Synthesis of 5,5 di-Substituted γ-Lactams through a Base Mediated Molecular Rearrangement

A Thesis

Submitted to

Indian Institute of Science Education and Research, Pune in partial fulfillment of therequirements for the BS-MS Dual Degree Programme

by

Devanandan K
ID: 20171026

Under the guidance of
Prof. Hosahudya N. Gopi

Indian Institute of Science Education and Research, Pune

Certificate

This is to certify that this dissertation entitled **"*Synthesis of 5,5 di-substituted γ-lactams through a base mediated molecular rearrangement*"** towards the partial fulfillment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents work carried out by **Devanandan K** at Indian Institute of Science Education and Research, Pune under the supervision of Prof. Hosahudya N. Gopi, Department of Chemistry, during the academic year 2021-2022.

10/4/2022

IISER Pune

Prof. Hosahudya N. Gopi

Research Supervisor

Professor, IISER Pune

Pune-411008, India

Declaration

I hereby declare that the matter embodied in the report entitled **"*Synthesis of 5,5 di-substituted γ-lactams through a base mediated molecular rearrangement*"** are the results of the investigations carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research, Pune under the supervision of Prof. Hosahudya N. Gopi, Department of Chemistry and the same has not been submitted elsewhere for any other degree.

10/04/22
IISER Pune

Devanandan K
20171026
BS-MS 5th year

Acknowledgments

First and foremost, I want to convey my gratitude to Prof. Hosahudya N. Gopi for allowing me to work in his peptide chemistry group. I am fortunate to have him as my MS project supervisor. I couldn't have finished the project without his ongoing encouragement, direction, and helpful recommendations. I'd also want to thank Prof. Raghavendra Kikkeri, TAC member for my MS thesis, for his encouragement, support, and helpful recommendations.

I'd want to take this opportunity to thank Manjeet Singh, Sachin Nalawade, Saikat Pahan, Souvik Roy, Souvik Panda, Sanjit Dey, Puneeth Kumar, and Sandip Tosarkar, all of my lab mates. All of you have taught me a lot. Thank you, especially Manjeet, for your never-ending efforts to teach me various tactics, reaction approaches, instrument operation, and so on. It was a pleasure to work with all of you. I should like to express my thanks to all of the operators who assisted me in obtaining all of the necessary spectroscopic data, as well as IISER Pune for offering such an exceptional research facility as well as all extracurricular activities. I did my best to explore and enjoy my time here.

Above all, I want to express my gratitude to my family. My father and mother are the two persons that are responsible for me being where I am now. I would be nowhere if it weren't for their love, care, and support. Thank you to each and every one of my buddies. This campus is so beautiful because of you. I wouldn't have been able to enjoy my time here as much as I did if it weren't for you.

Devanandan K

Contents

1. Abbreviations

aq. = Aqueous

Ala= Alanine

Bn = Benzyl

Boc = *tert*-Butoxy carbonyl

(Boc)2O = Di-*tert*-butyl-dicarbonate (Boc anhydride)

DCC = N,N'-Dicyclo hexyl carbodiimide

DCM = Dichloromethane

DIPEA = Diisoproylethyl amine

DMF = N,N'-Dimethyl formamide

dg-γ = dehydro gamma

Et = Ethyl

EtOAc = Ethyl Acetate

EtOH = Ethanol

g = gram

Gly = glycine

h = hours

HCl = Hydrochloric acid

HOBt = 1-Hydroxybenzotriazole

HBTU=(2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

Ileu = isoleucine

IBX = 2-iodoxybenzoic acid

IR = Infrared spectroscopy

Leu = Leucine

MeOH = Methanol

Me = Methyl

mg =Milligram

min = Minutes

mmol =millimoles

N = Normal

NHS = N-hydroxy succinimide

NMR = Nuclear Magnetic Resonancee

Phe = Phenyl alanine

Pro = Proline

RT= Room temperature

THF= Tetrahydrofuran

TFA = Trifluoro acetic acid

^{t}Bu = tertiary Butyl

Trp = Tryptohan

Val = Valine

1.Abstract

Proteins are huge macromolecules that play an vital role in all living beings. Proteins adopt a variety of shapes as a result of the folding process, which makes them unique in their functions. Foldamer chemistry research has recently revealed that the folding phenomenon can also occur in smaller peptides. These research also opened up a lot of possibilities for finding important structures in peptides carrying unnatural amino acids. The presence γ-amino acids in physiologically active peptide natural products underscores their significance. In our lab, we've done a lot of research on non-natural amino acids and how they might be used to design various secondary structures. Various studies on unnatural amino acids and its usage in creating diverse secondary structures and peptidomimetics are performed in our group. A recent investigation on the structural characteristics and reactivity of α,β-unsaturated amino acids (dehydro-amino acid) revealed that when a peptide was treated with a base, the double bond on the dehydro-amino acid shifted. Using this migration reaction, a strategy of direct transformation of non-helical α, γ-hybrid peptides composed of alternating α- and *E*-vinylogous amino acids into 12-helical structures has been noticed.[1] Also, *N*-protected α,β-unsaturated γ-amino amides have also been converted to γ-lactams using this base-mediated chemical rearrangement.[2] A strong base like KO^{t}Bu was used to carry out this rearrangement reaction. γ-lactams are known to show excellent anti-microbial activities and research have been conducted to use γ-lactams as inhibitors of various proteins.

Through this research, I am exploring the synthesis and studying the activity of 5,5 di-substituted γ-lactams as a potential anticancer drug targeted towards inhibition of cytochrome p53-MDM2 interaction. Cytochrome p53 is a tumor suppressor protein that induces cell death by apoptosis. MDM2, a negative regulator of p53, is overexpressed in many cancers, inhibiting p53 and encouraging uncontrolled cell division**.** I am examining the feasibility of using 5,5 di-substituted γ-lactams as a potential competitive inhibitor for MDM2.

3. Introduction

Biomolecules are organic molecules and especially a macromolecule, present in living organisms. They are the primary building block of a living organism and are heavily involved in the maintenance and metabolic processes. Nucleic acids, carbohydrates, lipids, and proteins are the four major types of biomolecules. Of all these biomolecules, proteins are the most abundant ones in a living system.

Proteins can be considered a linear polymer chain of amino acids linked through a peptide bond. There are 20 naturally occurring alpha-amino acids, and these add up linearly to form a linear peptide chain, which further folds into different three-dimensional structures. Generally, proteins fold and organises themselves into four different levels : Primary, Secondary, Tertiary and Quaternary structure. **Figure 1** shows the schematic representation.

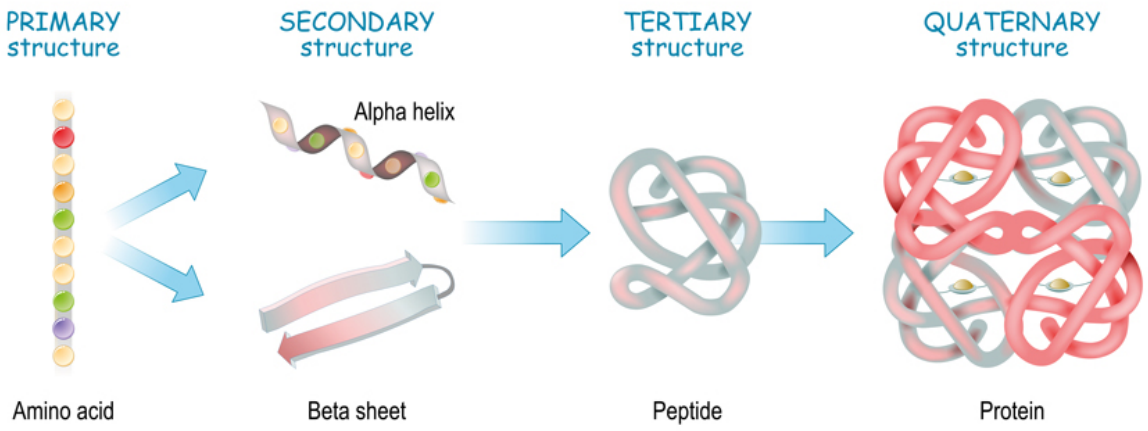


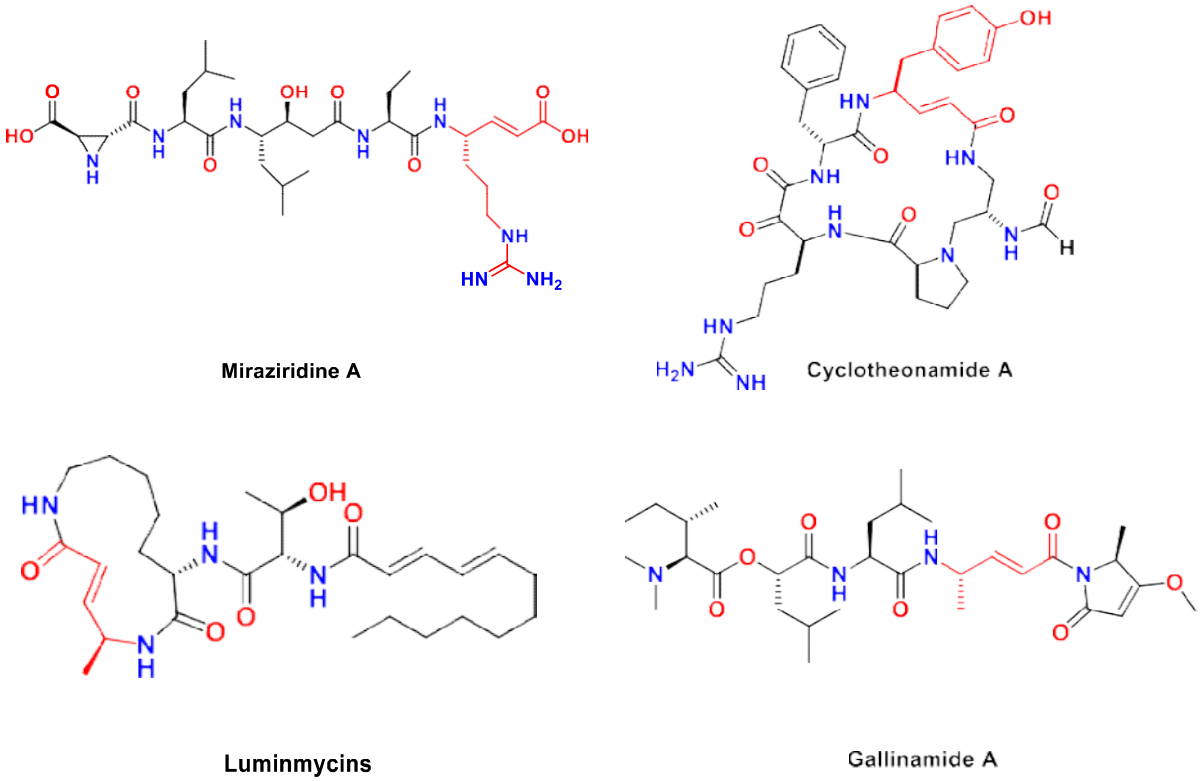
Figure 1 Levels of organization in protein.

The primary structure is the simplest level of protein structure which gives the amino acid sequence in a polypeptide chain. Due to interactions between atoms in the backbone, this primary structure folds locally to generate the secondary structure.

1. Helices 2. β-sheets 3. reverse turns are the three main secondary conformations of the protein.[3] The unique hydrogen bonding pattern between various functional groups(CO,NH) of the peptide chain distinguishes these secondary structures. The protein's tertiary structure is formed when the secondary structures fold into a three-dimensional structure with the least amount of energy. Bonding interactions between the side-chain groups stabilize this protein structure. Several tertiary structures or subunits come together due to electrostatic interaction to form the quaternary structure of the protein. The protein's three-dimensional structure is inevitable to its natural functioning. Being a polymer of 20 different amino acids, Peptide facilitates a diverse range of cellular processes like cell signaling, enzyme catalysis, transport of molecules, etc. Hence studying protein structure and function is still of high interest.

It is well known that using non-natural building blocks to replicate the structure of the protein will give a better understanding of its structure and function. The ability to construct functional macromolecules and surpass nature by producing molecules with more significant biological activity is enabled by mimicking protein secondary structure with abiotic oligomers. These abiotic oligomers can provide novel structures with higher affinity as biological ligands in suppressing protein-protein interactions. A number of organic molecules including non-natural amino acids have been investigated as building blocks for protein secondary structures in this context. Derivatives of γ- and β- amino acids are among the notable ones used as templates.[4]

α,β-unsaturated γ-amino acids are amino acids that aren't synthesized by ribosome and are used as organic templates to mimic naturally occurring peptides.[5] Several natural peptide products like Luminmycins (active proteasome inhibitors), Cyclotheonamides (potent inhibitors for serine protease and bovine β-trypsin),[6] Miraziridine A (inhibitors for a variety of proteases like trypsin-like proteases, Gallinamide A (anti-malarial agents),[7] and pepsin-like aspartyl protease[8] contain α,β-unsaturated γ-amino acids. **Scheme 1** shows structure of these natural peptide products.[9]

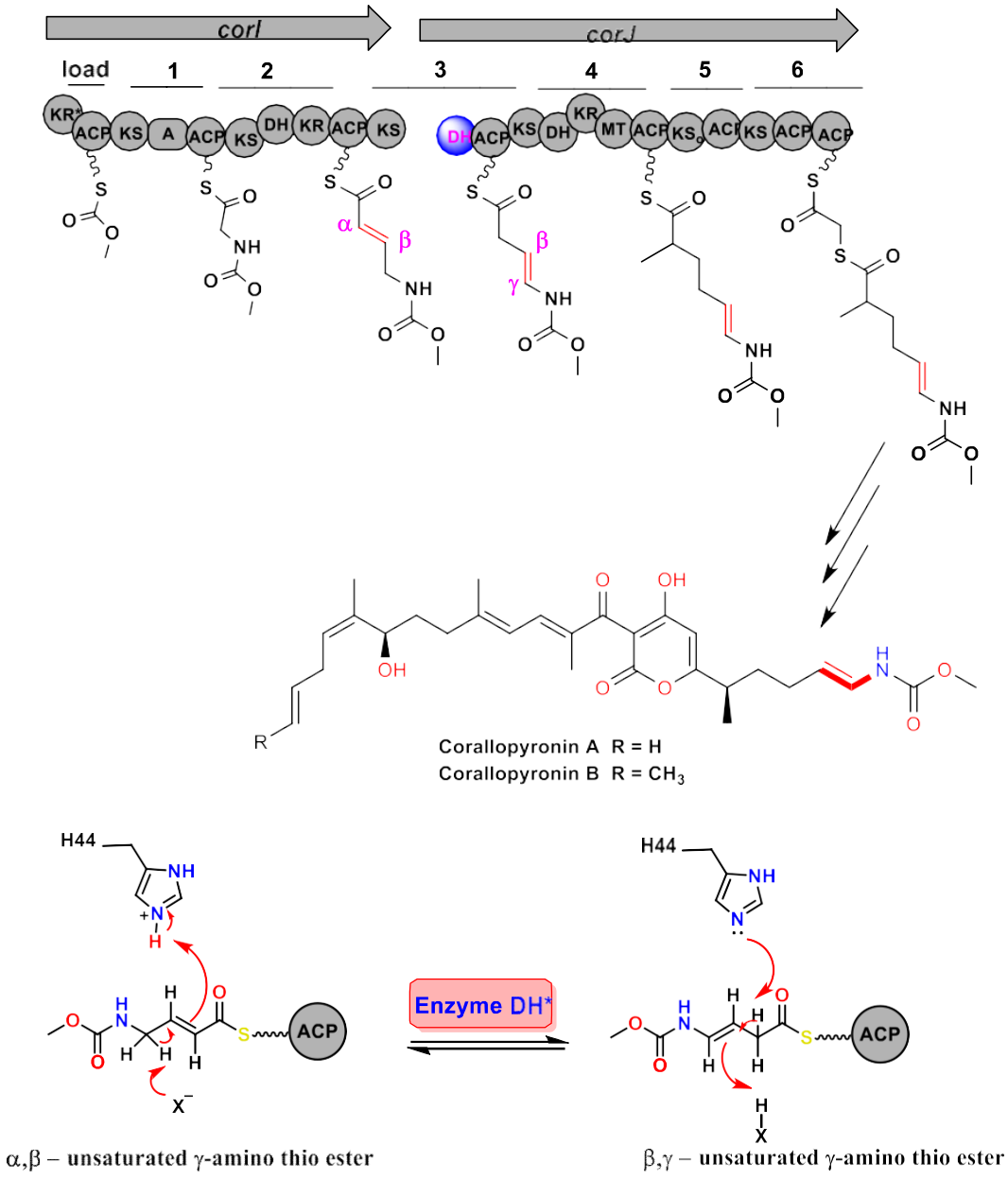


Scheme 1 Structure of natural peptide products containing dehydro γ-amino acids.

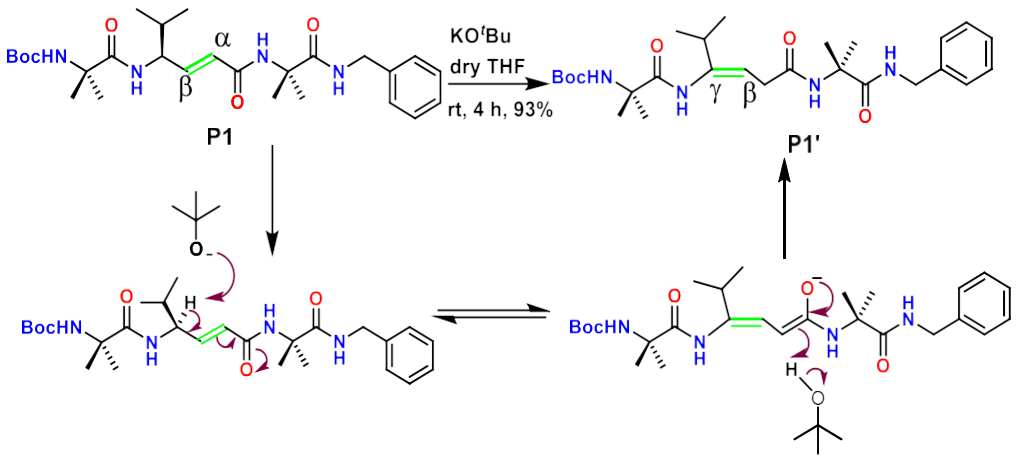
Dehydro-amino acids have been explored in our lab for their conformational features and chemical reactivity, as well as their use in the design of novel peptide foldamers, small molecule peptidomimetics, and double helices. In addition, our group has also observed a double bond migration from the α, β → β, γ position in α,β-unsaturated γ-amino amides in the presence of a base. Similar migration of double bonds have already been observed in several natural products containing *E*-vinylogous amino acids. One such example can be found in an intermediate step in the biosynthesis of Corallopyronin. Here, α, β → β, γ double bond migration was accomplished with the dehydratase enzyme.[10] A schematic representation is shown in the **Scheme 2**.

Inspired from these works, similar migration reactions were performed in our group. Through the various migration reactions conducted in different conditions, we were able to observe that the stronger the base, the easier it is to migrate the double bond. Hence, a stronger base like potassium tert-butoxide gave a better yield for these reactions. These base-mediated α, β → β, γ double-bond migrations were

seen to directly change non-helical, hybrid peptides consisting of alternating *E*- and α- vinylogous amino acids into 12-helical structures.[1] **Scheme 3** shows a schematic illustration of a probable process. When the same reaction was performed in smaller molecules as *N* protected unsaturated amino amides, the rearrangement lead to the formation of γ-lactams.[2]

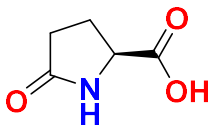


Scheme 2 Biosynthesis of corallopyronin along with α,β → β,γ double bond migration stepdone by the dehydratase enzyme.



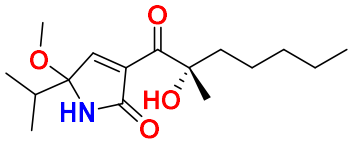
Scheme 3 Possible mechanism for α,β to β,γ double bond migration.

A lactam is a five-membered cyclic amide, primarily derived from an amino alkanoic acids.[11] A huge number of physiologically active natural and non-natural chemicals include the γ-lactam moiety.[12] These chemicals are capable of a wide range of biological functions in the treatment of cancer,[13] epilepsy,[14] Alzheimer's disease[15] human immune deficiency virus infection,[16] and bacterial and fungal infections[17]. Furthermore, γ-lactam derivatives have been demonstrated to be effective inhibitors of a variety of enzymes[18] and protein-protein interactions[19].Compounds containing γ-lactam have been utilized for producing constrained peptidomimetics in addition to their biological activity.[20] Few Medicinally active natural products containing γ-lactam are shown in **Scheme 4**.



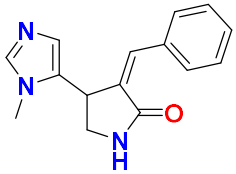
L-Pyroglutamic acid

Drug for neurodegenerative disorder



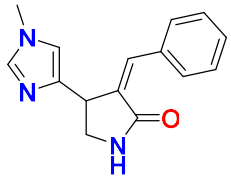
PI-091

Platelet aggregation inhibitors



(+/-) anantin

Shows antitumor activities

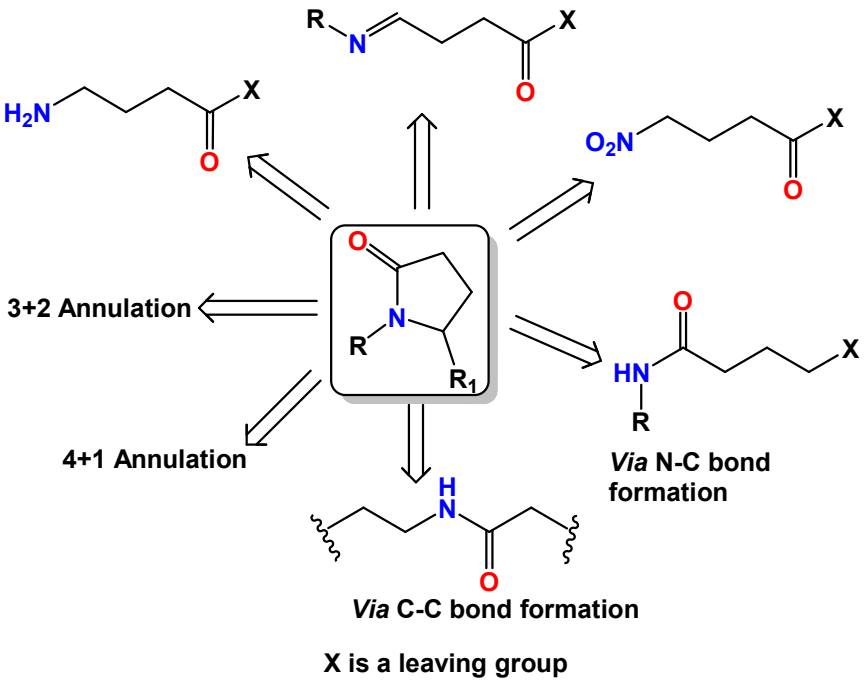


(+/-) isoanantin

Shows anti-inflammatory activity

Scheme 4 Structures of γ-lactam containing natural products.

Owing to the tremendous biological significance of γ-lactam moieties, extensive work has been conducted to synthesize these compounds.[12] Intramolecular condensation of carboxylic acid and amine functional groups of γ-amino acids is the most commonly used synthetic pathway.[21] Other than that, a series of transition metal-catalyzed C-N bond formation reactions such as Pd-catalyzed oxidative intramolecular allylic alkylations[22] and gold(I) catalyzed intramolecular cyclization of homopropargyl amides,[23] Ir (III) catalyzed C-H amidation.[24] C-C bond formation reactions such as Pd-catalyzed intramolecular reactions of N-allylpropiolamides,[25] Fe(X2) catalyzed cyclization of enynamides,[26] and gold(I) catalyzed cyclization of N-alkenyl β-ketoamides[27] and ring-closing metathesis[28] have been reported. Besides these cyclization strategies, (3+2) and (4+1) annulation reactions[29, 30] were also used in the construct biologically active γ-lactam products. These used strategies are schematically represented in **Scheme 5.**

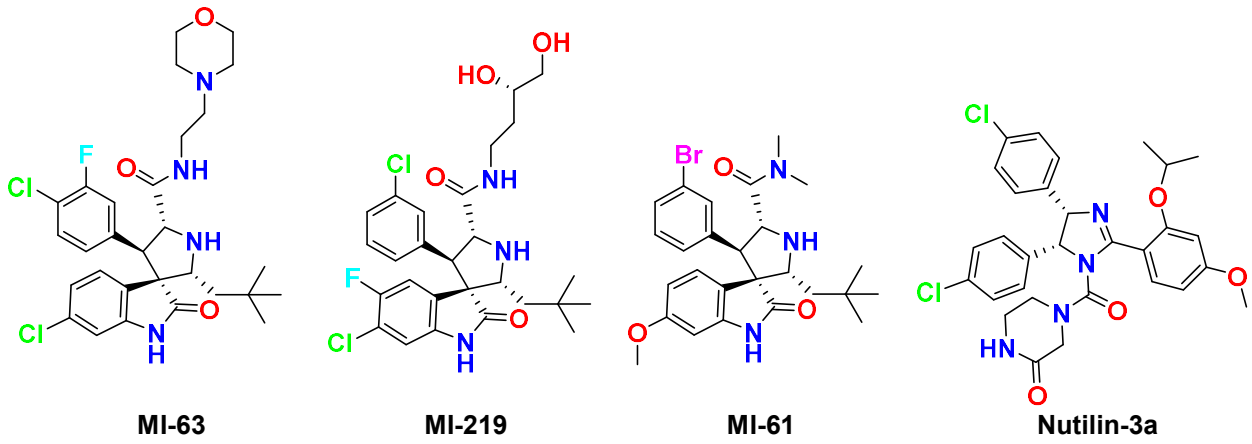


Scheme 5 Strategies used to synthesize the γ-lactams.

Studies have been conducted to check the viability of using γ-lactam moieties as potential inhibitors of p53–MDM2 interaction.[31] p53 or tumor protein p53 is a protein

found inside the nucleus and features a crucial role in regulating cell division and cell death. In response to different stress circumstances, such as DNA damage or oncogene activation, the tumour suppressor p53 initiates cell death via apoptosis. Loss of p53 tumor-suppressor action, whether caused by deletion or mutation of the TP53 gene or suppression of the p53 protein, promotes cancer growth.[32] The MDM2 protein acts as a p53 inhibitor. It reduces p53's transcriptional activity, promotes nuclear export, and promotes its destruction after binding to it.[33] Inhibiting the p53–MDM2 binding is an appealing method to activate p53-mediated apoptosis in tumours with upregulated MDM2 levels.[31] Researchers have discovered a number of small-molecular-weight chemicals and peptides that obstruct the p53–MDM2 interaction.[34] In tumour cells overexpressing MDM2, the peptidic inhibitors have an antiproliferative effect.

The use of a small chemical to inhibit the MDM2–p53 interaction is one appealing pharmacological strategy to p53 reactivation.[35] The Nutlins discovery provided essential proof of concept for this strategy.[36] Based on the crystal structure studies of the MDM2–p53 complex, a library of molecules containing γ-lactam in their backbone has been synthesized and studied as potential inhibitors. Structures of few molecules are shown in **Scheme 6.**



Scheme 6 Peptides that can act as potential P53-MDM2 interaction inhibitors.

In this study, the molecule MI-219 was identified as an ideal MDM2 inhibitor[37]. In

cells with wild-type p53, MI-219 breaks the MDM2–p53 interaction and turns on the p53 pathway, causing cell cycle arrest in all cells and selective apoptosis in tumor cells[37].

So, it was known that γ-lactam can serve as small molecules peptidomimetcs by acting as an inhibitor for p53-MDM2 interaction[20]. Here in this study, I aim to create a library of 5,5 di-substituted γ-lactams via a base facilitated molecular rearrangement and to check the viability of these molecules as potential p53-MDM2 inhibitors.

4. Materials and Methods

4.1 General experimental details

The amino acids and reagents used in each process, such as HBTU, EDC.HCl, TFA, DMF, and others, were bought from commercial sources. All of these reagents were utilized without being purified further. Prior to usage, solvents such as ethyl acetate, hexane, dichloromethane and methanol were distilled. Neutral silica (100-200 mesh) was used for column chromatography, which was also purchased from a commercial supplier. The ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were determined using a Bruker Avance 400 MHz or a JEOL 400 MHz spectrometer. The mass of materials was determined using high resolution mass spectrometry (HRMS).

4.2 Synthesis of Fmoc/Boc-amino NHS ester

Fmoc/Boc protected amino acid (25 mmol) was solvated in 50ml Tetrahydrofuran and to this solution N-hydroxysuccinimide (50 mmol) was added. After that, the reaction mixture was kept in ice cold condition and DCC (30mmol) was added. Product formation was confirmed using TLC. Once the reaction was completed after 12 hr, DCU formed in reaction was filtered out using a sintered funnel; the filtrate was collected and used for further reaction.

4.3 Synthesis of Boc/Fmoc Amino Alcohol

N-Boc-Amino NHS ester (20 mmol) is added into 50 ml THF allowed to dissolve. Temperature of the reaction mixture was brought down to 0°C. NaBH4 (25 mmol) was progressively added to this solution over a 10-minute period. For another 20 minutes, the reaction mixture was stirred. Product formation was confirmed using TLC. 10% HCl (10 percent by volume in water) was used to quench the reaction in an ice-cold environment (pH 2), on the reaction completion. Solvent was evaporated from the reaction mixture and the product was extracted three times with ethyl acetate (190 mL). The combined organic layer was washed in 40 mL of brine solution and dried on anhydrous Na_2SO_4. The organic layer was concentrated in vaccum to produce an oily product, which was then employed without purification in the next phase.

4.4 Synthesis of Boc/Fmoc Amino Aldehyde

IBX was added to a solution containing N-protected amino alcohol (10 mmol) in ethyl acetate (40 mL). The reaction was allowed to run for 8 hours at 70^{o}C under reflux conditions. After the completion of reaction, reaction mixture was cooled to room temperature and then filtered using sintered funnel. Residue was washed with EtOAc (25 mL), the filtrate was collected and evaporated under reduced pressure. This gave an oily product, which was then employed without purification in the next phase.

4.5 Synthesis of Boc-*N*- α, β-unsaturated γ-amino esters

Wittig ylide (11 mmol) was added to a solution containing N-Boc-Amino aldehyde (10 mmol) solvated in 50 mL of dry THF, at room temperature. The reaction mixture was agitated for roughly 8 hours at room temperature. TLC was used to track the reaction. After completion of the reaction, the solvent was evaporated, and the crude product was refined using pet ether/ethyl acetate column chromatography, yielding a (89%) pure product.

Characterization of Boc-*N*-dg-Ala-OEt (ethyl (*S, E*)-4-((tert-butoxycarbonyl) amino)pent-2-enoate).

Colorless cystalline solid, Yield= 2.85 g (93%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.83 (dd, *J* = 15.7, 5.0 Hz, 1H), 5.86 (dd, *J* = 15.7, 1.7 Hz, 1H), 4.59 (s, 1H), 4.36 (s, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 1.40 (s, 9H), 1.27 – 1.21 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 166.49, 155.02, 149.48, 120.20, 60.52, 28.42, 20.38, 14.28 .HRMS m/z calculated [M+H] is 244.1471, observed 244.1552.

Characterization of Boc-*N*-dg-Leu-OEt (ethyl (*S, E*)-4-((tert-butoxycarbonyl)amino)-6-methylhept-2-enoate).

Colorless cystalline solid, Yield= 2.73 g (89%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.81 (dd, *J* = 15.6, 5.5 Hz, 1H), 5.90 (dd, *J* = 15.6, 1.6 Hz, 1H), 4.53 – 4.42 (m, 1H), 4.39 – 4.26 (m, 1H), 4.18 (q, *J* = 6.8 Hz, 2H), 1.66 (dt, *J* = 13.4, 6.7 Hz, 1H), 1.43 (s, 9H), 1.37 (t, *J* = 7.4 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 166.57, 155.22, 149.04, 120.55, 60.55, 43.97, 28.48, 24.84, 22.83, 22.29, 14.35. HRMS m/z calculated [M+H] is 286.194, found 286.0604.

4.6 Synthesis of Boc-*N*-α,β-Unsaturated γ-Amino Amides

Hydrolysis of Boc-*N*- α,β-unsaturated γ-amino esters by NaOH

5 mmol N-protected,α,β-unsaturated γ-amino ester is dissolved in 20 mL distilled Methanol. 8 mL 1N NaOH is carefully added to the reaction mixture at room temperature. The reaction, which lasted 2 hours, was monitored using TLC. Once the reaction was completed, methanol was evaporated, and the reaction mixture was neutralised using 10% HCl solution (10 percent by volume in water). The neutralized product was then extracted in EtOAc three times (150 mL) and then the combined organic layer was washed with brine solution (3x30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated under low pressure.

Solution phase Coupling of Boc-*N*-α,β-unsaturated γ-amino acids with benzylamine

N-protected α,β-unsaturated γ-amino acid (3 mmol) was dissolved in 10 ml of DMF. Diisopropylethylamine (3 mmol) and benzylamine (3 mmol) were mixed into the reaction mixture and allowed to agitate for 10 minutes at ice-cold condition. The reaction was allowed to run for 12 hrs overnight. Meanwhile, TLC was done to keep track of the reaction progress. Following the reaction completion, the reaction mixture was diluted with Ethyl acetate three times(150 mL), and then the combined organic layer was washed with 10% HCl (3x50 mL), 10% Na_2CO_3 (1x50 mL), and brine solution (1x80 mL). The organic layer obtained was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. To obtain pure amides, the crude product was refined using column chromatography using a solvent system of petroleum ether/EtOAc

Characterization of Boc-*N*-dg-Ala-Bn (*tert*-butyl (*S,E*)-(5-(benzylamino)-5-oxopent-3-en-2-yl)carbamate).

White solid, yield = 0.813 g (89%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.31 – 7.24 (m, 5H), 6.74 (dd, *J* = 15.3, 5.5 Hz, 1H), 5.94 (s, 1H), 5.86 (dd, *J* = 15.3, 1.3 Hz, 1H), 4.56 (s, 1H), 4.47 (d, *J* = 5.8 Hz, 2H), 4.35 (dd, *J* = 9.7, 5.0 Hz, 1H), 1.41 (s, 9H), 1.23 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.46, 155.07, 138.18, 128.82, 128.01, 127.66, 77.33, 43.80, 28.47, 28.43, 20.71. MALDI m/z calculated [M+Na] is 327.1787 found 327.1796.

Characterization of Boc-*N*-dg-Leu-Bn (*tert*-butyl (*S, E*)-(1-(benzylamino)-6-methyl 1- oxohept-2-en-4-yl)carbamate).

White solid, yield = 0.935 g (90%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.13 (m,5H), 6.68 (dd, *J* = 15.2, 6.2 Hz, 1H), 6.01 (s, 1H), 5.88 (d, *J* = 15.2 Hz, 1H), 4.4(d, *J* = 5.8 Hz, 2H), 4.27 (d, *J* = 8.8 Hz, 1H), 1.65 (dt, *J* = 13.4, 6.8 Hz, 1H), 1.40 (s, 9H), 1.35 (t, *J* = 7.7 Hz, 2H), 0.90 (dd, *J* = 6.6, 1.5 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.50, 155.26, 144.91, 138.22, 128.79, 128.01, 127.62, 122.98, 79.66, 50.01, 44.08, 43.78, 28.47, 24.73, 22.71, 22.37. MALDI m/z calculated[M+Na] is 369.2256 found 369.2259.

Characterization of Boc-*N*-dg-Val-Bn ((*S,E*)-tert-Butyl (6-(Benzylamino)-2 methyl-oxohex-4-en-3-yl)-carbamate).

White solid, yield = 0.876 g (89%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.22 (m,5H), 6.75 (dd, *J* = 15.1, 6.3 Hz, 1H), 5.97 (s, 1H), 5.89 (dd, *J* = 15.2, 1.5 Hz, 1H), 4.61 (d, *J* = 9.3 Hz, 1H), 4.49 (d, *J* = 5.7 Hz, 2H), 4.09 (d, *J* = 8.3 Hz, 1H), 1.87 – 1.80 (m, 1H), 1.43 (s, 9H), 0.91 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.38, 143.47, 138.19, 128.85, 128.05, 127.70, 124.02, 57.04, 43.86, 32.45, 28.50, 18.99, 18.24. MALDI m/z calculated [M+Na] is 355.2100 found 355.1997.

Characterization of Boc-*N*-dg-Phe-Bn (tert-butyl (*S, E*)-(5-(benzylamino)-5 oxo-1 phenylpent-3-en-2-yl)carbamate).

White solid, yield = 1.083 g (95%), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 – 7.16 (m,12H), 6.87 – 6.73 (m, 1H), 5.79 (d, *J* = 15.2 Hz, 1H), 4.55 (s, 1H), 4.46 (d, *J* = 5.7 Hz, 2H), 2.84 (dd, *J* = 23.1, 6.6 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.16, 155.13, 138.11, 136.68, 129.57, 128.82, 128.62, 127.96, 127.68, 126.87, 77.32, 43.80, 28.40. HRMS m/z calculated [M+H] is 381.2100, observed 381.2178.

Characterization of Boc-*N*-dg-Trp-Bn (tert-butyl (*S,E*)-(5-(benzylamino)-1-(1H-indol 3-yl)-5-oxopent-3-en-2-yl)carbamate).

White solid, yield = 1.15 g (92%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 – 10.82 (m, 1H), 8.51 (t, *J* = 5.8 Hz, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.35 – 7.28 (m, 4H), 7.25 – 7.20 (m, 3H), 7.14 – 7.10 (m, 2H), 7.09 – 7.04 (m, 1H), 7.00 – 6.95 (m, 1H), 6.66 (dd, *J* = 15.4, 5.7 Hz, 1H), 5.96 (d, *J* = 15.4 Hz, 1H), 4.38 (dd, *J* = 13.6, 7.0 Hz, 1H), 4.30 (t, *J* = 5.2 Hz, 2H), 2.89 (d, *J* = 7.2 Hz, 2H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.67, 155.02, 143.30, 139.45, 136.15, 128.26, 127.28, 126.75, 123.40, 123.26, 120.84, 118.33, 118.23, 111.35, 110.58, 77.74, 51.91, 42.09, 30.35, 28.24. HRMS m/z calculated [M+H] is 420.2209, observed 420.2178.

Characterization of Boc-*N*-dg-Gly-Bn (*tert*-butyl (*E*)-(4-(benzylamino)-4 oxobut-2-en-1-yl)carbamate).

White solid, yield = 1.00 g (87%) , ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 (d, *J* = 9.8Hz, 1H), 7.36 – 7.25 (m, 5H), 6.65 (t, *J* = 9.8 Hz, 1H), 5.97 (s, 1H), 4.66 (q, *J* =8.0 Hz, 1H), 4.42 (d, *J* = 5.7 Hz, 2H), 2.92 (dd, *J* = 7.7, 1.1 Hz, 2H), 1.46 (s, 9H).
HRMS m/z calculated [M+H] is 291.163, observed 291.0876.

Characterization of Boc-*N*-dg-Ileu-Bn (*tert*-butyl (*E*)-(4-(benzylamino)-4 oxobut-2-en-1-yl)carbamate).

White solid, yield = 0.923 g (89%) , ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.26 (m, 6H), 6.76 (dd, *J* = 15.1, 5.6 Hz, 1H), 5.89 (d, *J* = 13.7 Hz, 1H), 4.62 (s, 1H), 4.50 (d, *J* = 5.5 Hz, 2H), 4.25 – 4.11 (m, 1H), 1.59 (q, *J* = 8.8, 6.8 Hz, 1H), 1.43 (s, 9H), 1.12 (ddd, *J* = 20.6, 15.0, 7.2 Hz, 2H), 0.90 (dd, *J* = 9.9, 7.1 Hz, 7H).
HRMS m/z calculated [M+H] is 347.2256, observed 347.2197.

Characterization of Boc-*N*-dg-Pro-Bn (*tert*-butyl (*E*)-2-(3-(benzylamino)-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate).

Pale yellow viscous liquid = 1.07 g (87%), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 7.26 (m, 5H), 6.74 (t, *J* = 14.9 Hz, 1H), 5.90 – 5.70 (m, 2H), 4.51 (s, 2H), 3.44 – 3.32 (m,2H), 2.04 (s, 1H), 1.87 – 1.70 (m, 4H), 1.41 (s, 9H).
HRMS m/z calculated [M+Na] is 353.1943, observed 353.1840.

Solution phase Coupling of Boc-*N*-α,β-unsaturated γ-amino acids with tryptamine

N-protected α,β-unsaturated γ-amino acid (3 mmol) was dissolved in 10 ml of DMF. Diisopropylethylamine (3 mmol) and tryptamine (3 mmol) were mixed into the reaction mixture and allowed to agitate for 10 minutes at ice-cold condition. The reaction was allowed to run for 12 hrs overnight. Meanwhile, TLC was done to keep track of the reaction progress. Following the reaction completion, the reaction mixture was diluted with Ethyl acetate three times(150 mL), and then the combined organic

layer was washed with 10% HCl (3x50 mL), 10% Na_2CO_3 (1x50 mL), and brine solution (1x80 mL). The organic layer obtained was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. To obtain pure amides, the crude product was refined using column chromatography using a solvent system of petroleum ether/EtOAc

Characterization of Boc-*N*-dg-Phe-Trp (*tert*-butyl (*S, E*)-(4-(benzylamino)-4-oxobut-2-en 1-yl)carbamate).

Pale yellow solid, yield = 1.543 g (89%), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.28 (s, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.27 (d, *J* = 6.9 Hz, 1H), 7.24 (s, 1H), 7.22 – 7.15 (m, 3H), 7.15 – 7.10 (m, 3H), 7.10 – 7.06 (m, 1H), 6.97 (d, *J* = 2.2 Hz, 1H), 6.71 (dd, *J* = 16.1, 3.8 Hz, 1H), 5.66 (s, 1H), 5.59 (t, *J* = 5.5 Hz, 1H), 4.53 (d, *J* = 12.8 Hz, 2H), 2.95 (t, *J* = 6.8 Hz, 2H), 2.87 – 2.80 (m, 2H), 1.36 (s, 9H).
HRMS m/z calculated [M+H] is 433.2365, observed 434.2267.

Characterization of Boc-*N*-dg-Trp-Trp (*tert*-butyl *(S,E)-(*4-(benzylamino)-4-oxobut-2-en 1-yl)carbamate).

Pale yellow solid, yield = 1.712 g (87%), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (s, 2H), 7.62 – 7.58 (m, 2H), 7.40 – 7.36 (m, 2H), 7.26 – 7.20 (m, 2H), 7.19 – 7.05 (m, 4H), 6.96 (s, 1H), 6.75 (d, *J* = 16.0 Hz, 1H), 5.65 (d, *J* = 15.7 Hz, 1H), 5.48 (t, *J* = 5.3 Hz, 1H), 4.65 (s, 1H), 3.63 (t, *J* = 5.6 Hz, 2H), 3.03 – 2.95 (m, 4H), 2.66 (d, *J* = 39.7 Hz, 2H), 1.42 (s, 9H).
HRMS m/z calculated [M+H] is 473.2474, observed 473.2552.

4.7 Synthesis of γ-lactams

Under N_2 atmosphere, KO^{t}Bu (9 mmol, 3 eq) was mixed to a solution containing N-protected,α,β-unsaturated γ-amino amide (3 mmol, 1 eq) in dry THF (15 mL) and agitated for about 8 hours at room temperature. TLC was used to track the reaction's progress. Following the reaction completion, solvent was evaporated and water (30 mL) was added to the reaction mixture. The aqueous layer of this mixture was extracted with EtOAc (30 mLx3), followed by a brine (30 mLx2) wash, drying over

anhydrous Na_2SO_4.Solvent in crude product was evaporated using vaccum evaporator and later purified using column chromatography using a solvent system of pet ether/ethyl acetate(75:25) to give pure compounds in 85% to 95% yields.

Characterization of Ala Lactam ((*tert*-Butyl (1-Benzyl-2-methyl-5-oxopyrrolidin-2yl)carbamate).

Colorless solid, yield = 0.565 g (93%), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.28 – 7.20(m, 5H), 4.80 (s, 1H), 4.62 – 4.49 (m, 1H), 4.25 (d, *J* = 12.4 Hz, 1H), 2.79 (s, 1H),2.53 – 2.38 (m, 2H), 2.05 (d, *J* = 13.2 Hz, 1H), 1.35 (s, 9H), 1.31 (s, 3H). ^{13}C NMR(101 MHz, Chloroform-*d*) δ 138.59, 128.62, 127.89, 127.19, 74.79, 42.43, 32.58,29.82, 28.34. HRMS m/z calculated [M+H] is 305.1787, observed 305.1365.

Characterization of Leu Lactam ((*tert*-Butyl (1-Benzyl-2-isobutyl-5-oxopyrrolidin-2-yl)carbamate).

Pale yellow viscous liquid, yield = 0.623 (90%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.18 (m, 5H), 4.82 (s, 1H), 4.49 – 4.26 (m, 2H), 2.80 (d, *J* = 20.4 Hz, 1H), 2.51 – 2.35 (m, 2H), 2.23 (ddd, *J* = 13.9, 10.7, 5.8 Hz, 1H), 1.69 – 1.64 (m, 1H), 1.51 – 1.38 (m, 2H), 1.32 (s, 9H), 0.94 (d, *J* = 6.3 Hz, 3H), 0.81 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 175.72, 138.50, 128.55, 128.14, 128.14, 127.12,48.39, 48.39, 42.86, 29.93, 29.75, 28.33, 24.45, 24.25, 23.96. HRMS m/z calculated [M+Na] is 369.2256, observed 369.2153.

Characterization of Phe Lactam ((*tert*-Butyl (1-Benzyl-2-methyl-5-oxopyrrolidin-2-yl)carbamate).

Colorless solid, yield = 0.699 (92%); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.67 (s, 1H), 7.86 (d, *J* = 7.3 Hz, 2H), 7.66 – 7.57 (m, 2H), 7.49 (t, *J* = 7.7 Hz, 4H), 7.42 (s, 1H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.29 (t, *J* = 7.3 Hz, 1H), 6.67 (s, 1H), 5.94 (s, 1H), 1.64 (s, 2H), 1.42 (s, 8H), 1.24 (t, *J* = 7.1 Hz, 2H).

HRMS m/z calculated [M+H] is 380.2100, observed 381.2178.

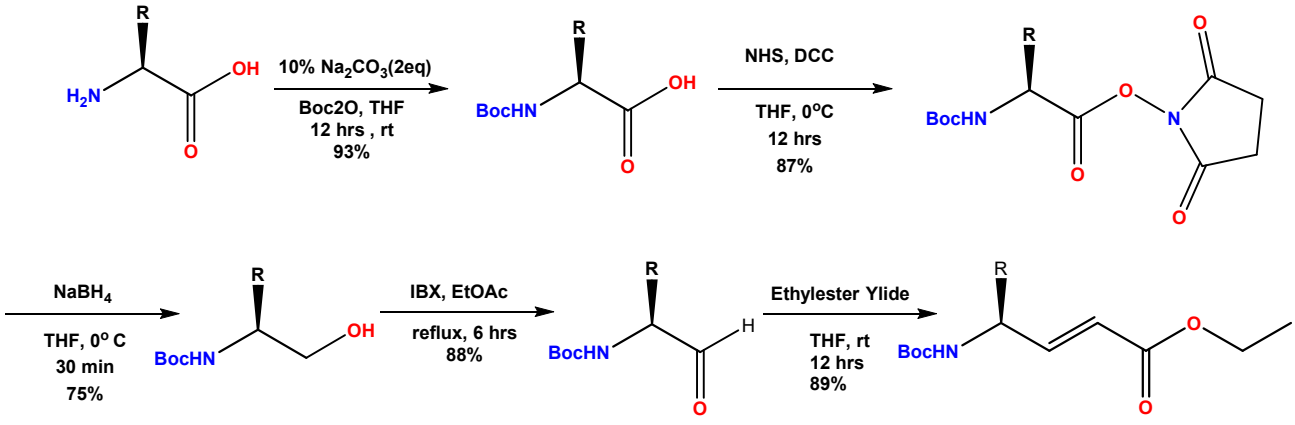
Characterization of Trp Lactam ((*tert*-butyl (2-((1H-indol-3-yl)methyl)-1-benzyl-5-oxopyrrolidin-2-yl)carbamate).

Brown solid, yield = 0.732 g (95%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.41 (d, *J* = 7.1 Hz, 3H), 7.34 (s, 3H), 7.24 – 7.18 (m, 1H), 7.06 (t, *J* = 8.0 Hz, 1H), 6.99 (t, *J* = 7.4 Hz, 1H), 6.88 (s, 1H), 4.46 (d, *J* = 6.4 Hz, 2H), 3.18 (d, *J* = 14.7 Hz, 1H), 3.05 (d, *J* = 14.8 Hz, 1H), 2.28 (d, *J* = 9.7 Hz, 2H), 2.11 (t, *J* = 9.7 Hz, 1H), 1.66 (t, *J* = 10.9 Hz, 1H), 1.27 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.40, 153.75, 138.83, 135.59, 128.25, 128.01, 126.56, 124.23, 120.94, 118.69, 117.99, 111.43, 107.25, 77.72, 42.40, 30.72, 29.40, 28.11. HRMS m/z calculated [M+Na] is 442.2209, observed 442.2187.

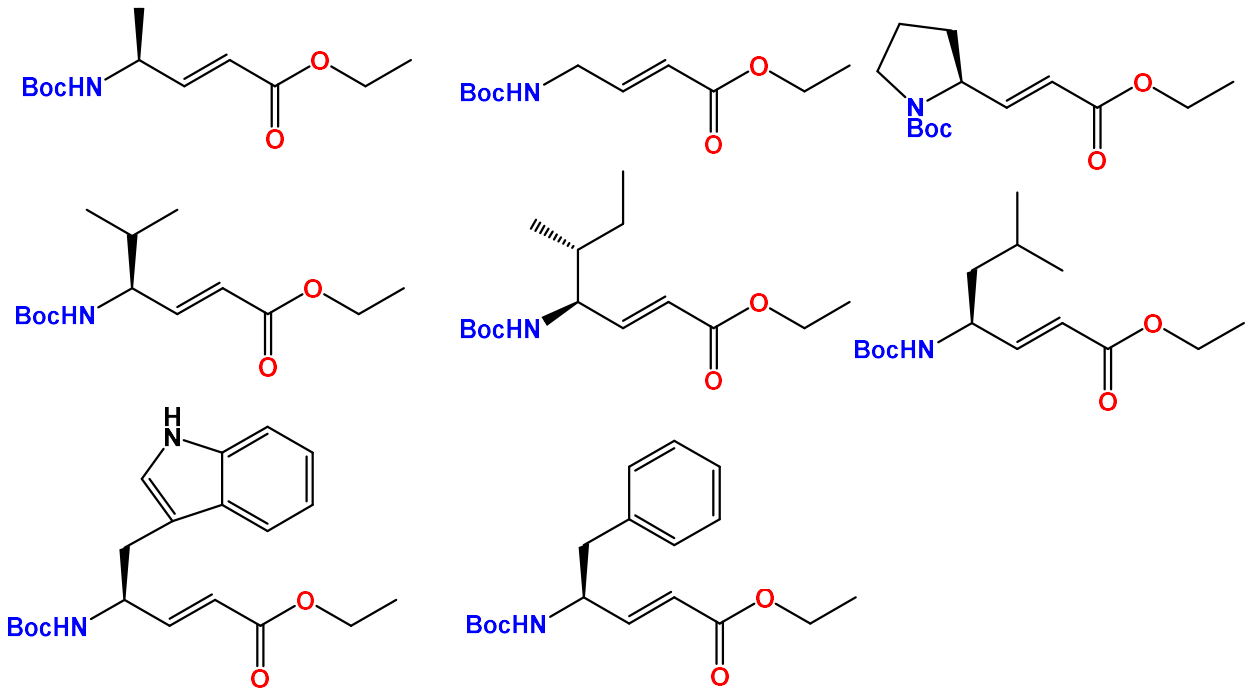
5. Results and Discussion

5.1 Reactivity and synthesis of α-β Unsaturated γ-amino esters

An array of α-β unsaturated γ-amino esters were synthesized using previously reported method. The production of dehydro γ-amino esters starting from α-amino acids is depicted schematically in **Scheme 7**. α-β unsaturated γ-amino ethyl esters of Leucine, Alanine, Valine, Phenyl alanine, Isoleucine, Tryptophan, Glycine and Proline were produced from N-Boc-amino aldehyde via the Wittig process, as reported earlier[11,12].



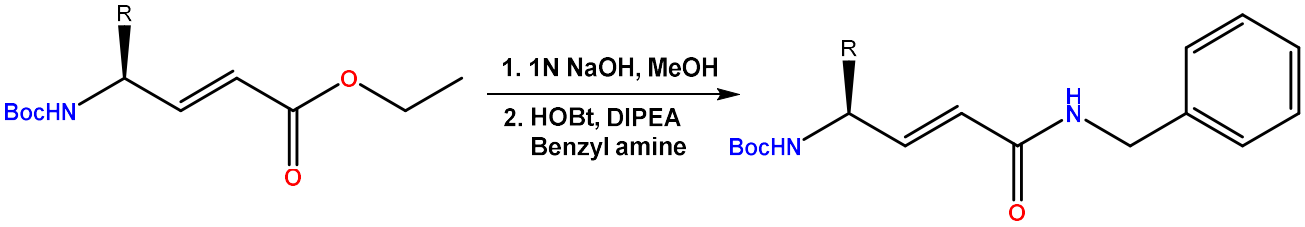
Scheme 7 Synthesis of α-β unsaturated γ-amino ethyl ester.



Scheme 8 Structures of α-β unsaturated γ-amino ethyl ester synthesized.

5.2 Synthesis and reactivity of α-β Unsaturated γ-amino amides

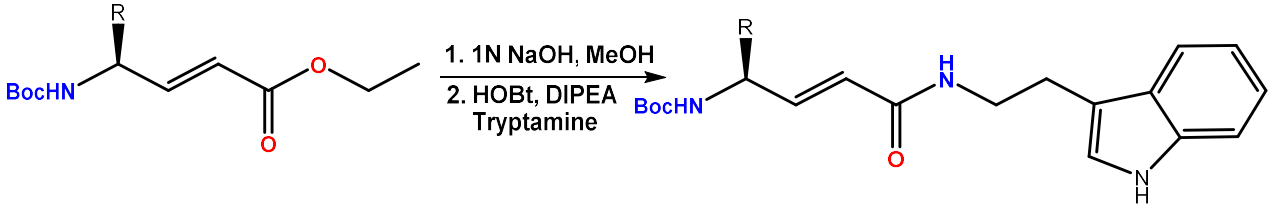
The saponification reaction was performed on the α-β unsaturated γ-amino esters. The resulting α-β unsaturated γ-amino acids were coupled with different amines through solution phase coupling to create the necessary peptides for the investigation. This procedure is depicted schematically in **Scheme 9.**



Scheme 9 Base hydrolysis followed by Coupling with benzyl amine.

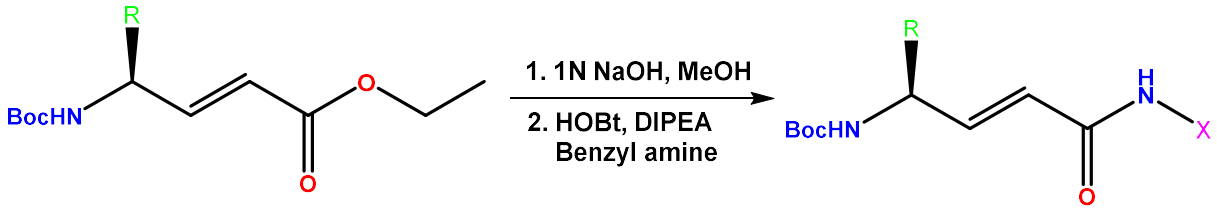
Several *N*-Boc α-β unsaturated γ-amino benzyl amine amides were successfully synthesized using this procedure.

N-Boc α-β unsaturated γ-amino tryptamine amides were also produced using the same method, which is shown in **Scheme 10.**



Scheme 10 Base hydrolysis followed by Coupling with Tryptamine.

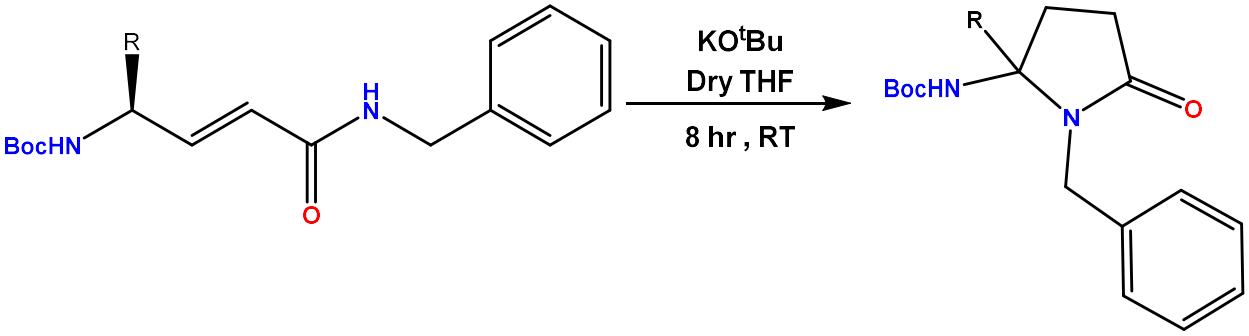
General scheme for solution phase coupling is as follows:



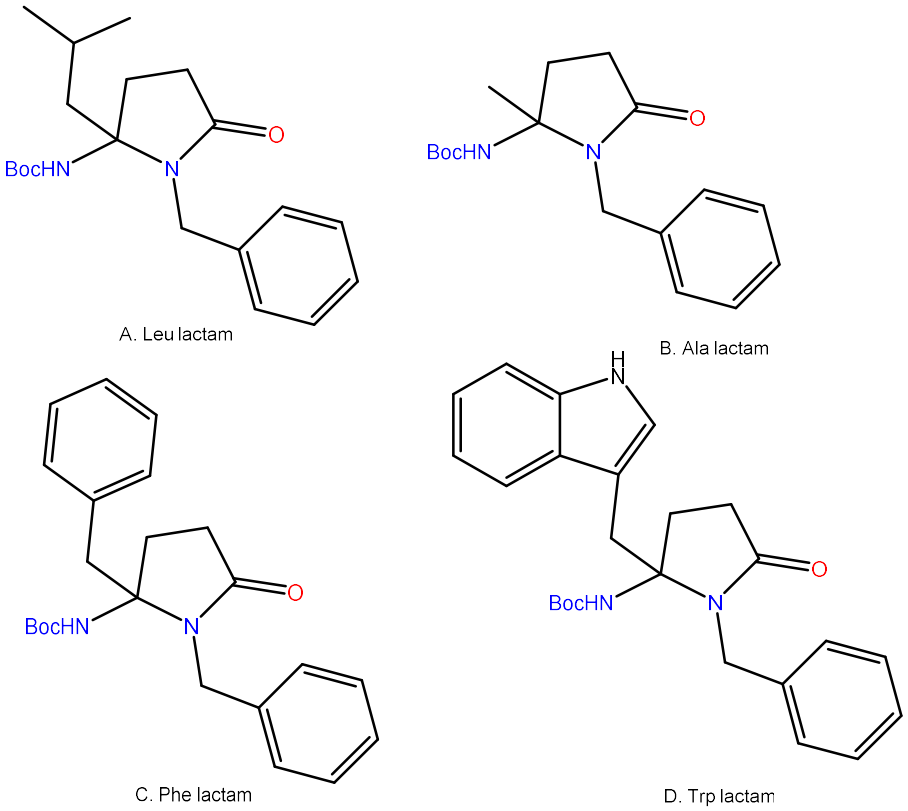
S. No.	R	X	Yield
1	$CH_2CH(CH_3)_2$	$CH_2C_6H_5$	90
2	$CH(CH_3)_2$	$CH_2C_6H_5$	89
3	CH_3	$CH_2C_6H_5$	89
4	H	$CH_2C_6H_5$	87
5	$CH(C_2H_5)(CH_3)$	$CH_2C_6H_5$	89
6	$CH_2C_6H_5$	$CH_2C_6H_5$	95
7	CH_2-Indole	$CH_2C_6H_5$	92
8	Pyrrolidine	$CH_2C_6H_5$	87
9	$CH_2C_6H_5$	$C_{10}H_{11}N$	89
10	CH_2-Indole	$C_{10}H_{11}N$	87

5.3 Synthesis and reactivity of 5,5-Disubstituted γ-Lactams

N-Boc α-β unsaturated γ-amino amides were then subjected to base mediated molecular rearrangement using potassium tert-butoxide. Lactams of Leucine, Alanine, Phenyl alanine and Tryptophan were synthesized successfully via this rearrangement reaction, as expected.



Scheme 11 Rearrangement of N-Boc-α,β Unsaturated γ-Amino Amide into N-Alkyl 5,5-Disubstituted γ-Lactams Mediated by KO^{t}Bu.



Scheme 12 Structures of N-Alkyl 5,5-Disubstituted γ-Lactams synthesized.

The formation of 5,5 disubstituted γ-lactam was confirmed by analyzing ^{1}H NMR. The ^{1}H NMR of Boc-*N*- α-β unsaturated γ-amino amides had a characteristic *dd* (doublet of doublet)and *d* (doublet) peaks of the two alkene hydrogen atoms. These characteristic peaks are absent in the 5,5 disubstituted γ-lactam as the alkene is reduced during the rearrangement reaction. Also, HRMS spectra of both the product and reactant shows same m/z values confirming the rearrangement reaction.

Compound 12D-Trp lactam single crystals were obtained. The X-ray diffracted structure is shown in **Figure 2**.

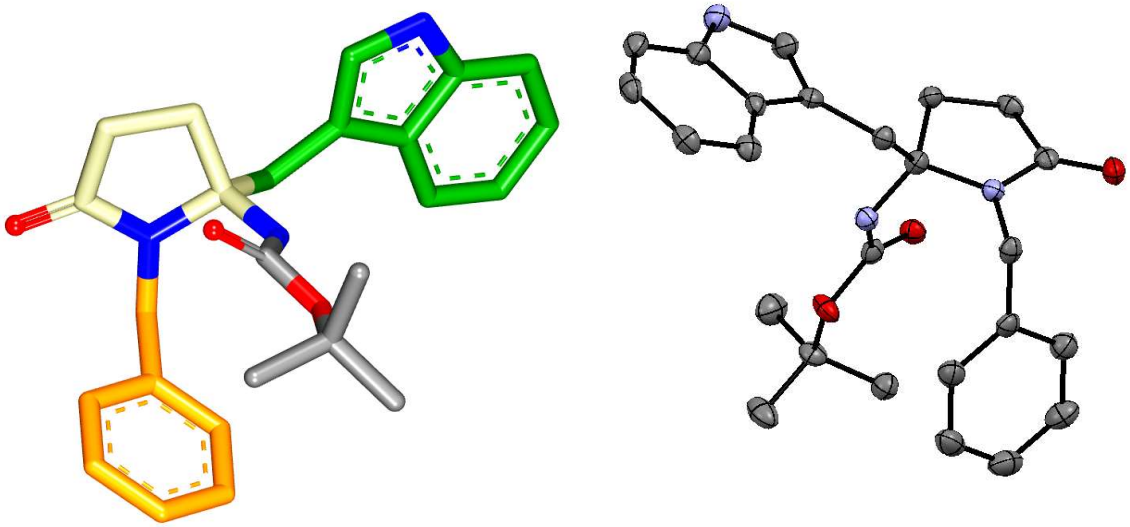
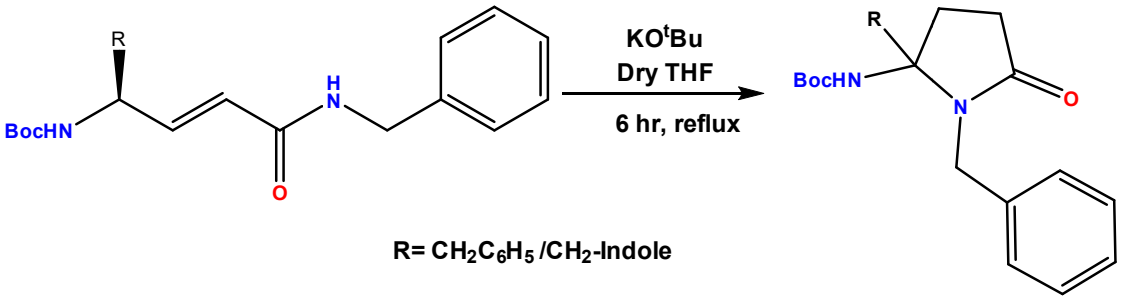


Figure 2. Crystal structure of Trp lactams (12D).

It was noted that Boc-*N*- α-β unsaturated γ-amino amides of Tryptophan and Phenyl alanine required reflux conditions at 40°C for the completion of this potassium tertiary butoxide mediated rearrangement.



R= $CH_2C_6H_5$ /CH_2-Indole

6. Conclusions

A library of Boc-*N*- α-β unsaturated γ-amino amides were successfully synthesized in excellent yields. These were then reacted with potassium tert-butoxide and formation of 5,5-disubstituted γ-lactams were observed, instead of double bond migration within the amino amides. Four different 5,5-disubstituted γ-lactams were successfully synthesized and characterized .

It is known from literature that γ-lactams can be used as drugs and inhibitors for several proteins. So, I am planning to do study the biological activity of 5,5-disubstituted γ-lactams in the near future. I believe that these 5,5-disubstituted γ-lactams can act as potential inhibitors of p53- MDM2 interaction and thereby serve as potential anticancer drug.

7.References

1. Veeresh, K.; Gopi, H. N. Design of Helical Peptide Foldamers through α,β → β,γ Double- Bond Migration. *Org. Lett*. **2019**, *21*, 4500−4504.
2. Ganesh Kumar, M., Veeresh, K., Nalawade, S.A., Nithun, R.V. and Gopi, H.N. *J. Org. Chem.* **2019**, *84*, 15145-15153.
3. Sun PD, Foster CE, Boyington JC. Overview of protein structural and functional folds. *Curr Protoc Protein Sci*. 2004;Chapter 17(1):1711-17.1.
4. (a) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366. (b) Horne, W. S.; Gellmann, S. H. *Acc. Chem. Res*., **2008**, *41*, 1399.
5. Michael W. Giuliano, , Stacy J. Maynard, Aaron M. Almeida, Li Guo, Ilia A. Guzei, Lara C. Spencer *J. Am. Chem. Soc.* **2014**, 136, 42, 15046–15053
6. Hagihara, M.; Schreiber, S. L. *J. Am. Chem. Soc*. **1992**, *114*, 6570.
7. Linington, R. G.; Clark, B. R.; Trimble, E. E.; Almanza, A.; Uren, L.-D.; Kyle, D. E.; Gerwick, W. H. *J. Nat. Prod*. **2009**, *72*, 14.
8. Nakao, Y.; Fujita, M.; Warabi, K.; Matsunaga, S.; Fusetani, N. *J. Am. Chem. Soc*. **2000**, *122*, 10462.
9. Schaschke, N.; Sommerhoff, C. P. *ChemMedChem* **2010**, *5*, 367.
10. Taft, F.; Brünjes, M.; Knobloch, T.; Floss, H. G.; Kirschning, A. *J. Am. Chem. Soc.* **2009**, *131*, 3812
11. Ai, Y.; Hu, Y.; Kang, F.; Lai, Y.; Jia, Y.; Huang, Z.; Peng, S.; Ji, H.; Tian, J.; Zhang, Y. *J. Med. Chem.* **2015**, *58*, 4506-4520
12. Kammerer, C.; Prestat, G.; Madec, D.; Poli, G. *Acc. Chem. Res*. **2014***, 47*, 3439−3447
13. Agatsuma, T.; Akama, T.; Nara, S.; Matsumiya, S.; Nakai, R.; Ogawa, H.; Otaki, S.; Ikeda, S.; Saitoh, Y.; Kanda, Y. *Org. Lett.* **2002**, *4*, 4387–4390.

14. Kwan, P.; Trinka, E.; Van Paesschen, W.; Rektor, I.; Johnson, M. E.; Lu, S. *Epilepsia* **2014**, *55*, 38–46.

15. Yang, Z. Q.; Shu, Y.; Ma, L.; Wittmann, M.; Ray, W. J.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T.; Kuduk, S. D. *ACS Med. Chem. Lett.* **2014**

16. Kazmierski, W. M.; Andrews, W.; Furfine, E.; Spaltenstein, A.; Wright, L. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 5689−5692.

17. Manam, R. R.; Teisan, S.; White, D. J.; Nicholson, B.; Grodberg, J.; Neuteboom, S. T. C.; Lam, K. S.; Mosca, D. A.; Lloyd, G. K.; Potts, B. C. M. *J. Nat. Prod*. **2005**, *68*, 240−243.

18. Yoon, C. H.; Nagle, A.; Chen, C.; Gandhi, D.; Jung, K. W. *Org. Lett*. **2003**, *5*, 2259–2262

19. Zhuang, C.; Miao, Z.; Zhu, L.; Dong, G.; Guo, Z.; Wang, S.; Zhang, Y.; Wu, Y.; Yao, J.; Sheng, C.; Zhang, W. *J. Med. Chem*. **2012**, *55*, 9630–9642

20. Geranurimi, A.; Lubell, W. D. *Org. Lett.* **2018**, *20*, 6126-6129

21. a) Martelli, G.; Orena, M.; Rinaldi, S. *Curr. Org. Chem*. **2014**, *18*, 1373–1481

22. Trend, R. M.; Ramtohul, Y. K.; Ferreira, E. M.; Stoltz, B. M. *Angew. Chem. Int. Ed*. **2003**, *42*, 2892-2895

23. Shu, C.; Liu, M.-Q.; Wang, S.-S.; Li, L.; Ye, L.-W. *J. Org. Chem.***2013**, *78*, 3292-3299.

24. Hong, S. Y.; Park, Y.; Hwang, Y.; Kim, Y. B.; Baik, M.-H.; Chang, S. *Science* **2018**, *359*, 1016-1021

25. Welbes, L. L.; Lyons, T. W.; Cychosz, K. A.; Sanford, M. S. *J. Am. Chem. Soc*. **2007**, *129*, 5836-5837

26. Yeh, M.-C. P.; Shiue, Y.-S.; Lin, H. –H.; Yu, T.-Y.; Hu, T.-C.; Hong, J.-J. *Org. Lett.* **2016**, *18*, 2407−2410.

27. Zhou, C.-Y.; Che, C.-M. *J. Am. Chem. Soc*. **2007**, *129*, 5828-5829

28. Lee, C.; Choi, E.; Cho, M.; Lee, B.; Oh, S. J.; Park, S.-K.; Lee, K.; Kim, H. M.; Han, G. *Bioorg. Med. Chem. Lett*. **2012**, *22*, 4189-4192.

29. N.; Arvedson, S.; Eisenberg, S.; Han, N.; Handley, M.; Huang, L.; Huang, Q.; Kiselyov, A.; Liu, Q.; Osslund, T.; Xu, S.; Zhang, J,Kendall, R.; Dominguez, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2905 —2909.

30. S. Gao, S.; Tu, Y. Q.; Hu, X.; Wang, S.; Hua, R.; Jiang, Y.; Zhao, Y. Fan, X. Zhang, S. *Org. Lett.* **2006**, *8*, 2373.

31. Chène P. Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. Nat Rev Cancer. 2003 Feb;3(2):102-9. doi: 10.1038/nrc991. PMID: 12563309.

32. Momand, J., Zambetti, G. P., Olson, D. C., George, D. & Levine, A. J. The Mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* **69**, 1237–1245 (1992).

33. Tao, W. & Levine, A. J. Nucleocytoplasmic shuttling of oncoprotein Hdm2 is required for Hdm2-mediated degradation of p53. *Proc. Natl Acad. Sci. USA* **96**, 3077–3080 (1999).

34. Vassilev LT (2004) *Cell Cycle* 3:419–421.

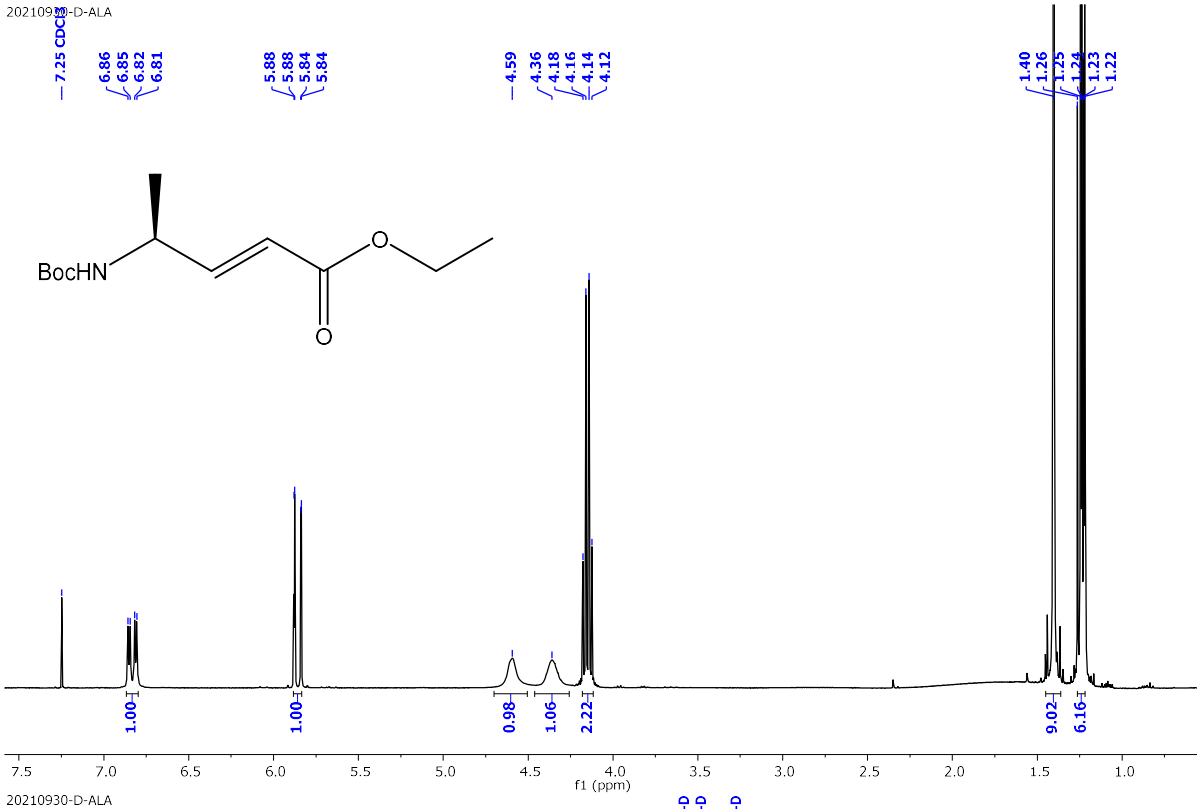
35. Chene P (2003) *Nat Rev Cancer* 3:102–109

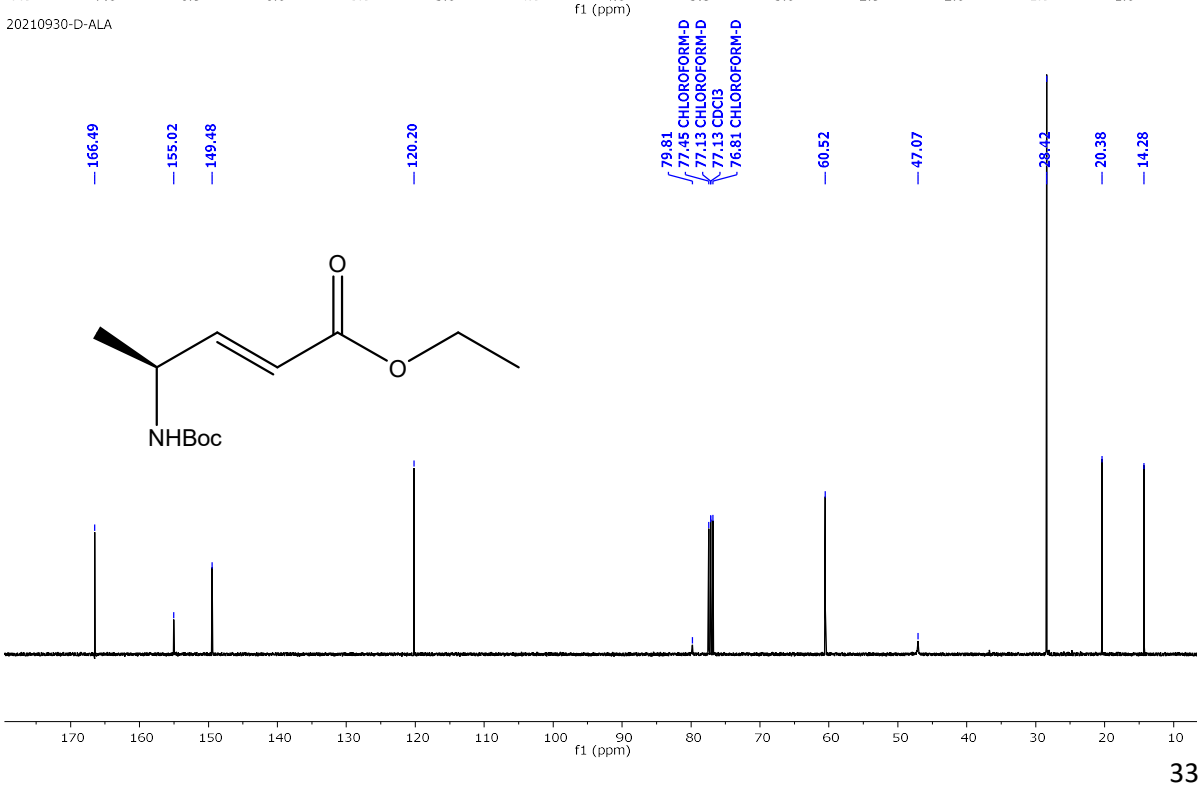
36. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, *et al.* (2004) *Science* 303:844–848.

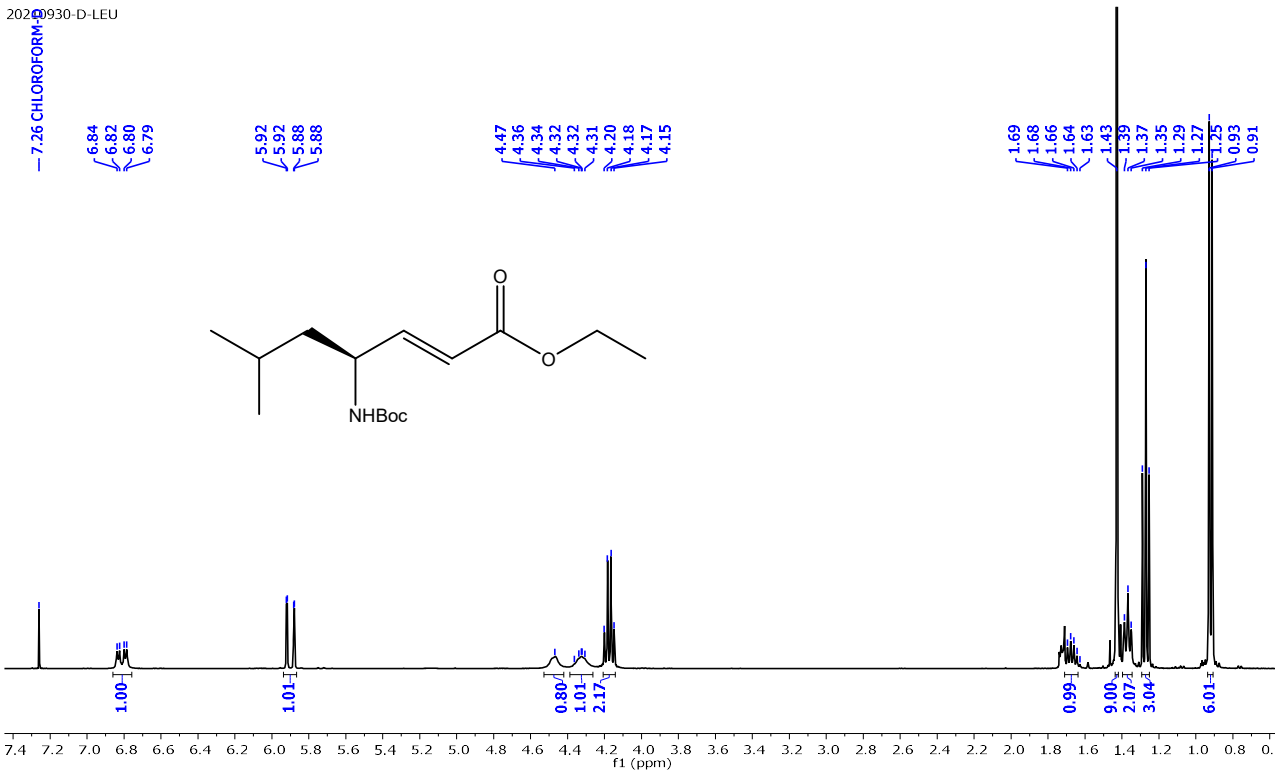
37. Shangary S, Qin D, McEachern D, Liu M, Miller RS, Qiu S, Nikolovska-Coleska Z, Ding K, Wang G, Chen J, Bernard D, Zhang J, Lu Y, Gu Q, Shah RB, Pienta KJ, Ling X, Kang S, Guo M, Sun Y, Yang D, Wang S.. Proc Natl Acad Sci U S A. 2008 Mar 11;105(10):3933-8. doi: 10.1073/pnas.0708917105. Epub 2008 Mar 3. PMID: 18316739; PMCID: PMC2268798..

Appendix

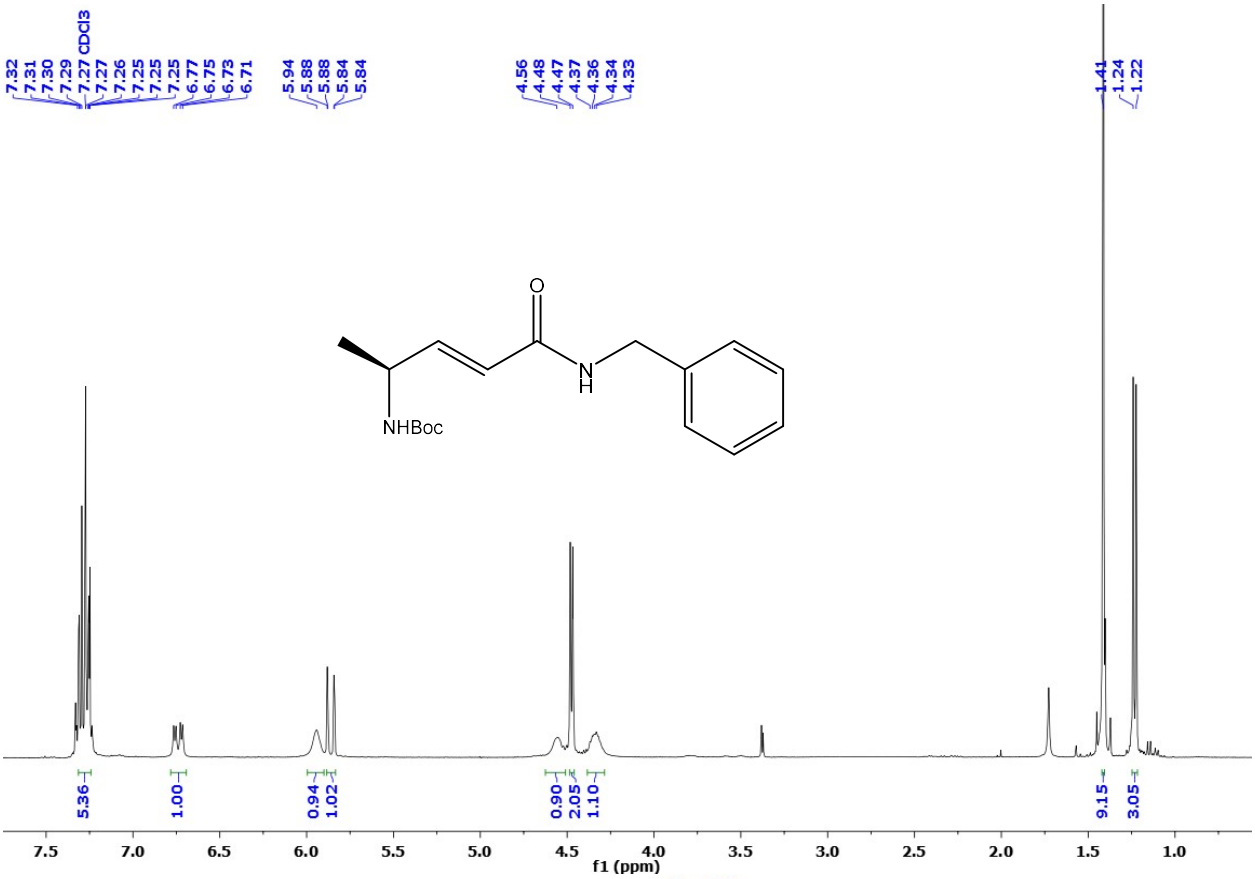
^{1}H and ^{13}C NMR spectra of compounds

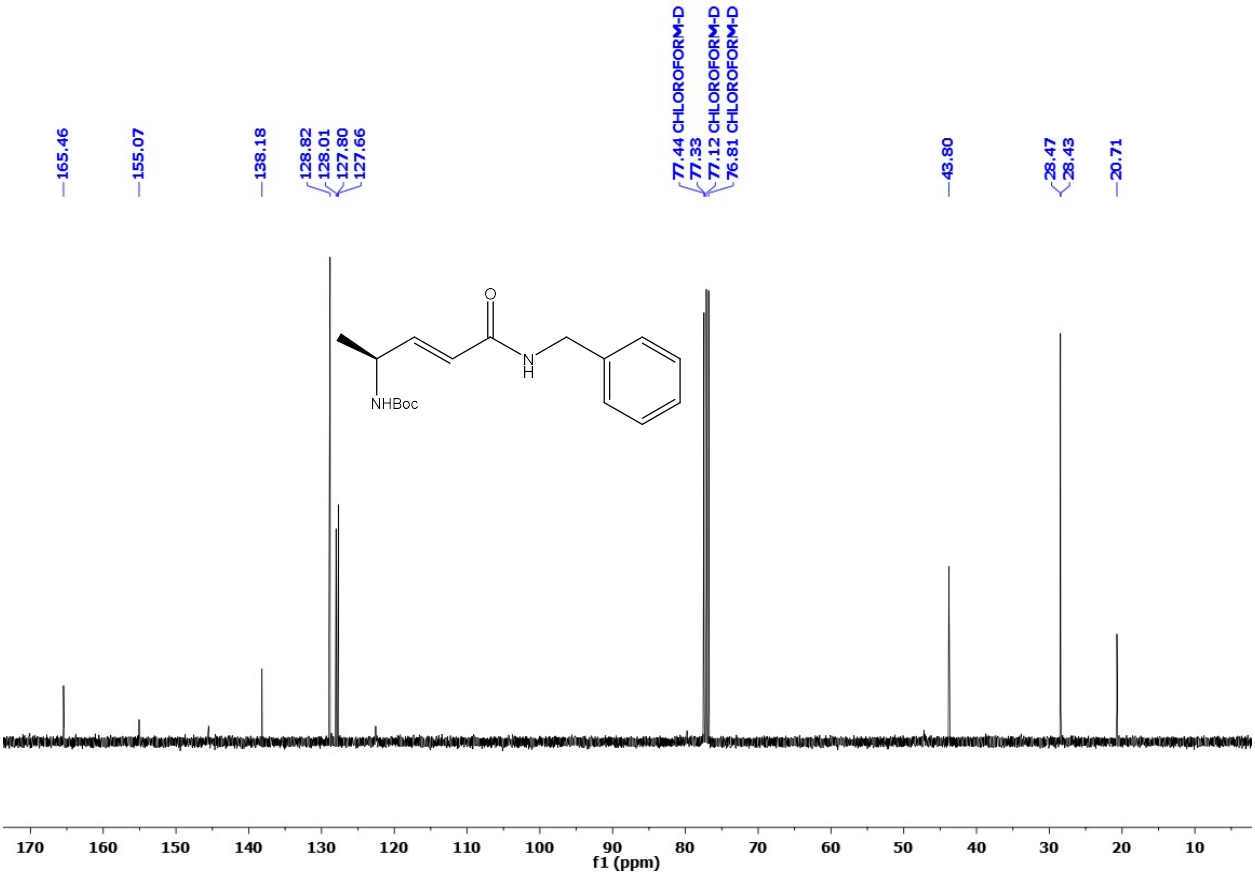


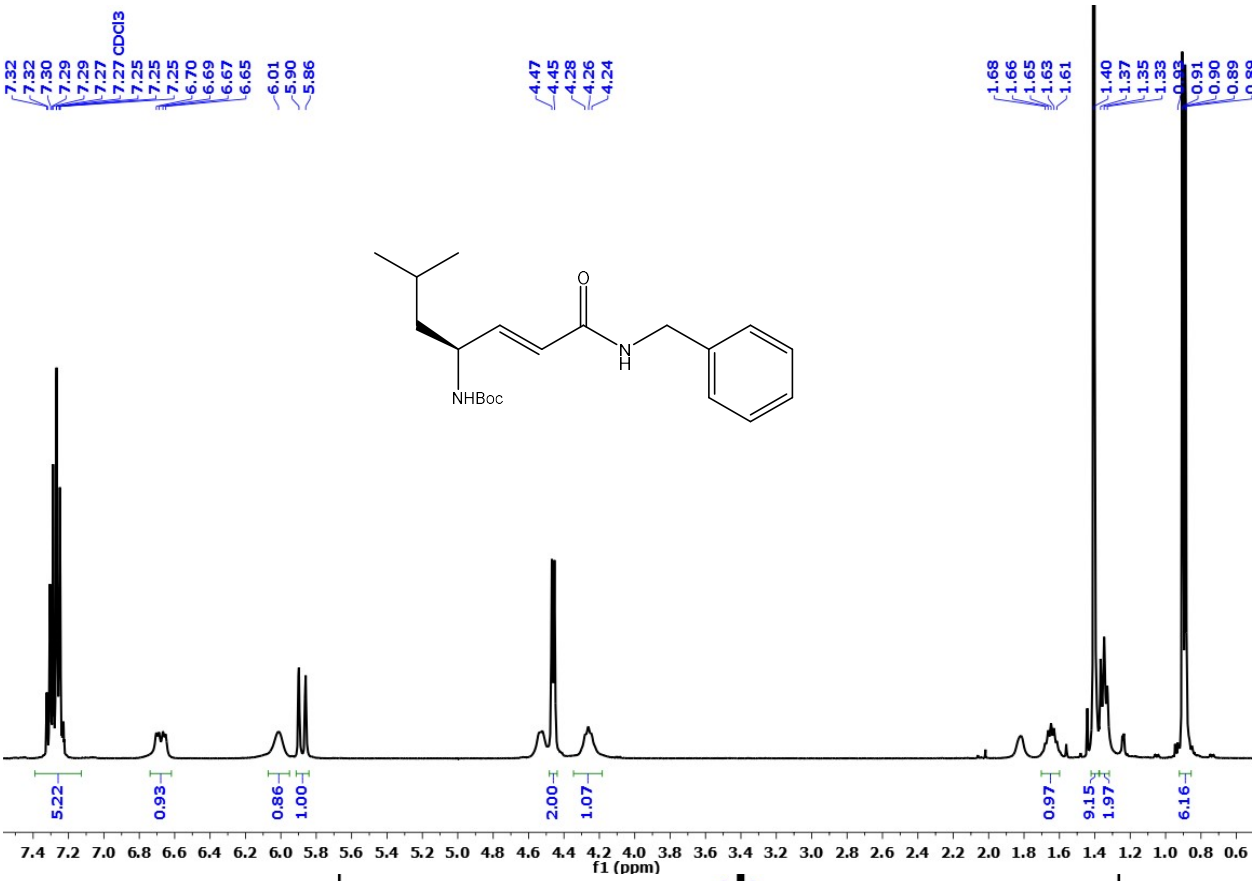


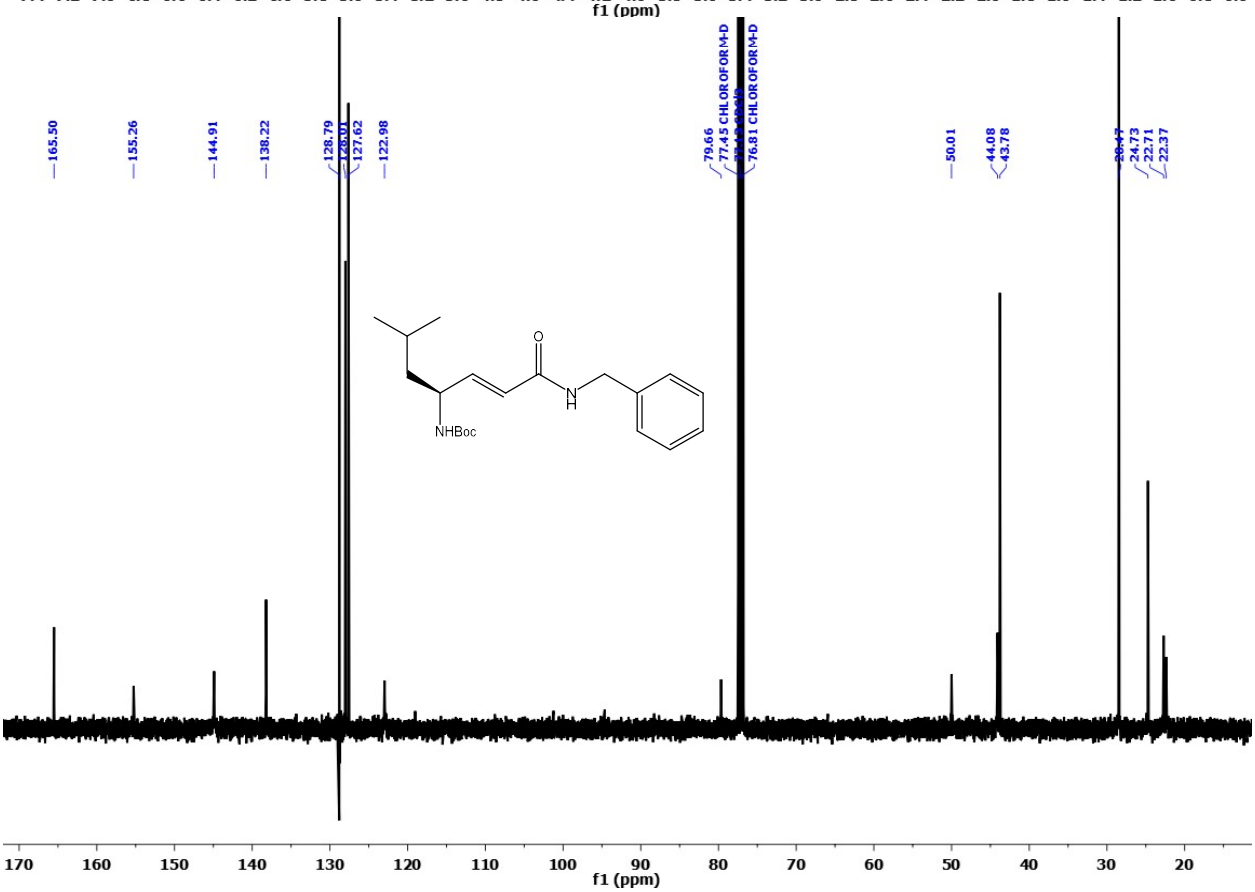

20210930-D-LEU
7.26 CHLOROFORM-D
6.84
6.82
6.80
6.79
5.92
5.92
5.88
5.88
4.47
4.36
4.34
4.32
4.32
4.31
4.20
4.18
4.17
4.15
1.69
1.68
1.66
1.64
1.63
1.43
1.39
1.37
1.35
1.29
1.27
1.25
0.93
0.91
O
O
NHBoc
1.00
1.01
0.80
1.01
2.17
0.99
9.00
2.07
3.04
6.01
f1 (ppm)

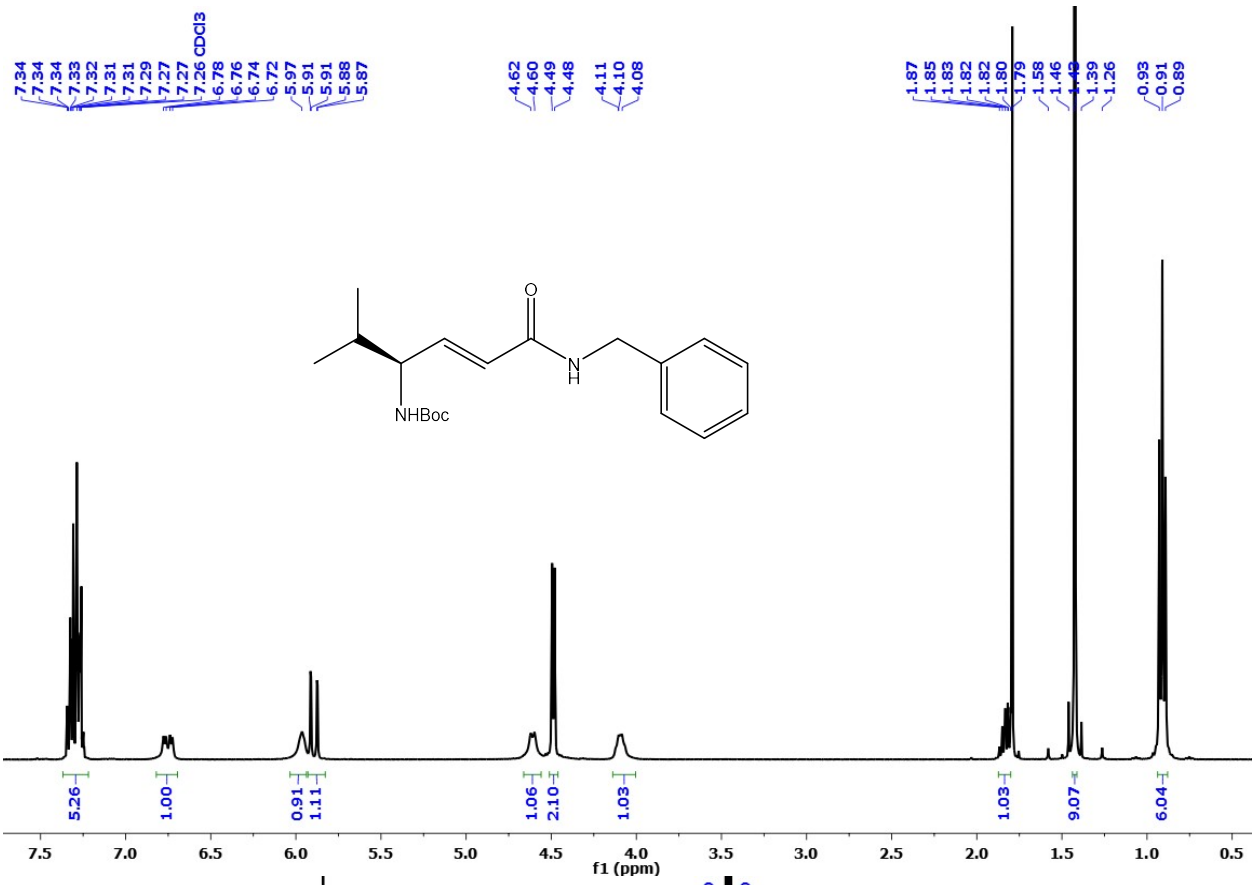

20210930-D-LEU
166.57
155.22
149.04
120.55
79.81
77.48 CHLOROFORM-D
77.16 CHLOROFORM-D
76.84 CHLOROFORM-D
60.55
43.97
28.48
24.84
22.83
22.29
14.35
O
O
NHBoc
f1 (ppm)

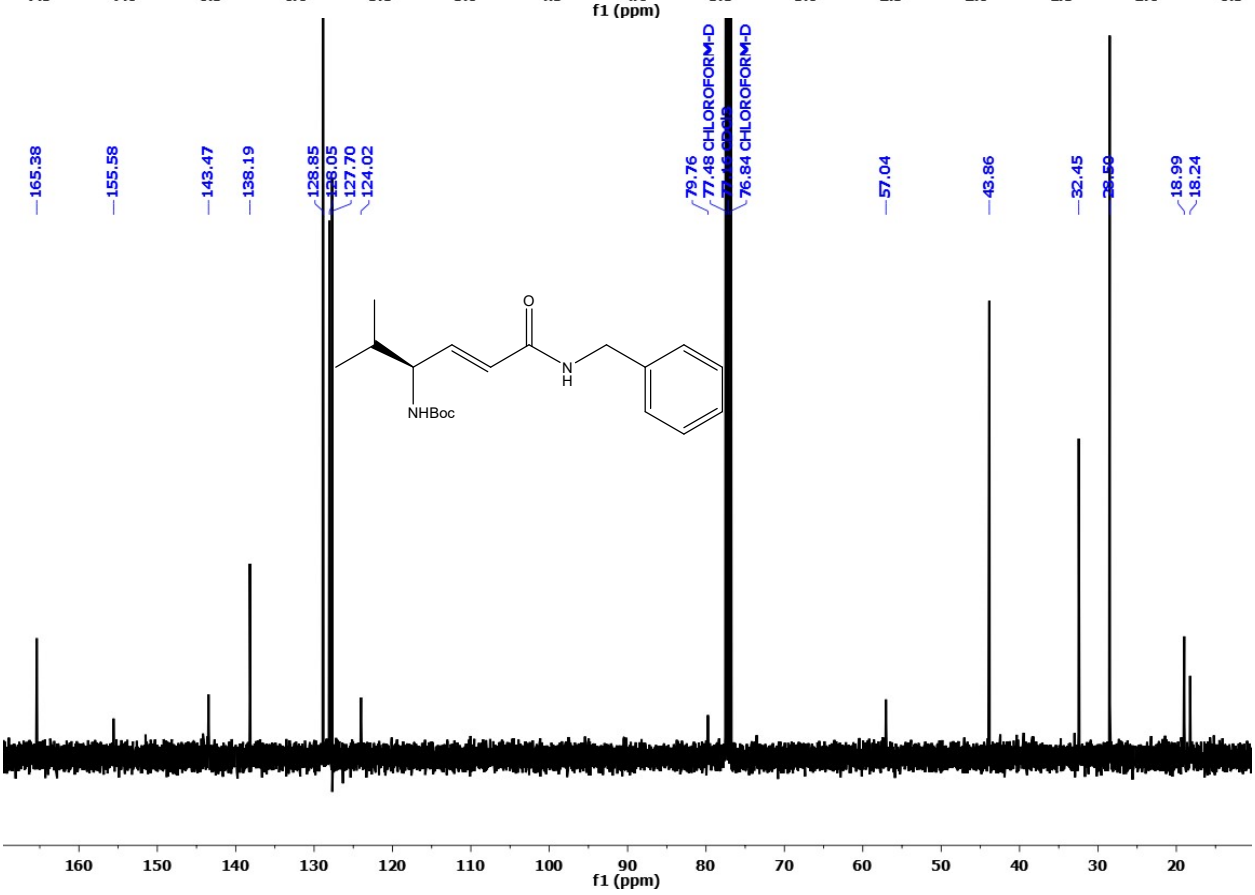

7.32
7.31
7.30
7.29
7.27 CDCl3
7.27
7.26
7.25
7.25
7.25
6.77
6.75
6.73
6.71
5.94
5.88
5.88
5.84
5.84
4.56
4.48
4.47
4.37
4.36
4.34
4.33
1.41
1.24
1.22
O
N
H
NHBoc
5.36
1.00
0.94
1.02
0.90
2.05
1.10
9.15
3.05
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

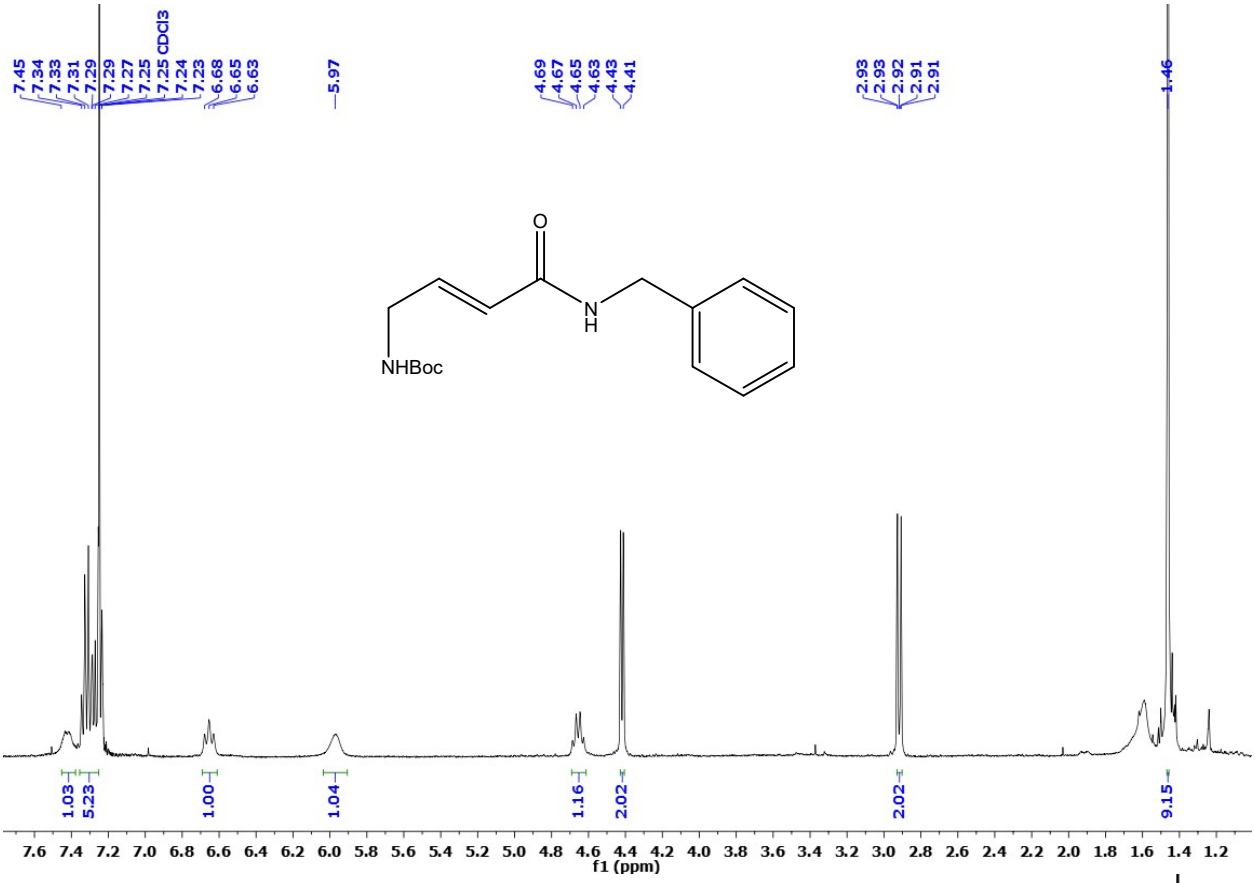

165.46
155.07
138.18
128.82
128.01
127.80
127.66
77.44 CHLOROFORM-D
77.33
77.12 CHLOROFORM-D
76.81 CHLOROFORM-D
43.80
28.47
28.43
20.71
O
N
H
NHBoc
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

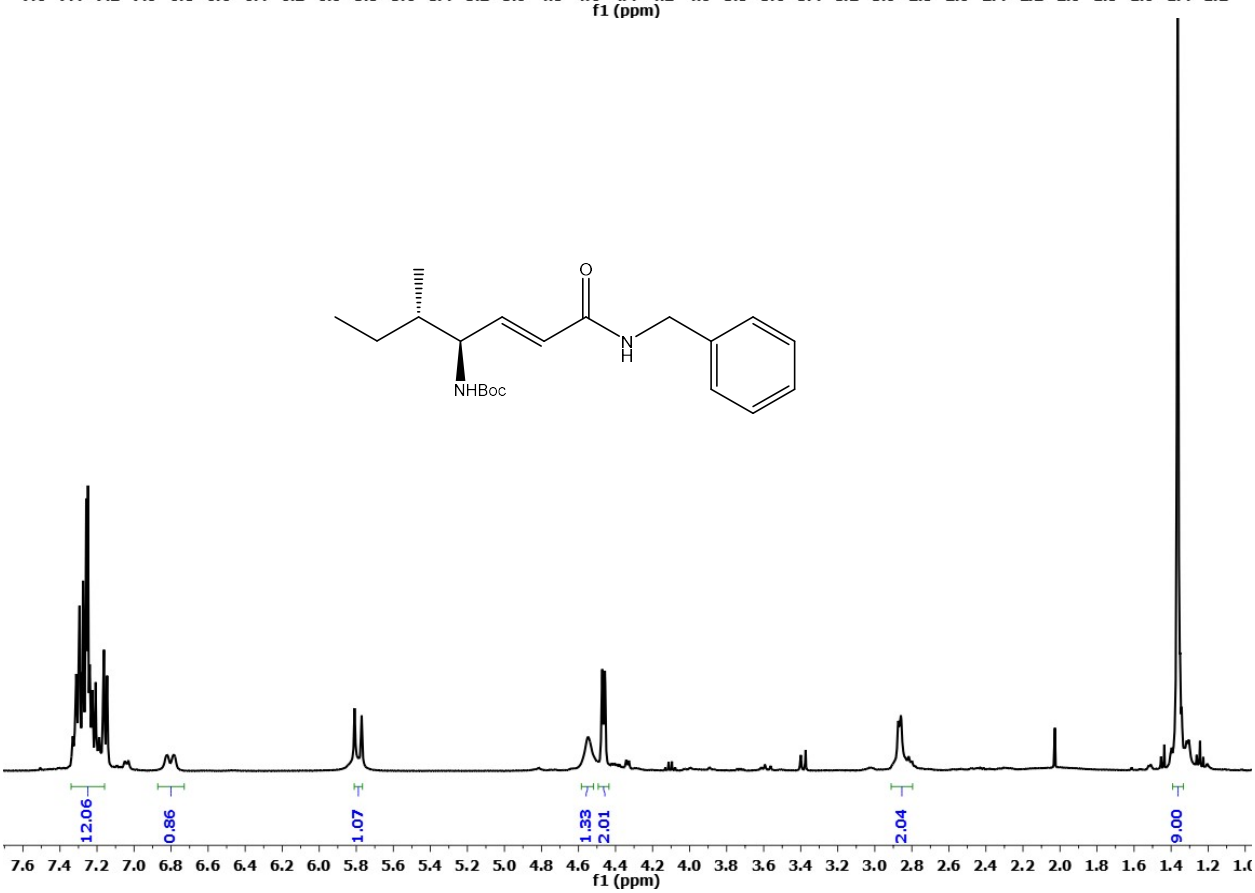

7.32
7.32
7.30
7.29
7.29
7.27
7.27 CDCl3
7.25
7.25
7.25
6.70
6.69
6.67
6.65
6.01
5.90
5.86
4.47
4.45
4.28
4.26
4.24
1.68
1.66
1.65
1.63
1.61
1.40
1.37
1.35
1.33
0.93
0.91
0.90
0.89
NHBoc
O
N
H
5.22
0.93
0.86
1.00
2.00
1.07
0.97
9.15
1.97
6.16
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6
f1 (ppm)

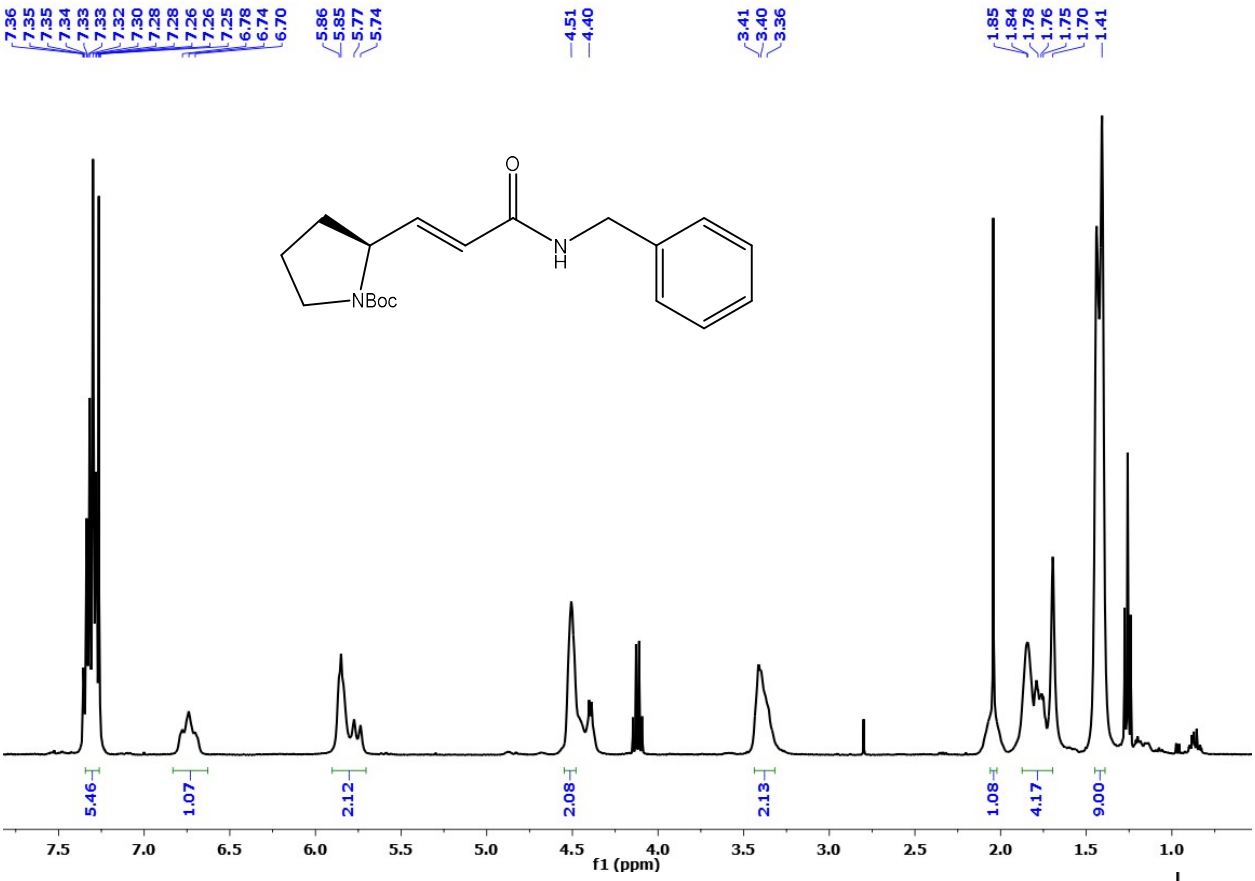

165.50
155.26
144.91
138.22
128.79
128.01
127.62
122.98
79.66
77.45 CHLOROFORM-D
76.81 CHLOROFORM-D
50.01
44.08
43.78
28.47
24.73
22.71
22.37
NHBoc
O
N
H
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

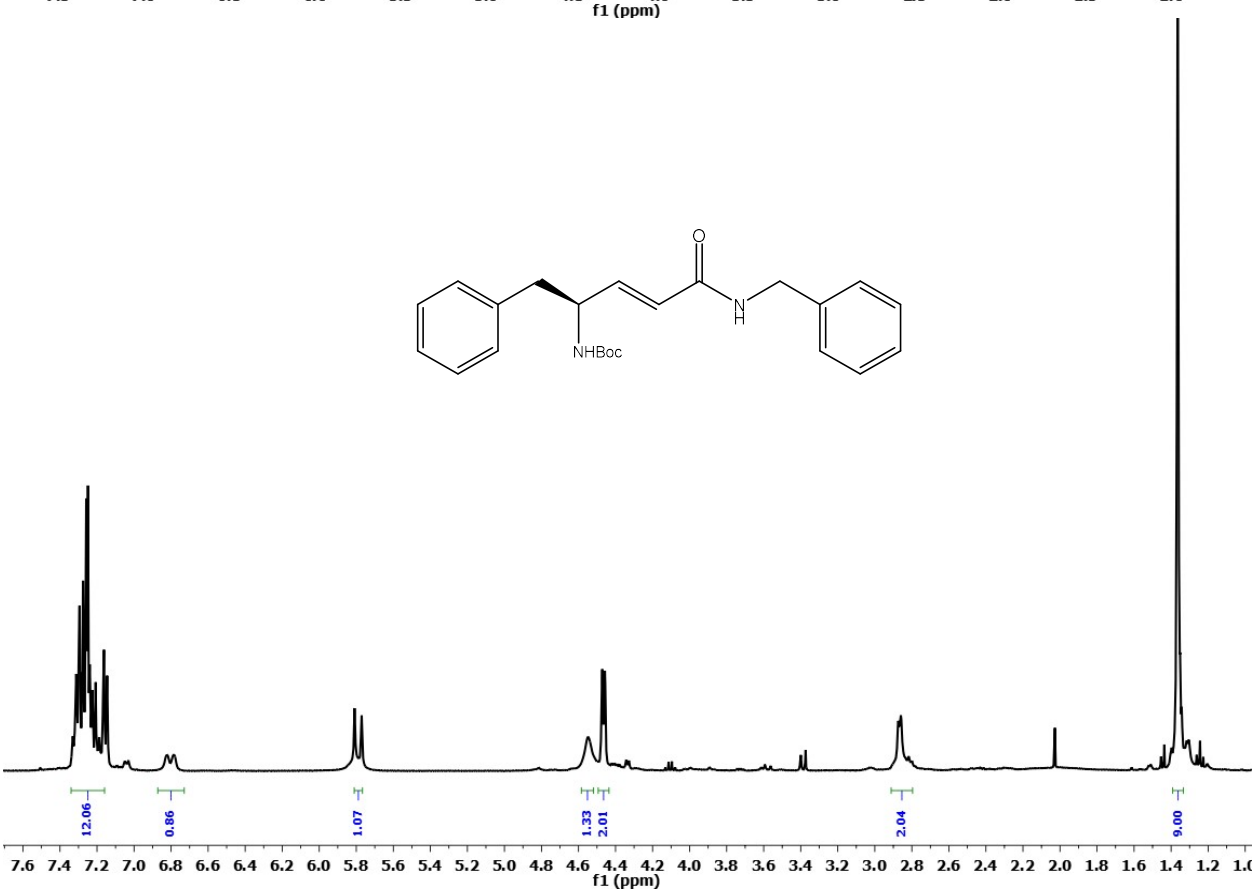

7.34
7.34
7.34
7.33
7.32
7.31
7.31
7.29
7.27
7.27
7.26 CDCl3
6.78
6.76
6.74
6.72
5.97
5.91
5.91
5.88
5.87
4.62
4.60
4.49
4.48
4.11
4.10
4.08
1.87
1.85
1.83
1.82
1.82
1.80
1.79
1.58
1.46
1.39
1.26
0.93
0.91
0.89
O
N
H
NHBoc
5.26
1.00
0.91
1.11
1.06
2.10
1.03
1.03
9.07
6.04
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

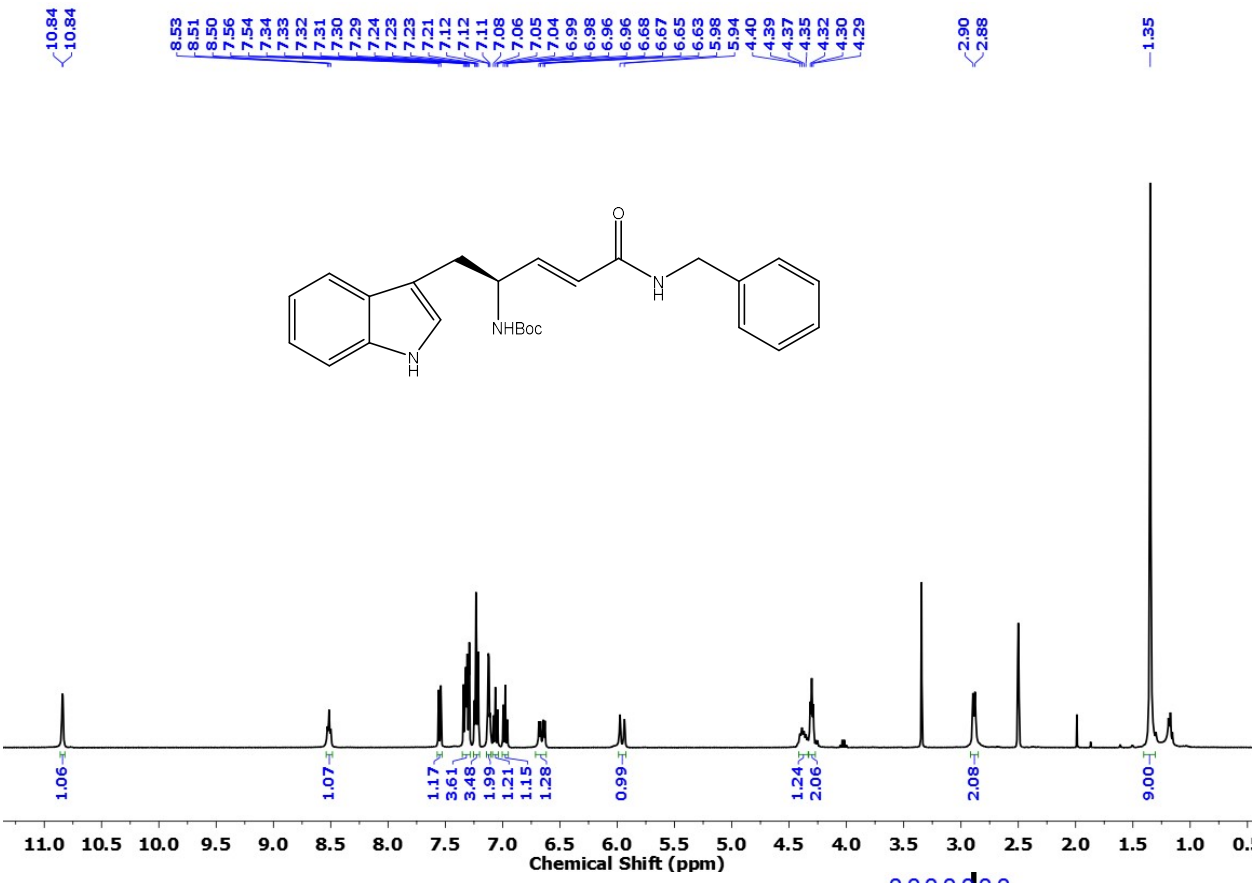

165.38
155.58
143.47
138.19
128.85
128.05
127.70
124.02
79.76
77.48 CHLOROFORM-D
76.84 CHLOROFORM-D
57.04
43.86
32.45
28.50
18.99
18.24
O
N
H
NHBoc
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

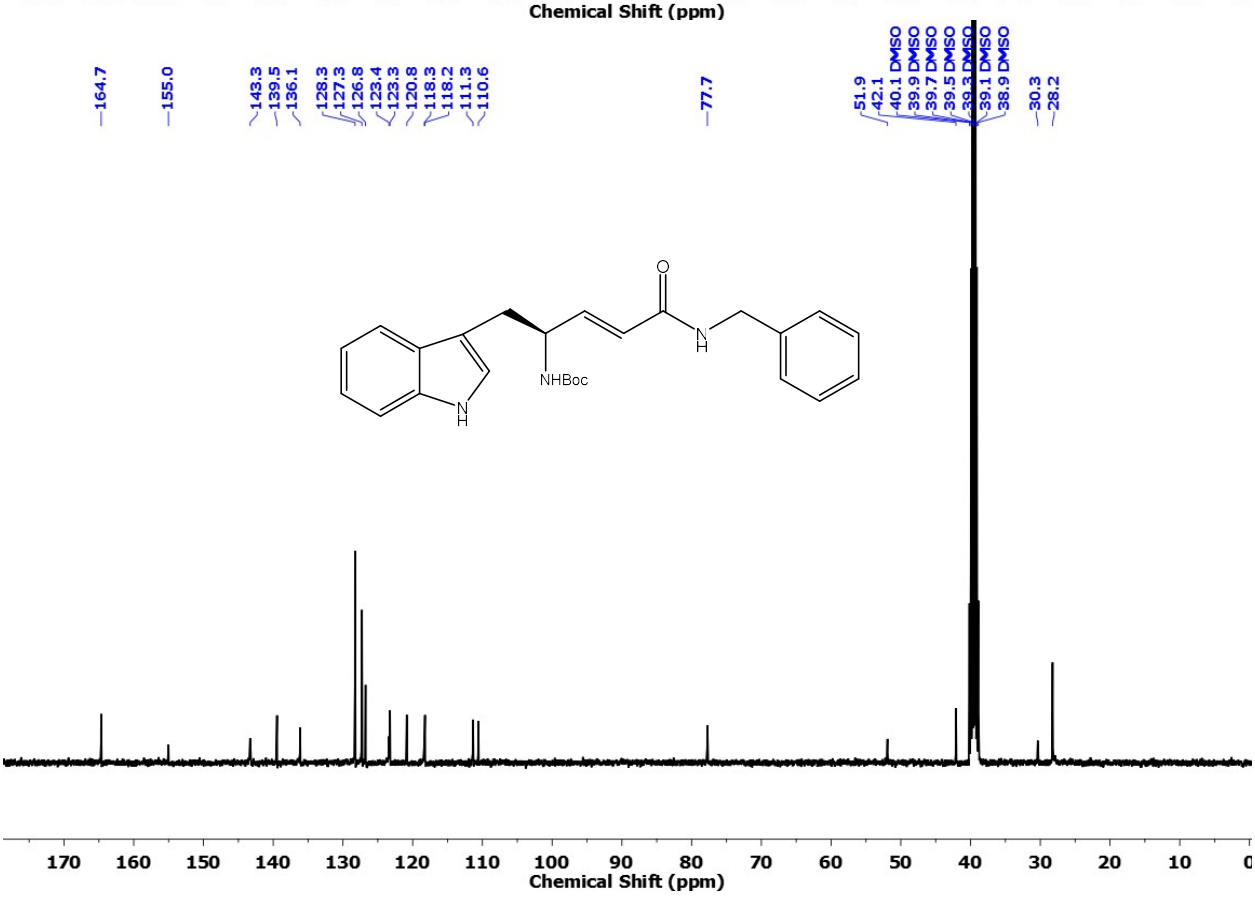

7.45
7.34
7.33
7.31
7.29
7.29
7.27
7.25
CDCl3
7.25
7.24
7.23
6.68
6.65
6.63
5.97
4.69
4.67
4.65
4.63
4.43
4.41
2.93
2.93
2.92
2.91
2.91
1.46
O
N
H
NHBoc
1.03
5.23
1.00
1.04
1.16
2.02
2.02
9.15
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
f1 (ppm)

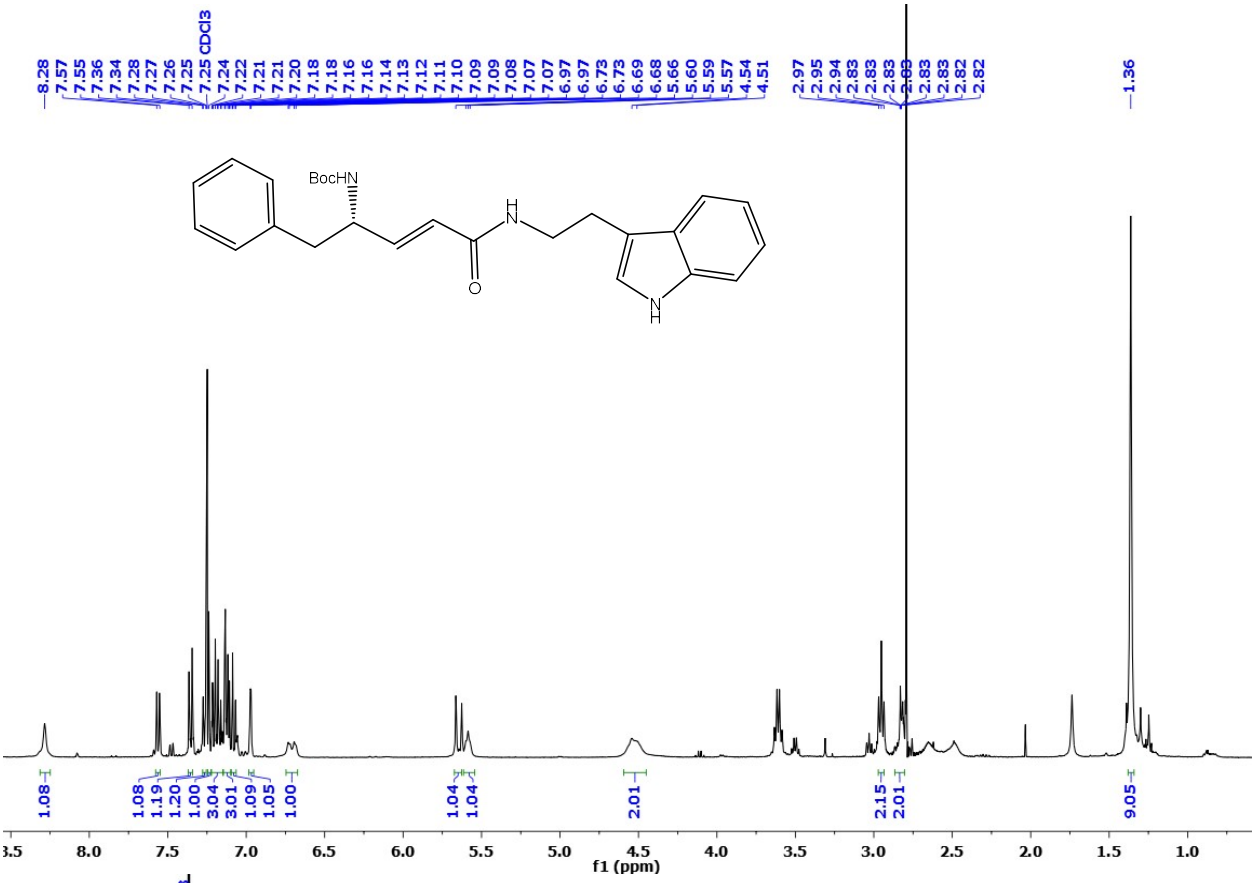

O
N
H
NHBoc
12.06
0.86
1.07
1.33
2.01
2.04
9.00
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
f1 (ppm)

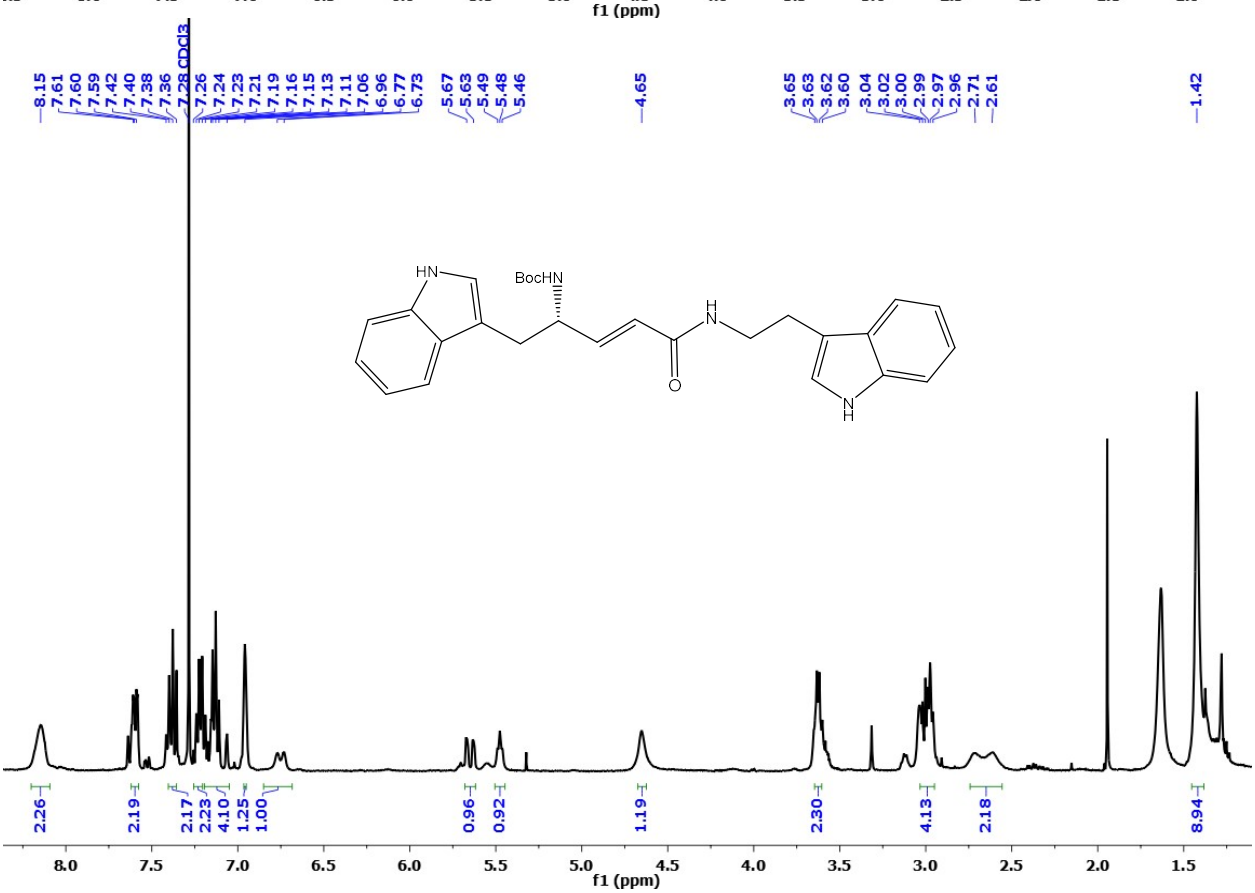

7.36
7.35
7.35
7.34
7.33
7.33
7.32
7.30
7.28
7.28
7.26
7.26
7.25
6.78
6.74
6.70
5.86
5.85
5.77
5.74
4.51
4.40
3.41
3.40
3.36
1.85
1.84
1.78
1.76
1.75
1.70
1.41
O
N
H
NBoc
5.46
1.07
2.12
2.08
2.13
1.08
4.17
9.00
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

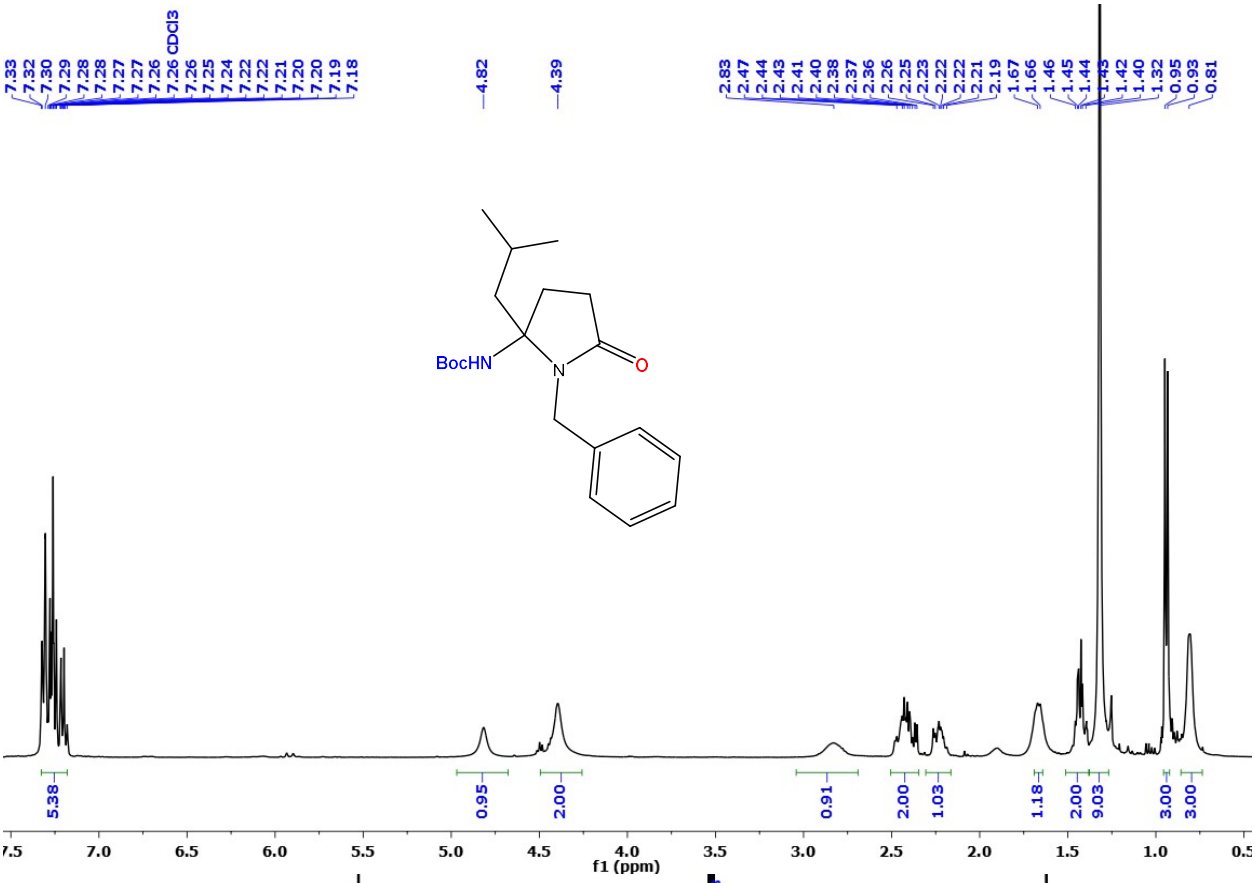

O
N
H
NHBoc
12.06
0.86
1.07
1.33
2.01
2.04
9.00
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
f1 (ppm)

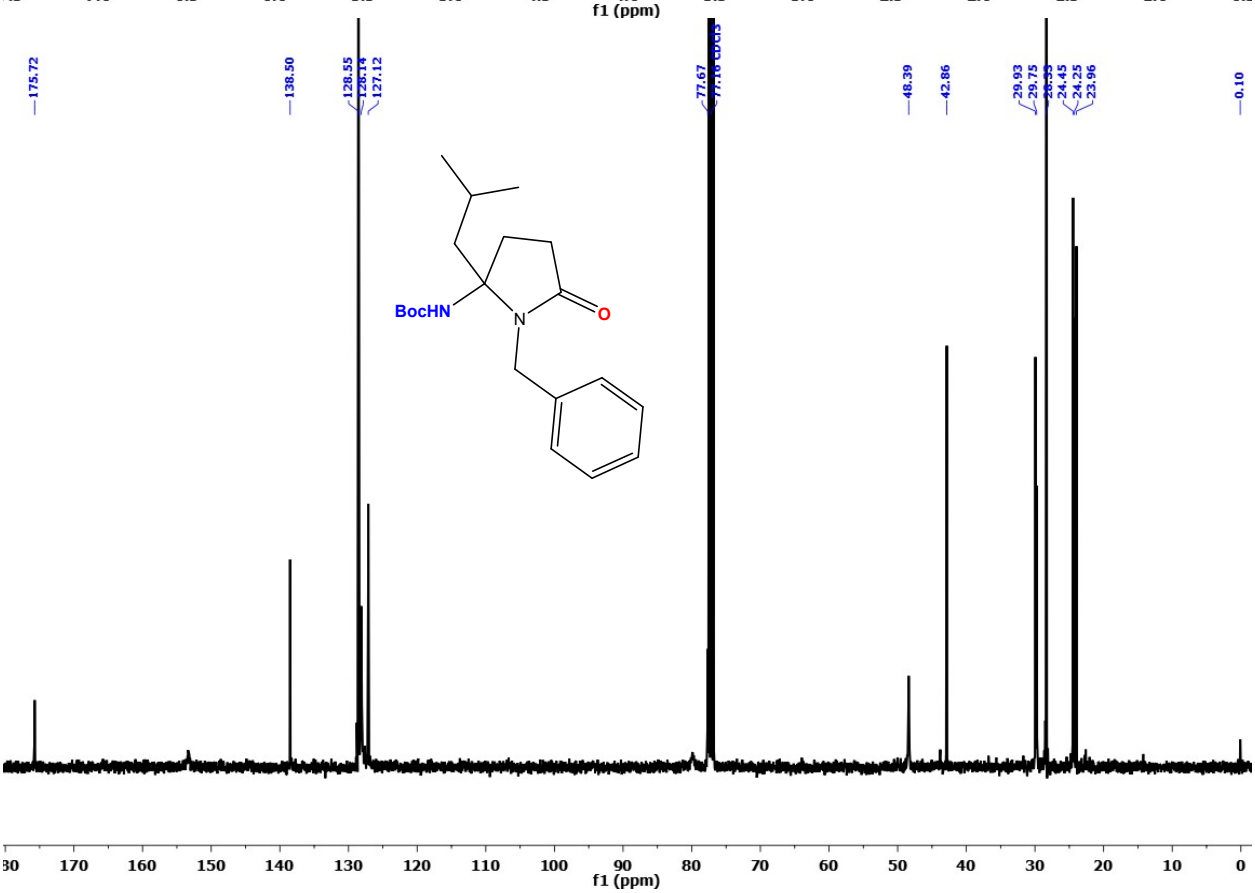

10.84
10.84
8.53
8.51
8.50
7.56
7.54
7.34
7.33
7.32
7.31
7.30
7.29
7.24
7.23
7.23
7.21
7.12
7.12
7.11
7.08
7.06
7.05
7.04
6.99
6.98
6.96
6.96
6.68
6.67
6.65
6.63
5.98
5.94
4.40
4.39
4.37
4.35
4.32
4.30
4.29
2.90
2.88
1.35
O
N
H
NHBoc
N
H
1.06
1.07
1.17
3.61
3.48
1.99
1.21
1.15
1.28
0.99
1.24
2.06
2.08
9.00
11.0 10.5 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
Chemical Shift (ppm)

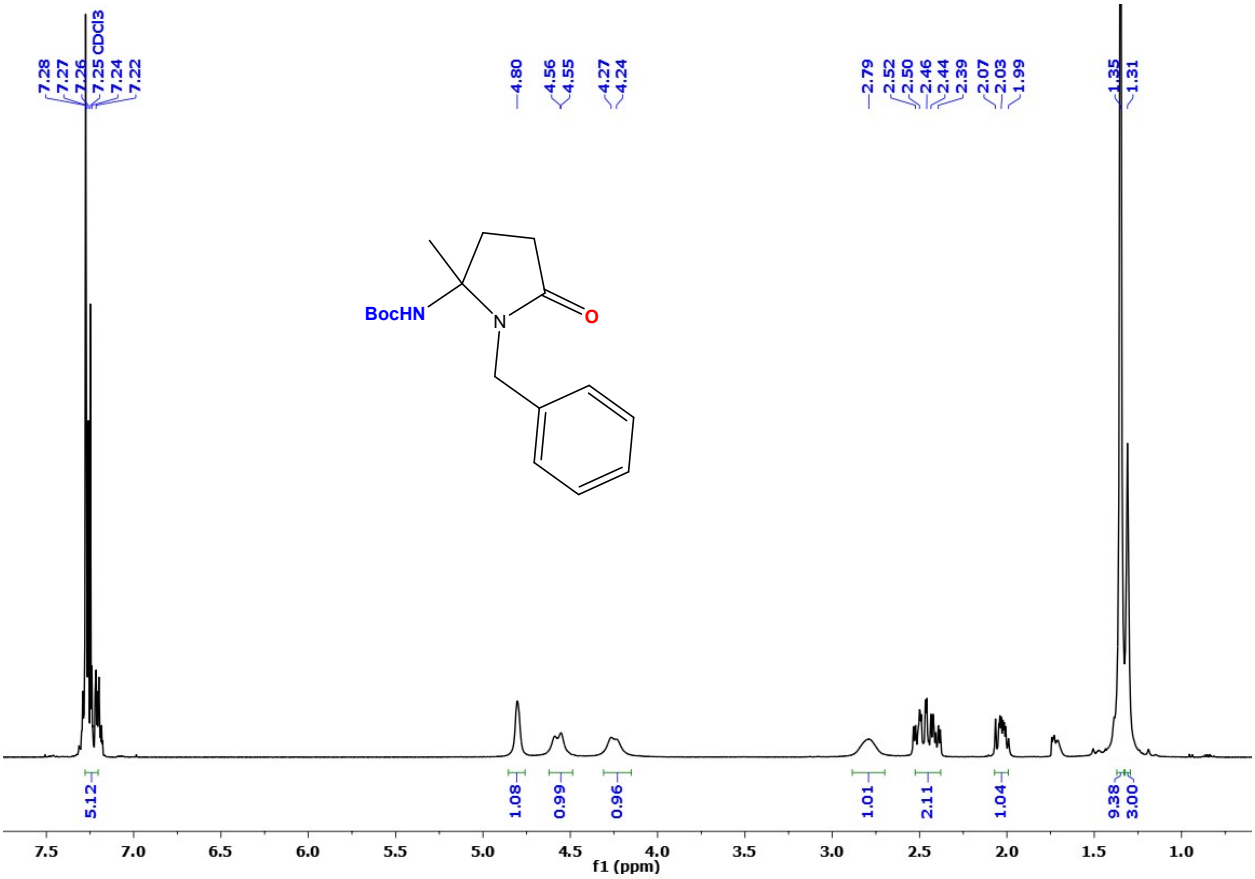

164.7
155.0
143.3
139.5
136.1
128.3
127.3
126.8
123.4
123.3
120.8
118.3
118.2
111.3
110.6
77.7
51.9
42.1
40.1 DMSO
39.9 DMSO
39.7 DMSO
39.5 DMSO
39.3 DMSO
39.1 DMSO
38.9 DMSO
30.3
28.2
O
N
H
NHBoc
N
H
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
Chemical Shift (ppm)

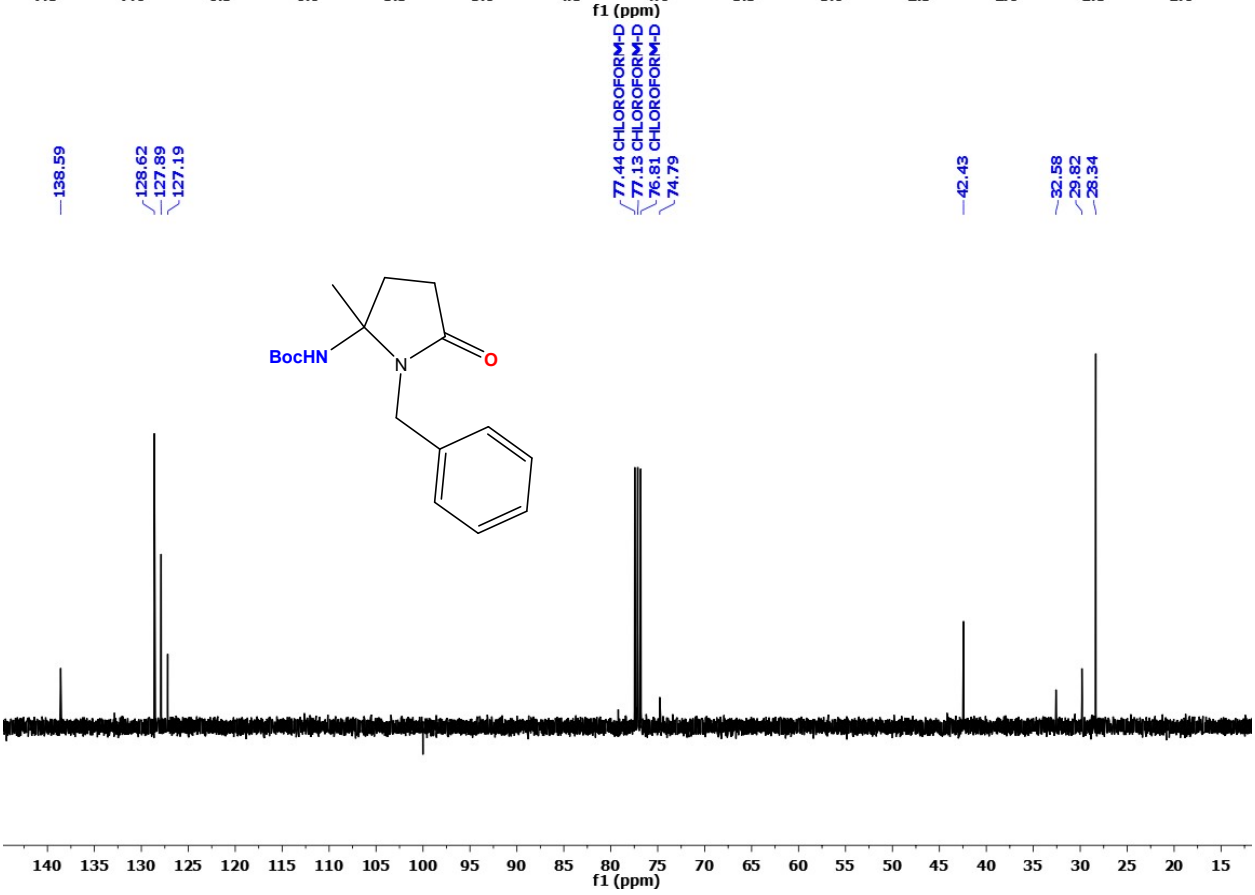

8.28
7.57
7.55
7.36
7.34
7.28
7.27
7.26
7.25
CDCl3
7.24
7.22
7.21
7.21
7.20
7.18
7.18
7.16
7.16
7.14
7.13
7.12
7.11
7.10
7.09
7.09
7.08
7.07
7.07
6.97
6.97
6.73
6.73
6.69
6.68
5.66
5.60
5.59
5.57
4.54
4.51
2.97
2.95
2.94
2.83
2.83
2.83
2.83
2.83
2.82
2.82
1.36
BocHN
H
N
O
N
H
1.08
1.08
1.19
1.20
1.00
3.04
3.01
1.09
1.05
1.00
1.04
1.04
2.01
2.15
2.01
9.05
f1 (ppm)

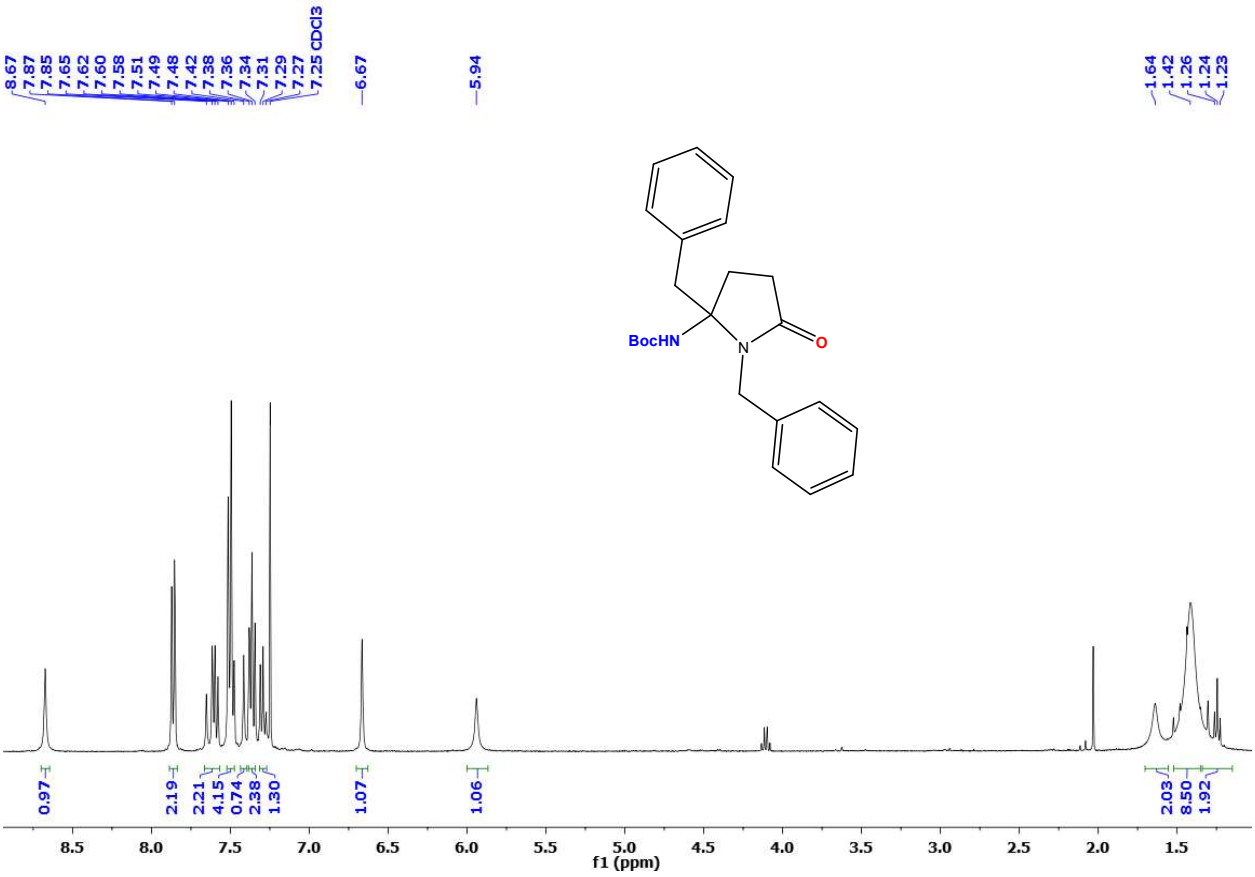

8.15
7.61
7.60
7.59
7.42
7.40
7.38
7.36
7.28
CDCl3
7.26
7.24
7.23
7.21
7.19
7.16
7.15
7.13
7.11
7.06
6.96
6.77
6.73
5.67
5.63
5.49
5.48
5.46
4.65
3.65
3.63
3.62
3.60
3.04
3.02
3.00
2.99
2.97
2.96
2.71
2.61
1.42
HN
BocHN
H
N
O
N
H
2.26
2.19
2.17
2.23
4.10
1.25
1.00
0.96
0.92
1.19
2.30
4.13
2.18
8.94
f1 (ppm)

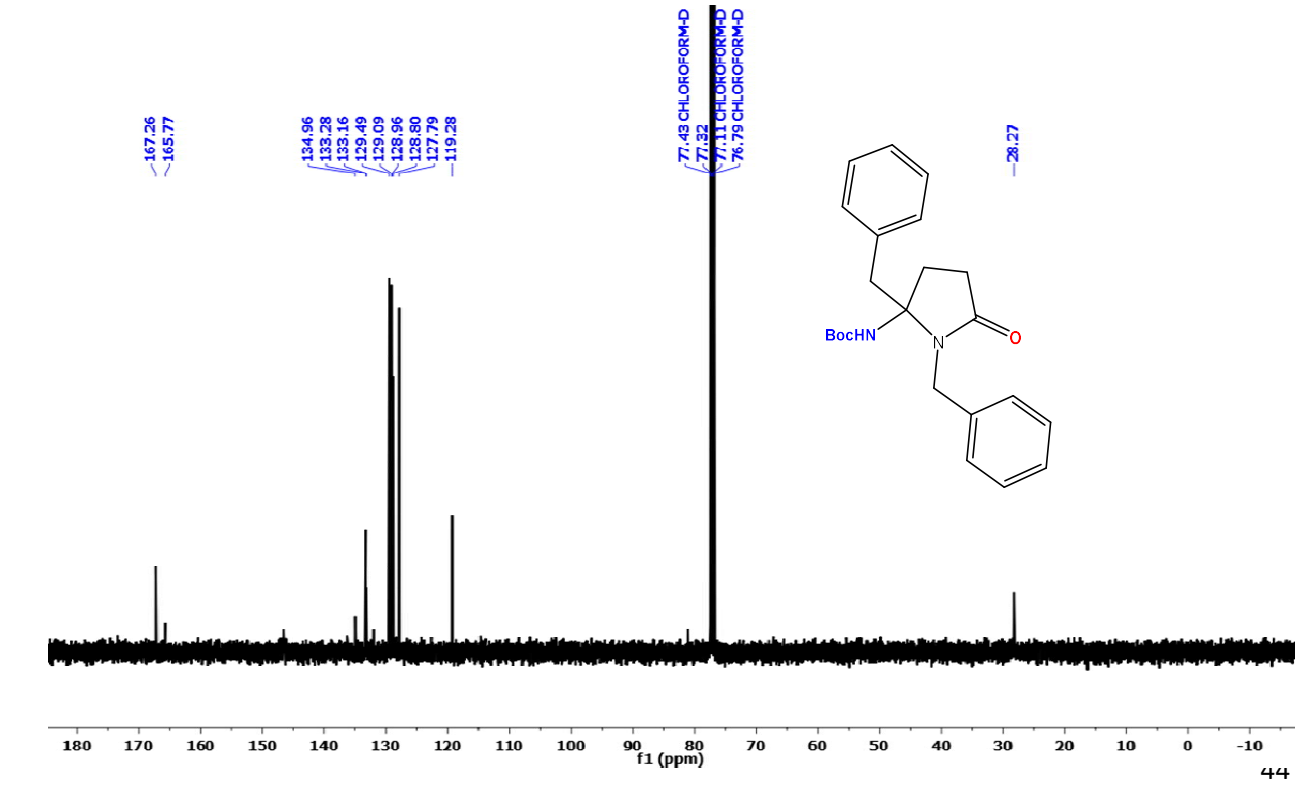

7.33
7.32
7.30
7.29
7.28
7.28
7.27
7.27
7.26
CDCl3
7.26
7.25
7.24
7.22
7.22
7.21
7.20
7.20
7.19
7.18
4.82
4.39
2.83
2.47
2.44
2.43
2.41
2.40
2.38
2.37
2.36
2.26
2.25
2.23
2.22
2.22
2.21
2.19
1.67
1.66
1.46
1.45
1.44
1.43
1.42
1.40
1.32
0.95
0.93
0.81
BocHN
N
O
5.38
0.95
2.00
0.91
2.00
1.03
1.18
2.00
9.03
3.00
3.00
f1 (ppm)

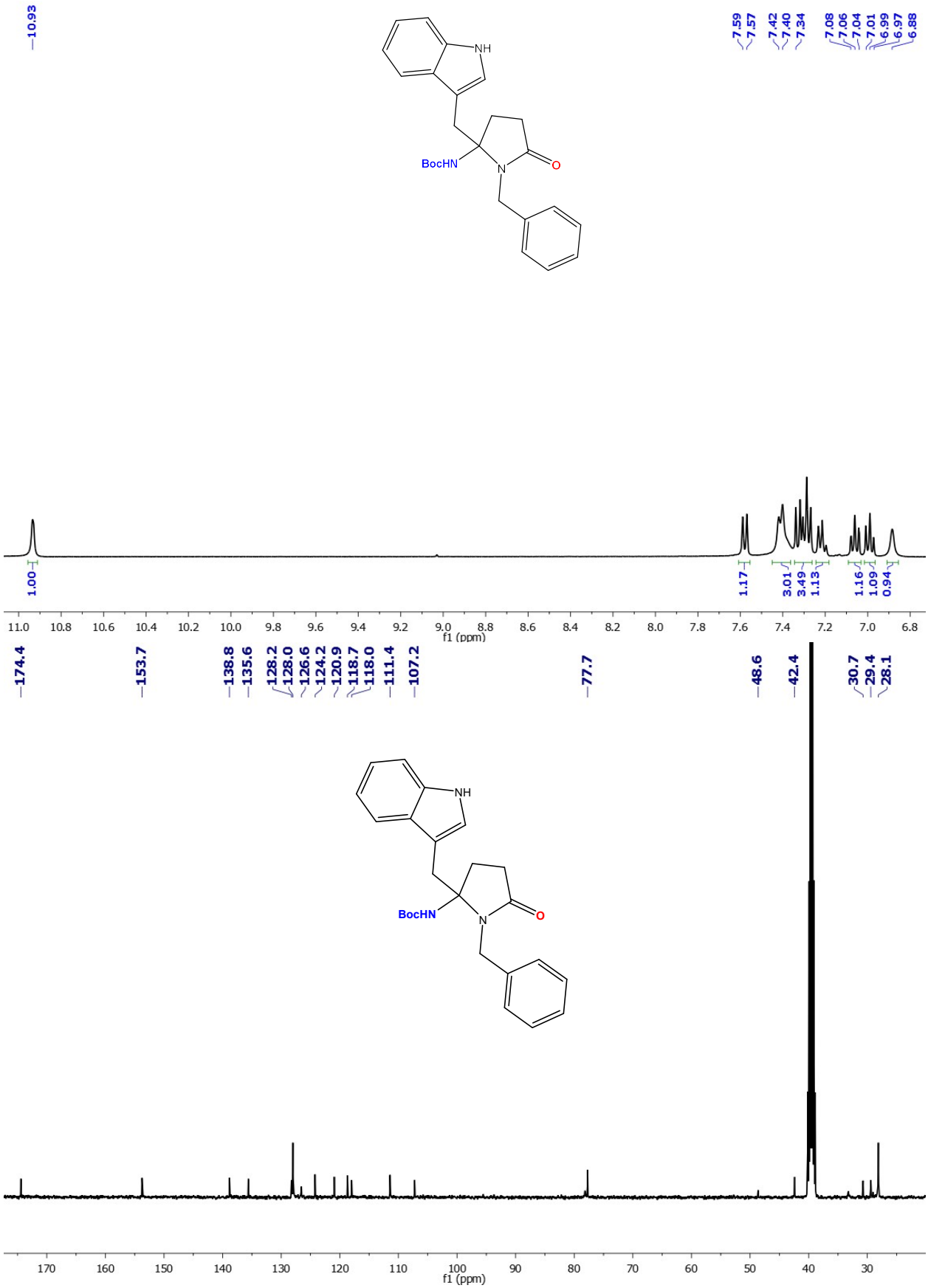

175.72
138.50
128.55
128.14
127.12
77.67
48.39
42.86
29.93
29.75
24.45
24.25
23.96
0.10
BocHN
N
O
f1 (ppm)


7.28
7.27
7.26
7.25 CDCl3
7.24
7.22
4.80
4.56
4.55
4.27
4.24
2.79
2.52
2.50
2.46
2.44
2.39
2.07
2.03
1.99
1.35
1.31
BocHN
O
N
5.12
1.08
0.99
0.96
1.01
2.11
1.04
9.38
3.00
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
f1 (ppm)

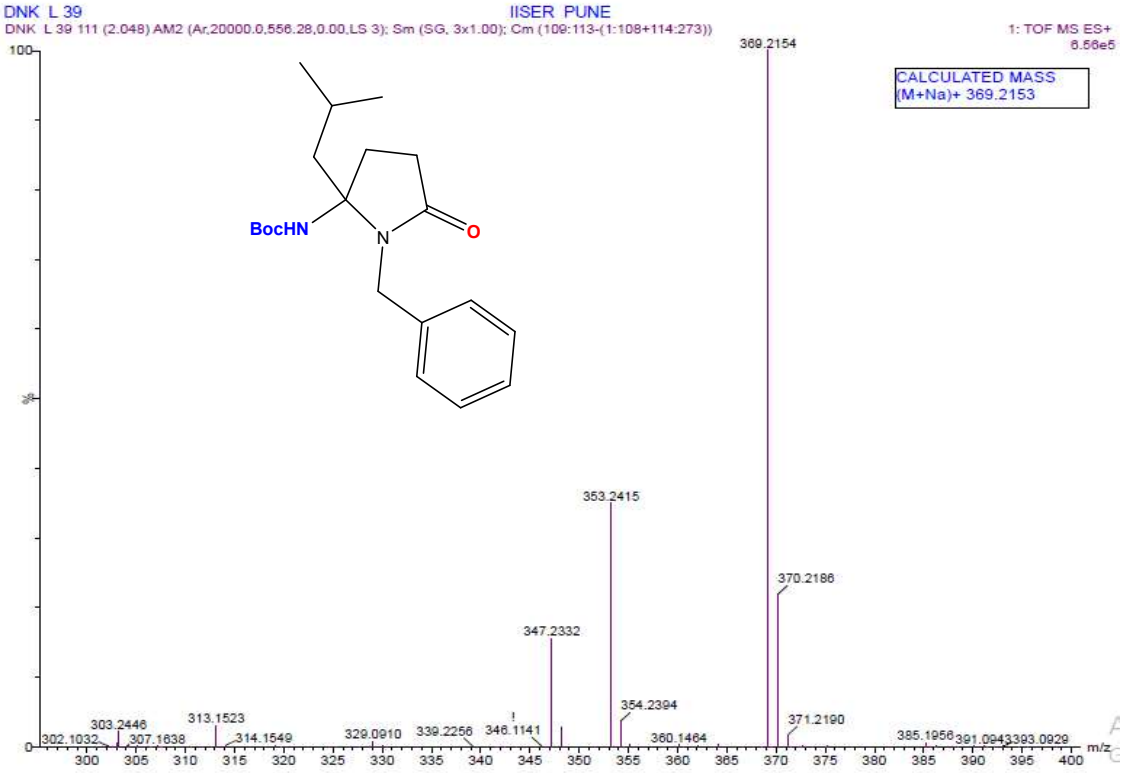

138.59
128.62
127.89
127.19
77.44 CHLOROFORM-D
77.13 CHLOROFORM-D
76.81 CHLOROFORM-D
74.79
42.43
32.58
29.82
28.34
BocHN
O
N
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
f1 (ppm)

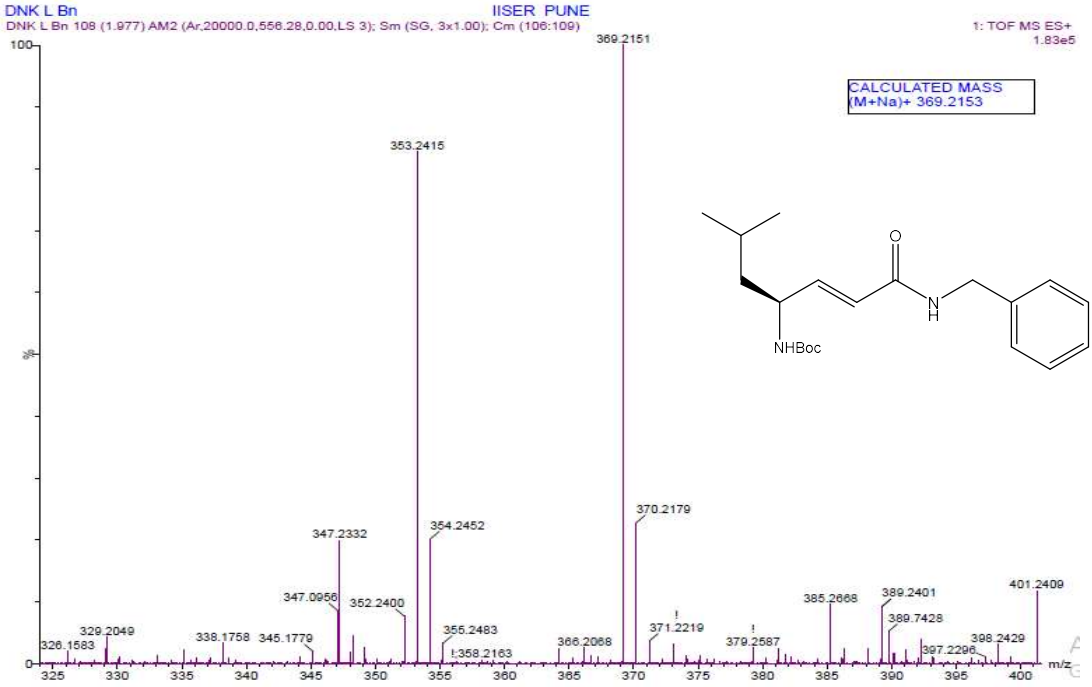

8.67
7.87
7.85
7.65
7.62
7.60
7.58
7.51
7.49
7.48
7.42
7.38
7.36
7.34
7.31
7.29
7.27
7.25 CDCl3
6.67
5.94
1.64
1.42
1.26
1.24
1.23
BocHN
N
O
0.97
2.19
2.21
4.15
0.74
2.38
1.30
1.07
1.06
2.03
8.50
1.92
f1 (ppm)

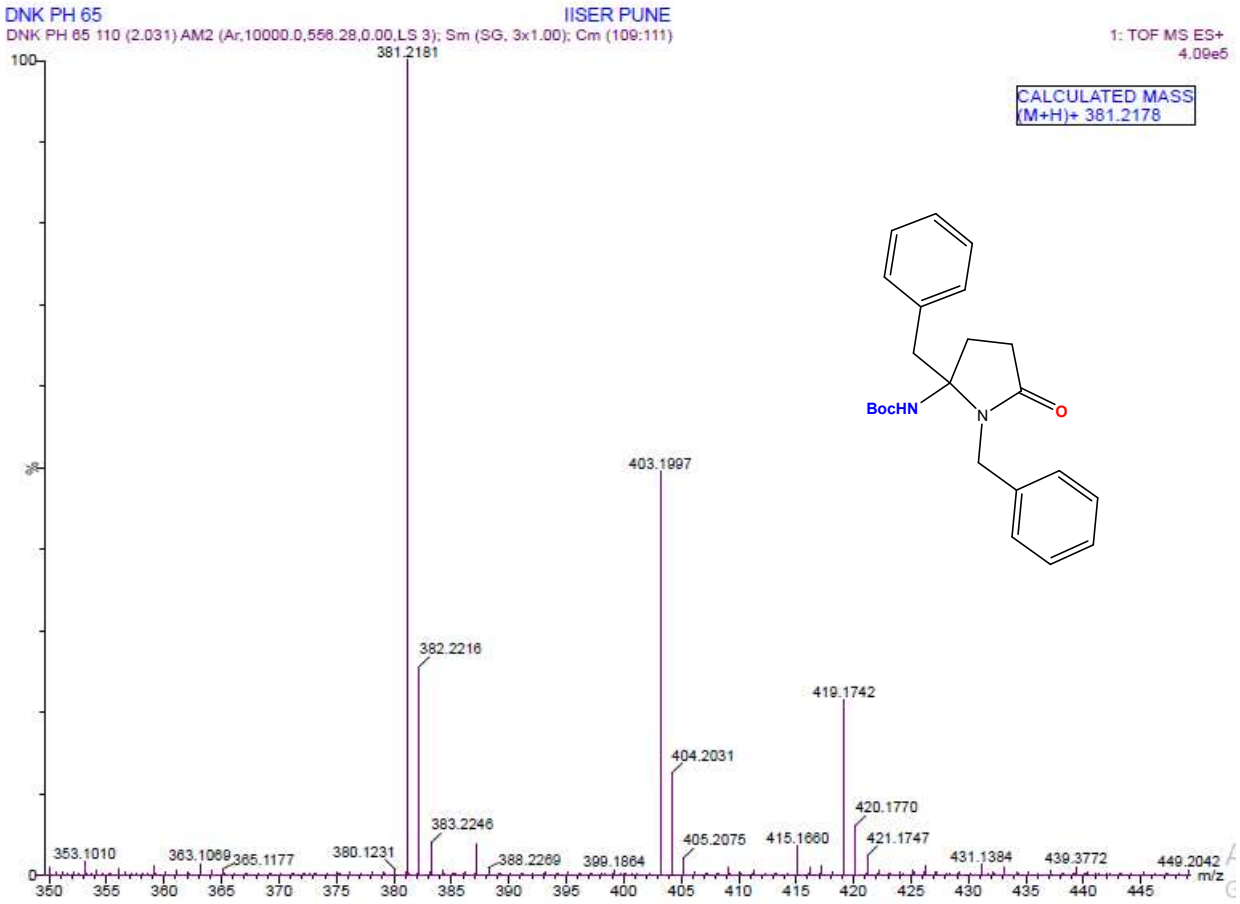

167.26
165.77
134.96
133.28
133.16
129.49
129.09
128.96
128.80
127.79
119.28
77.43 CHLOROFORM-D
77.32
77.11 CHLOROFORM-D
76.79 CHLOROFORM-D
28.27
BocHN
N
O
f1 (ppm)

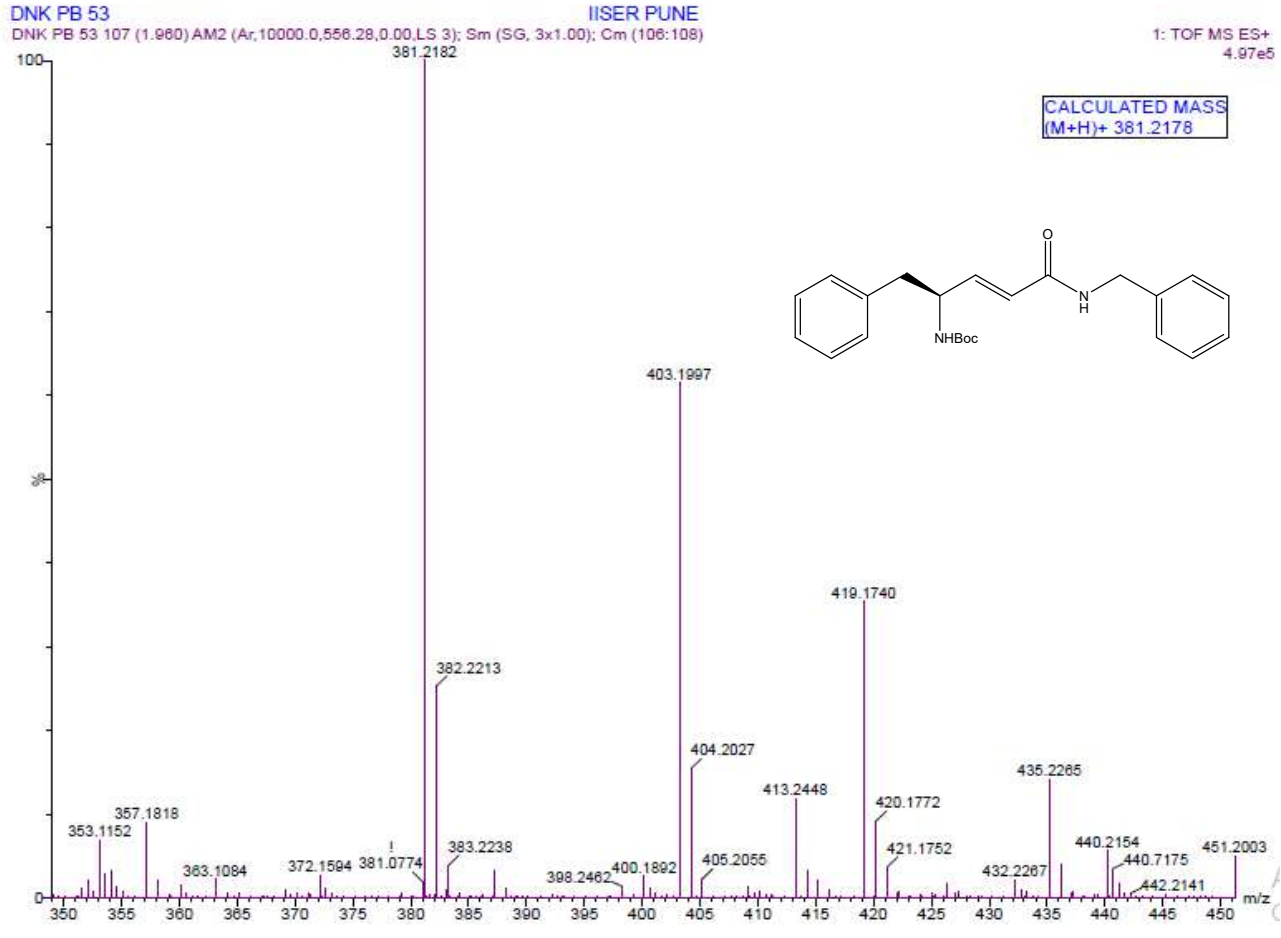

10.93
7.59
7.57
7.42
7.40
7.34
7.08
7.06
7.04
7.01
6.99
6.97
6.88
NH
BocHN
N
O
1.00
1.17
3.01
3.49
1.13
1.16
1.09
0.94
11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
f1 (ppm)
174.4
153.7
138.8
135.6
128.2
128.0
126.6
124.2
120.9
118.7
118.0
111.4
107.2
77.7
48.6
42.4
30.7
29.4
28.1
NH
BocHN
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)

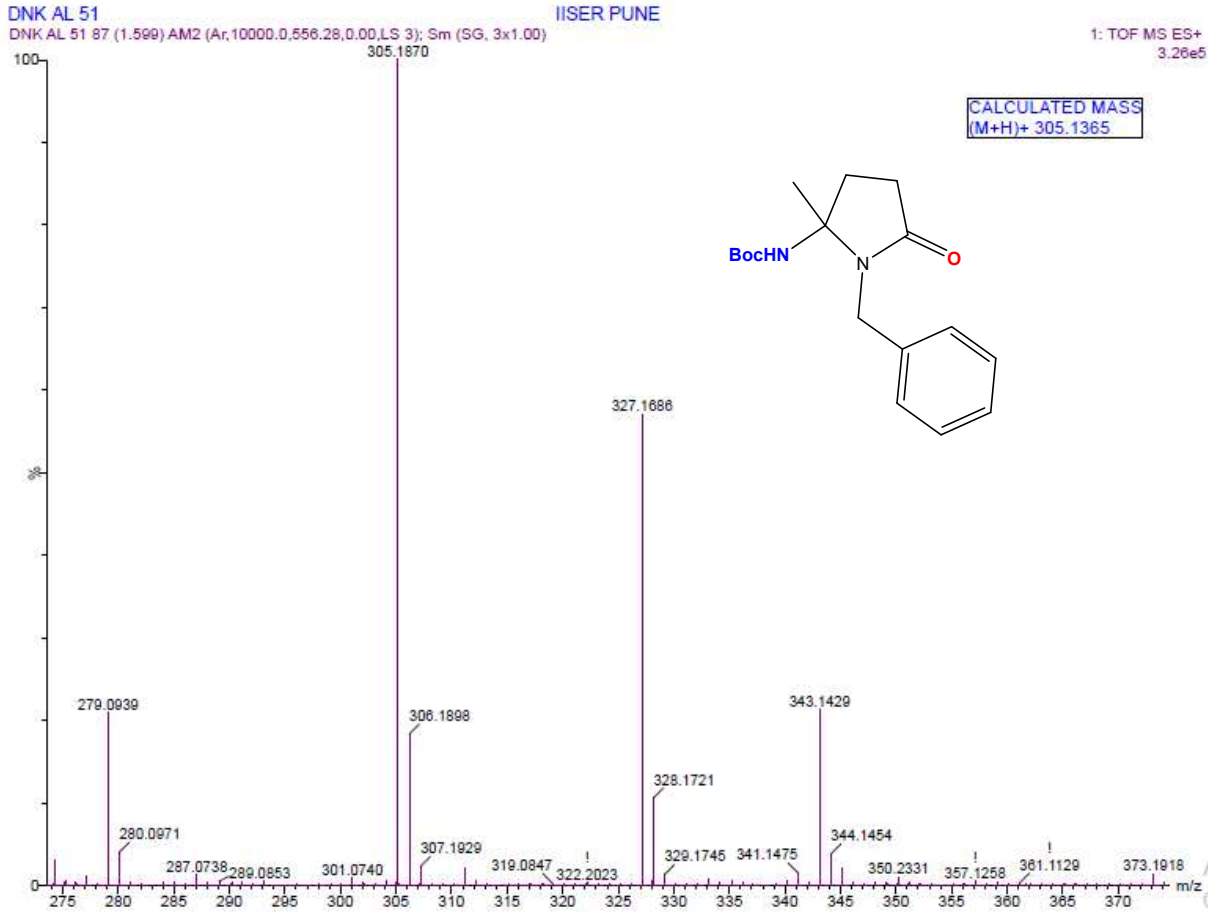
HRMS Spectra of compounds

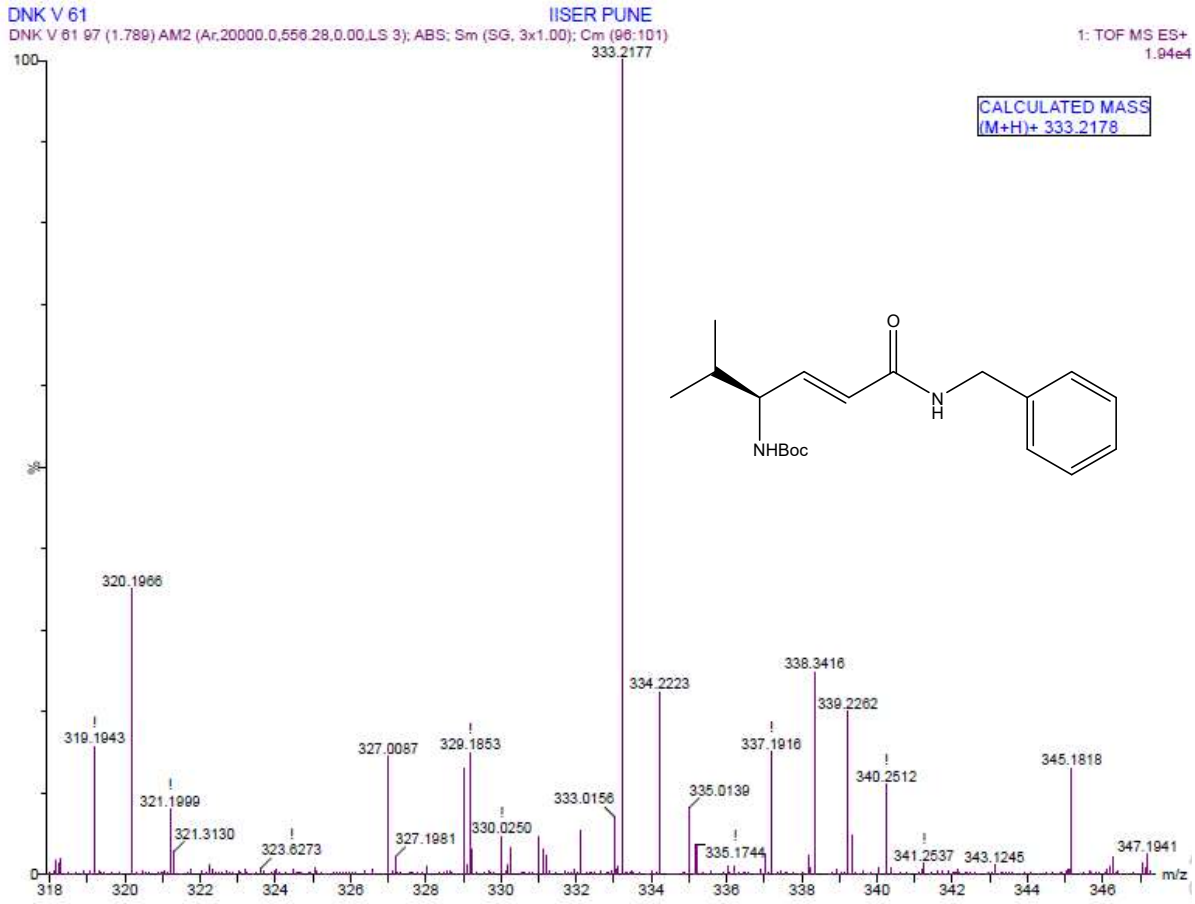





DNK PH 65
IISER PUNE
DNK PH 65 110 (2.031) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00); Cm (109:111)
1: TOF MS ES+
4.09e5
CALCULATED MASS
(M+H)+ 381.2178
BocHN
O
N
381.2181
403.1997
382.2216
419.1742
404.2031
420.1770
383.2246
421.1747
353.1010
363.1069
365.1177
380.1231
388.2269
399.1864
405.2075
415.1660
431.1384
439.3772
449.2042
m/z


DNK PB 53
IISER PUNE
DNK PB 53 107 (1.960) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00); Cm (106:108)
1: TOF MS ES+
4.97e5
CALCULATED MASS
(M+H)+ 381.2178
O
N
H
NHBoc
381.2182
403.1997
419.1740
382.2213
404.2027
435.2265
413.2448
420.1772
357.1818
353.1152
421.1752
440.2154
451.2003
383.2238
440.7175
363.1084
372.1594
381.0774
405.2055
432.2267
442.2141
398.2462
400.1892
m/z


DNK AL 51
IISER PUNE
DNK AL 51 87 (1.599) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
3.26e5
CALCULATED MASS
(M+H)+ 305.1365
BocHN
O
N
305.1870
327.1686
279.0939
306.1898
343.1429
328.1721
280.0971
344.1454
307.1929
287.0738
289.0853
301.0740
319.0847
322.2023
329.1745
341.1475
350.2331
357.1258
361.1129
373.1918
%
m/z


DNK V 61
IISER PUNE
DNK V 61 97 (1.789) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 3x1.00); Cm (96:101)
1: TOF MS ES+
1.94e4
CALCULATED MASS
(M+H)+ 333.2178
O
N
H
NHBoc
333.2177
320.1966
319.1943
338.3416
334.2223
339.2262
337.1916
327.0087
329.1853
345.1818
340.2512
321.1999
335.0139
333.0156
321.3130
330.0250
323.6273
327.1981
335.1744
341.2537
343.1245
347.1941
%
m/z