Synthesis and Characterization of Hybrid peptides Containing Gamma- and Vinylogous amino acids

A thesis submitted towards partial fulfillment of

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By

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CERTIFICATE

This is to certify that this dissertation entitled *" Synthesis and Characterization of Hybrid peptides Containing Gamma- and Vinylogous amino acids"* towards the partial fulfillment of the BS-MS dual degree programme at the Indian Institute of Science Education & Research, Pune represents original research carried out by Neha Agrawal at IISER Pune under the supervision of Dr. H. N. Gopi, Assistant Professor, Department of Chemistry, IISER Pune during the academic year 2011-2012.

Supervisor: Dr. H. N. Gopi

Date: 2/4/2012

Place: IISER, Pune

DECLARATION

I hereby declare that the matter embodied in the report entitled *"Synthesis and Characterization of Hybrid peptides Containing Gamma- and Vinylogous amino acids"* are the results of the investigations carried out by me at the Department of Chemistry, IISER Pune, under the supervision of Dr. H. N. Gopi and the same has not been submitted elsewhere for any other degree.

Name of the student: Neha Agrawal

(20071020)

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ABSTRACT

In contrast to the β- and α/β-peptides, the progress in the γ-, α/γ and β/γ-hybrid peptides is lagging behind. This is probably due to the difficulty in obtaining stereochemically pure double homologated γ^4-amino acids. In this project, we are describing the synthesis of stereochemically pure γ^4-amino acids and their utilization in the solid phase synthesis of hybrid octapeptides. The γ^4-amino acids were synthesized starting from the protected α-amino acids through Wittig reaction. The structural analysis of the peptides is under progress.

INTRODUCTION

The deconstruction of a protein leads to a limited number of secondary structural elements, such as strands, helices, and turns, which are assembled using loosely structured loops. These secondary structures are locally defined, meaning that there can be many different secondary motifs present in one single protein molecule. The helical secondary structures of α-peptides can be recognized as C_{13}-helix (or α- helix), C_{10}-helix (3_{10}-helix) and C_7-helix (or γ-helix) by the nature of the internal hydrogen bonding pattern [1, 2]. In all cases, the directionality of the hydrogen bond with respect to the chain direction (C ←N) is the same. Helices associate to form α-helical hairpins, coiled-coils, or helical bundles. Unlike helical geometries, the extended conformations can be classified as parallel and antiparallel β-sheets [1-3]. The two extended sheets are connected by a turn leading to β-hairpin structures. These occur widely in proteins and make up the fundamental building blocks of antiparallel β-sheets. The β-turns are the most economical polypeptide geometry that can link two extended segments of chain with reversal direction. The β-turn is the simplest defined loop structure with conformational characteristics determined by residues at two positions (i+1 and i+2) [4]. *De novo* design of existing or novel protein folds demands a thorough understanding of the rules that underlie protein structure and stability. Considerable attention has been paid over the past several decades on polypeptides composed of α-amino acids, with natural proteins and the use of stereochemically constrained amino acids and templates in the design of folded polypeptides [5]. Mimicking the protein secondary structures with non-natural amino acids or with organic templates is not only important to understand the protein folding problem but also from the perspective of medicinal chemistry [6]. In this regard a great deal of success has been achieved using the backbone homologated α-amino acids [7,8] .

In the past few years β-peptides composed of β-amino acids emerged as very promising tools in medicinal chemistry [9]. The β-amino acids are homologated species of α-amino acids [10]. Depending on the position of the side chain, they are classified as β^3- and β^2-amino acids (Figure 1). Double homologation of α-amino acids leads to $\gamma^{2,3}$-,

γ^3-, and γ^4-amino acids [11]. In contrast to α-peptides, β-peptides and higher homologue oligomers have proved the proteolytic and metabolic stability and the prospect of

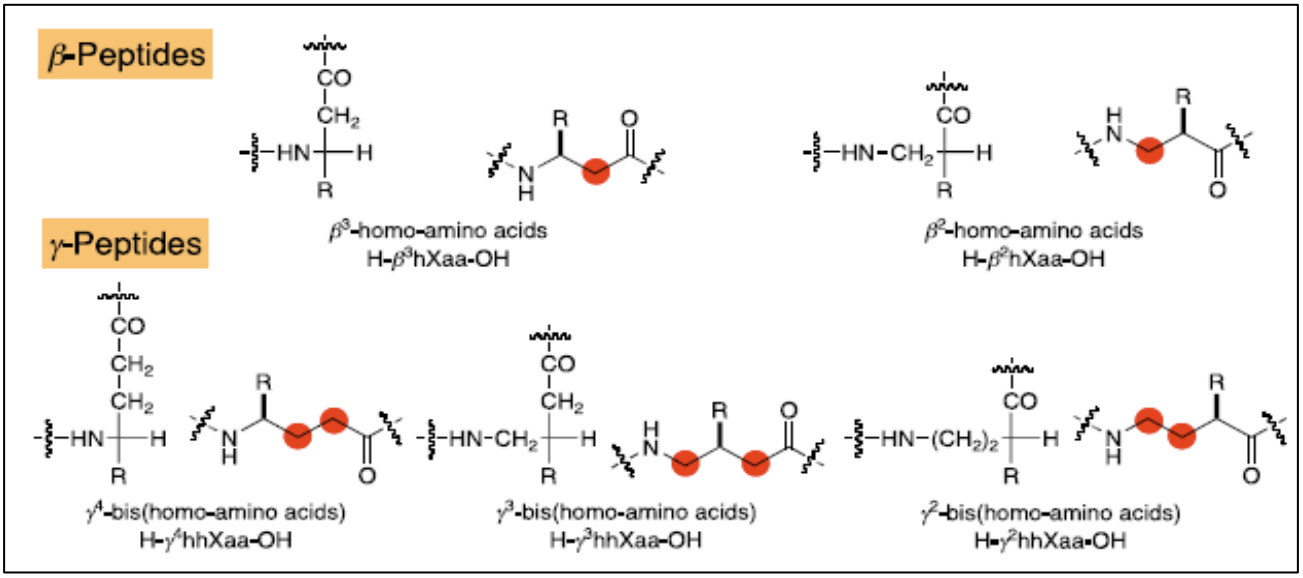


Figure 1: Different types of backbone homologated β-and γ-amino acids. The red circles on the amino acid structures indicating that the sites are free for further substitution and derivatization. The superscript numerical indicates the position of the side-chains.

intracellular delivery [12-14]. These properties make β-peptides and higher homologue oligomers very attractive from a biomedical perspective. β-Peptides have particular appeal for extending our understanding of protein structure and stabilization into the realm of folded, non-biological polymers, because β-amino acids represent the smallest step away from α-amino acids in "backbone space". Oligomers of β- and higher homologue peptides form unique, stable secondary structures [9,15]. Some of the representative examples are shown in Figure 2. The design of heterooligomers composed of α-and backbone homologated amino acids are more attractive compared to homooligomers due to the structural diversity and wide availability of side-chain diversity of α-amino acids. Balaram and colleagues and Gellman et al. showed variety of helical structures by the incorporation of β-amino acids as guests into the host α-peptides as well as 1:1 heterooligomers composed of α- and β-amino acids [16,17]. In contrast to the β-peptides and heterooligomers containing β-amino amino acids, the progress in field of γ-peptides (oligomers of double homologated α- amino acids) is

lagging behind. In addition, very little is known about the heterooligomers containing α- and γ-amino acids. This is probably due to the difficulty of obtaining stereochemically pure γ-amino acids. However, Hofmann and colleagues predicted the wide variety of helical structures heterooligomers containing 1:1 α- and γ-amino acids as well as 1:1 β- and γ-amino acids [18]

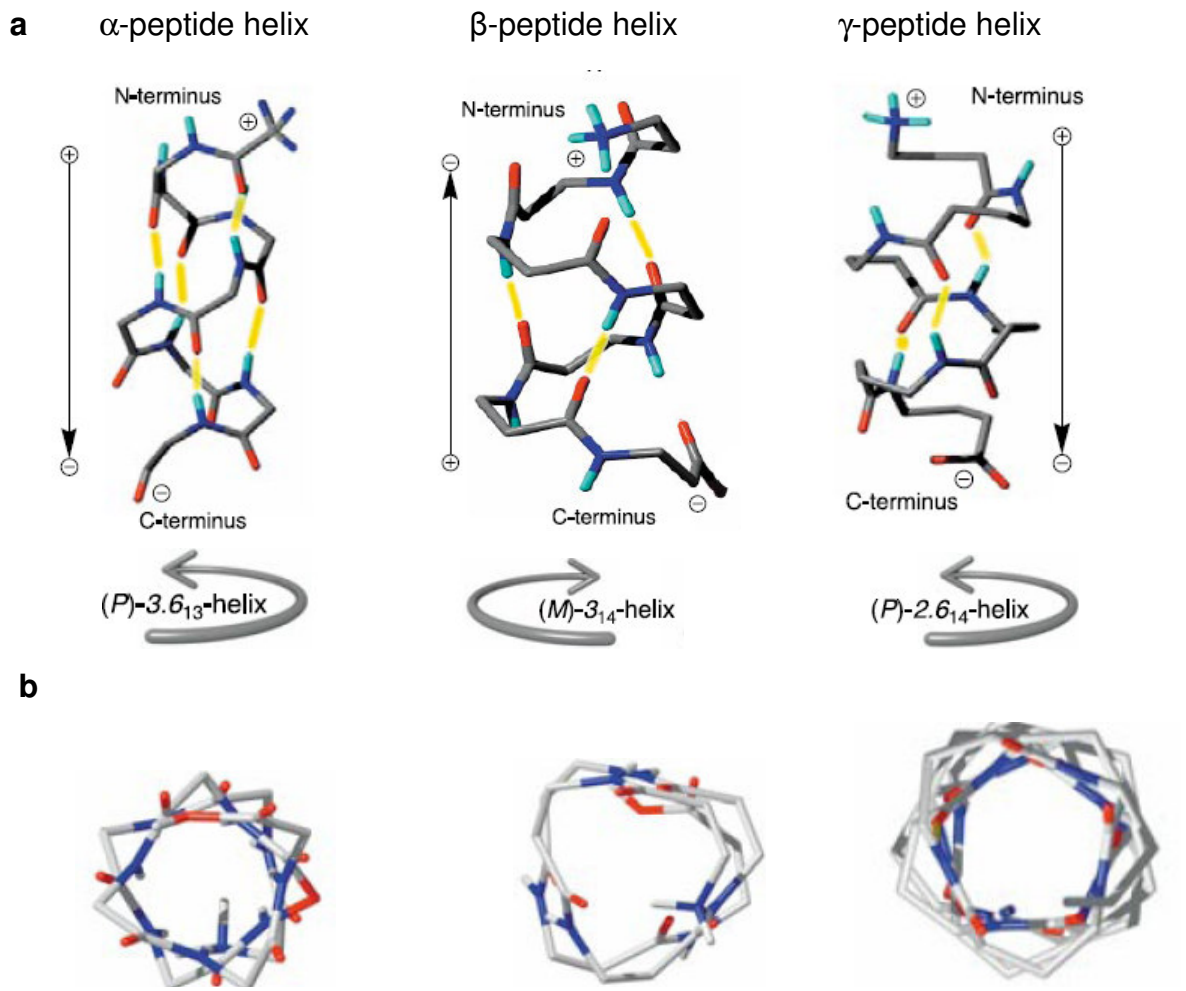


Figure 2a: Comparison of helices from α- , β- and γ-amino acids. The helical direction as well as macrodipole is also shown along with helical structure. **2b**: The top view of the helices from the α-, β-, and γ-amino acids.

using *ab initio* calculations (Figure 3). Intriguingly, theoretical calculations suggests stable C_{12}-helical conformations from 1:1 α/γ-hybrid peptides and C_{13}-helical

conformations from the heterooligomers of 1:1 combinations of β-and γ-amino acids (β/γ-hybrid peptides). According to the calculations α-helix can be generated without using α-amino acids. Recently, Gellman and colleagues reported 13-helix from the combination β- and stereochemically constrained cyclic γ-amino acids[19]. With this background, we asked whether it is possible to generate C_{12}-helix or the C_{13}-helix using α- and γ^4-amino acids or β^3-and γ^4- amino acids, respectively. In this project, we started the synthesis of stereochemically pure γ^4-amino acids starting from the α-amino acids and β-amino acids from aspartic acid to generate heterooligomers composed of α- and homologated amino acids. In this connection, two α/γ -hybrid octapeptides were synthesized using the combinations of α- and γ-amino acids. The synthetic details are given following sections.

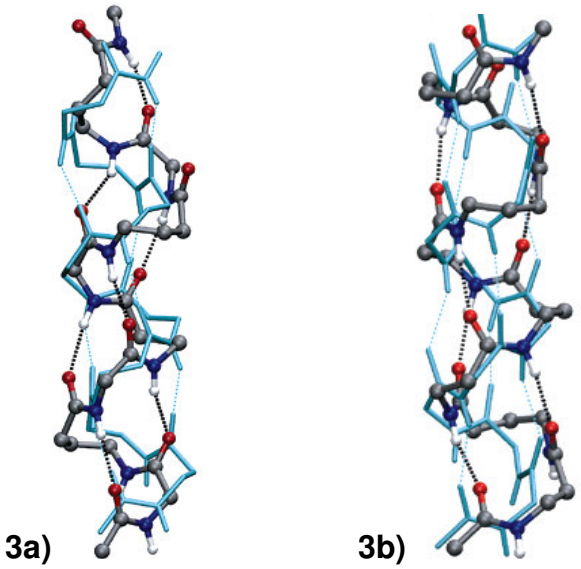
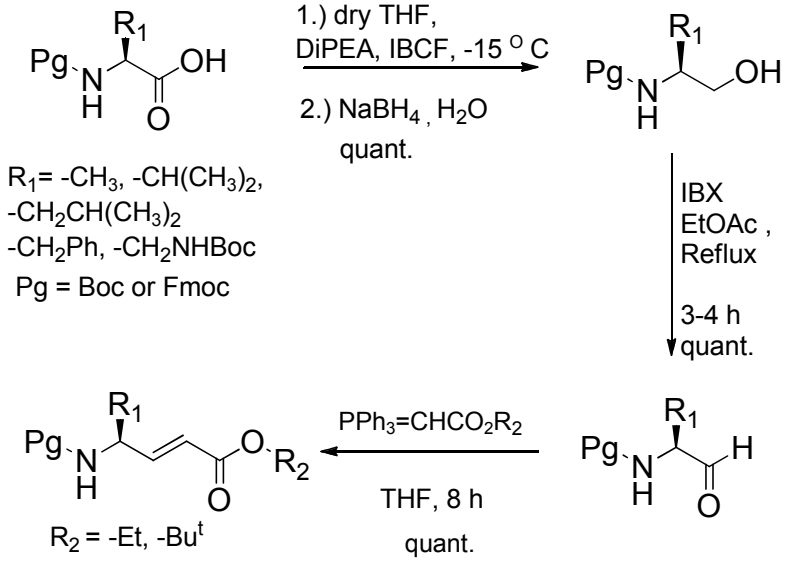


Figure 3a Stereo view 12-helix model of α/γ-hybrid peptide (gray, ball stick) and superimposition of 12-helix from β-peptides (blue). **3b** Stereo view of 13-helix model from β/γ-amino acids and superimposition of α-helix (blue)

RESULTS AND DISCUSSION

To understand the structural properties of α/γ-hybrid peptides, the γ-amino acids were synthesized starting from the α-amino acids. We adopted a well known Wittig reaction to synthesize the α, β-unsaturated γ-amino acids and subsequent transformation of unsaturated amino acids to γ-amino acids using catalytic hydrogenation [20]. The schematic representation of the synthesis of α, β-unsaturated γ-amino acids is shown in **Scheme 1**. Recently, this group has proved the high *E*- selectivity and the stereochemical purity of α, β-unsaturated γ-amino acids using Wittig reaction [21]. This procedure also provides the opportunity to study the conformational properties of unsaturated γ-amino acids, which have not been well characterized [22]. The *N*-protected α, β-unsaturated γ-amino acids were synthesized starting from the α-amino acids. The *N*-protected amino acids were converted to amino alcohols through the mild sodium borohydride reduction of corresponding mixed anhydrides. The mixed carbonic anhydrides were synthesized using isobutyl chloroformate (IBCF) and base DiPEA. The *N*-protected amino alcohols were subjected to oxidation reaction using IBX to get



Scheme 1: Synthesis of Boc/Fmoc-vinylogous amino ester from Boc/Fmoc-amino acid.

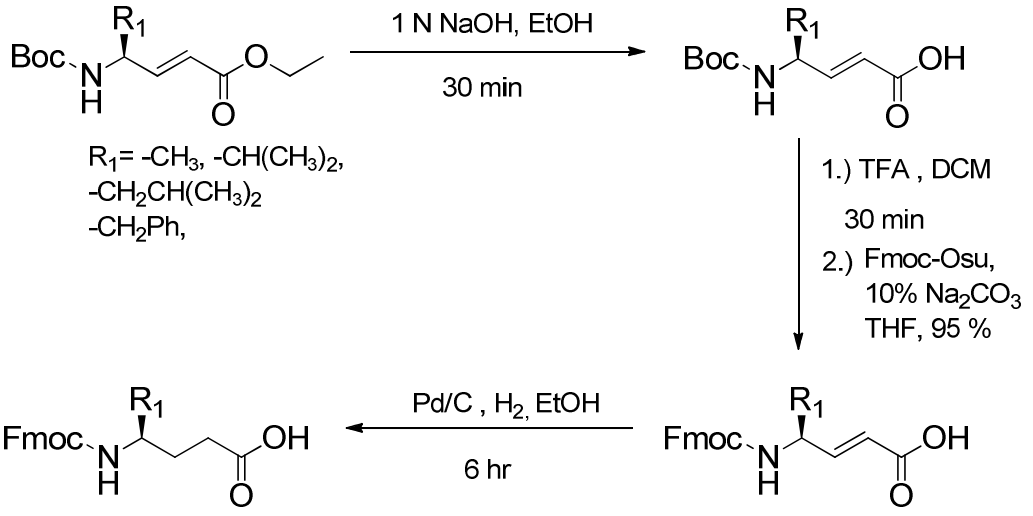
pure amino aldehydes. Further, the *N*-protected amino alcohols were subjected to Wittig reaction using the ylides either ethyl or *tert*-butyl (triphenyl phosphoranylidene) acetate. The Wittig ylides were generated from the reaction between triphenyl phosphine and ethyl bromo acetate or *tert*-butyl bromo acetate. Fmoc-Dap(Boc) aldehyde was synthesized following same protocol from commercially available Fmoc-Dap(Boc)-OH. It was subjected to Wittig reaction using the *tert*-butyl(triphenyl phosphoranylidene) acetate . The pure Wittig products were isolated with excellent *E*-selectivity after column chromatography. Using this protocol five different vinylogous

Table 1: List of α, β-unsaturated γ-amino acids synthesized using Wittig reaction

	R1	R2	Vinylogous amino esters	% Yield
1	$-CH_3$	-OEt	Boc-dgA-OEt	88
2	$-CH(CH_3)_2$	-OEt	Boc-dgV-OEt	92
3	$-CH_2-CH(CH_3)_2$	-OEt	Boc-dgL-OEt	90
4	$-CH_2-Ph$	-OEt	Boc-dgF-OEt	88
5	$-CH_2NHBoc$	$-OBu^t$	Fmoc-dgDap(Boc)-OBu^t	78

dg= α, β –dehydro γ-amino acid A = Ala, V = Val, L = Leu, and F = Phe.

amino esters were isolated. The list of the unsaturated esters along with their % of yield after the column purification is given in the Table 1. Further, we used these α, β-unsaturated γ-amino esters for the synthesis of γ-amino acids using catalytic hydrogenation. The Schematic representation of the transformation of unsaturated amino acids to saturated γ-amino acids is shown in **Scheme 2**. The pure ethyl esters of the unsaturated γ-amino acids were subjected to saponification using 1(N) NaOH and ethanol. The acid was isolated in pure form after the acidification using 5% HCl and aqueous work-up.



Scheme 2: Synthesis of Fmoc-γ^4-amino acids from the ethyl ester of Boc-vinylogous amino acid.

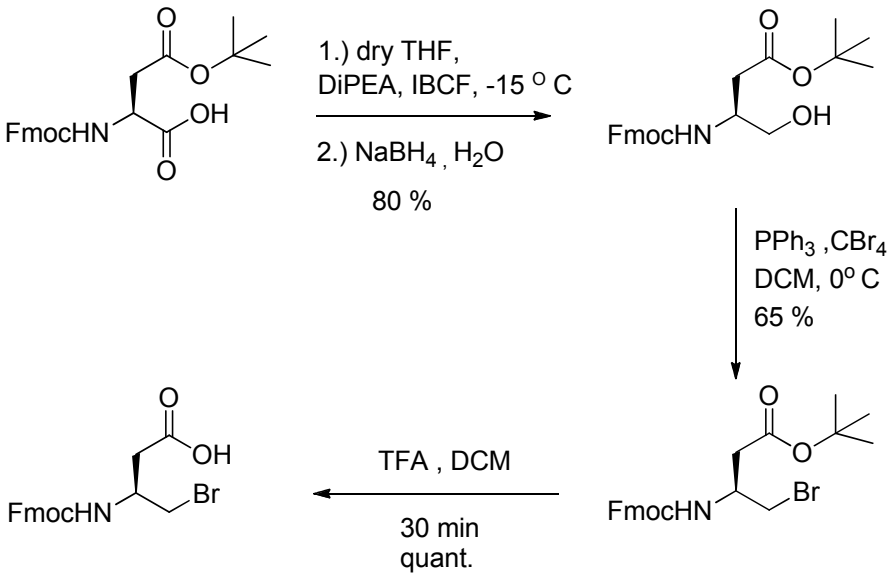
Further, the Boc-deprotection of unsaturated acid was carried out using 50% TFA in dichloromethane. The free unsaturated amino acid was again protected with Fmoc group under basic conditions to get amino acids that are compatible for the solid phase synthesis. The Fmoc-amino acid was further subjected to the catalytic hydrogenation using 10% Pd/C under the Table 2: List of Fmoc-γ-amino acids synthesized using the catalytic hydrogenation

	Vinylogous amino acid	γ-amino acid	% Yield
1	Boc-dgA-OEt	Fmoc-γAla-OH	87
2	Boc-dgV-OEt	Fmoc-γVal-OH	85
3	Boc-dgL-OEt	Fmoc-γLeu-OH	85
4	Boc-dgF-OEt	Fmoc-γPhe-OH	81

hydrogen atmosphere to obtain γ-amino acids. Both Fmoc-α, β-unsaturated γ-acids and the Fmoc-γ-amino acids can be used in the solid phase synthesis. The list of amino acids synthesized using this protocol along with the % of yield is given in the Table 2.

Further, in the case of Boc -and *tert*-butyl protected vinylogous amino acids, 50% TFA in DCM was used to remove the protecting groups and the free amine was again protected with solid phase synthesis compatible Fmoc-group under the basic conditions. The Fmoc- protected γ-amino acids and vinylogous amino acids were directly used for the solid phase synthesis.

In addition, to synthesize 13-helical peptides from the combination of β and γ-amino acids, we adopted new route for the synthesis of β-amino acids starting from commercially available Fmoc-Asp(OBu^t)-OH. The Schematic representation is shown **Scheme 3**. We anticipate that the bromine substituted Fmoc-β-Ala-OH can be functionalized to Fmoc-β-Ala-OH either using catalytic hydrogenation or by mild $NaBH_4$ reduction. This amino acid can be utilized for further derivatization using various nucleophiles such thymine, azide alkyl or aryl amines.



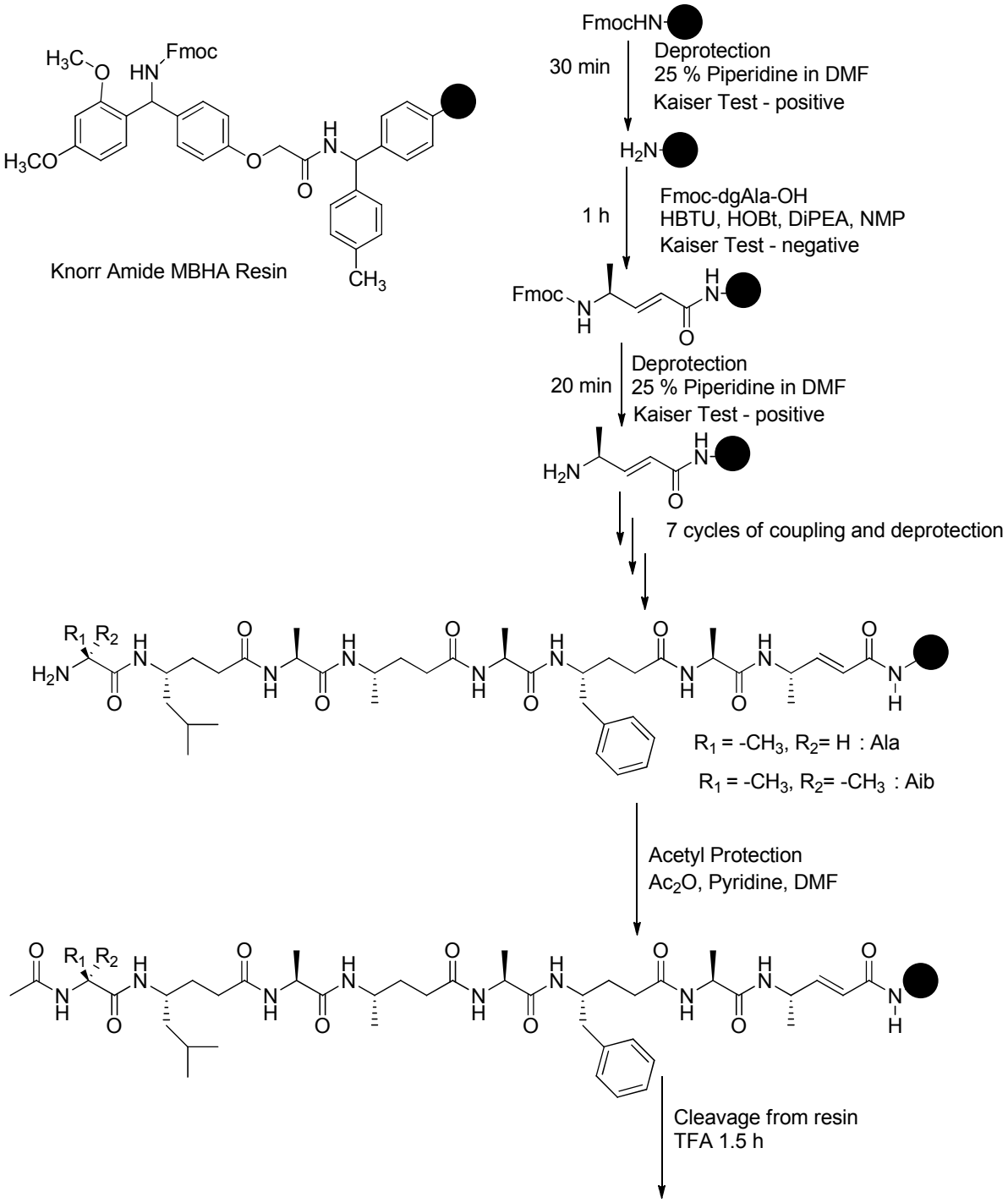
Scheme 3: Synthesis of functionalizable Fmoc-*N*-protected β-amino acid

The Fmoc-amino alcohol was synthesized using the mixed anhydride protocol as described above. The Fmoc-amino alcohol was subjected to the Appel reaction using PPh_3 and CBr_4 to generate alkyl bromide through the nucleophilic substitution reaction [22]. Further, the *tert*-butyl ester was removed using 50% TFA in DCM to get Fmoc-β-

amino acid. The transformation of bromo derivative to alkane is still under investigation. However, the bromo substituted Fmoc-β-amino acid can be directly used in the peptide synthesis.

Further, using the Fmoc- vinylogous amino acids and the Fmoc-γ-amino acids described in the **Scheme 2**, we designed two α/γ-hybrid octapeptides Ac-Ala-γ^4Leu-Ala-γ^4-Ala-Ala- γ^4Phe-Ala-dgAla-NH_2, (**P1**) and Ac-Aib-γ^4-Leu-Ala-γ^4Ala-Ala-γ^4Phe-Ala-dgAla-NH_2 (**P2**). Our ongoing research in the laboratory, suggests that incorporation of vinylogous amino acids at the C-terminal may lead to the stabilization of the helical peptides with the terminal C-H---O hydrogen bonding and also it can be useful for further derivatization like Michael addition and Diels- Alder reactions. For these reasons we inserted a α, β-dehydro γ-amino acid at the C-terminal of the peptides. The peptides were synthesized using Knorr amide MBHA resin using standard Fmoc chemistry [23]. The combination of HBTU/HOBt mixture was used as coupling reactions [24] The Fmoc was deprotected on the resin using 25% piperidine in DMF. The completion of the coupling reactions were monitored using Kaiser Test. After the completion of the synthesis, the *N*-terminal was protected with the acetyl group and the peptide was released from the resin using neat TFA. The solid phase synthesis is outlined in the **Scheme 4**. The MALDI-TOF analysis of the crude peptides suggests that **P1** and **P2** are major products in the synthesis. The Mass spectra of both the peptides are shown in Figure 4 and 5, respectively. After the purification of **P1** and **P2** using the reverse-phase HPLC, we will subject them for the structural analysis using both 2D NMR and single crystal X-ray crystallography. We anticipate the 12 membered H-bond stabilized helical structures from both the peptides **P1** and **P2**.

Schematic representation of the solid phase peptide



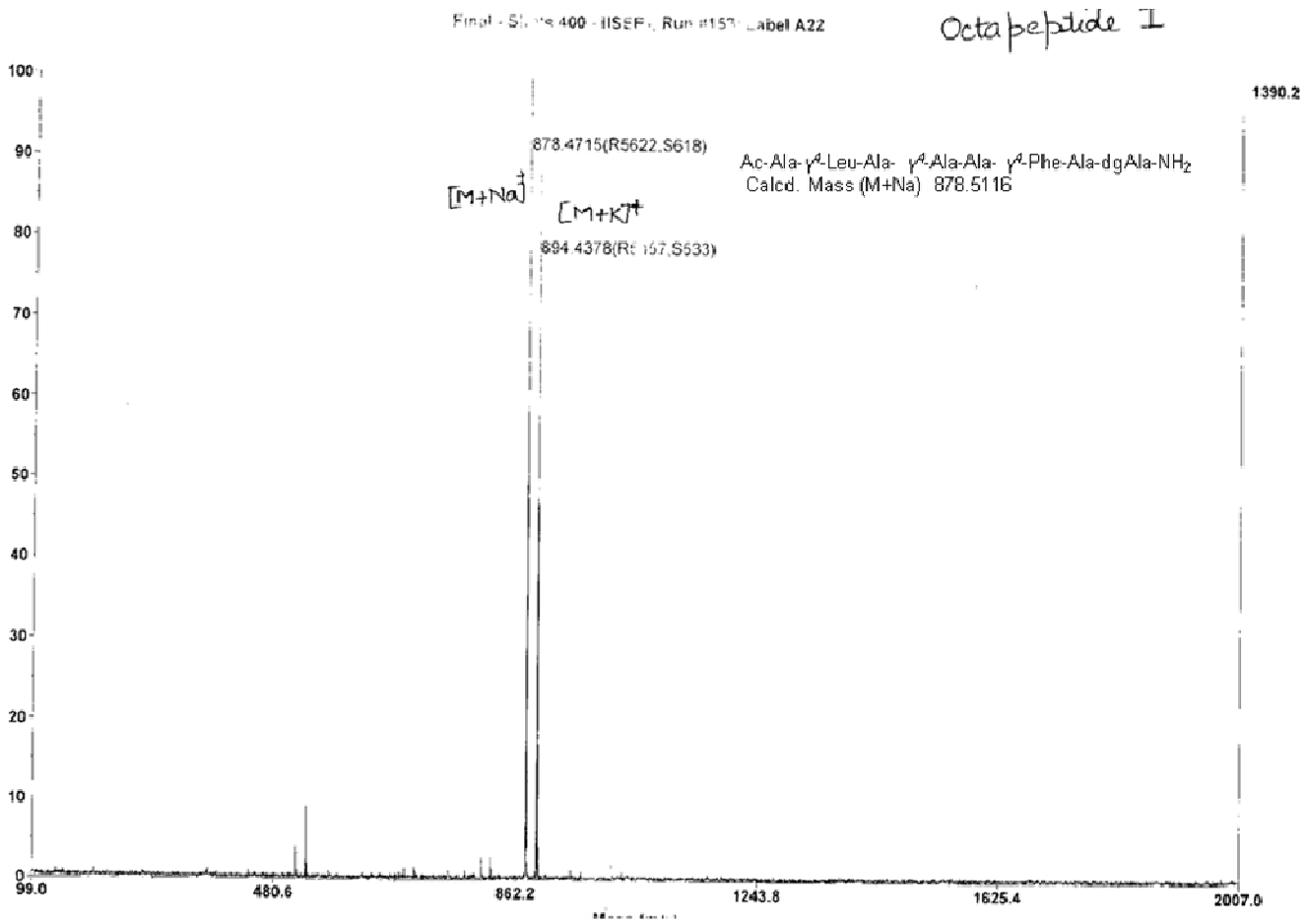
Ac-Ala-γ^4Leu-Ala-γ^4-Ala-Ala- γ^4Phe-Ala-dgAla-NH_2 (**P1**)

Ac-Aib-γ^4Leu-Ala-γ^4-Ala-Ala- γ^4Phe-Ala-dgAla-NH_2 (**P2**)

MALDI TOF/TOF Report of Peptide

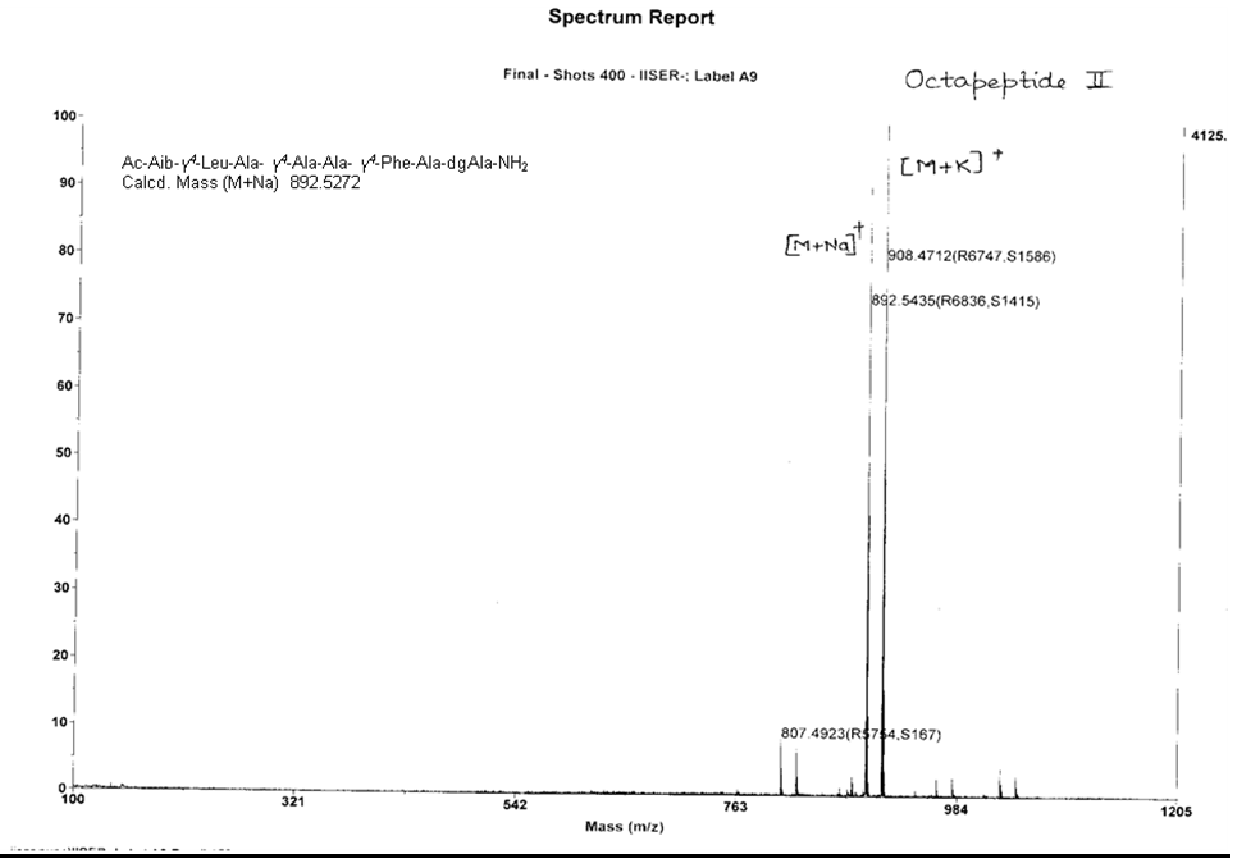
P1: Ac-Ala-γ^4Leu-Ala-γ^4-Ala-Ala- γ^4Phe-Ala-dgAla-NH_2

MALDI.TOF/TOF m/z Calcd. for $C_{49}H_{69}N_9O_9$ $(M+Na)^+$ 878.5116 Observed 878.4715



P2: Ac-Aib-γ^4Leu-Ala-γ^4-Ala-Ala- γ^4Phe-Ala-dgAla-NH_2

MALDI.TOF/TOF m/z Calcd. for $C_{50}H_{71}N_9O_9$ $(M+Na)^+$ 892.5272 Observed 892.5435



CONCLUSION

In conclusion, we demonstrated the synthesis of both Fmoc- vinylogous γ-amino acids as well as Fmoc-γ-amino acids starting from α-amino acids through Wittig reaction. Further, we also demonstrated the synthesis of funtionalizable Fmoc-bromo β-alanine from Fmoc-Asp(OBu^t). The vinylogous and γ-amino acids were incorporated in the designed octapeptides containing 1:1 alpha and gamma amino acids. The mass spectral analysis of the crude peptides after the SPPS suggests presence of the peptides as major components. The pure peptides after the HPLC purification will be subjected for the structural analysis. The combination of beta and gamma hybrid peptide is yet to start.

EXPERIMENTAL SECTION

Abbreviations.

1. Ala = Alanine
2. Boc = *tert*-butoxycarbonyl
3. Dap = Dipropionic acid
4. DCM = Dichloromethane
5 .DiPEA = diisopropylethyl amine
6. DCE = 1, 2-dichloroethane
7. DMF = *N*, *N*-dimethylamine
9. Fmoc = 9-fluronyl methoxy carbonyl
10. HBTU = (O-Benzotriazol-1-yl)-*N,N,N',N'*,-tetramethyl uranium hexaphosphate
11. HOBt = Hydroxy benzotriazole
12. IBCF = isobutyl chloroformate
12. Leu = Leucine
13 .NMP = N-methyl pyrrolidine
14. Phe = Phenylalanine
15 .TFA = Trifluoroacetic acid
17. THF = Tetrahydrofuran
18. Val = Valine

Materials and Methods.

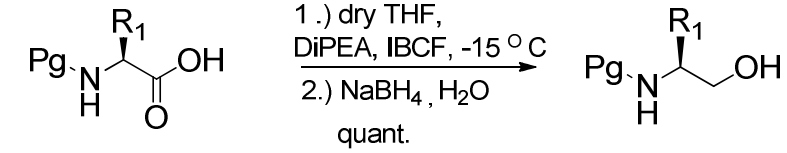
Materials obtained commercially were reagent grade unless otherwise stated. ^{1}H NMR spectra were recorded on *JEOL* 400MHz (100MHz for ^{13}C). Preparative scale reverse phase HPLC separations were performed on X Bridge PREP BEH 130 C18 column, using gradient mixtures of water 90% / Methanol 10% / 0.1%TFA (solvent system A) and water 10% / Methanol 90% / TFA 0.1% (solvent system B). Mass spectra were obtained from MALDI-TOF/TOF(Applied Biosystems) mass spectrometer.

Scheme I

Synthesis of **Boc-protected Vinylogous Amino Ester:**

1a) General procedure for the synthesis of Boc/Fmoc-*N*-protected amino alcohol:-

The N-protected amino acid (10 mmol) was dissolved in dry THF (20 mL) under nitrogen atmosphere, and cooled to -15 ^{o}C, and then treated with DiPEA (10.2 mmol , 1.32 g) followed by isobutyl chloroformate (10 mmol, 1.366 g). White hydrochloride salt of DiPEA was precipitated out immediately after the addition of isobutyl chloroformate. The reaction was continued to stir for another 10 mins. A solution of $NaBH_4$ (50 mmol, 1.8 g) in 10 mL of water was added with vigorous stirring under ice cold condition. Immediate evolution of gas was observed after the addition. THF was evaporated from the reaction mixture and diluted with EtOAc (150 mL). The organic layer was washed with 5 % HCl (5 % by volume in water , 2 X 50 mL), 5 % Na_2CO_3 solution in water (2 X 50 mL), followed by brine (50 mL). Organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The alcohol used were used for next step without purification.



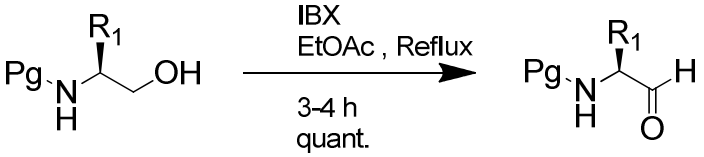
R_1= -CH_3, -$CH(CH_3)_2$,
-$CH_2CH(CH_3)_2$
-CH_2Ph, -CH_2NHBoc

Pg = Boc or Fmoc

1b) General procedure for the synthesis of Boc/Fmoc-*N*-protected amino aldehyde:-

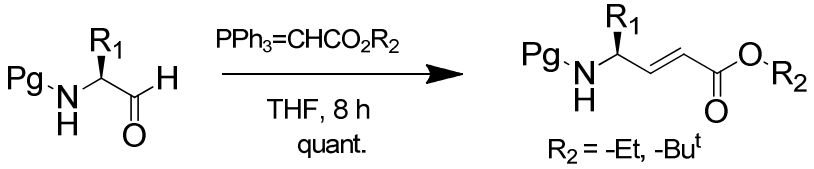
The N-protected amino alcohol (5 mmol) was dissolved in 50 mL of EtOAc and then IBX (10 mmol, 2.8 g) was added to it and refluxed. The reaction was monitored by TLC.

After completion of the reaction, the reaction mixture was filtered and the filtrate concentrated over reduced pressure to yield N-protected amino aldehyde. It was immediately used for next step without purification.



1c) <u>General procedure for the synthesis of Boc/Fmoc-protected Vinylogous Amino Ester:-</u>

The N-protected amino aldehyde (5 mmol) was dissolved in THF (20 mL). To this solution Wittig ylide (5.5 mmol) was added. The progress of the reaction was monitored by TLC. After completion of the reaction (8-12 hr) the THF was evaporated and product was purified by column chromatography using 5:95 ethyl acetate / pet ether solvent system.



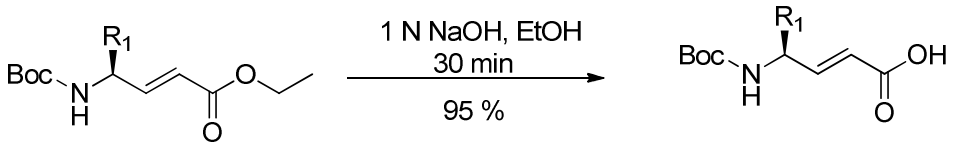
<u>Scheme II</u>

<u>General procedure for the synthesis of Fmoc- *γ*[4]- amino acids from esters of Boc-*α, β*- unsaturated *γ*- amino acids:-</u>

2a) Boc-*α, β*-unsaturated *γ*- amino acids :-

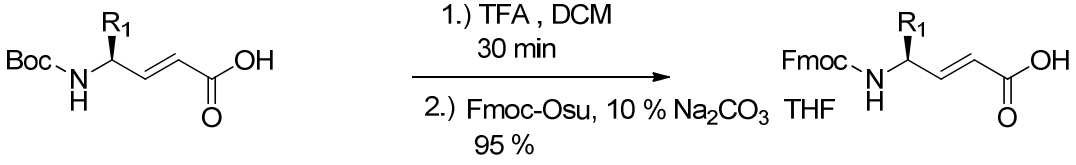
Ethyl ester of Boc-*α, β*-unsaturated *γ*- amino acid (2 mmol) was dissolved in 5 mL of ethanol. Then 5 mL of 1 N NaOH was added slowly to the reaction mixture. After completion of the reaction(~ 30-45 min), ethanol was evaporated from the reaction mixture and the residue was acidified using 5% HCl (5 % volume in water) at cold

condition. The product was extracted with EtOAc (3 X 40 mL). The combined organic layer was washed with brine (30 mL) and dried over anhydrous Na_2SO_4. The solvent was concentrated over reduced pressure to give Boc-*α, β*-unsaturated *γ*- amino acid as gummy product in the quantitative yield.



2b) Fmoc-*α,β*-unsaturated *γ*- amino acids :-

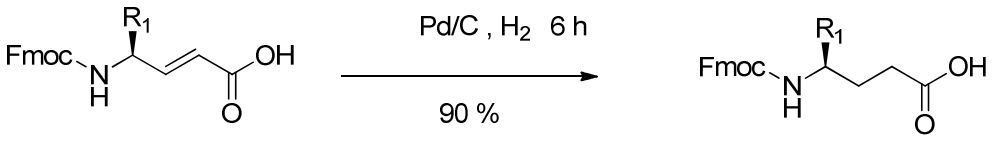
The Boc-*α, β*-unsaturated *γ*- amino acid (2 mmol) was dissolved in 5mL of DCM and cooled to 0 ^{o}C in the ice bath followed by the addition of 5 mL of neat TFA. After 30 min, TFA was removed from the reaction mixture using reduced pressure. Residue was dissolved in 20 mL water and pH was adjusted to ~10 by the slow addition of solid Na_2CO_3. The solution of Fmoc-Osu (2 mmol) in 15 mL of THF was added slowly to the reaction mixture. Reaction mixture was stirred overnight at RT. After completion of the reaction, the reaction mixture was acidified with 30 mL of 20 % HCl (20 % volume in water) in cold condition. Product was extracted with EtOAc (3 X 50 mL) . The combined organic layer was washed with brine (30 mL) and dried over anhydrous Na_2SO_4. The solvent was concentrated over reduced pressure to give gummy product which was recrystalized using EtOAc/ Pet Ether.



2c) Fmoc-*γ*^{4}- amino acids :-

Fmoc-*α, β*-unsaturated *γ*- amino acids (1 mmol) was dissolved in EtOH (40 mL) and was treated with 20 weight percentage of Pd/C. The hydrogen gas was supplied

through balloon. The reaction mixture was stirred for 6 hrs. The completion of reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was filtered on celite and washed with EtOH. The solvent was concentrated over reduced pressure and crude was purified by column chromatography using 35:65 ethyl acetate / pet ether solvent system.

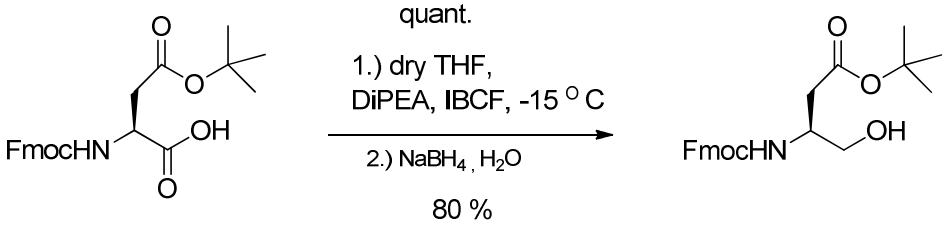


Scheme III

Synthesis of functionalized β-amino acids using Fmoc-Asp(OBut)-OH:

Synthesis of (*S*)-*tert*-butyl 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-hydroxybutanoate (Fmoc-Asp(OBu^t)-CH_2OH) :-

The product was synthesized following procedure described in 1b. The product was purified by column chromatography using 25:75 ethyl acetate / pet ether solvent system.

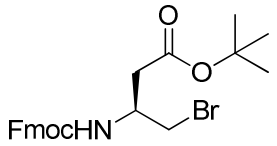


White powder: **^{1}H NMR** (400 MHZ , $CDCl_3$) δ 7.785-7.767 (d, *J*= 7.3 Hz, 2H, -Fmoc aromatic), 7.610-7.591 (d, *J*= 7.3 Hz, 2H, -Fmoc aromatic), 7.432- 7.394 (m , 2H, -Fmoc aromatic), 7.343-7.306 (m , 2H, -Fmoc aromatic), 5.500-5.479 (d, *J*=8.2 Hz, 1H, N*H*), 4.422-4.405 (d, *J*= 6.9 Hz, 2H, -CH_2(of Fmoc)), 4.244-4.209 (m, 1H, -C*H*(of Fmoc)) , 4.035 (m, NHC*H*CH_2OH), 3.748-3.725 (m, 2H, NHCHCH_2OH), 2.588-2.559 (t, J= 5.7 Hz, 1H, C$H_2CO_2Bu^t$), 2.415-2.385 (t, J= 6.0 Hz, 1H, C$H_2CO_2Bu^t$) 1.457 (s, 9H, -C(CH_3)$_3$ Boc) **^{13}C NMR** (100 MHz, $CDCl_3$) 169.924, 155.402, 143.742, 141.291, 127.714, 127.047, 125.016, 120.001, 81.652, 66.950, 48.386, 47.147, 38.146, 37.107,

35.934, 28.030; **MALDI TOF/TOF** m/z Calcd. for $C_{23}H_{27}NO_5$ (M+Na) 420.1787 Observed 420.2014

Synthesis of (*S*)-tert-butyl 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-bromobutanoate (Fmoc-Asp(OBu^t)-CH_2Br) : -

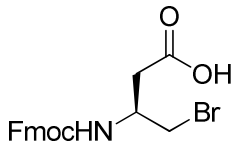
PPh_3 dissolved in 10 mL of DCM was added to a mixture of Fmoc-Asp(OBu^t)-CH_2OH (0.749g, 2 mmol, 1 eq) and CBr_4 (1.326 g, 4mmol, 2 eq) in DCM (15 mL) in ice cold condition. The reaction was stirred for 1 h in ice cold condition and allowed to come to r.t. and stirred for 2 h. Completion of reaction was monitored by TLC. On completion of the reaction solvent is concentrated over reduced pressure and purified by column chromatography with 5:95 ethyl acetate / pet ether solvent system to yield 0.564 g (61 %) of product as white powered solid. **^{1}H NMR** (400 MHZ , $CDCl_3$) δ 7.787-7.769 (d, *J*= 7.3 Hz, 2H, -Fmoc aromatic), 7.607-7.589 (d, *J*= 7.3 Hz, 2H, -Fmoc aromatic), 7.435- 7.397 (m , 2H, -Fmoc aromatic), 7.345-7.308 (m , 2H, -Fmoc aromatic), 5.418-5.397 (d, *J*=8.7 Hz, 1H, N*H*), 4.456-4.370 (m, 2H, -CH_2(of Fmoc)), 4.252-4.218 (m, 2H, -C*H*(of Fmoc) & NHC*H*CH_2OH), 3.749-3.559 (m, 2H, NHCHCH_2Br), 2.706-2.587 (m, 2H, -CH_2CO_2Bu^t), 1.457 (s, 9H, -C(CH_3)$_3$ Boc) **^{13}C NMR** (100 MHz, $CDCl_3$) 169.924, 155.402, 143.742, 141.291, 127.714, 127.047, 125.016, 120.001, 81.652, 66.950, 48.386, 47.147, 38.146, 37.107, 35.934, 28.030;; **MALDI TOF/TOF** m/z Calcd. for $C_{23}H_{26}BrNO_4$ $(M+Na)^+$ 482.0943 Observed 482.1720



Synthesis of (*S*)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-bromobutanoic acid (Fmoc-Asp(OH)-CH_2Br) : -

Fmoc-Asp(OBu^t)-CH_2Br (0.92 g, 2 mmol) was dissolved in 5mL of DCM and cooled to 0 ^oC in the ice bath followed by the addition of 5 mL of neat TFA. After 30 min, TFA was removed from the reaction mixture using reduced pressure. The product was

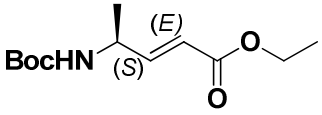
precipitated using pet ether to give a solid mass which is not stable in column chromatography. **MALDI TOF/TOF** m/z Calcd. for $C_{19}H_{18}BrNO_4$ (M+Na) 426.0317 Observed 426.1061



Spectroscopic data for N-protected vinylogous Amino Esters

(*S, E*)-ethyl 4-((tert-butoxycarbonyl)amino)pent-2-enoate (Boc-dgA-OEt): -

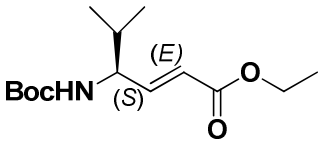
Colourless oil (Yield 2.13 g, 88 %), **^{1}H NMR** (400 MHz , $CDCl_3$) δ 6.886-6.835 (dd, *J*=15.6 Hz, 4.6 Hz, 1H, -CH-C*H*=CH), 5.902-5.886 (d, *J*= 14.2 Hz, 1H, -CH-CH=C*H*), 4.61 (br, 1H, -N*H*), 4.39 (br, 1H, -NH-C*H*-CH=CH), 4.202-4.148 (q, *J*= Hz, 2H, -OCH_2), 1.429 (s, 9H,-C(CH_3)$_3$ Boc), 1.28-1.24 (m, 6H, -OCH$_2$CH_3 & -CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) 166.481, 154.992, 149.491, 120.153, 79.793, 60.524, 47.061, 28.421, 20.383, 14.300; MALDI.TOF/TOF m/z Calcd. for $C_{12}H_{21}NO_4$ (M+Na) 266.1368 Observed 266.1047



(*S, E*)-ethyl 4-((tert-butoxycarbonyl)amino)-5-methylhex-2-enoate(Boc-dgV-OEt):-

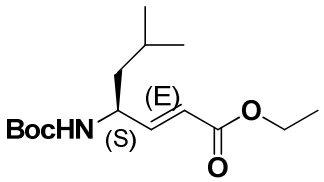
Colourless solid (Yield 2.47 g, 92 %); **^{1}H NMR**(400 MHz , $CDCl_3$) δ 6.889-6.837 (dd, *J*=15.8 Hz, 5 Hz, 1H, -CH-C*H*=CH), 5.943-5.900 (d, *J*= 14.2 Hz, 1H, -CH-CH=C*H*), 4.588-4.566 (d, *J*= 8.7 Hz, 1H, -N*H*), 4.224- 4.168 (m, 3H, -OCH_2CH$_3$ & -NH-C*H*-CH=CH), 1.91-1.83 (m, 1H, C*H*(CH_3)$_2$), 1.447 (s, 9H,-C(CH_3)$_3$ Boc), 1.310 (t, *J*= 7.1 Hz, 3H, -OCH$_2$CH_3), 0.95-0.90 (dd, *J*= 6.9 Hz, 6H, -CH(CH_3)$_2$) ; **^{13}C NMR** (100 MHz, $CDCl_3$) 166.272, 155.326, 147.365, 121.431, 79.679, 60.429, 56.615, 32.216,

28.326,18.839, 17.961, 14.214 **MALDI TOF/TOF** m/z Calcd. for $C_{14}H_{25}NO_4$ (M+Na) 294.1681 Observed 294.1938



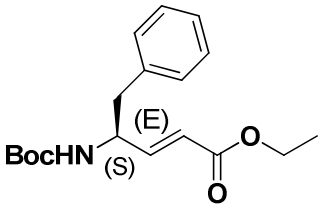
(*S, E*)-ethyl 4-((tert-butoxycarbonyl)amino)-6-methylhept-2-enoate (Boc-dgL-OEt):-

White crystalline solid (Yield 2.56 g, 90 %) ; **^{1}H NMR**(400 MHz, $CDCl_3$) δ 6.855-6.802 (dd, *J*= 15.6 Hz, 5.5 Hz, 1H, -CH-C*H*=CH), 5.934-5.895 (d, *J*= 15.6 Hz, 1H, -CH-CH=C*H*), 4.476-4.458 (d, 7.8 Hz, 1H, -N*H*), 4.358-4.327 (m, 1H, , -NH-C*H*-CH=CH), 4.216-4.162 (q, 2H, -OC$H_2$$CH_3$), 1.724-1. 658 (m, 1H, -CH_2C*H*(CH_3)$_2$), 1.442 (s, 9H, -C(CH_3)$_3$ Boc), 1.400-1.363 (t, *J* =7.3, 2H, CH_2CH(CH_3)$_2$), 1.305-1.268 (t, *J*= 7.3 Hz, 3H, -OCH_2CH_3), 0.942-0.926 (dd, *J*= 6.4 Hz, 6H, -CH_2CH(CH_3)$_2$) ; **^{13}C NMR** (100 MHz, $CDCl_3$) 166.415, 155.050, 148.890, 120.363, 79.669, 60.419, 49.749, 43.800, 28.326, 24.683, 22.691, 22.157, 14.214 **MALDI TOF/TOF** m/z Calcd. for $C_{15}H_{27}NO_4$ (M+Na) 308.1838 Observed 308.2026



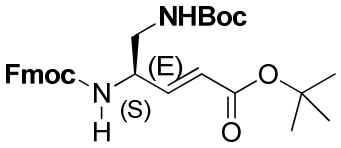
(*S,E*)-ethyl 4-((tert-butoxycarbonyl)amino)-5-phenylpent-2-enoate (Boc-dgF-OEt) :-

White solid (Yield 2.80 g, 88 %) ; **^{1}H NMR**(400 MHz, $CDCl_3$) δ 7.373-7.222 (5H, aromatic protons) , 6.988-6.936 (dd, *J*= 15.8 Hz, 4.8 Hz, 1H, -CH-C*H*=CH), 5.927-5.887 (d, *J*= 16 Hz, 1H, -CH-CH=C*H*), 4.670 (br, 1H, -N*H*), 4.596-4.579 (m, 1H, , -NH-C*H*-CH=CH), 4.256-4.203 (m, 2H, -OC$H_2$$CH_3$), 2.990-2.938 (m, 2H, NH-CHCH_2), 1.440 (s, 9H, -C(CH_3)$_3$ Boc), 1.342-1.305 (t, *J*= 7.3 Hz, 3H, -OCH_2CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) 166.138, 154.897, 147.856, 136.324, 129.363,128.543, 128.448, 126.827, 121.021, 79.803, 60.438, 52.200, 40.797, 28.249,14.186. **MALDI TOF/TOF** m/z Calcd. for $C_{18}H_{25}NO_4$ (M+Na) 342.1681 Observed 342.2072



(*R, E*)-*tert*-butyl 4- ((((9H-floren-9-yl)methoxy) carbonyl) amino)- 5-((*tert*-butoxycarbonyl) amino) pent-2-enoate (Fmoc-dgDap(Boc)-OBu^t) : -

Solid (Yield 3.96 g, 78 %) **^{1}H NMR** (400 MHZ , $CDCl_3$) δ 7.780-7.762 (d, *J*= 7.3 Hz, 2H, -Fmoc aromatic), 7.615-7.598 (d, *J*= 6.9 Hz, 2H, -Fmoc aromatic), 7.426- 7.389 (m , 2H, -Fmoc aromatic), 7.334-7.299 (m , 2H, -Fmoc aromatic), 6.761-6.709 (dd, *J*= 15.6 Hz, J= 5 Hz, 1H, -CH-C*H*=CH) , 5.950-5.911 (d, *J*= 15.6 Hz, 1H, -CH-CH=C*H*) , 5.730-5.713 (d, *J*=6 Hz, 1H, N*H* -Fmoc), 4.839 (br, 1H, N*H- Boc*), 4.401-4.384 (m, 3H, NH-C*H*-CH=CH, -CH_2(of Fmoc)), 4.235-4.200 (t, J= 6.9 Hz, 1H, -C*H*(of Fmoc)) , 3.359 (br, 2H, CH-CH_2-NH-Boc), 1.492 (s, 9H, -C(CH_3)$_3$ $CO_2C(CH_3)_3$), 1.454 (s, 9H, -C(CH_3)$_3$ Boc); **^{13}C NMR** (100 MHZ , $CDCl_3$) δ 165.175, 143.770, 143.446, 127.685, 127.056, 124.092, 124.396, 119.943, 80.785, 80.175, 66.950, 53.630, 47.156, 43.543, 28.259, 28.059. **MALDI TOF/TOF** m/z Calcd. for $C_{29}H_{36}NO_4$ (M+Na) 531.2466 Observed 531.3446

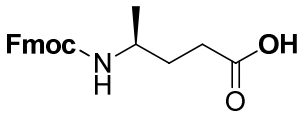


<u>Spectroscopic data for Fmoc-protected homologated γ^4-Amino Acids</u>

(*S*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pentanoic acid (Fmoc-γAla-OH):-

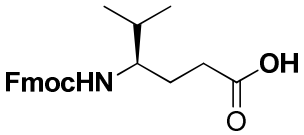
Solid (Yield 0.288 g, 87 %) **^{1}H NMR**(400 MHz, DMSO d-6) δ 7.897-7.878 (d, *J*= 7.8 Hz, 2H, -Fmoc aromatic), 7.704-7.686 (d, *J*= 6.9 Hz, 2H, -Fmoc aromatic), 7.427-7.391 (m, 2H, -Fmoc aromatic), 7.345- 7.308 (m , 2H, -Fmoc aromatic), 7.211-7.190 (d, *J*=8.2 Hz, 1H, N*H*), 4.332-4.188 (m, 3H, -CH_2 (of Fmoc) & -C*H*(of Fmoc)), 3.536-

3.467 (m, 1H, -NH-C*H*-CH_2-CH_2CO_2H), 2.210-2.0173 (m, 2H, -NH-CH-CH_2-C*H*$_2$$CO_2H$), 1.605 (br, 2H, -NH-C*H*-C*H*$_2$-CH_2CO_2H)) ; 1.042-1.026 (d, 3H,NH-CH-C*H*$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* 174.481, 155.603, 143.970, 140.767, 127.638, 127.085, 125.206, 120.163, 65.100, 46.823, 45.823, 45.945, 31.319, 30.709, 20.889 **MALDI TOF/TOF** m/z Calcd. for $C_{20}H_{21}NO_4$ (M+Na) 362.1368 Observed 362.1680



(*R*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-methylhexanoic acid (Fmoc-γVal-OH):-

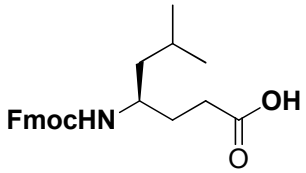
White solid (Yield 0.311 g, 85 %) **^{1}H NMR**(400 MHz, DMSO d-6) *δ* 12.006 (b, 1H, COO*H)* 896-7.878 (d, *J*= 7.3 Hz, 2H, -Fmoc aromatic), 7.715-7.697 (d, *J*= 6.9 Hz, 2H, -Fmoc aromatic), 7.426- 7.390 (m, 2H, -Fmoc aromatic), 7.338- 7.301 (m , 2H, -Fmoc aromatic), 7.120-7.098 (d, *J*=9.2 Hz, 1H, N*H*), 4.305-4.280 (m, 2H, -C*H*$_2$ (of Fmoc)), 4.213-4.195 (m, 1H, -C*H*(of Fmoc)), 3.251-3.14 (m, 1H, -NH-C*H*-CH_2-CH_2CO_2H), 2.244-2.096 (m, 2H, -NH-CH-CH_2-C*H*$_2$$CO_2H$), 1.701-1.472 (m, 3H, -NH-C*H*-C*H*$_2$-CH_2CO_2H) & NH-CHC*H*$(CH_3)_2$), 0.833-0.804 (t, 6H, CH(C*H*$_3$)$_2$); **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* 174.452, 156.280, 143.951, 140.767, 127.628, 127.037, 125.235, 120.143, 65.062, 55.423, 46.889, 31.930, 30.728, 26.457,19.058,18.333; **MALDI.TOF/TOF** m/z Calcd. for $C_{22}H_{25}NO_4$ $(M+Na)^+$ 390.1681 Observed 390.2271



(*R*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-methylheptanoic acid (Fmoc-γLeu-OH):-

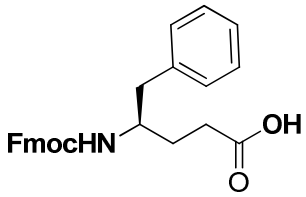
Solid (Yield 0.323 g, 85 %) **^{1}H NMR**(400 MHz, $CDCl_3$) *δ* 7.772-7.302 (m, 8H -Fmoc aromatic), 7.461-7.415 (m, 3H, -C*H*$_2$ (of Fmoc) & -C*H*(of Fmoc)), 4.226-4.160(m, 1H, N*H),* 3.718-3.672 (m, 1H, -NH-C*H*-CH_2-CH_2CO_2H), 2.348-2.310 (m, 2H, -NH-CH-CH_2-

C*H*$_2$CO$_2$H), 2.045-1.545 (m, 5H -NH-C*H*-C*H*$_2$-CH$_2$CO$_2$H), NH-CHC*H*$_2$CH(CH$_3$)$_2$ & NH-CHCH$_2$C*H*(CH$_3$)$_2$), 0.896-0.870 (d, 6H, CH(C*H*$_3$)$_2$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 178.676, 156.261, 143.723, 141.291, 127.628, 127.008, 124.596, 120.296, 66.216, 49.006, 47.366, 44.925, 30.671, 29.670, 24.769, 23.005, 22.099; **MALDI TOF/TOF** m/z Calcd. for $C_{23}H_{27}NO_4$ (M+Na) 404.1836 Observed 404. 1443

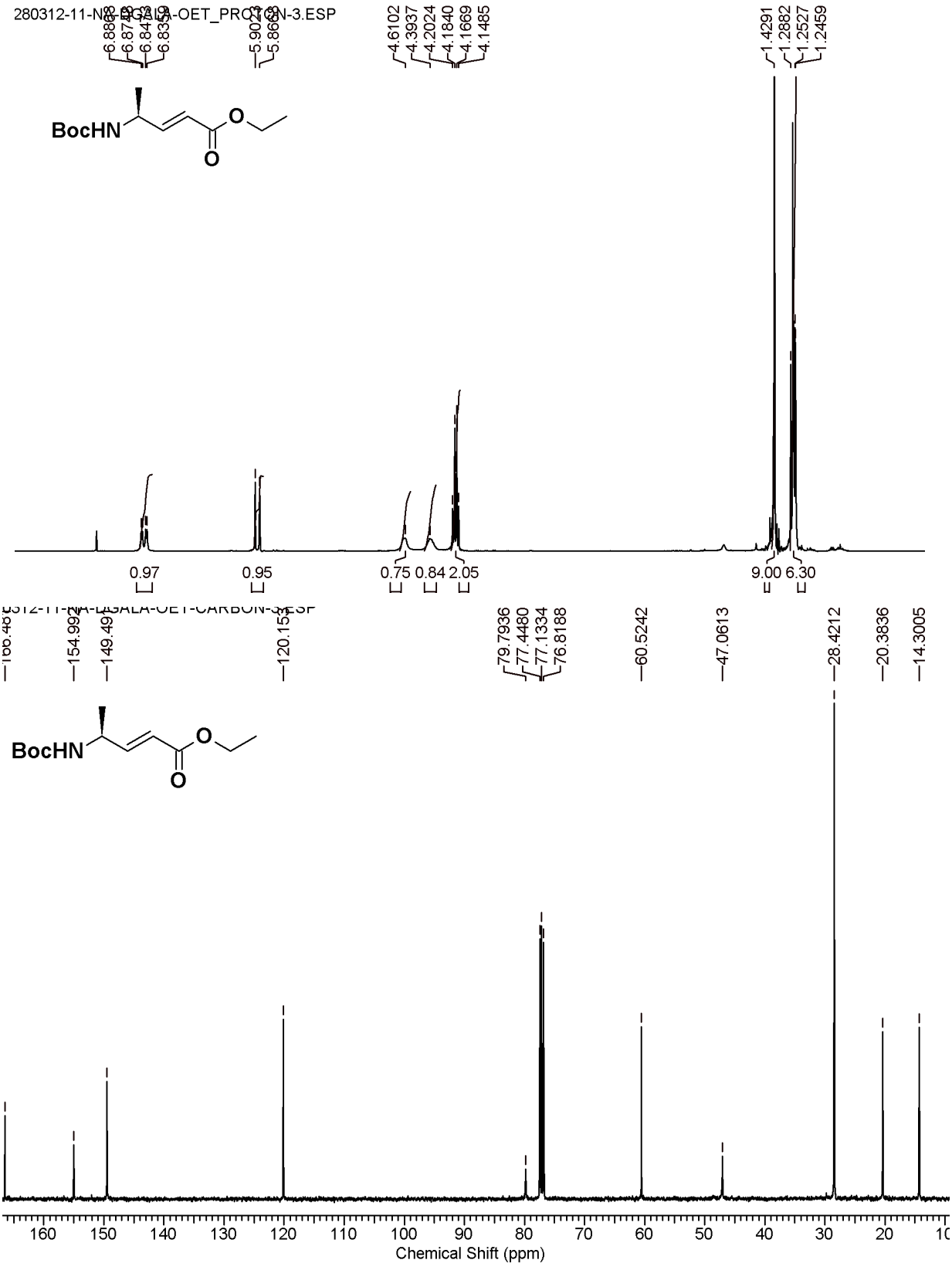


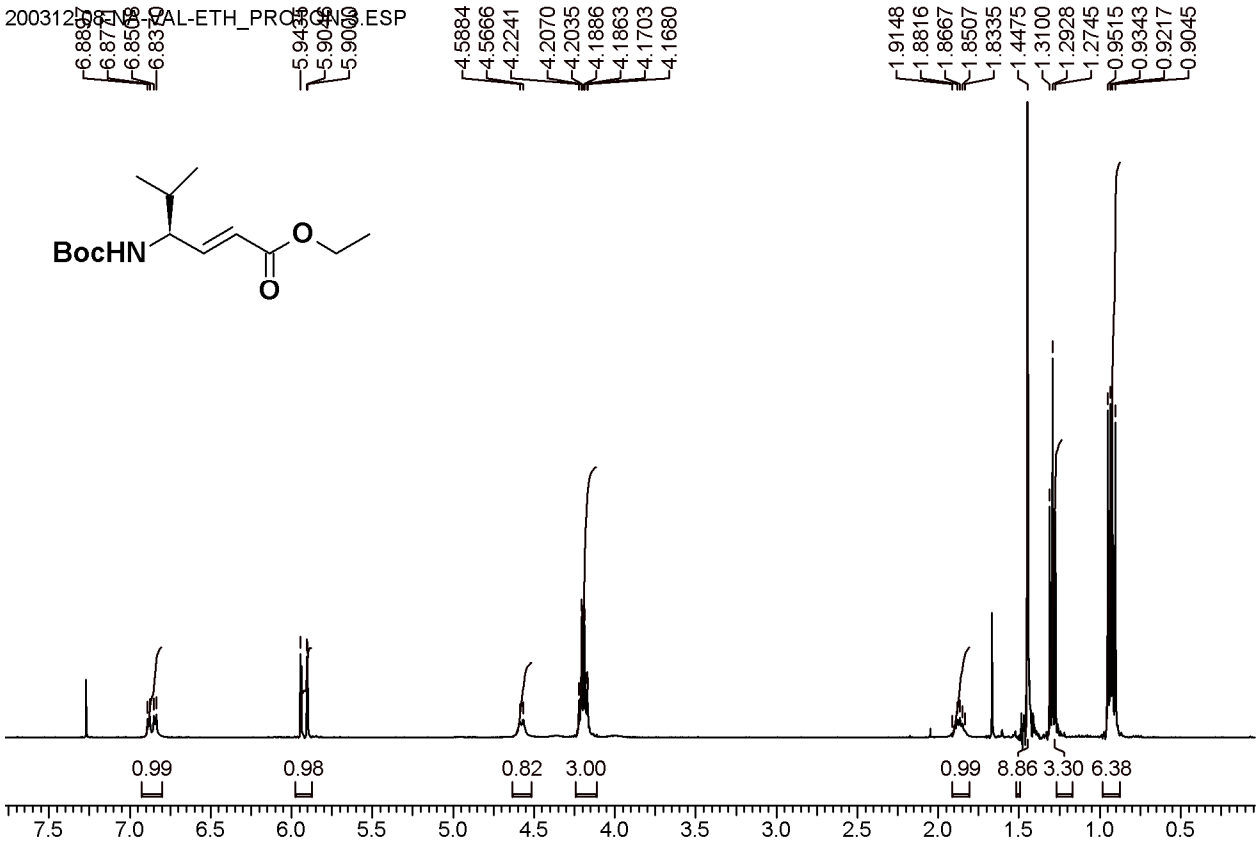
***(R)*-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-phenylpentanoic acid: (Fmoc-γPhe-OH) :-**

Solid (Yield 0.324 g, 81 %) **^{1}H NMR**(400 MHz, DMSO d-6) δ 11.663 (br, 1H, COO*H)* 7.894-7.875 (m, 2H, -Fmoc aromatic), 7.761-7.632 (d, 2H, -Fmoc aromatic), 7.424-7.170 (m, 10H, -Fmoc aromatic , -Ph protons & N*H*) , 4.234-4.219 (m, 2H, -C*H*$_2$ (of Fmoc)), 4.163-4.131(m,1H, -C*H*(of Fmoc)), 3.656 (br, 1H, -NH-C*H*-CH$_2$-CH$_2$CO$_2$H), 2.697-2.681(d, *J*= 6.4 Hz, 2H, NHCH-C*H*$_2$-Ph), 2.2930-2.162 (m, 2H, -NH-CH-CH$_2$-C*H*$_2$CO$_2$H), 1.173-1.556 (m, 2H, -NH-C*H*-C*H*$_2$-CH$_2$CO$_2$H)) ; **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.395, 155.736, 143.875, 140.748,138,955,129.211,128105,127.628, 127.066, 125.998, 125.244, 120.143, 65.091,51.781, 46.794, 30.528, 29.489 **MALDI TOF/TOF** m/z Calcd. for $C_{26}H_{25}NO_4$ (M+Na) 438.1681 Observed 438.1244



^{1}H and ^{13}C spectral data

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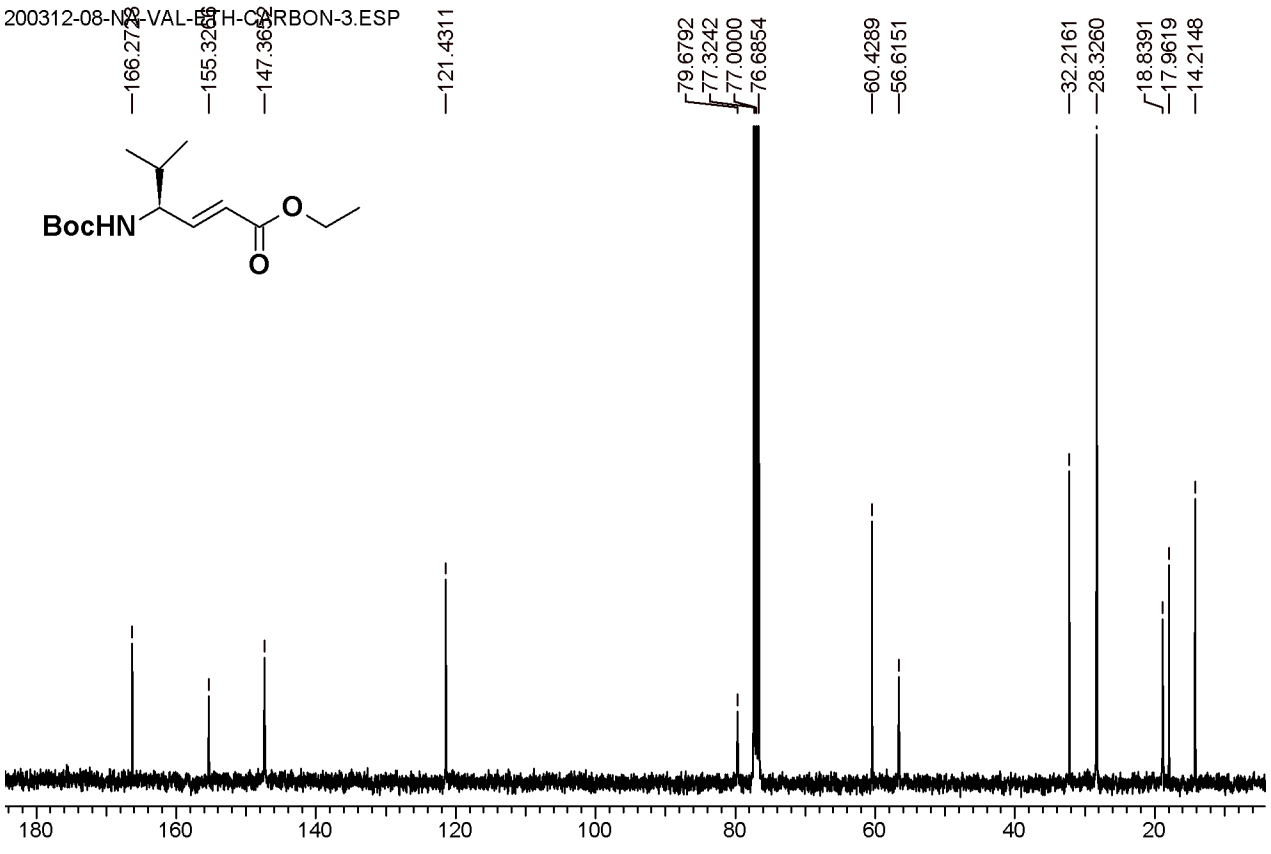
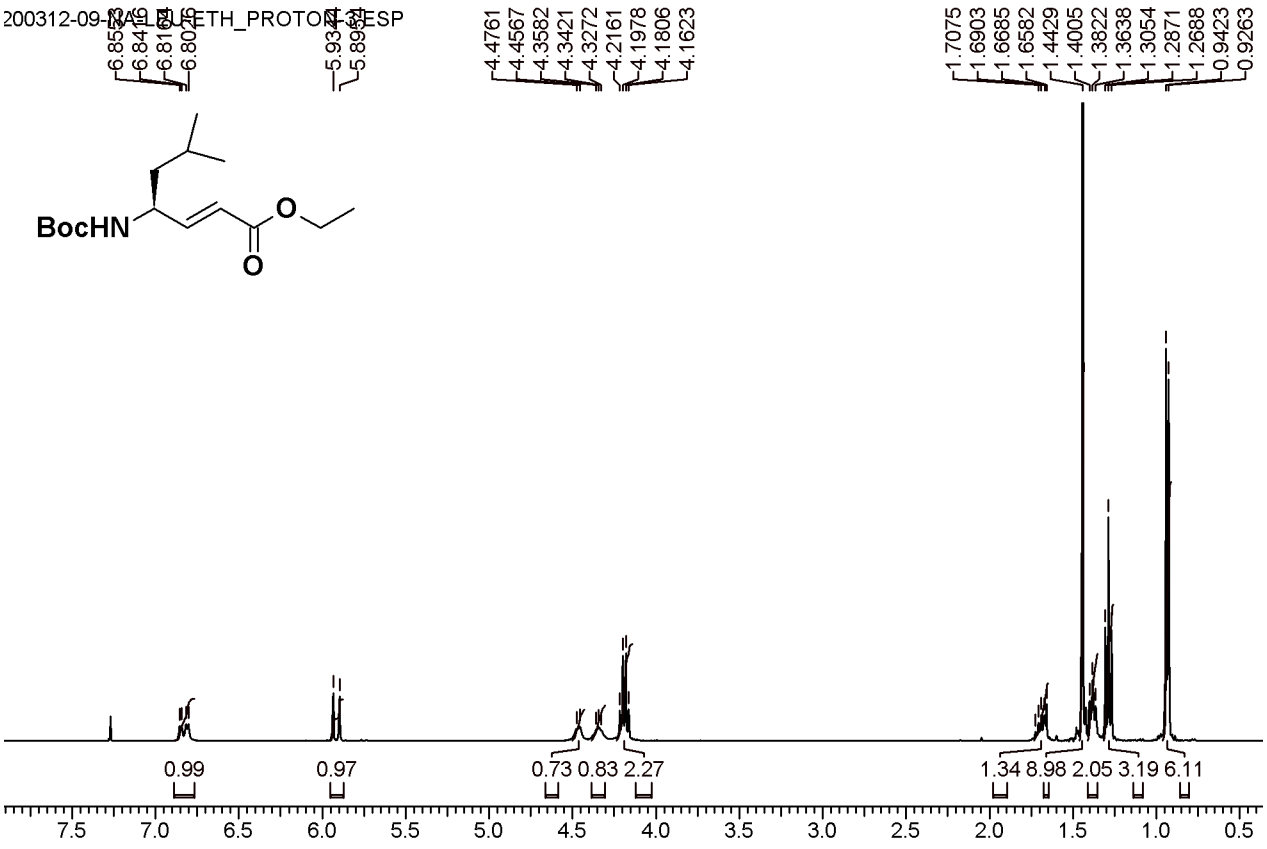
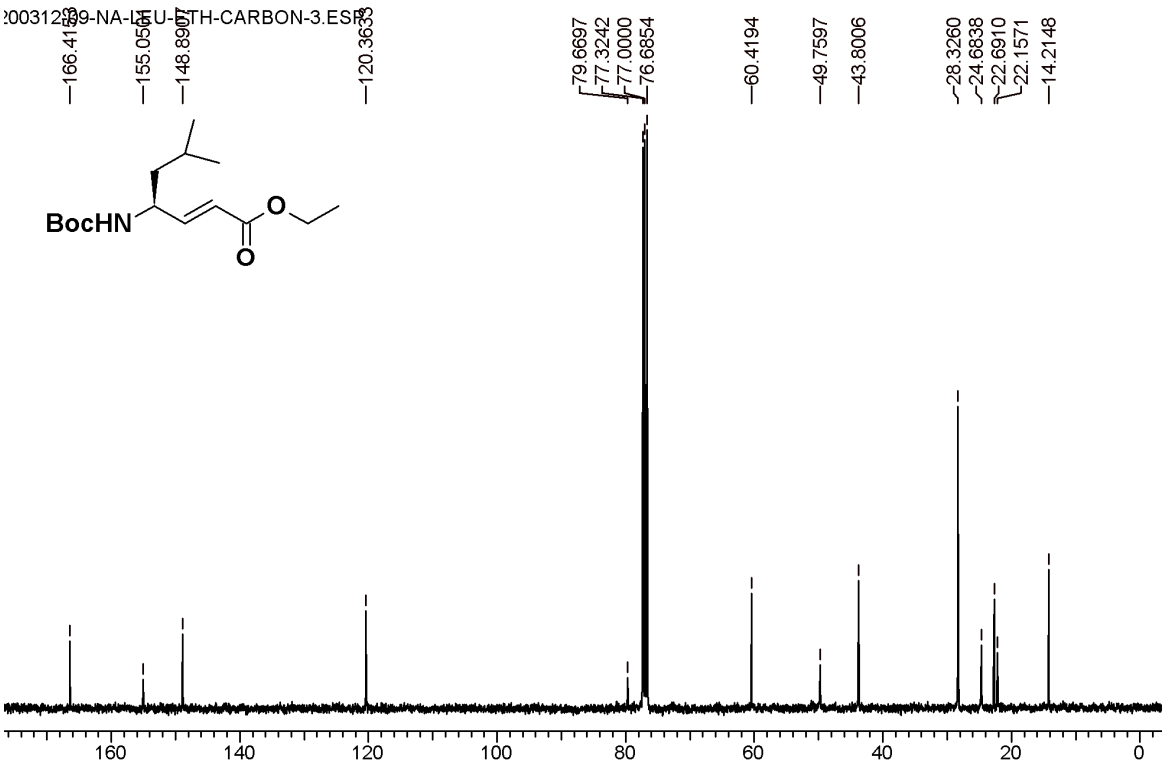

200312-08-NA-VAL-ETH-CARBON-3.ESP
166.274
155.326
147.365
121.4311
79.6792
77.3242
77.0000
76.6854
60.4289
56.6151
32.2161
28.3260
18.8391
17.9619
14.2148
BocHN
O
O
180
160
140
120
100
80
60
40
20


200312-09-NA-LEU-ETH_PROTON-1.ESP
6.8554
6.8416
6.8176
6.8027
5.9344
5.8952
4.4761
4.4567
4.3582
4.3421
4.3272
4.2161
4.1978
4.1806
4.1623
1.7075
1.6903
1.6685
1.6582
1.4429
1.4005
1.3822
1.3638
1.3054
1.2871
1.2688
0.9423
0.9263
BocHN
O
O
0.99
0.97
0.73
0.83
2.27
1.34
8.98
2.05
3.19
6.11
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


200312-09-NA-LEU-ETH-CARBON-3.ESP
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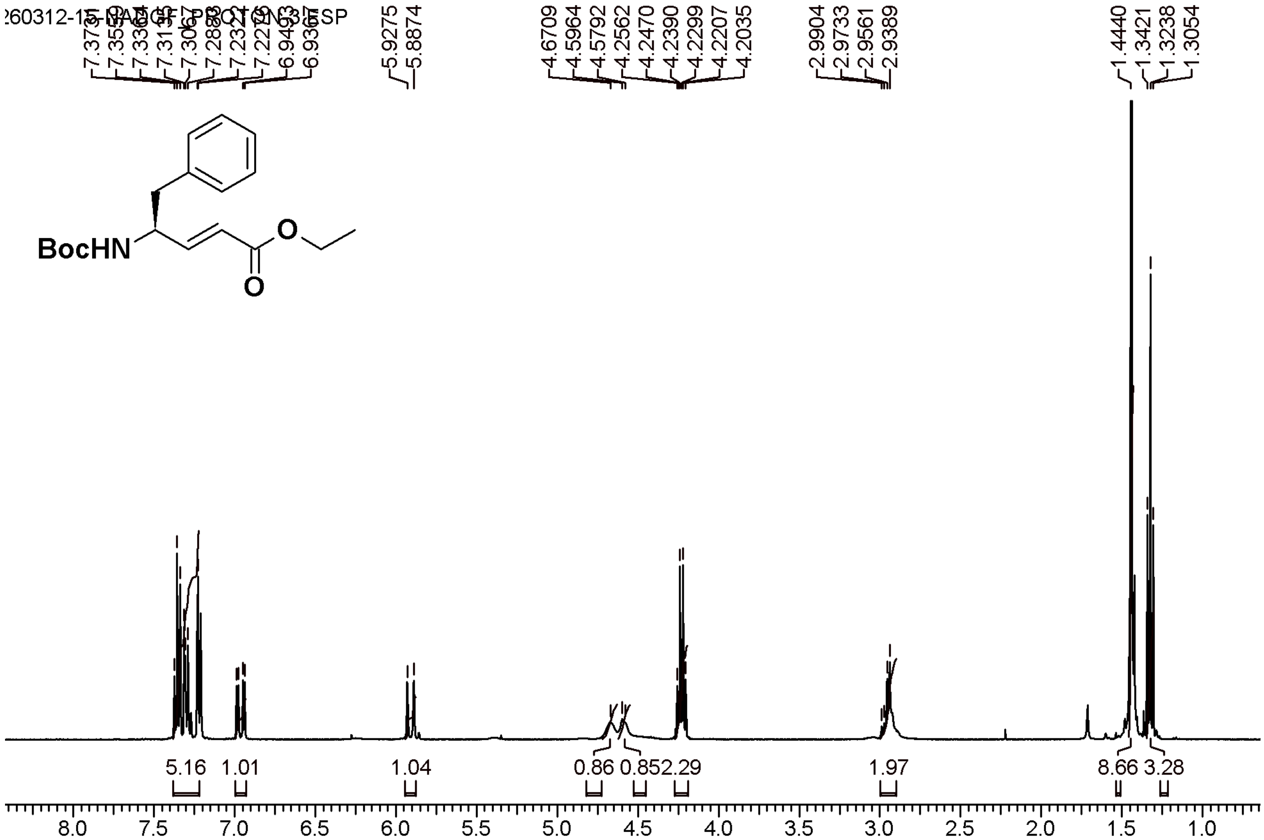
148.8907
120.3633
79.6697
77.3242
77.0000
76.6854
60.4194
49.7597
43.8006
28.3260
24.6838
22.6910
22.1571
14.2148
BocHN
O
O
160
140
120
100
80
60
40
20
0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4.6709
4.5964
4.5792
4.2562
4.2470
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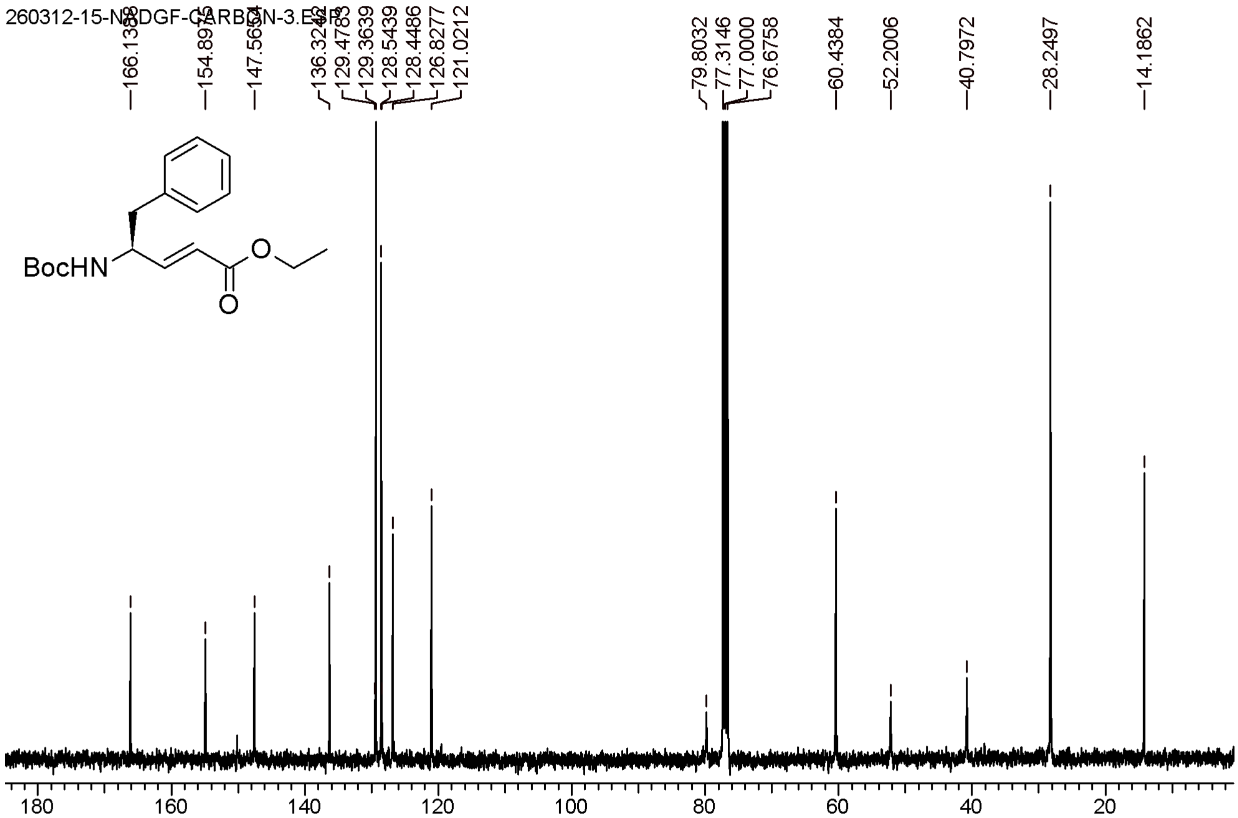
4.2299
4.2207
4.2035
2.9904
2.9733
2.9561
2.9389
1.4440
1.3421
1.3238
1.3054
BocHN
O
O
5.16
1.01
1.04
0.86
0.85
2.29
1.97
8.66
3.28
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0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128.5439
128.4486
126.8277
121.0212
79.8032
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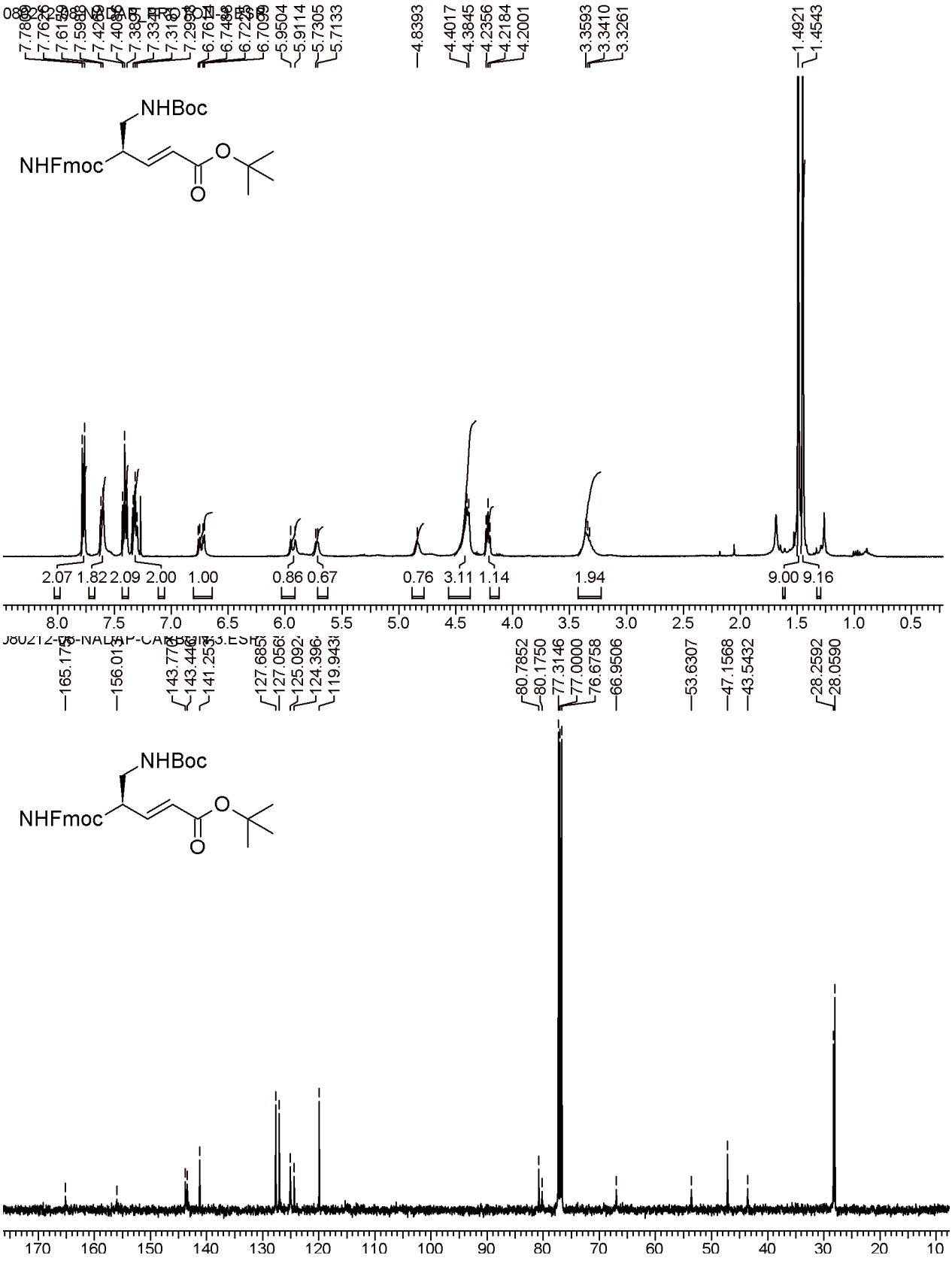
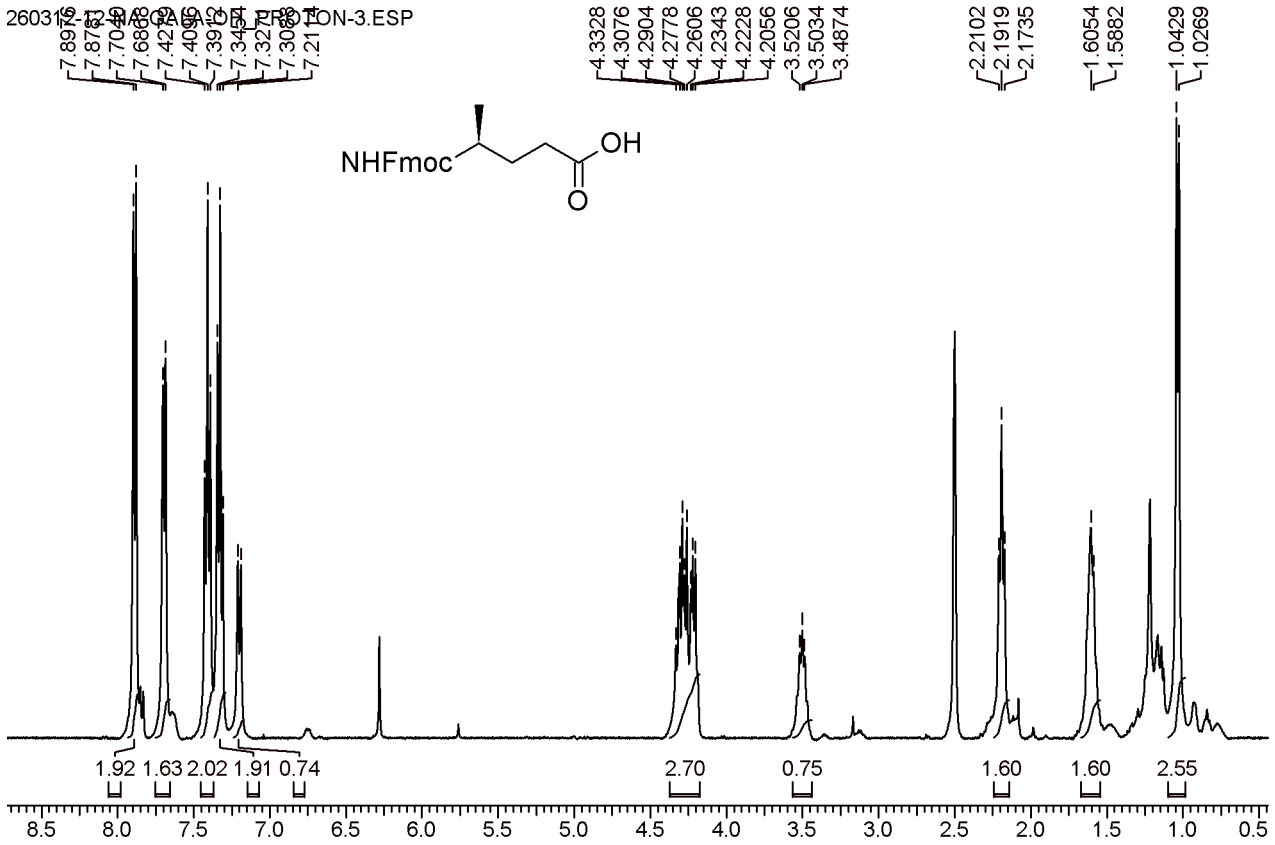
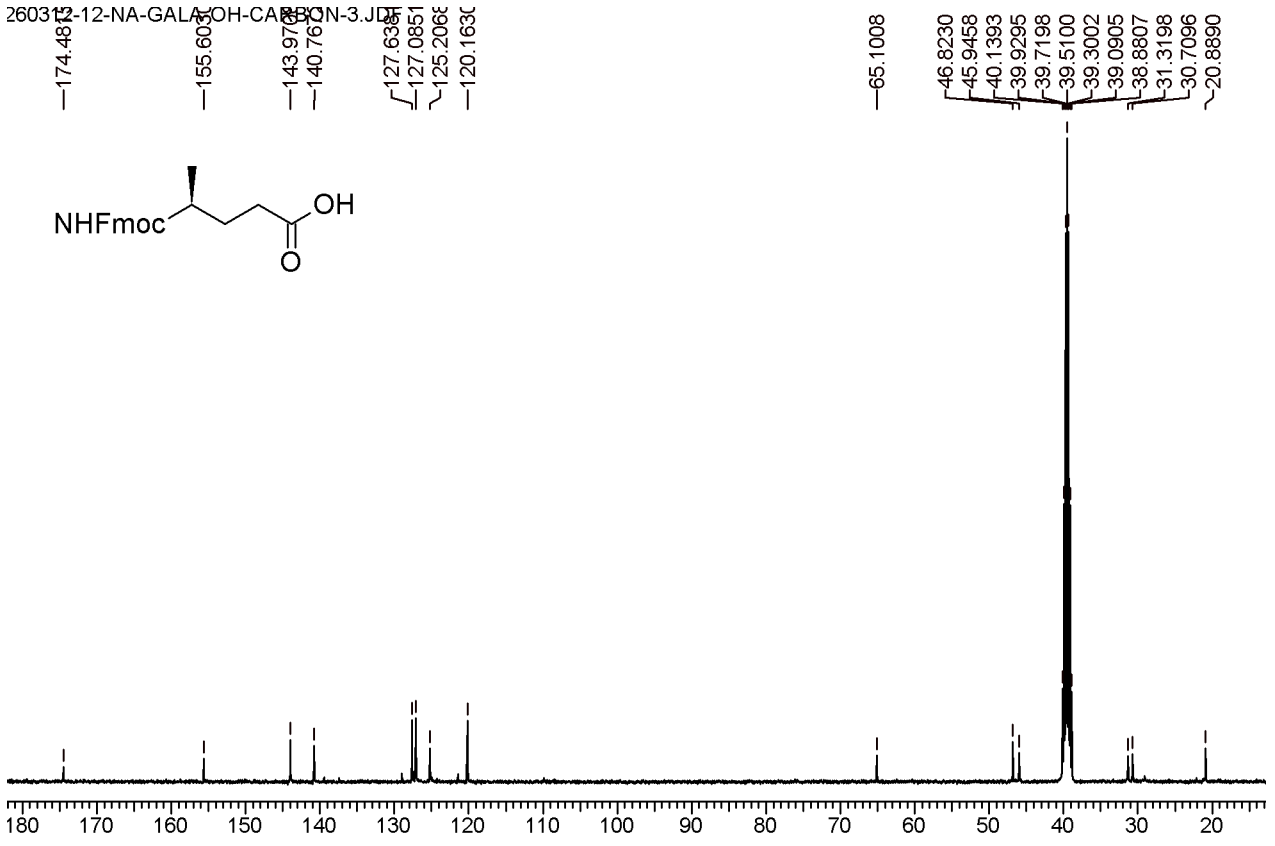
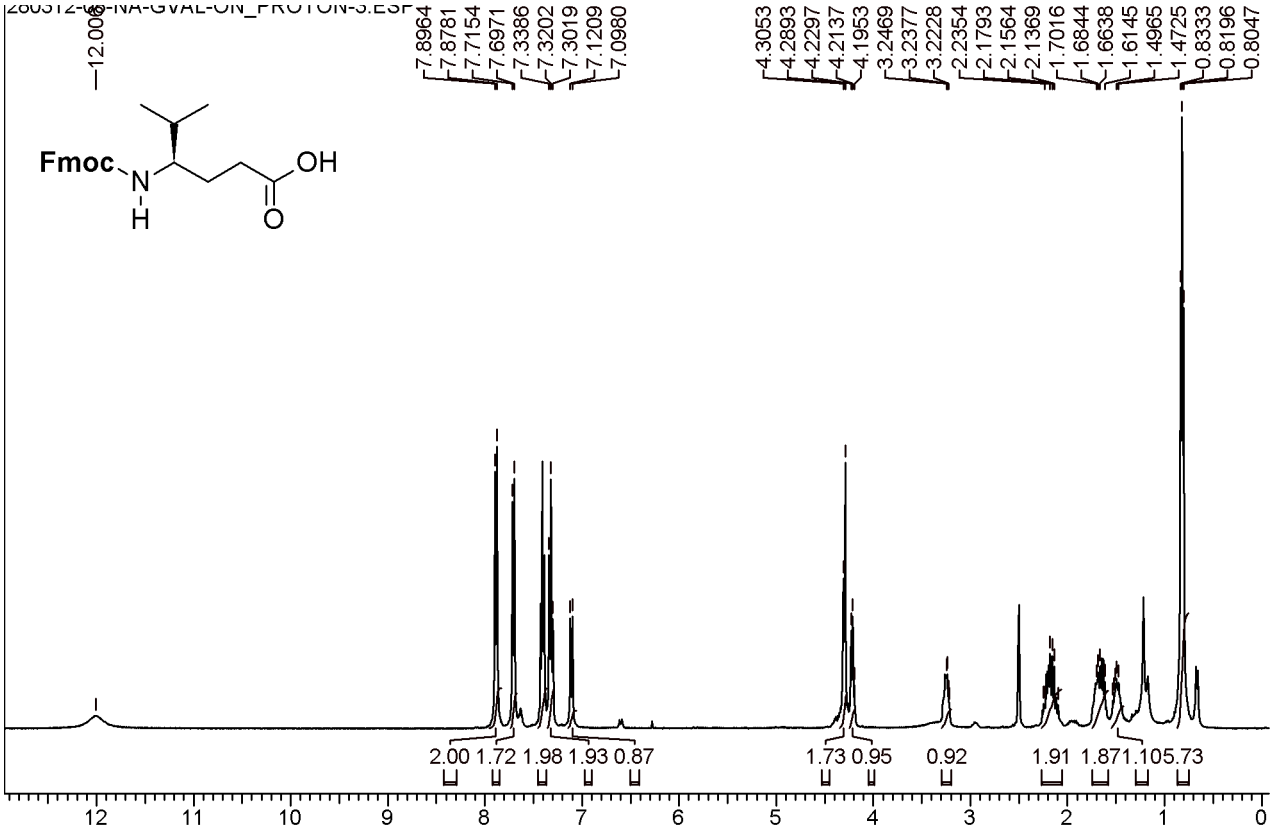
77.0000
76.6758
60.4384
52.2006
40.7972
28.2497
14.1862
BocHN
O
O
180 160 140 120 100 80 60 40 20


NHBoc
NHFmoc
O
O
2.07 1.82 2.09 2.00 1.00 0.86 0.67 0.76 3.11 1.14 1.94 9.00 9.16
NHBoc
NHFmoc
O
O


NHFmoc
OH
O
1.92 1.63 2.02 1.91 0.74
2.70
0.75
1.60
1.60
2.55


NHFmoc
OH
O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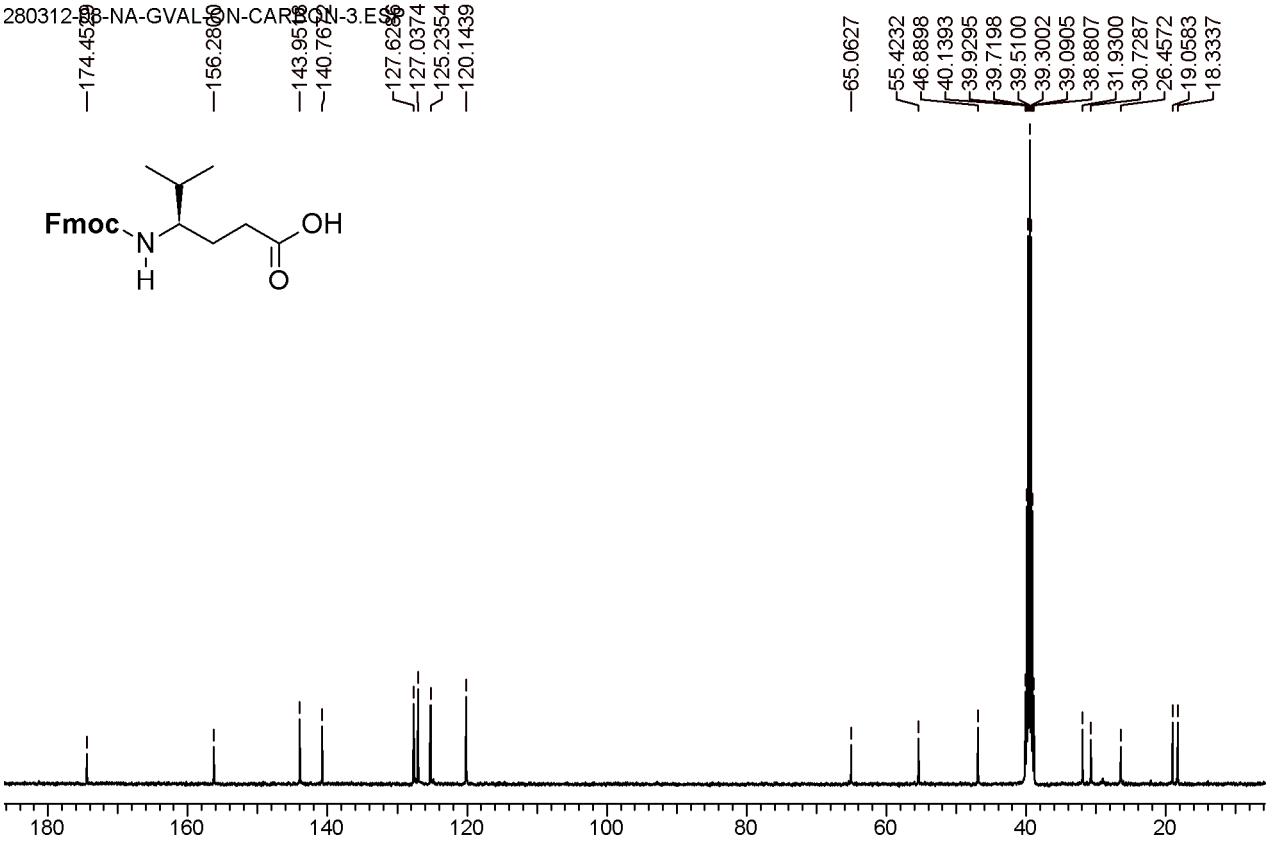
7.8964
7.8781
7.7154
7.6971
7.3386
7.3202
7.3019
7.1209
7.0980
4.3053
4.2893
4.2297
4.2137
4.1953
3.2469
3.2377
3.2228
2.2354
2.1793
2.1564
2.1369
1.7016
1.6844
1.6638
1.6145
1.4965
1.4725
0.8333
0.8196
0.8047
Fmoc
N
H
OH
O
2.00 1.72 1.98 1.93 0.87
1.73 0.95
0.92
1.91 1.87 1.10 5.73
12 11 10 9 8 7 6 5 4 3 2 1 0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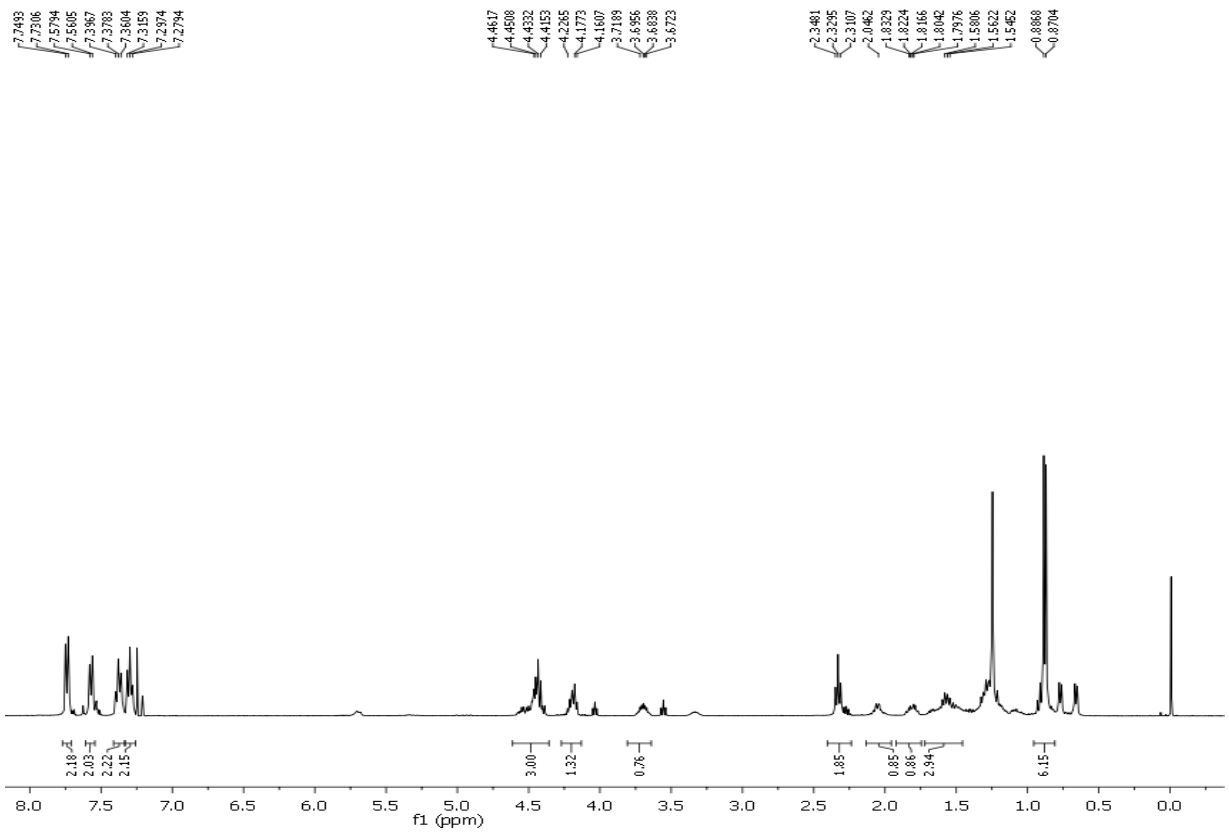
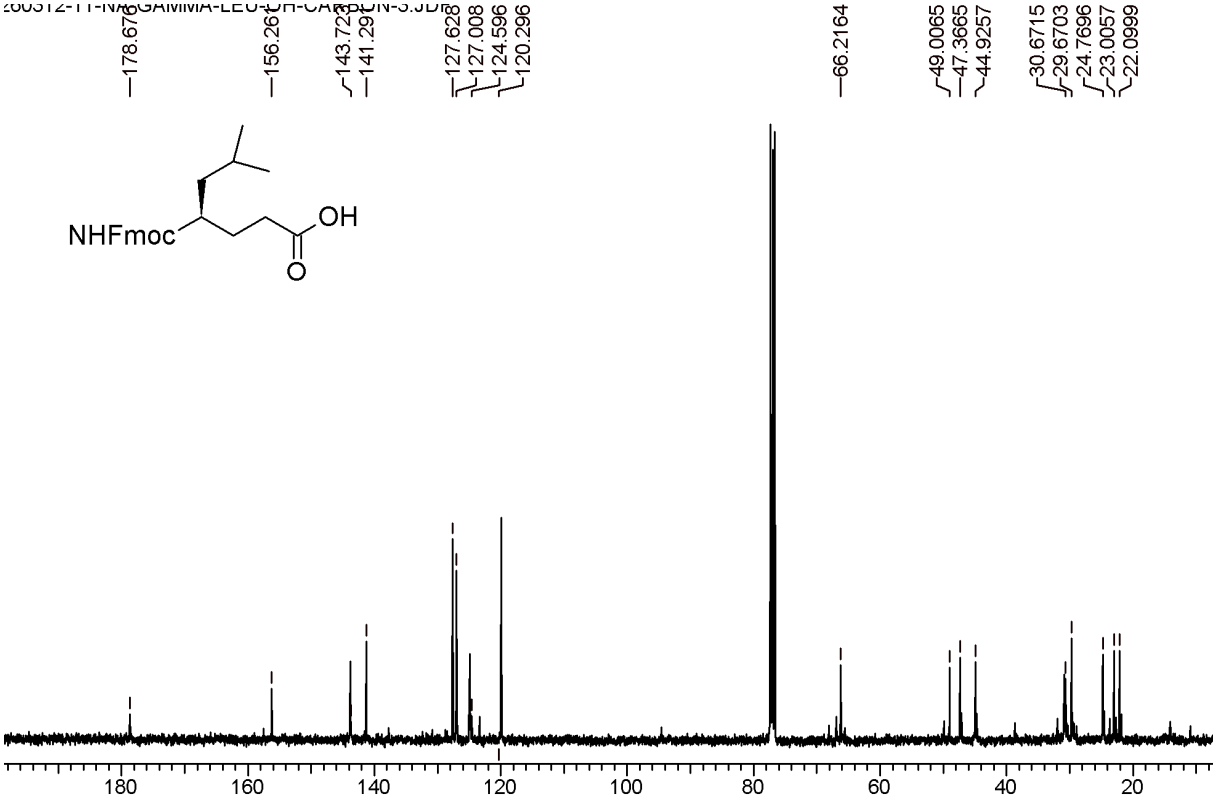
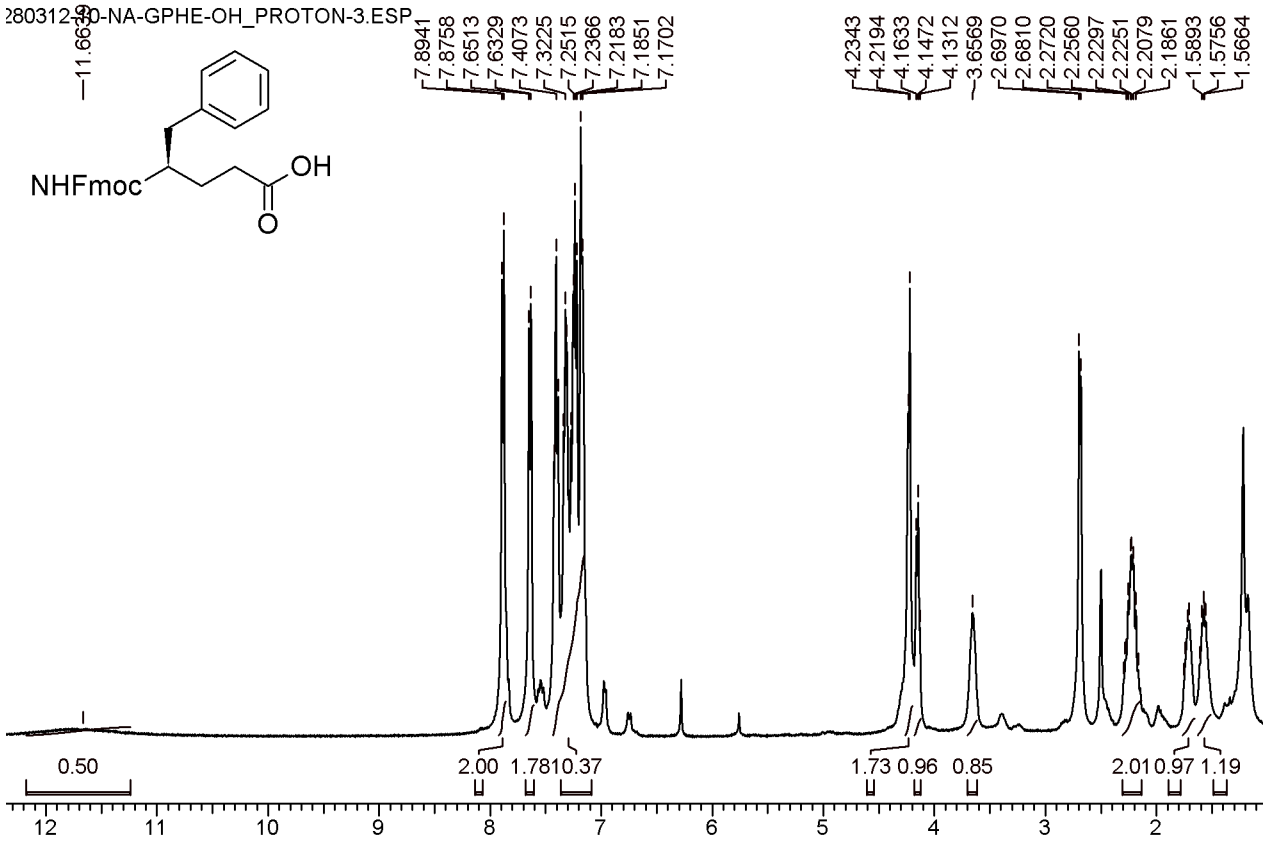
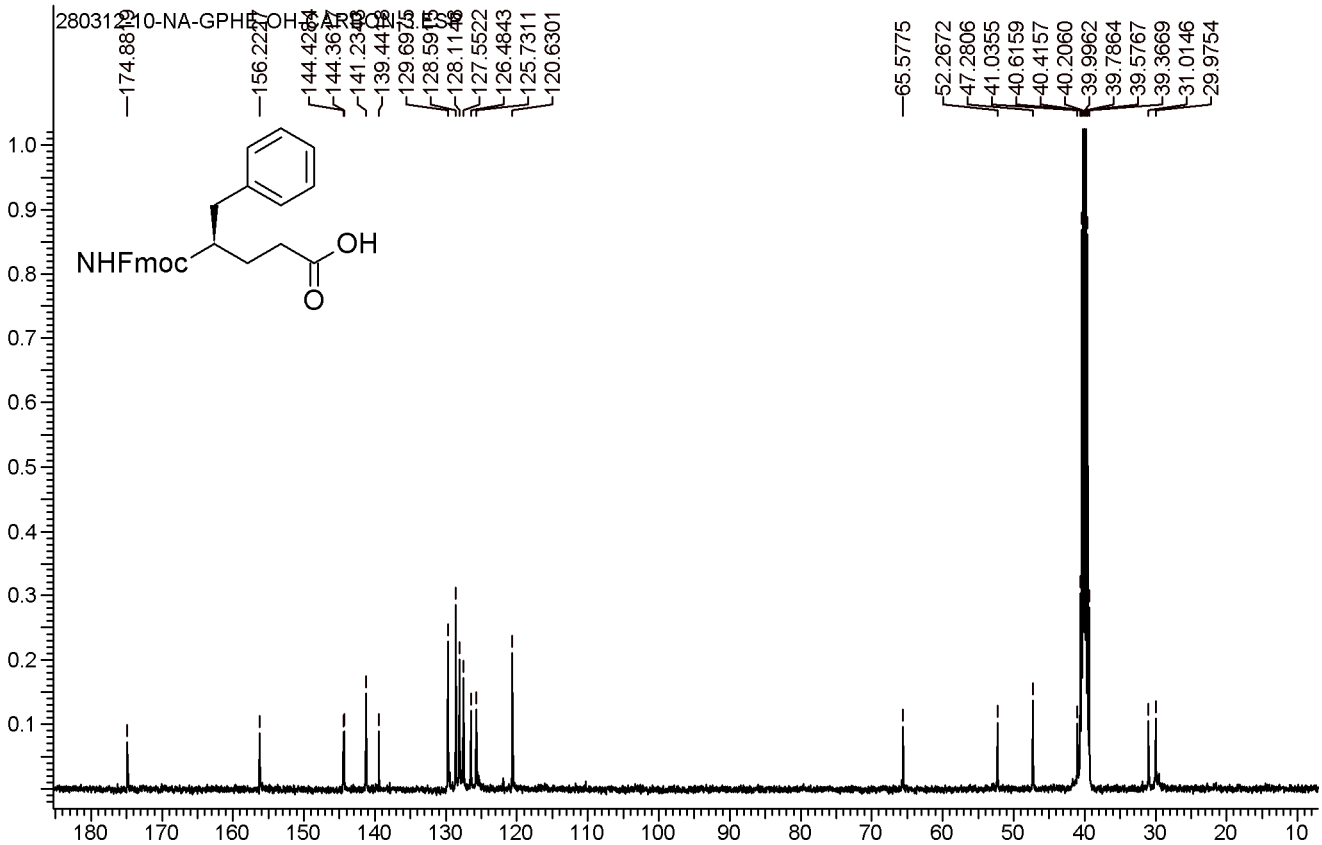
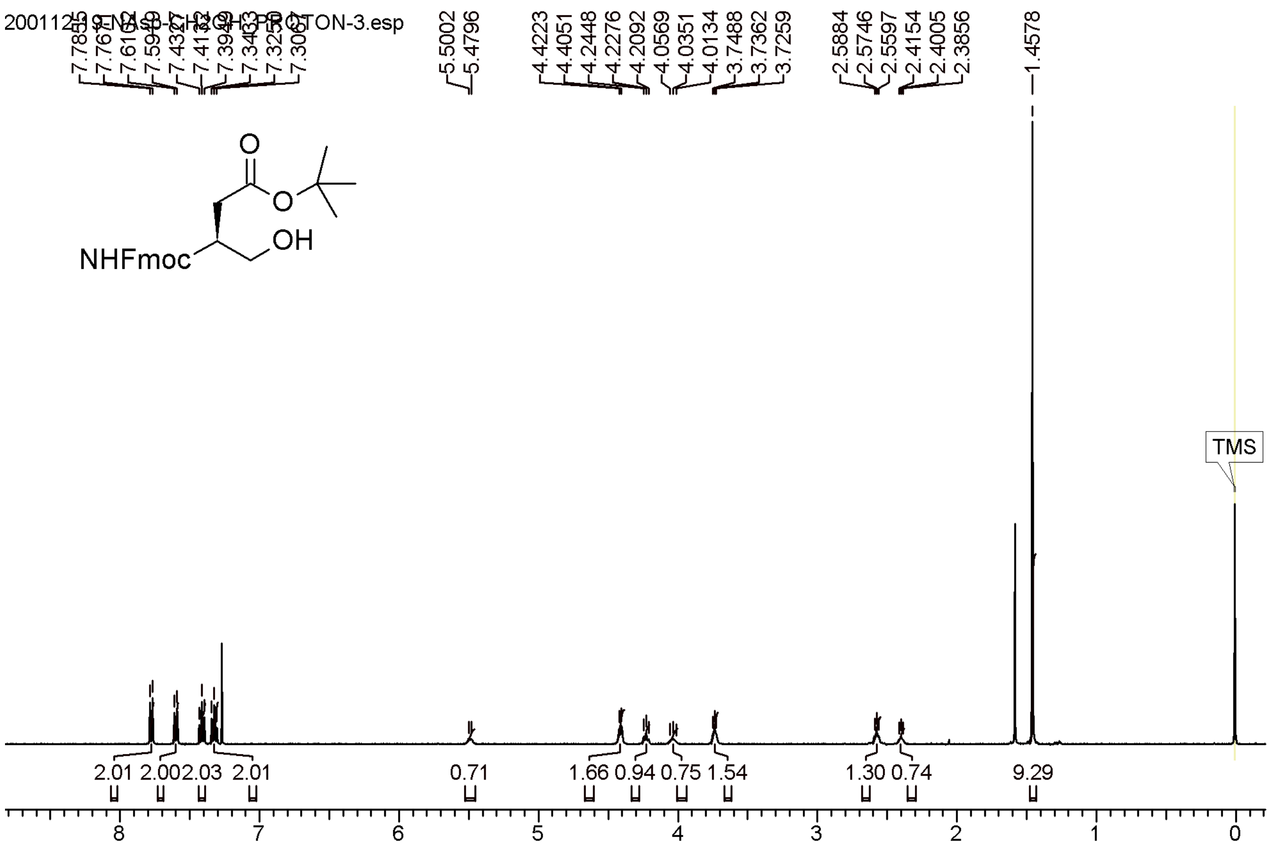
156.2800
143.9510
140.7672
127.6286
127.0374
125.2354
120.1439
65.0627
55.4232
46.8898
40.1393
39.9295
39.7198
39.5100
39.3002
39.0905
38.8807
31.9300
30.7287
26.4572
19.0583
18.3337
Fmoc
N
H
OH
O
180 160 140 120 100 80 60 40 20


f1 (ppm)


NHFmoc
OH
O


280312-10-NA-GPHE-OH_PROTON-3.ESP
11.6634
7.8941
7.8758
7.6513
7.6329
7.4073
7.3225
7.2515
7.2366
7.2183
7.1851
7.1702
4.2343
4.2194
4.1633
4.1472
4.1312
3.6569
2.6970
2.6810
2.2720
2.2560
2.2297
2.2251
2.2079
2.1861
1.5893
1.5756
1.5664
NHFmoc
OH
O
0.50
2.00
1.78
10.37
1.73
0.96
0.85
2.01
0.97
1.19
12
11
10
9
8
7
6
5
4
3
2


280312-10-NA-GPHE-OH_CARBON-3.ESP
174.8812
156.2227
144.4264
144.3614
141.2346
139.4415
129.6973
128.5913
128.1148
127.5522
126.4843
125.7311
120.6301
65.5775
52.2672
47.2806
41.0355
40.6159
40.4157
40.2060
39.9962
39.7864
39.5767
39.3669
31.0146
29.9754
NHFmoc
OH
O
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10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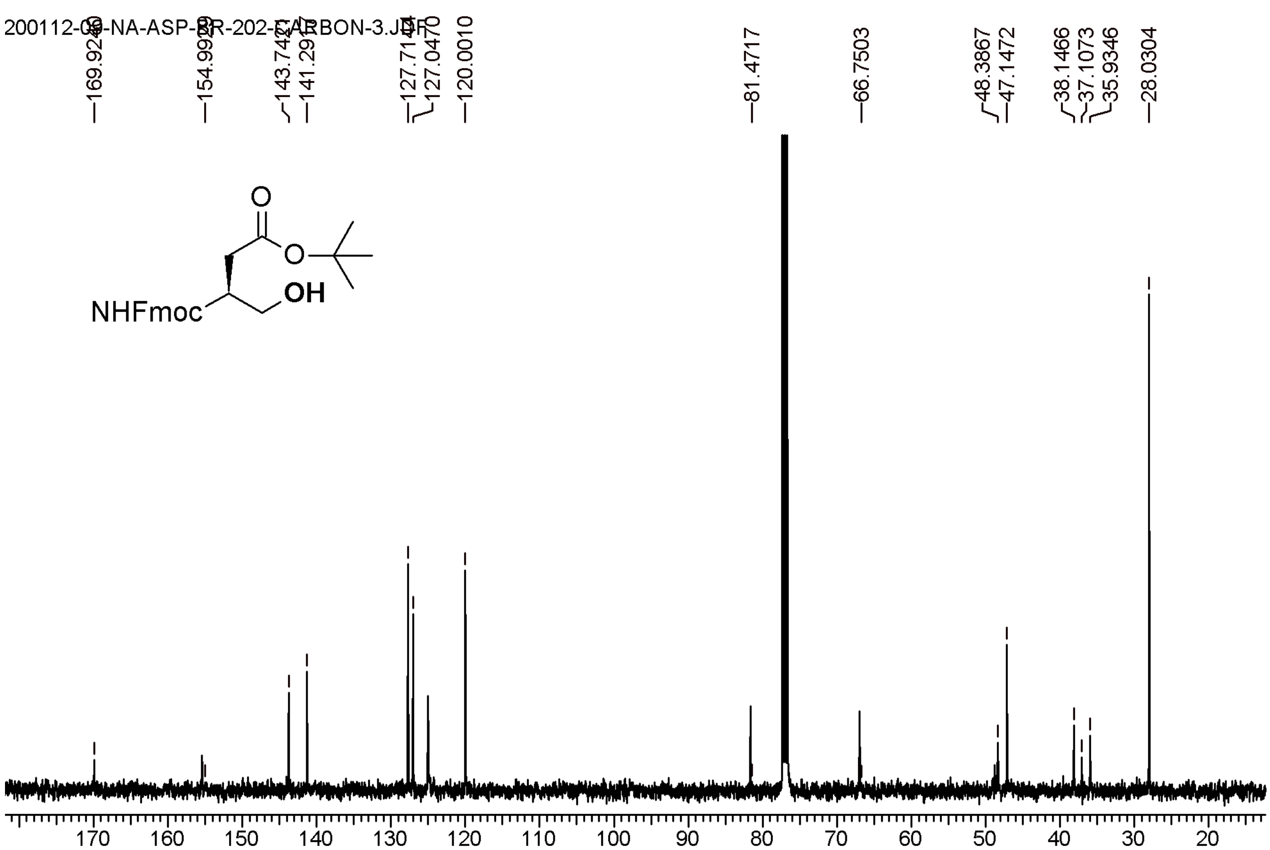
5.4796
4.4223
4.4051
4.2448
4.2276
4.2092
4.0569
4.0351
4.0134
3.7488
3.7362
3.7259
2.5884
2.5746
2.5597
2.4154
2.4005
2.3856
1.4578
NHFmoc
OH
TMS
2.01 2.00 2.03 2.01
0.71
1.66 0.94 0.75 1.54
1.30 0.74
9.29


120.0010
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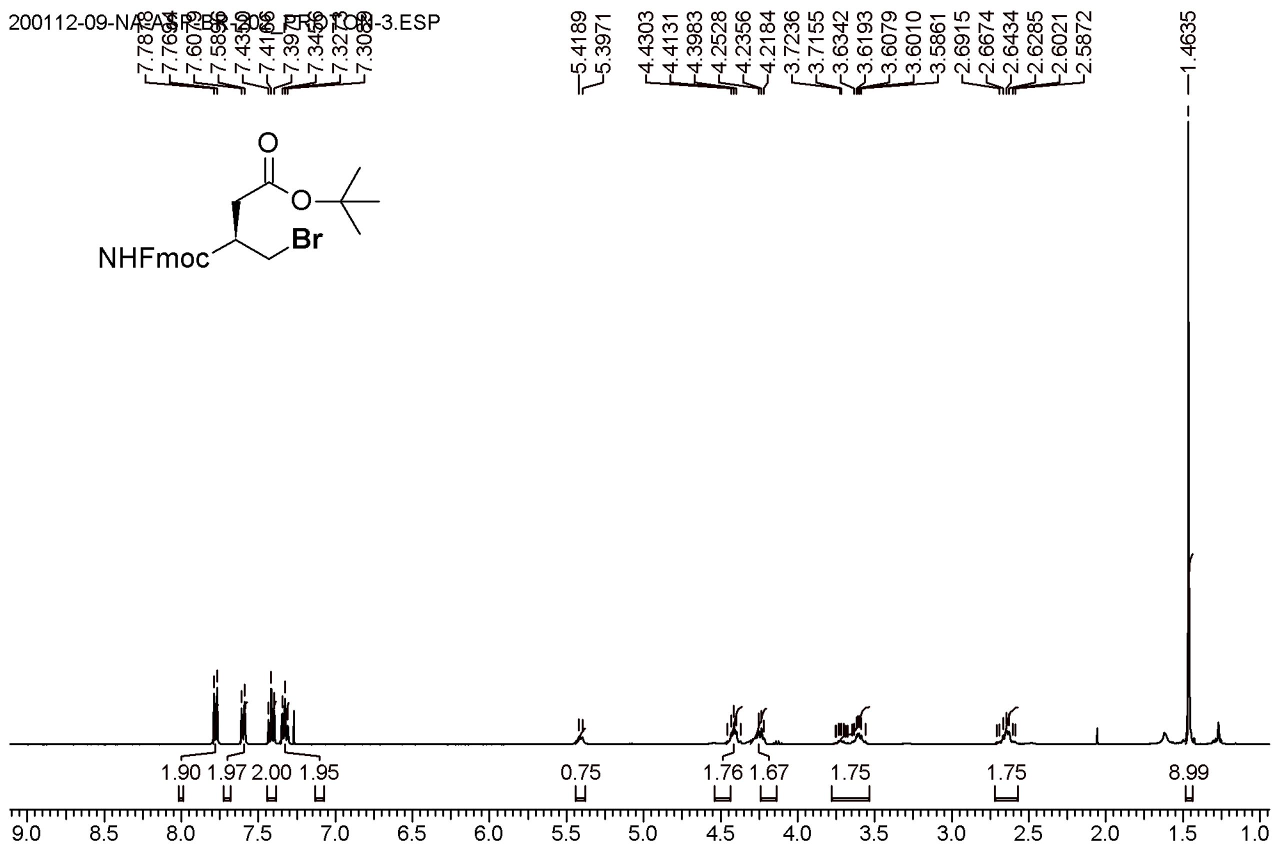
47.1472
38.1466
37.1073
35.9346
28.0304
NHFmoc
OH



NHFmoc
Br
O
1.90 1.97 2.00 1.95
0.75
1.76 1.67
1.75
1.75
8.99
5.4189
5.3971
4.4303
4.4131
4.3983
4.2528
4.2356
4.2184
3.7236
3.7155
3.6342
3.6193
3.6079
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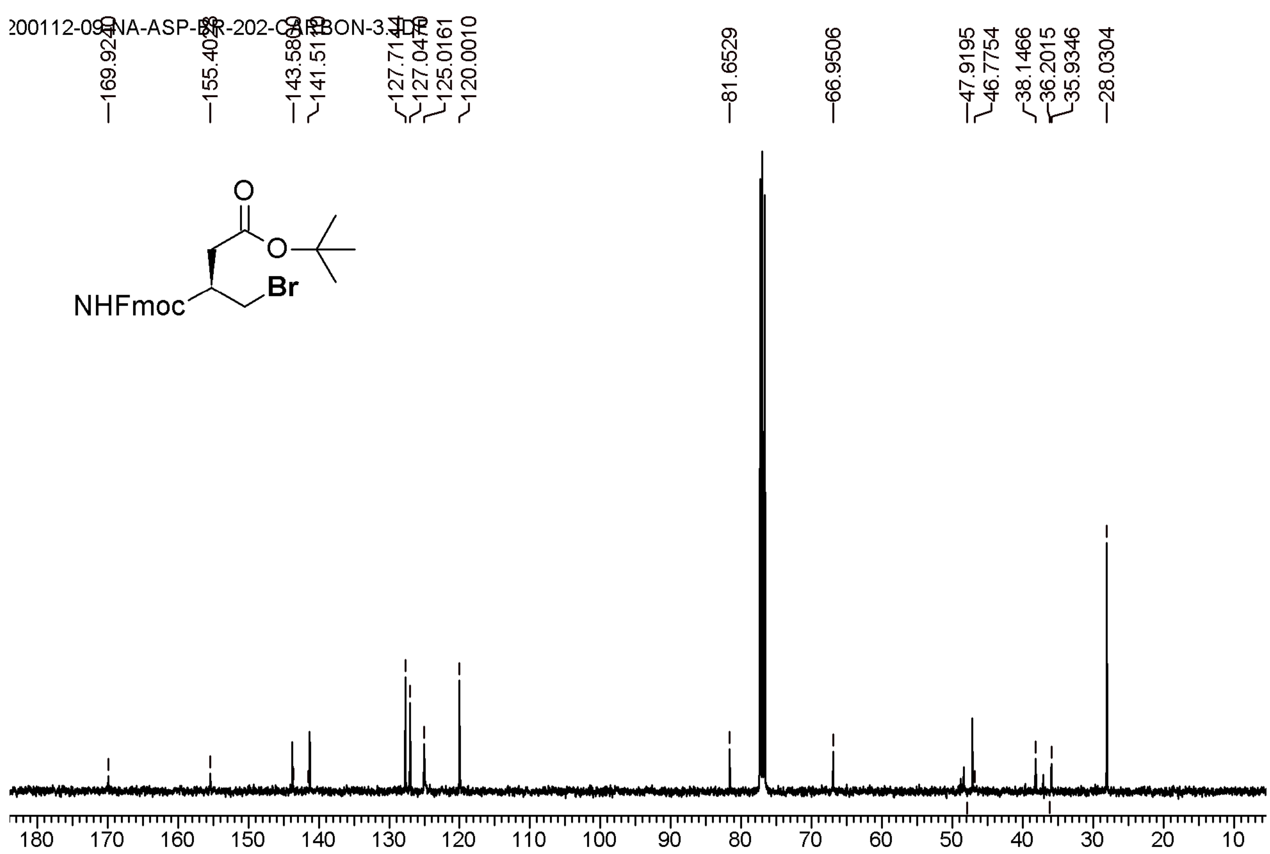
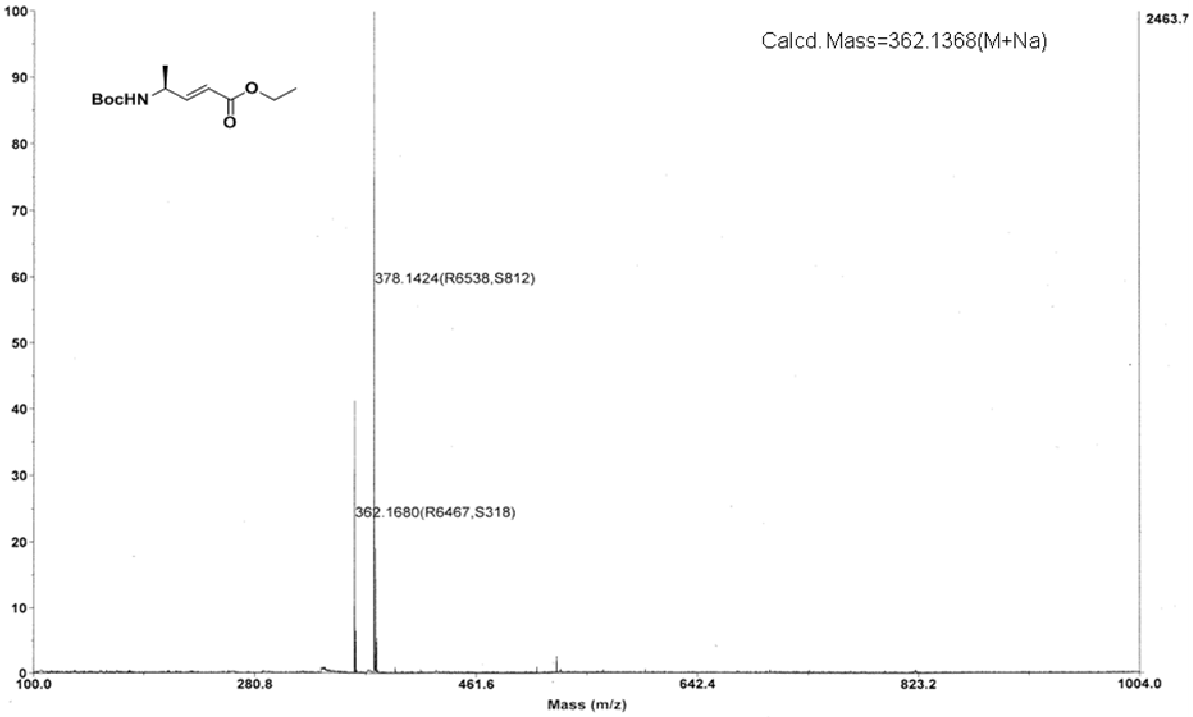
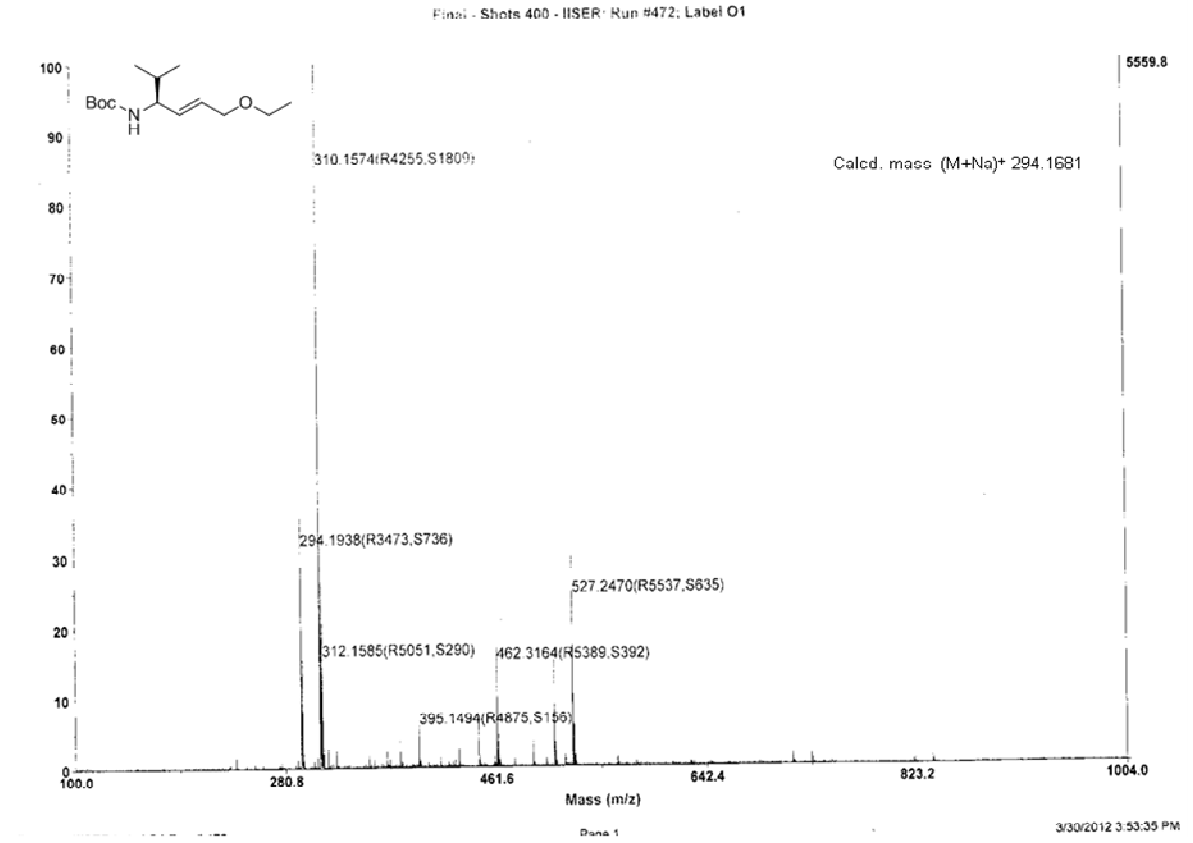
3.5861
2.6915
2.6674
2.6434
2.6285
2.6021
2.5872
1.4635

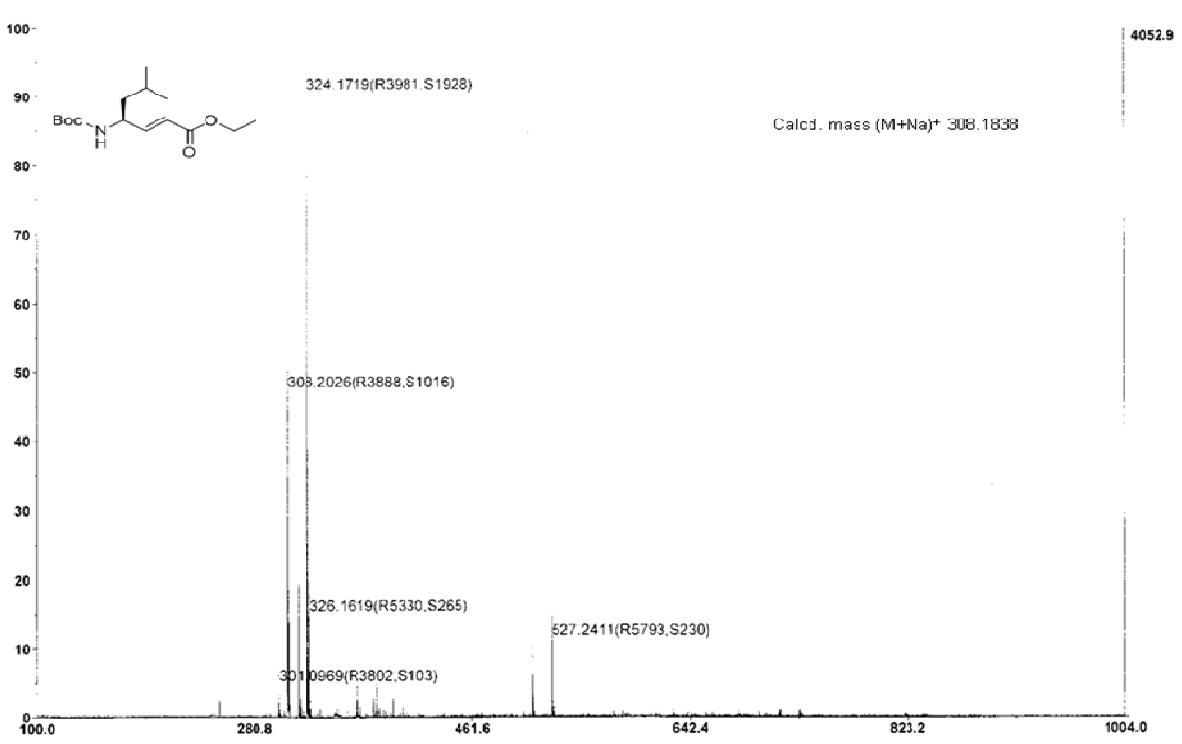


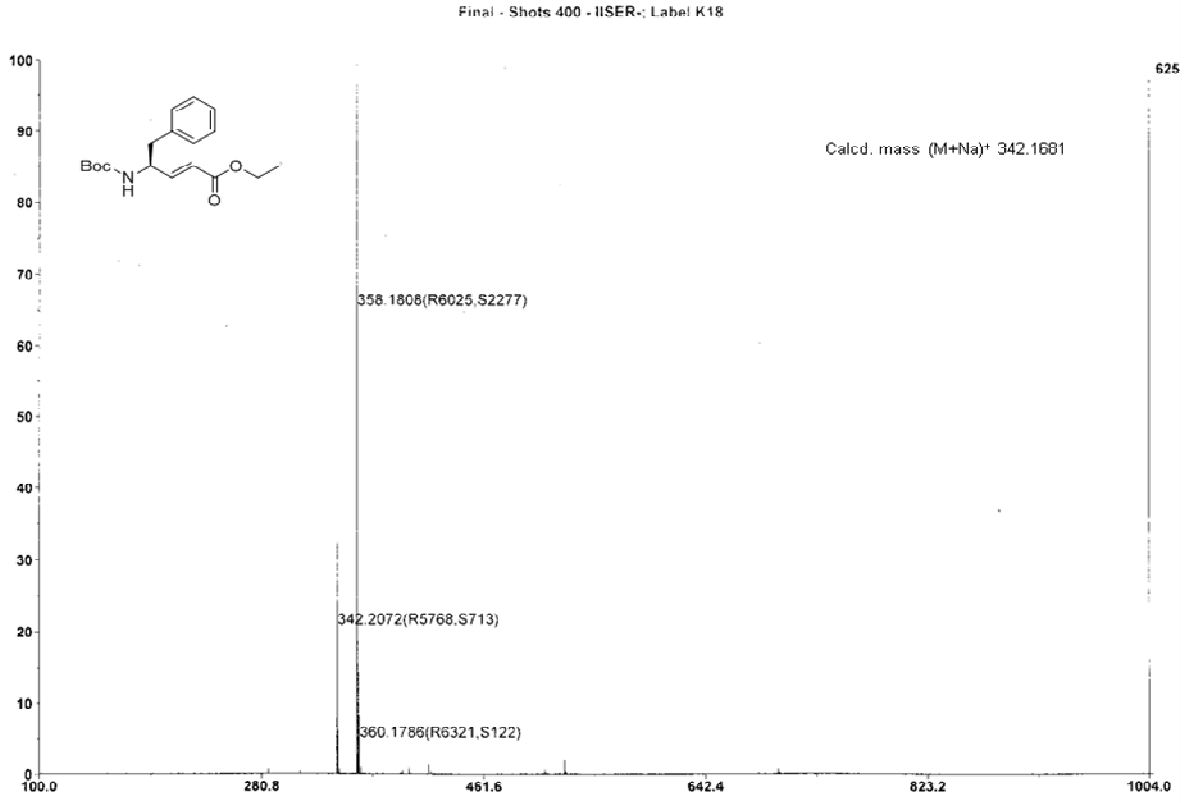
NHFmoc
Br
O
169.9240
155.4020
127.7140
127.0470
125.0161
120.0010
81.6529
66.9506
47.9195
46.7754
38.1466
36.2015
35.9346
28.0304

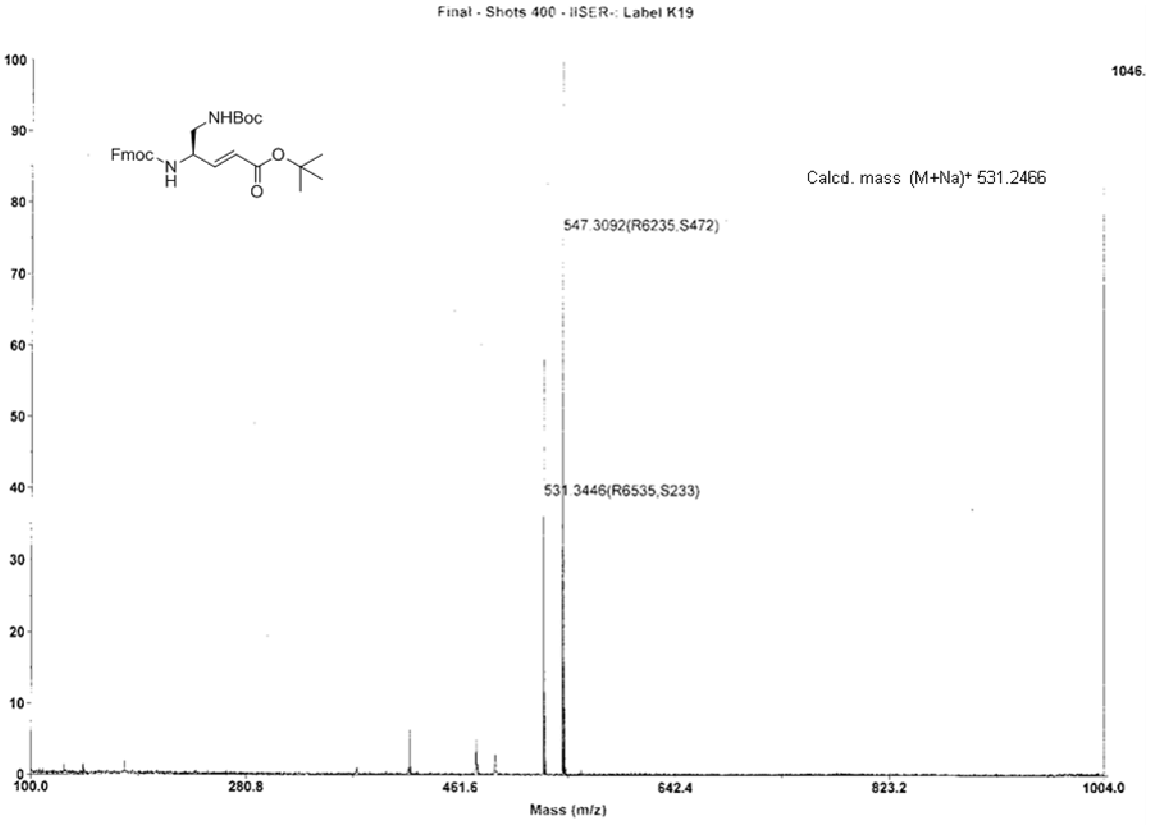
MALDI TOF/TOF data

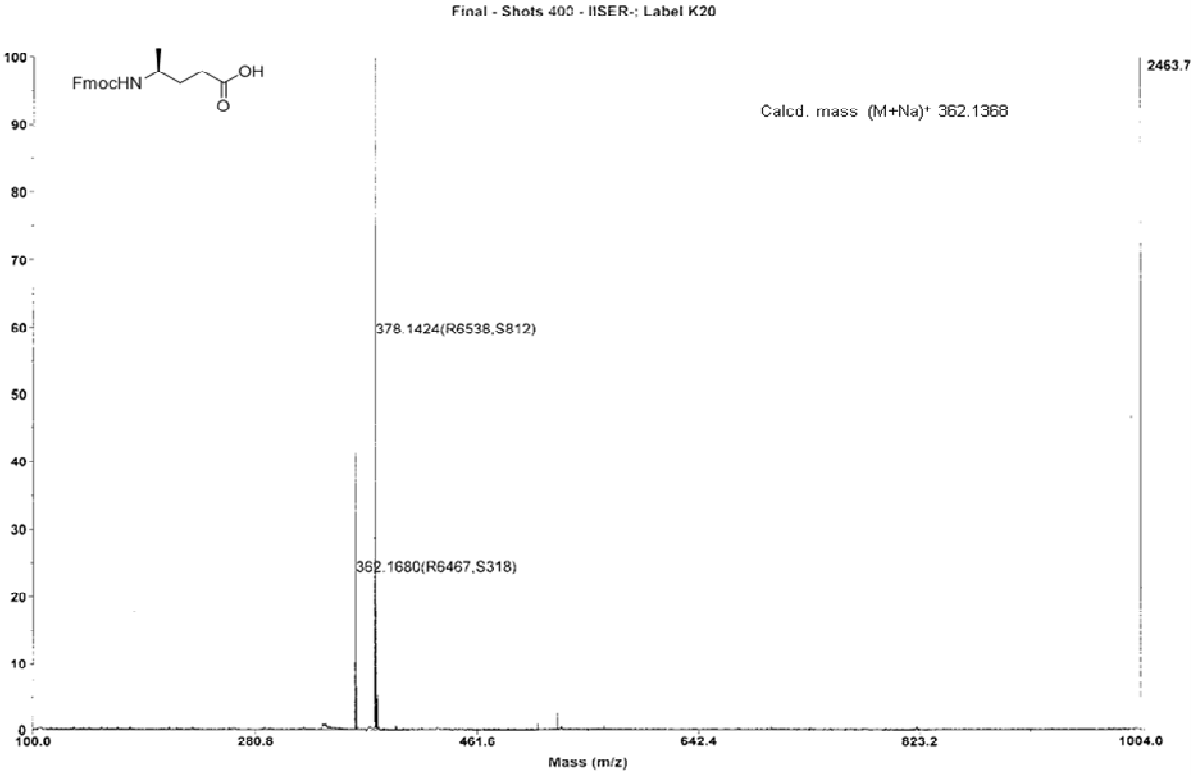


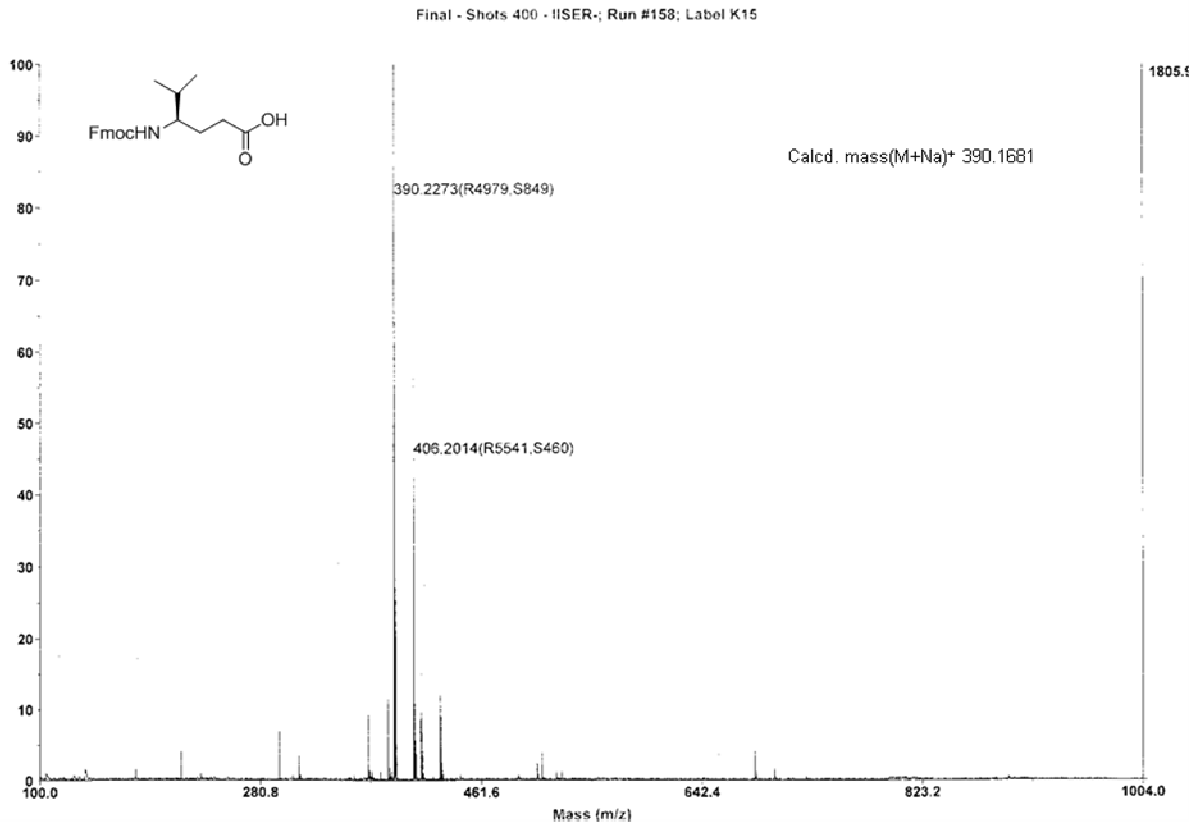


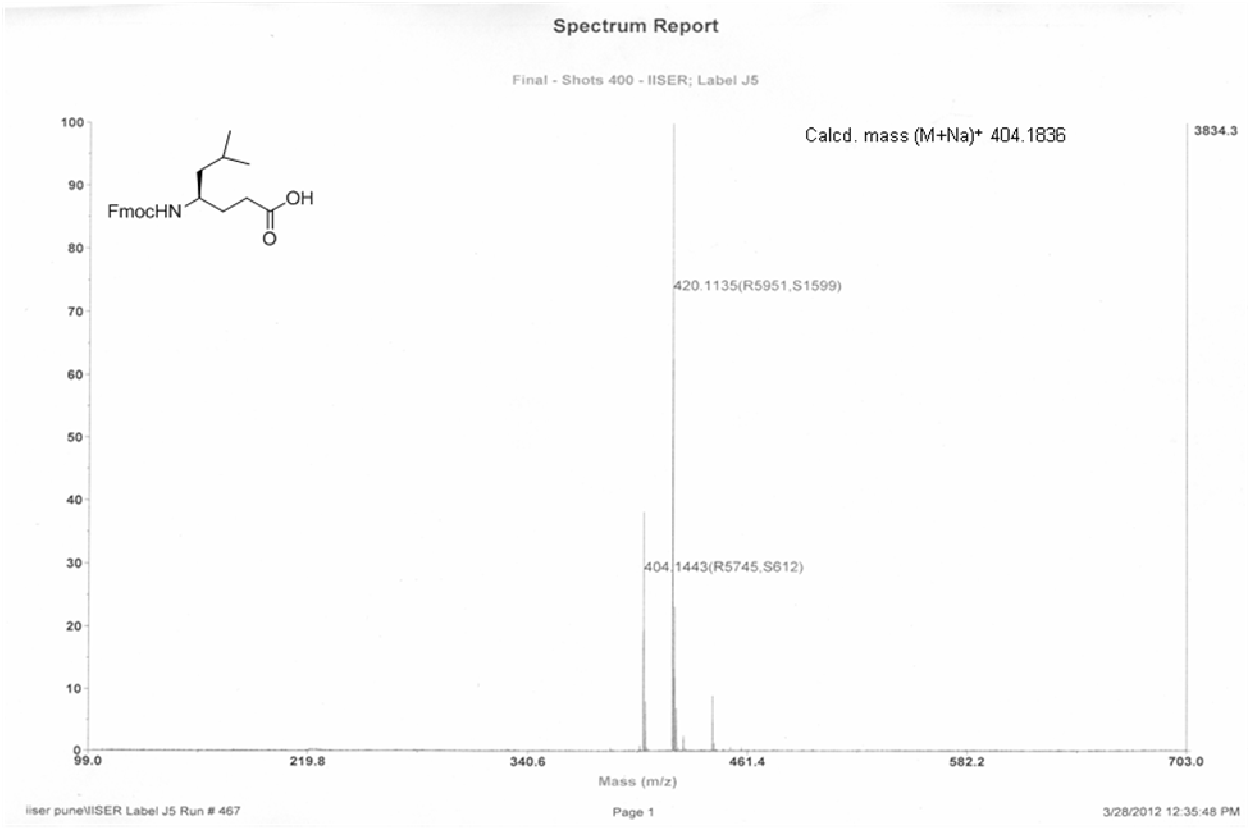

Boc
N
H
O
O
324.1719(R3981,S1928)
Calcd. mass (M+Na)+ 308.1838
4052.9
308.2026(R3888,S1016)
326.1619(R5330,S265)
527.2411(R5793,S230)
301.0969(R3802,S103)
100
90
80
70
60
50
40
30
20
10
0
100.0
280.8
461.6
642.4
823.2
1004.0

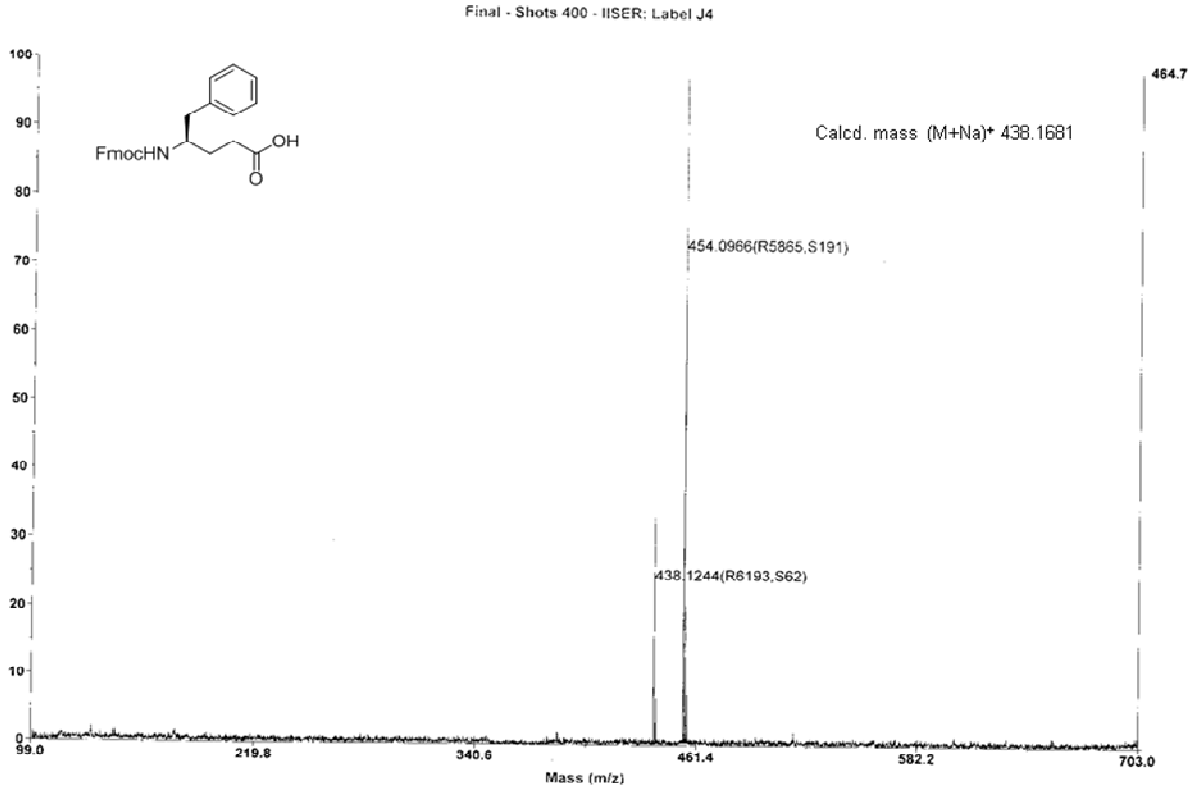

Final - Shots 400 - IISER-; Label K18
Boc
N
H
O
O
Calcd. mass (M+Na)+ 342.1681
625
358.1808(R6025,S2277)
342.2072(R5768,S713)
360.1786(R6321,S122)
100
90
80
70
60
50
40
30
20
10
0
100.0
280.8
461.6
642.4
823.2
1004.0

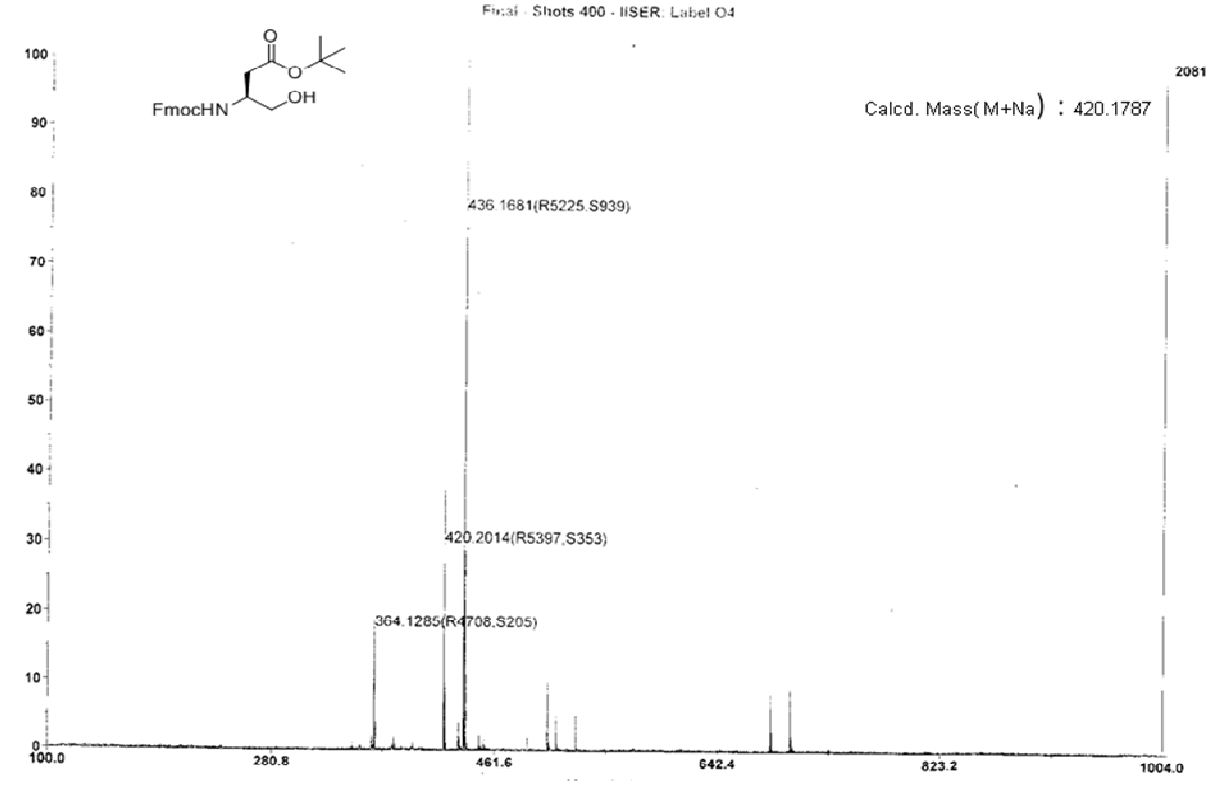

Final - Shots 400 - IISER-; Label K19
NHBoc
Fmoc
N
H
O
O
1046.
Calcd. mass (M+Na)+ 531.2466
547.3092(R6235,S472)
531.3446(R6535,S233)
100
90
80
70
60
50
40
30
20
10
0
100.0
280.8
461.6
642.4
823.2
1004.0
Mass (m/z)

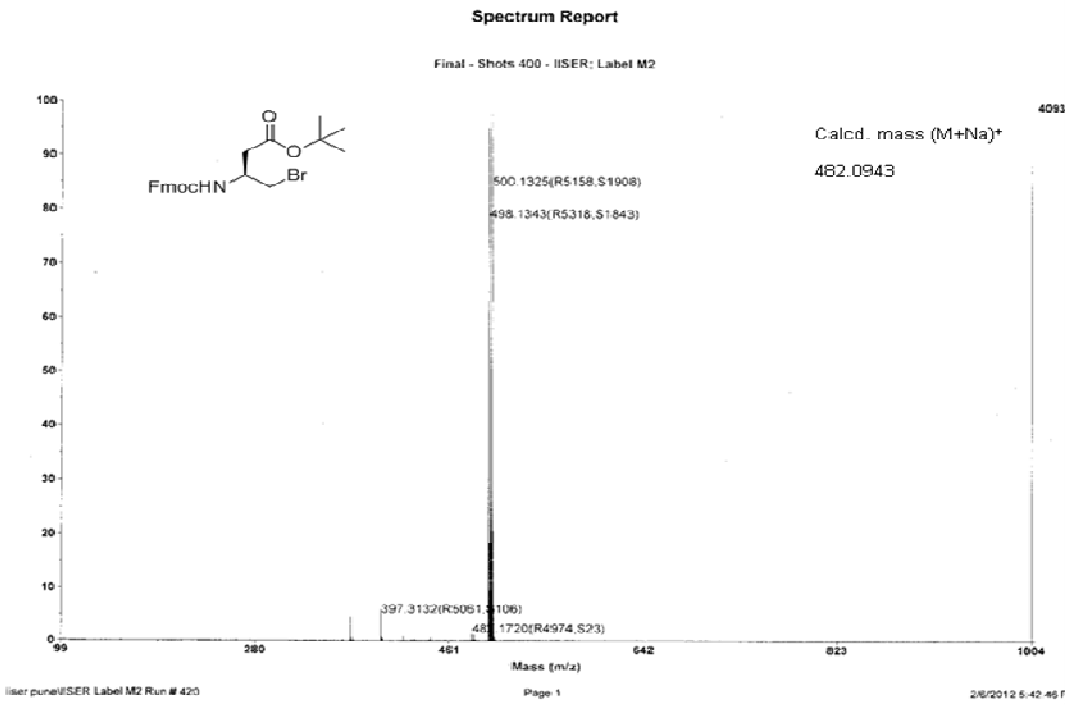

Final - Shots 400 - IISER-; Label K20
FmocHN
OH
O
2463.7
Calcd. mass (M+Na)+ 362.1368
378.1424(R6538,S812)
362.1680(R6467,S318)
100
90
80
70
60
50
40
30
20
10
0
100.0
280.8
461.6
642.4
823.2
1004.0
Mass (m/z)

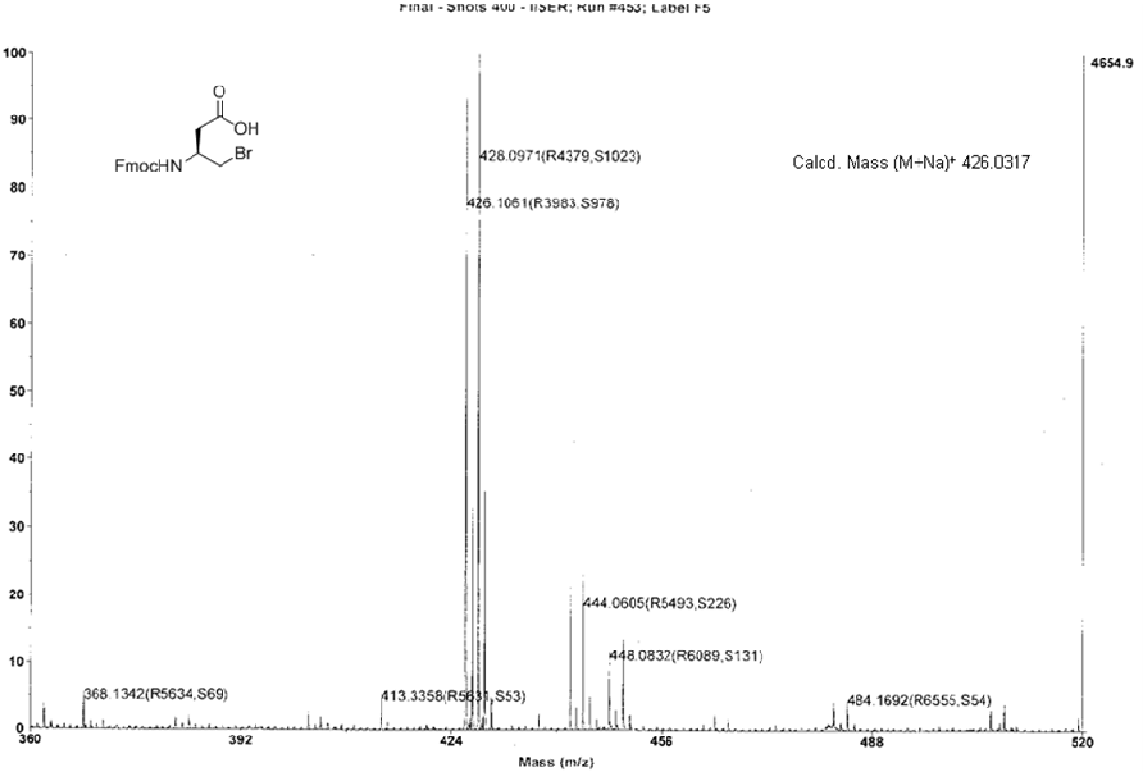

Final - Shots 400 - IISER-; Run #158; Label K15
FmocHN
OH
O
Calcd. mass(M+Na)+ 390.1681
390.2273(R4979,S849)
406.2014(R5541,S460)
1805.5
100
90
80
70
60
50
40
30
20
10
0
100.0
280.8
461.6
642.4
823.2
1004.0
Mass (m/z)


Spectrum Report
Final - Shots 400 - IISER; Label J5
FmocHN
OH
O
Calcd. mass (M+Na)+ 404.1836
420.1135(R5951,S1599)
404.1443(R5745,S612)
3834.3
100
90
80
70
60
50
40
30
20
10
0
99.0
219.8
340.6
461.4
582.2
703.0
Mass (m/z)
iiser puneIISER Label J5 Run # 467
Page 1
3/28/2012 12:35:48 PM


Final - Shots 400 - IISER: Label J4
FmocHN
OH
O
Calcd. mass (M+Na)+ 438.1681
464.7
454.0966(R5865,S191)
438.1244(R6193,S62)
100
90
80
70
60
50
40
30
20
10
0
99.0
219.8
340.6
461.4
582.2
703.0
Mass (m/z)


Final - Shots 400 - IISER: Label O4
FmocHN
OH
O
Calcd. Mass(M+Na) : 420.1787
2081
436.1681(R5225,S939)
420.2014(R5397,S353)
364.1285(R4708,S205)
100
90
80
70
60
50
40
30
20
10
0
100.0
280.8
461.6
642.4
823.2
1004.0


Spectrum Report
Final - Shots 400 - IISER; Label M2
FmocHN
Br
Calcd. mass (M+Na)+
482.0943
500.1325(R5158,S1908)
498.1343(R5318,S1843)
397.3132(R5061,S106)
Mass (m/z)
Page 1


FmocHN
OH
Br
428.0971(R4379,S1023)
426.1061(R3983,S978)
Calcd. Mass (M+Na)+ 426.0317
444.0605(R5493,S226)
448.0832(R6089,S131)
368.1342(R5634,S69)
484.1692(R6555,S54)
Mass (m/z)

RE

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