph was connected to a Varian 300 MS/MS which measured the mass of samples between 40 and 500 mass units with a resolution of 0.7 Da at 1250 Da/sec or 0.6 Da at 500 Da/sec and an ionizing potential of 70 eV at 200^{o}C and detector voltage of 1280V and accelerating voltage of constant @ 5kV. Mass spectral data are reported in ion counts and recorded in the following fashion: parent ion (relative intensity), *m/e* of significant fragments (relative intensity). The Ion Trap chromatograph used was a Bruker Amazon X Ion Trap with an ESI ionization, a -4500V capillary, -500V End Plate Offset and a 10psi, 5 l/min dry gas, 200^{o}C dry temp Nebulizer, infused @ 240µL/hour.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Varian AS500 using the ^{UNITY}INOVA NMR spectrometer system, VNMR 6.1C software and a switchable PFG NMR probe at room temperature (unless otherwise indicated). The solvent used was $CDCl_3$ unless otherwise stated. Chemical shifts are reported in parts per million (ppm) from an internal standard of tetramethylsilane (TMS). ^{13}C NMR spectra were also recorded using $CDCl_3$ as the solvent unless otherwise stated and TMS as the internal standard on the same spectrometer. The NMR data are reported as follows: chemical shift (for ^{1}H NMR signals: multiplicity, coupling constant in Hertz, integration).

Infrared (IR) spectra were measured on a Bruker IFS-66 Fourier transform infrared (FTIR) spectrometer with a resolution of 4 cm^{-1}. All spectra were determined using $CDCl_3$ unless otherwise noted, in the transmission mode using NaCl plates and are reported as wavenumbers. Ultraviolet spectra were measured using a Perkin-Elmer Lambda 11 spectrometer, in dichloromethane using a quartz cuvet, and are reported as wavelength of maximum absorption (in nm) with the corresponding molar absorptivity (ε).

A Varian Vista Pro Radial ICP-AES was used to detect trace amounts of palladium in select samples before cell testing was run. The Varian Vista Pro CCD simultaneous ECP-OES was used with a CETAC ASX-510 Auto sampler. Samples were dissolved in minimal amount of concentrated HCl and then diluted with deionized water. Low amounts of palladium were detected and reported in ppm and the samples were further purified before biological activity was tested.

All experiments were run under a positive pressure of nitrogen in flasks that were either flame or oven dried. Air and moisture sensitive reagents were transferred by syringe and introduced to the reaction flasks through rubber septa. Excess solvents were removed *in vacuo* at pressures obtained by a water aspirator drawing on a Buchi rotary evaporator. All compounds were stored at room temperature under atmospheric conditions or in the refrigerator unless noted otherwise. Diisopropylamine and benzene were distilled from phosphorus pentoxide. All other solvents were used as received.

Analytical thin layer chromatography (TLC) was performed on silica gel of 5-17 µm particle size, 60Å pore size, with a thickness of 250 µm, containing a 254 nm fluorescent indicator. The solvents used for chromatography are indicated in parentheses in the procedures and the relative concentrations are calculated by volume. Spots were viewed using ultraviolet light. Column chromatography was used to purify the reaction mixtures and was accomplished using 60-200 mesh grade silica gel and the solvent systems were determined by analytical TLC.

Tetrakis(triphenylphosphine)palladium(0)(**71**)[42] was synthesized using previously described methods. All other chemicals for which procedures are not listed were purchased from Aldrich.

4.2 Preparations

4.2.1 Experimental for Chapter Two

2-Propynol pyrrole-2-carboxylate **64**

To a flame dried, three-neck round bottom flask (25 mL), pyyrole-2-carboxylic acid (222 mg, 2.0 mmol) was added with a magnetic stir bar. Thionyl chloride (0.18 mL, 297 mg, 2.5 mmol) was added dropwise over 30 minutes. The solution was then heated by reflux at 80°C until it began to darken (approximately 30 minutes). The solution was then cooled in an ice bath to 0°C. Propargyl alcohol (0.15 mL, 140 mg, 2.5 mmol) was added dropwise slowly; maintaining the reaction temperature below 10°C. The reaction was then refluxed for 30 minutes at 75°C. Ice water (10 mL) was added to the mixture and extracted with diethyl ether (10 mL x 3). The combined organic extract was washed with saturated $NaHCO_3$ (10 mL x 1) and dried with $MgSO_4$. The mixture was then filtered through Celite by gravity and excess solvent removed *in vacuo.* A 30% yield of 2-propynol pyrrole-2-carboxylate was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.51.

UV: λ_{max} = 266 (ϵ = 141).

IR($CDCl_3$): 3456, 3307, 2924, 2253, 1703, 1553, 1417, 1309, 1161, 908, 731.

^{1}H NMR($CDCl_3$): δ 9.24(s, 1H, NH), 7.21-6.98(m, 2H, Ar-H), 6.28(dd, J= 2.5 Hz,2.5 Hz, 1H, Ar-H), 4.87 (d, J= 2.5 Hz, 2H, CH_2), 2.49 (t, J= 2.0 Hz, 2.0 Hz, 1H, CH).

^{13}C NMR($CDCl_3$): δ 160.3, 123.6, 116.4, 110.9(2C), 77.9, 75.1, 51.9.

MS: 149(47), 104(25), 94(100), 66(21), mass calcd for $C_8H_7O_2N$ *m/z* 149.0570, obsd *m/z* 149.1.

2-Propynol N-trimethylacetylpyrrole-2-carboxylate **88**

2-Propynol pyyrole-2-carboxylate (2.533g, 0.017mmol) and DMAP (0.2197g) were added to a 100 mL round bottom flask and purged with N_2. Dry dichloromethane (30 mL) was then added via syringe producing a brown solution. Triethylamine (3.6 mL) was then added and the solution was cooled to 0°C in an ice bath. Pivaloyl chloride (2.5 mL) was added over a 1 minute period and the solution was stirred at 0°C for approximately 10 minutes. The mixture was then warmed to room temperature and allowed to stir overnight. The following day the solvent was removed *in vacuo* and partitioned between 50 mL of saturated NH_4Cl and 50 mL of diethylether. The organic layers were washed with saturated sodium chloride (40 mL) and the aqueous layers were back extracted with diethyl ether. The solvent was removed *in vacuo* and the product purified by column chromatography (15% diethyl ether/ hexanes). A 26% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes):0.51.

UV: λ_{max} = 265 (ϵ = 28).

IR($CDCl_3$): 3457, 3307, 2950, 2253, 1701, 1553, 1450, 1435, 1416, 1310, 1163, 1127, 1108, 991, 908, 734, 734, 649.

^{1}H NMR($CDCl_3$): δ 7.03(d, J=33 Hz, 2H, Ar-H), 6.25 (s, 1H, Ar-H), 4.83(d, J=17.5 Hz, 2H, CH_2), 2.50(s, 1H, CH), 1.35(s, 9H, 3 CH_3).

^{13}C NMR($CDCl_3$): δ 182.32, 159.64, 125.15, 118.92, 116.33, 110.24, 75.19, 74.96, 52.09, 43.45, 27.72, 27.59, 26.98.

MS: 233(9), 176(92), 120(100), 57(33), mass calcd for $C_{13}H_{15}O_3N$ *m/z* 233.1151, obsd *m/z* 233.3.

2-Propynol pyridine-3-carboxylate **66**

To a flame dried, three-neck round bottom flask (25 mL), Nicotinic acid (246 mg, 2.0 mmol) was added with a magnetic stir bar. Thionyl chloride (0.18 mL, 297 mg, 2.5 mmol) was added dropwise over 30 minutes. The solution was then heated by reflux at 80^{o}C until it began to darken (approximately 30 minutes). The solution was then cooled in an ice bath to 0^{o}C. Propargyl alcohol (0.15 mL, 140 mg, 2.5 mmol) was added dropwise slowly; maintaining the reaction temperature below 10^{o}C. The reaction was then refluxed for 30 minutes at 75^{o}C. Ice water (10 mL) was added to the mixture and extracted with diethyl ether (10 mL x 3). The combined organic extract was washed with saturated $NaHCO_3$ (10 mL x 1) and dried with $MgSO_4$. The mixture was then filtered through Celite by gravity and excess solvent removed *in vacuo.* A 46% yield of 2-propynol pyridine-3 carboxylate was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.21.

UV: λ_{max} = 234 (ϵ = 14), 263 (14).

IR($CDCl_3$): 3307, 3154, 2952, 2253, 1731, 1592, 1421, 1371, 1277, 1110, 915, 746, 650.

^{1}H NMR($CDCl_3$): δ 9.27 (s, 1H, Ar-H), 8.82 (d, J= 4.0 Hz, 1-H, Ar-H), 8.34 (t, J= 2.0 Hz, 1H, Ar-H), 6.42 (m, 1H, Ar-H), 4.97 (d, J=2.5 Hz, 2H, CH_2), 2.56 (t, J= 2.5 Hz, 1H, CH).

^{13}C NMR($CDCl_3$): δ 164.5, 153.8, 151.1, 137.3, 137.2, 123.4, 77.2, 75.5, 52.8.

MS: 161(100), 106(97), 78(83), 51(70), mass calcd for $C_9H_6O_2N$ *m/z* 161.057, obsd *m/z* 161.1.

3-ethynyl-N-trimethylacetylaniline **68**

3-Ethynylaniline (1.56 mL, 0.017 mmol) and DMAP (0.2197g) were added to a 100mL round bottom flask and purged with N_2. Dry dichloromethane (30 mL) was then added via syringe producing a brown solution. Triethylamine (3. 6mL) was then added and the solution

was cooled to 0°C in an ice bath. Pivaloyl chloride (2.5 mL) was added over a 1 minute period and the solution was stirred at 0°C for approximately 10 minutes. The mixture was then warmed to room temperature and allowed to stir overnight. The following day the solvent was removed *in vacuo* and partitioned between 50 mL of saturated NH_4Cl and 50 mL of diethyl ether. The organic layers were washed with saturated sodium chloride (40 mL) and the aqueous layers were back extracted with diethyl ether. The solvent was removed *in vacuo* and the product purified by column chromatography (15% diethyl ether/ hexanes). A 75% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.61.

UV: λ_{max} = 236 (ϵ = 343).

IR($CDCl_3$): 3450, 3305, 3064, 2968, 2906, 2872, 2251, 1673, 1603, 1583, 1483, 1420, 1303, 908, 815, 733, 687, 650.

^{1}H NMR($CDCl_3$): δ 7.65(s, 1H, Ar-H), 7.59(d, J=4.5 Hz, 1H, Ar-H), 7.27(t, J=5 Hz, 1H, Ar-H), 7.23(d, J=6.5 Hz, 1H, Ar-H), 3.04(s, 1H, NH), 3.06(s, 1H, CH), 1.28(s, 9H, 3 CH_3's).

^{13}C NMR($CDCl_3$): δ 176.64, 138.05, 129.7, 127.0, 123.41, 122.0, 120.54, 83.14, 77.37, 39.65, 27.58(3C).

MS: 201(99), 117(97), 57(100), 41(43), 201(95), 117(81), 57(100), exact mass calcd for $C_{13}H_{15}ON$ *m/z* 201.1179, obsd *m/z* 201.1.

4-Ethynyl-N-trimethylacetylaniline **70**

4-Ethynylaniline (1.99g, 0.017 mmol) and DMAP (0.2197g) were added to a 100 mL round bottom flask and purged with N_2. Dry dichloromethane (30 mL) was then added via syringe producing a brown solution. Triethylamine (3.6 mL) was then added and the solution

was cooled to 0°C in an ice bath. Pivaloyl chloride (2.5 mL) was added over a 1 minute period and the solution was stirred at 0°C for approximately 10 minutes. The mixture was then warmed to room temperature and allowed to stir overnight. The following day the solvent was removed *in vacuo* and partitioned between 50 mL of saturated NH_4Cl and 50 mL of diethyl ether. The organic layers were washed with saturated sodium chloride (40 mL) and the aqueous layers were back extracted with diethyl ether. The solvent was removed *in vacuo* and the product purified by column chromatography (15% diethyl ether/ hexanes). A 45% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.58.

UV: λ_{max} = 269 (ϵ = 344).

IR($CDCl_3$): 3450, 3290, 3168, 3098, 2977, 2874, 2252, 2106, 1666, 1587, 1511, 1401, 1311, 1239, 1177, 908, 836, 732, 651.

^{1}H NMR($CDCl_3$): δ 7.51(d, J=8.5 Hz, 2H, Ar-H), 7.44(d, J=9 Hz, 2H, Ar-H), 3.04(s, 1H, NH), 1.73(s, 1H, CH), 1.27(s, 9H, 3CH_3's).

^{13}C NMR($CDCl_3$): δ 176.63, 138.48, 132.90(2C), 119.46(2C), 117.53, 83.38, 65.87, 39.73, 27.73, 27.61, 27.57, 26.50.

MS: 201(95), 117(81), 57(100), exact mass calcd for $C_{13}H_{15}ON$ *m/z* 201.1179, obsd *m/z* 201.1.

Tetrakis(triphenylphosphine)palladium(0) **71**

$PdCl_2$ (0.7236 g, 4.1 mmol) and PPh_3 (4.9090 g, 18.7 mmol) were mixed together in a round bottom flask (250 mL) with DMSO (55 mL). The contents were slowly heated using a heating mantle with a condenser over Ar. The solution turned bright yellow. The reaction continued to be heated for approximately 1 hour and 45 minutes until the precipitate fully

dissolved producing an orange transparent solution. The reaction was then removed from the heat and allowed to cool with vigorous stirring. When the yellow precipitate began to reform, hydrazine hydrate (1.32 mL, 1.36 g, 27.2 mmol) was added slowly via syringe. The precipitate went back into solution and the mixture turned dark brown during the addition. The precipitate then began to reform once again and resulted in a volumous yellow precipitate after further cooling. The contents were quickly filtered using a Buchner funnel and washed with absolute ethanol until the washing became colorless. It was then washed with diethyl ether (25 mL) and left to dry on vacuum for a few minutes. The catalyst was then stored under Ar/N_2 in the freezer. A percent yield of 92.4% was obtained.

The following compounds were synthesized using the general procedures described as either Method A or Method B.

Method A:

2-Bromoiodobenzene (0.13 mL, 283 mg, 1.0 mmol), $Pd(PPh_3)_4$ (23 mg, 0.02 mmol) and CuI (7.6 mg, 0.04 mmol) were combined in a heavy walled reaction vessel with dry diisopropylamine (5 mL). Alkyne (1.2 mmol) was then added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 5 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography.

Method B:

2-Bromoiodobenzene (0.13 mL, 283 mg, 1.0 mmol), $Pd(OAc)_2$ (4.49 mg, 0.02 mmol) and CuI (7.6 mg, 0.04 mmol) were combined in a heavy walled reaction vessel with dry diisopropylamine (5 mL). Alkyne (1.2 mmol) was then added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 5 hours at 110^oC. The reaction was then filtered over Celite and washed with diethylether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography.

1-bromo-2-(2-phenylethynyl)benzene **80**

Method A: 56 percent yield was observed using phenylacetylene (0.13 mL, 123 mg, 1.2 mmol), purified by column chromatography (100% hexanes).

Method B: 84 percent yield was observed using phenylacetylene (0.13 mL, 123 mg, 1.2 mmol), purified by column chromatography (100% hexanes).

TLC(100% Hexanes): 0.37.

UV: λ_{max} = 227 (ϵ = 45), 287 (47), 305 (38).

IR($CDCl_3$): 3154, 2253, 1492, 1466, 907, 732, 650.

^1H NMR($CDCl_3$): δ 7.62-7.55 (m, 3H, Ar-H), 7.36 (t, J= 3.5 Hz, 3H, Ar-H), 7.295 (t, J= 7.5 Hz, 2H, Ar-H), 7.18 (t, J= 7.0 Hz, 1H, Ar-H).

^{13}C NMR($CDCl_3$): δ 133.2, 132.5, 131.7(2C), 129.4, 128.7(2C), 128.7, 128.4, 127.0, 125.6, 122.9, 104.9, 93.9, 87.9.

MS: 255(100), 176(85), 151(64), 88(53), exact mass calcd for $C_{14}H_9Br$ *m/z* 255.9893, obsd *m/z* 255.9.

2-[(4-Aminophenyl)ethynyl]-1-bromobenzene **81**

Method A: 43 percent yield was observed using 4-ethynylaniline (0.10 mL, 117 mg, 1.2 mmol), purified by column chromatography (2:1; Ethyl Acetate: hexanes).

Method B: 20 percent yield was observed using 4-ethynylaniline (0.10 mL, 117 mg, 1.2 mmol), purified by column chromatography (2:1; Ethyl Acetate: hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.29.

UV: λ_{max} = 228 (ϵ = 27), 311 (31).

IR($CDCl_3$): 3400, 3036, 2925, 2854, 2252, 2215, 1620, 1584, 1515, 908, 731, 650.

^{1}H NMR($CDCl_3$): δ 7.60 (d, J= 8.0 Hz, 1H, Ar-H), 7.52 (d, J= 6.5 Hz, 1H, Ar-H), 7.40 (d, J= 8.5 Hz, 2H, Ar-H), 7.26 (t, J= 8.0 Hz, 1H, Ar-H), 7.13 (t, J= 6.5 Hz, 1H, Ar-H), 6.64 (d, J= 9.0 Hz, 2H, Ar-H), 3.86 (s, 2H, NH_2).

^{13}C NMR($CDCl_3$): δ 147.0, 133.1, 132.8, 132.3, 128.7, 126.9, 126.0, 125.3, 114.7(2C), 112.2, 104.9, 94.9, 86.2.

MS: 271(100), 191(22), 165(37), 95(31), exact mass calcd for $C_{14}H_{10}NBr$ *m/z* 271.0599, obsd *m/z* 271.0.

2-[(3-Aminophenyl)ethynyl]-1-bromobenzene **82**

Method A: 66 percent yield was observed using 3-ethynylaniline (0.10 mL, 117 mg, 1.2 mmol), purified by column chromatography (2:1; Ethyl Acetate: hexanes).

Method B: 30 percent yield was observed using 3-ethynylaniline (0.10 mL, 117 mg, 1.2 mmol), purified by column chromatography (2:1; Ethyl Acetate: hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.33.

UV: λ_{max} = 231 (ϵ = 2586), 286 (2265), 297 (2066).

IR($CDCl_3$): 3394, 2252, 1618, 1600, 1493, 1468, 1445, 911, 730.

^{1}H NMR($CDCl_3$): δ 7.61 (d, J=8.0 Hz, 1H, Ar-H), 7.54 (d, J= 7.0 Hz, 1H, Ar-H), 7.28 (t, J= 7.5 Hz, 1H, Ar-H), 7.18-7.13 (m, 2H, Ar-H), 6.98 (d, J= 7.0 Hz, 1H, (Ar-H), 6.90 (s, 1H, Ar-H), 6.68 (d, J= 8.0 Hz, 1H, Ar-H), 3.70 (s, 2H, NH_2).

^{13}C NMR($CDCl_3$): δ 146.3, 133.2, 132.4, 129.3, 129.3, 127.0, 125.6, 125.5, 123.6, 122.1, 117.8, 115.7, 94.2. 87.4.

MS: 271(100), 191(33), 165(65), 95(29), exact mass calcd for $C_{14}H_{10}NBr$ *m/z* 271.0599, obsd *m/z* 271.0.

1-Bromo-2-[(3-amino-N-trimethylacetylphenyl)ethynyl]benzene **87**

Method B: 14.5 percent yield was observed using **68** (252mg, 1.2 mmol), purified by column chromatography (10% Ethyl Acetate/ hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.59.

UV: λ_{max} = 234 (ϵ = 27560), 288 (18609).

IR($CDCl_3$): 3450, 3321, 2967, 2857, 2252, 1677, 1604, 1529, 1485, 1163, 908, 787, 734, 649.

^{1}H NMR($CDCl_3$): δ 7.83(dd, J=1 Hz, 1H, Ar-H), 7.74(s, 1H, Ar-H), 7.60-7.57(m, 1H, Ar-H), 7.51(d, J=6 Hz, 1H, Ar-H), 7.44(s, 1H, Ar-H), 7.31-7.25(m, 1H, Ar-H), 7.21-7.14(m, 1H, Ar-H), 6.98-6.95(m, 1H, Ar-H), 1.29(s, 9H, 3 CH_3's).

^{13}C NMR($CDCl_3$): δ 178.56, 140.34, 138.14, 133.30, 132.44, 129.46, 129.44, 129.07, 128.39, 127.52, 127.05, 122.75, 120.29, 93.41, 88.17,39.69, 27.61(3C)

1-Bromo-2-[(4-amino-N-trimethylacetylphenyl)ethynyl]benzene **86**

Method A: 12 percent yield was observed using **70** (252mg, 1.2 mmol), purified by column chromatography (10% Ethyl Acetate/ hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.61.

UV: λ_{max} = 240 (ϵ = 198), 305 (268), 328 (275).

IR($CDCl_3$): 3445, 3320, 3060, 2968, 2871, 2249, 2218, 1665, 1584, 1514, 1433, 1402, 1310, 1165, 1026, 908, 841, 733, 649.

^{1}H NMR($CDCl_3$): δ 7.83(d, J=8 Hz, 1H, Ar-H), 7.66-7.45(m, 4H, Ar-H), 7.25(t, J=7.5 Hz, 1H, Ar-H), 7.19-7.12 (m, 1H, Ar-H), 6.96 (t, J=7.5 Hz, 1H, Ar-H), 1.28(s, 9H, 3 CH_3's).

^{13}C NMR($CDCl_3$): δ 176.65, 140.24, 138.42, 132.67, 132.37, 132.35, 131.95, 129.61, 129.40, 129.20, 126.99, 119.59, 118.21, 93.76, 87.62, 44.93, 39.66, 27.53, 26.67.

1-Bromo-2-[2-(4-toluyl)ethynyl]benzene **83**

Method A: 75 percent yield was observed using 4-ethynyltoluene (0.13 mL, 116 mg, 1.2 mmol), purified by column chromatography (100% hexanes).

Method B: 32 percent yield was observed using 4-ethynyltoluene (0.13 mL, 116 mg, 1.2 mmol), purified by column chromatography (100% hexanes).

TLC(100% Hexanes): 0.45.

UV: λ_{max} = 239 (ϵ = 178), 288 (228), 307 (223).

IR($CDCl_3$): 3054, 3030, 2922, 2864, 2250, 2220, 1731, 1510, 1467, 1433, 907, 817, 732.

^{1}H NMR($CDCl_3$): δ 7.52 (d, J= 7.0 Hz, 1H, Ar-H), 7.55 (d, J= 6.0 Hz, 1H, Ar-H), 7.49 (d, J=7.5 Hz, 2H, Ar-H), 7.30 (t, J= 6.0 Hz, 2H, Ar-H), 7.18 (d, J= 7.5 Hz, 2H, Ar-H), 1.57 (t, J= 6.5 Hz, 3H, CH_3).

^{13}C NMR($CDCl_3$): δ 138.9, 133.1, 132.4, 131.5, 129.2(2C), 126.9, 125.6, 125.5, 119.8, 94.2, 87.4, 21.6.

MS: 271(100), 191(84), 165(74), 82(59), exact mass calcd for $C_5H_{11}Br$ *m/z* 270.0648, obsd *m/z* 271.6.

1-Bromo-2-[2-(3-toluyl)ethynyl]benzene **84**

Method A: 57 percent yield was observed using 3-ethynyltoluene (0.13 mL, 116 mg, 1.2 mmol), purified by column chromatography (100% hexanes).

Method B: 54 percent yield was observed using 3-ethynyltoluene (0.13 mL, 116 mg, 1.2 mmol), purified by column chromatography (100% hexanes).

TLC(100% Hexanes): 0.40.

UV: λ_{max} = 231 (ϵ = 28), 290 (43), 309 (40).

IR($CDCl_3$): 3058, 2923, 2855, 2249, 1601, 1487, 1467, 1433, 907, 732.

^{1}H NMR($CDCl_3$): δ 7.61 (d, J= 7.5 Hz, 1H, Ar-H), 7.54 (d, J= 6.5 Hz, 1H, Ar-H), 7.40- 7.17 (m, 6H, Ar-H), 2.36 (s, 3H, CH_3).

^{13}C NMR($CDCl_3$): δ 138.0, 133.2, 132.4, 132.2, 129.5, 129.3, 128.8, 128.3, 127.0, 125.6, 125.4, 122.6, 94.1, 87.6, 21.3.

MS: 272(95), 190(100), 165(97), 94(99), exact mass calcd for $C_5H_{11}Br$ *m/z* 270.0648, obsd *m/z* 272.2.

1-Bromo-2-[2-(3-fluorophenyl)ethynyl]benzene **85**

Method A: 56 percent yield was observed using 1-ethynyl-3-fluorobenzene (0.12 mL, 120 mg, 1.2 mmol) and $Pd(PPh_3)_4$ (69 mg, 0.06 mmol), purified by column chromatography (100% hexanes).

Method B: 11 percent yield was observed using 1-ethynyl-3-fluorobenzene (0.12 mL, 120 mg, 1.2 mmol) and $Pd(PPh_3)_4$ (69 mg, 0.06 mmol), purified by column chromatography (100% hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.42.

UV: λ_{max} = 285 (ϵ = 92).

IR($CDCl_3$): 3071, 2252, 1608, 15679, 1488, 1466, 1434, 908, 734.

^{1}H NMR($CDCl_3$): δ 7.63 (d, J= 9.0 Hz, 1H, Ar-H), 7.56 (dd, J= 1.5 Hz, 1.5 Hz, 1H, Ar-H), 7.38-7.28 (m, 4H, Ar-H), 7.21 (td, J= 1.5 Hz, 1H, Ar-H), 7.08 (tt, J=1 Hz, 1.5 Hz, 1 Hz, 1H, Ar-H).

^{13}C NMR($CDCl_3$): δ 163.4, 161.5, 133.1, 130.2, 129.6, 129.5, 128.4, 127.6, 125.8, 125.0, 118.4, 116.0, 92.5, 89.2.

MS: 274(100), 194(80), 175(66), 97(55), exact mass calcd for $C_{14}H_8FBr$ *m/z* 274.5487, obsd *m/z* 274.0

1-Bromo-2-[2-(3-pyridinyl)ethynyl]benzene **102**

Method A: 13 percent yield was observed using 3-ethynylpyyridine (132 mg, 1.2 mmol) and the reaction was run for 72 hours, purified by column chromatography (10% Ethyl Acetate/ hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.39.

UV: λ_{max} = 282 (ϵ =100), 328 (39).

IR($CDCl_3$): 3052, 2965, 2223, 1578, 1560, 1481, 1463, 1434, 1407, 1045, 1025, 908, 804, 754, 732, 703, 646.

^{1}H NMR($CDCl_3$): δ 8.82(s, 1H, Ar-H), 8.56(s, 1H, Ar-H), 7.82(d, J=8 Hz, 1H, Ar-H), 7.60(d, J=8 Hz, 1H, Ar-H), 7.54(dd, J=1.5 Hz, 1H, Ar-H), 7.29-7.25(m, 2H, Ar-H), 7.18(t, J=7.5 Hz, 1H, Ar-H)

^{13}C NMR($CDCl_3$): δ 152.32, 148.96, 140.35, 138.53, 132.77, 132.58, 132.15, 129.94, 128.52, 127.14, 123.04, 91.15, 90.36.

MS: 256(100), 177(99), 152(92), 125(96), 99(92), 62(80), exact mass calcd for $C_{13}H_8NBr$ *m/z* 256.9846, obsd *m/z* 256.6.

3-(2-Bromophenyl)-2-propynol pyrrole2-carboxylate **89**

Method A: 24% yield was observed using **64** (179 mg, 1.2 mmol) and the reaction was run for 48 hours, purified by column chromatography (10% Ethyl Acetate/ hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.54.

UV: λ_{max} = 262 (ϵ = 19).

IR($CDCl_3$): 3452, 3311, 2973, 2249, 1693, 1587, 1554, 1468, 1433, 1411, 1312, 1268, 1167, 1130, 1080, 992, 909, 734, 649, 606.

^{1}H NMR($CDCl_3$): δ 9.35(s, 1H, NH), 7.85(d, J=3 Hz, 1H, Ar-H), 7.67-7.45(m, 3H, Ar-H), 7.26-7.15(m, 3H, Ar-H), 6.99(dd, J=7 Hz, 1H, Ar-H), 6.27(s, 1H, Ar-H), 5.14(s, 2H, CH_2).

^{13}C NMR($CDCl_3$): δ 160.17, 140.81, 133.75, 132.73, 132.41, 129.89, 129.70, 129.42, 128.48, 128.36, 126.97, 87.78, 84.89, 52.51.

MS: 289(29), 193(75), 94(100), mass calcd for $C_{14}H_{10}O_2NBr$ *m/z* 288.9958, obsd *m/z* 289.

3-(2-Bromophenyl)-2-propynol N-trimethylacetylpyrrole2-carboxylate **90**

Method A: 18 percent yield was observed using **88** (280 mg, 1.2 mmol) and the reaction was run for 72 hours, purified by column chromatography (10% Ethyl Acetate/ hexanes).

TLC(2:1, Ethyl Acetate:Hexanes): 0.57.

UV: λ_{max} = 262 (ϵ = 241).

IR($CDCl_3$): 3455, 3319, 2978, 2252, 1701, 1555, 1436, 1310, 1160, 1107, 1054, 961, 908, 732, 649.

^{1}H NMR($CDCl_3$): δ 7.80(d, J=5.5 Hz, 1H, Ar-H), 7.56(t, J=6 Hz, 2H, Ar-H), 7.25-7.14(m, 2H, Ar-H), 7.00-7.93 (m, 1H, Ar-H), 6.28(d, J=36.5 Hz, 1H, Ar-H), 5.13(s, 2H, CH_2), 1.25(s, 9H, 3 CH_3's).

^{13}C NMR($CDCl_3$): δ 172.86, 160.09, 140.14, 132.07, 131.90, 129.78, 129.31, 123.59, 123.54, 116.16, 101.14, 98.82, 87.80, 84.79, 52.37, 44.68, 27.47, 26.67, 24.59.

2-[2-(3-Pyridinyl)ethynyl]-1-[2-(trimethylsilyl)ethynyl]benzene **92**

(2-Bromophenylethynyl)trimethylsilane (0.213 mL, 253 mg, 1.0 mmol), $Pd(PPh_3)_4$ (23 mg, 0.02 mmol) and CuI (7.6 mg, 0.04 mmol) were combined in a heavy walled reaction vessel with dry diisopropylamine (5 mL). 3-ethynylpyridine (132 mg, 1.2 mmol) was then added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 130^{o}C. The reaction was then filtered over Celite and washed with diethylether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). An 11 % yield was obtained as determined by GC.

TLC(100% Hexanes): 0.51.

UV: λ_{max} = 255 (ϵ = 242), 297 (229).

IR($CDCl_3$): 3061, 2960, 2899, 2224, 2158, 1563, 1484, 1442, 1407, 1250, 909, 873, 845, 734, 646.

^{1}H NMR($CDCl_3$): δ 8.80(d, J= 1 Hz, 1H, Ar-H), 8.55 (dd, J=1.5 Hz, 1H, Ar-H), 7.82 (dt, J=1.5 Hz, 1, 1H, Ar-H), 7.53-7.51 (m, 2H, AR-H), 7.32-7.27(M, 3H, AR-H), 0.27-0.26 (M, 9H, TMS).

^{13}C NMR($CDCl_3$): δ 152.32, 148.70, 138.42(2C), 132.36(2C), 131.80(2C), 128.42, 128.28, 125.78, 125.26, 123.02, 120.48, 103.17, 99.04, 91.40, 89.796, 44.98(3C).

MS: 275(100), 244(92), 261(86), 259(77), 217(56), exact mass calcd for $C_{18}H_{17}NSi$ *m/z* 276.1243, obsd *m/z* 275.3.

ICP-AES: 0.1189ppm (traces of Pd)

2-(2-Phenylethynyl)-1-[2-(3-pyridinyl)ethynyl]benzene **93**

80 (186 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, 3-ethynylpyridine (132 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 13% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.43.

UV: λ_{max} = 275 (ϵ = 34).

IR($CDCl_3$): 3057, 2923, 2216, 2223, 1597, 1560, 1494, 1481, 1442, 1406, 1022, 755, 702, 690.

^{1}H NMR($CDCl_3$): δ 8.82 (s, 1H, Ar-H), 8.55 (s, 1H, Ar-H), 7.82 (d, J= 8 Hz, 1H, AR-H), 7.58-7.55 (m, 4H, Ar-H), 7.36-7.25 (m, 6H, Ar-H).

^{13}C NMR($CDCl_3$): δ 152.21, 148.67, 138.48, 131.91(2C), 131.59(2C), 128.61, 128.57(2C), 128.48(2C), 128.07, 125.97, 124.97, 123.11, 123.04, 93.85, 91.53, 89.93, 87.96.

MS: 279(100), 277(74), 250(54), exact mass calcd for $C_{21}H_{13}N$ *m/z* 279.1020, obsd *m/z* 279.1.

ICP-AES: 0.0042ppm (traces of Pd)

2-[2-(4-Aminophenyl)ethynyl]-1-[2-(3-pyridinyl)ethynyl]benzene **94**

81 (234 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, 3-ethynylpyridine (132 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 12% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.45.

UV: λ_{max} = 288 (ϵ = 33), 307 (33), 328 (24).

IR($CDCl_3$): 3333, 3056, 2964, 2253, 2212, 1606, 1515, 1481, 1436, 1260, 1181, 1119, 1024, 723, 696, 644.

^{1}H NMR($CDCl_3$): δ 8.80 (s, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 8.23(d, J=7.5 Hz, 1H, Ar-H), 7.61-7.52 (m, 3H, Ar-H), 7.35-6.89 (m, 5H, Ar-H), 6.67(d, J=8 Hz, 1H, Ar-H), 3.72(s, 2H, NH_2).

^{13}C NMR($CDCl_3$): δ 152.29, 148.94, 13.60, 133.20, 131.69, 131.59, 129.96, 129.32, 129.27, 128.45, 128.31, 127.14, 127.00, 122.08, 117.80, 115.33, 94.19, 91.75, 89.5, 88.76.

ION TRAP MS(+Na): 316(100), 293(79), 127(58),exact mass calcd for $C_{21}H_{14}N_2$ *m/z* 294.1167 (+23=317), obsd *m/z* 316.6.

2-[2-(3-Aminophenyl)ethynyl]-1-[2-(3-pyridinyl)ethynyl]benzene **95**

82 (234 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, 3-ethynylpyridine (132 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 14% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.32.

UV: λ_{max} = 230 (ϵ = 780), 248 (621), 285 (875).

IR($CDCl_3$): 3291, 3058, 2965, 2928, 2253, 2222, 1609, 1581, 1483, 1437, 1263, 1184, 1119, 1025, 909, 723, 695, 541.

^{1}H NMR($CDCl_3$): δ 8.81(s, 1H, Ar-H), 8.58(d, J=4 Hz, 1H, Ar-H), 7.86 (dt, J=1.5 Hz, 1H, Ar-H), 7.64-7.50 (m, 4H, Ar-H), 7.39-7.12(m, 4H, Ar-H), 6.64 (dt, J=2 Hz, 1H, Ar-H), 5.97 (s, 2H, NH_2).

^{13}C NMR($CDCl_3$): δ 152.28, 148.93, 138.56, 133.35, 132.95, 132.6, 132.55, 132.33, 129.95, 128.69, 128.45, 128.25, 127.64, 127.14, 126.94, 125.67, 114.73, 94.73, 94.91, 91.13, 90.32, 86.17.

ION TRAP MS(+Na): 316(100), 293(32), 229(30), 127(37),exact mass calcd for $C_{21}H_{14}N_2$ *m/z* 294.1167 (+23=317), obsd *m/z* 316.6.

2-[2-(3-Amino-N-trimethylacetylphenyl)ethynyl]-1-[2-(3-pyridinyl)ethynyl]benzene **100**

87 (257 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, **68** (238 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 9% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.21.

UV: λ_{max} = 252 (ϵ = 37), 274 (38), 328 (27).

IR($CDCl_3$): 3340, 3305, 3065, 2963, 2928, 2253, 2221, 1609, 1584, 1483, 1436, 1184, 1074, 1025, 905, 908, 786, 765, 723, 734, 650, 473.

^{1}H NMR($CDCl_3$): δ 8.82 (s, 1H, Ar-H), 8.55 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.79 (d, J=3.5 Hz, 1H, Ar-H), 7.56 (dd, J=4.5 Hz, 4H, Ar-H), 7.37-7.26 (m, 4H, Ar-H), 4.09 (s, 1H, N-H), 1.27(s, 9H, 3CH_3's).

^{13}C NMR($CDCl_3$): δ 176.66, 171.15, 152.31, 148.68, 138.29, 131.96, 131.88, 129.11, 128.59, 128.16, 127.33, 125.98, 125.12, 123.78, 123.24, 122.96, 120.33, 93.49, 91.58, 90.07, 88.18, 60.40, 31.60, 31.62, 27.61.

ION TRAP MS(+Na): 425(57), 401(100), 341(36), 301(53),exact mass calcd for $C_{26}H_{22}N_2O$ *m/z* 378.9732 (+23=401), obsd *m/z* 401.2.

2-[2-(4-Amino-N-trimethylacetylphenyl)ethynyl]-1-[2-(3-pyridinyl)ethynyl]benzene **99**

86 (257 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, **70** (238 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 4% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.36.

UV: λ_{max} = 287 (ϵ =46), 307 (41).

IR($CDCl_3$): 3350, 3405, 3064, 2955, 2929, 2253, 2223, 1609, 1574, 1473, 1437, 1174, 1074, 1025, 905, 908, 789, 768, 726, 734, 651, 473.

^{1}H NMR($CDCl_3$): δ 1.38(s, 1H, Ar-H), 8.57(d, J=4 Hz, 1H, Ar-H), 7.83(dd, J=8 Hz, 1H, Ar-H), 7.63-7.49(m, 4H, Ar-H), 7.30-7.12(m, 5H, Ar-H), 4.09(s, 1H, NH), 1.25(s, 9H, 3 CH_3's).

^{13}C NMR($CDCl_3$): δ 171.15, 152.27, 152.25, 148.94, 148.56, 138.61, 133.3, 132.59, 131.72, 129.99, 128.85, 128.48, 127.17, 125.70, 124.73, 123.15, 122.53, 120.20, 92.70, 91.19, 90.32, 60.40, 31.63, 22.69, 21.04.

ION TRAP MS(+Na): 401(68), 379(100), 279(36), 123(46),exact mass calcd for $C_{26}H_{22}N_2O$ *m/z* 378.9732 (+23=401), obsd *m/z* 401.2.

1-[2-(3-Pyridinyl)ethynyl]-2-[2-(4-toluyl)ethynyl]benzene **96**

83 (243 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, 3-ethynylpyridine (132 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 2.5% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.48.

UV: λ_{max} = 232 (ϵ =40), 276 (72).

IR($CDCl_3$): 3028, 2922, 2855, 2215, 1716, 1561, 1510, 1483, 1407, 1023, 908, 816, 733, 703, 646.

^{1}H NMR($CDCl_3$): δ 8.81 (d, J=1 Hz, 1H, Ar-H), 8.56 (d, J=5.5 Hz, 1H, Ar-H), 7.83 (dt, J= 2 Hz, 1.5 Hz, 1H, Ar-H), 7.57 (dd, J= 2 Hz, 2H, Ar-H), 7.45 (d, J=7.5 Hz, 2H, Ar-H), 7.35-7.27 (m, 3H, Ar-H), 7.16 (d, J=8 Hz, 2H, Ar-H), 2.37 (s, 3H, CH_3).

^{13}C NMR($CDCl_3$): δ 152.2, 148.8, 148.5, 138.8, 138.5, 133.3, 132.5, 131.9, 131.8, 131.5, 129.9, 129.2, 128.5, 127.8, 127.1, 126.3, 124.9, 123.1, 94.1, 91.6, 89.8, 87.4.

MS: 293(100), 292(48), 291(23), exact mass calcd for $C_{22}H_{15}N$ *m/z* 293.1216, obsd *m/z* 293.2.

1-[2-(3-Pyridinyl)ethynyl]-2-[2-(3-toluyl)ethynyl]benzene **97**

84 (243 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, 3-ethynylpyridine (132 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 12% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.47.

UV: λ_{max} = 274 (ϵ = 40), 307 (32).

IR($CDCl_3$): 3033, 2923, 2856, 2247, 2222, 1600, 1581, 1561, 1489, 1482, 1407, 1024, 948, 804, 784, 757, 647.

^{1}H NMR($CDCl_3$): δ 8.82 (s, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 7.85 (t, J= 8.5 Hz, 1H, Ar-H), 7.57 (d, J=7.5 Hz, 2H, Ar-H), 7.38-7.22 (m, 6H, Ar-H), 7.16 (d, J= 7.5 Hz, 1H, Ar-H), 2.33 (s, 3H, CH_3).

^{13}C NMR($CDCl_3$): δ 152.23, 148.66, 138.48, 138.12, 133.34, 132.55, 132.24, 131.85, 131.82, 129.52, 128.61, 128.37, 127.98, 127.13, 126.67, 125.00, 122.84,94.13, 91.61, 89.92, 87.67, 21.25.

MS: 293(100), 292(49), 291(24), exact mass calcd for $C_{22}H_{15}N$ *m/z* 293.1216, obsd *m/z* 293.1.

ICP-AES: 0.0066ppm (traces of Pd)

2-[2-(3-Fluorophenyl)ethynyl]-1-[2-(3-pyridinyl)ethynyl]benzene **98**

85 (246 mg, 1.0 mmol) was dissolved in dry diisopropylamine (5 mL) in a heavy walled reaction vessel with a stir bar. $Pd(PPh_3)_4$ (48.5 mg, 0.04 mmol) was then added and stirred for ten minutes. CuI (26.6 mg, 0.14 mmol) was then added and allowed to stir for an additional 10 minutes. Lastly, 3-ethynylpyridine (132 mg, 1.2 mmol) was added and the mixture was degassed for two minutes with N_2 gas. The reaction vessel was tightly capped and heated for 72 hours at 110^{o}C. The reaction was then filtered over Celite and washed with diethyl ether (10 mL x 3). The mixture was then washed with saturated $NaHCO_3$ (10 mL) and the organic layer was dried with $MgSO_4$. The solvent was removed *in vacuo* and the product purified by column chromatography (10% Ethyl Acetate/ hexanes). A 25% yield of was obtained as determined by GC.

TLC(2:1, Ethyl Acetate:Hexanes): 0.43.

UV: λ_{max} = 273 (ϵ =63), 328 (69).

IR($CDCl_3$): 3065, 2926, 2251, 2224, 1607, 1579, 1491, 1407, 1263, 1205, 1024, 908, 785, 731, 649.

^{1}H NMR($CDCl_3$): δ 8.80(d, J=1 Hz, 1H, Ar-H), 8.56 (dd, J= 1 Hz, 2H, Ar-H), 7.82 (dt, J= 2 Hz, 1.5 Hz, 1H, Ar-H), 7.57 (q, J= 4 Hz, 4.5 Hz, 2H, Ar-H), 7.35-7.22 (m, 5H, Ar-H), 7.07-7.03 (m, 1H, Ar-H).

^{13}C NMR($CDCl_3$): δ 163.38, 161.42, 152.20, 148.78, 138.45, 132.00, 130.12, 130.05, 128.61, 128.43, 127.48, 127.46, 125.13, 124.92, 118.42, 118.24, 116.05, 92.41, 91.32, 90.06, 88.80.

MS: 297(100), 296(67), 295(23), exact mass calcd for $C_{21}H_{12}F$ *m/z* 296.1179, obsd *m/z* 297.1.

4.2.2 Experimental for Chapter Three

Cell culture

Human alveolar type 11-like epithelial A549 cells (ATCC# CCL-185, ATCC ampule passage no. 80; American Type Culture Collection, Manassas, VA, USA) were maintained in Costar 0.2 μm vent cap cell culture flasks (Corning, Corning, NY, USA) with standard Dulbecco's modified Eagle's medium nutrient mixture F-12 Ham (Sigma-Aldrich, Oakville, ON, Canada) supplemented with 10% iron-fortified bovine calf serum (SAFC Biosciences, Lenexa, KS, USA), 2 mM l-glutamine (Gibco, Carlsbad, CA), and antibiotic/antimycotic (100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B; Gibco). Cultures were incubated at 37ºC in a humidified atmosphere of 5 % CO_2 in air and sub-cultured when 80% confluent. Prior to plating, cell counts and viabilities were assessed using a Vi-Cell XR Cell Viability Analyzer (Beckman Coulter, Mississauga, ON, Canada). Experiments were performed using serum-free media.

H9c2(2-1) is a subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue.

Cytotoxic Properties of Activated Enediynes - Cell Viability

Before the cell testing could take place, approximately 10,000 A549 or H9c2 cells/ well were seeded into sterile flat-bottom 96-well plates (Corning, clear), and incubated for 24 hours in serum media. To determine the cytotoxicity of the UV active drugs, cells were tested as control, control and Tween 80, or enediyne -containing media (range 7-15 μM final concentration). Each enediyne was first dissolved in 200μL of the surfactant Tween 80 (a mono-fatty acid ester of polyoxyethylene sorbitan) before being placed in the serum-free media. After 3 hours and 50 minutes of pre-incubation of the A549 or H9c2 cells with the enediynes, the drugs were activated with a 10 min or 6.5 min exposure to 302nm UV light, respectively (UVB Transilluminator, 8 watts, 115 V, ~60 Hz and 0.7 Amps, Toronto, Canada) and then incubated for a further 16 hours, for a 24 hour total incubation period (including 4 hours of MTT). Control cells were exposed to the enediynes alone or UV light alone.

The MTT (method of transcriptional and translational) assay was used to assess cell death. This test works by standard colorimetric assay (an assay which measures changes in color) and measures the activity of enzymes that reduce MTT to formazan, giving a purple color. It can also be used to determine cytotoxicity of potential medicinal agents and other toxic materials, since those agents would result in cell toxicity and therefore metabolic dysfunction and decreased performance in the assay. Yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) was used due to its ability to reduce to purple formazan in living cells. After the cells were incubated for the further 16 hours, culture media (150 μL) was replaced with fresh media containing 10 % yellow MTT (Thiazolyl Blue Tetrazolium Bromide; Sigma-Aldrich) reagent and cells were incubated under standard conditions for an additional 4 hours. The culture media were aspirated and 50 μL

dimethylsulfoxide was added per well to solubilize the formazan crystals. Following agitation, absorbance was measured spectrophotometrically at a wavelength of 570 nm (650 nm background correction wavelength) using a PowerWave XS Microplate Spectrophotometer (BioTek, Winooski, VT, USA). Viabilities of challenged cells were assessed relative to control cells.

4.2.3 Experimental for Chapter Four

All of the standard calibration curves were prepared by Dr. Christine Gottardo except for 5-phenyl-2-furaldehyde and 2-furaldehyde. All of the compounds presented in Chapter Four have been previously characterized and found in the literature except for the following:[45-47,56]

General Procedures for Direct Arylation:

Method A: Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110^{o}C for five hours. The mixture was cooled and filtered over Celite using CH_2Cl_2. The organic layer was removed *in vacuo*. GC samples were prepared by adding CH_2Cl_2 (5.0 mL) and the internal standard, 1,2-dichlorobenzene (7.6 μL).

Method B: Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol), benzoquinone (2.0 mmol, 0.216 g), pivaloyl acid (6.0 mmol, 0.613 g) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110^{o}C for five hours. The mixture was cooled and filtered over Celite using CH_2Cl_2. The organic layer was removed *in*

vacuo. GC samples were prepared by adding CH_2Cl_2 (5.0 mL) and the internal standard, 1,2-dichlorobenzene (7.6 μL).

Method C: Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol), CsOPiv (0.4 mmol, 0.094 g), 4-methyl-3-nitropyridine (0.1 mmol, 0.014 g), pivaloyl acid (6.0 mmol,0.613 g) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110°C for five hours. The mixture was cooled and filtered over Celite using CH_2Cl_2. The organic layer was removed *in vacuo*. GC samples were prepared by adding CH_2Cl_2 (5.0 mL) and the internal standard, 1,2-dichlorobenzene (7.6 μL).

Method D: Heteroaromatic compound (1.0 mmol), oxidant (6.0 mmol), benzoquinone (2.0 mmol, 216 mg), pivaloyl acid (6.0 mmol, 613 mg) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110°C for five hours. The mixture was cooled and filtered over Celite using CH_2Cl_2. The organic layer was removed *in vacuo*. GC samples were prepared by adding CH_2Cl_2 (5.0 mL) and the internal standard, 1,2-dichlorobenzene (7.6 μL).

Method E: Heteroaromatic compound (1.0 mmol), oxidant (1.5 mmol), benzoquinone (2.0 mmol, 216 mg), pivaloyl acid (6.0 mmol, 613 mg) and Pd-catalyst (0.05 mmol) were combined in a vial containing dry benzene (2.7 mL), sealed and heated to 110°C for five hours. The mixture was cooled and filtered over Celite using CH_2Cl_2. The organic layer was removed *in vacuo*. GC samples were prepared by adding CH_2Cl_2 (5.0 mL) and the internal standard, 1,2-dichlorobenzene (7.6 μL).

General Procedure for Photochemical Direct Arylation:

Method F: Heteroaromatic compound (1.0 mmol), oxidant (3.0 mmol), benzoquinone (2.0 mmol, 0.216 g), pivaloyl acid (6.0 mmol, 0.613 g) and Pd-catalyst (0.05 mmol) were combined in a quartz tube containing dry benzene (2.7 mL). The system was purged with Nitrogen for 2 minutes, sealed with a septum and irradiated with UV light for 5 hours. The mixture was cooled and filtered over Celite using CH_2Cl_2. The organic layer was removed *in vacuo*. GC samples were prepared by adding CH_2Cl_2 (5.0 mL) and the internal standard, 1,2-dichlorobenzene (7.6 μL).

GC Analysis:

GC analyses were performed on an Agilent 6850 using an Agilent DB-5HT, 30m long, 0.25mm diameter, 0.10μm film thickness, with temperature limits of -20° to 300°C column. Calibration curves were constructed using 1,2-dichlorobenzene as an internal standard and a pure sample of the phenyl substituted heteroaromatic compound obtained either directly from Sigma-Aldrich (5-phenyl-2-furaldehyde) or synthesized through a Suzuki coupling from the correctly substituted heteroaromatic boronic acid ester and iodobenzene.

2-Phenylfuran[45,46]:

2-Furanylboronic acid (1.05 mmol, 0.117 g), iodobenzene (1.0 mmol, 0.204 g, 0.11 mL), $Pd(OAc)_2$ (0.02 mmol, 0.005 g) and K_2CO_3 (2.5 mmol, 0.345 g) were combined in acetone / water (2.0 mL/ 2.5 mL) and placed in a vial. The vial was heated for 45 minutes at 65 ^oC. The cooled solution was extracted with diethyl ether (4 x 10 mL). The combined organic extracts

were washed with water (10 mL), dried and removed *in vacuo*. The residue was purified by MPLC, eluting with hexanes (100%). The reaction yielded 0.075 g (36%) of a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.47 (dd, J=2.4, 1.6 Hz, 1H), 6.65 (d, J=2.8 Hz, 1H), 7.24 (d, J=6.8 Hz, 1H), 7.38 (t, J=6.4 Hz, 2H), 7.47 (d, J=1.2 Hz, 1H), 7.67(d, J=5.6 Hz, 2H).

^{13}C NMR (125 MHz, $CDCl_3$): δ106.5, 111.4, 121.2, 122.7, 124.2, 127.4, 144.9, 153.7.

MS (EI, 70eV): *m/z* (%): 115.1 (98), 144.1 (100) [M^+]. Calcd mass: 145.13.

2-methyl-5-phenylfuran[47]:

5-Methylfuranyl-2-boronic acid pinacol ester (1.1 mmol, 0.229 g), iodobenzene (1.0 mmol, 0.204 g, 0.11 mL), K_2CO_3 (2.5 mmol, 0.345 g), $Pd(OAc)_2$ (0.02 mmol, 0.005 g) and tetrabutylammonium bromide (1.0 mmol, 0.322 g) were combined in deionized water (2.0 mL) and stirred at room temperature for five hours. The reaction mixture was diluted with water (5mL) and extracted with EtOAc (4 x 10 mL). The combined organic layers were stirred over charcoal (0.75 g) for 30 minutes and sodium sulfate was added. The dried organic layer was filtered and the excess solvent removed *in vacuo*. The residue was purified by MPLC, eluting with hexanes (100%). The reaction yielded 0.098 g (62%) of a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.43 (s, 3H), 6.11 (d, J=3.5 Hz, 1H), 6.58 (d. J=3.5 Hz, 1H), 7.36 (t, J=8.0 Hz, 1H), 7.45 (D, J=8.1 Hz, 2H), 7.71(d, J=8.0 Hz, 2H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 105.8, 108.1, 123.8, 126.4, 128.8, 131.7, 149.8, 152.0.

MS (EI, 70ev) *m/z (%)*:115.1 (35), 157.1 (70%), 158.1 (100) [M^+]. Calcd mass: 158.07.

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APPENDIX I

The following tables correspond to the values obtained for the A549 cells treated with ultraviolet light. The absorbances were measured spectrophotometrically at a wavelength of 490nm (650 nm back ground correction wavelength) using a PowerWave XS Microplate Spectrophotometer (BioTek, Winooski, VT, USA).

[Plate: M#1 490]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.049	0.049	0.049	0.05	0.049	0.049	0.05	0.05	0.049	0.049	0.05
B	0.049	0.085	0.101	0.06	0.058	0.077	0.054	0.063	0.05	0.05	0.048	0.049
C	0.049	0.064	0.074	0.08	0.079	0.082	0.062	0.054	0.049	0.056	0.051	0.049
D	0.05	0.051	0.069	0.052	0.053	0.052	0.051	0.048	0.048	0.053	0.057	0.049
E	0.048	0.064	0.058	0.053	0.051	0.061	0.054	0.05	0.049	0.051	0.141	0.048
F	0.049	0.105	0.087	0.213	0.118	0.079	0.066	0.056	0.053	0.061	0.139	0.049
G	0.049	0.055	0.06	0.061	0.057	0.055	0.05	0.051	0.05	0.104	0.139	0.049
H	0.049	0.049	0.049	0.048	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.051

[Plate: M#2 650]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.045	0.046	0.045	0.046	0.046	0.045	0.046	0.045	0.047	0.046	0.046	0.045
B	0.045	0.064	0.075	0.044	0.043	0.056	0.045	0.051	0.044	0.043	0.042	0.045
C	0.045	0.048	0.056	0.061	0.062	0.061	0.046	0.045	0.042	0.043	0.042	0.045
D	0.047	0.042	0.051	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.045	0.045
E	0.045	0.045	0.043	0.042	0.041	0.043	0.043	0.042	0.042	0.042	0.051	0.045
F	0.045	0.08	0.064	0.168	0.092	0.055	0.052	0.046	0.043	0.044	0.05	0.045
G	0.045	0.043	0.045	0.045	0.042	0.042	0.042	0.042	0.042	0.08	0.072	0.045
H	0.045	0.045	0.046	0.046	0.045	0.045	0.045	0.045	0.046	0.046	0.046	0.047

[Plate: Delta OD]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	4.00E-03	3.00E-03	4.00E-03	3.00E-03	4.00E-03	4.00E-03	3.00E-03	5.00E-03	3.00E-03	3.00E-03	3.00E-03	5.00E-03
B	4.00E-03	0.021	0.026	0.016	0.015	0.021	9.00E-03	0.012	6.00E-03	7.00E-03	6.00E-03	4.00E-03
C	4.00E-03	0.016	0.018	0.019	0.017	0.021	0.016	9.00E-03	7.00E-03	0.013	9.00E-03	4.00E-03
D	3.00E-03	9.00E-03	0.018	1.00E-02	0.011	1.00E-02	9.00E-03	6.00E-03	6.00E-03	0.011	0.012	4.00E-03
E	3.00E-03	0.019	0.015	0.011	1.00E-02	0.018	0.011	8.00E-03	7.00E-03	9.00E-03	0.09	3.00E-03
F	4.00E-03	0.025	0.023	0.045	0.026	0.024	0.014	0.01	0.01	0.017	0.089	4.00E-03
G	4.00E-03	0.012	0.015	0.016	0.015	0.013	8.00E-03	9.00E-03	8.00E-03	0.024	0.067	4.00E-03
H	4.00E-03	4.00E-03	3.00E-03	2.00E-03	4.00E-03	4.00E-03	4.00E-03	4.00E-03	3.00E-03	3.00E-03	3.00E-03	4.00E-03

The following tables correspond to the values obtained for the A549 cells not treated with ultraviolet light. The absorbances were measured spectrophotometrically at a wavelength of 490nm (650 nm back ground correction wavelength) using a PowerWave XS Microplate Spectrophotometer (BioTek, Winooski, VT, USA).

[Plate: M#1 490]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.049	0.049	0.048	0.05	0.048	0.048	0.049	0.049	0.049	0.049	0.049
B	0.048	0.108	0.072	0.073	0.075	0.062	0.071	0.062	0.051	0.064	0.063	0.049
C	0.048	0.059	0.056	0.051	0.052	0.051	0.054	0.048	0.048	0.058	0.052	0.047
D	0.047	0.052	0.057	0.056	0.054	0.05	0.051	0.048	0.047	0.05	0.048	0.048
E	0.048	0.082	0.069	0.074	0.052	0.133	0.048	0.064	0.051	0.051	0.144	0.048
F	0.048	0.061	0.067	0.056	0.053	0.062	0.049	0.048	0.051	0.052	0.129	0.049
G	0.048	0.123	0.175	0.126	0.143	0.066	0.055	0.062	0.047	0.051	0.115	0.049
H	0.048	0.049	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.049	0.049
[Plate: M#2 650]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.045	0.046	0.046	0.046	0.046	0.045	0.045	0.046	0.046	0.047	0.046	0.045
B	0.045	0.088	0.054	0.056	0.06	0.047	0.056	0.051	0.045	0.049	0.051	0.045
C	0.045	0.049	0.044	0.043	0.042	0.042	0.043	0.042	0.042	0.046	0.043	0.045
D	0.044	0.042	0.044	0.043	0.042	0.042	0.042	0.043	0.042	0.042	0.042	0.045
E	0.045	0.061	0.052	0.056	0.042	0.104	0.042	0.052	0.043	0.042	0.052	0.045
F	0.045	0.045	0.049	0.044	0.042	0.045	0.042	0.043	0.043	0.043	0.051	0.045
G	0.045	0.091	0.119	0.103	0.114	0.048	0.045	0.05	0.043	0.043	0.049	0.045
H	0.045	0.045	0.046	0.046	0.045	0.045	0.045	0.045	0.046	0.046	0.046	0.045
[Plate: Delta OD]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	4.00E-03	3.00E-03	3.00E-03	2.00E-03	4.00E-03	3.00E-03	3.00E-03	3.00E-03	3.00E-03	2.00E-03	3.00E-03	4.00E-03
B	3.00E-03	0.02	0.018	0.017	0.015	0.015	0.015	0.011	6.00E-03	0.015	0.012	4.00E-03
C	3.00E-03	1.00E-02	0.012	8.00E-03	1.00E-02	9.00E-03	0.011	6.00E-03	6.00E-03	0.012	9.00E-03	2.00E-03
D	3.00E-03	1.00E-02	0.013	0.013	0.012	8.00E-03	9.00E-03	5.00E-03	5.00E-03	8.00E-03	6.00E-03	3.00E-03
E	3.00E-03	0.021	0.017	0.018	1.00E-02	0.029	6.00E-03	0.012	8.00E-03	9.00E-03	0.092	3.00E-03
F	3.00E-03	0.016	0.018	0.012	0.011	0.017	7.00E-03	5.00E-03	8.00E-03	9.00E-03	0.078	4.00E-03
G	3.00E-03	0.032	0.056	0.023	0.029	0.018	0.01	0.012	4.00E-03	8.00E-03	0.066	4.00E-03
H	3.00E-03	4.00E-03	2.00E-03	2.00E-03	3.00E-03	3.00E-03	3.00E-03	3.00E-03	2.00E-03	2.00E-03	3.00E-03	4.00E-03

APPENDIX II

The following tables correspond to the values obtained for the H9c2 cells treated with ultraviolet light. The absorbances were measured spectrophotometrically at a wavelength of 490nm (650 nm back ground correction wavelength) using a PowerWave XS Microplate Spectrophotometer (BioTek, Winooski, VT, USA).

[Plate: M#1 490]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.048	0.048	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.05	0.049
B	0.049	0.093	0.096	0.103	0.103	0.098	0.093	0.079	0.068	0.082	0.074	0.05
C	0.049	0.071	0.068	0.074	0.067	0.069	0.071	0.069	0.06	0.084	0.073	0.049
D	0.048	0.071	0.075	0.074	0.076	0.09	0.076	0.07	0.062	0.082	0.072	0.048
E	0.048	0.1	0.106	0.085	0.13	0.082	0.083	0.07	0.182	0.086	0.194	0.048
F	0.048	0.093	0.105	0.095	0.109	0.074	0.073	0.065	0.201	0.074	0.195	0.049
G	0.048	0.125	0.109	0.141	0.123	0.081	0.091	0.087	0.304	0.107	0.229	0.049
H	0.049	0.049	0.049	0.048	0.048	0.048	0.048	0.049	0.049	0.049	0.049	0.049
[Plate: M#2 650]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.045	0.046	0.045	0.046	0.045	0.046	0.045	0.045	0.046	0.046	0.047	0.046
B	0.045	0.052	0.052	0.06	0.055	0.051	0.053	0.047	0.046	0.047	0.045	0.046
C	0.045	0.046	0.045	0.046	0.045	0.045	0.046	0.045	0.044	0.048	0.048	0.045
D	0.045	0.045	0.046	0.047	0.047	0.053	0.047	0.045	0.046	0.047	0.048	0.045
E	0.045	0.064	0.067	0.049	0.06	0.048	0.048	0.046	0.066	0.049	0.064	0.045
F	0.045	0.058	0.066	0.056	0.052	0.046	0.045	0.044	0.064	0.045	0.065	0.045
G	0.045	0.088	0.07	0.091	0.057	0.05	0.055	0.058	0.109	0.067	0.091	0.046
H	0.045	0.046	0.046	0.046	0.045	0.045	0.045	0.045	0.046	0.046	0.046	0.045
[Plate: Delta OD]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	4.00E-03	2.00E-03	3.00E-03	3.00E-03	4.00E-03	3.00E-03	4.00E-03	4.00E-03	3.00E-03	3.00E-03	3.00E-03	3.00E-03
B	4.00E-03	0.041	0.044	0.043	0.048	0.047	0.04	0.032	0.022	0.035	0.029	4.00E-03
C	4.00E-03	0.025	0.023	0.028	0.022	0.024	0.025	0.024	0.016	0.036	0.025	4.00E-03
D	3.00E-03	0.026	0.029	0.027	0.029	0.037	0.029	0.025	0.016	0.035	0.024	3.00E-03
E	3.00E-03	0.036	0.039	0.036	0.07	0.034	0.035	0.024	0.116	0.037	0.13	3.00E-03
F	3.00E-03	0.035	0.039	0.039	0.057	0.028	0.028	0.021	0.137	0.029	0.13	4.00E-03
G	3.00E-03	0.037	0.039	0.05	0.066	0.031	0.036	0.029	0.195	0.04	0.138	3.00E-03
H	4.00E-03	3.00E-03	3.00E-03	2.00E-03	3.00E-03	3.00E-03	3.00E-03	4.00E-03	3.00E-03	3.00E-03	3.00E-03	4.00E-03

The following tables correspond to the values obtained for the H9c2 cells not treated with ultraviolet light. The absorbances were measured spectrophotometrically at a wavelength of 490nm (650 nm back ground correction wavelength) using a PowerWave XS Microplate Spectrophotometer (BioTek, Winooski, VT, USA).

[Plate: M#1 490]

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.049	0.048	0.048	0.049	0.048	0.048	0.049	0.049	0.049	0.049	0.049
B	0.048	0.078	0.074	0.072	0.072	0.078	0.068	0.067	0.061	0.069	0.064	0.049
C	0.048	0.174	0.251	0.109	0.187	0.162	0.182	0.11	0.059	0.077	0.064	0.048
D	0.048	0.051	0.072	0.072	0.099	0.102	0.13	0.086	0.059	0.074	0.064	0.048
E	0.048	0.105	0.131	0.082	0.163	0.087	0.085	0.068	0.063	0.074	0.259	0.048
F	0.048	0.076	0.074	0.105	0.13	0.082	0.083	0.071	0.259	0.109	0.295	0.048
G	0.048	0.416	0.215	0.31	0.217	0.083	0.093	0.077	0.276	0.076	0.238	0.048
H	0.049	0.048	0.049	0.048	0.048	0.048	0.048	0.049	0.048	0.049	0.049	0.05

[Plate: M#2 650]

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.045	0.046	0.046	0.046	0.045	0.045	0.045	0.045	0.047	0.047	0.046	0.046
B	0.045	0.05	0.046	0.045	0.045	0.047	0.046	0.046	0.045	0.045	0.044	0.045
C	0.045	0.156	0.204	0.088	0.133	0.139	0.123	0.082	0.045	0.046	0.044	0.045
D	0.045	0.044	0.045	0.047	0.062	0.068	0.103	0.058	0.044	0.045	0.044	0.045
E	0.045	0.069	0.084	0.048	0.086	0.056	0.052	0.045	0.045	0.046	0.077	0.045
F	0.045	0.047	0.045	0.065	0.062	0.053	0.05	0.047	0.073	0.07	0.071	0.045
G	0.045	0.298	0.174	0.281	0.124	0.055	0.058	0.051	0.07	0.047	0.07	0.045
H	0.045	0.046	0.046	0.046	0.046	0.045	0.045	0.045	0.046	0.046	0.046	0.046

[Plate: Delta OD]

	1	2	3	4	5	6	7	8	9	10	11	12
A	4.00E-03	3.00E-03	2.00E-03	2.00E-03	4.00E-03	3.00E-03	3.00E-03	4.00E-03	2.00E-03	2.00E-03	3.00E-03	3.00E-03
B	3.00E-03	0.028	0.028	0.027	0.027	0.031	0.022	0.021	0.016	0.024	0.02	4.00E-03
C	3.00E-03	0.018	0.047	0.021	0.054	0.023	0.059	0.028	0.014	0.031	0.02	3.00E-03
D	3.00E-03	7.00E-03	0.027	0.025	0.037	0.034	0.027	0.028	0.015	0.029	0.02	3.00E-03
E	3.00E-03	0.036	0.047	0.034	0.077	0.031	0.033	0.023	0.018	0.028	0.182	3.00E-03
F	3.00E-03	0.029	0.029	0.04	0.068	0.029	0.033	0.024	0.186	0.039	0.224	3.00E-03
G	3.00E-03	0.118	0.041	0.029	0.093	0.028	0.035	0.026	0.206	0.029	0.168	3.00E-03
H	4.00E-03	2.00E-03	3.00E-03	2.00E-03	2.00E-03	3.00E-03	3.00E-03	4.00E-03	2.00E-03	3.00E-03	3.00E-03	4.00E-03