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Unnatural amino acids designed for click chemistry and their application to the modification of peptides & nitrene transfer reactions catalyzed by metalloporphyrins

Jeremiah David Harden
University of Tennessee

To the Graduate Council:

I am submitting herewith a dissertation written by Jeremiah David Harden entitled "Unnatural amino acids designed for click chemistry and their application to the modification of peptides & nitrene transfer reactions catalyzed by metalloporphyrins." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

Shane Foister, Major Professor

We have read this dissertation and recommend its acceptance:

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a dissertation written by Jeremiah David Harden entitled "Unnatural Amino Acids Designed for Click Chemistry and Their Application to the Modification of Peptides & Nitrene Transfer Reactions Catalyzed by Metalloporphyrins" I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

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We have read this dissertation
and recommend its acceptance:

George Kabalka_____

Ben Xue_____

Tim Sparer_____

Accepted for the Council:

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Unnatural Amino Acids Designed for Click Chemistry and Their Application to the Modification of Peptides

&

Nitrene Transfer Reactions Catalyzed by Metalloporphyrins

A Dissertation
Presented for the
Doctor of Philosophy
Degree
University of Tennessee, Knoxville

Jeremiah David Harden
May 2009

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I would like to thank all of my friends and family for not realizing, or at least being endlessly kind enough not to point out, that I am completely and utterly out of my mind.

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To the rest of my family, you have all be a supply of endless support. Memom, Janet, and Shirley, thank you for all of your visits and concern, you were always supportive, yet even more so towards the end when I needed it most. Grandmom and Granddad who always knew I could do whatever I wanted and told me so all the time. Justin and Joshua, my brothers, you have always been a pain in my ass, but you have also been the best brothers and best friends anyone could ever want; I love both of you.

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Abstract

The field of peptidomimetics has rapidly grown into an area of great interest for the design and synthesis of pharmaceutical drug targets. The large array of natural peptides with biological function as well as the growing understanding of the roles of these peptides in biological events has led to a large interest in these compounds as drug candidates. The majority of peptide and peptide-like molecules have not found widespread pharmaceutical utility; however, due to there lability in biological systems. This major drawback leads to the necessity for the development of peptide-like molecules with increased stability under biological conditions. To this end there has been an increased interest in the development of unnatural amino acids for the synthetic modification of peptides and proteins.

Presented in this dissertation is the synthesis of a series of unnatural amino acids designed for applications to [3+2] click cycloaddition reactions. It also covers the introduction of these amino acids into the sequence of peptides for the purpose of labeling the peptide with aryl triazole chromophores in an attempt to analyze the electron transfer capabilities of aryl triazoles. The information from the fluorescent studies of these peptides will provide a basis for the design of fluorophoric peptide probes that can be introduced into a peptide at any time under labile conditions. This methodology provides a powerful tool for the analysis of peptide structure and the analysis of peptide-macromolecular interactions.

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List of Abbreviations

Boc	Tert-Butyl Carbamate
BPB	Benzylproline Benzophenone
Co(Por)	Cobalt (porphyrin)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIEPA	Diisolpropylethylamine
DMAP	Dimethylaminopyridine
DPPA	Diphenylphosphorylazide
EDTA	Ethylenediaminetetraacetic Acid
Fe(Por)Cl	Iron (Porphyrin) chloride
Fmoc	9-fluorenylmethoxycarbonyloxy
FmocOSu	N-9-fluorenylmethoxycarbonyloxy Succinamide
HATU	O-(7-Azabenzotriazole-1-yl)-N, N,N'N'-tetramethyluronium hexafluorophosphate
HBTU	O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium Hexafluorophosphate
HCTU	O-(6-Chloro-1-hydroxibenzotriazol-1-yl) -1,1,3,3- tetramethyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
KOH	Potassium Hydroxide
NMP	N-Methylpyrolidinone
OEP	Octaethylporphyrin
ORC	On-Resin Click
PhI	Iodobenzene
PhI=NTs	[N-(p-toluenesulfonyl)imino]phenyliodinane
PhI(OAc)2	IodoBenzene Diacetate
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TMS	Trimethylsilane
TPP	Tetraphenylporphyrin

Part 1: Clickable Unnatural Amino Acids and Their Application to the Modification of Peptides

Chapter 1: Introduction

Proteins are a key class of macromolecules that perform functional and structural tasks in virtually all physiological processes. They are composed of linear sequences of amino acids that fold into specific three dimensional structures with exquisite control over reactive group placement. Protein-macromolecule interactions implicated in disease have long been the highest goal of drug discovery; however, the complexities of protein structure, the conformational flexibility, and the poor pharmacology of peptide fragments have hindered this approach to therapeutic development.[1]

Peptidomimetics is the term used to describe the design of modified peptides and peptide mimic compounds, and they address both the complexity and flexibility of protein structure by attempting to restrain peptide fragments in conformations similar to that of their intact protein's structure.[2] The reduced size and chemical diversity of peptidomimetics relative to natural peptides make them more pharmacologically desirable. An enormous number of biological and physiological processes are controlled by peptides and short proteins.[3, 4] Successful peptidomimetics offer the possibility of disrupting pathological protein-protein interfaces by competing with one of the interacting species.[5-7] Traditionally peptidomimetics have been synthesized in non-aqueous solvents; however, this often hinders attempts to isolate the biologically-relevant conformations and can lead to peptidomimetics that are constrained in inactive states or that are largely disordered in solution.[7] In many cases the final peptidomimetics constructed in this fashion are only mildly water soluble, which limits their utility as drug candidates.

To address this problem, synthetic transformations compatible with naturally occurring sidechain functional groups and aqueous solvents present the possibility of novel peptidomimetics with greater therapeutic potential. Alkynes, nitriles and azides are energetic functional groups with narrow reactivity distributions under physiological conditions.[8] When co-localized, alkynes and nitriles react rapidly, reliably, and irreversibly with azides in a [3+2] cycloaddition, click reaction, to form triazoles and tetrazoles respectively (Figure 1-1).

Realizing the biological utility of [3+2] click cycloaddition reactions has led our group to the design and syntheses of amino acids bearing "clickable" functionalities (Figure 1-2). Our major focus involves the development, characterization, and activity analysis of a variety of novel peptidomimetics, which incorporate a developed library of "clickable" amino acids. It is well known that many biologically active peptides assume unique secondary structure upon interaction with their targets; however, the conformation is lost in the absence of the target which makes characterization of the active structure difficult.[9, 10] The developed peptidomimetic libraries can then be used as functional probes for investigating the conformational requirements of active peptidomimetic compounds in systems where active species determination is not otherwise possible.

The major peptidomimetic approach of this method involves spacing two "clickable" amino acids at well defined intervals within a peptide sequence. The remaining residues may be modified according to a specific natural peptide sequence or may be used as sites of adding diversity elements through the use of standard split-pool

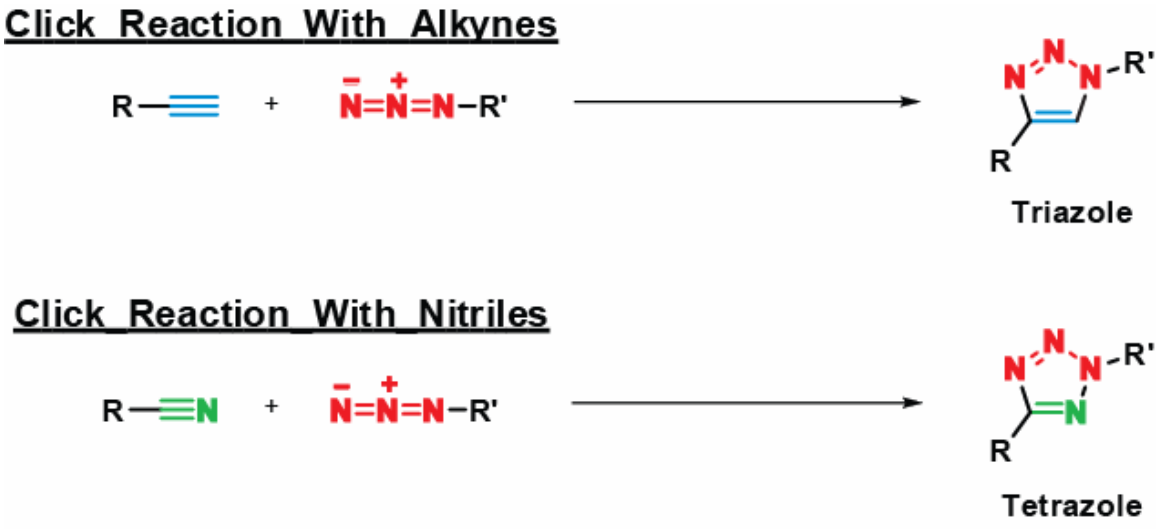


Figure 1-1. General Reaction Schemes for Alkynes and Nitriles with Azides to Give Triazoles and Tetrazoles

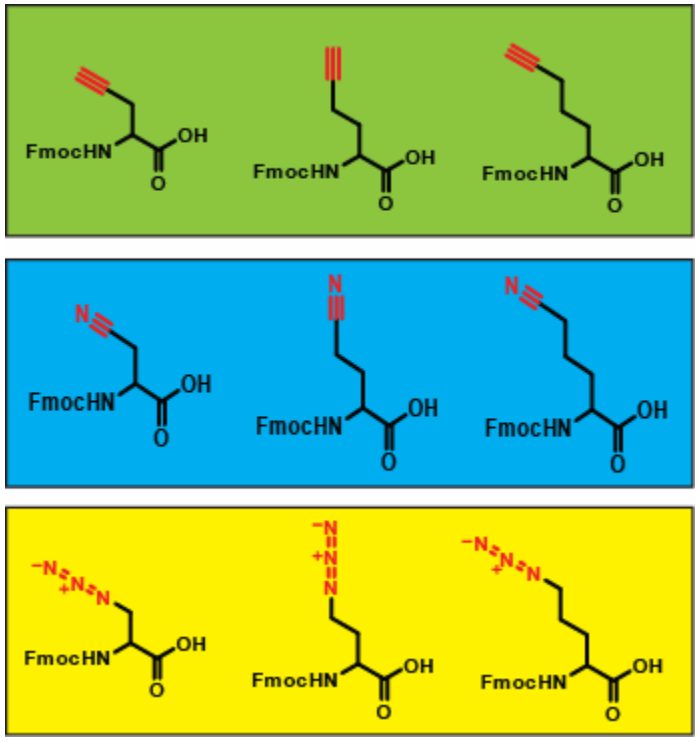


Figure 1-2. Unnatural “Clickable” Amino Acid Analogues for Peptidomimetic Applications

synthesis. These peptidomimetic compounds can then be equilibrated with specific macromolecular targets. Equilibrating our modified peptides with a biological target or with a structure inducing co-solvent can cause the peptide to assume its bioactive conformation. This will bring the click functionalities into contact leading to the irreversible click reaction that will lock the peptide into its active conformation (Figure 1-3).

This method of peptidomimetic design allows for the enforcement of a multitude of variations in secondary structures such as α-helices and β-sheets, providing peptides with a broad range of applications including antimicrobials, and therapeutic treatments of neurodegenerative diseases. This strategy can be applied to the construction of antimicrobial α-helical and β-sheet peptides. Antimicrobial peptides with α-helical structures and positive net charges have been a focus of interest for the development of antibiotic agents against Gram-negative bacteria.[10] The use of a short peptide sequence may provide greater selectivity for the bacteria cells, and usually decreases unwanted toxic side effects of the peptidomimetic compound.[11] It has been found that bacteria are less likey to develop resistance to antimicrobial peptides compared to other antimicrobial treatments.[12-14] For this reason, many peptidomimetics may also provide methods of overcoming drug resistant strains of bacteria. Once constructed, the new α-helical and β-sheet peptides can be characterized and then assayed against a panel of clinically relevant pathogens in order to assess their antibiotic profiles.

One well accepted approach for the discovery of drugs for neurodegenerative diseases is the design of soluble β-sheet peptidomimetics.[15] Many peptidomimetics

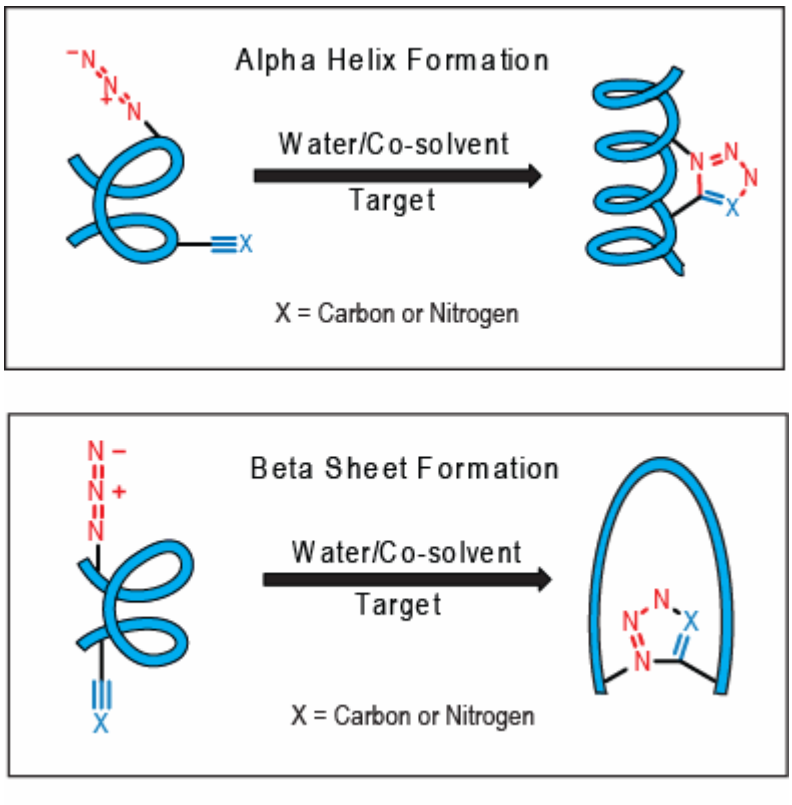


Figure 1-3. Conformational Control of Peptides via Intramolecular Click Chemistry

are known to inhibit plaque formation of amyloid proteins, which is believed to be a major cause of some neurodegenerative diseases, show secondary sheet and turn sructures.[15] Our peptidomimetic approach would allow for the rapid preparation of modified peptides that would lock into these conformations. The sequences of these peptides would also be tunable to provide the solubility, and bioactivity necessary to make them effective therapeutic agents for neurodegenerative diseases.

This chemistry not only provides a means of constraining and influencing peptidomimetic structure, but placement of clickable amino acids in the sequences of peptides then allows for the possible attachment of fluorescent probes to the constructed peptidomimetics. This methodology would provide a tool for the analysis of binding behaviors of constructed peptidomimetics, as well as, the active and inactive structures of the peptidomimetics. Incorporation of fluorescent probes directly to a peptide via click chemistry provides a variety of possibilities for studying peptide folding both in solution and during interactions with their macromolecular targets. This approach lends flexibility to the analysis of peptide binding interactions.

Chapter 2: Synthesis of Unnatural Amino Acids Bearing High Energy Side Chain Functionalities for Applications to Click Chemistry

Background and Significance

The field of peptidomimetics has rapidly grown into an area of great interest for the design and synthesis of pharmaceutical drug targets. The large array of natural peptides with biological function as well as the growing understanding of the roles of these peptides in biological events has led to a large interest in these compounds as drug candidates. The majority of peptide and peptide-like molecules have not found widespread pharmaceutical utility, due to their limited stability in biological systems.[16] Small peptides, up to a few hundred Daltons, usually have half-lifes of only a few seconds to a few minutes, and are rapidly metabolized, usually to their constituent amino acids.[17] Larger peptides, a thousand Daltons or more, have been shown to have improved half-lifes, but neither large nor short peptides have lifetimes long enough to be effective if administered orally. This major drawback leads to the necessity for the development of peptide-like molecules with increased stability under biological conditions. To this end there has been an increased interest in the development of unnatural amino acids for the synthetic modification of peptides and proteins.

Realizing the biological utility of [3+2] cycloaddition “click” reactions has led our research group to the design and synthesis of amino acids bearing “clickable” functionalities. Chapter 2 outlines the synthesis of a sizable library of unnatural chiral amino acids bearing highly energetic nitrile, azide, and alkyne functional groups. These

unnatural amino acids will provide the base for a wide range of peptidomimetic applications involving click chemistry.

Synthesis of Amino Acids Bearing Nitrile Side Chains

Nitrile substituted amino acids can function as click partners with azides for [3+2] click cycloadditions. These unnatural amino acids were originally envisioned as the primary coupling partners for click reactions with azides, however, they were found to react sluggishly and less cleanly than anticipated, and so they were substituted by alkynes until more beneficial reaction conditions could be found. The most direct method for the synthesis of amino acids bearing nitrile side chains involves the conversion of the amide side chains of asparagine and glutamine into nitriles via isocyanate formation. This has been accomplished most commonly with N,N'-dicyclohexylcarbodiimide (DCC) in pyridine.[18] We have found that the same conversion can be carried out just as easily with trichloroacetic anhydride or acetic anhydride in pyridine.[19]

The synthetic route to the 9-fluorenylmethoxycarbonyloxy (Fmoc) protected amino acid nitriles can be carried out as shown in Figure 2-1. The commercially available chiral amino acids asparagine **1** and glutamine **2** were easily protected with di-tert-butyl dicarbonate (Boc Anhydride) under basic aqueous conditions to give the tert-butyl carbamate (Boc) protected amino acids **3** and **4** Figure 2-1. Reaction of the Boc amino acids with acetic anhydride in pyridine gives the corresponding Boc amino acid nitriles **5** and **6**. The amino acid nitriles can then be deprotected with trifluoroacetic acid (TFA) in dichloromethane (DCM) to give the unprotected amino acid. The free amino

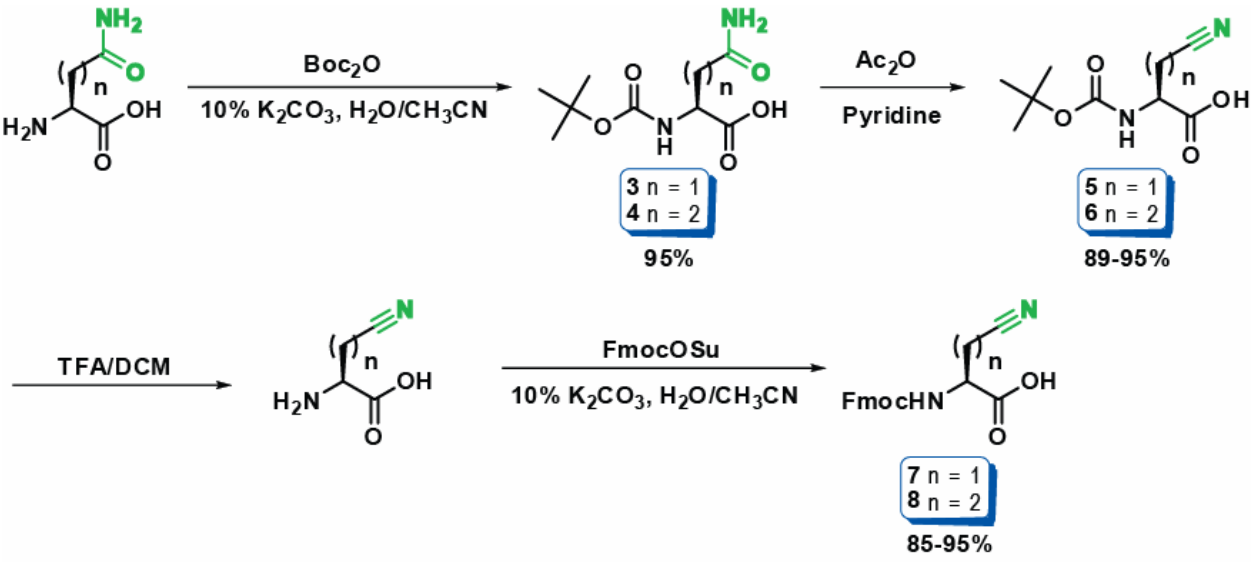


Figure 2-1. Scheme for the Synthesis of Unnatural Amino Acid Nitriles Fmoc-2-Amino-3-Cyanopentanoic Acid **7** and Fmoc-2-Amino-3-Cyanobutanoic Acid **8**

acid can be 9-Fluorenylmethoxycarbonyloxy (Fmoc) protected by reaction with N-(9-fluorenylmethoxycarbonyloxy) succinimide (FmocOSu) in aqueous potassium carbonate to give the desired Fmoc-2-amino-3-cyanopentanoic acid **7** and Fmoc-2-amino-4-cyanobutanoic acid **8 (**Figure 2-1). The synthetic sequence is robust enough to allow all steps to be carried out with minimal purification requiring only one purification by column chromatography on the final Fmoc protected amino acids. This synthetic strategy also allows for the synthesis of both L and D conformations of the amino acid nitriles because the enantiomeric purity of the starting materials can be carried through the entire synthesis.

Synthesis of Amino Acids Bearing Azide Side Chains

Since azides are the required coupling partners for both nitriles and alkynes, the ability to introduce azides into a peptide sequence was crucial for any envisioned click applications for unnatural peptides. For this reason the procedures and methods for the synthesis of these compounds needed to be both effective and straightforward.

Amino acids bearing azide functionalized side chains linked to the backbone with either one or two carbons as spacers can be synthesized from the L or D forms of asparagine and glutamine respectively. The commercially available chiral amino acids asparagine **1** and glutamine **2** can be Boc protected with Boc Anhydride under basic aqueous conditions to give the Boc amino acids **3** and **4** (Figure 2-2)**.** The protected amino acids can then be converted to the corresponding amines, **9** and **10**, via the reaction with phenyl iodobistrifluoroacetate or phenyl iodo diacetate in a 1:1 mixture of

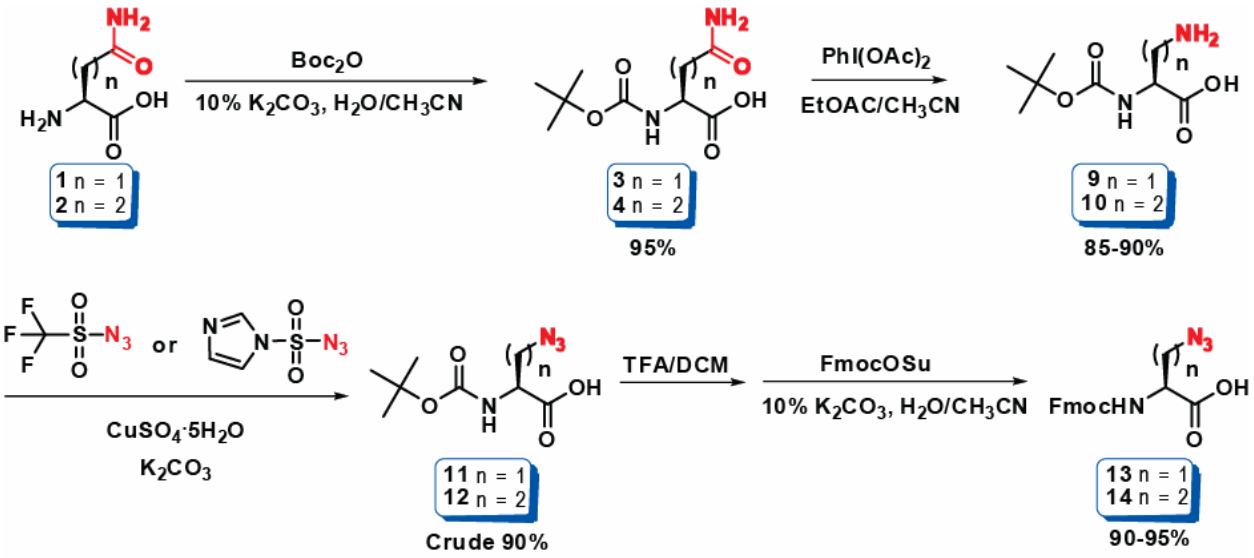


Figure 2-2. Synthetic Scheme for Fmoc -2-Amino-3-Azidopropanoic Acid **13** and Fmoc-2-Amino-4-Azidobutanoic Acid **14**

ethyl acetate and acetonitrile at 0°C (Figure 2-2).[20, 21] The amines are easily recovered as white solids by filtration from the reaction solution and can be used without any further purification. The sidechain amine functionalities can then be converted to azides by diazo transfer to provide amino acids **11** and **12 (**Figure 2-2).

Initially this transformation was carried out using triflyl azide as the azo transfer reagent.[22-24] The reaction was carried out in basic aqueous solution with copper sulfate as a catalyst. However, the triflyl azide reagent suffered from several drawbacks.[25] The reagent itself is very volatile so it could not be concentrated or left in the open, and reaction vessels needed to be sealed for long reaction times. The reagent had to be made and stored as a solution in toluene, and the shelf-life of the azide reagent was not suitable for storage of large quantities. This led to the reagent having to be made frequently in small batches. For these reasons the triflyl azide reagent was later replaced with the more robust and economical imidazole-1-sulfonyl azide.[26] This reagent can be isolated as a hydrochloride salt which allows for isolation and characterization. The formation of the reagent as a salt also allows larger quantities to be made and stored with little to no degradation of the reagent. It is used in a similar fashion to the triflic azide, except that the imidizole-1-sulfonyl azide diazo transfer can be run in methanol which provides better results and easier cleanup. Also, it requires an extra equivalent of base to neutralize the HCl. Both the triflyl and imidzole-1-sulfonyl azide reagents provided the corresponding azides **13** and **14** in good to excellent yield. From here the amino acid azides could be Boc deprotected in TFA/DCM to give the free amino acids. The free amino acids were then Fmoc protected in basic aqueous solution with FmocOSu as the reagent. Isolation of the amino acids by column chromatography

over silica gel provided pure products Fmoc-2-amino-3-azidopropanoic acid **13** and Fmoc-2-amino-4-azidobutanoic acid **14** as white to off-white solids (Figure 2-2).

Amino acid azides bearing three and four carbon side chains can be directly synthesized from the diprotected amino acids ornithine **15** and lysine **16** (Figure 2-3). The side chain Boc groups were removed with 25% TFA/DCM to give the free amines. The free amines were converted to the Fmoc amino acid azides via azo transfer. The formation of these amino acid azides proceeds well; however, the reaction cannot be run in base for extended periods of time due to loss of the Fmoc protecting group. The final amino acids were purified over silica gel to provide Fmoc-2-amino-5-azidopentanoic acid **17** and Fmoc-2-amino-6-azidohexanoic acid **18** (Figure 2-3).

Since deprotection of the Fmoc protected amines occationally occured during the azo transfer, an alternate route for synthesis of the long chain azides was desirable. Copper has been used as a coordination metal with amino acids for many applications including side chain functionalization.[27, 28] This approach was applied to the azide synthesis using commercially available L-lysine hydrochloride (Figure 2-4). The lysine hydrochloride was coordinated to copper in aqueous base using copper sulfate pentahydrate as the copper source to produce the amino acid complex **19**. After two to three hours the azo transfer reaction was then set in the same pot by addition of an extra catalytic amount of copper sulfate pentahydrate and excess potassium carbonate. This was followed by the addition of imidazole-1-sulfonyl azide and the reaction was stirred at room temperature overnight (Figure 2-4). The copper complexed lysine azide **20** was recovered as a light blue solid by filtration from the reaction solution. The solid was dried, crushed and redissolved in a saturated ethylenediaminetetraacetic acid

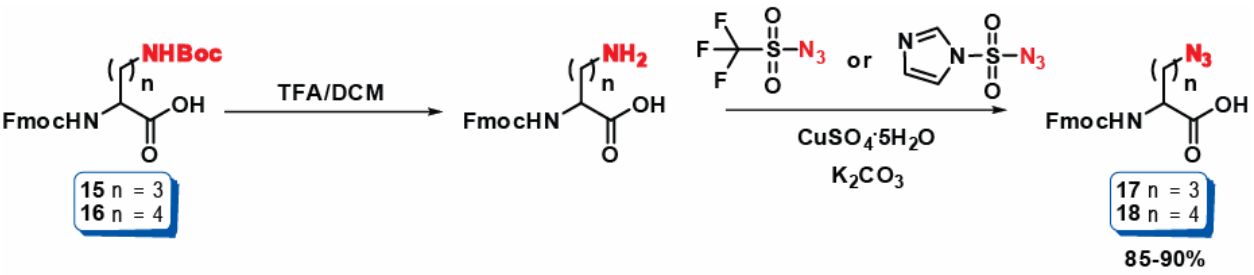


Figure 2-3. Synthesis of Fmoc-2-Amino-5-Azidopentanoic Acid **17** and Fmoc-2-Amino-6-Azidohexanoic Acid **18**

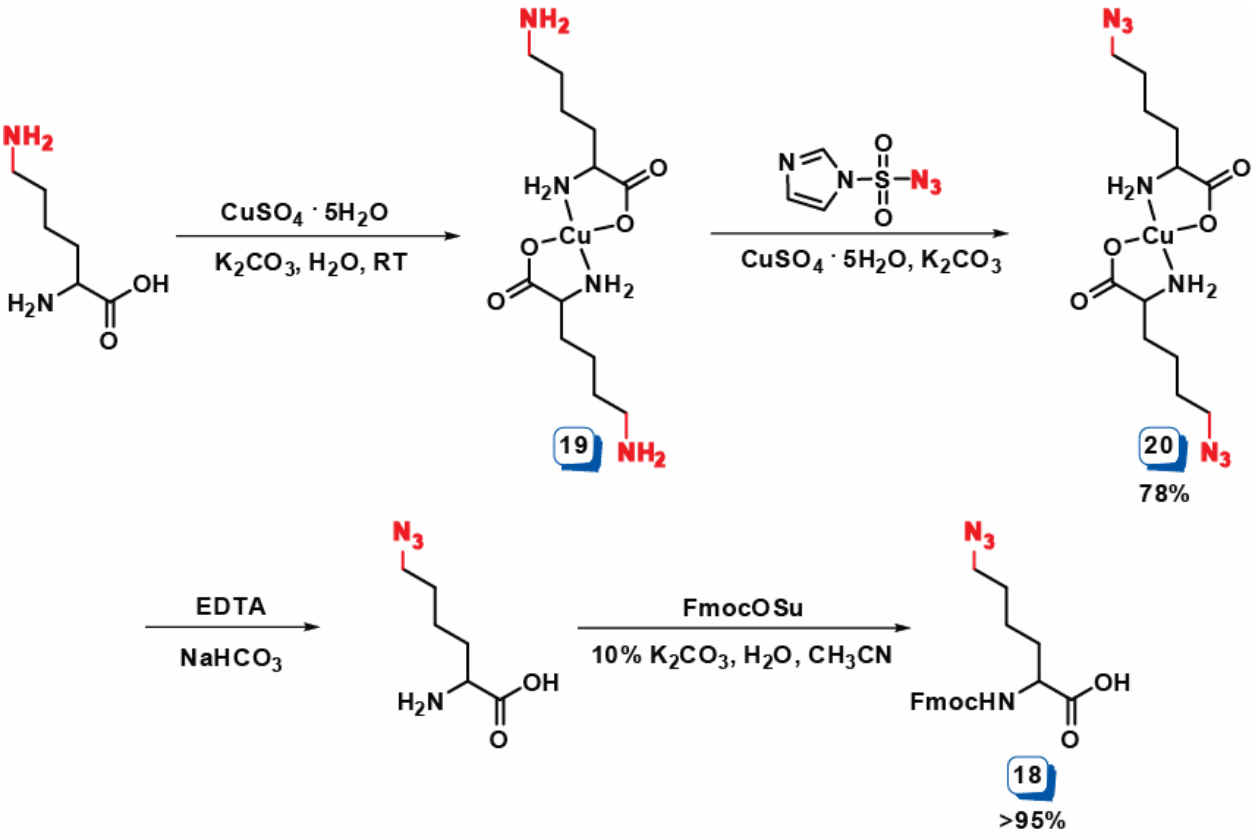


Figure 2-4. Synthetic Scheme for the Synthesis of Fmoc-2-Amino-6-Azidohexanoic Acid Using Copper Complex

(EDTA) solution and the mixture was shaken vigorously for 1-2 hours. During this time the solution turned a very deep blue color indicating the formation of the copper EDTA complex. When this method is used for side chain protection the product amino acid is often filtered from the copper EDTA solution[28]; however, the lysine azide was completely soluble in the aqueous solution. Rather than attempting a tedious purification of the amino acid azide from the solution of copper and base, the Fmoc protection was attempted on the resulting crude solution. The solution was transferred to a larger flask and the pH was adjusted to approximately 8 using solid potassium carbonate. A solution of FmocOSu in acetonitrile was then added at 0^{o}C and then the reaction was stirred at room temperature for 4-6 hours. The Fmoc protection proceeded efficiently on the crude solution and the protected amino acid **18** was removed from the solution by acidification of the reaction solution to a pH of 2 followed by extraction with ethyl acetate (Figure 2-4). Occationally the products were sufficiently pure from extraction alone; however, when needed the products were purified by flash chromatography over silica gel.

Synthesis of Amino Acids Bearing Alkyne Side Chains

The synthesis of chiral amino acid alkynes involved a more elaborate approach than that of the nitriles and azides. Alkyene amino acids cannot be readily synthesized directly from chiral amino acid starting materials. This means that the chirality must be installed during the synthesis, which can lead to mixtures of stereoisomers, as well as tedious isomeric separations.[29, 30] Many effective methods have emerged for the asymmetric synthesis of α-amino acids.[31] One of the most direct and general methods

involves the alkylation of a chiral auxiliary of glycine.[31] There are examples of auxiliaries that have been employed for the synthesis of chiral amino acids; however, many of these also possess negative qualities as well.[32, 33] The use of chiral auxiliaries almost always lengthens the synthesis, usually because the auxiliary itself must be synthesized. The nickel benzylproline benzophenone glycine complex **21** was chosen as an appropriate auxiliary for several attractive reasons (Figure 2-5). The complex itself can be synthesized from mostly low cost materials, including the chiral installation which consists of commercially available L or D proline, which is of moderate price. [34, 35] The synthesized benzylproline benzophenone ligand is recoverable in high yields from the hydrolysis of the nickel complex, and is also reusable which cuts down on synthesis steps and cost after the initial synthesis. The side chain functionality of the glycine residue is achieved by alkylation of the nickel complex with alkyl bromides under basic conditions. The alkylation of the complex can be easily accomplished in high yield and with excellent selectivity.

The synthesis of complex **21** can be accomplished in three synthetic steps and the intermediate steps can be sufficiently purified without the need for column chromatography (Figure 2-6). The formation of the complex begins with the synthesis of S-benzyl proline from L-proline and benzyl chloride. The reaction provides a moderate yield, but it is robust and resilient enough to allow for large multigram scale up. The second step involves the reaction of benzyl proline with 2-amino benzophenone to yield the benzylproline benzophenone (BPB) ligand **22** (Figure 2-6)

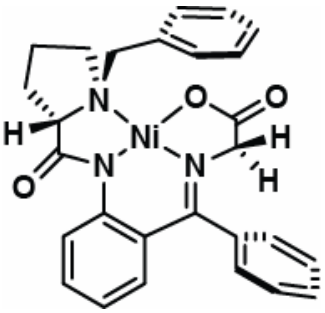


Figure 2-5. Structure of Nickel (II) Complex **21** of Glycine Schiff Base with Benzylproline Benzophenone

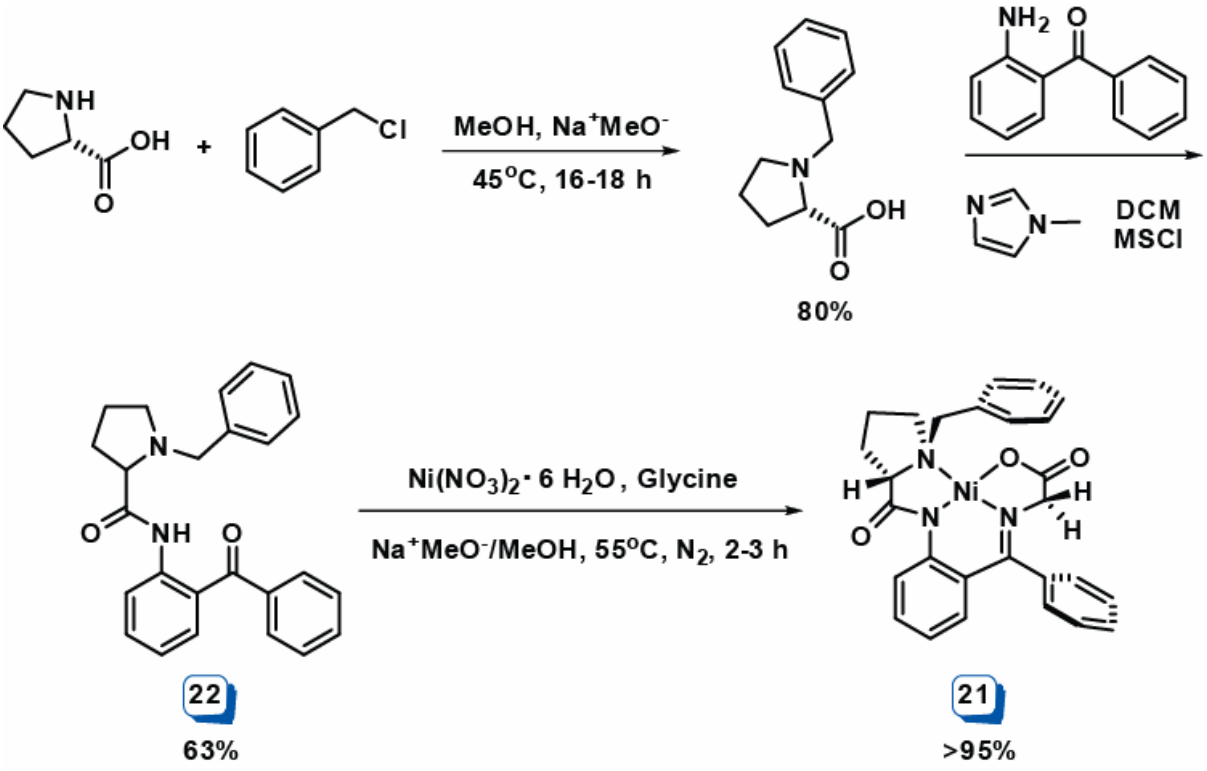


Figure 2-6. Synthetic Scheme for the Synthesis of the Nickel (II) Benzylproline Benzophenone Glycine Complex **21**

The ligand can be separated from the reaction by crystallization from methanol and can be used for the synthesis of the final complex without further purification. Benzylproline benzophenone, glycine, and nickel nitrate hexahydrate are combined and stirred in refluxing methanol followed by potassium hydroxide (KOH) addition to produce the deep red complex **21** (Figure 2-6). The reaction is quenched by the slow dropwise addition of acetic acid followed by water. The product is extracted with DCM and concentrated to yield a fluffy, red foam that can be alkylated without purification. If desired, the complex can be purified by flash chromatography over silica gel with a 3:1 DCM to acetone eluent system or it can be recrystalized from acetonitrile. The complex can be made quickly and efficiently in large quantities and the various components are all stable enough to be synthesized in bulk and stored indefinitely at room temperature.

The alkylation of the complex can be easily accomplished in acetonitrile with an alkyl halide and using powdered KOH as a base.[34, 36] The synthesis of the alkyne amino acid with a three carbon side chain was accomplished with the commercially available propargyl bromide. The four and five carbon side chain amino acid alkynes required the synthesis of reactive alkyl halides. Both the four and five carbon alkynes can be synthesized from butyne-1-ol and pentyne-1-ol respectively. Tosylation of the alcohol followed by protection of the terminal alkynes with trimethylsilane (TMS) gives the protected tosylates. These tosylates can be converted to the TMS protected bromides by reaction with lithium bromide in dry actone for 18-36 hours. The protected bromides can then be used for the alkylation of the nickel glycine complex **21**. The alkylation of the nickel glycine complex with the protected bromobutyne has consistently

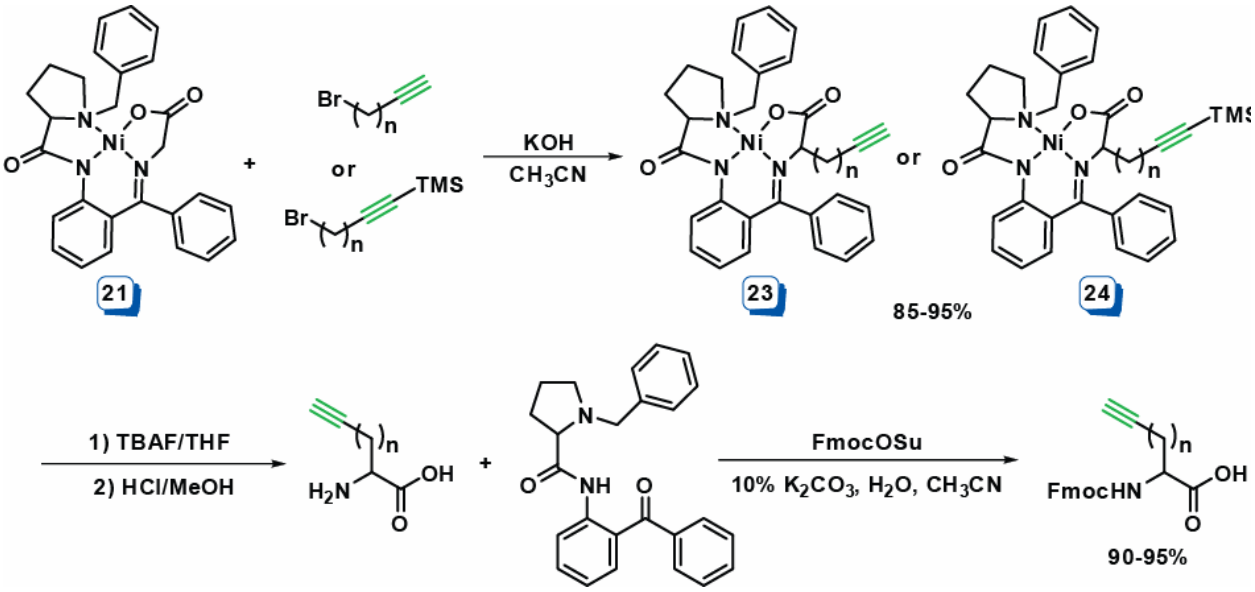


Figure 2-7. Scheme for the Synthesis of Alkyne Amino Acids Using the Nickel (II) Benzylproline Benzophenone Glycine Complex

given a breakdown product of the complex and little to no desired alkylation product under a variety of conditions. More studies are required to determine the difficulties in producing this particular alkylated complex. Instead, the commercially available propargyl bromide was used and yields the propargyl glycine complex directly; however the TMS protected alkyne bromide provides a mixture of TMS protected alkyne glycine complex **23** and deprotected alkyne glycine complex **24** (Figure 2-7). Treatment of the mixture with tetrabutylammonium fluoride converts all of the protected complex to the deprotected complex. All of the reactions produce a small amount of the undesired stereochemical product. The undesired stereochemical product and side products are removed by column chromatography using 4:1 DCM to acetone as an eluent to provide pure alkylated complexes.

The alkyated complexes can then be hydrolyzed by stirring in 50:50 5M hydrochloric acid and methanol to produce the free amino acid and regenerating the BPB ligand. The BPB ligand can be extracted out with DCM. The free amino acid is then dried and the nickel is removed by purification over ion exchange resin to provide pure alkyne amino acid which can then be reacted with FmocOSu to provide the Fmoc amino acid alkyne (Figure 2-7).

Experimental

General Considerations. Amino acid starting materials were purchased from Advanced Chem Tech. Reagents were used as recieved without additional purification. Dry solvents were obtained from a Grubb's solvent system and used immediately or stored over activated 4 Å molecular sieves. Proton and carbon nuclear magnetic

resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Bruker 400 MHz or a Varian 300 mHz spectrometer and referenced with respect to internal TMS standard or residual solvent. Infrared spectra were obtained using a Bomen B100 FT-IR spectrometer. IR samples were prepared as films on a sodium chloride plate by evaporating DCM solutions. High resolution mass spectroscopy (HRMS) was performed at the Mass Spectrometry Center located in the Chemistry Department of the University of Tennessee on a JOEL JMS-T100CC ACCU-TOF instrument using either direct analysis in real time (DART) ionization or by matrix assisted laser deposition ionization (MALDI). Thin layer chromatography (TLC) was carried out on E. Merck Silica Gel 60 F-254 TLC plates. Ion exchange chromatography was performed using Dowex 50WX2-100 ion exchange resin. Selected amino acid proton spectra are listed in the appendix of this section.

Boc-L-2-amino-3-cyanopropanoic acid. (Figure 2-1, Entry 5) Boc-D-asparagine-OH (3.40 g, 15.0 mmol, 1.00 equiv.) was weighed out into a roundbottom flask with a stirbar and dissolved in 50 mL of pyridine. Once the acid was fully dissolved acetic anhydride (1.70 mL, 18.0 mmol, 1.20 equiv.) was added all at once at room temperature. The reaction mixture was stirred at room temperature overnight. Depending on the purity of the commercially acquired amino acid a slight red hue was occasionally observed; however, no effect on the reaction was observed. The reaction was concentrated in vacuo and taken up in ethyl acetate. The ethyl acetate layer was rinsed three times with 30 mL portions of 2 M acetic acid, followed by rinses with water (2 x 20mL) and brine (2 x 20mL). The ethyl acetate layer was collected, dried over

anhydrous sodium sulfate, and concentrated in vacuo to yield a crude viscous oil which was carried on without futher purification. Crude Yield = 87%

Fmoc-L-2-amino-3-cyanopropanoic acid. (Figure 2-1, Entry 7) Boc-L-2-amino-3-cyanopropanoic acid (2.79 g, 13.0 mmol, 1.00 Equiv.) was dissolved in 25% trifluoroacetic acid in DCM and stirred at room temperature for 30-45 minutes. The reaction was concentrated in vacuo, redissolved in DCM and reconcentrated. The residue was dissolved in 75 mL of 10% potassium carbonate solution and cooled to 0 ^{o}C in an ice bath. FmocOSu (4.71 g, 14.0 mmol, 1.10 Equiv.) was dissolved in a minimal amount of acetonitrile and the solution was added to the reaction dropwise at 0 ^{o}C. The reaction was stirred at 0 ^{o}C for 30 min. then it was stirred at room temperature for 3-3.5 hours. The reaction was filtered through celite, and the aqueous filtrate was collected. The aqueous layer was suspended with ethyl acetate in an Erlenmeyer flask. The aqueous layer was acidified to a pH of 2-3 using 2 M HCL. The ethyl acetate was collected and the aqueous layer was extracted twice with ethyl acetate (40 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by column chromatography over silica gel eluting with 2 hexane: 1 ethyl acetate: 0.1 acetic acid. The yield of purified amino acid was 3.4 g (80% overall). ^{1}H NMR (400 MHz, CD_3OD): δ 2.61 (ddd, 1H, J = 2.5, J = 7.5, J = 17), 2.69 (ddd, 1H, J = 2.5 Hz, J = 5.1 Hz, J = 17 Hz), 4.09 (t, 1H, J = 7.1 Hz), 4.20 (m, 3H), 7.17 (t, 2H, J = 7.3 Hz), 7.25 (t, 2H, J = 7.3 Hz), 7.54 (d, 2H, J = 7.0 Hz), 7.65 (d, 2H, J = 7.7 Hz). ^{13}C (100 MHz, CD_3OD): δ 23.7, 55.3, 69.2, 73.2, 81.4, 122.0, 127.4, 129.2, 129.8, 143.6, 146.3, 159.4, 174.8. HRMS-DART ([M]-): for $C_{19}H_{16}N_2O_4$ Calculated 335.1038, Found 335.1041.

Boc-L-2-amino-4-cyanobutanoic acid. (Figure 2-1, Entry 6) Boc-L-glutamine-OH (2.00 g, 8.60 mmol, 1.00 equiv) was weighed out into a roundbottom flask with a stirbar and dissolved in 50 mL of pyridine. Once the acid was fully dissolved, acetic anhydride (0.98 mL, 10.3 mmol, 1.20 equiv.) was added all at once at room temperature. The reaction mixture was stirred at room temperature overnight. Depending on the purity of the commercially acquired amino acid a slight red hue was occasionally observed; however no effect on the reaction was observed. The reaction was concentrated in vacuo and taken up in ethyl acetate. The ethyl acetate layer was rinsed three times with 30 mL portions of 2M acetic acid, followed by rinses with water (2 x 20mL) and brine (2 x 20mL). The ethyl acetate layer was collected, dried over anhydrous sodium sulfate, and concentrated in vacuo to yield a crude viscous oil which was carried on without further purification. Crude Yield 83%.

Fmoc-L-2-amino-4-cyanobutanoic acid. (Figure 2-1, Entry 8) Boc-L-2-amino-4-cyanobutanoic acid (7.30 mmol, 1.00 equiv.) was dissolved in 25% TFA acid in DCM and stirred at room temperature for 30-45 minutes. The reaction was concentrated in vacuo, redissolved in DCM and reconcentrated. The residue was dissolved in 75 mL of 10% potassium carbonate solution and cooled to 0 ^{o}C in an ice bath. FmocOSu (7.26 g, 18.3 mmol, 2.50 equiv.) was dissolved in a minimal amount of acetonitrile and the solution was added to the reaction dropwise at 0 ^{o}C. The reaction was stirred at 0 ^{o}C for 30 min. then it was stirred at room temperature for 3-3.5 hours. The reaction was filtered through celite, and the aqueous filtrate was collected. The aqueous layer was suspended with ethyl acetate in an Erlenmeyer flask. The aqueous layer was acidified to a pH of 2-3 using 2 M HCL. The ethyl acetate was collected and the aqueous layer

was extracted twice with ethyl acetate (40 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by column chromatography over silica gel eluting with 2 hexane: 1 ethyl acetate: 0.1 acetic acid to provide 2.25 g (75.7% overall). ^{1}H NMR (400MHz, CD_3OD): δ 2.44-2.51 (q, 2H, J = 7.0 Hz), 2.59-2.65 (t, 2H, J = 6.9 Hz), 4.21-4.41 (m, 2H), 4.49-4.56 (d, 2H, J = 6.8 Hz), 7.29-7.34 (t, 2H, J = 7.4 Hz), 7.36-7.43 (t, 2H, J = 7.4 Hz), 7.60-7.65 (d, 2H, J = 7.6 Hz), 7.77-7.78 (d, 2H, J = 7.6 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 28.8, 29.5, 47.8, 66.9, 72.4, 121.0, 126.1, 128.3, 129.0, 142.6, 144.4, 158.0, 171.5. HRMS-DART ([M]-): for $C_{20}H_{18}N_2O_4$ Calculated 349.1188, Found 349.1184.

Boc-L-2-amino-3-aminopropanoic acid. (Figure 2-2, Entry 9) Boc-L-asparagine (2.00 g, 8.60 mmol, 1.00 equiv.) was weighed out into a 100 mL roundbottom flask with a stirbar. Ethyl acetate (20 mL), acetonitrile (20 mL), and 1-2 mL of water were added and the solution was stirred at room temperature until all of the amino acid was dissolved. The solution was cooled to 0 ^{o}C in an ice water bath. Once the solution reached 0 ^{o}C, phenyl iododiacetate (3.32 g, 10.3 mmol, 1.20 equiv.) was added to the reaction all at once. The reaction was stirred at 0 ^{o}C for 30 min. and then warmed to room temperature. The reaction was then stirred at room temperature for five to six hours. During the reaction time some product occasionally precipitated from the reaction solution. The ethyl acetate and acetonitrile were then removed in vacuo. The concentrated solution was then cut with pure acetonitrile until precipitation was observed. Once precipitation began another 5-8 mL of acetonitrile was added and the solution was cooled to 0 ^{o}C to ensure complete precipitation of the product. The white solid was collected on a fritted filter funnel and dried on a vacuum line to afford 1.45 g of

the desired amine (82%). HRMS-DART ([M]+): for $C_8H_{16}N_2O_4$ Calculated 205.1188, Found 205.1191.

Boc-L-2-amino-3-azidopropanoic acid. (Figure 2-2, Entry 11) Boc-L-2-amino-3-aminopropanoic acid (1.45 g, 7.10 mmol, 1.00 equiv.) was weighed into a roundbottom flask and suspended in methanol. Potassium carbonate (2.65 g, 19.1 mmol, 2.70 equiv.) and copper sulfate (0.0180 g, 0.0710 mmol, 0.0100 equiv.) were added to the solution. The mixture was stirred until all of the materials had mostly dissolved. Imidazole sulfonyl azide (2.08 g, 10.0 mmol, 1.40 equiv.) was then added to the reaction. The reaction solution was stirred at room temperature for 6-8 hours until TLC showed the consumption of all starting material. The organic layer was removed on a rotary evaporator and the residue was taken up in aqueous potassium carbonate, extracted with ethyl acetate. The ethyl acetate was discarded and the aqueous layer was acidified to a pH of approximately 2. The organic layer was extracted with ethyl acetate (3 x 30 mL). The ethyl acetate extracts were combined, dried over sodium sulfate, and concentrated on a rotovap. The crude material was carried on without further purification. Crude Yield 91%.

N-Fmoc-L-2-amino-3-azidopropanoic acid. (Figure 2-2, Entry 13) The residue of Boc-L-2-amino-3-azidopropanoic acid (6.40 mmol, 1.00 equiv.) was redissolved in 25% TFA acid in DCM. The solution was stirred for approximately 30 min. The reaction was concentrated in vacuo, redissolved in DCM and reconcentrated. The residue was dissolved in 10% potassium carbonate solution and cooled to 0 ^{o}C in an ice bath. Fmoc-OSu (2.39 g, 7.10 mmol, 1.10 equiv.) was dissolved in a minimal amount of acetonitrile, and the solution was added to the reaction dropwise at 0 ^{o}C. The reaction

was stirred at 0 ^{o}C for 30 min; then stirred at room temperature for 4-5 hours. The reaction was filtered through celite, and the aqueous filtrate was collected. The aqueous layer was suspended with ethyl acetate in an Erlenmeyer flask. The aqueous layer was acidified to a pH of 2-3 using 2 M HCL. The ethyl acetate was collected and the aqueous layer was extracted twice with ethyl acetate (40 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by column chromatography over silica gel eluting with 2 hexane: 1 ethyl acetate: 0.1 acetic acid to provide approximately 1.83 g the desired product (88%). ^{1}H NMR (400MHz, CD_3OD): δ 3.70 (m, 2H), 4.25 (t, 1H, J = 6.8 Hz), 4.38 (d, 2H, J = 6.8 Hz), 4.43 (t, 1H, J = 5.25 Hz), 7.32 (t, 2H, J = 7.5 Hz), 7.40 (t, 2H, J = 7.5 Hz), 7.69 (m, 2H), 7.80 (d, 2H, J = 7.5 Hz). ^{13}C NMR (100MHz, CD_3OD): δ 51.5, 54.1, 66.9, 119.6, 124.9, 126.9, 127.5, 141.3, 143.9, 157.2, 171.3. HRMS-DART ([M]-): for $C_{18}H_{16}O_4N_4$, Calculated 351.1093, Found 351.1089.

Boc-D-2-amino-3-aminopropanoic acid. (Figure 2-2, Entry 9) Boc-D-asparagine (2.00 g, 8.60 mmol, 1.00 equiv.) was weighed out into a 100 mL roundbottom flask with a stirbar. Ethyl acetate (20 mL), acetonitrile (20 mL), and 1-2 mL of water were added and the solution was stirred at room temperature until all of the amino acid was dissolved. The solution was cooled to 0 ^{o}C in an ice water bath. Once the solution reached 0 ^{o}C, phenyl iododiacetate (3.32 g, 10.0 mmoles, 1.20 equiv.) was added to the reaction all at once. The reaction was stirred at 0 ^{o}C for 30 min. and then warmed to room temperature. The reaction was then stirred at room temperature for five to six hours. During the reaction time some product occasionally precipitated from the reaction solution. The ethyl acetate and acetonitrile were then removed in vacuo.

The concentrated solution was then cut with pure acetonitrile until precipitation was observed. Once precipitation began another 5-8 mL of acetonitrile was added and the solution was cooled to 0 ^{o}C to ensure complete precipitation of the product. The white solid was collected on a fritted filter funnel and dried on a vacuum line to afford approximately 1.62 g of the desired amine (80%). HRMS-DART ([M]+): for $C_8H_{16}N_2O_4$ Calculated 205.1188, Found 205.1184.

Boc-D-2-amino-3-azidopropanoic acid. (Figure 2-2, Entry 11) Boc-D-2-amino-3-aminopropanoic acid (1.45 g, 6.70 mmol, 1.00 equiv.) was weighed into a round bottom flask and suspended in methanol. Potassium carbonate (2.47 g, 18.0 mmol, 2.70 equiv.) and copper sulfate (0.0170 g, 0.0600 mmol, 0.0100 equiv.) were added to the solution. The mixture was stirred until all of the materials had mostly dissolved. Imidazole sulfonyl azide (1.67 g, 7.94 mmol, 1.20 Equiv.) was then added to the reaction. The reaction solution was stirred at room temperature for 6-8 hours until TLC showed the consumption of all starting material. The organic layer was removed on a rotovap and the residue was taken up in aqueous potassium carbonate, extracted with ethyl acetate. The ethyl acetate was discarded and the aqueous layer was acidified to a pH of approximately 2. The organic layer was extracted with ethyl acetate (3 x 30 mL). The ethyl acetate extracts were combined, dried over sodium sulfate, and concentrated on a rotovap. Crude Yield 87%.

N-Fmoc-D-2-amino-3-azidopropanoic acid. (Figure 2-2, Entry 13) The residue of Boc-D-2-amino-3-azidopropanoic acid (5.80 mmol, 1.00 equiv.) was redissolved in 25% TFA acid in DCM. The solution was stirred for approximately 30 min. The reaction was concentrated in vacuo, redissolved in DCM and reconcentrated. The residue was

dissolved in 10% potassium carbonate solution and cooled to 0°C in an ice bath. Fmoc-OSu (2.16 g, 6.40 mmol, 1.10 equiv.) was dissolved in minimal acetonitrile and the solution was added to the reaction dropwise at 0 °C. The reaction was stirred at 0 °C for 30 min. then it was stirred at room temperature for 4-5 hours. The reaction was filtered through celite, and the aqueous filtrate was collected. The aqueous layer was suspended with ethyl acetate in an Erlenmeyer flask. The aqueous layer was acidified to a pH of 2-3 using 2 M HCL. The ethyl acetate was collected and the aqueous was extracted twice with ethyl acetate (40 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by column chromatography over silica gel eluting with 2 hexane: 1 ethyl acetate: 0.1 acetic acid to provide 1.69 g the desired product (89%). ^{1}H NMR (400MHz, CD_3OD): δ 3.70 (m, 2H), 4.25 (t, 1H, J = 6.8 Hz), 4.38 (d, 2H, J = 6.8 Hz), 4.43 (t, 1H, J = 5.25 Hz), 7.32 (t, 2H, J = 7.5 Hz), 7.40 (t, 2H, J = 7.5 Hz), 7.69 (m, 2H), 7.80 (d, 2H, J = 7.5 Hz). ^{13}C NMR (100MHz, CD_3OD): δ 51.5, 54.1, 66.9, 119.6, 124.9, 126.9, 127.5, 141.3, 143.9, 157.2, 171.3. HRMS-DART ([M]-): for $C_{18}H_{16}O_4N_4$, Calculated 351.1093, Found 351.1088.

Boc-L-2-Amino-4-Aminobutanoic Acid. (Figure 2-2, Entry 10) Boc-L-glutamine (2.00 g, 0.00861 moles, 1.00 equiv.) was weighed out into a 100 mL roundbottom flask with a stirbar. Ethyl acetate (20 mL), acetonitrile (20 mL), and 1-2 mL of water were added and the solution was stirred at room temperature until all of the amino acid was dissolved. The solution was cooled to 0 °C in an ice water bath. Once the solution reached 0 °C, phenyl iododiacetate (3.30 g, 10.3 mmoles, 1.20 equiv.) was added to the reaction all at once. The reaction was stirred at 0 °C for 30 min. and then warmed to

room temperature. The reaction was then stirred at room temperature for five to six hours. During the reaction time, some product occasionally precipitated from the reaction solution. The ethyl acetate and acetonitrile were then removed in vacuo. The concentrated solution was then cut with pure acetonitrile until precipitation was observed. Once precipitation began another 5-8 mL of acetonitrile was added and the solution was cooled to 0 ^{o}C to ensure complete precipitation of the product. The white solid was collected on a fritted filter funnel and dried on a vacuum line to afford 1.42 g of the desired amine (76%). HRMS-DART ([M]+): for $C_9H_{18}N_2O_4$ Calculated 219.1344, Found 219.1343.

Boc-L-2-amino-4-azidobutanoic acid. (Figure 2-2, Entry 12) Boc-L-2-amino-4-aminobutanoic acid (1.42 g, 6.5 mmol, 1.0 equiv.) was weighed into a roundbottom flask and suspended in methanol. Potassium carbonate (2.44 g, 18.0 mmol, 2.70 equiv.) and copper sulfate (0.0160 g, 0.0650 mmol, 0.0100 equiv.) were added to the solution. The mixture was stirred until all of the materials had mostly dissolved. Imidazole sulfonyl azide (1.65 g, 7.80 mmol, 1.20 equiv.) was then added to the reaction. The reaction solution was stirred at room temperature for 6-8 hours until TLC showed the consumption of all starting material. The organic layer was removed on a rotovap and the residue was taken up in aqueous potassium carbonate, and extracted with ethyl acetate (3 x 30 mL). The ethyl acetate was discarded and the aqueous layer was acidified to a pH of approximately 2. The organic layer was extracted with ethyl acetate (3 x 30 mL). The ethyl acetate extracts were combined dried over sodium sulfate, and concentrated on a rotovap. Crude Yield 93%.

Fmoc-L-2-amino-4-azidobutanoic acid. (Figure 2-2, Entry 14) The residue of Boc-L-2-amino-4-azidobutanoic acid (6.10 mmol, 1.00 equiv.) was redissolved in 25% TFA acid in DCM. The solution was stirred for approximately 30 min. The reaction was concentrated in vacuo, redissolved in DCM and reconcentrated. The residue was dissolved in 10% potassium carbonate solution and cooled to 0 ^{o}C in an ice bath. Fmoc-OSu (2.25 g, 6.60 mmol, 1.10 equiv.) was dissolved in a minimal amount of acetonitrile and the solution was added to the reaction dropwise at 0 ^{o}C. The reaction was stirred at 0 ^{o}C for 30 min., and then stirred at room temperature for 4-5 hours. The reaction was filtered through celite, and the aqueous filtrate was collected. The aqueous layer was suspended with ethyl acetate in an Erlenmeyer flask. The aqueous layer was acidified to a pH of 2-3 using 2 M HCL. The ethyl acetate was collected and the aqueous layer was extracted twice with ethyl acetate (40 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by column chromatography over silica gel eluting with 2 hexane: 1 ethyl acetate: 0.1 acetic acid to provide 1.99 g of the desired product (89%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.78-1.92 (m, 1H), 1.99-2.12 (m, 2H), 3.27-3.42 (m, 2H), 4.12-4.19 (t,1H, J = 7 Hz), 4.18-4.24 (m, 1H), 4.29-4.34 (d, 2H, J = 7 Hz), 7.21-7.27 (t, 2H, J = 7.45 Hz), 7.29-7.35 (t, 2H, J = 7.48 Hz), 7.57-7.63 (t, 2H, J = 6.2 Hz), 7.70-7.75 (d, 2H, J = 7.5 Hz). ^{13}C NMR (100MHz, CD_3OD): δ 31.8, 48.4, 52.7, 67.9, 120.9, 126.2, 128.1, 128.7, 142.6, 145.1, 145.3, 158.6, 175.1. HRMS-DART ([M]-): for $C_{19}H_{18}N_4O_4$, Calculated 365.1250, Found 365.1232.

Boc-D-2-amino-4-aminobutanoic acid. (Figure 2-2, Entry 10) Boc-D-glutamine (2.00 g, 8.60 mmoles, 1.00 equiv.) was weighed out into a 100 mL roundbottom flask

with a stirbar. Ethyl acetate (20 mL), acetonitrile (20 mL), and 1-2 mL of water were added and the solution was stirred at room temperature until all of the amino acid was dissolved. The solution was cooled to 0 ^{o}C in an ice water bath. Once the solution reached 0 ^{o}C, phenyl iododiacetate (3.30 g, 10.3 mmoles, 1.20 equiv.) was added to the reaction all at once. The reaction was stirred at 0 ^{o}C for 30 min. and then warmed to room temperature. The reaction was then stirred at room temperature for five to six hours. During the reaction some product occasionally precipitated from the reaction solution. The ethyl acetate and acetonitrile were then removed in vacuo. The concentrated solution was then cut with pure acetonitrile until precipitation was observed. Once precipitation began another 5-8 mL of acetonitrile was added and the solution was cooled to 0 ^{o}C to ensure complete precipitation of the product. The white solid was collected on a fritted filter funnel and dried on a vacuum line to afford 1.57 g of the desired amine (86%). HRMS-DART ([M]+): for $C_9H_{18}N_2O_4$ Calculated 219.1344, Found 219.1347.

Boc-D-2-amino-4-azidobutanoic acid. (Figure 2-2, Entry 12) Boc-D-2-amino-4-aminobutanoic acid (1.42 g, 6.50 mmol, 1.00 equiv.) was weighed into a roundbottom flask and suspended in methanol. Potassium carbonate (2.44 g, 18.0 mmol, 2.70 equiv.) and copper sulfate (0.0160 g, 0.0650 mmol, 0.0100 equiv.) were added to the solution. The mixture was stirred until all of the reagents had mostly dissolved. Imidazole sulfonyl azide (1.65 g, 7.80 mmol, 1.20 equiv.) was then added to the reaction. The reaction solution was stirred at room temperature for 6-8 hours until TLC showed the consumption of all starting material. The organic layer was removed on a rotovap and the residue was taken up in aqueous potassium carbonate, extracted with

ethyl acetate. The ethyl acetate was discarded and the aqueous layer was acidified to a pH of approximately 2. The organic layer was extracted 3 times with ethyl acetate. The ethyl acetate extracts were combined dried over anhydrous sodium sulfate, and concentrated on a rotovap. Crude Yield 93%.

Fmoc-D-2-amino-4-azidobutanoic acid. (Figure 2-2, Entry 14) The residue of Boc-D-2-amino-4-azidobutanoic acid (6.10 mmol, 1.00 equiv.) was redissolved in 25% TFA in DCM. The solution was stirred for approximately 30 min. The reaction was concentrated in vacuo, redissolved in DCM and reconcentrated. The residue was dissolved in 10% potassium carbonate solution and cooled to 0 ^{o}C in an ice bath. Fmoc-OSu (2.25 g, 6.60 mmol, 1.10 equiv.) was dissolved in a minimal amount of acetonitrile and the solution was added to the reaction dropwise at 0 ^{o}C. The reaction was stirred at 0 ^{o}C for 30 min. then it was stirred at room temperature for 4-5 hours. The reaction was filtered through celite, and the aqueous filtrate was collected. The aqueous layer was suspended with ethyl acetate in an Erlenmeyer flask. The aqueous layer was acidified to a pH of 2-3 using 2 M HCL. The ethyl acetate was collected and the aqueous layer was extracted twice with ethyl acetate (40 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by column chromatography over silica gel eluting with 2 hexane: 1 ethyl acetate: 0.1 acetic acid to provide 1.99 g of the desired product (89%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.78-1.92 (m, 1H), 1.99-2.12 (m, 2H), 3.27-3.42 (m, 2H), 4.12-4.19 (t,1H, J = 7 Hz), 4.18-4.24 (m, 1H), 4.29-4.34 (d, 2H, J = 7 Hz), 7.21-7.27 (t, 2H, J = 7.45Hz), 7.29-7.35 (t, 2H, J = 7.48 Hz), 7.57-7.63 (t, 2H, J = 6.2 Hz), 7.70-7.75 (d, 2H, J = 7.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 31.8, 48.4, 52.7, 67.9, 120.9, 126.2,

128.1, 128.7, 142.6, 145.1, 145.3, 158.6, 175.1. HRMS-DART ([M]-): for $C_{19}H_{18}N_4O_4$, Calculated 365.1250, Found 365.1230.

Fmoc-D-2-amino-5-azidopentanoic acid. Fmoc-D-ornithine-Boc-OH (1.41 g, 2.50 mmol, 1.00 equiv.) was weighed out into a roundbottom flask with a stirbar. The amino acid was dissolved in 25% TFA in dichloromethane. The solution was stirred for approximately 30 min. The reaction was concentrated in vaccuo, redissolved in DCM and reconcentrated. The residue of Fmoc-ornithine-OH (1.10 g, 2.50 mmol, 1.00 equiv.) was dissolved in ~75mL of 10% potassium carbonate solution. Copper sulfate pentahydrate (0.126 g, 0.500 mmol, 0.200 equiv.) was added to the solution and the mixture was stirred until most of the copper sulfate was dissolved. The mixture was cooled to 0 ^{o}C and then triflyl azide in toluene (10.5 mL, 1.00 M, 10.1 mmol, 4.00 equiv.) was added slowly to the reaction mixture. The reaction was stirred at room temperature for 30 min. and then for another 6-8 hours at room temperature. The reaction was then suspended with ethyl acetate and acidified to a pH of 2. The reaction was extracted twice more with ethyl acetate. The organic extracts were combined, dried over anhydrous sodium sulfate, and concentrated to provide 0.900 g of the product as a white solid (91%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.51-1.92 (m, 4H), 3.24-3.27 (m, 2H), 3.98-407(m, 1H), 4.12-4.19 (m, 1H), 4.245-4.404 (m, 2H), 7.22-7.29 (t, 2H, J = 7.4 Hz), 7.30-7.37 (t, 2H, J = 7.4 Hz), 7.53-7.63 (m, 2H), 7.71-7.76 (d, 2H, J = 7.4) ^{13}C NMR (100 MHz, CD_3OD): δ 22.4, 30.5, 48.4, 52.1, 67.7, 120.9, 126.1, 128.1, 128.7, 142.6, 145.2, 174.4. HRMS-DART ([M]-): for $C_{20}H_{20}N_4O_4$, Calculated 379.1406, Found 379.1410.

Fmoc-L-2-amino-6-azido-hexanoic acid. Fmoc-lysine-OH (1.00 g, 2.70 mmol, 1.00 equiv.) was dissolved in ~75mL of 10% potassium carbonate solution. Copper sulfate pentahydrate (0.136 g, 0.540 mmol, 0.200 equiv.) was added to the solution and the mixture was stirred until most of the copper sulfate was dissolved. The mixture was cooled to 0 ^{o}C and then triflyl azide in toluene (11.0 mL, 1.00 M, 11.0 mmol, 4.00 equiv.) was added slowly to the reaction mixture. The reaction was stirred at room temperature for 30 min. and then for another 6-8 hours at room temperature. The reaction was then suspended with ethyl acetate and acidified to a pH of 2. The reaction was extracted twice more with ethyl acetate. The organic extracts were combined, dried over anhydrous sodium sulfate, and concentrated to provide 1.01 g of the product as a white solid (94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42-1.53 (m, 2H), 1.56-1.68 (m, 2H), 1.68-1.80 (m, 1H), 1.88-2.00 (m, 1H), 3.25-3.53 (t, 1H, J = 6.6 Hz), 4.20-4.27 (t, 1H, J = 7.2 Hz), 4.40-4.47 (d, 2H, J = 6.6 Hz), 7.29-7.36 (t, 2H, J = 7.2 Hz), 7.37-7.44 (t, 2H, J = 7.3 Hz), 7.55-7.67 (d, 2H, J = 7.4 Hz), 7.74-7.83 (d, 2H, J = 7.2 Hz). ^{13}C NMR (121MHz, $CDCl_3$): δ 22.3, 28.2, 31.8, 47.1, 51.0, 67.1, 119.9, 125.0, 127.0, 127.7, 141.3, 143.6, 156.1. HRMS-DART ([M]-): for $C_{21}H_{22}N_4O_4$, Calculated 393.1563, Found 393.1565.

Copper (II) lysine monohydrochloride complex. Copper (II) sulfate pentahydrate (0.984 g, 3.30 mmol, 1.20 equiv.) and potassium carbonate (1.82 g, 13.0 mmol, 4.00 equiv.) were weighed into a 100 mL roundbottom flask. Water (8 mL) was added and the solution was stirred until most all of the potassium carbonate was dissolved. L-Lysine mono hydrochloride was added and the reaction was stirred at room temperature for 1-2 hours. The azide reaction was continued in same pot.

Copper (II) 2-amino-6-azidohexanoic acid complex. Copper (II) sulfate pentahydrate (0.218 g, 0.850 mmol, 0.260 equiv.) and potassium carbonate (1.82 g, 13.0 mmol, 4.00 equiv.) were added to the solution. The solution was stirred for 5-10 min. and then imidazolium-1-sulfonyl azide (1.99 g, 11.5 mmol, 3.50 equiv.) was added to the reaction. The reaction was stirred at room temperature overnight. The desired product was filtered out of the reaction solution as a pale blue powder (0.972 g, 73% yield).

Fmoc-2-Amino-6-Azidohexanoic Acid. The blue copper (II) 6-azido-2-amino-hexanoic acid complex was dried and crushed into a powder. The powdered complex was placed into a 50 mL roundbottom flask. A saturated aqueous solution of EDTA was prepared by adding (2.53 g, 8.60 mmol) of EDTA to a solution of 1.42 g of sodium bicarbonate in 20 mL of water slowly in batches. The solution was stirred vigorously until all of the EDTA had dissolved. The EDTA solution was added to the copper (II) 6-azido-2-amino-hexanoic acid complex and the solution was shaken for approximately 1 hour. During this time the solution turned a deep blue color. The resulting solution was filtered and transferred to a 250 mL roundbottom flask. The pH was tested and adjusted to around a pH of 8 using 10% potassium carbonate solution. A solution of FmocOSu in acetonitrile was made. The solution of 2-amino-6-azido-hexanoic acid was cooled to 0 ^{o}C in an ice water bath and the FmocOSu solution was added dropwise. The reaction was stirred at 0 ^{o}C for 30 min. and then warmed to room temperature. The reaction was stirred for another 4-6 hours at room temperature. The reaction was filtered through celite, and the aqueous filtrate was collected. The aqueous layer was suspended with ethyl acetate in an erlenmeyer flask. The aqueous layer was acidified

to a pH of 2-3 using 2M HCL. The ethyl acetate was collected and the aqueous layer was extracted twice with ethyl acetate (40 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by column chromatography over silica gel eluting with 2 hexane: 1 ethyl acetate: 0.1 acetic acid to provide 0.930 g of the desired product (97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42-1.53 (m, 2H), 1.56-1.68 (m, 2H), 1.68-1.80 (m, 1H), 1.88-2.00 (m, 1H), 3.25-3.53 (t, 1H, J = 6.6 Hz), 4.20-4.27 (t, 1H, J = 7.2 Hz), 4.40-4.47 (d, 2H, J = 6.6 Hz), 7.29-7.36 (t, 2H, J = 7.2 Hz), 7.37-7.44 (t, 2H, J = 7.3 Hz), 7.55-7.67 (d, 2H, J = 7.4 Hz), 7.74-7.83 (d, 2H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.3, 28.2, 31.8, 47.1, 51.0, 67.1, 119.9, 125.0, 127.0, 127.7, 141.3, 143.6, 156.1. HRMS-DART ([M]-): for $C_{21}H_{22}N_4O_4$, Calculated 393.1563, Found 393.1565.

S-N-Benzyl Proline. L-Proline (20.0 g, 174 mmol, 1.00 equiv.) was suspended in 95 mL of dry methanol in a 500 mL roundbottom flask. The roundbottom flask was fitted with a pressure equalizing addition funnel and the system was purged with nitrogen for 15-20 min. The addition funnel was charged with 5.3 M sodium methoxide in methanol (65.5 mL, 347 mmol, 2.00 equiv.). The sodium methoxide solution was added to the suspension of L-proline dropwise at room temperature. The addition funnel was rinsed three times with 5 mL of dry methanol. The reaction was then heated to 48 ^{o}C. Once the solution had reached 48 ^{o}C benzyl chloride (20.5 mL, 117 mmol, 1.10 equiv.) was added to the reaction dropwise under nitrogen. The reaction was stirred overnight at 48 ^{o}C. The reaction was cooled to room temperature and quenched by dropwise addition of conc. hydrochloric acid to a pH of 4. Chloroform (200 mL) was then added and the mixture was stirred at room temperature for 1 hour. The precipitate was filtered, rinsed

well with chloroform and discarded. The filtrate was concentrated on a rotovap. The residue was taken up in acetone and mixed vigorously with a spatula leading to the formation a fine white precipitate. The precipitate was collected on a filter funnel and rinsed thoroughly with acetone to provide 28 g of product (79%). The precipitated benzyl proline was used without further purification. ^{1}H NMR ($CDCl_3$): δ 1.93-2.06 (2H, m), 2.27-2.35 (2H, m), 2.90 (1H, dd, J = 18.3, 9.9 Hz), 3.64 (1H, ddd J = 9.9, 7.1, 3.6 Hz), 3.82 (1H, dd, J = 14.1, 7.1 Hz) 4.21, 4.34 (2H, AB, J = 12.9 HZ), 7.37-7.42 (5H, m). HRMS-DART ([M]+): for $C_{12}H_{15}NO_2$, Calculated 206.1181, Found 206.1187.

Benzyl Proline Benzophenone. (Figure 2-5, Entry 19) Benzyl proline (6.94 g, 34.0 mmol, 1.00 equiv.) was weighed out into a roundbottom flask with a stirbar. It was suspended in DCM, and n-methyl imidazole (5.93 mL, 74.4 mmol, 2.20 equiv.) was then added. The mixture was cooled to 0 ^{o}C in an ice water bath. Mesyl chloride (2.63 mL, 34.0 mmol, 1.00 equiv.) was added to the reaction dropwise under nitrogen. The reaction was removed from the ice bath, and 2-aminobenzophenone (6.00 g, 30.4 mmol, 0.900 equiv.) was added. The reaction was heated to 45 ^{o}C and was stirred at that temperature overnight. The reaction was quenched with saturated ammonium chloride solution. The aqueous phase was then extracted three times with dichloromethane. The organic extracts were combined, dried over sodium sulfate, and concentrated on a rotovap. The resulting residue was taken up in methanol sonicated for 20-30 min. and then placed in a freezer for 2-3 hours. The precipitated ligand was collected by filtration and rinsed thoroughly with cold methanol to give 7.8 g of BPB as an off-yellow solid (61%). The recovered ligand was used without further purification. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.73–1.86 (2H, m), 1.96 (1H, ddd, J = 4.4, 8.4, 16.8 Hz),

2.25 (1H, ddd, *J* = 9.8, 13.0, 18.1 Hz), 2.41 (1H, dt, *J* = 6.8, 9.6 Hz), 3.21 (1H, ddd, *J* = 2.4, 6.8, 9.2 Hz), 3.32 (1H, dd, *J* = 4.7, 10.2 Hz), 3.59 (1H, d, *J* = 12.8 Hz), 3.92 (1H, d, *J* = 12.8 Hz), 7.07–7.62 (12H, m), 7.78 (1H, d, *J* = 7.9 Hz), 8.56 (1H, d, *J* = 8.5 Hz), 11.52 (1H, s); ^{13}C NMR *δ* (100 MHz, $CDCl_3$): 24.1, 30.9, 53.8, 59.8, 68.2, 121.4, 122.1, 125.2, 127.0, 128.1, 128.2, 129.0, 130.1, 132.5, 132.5, 133.3, 138.1, 138.5, 139.1, 174.6, 198.0. HRMS-DART ([M]+): for $C_{25}H_{24}N2O_2$, Calculated 385.1916, Found 385.1918.

Nickel Benzylproline Benzophenone Glycine Complex. (Figure 2-5, Entry 18) A solution of benzyl proline benzophenone (6.50 g, 17.0 mmol, 1.00 equiv.), nickel nitrate hexahydrate (9.21 g, 34.0 mmol, 2.00 equiv.), and glycine (6.36 g, 85.0 mmol, 5.00 equiv.) in dry methanol was heated to 65 ^{o}C under nitrogen. A solution of potassium hydroxide (6.66 g, 119 mmol, 7.00 equiv.) in dry methanol was added all at once to the heated solution. The resulting solution was stirred at 65 ^{o}C under nitrogen for 3-4 hours. The reaction was neutralized with conc. acetic acid and then diluted to three times the volume with slow dropwise addition of water. The resulting slurry was extracted three times with DCM. The organic extracts were combined, dried over sodium sulfate, and concentrated on a rotovap. Drying on a high vacuum line provided 8.11 g of the product as a fluffy, foamy red solid which could be used without further purification (94%). HRMS-DART ([M]+): for $C_{27}H_{25}N_3NiO_3$ Calculated 498.1327, Found 498.1320.

Nickel Benzylproline Benzophenone Propargyl Glycine. Nickel benzylproline benzophenone (2.70 g, 5.40 mmol, 1.00 equiv.) was placed in a roundbottom flask with a stirbar. The flask was purged with nitrogen and then acetonitrile (20 mL) was added

to the flask via syringe. Powdered sodium hydroxide (0.790 g, 14.0 mmol, 2.50 equiv.) was then added to the solution and the mixture was stirred under nitrogen for 10-15 min. Propargyl bromide (0.870 mL, 8.10 mmol, 1.50 equiv.) was then added to the reaction via syringe. The reaction was stirred at room temperature under nitrogen for 2-3 hours. The reaction was quenched by the addition of 0.1 M hydrochloric acid (150 mL). The reaction was extracted three times with 20 mL of DCM. The DCM extracts were combined, dried over anhydrous sodium sulfate and concentrated to yield the deep red product complex. The complex was purified by chromatography over silica gel using 4 DCM: 1 acetone as an eluent to provide 2.62 g of the deep red product complex (90 %). HRMS-DART ([M]+): for $C_{30}H_{27}N_3NiO_3$ Calculated 536.1484, Found 536.1478.

Propargyl Glycine. Methanol (16mL) and 2 M hydrochloric acid were added to a roundbottom flask with a stirbar. The solution was heated to 60 ^{o}C in an oil bath. The nickel benzylproline benophenone propargyl glycine complex was dissolved in 12 mL of a 2 methanol to 1 DCM solution. The nickel benzylproline benophenone propargyl glycine solution was added dropwise to the heated acid solution. The resulting red solution was stirred at 60 ^{o}C for 1-2 hours. As the hydrolysis occurred the red solution slowly turned to an emerald green colored solution. Once the entire complex had been consumed as determined by TLC the reaction was cooled to room temperature, and the methanol and water were removed on a rotovap. The residue was taken back up in 25% aqueous ammonium hydroxide and reconcentrated. The residue was taken back up in water. The aqueous layer was extracted 3-4 times with DCM to remove the liberated Benzyl Proline Benzophenone which was collected and recrystallized to be reused for complex formation. The aqueous layer was concentrated to ~ 10 mL. The

amino acid was purified by ion exchange chromatography using Dowex 50X ion exchange resin and using pure water as a flush and 25% aqueous ammonium hydroxide to recover 0.540 g of the purified amino acid (94%).

Fmoc-Propargyl Glycine. The purified propargyl glycine (400 mg, 3.50 mmol, 1.00 equiv.) was dissolved in 7-8 mL of 10% potassium carbonate solution. FmocOSu (1.24 g, 3.70 mmol, 1.10 equiv.) was dissolved in 7-8 mL of acetonitrile. The propargyl glycine solution was cooled to 0 ^{o}C in an ice water bath. Once the propargyl glycine solution was cooled, the FmocOSu solution was added dropwise. The reaction was stirred for 30 min. at 0 ^{o}C and then warmed to room temperature. The reaction was stirred at room temperature for another 4-6 hours. Once most all of the starting amine was consumed by TLC analysis the reaction was quenched with water and the acetonitrile was removed on a rotovap. The basic solution was extracted once with 20 mL of ethyl acetate. The aqueous layer was collected, acidified to a pH of 2 with 3 M HCl, and extracted 3 times with ethyl acetate (40 mL). The acidic ethyl acetate extractions were combined, washed with water twice, dried over anhydrous sodium sulfate and concentrated on a rotovap. The product's purity was then checked by TLC and HPLC. If needed the product was purified by flash chromatography over silica gel using 3 hexane: 1 ethyl acetate: 0.1 acetic acid as an eluent. ^{1}H NMR (400 MHz, CD_3OD): δ 2.32-2.33 (t, 1H, J = 2.6 Hz), 2.62-2.69 (ddd, 1H, J = 2.55 Hz, J = 7.4 Hz, J = 16.8 Hz), 2.70-2.77 (ddd, 1H, J = 2.52 Hz, J = 5.22 Hz, J = 16.9 Hz), 4.18-4.23 (t, 1H, J = 5.6 Hz), 4.28-4.36 (m, 1H), 7.24-7.32 (t, 2H, J = 7.4 Hz), 7.33-7.39 (t, 2H, J = 7.4 Hz), 7.62-7.69 (d, 2H, J = 6.9 Hz), 7.74-7.79 (d, 2H, J = 7.4 Hz). ^{13}C NMR (100 MHz,

CD_3OD): δ 22.6, 48.3, 54.2, 68.1, 72.0, 80.3, 120.9, 126.2, 128.1, 128.7, 197.5, 207.1. HRMS-DART ([M]-): for $C_{20}H_{17}NO_4$ Calculated 334.108, Found 334.107.

Nickel Benzylproline Benzophenone Pentynyl Glycine. Nickel benzylproline benzophenone (2.37 g, 4.80 mmol, 1.00 equiv.) was placed in a roundbottom flask with a stirbar. The flask was purged with nitrogen and then acetonitrile (20 mL) was added to the flask via syringe. Powdered sodium hydroxide (0.690 g, 11.9 mmol, 2.50 equiv.) was then added to the solution and the mixture was stirred under nitrogen for 10-15 min. TMS-pentynyl bromide (1.25 g, 5.70 mmol, 1.20 equiv.) was then added to the reaction via syringe. The reaction was stirred at room temperature under nitrogen for 2-3 hours. The reaction was quenched by the addition of 0.1 M hydrochloric acid (150 mL). The reaction was extracted three times with 20 mL of DCM. The DCM extracts were combined, dried over anhydrous sodium sulfate and concentrated to yield the deep red product complex. The complex was purified by chromatography over silica gel using 4 DCM: 1 Acetone as an eluent to provide 2.72 g of product complex (90%). HRMS-DART ([M]+) for $C_{32}H_{31}N3NiO_3$, Calculated 564.1797, Found 564.1772.

Pentynyl Glycine. Methanol (16mL) and 2 M hydrochloric acid were added to a roundbottom flask with a stirbar. The solution was heated to 60 ^oC in an oil bath. The nickel benzylproline benophenone pentynyl glycine complex was dissolved in 12 mL of a 2 methanol to 1 DCM solution. The nickel benzylproline benophenone pentynyl glycine solution was added dropwise to the heated acid solution. The resulting red solution was stirred at 60 ^oC for 1-2 hours. As the hydrolysis occurred the red solution slowly turned to an emerald green colored solution. Once the entire complex had been consumed as determined by TLC, the reaction was cooled to room temperature, and

the methanol and water were removed on a rotovap. The residue was taken back up in 25% aqueous ammonium hydroxide and reconcentrated. The residue was taken back up in water. The aqueous layer was extracted 3-4 times with DCM to remove the liberated Benzyl Proline Benzophenone which was collected and recrystallized to be reused for complex formation. The aqueous layer was concentrated to ~10 mL. The amino acid was purified by ion exchange chromatography using Dowex 50X ion exchange resin and using pure water as a flush and 25% aqueous ammonium hydroxide to recover 0.700 g of the purified amino acid (92%).

Fmoc-Pentynyl Glycine. The purified pentynyl glycine (400 mg, 0.00350 mol, 1.00 equiv.) was dissolved in 7-8 mL of 10% potassium carbonate solution. FmocOSu (1.24 g, 0.00371 mol, 1.05 equiv.) was dissolved in 7-8 mL of acetonitrile. The pentynyl glycine solution was cooled to 0 ^{o}C in an ice water bath. Once the pentynyl glycine solution was cooled, the FmocOSu solution was added dropwise. The reaction was stirred for 30 min. at 0 ^{o}C and then warmed to room temperature. The reaction was stirred at room temperature for another 4-6 hours. Once most all of the starting amine was consumed by TLC analysis, the reaction was quenched with water and the acetonitrile was removed on a rotovap. The basic solution was extracted once with 20 mL of ethyl acetate. The aqueous layer was collected, acidified to a pH of 2 with 3M HCl, and extracted 3 times with ethyl acetate (40 mL). The acidic ethyl acetate extractions were combined, washed with water twice, dried over anhydrous sodium sulfate and concentrated on a rotovap. The product's purity was then checked by TLC and HPLC. If needed the product was purified by flash chromatography over silica gel using 3 hexane: 1 ethyl acetate: 0.1 acetic acid as an eluent to provide 0.900 g of

desired product (87%). ^{1}H NMR (300 MHz, DMSO): δ 1.41-1.58 (m, 2H), 1.59-1.76 (m, 1H), 1.76-1.89 (m, 1H), 2.10-2.22 (m, 2H), 3.91-4.02 (m, 1H), 4.18-4.26 (t, 1H, J = 6.0 Hz), 4.26-4.34 (d, 1H, J = 5.8 Hz), 7.28-7.37 (t, 2H, J = 7.1 Hz), 7.37-7.45 (t, 2H, J = 7.4 Hz), 7.67-7.77 (t, 2H, J = 7.6 Hz), 7.85-7.92 (d, 2H, J = 7.1 Hz). ^{13}C NMR (75 MHz, DMSO): δ 17.3, 22.8, 24.7, 46.6, 53.4, 65.1, 71.4, 121.4, 132.0, 132.1, 161.2, 185.3, 214.3. HRMS-DART ([M]-): for $C_{22}H_{21}NO_4$ Calculated 362.1392, Found 362.1394.

Chapter 3: Synthesis of Aryl Triazoles and Tryptophan Containing Peptides Bearing Aryl Triazoles

Background and Significance

Fluorescent probe molecules have become powerful tools for the monitoring of molecular structure and the activity of biomolecules. These processes have been applied successfully to nucleic acid detection of the presence of specific DNA or RNA target by changes in fluorescence.[37] However, the design of peptide-based probes (peptide beacons) that report on binding to, and dissociation from, specific protein targets has been more challenging due to the lack of a universal peptide recognition code.[38, 39] Many of the successful approaches to peptide-beacons rely on strategic positioning of interacting fluorescent dyes so that changes in the peptide structure, due to target binding and dissociation, lead to changes in the fluorescence intensity, via photo-induced electron transfer (PET), or shifts in the fluorescence wavelength, via fluorescence resonance energy transfer (FRET).

We then looked at the possibility of using our clickable amino acids used to design a method of introducing fuorophoric probe molecules. This chemistry provides the capability to place clickable amino acids into the sequences of peptides and peptidomimetics. Fluorescent probe molecules could then be attached to the constructed peptidomimetics via click chemistry. For this to be a valid approach for peptide labeling, the constructed triazole attachments would have to function as interactive fluorescent dyes. The best possibility for the generation of triazole

chromophores was to synthesize and analyze the absorbance and fluorescence character of a variety of triazoles and triazole containing peptides.

A series of aryl triazoles and triazole containing peptides were synthesized on-resin for the analysis of their absorbance and fluorescent properties, which is discussed in Chapter 4. These studies are to analyze the potential use of triazoles as fluorescent probes for peptide structure and binding. The on-resin approach was chosen for the ease of the synthesis, the ability to use an excess of reagent, and for the ease of purification. In many cases the products were pure enough for study without any purification after cleavage from resin.

Synthesis of Simple Aryl Triazoles

The synthesis of the aryl triazoles was accomplished on modified Wang resin and is outlined in Figure 3-1. The resin was converted to a more reactive resin by the attachment of trichloroacetonitrile. The trichloroacetimidate resin (TCA resin) was prepared by the reaction of commercial Wang resin with a large excess of trichloroacetonitrile and a catalytic amount of 1, 8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry dichloromethane (DCM). The resin was then drained, rinsed well with DCM, dried and stored. The dried TCA resin was converted to an alkyne functionalized resin by the reaction with propargyl alcohol and a catalytic amount of boron trifluoride diethyl etherate in a 50:50 mixture of dry DCM and dry tetrahydrofuran (THF). The aryl triazoles were then assembled by on-resin click with a variety of aryl azides with the propargyl Wang resin **1**. The click reactions were carried out on the resin bound substrate with copper iodide and piperidine. All of the on-resin click (ORC) reactions

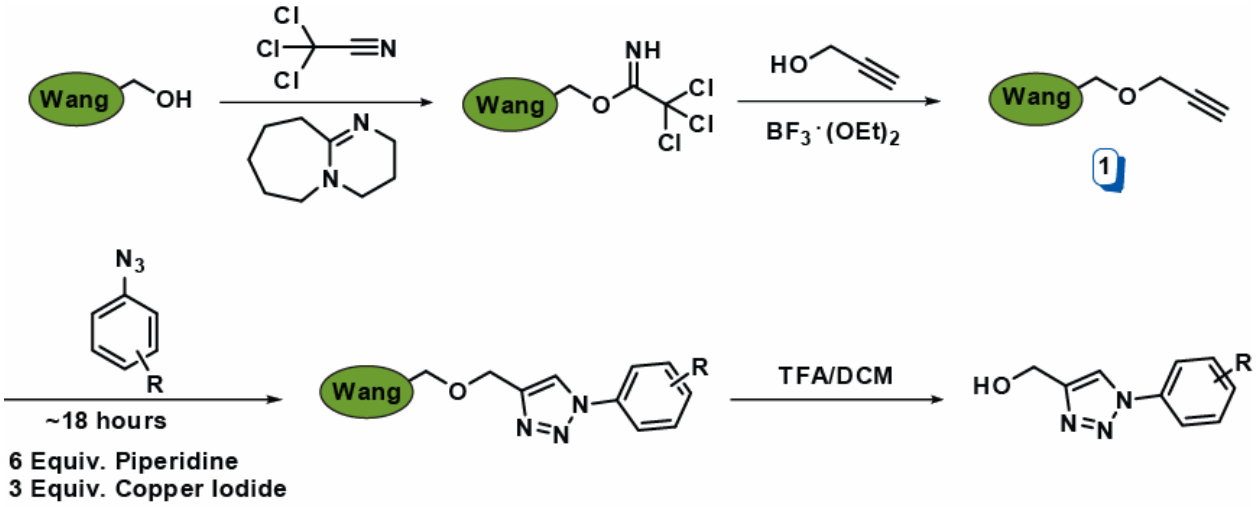


Figure 3-1. General Reaction Scheme for the Synthesis of Aryl Triazoles on TCA Modified Wang Resin

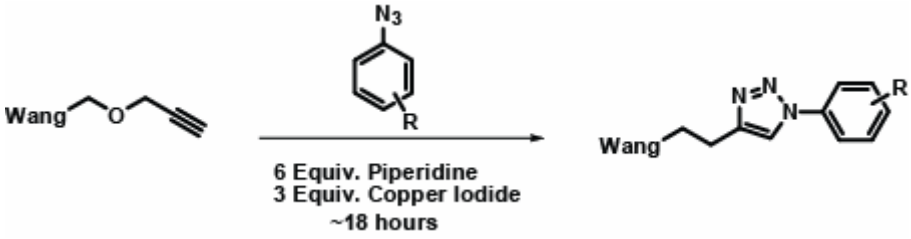
proceeded efficiently to give products that in many cases required no further purification. A list of synthesized aryl triazoles is provided in Table 3-1.

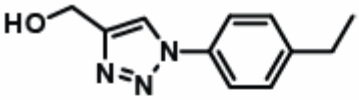
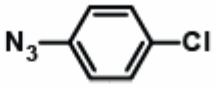
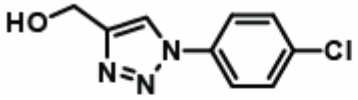
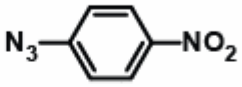
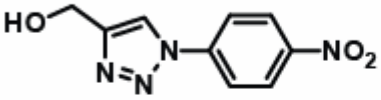
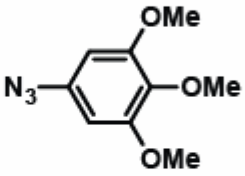
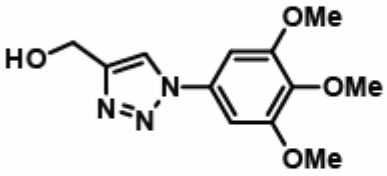
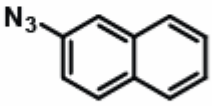
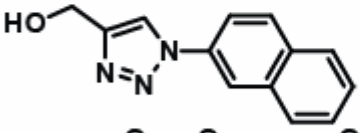
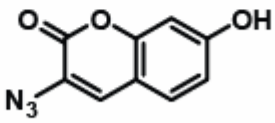
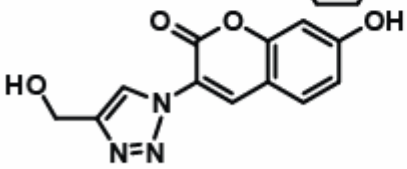
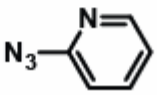
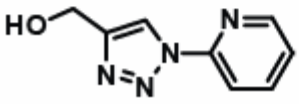
Synthesis of Small Aryl Triazole Functionalized Peptides

An initial series of short peptides was synthesized in order to test the feasibility of using the aryl triazoles as fluorophore partners for tryptophan residues. A standard peptide **2** containing no aryl triazole was synthesized on Knorr Rink resin using standard solid phase peptide synthesis (Figure 3-2). The remaining test peptides were all assembled from an aryl triazole base structure. The aryl triazole was assembled utilizing the propargyl modified Wang resin as shown in Figure 3-3.

The two aryl triazole functionalized peptides **3** and **4** were synthesized by clicking p-nitrophenyl azide (Table 3-1, Entry 3) to the previously prepared propargyl resin to give the triazole functionalized resin **5** as shown in Figure 3-3. Reduction of the nitro group gave the aryl amine **6**. The first amino acid residue was coupled using 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) to activate the acid. The amino acid coupling to the aryl amine was sluggish even with the use of the stronger coupling reagent HATU. The coupling of the first amino acid was performed three times to ensure efficient loading before moving to the next residue. The remaining portion of each peptide sequence was synthesized using standard solid phase peptide conditions. The entire synthetic scheme for the two peptides is outlined in Figure 3-3. The two peptides, **3** and **4**, were synthesized using the same aryl triazole, however the proline residue in peptide **3** was substituted for a glycine in peptide **4.**

Table 3-1. Click Reactions of Aryl Azides with Propargyl Wang Resin



Entry	Aryl Azide	Product	Yield
1			90%
2			81%
3			47%
4			88%
5			93%
6			85%
7			79%

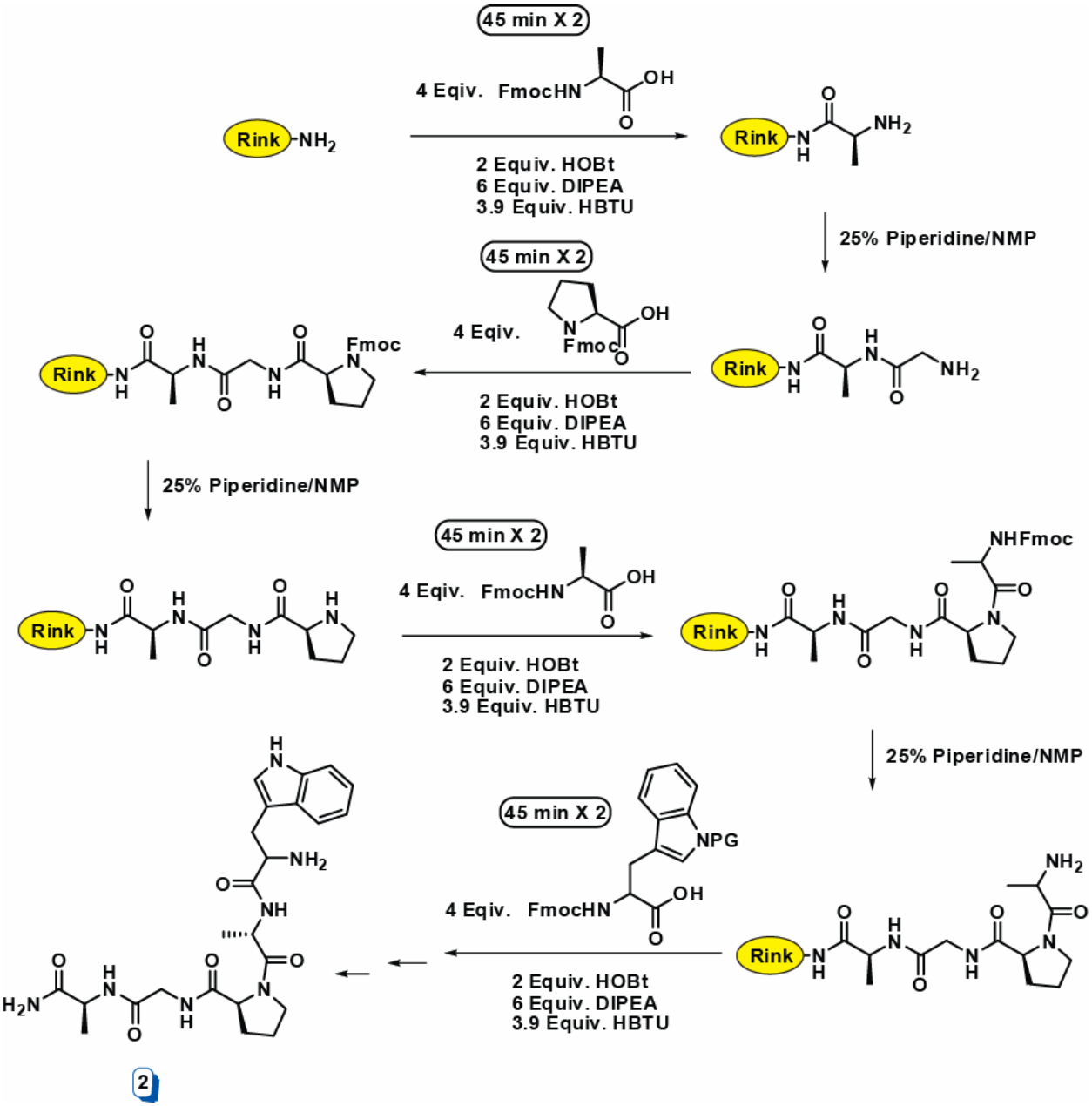


Figure 3-2. Synthetic Scheme for the Synthesis of the Natural Non-Triazole Standard Peptide

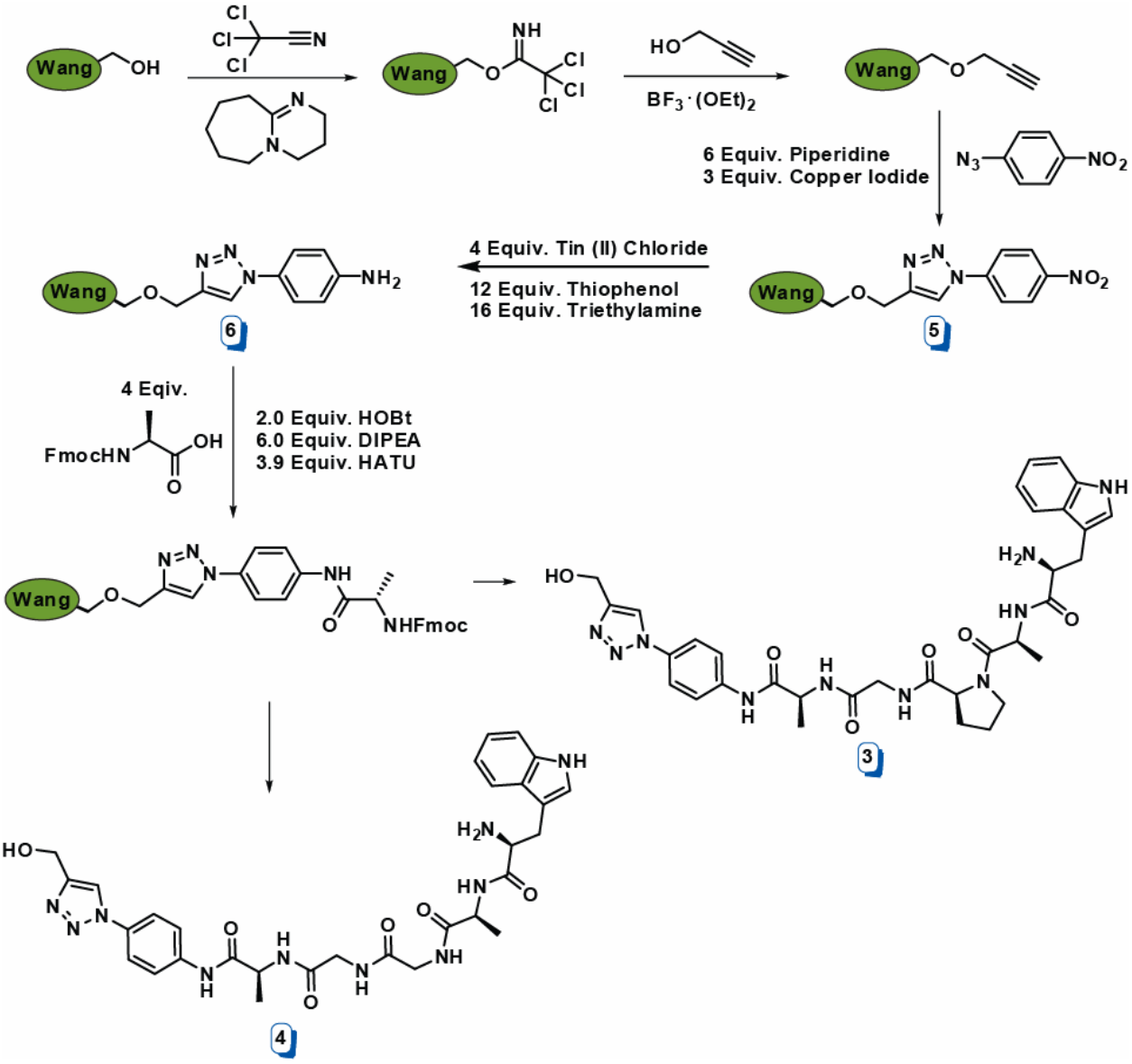


Figure 3-3. Scheme for the Synthesis of Triazole Containing Peptides **3** and **4**

The last peptide was synthesized with a biaryl triazole. The synthesis of this peptide involved the substitution of TCA resin with p-iodophenol. The p-iodophenol resin was then coupled with trimethylsilylacetylene (TMS) via sonogishira coupling. The TMS protected phenyl acetylene resin was then deprotected with tetrabutylammonium fluoride and then clicked with the p-nitrophenyl azide. Reduction of the azide followed by amino acid coupling provided the biaryl triazole peptide **7.** The synthetic scheme for the biaryl triazole peptide **7** is shown in Figure 3-4.

Synthesis of Longer Peptides Containing Side Chain Aryl Triazoles

The two base peptides **8** and **9** were synthesized using a Protein Technologies Inc. Prelude peptide synthesizer (Figure 3-5). The program was set to couple using standard peptide coupling conditions and was set to couple each amino acid residue twice. Once the base sequence was synthesized the last alkyne containing residue was added manually to the resin using a 1:3 mixture of HATU to O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) respectively as the coupling reagents to provide peptides **10** and **11**. Once the final residue was in place, the two peptide resins were split into two batches. Each peptide was then clicked with two different azides. One batch of each peptide was set to click with p-chlorophenyl azide to give peptides **12** and **13.** The second batch of each peptide was set to click with the coumarin, 3-azido-7-hydroxy-2H-chromen-2-one, to give peptides **14** and **15.** After the click reaction, all of the peptides were 9-fluorenylmethoxycarbonyloxy (Fmoc) deprotected, cleaved from resin and purified by

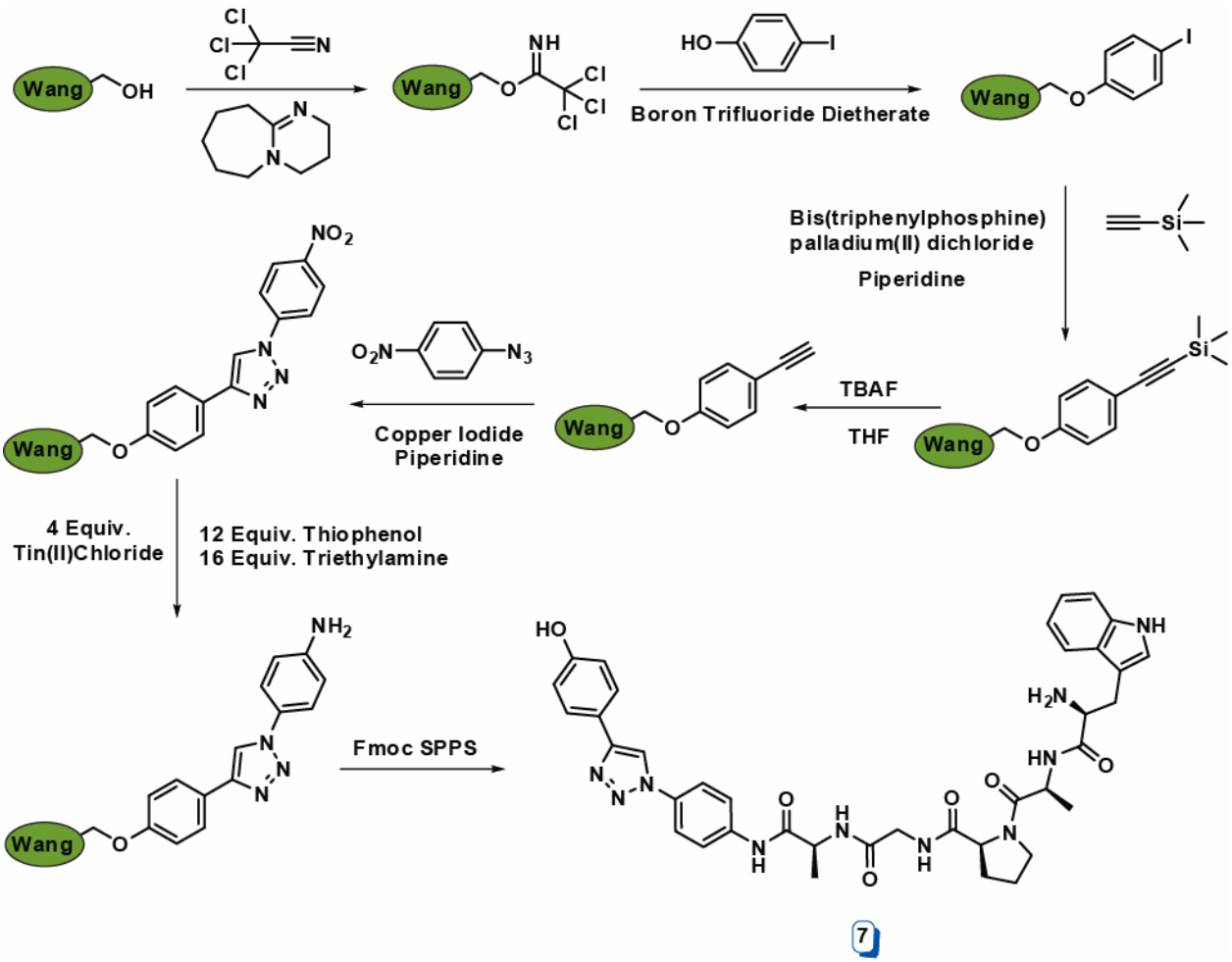


Figure 3-4. Synthetic Scheme for the Synthesis Biaryl Triazole Peptide **7**

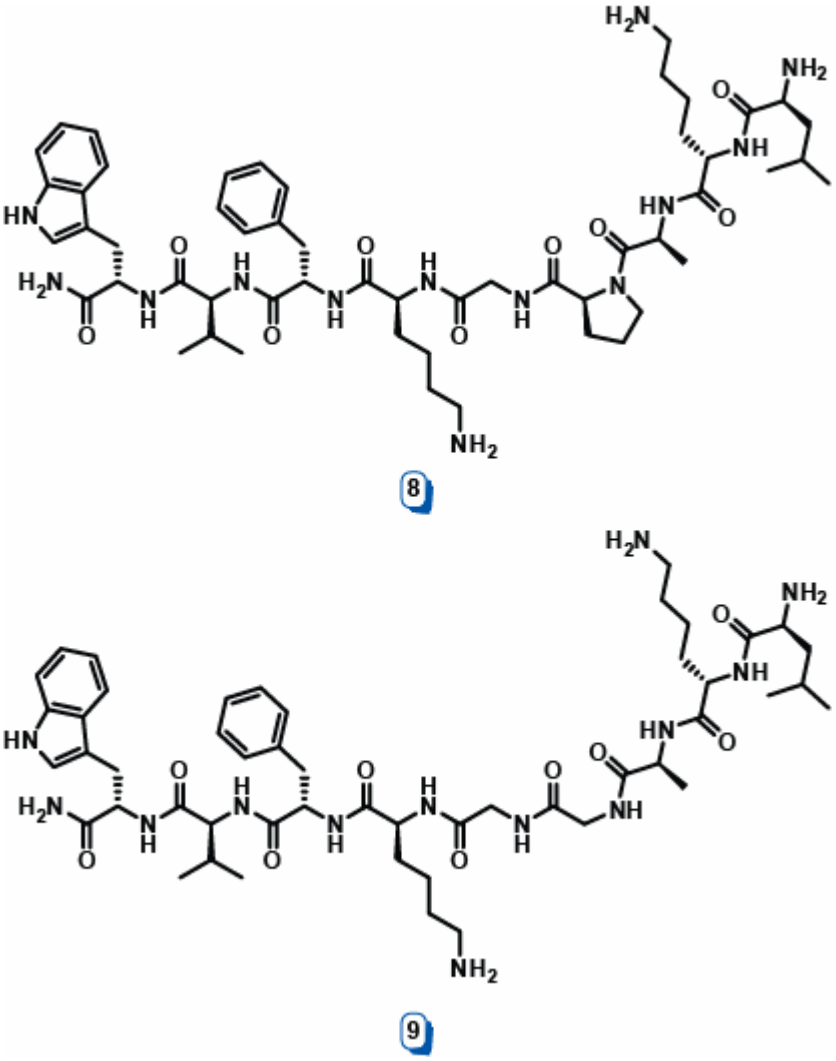


Figure 3-5. Structure of Sequences of Longer Peptides

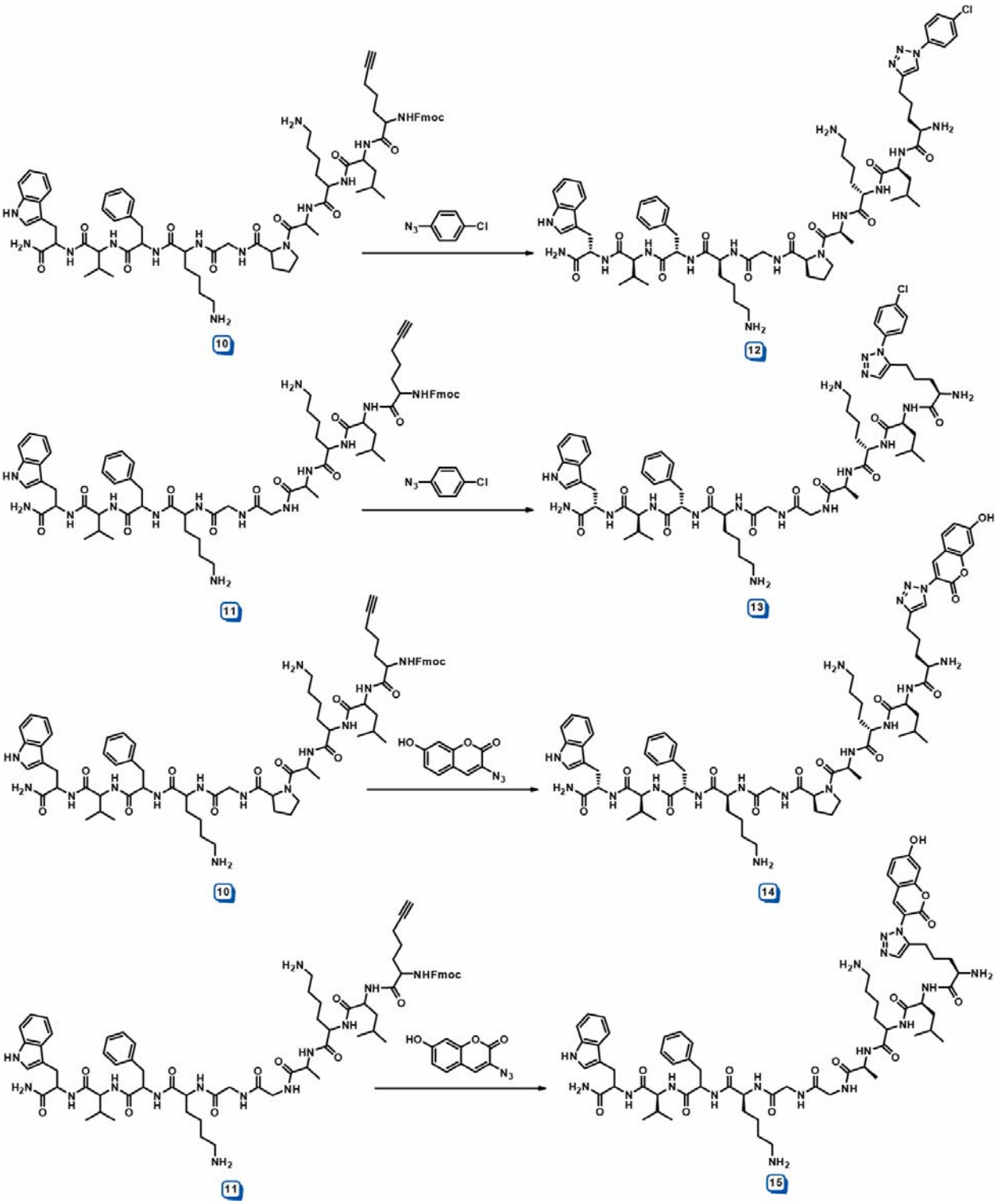


Figure 3-6. Formation of Clicked p-Chlorobenzyl Triazole Peptides **12** and **13** and Coumarin Triazole Peptides **14** and **15**

either preparatory HPLC or by solid phase extraction. The formation of the clicked peptides **12, 13, 14,** and **15** is shown in Figure 3-6.

Experimental

General Considerations. Amino acid starting materials, coupling reagents, and solid phase synthesis solvents were purchased from Advanced Chem Tech. and used as received. Reagents were used as is without additional purification. Unnatural amino acids were synthesized as described in Chapter 2. Dry solvents were obtained from a Grubb's Solvent system and used immediately or stored over activated 4Å molecular sieves. Samples were purified by HPLC using a Hewlett Packard Series 1100 HPLC fitted with a Varian Dynamax Microsorb 100-5 C8 column or by solid phase extraction using Peninsula Labs C18 Sep-columns. High resolution mass spectroscopy (HRMS) was performed in the Mass Spectrometry Center located in the Chemistry Department of the University of Tennessee on a JOEL JMS-T100CC ACCU-TOF using DART ionization or on a MALDI-TOF.

General Conditions for Standard Solid Phase Peptide Synthesis. The resin to be coupled (1.0 Equiv.) was allowed to swell in dichloromethane (DCM) for 5-10 min and then drained. The amino acid (4.0 Equiv.) to be coupled was activated by dissolving in N-Methylpyrolidinone (NMP) and the adding diisopropylethylamine (DIPEA) (8.0 Equiv.), HBTU (3.9 Equiv.) and N-Hydroxybenzotriazole (HOBt) (2 Equiv.). The amino acid solution was allowed to mix for 15-20 min. The activated amino acid solution was added to the resin and the mixture was shaken for 30-45 min.

The resin was drained rinsed with NMP, DCM, and methanol. The process was repeated once.

Trichloroacetimidate Wang Resin. TCA Wang Resin (2.00 g, resin loading 0.650 mmol/g, 1.30 mmol, 1.00 equiv.) was weighed out into a dry roundbottom flask with a stirbar. The flask was capped with a septum and purged well with nitrogen. Dry DCM (20 mL) was added to the flask via syringe and the mixture was stirred under nitrogen for 15 to 20 min. in order to swell the resin. The resin mixture was then cooled to 0 ^{o}C in an ice bath. TCA (4.65 mL, 52.0 mmol, 40.0 equiv.) was then added to the reaction flask via syringe followed by a solution of 1, 8-diazabicyclo[5.4.0]undec-7-ene (0.195 mL, 1.29 mmol, 0.990 equiv.) in 4 mL of dry DCM. The reaction was stirred under nitrogen for approximately 1 hour. The solution was then filtered through a solid phase synthesis vessel in order to collect the resin. The resin was rinsed well with DCM and methanol and then dried and stored.

Propargyl Wang Resin. TCA Wang resin (0.400 g, 0.254 mmol, 1.00 equiv) was weighed out into a 20 mL scintillation vial. A stir bar was added and the vial was fitted with a septum and purged well with nitrogen. A 50:50 mixture of dry THF and dry DCM (6 mL) was added to the resin and the mixture was allowed to stir for 20-30 min. in order to swell the resin. Propargyl alcohol was then added to the resin solution which was allowed to stir for another 5-10 min. A second vial was fitted with a septum and purged with nitrogen. 3 mL of dry tetrahydrofuran (THF), 3 mL of dry DCM, and 0.12 mL of boron trifluoride diethyl etherate were added to the second vial via syringe and the solution was mixed well. The boron trifluoride diethyl etherate solution was then added dropwise to the solution of resin. The reaction was allowed to stir for

approximately 1 hour at room temperature. The resin was collected in a solid phase synthesis vessel and rinsed well with DCM and methanol. The resin was dried well and stored for later use.

General Conditions for the On-Resin Click (ORC) Reaction of Aryl Azides with Propargyl Wang Resin. Propargyl Wang resin (0.025 g, 0.017 mmol, 1 equiv.) was weighed out into a peptide synthesis vessel. 2-5 mL of N-methyl pyrolidinone was added to the resin which was allowed to swell in the solvent for 20-30 min. In a scintillation vial, copper iodide (0.00940 g, 0.0500 mmol, 3.00 equiv.) was suspended in ~1 mL of NMP. Piperidine (9.80 uL, 0.100 mmol, 6.00 equiv.) was added to the suspension and the solution was mixed until all of the copper iodide had dissolved and the solution had turned a deep greenish brown. The aryl azide (0.0500 mmol, 3.00 equiv.) was then added to the copper iodide solution and mixed until dissolved. The propargyl wang resin was drained and the copper iodide solution was added. The vial was rinsed with ~1 mL of NMP and the rinse was also added to the resin. The reaction was shaken overnight (16-18 hours). Once finished, the resin was drained and rinsed with NMP, methanol, and DCM. The resin was allowed to swell in DCM for 20-30 min. The product was then cleaved from the resin using a cocktail of 75% trifluoroacetic acid (TFA) in DCM with 5-8 drops of triisopropyl silane. The mixture was shaken for 45 min to 1 hour and then drained and repeated twice more. In cases where purification was needed the compounds were purified by HPLC on a C8 column using an acetonitrile and 0.1% aqueous TFA eluent system. The HPLC was run using a gradient from 5% acetonitrile to 95% acetonitrile over 15 min.

(1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methanol. (Table 3-1, Entry 1) was synthesized by the reaction of p-azido ethylbenzene with propargyl Wang resin. The product was recovered from resin in 90% yield. HRMS-DART ($[M]^+$): Calculated for $C_{11}H_{13}N_3O$, 204.1137, Found 204.1131.

(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methanol. (Table 3-1, Entry 2) was synthesized from the reaction of p-azido chlorobenzene with propargyl Wang resin. The product was recovered from resin in 81% yield. HRMS-DART ($[M]^+$): Calculated for $C_9H_8ClN_3O$, 210.0434, Found 210.0432.

(1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol. (Table 3-1, Entry 3) was synthesized from the reaction of p-azido nitrobenzene with propargyl Wang resin. The product was purifed by preparatory HPLC. The product was recovered from column in 47% yield. HRMS-DART ($[M]^+$): Calculated for $C_9H_8N_4O_3$, 221.0675, Found 221.0671.

(1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methanol. (Table 3-1, Entry 4) was synthesized from the reaction of 3,4,5,-trimethoxyphenyl azide with propargyl Wang resin. The product was recovered from resin in 88% yield. HRMS-DART ($[M]^+$): Calculated for $C_{12}H_{15}N_3O_4$, 266.1141, Found 266.1133.

(1-(naphthalen-2-yl)-1H-1,2,3-triazol-4-yl)methanol. (Table 3-1, Entry 5) was synthesized from the reaction of 2-Azido napthylene with propargyl Wang resin. The product was recovered from resin in 93% yield. HRMS-DART ($[M]^+$): Calculated for $C_{11}H_{13}N_3O$, 226.0980, Found 226.0982.

7-hydroxy-3-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)-2H-chromen-2-one. (Table 3-1, Entry 6) was synthesized from the reaction of 3-azido-7-hydroxy-2H-

chromen-2-one with propargyl Wang resin. The product was recovered from resin in 85% yield. HRMS-DART ($[M]^-$) Calculated for $C_{11}H_{13}N_3O$, 258.0515, Found 258.0520.

(1-(pyridin-2-yl)-1H-1,2,3-triazol-4-yl)methanol. (Table 3-1, Entry 7) was synthesized from the reaction of 2-azidopyridine with propargyl Wang resin. The product was recovered from resin in 79% yield. HRMS-DART ($[M]^-$): Calculated for $C_{11}H_{13}N_3O$, 175.0620, Found 175.0616.

Synthesis of Peptide 2 (Trp-Ala-Pro-Gly-Ala). The peptide was synthesized on Knorr Rink resin using standard solid phase peptide synthesis (SPPS) conditions. Each amino acid residue was added by activating the acid in an NMP solution containing 4 equivalents of amino acid, 3.9 equivalents of O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 2 equivalents of N-Hydroxybenzotriazole (HOBt), and 8 equivalents of diisopropylethylamine (DIPEA). The activated solution was then added to the resin and the mixture was shaken for 30-45 min. MS MALDI (H^+): Calculated for $C_{24}H_{33}N_7O_5$, 500.26214, Found 500.26.

Synthesis of Peptides 3 and 4 (Trp-Ala-Pro-Gly-Ala-MonoArylTriazole) and (Trp-Ala-Gly-Gly-Ala-MonoArylTriazole). (1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol resin (0.250 g, loading 0.650 mmol/g, 0.160 mmol, 1.00 equiv.) was weighed out into a peptide synthesis vessel. Tin (II) chloride dehydrate (0.147 g, 0.650 mmol, 4.00 equiv.) was weighed out into a scintillation vial and 2 mL of THF was added followed by thiophenol (0.201 mL, 1.95 mmol, 12.0 equiv.). Triethylamine (0.343 mL, 2.40 mmol, 15.0 equiv.) was then added to the solution. The solution was mixed well and the added to the(1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol resin. The reaction was allowed to shake for 16-18 hours. The resin was drained and then rinsed

well with water, methanol, NMP, DCM, and ether. Peptides **3** and **4** were synthesized using standard Fmoc solid phase peptide synthesis. The peptides were synthesized off of the aryl amine that was obtained from the reduction of (1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol**.** The peptides were cleaved from resin using a cocktail of 75% TFA in DCM with 1% triisopropylsilane. The cleaved peptides were purified by HPLC on a C8 column using an acetonitrile and 0.1% aqueous TFA eluent system. Peptide **3** MS MALDI (Na^+): Calculated for $C_{30}H_{36}N_{10}O_6$, 655.8323, Found 655.83. Peptide **4** MS MALDI (H^+): Calculated for $C_{33}H_{40}N_{10}O_6$, 673.3215, Found 673.32.

Synthesis of Peptide 7 (Trp-Ala-Pro-Gly-Ala-BiArylTriazole). The previously prepared TCA wang resin (0.400 g, 0.250 mmol, 1.00 equiv.) was weighed out into a 20 mL scintillation vial. A stir bar was added and the vial was fitted with a septum and purged well with nitrogen. A 50:50 mixture of dry THF and dry DCM (6 mL) was added to the resin and the mixture was allowed to stir for 20-30 min. in order to swell the resin. A solution of p-iodophenol in DCM was then added to the resin solution which was allowed to stir for another 5-10 min. A second vial was fitted with a septum and purged with nitrogen. Dry THF (3 mL), dry DCM (3 mL), and 0.12 mL of boron trifluoride diethyl etherate were added to the second vial via syringe and the solution was mixed well. The boron trifluoride diethyl etherate solution was then added dropwise to the solution of resin. The reaction was allowed to stir for approximately 1 hour at room temperature. The resin was collected in a solid phase synthesis vessel and rinsed well with DCM and methanol. The resin was dried well and stored for later use.

The prepared p-iodophenol resin (0.250 g, loading 0.65 mmol/g, 0.16 mmol, 1 equiv.) was weighed out into a scintillation vial. The resin was allowed to swell in

tetrahydrofuran (THF) for 5-10 min. Bis(triphenylphosphine)palladium(II) dichloride (0.057 g, 0.08 mmol, 0.5 equiv.) was dissolved in THF (2 mL). Piperidine (80 uL, 0.813 mmol, 5 equiv.) was added to the solution and the solution was mixed for 5-10 min. The catalyst solution was then added to the p-iodophenol resin and the mixture was stirred for 20-30 min. TMS (0.231 mL, 1.63 mmol, 10.0 equiv.) was diluted in 1 mL of THF and the solution was added slowly to the resin mixture. The reaction was allowed to stir for 30-45 min. The reaction was then drained through a peptide synthesis vessel in order to collect the resin.

The protected alkyne resin was rinsed well with THF, DCM, and methanol, then deprotected by shaking the resin in a solution of 0.170 g of tetrabutylammonium fluoride in 2 mL of THF for 1-2 hours. The resin was then drained and rinsed well with DCM. The deprotected alkyne was the reacted with p-azido nitrobenzene using the general on-resin click conditions in order to provide the nitro substituted biaryl triazole.

The resin was weighed out into a peptide synthesis vessel. Tin (II) chloride dehydrate (0.147 g, 0.65 mmol, 4 equiv.) was weighed out into a scintillation vial and 2 mL of THF was added followed by thiophenol (0.200 mL, 1.95 mmol, 12.0 equiv.). Triethylamine (0.343 mL, 2.44 mmol, 15.0 equiv.) was then added to the solution. The solution was mixed well and the added to the (1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol resin. The reaction was allowed to shake for 16-18 hours. The resin was drained and then rinsed well with water, methanol, NMP, DCM, and ether.

Peptide **7** was then synthesized on the biaryl triazole resin using standard Fmoc solid phase petide synthesis. The peptide was cleaved from resin using a cocktail of 75% TFA in DCM with 1% triisopropylsilane. The cleaved peptides were purified by

HPLC on a C8 column using an acetonitrile and 0.1% aqueous TFA eluent system. MS MALDI (H^+): Calculated for $C_{38}H_{42}N_{10}O_6$, 735.3367, Found 735.33.

Click Reactions of Peptides 10 and 11 with p-Chlorophenyl Azide and 3-Azido-7-Hydroxy-2H-Chromen-2-one. The batches of resins containing peptides **10** and **11** were each split into two batches of 100mg of resin. One 100 mg batch of peptides **10** and **11** were reacted with p-azido chlorobenzene using the general on-resin click (ORC) condition to give peptides **12** and **13 (p-Chloroclicked PentynylGlycine-Val-Lys-Ala-Pro-Gly-Lys-Phe-Val-Trp)** and **(p-Chloroclicked PentynylGlycine-Val-Lys-Ala-Gly-Gly-Lys-Phe-Val-Trp)** The second 100 mg batches of the two peptides were reacted with 3-Azido-7-Hydroxy-2H-Chromen-2-one using the same on-resin click (ORC) conditions to yield peptides **14** and **15 (Coumarin Clicked PentynylGlycine-Val-Lys-Ala-Pro-Gly-Lys-Phe-Val-Trp)**. **(Coumarin Clicked PentynylGlycine-Val-Lys-Ala-Gly-Gly-Lys-Phe-Val-Trp)**. The peptides were cleaved from resin using a cocktail of 75% TFA in DCM with 7-8 drops of triisopropylsilane. The cleaved peptides were purified by HPLC on a C8 column using an acetonitrile and 0.1% aqueous TFA eluent system. Peptide **12** MS MALDI (H^+): Calculated for $C_{66}H_{94}N_{17}O_{10}$, 1320.7136, Found 1320.70. Peptide **13** MS MALDI (H^+): Calculated for $C_{63}H_{90}N_{17}O_{10}$, 1280.6823, Found 1280.69. Peptide **14** MS MALDI (H^+): Calculated for $C_{69}H_{95}N_{17}O_{13}$, 1370.7374, Found 1370.72. Peptide **15** MS MALDI (Na^+): Calculated for $C_{66}H_{91}N_{17}O_{13}$, 1352.6880, Found 1352.69.

Chapter 4: Absorbance and Fluorescence Studies of Aryl Triazoles and Tryptophan Containing Peptides Bearing Aryl Triazoles

Backround and Significance

Fluorescence spectroscopy provides a variety of tools to measure distances, monitor dynamics, or observe molecular interactions with sensitivity far beyond that of other biophysical methods.[40] The use of fluorophore donor-acceptor pairs as sensitive molecular reporters, termed molecular beacons, are an attractive approach to monitor molecular interactions. Molecular beacons rely on electron transfer processes which are the distance-dependant interactions between a fluorophore donor with a quenching or resonating acceptor molecule or fragment.[41] The interactions are observed as fluorescence changes, such as fluorescence resonance energy transfer (FRET) or resonance energy transfer (RET).[40] These processes are capable of providing information on the nanometer length scale, well below the detection limit of optical techniques.[40]

The basic principle of RET consists of a nonradiative transfer of energy from a chromophore in an excited state, called a donor, to an acceptor molecule.[42] A result of this interaction is a decrease in the fluorescence of donor molecule.[43] The energy transfer for RET (E_{RET}) is inversely proportional to the sixth power of the distance between the donor and acceptor chormophores

$$E_{RET} = \frac{1}{1 + R^6 / R_0^6}$$

where R_o is the distance at which there is 50% energy transfer from the donor chromophore to the acceptor chromophore.[43] This distance is usually between 2-5 nm and the maximum distance for any observable energy transfer is usually around 10 nm. The FRET methodology has become a common approach to the study of protein-protein interactions.

Molecular beacons have found much success in the field of oligonucleotides.[41, 44] Oligonucleotide molecular beacons utilize the incorporation of a fluorophore-quencher pair at opposing ends of a nucleotide strand. The nucleotides form a stem-loop structure which places the fluorophore and quencher in close proximity, thus exhibiting no overall fluorescence. When the nucleotide strand is equilibrated with its target, the helix of the stem is opened in order to bind its target, and the fluorophore-quencher pair are moved apart which restores the fluorescence of the fluorophore (Figure 4-1). This technique is finding more use as an efficient method to monitor specific binding interactions between nucleotides and DNA or RNA targets.[41, 44]

While the application of molecular beacons has mostly been limited to the field of oligonucleotides, this technology is beginning to find uses in the field of peptides. The use of fluorophoric probes can provide crucial information about peptide structure and folding (Figure 4-1). The spacing of a fluorophore and either a quenching molecule or a FRET acceptor within a peptide sequence can provide vital information about peptide conformations and secondary structures, as well as providing information on target binding. When the fluorescent dye molecules are close together, electron transfer takes place, which leads to either a shift in the fluorescent emission (FRET) or quenching of the fluorescent signal. When the fluorescent dyes are moved far enough away from

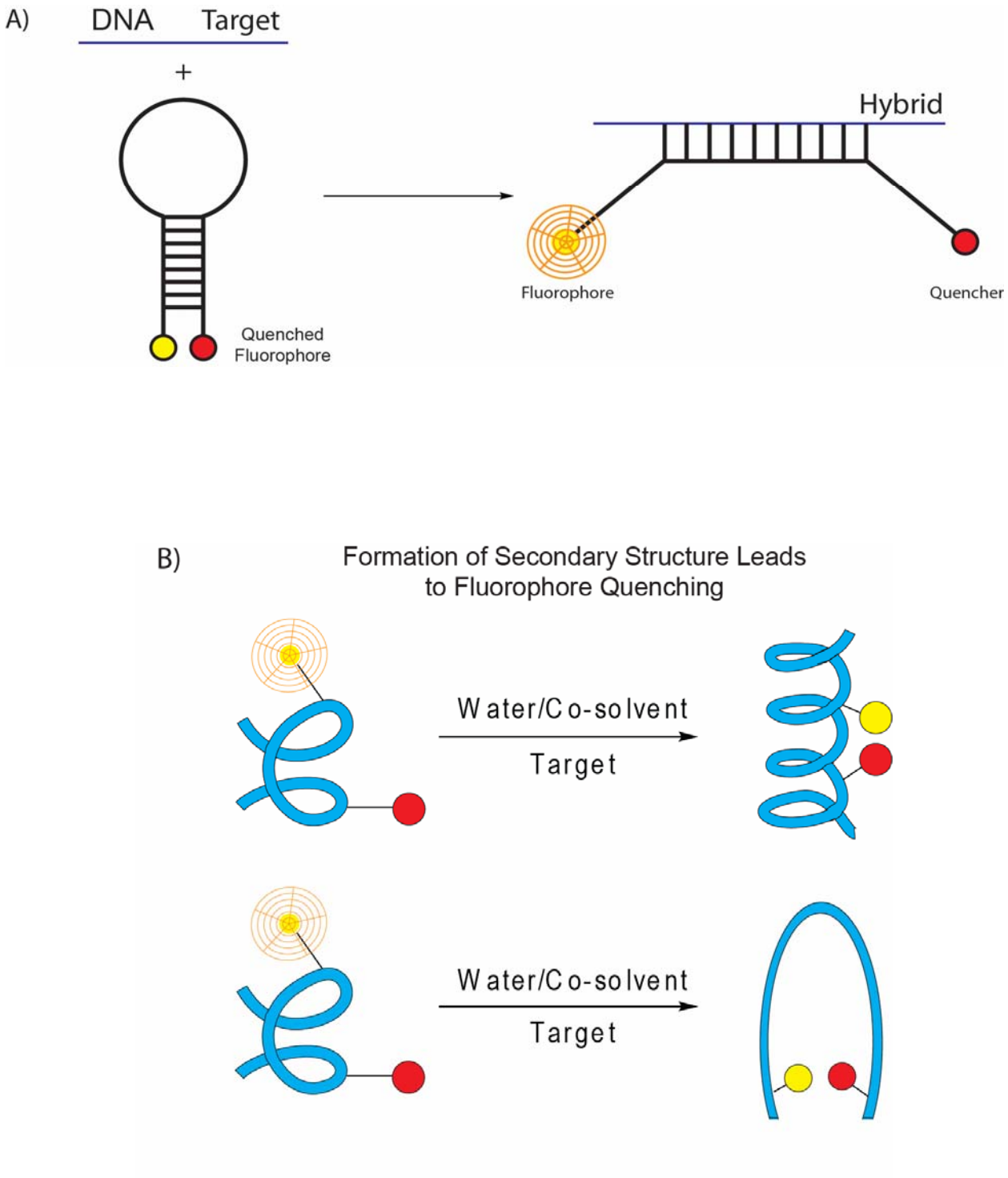


Figure 4-1 General Scheme for the Activity of Molecular Beacons and Peptide Beacons. A) General Scheme for the Fluorescent Activation of Molecular Beacons Upon Target Binding B) General Scheme for the Fluorescence Quenching of Peptide Beacons Upon Formation of Secondary Structure

each other, then normal fluorescence is regained. This approach has been used by Plaxco et al., who have recently found success in labeling the ends of a six-residue peptide epitope of HIV protein p17.[45] The residue is consistently quenched in the absence of its target antibody due to the molecular mobility. When bound to its target, however, the epitope adopts a very extended and rigid conformation that segregates the quencher from the fluorophore which enhances the fluorescence.[45]

In order to develop a versatile method for the introduction of fluorophores into peptides, our click peptide motif was used for the incorporation of probe compounds into peptides. This would provide a reliable and tunable method for the synthesis of peptide beacons. Tryptophan is naturally fluorescent, and is therefore a readily available fluorophore for testing the FRET capability of various triazoles. Initially it was decided that a series of four small peptides could be used to determine the feasibility of using aryl triazoles as charge transfer companions for tryptophan. The use of triazoles allows for the introduction of fluorophores into peptides through the use of clickable unnatural amino acids. This chemistry will allow for a wide variety of tunable probe molecules to be incorperated into any peptide sequence.

Absorbance and Fluorescent Properties of Initial Small Peptides

It is known that tryptophan fluorescence is dependant on the protein or peptide in which it is contained.[46] This is partially due to the fact that the fluorescence of tryptophan can be internally quenched by carbonyl groups.[47-50] Because of this, a sample peptide with no triazole would be needed to provide a standard for tryptophan fluorescence in the sequence used. The first series of peptides are shown in Figure 4-2 and their synthesis

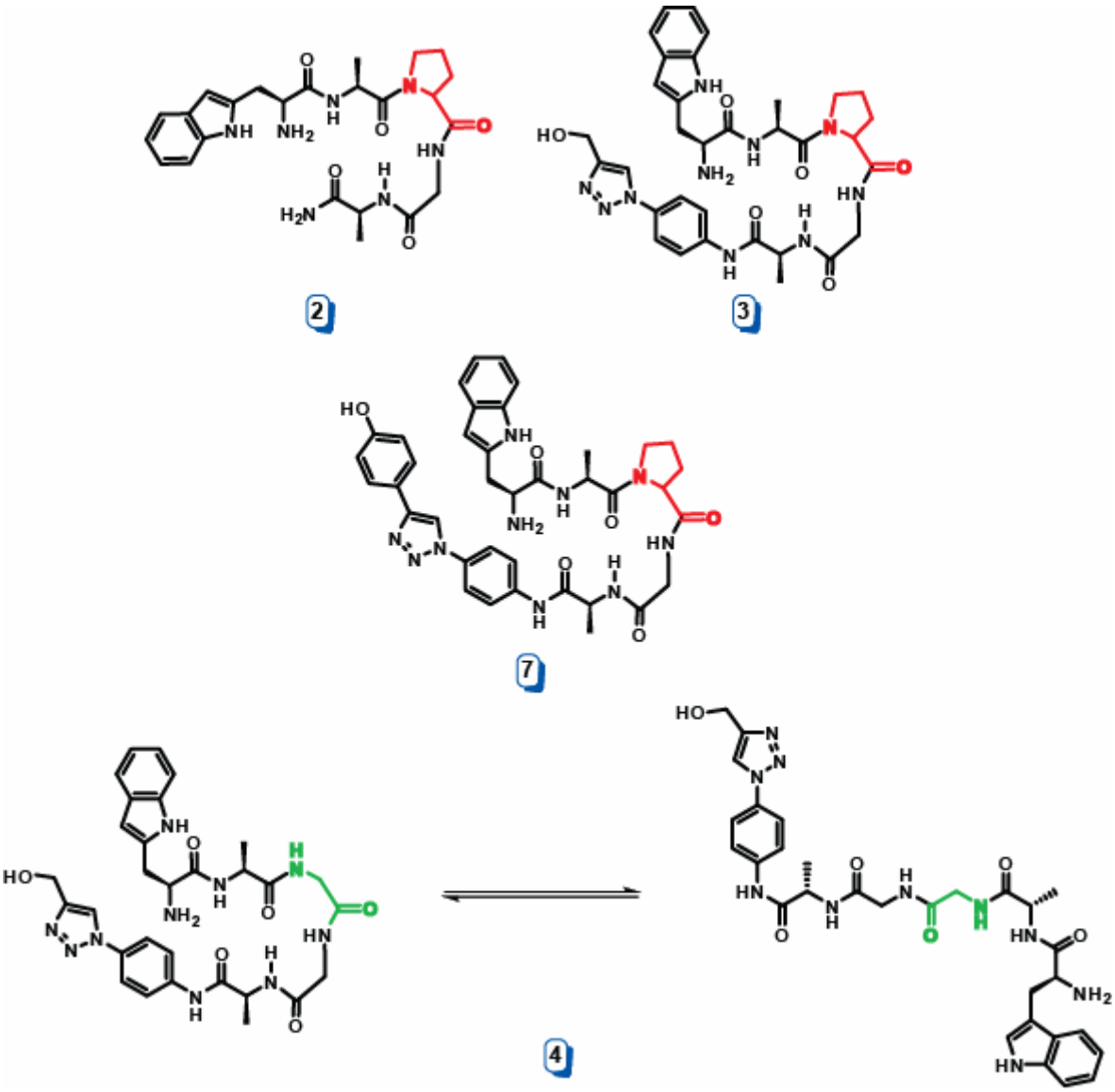


Figure 4-2. Initial Set of Small Peptides Tested for Electron Transfer Properties

is discussed in Chapter 3. The initial peptide **2** bearing no triazole was constructed as a control to provide the fluorescence intensity of tryptophan for the amino acid sequence used. It was then used as a relative control for full tryptophan fluorescence for comparison to the aryl triazole containing sequences used. The mono aryl and biaryl triazole peptides, **3** and **7** respectively, containing a proline turn, were both expected to provide either quenching or FRET activity due to the proximity of the aryl triazoles to the tryptophan. A short six to seven residue sequence coupled with the forced proline turn was assumed to provide the suitable distance of 2-10 nanometers between the triazole and tryptophan residue for any electron transfer phenomena to be observed.[40, 44, 51, 52] The mono aryl peptide **4** was constructed by replacing the proline turn of peptide **3** with a glycine residue. It was expected that the removal of the proline turn would add flexibility to the peptide and allow it to extend in water, moving the aryl triazole and tryptophan residues apart and thus restoring the tryptophan fluorescence of the peptide.

The fluorescence was measured on the peptides at a concentration of 10 uM by excitation of the tryptophan residue at 280 nm and is shown in Figure 4-3**.** It was observed that both proline containing aryl triazole peptides **3** and **7** demonstrated fluorescence quenching, meaning that the triazoles were involved in FRET. This means that it is possible that the proline turn did bring the triazoles in close proximity to the tryptophan residue. The fluorescence intensity of the tryptophan was decreased to almost half of that seen in the non triazole containing peptide **2**. Unexpectedly, however, the glycine containing peptide **4** showed fluorescence quenching comparable to that of the two peptides containing a proline turn. This indicated that either the alanine did not provide enough flexibility to move the triazole away from the tryptophan

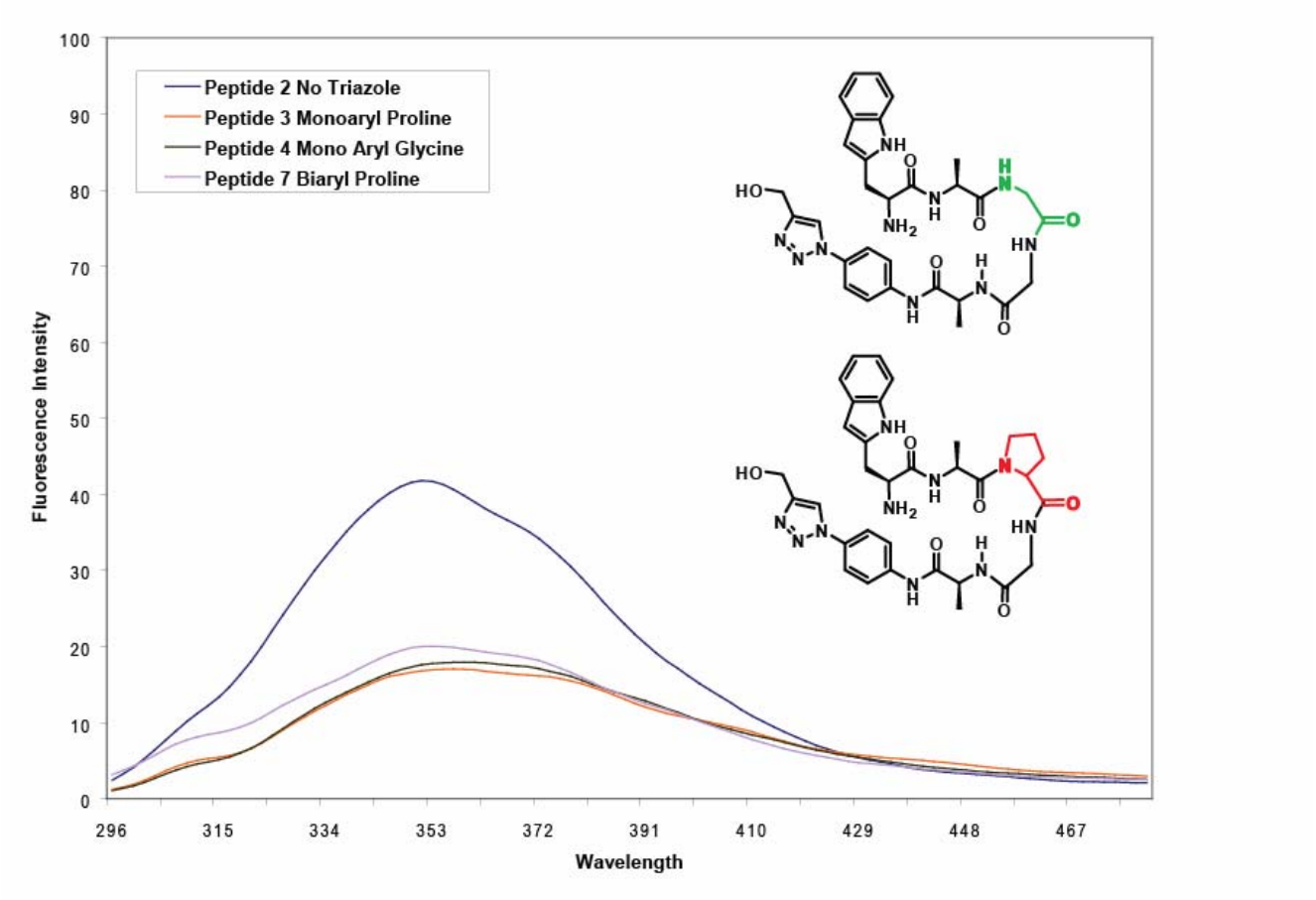


Figure 4-3. Stack Plot of Fluorescence for Short Peptides **2**, **3**, **4**, and **7**

residue (Figure 4-2), or that the quenching of the tryptophan fluorescence was not the result of a true electron transfer process.

Upon reflection of the peptide design, it was noticed that the construction of the peptides placed the aryl triazoles in the backbone portion of the sequence. It is possible that having the aryl triazole incorporated into the backbone of the sequence made the tryptophan residue incapable of being moved far enough away from the triazole to restore the tryptophan fluorescence. It was decided that a longer peptide sequence and incorporation of the aryl triazole as a side chain substituent would help to ensure the separation between it and the tryptophan residue. It was also decided that a series of small molecule aryl triazoles should be analyzed to determine the tunabilty of the absorbance and fluorescence behavior of the triazoles.

Absorbance and Fluorescent Properties of Small Molecule Aryl Triazoles

A library of chromophores with a wide variety of electronic properties would provide the opportunity to match probes to specific uses. For example, the studies presented rely on the natural fluorescence of tryptophan; however, it might be useful to have fluorophores which have higher fluorescent emissions or which have higher quantum yields and produce fluorescence at lower concentrations. In order to investigate the tunability of the triazole chromophores, a series of small molecule aryl triazoles was constructed on the solid phase using propargyl modified Wang resin. The absorption was measured to determine the wavelength at which to apply the fluorescence excitation, as well as, to determine the molar extinction coefficients for calculating more

accurate solution concentrations. The fluorescence data was used to determine whether or not the triazole itself possessed any fluorescent character. Triazoles with no fluorescence of their own could be used as possible quenching chromophores, while fluorescent triazoles could be used with another fluorophore as part of a FRET pair.

The first two triazoles synthesized were the mono aryl triazole present in peptides **3** and **4** and the biaryl triazole present in initial peptide **7**. These were synthesized using the p-nitrophenyl azide as described in Chapter 2. The triazole was cleaved; however, after the first alanine residue was coupled and deprotected. The absorbance data showed that both of these pieces had a peak absorbance at around 260 nm, which overlaps that of tryptophan (Figure 4-4). Neither of the pieces alone displayed any fluorescence even in solutions as concentrated as 200 uM. Since the triazole pieces absorb in the same region as the tryptophan, the observed quenching could be a product of the overlapping absorbances rather then electron transfer.

In order to verify that the quenching phenomena observed in the peptides was indeed due to electron transfer and not just the overlapping absorbance of the triazoles with tryptophan, the fluorescence of a series of standard solutions of the two aryl triazole pieces with tryptophan in a 1:1 ratio was examined. Solutions of triazole and tryptophan as well as a solution of only tryptophan were made at 50 uM and then serially diluted by half to produce solutions of 25 uM, 12.5 uM and 6.25 uM. The fluorescence of each solution was then taken and the spectra were compared to ensure that the quenching observed was not just a product of the overlap of the aryl triazoles absorbance with that of tryptophan. The obtained data shows no quenching of tryptophan fluorescence in the solutions of free triazole, with tryptophan at any of the

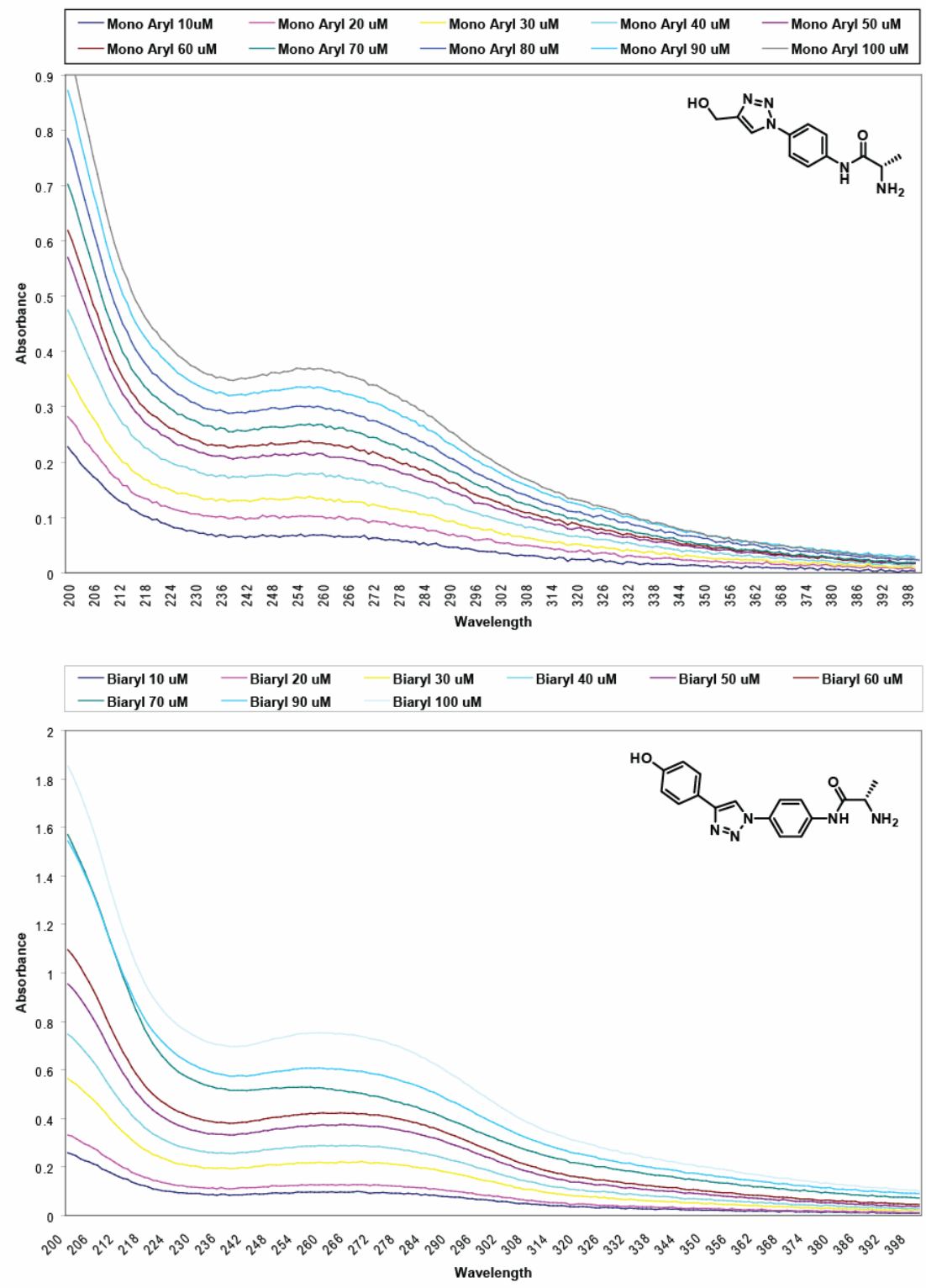


Figure 4-4. Graphs of Monoaryl and Biaryl Triazole Absorbance Data

concentrations (Figure 4-5). This would indicate that the quenching observed in peptides **2**, **3**, **4**, and **7** was indeed be due to electron transfer and not just a result of the triazole absorbances overlapping that of tryptophan.

In order to analyze the effects of substituted aryl rings on the absorbance and fluorescence characteristics of triazoles a series of small molecule triazoles was constructed (Table 3-1). Of the seven constructed triazoles the p-ethylphenyl triazole (Table 3-1, Entry 1), the p-chlorophenyl triazole (Table 3-1, Entry 2), and the 2-pyridyl triazole (Table 3-1, Entry 7) had the lowest absorbances. The 2-pyridyl triazole showed only mild absorbance from 200nm to 240nm and both the p-chlorophenyl triazole and p-ethylphenyl triazole showed a peak absorbance at 247nm (Figure 4-6). The 3,4,5,-trimethoxyphenyl triazole (Table 3-1, Entry 4) showed slightly increased absorbance at 253 nm, while the p-nitrophenyl triazole (Table 3-1, Entry 3) provided a considerable increase to 283 nm (Figure 4-7). The napthyl triazole (Table 3-1, Entry 5) exhibited three absorbance bands at 215 nm, 243, nm and 280 nm, and the coumarin triazole (Table 3-1, Entry 6) provided the largest absorbance at 343nm which falls in range of tryptophan emission (Figure 4-8).

The fluorescent properties for each of the triazole pieces were then examined by excitation of each triazole at its recorded absorbance values. Of the seven triazoles, the p-ethylphenyl, 2-pyridyl, and p-chlorophenyl triazoles showed little to no fluorescence in solutions up to 100 uM (Figure 4-9). The p-nitrophenyl triazole showed a moderate fluorescence band at around 378 nm, and the 3,4,5-trimethoxyphenyl triazole demonstrated a greater fluorescence band at around 378 nm (Figure 4-10). Both the napthyl and coumarin triazoles were highly fluorescent even at 1 uM

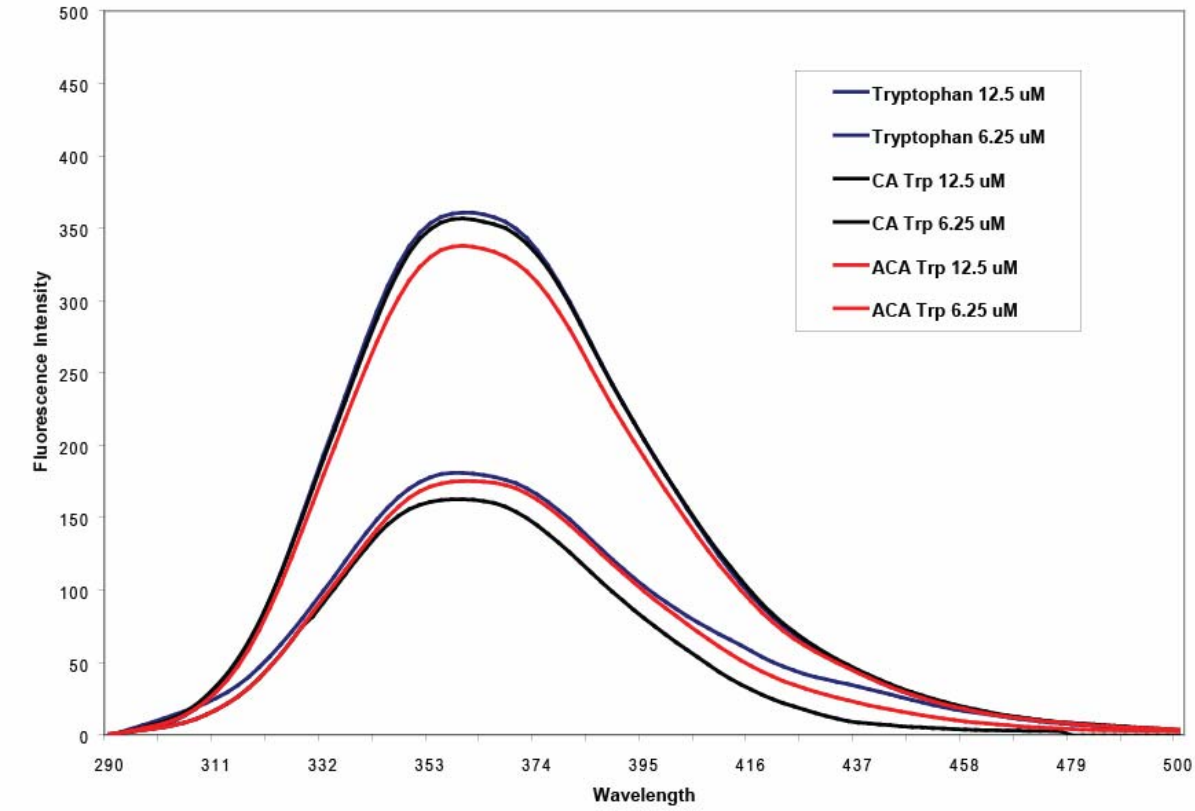


Figure 4-5. Fluorescence of Standard Solution Mixtures

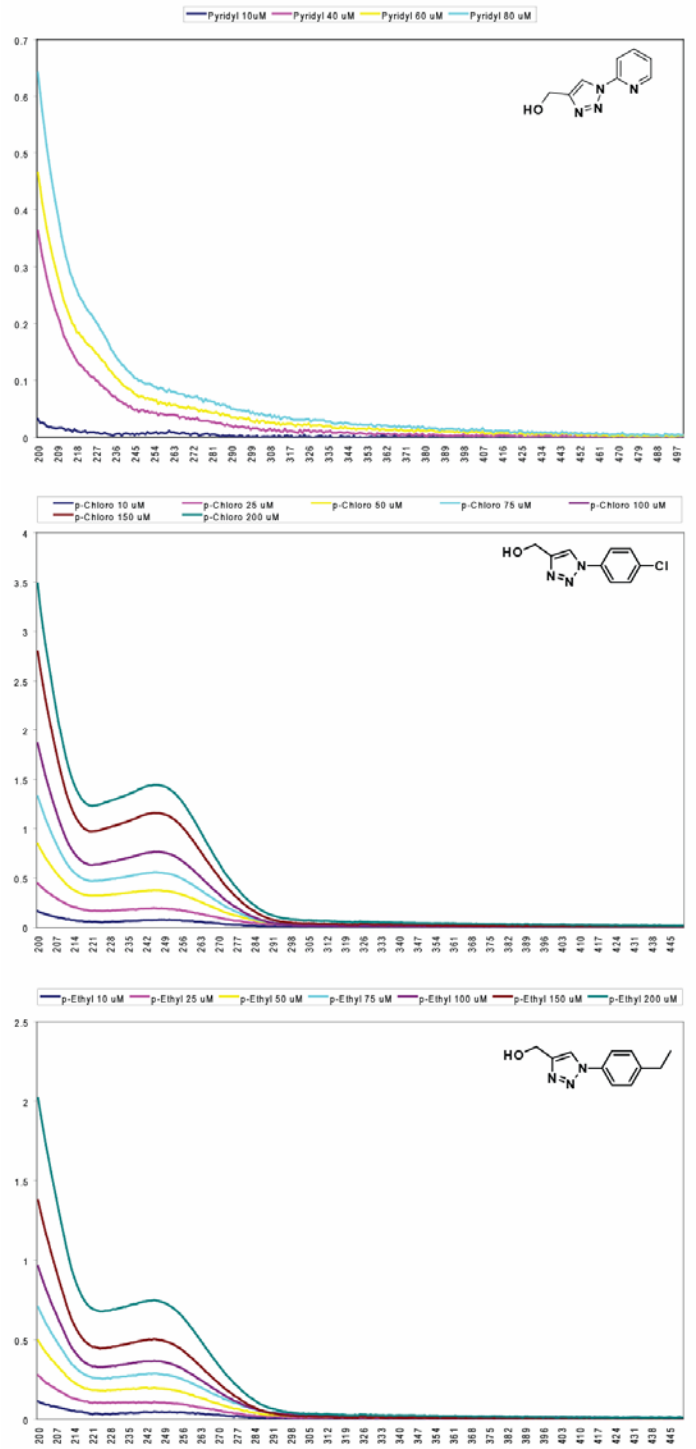


Figure 4-6. Absorbance Data for 2-Pyridyl, p-Ethylphenyl, and p-Chlorophenyl Triazoles

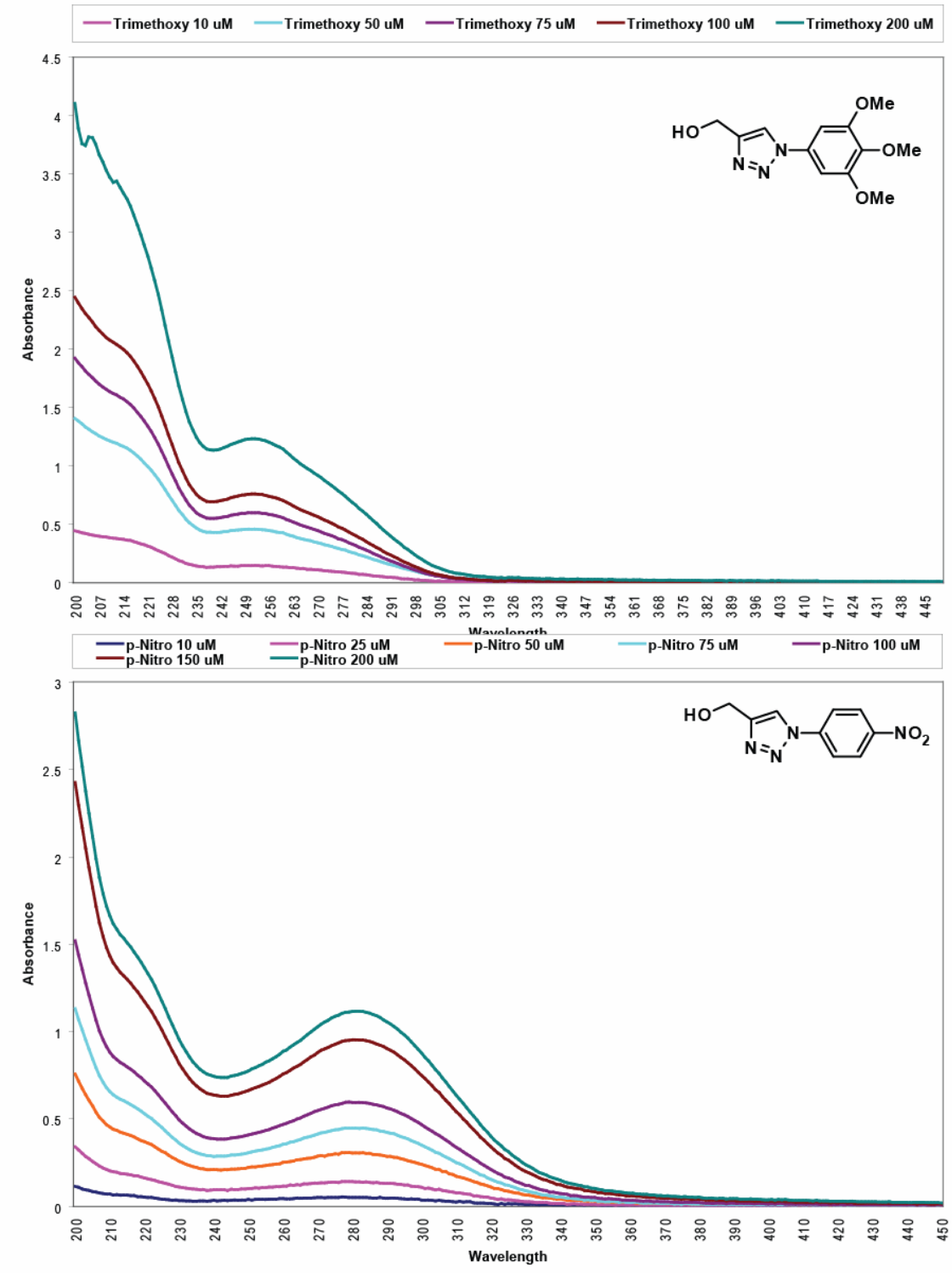


Figure 4-7. Absorbance Data for p-Nitrophenyl and 3,4,5-Trimethoxy Triazoles

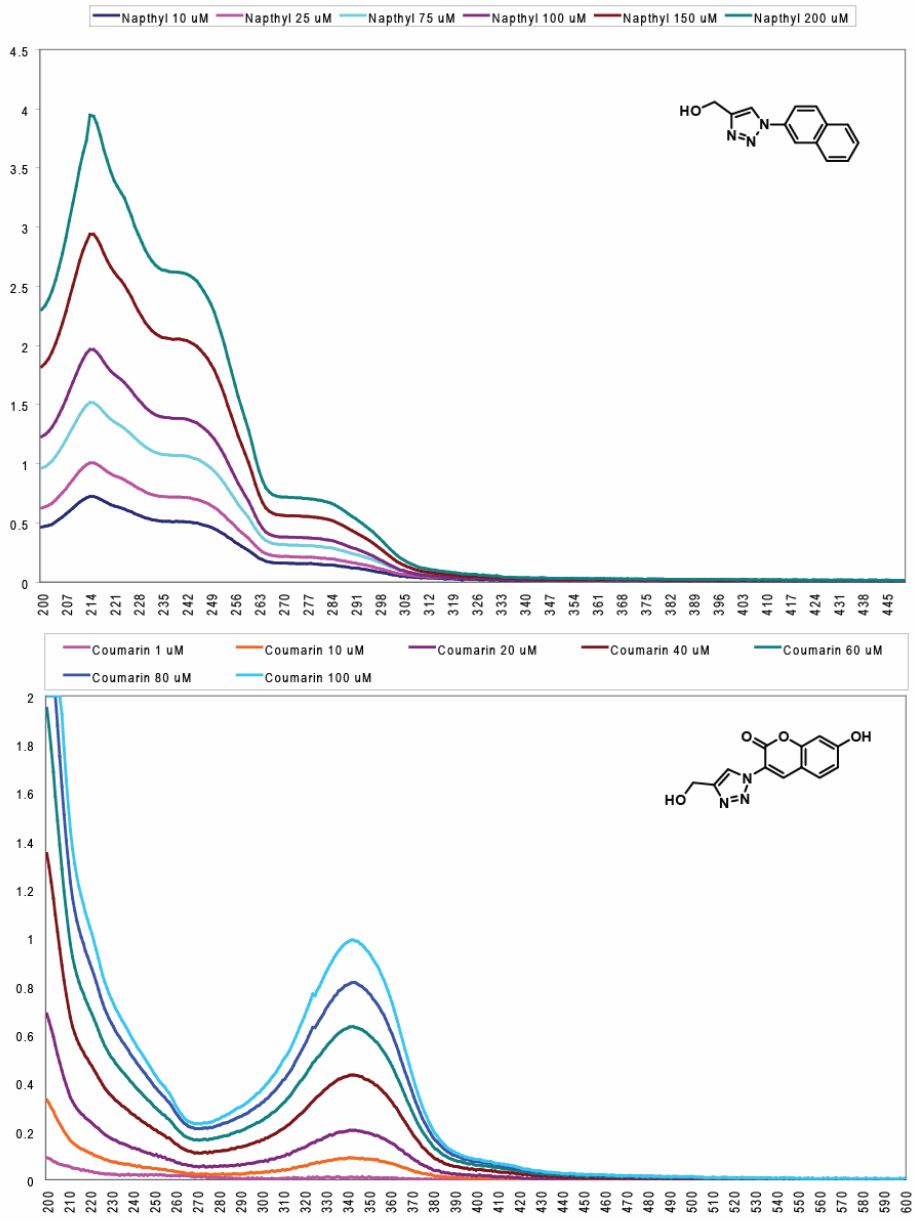


Figure 4-8. Absorbance Data for Napthyl and Coumarin Triazoles

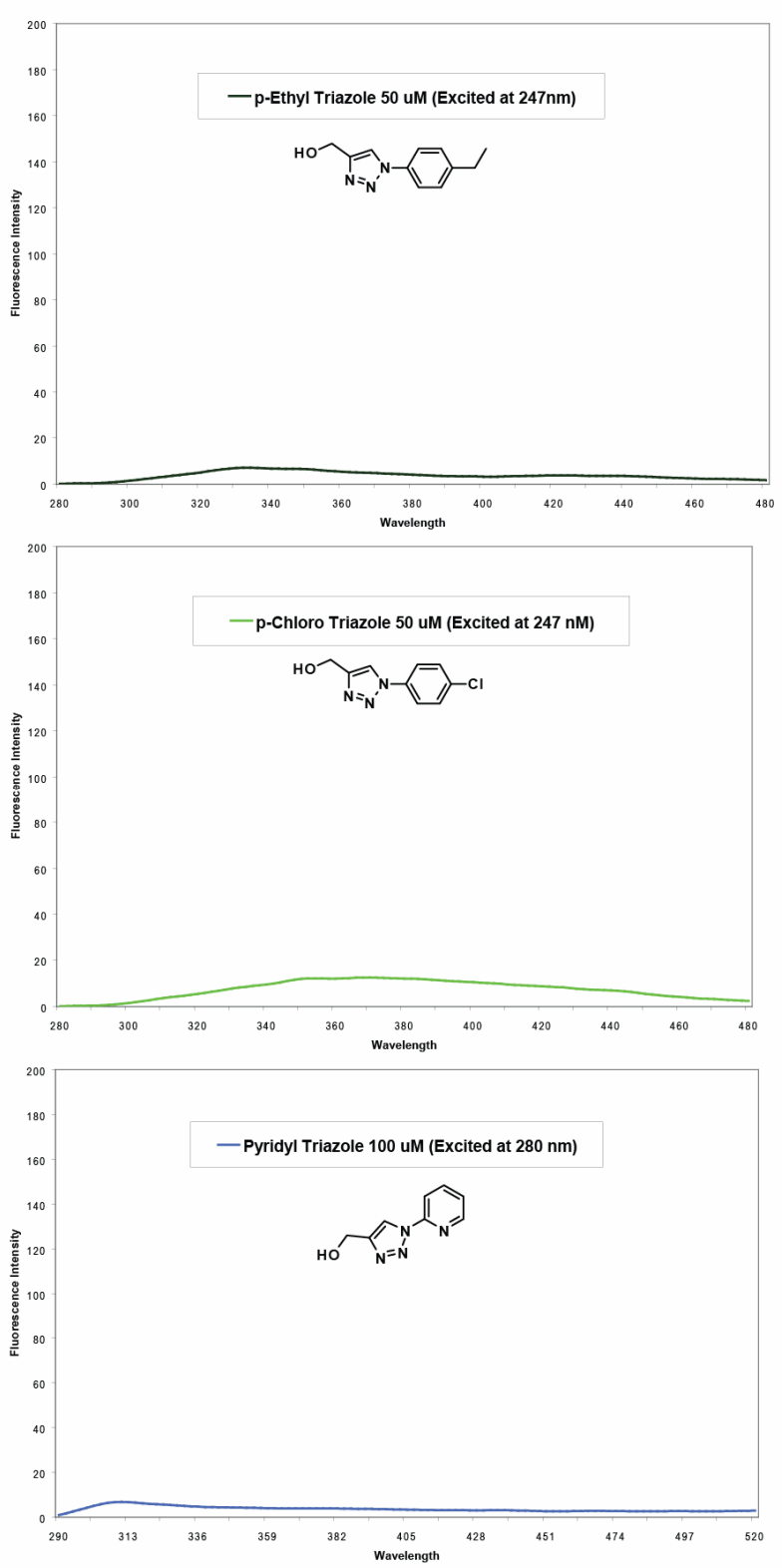


Figure 4-9. Fluorecence Emission Spectra for 2-Pyridyl, p-Chlorophenyl, and p–Ethylphenyl Triazoles

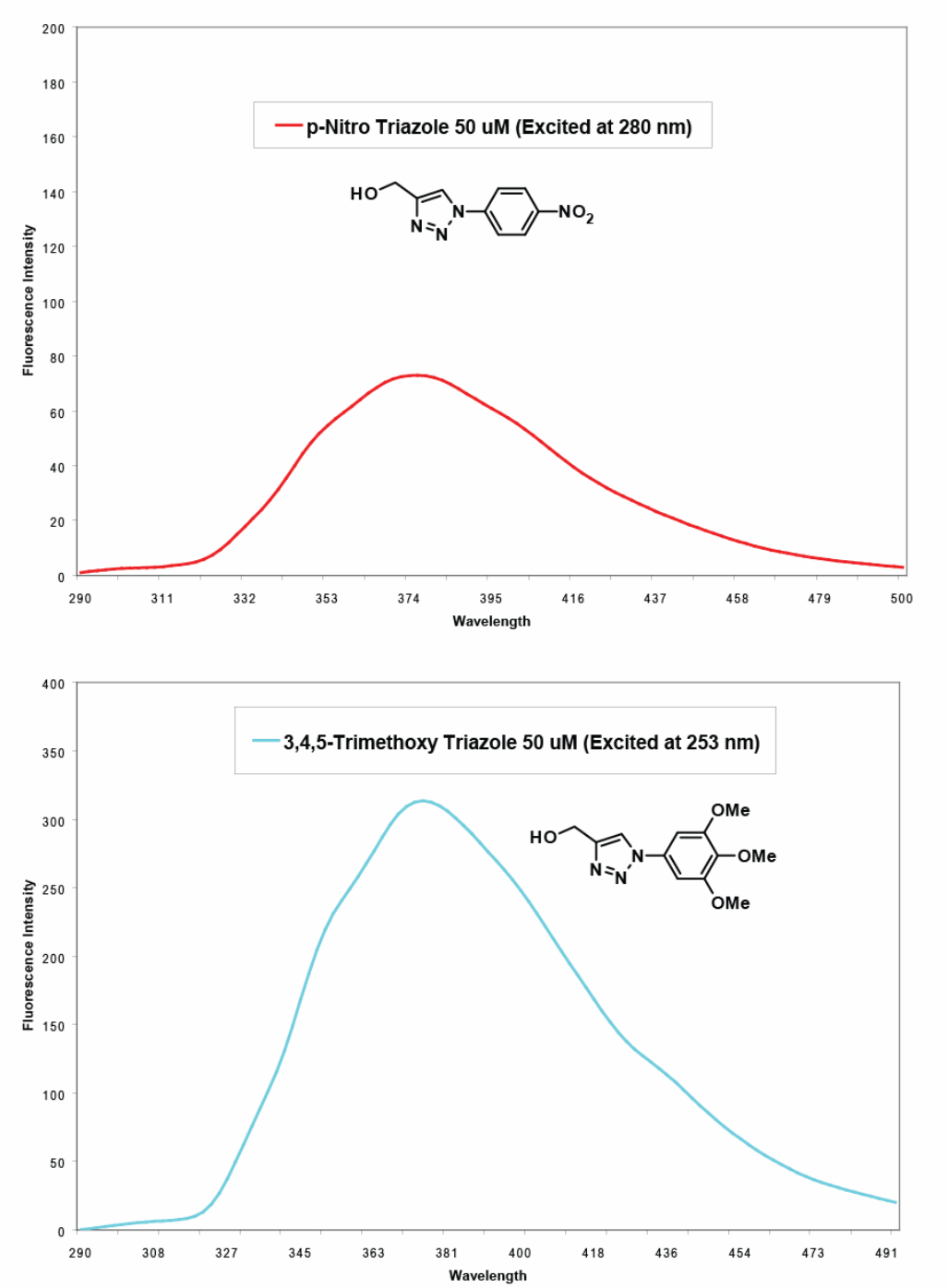


Figure 4-10. Fluorescence Emission Spectra for p-Nitrophenyl, and 3,4,5-Trimethoxyphenyl Triazoles

concentrations (Figure 4-11). The coumarin triazole demonstated the highest fluorescence with a strong fluorescence band at around 470 nm. Excitation of the napthyl triazole at the three absorbances indicated that the fluorescence at 380 nm resulted from the absorbance at 243 nm (Figure 4-11).

Of the screened triazoles, the coumarin triazole was the most likely to provide any kind of FRET activity due to the fact that its absorbance overlapped with the emission of tryptophan at around 340 nm, and it had a much higher emission band than tryptophan at around 480 nm. For this reason the coumarin triazole was chosen to be attached to one of the longer peptides to investigate possible FRET interactions between tryptophan and the coumarin triazole.

Absorbance and Fluorescence Properties of Long Aryl Triazole Peptides

To determine if increasing the distance between the tryptophan residue and the aryl triazole would provide a system where the quenching and fluorescence could be controlled by the rigidity of the sequence, two sets of longer ten residue peptides were synthesized. In order to add to the distance and mobility, the aryl triazole moiety was constructed as a side chain functionality rather than being contained in the backbone of the peptides. The peptides were again synthesized as pairs with one of each pair containing a proline residue and the other containing a glycine residue in its place. The peptides contained an unnatural alkyne amino acid as the final residue which allowed for triazole functionalization with any desired aryl azide via click chemistry. Incorporation of one of the aryl triazole pieces with no fluorescent character was

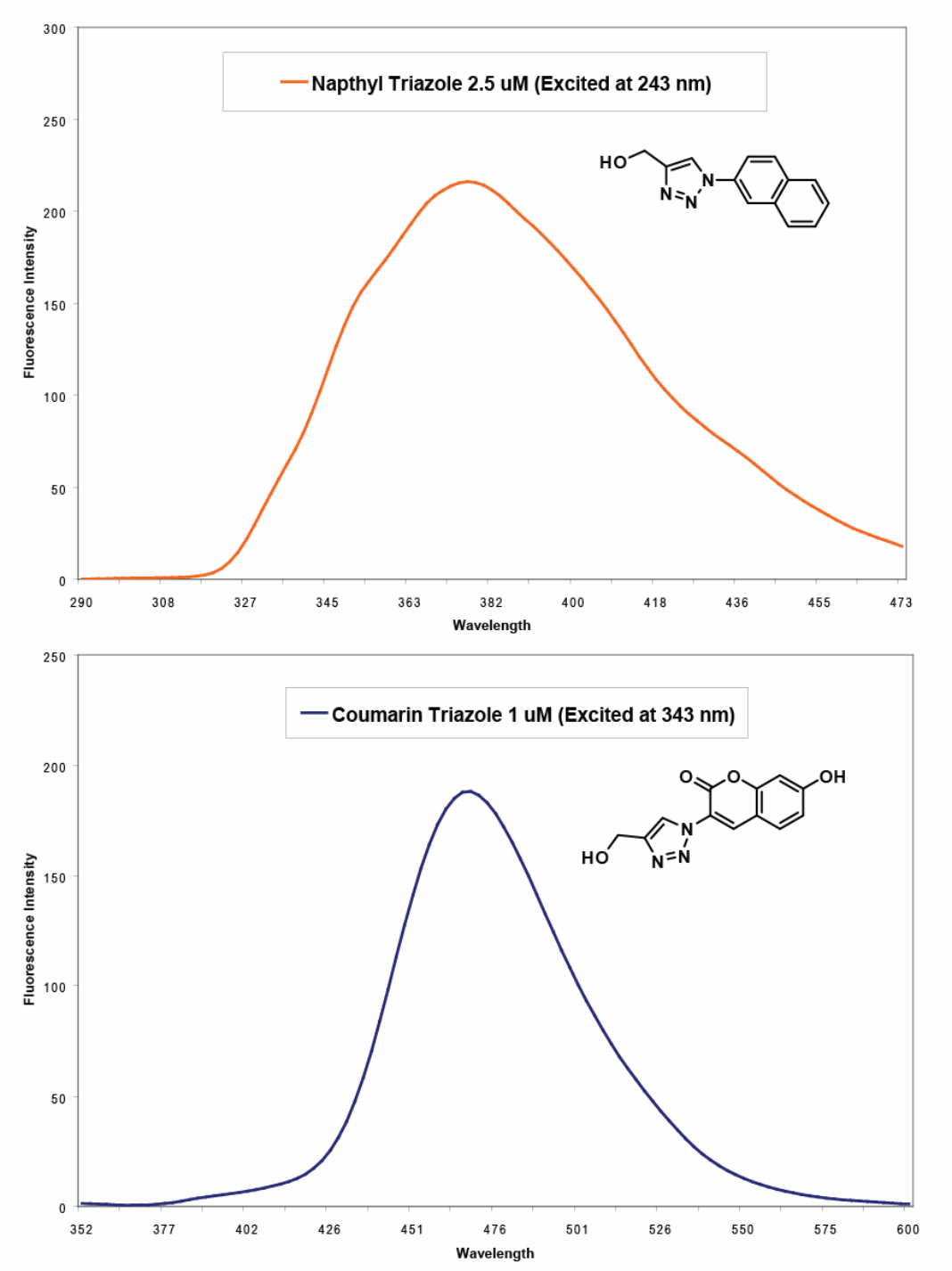


Figure 4-11. Fluorescence Emission Spectra for Napthyl, and Coumarin Triazoles

expected to produce a quenching compound, while incorporation of the coumarin triazole which had absorbance at the same wavelength as the tryptophan fluorescence was expected to be a possible fluorescent FRET companion for tryptophan.

The four peptides are pictured in Figure 3-6 in Chapter 3. The p-chlorophenyl azide was chosen to form the quenching peptide because its absorbance was at a lower wavelength than tryptophan and because it had very little fluorescence of its own. The fluorescence of all of the peptides were examined in aqueous solution as well as in varying solutions of trifluoroethanol (TFE) in water. TFE solutions add hydrophobic interactions and cause peptides to fold into secondary structure, so this was employed as a secondary method of bringing the two fluorophoric ends of the peptides closer together. The fluorescent analysis of the coumarin peptides was slightly inhibited by the fact that the coumarin moiety had a mild fluorescence absorbance at 280 nm which was the excitation wavelength used for tryptophan. There was still, however, a noticeable difference in the fluorescence between the proline containing peptide and the glycine containing peptide (Figure 4-12). The glycine containing peptide showed a moderate tryptophan fluorescence peak at 318 nm as well as the coumarin fluorescence at 455 nm. In the proline containing peptide, the typtophan fluorescence was weakened and leveled off and the coumarin fluorescence peak was intensified (Figure 4-12). Interestingly, increasing the hydrophobicity with the addition of TFE provided almost no change in the fluorescence data. It could be that the ends of the peptides, where the fluorophore groups are still mobile enough that changes in the hydrophobicity made little change in fluorescence compared to the forcing of a turn by the addition of proline. It is also possible that the proline induced turn is far more important to the secondary

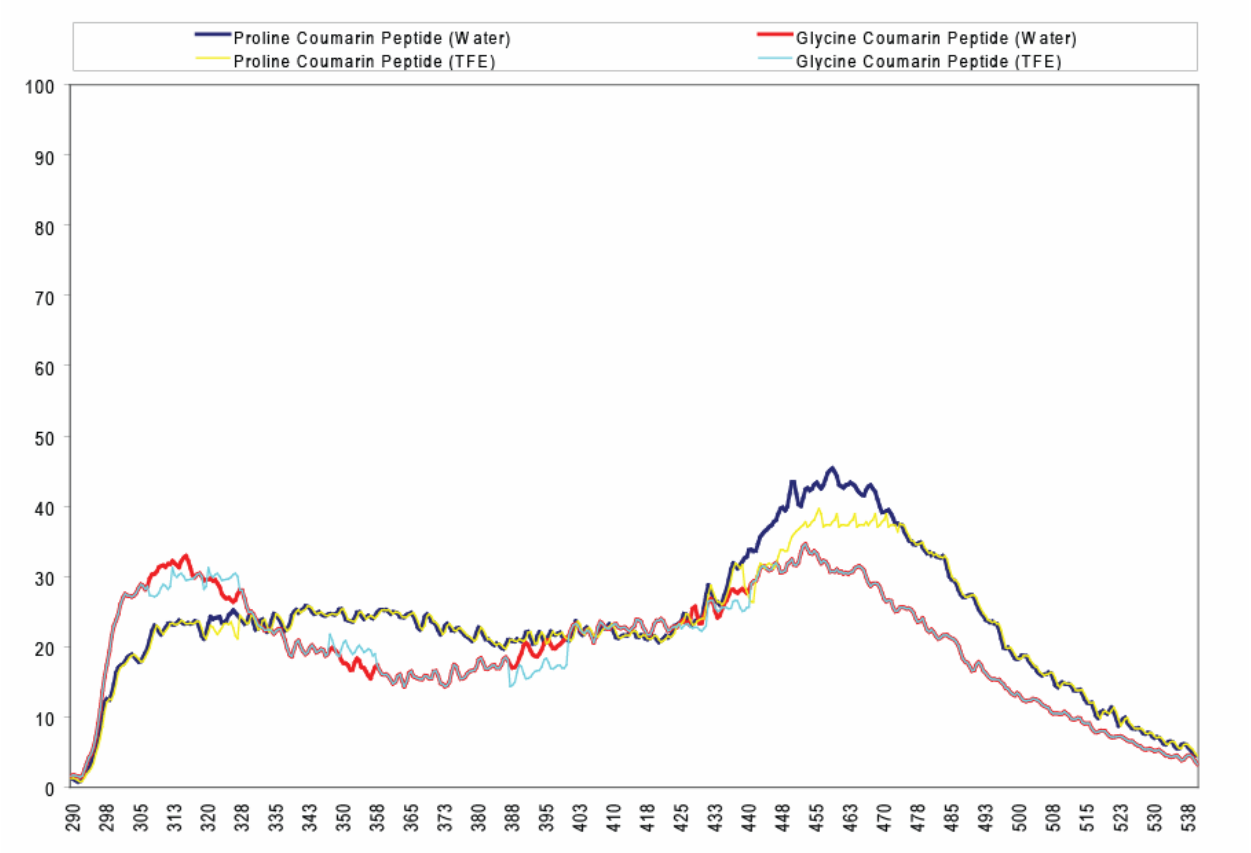


Figure 4-12. Fluorescence Graph of the Proline and Glycine Coumarin Peptides

structure than the hydrophobic interctions provided by the TFE solution. This data also strongly suggest that modification of the coumarin triazole moiety could provide a comparable FRET partner for tryptophan. The overlapping absorbances make this particular system less useful; however, chemical modification of the coumarin fluorophore could be used to adjust its fluorescence properties, or a fluorescence donor could be employed.

The fluorescence data for the p-chlorophenyl triazole was more distinct and informative due to the fact that the triazole had no absorbance or fluorescence that interfered with the fluorescence readings of tryptophan (Figure 4-13). The glycine containing peptide exhibited considerable yet broad tryptophan fluorescence peak at around 314 nm (Figure 4-13). The fluorescence signal was seen both in water and in TFE solution. The proline containing peptide also showed a tryptophan fluorescence at 314nm, but the intensity was greatly diminished compared to that of the glycine containing peptide. The TFE solutions still provided little improvement to the quenching. Unlike the coumarin peptide, however, the TFE did produce a slight difference in the fluorescence reducing it by almost 10 fluorescent units.

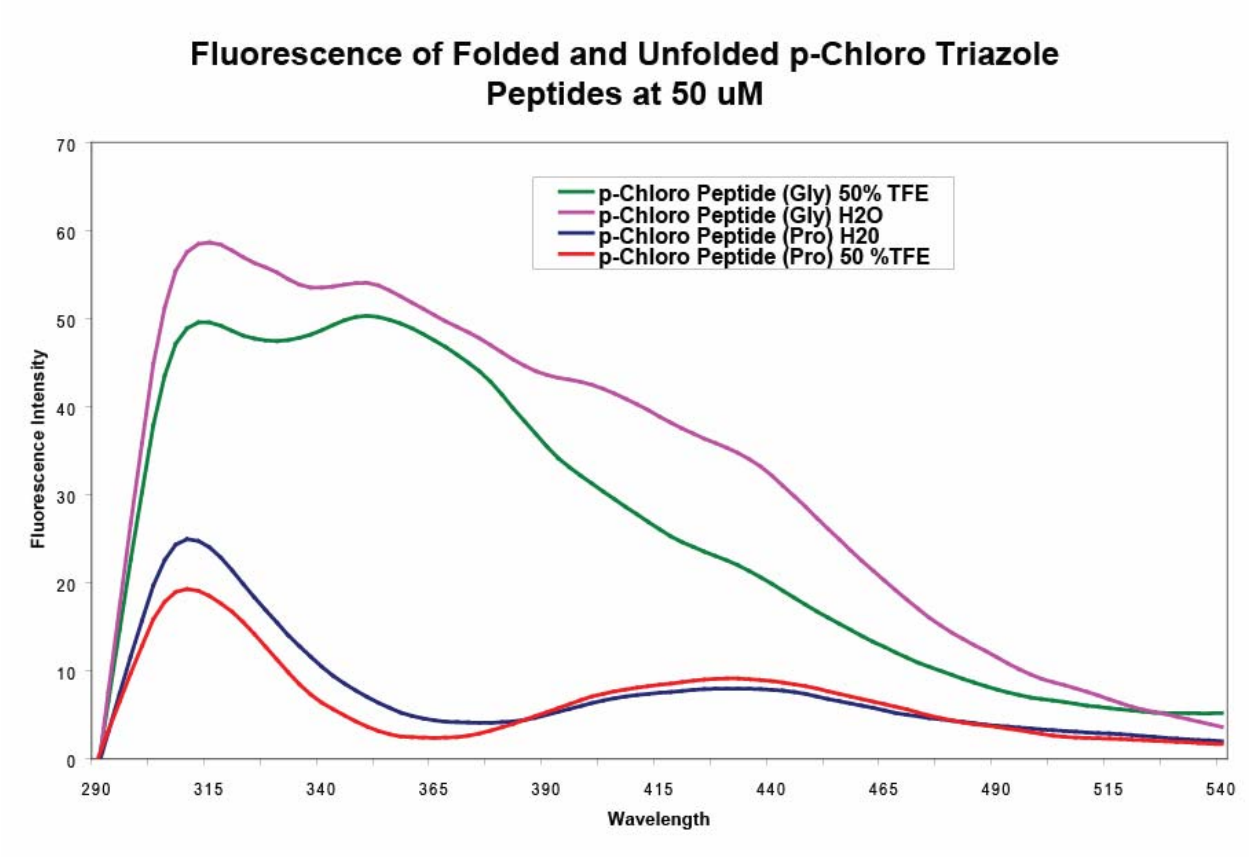


Figure 4-13. Fluorescence Graph of the Proline and Glycine p-Chloro Triazole Peptides

Experimental

Absorption measurements were taken on a Thermo Science Evolution 600 UV-Visual Spectrometer in water at 20°C. Initial absorption measurements were taken for all small molecules and peptides in order to determine the molar absorption coefficients (ε_{280}). The molar absorption coefficients were taken at 280 nm the wavelength for the excitation of tryptophan. For the small click reactions the molar absorption coefficient was also measured at the wavelength of the maximum absorption. The molar absorption coefficients were then used to measure the concentration for all of the working analytical samples used for measurements.

Fluorescence measurements were taken on a Perkin-Elmer LS 55 Spectrofluorimeter. Samples were prepared to the desired concentrations in either deionized water or a mixture of deionized water and trifluoroethanol. Fluorescence measurements for all compounds were taken at several concentrations for correlation. The concentrations of samples were determined and adjusted based on the molar absorption coefficients. All measurements involving tryptophan fluorescence were excited at 280 nm and the emission was recorded from 250 nm to 550 nm. Measurements on small triazole compounds were excited at the wavelength, or wavelengths, that corresponded to the specific absorption values for the compound. The emission was recorded from 250 nm to 850 nm.

Conclusions and Future Developments

The data obtained from the synthesized peptides provides a basis for the use of aryl triazoles, formed via click chemistry, as fluorescent dye compounds for the use as probes of peptide and protein structure. The non-fluorescent aryl triazoles have been proven to act as quenching chromophores for the natural fluorescence of tryptophan residues. The ease of synthesis and availability of starting materials for aryl azides provides the possibility for the synthesis of a large library of aryl triazoles. The applications of these compounds are even further expanded by the ability to incorporate the chromophore into the peptide at any time during the synthesis through the use of click chemistry, and the library of azide and alkyne functionalized amino acids provides the ability to incorporate triazole moieties at almost any position of a peptide sequence. This chemistry provides a basis for the formation of a modular approach to the formation of peptide beacons. With modified coumarin azides it should also be possible to find an appropriate fluorophoric compound to function as an operational FRET partner for tryptophan.

This chemistry does not need to be limited to the use of the natural fluorescence of tryptophan residues. The ability to incorporate multiple “clickable” amino acids into a peptide allows for the introduction of both a synthetic fluorophore as well as a synthetic electron transfer partner. The ability to rapidly and efficiently incorporate fluorophoric probe compounds would allow for a modular analysis of active peptide structure and binding modes.

This methodology could be applied to monitoring the conditions of intramolecular click reactions of peptides. In many cases it is desirable to lock peptides into their bioactive conformations. This can be readily achieved by click chemistry, however monitoring these reactions can be costly and tedious. For intramolecular click reactions, the mass of the product does not change from that of the starting material. This means that these reactions must be monitored by both mass and HPLC, which is time consuming. Since the click reaction to close a peptide forms a triazole, placement of a complimentary fluorophore into the sequence of the peptide would produce a method for internal fluorescence quenching to monitor intramolecular peptide click.

It has been shown that aryl triazoles work as companion chromophores with tryptophan for the purpose of constructing peptide beacons. These compounds are capable of taking place in electron transfer processes, thus making them useful as probes for analyzing the activity and structure of peptides. The advantages of these groups are that they can be introduced into a peptide at any time under mild conditions. The availability of aryl azides and methods of synthesizing aryl azides coupled with click functionalized unnatural amino acids provides a method of incorporating probes into any position of a peptide sequence. This methodology will provide a multitude of possibilities for analyzing peptidomimetic function and structure.

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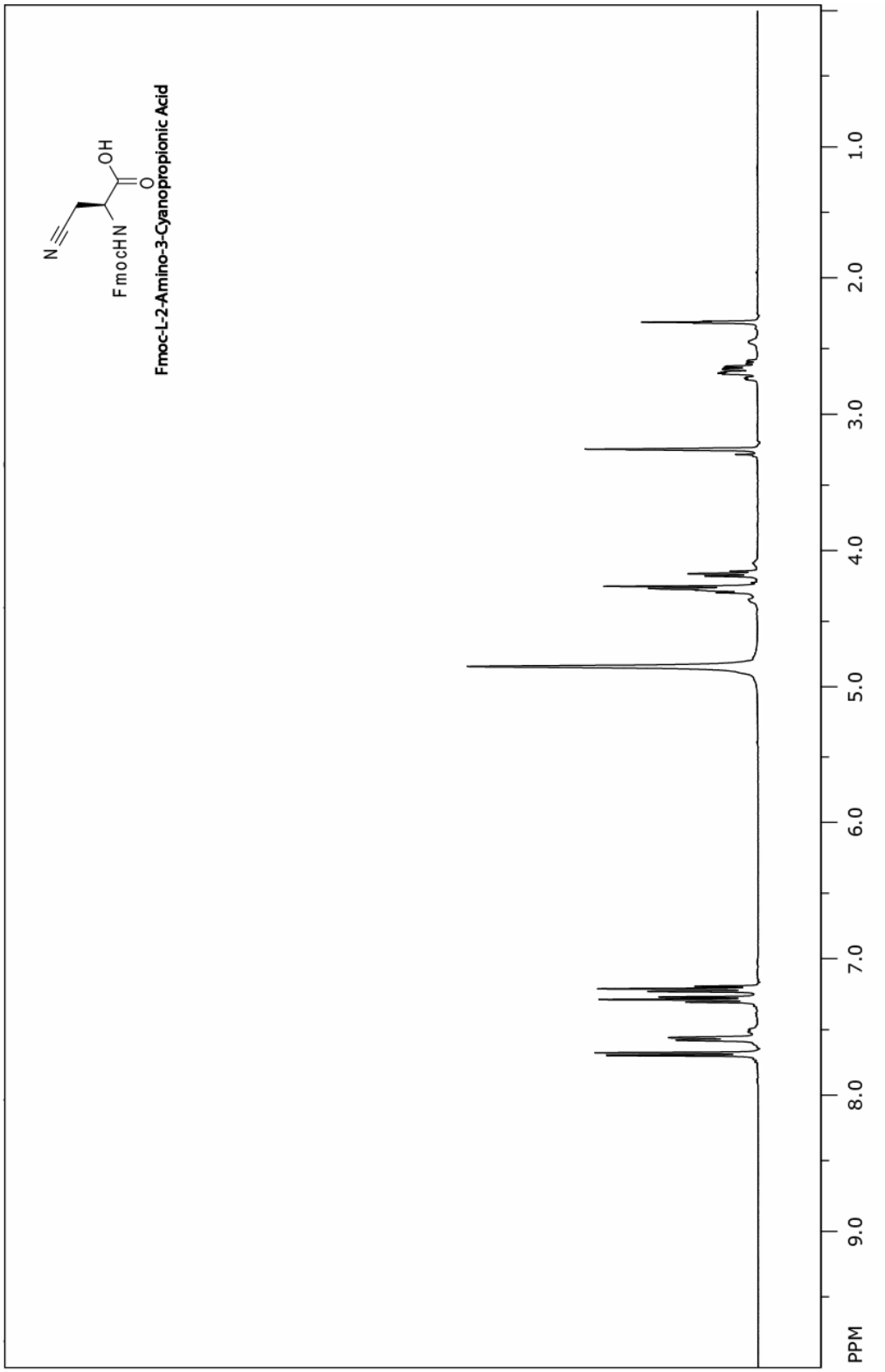
Appendix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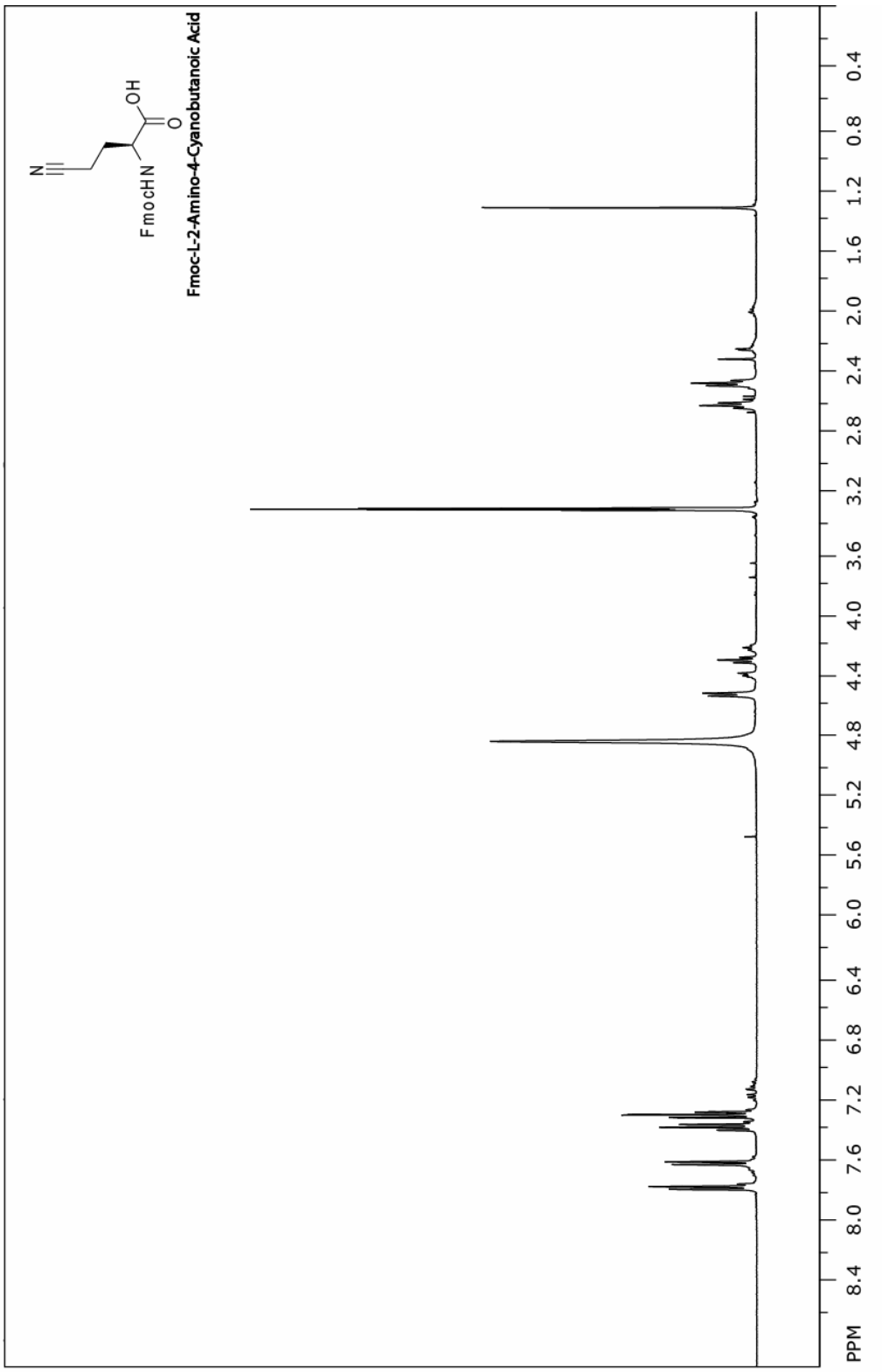
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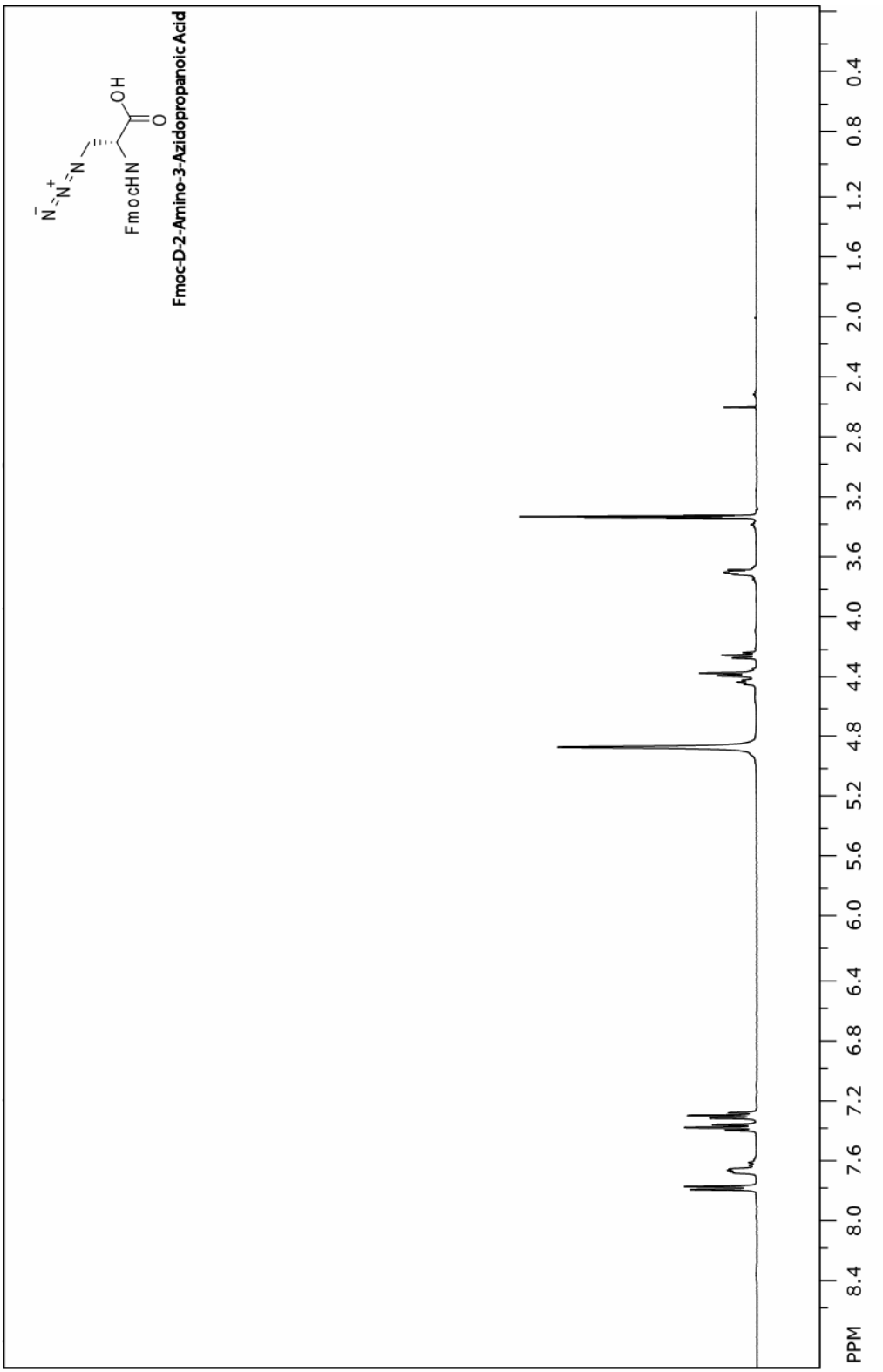
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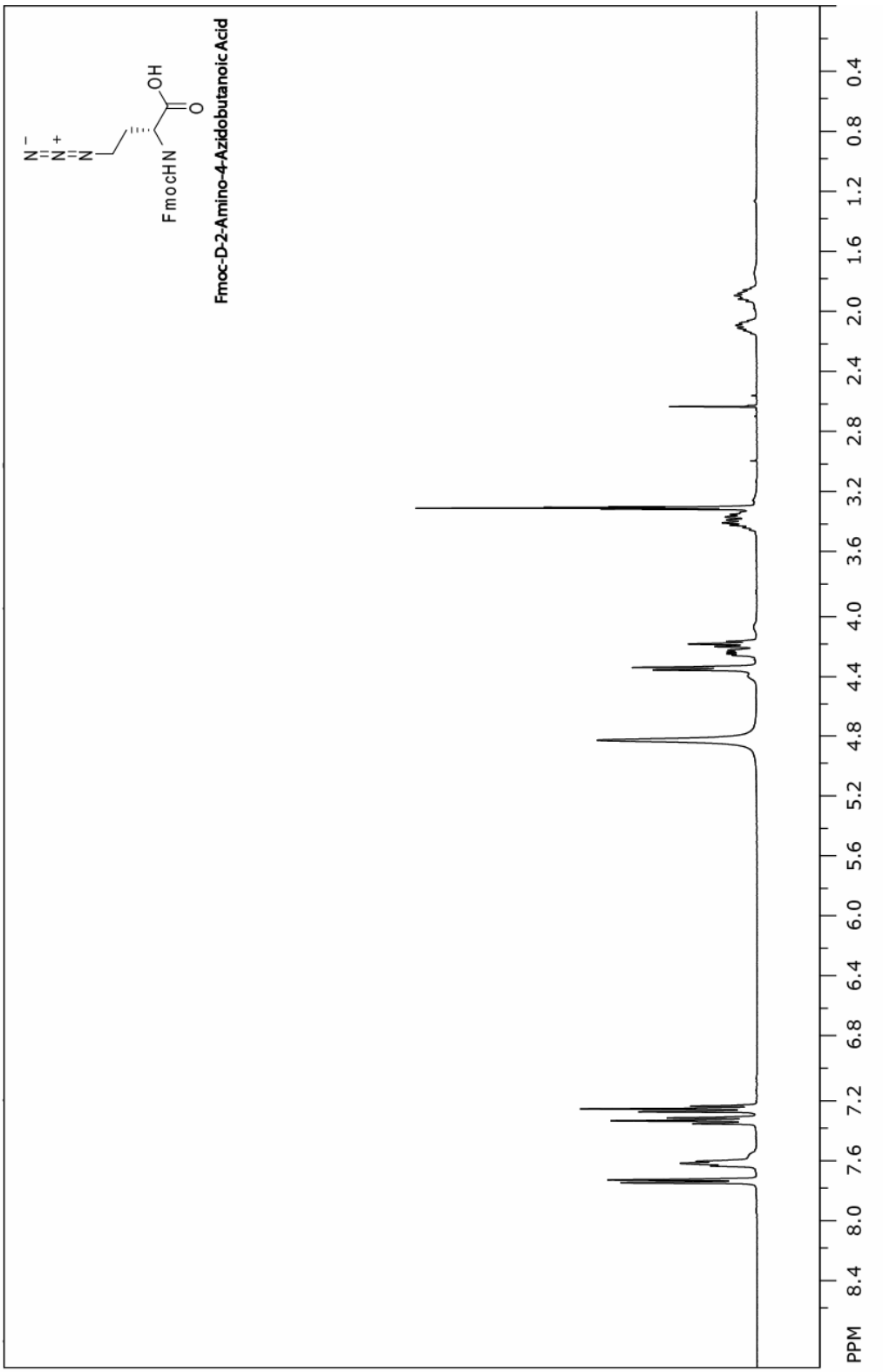
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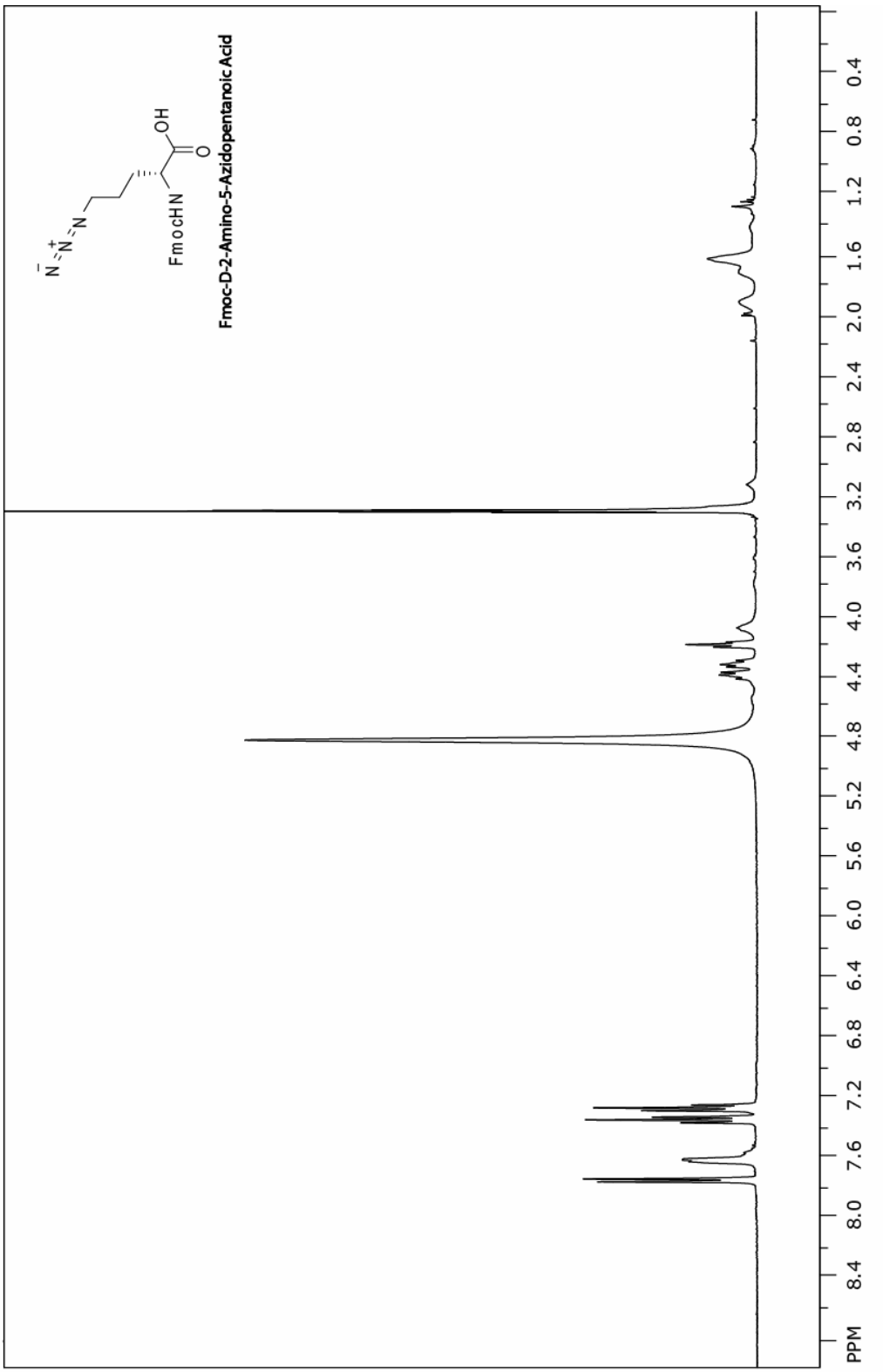
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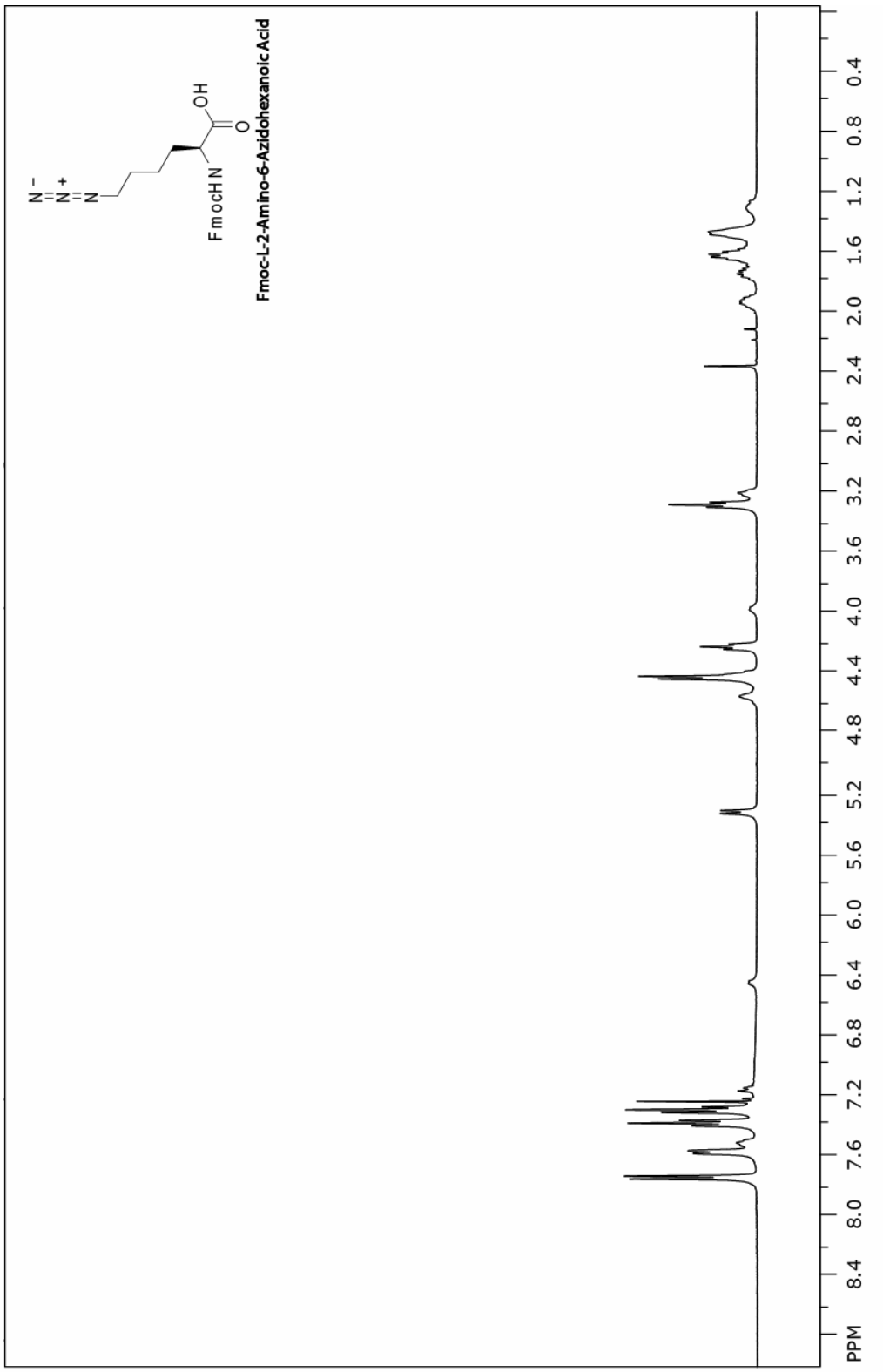
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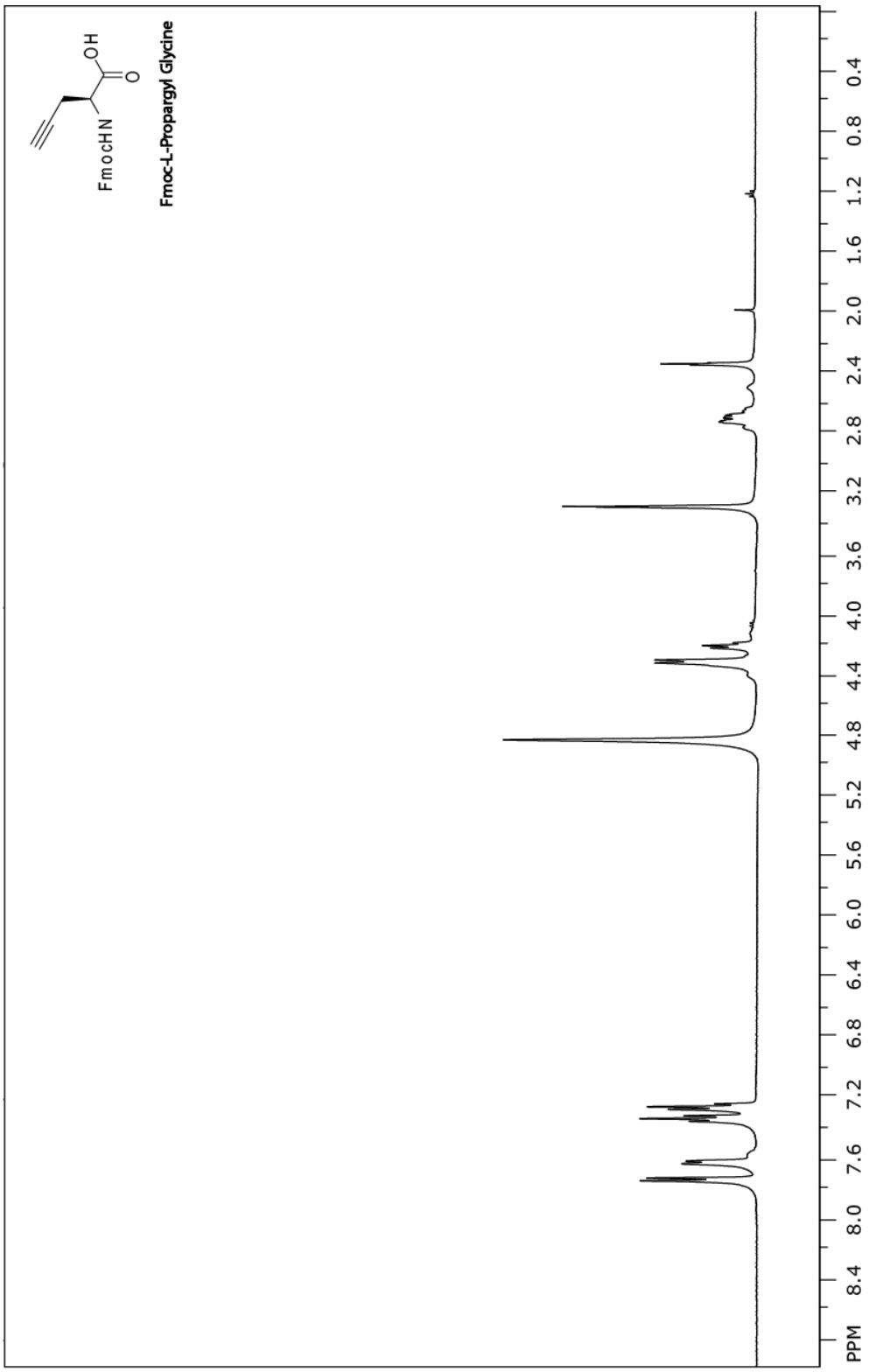
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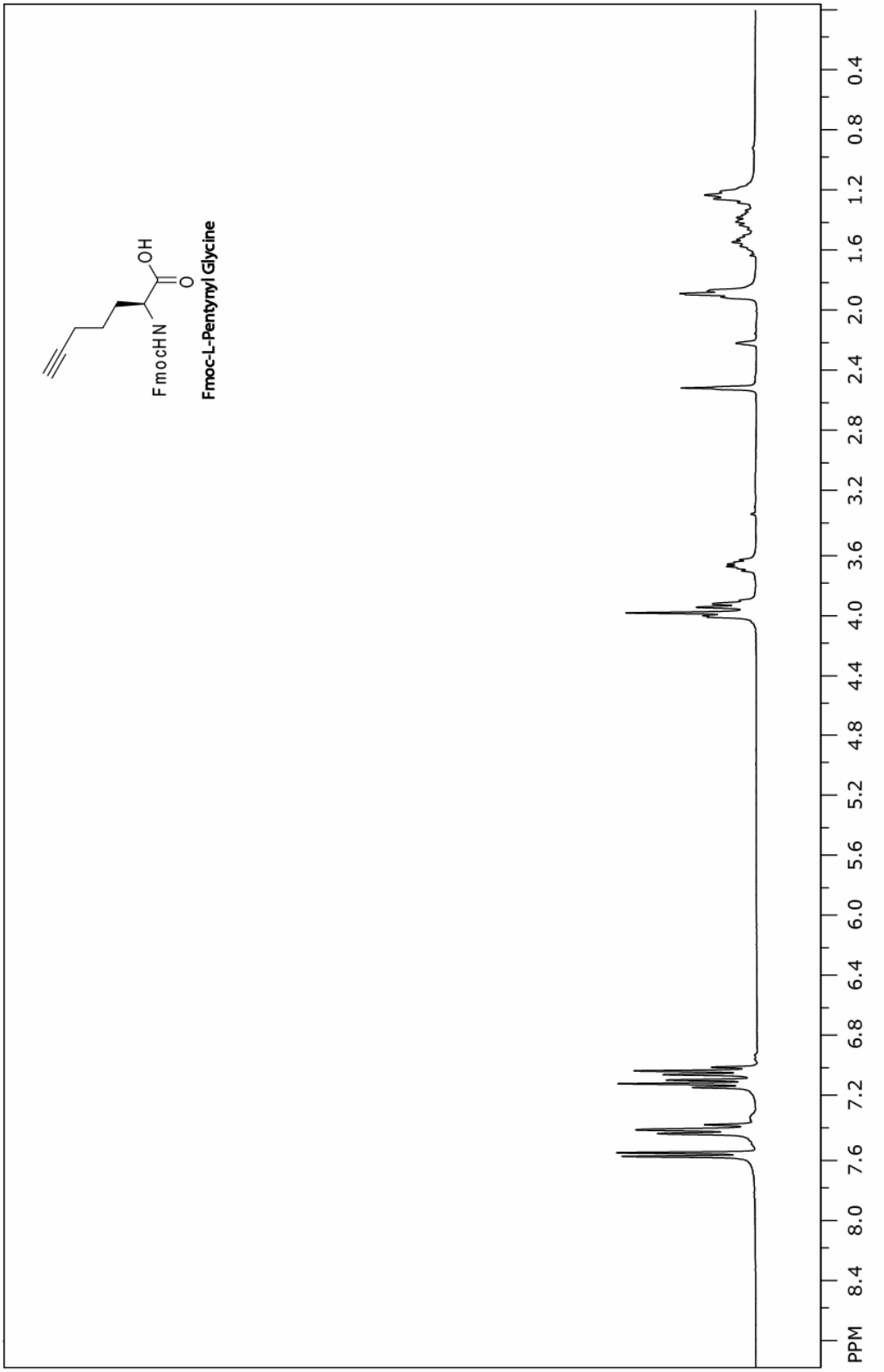
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Section 2: Nitrene Transfer Reactions Catalyzed by Metalloporphyrins

Abstract

Porphyrins and related tetrapyrrolic macrocycles are an important class of biologically relevant molecules that have found a broad spectrum of applications in various fields such as catalysis, materials and medicine. As a result, intense efforts have been made to synthesize porphyrins with tunable electronic and steric substituents. However, the classic synthesis of porphyrins typically gives low yields, requires tedious purification, and restrains effective preparation of porphyrin derivatives with functional and sensitive groups. Therefore, it is highly desirable to develop new strategies and alternative methods for efficient porphyrin synthesis. By applying palladium-catalyzed amination and etheration to halogenated porphyrin precursors, our research group has successfully developed a series of general and efficient methods for the synthesis of novel porphyrins that contain nitrogen, oxygen, sulfur, and other functionalities. We continued to expand this porphyrin synthetic strategy through application of other types of metal-based catalytic reactions.

Despite the well-known advantages of aziridines, they have received limited application in synthesis due to the difficulty in preparation and storage.[53] Several factors have hindered the area of catalytic aziridination. One of the major drawbacks is that most catalytic aziridination systems employ the use of the reagent [*N*-(*p*-toluenesulfonyl)imino]phenyliodinane. The drawbacks of using [*N*-(*p*-toluenesulfonyl)imino]phenyliodinane are that it is commercially unavailable, it can be costly to make, it is insoluble in many common solvents. To this end we have investigated the ability of metalloporphyrins to catalyze the aziridination of alkenes with a variety of alternate nitrene sources.

Chapter 5: Iron (III) Porphyrin Catalyzed Aziridination of Alkenes using Bromamine-T as Nitrene Source

This Chapter is a lightly revised version of a paper by the same name published in the journal *Organic Letters* in 2004 by Renu Vyas, Guang-Yao Gao, Jeremiah D. Harden, and X. Peter Zhang. The major changes include 1) the exclusion of the abstract to the paper, 2) the reformatting of selected text to fit the guidelines of this dissertation, 3) reproduction and reformatting of the figures and tables, and 4) combination of the references in this work with those from the other chapters in this section.

Renu Vyas, Guang-Yao Gao, Jeremiah D. Harden, and X. Peter Zhang. Iron (III) Porphyrin Catalyzed Aziridination of Alkenes using Bromamine-T as Nitrene Source. *Organic Letters*, **2004** 6(12), 1907.

My use of we and us refers to the other coauthors and myself. My primary contributions to this paper include 1) synthesis of the Bromamine-T reagent 2) repetition and verification of selected substrate reactions, and 3) characterization of some of the obtained products.

Aziridination of alkenes mediated by a transition metal complex is one of the most attractive methods for the efficient and selective construction of synthetically and biologically important aziridines.[54-58] The reagent [*N*-(*p*-toluenesulfonyl) imino]phenyliodinane (PhI=NTs) and related iminoiodane derivatives, aza-analogues of iodosylbenzene, have been extensively used as primary nitrene sources for catalytic aziridination.[59-61] Although great progress has been made with PhI=NTs in a number of metal-catalyzed systems including asymmetric aziridination,[54-58, 62-65] the reagent suffers from several drawbacks including its commercial unavailability, high cost, and insolubility along with generation of the heavy PhI byproduct.[66-68] Consequently, there has been growing interest in developing metal-catalyzed aziridination of alkenes with alternative nitrene sources such as chloramine-T,[69-76] bromamine-T,[77-80] and azides. [81, 82]

Metalloporphyrins have played a pivotal role in the development of several important catalytic atom/group transfer reactions, including aziridination of alkenes. Metalloprophyrins were the first class of transition metal complexes that were demonstrated to catalyze the aziridination of alkenes.[83, 84] Following this breakthrough, porphyrin complexes of several metal ions such as Fe, Mn, and Ru have been disclosed to have catalytic activity for aziridination when PhI=NTs was used as the nitrene source.[85-89] As a part of our program of metalloporphyrin-based atom/group transfer catalysts,[90-94] we became interested in developing practical catalytic aziridination processes with alternative nitrene sources. To this end we developed iron(III) porphyrin complexes Fe(Por)Cl (Figure 5-1) that are efficient and general catalysts for the aziridination of a wide variety of alkenes using bromamine-T as a nitrene source (Figure 5-2).[95] The reactions can be performed under mild conditions with alkenes as limiting reagents, and they proceed with moderate to low stereospecificity for 1,2-disubstituted alkenes.

First the catalytic aziridination was surveyed using various metalloporphyrins with different nitrene sources. These reactions were carried out at room temperature in acetonitrile in the presence of 5 Å molecular sieves using 5 mol % catalyst using a styrene to nitrene source mole ratio of 5: 1 (Table 5-1). While chloramine-T was found to be an ineffective nitrene source under these conditions, bromamine-T was found to be a superior nitrene source compaired to PhI=NTs, affording the desired aziridine in 60% isolated yield. Other metal complexes of tetraphenylporphyrin (TPP) such as Mn(TPP)Cl, Ru(TPP)(CO) and Co(TPP) afforded the desired aziridine, but with less efficiency. Although porphyin complexes of Fe, Mn, and Ru

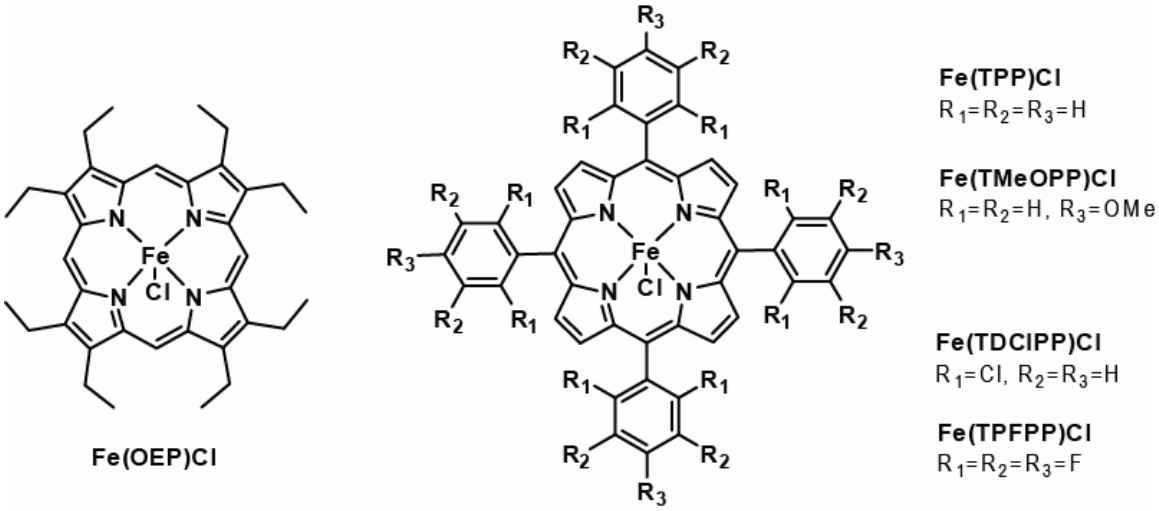


Figure 5-1. Stuctures of Iron (III) Porphyrin Complexes

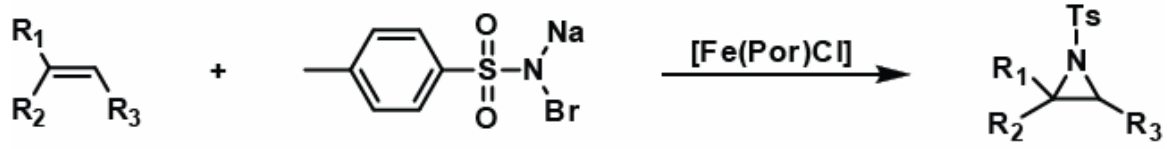
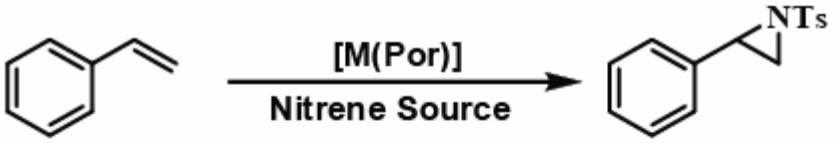


Figure 5-2. Aziridination of Alkenes Catalyzed by Iron (III) Porphyrin Fe(TPP)Cl

Table 5-1. Aziridination of Styrene by Metalloporphyrins[a]



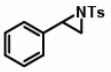
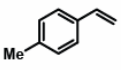
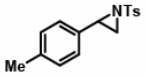
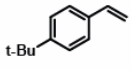
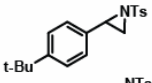
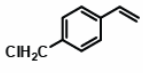
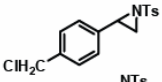
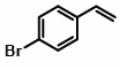
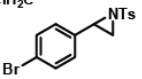
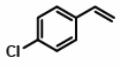
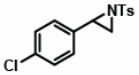
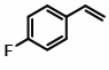
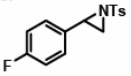
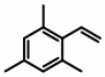
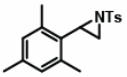
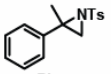
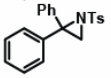
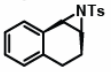
Entry	Nitrene Source	Metalloporphyrin[b]	Yield (%)[c]
1	PhI=NTs	Fe(TPP)Cl	31[d]
2	Chloramine-T	Fe(TPP)Cl	5
3	Bromamine-T	Fe(TPP)Cl	60
4	Bromamine-T	Mn(TPP)Cl	12
5	Bromamine-T	Co(TPP)Cl	27
6	Bromamine-T	Ru(TPP)CO	47
7	Bromamine-T	Fe(OEP)Cl	0
8	Bromamine-T	Fe(TMeOPP)Cl	25
9	Bromamine-T	Fe(TDClPP)Cl	38
10	Bromamine-T	Fe(TPFPP)Cl	80

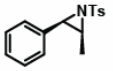
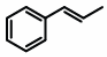
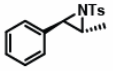
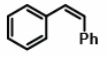
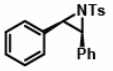
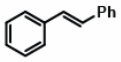
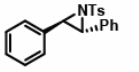
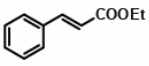
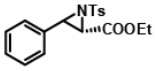
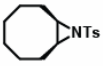
[a] Reactions were carried out for 12 h at room temperature in CH3CN under N2 in the presence of 5 Å molecular sieves with a styrene to nitrene source mole ratio of 5:1. [b] Catalyst loading: 5 mol %; see Figure 5-1 for structures of metalloporphyrins. [c]Yields represent isolated yields of >95% purity as determined by ^{1}H NMR. [d] From ref 11.

have been known to catalyze aziridination,[83-89] to the best of our knowledge this was the first example of cobalt porphyrins catalyzing the aziridination of alkenes. It was also seen that the catalytic activity of Fe(Por)Cl with bromamine-T has a dramatic ligand dependence. Both Fe(MeOPP)Cl and Fe(TDClPP)Cl were less effective than Fe(TPP)Cl and no activity was observed with Fe(OEP)Cl. However, the electron-deficient Fe(TPFPP)Cl improved the isolated yield of the desired aziridine to 80%(Table 5-1).

The substrate scope of the aziridination reactions by Fe(TPP)Cl with bromamine-T was explored using a variety of alkenes under the same conditions (Table 5-2). The catalytic system is suitable for a variety of styrene derivatives with *para*-substituted alkyl and halogen groups, affording the corresponding aziridines in yields similar to or better than that of the styrene reaction (Table 5-2). Sterically hindered derivatives such as 2,4,6-timethylstyrene could also be aziridinated, albeit in a lower yield. Both α- and β-subtituted styrenes including ethyl *trans*-cinnamate could be successfully converted to the desired aziridines in moderate to good yields (Table 5-2). The stereospecificity ranged from low to moderate. Like the ethyl *trans*-cinnamate, the α,β-unsaturated ester *tert*-butyl acrylate could also be aziridinated in moderate yield. Cyclic alkenes with different ring sizes as well as straight-chain alkenes were also suitable substrates (Table 5-2). *Exo*-methylene carbocycles such as methylenecyclohexene, however, could only be converted to the corresponding aziridine in trace amounts under these conditions (Table 5-2).

Table 5-2. Aziridination of Alkenes with Bromamine-T Catalyzed by Fe(TPP)Cl[a]

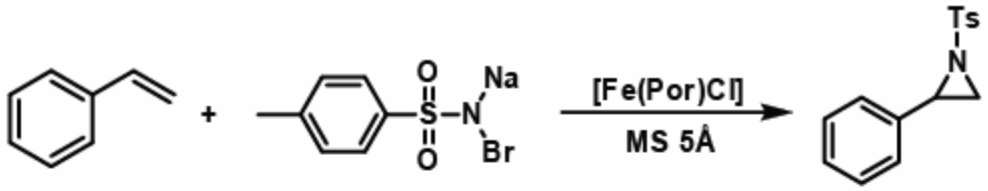
Entry	Substrate	Product	Yield A (%)[b]	Yield B (%)[c]
1			60	77
2			60	75
3			68	--
4			73[g]	--
5			44	--
6			52	80
7			55	--
8			46	--
9			42	--
10			72	--
11			28	48

Entry	Substrate	Product	Yield A (%)[b]	Yield B (%)[c]
12			38 (61:39)[d]	54 (56:44)[d]
13			64 (18:82)[d]	75 (18:82)[d]
14			64 (69:31)[d]	68 (60:40)[d]
15			72 (58:42)[d]	70 (50:50)[d]
16			42 (31:69)[d]	--
17			36[e]	--
18			48	47[f]
19			42	45[f]
20			53	60
21			55	60
22			0	50

[a] Carried out at room temperature in CH_3CN for 12 hours under N_2 in the presence of 5 Å molecular sieves using 5 mol% Fe(TPP)Cl. Concentration: 0.1 mmol alkene/2mL CH_3CN. [b] Isolated yields with Bromamine-T as limiting reagent (alkene/Bromamine-T = 5:1). [c] Isolated yields with alkene as limiting reagent (alkene/Bromamine-T = 1:2). [d] *cis*:*trans ratios*. [e] Performed at 60 °C. [f] Fe(TPFPP)Cl was used. [g] Contaminated with small amount of side product.

To further improve the Fe(TPP)Cl/bromamine-T aziridination system, detailed optimization of various reaction parameters was carried out using styrene as the model substrate (Table 5-3). Acetonitrile appeared to be the best solvent choice, as other solvents such as toluene, tetrahydrofuran, and dichloromethane gave none or only trace amounts of the desired aziridine (Table 5-3). The reaction time could be shortened to 6 hours without dramatically lowering the yield, however shorter times led to considerable loss in yield (Table 5-3). An increase in reaction temperature typically led to higher yields, for example the isolated yields at 40^{o}C and 80^{o}C were 64% and 80% respectively (Table 5-3). However temperatures in excess of 100^{o}C resulted in a dramatic decrease in the yield of the desired aziridine and often led to the formation of otherwise unseen side products (Table 5-3). There was an observed decrease in yield if the catalyst loading was decreased below 5 mol%, as well as when the styrene to bromamine-T ratio increased over 5:1 respectively (Table 5-3). Alternatively the results improved if styrene was employed as the limiting reagent (Table 5-3). However, increase in the styrene to bromamine-T ratio over 1:2 respectively led to the formation of side products and a decrease in the yield of the desired aziridine (Table 5-3). The best isolated yield (82%) of the N-(p-tolylsulfonyl)-2-phenylaziridine was achieved when a higher catalyst loading was employed at room temperature with a 1:2 ratio of styrene to bromamine-T respectively (Table 5-3). On the basis of the optimization results the aziridination of representative examples of alkenes with bromamine-T was carried out again at room temperature using the alkenes as the limiting reagents and employing 5 mol% of Fe(TPP)Cl. As summarized in Yield B of Table 5-2 the yields were improved in

Table 5-3. Aziridination of Styrene with Bromamine-T Catalyzed by Fe(TPP)Cl under various conditions[a]



Entry	S:BT[b]	[Fe] (mol %)	Solvent	Temp. (°C)	Time (h)	Yield (%)[c]
1	5:1	5	Toluene	23	12	0
2	5:1	5	THF	23	12	0
3	5:1	5	CH_2Cl_2	23	12	5
4	5:1	5	CH_3CN	23	12	60
5	5:1	5	CH_3CN	23	6	64
6	5:1	5	CH_3CN	23	2	42
7	5:1	5	CH_3CN	40	12	65
8	5:1	5	CH_3CN	80	12	80
9	5:1	5	CH_3CN	100	12	39
10	5:1	2	CH_3CN	23	12	10
11	5:1	10	CH_3CN	23	12	70
12	10:1	5	CH_3CN	23	12	55
13	100:1	5	CH_3CN	23	12	45
14	1:1.5	5	CH_3CN	23	12	62
15	1:2	5	CH_3CN	23	12	77
16	1:2	5	CH_3CN	80	12	67
17	1:2	10	CH_3CN	23	12	82
18	1:5	5	CH_3CN	23	12	52

[a] Reactions were carried out under N_2 in the presence of 5 Å molecular sieves with a concentration of 0.1 mmol styrene/2 mL solvent. [b] S:BT = the mole ratio of styrene substrate to bromamine-T. [c] Yields represent isolated yields of >95% purity as determined by ^1H NMR.

all of the examined cases. For example, a large increase in yields were obtained for styrene derivatives (Table 5-2, entries 1, 2, and 6). For β-substituted styrenes, good yields were achieved in most cases, but stereospecificities remained almost the same (Table 5-2, entries 11-15). Similar improvement in yield was also observed for cyclic and straight-chain alkenes (Table 5-2, entries 18-21). Under the optimized conditions the previously unconverted methylenecyclohexene provided the desired spirocyclic aziridine in 50% yield (Table 5-2, entry 22).

The catalytic aziridinations by iron (III) porphyrins with bromamine-T are assumed to proceed via a mechanism similar to that proposed for other metalloporphyrin systems with PhI=NTs.[83-89] As shown in Figure 5-3, reaction of bromamine-T with an iron (III) porphyrin generates the iron-nitrene intermediate **A** with the associated generation of NaBr. Nitrene transfer from intermediate **A** to an alkene substrate produces the aziridine product and regenerates the iron (III) porphyrin catalyst to turn over the catalytic cycle. However, the low to moderate stereoselectivity observed for the 1,2-disubstituted olefins could suggest a secondary nitrene or radicle intermediate.

Bromamine-T has been shown to be an effective nitrene source for the aziridination of alkenes by porphyrin complexes of transition metal ions such as Mn, Ru, Co, and Fe. The utility of the Fe(TPP)Cl catalytic aziridination system employing alkenes as limiting reagents under mild and practical conditions was demonstrated. The catalytic system is general and suitable for a wide range of alkene substrates, providing the desired aziridines in good yields, albeit with poor stereospecificity.

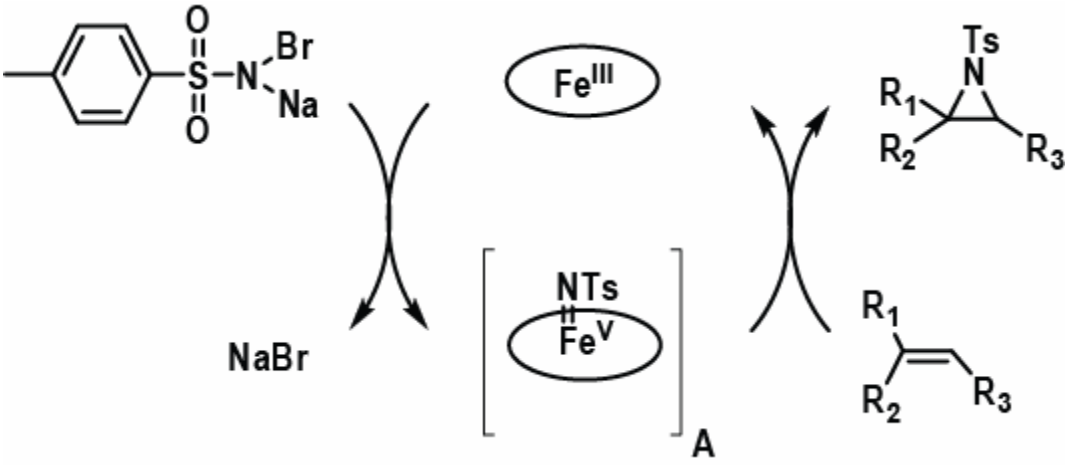


Figure 5-3. Possible Mechanism for the Aziridination of Alkenes Catalyzed by Iron(III) Porphyrin with Bromamine-T

Experimental

General Considerations. All reactions were carried out under a nitrogen atmosphere in an oven dried Schlenk tube. All olefins were purchased from Acros or Aldrich Chemicals and used without further purification. Anhydrous acetonitrile was purchased from Aldrich Chemicals. All metalloporphyrins were purchased from Strem or Midcentury Chemicals. Bromamine-T was prepared from the commercially available Chloramine-T according to the literature procedure.[96-98] Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Varian Mercury 300 spectrometer and referenced with respect to internal TMS standard or residual solvent. Infrared spectra were obtained using a Bomen B100 FT-IR spectrometer. Samples were prepared as films on a sodium chloride plate by evaporating THF solutions. High resolution mass spectroscopy (HRMS) was performed by the Mass Spectrometry Center located in the Chemistry Department of the University of Tennessee on a VG Analytical hybrid high performance ZAB-EQ (B-E-Q geometry) instrument using electron impact (EI) ionization technique with a 70 eV electron beam. Thin layer chromatography was carried out on E. Merck Silica Gel 60 F-254 TLC plates.

General procedure for aziridination of olefins. An oven dried Schlenk tube equipped with a stirring bar was degassed on a vacuum line and purged with nitrogen. The tube was charged with metalloporphyrin (5 mol%), Bromamine-T (0.1 mmol) and activated 5Å molecular sieves. The tube was equipped with a Teflon screw cap, and evacuated on a vacuum line for 30 min. The Teflon screw cap was replaced with a rubber septum then 2 mL of solvent and the olefin substrate (0.5mmol) were then added

successively. The tube was purged with nitrogen for 1 minute and the contents were stirred at room temperature for 12 hours. After completion of the reaction the solvent was concentrated under reduced pressure. The solid residue was purified by flash chromatography (silica gel, 15% ethyl acetate: hexanes) to afford pure product.

N-(p-Tolylsulfonyl)-2-phenylaziridine.[63] (Table 5-2, Entry 1) was synthesized from styrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (d, 2H, *J* = 8.1Hz), 7.28 (m, 7H), 3.79 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.98 (d, 1H, *J* = 7.2 Hz), 2.42 (s, 3H), 2.39 (d, 1H, *J* = 4.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.6, 135.0, 129.7, 128.5, 128.2, 127.9, 126.5, 41.0, 35.9, 21.6. FT-IR (film, cm^{-1}): 3575, 1215, 1150, 916. HRMS-EI ($[M]^+$): Calculated for $C_{15}H_{15}NO_2S$, 273.0823, Found 273.0821.

N-(p-Tolylsulfonyl)-2-(p-methylphenyl)aziridine.[63] (Table 5-2, Entry 2) was synthesized from p-methylstyrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.1 Hz), 7.32 (d, 2H *J* = 7.8 Hz), 7.09 (s, 4H), 3.74 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.96 (d, 1H, *J* = 7.2 Hz), 2.42 (s, 3H), 2.36 (d, 1H, *J* = 4.2 Hz), 2.31 (s, 3H). FT-IR (film, cm^{-1}): 3057, 1600, 1150, 916. HRMS-EI ($[M]^+$): Calculated for $C_{16}H_{17}NO_2S$, 287.0430, Found 287.0441.

N-(p-Tolylsulfonyl)-2-(p-*tert*-butylphenyl)aziridine. (Table 5-2, Entry 3) was synthesized from p-*tert*-butyltyrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.1 Hz), 7.28-7.33 (m, 4H), 7.12 (d, 2H, *J* = 8.1 Hz), 3.74 (dd, 1H, *J* = 7.2, 4.8 Hz), 2.94 (d, 1H, *J* = 7.2 Hz), 2.42 (s, 3H), 2.36 (d, 1H, *J* = 4.2 Hz), 1.28 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.0, 138.0, 135.0, 129.3, 127.5, 125.8, 40.5, 39.8, 35.3, 30.8, 21.2. HRMS-EI($[M]^+$): Calculated for $C_{19}H_{22}NO_2S$, 329.1450, Found 329.1454.

N-(p-Tolylsulfonyl)-2-(p-chloromethylphenyl)aziridine.[99] (Table 5-2, Entry 4) was synthesized from p-vinylbenzyl chloride as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, 2H, *J* = 8.1 Hz), 7.31 (m, 4H), 7.19 (d, 2H, *J* = 7.8 Hz), 4.52 (s, 2H), 3.74 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.96 (d, 1H, *J* = 6.9 Hz), 2.42 (s, 3H), 2.34 (d, 1H, *J* = 4.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.0, 131.0, 129.7, 128.8, 128.0, 127.9, 126.9, 126.8, 45.7, 40.5, 36.0, 21.6. FT-IR (film, cm^{-1}): 3575, 2927, 2858, 1594, 1324, 1160, 1091, 911, 814. HRMS-EI ($[M]^+$): Calculated for $C_{15}H_{12}NO_2SCl$, 285.0824, found 285.0817.

N-(p-Tolylsulfonyl)-2-(p-bromophenyl)aziridine.[100] (Table 5-2, Entry 5) was synthesized from p-bromostyrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.4 Hz), 7.40 (d, 2H, *J* = 8.7 Hz), 7.31 (d, 2H, *J* = 8.7 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 3.70 (dd, 1H, J = 7.2, 4.2 Hz), 2.95 (d, 1H, *J* = 7.2 Hz), 2.42 (s, 3H), 2.32 (d, 1H, *J* = 4.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 136.0, 134.0, 131.7, 131.6, 129.7, 128.1, 127.9, 40.2, 35.9, 21.0. HRMS-EI ($[M]^+$): Calculated for $C_{15}H_{14}NO_2SBr$, 350.9929, Found 350.9926.

N-(p-Tolylsulfonyl)-2-(p-chlorophenyl)aziridine.[63] (Table 5-2, Entry 6) was synthesized from p-chlorostyrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.1 Hz), 7.32 (d, 2H, *J* = 8.1 Hz), 7.25 (d, 2H, *J* = 8.4 Hz), 7.12 (d, 2H, *J* = 8.7 Hz), 3.71 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.96 (d, 1H, *J* = 7.2 Hz), 2.43 (s, 3H), 2.33 (d, 1H, *J* = 4.2 Hz). FT-IR (film, cm^{-1}): 3575, 2361, 1771, 1540, 1092, 900. HRMS-EI ($[M]^+$): Calculated for $C_{15}H_{14}NO_2SCl$, 307.0434, Found 307.0431.

N-(p-Tolylsulfonyl)-2-(p-fluorophenyl)aziridine.[100] (Table 5-2, Entry 7) was synthesized from p-fluorostyrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d,

2H, J = 8.1 Hz), 7.33 (d, 2H, J = 8.4 Hz), 7.19 (m, 2H), 6.96 (m, 2H), 3.74 (dd, 1H, J = 7.2, 4.2 Hz), 2.95 (d, 1H, J = 7.2Hz), 2.42 (s, 3H), 2.33 (d, 1H, J = 4.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 132.0, 130.0, 128.5, 128.1, 115.9, 115.6, 40.5, 36.2, 21.9. HRMS-EI ($[M]^+$): Calculated for $C_{15}H_{14}NO_2SF$, 291.079, Found 291.741.

N-(p-Tolylsulfonyl)-2-(2',4',6'-trimethylphenyl)aziridine. (Table 5-2, Entry 8) was synthesized from 2,4,6-trimethylstyrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.1 Hz), 7.32 (d, 2H, *J* = 8.1 Hz), 6.77 (s, 2H), 3.83 (dd, 1H, *J* = 6.9, 4.2 Hz), 2.91 (d, 1H, 6.9 Hz), 2.44 (s, 3H), 2.27, (s, 6H), 2.21(s, 3H), 2.14 (d, 1H, *J* = 4.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.9, 137.7, 137.6, 135.2, 130.6, 129.9, 128.5, 128.4, 38.9, 35.0, 21.2, 20.3, 19.0. HRMS-EI ($[M]^+$): Calculated for $C_{18}H_{17}NO_2S$, 315.1293, Found 315.1297.

N-(p-Tolylsulfonyl)-2-methyl-2-phenylaziridine.[63] (Table 5-2, Entry 9) was synthesized from α-methylstyrene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 6.6 Hz), 7.23-7.38 (m, 7H), 2.94 (s, 1H), 2.51 (s, 1H), 2.41 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 142.0, 139.7, 130.0, 129.3, 128.8, 128.5, 127.6, 52.0, 42.0, 21.9, 21.5. FT-IR (film, cm^{-1}): 3020, 2930, 1600, 1220, 1100, 920. HRMS-EI ($[M]^+$): Calculated for $C_{16}H_{17}NO_2S$, 287.0980, Found, 287.0976.

N-(p-Tolylsulfonyl)-2,2-diphenylaziridine.[84] (Table 5-2, Entry 10) was synthesized from 1,1-dipenylethylene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, 2H, *J* = 7.8 Hz), 7.18-7.32 (m, 12H), 3.01 (s, 2H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.4, 138.3, 129.8, 129.1, 128.6, 128.5, 128.3, 128.2, 128.1, 57.4, 40.8, 22.0. HRMS-EI ($[M]^+$): Calculated for $C_{21}H_{19}NO_2S$, 349.1137, Found 349.1142.

N-(p-Tolylsulfonyl)amino-1,2,3,4-tetrahydronapthalene-1,2-imine.[63] (Table 5-2, Entry 11) was synthesized from 1,2-dihydronapthalene as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 2H, *J* = 8.1 Hz), 7.01-7.30 (m, 6H), 3.80 (d, 1H, *J* = 6.9 Hz), 3.54 (d, 1H, *J* = 6.9 Hz), 2.74 (dt, 1H, *J* = 15, 6.3 Hz), 2.54 (dd, 1H, *J* =15, 6.0 Hz), 2.40 (s, 3H), 2.25 (dd, 1H, *J* = 14.1, 6.6 Hz), 1.66 (dt, 1H, *J* = 13.2, 5.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 138.0, 135.0, 129.9, 129.4, 128.4, 128.2, 127.4, 126.0, 39.6, 35.6, 21.9, 21.0, 20.3. HRMS-EI ($[M]^+$): Calculated for $C_{17}H_{17}NO_2S$, 299.0980; Found 299.0994.

N-(p-Tolylsulfonyl)-2-methyl-3-phenylaziridine.[63] (mixture of *cis*- and *trans*-isomers) (Table 5-2, Entry 12, 13) was synthesized from cis-β-methylstyrene (Entry 12) or *trans*-β-methylstyrene (Entry 13) as a substrate and was a mixture of *cis*- and *trans*-isomers. For *cis*- isomers: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 8.4 Hz), 7.17-7.32 (m, 7H), 3.90 (d, 1H, *J* = 7.5 Hz), 3.18 (dq, 1H, *J* = 7.2, 5.7 Hz), 2.42 (s, 3H), 1.01 (d, 3H, *J* = 5.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 137.0, 132.0, 129.2, 129.0, 127.3, 45.6, 41.4, 24.0, 11.5. FT-IR (film, cm^{-1}): 3013, 1600, 1414, 1100, 920. HRMS-EI ($[M]^+$): calcd for $C_{16}H_{17}NO_2S$, 287.0974; found 287.0974. For *trans*- isomers: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.7 Hz), 7.24-7.27 (m, 5H), 7.15 (m, 2H), 3.80 (d, 1H, *J* = 3.9 Hz), 2.89 (dq, 1H, *J* = 6.6, 4.4 Hz), 2.37 (s, 3H), 1.83 (d, 3H, *J* = 6.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.0, 137.0, 135.0, 129.0, 128.0, 127.0, 126.0, 49.0, 21.0, 14.0. HRMS-EI ($[M]^+$): Calculated for $C_{16}H_{17}NO_2S$, 287.0974, found 287.0973.

N-(p-Tolylsulfonyl)-2,3-diphenylaziridine.[63] (mixture of *cis*- and *trans*-isomers) (Table 5-2, Entry 14, 15) was synthesized from *cis*-stilbene (Entry 14) or *trans*-

stilbene (Entry 15) as a substrate and the product was a mixture of *cis*- and *trans*-isomers. For *cis*- isomers: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, 2H, *J* = 8.1 Hz), 7.34 (d, 2H, *J* = 8.1 Hz), 7.01-7.11 (m, 10H), 4.20 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 129.8, 129.3, 128.6, 128.4, 128.2, 127.9, 127.7, 127.4, 47.4, 21.6. FT-IR (film, cm^{-1}): 3017, 3001, 1320, 974, 819. HRMS-EI ($[M]^+$): calcd for $C_{20}H_{16}NO_2S$, 349.1137; found 349.1133. For *trans*- isomers: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, 2H, *J* = 8.1 Hz), 7.32-7.42 (m, 10H), 7.18 (d, 2H, *J* = 8.4 Hz), 4.24 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3017, 3000, 1322, 927, 707. HRMS-EI ($[M]^+$): Calculated for $C_{20}H_{16}NO_2S$, 349.1137, Found 349.1132.

N-(p-Tolylsulfonyl)-3-phenyl-2-aziridinecarboxylic acid, ethyl ester.[101, 102] (mixture of *cis*- and *trans*- isomers) (Table 5-2, Entry 16) was synthesized from ethyl *trans*-cinnamate as a substrate and the product was a mixture of two isomers. For *cis*-isomers: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, 2H, *J* = 8.1 Hz), 7.35 (d, 2H, *J* = 8.1 Hz), 7.25-7.32 (m, 5H), 4.10 (d, 1H, *J* = 7.2 Hz), 3.90 (m, 2H), 3.66 (d, 1H, *J* = 7.8Hz), 2.43 (s, 3H), 0.92 (t, 3H, *J* = 7.2 Hz). For *trans*- isomers: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, 2H, *J* = 8.1 Hz), 7.25-7.32 (m, 7H), 4.42 (d, 1H, *J* = 4.2 Hz), 4.30 (m, 2H), 3.49 (d, 1H, *J* = 4.2 Hz), 2.40 (s, 3H), 1.33 (t, 3H, *J* = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 144.0, 136.0, 132.0, 129.8, 128.8, 128.5, 127.4, 127.3, 61.5, 47.0, 45.2, 43.3, 21.6, 13.7. FT-IR (film, cm^{-1}): 3068, 1749, 1333, 1162, 554, 421. HRMS-EI ($[M]^+$): Calculated for $C_{18}H_{19}NO_4S$, 345.1035, Found 345.1053.

N-(p-Tolylsulfonyl)-2-aziridinecarboxylic acid, *tert*-butyl ester.[103] (Table 5-2, Entry 17) was synthesized from *tert*-butyl acrylate as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 2H, *J* = 8.4 Hz), 7.34 (d, 2H, *J* = 8.1 Hz), 3.20 (q, 1H, *J* = 4.2 Hz),

2.66 (d, 1H, *J* = 6.6 Hz), 2.48 (d, 1H, *J* = 4.2 Hz), 2.44 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.0, 134.0, 128.0, 83.0, 35.0, 31.0, 27.0, 21.0. FT-IR (film, cm^{-1}): 3523, 2900, 1740, 1600, 1165. HRMS-EI ($[M]^+$): Calculated for $C_{14}H_{19}NO_2S$, 297.1035, Found 297.1025.

N(p-Tolylsulfonyl)-6-azabicyclo[3.1.0]hexane.[64] (Table 5-2, Entry 18) was synthesized form cyclopentene as a substrate. ^1H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, 2H, *J* = 8.1 Hz), 7.28 (d, 2H, *J* = 7.8 Hz), 3.31 (s, 2H), 2.42 (s, 3H), 1.92 (m, 4H), 1.6 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 135.0, 129.5, 127.5, 46.7, 26.9, 21.6, 19.4. HRMS-EI ($[M]^+$): Calculated for $C_{12}H_{15}NO_2S$, 237.0824, Found 237.0828.

N-(p-Tolylsulfonyl)-7-azabicyclo[4.1.0]heptane.[63] (Table 5-2, Entry 19) was synthesized from cyclohexene as a substrate. ^1H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, 2H, *J* = 7.8 Hz), 7.30 (d, 2H, *J* = 8.1 Hz), 2.95 (s, 2H), 2.42 (s, 3H), 1.75 (m, 4H), 1.39 (m, 2H), 1.20 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.0, 141.0, 129.0, 127.0, 39.3, 22.3, 21.9, 18.9. FT-IR (film, cm^{-1}): 3000, 1600, 1392, 1155, 920. HRMS-EI ($[M]^+$): Calculated for $C_{13}H_{17}NO_2S$, 251.0723, Found 251.0731.

N-(p-Tolylsulfonyl)-9-azabicyclo[6.1.0]nonane.[64] (Table 5-2, Entry 20) was synthesized from cyclooctene as a substrate. ^1H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 2H, *J* = 8.4 Hz), 7.30 (d, 2H, *J* = 7.8 Hz), 2.75 (d, 1H, *J* = 4.2 Hz), 2.42 (s, 3H), 1.98 (d, 1H, *J* = 13.5 Hz), 1.55-1.23 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 129.6, 127.5, 43.9, 26.3, 26.1, 25.1, 21.6. HRMS-EI ($[M]^+$): Calculated for $C_{15}H_{21}NO_2S$, 279.1293; Found 279.1297.

N-(p-Tolylsulfonyl)-9-heptylaziridine.[104] (Table 5-2, Entry 21) was synthesized from 1-nonene as a substrate. ^1H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 2H, *J* = 6.6 Hz),

7.30 (d, 2H, *J* = 8.1 Hz), 2.68 (m, 1H), 2.62 (d, 1H, *J* = 6.9 Hz), 2.42 (s, 3H), 2.03(d, 1H, *J* = 4.5 Hz), 1.2-1.4 (m, 12H), 0.84 (t, 3H, *J* = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 135.0, 129.5, 127.9, 40.4, 33.7, 31.6, 31.2, 29.0, 28.9, 26.7, 22.5, 21.6, 14.0. HRMS-EI ($[M]^+$): Calculated for $C_{16}H_{25}NO_2S$, 295.1606, Found 295.1610.

N-(p-Tolylsulfonyl)-1-azaspiro[2.5]octane.[105] (Table 5-2, Entry 22) was synthesized from methylene cyclohexane as a substrate. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.1 Hz), 7.31 (d, 2H, *J* = 8.1 Hz), 2.43 (s, 3H), 2.40 (s, 2H), 1.93 (s, 2H), 1.7-2.0 (m, 6H), 1.2-1.5 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 129.0, 126.8, 53.5, 40.6, 32.6, 24.9, 24.7, 21.1. FT-IR (film, cm^{-1}): 2900, 1600, 1300, 900. HRMS-EI ($[M]^+$): Calculated for $C_{14}H_{19}NO_2S$, 265.1137, Found 265.1140.

Chapter 6: Cobalt-Catalyzed Efficient Aziridination of Alkenes

This chapter is a revised version of a paper by the same name published in the journal *Organic Letters* in 2005 by authors Guang-Yao Gao, Jeremiah D. Harden, and X. Peter Zhang. The major changes include 1) the exclusion of the abstract to the paper, 2) the reformatting of selected text to fit the guidelines of this dissertation, 3) reproduction and reformatting of the figures and tables, and 4) combination of the references in this work with those from the other chapters in this section.

Guang-Yao Gao, Jeremiah D. Harden, and X. Peter Zhang. Cobalt Catalyzed Efficient Aziridination of Alkenes. *Organic Letters* **2005**, 7(15), 3191.

My use of we and us refers to the other coauthors and myself. My primary contributions to this paper include 1) synthesis of the Co(TDClPP) porphyrin catalyst 2) synthesis of the Bromamine-T reagent 3) execution and analysis of screening reaxction, 4) execution and analysis of substrate reactions, and 5) characterization of some of the obtained products.

Aziridines are a class of synthetically and biologically important three-membered heterocyclic compounds that have found many applications.[58, 106-108] Among synthetic methodologies, transition metal complex mediated aziridination of alkenes with a nitrene source represents a direct and powerful approach for the construction of the aziridine rings.[54-57] The most widely used nitrene sources for aziridination are the reagent [*N*-(*p*-toluenesulfonyl)imino]phenyliodinane (PhI=NTs) and related iminoiodane derivatives.[59-61] To overcome several limitations associated with the use of PhI=NTs,[66-68] alternative nitrene sources such as chloramine-T,[73, 95, 109] bromamine-T,[77, 78] and tosyl azide[82, 110] have also been actively pursued. With these nitrene sources, complexes of Mn, Fe, Ru, Rh, and Cu that are supported by different ligands have been identified to catalyze aziridination.[54-63, 65-68, 73, 77, 78, 82, 95, 106-110]

The unique ligand environment and metal coordination mode of metalloporphyrins render them a class of attractive catalysts for aziridination and related atom/group transfer reactions.[111] Indeed, the first transition metal complexes that were

discovered for aziridination catalytic activity are metalloporphyrins.[83, 84] To date, porphyrin complexes of Fe, Mn, and Ru have been known to catalyze aziridination.[85-89] As part of our ongoing efforts in developing metalloporphyrin-based practical atom/group transfer catalytic systems,[90-94, 112-114] we reveal herein the first cobalt-based catalytic system that is efficient for the aziridination of different alkenes.[115-117] Cobalt(II) porphyrins (Figure 6-1) were shown to be effective catalysts for the aziridination of a wide variety of alkenes with bromamine-T (Figure 6-2). Using styrene as a model substrate, we first evaluated the catalytic aziridination activities of Co complexes supported by various porphyrins under practical conditions (room temperature, one-pot protocol, and styrene as the limiting reagent). The results are summarized in Table 6-1. Although the Co complex of the most common porphyrin Co(TPP) could aziridinate styrene in a low yield, the Co complexes of electron-rich porphyrins such as Co(TTMeOPP) and Co(TMeOPP) furnished no or only a trace amount of the desired product (Table 6-1, entries 1-3). The production of aziridine, however, was tripled when the reaction was catalyzed by the Co complex of an electron-deficient porphyrin Co(TPFPP) (Table 6-1, entry 4). Significant further improvement was achieved with the Co complex of an electron-deficient and sterically hindered porphyrin Co(TDClPP) as the catalyst, producing the desired aziridine in 83% isolated yield (Table 6-1, entry 5). Whereas change in the ratio of styrene to bromamine-T from 1:2 to 1:1.2 had no significant influence on the catalytic reaction, an excess of styrene resulted in a

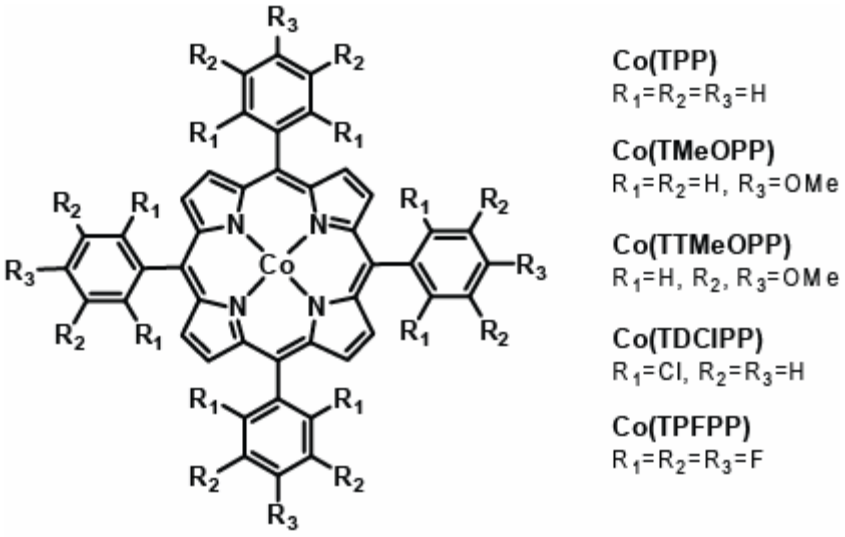


Figure 6-1. Structures of Cobalt (II) Porphyrin Complexes

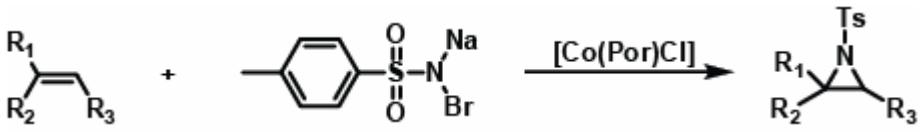


Figure 6-2. Aziridination of Alkenes with Bromamine-T Catalyzed by Cobalt (II) Porphyrin Complexes

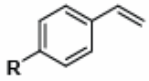
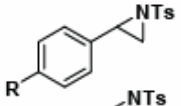
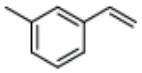
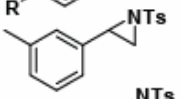
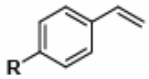
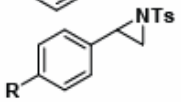
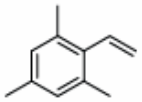
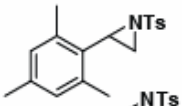
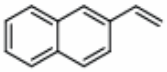
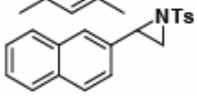
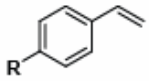
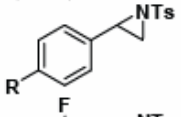
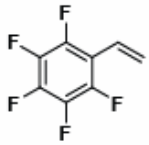
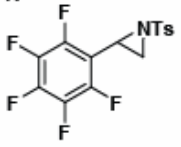
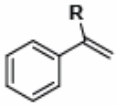
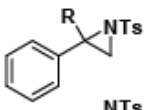
Table 6-1. Aziridination of Styrene by Cobalt Porphyrins[a]

Entry	S:BT[b]	[Co]Por[c]	Mol %	Solvent	Temp. (°C)	Time (h)	Yield (%)[d]
1	1:2	Co(TPP)	5	CH_3CN	23	18	18
2	1:2	Co(TMeOPP)	5	CH_3CN	23	16	<5
3	1:2	Co(TTMeOPP)	5	CH_3CN	23	20	0
4	1:2	Co(TPFPP)	5	CH_3CN	23	17	53
5	1:2	Co(TDClPP)	5	CH_3CN	23	18	83
6	1:1.2	Co(TDClPP)	5	CH_3CN	23	18	75
7	5:1	Co(TDClPP)	5	CH_3CN	23	17	67
8	1:2	Co(TDClPP)	5	THF	23	20	0
9	1:2	Co(TDClPP)	5	CH_2Cl_2	23	17	<5
10	1:2	Co(TDClPP)	5	$CH_3C_6H_5$	23	19	0
11	1:2	Co(TDClPP)	5	CH_3CN	40	17	84
12	1:2	Co(TDClPP)	5	CH_3CN	82	18	66
13	1:2	Co(TDClPP)	2	CH_3CN	23	17	80
14	1:2	Co(TDClPP)	5	CH_3CN	23	7	71
15	1:2	Co(TDClPP)	10	CH_3CN	23	7	82

[a] Carried out under N_2 in the presence of 5 Å molecular sieves with a concentration of 0.1 mmol styrene/2 mL of solvent. [b] The mole ratio of styrene substrate to bromamine-T. [c] See Figure 6-1. [d] Isolated Yields.

relatively lower yield (Table 6-1, entries 6 and 7). Acetonitrile appeared the solvent of choice for the catalytic reaction, as the uses of other solvents such as tetrahydrofuran, methylene chloride, or toluene gave no or only a trace amount of the desired product (Table 6-1, entries 8-10). Although a slightly better yield was obtained at 40°C, further increase in reaction temperature caused a lower yield (Table 6-1, entries 11 and 12). The room temperature reaction could be effectively carried out at a lower catalyst loading without affecting the yield (Table 6-1, entry 13). A relatively lower yield was observed when the reaction time was shortened (Table 6-1, entry 14). The employment of higher catalyst loading, however, could allow the reaction to be finished in a short time without decrease of the yield (Table 6-1, entry 15). Using the above optimized reaction conditions, the new Co(TDClPP)-based catalytic system was found to be suitable for many different types of alkene substrates (Table 6-2). In addition to styrene, derivatives of styrene with alkyl substituents could be equally aziridinated to afford the desired products in high yields (Table 6-2, entries 1-4). Functional groups in styrene derivatives could be well tolerated to cleanly generate the corresponding aziridines (Table 6-2, entries 5-7). Sterically hindered derivatives such as 2,4,6-trimethylstyrene as well as 2-vinylnaphthalene could also be catalytically aziridinated, albeit in lower yields (Table 6-2, entries 8 and 9). Halogenated styrenes including highly electron-deficient pentafluorostyrene could be successfully converted to the desired aziridines in good to high yields (Table 6-2, entries 10-13). To the best of our knowledge, this represents the first example of aziridination of pentafluorostyrene. In addition, both R-substituted and *α*-substituted (cyclic and acyclic) styrenes were suitable substrates for the catalytic process (Table 6-2, entries 14-21). Whereas a competitive amidation was presumably

Table 6-2. Aziridination of Different Alkenes by Co(TDClPP)

Entry	Substrate	Product	Yield (%)[b]
1			R = H: 83
2			R = Me: 76
3			R = t-Bu: 81
4			89
5			R = CH_2Cl: 86
6			R = CF_3: 90
7			R = CO_2CH_3: 92
8			61
9			53
10			R = Br: 70
11			R = Cl: 71
12			R = F: 86
13			61
14			R = Me: 70
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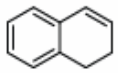
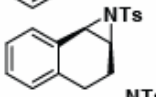
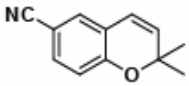
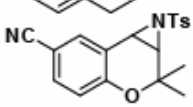
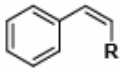
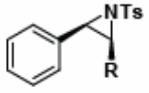
16			33
17[c]			66
18			R = Me: 94[e]
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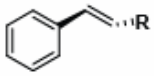
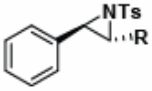
20			R = Me: 87[g]
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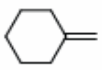
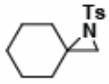
25			56
26			67

[a] Carried out at room temperature in CH_3CN overnight under N_2 with alkenes as limiting reagent (alkene/bromamine-T = 1:2) using 5 mol% Co(TDClPP) in the presence of 5 Å molecular sieves at a concentration of 0.2 mmol/4-5 mL of CH_3CN. [b] Isolated Yields. [c] Performed with alkene/bromamine-T = 5:1. [d] Performed with alkene/bromamine-T = 1:3 using 10 mol% Co(TDClPP). [e] *cis*:*trans* = 9:91. [f] *cis*:*trans* = 47:53. [g] *cis*:*trans* = 8:92. [h] *cis*:*trans* = 58:42.

responsible for the low aziridination yield of 1,2-dihydronaphthalene (Table 6-2, entry 16), excellent yields were obtained for acyclic *α*-substituted styrenes in both *cis*- and *trans*- forms (Table 6-2, entries 18-21). For the latter substrates, the catalytic reactions by Co(TDClPP) appeared to lack stereospecificity, although high *trans*-stereoselectivity was observed for both *cis*- and *trans*-*α*-methylstyrenes. In addition to aromatic and conjugated alkenes, acyclic and cyclic aliphatic alkenes with different ring sizes are suitable substrates for the catalytic system (Table 6-2, entries 22-25). Under similar conditions, *exo*methylene carbocycles such as methylenecyclohexane could also be successfully aziridinated to afford the desired spirocyclic aziridine in 67% isolated yield (Table 6-2, entry 26).

In summary, we have developed the first Co-based catalytic system that is efficient for aziridination of various alkenes.[115-117] We demonstrated that the new Co(TDClPP)/bromamine-T catalytic system can be effectively operated under mild and practical conditions with alkenes as limiting reagents and is generally suitable for a wide variety of alkene substrates. Further improvement of the catalytic system including stereospecificity and the development of its asymmetric variant are in progress. The successful demonstration of the catalytic capability of Co(II) porphyrins for aziridination will also likely stimulate future studies to address some interesting mechanistic issues.

Experimental Section

General Considerations. All reactions were carried out under nitrogen atmosphere in an oven dried Schlenk tube. All olefins were purchased from Acros or Aldrich Chemicals and used without further purification. Acetonitrile was dried with

calcium hydride in reflux. All metalloporphyrins were purchased from Strem or Midcentury Chemicals. Bromamine-T was prepared from Chloramine-T according to the literature procedure and dried at 80 ^{o}C in vacuum overnight before use.[96-98] Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Varian Mercury 300 spectrometer and referenced with respect to residual solvent. Infrared spectra were obtained using a Bomen B100 Series FT-IR spectrometer. Samples were prepared as films on a NaCl plate with chloroform as solvent. Thin layer chromatography was carried out on E. Merck Silica Gel 60 F-254 TLC plates.

General Procedure for Aziridination of Alkenes. An oven dried Schlenk tube equipped with stirring bar was degassed on vacuum line and purged with nitrogen. The tube was charged with metalloporphyrin (5 mol %), Bromamine-T (0.4 mmol) and activated 5 Å molecular sieves (500 mg). The tube was capped with a Teflon screw cap, evacuated on vacuum line for 30-45 min. The Teflon screw cap was replaced with a rubber septum and 3-5 mL of solvent and substrate (0.2 mmol) were then added successively. The tube was purged with nitrogen for 1-2 min and the contents were stirred overnight at ambient temperatures. After completion of the reaction, molecular sieves were removed by filtration and the filtrate was concentrated under vacuum. The solid residue was purified by flash chromatography (silica gel, ethyl acetate:hexanes (V:V) = 3:7) to afford the pure product.

***N*-(*p*-Tolylsulfonyl)-2-phenylaziridine.**[63, 113] (Table 6-2, Entry 1) was synthesized from styrene as substrate and the product obtained as a yellow oil (45 mg, yield 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, 2H, *J* = 8.1Hz), 7.17-7.31 (m, 7H), 3.73 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.94 (d, 1H, *J* = 7.2 Hz), 2.39 (s, 3H), 2.35 (d, 1H, *J* = 4.5

Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.6, 140.5, 135.0, 134.8, 129.7, 128.5, 128.3, 127.9, 126.5, 41.0, 35.9, 21.6. FT-IR (film, cm^{-1}): 3064, 3034, 2924, 2855, 1597, 1495, 1459, 1385, 1324,1292, 1232, 1190, 1160, 978, 909, 815, 775, 758, 715, 696, 665.

***N*-(*p*-Tolylsulfonyl)-2-(*p*-methylphenyl)aziridine.**[63, 113] (Table 6-2, Entry 2) was synthesized from *p*-methylstyrene as substrate and the product obtained as a yellow oil (43 mg, yield 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.1 Hz), 7.31 (d, 2H, *J* = 8.4. Hz), 7.08 (s, 4H), 3.73 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.95 (d, 1H, *J* = 7.2 Hz), 2.41 (s, 3H), 2.36 (d, 1H, *J* = 4.5 Hz), 2.29 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.6, 140.5, 138.1, 134.9, 131.9, 129.7, 129.2, 127.9, 126.4, 41.0, 35.8, 21.6, 21.1. FT-IR (film, cm^{-1}): 2922, 2857, 1597, 1517, 1494, 1453, 1381, 1324, 1292, 1232, 1186, 1160, 1118, 1093, 1019, 979, 911, 815, 731, 716, 693, 665.

***N*-(*p*-Tolylsulfonyl)-2-(*p*-*tert*-butylphenyl)aziridine.**[113] (Table 6-2, Entry 3) was synthesized from *p*-*tert*-butylstyrene as substrate and the product obtained as a yellow oil (53 mg, yield 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.1 Hz), 7.29-7.33 (m, 4H), 7.13 (d, 2H, *J* = 8.1 Hz), 3.75 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.94 (d, 1H, *J* = 7.2 Hz), 2.42 (s, 3H), 2.37 (d, 1H, *J* = 4.8 Hz), 1.28 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.4, 147.4, 144.6, 134.9, 131.9, 129.7, 127.9, 126.2, 125.8, 40.9, 35.8, 34.5, 31.2, 21.6. FT-IR (film, cm^{-1}): 3281, 2962, 2869, 1688, 1599, 1511, 1459, 1410, 1332, 1271, 1161, 1093, 1019, 958, 834, 757, 725, 705, 663.

***N*-(*p*-Tolylsulfonyl)-2-(3-methylphenyl)aziridine.**[113, 118] (Table 6-2, Entry 4) was synthesized from 3-methylstyrene as substrate and the product obtained as a yellow oil (51 mg, yield 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.4 Hz), 7.31 (d, 2H*J* = 8.7. Hz), 7.01-7.16 (m, 4H), 3.73 (dd, 1H, *J* = 7.2, 4.5 Hz), 2.94 (d, 1H, *J* = 6.9 Hz),

2.42 (s, 3H), 2.36 (d, 1H, *J* = 4.5 Hz), 2.29 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.6, 138.3, 134.8, 129.7, 129.0, 128.4, 127.9, 127.1, 123.6, 41.0, 35.9, 21.6, 21.2. FT-IR (film, cm^{-1}): 3292, 2922, 2362, 1688, 1596, 1492, 1453, 1382, 1325, 1292, 1215, 1184, 1160, 1093, 1039, 1019, 981, 929, 867, 816, 787, 720, 692, 666.

***N*-(*p*-Tolylsulfonyl)-2-(*p*-chloromethylphenyl)aziridine.**[99, 113] (Table 6-2, Entry 5) was synthesized from *p*-vinylbenzyl chloride as substrate and the product obtained as a yellow oil (55 mg, yield 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.4 Hz), 7.30 (m, 4H), 7.20 (d, 2H, *J* = 7.8 Hz), 4.54 (s, 2H), 3.76 (dd, 1H, *J* = 4.5, 7.2 Hz), 3.00 (d, 1H, *J* = 6.9 Hz), 2.44 (s, 3H), 2.36 (d, 1H, *J* = 4.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.3, 135.4, 129.8, 128.8, 128.0, 127.9, 126.9, 45.7, 40.5, 36.0, 21.6. FT-IR (film, cm^{-1}): 2362, 1596, 1519, 1493, 1453, 1383, 1324, 1268, 1187, 1161, 1093, 1019, 981, 909, 815, 750, 715, 674.

***N*-(*p*-Tolylsulfonyl)-2-(4-trifluoromethylphenyl)aziridine.**[113, 118] (Table 6-2, Entry 6) was synthesized from 4-(trifluoromethyl)styrene as substrate and the product obtained as a yellow oil (61 mg, yield 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 8.1 Hz), 7.54 (d, 2H, *J* = 8.1 Hz), 7.34 (d, 4H, *J* = 8.1 Hz), 3.80 (dd, 1H, *J* = 4.2, 7.2 Hz), 3.01 (d, 1H, *J* = 7.2 Hz), 2.44 (s, 3H), 2.37 (d, 1H, *J* = 4.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.4, 145.0, 139.2, 134.6, 129.8, 127.9, 126.9, 125.6, 125.5, 125.4, 40.1, 36.2, 21.6. ^{19}F NMR (75 MHz, $CDCl_3$): -63.1. FT-IR (film, cm^{-1}): 2926, 2362, 1621, 1597, 1494, 1454, 1421, 1383, 1325, 1233, 1163, 1121, 1093, 1067, 1018, 982, 911, 840, 817, 751, 697, 661.

***N*-(*p*-Tolylsulfonyl)-2-(4-acetoxylphenyl)aziridine.**[88, 101] (Table 6-2, Entry 7) was synthesized from 4-acetoxystyrene as substrate and the product obtained as a

yellow oil (61 mg, yield 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 8.1 Hz), 7.33 (d, 2H, *J* = 8.1 Hz), 7.21 (d, 4H, *J* = 8.4 Hz), 7.01 (d, 4H, *J* = 8.4 Hz), 3.75 (dd, 1H, *J* = 4.2, 7.2 Hz), 2.97 (d, 1H, *J* = 7.2 Hz), 2.43 (s, 3H), 2.35 (d, 1H, *J* = 4.5 Hz), 2.27 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.4, 150.5, 144.7, 134.7, 132.6, 129.8, 127.9, 127.6, 121.7, 40.4, 36.0, 21.6, 21.0. FT-IR (film, cm^{-1}): 3583, 2925, 1762, 1597, 1510, 1453, 1371, 1325, 1194, 1162, 1093, 1016, 982, 911, 852, 816, 724, 710, 694, 666.

***N*-(*p*-Tolylsulfonyl)-2-(2',4',6'-trimethylphenyl)aziridine.**[113] (Table 6-2, Entry 8) was synthesized from 2,4,6-trimethylstyrene as substrate and the product obtained as a yellow oil (39 mg, yield 61%). ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.88 (d, 2H, *J* = 8.4 Hz), 7.35 (d, 2H, *J* = 7.8 Hz), 6.79 (s, 2H), 3.86 (t, 1H, *J* = 4.8 Hz), 2.93 (d, 1H, *J* = 7.5 Hz), 2.46 (s, 3H), 2.31 (s, 6H), 2.24 (s, 3H), 2.16 (d, 1H, *J* = 4.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.9, 139.8, 137.4, 135.2, 129.7, 129.1, 128.8, 128.2, 127.1, 38.9, 35.4, 21.7, 20.9, 20.1. FT-IR (film, cm^{-1}): 3583, 3282, 2923, 1711, 1610, 1449, 1331, 1160, 1092, 934, 852, 814, 754, 665.

***N*-(*p*-Tolylsulfonyl)-2-(2-naphthyl)aziridine.**[81, 113] (Table 6-2, Entry 9) was synthesized from 2-vinylnapthalene as substrate and the product obtained as a yellow oil (34 mg, yield 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (d, 2H, *J* = 8.1 Hz), 7.71-7.79 (m, 4H), 7.46 (m, 2H), 7.30 (d, 2H, *J* = 8.4 Hz), 7.26 (s, 1H), 3.80 (d, 1H, *J* = 7.2, 4.5 Hz), 3.05 (d, 1H, *J* = 7.2 Hz), 2.47 (d, 1H, *J* = 4.5 Hz), 2.40 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.7, 134.9, 133.1, 133.0, 132.4, 129.8, 128.5, 127.9, 127.8, 127.7, 126.4, 126.3, 126.1, 123.6, 41.3, 36.0, 21.6. FT-IR (film, cm^{-1}): 3583, 3056, 2923, 2362, 1685, 1597, 1509, 1453, 1398, 1324, 1160, 1093, 1019, 953, 920, 858, 816, 751, 722, 666.

***N*-(*p*-Tolylsulfonyl)-2-(*p*-bromophenyl)aziridine.**[100, 113] (Table 6-2, Entry 10) was synthesized from 4-bromostyrene as substrate and the product obtained as a yellow oil (49 mg, yield 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, 2H, *J* = 8.4 Hz), 7.40 (d, *J* = 8.4 Hz), 7.31 (d, 2H, *J* = 8.4 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 3.70 (dd, 1H, *J* = 4.2, 7.2 Hz), 2.95 (d, 1H, *J* = 7.2 Hz), 2.42 (s, 3H), 2.32 (d, 1H, *J* = 4.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.8, 134.7, 134.1, 131.7, 129.7, 128.2, 127.9, 122.3, 40.2, 35.9, 21.6. FT-IR (film, cm^{-1}): 2922, 1491, 1453, 1407, 1377, 1325, 1229, 1186, 1161, 1115, 1093, 1071, 1012, 981, 910, 816, 769, 727, 705, 693, 665.

***N*-(*p*-Tolylsulfonyl)-2-(*p*-chlorophenyl)aziridine.**[63, 113] (Table 6-2, Entry 11) was

synthesized from 4-chlorostyrene as substrate and the product obtained as a yellow oil (44 mg, yield 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 8.1 Hz), 7.27 (d, 2H, *J* = 8.7 Hz), 7.16 (d, 2H, *J* = 8.4 Hz), 3.73 (dd, 1H, *J* = 4.2, 7.2 Hz), 2.97 (d, 1H, *J* = 6.9 Hz), 2.44 (s, 3H), 2.34 (d, 1H, *J* = 4.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.8, 134.7, 134.1, 133.6, 129.8, 128.7, 127.9, 40.2, 36.0, 21.6. FT-IR (film, cm^{-1}): 2924, 2361, 1597, 1494, 1454, 1414, 1378, 1325, 1306, 1230, 1188, 1162, 1092, 1016, 981, 911, 816, 776, 729, 708, 694, 669.

***N*-(*p*-Tolylsulfonyl)-2-(*p*-fluorophenyl)aziridine.**[100, 113] (Table 6-2, Entry 12) was synthesized from 4-fluorostyrene as substrate and the product obtained as a yellow oil (50 mg, yield 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, 2H, *J* = 8.1 Hz), 7.31 (d, 2H, *J* = 8.4 Hz), 7.16 (m, 2H), 6.96 (m, 2H), 3.74 (dd, 1H, *J* = 4.5, 7.2 Hz), 2.94 (d, 1H, *J* = 7.2 Hz), 2.42 (s, 3H), 2.32 (d, 1H, *J* = 4.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.8, 134.7, 130.7, 129.8, 128.3, 128.2, 127.9, 115.7, 115.4, 40.2, 36.0, 21.6. FT-IR (film,

cm^{-1}): 3583, 3069, 2924, 1600, 1513, 1454, 1379, 1325, 1235, 1188, 1162, 1093, 1017, 982, 912, 839, 818, 720, 692, 665.

***N*-(*p*-Tolylsulfonyl)-2-(pentafluorophenyl)aziridine.**[119, 120] (Table 6-2, Entry 13) was synthesized from pentafluorostyrene as substrate and the product obtained as a yellow oil (45 mg, yield, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, 2H, *J* = 8.1 Hz), 7.34 (d, 2H, *J* = 8.1 Hz), 3.77 (dd, 1H, *J* = 4.5, 7.2 Hz), 3.01 (d, 1H, *J* = 7.2 Hz), 2.77 (d, 1H, *J* = 4.2 Hz), 2.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.2, 134.1, 129.8, 128.3, 32.4, 31.9, 21.7. ^{19}F NMR (75 MHz, $CDCl_3$): -142.6, -153.1, -161.8. FT-IR (film, cm^{-1}): 2926, 1656, 1597, 1525, 1504, 1456, 1379, 1333, 1307, 1230, 1186, 1164, 1131, 1093, 1023, 975, 943, 873, 816, 778, 747, 711, 696, 673.

***N*-(*p*-Tolylsulfonyl)-2-methyl-2-phenylaziridine.**[63, 113] (Table 6-2, Entry 14) was synthesized from α-methylstyrene as substrate and the product obtained as a yellow oil (42 mg, yield, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, 2H, *J* = 8.4 Hz), 7.28-7.36 (m, 7H), 2.95 (s, 1H), 2.51 (s, 1H), 2.42 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 141.0, 137.6, 129.5, 128.4, 127.7, 127.5, 126.5, 51.8, 41.8, 21.6, 20.9. FT-IR (film, cm^{-1}): 3276, 2362, 1598, 1495, 1447, 1326, 1159, 909, 815, 765, 703, 666.

***N*-(*p*-Tolylsulfonyl)-2,2-diphenylaziridine.**[84, 113] (Table 6-2, Entry 15) was synthesized from 1,1-diphenylethylene as substrate and the product obtained as a yellow oil (57 mg, yield 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (d, 2H, *J* = 7.8 Hz), 7.00-7.14 (m, 12H), 2.83 (s, 2H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.4, 129.7, 129.4, 128.8, 128.6, 128.2, 128.1, 127.9, 40.8, 21.6. FT-IR (film, cm^{-1}): 3273, 3061, 2362, 1722, 1597, 1557, 1542, 1492, 1448, 1398, 1327, 1279, 1159, 1090, 1020, 943, 917, 814, 756, 700, 673.

***N*-(*p*-Tolylsulfonyl)amino-1,2,3,4-tetrahydronapthalene-1,2-imine.**[63, 113] (Table 6-2, Entry 16) was synthesized from 1,2-dihydronapthalene as substrate and the product obtained as a yellow oil (20 mg, yield 33%). ^{1}H NMR (300 MHz, CDCl3): ẟ7.82 (d, 2H, *J* = 8.1 Hz), 7.30 (d, 2H, *J* = 8.1 Hz), 7.03-7.25 (m, 4H), 3.81 (d, 1H, *J* = 7.2 Hz), 3.55 (d, 1H, *J* = 6.9 Hz), 2.72 (dt, 1H, *J* = 13.5, 6.3 Hz), 2.5 (dd, 1H, *J* = 13.5, 5.4 Hz), 2.42 (s, 3H), 2.24 (dd, 1H, *J* = 14.8, 6.3 Hz), 1.62 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144, 136.6, 135.5, 129.7, 129.4, 128.5, 128.4, 127.6, 126.3, 114.3, 42.0, 41.7, 24.7, 21.6, 19.9. FT-IR (film cm^{-1}): 3585, 3026, 2925, 2854, 1598, 1494, 1433, 1398, 1321, 1229, 1157, 1091, 1028, 989, 945, 908, 877, 814, 754, 731, 715, 670.

2,2-Dimethyl-1-(toluene-4-sulfonyl)-1,1a,2,7b-tetrahydro-3-oxa-1-azacyclo-propa[a]naphthalene-6-carbonitrile.[62, 82, 113] (Table 6-2, Entry 17) was synthesized from 2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (185 mg, 1.0 mmol) and bromamine-T (54.4 mg, 0.2 mmol) and the product obtained as a yellow oil (47 mg, yield 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 2H, *J* = 8.1 Hz), 7.53 (d, 1H, *J* = 1.8 Hz), 7.46 (dd, 2H, *J* = 8.4, 1.8 Hz), 7.31 (d, 2H, *J* = 8.1 Hz), 6.80 (d, 1H, *J* = 8.4 Hz), 3.86 (d, 1H, *J* = 7.2 Hz), 3.35 (d, 1H, *J* = 7.5 Hz), 2.43 (s, 3H), 1.29 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.2, 134.4, 134.2, 133.2, 129.8, 128.0, 119.3, 119.2, 104.7, 73.2, 49.2, 38.7, 25.8, 23.8, 21.7. FT-IR (film, cm^{-1}): 2980, 2925, 2854, 2360, 2227, 1615, 1598, 1579, 1492, 1461, 1328, 1276, 1251, 1208, 1159, 1092, 1025, 962, 935, 874, 850, 833, 784, 768, 717, 674.

***trans*-*N*-(*p*-Tolylsulfonyl)-2-methyl-3-phenylaziridine.**[63, 113] (Table 6-2, Entry 18) was synthesized from *cis*-β-methystyrene as substrate and the product obtained as a yellow oil (54 mg, yield 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.4 Hz),

7.24-7.30 (m, 5 H), 7.15-7.19 (m, 2H), 3.81 (d, 1H, *J* = 4.2 Hz), 2.93 (dq, 1H, *J* = 6.3, 4.8 Hz), 2.40 (s, 3H), 1.85 (d, 3H, *J* = 5.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.9, 137.8, 135.5, 129.5, 128.5, 128.0, 127.1, 126.2, 49.1, 49.0, 21.5, 14.1. FT-IR (film, cm^{-1}): 2929, 1598, 1497, 1455, 1413, 1383, 1321, 1239, 1205, 1184, 1159, 1091, 1059, 1037, 971, 890, 815, 749, 697, 685.

***(cis- & tran)-N-(p-*Tolylsulfonyl)-2,3-diphenylaziridine.**[63, 113]* (Table 6-2, Entry 19) was synthesized with *cis*-stilbene (36 μL, 0.2 mmol) with bromamine-T (163.0 mg, 0.6 mmol) in the presence of 10 mole% Co(DClTPP) (19 mg, 0.2 mmol), and the products obtained as a mixture of *cis*- and *trans*- aziridine (65 mg, yield 92%). For *cis*-*N*-(*p*-Tolylsulfonyl)-2,3-diphenylaziridine ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, *J* = 8.4 Hz), 7.47 (d, 2H, *J* = 8.7 Hz), 7.03-7.13 (m, 10H), 4.23 (s, 2H), 2.45 (s, 3H). For *trans*-*N*-(*p*-Tolylsulfonyl)-2,3-diphenylaziridine ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, 2H, *J* = 8.4 Hz), 7.34-7.44 (m, 10H), 7.20 (d, 2H, *J* = 8.4 Hz), 4.27 (s, 2H), 2.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.7, 143.9, 137.3, 137.0, 134.8, 133.0, 132.0, 129.8, 129.4, 128.6, 128.4, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 126.5, 50.3, 47.4, 21.7, 21.6. FT-IR (film, cm^{-1}): 3061, 3031, 2923, 2854, 1598, 14957, 1452, 1401, 1327, 1160, 1090, 1026, 908, 813, 785, 760,697, 674.

***trans-N-(p-*Tolylsulfonyl)-2-methyl-3-phenylaziridine.**[63, 113]* (Table 6-2, Entry 20) was synthesized from *trans*-β-methystyrene as substrate and the product obtained as a yellow oil (51 mg, yield 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.4 Hz), 7.24-7.30 (m, 5 H), 7.15-7.19 (m, 2H), 3.81 (d, 1H, *J* = 4.2 Hz), 2.93 (dq, 1H, *J* = 6.3, 4.8 Hz), 2.40 (s, 3H), 1.85 (d, 3H, *J* = 5.7 Hz). ^{13}C NMR(75 MHz, $CDCl_3$): δ 143.9, 137.8, 135.5, 129.5, 128.5, 128.0, 127.1, 126.2, 49.1, 49.0, 21.5, 14.1. FT-IR (film, cm^{-1}

^{1}): 3583, 2928, 1598, 1497, 1456, 1413, 1383, 1320, 1238, 1205, 1184, 1159, 1091, 1059, 1037, 971, 890, 815, 748, 697, 685, 665.

(cis- & trans)-N-(p-Tolylsulfonyl)-2,3-diphenylaziridine.[63, 113] (Table 6-2, Entry 21) was synthesized with *trans*-stilbene (36 mg, 0.2 mmol) with bromamine-T (163.0 mg, 0.6 mmol) in the presence of 10 mole% Co(DClTPP) (19 mg, 0.2 mmol), and the products obtained as a mixture of *cis*- and *trans*- aziridine (66 mg, yield 94%). For *cis*-*N*-(*p*-Tolylsulfonyl)-2,3-diphenylaziridine ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, *J* = 8.4 Hz), 7.47 (d, 2H, *J* = 8.7 Hz), 7.03-7.13 (m, 10H), 4.23 (s, 2H), 2.45 (s, 3H). For *trans*-*N*-(*p*-Tolylsulfonyl)-2,3-diphenylaziridine ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, 2H, *J* = 8.4 Hz), 7.34-7.44 (m, 10H), 7.20 (d, 2H, *J* = 8.4 Hz), 4.27 (s, 2H), 2.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.7, 143.9, 137.3, 137.0, 134.8, 133.0, 132.0, 129.8, 129.4, 128.6, 128.4, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 126.5, 50.3, 47.4, 21.7, 21.6. FT-IR (film, cm^{-1}): 3583, 3273, 3062, 3032, 1723, 1598, 1495, 1451, 1399, 1327, 1160, 1092, 1025, 906, 813, 785, 752, 699, 669.

N-(p-Tolylsulfonyl)-6-(azabicyclo)[3.1.0]hexane. (Table 6-2, Entry 22) was synthesized from cyclopentene as substrate and the product obtained as a yellow oil (29 mg, yield 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 2H, J = 8.4 Hz), 7.31 (d, 2H, *J* = 8.1 Hz), 3.33 (s, 2H), 2.44 (s, 3H), 1.95 (m, 2H), 1.61 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.1, 135.9, 129.6, 127.6, 46.7, 26.9, 21.6, 19.5. FT-IR (film, cm^{-1}): 3271, 2958, 2925, 2854, 1598, 1494, 1439, 1367, 1320, 1303, 1157, 1093, 1075, 1009, 977, 931, 874, 832, 815, 723, 673.

N-(p-Tolylsulfonyl)-7-azabicyclo[4.1.0]heptane.[64, 113] (Table 2, Entry 23) was synthesized from cyclohexene as substrate and the product obtained as a yellow oil (33

mg, yield 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, 2H, *J* = 7.8 Hz), 7.30 (d, 2 H, *J* = 8.1 Hz), 2.96 (s, 2H), 2.42 (s, 3H), 1.77 (m, 4H), 1.39 (m, 2H), 1.20 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 143, 141, 129, 127, 39.3, 22.3, 21.9, 18.9. FT-IR (film, cm^{-1}): 3280, 2927, 2856, 2361, 2340, 1598, 1494, 1440, 1399, 1320, 1239, 1184, 1157, 1091, 1020, 965, 921, 847, 816, 793, 724, 667.

***N*-(*p*-Tolylsulfonyl)-9-azabicyclo[6.1.0]nonane.**[63, 113] (Table 6-2, Entry 24) was synthesized from cyclooctene as substrate and the product obtained as a yellow oil (44 mg, yield 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, 2H, *J* = 8.1 Hz), 7.31 (d, 2H, *J* = 7.8 Hz), 2.76 (d, 1H, *J* = 9.9 Hz), 2.43 (s, 3H), 1.99 (d, 1H, *J* = 14.1 Hz), 1.55-1.23 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.0, 135.8, 129.6, 127.5, 43.9, 26.3, 26.1, 25.2, 21.6. FT-IR (film cm^{-1}): 2927, 2857, 1598, 1494, 1468, 1449, 1425, 1320, 1158, 1092, 1016, 985, 933, 889, 869, 856, 825, 815, 796, 764, 750, 720, 668.

***N*-(*p*-Tolylsulfonyl)-2-heptylaziridine.**[104, 113] (Table 6-2, Entry 25) was synthesized from 1-nonene as substrate and the product obtained as a yellow oil (33 mg, yield 56%). ^{1}H NMR (300 MHz, CDCl3): δ 7.82 (d, 2H, *J* = 6.6 Hz), 7.33 (d, 2H, *J* = 8.1 Hz), 2.68 (m, 1H), 2.63 (d, 1H, *J* = 6.9 Hz), 2.44 (s, 3H), 2.05 (d, 1H, *J* = 4.2 Hz), 1.2 (m, 12H), 0.87 (t, 3H, *J* = 6.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.4, 129.6, 128.0, 40.5, 33.8, 31.6, 31.3, 29.1, 28.9, 26.8, 22.6, 21.6, 14.1. FT-IR (film, cm^{-1}): 2926, 2856, 1725, 1598, 1494, 1458, 1401, 1325, 1232, 1161, 1092, 1020, 929, 869, 815, 768, 747, 715, 694, 662.

***N*-(*p*-Tolylsulfonyl)-1-azaspiro[2.5]octane.**[105, 113] (Table 6-2, Entry 26) was synthesized from methylene cyclohexane as substrate and the product obtained as a yellow oil (36 mg, yield 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 2H, *J* = 7.8 Hz),

7.30 (d, 2H, J = 8.1 Hz), 2.43 (s, 3H), 2.40 (s, 2H), 1.72-1.97 (m, 6H), 1.50 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.7, 137.7, 129.4, 127.3, 54.0, 41.0, 33.0, 25.4, 25.2, 21.6. FT-IR (film, cm^{-1}): 3287, 2934, 2857, 1598, 1495, 1448, 1386, 1318, 1252, 1209, 1158, 1129, 1092, 1002, 943, 867, 840, 816, 793, 723, 664.

Chapter 7: Cobalt-Catalyzed Intermolecular C-H Amination with Bromamine-T as a Nitrene Source

This Chapter is a lightly revised version of a paper by the same name published in the journal *Chemical Communications* in 2007 by authors Jeremiah D. Harden, Joshua V. Ruppel, Guanyo-Gao and X. Peter Zhang. The major changes include 1) the exclusion of the abstract to the paper, 2) the reformatting of selected text to fit the guidelines of this dissertation, 3) reproduction and reformatting of the figures and tables, and 4) combination of the references in this dissertation with those from the other chapters in this section.

Jeremiah D. Harden, Joshua V. Ruppel, Guang-Yao Gao and X. Peter Zhang. Cobalt Catalyzed Intermolecular C-H Amination with Bromamine-T as a Nitrene Source. *Chemical Communications* **2007**, 4644.

My use of we and us refers to the other coauthors and myself. My primary contributions to this paper include 1) synthesis of several of the porphyrin catalysts 2) synthesis of the Bromamine-T reagent 3) execution and analysis of all screening reactions, 4) execution and analysis of all substrate reactions, 5) characterization of all products, and 6) writing of the majority of the text.

Cobalt, supported by porphyrin ligands, is capable of catalyzing intermolecular nitrene insertion of sp^3 C–H bonds with bromamine-T as the nitrene source, forming the desired tosyl-protected amines with NaBr as the by-product. Nitrogen containing molecules are abundant in nature and their importance in biology and medicine has been widely documented. Among modern amination methods,[121-124] metal-catalyzed nitrene insertion provides an attractive strategy for direct transformation of ubiquitous C–H bonds to value-added amine functionalities through the use of appropriate nitrene sources.[54, 59, 60, 125] The reagent PhI=NTs and related iminoiodane derivatives have been widely used as primary nitrene sources,[54, 59, 60, 125-129] resulting in significant advances in amination of C–H bonds.[85, 89, 130-134] In view of some limitations associated with the use of the hypervalent iodine reagents, including their instability and the

generation of ArI as a byproduct, considerable efforts have been made to develop alternative nitrene sources for catalytic C–H amination. One such remarkably successful effort has been the approach of in situ iminoiodane generation from amides using terminal oxidants such as the combination of sulfamates with $PhI(OAc)_2$ or sulfonamides with PhI=O.[66, 126-128, 135-137] In addition, efforts have been devoted to employ other potential nitrene sources that obviate the need of terminal oxidants for the catalytic amination process, including chloramine-T,[134, 138] bromamine-T,[77] tosyloxycarbamates,[139-141] and azides.[81, 142] Using these nitrene sources, complexes of manganese, iron, ruthenium, rhodium, and copper supported by various ligands have been identified to catalyze C–H amination reactions, with dirhodium tetracarboxylates being the most widely used catalysts.[54, 59, 60, 66, 85, 89, 125-127, 129-137] In the light of various remaining challenges in intermolecular C–H amination,[54, 59, 60, 66, 85, 89, 125-127, 129-137] it is desirable to search for new catalytic systems based on other metal ions in combination with alternative nitrene sources. To this end, silver[143] - and cobalt[115, 144] -based systems were recently reported to aminate C–H bonds with PhI=NTs and aryl azides, respectively.

Attracted by their practical attributes as alternative nitrene sources, we recently initiated a research project on the development of catalytic nitrene transfer processes using bromamine-T and azides. In connection with our studies on catalytic carbene transfer reactions by cobalt porphyrins [Co(Por)],[93, 94, 112, 145, 146] we documented new Co-based catalytic systems that can catalyze aziridination of alkenes with diphenylphosphoryl azide (DPPA)[147] and bromamine-T.[148] The Co(Por)/bromamine-T system, which proved to be superior to the Fe(Por)Cl/bromamine-T system,[148] can be

effectively operated under mild conditions with alkenes as the limiting reagent, generating NaBr as the by-product, and is generally suitable for a wide range of alkene substrates. While the desired aziridines were formed in high yields for most of the substrates, low aziridination yields were obtained from certain substrates containing activated C–H bonds, such as 1,2-dihydronaphthalene. At that time, without being able to obtain concrete experimental data, we presumed a competitive amination reaction to be the cause of low yields. Since then, we have carried out a thorough study on the possibility of catalytic C–H amination by Co(Por) with bromamine-T as the nitrene source (Figure 7-1). Herein, we wish to report our findings.

Using indan as a model substrate, we systematically evaluated its catalytic C–H nitrene insertion with bromamine-T under different conditions by cobalt complexes of porphyrin ligands possessing varied electronic and steric environments (Figure 7-2). Selected results are summarized in Table 7-1. While most of the tested cobalt complexes exhibited poor catalytic activity toward C–H amination, the cobalt complex of electron-deficient porphyrin Co(TPFPP) was found to aminate one of the benzylic C–H bonds of indan effectively to form the desired product 2a in 66% yield at room temperature (Table 7-1, entries 9–13). Further experiments indicated the use of electron-deficient as well as more sterically demanding porphyrin TDClPP could further improve the cocatalyzed amination reaction, producing 2a in 75% yield (Table 7-1, entry 3). This reaction was performed with a 1a : bromamine-T ratio of 10 : 1 since the use of other reactant ratios appeared to give lower yields of 2a (Table 7-1, entries 1–3). Among the solvents examined, CH_3CN proved to be the best for the catalytic process (Table 7-1, entries 3–7). Although the employment of lower catalyst loading could result

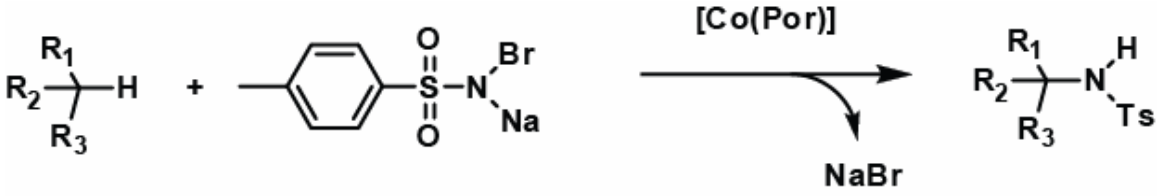


Figure 7-1. Cobalt Catalyzed C-H Amination with Bromamine-T

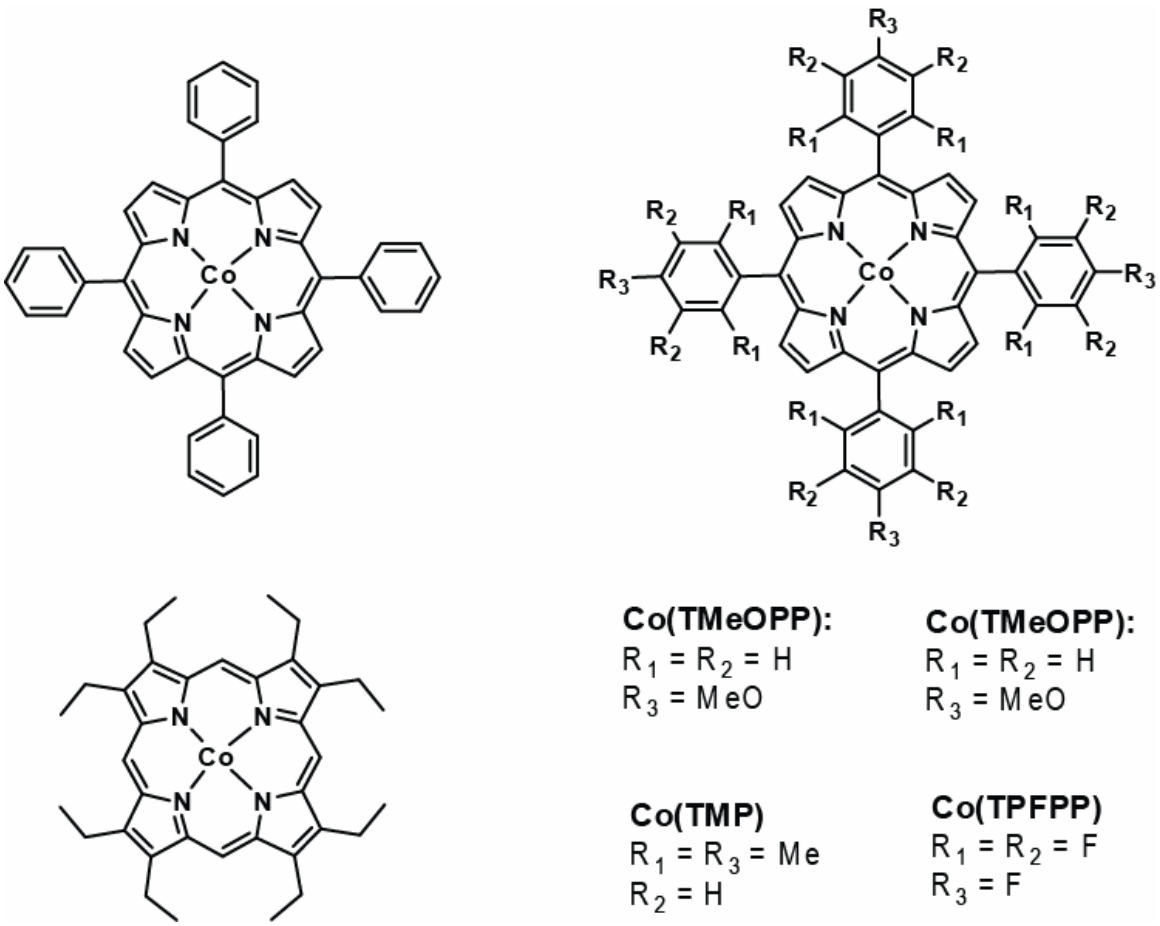
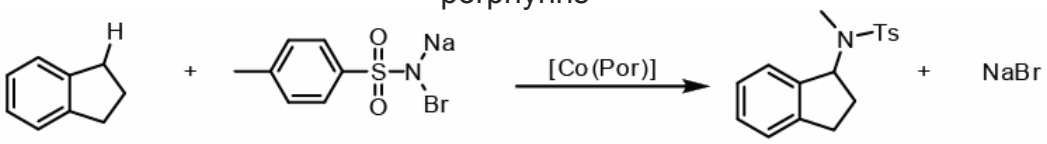


Figure 7-2. Structures of Cobalt (II) Complexes of Porphyrins

Table 7-1. Intermolecular C–H amination of indan with bromamine-T by cobalt(II) porphyrins[a]



Entry	S:BT[b]	[Co(Por)][c]	mol (%)	Solvent	Time (h)	Yield (%)[d]
1	1:1	Co(TDClPP)	5	CH_3CN	19	43
2	1:5	Co(TDClPP)	5	CH_3CN	17	30
3	10:1	Co(TDClPP)	5	CH_3CN	18	75
4	10:1	Co(TDClPP)	5	THF	15	15
5	10:1	Co(TDClPP)	5	Toluene	18	14
6	10:1	Co(TDClPP)	5	Chlorobenzene	20	12
7	10:1	Co(TDClPP)	5	Dichloromethane	18	<2
8	10:1	Co(TDClPP)	2	CH_3CN	20	15
9	10:1	Co(TPP)	5	CH_3CN	19	<2
10	10:1	Co(TMP)	5	CH_3CN	18	<2
11	10:1	Co(TPFPP)	5	CH_3CN	18	66
12	10:1	Co(OEP)	5	CH_3CN	18	7
13	10:1	Co(TMeOPP)	5	CH_3CN	20	20

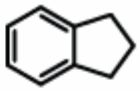
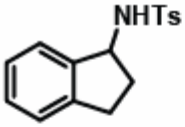
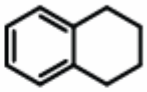
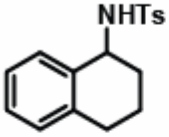
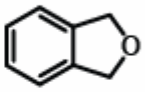
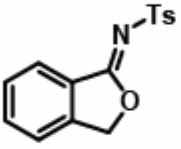
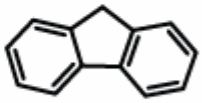
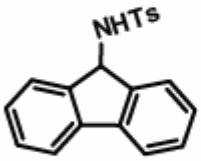
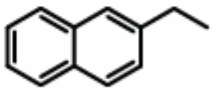
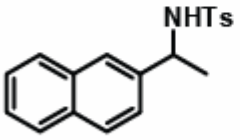
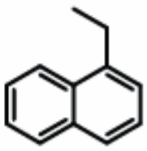
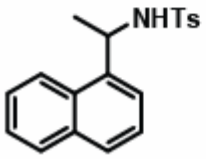
[a]Reactions were carried out at room temperature under N2 with bromamine-T in the presence of 5 Å molecular sieves at a concentration of 2.0 mmol substrate/3–5 mL solvent. [b]The mole ratio of indan substrate to bromamine-T. [c] See Fig. 7-1 for structures. [d] Yields represent GC yields using tridecane as a standard.

in the formation of the desired amination product (Table 7-1, entry 8), 5 mol% of Co(TDClPP) was needed for an effective transformation (Table 7-1, entry 3).

Under the optimized catalytic conditions derived from the above experiments for the amination of indan, the Co(TDClPP)/bromamine-T catalytic systems could also be applied to aminate other substrates at room temperature (Table 7-2). While the isolated yield of 2a was 73% (Table 7-2, entry 1), the corresponding amination product 2b from 1,2,3,4-tetrahydronaphthalene (1b) was isolated in 66% yield (Table 7-2, entry 2). In both of the cases, high chemoselectivity toward benzylic C–H bonds was observed. The benzylic C–H bond of the cyclic ether 1c seemed to be similarly aminated with bromamine-T. The reaction, however, didn't afford the corresponding amine product. Instead, the corresponding imine product 2c was isolated in 50%yield (Table 7-2, entry 3), presumably formed from the initial amine via a secondary reaction.[149] Although fluorene 1d contains more activated benzylic C–H bonds, its catalytic amination reaction by Co(TDClPP) gave the C–H insertion product 2d in a much lower yield (Table 7-2, entry 4). Steric hindrance is likely responsible for the adverse outcome. When substrates that contain both secondary benzylic and primary C–H bonds such as 1- and 2-ethylnaphthalene (1e and 1f) were used, the corresponding amination products of the benzylic C–H bonds 2e and 2f were produced as the major products, albeit in lower yields (Table 7-2, entries 5–6), affirming the high chemoselectivity towards benzylic C–H bonds.

In summary, we have demonstrated the capability of cobalt porphyrins in catalyzing intermolecular nitrene insertion of sp^3 C–H bonds with bromamine-T at room

Table 7-2. Co(TDClPP)-Catalyzed Intermolecular C–H Amination Reactions with Bromamine-T[a]

Entry	Substrate	Product	Yield
1	**1a** 	**2a** 	73
2	**1b** 	**2b** 	66
3	**1c** 	**2c** 	50
4	**1d** 	**2d** 	14
5	**1e** 	**2e** 	34
6	**1f** 	**2f** 	34

[a]Reactions were carried out at room temperature in CH_3CN under N_2 with bromamine-T as the limiting reagent (substrate/bromamine- T = 10 : 1) using 5 mol% Co(TDClPP) in the presence of 5 Å molecular sieves at a concentration of 2.0 mmol substrate/3–5 mL CH3CN. [b]Yields represent isolated yields in 95% purity as determined by ^{1}H NMR.

temperature. In addition to mild conditions, the cobalt-based catalytic system enjoys the use of bromamine-T as the nitrene source since it produces innocent NaBr as the by-product. Furthermore, the current Co(TDClPP)/ bromamine-T catalytic intermolecular amination system has high chemoselectivity towards benzylic C–H bonds. Efforts are underway to design and synthesize new porphyrin ligands to further improve the scope and efficacy of the Co-catalyzed intermolecular C–H amination system.

Experimental Section

General Considerations. All reactions were carried out under nitrogen atmosphere in an oven dried Schlenk tube. All alkanes were purchased from Acros or Aldrich Chemicals and used without further purification. Acetonitrile and methylene chloride were dried by refluxing over calcium hydride. Toluene and tetrahydrofuran were dried by refluxing over sodium benzophenone. Metalloporphyrins were purchased from Strem or Midcentury Chemicals. Bromamine-T was prepared from Chloramine-T according to the literature procedure and dried at 80ºC in vacuum overnight before use.[150] Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Varian Mercury 300 or Varian Inova400 spectrometer and referenced with respect to residual solvent. Infrared spectra were measured with a Nicolet Avatar 320 spectrometer with a Smart Miracle accessory. High resolution mass spec. (HRMS) data was obtained on an Agilent 1100 LC/MS ESI/TOF mass spectrometer with electrospray ionization. Thin layer chromatography was carried out on E. Merck Silica Gel 60 F-254 TLC plates.

General Procedure for Amination. An oven dried Schlenk tube equipped with a stirring bar was degassed on vacuum line and purged with nitrogen. The tube was

charged with metalloporphyrin (5 mol %), Bromamine-T (0.2 mmol) and activated 5 Å molecular sieves (200 mg). The tube was capped with a Teflon screw cap and evacuated on vacuum line for 30-45 min. The Teflon screw cap was replaced with a rubber septum and 0.5 mL of solvent and substrate (2 mmol) followed by the remaining solvent (total 2 mL) were then added successively. The tube was purged with nitrogen for 1-2 min and the contents were stirred overnight at ambient temperatures. After completion of the reaction, molecular sieves were removed by filtration and the filtrate was concentrated under vacuum. The residue was purified by flash chromatography, and the fractions containing product were collected and concentrated by rotary evaporation to afford the pure compound.

N-(p-Toluenesulfonyl)-1-aminoindan.[77, 89, 151-153] (Table 7-2, Entry 1) 1-5 was synthesized using indan as the substrate and the product was obtained as a tan-white solid (42.1 mg, 72.8%). ^{1}H NMR (300 MHz, CDCl3): δ 7.84 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.07-7.22 (m, 4H), 4.84 (dd, *J*1 = 7.5 Hz, *J*2 = 15.9 Hz, 1H), 4.71 (d, *J* = 8.7 Hz, 1H), 2.85-2.97 (m, 1H), 2.67-2.78 (m, 1H), 2.45 (s, 3H), 2.28-2.39 (m, 1H), 1.68-1.81 (m, 1H). 13C NMR (75 MHz, CDCl3): δ 143.43, 142.79, 141.96, 138.16, 129.75, 128.24, 127.10, 126.81, 124.76, 124.06, 58.68, 34.66, 29.92, 21.53. IR (neat, cm–1): 3257 (N-H), 1158 (S=O). HRMS (ESI): Calculated For C16H21N2O2S ([M+NH4]+) m/z 305.13183, Found 305.13292.

N-(p-Toluenesulfonyl)-1-amino-1,2,3,4-tetrahydronaphthalene.[73, 77, 150, 151] (Table 7-2, Entry 2) 1,2,4- 6 was synthesized using 1,2,3,4-tetrahydronaphthalene as the substrate and the product was obtained as a tan-white solid (40.1 mg, 66%) 1H NMR (300 MHz, CDCl3): δ 7.82 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 7.8 Hz, 2H), 7.12 (m,

2H), 7.05 (t, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 4.62 (d, *J* = 7.8 Hz, 1H), 4.44 (m, 1H), 2.71 (m, 2H), 2.46 (s, 3H), 1.72-1.86 (m, 4H). 13C NMR (75 MHz, CDCl3): δ 143.40, 137.55, 135.57, 129.76, 129.21, 128.76, 127.64, 127.14, 126.30, 51.89, 30.75, 28.86, 21.56, 19.08. IR (neat, cm–1) 3258 (N-H), 1153 (S=O). HRMS (ESI): Calculated for C17H19NO2SNa ([M+Na]+) m/z 324.10287, Found 324.10191.

N-(isobenzofuran-1(3H)-ylidene)-4-methylbenzenesulfonamide. (Table 7-2, Entry 3) was synthesized using phthalan as the substrate and the product was recovered as a dark tan solid (29.1 mg, 50%). ^{1}H NMR (400 MHz, CDCl3): δ 7.97 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.68 (t, *J* = 7.2 Hz, 1H), 7.53-7.46 (m, 2H), 7.31 (d, *J* = 7.6 Hz, 1H), 5.59 (s, 2H), 2.42 (s, 3H). 13C NMR (100 MHz, CDCl3): δ 134.51, 129.39, 129.25, 127.52, 125.66, 121.42, 76.25, 29.68. IR (neat, cm–1): 1634 (C=N), 1297 (C-O), 1152 (S=O). HRMS (ESI): Calculated for C15H14NO3S ([M+H]+) m/z 288.06889, Found 288.06848.

N-(p-Toluenesulfonyl)-1-aminofluorene.[154] (Table 7-2, Entry 4) 7 was synthesized using fluorene as the substrate and the product was recovered as a dark tan solid (9.4 mg, 14%). ^{1}H NMR (300 MHz, CDCl3): δ 7.97 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.42 (d, *J* = 7.8 Hz, 2H), 7.36 (m, 2H), 7.23 (m, 4H), 5.41 (d, *J* = 9.6 Hz, 1H), 4.75 (d, *J* = 9.3 Hz, 1H), 2.51 (s, 3H). 13C NMR (CDCl3): δ 144.06, 143.55, 138.60, 130.22, 129.24, 128.11, 127.58, 125.43, 120.19, 58.62, 21.89. IR (neat, cm–1): 3303 (N-H), 1155 (S=O). HRMS (ESI): Calculated for C20H21N2O2S ([M+NH4]+) m/z 353.13183, Found 353.13296.

N-(p-Toluenesulfonyl)-1-amino-2-ethylnaphthalene.[89, 150, 153, 155, 156] (Table 7-2, Entry 5) was synthesized using 2-ethylnaphthalene as the substrate and the product

was recovered as a dark tan solid (22.1 mg, 34%). ^{1}H NMR (300 MHz, CDCl3): δ 7.75 (t, *J*1 = 6 Hz, *J*2 = 3.3 Hz, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.57 (d, *J* = 10 Hz, 2H), 7.41-7.47 (m, 3H), 7.20 (d, *J* = 8.7 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 4.92 (d, *J* = 7.2 Hz, 1H), 4.64 (dt, *J*1 = 13.5 Hz, *J*2 = 6.6 Hz, 1H), 2.25 (s, 3H), 1.51 (d, *J* = 6.6 Hz, 3H). 13NMR (75 MHz, CDCl3): δ 143.11, 138.98, 137.50, 133.03, 132.67, 129.28, 128.44, 127.49, 127.06, 126.17, 125.96, 125.03, 124.03, 53.78, 23.44, 21.31. IR (neat, cm–1): 3267 (N-H), 1155 (S=O). HRMS (ESI): Calculated for C19H23N2O2S ([M+NH4]+) m/z 343.14748, Found 343.14775.

N-(p-Toluenesulfonyl)-1-amino-1-ethylnaphthalene.[153, 157] (Table 7-2, Entry 6) 5,10 was synthesized using 1-ethylnaphthalene as the substrate and the product was recovered as a dark tan solid (21.4 mg, 33%). ^{1}H NMR (300 MHz, CDCl3): δ 7.88 (d, *J* = 7.2 Hz, 1H), 7.80 (d, *J* = 6 Hz, 1H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.44 (m, 2H), 7.35 (m, 2H), 7.07 (d, *J* =8.1 Hz, 2H), 5.28 (dt, *J*1 = 13.5 Hz, *J*2 = 6.6 Hz, 1H), 5.0 (d, *J* = 6.9 Hz, 1H), 2.33 (s, 3H), 1.59 (d, *J* = 6.9 Hz, 3H). 13C NMR (75 MHz, CDCl3): δ 143.07, 137.56, 137.38, 133.77, 130.10, 129.26, 128.82, 128.14, 127.04, 126.27, 125.63, 125.21, 123.33, 122.59, 49.76, 23.20, 21.40. IR (neat, cm–1): 3268 (N-H), 1155 (S=O). HRMS (ESI): Calculated for C19H23N2O2S ([M+NH4]+) m/z 343.14748, Found 343.14831.

Chapter 8: Cobalt-Catalyzed Aziridination with Diphenylphosphoryl Azide (DPPA): Direct Synthesis of N- Phosphorous – Substituted Aziridines from Alkenes

This Chapter is a lightly revised version of a paper by the same name published in the *Journal of Organic Chemistry* in 2006 by Guang-Yao Gao, Jess E. Jones, Renu Vyas, Jeremiah D. Harden, and X. Peter Zhang. The major changes include 1) the exclusion of the abstract to the paper, 2) the reformatting of selected text to fit the guidelines of this work, 3) reproduction and reformatting of the figures and tables, and 4) combination of the references in this work with those from the other chapters in this section.

Guang-Yao Gao, Jess E. Jones, Renu Vyas, Jeremiah D. Harden, and X. Peter Zhang. Cobalt-Catalyzed Aziridination with Diphenylphosphoryl Azide (DPPA): Direct Synthesis of N-Phosphorous-Substituted Aziridines from Alkenes. *Journal of Organic Chemistry* **2006,** 71, 6655.

My use of we and us refers to the other coauthors and myself. My primary contributions to this paper include 1) synthesis of porphyrin catalyst, and 2) repetition and verification of select reactions.

Aziridines, the smallest nitrogen-containing heterocyclic compounds, have received considerable research interests because of their fundamental and practical importance.[55, 106-108] In addition to being an important motif in many biologically and pharmaceutically interesting compounds, aziridines are notably known as a class of versatile synthons for preparation of functionalized amines. Of known synthetic strategies, catalytic nitrene transfer from a suitable nitrene source to alkene substrates by transition metal complexes is considered the most attractive approach for the construction of the three-membered ring structures.[54, 56, 57] Compared to the considerable advances in analogous epoxidation and cyclopropanation reactions, catalytic aziridination is much less developed, presumably due to the lack of suitable nitrene sources. [*N*-(*p*-Toluenesulfonyl)imino]phenyliodinane (PhI=NTs) has been extensively used as the primary nitrene source for metal-catalyzed aziridination.[59-61]

While notable results have been obtained with PhI=NTs in several metal catalyzed systems,[62-64] the nitrene source suffers from several drawbacks: commercial unavailability, costly synthesis, short shelf life, insolubility in common solvents, and generation of PhI as a byproduct.[67, 68, 153] To this end, recent efforts have been made to develop alternative nitrene sources that can be used in metal-catalyzed aziridination, including chloramine-T,[73, 95] bromamine-T,[77, 78] and tosyl azide.[81, 110]

Supported by different ligands, complexes of several transition metals, including Mn, Fe, Cu, Ru, and Rh, have been demonstrated to catalyze aziridination of alkenes with these nitrene sources.[54, 56-65, 67, 68, 73, 77, 78, 81, 95, 106-108, 110, 153] Most of these catalytic systems lead to the formation of *N*-sulfonylated aziridines. Different from *N*-sulfonylated aziridines in which the deprotection of *N*-sulfonyl groups normally requires harsh conditions,[158, 159] *N*-phosphorylated and *N*-phosphinylated aziridines offer advantages as synthetic building blocks since the phosphoryl and phosphinyl groups bring suitable activation to the aziridine ring and can be easily deprotected.[160-170] Although several methods are available for the preparation of *N*-phosphorus-substituted aziridines,[160-168, 171] their direct synthesis from alkenes via transition metal-catalyzed aziridination has not been developed.[172]

Attracted by their unique ligand environments and metal coordination modes,[173] we[90-94, 112-114, 148, 174, 175] and others[83, 84, 86-89, 176] have been interested in developing metalloporphyrin-based catalytic systems for aziridination. Previously, we reported that iron and cobalt porphyrins can effectively catalyze aziridination reactions of a wide variety of alkenes with bromamine-T as an alternative nitrene source.[113, 148] We reveal herein that cobalt(II) porphyrin complex Co(TPP) (Figure 8-1) can catalyze aziridination

of alkenes using diphenylphosphoryl azide (DPPA) as a convenient new nitrene source,[61, 177-179] leading to the formation of *N*-phosphorylated aziridines with dinitrogen as the byproduct (Figure 8-2). To the best of our knowledge, this represents one of the few catalytic aziridination systems that use cobalt-based catalysts[113, 148, 180] or that employ azides as nitrene sources.[81, 110, 115]

To explore the possibility of DPPA as an effective nitrene source, we initially carried out systematic studies on aziridination of styrene as a model reaction using different metalloporphyrins as potential catalysts under various conditions. Among porphyrin complexes of different metal ions that were evaluated, cobalt ion seems essential to successful aziridination of styrene with DPPA. While Co(TPP) could catalyze the formation of the desired product (Table 8-1), employment of other metalloporphyrins, including Cr(TPP)Cl, Mn(TPP)Cl, Fe(TPP)Cl, Ni(TPP), and Ru(TPP)(CO) (Figure 8-1), produced only trace amounts of the *N*-phosphorylated aziridine. It should be noted that no aziridines were observed without a catalyst. Strong solvent effects were also noticed for the aziridination process. Of the common solvents used, chlorobenzene appeared the solvent of choice for the reaction, forming the desired *N*-phosphorylated aziridine as the major product. Use of other solvents, including acetonitrile, dichloromethane, dimethylformamide, tetrahydrofuran, and toluene, gave little or no amount of the desired product.

As summarized in Table 8-1, a styrene to DPPA ratio of 5:1 gave the best result for the Co(TPP)-catalyzed aziridination in chlorobenzene. An increase or decrease in the ratio lowered the yield of the desired product (entries 1-4). Although the catalytic

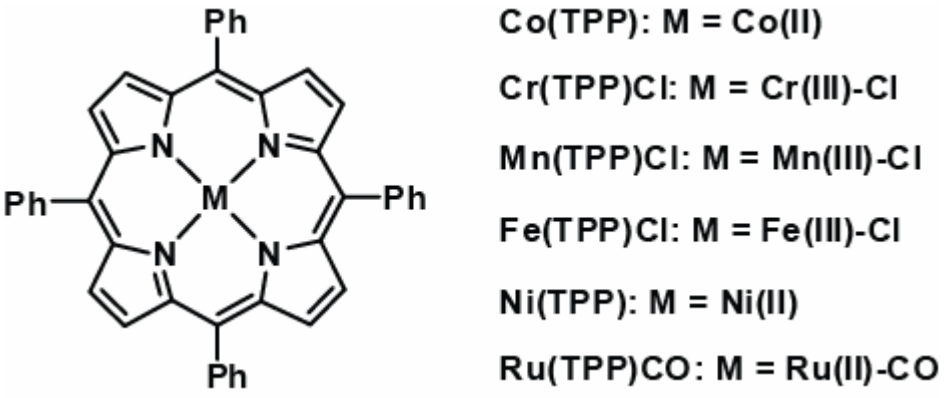


Figure 8-1. Structures of Several Common Metal Complexes of Tetraphenylporphyrin

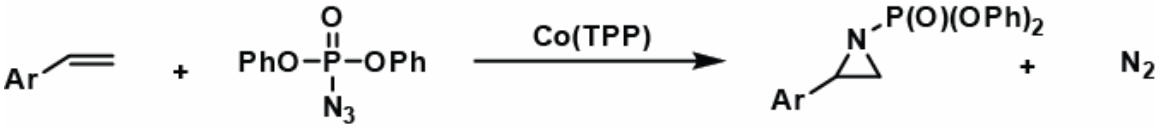
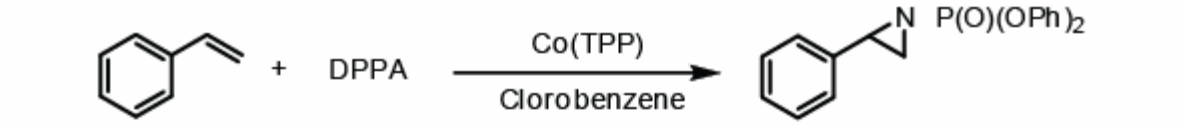


Figure 8-2. Aziridination of Alkenes with DPPA Catalyzed by Cobalt (II) Tetraphenylporphyrin complex

Table 8-1. Aziridination of Styrene with DPPA catalyzed by Cobalt (II) Tetraphenylporphyrin Complex Under Various Conditions[a]



Entry	Cat (mol %)	S:A[b]	Additv (mol %)	Temp (C°)	Time (h)	Yield (%)[c]
1	10	1:2	None	100	17	0
2	10	3:1	None	100	17	32
3	10	5:1	None	100	17	50
4	10	10:1	None	100	17	33
5	10	5:1	None	100	40	29
6	10	5:1	None	120	17	54
7	10	5:1	None	120	7	17
8	10	5:1	None	120	40	0
9	10	5:1	None	80	17	19
10	10	5:1	None	80	46	56
11	5	5:1	None	100	17	20
12	5	5:1	None	120	17	27
13	10	5:1	DMAP (10)	100	17	0
14	10	5:1	DMAP (10)	80	17	0
15	10	5:1	DMAP (10)	100	6	0
16	10	5:1	THF (100)	100	17	35
17	10	5:1	CH_3CN (100)	100	17	43
18	10	5:1	Ph_3P (10)	100	17	42

[a] Reactions were carried out in chlorobenzene under N_2 in the presence of 5Å molecular sieves using Co(TPP) as a catalyst with or without additives. Concentration: [DPPA] = 0.1M [b] Mole ratio of Styrene to DPPA. [c] Isolated Yields.

reaction could proceed with a nearly complete conversion of DPPA overnight at 100 °C, the *N*-phosphorylated aziridine was isolated in only 50% yield (entry 3) due to formation of some unidentified side products during reaction (and possibly during product isolation with silica gels). While a slight increase in yield was obtained at a higher temperature (entry 6), prolonged heating resulted in a yield reduction (entries 5 and 8). A reduced yield was also observed at a shorter reaction time (entry 7) or at a lower reaction temperature (entry 9). However, a reaction that was carried out at lower temperature for longer time gave the desired product in an improved yield (entry 10). Reduction in catalyst loadings dropped the yields for overnight reactions (entries 11 and 12). A negative additive effect was observed for the catalytic process. Addition of a small amount of DMAP completely shut down the reaction (entries 13-15). The negative effect was reduced with weaker coordinative additives, such as Ph_3P, THF, and CH_3CN (entries 16-18). As aforementioned, no desired products were observed when the reactions were performed with THF or CH_3CN as the solvent. Block of coordination sites of cobalt center by the potentially coordinative additives might be responsible for the observed negative effects.

The substrate scope of the Co(TPP)-based aziridination with DPPA was then investigated with different alkenes. The results of a series of styrene derivatives are summarized in Table 8-2. Under the above-mentioned typical reaction conditions, *m*methylstyrene was a better substrate than styrene, while aziridination of *p-tert*-butyl styrene gave a slightly lower yield (entries 1-3). The Co-based aziridination system appeared equally suitable to styrene derivatives having electron-withdrawing substituents, such as halogen and trifluoromethyl groups (entries 4-7). Even the highly

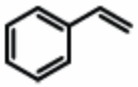
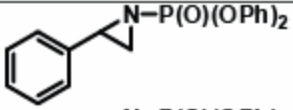
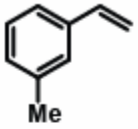
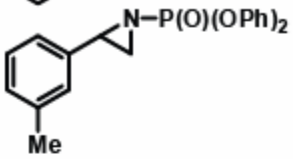
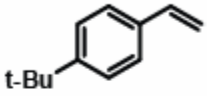
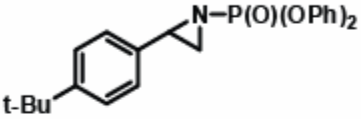
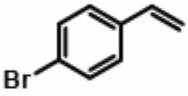
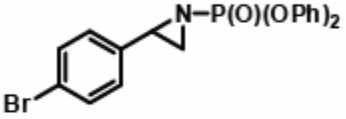
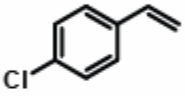
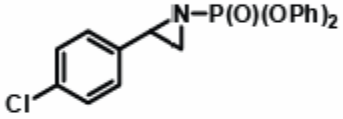
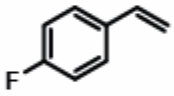
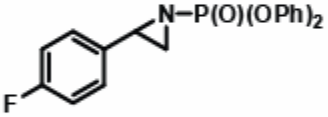
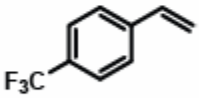
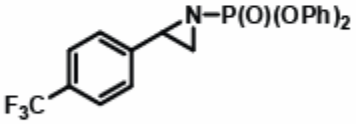
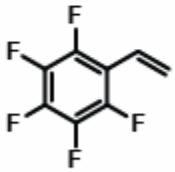
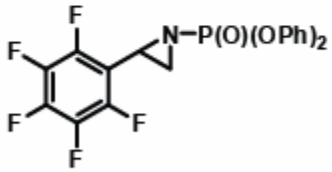
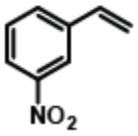
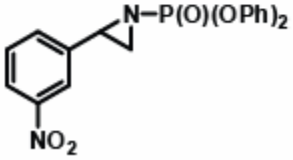
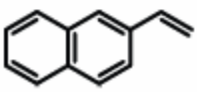
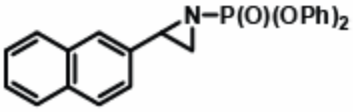
electron-deficient pentafluorostyrene could be aziridinated with DPPA, albeit in lower yields (entry 8).[181] While a low yield was obtained for the aziridination of 2-vinylnaphthalene (entry 10), the reaction of *m*-nitrostyrene produced the desired *N*-phosphorylated aziridine in the highest yield (entry 9). All the aziridination products were isolated in high purity and characterized by ^{1}H, ^{13}C, and ^{31}P NMR, FTIR, and high-resolution MS spectroscopy.

As exemplified with the *N*-phosphorylated aziridine from styrene (Figure 8-3), each of the three aziridine-ring hydrogens exhibits a characteristic doublet of doublet of doublets (ddd) peak pattern in the ^{1}H NMR spectrum between 2.2 and 3.8 ppm, which results from the coupling among the three hydrogens that was further split by the phosphorus atom.

The catalytic aziridination by Co(TPP) with DPPA can be assumed to proceed via a mechanism similar to that proposed for other metalloporphyrin-based systems with PhI=NTs. As proposed in Figure 8-4, this mechanism requires, however, the involvement of a cobalt-nitrene intermediate **A**, which has not been known previously. More experimental work is obviously necessary before there is further discussion of the reaction mechanism for this new catalytic process.

In summary, we have demonstrated the first application of the common reagent DPPA as a new nitrene source for catalytic aziridination by Co(TPP), forming synthetically valuable *N*phosphorylated aziridines directly from the correspondingalkenes. Considering its low cost, commercial availability, and high stability, DPPA and related phosphoryl azides may find broad applications in metal-catalyzed aziridination and other nitrene-transfer processes. In addition to our ongoing

Table 8-2. Aziridination of Styrene Derivatives with DPPA Catalyzed by Cobalt (II) Tetraphenylporphyrin Complex[a]

Entry	Substrate	Product	Yield (%)[b]
1			50
2			64
3			43
4			54
5			52
6			45
7			60
8			36
9			68
10			24

[a] Reactions were carried out overnight at 100°C in chlorobenzene under N_2 in the presence of 5Å molecular sieves using 10 mol % Co(TPP). Concentration: [DPPA] = 0.1M; Alkene:DPPA = 5:1.
[b] Isolated Yields.

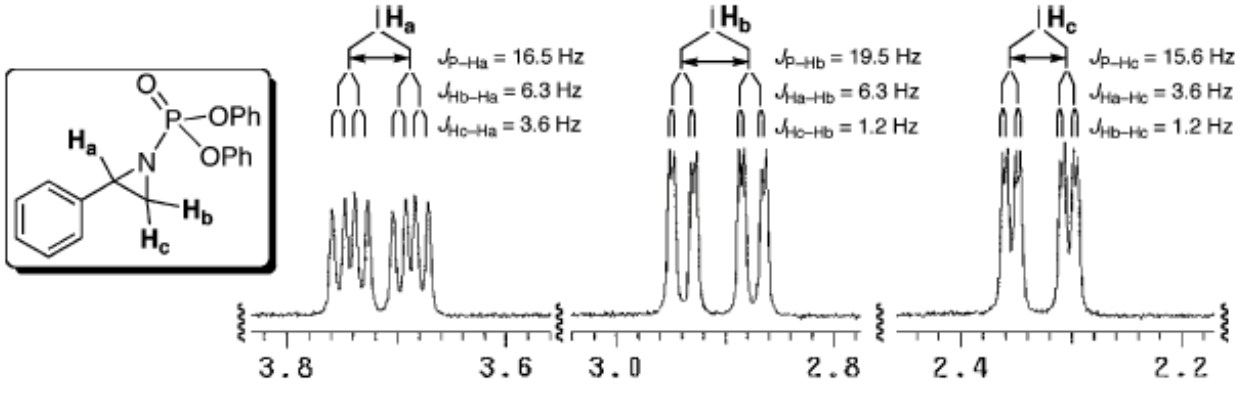


Figure 8-3. Characteristic ^{1}H NMR splitting pattern aziridine-ring hydrogens of N-Phosporylated Aziridines

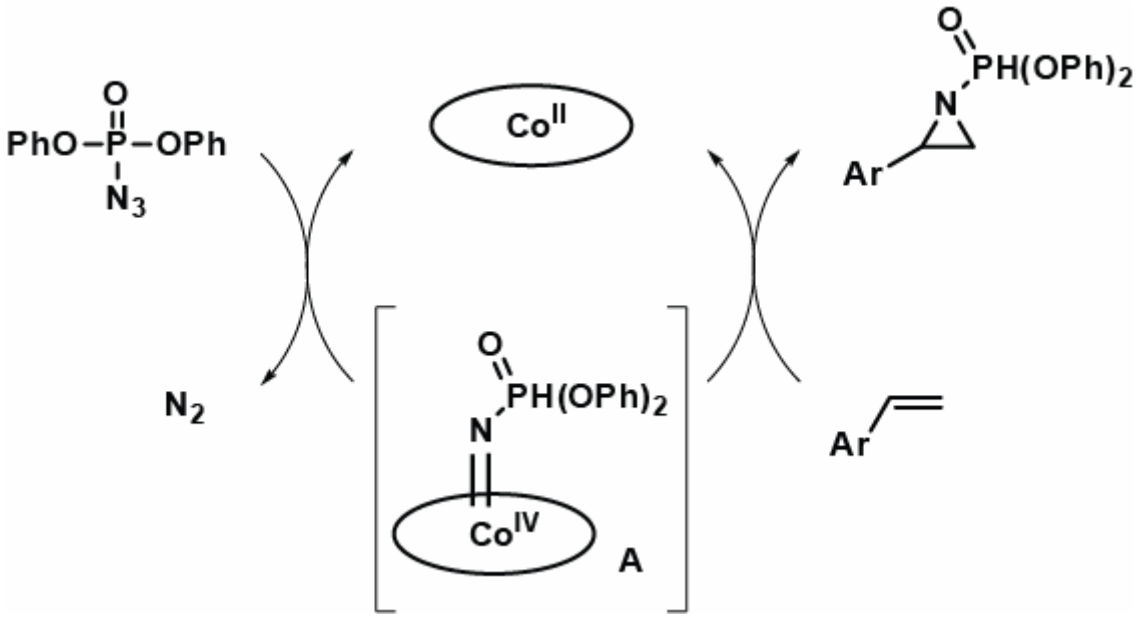


Figure 8-4. Proposed Aziridination Mechanism with DPPA Catalyzed by Cobalt (II) Tetraphenylporphyrin

efforts to understand the mechanism of the Co-catalyzed aziridination, further improvement of its catalytic efficiency and the development of its asymmetric variant are in progress in our laboratory.

Experimental Section

General Considerations. All reactions were carried out under a nitrogen atmosphere in an oven dried Schlenk tube. Diphenylphosphoryl azide (DPPA), the metalloporphyrins, and the olefins are commercially available and were used without further purification. Chlorobenzene was dried with calcium hydride in reflux. Proton, carbon, phosphorous, and fluorine nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and ^{19}F NMR) were recorded on a 300 MHz spectrometer and referenced with respect to residual solvent. Thin layer chromatography was carried out on Silica Gel 60 F-254 TLC plates.

General Procedure for Aziridination of Alkenes. An oven dried Schlenk tube equipped with a stirring bar was degassed on a vacuum line and purged with nitrogen. The tube was charged with metalloporphyrin (10 mol %) and activated 5Å molecular sieves (200 mg). The tube was capped with a Teflon screw cap, then evacuated on a vacuum line for 30-45 min. The Teflon screw cap was replaced with a rubber septum and 2 mL of solvent, diphenylphosphoryl azide (0.2 mmol) and substrate (1.0 mmol) were then added successively. The tube was purged with nitrogen for 1-2 min and the contents were stirred and heated at 80-100 ^{o}C, overnight. After completion of the reaction, the mixture was cooled down to room temperature. The molecular sieves were removed by filtration and the filtrate was concentrated under vacuum. The solid

residue was purified by flash chromatography (silica gel, ethyl acetate:hexanes (V:V) = 3:7) to afford the pure product.

(2-Phenylaziridin-1-yl)phosphonic acid diphenyl ester. (Table 8-2, Entry 1) was synthesized from the reaction of styrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.27-7.00 (m, 15H), 3.63 (ddd, 1H, J) 16.5, 6.3, 3.6 Hz), 2.82 (ddd, 1H, J) 19.5, 6.3, 1.2 Hz), 2.24 (ddd, 1H, J) 15.6, 3.6, 1.2 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 129.7, 129.6, 128.5, 128.1, 126.2, 125.2, 120.5, 120.4, 120.3, 39.0, 38.9, 35.0, 34.9. ^{31}P NMR (121 MHz, CDCl3): δ 6.11 (s). FT-IR (film, cm-1): 1590, 1191, 941, 669. HRMS-EI ([M]+) for C20H18NO3P, Calculated 351.1024, Found 351.1022.

(2-m-Tolylaziridin-1-yl)phosphonic acid diphenyl ester. (Table 8-2, Entry 2) was synthesized from the reaction of m methylstyrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.28-6.95 (m, 14H), 3.61 (ddd, 1H, J) 16.5, 6.0, 3.3 Hz), 2.80 (dd, 1H, J) 19.2, 6.0 Hz), 2.22 (m, 4H). ^{13}C NMR (75 MHz, CDCl3): δ 129.7, 129.6, 128.8, 128.3, 126.8, 125.2, 123.4, 120.5, 120.4, 120.3, 39.0, 38.9, 34.9, 34.8, 21.3. ^{31}P NMR (121 MHz, CDCl3): ä 6.19 (s). FT-IR (film, cm-1): 1711, 1585, 1482, 1190, 1010, 932, 762. HRMS-EI ([M]+) for C21H20NO3P, Calculated 365.1181, Found 365.1174.

[2-(p-tert-Butylphenyl)aziridin-1-yl]phosphonic acid diphenyl ester. (Table 8-2, Entry 3) was synthesized from the reaction of p-tertbutylstyrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.28-7.00 (m, 14H), 3.62 (ddd, 1H, J) 16.5, 6.0, 3.6 Hz), 2.80 (ddd, 1H, J) 19.8, 6.6, 1.2 Hz), 2.24 (ddd, 1H, J) 15.3, 3.6, 1.2 Hz), 1.24 (s, 9H). ^{13}C NMR (75 MHz, CDCl3): δ 129.7, 129.6, 125.9, 125.4, 125.2, 120.5, 120.4, 120.3, 38.9, 38.8, 34.9, 34.8, 34.5, 31.3. ^{31}P NMR (121 MHz,

CDCl3): δ 6.24 (s). FT-IR (film, cm-1): 1592, 1490, 1193, 943, 773, 689. HRMS-EI ([M]+) for C24H26NO3P, Calculated 407.1650, Found 407.1658.

[2-(p-Bromophenyl)aziridin-1-yl]phosphonic acid diphenyl ester. (Table 8-2, Entry 4) was synthesized from the reaction of p-bromostyrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.35 (d, 2H, J) 8.4 Hz), 7.28-7.07 (m, 10H), 7.01 (d, 2H, J) 8.4 Hz), 3.57 (ddd, 1H, J) 16.2, 6.0, 3.3 Hz), 2.80 (ddd, 1H, J = 18.9, 6.0, 1.2 Hz), 2.19 (ddd, 1H, J) 15.3, 3.3, 1.2 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 129.7, 129.6, 127.9, 125.3, 120.4, 120.3, 120.3, 38.3, 38.2, 35.0, 34.9. ^{31}P NMR (121 MHz, CDCl3): δ 5.76 (s). FT-IR (film, cm-1): 1591, 1489, 1283, 1192, 1163, 1072, 1006, 945, 827, 774. HRMS-EI ([M]+) for C20H17BrNO3P, Calculated 429.0129, Found 429.0127.

[2-(p-Chlorophenyl)aziridin-1-yl]phosphonic acid diphenyl ester. (Table 8-2, Entry 5) was synthesized from the reaction of p-chlorostyrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.28-7.07 (m, 14H), 3.57 (ddd, 1H, J) 16.2, 6.0, 3.3 Hz), 2.80 (ddd, 1H, J) 19.2, 6.0, 1.2 Hz), 2.19 (ddd, 1H, J) 15.3, 3.3, 1.2 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 129.7, 129.6, 128.6, 127.5, 125.3, 120.4, 120.3, 120.2, 38.3, 38.2, 35.0, 34.9. ^{31}P NMR (121 MHz, CDCl3): δ 5.79 (s). FT-IR (film, cm-1): 1592, 1490, 1193, 943, 773, 689. HRMS-EI ([M]+) for C20H17ClNO3P, Calculated 385.0635, Found 385.0629.

[2-(p-Fluorophenyl)aziridin-1-yl]phosphonic acid diphenyl ester. (Table 8-2, Entry 6) was synthesized from the reaction of p-fluorostyrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.28-7.04 (m, 12H), 6.92 (t, 2H, J) 8.7 Hz), 3.59 (ddd, 1H, J) 16.5, 6.0, 3.6 Hz), 2.80 (ddd, 1H, J) 19.5, 6.3, 1.2 Hz), 2.19

(ddd, 1H, J) 15.0, 3.3, 0.6 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 129.7, 129.6, 127.8, 127.7, 125.3, 120.4, 120.3, 120.2, 115.6, 115.3, 38.3, 38.2, 35.0, 34.9. ^{31}P NMR (121 MHz, CDCl3): δ 5.96 (s). FT-IR (film, cm-1): 1592, 1490, 1224, 1192, 932, 835, 689. HRMS-EI ([M]+) for C20H17FNO3P, Calculated 369.0930, Found 369.0946.

[2-(p-Trifluoromethylphenyl)aziridin-1-yl]phosphonic acid diphenyl ester. (Table 8-2, Entry 7) was synthesized from the reaction of p-trifluoromethylstyrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.48 (d, 2H, J) 7.80 Hz),7.26 (d, 2H, J) 7.5 Hz), 7.23-7.04 (m, 10H), 3.65 (ddd, 1H, J) 16.2, 6.0, 3.6 Hz), 2.84 (dd, 1H, J) 19.2, 6.3 Hz), 2.22 (dd, 1H, J) 15.3, 3.3 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 129.8, 129.7, 126.5, 125.5, 125.4, 125.3, 120.3, 120.3, 120.2, 38.3, 38.2, 35.0, 35.0. ^{31}P NMR (121 MHz, CDCl3): δ 5.63 (s).19F NMR (280 MHz, CDCl3): ä 62.95. FT-IR (film, cm-1): 1621, 1592, 1490, 1326, 1193, 1165, 1068, 1005, 947, 904, 774, 689. HRMS-EI ([M]+) for C21H17F3NO3P, Calculated 419.0898, Found 419.0894.

(2-Pentafluorophenylaziridin-1-yl)phosphonic acid diphenyl ester. (Table 8-2, entry 8) was synthesized from the reaction of pentafluorostyrene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.29-7.07 (m, 10H), 3.71 (ddd, 1H, J) 16.5, 6.3, 3.6 Hz), 2.86 (dd, 1H, J) 18.9, 6.3 Hz), 2.76 (dd, 1H, J) 15.6, 3.6 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 129.8, 129.7, 125.4, 125.3, 120.2, 120.1, 120.0, 30.9, 30.0. ^{31}P NMR (121 MHz, CDCl3): δ 5.35 (s). 19F NMR (280 MHz, CDCl3): ä -140, -152, -160. FT-IR (film, cm-1): 1687, 1520, 1476, 1176, 980. HRMSEI ([M]+) for C20H13F5NO3P, Calculated 441.0553, Found 441.0559.

[2-(m-Nitrophenyl)aziridin-1-yl]phosphonic acid diphenyl ester. (Table 8-2, entry 9) was synthesized from the reaction of m-nitrostyrene with DPPA and obtained

as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 8.05 (dd, 1H, J) 8.1, 0.9 Hz), 7.98 (br s, 1H), 7.49-7.04 (m, 10H), 3.70 (ddd, 1H, J) 16.2, 6.0, 3.3 Hz), 2.89 (dd, 1H, J) 18.6, 6.0 Hz), 2.25 (dd, 1H, J) 15.3, 3.3 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 132.3, 129.8, 129.7, 129.5, 125.4, 123.0, 121.1, 120.3, 120.2, 37.9, 37.8. ^{31}P NMR (121 MHz, CDCl3): δ 5.27 (s). FT-IR (film, cm-1): 1591, 1531, 1488, 1350, 1190, 950. HRMS-EI ([M - H]+) for C20H17N2O5P, Calculated 395.0797,Found 395.0789.

[2-(2-Naphthalenyl)aziridin-1-yl]phosphonic acid diphenyl ester. (Table 8-2, entry 10) was synthesized from the reaction of 2-vinylnaphthalene with DPPA and obtained as a yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ 7.77-7.64 (m, 4H), 7.42-7.39 (m, 2H), 7.28-7.05 (m, 10H), 3.80 (ddd, 1H, J) 16.5, 6.0, 3.6 Hz), 2.89 (ddd, 1H, J) 19.2, 6.0, 1.2 Hz), 2.34 (ddd, 1H, J) 15.3, 3.3, 1.2 Hz). ^{13}C NMR (75 MHz, CDCl3): δ 129.7, 129.6, 128.3, 127.7, 127.7, 126.3, 126.1, 125.8, 125.2, 123.4, 120.5, 120.4, 120.3, 39.2, 34.9. ^{31}P NMR (121 MHz, CDCl3): δ 6.13 (s). FT-IR (film, cm-1): 1593, 1489, 1193, 936, 689. HRMS-EI ([[M]+) for C24H20NO3P, Calculated 401.1181, Found 401.1183.

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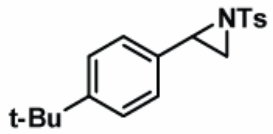
180. Under microwave conditions, $CoCl_2$ was to catalyze aziridination of styrene with bromamine-T to form the desired aziridine in 56% yield. See refs. 48 and 49.

181. To the best of our knowledge the only other example of aziridination of pentafluorostyrene was previously reported by us with a cobalt porphyrin/bromamine-T catalytic system. See ref 21.

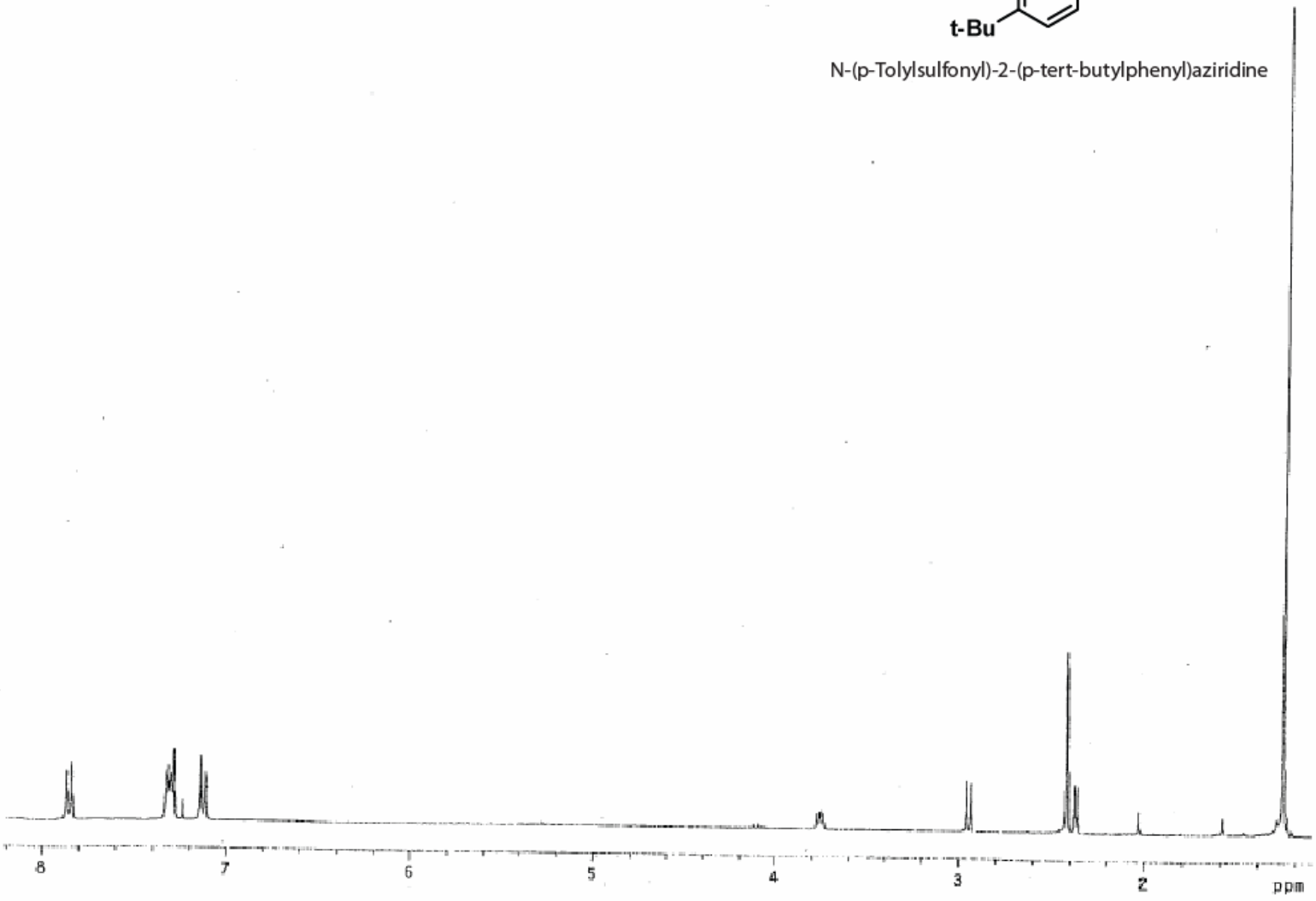
Appendix

OYG-VI-169

Pulse Sequence: s2pul

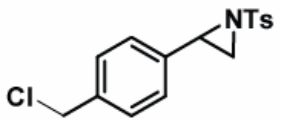


N-(p-Tolylsulfonyl)-2-(p-tert-butylphenyl)aziridine

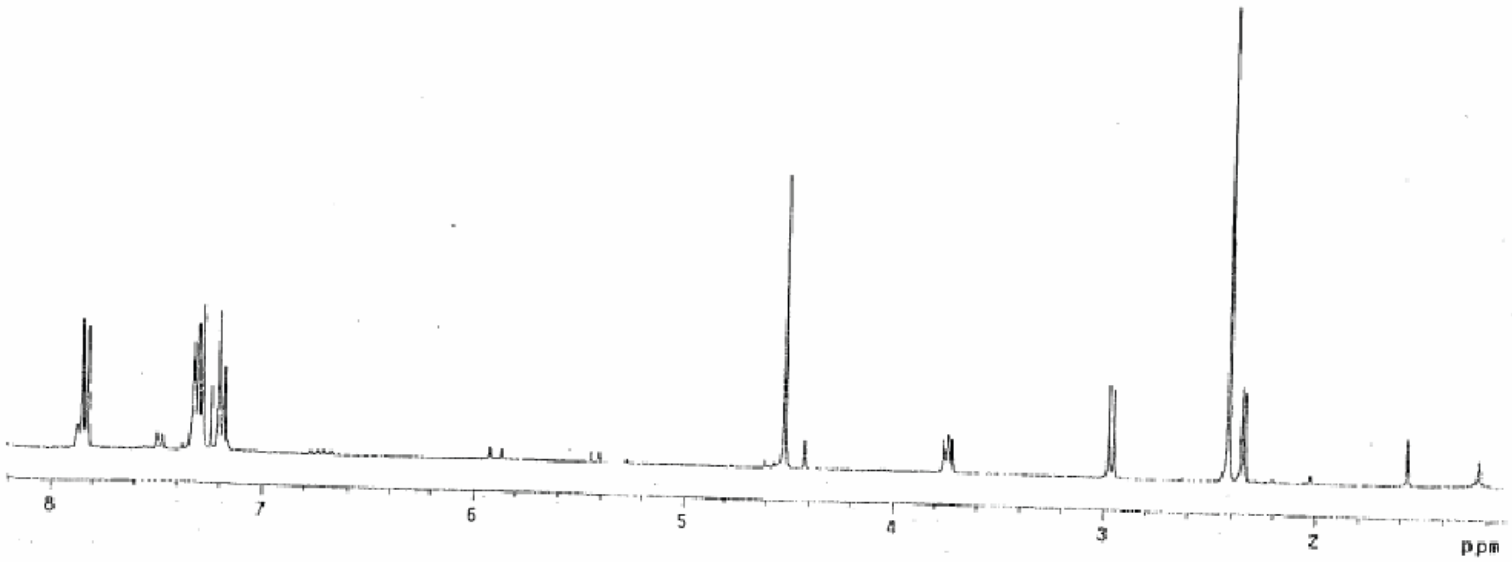


OYO-VI-178-2

Pulse Sequence: s2pul

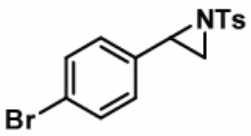


N-(p-Tolylsulfonyl)-2-(p-chloromethylphenyl)aziridine

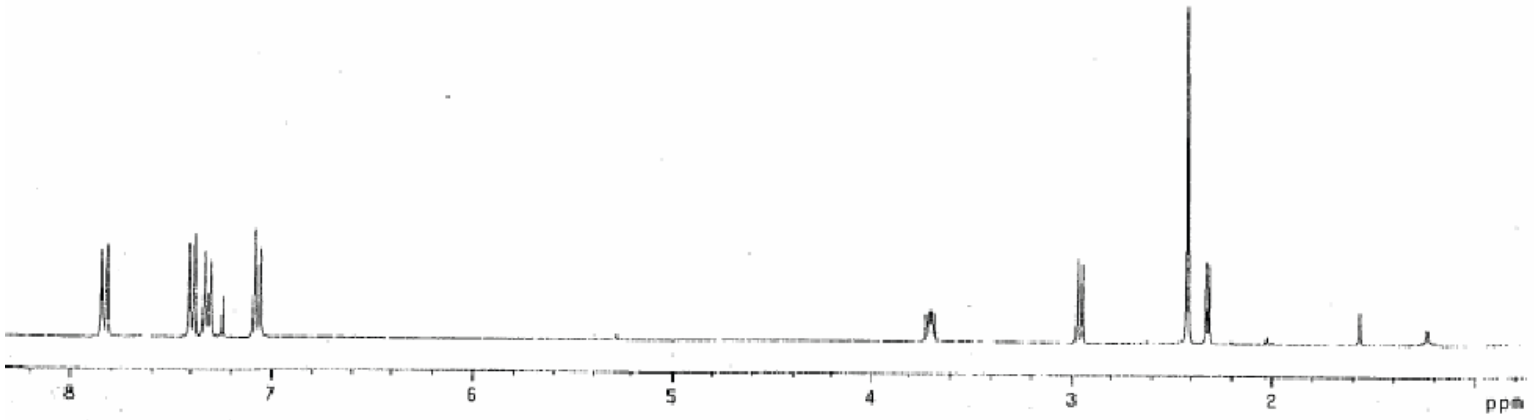


GYC-VI-167

Pulse Sequence: s2pul

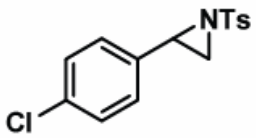


N-(p-Tolylsulfonyl)-2-(p-bromophenyl)aziridine

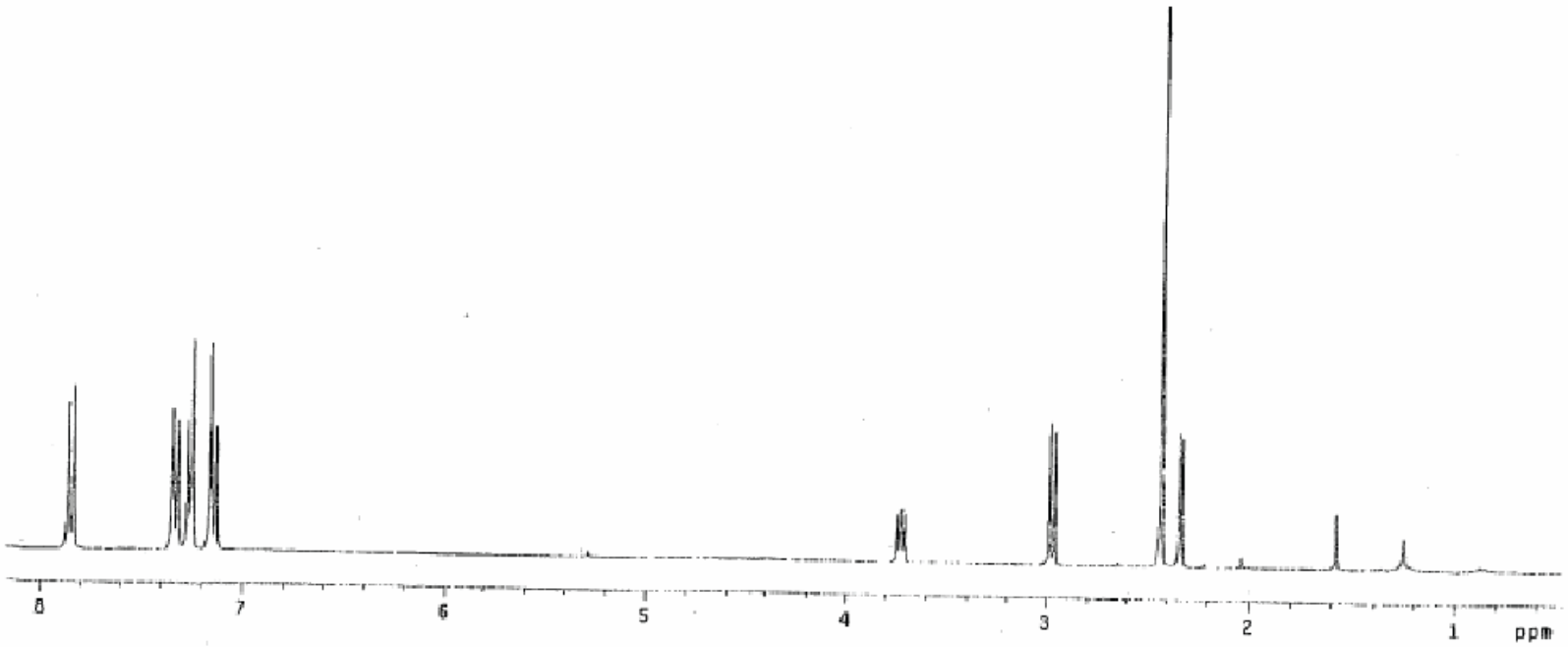


GYG-VI-168

Pulse Sequence: s2pul

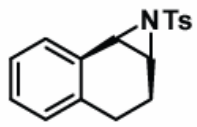


N-(p-Tolylsulfonyl)-2-(p-chlorophenyl)aziridine

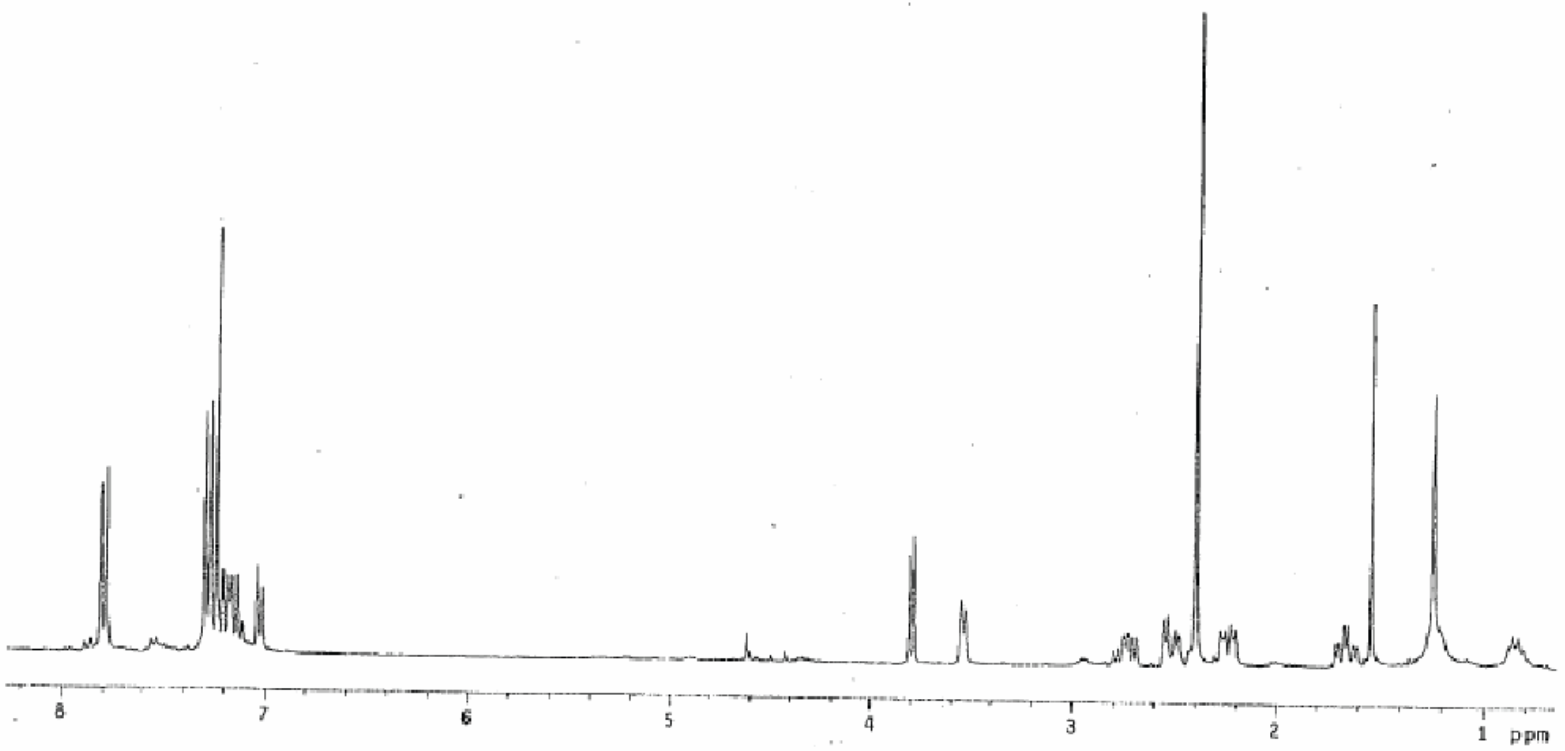


GYO-WJ-1X1

Pulse Sequence: s2pul

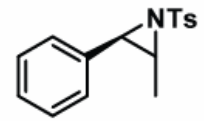


N-(p-Tolylsulfonyl)amino-1,2,3,4-tetrahydronapthalene-1,2-imine

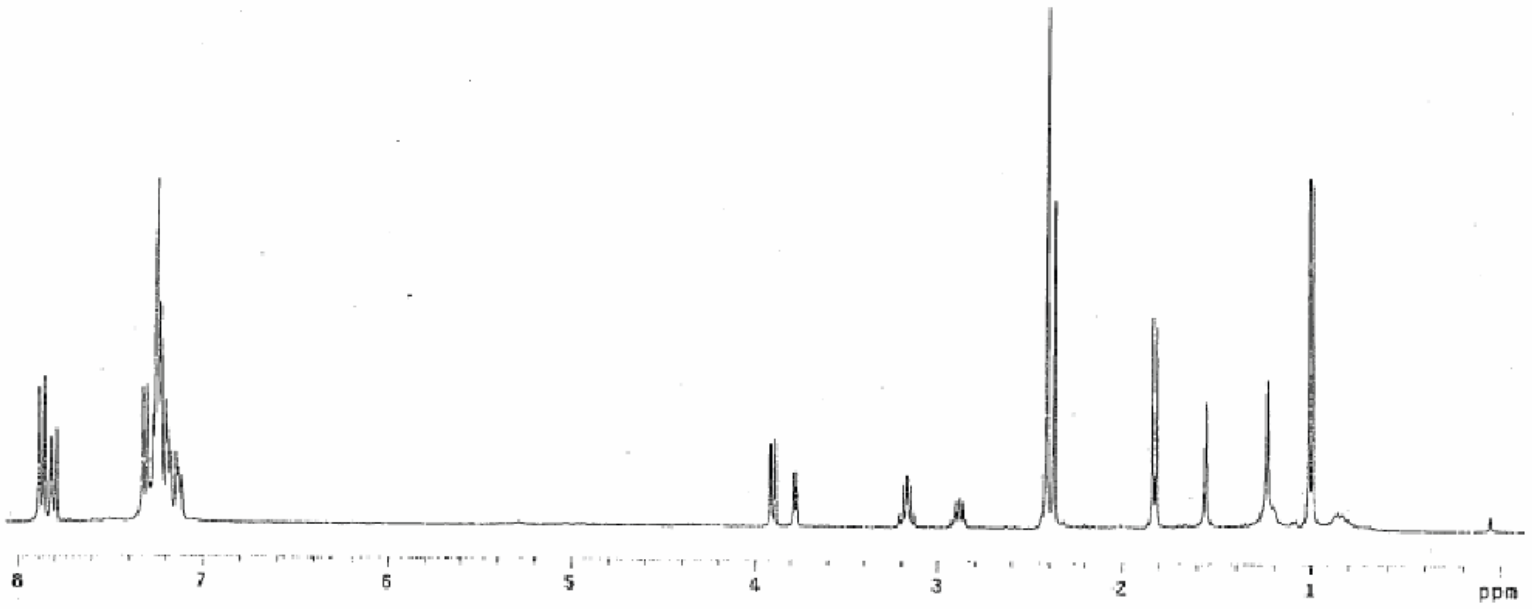


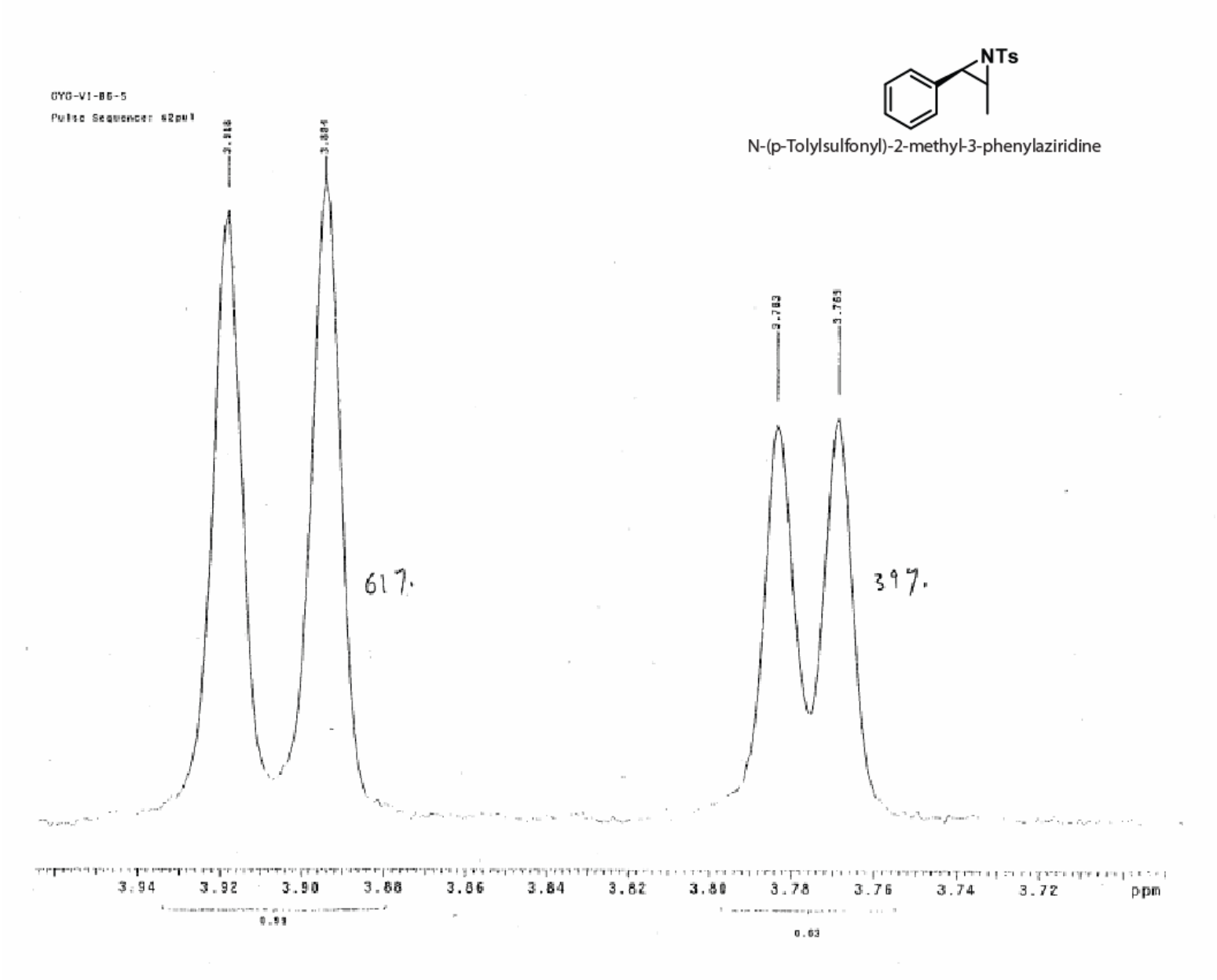
GVG-VI-88-S

Pulse Sequence: s2pul



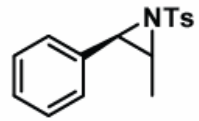
N-(p-Tolylsulfonyl)-2-methyl-3-phenylaziridine



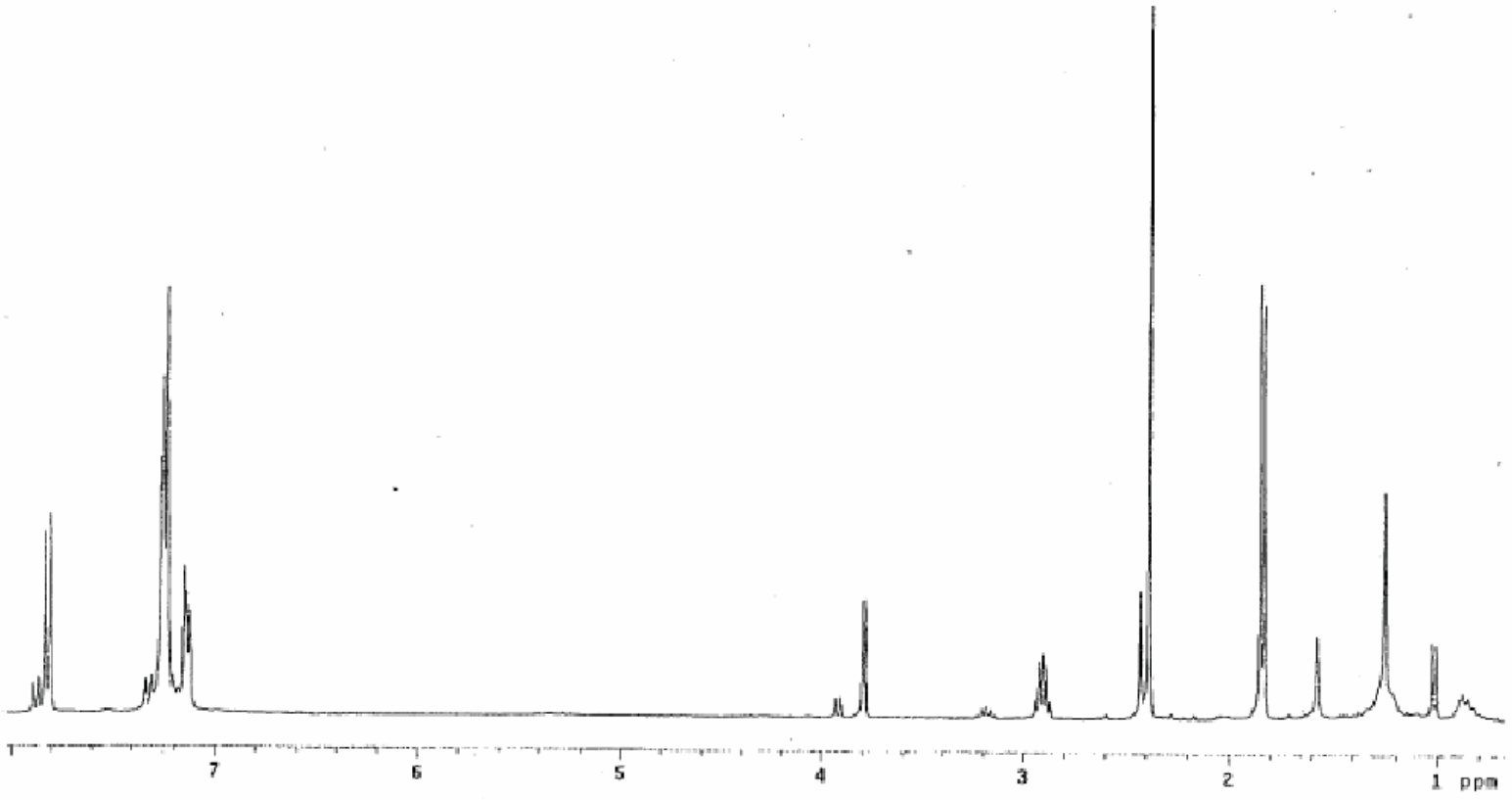

NTs
N-(p-Tolylsulfonyl)-2-methyl-3-phenylaziridine
GYG-V1-86-5
Pulse Sequence: s2pul
3.918
3.884
3.783
3.761
61%
39%
3.94
3.92
3.90
3.88
3.86
3.84
3.82
3.80
3.78
3.76
3.74
3.72
ppm
0.99
0.63

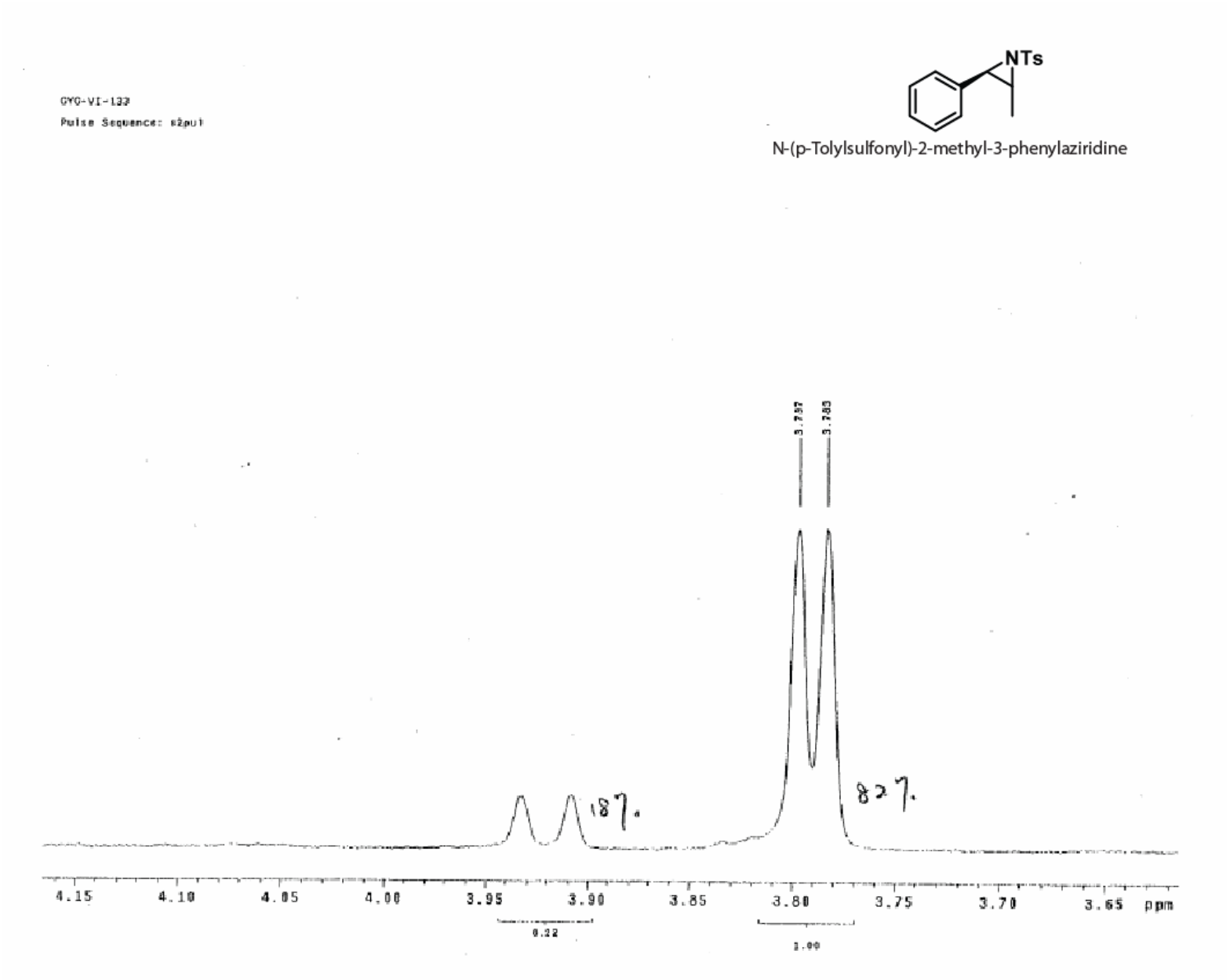
GMD-VI-133

Pulse Sequence: s2pul



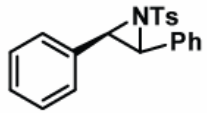
N-(p-Tolylsulfonyl)-2-methyl-3-phenylaziridine



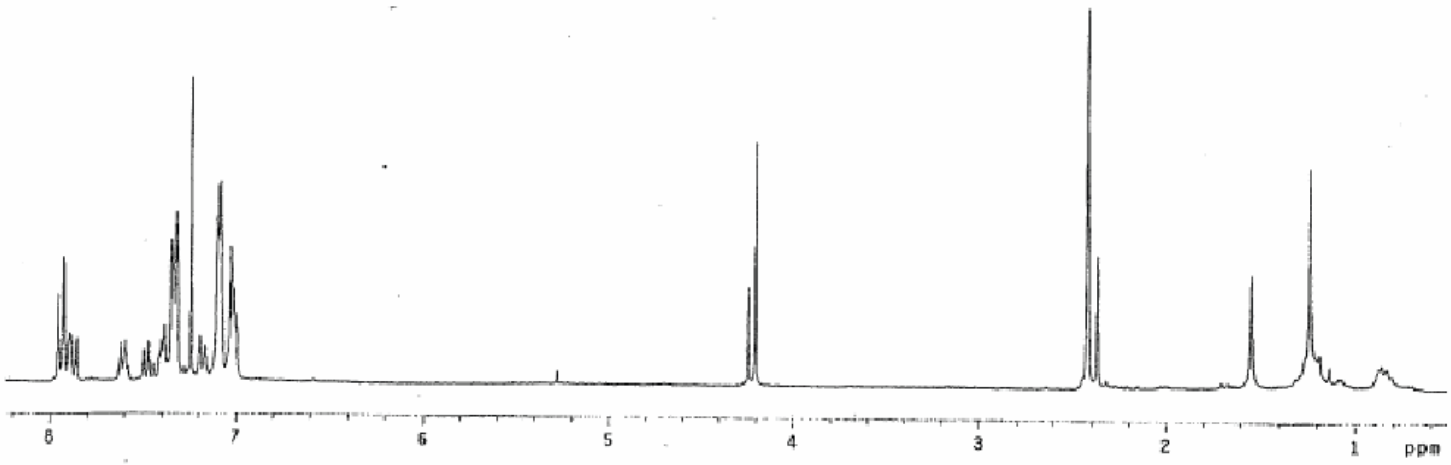

GYC-VI-132
Pulse Sequence: s2pul
NTs
N-(p-Tolylsulfonyl)-2-methyl-3-phenylaziridine
3.797
3.785
18%
82%
4.15
4.10
4.05
4.00
3.95
3.90
3.85
3.80
3.75
3.70
3.65
ppm
0.22
1.00

GYO-VI-192

Pulse Sequence: s2pul

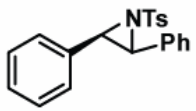


N-(p-Tolylsulfonyl)-2,3-diphenylaziridine

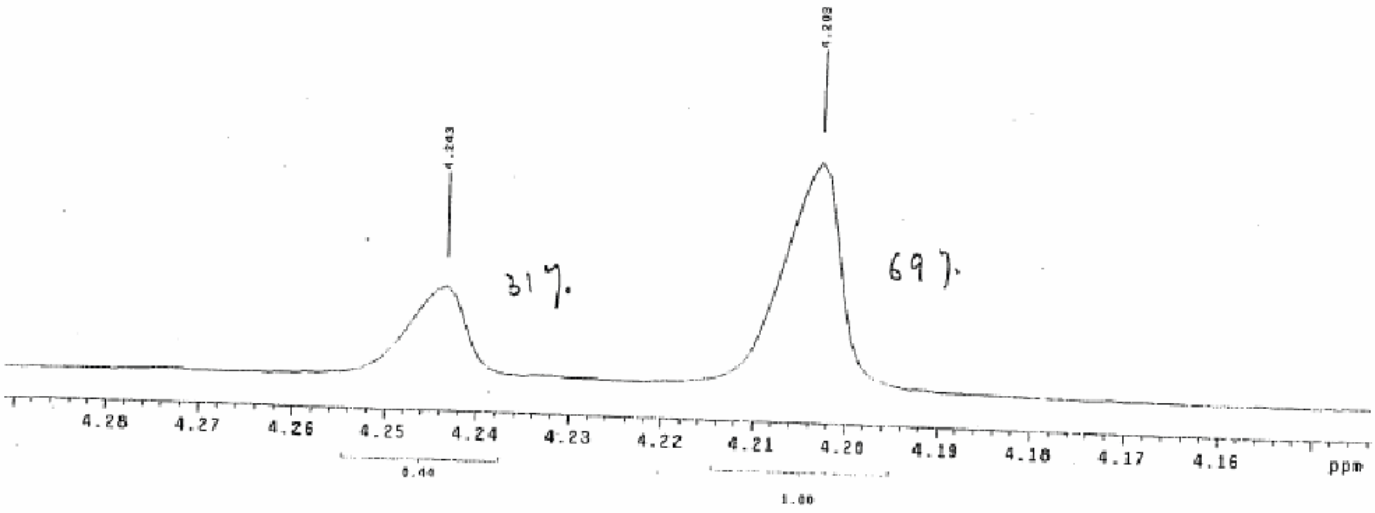


GYD-VI-132

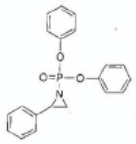
Pulse Sequence: s2pul



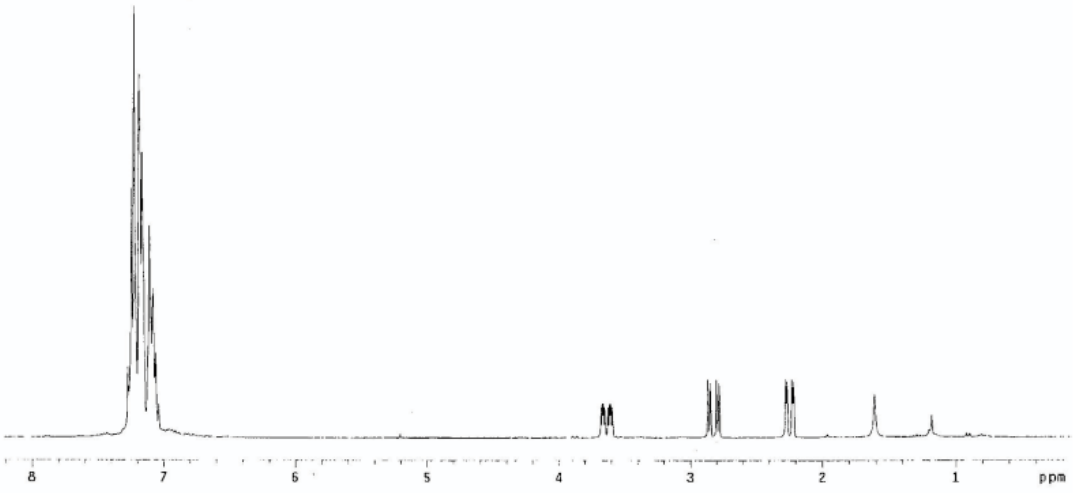
N-(p-Tolylsulfonyl)-2,3-diphenylaziridine



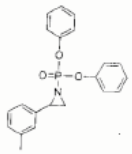
GYG-VI-194
Pulse Sequence: s2pul



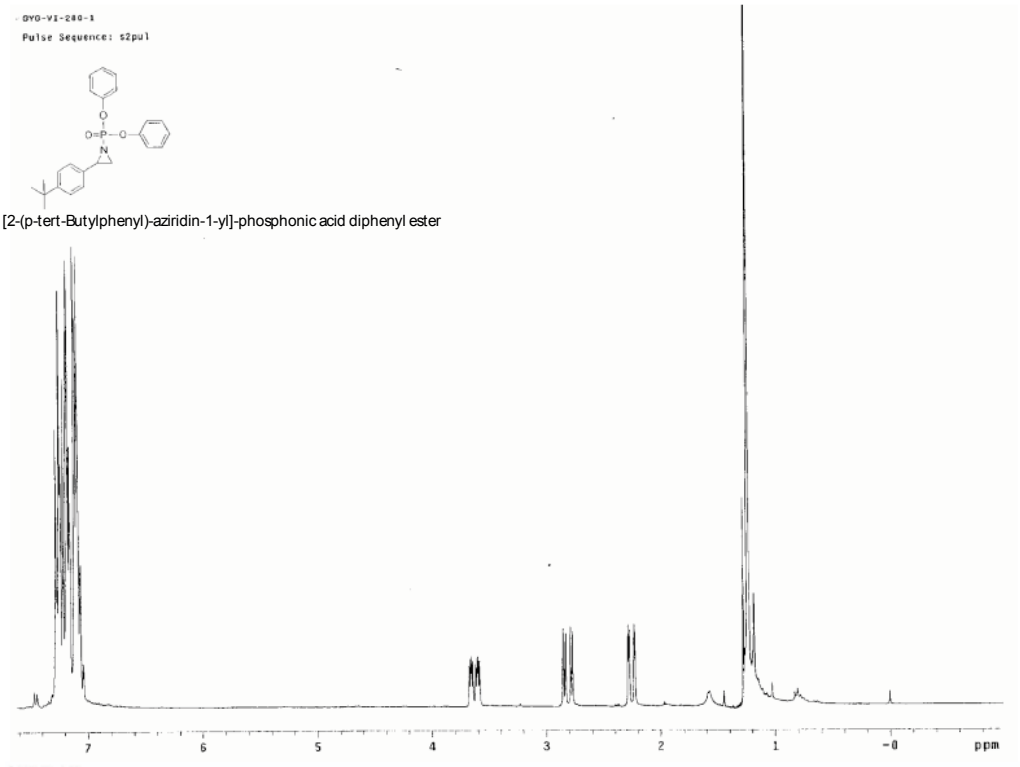
(2-Phenyl-aziridin-1-yl)-phosphonic acid diphenyl ester



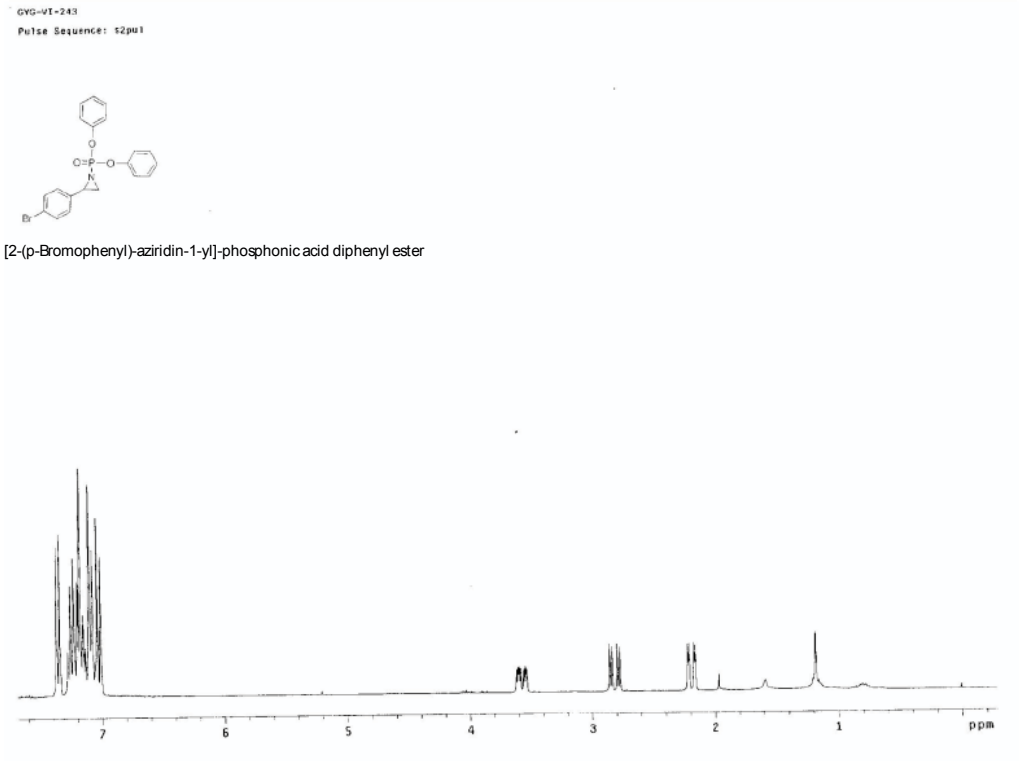
GYG-VI-259-1
Pulse Sequence: s2pul



(2-m-Tolyl-aziridin-1-yl)-phosphonic acid diphenyl ester

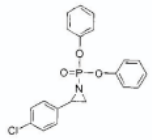


[2-(p-tert-Butylphenyl)-aziridin-1-yl]-phosphonic acid diphenyl ester

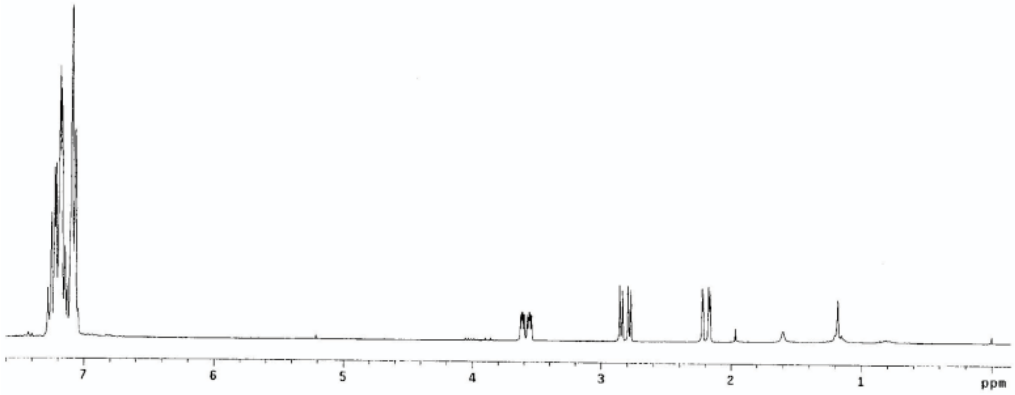


[2-(p-Bromophenyl)-aziridin-1-yl]-phosphonic acid diphenyl ester

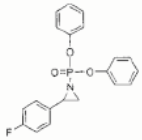
GYG-VI-242
Pulse Sequence: s2pul



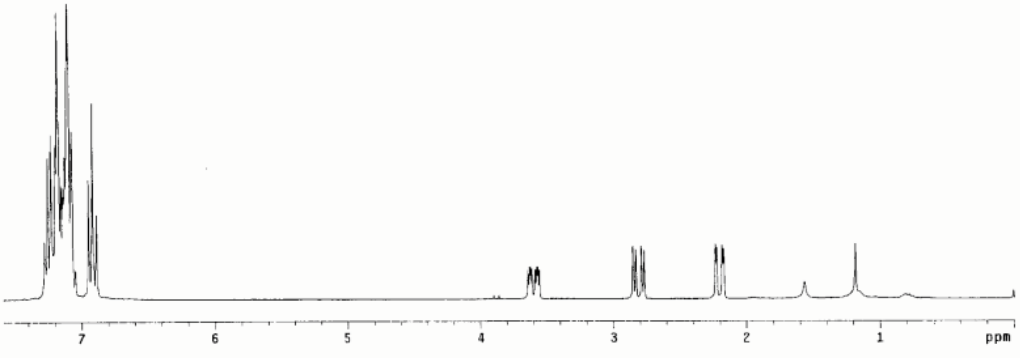
[2-(p-Chlorophenyl)-aziridin-1-yl]-phosphonic acid diphenyl ester



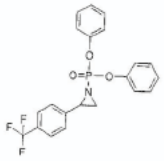
GYG-VI-246
Pulse Sequence: s2pul



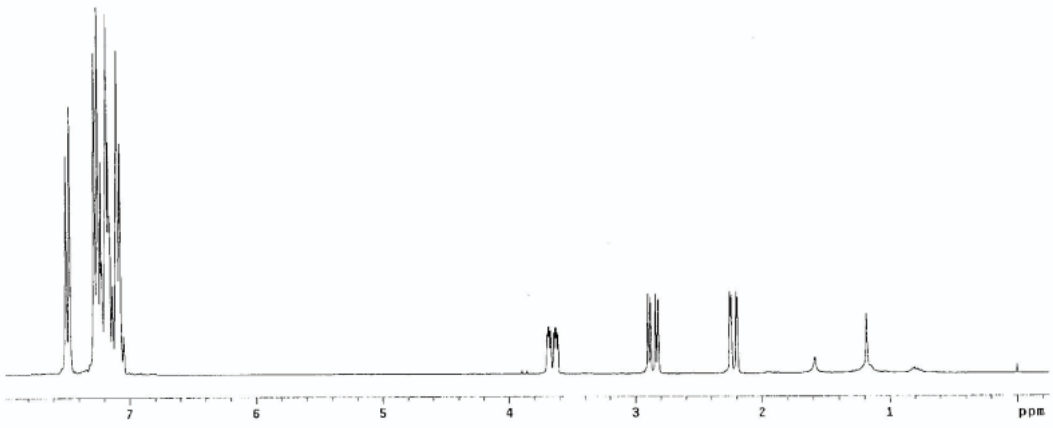
[2-(p-Fluorophenyl)-aziridin-1-yl]-phosphonic acid diphenyl ester



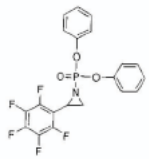
GYG-VI-247
Pulse Sequence: s2pul



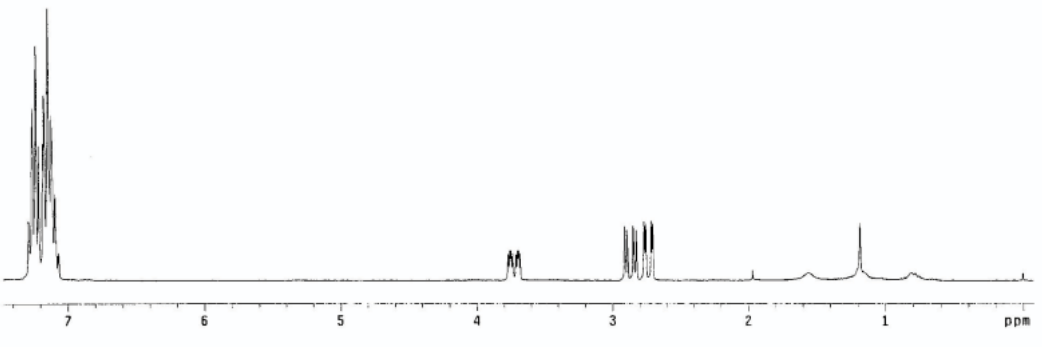
[2-(p-Trifluoromethyl-phenyl)-aziridin-1-yl]-phosphonic acid diphenyl ester

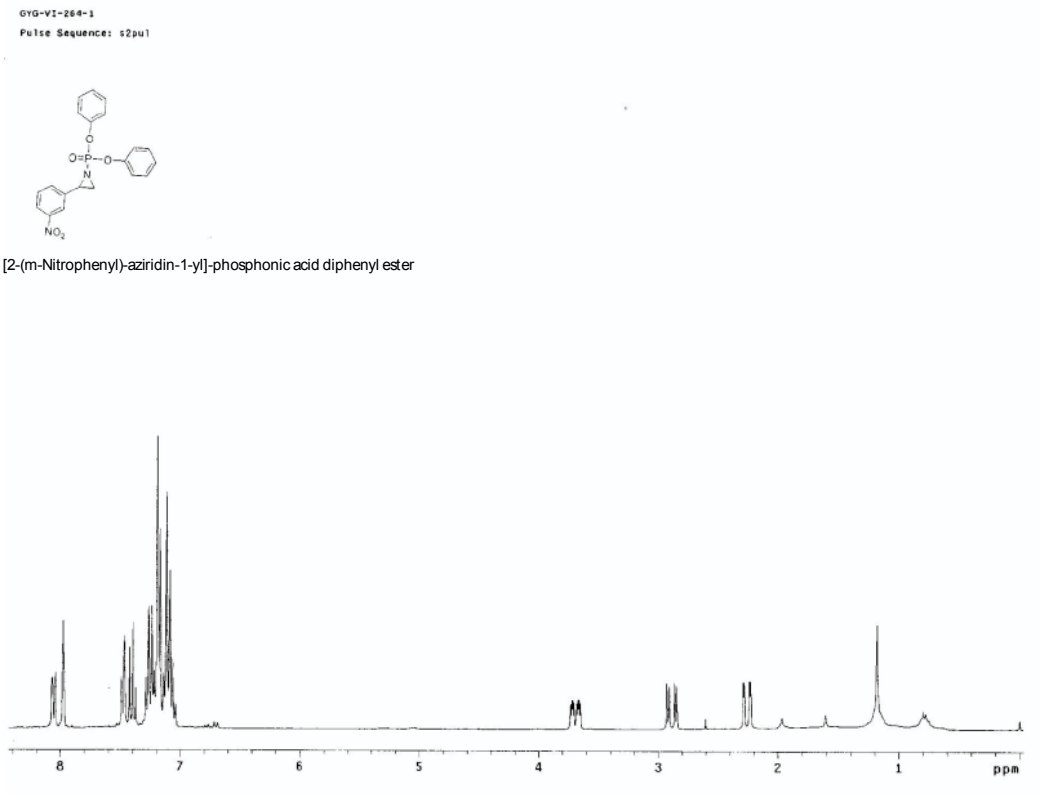


GYG-VI-248
Pulse Sequence: s2pul

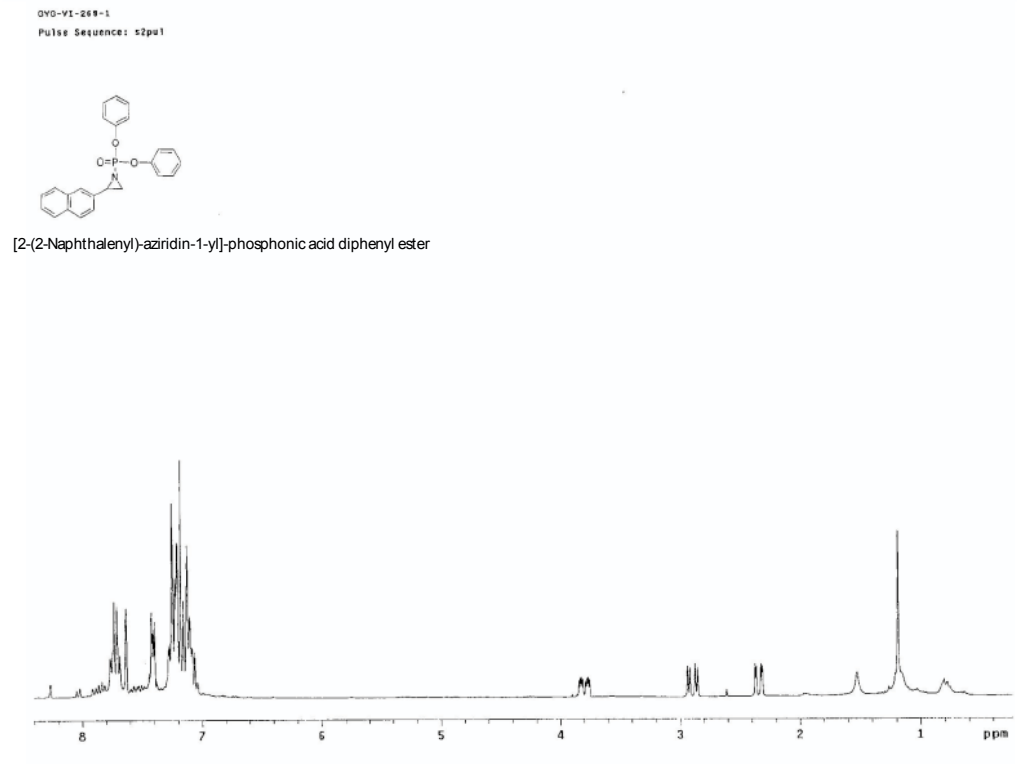


(2-Pentafluorophenyl-aziridin-1-yl)-phosphonic acid diphenyl ester

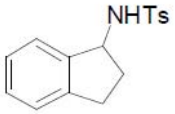




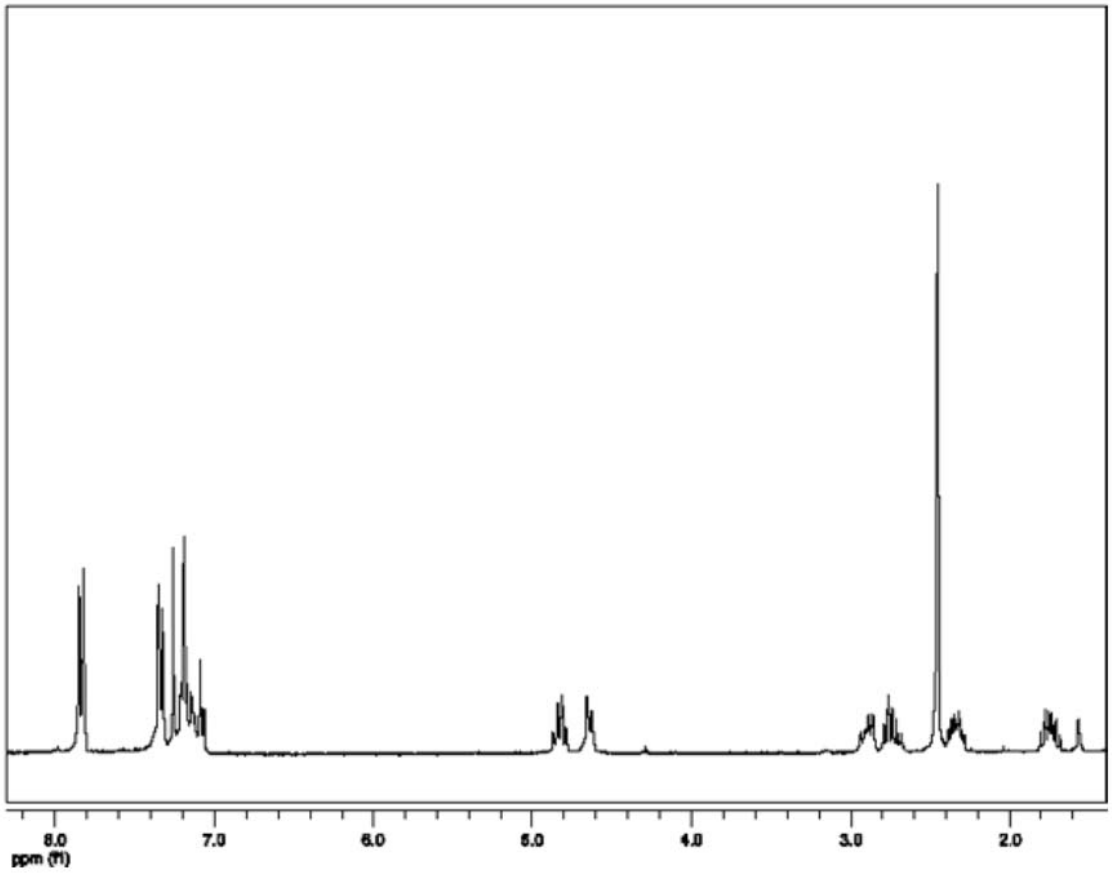
[2-(m-Nitrophenyl)-aziridin-1-yl]-phosphonic acid diphenyl ester

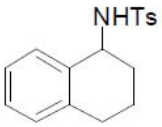


[2-(2-Naphthalenyl)-aziridin-1-yl]-phosphonic acid diphenyl ester

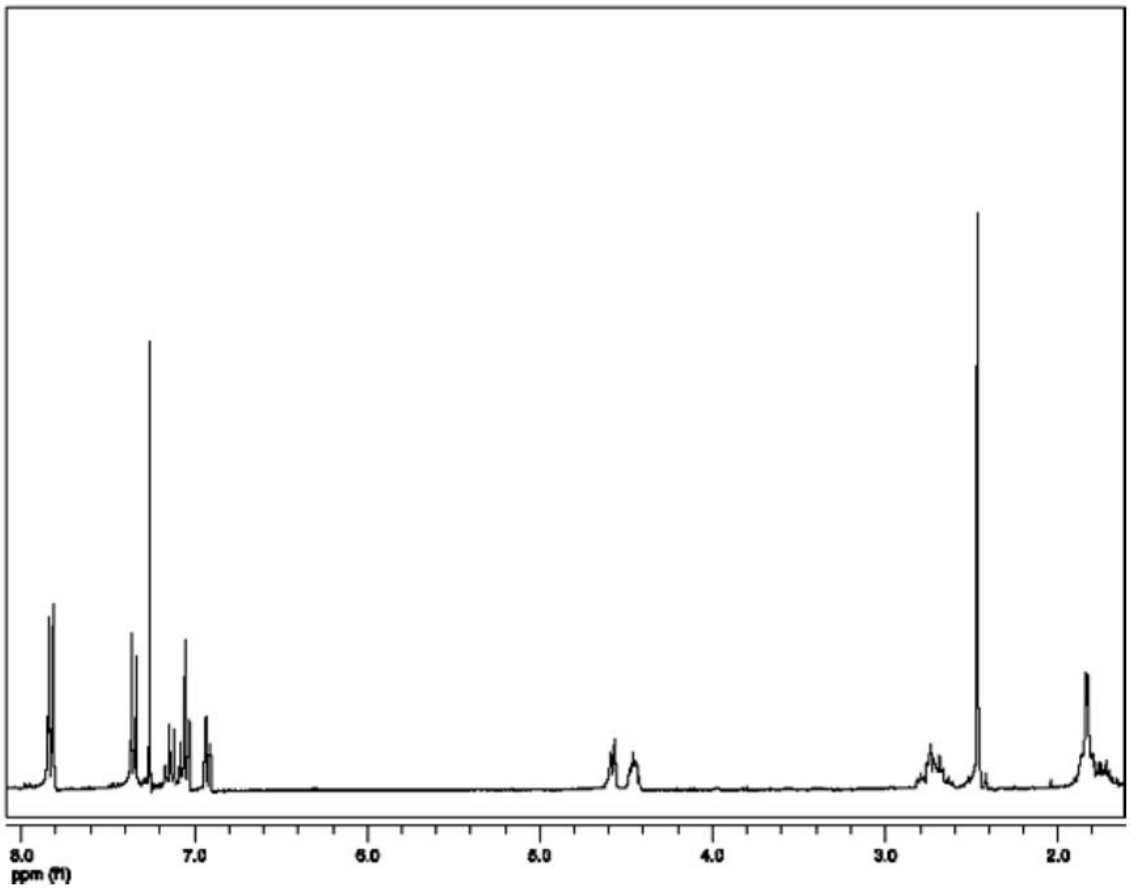


N-(p-Toluenesulfonyl)-1-aminoindan
Proton

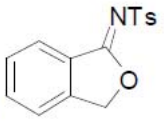


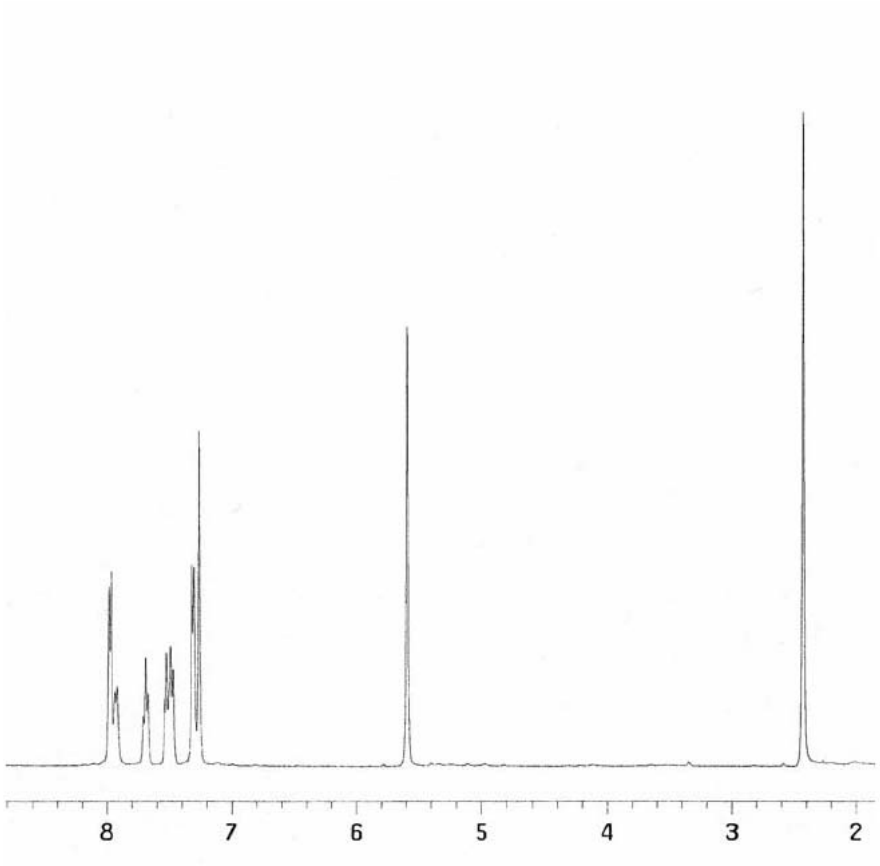


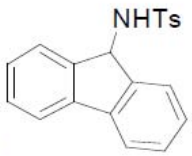
N-(p-Toluenesulfonyl)-1-amino-1,2,3,4 tetrahydronaphthalene



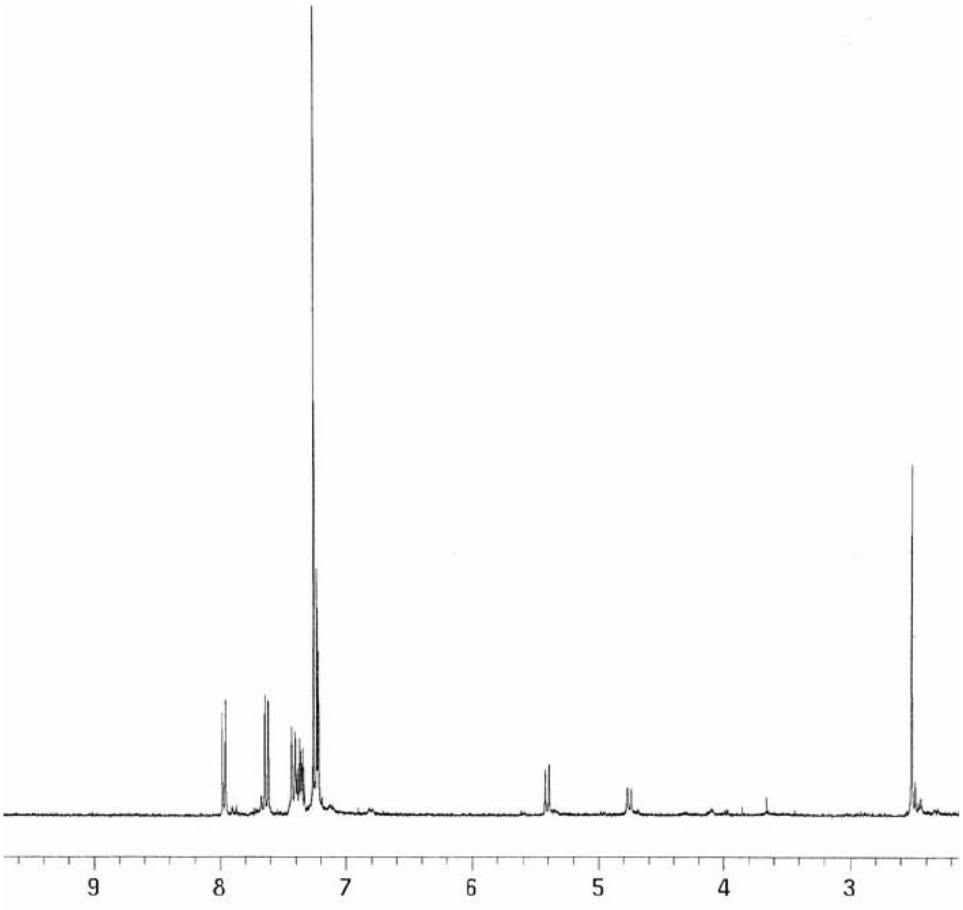
N-(isobenzofuran-1(3H)-ylidene)-4-methylbenzenesulfonamide



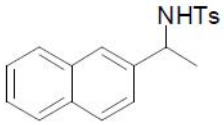


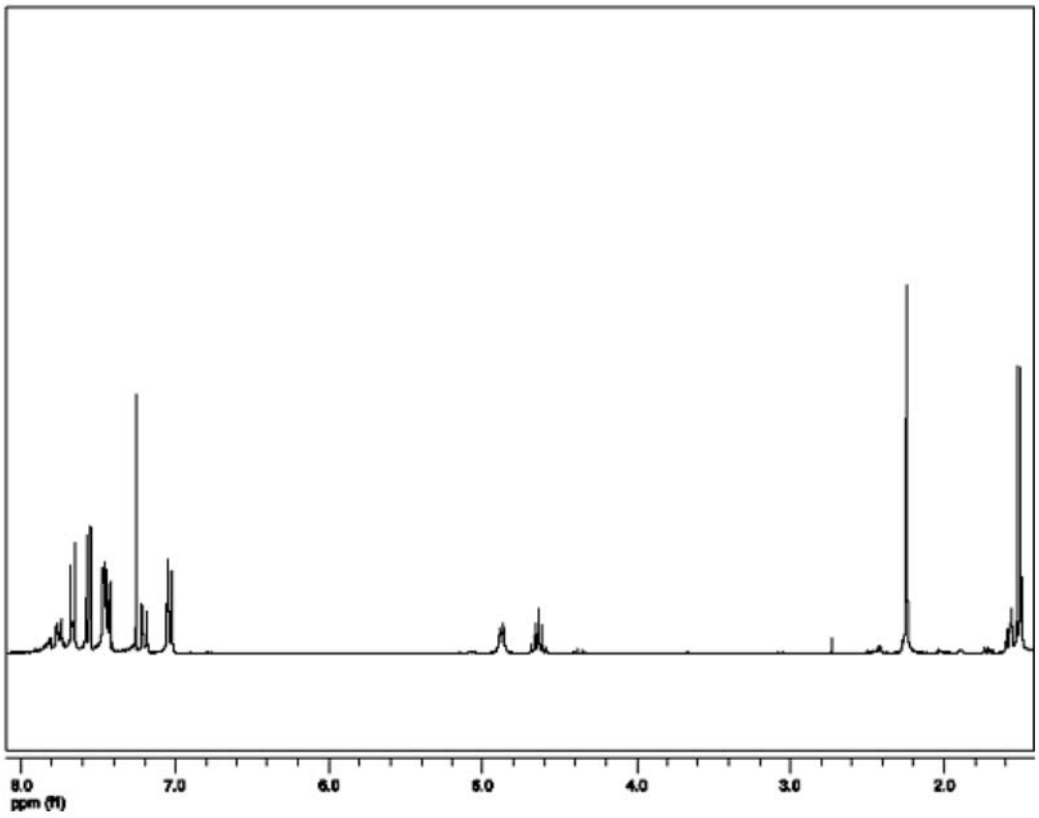


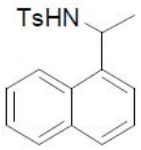
N-(p-Toluenesulfonyl)-1-aminofluorene



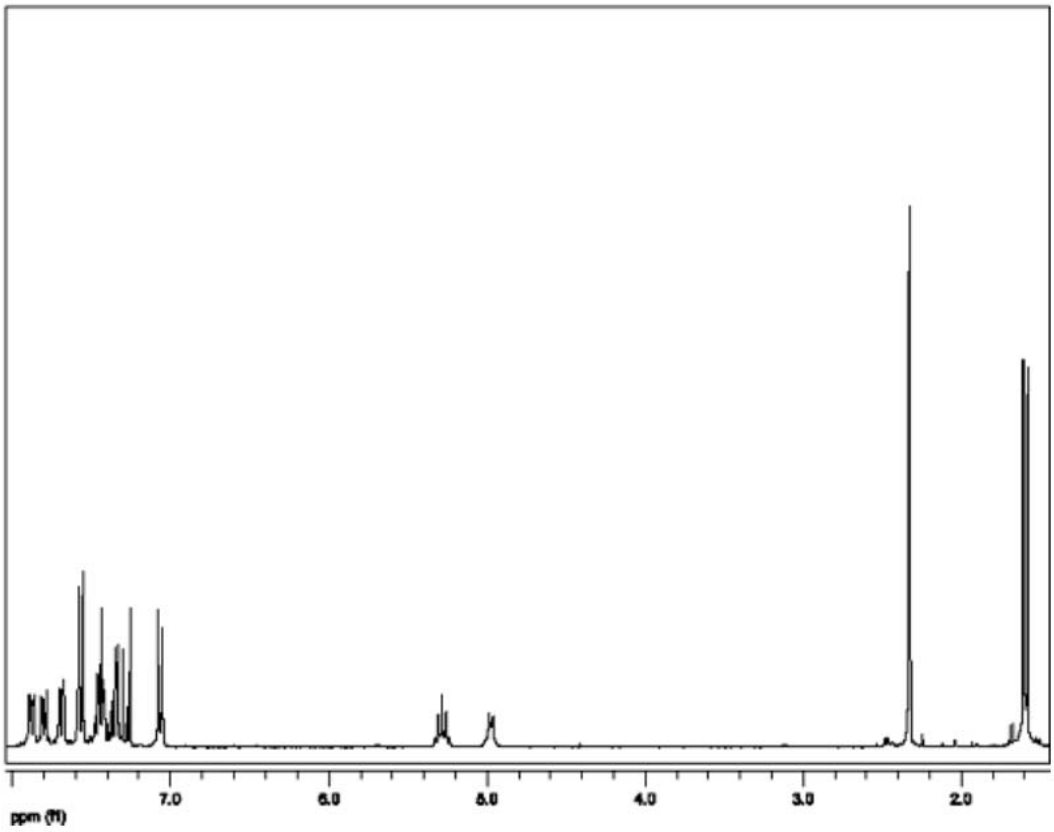
N-(p-Toluenesulfonyl)-1-amino-2-ethylnaphthalene







N-(p-Toluenesulfonyl)-1-amino-1-ethylnaphthalene



Vita

Jeremiah D. Harden was born in Birmingham, Alabama in 1978. He was raised by his loving parents in Alabaster, Alabama where he attended Thompson High School. After graduation he persued his college career at Berea College in Kentucky. Years later he finished his college education with a Bachelor of Science degree in chemistry in 2003 from the Universtity of Montevallo in Montevallo, Alabama. Jeremiah then moved to Tennessee where he earned his Ph.D. in organic chemistry in the fall of 2008.