5-2013

1,2,4-Triazine-Accelerated Azide-Alkyne Cycloaddition and Synthesis of Metalloenzyme Inhibitors

Belinda Shea Lady
blady@utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Belinda Shea Lady entitled "1,2,4-Triazine-Accelerated Azide-Alkyne Cycloaddition and Synthesis of Metalloenzyme Inhibitors." 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:

Michael D. Best, Jimmy W. Mays, Thomas A. Zawodzinski

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

1,2,4-Triazine-Accelerated Azide-Alkyne Cycloaddition and Synthesis of Metalloenzyme Inhibitors

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Belinda Shea Lady

May 2013

To those who have
challenged, supported, and motivated me.

Acknowledgements

First, I would like to thank my parents for instilling in me the value of an education (although they probably didn't think I would spend the past 22 years in school!) and for all their love, support, and encouragement along the long path. To my sister, thanks for serving as my competition, feeding my desire to be as smart as you, and for always wanting to know what I'm working on. To my brothers, thanks for your support and making me tough.

My journey here would not have been possible without all the guidance and lessons learned throughout my high school and college career. Thanks to my math and science teachers along the way who sparked my interest in science in general. I must acknowledge Dr. Darwin Dahl and Dr. Les Pesterfield for without their teaching I may never have become a chemistry major.

I would like to thank my committee members for their time and participation on my committee. I am privileged to have a distinguished committee, so thank you Dr. Michael Best, Dr. Jimmy Mays, and Dr. Thomas Zawodzinski. Also, thanks to my advisor, Prof. Shane Foister for giving me the basis to become an independent researcher. Although not on my committee, I would also like to acknowledge Dr. Craig Barnes. Thanks for advice and assistance and for allowing me to take part in the redevelopment of the general chemistry undergraduate program over the past year.

To the chemistry department at UTK, thank you for the financial support during my time here. Thank you to everyone in the front office, business office, electronics shop, and general chemistry stockroom that help make things run smoother on a day-to-day basis.

Last but not least, I would like to thank the former members of the Foister group that I was privileged to work with. Dave and Jeremiah, you may have scared me a little in my first year, but thanks for showing me all the shortcuts to take and telling funny stories. Thanks Rachel for all the late night studying, food runs, and random walks / naps in front of Ayres Hall. To the undergraduates that I have mentored in the lab, thanks for helping me to realize how much I enjoy teaching. Kelly, Britta, Matt, Kent, and Jillian, I hope that all your hard work and efforts reward you.

Very special thanks to Ashleigh Prince and Ashesh Belapure. I was fortunate to work with both of you for the past five years. Despite some of the not so fortunate events that have occurred, I am glad that our paths have crossed. You both have been excellent labmates and friends. Words cannot express the gratitude I have for all the advice you have given me over the years and for keeping me calm and focused. Also, I am very appreciative of you both for your editing of this dissertation. In addition, thanks to Amber for always lending an ear, giving me encouragement along the way, and helping me see my passion for teaching.

Abstract

The work of this dissertation describes the design and synthesis of 1,2,4-triazine ligands and other N-containing heterocycles and their use in the copper-catalyzed azide-alkyne cycloaddition (CuAAC). A variety of ligands were synthesized to probe the steric and electronic demands required for use in the CuAAC reaction. Substituents on the 1,2,4-triazine were systematically altered and the core 1,2,4-triazine modified to determine the most active ligand. Additional experiments explored the variability in the reaction conditions, such as solvent choice, use of reducing agents, and optimal stoichiometry. Under optimum conditions 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine and copper (II) tetrafluoroborate in the presence of triethylamine was found to be an effective accelerant producing 97% of the desired 1,2,3-triaozle in 1 hour. A broad substrate scope was conducted with an assortment of azides and alkynes.

The use of 1,2,4-triazine-accelerated CuAAC was applied to the synthesis of solid-supported catalysts on both polystyrene and silica. Immobilized catalysts provide advantages over their soluble counterparts in that they can be recycled and can prevent metal contamination of 1,2,3-triazole products. Results indicated that 1,2,4-triazines appended to solid supports were more effective when compared to 1,2,3-triazole control catalysts. In addition, less metal leaching occurred with triazine supports as compared to triazole controls.

The optimal ligand from the homogeneous screening was then used in the synthesis of a library of small molecules containing 1,2,3-triazoles and/or 1,2,4-triazoles. Upon synthesis, compounds were screened for activity against various histone deacetylase (HDAC) enzymes for both activity and selectivity. Although successfully synthesized, the molecules did not prove to be active against the selected metalloenzyme.

Table of Contents

List of Tables

List of Figures

List of Schemes

Abbreviations and Acronyms

ACN	Acetonitrile
AcOH	Acetic Acid
Br_2	Bromine
$CHCl_3$	Chloroform
DCE	Dichloroethane
DCM	Dichloromethane
DMF	N,N-Dimethylfomamide
CuAAC	Copper-Catalyzed Azide-Alkyne Cycloaddition
$Cu(BF_4)_2 \bullet 6H_2O$	Copper tetrafluoroborate hexahydrate
CuCN	Copper cyanide
CuI	Copper iodide
$[Cu(MeCN)_4]^+BF_4^-$	Tetrakis(acetonitrile-N)copper(I) tetrafluoroborate
$CuSO_4 \bullet 5H_2O$	Copper sulfate pentahydrate
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
GC-MS	Gas chromatography – mass spectroscopy
H_2SO_4	Sulfuric acid
HBTU	O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate
HCl	Hydrochloric acid
HDAC	Histone deacetylase
HDACi	Histone deaceytlase inhibitor
HNO_3	Nitric acid
HOBt	1-Hydroxybenzotriazole hydrate
HPLC	High-pressure liquid chromatography
IC_{50}	Inhibitory concentration of half-maximum (50%) activity
ICP-OES	Inductively coupled plasma – optical emission spectroscopy
IR	Infrared spectroscopy
KBr	Potassium bromide
KCN	Potassium cyanide
$KMnO_4$	Potassium permanganate
KOH	Potassium hydroxide
LC/MS	Liquid chromatography-mass spectroscopy
MeCN	Acetonitrile
MeOH	Methanol
MMP	Matrix metalloproteinase
MW	Microwave
$NaHCO_3$	Sodium bicarbonate

NaN_3	Sodium azide
$NaNO_2$	Sodium nitrite
NH_4OAc	Ammonium acetate
NMP	N-Methylpyrolidinone
NMR	Nuclear magnetic resonance
$Pd(OAc)_2$	Palladium(II) acetate
$Pd(PPh_3)_2Cl_2$	Bis(triphenylphosphine)palladium(II) chloride
SAHA	Suberoylanilide hydroxamic acid
SAR	Structure Activity Relationship
SeO_2	Selenium dioxide
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBTA	Tris(benzyltriazolylmethyl)amine
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TSA	Trichostatin A
$SnCl_2 \bullet 2H_2O$	Tin(II) chloride dihydrate
UV-Vis	Ultraviolet – visible spectroscopy

Chapter 1.
Azide-Alkyne Cycloaddition and 1,2,3-Triazoles

1.1. Click Chemistry

In the realm of synthesis, there are two broad schools of thought. One approach is to construct products using combinations of novel reactions regardless of how difficult the target might be, as long as a desired activity is present. Natural product synthesis usually evokes this process to make the difficult molecules or metabolites, naturally occurring in plants, marine species, or other native environments. Sometimes these methods are not conducive to scale-up, commercialization, or synthesis of corresponding analogues. A second approach is to restrict drug discovery targets to molecules that are easy to make and through methods that can be rapidly modified to introduce diversity. Researchers estimate that there are on order of between 10^{62} and 10^{63} druglike candidates.[1] Druglike candidates are defined as having less than 30 non-hydrogen atoms, containing C,H, N, O, P, Cl, Br and stable at ambient temperature, in the presence of oxygen and water.[1] With the amount of probable druglike molecules, it is reasonable to attempt synthesis of molecules that can easily be made. This is a shift to focus more on the activity than the structure of the desired molecule. If the synthesis can be easily modified for diversity elements, one can more rapidly complete a structure-activity relationship (SAR) study.

From this idea, was born the “click chemistry” philosophy described by K.B Sharpless et al.[2] Reactions that fit into the click chemistry philosophy should be “modular, wide in scope, high yielding, generate only inoffensive byproducts removed by nonchromatographic methods, and stereospecific.” Reaction conditions should be insensitive to water and oxygen, use no solvent, a benign solvent, or an easily removed solvent, and be amenable to simple product isolation. Starting materials should be readily available to further expedite the process.[2] This process should be as simple as joining two units together, such as fitting two puzzle pieces together. The reactive fragments should only react with one another and the reaction conditions should be tolerable to a wide array of functional groups, without the need for protecting group chemistry.

The constraints of click chemistry may seem demanding, but several reactions fit into this category. Figure 1.1 shows examples of click chemistry which include oxidative

additions to carbon-carbon multiple bonds such as epoxidation, aziridination, and dihydroxylation. Michael additions of nucleophiles to unsaturated systems also fit into this category, along with nucleophilic ring opening of strained rings, such as aziridines or epoxides. Non-aldol additions to carbonyls such as in formation of ureas, amides, or oxime ethers are also consistent with the constraints of click chemistry. Non-aldol carbonyl chemistry to form aromatic systems also falls within the definition of click reactions. Finally, several cycloaddition reactions, including Diels-Alder transformations and 1,3-dipolar cycloaddition reactions fit into the framework of click chemistry. With the exception of Diels-Alder, the majority of these reactions take advantage of carbon-heteroatom linkages. These heteroatom linkages can be described as mimics of nature, as a large number of diverse compounds (nucleic acids, proteins, polysaccharides) are generated from only a small number of naturally occurring building blocks.

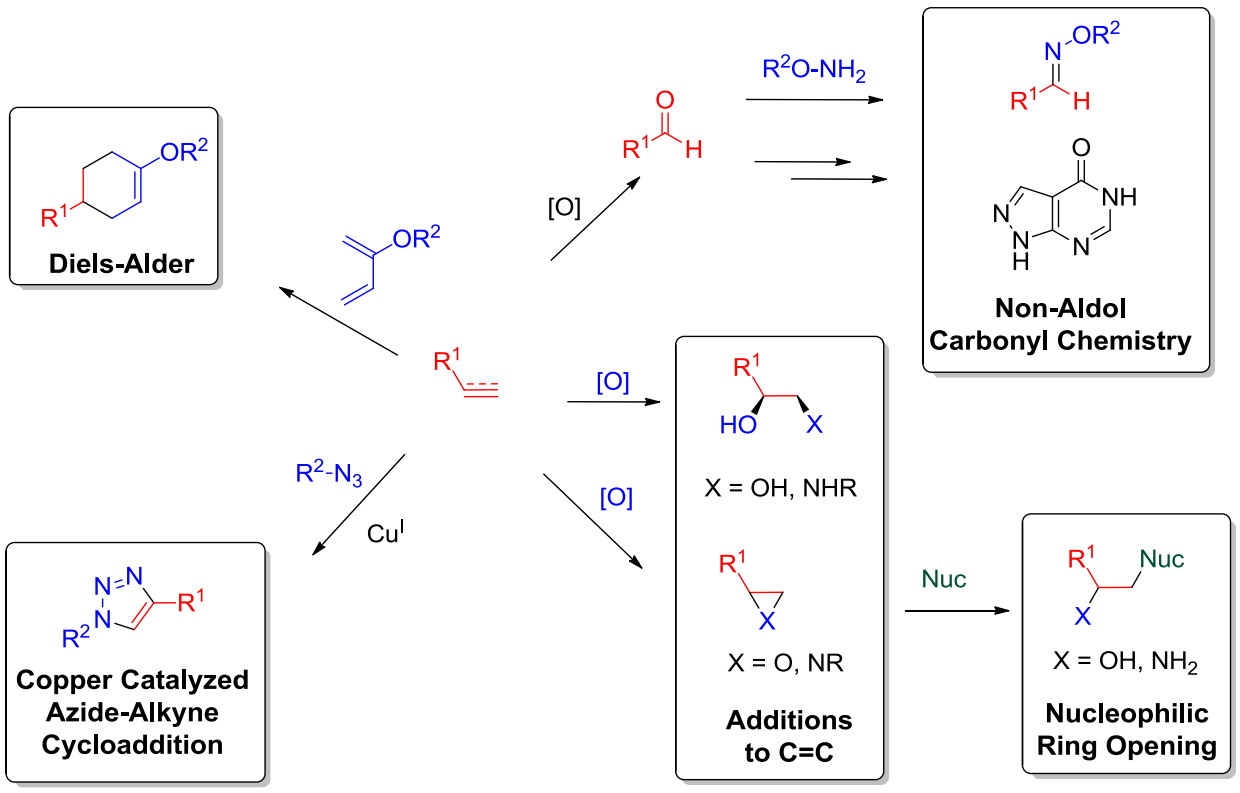


Figure 1.1 Representation of reactions conforming to "click" guidelines. Adapted from Moses et. al[3] & Kolb et. al[4]

Among these reactions, one has emerged as the centerpiece of “click chemistry” sometimes being incorrectly defined as click chemistry, in itself. Since the discovery of the copper catalyzed variant of the Husigen azide-alkyne cyclocaddition (CuAAC), the field of click chemistry has expanded to include drug discovery,[4] biological applications,[5] materials science and polymer chemistry[6,7] and has produced a number of reviews in various areas.[3,8,9] Why has this reaction had such a dramatic impact on the way scientists approach these fields? This reaction is highly regioselective, involves two relatively unreactive starting materials, and forms a stable product that has implications in biological systems.

1.2. 1,2,3-Triazoles as Pharmacophores

The field of drug discovery has been greatly influenced by the discovery of a copper-catalyzed azide-alkyne cycloaddition (CuAAC). This has allowed the rapid synthesis of a number of 1,2,3-triazole containing molecules for a wide variety of applications. Several reviews have summarized the growing trend of triazoles as important pharmacophores and their use in biological applications.[10-14]

One reason for the growing use of triazoles stems from the suggestion as amide bond replacements.[15] Evidence suggests that 1,2,3-triazoles can be both electronically and spatially similar to amide bonds. Amide bonds, in either the E or Z conformation, can be related to disubstituted 1,2,3-triazoles, either in a 1,4- or 1,5-substitution pattern (Figure 1.2).[12] The 1,4-disubstituted-1,2,3-triazoles mimic the Z-amide bond. Electronically, N(2) or N(3) of the triazole can serve as a hydrogen bond acceptor similar to the carbonyl oxygen of an amide. Both the amide and the triazole can also serve as hydrogen bond donors with the C(5)-H of the triazole mimicking the NH of an amide. Both also have electrophilic carbons (C-O vs. C-N). Although similar, the triazole has a larger dipole moment, making its hydrogen bond donor and acceptor abilities greater than those of the amide. This could also contribute to its increased activity in biological systems.

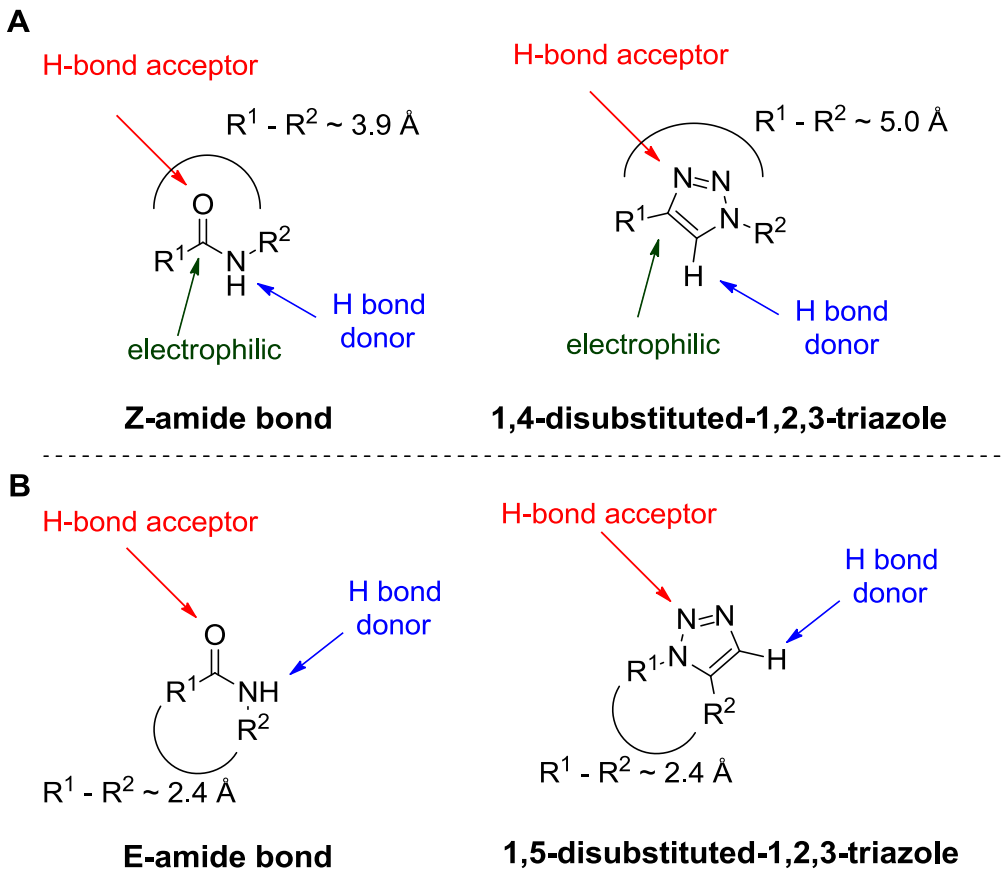


Figure 1.2 Triazoles as isosteres for amide bonds. Modified from Tron et. al.[12] Comparison between **A)** Z-amide bond and 1,4-disubstituted-1,2,3-triazole and **B)** E-amides and 1-,5-disubstituted-1,2,3-triazole.

The geometry and spatial arrangement of atoms in a triazole ring are similar to that of the amide bond as well, with the triazole spacing being slightly longer by 1.1 Å.[12] In addition, triazoles have also been demonstrated to retain conformational geometries in secondary structures when replacing amide bonds in α-helices[16] and β-turns.[17]

There are two significant differences between amide bonds and triazoles, both of which tip the scales in favor of the triazole: stability and ease of synthesis. Triazoles are stable under a wide array of conditions, including resistance to hydrolysis, oxidation, and reduction, conditions by which the amide bond is susceptible. Triazoles are compatible with either acidic or basic media, and degradation by metabolic pathways does not interfere with the triazole scaffold. With the discovery of copper-catalyzed azide-alkyne cycloaddition and the analogous ruthenium-catalyzed azide-alkyne cycloaddition, the

synthesis of 1,2,3-triazoles is much less laborious and has abounded in a number of biologically relevant molecules being synthesized. Because triazole synthesis is amenable to a variety of functional groups, diverse libraries of compounds can be quickly and efficiently synthesized to screen for a wide array of biological activities.

The triazole core is present in a variety of drug leads (Figure 1.3) such as antiviral,[18] anti-HIV,[19] antituberculosis activity,[20,21] antifungal,[22] anti-inflammatory,[23] and antiobesity.[24] Additional uses of 1,2,3-triazoles are in the design of histone deacetylase (HDAC) inhibitors,[25] or resveratrol mimics,[26] and anti-cancer therapeutics.[27] Some triazole containing compounds have undergone clinical trials or are commercially available (Figure 1.4). The synthesis of additional triazole molecules is now greatly aided by the copper-catalyzed azide-alkyne cycloaddition.

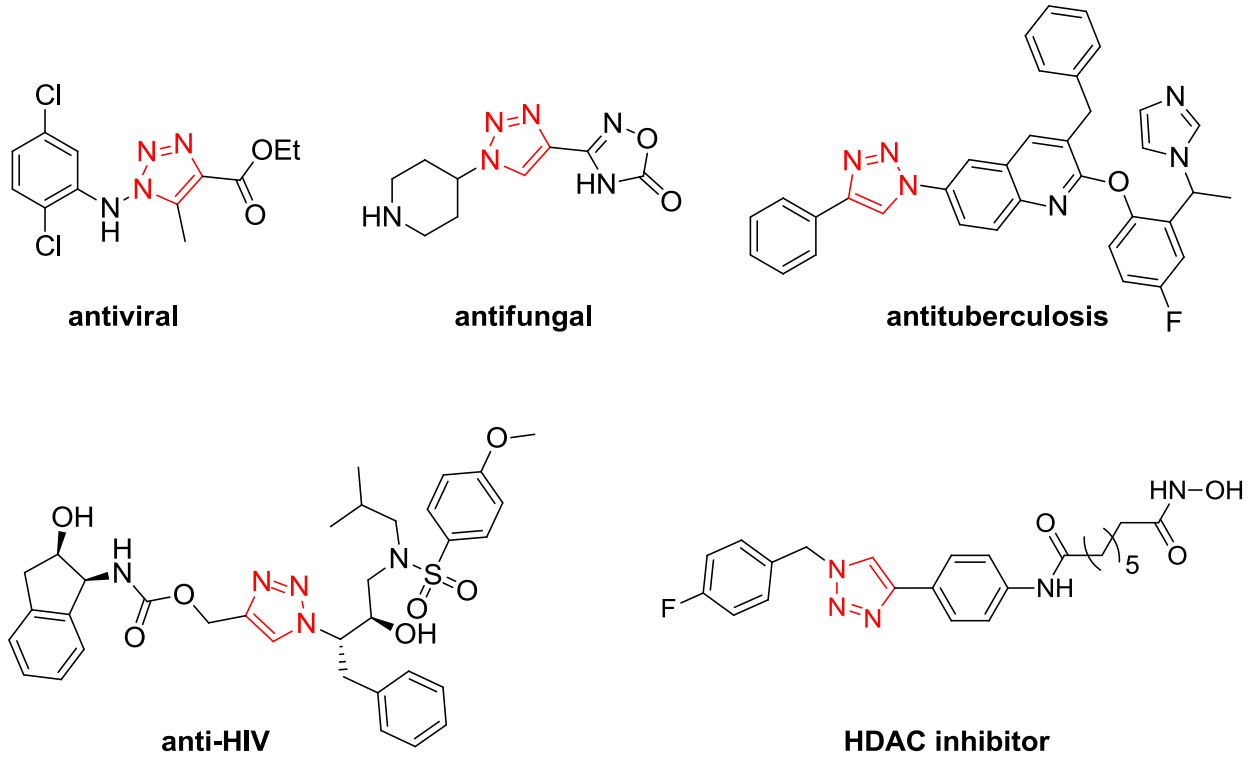


Figure 1.3 Selected molecules containing 1,2,3-triazoles and their potential applications.

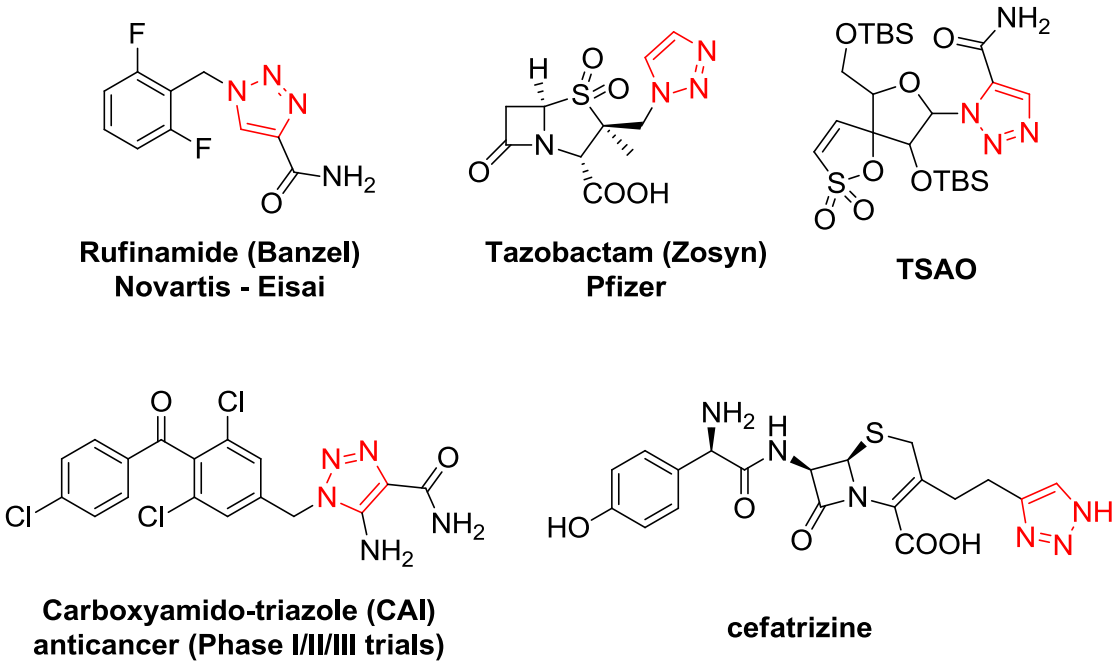


Figure 1.4 Potential pharmaceuticals containing a 1,2,3-triazole.[11] Generic and trade names listed for commercial products along with supplier.

1.3. Azide-Alkyne Cycloadditions

The azide-alkyne cycloaddition has made it possible to synthesize a large number of 1,2,3-triazoles. Even prior to the discovery of the copper catalyzed variant, thousands of 1,4-disubstituted-1,2,3-triazoles had been synthesized. The azide-alkyne cycloaddition falls into the family of 1,3-dipolar cycloadditions, which was the topic of extensive research by Rolf Huisgen and colleagues.[28] 1,3-Dipolar cycloadditions provide a general method of synthesizing 5-membered heterocycles, starting from a 1,3-dipole, containing both electrophilic and nucleophilic atoms and a dipolarophile, containing a multiple bond. The azide-alkyne cycloaddition focuses on the azide as the dipole and alkyne as the dipolarophile. The first 1,2,3-triazole synthesis was performed by A. Michael in 1893.[29] Further work was explored by Huisgen throughout the 1950s-1970s. The original reaction between azides and alkynes was thermally controlled, leading to prolonged reaction times, low yields, and low selectivity. Changes to the electronics of the azide or alkyne contribute to the overall reactivity, making the scope of the reaction limited. With cycloadditions, there are two possible overlaps between the dipole HOMO

and dipolarophile LUMO or dipole LUMO and dipolarophile HOMO. Because the difference in the molecular orbitals is small, a mixture of regioisomers is formed.[9] Electron deficient alkynes could be used to tune the reaction to achieve better regioselectivity, but this again limits the overall diversity attached to the triazole scaffold.

Although the triazole formation could be slow and non-regiospecific, there was still a desire to further investigate the azide-alkyne cycloaddition. Organic azides are unique dipoles in that they can be made, handled, and stored as stable compounds. Azides and alkynes are relatively non-polar, and can remain hidden from and unreactive with most other functionalities, allowing their installation early on in a synthetic scheme. The reaction would also be atom-economical, employing accessible dipoles and dipolarophiles. Finally, the triazole product is a stable aromatic structure (serving as an amide bond replacement).[9] If the azide-alkyne reaction could be catalyzed and be tuned for regioselectivity, it had potential to find a place in the “click chemistry” world.

In 2001, the groups of Meldal[30] and Fokin and Sharpless[31] accomplished this feat and discovered the copper-catalyzed variant of the azide-alkyne cycloaddition, which exclusively produced the 1,4-disubstituted triazole isomer. The analogous ruthenium catalyzed reaction to produce the 1,5-disubstituted isomer (or 1,4,5-trisubstituted isomer if internal alkynes are used) was also discovered by Fokin and Sharpless.[32] An additional method of producing 1,2,3-triazoles was also sought after especially for biological applications in which copper toxicity limits applications with living cells. Several researchers have explored the use of strained cyclooctyne systems to perform selective cycloaddition reactions in living systems.[33,34] This copper free process has allowed the selective modification of biomolecules to study proteins, lipids, bactereriophages, and small organisms, such as zebrafish.[34] Although this method can produce two regioisomers, this would not have an adverse effect on the biological applications. The four methods of producing triazoles from azides and alkynes are illustrated in Figure 1.5.

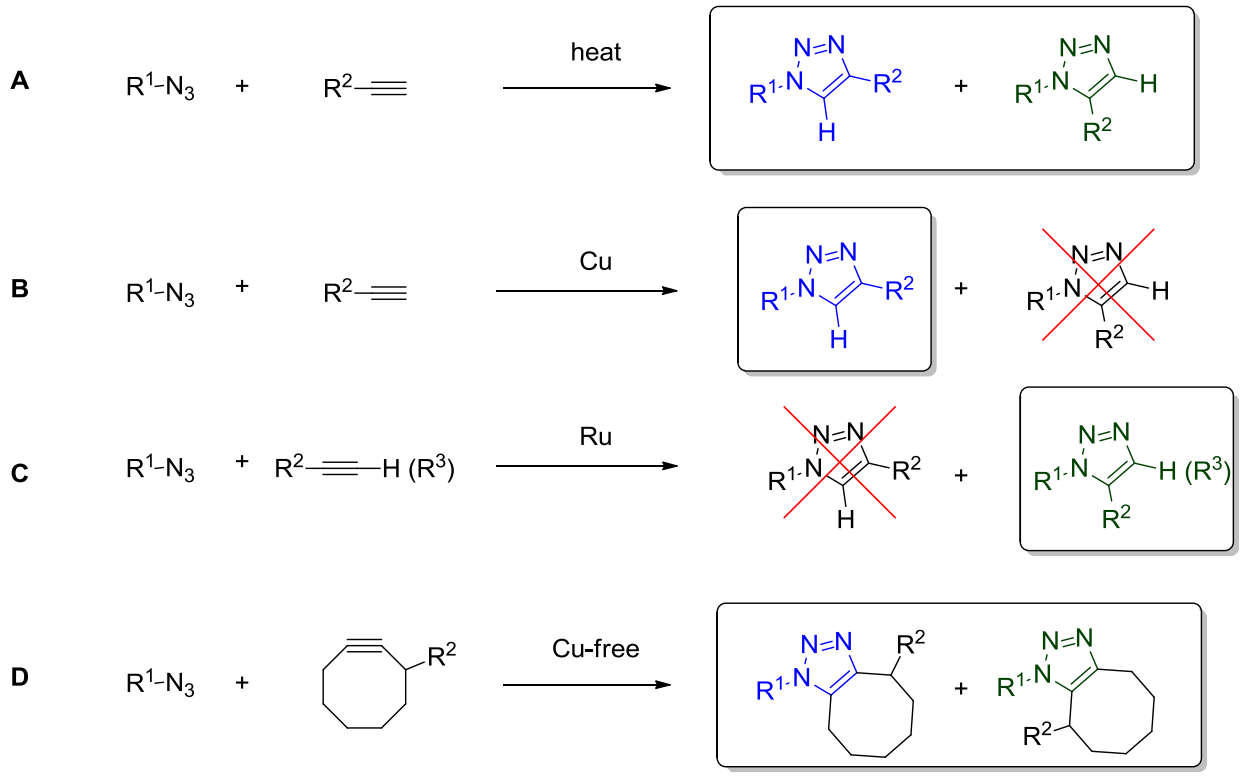


Figure 1.5 Synthesis of 1,2,3-triazole regioisomers. Synthesis can occur through (A) thermal cycloaddition, yielding a mixture of regioisomers, through (B) copper-catalyzed variant producing 1,4-disubstituted-1,2,3-triazoles, or through (C) ruthenium-catalyzed variant producing 1,5-disubstituted or 1,4,5-trisubsituted triazoles or (D) copper-free cycloaddition of strained alkynes

1.4. Copper-Catalyzed Azide-Alkyne Cycloaddition

In addition to providing a regioselective path to 1,2,3-triazoles, the copper-catalyzed variant also accelerates the cycloaddition on the order of 10^6 relative to the uncatalyzed reaction.[9] This allows the reaction to now take place within a reasonable time frame, minutes or hours as opposed to days, and at room temperature. The catalyzed variant is also not limited by sterics or electronics of the azide or alkyne component, and most organic functional groups are not affected, eliminating the need for protecting groups. Due to the rate acceleration and the high regioselectivity, azide-alkyne cycloaddition has become a leading method for covalent assembly of molecules, large and small.

The copper-catalyzed reaction is fairly insensitive to reaction conditions, as long as a source of Cu^I is present. Both aqueous and organic solvents may be employed and the

reaction proceeds with solution-phase or solid-supported reagents. An important note is that Cu^{I} must be present or generated in situ and should be kept at a high level with regards to the inactive Cu^{II} or Cu^{0} oxidation states. An extensive list of Cu^{I} sources has been compiled and summarized by Meldal.[8] The Cu^{I} can come from three main categories, from a Cu^{I} salt, from a Cu^{II} salt and a reducing agent, or through oxidation of Cu^{0} found in copper wire, turnings, or powders. Of these methods, the most commonly employed is the use of copper (II) sulfate in the presence of a reducing agent, commonly sodium ascorbate. These reaction conditions allow for less air-susceptibility, not requiring dry solvents, or inert atmospheres to carry out the reaction. Copper sulfate in the presence of a reducing agent and alkyne can form the active copper-acetylide species required for the reaction immediately, but this process is not as facile for copper halides such as CuI or CuBr. These salts typically require additional amine base or high temperatures to form copper-acetylides. The copper halides favor anhydrous conditions and tend to have better solubility in solvents of intermediate polarity such as acetonitrile, tetrahydrofuran, and dimethylsulfoxide. The final method is the use of elemental copper. The downside to this method is the increased reaction time, since the active species must be formed first. The advantage lies in product isolation in which solid copper can be easily removed.

Recent reports have attempted to explain catalysis of azide-alkyne cycloaddition by copper (II) salts with the argument that these reactions are catalyzed by copper (II) and not the more common copper (I) state.[35] According to the experts in the field,[9] these reports are not accurate. The most likely explanation for these reports is that reduction of the starting copper (II) salt is occurring to produce copper (I). Since Cu^{II} serves as a good oxidizing agent, it is likely that solvent or even starting alkynes are being oxidized, resulting in the reduced and catalytically active Cu^{I} species. Even a small amount of copper (I) in the reaction mixture is sufficient to catalyze the cycloaddition.[8] Although there has been much success in the field of copper-catalyzed azide-alkyne cycloaddition, there was a desire to find better conditions, such that less copper could be employed and separation from copper salts was not as tedious or cumbersome.

1.5. Ligand-Accelerated CuACC (Homogeneous & Heterogeneous Applications)

Although not a necessity, many researchers have chosen to include a ligand to enhance either the reaction rate or to protect the active Cu^{I} from oxidation. Additional benefits from utilizing ligands are reduced catalyst loadings and improved product purity with less copper contamination. Ligands can be especially useful for demanding applications. The added ligands can either be soluble homogeneous ligands or ligands attached to an insoluble support. A large number of ligands have been synthesized, generally including heteroatoms, and reviewed.[36] Nitrogen based ligands make up the majority of those explored for azide-alkyne cycloaddition. The simplest and earliest additives employed were amines such as 2,6-lutidine, triethylamine or diisopropylethylamine (DIEA), or proline.[37] Other early ligands involved nitriles, guanidines, imines, or halides. With the early ligands, two main problems arose. Some of these ligands (halides and amines) were too labile and did not protect the copper from oxidation to an inactive state. Other ligands (nitriles) were found to bind the metal too tightly, resulting in decreased reactivity of the needed copper center.[8] Sharpless and Fokin[38] realized during kinetic investigations that reaction rates of selected substrates were extremely high, almost appearing autocatalytic; the triazole product could be catalyzing its own formation. Since triazoles had earlier been difficult to synthesize, using triazoles as ligands for product triazole formation had previously been unexplored. This work began a new approach in designing ligands for CuAAC. TBTA (Figure 1.6, **1-1**) was found to be an effective ligand.[38] From this work, stemmed several additional ligands. Finn and coworkers kept the central amine framework the same and changed the surrounding heterocycle. The 1,2,3-triazole was exchanged for benzimidazole, benzothiazole, pyridine, and related derivatives. A systematic exploration of various hybrid ligands was undertaken to determine optimal reaction conditions, mechanistic insights, and dependence on solvent and ligand metal stochiometries.[39] This led to hybrid or mixed ligands similar to TBTA, but with more than one different heterocycle attached to the core amine. $Bim(Py)_2$ (**1-3**) was found to be the best ligand for strict organic conditions.

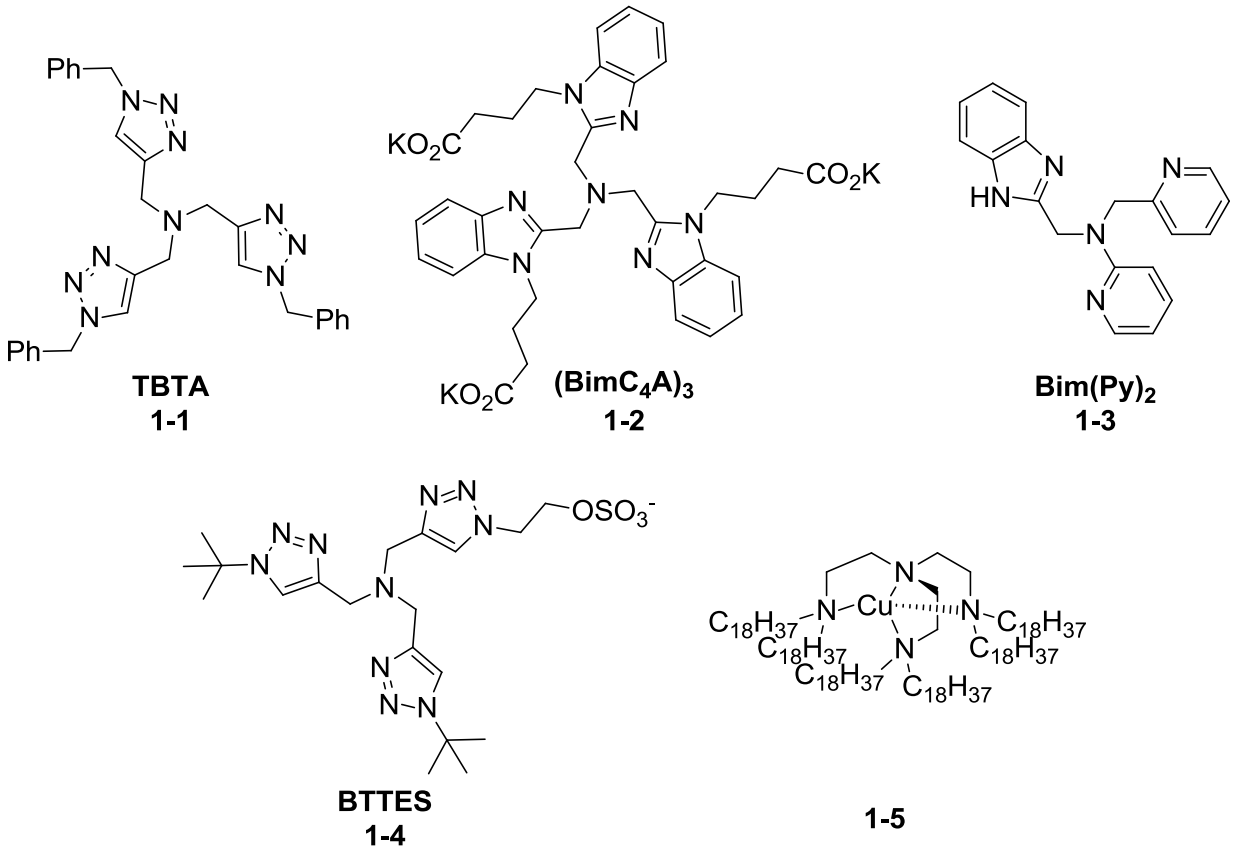


Figure 1.6 Heterocyclic ligands used for azide-alkyne cycloaddition.

Inspired by this work and the results observed, other researchers began to investigate tripodal ligands (Figure 1-7). One downfall of the TBTA system was its poor water solubility. This leads to difficulty in biological applications as an increased copper loading is necessary, resulting in toxicity within living systems. As a potential solution, researchers designed additional analogues of TBTA and examined their water solubility, azide-alkyne reaction rate, and cytotoxicity.[40] Addition of BTTES (**1-4**) premixed with copper sulfate (and reduced with sodium ascorbate) was found to be a more effective accelerant of azide-alkyne cycloaddition compared to TBTA-Cu(I) catalyst. In addition, cell death was suppressed in BTTES-Cu(I) treated cells compared to Cu(I) treated cells, showing a decreased toxicity. Additional work looked at catalysts containing long alkyl amines around a central nitrogen atom. This [Cu(C18$_6$tren)]Br catalyst (**1-5**) had been

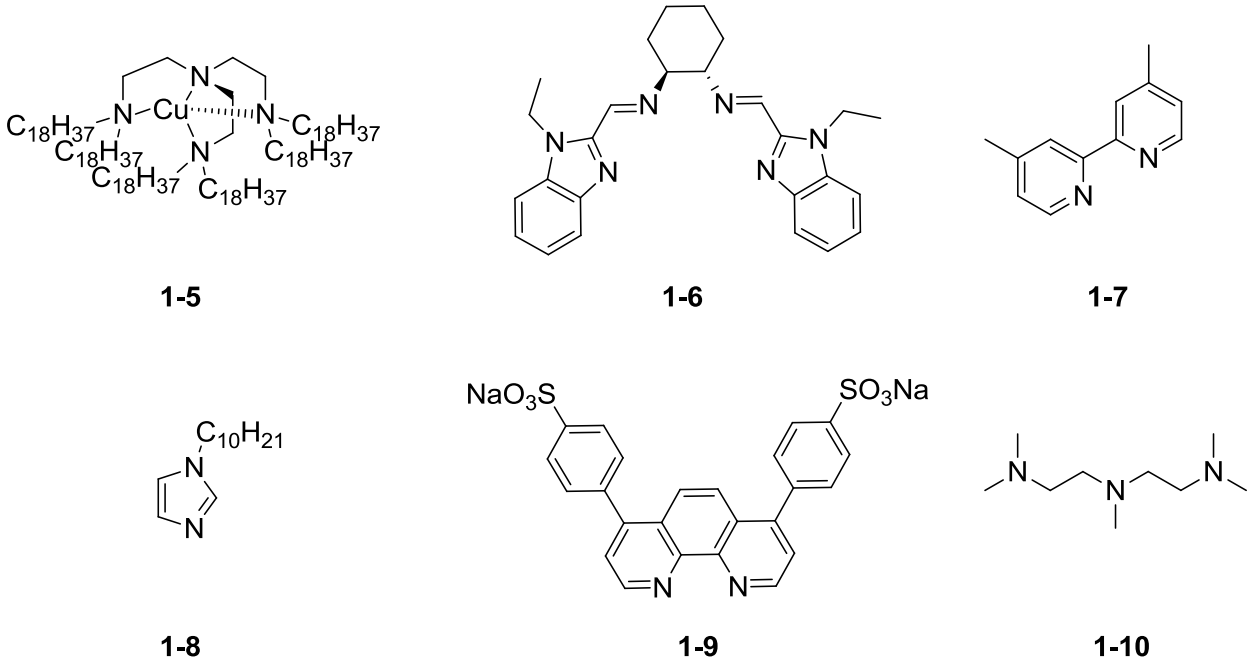


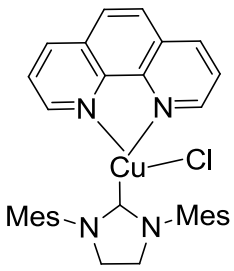
Figure 1.7 Additional nitrogen containing ligands used for azide-alkyne cycloadditions.

previously prepared and used in atom transfer radical polymerization (ATRP) reactions.[41] Because of the increased stability towards aerobic conditions and its ability to be isolated and handled without air-free techniques, it was screened as a catalyst for azide-alkyne cycloaddition. This proved to be effective and reusable up to three times without a loss in activity. Additional benzimidazole ligands were synthesized by Li and Hor[42] with **1-6** showing good activity for both azide-alkyne cycloaddition and N-arylation reactions.

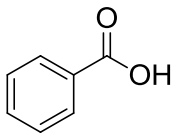
Other nitrogen containing ligands have been explored including bipyridines (**1-7**), phenanthrolines, and imidazoles. Imidazoles, such as **1-8** (Figure 1.7) with a long alkyl linker were found to give cycloaddition products in good yields when performed neat or in water.[43] Sulfonated bathophenanthroline (**1-9**) has proven to be superior to TBTA under some conditions and has proved useful in bioconjugation contexts.[44]

Ligands can also be extended to include other heteroatoms, such as phosphorus, sulfur, oxygen, or carbon. NHCs (N-heterocyclic carbenes) contain nitrogens but are actually coordinated through a carbon (or more specifically a carbene). Most of the studies with NHC-containing ligands focus on methodology, and have not fully been explored within the context of complex systems, although promising results are present. The best NHC ligand has resulted from the addition of an aromatic nitrogen based ligand, such as phenanthroline[45] to combine the best of both features in one molecule. Figure 1-8 illustrates a mixed NHC-phenanthroline copper complex (**1-11**) that was used in the synthesis of potential metal chelators. In general, there remains work in this field to match the abilities of other ligands in azide-alkyne cycloadditions.

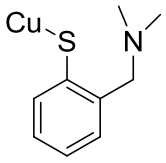
Additional ligands involving other heteroatoms are relatively scarce. Two notable phosphorus complexes are $[CuBr(PPh_3)_3]$ and $[Cu(phen)(PPh_3)]NO_3$.[46,47] The former adhered to strict "click" conditions, using low catalyst loadings, benign solvents, short reaction times, and no additional purifications. Oxygen ligands have also been used, most notable are carboxylic acids. Copper (I) acetate has been shown to outperform other Cu^I salts in the absence of other additives. The theory behind this activity was the ability of the acetate to effectively coordinate the copper. Other carboxylic acids such as benzoic acids (**1-12**)[48] have been shown to act as ligands. Finally, sulfur ligands have displayed good activity even if there is not much interest in this area. This ligand (**1-13**) was successfully used to decorate dendrimers with a derivatized sugar azide.[49]



1-11



1-12



1-13

Figure 1.8 Catalysts invovling metal-coordination atoms other than nitrogen.

Attempts have been made to compare the various ligand-accelerated reactions, but because of the wide variety of reaction conditions employed it has been difficult to carry out a direct comparison. Despite the ability of several ligands to accelerate the cycloaddition of simple azide alkyne systems, there remains a need to identify and investigate ligands for increasingly demanding applications.

An additional growing area of research is the use of heterogeneous materials to catalyze the azide-alkyne cycloaddition. Heterogeneous ligands and catalysts can have distinct advantages over their homogeneous counterparts. The main advantages with using solid supports are the ease in product isolation, decreased metal contamination of products, and potential recyclability of the catalyst. In addition, a wide range of insoluble supports are commercially available. Some heterogeneous materials can be directly used in the presence of metal to catalyze the desired cycloaddition reaction. Other materials can be easily modified to covalently attach a metal binding ligand, and then used for catalytic applications. Surfaces that have been used to catalyze the cycloaddition include copper clusters[50], copper-aluminum hydrotalcite,[51] copper zeolites,[52] copper nanoparticles on alumina[53] and copper in charcoal.[54] Although these surfaces can coordinate copper, there was an effort to design more steric and electronically demanding ligands to match those of homogeneous ligands. Fokin and Chan[55] developed a solid supported version of TBTA (**1-14**) to lead the way. The structures of a selection of solid supported catalysts are illustrated in Figure 1.9. These include other ligands attached to polystyrene resins[56] and silica.[57,58] These heterogeneous catalysts have proven to be useful from a synthetic standpoint. There still remain some disadvantages in terms of cost of support-bound catalysts, increased reaction times, and increased temperatures. There is still a need to develop better heterogeneous catalysts for CuAAC.

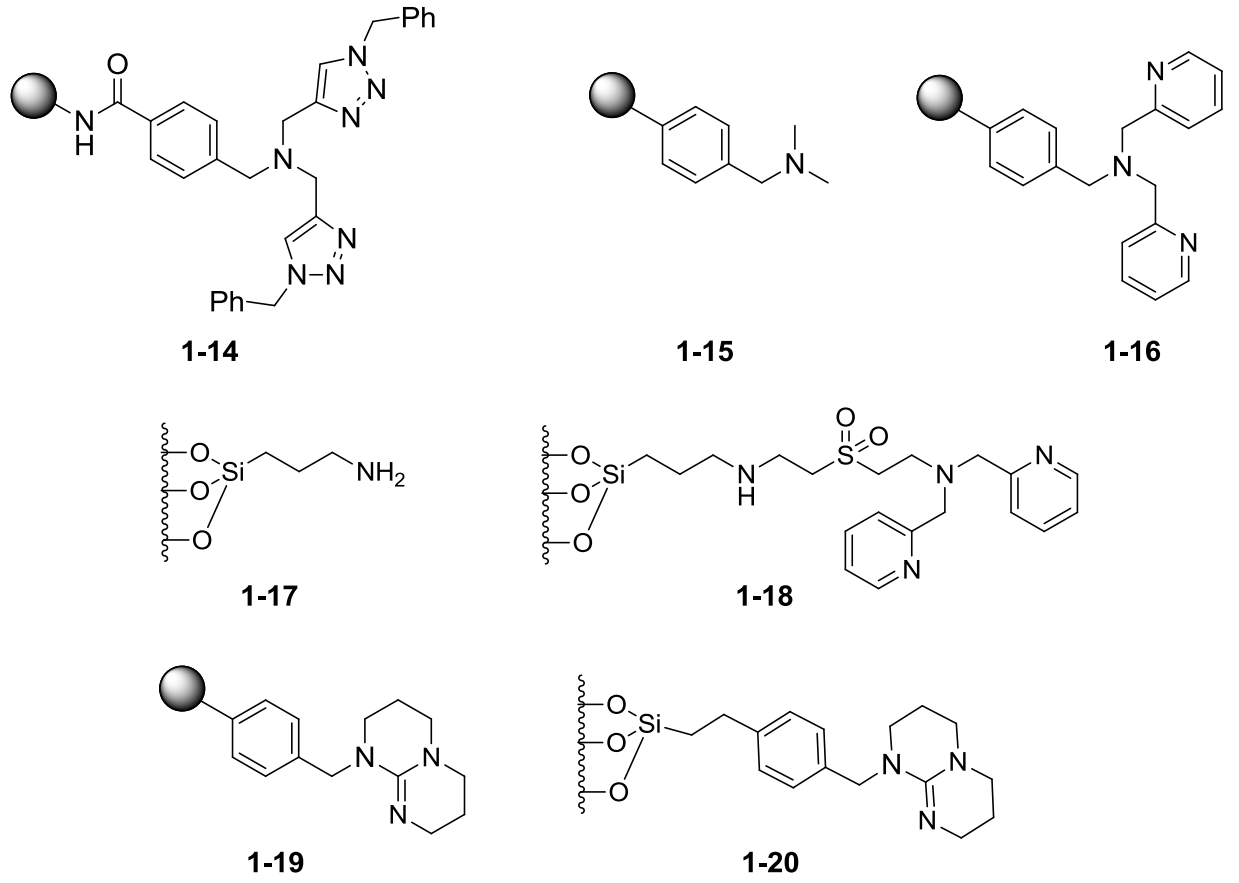


Figure 1.9 Examples of heterogeneous ligands used to accelerate CuAAC.

1.6. Mechanistic Considerations

From a mechanistic perspective, it is important to realize that the reaction is fairly insensitive to reaction conditions, performing in the presence of Cu^{I}, with aqueous or organic solvents, and working in either solution- or solid-phase environments. Many reports exist in an effort to explain the mechanism of the azide-alkyne cycloaddition. Experimental evidence has been collected with regards to copper-catalyzed cycloadditions in both the presence and the absence of additional ligands.

Using pseudo-first order kinetics or through DFT calculations, several mechanistic theories have been presented to explain experimental observations. A point of dispute between researchers is the nature of the copper intermediate. Copper acetylides and copper π-complexes have both been proposed as intermediates. Cantillo and

coworkers recently performed a computational analysis using DFT to compare the various proposed mechanisms and to compare catalyzed and uncatalyzed pathways.[59] As can be expected, in the uncatalyzed version, no significant energy difference exists between the two possible activation energy barriers leading to 1,4- or 1,5-disubstituted-1,2,3-triazoles. This explains the lack of regioselectivity in the uncatalyzed version, and the high activation energy of 36 kcal/mol for both pathways explains the sluggish reaction. From their research, activation energy barriers were calculated for the following proposed intermediates: mononuclear copper acetylides, dinuclear copper acetylides, mononuclear and dinuclear pi copper complexes, and tetranuclear copper acetylides. These values are summarized in Table 1.1.

The lowest activation energy barrier points to the copper acetylides with two metal centers as being a plausible intermediate, which was previously proposed by Fokin et. al through kinetic studies.[60] Figure 1.11 highlights the catalytic cycle for the copper-catalyzed azide-alkyne cycloaddition. The initial step begins with coordination of the copper with the alkyne to form the π-alkyne copper complex. The coordination of the copper lowers the pK_a of the alkyne proton by approximately 10 units, making it much more acidic. The alkyne is deprotonated and the copper acetylide forms. It is proposed that a second copper interacts with the copper acetylide through a second π-complex. The azide then becomes activated by coordination to the copper atom. An unusual six-membered metallocycle is formed. The ring contraction step is highly favored and occurs rapidly to form the copper-triazolyl species. Proteolysis is the final step in which the copper is regenerated and the triazole product formed.

Depending on the conditions employed, byproducts can result during the azide-alkyne cycloaddition. Common byproducts are shown in Figure 1.12. These byproducts are a result of oxidative couplings which are also catalyzed by copper (I) species. Fortunately, methods exist to minimize the amount of byproducts formed by addition of reducing agents or metal-binding ligands.

Table 1.1 Comparison of activation energy barriers for proposed intermediates in CuAAC.

	Activation Energy Barrier (1,4-disubstituted-1,2,3-triazole) kcal/mol	Activation Energy Barrier (1,5-disubstituted-1,2,3-triazole) kcal/mol
uncatalyzed	36.1	35.6
mononuclear copper acetylide	27.1	40.1
mononuclear π complex	29.1	34.0
dinuclear π complex	21.7	27.6
tetranuclear copper acetylide	28.5	38.7
dinuclear copper acetylides	16.0	20.4

1.7. 1,2,4-Triazines as Ligands

Previous challenges in our group had included synthesis of 1,2,3-triazoles, even in the presence of a supporting ligand. We embarked on a project to pursue ligands to improve the efficiency of the copper-catalyzed azide-alkyne cycloaddition. Our inspiration for using 1,2,4-triazines was their known ability to coordinate various transition metals and several complexes, especially with copper, had been synthesized and studied.[61-64] Through these studies 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine was shown to coordinate a copper atom through coordination of N2 of the triazine and the nitrogen of the pyridine substituent (Figure 1.12). It could be hypothesized that coordination could occur through the N4 position of the 1,2,4-triazine, but the substituent at position 5 creates steric bulk disfavoring that conformation. In addition, to the binding of copper, it is proposed that triazines can stabilize copper (I) oxidation state by disfavoring disproportionation.[65,66] Based on the literature precedents, 1,2,4-triazines were ligands worthy of exploring.

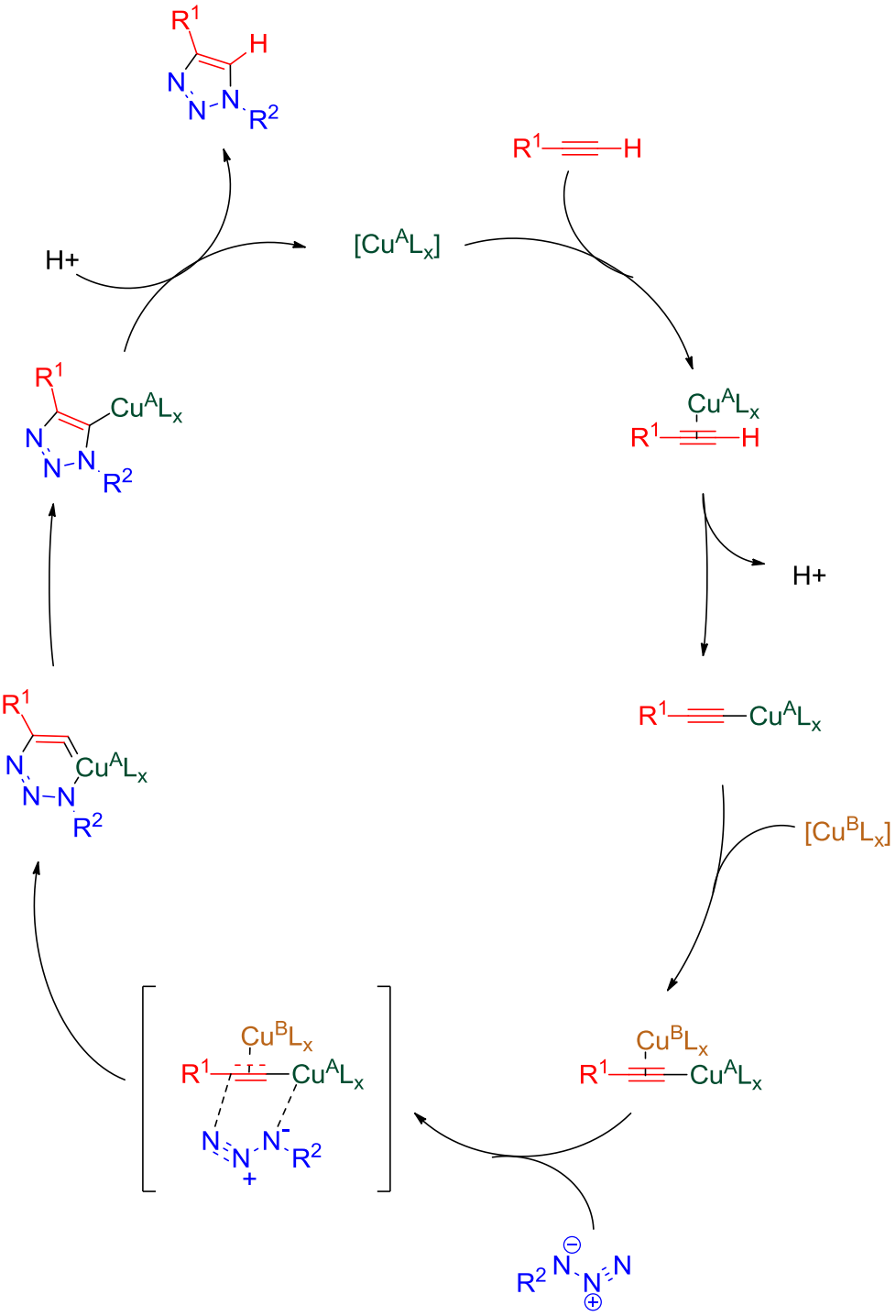


Figure 1.10 Proposed catalytic cycle of CuAAC.[67]

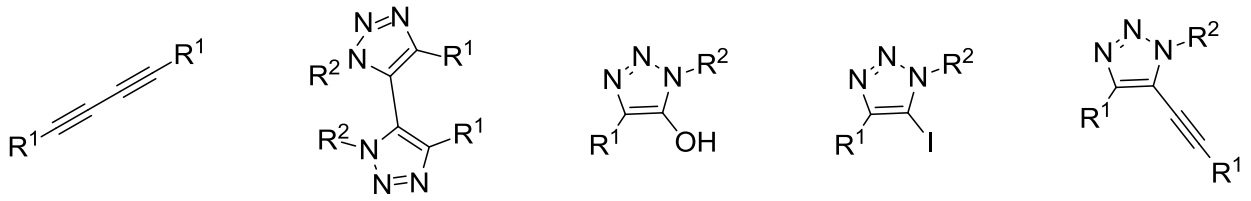


Figure 1.11 Possible byproducts observed during CuAAC.

1.8. Scope of this Work

This dissertation describes the research towards using 1,2,4-triazines as ligands for the copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC). Chapter 2 details the design and synthesis of 1,2,4-triazines and similar nitrogen containing heterocycles. Chapter 3 discusses the catalytic activity of both homogeneous 1,2,4-triazines and heterogeneous 1,2,4-triazines, with regards to CuAAC. An extension of the catalytic activity was then applied to a small study on one-pot Sonogashira-CuAAC reactions. Chapter 4 details the synthesis of small molecules as potential metalloenzyme inhibitors using 1,2,4-triazines as ligands. The activity of these synthesized inhibitors in fluorescence assays against various histone deacetylase (HDAC) isoforms will also be discussed.

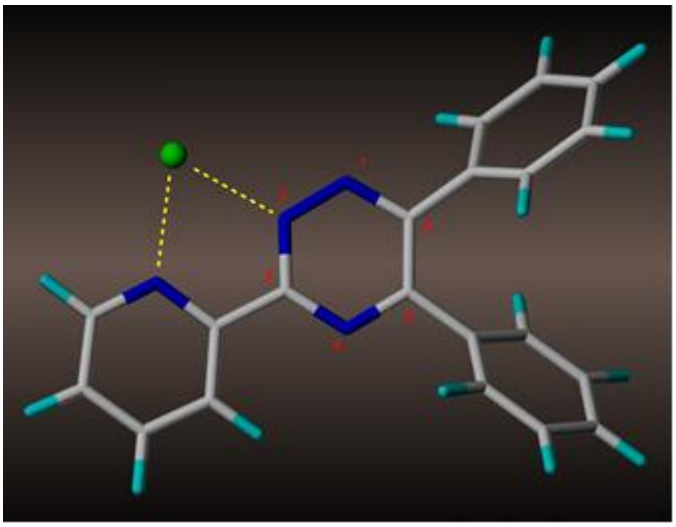


Figure 1.12 Coordination mode of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine

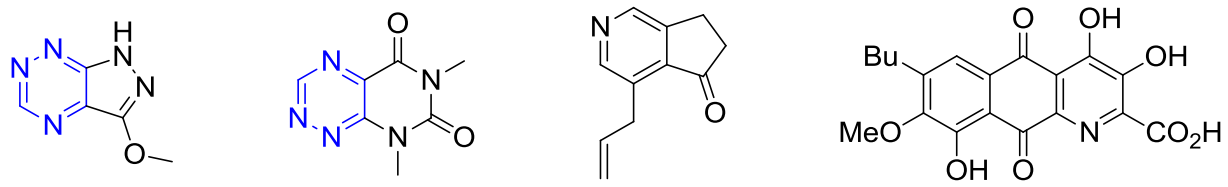
Chapter 2.

Design & Synthesis of 1,2,4-Triazine Ligands

2.1. Background & Significance

1,2,4-Triazines are well established compounds with a number of publications describing the preparation, physical or theoretical properties, and applications, including a thorough review by Lindsley et al.[68] The 1,2,4-triazine core is scarce in natural products with compounds **2-1**[69] and **2-2**[70] (Figure 2.1) highlighting two rare naturally occurring examples. Despite the lack of natural occurring sources, the triazine core has found utility as an intermediate in the synthesis of natural products. It is especially useful in the synthesis of pyridine ring systems by undergoing Diels-Alder reactions with numerous dienophiles, as can be seen by the synthesis of the pyridine alkaloids of the Louisianin family (**2-3**)[71] and phomazarin (**2-4**).[72] The potential uses of triazines are encompassing of many areas including molecular probes (**2-5**),[73] potential therapeutics for Parkinson's disease (**2-6**),[74] or cancer treatments (**2-7**).[75] Additional applications of 1,2,4-triazines include use as metal chelators (**2-8**),[76] dyes, as backbone of polymers (**2-9**),[68] or as agrochemicals such as the herbicide (**2-10**).[77] A selection of molecules with a 1,2,4-triazine scaffold are included in Figure 2.2.

Of the potential uses of triazines, the metal chelation abilities sparked our interest in the triazine scaffold. Several metal complexes have been synthesized, their crystal structures analyzed and properties investigated, including complexes of copper,[63] iron,[78] and even lanthanide metals.[79] Of particular interest were copper complexes of 1,2,4-triazines.[61] Copper complexes of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazines showed a high Cu^{II}-Cu^{I} redox potential, which is synonymous with destabilization of Cu^{II}. Prior to



2-1 **2-2** **2-3** **2-4**

Figure 2.1 Natural products containing or synthesized from 1,2,4-triazine.

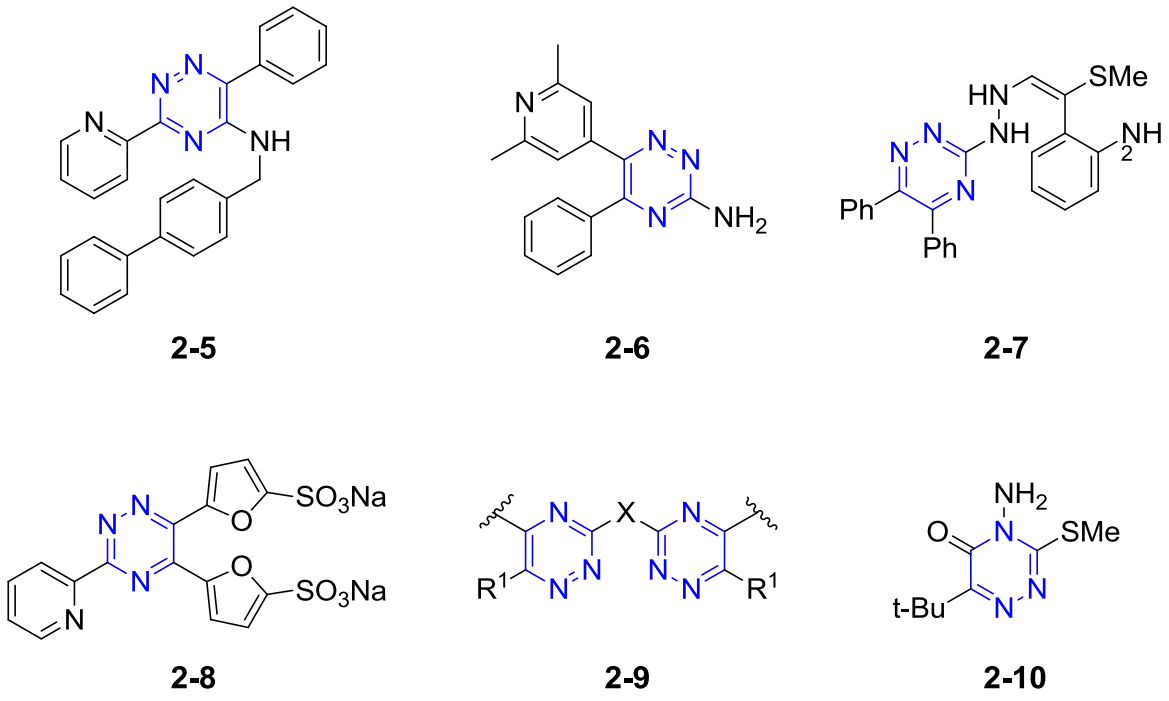


Figure 2.2 Selected molecules containing 1,2,4-triazine scaffold.

this research project, an underlying interest was the synthesis of 1,2,3-triazoles for bioorganic research, but that project had struggled due to a lack of an efficient way to synthesize the molecules of interest. Our goal became to investigate the abilities of 1,2,4-triazines to accelerate copper-catalyzed azide-alkyne cycloaddition, and use that to synthesize medicinally relevant compounds. Literature is abundant on the uses of triazines as metal chelators and the use of various nitrogen ligands as ligands for CuAAC, but prior to this work these two had not been merged. Further support for this project was gained since the precursors needed for synthesis of triazines were readily available and overlapped with precursors needed for other ongoing projects within the group (Figure 2.3).

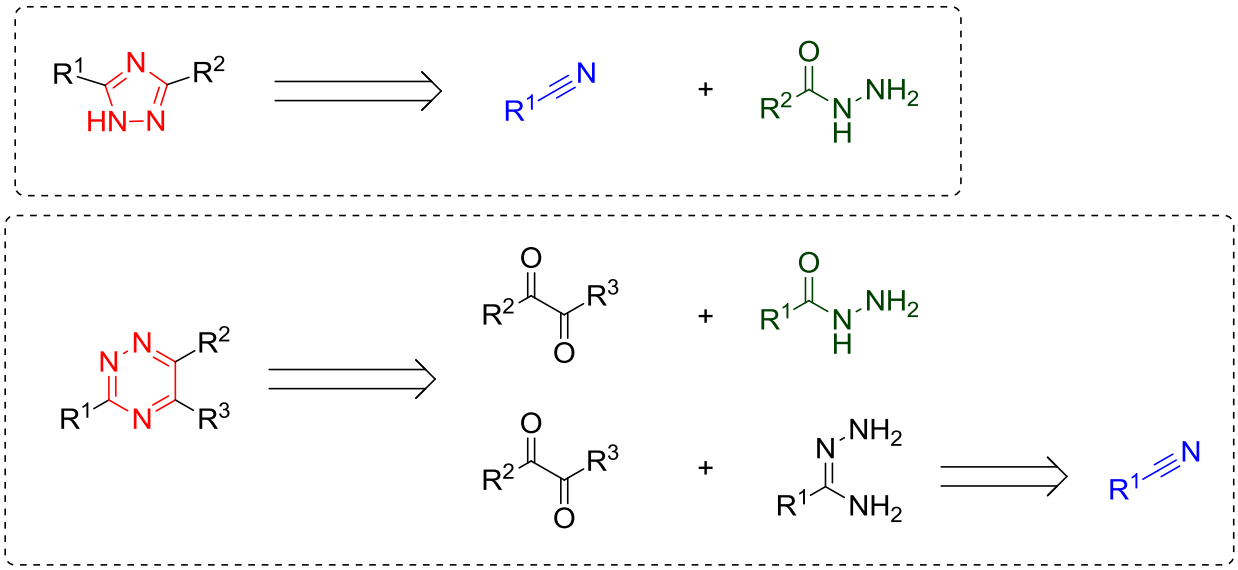


Figure 2.3 Comparison of retrosynthetic pathways for 1,2,4-triazines and 1,2,4-triazoles.

2.2. Synthetic Strategies for 1,2,4-Triazine Synthesis

The general synthesis of 1,2,4-triazines can be classified into 5 broad categories (Figure 2.4).[68] The most common of these methods is the **ring closure** in which one, two, or three N-C bonds are made. Several modifications and variations exist for ring-closure reactions, making 1,2,4-triazines accessible from a number of starting materials. A second method, **ring transformation**, begins with a nitrogen containing heterocycle that is either rearranged or reacted to form 1,2,4-triazines. Substituted pyrazoles, imidazoles and tetrazines are common starting materials for ring transformations. A third method of **substituent modifications** alters a substituent on the preformed 1,2,4-triazine. Depending on the starting nature of the triazine, nucleophilic or electrophilic substitution can occur. The starting materials and reagents can be tuned for selective substitution. **Annulation** of a second heterocycle onto an existing 1,2,4-triazine nucleus is a common method employed for 1,2,4-triazines fused to other heterocycles, such as imidazoles, pyrazoles, or triazoles. Finally, a method commonly employed with di- or tetrahydrotriazines or triazine-ones is to **aromatize** through elaborate oxidative methods or elimination to afford aromatic 1,2,4-triazines.

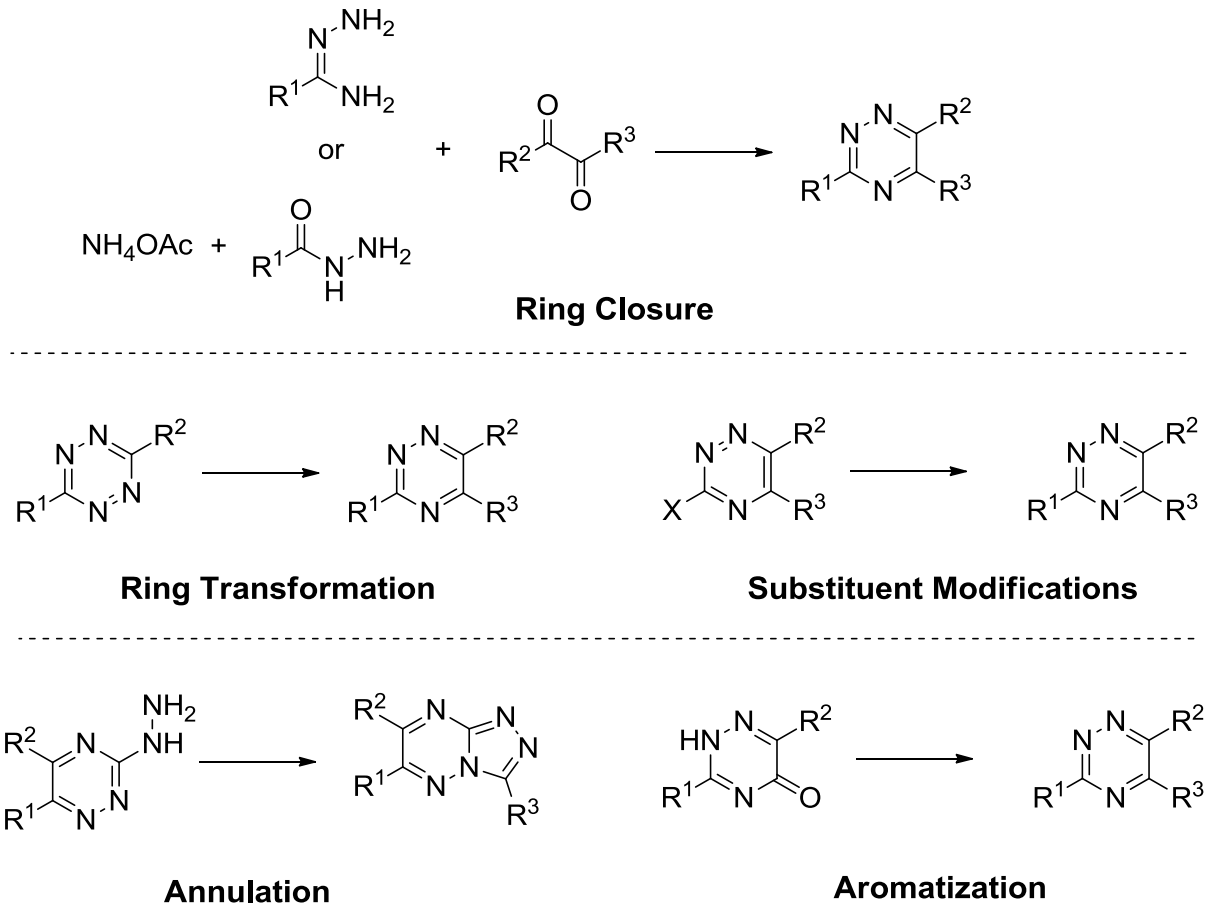


Figure 2.4 General synthetic strategies for the synthesis of 1,2,4-triazines.

From our perspective, there were three major areas that could be manipulated when synthesizing 1,2,4-triazines: the substituent at the 3 position, substituents at the 5,6 positions, and the core triazine (Figure 2.5). The ring closure method would provide us with the most versatility to modify these three areas. Ring transformations and substituent modifications would allow us to diversify triazines synthesized through ring closure reactions. Our goal was to construct a library of triazines consisting of various stereoelectronic features and analyze each for catalytic activity in CuAAC reaction. From this, we hoped to gain the structural and electronic requirements necessary for activity.

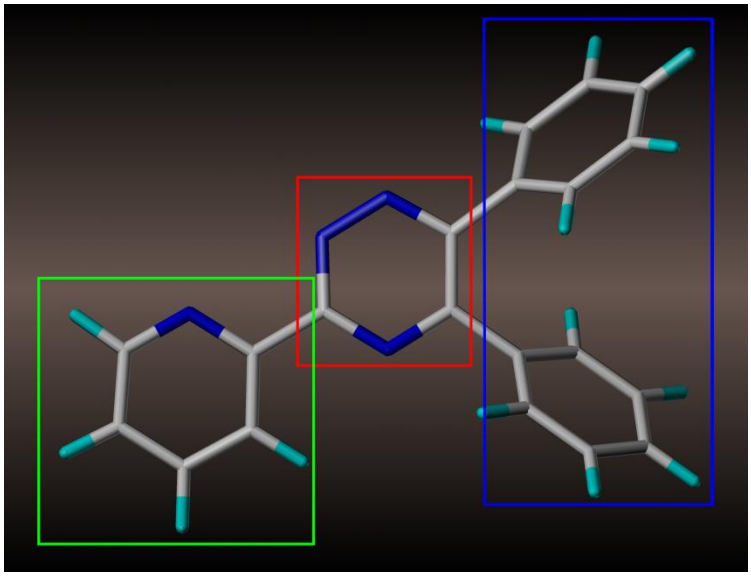


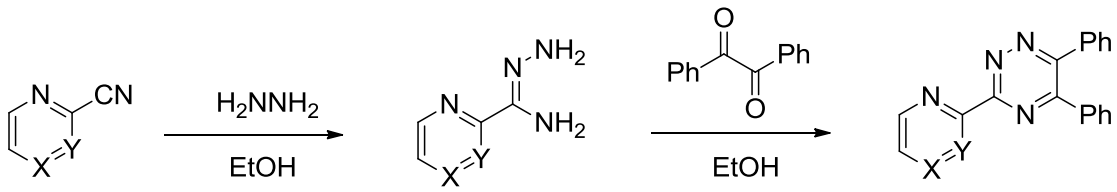
Figure 2.5 Areas of 1,2,4-triazine that could be manipulated.

2.3. Ring Closure Reactions

The most common method to form 1,2,4-triazines is through ring closure reactions by forming three nitrogen-carbon bonds or forming two nitrogen-carbon bonds depending on the starting materials employed. Substituents at the 3, 5, and 6 position can be varied and a combinatorial library quickly assembled by altering the two starting materials.

2.3.1. Synthesis of 3-substituted-5,6-diphenyl-1,2,4-triazine

Our initial synthetic goal was to vary the 3-position of the 1,2,4-triazine while keeping the 5,6-positions constant. The formation of two N-C bonds involved a two-step procedure (Scheme 2.1), in which nitriles are first converted to amidrazones using hydrazine hydrate at room temperature. After isolation, the amidrazone is reacted with a diketone, benzil, to afford the desired 1,2,4-triazine in approximately 75% yield. This method provided rapid access to 1,2,4-triazines in good yield (70% over two steps) and purity. Both the amidrazone intermediate and triazine product were isolated by filtration resulting in an easy workup. Various heterocyclic nitriles were available and subjected to reaction with hydrazine and condensation with benzil to form a series of 1,2,4-triazines with varying substituents at the 3-position.



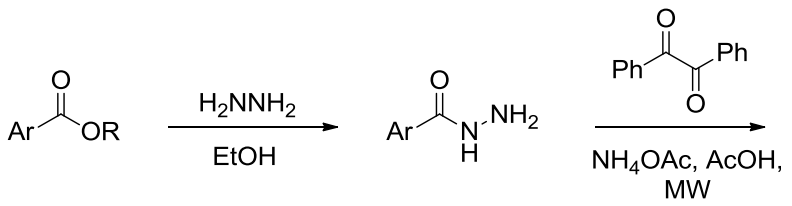
2-11	X = CH, Y = CH	**2-14**	X = CH, Y = CH	**2-17**	X = CH, Y = CH
2-12	X = CH, Y = N	**2-15**	X = CH, Y = N	**2-18**	X = CH, Y = N
2-13	X = N, Y = CH	**2-16**	X = N, Y = CH	**2-19**	X = N, Y = CH

Scheme 2.1 Synthesis of 3-substituted-5,6-diphenyl-1,2,4-triazines.

In some instances, the nitrile was not available, but instead the corresponding carboxylic acids or esters were available. For those cases, a second method was utilized to react a hydrazide with a diketone in the presence of ammonium acetate. This reaction can be performed thermally, but this leads to slow and sometimes incomplete conversion. Initially, this reaction was performed in a conventional microwave using phenyl hydrazide and benzil, with low yields (51%) still being the drawback. Literature suggested that these reactions could be performed in a microwave reactor to achieve higher yields (> 79%) and less side product formation (>75% purity).[80]

Upon acquiring a Microwave Synthesizer, we attempted this protocol with heteroaromatic compounds to construct triazines containing substituents, such as indole, benzofuran, benzothiophene, and benzothiazole. The carboxylic acid or ester was converted to the hydrazide using hydrazine hydrate under reflux or microwave conditions. The hydrazide was then reacted with a diketone in the microwave in the presence of ammonium acetate to afford the desired triazine (Scheme 2.2). The reaction to synthesize indole triazine resulted in a higher yield (63%) compared to the other heterocyclic triazines, including benzothiophene substituted triazine (28%), benzofuran triazine (42%), and benzothiazole triazine (34%). This was due to by-product formation. While these byproducts were not isolated and characterized, additional UV-active spots were observed via TLC. In an attempt to decrease the side product formation, the hydrazine was reacted with the diketone at 60°C to form the imine. Once the hydrazide was consumed, the reaction was completed in the microwave. Unfortunately, this didn't increase the yields significantly. Decreasing the

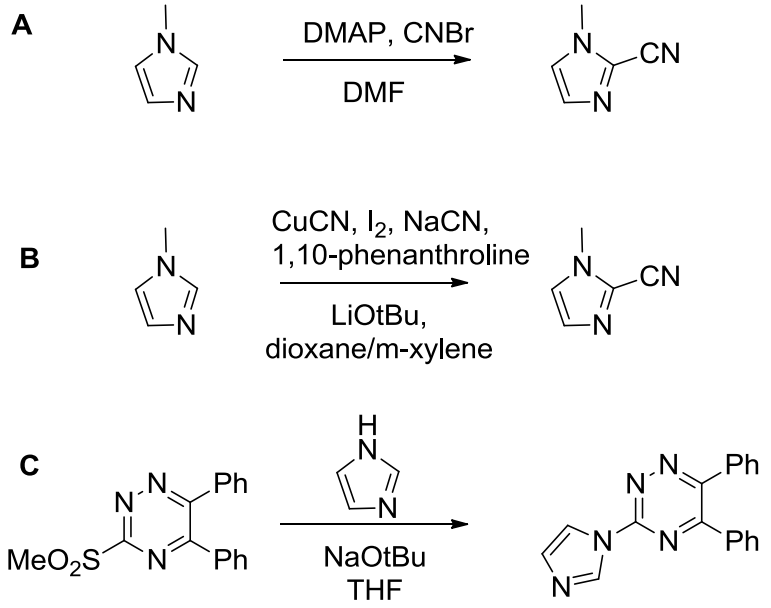
substrate concentration in the reaction mixture or shorter reaction times could help limit the side product formation.



2-20	Ar = Ph, R = OMe	**2-25**	Ar = Ph	**2-30**	Ar = Ph
2-21	Ar = 2-benzothiophene, R = H	**2-26**	Ar = 2-benzothiophene	**2-31**	Ar = 2-benzothiophene
2-22	Ar = 2-benzofuran, R = H	**2-27**	Ar = 2-benzofuran	**2-32**	Ar = 2-benzofuran
2-23	Ar = 2-indole, R = H	**2-28**	Ar = 2-indole	**2-33**	Ar = 2-indole
2-24	Ar = 2-benzothiazole, R =OEt	**2-29**	Ar = 2-benzothiazole	**2-34**	Ar = 2-benzothiazole

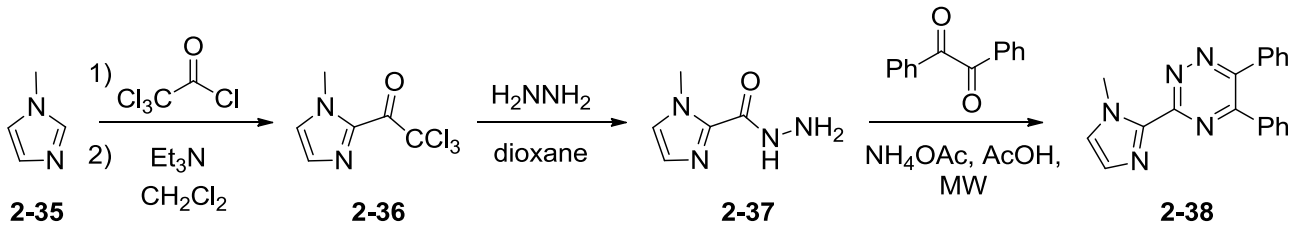
Scheme 2.2 Synthesis of 1,2,4-triazines from carboxylic acids or esters.

These two methods afforded several heterocycles appended to the 3 position of the 1,2,4-triazine. Based on initial screening results (discussed in the following chapter), the 3-position was the most influential portion of the molecule. There was a desire to substitute other nitrogen based heterocycles, such as an imidazole or a 1,2,4-triazole, both known to coordinate metals. To synthesize the imidazole derivative, the initial approach was to make the 2-cyano-methylimidazole derivative starting from methyl imidazole. Method A[81] (Scheme 2.3) used a mixture of cyanogen bromide and dimethylaminopyridine which resulted in desired product but in a low yield. Conditions were slightly altered but sufficient yields were not achieved. Method B[82] used copper cyanide and sodium cyanide as the nitrile source and relied on the addition of phenanthroline. This resulted in only recovered starting material. This was likely due to low solubility in the recommended solvent of dioxanes and m-xylene. Since installation of the nitrile functional group was problematic, other alternatives were sought after. Literature methods show that 1,2,4-triazines are electrophilic at the 3-position when a good leaving group such as methanesulfonyl was present, and considering that the other two positions (5,6) are occupied by phenyl rings.[83] Imidazole could be used as a nucleophile to replace the SO_2Me group. Although this would lead to the formation of an N-C bond with the triazine, it was worth the attempt, but this method also proved futile.

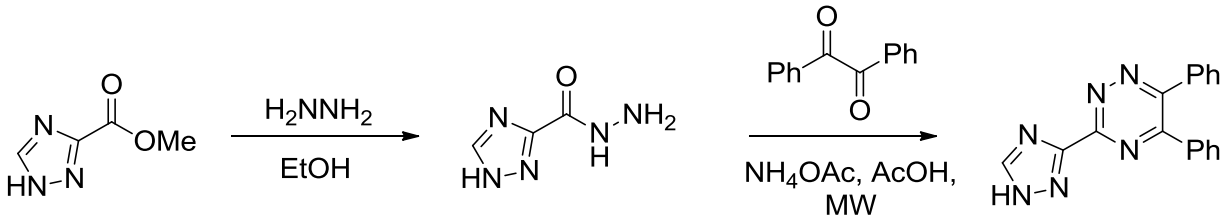


Scheme 2.3 Attempted routes to synthesize 3-methylimidazoyl triazine.

The final attempt was to synthesize the hydrazide substituted imidazole since this could be used in place of the amidrazone to produce the triazine (Scheme 2.4). Methyl imidazole was first converted to the trichloroacetyl methyl imidazole.[84] This intermediate could then be reacted with hydrazine hydrate to afford the desired hydrazide. The hydrazide was then condensed with benzil in the presence of acetic acid and ammonium acetate to form the desired imidazole substituted triazine (**2-38**) in 52% yield. The last heterocycle attempted was to position a 1,2,4-triazole in the 3-position (Scheme 2.5), beginning from commercially available methyl-1,2,4-triazole-3-carboxylate. Although a single product was isolated after silica gel column chromatography, the low solubility of the product made it difficult to fully characterize by NMR. For this reason it was not screened for catalytic activity.



Scheme 2.4 Successful synthesis of 3-methylimidazoyl triazine.



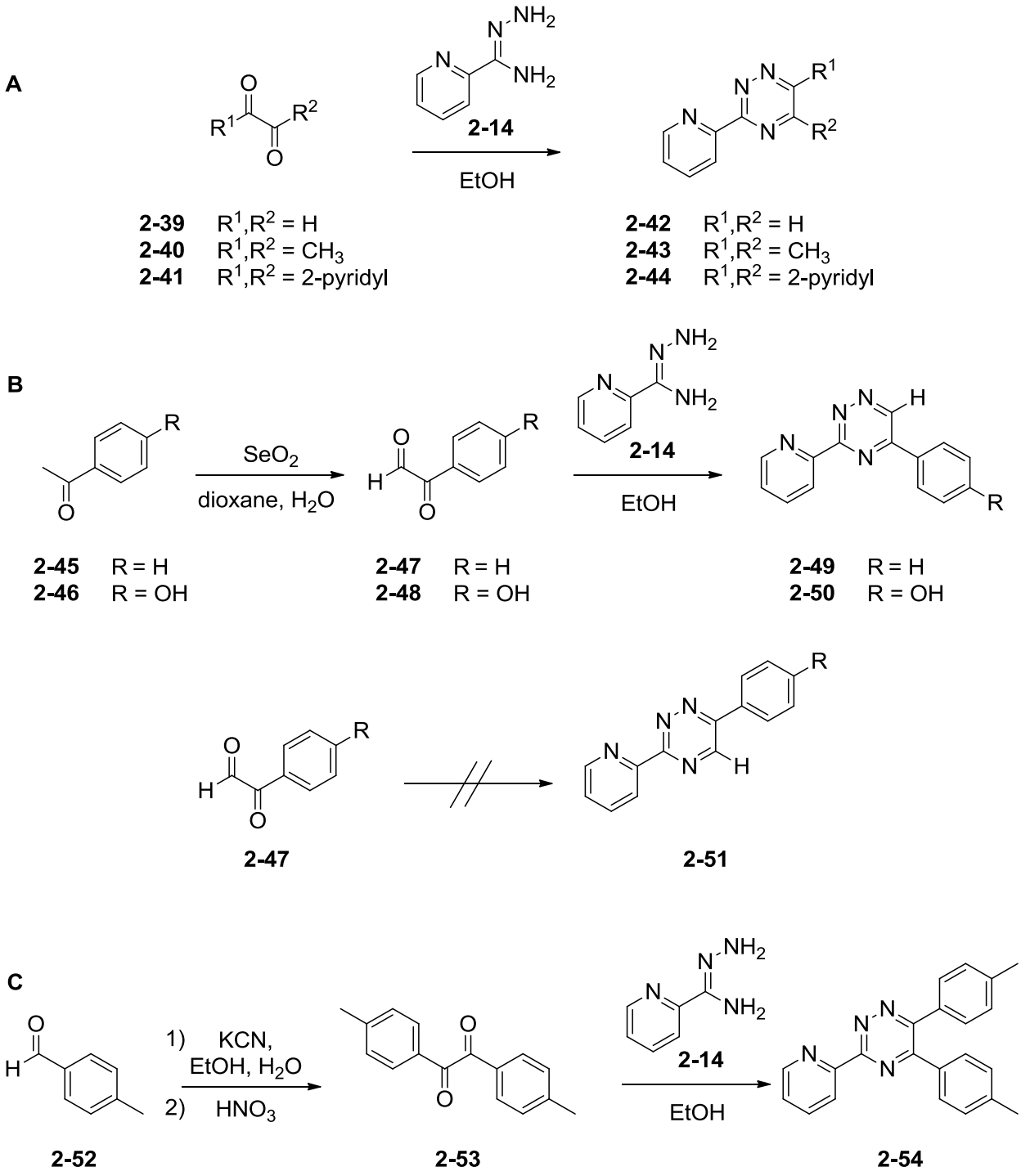
Scheme 2.5 Attempted synthesis of 3-(1,2,4-triazol-3-yl)-triazine.

2.3.2. Synthesis of 3-pyridyl-5,6-disubstituted-1,2,4-triazines

Up to this point only the substituent at the 3-position had been altered. It was desirable to attempt to tune the activity by altering the substituents at the 5,6 positions. Other commercial dicarbonyls available were glyoxal, and 2,3-butanedione. These were condensed with 2-pyridyl amidrazone (**2-14**) to yield triazines **2-42** and **2-43** in 83% and 90% yield (Scheme 2.6A). This allowed exploration of the effect of non-aryl substituents and the influence of steric bulk at these positions. Using pyridil as the diketone allowed us to construct the tripyridine triazine **2-44** in 95% yield.

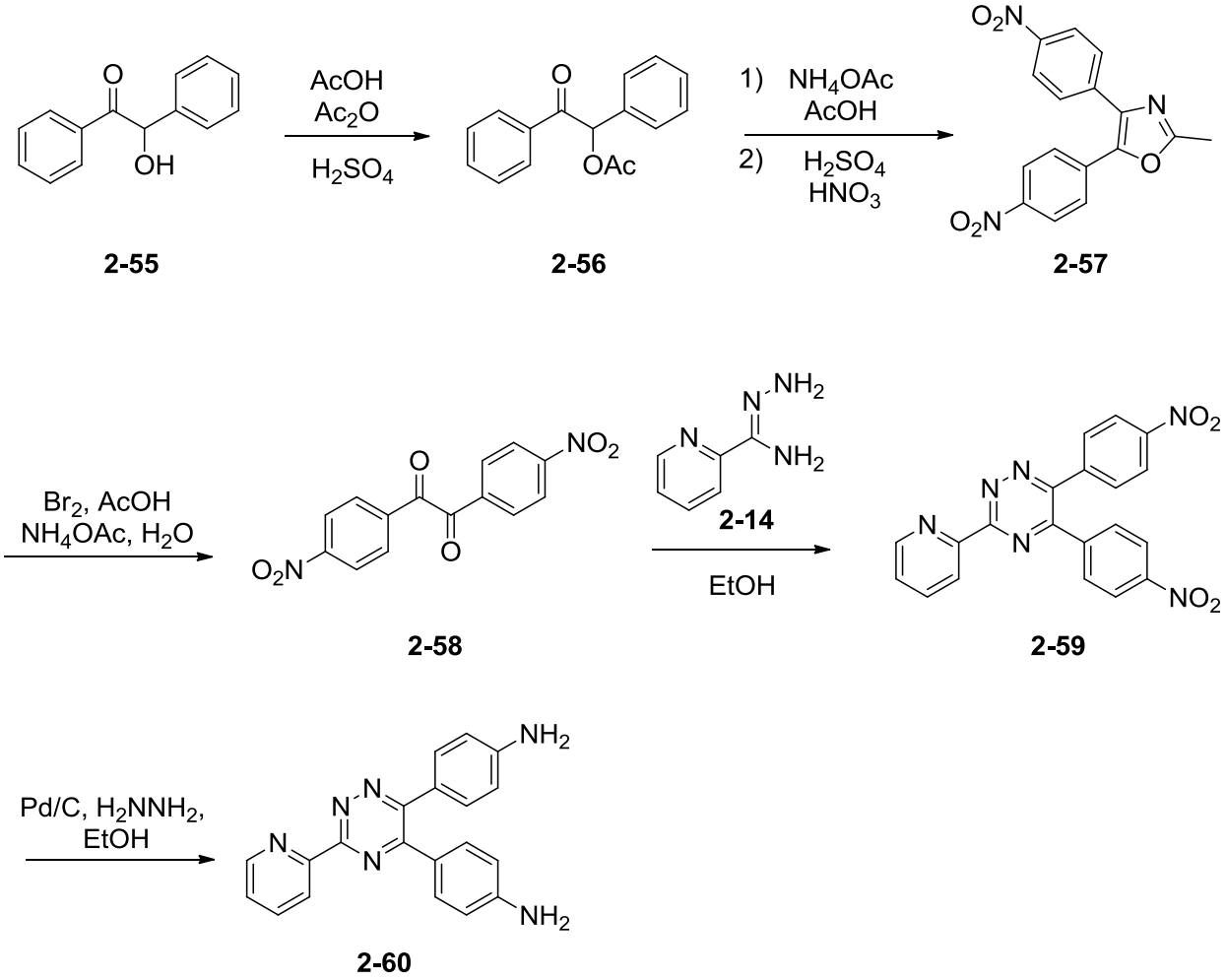
It was also advantageous to explore the effect of substituents at the 5 and 6 positions independently. Thus far, we had only used symmetric diketones, so the 5 and 6 positions were identical. By employing ketoaldehydes, we had hoped to synthesize the triazine with substitution at position 5 in one isomer and substitution at the 6 position in the other isomer (Scheme 2.6B). The ketoaldehydes were synthesized from substituted acetophenones using selenium dioxide. Condensation with the amidrazone (**2-14**) under neutral conditions resulted in a single isomer (**2-49**) in 82% yield.[85] The most nucleophilic nitrogen is the terminal nitrogen of the hydrazono group and this will react with the more electrophilic carbon of the aldehyde, leading to the substituent at the 5 position. Attempts to synthesize the other isomer were not successful, although literature methods reported that this should be the predominant isomer under acidic conditions.[86]

In addition to sterics, electronic properties of the triazine substituents were investigated by installation of electron withdrawing and electron donating substituents. These diketones were not on hand, so the diketones were first synthesized through a Benzoin



Scheme 2.6 Synthesis of 3-(2-pyridyl),5,6-disubsituted triazines. From A) commercial dicarbonyls, B) from substituted acetophenones, and C) from substituted benzaldehyde

Condensation (Scheme 2.6C). The substituted aldehyde was exposed to KCN in refluxing EtOH and H_2O, followed by refluxing nitric acid. Although this worked for the methyl substituent, the Benzoin Condensation failed with strongly withdrawing nitro groups. An alternate synthesis involved formation of an oxazole and subsequent nitration, and oxidative opening of the oxazole to lead to the formation of the desired diketone (Scheme 2.7). Reaction with amidrazone (**2-14**) yielded the nitro substituted 1,2,4-triazine derivative (**2-59**). Although the reaction between amidrazone and diketone produced 93% product, the yield over four steps from starting benzoin was a minuscule 15%. Reduction of the nitro groups resulted in the amine derivative, but difficulties in isolation resulted in only 80% purity of **2-60**.



2-55 **2-56** **2-57**

2-58 **2-59**

2-60

Scheme 2.7 Synthesis of nitro and amine substituted 1,2,4-triazines.

Now that we had changed the size of the functional groups and the electronics of the groups attached to the 1,2,4-triazine, we next explored syntheses to impact the shape of the molecule. At first glance, one might think the molecules synthesized previously are planar or flat. The 3-substituent lies within the same plane as the 1,2,4-triazine, but the 5 and 6 positions are slightly twisted out of this plane with a dihedral angle of 63° between the two phenyl substituents.[87] To illustrate this, we examined the lowest energy conformation of 2 selected 1,2,4-triaines (Figure 2.6). Sybyl modeling software was used to obtain a rough estimation of the geometry of 1,2,4-triazines in the lowest energy conformation. In 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine (**2-17**) the lowest energy conformation showed twisted phenyl rings as was expected. If the 5,6 substituents were connected (**2-62**) the lowest energy conformation was entirely planar. To see if this would alter the catalytic activity, we chose to use diketones that would "lock" these substituents into the same plane. Three diketones, phenanthrene-9,10-dione (**2-61**), 1,10-phenanthroline-5,6-dione (**2-64**), and isatin (**2-66**) were reacted with amidrazone **2-14** to afford the corresponding triazines in 74%, 75% and 59% yield (Scheme 2.8).

Encouraged by some of the results from catalytic screening, our next goal was to incorporate two 1,2,4-triazines within one molecule, bridged by a pyridine ring. The most straight forward route was to construct a bis-amidrazone and react it with two equivalents of benzil. (Scheme 2.9) 2,6-Dicyanopryridine was synthesized starting from pyridine dicarboxylic acid. The acid was converted into the bisamide followed by dehydrating conditions to give the desired 2,6-dicyanopyridine. Reaction with hydrazine hydrate resulted in the bis-amidrazone which was reacted with benzil to form the desired bis-triazine (**2-72**) in 92% yield. The overall yield through 5 steps was only 16%, but each intermediate was isolated through either filtration or aqueous extraction, eliminating the need for column chromatography and allowing the synthesis to be completed rather quickly.

Having synthesized the bistriazine, we were curious if another nitrogen heterocycle could be appended in close proximity to the triazine core. One idea was to synthesize a 1,2,3-triazole triazine. Conversion of an aromatic amine to an azide occurs easily under

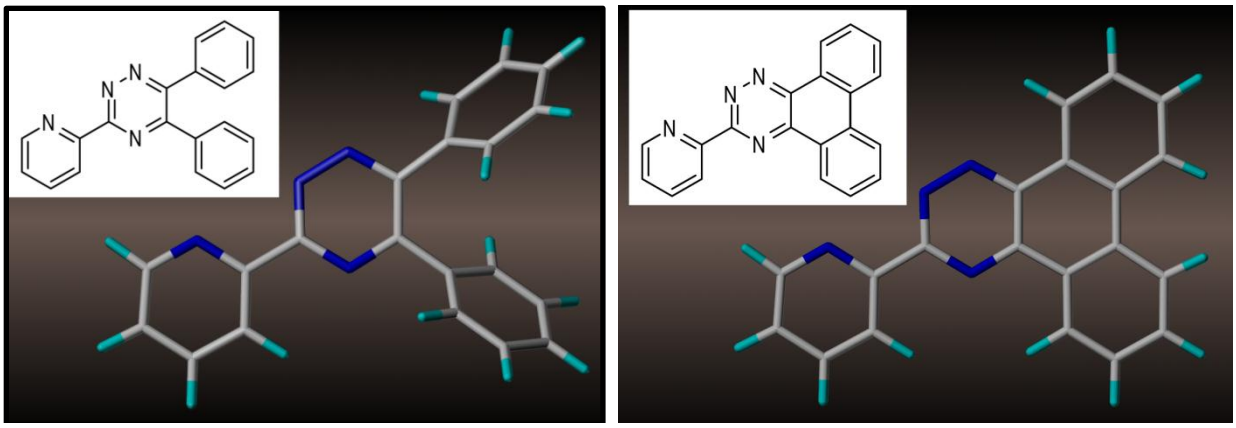
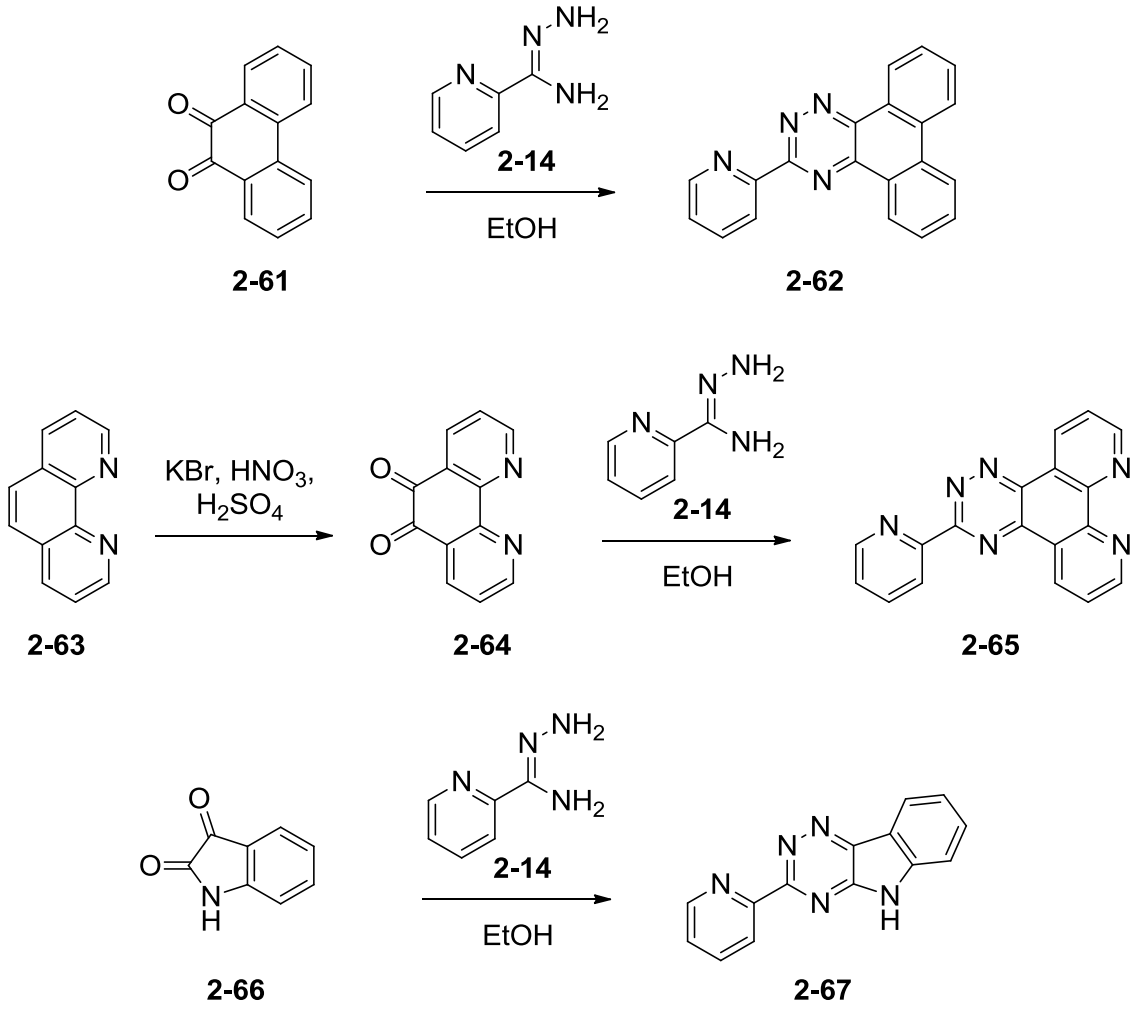
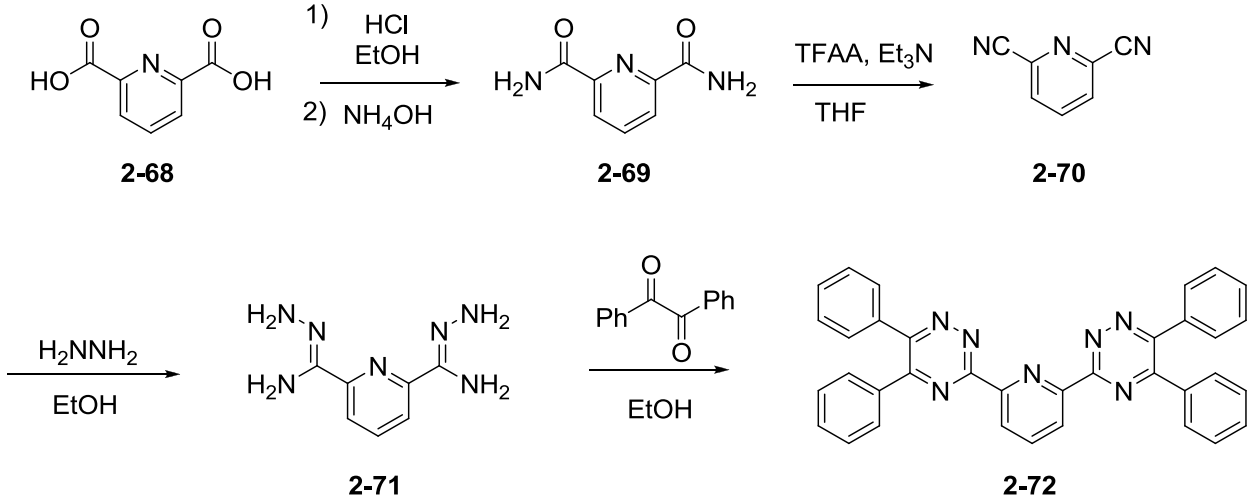


Figure 2.6 Illustration showing modeling of 1,2,4-triazines in Sybyl. Two phenyl substituents are twisted in 5,6-diphenyl-1,2,4-triazine (2-17), but are planar in "fused" triazine (2-62).



Scheme 2.8 Synthesis of triazines using cyclic or fused diketones.

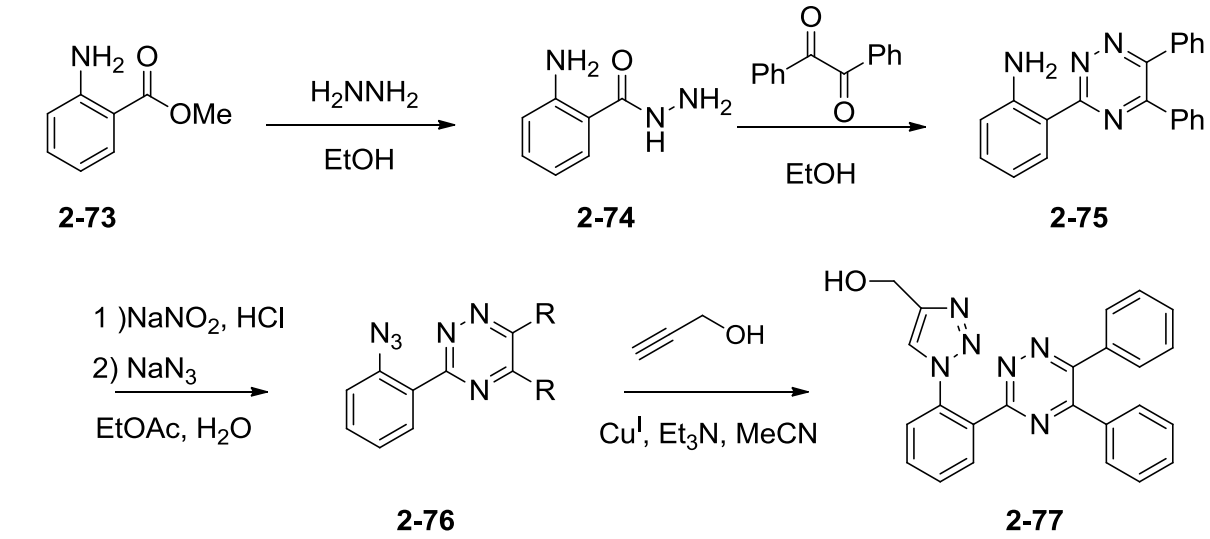


2-68 **2-69** **2-70**

2-71 **2-72**

Scheme 2.9 Synthesis of bis1,2,4-triazine.

diazotization conditions, using sodium nitrite and sodium azide in acidic media (Scheme 2.10).[88] For this reason, we began with the amino methyl benzoate and converted it into the corresponding hydrazide followed by condensation with benzil and ammonium acetate under microwave conditions. The amine was left unprotected as the hydrazide amine should be much more reactive than the aromatic amine. After converting the amine to the azide, a CuAAC reaction with propargyl alcohol installed the 1,2,3-triazole, to provide an additional metal binding functional group with an overall yield of 19% over 4 steps.

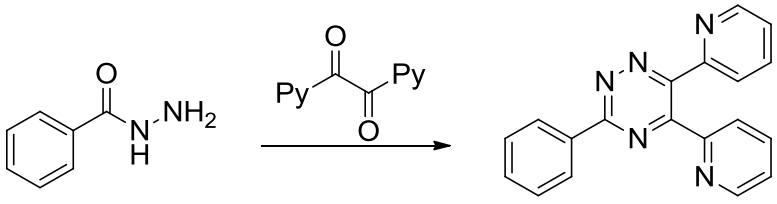


2-73 **2-74** **2-75**

2-76 **2-77**

Scheme 2.10 Synthesis of triazole triazine.

One final ring closure reaction was conducted between phenyl hydrazide (**2-20**) and pyridil (Scheme 2.11) to give a triazine with pyridyl substituents in the 5- and 6-positions, and a phenyl in the 3-position. The triazine synthesis was carried out in the microwave to yield the product (**2-78**) in 40% yield after column chromatography. This does not fit into either of the categories discussed earlier, since all three substituents are being modified. The series of triazines including **2-17**, **2-44**, and **2-78** has the pattern of increasing the pyridine substituents from 1 to 2 to 3. The addition of this compound could help us to better understand the dependence on the location of the pyridine substituent.



2-20 **2-78**

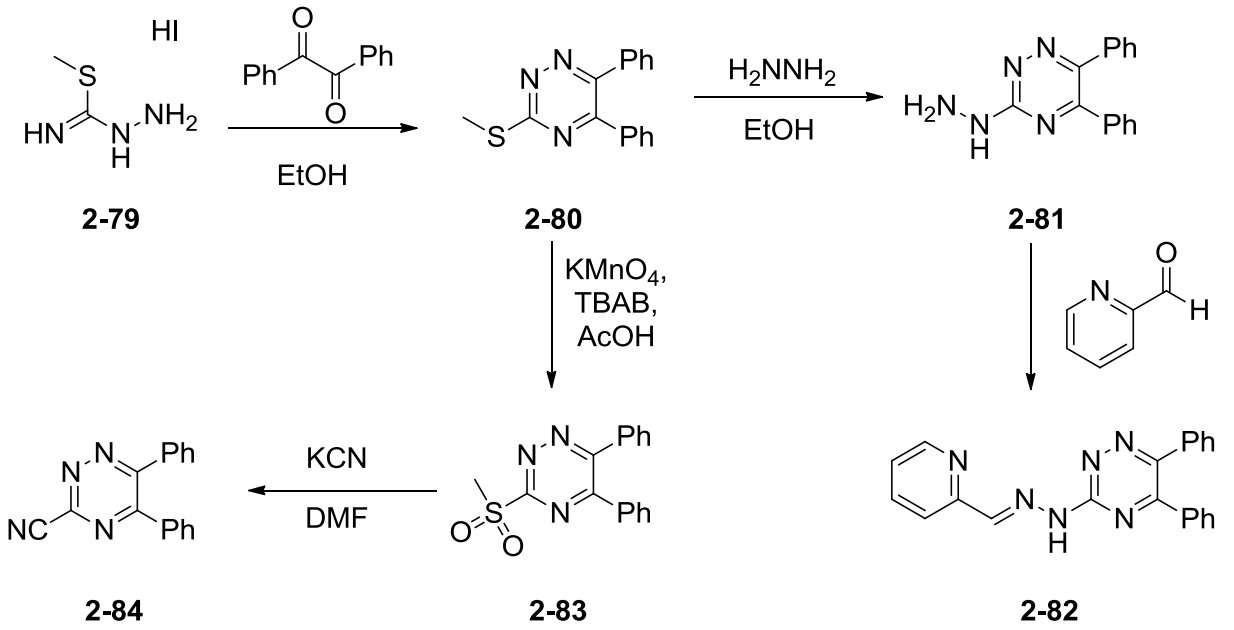
Scheme 2.11 Synthesis of 3-phenyl-5,6-di(2-pyridyl)-1,2,4-triazine.

2.4. Substituent Modifications

Substituent modifications were at first an appealing route to install additional heterocycles onto synthesized triazines. Depending on the existing substitution pattern, the reactivity pattern for 1,2,4-triazines is C5 > C3 > C6.[83] A good leaving group can be installed during the initial 1,2,4-triazine formation and displaced at a later point. From the observed activities of triazines, we were interested in substituting additional heterocycles at the 3-position. This was in response to work by Finn and colleagues in their synthesis of tailored tripodal ligands.[39] Although we had successfully synthesized a 1,2,3-triazole and a second 1,2,4-triazine in close proximity to the 1,2,4-triazine core, we wanted to install a 1,2,3-triazole, 1,2,4-triazole or 1,2,4-triazine directly attached to the triazine core.

Initially, we synthesized the 3-thiomethyl-1,2,4-triazine through a ring closure reaction between benzil and S-methylisothiosemicarbazide hydroiodide salt **2-79** in 89% yield (Scheme 2.12). The methylthiol group was easily displaced by hydrazine hydrate, which

was reacted with an aldehyde to form the imine containing triazine (**2-82**) in 80% yield. We then displaced the thiomethyl group using sodium azide as the nucleophile, but all attempts at a cycloaddition with propargyl alcohol resulted in recovery of starting material. The azide is in equilibrium with the tetrazole formed by reaction with the triazine core, with the tetrazole being the dominant form, preventing the cycloaddition reaction.[89]



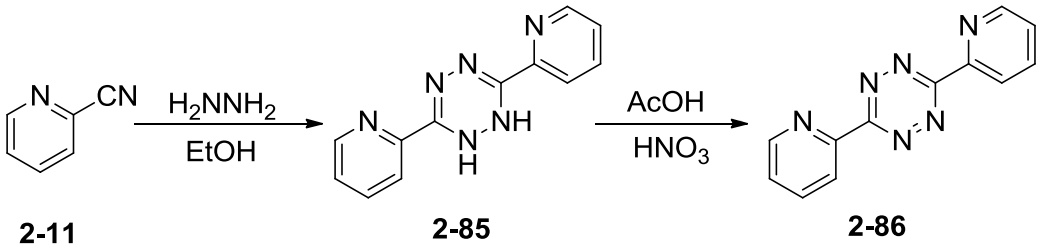
Scheme 2.12 Synthesis of 1,2,4-triazines by substituent modification.

With the attempt to install a 1,2,3-triazole halted, we moved on to the next heterocycle. The substitution by cyanide was not successful from thiomethyl triazine (**2-80**). This required us to oxidize the thiomethyl substituent to the mesyl group, which would serve as a better leaving group (**2-83**). The substitution proceeded in decent yield, but unfortunately the reactivity observed was not as expected. Instead of the nucleophile reacting at the nitrile carbon, the triazine carbon proved to be more susceptible to nucleophilic attack. This led to the displacement of the nitrile functional group by nucleophiles such as hydrazine, 2-pyridyl hydrazide, and n-butanol.[90] Attempts to include a 1,2,3-triazole, 1,2,4-triazole, or a 1,2,4-triazine directly connected to the 3-position of the existing 1,2,4-triazine were all unsuccessful.

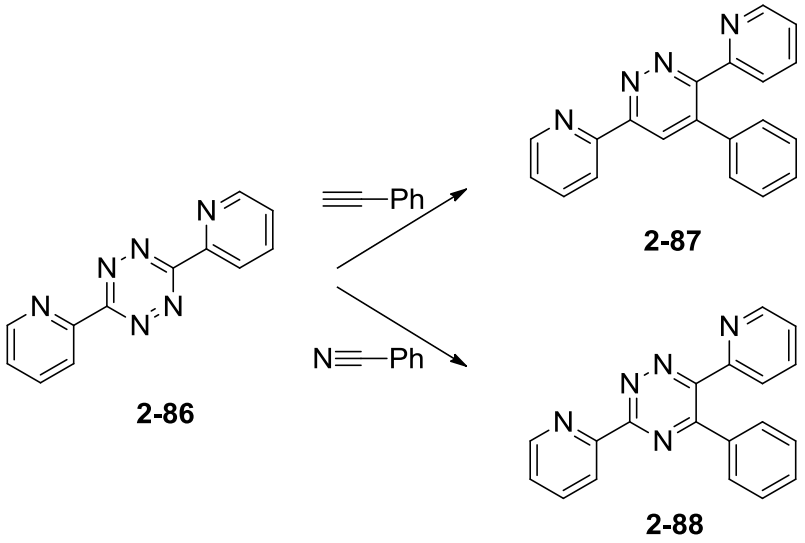
2.5. Ring Transformations

Despite unsuccessful attempts in the synthesis of the heterocycles above, there were still other triazines and related heterocycles that could be synthesized. Ring transformations are a less common way to make 1,2,4-triazines. This method was not fully explored to make 1,2,4-triazines, but rather as a way to make other nitrogen containing heterocycles. In an attempt to synthesize one of the molecules above, we heated 2-cyanopyridine in the presence of hydrazine hydrate. This resulted in an orange solid, but did not match the expected spectral data. It was confirmed that we had synthesized the dihydrotetrazine (**2-85**), rather than the expected triazine (Scheme 2.13). To our advantage, this material could be oxidized to the aromatic tetrazine (**2-86**) and then reacted with an alkyne in an inverse electron-demand Diels-Alder to form the pyridazine (**2-87**) in 77% yield. Changing the dienophile from an alkyne to a nitrile would result in a 1,2,4-triazine (**2-88**), but this reaction wasn't fully explored. With this route, we were also interested in observing if the presence of all three nitrogens of the core triazine were necessary for activity. The bipyridine derivative (**2-89**) was synthesized utilizing aza-Diels-Alder to expel nitrogen (N_2) and form the bipyridine in 66% yield.[91]

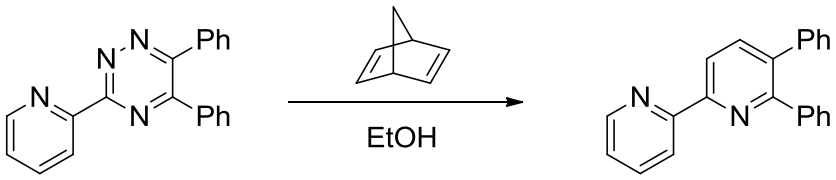
Finally, we included a few additional heterocycles that contained aromatic nitrogens but were not classified as triazines (Scheme 2.14). Reaction of amidrazone **2-14** with an aldehyde resulted in non-cyclic triazine derivatives (**2-92**, **2-93**) in excess of 80% yield. This would allow us to probe the catalytic activity and observe if a closed aromatic ring was necessary for activity. Quinoxalines (**2-94**) are also interesting scaffolds and could be made from diketones and 1,2-phenylenediamines. The position of the nitrogen atoms appeared able to bind metal. Finally, pyridil was reacted with an aromatic amine to form the imine (**2-95**) in 53% yield after column chromatography. Due to steric bulk, the amine only added to one carbonyl of the diketone, even with excess amine and applied heat. The proximity of the pyridine from the diketone and the imine from the aromatic amine appear to be comparable to the proximity of the pyridine and 1,2,4-triazine core in **2-17**.



2-11 **2-85** **2-86**

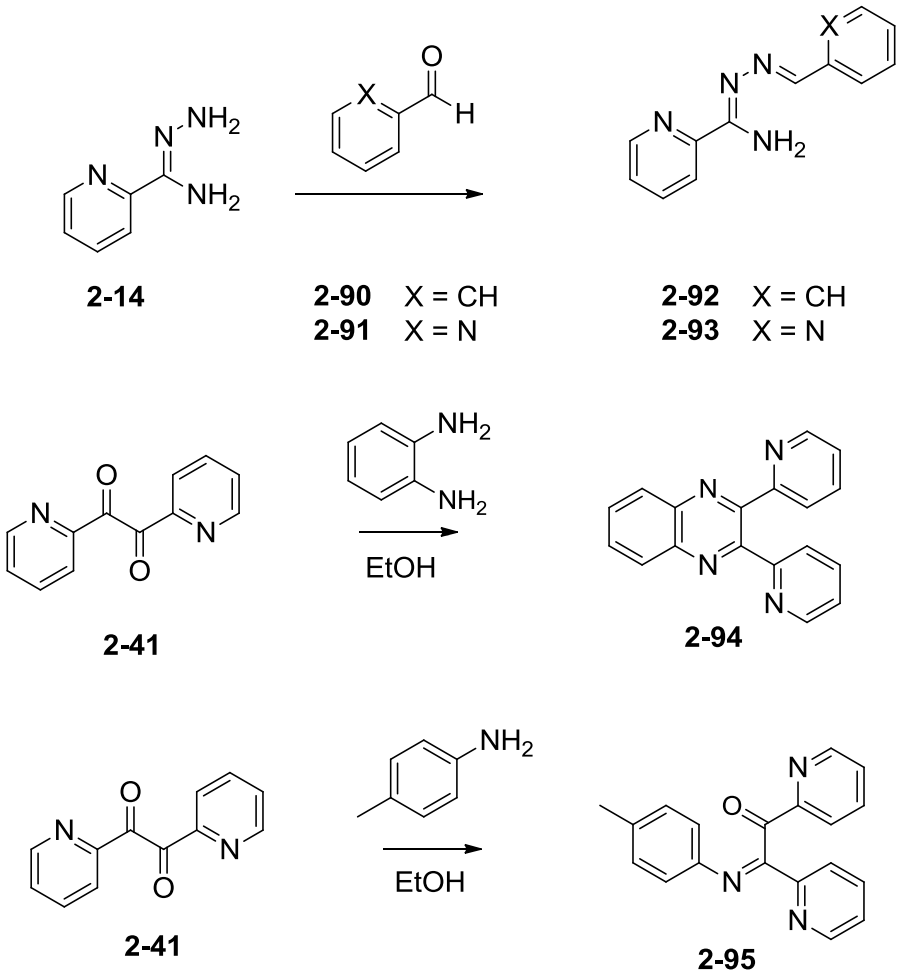


2-86 **2-87** **2-88**



2-17 **2-89**

Scheme 2.13 Diels-Alder reactions of tetrazines and triazines.



Scheme 2.14 Non-1,2,4-triazine heterocycles containing sp^2 nitrogens.

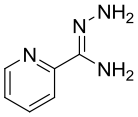
2.6. Conclusions

A series of 1,2,4-triazines was synthesized using ring closure and substituent modifications. Ring closure reactions between amidrazones and dicarbonyls were the most effective method of synthesizing 1,2,4-triazines, resulting in yields between 70 – 90%, most of which could be isolated by filtration. Ring closure reactions between hydrazides and diketones in the presence of ammonium acetate in the microwave was used to synthesize additional 1,2,4-triazines. Yields were much lower, in the range of 28 – 68%, and triazines required chromatographic purification. Substituent modification reactions of preformed triazines led to imine substituted triazines, but failed to provide 1,2,3-triazoles or 1,2,4-triazines substituted in the 3-position.

Additional sp^2 nitrogen containing ligands, such as pyridines, pyridazines, imines, and quinoxalines were also synthesized using ring closure and ring transformations methods. Overall modifications of 1,2,4-triazines were made to the 3-position, 5 and 6 positions, and to the core 1,2,4-triazine. The activity of the synthesized triazine ligands will be explored in Chapter 3.

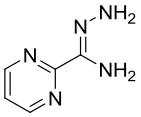
2.7. Experimental

Materials and General Methods. Reagents and solvents were purchased from various commercial sources and used without further purification unless otherwise stated. Anhydrous solvents were purified using a Grubbs solvent system. Analytical thin-layer chromatography (TLC) was performed using aluminum backed silica gel TLC plates with UV indicator from Sorbent Technologies. Flash column chromatography was performed using 40-63 μm (230 x 400 mesh) silica gel from Sorbent Technologies. ^{1}H and ^{13}C NMR were recorded at 600 MHz and 151 MHz, respectfully, on a Varian Inova spectrometer or at 500 MHz and 125 MHz, on a Varian spectrometer. All chemical shifts were reported in δ units relative to tetramethylsilane using the corresponding deuterated solvent as a reference point. High resolution mass spectra (ESI) were obtained on a JEOL AccuTOF DART spectrometer. Infrared spectra were recorded on a Varian 4100 FT-IR using KBr pellets or KBr salt plates. Absorption spectra were collected on a Thermo Scientific Evolution 600. High pressure liquid chromatography (HPLC) was performed using a Beckman Coulter System equipped with a UV-Vis detector, autosampler, Varian C18 column, and a mobile phase composed of acetonitrile and trifluoroacetic acid. Microwave reactions were performed in sealed vials using a Biotage Initiator Microwave Synthesizer.

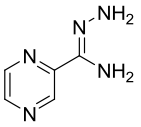


picolinohydrazonamide (2-14). 2-7 (5g, 48 mmol) was dissolved in EtOH (6mL) and hydrazine hydrate (3.26 mL, 67 mmol) and stirred at room temperature overnight. The pale white precipitate was filtered and washed with Et_2O in 94% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.52 (m, 1H), 8.01 (d, 1H, J = 7.8 Hz), 7.69 (t, 1H, J = 7.8 Hz), 7.26 (m, 1H), 5.27 (br, 2H), 4.59 (b, 2H). ^{13}C NMR ($CDCl_3$,

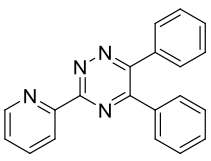
151 MHz) δ 150.8, 148.7, 147.9, 136.4, 123.8, 119.7. HRMS (ESI) m/z calculated 137.08272 ($C_6H_9N_4$, $[M+H]^+$), m/z observed 137.08324 ($C_6H_9N_4$, $[M+H]^+$).



pyrimidine-2-carbohydrazonamide (2-15). **2-8** (250 mg, 2.38 mmol) was dissolved in EtOH (0.3 mL) and hydrazine hydrate (0.163 mL, 3.33 mmol) and stirred overnight at room temperature. Reaction was monitored by TLC. The reaction was concentrated *in vacuo* to yield a yellow oil in quantitative yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.77 (d, 2H, J = 4.8 Hz), 7.26 (t, 1H, J = 4.8 Hz), 5.17 (b, 2H), 4.83 (b, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 158.8, 157.2, 146.6, 120.6. HRMS (ESI) m/z calculated 138.07797 ($C_5H_8N_5$, $[M+H]^+$), m/z observed 138.07782 ($C_5H_8N_5$, $[M+H]^+$).

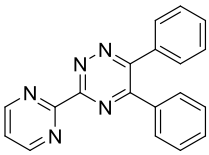


pyrazine-2-carbohydrazonamide (2-16). **2-9** (250 mg, 2.38 mmol) was dissolved in EtOH (0.3 mL) and hydrazine hydrate (0.163 mL, 3.33 mmol) and stirred overnight at room temperature. Reaction was monitored by TLC. The pale white precipitate was filtered and washed with EtOH resulting in a 60% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.27 (s, 1H), 8.51 (m, 1H), 8.44 (m, 1H), 5.12 (b, 2H), 4.72 (b, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 146.6, 146.4, 144.1, 142.8, 142.3. HRMS (ESI) m/z calculated 138.07797 ($C_5H_8N_5$, $[M+H]^+$), m/z observed 138.07783 ($C_5H_8N_5$, $[M+H]^+$).

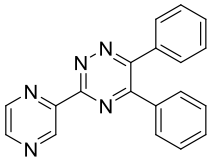


5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine (2-17). **2-10** (500 mg, 3.67 mmol) and benzil (772 mg, 3.67 mmol) were added to 10 mL of EtOH. The slurry was heated at 80°C for 6 h with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with EtOH and Et_2O resulting in a 76% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.91 (d, 1H, J = 4.2 Hz), 8.70 (d, 1H, J = 7.8 Hz), 7.91 (t, 1H, J = 7.8 Hz), 7.68 (d, 2H, J = 7.8 Hz), 7.64 (d, 2H, J = 7.8 Hz), 7.46 (t, 1H, J = 6.6 Hz), 7.43-7.31 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 160.7, 156.5, 156.3, 152.9, 150.5, 137.0, 135.6, 135.3, 130.7, 129.9, 129.7, 129.6, 128.6. 128.5, 125.4, 124.1. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 387 (454.7). HRMS

(ESI) m/z calculated 311.12967 ($C_{20}H_{15}N_4$, $[M+H]^+$), m/z observed 311.12831 ($C_{20}H_{15}N_4$, $[M+H]^+$).



5,6-diphenyl-3-(pyrimidin-2-yl)-1,2,4-triazine (2-18). **2-11** (100 mg, 0.730 mmol) and benzil (153 mg, 0.730 mmol) were added to 2 mL of EtOH. The slurry was heated for 6 h at 80°C. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting yellow precipitate was filtered and washed with EtOH resulting in a 75% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.10 (d, 2H, J = 4.8 Hz), 7.68-7.64 (m, 4H), 7.51 (t, 1H, J = 4.8 Hz), 7.46-7.43 (m, 2H), 7.41-7.39 (m, 2H), 7.38-7.35 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.8, 160.2, 158.3, 157.4, 156.7, 135.5, 135.2, 131.0, 130.1, 129.9, 128.8, 121.9. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 386 (460.4). HRMS (ESI) m/z calculated 312.12492 ($C_{19}H_{14}N_5$, $[M+H]^+$), m/z observed 312.12414 ($C_{19}H_{14}N_5$, $[M+H]^+$).

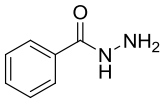


5,6-diphenyl-3-(pyrazin-2-yl)-1,2,4-triazine (2-19). **2-12** (100 mg, 0.730 mmol) and benzil (153 mg, 0.730 mmol) were added to 2 mL of EtOH. The slurry was heated overnight at 80°C. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to quantitatively yield the product as a yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.91 (s, 1H), 8.87 (s, 1H), 8.78 (s, 1H), 7.69 (d, 2H, J = 7.2 Hz), 7.65 (d, 2H, J = 7.8 Hz), 7.48-7.45 (m, 2H), 7.42-7.37 (m, 4H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 159.6, 157.2, 156.7, 148.6, 146.3, 145.7, 145.0, 135.4, 135.1, 131.2, 130.2, 130.1, 129.8, 128.9, 128.8. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 386 (523.6). HRMS (ESI) m/z calculated 312.12492 ($C_{19}H_{14}N_5$, $[M+H]^+$), m/z observed 312.12346 ($C_{19}H_{14}N_5$, $[M+H]^+$).

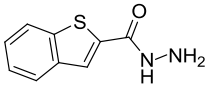
General Hydrazide Method A: Methyl (or Ethyl) Ester (1 eq) was dissolved in hydrazine hydrate (2.5 eq) and MeOH (or EtOH) (3.5 M) and refluxed overnight. MeOH

(or EtOH) and excess hydrazine hydrate were removed *in vacuo*. The precipitated product was dried under vacuum.

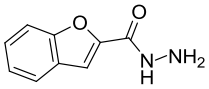
General Hydrazide Method B: Carboxylic acid (1 eq) was dissolved in xylenes (0.6 M) and hydrazine hydrate (10 eq) was added to the mixture. This was microwaved at 180°C for 6 hours. The resulting mixture was extracted with DCM and concentrated *in vacuo*.



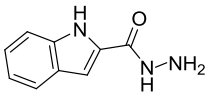
benzohydrazide (2-25). Prepared using general hydrazide method A and isolated as a white solid. ^{1}H NMR (CD_3CN, 600 MHz) δ 8.23 (br), 7.76 (d, 2H, J = 7.2 Hz), 7.52 (m, 1H), 7.45 (t, 2H, J = 7.2 Hz) ^{13}C NMR (CD_3CN, 151 MHz) δ 168.3, 134.3, 132.3, 129.4, 127.8. HRMS (ESI) m/z calculated 137.07149 ($C_7H_9N_2O$, $[M+H]^+$), m/z observed 137.07190 ($C_7H_9N_2O$, $[M+H]^+$).



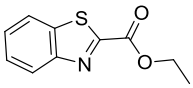
benzo[b]thiophene-2-carbohydrazide (2-26). Prepared using general hydrazide method B and isolated as a brown solid in 54% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 10.03 (bs, 1H), 8.00 – 8.02 (m, 2H), 7.91 (d, 1H, J = 7.2 Hz), 7.41 – 7.46 (m, 2H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 161.3, 139.9, 139.2, 138.4, 126.0, 125.0, 124.9, 124.1, 122.7. HRMS (ESI) m/z calculated 193.04356 ($C_9H_9N_2OS$, $[M+H]^+$), m/z observed 193.04230. ($C_9H_9N_2OS$, $[M+H]^+$).



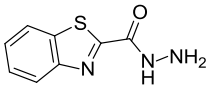
benzofuran-2-carbohydrazide (2-27). Prepared using general hydrazide method B and isolated as a white solid in 63% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 10.02 (bs, 1H), 7.75 (d, 1H, J = 7.8 Hz), 7.63 (d, 1H, J = 8.4 Hz), 7.51 (s, 1H), 7.44 (t, 1H, J = 7.8 Hz), 7.32 (t, 1H, J = 7.8 Hz), 4.85 (b, NH_2). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 157.8, 154.1, 148.4, 127.0, 126.6, 123.6, 122.6, 111.7, 108.7. HRMS (ESI) m/z calculated 177.06640 ($C_9H_9N_2O_2$, $[M+H]^+$), m/z observed 177.06594. ($C_9H_9N_2O_2$, $[M+H]^+$).



1H-indole-2-carbohydrazide (2-28). Prepared using general hydrazide method B and isolated as a pale yellow solid in 64% yield. Used crude for next step. HRMS (ESI) m/z calculated 176.08239 ($C_9H_{10}N_3O$, $[M+H]^+$), m/z observed 176.08134. ($C_9H_{10}N_3O$, $[M+H]^+$).

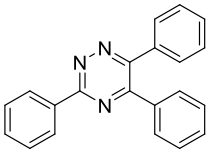


ethyl benzo[d]thiazole-2-carboxylate (2-24). 2-aminothiophenol (1eq) was dissolved in diethyloxalate (2 eq) and heated at 120°C overnight. Reaction progress was followed by TLC. Upon consumption of starting material, reaction mixture was poured into a mixture of HCl, H_2O, and EtOH. Two layers formed. Stir biphasic solution. Once solid formation begins, place in chilled water bath. Filter and wash solid with small amounts of cold water. Isolated in 72% yield as a pale green solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.25 (d, 1H, J = 8.4 Hz), 7.98 (d, 1H, J = 7.8 Hz), 7.58 (t, 1H, J = 7.8 Hz), 7.54 (t, 1H, J = 7.8 Hz), 4.56 (q, 2H, J = 7.2 Hz), 1.49 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 160.8, 158.7, 153.4, 136.9, 127.7, 127.2, 125.7, 122.2, 63.3, 14.4. HRMS (ESI) m/z calculated 208.04322 ($C_{10}H_{10}NO_2S$, $[M+H]^+$), m/z observed 208.04252. ($C_{10}H_{10}NO_2S$, $[M+H]^+$).

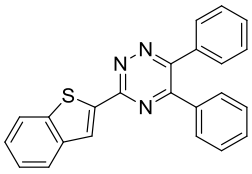


benzo[d]thiazole-2-carbohydrazide (2-29). Ester 2-24 (1 eq) was dissolved in EtOH (0.4 M) and to this mixture was added hydrazine hydrate (1.5 eq). This solution was heated overnight at 80°C with complete dissolution. Reaction progress was followed by TLC. Upon removal of solution from heat, yellow precipitate formed. Filter and wash with small portions of EtOH. Resultant solid dried under vacuum. Collected yellow solid in 81% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 10.46 (s, 1H) 8.22 (d, 1H, J = 7.8 Hz), 8.11 (d, 1H, J = 7.8 Hz), 7.62 (t, 1H, J = 7.8 Hz), 7.57 (t, 1H, J = 7.8 Hz), 4.72 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 163.7, 158.1, 152.8, 135.7, 127.0, 126.7, 123.9, 122.9. HRMS (ESI) m/z calculated 194.03881 ($C_8H_8N_3OS$, $[M+H]^+$), m/z observed 194.03803. ($C_8H_8N_3OS$, $[M+H]^+$)

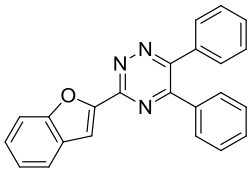
General Triazine Method B: Heterocyclic hydrazide (1.1 eq) was dissolved in AcOH (0.25 M). To this mixture was added benzil (1 eq) and NH_4OAC (10 eq). This solution was heated in a sealed vial using a microwave synthesizer at 180°C.



3,5,6-triphenyl-1,2,4-triazine (2-30). 2-16 (142 mg, 1.05 mmol), benzil (200 mg, 0.95 mmol), and ammonium acetate (732 mg, 9.50 mmol) were suspended in AcOH (2 mL). The suspension was heated in a conventional microwave at 750W and monitored by TLC until benzil no longer remained. The reaction mixture was concentrated *in vacuo* and precipitated out of EtOH. The product was isolated as a pale yellow solid in 51% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.67 (s, 1H), 7.67 (d, 2H, J = 7.2 Hz), 7.62 (d, 2H, J = 7.2 Hz), 7.55-7.54 (m, 3H), 7.44-7.34 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.3, 155.48, 155.46, 135.9, 135.5, 134.8, 131.5, 130.7, 129.8, 129.5, 129.4, 128.8, 128.6, 128.5, 128.3. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 385 (480.9). HRMS (ESI) m/z calculated 310.13442 ($C_{21}H_{16,}N_3$, $[M+H]^+$), m/z observed 310.13349 ($C_{21}H_{16}N_3$, $[M+H]^+$).

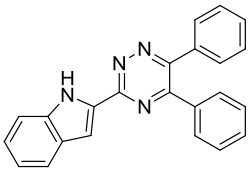


3-(benzo[b]thiophen-2-yl)-5,6-diphenyl-1,2,4-triazine (2-31). Prepared using general triazine method B. The solid formed upon heating was centrifuged, washed with AcOH, and purified over silica gel, eluting with a gradient of hexanes and ethyl acetate to afford a yellow solid in 28% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.53 (s, 1H), 7.91 – 7.94 (m, 2H) 7.66 – 7.70 (m, 2H), 7.60 – 7.62 (m, 2H), 7.37 – 7.50 (m, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.3, 155.9, 155.6, 142.4, 140.1, 139.8, 135.63, 135.56, 131.0, 130.0, 129.8, 129.6, 128.8, 128.7, 127.3, 126.4, 125.2, 125.0, 122.9. HRMS (ESI) m/z calculated 366.10649 ($C_{23}H_{16}N_3S$, $[M+H]^+$), m/z observed 366.10475. ($C_{23}H_{16}N_3S$, $[M+H]^+$).

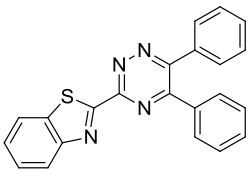


3-(benzofuran-2-yl)-5,6-diphenyl-1,2,4-triazine (2-32). Prepared using general triazine method B. The fine solid formed upon heating was centrifuged, washed with AcOH, and purified over silica gel,

eluting with a gradient of hexanes and ethyl acetate to afford a pale yellow solid in 42% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.97 (s, 1H), 7.75 (d, 1H, J = 7.8 Hz) 7.71 (d, 1H, J = 8.4 Hz), 7.67 (d, 2H, J = 7.2 Hz), 7.62 (d, 2H, J = 7.2 Hz), 7.42 – 7.49 (m, 3H), 7.37 – 7.41 (m, 4H), 7.33 (t, 1H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 156.5, 156.13, 156.07, 155.8, 151.4, 135.6, 135.4, 131.0, 130.0, 129.9, 129.7, 128.8, 128.3, 126.9, 123.8, 122.5, 112.5, 111.3. HRMS (ESI) m/z calculated 350.12934 ($C_{23}H_{16}N_3O$, $[M+H]^+$), m/z observed 350.12765. ($C_{23}H_{16}N_3O$, $[M+H]^+$).

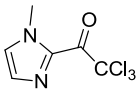


2-(5,6-diphenyl-1,2,4-triazin-3-yl)-1*H*-indole (2-33). Prepared using general triazine method B. The fine solid formed upon heating was centrifuged, washed with AcOH, and purified over silica gel, eluting with a gradient of hexanes and ethyl acetate to afford a bright yellow solid in 63% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.57 (s, 1H), 7.76 (d, 1H, J = 8.4 Hz) 7.68 (s, 1H), 7.66 (d, 2H, J = 7.2 Hz), 7.59 (d, 2H, J = 7.2 Hz), 7.32 (t, 1H, J = 7.2 Hz), 7.17 (t, 1H, J = 7.2 Hz), 7.46 – 7.51 (m, 2H), 7.41 – 7.45 (m, 1H), 7.37 – 7.41 (m, 4H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 157.0, 156.2, 155.4, 137.7, 135.8, 135.6, 133.0, 131.0, 130.0, 129.7, 129.6, 128.9, 128.7, 124.9, 122.3, 120.9, 111.9, 107.1. HRMS (ESI) m/z calculated 349.14532 ($C_{23}H_{17}N_4$, $[M+H]^+$), m/z observed 349.14435. ($C_{23}H_{17}N_4$, $[M+H]^+$).

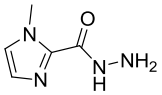


2-(5,6-diphenyl-1,2,4-triazin-3-yl)benzo[*d*]thiazole (2-34). Prepared using general triazine method B. The solution was diluted with water and extracted with DCM (3x). The organic layer was concentrated *in vacuo* and the resultant material was purified over silica gel, eluting with a gradient of hexanes and ethyl acetate to afford a yellow solid in 34% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.32 (d, 1H, J = 8.4 Hz), 8.03 (d, 1H, J = 7.8 Hz), 7.71 (d, 2H, J = 7.8 Hz), 7.65 (d, 2H, J = 8.4 Hz), 7.58 (t, 1H, J = 7.2 Hz, 8.4 Hz), 7.52 (t, 1H, J = 7.2 Hz, 8.4 Hz), 7.45 – 7.49 (m, 2H), 7.38-7.42 (m, 4H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 164.1, 157.9, 157.5, 156.8, 154.6, 136.8, 135.09, 135.07, 131.3, 130.3, 130.2, 129.8, 128.9, 128.8, 127.0, 126.9, 125.3, 122.1. HRMS (ESI) m/z

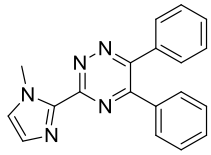
calculated 367.10174 ($C_{22}H_{15}N_4S$, $[M+H]^+$), m/z observed 367.09878 (7.98 ppm). ($C_{22}H_{15}N_4S$, $[M+H]^+$).



2,2,2-trichloro-1-(1-methyl-1H-imidazol-2-yl)ethanone (2-36). Methyl imidazole (1 mmol) was dissolved in DCM (1.3 mL). In a separate flask, combine DCM (1.3 mL) and trichloroacetylchloride (1.1 mmol). To a stirred solution of methyl imidazole in DCM, and the trichloroacetylchloride in DCM dropwise. Solution turned cloudy upon first drops, but quickly turned transparent yellow. Upon stirring solution became cloudy yellow. Stir at room temperature overnight. Cool reaction mixture in ice bath and dropwise add triethylamine (1.1 mmol). Solution turned from thick bright yellow to dark brown upon addition. Concentrate *in vacuo* and resulting residue purified via silica gel chromatography using gradient elution of hexanes and DCM. Isolated as a white solid in 72% yield.

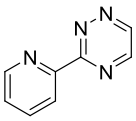


1-methyl-1H-imidazole-2-carbohydrazide (2-37). Dissolve **2-36** (1 mmol) in dioxanes (5 mL). Cool solution in ice bath, and add hydrazine hydrate (2 mmol) to cooled solution. Remove ice bath and stir solution at room temperature overnight. Reaction progress followed by TLC. Upon completion, reaction mixture was concentrated *in vacuo* to yield an off-white solid in 90% yield.

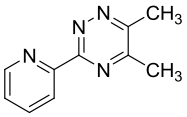


3-(1-methyl-1*H*-imidazol-2-yl)-5,6-diphenyl-1,2,4-triazine (2-38). Benzil (1 mmol) and hydrazide 2-37 (1.05 mmol) were combined in AcOH (4 mL) in a microwave vessel. Reaction mixture was stirred at 60°C overnight. TLC shows consumption of starting material. Add NH_4OAC (10 mmol) and microwave at 180°C for 10 min. Starting materials consumed by TLC. Add H_2O and extract with DCM. Combine organics and wash with H_2O. Concentrate *in vacuo* and purify over silica gel eluting with hexanes, ethyl acetate, and MeOH. Isolated a yellow solid in 52% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.67 – 7.69 (m, 2H), 7.61 – 7.62 (m, 2H), 7.42 – 7.45 (m, 2H), 7.38 – 7.40 (m, 2H), 7.32 – 7.36 (m, 3H), 7.15 (s, 1H), 4.28 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 156.6, 156.0, 155.6, 141.9, 135.5, 135.4, 131.0,

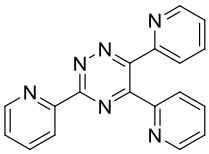
130.6, 130.2, 129.9, 129.6, 128.8, 128.6, 126.2, 37.4. HRMS (ESI) m/z calculated 314.14057 ($C_{19}H_{16}N_5$, $[M+H]^+$), m/z observed 314.14014. ($C_{19}H_{16}N_5$, $[M+H]^+$).



3-(pyridin-2-yl)-1,2,4-triazine (2-42). **2-14** (500 mg, 3.67 mmol) and **2-39** (40% by wt in water, 533 mg, 3.67 mmol) were added to 10 mL of EtOH. The slurry was heated overnight at 60°C with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* to yield an orange-brown solid in 83% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.29 (s, 1H), 8.89 (d, 1H, J = 4.2 Hz), 8.82 (s, 1H), 8.68 (d, 1H, J = 7.8 Hz), 7.93 (m, 1H), 7.49 (t, 1H, J = 6.6 Hz) ^{13}C NMR ($CDCl_3$, 151 MHz) δ 163.6, 152.5, 150.7, 149.5, 148.8, 137.4, 126.0, 124.2. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 388 (376.0). HRMS (ESI) m/z calculated 159.06707 ($C_8H_7N_4$, $[M+H]^+$), m/z observed 159.06661 ($C_8H_7N_4$, $[M+H]^+$).

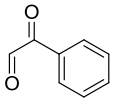


5,6-dimethyl-3-(pyridin-2-yl)-1,2,4-triazine (2-43). **2-14** (600 mg, 4.17 mmol) and **2-40** (377 mg, 4.38 mmol) were added to 10 mL of EtOH. The slurry was heated at 80°C overnight with complete dissolution. Progress of the reaction was monitored by TLC. After concentration *in vacuo*, the crude product was purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a pale brown solid in 90.0% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.88 (m, 1H), 8.65 (d, 1H, J = 8.4 Hz), 7.90 (t, 1H, J = 7.8 Hz), 7.46-7.44 (m, 1H), 2.78 (s, 3H), 2.70 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.5, 159.6, 157.0, 153.0, 150.3, 137.1, 125.2, 123.8, 22.1, 19.7. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 372 (378.1). HRMS (ESI) m/z calculated 187.09782 ($C_{10}H_{11}N_4$, $[M+H]^+$), m/z observed 187.09857 ($C_{10}H_{11}N_4$, $[M+H]^+$).

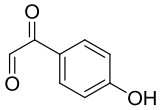


3,5,6-tri(pyridin-2-yl)-1,2,4-triazine (2-44). **2-14** (100 mg, 0.730 mmol) and **2-44** (150 mg, 0.730 mmol) were added to 2 mL of EtOH. The slurry was heated overnight at 80°C with complete dissolution.

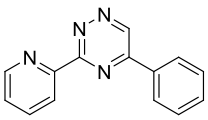
Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with EtOH resulting in a 95% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.94 (d, 1H, J = 3.6 Hz), 8.75 (d, 1H, J = 7.8 Hz), 8.36-8.35 (m, 2H), 8.27-8.25 (m, 2H), 7.96 (dt, 1H, J = 7.8, 1.8 Hz), 7.93-7.87 (m, 2H), 7.52-7.49 (m, 1H), 7.32-7.29 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.5, 156.37, 156.35, 154.9, 154.8, 152.8, 150.8, 148.93, 148.92, 137.4, 137.21, 137.19, 125.9, 125.1, 124.70, 124.66, 124.5, 124.0. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 382 (495.9). HRMS (ESI) m/z calculated 313.12017 ($C_{18}H_{13}N_6$, $[M+H]^+$), m/z observed 313.11959 ($C_{18}H_{13}N_6$, $[M+H]^+$).



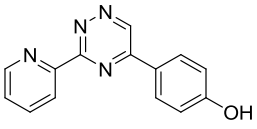
2-oxo-2-phenylacetaldehyde (2-47). Selenium dioxide (924 mg, 8.32 mmol) was suspended in dioxane (4.2 mL) and water (0.20 mL) and heated at 55°C until mostly dissolved. **2-45** (1 g, 8.32 mmol) was then added and the reaction was refluxed for 4 h. After cooling to room temperature, the reaction was filtered and the filtrate was concentrated *in vacuo*. Purification via silica gel chromatography with gradient elution using EtOAc and hexanes was used to further purify the reaction mixture. The crude product was used in further reactions. MS (ESI) m/z calculated 135.04460 ($C_8H_7O_2$, $[M+H]^+$), m/z observed 135.042 ($C_8H_7O_2$, $[M+H]^+$).



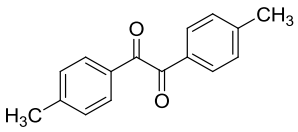
2-(4-hydroxyphenyl)-2-oxoacetaldehyde (2-48). Selenium dioxide (814 mg, 7.34 mmol) was suspended in dioxane (3.7 mL) and water (0.18 mL) and heated at 55°C until mostly dissolved. **2-46** (3 g, 15.0 mmol) was then added and the reaction was refluxed for 4 h. After cooling to room temperature, the reaction was filtered and the filtrate was concentrated *in vacuo*. Purification via silica gel chromatography with gradient elution using EtOAc and hexanes was used to further purify the reaction mixture. The crude product was used in further reactions. MS (ESI) m/z calculated 151.03952 ($C_8H_7O_3$, $[M+H]^+$), m/z observed 151.038 ($C_8H_7O_3$, $[M+H]^+$).



5-phenyl-3-(pyridin-2-yl)-1,2,4-triazine (2-49). **2-47** (246 mg, 1.84 mmol) and **2-14** (250 mg, 1.84 mmol) were added to 5 mL of EtOH. The slurry was heated for 4 h at 80°C with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a white solid in 82% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.72 (s, 1H), 8.94 (d, 1H, J = 4.2 Hz), 8.68 (d, 1H, J = 7.8 Hz), 8.31 (d, 2H, J = 8.4 Hz), 7.95 (t, 1H, J = 7.8 Hz), 7.63-7.59 (m, 3H), 7.50 (m, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 162.9, 156.0, 153.2, 150.7, 145.5, 137.3, 133.6, 132.8, 129.6, 128.0, 125.7, 124.3. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 382 (486.2). HRMS (ESI) m/z calculated 235.09837 ($C_{14}H_{11}N_4$, $[M+H]^+$), m/z observed 235.09829 ($C_{14}H_{11}N_4$, $[M+H]^+$).

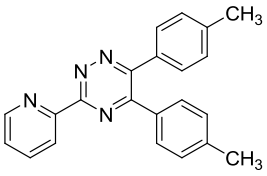


4-(3-(pyridin-2-yl)-1,2,4-triazin-5-yl)phenol (2-50). **2-48** (150 mg, 1.0 mmol) and **2-14** (136 mg, 1.0 mmol) were added to 3 mL of EtOH. The slurry was heated overnight at 80°C with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and concentrated *in vacuo* yielding an orange solid in 90% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.56 (s, 1H), 8.89 (d, 1H, J = 4.2 Hz), 8.72 (d, 1H, J = 7.8 Hz), 8.10 (d, 2H, J = 8.4 Hz), 8.00 (t, 1H, J = 8.4 Hz), 7.56 (m, 1H), 6.96 (d, 2H, J = 4.8 Hz), 3.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 162.5, 161.6, 156.1, 153.1, 149.9, 145.1, 130.2, 126.0, 124.8, 124.7, 116.9. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 385 (1054.7). HRMS (ESI) m/z calculated 251.09329 ($C_{14}H_{11}N_4O$, $[M+H]^+$), m/z observed 251.09254 ($C_{14}H_{11}N_4O$, $[M+H]^+$).

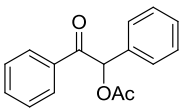


1,2-di-p-tolylethane-1,2-dione (2-53). **2-52** (5 g, 41.6 mmol) and KCN (1.35 g, 20.8 mmol) was dissolved in EtOH (8 mL) and water (4 mL). The mixture was heated under reflux for 0.5 h. After cooling to room temperature, the reaction mixture was poured into an ice bath and the crude benzoin intermediate was precipitated. The solid was then filtered and further washed with water. The crude benzoin intermediate was then refluxed in nitric acid (8

mL) for 2 h. The reaction mixture was poured into an ice bath and the crude benzil derivative was precipitated and filtered. Recrystallization from EtOH yielded a yellow solid in 39% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.86 (d, 4H, J = 7.8 Hz), 7.30 (d, 4H, J = 7.8 Hz), 2.42 (s, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 194.6, 146.2, 130.9, 130.2, 129.8, 22.1. HRMS (ESI) m/z calculated 239.10720 ($C_{16}H_{15}O_2$, $[M+H]^+$), m/z observed 152.08191 239.10710 ($C_{16}H_{15}O_2$, $[M+H]^+$).

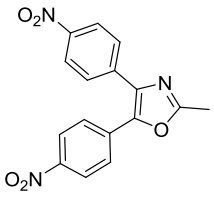


3-(pyridin-2-yl)-5,6-di-p-tolyl-1,2,4-triazine (2-54). 2-14 (284 mg, 2.10 mmol) and **2-52** (500 mg, 2.10 mmol) were added to EtOH (12 mL). The slurry was heated at 80°C for 6 h with complete dissolution. Progress of the reaction was monitored by TLC. After concentration *in vacuo*, the crude product was purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a pale yellow solid in 41.1% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.92 (d, 1H, J = 4.8 Hz), 8.70 (d, 1H, J = 7.8 Hz), 7.93 (t, 1H, J = 7.8 Hz), 7.62 (d, 2H, J = 8.4 Hz), 7.54 (d, 2H, J = 8.4 Hz), (m, 1H), 7.21 (d, 2H, J = 7.8 Hz), 7.19 (d, 2H, J = 7.8 Hz), 2.40 (s, 3H), 2.38 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 160.6, 156.5, 156.3, 153.2, 150.6, 141.3, 140.0, 137.2, 133.1, 132.8, 130.1, 129.6, 129.5, 129.4, 125.4, 124.2, 21.7, 21.6. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 400 (445.5). HRMS (ESI) m/z calculated 339.16097 ($C_{22}H_{19}N_4$, $[M+H]^+$), m/z observed 339.15939 ($C_{22}H_{19}N_4$, $[M+H]^+$).

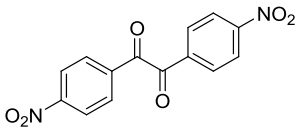


2-oxo-1,2-diphenylethyl acetate (2-56). 2-55 (4 g, 18.84 mmol) and acetic anhydride (4.33g, 42.40 mmol) were dissolved in AcOH (4 mL). Concentrated HCl (0.4 mL) was added dropwise at room temperature. The reaction was then heated at 100°C for 30 min. After cooling to room temperature, 50 mL of water was added and the reaction continued to stir at room temperature for an additional 2 h. The precipitate was filtered and purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a white solid in 63% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.93 (d, 2H, J = 7.8 Hz), 7.51 (t, 1H, J = 7.8 Hz), 7.47 (d, 2H, J = 7.8 Hz), 7.41-7.34 (m, 5H), 6.87 (s, 1H), 2.21 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ

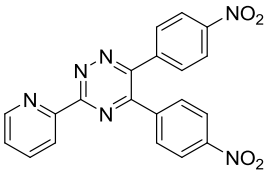
193.8, 170.6, 134.7, 133.7, 133.6, 129.5, 129.3, 128.9, 128.83, 128.76, 77.8, 20.9. HRMS (ESI) m/z calculated 255.10212 ($C_{16}H_{15}O_3$, $[M+H]^+$), m/z observed 255.10306 ($C_{16}H_{15}O_3$, $[M+H]^+$).



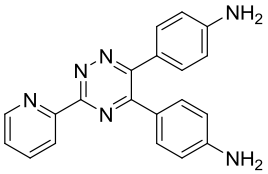
2-methyl-4,5-bis(4-nitrophenyl)oxazole (2-57). **2-56** (1 g, 3.93 mmol) and ammonium acetate (454 mg, 5.90 mol) were dissolved in AcOH (1.3 mL) and refluxed for 2 h. After warming to room temperature, the crude reaction was poured over ice and extracted with Et_2O. The organic layer was washed with water and brine, then dried with sodium sulfate. Concentration *in vacuo* produced the intermediate oxazole as a brown oil. This oil was dissolved in sulfuric acid (3 mL) and nitric acid (3 mL) was added dropwise at room temperature. The reaction was then heated at 55°C for 15 min. Progress of the reaction was monitored by TLC. After warming to room temperature, ice was added to precipitate the product. Recrystalization from AcOH producted yellow solid in 59% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.26 (t, 4H, J = 8.4 Hz), 7.82 (d, 2H, J = 8.4 Hz), 7.74 (d, 2H, J = 8.4 Hz), 2.62 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 162.4, 147.9, 147.7, 145.0, 138.3, 136.3, 134.3, 128.9, 127.2, 124.5, 124.3, 14.2. HRMS (ESI) m/z calculated 326.07770 ($C_{16}H_{12}N_3O_5$, $[M+H]^+$), m/z observed 326.07637 ($C_{16}H_{12}N_3O_5$, $[M+H]^+$).



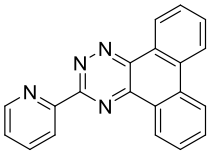
1,2-bis(4-nitrophenyl)ethane-1,2-dione (2-58). **2-57** (600 mg, 1.85 mmol) and sodium acetate (1.2 g, 14.8 mmol) were dissolved in a combination of AcOH (6.6 mL) and water (0.67 mL). Bromine (591 mg, 7.4 mmol) was then added and the reaction was refluxed for 2 h. Progress of the reaction was monitored by TLC. After cooling to room temperature, the reaction was poured over ice and filtered. Recrystalization from AcOH produced a yellow solid in 42% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.39 (d, 4H, J = 8.4 Hz), 8.21 (d, 4H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 190.4, 151.7, 136.9, 131.4, 124.4. HRMS (ESI) m/z calculated 301.04606 ($C_{14}H_9N_2O_6$, $[M+H]^+$), m/z observed 301.04552 ($C_{14}H_9N_2O_6$, $[M+H]^+$).



5,6-bis(4-nitrophenyl)-3-(pyridin-2-yl)-1,2,4-triazine (2-59). 2-14 (136 mg, 1.0 mmol) and **2-58** (300 mg, 1.0 mmol) were added to 6 mL of EtOH. The slurry was heated overnight at 80°C with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with EtOH resulting in a 93% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.96 (d, 1H, J = 4.2 Hz), 8.75 (d, 1H, J = 7.8 Hz), 8.30-8.27 (m, 4H), 7.99 (dt, 1H, J = 7.8, 1.2 Hz), 7.86 (d, 2H, J = 7.2 Hz), 7.82 (d, 2H, J = 6.6 Hz), 7.56-7.54 (m, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.6, 154.7, 154.6, 152.0, 151.0, 149.5, 149.1, 141.1, 140.7, 137.5, 131.2, 130.8, 126.3, 124.8, 124.33, 124.28. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 404 (546.5). HRMS (ESI) m/z calculated 401.09983 ($C_{20}H_{13}N_6O_4$, $[M+H]^+$), m/z observed 401.09918 ($C_{20}H_{13}N_6O_4$, $[M+H]^+$).

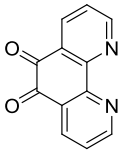


4,4'-(3-(pyridin-2-yl)-1,2,4-triazine-5,6-diyl)dianiline (2-60). 2-59 (200 mg, 0.5 mmol), and 5% palladium/carbon (2 mg), and hydrazine hydrate (1 mL) were added to 2.5 mL of EtOH. The reaction was refluxed for 6 h and progress was monitored by TLC. Upon completion, reaction was filtered through celite and eluted with MeOH. Concentration *in vacuo* yielded the crude product as an orange solid in 85% yield. HPLC purity: 80%. UV-Vis (9:1 MeCN:DMSO) λ_{max} nm (ε M^{-1} cm^{-1}): 475 (483.0). HRMS (ESI) m/z calculated 341.15147 ($C_{20}H_{17}N_6$, $[M+H]^+$), m/z observed 341.15043 ($C_{20}H_{17}N_6$, $[M+H]^+$).

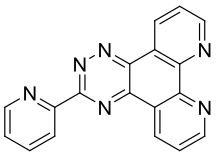


3-(pyridin-2-yl)phenanthro[9,10-e][1,2,4]triazine (2-62). 2-14 (500 mg, 3.67 mmol) and **2-61** (764 mg, 3.67 mmol) were added to 10 mL of ACN. The slurry was heated at 80°C overnight with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with ACN and Et_2O resulting in a 75% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.55 (d, 1H, J =

7.8 Hz), 9.49 (d, 1H, J = 7.8 Hz), 9.01 (d, 1H, J = 4.8 Hz), 8.90 (d, 1H, J = 7.8 Hz), 8.61 (d, 2H, J = 7.8 Hz), 8.01 (t, 1H, J = 7.8 Hz), 7.91 (t, 1H, J = 7.8 Hz), 7.86 (t, 1H, J = 7.8 Hz), 7.82 (t, 1H, J = 7.2 Hz), 7.78 (t, 1H, J = 7.2 Hz), 7.53 (m, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 160.67, 153.7, 150.6, 145.6, 143.3, 137.2, 133.9, 132.6, 131.4, 131.1, 128.7, 128.1, 127.9, 127.5, 127.2, 125.31, 125.28, 124.3, 123.1, 123.0. UV-Vis (DCE) λ_{max} nm (ε M^{-1} cm^{-1}): 424 (381.7). HRMS (ESI) m/z calculated 309.11402 ($C_{20}H_{13}N_4$, $[M+H]^+$), m/z observed 309.11249 ($C_{20}H_{13}N_4$, $[M+H]^+$).

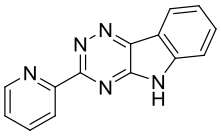


1,10-phenanthroline-5,6-dione (2-64). 2-63 (500 mg, 2.78 mmol) and KBr (700 mg, 5.88 mmol) was added to a mixture of concentrated sulfuric acid (5 mL) and nitric acid (2.5 mL) at -5°C. The mixture was stirred at that temperature for 15 min and then brought to reflux and heated for 4 h. The yellow solution was allowed to cool and poured over 50 g of ice and carefully brought to pH 9 by addition of 5 M sodium hydroxide. The suspension was extracted with $CHCl_3$, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to yield fine yellow needles in 84% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.14 (d, 2H, J = 4.8 Hz), 8.52 (d, 2H, J = 7.8 Hz), 7.60 (dd, 2H, J = 7.8, 4.8 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 178.7, 156.4, 152.9, 137.3, 128.1, 125.6. HRMS (ESI) m/z calculated 211.05075 ($C_{12}H_7N_2O_2$, $[M+H]^+$), m/z observed 211.05055 ($C_{12}H_7N_2O_2$, $[M+H]^+$).

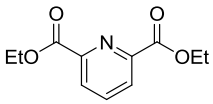


3-(pyridin-2-yl)-[1,2,4]triazino[5,6-f][1,10]phenanthroline (2-65). 2-14 (64 mg, 0.476 mmol) and **2-64** (100 mg, 0.476 mmol) were added to 4 mL of DMF. The slurry was heated at 80°C overnight with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with DMF and Et_2O resulting in a 74% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 9.71 (d, 1H, J = 7.8 Hz), 9.66 (d, 1H, J = 7.2 Hz), 9.35 (s, 1H), 9.31 (s, 1H), 8.96 (s, 1H), 8.84 (d, 1H, J = 7.8 Hz), 8.17 (t, 1H, J = 7.2 Hz), 8.05 (s, 2H), 7.72 (s, 1H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 160.9, 154.4, 153.2, 152.8, 150.4, 149.3, 147.1, 144.5, 142.6, 137.6, 133.9, 132.1, 126.0, 125.4, 125.1, 124.84, 124.78, 124.5. UV-Vis (9:1

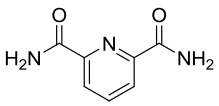
DCE:MeOH) λ_{max} nm (ε M^{-1} cm^{-1}): 423 (376.6). HRMS (ESI) m/z calculated 311.10452 ($C_{18}H_{11}N_6$, $[M+H]^+$), m/z observed 311.10452 ($C_{18}H_{11}N_6$, $[M+H]^+$).



3-(pyridin-2-yl)-5H-[1,2,4]triazino[5,6-b]indole (2-67). 2-14 (500 mg, 3.67 mmol) and **2-66** (540 mg, 3.67 mmol) were added to 10 mL of EtOH. The slurry was heated at 80°C overnight with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting orange precipitate was filtered and washed with EtOH and Et_2O resulting in a 59% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 8.67 (d, 1H, J = 4.2 Hz), 8.50-8.47 (m, 2H), 8.02 (bs, 1H), 7.90 (dt, 1H, J = 9, 1.8 Hz), 7.50 (m, 1H), 7.32 (t, 1H, J = 7.8 Hz), 7.11 (t, 1H, J = 7.8 Hz), 6.90 (d, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 166.9, 160.6, 149.6, 148.9, 146.3, 142.4, 137.0, 131.8, 128.9, 126.1, 123.0, 122.7, 118.5, 110.3 MS (ESI) m/z calculated 248.09362 ($C_{14}H_{10}N_5$, $[M+H]^+$), m/z observed 248.09721 ($C_{14}H_{10}N_5$, $[M+H]^+$).

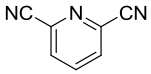


diethyl pyridine-2,6-dicarboxylate. 2-68 (2 g, 11.97 mmol) was suspended in EtOH (5 mL) and HCl (0.5 mL). The suspension was heated in a conventional microwave oven at 750W and monitored by TLC until starting material no longer remained. The reaction mixture was concentrated *in vacuo* and redissolved in water. After adjusting the pH to 8 with 10 M sodium hydroxide, the reaction was extracted with EtOAc (x3) and rinsed with brine. The solution was dried with sodium sulfate and concentrated *in vacuo* to yield a white solid in 56% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 8.28 (d, 2H, J = 6.6 Hz), 8.00 (t, 1H, J = 6.6 Hz), 4.48 (d, 4H, J = 6.0 Hz), 1.45 (s, 6 H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 164.8, 148.8, 138.3, 128.0, 62.5, 14.4. HRMS (ESI) m/z calculated 224.09228 ($C_{11}H_{14}NO_4$, $[M+H]^+$), m/z observed 224.09179 ($C_{11}H_{14}NO_4$, $[M+H]^+$).

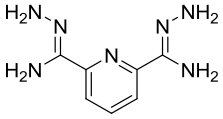


pyridine,2-6-dicarboxamide (2-69). Ammonium hydroxide (2.5 mL) was added to **2-24** (1.5 g, 6.72 mmol) and warmed at 35°C for 1 h with

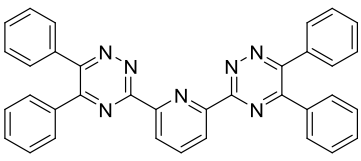
vigorous stirring. After cooling to 0°C, the precipitate was filtered and washed with water. The resulting white solid was dried under vacuum in an 80% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.87 (s, 2H), 8.19-8.14 (m, 3H), 7.70 (s, 2H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 165.4, 149.1, 139.1, 124.1. HRMS (ESI) m/z calculated 166.06165 ($C_7H_8N_3O_2$, $[M+H]^+$), m/z observed 166.06152 ($C_7H_8N_3O_2$, $[M+H]^+$).



pyridine-2,6-dicarbonitrile (2-70). Anhydrous THF (5 mL) and triethylamine (1.58 mL, 11.31 mmol) were added to **2-69** (850 mg, 5.14 mmol) under nitrogen atmosphere. The suspension was cooled to 0°C and trifluoroacetic anhydride (1.57 mL, 11.31 mmol) was added dropwise. After 10 min, the solution was allowed to stir at room temperature for 3 h. The reaction was then quenched with 5 mL of saturated sodium bicarbonate. The resulting precipitate was filtered and washed with water. The resulting white solid was dried under vacuum in a 52% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.06 (t, 1H, J = 7.8 Hz), 7.92 (d, 2H, J = 7.8 Hz) ^{13}C NMR ($CDCl_3$, 151 MHz) δ 138.7, 135.4, 131.0, 115.4. HRMS (ESI) m/z calculated 130.04052 ($C_7H_4N_3$, $[M+H]^+$), m/z observed 130.03995 ($C_7H_4N_3$, $[M+H]^+$).

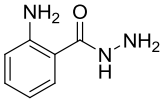


pyridine-2,6-bis(carbohydrazonamide) (2-71). 2-26 (288 mg, 2.23 mmol) was dissolved in EtOH (0.5 mL) and hydrazine hydrate (0.5 mL, 8.93 mmol) and stirred overnight at room temperature. EtOH and excess hydrazine hydrate were removed *in vacuo*. The white solid was dried under vacuum and then stored at 0°C in a 73% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 7.93 (d, 2H, J = 7.8 Hz), 7.66 (t, 1H, J = 7.8 Hz), 6.18 (br), 3.29 (br). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 149.6, 146.0, 136.0, 119.1. HRMS (ESI) m/z calculated 194.11542 ($C_7H_{12}N_7$, $[M+H]^+$), m/z observed 194.11599 ($C_7H_{12}N_7$, $[M+H]^+$).

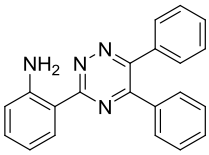


2,6-bis(5,6-diphenyl-1,2,4-triazin-3-yl)pyridine (2-72). 2-71 (288 mg, 1.49 mmol) and benzil (642 mg, 3.05 mmol) were added to 4 mL of EtOH. The slurry was heated

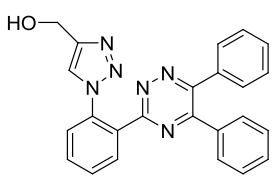
overnight at 80°C with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with EtOH and Et_2O resulting in a 92% yield. ^1H NMR (CD_2Cl_2, 600 MHz) δ 8.86 (d, 2H, J = 7.8 Hz), 8.23 (t, 1H, J = 7.8 Hz), 7.73 (d, 4H, J = 7.2 Hz), 7.66 (d, 4H, J = 7.2 Hz), 7.51-7.48 (m, 4 H), 7.47-7.40 (m, 8H). ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 160.9, 156.9, 156.5, 153.8, 138.5, 135.9, 135.7, 131.0, 130.1, 130.0, 129.8, 128.84, 128.75, 125.9. UV-Vis (9:1 DCE:MeOH) λ_{max} nm (ε M^{-1} cm^{-1}): 394 (1039.0). HRMS (ESI) m/z calculated 542.20932 ($C_{35}H_{24}N_7$, $[M+H]^+$), m/z observed 542.21167 ($C_{35}H_{24}N_7$, $[M+H]^+$).



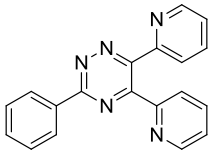
2-aminobenzohydrazide (2-74). Prepared using general hydrazide method A and isolated as a pale brown solid. ^1H NMR (CD_3CN, 600 MHz) δ 8.04 (br) 7.34 (d, 2H, J = 7.8 Hz), 7.18 (t, 1H, J = 7.2 Hz). 6.71 (d, 1H, J = 7.2 Hz), 6.59 (t, 1H, J = 7.2 Hz), 4.79 (br). ^{13}C NMR (D_2O, 151 MHz) δ 170.8, 146.5, 132.8, 128.3, 118.5, 117.9, 117.3. HRMS (ESI) m/z calculated 152.08239 ($C_7H_{10}N_3O$, $[M+H]^+$), m/z observed 152.08191 ($C_7H_{10}N_3O$, $[M+H]^+$).



2-(5,6-diphenyl-1,2,4-triazin-3-yl)aniline (2-75). 2-74 (230 mg, 1.52 mmol), benzil (306 mg, 1.46 mmol), and ammonium acetate (1.02 g, 13.2 mmol) were suspended in AcOH (3 mL). The suspension was heated in a conventional microwave at 750W and monitored by TLC until benzil no longer remained. The reaction mixture was concentrated *in vacuo* and precipitated out of EtOH. The product was isolated as an orange solid in 42% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 8.61 (d, 1H, J = 7.8 Hz), 7.66 (d, 2H, J = 7.8 Hz), 7.60 (d, 2H, J = 7.2 Hz), 7.46-7.35 (m, 6H), 7.29 (t, 1H, J = 7.2 Hz), 6.84-6.79 (m, 2H), 6.39 (s, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 163.3, 155.3, 154.0, 149.3, 136.2, 135.8, 132.3, 131.0, 130.9, 130.0, 129.7, 129.6, 128.8, 128.7, 117.5, 117.2, 115.8. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 373 (5762.7). HRMS (ESI) m/z calculated 325.14532 ($C_{21}H_{17}N_4$, $[M+H]^+$), m/z observed 325.14438 ($C_{21}H_{17}N_4$, $[M+H]^+$).

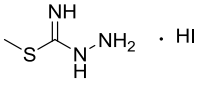


(1-2-(5,6-diphenyl-1,2,4-triazin-3-yl)phenyl)-1H-1,2,3-triazol-4-yl)methanol (2-77). 2-75 (50 mg, 0.154 mmol) was dissolved in 2 mL EtOAc. 1 mL of 1.5 M HCl was added and the solution was cooled to 0°C in an ice bath. Sodium nitrite (21 mg, 0.308 mmol) was dissolved in 0.5 mL water and added dropwise to the reaction solution while stirring at 0°C. After 15 min, sodium azide (40 mg, 0.616 mmol) was dissolved in 0.5 mL water and added dropwise to the reaction solution while stirring at 0°C. After slowly warming to room temperature, the reaction was monitored by TLC. Upon completion, the reaction was extracted with EtOAc (x3), washed with 1 M HCl, water, and brine, and dried with sodium sulfate. After concentration *in vacuo*, the crude material was dissolved in 0.4 mL ACN. Propargyl alcohol (4.3 mg, 0.077 mmol) and triethylamine (10.7 µL, 0.077 mmol) were added to the reaction solution. 5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine (0.2 mg, 0.77 µmol) and tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (0.2 mg, 0.77 µmol) were then added to the reaction solution. The reaction was stirred at room temperature overnight. After concentration *in vacuo*, the crude product was purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a pale yellow solid in 57% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.47 (d, 1H, J = 7.2), 7.83 (s, 1H), 7.77-7.72 (m, 2H), 7.59-7.56 (m, 3H), 7.41-7.29 (m, 9H), 4.73 (s, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.1, 155.4, 155.3, 136.1, 135.1, 135.0, 132.2, 131.9, 131.5, 130.9, 130.4, 129.8, 129.7, 129.5, 128.6, 128.4, 127.0, 56.5. HRMS (ESI) m/z calculated 407.16203 ($C_{24}H_{19}N_6O$, $[M+H]^+$), m/z observed 407.16117 ($C_{24}H_{19}N_6O$, $[M+H]^+$).

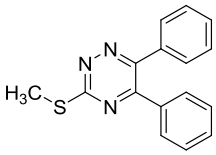


3-phenyl-5,6-di(pyridin-2-yl)-1,2,4-triazine (2-78). Phenyl hydrazide, **2-20** (1 mmol), was dissolved in AcOH (4 mL) in a microwave vial. To this was added 2-2'-pyridil (1 mmol) and ammonium acetate (10 mmol). The reaction mixture was sealed and microwaved for 4 hours at 180°C. Consumption of starting materials was confirmed by TLC. H_2O and DCM were added to the reaction mixture and transferred to a separatory funnel. The aqueous layer was neutralized with NaOH, and extracted with DCM (3x). Combined organics were dried over sodium sulfate, filtered, and concentrated in vacuo. The

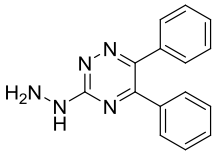
resultant solid was purified over silica eluting with hexanes and ethyl acetate to yield a yellow solid in 40% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.68 – 8.70 (m, 2H), 8.35 (t, 2H, J = 4.2 Hz), 8.26 (dd, 2H, J = 7.8 Hz, J = 4.2 Hz), 7.91 (tq, 2H, J = 7.8 Hz, J = 1.8 Hz), 7.56 – 7.60 (m, 3H), 7.32 (ddd, 1H, J = 7.2 Hz, J = 4.8 Hz, J = 1.2 Hz), 7.30 (ddd, 1H, J = 7.2 Hz, J = 4.8 Hz, J = 1.2 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.9, 155.5, 155.4, 155.2, 155.0, 148.83, 148.76, 137.10, 137.07, 134.7, 132.0, 129.0, 128.7, 124.64, 124.61, 124.1, 123.7. HRMS (ESI) m/z calculated 312.12492 ($C_{19}H_{14}N_5$, $[M+H]^+$), m/z observed 312.12461. ($C_{19}H_{14}N_5$, $[M+H]^+$).



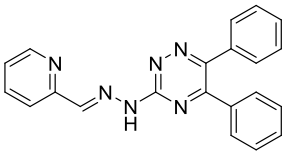
methyl hydrazinecarbimiodthioate hydroiodide (2-79). Thiosemicarbazide (500 mg, 5.49 mmol) was dissolved in 10 mL EtOH and heated at 60°C for 30 min. Iodomethane (0.38 mL, 6.06 mmol) was added portion wise and continue stirring at 60°C for an additional 30 min. After cooling to room temperature, the mixture was concentrated *in vacuo* resulting in a pale brown solid in quantitative yield. The recovered solid was used in further reactions without additional purification.



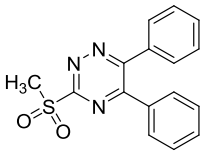
3-(methylthio)-5,6-diphenyl-1,2,4-triazine (2-80). 2-79 (500 mg, 2.15 mmol) and benzil (451 mg, 2.15 mmol) were dissolved in 10 mL of EtOH and stirred overnight at room temperature. Progress of the reaction was monitored by TLC. The reaction was quenched by addition of saturated sodium bicarbonate and solid sodium sulfate and stirring 30 min. The mixture was filtered and DCM was added to the resulting precipitate. The DCM solution was filtered and the corresponding filtrate concentrated *in vacuo*. The product was isolated as a yellow solid in 89% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.55 (d, 2H, J = 8.4 Hz), 7.52 (d, 2H, J = 7.8 Hz), 7.44-7.39 (m, 2H), 7.37-7.32 (m, 4H), 2.76 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 171.3, 155.6, 153.9, 135.54, 135.52, 131.0, 130.0, 129.57, 129.56, 128.8, 128.7, 14.2. HRMS (ESI) m/z calculated 280.09084 ($C_{16}H_{14}N_3S$, $[M+H]^+$), m/z observed 280.08966 ($C_{16}H_{14}N_3S$, $[M+H]^+$).



3-hydrazinyl-5,6-diphenyl-1,2,4-triazine (2-81). 2-80 (200 mg, 0.717 mmol) and hydrazine hydrate (336 mg, 4.30 mmol) were dissolved in 2 mL of EtOH and heated at 80°C overnight. Progress of the reaction was monitored by TLC. The reaction mixture was concentrated *in vacuo* and dried under vacuum yielding a green-yellow solid in 84% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.49-7.48 (m, 2H), 7.43-7.41 (m, 3H), 7.36-7.32 (m, 5H), 6.60 (br, 1H), 4.17 (br, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 162.42, 157.3, 151.4, 136.2, 136.1, 130.7, 129.8, 129.5, 128.8, 128.6. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 353 (2894). HRMS (ESI) m/z calculated 264.12492 ($C_{15}H_{14}N_5$, $[M+H]^+$), m/z observed 264.12513 ($C_{15}H_{14}N_5$, $[M+H]^+$).

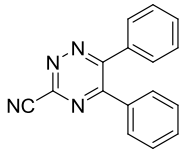


5,6-diphenyl-3-(2-(pyridin-2-ylmethylene)hydrazinyl)-1,2,4-triazine (2-82). 2-81 (75 mg, 0.285 mmol) and 2-pyridinecarbaldehye (31 mg, 0.285 mmol) were dissolved in 3 mL of EtOH and refluxed for 4 h. After cooling to room temperature, the resulting precipitate was filtered and rinsed with EtOH yielding a yellow solid in 80% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.60 (d, 1H, J = 4.8 Hz), 8.34 (s, 1H), 8.00 (d, 1H, J = 8.4 Hz), 7.87 (t, 1H, J = 7.8 Hz), 7.05-7.36 (m, 12H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 158.4, 156.6, 153.6, 151.4, 149.4, 144.3, 136.8, 136.0, 135.8, 130.3, 129.5, 129.1, 128.5, 128.30, 128.29, 123.8, 119.5. UV-Vis (9:1 DCE/MeOH) λ_{max} nm (ε M^{-1} cm^{-1}): 376 (13290). HRMS (ESI) m/z calculated 353.15147 ($C_{21}H_{17}N_6$, $[M+H]^+$), m/z observed 353.14993 ($C_{21}H_{17}N_6$, $[M+H]^+$).

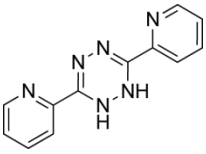


3-(methylsulfonyl)-5,6-diphenyl-1,2,4-triazine (2-83). Dissolved **2-80** (2.5 g, 8.9 mmol) and tetrabutylammonium bromide (287 mg, 0.89 mmol) in 60 mL of benzene and 18 mL of AcOH. Added potassium permanganate (2.8 g, 17.9 mmol) as a solution in 90 mL of water to the reaction and stirred at room temperature overnight. Added saturated sodium bisulfite (approx 5 mL) until color disappeared. Neutralized solution with solid potassium carbonate. Dried organic layer with anhydrous sodium sulfate and concentrated *in vacuo* yielding a

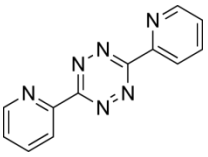
yellow solid in 77% yield. Compound was used immediately or carefully stored away from light and in the freezer to prevent decomposition. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.68 (d, 2H, J = 7.2 Hz), 7.62 (d, 2H, J = 7.2 Hz), 7.52-7.49 (m, 2H), 7.43 (t, 2H, J = 7.8 Hz), 7.37 (t, 2H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 164.5, 159.7, 158.1, 134.2, 133.8, 132.33, 131.0, 130.4, 129.7, 129.4, 129.0, 128.5. HRMS (ESI) m/z calculated 312.08067 ($C_{16}H_{14}N_3O_2S$, $[M+H]^+$), m/z observed 312.08136 ($C_{16}H_{14}N_3O_2S$, $[M+H]^+$).



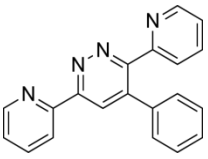
5,6-diphenyl-1,2,4-triazine-3-carbonitrile (2-84). Dissolved **2-83** (500 mg, 1.61 mmol) and finely crushed KCN (210 mg, 3.22 mmol) in 16 mL of DMF and stirred at room temperature. Progress of the reaction was closely monitored by TLC and not allowed to stir overnight. When reaction reached completion, added a small amount of water and extracted with DCM. Purification by alumina chromatography with gradient elution using EtOAc and hexanes yielded a yellow solid in 75% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.63 (d, 4H, J = 7.8 Hz), 7.51 (t, 2H, J = 7.8 Hz), 7.43 (t, 2H, J = 7.8 Hz), 7.39 (t, 2H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 158.3, 156.5, 144.8, 134.1, 133.6, 132.3, 131.2, 130.1, 129.8, 129.2, 129.1. MS (ESI) m/z calculated 259.09837 ($C_{16}H_{11}N_4$, $[M+H]^+$), m/z observed 259.09377 ($C_{16}H_{11}N_4$, $[M+H]^+$).



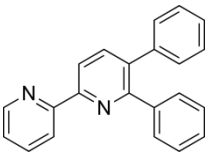
3,6-di(pyridin-2-yl)-1,2-dihydro-1,2,4,5-tetrazine (2-85). 2-cyanopyridine (1.0 g, 10.5 mmol) and hydrazine hydrate (2 mL, 42 mmol) was dissolved in 8 mL of EtOH and refluxed overnight. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting orange precipitate was filtered and washed with Et_2O resulting in a 42% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.58-8.57 (m, 4H), 8.05 (d, 2H, J = 7.8 Hz), 7.75 (dt, 2H, J = 7.2, 1.8 Hz), 7.40-7.34 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.5, 147.7, 146.7, 136.9, 125.0, 121.4. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 382 (350.3). HRMS (ESI) m/z calculated 239.10452 ($C_{12}H_{11}N_6$, $[M+H]^+$), m/z observed 239.10400 ($C_{12}H_{11}N_6$, $[M+H]^+$).



3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (2-86). To a stirring suspension of **2-85** (500 mg, 2.1 mmol) in 2.5 mL of AcOH at 0°C was added dropwise 0.4 mL of concentrated nitric acid. The slurry was allowed to warm to room temperature overnight. The mixture was poured over cold water and made alkaline by addition of sodium carbonate solution. The resulting purple precipitate was filtered, washed with water, and dried under vacuum in 66% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.00 (d, 2H, J = 3.6 Hz), 8.76 (d, 2H, J = 8.4 Hz), 8.02 (dt, 2H, J = 8.4, 1.8 Hz), 7.60-7.58 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 164.0, 151.2, 150.3, 137.6, 126.7, 124.7. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 539 (351.9). HRMS (ESI) m/z calculated 237.08887 ($C_{12}H_9N_6$, $[M+H]^+$), m/z observed 237.08985 ($C_{12}H_9N_6$, $[M+H]^+$).

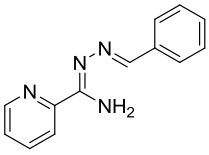


4-phenyl-3,6-di(pyridin-2-yl)pyridazine (2-87). **2-86** (260 mg, 0.85 mmol) and phenylacetylene (173 mg, 1.69 mmol) was dissolved in 5 mL of toluene. The reaction was refluxed for 8 h with progress of the reaction monitored by TLC. Upon disappearance of the starting materials, the reaction was concentrated *in vacuo* and purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a pale brown solid in 77% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.81 (d, 1H, J = 8.4 Hz), 8.74 (d, 1H, J = 4.8 Hz), 8.67 (s, 1H), 8.48 (d, 1H, J = 4.8 Hz), 7.94-7.89 (m, 2H), 7.80 (dt, 1H, J = 9.0, 1.8 Hz), 7.43-7.41 (m, 1H), 7.34-7.30 (m, 3H), 7.29-7.26 (m, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 158.5, 157.9, 156.0, 153.6, 149.6, 149.2, 140.7, 137.4, 137.1, 136.7, 129.1, 128.57, 128.55, 125.8, 125.04, 124.95, 123.5, 122.1. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 350 (344.8). HRMS (ESI) m/z calculated 311.12967 ($C_{20}H_{15}N_4$, $[M+H]^+$), m/z observed 311.12827 ($C_{20}H_{15}N_4$, $[M+H]^+$).

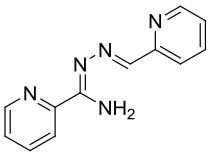


5,6-diphenyl-2,2'-bipyridine (2-89). **2-17** (250 mg, 0.81 mmol) and 2,5-norbornadiene (746 mg, 8.1 mmol) were added to 5.4 mL EtOH and refluxed overnight. Progress of the reaction was monitored by TLC. After concentration *in vacuo*, the crude product was purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a white solid in

66% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.71 (d, 1H, J = 4.8 Hz), 8.60 (d, 1H, J = 7.8 Hz), 8.45 (d, 1H, J = 7.8 Hz), 7.87 (d, 1H, J = 8.4 Hz), 7.82 (dt, 1H, J = 7.8, 1.2 Hz), 7.49-7.48 (m, 2H), 7.33-7.27 (m, 7H), 7.25-7.23 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 156.4, 156.2, 154.7, 149.2, 140.5, 140.1, 139.7, 137.0, 136.2, 130.3, 129.7, 128.5, 127.97, 127.96, 127.4, 123.9, 121.6, 119.3. UV-Vis (DCE) λ_{max} nm (ε M^{-1} cm^{-1}): 350 (240.5). HRMS (ESI) m/z calculated 309.13917 ($C_{22}H_{17}N_2$, $[M+H]^+$), m/z observed 309.13781 ($C_{22}H_{17}N_2$, $[M+H]^+$).

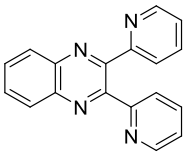


benzylidenepicolinohydrazonamide (2-92). **2-14** (250 mg, 1.84 mmol) and **2-89** (390 mg, 3.67 mmol) were added to 5 mL of EtOH. The reaction was heated overnight at 80°C with progress of the reaction monitored by TLC. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by silica gel chromatography with gradient elution using EtOAc and hexanes yielded a yellow solid in 87% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.61 (d, 1H, J = 4.8 Hz), 8.57 (s, 1H), 8.35 (d, 1H, J = 8.4 Hz), 7.84-7.82 (m, 2H), 7.80-7.78 (m, 1H), 7.44-7.40 (m, 3H), 7.40-7.37 (m, 1H), 6.57 (br, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 157.2, 156.1, 150.4, 148.5, 136.7, 135.4, 130.3, 128.8, 128.1, 125.3, 121.5. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 332 (26568). HRMS (ESI) m/z calculated 225.11402 ($C_{13}H_{13}N_4$, $[M+H]^+$), m/z observed 225.11336 ($C_{13}H_{13}N_4$, $[M+H]^+$).

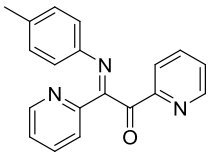


(pyridin-2-ylmethylene)picolinohydrazonamide (2-93). **2-14** (250 mg, 1.84 mmol) and **2-90** (394 mg, 3.67 mmol) were added to 5 mL of EtOH. The reaction was heated overnight at 80°C with progress of the reaction monitored by TLC. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* Purification by silica gel chromatography with gradient elution using EtOAc and hexanes yielded a yellow solid in 84% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.67 (d, 1H, J = 4.2 Hz), 8.63 (s, 1H), 8.60 (d, 1H, J = 4.2 Hz), 8.38 (d, 1H, J = 8.4 Hz), 8.08 (d, 1H, J = 8.4 Hz), 7.80 (dt, 1H, J = 7.2, 1.2 Hz), 7.31 (dt, 1H, J = 7.2, 1.8 Hz), 7.40-7.38 (m, 1H), 7.29-7.27 (m, 1H), 7. 6.63 (br, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 157.9, 156.3, 154.4, 150.2, 149.8, 148.5, 136.8, 136.4, 125.5, 124.7, 121.8, 121.7.

UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 332 (32452). HRMS (ESI) m/z calculated 226.10927 ($C_{12}H_{12}N_5$, $[M+H]^+$), m/z observed 226.11020 ($C_{12}H_{12}N_5$, $[M+H]^+$).



2,3-di(pyridin-2-yl)quinoxaline (2-94). 1,2-phenylenediamine (50 mg, 0.472 mmol) and pyridil (**2-41**) (100 mg, 0.472 mmol) were dissolved in 2.4 mL of EtOH and refluxed for 6 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* yielding a pale brown solid in quantitative yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.38 (d, 2H, J = 4.8 Hz), 8.23 (dd, 2H, J = 3.6, 2.4 Hz), 7.96 (d, 2H, J = 7.8 Hz), 7.84-7.80 (m, 4H), 7.24 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 157.6, 152.6, 148.8, 141.3, 136.8, 130.6, 129.6, 124.4, 123.1. UV-Vis (MeCN) λ_{max} nm (ε M^{-1} cm^{-1}): 332 (8345). HRMS (ESI) m/z calculated 285.11402 ($C_{18}H_{13}N_4$, $[M+H]^+$), m/z observed 285.11511 ($C_{18}H_{13}N_4$, $[M+H]^+$).



1,2-di(pyridin-2-yl)-2-(p-tolylimino)ethanone (2-95). 2,2'-pyridil (1 mmol) was dissolved in EtOH (4 mL). To this was added p-toluidine (2 mmol). The reaction mixture was stirred at room temperature overnight. Consumption of 2,2'-pyridil was confirmed by TLC. Reaction mixture was concentrated *in vacuo* to remove EtOH. Residue was purified over silica eluting with hexanes and ethyl acetate to yield a yellow solid in 53% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.57 (dq, 1H, J = 4.5 Hz, J = 1.0 Hz), 8.46 (dq, 1H, J = 5.0, 1.0 Hz), 8.36 (dt, 1H, J = 8.0 Hz, J = 1.0 Hz), 7.97 (dt, 1H, J = 8.0 Hz, J = 1.0 Hz), 7.83 (td, 1H, J = 7.5 Hz, J = 1.5 Hz), 7.78 (td, 1H, J = 7.5 Hz, J = 1.5 Hz), 7.37 (qd, 1H, J = 5.0 Hz, J = 1.0 Hz), 7.30 (qd, 1H, J = 5.0 Hz, J = 1.0 Hz), 6.94 (d, 1H, J = 7.5 Hz), 6.85 (d, 1H, J = 8.5 Hz), 2.21 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.8, 167.0, 155.4, 152.9, 149.7, 148.9, 137.0, 136.9, 129.3, 127.4, 125.1, 122.0, 121.6, 120.3HRMS (ESI) m/z calculated 302.1293 ($C_{19}H_{16}N_3O$, $[M+H]^+$), m/z observed 302.13002 ($C_{19}H_{16}N_3O$, $[M+H]^+$).

Chapter 3.
Catalytic Activity of 1,2,4-Triazine Ligands

3.1. Background & Significance

Despite the ability of copper to catalyze the azide-alkyne cycloaddition, more demanding applications have arisen, which have led to the synthesis of ligands to further accelerate the cycloaddition reaction. Ligands can be designed to tailor to the conditions required. With this in mind, we synthesized a series of 1,2,4-triazine ligands to study their ability to catalyze the CuAAC reaction. Depending on the substitution pattern of the 1,2,4-triazine, the ligands could be potent accelerants using Cu^{I} or Cu^{II} salts in the presence of a reducing agent. Optimal conditions were determined using a model reaction between benzyl azide and phenylacetylene, including amount of base, catalyst loading, and stoichiometry between ligand and copper. Additional azide and alkyne substrates were then examined. Finally, a study of the catalytic activity of various 1,2,4-triazines outlined the structural and electronic characteristics necessary to catalyze the reaction.

As an application, the 1,2,4-triazine ligand was used to attach an alkyne bearing triazine to different solid supports, polystyrene and silica. These heterogeneous ligands were also screened for CuAAC activity, and were found to be effective accelerants. The recyclability and the copper retention were also explored, showing some catalysts were reusable up to 3 times without loss of activity or significant copper leaching.

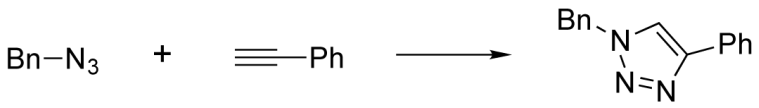
3.2. Optimization of Reaction Conditions for 1,2,4-Triazine Accelerated CuAAC

Our initial screening for catalytic activity involved the use of several heterocycles. We initially screened 1,2,4-triazoles (substituted at both the 1 and 4 positions), 1,2,3-triazoles, and 1,2,4-triazines. Of these, only 1,2,4-triazines produced an efficient yield of the desired 1,2,3-triazole product under ambient conditions. 1,2,4-Triazoles were somewhat effective, but required heat in order to form the active copper complex.

With this knowledge in hand we moved on to further optimize and explore the conditions under which 1,2,4-triazines could effectively accelerate CuAAc. We began by evaluating the model reaction between benzyl azide and phenylacetylene in acetonitrile. We initially tested the catalyst loading and the use of Cu^{I} or Cu^{II} salts. Reactions were conducted at room temperature for 1 hour, diluted, and analyzed by LC-MS for yield of

triazole product. With addition of ligand, **2-17**, a significant enhancement in product formation was seen over the control reaction which had copper but no ligand (Table 3.1 Entry 3 vs. Entry 6). The best results were observed with 1 mole percent of ligand and copper using either Cu^{I} or Cu^{II} salts (Entries 3, 7, 8). In order to prevent oxidation to Cu^{II}, a nitrogen base was also added. This served as a reducing agent to convert the starting Cu^{II} to Cu^{I}. Cu^{II} salts generally have better solubility than the analogous Cu^{I} salt. Although $[Cu(MeCN)_4]^+BF_4^-$ was initially chosen due to its solubility, the compound was hydroscopic and unless stored tightly or free from air and water, it became less soluble when dissolved for subsequent catalytic trials. Because of this decreased solubility, we began to use $Cu(BF_4)\cdot 6H_2O$ in its place. It had a much better solubility. Additional Cu^{II} salts could also be used without significant loss of catalytic activity (Entry 7). After the initial LC-MS analysis, work was continued to optimize the reaction conditions. Product 1,2,3-triazoles were isolated after 1 hour and percent yields reported based on the isolated products rather than LC-MS yields.

Table 3.1 Optimization of CuAAC reaction conditions using 1,2,4-triazines.[a]



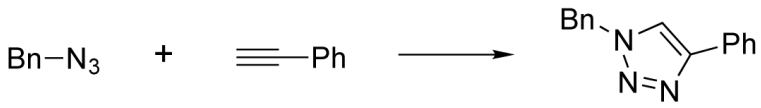
Entry	Copper Source	mol% Copper	mol% Triazine	Percent Yield after 1 h
1	$[Cu(MeCN)_4]^+BF_4^-$	2	2	66.6 ± 5.7
2	$[Cu(MeCN)_4]^+BF_4^-$	1	2	65.5 ± 3.5
3	$[Cu(MeCN)_4]^+BF_4^-$	1	1	75.1 ± 1.4
4	$[Cu(MeCN)_4]^+BF_4^-$	1	0.5	44.0 ± 2.8
5	$[Cu(MeCN)_4]^+BF_4^-$	0.5	0.5	25.1 ± 1.3
6	$[Cu(MeCN)_4]^+BF_4^-$	1	0	12.0 ± 0.6[b]
7	$Cu(OAc)_2 \cdot H_2O$	1	1	62.4 ± 8.2
8	$Cu(BF_4)_2 \cdot 6H_2O$	1	1	72.0 ± 3.2

[a]General conditions: benzyl azide (1 eq), phenylacetylene (1 eq), triethylamine (2 eq), copper source, and 5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine **(2-17)**. Reactions were run at room temperature in 0.2 M acetonitrile with respect to substrates. Percent yield determined by HPLC-MS.
[b]General conditions with following exception: triethylamine (1 eq)

Simultaneously we examined the effect of substrate concentration and the amount of nitrogen base (or reducing agent) required (Table 3.2). Since the pyridine functional group is capable of binding copper, we wanted to compare the activity of pyridine to that of the synthesized triazine containing a pyridine functional group. We were surprised to observe that the addition of pyridine did not result in product formation. Pyridine may have been binding to the copper, but possibly it remained in the inactive Cu^{II} state. Alkyl amines such as triethylamine (Et_3N) are employed to reduce Cu^{II} to the active Cu^{I} species.[92] The addition of Et_3N was deemed necessary for this reaction (entry 4), but Et_3N without triazine **2-17** (Table 3.1, Entry 6) resulted in very minimal product formation. From the studies on substrate concentration, very little change occurred when the concentration was decreased to 0.1 M. The reaction was still viable at 0.05 M, but longer reaction times (3 hrs) were necessary with a decreased overall yield of 64% (Table 3.2 Entries 4-6). We also examined if stoichiometric or catalytic amounts of base were required. Typical azide-alkyne cycloadditions use excess reducing agent when compared to loading of copper catalyst. If the triethylamine is acting as a reducing agent alone, the cycloaddition should still work with decreased amounts of amine. At 0.5 eq of triethylamine, high yields (97%) were observed within an hour (Table 3.2, Entry 7). With 0.06 equivalents, the reaction still progressed but at a much slower rate. After 1 hour, very little product had formed. Upon stirring for an additional 5 hours, 91% of the triazole product was isolated (Table 3.2, Entry 8).

We also evaluated the solvent scope (Table 3.3). Nonpolar, aprotic solvents appeared to be optimal solvents giving the highest isolated yields of 1,2,3-triazole (Entries 1-4, 6-7) Reactions carried out in polar protic and polar aprotic solvents were met with some difficulty. Solvents such as dimethylformamide (DMF) and isopropanol had significantly lower yields. The major reason for this was the result of side product formation of bis-triazole (**3-4**).[93] This reduced the triazole product yield and also made isolation of the pure triazole product difficult. One surprising note from this data set was when tetrahydrofuran was employed as the solvent. Initially, the reaction did not progress and the solution remained green in color. The reaction was left stirring overnight and eventually the solution turned dark and product formation was evident by TLC. Possible

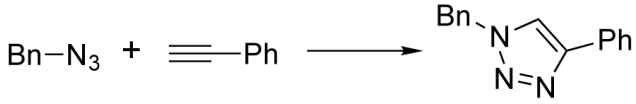
Table 3.2 Evaluation of Base and Substrate Concentration in CuAAC.[a]



Entry	Base (Equivalents)	Substrate Concentration	Percent Yield (reaction time)
1	None	0.2 M	< 5 % (1 hr)[b]
2	Pyridine (1)	0.2 M	< 5 % (1 hr)[b]
3	2,6-Lutidine (1)	0.2 M	11 % (1 hr)[b]
4	Et_3N (1)	0.2 M	97 % (1 hr)
5	Et_3N (1)	0.1 M	94 % (1 hr)
6	Et_3N (1)	0.05 M	64 % (3 hr)
7	Et_3N (0.5)	0.1 M	97 % (1 hr)
8	Et_3N (0.06)	0.1 M	91 % (5.5 hr)

[a]General conditions: Isolated yields for reactions between benzyl azide (1.05 mmol), phenylacetylene (1 mmol), organic base, and copper(II) tetrafluoroborate hexahydrate (0.01 mmol), and 5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine **(2-17)** (0.01 mmol). Reactions were run at room temperature in DCE at concentration listed with respect to substrates.
[b]General conditions except MeCN used as solvent and yields determined by HPLC-MS.

Table 3.3 Evaluation of Solvent for CuAAC.[a]



Entry	Solvent	Isolated Percent Yield	Entry	Solvent	Isolated Percent Yield
1	Dichloromethane	92	6	Dioxane	83
2	Toluene	91	7	Cyclohexane	85
3	Ethyl Acetate	90	8	Tetrahydrofuran	86[b]
4	Acetone	79	9	Dimethylsulfoxide	13
5	Dimethylformamide	61	10	Isopropanol	45

[a]General conditions: azide (1 eq), alkyne (1 eq), triethylamine (1 eq), tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (0.01 eq), and 5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine **(2-17)** (0.01 eq) in 20% ACN: 80% solvent at room temperature for 1 hr.
[b]No reaction after 1 hr. Yield represents reaction after 24 hrs.

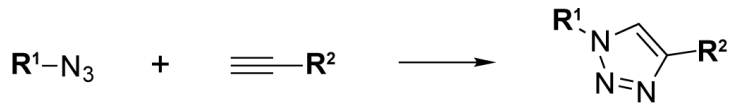
competition between the THF and triazine ligand for access to the copper or solvation of the copper by THF may have caused the delay in the reaction.

3.3. Substrate Scope

Having optimized the general conditions, we moved on to test the substrate compatibility by examining various azides and alkyne substrates with various stereoelectronic properties (Table 3.4). Electron rich and electron poor aromatic azides, primary and secondary alkyl azides, and benzylic azides all showed good product yields (> 80%). Sterically hindered aromatic azides also produced the corresponding triazoles in high yield. Alkyl azides containing an acid functional group surprisingly did not undergo the cycloaddition in good yield (Entry 11, 47%). Even with an additional equivalent of base, this reaction did not proceed well. Since other alkyl azides had produced triazole in efficient yield, the acid functional group was most likely the cause of the poor yield in this case. The only azide found to be unreactive was the tertiary adamantyl azide which produced very little product even after prolonged reaction times (Entry 10).

While azides were not particularly sensitive to the reaction conditions, alkynes proved to be more problematic. Aromatic alkynes containing ether or ester functional groups resulted in a second UV active spot observable via TLC. This was determined by NMR to be the bis-triazole byproduct, not easily separated from the product triazole, resulting in reduced yields or purity. Luckily, this byproduct could be circumvented by use of a different solvent, particularly DCM. DCM or DCE were substituted for MeCN to prevent side product formation. The yields for triazoles synthesized by varying the stereoelectronics of alkynes were more varied as compared to the azide counterpart, ranging from 68 – 95%. The only alkyne that did not perform particularly well was the alkyl decyne. This resulted in only 23% yield after 1 hour. Possible options to increase the yield would be to heat the reaction or increase the reaction time. Despite the lower yields with some substrates, reasonable yields were observed with a variety of azide and alkyne substrates.

Table 3.4 Substrate scope for 1,2,4-triazine accelerated CuAAC.[a]



3-1 **3-2** **3-3**

Entry	R^1	R^2	Product	Isolated Percent Yield
1	Bn (3-1a)	Ph (3-2a)	3-3a	97[c]
2	Ph (3-1b)	Ph (3-2a)	3-3b	95[b]
3	4-OMePh (3-1c)	Ph (3-2a)	3-3c	83[b]
4	4-CO_2MePh (3-1d)	Ph (3-2a)	3-3d	91[b]
5	2,4-diMePh (3-1e)	Ph (3-2a)	3-3e	90
6	2,6-diMePh (3-1f)	Ph (3-2a)	3-3f	83
7	$CH_3(CH_2)_6$ (3-1g)	Ph (3-2a)	3-3g	88
8	$CH_3(CH_2)_4CHCH_3$ (3-1h)	Ph (3-2a)	3-3h	80
9	2-pyridyl-CH_2 (3-1i)	Ph (3-2a)	3-3i	88[c]
10	1-adamantyl (3-1j)	Ph (3-2a)	3-3j	5
11	$(CH_2)_5COOH$ (3-1k)	Ph (3-2a)	3-3k	47
12	Bn (3-1a)	CH_2OH (3-2b)	3-3l	76
13	Bn (3-1a)	$C(CH_3)_2OH$ (3-2c)	3-3m	80
14	Bn (3-1a)	1-OHC_6H_{10} (3-2d)	3-3n	79
15	Bn (3-1a)	2,4-diMePh (3-2e)	3-3o	68
16	Bn (3-1a)	4-OMePh (3-2f)	3-3p	87[c]
17	Bn (3-1a)	4-CO_2MePh (3-2g)	3-3q	86[c]
18	Bn (3-1a)	2-CO_2MePh (3-2h)	3-3r	89[c]
19	Bn (3-1a)	2-pyridyl (3-2i)	3-3s	92[c]
20	Bn (3-1a)	$(CH_2)_7CH_3$ (3-2j)	3-3t	23[c]
21	Bn (3-1a)	4-CNPh (3-2k)	3-3u	95[c]

[a]General conditions: azide (1 eq), alkyne (1 eq), triethylamine (1 eq), tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (0.01 eq), and 5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine **(2-17)** (0.01 eq). Reactions were run at room temperature for 18 h in 0.2 M acetonitrile with respect to substrates.

[b]Isolated by filtration.

[c]General conditions with following exceptions: Rxn carried out in DCE with copper (II) tetrafluoroborate (0.01 mmol) for 1 hr at room temperature.

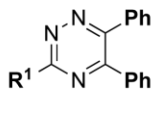
3.4. Ligand Scope

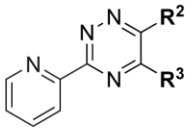
As we were optimizing the conditions for triazine-accelerated CuAAC, we were also synthesizing additional 1,2,4-triazines in hopes of finding more active ligands. The optimization conditions were all conducted with 5,6-diphenyl-3-(2-pyridyl)-1-2-4-triazine which gave 97% isolated yield of product triazole after 1 hour under the best conditions.

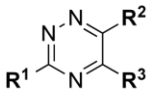
The results from screening of various ligands are compiled in Table 3.5. Since the pyridyl substituent was active, we also screened the pyrimidine and pyrazine substituted triazines. These turned out to be completely inactive (Entries 2-3), showing that more nitrogens isn't always better. We also wanted to investigate if the pyridine was necessary for activity. If pyridine was better than pyrimidine and pyrazine, would a phenyl substituent be better than pyridine? When the pyridine was replaced by phenyl, the activity significantly decreased to 2% isolated yield (Entry 4). This seemed reasonable as coordination of the copper occurs through the pyridine nitrogen. The 2-pyridyl substituent at the 3 position seemed to be necessary for activity, so we decided to investigate other N, O, or S containing heterocycles that could potentially replace the pyridine ring. Of the five heterocycles synthesized, benzothiophene, benzofuran, indole, and benzothiazole were all inactive, producing only trace amounts of triazole product. The only substituent at the 3-position that showed intermediate activity was the replacement of the pyridine with methyl imidazole to yield 50% of triazole product in 1 hour (Entries 5-9).

The pyridine substituent at the 3 position appeared to be irreplaceable, so we decided to move onto the 5,6 position to see if the activity could be tuned with respect to those substituents. Other commercial dicarbonyls allowed us to investigate the steric bulk. The replacement of the phenyl rings with H decreased the yield, only slightly, from 97% to 91%. The methyl substituents at 5 and 6 positions did not fare as well. This product was not an easily handled powder like the other triazines, but instead formed a semi-solid oily residue, making it slightly less soluble, and more difficult to measure out. Replacement of the phenyl rings with pyridine to make 3,5,6-tripyridyl-1,2,4-triazine also did not increase the activity, rather decreasing it, much to our disappointment (Entry 12).

Table 3.5 Evaluation of 1,2,4-triazines and other heterocycles for CuAAC.[a]



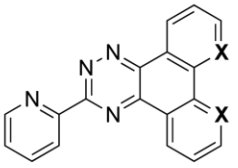




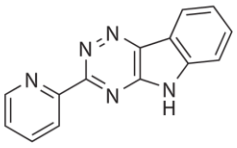
2-17 R^1 = 2-pyridine
2-18 R^1 = 2-pyrimidine
2-19 R^1 = 2-pyrazine
2-30 R^1 = Ph
2-31 R^1 = 2-benzothiophene
2-32 R^1 = 2-benzofuran
2-33 R^1 = 2-indole
2-34 R^1 = 2-benzothiazole
2-35 R^1 = 2-(1-methylimidazole)

2-42 $R^2 = R^3$ = H
2-43 $R^2 = R^3 = CH_3$
2-44 $R^2 = R^3$ = 2-pyr
2-49 R^2 = H, R^3 = Ph
2-50 R^2 = H, R^3 = 2-OH-Ph
2-54 $R^2 = R^3 = 4\text{-}CH_3\text{-Ph}$
2-59 $R^2 = R^3 = 4\text{-}NO_2\text{-Ph}$
2-60 $R^2 = R^3 = 4\text{-}NH_2\text{-Ph}$

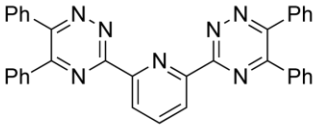
2-75 R^1 = 2-aniline, $R^2 = R^3$ = Ph
2-78 R^1 = Ph, $R^2 = R^3$ = 2-pyr
2-81 $R^1 = NHNH_2$, $R^2 = R^3$ = Ph
2-82 R^1 = NHNCH-(2-pyr), $R^2 = R^3$ = Ph



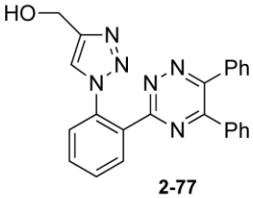
2-62 X = CH
2-65 X = N



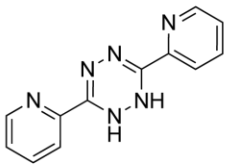
2-67



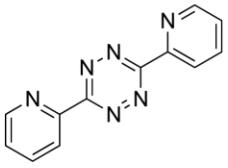
2-72



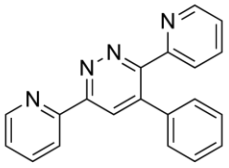
2-77



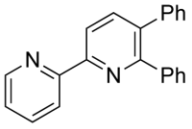
2-85



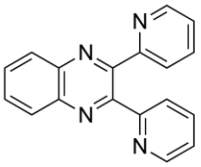
2-86



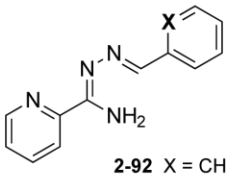
2-87



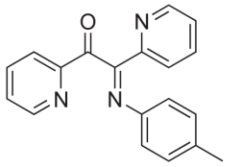
2-89



2-94



2-92 X = CH
2-93 X = N



2-95

Entry	Ligand	Percent Yield	Entry	Ligand	Percent Yield	Entry	Ligand	Percent Yield
1	2-17	97	12	2-44	7[c]	24	2-78	6
2	2-18	29[b]	13	2-49	97	25	2-81	26[b]
3	2-19	24[b]	14	2-50	18	26	2-82	7[c]
4	2-30	2[c]	15	2-54	86[b]	27	2-85	20
5	2-31	7[c]	16	2-59	61[b]	28	2-86	33[c]
6	2-32	1	17	2-60	61[b]	29	2-87	63
7	2-33	8	18	2-62	96	30	2-89	1[c]
8	2-34	14	19	2-65	96	31	2-92	19[b]
9	2-35	50	20	2-67	1	32	2-93	19[b]
10	2-42	91	21	2-72	0[c]	33	2-94	12[c]
11	2-43	45[b]	22	2-75	12[b]	34	2-95	95 (4 hrs)
			23	2-77	10[b]	35	NA	1

[a]General conditions: benzyl azide (1.05 mmol), phenylacetylene (1 mmol), triethylamine (1 mmol), copper tetrafluoroborate hexahydrate (0.01 mmol) and ligand (0.01 mmol). Reactions were run at 0.2 M with respect to substrates in dichloroethane at room temperature for 1 hour. Isolated yields reported.
[b]General conditions except tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (0.01 mmol). Reactions were run at room temperature in 0.2 M acetonitrile with respect to substrates. Percent yield determined by LC-MS.
[c]General conditions except percent yield determined by HPLC.

Through our screening it was identified that both phenyl rings were not required as a 6-phenyl-1,2,4-triazine displayed similar activity to the initial ligand **2-17** (Entry 13). The triazine containing an alcohol functional group (**2-50**) was not active. This compound wasn't very soluble in DCM or DCE, which could have led to the decreased catalytic activity. Use of a different solvent for this triazine ligand could improve the activity. The addition of the nitro and amine substituents did not have a positive impact on the catalytic activitiy (Entries 16-17). The activity decreased for both triazines. Neither of these triazines were particularly soluble, so the decreased solubility could have led to their decreased activity.

Locking the conformation of the 5,6 substituents gave similar results (96%) to the original triazine **2-17** (Entries 18-19). Surprisingly, the addition of nitrogens in **2-65** did not have an adverse effect on the catalytic activity. Since these nitrogens are pointed away from the metal binding region of the molecule, it could be proposed that these additional nitrogens wouldn't interfere with copper coordination and access by the azide or alkyne components. Contrary to this, the nitrogen in **2-67** is closer to the metal binding center and is also more basic than the pyridine nitrogen. This triazine resulted in only a trace amount of product formed (Entry 20).

Somewhat of a disappointment were the results from the bis-triazine and the 1,2,3-triazolyl-1,2,4-triazine. (Entries 21, 23) Neither of these proved to be active for the azide-alkyne cycloaddition. The metal binding site is more crowded now, so this might either lead to no binding of the copper by the triazine, or the alkyne cannot access the copper once bound. Either of these would prevent the cycloaddition.

Of the remaining ligands, pyridazine (**2-87**) showed some modest activity with 63% isolated yield over 1 hour (Entry 29). The imine (**2-95**) resulted in 95% yield but the reaction progressed for an additional 4 hours. After 1 hour, the reaction was not complete, so longer reaction times were necessary. It would be of interest to explore this scaffold further.

3.5. Mechanistic Considerations

A limited amount of data was collected in order to explore the copper dependence on 1,2,4-triazine accelerated CuAAC. The first method was to examine the UV-Vis absorption spectra of 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine (**2-17**) with copper (I) and copper (II) salts, in the presence or absence of triethylamine (Figure 3.1). For ligand and copper (II) containing spectra, an absorbance was present around 700 nm. This is characteristic of the d-d transitions in copper (II) complexes. This absorbance band was diminished with the addition of base, and a new absorbance peak around 480 nm was present. The spectra obtained from a solution containing ligand, copper, and base are superimposable when comparing copper (I) to copper (II) salts. The absorbance spectrum of copper (I) and ligand alone is similar to the spectrum of the same solution with base added. The intensity of the peaks may differ, but the general features are similar. From this data we could confidently state that the Cu^{II} was being reduced to generate the active Cu^{I} species.

Although from our optimization studies (Table 3.2), it was shown that a catalytic amount of base was sufficient to convert Cu^{II} into Cu^{I} and yield the desired triazole product, the UV-Vis scan did not show the Cu^{I} species. The experiment with catalytic base required longer reaction times (> 5 hr) to yield product triazoles in moderate yield. For the UV-Vis experiment, scans were conducted immediately after mixing the ligand, copper, and base. For a better comparison, the UV-Vis spectrum should be taken at various time points. The conversion of Cu^{II} to Cu^{I} is not instantaneous with catalytic amount of base, especially since the reaction mixtures were not being stirred within the UV-Vis.

The addition of substrates did not affect the features of the absorption spectra as can be seen when comparing Figure 3.1B to Figure 3.1A. The intensity of the spectral features may have increased slightly, but there was no shift in the wavelength at which the absorption took place.

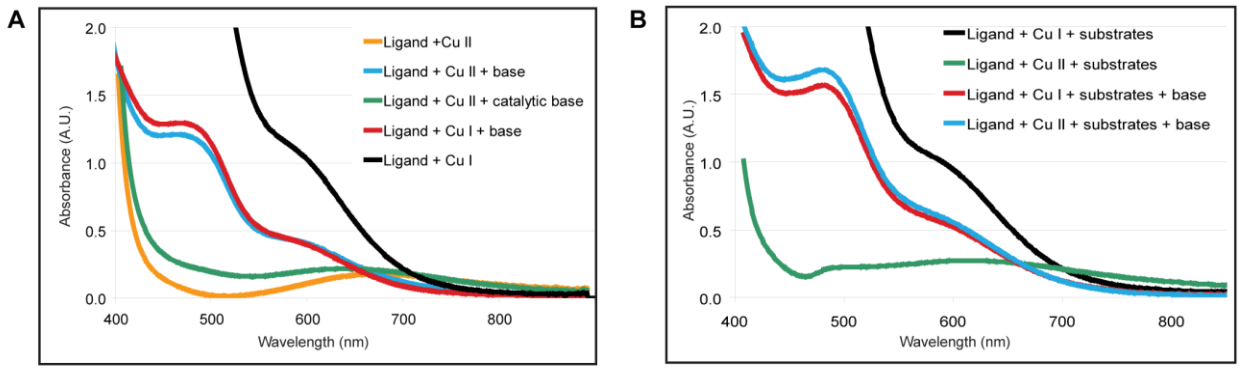
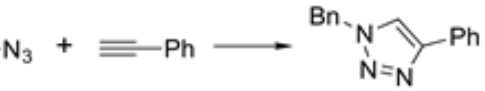


Figure 3.1 UV-Vis spectra of 1,2,4-triazine-accelerated CuAAC. 5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine (0.01 eq) (2-17) in acetonitrile with either tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (0.01 eq) or copper(II) tetrafluoroborate hexahydrate (0.01 eq). The addition of triethylamine (0.01 eq or 1.0 eq) was studied without (A) and with (B) benzyl azide (1.0 eq) and phenylacetylene (1.0 eq).

A preliminary kinetics investigation was undertaken using ^{1}H NMR to monitor the reaction between phenylacetylene and benzyl azide. (Figure 3.2) The initial observations revealed a reaction order of zero with respect to the azide substrate. When concentration of azide was doubled the reaction rate was unaffected. When the copper and ligand concentrations were doubled, the reaction rate also doubled. Changing the concentration of alkyne was more difficult, as doubling the concentration led to the formation of precipitate. Changes to the solvent could potentially eliminate this issue. A more thorough investigation and mathematical processing of the data is required to provide more concrete evidence about the nature of the mechanism.

Preliminary electrochemical experiments were also conducted.[66] Unfortunately, the addition of ligand, copper, and base resulted in a very dark brown solution, which left residual components in the frit of the electrode. After a series of experiments, the contamination of the frit began to influence the spectral features. A consistent baseline and reproducible spectra were no longer possible to collect. To prevent the contamination of the frit, more dilute solutions were attempted, but these were too dilute to measure electrochemical potentials.

A



B

Time (min)	Standard % yield	Azide = [0.4] % yield (ratio)	Alkyne = [0.1] % yield (ratio)	Catalyst = [0.004] % yield (ratio)
6	15	11 (0.7)	16 (1.1)	37 (2.5)
7	17	14 (0.9)	20 (1.2)	43 (2.6)
8	19	17 (0.9)	24 (1.3)	49 (2.6)
9	21	21 (1.0)	27 (1.3)	54 (2.5)
10	25	23 (0.9)	32 (1.3)	58 (2.3)
11	27	26 (1.0)	36 (1.3)	62 (2.3)
12	31	29 (0.9)	39 (1.3)	67 (2.2)
13	32	29 (0.9)	44 (1.3)	72 (2.2)
14	37	34 (0.9)	47 (1.3)	81 (2.2)
15	39	38 (1.0)	50 (1.3)	87 (2.3)
16	41	41 (1.0)	54 (1.3)	93 (2.3)
17	47	44 (1.0)	57 (1.2)	97 (2.1)

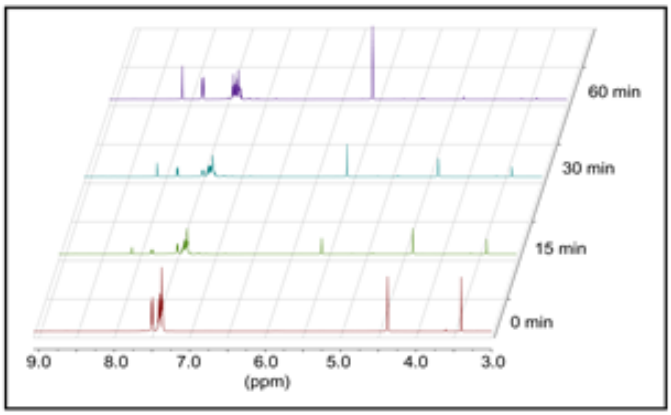


Figure 3.2 Kinetic NMR Data of 1,2,4-Triazine-Accelerated CuAAC Reaction. (A) Standard conditions: benzyl azide (0.2 M), phenylacetylene (0.2 M), triethylamine (0.4 M), $Cu(MeCN)_4BF_4$ (0.002 M), and 5,6-diphenyl-3-(pyridin-2-yl)-1,2,4-triazine (2-17) (0.002 M) in deuterated acetonitrile. Concentrations for additional reactions were altered as indicated above. Ratio is percent yield of the indicated reaction divided by that of the standard reaction. (B) Kinetic array for 1,2,4-triazine-accelerated CuAAC reaction under standard conditions. Disappearance of alkyne (3.4 ppm) and benzylic azide protons (4.4 ppm) as benzylic triazole protons (5.6 ppm) appear over time.

Of the limited amount of data collected, the most useful data was the UV-Vis spectra. This confirmed that the initial Cu^{II} salts were being converted to the active Cu^{I} species upon addition of base. For a more complete mechanistic evaluation, additional data is required.

3.6. Other Catalytic Applications

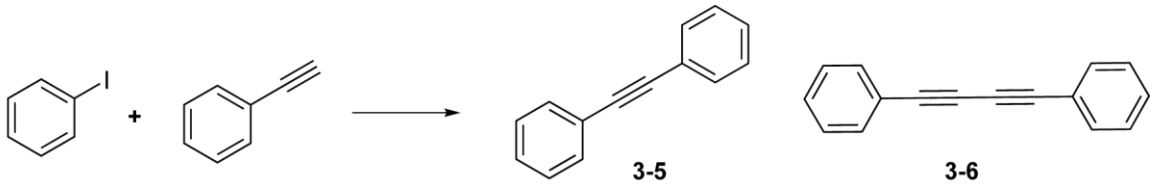
Since 3-(2-pyridyl)-1,2,4-triazines are known to bind various metals, we decided to explore other metal catalyzed reactions to see if triazine presence could accelerate these reactions. One reaction that we were interested in would be to perform a one-pot Sonogashira-CuAAC reaction. The reaction would begin with an aryl iodide, which would be converted into a terminal alkyne. The terminal alkyne would then be reacted with an azide under 1,2,4-triazine-accelerated CuAAC conditions previously developed. Many reports exist on the in situ preparation of azides from bromides for a one-pot

CuAAC reaction,[94,95] or the in situ preparation of alkynes from iodides for a one pot Sonogashira-CuAAC reaction.[96] From our experience, synthesis of aryl alkynes is usually a lengthier process than for the complimentary azides needed for CuAAC reactions. Alkynes usually are purified by silica gel chromatography to remove excess palladium and copper salts after the reaction. Our goal was to conduct the Sonogashira and azide-alkyne cycloaddition in one pot, in the hopes of speeding up the process and isolation of the final product.

We began the investigation by examining the model reaction between iodobenzene and phenylacetylene (Table 3.6). Entry 1 represents the standard conditions employed for Sonogashira conditions. This reaction proceeds at room temperature, but did produce some oxidative Glaser coupling byproduct. Addition of 1,2,4-triazine (**2-17**) to bis(triphenylphosphine)palladium(II) chloride resulted in decreased yield of the desired internal alkyne (**3-5**) and no change in the selectivity of **3-5** over **3-6**. Other available palladium sources known to bind 1,2,4-triazines were palladium (II) acetate. On its own this palladium source did not catalyze the Sonogashira reaction (Entry 3). Addition of 1,2,4-triazine (2-17) increases the yield, but not to that of an appreciable amount. We next evaluated if the yield could be improved with addition of heat or a change in solvent. From optimization experiments with CuAAC, THF was observed to delay the reaction. Changing the solvent and increasing the temperature proved to be very beneficial. Both acetonitrile and toluene (Entries 10 and 12) displayed modest yields with acetonitrile showing the best selectivity thus far, with a ratio of nearly 13 : 1 desired product to Glaser byproduct. Since the palladium exists as a trimer, the stoichiometry of the added ligand was adjusted to find the optimal ratio between palladium and triazine (Entry 10, 13).

From these initial results, it appeared that this method could be viable. We then tried to extend this method to a one-pot reaction in which both the Sonogashira and azide alkyne cycloaddition would take place in one pot. Unfortunately, our attempts at a one-pot Sonogashira-CuAAC only resulted in approximately a 12% isolated yield (Table 3.7). This product was darker in color than the normal product triazole, indicating likely palladium contamination. Unreacted starting material was a culprit in the low yields.

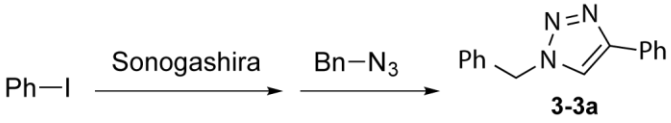
Table 3.6 Initial studies of 1,2,4-triazines as additives in Sonogashira reaction.[a]



Entry	Palladium Source	Ligand (eq)	Solvent	Percent Yield of **3-5**	Percent Yield of **3-6**	Ratio of **3-5** : **3-6**
1	$Pd(PPh_3)_2Cl_2$	None	THF	85	15	5.7
2	$Pd(PPh_3)_2Cl_2$	**2-17** (0.080)	THF	54	10	5.4
3	$Pd_3(OAc)_6$	None	THF	<1	7	0.4
4	$Pd_3(OAc)_6$	**2-17** (0.080)	THF	12	4	3.4
5	$Pd_3(OAc)_6$	None	THF	3[b]	4	0.6
6	$Pd_3(OAc)_6$	**2-17** (0.015)	THF	4[b]	3	1.3
7	$Pd_3(OAc)_6$	**2-17** (0.025)	THF	5[b]	2.5	2.0
8	$Pd_3(OAc)_6$	**2-17** (0.040)	THF	7[b]	3	2.5
9	$Pd_3(OAc)_6$	**2-17** (0.080)	THF	14[b]	4	3.6
10	$Pd_3(OAc)_6$	**2-17** (0.080)	MeCN	77[b]	6	12.8
11	$Pd_3(OAc)_6$	**2-17** (0.080)	DCE	36[b]	5	7.2
12	$Pd_3(OAc)_6$	**2-17** (0.080)	Toluene	70[b]	7	10
13	$Pd_3(OAc)_6$	**2-17** (0.030)	MeCN	69[b]	7	9.8

[a]General conditions: iodobenzene (1 eq), phenylacetylene (1.25 eq), triethylamine (1.25 eq), copper iodide (0.015 eq), ligand, and palladium source (0.025 eq) in 0.4 M tetrahydrofuran at room temperature for 5.5 h. Percent yields determined by HPLC.
[b]General conditions except at 60°C for 5.5 h. Percent yields determined by HPLC.

Table 3.7 Evaluation of one-pot Sonogashira-CuAAC reaction.[a]



3-3a

Entry	Palladium Source	Ligand (eq)	Percent Yield of **3-3a**[a]
1	$Pd(PPh_3)_2Cl_2$	None	0
2	$Pd_3(OAc)_6$	**2-17** (0.08)	13
3	$Pd(PPh_3)_2Cl_2$	**2-17** (0.08)	0[b]

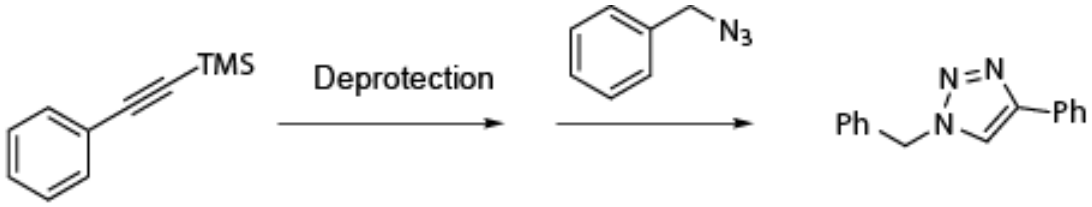
[a]General conditions: iodobenzene (1 eq), TMS-acetylene (1.25 eq), triethylamine (2.25 eq), palladium source (0.025) copper iodide (0.015), and ligand dissolved in MeCN. Reaction heated at 60°C for 5.5 h. tetrabutylammonium fluoride (0.4 eq), benzyl azide (1 eq) were added and the reaction heated at 60°C overnight. Yields are of isolated products.
[b]General conditions except copper (II) tetrafluoroborate hexahydrate (0.01 eq) and ligand (2-17) added with benzyl azide in second step.

From here, we decided to examine the deprotection and cycloaddition steps. In the examples above, the aryl iodide was reacted with a TMS-protected alkyne. This alkyne must be deprotected before the azide-alkyne cycloaddition can take place. Since the Sonogashira reaction was resulting in product, we wanted to isolate the other portions of the reaction, to determine where the reaction was stalling. For this, TMS-protected phenylacetylene was synthesized and used to screen various deprotection conditions. After addition of benzyl azide, triazole product formation was monitored (Table 3.8). We also decided to take advantage of the Microwave Synthesizer and performed the reactions in Table 3.8 at 120°C in the microwave. We explored various copper sources for the cycloaddition and observed that the reaction required larger equivalents of copper compared to the standard reaction between phenylacetylene and benzyl azide. Increasing the amount of ligand and copper increased the yield, but in the microwave the difference between triazine-accelerated and control reaction wasn't significant (98% to 86%, Entries 11, 12).

Having observed good yields for triazole formation, we decided to move forward and once again try a one-pot approach. Using the optimal deprotection conditions, iodobenzene was converted into TMS-phenylacetylene. Again a problem arose in the deprotection and no triazole product was formed. Protected phenylacetylene was the main component in the reaction mixture. This made us begin to question if palladium was possibly interfering with the deprotection step. First, we examined the presence of bis(triphenylphosphine)palladium (II) chloride during the deprotection of TMS-phenylacetylene with tetrabutylammonium fluoride. There was a dependence on the solvent used for the deprotection as shown in Table 3.9.

A few more parameters were altered, and now we were able to remove the protecting group from the alkyne, but we still were not observing triazole product formation (Table 3.10). Instead of recovering protected alkyne, we had a free terminal alkyne, but no cycloaddition was taking place. The problem appeared to be the addition of palladium acetate. We then examined the CuAAC reaction in the presence of palladium to determine if the cycloaddition reaction was being hindered by the presence of palladium. Using benzyl azide and phenylacetylene as the model system, we added palladium ($Pd_3(OAc)_6$) and tetrabutylammonium fluoride to the reaction mixture. After an

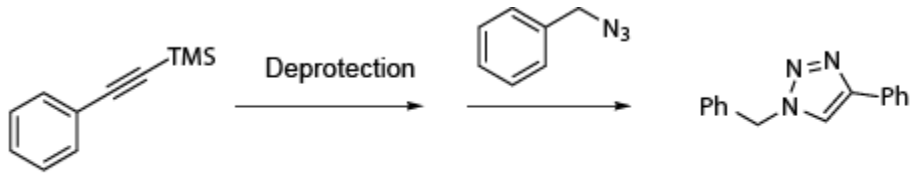
Table 3.8 Optimization of deprotection of TMS-phenylacetylene and concurrent cycloaddition.[a]



Entry	Deprotection Conditions	Ligand (eq)	Cu^{I} (eq)	Percent Yield of 3-3a
1	K_2CO_3, MeOH	**2-17** (0.1)	CuI (0.1)	84
2	TBAF, ACN	**2-17** (0.1)	CuI (0.1)	81
3	MeOH	**2-17** (0.1)	CuI (0.1)	90
4	K_2CO_3, MeOH	None	CuI (0.1)	90
5	K_2CO_3, MeOH	**2-17** (0.01)	$Cu(BF_4)_2$ (0.01)	35[b]
6	K_2CO_3, MeOH	**2-17** (0.01)	CuI (0.01)	53
7	K_2CO_3, MeOH	None	CuI (0.01)	27
8	MeOH	None	CuI (0.01)	65[c]
9	K_2CO_3, MeOH	**2-17** (0.02)	CuI (0.02)	84
10	K_2CO_3, MeOH	None	CuI (0.02)	74
11	K_2CO_3, MeOH	**2-17** (0.05)	CuI (0.05)	98
12	K_2CO_3, MeOH	None	CuI (0.05)	86

[a]General conditions: benzyl azide (1 eq), TMS-phenylacetylene (1 eq), triethylamine (1 eq), copper source, and ligand microwaved for 120°C for 20 minutes. Percent yields determined by HPLC.
[b]General conditions except at room temp for 1 h. Percent yields determined by HPLC.
[c]Additional byproduct formed.

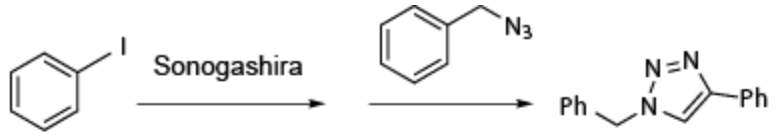
Table 3.9 Deprotection of TMS-phenylacetylene in the presence of palladium.



Entry	Deprotection Conditions	Ligand (eq)	Palladium(eq)	Percent Yield of 3-3a[a]
1	TBAF, ACN	**2-17** (0.025)	$Pd(PPh_3)_2Cl_2$ (0.025)	51
2	TBAF, MeOH	**2-17** (0.025)	$Pd(PPh_3)_2Cl_2$ (0.025)	78

[a]General conditions: benzyl azide (1 eq), TMS-phenylacetylene (1 eq), triethylamine (1 eq), copper iodide (0.025), palladium, and ligand microwaved for 120°C for 20 minutes. Percent yields determined by HPLC.

Table 3.10 Re-evaluation of triazine ligand in one pot Sonogashira-CuAAC reaction.



Entry	Palladium Source	Ligand (eq)	Percent Yield of 3-3a[a]
1[a]	$Pd_3(OAc)_6$	**2**-**17** (0.05)	0[b]
2[a]	$Pd(PPh_3)_2Cl_2$	**2**-**17** (0.05)	0[b]

[a]General conditions: iodobenzene (1 eq), TMS-acetylene (1.25 eq), triethylamine (1.25 eq), palladium source (0.025) copper iodide (0.025), and ligand dissolved in MeOH. Reaction microwaved at 120°C for 20 minutes. tetrabutylammonium fluoride (0.4 eq) and benzyl azide (1 eq) were added and the reaction microwaved at 120°C for an additional 20 minutes. Percent yield determined by HPLC.
[b]No triazole product formed.

hour at room temperature, this reaction showed very little progress. After a prolonged time, product formation began to occur, but additional byproducts were observed by TLC. This combination was causing a sluggish cycloaddition reaction. Future options would be to try a different palladium source. The triazine may be binding too tightly to the palladium and not available to bind to copper to catalyze the cycloaddition reaction.

The last reaction attempted was to use the standard sonogashira conditions (bis(triphenylphosphine)palladium(II) chloride and copper iodide. The deprotection was performed using tetrabutylammonium fluoride and the cycloaddition reagents were copper (II) sulfate and sodium ascorbate. These conditions led to product formation in a 58% yield. This reaction was conducted in order to check for the azide reduction to amine. No free amine was detected, so it was not believed that azide decomposition was leading to any prior unreactivities. At this point, it was deemed necessary to place this work aside and focus on applications of 1,2,4-triazines in heterogeneous systems and in synthesis of metalloenzyme inhibitors.

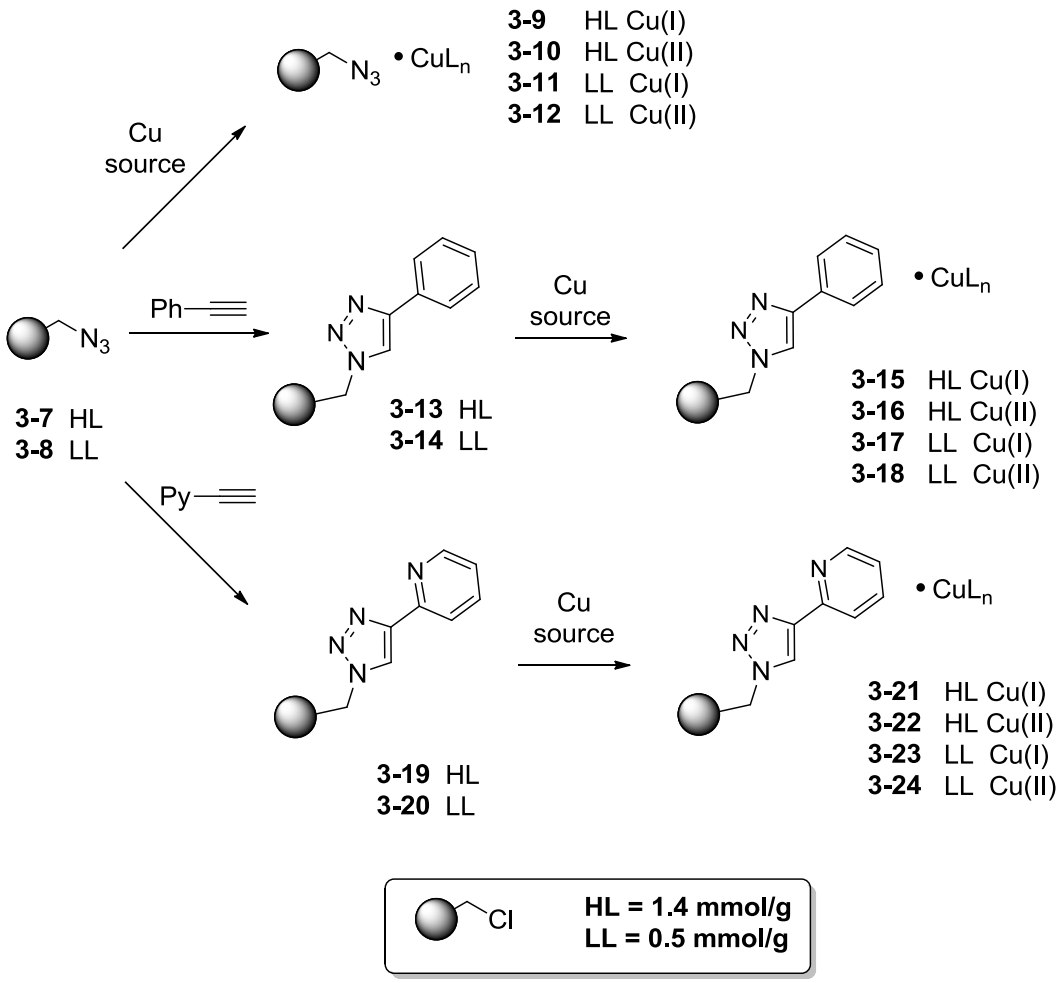
3.7. Solid-Supported 1,2,4-Triazines

After observing promising activity with the homogeneous triazines, it was decided to make heterogeneous versions of the triazine catalyst by covalently linking the 1,2,4-triazines to both silica and polystyrene supports. This work was discussed in detail by Prince.[97] I will highlight the key schemes used to continue this work, and present the catalytic activity, recyclability, and copper retention for these heterogeneous supports.

3.7.1. Overview of Synthesis of Solid-Supported 1,2,4-Triazines and Control 1,2,3-Triazole Resins

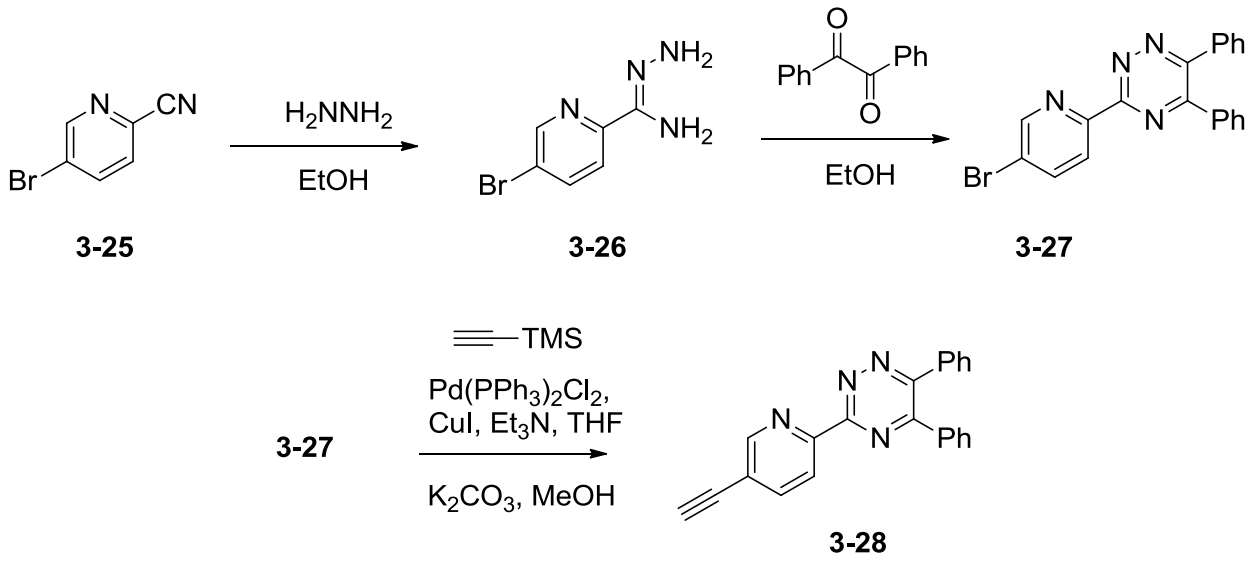
Commercial Merrifield resin was chosen as a polystyrene support due to its ability to swell in a number of solvents, low cost, and easy availability. For the synthesis, we used a high loading resin (1.4 mmol/g) and a low loading resin (0.5 mmol/g). Since a triazine alkyne was synthesized to attach to the solid support, the polystyrene resin was first converted from a chloride to an azide using sodium azide. At this time, we synthesized a series of controls (Scheme 3.1). Azide functional groups are known to bind copper, so we first coordinated copper (I) and copper (II) to azide derivatized polystyrene. Additionally, since triazoles are known to catalyze azide-alkyne cycloaddition, we wanted to ensure that the activity being observed was due to the triazine ligand, and not due to the presence of the 1,2,3-triazole linker. Therefore, we synthesized two 1,2,3-triazoles by reacting the azide resin with phenylacetylene and 2-ethynyl pyridine. The pyridine derivative would install an additional nitrogen that could be used to bind metal. Since we had both high loading and low loading resin each reaction was carried out with each support. Each product was then coordinated with a copper (I) or copper (II) salt to afford 12 control compounds (Scheme 3.1).

A similar process was carried out using triazine alkynes and the azido resin to form the 1,2,4-triazine supported catalysts. The alkyne of the 1,2,4-triazine was located either on an aromatic ring at the 3-position or at the 5-position. To synthesize the alkyne precursor at the 3-position, commercially available 5-bromo-2-cyano-pyridine was

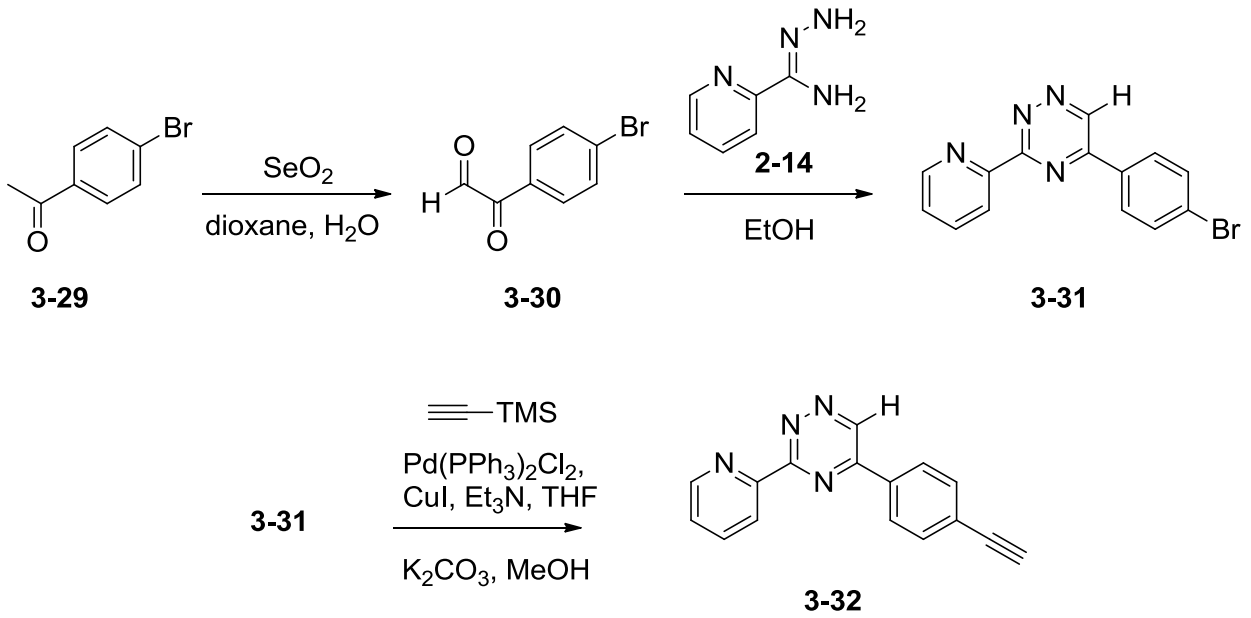


Scheme 3.1 Synthesis of solid-supported control 1,2,3-triazoles.

converted into the amidrazone (**3-26**). This was condensed with benzil to afford the 1,2,4-triazine (**3-27**). The bromide was then converted to the alkyne using the Sonogashira coupling reaction (Scheme 3.2). The alkyne was produced in 45% yield over 3 steps. For synthesis of the 5-substituted alkyne triazine, 4-bromoacetophenone was converted to the ketoaldehyde (**3-30**) through oxidation with selenium dioxide. This was condensed with the pyridine amidrazone to yield the 1,2,4-triazine (**3-31**) with a bromide on the aryl ring at the 5-position. This bromide was then converted to the alkyne through the Sonogashira coupling reaction (Scheme 3.3). The overall yield for this alkyne triazine was 20% over 3 steps.



Scheme 3.2 Synthesis of alkyne on 3-substituent of 1,2,4-triazine.



Scheme 3.3 Synthesis of alkyne on 5-substituent of 1,2,4-triazine.

The alkyne containing triazines were then subjected to a cycloaddition reaction with the azide containing solid supports. Triazine **3-28** was attached to both HL and LL polystyrene supports. Triazine **3-32** was attached only to the HL polystyrene support.

The alkyne triazine precursor synthesis was lower yielding (and with regards to catalytic activity was not superior to triazine 3-28). After carrying out the cycloaddition reaction, it was observed that the reaction did not go to completion, leaving some unreacted azides on the solid support. This resin was reacted with tin chloride to reduce the unreacted azides to free amines. Both the azide and amine containing resins were then complexed with copper (I) and copper (II). This resulted in 12 triazine containing heterogeneous ligands. A summary of the triazine ligands to be discussed in the results is presented in Figure 3.3 and Figure 3.4.

3.7.1. Catalytic Activity of Solid Supported 1,2,4-Triazines

With the synthesis of triazine catalysts and appropriate controls completed, we began to evaluate their catalytic activity. To determine the copper loading, each heterogeneous support was heated in a solution of either copper (I) or copper (II) for two hours. The heterogeneous support was filtered and washed thoroughly. The filtrate was concentrated and rediluted in 2% nitric acid. The filtrate was then analyzed for copper content using ICP-OES. By difference the copper loading was calculated. The loading for copper (I) and copper (II) is illustrated in Table 3.11. The triazine ligands (Entries 1-5) were all efficient at binding copper to a much better extent than the azide derivative (Entry 6) or the simple 1,2,3-triazole with a phenyl substituent (Entries 7-8). The copper binding ability of the pyridine substituted 1,2,3-triazole was surprisingly greater than the binding ability of the 1,2,4-triazines when the high loading polystyrene resin was used (Entry 9). When low loading resin was used a decrease in copper loading was observed with both triazines and triazoles. In general copper (II) catalysts had a slightly higher copper content than their copper (I) counterparts. Supports with residual amine showed slightly more copper loading when compared to the analogous residual azide supports.

Once the copper loading was determined, the various functionalized supports were screened for activity using the model system of benzyl azide and phenylacetylene. DCE was added to 5 mg of the appropriate resin followed by the addition of azide, alkyne, and triethylamine. Reactions were allowed to proceed for 4 hours at 60°C and the 1,2,3-triazole product was separated from the resin by filtration. Concentration *in vacuo* afforded the analytically pure triazole. The trend in percent yields was similar to that

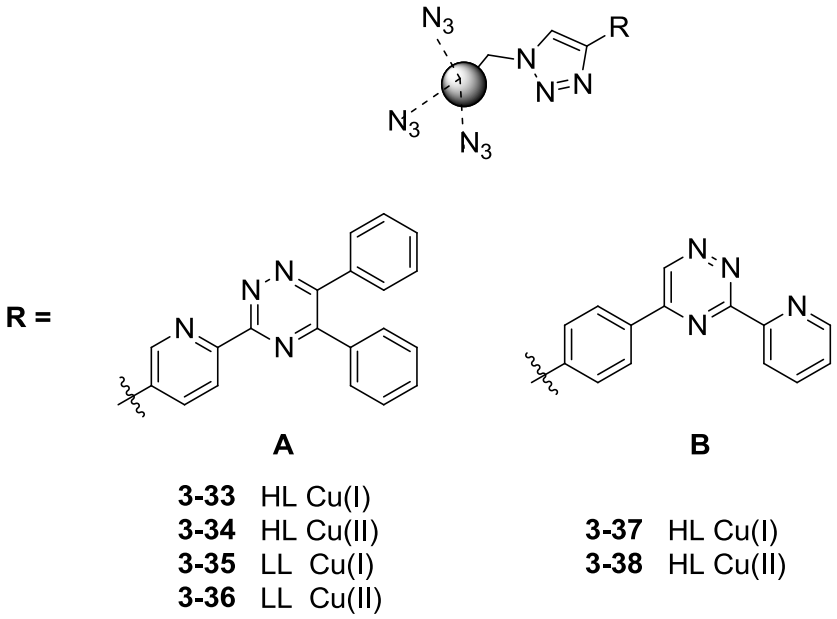


Figure 3.3 Summary of triazine ligands attached to polystyrene resins containing unreacted azides.

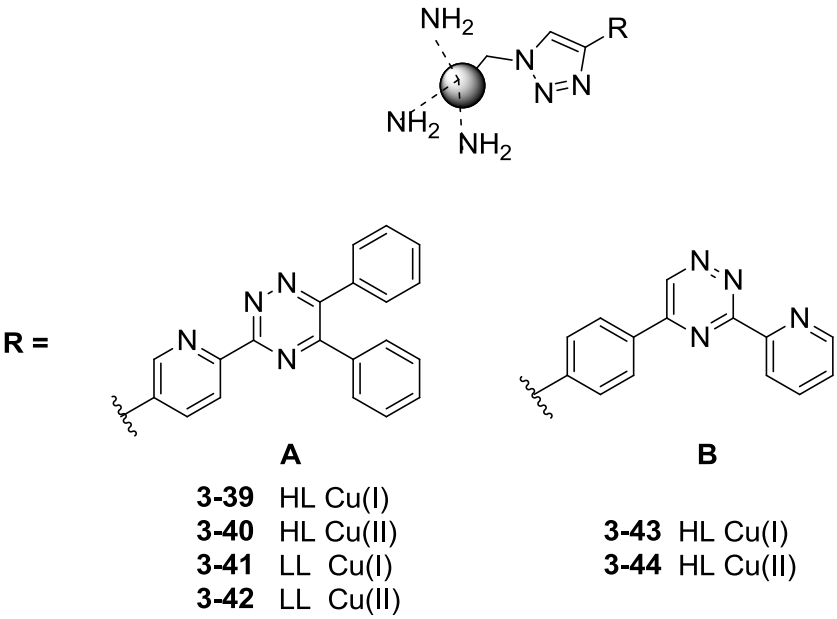
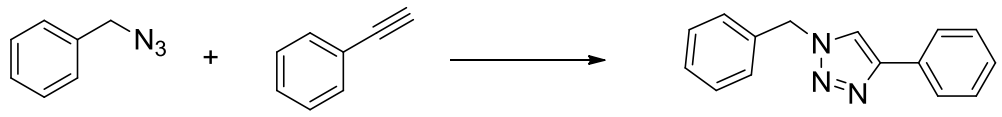


Figure 3.4 Summary of triazine ligands attached to polystyrene containing free amines.

Table 3.11 Copper loading and catalytic activity of various polystyrene supports.



	Entry	Catalyst	Cu(I) loading[a]	Cu(I) Percent Yield[b]	Catalyst	Cu(II) loading[a]	Cu(II) Percent Yield[b]
Triazine A (N_3) HL	1	3-33	0.41	75	3-34	0.43	88
Triazine B (N_3) HL	2	3-37	0.38	65	3-38	0.45	79
Triazine A (NH_2) HL	3	3-39	0.46	80	3-40	0.48	87
Triazine B (NH_2) HL	4	3-43	0.47	67	3-44	0.59	88
Triazine A (NH_2) LL	5	---	---	---	3-42	0.12	83
Azide HL	6	3-9	0.08	0	3-10	0.00	0
Ph Triazole HL	7	3-15	0.00	0	3-16	0.16	73
Ph Triazole LL	8	---	---	---	3-18	0.01	3
Py Triazole HL	9	3-21	0.52	99	3-22	0.61	99
Py Triaozle LL	10	3-23	0.21	87	3-24	0.20	99

[a]General conditions: Polystyrene resin (1 equiv) and copper source (1.1 equiv) were heated at 60°C for 2 h in a 1:1 mixure of ACN:DCE at 0.01 M. The resin was filtered and washed thoroughly. The filtrate was used to calculate loading (mmol/g) by difference using ICP-OES.
[b]General Condtions: Percent yield determined from the reaction of benzyl azide (0.25 mmol, 1 equiv), phenylacetylene (1 equiv), triethylamine (1 equiv), and resin (5 mg) in DCE at 0.2 M. The reactions were heated at 60°C for 4 h, filtered, and concentrated *in vacuo*. Percent yield was determined based on mass of isolated 1,2,3-triazole.

observed for the copper loading. Copper (II) catalysts outperformed the copper (I) derivatives in all cases with about a 10% difference in the isolated yields. The activity between triazines substituted through the 3-position was very similar to the activity of triazines attached through the 5-position with a slight favoring of the 3-substituted triazine linker.

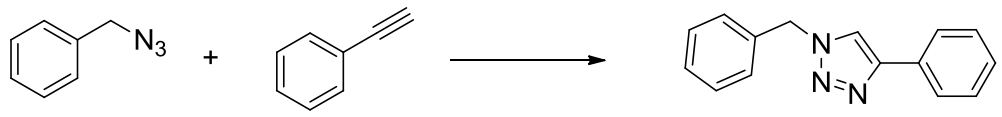
One surprising note was the activity of the 1,2,3-triazole with a phenyl substituent (Entry 7) on a high loading resin. This was only slightly less active than the 1,2,4-triazine supports. When the 1,2,3-triazole supported on a low loading resin was screened, there was no appreciable activity (Entry 8). It was hypothesized that the higher density of triazoles on the high loading resin was catalyzing the azide-alkyne cycloaddition.[38,55] This was supported by the absence of activity for the low loading triazole resin. Additionally, the pyridine substituted 1,2,3-triazole also displayed a high activity, recovering 99% product from the high loading resin and 99% using a low loading resin

with copper (II) (Entries 9-10). The increased activity at using the low-loading resin was surprising, but can be explained since the pyridine adjacent to the triazole is able to chelate the copper to assist in the azide-alkyne cycloaddition.

Since the initial results were promising, we next investigated if the solid supports could be reused for multiple catalytic cycles. We were interested to see if the simple phenyl or pyridyl substituted 1,2,3-triazoles were active for multiple cycles. After each cycloaddition reaction, catalyst resin was filtered, rinsed, and reused without any additional treatment. Catalysts were reused for up to 4 consecutive reactions. The isolated product yields were reported (Table 3.12). For these studies only the triazine appended to the solid support through the 3-position was examined. Since there was not an appreciable difference in the activity between the two triazines, we elected to continue studies using the support which was easier to synthesize (the triazine with an alkyne at the 3-position). For the two triazine ligands (Entries 1-2) moderate yields were displayed for two cycles with the copper (I) catalyst, while moderate yields were illustrated for three cycles for the copper (II) catalysts. The 1,2,3-triazole substituted with a phenyl ring had showed modest activity after one cycle with 73% isolated triazole product, but trying to reuse the catalyst resulted in very minimal product formation (Entry 3). The 1,2,3-triazole substituted with a pyridyl ring was active for two consecutive cycles when the high loading resin was used, but failed to give any substantial product in cycle 3 (Entries 4-5). The low loading resin with a pyridyl 1,2,3-triazole (Entries 6- 7) was only active for one cycle.

Directly related to the recyclability of the resin, is the copper retained by the heterogeneous catalyst. Since one advantage of heterogeneous catalysts is to prevent copper contamination in the products, we examined the percentage of copper leached after consecutive reactions. The isolated triazole product was diluted in 2% nitric acid and the amount remaining on the solid support was determined by difference from the ICP-OES results. The copper retention studies (Table 3.13) follow the same pattern seen in the recyclability studies. As the yield decreased, it can be seen that the percentage of copper remaining on the polystyrene support was also decreasing. For the 1,2,3-triazoles without triazines appended, the copper retention decreased to a range of 11-50% after one cycle. Triazines, by comparison, retained between 80 and

Table 3.12 Recyclability of polystyrene supported catalysts.[a]



	Entry	Catalyst	Cycle 1 % yield	Cycle 2 % yield	Cycle 3 % yield	Cycle 4 % yield
Triazine A (NH_2) HL - Cu(I)	1	3-39	81	72	54	29
Triazine A (NH_2) HL - Cu(II)	2	3-40	76	83	77	50
Ph Triazole HL - Cu(II)	3	3-16	73	4	2	N.D.
Py Triazole HL - Cu(I)	4	3-21	99	95	13	N.D.
Py Triazole HL - Cu(II)	5	3-22	99	98	12	N.D.
Py Triaozle LL - Cu (I)	6	3-23	87	6	N.D.	N.D.
Py Triaozle LL - Cu (II)	7	3-24	99	3	N.D.	N.D.

[a]General condtions: Percent yield determined from the reaction of benzyl azide (1.0 mmol, 1 equiv), phenylacetylene (1 equiv), triethylamine (1 equiv), and resin (20 mg) in DCE at 0.2 M. The reactions were heated at 60°C for 4 h, filtered, and concentrated *in vacuo*. Percent yield was determined based on mass of isolated 1,2,3-triazole. Resin was collected, dried, and re-used without additional treatment.

Table 3.13 Copper retention by polystyrene supported catalysts.[a]

	Entry	Catalyst	Cycle 1 % copper	Cycle 2 % copper	Cycle 3 % copper	Cycle 4 % copper
Triazine A (NH_2) HL - Cu(I)	1	3-39	84	71	53	28
Triazine A (NH_2) HL - Cu(II)	2	3-40	95	82	64	45
Ph Triazole HL - Cu(II)	3	3-16	48	34	24	N.D.
Py Triazole HL - Cu(I)	4	3-21	47	29	N.D.	N.D.
Py Triazole HL - Cu(II)	5	3-22	58	27	N.D.	N.D.
Py Triaozle LL - Cu (I)	6	3-23	40	N.D.	N.D.	N.D.
Py Triaozle LL - Cu (II)	7	3-24	11	N.D.	N.D.	N.D.

[a]General condtions: Benzyl azide (1.0 mmol, 1 equiv), phenylacetylene (1 equiv), triethylamine (1 equiv), and resin (20 mg) were heated at 60°C for 4 h in DCE at 0.2 M. The reactions were filtered, and concentrated *in vacuo*. The isolated 1,2,3-triazoles were dissolved in 2% nitric acid (4 mL), heated to 60°C for 10 min, and left undisturbed overnight. The suspension was then filtered and the filtrate used to calculate the percent of copper retained by the resin using ICP-OES.

90% of the original loaded copper. This data makes sense in that as copper is leached the catalyst support will not be as effective in a catalytic reaction, since the level of copper has decreased dramatically. These results were encouraging in that the triazines could be reused, while the non-triazine controls were more limited.

Additional results not presented here showed that 1,2,4-triazines attached to silica supports displayed higher isolated yields of 1,2,3-triazole product, and could be used for up to four cycles while maintaining greater than 90% yields, without significant copper leaching. Some catalysts were even effective without the addition of triethylamine.

As an extension to this work, an evaluation of solvent was conducted with selected polystyrene and silica catalysts. Organic solvents such as MeCN, DCE, t-BuOH, and toluene had been previously evaluated.[97] Silica catalysts performed much better over a wider array of solvents. The polystyrene catalysts were not as effective in solvents such as tert-butanol or toluene. An additional experiment was conducted to see the activity of the solid supports in water. Surprisingly, both the polystyrene and silica catalysts performed well in the presence of water with isolated yields of 98-99% (Table 3.14 Entry 5). We had not explored the use of water as a solvent with homogeneous ligands, since the substrates did not dissolve in aqueous medium. In this case, the reaction was stirred to ensure reaction of the nonpolar substrates with the triazine catalyst. The isolation of the product was also rather simple, as the reaction was filtered, and the resin rinsed with DCM to dissolve the product triazole. Using water would be more advantageous especially from a “click” perspective. Additional experiments should be carried out with the solid supports in water, specifically a study to look at recyclability of catalysts and the copper retention when water is used for the reaction media. The dependence of base when reactions are carried out in water would also be a worthwhile experiment. Finally, since the homogenous ligands were not used with water, it would be advantageous to test those ligands in aqueous conditions to see if similar results are obtained.

Table 3.14 Evaluation of solvents with various polystyrene and silica catalysts.

Entry	Solvent	Isolated Percent Yield[a]	Isolated Percent Yield[b]
1	acetonitrile	89	88
2	dichloroethane	69	86
3	tert-butanol	5	79
4	toluene	3	83
5	water	99	98

[a]General condtions: Percent yield determined from the reaction of benzyl azide (0.25 mmol, 1 equiv), phenylacetylene (1 equiv), triethylamine (1 equiv), and polystyrene catalyst **3-40** (5 mg) at 0.2 M. The reactions were heated at 60°C for 4 h, filtered, and concentrated *in vacuo*. Percent yield was determined based on mass of isolated 1,2,3-triazole.
[b]General conditions with the following exception: silica catalyst (10 mg)

3.8. Conclusions

In summary, several parameters were evaluated with respect to the use of homogeneous triazines as ligands for CuAAC. Optimization studies, evaluation of substrate scope, and ligand scope were all conducted to explore the feasibility of 1,2,4-triazines as ligands for CuAAC. Optimization studies revealed that homogeneous 3-(2-pyridyl)-1,2,4-triazines with either copper (I) or copper (II) salts could be used effectively in the presence of a reducing agent, triethylamine, giving isolated 1,2,3-triazole product in 97% yield. Best results were observed with an excess of reducing agent (0.5 to 1.0 equivalents) although 0.06 equivalents of triethylamine produced reasonable yields (91%), but required longer reaction times (5 hours). The substrate concentration could be reduced to 0.1 M without adverse effects to the catalytic activity, but further decreasing to 0.05 M decreased the catalytic activity greatly.

The scope of the reaction seems to encompass a wide array of substrate azide and alkynes. Aryl and alkl azides in general underwent cycloadditions with phenylacetylene in good yields (>80%). Alkyl acids only gave modest triazole products, and tertiary azides, such as adamantly azide resulted in minimal product formation. Variations in azides were more tolerable compared to their alkyne counterparts. Alkyne substituents were more apt to formation of bistriazole products, requiring modifications to the solvent

choice, using DCM in place of MeCN. Yields with alkynes, in general, were slightly lower ranging from 68 – 95%. The exception was the long alkyl alkyne, decyne which showed poor results. With regards to solvent, the choices were somewhat limited, working best in nonpolar non-coordinating solvents. Solvents that could coordinate metals such as DMSO, DMF, or THF led to decreased yields, increased by-product formation, or longer reaction times.

A ligand scope was also conducted in search of more active nitrogen containing ligands. The 3-(2-pyridyl)-substituent was deemed necessary for activity. Triazines lacking this group were inactive with isolated yields less than 10%, with the exception of the imidazoyl substitutent that produced 50% of triazole product in 1 hour. Modifications to the 5,6-substitutents proved to be less detrimental to the activity, but did not surpass the activity of the triazine used for optimization studies (**2-17**). Replacing the 1,2,4-triazine core with other heterocycles such as tetrazines, pyridines, or imines did not yield triazole product in a catalytic fashion. The two exceptions were pyridizine (**2-87**) which resulted in 63% yield and imine (**2-95**) which resulted in 95% yield of the triazole product. The imine ligand did require longer reaction times compared to 3-(2-pyridyl)-1,2,4-triazines.

Two applications utilizing the homogenous triazine ligand were evaluation in other metal catalyzed reactions, such as the Sonogashira reaction and use in synthesis of solid supported catalysts. The application of triazines to a one-pot Sonogashira-azide-alkyne cycloaddition reaction was not fully optimized. Further work is still needed to make this route viable. Options include changing the palladium source and possibly the copper salt.

The second application, the synthesis and catalytic activity of the solid-supported triazines proved to be much more valuable. Good to excellent yields were observed with polystyrene and silica catalysts containing 1,2,4-triazines. Although heat was needed for the reaction to proceed, the high yields, easy product isolation, and copper retention by the support, made this an efficient and viable synthetic route. 1,2,4-triazines were more effective at binding copper and were able to be recycled compared to 1,2,3-triazoles which were synthesized as controls. In addition, both polystyrene and silica supported

1,2,4-triazines worked efficiently in water, affording >98% yield of 1,2,3-triazole product. Further exploration of this method to the application of more challenging substrates would be a worthwhile expedition.

3.9. Experimental

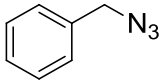
Materials and General Methods. Reagents and solvents were purchased from various commercial sources and used without further purification unless otherwise stated. Anhydrous solvents were purified using a Grubbs solvent system. Analytical thin-layer chromatography (TLC) was performed using aluminum backed silica gel TLC plates with UV indicator from Sorbent Technologies. Flash column chromatography was performed using 40-63 μm (230 x 400 mesh) silica gel from Sorbent Technologies. ^{1}H and ^{13}C NMR were recorded at 600 MHz and 151 MHz, respectfully, on a Varian Inova spectrometer or at 500 MHz and 125 MHz on a Varian spectrometer. All chemical shifts were reported in δ units relative to tetramethylsilane or the corresponding deuterated solvent. High resolution mass spectra (ESI) were obtained on a JEOL AccuTOF DART spectrometer. Liquid chromatography mass spectra were obtained on a QSTAR XL Hybrid LC/MS/MS equipped with a Vydac C18 column and a mobile phase composed of acetonitrile and formic acid. Infrared spectra were recorded on a Varian 4100 FT-IR using KBr pellets or KBr salt plates. Absorption spectra were collected on a Thermo Scientific Evolution 600. High pressure liquid chromatography (HPLC) was performed using a Beckman Coulter System equipped with a UV-Vis detector, autosampler, Varian C18 column, and a mobile phase composed of acetonitrile and trifluoroacetic acid. Copper was quantified by inductivel coupled plasma – optical emission spectroscopy (ICP-OES) using a Perkin Elmer Optima 2100 DV.

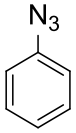
WARNING: Low molecular weight azides are potentially explosive. Appropriate safety measures should always be taken when handling these compounds.

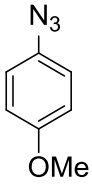
LC/MS Methodology. All reactants and reagents were added as stock solutions unless otherwise noted. The concentrations of these stock solutions were determined by UV-Vis spectroscopy. Reactants and reagents were added in the following order: excess solvent, azide, alkyne, base, ligand and copper source. After 1 h the reactions were diluted in acetonitrile to a final concentration range of 15 – 25 μM and analyzed by LC-MS using a Vydac C18 column. The isocratic mobile phase was composed of 45% acetonitrile and 0.5% formic acid. Percent yields were determined based on product formation using an external calibration curve. All reactions containing a standard deviation were run in triplicate unless otherwise noted.

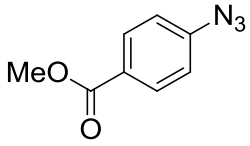
General Azide Method A: Aromatic Azides. The aniline derivative (approx 2 g, 16.24 mmol) was dissolved in 80 mL of EtOAc. 40 mL of 1.5 M HCl was added and the solution cooled to 0°C in an ice bath. Sodium nitrite (1.68 g, 24.36 mmol) was dissolved in 20 mL water and added dropwise to the reaction solution while stirring at 0°C. After 15 min, sodium azide (3.17 g, 48.72 mmol) was dissolved in 20 mL water and added dropwise to the reaction solution while stirring at 0°C. After slowly warming to room temperature, the reaction was monitored by TLC. Upon completion, the reaction was extracted with EtOAc (x3) and washed with 1 M HCl, water, and brine. The solution was then dried with sodium sulfate and concentrated *in vacuo* to yield the corresponding aromatic azide. Yields: 80 – 94%.

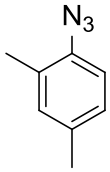
General Azide Method B: Aliphatic/Benzylic Azides. The bromine derivative (approx 2 g, 11.17 mmol) was dissolved in 25 mL of dimethylformamide. Sodium azide (799 mg, 12.28 mmol) was added and the reaction was stirred overnight at room temperature. Progress of the reaction was monitored by TLC and GC-MS. Upon completion, the reaction was quenched with 50 mL of water (exothermic) and stirred until reaching room temperature. The solution was then extracted with diethyl ether (x3) and washed with water (x2), copper sulfate (x1), and brine. The solution was then dried with sodium sulfate and concentrated *in vacuo* to yield the corresponding aliphatic azide. Yields: 79 – 98%.

 **(azidomethyl)benzene (3-1a).** Prepared using general azide method B and isolated as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.40-7.37 (m, 2H), 7.35-7.31 (m, 3H), 4.33 (s, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 135.5, 129.0, 128.44, 128.35, 54.9. IR (film) ν_{max} 2099 cm^{-1}. MS (ESI) m/z calculated 106.066 (C_7H_8N, $[M-N_2+H]^+$), m/z observed 106.064 (C_7H_8N, $[M-N_2+H]^+$).

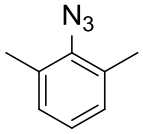
 **azidobenzene (3-1b).** Prepared using general azide method A and isolated as a brown oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.35 (t, 2H, J = 7.8 Hz), 7.14 (t, 1H, J = 7.8 Hz), 7.03 (d, 2H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 140.0, 129.8, 124.9, 119.1. IR (film) ν_{max} 2129 cm^{-1}. MS (ESI) m/z calculated 92.050 (C_6H_6N, $[M-N_2+H]^+$), m/z observed 92.048 (C_6H_6N, $[M-N_2+H]^+$).

 **1-azido-4-methoxybenzene (3-1c).** Prepared using general azide method A and isolated as a brown solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.95 (d, 2H, J = 9 Hz), 6.89 (d, 2H, J = 9 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 157.0, 132.3, 120.0, 115.1, 55.6. IR (film) ν_{max} 2110 cm^{-1}. MS (ESI) m/z calculated 122.061 (C_7H_8NO, $[M-N_2+H]^+$), m/z observed 122.060 (C_7H_8NO, $[M-N_2+H]^+$).

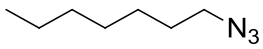
 **methyl 4-azidobenzoate (3-1d).** Prepared using general azide method A and isolated as an orange-brown solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.05 (d, 2H, J = 7.4 Hz), 7.07 (d, 2H, J = 7.4 Hz), 3.91 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 166.4, 144.9, 131.6, 126.8, 119.0, 52.3. IR (film) ν_{max} 2125 cm^{-1}. MS (ESI) m/z calculated 150.056 ($C_8H_8NO_2$, $[M-N_2+H]^+$), m/z observed 150.056 (C_7H_7NO, $[M-N_2+H]^+$).

 **1-azido-2,4-dimethylbenzene (3-1e).** Prepared using general azide method A and isolated as a brown oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.03-6.97 (m, 3H),

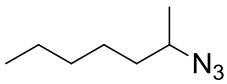
2.29 (s, 3H), 2.17 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 135.6, 134.2, 131.9, 129.3, 127.6, 117.8, 20.8, 17.2. IR (film) ν_{max} 2122 cm^{-1}. MS (ESI) m/z calculated 120.081 ($C_8H_{10}N$, $[M-N_2+H]^+$), m/z observed 120.081 ($C_8H_{10}N$, $[M-N_2+H]^+$).



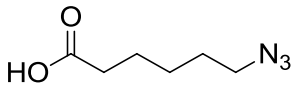
2-azido-1,3-dimethylbenzene (3-1f). Prepared using general azide method A and isolated as a brown oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.02-7.00 (m, 3H), 2.36 (s, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 137.0, 132.1, 128.9, 125.7, 18.2. IR (film) ν_{max} 2129 cm^{-1}. MS (ESI) m/z calculated 120.081 ($C_8H_{10}N$, $[M-N_2+H]^+$), m/z observed 120.081 ($C_8H_{10}N$, $[M-N_2+H]^+$).



1-azidoheptane (3-1g). Prepared using general azide method B and isolated as a clear oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 3.26 (t, 2H, J = 6.6 Hz), 1.62-1.58 (m, 2H), 1.38-1.27 (m, 8H), 0.89 (t, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 51.5, 31.7, 28.8, 26.7, 22.6, 14.1, 14.0. IR (film) ν_{max} 2099 cm^{-1}. MS (ESI) m/z calculated 114.128 ($C_7H_{16}N$, $[M-N_2+H]^+$), m/z observed 114.128 ($C_7H_{16}N$, $[M-N_2+H]^+$).

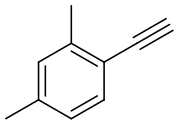


2-azidoheptane (3-1h). Prepared using general azide method B and isolated as a clear oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 3.43-3.40 (m, 1H), 1.52-1.27 (m, 8H), 1.25 (d, 3H, J = 6 Hz), 0.89 (t, 3H, J = 6 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 58.2, 36.3, 31.7, 25.9, 22.7, 19.6, 14.1. IR (film) ν_{max} 2110 cm^{-1}. MS (ESI) m/z calculated 114.128 ($C_7H_{16}N$, $[M-N_2+H]^+$), m/z observed 114.129 ($C_7H_{16}N$, $[M-N_2+H]^+$).

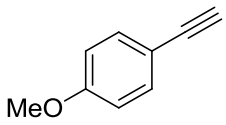


6-azidohexanoic acid (3-1k). Prepared using general azide method B and isolated as a clear oil in 98.1% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 3.29 (t, 2H, J = 7.2), 2.38 (t, 2H, J = 7.2 Hz), 1.70-1.60 (m, 4H), 1.47-1.42 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 179.50, 51.32, 33.94, 28.65, 26.28, 24.29. IR (film) ν_{max} 2098.55 cm^{-1}. MS (ESI) m/z calculated 130.087 ($C_6H_{12}NO_2$, $[M-N_2+H]^+$), m/z observed 130.087 ($C_6H_{12}NO_2$ $[M-N_2+H]^+$).

General Alkyne Method. Aryl iodide (10 mmol), dichlorobis-(triphenylphosphine)palladium(II) (0.25 mmol), and CuI (0.15 mmol) were combined in a round bottom flask and flushed with N_2. Anhydrous THF was added (12.5 mL) followed by a dropwise addition of triethylamine (15 mmol) and trimethylsilylacetylene (15 mmol) in anhydrous THF (12.5 mL). Reaction was stirred at room temperature and monitored by TLC. Upon completion, solvent was removed *in vacuo* and purified by silica gel chromatography to yield the corresponding trimethylsilyl protected alkyne. To the protected alkyne (10 mmol) in MeOH (20 mL) was added solid K_2CO_3 (1.5 mmol) and the resulting suspension stirred at room temperature, with reaction progress monitored by TLC. Upon completion, the reaction was concentrated *in vacuo*, and the residue brought up in DCM and water. The aqueous layer was extracted with DCM (x3). The combined organic layers were washed with water (x1), sat'd $NaHCO_3$, and brine. The solution was dried over sodium sulfate, concentrated *in vacuo* and purified by silica column chromatography to yield corresponding terminal alkyne.

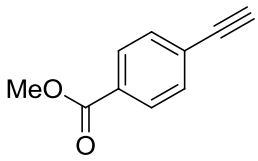


1-ethynyl-2,4-dimethylbenzene (3-2e). Prepared using general alkyne method from 1-iodo 2,4-dimethylbenzene. Purified by silica chromatography using gradient elution of hexanes and ethyl acetate to yield a red oil in 61% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.35 (d, 1H, J = 7.8 Hz), 7.02 (s, 1H), 6.95 (d, 1H, J = 7.8 Hz), 3.22 (s, 1H), 2.42 (s, 3H), 2.32 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 140.7, 139.0, 132.6, 130.4, 126.5, 119.0, 82.9, 80.3, 21.6, 20.6. HRMS (ESI) m/z calculated 131.08608 ($C_{10}H_{11}$, $[M+H]^+$), m/z observed 131.08606. ($C_{10}H_{11}$, $[M+H]^+$).

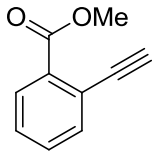


1-ethynyl-4-methoxybenzene (3-2f). Prepared using general alkyne method from 4-iodoanisole. Purified by silica chromatography using gradient elution of hexanes and ethyl acetate to yield a yellow oil in 65% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43 (d, 2H, J = 7 Hz), 6.85 (d, 2H, J = 7 Hz), 3.82 (s, 3H), 2.99 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.1, 133.7, 114.3, 114.1,

83.8, 75.9, 55.4. HRMS (ESI) m/z calculated 133.06534 (C_9H_9O, $[M+H]^+$), m/z observed 133.06486. (C_9H_9O, $[M+H]^+$).



methyl 4-ethynylbenzoate (3-2g). Prepared using general alkyne method from methyl-4-iodobenzoate. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield an off-white solid in 91% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.00 (d, 2H, J = 8.4 Hz), 7.55 (d, 2H, J = 8.4 Hz), 3.92 (s, 3H), 3.23, (s, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 166.6, 132.2, 130.3, 129.6, 126.9, 82.9, 80.2, 52.4. HRMS (ESI) m/z calculated 161.06025 ($C_{10}H_9O_2$, $[M+H]^+$), m/z observed 161.05971. ($C_{10}H_9O_2$, $[M+H]^+$).



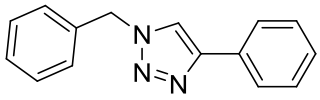
methyl 2-ethynylbenzoate (3-2h). Prepared using general alkyne method from methyl-2-iodobenzoate. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield an orange oil in 88% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.94 (dd, 1H, J = 7.8, 1.2 Hz), 7.63 (dd, 1H, J = 7.8, 1.2 Hz), 7.48 (td, 1H, J = 7.8, 1.2 Hz), 7.41 (td, 1H, J = 7.8, 1.2 Hz), 3.94 (s, 3H), 3.40 (s, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 166.6, 135.1, 132.6, 131.9, 130.4, 128.6, 122.8, 82.4, 82.2, 52.3. HRMS (ESI) m/z calculated 161.06025 ($C_{10}H_9O_2$, $[M+H]^+$), m/z observed 161.06039. ($C_{10}H_9O_2$, $[M+H]^+$).

General Triazole Method A: Azide (1 mmol), alkyne (1 mmol), and triethylamine (1 mmol) were dissolved in acetonitrile (5 mL). **(1)** (10 μmol) and tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (10 μmol) were added to the reaction solution and stirred at room temperature for 18 hours. Concentration *in vacuo* and purification yielded desired 1,2,3-triazole product.

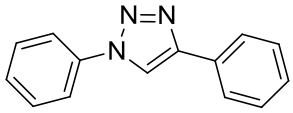
General Triazole Method B: Azide (1 mmol), alkyne (1 mmol), and triethylamine (1 mmol) were dissolved in dichloromethane (5 mL). **(1)** (10 μmol) and copper(II) tetrafluoroborate hexahydrate (10 μmol) were added to the reaction solution and stirred

at room temperature for 18 hours. Concentration *in vacuo* and purification yielded desired 1,2,3-triazole product.

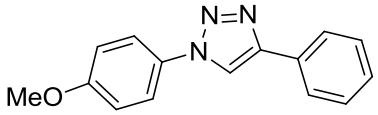
General Triazole Method C: Azide (1 mmol), alkyne (1 mmol), and triethylamine (1 mmol) were dissolved in dichloroethane (5 mL). **(1)** (10 μmol) and copper(II) tetrafluoroborate hexahydrate (10 μmol) were added to the reaction solution and stirred at room temperature for 1 hour. Reaction mixture was poured directly onto silica and purification yielded desired 1,2,3-triazole product.



1-benzyl-4-phenyl-1H-1,2,3-triazole (3-3a). Prepared using general triazole method C. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate as a white solid in 97% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.80 (d, 2H, J = 8.4 Hz), 7.66 (s, 1H), 7.41-7.37 (m, 5H), 7.32-7.31 (m, 3H), 5.58 (s, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.4, 134.8, 130.7, 129.3, 128.9, 128.3, 128.2, 125.8, 119.6, 54.4. HRMS (ESI) m/z calculated 236.11877 ($C_{15}H_{14}N_3$, $[M+H]^+$), m/z observed 236.11898 ($C_{15}H_{14}N_3$, $[M+H]^+$).

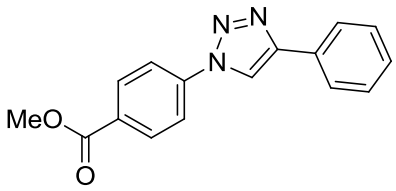


1,4-diphenyl-1H-1,2,3-triazole (3-3b). Prepared using general triazole method A. Precipitated from 5% nitric acid as a brown solid in 95% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.20 (s, 1H), 7.93 (d, 2H, J = 6.6 Hz), 7.81 (d, 2H, J = 7.8 Hz), 7.55 (t, 2H, J = 7.8 Hz), 7.48-7.46 (m, 3H), 7.38 (t, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.6, 137.2, 130.4, 129.9, 129.1, 128.9, 128.6, 126.0, 120.7, 117.7 . HRMS (ESI) m/z calculated 222.10312 ($C_{14}H_{12}N_3$, $[M+H]^+$), m/z observed 222.10280 ($C_{14}H_{12}N_3$, $[M+H]^+$).

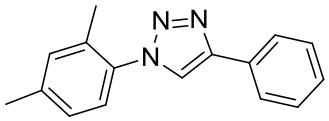


1-(4-methoxyphenyl)-4-phenyl-1H-1,2,3-triazole (3-3c). Prepared using general triazole method A. Precipitated from 5% nitric acid as a brown solid in 83% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.11 (s, 1H), 7.91 (d, 2H, J = 7.2 Hz), 7.69 (d, 2H, J = 7.8 Hz), 7.46

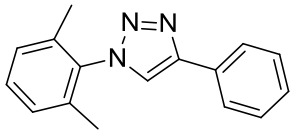
(t, 2H, J = 7.2 Hz), 7.37 (t, 1H, J = 7.2 Hz), 7.05 (d, 2H, J = 7.8 Hz) 3.88 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 160.0, 148.4, 130.7, 130.5, 129.0, 128.5, 126.0, 122.3, 118.0, 115.0, 55.8. HRMS (ESI) m/z calculated 252.11369 ($C_{15}H_{14}N_3O$, $[M+H]^+$), m/z observed 252.11371 ($C_{15}H_{14}N_3O$, $[M+H]^+$).



methyl 4-(4-phenyl)-1H-1,2,3-triazol-1-yl)benzoate (3-3d). Prepared using general triazole method A. Precipitated from 5% nitric acid as a yellow solid in 91% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 9.46 (s, 1H), 8.21 (d, 2H, J = 9 Hz), 8.15 (d, 2H, J = 8.4 Hz), 7.97 (d, 2H, J = 7.8 Hz), 7.52 (t, 2H, J = 7.8 Hz), 7.41 (t, 1H, J = 7.8 Hz), 3.91 (s, 1H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 165.4, 147.6, 139.8, 131.1, 130.0, 129.4, 129.1, 128.4, 125.4, 119.8, 119.7, 52.4. HRMS (ESI) m/z calculated 280.10860 ($C_{16}H_{14}N_3O_2$, $[M+H]^+$), m/z observed 280.10883 ($C_{16}H_{14}N_3O_2$, $[M+H]^+$).

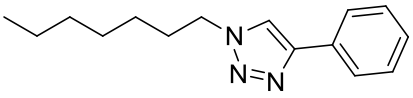


1-(2,4-dimethylphenyl)-4-phenyl-1H-1,2,3-triazole (3-3e). Prepared using general triazole method A. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate as a white solid in 90% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.92 (d, 3H, J = 9 Hz), 7.46 (t, 2H, J = 7.8 Hz), 7.36 (t, 1H, J = 7.2 Hz) 7.27 (d, 1H, J = 7.8 Hz), 7.20 (s, 1H), 7.15 (d, 1H, J = 7.8 Hz), 2.41 (s, 3H), 2.23 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 147.6, 140.1, 134.3, 133.6, 132.2, 130.6, 129.0, 128.4, 127.6, 125.92, 125.91, 121.3, 21.3, 18.0. HRMS (ESI) m/z calculated 250.13442 ($C_{16}H_{16}N_3$, $[M+H]^+$), m/z observed 250.13462 ($C_{16}H_{16}N_3$, $[M+H]^+$).

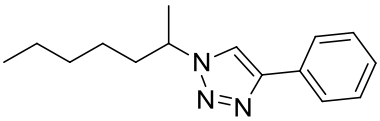


1-(2,6-dimethylphenyl)-4-phenyl-1H-1,2,3-triazole (3-3f). Prepared using general triazole method A. Purified by silica column chromatography using a gradient elution of hexanes and ethyl acetate as a white solid in 83% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.94 (d, 2H, J

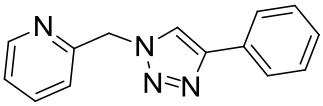
= 8.4 Hz), 7.87 (s, 1H), 7.47 (t, 2H, J = 7.2 Hz), 7.37 (t, 1H, J = 7.8 Hz), 7.34 (t, 1H, J = 7.2 Hz), 7.21 (d, 2H, J = 7.8 Hz), 2.07 (s, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 147.8, 136.1, 135.6, 130.6, 130.2, 129.1, 128.6, 128.5, 125.9, 121.4, 17.6. HRMS (ESI) m/z calculated 250.13442 ($C_{16}H_{16}N_3$, $[M+H]^+$), m/z observed 250.13497 ($C_{16}H_{16}N_3$, $[M+H]^+$).



1-heptyl-4-phenyl-1H-1,2,3-triazole (3-3g). Prepared using general triazole method A. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate as a white solid in 88% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.84 (d, 2H, J = 7.8 Hz), 7.74 (s, 1H), 7.42 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 7.8 Hz), 4.39 (t, 2H, J = 7.2 Hz), 1.97-1.92 (m, 2H,), 1.35-1.27 (m, 8H), 0.88 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 147.9, 130.9, 128.9, 128.2, 125.8, 119.5, 50.6, 31.7, 30.5, 28.8, 26.6, 22.7, 14.2. HRMS (ESI) m/z calculated 244.18137 ($C_{15}H_{22}N_3$, $[M+H]^+$), m/z observed 244.18152 ($C_{15}H_{22}N_3$, $[M+H]^+$).

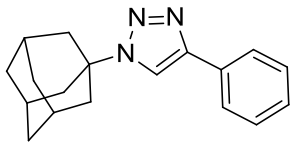


1-(heptan-2-yl)-4-phenyl-1H-1,2,3-triazole (3-3h). Prepared using general triazole method A. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate as a white solid in 80% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.85 (d, 2H, J = 7.8 Hz), 7.74 (s, 1H), 7.42 (t, 2H, J = 7.8 Hz), 7.32 (t, 1H, J = 7.2 Hz), 4.74-4.68 (m, 1H), 1.97-1.92 (m, 1H), 1.86-1.82 (m, 1H), 1.60 (d, 3H, J = 6.6 Hz), 1.28-1.18 (m, 6H), 0.86 (t, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 147.7, 131.0, 128.9, 128.1, 125.8, 117.4, 57.7, 37.4, 31.5, 25.8, 22.6, 21.6, 14.1. HRMS (ESI) m/z calculated 244.18137 ($C_{15}H_{22}N_3$, $[M+H]^+$), m/z observed 244.18197 ($C_{15}H_{22}N_3$, $[M+H]^+$).

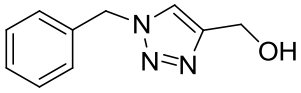


2-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)pyridine (3-3i). Prepared using general triazole method C. Purified by silica column chromatograph using gradient elution of hexanes and ethyl acetate and isolated as white solid in 88% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.62 (d, 1H, J = 5.0 Hz), 7.94

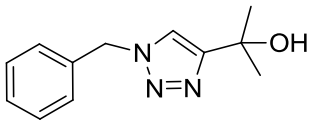
(s, 1H), 7.83 (d, 2H, J = 7.5 Hz), 7.70 (t, 1H, J = 7.5 Hz), 7.41 (t, 2H, J= 8.0 Hz), 7.32 (t, 1H, J= 7.0 Hz) 7.23 – 7.29 (m, 2H), 5.71 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.7, 149.9, 148.4, 137.6, 130.7, 129,0, 128.3, 125.9, 123.6, 122.6, 120.3, 55.9. HRMS (ESI) m/z calculated 237.11402 ($C_{14}H_{13}N_4$, $[M+H]^+$), m/z observed 237.11389 ($C_{14}H_{13}N_4$, $[M+H]^+$).



1-(adamantan-1-yl)-4-phenyl-1H-1,2,3-triazole (3-3j). Prepared using general triazole method A. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate as a white solid in 5% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.85-7.82 (m, 3H), 7.41 (t, 2H, J = 6.0 Hz), 7.31 (t, 1H, J = 6.0 Hz), 2.30 (s, 9 H), 1.82 (s, 6 H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 146.9, 131.3, 128.9, 128.0, 125.8, 116.1, 59.7, 43.2, 36.1, 29.6. HRMS (ESI) m/z calculated 280.18137 ($C_{10}H_{20}N_3O$, $[M+H]^+$), m/z observed 280.18240 ($C_{10}H_{20}N_3O$, $[M+H]^+$).

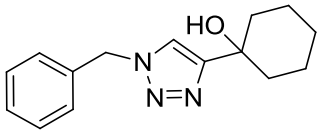


(1-benzyl-1H-1,2,3-triazol-4-yl)methanol (3-3l) Prepared using general triazole method A. Purified by silica column chromatography using gradient elution of ethyl acetate and methanol to yield a white solid in 76% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.44 (s, 1H), 7.37 (m, 3H), 7.27 (d, 2H, J = 9 Hz), 5.51 (s, 2H), 4.76 (s, 2H), 2.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.2, 134.6, 128.3, 129.0, 128.3, 121.7, 56.7, 54.4. HRMS (ESI) m/z calculated 190.09804 ($C_{10}H_{12}N_3O$, $[M+H]^+$), m/z observed 190.09876. ($C_{10}H_{12}N_3O$, $[M+H]^+$).

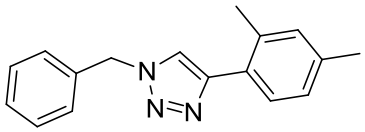


2-(1-benzyl-1H-1,2,3-triazol-4-yl)propan-2-ol (3-3m) Prepared using general triazole method A. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 80.0% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.39-7.36 (m, 3H), 7.34 (s, 1H), 7.28 (d, 2H, J = 7.2 Hz), 5.50 (s, 2H), 2.41 (s, 1H), 1.61 (s, 6H). ^{13}C

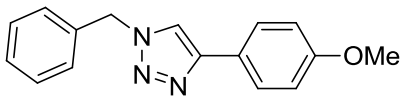
NMR ($CDCl_3$, 151 MHz) δ 156.1, 134.7, 129.3, 128.9, 128.3, 119.0, 68.7, 54.3, 30.6. HRMS (ESI) m/z calculated 218.12934 ($C_{12}H_{16}N_3O$, $[M+H]^+$), m/z observed 218.13039. ($C_{12}H_{16}N_3O$, $[M+H]^+$).



1-(1-benzyl-1*H*-1,2,3-triazol-4-yl)cyclohexanol (3-3n) Prepared using general triazole method A. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 79% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.37 (d, 3H, J = 9 Hz), 7.34 (s, 1H), 7.27 (d, 2H, J = 9 Hz), 5.50 (s, 2H), 2.24 (s, 1H), 1.94 (t, 2H, J = 10.8 Hz), 1.85 (d, 2H, J = 13.2 Hz), 1.69-1.75 (m, 2H), 1.59-1.65 (m, 1H), 1.53-1.55 (m, 2H), 1.31-1.37 (m, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 156.2, 134.8, 129.3, 128.9, 128.3, 119.5, 69.8, 54.3, 38.3, 25.5, 22.1. HRMS (ESI) m/z calculated 258.16064 ($C_{15}H_{20}N_3O$, $[M+H]^+$), m/z observed 258.16137. ($C_{15}H_{20}N_3O$, $[M+H]^+$).

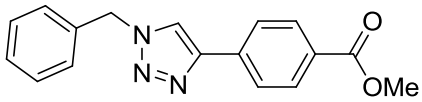


1-benzyl-4-(2,4-dimethylphenyl)-1H-1,2,3-triazole (3-3o) Prepared using general triazole method A. Purified by silica column chromatography using gradient elution with hexanes and ethyl acetate to yield a white solid in 68.4% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.63 (d, 1H, J = 7.8 Hz), 7.52 (s, 1H), 7.36-7.40 (m, 3H), 7.31 (d, 2H, J = 7.8 Hz), 7.1 (m, 2H), 5.60 (s, 2H), 2.39 (s, 3H), 2.34 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 147.8, 135.4, 135.0, 131.7, 129.3, 128.9, 128.8, 128.1, 127.2, 126.9, 121.5, 54.3, 21.4, 21.3. HRMS (ESI) m/z calculated 264.15007 ($C_{17}H_{18}N_3$, $[M+H]^+$), m/z observed 264.15016. ($C_{17}H_{18}N_3$, $[M+H]^+$).

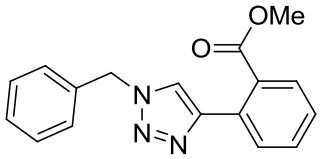


1-benzyl-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (3-3p) Prepared using general triazole method B. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 87% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.72 (d, 2H, J = 8.4 Hz), 7.57 (s, 1H), 7.35-7.41 (m, 3H), 7.30-7.32 (m, 2H), 6.93 (d, 2H, J = 9 Hz), 5.57 (s, 2H),

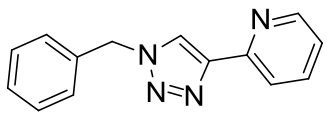
3.83 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 159.7, 148.3, 134.9, 129.3, 128.9, 128.2, 127.2, 123,4, 118.8, 114.3, 55.5, 54.4. HRMS (ESI) m/z calculated 266.12934 ($C_{16}H_{16}N_3O$, $[M+H]^+$), m/z observed 266.13042. ($C_{16}H_{16}N_3O$, $[M+H]^+$).



methyl 4-(1-benzyl-1H-1,2,3-triazol-4-yl) benzoate (3-3q) Prepared using general triazole method B. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 86% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.07 (d, 2H, J = 8.4 Hz), 7.88 (d, 2H, J = 8.4 Hz), 7.74 (s, 1H), 7.36-7.42 (m, 3H), 7.32-7.33 (m, 2H), 5.59 (s, 2H), 3.92 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 166.9, 147.3, 135.0, 134.6, 130.3, 129.7, 129.4, 129.1, 128.3, 125.6, 120.5, 54.5, 52.3. HRMS (ESI) m/z calculated 294.12425 ($C_{17}H_{16}N_3O_2$, $[M+H]^+$), m/z observed 294.12490. ($C_{17}H_{16}N_3O_2$, $[M+H]^+$).

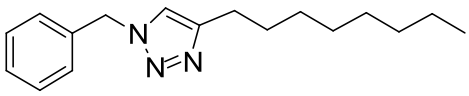


methyl 2-(1-benzyl-1H-1,2,3-triazol-4-yl)benzoate (3-3r) Prepared using general triazole method B. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield an off-white solid in 89% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.81 (d, 1H, J = 7.8 Hz), 7.77 (d, 1H, J = 7.8 Hz), 7.72 (s, 1H), 7.54 (td, 1H, J = 7.8, 1.2 Hz), 7.35-7.41 (m, 4H), 7.31-7.32 (m, 2H), 5.59 (s, 2H), 3.72 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 168.9, 146.3, 135.0, 131.6, 130.5, 130.4, 130.3, 129.3, 128.9, 128.2, 122.7, 54.3, 52.3. HRMS (ESI) m/z calculated 294.12425 ($C_{17}H_{16}N_3O_2$, $[M+H]^+$), m/z observed 294.12508. ($C_{17}H_{16}N_3O_2$, $[M+H]^+$).

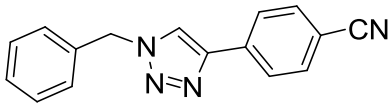


2-(1-benzyl-1H-1,2,3-triazol-4-yl)pyridine (3-3s). Prepared using general triazole method C. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 92% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.45 (d, 1H, J = 4.0 Hz), 8.18 (d, 1H, J = 8.0 Hz), 8,04 (s, 1H), 7.77 (tt, 1H, J = 8.0, 2.0 Hz), 7.30-7.40 (m, 5H), 7.21 (t, 1H, J = 6.5 Hz), 5.58 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 150.4, 149.5, 148.9, 137.0, 134.5,

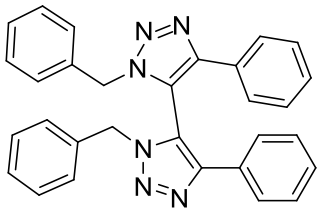
129.3, 123.0, 122.0, 120.4, 54.6. HRMS (ESI) m/z calculated 237.11402. ($C_{14}H_{13}N_4$, $[M+H]^+$), m/z observed 237.11410. ($C_{14}H_{13}N_4$, $[M+H]^+$).



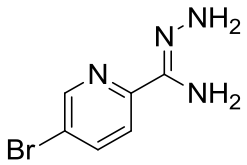
1-benzyl-4-octyl-1H-1,2,3-triazole (3-3t). Prepared using general triazole method C. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 23% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34 – 7.39 (m, 2H), 7.24 – 7.26 (m, 3H), 7.17 (s, 1H), 5.49 (s, 2H), 2.68 (t, 2H, J = 8.0 Hz), 1.63 (q, 2H, 7.5 Hz), 1.2 – 1.4 (m, 10H), 0.869 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ149.2, 135.2, 129.2, 128.7, 128.1, 120.6, 105.2, 54.1, 32.0, 29.6, 29.4, 29.3, 25.9, 22.8, 14.2. HRMS (ESI) m/z calculated 272.21267 ($C_{17}H_{26}N_3$, $[M+H]^+$), m/z observed 272.21304 ($C_{17}H_{26}N_3$, $[M+H]^+$).



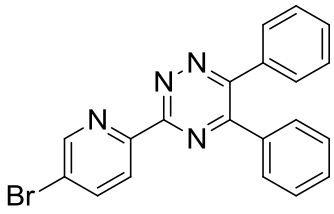
4-(1-benzyl-1H-1,2,3-triazol-4-yl)benzonitrile (3-3u). Prepared using general triazole method C. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 95% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (d, 2H, J = 9.0 Hz), 7.74 (s, 1H), 7.69 (d, 2H, J = 8.5 Hz), 7.37 – 7.43 (m, 3H), 7.31 – 7.34 (m, 2H), 5.60 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 146.5, 135.1, 134.4, 132.8, 129.4, 129.2, 128.3, 126.2, 120.7, 118.9, 111.7, 54.6. HRMS (ESI) m/z calculated 261.11402 ($C_{16}H_{13}N_4$, $[M+H]^+$), m/z observed 261.11498. ($C_{16}H_{13}N_4$, $[M+H]^+$).



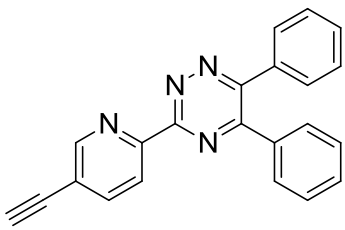
3,3'-dibenzyl-5,5'-diphenyl-3H,3'H-4,4'-bi(1,2,3-triazole) (3-4). Isolated as by-product when using general triazole method A. Purified by silica column chromatography using gradient elution of hexanes and ethyl acetate to yield a white solid in 3% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.42–7.46 (m, 4H), 7.22–7.25 (m, 2H), 7.18–7.22 (m, 4H), 7.11–7.16 (m, 2H), 7.08 (t, 4H, J = 7.2 Hz), 6.80 (d, 4H, J = 7.2 Hz), 4.69 (d, 2H, J = 15 Hz), 4.62 (d, 2H, J = 15 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.0, 133.1, 129.4, 129.1, 129.0, 128.92, 128.86, 128.3, 126.0, 120.0, 52.8. HRMS (ESI) m/z calculated 469.21407 ($C_{30}H_{25}N_6$, $[M+H]^+$), m/z observed 469.21239. ($C_{30}H_{25}N_6$, $[M+H]^+$).



5-bromopicolinohydrazonamide (3-26). To a stirring suspension of **3-25** (1.0 g, 5.5 mmol) in 5 mL of EtOH was added hydrazine hydrate (0.584 mL, 7.7 mmol). The suspension was stirred at room temperature for 72 h and progress of the reaction was monitored by TLC. The pale yellow precipitate was then filtered and washed with Et_2O in 79% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 8.57 (s, 1H), 7.91 (dd, 1H, J = 8.4, 2.4 Hz), 7.81 (dt, 1H, J = 9.0, 2.4 Hz), 5.16 (bs, 1H), 4.60 (bs, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 149.6, 149.0, 147.9, 139.2, 121.14, 121.08. HRMS (ESI) m/z calculated 214.99323 ($C_6H_8BrN_4$, $[M+H]^+$), m/z observed 214.99251 ($C_6H_8BrN_4$, $[M+H]^+$).

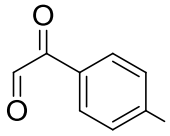


3-(5-bromopyridin-2-yl)-5,6-diphenyl-1,2,4-triazine (3-27). **3-26** (800 mg, 3.72 mmol) and benzil (782 mg, 3.72) were added to 15 mL of EtOH. The slurry was heated overnight at 80°C with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with EtOH and Et_2O resulting in a 91% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 8.97 (s, 1H), 8.62 (dd, 1H, J = 9.0,1.8 Hz), 8.07 (dt, 1H, J = 8.4, 3.0 Hz), 7.67 (d, 2H, J = 6.6 Hz), 7.64 (d, 2H, J = 7.2 Hz), 7.47-7.44 (m, 2H), 7.41-7.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 160.4, 156.8, 156.6, 151.8, 151.5, 139.9, 135.6, 135.3, 131.0, 130.1, 130.0, 129.7, 128.81, 128.79, 125.4, 123.6. MS (ESI) m/z calculated 389.04018 ($C_{20}H_{14}BrN_4$, $[M+H]^+$), m/z observed 389.02638 ($C_{20}H_{14}BrN_4$, $[M+H]^+$).

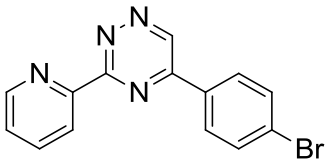


3-(5-ethynylpyridin-2-yl)-5,6-diphenyl-1,2,4-triazine (4-16). **4-15** (1.0 g, 2.57 mmol), dichlorobis(triphenylphosphine)palladium(II) (90 mg, 0.13 mmol), copper iodide (24 mg, 0.13 mmol), and anhydrous THF (13 mL) were combined and placed under nitrogen. Triethylamine (780 mg, 7.71 mmol) and ethynyltrimethylsilane (757 mg, 7.71 mmol)

were added and the reaction was heated overnight at 80°C. Reaction progress was monitored by TLC. After concentration *in vacuo*, the crude intermediate was purified by silica gel chromatography with gradient elution using EtOAc and hexanes. The intermediate was then dissolved in 8 mL of EtOH, 4 mL of MeOH, and 4 mL DCM. Potassium carbonate (0.1 eq) was added the reaction was allowed to stir overnight at room temperature with reaction progress monitored by TLC. Upon completion, the reaction was concentrated *in vacuo* and purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a yellow solid in 62% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.00 (s, 1H), 8.70 (d, 1H, J = 8.4 Hz), 8.01 (dd, 1H, J = 8.4, 1.8 Hz), 7.67 (d, 2H, J = 7.2 Hz), 7.63 (d, 2H, J = 7.2 Hz), 7.47-7.44 (m, 2H), 7.41-7.36 (m, 4H), 3.38 (s, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 160.4, 156.7, 156.6, 153.5, 152.2, 140.3, 135.7, 135.3, 131.0, 130.1, 130.0, 129.8, 128.80, 128.78, 123.5, 121.2, 82.8, 80.5. MS (ESI) m/z calculated 335.12967 ($C_{22}H_{15}N_4$, $[M+H]^+$), m/z observed 335.12451 ($C_{22}H_{15}N_4$, $[M+H]^+$).

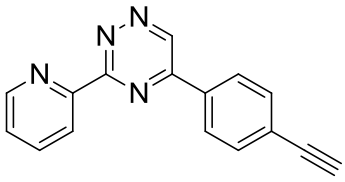


2-(4-bromophenyl)-2-oxoacetaldehyde (3-30). Selenium dioxide (1.67 g, 15 mmol) was suspended in dioxane (7.5 mL) and water (0.36 mL) and heated at 55°C until mostly dissolved. 1-(4-bromophenyl)ethanone (3 g, 15.0 mmol) was then added and the reaction was refluxed for 3 h. After cooling to room temperature, the reaction was filtered and the filtrate was concentrated *in vacuo*. The crude product was used in further reactions. MS (ESI) m/z calculated 212.95512 ($C_8H_6BrO_2$, $[M+H]^+$), m/z observed 212.986 ($C_8H_6BrO_2$, $[M+H]^+$).



5-(4-bromophenyl)-3-(pyridin-2-yl)-1,2,4-triazine (3-31). 3-30 (782 mg, 3.7 mmol) and picolinohydrazonamide (500 mg, 3.7 mmol) were added to 14 mL of EtOH. The slurry was heated overnight at 80°C with complete dissolution. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with EtOH resulting in a 46% yield. ^{1}H

NMR ($CDCl_3$, 600 MHz) δ 9.70 (s, 1H), 8.94 (d, 1H, J = 4.2 Hz), 8.68 (d, 1H, J = 7.8 Hz), 8.20 (d, 2H, J = 7.8 Hz), 7.96 (t, 1H, J = 7.8 Hz), 7.75 (d, 2H, J = 6.6 Hz), 7.51 (t, 1H, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 132.6, 132.48, 132.45, 132.1, 131.7, 131.5, 131.2, 130.2, 88.9, 87.5. MS (ESI) m/z calculated 313.00888 ($C_{14}H_{10}BrN_4$, $[M+H]^+$), m/z observed 313.00791 ($C_{14}H_{10}BrN_4$, $[M+H]^+$).



5-(4-ethynylphenyl)-3-(pyridin-2-yl)-1,2,4-triazine (3-32). **3-31** (594 mg, 1.9 mmol), dichlorobis(triphenylphosphine)palladium(II) (67 mg, 0.1 mmol), copper iodide (18 mg, 0.1 mmol), and anhydrous THF (7.5 mL) were combined and placed under nitrogen. Triethylamine (577 mg, 5.7 mmol) and ethynyltrimethylsilane (560 mg, 5.7 mmol) were added and the reaction refluxed overnight. Reaction progress was monitored by TLC. After concentration *in vacuo*, the crude intermediate was purified by silica gel chromatography with gradient elution using EtOAc and hexanes. The intermediate was then dissolved in a 0.5 M alcohol solution (2 EtOH : 1 MeOH) and 0.1 eq of potassium carbonate was added. The reaction was allowed to stir at room temperature and reaction progress was monitored by TLC. Upon completion, the reaction was concentrated *in vacuo* and purified by silica gel chromatography with gradient elution using EtOAc and hexanes to yield a pale brown solid in 44% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 9.72 (s, 1H), 8.95 (d, 1H, J = 4.8 Hz), 8.69 (d, 1H, J = 7.8 Hz), 8.30 (d, 2H, J = 8.4 Hz), 7.96 (dt, 1H, J = 7.8, 1.8 Hz), 7.71 (d, 2H, J = 8.4 Hz), 7.52–7.50 (m, 1H), 3.3 (s, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 163.0, 155.1, 153.1, 150.7, 145.3, 137.3, 133.7, 133.2, 127.9, 126.8, 125.9, 124.4, 82.9, 80.8. HRMS (ESI) m/z calculated 259.09837 ($C_{16}H_{11}N_4$, $[M+H]^+$), m/z observed 259.09788 ($C_{16}H_{11}N_4$, $[M+H]^+$).

General 1,2,4-Triazine-Accelerated Sonogashira Reaction. Iodobenzene (0.10 mmol), phenylacetylene (0.10 mmol), and triethylamine (0.10 mmol) were added to the triazine ligand (variable) and palladium source (variable) at 0.4 M, with respect to

substrates, in solvent. Reactions were heated 5.5 hours at 60°C, cooled to room temperature, and analyzed by HPLC using a C18 column with an isocratic mobile phase of 80% acetonitrile. Percent yields were determined using an external calibration curve.

Polystyrene-Supported Azide Method. Merrifield resin (14 mmol), sodium azide (42 mmol), tetrabutylammonium iodide (14 mmol) were added to 60 mL of NMP and 10 mL of water. Reaction was heated at 80°C overnight. After cooling to room temperature, the resin was filtered, washed with NMP, water, DCM, MeOH, and Et_2O, and air dried.

Heterogeneous CuAAC Method A. A solution of phenylacetylene (1.07 mmol), triethylamine (1.07 mmol), **2-17** (9.72 µmol), and tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (9.72 µmol) dissolved in 1 mL of ACN and 4 mL of DCE was added to the supported azide (0.972 mmol). Reaction was heated at 60°C for 4 h. After cooling to room temperature, the support was filtered and washed with DCE, water, MeOH, and Et_2O and air dried.

Heterogeneous CuAAC Method B. The corresponding alkyne-substituted 1,2,4-triazine (2.38 mmol), triethylamine (2.38 mmol), **2-17** (22.0 µmol), and tetrakis(acetonitrile-N)copper(I) tetrafluoroborate (22.0 µmol) were dissolved in 4.4 mL of ACN and 8.8 mL of DCE and added to the supported azide (2.16 mmol). Reaction was heated at 60°C for 18 h. After cooling to room temperature, an aliquot of the reaction solution was appropriately diluted and analyzed by HPLC to determine loading of the 1,2,4-triazine. The support was then filtered, washed with DCE, water, MeOH, and Et_2O and air dried.

Heterogeneous CuAAC Method C. A solution of 2-ethynyl pyridine (1.07 mmol), triethylamine (1.07 mmol), **2-17** (9.72 µmol), copper(II)tetrafluoroborate (9.72 µmol) dissolved in 1 mL of ACN and 4 mL of DCE was added to the supported azide (0.972

mmol). Reaction was heated at 60°C for 4 h. After cooling to room temperature, the support was filtered and washed with DCE, water, MeOH, and Et_2O and air dried.

Polystyrene-Supported Complexation Method. A 10 mM stock solution of the corresponding copper source in 1:1 ACN:DCE was prepared. Copper (0.0594 mmol) was added to the resin (0.054 mmol) and heated at 60°C for 2 h. An equivalent volume of copper stock solution was concentrated and analyzed by ICP-OES as a control. After cooling to room temperature, the resin was filtered, washed with ACN, DCM, MeOH, and Et_2O and air dried. The filtrate was concentrated *in vacuo*, analyzed by ICP-OES, and compared to the ICP-OES value of the aforementioned control solution.

Silica-Supported Complexation Method. A 10 mM stock solution of the corresponding copper source in ACN was prepared. Copper (0.0226 mmol) was added to the silica (0.0205 mmol) and heated at 60°C for 2 h. An equivalent volume of copper stock solution was concentrated and analyzed by ICP-OES as a control. After cooling to room temperature, the silica was filtered, washed with ACN, DCM, MeOH, and Et_2O and air dried. The filtrate was concentrated *in vacuo*, analyzed by ICP-OES, and compared to the ICP-OES value of the aforementioned control solution.

ICP-OES Methodology. The filtrate recovered from complexation reactions was redissolved in 2% nitric acid and 1% MeOH to a final concentration of 75 ppm copper (assuming all copper from the reaction was still present). Samples from the initial 10 mM copper stock solutions were treated in the same manner. All samples were heated at 60°C for 10 min and left undisturbed at room temperature overnight to ensure all copper was dissolved. Quantification of copper was determined using an external calibration curve ranging from 75 ppm – 1 ppm at a wavelength of 324.75 nm.

General Heterogeneous 1,2,4-Triazine-Accelerated CuAAC Reaction. Benzyl azide (0.25 mmol), phenylacetylene (0.25 mmol), and triethylamine (0.25 mmol) were added to the heterogeneous catalyst (1 mol% copper) at 0.2 M, with respect to substrates, in acetonitrile. Reactions were heated at 60°C for 4 h, cooled to room temperature, and filtered to remove catalyst. The filtrate was concentrated *in vacuo* to yield 1,2,3-triazole as a white solid.

Recyclability of Heterogeneous Catalysts. After CuAAC reaction was complete and filtered, the catalyst was rinsed with dichloromethane, methanol, and ether. Catalyst was air dried and reused without additional treatment.

Copper Retention of Heterogeneous Catalysts. A solution of 2% HNO_3 (4 mL) and MeOH (0.04 mL) were added to isolated 1,2,3-triazole products. After heating at 60°C for approximately 10 min, the suspension was left standing at room temperature overnight. After filtration, the filtrate was analyzed by ICP-OES in order to calculate the percent of copper retained by the catalyst. Copper was quantified using an external calibration curve ranging from 75 ppm – 1 ppm at a wavelength of 324.75 nm.

Chapter 4.
Design and Synthesis of Metalloenzyme Inhibitors

4.1. Background and Significance of Histone Deacetylase (HDAC)

Cancer has been a difficult target for researchers, owing to its ability to avoid therapeutic strategies, propagate through the body, and circumvent normal cell cycle regulatory processes.[98] Through years of research much information has been gained about causes and effects of cancer, but a cure for all cancer subtypes is still out of reach.

Mutations to healthy cells in which the genetic information is permanently changed is one target of cancer therapy. Another is the regulation of non-mutated DNA through epigenetic modifications. Epigenetics look at inheritable changes in gene expression without altering the coding sequence.[99] Epigenetics encompasses a wide range of regulating mechanisms, including DNA methylation, post-translational modification of histone proteins,[99] chromatin remodeling,[100] transcription factors,[101] and noncoding RNA.[102] Post-translational modification of histone and non-histone proteins has drawn much attention over the past 15 years, specifically with regards to histone deacetylase enzymes. Why are histone proteins important and how do chemical modifications effect regulation of DNA?

Histones are chromatin-associated proteins where chromatin is the tightly packaged genetic material made of repeating nucleosome units. This condensed package regulates transcription, DNA replication and repair, mitosis, and apoptosis.[99] Within each nucleosome unit is DNA that has been wound around a histone octamer, where histones are small basic proteins, containing an abundance of lysine and arginine residues (Figure 4.1). The location of the lysine tails is of upmost importance in regulating transcription. The lysine side chains extend toward the outer edges of the nucleosome, leaving them available for an array of post-translational modifications, such as acetylation, methylation, or phosphorylation, to name a few. Histones can be either acetylated or deacetylated, in processes controlled by the action of HAT (Histone Acetyl Transferase) or HDAC (Histone Deacetylase) enzymes. As the name suggests, one is responsible for transferring the acetyl group to the lysine of the histone protein and the other removes the acetyl group. The two states control the accessibility of DNA and therefore, transcription.

In the deacetylated form (Figure 4.1), the lysine residues will be positively charged amines at physiological pH. This will lead to an electrostatic interaction with the negatively charged phosphates of the DNA backbone. This causes the chromatin to condense, leaving it inaccessible to transcription factors. In the acetylated form, the lysine residues are now neutral weakening the interaction between DNA and histone. The chromatin adopts a relaxed state, allowing space for transcriptional factors, and thereby activating transcription. Although the name implies the deacetylase activity only occurs at histone proteins, there are at least 50 non-histone substrates which have been linked to the HDAC enzyme.[103] The equilibrium between these acetylation and deacetylation mechanisms is required for the delicate balance of a host of nuclear processes. The improper regulation has been implicated in a variety of cancer subtypes,[104] as well as neurodegenerative diseases,[105] and immune disorders.[106]

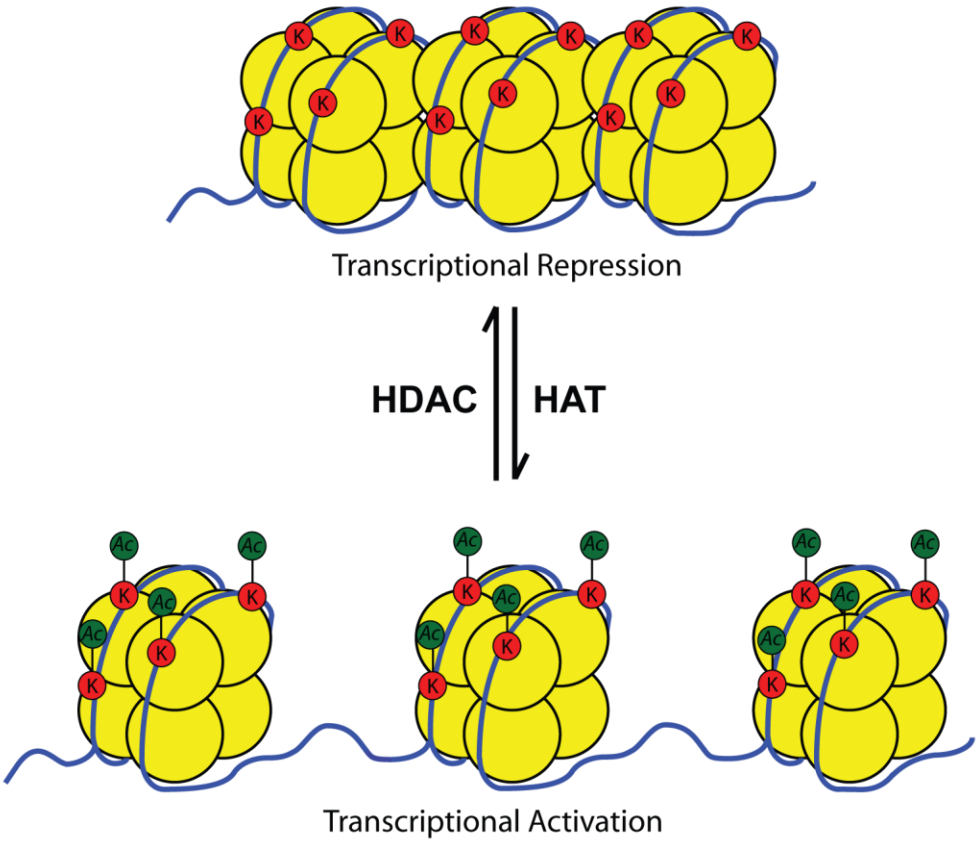


Figure 4.1 Equilibrium between transcriptional repression and transcriptional activation.

Overexpression of HDACs has been found in a variety of cancers. This can lead to silencing of guardian proteins and mediate tumor cell proliferation.[107] Studies on inhibition of HDACs have shown a promising ability to affect processes that have gone awry in neoplastic cells, including an ability to induce cell cycle arrest, or induce apoptosis by expression of tumor suppressor proteins.[107,108]

For this reason, much research has been devoted to development of histone deacetylase inhibitors, with end goals as promising pharmaceuticals, especially in the fields of anti-cancer agents, neuroprotective agents, or anti-inflammatory agents. The first interest in histone deacetylase inhibitors (HDACi) was sparked about 35 years ago during research to elucidate why DMSO caused terminal differentiation in erythroleukemia cells.[109,110] Since that time a number of researchers have developed interest in design, synthesis, and studies of histone deacetylase inhibitors.

In order to target HDACs effectively, it is important to know about their structure and physiological relevance. Fortunately, some structural and functional knowledge has been determined. Eighteen known HDAC isoforms exist and they are divided between four structural categories depending on their sequence similarities and cellular locations. Class I, II, and IV are all zinc dependent while Class III is mediated by nicotinamide adenine dinucleotide (NAD+) and often referred to as sirtuins. Sirtuins will not be focused on for this work. Class I includes HDAC isoforms HDAC-1,-2, -3, and -8, and are found within the nucleus of many cell types. Class II is further broken down into IIa (HDAC-4,-5,-7,and-9) and IIb (HDAC-6,-10). Class IIa are able to shuttle between nuclear and cytoplasmic locations while IIb are primarily located in the cytoplasm. Class IIb is unique due to the presence of two active enzyme sites. Class IV consists of HDAC-11 and exhibits properties of both Class I and II HDACs. Very little is known about this isoform.

The specific functions of each HDAC are not completely understood, but mammalian studies using knockout and transgenic mice, along with siRNA techniques have highlighted some key roles for the various isoforms. The information about the various HDAC isoforms is highlighted in Table 4.1.

Table 4.1 Features of the various isoforms of HDAC Class I, II and IV. Modified from Fattori, et al.,[111] Marks, et al.,[112] and Oyelere, et al.[98]

	Primary Location	Amino Acid Residues	Physiological role	Crystal Structure
HDAC1	nucleus	483	proliferation, development, gene regulation	0
HDAC2	nucleus	488	cardiac development, proliferation	1
HDAC3	nucleus	428	regulation of mitosis	0
HDAC8	nucleus	377	muscle contraction	21
HDAC4	nucleus / cytoplasm	1084	bone development, neuronal cell death mediator	8
HDAC5	nucleus / cytoplasm	1122	cardiac development	0
HDAC7	nucleus / cytoplasm	855	regulation of apoptosis in developing T-cells	3
HDAC9	nucleus / cytoplasm	1011	cardiac development	1
HDAC6	cytoplasm	1215	controls status of tubulin, microtubules, and HSP90	0
HDAC10	cytoplasm	669	NA	0
HDAC11	nucleus	347	NA	0

Some structural information regarding the zinc-dependent HDACs has become available in the past ten years. The first structural insights were gained from the crystal structure of a histone deacetylase-like protein (HDLP) complexed with inhibitors and isolated from a bacteria species with approximately 35% similarity to human HDAC-1.[113] The first human HDAC structure to be co-crystallized with inhibitors was HDAC-8. From the table above, it can be seen that an abundance of HDAC-8 crystal structures have been determined while some HDACs such as HDAC-1, HDAC-6, have shown limited success in obtaining crystal structures, although structures of bacterial homologues exist. The reason for this disproportionality is that most HDACs occur as high molecular weight multiprotein complexes, rather than individual monomeric proteins making it difficult to obtain a crystal structure of the HDAC protein.[111]

From the crystal structure of HDAC-8 and HDLP, some general features of HDAC active sites have been inferred. The active site contains a hydrophobic tunnel of 11-14 Å, with the exact length varying between isoforms. Contained within this tunnel are residues which are conserved across the class I HDACs (Phe152, Phe208, His180, Gly151, and Tyr306). The catalytic site lies at the bottom of the tunnel containing a Zn^{2+} ion coordinated to Asp178, His180 and Asp267.[114] Tyrosine, histidine, and aspartic acid residues within the catalytic site interact with one another, substrate, or inhibitors. The proposed interaction between the zinc ion, amino acids, and either the substrate or a known inhibitor is shown in Figure 4.2.

The structural insight gained has been used recently to develop the new inhibitors for HDAC activity. This was not always the case in designing inhibitors. Prior to the availability of crystal structure evidence, several inhibitors had been designed or isolated from natural products. The design of early inhibitors, coupled with the structural information known, has allowed researchers to investigate structure-activity relationships between a variety of structural scaffolds.

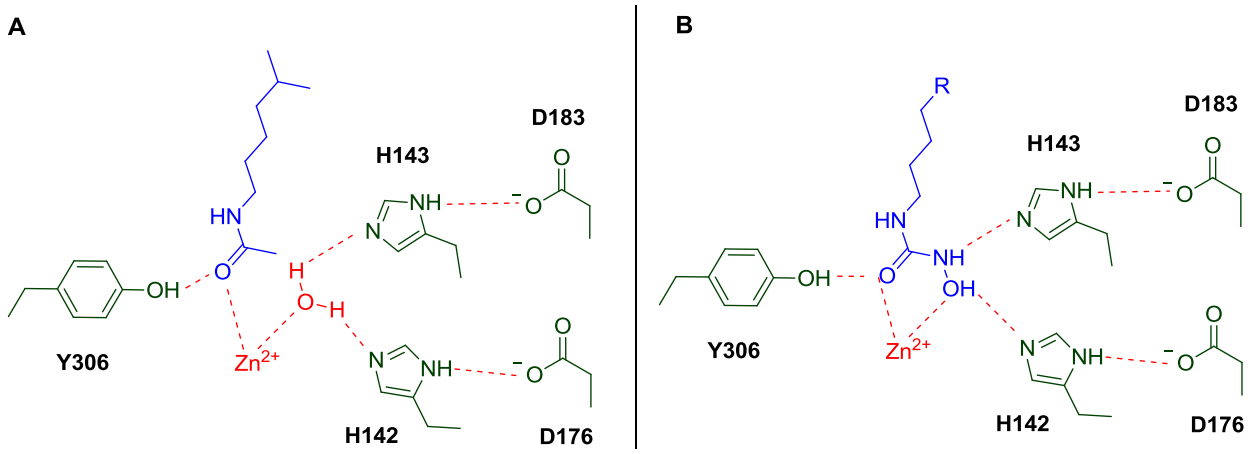


Figure 4.2 Representation of binding with A) substrate (proposed) and with B) hydroxamate inhibitor. Amino acids are shown in green, substrate, or hydroxamate inhibitor in blue, and interactions in red.[114]

4.2. Histone Deacetylase Inhibitors (HDACi)

There is a common theme in a large majority of HDAC inhibitors to begin with a common scaffold, consisting of a zinc-chelating functional group, linker region to occupy the hydrophobic tunnel, and a surface recognition unit, or cap group (Figure 4.3). This scaffold mimics the natural substrate, an acetylated lysine residue. The zinc binding group can further divide inhibitors into hydroxamic acid and non-hydroxamic acid inhibitors. Some non-hydroxamic acid inhibitors include small carboxylic acids, benzamides, ketones, cyclic peptides, and depsipeptides. A collection of inhibitors is shown in Figure 4.4. A significant amount of the literature and research has focused on hydroxamate inhibitors, but an increasing amount of work has surfaced in an attempt to determine other zinc binding groups of comparable potency and increased selectivity.

A comprehensive list of molecules designed and synthesized for HDAC inhibition is outside the scope of this work, and has been the focus of other reviews,[99,108,111] but a brief highlighting of some of the common or clinically relevant molecules will be discussed here.

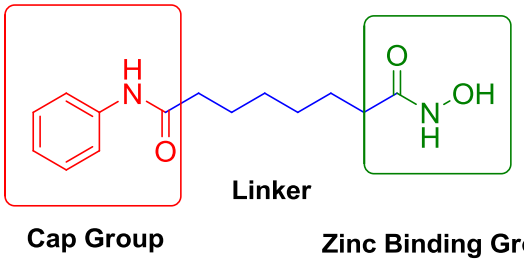


Figure 4.3 The general regions for consideration in design of HDAC inhibitors

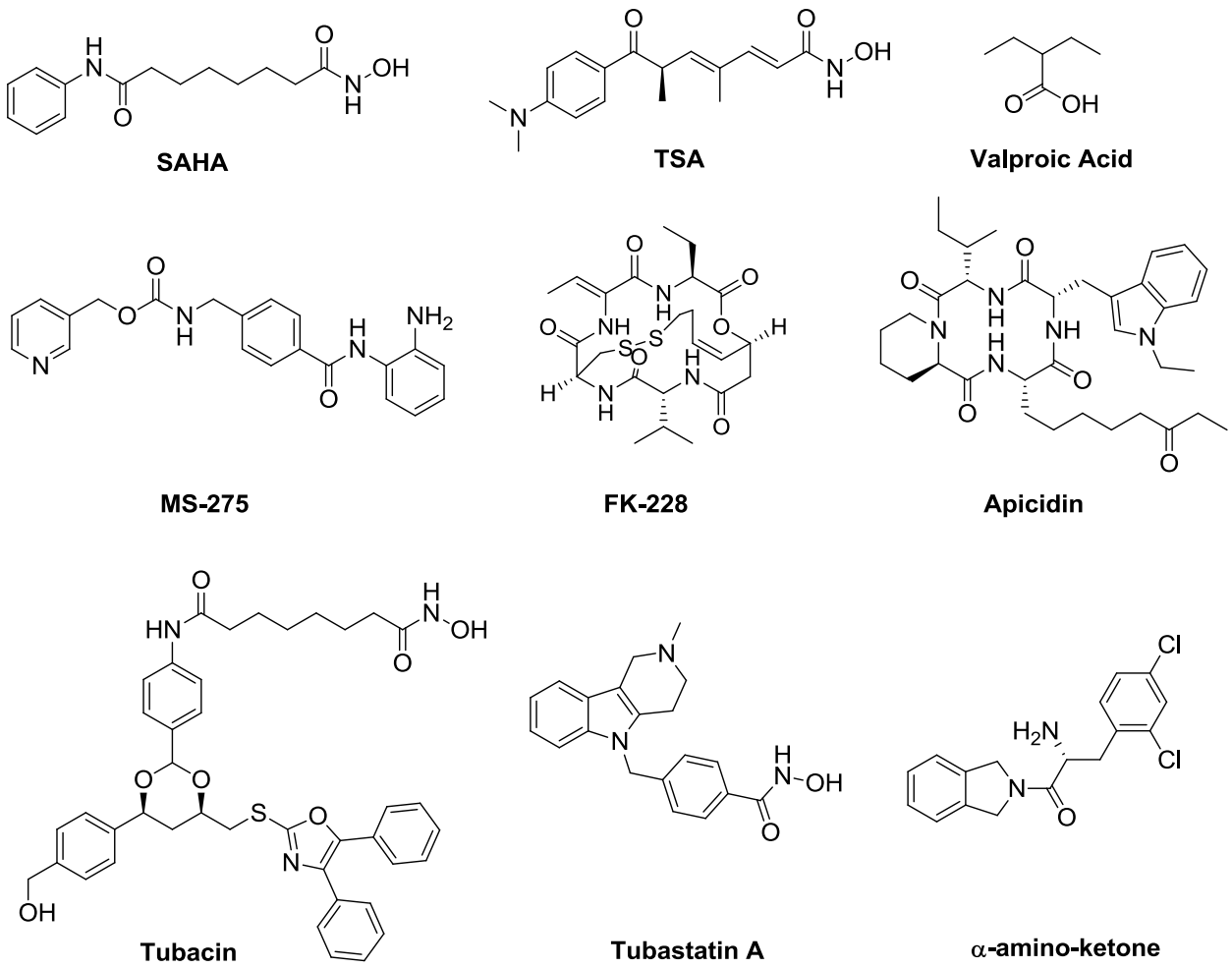


Figure 4.4 Selected HDAC inhibitors

Some of the most recognizable inhibitors are SAHA (suberoylanilide hydroxamic acid) and TSA (trichostatin A). SAHA is one of many HDAC inhibitors that have entered clinical trials. SAHA is unique in that is one of two HDAC inhibitors approved by the FDA for treatment of cutaneous T-cell lymphoma (CTCL) with the other being FK-228.[115] SAHA is the standard by which most HDAC inhibitors are compared and has been mimicked and modified in efforts to discover potent and selective inhibitors. Typical modifications involve changing the linker region, in distance by adding methylene groups, in rigidity by adding unsaturation, aromatic groups, or cinnamoyl linkers, or in hydrophobicity, by modifying linker substituents. Reviews comparing the activity of the various modified structures have been written.[111,116] The cap group is also amenable to modifications. This area seems to be accepting of the most diversity. It is not restricted by size of the tunnel, as the linker region is, allowing extensive substitution. By these modifications, the activity of the molecule can be tuned. Although some more potent molecules have been discovered, a downfall to this is the decrease in isoform selectivity, or off-target toxicity. TSA, a natural product, is a strong nanomolar inhibitor that suffers from the drawback of being too potent for multiple isoforms.

In general, most of the inhibitors thus far have been pan-inhibitors unable to distinguish between the multiple isoforms. SAHA and TSA fall into this category. Because of the conserved sequence homology between HDAC isoforms, design and synthesis of isoform selective inhibitors has been difficult. Some inhibitors have emerged to have a slight selectivity for one class over the others. Class specificity is the first target of selectivity, and then isoform specificity. FK228 (Figure 4.4), the other FDA approved HDACi, is a prodrug depsipeptide that releases an alkyl thiol in vivo by reduction of the disulfide bond[117] and has shown selectivity for Class I HDACs. MS-275 and other related benzamides have also shown promise as Class I selective HDAC inhibitors. The selectivity has been a trade-off for potency as the IC_{50} now resides in the micromolar region instead of nanomolar region of hydroxamic acids. With the decreased potency, there has also been a decrease in the off-target side effects such as cardiac toxicity.[98]

Tubacin, although not a good drug candidate, (Figure 4.4) was found to be an inhibitor of HDAC6 and also led to a linking of HDAC-6 to tubulin deacetylase activity. This

paved the way for Tubastatin A to be discovered with a nanomolar inhibition and 1000-fold selectivity for HDAC6.

In addition to hydroxamic acids, carboxylic acids such as butyric acid and valproic acids were observed to inhibit cancer cell growth and induce apoptosis, but in general carboxylic acids were not particularly potent (requiring millimolar concentrations), not selective, and had low bioavailability.[99] The downfalls to HDAC inhibitors thus far are their ineffectiveness against solid tumors. Majority of patients did not respond to treatment and side effects were high. Some patients did experience stabilization of their disease, so there is opportunity for inhibitors to be used in combination with other chemotherapeutic drugs for treatment of solid tumors.

Some of the keys to overcoming the barriers that have prevented more HDAC inhibitors from progression through clinical trials, are targeted therapy and selective delivery.[98] This is of course easier to suggest than it is to carry out. If HDACs could be targeted by an inhibitor for a single isoform, the effectiveness should increase and off-target toxicity should decrease. Designing tools to selectively deliver these molecules to the location of interest is another pathway that should be explored.

4.3. Strategies for Synthesis

The use of triazoles for biological applications has greatly emerged with the discovery of copper-catalyzed azide-alkyne cycloaddition. The field of HDAC inhibitors is no exception. As a mimic of SAHA, three independent research groups incorporated a triazole in order to increase the affinity in the inhibitor cap region with the HDAC enzyme active site. The major goal was to create isoform selectivity, since a major downfall of most HDAC inhibitors is a lack of specificity between various HDAC isoforms. Researchers at University of Illinois, Chicago[25] reconfigured the cap group of SAHA by incorporating a triazole attached to the phenyl group of SAHA. They examined both 1,4 and 1,5-disubstituted triazoles (Figure 4.3, **4-1**) in their efforts to find selective and potent HDAC inhibitors. Two additional research groups decided to substitute a 1,2,3-triazole as an isostere of the amide bond (**4-2**) in order to increase affinity in the cap region of HDAC enzymes.[118,119] Both references report similar findings that replacement

of the amide with a triazole led to an increase in the HDAC inhibition. Further modifications to the cap group revealed even more potent inhibitors. These compounds still contained a hydroxamic acid as the zinc binding group. While hydroxamic acids are among the most common zinc binding group used in HDAC inhibitors, they can suffer from short clearance in vivo and often lack isoform specificity. There are many other zinc metalloenzymes so lack of specificity could lead to adverse side effects. This has led researchers to investigate non-hydroxamic zinc binding groups, although finding a similar strong affinity group is not an easy task.

To this end, researchers manipulated an oxazole scaffold, which was shown to have HDAC and antitumor activity,[120] to incorporate a 1,2,3-triazole (**4-3**).[121] This molecule doesn't have a strict zinc binding functionality. While not screened directly against HDAC activity, it did show antiproliferative activity against several cancer cell lines. The final inspiration for this work incorporated the triazole into the linker and moved it in closer proximity to the hydroxamic acid. While the triazole adjacent to the hydroxamic acid was not particularly active, moving the triazole within the linker displayed similar activity as SAHA.[122]

From the previous work and as a further modification of this scaffold, we decided to incorporate a 1,2,3-triazole into the zinc binding domain. By examining electrostatic potential maps of hydroxamic acids in relation to the endogenous lysine substrate and to 1,2,3-triazoles (Figure 4.6), it was hypothesized that the latter could serve as an efficient, stable replacement of the previously known zinc binding group. The nitrogen of the triazole could serve as a hydrogen bond acceptor similar to that of the hydroxamic acid carbonyl. The alcohol appended to the triazole could serve as a hydrogen bond donor or acceptor similar to that of the hydroxamic acid oxygen. Due to the structural similarities and increased stability of 1,2,3-triazoles, their incorporation into a histone deacetylase inhibitor seemed a favorable approach.

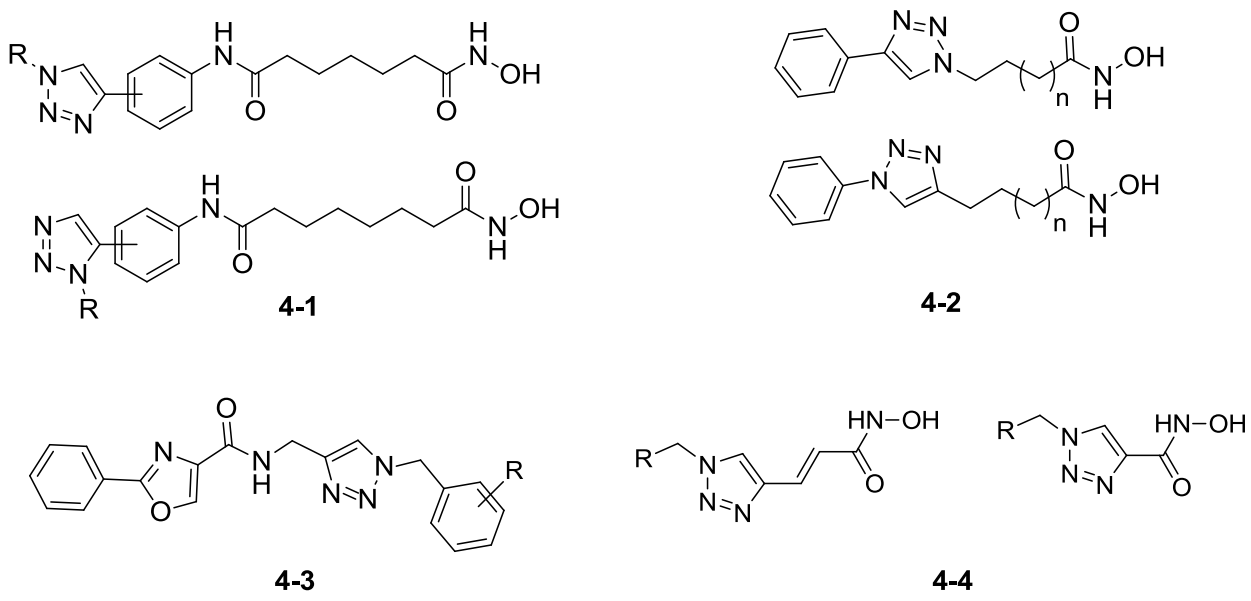


Figure 4.5 Molecules synthesized for HDAC inhibition involving 1,2,3-triazoles.

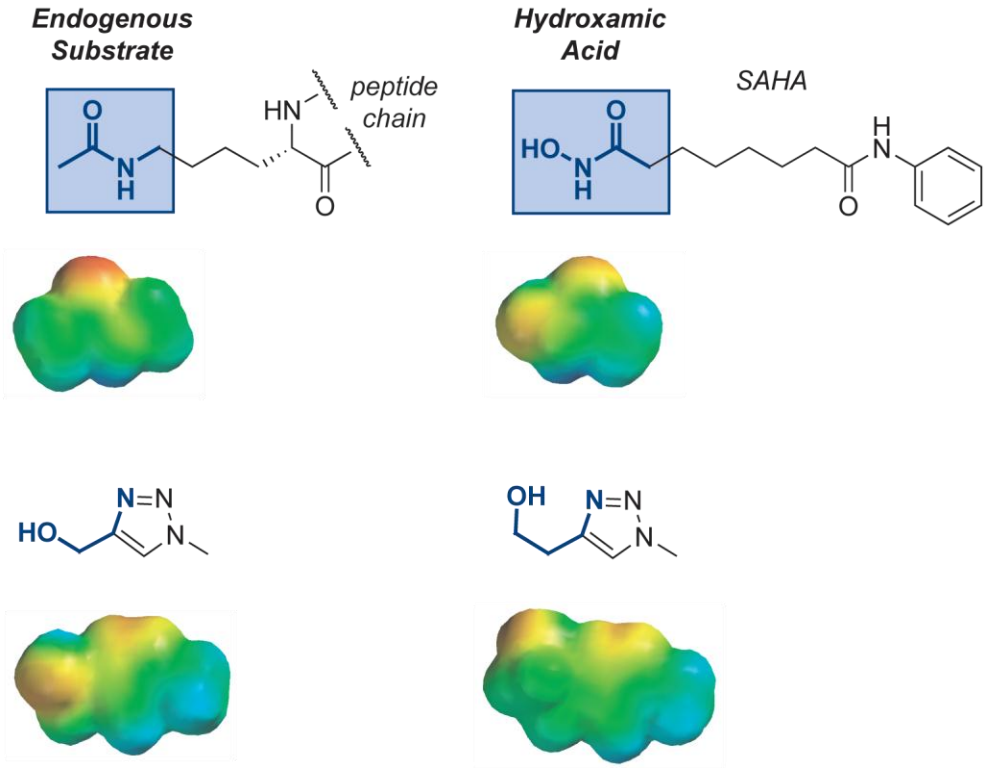


Figure 4.6 Electrostatic potential map comparing 1,2,3-triazoles to hydroxamic acids.

4.3.1. Solid Phase Approach

The original synthetic scheme utilized solid phase organic synthesis. The envisioned plan was to construct the HDAC inhibitor beginning with the zinc binding group and concluding with the cap group. Three different resins (Figure 4.7) were to be employed: Wang resin, Weinreb amide resin, and Fmoc-Hydroxylamine 2-chlorotrityl resin with each resulting in a different functional group upon cleavage (alcohol or acids, aldehydes, and hydroxamic acids). Various alkynes were chosen to anchor to the solid support. Known protocols were chosen to attach propargyl alcohol, homopropargyl alcohol, and propionic acid to Wang resin.[123] This resulted in terminal alkynes on a solid support. Two consecutive CuAAC reactions were then carried out first with a diazide and then with an aryl alkyne. This scheme could potentially be rapidly modified with various diazides to adjust the linker length between cap group and zinc binding group. In addition the aryl alkyne could be easily changed to rapidly assemble a small library of compounds. Upon cleavage from the solid support, this would result in terminal alcohols or acids appended to a 1,2,3-triazole. This scheme is illustrated in Scheme 4.1.

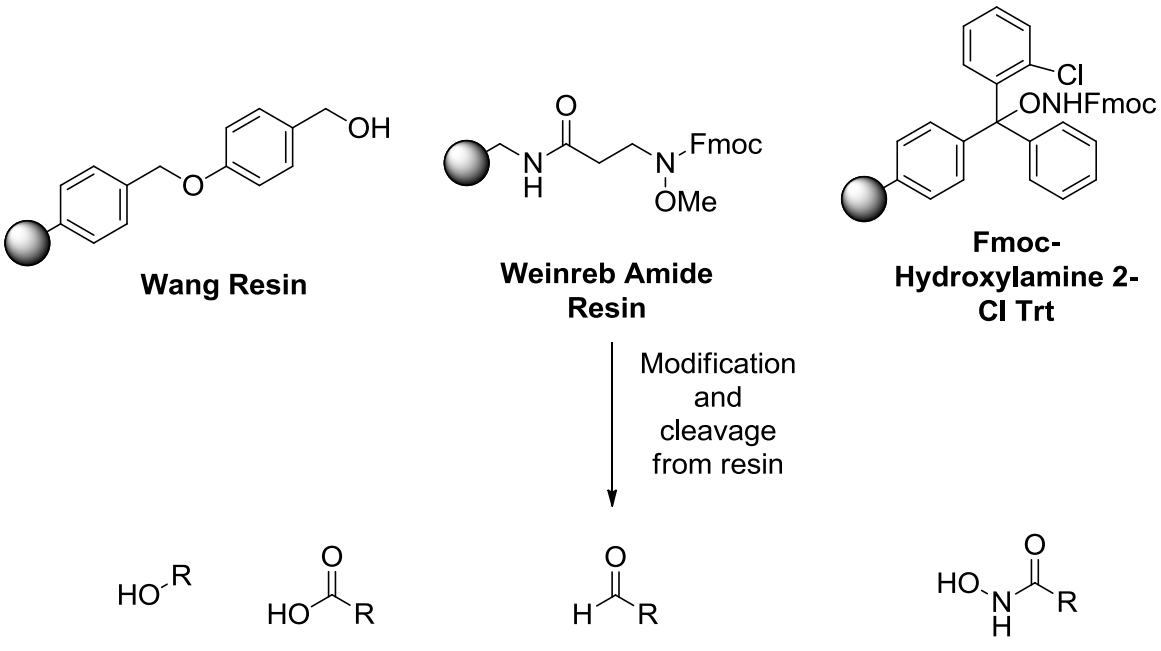
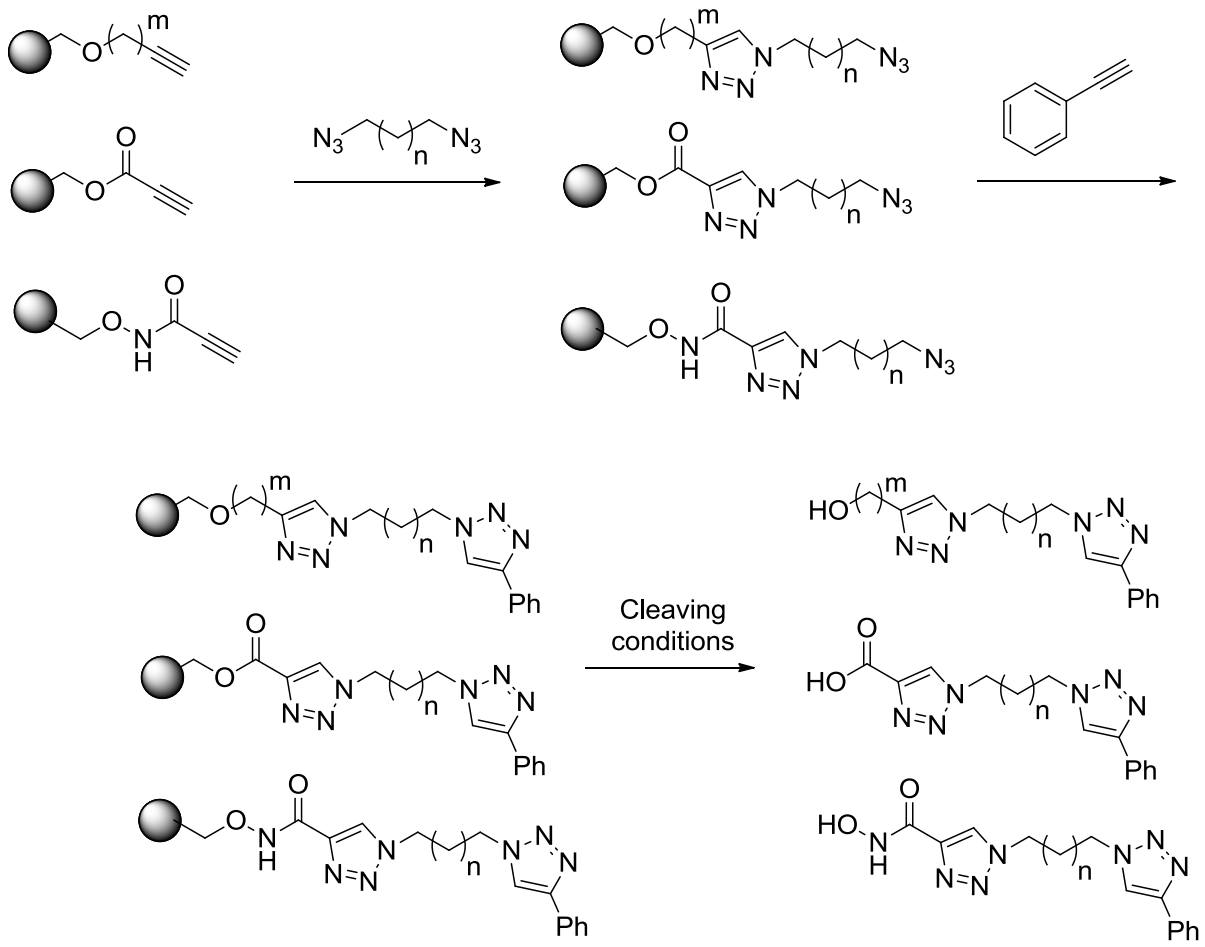


Figure 4.7 Different solid phase resins for HDAC inhibitor synthesis.



Scheme 4.1 Solid phase synthetic scheme for HDAC inhibitors.

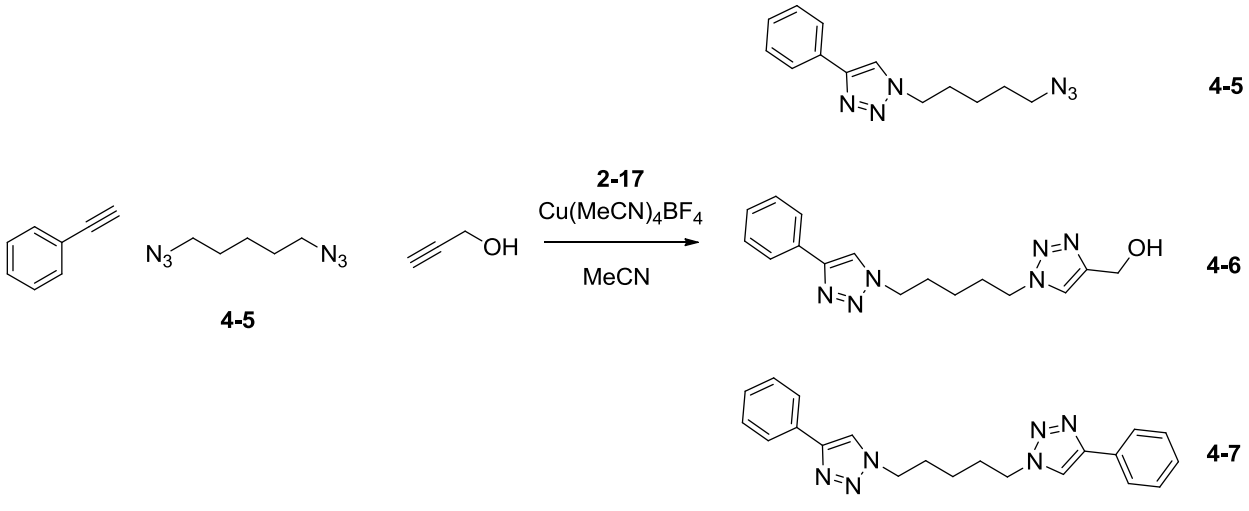
In addition, synthesis of a hydroxamic acid substituted triazole was also planned using 2-chlorotrityl resins and propionic acid. The CuAAC steps would proceed as described above to furnish the desired compound. Finally, an aldehyde substituted triazole was proposed by using Weinreb amide resin loaded with propionic acid. As a control, a traditional hydroxamic acid lacking a 1,2,3-triazole was also envisioned via a solid phase route.

Although some of the solid phase syntheses appeared promising, as confirmed by TLC, HPLC, and HRMS, difficulties arose in obtaining a sufficient amount of pure material. Products cleaved from the solid support contained trace byproducts that would need to be removed either through recrystallization or column chromatography. This did not result in enough product material for full characterization and enzyme testing. It was

perceived to be a more expensive route to scale up the solid phase approach, and therefore, a complementary solution phase approach was designed.

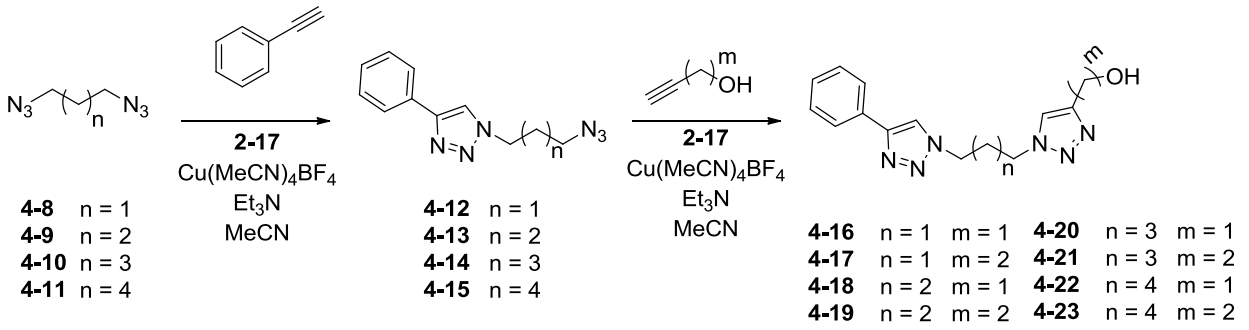
4.3.2. Solution Phase Approach

The solution phase approach would utilize many of the same precursors used for the solid phase approach, but it would eliminate the loading of propargyl alcohols onto a solid support. This method could be readily scaled up and purification performed after each step if desired. Although this resulted in a longer synthesis, the solution phase route allowed for the synthesis of pure material in a quantity that could be fully characterized and tested. Two routes were planned in order to obtain the final product. The initial solution phase scheme was to synthesize the desired HDAC inhibitor in a one-pot reaction involving two alkynes and one diazide linker (Scheme 4.2). This approach successfully led to the formation of the desired product (**4-6**) but also resulted in two additional byproducts, the unreacted triazole azide (**4-5**) and the symmetric bistriazole (**4-7**). This decreased the yield of the desired product to 38%. Separation of the three products was achieved by column chromatography and identity of the three products formed was confirmed by NMR and HRMS.



Scheme 4.2 One pot procedure for synthesis of HDAC inhibitors.

A two-step sequence was also investigated simultaneously (Scheme 4.3). Excess diazide was reacted with phenylacetylene to obtain the desired azido triazole. This was then reacted with propargyl alcohol to produce the desired product. This route involved two steps with chromatography after each one. This allowed for better control and less side product formation so this route was taken to synthesize a small library of compound in which the linker diazide length and alkynol were varied.



Scheme 4.3 Two-step synthesis for HDAC inhibitors containing 1,2,3-triazoles.

Through this route we were able to synthesize a series of 8 compounds containing 1,2,3-triazoles in both the cap group region as well as in the zinc binding group domain. The initial step of converting dibromides to diazides was high yielding (> 86%). The synthesis of monoazide triazoles resulted in lower yields ranging from 27% of **4-12** to 68% of **4-14**. The decreased yields resulted from formation of the bistriazole (**4-7**) where phenylacetylene reacted with both of the azides on a single alkyl linker. For the bistriazoles, **4-16** to **4-23**, better isolated yields resulted, since only one azide was available to react. Initially, column chromatography was used to separate the product triazoles from any residual copper or other slight byproducts. Upon scale-up, this led to some difficulties in that the bistriazoles were not very soluble in organic solvents such as DCM or MeOH, leading to broad elution from silica gel chromatography. Since the triazoles were not soluble, but the copper salts were, we could isolate the triazole product by filtration in both higher yield and purity than was observed from column chromatography. By filtration we recovered 82% yield of **4-16**. Yields of compounds

isolated by chromatography ranged from 48% (**4-17**) to ~80% (**4-18**, **4-19**, **4-20**, **4-23**). Yields over 3 steps ranged from 18% for compound **4-20** to 51% for compound **4-18**.

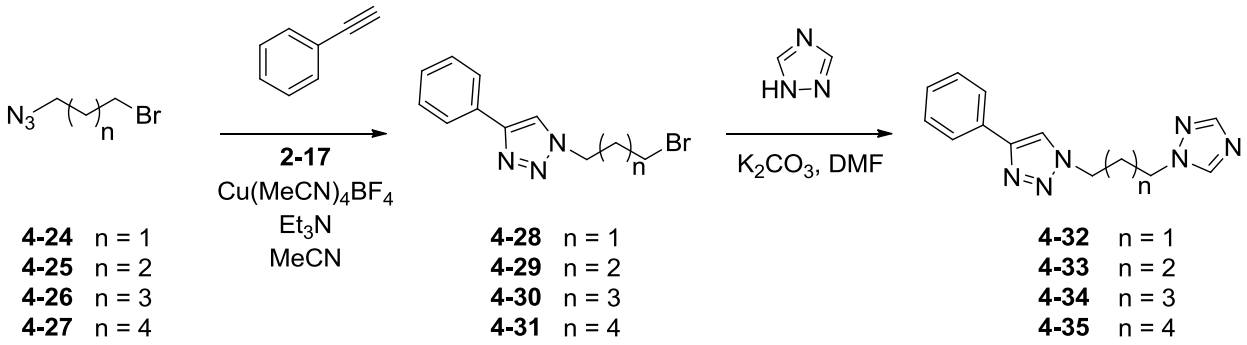
This solution phase scheme allowed access to the alcohol substituted triazoles, but did not allow for synthesis of the aldehyde or acid substituents for the triazole zinc binding group. Due to the primary alcohol functional group appended to the triazole, it was hypothesized that this could be oxidized to the corresponding acid or aldehyde, depending on the reagents employed. A series of test reactions were conducted with a simplified triazole (**3-3l**) before using the more complicated bistriazoles (**4-16** to **4-23**). Two methods were utilized: Jones Reagent to oxidize the alcohol to the carboxylic acid or Swern oxidation to afford the aldehyde. Both methods resulted in consumption of the starting material in the model systems. When applied to the more complicated bistriazoles, neither of these methods proved to be effective leading to recovery of only starting materials. Additional oxidants, such as TEMPO and oxone, were also tried, but these failed. The low solubility of these bistriazoles in organic solvents is a possible reason that the oxidations failed to work on this system. Because of the struggles with this chemistry, this route was abandoned and the focus moved to other potential zinc binding domains.

Since the 1,2,3-triazoles had been successfully synthesized, we decided to incorporate a 1,2,4-triazole to test their ability to function as a zinc binding group. Both are five membered rings with three nitrogen atoms, but we felt that the difference in geometry might make a difference in the zinc binding ability. Furthermore, work by lab mates had previously explored synthesis of 1,2,4-triazoles, and several intact 1,2,4-triazoles were commercially available. This synthesis could rely on some of the starting materials used for the 1,2,3-triazole synthetic scheme so it was a logical progression.

In this scheme (Scheme 4.4), dibromides were used for a linker as before, but were converted to monoazides instead of diazides in the initial step. The monoazides were not purified prior to use, so a lower overall yield was observed in these reactions. A CuAAC reaction resulted in an aryl triazole with a terminal bromide at the end of an alkyl linker (compounds **4-28** to **4-31**). These compounds were isolated in ~30% yield, with the low yield attributed to using the crude azidobromoalkane. The bromide was then

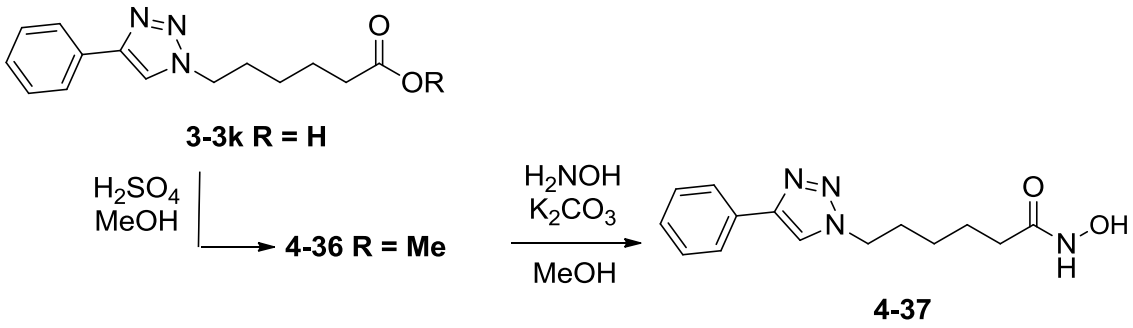
displaced by a suitable nucleophile. In this case, commercial triazoles were chosen as the nucleophiles. Previous work in our group had illustrated this reaction was indeed regioselective, reacting preferentially at the N1, and using the intact triazole was easier than synthesizing the 5-membered heterocycle.

This led to the quick synthesis of 4 additional molecules containing both 1,2,3- and 1,2,4-triazoles using commercially available 1,2,4-triazole. The displacement of the bromide with 1,2,4-triazole resulted in the product in 76 – 84% yield, with the 4-carbon linker resulting in only 44% yield. We also tried using 3-amino-1,2,4-triazole as the nucleophile, but this led to a 50/50 mixture of products by NMR. The two products observed were regioisomers as two nucleophilic sites existed in the starting material.



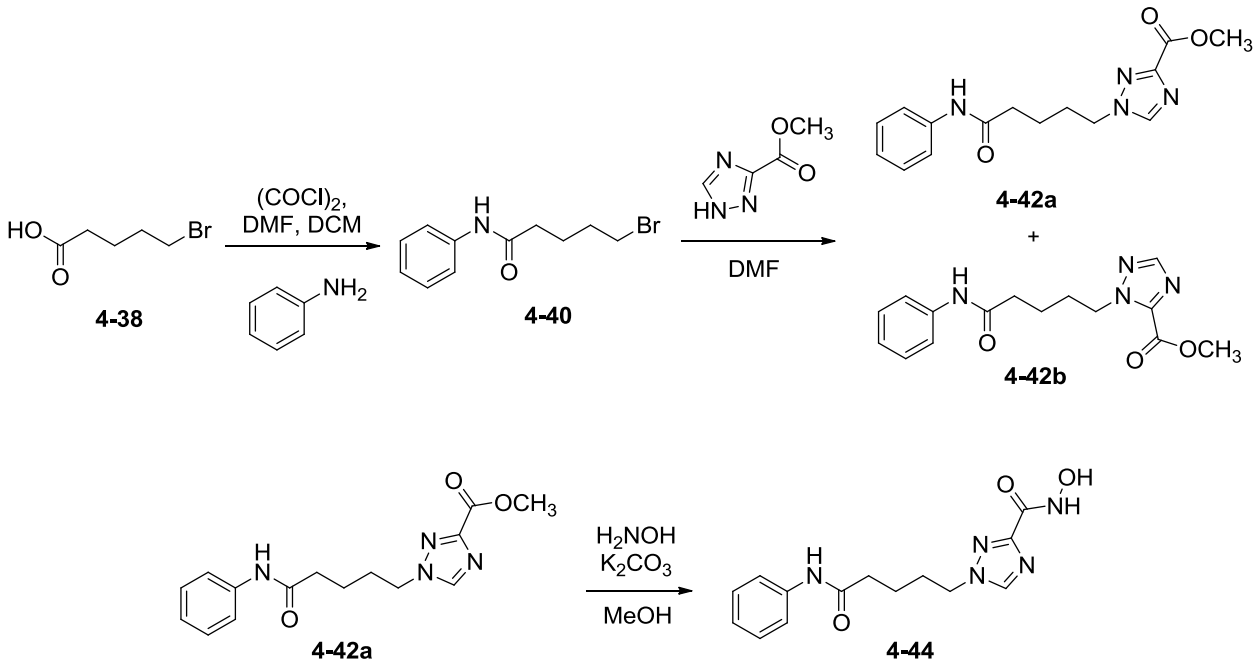
Scheme 4.4 Synthetic scheme for synthesis of 1,2,4-triazole containing HDAC inhibitors.

As a final compound in this series, we chose to synthesize a previously published HDAC inhibitor molecule containing the triazole in the cap region with the standard hydroxamic acid as the zinc binding group to serve as a synthesized positive control (Scheme 4.5). The precursor for this molecule had been synthesized during the catalytic studies from Chapter 3. The acid functional group was converted into the methyl ester in 85% yield, and conversion to the hydroxamic acid under MW irradiation resulted in the product in 60% yield.



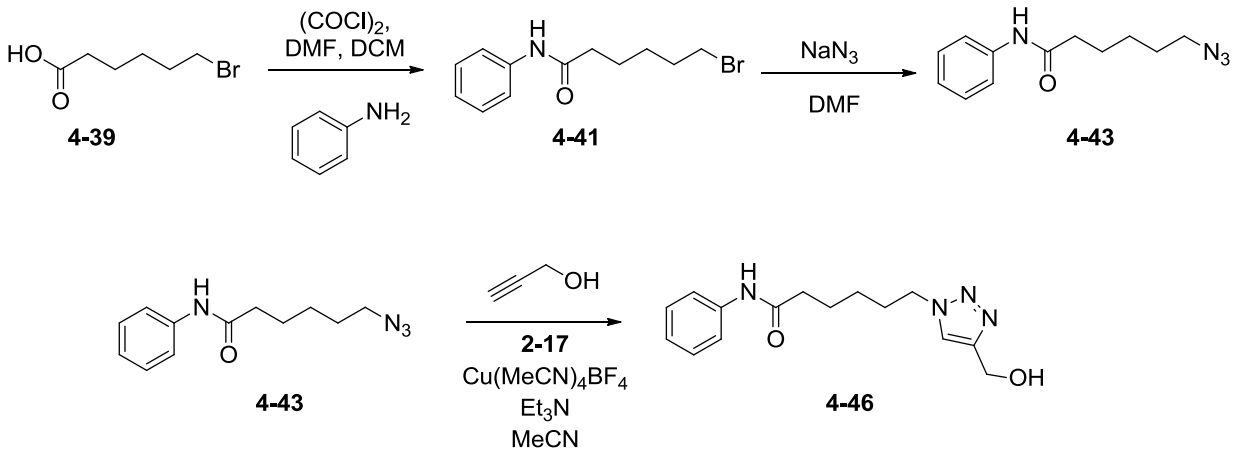
Scheme 4.5 Synthesis of previously published HDAC Inhibitor.

It was also of interest to explore other 1,2,4-triazoles positioned in the zinc binding domain. For the next series of molecules, we decided to leave the amide bond of SAHA intact, and instead just manipulate the zinc binding domain (Scheme 4.6). First, the amide bond was synthesized in 82% yield by reacting aniline with 5-bromovaleric acid that had been converted to the more active acyl chloride with DMF and oxalyl chloride. The resultant bromoanilide could then be subjected to a solution of the desired triazole with K_2CO_3 in DMF. The triazole chosen as the nucleophile was 3-methyl-1,2,4-triazole carboxylate. This reaction proceeded to produce the 1,2,4-triazole with a methyl ester appended to the three position. Two regioisomers were possible, but only one of the two was isolated in 39% yield. The position of the substituent was confirmed by 2D NMR, by examining the HMBC correlations. The lower yield for this step can be attributed to difficulty in removing all the DMF from the product. In the final step, methyl esters are easily converted to the hydroxamic acid using hydroxylamine hydrochloride and K_2CO_3 in MeOH under microwave conditions. This reaction proceeded smoothly and product was easily isolated with an aqueous workup in 58% yield. Prior experience with 1,2,4-triazoles had shown that the functional groups attached are not nearly as reactive when compared to other aromatic systems. We hoped to use this to our advantage to create a hydroxamic acid-like compound that could match the potency, but with decreased toxicity as compared to standard hydroxamic acids.



Scheme 4.6 Synthesis of 1,2,4-triazole hydroxamic acid compound.

Since the 1,2,4-triazole compound above was synthesized using a bromoanilide, it was decided that a similar process should be used to make a 1,2,3-triazole compound containing an amide in the cap region. This would be complementary to the 1,2,4-triazole compound, so the activities could be directly correlated. The amide bond was made between aniline and 6-bromohexanoic acid using DMF and oxalyl chloride in 85% yield. The bromide was displaced with sodium azide and an azide-alkyne cycloaddition performed using propargyl alcohol to afford the desired triazole compound in 40% yield over the two steps (Scheme 4.7).



Scheme 4.7 Synthesis of 1,2,3-triazole anilide.

4.4. Screening of Synthesized Small Molecules for HDAC inhibition

Upon synthesis, the molecules were subjected to a commercial high throughput assay to determine their relative activities for three different HDAC isoforms, HDAC-1, -6, and -8 using human recombinant HDAC enzymes. A comparison of HDAC-1 and HDAC-6 will allow us to determine if any selectivity is observed between different classes of enzymes while two isoforms from Class I (HDAC-1 and -8) were screened to see if any selectivity between isoforms existed.

The commercial assay is designed with a fluorogenic substrate based on p53 residues 379-382 that includes an acetylated lysine side chain. When incubated with HDAC, the acetyl group is removed, leaving a free amine to react with the proprietary developer which generates a fluorophore. Fluorescence of samples containing inhibitor is compared to samples with no inhibitor to determine percent inhibition. If HDAC enzyme is active, a large fluorescence signal is observed. If reduced fluorescence is observed compared to the no inhibitor control, then inhibition of the HDAC enzyme is occurring.

4.4.1. General procedure for performing enzyme inhibition assays:

Enzyme kits were purchased from EnzoLife Sciences. Upon receipt of enzyme kit, components were defrosted and aliquotted into microcentrifuge tubes and refrozen with

liquid nitrogen to avoid multiple freeze/thaw cycles. All components were stored at -80°C until used.

HDAC Assay Buffer II was defrosted and allowed to warm to room temperature. Test inhibitors were diluted to 10 mM with DMSO. Test inhibitors were further diluted down with HDAC Assay Buffer II to a concentration of 100 µM, which is 5X the concentrations used in the assay. A known inhibitor, Trichostatin A, was diluted with DMSO to an initial concentration of 2 µM with DMSO. Further dilutions were performed with HDAC Assay Buffer II to a final concentration of 31.25 nM. Finally, DMSO was diluted to 1% with HDAC Assay Buffer II to match the concentration of DMSO found in the inhibitor samples. 10 µL of each test inhibitor was added to low retention microcentrifuge tubes. For the no-inhibitor control, 10 µL of DMSO was instead added. Microcentrifuge tubes were sealed and added to incubator at 33°C. Fluor de Lys-SIRT1 Deacetylase Substrate was diluted with HDAC Assay Buffer II to give a substrate concentration of 20 µM and placed in incubator at 33°C for 10 minutes. HDAC Enzyme was thawed and chilled enzyme was diluted into warm HDAC Assay Buffer II. 15 µL of HDAC Enzyme was then added to all samples, except the no enzyme control, mixed, and incubated at 33°C. After 10 minutes, 25µL of the diluted substrate was added to all samples, mixed thoroughly, and incubated for 40 minutes at 33°C. While samples were incubating, Fluor de Lys-Developer II Concentrate was thawed and diluted 5-fold with HDAC Assay Buffer I. Trichostatin A was then diluted 100-fold in the 1x developer just prepared. Diluted developer was stored on ice until ready for use. After the HDAC, inhibitor, substrate mixture was allowed to react for the desired time, 50 µL of developer was added to all samples. Samples were then incubated at room temperature for 45 minutes. Samples were then transferred to a white ½ volume microplate and fluorescence measured using a Biotek Synergy platereader with excitation filters of 360/40 and emission filters of 440/30. Samples were read with a top 50% mirror or dichroic 410 mirror.

Data analysis: Fluorescence reading from no enzyme control well (background) was subtracted from all sample readings. The sample fluorescence values were compared to the no inhibitor control

4.5. Analysis of Enzyme Inhibition Results

The inhibition of each HDAC enzyme was evaluated with each of the 14 synthetic compounds. To our dismay and disappointment, none of the 1,2,3-triazole zinc binding groups showed any inhibition with regards to any of the enzymes studied (Figure 4.8). The samples containing test compounds were not statistically different from the sample with no inhibitor present. The positive control with TSA, showed approximately 50% inhibition in all three enzymes. The binding potential of the triazole with zinc may not be strong enough to bind the inhibitor in the pocket. In comparison to the hydroxamic acid, the hydrogen bond with the NH would be missing in the 1,2,3-triazole inhibitors we designed. This hydrogen bonding interaction could be replaced with a H-bond between the C(5)-H of the triazole and the imidazole of the histidine residue (Figure 4.9). An additional hypothesis is that the 1,2,3-triazole may be too bulky to appropriately fit into the catalytic pocket. Furthermore, the 1,2,3-triazole might also be too polar for the hydrophobic channel leading to the catalytic zinc binding domain. Triazoles are known to be much more polar than their amide bond counterparts[12] which may prevent access to the catalytic pocket.

To test these hypotheses, the compounds could be mixed with a zinc (II) salt to see if a complex forms. The zinc triazole complex may require increased temperature, which would not be biologically relevant. To test the other theory, modeling could be utilized to predict the lowest energy conformation of the molecule. Some preliminary modeling studies showed that especially with the longer linker, the molecule would adopt an almost U-shaped conformation. Since these were preliminary studies, we weren't confident in the parameters used to optimize the geometry. We initially thought this was due more to inexperience with modeling software than the actual geometry. A closer look at the modeling studies could be of value.

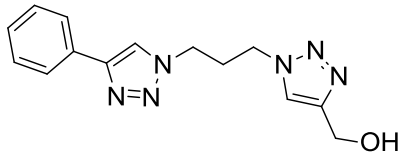
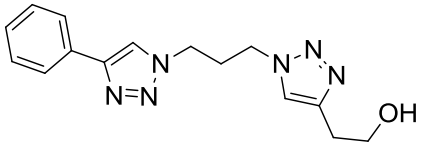
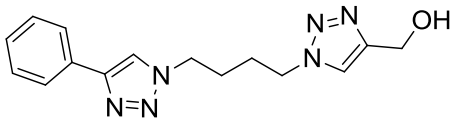
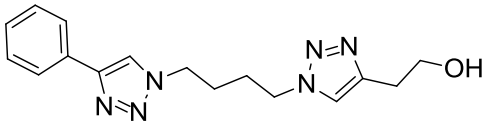
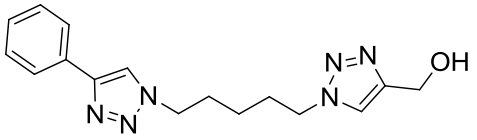
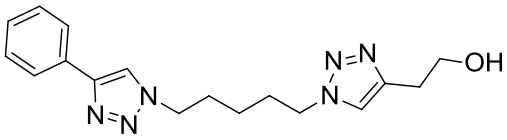
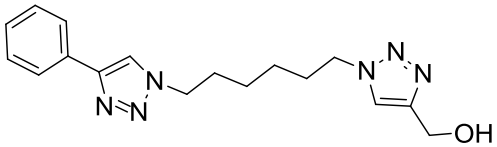
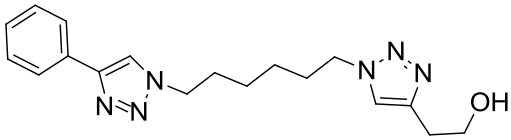
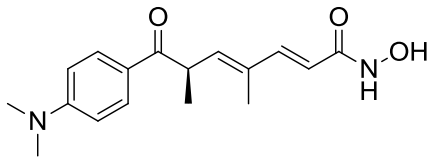
	% Inhibition HDAC-1	% Inhibition HDAC-6	% Inhibition HDAC-8
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	41 %	54 %	52 %

Figure 4.8 Analysis of 1,2,3-triazoles as zinc binding groups for HDAC inhibition.

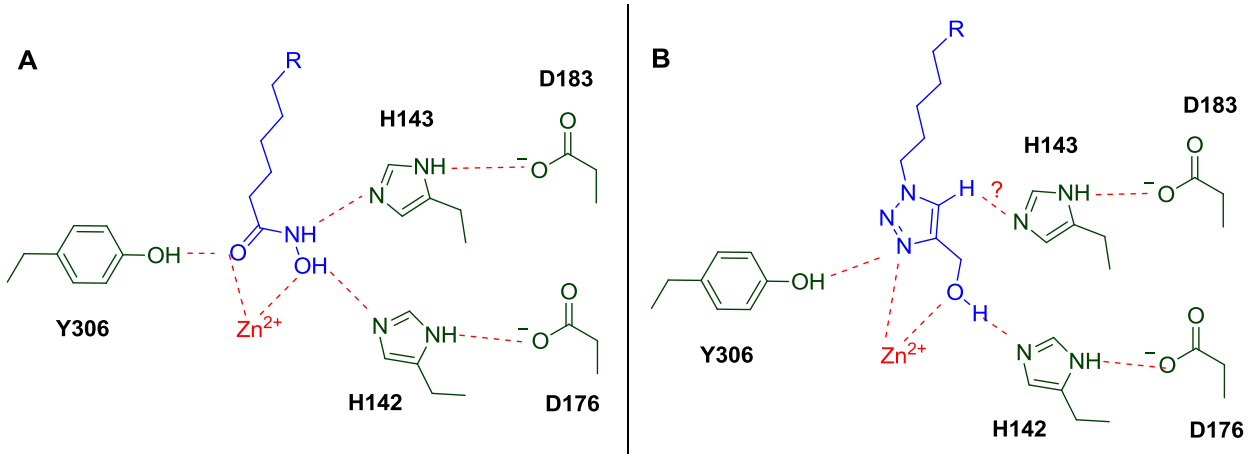


Figure 4.9 Comparison of interaction with A) hydroxamic acid and B) proposed inhibitor.

The terminal 1,2,4-triazoles as a zinc binding group did not fare any better in the enzyme assay (Figure 4.10). There was no distinguishable activity for the four 1,2,4-triazoles synthesized. Even the hydroxamic acid substituted 1,2,4-triazole did not show any inhibitory activity of any of the three HDAC isoforms tested. In hindsight, this followed similar results observed by Shen et. al.[122] Their research described attachment of a hydroxamic acid to a 1,2,3-triazole, but their compounds were not significantly active for HDAC inhibition, with the largest percentage of inhibited HDAC activity being 20% with 500 nM of test compound. Of the synthesized inhibitors, the only one that showed any activity against HDAC was the control compound that was synthesized to match a previously reported compound.[118] As with the 1,2,3-triazoles, the increased polarity of the zinc binding group or the size could be preventing the synthesized compounds from interacting with the enzyme active site. Further modeling or evaluation against other metalloenzymes could be a worthwhile pursuit.

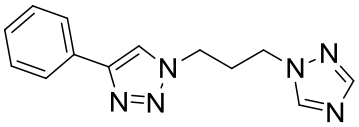
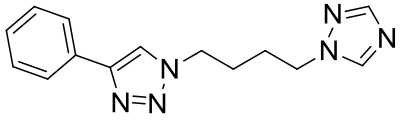
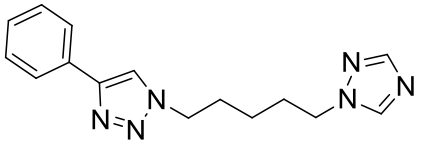
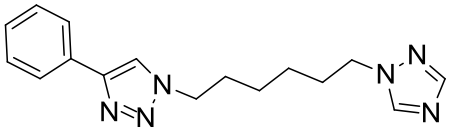
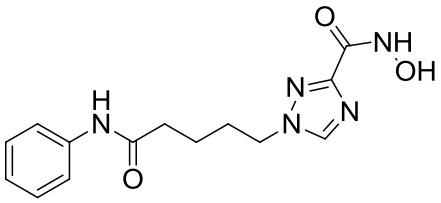
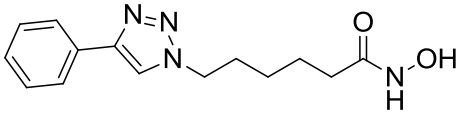
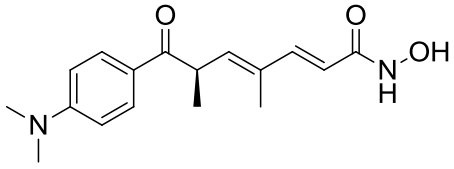
	% Inhibition HDAC-1	% Inhibition HDAC-6	% Inhibition HDAC-8
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	< 5%	< 5%	< 5%
	95%	97%	94%
	41 %	54 %	52 %

Figure 4.10 Analysis of 1,2,4-triazoles as zinc binding groups for HDAC inhibition.

4.6. Conclusions

A small library of compounds was designed and synthesized as inhibitors of various HDAC isoforms. The goal of the research was to investigate the potential of 1,2,3- or 1,2,4-triazoles as zinc binding groups. A solid phase route was originally carried out, but led to additional byproducts that were difficult to separate on such a small scale. A similar solution phase synthesis was designed and successfully carried out. This led to the synthesis of a series of molecules containing 1,2,3-triazoles in both the cap group and zinc binding domain. Additional molecules containing a 1,2,4-triazole in the zinc binding domain and a 1,2,3-triazole in the cap group were also synthesized. In an effort to match the zinc binding ability of hydroxamic acids, a compound containing a 1,2,4-triazole substituted with a hydroxamic acid was also designed and synthesized. A control compound that had been previously reported with activity against HDAC was also synthesized to serve as a positive control.

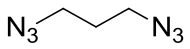
Despite evidence that would suggest triazoles as effective zinc binding groups, the designed molecules did not show activity for the enzymes tested. The molecules were screened against two enzymes from Class I (HDAC-1 and HDAC-8) to look for isoform specificity and one enzyme from Class II-b (HDAC-6) to examine class specificity. The only molecule to show activity was an already published inhibitor that was synthesized as a control. Future work could include more modeling studies in the design process or the screening of these molecules against other metalloenzymes, specifically ones that had wider enzyme active sites or that involved more polar amino acid residues.

4.7. Experimental

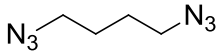
Materials and General Methods. Reagents and solvents were purchased from various commercial sources and used without further purification unless otherwise stated. Anhydrous solvents were purified using a Grubbs solvent system. Analytical thin-layer chromatography (TLC) was performed using aluminum backed silica gel TLC plates with UV indicator from Sorbent Technologies. Flash column chromatography was performed using 40 – 63 μm (230 x 400 mesh) silica gel from Sorbent Technologies.

Microwave reactions were performed in sealed vials using a Biotage Initiator Microwave. ^{1}H and ^{13}C NMR were recorded at 600 MHz and 151 MHz, respectively, on a Varian Inova spectrometer or at 500 MHz and 125 MHz, respectively, on a Varian VNMRS spectrometer. All chemical shifts were reported in δ units relative to tetramehtylsilane or the corresponding deuterated solvent. High resolution mass spectra (HRMS) were obtained on a JEOL AccuTOF DART spectrometer. Infrared spectra were recorded on a Varian 4100 FT-IR using KBr pellets or KBR salt plates. Absorption spectra were collected on a Thermo Scientific Evolution 600. High pressure liquid chromatography (HPLC) was performed using a Beckman Coulter System equipped with a UV-Vis detector, autosampler, Varian C18 column, and a mobile phase composed of acetonitrile and trifluoracetic acid.

General Diazide Procedure: Dibromide (4 mmol) was dissolved in dimethylformamide and water (9:1 mL). Sodium azide (9 mmol) was added as a solid and the solution stirred overnight at room temperature. Progress of the reaction was monitored by GC-MS. Upon completion, the reaction was quenched with 30 mL of water (exothermic) and stirred until reaching room temperature. The solution was then extracted with diethyl ether (3x). The combined organics were washed with water (2x), copper sulftate (1x) and brine (1x). The solution was dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield the desired diazide.

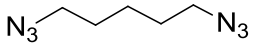


1,3-diazidopropane (4-8). Prepared using the General Diazide Procedure from 1,3-dibromopropane. Isolated as a pale yellow oil in 86% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.42 (t, 4H, J = 6.6 Hz), 1.84 (q, 2H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 48.6, 28.5. MS (ESI) m/z calculated 70.05 ($C_3H_6N_2$, $[M-N_2-N_2]^+$), m/z observed 70.10. ($C_6H_6N_2$, $[M-N_2-N_2]^+$).

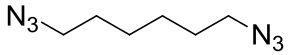


1,4-diazidobutane (4-9). Prepared using the General Diazide Procedure from 1,4-dibromobutane. Isolated as a pale yellow oil in 98% yield. ^{1}H NMR

($CDCl_3$, 600 MHz) δ 3.33 (t, 4H, J = 6.0 Hz), 1.69 (q, 4H, J = 3.0 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 51.1, 26.3. MS (ESI) m/z calculated 84.07 ($C_4H_8N_2$, $[M-N_2-N_2]^+$), m/z observed 84.10. ($C_4H_8N_2$, $[M-N_2-N_2]^+$).

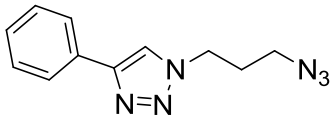


1,5-diazidopentane (4-10). Prepared using the General Diazide Procedure from 1,5-dibromopentane. Isolated as a pale yellow oil in 90% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 3.29 (t, 4H, J = 6.6 Hz), 1.63 (q, 4H, J = 6.6 Hz), 1.44 – 1.50 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 51.4, 28.6, 24.1. MS (ESI) m/z calculated 98.08 ($C_5H_{10}N_2$, $[M-N_2-N_2]^+$), m/z observed 98.10. ($C_5H_{10}N_2$, $[M-N_2-N_2]^+$).



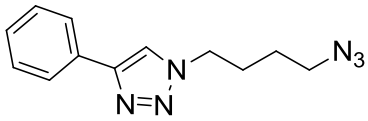
1,6-diazidohexane (4-11). Prepared using the General Diazide Procedure from 1,6-dibromohexane. Isolated as a pale yellow oil in 98% yield. ^1H NMR ($CDCl_3$, 600 MHz) δ 3.28 (t, 4H, J = 6.6 Hz), 1.59 – 1.65 (m, 4H), 1.39 – 1.44 (m, 4H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 51.5, 28.9, 26.5. MS (ESI) m/z calculated 112.10 ($C_6H_{12}N_2$, $[M-N_2-N_2]^+$), m/z observed 112.10. ($C_6H_{12}N_4$, $[M-N_2-N_2]^+$).

General Procedure for Azido 1,2,3-triazoles: Diazide (2.5 mmol) was dissolved in acetonitrile (10 mL). Phenylacetylene (1 mmol) and triethylamine (1 mmol) were added. A solution of triazine **2-17** (0.01 mmol) in acetonitrile was added, followed by a solution of tetrakis(acetonitrle-N)copper(I) tetrafluoroborate (0.01 mmol) in acetonitrile. The resulting solution was stirred at room temperature overnight. Reaction progress was followed by TLC. Upon completion, the mixture was concentrated *in vacuo* and purified via silica gel chromatography eluting with a gradient of hexanes and ethyl acetate to yield the desired azidoalkyl phenyl-1,2,3-triazole.

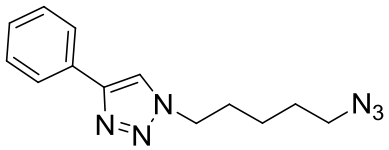


1-(3-azidopropyl)-4-phenyl-1H-1,2,3-triazole (4-12). Prepared using General Procedure for Azido 1,2,3-triazoles from 1,3-diazidopropane (**4-8**) and isolated as pale yellow solid in 27% yield. ^1H NMR (d_6-DMSO, 600 MHz) δ 8.61 (s, 1H), 7.84 (d, 2H, J = 8.4

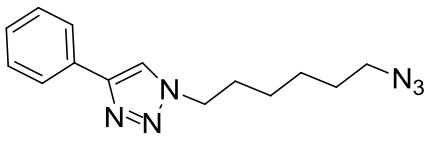
Hz), 7.45 (t, 2H, J = 7.2 Hz), 7.33 (t, 1H, J = 7.2 Hz), 4.47 (t, 2H, J = 6.6 Hz), 3.42 (t, 2H, J = 6.6 Hz), 2.13 (q, 2H, J = 6.6 Hz). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 146.4, 130.8, 128.9, 127.9, 125.2, 121.5, 47.9, 47.0, 29.0. HRMS (ESI) m/z calculated 229.12017 ($C_{11}H_{13}N_6$, $[M+H]^+$), m/z observed 229.11965. ($C_{11}H_{13}N_6$, $[M+H]^+$).



1-(4-azidobutyl)-4-phenyl-1H-1,2,3-triazole (4-13). Prepared using General Procedure for Azido 1,2,3-triazoles from 1,4-diazidobutane (**4-9**) and isolated as an off-white solid in 60% yield ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.59 (s, 1H), 7.84 (d, 2H, J = 7.2 Hz), 7.45 (t, 2H, J = 7.2 Hz), 7.33 (t, 1H, J = 7.2 Hz), 4.44 (t, 2H, J = 7.2 Hz), 3.39 (t, 2H, J = 6.6 Hz), 1.93 (q, 2H, J = 7.2 Hz), 1.54 (q, 2H, J = 6.6 Hz). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 146.3, 130.8, 128.9, 127.8, 125.1, 121.3, 50.0, 49.0, 26.9, 25.4. HRMS (ESI) m/z calculated 243.13582 ($C_{12}H_{15}N_6$, $[M+H]^+$), m/z observed 243.13586. ($C_{12}H_{15}N_6$, $[M+H]^+$).



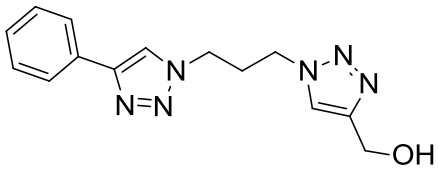
1-(5-azidopentyl)-4-phenyl-1H-1,2,3-triazole (4-14). Prepared using General Procedure for Azido 1,2,3-triazoles from 1,5-diazidopentane (**4-10**) and isolated as an off-white solid in 68% yield**.** ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.58 (s, 1H), 7.84 (d, 2H, J = 7.8 Hz), 7.44 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 7.2 Hz), 4.40 (t, 2H, J = 7.2 Hz), 3.32 (t, 2H), 1.89 (q, 2H, J = 7.2 Hz), 1.57 (q, 2H, J = 7.2 Hz), 1.32 (q, 2H, J = 7.2 Hz). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 146.3, 130.9, 128.9, 127.8, 125.1, 121.3, 50.4, 49.3, 29.1, 27.6, 23.1. HRMS (ESI) m/z calculated 257.15147 ($C_{13}H_{17}N_6$, $[M+H]^+$), m/z observed 257.15147. ($C_{13}H_{17}N_6$, $[M+H]^+$).



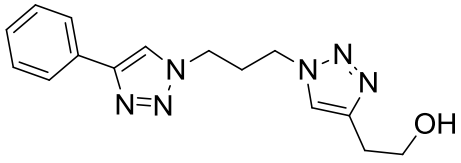
1-(6-azidohexyl)-4-phenyl-1H-1,2,3-triazole (4-15). Prepared using General Prodedure for Azido 1,2,3-triazoles from 1,6-diazidohexane (**4-11**) and isolated as an off-white solid in 47% yield**.** ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.58 (s, 1H), 7.84 (d,

2H, J = 8.4 Hz), 7.44 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 7.8 Hz), 4.39 (t, 2H, J = 6.6 Hz), 3.31 (t, 2H, J = 6.6 Hz), 1.87 (q, 2H, J = 7.2 Hz), 1.52 (q, 2H, J = 7.2 Hz), 1.36 (q, 2H, J = 7.2 Hz), 1.29 (q, 2H, J = 7.2 Hz). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 146.3, 130.9, 128.9, 127.8, 125.1, 121.2, 50.5, 49.4, 29.4, 28.0, 25.5, 25.4. HRMS (ESI) m/z calculated 271.16712 ($C_{14}H_{19}N_6$, $[M+H]^+$), m/z observed 271.16567. ($C_{14}H_{19}N_6$, $[M+H]^+$).

General Procedure for Bistriazoles: Azidoalkyl-phenyl 1,2,3-triazole (1.0 mmol) was dissolved in acetonitrile (2.5 mL). Propargyl or homopropargyl alcohol (1 mmol) and triethylamine (1 mmol) were added. A solution of triazine **2-17** (0.01 mmol) in acetonitrile (750 µL) was added, followed by a solution of tetrakis(acetonitrle-N)copper(I) tetrafluoroborate (0.01 mmol) in acetonitrile (750 µL). The resulting solution was stirred 60°C overnight. Reaction progress was followed by TLC. Upon completion, the mixture was concentrated *in vacuo* and purified via silica gel chromatography eluting with a gradient of ethyl acetate and methanol to yield the desired bistriazoles.

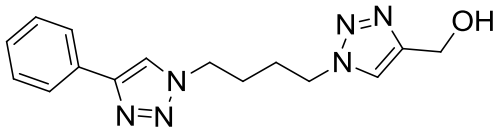


(1-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-1,2,3-triazol-4-yl)methanol (4-16). Prepared using General Procedure for Bistriazoles from **4-12** and propargyl alcohol with following modification: isolated by filtration to yield a white solid in 82% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.59 (s, 1H), 8.02 (s, 1H), 7.84 (d, 2H, J = 7.8 Hz), 7.45 (t, 2H, J = 7.8 Hz), 7.34 (t, 1H, J = 7.8 Hz), 5.17 (t, 1H, J = 6.0 Hz), 4.51 (d, 2H, J = 6.0 Hz), 4.42 (q, 4H, J = 7.2 Hz), 2.46 (q, 2H, J = 7.2 Hz). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 148.1, 146.4, 130.7, 128.9, 127.9, 125.1, 122.9, 121.5, 55.1, 46.9, 46.5, 30.2. HRMS (ESI) m/z calculated 285.14638 ($C_{14}H_{17}N_6O$, $[M+H]^+$), m/z observed 285.14514. ($C_{14}H_{17}N_6O$, $[M+H]^+$).

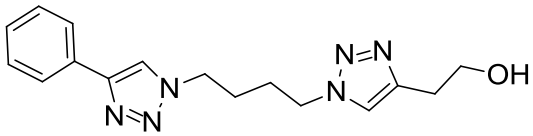


2-(1-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)-1H-1,2,3-triazol-4-yl)ethanol (4-17). Prepared using General Procedure for Bistriazoles from **4-12** and

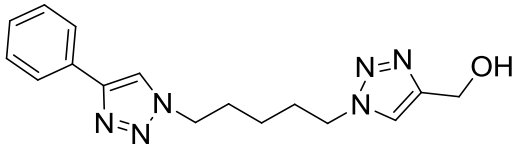
homopropargyl alcohol and isolated as a white solid in 76% yield. ^{1}H NMR (d_6-DMSO, 500 MHz) δ 8.58 (s, 1H), 7.90 (s, 1H), 7.83 (d, 2H, J = 7.0 Hz), 7.45 (t, 2H, J = 7.5 Hz), 7.33 (tt, 1H, J = 7.8 Hz, J = 1.5 Hz), 4.67 (t, 1H, J = 5.5 Hz), 4.44 (t, 2H, J = 7.0 Hz), 4.39 (t, 2H, J = 7.0 Hz), 3.62 (q, 2H, J = 7.0 Hz), 2.76 (t, 2H, J = 7.0 Hz), 2.45 (q, 2H, J = 7.0 Hz). ^{13}C NMR (d_6-DMSO, 125 MHz) δ 146.4, 144.4, 130.7, 128.9, 127.8, 125.1, 122.6, 121.5, 60.3, 46.9, 46.5, 30.1, 29.2. HRMS (ESI) m/z calculated 299.16203 ($C_{15}H_{19}N_6O$, $[M+H]^+$), m/z observed 299.16128. ($C_{15}H_{19}N_6O$, $[M+H]^+$).



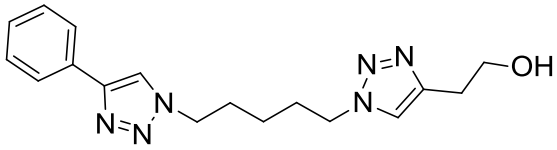
(1-(4-(4-phenyl-1H-1,2,3-triazol-1-yl)butyl)-1H-1,2,3-triazol-4-yl)methanol (4-18). Prepared using General Procedure for Bistriazoles from **4-13** and propargyl alcohol and isolated as a white solid in 48% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.56 (s, 1H), 7.96 (s, 1H), 7.83 (d, 2H, J = 7.2 Hz), 7.44 (t, 2H, J = 7.2 Hz), 7.33 (t, 1H, J = 7.2 Hz), 5.15 (t, 1H, J = 6.0 Hz), 4.50 (d, 2H, J = 6.0 Hz), 4.43 (t, 2H, J = 6.6 Hz), 4.38 (t, 2H, J = 6.6 Hz) 1.81 – 1.84 (m, 4H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 148.0, 146.3, 130.8, 128.9, 127.8, 125.1, 122.7, 121.4, 55.1, 48.9, 48.6, 26.9, 26.8. HRMS (ESI) m/z calculated 299.16203 ($C_{15}H_{19}N_6O$, $[M+H]^+$), m/z observed 299.16125. ($C_{15}H_{19}N_6O$, $[M+H]^+$).



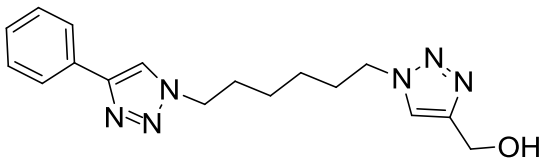
2-(1-(4-(4-phenyl-1H-1,2,3-triazol-1-yl)butyl)-1H-1,2,3-triazol-4-yl)ethanol (4-19). Prepared using General Procedure for Bistriazoles from **4-13** and homopropargyl alcohol and isolated as a white solid in 64% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.56 (s, 1H), 7.85 (s, 1H), 7.83 (d, 2H, J = 6.6 Hz), 7.44 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 7.8 Hz), 4.67 (t, 1H, J = 5.4 Hz), 4.43 (t, 2H, J = 6.6 Hz), 4.35 (t, 2H, J = 6.6 Hz), 3.61 (q, 2H, J = 6.6 Hz), 2.75 (t, 2H, J = 6.6 Hz), 1.77 – 1.87 (m, 4H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 146.3, 144.3, 130.8, 128.9, 127.8, 125.1, 122.6, 121.3, 60.4, 48.9, 48.4, 29.2, 26.8, 26.7. HRMS (ESI) m/z calculated 313.17768 ($C_{16}H_{21}N_6O$, $[M+H]^+$), m/z observed 313.17625. ($C_{16}H_{21}N_6O$, $[M+H]^+$).



(1-(5-(4-phenyl-1H-1,2,3-triazol-1-yl)pentyl)-1H-1,2,3-triazol-4-yl)methanol (4-20). Prepared using General Procedure for Bistriazoles from **4-14** and propargyl alcohol and isolated as a white solid in 84% yield. ^{1}H NMR (d_6-DMSO, 500 MHz) δ 8.56 (s, 1H), 7.95 (s, 1H), 7.83 (d, 2H, J = 7.0 Hz), 7.45 (t, 2H, J = 7.5 Hz), 7.33 (tt, 1H, J = 7.5 Hz, J = 1.5 Hz), 5.14 (t, 1H, J = 6.0 Hz), 4.49 (d, 2H, J = 6.0 Hz), 4.39 (t, 2H, J = 7.0 Hz), 4.33 (t, 2H, J = 7.0 Hz), 1.82 – 1.94 (m, 4H), 1.21 – 1.28 (m, 2H). ^{13}C NMR (d_6-DMSO, 125 MHz) δ 147.9, 146.2, 130.8, 128.9, 127.8, 125.1, 122.5, 121.2, 55.0, 49.3, 48.9, 29.1, 29.0, 22.8. HRMS (ESI) m/z calculated 313.17768 ($C_{16}H_{21}N_6O$, $[M+H]^+$), m/z observed 313.17693. ($C_{16}H_{21}N_6O$, $[M+H]^+$).

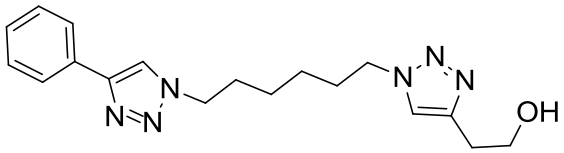


2-(1-(5-(4-phenyl-1H-1,2,3-triazol-1-yl)pentyl)-1H-1,2,3-triazol-4-yl)ethanol (4-21). Prepared using General Procedure for Bistriazoles from **4-14** and homopropargyl alcohol and isolated as a white solid in 64% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.55 (s, 1H), 7.82 – 7.85 (m, 3H), 7.45 (t, 2H, J = 7.8 Hz), 7.33 (tt, 1H, J = 7.8 Hz, J = 1.2 Hz), 4.66 (t, 1H, J = 5.4 Hz), 4.38 (t, 2H, J = 7.2 Hz), 4.29 (t, 2H, J = 7.2 Hz), 3.60 (q, 2H, J = 6.6 Hz), 2.73 (t, 2H, J = 6.6 Hz), 1.89 (q, 2H, J = 7.2 Hz), 1.84 (q, 2H, J = 7.2 Hz), 1.21 – 1.27 (m, 2H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 146.2, 144.3, 130.8, 128.9, 127.8, 125.1, 122.2, 121.2, 60.4, 49.2, 48.8, 29.2, 29.1, 29.0, 22.8. HRMS (ESI) m/z calculated 327.19333 ($C_{17}H_{23}N_6O$, $[M+H]^+$), m/z observed 327.19218. ($C_{17}H_{23}N_6O$, $[M+H]^+$).



(1-(6-(4-phenyl-1H-1,2,3-triazol-1-yl)hexyl)-1H-1,2,3-triazol-4-yl)methanol (4-22). Prepared using General Procedure for Bistriazoles from **4-15** and propargyl alcohol and isolated as a white solid in 87% yield. ^{1}H NMR (d_6-DMSO, 500 MHz) δ 8.57 (s, 1H), 7.95 (s,1H), 7.83 (d, 2H, J = 7.0 Hz), 7.44 (t, 2H, J = 7.5 Hz), 7.32 (tt, 1H, J = 7.5 Hz, J = 1.5 Hz), 5.13 (t, 1H, J = 5.5 Hz), 4.37 (t,

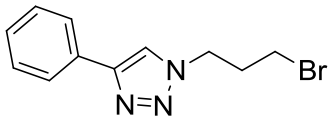
2H, J = 7.5 Hz), 4.31 (t, 2H, J = 7.0 Hz), 1.85 (q, 2H, J = 7.0 Hz), 1.80 (q, 2H, J = 7.0 Hz), 1.25 – 1.31 (m, 4H). ^{13}C NMR (d_6-DMSO, 125 MHz) δ 147.9, 146.2, 130.8, 128.9, 127.7, 125.1, 122.5, 121.2, 55.1, 49.4, 49.0, 29.5, 29.4, 25.25, 25.23. HRMS (ESI) m/z calculated 327.19333 ($C_{17}H_{23}N_6O$, $[M+H]^+$), m/z observed 327.19240. ($C_{17}H_{23}N_6O$, $[M+H]^+$).



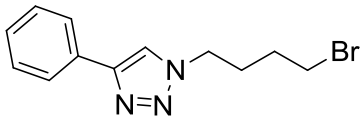
2-(1-(6-(4-phenyl-1H-1,2,3-triazol-1-yl)hexyl)-1H-1,2,3-triazol-4-yl)ethanol (4-23). Prepared using General Procedure for Bistriazoles from **4-15** and homopropargyl alcohol and isolated as a white solid in 81% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 8.57 (s, 1H), 7.82 – 7.85 (m, 3H), 7.44 (t, 2H, J = 7.8 Hz), 7.32 (t, 1H, J = 7.8 Hz), 4.67 (t, 1H, J = 5.4 Hz), 4.37 (t, 2H, J = 7.2 Hz), 4.27 (t, 2H, J = 6.6 Hz), 3.61 (q, 2H, J = 6.6 Hz), 2.74 (t, 2H, J = 6.6 Hz), 1.85 (q, 2H, J = 7.2 Hz), 1.85 (q, 2H, J = 7.2 Hz), 1.78 (q, 2H, J = 7.2 Hz), 1.26 – 1.31 (m, 4H). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 146.3, 144.3, 130.9, 128.9, 127.8, 125.1, 122.2, 121.2, 60.4, 49.4, 49.0, 29.5, 29.4, 29.2, 25.29, 25.26. HRMS (ESI) m/z calculated 341.20898 ($C_{18}H_{25}N_6O$, $[M+H]^+$), m/z observed 341.21026. ($C_{18}H_{25}N_6O$, $[M+H]^+$).

General procedure for azido bromo-alkanes: Dibromide (1.2 mmol) was dissolved in dimethylformamide and water (9:1 mL). Sodium azide (1 mmol) was added as a solid and the solution stirred overnight at room temperature. Progress of the reaction was monitored by GC-MS. This reaction resulted in a mixture of unreacted dibromide, the desired azidobromoalkane, and diazidoalkane. The reaction was quenched with 20 mL of water (exothermic) and stirred until reaching room temperature. The solution was then extracted with diethyl ether (3x). The combined organics were washed with water (2x), copper sulftate (1x) and brine (1x). The solution was dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield the crude azidobromoalkane as a pale yellow oil. This solution was used in the next step without further purification.

General procedure for bromo 1,2,3-triazoles: Using the crude azidobromoalkane, it was assumed (based on GC-MS results) that ~30% of the mixture was desired azidobromoalkane. Excess azidobromoalkane (1 mmol) was measured out (mmol were calculated by multiplying mass by 0.3 and using molecular weight of desired azidobromoalkane) and dissolved in acetonitrile (8 mL).). Phenylacetylene (3.3 mmol) and triethylamine (3.3 mmol) were added. A solution of triazine **2-17** (0.033 mmol) in acetonitrile (4 mL) was added, followed by a solution of tetrakis(acetonitrle-N)copper(I) tetrafluoroborate (0.033 mmol) in acetonitrile (4 mL). The resulting solution was stirred at room temperature overnight. Reaction progress was followed by TLC. Upon completion, the mixture was concentrated *in vacuo* and purified via silica gel chromatography eluting with a gradient of hexanes and ethyl acetate to yield the desired bromoalkyl phenyl-1,2,3-triazole.

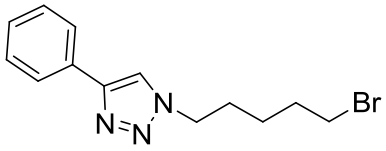


1-(3-bromopropyl)-4-phenyl-1H-1,2,3-triazole (4-28). Prepared using General Procedure for bromo-1,2,3-triazoles from 1-azido-3-bromopropane **4-24** and isolated as a white solid in 28% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.84 (d, 2H, J = 7.8 Hz), 7.82 (s, 1H), 7.43 (t, 2H, J = 7.8 Hz), 7.34 (t, 1H, J = 7.8 Hz), 4.61 (t, 2H, J = 6.6 Hz), 3.40 (t, 2H, J = 6.0 Hz), 2.52 (q, 2H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 147.9, 130.6, 129.0, 128.4, 125.9, 120.4, 48.3, 32.7, 29.7. HRMS (ESI) m/z calculated 266.02929 ($C_{11}H_{13}BrN_3$, $[M+H]^+$), m/z observed 266.02908. ($C_{11}H_{12}BrN_3$, $[M+H]^+$).

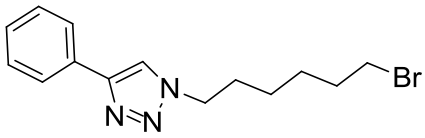


1-(4-bromobutyl)-4-phenyl-1H-1,2,3-triazole (4-29). Prepared using General Procedure for bromo-1,2,3-triazoles from 1-azido-4-bromobutane **4-25** and isolated as a white solid in 34% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.83 (d, 2H, J = 7.2 Hz), 7.77 (s, 1H), 7.43 (t, 2H, J = 7.8 Hz), 7.34 (t, 1H, J = 7.8 Hz), 4.45 (t, 2H, J = 6.6 Hz), 3.44 (t, 2H, J = 6.6 Hz), 2.14 (q, 2H, J = 7.2 Hz), 1.92 (q, 2H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.1, 130.7, 129.0, 128.3, 125.8, 119.5, 49.5, 32.6, 29.4, 28.9. HRMS (ESI) m/z

calculated 280.04494 ($C_{12}H_{15}BrN_3$, $[M+H]^+$), m/z observed 280.04348. ($C_{12}H_{15}BrN_3$, $[M+H]^+$).



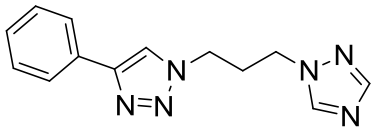
1-(5-bromopentyl)-4-phenyl-1H-1,2,3-triazole (4-30). Prepared using General Procedure for bromo-1,2,3-triazoles from 1-azido-5-bromopentane **4-26** and isolated as a white solid in 29% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.83 (d, 2H, J = 7.8 Hz), 7.75 (s, 1H), 7.43 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 7.8 Hz), 4.42 (t, 2H, J = 7.2 Hz), 3.40 (t, 2H, J = 6.6 Hz), 2.00 (q, 2H, J = 7.2 Hz), 1.92 (q, 2H, J = 7.2 Hz), 1.50 – 1.56 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.0, 130.8, 129.0, 128.3, 125.8, 119.6, 50.2, 33.3, 32.1, 29.7, 25.2. HRMS (ESI) m/z calculated 294.06059 ($C_{13}H_{17}BrN_3$, $[M+H]^+$), m/z observed 294.05971. ($C_{13}H_{17}BrN_3$, $[M+H]^+$).



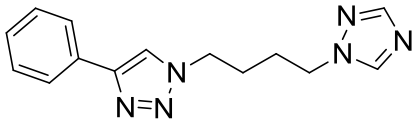
1-(6-bromohexyl)-4-phenyl-1H-1,2,3-triazole (4-31). Prepared using General Procedure for bromo-1,2,3-triazoles from 1-azido-6-bromohexane **4-27** and isolated as a white solid in 32% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.83 (d, 2H, J = 7.8 Hz), 7.75 (s, 1H), 7.43 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 7.2 Hz), 4.41 (t, 2H, J = 7.2 Hz), 3.40 (t, 2H, J = 6.6 Hz), 1.98 (q, 2H, J = 7.8 Hz), 1.86 (q, 2H, J = 7.2 Hz), 1.52 (q, 2H, J = 7.2 Hz), 1.39 (q, 2H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 148.0, 130.8, 129.0, 128.3, 125.8, 119.5, 50.3, 33.7, 32.5, 30.3, 27.6, 25.8. HRMS (ESI) m/z calculated 308.07624 ($C_{14}H_{19}BrN_3$, $[M+H]^+$), m/z observed 308.07481. ($C_{14}H_{19}BrN_3$, $[M+H]^+$).

General procedure for 1,2,4-triazolyl-12,3-triazoles: Bromoalkyl phenyl-1,2,3-triazole (1 mmol) was dissolved in DMF (4 mL). 1H-1,2,4-triazole (1 mmol) was added along with solid K_2CO_3 (2 mmol). This heterogeneous mixture was stirred at room temperature overnight. Reaction progress was followed by TLC. Reaction mixture was diluted with water and extracted with ethyl acetate (3x). Combined organics were washed with water (1x) and saturated sodium chloride (1x). The organic layer was dried over sodium

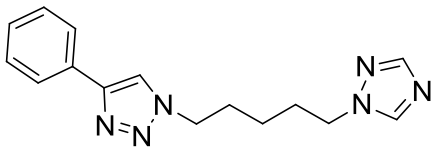
sulfate, filtered, and concentrated *in vacuo* to yield the desired 1,2,4-triazolyl-1,2,3-triazole.



1-(3-(1H-1,2,4-triazol-1-yl)propyl)-4-phenyl-1H-1,2,3-triazole (4-32). Prepared using the general procedure for 1,2,4-triazolyl-1,2,3-triazoles from bromide **4-28** and 1H-1,2,4-triazole. Isolated as a white solid in 76% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.17 (s, 1H), 8.00 (s, 1H), 7.83 (m, 3H), 7.44 (t, 2H, J = 7.8 Hz), 7.35 (t, 1H, J = 7.8 Hz), 4.43 (t, 2H, J = 6.6 Hz), 4.25 (t, 2H, J = 6.6 Hz), 2.55 (q, 2H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 152.6, 148.2, 144.4, 130.4, 129.1, 128.5, 125.9, 120.3, 46.8, 45.9, 30.4. HRMS (ESI) m/z calculated 255.13582 ($C_{13}H_{15}N_6$, $[M+H]^+$), m/z observed 255.13509. ($C_{13}H_{15}N_6$, $[M+H]^+$).

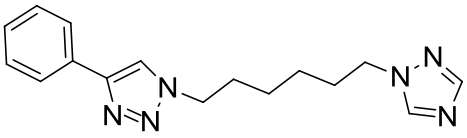


1-(4-(1H-1,2,4-triazol-1-yl)butyl)-4-phenyl-1H-1,2,3-triazole (4-33). Prepared using the general procedure for 1,2,4-triazolyl-1,2,3-triazoles from bromide **4-29** and 1H-1,2,4-triazole. Isolated as a white solid in 44% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.05 (s, 1H), 7.95 (s, 1H), 7.82 (d, 2H, J = 7.8 Hz), 7.72 (s, 1H), 7.43 (t, 2H, J = 7.8 Hz), 7.34 (t, 1H, J = 7.8 Hz), 4.44 (t, 2H, J = 6.0 Hz), 4.22 (t, 2H, J = 6.0 Hz), 1.97 – 1.99 (m, 4H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 152.4, 148.2, 143.2, 130.6, 129.0, 128.4, 125.8, 119.6, 49.6, 48.8, 27.4, 27.0. HRMS (ESI) m/z calculated 269.15147 ($C_{14}H_{17}N_6$, $[M+H]^+$), m/z observed 269.15093. ($C_{14}H_{17}N_6$, $[M+H]^+$).

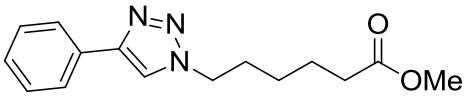


1-(5-(1H-1,2,4-triazol-1-yl)pentyl)-4-phenyl-1H-1,2,3-triazole (4-34). Prepared using the general procedure for 1,2,4-triazolyl-1,2,3-triazoles from bromide **4-30** and 1H-1,2,4-triazole. Isolated as a white solid in 76% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.03 (s, 1H), 7.93 (s, 1H), 7.82 (d, 2H, J = 7.2 Hz), 7.72 (s, 1H), 7.43 (t, 2H, J = 7.2 Hz), 7.34 (t, 1H, J = 7.2 Hz), 4.39 (t, 2H, J = 6.6 Hz), 4.16 (t, 2H, J = 6.6 Hz), 1.93 –

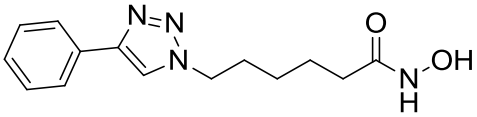
2.02 (m, 4H), 1.33 – 1.40 (m, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 152.2, 148.0, 143.2, 130.7, 129.0, 128.3, 125.8, 119.6, 50.1, 49.4, 29.9, 29.3, 23.6. HRMS (ESI) m/z calculated 283.16712 ($C_{15}H_{19}N_6$, $[M+H]^+$), m/z observed 283.16605. ($C_{15}H_{19}N_6$, $[M+H]^+$).



1-(6-(1H-1,2,4-triazol-1-yl)hexyl)-4-phenyl-1H-1,2,3-triazole (4-35).). Prepared using the general procedure for 1,2,4-triazolyl-1,2,3-triazoles from bromide **4-31** and 1H-1,2,4-triazole. Isolated as a white solid in 84% yield. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.03 (s, 1H), 7.93 (s, 1H), 7.83 (d, 2H, J = 7.2 Hz), 7.73 (s, 1H), 7.42 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 7.8 Hz), 4.39 (t, 2H, J = 6.6 Hz), 4.15 (t, 2H, J = 7.2 Hz), 1.95 (q, 2H, J = 7.2 Hz), 1.89 (q, 2H, J = 6.6 Hz), 1.32 – 1.42 (m, 4H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 152.1, 148.0, 143.0, 130.7, 129.0, 128.3, 125.8, 119.5, 50.2, 49.5, 30.2, 29.7, 26.02, 25.99. HRMS (ESI) m/z calculated 297.18277 ($C_{16}H_{21}N_6$, $[M+H]^+$), m/z observed 297.18213. ($C_{16}H_{21}N_6$, $[M+H]^+$).

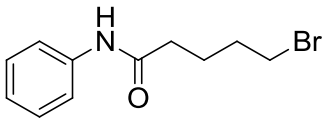


methyl 6-(4-phenyl-1H-1,2,3-triazol-1-yl)hexanoate (4-36). 1,2,3-Triazoyl carboxylic acid **3-3k** (1 mmol) was dissolved in methanol (2.5 mL) with a catalytic amount of H_2SO_4 (100 µL). The mixture was refluxed overnight and reaction progress monitored by TLC. Solution was concentrated *in vacuo*, and residue redissolved in DCM and water. The aqueous layer was made neutral by dropwise addition of 6 M NaOH and extracted with DCM (3x). The combined organics were washed with 2 M NaOH (1x), H_2O (1x), and saturated NaCl (1x). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield an off-white solid in 85% yield.

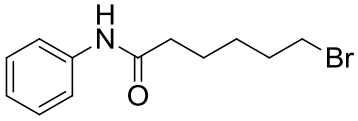


N-hydroxy-6-(4-phenyl-1H-1,2,3-triazol-1-yl)hexanamide (4-37). Triazolyl methyl ester **4-36** (1 mmol) was added to a microwave vial. To this was added methanol (5 mL), hydroxylamine hydrochloride (3 mmol) and crushed NaOH

(6 mmol). The heterogeneous mixture was microwaved at 120°C for 30 minutes. Starting material consumption was confirmed by TLC and methanol was removed *in vacuo*. Water and DCM were added to the residue and the aqueous layer acidified to pH of 4-5 with glacial acetic acid. The water layer was then extracted with DCM (3x). Combined organics were washed with ammonium chloride and saturated NaCl. The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield an off-white solid in 60% yield.

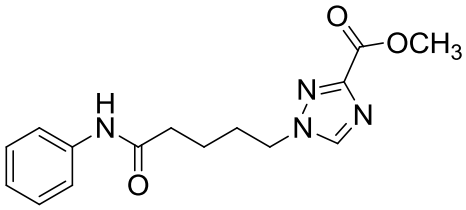


5-bromo-N-phenylpentanamide (4-40). 5-bromopentanoic acid (1 mmol) was dissolved in DCM (2.85 mL) and cooled to 0°C in ice bath. A solution of oxalyl chloride in DCM (2M, 1.1 mmol) was added dropwise along with a catalytic amount of DMF (0.1 mmol). The ice bath was removed and the solution stirred at room temperature for 1 hour. TLC confirmed consumption of starting material. Volatiles were removed under a stream of N_2. The resulting residue was dissolved in DCM (4.25 mL) and cooled to 0°C in an ice bath. Dropwise add a solution of DIEA (2.1 mmol) and aniline (1.2 mmol) dissolved in DCM (0.5 mL). Remove ice bath and stir overnight at room temperature. Reaction progress was monitored by TLC. Upon completion, water was added and extracted with DCM (3x). The combined organics were washed with 1 M HCl, saturated sodium bicarbonate, and saturated NaCl. The organic layer was then dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield a pale pink solid in 84% yield.

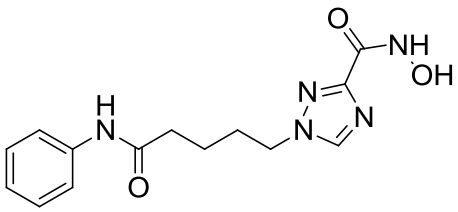


6-bromo-N-phenylhexanamide (4-41). 5-bromopentanoic acid (1 mmol) was dissolved in DCM (2.85 mL) and cooled to 0°C in ice bath. A solution of oxalyl chloride in DCM (2M, 1.1 mmol) was added dropwise along with a catalytic amount of DMF (0.1 mmol). The ice bath was removed and the solution stirred at room temperature for 1 hour. TLC confirmed consumption of starting material. Volatiles were removed under a stream of N_2. The resulting residue was dissolved in DCM (4.25 mL) and cooled to 0°C in an ice bath. Dropwise add a solution of DIEA (2.1 mmol) and aniline (1.2 mmol) dissolved in

DCM (0.5 mL). Remove ice bath and stir overnight at room temperature. Reaction progress was monitored by TLC. Upon completion, water was added and extracted with DCM (3x). The combined organics were washed with 1 M HCl, saturated sodium bicarbonate, and saturated NaCl. The organic layer was then dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield an off-white solid in 82% yield.

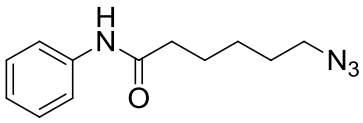


methyl 1-(5-oxo-5-(phenylamino)pentyl)-1H-1,2,4-triazole-3-carboxylate (4-42). 5-bromo-N-phenylpentanamide **4-40** (1 mmol) was dissolved in DMF (4 mL). Methyl 1-H-1,2,4-triazole-3-carboxylate and K_2CO_3 were added as solids. The heterogeneous mixture was stirred overnight at room temperature. Reaction progress was monitored by TLC. Upon completion, a small amount of water was added and extracted with ethyl acetate (3x). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. Crude material still contained DMF. Purify via silica gel chromatography eluting with gradient of hexanes and ethyl acetate to yield a white solid in 39% yield. Experiments on 600 NMR – HMBC in particular showed correlation between CH of triazole and CH_2 of alkyl linker. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.18 (s, 1H), 7.49 (d, 2H, J = 7.5 Hz), 7.35 (br, 1H), 7.31 (t, 2H, J = 7.5 Hz), 7.10 (t, 1H, J = 7.5 Hz), 4.28 (t, 2H, J = 7.0 Hz), 3.91 (s, 3H), 2.40 (q, 2H, J = 7.0 Hz), 2.04 (q, 2H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 160.3, 155.1, 144.7, 137.8, 129.2, 124.6, 120.0, 52.9, 50.4, 36.6, 29.3, 22.2. HRMS (ESI) m/z calculated 303.1457 ($C_{15}H_{19}N_4O_3$, $[M+H]^+$), m/z observed 304.14590. ($C_{15}H_{19}N_4O_3$, $[M+H]^+$).

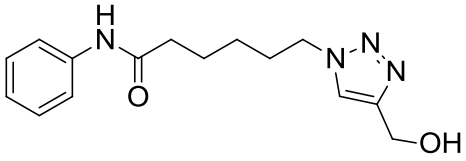


N-hydroxy-1-(5-oxo-5-(phenylamino)pentyl)-1H-1,2,4-triazole-3-carboxamide (4-44). Methyl ester **4-42** (1 mmol) was suspended in methanol (5 mL) in a microwave vial. Hydroxylamine hydrochloride (3 mmol) and crushed NaOH (6 mmol) were added as solids and the resulting heteroenous mixture was microwaved for 1 hour at 120°C. Starting material consumption was confirmed by TLC and methanol was removed *in vacuo*. Water and

ethyl acetate were added to the residue and the aqueous layer acidified to pH of 4-5 with glacial acetic acid. The water layer was then extracted with ethyl acetate (3x). Combined organics were dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield a white solid in 58% yield. ^{1}H NMR (d_6-DMSO, 600 MHz) δ 11.22 (s, 1H), 9.88 (s, 1H), 9.10 (s, 1H), 8.62 (s, 1H), 7.57 (d, 2H, J = 7.8 Hz), 7.27 (t, 2H, J = 7.2 Hz), 7.01 (t, 1H, J = 7.8 Hz), 4.24 (t, 2H, J = 6.6 Hz Hz), 2.34 (t, 2H, J = 7.2 Hz), 1.84 (q, 2H, J = 6.6 Hz), 1.54 (q, 2H, J = 7.2 Hz). ^{13}C NMR (d_6-DMSO, 151 MHz) δ 170.8, 156.8, 155.7, 144.9, 139.2, 128.6, 123.0, 119.0, 49.0, 35.6, 28.8, 22.0. HRMS (ESI) m/z calculated 303.1331 ($C_{14}H_{17}N_5O_3$, $[M+H]^+$), m/z observed 303.13299. ($C_{14}H_{17}N_5O_3$, $[M+H]^+$).



6-azido-N-phenylhexanamide (4-43). Bromoamide **4-41** (1 mmol) was dissolved in dimethylformamide and water (2.25 mL : 0.25 mL). Sodium azide (1.25 mmol) was added as a solid and the solution stirred overnight at room temperature. Progress of the reaction was monitored by TLC. Upon completion, the reaction was quenched with 30 mL of water (exothermic) and stirred until reaching room temperature. The solution was then extracted with DCM (3x). The combined organics were washed with water (3x), copper sulftate (1x) and brine (1x). The solution was dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield the desired azide as a yellow-orange oil in 75% yield.



6-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)-N-phenylhexanamide (4-46). Azide **4-41** (1 mmol) was dissolved in acetonitrile (3.0 mL). Propargyl alcohol (1.05 mmol) and triethylamine (1 mmol) were added. A solution of triazine **2-17** (0.01 mmol) in acetonitrile (1000 µL) was added, followed by a solution of tetrakis(acetonitrle-N)copper(I) tetrafluoroborate (0.01 mmol) in acetonitrile (1000 µL). The resulting solution was stirred 60°C overnight. Reaction progress was followed by TLC. Upon completion, the mixture was concentrated *in vacuo* and purified via silica gel chromatography eluting with a gradient of ethyl acetate and methanol to yield the desired 1,2,3-triazole as an off-white solid in 53% yield.

Chapter 5.
Conclusions and Future Work

5.1. Concluding Remarks

In this dissertation, efforts to design new ligands for copper-catalyzed azide–alkyne cycloadditions were discussed. A series of 1,2,4-triazine ligands was successfully synthesized by varying the substituents at the 3-position and 5- and 6-positions. The triazine core scaffold was replaced with other nitrogen containing heterocycles. These compounds were then screened for CuAAC activity. Work is still needed to make this a true “click” reaction as our isolation technique still involved chromatographic separation. The use of water as a solvent was not explored in our optimization conditions, and should be given more focus in the future.

1,2,4-Triazine ligands were also attached to solid supports to create heterogeneous catalysts for CuAAC. Both polystyrene and silica catalysts were effective catalysts with simple isolation of triazole products. The use of water proved to be beneficial for both heterogeneous systems. This should also be explored more in the future. Decreased temperatures might be achievable by altering the solvent or other parameters. Recyclability studies and copper retention were not examined with respect to reactions carried out in water. These results would give additional information on the future viability of these supports.

Finally, 1,2,4-triazine ligands were used to synthesize a library of compounds containing 1,2,3- or 1,2,4-triazoles as mimics of hydroxamic acid zinc-binding groups. Although, the synthesis was successful, the HDAC inhibition data was not promising. Modeling studies could help to design a better scaffold of inhibitors for future work. Additional metalloenzymes could also be screened for activity.

References

(1) Bohacek, R. S.; McMartin, C.; Guida, W. C. *Med. Res. Rev.* 1996, *16*, 3.

(2) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* 2001, *40*, 2004.

(3) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* 2007, *36*, 1249.

(4) Roper, S.; Kolb, H. C. *Methods and Principles in Medicinal Chemistry* 2006, *34*, 313.

(5) Mamidyala, S. K.; Finn, M. G. *Chem. Soc. Rev.* 2010, *39*, 1252.

(6) Meldal, M. *Macromol. Rapid Commun.* 2008, *29*, 1016.

(7) Lutz, J.-F. *Angewandte Chemie International Edition* 2007, *46*, 1018.

(8) Meldal, M.; Tornoe, C. W. *Chem. Rev.* 2008, *108*, 2952.

(9) Finn, M. G.; Fokin, V. V. *Catal. Precious Met.* 2010, 235.

(10) Kharb, R.; Sharma, P. C.; Yar, M. S. *Journal of Enzyme Inhibition and Medicinal Chemistry* 2011, *26*, 1.

(11) Agalave, S. G.; Maujan, S. R.; Pore, V. S. *Chemistry--An Asian Journal* 2011, *6*, 2696.

(12) Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* 2008, *28*, 278.

(13) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* 2003, *8*, 1128.

(14) Angell, Y. L.; Burgess, K. *Chem. Soc. Rev.* 2007, *36*, 1674.

(15) Brik, A.; Alexandratos, J.; Lin, Y.-C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C.-H. *ChemBioChem* 2005, *6*, 1167.

(16) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* 2004, *126*, 15366.

(17) Oh, K.; Guan, Z. *Chemical Communications (Cambridge, United Kingdom)* 2006, 3069.

(18) Jordao, A. K.; Afonso, P. P.; Ferreira, V. F.; de Souza, M. C. B. V.; Almeida, M. C. B.; Beltrame, C. O.; Paiva, D. P.; Wardell, S. M. S. V.; Wardell, J. L.; Tiekink, E. R. T.; Damaso, C. R.; Cunha, A. C. *European Journal of Medicinal Chemistry* 2009, *44*, 3777.

(19) Giffin, M. J.; Heaslet, H.; Brik, A.; Lin, Y.-C.; Cauvi, G.; Wong, C.-H.; McRee, D. E.; Elder, J. H.; Stout, C. D.; Torbett, B. E. *J. Med. Chem.* 2008, *51*, 6263.

(20) Upadhayaya, R. S.; Kulkarni, G. M.; Vasireddy, N. R.; Vandavasi, J. K.; Dixit, S. S.; Sharma, V.; Chattopadhyaya, J. *Bioorganic & Medicinal Chemistry* 2009, *17*, 4681.

(21) Gill, C.; Jadhav, G.; Shaikh, M.; Kale, R.; Ghawalkar, A.; Nagargoje, D.; Shiradkar, M. *Bioorganic & Medicinal Chemistry Letters* 2008, *18*, 6244.

(22) Sangshetti, J. N.; Nagawade, R. R.; Shinde, D. B. *Bioorganic & Medicinal Chemistry Letters* 2009, *19*, 3564.

(23) Wuest, F.; Tang, X.; Kniess, T.; Pietzsch, J.; Suresh, M. *Bioorganic & Medicinal Chemistry* 2009, *17*, 1146.

(24) Poulsen, S.-A.; Wilkinson, B. L.; Innocenti, A.; Vullo, D.; Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 2008, *18*, 4624.

(25) Chen, Y.; Lopez-Sanchez, M.; Savoy, D. N.; Billadeau, D. D.; Dow, G. S.; Kozikowski, A. P. *J. Med. Chem.* 2008, *51*, 3437.

(26) Pagliai, F.; Pirali, T.; Del Grosso, E.; Di Brisco, R.; Tron, G. C.; Sorba, G.; Genazzani, A. A. *J. Med. Chem.* 2006, *49*, 467.

(27) Kamal, A.; Shankaraiah, N.; Devaiah, V.; Reddy, K. L.; Juvekar, A.; Sen, S.; Kurian, N.; Zingde, S. *Bioorganic & Medicinal Chemistry Letters* 2008, *18*, 1468.

(28) Huisgen, R. *Angewandte Chemie* 1963, *75*, 604.

(29) Michael, A.; Luehn, F.; Higbee, H. H. *American Chemical Journal* 1898, *20*, 377.

(30) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* 2002, *67*, 3057.

(31) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* 2002, *41*, 2596.

(32) Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* 2005, *127*, 15998.

(33) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *Journal of the American Chemical Society* 2004, *126*, 15046.

(34) Jewett, J. C.; Bertozzi, C. R. *Chem. Soc. Rev.* 2010, *39*, 1272.

(35) Reddy, K. R.; Rajgopal, K.; Kantam, M. L. *Synlett* 2006, 957.

(36) Diez-Gonzalez, S. *Catal. Sci. Technol.* 2011, *1*, 166.

(37) Rodionov, V. O.; Presolski, S. I.; Gardinier, S.; Lim, Y.-H.; Finn, M. G. *J. Am. Chem. Soc.* 2007, *129*, 12696.

(38) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* 2004, *6*, 2853.

(39) Presolski, S. I.; Hong, V.; Cho, S.-H.; Finn, M. G. *Journal of the American Chemical Society* 2010, *132*, 14570.

(40) Soriano del Amo, D.; Wang, W.; Jiang, H.; Besanceney, C.; Yan, A. C.; Levy, M.; Liu, Y.; Marlow, F. L.; Wu, P. *Journal of the American Chemical Society* 2010, *132*, 16893.

(41) Candelon, N.; Lastecoueres, D.; Diallo, A. K.; Ruiz Aranzaes, J.; Astruc, D.; Vincent, J.-M. *Chem. Commun.* 2008, 741.

(42) Li, F.; Hor, T. S. A. *Chem.--Eur. J.* 2009, *15*, 10585.

(43) Asano, K.; Matsubara, S. *Organic Letters* 2010, *12*, 4988.

(44) Sen Gupta, S.; Kuzelka, J.; Singh, P.; Lewis, W. G.; Manchester, M.; Finn, M. G. *Bioconjugate Chemistry* 2005, *16*, 1572.

(45) Teyssot, M. L.; Nauton, L.; Canet, J.-L.; Cisnetti, F.; Chevry, A.; Gautier, A. *Eur. J. Org. Chem.* 2010, 3507.

(46) Lal, S.; Diez-Gonzalez, S. *J. Org. Chem.* 2011, *76*, 2367.

(47) Wang, D.; Zhao, M.; Liu, X.; Chen, Y.; Li, N.; Chen, B. *Org. Biomol. Chem.* 2012, *10*, 229.

(48) Shao, C.; Wang, X.; Xu, J.; Zhao, J.; Zhang, Q.; Hu, Y. *The Journal of Organic Chemistry* 2010, *75*, 7002.

(49) Fabbrizzi, P.; Cicchi, S.; Brandi, A.; Sperotto, E.; van Koten, G. *Eur. J. Org. Chem.* 2009.

(50) Duran Pachon, L.; van Maarseveen, J. H.; Rothenberg, G. *Adv. Synth. Catal.* 2005, *347*, 811.

(51) Namitharan, K.; Kumarraja, M.; Pitchumani, K. *Chem.--Eur. J.* 2009, *15*, 2755.

(52) Chassaing, S.; Kumarraja, M.; Sido, A. S. S.; Pale, P.; Sommer, J. *Org. Lett.* 2007, *9*, 883.

(53) Kantam, M. L.; Jaya, V. S.; Sreedhar, B.; Rao, M. M.; Choudary, B. M. *J. Mol. Catal. A: Chem.* 2006, *256*, 273.

(54) Lipshutz, B. H.; Taft, B. R. *Angew. Chem., Int. Ed.* 2006, *45*, 8235.

(55) Chan, T. R.; Fokin, V. V. *QSAR Comb. Sci.* 2007, *26*, 1274.

(56) Girard, C.; Oenen, E.; Aufort, M.; Beauviere, S.; Samson, E.; Herscovici, J. *Org. Lett.* 2006, *8*, 1689.

(57) Miao, T.; Wang, L. *Synthesis* 2008, *3*, 363.

(58) Coelho, A.; Diz, P.; Caamano, O.; Sotelo, E. *Adv. Synth. Catal.* 2010, *352*, 1179.

(59) Cantillo, D.; Avalos, M.; Babiono, R.; Cintas, P.; Jimenez, J. L.; Palacios, J. C. *Org. Biomol. Chem.* 2011, *9*, 2952.

(60) Rodionov Valentin, O.; Fokin Valery, V.; Finn, M. G. *Angewandte Chemie (International ed. in English)* 2005, *44*, 2210.

(61) Uma, R.; Palaniandavar, M.; Butcher, R. J. *J. Chem. Soc. Dalton Trans.* 1996, 2061.

(62) Zou, X.; Cai, J.; Feng, X.; Hu, X.; Yang, G.; Zhang, H.; Ji, L. *Transistion Metal Chem.* 2001, *26*, 704.

(63) Machura, B.; Switlicka, A.; Kruszynski, R.; Mrozinski, J.; Klak, J.; Kusz, J. *Polyhedron* 2008, *27*, 2959.

(64) Malecki, J. G.; Machura, B.; Switlicka, A. *Struct Chem* 2011, *22*, 77.

(65) Soudi, A. A.; Farzin, M.; Morsali, A.; Kempe, R.; Hertle, I. *J. Coordination Chem.* 2005.

(66) El Jammal, A.; Graf, E.; Gross, M. *J. Electroanal. Chem.* 1986, *214*, 507.

(67) Ahlquist, M.; Fokin, V. V. *Organometallics* 2007, *26*, 4389.

(68) Lindsley, C. W.; Layton, M. E. *Sci. Synth.* 2004, *17*, 357.

(69) Kelly, T. R.; Elliott, E. L.; Lebedev, R.; Pagalday, J. *Journal of the American Chemical Society* 2006, *128*, 5646.

(70) Barraclough, P.; Firmin, D.; Iyer, R.; King, W. R.; Lindon, J. C.; Nobbs, M. S.; Smith, S.; Wharton, C. J.; Williams, J. M. *J. Chem. Soc., Perkin Trans. 2* 1988, 1839.

(71) Catozzi, N.; Edwards, M. G.; Raw, S. A.; Wasnaire, P.; Taylor, R. J. K. *The Journal of Organic Chemistry* 2009, *74*, 8343.

(72) Boger, D. L.; Hong, J.; Hikota, M.; Ishida, M. *Journal of the American Chemical Society* 1999, *121*, 2471.

(73) Theriault, J. R.; Felts, A. S.; Bates, B. S.; Perez, J. R.; Palmer, M.; Gilbert, S. R.; Dawson, E. S.; Engers, J. L.; Lindsley, C. W.; Emmitte, K. A. *Bioorganic & Medicinal Chemistry Letters* 2012, *22*, 76.

(74) Congreve, M.; Andrews, S. P.; Doré, A. S.; Hollenstein, K.; Hurrell, E.; Langmead, C. J.; Mason, J. S.; Ng, I. W.; Tehan, B.; Zhukov, A.; Weir, M.; Marshall, F. H. *Journal of Medicinal Chemistry* 2012, *55*, 1898.

(75) Abdel-Rahman, R. M. *Pharmazie* 2001, *56*, 18.

(76) Hennessy, D. J.; Reid, G. R.; Smith, F. E.; Thompson, S. L. *Can. J. Chem.* 1984, *62*, 721.

(77) Abdel-Rahman, R. M. *Pharmazie* 2001, *56*, 195.

(78) Stookey, L. L. *Analytical Chemistry* 1970, *42*, 779.

(79) Panayiotidou, L.; Stylianou, M.; Arabatzis, N.; Drouza, C.; Lianos, P.; Stathatos, E.; Keramidas, A. D. *Polyhedron*.

(80) Shipe, W. D.; Yang, F.; Zhao, Z.; Wolkenberg, S. E.; Nolt, M. B.; Lindsley, C. W. *Heterocycles* 2006, *70*, 655.

(81) Whitten, J. P.; McCarthy, J. R.; Matthews, D. P. *Synthesis* 1988, 470.

(82) Do, H.-Q.; Daugulis, O. *Organic Letters* 2010, *12*, 2517.

(83) France-Aimee, A.; Suzenet, F.; Keromnes, A.; Lebret, B.; Guillaumet, G. *Synthesis* 2004, *17*, 2893.

(84) Jaramillo, D.; Liu, Q.; Aldrich-Wright, J.; Tor, Y. *The Journal of Organic Chemistry* 2004, *69*, 8151.

(85) Neunhoeffer, H.; Hennig, H.; Fruehauf, H. W.; Mutterer, M. *Tetrahedron Lett.* 1969, 3147.

(86) Neunhoeffer, H.; Motitschke, L.; Hennig, H.; Ostheimer, K. *Justus Liebigs Ann. Chem.* 1972, *760*, 88.

(87) Eltayeb, N. E.; Teoh, S. G.; Fun, H.-K.; Ibrahim, K.; Rosli, M. M. *Acta. Cryst.* 2007, *E63*, m1182.

(88) Smith, P. A. S.; Boyer, J. H. *Organic Syntheses* 1951, *31*, 14.

(89) Deev, S. L.; Shenkarev, Z. O.; Shestakova, T. S.; Chupakhin, O. N.; Rusinov, V. L.; Arseniev, A. S. *J. Org. Chem.* 2010, *75*, 8487.

(90) Huang, J. J. *J. Org. Chem.* 1985, *50*, 2293.

(91) Boger, D. L. *Chem. Rev.* 1986, *86*, 781.

(92) Tang, H.; Radosz, M.; Shen, Y. *Macromol. Rapid Commun.* 2006, *27*, 1127.

(93) Angell, Y.; Burgess, K. *Angew. Chem., Int. Ed.* 2007, *46*, 3649.

(94) Ozcubukcu, S.; Ozkal, E.; Jimeno, C.; Pericas, M. A. *Org. Lett.* 2009, *11*, 4680.

(95) Smith, N. M.; Greaves, M. J.; Jewell, R.; Perry, M. W. D.; Stocks, M. J.; Stonehouse, J. P. *Synlett* 2009, *9*, 1391.

(96) Lorincz, K.; Kele, P.; Novak, Z. *Synthesis* 2009, *20*, 3527.

(97) Prince, A. L. *PhD diss., University of Tennessee* 2011.

(98) Gryder, B. E.; Sodji, Q. H.; Oyelere, A. K. *Future Medicinal Chemistry* 2012, *4*, 505.

(99) Zheng, Y. G.; Wu, J.; Chen, Z.; Goodman, M. *Med. Res. Rev.* 2008, *28*, 645.

(100) Cairns, B. R. *Nature (London, United Kingdom)* 2009, *461*, 193.

(101) Schulz Wolfgang, A.; Hoffmann Michele, J. *Epigenetics* 2007, *2*, 37.

(102) Morris, K. V. *Epigenetics* 2009, *4*, 296.

(103) Witt, O.; Deubzer, H. E.; Milde, T.; Oehme, I. *Cancer Letters (Shannon, Ireland)* 2009, *277*, 8.

(104) Rodriguez-Paredes, M.; Esteller, M. *Nature Medicine (New York, NY, United States)* 2011, *17*, 330.

(105) Kazantsev, A. G.; Thompson, L. M. *Nature Reviews Drug Discovery* 2008, *7*, 854.

(106) Georgopoulos, K. *Nature Immunology* 2009, *10*, 13.

(107) Huang, B. H.; Laban, M.; Leung, C. H. W.; Lee, L.; Lee, C. K.; Salto-Tellez, M.; Raju, G. C.; Hooi, S. C. *Cell Death and Differentiation* 2005, *12*, 395.

(108) Monneret, C. *Anti-Cancer Drugs* 2007, *18*, 363.

(109) Friend, C.; Scher, W.; Holland, J. G.; Sato, T. *Proc. Nat. Acad. Sci. U. S.* 1971, *68*, 378.

(110) Marks, P. A. *Oncogene* 2007, *26*, 1351.

(111) Paris, M.; Porcelloni, M.; Binaschi, M.; Fattori, D. *Journal of Medicinal Chemistry* 2008, *51*, 1505.

(112) Dokmanovic, M.; Clarke, C.; Marks, P. A. *Molecular Cancer Research* 2007, *5*, 981.

(113) Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature (London)* 1999, *401*, 188.

(114) Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B.-C.; Verner, E.; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. *Structure (Cambridge, MA, United States)* 2004, *12*, 1325.

(115) Mann, B. S.; Johnson, J. R.; Cohen, M. H.; Justice, R.; Pazdur, R. *Oncologist* 2007, *12*, 1247.

(116) Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* 2003, *46*, 5097.

(117) Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K.-H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Research* 2002, *62*, 4916.

(118) Pirali, T.; Pagliai, F.; Mercurio, C.; Boggio, R.; Canonico, P. L.; Sorba, G.; Tron, G. C.; Genazzani, A. A. *J Comb Chem* 2008, *10*, 624.

(119) Chen, P. C.; Patil, V.; Guerrant, W.; Green, P.; Oyelere, A. K. *Bioorganic & Medicinal Chemistry* 2008, *16*, 4839.

(120) Fennell, K. A.; Miller, M. J. *Organic Letters* 2007, *9*, 1683.

(121) Stefely, J. A.; Palchaudhuri, R.; Miller, P. A.; Peterson, R. J.; Moraski, G. C.; Hergenrother, P. J.; Miller, M. J. *Journal of Medicinal Chemistry* 2010, *53*, 3389.

(122) Shen, J.; Woodward, R.; Kedenburg, J. P.; Liu, X.; Chen, M.; Fang, L.; Sun, D.; Wang, P. G. *Journal of Medicinal Chemistry* 2008, *51*, 7417.

(123) Gao, D. A.; Xiong, Z.; Heim-Riether, A.; Amodeo, L.; August, E. M.; Cao, X.; Ciccarelli, L.; Collins, B. K.; Harrington, K.; Haverty, K.; Hill-Drzewi, M.; Li, X.; Liang, S.; Margarit, S. M.; Moss, N.; Nagaraja, N.; Proudfoot, J.; Roman, R.; Schlyer, S.; Keenan, L. S.; Taylor, S.; Wellenzohn, B.; Wiedenmayer, D.; Li, J.; Farrow, N. A. *Bioorganic & Medicinal Chemistry Letters* 2010, *20*, 5039.

Vita

Belinda Shea Lady was born and raised in Bowling Green, Ky. After graduating from Warren East High School, she enrolled at Western Kentucky University where she received a Bachelor of Science degree in Chemistry, graduating summa cum laude. From there she pursued a Ph.D. in organic chemistry under the guidance of Shane Foister at the University of Tennessee, Knoxville