LYCORINE – AMINO ACID CONJUGATE AS A POTENTIAL ANTICANCER AGENT

by

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DEDICATION

To my Mom, friends and family.

ACKNOWLEDGEMENTS

When I started this project I have to be honest and say that I underestimated the challenge. My journey into the world of chemistry started in my undergraduate organic chemistry class. Historically I was a poor student, making mostly C's and B's. Organic chemistry 1 was the first difficult class I ever made an A in, and from that point on I was hooked. I spent hundreds of hours throughout that semester and the following semester studying organic chemistry. After I made my A in organic chemistry 2 I was hired as a Supplemental Instructor(SI), going back to the beginning of O-chem 1 and helping new students understand the difficult material. After a year of helping teach o-chem lecture as an SI, I began teaching the experimental side of chemistry as a lab instructor for organic chemistry. At that point I felt like I "understood" chemistry, because on paper I knew all the theory, and even in the teaching labs I knew all the techniques. I taught labs for the last year of my undergraduate degree, which was when I made the decision to stay at Texas State for my masters in chemistry. I did some research into what kind of chemistry was actually being done at Texas State, and found the only lab that actually interested me was the medicinal chemistry research lab, run by Dr. Kornienko. During a meeting with Dr. Kornienko I was told that this lab is usually more of a challenge to the straight "A" students than to the students who are ok with failure. I was told that things often don't work and this can be extremely frustrating to the students who are used to succeeding in class without long stretches of failure. This seemed perfect for me. I was also told that I

would need to run close to one hundred columns before I knew practically anything about column chromatography. I experienced all of these to be true very quickly. I failed, often. For the entire first year of the masters I ran reaction after reaction and column after column and rarely got higher than 40-50% yields. I pretty much never got pure product, either. After about a year of failure I finally started to see some success. Followed by more failure. I experienced this back and forth between short periods of success followed by long stretches of failure many times throughout the course of this project. The highs of succeeding were extremely high, and the lows of failure were equally low. Throughout the masters I was extremely fortunate to have Dr. Ramesh Dasari there to patiently explain to me how the process should work, often times he would have to explain 5 or 6 times before I finally got it. I have to give Dr. Ramesh a huge amount of credit in helping me through this entire experience.

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LIST OF ABBREVIATIONS

Abbreviation	Description
BBB	Blood Brain Barrier
CNS	Central Nervous System
DCM	Dichloromethane
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
GI_{50}	Inhibitory Growth Concentration
LAT1	Large Amino Acid Transporter
NMR	Nuclear Magnetic Resonance
TEA	Triethylamine
TFA	Trifluoroacetic Acid
TLC	Thin Layer Chromatography

I. INTRODUCTION

Cancer

Cancer prevalence in the United States is quite significant with roughly 1.7 million new cancer incidences and almost 600,000 cancer related deaths expected in 2015 alone.[1] The most common cancers in the United States are breast, lung, prostate, colon/rectal, and melanoma.[2] There are many chemotherapeutic options currently available, but often come with negative side effects. Cancer cells are human cells, so often the same drugs that kills cancer cells will also kill healthy cells. The traditional chemotherapeutic approach has been to systemically administer anti-cancer agents, and hope that they kill off the cancer before they kill off too many of the healthy cells. For this reason, it is important for chemotherapeutic agents to show selectivity for killing cancer cells more quickly and often than they kill healthy human cells. Although development of chemotherapeutic drugs has been ongoing for decades, an aging population coupled with the typical sedentary lifestyle of developed nations has seen the incidence of cancer increasing. [3] One of the additional dangers of cancer is complications like metastases, which is the spread of malignant cells to different parts of the body from the original site of cancer. Across the 5 most common cancers, the rates of brain metastases is as high as 16.5% over the course of 5 years.[4] Clearly new and more effective chemotherapeutic agents are needed.

Natural Products Are Excellent Sources of New Drugs

There are many reasons that make natural products or their derivatives good drug candidates. Nature has been selecting for stable, biologically compatible molecules since the first single celled organisms evolved over 3 billion years ago. Natural products often

contain what are known as “privileged structures” which are common substructures found on the molecule that allow it to interact with structurally and functionally different enzymes or receptors. [5] Statistically, natural products or their derivatives are the most common drugs on the market with roughly 80% of commercial drugs being a natural product or a derivative of a natural product. [6] Taxol is one particular drug which is a successful natural product cancer agent. It is extracted from the pacific yew tree.

Alkaloid Lycorine is a Promising Lead to Combat Drug Resistant Cancers

Lycorine is one of many alkaloids found in the *Amaryllidaceae* family of flowers. Extracts from these flowers have been used as cancer therapy for thousands of years, although it was not until the last century that it was determined which alkaloids have anti-cancer properties. [7] Lycorine is a promising lead for cancer therapy for several reasons. The first reason is that Lycorine has a low micromolar GI_{50} at around 6 µM. [8] GI_{50} is the inhibitory growth concentration; it is the concentration required to inhibit 50% of the growth of cells. [9] This particular GI_{50} is an average of the GI_{50} values found across a variety of cancer cells.[10] A low micromolar GI_{50} value is important, because while optimizing the molecule there is a chance of the potency going down. This reduction in potency can be worthwhile if the derivatization allows the molecule access to an area of the body it did not previously have, or if the new analogue is more selective for cancer cells than the original drug. The potential reduction in potency is the reason why a starting or “lead” compound must have an initially low GI_{50}.

Lycorine is also a desirable natural product for cancer therapy because it does not work through the induction of apoptosis. [11] Apoptosis is a common form of programmed cell death. The fact that apoptosis is a genetically directed program is very important in

understanding cancers and the therapeutic options available for treatment. Most cytotoxic anti-cancer agents work by inducing apoptosis, and many malignant cancers are drug-resistant because they have mutations in the genes responsible for apoptosis. Thus, these cancers are resistant to conventional cytotoxic cancer therapies.[12] Mutations in genes responsible for apoptosis result in a cell which cannot undergo apoptosis, and is a compelling explanation for the failure of many cytotoxic cancer drugs in eliminating aggressive cancers.[13] For this reason, a non-apoptotic cancer drug is desirable. Glioblastoma is a particularly aggressive form of brain cancer which is apoptosis-resistant. The prognosis after diagnosis of glioblastoma is quite dismal. The median survival time without treatment is 4 and ½ months, while the median survival time with treatment is 15 months. It is clear that there is a need for therapy for this cancer. [13a]

Finally, lycorine is a good choice as a cancer therapy because it has a good therapeutic ratio. The therapeutic ratio of a molecule refers to the ratio of cancerous cells destroyed versus healthy ones. Lycorine is about 15 times more selective for cancer cells than for healthy cells. [7]

Mechanism of Action of Lycorine

Lycorine inhibits peptide bond formation during translation. [14] Proteins are important biological molecules, involved in mechanisms ranging from signal transduction to the replication of DNA. [15] Lycorine binds to the peptidyl tranferase center of the ribosome and blocks the ability for the incoming amino acid to form a peptide bond with the growing amino acid chain. [16] If the amino acid chain cannot be synthesized, then the protein cannot adopt its functional tertiary conformation and thus cannot perform its function. This disrupts nearly every aspect of cellular function. It is important to note that

healthy cells require proteins for the same functional reasons that cancerous cells do, this is another reason why the selectivity of lycorine is important.

Transport Proteins

Transport proteins are incredibly crucial for the uptake of nutrients into all cells. Two important macronutrients are carbohydrates and amino acids. Both of these molecules tend to be polar, and would struggle to diffuse across a biological membrane which is non-polar. Nature has devised these transport proteins in order to ensure uptake of polar molecules like carbohydrates and amino acids into cells. These transport proteins tend to be overexpressed in aggressive and mature cancers due to the fact that aggressive cancers have high metabolisms to support their aggressive proliferation. [17] The Large Amino Acid transporter, or LAT1, is a heterodimeric membrane transport protein which preferentially transports branched chain, and aromatic amino acids. The LAT1 is a membrane spanning protein which forms a pore in the membrane and allows amino acids passage through. [18] The LAT1 is expressed in all cells, but tends to be over expressed in endothelial cells which are associated with brain capillaries and the BBB.

LAT1 Can Be Exploited for Selective Drug Delivery

There are many examples of drugs which exploit the LAT1 for drug delivery. In Figure 1.1, a series of amino acid prodrugs can be seen. L-DOPA is one of the most well-known prodrugs which exploits the LAT1 for delivery through the BBB into the central nervous system. [20] L-DOPA is a prodrug which is decarboxylated to dopamine within the central nervous system, and serves as a therapy for patients with Parkinson's disease. [21] Dopamine itself is too hydrophilic to cross the blood brain barrier, but when the

carboxyl group is present on the alpha carbon of the corresponding amine group, it becomes a substrate for the LAT1. It was observed that when competitive inhibition studies were performed, and the concentration of the known LAT1 substrates increased, the cellular uptake of L-DOPA decreased or halted all together. [22] This was convincing evidence that L-DOPA competes for binding of LAT1. Another example of a prodrug which acts as a substrate to the LAT1 is Ketoprofen-Tyrosine. [23] Ketoprofen is a non-steroidal anti-inflammatory drug which does not bind to LAT1 on its own, but after being conjugated to tyrosine becomes a substrate for LAT1. [23] Ketoprofen-Tyrosine was evaluated as a substrate for LAT1 by In Situ Rat Brain Perfusion, and competitive inhibition studies. [24]

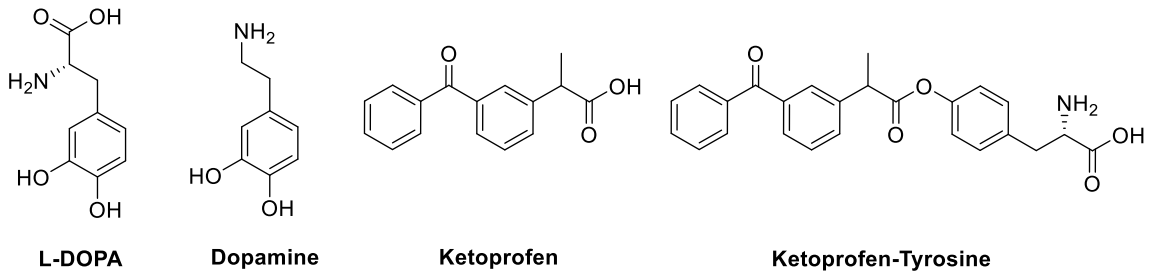


Fig. 1.1 Amino Acid Prodrugs Which Exploit the LAT1 and their Leads

Research Goals

Based on the literature precedent that an amino acid moity can function as a prodrug which can help shuttle a molecule through the LAT1, and the fact that lycorine is such an attractive candidate for cancer therapy, the goal of this project is to couple the amino acid tyrosine to Lycorine. In Figure 1.2, the structure of DHT-1 is seen. DHT-1 is the final conjugate of tyrosine and lycorine, coupled via a triazole bridge attached at the C-2 hydroxyl on the Lycorine portion, and the phenol-like hydroxyl on the tyrosine

portion. The substitution positions of lycorine and tyrosine can be seen in Figure 1.3. DHT-1 should be selective for cancer cells for a number of reasons. It will have the inherent selectivity due to the therapeutic ratio of lycorine, and it will be shuttled into cancer cells at a higher rate than normal cells due to the fact that it will be a substrate for the LAT1. Additionally, it has potential as a therapy for cancers of the CNS. The BBB, which surrounds and protects the CNS, has been shown to highly express LAT1 along the endothelial cells, which make up its border. The brain requires amino acids for protein synthesis just like all cells, and expresses LAT1 along the BBB to ensure hydrophobic amino acids have a way to get into the brain.

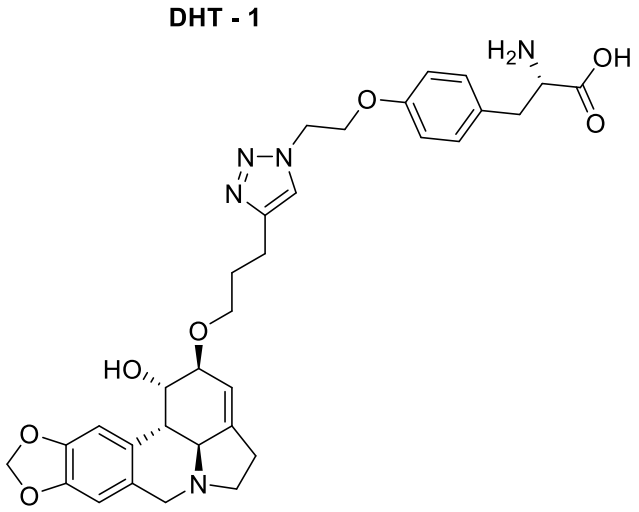


Fig. 1.2 Structure of "DHT-1"

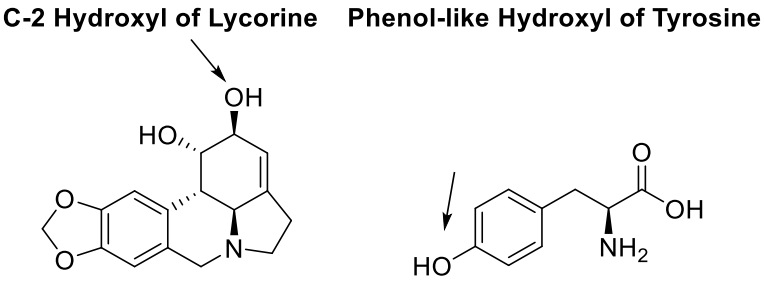
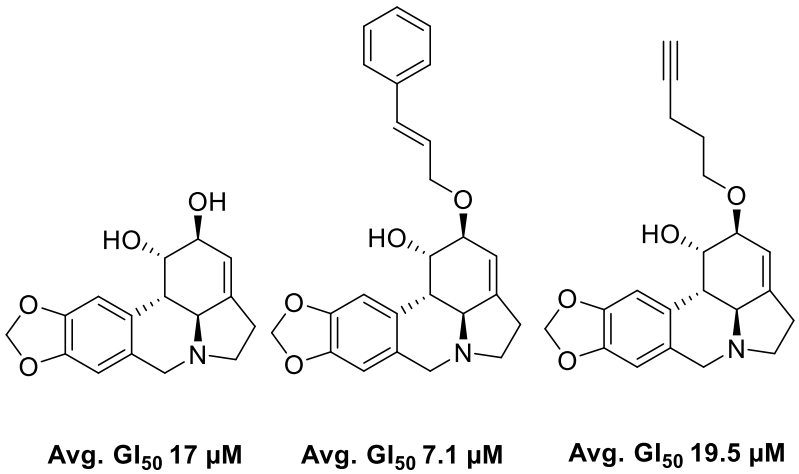


Fig. 1.3 Locations of Substitutions On Tyrosine and Lycorine

Substitution Locations on Tyrosine and Lycorine

The position of the substitution of lycorine and tyrosine were carefully chosen. It has been shown that when lycorine is substituted at the C-2 hydroxyl it retains much of its original potency. [25] Figure 1.4 shows a series of C-2 substituted lycorine analogues which retain lycorine's low μM potency.



Avg. GI_{50} 17 μM **Avg. GI_{50} 7.1 μM** **Avg. GI_{50} 19.5 μM**

Fig. 1.4 C2 Lycorine Derivatives and Their Potencies

Likewise, there are many examples of drugs which are structurally similar to tyrosine, and which are substrates for the LAT1. [26] Looking at various substrates of the LAT1, it is clear that the hydrophobic portion of the molecule can be substituted without

interfering with LAT1's ability to bind and transport. [26-28] Figure 1.5 shows a series of LAT1 substrates which have similar structure to tyrosine.

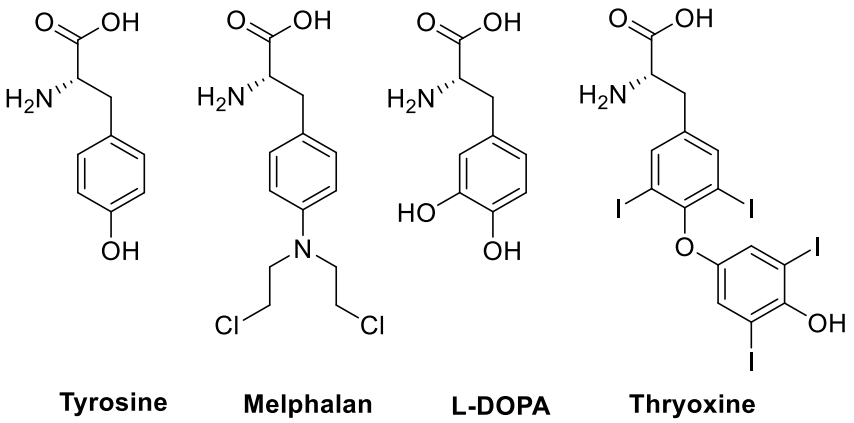


Fig. 1.5 Substrates for LAT1

When experimenters combined ligand-based molecular modeling methods, competitive inhibition studies, and structure activity relationships, they determined relationship between L-Leucine uptake and the uptake of various LAT1 substrates. Figure 1.6 shows the difference in inhibition of L-leucine uptake into the cell by various LAT1 substrates which retained or disrupted the alpha amino acid functionality. They found that molecules which retained the alpha amino acid functionality were consistently better LAT1 substrates than molecules without this functional group organization. [29]

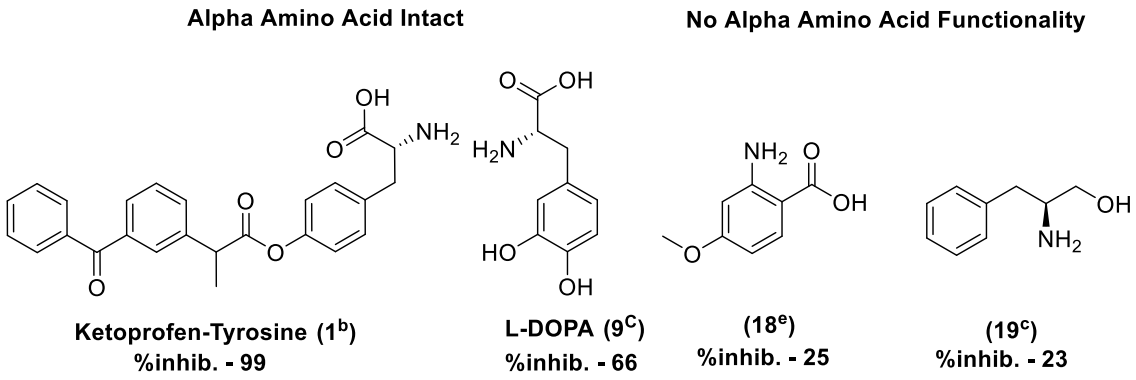
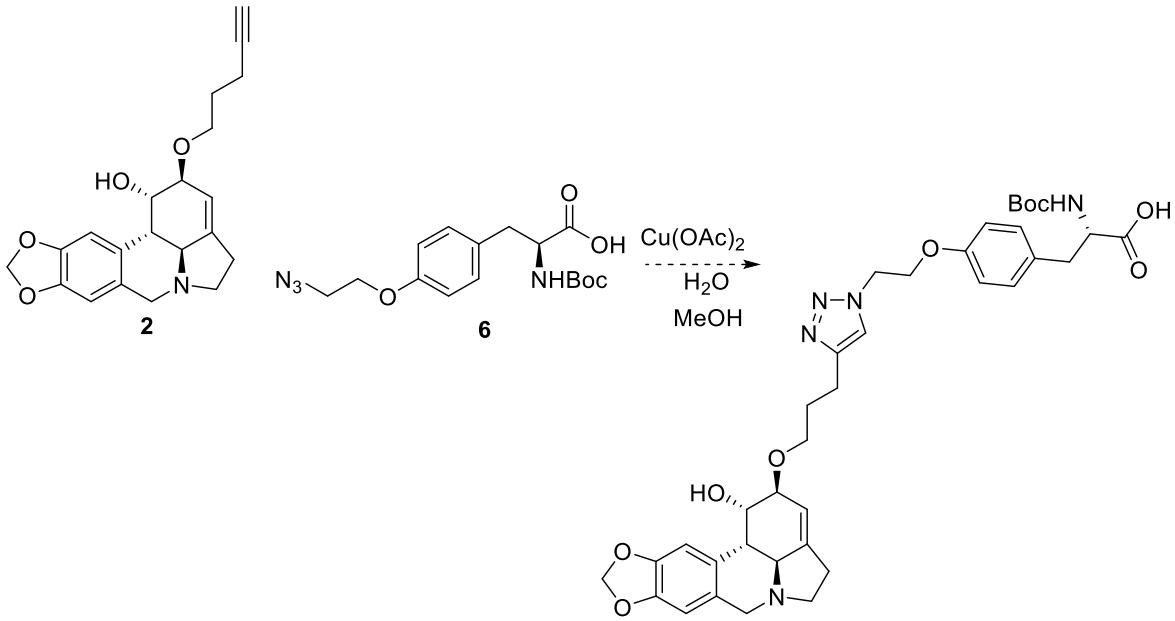


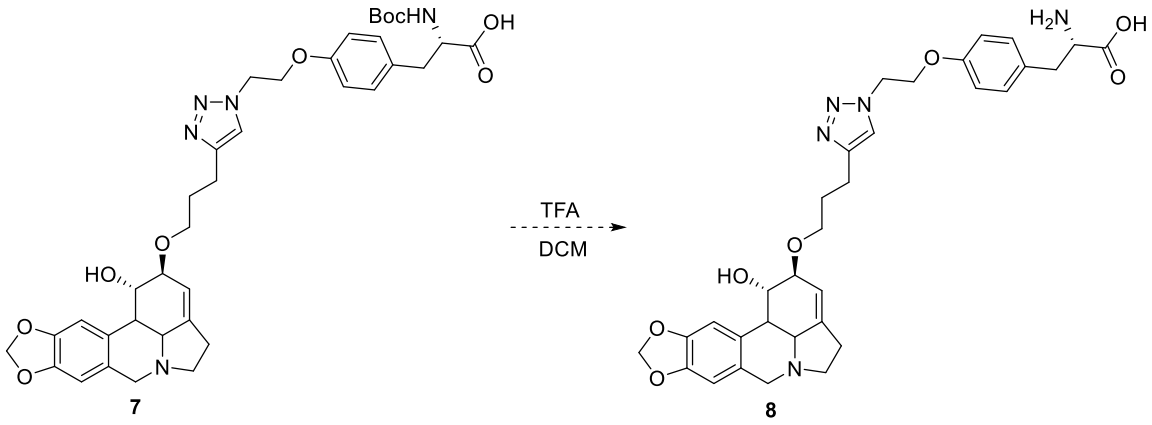
Fig. 1.6 Inhibition of LAT1 uptake of L-Leucine by various substrates

II. SYNTHETIC PLAN

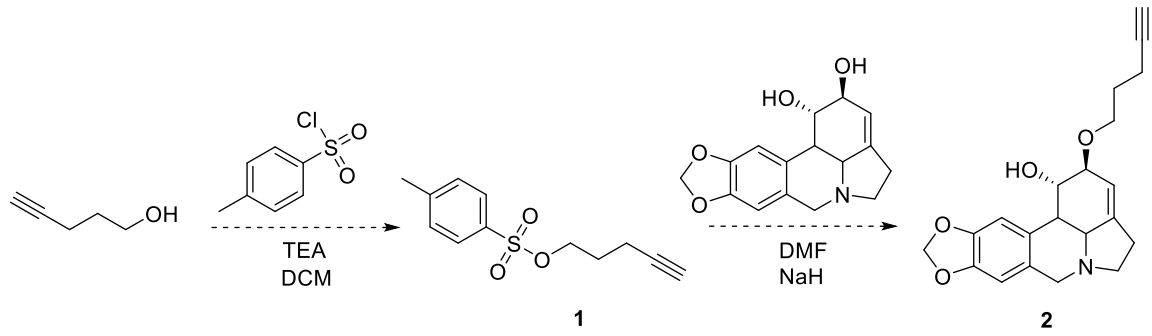
The synthesis of DHT-1 would be carried out by first synthesizing the alkyne **2**, and azide **6** building blocks, and coupling them together via copper catalyzed click reaction to afford compound **8** (Scheme 2.1). After the synthesis of compound **8**, BOC deprotection using trifluoroacetic acid would be performed to yield compound **9**, or DHT-1 (Scheme 2.2). The building blocks themselves would be synthesized first. Tosylation of 4-pentynol would produce **1** (Scheme 2.3). Lycorine would then be treated with compound **1** and through the Williamson Ether Synthesis would afford compound **2**. Next, compound **3** was synthesized by displacing the bromine of 2-bromoethanol with sodium azide. (Scheme 2.4) Compound **3** was treated with *p*-toluenesolfonic acid to afford the tosyl-ester form of the azide, **4**. *N*-(*tert*-Butoxycarbonyl)-L-tyrosine methyl ester was treated with compound **4** and underwent the Williamson Ether synthesis, creating compound **5**. Finally, compound **5** was hydrolyzed using LiOH to afford compound **6**.



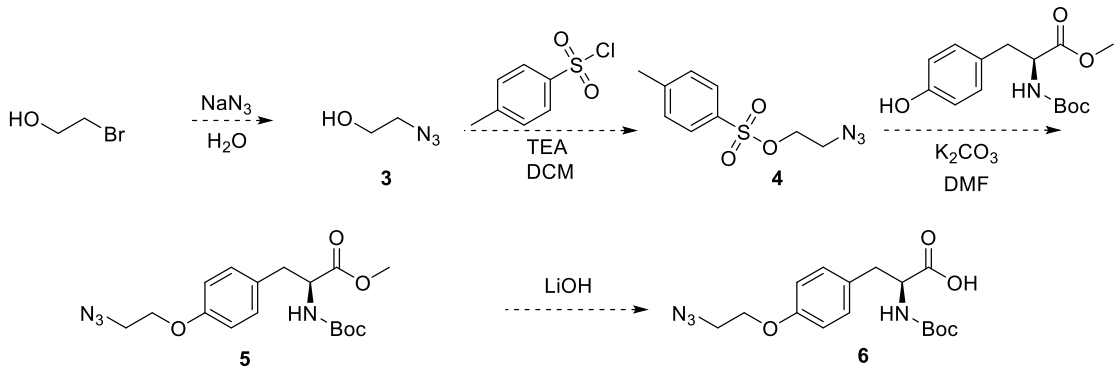
Scheme 2.1 Click Reaction



Scheme 2.2 Boc Deprotection



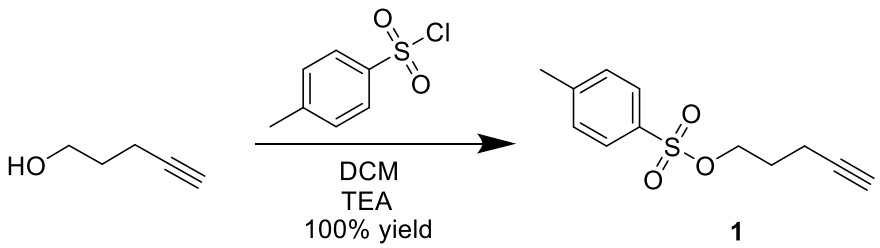
Scheme 2.3 Lycorine Alkyne Synthesis



Scheme 2.4 Tyrosine Azide Synthesis

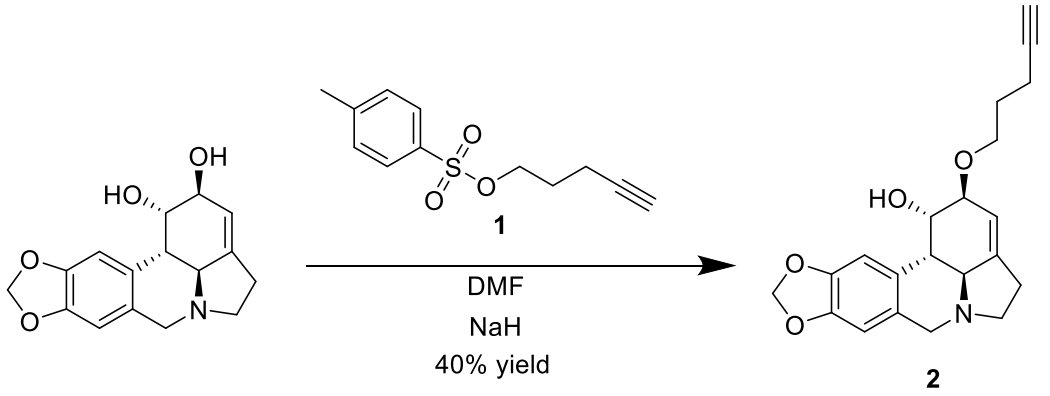
III. RESULTS AND DISCUSSION

Synthesis



Scheme 3.1 Compound 1

Compound 1 (Scheme 3.1) was synthesized via the tosylation of 4-pentynol. This was a simple first reaction to perform. The reaction was carried out according to conditions found in the chemistry literature, using close to 1:1 ratio of 4-pentynol, triethylamine, and *p*-toluenesulfonic acid. Full conversion and 100% yield were both observed each time this reaction was performed and purification was not required.



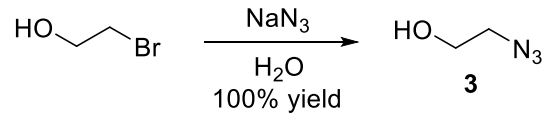
Scheme 3.2 Compound 2

Compound 2 (Scheme 3.2) was synthesized via the Williamson Ether synthesis of lycorine and **1**. This reaction was by far the most difficult in terms of getting a reasonable yield. It was difficult to get this compound in high yield for several reasons. First, even when less than 1 molar equivalent of **1** was used, di-product would form --

both the C-1 and C-2 hydroxyls of lycorine would be substituted with the ether**.** This required incremental additions of **1** to the reaction mixture at room temperature. Initially, half molar equivalents of **1** were added over the course of multiple days but di-product was still observed. Eventually quarter molar equivalents of **1** were added over multiple days. Even when quarter molar equivalents were used, di-product was observed. Eventually, di-product formation was accepted as an inevitability in this reaction and a reduced yield was observed each time.

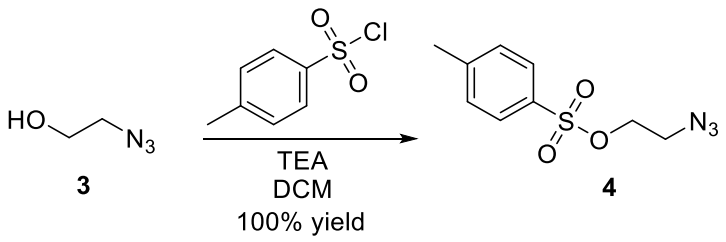
In addition to di-product formation, this reaction was highly sensitive to moisture. Before the reaction was set up, all glassware and septa were carefully washed and allowed to dry in oven over the course of 5 h. After drying in the oven, the flask was quickly capped and flushed with nitrogen. Throughout the course of the reaction nitrogen continued to flush the flask in order to keep conditions in the flask completely dry. Even when these careful steps were taken to ensure the reaction flask was completely dry, full conversion of lycorine was never observed.

Finally, reduced yield was observed due to the difficulty of purifying lycorine compounds. There are multiple hydrogen bond donors and hydrogen bond acceptors on **2** and it was observed that **2,** as well as lycorine containing by-products tend to stick to silica gel during purification using column chromatography. Although careful experimentation was carried out to determine the best solvent system for purification of the mono O-alkyl and di O-alkyl products, it was very difficult to produce pure **2** reliably. More often, it was observed that the mono and di products eluded the column together for at least two to three fractions. The best yield observed was 40%



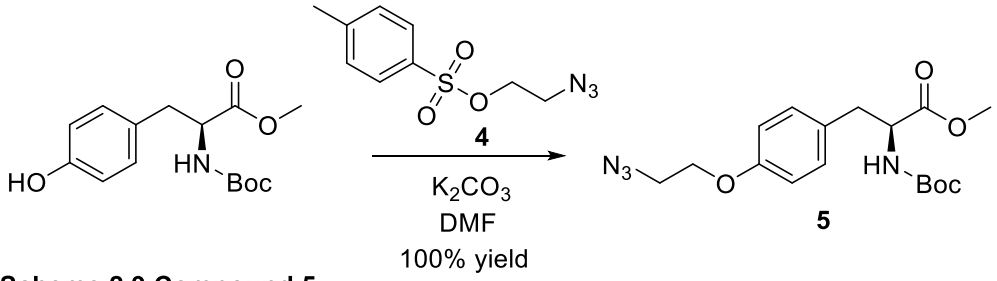
Scheme 3.3 Compound 3

Compound 3 (Scheme 3.3) was synthesized via azidation of 2-bromoethanol with sodium azide. The experimental procedure found in the literature claimed full conversion with 1.5 equivalents of sodium azide at room temperature, [36] but this was not observed. After checking NMR of crude reaction mixture, there was only 50% conversion at 1.5 molar equivalents and room temperature. The reaction was run again using 3 molar equivalents of sodium azide, but full conversion was still not observed. It seemed that sodium azide was not completely dissolving in H_2O at room temperature so the decision was made to increase the temperature. The third time the reaction was run, it was placed in an oil bath and brought to a temperature of 70 °C. This time the NMR of crude reaction mixture showed full conversion to compound **3** when using increased temperature and 3 molar equivalents of sodium azide**.**



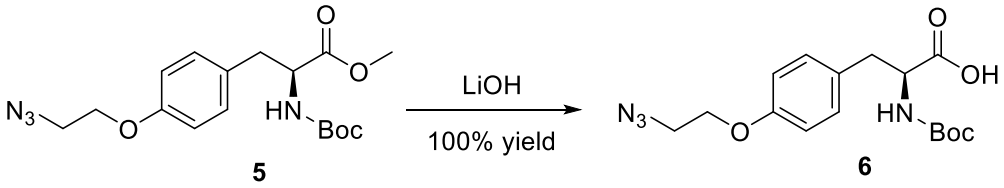
Scheme 3.4 Compound 4

Compound 4 (Scheme 3.4) was synthesized using the same conditions as the tosylation of compound **1**. Full conversion and 100% yield was observed on the first attempt performing this reaction with no purification required.



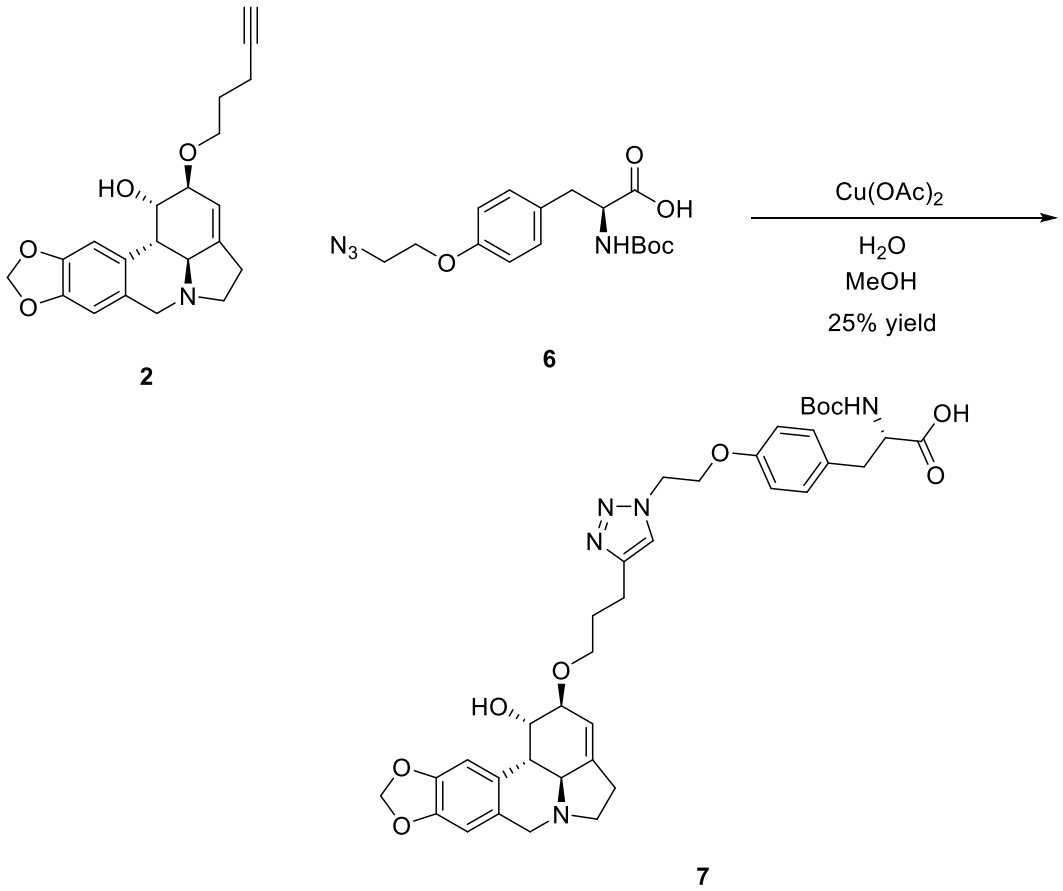
Scheme 2.9 Compound 5

Compound 5 (Scheme 3.5) was synthesized via Williamson Ether synthesis. Although some experimentation was required to achieve full conversion of *N*-(*tert*-butoxycarbonyl)-L-tyrosinemethyl ester, it was quite simple when compared to the *O*-alkylation of lycorine. There is only one reactive hydroxyl on *N*-(*tert*-butoxycarbonyl)-L-tyrosinemethyl ester, and all that was required to achieve full conversion was to increase the molar equivalents of **4.** It was observed that when three molar equivalents of **4** were used, full conversion and 100% yield was achieved.



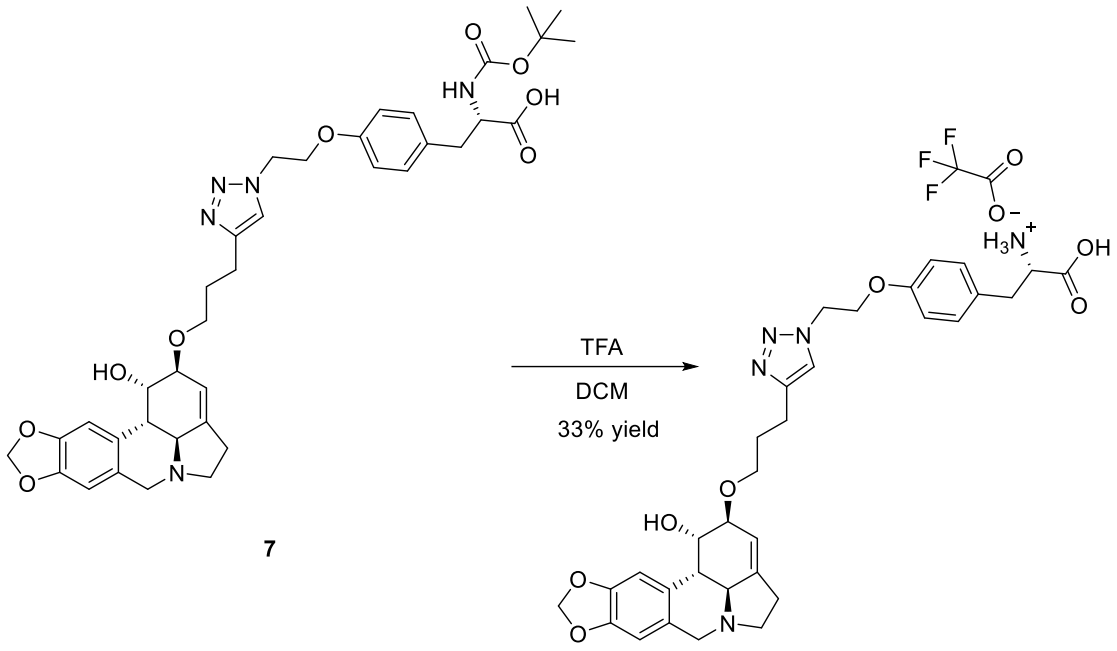
Scheme 3.6 Compound 6

Compound 6 (Scheme 3.6) was observed after the hydrolysis of **5**. The experimental procedure found in the literature worked well on the first attempt and full conversion was observed.



Scheme 3.7 Compound 7

Compound 7 (Scheme 3.7) was synthesized via Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) between **2** and **6**. Although there are multiple reaction conditions possible for this reaction, $Cu(OAc)_2$ in H_2O and MeOH was chosen due to the ease of set up and comparable yields to more complicated reaction conditions. The first time this reaction was run, 25% yield was observed. Although solvent ratios and the amount of copper catalyst were varied over the course of four attempts, a greater yield was not observed.



7

8

Scheme 3.8 Compound 8

Compound 8 (Scheme 3.8) was obtained after BOC deprotection of **7.** BOC de-protection is a highly exothermic and irreversible process initiated when the carbonyl oxygen on the BOC protecting group is protonated by a suitable acid. In this case, trifluoroacetic acid was used. Protonation via TFA causes the group to fall apart irreversibly. First *tert*-butyl cation is lost which leaves carbamic acid still attached to the parent molecule and producing isobutylene. After decarboxylation, the free amino acid is left. The free amino acid is protonated and forms the TFA salt. [34] Both isobutylene and CO can be removed by heat and vacuum, while the free TFA can be removed via lyophilization. In theory no purification should be required after the de-protection of the BOC group. In practice, the NMR of the crude product contained many impurities, so purification was required via TLC.

Biological Evaluation

The method for testing DHT-1 is the MTT calorimetric assay. This assay works by first exposing the live cancer cells to the compound 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT, as well as the drug to be tested. When a cell is living and performing metabolic processes, MTT is reduced by the mitochondria to (*E,Z*)-5-(4,5-dimethylthazol-2-yl)-1,3-diphenylformazan, or formazan. [30] MTT is a pale yellow, while Formazan is a bright purple. [31] Thus, if the cell becomes purple after exposure to MTT and the drug in question, the mitochondria are still functioning in the cell. If the cells stay a pale yellow, then mitochondrial function has ceased. IC_{50} can be determined by noting the concentration required to achieve a 50% reduction in proliferation of cells after a predetermined amount of time.

DHT-1 was tested for selectivity against cancers which overexpress LAT1 by comparing its activity against cell lines which do not overexpress LAT1. It has been shown that U87 glioblastoma, [31][32] and HeLa cervical adenocarcinoma both overexpress LAT1. [33] MCF7 breast cancer has not been shown to over express LAT1 but is a tumorgenic cancer cell line. DHT-1 was tested for activity against the former three cell lines as well as MCF10A and ARPE cells. MCF10A is a human breast epithelial cell line, while ARPE is a human retinal pigment epithelial cell line. These cell lines will serve as controls for selectivity due to the fact that they are non-tumorgenic and because they do not over express LAT1. As previously mentioned, Lycorine has a natural therapeutic ratio of 15:1 against cancer cells. If the ratio is found to be much higher than 15:1 after testing DHT-1, then it could be indicative of LAT1-related uptake into cancer cells.

Table 1 IC_{50} of DHT-1 vs Lycorine Against Various Cell Lines

Compound	Date	Cell Line	IC_{50} (µM)
DHT-1	102816	MCF7	198.55
Lyc	102816	MCF7	16.52
DHT-1	102816	MCF10A	217.14
Lyc	102816	MCF10A	16.94

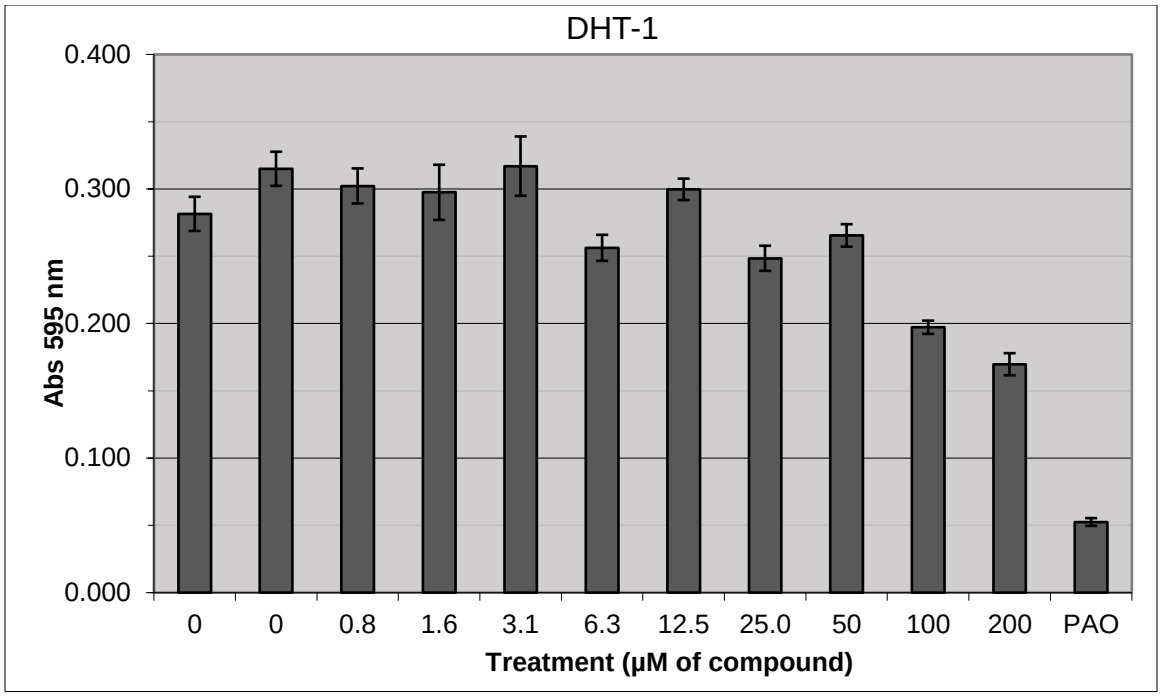


Fig. 1.7 Dose dependent growth inhibition upon the MCF-7 cell treatment with DHT-1.

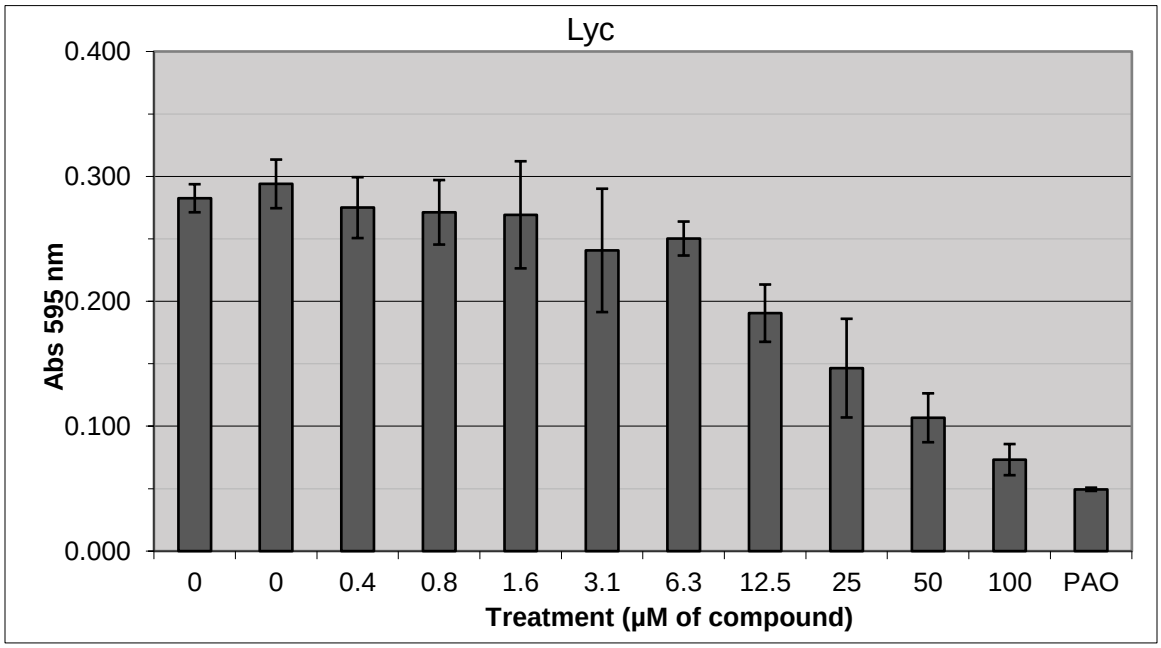


Fig. 1.8 Dose dependent growth inhibition upon the MCF-7 cell treatment with lycorine.

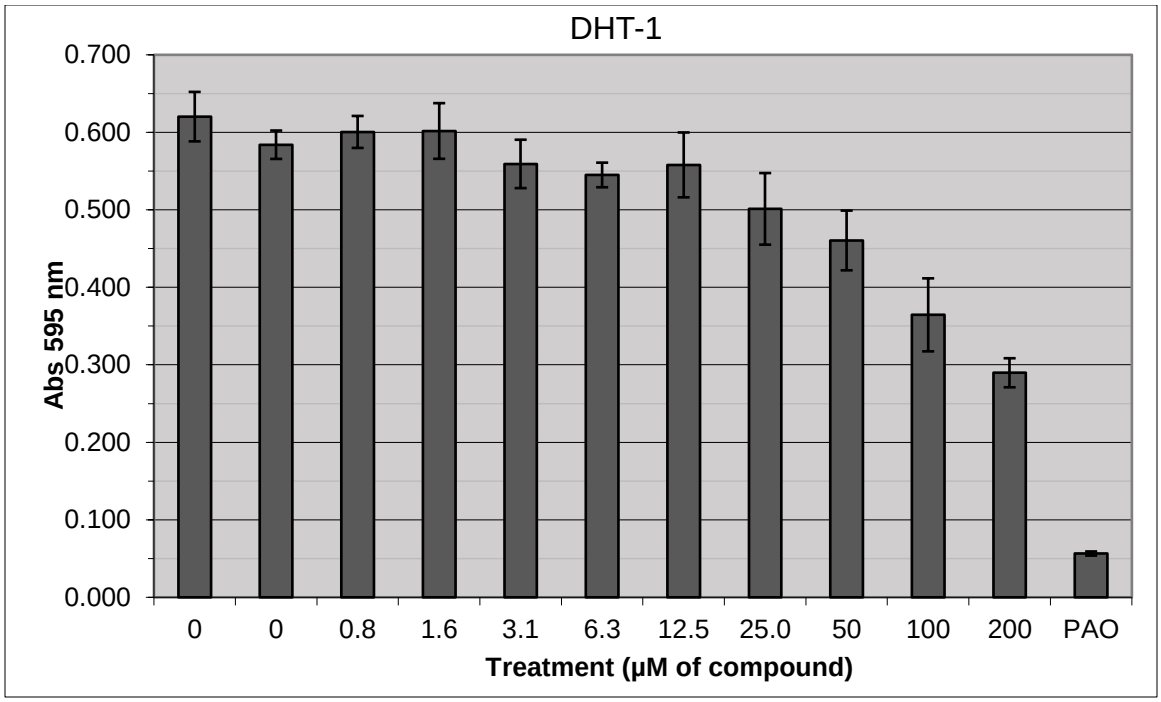


Fig. 1.9 Dose dependent growth inhibition upon the MCF-10A cell treatment with DHT-1

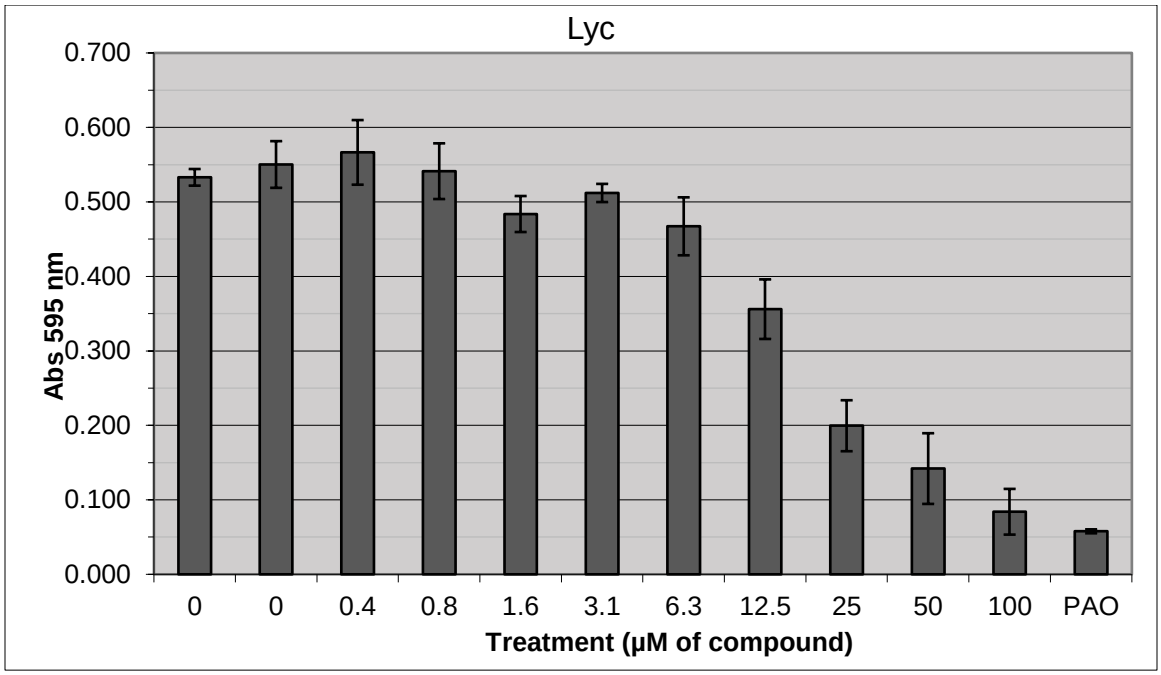


Fig. 1.10 Dose dependent growth inhibition upon the MCF-10A cell treatment with lycorine

Shown above is the testing data of DHT-1 vs Lycorine on MCF7 and MCF10A cell lines. DHT-1 is not selective for either of these cells lines, with an IC_{50} over 10 times higher than lycorine . To be finished when the complete biological data are received.

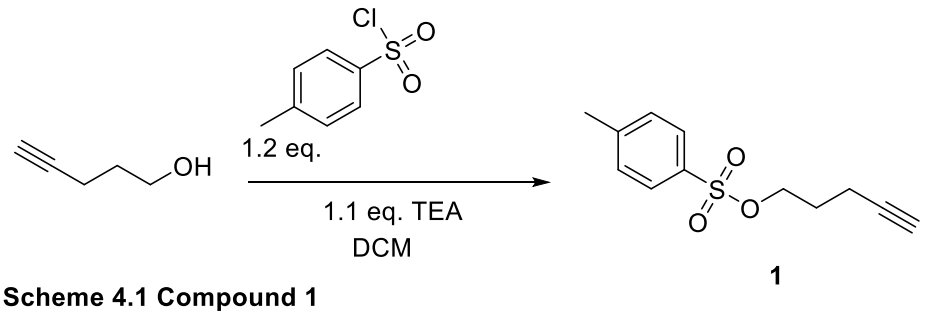
IV. CONCLUSIONS

Based on the fact that aggressive cancer cells overexpress LAT1 it was decided to synthesize a cancer agent, which would take advantage of this fact. We hypothesized and successfully synthesized a compound which was a conjugate of the natural product, lycorine, with the amino acid, tyrosine. This is the first time a non-apoptotic cancer drug has been conjugated to an amino acid in an attempt to explore LAT1-based targeting of cancer cells. The synthesized conjugate was tested against various cancer cell lines, which varied in their expression of LAT1 in order to determine the selectivity and anti-cancer activity against the cells. Unfortunately, the results of the testing showed that we did not achieve selectivity with DHT-1. It is possible that the molecule is not a substrate for LAT1 in which case its polarity would likely prevent it from entering into the cell at all. It is also possible that the amino acid portion of DHT-1 prevented the entire molecule from binding the peptidyl tranferase center in the ribosome and thus, losing its ability to disrupt protein synthesis. In any case, there is more work to be done with lycorine – tyrosine conjugates. In the future, more analogues could be created varying different parameters of the molecule, from distance of the bridging portion of the molecule, to creating a cleavable molecule which would allow Lycorine to break away from the amino acid portion of the molecule and thus, retain its original potency while still taking advantage of the LAT1.

V. EXPERIMENTAL

All reagents, solvents, and catalysts were purchased from commercial sources and used without purification. All reactions were performed in oven-dried flasks open to the atmosphere or under nitrogen and monitored by thin layer chromatography (TLC) on TLC precoated (250 μm) silica gel XHL glass-backed plates (Sorbent Technologies.). Visualization was accomplished with UV light. Flash column chromatography was performed on silica gel (32−63 μm, 60 Å pore size). ^{1}H NMR spectra were recorded on a Bruker 400 spectrometer. Chemical shifts (δ) are reported in ppm relative to the TMS internal standard. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet).

Compound 1



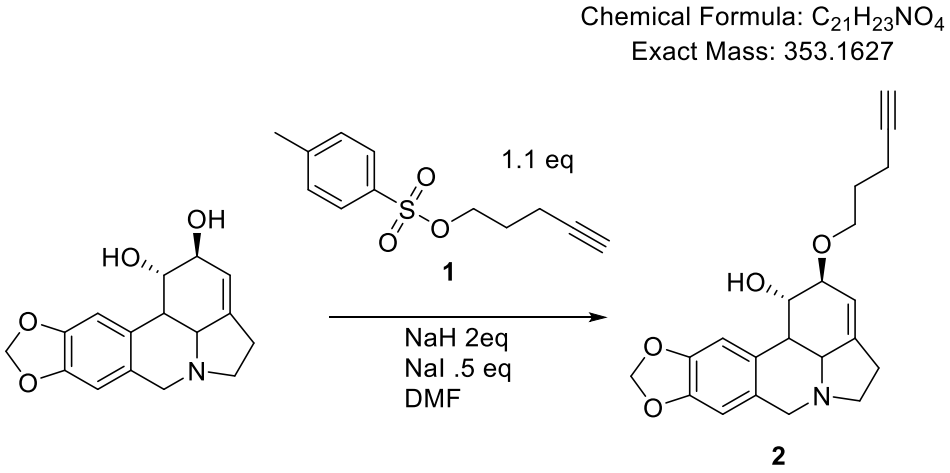
Scheme 4.1 Compound 1

One gram of 4-pentynol (11.8 mmol) was added to 3.5 mL DCM in a 25 mL round bottom flask submerged in 0 °C ice bath with stirring. Next, 2.14 mL (15.3 mmol) of triethylamine was added dropwise followed by 3.4 g (11.7 mmol) of 4-toluenesulfonyl chloride. Reaction was left to stir for 2 h and TLC showed full conversion of the starting material to **1**. Aqueous work up was followed by organic extraction repeated three times.

The organic solvent chosen for extraction was DCM. The organic phase was dried over Na_2SO_4, evaporated down and purified using column chromatography. 100% yield was achieved. ^{1}H NMR data was consistent with the literature data [33a]. Specifically;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (dt, *J* = 3.5, 1.1 Hz, 1H), 7.36 – 7.31 (m, 1H), 4.13 (q, *J* = 6.0 Hz, 1H), 2.47 – 2.39 (m, 2H), 2.26 – 2.20 (m, 1H), 1.89 – 1.79 (m, 2H).

Compound 2



Scheme 4.2 Compound 2

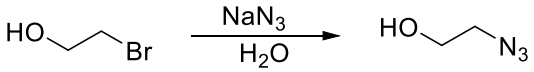
Lycorine (50 mg, 0.17 mmol) was added to 2 mL DMF in a 10 mL round bottom flask and allowed to fully dissolve with stirring at room temperature. To this solution was added NaH (13 mg, 0.18 mmol), NaI (13 mg, 8.5 mmol), and **1** (62 mg, 0.20 mmol). The reaction was allowed to stir over the following two days at room temperature. TLC showed minimum conversion of Lycorine to the di-substituted product (R_f=0.55 in 10% hexanes/ethyl acetate) and mostly formation of **2** (R_f=0.35 in 10% hexanes/ethyl acetate). Aqueous work up was followed by organic extraction repeated three times. The organic solvent chosen for extraction was ethyl acetate. The organic phase was dried over Na_2SO_4

and evaporated down and further purified via column chromatography. 40% yield was achieved.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.65 (s, 1H), 5.94 (d, *J* = 5.5 Hz, 2H), 5.61 (s, 1H), 4.66 (d, *J* = 8.9 Hz, 1H), 3.96 (s, 1H), 3.82 – 3.67 (m, 3H), 2.71 (d, *J* = 10.5 Hz, 3H), 2.33 – 2.23 (m, 2H), 1.98 (t, *J* = 2.6 Hz, 1H), 1.84 – 1.70 (m, 3H), 1.25 (t, *J* = 7.1 Hz, 4H). MS M/Z for $C_{21}H_{23}NO_4$ (M+H) calc. 353.12 found 353.1

Amino Acid – Azide synthesis

Compound 3

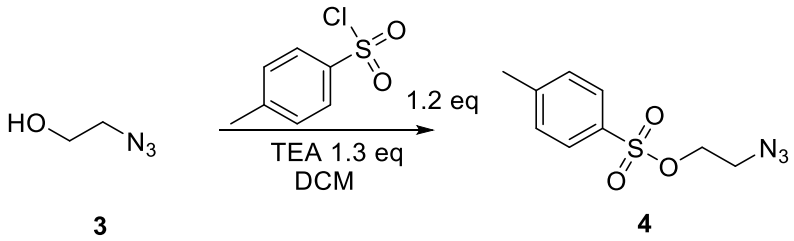


3

Scheme 4.3 Compound 3

2 g of **5** (16 mmol) was added to 15 mL H_2O followed by 6.24 g of NaN_3 (96 mmol) and left to stir with 70 °C heating for 4 h. TLC showed full conversion of the starting material to **6**. Aqueous work up was followed by organic extraction repeated three times. The organic solvent chosen for extraction was diethyl ether. The organic phase was dried over Na_2SO_4, and evaporated down. Further purification was not required. 100% yield was achieved. ^{1}H NMR data was consistent with the literature data [34]. Specifically; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 – 3.74 (m, 1H), 3.45 – 3.40 (m, 1H), 2.82 (s, 0H).

Compound 4

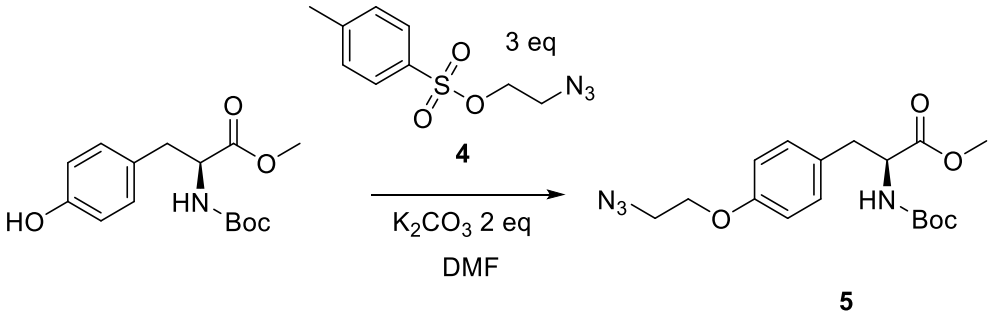


Scheme 4.4 Compound 4

1.88 g of **3** (21.6 mmol) was added to 3.5 mL DCM in a 25 mL round bottom flask submerged in 0 °C ice bath with stirring. Next 3.81 mL (28 mmol) of triethylamine was added dropwise followed by 5.76 g (25.9 mmol) of 4-toluenesulfonyl chloride. Reaction was left to stir for 2 h and TLC showed full conversion of the starting material to **4**. Aqueous work up was followed by organic extraction repeated three times. The organic solvent chosen for extraction was DCM. The organic phase was dried over Na_2SO_4, evaporated down and purified using column chromatography. 100% yield was achieved. ^{1}H NMR data was consistent with the literature data [35]. Specifically;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, 1H), 7.38 – 7.34 (m, 1H), 4.18 – 4.14 (m, 1H), 3.50 – 3.46 (m, 1H), 2.45 (t, *J* = 2.1 Hz, 2H).

Compound 5

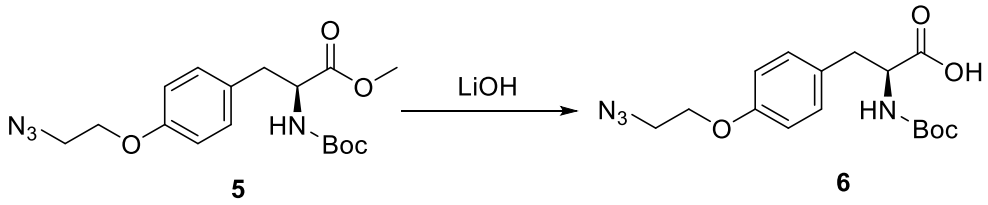


Scheme 4.5 Compound 5

100 mg (0.33 mmol) of *N*-(*tert*-Butoxycarbonyl)-L-tyrosinemethyl ester was dissolved in 2 mL DMF in a 10 mL round bottom flask with stirring and placed in 0 °C ice bath. To this solution was added K_2CO_3 (93 mg, 0.676 mmol) and **4** (81 mg, 0.4 mmol). Reaction was left to stir overnight and TLC showed full conversion to **5** the following morning. Aqueous work up was followed by organic extraction repeated three times. The organic solvent chosen for extraction was chloroform. Organic layer was evaporated and dried over Na_2SO_4. Crude mixture was purified via column chromatography. 100% yield was achieved. ^{1}H NMR data was consistent with the literature data [36]. Specifically;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.87 – 6.67 (m, 1H), 6.86 – 6.76 (m, 1H), 4.97 (s, 0H), 4.52 (s, 0H), 4.15 – 4.04 (m, 1H), 3.55 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.01 (dt, *J* = 13.8, 8.1 Hz, 1H), 1.43 (d, *J* = 22.4 Hz, 5H).

Compound 6



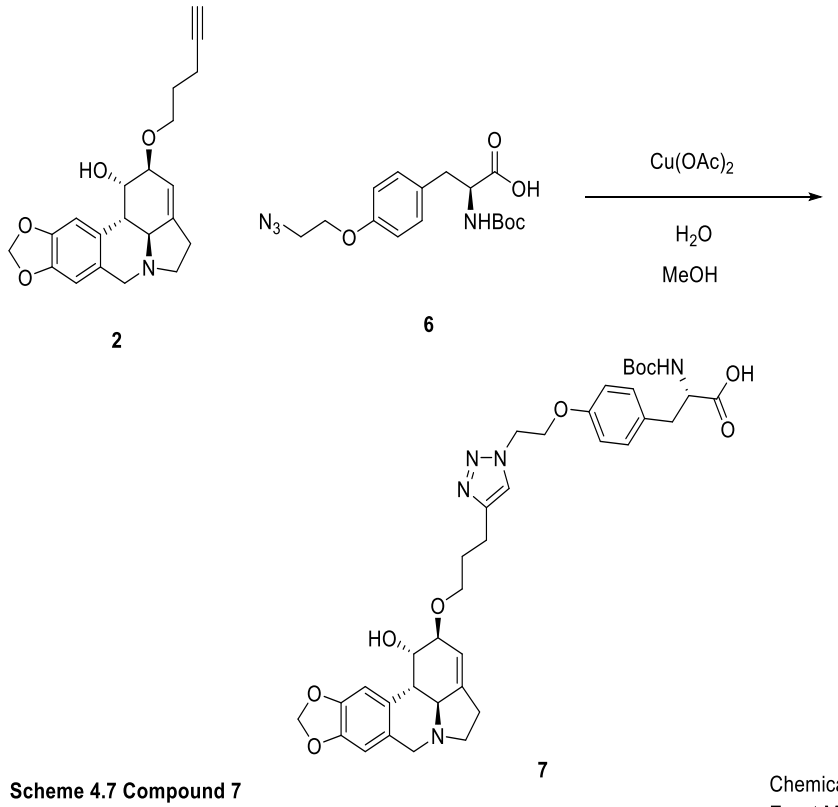
5 **6**

Scheme 4.6 Compound 6

1 g (2.6 mmol) of **5** was dissolved into 3.5 mL THF in a 25 mL round bottom flask. To this solution was added 14 mL 1M LiOH and left to stir for 2 h. TLC showed full conversion to **6.** The reaction was neutralized using 14 mL 1M HCl. Aqueous work up was followed by organic extraction repeated three times. The organic solvent chosen for extraction was ethyl acetate. The organic fraction was evaporated down and dried over Na_2SO_4. Further purification was not required. 100% yield was achieved. ^{1}H NMR data was consistent with the literature data [36]. Specifically: ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.10 (t, *J* = 9.5 Hz, 1H), 6.89 – 6.80 (m, 1H), 4.97 (d, *J* = 7.4 Hz, 0H), 4.56 (d, *J* = 5.2 Hz, 0H), 4.11 (dd, *J* = 10.3, 5.1 Hz, 1H), 3.59 – 3.55 (m, 1H), 3.13 (dd, *J* = 13.7, 5.1 Hz, 0H), 3.03 (dd, *J* = 13.7, 5.7 Hz, 0H), 1.46 – 1.31 (m, 5H).

Compound 7



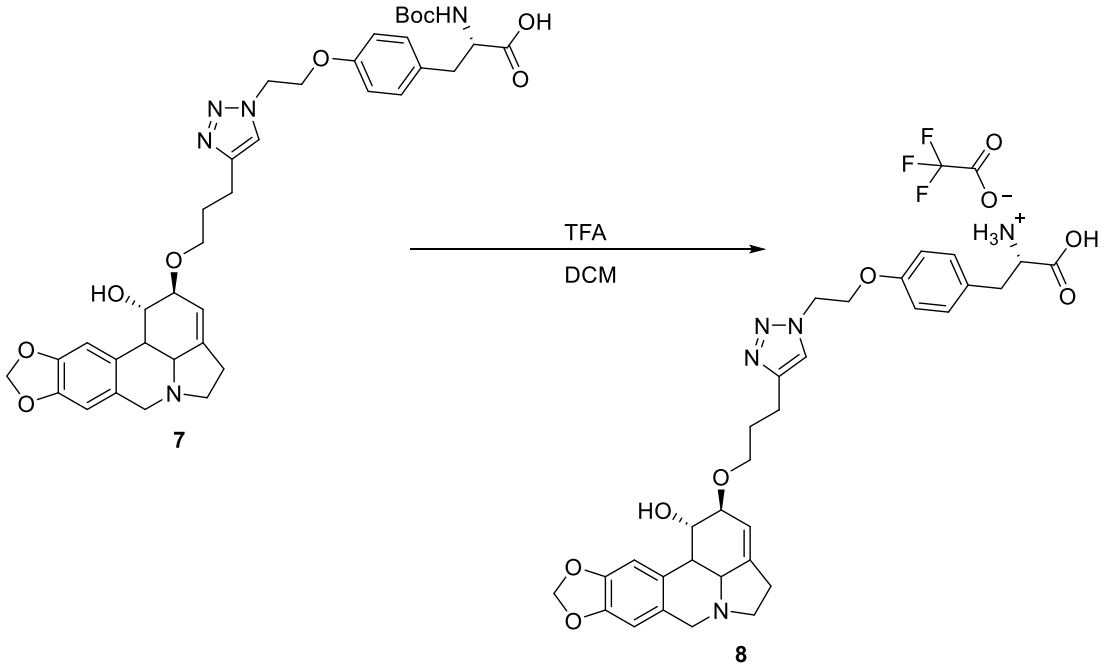
Scheme 4.7 Compound 7

Chemical Formula: $C_{37}H_{45}N_5O_9$
Exact Mass: 703.3217

2 (10 mg, 0.02 mmol) and **6** (10 mg, 0.02 mmol) were placed into a 10 mL round bottom flask and dissolved in a 1:4 mixture of H_2O:methanol with stirring. Aluminum foil was wrapped around flask to keep reaction conditions completely dark. Copper acetate (20 mg, 0.11 mmol) was added to the flask and left to stir overnight. TLC showed significant conversion to **7** and the reaction was purified directly via preparatory TLC. No work up was required. 25% yield was achieved. ^{1}H NMR (400 MHz, DMSO) δ 7.90 (s, 1H), 7.10 (d, *J* = 8.5 Hz, 2H), 6.92 (s, 1H), 6.80 (d, *J* = 8.6 Hz, 2H), 6.67 (s, 1H), 5.94 (s, 2H), 5.43

(s, 1H), 4.67 (t, *J* = 5.2 Hz, 2H), 4.45 (s, 1H), 4.32 (t, *J* = 5.2 Hz, 2H), 2.66 (dd, *J* = 14.3, 6.8 Hz, 3H), 2.51 – 2.47 (m, 9H), 2.51 – 2.47 (m, 10H), 1.86 – 1.77 (m, 2H), 1.31 (s, 8H). MS M/Z $C_{37}H_{45}N_5O_9$ (M+H) calc. 703.31 found 703.2

Compound 8

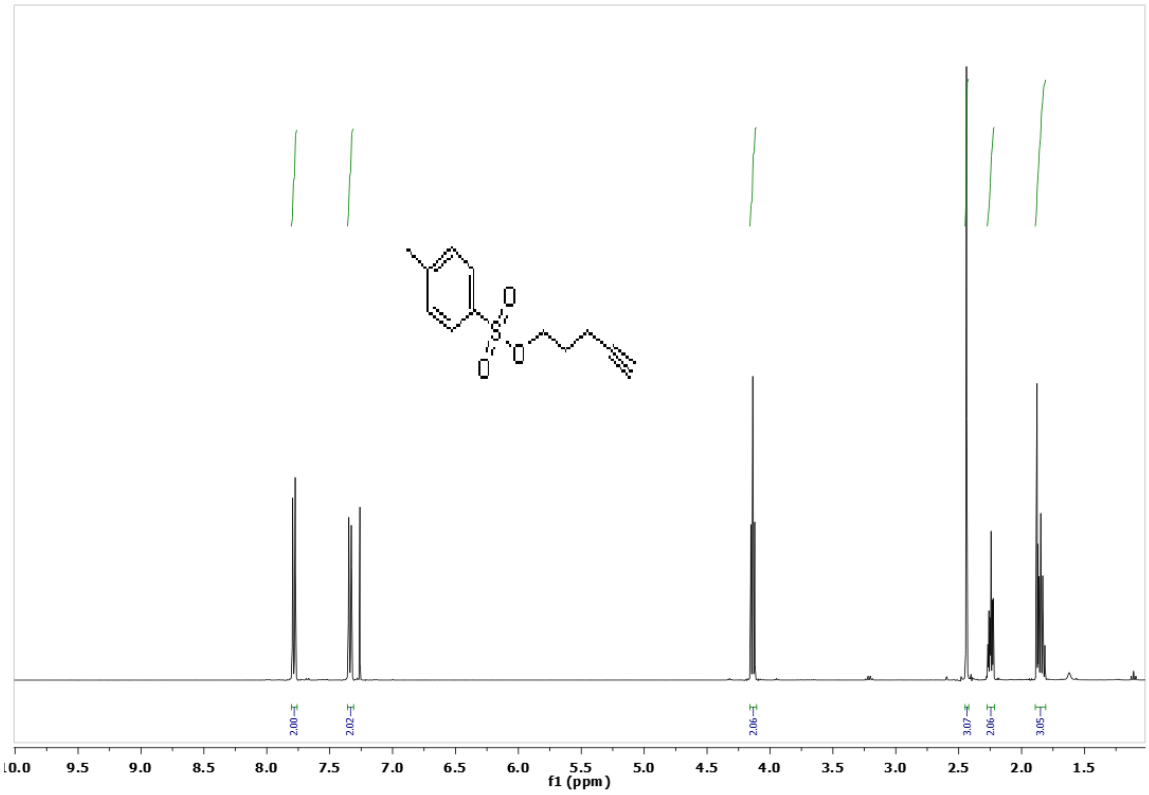


Scheme 4.8 Compound 8

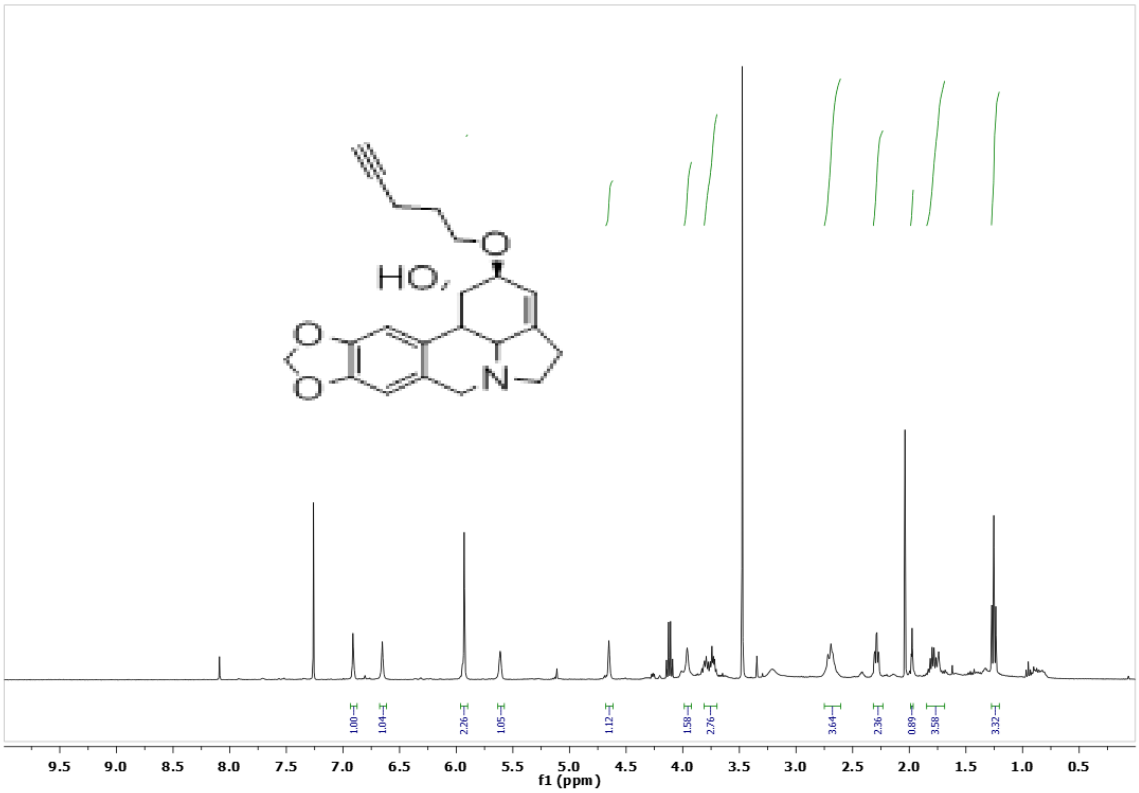
7 (6 mg, 0.08 mmol) was placed into a 5 mL round bottom flask and dissolved into 1.5 mL 1:1 mixture of DCM:TFA. Reaction was left to stir for 4 h. TLC showed full de-protection to **8**. DCM and TFA were evaporated off using rotovap and high vacuum. The reaction was purified via preparatory TLC. 33% yield was achieved. ^{1}H NMR (400 MHz, D_2O) δ 7.89 (s, 1H), 7.22 (d, *J* = 8.7 Hz, 2H), 6.96 – 6.87 (m, 4H), 6.05 (dd, *J* = 8.8, 1.0 Hz, 3H), 5.78 (s, 1H). MS M/Z for $C_{32}H_{38}N_5O_7$ (M+H) calc. 604.27 found 604.1

APPENDIX SECTION

Compound 1



Compound 2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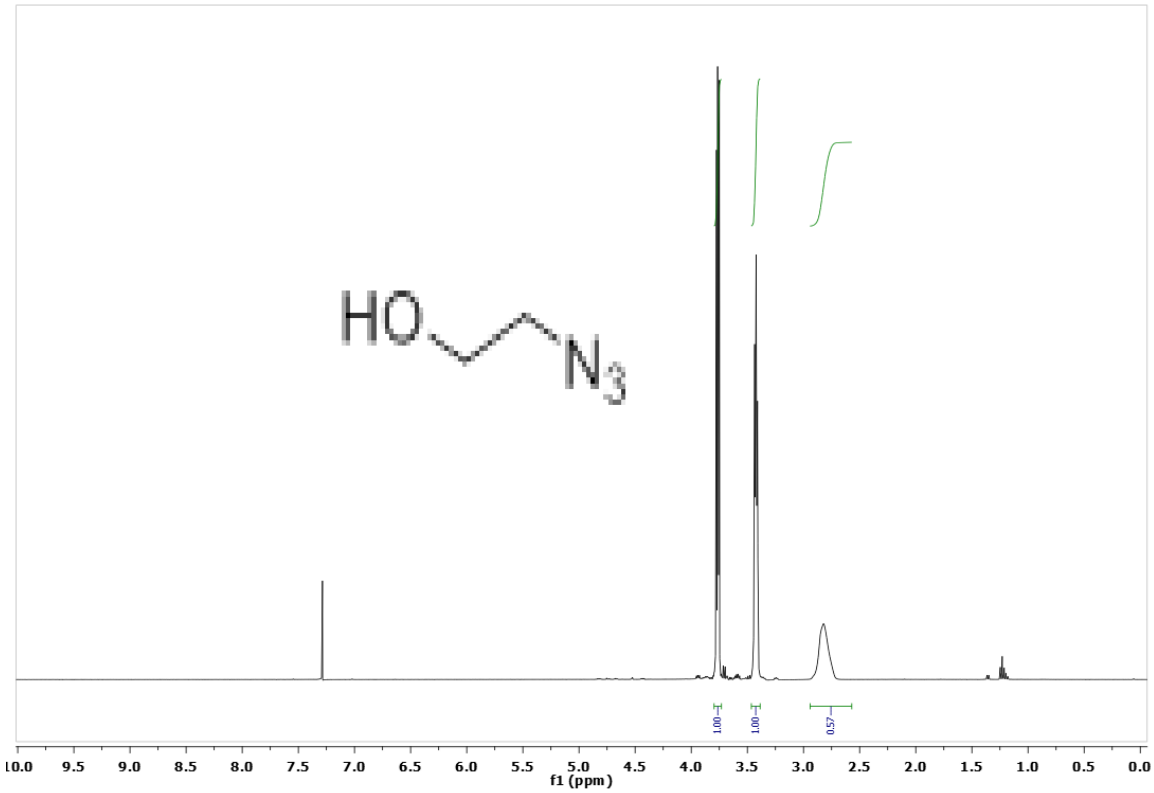
O
O
O
N
1.00
1.04
2.26
1.05
1.12
1.58
2.76
3.64
2.36
0.89
3.58
3.32
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

Compound 3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N3
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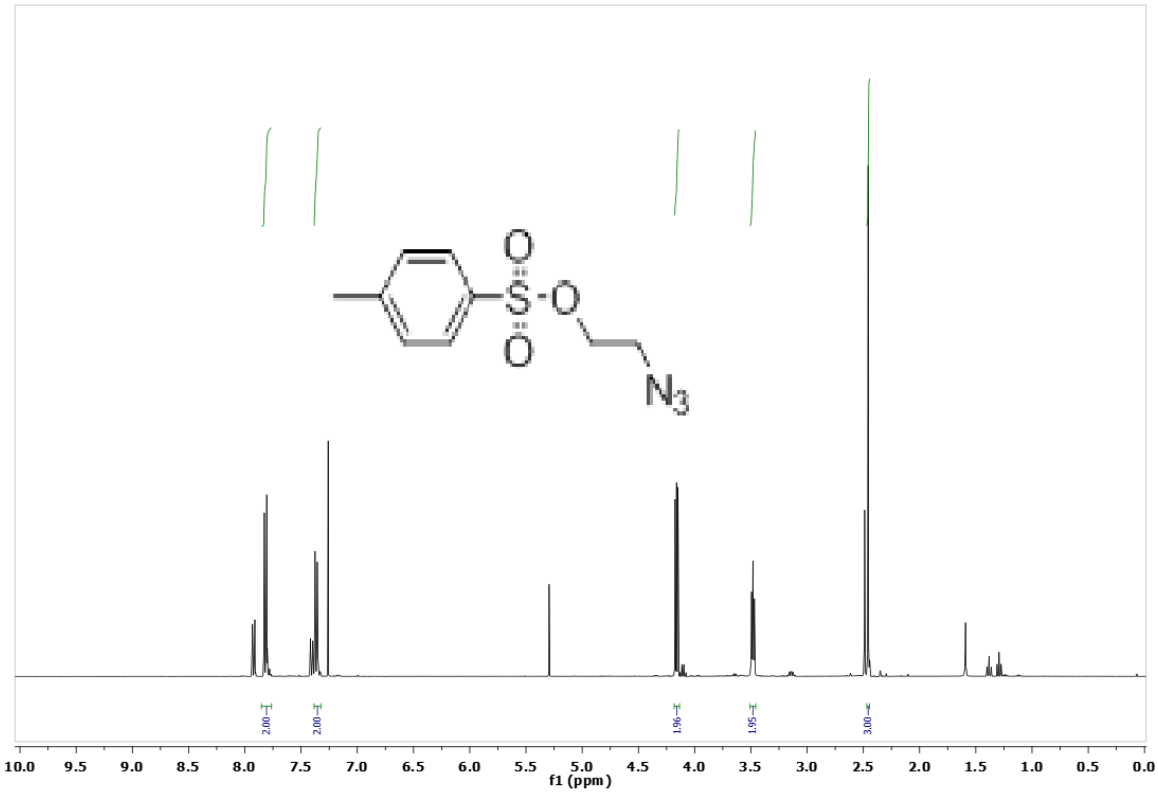
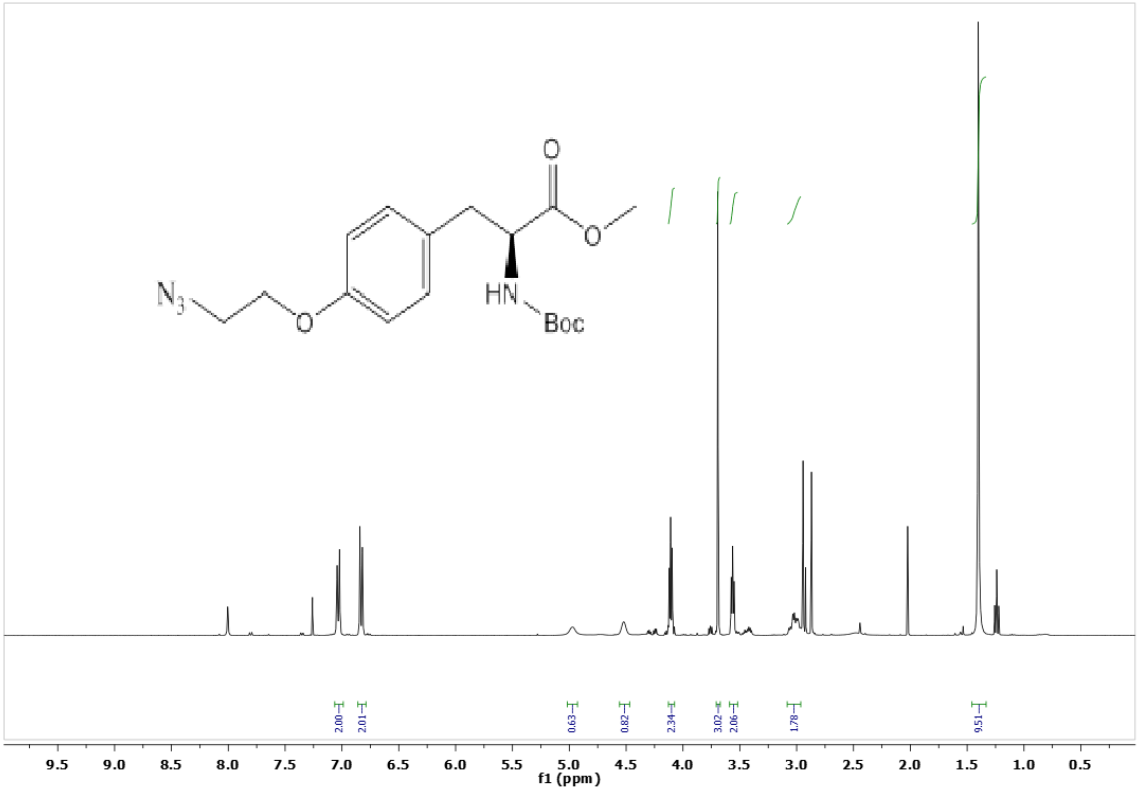
1.00
0.57
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

Compound 4


O
S
O
O
N3
2.00
2.00
1.96
1.95
3.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

Compound 5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O
HN
Boc
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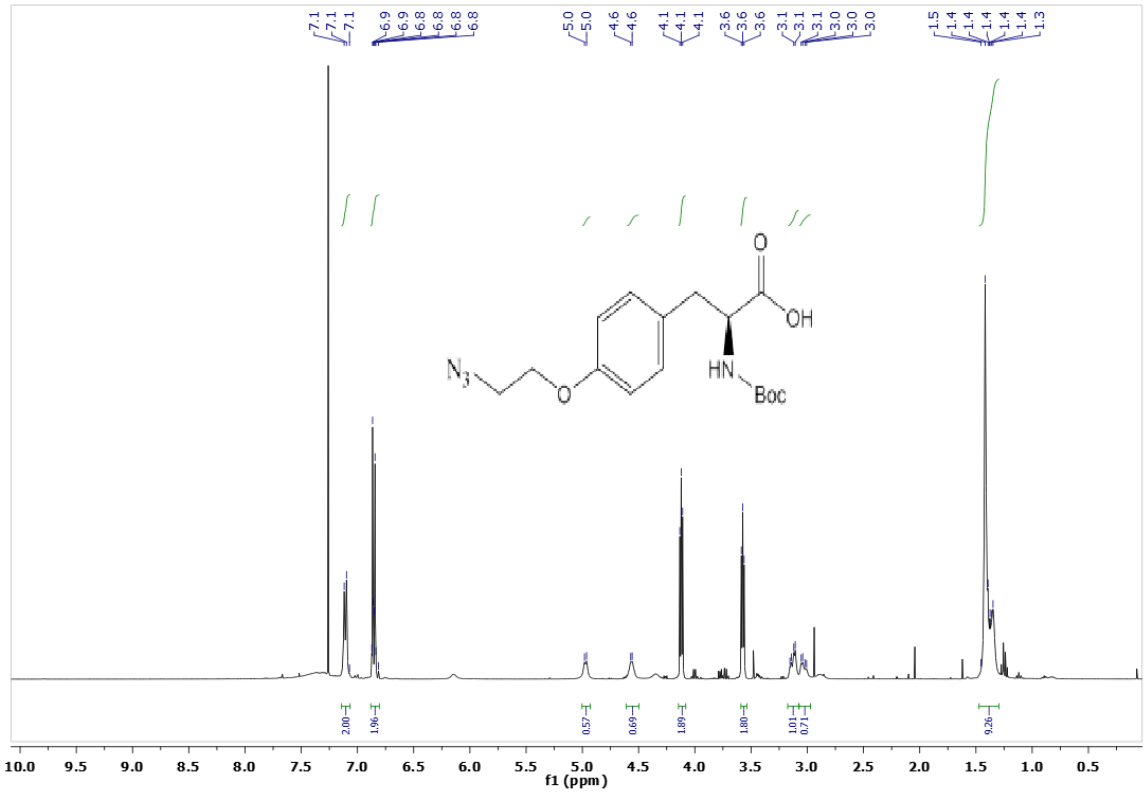
2.01
0.63
0.82
2.34
3.02
2.06
1.78
9.51
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

Compound 6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7.1
7.1
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6.9
6.8
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3.6
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1.4
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1.4
1.4
1.4
1.3
O
OH
HN
Boc
O
N3
2.00
1.96
0.57
0.69
1.89
1.80
1.01
0.71
9.26
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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3.5
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2.5
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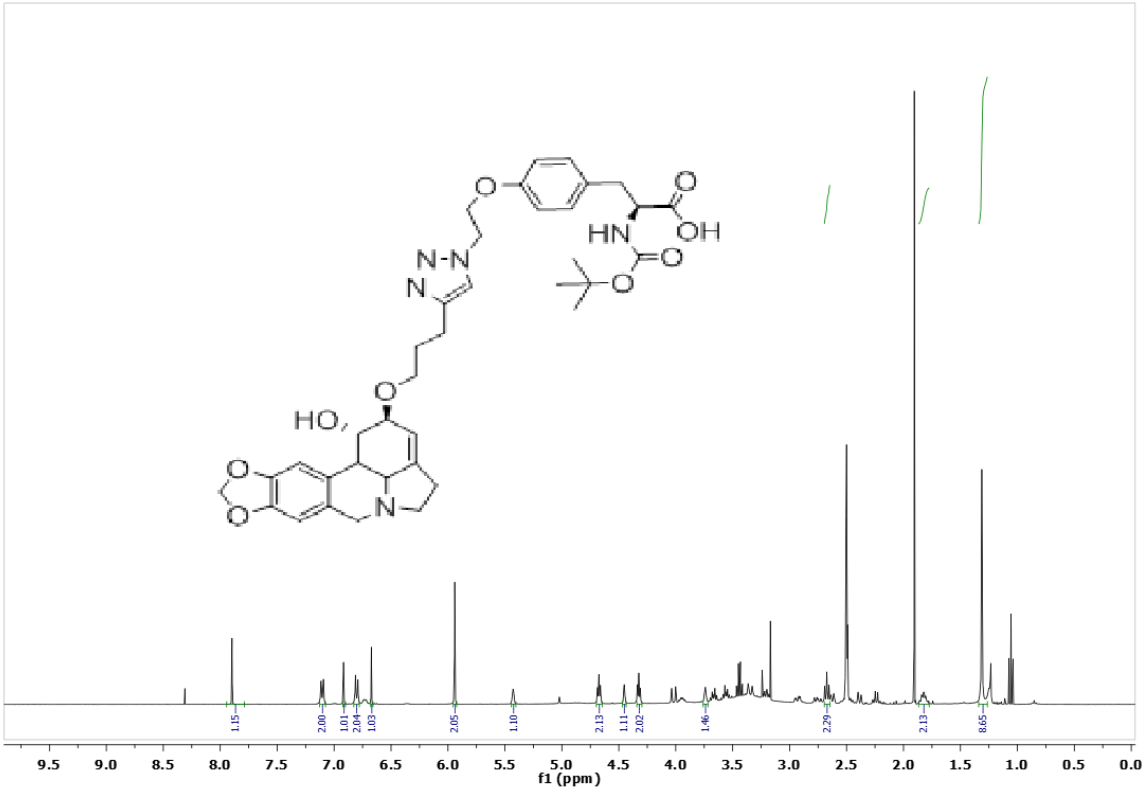
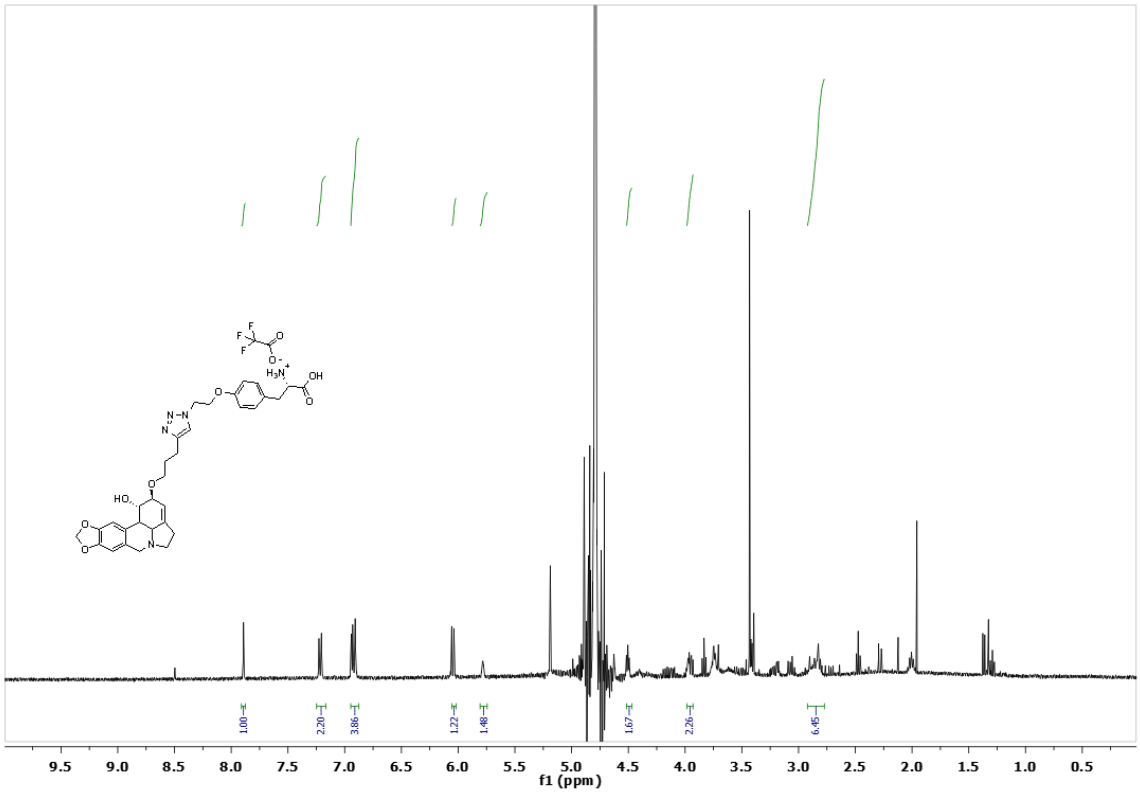
1.5
1.0
0.5
f1 (ppm)

Compound 7



Compound 8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2.20
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1.67
2.26
6.45
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f1 (ppm)

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