ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF CARBORANE-APPENDED BIOMOLECULES

Tirtha Raj Sibakoti, MS
Department of Chemistry and Biochemistry
Northern Illinois University, 2015
Dr. Narayan S. Hosmane, Director

A multi-step organic synthetic methodology to attach multiple boron-containing carborane cages (boron source) to suitable biocompatible organic compounds (carriers) that can be used for boron neutron capture therapy (BNCT) applications has been established. The method involves alkylation of the selected organic compounds followed by conjugation with a polyhedral carborane cage using a click reaction. To date, carborane derivatives of 4-nitroimidazole, 2-nitroimidazole, phenothiazine, 1, 3, 4-thiadiazole-2, 5-dithiol, adenine, 6-chloropurine and 3’, 5’-dihydroxyacetophenone have been successfully synthesized. In addition, different coumarin derivatives were successfully synthesized using click chemistry. All products were purified and isolated in good yields and characterized using Fourier-Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), elemental analysis and Mass Spectrometry (MS). These products could be made water soluble by decapitation of the carborane cage in order for their biological evaluation.
SYNTHESIS AND CHARACTERIZATION OF
CARBORANE-APPENDED BIOMOLECULES

BY
TIRTHA RAJ SIBAKOTI
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Thesis Director:
Narayan S. Hosmane
DEDICATION

This thesis is dedicated to my lovely parents Krishna Prasad Sibakoti and Rama Kumari Sibakoti
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I would like to dedicate this thesis to my parents (Krishna Prasad Sibakoti and Rama Kumari Sibakoti) for their treasurable love and overwhelming support.

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CHAPTER 1

BACKGROUND

The objective of this thesis was to develop a multi-step organic synthetic methodology to synthesize carborane-appended biomolecules as drug delivery agents that would deliver a sufficient amount of boron within tumor cells with least toxicity\(^1\). However, this work mostly focused on the synthesis of novel carborane-appended biomolecules by applying click chemistry\(^2,3\). Various organic compounds with different functional groups were selected as a carrier because of their biocompatibility, lower toxicity and ease of functionalization. The selected starting materials chosen as carrier compounds are 4-nitroimidazole, 2-nitroimidazole, phenothiazine, 1,3,4-thiadiazole-2,5-dithiol, adenine, guanine, 6-chloropurine, 3’,5’-dihydroxyacetophenone and coumarin derivatives. These compounds are of different nature, and they would potentially target different receptors found on the surface of a tumor cell; however, they all could increase the uptake of boron in some ways. They all also have a very good binding affinity.

A synthetic methodology was developed to accomplish this goal. The initial step was to perform the alkylation reaction of these selected compounds using propargyl bromide, appropriate base and solvent, and then directly attach the decaborane on them. However, this direct approach did not work as planned. Thus, determining different routes in order to attach boron on the carrier compounds was important. Fortunately, click chemistry was taken as the next approach and was successful. Click chemistry, previously known as Cu(I)I-catalyzed azide-
alkyne cycloaddition reaction, is a simple reaction between terminal alkyne and azide functional 
groups resulting in cyclic triazole ring with the use of a catalyst. In this case, 1-methyl-o-
carborane was selected as a boron source, and following the literature, alkyl azide group was 
incorporated into it. Carboranes are a class of heteroboranes that contains both boron (10 atoms) 
and carbon atoms in clusters that can enhance the uptake of boron in a cell; they can easily be 
functionalized and also they can be decapitated to make the carboranyl derivative water-soluble. 
The combination of selected carrier compounds and carborane are complementary as the 
hydrophilic selected carriers compensate for hydrophobicity of carboranes\(^2\). This newly 
developed methodology has been successful in incorporating carborane into most of the carrier 
agent. The compounds were purified and isolated in good yields and characterized using Fourier-
Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), Mass 
Spectrometry (MS) and elemental analysis.

In addition, all the compounds could be made water soluble by decapitating the carborane 
cage in order for bio-distribution studies to investigate their application in medicinal chemistry, 
particularly in boron neutron capture therapy (BNCT) for cancer treatment. Once the compounds 
are water soluble, biological distribution studies will be performed in the near future in order to 
calculate the boron uptake and determine if they are potential cancer therapeutics. Cytotoxicity 
of few compounds has been tested and found to be non-toxic in the various cancer cell lines. 
Furthermore, suitable compounds could be encapsulated with the liposomes to make the drug 
delivery feasible and effective. Various other organic compounds could also be selected as a 
starting material in order to synthesize more carborane-appended biomolecules with the 
established methodology.
CHAPTER 2
CARBORANES

2.1 Introduction

Carboranes (C\(_2\)B\(_{10}\)H\(_{12}\)) belong to the heteroboranes family, which consists of two carbon atoms, ten boron atoms and twelve hydrogen atoms\(^1\). The carboranes are polyhedral clusters which exhibit icosahedral structure (20 faces, 30 edges and 12 vertices)\(^4\). The presence of two carbon atom in carboranes allows various organic modifications. The hydrogens bonded to the carbons in the carborane cage are acidic, allowing their easy removal using strong bases, thus yielding several derivatives of clusters. These icosahedral dicarbaboranes can be easily functionalized and have been frequently applicable in medicinal chemistry\(^5\). Therefore, carboranes are of great interest for boron chemists to look into for their research. The most common applications can be observed in materials science, novel drug discovery, catalysis and polymers.

2.2 Nomenclature and Different Forms

In general, nomenclature of carboranes is represented by C\(_2\)B\(_n\)H\(_{n+2}\), where \(n\) is an integer\(^4\). Carboranes with \(n\) ranging from 3 to 12 have been synthesized and characterized. The numbering of the atoms in carboranes starts with the atom of lowest coordination with fewest bonds, and numbering polyhedral vertex atoms in a clockwise direction (carbon atoms being given the lowest possible numbers). The systematic (IUPAC) name of carboranes is given as closo-dicarbadodecaborane.
Carboranes exist in three different isomeric forms as ortho-, meta-, and para- isomers, which only differ in the relative position of carbon atoms in the cluster as shown in Figure 1. Most commonly, ortho- and meta- forms of these clusters are used to obtain their derivatives. Ortho-carboranes are the most extensively studied of all forms of carboranes.

Figure 1: Structure of ortho-, meta-, and para- carborane isomers

Generally, structures of carboranes can be predicted based on electron counting rules known as “Wade’s rules”. Application of Wade’s rules will provide a relatively simple relationship between the number of skeletal bonding electron pairs and the geometry of a carborane molecule, which is shown in Table 1 below, where $x$ is occupied vertices.
Carboranes are classified into three geometric categories; closo, nido and arachno. The closo (closed) carboranes are more thermally stable and less air sensitive compared to nido (open) and arachno forms as they lack closed polyhedral structures\textsuperscript{6, 7, 8}. The skeletal atoms occupy all corners of a polyhedron in closo structures, while in nido, one corner of the polyhedron is vacant as shown in Figure 2 below (n=12). Additionally, if two vertices are removed from the closo structure, it will result in the arachno form of carborane\textsuperscript{9}.

Table 1

Summary of Electron-counting Rules

<table>
<thead>
<tr>
<th>Structure types</th>
<th>Skeletal electron pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>closo</td>
<td>x + 1</td>
</tr>
<tr>
<td>Nido</td>
<td>x + 2</td>
</tr>
<tr>
<td>Arachno</td>
<td>x + 3</td>
</tr>
</tbody>
</table>
Figure 2: Schematic polyhedral atomic structures of closo, nido, and arachno carboranes showing conventional numbering schemes.
2.3 Synthesis of Carboranes

The first carboranes were synthesized by the reaction between pentaborane (B₅H₉) and acetylene (C₂H₂) in an electric glow discharge. This reaction is shown in equation 1.

\[ \text{B}_5\text{H}_9 + \text{C}_2\text{H}_2 \rightarrow 1,5\text{-C}_2\text{B}_3\text{H}_5 + 1,6\text{-C}_2\text{B}_4\text{H}_6 + 2,4\text{-C}_2\text{B}_5\text{H}_7 \ldots \ldots \ldots (1) \]

According to the literature published, carboranes can be synthesized by reacting decaborane (B₁₀H₁₄) with acetylene in presence of Lewis base and appropriate solvent to yield ortho-carborane along with a release of hydrogen gas. Thermal isomerization of the ortho isomer in an autoclave under inert conditions can yield meta isomer around 500°C and para isomer around 700°C as shown in Scheme 1.

Scheme 1: Synthesis of the carborane isomers [12]
Carborane is a source of boron in this work. Commercially available 1-methyl-o-carborane was used for the purpose of synthesis of carboranylazide that will be incorporated into various organic compounds in order to obtain their carborane-appended derivatives. These carborane-appended biomolecules could be applicable in cancer treatment by using a popular technique called BNCT that utilizes boron drugs and a neutron source for selectively killing cancer cells via production of highly energetic alpha particles and gamma rays through nuclear fission reaction. Various carborane-appended biomolecules have been synthesized as of today; however, this has not been successfully applicable in BNCT cancer treatment. Sodium borocaptate (Na$_2$B$_{12}$H$_{11}$SH), most popularly known as BSH, is one of the FDA approved boron drugs available in the market; however, BSH has toxicity issues. Therefore, in order to design a new boron drug for effective BNCT, more emphasis should be given to the boron content, carrier biocompatibility, water solubility and biological activities before getting into clinical trials. Carboranes are a very important source of boron in BNCT because of their high boron content (10 boron atoms) and their ease of functionalization.

2.4 Decapitation and Bio-distribution

Commercially available ortho-carborane clusters can be decapitated in the presence of an appropriate base to the monoanionic [nido- 7, 8-C$_2$B$_9$H$_{12}$]$^-$ by the removal of one boron atom as illustrated in scheme 3. In order to produce dianionic species [nido- 7, 8-C$_2$B$_9$H$_{12}$]$_2^-$, a somewhat acidic hydrogen bridge in [nido- 7, 8-C$_2$B$_9$H$_{12}$]$^-$ can be removed by treating with a strong base such as butyllithium, sodium hydroxide or potassium hydroxide as shown in Scheme 2 below$^{13}$. 
Scheme 2: Conversion of closo-carborane to nido- form

After the synthesis of carborane-appended biomolecules and their cage opening, a biological distribution study needs to be performed in order to check their cytotoxicity and other biological activities in cell lines and for further animal studies. Bio-distribution studies provides information about the uptake of boron by cell which is an important parameter to be determined before BNCT applications.
CHAPTER 3
BORON NEUTRON CAPTURE THERAPY (BNCT)

3.1 Introduction

Neutron capture therapy (NCT) was introduced in 1936, four years after Sir James Chadwick discovered the neutron. Boron (B) and Gadolinium (Gd) are two ideal candidates that can be used as a capture agent for NCT because of their high neutron capture cross section. Neutron capture therapy had been utilized in the treatment of various types of cancers. Chemotherapy, radiation therapy, and surgery have had some success in treating cancer; however, these treatments do not selectively kill the cancer cells. Therefore, a technique that can selectively destroy the cancer cell is required. Thus, doing more research in other potential cancer therapies is important, one of which is BNCT that utilizes boron-10 nuclei.

A few years after Sir James Chadwick discovered neutrons, H.J. Taylor (in 1935) discovered that boron-10 could capture the thermal neutrons. Additionally, one year later, G.L. Locher understood the therapeutic potential of this finding and proposed that neutron capture could be used to treat tumors, which led to BNCT. This technique is binary radiation therapy, because it utilizes $^{10}$B enriched drug and thermal neutrons at the same time. Boron has two isotopes in nature, $^{10}$B (20% abundance) and $^{11}$B (80% abundance), so its ready availability makes it an ideal candidate for BNCT. In BNCT, a $^{10}$B enriched compound is transported into cancer cells where it absorbs low energy thermal neutrons upon irradiation to form an excited but less stable (10^{-12} sec) $^{11}$B nucleus. This $^{11}$B nucleus will quickly undergo nuclear fission reaction and disintegrate into highly energetic alpha particles and recoiled lithium ions along with low energy of gamma ray as
shown in Figure 3. The unique properties of this technique is that path length of the radiation (~9 µm) corresponds to the dimensions of a single cell, hence selectively killing targeted tumor tissues while sparing neighboring healthy ones. In order for BNCT to work effectively, it has to meet several requirements such as concentration of boron within tumor cells, minimal toxicity and water solubility. There are only limited boron drug delivery agents available in the market today, and they are not very effective, so it should be a priority to overcome such drawbacks and find alternatives.

Figure 3: Boron Neutron Capture reaction: Emission of 1.47MeV $\alpha$-particle, 0.84MeV Li-particle, and 0.48 MeV $\gamma$-rays

3.2 Why Boron?

In 1808, Gay-Lussac and Thenard first isolated boron, and Sir Humphry Davy discovered this element independently when he reacted boric acid with potassium. Boron is classified as metalloid because of its resemblance as both metal and non-metal. It is an only non-metal element located in the group 13 and is the fifth element of the periodic table. Boron has two isotopes, $^{10}$B and $^{11}$B, which differ from each other by 1 neutron. $^{10}$B is 20% abundant in nature while $^{11}$B is 80% abundant. $^{10}$B has five neutrons, whereas $^{11}$B has 6 neutrons as shown in Figure 4 below.
Why is boron an ideal candidate for neutron capture reaction? In neutron capture reaction, neutrons get absorbed initially, which forms a heavier nucleus and the neutron capture cross-section is the parameter used to measure the probability of neutrons absorption.

Figure 4: Isotopes of Boron [18]

The higher the neutron’s capture cross-section, probability that the nuclide will absorb the neutron is also higher. Boron has a neutron capture cross section of approximately 4000 barns. As shown in Table 2 below, $^{10}$B is the most suitable nuclide for neutron capture therapy because of its higher neutron capture cross-section. $^{10}$B is non-radioactive and is 20% naturally abundant which makes it readily available. Alpha particles and lithium produced after neutron capture reaction have high linear energy transfer (LET). Moreover, the path length of the radiation corresponds to the diameter of a cell size, thus radiation effect can be localized to the targeted tumor cell while sparing healthy ones. Additionally, boron chemistry also allows incorporation of different chemical structures into it far more easily.
Table 2

Thermal Neutron Capture Cross-section Values of Potential Nuclides for Neutron Capture Therapy

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Cross section (barns)</th>
</tr>
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<tbody>
<tr>
<td>$^{10}$B</td>
<td>3837.00</td>
</tr>
<tr>
<td>$^{11}$B</td>
<td>0.01</td>
</tr>
<tr>
<td>$^{12}$C</td>
<td>0.00</td>
</tr>
<tr>
<td>$^{1}$H</td>
<td>0.33</td>
</tr>
<tr>
<td>$^{14}$N</td>
<td>1.70</td>
</tr>
<tr>
<td>$^{35}$Cl</td>
<td>43.60</td>
</tr>
<tr>
<td>$^{23}$Na</td>
<td>0.53</td>
</tr>
<tr>
<td>$^{157}$Gd</td>
<td>254000.00</td>
</tr>
<tr>
<td>$^{153}$Gd</td>
<td>0.02</td>
</tr>
</tbody>
</table>

3.3 Neutron Source

In the early days, low energy thermal neutron was used for the irradiation. Later in Japan, an epithermal neutron beam was used as it had better tissue penetrating properties$^1$. Therefore, epithermal neutrons have been preferred in BNCT clinical trials. An epithermal neutron beam has a kinetic energy slightly less than one kilo-electron volt. Schematic of epithermal neutron beam is shown below in Figure 5.

![Schematic of epithermal neutron beam](image)

Figure 5: Pathway of neutrons [18]

As observed in the diagram above, fast neutrons are in the nuclear reactor which then passed through a moderator material, rich in hydrogen. This moderator helps to slow down the
fast neutrons into the epithermal neutron. Finally, the neutron is absorbed by a $^{10}$B compound placed in the tumor, thus killing the cells.

3.4 How BNCT Works

The schematic diagram of how BNCT works is illustrated in the Figures 6 and 7 shown below. Initially compound containing Boron-10, is transported into the tumor cells using a suitable delivery agent, which is then followed by irradiation of neutrons$^{17}$. Therefore, this technique is referred to as a binary radiation therapy. Upon irradiation, neutrons are absorbed by $^{10}$B which becomes excited to $^{11}$B. These $^{11}$B isotopes of boron are less stable and only remain for $10^{-12}$ s. $^{11}$B undergoes fission reaction and rapidly disintegrates into highly energetic helium ions (alpha particles) and highly recoiled lithium-7, along with gamma rays$^{18}$. The radiation path-length (~9 μm) of these particles corresponds to the diameter of cell size, meaning it is compatible within a cell and could destroy targeted cells only. Only the cell concentrated with $^{10}$B is destroyed as shown in Figure 6. In order for BNCT to work effectively, $10^9$ $^{10}$B atoms (~30μg/g tumor tissue) must be delivered to tumor cells$^{17}$. On the other hand, $10^{12}$ neutrons per square centimeter are needed. This is why this technique is unique and could be effective if requirements are met.
Figure 6: Schematic diagram of BNCT reaction in a cell. $^{10}$B absorbs thermal neutrons and produces Li particles and alpha particle [18]

Figure 7: Schematic diagram of localized effect in a tumor cell. A tumor is selectively killed sparing healthy cells [18]
3.5 BNCT Procedure

The invasive nature of the cancer makes the treatment difficult. BNCT is one of the unique and promising methods of cancer treatment, as it could selectively destroy the tumors. Before BNCT, surgery and chemotherapy should be performed in either order as illustrated in Figure 8. Generally, surgery is recommended to be performed 3-4 weeks prior to doing BNCT. Initially, the drug will be infused into the patient’s body through an effective means and it will take two hours for the completion of drug infusion, and then BNCT can start after approximately 45 minutes after the end of infusion. Usually, one session of BNCT takes approximately one hour\textsuperscript{19}. There are various challenges that lie in doing BNCT, especially in regards to targeting the tumor cell, matching the boron concentration requirement, and irradiating with a sufficient amount of neutrons required for a nuclear fission reaction.

![Figure 8: Treatment strategies for malignancies](image-url)
3.6 Boron Delivery Agents

Various challenges lie in fulfilling the requirement for BNCT to work effectively. Firstly and most significantly, boron concentration of 30-35µg $^{10}$B/g tumor tissue is required\textsuperscript{20}. Low systemic toxicity, high tumor uptake, rapid clearance from blood and normal tissue and retention during irradiation are among others. Unfortunately, there are no drug delivery agents which meet all these criteria. Boric acid and its derivative were initially used in clinical trials of the 1950s and early 1960s; however, these molecules were not selective, had poor tumor retention, and low tumor/brain ratios\textsuperscript{21}. Boronophenylalanine (BPA) and sodiumborocaptate (BSH)are the only FDA approved boron drug in the market as of now. BPA has low boron content, while BSH has thiol group which could be linked with another thiol group and form disulfide linkage and can damage DNA. The structures of BPA and BSH are shown in Scheme 3 below.

Scheme 3: Structure of BPA (boronphenylalanine) and BSH (Sodium borocaptate)
Therefore, boron enriched compound is required for the effective treatment of cancer. It is our primary goal to design a new drug by considering the requirements of an effective boron delivery agent for BNCT. There are two different and important category in this aspects, one being boron delivery agent and another boron source which is carborane in our work.
CHAPTER 4
SYNTHESIS OF CARBORANYL AZIDE

The boron source used in our work is commercially available 1-methyl-o-carborane. Multi-step synthesis was performed with this compound to obtain carboranyl azide (compound 2) required for click reaction. Initially, our starting material was treated with 1,4-diiodobutane in presence of base, butyl-lithium to produce an intermediate 1, which was then reacted with anhydrous sodium azide in presence of acetone to give compound 2 as shown in Scheme 4 below. Compound 2 is our boron source used for the synthesis of carborane-appended biomolecules in this work.

Scheme 4: A reaction scheme showing synthesis of carborane-appended azide compounds
CHAPTER 5
SYNTHESIS OF ALKYNYL COMPOUNDS

Alkylation reaction was performed with all of our selected organic compounds in order to synthesize their alkynyl derivatives required for click reaction. Various organic compounds with different organic moieties are selected as a starting material. Organic compounds containing imidazole, secondary amine, thiols, nucleobases and dihydroxy group are selected because of their biocompatibility, less toxicity and ease of functionalization. Structures and names of the selected starting organic compounds are shown in Scheme 5 below. The properties of these selected compounds are discussed below.

Scheme 5: Structures and names of selected organic compounds
Imidazole is a heterocyclic compound that resembles a five-membered ring structure composed of three carbon atoms and two nitrogen atoms at non-adjacent positions and has a colorless to pale yellow appearance. Out of two nitrogen atoms in imidazole, one is slightly acidic and another is basic. They are usually soluble in organic compounds such as chloroform. The derivatives of imidazole are widely applicable in the synthesis of organic compounds especially in pharmaceuticals. Therefore, nitro derivatives of imidazole have been selected in order to incorporate them with carborane in order to investigate their potential use as boron delivery agents in BNCT.

Nitroimidazoles, generally known as azomycins possess significant medicinal applications as they could be attached with different functional groups and their derivatives could be synthesized to evaluate their tumor imaging properties. 4-nitroimidazole is an organic compound with the formula C\textsubscript{3}H\textsubscript{3}N\textsubscript{3}O\textsubscript{2} and is one of the most common isomers of imidazole containing one nitro group. 4-nitroimidazole was treated with propargyl bromide and anhydrous potassium carbonate in acetone in order to synthesize a terminal alkyne derivative (compound 3) required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 6 below.

Scheme 6: Alkylation reaction of 4-nitroimidazole using propargyl bromide
Similarly, 2-nitroimidazole is an organic compound with the formula C$_3$H$_3$N$_3$O$_2$ and is another common isomer of imidazole containing one nitro group. Generally, 2-nitroimidazole is soluble in organic solvents such as DMSO or ethanol or chloroform. 2-nitroimidazole was treated with propargyl bromide and anhydrous potassium carbonate in acetone in order to synthesize terminal alkyne derivative required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 7 below.

![Scheme 7: Alkylation reaction of 2-nitroimidazole using propargyl bromide](image)

Phenothiazine is a tricyclic organic compound with the formula S(C$_6$H$_4$)$_2$NH which belongs to thiazine-class of heterocyclic compounds. This compound was first prepared in 1883 by Bernthsen via the reaction between diphenylamine and sulfur; however, cyclization of 2-substituted diphenyl sulfides is more popular recently. It is yellow in appearance and mostly soluble in benzene, ether and acetic acid. Phenothiazine derivatives have been found to have significant uses as drugs. Thus, phenothiazine was selected as a starting material in our research to attach carborane in to it for evaluating their use as a boron drug.
Phenothiazine was treated with propargyl bromide and anhydrous potassium carbonate in acetone in order to synthesize terminal alkyne derivative required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 8 below.

Scheme 8: Alkylation reaction of Phenothiazine using propargyl bromide

1, 3, 4-thiadiazole-2, 5-dithiol is another organic compound with the chemical formula of C\(_2\)H\(_2\)N\(_2\)S\(_3\) and selected in this work because this moiety exhibits various physiological activities. This compound contains thiadiazole rings, which has three donor centers for the two N atoms and the heterocyclic S atoms, and two thiols which are equivalent sterically\(^{25}\). Thus, it makes this compound highly nucleophilic, and it is interesting to introduce other functional groups in order to synthesize their derivatives. Synthesis of carborane-appended derivative of this compound is promising and could produce a new compound with broad spectrum of biological activity according to our hypothesis. Our hypothesis is to introduce terminal alkyne group in to each thiol end of this compound and incorporate carboranylazide (Compound 2) via a click reaction in order to derive a boronated biomolecule.

1, 3, 4-thiadiazole-2, 5-dithiol was treated with propargyl bromide and anhydrous potassium tert-butoxide (2 equivalent) in acetonitrile in order to synthesize the terminal alkyne
derivative required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 9 below.

![Scheme 9: Alkylation reaction of 1, 3, 4-thiadiazole-2, 5-dithiol using propargyl bromide](image)

Adenine, one of the most important organic molecules for life, is one of the purine nucleobase with chemical formula C$_5$H$_5$N$_5$ which is significantly important in a variety of roles in biochemistry. Adenine is used in forming nucleotides of the nucleic acids, binds to thymine and uracil in DNA and RNA respectively. Adenine can also be attached with ribose and deoxyribose to form adenosine and deoxyadenosine (nucleosides) respectively. In addition, adenosine triphosphate (ATP), a nucleotide, can be formed when adenosine is attached with three phosphate groups which is useful in cellular metabolism. Adenine was first named by Albrecht Kossel in 1885 and in 1961 by Joan Oro when he synthesized adenine from the polymerization of ammonia with five hydrogen cyanide (HCN) molecules in aqueous solution.

Derivatives of adenine containing compound are found to be applicable in medicinal chemistry because of their reaction with receptors$^{26}$. Therefore, we have selected adenine as one of our strating organic compounds to attach carborane. Adenine was treated with propargyl bromide and anhydrous potassium carbonate in dimethylformamide (DMF) in order to
synthesize terminal alkyne derivative required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 10 below.

![Scheme 10](image)

Scheme 10: Alkylation reaction of adenine using propargyl bromide

Similarly, guanine another purine nucleobase with the chemical formula C₅H₅N₅O consists of a fused pyrimidine-imidazole ring system with conjugated double bonds. Guanine along with adenine is also present in both DNA and RNA, where it binds to cytosine through three hydrogen bonds. It was named in 1846, two years after its reported isolation from the excreta of sea birds. Guanine can be synthesized in trace amounts by the polymerization of ammonium cyanide (NH₄CN)²⁷.

Guanine derivatives are also significantly important in novel drug discoveries. Thus, guanine is also our selected compound to test proposed hypothesis in order to synthesize its carborane-appended derivatives. Guanine was treated with propargyl bromide and anhydrous potassium carbonate in dimethylformamide (DMF) in order to synthesize terminal alkyne derivative required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 11 below.

6-chloropurine is a purine derivative with a formula C₅H₅ClN₄ and soluble in dimethylformamide (DMF) and ether. The derivative of this compound has also been found
applicable in medical science. The alkylation reaction of this compound is a feasible way to obtain one of its derivatives among others. 6-chloropurine was treated with propargyl bromide and anhydrous potassium carbonate in dimethylformamide (DMF) in order to synthesize terminal alkyne derivative required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 12 below.

3’, 5’-dihydroxyacetophenone, white to light yellow crystal powder in appearance, is a dihydroxy derivative of acetophenone. This compound has also been applicable in medicinal chemistry as it shows some antitumor activity. Thus, we have used this compound in order to derive its derivatives that could be useful in cancer treatment. 3’, 5’-dihydroxyacetophenone was
treated with propargyl bromide and anhydrous potassium carbonate in dimethylformamide (DMF) in order to synthesize terminal alkyne derivative required for its click reaction with carboranyl azide. The reaction scheme is shown in Scheme 13 below.

Scheme 13: Alkylation reaction of 3’, 5’-dihydroxyacetophenone using propargyl bromide
CHAPTER 6
CLICK CHEMISTRY

Click chemistry was first invented by Nobel laureate K. Barry Sharpless in 1998. In 2001, Sharpless and his Scripps Research Institute colleagues Hartmuth and M.G Finn described the concept in depth. The most popular concept of click chemistry lies within the Huisgen 1, 3-dipolar cycloaddition of alkynes to azides to form 1, 4-disubstituted-1, 2, 3-triazoles using cupper catalyst at room temperature. Click chemistry had broad application most importantly in the drug’s discovery. It is a very successful organic reaction in eliminating a complicated tradition drug discovery process.

Click reaction is a simple, wide in scope and high yielding reaction between azide and alkyne which was performed using Cu(I)I (copper (I) iodide) as a catalyst, and a 50:50 mixture of acetonitrile (CH₃CN) and tetrahydrofuran (THF) as solvent whereas N,N-diisopropylethylamine (DIPEA) was used as a proton scavenger helping to avoid unwanted side-reactions. In this research work, click chemistry was used for attaching boron source in to the various organic compounds. We have preferred Cu (I)-catalyzed reaction rather than Ru (II)-catalyzed reaction because Cu (I) catalyst work effectively with terminal alkynes. If you have alkyne and azide available, click reaction can be completed in a one-pot process so it is feasible and quick reaction.

A general scheme of the click reaction is shown below in Scheme 14. Basically, it is a cupper (I)-catalyzed union of compound containing azide functional group and terminal alkynes.
yielding a compound containing cyclic triazole ring. This reaction has succeeded so far in making connections between structures that bear wide variety of functional groups; thus it is a demanding approach applicable in this research.

![Scheme 14: A general scheme of click reaction](image)

The proposed mechanism of click reaction is shown in scheme 16 below. The interesting fact of the mechanistic scheme is that the regiospecific pathway of this mechanism is not limited to azides, but also works well with nitrile oxides\textsuperscript{28}. The barrier for this catalytic process has been known to be considerable lower than one for the uncatalyzed reaction. The mechanism of click reaction (Scheme 15) begins with the coordination of the alkyne to the Cu(I) species. Then the alkyne is converted to the acetylide forming Cu acetylide species as bonafide intermediates. This conversion process is believed to be involved in many C-C bond forming reactions. Now, azide replaces one of the ligands and binds to the copper atom via the nitrogen proximal to carbon, forming another intermediate. The distal nitrogen of the azide from this intermediate then attacks the C-2 carbon of the acetylide, forming the unusual six-membered copper (III) metallacycle. This process is known to be endothermic. The ring contraction of this metallacycle will from triazolyl-copper derivative. It is calculated that the barrier for ring contraction is very low. Finally, proteolysis of the triazolyl-copper derivative will give the desired triazole product. This is how this catalytic cycle of click reaction is completed. It is also known that the rate
acceleration of the copper(I)-catalyzed process is enormous, 7 to 8 orders of magnitude, as compared to the purely thermal cyclo-addition.

Scheme 15: A proposed mechanism for click reaction [28]
CHAPTER 7
SYNTHESIS OF CARBORANE-APPENDED BIOMOLECULES

In order to synthesize carborane-appended biomolecules, carboranyl azide was synthesized first, and alkylation reaction was performed with the selected organic compounds to synthesize organic compounds with terminal alkyne groups. Click chemistry was used to incorporate azide into alkyne to give carborane-appended biomolecules. Click reaction was carried out between alkynyl compounds (3, 4, 5, 6, 7, 8, 9 and 10) and carboranyl azide (2) to give carborane-appended biomolecules as compound 11, 12, 13, 14, 15, 16, 17 and 18 respectively. All the details including their reaction schemes are discussed in the sections below.

Terminal alkyne containing 4-nitroimidazole derivative (Compound 3) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using cupper catalyst to give carborane-appended compound 11 in good yield. The reaction scheme is shown in scheme 16.

Terminal alkyne containing 2-nitroimidazole derivative (Compound 4) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using cupper catalyst to give carborane-appended compound 12 in good yield. The reaction scheme is shown in scheme 17.

Terminal alkyne containing phenothiazine derivative (Compound 5) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using
cupper catalyst to give carborane-appended compound 13 in good yield. The reaction scheme is shown in scheme 18.

Scheme 16: Click reaction of 4-nitroimidazole derivative (3) with carboranyl azide (2)
Scheme 17: Click reaction of 2-nitroimidazole derivative (4) with carboranyl azide (2)
Scheme 18: Click reaction of phenothiazine derivative (5) with carboranyl azide (2)
Terminal alkynes containing 1, 3, 4-thiadiazole-2, 5-dithioldervative (Compound 6) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using cupper catalyst to give carborane-appended compound 14 in good yield. This is the first compound which contains two carboranes thus doubling the boron content. The reaction scheme is shown in Scheme 19.

Scheme 19: Click reaction of 1, 3, 4-thiadiazole-2, 5-dithiol derivative (6) with carboranyl azide (2)
Terminal alkynes containing adenine derivative (Compound 7) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using cupper catalyst to give carborane-appended compound 15 in good yield. The reaction scheme is shown in Scheme 20.

Scheme 20: Click reaction of adenine derivative (7) with carboranyl azide (2)
Terminal alkynes containing guanine derivative (Compound 8) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using cupper catalyst to give carborane-appended compound 16. This is another compound which contains two carboranes hence doubling the boron content. However; this reaction was not as successful as we have expected. The reaction scheme is shown in Scheme 21.

Scheme 21: Click reaction of guanine derivative (8) with carboranyl azide (2)
Terminal alkynes containing 6-chloropurine derivative (Compound 9) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using cupper catalyst to give carborane-appended compound 17 in good yield. The reaction scheme is shown in Scheme 22.

Scheme 22: Click reaction of 6-chloropurine derivative (9) with carboranyl azide (2)
Terminal alkynes containing 3’, 5’-dihydroxyacetophenonedervative (Compound 10) was treated with carboranyl azide compound (Compound 2) in order to attach them via click chemistry using cupper catalyst to give carborane-appended compound 18 in good yield. Again, this is one of the compounds which contain two carboranes hence doubling the boron content. The reaction scheme is shown in Scheme 23.

Scheme 23: Click reaction of 3’, 5’-dihydroxyacetophenone derivative (10) with carboranyl azide (2)
Overall, click chemistry was used in order to synthesize the carborane-appended derivative of the entire selected starting compound. We were successful in most of the compounds except for guanine. Compound 11-18 were mostly characterized by NMR ($^1$H, $^{13}$C, $^{11}$B) and some of them by either FTIR or MS (see Appendix). In addition, elemental analysis was also performed with some of the compounds in order to check their purity (see Chapter 10). Moreover, carborane-appended compounds of coumarin derivatives were also synthesized using the same approach which is discussed in chapter 8.
CHAPTER 8
COUMARINS

8.1 Introduction

Coumarin belongs to the benzopyrone group of organic compounds and is widely found in nature. This class of chemical compound has been generally used as fragrance, usually in perfumes and fabric conditioners. They are also useful in diverse biological activities. Coumarin was first synthesized in 1868, and since then it has been used as a precursor reagent in various syntheses such as the anticoagulant warfarin by pharmaceutical industries\textsuperscript{29}. It is therefore, various coumarins derivatives have intrigued medicinal chemists for their applicability as drugs.

8.2 Coumarin Derivatives

It is known that coumarin derivatives are applicable in various therapeutics such as CNS stimulants, anti-coagulants and antitumor\textsuperscript{29}. Therefore, it is desired to synthesize coumarin derivatives and attach polyhedral carborane cage into it for BNCT application. The coumarin derivatives we have synthesized are bio-compatibles and easy to functionalize. We have used commercially available resorcinol as our starting material and treated them with ethyl-2-ethylacetoacetate or ethylacetoacetate to yield coumarin derivatives compounds \textbf{19} and \textbf{22} respectively. The reaction schemes are shown in Scheme 24 and Scheme 25 respectively.
8.3 Alkylation Reactions

Thus derived coumarins derivatives (19 and 22) were treated with propargyl bromide and anhydrous potassium carbonate to synthesize compound 20 and 23 respectively as an alkyne source for click reaction. The general scheme of the alkylation reaction is shown in Scheme 26 and Scheme 27.
Scheme 26: Alkylation of coumarin derivative (19) using propargyl bromide

Scheme 27: Alkylation of coumarin derivative (22) using propargyl bromide

8.4 Click Reactions

In order to attach the boron source (Compound 2) onto the alkylated derivatives of coumarins (Compounds 20 and 23), a click reaction was performed using Cu(I) catalyst for three days at room temperature to yield compound 21 and 24 respectively. The cytotoxicity of these compounds have been tested and both of them are found to be non-toxic. The general scheme of the click reactions performed is shown in Scheme 28 and Scheme 29 respectively.
Scheme 28: Click reaction of alkylated coumarin derivative (20) with carboranyl azide (2)
Scheme 29:  Click reaction of alkylated coumarin derivative (23) with carboranyl azide (2)

Again, click chemistry was used in order to synthesize the carborane-appended derivatives of the coumarins. Compounds 21 and 24 were characterized by NMR ($^1$H, $^{13}$C, $^{11}$B) (see Appendix).
8.5 Cytotoxicity

Cytotoxicity study of compounds 21 and 24 were carried out in a triple negative breast cancer cell line MDA-MB-231 (Credit: Shirisha Gurrapu, University of Minnesota Duluth). The compounds were dissolved in DMSO and IC\textsubscript{50} was tested starting from 100 µM to 0.78 µM. The IC\textsubscript{50} values of both compounds are greater than 100 µM confirming that the compounds are non-toxic. The data analysis was performed by using GraphPad Prism 6 software which is shown in Figure 9 and Figure 10 below. Both compounds (21 and 24) were tested three times for their consistency (only one shown). The procedure of the experiment is discussed in Experimental section (Chapter 10). This will lead to the bio-distribution studies of these compounds.

Figure 9: Cytotoxicity result of Compound 21 (IC\textsubscript{50} >100 µM)
Figure 10:  Cytotoxicity result of Compound 24 (IC$_{50}$ >100 µM)
Firstly, making the synthesized compounds water soluble is one of the critical requirements to be fulfilled before transporting them into the body. It is a priority to make all the synthesized compounds water soluble by decapitation of the carborane cage in order to perform their biological evaluation. To make these compounds water soluble, several reactions have been performed in order to investigate and optimize their reaction conditions. This work is currently under way as well.

The reaction scheme of decapitating the carborane cage of compound 11 is shown in Scheme 30 below.

Similarly, the reaction scheme of decapitating the carborane cage of compound 14 is shown in Scheme 31 below.
Scheme 30: Decapitation of carborane-appended 4-nitroimidazole compound (11)
Scheme 31: Decapitation of carborane-appended 1, 3, 4-thiadiazole-2, 5-dithiol derivative (compound 11)

The Water Solubility table of decapitated compounds (25 and 26) along with their comparison to carborane-appended compounds (11 and 14) is shown in Table 3 below. The experiment was started with dissolving 0.1 mg of compound 11 in 10 mL of deionized water and while increasing the mass gradually it was found that 0.17 mg of compound 11 dissolved completely in 10 mL of deionized water. When the mass of compound 11 was at 0.2 mg, it was observed that the compound 11 did not dissolve completely, thus providing the evidence that the solubility limit is 0.17mg/10 mL (0.017mg/1mL or 42 µM).
Table 3
Solubility (µM) table of decapitated compounds

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Molecular Weight (g/mol)</th>
<th>Solubility (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>406.38</td>
<td>42</td>
</tr>
<tr>
<td>25</td>
<td>527.58</td>
<td>378</td>
</tr>
<tr>
<td>14</td>
<td>736.98</td>
<td>55</td>
</tr>
<tr>
<td>26</td>
<td>752.54</td>
<td>531</td>
</tr>
</tbody>
</table>

The solution was kept in ultra-sonication bath for half hour. Likewise, 1 mg of compound 25 was treated with 10 mL of deionized water, and upon gradual increase in the mass it was found that 2 mg of compound 25 dissolves completely in 10 mL of deionized water. When the mass of compound 25 was at 2.5 mg, it was observed that the compound 25 did not dissolve completely. Therefore, it is known that maximum solubility limit of compound 25 is 2mg/10mL (0.2mg/1mL or 378 µM). This solution was also kept in ultra-sonication bath for half hour. Again, another experiment was started with dissolving 0.1 mg of compound 14 in 10 mL of deionized water and while increasing the mass gradually it was found that 0.4 mg of compound 11 dissolved completely in 10 mL of deionized water. When the mass of compound 14 was at 0.45 mg, it was observed that the compound 14 did not dissolve completely. Thus, it is known that maximum solubility limit of compound 14 is 0.4mg/10mL (0.040mg/1mL or 55 µM).
The solution was kept in ultra-sonication bath for half hour. Likewise, 1 mg of compound 26 was treated with 10 mL of deionized water, and upon gradual increase in the mass it was found that 5 mg of compound 26 dissolves completely in 10 mL of deionized water. When the mass of compound 26 was at 5.5 mg, it was observed that the compound 26 did not dissolve completely. Therefore, it is known that maximum solubility limit of compound 26 is 5mg/10mL (0.5mg/1mL or 531 µM). The solution was kept in ultra-sonication bath for half hour.

Overall, the solubility of decapitated compounds was tested by dissolving them in deionized water and visually observing them. The calculated solubility (µM) has proven that the solubility of the compounds 11 and 14 were increased by almost 10 folds after the decapitation.
CHAPTER 10
EXPERIMENTAL

All reactions were generally performed under argon atmosphere in an oven-dried flask using standard Schlenk techniques. Solvents and reagents were added by syringes as required. All solvents were dried using standard procedure. Reagents were used as purchased without further purification unless indicated otherwise. The progresses of all reactions were monitored using thin layer chromatography (TLC). All the products synthesized were purified by column chromatography on silica gel (70-230 mesh). Compounds 5 and 13 were purified by adding a drop of triethyl amine (TEA) or ammonium hydroxide (NH$_4$OH) in the silica while silica was degrading the compound. Both $^1$H NMR and $^{13}$C NMR were recorded on a burker NMR spectrometer at 300 MHz frequency. The chemical shifts were reported relative to CDCl$_3$ ($^1$H: $\delta=$ 7.24 ppm, $^{13}$C: $\delta=$ 77.23 ppm), CD$_3$CN ($^1$H: $\delta=$ 1.94 ppm, $^{13}$C: $\delta=$ 118.69, 1.39 ppm) and DMSO ($^1$H: $\delta=$ 2.50(5) ppm, $^{13}$C: $\delta=$ 39.51 (7)). The $^{11}$B NMR spectra were also recorded at 300 MHz. On the other hand, infrared (IR) spectra of compounds 11, 12, 13 and 14 were recorded on an ATR FT-IR Spectrophotometer. All the compounds were completely dried under high vacuum. Mass spectral analyses of compounds 11, 12, 13 and 14 shown were performed in UIUC using high resolution mass spectrometry.
The reaction scheme (Scheme 4) is started with 3.17g (20.00 mmol) of 1-methyl-o-carborane ($C_3H_{14}B_{10}$) in an oven-dried 250-mL round-bottom, three-necked flask equipped with a magnetic stirring bar and the compound was dissolved in 100 mL of a diethyl ether/toluene ($v/v=2/1$). The reaction mixture was then cooled to -78 °C using dry ice, and then 12.6 mL (20.16 mmol) of the tert-butyllithium was added drop-wise to the mixture with the help of a syringe. The mixture was maintained at -78 °C for nearly 30 min and then allowed slowly to warm to room temperature. The reaction mixture was stirred at room temperature for 4 hours and cooled to 0 °C using ice. Approximately 3.0 mL (22.75 mmol) of 1,4-diiodobutane was added to the mixture using a syringe, and the reaction mixture was stirred for 30 min at 0 °C, slowly warmed to room temperature, stirred for additional 2 hours and then refluxed for nearly 4 hours. After reflux was done, the mixture was cooled to 0 °C and then quenched with deionized (DI) water. Solvents were evaporated using a rotary evaporator, and the extraction was carried out using diethyl ether and DI H$_2$O. All the extracts from aqueous layer were combined with organic layer, and then solvents were removed, yielding 2.8 g (82% yield) of yellow sticky residue, compound 1. The purity of compound 1 was monitored with TLC, developed with n-hexane/ethyl acetate ($v/v=6/1$) and purified using silica-gel column chromatography.

2.00 g (5.88 mmol) of compound 1 was mixed with 3.90 g (59.39 mmol) of sodium azide and 120 mL of anhydrous acetone and refluxed in the dark for 3 days in a 250-mL round-bottom flask equipped with a magnetic stirring bar. After 3 days, the reaction mixture was cooled to room temperature, solvents were removed using a rotary evaporator and the residue was extracted using diethyl ether. The diethyl ether was then removed from the extract to give the crude product that was later purified by TLC (SiO2, developed with n-hexane/ethyl acetate in 5:1
ratio) and silica-gel column chromatography to yield 1.35 g (90% yield) of compound 2 as waxy, pale yellow solid. Compounds 1 and 2 were characterized by NMR (\(^1\)H, \(^{13}\)C, \(^{11}\)B) and FTIR (see Appendix).

Compounds 3, 4, 5, 6, 7, 8, 9 and 10 were synthesized by treating the commercially available organic compounds (4-nitroimidazole, 2-nitroimidazole, phenothiazine and 1,3,4-thiadiazole-2,5-dithiol, adenine, guanine, 6-chloropurine and 3’,5’-dihydroxyacetophenone) individually with propargyl bromide in presence of strong base potassium carbonate or potassium tert-butoxide (K\(_2\)CO\(_3\)/KOTBu) and dry solvent acetone/acetonitrile or N,N-dimethylformamide (DMF) in a 100 mL round bottom flask equipped with magnetic stirring bar (see Schemes 6-13).

Compound 3 was synthesized by treating 340 mg (3.007 mmol) of commercially available 4-nitroimidazole with 435 mg (3.148 mmol) of anhydrous K\(_2\)CO\(_3\) using 10 mL of dry acetone under argon atmosphere using schlenk lines. Approximately 0.4 mL of propargyl bromide was added to the reaction mixture using a syringe, and the reaction was stirred overnight at room temperature. The reaction mixture was diluted with 10 mL of ethyl acetate, products were filtered and solvent was removed to collect white solid powder-like product. The purity of compound 3 was monitored with thin-layer chromatography, developed with n-hexane/ethyl acetate (v/v=1/1) and purified using silica-gel column chromatography yielding 400 mg (2.65 mmol), 88% yield of the product. The identification of this compound was confirmed by characterization with \(^1\)H and \(^{13}\)C NMR (see Appendix).

Similarly, compound 4 was synthesized by treating 100 mg (0.88 mmol) of commercially available 2-nitro imidazole with 140 mg (1.013 mmol) of anhydrous K\(_2\)CO\(_3\) using 10 mL of dry
acetone under argon atmosphere using schlenk lines. Approximately 0.2 mL of propargyl bromide was added to the reaction mixture using syringe, and reaction was stirred overnight at room temperature. The reaction mixture was then diluted with 10 mL of ethyl acetate, products were filtered and solvent was removed to collect yellowish sticky residue that solidifies upon standing. The purity of compound 4 was monitored with thin-layer chromatography, developed with n-hexane/ethyl acetate (v/v=1/1) and purified using silica-gel column chromatography yielding 95 mg (0.63 mmol), 70% yield of the product. The identification of this compound was confirmed by characterization with $^1$H and $^{13}$C NMR (see Appendix).

Similarly, compound 5 was synthesized by treating 500 mg (2.51 mmol) of commercially available phenothiazine with 470 mg (3.40 mmol) of anhydrous K$_2$CO$_3$ using 25 mL of dry acetonitrile under argon atmosphere using schlenk lines. Approximately 3.4 mL of propargyl bromide was added to the reaction mixture using syringe and reaction was stirred overnight at room temperature. Solvents were evaporated using a rotary evaporator, and the extraction was carried out using dichloromethane (DCM) and DI H$_2$O. Firstly, organic layer was separated and the aqueous layer was extracted with dichloromethane (3× 50 mL). All the extracts from the aqueous layer were combined with the organic layer, and then solvents were removed. The purity of compound 5 was monitored with thin-layer chromatography, developed with n-hexane/ethyl acetate (v/v=1/1) and purified using silica-gel column chromatography, yielding 620 mg (2.74 mmol), 80% yield of brown solid powder as a product$^{25}$. The identification of compound was confirmed by characterization with $^1$H and $^{13}$C NMR (see Appendix).

Similarly, compound 6 was synthesized by treating 500 mg (3.327 mmol) of commercially available 1,3,4-thiadiazole-2,5-dithiol with 750 mg (6.684 mmol) with anhydrous
KOTBu using 15 mL of dry acetonitrile under argon atmosphere using Schlenk lines.
Approximately 0.4 mL of propargyl bromide was added to the reaction mixture using a syringe, and reaction was stirred overnight at room temperature. Solvents were evaporated using a rotary evaporator and the extraction was carried out using diethyl ether and milli-Q H$_2$O. Firstly, organic layer was separated and the aqueous layer was extracted with diethyl ether (3× 50 mL). All the extracts from the aqueous layer were combined with the organic layer, and then solvents were removed. The purity of compound 6 was monitored with thin-layer chromatography, developed with n-hexane/ethyl acetate (v/v=1/1) and purified using silica-gel column chromatography, yielding 680 mg (2.87 mmol), 86% yield of brown solid powder as a product. The identification of compound was confirmed by characterization with $^1$H and $^{13}$C NMR (see Appendix).

Compound 7 was synthesized by treating 100 mg (0.74 mmol) of commercially available adenine with 102.3 mg (0.74 mmol) of anhydrous potassium carbonate and 0.1 mL of propargyl bromide while dissolving in 25 mL of DMF. The reaction mixture was refluxed overnight. Solvents were removed, and then extraction was performed using 30 mL DI water and ethyl acetate. The residue was washed with water and dried under sodium sulfate. The purification was done by column chromatography developed in 30% hexane and 70% ethyl acetate. The product was dried under high-vacuum for 3 days to give compound 7 in 65% yields as white powder. Compound 7 was characterized by doing $^1$H and $^{13}$C NMR using 300 MHZ Burker NMR (see Appendix).

Similarly, compound 8 was synthesized by treating 100 mg (0.66 mmol) of commercially available guanine with 182mg (1.32 mmol) of anhydrous potassium carbonate and 0.15 mL of
propargyl bromide while dissolving in 25 mL of DMF. The reaction mixture was refluxed overnight. Solvents were removed, and then extraction was performed using 30 mL DI water and ethyl acetate. The residue was washed with water and dried under sodium sulfate. The purification was done by column chromatography developed in 10% hexane and 90% ethyl acetate. The product was dried under high-vacuum for 3 days to give compound 8 in 60% yields as brownish powder. Compound 8 was characterized by doing $^1$H and $^{13}$C NMR using 300 MHZ Burker NMR (see Appendix).

Compound 9 was synthesized by treating 200 mg (1.3 mmol) of 6-chloropurine with 180 mg (1.3 mmol) of anhydrous potassium carbonate and 0.1 mL of propargyl bromide in 10 mL dry DMF. Two separate reactions were performed at the same time, one at room temperature and the other at reflux for 24 hrs. Extraction was carried out using 20 mL DI water and ethyl acetate. Two spots were observed in thin layer chromatography, so prep TLC was done to separate them. Column chromatography was also performed for further purification using 30% hexane and 70% ethyl acetate. The residue was dried under high-vacuum to give compound 9 in 85% yields as a dark brown crystalline powder. The results from the reaction performed at RT were better while compared to one with reflux. Compound 9 was characterized by doing $^1$H and $^{13}$C NMR using 300 MHZ Burker NMR (see Appendix).

Likewise, compound 10 was synthesized by treating 200 mg (1.31 mmol) of 3’,5’-dihydroxyacetophenone with 362 mg (2.62 mmol) of anhydrous potassium carbonate and 0.25 mL of propargyl bromide in 20 mL dry DMF. The reaction mixture was stirred at RT for 24 hours, and then solvents were evaporated under reduced pressure. Extraction was carried out using 40 mL DI water and diethyl ether (3 folds). All the organic phases were combined and
evaporated, and then washed with water and dried over anhydrous sodium sulfate. Compound 10 was also purified by doing silica gel column chromatography developed in 40% hexane and 60% ethyl acetate. The residue obtained was dried under high-vacuum for 3 days to give compound 10 in 80% yields as brownish-yellow crystalline substance (see Appendix).

Click reaction was carried out between alkynyl compounds (3, 4, 5, 6, 7, 8, 9 and 10) and carboranyl azide (Compound 2) to give carborane-appended compounds (11, 12, 13, 14, 15, 16, 17 and 18) respectively (see scheme 16-23).

Compound 11 (C_{13}H_{26}N_{6}B_{10}O_{2}) was synthesized by treating 300 mg (1.985 mmol) of compound 3 with 255 mg (1.00 mmol) of compound 2 using 10 mL dry acetonitrile/THF (v/v = 1/1) and under argon atmosphere using schlenk lines. Approximately 210 mg (1.10 mmol) of Cu(I)I was added to the reaction mixture as a catalyst and 2 mL (10 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Solvents were evaporated using a rotary evaporator. The purity of compound 11 was monitored with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column chromatography, yielding 345 mg (0.85 mmol), 85% yield of dark-brown solid powder as a product. The identification of this compound was confirmed by characterization with NMR (\textsuperscript{1}H, \textsuperscript{13}C and \textsuperscript{11}B), FTIR, MS and elemental analysis (see Appendix).

Elemental analysis calculated for C_{13}H_{26}N_{6}B_{10}O_{2}C 38.7%, H 6.4%, N 20.6%; found C 38.8%, H 6.2% N 20.3%.

Similarly, compound 12 (C_{13}H_{26}N_{6}B_{10}O_{2}) was synthesized by treating 80 mg (0.53 mmol) of compound 4 with 65 mg (0.26 mmol) of compound 2 using 5 mL dry CH\textsubscript{3}CN/THF (v/v = 1/1) under argon atmosphere using schlenk lines. Approximately 55 mg (0.29 mmol) of
Cu(I)I was added to the reaction mixture as a catalyst, and 0.5 mL (2.5 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Solvents were evaporated using a rotary evaporator. The purity of compound 12 was monitored with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column chromatography, yielding 90 mg (0.22 mmol), 86% yield of pale brown, solid powder as a product. The identification of this compound was confirmed by characterization with NMR (1H, 13C and 11B), FTIR, MS and elemental analysis (see Appendix).

Elemental analysis calculated for C13H26N6B10O2C 38.7%, H 6.4%, N 20.6%; found C 37.96%, H 6.35% N 20.2%.

Similarly, compound 13 (C22H30N4B10S) was synthesized by treating 250 mg (1.053 mmol) of compound 5 with 128 mg (0.501 mmol) of compound 2 using 8 mL dry CH3CN/THF (v/v = 1/1) under argon atmosphere using schlenk lines. Approximately 105 mg (0.55 mmol) of Cu(I)I was added to the reaction mixture as a catalyst, and 0.9 mL (5.02 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Solvents were evaporated using a rotary evaporator. The purity of compound 13 was monitored with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column chromatography, yielding 220 mg (0.449 mmol), 90% yield of pale black, solid powder as a product. The identification of compound was confirmed by characterization with NMR (1H, 13C and 11B), FTIR, MS and elemental analysis (see Appendix).

Elemental analysis calculated for C22H30N4B10S C 53.8%, H 6.16%, N 11.5%; found C 58.13%, H 7.43% N 11.73%.
Similarly, compound \textbf{14} \((C_{22}H_{48}N_{4}B_{10}S_{3})\) was synthesized by treating 245 mg (1.082 mmol) of compound \textbf{6} with 277 mg (1.083 mmol) of compound \textbf{2} using 10 mL dry CH\textsubscript{3}CN/THF (v/v = 1/1) under argon atmosphere using schlenk lines. Approximately 230 mg (1.207 mmol) of Cu(I)I was added to the reaction mixture as a catalyst, and 1.9 mL (10.83 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Solvents were evaporated using a rotary evaporator. The purity of compound \textbf{14} was also monitored with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column chromatography, yielding 705 mg (0.957 mmol), 88\% yield of bright brown powder as a product. The identification of this compound was confirmed by characterization with NMR (\textsuperscript{1}H, \textsuperscript{13}C and \textsuperscript{11}B), FTIR, ESI-TOF MS and elemental analysis (see Appendix).

Elemental analysis calculated for C\textsubscript{22}H\textsubscript{48}N\textsubscript{4}B\textsubscript{10}S\textsubscript{3} C 36\%, H 6.5\%, N 15.2\%; found C 36.8\%, H 6.1\% N 17.3\%.

Compound \textbf{15} \((C_{15}H_{28}N_{8}B_{10})\) was synthesized by treating 100 mg (0.577 mmol) of compound \textbf{7} with 150 mg (0.577 mmol) of compound \textbf{2} using 20 mL dry CH\textsubscript{3}CN/THF (v/v = 1/1) under argon atmosphere using schlenk lines. Approximately 120 mg (0.627 mmol) of Cu(I)I was added to the reaction mixture as a catalyst, and 1 mL (5.77 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Solvents were evaporated using a rotary evaporator. The purity of compound \textbf{15} was monitored with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column chromatography, yielding 75\% yield of dark brownpowder as a
product. The identification of compound was confirmed by characterization with NMR ($^1$H, $^{13}$C and $^{11}$B) and elemental analysis (see Appendix).

Elemental analysis calculated for C$_{15}$H$_{28}$N$_8$B$_{10}$C 42%, H 6.59%, N 26.13%; found C 44.33%, H 7.19% N 27.76%.

Similarly, compound 16 (C$_{25}$H$_{51}$N$_{11}$B$_{20}$) was synthesized by treating 200 mg (0.88 mmol) of compound 8 with 450 mg (1.76 mmol) of compound 2 using 40 mL dry CH$_3$CN /THF (v/v = 1/1) under argon atmosphere using schlenk lines. Approximately 185 mg (0.968 mmol) of Cu (I) was added to the reaction mixture as a catalyst, and 1.53 mL (8.8 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Solvents were evaporated using a rotary evaporator. The purity of compound 16 was monitored with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column chromatography, yielding 68% yield of dark brown powder as a product. The identification of compound was confirmed by characterization with NMR ($^1$H, $^{13}$C and $^{11}$B). However, NMR results were not accurate to confirm the identity of compound 16.

Compound 17 (C$_{15}$H$_{26}$N$_7$B$_{10}$Cl) was synthesized by treating 90 mg (0.47 mmol) of compound 9 with 120 mg (0.47 mmol) of compound 2 using 40 mL dry CH$_3$CN /THF (v/v = 1/1) under argon atmosphere using schlenk lines. Approximately 100 mg (0.517 mmol) of Cu (I) was added to the reaction mixture as a catalyst, and 1 mL (4.7 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Solvents were evaporated using a rotary evaporator. Extraction was performed with 40 mL of DI water and diethyl ether. The purity of compound 17 was checked with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column
chromatography, yielding 84% yield of white solid as a product. The identification of compound was confirmed by characterization with NMR (\(^1^H, \^{13}^C\) and \(^{11}B\)) and elemental analysis (see Appendix).

Elemental analysis calculated for C\(_{15}\)H\(_{26}\)N\(_7\)B\(_{10}\)Cl C 43.6%, H 6.35%, N 23.75%; found C 39.8%, H 6.4% N 24.3%.

Compound 18 (C\(_{28}\)H\(_{54}\)B\(_{20}\)O\(_3\)) was synthesized by treating 100 mg (0.44 mmol) of compound 10 with 225 mg (0.88 mmol) of compound 2 using 40 mL dry CH\(_3\)CN/THF (v/v = 1/1) under argon atmosphere using schlenk lines. Approximately 92 mg (0.484 mmol) of Cu (I) I was added to the reaction mixture as a catalyst, and 0.8 mL (4.4 mmol) of DIPEA was also added in the mixture. Click reaction was carried out by stirring the reaction mixture at room temperature for 3 days. Extraction was performed with 50 mL of DI water and diethyl ether. The purity of compound 18 was also checked with TLC, developed with n-hexane/ethyl acetate (v/v=1/2) and purified using silica-gel column chromatography, to give 78% yield of a brown solid as a product. The identification of compound was confirmed by characterization with NMR (\(^1^H, \^{13}^C\) and \(^{11}B\)) and elemental analysis (see Appendix).

Elemental analysis calculated for C\(_{28}\)H\(_{54}\)B\(_{20}\)O\(_3\) C 51.3%, H 8.2%; found C 51.8%, H 8.4%.

Compound 19 (C\(_{12}\)H\(_{12}\)O\(_3\)) was synthesized by treating 1g (9.08 mmol) of commercially available Resorcinol with 1.2 equivalents (1.76 mL) of ethyl-2-ethylacetoacetate and 5 mL of dry acetone, and 5 mL of concentrated H\(_2\)SO\(_4\) was added drop-wise, while stirring the reaction mixture at RT for 3 hours (see Scheme 24). The progress of the reaction was monitored using thin-layer chromatography using 5% methanol in 95% chloroform. TLC showed a single spot,
so the product was cooled using ice and sonication was done for 30 minute. The reaction mixture was filtered and residue was collected and kept under high-vacuum overnight yielding a dry white crystalline solid in 90% percent yield. The identity of compound 19 was confirmed by performing $^1$H and $^{13}$C NMR using DMSO (see Appendix).

To a solution of 1g (4.9 mmol) coumarins (compound 19) in dry acetone (10mL), 1.5 equivalent of anhydrous potassium carbonate (7.4 mmol), and 1.2 mL propargyl bromide (7.4 mmol) were added (see Scheme 25). The resultant mixture was stirred and refluxed at 60 °C for 24 hours, then the mixture was cooled and filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator. The residue was then treated with 20 mL of deionized water and extracted with ethyl acetate. All organic phase extracts were combined, washed with DI water, dried over anhydrous magnesium sulfate and then filtered. The obtained crude product was purified by crystallization from hexane/ethyl acetate mixture to give compound 20 (C$_{15}$H$_{14}$O$_3$) in 80% yield. The identity of compound 20 was confirmed by performing $^1$H and $^{13}$C NMR using DMSO (see Appendix).

Compound 21 (C$_{22}$H$_{35}$N$_3$B$_{10}$O$_3$) was synthesized by treating 200 mg (0.82 mmol) of compound 20 with 210 mg (0.82 mmol) of compound 2 using 15 mL dry CH$_3$CN/THF (v/v = 1/1) under argon atmosphere using schlenk lines (see Scheme 26). Approximately 172 mg (0.902mmol) of Cu(I)I was added to the reaction mixture as a catalyst, and 1.43mL (8.2mmol) of DIPEA was also added in the mixture as a proton scavenger. The reaction mixture was stirred at room temperature for 3 days. The progress of the reaction was frequently monitored with TLC. Extraction was performed using 20 mL of DI water and ethyl acetoacetate. Solvents were evaporated using a rotary evaporator. Initially, recrystallization using hexane and ethyl acetate
was performed for the purification of compound 21; however, it was unsuccessful. Therefore, silica-gel column chromatography developed with n-hexane/ethyl acetate (v/v=1/2) was performed for further purification, yielding 82% of bright brownish powder as a product. The identification of compound was confirmed by characterization with NMR (\(^1\)H, \(^{13}\)C N and \(^{11}\)B) (see Appendix).

Compound 22 (C\(_{10}\)H\(_8\)O\(_3\)) was synthesized by treating 1g (9.08 mmol) of commercially available Resorcinol with 1.2 equivalent (10.9 mmol, 1.4 mL) of ethyl acetoacetate and 5 mL of dry acetone, and 5 mL of concentrated H\(_2\)SO\(_4\) was added drop-wise while stirring the reaction mixture at 60 °C for 2 hours (see Scheme 27). The progress of the reaction was monitored using thin layer chromatography using 5% methanol in 95% chloroform. TLC showed a single spot, so the product was cooled using ice and sonication was done for 30 min. The reaction mixture was filtered and residue was collected and kept under high-vacuum overnight yielding a dry brown crystalline solid in 90% yield. The identity of compound 22 was confirmed by performing \(^1\)H and \(^{13}\)C NMR using DMSO (see Appendix).

To a solution of 1g (5.7 mmol) coumarins (compound 22) in dry acetone (10 mL), 1.5 equivalent of anhydrous potassium carbonate (8.5 mmol), and 1.4 mL propargyl bromide (8.5 mmol) were added (see Scheme 28). The resultant mixture was stirred and refluxed at 70 °C overnight, then the mixture was cooled and filtered, and the solvent was evaporated under reduced pressure. The residue was then treated with 20 mL of deionized water and extracted with ethyl acetate. All organic phase extracts were combined, washed with DI water, dried over anhydrous magnesium sulfate and filtered. The obtained crude product was purified by recrystallization using hexane and ethyl acetate to give compound 23 (C\(_{13}\)H\(_{10}\)O\(_3\)) in 85% yields.
The identity of compound 23 was confirmed by performing $^1$H and $^{13}$C NMR using DMSO (see Appendix).

Compound 24 (C$_{20}$H$_{31}$N$_3$B$_{10}$O$_3$) was synthesized by treating 300 mg (1.4mmol) of compound 23 with 358 mg (1.4mmol) of compound 2 using 40 mL dry CH$_3$CN/THF (v/v = 1/1) and under argon atmosphere using Schlenk lines (see Scheme 29). Approximately 295 mg (1.55mmol) of Cu(I)I was added to the reaction mixture as a catalyst and 2.5 mL (15mmol) of DIPEA was also added in the mixture to help avoid unwanted reactions. The reaction mixture was stirred at room temperature for 3 days. The reaction’s reaction was frequently monitored with thin layer chromatography. Extraction was performed using 40 mL of DI water and ethyl acetoacetate. Solvents were evaporated using rotary evaporator. The purity of compound 24 was successful with recrystallization using hexane and ethyl acetate, while further purification was carried out by doing silica-gel column chromatography developed with n-hexane/ethyl acetate (v/v=1/2), yielding 87% of white solid powder as a product. The identification of compound was confirmed by characterization with NMR ($^1$H, $^{13}$C N and $^{11}$B) (see Appendix).

Compounds 25 and 26 were synthesized by treating compounds 11 and 14 with a strong base (NaOH, KOH and CsOH) and 95% ethanol or THF as a solvent, and refluxed the reaction mixture for three days stirring under argon (see Schemes 30 and 31).

The first attempt was using NaOH as a base to decapitate the carborane cage. A solution was prepared by dissolving 2g of NaOH in 60 mL of 95% ethanol, and the resulting solution was then added to 60 mg of carborane-appended compound (11 or 14). The reaction mixture was constantly stirred in an ultrasonic bath for approximately thirty minutes, and then heat was applied to the resulting mixture, which was then refluxed for three days. The mixture was then
cooled to 0°C, and the solution was neutralized with aqueous HCl to a pH of ~5.0 in order to remove unreacted salts. The solution was slowly washed with cold water to remove NaCl, and solvents were removed by rotary evaporator under reduced pressure. Then, the resulting compound was dried under high vacuum. Extraction was performed using 20 mL of DI water and DCM. The resulting residue was purified by doing column chromatography developed in hexane and ethyl acetate. However, the outcome of characterization was not positive as confirmed by NMR results, so other attempts were made using CsOH or KOH as a base.

Compound 25 was synthesized by treating 65 mg (0.16 mmol) of compound 11 with 50 mg (0.32 mmol) of CsOH (cesium hydroxide) in 10 mL of ethanol, while refluxing the mixture for three days. The obtained crude mixture was cooled to 0 °C using ice and solvents were evaporated under reduced pressure. The compound was extracted by using 20 mL DI water and diethyl ether. Column chromatography was performed for compounds purification, by initially washing with 100% hexane and then eluting with 60% ethyl acetate. Compound 25 was characterized by $^1$H, $^{13}$C and $^{11}$B NMR (see Appendix).

Compound 26 was synthesized by treating 250 mg (0.34 mmol) of compound 14 with 381.5 mg (6.8 mmol) of potassium hydroxide in 5 mL of THF and 10 mL of ethanol, while refluxing the mixture for 24 hours. The obtained reaction mixture was then cooled to RT, and then cooled by using dry ice. The insoluble portion of the mixture was filtered through a Buchner funnel, and then the filtrate was concentrated and dried under high vacuum. The compound was purified by performing column chromatography developed in DCM and acetone, a separation starting with 10% acetone and slowly increasing the concentration of acetone over time, acetone being eluent in this case. The purity of compound was not confirmed, so recrystallization was
performed for further purification using water and ethanol. This compound was characterized by \textsuperscript{1}H, \textsuperscript{13}C and \textsuperscript{11}B NMR (see Appendix).

In order to perform the cytotoxicity studies of the compounds 21 and 24 MDA-MB-231 culture media consists of DMEM, 10\% FBS and 1\% penicillin-streptomycin was used. Cells were seeded at 5x10^4 cells/mL in 96-well plates and incubated at 37°C and 5\% CO\textsubscript{2} atmosphere for 18-24 hours. The stock solutions of test compounds were prepared in DMSO at 1000x concentration and further diluted in growth media. 100µL of this solution was added into 96-well plate in replicate and serial dilution was done. The plated were incubated for 72 hours and 10µL of MTT (5mg of MTT in 1mL of 1X PBS) was added in each well and incubated for 4 hours. 100µL of SDS-HCl (1g SDS in 10 mL 0.01N HCl) was added into wells to dissolve formazan precipitate and further incubated for 4 hours. Absorbance was recorded at 570 nm and \% survival was calculated using growth media as control [\%survival = (absorbance of test compound/absorbance of control)*100].
CHAPTER 11

SUMMARY OF RESULTS

All the compounds were isolated in good yield. All the compounds were assigned their number for their easy identification purpose starting from 1 to 26. Their respective molecular formula, molecular weight and percent yields are shown in the Tables 4-8 below.

Table 4
Summary of results for compounds 1 and 2 (Carboranes)

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<th>Molecular Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>% Yield</th>
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<td>C\textsubscript{7}H\textsubscript{21}B\textsubscript{10}I</td>
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Table 5
Summary of results for compounds 3 to 10 (Alkynyl Derivatives)

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<th>% Yield</th>
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<td>5</td>
<td>C\textsubscript{15}H\textsubscript{11}NS</td>
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<td>6</td>
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Table 6
Summary of results for compounds 11 to 18 (Carborane-Appended Biomolecules)

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Table 7
Summary of results for compounds 19 to 24 (Coumarins Derivatives)

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Table 8
Summary of results for compounds 25 and 26 (Decapitated Compounds)

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<th>Molecular Weight (g/mol)</th>
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<td>527.58</td>
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<tr>
<td><strong>26</strong></td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;46&lt;/sub&gt;N&lt;sub&gt;8&lt;/sub&gt;B&lt;sub&gt;18&lt;/sub&gt;S&lt;sub&gt;3&lt;/sub&gt;K</td>
<td>752.54</td>
<td>75</td>
</tr>
</tbody>
</table>
CHAPTER 12
DISCUSSION AND CONCLUSIONS

When commercially available organic compounds; 4-nitroimidazole, 2-nitroimidazole, phenothiazine, 1,3,4-thiadiazole-2,5-dithiol, adenine, guanine, 6-chloropurine and 3’,5’-dihydroxyacetophenone were treated with propargyl bromide in the presence of strong base K$_2$CO$_3$ or KOtBu and dry acetone/acetonitrile or DMF/THF as solvent, compounds 3, 4, 5, 6, 7, 8, 9 and 10 were obtained in quantitative yield (see Schemes 6-13). Likewise, carboranyl azide (compound 2) was synthesized in a good yield following the protocol mentioned in the literature procedure\(^2\) (see Scheme 4). Compounds 11, 12, 13, 14, 15, 16, 17 and 18 (see Schemes 16-23) were synthesized by the reaction between carboranyl azide (compound 2) and compounds 3, 4, 5, 6, 7, 8, 9 and 10 respectively via click reactions. Similarly, compounds 19 and 22 are synthesized by treating resorcinol with ethyl-2-ethylacetoacetate and ethyl acetoacetate respectively. Compounds 20 and 23 are the alkylation product of 19 and 22 respectively while compound 21 and 24 are the click were synthesized by performing click reactions between compound 2 and compound 20 and 23 respectively (see Schemes 24-29). Moreover, compound 25 and 26 (see Schemes 30 and 31) were synthesized by decapitating compounds 11 and 14 respectively using CsOH/KOH as a strong base and 95% ethanol/THF as solvent to make them water soluble for their biological distribution studies. This work is currently underway as well.

Click reaction is a simple, wide in scope and high yielding reaction between azide and alkyne which was performed using Cu(I)I as catalyst, and 50:50 mixture of CH$_3$CN and THF as
solvent whereas N,N-Diisopropylethylamine (DIPEA) was used as a proton scavenger helping to avoid unwanted side-reactions. Most of the synthesized compounds were purified and isolated in good yields, and characterized by NMR spectra ($^1$H, $^{13}$C, and $^{11}$B), FTIR spectra, high resolution mass spectral analysis and elemental analysis where possible. The FTIR spectra of compounds containing carborane cage showed strong bonds between 2580 and 2586 cm$^{-1}$ corresponding to $\nu$ (B-H). The presence of carborane cage was also evident from $^1$H, $^{13}$C, and $^{11}$B NMR spectra. Mass spectral data of compounds 11, 12, 13 and 14 confirmed their identifications. High resolution mass spectral data for the carborane-appended compounds 11, 12, 13, 14 showed prominent peaks at m/z 407.3199 (M+H), 407.200 (M+H), 494.3407 (M+3H) and 738.5232 (M+H) respectively (see appendix). The elemental analysis was performed with compounds 11, 12, 13, 14, 15, 16, 17 and 18 which are discussed in experimental section.

The click reaction (Cu(I) I-catalyzed azide-alkyne cycloaddition reaction) has been found to be a facile approach for the synthesis of carborane-appended biocompatible compounds potentially suitable for BNCT applications. Carborane-appended derivatives of various organic compounds (4-nitroimidazole, 2-nitroimidazole, phenothiazine, 1, 3,4-thiadiazole-2, 5-dithiol, adenine, 6-chloropurine, 3’,5’-dihydroxyacetophenone and coumarin compounds) were synthesized in very good yields. A multi-step organic synthetic methodology was established to attach a polyhedral carborane cage to these compounds. This methodology includes the alkylation reaction of the selected organic compounds followed by their conjugation with carboranyl azide compound via click reaction. Compounds 11 and 14 were made water soluble by the decapitation of their carborane cage. Cytotoxicity study of the compounds 21 and 24 were
performed and found to be non-toxic. Bio-distribution studies of these compounds will provide the idea about their potential applicability in cancer therapeutics.
CHAPTER 13

FUTURE DIRECTIONS

In the future, more similar studies will be performed by selecting various other organic compounds as a starting material. All of the synthesized compounds could be made water soluble in order for their biological distribution studies. The cytotoxicity of the remaining compounds will also be tested in order to determine their biological activity. It is also possible to encapsulate the products with liposomes for their effective delivery into the body. Moreover, since all the products synthesized contain natural boron (20\% ¹⁰B and 80\% ¹¹B), this work will be repeated using ¹⁰B enriched carborane before BNCT.
REFERENCES


25. Zhivotova, T. S. "Reaction of 1, 3, 4-thiadiazol-2, 5-dithiol with N-acryloyl-substituted derivatives of several alkaloids." Chemistry of natural compounds45.6 (2009): 846-848.


APPENDIX A

LIST OF NMR, FTIR, AND MS SPECTRUMS OF
ALL SYNTHESIZED COMPOUNDS
Compounds 1 & 2: $^{13}$C, $^1$H and $^{11}$B NMR Spectrum
Compound 3: $^{13}$C and $^1$H Spectrum
Compound 4: $^{13}\text{C}$ and $^1\text{H}$ Spectrum
Compound 5: $^{13}$C and $^1$H Spectrum
Compound 6: $^{13}$C and $^1$H Spectrum
Compound 7: $^{13}$C and $^1$H Spectrum
Compound 8: $^{13}$C and $^1$H Spectrum
Compound 9: $^{13}$C and $^1$H Spectrum
Compound 10: $^{13}$C and $^1$H Spectrum
Compound 11: NMR ($^{13}$C, $^1$H, $^{11}$B), FTIR and MS Spectrum
Compound 12: NMR ($^{13}$C, $^1$H, $^{11}$B), FTIR and MS Spectrum
Compound 13: NMR ($^{13}$C, $^1$H, $^{11}$B), FTIR and MS Spectrum
Compound 14: NMR (\textsuperscript{13}C, \textsuperscript{1}H, \textsuperscript{11}B), FTIR and MS Spectrum
Compound 15: $^{13}$C, $^1$H, and $^{11}$B NMR Spectrum
Compound 17: $^{13}$C, $^1$H, and $^{11}$B NMR Spectrum
Compound 18: $^{13}$C, $^1$H, and $^{11}$B NMR Spectrum
Compound 19: $^{13}$C and $^1$H NMR Spectrum
Compound 20: $^{13}$C and $^1$H NMR Spectrum
Compound 21: $^{13}$C, $^1$H, and $^{11}$B NMR Spectrum
Compound 22: $^{13}$C and $^1$H NMR Spectrum
Compound 23: $^{13}$C and $^1$H NMR Spectrum
Compound 24: $^{13}$C, $^1$H, and $^{11}$B NMR Spectrum
Compound 25: $^{13}$C, $^1$H, and $^{11}$B NMR Spectrum
Compound 26: $^{13}$C, $^1$H, and $^{11}$B NMR Spectrum