Microwave Assisted Intramolecular Heck Cyclization of Aryl Chlorides

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Lauren Marie Chapman

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In the field of

Chemistry

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ABSTRACT OF THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry in the graduate school of Arts and Sciences of Northeastern University, May 2009
ABSTRACT

Since its discovery in the 1970’s, the Heck reaction has become an incredibly powerful synthetic tool in organic chemistry. Under the influence of a palladium catalyst, the Heck reaction forms carbon-carbon bonds between an alkene or an alkyne, and an unsaturated halide. Because the Heck reaction proceeds under mild conditions, functional group tolerance is quite high. As such, the intramolecular Heck reaction in particular has been used extensively to construct complex molecular frameworks and natural products, such as those pioneered by L. E. Overman et al. Challenging Heck reactions of this nature have traditionally been accomplished with iodides and bromides.

Aryl chlorides have not typically been used in the Heck reaction because oxidative addition occurs more easily with bromides and iodides. However, the use of aryl chlorides is highly desirable, as they are more cost effective and readily available over alternative reagents. Within the last decade, G. C. Fu and others have made significant advances in activating aryl chlorides for use in the Heck reaction.

We have investigated the utility of the intramolecular Heck reaction with aryl chlorides as substrates under microwave irradiation. Herein we report that the product mixture of this reaction is influenced by three key reaction conditions; solvent, catalyst loading, and identity of the phosphine ligands. Prepared from simple, commercially available reagents, tricyclic 1,3-dienes, allenes, and single olefins were isolated in good yields. Additionally, the success of this methodology extends to a more general bicyclic scaffold, where allene products were also obtained. At the present time, and to the best of our knowledge, this work represents the first example of allenic compounds prepared under these conditions.
ACKNOWLEDGEMENTS

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I give sincere thanks to Dr. Alexandros Makriyannis for allowing me to utilize space within his laboratory, and access to microwave technology. His kind generosity, enabled me to advance this project to the next level. Especially to Dr. Kiran Vemuri, who always welcomed me in the Center for Drug Discovery, and aside from helping me with chemistry, taught me many valuable life lessons that I will never forget. To my thesis advisor at Northeastern, Dr. Graham Jones, who worked to ensure the success of carrying this project forward, and has always supported my academic achievements as a student. Without his help, this thesis, as well as my extraordinary experience at Northeastern would not have been possible.
I am in debt to the Department of Chemistry and Chemical Biology for affording me the opportunity to earn a Masters degree in five years. Thank you to the countless professors who have helped me to learn not just the technical information, but practical lessons one needs to be successful in the pharmaceutical industry.

And finally, to my friends and family, who have always been the foundation of my success in life. My deepest gratitude to Kevin Eldridge for his infinite patience, understanding, and unconditional love despite the many stressful times that come of the rigors of pursuing a double major as an undergraduate, and a Master’s degree as a graduate student. Thank you for everything, I would not be the person I am without you.
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>AD$_2$BuP</td>
<td>di(1-adamantyl)-$n$-butylphosphine</td>
</tr>
<tr>
<td>AD$_2$BnP</td>
<td>di(1-adamantyl)benzylphosphine</td>
</tr>
<tr>
<td>Ag$_2$CO$_3$</td>
<td>silver carbonate</td>
</tr>
<tr>
<td>Ar</td>
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</tr>
<tr>
<td>AuBr$_3$</td>
<td>gold-III bromide</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Cbz</td>
<td>benzyloxy carbonyl</td>
</tr>
<tr>
<td>CF$_3$</td>
<td>trifluoromethyl</td>
</tr>
<tr>
<td>Cl</td>
<td>chloride</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
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<tr>
<td>CPME</td>
<td>cyclopentyl methyl ether</td>
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<td>Cs$_2$CO$_3$</td>
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<td>CsF</td>
<td>cesium fluoride</td>
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<tr>
<td>CV</td>
<td>column volumes</td>
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<td>Cys</td>
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<tr>
<td>DAD</td>
<td>diode array detection</td>
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<tr>
<td>DavePhos</td>
<td>2-Dicyclohexylphosphino-2′-(N,N-dimethylamino)biphenyl</td>
</tr>
<tr>
<td>DCE</td>
<td>dichloroethane</td>
</tr>
<tr>
<td>DCM</td>
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</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>ECM</td>
<td>extracellular matrix</td>
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<td>---------</td>
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<td>ELSD</td>
<td>evaporative light scattering detection</td>
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<td>HBTU</td>
<td>O-Benzotriazole-N,N,N’,N’-tetramethyl-uronium-hexafluoro-phosphate</td>
</tr>
<tr>
<td>His</td>
<td>histidine</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple bond correlation</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td>HSQC</td>
<td>heteronuclear single quantum coherence</td>
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<tr>
<td>IC$_{50}$</td>
<td>concentration of a drug that is required for 50% inhibition <em>in vitro</em></td>
</tr>
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<td>IPA</td>
<td>isopropyl alcohol</td>
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<td>LC/MS</td>
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<td>MeOH</td>
<td>methanol</td>
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<td>Mg$_2$SO$_4$</td>
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</tr>
<tr>
<td>MMPs</td>
<td>matrix metalloprotinases</td>
</tr>
<tr>
<td>MW</td>
<td>microwave</td>
</tr>
<tr>
<td>Na$_2$CO$_3$</td>
<td>sodium carbonate</td>
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<tr>
<td>Symbol</td>
<td>Definition</td>
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</tr>
<tr>
<td>NaHCO₃</td>
<td>sodium bicarbonate</td>
</tr>
<tr>
<td>NMR</td>
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<tr>
<td>NOE</td>
<td>nuclear overhauser enhancement</td>
</tr>
<tr>
<td>OCH₃</td>
<td>methoxy</td>
</tr>
<tr>
<td>Pd</td>
<td>palladium</td>
</tr>
<tr>
<td>Pd₂(dba)₃</td>
<td>tris(dibenzylideneacetone)dipalladium</td>
</tr>
<tr>
<td>Pd(dppf)Cl₂</td>
<td>[1,1’-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)</td>
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<td>Pd(MeCN)₂</td>
<td>Bis(acetonitrile)dichloropalladium(II)</td>
</tr>
<tr>
<td>Pd(OAc)₂</td>
<td>palladium acetate</td>
</tr>
<tr>
<td>Pd(Ph₃P)₄</td>
<td>palladium tetrakis</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PtO₂</td>
<td>platinum oxide</td>
</tr>
<tr>
<td>r.t.</td>
<td>room temperature</td>
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<td>SAR</td>
<td>structure activity relationship</td>
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<td>S-Phos</td>
<td>2-Dicyclohexylphosphino-2′,6′-dimethoxybiphenyl</td>
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<td>STAB</td>
<td>sodium triacetoxyborohydride</td>
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<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<tr>
<td>TIC</td>
<td>total ion count</td>
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<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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<td>X-Phos</td>
<td>2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl</td>
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Chapter 1: Introduction and Background
1.1 The Heck Reaction

Since its discovery in the early 1970’s, the Heck reaction has become an extraordinarily powerful synthetic tool in organic chemistry. The palladium catalyzed coupling of aryl halides with alkenes and alkynes has been used as an efficient means to prepare a wide variety of compounds. The reaction typically proceeds under very mild, and nearly neutral conditions, thus many sensitive functional groups are tolerated well.\(^1\) For this reason, the Heck reaction is among the most reliable methods for carbon-carbon bond formation, and is used widely in drug discovery.\(^1,2\) Because of its synthetic importance, organic chemists have continued to explore new applications to the Heck reaction, building upon its potential to yield versatile products.

Also of great utility is the intramolecular Heck reaction, which has proven a vital bridge to access many complex molecular frameworks, and heterocyclic ring systems such as those pioneered by L. E. Overman et al. throughout the years.\(^2-4\) A broad spectrum of ring formations, ranging from small to macrocyclic, has been accomplished through this remarkable transformation. In addition, asymmetric intramolecular Heck reactions provide a synthetic strategy for installing sterically hindered tertiary and quaternary stereocenters.\(^3\) To date, some of the most challenging examples of intramolecular Heck cyclizations are accomplished with aryl iodides and bromides to form natural products. Such scaffolds are of interest to both industrial and academic communities for their bioactive properties.
Figure 1.1: Synthesis of (-)-morphine through intramolecular Heck cyclization, Overman, L. E. Pure & Appl. Chem., 1994, 66(7), 1423.

1.2 Aryl Chlorides as Substrates

Because oxidative addition occurs more readily with aryl bromides and iodides, aryl chlorides were traditionally uncommon in the Heck reaction. However, the use of aryl chlorides is highly desirable, as they are more cost effective and readily available over alternative reagents. Thus, recent advances in the methodology of the Heck reaction have expanded the scope to include aryl chlorides as suitable substrates under special reaction conditions. Work by G. C. Fu and others has led to the development of a unique palladium-phosphine ligand complex, palladium tri-\(t\)-butyl phosphine, for efficient catalysis of Heck couplings between activated aryl chlorides and reactive olefins.
Electron rich phosphine ligands of this nature are understood to facilitate reductive elimination of the palladium species in the catalytic cycle, thereby promoting the efficiency of this reaction. As such, these ligands are now widely used in the context of aryl chloride substrates. While many of the advances in accomplishing Heck reactions with aryl chlorides report coupling at room temperature, it is important to note that the olefins employed in these examples are nearly all activated, monosubstituted styrene and acrylic acid derivatives. For this reason, we felt that an opportunity remained to expand the scope of this reaction by utilizing more challenging unsaturated substrates. Thus, we endeavored to investigate the formation of ring-constrained analogues by intramolecular Heck reaction of electron-deficient aryl chlorides.

Figure 1.2: Examples of Heck reactions with aryl chlorides as substrates, Littke, F.; Fu, G. J. Am. Chem. Soc., 2001, 123(29), 6989.
1.3 Introduction to the Project

The core tricyclic scaffold presented in the following report was first discovered as part of a drug discovery program for the treatment of diseases affecting the central nervous system (CNS). The particular cyclization method used to form the final diene product of this compound series was of considerable synthetic interest. On this basis, development of this methodology was decidedly transformed into an academic project, and the subject of this dissertation.

![Chemical Structure](attachment:image.png)

Figure 1.3: General scheme to study the efficiency of the Heck reaction performed with piperdine-based aryl chloride substrates

The goals from the outset of the project were to determine optimal reaction conditions, expand the scope with a variety of functional groups at key locations, and to study the efficiency of aryl chlorides as Heck substrates. As the screen for optimal condition parameters was conducted, we found that allenic products could be obtained by modulating the identity of the electron rich phosphine ligands in coordination to palladium. Furthermore, we discovered that single olefins resulted from reaction in protic solvents such as ethanol, and 2-propanol (IPA). The success of this methodology led to the development of an even more general bicyclic scaffold, where the effect of a lower
catalyst loading was also explored. Throughout the project, microwave irradiation was employed to attain short reaction times, while maintaining high yields. At the present time, and to the best of our knowledge, this work represents the first example of allenic compounds prepared under these conditions.

1.4 Microwave Technology

The use of microwave technology in organic synthesis is highly advantageous in several ways. First, conducting a reaction in the microwave is an excellent alternative to conventional thermal heating, as it exposes the mixture to higher temperatures and pressures, resulting in dramatically shorter reaction times. In both academic and industrial settings, this allows for fast turnaround and expedited synthesis of diverse compound libraries, especially in cases equipped with automated systems. Second, transfer of kinetic energy occurs uniformly within the reaction mixture, as opposed to thermal heating, where energy transfer occurs on the basis of direct contact with the heat source. Finally, microwave technology may enhance the duration of action of some catalysts, and often reduces the formation of many side products.\textsuperscript{10}

Reports by various groups over the years have detailed the many benefits of microwave chemistry for accomplishing challenging Heck reactions.\textsuperscript{10-13} Many of these reports demonstrate ligand-free, or solvent-free conditions, but none have attempted coupling with aryl chlorides as substrates in this context. The experimental methods in this dissertation utilize microwave technology to achieve effective coupling of aryl chlorides in the intramolecular Heck cyclization.
1.5 Mechanism of the Intramolecular Heck Cyclization

Though current research is still focused on elucidating the details of various intramolecular Heck mechanisms, the general catalytic cycle of the reaction is well understood.\(^1\)\(^6\) The reaction begins with oxidative addition of palladium into the carbon-halogen bond. Next, coordination of the unsaturated system with palladium results in migratory insertion of the alkene or alkyne, followed by \(\beta\)-hydride elimination, and the consequential release of an oxidized palladium species. The catalytic cycle concludes with reductive elimination, and the regeneration of Pd\(^0\).

Figure 1.4: Catalytic cycle of the Heck Reaction, Littke, F.; Fu, G. J. Am. Chem. Soc., 2001, 123(29), 6989.

The hypothesis for formation of the diene system presented in this dissertation is believed to proceed through an allenic intermediate.\(^1\)\(^4\) Re-coordination of the initially released oxidized palladium species results in a subsequent syn \(\beta\)-hydride elimination, and final release of the hydrochloride palladium species, as shown in figure 1.5. Subsequent reductive elimination results in regeneration of Pd\(^0\) and formation of HCl. Thus, it is proposed that the cyclized substrate therefore undergoes an overall olefin migration,
furnishing 1,3-diene products. It is detailed herein that under certain reaction conditions, the allenic “intermediate” is in fact captured and able to be isolated as a stable product of the intramolecular Heck reaction.

Figure 1.5: Proposed mechanism for formation of tricyclic 1,3 diene products
Chapter 2: Results and Discussion
2.1 Optimization of Reaction Conditions

Because oxidative addition of palladium occurs more readily in electron deficient aromatic systems, development of this methodology began by substituting the aryl ring with an electron-withdrawing trifluoromethyl (CF$_3$) substituent. With this intermediate in hand, we endeavored to find optimal reaction conditions that would both minimize reaction time and maximize yield. Initial experiments conducted on the bench proved that conversion to product proceeded very slowly, with at least half of the starting material still present by LC/MS after 72 hours of thermal heating to 90°C. To rectify this issue, we turned to microwave irradiation, under which the reaction proceeded to completion in just 20 minutes at 140°C. This was extremely fortuitous, as it facilitated the rapid screening of a number of catalytic systems. Several combinations of palladium sources and ligands were explored, as well as a number of bases (both inorganic and organic), and solvents. Drawing from previously reported Heck conditions, we used cesium carbonate and dioxane to identify optimal catalytic systems. Once these were established, each of our best ligands were used in conjunction with palladium acetate (our most successful catalytic source) to evaluate the success of different bases and solvents, as summarized in Table 2.1.
Surprisingly, the conditions reported by Fu et al. failed to give any conversion to product. In fact, all instances where tris(dibenzylideneacetone)dipalladium was employed as a catalytic source (even in conjunction with cesium carbonate) were unsuccessful. Furthermore, dicyclohexylmethylamine (Cy₂MeN) proved detrimental to the reaction, as no product conversion was observed when used with Pd(OAc)₂/AD₂BuP in dioxane, a combination that otherwise gives optimal results with Cs₂CO₃ as a base. It was also surprising to find that the reaction proceeded just as well in ethyl acetate (EtOAc), as in
dioxane. Because EtOAc is regarded as more environmentally friendly and readily available, we chose to include this solvent in our final optimized reaction conditions.

During the course of conditions optimization screening, we evaluated the success of each reaction by LC/MS analysis. Therefore, the percent conversions reported in table 2.1 reflect relative concentrations of starting material versus product, as determined by the intensity of the corresponding major peaks in each chromatogram. Reactions that showed full conversion to the expected product mass were worked up, purified, and further analyzed by $^1$H NMR spectroscopy. Over the course of this process, we discovered that two distinct spectral patterns were consistently obtained with an apparent correlation to the identity of the phosphine ligands used in the reaction. Generally, we observed that reaction with 2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (X-Phos) gave allenic products, while reaction with Di(1-adamantyl)-n-butylphosphine (AD$_2$BuP) tended to give diene products. Nearly all reactions with bis(tri-$t$-butyl phosphine) gave diene products as well. Figure 2.1 summarizes this trend, as well as our optimal reaction parameters, which were used in all subsequent reactions for the development of this methodology.

Figure 2.1: Optimal reaction conditions established for tricyclic system
2.2 Aryl Substitution

In an effort to explore the effects of different substituents on the aryl ring, we chose three key electronic cases to evaluate the success of this methodology. Thus, electron-withdrawing, as well as electron-donating groups were investigated in addition to no substitution, which we regard as the “neutral” case. The reaction proceeded quickly in the context of CF$_3$ substitutions, and we obtained good to excellent isolated yields with this series of compounds. This success was generally consistent, and sometimes even better, in the absence of aryl substitution.

Because palladium is coordinated to very electron-rich ligands in these reactions, oxidative addition is more facile when the complex approaches an electron-deficient system.$^{15}$ Highly electronegative substituents like CF$_3$ decrease the electron density of the aromatic ring by drawing electrons toward the fluorine atoms. Thus, the C-Cl bond becomes activated toward oxidative addition by the presence of electron-withdrawing substituents. Conversely, aromatic systems substituted with groups that increase the electron density of the ring are more sterically demanding, and oxidative addition is more challenging.$^6$ For this reason, we were not very optimistic about installing electron-donating groups on the aryl ring, but were surprised to obtain decent yields with methoxy (OCH$_3$) substituents, particularly when X-phos was used as a ligand. An overview of the electronic effects of these various aryl substitutions is given in table 2.2.
As was observed during the conditions optimization screen, we again found that the identity of the phosphine ligands used in the reaction was influential in determining the final structure of the isolated product. For this reason, we conducted experiments for each aryl substitution three times, using X-Phos, AD$_2$BuP, and Pd(t-Bu$_3$P)$_2$ in each electronic case. Table 2.2 details the structural results obtained from these trials. In a general sense, X-Phos exclusively gave allene products, while Pd(t-Bu$_3$P)$_2$ afforded diene products in most cases. Conversely, AD$_2$BuP was observed to give both products in different substitution contexts. The data collected therefore suggests that the electronic effects of the various aryl substituents may play a role (in conjunction with the ligands coordinated to palladium) in directing the final product structure. Accordingly, AD$_2$BuP affords both
allene and diene products depending on the nature of the aryl substituent on the Heck substrate. This is a preliminary conclusion, and clearly requires validation by further investigation with a wider variety of aryl substitution profiles.

In all cases discussed thus far, aryl substitutions were evaluated in the meta position, with respect to the chlorine atom on the aryl ring. In addition, we explored the effects of the CF₃ group in the para position. We expected this reaction to be markedly more successful because the electron-withdrawing effect would presumably be more pronounced when placed para to the site of oxidative addition. In fact, we found that compounds of this type give similar yields to m-CF₃ substrates, in the mid to high 70’s. Another interesting case that was tried in this context was p-OCF₃, which gave a moderate 52% yield; not as good as electron-withdrawing CF₃, yet significantly more successful than the electron-donating OCH₃ group.

2.3 Acetylene Substitution

2.3.1 Cyclohexylacetylene

Next, we were interested to see how isomerization to the diene product would be affected by varying the side chain off the acetylene moiety, where the β-hydride elimination events occur. We began by substituting with cyclohexylacetylene at the R₂ position shown in table 2.3. The formation of compounds with this moiety were especially interesting because the allene, if formed, would be placed between two ring junctures. Alternatively, the terminal olefin of the diene would interrupt the chair conformation of the aliphatic cyclohexyl ring. We were pleased to find excellent yields for both ligand
systems in the case of CF₃ substitution on the aryl ring. Because the diene product was substantially favored, we believe the structural configuration of the allene between the two ring junctures is energetically unfavorable.

It is noteworthy that the isolated yields of the simple aryl substitution experiments were comparable between the electron-withdrawing and neutral cases. However, these experiments show that there is a far greater disparity in success of the reaction when the structure is varied at the site of hydride elimination/isomerization. Here we observe more clearly that an electron deficient aryl ring is activated toward oxidative addition, and accordingly participates in the intramolecular Heck reaction more efficiently.

2.3.2 4-Phenyl-1-butyne
We hypothesized that formation of the diene would be favored when a system in resonance with the aryl ring could be created. To test this, we installed 4-Phenyl-1-butyne, which we postulated would form a diene in full conjugation with both aromatic ring systems. In fact, compounds baring this moiety were isolated as mixtures from reactions using both X-Phos and AD₂BuP. This result was quite surprising, as we did not expect allene product to form under the influence of AD₂BuP ligands based on our previous observations, especially given our reasoned driving force for formation of the diene product. It is noteworthy however, that the predominant product formed was in fact diene, isolated from the allene structural isomer in a 2:1 ratio.
The allene still formed as the major product in reaction with X-Phos, a result that agrees with our earlier findings during simple aryl substitution experiments. In this context we also observed some formation of the diene isomer, with a final ratio of 4:1 allene/diene products. This could possibly be attributed to our earlier postulation that it is more favorable to form a fully conjugated system, thereby promoting some isomerization to diene. However, this explanation seems contrary to our observations of the same reaction with AD$_2$BuP, as discussed previously. For this reason, it is clear that the formation of the final product is not exclusively determined by the identity of the phosphine ligands. Instead, it seems likely that palladium itself plays a larger role in influencing the final product structure.

Table 2.3: Establishing reaction scope with acetylene substitution
2.4 Aldehyde Substitution

We attempted to vary the position opposite the acetylene moiety by using paraformaldehyde in the Mannich reaction (see chapter 3 for synthesis of Heck intermediates). All yields for even aryl CF$_3$ compounds in this series of reactions were less than 10%. Thus, it appears that some steric bulk in this position is necessary for the reaction to be successful. This observation is likely due to the fact that more sterically demanding substituents would lock the conformation of the acetylene moiety into an orientation that is within advantageous proximity to the C-Cl bond for efficient participation in the Heck reaction.

2.5 Characterization of the Products

Diene product gave three distinct signals in routine $^1$H NMR spectra, with chemical shifts within the olefin region between 5-7ppm. These signals, highlighted in figure 2.2 accurately represent the positions of protons d, e, and f corresponding to the doublet, and two doublet of doublets respectively, from left to right.
Figure 2.2: $^1$H NMR of compound 2 showing signature 1,3 diene signals

Of note are the minor signals that are most visible in the enhanced selection of the spectra. These signals correspond to formation of a minor 2,4 diene isomer illustrated in figure 2.3. This structural variant was isolated from the major product by flash chromatography and characterized by $^1$H NMR separately (figure 2.3).
Figure 2.3: $^1$H NMR of minor isomer showing migrated 2,4 diene signals

One thing that could not be deduced from simple proton NMR spectra was the orientation of the olefin side chain with respect to the aryl ring. To determine this, proton g was selectively irradiated in a nuclear overhauser enhancement (NOE) experiment. As shown in figure 2.3, the signal corresponding to olefin proton e is markedly enhanced, indicating that there is a close spatial relationship between the two. This information establishes the olefin geometry, as the resonance for proton d would be expected to show enhancement if the side chain were oriented in the other direction.
Additionally, selective irradiation of proton g also gives information about the stereochemistry at centers g and h. Because no enhancement is observed for resonance h in the spectra above, it may be concluded that protons g and h are oriented trans across the central ring of the tricyclic system. To confirm this, another selective NOE was preformed with resonance h irradiated. While weak enhancements were observed for nearby protons, c, j, l, and q, there is clearly no significant enhancement of g, as would be expected if g and h were located on the same face of the ring.
Figure 2.5: NOE spectrum of compound 2 with selective irradiation of proton “h”

The final stereochemical issue left outstanding is the orientation of the isopropyl group at
the end of the olefin side chain. With only two possible cis and trans conformations,
molecular mechanics was employed to confirm that the lowest energy conformer is
indeed the trans product, as depicted in the ball and stick model in figure 2.6.
With the diene product extensively characterized, we were confident that the alternative NMR spectrum obtained from reaction of 1 with X-Phos ligands had to be a structural variant of the same mass. In an effort to elucidate the structure of this apparent isomer, we turned to the mechanism proposed at the outset of this investigation. After migratory insertion of the acetylene into the palladium-carbon bond, the resulting Pd$^{\text{II}}$-Cl species undergoes elimination with the only available adjacent β-hydride to form an allenic intermediate. Though we initially believed that this allene would be unstable, we considered the possibility that in fact this structure could be isolated. From a spectroscopic standpoint, the triplet signal shown in figure 2.7 is consistent with an allenic moiety, as the only olefin proton would be spilt twice by the adjacent methylene.
Figure 2.7: $^1$H NMR of neutral tricyclic allene

Definitive evidence of the allenic structure was obtained from a heteronuclear multiple bond correlation (HMBC) experiment, which gives information about proton-carbon associations 2, 3 or sometimes 4 bonds away from each other. The 2D HMBC spectrum in figure 2.8 shows long range couplings between the carbon at 201.7ppm and three key protons, e, g and j. Such a highly deshielded chemical shift of 201.7ppm in the $^{13}$C spectrum is in fact quite characteristic of an allenic central carbon. Therefore, in conjunction with HSQC data, indicating an absence of protons on carbon A, the structure of the allene was confirmed.
It is noteworthy that as with the diene, a minor signal was observed in the $^1$H NMR spectrum at a slightly different chemical shift than the primary “allenic triplet.” This signal corresponds to a minor diastereomer of the allene, possessing the opposite spatial orientation of the aliphatic side chain protruding from the allenic moiety.

Figure 2.8: HMBC spectrum of neutral allene showing signature carbon at 201.7 ppm
2.6 Reaction in Protic Solvents

2.6.1 Reaction in Ethanol

During our solvent screen to determine optimal conditions, we discovered a third structural variant that could be isolated when the reaction was run in protic solvents. We chose to attempt the reaction in ethanol because it is a particularly high microwave absorbing solvent (and therefore most efficient for use with microwave technology). In ethanol, two additional mass units than were expected for typical allene/diene products were detected by LC/MS analysis. Upon isolation and NMR characterization, we found that the chemical shift of what looked at first to be the signature allenic triplet at 5.43ppm was now shifted farther downfield at nearly 6.0ppm.
Figure 2.10: LC/MS trace for single olefin

Figure 2.11: LC/MS trace for allene product
Since the formation of a single olefin is often more synthetically useful, (particularly at a ring juncture where the formation of a quaternary stereogenic center is possible) we endeavored to explore this new product quite extensively. Table 2.4 summarizes the experiments that we ran to establish the scope of this new reaction. While most of our substrates were aryl CF$_3$ substituted, we also tried some cases with OCH$_3$ in an effort to discover whether or not this system would tolerate the most challenging electronic case. Interestingly, our best results were obtained when substituted with 5-ethynyl-1-methyl-
1H-imidazole on an Ar-OCH₃ substrate. This same reaction was not attempted with an Ar-CF₃ substrate due to material availability and time constraints, however it would be interesting to see if higher yields could be attained with this combination.

2.6.2 Reaction in 2-Propanol

Seeing as the yields in reaction with ethanol were generally poor, we turned to 2-propanol (IPA) as an alternative protic solvent. Reaction in IPA gave dramatically higher isolated yields as summarized in table 2.6.2. Higher yields were actually obtained in the
absence of substituents on the aryl ring, though the figures are generally comparable to those compounds that contain a m-CF$_3$ group. This deviation from the general trend was also observed when AD$_2$BuP was used as a ligand in the simple aryl substitution experiments. Thus, the data suggests that when the acetylene moiety is 5-Methyl-1-hexyne, and the ligand is AD$_2$BuP, the reaction proceeds in higher yield for neutral cases. Again, this reaction was not performed under the influence of X-Phos ligands due to time constraints, but would be advantageous in providing further evidence in support of, or against this apparent finding.

Table 2.5: Reaction scope of single olefin variants in 2-Propanol

<table>
<thead>
<tr>
<th>$R_1$</th>
<th>$R_2$</th>
<th>Ligand</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td></td>
<td>AD$_2$BuP</td>
<td>78%</td>
</tr>
<tr>
<td>CF$_3$</td>
<td></td>
<td>AD$_2$BuP</td>
<td>70%</td>
</tr>
</tbody>
</table>

2.6.3 Proposed Mechanism

Given that this new product was isolated only in instances where a protic solvent was used, we turned to our originally proposed reaction mechanism in order to elucidate how exactly this structural variant was formed. After migratory insertion of the acetylene into the palladium-carbon bond, the newly formed Pd$^{II}$-Cl species normally undergoes syn $\beta$-hydride elimination to form the allene. We postulate that in ethanol, the electrons of the
The sharp increase in insolated yield for reactions run in IPA, as compared to those run in ethanol, supports this mechanism. As shown in figure 2.14, the nucleophilic hydride still originates from the solvent, but generates acetone instead of formaldehyde when the carbonyl is formed. We believe the formation of a more stable byproduct is responsible for the higher success rate of the reaction in IPA.
2.7 Bicyclic Scaffold Variation

2.7.1 Introduction

This methodology was so successful, that we wanted to expand the scope not just of the substitutions, but also of the core scaffold itself. There are several advantages to the bicyclic scaffold we chose to pursue. First, it provides an alternative to the constrained tricyclic system explored thus far. Second, a new point of versatility is introduced in the addition of another aromatic ring. Finally, we were able to avoid a problematic hydrogenation described in chapter 3, by simplifying the synthetic route to access the Heck intermediates.

Figure 2.14: Proposed mechanism of product formation in 2-propanol
2.7.2 Effect of Catalytic Loading

Because this was an entirely new scaffold, we wanted to use this opportunity to see if we could lower the catalyst loading from 15mol% to 5mol%. This lower loading was attempted during the conditions optimization screen of catalysts for the tricyclic scaffold, but was largely unsuccessful, with little to no product formation detected by LC/MS analysis.

Fortuitously, we were able to obtain yields of marketed improvement over the more challenging tricyclic counterparts. However, we found that the reaction often gave a mixture of allene and diene product for both ligands when 15mol% palladium was used. Conversely, we were successful in modulating the product structure exclusively when the palladium catalyst loading was reduced to 5mol%. In this context, we observed the same general trend as previously established; AD$_2$BuP gives diene, and X-Phos gives allene product. While exclusive product formation, and a lower catalyst loading are ideal, the isolated yields of 5 mol% reactions for each ligand suffered by 20% or more. This sacrifice in yield is most pronounced in the case of X-Phos, whereas yields for AD$_2$BuP are still well within an acceptable range, particularly for applications in drug discovery.
Table 2.6: Variation in catalyst loading for bicyclic scaffold

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD$_2$BuP</td>
<td>2:1 Allene/Diene</td>
<td>100%</td>
</tr>
<tr>
<td>X-Phos</td>
<td>2:1 Allene/Diene</td>
<td>72%</td>
</tr>
<tr>
<td>Pd(t-Bu$_3$P)$_2$</td>
<td>Diene</td>
<td>37%</td>
</tr>
<tr>
<td>AD$_2$BuP</td>
<td>Diene</td>
<td>88%</td>
</tr>
<tr>
<td>X-Phos</td>
<td>Allene</td>
<td>38%</td>
</tr>
</tbody>
</table>

It is noteworthy that while a reduction in catalyst loading helped avoid isomeric mixtures, it also changed the identity of the major product in the case of AD$_2$BuP. With 15mol% Pd, this ligand predominantly gives allene product, while a lower loading switches completely to give diene product only. This observation further supports the earlier assertion that palladium itself likely plays a major role in determining the final structure of the products.

2.7.3 Aldehyde Substitution

As stated previously, substitution with paraformaldehyde in the position opposite the acetylene moiety was unsuccessful on the tricyclic scaffold. However, because we were able to lower the catalyst loading with the bicyclic scaffold when it had originally failed
with the tricyclic compounds, we reasoned that paraformaldehyde substitution on this new series would be more promising. Indeed, we found that yields were generally much better than our previous attempts, especially in the case of AD$_2$BuP, albeit with isomeric mixtures at 15mol% Pd. Again, we observe a drop in yield by about 20% when the catalyst loading is decreased to 5mol%. These findings are summarized in table 2.7.

Table 2.7: Aldehyde substitution for bicyclic scaffold

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD$_2$BuP</td>
<td>3:1 Allene/Diene</td>
<td>49%</td>
</tr>
<tr>
<td>X-Phos</td>
<td>Allene</td>
<td>20%</td>
</tr>
<tr>
<td>Pd(t-Bu$_3$P)$_2$</td>
<td>Diene</td>
<td>5%</td>
</tr>
<tr>
<td>AD$_2$BuP</td>
<td>Allene</td>
<td>25%</td>
</tr>
<tr>
<td>X-Phos</td>
<td>Allene</td>
<td>6%</td>
</tr>
</tbody>
</table>

Due to availability of reagents and time constraints, we were not able to explore further substitution on either aryl ring. However, substitution in these positions may provide an opportunity to elucidate the parameters that ultimately affect the structure of the final/major product.
Chapter 3: Experimental Methods
3.1 Introduction

3.1.1 Tricyclic Scaffold

Synthesis of the tricyclic Heck substrates was accomplished in a straightforward three-step process, consisting of an initial Suzuki coupling, followed by selective hydrogenation of the pyridine ring, and a gold-III-catalyzed Mannich reaction in water.\(^{16}\)

![Synthetic Route to Tricyclic Heck Substrates](image)

Figure 3.1: Synthetic Route to Tricyclic Heck Substrates

Nearly all Suzuki reactions proceeded to completion, resulting in good to excellent yields, and crystalline products. However, the selective hydrogenation of the pyridine ring was consistently problematic. Initially, this reaction was attempted at high atmospheric hydrogen pressures on a parr shaker. The product obtained was fully hydrogenated across both ring systems. As a result, the pressure was reduced, but in all cases reaction on the parr shaker was unsuccessful. Next we turned to a simple balloon...
atmosphere of hydrogen on the bench. After about 2 hours, we detected formation of a significant byproduct by LC/MS. As the starting material was consumed, a small peak in the chromatogram grew over time, corresponding to 34 mass units less than the expected product mass. Upon isolation and characterization, we determined that the byproduct was in fact the result of dechlorination.

Since methanol is an active solvent in hydrogenation, we attempted the reaction in a solution of 9:1 ethyl acetate/acetic acid. Though this slowed the reaction, a considerable amount of starting material was still lost to the formation of the dechlorinated byproduct. In an effort to screen a number of catalysts and solvent systems, we turned to an internal resource within Merck. The reaction was submitted to the hydrogenation lab for optimization in Rahway, New Jersey. After running a comprehensive screen, the group determined that the final conditions we arrived at in fact gave optimal yields. Thus, we were forced to accept poor yields and carry the synthesis forward by scaling up this intermediate. Because of this issue, we were eager to pursue an alternate scaffold that would avoid this problematic step.

Fortunately, the Mannich reaction proceeded to very high yields, and proved quite robust across many different substitution profiles. A simple aqueous extraction from ethyl acetate cleared the reaction of most impurities. Since many alkyl groups were attached to the substrate at this point, most of the product from these reactions eluted from a flash column fairly quickly, helping to expedite the development of this methodology.
3.1.2 Bicyclic Scaffold

With the prospect of a new core scaffold, we sought to avoid the aforementioned problematic hydrogenation. In so doing, we replaced both Suzuki and hydrogenation steps with a reductive amination between benzyl amine and 2-chlorobenzaldehyde, followed by the gold-catalyzed Mannich reaction as before. This afforded our bicyclic Heck intermediates in only two synthetic steps as shown in figure 3.2.

Figure 3.2: Synthesis of bicyclic Heck substrates
3.2 Reagents & Instrumentation

All reagents used in preparation of Heck substrates were obtained from commercial sources. Product compounds were purified using flash chromatography on Biotage SP1 systems equipped with silica gel columns of various size, depending on scale of the reaction. Nearly all columns used were in the range between 25+S and 40+M models, as manufactured by Biotage.

All reactions were monitored by an LC/MS system equipped with diode array detection (DAD) to visualize product formation. Compounds without active chromophores were detected by total ion count (TIC) traces recorded by the MS component. The majority of compounds were synthesized at Merck Research Laboratories, where analysis of products was preformed with an Agilent 1200 series HPLC system coupled to an Agilent LC/MSD SL mass spectrometer. This system was routinely run over the course of 2.3 minutes, with an eluent 50-98% acetonitrile/water and 0.05% trifluoroacetic acid. A select number of tricyclic compounds, and all bicyclic products were synthesized and analyzed in the Center for drug discovery at Northeastern University, where a Waters LC/MS system was utilized, equipped with 2424 ELSD and 2996 PDA detectors, coupled to a ZQ micromass single quad mass spectrometer. Finally, the structures of all product compounds reported herein were determined by spectra acquired by a Varian Inova 600MHz NMR system.
3.3 Experimental Methods and Procedures

3.3.1 General Procedure for Suzuki Coupling

2-chloropyridine (1.00 eq) and the corresponding substituted 2-chloroboronic acid (1.20 eq) were combined in a 0.100 M solution of anhydrous ethanol and sealed in a 20 ml microwave vial. The reaction mixture was irradiated in the Biotage initiator microwave system for 30 minutes at 140°C. Reaction completion was generally characterized by transformation to a deep orange or red color after irradiation. The reaction was filtered from the solid residue, which was washed with ethyl acetate and filtered again over Whatman filtration paper. Solvent was removed by rotary evaporation, and the crude residue purified on a 40+M silica gel column 2-15% EtOAc/Hexanes over 50 CV. Purity of fractions containing product was monitored by TLC (20% EtOAc/Hex). Collected material is a white, crystalline solid.

3.3.2 General Procedure for Hydrogenation

Products of the previous Suzuki coupling were dissolved in a 0.100 M solution of 9:1 ethyl acetate/acetic acid and degassed with nitrogen in a three-necked flask for 10 minutes. To the dry solvent was added 10 mol% PtO₂ and placed under a balloon of atmosphere of H₂ gas. The reaction mixture was allowed to stir at room temperature for several hours, during which time product formation was monitored by LC/MS analysis. When byproduct formation approached a 1:1 ratio with desired product (typically 2-4 hours), the reaction was quenched with Na₂CO₃, and extracted from ethyl acetate. The organic layer was dried over MgSO₄ and filtered. Solvent was removed by rotary evaporation, and the crude residue purified via flash chromatography on a 40+M biotage
column. Product was identified in fractions by LC/MS and ninhydrin stain. Collected material is a clear oil.

3.3.3 General Procedure for Reductive Amination
2-chlorobenzaldehyde (1.00eq), benzylamine (1.20eq), and sodium triacetoxyborohydride (1.50eq) were combined in a 250 ml round bottom flask and stirred in a 0.100M solution of DCE at room temperature for 12 minutes. The reaction mixture was quenched with NaHCO₃, and extracted from a partition between water and ethyl acetate. The organic layer was collected, and solvent was removed via rotary evaporation. The crude residue was purified by flash chromatography on a 25+M biotage column gradient: 0-30% DCM/MeOH over 60 CV. Product was identified by LC/MS and ninhydrin stain. Collected material is a white crystalline solid.

3.3.4 General Procedure for the Mannich Reaction
The hydrogenated product was combined with 10mol% AuBr₃, and the corresponding acetylene and aldehyde (2.50 eq each) in a 0.100M solution of deionized water, and sealed in a 20 ml microwave vial. The reaction mixture was stirred in an oil bath at 75°C overnight (18 hours). Product formation was confirmed by LC/MS analysis, upon which the reaction mixture was diluted with ethyl acetate and extracted from brine. The organic layer was collected and dried over Mg₂SO₄. Solvent was via rotary evaporation. The crude residue was purified via flash chromatography on a 25+S Biotage column, gradient: 0% 3CV, 0-10% 40CV. Collected material is a yellow oil.
3.3.5 General Procedure for the Intramolecular Heck Cyclization

To the dry Mannich product was added 15mol% Pd(OC)\(_2\), 30mol% ligand, and Cs\(_2\)CO\(_3\) (1.50 eq). The mixture was dissolved in a 0.100M solution of EtOAc, sealed in a microwave vial, and irradiated at 140\(^\circ\)C for 30 minutes. Reaction completion was generally characterized by transformation to a deep brown or black color after irradiation. The reaction was filtered over celite and solvent removed from the filtrate by rotary evaporation. The crude residue was purified via flash chromatography on a 25+S Biotage column, gradient: 0-15% 30CV. Collected material is a yellow oil.

3.4 NMR Characterization Data

3.4.1 \(^1\)H NMR Data for Allene Heck Products

6-Isobutyl-7-(4-methyl-pent-1-enylidene)-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline \(^1\)H NMR (600 MHz, cdcl3) δ 7.47 (dd, \(J = 7.0, 2.2, 1\)H), 7.22 – 7.18 (m, 1H), 7.15 (dt, \(J = 10.6, 3.6, 2\)H), 5.41 (t, \(J = 7.4, 1\)H), 3.97 (s, 1H), 3.53 (dd, \(J = 10.4, 3.5, 1\)H), 2.73 – 2.66 (m, 2H), 2.11 (d, \(J = 17.1, 1\)H), 2.01 (dt, \(J = 14.0, 3.8, 3\)H), 1.94 (s, 1H), 1.69 (dt, \(J = 19.3, 6.4, 4\)H), 1.62 (s, 2H), 1.54 – 1.44 (m, 4H), 1.44 – 1.38 (m, 2H), 0.93 (t, \(J = 6.7, 7\)H), 0.91 (d, \(J = 6.6, 4\)H), 0.84 (d, \(J = 6.7, 3\)H).

6-Isobutyl-7-(4-methyl-pent-1-enylidene)-10-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline \(^1\)H NMR (600 MHz, cdcl3) δ 7.55 (d, \(J = 8.2, 2\)H), 7.43 (s, 2H), 7.36 (d, \(J = 8.1, 2\)H), 5.45 (t, \(J = 7.5, 2\)H), 3.86 (s, 2H), 3.49 (dd, \(J = 10.1, 4.1, 2\)H), 2.70 – 2.57 (m, 4H), 2.09 (s, 2H), 2.03 (t, \(J = 7.0, 4\)H), 1.84 (s, 2H), 1.69 (ddd, \(J = 41.7, 13.7, 7.0\), 1H).
24.2, 17.5, 9H), 1.54 (s, 2H), 1.48 (ddd, \(J = 13.2, 9.0, 4.2, 5H\), 1.36 – 1.28 (m, 3H), 1.23 (s, 1H), 0.94 (t, \(J = 6.4\), 13H), 0.91 (d, \(J = 6.6\), 7H), 0.84 (d, \(J = 6.7\), 7H).

6-Isobutyl-7-(4-methyl-pent-1-enylidene)-9-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline \(^1\)H NMR (600 MHz, CDCl3) \(\delta\) 7.70 (s, 3H), 7.35 (d, \(J = 8.2\), 4H), 7.29 (dd, \(J = 8.1, 4.9\), 5H), 6.61 (d, \(J = 11.0\), 1H), 6.33 (dd, \(J = 14.6, 11.3\), 1H), 5.91 (dd, \(J = 14.9, 6.4\), 1H), 5.57 (t, \(J = 7.7\), 1H), 5.46 (t, \(J = 7.4\), 3H), 3.87 (d, \(J = 6.3\), 4H), 3.77 (s, 1H), 3.68 (d, \(J = 6.5\), 1H), 3.50 (dd, \(J = 9.5, 4.6\), 4H), 2.80 – 2.56 (m, 10H), 2.41 (dq, \(J = 13.4, 6.8\), 1H), 2.15 (s, 3H), 2.12 – 1.97 (m, 11H), 1.86 (s, 3H), 1.79 – 1.60 (m, 22H), 1.57 (s, 10H), 1.48 (dd, \(J = 11.9, 7.7\), 15H), 1.38 – 1.21 (m, 6H), 1.04 (d, \(J = 6.8\), 6H), 1.00 (d, \(J = 6.7\), 4H), 0.98 (d, \(J = 6.7\), 4H), 0.94 (d, \(J = 6.9\), 9H), 0.93 (d, \(J = 6.8\), 9H), 0.91 (d, \(J = 6.6\), 8H), 0.89 (s, 5H), 0.86 (d, \(J = 5.1\), 6H), 0.84 (s, 6H), 0.79 (d, \(J = 6.2\), 3H).

6-Isobutyl-7-(3-phenyl-propenylidene)-9-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline \(^1\)H NMR (600 MHz, CDCl3) \(\delta\) 7.75 (s, 3H), 7.39 (d, \(J = 6.9\), 5H), 7.31 (dd, \(J = 7.9, 5.6\), 8H), 7.27 (d, \(J = 7.2\), 6H), 7.25 – 7.23 (m, 8H), 7.19 (d, \(J = 7.2\), 3H), 5.84 (t, \(J = 7.2\), 1H), 5.69 (t, \(J = 7.2\), 3H), 3.91 (s, 3H), 3.49 (dd, \(J = 11.7, 7.2\), 11H), 2.51 (s, 6H), 2.14 (s, 2H), 2.07 (s, 3H), 1.90 (s, 3H), 1.75 – 1.62 (m, 13H), 1.58 (d, \(J = 5.7\), 7H), 1.54 – 1.42 (m, 13H), 1.38 – 1.31 (m, 4H), 1.04 (d, \(J = 5.8\), 2H), 0.93 (d, \(J = 6.6\), 8H), 0.91 (d, \(J = 6.7\), 4H), 0.86 (d, \(J = 6.7\), 9H), 0.85 (d, \(J = 6.6\), 5H).
6-Isobutyl-7-(4-methyl-pent-1-enylidene)-10-trifluoromethoxy-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) δ 7.46 (d, $J = 8.6$, 4H), 7.03 – 6.98 (m, 13H), 6.53 (d, $J = 11.0$, 1H), 6.37 – 6.28 (m, 1H), 5.84 (dd, $J = 14.9$, 6.6, 1H), 5.53 (t, $J = 7.6$, 1H), 5.41 (t, $J = 7.4$, 4H), 3.86 (s, 5H), 3.47 (dd, $J = 9.9$, 4.6, 5H), 2.66 – 2.57 (m, 10H), 2.09 (s, 4H), 2.02 (t, $J = 7.5$, 12H), 1.85 (s, 4H), 1.74 – 1.57 (m, 30H), 1.52 – 1.43 (m, 18H), 1.34 (ddd, $J = 33.9$, 19.5, 9.5, 9H), 1.23 (s, 12H), 0.93 (t, $J = 6.4$, 24H), 0.91 (d, $J = 6.6$, 17H), 0.85 (d, $J = 6.6$, 15H).

3.4.2 $^1$H NMR Data for Diene Heck Products

6-Isobutyl-7-(4-methyl-pent-2-enylidene)-9-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) δ 7.79 (s, 1H), 7.39 (d, $J = 7.9$, 1H), 7.28 (d, $J = 8.2$, 1H), 6.61 (d, $J = 10.9$, 1H), 6.40 – 6.27 (m, 1H), 5.91 (dd, $J = 15.0$, 6.5, 1H), 3.87 (d, $J = 9.7$, 1H), 3.69 (d, $J = 5.1$, 1H), 2.82 – 2.71 (m, 1H), 2.66 (s, 1H), 2.41 (qt, $J = 13.0$, 6.6, 1H), 2.16 (d, $J = 12.0$, 1H), 1.81 – 1.34 (m, 13H), 1.04 (d, $J = 6.8$, 6H), 0.96 (d, $J = 5.9$, 3H), 0.79 (d, $J = 6.3$, 3H).

6-Isobutyl-7-(4-methyl-penta-1,3-dienyl)-9-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) δ 7.35 (d, $J = 8.2$, 1H), 7.30 (s, 1H), 7.22 (d, $J = 8.2$, 1H), 6.32 (dd, $J = 15.1$, 10.8, 1H), 5.98 (dd, $J = 15.2$, 8.8, 1H), 5.77 (d, $J = 11.4$, 1H), 3.37 (d, $J = 10.8$, 1H), 3.24 (d, $J = 8.7$, 1H), 2.78 (d, $J = 10.0$, 1H), 2.69 – 2.56 (m, 3H), 2.16 (s, 2H), 1.86 (s, 1H), 1.75 (d, $J = 13.7$, 6H), 1.53 (s, 5H), 1.23 (s, 6H), 0.87 (d, $J = 6.5$, 3H), 0.82 (d, $J = 6.6$, 4H), 0.05 (s, 3H).
3.4.3 $^1$H NMR Data for Single Olefin Heck Products

6-Isobutyl-7-(4-methyl-pentylidene)-9-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) $\delta$ 7.73 (s, 4H), 7.38 (d, $J = 8.1$, 4H), 7.27 (d, $J = 8.3$, 3H), 5.99 (t, $J = 7.2$, 3H), 5.57 (t, $J = 7.6$, 1H), 5.46 (t, $J = 7.5$, 1H), 3.73 (dd, $J = 9.9$, 3.3, 3H), 3.70 (d, $J = 7.4$, 3H), 3.50 (s, 2H), 2.71 (d, $J = 11.1$, 5H), 2.64 (dd, $J = 12.3$, 7.5, 6H), 2.27 (dt, $J = 17.0$, 6.5, 4H), 2.15 (t, $J = 16.2$, 8H), 2.07 – 2.01 (m, 4H), 1.72 – 1.57 (m, 27H), 1.54 – 1.36 (m, 20H), 1.36 – 1.26 (m, 7H), 1.05 (dd, $J = 16.1$, 6.7, 5H), 0.99 (dd, $J = 13.2$, 6.6, 7H), 0.94 (t, $J = 6.0$, 17H), 0.91 (dd, $J = 6.6$, 1.8, 25H), 0.87 – 0.83 (m, 7H), 0.80 (d, $J = 6.3$, 10H).

6-Isobutyl-9-methoxy-7-(3-methyl-3H-imidazol-4-ylmethylene)-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) $\delta$ 7.49 (s, 1H), 7.14 (d, $J = 5.6$, 3H), 7.10 (s, 1H), 6.84 (d, $J = 8.6$, 2H), 6.55 (s, 1H), 5.27 (s, 3H), 4.09 (d, $J = 7.1$, 1H), 4.03 (s, 1H), 3.88 (s, 2H), 3.82 (s, 5H), 3.65 (s, 2H), 3.62 (s, 4H), 2.66 (s, 2H), 2.59 (s, 2H), 2.02 (s, 2H), 2.00 (s, 6H), 1.72 (s, 4H), 1.61 (s, 4H), 1.58 (s, 4H), 1.45 (s, 2H), 1.24 (d, $J = 7.1$, 6H), 0.91 (dd, $J = 14.5$, 7.0, 3H), 0.85 (t, $J = 6.5$, 7H), 0.79 (d, $J = 6.3$, 4H).

7-(2,2-Dimethyl-propylidene)-6-isobutyl-9-methoxy-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) $\delta$ 7.06 (d, $J = 8.5$, 1H), 6.98 (d, $J = 2.5$, 1H), 6.75 (d, $J = 8.4$, 1H), 5.89 (s, 1H), 4.04 (s, 1H), 3.85 (s, 1H), 3.80 (s, 4H), 2.64 (s, 2H), 1.92 (s, 3H), 1.84 (s, 2H), 1.56 (s, 4H), 1.43 (s, 2H), 1.21 (s, 11H), 1.09 (d, $J = 18.3$, 2H), 0.90 (d, $J = 6.5$, 4H), 0.87 (d, $J = 6.6$, 4H).
7-Benzylidene-6-isobutyl-9-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) $\delta$ 7.89 (s, 1H), 7.46 (d, $J = 8.0$, 1H), 7.34 (dt, $J = 14.6$, 7.4, 5H), 7.25 (d, $J = 8.2$, 1H), 7.05 (s, 1H), 4.09 – 4.02 (m, 1H), 3.84 (d, $J = 2.4$, 1H), 2.71 – 2.63 (m, 1H), 2.56 (dd, $J = 10.2$, 7.9, 1H), 2.10 (d, $J = 9.4$, 1H), 1.79 (d, $J = 9.7$, 1H), 1.61 (s, 3H), 1.49 (dd, $J = 15.1$, 8.4, 3H), 1.13 (dd, $J = 14.5$, 6.6, 1H), 0.75 (d, $J = 6.4$, 3H), 0.54 (d, $J = 6.2$, 3H).

6-Isobutyl-7-(3-phenyl-propylidene)-9-trifluoromethyl-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinoline $^1$H NMR (600 MHz, cdcl3) $\delta$ 7.74 (d, $J = 6.0$, 1H), 7.40 (d, $J = 8.3$, 1H), 7.28 (dd, $J = 7.5$, 5.4, 3H), 7.20 (dd, $J = 16.5$, 7.7, 3H), 6.06 (t, $J = 7.2$, 1H), 3.71 (dd, $J = 15.3$, 5.7, 2H), 2.85 – 2.75 (m, 2H), 2.60 (tdd, $J = 16.0$, 11.9, 4.7, 3H), 2.48 (ddd, $J = 14.7$, 9.6, 7.1, 1H), 2.17 – 2.10 (m, 1H), 1.73 – 1.54 (m, 6H), 1.52 – 1.41 (m, 4H), 1.03 (dd, $J = 12.6$, 6.3, 1H), 0.92 (t, $J = 4.9$, 3H), 0.86 – 0.81 (m, 1H), 0.79 (d, $J = 6.2$, 3H).
Chapter 4: Perspective and Future Directions
4.1 Allenes in Pharmaceuticals

Allenes are an interesting class of reactive compounds that have recently found their way into a number of biologically active compounds. Found in many natural products, allenes have traditionally been incorporated into steroids, prostaglandins, amino acids, and nucleosides within the last 30 years. Additionally, synthetic allenes have also been very effective as anti-virals, decarboxylase inhibitors, and DNA damaging agents in the case of enediynes, which are believed to function through an allenic intermediate. The activity of many of these pharmacologically active allenes has been ascribed to their role as Michael acceptors with respect to nucleophilic enzyme side chains. This makes an allene moiety a good candidate for a reactive center capable of exploiting the nucleophilic sulfur within cysteine residues.

The incorporation of allenes into new pharmacophores incurs an additional advantage in their novelty. Since allenes are relatively new in pharmaceuticals, they offer a convenient way to break patent space around intellectual property established for compounds targeting the same, or a similar biological pathway. For this reason, the methodology for synthesizing allenes reported herein may be useful in both academic and industrial drug discovery efforts.

4.2 Cysteine Proteases

Cysteine proteases represent a high impact biological target with potential to modulate the effects of many debilitating diseases including Alzheimer’s, arthritis, cancer, stroke and even many viral infections. Cathepsin B is one of many classes of cysteine
proteases that are involved in tumor metastasis of particularly aggressive cancers. Hyperactivity of Cathepsin B has been well documented in many cases of esophageal, brain, and ovarian cancers,\textsuperscript{20} where the five-year survival rate is less than 50%. Given such poor prognoses, a class of drugs that can effectively inhibit Cathepsin B is highly desirable in order to increase the duration, and quality of many patients’ lives.

As a class, proteases are enzymes that break down proteins by cleaving peptide bonds. Under normal conditions, these enzymes are essential for breaking down a variety of proteins, and thereby play a key role in cellular regulation. However, cancer cells, characterized by their abnormal activity, employ proteases to initiate invasion into healthy tissue. Tumor invasiveness is positively correlated with a cells’ ability to digest the extracellular matrix (ECM) by secreting proteolytic enzymes. Cathepsin B in particular, is a lysosomal enzyme that is secreted by cancer cells to degrade the extracellular environment by cleaving proteins such as collagen, and fibronectin.\textsuperscript{21}

Cathepsin B plays a central role in an intricate enzyme cascade that ultimately leads to cell proliferation and tumor metastasis. It is activated by the release of an inactivating peptide shown in figure 4.1, which denatures when it enters the extracellular environment.\textsuperscript{21} In its active form, Cathepsin B functions like a bridge, activating another set of enzymes, matrix metalloprotinases (MMPs), which assist in the destruction of the ECM. Together, Cathepsin B and MMPs are largely responsible for tumor invasion by ECM degradation, cell proliferation, and stimulating angiogenesis; all hallmark behaviors of aggressive cancers.
With a strategic target in mind, a mechanism of action-based inhibitor may be designed by first looking at how normal proteolytic activity of Cathepsin B proceeds. As shown in figure 4.2, a cysteine residue with a nucleophilic sulfur resides in the active site of this particular protease. In processing peptides, the nucleophilic sulfur of Cys\textsubscript{29} attacks the carbonyl of a peptide bond. After abstraction of a proton on the positively charged His\textsubscript{199}, the carbonyl reforms and the amine derivative is cleaved from the enzyme. Hydrolysis of
the resulting complex occurs when water attacks the carbonyl again, releasing the enzyme and the free acid metabolite.

![Figure 4.2: Mechanism of proteolysis in Cathepsin B](image)

A logical beginning in the design of an efficacious inhibitor examines the features of Cathepsin B’s endogenous ligand, Cystatin C. Many attempts by other researchers to develop inhibitors of this class have employed peptidomimetic approaches. However, these strategies have been met with limited success due to the high peptide character of the resulting compounds, which opens opportunity for proteases of similar classes to break them down at other sites. Also, highly peptidic compounds are typically associated with poor oral bioavailability. Nonetheless, key structural information can be derived from these modestly potent inhibitors. A survey of the structures shown in figure 4.3 reveals two common residues among a diversified series of compounds, Phenylalanine and Leucine. In fact, subsequent studies have confirmed that indeed Cathepsin B has high affinity for Phenylalanine at the P2 position within the active site of the enzyme. Leucine is also well tolerated as an alternative according to binding affinity data reported throughout the literature.
While others have utilized this information to construct non-peptidic cysteine protease inhibitors before, few have achieved excellent potencies against cathepsin B. A most notable case is exemplified by the acylamino azetidinone series introduced by Zhou et al. Their compounds featured just one amino acid, Phenylalanine, for binding affinity, and the β-lactam moiety as a novel pharmacophore. While very potent against Cathepsins L, K, and S, in comparison these compounds did not show effective inhibition against Cathepsin B Table 4.1.
4.3 Allenic Hybrids as Cathepsin B Inhibitors

It is proposed herein that stronger, more effective inhibition of Cathepsin B may be achieved by installing a more reactive center toward nucleophilic attack on a non-peptidic scaffold, imbued only with Phenylalanine or Leucine for enzyme affinity.

The core structure of the proposed inhibitor series is presented in Figure 4.4. It includes a Phenylalanine (or alternatively, a Leucine) residue, as well as the reactive allene moiety spaced from the amino acid in a similar fashion as the β-lactam on the aforementioned azetidinones. Here, the expected mechanism of inhibition is shown, where nucleophilic attack by the Cys\textsubscript{29} sulfur occurs at the central carbon of the allene, acting as a Michael acceptor. A proton is abstracted from His\textsubscript{199}, as in the proteolytic mechanism presented.
before, only the complex that forms here is not able to be hydrolyzed by water because there is no reformed carbonyl arising from a peptide bond. This accounts for an irreversible mechanism-of-action based inhibition of Cathepsin B. Though the pharmaceutical industry has been reluctant to develop irreversible inhibitors, it is important to note that this class of proposed compounds will be used as a potential therapy for cancers with poor prognoses. To this end, any rate of success in extending a patient’s life with this method will certainly be considered valuable.

The synthesis of these compounds is fairly straightforward, and may be accomplished in just three or four steps. Figure 4.5 depicts the primary synthetic route by which these compounds may be prepared; first an amide bond formation between two readily available reagents; a benzyl carbamate protected L-Phenylalanine, and 2-chlorobenzylamine, coupled with HBTU. Next, a gold catalyzed Mannich reaction, which proceeds over 18 hours at 75°C in water. The third step incorporates the intramolecular Heck cyclization methodology for forming allenes from aryl chlorides presented in this dissertation. Finally, Birkofer’s conditions may be used in an optional de-protection to give the free amine. The mild nature of this last step was selected to efficiently cleave

Figure 4.4: General scaffold and mechanism of action of proposed compound series
the benzyl carbamate group, while preserving the allene. Alternatively, the Mannich reaction may proceed first, followed by amide bond formation, and then the heck and optional de-protection. This approach may prove useful in avoiding unforeseen difficulties with purification of the first intermediate.

![Chemical structures](image)

Figure 4.5: Proposed synthesis of allenic hybrid inhibitors

This allenic hybrid scaffold features several points of variability with which structure-activity relationships (SAR) may be discerned. First, the benzyl carbamate group protecting the amino acid may be left in tact on the final compounds, or cleaved to expose the free amine. Both forms of inhibitors were typically tested in the literature, and one or the other may prove significantly more efficacious. Secondly, a great deal of versatility can be afforded by combinations of different $R_1$ and $R_2$ substitutions on the acetylenes and aldehydes used in the Mannich reaction. Most importantly, the choice of $R_2$ will serve to modulate the reactivity of the allene towards nucleophilic attack. This
should prove most useful in modulating specificity for Cathepsin B over other cysteine proteases. The list of R groups in figure 4.5 is derived from both common substitutions on existing cysteine protease inhibitors, and commercially available reagents. Lastly, a number of substituted 2-chlorobenzylamines are commercially available, providing another point from which to build functionality on the aryl ring.

In summary, Cathepsin B is a class of cysteine proteases that are centrally involved in the biochemical pathway leading to metastasis of aggressive cancers. These proteases are secreted by tumor cells to digest the ECM of surrounding tissue, promoting invasion, and cell proliferation. Rational drug design of efficacious Cathepsin B inhibitors may be used as an anti-cancer therapy that will hopefully improve the generally poor prognosis of ovarian, brain, and esophageal cancers.

With a novel methodology in hand for making versatile allenes, a facile synthesis of compounds that will participate in irreversible enzyme inhibition as a Michael acceptor is proposed. Such compounds, with minimal peptide character, would be expected to escape degradation from related proteases, provide better oral bioavailability, and thus achieve a better pharmacological profile overall. Finally, the allenic moiety provides a convenient avenue around existing patent space, and allows for new intellectual property to be claimed for successful drug candidates.
References


For more reading on the intramolecular Heck reaction, and allene synthesis please see:


