NORTHERN ILLINOIS UNIVERSITY

The Synthesis of Carborane-Appended Dopamine and L-Dopa for Boron Neutron Capture Therapy

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Abstract

With a series of reactions, biological molecules Dopamine and L-Dopa were conjugated with carborane in an effort to synthesize new boron delivery agents for a bimodal cancer treatment known as Boron Neutron Capture Therapy (BNCT). The products of these reactions were purified using column chromatography, and characterized with Fourier-Transform Infrared Spectroscopy (FT-IR) and Nuclear Magnetic Resonance (NMR) Spectroscopy. The successful synthesis of carborane-appended dopamine and L-Dopa has been supported with characterizations performed thus far. In the future, the compounds will undergo further analysis with elemental analysis, mass spectroscopy, and biodistribution studies to determine cytotoxicity and cellular uptake in the future.
Introduction

Boron Neutron Capture Therapy (BNCT) is a bimodal cancer treatment which is based on the selective accumulation of Boron-10 ($^{10}$B) atoms into cancer cells using biologically compatible boron delivery agents.\(^1\) $^{10}$B can be irradiated with a beam of low-energy neutrons to produce $^{11}$B atoms. These atoms break down into Helium-4 ($^4$He) alpha particles and Lithium-7 ($^7$Li) atoms, and produce 2.4 MeV of energy.\(^1\) The high linear energy transfer (LET) alpha particles and $^7$Li particles travel approximately 5-9 µm, which is the diameter of one cell.\(^1,2\) The distance traveled by these particles results in the destruction of only the cells containing a sufficient amount of $^{10}$B atoms (Figure 1).\(^1,2\) As cancer cells are selectively destroyed, the surrounding healthy cells are not affected.\(^3\) This greatly reduces the side effects associated with traditional cancer treatment methods such as chemotherapy and radiation. With the use of BNCT, patients will experience a better quality of life during their treatment.\(^3\) Therefore, selective accumulation of boron containing compounds into cancerous cells only is very desirable, and has become the focus of current research with BNCT.\(^3\)

![Figure 1: BNCT Mechanism](image)

Currently, there are only two boron delivery agents that have been approved by the FDA for clinical use. These compounds include boronophenylalanine fructose (BPA) and sodium...
Borocaptate (BSH) (Figure 2). BPA is a derivative of amino acid tyrosine, and has been used to treat skin cancers, whereas BSH is a boron cluster which has been used to treat brain tumors. Although successful, these compounds have limitations. BSH contains a sulfur atom, which can bond to other sulfur atoms to form disulfide bridges. This causes the formation of dangerous radicals in the body, and can cause negative side effects. BPA has only one Boron atom per molecule, and therefore requires high dosages for substantial boron accumulation into the cells. In order to successfully destroy a cancer cell, the boron content of that cell must be between 20-35 \( \mu g/g \) of tumor tissue, but a low concentration of \( ^{10}B \) must be maintained in surrounding healthy cells. Therefore, current research has been focused on the synthesis of new boron delivery agents that have a high concentration of boron atoms and are biologically compatible.

![Figure 2: (a) BSH (b) BPA](Image)

Carborane is a biologically compatible molecule with a cage structure that contains ten boron atoms. Because of its properties, carborane would be an excellent boron carrier, and would allow faster accumulation of the necessary amount of boron in cancer cells. However, carborane cannot be taken up by cells on its own. Cancer cells take up large amounts of nutrients such as glucose, amino acids, and neurotransmitters to facilitate their rapid growth, but would not take up an individual carborane molecule. Therefore, by conjugating carborane with a molecule that would
normally be taken up by cancer cells, the carborane cage can be disguised using the “Trojan Horse Method” and will be able to enter the cell. Although all cells will take up the carborane appended biomolecule, cancer cells will take up much larger amounts. Healthy cells will then metabolize the small amount of boronated compound, and it will safely exit the body. As cancerous cells will have a higher concentration of the boronated compound, some will still remain after it has been removed from the healthy cells. It is at that time that the neutron beam will irradiate the cancerous cells, and the reaction to destroy only those cells will occur.

To successfully disguise the carborane cage and facilitate uptake by cancerous cells, carborane will be conjugated with Dopamine and L-Dopa. Dopamine is a neurotransmitter that plays a role in several functions such as movement, memory, the reward system, sleep, and mood, and is found in many locations throughout the body. L-Dopa is a precursor of Dopamine, and, unlike dopamine, can cross the blood-brain-barrier (BBB). To treat brain tumors, the boron carrier must be able to pass through the BBB, which is very selective. It is freely permeable to water, and is able to uptake molecules such as glucose, L-Dopa, and amino acids through selective transporters. Generally, the solubility of a compound in lipids positively correlates with the ability of a compound to pass through the blood brain barrier. Water and ethanol have high lipid solubility and can pass freely through the BBB, while L-Dopa and glucose have lower lipid solubility and require specific carriers to facilitate their passage (Figure 3). Although L-Dopa enters the brain quickly, enzymes called L-Dopa decarboxylase and monoamine oxidase break down L-Dopa and limit the amount that is able to enter the central nervous system. Because of this, large doses of the synthesized carborane-appended L-Dopa would be needed for effective BNCT treatment. However, a drug called carbidopa reduces decarboxylation of L-Dopa, and could be administered with L-Dopa to increase uptake.
As the structures of both Dopamine and L-Dopa are very similar to that of BPA, the conjugation of Dopamine and L-Dopa with carborane could be viable options for the synthesis of boron delivery agents for BNCT. The synthesis of these new boron delivery agents would decrease side effects, as there would be no possibility of the formation of disulfide bridges, and would allow for high concentrations of boron to be taken up by the cells. Through the continuation of research, BNCT will improve and will become a safe and widely used method of treatment for many types of cancers.

**Materials and Methods**

Each reaction done in this research project has included the use of various laboratory equipment such as round bottom flasks, magnetic stir bar and hot plate, TLC paper, silica-gel column, ultraviolet lamp, rotary evaporator, and distilling apparatus including heating tape. The reactions were performed under argon or nitrogen gas using Schlenk lines to ensure an inert environment and ideal reaction conditions. The chemicals involved in these reactions were used
without further purification and include previously prepared and purified 1-iodobutyl-\textit{o}-carborane, dopamine HCl, L-Dopa, sodium hydroxide, hexane, hydrochloric acid, methanol, and dimethylformamide. The method by which 1-iodobutyl-\textit{o}-carborane was prepared can be seen in Scheme 1.

\textit{Scheme 1: Synthesis of 1-iodopropyl-\textit{o}-carborane}

\textbf{1. Synthesis of Carborane Appended Dopamine}

\textit{Scheme 2: Synthesis of carborane-appended dopamine using dimethylformamide.}
Sodium hydroxide (160.3 mg, 4.00 mmol) was added to 100 mL DMF in an oven-dried 250 mL round-bottom flask, and was sonicated for 30 minutes. After the sodium hydroxide had dissolved, dopamine hydrochloride (39.8 mg, 0.210 mmol) and 1-iodopropyl-o-carborane (129.7 mg, 0.422 mmol) were added to the flask. The mixture was refluxed for 48 hours at 80°C. Following the reflux, the dimethylformamide solvent was removed in small fractions using distillation, leaving the crude product. This crude product was then purified using silica gel column chromatography (SiO₂, eluted with hexane and dimethylformamide) and TLC (SiO₂, developed with hexane, dimethylformamide). The solvent was then again removed from the purified product using distillation, and the product was dried under vacuum for 10 hours. The product was then characterized using Fourier-Transform Infrared Spectroscopy shown in Figure 4, and ¹H, ¹¹B, and ¹³C NMR in dimethyl sulfoxide (DMSO) solvent shown in Figures 2, 3, and 4. In an effort to remove some of the excess salt that was formed during the reaction, a small portion of the product was washed with water. The washed product as well as the unwashed product were taken for characterization using elemental analysis, and the results were compared with the elemental analysis of unreacted dopamine hydrochloride (Table 1).

2. Synthesis of Carborane Appended L-Dopa

a. Protection of Carboxylic Acid using Fischer Esterification

\[
\begin{align*}
\text{L-Dopa} & \quad \xrightarrow{\text{HCl, MeOH}} \quad \text{L-Dopa}^+ \times \text{OCH}_3 \\
\end{align*}
\]

*Scheme 3: Fischer Esterification reaction*
**Reaction I:**

First, 15 mL of methanol was added to a 50 mL round bottom flask. 49.8 mg of L-Dopa was measured using a laboratory balance and then was added to the flask. This was cooled with ice for five minutes before 1 mL concentrated HCl was added dropwise into the solution with continued cooling and stirring. The mixture was then allowed to return to room temperature before beginning reflux at 50°C. The reflux was continued overnight, and then the solvent was removed with rotary evaporation. The sticky yellow product was analyzed with Fourier Transform Infrared Spectroscopy, $^1$H NMR, and $^{13}$C NMR as seen in Figures 5, 6, and 7, respectively. Following this initial analysis, the product was dried further under vacuum conditions to produce an off-white, fluffy solid. This product was again analyzed using $^1$H NMR, $^{13}$C NMR, and FTIR as seen in Figures 8, 9, and 10, respectively.

**Reaction II:**

In order to produce a more substantial amount of product for use in the reaction depicted in Scheme 3, a larger scale reaction was completed. 60 mL methanol was added to a 250 mL round bottom flask. Then, 200.7 mg L-Dopa was added to the flask with a magnetic stir bar. This mixture was placed on ice to cool and stir for approximately five minutes before the addition of 4.2 mL concentrated HCl in a dropwise manner. The clear solution was allowed to stir on ice for approximately five minutes before being allowed to return to room temperature while stirring. The solution was then heated to 50°C to reflux overnight. The following day, the solvent was removed with rotary evaporation to produce a sticky yellow product as observed in Reaction I. This product was then dried under vacuum conditions overnight to produce an off-white, fluffy solid.
b. Addition of Carborane Cage

Scheme 4: Synthesis of carborane-appended L-Dopa

To finish the synthesis of carborane appended L-Dopa, the second part of the procedure was performed as follows. Sodium hydroxide (42.4 mg, 1.00 mmol) was added to 50 mL DMF in an oven-dried 250 mL round-bottom flask and was sonicated for 30 minutes. 50 mL DMF was also added to the flask containing the protected L-Dopa (281.4 mg, 1.33 mmol). Following the dissolution of the sodium hydroxide, the solution containing the protected L-Dopa and was added to the sodium hydroxide and DMF solution. Then, 1-iodopropyl-o-carborane (312.7 mg, 1.01 mmol) was added to the flask. The solution was refluxed for 16 hours at 80°C before the solvent was removed through distillation. The product obtained from this reaction was then characterized using FTIR and $^1$H, $^{13}$C, and $^{11}$B NMR (Figures 11, 12, 13, and 14).
Results

1. Synthesis of Carborane Appended Dopamine

**Figure 4:** FTIR spectra of unreacted dopamine HCl (red) compared with purified carborane appended dopamine (blue).

**Figure 5:** $^1$H NMR of purified carborane-appended dopamine.
Figure 6: $^{13}$C NMR of purified carborane-appended dopamine.

Figure 7: $^{11}$B NMR of purified carborane-appended dopamine.
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<tr>
<th>Sample</th>
<th>Expected</th>
<th>Observed</th>
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<td>Dopamine Hydrochloride</td>
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<td></td>
<td>C: 50.67%</td>
<td>C: 50.77%</td>
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<tr>
<td></td>
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<tr>
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<tr>
<td>Carborane-Appended Dopamine</td>
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<td></td>
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<tr>
<td>(Before wash with DI water)</td>
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<tr>
<td>Carborane-Appended Dopamine</td>
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<tr>
<td>(After wash with DI water)</td>
<td>C: 43.10%</td>
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Table 1: Elemental Analysis results for procedure outlined in Scheme 2.

2. **Synthesis of Carborane Appended L-Dopa**

   a. **Protection of Carboxylic Acid using Fischer Esterification**

![Figure 8: Fourier Transform Infrared Spectroscopy of L-Dopa (Red) and L-Dopa with a methyl group protecting the carboxylic acid (Blue) (Reaction I)) before drying.](image-url)
Figure 9: $^1$H NMR of protected L-Dopa before drying (Reaction I).

Figure 10: $^{13}$C NMR of protected L-Dopa before drying (Reaction I).
Figure 11: $^1$H NMR of protected L-Dopa following drying under vacuum conditions (Reaction 1).

Figure 12: $^{13}$C NMR of protected L-Dopa following drying under vacuum conditions (Reaction 1).
Figure 13: FTIR of protected L-Dopa following drying under vacuum conditions (Reaction I).

b. Addition of Carborane Cage

Figure 14: FTIR of L-Dopa (red), methyl protected L-Dopa (dark blue), and carborane-appended L-Dopa (bright blue).
Figure 15: $^1$H NMR of carborane-appended L-Dopa.

Figure 16: $^{13}$C NMR of carborane-appended L-Dopa
Discussion

1. Synthesis of Carborane Appended Dopamine

When the product obtained as described in Scheme 2 was purified and the solvent was removed with distillation, it was dark brown in color and very waxy. Initially, it was hypothesized that there could be a small amount of DMF left in the product. After many hours of drying under vacuum, a brown clumpy solid was formed. In Figure 4, the FTIR spectra of dopamine hydrochloride and the purified carborane appended dopamine product are compared. The large peak at 3400 to 3000 cm\(^{-1}\) which represent the OH groups on the dopamine hydrochloride shifted to the left and centered at 3500 cm\(^{-1}\). This indicates that the OH groups were removed, and the peak at 3500 cm\(^{-1}\) is representative of the NH\(_2\) group in the carborane appended dopamine. Additionally, a strong peak appeared at 2500 cm\(^{-1}\) in the spectrum of the
product, which is indicative of a B-H bond. This suggests that the carborane cage was successfully attached to the dopamine.

The $^{11}\text{B}$ NMR shown in Figure 7 further supports this conclusion, as there are ten peaks in the spectrum, which correspond to the ten boron atoms in a carborane cage. The $^{1}\text{H}$ NMR and $^{13}\text{C}$ NMR seen in Figures 5 and 6, respectively, are not as promising, as there are not as many signals as expected. Additionally, the results from elemental analysis can be seen in Table 1. The values obtained from the analysis of the carborane-appended dopamine product are slightly different from the expected values, but do not give reason to believe that the synthesis of the compound was unsuccessful. One of the most significant differences was in the nitrogen content of the carborane-appended dopamine before being washed with water. Rather than the expected 2.79%, the nitrogen content was 12.05%. Additionally, before being washed with water, the carborane-appended dopamine exhibited a carbon content of 39.88%. After being washed with water, the carbon content increased to 49.37% as compared to the 43.10% that is expected. These discrepancies, although larger than would be normally accepted, can be attributed to the calibration curve that was produced by the elemental analysis apparatus. The calibration curve was significantly deviant from the normal range, and contributed to the value differences between expected and observed. Because of this, the results from elemental analysis are inconclusive. This method of characterization will therefore be performed again to obtain more credible results.

2. Synthesis of Carborane Appended L-Dopa

a. Protection of Carboxylic acid with Fischer Esterification

After completion of the reaction outlined in Scheme 3 aimed to protect the carboxylic acid group with a methyl group, a sticky, yellow product was obtained. The product was characterized
using FTIR and compared with the FTIR of the starting material L-Dopa, as seen in Figure 8. There are some differences in the spectra indicating that a favorable reaction did occur. Because the desired product has only one additional carbon that has replaced a hydroxyl group on the carboxylic acid, it is difficult to determine the success of the reaction solely from the FTIR Spectra. However, the desired peaks are present. In the spectrum of the product following drying under vacuum condition (Figure 13), a stretch at 1735 cm\(^{-1}\) indicates a carbonyl carbon, while stretches at 1287 cm\(^{-1}\) and 1117 cm\(^{-1}\) indicate a C-O bond. Additionally, the large stretch at 3214 cm\(^{-1}\) indicates the OH groups and NH\(_3\) groups that are still present on the L-Dopa.

In the \(^{13}\)C NMR spectra in Figure 12, the peaks at 110-120 ppm indicate a C-O bond. Likewise, the peak at approximately 3.8 ppm indicates the presence of an RO-CH\(_3\) bond in the \(^1\)H NMR spectra (Figure 11). Additionally, in the \(^1\)H NMR (Figure 11), the peak at 10-13 ppm that would represent a carboxylic acid is not present. All of these peaks indicate that the reaction was successful in protecting the carboxylic acid with a methyl group.

**b. Addition of Carborane Cage**

From the characterizations obtained on the product from the reaction outlined in Scheme 3, it is promising that the desired compound, carborane-appended L-Dopa, was synthesized. In Figure 14, there is a characteristic peak at approximately 2600 cm\(^{-1}\) indicating the presence of B-H bonds. Additionally, the peak at approximately 3500 cm\(^{-1}\) is sharp, which means that there is an amine in the compound. The absence of the broad –OH peak at this location indicates that the carborane cages were attached to the –OH groups on the L-Dopa. The \(^{11}\)B NMR spectrum in Figure 17 shows 10 peaks for the 10 boron atoms in a carborane cage. Additionally, the \(^{13}\)C NMR in Figure 16 has a peak at approximately 220 ppm indicating a carbonyl bond. The small peaks at approximately
125 ppm indicate aromatic carbons, which are found in the ring. These peaks are all positive indications of the successful synthesis of carborane-appended L-Dopa.

**Conclusion**

As the synthesis of these molecules has been determined to be successful thus far, it will be necessary to continue research on this subject in order to fully characterize the compounds and determine their biological compatibility. Mass spectrometry and additional trials in elemental analysis will further solidify the identities of the compounds, and biodistribution studies will be conducted to determine each compound’s cytotoxicity and cellular uptake in cancer cell lines. With positive results in these areas, clinical trials would follow. With the successful synthesis of these boron delivery agents, Boron Neutron Capture Therapy will continue to make advancements, and the use of BNCT as a cancer treatment will become more widespread. Patients will benefit from advancements in this area by experiencing fewer side effects than with traditional cancer therapies, and fewer treatments overall.\(^7\) In addition to this avenue of research, future directions could include increasing tumor cell specificity and tissue selectivity for even more reduced side effects on healthy cells, exploring the development of neutron beams specifically for BNCT to improve treatment success, and investigating treatment planning including specific drug doses and timing of treatment for patients on an individual basis.\(^8,9\) Although there is much research to be done in this field, BNCT is a promising method of cancer therapy that will greatly benefit patients suffering from many different types of cancers.
References


