Base-Mediated Molecular Rearrangement in Alpha-Beta Unsaturated Gamma Amino Acids

A Thesis

Submitted to

Indian Institute of Science Education and Research Pune in partial fulfilment of the requirements for the BS-MS Dual Degree Programme

by

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ID: 20161042

Under the guidance of

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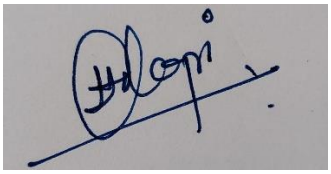
Indian Institute of Science Education and Research, Pune

Certificate

This is to certify that this dissertation entitled **"*Base-Mediated Molecular Rearrangement in Alpha-Beta Unsaturated Gamma Amino Acids*"** towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents work carried out by **Nithun Raj V** at Indian Institute of Science Education and Research, Pune under the supervision of Prof. Hosahudya N. Gopi, department of chemistry, during the academic year 2020-2021.



Date 31/7/21

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Declaration

I hereby declare that the matter embodied in the report entitled ***"Base-Mediated Molecular Rearrangement in Alpha-Beta Unsaturated Gamma Amino Acids"*** 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.

1/08/21
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20161042
BS-MS 5th year

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Nithun Raj V

Contents

1. Abbreviations

Aib = α-Amino isobutyric acid

aq. = Aqueous

Ala= Alanine

Bn = Benzyl

Boc = *tert*-Butoxycarbonyl

$(Boc)_2O$ = Di-*tert*-butyl-dicarbonate (Boc anhydride)

DCC = N,N'-Dicyclohexylcarbodiimide

DCM = Dichloromethane

DIPEA = Diisoproylethyl amine

DMF = N,N'-Dimethyl formamide

dg-γ = dehydro gamma

EDC.HCl = 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

EtOAc = Ethyl Acetate

EtOH = Ethanol

g = gram

h = hours

HCl = Hydrochloric acid

HOBt = 1-Hydroxybenzotriazole

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

IBX = 2-iodoxybenzoic acid

IR = Infrared spectroscopy

Leu = Leucine

LAH = Lithium Aluminium Hydride

MeOH = Methanol

MALDI-TOF/TOF = Matrix-Assisted Laser Desorption/Ionization-Time of Flight

Me = Methyl

mg = Milligram

min = Minutes

mmol = millimoles

N = Normal

NHS = N-hydroxy succinimide

NMR = Nuclear Magnetic Resonance

Phe = Phenyl alanine

Phegly= Phenyl glycine

RP- HPLC = Reversed Phase-High Performance Liquid Chromatography

RT = Room Temperature

THF= Tetrahydrofuran

TFA = Trifluoro acetic acid

^{t}Bu = tertiary Butyl

2. Abstract

Proteins are large biomolecules which performs considerable function in all living organisms. Various structures adopted by protein via folding phenomenon make them specific to their functions. Recent studies in foldamer chemistry shows that the folding phenomenon exists not only in proteins but also in peptides. These studies also gave a wide scope to search significant structures that can be formed by peptides containing non-natural amino acids. The existence of different biologically active peptide natural products where non-natural amino acids like γ-amino acids are present also states its significance. Several studies on non-natural amino acids and their utility on designing various secondary structures are done in our lab. The study on conformational properties and chemical reactivity of α,β-unsaturated γ-amino acids (dehydro γ-amino acid) which was previously conducted shown that double bond on dehydro γ-amino acid in a peptide got migrated on treatment with a base. By exploiting the migration reaction, a method 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 observed.[1] This base-mediated chemical rearrangement has also been used to turn *N*-protected α,β unsaturated γ-amino amides into γ-lactams.[2] These transformations are carried out by the strong base KO^{t}Bu, which makes it challenging to utilize in the presence of chiral amino acids. We wanted to see if mild bases like NaOH could be used to convert *N*-protected α,β unsaturated γ-amino amides into γ-lactams. In this study, we examined whether the double bonds can migrate in the presence of NaOH by subjecting various types unsaturated amino acid esters and terminal amides. Among the various amino acids tested, we found that the double bond migration was observed case of dehydro γ-Phenylglycine. Further various peptides composed of dg- γ-Phenylglycine and chiral amino acids were synthesized and double bond migration was studied using base NaOH without affecting the chiral integrity of the molecule. Similar type of NaOH mediated double bond migration was not observed in the peptides consists of other unsaturated amino acids. Overall, the results obtained in this project revealed that α,β-unsaturated γ-amino acids cannot used for the alkaline hydrolysis. In addition, it has been proved all unsaturated amino acids are not same and the acidity of γ-H play a crucial role in the double bond migration in unsaturated amino acids. The conformational studies of all synthesized peptides are in progress.

3. Introduction

Molecules that constitute all living organism are termed as biomolecules. Mainly there exist four different biomolecules termed as nucleic acids, lipids, carbohydrates, and proteins. Proteins are vital biomolecules which perform numerous functions in all living organism. The basic structure of protein can be considered as a linear chain of L-amino acids. As a result of interactions among and in between several linear chains of amino acids proteins fold into several three-dimensional structures based on the interaction. These three-dimensional structures are must requirement for a protein to perform its biological functions like enzyme catalysis, cell signaling, muscle contraction, transport of molecules, etc.

The protein structure is organized into four different levels namely 1. Primary 2. Secondary 3. Tertiary and 4. Quaternary structure. **Figure 1** shows the schematic representation. Primary structure gives the sequence of amino acids in the peptide chain which are spontaneously fold into three-dimensional structures to form the secondary structures of the protein. i. Helices ii. β-sheets and iii. reverse turns are the three different major secondary conformations of protein. These secondary structures are differentiated by the unique hydrogen bonding pattern between the NH and CO groups of the polypeptide chain. The secondary structures fold into three-dimensional structure with minimum energy to give the tertiary structure of the protein. Different individual tertiary structures come closer and arrange according to electrostatic interaction will gave a quaternary structure of the protein.

The idea of chemist to use non-natural building blocks to mimic the protein structure in order to study their structure and function resulted in developing functional molecules with superior biological activity. Further several non-natural building blocks (non-natural amino acids) were explored as elementary unit in the construction of secondary structures of protein. Considering different abiotic amino acids utilized, oligomers of β-amino acid and γ-amino acid are the most widely studied.[3] Foldamers of β-amino acid and γ-amino acid were well explored by several groups, different secondary structures were observed in these hybrid peptides. These hybrid peptides with β-amino acid were biologically stable than natural peptides hence are utilized to design biologically active sequences such as antimicrobials,[4] cancer-cell growth inhibitors,[5] etc.

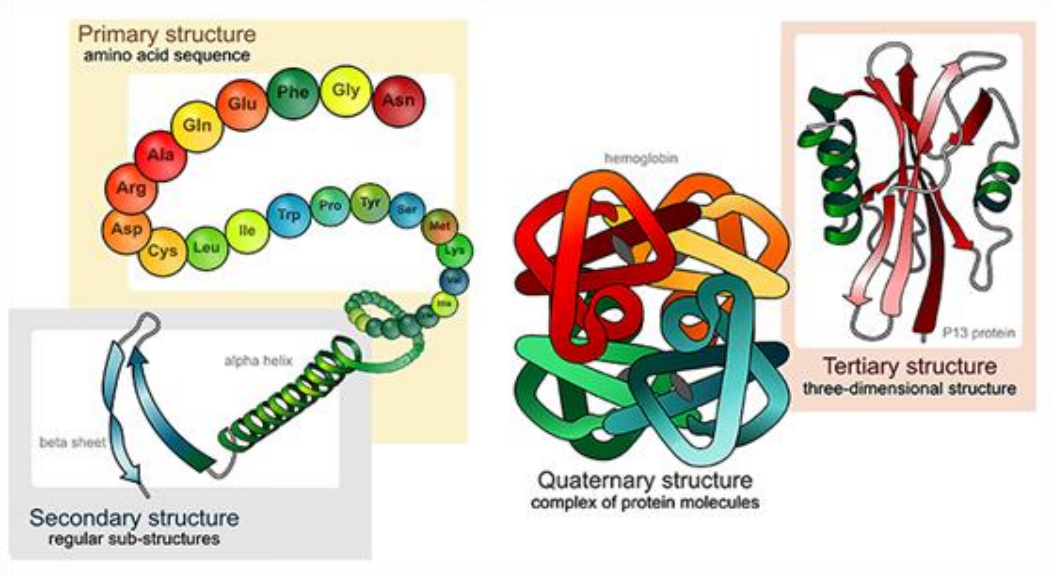
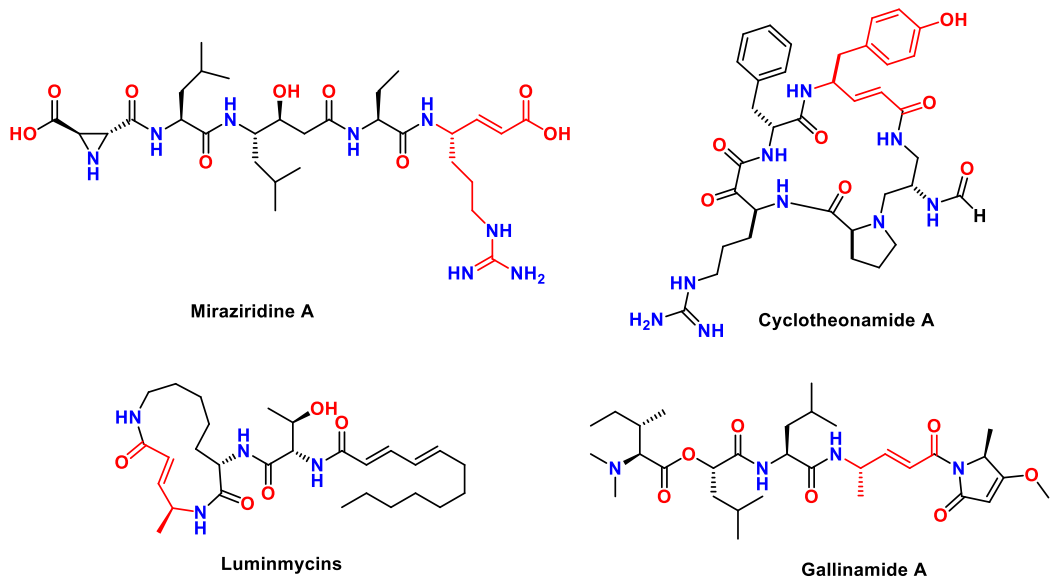
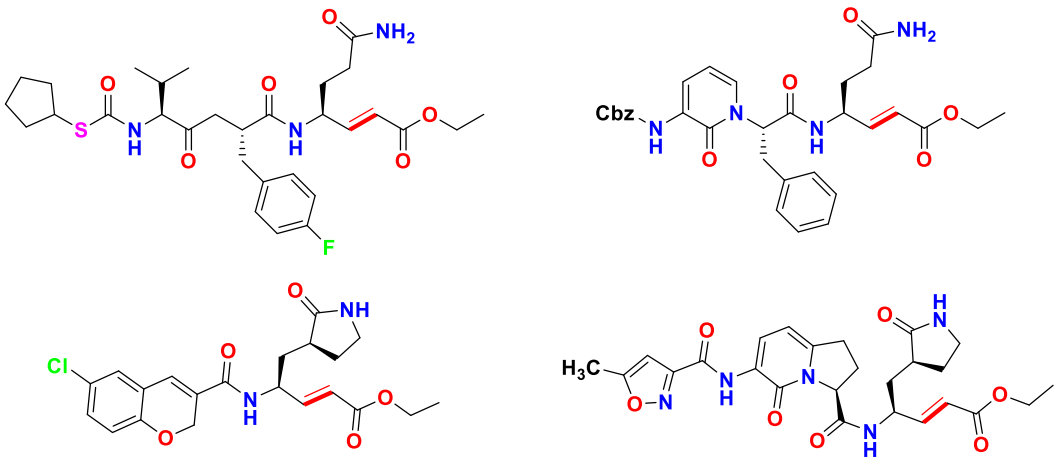


Figure 1 Levels of organization in protein.

α,β-Unsaturated γ-amino acids (vinylogous amino acids) are non-ribosomal amino acids that have a lot of importance but haven't been studied much in terms of foldamer design. Several natural peptide products contain α,β-Unsaturated γ-amino acids like Cyclotheonamides (potent inhibitors for serine protease and bovine β-trypsin[6]), Luminmycins (active proteasome inhibitors), Gallinamide A (anti-malarial agents[7]), Miraziridine A (inhibitors for variety of proteases like trypsin-like protease, papain-like cysteine protease, and pepsin-like aspartyl protease[8]). The **Scheme 1** shows the structure of these natural peptide products. Dehydro γ -amino acids were used to create peptide-based inhibitors for cysteine and serine proteases. **Scheme 2** gives the sequences of some of these inhibitors.[9] In our lab, we have studied the conformational properties and chemical reactivity of dehydro γ-amino acids and its availability in designing novel peptide foldamers, small molecule peptidomimetics, and β-double helices.



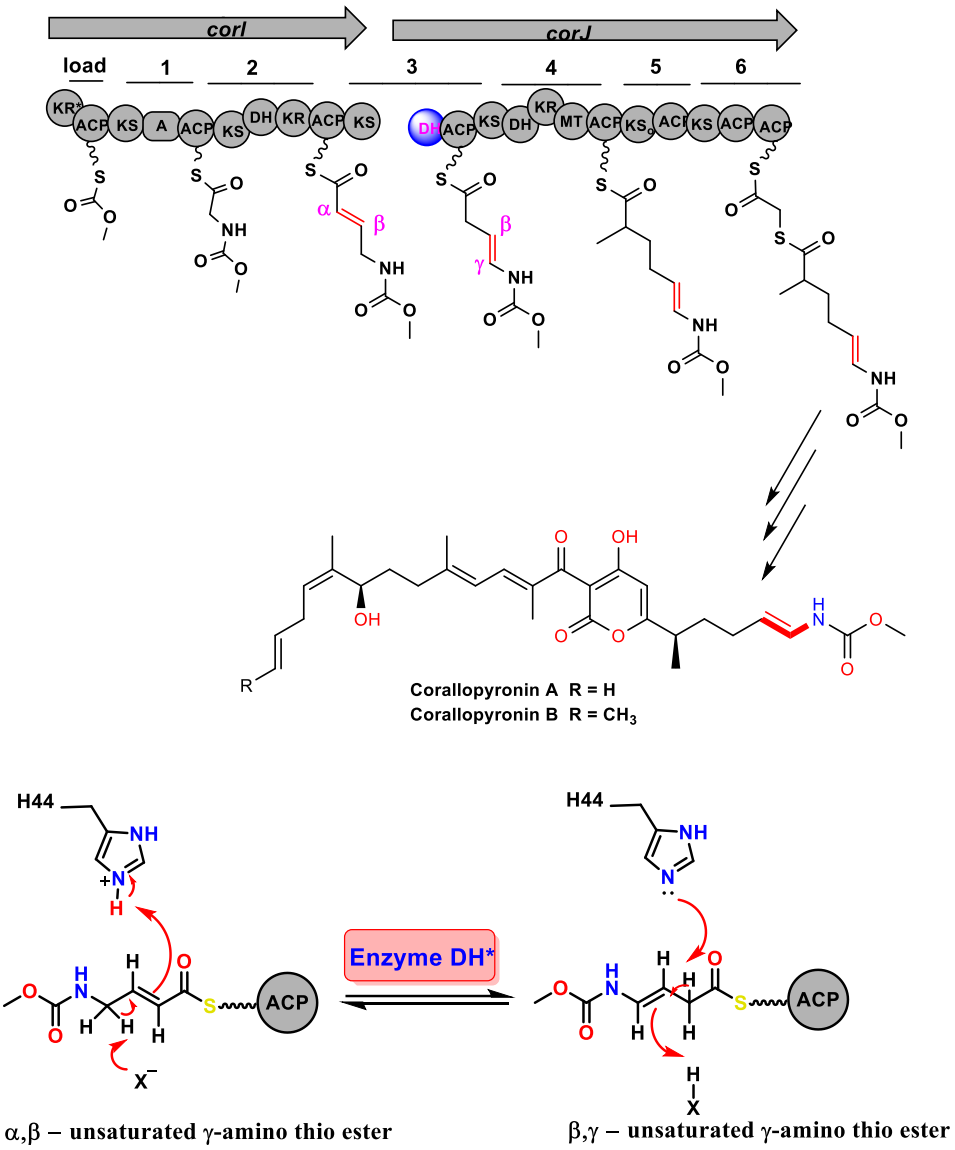
Scheme 1 Structure of these natural peptide products containing dehydro γ-amino acids



Scheme 2 structure synthetic protease inhibitors containing dehydro γ-amino acids

In addition, our group has also observed the double bond migration in peptides and γ -lactams formed from amino amides involving multiple double bond migrations in the presence of a base. Many natural products containing *E*-vinylogous amino acids have shown double bond migration from the α, β → β, γ position. For example, one of the intermediate steps in the biosynthesis of corallopyronin (a polyketide) involves the migration of double bonds from the α, β → β, γ position

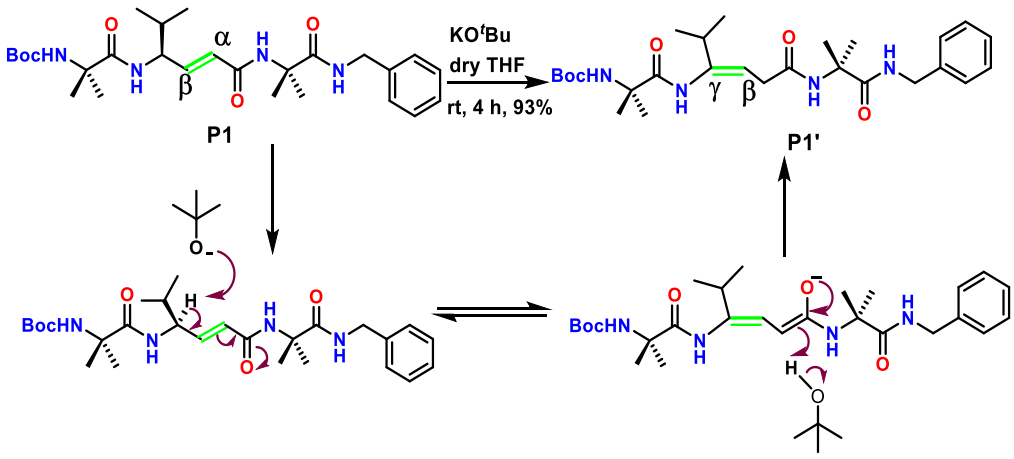
on the intermediate compound, *E*-vinylogous amino acid. The α, β → β, γ double bond migration step was done by the dehydratase enzyme.[10] Schematic representation is shown in the **Scheme 3**.



Scheme 3 Biosynthesis of corallopyronin along with α, β → β, γ double bond migration step done by the dehydratase enzyme

It was a necessity to study these reactions observed and hence a systematic investigation was conducted on these α, β → β, γ double bond migration. It was observed, as stronger the base the reaction becomes much easier to migrate the double bond. These base-mediated α, β → β, γ double-bond migrations were seen to directly change non-helical, hybrid peptides consisting of

alternating α- and *E*-vinylogous amino acids into 12-helical structures.[1] Schematic representation showing plausible mechanism is shown in **Scheme 4**. This base-mediated chemical rearrangement has also been used to turn *N*-protected α-β unsaturated γ-amino amides into γ-lactams.[2]



Scheme 4 Possible mechanism for α,β→β,γ double bond migration in **P1**.

KO^{t}Bu is a strong base which pKa value of conjugate acid around 17. It's widely used in several organic reactions. Even though it is difficult to use KOtBu in the presence chiral amino acids. It can abstract the α-proton of all amino acid resulting a racemic mixture. Hence milder bases like NaOH may useful to selectively introduce double bond migrations in the peptides without affecting chiral integrity of the molecules. In this study we sought to examine whether the double bonds can migrate in the presence of NaOH by subjecting various types of unsaturated amino acid esters and hybrid peptides with dehydro γ-amino acids to NaOH hydrolysis.

4. Materials and Methods

4.1 General experimental details

The reagents used in each reaction like HBTU, EDC.HCl, TFA, DMF, etc. and the amino acids were purchased from the commercial sources available. All these reagents used without further purification. Solvents like ethyl acetate, hexanes, methanol, etc. were distilled prior to use. For column chromatography, neutral alumina (100-200 mesh) was employed, which was likewise bought from a commercial source. A Bruker Avance 400 MHz or JEOL 400 MHz spectrometer

was used to measure ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra. High resolution mass spectrometry (HRMS) was used to examine the mass of samples. The X-ray data were acquired using a Bruker APEX(II) DUO CCD diffractometer at 100 K temperature, Mo Kα radiation (λ = 0.71073 Å).

4.2 Boc-*N*- α, β-unsaturated γ-amino esters general synthetic scheme

Wittig ylide (11 mmol) was added at RT after N-Boc-Amino aldehyde (10 mmol) was dissolved in 30 mL of dry THF. At room temperature, the reaction mixture was stirred for about 5 hours. TLC was used to monitor the reaction completion. After completion, the solvent was evaporated, and the crude product was purified using EtOAc/pet ether column chromatography to yield (89%) pure product.

4.2.1 Characterization of Boc-*N*- α, β-unsaturated γ-Phegly-OEt

White powder, Yield= 2.73 g (89%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.27 (m, 5H), 7.05 (dd, *J* = 15.6, 5.1 Hz, 1H), 5.98 (dd, *J* = 15.6, 1.8 Hz, 1H), 5.44 (s, 1H), 4.91 (s, 1H), 4.23 – 4.16 (m, 2H), 1.44 (s, 9H), 1.28 (t, *J* = 7.1 Hz, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 166.18, 154.79, 147.06, 139.28, 129.03, 128.29, 128.18, 127.21, 126.08, 121.55, 60.58, 33.72, 28.35, 28.13, 14.23. HRMS m/z calculated [M+Na] is 328.15 found 328.153. IR data shows C=O peak of ester at 1711.85 cm^{-1}, N-H stretching 3349.14 cm^{-1}.

4.3 Treating Boc-dg-γ-phegly-OEt with 1*N* NaOH

In 20 mL of distilled MeOH, 1.53 g (5 mmol) of Boc-dehydro-phenyl glycine ester is dissolved. At room temperature, 5 mL of 1*N* NaOH is gently added to the reaction mixture. TLC was used to monitor the reaction, which lasted 5 hours. After that, the solvent was evaporated and the reaction mixture was neutralized with 0.5*N* HCl. The product was extracted in EtOAc (150 mL) and then washed with brine solution (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under low pressure.

4.3a Synthesis of N-benzyl-4-oxo-4-phenylbutanamide (Product 2a)

The product after hydrolysis measured around 0.37 g (2 mmol) to that 0.76 g (2 mmol) HBTU was added. Along with DIPEA 1.04 ml (6 mmol) all these was dissolved in 10 ml of DMF. After mixing all about 5 min at ice-cold condition, benzylamine (0.786 mL, 6 mmol) was added. Overnight, the reaction was stirred. TLC kept track of the reaction progress. Following completion,

the reaction mixture was diluted with ethyl acetate (150 mL), followed by sequential washings with 10% Na_2CO_3 (3 × 50 mL), 10% HCl (3 × 50 mL), and brine solution (3 × 50 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated under decreased pressure. The crude product was purified via column chromatography using petroleum ether/EtOAc and pure product obtained.

White solid yield= 0.448 g (84%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 – 7.96 (m, 2H), 7.60 – 7.54 (m, 1H), 7.46 (dd, *J* = 8.3, 6.9 Hz, 2H), 7.38 – 7.26 (m, 5H), 6.13 (s, 1H), 4.45 (d, *J* = 5.7 Hz, 2H), 3.40 (t, *J* = 6.5 Hz, 2H), 2.66 (t, *J* = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 199.09, 171.96, 138.27, 136.56, 133.31, 128.71, 128.64, 128.11, 127.76, 127.47, 43.72, 34.09, 30.28. HRMS m/z calculated [M+H] is 268.13 found 268.134. IR data C=O stretching (secondary amide) 1684.29 cm^{-1}, C=O stretching 1633.36 cm^{-1} N-H stretching 3287.48 cm^{-1}.

4.3b Synthesis of methyl 4-oxo-4-phenylbutanoate (Product 2b)

The hydrolyzed product (0.37g, 2 mmol) that has to be methylated is dissolved in a 3 mL dimethylformamide (DMF) solvent. 0.6 g K_2CO_3 and 0.32 mL CH_3I are added, and the mixture is allowed to react for 8 hours. TLC was used to monitor the reaction completion. The reaction mixture was extracted with EtOAc (3 × 50 mL) and washed with 10% HCl (3 × 30 mL), 10% Na_2CO_3 (3 × 30 mL), and brine solution (3 × 30 mL) after completion of the reaction. The organic layer was passed over anhydrous Na_2SO_4 and concentrated under decreased pressure. The crude product was purified using petroleum ether/EtOAc by column chromatography.

Colorless semiliquid, yield=0.34 g (88%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.60 – 7.54 (m, 1H), 7.50 – 7.44 (m, 2H), 3.71 (s, 3H), 3.32 (t, *J* = 6.7 Hz, 2H), 2.77 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 198.08, 173.40, 136.54, 133.27, 128.64, 128.05, 51.86, 33.42, 29.71, 28.03. HRMS m/z calculated [M+Na] is 215.07 found 215.068. IR data C=O stretching 1687.47 cm^{-1} and 1738.07 cm^{-1} (ester).

4.4 Synthesis of Boc-dg-γ-Phegly-OH

Wittig ylide with tert-butyl ester (11 mmol) was added at RT after *N*-Boc-Amino aldehyde (10 mmol) was dissolved in 30 mL of dry THF. At room temperature, the reaction mixture was stirred for roughly 2 hours. TLC was used to monitor the reaction completion. After completion, the solvent was evaporated, and the crude product was refined using EtOAc/pet ether column

chromatography to yield (89%) pure product. To the product 10 ml 1:1 mixture of TFA and DCM is added. Reaction monitored via TLC. After completion TFA is evaporated via rotatory evaporator at low pressure. To the product 2 g of Na_2CO_3 is added and both dissolved in 30 ml of THF and 30 ml of water. To the reaction mixture 2.5 ml of Boc-anhydride was added, TLC was used to monitor the reaction. The reaction mixture was extracted with EtOAc (3 × 50 mL) and washed with 0.5*N* HCl (3 × 50 mL) and saturated NaCl solution (3 × 30 mL) after completion of the reaction. The organic layer was dried by anhydrous Na_2SO_4 and concentrated under decreased pressure.

White solid, Yield= 2.5 g (89%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.26 (m, 5H), 7.20 – 7.10 (m, 1H), 6.00 (dd, *J* = 15.6, 1.7 Hz, 1H), 5.47 (s, 1H), 4.92 (s, 1H), 1.44 (s, 9H)

HRMS m/z calculated [M+Na] is 300.12 found 300.122

4.5 Synthesis of peptide P1 to P3

4.5.1 Synthesis of **P1**

In 10 mL of DMF, Boc-dg- γ-Phegly-OH (1.38 g, 5 mmol) and HBTU (1.89 g, 5 mmol) were dissolved, then DIPEA (1.73 mL, 10 mmol) was added. To this mixture benzylamine (1.31 mL, 10 mmol) was added after agitating the reaction mixture for roughly 5 minutes at ice-cold temperature. After addition of benzylamine stirring was carried out for 12 hours. Work up was done by diluting the reaction mixture with ethyl acetate (150 mL), followed by sequential washings with 10% HCl (3 × 50 mL), 10% Na_2CO_3 (3 × 50 mL) and brine solution (3 × 50 mL) when the reaction was completed. The organic layer was dried over anhydrous sodium sulphate and concentrated under decreased pressure. The obtained crude compound was dissolved in very little amount of ethyl acetate and precipitated out by adding petroleum ether to get a pure, white solid compound.

White powder Yield= 1.52 g (83%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.26 (m, 9H), 7.25 (s, 1H), 6.99 (dd, *J* = 15.2, 5.6 Hz, 1H), 5.91 (dd, *J* = 15.2, 1.7 Hz, 1H), 5.82 (s, 1H), 5.41 (s, 1H), 4.93 (s, 1H), 4.50 (d, *J* = 5.7 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.02, 154.87, 143.37, 139.65, 137.95, 128.95, 128.77, 128.05, 127.99, 127.65, 127.14, 43.83, 28.37. HRMS m/z calculated [M+Na] is 389.18 found 389.184

4.5.2 Synthesis of peptide **P2**

Peptide **P1** synthesized was utilized in the synthesis. P1 (0.73 g, 2 mmol) was dissolved in 10 ml of 1:1 mixture of TFA and DCM. Reaction was monitored by TLC. After completion TFA was pull out by repeated evaporation under decreased pressure. The product was then added to the mixture of Boc-Leu-OH (0.5 g, 2 mmol), HBTU (0.83 g, 2 mmol), DIPEA (2 ml, 11.5 mmol) in DMF which was stirred about 5 min at ice-cold temperature. TLC kept track of the coupling. The reaction mixture was extracted with EtOAc (3 × 50 mL) and washed with 10% HCl (3 × 50 mL), 10% Na_2CO_3 (1 × 50 mL), and brine solution (1 × 80 mL) after completion of the reaction. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under decreased pressure. The crude product was purified using petroleum ether/EtOAc column chromatography.

White solid yield= 0.78 g (82%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.26 (m, 9H), 7.25 – 7.20 (m, 2H), 7.17 (d, *J* = 1.6 Hz, 1H), 6.98 (dd, *J* = 15.2, 5.3 Hz, 1H), 5.94 (d, *J* = 15.6 Hz, 1H), 5.69 (s, 1H), 4.93 (s, 1H), 4.49 (d, *J* = 5.1 Hz, 2H), 4.24 (s, 1H), 1.69 (s, 1H), 1.47 (dd, *J* = 16.6, 7.2 Hz, 2H), 1.37 (s, 9H), 0.94 (d, *J* = 6.6 Hz, 3H), 0.86 (t, *J* = 6.0 Hz, 3H).

HRMS m/z calculated [M+Na] is 502.27 found 502.267

4.5.3 Synthesis of peptide P3

Synthesis of Boc-Leu-Benzyl amine (4A) was done by similar procedure in the synthesis of peptide **P1**. Instead of Boc-dg-γ-Phegly-OH (1.38 g, 5 mmol) here Boc-Leu-OH (1.15 g, 5 mmol) was used. Boc deprotection was done by TFA in a similar way described in previous procedure. Boc deprotected 4A (1.12 g, 5.1 mmol) was used instead of benzyl amine and also EDC.HCl in place of HBTU repeated the procedure for the synthesis of **P1**. Tripeptide Boc-dg- γ -Phegly-Leu-Bn (4B) were isolated. 4B (1 g, 2 mmol) taken instead of **P1** and EDC.HCl in place of HBTU repeated the procedure for the synthesis of **P2**. White colored powder like product were isolated.

White solid yield= 0.86g (73%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (s, 1H), 7.51 (d, *J* = 8.1 Hz, 1H), 7.37 – 7.26 (m, 5H), 7.26 – 7.18 (m, 6H), 7.03 (d, *J* = 6.7 Hz, 1H), 4.87 (d, *J* = 7.2 Hz, 1H), 4.45 (d, *J* = 9.2 Hz, 1H), 4.34 (dd, *J* = 15.1, 5.6 Hz, 1H), 4.24 (d, *J* = 7.6 Hz, 1H), 3.05 (dd, *J* = 16.0, 8.0 Hz, 2H), 1.60 (s, 2H), 1.45 (s, 9H), 1.25 (s, 4H), 1.00 – 0.89 (m, 12H). HRMS m/z calculated [M+Na] is 615.35 found 615.35

4.8 General procedure for hydrolysis reaction by NaOH

In 30 mL of ethanol, the desired molecule was dissolved. 2 equivalents of 1*N* NaOH were added to the solution. At room temperature, the reaction was kept for 4 to 6 hours. TLC was used to observe the reaction. Ethanol was evaporated after completion, and the reaction mixture was diluted with EtOAc (3 × 50 mL) and washed with 10% HCl (3 × 50 mL) and brine solution (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under decreased pressure.

4.8.1 Characterization data for lactam formed **5A**

White solid yield= 0.22g in from 1 mmol scale (60%) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.34 (m, 3H), 7.27 (d, *J* = 1.6 Hz, 1H), 7.23 (d, *J* = 12.2 Hz, 6H), 5.10 (s, 1H), 4.94 (d, *J* = 15.3 Hz, 1H), 3.88 (d, *J* = 15.3 Hz, 1H), 2.86 (d, *J* = 15.0 Hz, 2H), 1.63 – 1.60 (m, 2H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 142.69, 137.78, 129.24, 128.48, 128.38, 127.11, 124.89, 80.23, 44.30, 29.33, 28.11. HRMS m/z calculated [M+Na] is 389.18 found 389.184

4.8.2 Characterization data for **P2'**

White solid yield =94% ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.94 (s, 1H), 7.41 – 7.26 (m, 9H), 7.26 – 7.21 (m, 2H), 6.03 (t, *J* = 7.8 Hz, 1H), 4.90 (d, *J* = 7.4 Hz, 1H), 4.42 (d, *J* = 5.9 Hz, 2H), 4.23 (s, 1H), 3.10 (d, *J* = 7.8 Hz, 2H), 1.78 – 1.72 (m, 2H), 1.54 (dd, *J* = 9.4, 7.9 Hz, 1H), 1.43 (s, 9H), 0.98 (dd, *J* = 12.2, 6.1 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.31, 170.70, 138.50, 137.06, 135.42, 128.57, 128.54, 127.62, 127.18, 125.86, 119.16, 43.58, 37.17, 28.29, 24.96, 22.93, 21.96. HRMS m/z calculated [M+H] is 480.28 found 480.28.

4.8.3 Characterization data for **P3'**

White solid yield =92% ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.30 (s, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.33 (dd, *J* = 15.8, 3.5 Hz, 5H), 7.25 – 7.17 (m, 5H), 7.00 (s, 1H), 6.08 (t, *J* = 8.1 Hz, 1H), 4.87 (d, *J* = 7.2 Hz, 1H), 4.47 (dd, *J* = 13.8, 7.2 Hz, 2H), 4.35 (dd, *J* = 15.1, 5.6 Hz, 1H), 4.25 (t, *J* = 7.8 Hz, 1H), 3.05 (dd, *J* = 15.6, 8.1 Hz, 2H), 1.65 – 1.59 (m, 2H), 1.45 (s, 9H), 1.25 (s, 4H), 0.99 – 0.89 (m, 13H). HRMS m/z calculated [M+H] is 593.3 found 593.37.

4.9 General procedure for synthesis of peptides B1 to B3

Boc-Aib-OH (6.1 g, 30 mmol) was dissolved in 60 ml THF, then NHS (6.9 g, 60 mmol) and DCC (7.8 g, 32 mmol) were added, and the mixture was agitated overnight at 0 °C initially. TLC was used to observe the reaction. After the reaction was finished, the filtrate was filtered out and concentrated under reduced pressure. After that, the product was dissolved in 80 mL of THF, water, and Na_2CO_3 (6 g, 30 mmol). At room temperature, H_2N-Leu-OH was added to the mixture and stirred for 12 hours. TLC confirmed the reaction occurrence. Following completion, the reaction mixture was diluted with ethyl acetate (150 mL), followed by washings with 10 % HCl (3 × 50 mL) and brine solution (3 × 50 mL). The organic layer was dried by Na_2SO_4 and concentrated under decreased pressure.

Boc deprotection of α-β unsaturated γ-amino esters was done by TFA by same procedure said earlier. These free amines are coupled with product isolated by coupling procedure explained in synthetic procedure for peptide **P1**. Each peptide is purified by column chromatography and then subjected to NaOH hydrolysis to get peptide acids **B1**, **B2** and **B3**.

4.9.1 Characterization of **B1**

White solid yield= 80% ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.95 – 6.88 (m, 1H), 6.53 (d, *J* = 18.1 Hz, 1H), 5.80 (dd, *J* = 15.6, 3.8 Hz, 1H), 4.94 (s, 1H), 4.68 – 4.60 (m, 1H), 4.46 – 4.38 (m, 1H), 1.51 – 1.45 (m, 6H), 1.42 (s, 9H), 1.23 (d, *J* = 15.9 Hz, 2H), 0.98 – 0.82 (m, 16H).
HRMS m/z calculated [M+H] is 478.29 found 478.289

4.9.2 Characterization of **B2**

White solid yield= 82% ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (d, *J* = 8.6 Hz, 1H), 7.19 (ddd, *J* = 6.2, 3.0, 1.3 Hz, 5H), 6.93 (dd, *J* = 5.0, 3.4 Hz, 1H), 6.62 (s, 1H), 6.40 (s, 1H), 5.79 (dd, *J* = 15.7, 1.7 Hz, 1H), 4.94 (s, 1H), 4.37 (d, *J* = 3.7 Hz, 1H), 1.44 (s, 9H), 1.42 (s, 6H), 1.28 – 1.22 (m, 2H), 0.89 – 0.83 (m, 9H). HRMS m/z calculated [M+H] is 512.27 found 512.27

4.9.3 Characterization of **B3**

White solid yield= 86% ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (s, 1H), 6.94 (dd, *J* = 25.9, 4.8 Hz, 1H), 6.54 (d, *J* = 38.3 Hz, 1H), 5.89 (ddd, *J* = 53.2, 15.7, 1.8 Hz, 1H), 4.96 (s, 1H), 4.68 (d, *J*

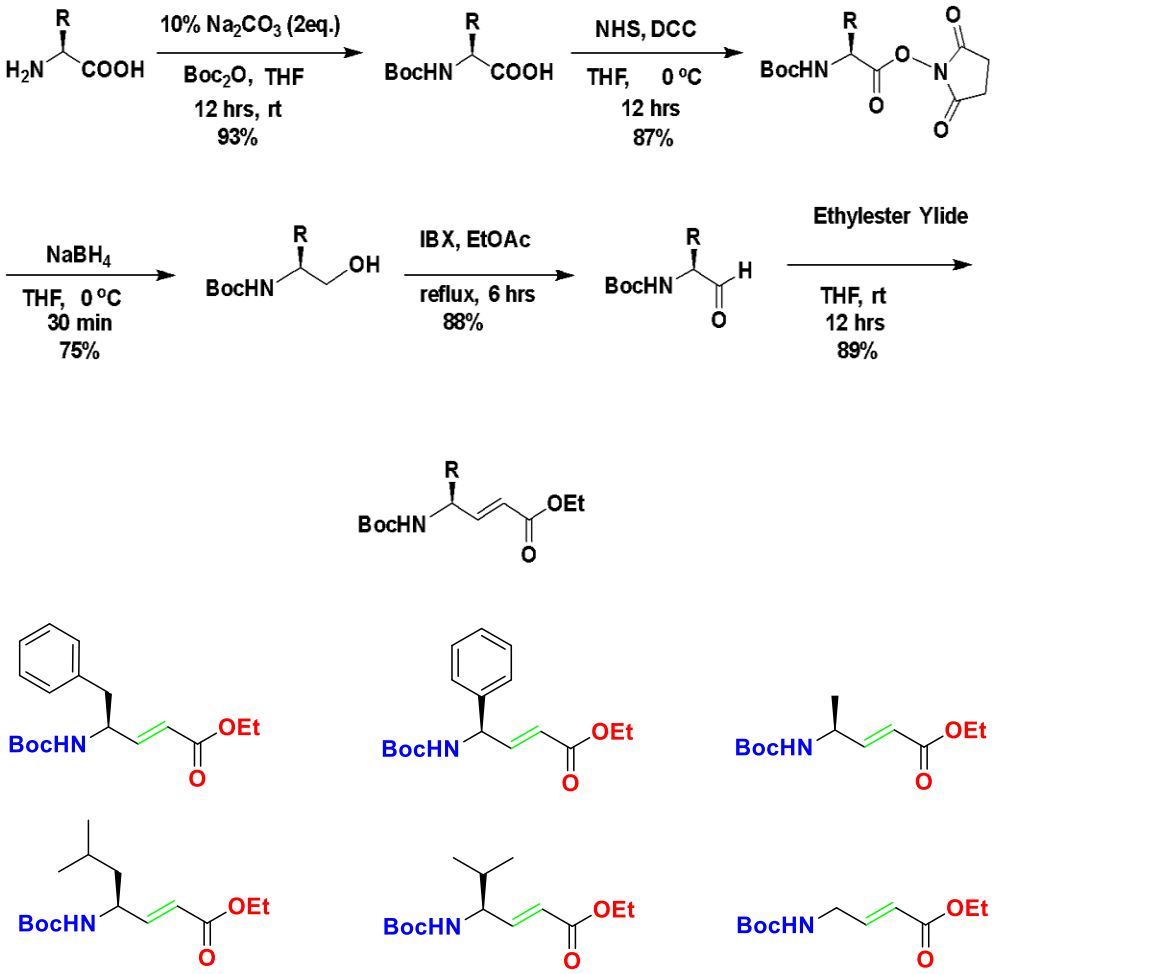
= 13.4 Hz, 1H), 4.48 – 4.37 (m, 1H), 1.52 – 1.44 (m, 6H), 1.42 (d, *J* = 3.2 Hz, 9H), 1.31 (dd, *J* = 10.4, 7.1 Hz, 3H), 1.20 (d, *J* = 6.1 Hz, 2H), 0.96 – 0.88 (m, 7H). HRMS m/z calculated [M+H] is 436.24 found 436.242

5. Results and Discussion

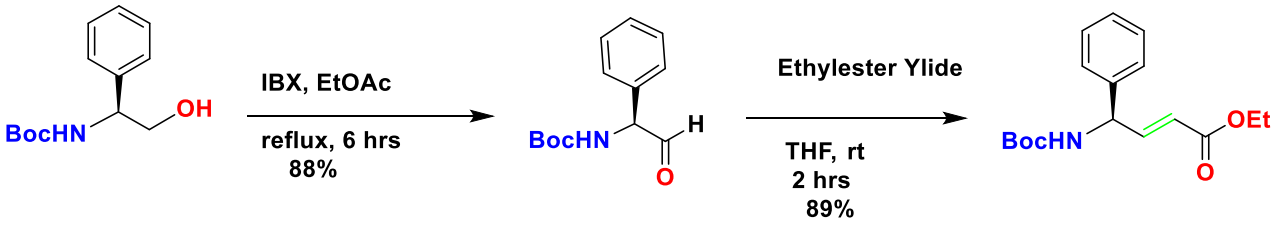
5.1 Synthesis and reactivity of α-β Unsaturated γ-amino acids

α-β Unsaturated γ-amino acids were produced using a well-known method. **Scheme 5** shows a schematic illustration of the synthesis of dehydro γ-amino acids starting from α-amino acids. α-β Unsaturated γ-Leucine, Valine, Phenyl alanine, Phenyl glycine, Alanine, and glycine were produced from N-Boc-amino aldehyde using the Wittig process, as previously reported.[11,12] The synthesis of dehyro γ-amino acid of phenylglycine were not happening by the normal procedure. The conversion from Boc-phegly-alcohol to aldehyde required a high temperature and it react completely with ethyl ester ylide in an hour (**Scheme 6**). The α-β unsaturated γ-amino esters were further subjected to saponification reaction. α-β unsaturated γ-amino acids formed were utilized in the coupling reaction in order to make desired peptides for the study.

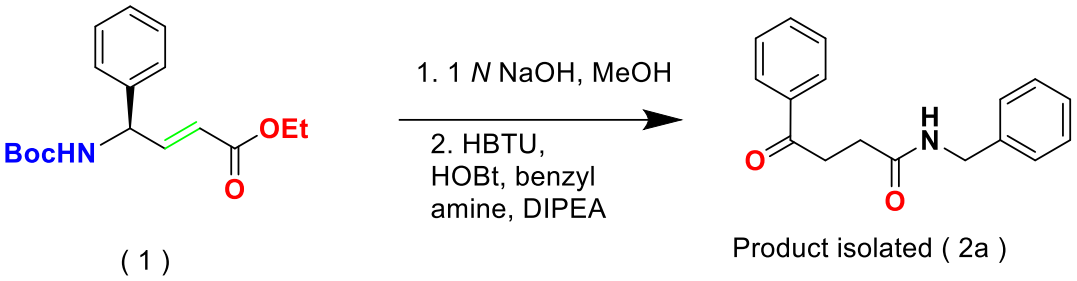
Interestingly, in contrast to the various other amino acids shown in the **Scheme 5**, dehydro phenylglycine undergo double bond migration in the presence 1 *N* NaOH during ester hydrolysis. The double bond migrated product further undergone imine-enamine hydrolysis to form the γ -keto caraboxylic acid. γ -keto caraboxylic acid was isolated and coupled with the benzyl amine (**Scheme 7**). The was product isolated, purified via column chromatography and characterized by ^{1}H, ^{13}C NMR, HRMS and IR. Single crystals of the product were also able to obtain and got diffracted. The X-ray structure of the product is shown in the **Figure 2**.



Scheme 5 Synthesis of α-β unsaturated γ-amino acid



Scheme 6 Synthesis of dehydro- γ-phenyl glycine



Scheme 7 Base hydrolysis followed by Coupling reaction

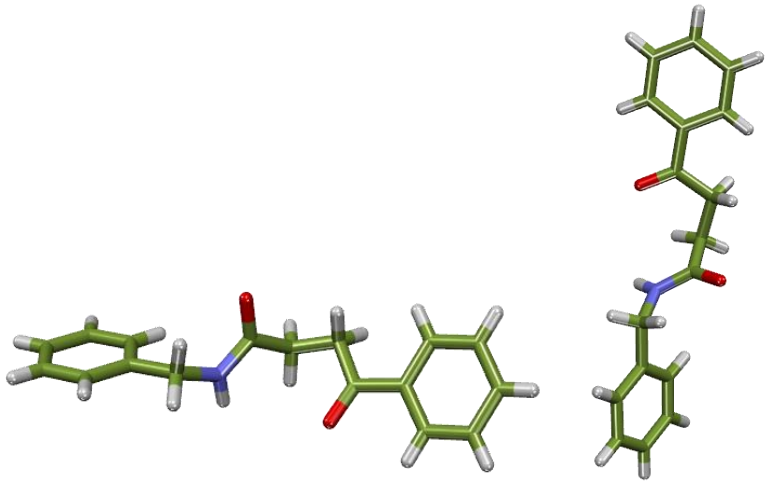
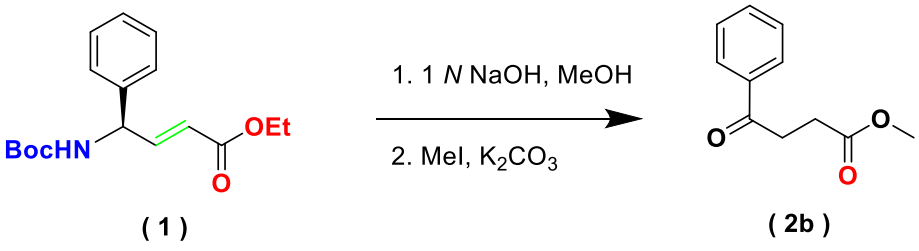


Figure 2 Crystal structure of the product isolated.

The product isolated was not expected. The product formed was gamma ketone of the phenyl glycine which shows that migration of double bond occurs even with NaOH. In order to make sure that the formation of gamma ketone occurred at the stage of base hydrolysis, the reaction was carried out again and product isolated after methylation reaction as shown in **Scheme 8**.



Scheme 8 Base hydrolysis followed by methylation

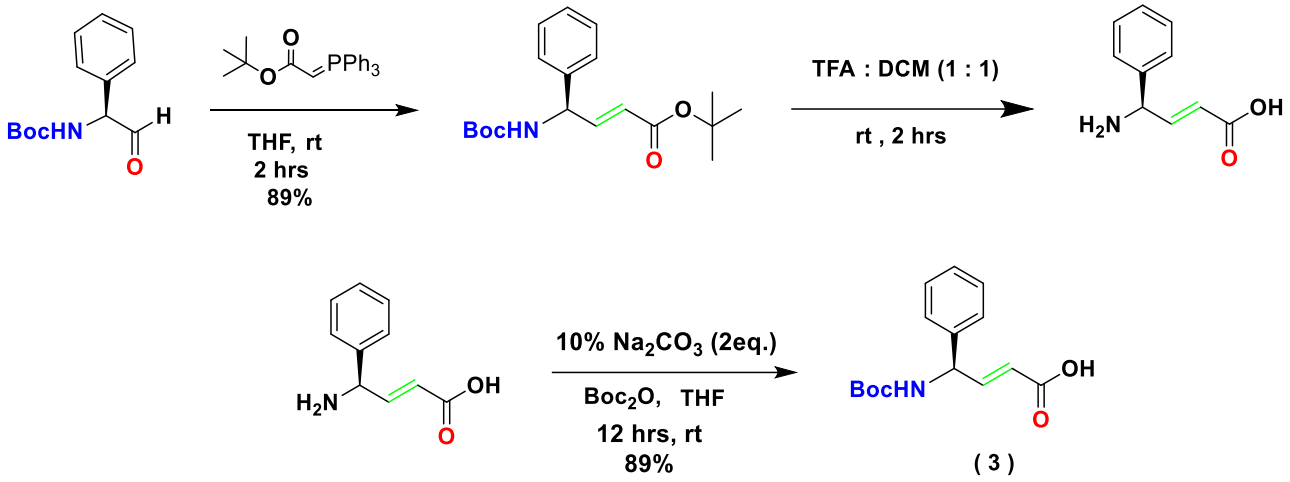
As expected, gamma keto acid of phenyl glycine amino acid was formed and its methylated product was isolated which is characterized via ^{1}H, ^{13}C NMR, HRMS and IR.

Hydrolysis reaction of all other α-β unsaturated γ-amino esters were studied. Double bond migration was not observed in any other α-β unsaturated γ-amino esters. **Table 1** show result of hydrolysis reaction.

S. No	**Reactant**	**Result observed on reacting with 1 *N* NaOH in MeOH**
1	α-β unsaturated γ-leucine ethyl ester	Hydrolyzed product
2	α-β unsaturated γ-phenylalanine ethyl ester	Hydrolyzed product
3	α-β unsaturated γ-valine ethyl ester	Hydrolyzed product
4	α-β unsaturated γ-phenylglycine ethyl ester	Migrated product along with hydrolysis
5	α-β unsaturated γ-alanine ethyl ester	Hydrolyzed product
6	α-β unsaturated γ-glycine ethyl ester	Hydrolyzed product

Table 1 Result of hydrolysis reaction on 1 *N* NaOH

In order to avoid usage of NaOH in the synthesis of peptide an alternate way was figured out as shown in **Scheme 9**.

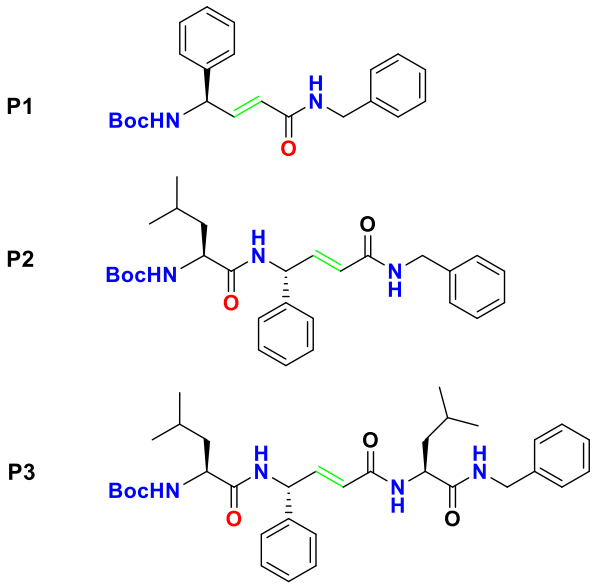


Scheme 9 Synthesis of Boc-dg-γ-Phegly-OH.

Boc-dg- γ -Phegly-OH was successfully synthesized and characterized by ^{1}H, ^{13}C NMR and HRMS.

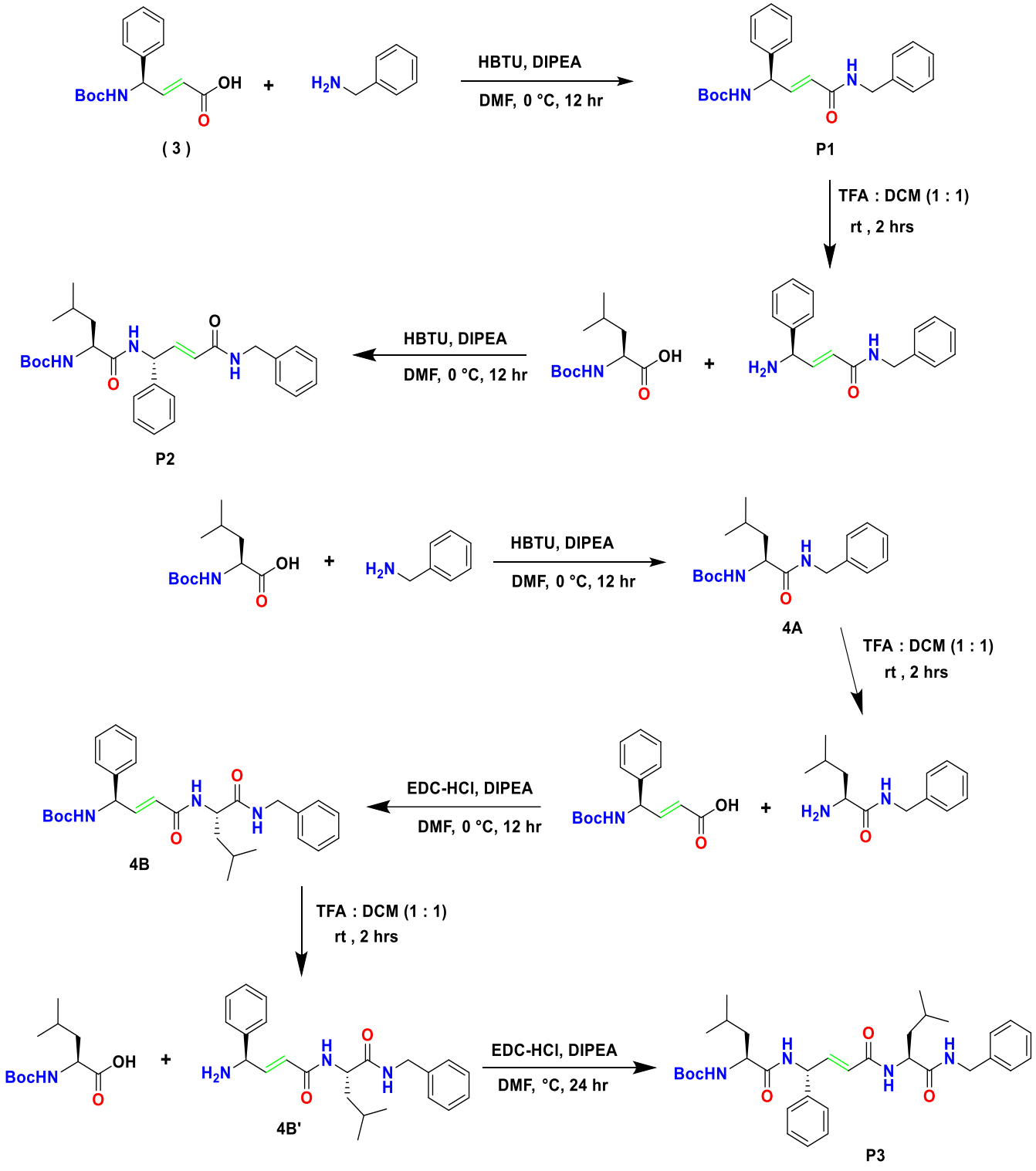
5.2 Synthesis of Peptides P1 to P3

Hybrid peptides containing α-β unsaturated γ-phenylglycine have been designed to investigate the possibility of α, β → β, γ double bond migration by base NaOH. In the case of **P1** the N-terminal of α,β unsaturated γ-phenylglycine is protected with the Boc and C-terminal with the benzylamine group. Terminal protection in **P2** and **P3** were also done similarly along with the integration of chiral amino acid (L-leu) in between. The sequence of peptides is shown in the **Scheme 10**.



Scheme 10 Sequences of hybrid peptides composed of α-L-leu and α,β-unsaturated γ-phenylglycine

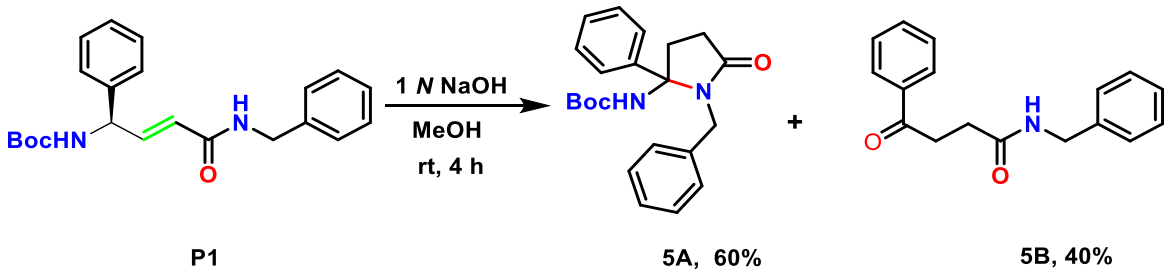
Synthesis of hybrid peptides **P1** to **P3** is carried out by conventional solution phase synthesis. *N*-terminus protection was done by tert-butyloxycarbonyl group. Deprotections for N-terminal were carried out with trifluoroacetic acid, couplings were mediated either by HBTU or EDC-HCl. The synthetic strategy for peptides **P1** to **P3** is shown in **Scheme 11**. **P1** is prepared by coupling benzyl amine with Boc-*E*-d^{2,3}γPhegly-OH. In order to prepare **P2** boc deprotection done in **P1** followed by coupling with Boc-L-Leu-OH. To synthesize peptide P**3**, starting materials, Boc-Leu-benzylamine (**4A**) was prepared by coupling the Boc-Leu-OH with benzylamine, which subjected to TFA deprotection and then coupled with Boc-*E*-d^{2,3}γPhegly-OH. Boc-Leu-OH is further introduced in the peptide in a similar manner in **P2**. All three peptides were purified through column chromatography.



Scheme 11 Schematic representation of synthetic strategy for the synthesis of **P1** to **P3**

5.3 α,β→β,γ Double bond migration by base NaOH and chemical reactivity

We first treated peptide **P1** with 1*N* NaOH in ethanol as a solvent to explore the possibilities of α,β→β,γ double bond migration by base NaOH. TLC was used to monitor the reaction, and it took 4 hours for the starting material to completely vanish, resulting in the development of two spots. Both the products were separated and purified via column chromatography, characterized by via ^{1}H, ^{13}C NMR and HRMS. **Scheme 12** depicts the reaction conditions as well as the product structure. Product **5A** single crystals were obtained, and their X-ray diffracted structure is depicted in **Figure 3**.



Scheme 12 Product on treating P1 with NaOH

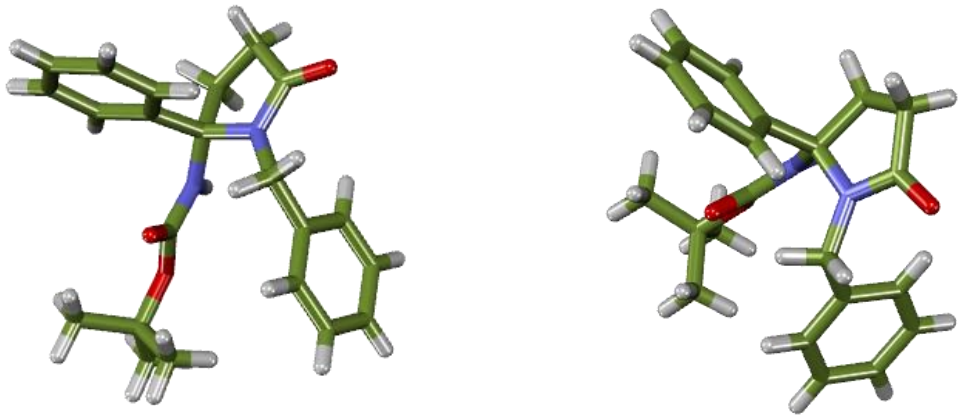
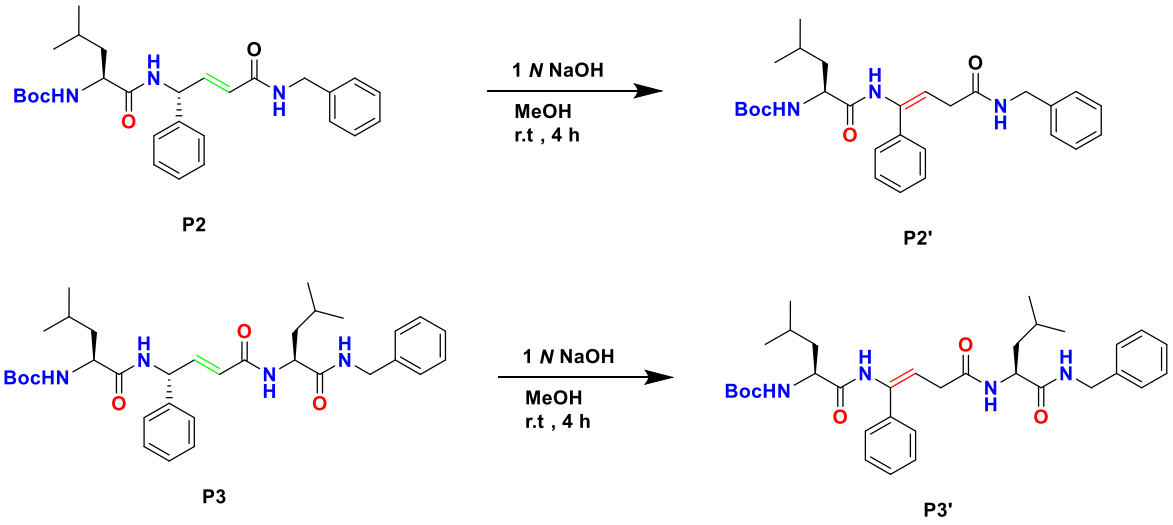


Figure 3 Crystal structure of **5A**

As expected NaOH abstracted the α-H from dehydro- γ-phenyl glycine resulting in the migration of double bond proceeded by cyclisation to give γ-lactam (**5A**), some of the molecules undergone imine-enamine hydrolysis due to the presence of water to form **5B** which was isolated earlier.

P2 and **P3** subjected to 1 *N* NaOH in a similar manner as **P1** and reaction monitored by TLC. Reaction was completed in 4 hours at room temperature. **P2'** and **P3'** represents the double bond migrating products, were isolated with a high yield of 92%. The typical *trans* double bond protons and Cγ H signal have vanished in ^{1}H NMR of **P2'** and **P3'**. In addition, the ^{1}H NMR spectra revealed a novel triplet and a doublet, confirming double bond migration in the product. The migration in **P2** and **P3** is depicted in **Scheme 13**.

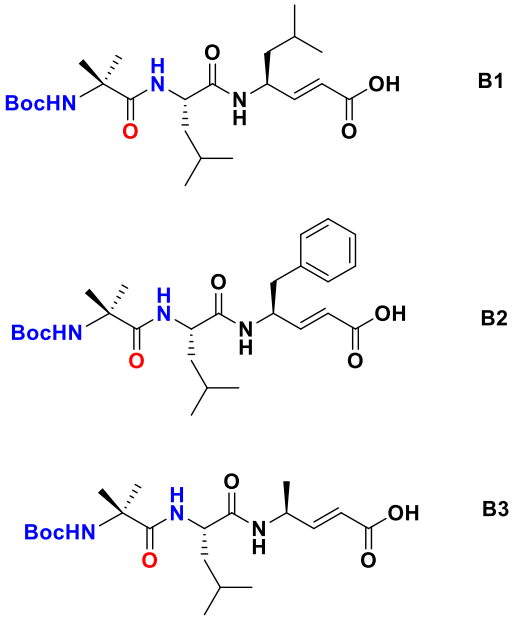


Scheme 13 α,β→β,γ Double bond migration in peptides **P2** and **P3**

α,β→β,γ Double bond migration was observed in peptides **P2** and **P3** with 1 *N* NaOH. The chirality of the α-amino acids is probably unaffected, however we are investigating purity and chirality of the peptide using HPLC. We are expecting peptide **P3'** can have a similar hydrogen bonding pattern as that of previously reported peptides and fold into 12 helix. The conformational studies of the peptides need to be done.

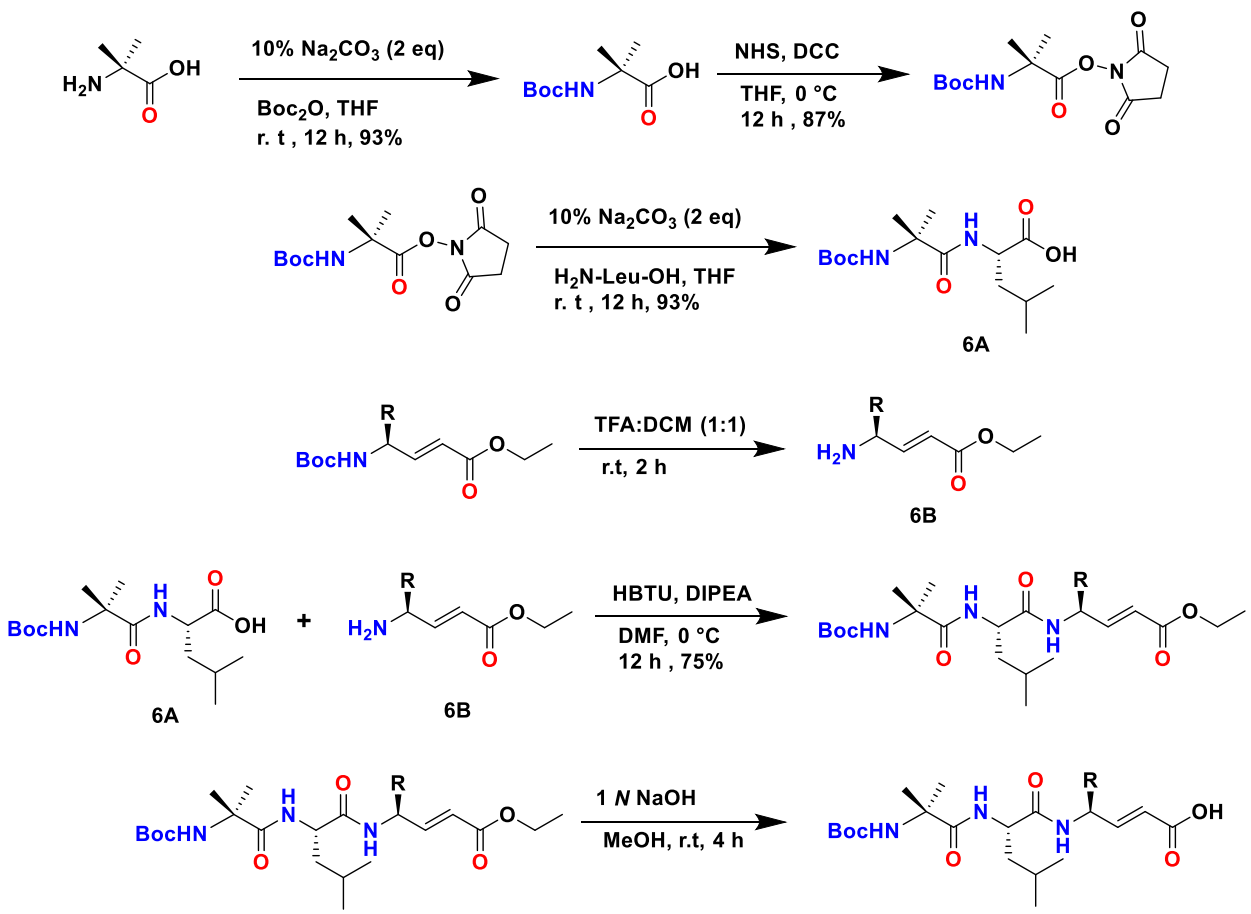
5.4 Synthesis of peptides B1 to B3

In order to understand the whether or not unsaturated amino acids can undergo double bond migration similar to observed in the phenylglycine, we have designed three peptides as shown in **Scheme 14**. Synthetic details of these peptides are shown in **Scheme 15**. Conformational studies of these peptides are also in progress.



Scheme 14 Sequence of peptides

All the peptides **B1** to **B3** are successfully synthesized and characterized by ^{1}H, ^{13}C NMR and HRMS. In a sharp contrast to the results observed in the case of unsaturated phenylglycine, no double bond migration was observed in these peptides.



Scheme 15 Synthetic scheme for peptides **B1** to **B3**.

6. Conclusions

The complete double bond migration was observed in the alkaline hydrolysis of α-β unsaturated γ-phenylglycine. Upon hydrolysis of α-β unsaturated γ-phenylglycine gave γ-keto acid instead of ester hydrolysis product. In sharp contrast no other unsaturated amino acid gave γ-keto-acids under identical conditions. So, unsaturated Phenylglycine cannot subjected for alkaline hydrolysis. For incorporation this amino acid into the peptide sequence, we have shown the different method. The amide of unsaturated Phenylglycine amino acid gave lactam in monomer and double bond migrated product in peptides. The acidity of the γ-H plays a crucial role in the base mediated double bond migration. Overall, the study suggested that the reactivity all unsaturated amino acids are not same.

7. References

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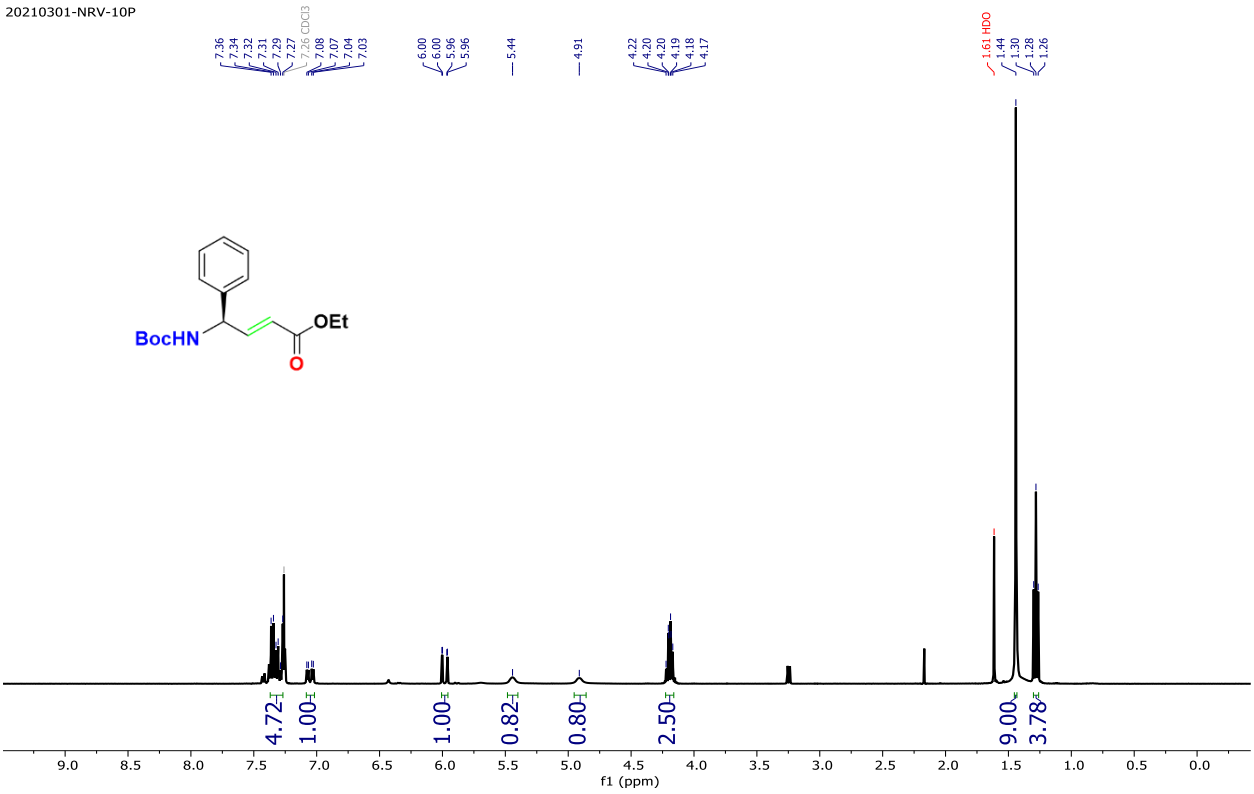
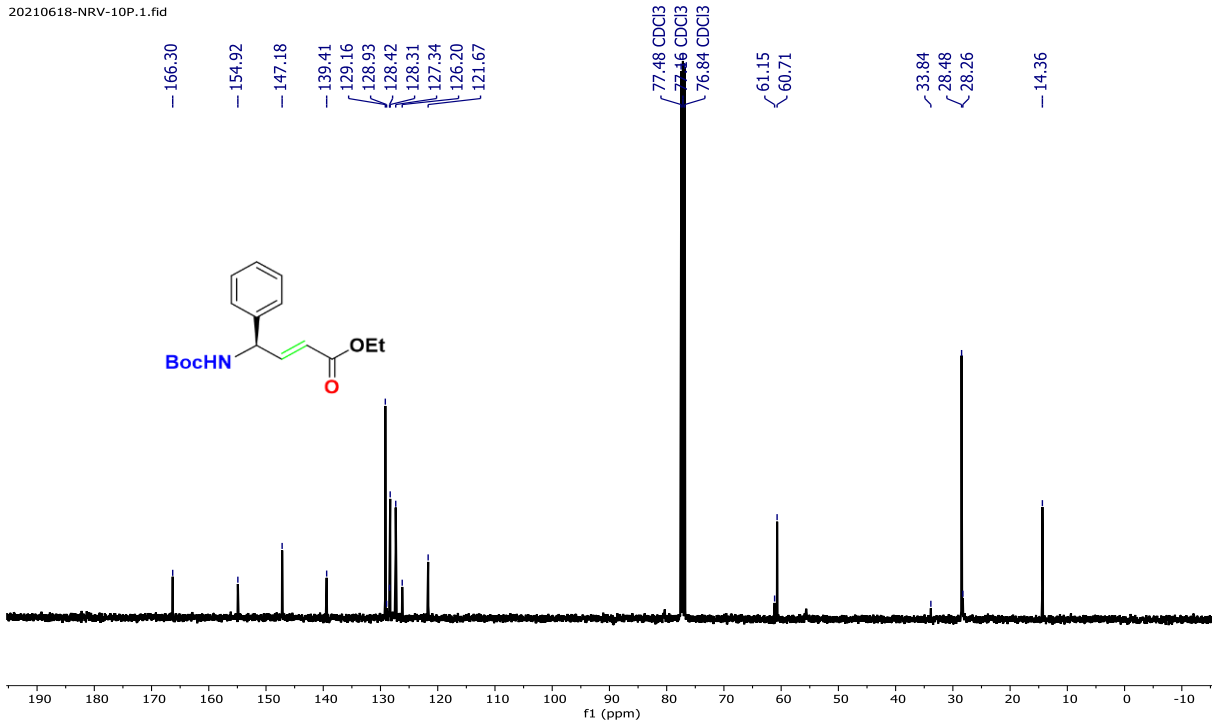
8. Appendix

7.1 ^{1}H, ^{13}C NMR Spectra of Compounds





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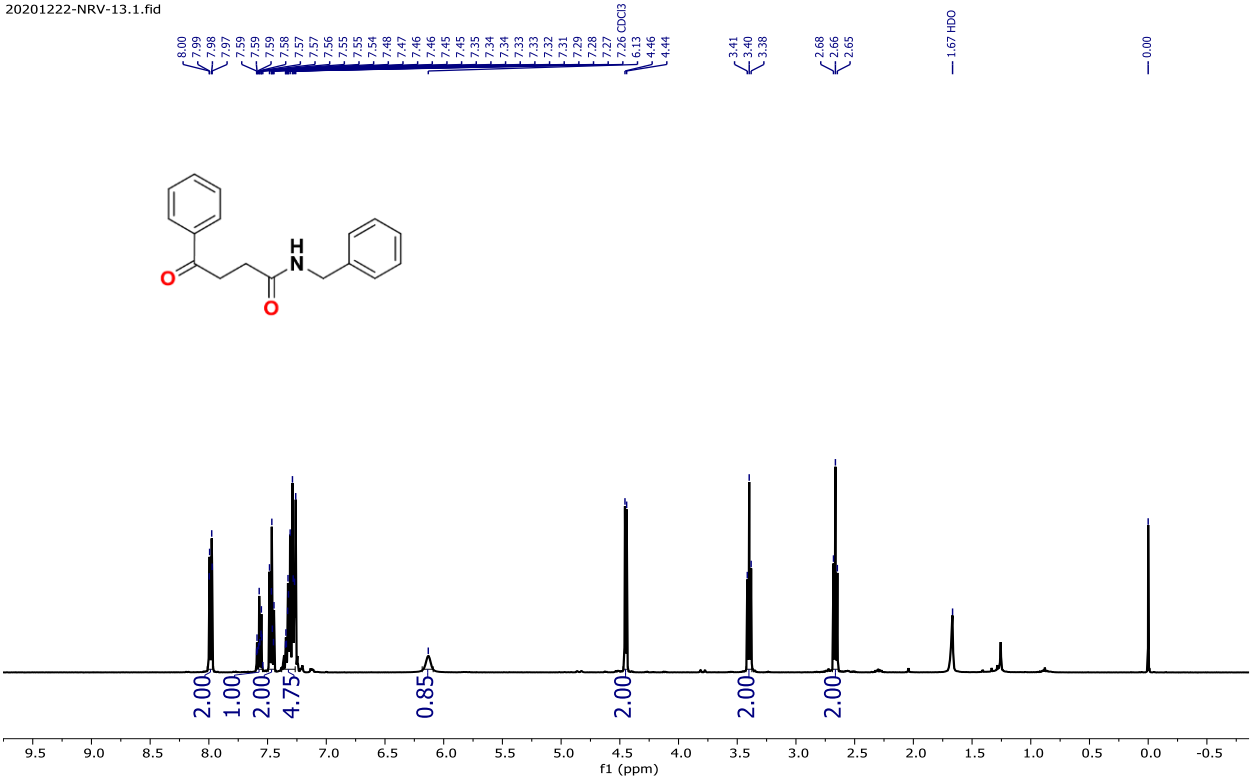

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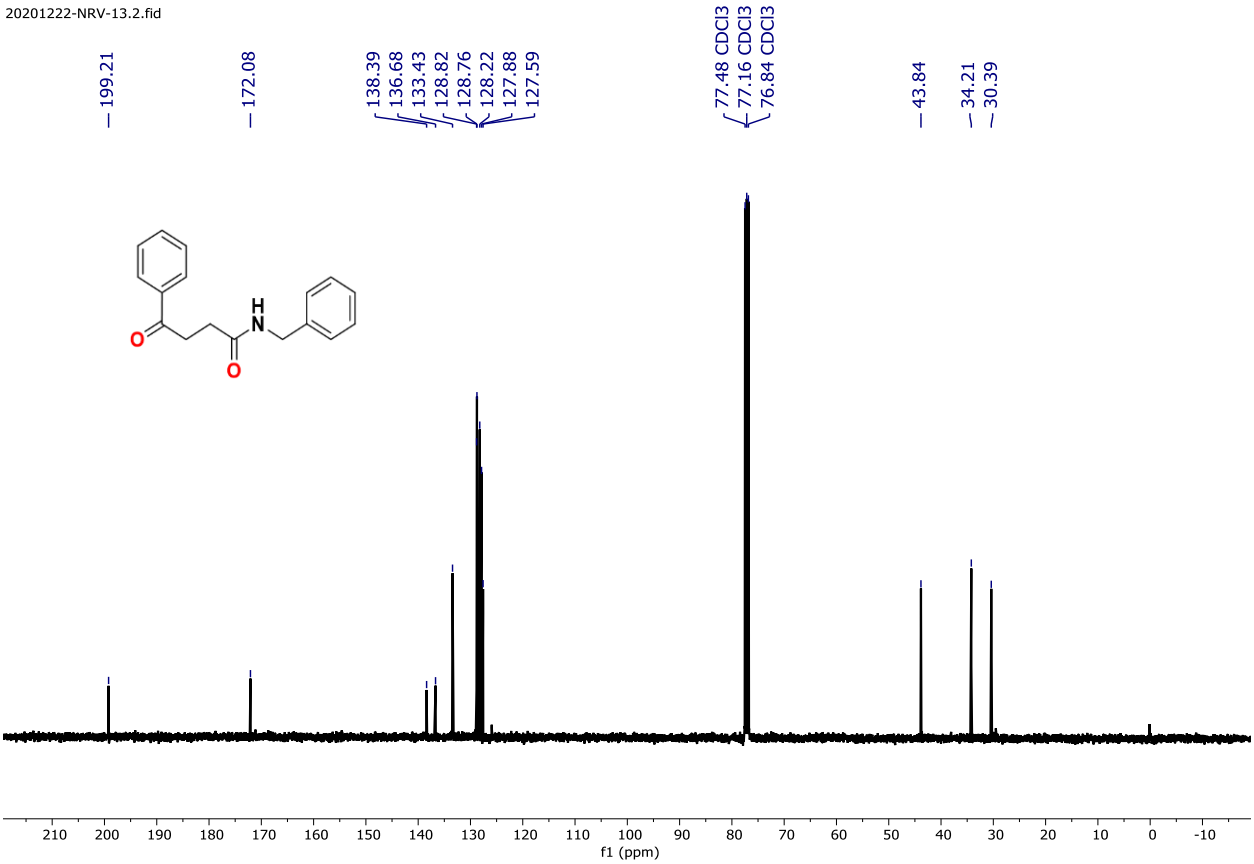

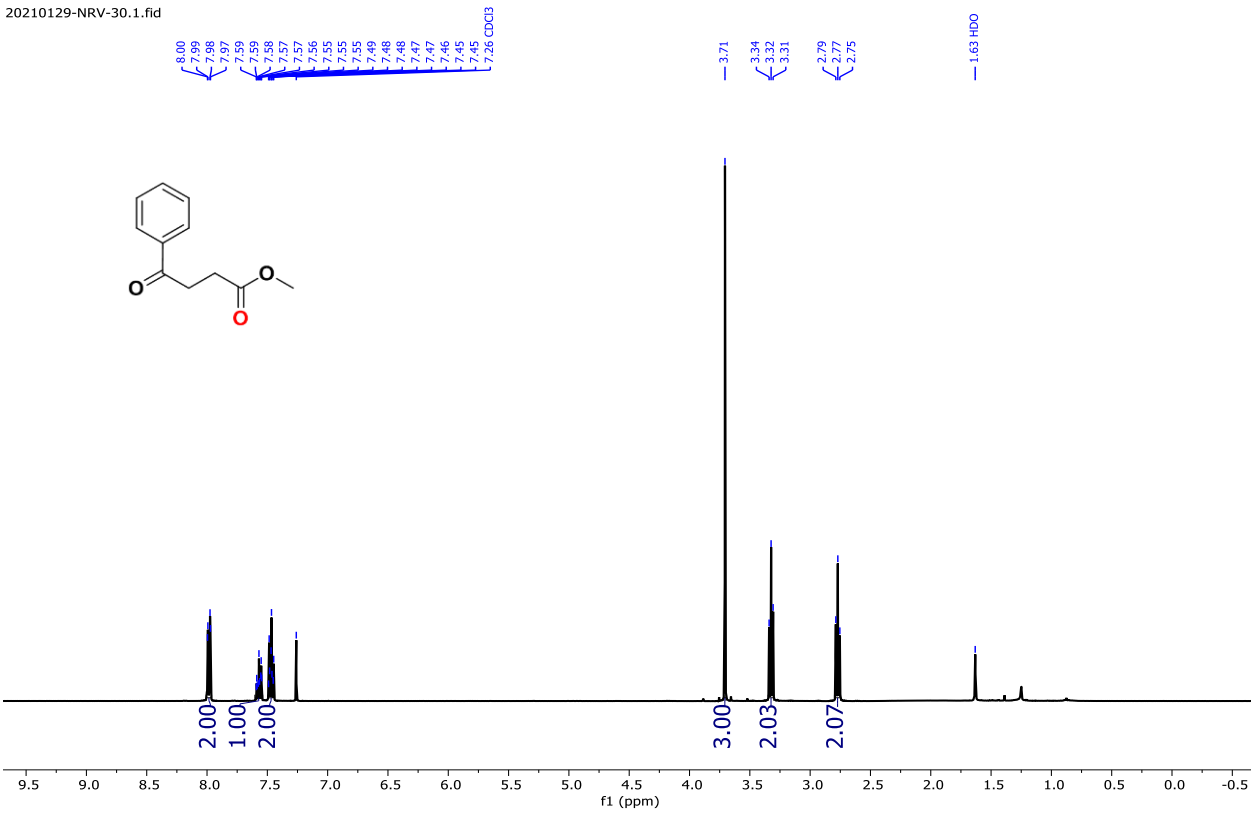
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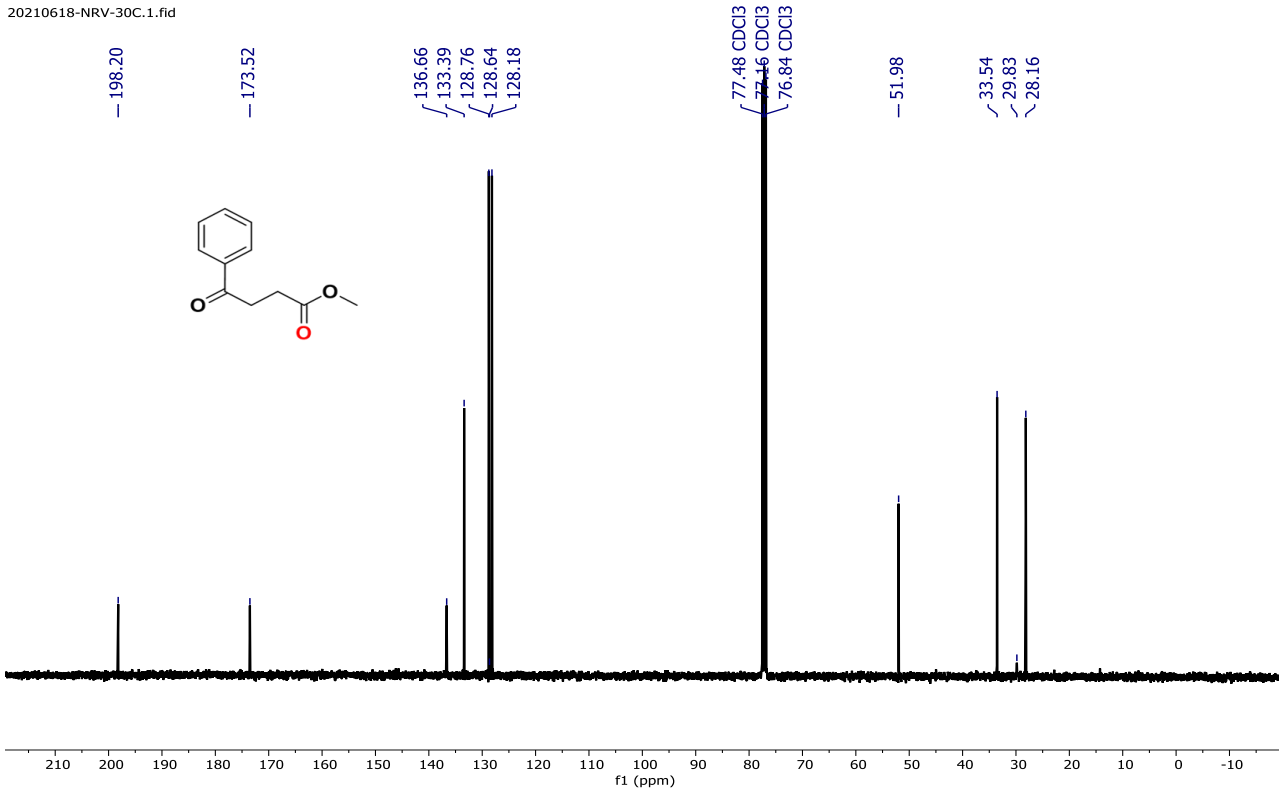
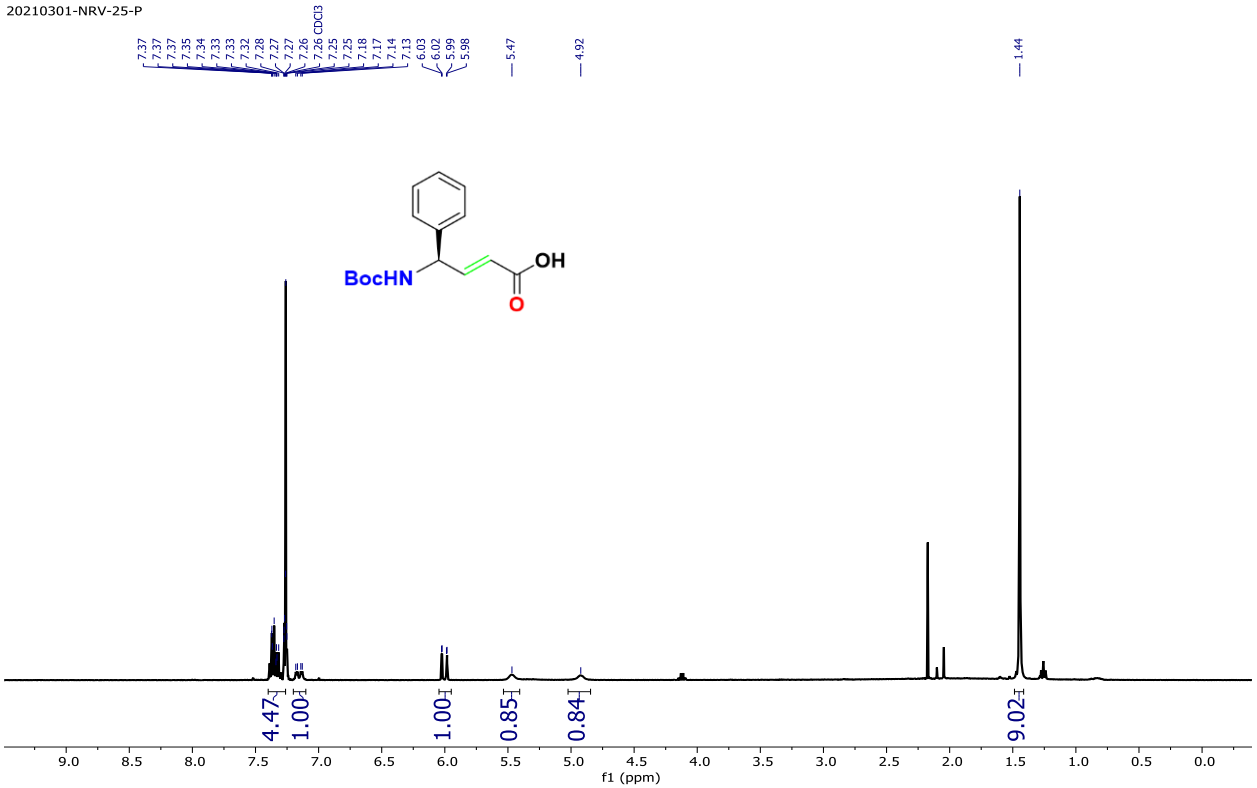
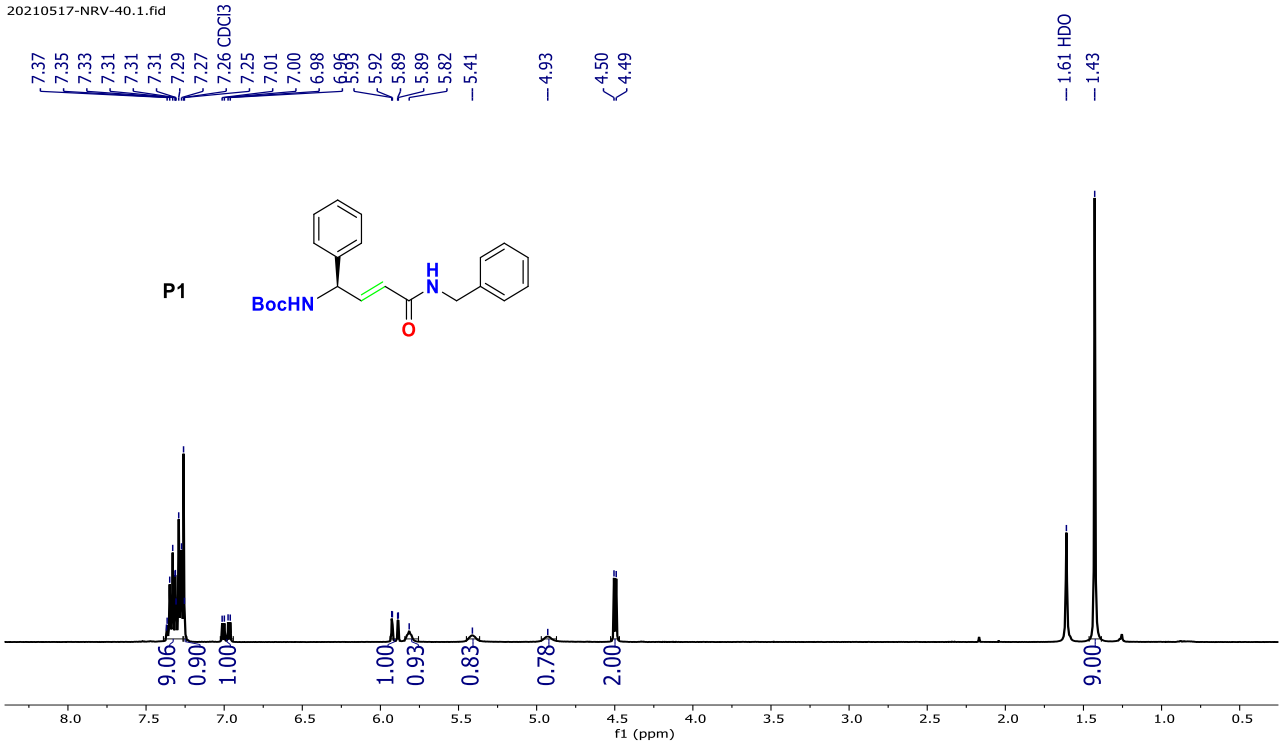
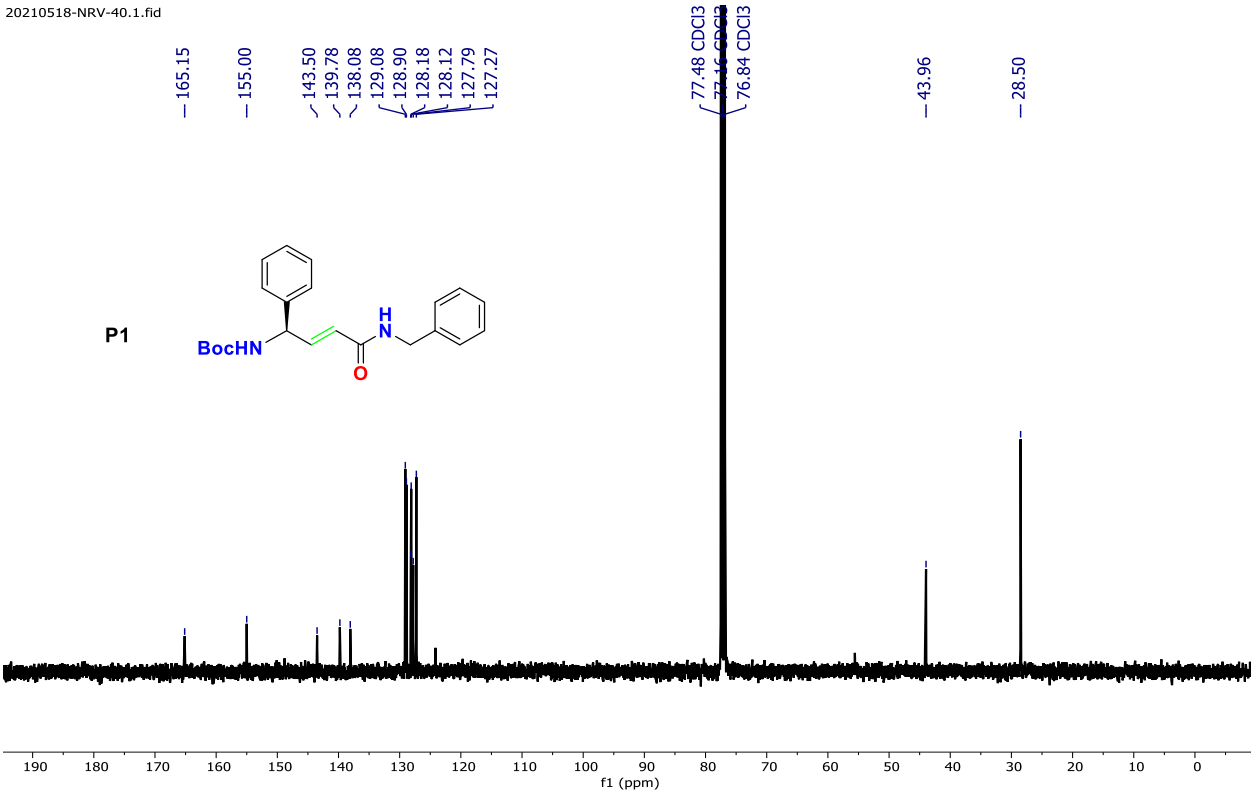
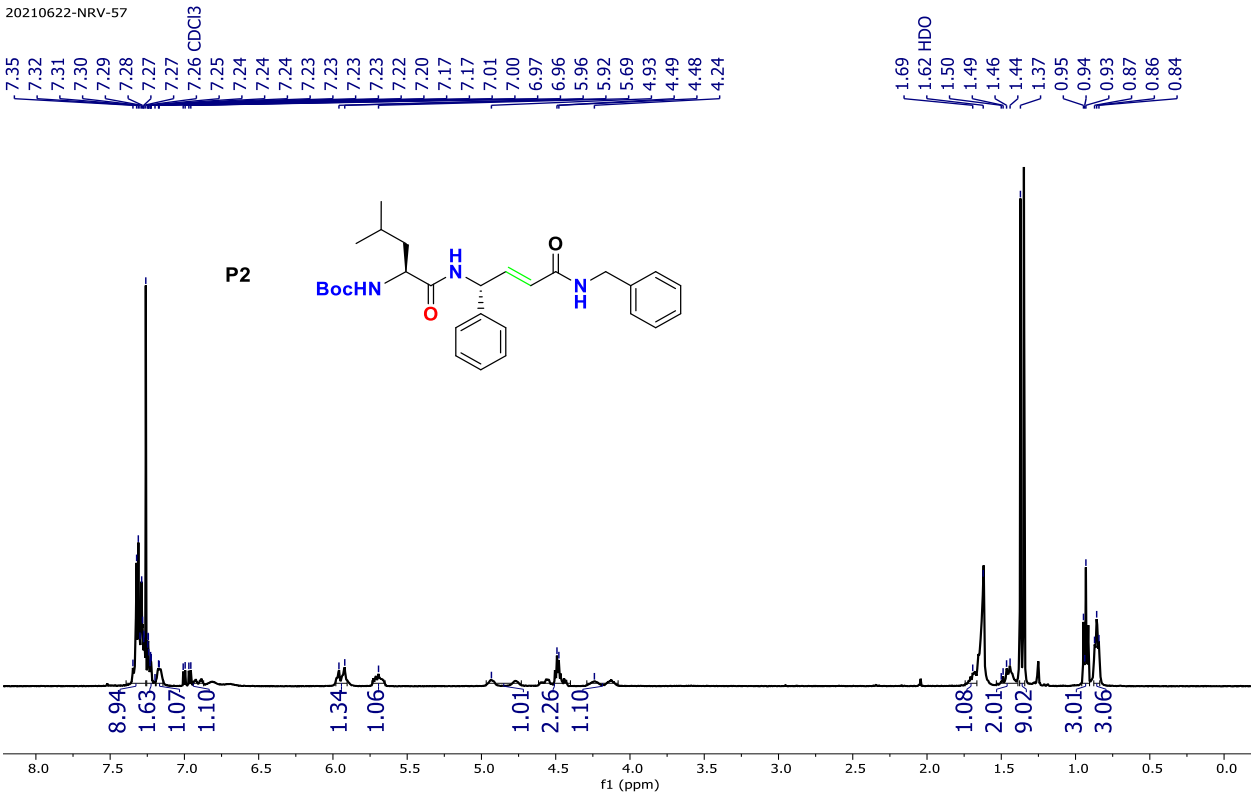
3.34
3.32
3.31
2.79
2.77
2.75
1.63 HDO
2.00
1.00
2.00
3.00
2.03
2.07
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

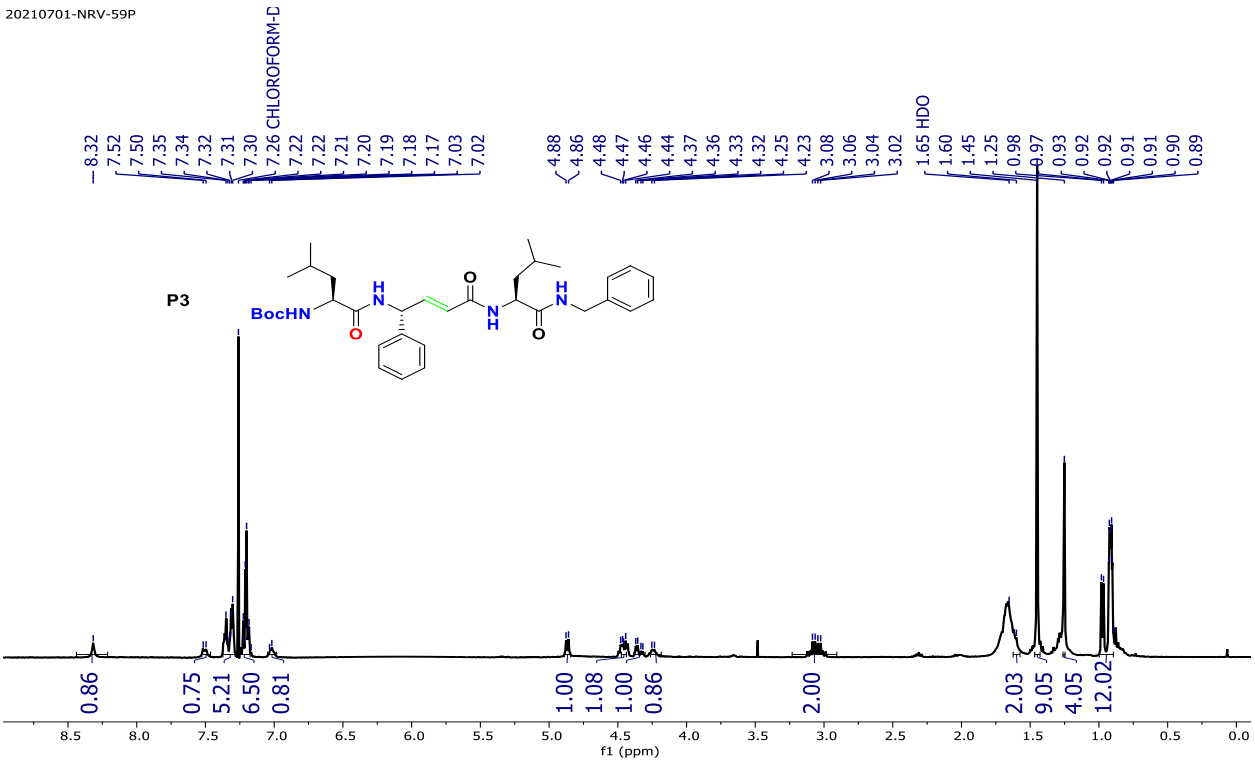

20210618-NRV-30C.1.fid
198.20
173.52
136.66
133.39
128.76
128.64
128.18
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
51.98
33.54
29.83
28.16
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

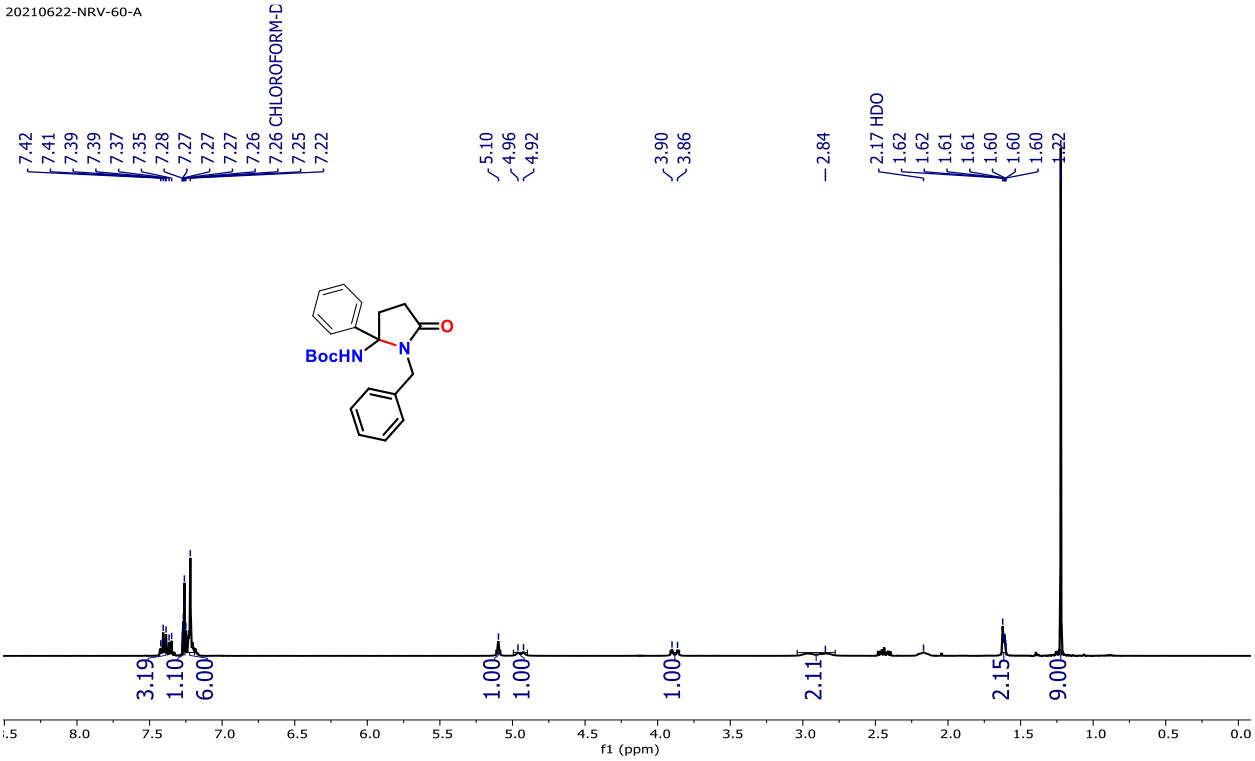

20210301-NRV-25-P
7.37
7.37
7.37
7.35
7.34
7.33
7.33
7.32
7.28
7.27
7.27
7.26
7.26 CDCl3
7.25
7.25
7.18
7.17
7.14
7.13
6.03
6.02
5.99
5.98
5.47
4.92
1.44
BocHN
OH
O
4.47
1.00
1.00
0.85
0.84
9.02
f1 (ppm)


20210517-NRV-40.1.fid
7.37
7.35
7.33
7.31
7.31
7.31
7.29
7.27
7.26 CDCl3
7.25
7.01
7.00
6.98
6.96
6.93
5.92
5.89
5.89
5.82
5.41
4.93
4.50
4.49
1.61 HDO
1.43
P1
BocHN
H
N
O
9.06
0.90
1.00
1.00
0.93
0.83
0.78
2.00
9.00
f1 (ppm)

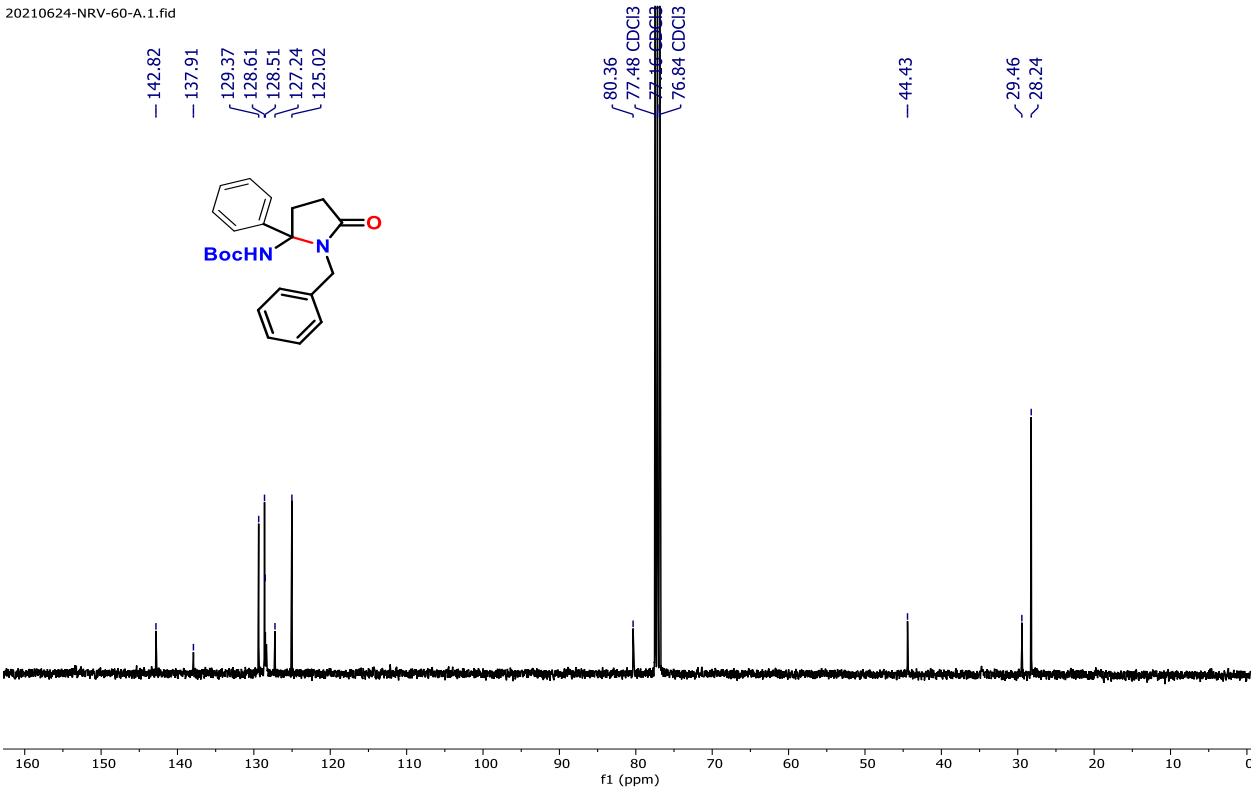

20210518-NRV-40.1.fid
165.15
155.00
143.50
139.78
138.08
129.08
128.90
128.18
128.12
127.79
127.27
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
43.96
28.50
P1
BocHN
H
N
O
f1 (ppm)


20210622-NRV-57
7.35
7.32
7.31
7.30
7.29
7.28
7.27
7.27
7.26 CDCl3
7.25
7.24
7.24
7.24
7.23
7.23
7.23
7.23
7.22
7.20
7.17
7.17
7.01
7.00
6.97
6.96
5.96
5.92
5.69
4.93
4.49
4.48
4.24
1.69
1.62 HDO
1.50
1.49
1.46
1.44
1.37
0.95
0.94
0.93
0.87
0.86
0.84
P2
BocHN
H
N
O
O
N
H
8.94
1.63
1.07
1.10
1.34
1.06
1.01
2.26
1.10
1.08
2.01
9.02
3.01
3.06
f1 (ppm)

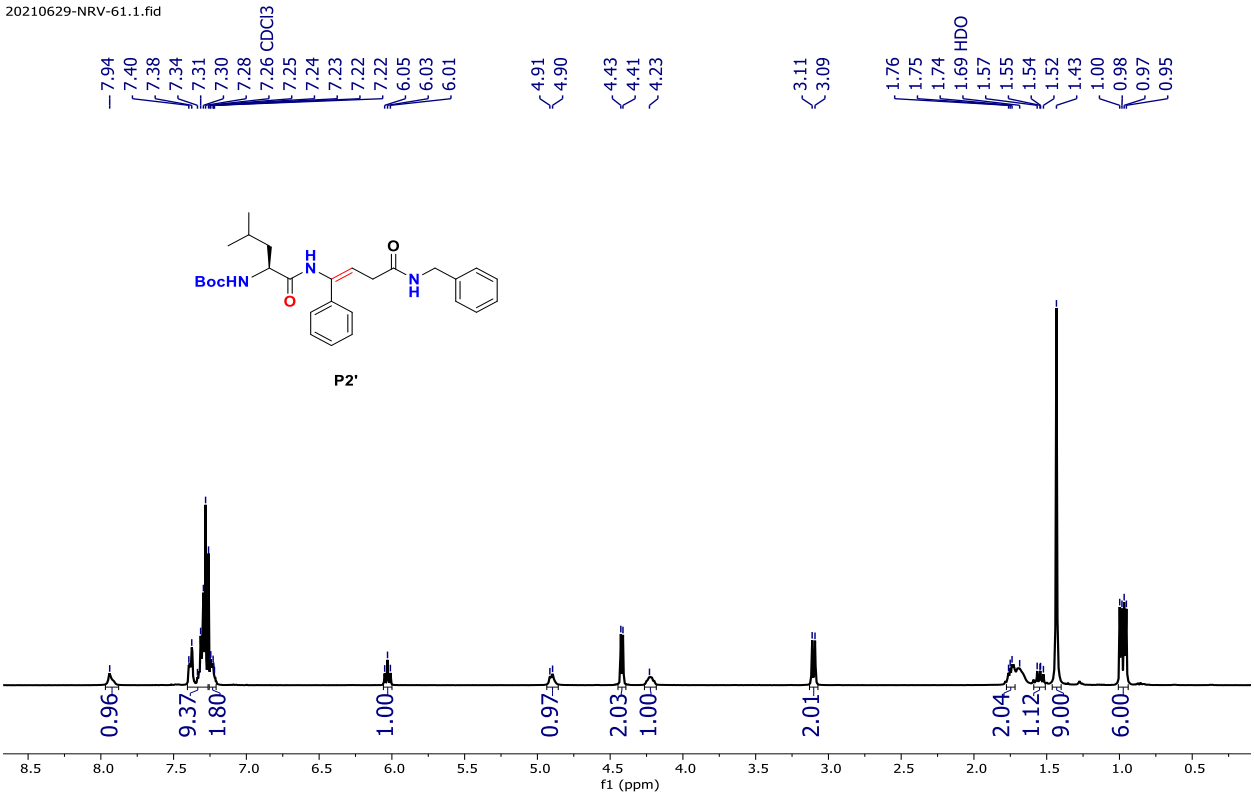

20210701-NRV-59P
CHLOROFORM-D
HDO
P3
BocHN
f1 (ppm)

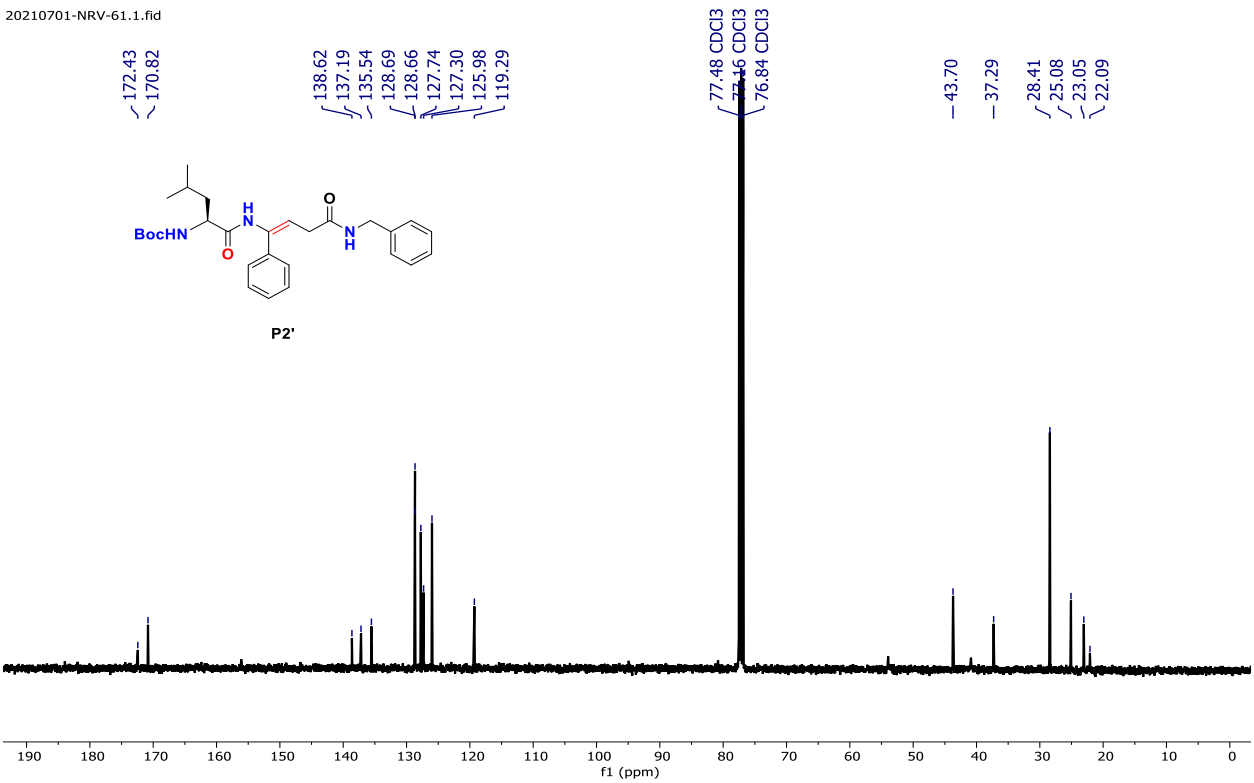

20210622-NRV-60-A
CHLOROFORM-D
HDO
BocHN
f1 (ppm)

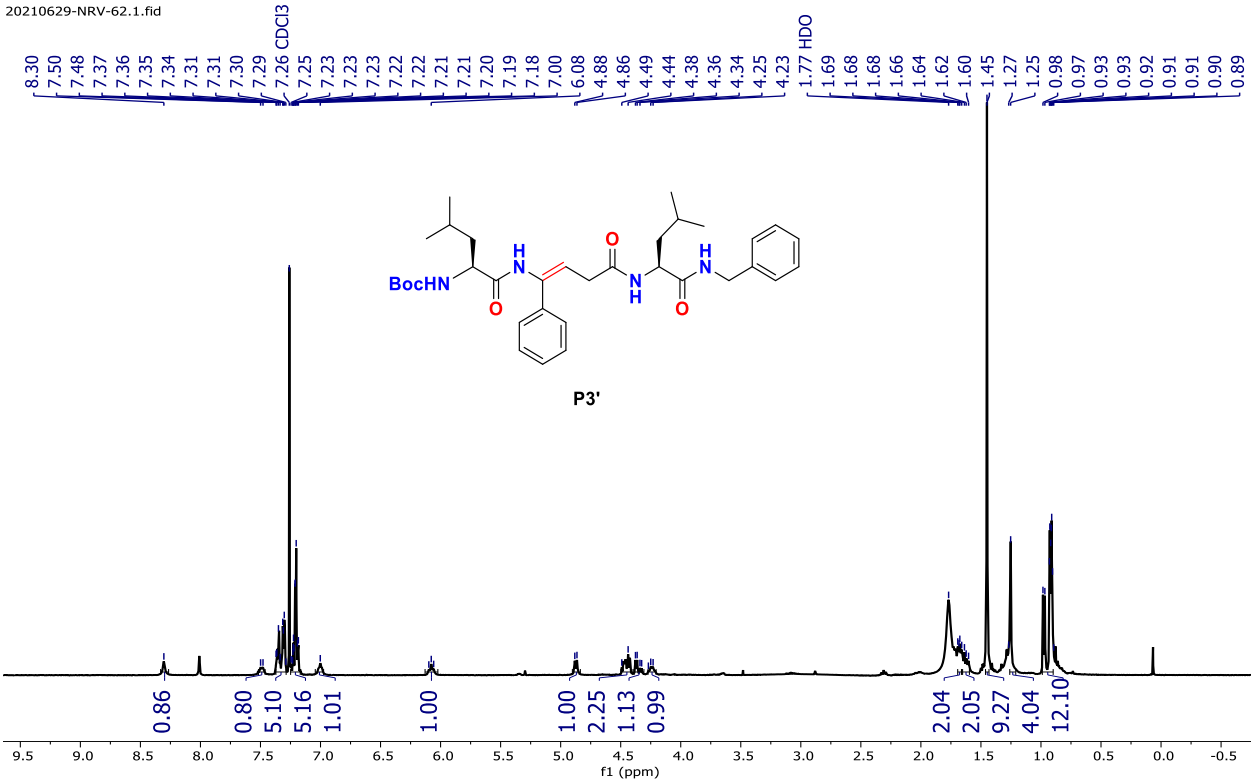
20210624-NRV-60-A.1.fid

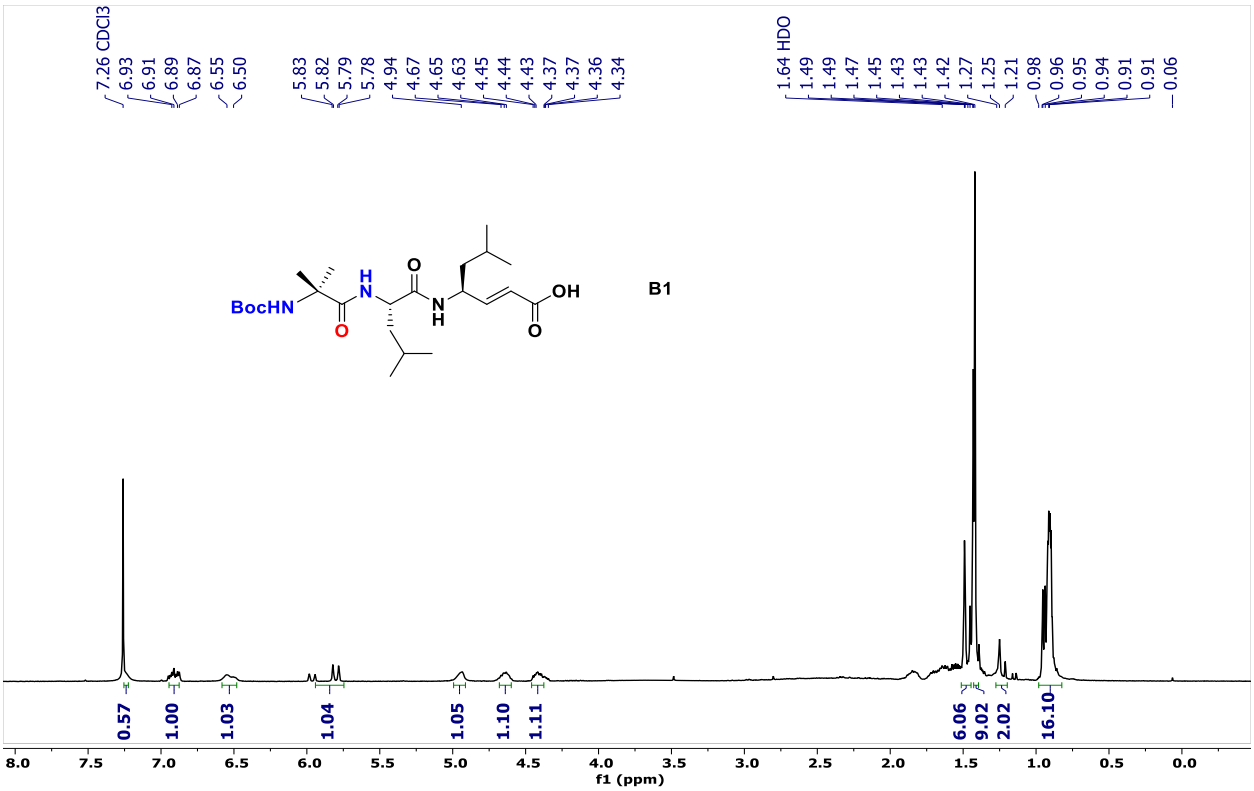


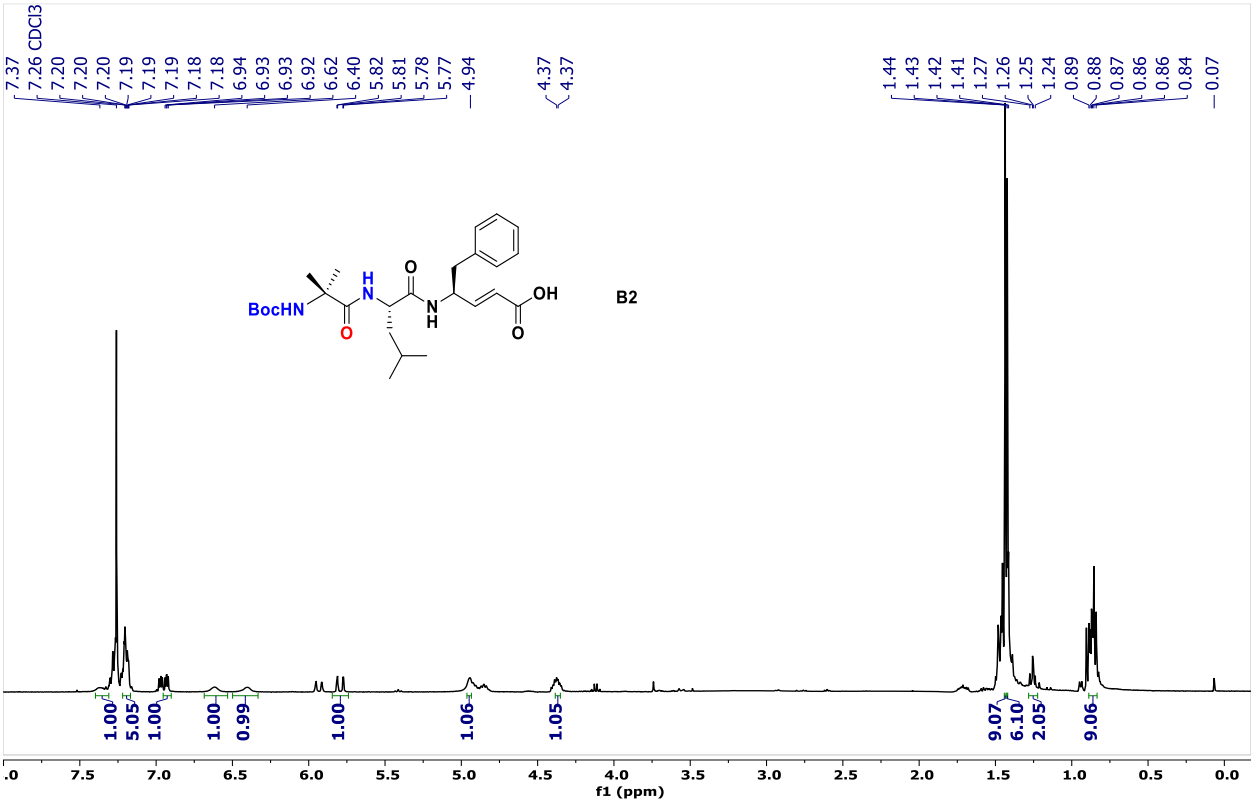
20210629-NRV-61.1.fid

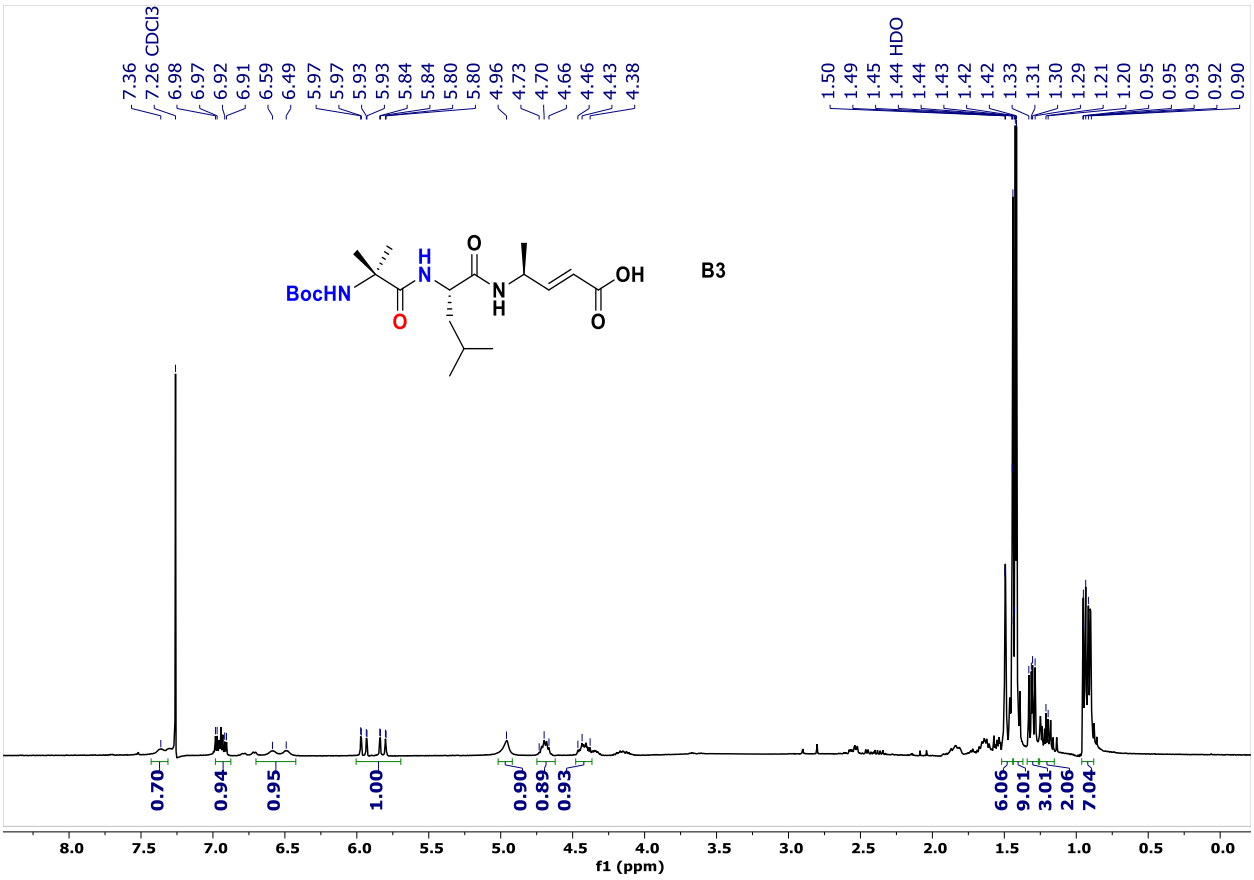



20210701-NRV-61.1.fid
172.43
170.82
138.62
137.19
135.54
128.69
128.66
127.74
127.30
125.98
119.29
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
43.70
37.29
28.41
25.08
23.05
22.09
BocHN
H
N
O
O
N
H
O
P2'
f1 (ppm)

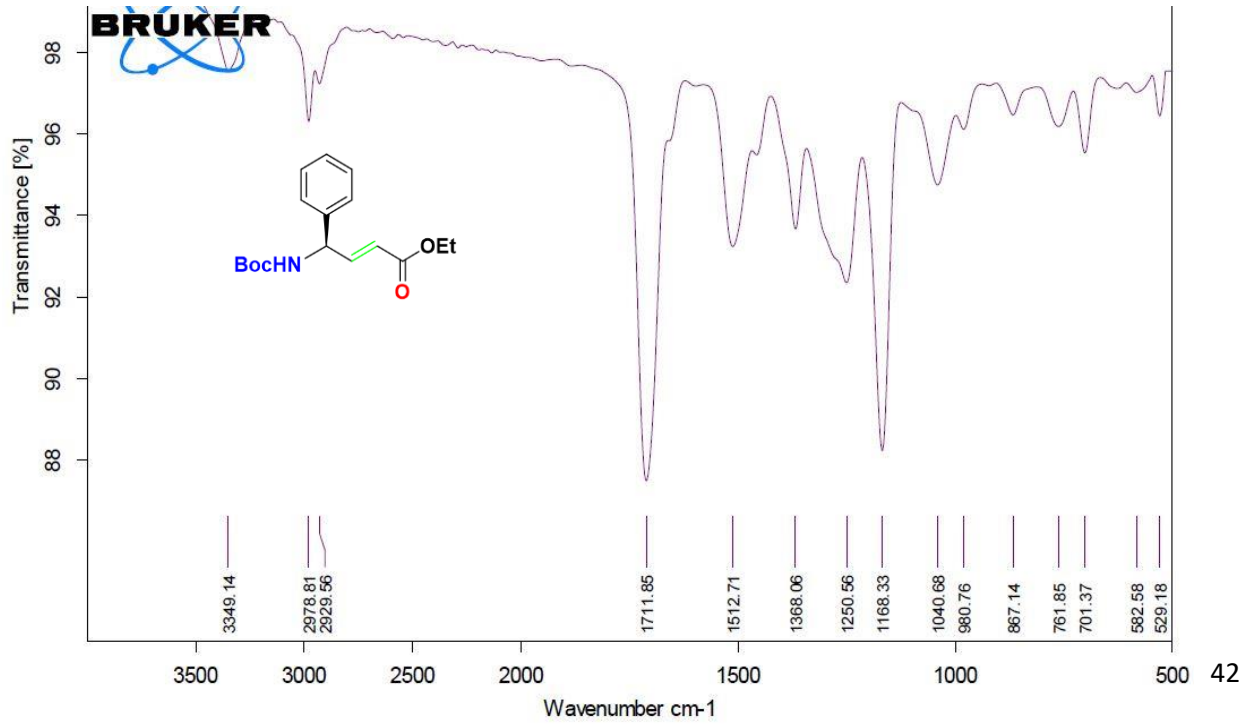

20210629-NRV-62.1.fid
7.26 CDCl3
1.77 HDO
BocHN
H
N
O
O
N
H
O
H
N
O
P3'
0.86
0.80
5.10
5.16
1.01
1.00
1.00
2.25
1.13
0.99
2.04
2.05
9.27
4.04
12.10
f1 (ppm)

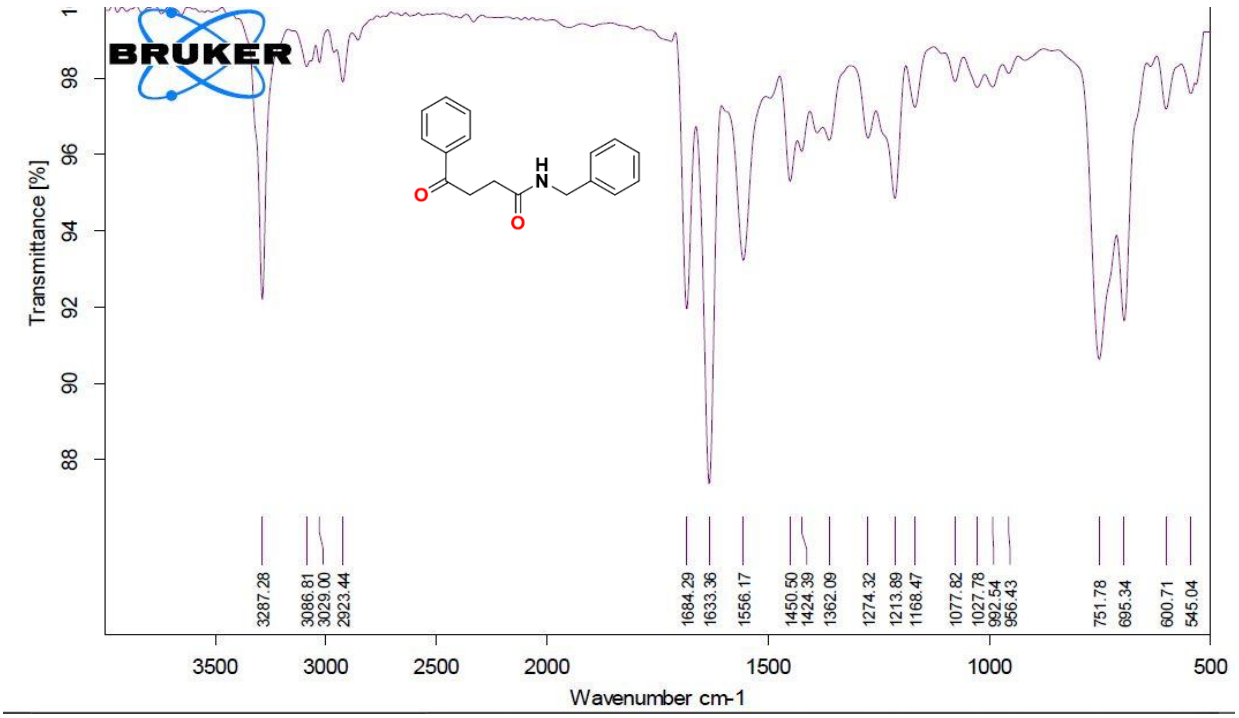

7.26 CDCl3
6.93
6.91
6.89
6.87
6.55
6.50
5.83
5.82
5.79
5.78
4.94
4.67
4.65
4.63
4.45
4.44
4.43
4.37
4.36
4.34
1.64 HDO
1.49
1.49
1.47
1.45
1.43
1.43
1.42
1.27
1.25
1.21
0.98
0.96
0.95
0.94
0.91
0.91
0.06
BocHN
O
H
N
O
N
H
OH
O
B1
0.57
1.00
1.03
1.04
1.05
1.10
1.11
6.06
9.02
2.02
16.10
8.0
7.5
7.0
6.5
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)

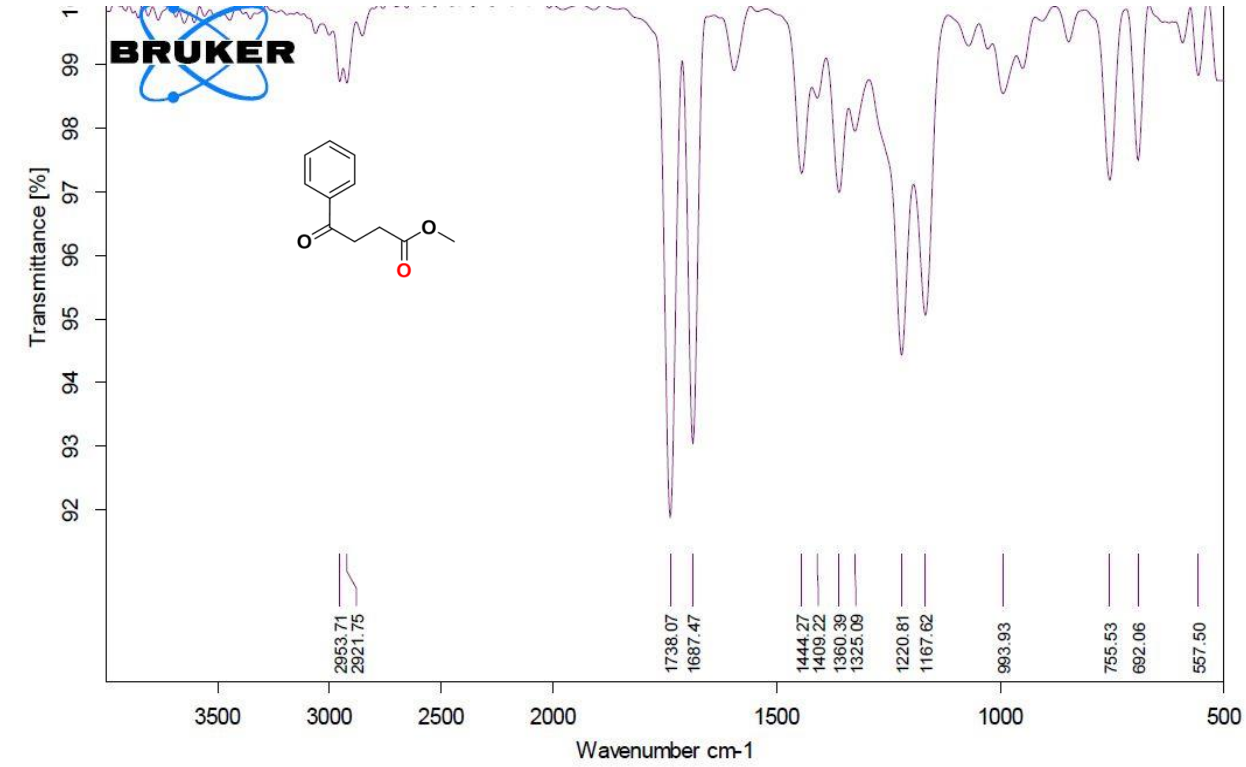

7.37
7.26 CDCl3
7.20
7.20
7.20
7.19
7.19
7.19
7.18
7.18
6.94
6.93
6.93
6.92
6.62
6.40
5.82
5.81
5.78
5.77
4.94
4.37
4.37
1.44
1.43
1.42
1.41
1.27
1.26
1.25
1.24
0.89
0.88
0.87
0.86
0.86
0.84
0.07
BocHN
O
H
N
O
N
H
OH
O
B2
1.00
5.05
1.00
1.00
0.99
1.00
1.06
1.05
9.07
6.10
2.05
9.06
.0
7.5
7.0
6.5
6.0
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)



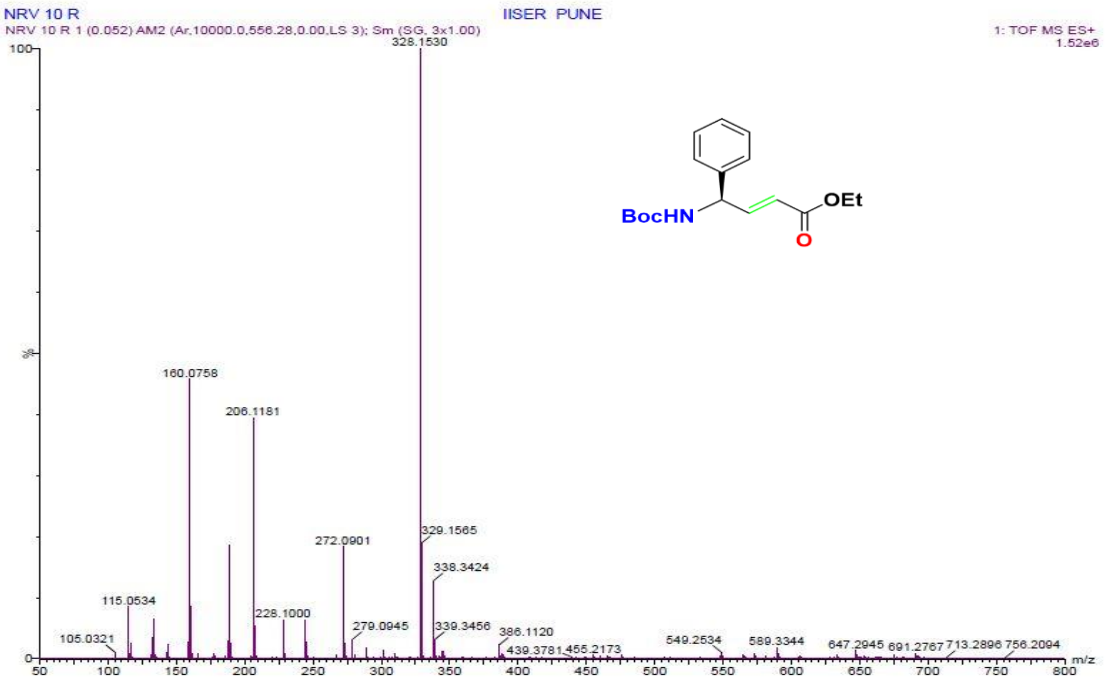
7.2 I R spectrum of compounds

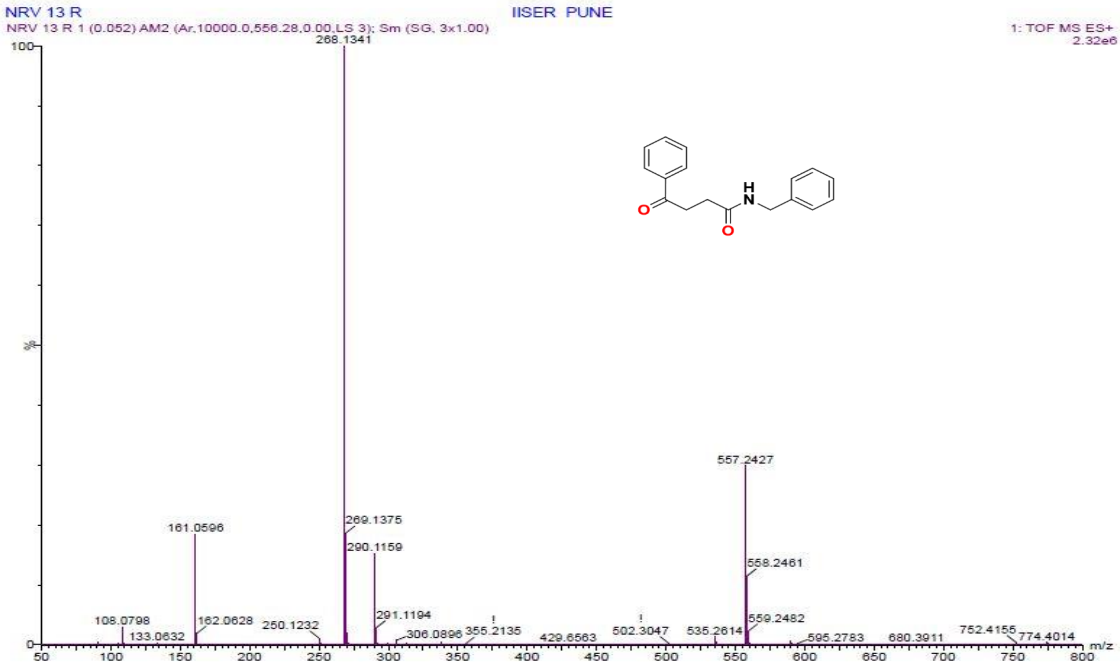


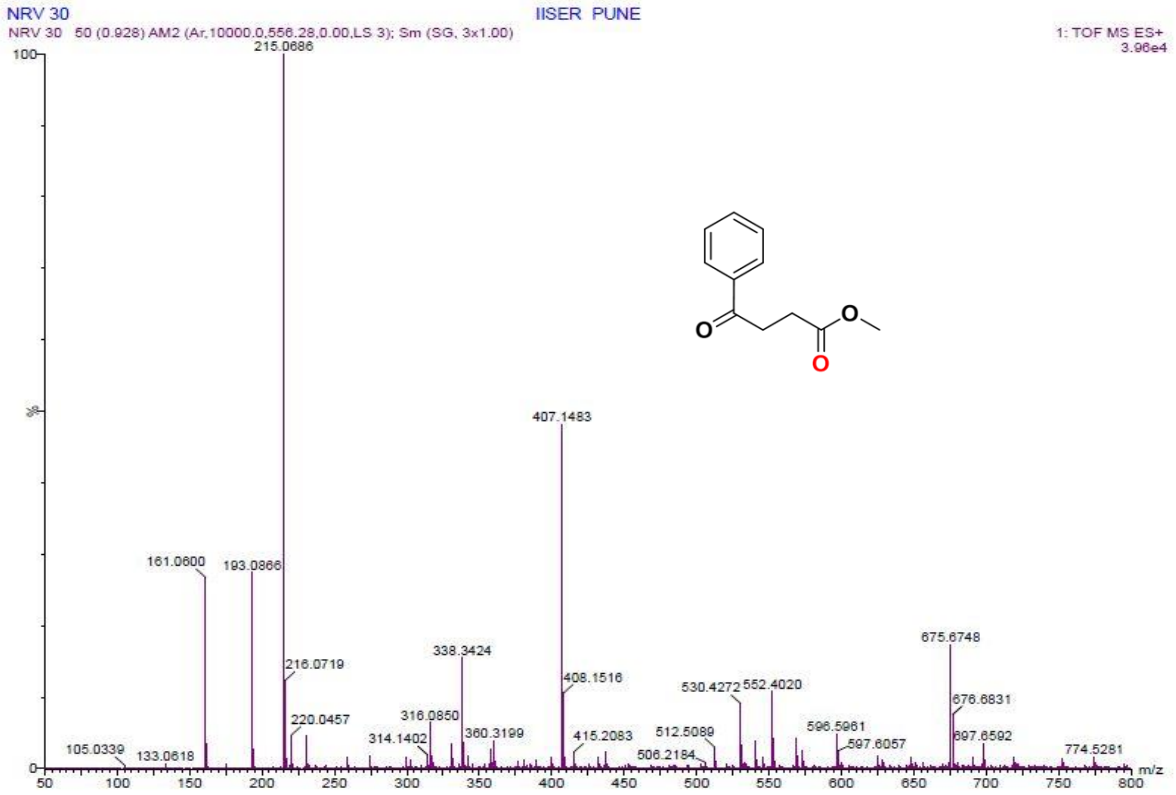

BRUKER
Transmittance [%]
100
98
96
94
92
90
88
3287.28
3086.81
3029.00
2923.44
1684.29
1633.36
1556.17
1450.50
1424.39
1362.09
1274.32
1213.89
1168.47
1077.82
1027.78
992.54
956.43
751.78
695.34
600.71
545.04
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

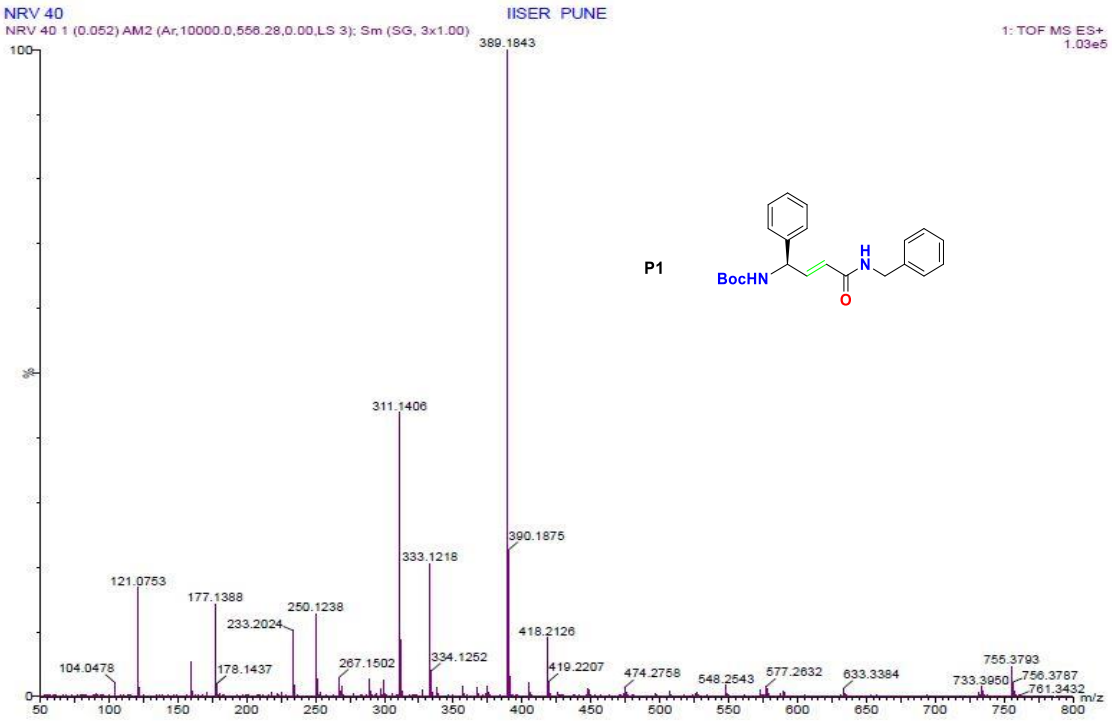

BRUKER
Transmittance [%]
100
99
98
97
96
95
94
93
92
2953.71
2921.75
1738.07
1687.47
1444.27
1409.22
1360.39
1325.09
1220.81
1167.62
993.93
755.53
692.06
557.50
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

7.3 HRMS Spectra of Compounds

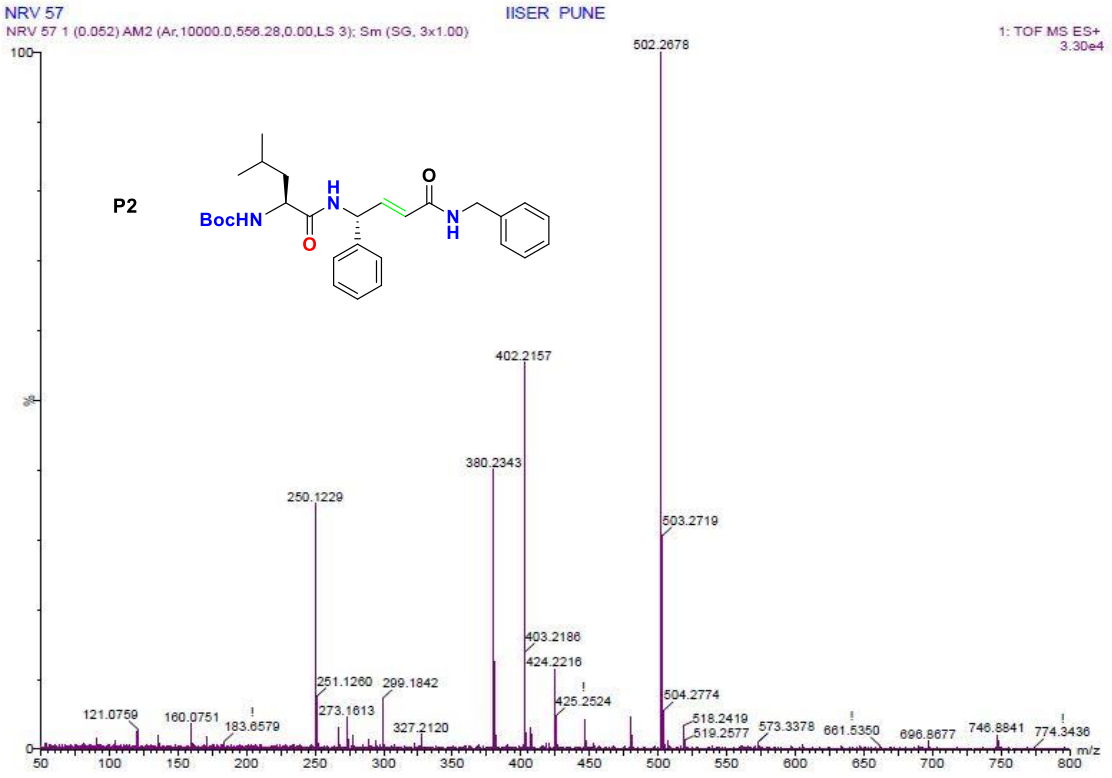


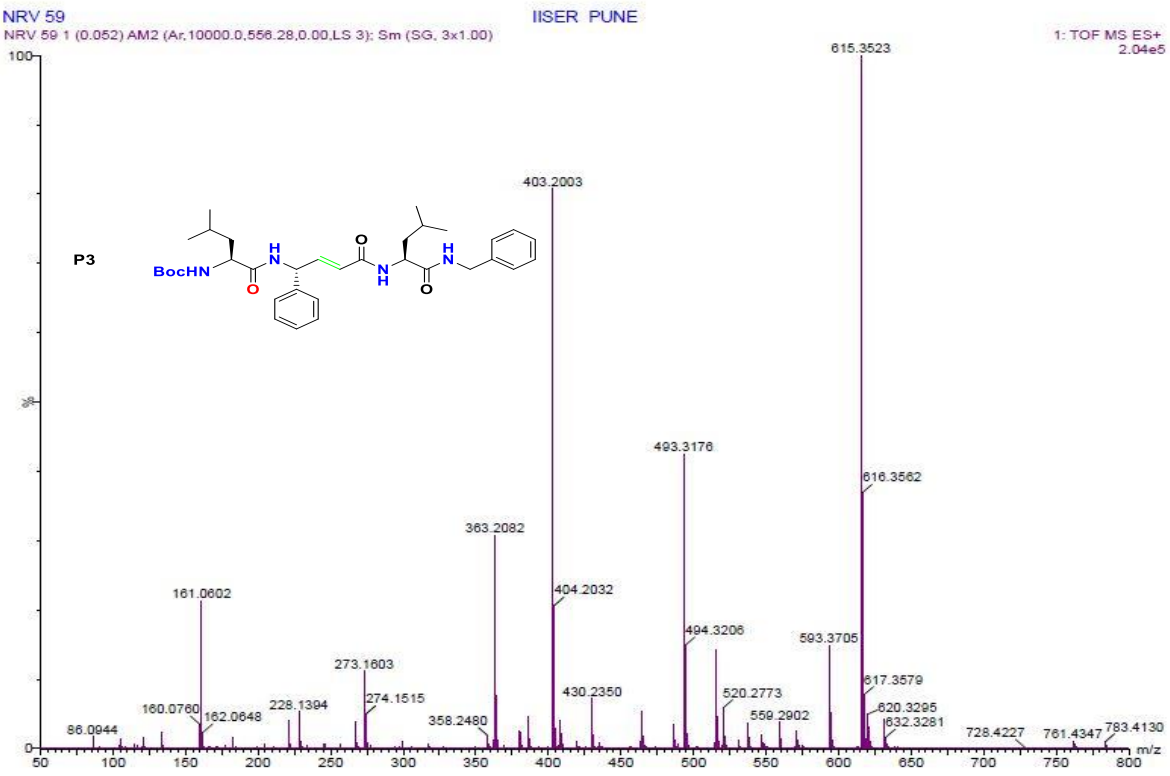


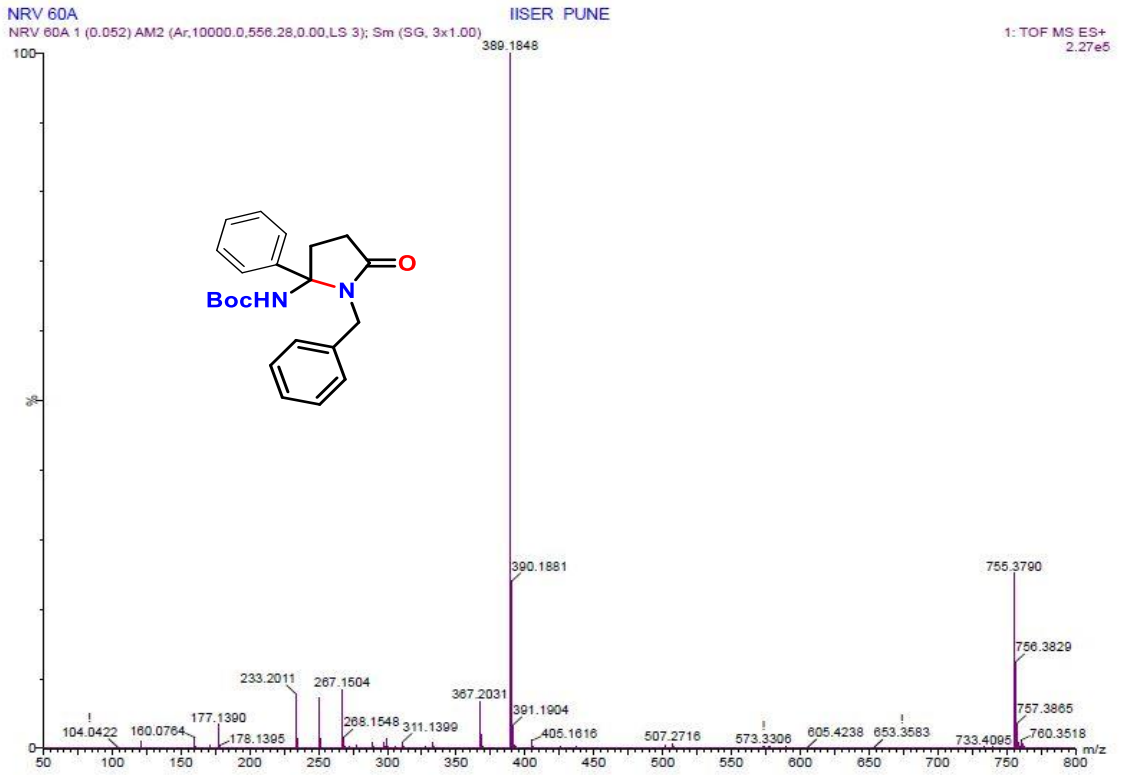

NRV 30
IISER PUNE
NRV 30 50 (0.928) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
3.96e4
215.0686
407.1483
161.0600
193.0866
216.0719
338.3424
408.1516
220.0457
316.0850
360.3199
314.1402
415.2083
512.5089
506.2184
530.4272
552.4020
675.6748
676.6831
596.5961
597.6057
697.6592
774.5281
105.0339
133.0618
m/z

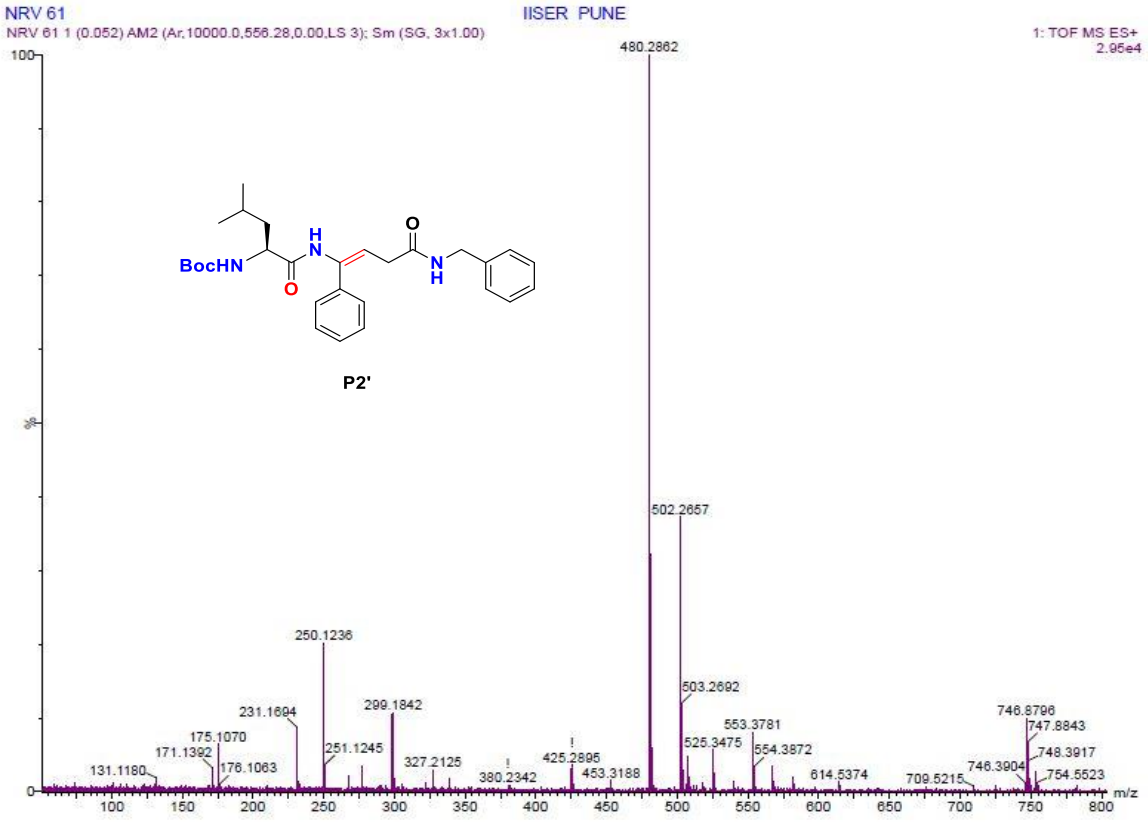

NRV 40
IISER PUNE
NRV 40 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
1.03e5
389.1843
311.1406
390.1875
333.1218
121.0753
177.1388
250.1238
233.2024
418.2126
104.0478
178.1437
267.1502
334.1252
419.2207
474.2758
548.2543
577.2632
633.3384
755.3793
733.3950
756.3787
761.3432
m/z

P1

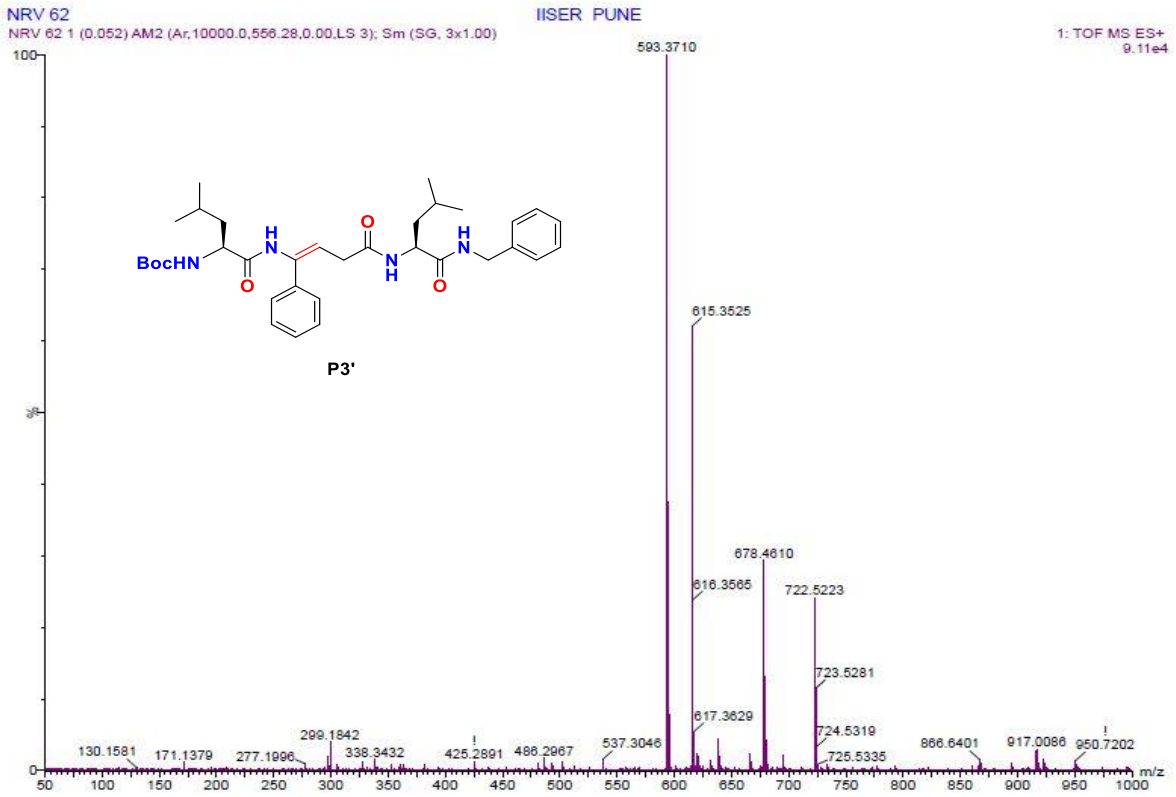

NRV 57
IISER PUNE
NRV 57 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
3.30e4
P2
BocHN
502.2678
402.2157
380.2343
250.1229
503.2719
403.2186
424.2216
251.1260
299.1842
425.2524
504.2774
273.1613
121.0759
160.0751
183.6579
327.2120
518.2419
519.2577
573.3378
661.5350
696.8677
746.8841
774.3436
m/z

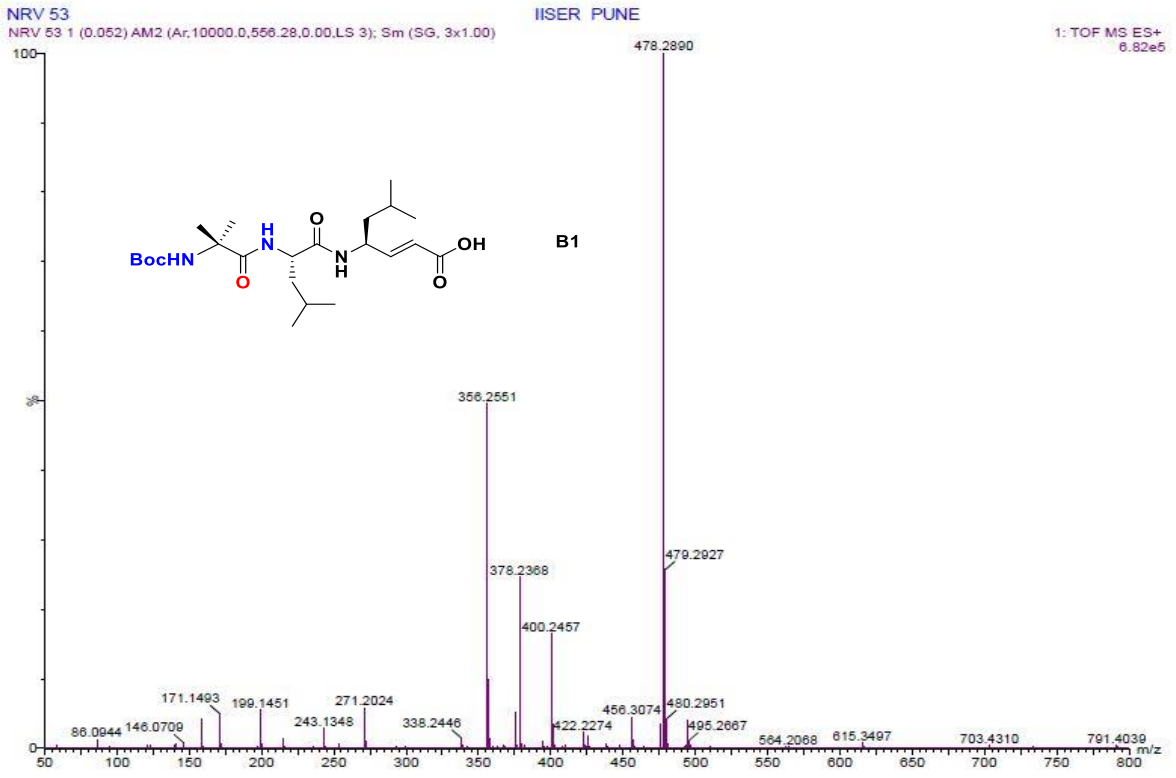

NRV 59
IISER PUNE
NRV 59 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
2.04e5
P3
BocHN
615.3523
403.2003
493.3176
616.3562
363.2082
161.0602
404.2032
494.3206
593.3705
273.1603
617.3579
274.1515
430.2350
520.2773
160.0760
162.0648
228.1394
358.2480
559.2902
620.3295
632.3281
86.0944
728.4227
761.4347
783.4130
m/z

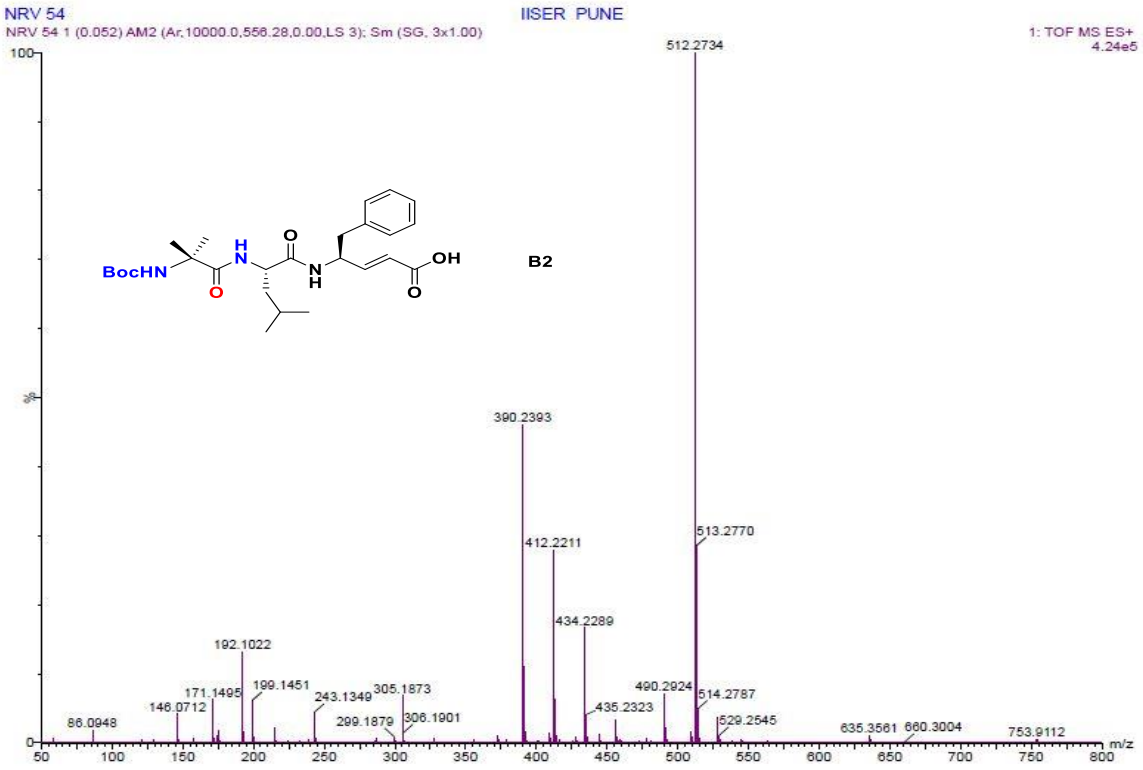

NRV 60A
IISER PUNE
NRV 60A 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
2.27e5
BocHN
O
N
389.1848
390.1881
755.3790
756.3829
757.3865
760.3518
233.2011
267.1504
367.2031
391.1904
177.1390
268.1548
311.1399
104.0422
160.0764
178.1395
405.1616
507.2716
573.3306
605.4238
653.3583
733.4095
m/z

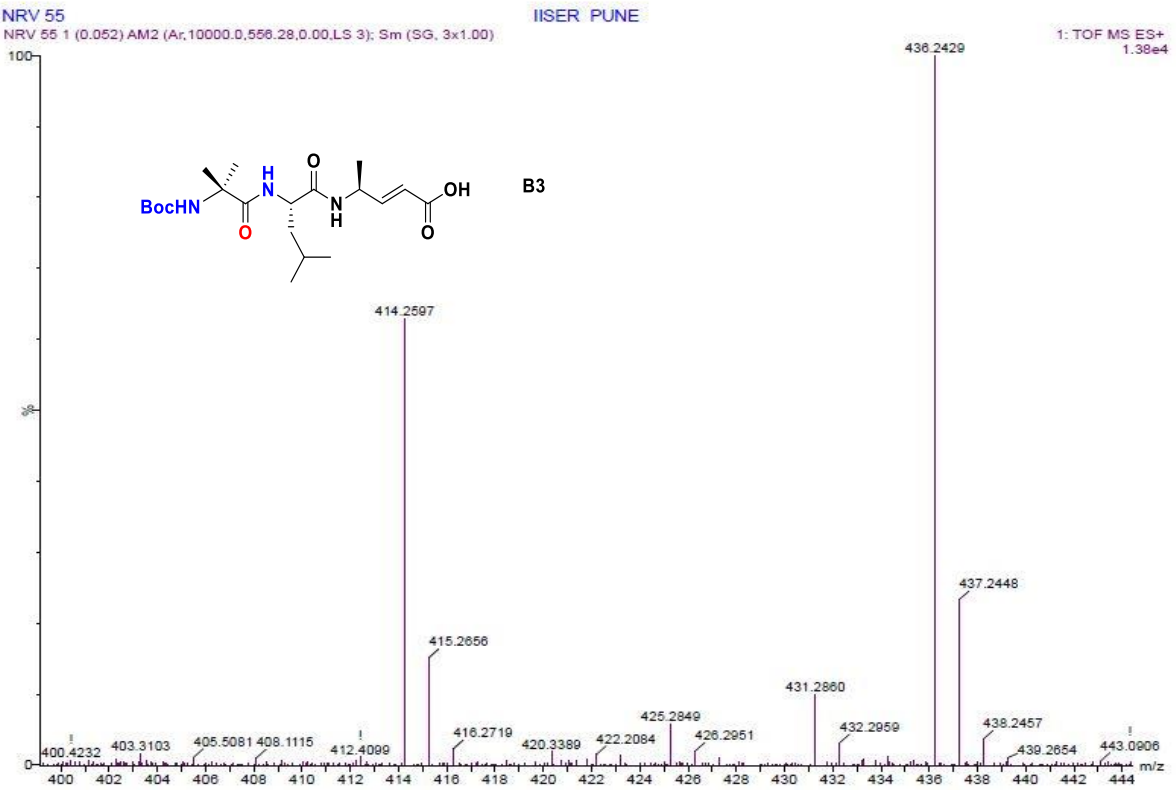

NRV 61
IISER PUNE
NRV 61 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
2.95e4
BocHN
H
N
O
O
N
H
480.2862
502.2657
503.2692
250.1236
299.1842
231.1694
175.1070
171.1392
131.1180
176.1063
251.1245
327.2125
380.2342
425.2895
453.3188
525.3475
553.3781
554.3872
614.5374
709.5215
746.3904
746.8796
747.8843
748.3917
754.5523
m/z

P2'


NRV 62
NRV 62 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
IISER PUNE
1: TOF MS ES+
9.11e4
BocHN
P3'
593.3710
615.3525
678.4610
616.3565
722.5223
723.5281
617.3629
724.5319
725.5335
299.1842
130.1581
171.1379
277.1996
338.3432
425.2891
486.2967
537.3046
866.6401
917.0086
950.7202
%
m/z


NRV 53
NRV 53 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
IISER PUNE
1: TOF MS ES+
6.82e5
BocHN
OH
B1
478.2890
356.2551
479.2927
378.2368
400.2457
171.1493
199.1451
271.2024
243.1348
338.2446
456.3074
480.2951
422.2274
495.2667
86.0944
146.0709
564.2068
615.3497
703.4310
791.4039
%
m/z


NRV 54
NRV 54 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
IISER PUNE
1: TOF MS ES+
4.24e5
BocHN
B2
512.2734
390.2393
412.2211
513.2770
434.2289
192.1022
199.1451
171.1495
146.0712
243.1349
305.1873
86.0948
299.1879
306.1901
490.2924
514.2787
435.2323
529.2545
635.3561
660.3004
753.9112
m/z


NRV 55
NRV 55 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
IISER PUNE
1: TOF MS ES+
1.38e4
BocHN
B3
436.2429
414.2597
437.2448
415.2656
431.2860
425.2849
432.2959
438.2457
416.2719
420.3389
422.2084
426.2951
400.4232
403.3103
405.5081
408.1115
412.4099
439.2654
443.0906
m/z