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Bioorthogonal Reactions: Synthesis and Evaluation of Different Ligands in Copper Catalyzed Azide-Alkyne1,3-Dipolar Cycloaddition (CuAAC)

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Bioorthogonal Reactions: Synthesis and Evaluation of Different Ligands in Copper CatalyzedAzide-Alkyne1,3- Dipolar Cycloaddition (CuAAC)

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by

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Abstract

The Copper CatalyzedAzide-Alkyne1,3-Dipolar Cycloaddition (CuAAC) reaction has unique features that qualify it to be one of the best click reactions. Its applications have been shown in different aspects and for multiple purposes. The oxidative degradation of biological systems (labile proteins and live cells) is, however, generally recognized as the major problem when using this reaction in living systems. Reactive oxidation species can be easily produced in the presence of copper(II), ascorbate and air, and this is the main cause of toxicity. However, the uses of ligands have shown a major impact on reducing copper toxicity, protecting Cu(I) from the redox potential, and increasing the reaction rate. The aim of this study is to synthesize three different water-soluble ligands for evaluation in CuAAC reactions and to see if they can facilitate CuAAC in aqueous systems. These ligands were chosen because of their low cost and the simplicity of the production process. The key finding of this study is that the three different ligands were able to be synthesized from the same starting materials with small alterations to the process. The three ligands were also evaluated in CuAAC reactions, and it was found that the first ligand with –OH achieved higher efficiency in enhancing the reaction rate than the other ligands.

Acknowledgment

It is a pleasure to thank those who supported me and encouraged me to be successful during my studies. I owe my deepest gratitude to my god for his blessings and aid in overcoming all the difficulties and challenges I faced in pursuing my goals. I am greatly indebted to my advisor, Professor Shi for his support and guidance. I also would like to express my gratitude to my committee members, Dr. Durham and Dr. Allison. I am grateful to both postdoctoral follows, Drs. Guanghui Zong and Xianwei Sun who taught me how to work in the lab. Finally, it is an honor for me to thank all my friends, especially Sefat Alwarsh, and my family for their support and prayers.

Dedication

This edition of Synthesis and Evaluation of Different Ligands in Copper CatalyzedAzide-Alkyne1, 3 Dipolar Cycloaddition (CuAAC) is dedicated to Imam Almahdi and to the family of the prophet Mohammad.

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Chapter 1: Introduction

1.1 Overview

1.1.1 Bioorthogonal reactions

Researchers in chemistry and biology, finding that they share some common interests, have increasingly begun sharing information and developing new methods for studying biomolecules, such as proteins inside living systems. Because of the fast development of interdisciplinary research, scientists have successfully achieved an innovation called bioorthogonal chemistry. Bioothogonal chemistry is a modern technology defined as chemical reactions, whose components, under physiological conditions, must react selectively and rapidly with each other, without interfering with the original biological processes and without causing toxicity to cells and living organisms.[1] One type of the most well-known bioorhogonal reactions is the so-called click chemistry.

1.1.2 “Click" Reactions

A click reaction is a synthetic method that refers to the ligation of two building blocks (biomolecules) in order to form very selective products in high yield. The applications of the click reaction have been shown in the discovery of bioactive molecules and many other aspects of drug discovery.[2]

A perfect click reaction should meet some particular standards. First, the reaction should be chemoselective and orthogonal (or inert) to many functional groups present in biomolecules. Second, it should be rapid and effective under physiological conditions. Finally, it should minimize steps of protection and deprotection.[3] Sharpless and co-workers listed the criteria for "click chemistry", in 2001, as *"The reaction must be modular, wide in scope, give very high*

yields, generate only inoffensive by-products that can be removed by non-chromatographic methods, and be stereospecific (but not necessarily enantioselective). The required process characteristics include simple reaction conditions, readily available starting materials and reagents, the use of no solvents or a solvent that is benign (such as water) or easily removed, and simple product isolation".[3]

Click reactions are classified into four categories: (i) cycloaddition of unsaturated species such as the Diels-Alder transformation between diene and dienophile, and 1,3-dipolar cycloaddition, such as a reaction of alkyne and azide to produce triazole. (ii) nucleophilic substitution chemistry including ring-opining reactions such as epoxide. (iii) non-aldo carbonyl group reactions such as formation of hydrazones and urea. (iv) addition reactions to carbon-carbon multiple bond such as Michael addition. (**Figure 1**).[2] Despite the many click reactions developed recently, none can satisfy all the criteria of the perfect click reaction. Copper catalyzed azide-alkyne 1,3- dipolar cycloaddition (CuAAC) has, however, been considered as one of the best click reactions because it meets most of the criteria of a perfect click reaction.[1]

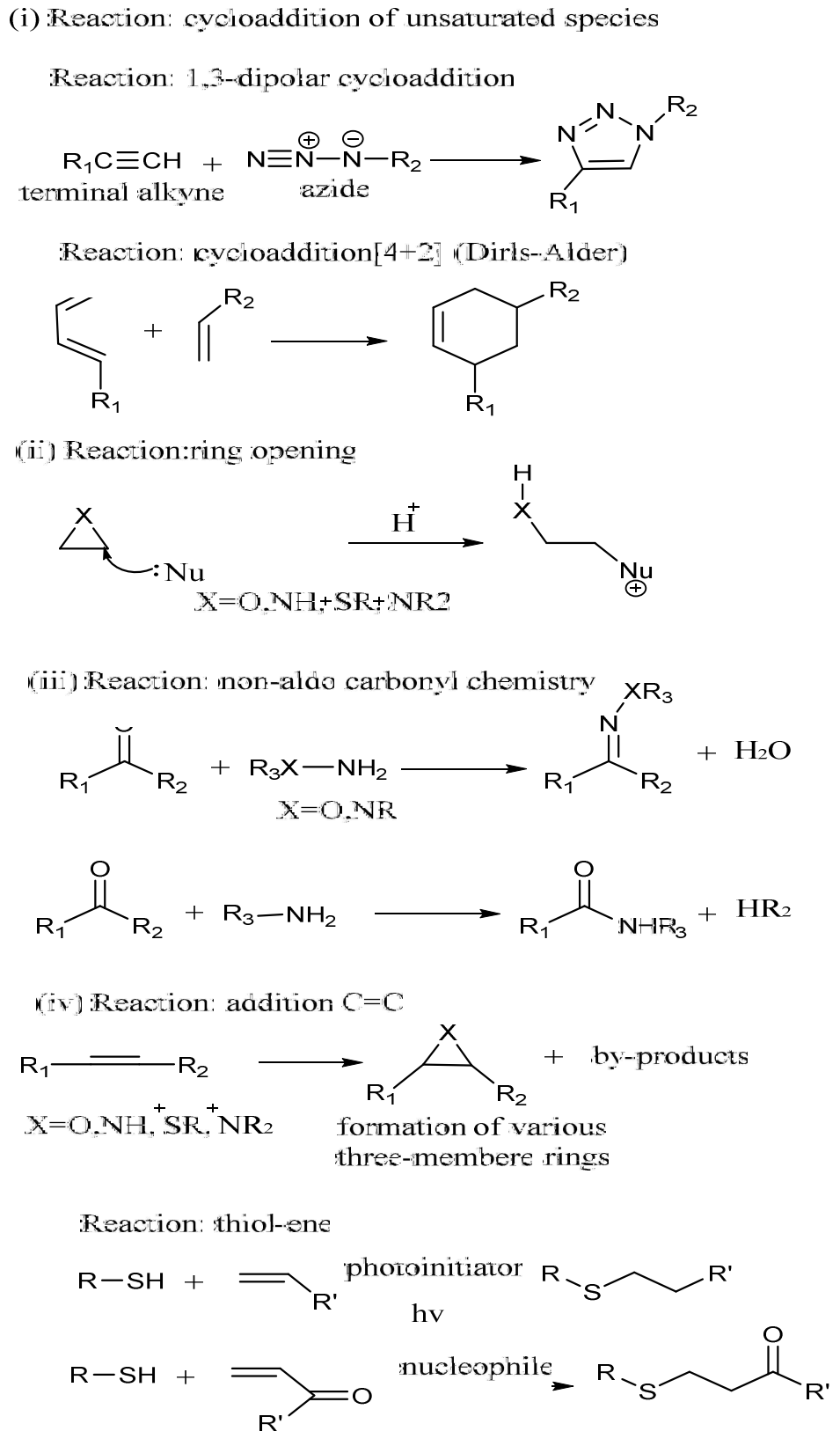

Figure 1. *Different types of click reactions.*

1.1.3 Copper (I)- Catalyzed Azide-Alkyne Cycloaddition(CuAAC) Reaction

The first discovery of a 1,3-dipolar cycloaddition reaction between azide and alkyne was by Rolf Huisgen at the end of the 19th century.[1] The principle of the reaction performs on cycloaddition between azide and alkyne producing two mixture regioisomers of triazoles, the 1,4- and the 1,5-regioisomers, which need to be separated using a classical chromatographic procedure. Due to the kinetic stability of the reactants, azide and alkyne, the reaction is typically very slow. Also, this transformation requires high pressure or temperature in addition to a long period of time to complete.[1] In order to solve this problem, in 2002, [1,3,4] Sharpless and Meldal reported in different studies that using copper as a catalyst evoked a noticeable enhancement in the reaction rate, 10^7 rate enhancement over non catalyzed reaction, at room temperature, and gave only one product, the 1,4-regioisomers **(Figure 2).**[3]

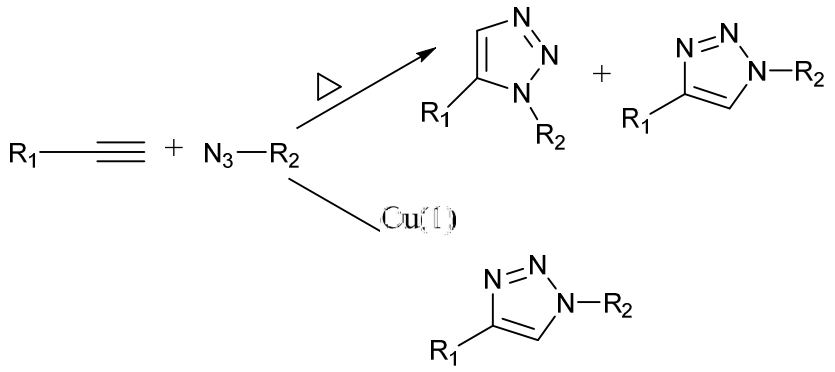

Figure 2. *The1,3-dipolar cycloaddition between azides and alkynes.*

In the1960s, Witting and Krebs modified the method and developed strain-promoted azide- alkyne cycloaddition, by using a strained cyclooctyne.[1] The aim of doing this modification was to improve the kinetics of the reaction and avoid using a metal such as copper as a catalyst. The study reported that strained cyclooctyne is the smallest stable cycloalkyne, but it is very active when it combines with phenylazide. A strained cyclooctyne seems to be effective and

advantageous because of its ability in identifying biological samples without cytotoxic effects. Although the improvements were noticeable, this type of reaction is not as fast as CuAAC. Many researchers have shown that CuAAC is considerably faster in an aqueous solution than strain–promoted azide- alkyne cycloaddition (SPAAC), 10-100 times faster, in fact.[5] In addition, the CuAAC reaction is an attractive candidate for labeling biomolecules in vivo because this reaction involves a terminal alkyne that represents an excellent bioorthogonal handle.[5]

Another famous bioorthognal reaction, known as the Staudinger ligation was notified by Saxon and Bertozzi in 2000. [1,6,7] This type of reaction proceeds by covalently linking azide and ester-functionalized triphenylphosphine by an amide bond **(Figure 3)**.[6] In spite of high specificity for the azide group, the reaction is considered kinetically slow. It is actually 25 times slower than CuAAC reaction .[8] Undesirable side reactions, due to the oxidation of phosphine reagents in air, may affect selectivity.[1, 7]

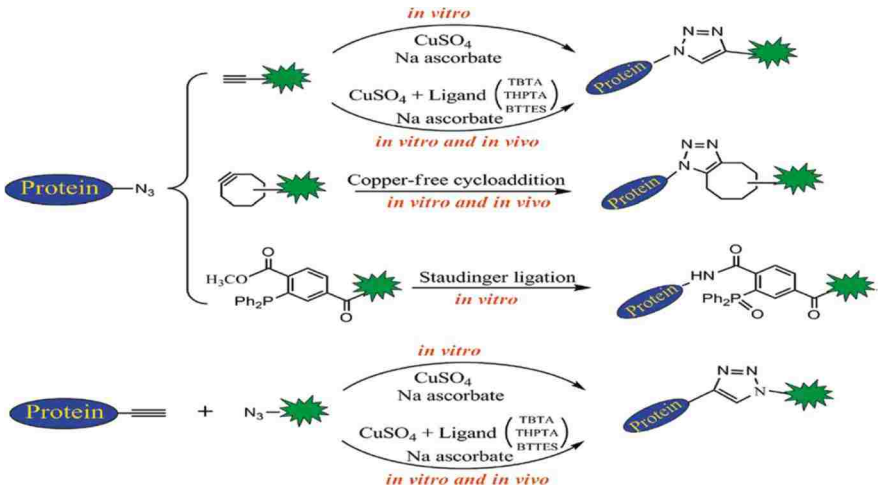

Figure 3. *Azide and alkyne participate in biorthogonal reactions on protiens, one of which The Staudinger ligation.*[6]

1.2 Mechanism of CuAAC Reaction

Any mechanism aiming to describe the CuAAC reaction should provide all experimental evidence to explain some key features related to the reaction: including the unique method of its transformation, that is, showing the orthogonality of azide and terminal alkyne with most other functional groups.[9] In addition, the reaction should proceed well in various solvents, and in a wide range of pH[10] and temperatures. Based on kinetic and computational studies, Finn et al suggested a stepwise mechanism of the CuAAC reaction that showed all of its key features.[9]

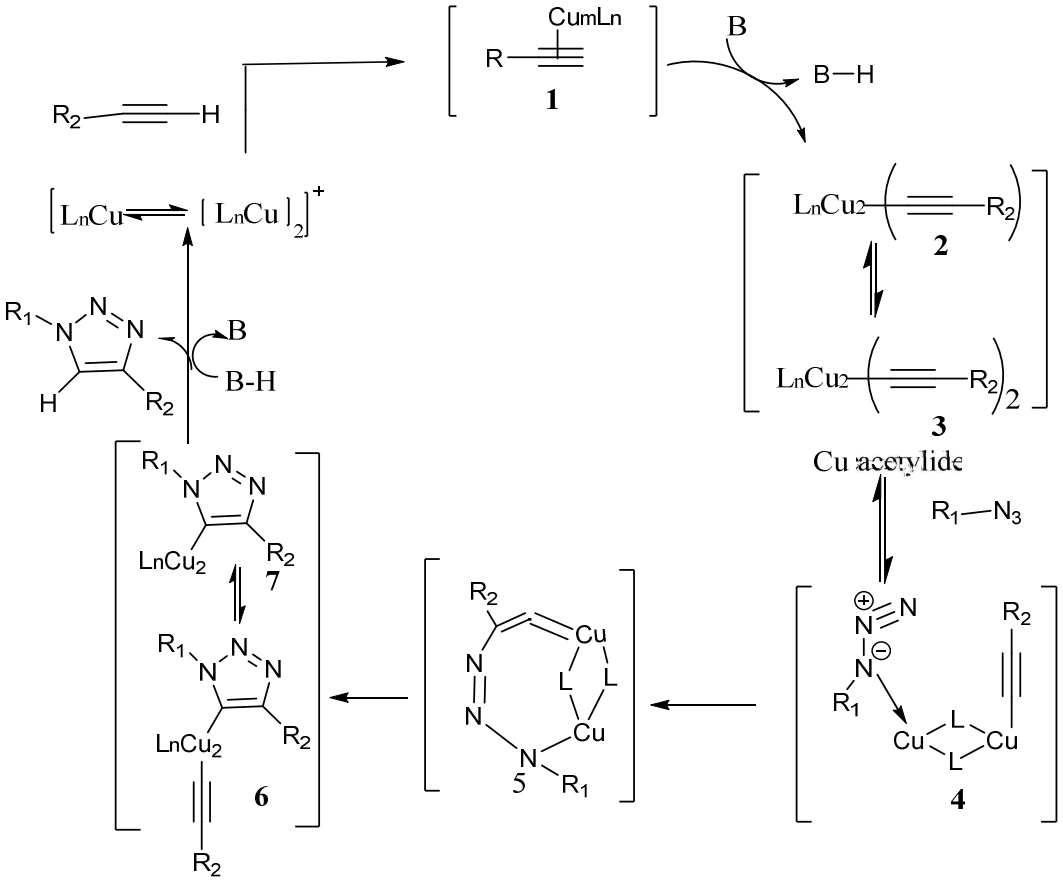

Figure 4. *The catalytic cycle of CuAAC.*

It is reported that the thermal dipolar cycloaddition of alkynes and azides proceeds through a concerted mechanism. However, for any concerted process, the lowest activation

barrier found is 23.7 kcal/mol, which is too high for the reaction to occur at room temperature. In contrast, a stepwise mechanism using Cu(I) as the catalyst decreases the activation barrier associated with the concerted process by 11 kcal/mol when compared to the concerted process, which demonstrates the reason for the enhancement of the reaction rate under the Cu(I) catalyst. The mechanism of the reaction starts by the formation of Alkyne-Cu(I) π-complex **1 (Figure 4).** The formation of copper acetylide **2** is in equilibrium with **3**. DFT calculations proposed that coordination of Cu(I) to the terminal alkyne creating **1** decreases the pKa of the acetylenic proton by up to 9.8 pH units,[9,11] which makes deprotonation possible in aqueous systems without needing to add a strong base. In spite of the significant influence on pKa, the Cu(I)-acetylide species were found even at acidic pH in water solution. Recent results showed the existence of distinct Cu(I)-acetylide species, but the most dominant species were determined based on reaction conditions. The existence of multiple Cu(I)-acetylide species may even consist of π-complexes, further complicates matters.[9]

After the production of the active Cu(I)-acetylide species **2**, the attack of azide causes displacement of the labile ligand, or ligand exchange at the copper center with azide, producing a Cu(I)-acetylide azide complex **4**.[12] Subsequently, the cyclization of the dimeric Cu(I) complexes, which is assumed to be similar to the cyclization of the monomeric Cu(I) species, takes place at this position. Then, metallocycle **5** is generated by the activation of azide, which is caused by the coordination of azide to the copper center, toward the nucleophilic attack of a lone pair of electron on N (3) at acetylide carbon C (4). This mechanism shows the importance of electron-withdrawing substituent on alkyne and the way in which it participates in accelerating the reaction rate of CuAAC. Moreover, the transformation of **5** into triazole- copper derivative **7** is

rapid, followed by the formation of **6** occurring by protonation of **7,** or by isolation of the product and regeneration of catalyst.[9,11]

Despite the fact that many mechanisms have proposed to describe the CuAAC reaction, not all of them have the ability to understand the reaction regarding active Cu (I)-acetylide complexes that exist in the reaction mixtures. The Cu(I)-acetylide species plays a crucial role in determining the rate of the CuAAC reaction. Kinetics studies of CuAAC reactions found that when the concentration of copper is low, the rate of the catalytic process in Cu(I) is the second order. On the other hand, increasing the concentration of copper, leads to the formation of less reactive species including metal aggregates. Under an excessive amount of copper, the reaction was in between a first and a second order in alkyne concentration. Because of that, two pathways were suggested to describe the mechanism involving one and two acetylenes. The pathway requiring two acetylenes is favored, but it is inhibited at higher concentration. The higher concentration of alkyne may negatively influence the coordination of the copper ion because of the tendency of Cu(I) to alkyne ligand more than azide ligand. Consequently, it prevents azide from binding to the metal and reduces the overall reaction rate. To support this information, commercially available Cu(I)–acetylides, show no catalytic activity because alkyne might affectively saturate copper ion. That emphasizes the significance of labile ligand dissociation from catalysts. Some conclusions are drawn based on the evidence that is currently available. Some requirements that copper acetylide species should achieve for successful catalysis are proposed as follows: two metal centers, labile ligands allowing the binding of azide, and one or two alkyne ligands (**Figure 4**).[9]

.

1.3 Applications of the CuAAC Reaction

The development of the CuAAC reaction is significant because it has numerous applications in many distinct fields, including chemical biology, polymer chemistry, and organic synthesis.[1,2] The CuAAC reaction has unique properties such as simplicity and tolerance to the aqueous environment. In addition, triazole ring inertness qualifies this protocol to be used in the linking of two or more molecular entities. For example, using this reaction contributes to generation of dimers, chimeras and multivalence drugs. The strategy of homodimers proved to be successful in increasing the strength of biological activity of compounds. The first try using click reactions with this hypothesis made derivatives of Vitamin D_3. The CuAAC reaction was used to connect two side chains of Vitamin D_3 and form the desired product in high yield and with very good regioselective control, that is, the 1, 4-disubsitituted triazole dimers. (e.g., **8**) (**Figure 5).**[3] The idea of using triazole as a linking agent in dimer/polymer has extended to the formation of chimeric drugs capable of two different activities. For instance, CuAAC was successfully utilized to combine an antibiotic with a linezolid and a macrolide (e.g., **9**) (**Figure 6).**[3]

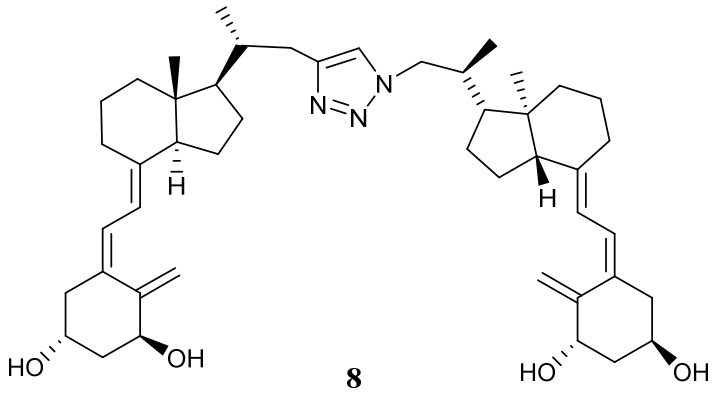

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Figure 5. *An example of homodimers.*

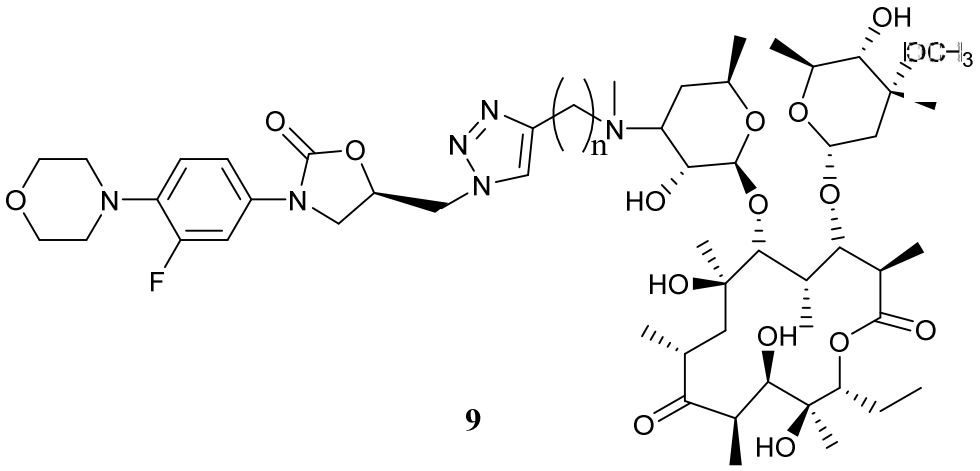

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Figure 6. *An example of heterodimers linked by triazole rings.*

1.3.1 Lipids and Sugars

The applications of the CuAAC reaction continue to be used for a variety of purposes in new research fields. The reaction has been successfully utilized to link lipids to proteins. For example, phosphatidylethanolamine was transformed in an azide derivative that was successful in reacting with a terminal alkyne-modified protein (e.g.**, 10** in **Figure 7).**[2]

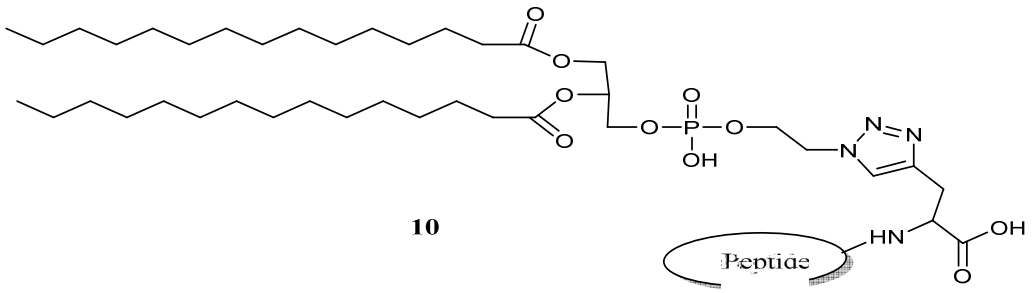

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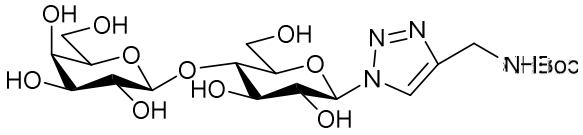
Figure 7. *Phosphatidylethanolamine.*

Click reactions have had a great impact not only on lipids but also on sugars. The applications of CuAAC have been extended to the preparation of carbohydrate derivatives, including production of N-glycosyl triazoles, either by linking simple acetylene (or its derivatives) with a variety of chemical entities including amino acids or sugars (e.g., **11** in

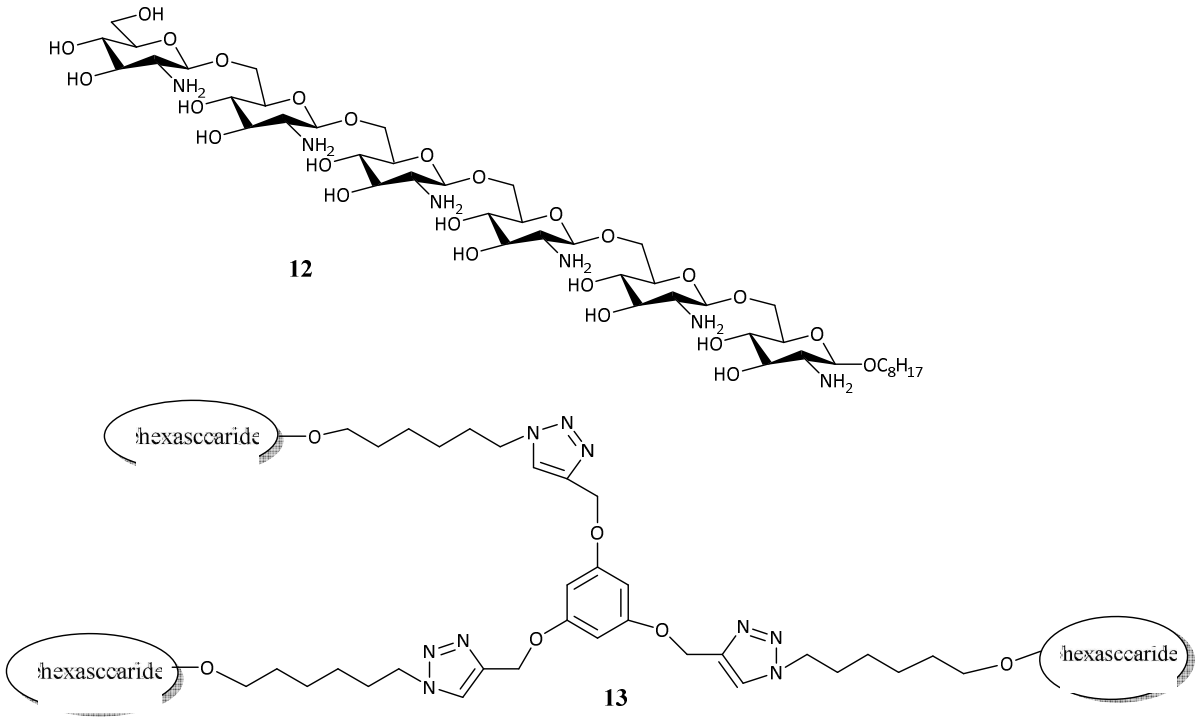
Figure 8).[2] CuAAC has also been found in the production of oligosaccharides, macrocycles and glyco-polycycles, glycopeptides, and glyco-clusters.[2]



11

Figure 8. *Mimetic glycosides produced by cycloaddition reaction between azide and alkyne derivatives.*

The CuAAC reaction has been utilized to attach numerous sugars to a central aromatic scaffold, or to different sugar scaffolds, generating compounds with antitumoral potential as "anticancer drugs". For example, to achieve multivalency, a C3 symmetric (1-6)-N-acetyl-β-$_D$-glucosamine octadecasaccharide (e.g., **13**) was generated by incorporating three antitumoral, β-$_D$-glucosamine hexamers (e.g., **12**), via triazole- linkages **(Figure 9)**.[3]



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13

Figure 9*. Multivalent scaffold via CuAAC.*

1.3.2 Click reactions and Drug Discovery

The applications of click chemistry include the optimization and the discovery of bioactive compounds, which are especially useful in the development of new drugs directed toward diverse therapeutic targets. For instance, click reactions have been used to design new neuraminidase inhibitors. These inhibitors are designed to obtain drugs that are effective in the treatment of avian influenza virus (AIV). Recently, Linhardt et al. synthesized the derivatives of non-hydrolysable1,2,3-triazol-linked sialic acid (e.g., **15**) by click reaction using alpha sialic acid azide (e.g., **14**) with terminal alkynes for inhibiting neuraminidase (**Figure 10**).[2] In order to optimize the affinity of a neuraminidase inhibitor, a sialic acid disaccharide mimic (e.g., **16**) and a model dendrimer of sialic acid (e.g., **17**) (examples of multivalent derivatives) were synthesized using the CuAAC reaction **(Figure 11).**[2]

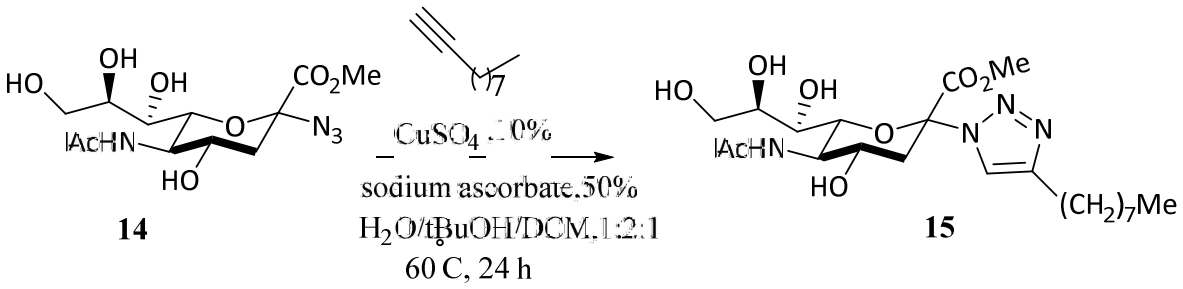


Figure 10. *Generation of 1,2,3-triazole-linked sialic acid.*

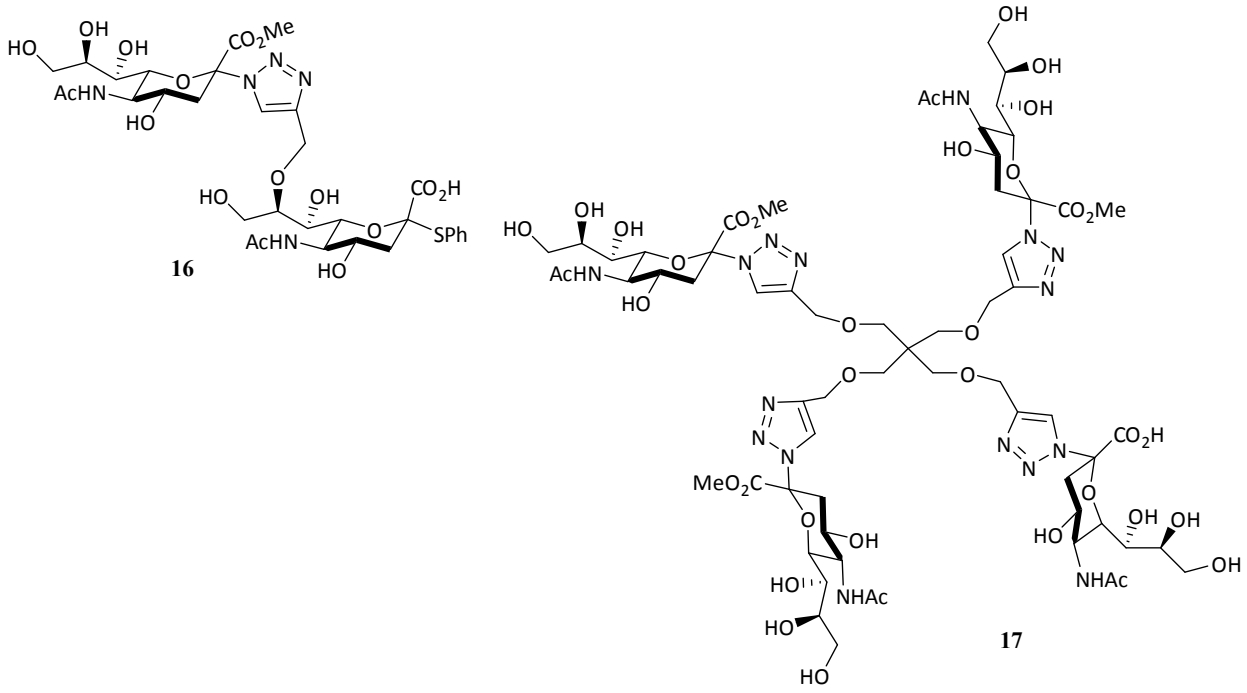


Figure 11. *Sialic acid disaccharide mimic* ***16*** *and dendrimer of sialic acid* ***17*** *as neuraminidase inhibitors.*

1.3.3 Click chemistry and the development of neoglycopolymers

Click chemistry reactions have had great applications in synthesizing biopolymers with diverse structures by creating polymeric biomaterials via a triazole bridge. This reaction occurs by the link of azide- and alkyne –containing molecules, such as proteins, sugars, peptides, nucleic acids, viruses and cells (**Figure 12)**.[2]

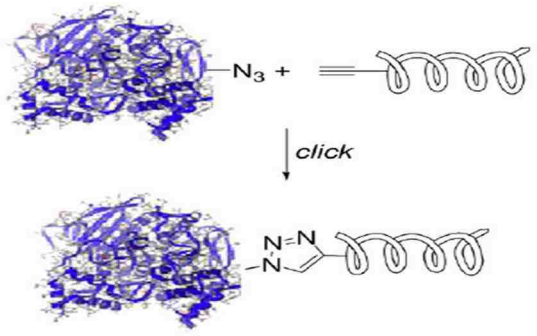


Figure 12*. Synthesis of biomolecular structures by click chemistry.*[2]

One of the powerful applications of bioorthogonal reactions in a living system is its use in tagging distinct classes of biomolecules in cells. There is a strategy called genetic code expansion, which can be coupled with bioorthognal chemistry to label proteins in the living system. Protein labeling requires unique chemical groups that work as handles to incorporate new functional modules into target proteins without reacting with other biomolecules in cells under physiological conditions.[5] These chemical groups must be both stable in aqueous systems and nontoxic. As a powerful bioorthognal reaction, CuAAC has met the above requirements for labeling proteins bearing a terminal alkyne as a bioorthogonal handle that is easy to incorporate into biomolecules.[5]

Not only proteins but also DNA can be labeled and detected using click reactions in the presence of a ligand, TBTA, which functions by stabilizing the oxidation state of Cu(I). However, DNA fragmentation was observed in the absence of a ligand. Click reactions are also used to synthesize DNA by incorporation of modified uridine nucleotide **18** into certain types of azide labels**,** one of which is coumarin **19. 18** was made from phosphoramidite chemistry. These types of azides provide high-density functionalization of alkyne-modified DNA (**Figure 13).** [13]

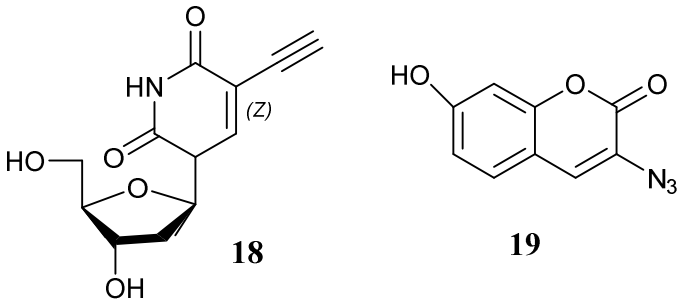


Figure 13. *Alkyne modified uridine nucleosides**18** and azide labels**19** used in the high-density functionalization of alkyne modified DNA.*

1.4 The advantages of CuAAC Reaction

Copper-catalyzed azide-alkyne 1, 3-dipolar cycloaddition is the most powerful and popular bioorthogonal reaction because it has most of the features of a perfect click reaction such as selectivity, efficiency and simplicity.[1] Triazoles, generated from CuAAC, have unique properties of stability and chemoselectivity.[8] They are stable under different conditions, such as acidic or basic hydrolysis, oxidation and reduction. Due to the high dipole moment of heterocycle, triazoles can easily contribute to the formation of hydrogen bonding and dipole-dipole interactions.[3] The reactive groups of azide and alkyne involved in CuAAC have beneficial and effective features that qualify them for labeling proteins: their small size, stability and inactivity under physiological conditions. Their small size also qualifies them to be easily and selectively incorporated into biomolecules by cellular metabolism pathways without interfering with other biomolecules in cells.[8]

Transformations in bioorthognal reactions can be classified into two categories: polar reactions and cycloaddition chemistries (**Figure 14)**.[14] A polar reaction is classified as the reaction between an electrophile and a nucleophile; aldehydes and ketones are more frequently used in polar reactions for bimolecular labelling. They act as electrophiles, which might be selectively linked with alpha-effect nucleophiles, such as hydrazides and aminooxy compounds, to produce stable Schiff bases. However, since the aldehydes are found in glucose and most intracellular metabolites, and also ketones are present in microbial natural products and in mammalian hormones, these naturally-occurring molecules might affect selectivity and lead to undesirable by-products. The second category is cycloaddition reactions, especially dipolar cycloaddition, such as CuAAC, that involves azide and terminal alkynes.[14] This transformation is different from most catalytic processes because it has several key unique features: 1) most of

other functional groups coexisting with alkynes and azides do not require to be protected; 2) CuAAC can occur in various solvent systems, including a mixture of water/ alcohol (most commonly used), acetone, tetrahydrofuran, dimethylsulfosides, dimethylformamide and acetonitrile, or even water without organic cosolvents; 3) CuAAC proceeds well at wide range of temperatures (0℃-160℃) and pH values (4-12); 4) the pure product is normally obtained by doing simple extraction or filtration, without the need to use column chromatography.[11]

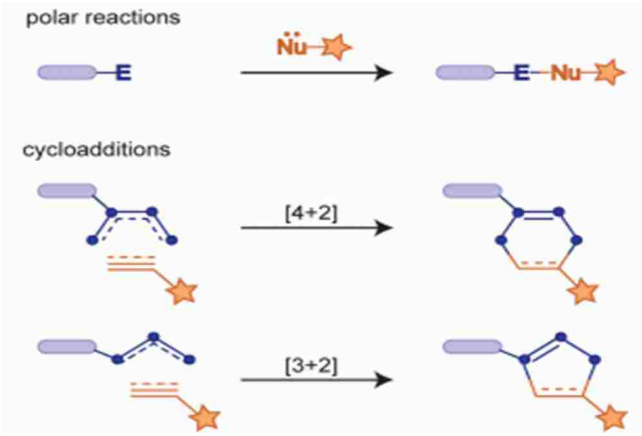


Figure 14 *Two types of transformations in the bioorthogonal toolkit: polar reactions between nucleophiles and electrophiles and cycloaddition.*[14]

1.5 The Disadvantages of CuAAC Reaction

Despite the fact that using copper can significantly accelerate the reaction rate of CuAAC, there is a negative side effect in living systems due to its toxicity. The toxicity of copper was observed in some mammalian cells and caused cell death.[1] Copper toxicity was observed not only in mammalian cells but also in eukaryotic and prokaryotic cells because the oxidative damage of copper impairs many biomolecules in living systems. Recently, some studies have indicated a critical lethal impact of copper occurring inside microorganisms.[5] For example, destruction of Fe-S cluster-containing enzymes found in bacterial cytoplasm is caused by the highly thiophilic Cu(I) ion. The same studies also found that some chelators, such as

bathocuproine sulphonate BPS, have the ability to isolate copper ions, and can inhibit its tendency to harm intercellular Fe-S clusters.[5]

1.6 Catalysts, Solvents, and Additives in the 1,3-Dipolar cycloaddition

Diverse copper sources have been used to catalyze the CuAAC reaction. Copper(I) sources, such as (CuBr), (CuI) or coordinated complexes $Cu(CH_3CN)_4PF_6$, $CuBr(PPh_3)_4$ or $CuIP(OEt)_3$, can be directly utilized to catalyze the reaction. The coordination complexes have been shown to be effective particularly in organic solvents. It requires both a stoichiometric amount of a copper(I) salt and an excess amount of a base, such as a tertiary amine (TEA, DIPEA). Problems with Cu(I) salts are associated with their inherent thermodynamic instability that makes them easily oxidized into inactive Cu(II).[3] In addition, forming Cu(II) salts from oxidation of Cu(I) salts leads to side reactions. Cu(II), being an oxidant, diminishes the reaction rate of CuAAC. To avoid any side reaction, the CuAAC reaction should occur in an oxygen-free environment. Alternatively, the addition of a reducing agent, usually ascorbic acid or its salt together with copper(II) salts in the reaction of CuAAc can aid in avoiding this problem. It has been found that the presence of a sacrificial reducing reagent, such as sodium ascorbate, reduces copper (II) to copper(I) constantly and maintains the catalytic species at high levels. Ascorbic acid plays a significant role in bioconjugate reactions; its combination with copper(II) salts, such as a $CuSO_4.5H_2O$ or $Cu(OAc)_2$, is commonly used in CuAAC standard catalytic systems. The use of aqueous solvents such as a mixture of *tert*-butanol and water makes the use of a base unnecessary in producing copper acetylide species.[10]

Copper(I) plays an important role in accelerating the reaction rate of CuAAC, but its toxicity is still a critical issue when applied in a living system. Some researchers have discovered an alternative strategy that helps improve the catalytic effect of Cu(I). They stated that the uses

of ligands have a major impact on increasing the reaction rate, reducing copper toxicity and protecting Cu(I) from oxidation by oxygen.[6] In other words, a ligand has the ability to coordinate with copper, which helps stabilize Cu(I) oxidation state.[15] Moreover, Cu(I)-ligand complexes can maintain an appropriate concentration of Cu(I) that is responsible for the catalytic activity throughout the reaction. Many ligands have been developed to date. For example, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl) methyl] amine TBTA (e.g., **20**), a ligand developed by Fokin et al, has shown a remarkable enhancement in accelerating the reaction rate. On the other hand, tris(3-hydroxypropyltriazolylmethyl) amine THPTA **(**e.g., **21)**, a water-soluble ligand developed by Finn et al used in living mammalian cells for faster labelling of surface glycans. Later on, fourteen THPTA analogues were developed by Wu and coworkers, and they discovered a new ligand, BTTES **(**e.g., **22)**, that showed many advantages (**Figure 15).**[7] One major advantage of the ligand is to accelerate CuAAC reaction without toxic effects on the living system.[6] Both BTTAA **(**e.g., **23**) and BPS (e.g., **24**) are water soluble, so they dramatically speed up the reaction rate of CuAAC. Their applications have been found to label-cell surface proteins and glycans in live embryos of zebrafish.[5]

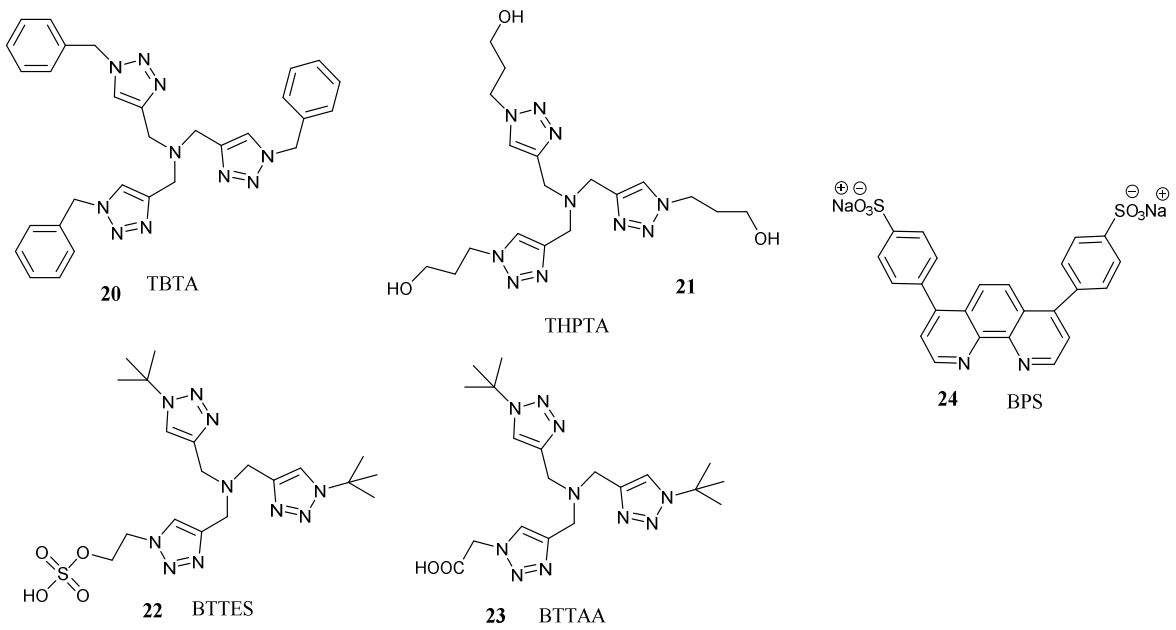


Figure 15. *Cu(I) ligands used to enhance CuAAC reaction.*

In vivo and in vitro experiments have been done to evaluate and compare the efficacy of various complexes, that is, uncoordinated Cu(I) and ligand-coordinate Cu(I) complexes. The study focused on demonstrating reasons beyond the toxicity. It states that uncoordinated Cu(I) is very active in producing damaging hydroxyl radicals.[5] In other words, the toxicity of copper is due to oxidative damage caused by reactive oxygen species (ROS), including: superoxide, hydrogen peroxide and hydroxyl radicals according to equations 1-4 involving the Fenton reaction (**Figure 16**).[15] The toxicity of copper complexes depends on two factors: the number of ligands coordinated and the reduction potential. Fenton chemistry requires the estimated reduction potential of Cu(II)/Cu(I) to be within -160 to 460 mV in order to generate the reactive oxygen species that lead to toxicity.[15] It has been theorized that the tendency of Cu(I) complexes in generating ROS should be found to be low at the high redox potential.[5] However, Fenton reactions can be controlled by ascorbate acid.[15]

Ligand-coordinated Cu(I) complexes, on the other hand, generated much lower levels of oxidative species resulting in low toxicity. In other words, the coordination of ligand to the Cu(I) ion assists to control the redox potential.[16]

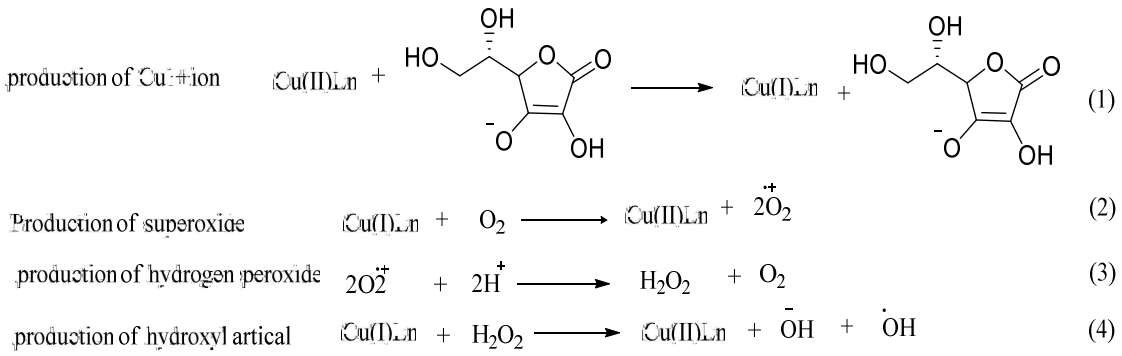
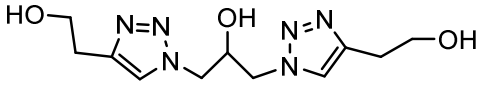


Figure 16. *The Fenton reaction.*

Although these recently developed ligands are useful in accelerating the reaction rate, some have also shown shortcomings. For instance, TBTA, which is the most extensively-used ligand for bioorthogonal CuAAC reactions, has low solubility in aqueous buffers. As a result, the ligation between azide and alkyne cannot be completed at micromolar concentrations. Moreover, some studies found that ligand-free Cu(I) and TBTA-complexed Cu(I) show toxicity to *E.coil* cells. In contrast, both BPS and BTTPS-complexed Cu(I) showed low toxicity because negatively- charged sulfate groups prevent their cellular entry. This makes them unsuitable catalysts for intracellular applications. L-histidine was demonstrated to reduce copper toxicity in *E.coil* and mammalian cells. However, its ability to increase the reaction rate of CuAAC is considerably slower than tris (triazolymethyl) amine-based ligands. Moreover, there is a possibility that this ligand acts as a solubilizing agent for Cu(I), which may facilitate the damage of primary Fe-S clusters of aldeydrates.[5]

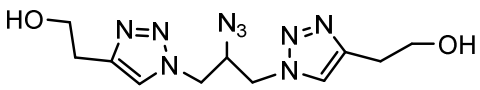
Goal and Objective of this Thesis

The CuAAC reaction has unique features that qualify it to be the best click reaction. Its applications have been shown in different aspects and for multiple purposes. The oxidative degradation of biological systems (labile proteins and live cells) is, however, generally recognized as the major problem when using this reaction in living systems. Reactive oxidation species can be easily produced in the presence of copper, ascorbate and air, and this is the main cause of toxicity. However, the uses of ligands have a major impact on reducing copper toxicity, protecting Cu(I) from redox potential, and increasing the reaction rate. [6] The aim of this study is to synthesize three different water-soluble ligands for evaluation in CuAAC reactions and to see if they could be used in aqueous and even biological systems. These ligands were chosen because of their low cost and the simplicity of the process. The ligand#1 can be obtained after three steps, whereas, the ligand #2 and ligand #3 require about five steps for production. The three different ligands were synthesized from the same starting materials with some alterations to the process.



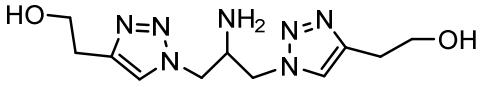
Ligand #1

25



Ligand #2

26



Ligand #3

27

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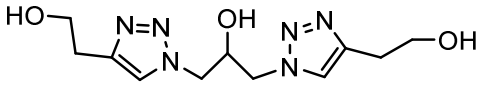
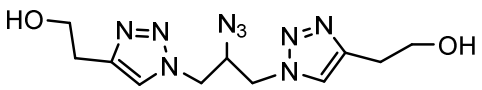
Chapter 2-Results and Discussions

The aim of this study is to synthesize three different water-soluble ligands for evaluation in CuAAC reactions and to see if they could be used in aqueous systems. These ligands were chosen because of their low cost and the simplicity of the process. The ligand #1 is obtained after three steps, whereas, the ligand #2 and ligand #3 require about five steps to be produced **(Figure 17**). The three different ligands were able to be efficiently synthesized from the same starting materials with small alterations to the process.



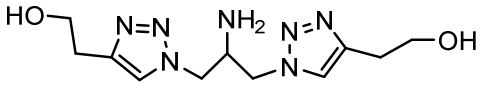
Ligand #1

25



Ligand #2

26



Ligand #3

27

The Synthetic Route of three ligands

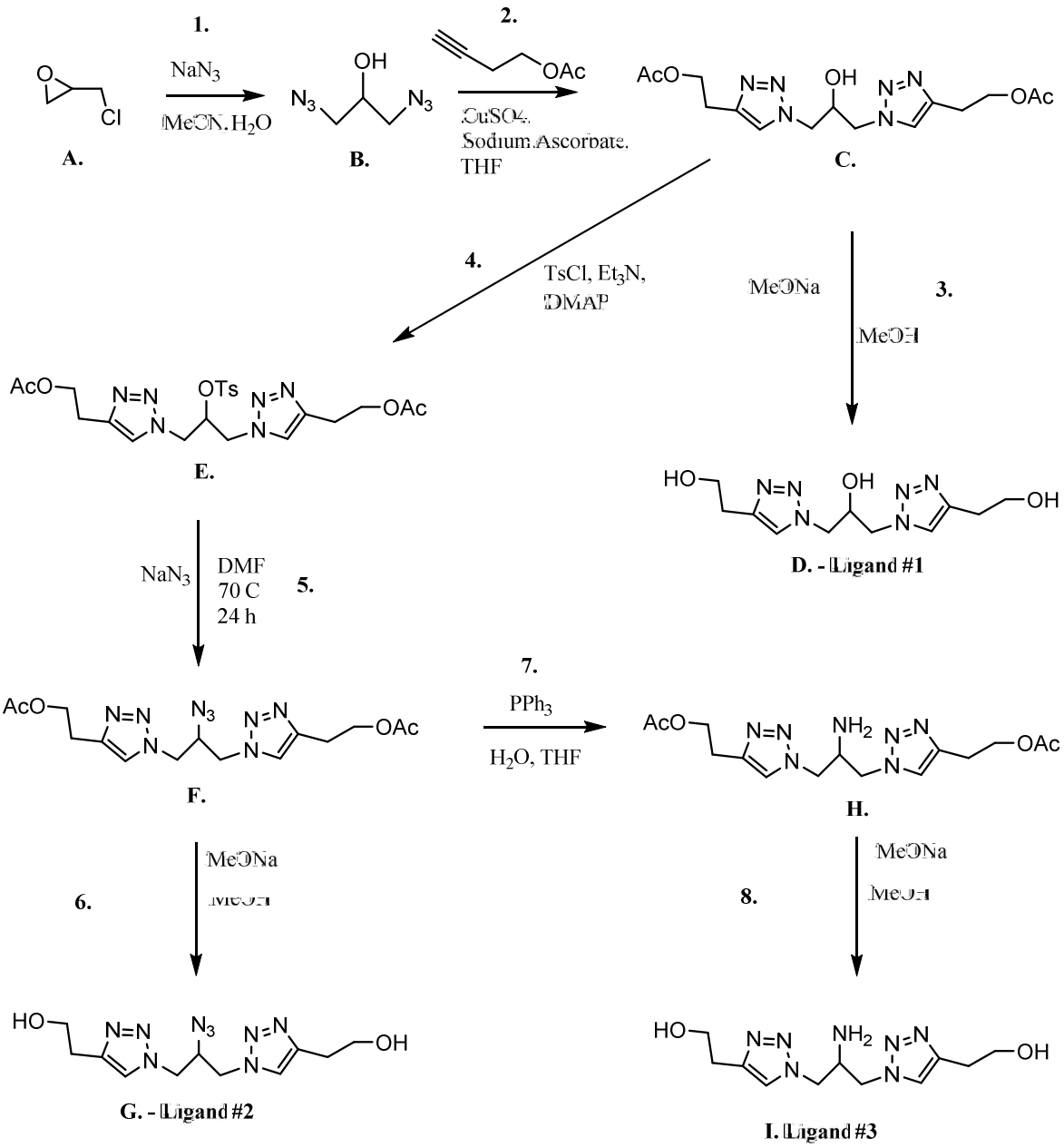
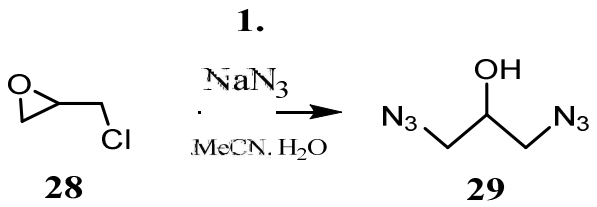


Figure 17. *The Synthetic route of three ligands*

2.1 Synthesis of ligands

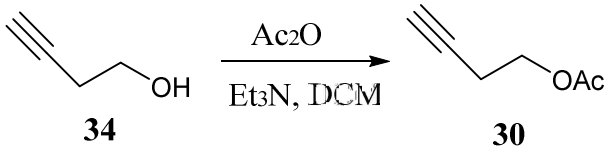
2.1.1 Ligand #1

Ligand #1 containing a hydroxyl group required three steps to be synthesized. The first step is called azidolysis of epoxide. This type of reaction occurred by using sodium azide in aqueous acetonitrile at 90 °C.

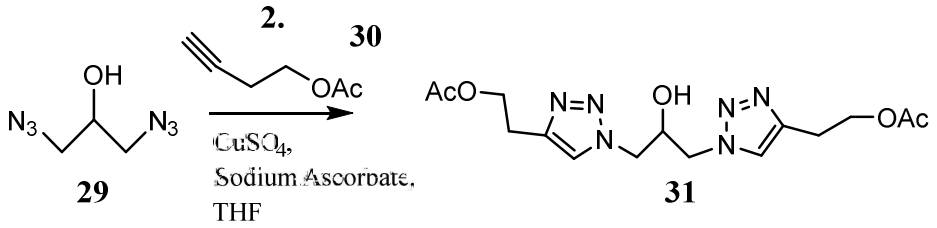


The aqueous-organic medium of sodium azide in water and epoxide in organic solvents were used. They could drive the reaction to favorable homogenous conditions because of good solubility of sodium azide in water and the epoxide in organic solvents.[1] The reaction proceeded as a double S_N2 reaction so that the sodium azide could nucleophilically attack the C-Cl bond and also open the epoxide ring.[2]

In order to do a click reaction, preparation of 3-butanol-1- acetate **30** by acetylation of primary alcohol **34** was necessary. The reaction proceeded by the addition of acetic anhydride in the presence of triethylamine in DCM. [3,4]

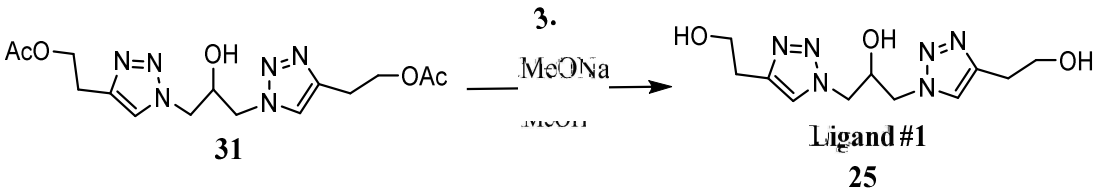


The second step was a click reaction between azide **29** and 3-butanol-1- acetate **30** that produced the desired compound **31**. From this common precursor **31**, ligand #1 **25**, ligand #2 **26**, and ligand #3 **27** were synthesized.



29 **31**

The final ligand #1 **25** was produced by transesterification between ester **31** to alcohol **25** using methanol and a catalytic amount of sodium methoxide.



31 **Ligand #1**
25

The reaction mechanism started with the attack of a methoxide anion to a carbonyl group on the ester, generating a tetrahedral intermediate, kicking out acetyl groups, and producing alcohol groups (**Figure 18**).[2]

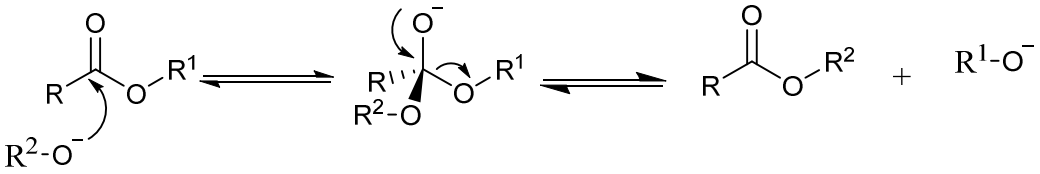
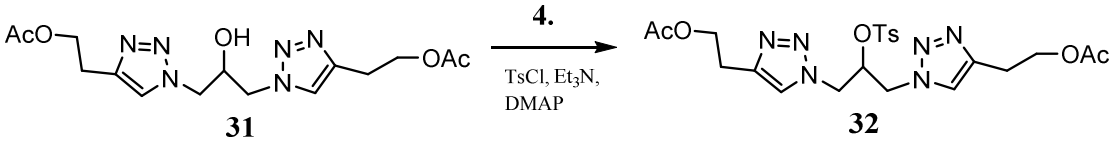


***Figure 18**. The mechanism of the transesterification reaction.*

2.1.2 Synthesis of Ligand #2

Ligand #2 production required three steps after the click reaction. The first step was conversion of alkyl alcohol **31** to alkyl tosylate **32** by the addition of tosyl chloride in the presence of triethyl amine and DMAP.



This type of reaction proceeded by the attack of an electron pair of an oxygen in –OH to the electrophilic center of tosyl sulfur. That displaced chloride functions as a good leaving group to form tosylate.[2] The addition of a catalytic amount of DMAP is crucial for the formation of the product.[4] The reaction should occur in the presence of a base condition, such as trimethylamine. That was necessary to dispose of the HCl byproduct (**Figure 19**).

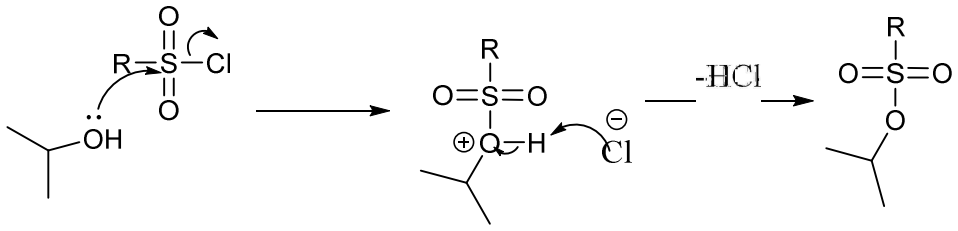
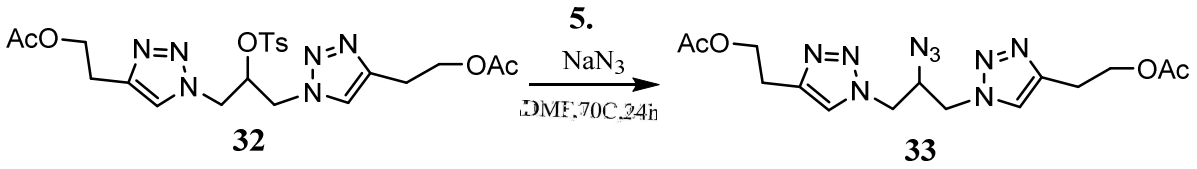
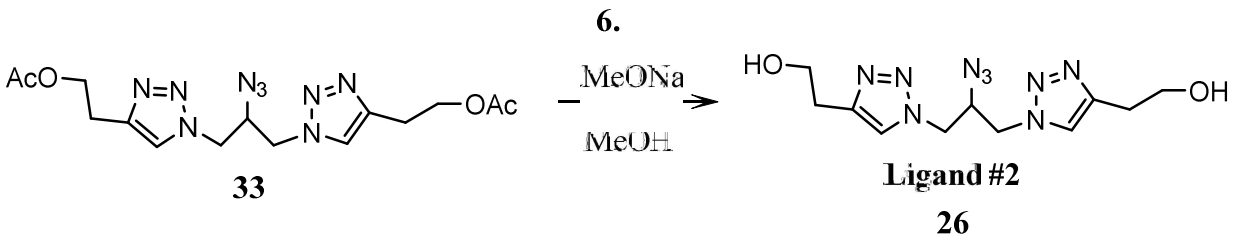


Figure 19*. The conversion of alcohol to tosylate group.*

The next step was to replace the tosylate group **32** with azide **33** by the addition of sodium azide in DMF at 70 ºC. [6,7] This type of reaction proceeds via the S_N2 mechanism. It is known that DMF, a polar aprotic solvent with a high boiling point, is usually used to facilitate the S_N2 reaction mechanism.

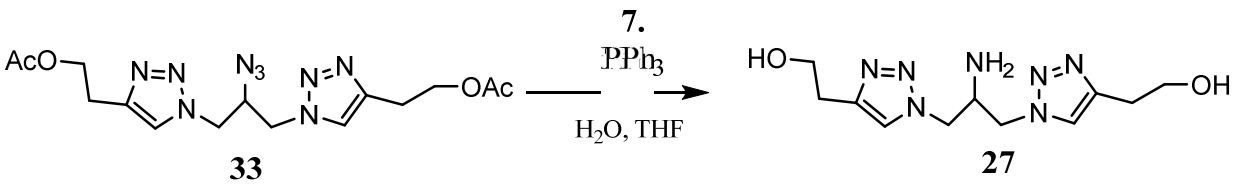


The final step to produce ligand #2 was transesterification to alcohol **26** using the same conditions that are mentioned in the third step of synthesizing ligand #1.



2.1.3 Synthesis of Ligand #3

Ligand #3 **27** was synthesized from compound **33** using the Staudinger ligation. This reaction is named after Hermann Staudinger, who discovered this reaction in 1919.



It has been reported that the reaction occurs between triarylphosphine **35** with azide **36** to generate a phosphazide **38.** Then, a subsequent loss of nitrogen gas proceeds via the 4-membered-ring transition state **39** and forms iminophosphorane **37a**. The high nucleophilicity of nitrogen atoms in iminophosphorane **37a** provides its ability to react with an electrophilic reagent. Moreover, performing this reaction in an aqueous solvent leads to the hydrolysis of **37a** to produce primary amine **40** (**Figure 20**).[8]

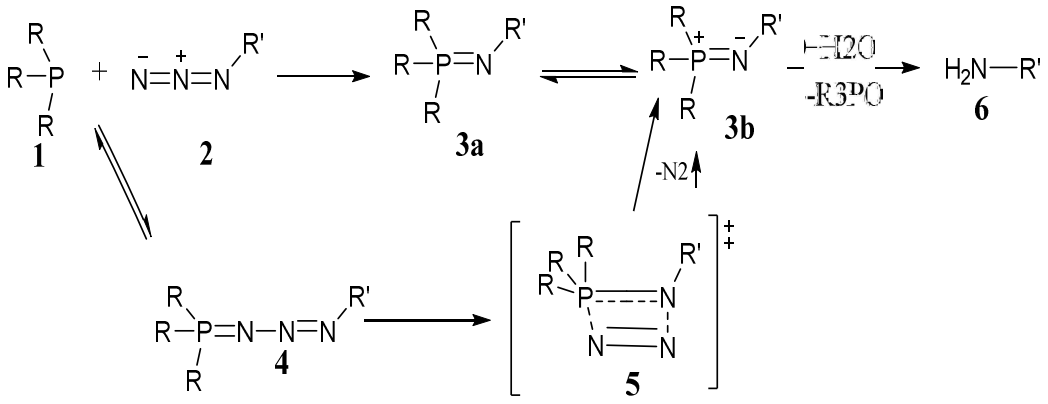


Figure 20*. The Staudinger ligation mechanism*

It has been noticeable in this study that both conversion of esters to alcohols and reducing azide to amine have occurred in one-step after leaving the reaction 48 h. To ensure the obtained result, the same reactions were repeated several times. A crude NMR spectrum proves that acetyl groups have been removed, in addition to reducing the azide. The purpose of taking a crude NMR spectrum was to determine in which stage the acetyl protecting groups were removed, during the reaction time or after completing the work up. The spectrum showed that the acetyl protecting groups were removed during the reaction. Many papers reported this reaction should occur in two steps. First is the addition of a base, such as MeONa or K_2CO_3[9] in methanol. This catalyst can deprotonate methanol to give methoxide, which attacks a carbonyl group to remove acetyl protecting groups. The second step is to reduce azide to primary amine using PPh_3 in H_2O: THF.[10] The reaction that caused acetyl groups to be removed could be hydrolysis of ester. That is because the reaction existed in a system that contained H_2O under basic conditions. It is believed that the PPh_3 might participate in the deprotonation of water to generate–OH, which is crucial for the process of hydrolysis (**Figure 21**).

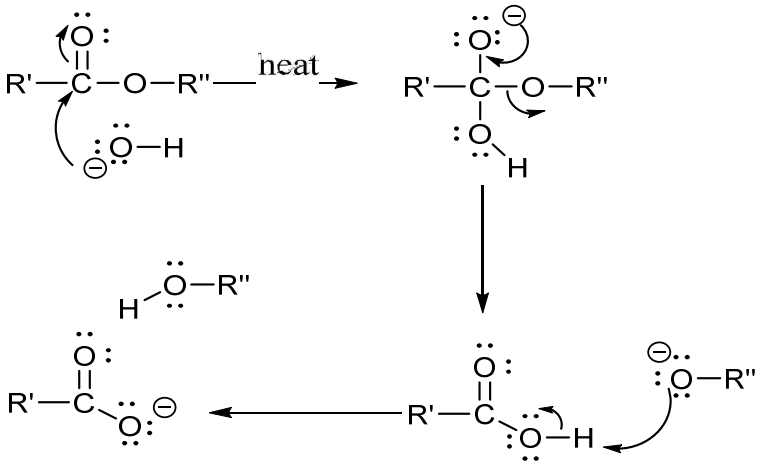
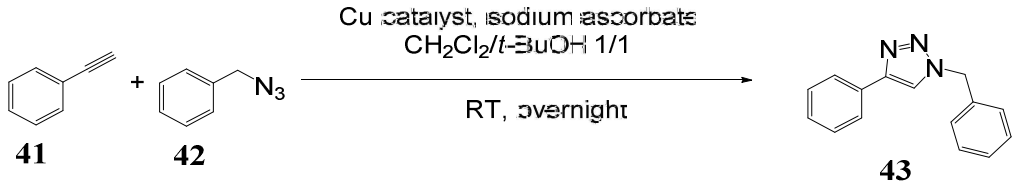


Figure 21. *The ester hydrolysis mechanism.*

2.2 Qualitative Evaluation of three ligands in Copper Catalyzed Alkyne-Azide Cycloaddition (CuAAC)

2.2.1 Evaluation of the three ligands

The three ligands were applied in the CuAAC reaction in order to evaluate their effectiveness in accelerating the reaction rate. A model reaction between phenyl acetylene **41** with benzyl azide **42** in **1:1** t-BuOH/DCM was used.[11] The reaction proceeded with 5 mol% of $CuSO_4$ and 10 mol% of a reducing agent sodium ascorbate in the presence of ligands. Sodium ascorbate assists in eliminating the need for inert atmospheres. Four click reactions in different vials were set up at the same time at room temperature overnight (**Table 1a).** The reactions were qualitatively characterized by checking TLC plates at different times (**Table 2b**).



a)

Entry	phenyl acetylene (mg)	Benzyl Azide (mg)	$CuSO_4$ (0.1 M)	Sodium ascorbate (1 M)	Ligand (0.1 e.q)
W	10	15.6 (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	_
1	10	15.6 (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	Ligand#1 2.8 mg
2	10	15.6 (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	Ligand#2 2.8 mg
3	10	15.6 (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	Ligand#3 2.8 mg

b)

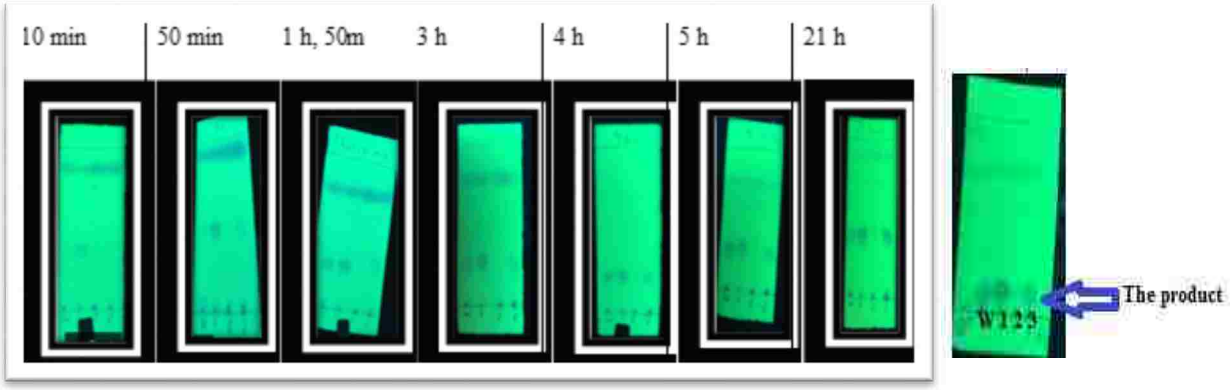


Table 3. **a**) *Evaluation of the three ligands in four click reactions*; **b**) **(**On the left, W: without ligand and #1: with ligand#1; In the middle#2: with ligand#2; on the right#3: with ligand#3).

The results showed that ligand #1 with –OH group **25** had significantly accelerated the reaction rate more than the others. In the TLC plates, the dark spots represented the product **43**, which started to appear within 10min or less when ligand #1 **25** was used (e.g., **1** in **Table 1b**). After 50min, both the reaction without ligand and the reaction with ligand #3 **26** containing –NH_2 started to proceed (e.g., **W,3** in **Table 1b**). It is possible that the reason for the relatively higher efficiency of the ligand #1 **25** with-OH is because of its ability to bind to the metal center and stabilize the catalytic activity of Cu(I). In other words, the ligand with –OH **25** can keep its coordination with the metal by providing weakly binding arms with a high local concentration that allows the coordination sites to be opened for alkyne and azide at the same time.[12] On the other hand, the ligand #3 **27** with a primary amine is a strong binder, which binds tightly to the center of the metal and might diminish the catalytic activity of Cu(I).[11] Surprisingly, the click reaction with ligand #2 **26** containing-N_3 failed to show any triazole product (e.g., **2** in **Table 1b**). To confirm that, the reaction containing ligand #2 was repeated again, but the result was still the same. Since ligand #2 **26** contains azide, and one of the two starting materials was also azide,

it is possible that ligand #2 can participate in the click reaction and serve as a starting material, but not as a ligand.

2.2.2 Evaluation of ligand#1 using different concentrations of catalysts

Since ligand #1 **25** has a major impact on accelerating the reaction rate, various click reactions were conducted using different equivalents of sodium ascorbate and copper sulfate with or without ligand #1 (**Tables 2,3**).

Entry	phenyl acetylene (mg)	Benzyl Azide (mg)	$CuSO_4$ (0.1 M)	Sodium ascorbate (1 M)	Ligand#1 (0.1 e.q)
1	10	15.6 (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	2.8 mg
2	10	15.6 (1.2eq)	245 µL (0.25eq)	9.8 µL (0.1eq)	2.8 mg
3	10	15.6 (1.2eq)	245 µL (0.25eq)	49 µL (0.5eq)	-
4	10	15.6 (1.2eq)	245 µL (0.25eq)	49 µL (0.5eq)	2.8 mg

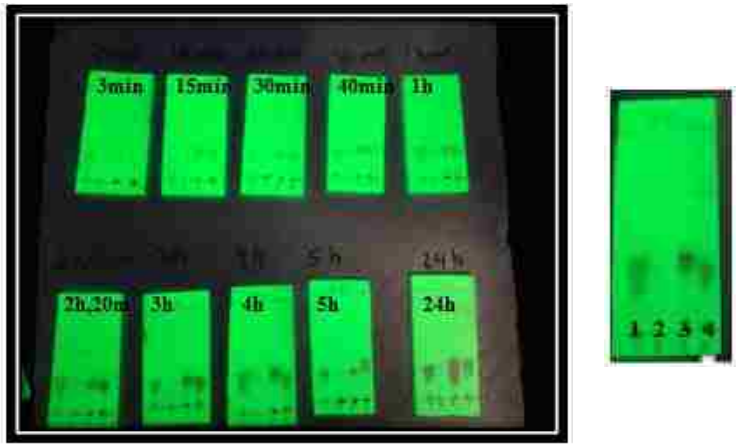


Table 4. *Evaluation of ligand#1 using different concentrations of catalysts.* **(**On the left#1: $CuSO_4$(0.05 eq) and NaAse (0.1 eq); On the left#2: $CuSO_4$(0.25 eq) and NaAse (0.1 eq) ;In the middle#3: $CuSO_4$(0.25 eq) and NaAse (0.5 eq), without ligand; On the right#4 $CuSO_4$(0.25 eq) and NaAse (0.5 eq) with ligand).

Entry	phenyl acetylene (mg)	Benzyl Azide (mg)	$CuSO_4$ (0.1 M)	Sodium ascorbate (1 M)	Ligand#1 (0.1 e.q)
1	10	15.6 (1.2eq)	49 µL (0.05eq)	49 µL (0.5eq)	2.8 mg
2	10	15.6 (1.2eq)	245 µL (0.25eq)	9.8 µL (0.1eq)	2.8 mg
3	10	15.6 (1.2eq)	245 µL (0.25eq)	49 µL (0.5eq)	2.8 mg

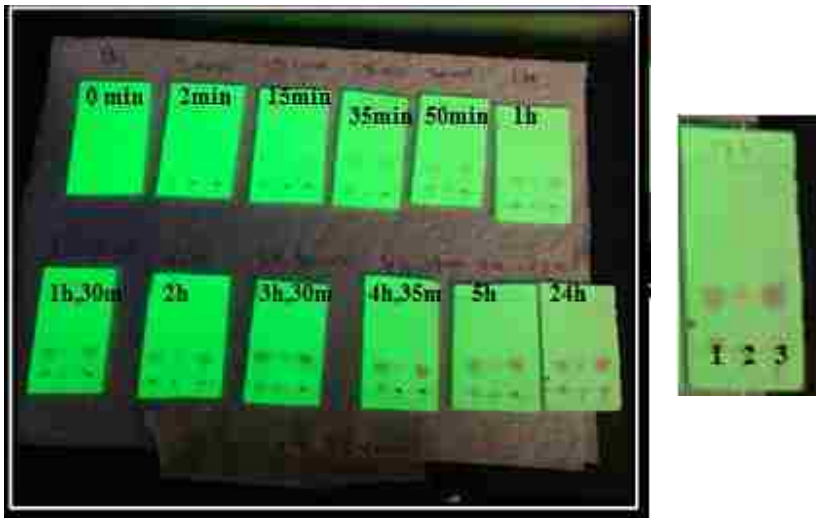
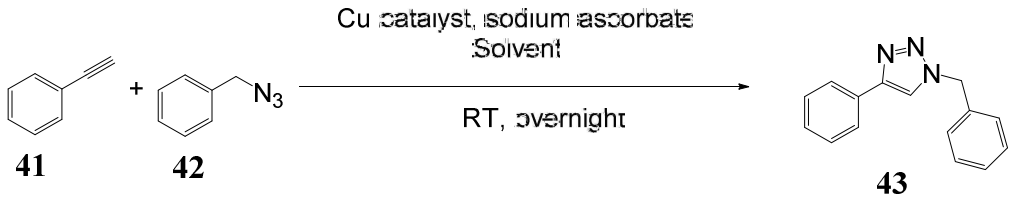


Table 5.*Evaluation of ligand#1 using different concentrations of catalysts.* **(**On the left#1: $CuSO_4$(0.05 eq) and NaAse (0.5); In the middle: $CuSO_4$(0.25 eq) and NaAse (0.1 eq); On the right: $CuSO_4$(0.25 eq) and NaAse (0.5 eq)).

A conclusion can be drawn that using different equivalents of catalysts, either by increasing the sodium ascorbate amount or increasing both the amount of copper sulfate and sodium ascorbate in the presence of ligand #1 **25**, the results were similar (e.g., **1,3** in **Table 3**). By making a comparison between the reactions containing 0.25 eq of copper and 0.5 eq of sodium ascorbate with and without ligand #1 (e.g., **3,4** in **Table 2**), the difference was not that much at the beginning. Ligand #1 showed a better result at low equivalence of both copper sulfate and sodium ascorbate (e.g., **1** in **Table 2**). By increasing only, the equivalent of copper sulfate in a constant amount of sodium ascorbate, the reaction did not proceed (e.g., **2** in **Table 3**). It seems that using an excessive amount of copper (II) with less sodium ascorbate inhibited

the reaction even in the presence of ligand #1 because copper (II) is inactive and requires a sufficient amount of sodium ascorbate to reduce it to copper (I).

2.2.3 Evaluation of ligand #1 using different solvents



41 **42** **43**

After testing ligand #1 **25** in different conditions, the best condition, using $CuSO_4$ (0. 05eq) and (0.1eq) of sodium ascorbate, was chosen to test the ligand in different solvents, or test the ligand in aqueous systems. Two solvent systems, **2:1** t-BuOH: H_2O and pure water were used to evaluate the speed of the reaction after adding ligand #1. Consequently, based on the photos taken at different times of TLC plates (**Table 4**), the results showed that the ligand #1 works in organic solvents better than in aqueous solvents. The reason for this might be the low solubility of the starting materials, phenyl acetylenes and benzyl azide in water, which makes the reaction unable to proceed.

	phenyl acetylene (mg)	Benzyl Azide (mg)	$CuSO_4$ (0.1 M)	Sodium ascorbate (1M)	Solvent	Ligand (0.1 e.q)
(A)	10	15.6 mg (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	t-BuOH/H_2O (2:1)	–
(B)	10	15.6 mg (1.2eq)	49 µL (0.05 eq)	9.8 µL (0.1eq)	t-BuOH/H_2O (2:1)	2.8 mg
(C)	10	15.6 mg (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	Pure Water 2 ml	–
(D)	10	15.6 mg (1.2eq)	49 µL (0.05eq)	9.8 µL (0.1eq)	Pure water 2 ml	2.8 mg

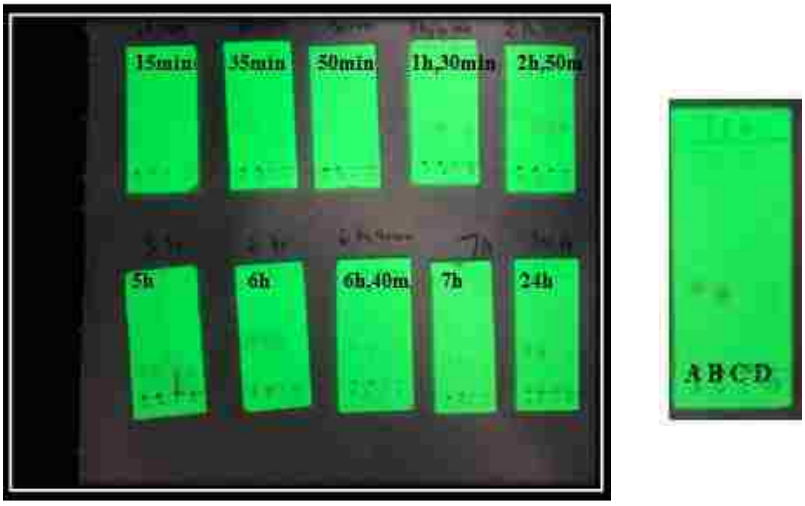


Table 6. *Evaluate ligand#1 using different solvents.* (A): without ligand, t-BuOH:H_2 O;(B): with ligand#1, t-BuOH: H_2O; (C): without ligand, H_2O; (D): with ligand, H_2O.

2.2.3 Comparison between ligand #1 and TBTA

The last step was to compare ligand #1 **25** with TBTA, the ligand that was commercially available, using $CuSO_4$ (0. 01eq), (0.04eq) of sodium ascorbate, and (0.01eq) of ligands. It was found that TBTA was more effective in accelerating the reaction rate than ligand #1 **25** when the **2:1** t-BuOH/water was used as the solvent (**Table 5**).

	phenyl acetylene (mg)	Benzyl Azide (mg)	$CuSO_4$ (0.1 M)	Sodium ascorbate (1 M)	Ligand (0.01 e.q)
1	10	15.6 mg (1.2eq)	9.8µl (0.01eq)	3.9µL (0.04eq)	_
2	10	15.6 mg (1.2eq)	9.8µl (0.01eq)	3.9µL (0.04eq)	Ligand#1
3	10	15.6 mg (1.2eq)	9.8µl (0.01eq)	3.9µl (0.04eq)	TBTA

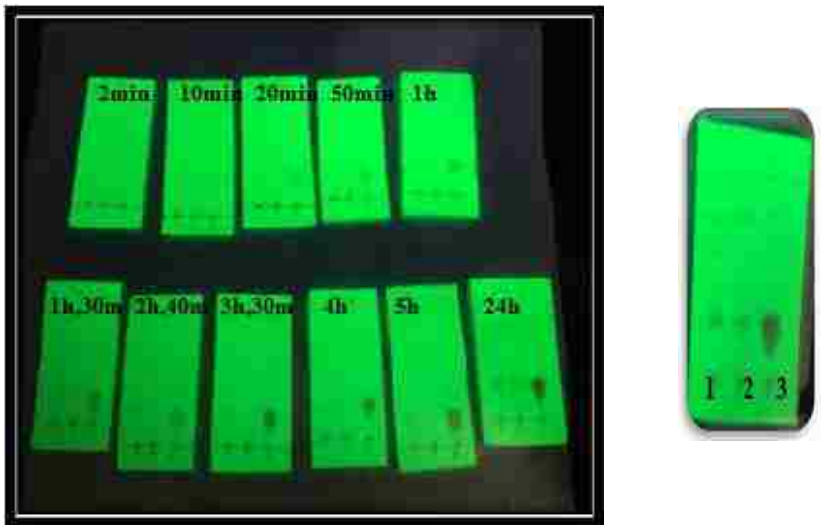


Table 7.*Comparison between ligand#1 and TBTA.* **(**On the left#1: CuSO4(0.01 eq), NaAse (0.04 eq), without ligand; In the middle#2: $CuSO_4$(0.01 eq),NaAse(0.04 eq),with ligand#1(0.01eq); On the right#3 $CuSO_4$ (0.01 e.q),NaAse(0.04 eq), with TBTA(0.01eq)).

Some studies have reported the reason for the efficiency of TBTA **20** in accelerating the reaction rate is due to its tetradentate binding ability to fully cover the copper(I) center.[11] As a result, that geometry can maintain the stability of Cu(I) and not allow free binding sites to be available for interactions. The TBTA **20** structure is composed of a tertiary amine and 1,2,3-triazole, which makes this ligand so efficient. Nitrogen can provide additional electron density to the copper(I) center, which participates in accelerating the catalysis. In addition, TBTA can also

be easily removed to allow the copper(I)-acetylide/ligand complex to form.[11] Therefore, the central tertiary nitrogen atom in TBTA is critical for catalysis,[12] when compared to ligand #1.

2.3 Quantitative Evaluation of Ligand #1 Catalyzed Alkyne-Azide Cycloaddition (CuAAC)

General methods for setup and evaluation of CuAAC reactions

First, a solution of benzyl azide (0.080 mmol) and phenylacetylene (0.12 mmol) in 1:1 t-BuOH/CH_2Cl_2 was added to $CuSO_4$, sodium ascorbate (NaAs) and Ligand (if applicable) to create the reaction mixture. Then the reaction mixture was stirred at 25 ^oC for 24 hours.

Second, the samples were prepared for ^1H NMR**.** The solvents for each reaction were then removed by blowing with nitrogen gas and the residue was dried in a high vacuum for 30 min.

Third, the standard sample was prepared for ^1H NMR. The standard was 18.9 mg triazole product (theoretical yield, synthesized beforehand).

All of the samples were dissolved in $CDCl_3$ (0.55 mL) followed by adding the same amount of CH_2Br_2 to each parallel experiment. The resulting solutions were ready for ^1H NMR analysis after filtration.

Finally, the yield of the reaction was determined. The ^1H NMR was run first for the standard sample. The integration of triazole-*H* signal was calibrated as 1.00, so the integration of C*H*$_2$Br$_2$ signal would be obtained (e.g., 2.00). For the other samples, the integration of C*H*$_2$Br$_2$ signal was always calibrated the same as shown in the standard sample (e.g., 2.00). The yield for each reaction was calculated using the following equation: yield = integration of triazole-*H* signal/ integration of C*H*$_2$Br$_2$ signal (e.g., 2.00) (**Table 6**).

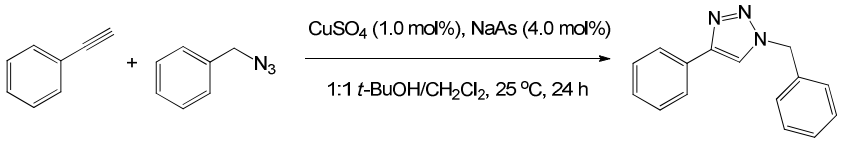


Table 6. *Effect of ligand#1on CuAAC* [a]

Entry	BnN_3 (mmol)	Phenylacetylene (mmol)	0.16 M Ligand in DMSO (µL)	DMSO (µL)	Yield,[b] %
1	0.080	0.12	-	-	26.4
2	0.080	0.12	-	50	71.7
3	0.080	0.12	50	-	98.6

[a] Co-solvent 0.2 mL t-BuOH and 0.2 mL CH_2Cl_2 were used for each reaction; [b] Determined by 1H NMR after 24 h based on triazole-*H* signal.

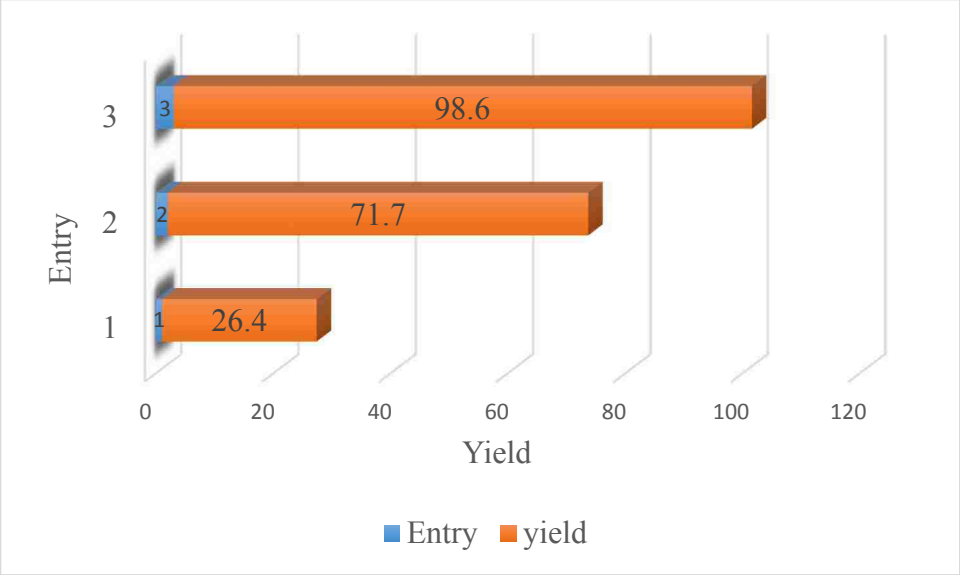


Figure 22. . *The yields of different click reactions:1) without ligand#1 and without DMSO, 2) with DMSO and without ligand#1,3) with ligand#1 and with DMSO*

In table **6** and figure **22**, the entry **1** represents the click reaction without ligand #1 and without adding DMSO, and the result gave 26% yield for the product. However, in entry **2**, when a small amount of DMSO was used in the reaction, the yield was increased by up to 71.7%. Moreover, in entry **3**, the ligand #1 dissolved in DMSO further increased the reaction rate and gave a 98.6% yield of the product. The amount of DMSO used in entry **2** was the same amount of DMSO used to dissolve ligand #1 in entry **3**. The reason for adding the same amount of DMSO in entry **2** and entry **3** was to make an accurate comparison between the click reactions

with and without ligand. Consequently, the reaction with ligand #1showed a higher yield than the one without.

Conclusion

In this study, three different water-soluble ligands were synthesized and evaluated in the CuAAC reaction. It was found that ligand #1 with –OH is more efficient in accelerating the reaction rate of CuAAC compared to ligand #2 and ligand #3. Because of that, ligand #1 was tested in different solvents and different amounts of catalysts. As a result, ligand #1 showed better activity at low amounts of catalysts and in organic systems than aqueous systems. When ligand #1 was compared with TBTA, the most extensively used ligand, TBTA provided a better result in accelerating the reaction rate than ligand #1.

References

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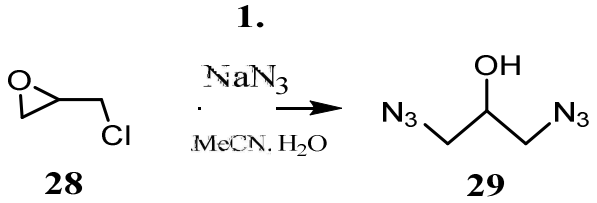
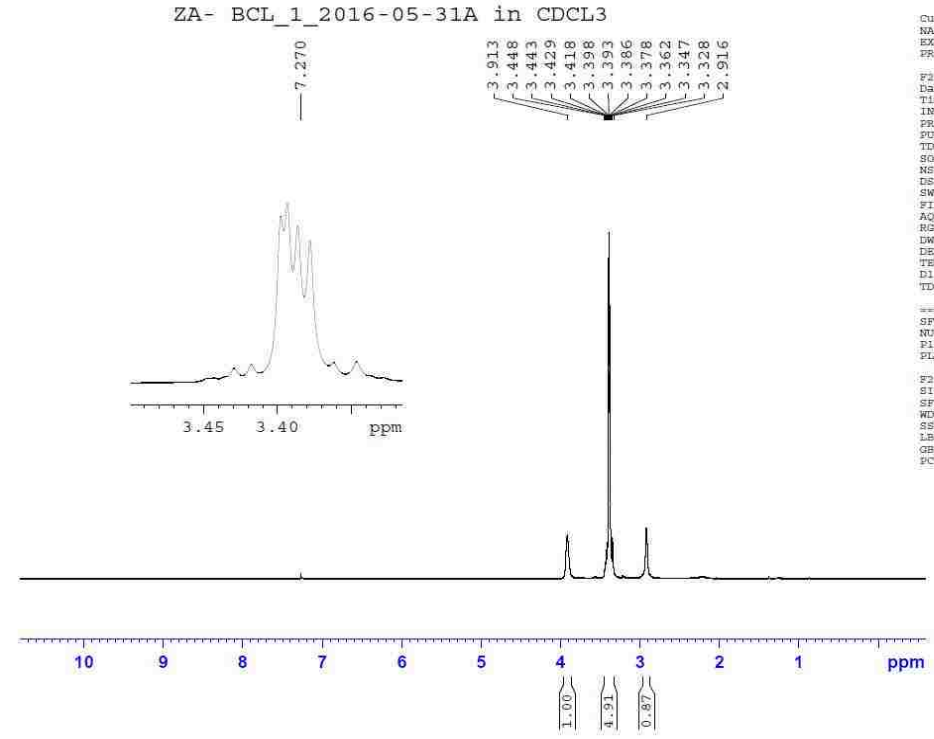
Chapter 3- Experiments and synthesis

Synthesis



Following the procedure proposed in the literature[1], epichlorohydrin **28** (1 g, 10.80 mmol, 1eq) was added to a solution of sodium azide (2.1 g, 32.30mmol, 3 eq) in 20 ml of (MeCN: H_2O) (7:3), and the reaction mixture was stirred overnight at 90 °C. After the reaction was completed, the solvent (acetonitrile) was subsequently evaporated under reduced pressure. The aqueous layer was extracted with ethyl acetate three times, and the combined organic layer was dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography to obtain colorless oil 1, 3-diazido-2-propanol **29,** (1.078g, 6.65 mmol,62%). ^{1}H NMR (400 MHz, $CDC1_3$) *&* 3.91 (s, 1 H), 3.45-3.33 (m, 4H), 2.92(s, OH); 13C NMR (101 MHz, $CDCI_3$) *&* 69.5, 53.8.

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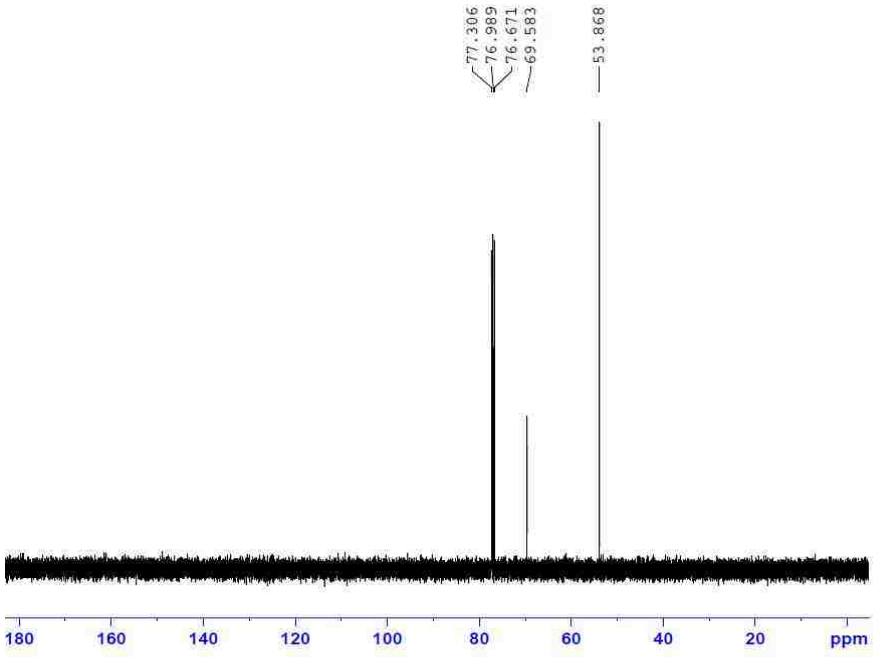


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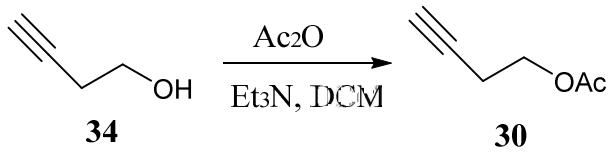

34 **30**

Following the procedure proposed in the literature,[2] trimethylamine Et_3N (6.31ml,44.94 mmol, 1.05 eq) and acetic anhydride Ac_2O (4.59g or 4.24ml, 44.96mmol, 1.05 eq) were added to a solution 3-butyn-1-ol **34** (3g,42.80mmol, 1 eq) in DCM (30 ml), and stirred at RT for 60 min. After the reaction completion, it was quenched with aq. sodium bicarbonate and extracted with DCM. The combined organic layer was dried over sodium sulfate and filtered. Then, the filtrate subsequently evaporated under reduced pressure. To remove the rest of triethylamaine, 10ml of HCl(0.5M) was added after dissolving the product in 50ml of DCM. The organic layer was washed again with HCl, and dried over sodium sulfate and filtrated. The filtrate was concentrated under reduced pressure to obtain colorless liquid 3-butanol-1- acetate **30** (2.89g, 60%). ^{1}H NMR (400 MHz, $CDC1_3$) δ 4.14 (t, 2 H, J=6.8, Hz), 2.40 (t, 2H, J=6.8 Hz), 2.05(s, 3H), 1.98 (s, 1H); ^{13}C NMR (101 MHz,

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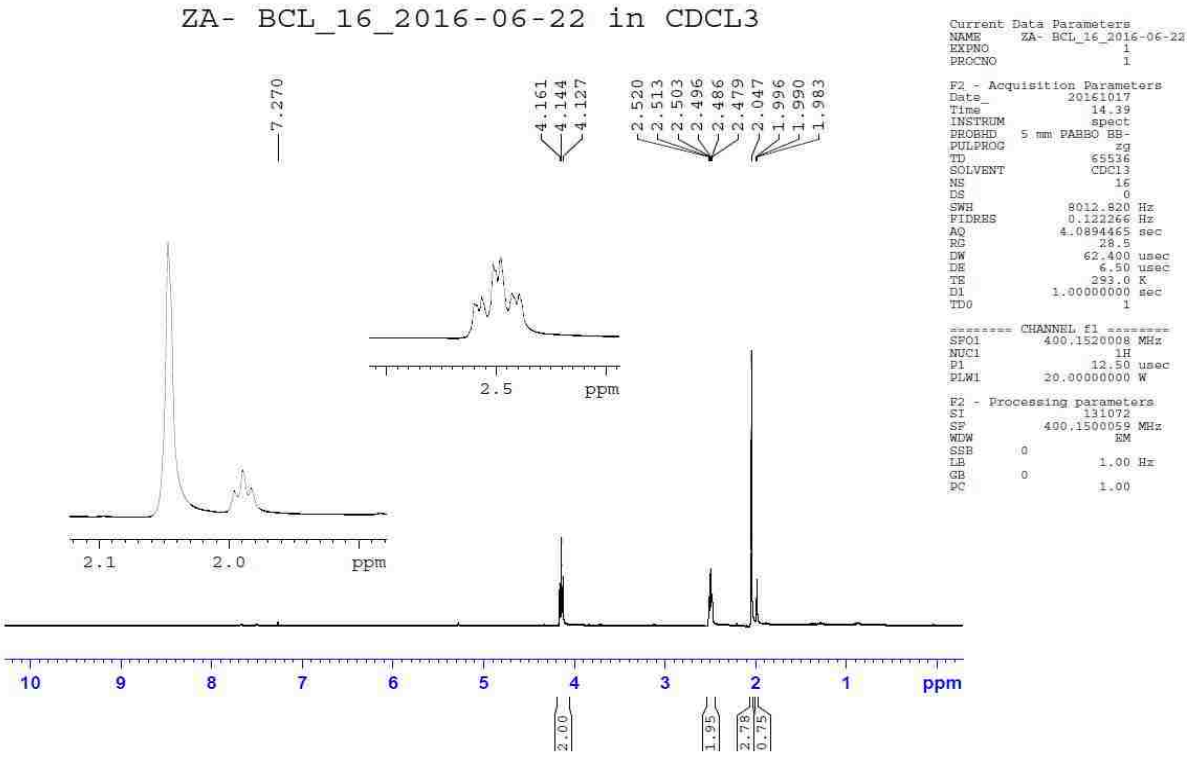


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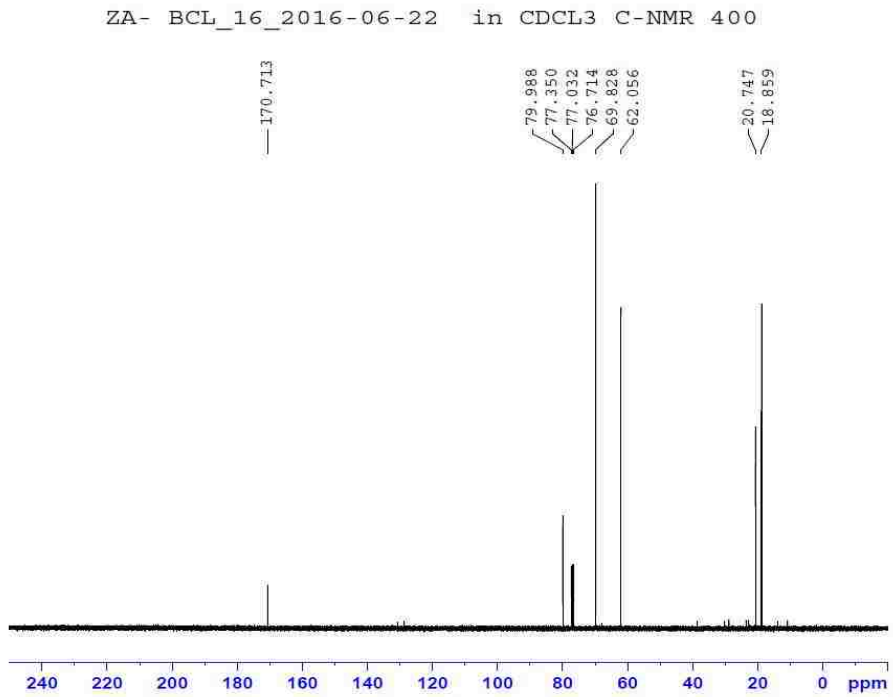


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PLW1                 65.00000000 W

======== CHANNEL f2 ========
SFO2                  400.1520008 MHz
NUC2                          1H
CPDPRG[2                 waltz16
PCPD2                     90.00 usec
PLW2                 20.00000000 W
PLW12                 0.29877001 W
PLW13                 0.24200000 W

F2 - Processing parameters
SI                        1048576
SF                    100.6178022 MHz
WDW                           EM
SSB                0
LB                         0.30 Hz
GB                 0
PC                         1.40
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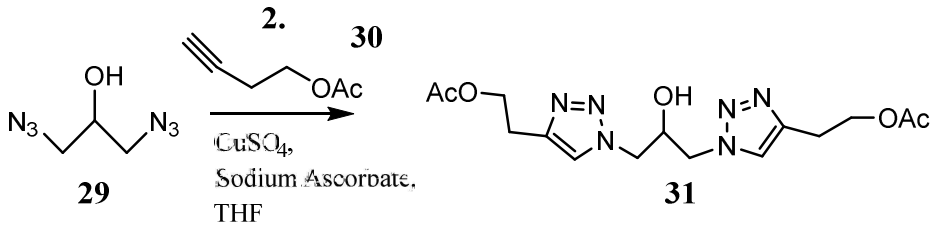
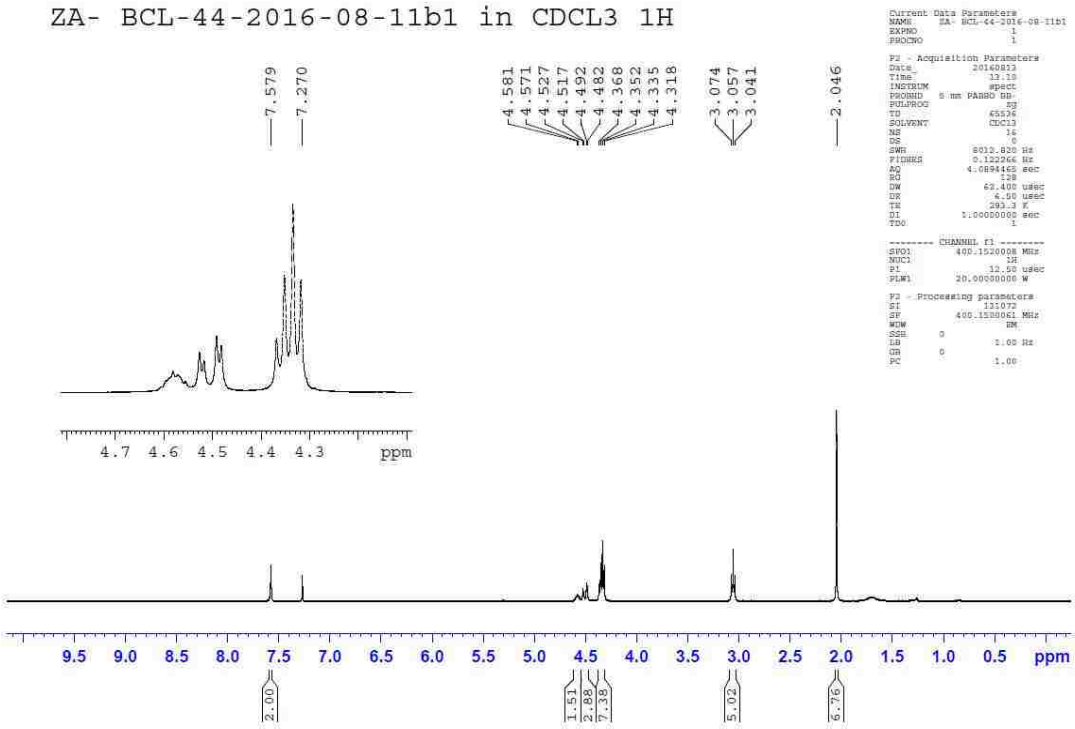


Following the procedure in the literature,[3] 3-butanol-1- acetate **30** (0.95g, 8.4mmol, 3 eq) was added slowly to a solution of 1, 3-diazido-2-propanol **29** (0.400 g, 2.8mmol, 1 eq), copper sulfate (0.032g, 0.2mmol,0.072 eq)) and sodium ascorbate (0.159g, 0.8mmol,0.287 eq) in anhydrous THF(30ml), and stirred at 70 ^{o}C overnight under inert atmosphere. After the reaction completion, the solid was removed by filtration, and the solvent was subsequently evaporated under reduced pressure. The product was washed with 1 ml of DCM and dried again under reduced pressure and a high vacuum to obtain pale yellow solid **31** (0.92g, 89%). ^{1}H NMR (400 MHz, CDC1$_3$) δ 7.58 (s, 2 H), 4.57-4.58(m, 1 H), 4.50 (dd, 2 H, J= 4.0, 14 Hz), 4.50(dd, 6 H, J=6.4, 13.2 Hz), 3.06 (t, 4 H, J=6.4 Hz), 2.05 (s, 6 H); ^{13}C NMR (101 MHz, CDCI$_3$) δ 170.9, 144.1, 123.6, 68.6, 62.9, 52.8, 25.3, 20.9*).*

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Current Data Parameters
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EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20160813
Time_             13.10
INSTRUM           spect
PROBHD  5 mm PABBO BB-
PULPROG             zg
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    0
SWH            8012.826 Hz
FIDRES         0.122266 Hz
AQ             4.0894465 sec
RG                  128
DW                62.400 usec
DE                 6.50 usec
TE               293.3 K
D1             1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1          400.1520008 MHz
NUC1                  1H
P1                 12.50 usec
PLW1          20.00000000 W

F2 - Processing parameters
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SF            400.1500061 MHz
WDW                   EM
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PC                 1.00
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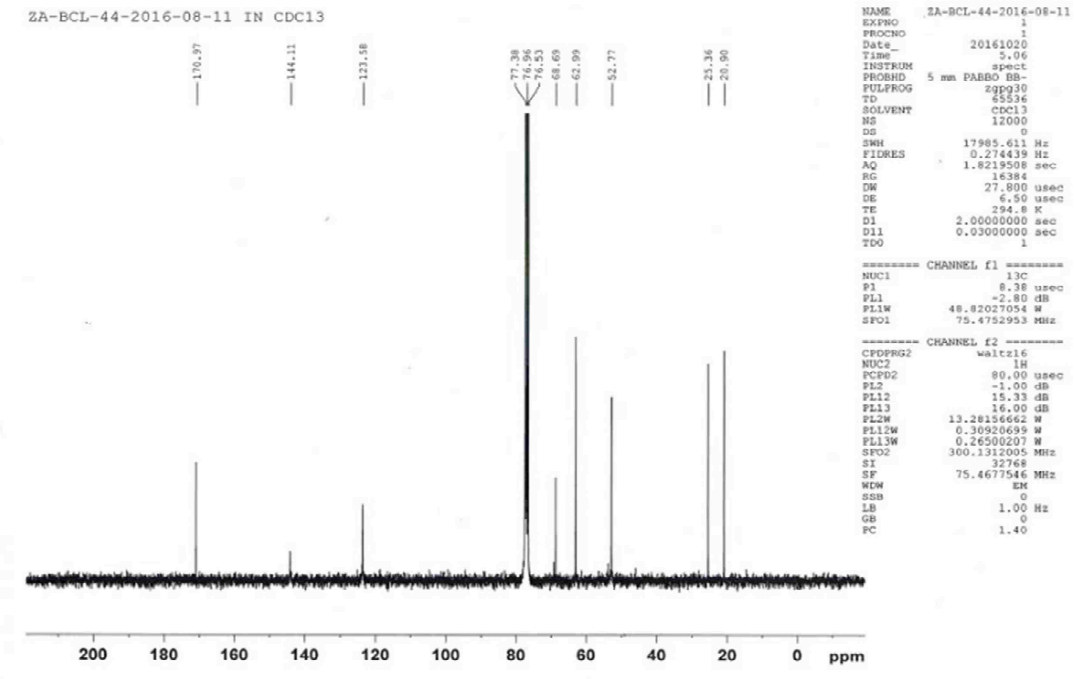


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PROCNO                1
Date_          20161020
Time               5.06
INSTRUM           spect
PROBHD  5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                12000
DS                    0
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ             1.8219508 sec
RG                 16384
DW                27.800 usec
DE                 6.50 usec
TE               294.8 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                 8.38 usec
PL1               -2.80 dB
PL1W          48.82027054 W
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -1.00 dB
PL12              15.33 dB
PL13              16.00 dB
PL2W          13.28156662 W
PL12W          0.30920699 W
PL13W          0.26500207 W
SFO2          300.1312005 MHz
SI                32768
SF            75.4677546 MHz
WDW                   EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
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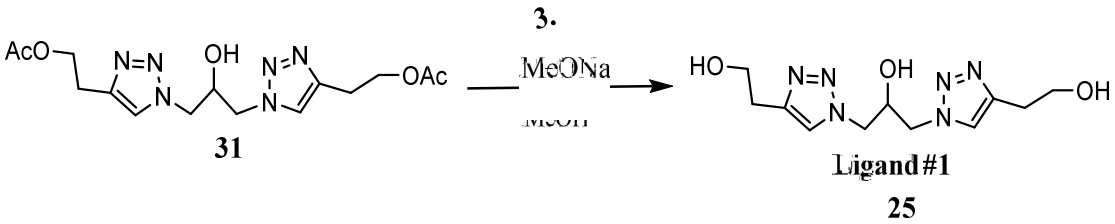
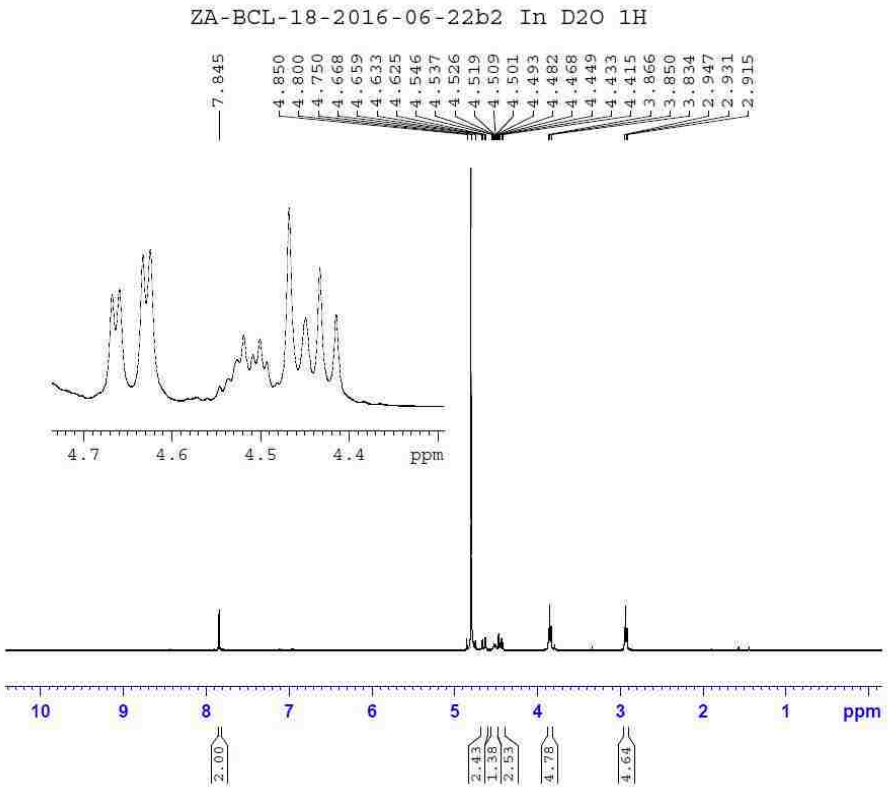


Following the procedure in the literature,[4] sodium methoxide (0.0014g,0.025mmol ,0.1eq) was added to a solution of **31**(0.1g, 0.27mmol, 1eq) in methanol(5ml), and stirred for three hours in room temperature. After the reaction completion, it was quenched with 0.5 ml, aq. $NaHCO_3$. The solid was removed by filtration and the solvent was subsequently evaporated under reduced pressure to obtain **25** (0.076g, 98%). The product was confirmed by NMR. ^{1}H NMR (400 MHz, D_2O) δ 7.85 (s, 2 H), 4.64 (dd, 2 H, J= 3.6, 14 Hz), 4.55-4.48(m, 1 H), 4.44 (dd, 2H, J=7.6, 14 Hz), 3.85(t, 4 H, J=6.4 Hz), 2.93 (t, 6 H, J=6.4 Hz); ^{13}C NMR (101 MHz, D_2O) δ 145.2, 124.7, 68.5, 60.5, 52.9, 27.7)

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Current Data Parameters
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EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
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Time                 15.02
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG                zg
TD                  65536
SOLVENT               D2O
NS                     16
DS                      0
SWH               8012.820 Hz
FIDRES            0.122266 Hz
AQ                4.0894465 sec
RG                     64
DW                62.400 usec
DE                 6.50 usec
TE                 293.1 K
D1              1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1           400.1520008 MHz
NUC1                   1H
P1                  12.50 usec
PLW1           20.00000000 W

F2 - Processing parameters
SI                  131072
SF             400.1499614 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.00
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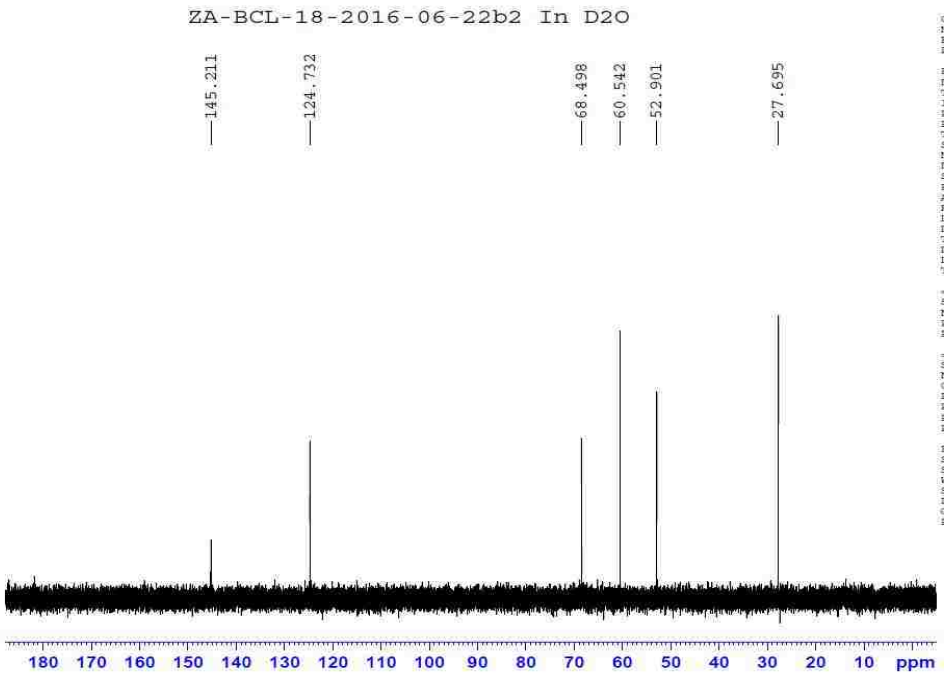


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Current Data Parameters
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EXPNO                 3
PROCNO                1

F2 - Acquisition Parameter
Date_              20160731
Time                 18.32
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg
TD                 524288
SOLVENT               D2O
NS                     64
DS                      0
SWH              27173.912
FIDRES            0.051830
AQ                9.6468992
RG                    144
DW                18.400
DE                 6.50
TE                 294.0
D1              2.00000000
D11             0.03000000
TD0                    1

======== CHANNEL f1 ========
SFO1           100.6293686
NUC1                  13C
P1                  10.00
PLW1           65.00000000

======== CHANNEL f2 ========
SFO2           400.1520008
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              90.00
PLW2           20.00000000
PLW12           0.29877001
PLW13           0.24200000

F2 - Processing parameter
SI                 1048576
SF             100.6177975
WDW                    EM
SSB       0
LB                  0.30
GB        0
PC                  1.40
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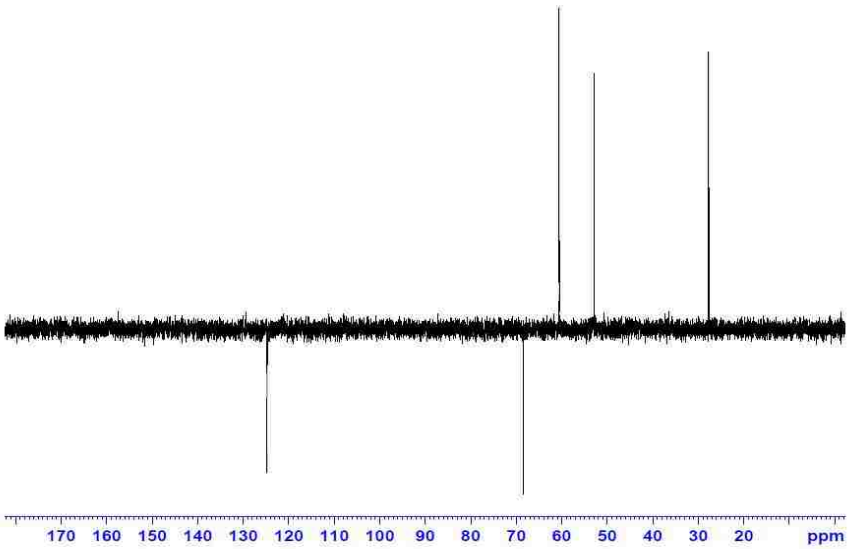
ZA-BCL-18-2016-06-22b2 In D2O DEPT 135

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Current Data Parameters
NAME          ZA-BCL-18-2016-06-22b2
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_              20160731
Time                 18.18
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG            dept135
TD                   65536
SOLVENT                D2O
NS                      64
DS                       8
SWH              27173.912 Hz
FIDRES            0.414641 Hz
AQ                1.2058624 sec
RG                     2050
DW                  18.400 usec
DE                   6.50 usec
TE                  293.5 K
CNST2           145.00000000
D1                2.00000000 sec
D2                0.00344828 sec
D12               0.00002000 sec
TD0                       1

========= CHANNEL f1 =========
SFO1            100.6293686 MHz
NUC1                     13C
P1                     10.00 usec
P2                     20.00 usec
PLW1           65.00000000 W

========= CHANNEL f2 =========
SFO2            400.1520008 MHz
NUC2                     1H
CPDPRG[2]           waltz16
P3                     11.00 usec
P4                     22.00 usec
PCPD2                  90.00 usec
PLW2           20.00000000 W
PLW12           0.29877001 W

F2 - Processing parameters
SI                     32768
SF              100.6177975 MHz
WDW                       EM
SSB          0
LB                      1.00 Hz
GB           0
PC                      1.40
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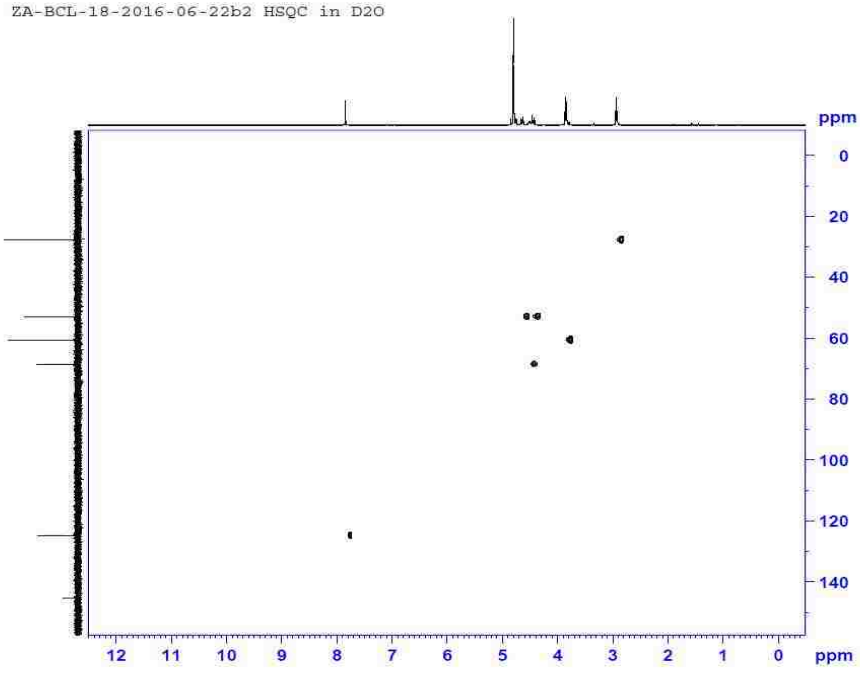
ZA-BCL-18-2016-06-22b2 HSQC in D2O

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Current Data Parameters
NAME          ZA-BCL-18-2016-06-22b2
EXPNO                 4
PROCNO                1

F2 - Acquisition Parameters
Date_              20160803
Time                 15.38
INSTRUM              spect
PROBHD    5 mm PABBO BB-
PULPROG          hsqcetgpsi
TD                   1024
SOLVENT                D2O
NS                       8
DS                      16
SWH              5197.505 Hz
FIDRES           5.075689 Hz
AQ                0.0985008 sec
RG                     2050
DW                  96.200 usec
DE                   6.50 usec
TE                  293.2 K
CNST2           145.0000000
D0                0.00000030 sec
D1                1.50000000 sec
D4                0.00172414 sec
D11               0.03000000 sec
D16               0.00020000 sec
D24               0.00150000 sec
IN0               0.00003000 sec
ZGOPTNS

======== CHANNEL f1 ========
SFO1            400.1324004 MHz
NUC1                     1H
P1                     12.50 usec
P2                     25.00 usec
PL6              1000.00 usec
PLW1           20.00000000 W

======== CHANNEL f2 ========
SFO2            100.6253021 MHz
NUC2                     13C
CPDPRG[2                 garp
P3                     10.00 usec
P4                     20.00 usec
PCPD2                  80.00 usec
PLW2           65.00000000 W
PLW12           1.21553997 W

====== GRADIENT CHANNEL =====
GPNAM[1]          SMSQ10.100
GPNAM[2]          SMSQ10.100
GPZ1                   80.00 %
GPZ2                   20.10 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                      128
SFO1            100.6263 MHz
FIDRES          130.209228 Hz
SW                160.431 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                     1024
SF              400.1320000 MHz
WDW                    QSINE
SSB                       2
LB           0 Hz
GB           0
PC                      1.40

F1 - Processing parameters
SI                     1024
MC2          echo-antiecho
SF              100.6177975 MHz
WDW                    QSINE
SSB                       2
LB           0 Hz
GB           0
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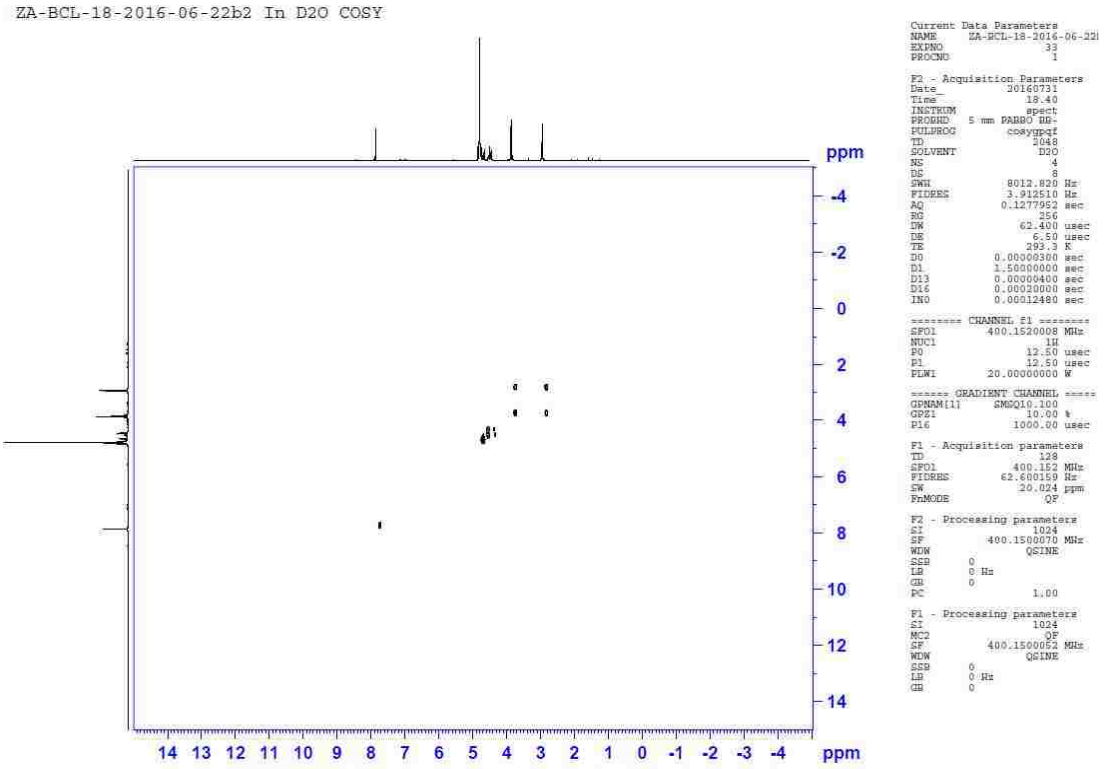
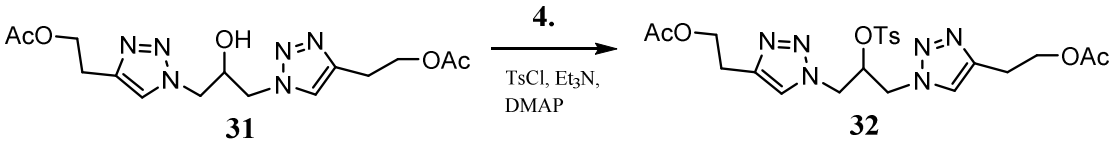
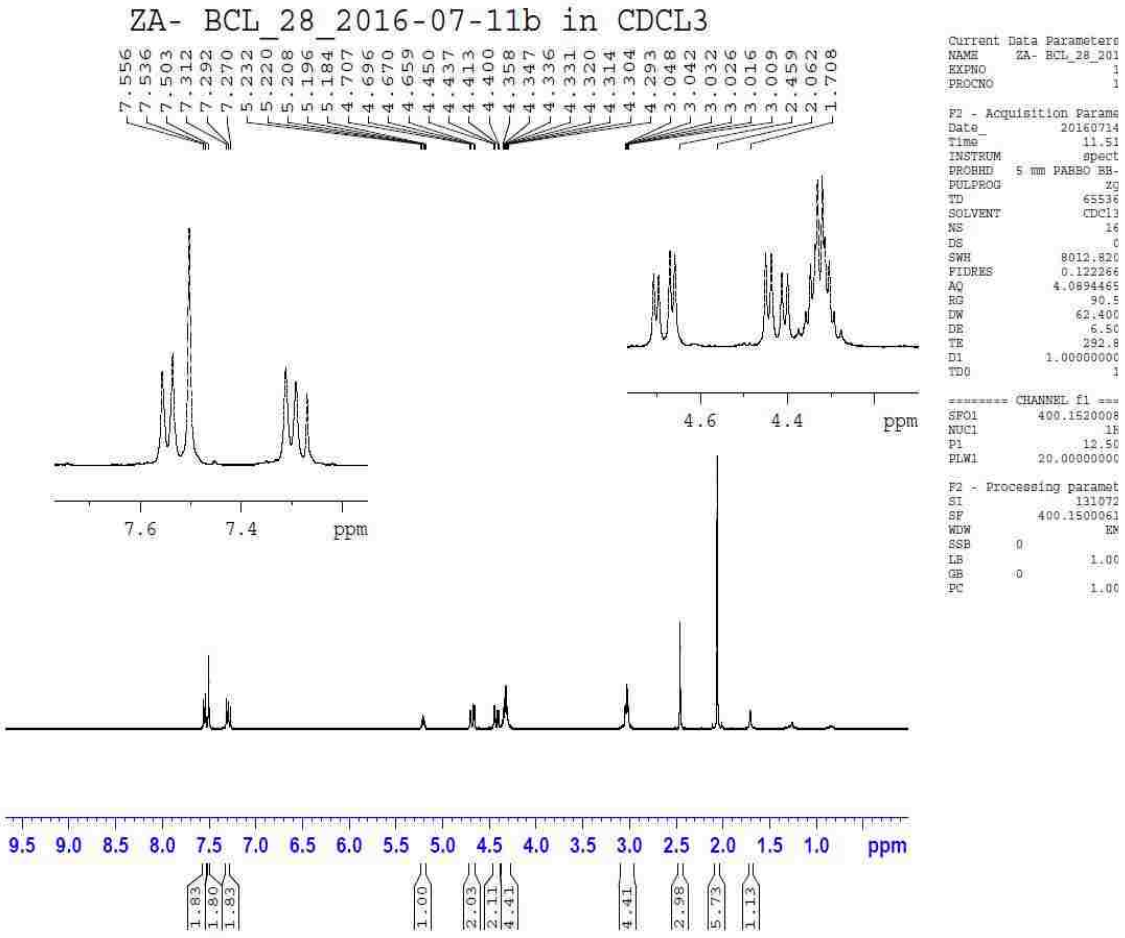


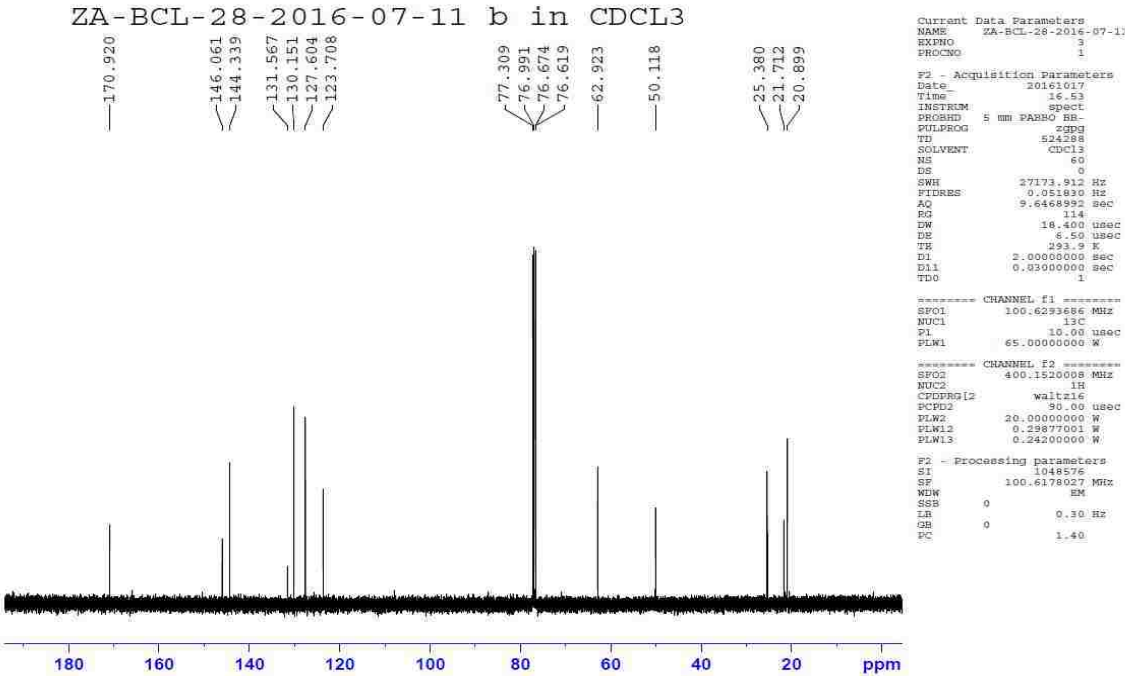


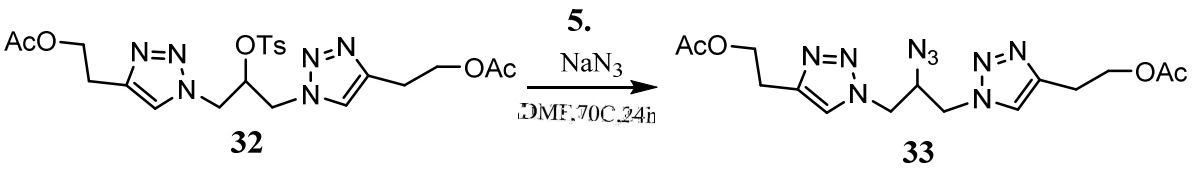
31 **32**

Following the procedure in the literature,[5] Et_3N (0.38ml, 2.76mmol, 2eq) and DMAP (0.016g, 0.13mmol, 0.1eq) were added to a solution of **31** (0.500g, 1.36mmol, 1eq) in dry DCM (10ml). Finally, TsCl (0.52g,2.72mmol, 2eq) was added slowly at different times to the solution and stirred for 24 hours at room temperature under nitrogen gas. After completion of the reaction, it was quenched with 15ml, aq. $NaHCO_3$. The aqueous layer was extracted three times with DCM. The organic layer was combined, dried over sodium sulfate, and filtered. The filtrate was evaporated under reduced

pressure. The product was purified by using column chromatography to obtain **32 (0.59g, 83%)**. The product was confirmed by NMR. ^{1}H NMR (400 MHz, CDC1$_3$) δ 7.54(d, 2H, J= 8 Hz), 7.50(s, 2 H), 7.30(d, 2H, J=8 Hz), 5.21(q, 1 H, J=4.8, 9.6 Hz), 4.69(dd, 2 H, J= 4.4, 14.8 Hz), 4.42(dd, 2H, J=5.2, 14.8 Hz), 4.35-4.29(m,4H)), 3.03 (t, 4 H, J=6.4 Hz), 2.46(s, 3H), 2.06 (s, 6H); ^{13}C NMR (101 MHz, CDCI$_3$) δ 170.9, 146.1, 144.3, 131.6, 130.1,127.6,123.7, 62.9, 50.1, 25.3, 21.7, 20.9*)*.

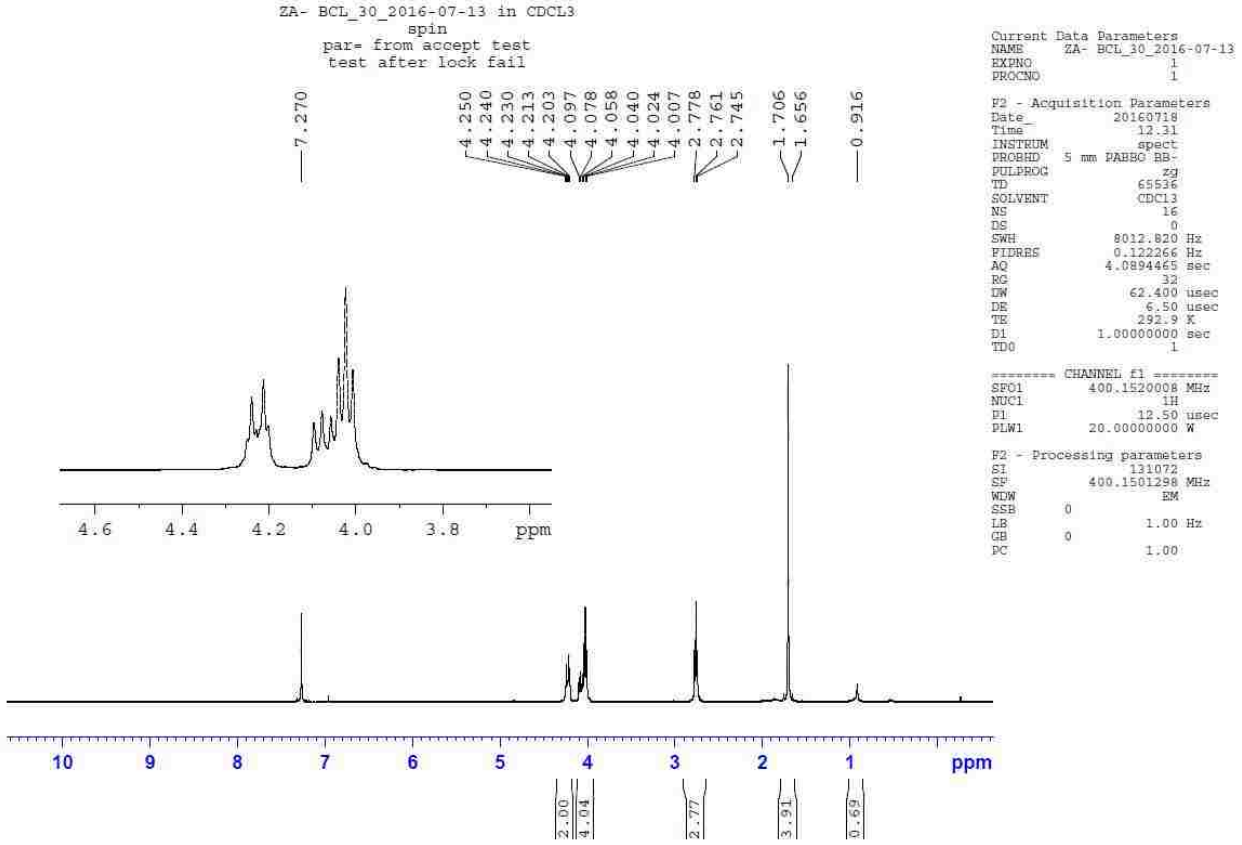


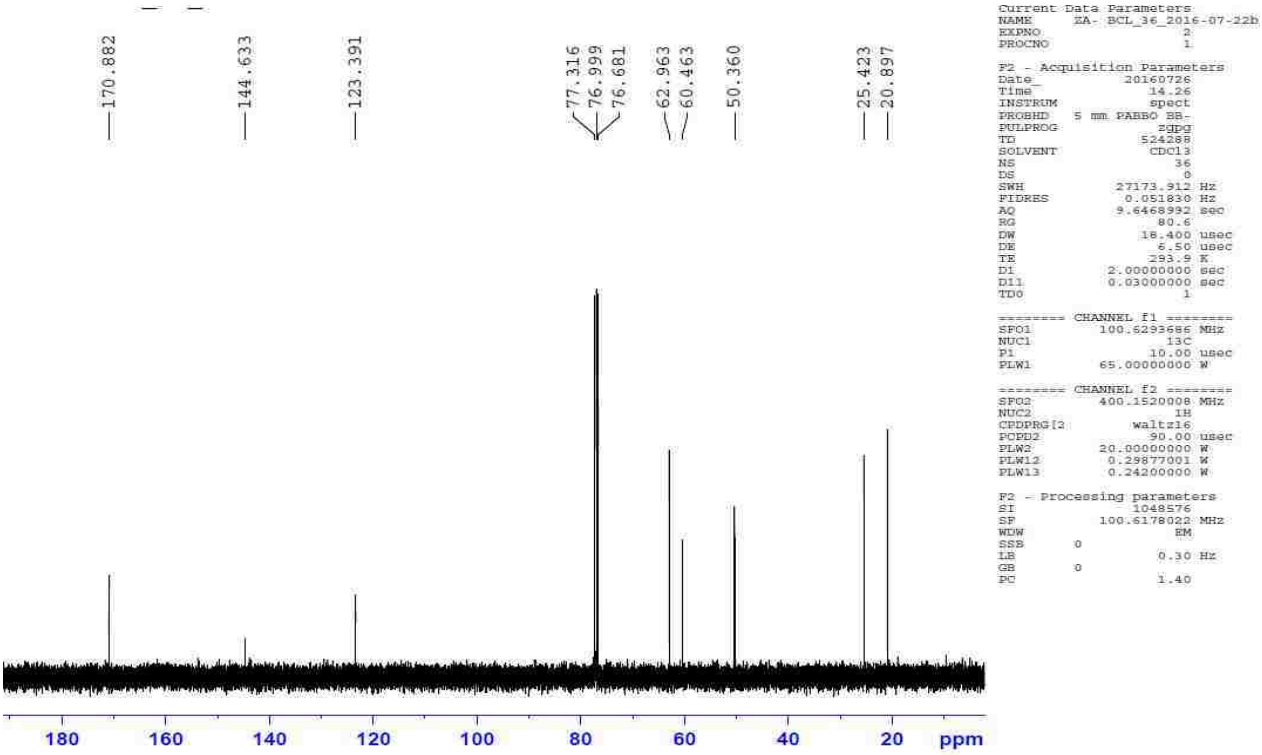




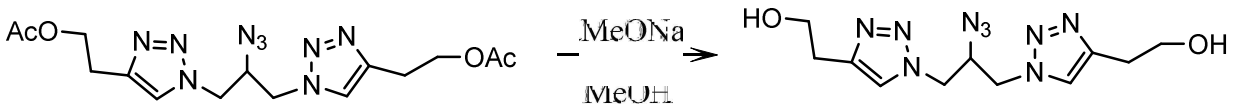
Following the procedure in the literature,[1] to a solution of the starting material **32** (0.17g, 0.32mmol, 1eq) in DMF (9ml), NaN_3 (0.063g,0.98mmol, 3eq) was added. The reaction mixture was stirred for 24 hours at 70 °C. After the reaction was completed, it was quenched with 25 ml of water. The aqueous layer was extracted three times with EtOAc (4*15 ml). The organic layer extracted was washed with 15 ml of water and 15ml of brine. The combined organic layer was dried over sodium sulfate and filtered. Then, the filtrate was evaporated under reduced pressure to get **33** (0.091,94%).

The product was confirmed by NMR. ^{1}H NMR (400 MHz, CDC1$_3$) δ 7.58 (s, 2 H), 4.56-4.51 (m, 3 H), 4.39(t, 2H, J=7.6 Hz),4.33(t, 4H, J=6.4 HZ), 3.07 (t, 4 H, J=6.4 Hz), 2.02 (s, 6 H); ^{13}C NMR (101 MHz, CDCI$_3$) δ 170.9, 144.6, 123.3, 62.9, 60.4, 50.3, 25.4, 20.8*).*



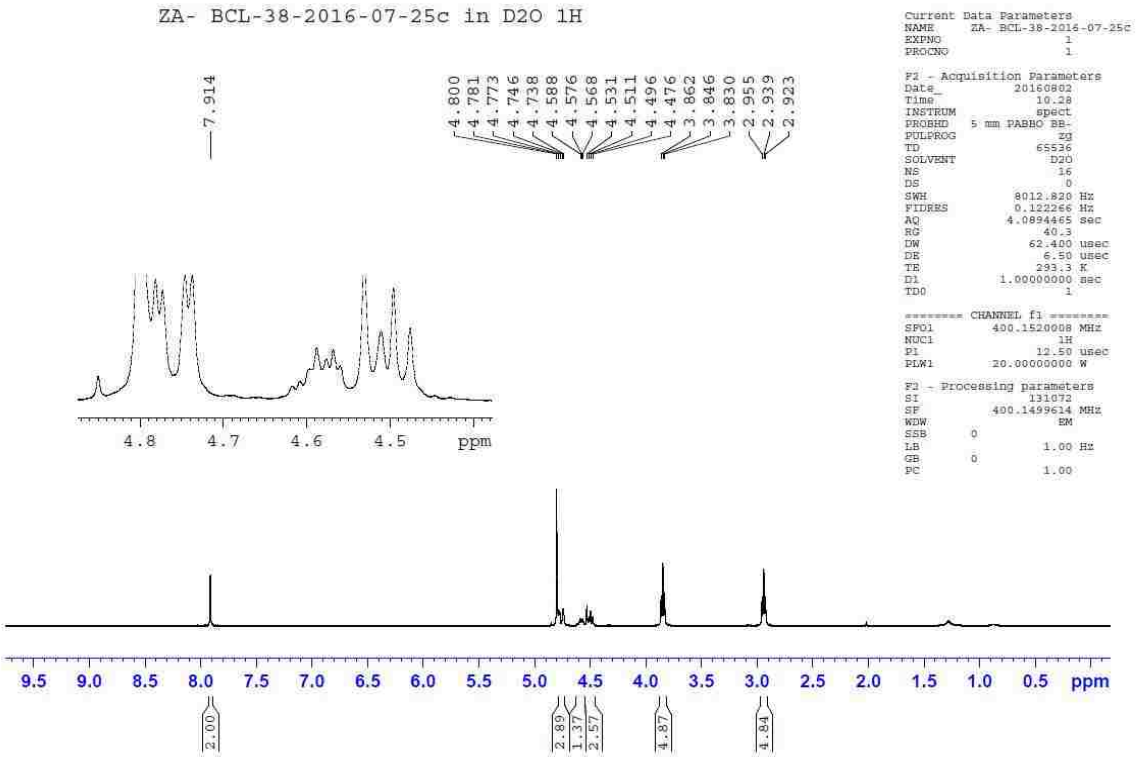


6.



33 **Ligand #2**
26

Following the procedure in the literature,[4] to o a solution of **33** (0.091g, 0.23mmol, 1eq) in methanol (4ml), sodium methoxide (0.0013g,0.024mmol ,0.1eq) was added and stirred for three hours at room temperature. After the reaction was compleled, it was quenched with a little Amber lite-exchange resins. The resins were removed by filtration and the obtained solution was concentrated under reduced pressure to get **26** (0.04g, 57%). The product was confirmed by NMR. ^{1}H NMR (400 MHz, D_2O) δ 7.91 (s, 2 H), 4.78-4.73 (dd, 2 H, J= 3.2, 14 Hz), 4.58-4.56 (m, 1 H), 4.53-4.47 (dd, 2H, J= 8, 14 Hz), 3.84(t, 4 H, J=6.4 Hz), 2.93 (t, 6 H, J=6.4 Hz); ^{13}C NMR (101 MHz, D_2O) δ &145.5, 1246, 60.9, 60.4, 50.8, 27.6).


ZA- BCL-38-2016-07-25c in D2O 1H
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4.800
4.781
4.773
4.746
4.738
4.588
4.576
4.568
4.531
4.511
4.496
4.476
3.862
3.846
3.830
2.955
2.939
2.923
4.8
4.7
4.6
4.5
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.00
2.89
1.37
2.57
4.87
4.84
Current Data Parameters
NAME ZA- BCL-38-2016-07-25c
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
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Time 10.28
INSTRUM spect
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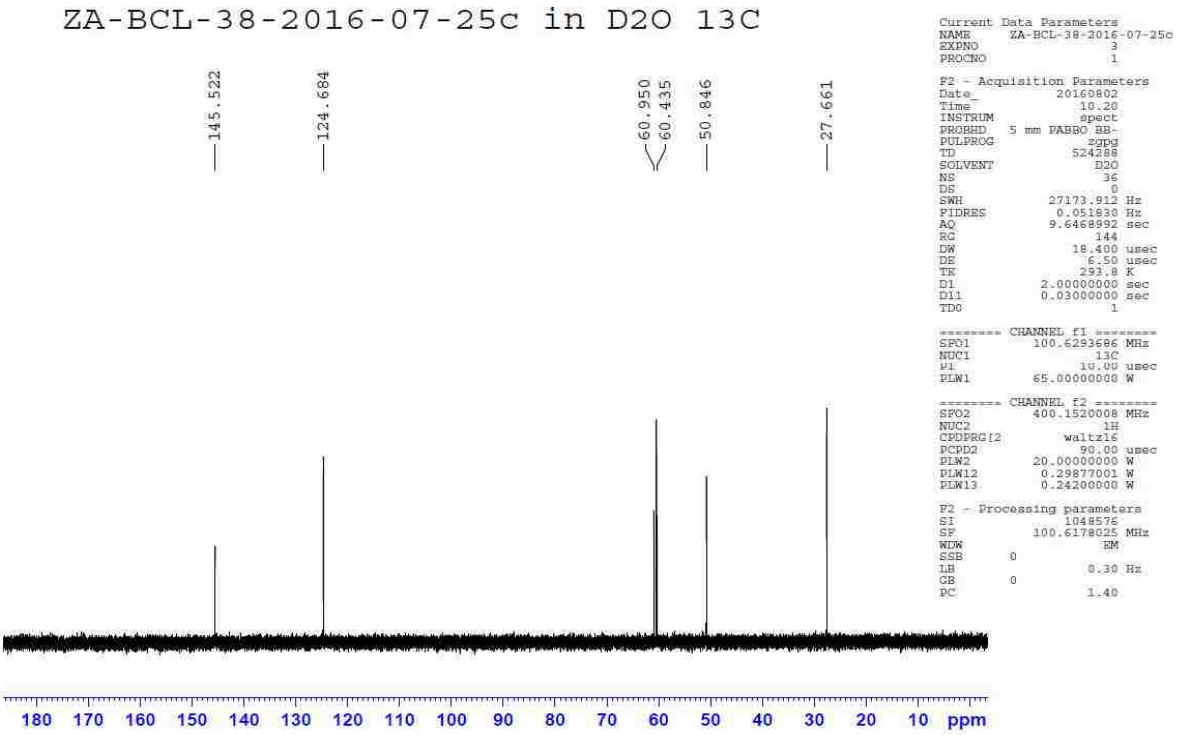
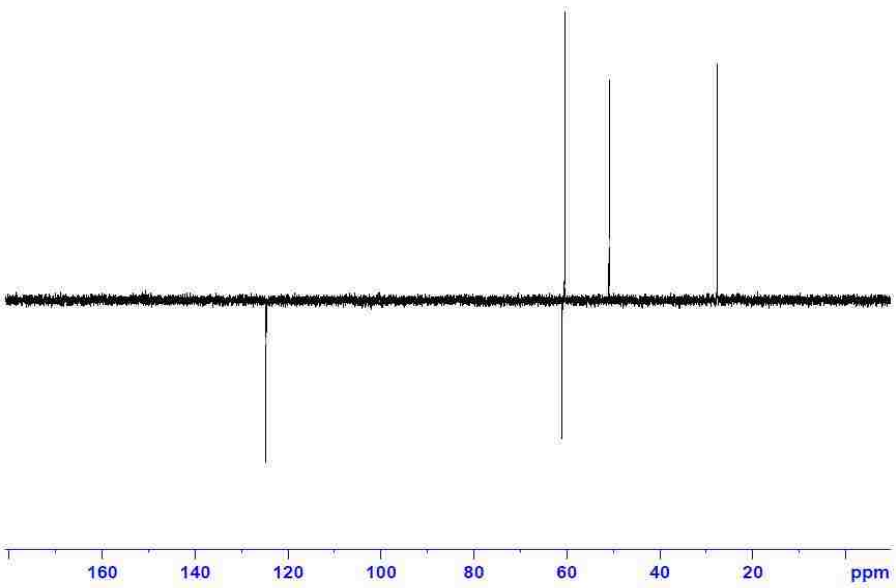
PULPROG zg
TD 65536
SOLVENT D2O
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 40.3
DW 62.400 usec
DE 6.50 usec
TE 293.3 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.1520008 MHz
NUC1 1H
P1 12.50 usec
PLW1 20.00000000 W
F2 - Processing parameters
SI 131072
SF 400.1499614 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00


ZA-BCL-38-2016-07-25c in D2O 13C
145.522
124.684
60.950
60.435
50.846
27.661
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
Current Data Parameters
NAME ZA-BCL-38-2016-07-25c
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160802
Time 10.20
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg
TD 524288
SOLVENT D2O
NS 36
DS 0
SWH 27173.912 Hz
FIDRES 0.051830 Hz
AQ 9.6468992 sec
RG 144
DW 18.400 usec
DE 6.50 usec
TE 293.8 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6293586 MHz
NUC1 13C
P1 10.00 usec
PLW1 65.00000000 W
======== CHANNEL f2 ========
SFO2 400.1520008 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 20.00000000 W
PLW12 0.29877001 W
PLW13 0.24200000 W
F2 - Processing parameters
SI 1048576
SF 100.6178025 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

ZA-BCL-38-2016-07-25c in D2O DEPT 135

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Current Data Parameters
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EXPNO                        11
PROCNO                        1

F2 - Acquisition Parameters
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Time                      10.46
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PULPROG                dept135
TD                        65536
SOLVENT                     D2O
NS                           48
DS                            8
SWH                   27173.912 Hz
FIDRES                 0.414641 Hz
AQ                    1.2058624 sec
RG                         2050
DW                      18.400 usec
DE                       6.50 usec
TE                      293.5 K
CNST2                145.0000000
D1                    2.00000000 sec
D2                    0.00344828 sec
D12                   0.00002000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1                 100.6293686 MHz
NUC1                         13C
P1                        10.00 usec
P2                        20.00 usec
PLW1                 65.00000000 W

======== CHANNEL f2 ========
SFO2                 400.1520008 MHz
NUC2                         1H
CPDPRG[2                 waltz16
P3                        11.00 usec
P4                        22.00 usec
PCPD2                     90.00 usec
PLW2                 20.00000000 W
PLW12                 0.29877001 W

F2 - Processing parameters
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SF                   100.6177975 MHz
WDW                           EM
SSB                  0
LB                        1.00 Hz
GB                   0
PC                        1.40
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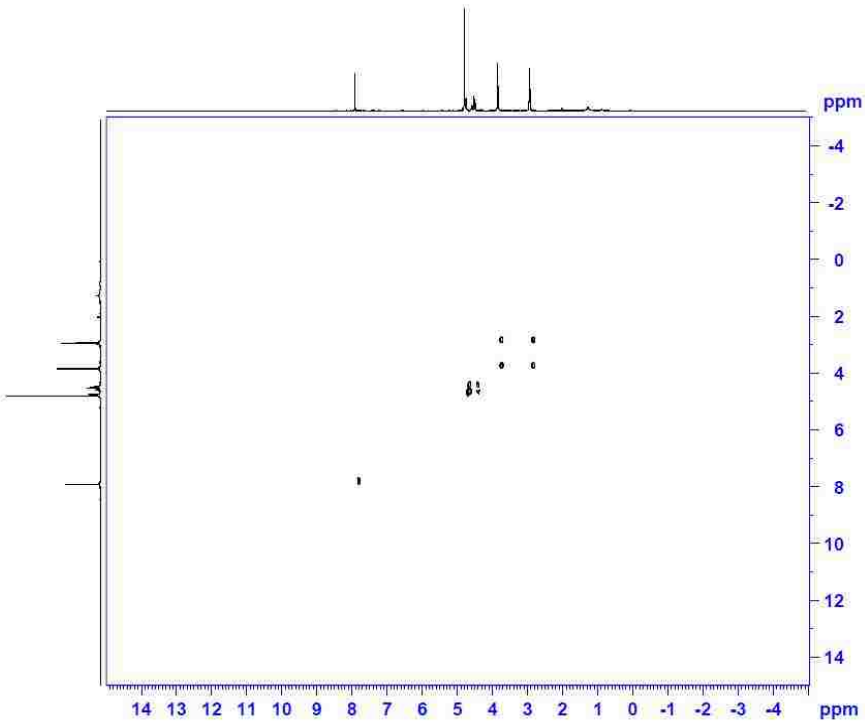
ZA-BCL-38-2016-07-25c in D2O COSY

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EXPNO                        33
PROCNO                        1

F2 - Acquisition Parameters
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Time                      11.04
INSTRUM                   spect
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PULPROG                cosygpqf
TD                         2048
SOLVENT                     D2O
NS                            8
DS                            8
SWH                   8012.820 Hz
FIDRES                3.912510 Hz
AQ                    0.1277952 sec
RG                          144
DW                      62.400 usec
DE                       6.50 usec
TE                      293.0 K
D0                 0.00000300 sec
D1                 1.50000000 sec
D13                0.00000400 sec
D16                0.00020000 sec
IN0                0.00012480 sec

======== CHANNEL f1 ========
SFO1                 400.1520008 MHz
NUC1                         1H
P0                        12.50 usec
P1                        12.50 usec
PLW1                 20.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]       SMSQ10.100
GPZ1                      10.00 %
P16                     1000.00 usec

F1 - Acquisition parameters
TD                          128
SFO1                 400.152 MHz
FIDRES                62.600159 Hz
SW                       20.024 ppm
FnMODE                       QF

F2 - Processing parameters
SI                          1024
SF                   400.1500070 MHz
WDW                        QSINE
SSB                  0
LB                   0 Hz
GB                   0
PC                        1.00

F1 - Processing parameters
SI                          1024
MC2                          QF
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WDW                        QSINE
SSB                  0
LB                   0 Hz
GB                   0
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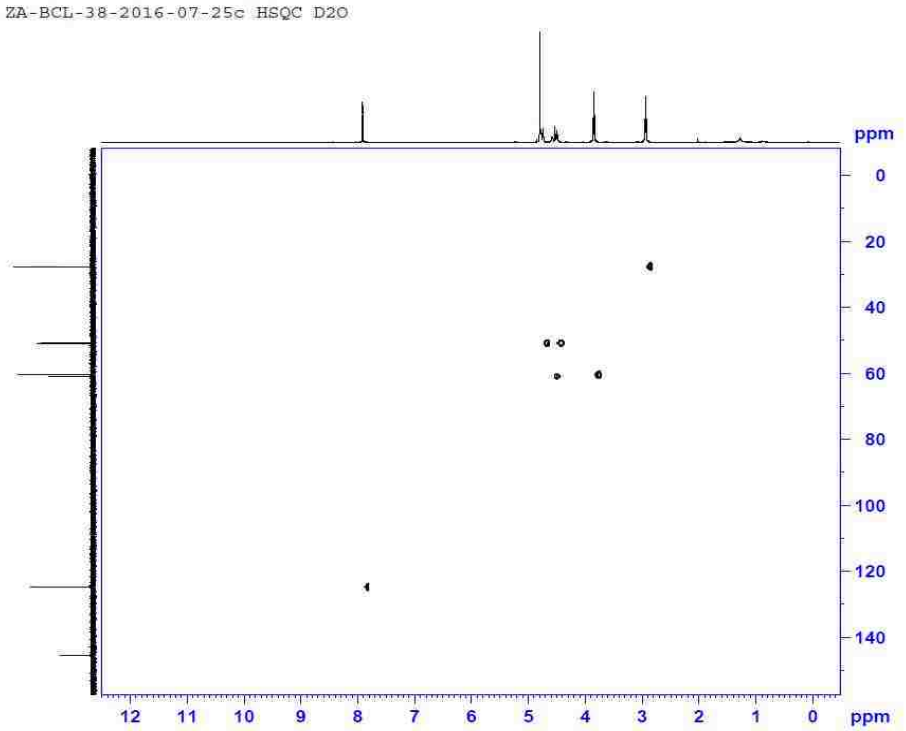
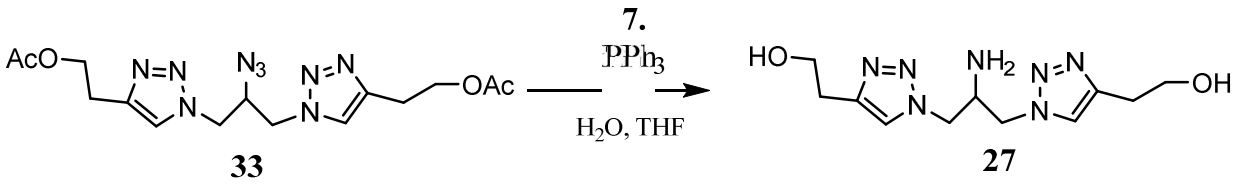
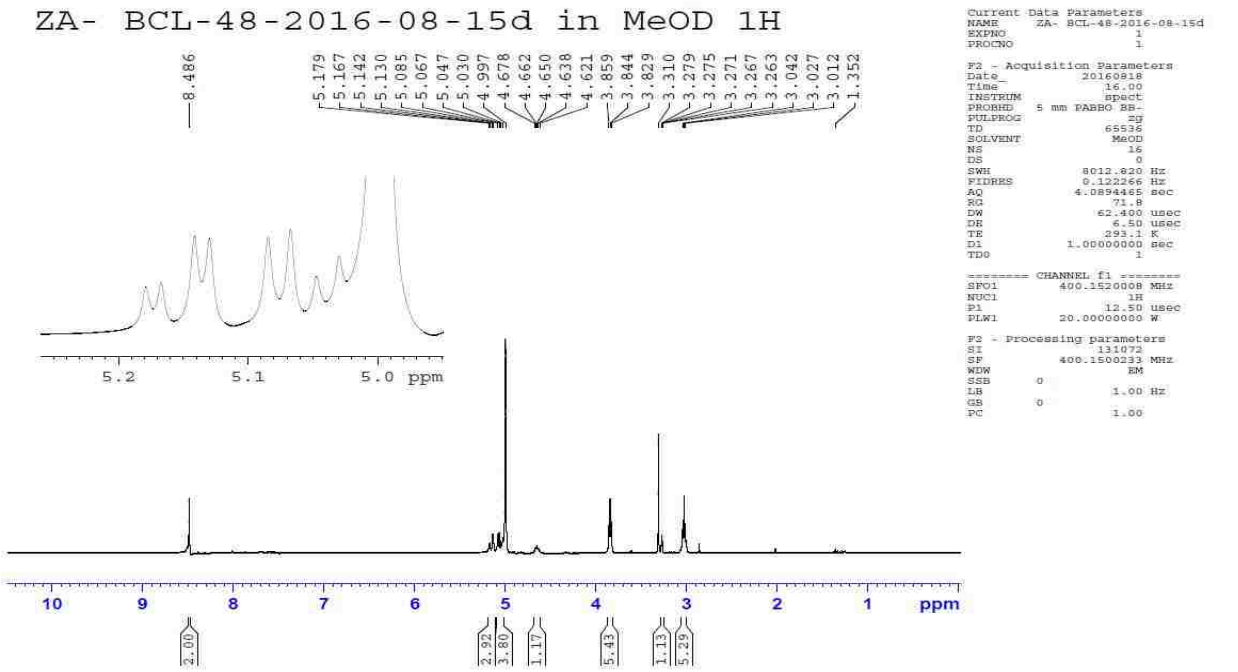


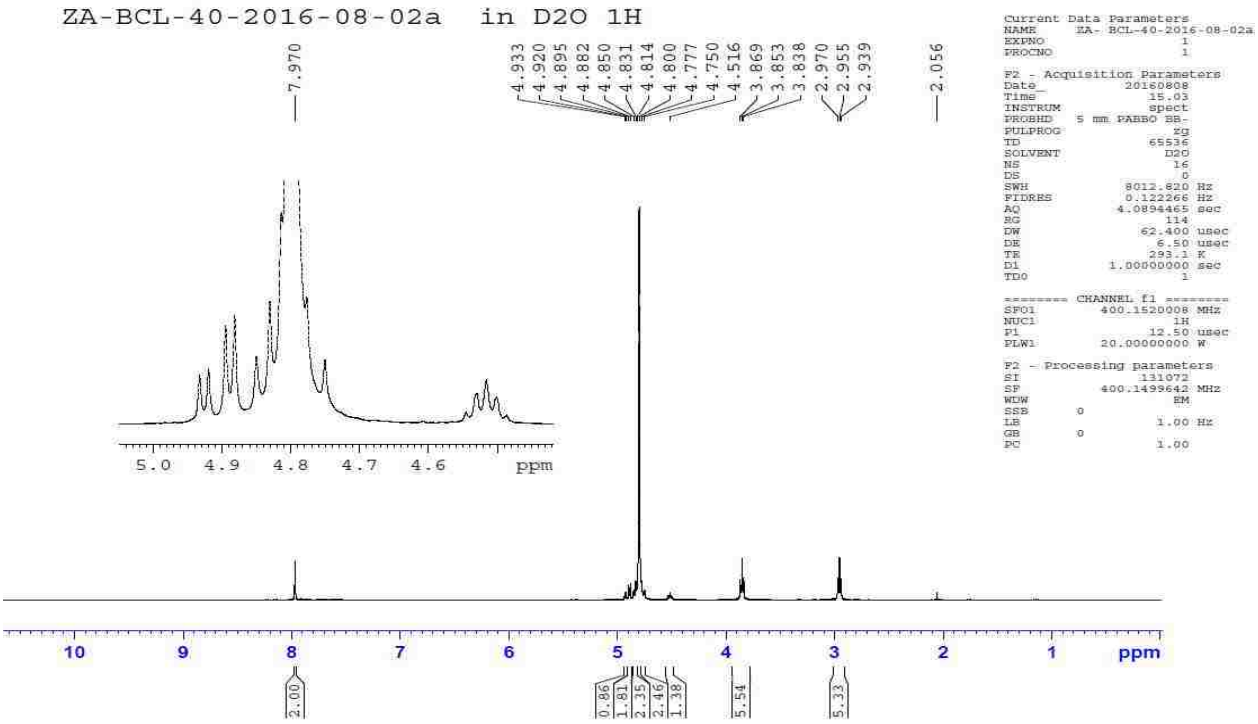


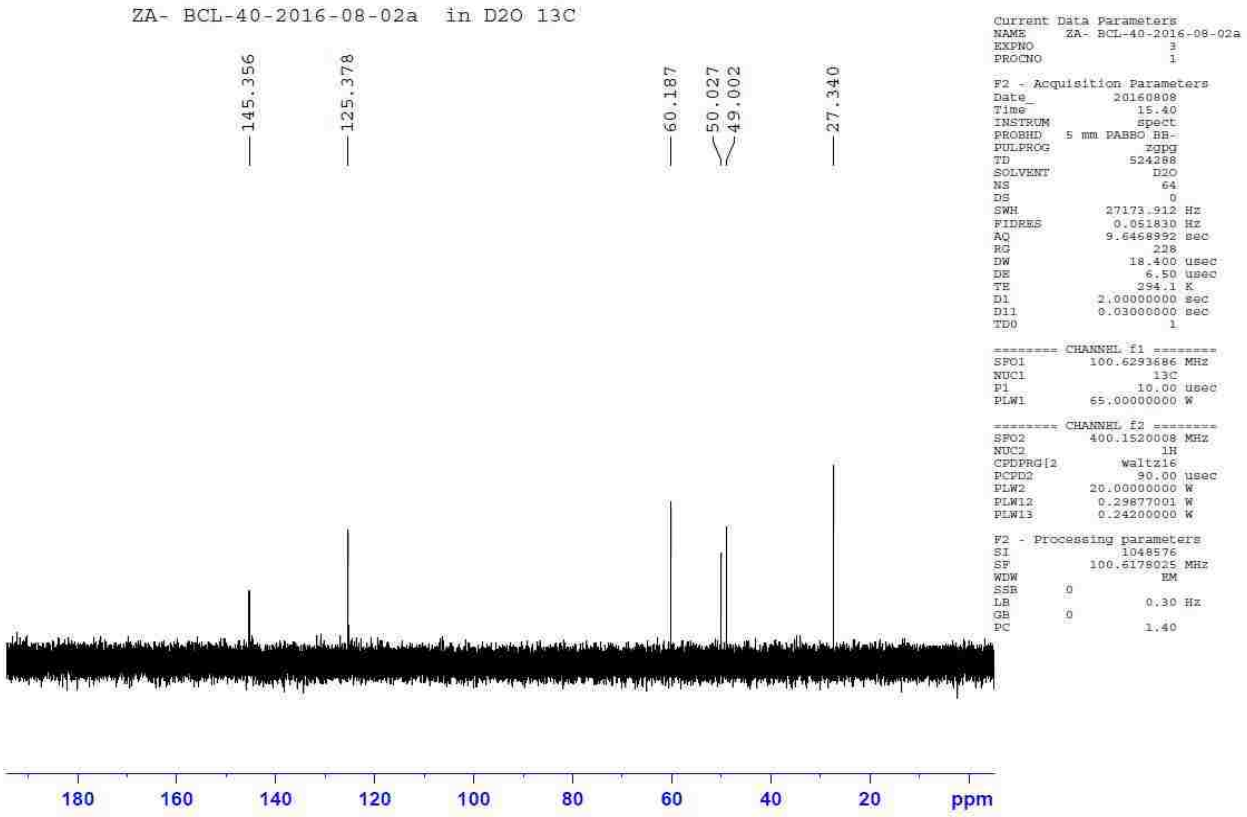
33 **27**

Following the procedure in the literature,[6] to o a solution of the starting material **33** (0.170g,0.43mmol,1eq**)** in 4ml of (THF: water) (10:1), PPh_3(0.23g, 0.87mmol, 2eq) was added. It was stirred for two days at 40 °C. After completion of the reaction, it was quenched with 1 ml of HCl (0.5 M). The aqueous layer was extracted three times with EtOAc (3*15 ml). The organic layer extracted had the byproducts and the water layer had the product. The water layer was evaporated under reduced pressure to get **27** (0.092g, 85%) The product was confirmed by NMR using D_2O as a solvent. ^{1}H NMR (400 MHz, MeOD) δ 8.48(s, 2 H), 5.15(dd, 2 H, J= 4.8,

14.8 Hz), 5.05(dd, 2H, J= 7.2, 15.2 Hz), 4.67-4.62 (m, 1H), 3.84(t, 4 H, J=6Hz), 3.02 (t, 4 H, J=6 Hz); ^{13}C NMR (101 MHz, D_2O) δ 145.3, 125.3, 60.1, 50.02, 49.0, 27.3)





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EXPNO                          3
PROCNO                         1

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Time                   15.40
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PROBHD    5 mm PABBO BB-
PULPROG                zgpg
TD                     524288
SOLVENT                 D2O
NS                        64
DS                         0
SWH                27173.912 Hz
FIDRES              0.051830 Hz
AQ                  9.6468992 sec
RG                       228
DW                    18.400 usec
DE                     6.50 usec
TE                    294.1 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1               100.6293686 MHz
NUC1                       13C
P1                       10.00 usec
PLW1               65.00000000 W

======== CHANNEL f2 ========
SFO2               400.1520008 MHz
NUC2                       1H
CPDPRG[2                waltz16
PCPD2                    90.00 usec
PLW2               20.00000000 W
PLW12               0.29877001 W
PLW13               0.24200000 W

F2 - Processing parameters
SI                      1048576
SF                 100.6178025 MHz
WDW                        EM
SSB          0
LB                       0.30 Hz
GB           0
PC                       1.40
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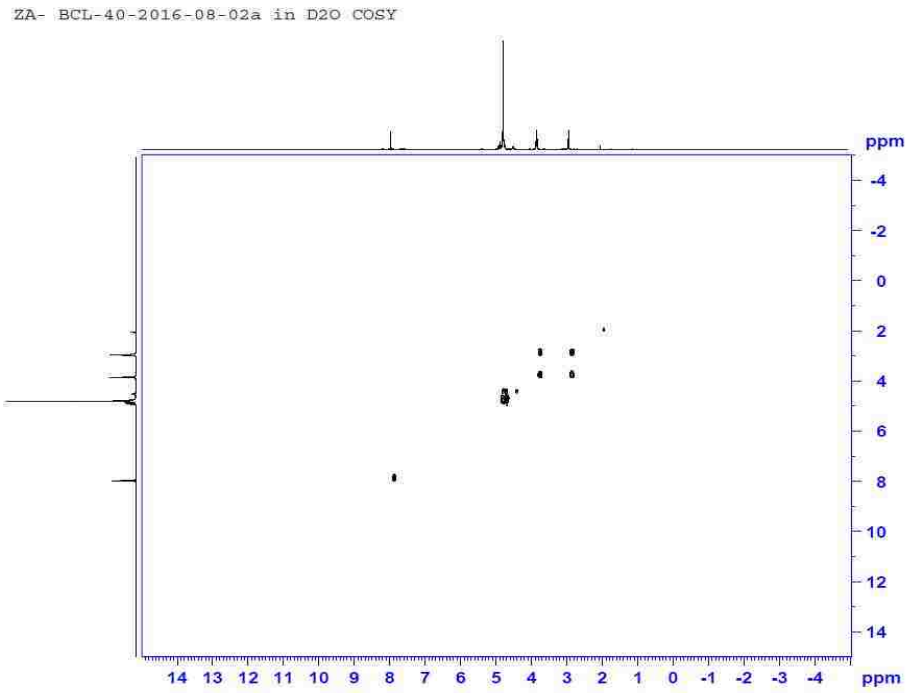


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PROCNO                         1

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PULPROG             cosygpqf
TD                     2048
SOLVENT                 D2O
NS                        8
DS                        8
SWH                8012.820 Hz
FIDRES             3.912510 Hz
AQ                 0.1277952 sec
RG                      144
DW                    62.400 usec
DE                     6.50 usec
TE                    292.8 K
D0                 0.00000300 sec
D1                 1.50000000 sec
D13                0.00000400 sec
D16                0.00020000 sec
IN0                0.00012480 sec

======== CHANNEL f1 ========
SFO1               400.1520008 MHz
NUC1                       1H
P0                       12.50 usec
P1                       12.50 usec
PLW1               20.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]         SMSQ10.100
GPZ1                    10.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1                400.152 MHz
FIDRES             62.600159 Hz
SW                     20.024 ppm
FnMODE                   QF

F2 - Processing parameters
SI                       1024
SF                400.1500070 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
PC                       1.00

F1 - Processing parameters
SI                       1024
MC2                        QF
SF                400.1500052 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
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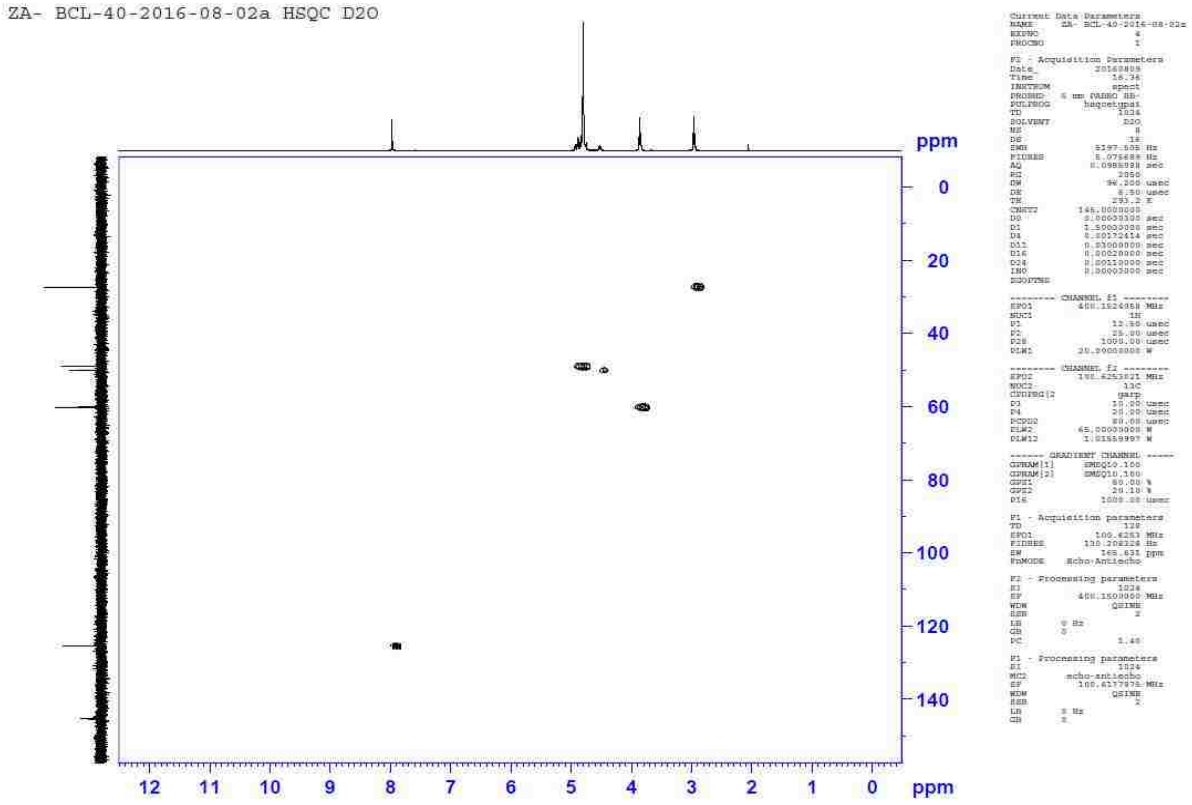
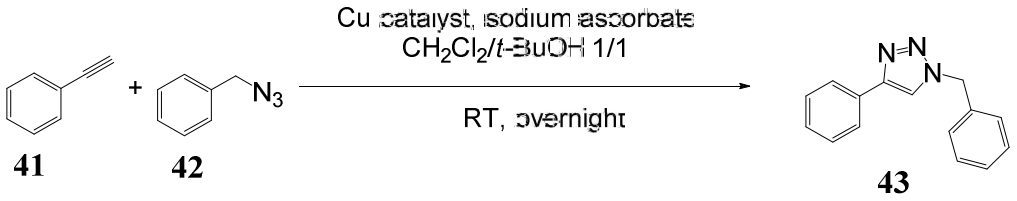
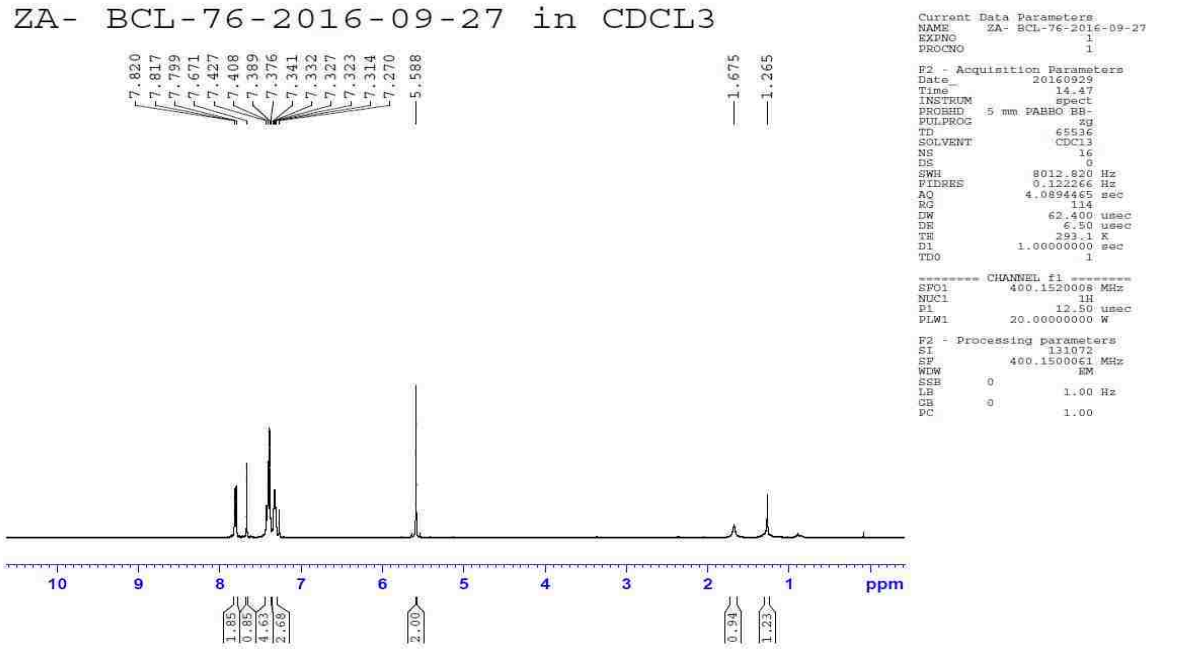


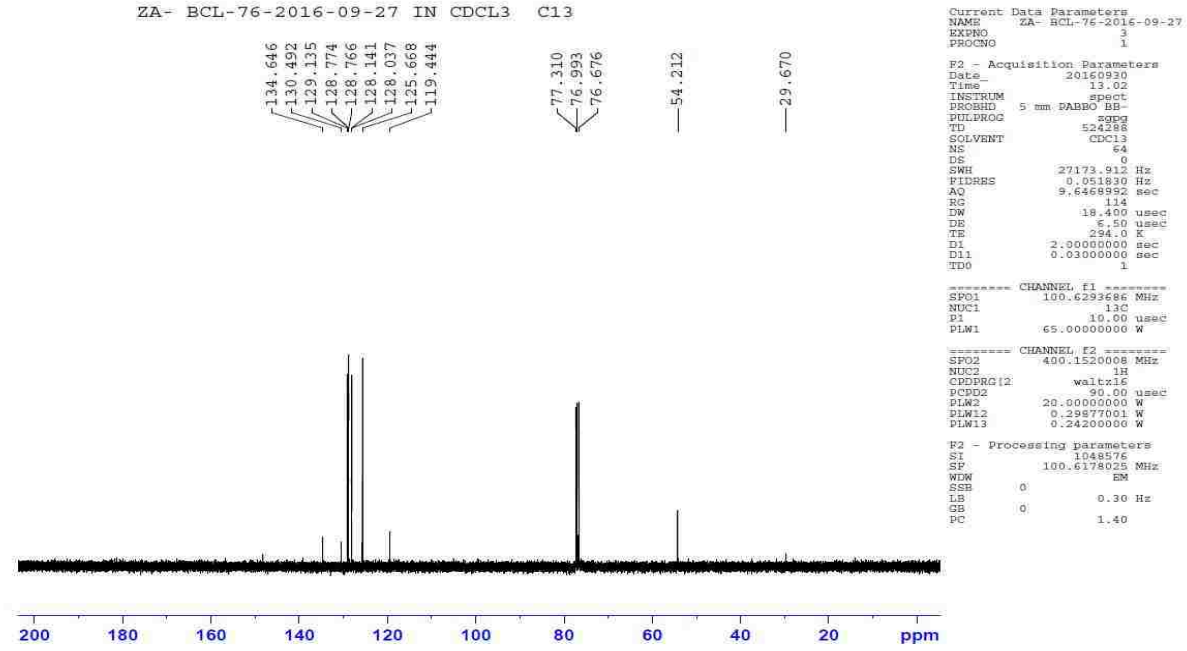


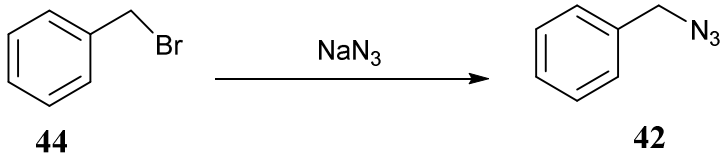
41 **42** **43**

Following the procedure in the literature.[7] to o a solution of phenyl acetylene **41** (0.010g, 0.097mmol, 1eq) and benzyl azide **42** (0.0156g,0.117mmol, 1.2eq) in 2ml of (DCM/t-BuOH) (1/1), $CuSO_4$(0.007g,49µl, 0.0048mmol, 0.05eq) and Na ascorbate (0.002g. 9.8µl, 0.0097mmol, 0.1eq) were added. The reaction mixture was stirred for 24 h at room temperature. After the reaction completion, 3 ml of water was added. The aqueous layer was extracted three times with DCM (3*5 ml). The organic layer extracted was dried with sodium sulphate. The filtrate was evaporated under reduced pressure to get the product **43** (0.019g, 83%). The product was confirmed by NMR. ^{1}H NMR (400 MHz, $CDCL_3$) δ 7.81(d, 2 H, J= 8.4 Hz), 7.67(s, 1H), 7.43-

7.38(m, 5H), 7.34-7.31(m, 3H), 5.59(s, 2H); ^{13}C NMR (101 MHz, $CDCL_3$) δ 134.6, 130.5, 129.1, 128.8, 128.7, 128.1, 128.0, 125.6, 119.4, 54.2, 29.6).

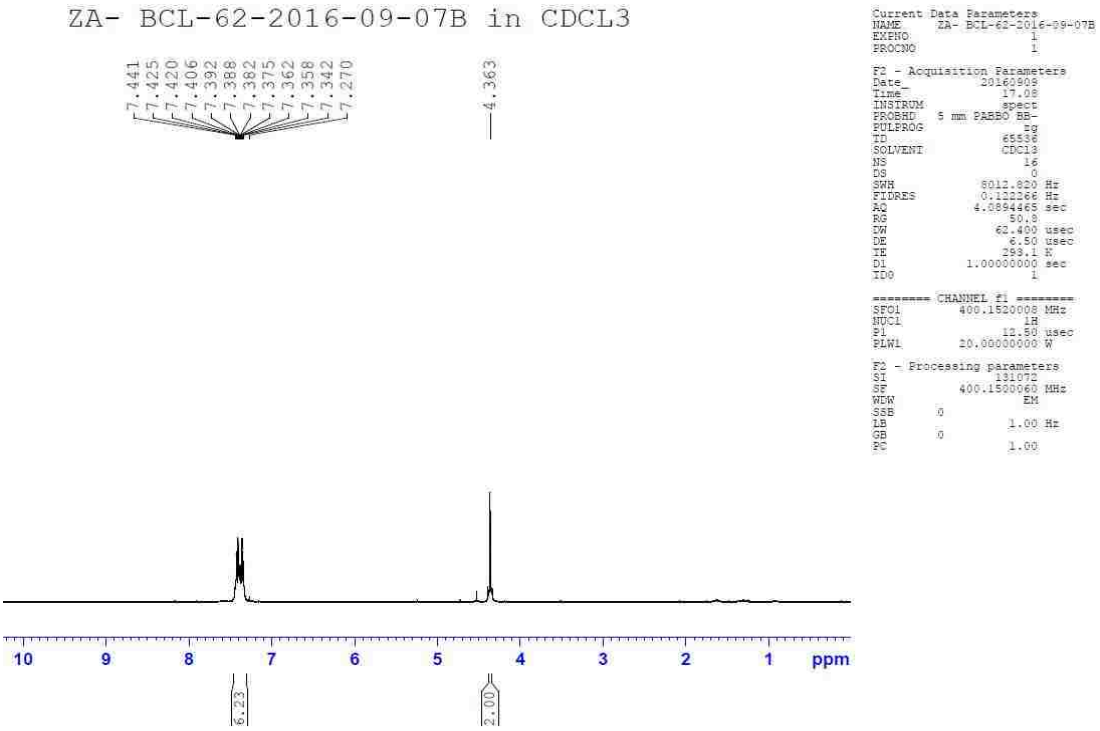




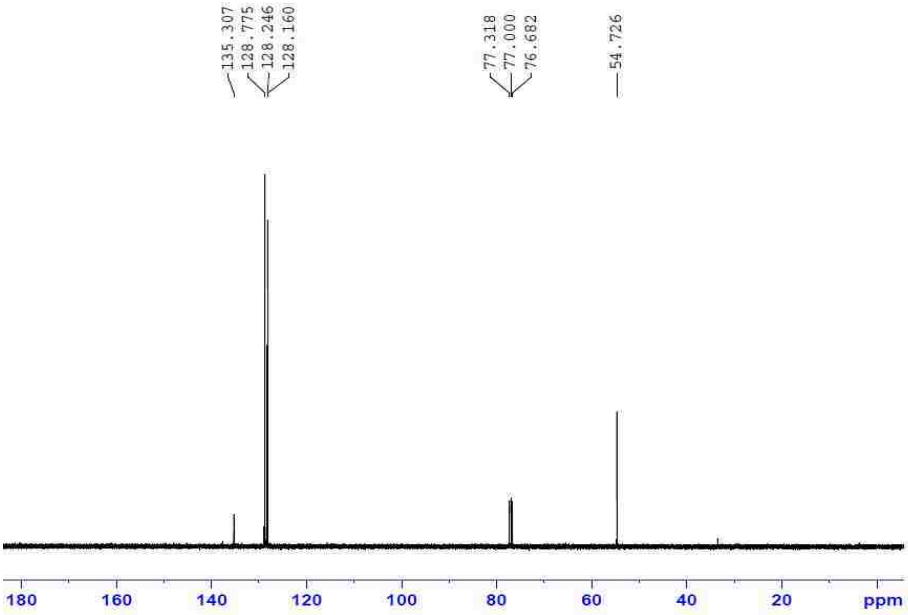


44 **42**

Following the procedure in the literature,[8] to a solution of the starting material benzyl bromide **44** (1.08g, 6.3mmol) in DMF(20ml), NaN_3(0.63g, 9.69mmol) was added. The reaction mixture was stirred for 24 hours at 80°C. After the reaction was completed, it was quenched with 45 ml of water. The aqueous layer was extracted by washing the water layer three times with diethylether (3*45 ml). The organic layer extracted was washed with (5*50) ml of water. The combined organic layer was dried over sodium sulfate and filtered. Then, the filtrate was concentrated under reduced pressure to get benzyl azide **42** (1.15g, 95%). NMR. ^{1}H NMR (400 MHz, $CDCL_3$) δ 7.44-7.27(m, 5H), 4.36(s, 2H); 13C NMR (101 MHz, $CDCL_3$) δ 135.3, 128.7, 128.1, 128.2, 54.7).



ZA- BCL-62-2016-09-07B IN CDCL3 C13

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PROCNO                         1

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Date_                   20160909
Time                       17.21
INSTRUM                    spect
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PULPROG                    zgpg
TD                       524288
SOLVENT                    CDCl3
NS                            16
DS                             0
SWH                    27173.912 Hz
FIDRES                     0.051830 Hz
AQ                         9.6468992 sec
RG                           114
DW                        18.400 usec
DE                         6.50 usec
TE                        293.4 K
D1                     2.00000000 sec
D11                    0.03000000 sec
TD0                            1

======== CHANNEL f1 ========
SFO1                 100.6293686 MHz
NUC1                         13C
P1                        10.00 usec
PLW1                65.00000000 W

======== CHANNEL f2 ========
SFO2                 400.1320008 MHz
NUC2                         1H
CPDPRG[2                 waltz16
PCPD2                     90.00 usec
PLW2                20.00000000 W
PLW12                0.29877001 W
PLW13                0.24200000 W

F2 - Processing parameters
SI                          1048576
SF                   100.6178087 MHz
WDW                            EM
SSB                 0
LB                          0.30 Hz
GB                  0
PC                          1.40
```

References

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Chapter 4- Conclusion and Future Work

Conclusion

Copper-catalyzed azide-alkyne 1, 3-dipolar cycloaddition CuAAC is the most powerful and popular bioorthogonal reaction because it meets most of the features of a click reaction. The applications of CuAAC have been shown in many distinct fields, including chemical biology, polymer chemistry, and organic synthesis. However, the copper toxicity is still a critical issue which has been observed in different types of cells. Some researchers stated that the uses of ligands have a major impact on increasing the reaction rate, reducing copper toxicity and protecting Cu(I) from oxidation by oxygen. Therefore, in this study, three different water-soluble ligands were synthesized and evaluated in the CuAAC reaction. It was found that ligand#1 with –OH achieved higher efficiency in enhancing the reaction rate than ligand#2 and ligand#3. Because of that, ligand #1 was tested in different solvents and different amounts of catalysts. To conclude, ligand#1 showed better results at a low amount of catalysts and in organic systems than aqueous systems. When ligand#1 was compared with TBTA, the most extensively used ligand, TBTA provides a better result in accelerating the reaction rate than logand#1.

Future work

For future work, the Staudinger ligation will be examined in a reaction with a substrate that has an ester group only. The purpose of that is to see if the Staudinger ligation has the ability to remove the acetyl group or not. Moreover, different studies need to be conducted in order to evaluate these ligands accurately. First, the ligands will be tested in click reactions that contain water-soluble starting materials in different solvents. Second, the structure of ligand#1 will be redesigned to mimic the standard structure of TBTA, Finally, appropriate techniques will be used to obtain accurate quantitative data.