Supporting Information

Crystal structure analysis of helix-turn-helix type motifs in α,γ-hybrid peptides

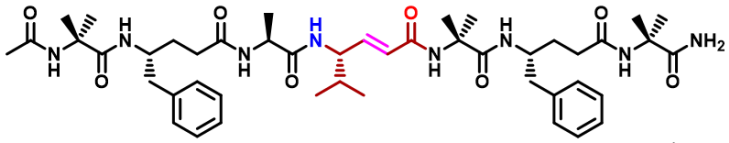
Sachin A. Nalawade, Mothukuri Ganesh Kumar, DRGKoppalu R. Puneeth Kumar, Manjeet Singh, Sanjit Dey, and Hosahudya N. Gopi*

Department of Chemistry, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pashan, Pune-411 008, India; E-mail: hn.gopi@iiserpune.ac.in

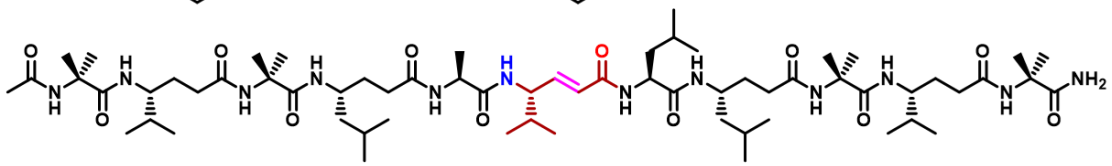
Table of Contents

1) Sequences of Peptides P1-P3

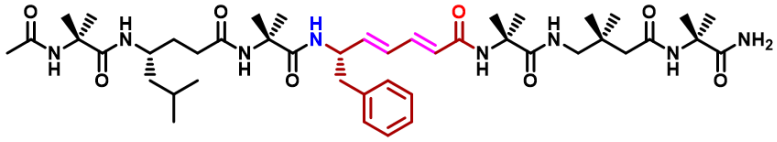
P1



P2



P3



Scheme S1: Sequences of Peptides **P1**, **P2** and **P3**.

2) ORTEP Diagrams of Peptides P1, P2 and P3:

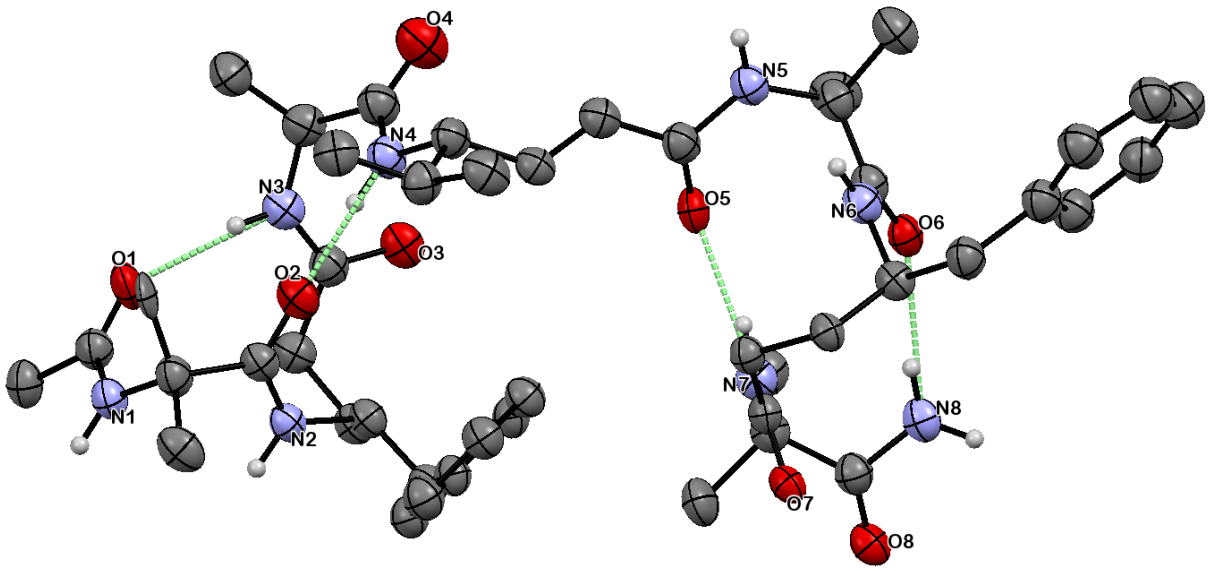


Fig S1: ORTEP diagram of **P1** [Ac-Aib-*γ*Phe-Ala-(*E*)d*γ*Val-Aib-*γ*Phe-Aib-$CONH_2$], with labelled heteroatoms. H-bonding is represented by dotted lines in light green colour. The side-chain and backbone H-atoms are not shown for clarity (except N-H). Ellipsoids are drawn at 50% probability. [(*E*)d*γ*Val = (*E*)-α, β-unsaturated γ-Valine[1]] (**CCDC no : 2226520**)

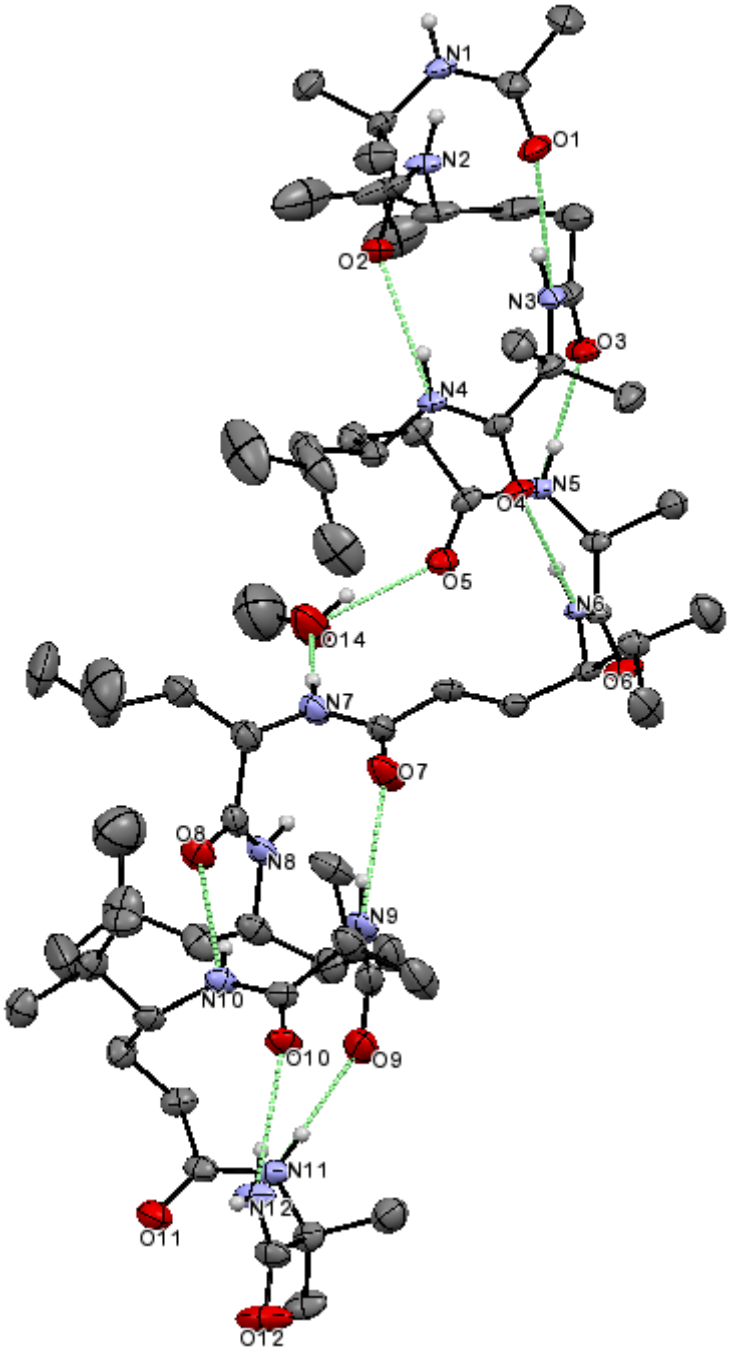


Fig S2: ORTEP diagram of **P2** [Ac-Aib-*γ*Val-Aib-*γ*Leu-Ala-(*E*)d*γ*Val-Leu-*γ*Leu-Aib-*γ*Val-Aib-$CONH_2$], with labelled heteroatoms. H-bonding is represented by dotted lines in light green colour. The side-chain and backbone H-atoms are not shown for clarity (except N-H). Ellipsoids are drawn at 50% probability. [(*E*)d*γ*Val = (*E*)-α, β-unsaturated γ-Valine[1]] (**CCDC no : 2226522**)

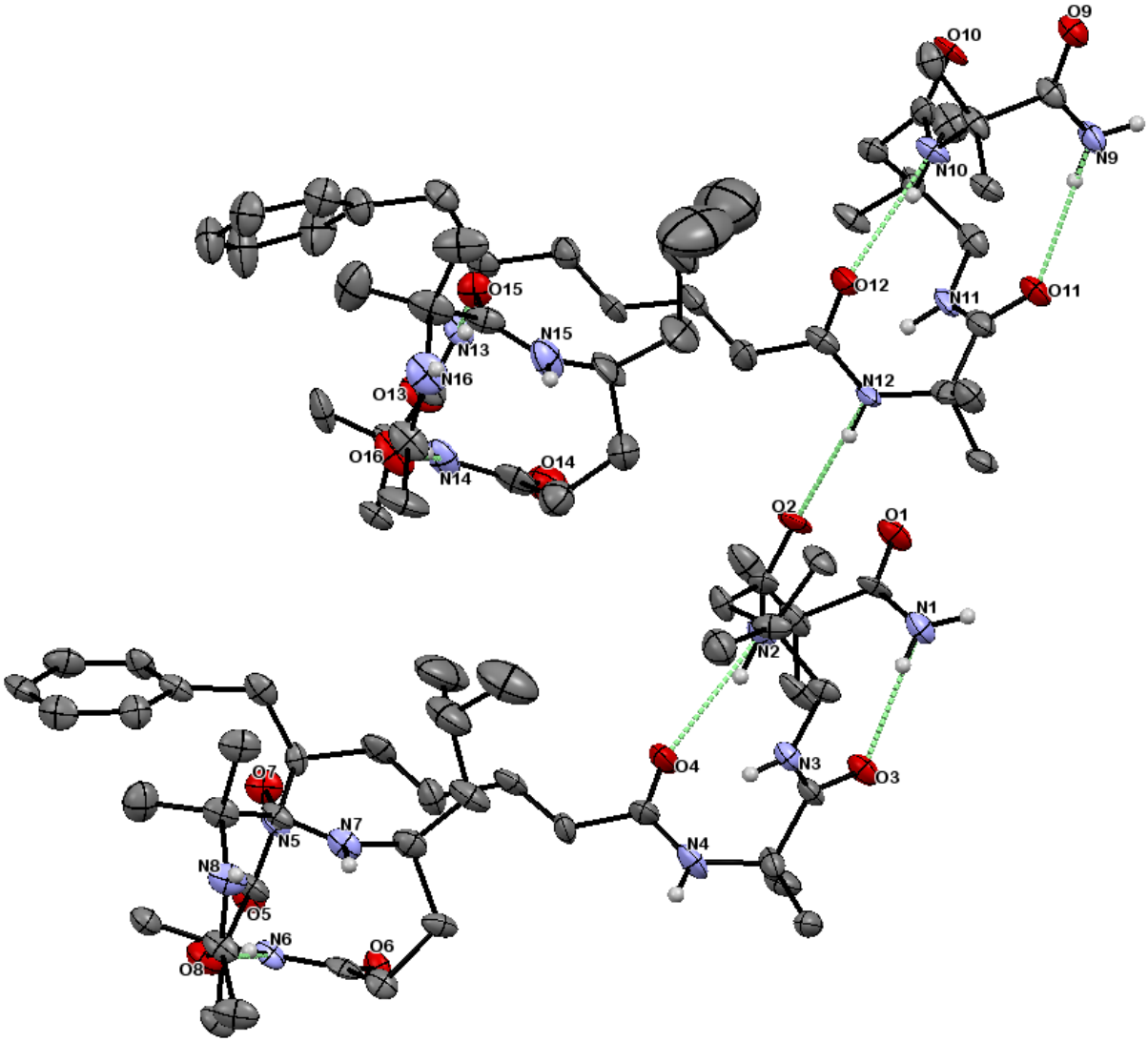


Fig S3: ORTEP diagram of **P3** [Ac-Aib-*γ*Leu-Aib-(*E*,*E*)-dd*ε*Phe-Aib-Adb-Aib-$CONH_2$], with labelled heteroatoms. H-bonding is represented by dotted lines in light green colour. The side-chain and backbone H-atoms are not shown for clarity (except N-H). Ellipsoids are drawn at 50% probability. [(*E*,*E*)dd*ε*Phe = (*E*,*E*)-αβ, γδ-unsaturated ε-Phenylalanine[2]; Adb = 4-amino-3,3-dimethyl-butanoic acid[3]] (**CCDC no : 2226523**)

3) Crystallographic Information of Peptides P1–P3:

Crystal structure analysis of Ac-Aib-*γ*Phe-Ala-(*E*)d*γ*Val-Aib-*γ*Phe-Aib-$CONH_2$ (P1): Colourless rod shape Crystals of **P1** were grown by slow evaporation from an aqueous solution of methanol. A good quality single crystal (0.13 × 0.08 × 0.05mm) was mounted on the head of a goniometer using a loop with a small amount of paraffin oil. The X-ray diffraction data of a single crystal were collected at 100K temperature on a Bruker APEX(II) DUO CCD

diffractometer using Mo Kα radiation (λ = 0.71073 Å), ω-scans (2θ = 39.992), for a total of 17071 independent reflections. Space group C 2, a = 33.21(5), b = 10.160(13), c = 16.17(2), β = 112.22(3), V = 5051(11) Å^3, Monoclinic, Z = 4 for chemical formula C_{46} H_{68} N_8 O_8 with one molecule in asymmetric unit; ρ calcd = 1.132 gcm^{-3}, μ = 0.078 mm^{-1}, F (000) = 1856.0, Rint= 0.2784. The structure was obtained by direct methods using SHELXL-97.[4] The final R value was 0.1038 (wR2 = 0.2341) 4549 observed reflections ($F0 \geq 4\sigma$ (|F0|)) and 571 variables, S = 0.857. The largest difference peak and hole were 0.327 and -0.253 eÅ^3, respectively.

The diffracted single crystal was a small-sized and the quality of diffraction was poor. Several datasets were collected on single crystals from different groups and one of the highest quality is reported herein.

There is some partially occupied solvent molecule also present in the asymmetric unit. A significant amount of time was invested in identifying and refining the disordered molecule. Option SQUEEZE of program PLATON[5] was used to correct the diffraction data for diffuse scattering effects and to remove the contributions of the disordered solvent molecules from the structure factors. PLATON calculated the upper limit of volume that can be occupied by the solvent to be 461 Å^3 of the unit cell volume. The program calculated 148 electrons in the unit cell for the diffuse species. No data are given for the diffusely scattering species. Outputs of the SQUEEZE report are appended in CIF file **P1**.

Crystal structure analysis of Ac-Aib-γVal-Aib-γLeu-Ala-(*E*)dγVal-Leu-γLeu-Aib-γVal-Aib-CONH$_2$ (P2): Colourless plate shape Crystals of **P2** were grown by slow evaporation from the aqueous solution of methanol. A good quality single crystal (0.25 × 0.12 × 0.1 mm) was mounted on the head of a goniometer using a loop with a small amount of paraffin oil. The X-ray diffraction data of a single crystal were collected at 100K temperature on a Bruker APEX(II) DUO CCD diffractometer using Mo Kα radiation (λ = 0.71073 Å), ω-scans (2θ = 56.65), for a total of 43572 independent reflections. Space group P 21, a = 14.637(8), b = 19.378(9), c = 16.197(7), β = 104.947(16), V = 4439(4) Å^3, Monoclinic, Z = 2 for chemical formula C_{64} H_{116} N_{12} O_{12}, C H_4 O with one methanol molecule in asymmetric unit; ρ calcd = 0.956 gcm^{-3}, μ = 0.067mm^{-1}, F (000) = 1396, Rint= 0.0384. The crystal structure was solved by direct methods using SHELXL-97.[4] The final R value was 0.0531 (wR2 = 0.1158) 21737 observed reflections ($F0 \geq 4\sigma$ (|F0|)) and 837 variables, S = 1.053. The largest difference peak and hole were 0.442 and -0.275 eÅ^3, respectively.

There is some partially occupied solvent molecule also present in the asymmetric unit. A significant amount of time was invested in identifying and refining the disordered molecule. The solvent masks option in program Olex2-1.2[6] serves as an alternative to SQUEEZE which is implemented in PLATON[5]. The solvent masks option in program Olex2-1.2[6] was used to correct the diffraction data for diffuse scattering effects and to remove the contributions of the disordered solvent molecules from the structure factors. The Olex2-solvent mask calculated the upper limit of volume that can be occupied by the solvent to be 1193 $Å^3$, or 26.9% of the unit cell volume. The program calculated 159 electrons in the unit cell for the diffuse species. No data are given for the diffusely scattering species. Outputs of the Olex2-solvent mask (SQUEEZE) report are appended in CIF file **P2**.

Crystal structure analysis of Ac-Aib-*γ*Leu-Aib-(*E*, *E*)dd*γ*Phe-Aib-Adb-Aib-$CONH_2$ (P3): Colourless needle shape Crystals of **P3** were grown by slow evaporation from the aqueous solution of methanol. A good quality single crystal (0.12 × 0.09 × 0.07 mm) was mounted on the head of a goniometer using a loop with a small amount of paraffin oil. The X-ray diffraction data of a single crystal were collected at 100K temperature on a Bruker APEX(II) DUO CCD diffractometer using Mo Kα radiation (λ = 0.71073 Å), *ω*-scans (2*θ* = 56.666), for a total of 66655 independent reflections. Space group P 21, a = 17.73(10), b = 10.52(6), c = 28.85(16), *β* = 99.42(8), V = 5309(50) $Å^3$, Monoclinic, Z = 4 for chemical formula C_{45} H_{72} N_8 O_8 with two molecule in asymmetric unit; *ρ* calcd = 1.067 gcm^{-3}, *μ* = 0.074 mm^{-1}, F (000) = 1848, Rint= 0.1393. The structure was obtained by direct methods using SHELXL-97.[4] The final R value was 0.1070 (wR2 = 0.2415) 22799 observed reflections ($F0 \geq 4\sigma$ (|F0|)) and 1109 variables, S = 1.028. The largest difference peak and hole were 0.949 and -0.386 $eÅ^3$, respectively.

The diffracted single crystal was a small-sized and the quality of diffraction was poor. Several datasets were collected on single crystals from different groups and one of the highest quality is reported herein.

There is some partially occupied solvent molecule also present in the asymmetric unit. A significant amount of time was invested in identifying and refining the disordered molecule. Option SQUEEZE of program PLATON[5] was used to correct the diffraction data for diffuse scattering effects and to remove the contributions of the disordered solvent molecules from the structure factors. PLATON calculated the upper limit of volume that can be occupied by the solvent to be 732 $Å^3$ of the unit cell volume. The program calculated 203 electrons in

the unit cell for the diffuse species. No data are given for the diffusely scattering species. Outputs of the SQUEEZE report are appended in CIF file **P3**.

4) Backbone Torsional Angles and H-bond Parameters of Peptides P1-P3:

Table S1: Backbone Torsional angle variables (in deg) for peptide P1

Torsion points	Torsion angle [°]	Angle of Amino acid	Amino Acids
C(1)-C(2)-N(1)-C(3)	-179(2)	-	$NH\text{-}COCH_3$
C(2)-N(1)-C(3)-C(6)	-54(3)	ϕ	**Aib1**
N(1)-C(3)-C(6)-N(2)	-53(3)	Ψ	
C(3)-C(6)-N(2)-C(7)	178(2)	ω	
C(6)-N(2)-C(7)-C(15)	-119(3)	ϕ	**γPhe (2)**
N(2)-C(7)-C(15)-C(16)	58(3)	θ_1	
C(7)-C(15)-C(16)-C(17)	61(3)	θ_2	
C(15)-C(16)-C(17)-N(3)	-128(3)	Ψ	
C(16)-C(17)-N(3)-C(18)	-171(3)	ω	
C(17)-N(3)-C(18)-C(20)	-70(3)	ϕ	**Ala (3)**
N(3)-C(18)-C(20)-N(4)	-34(3)	Ψ	
C(18)-C(20)-N(4)-C(21)	-172(2)	ω	
C(20)-N(4)-C(21)-C(25)	**-81(3)**	ϕ	**dγVal (4)**
N(4)-C(21)-C(25)-C(26)	**122(3)**	θ_1	
C(21)-C(25)-C(26)-C(27)	**-176(2)**	θ_2	
C(25)-C(26)-C(27)-N(5)	**-169(3)**	Ψ	
C(26)-C(27)-N(5)-C(28)	**-178(2)**	ω	
C(27)-N(5)-C(28)-C(31)	-53(3)	ϕ	**Aib (5)**
N(5)-C(28)-C(31)-N(6)	-49(3)	Ψ	
C(28)-C(31)-N(6)-C(32)	-178(2)	ω	
C(31)-N(6)-C(32)-C(40)	-125(2)	ϕ	**γPhe (6)**
N(6)-C(32)-C(40)-C(41)	56(3)	θ_1	
C(32)-C(40)-C(41)-C(42)	66(3)	θ_2	
C(40)-C(41)-C(42)-N(7)	-111(3)	Ψ	
C(41)-C(42)-N(7)-C(43)	-179(2)	ω	

C(42)-N(7)-C(43)-C(46)	-55(3)	ϕ	**Aib (7)**
N(7)-C(43)-C(46)-N(8)	-43(3)	Ψ	

Table S2: Hydrogen bonding Parameters of Peptide **P1**

Intra-molecular H-bonds of peptide **P1**				
D-H.....A	**d(D-H)**	**d(H.....A) (Å)**	**d(D.....A) (Å)**	**<(N-H.....O) (°)**
N(3)-H(3A)**.....**O(1)	0.88	2.04	2.91(3)	169
N(4)-H(4)**.....**O(2)	0.88	1.85	2.70(3)	163
N(7)-H(7)**.....**O(5)	0.88	1.98	2.85(3)	170
N(8)-H(8B)**.....**O(6)	0.88	2.11	2.97(3)	166
Intermolecular H-bonds of peptide **P1**				
N(1)-H(1)**.....**O(8)#	0.88	2.01	2.85(3)	160
N(8)-H(8A)**.....**O(6)*	0.88	2.12	2.87(3)	142

[D = Donor, A = Acceptor, H = Hydrogen, d = distance in Å, < = angle in (°)]

Symmetry operations used to generate equivalent atoms:

-x+1/2,y+1/2,-z+1 * -x+1,y,-z+1

Table S3: Backbone Torsional angle variables (in deg) for peptide **P2**

Torsion points	**Torsion angle [°]**	**Angle of Amino acid**	**Amino Acids**
C(1)-C(2)-N(1)-C(4)	-175.4(3)	-	NH-$COCH_3$
C(2)-N(1)-C(4)-C(6)	-59.4(4)	ϕ	**Aib1**
N(1)-C(4)-C(6)-N(2)	-38.9(3)	Ψ	
C(4)-C(6)-N(2)-C(10)	-174.6(3)	ω	
C(6)-N(2)-C(10)-C(11)	-123.9(3)	ϕ	
N(2)-C(10)-C(11)-C(12)	48.0(3)	θ_1	
C(10)-C(11)-C(12)-C(13)	65.2(3)	θ_2	

C(11)-C(12)-C(13)-N(3)	-117.6(3)	Ψ	**γVal (2)**
C(12)-C(13)-N(3)-C(14)	-174.4(3)	ω	
C(13)-N(3)-C(14)-C(17)	-57.9(4)	ϕ	**Aib (3)**
N(3)-C(14)-C(17)-N(4)	-45.1(3)	Ψ	
C(14)-C(17)-N(4)-C(18)	-172.9(2)	ω	
C(17)-N(4)-C(18)-C(23)	-119.1(3)	ϕ	**γLeu (4)**
N(4)-C(18)-C(23)-C(24)	49.2(3)	θ_1	
C(18)-C(23)-C(24)-C(25)	61.5(4)	θ_2	
C(23)-C(24)-C(25)-N(5)	-129.0(3)	Ψ	
C(24)-C(25)-N(5)-C(26)	-164.4(2)	ω	
C(25)-N(5)-C(26)-C(28)	-68.8(3)	ϕ	**Ala (5)**
N(5)-C(26)-C(28)-N(6)	-30.0(4)	Ψ	
C(26)-C(28)-N(6)-C(29)	-170.1(2)	ω	
C(28)-N(6)-C(29)-C(33)	**-110.1(3)**	ϕ	**dγVal(6)**
N(6)-C(29)-C(33)-C(34)	**5.3(4)**	θ_1	
C(29)-C(33)-C(34)-C(35)	**-177.4(3)**	θ_2	
C(33)-C(34)-C(35)-N(7)	**179.0(3)**	Ψ	
C(34)-C(35)-N(7)-C(36)	**-171.2(3)**	ω	
C(35)-N(7)-C(36)-C(41)	-70.7(4)	ϕ	**Leu (7)**
N(7)-C(36)-C(41)-N(8)	-29.9(4)	Ψ	
C(36)-C(41)-N(8)- C(42)	-178.8(3)	ω	
C(41)-N(8)-C(42)-C(47)	-125.9(3)	ϕ	**γLeu (8)**
N(8)-C(42)-C(47)-C(48)	49.5(4)	θ_1	
C(42)-C(47)-C(48)-C(49)	60.0(4)	θ_2	
C(47)-C(48)-C(49)-N(9)	-114.4(4)	Ψ	
C(48)-C(49)-N(9)-C(50)	-173.9(3)	ω	
C(49)-N(9)-C(50)-C(53)	-54.9(4)	ϕ	**Aib (9)**
N(9)-C(50)-C(53)-N(10)	-41.4(4)	Ψ	
C(50)-C(53)-N(10)-C(54)	-171.5(3)	ω	
C(53)-N(10)-C(54)-C(58)	-127.8(3)	ϕ	
N(10)-C(54)-C(58)-C(59)	52.8(3)	θ_1	
C(54)-C(58)-C(59)-C(60)	61.0(3)	θ_2	

C(58)-C(59)-C(60)-N(11)	-120.3(3)	Ψ	**γVal (10)**
C(59)-C(60)-N(11)-C(62)	-172.2(3)	ω	
C(60)-N(11)-C(62)-C(64)	-57.1(4)	ϕ	**Aib (11)**
N(11)-C(62)-C(64)-N(12)	-42.0(4)	Ψ	

Table S4: Hydrogen bonding Parameters of Peptide **P2**

Intra-molecular H-bonds of peptide **P2**				
D-H.....A	**d(D-H)**	**d(H.....A) (Å)**	**d(D.....A) (Å)**	**<(N-H.....O) (°)**
N(3)-H(3)**.....**O(1)	0.88	2.05	2.911(3)	165
N(4)-H(4)**.....**O(2)	0.88	2.04	2.902(3)	167
N(5)-H(5)**.....**O(3)	0.88	1.93	2.807(3)	175
N(6)-H(6)**.....**O(4)	0.88	2.03	2.872(3)	160
N(9)-H(9)**.....**O(7)	0.88	2.01	2.881(3)	168
N(10)-H(10A)**.....**O(8)	0.88	2.05	2.875(3)	156
N(11)-H(11)**.....**O(9)	0.88	2.04	2.918(3)	172
N(12)-H(12D)**.....**O(10)	0.88	1.99	2.850(3)	166
Intermolecular H-bonds of peptide **P2**				
N(1)-H(1)**.....**O(6)#	0.88	2.01	2.875(3)	169
N(2)-H(2)**.....**O(12)*	0.88	2.07	2.870(3)	151
N(12)-H(12C)**.....**O(6)$	0.88	2.12	2.993(3)	171

Symmetry operations used to generate equivalent atoms: # x+1,y,z * x+1,y,z-1 $ x,y,z+1

Table S5: Backbone Torsional angle variables (in deg) for peptide **P3**

Backbone Torsional angle of Peptide **P3** (**Molecule 1**)			
Torsion points (**Molecule 1**)	**Torsion angle [°]** (**Molecule 1**)	**Angle of Amino acid**	**Amino Acids** (**Molecule 1**)
N(1)-C(1)-C(2)-N(2)	52.3(9)	Ψ	
C(1)-C(2)-N(2)-C(5)	48.6(10)	ϕ	**Aib7**
C(2)-N(2)-C(5)-C(6)	175.4(6)	ω	

N(2)-C(5)-C(6)-C(7)	105.3(8)	Ψ	**Adb (6)**
C(5)-C(6)-C(7)-C(10)	-56.4(10)	θ_2	
C(6)-C(7)-C(10)-N(3)	-54.2(9)	θ_1	
C(7)-C(10)-N(3)-C(11)	129.7(8)	ϕ	
C(10)-N(3)-C(11)-C(12)	175.7(7)	ω	**Aib (5)**
N(3)-C(11)-C(12)-N(4)	33.9(10)	Ψ	
C(11)-C(12)-N(4)-C(15)	61.6(11)	ϕ	
C(12)-N(4)-C(15)-C(16)	**168.3(7)**	ω	**(*E*,*E*)ddεPhe (4)**
N(4)-C(15)-C(16)-C(17)	**179.3(8)**	Ψ	
C(15)-C(16)-C(17)-C(18)	**178.5(8)**	θ_4	
C(16)-C(17)-C(18)-C(19)	**-179.8(8)**	θ_3	
C(17)-C(18)-C(19)-C(20)	**178.8(8)**	θ_2	
C(18)-C(19)-C(20)-N(5)	**3.3(12)**	θ_1	
C(19)-C(20)-N(5)-C(28)	**-101.7(8)**	ϕ	
C(20)-N(5)-C(28)-C(29)	-172.1(6)	ω	**Aib (3)**
N(5)-C(28)-C(29)-N(6)	-42.5(10)	Ψ	
C(28)-C(29)-N(6)-C(32)	-55.0(9)	ϕ	
C(29)-N(6)-C(32)-C(33)	-173.0(6)	ω	**γLeu(2)**
N(6)-C(32)-C(33)-C(34)	-116.6(7)	Ψ	
C(32)-C(33)-C(34)-C(35)	63.3(9)	θ_2	
C(33)-C(34)-C(35)-N(7)	46.5(9)	θ_1	
C(34)-C(35)-N(7)-C(40)	-123.8(7)	ϕ	
C(35)-N(7)-C(40)-C(41)	-171.5(6)	ω	**Aib(1)**
N(7)-C(40)-C(41)-N(8)	-37.9(8)	Ψ	
C(40)-C(41)-N(8)-C(44)	-56.7(9)	ϕ	
C(41)-N(8)-C(44)-C(45)	173.9(7)	-	NH-$COCH_3$

Backbone Torsional angle of Peptide **P3** (**Molecule 2**)

Torsion points (Molecule 2)	**Torsion angle [°] (Molecule 2)**	**Angle of Amino acid**	**Amino Acids** (**Molecule 2**)
N(9)-C(46)-C(47)-N(10)	-51.0(9)	Ψ	**Aib7**
C(46)-C(47)-N(10)-C(50)	-49.6(10)	ϕ	

C(47)-N(10)-C(50)-C(51)	-176.0(6)	ω	**Adb (6)**
N(10)-C(50)-C(51)-C(52)	-108.6(8)	Ψ	
C(50)-C(51)-C(52)-C(55)	58.8(9)	θ_2	
C(51)-C(52)-C(55)-N(11)	56.9(8)	θ_1	
C(52)-C(55)-N(11)-C(56)	-129.9(8)	ϕ	
C(55)-N(11)-C(56)-C(57)	-176.7(7)	ω	**Aib (5)**
N(11)-C(56)-C(57)-N(12)	-35.4(9)	Ψ	
C(56)-C(57)-N(12)-C(60)	-57.2(11)	ϕ	
C(57)-N(12)-C(60)-C(61)	**-177.9(7)**	ω	**(*E*,*E*)dd*ε*Phe (4)**
N(12)-C(60)-C(61)-C(62)	**-160.4(9)**	Ψ	
C(60)-C(61)-C(62)-C(63)	**-178.3(9)**	θ_4	
C(61)-C(62)-C(63)-C(64)	**-174.9(10)**	θ_3	
C(62)-C(63)-C(64)-C(65)	**-176.1(8)**	θ_2	
C(63)-C(64)-C(65)-N(13)	**28.6(11)**	θ_1	
C(64)-C(65)-N(13)-C(73)	**-115.2(8)**	ϕ	
C(65)-N(13)-C(73)-C(74)	-166.9(7)	ω	**Aib (3)**
N(13)-C(73)-C(74)-N(14)	-46.5(10)	Ψ	
C(73)-C(74)-N(14)-C(77)	-52.0(11)	ϕ	
C(74)-N(14)-C(77)-C(78)	-175.0(7)	ω	**γLeu(2)**
N(14)-C(77)-C(78)-C(79)	-120.6(9)	Ψ	
C(77)-C(78)-C(79)-C(80)	67.8(12)	θ_2	
C(78)-C(79)-C(80)-N(15)	43.0(12)	θ_1	
C(79)-C(80)-N(15)-C(85)	-119.8(9)	ϕ	
C(80)-N(15)-C(85)-C(86)	-172.9(7)	ω	**Aib(1)**
N(15)-C(85)-C(86)-N(16)	-38.6(10)	Ψ	
C(85)-C(86)-N(16)-C(89)	-55.2(11)	ϕ	
C(86)-N(16)-C(89)-C(90)	-177.3(8)	-	$NH\text{-}COCH_3$

[(*E*,*E*)dd*ε*Phe = (*E*,*E*)-αβ, γδ-unsaturated ε-Phenylalanine[2]; Adb = 4-amino-3,3-dimethylbutanoic acid[3]]

Table S6: Hydrogen bonding Parameters of Peptide **P3**

Intra-molecular H-bonds of peptide **P3 (Molecule 1)**				
D-H.....A	**d(D-H)**	**d(H.....A) (Å)**	**d(D.....A) (Å)**	**<(N-H.....O) (°)**
N(6)-H(6)**.....**O(8)	0.88	2.00	2.876(15)	172
N(5)-H(5)**.....**O(7)	0.88	2.12	2.962(18)	161
N(2)-H(2)**.....**O(4)	0.88	2.03	2.885(16)	162
N(1)-H(1B)**.....**O(3)	0.88	2.16	3.024(17)	168
Intermolecular H-bonds of peptide **P3 (Molecule 1)**				
N(8)-H(8)**.....**O(5)$	0.88	2.17	3.006(17)	158
N(4)-H(4)**.....**O(10)*	0.88	2.04	2.918(14)	177
N(1)-H(1A)**.....**O(9)#	0.88	2.16	3.012(14)	162
N(12)-H(12)**.....**O(2)	0.88	2.05	2.928(14)	175
N(9)-H(9A)**.....**O(1)@	0.88	2.10	2.959(14)	166

Symmetry operations used to generate equivalent atoms: # -x,y+1/2,-z+2 * x+1,y,z

$ x,y-1,z @ -x,y-1/2,-z+2

Intra-molecular H-bonds of peptide **P3 (Molecule 2)**				
N(14)-H(14)**.....**O(16)#	0.88	1.99	2.867(16)	172
N(13)-H(13)**.....**O(15)*	0.88	2.08	2.903(17)	156
N(10)-H(10)**.....**O(12)$	0.88	1.99	2.854(16)	166
N(9)-H(9B)**.....**O(11)$	0.88	2.15	3.024(17)	172
Intermolecular H-bonds of peptide **P3 (Molecule 2)**				
N(16)-H(16)**.....**O(13)$	0.88	2.01	2.873(17)	167
N(12)-H(12)**.....**O(2)	0.88	2.05	2.928(14)	175
N(9)-H(9A)**.....**O(1)@	0.88	2.10	2.959(14)	166
N(4)-H(4)**.....**O(10)*	0.88	2.04	2.918(14)	177
N(1)-H(1A)**.....**O(9)#	0.88	2.16	3.012(14)	162

Symmetry operations used to generate equivalent atoms: # -x,y+1/2,-z+2 * x+1,y,z

$ x,y-1,z @ -x,y-1/2,-z+2

5) Materials, Methods, and General Experimental Details:

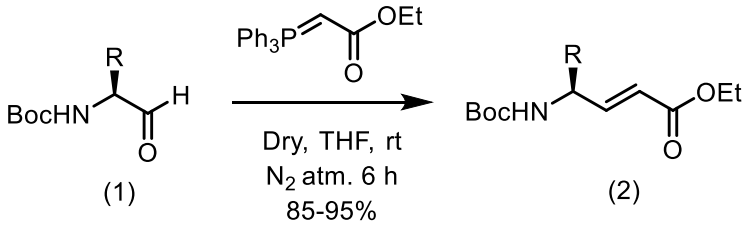
All amino acids, Di-tert-butyl dicarbonate, N,O-Dimethylhydroxylamine hydrochloride, DCC, HOBt, DIPEA, $LiAlH_4$, PPh_3, Ethyl bromoacetate, Pd/C (Palladium on carbon), Sodium hydroxide (NaOH), FMOC-Succinimide (FMOC-OSu), DIBAL-H, BF_3OEt_2, 2-iodoxybenzoic acid (IBX), Acetic anhydride, Knorr-Amide resin, MeOH, THF, DCM, and Ethyl acetate, were purchased from the commercial sources. THF was dried over sodium metal and distilled before use. DCM was also dried using CaH_2 and distilled under an inert atmosphere before use. MeOH, Pet-ether, and Ethyl acetate were distilled before use. Column chromatography was performed on silica gel (230-400 mesh). Final peptides were purified on reverse phase HPLC (C-18 column, MeOH/H2O 70:30-95:5 as gradient with flow rate 2.00 mL/min). ^{1}H NMR (1D and 2D NMR) spectra were recorded on 600 MHz, 500 MHz, and 400 MHz (or ^{13}C on 100 MHz) using residual solvents as internal standards (CD_3OH*δH* 3.31 *ppm*, *δC* 49.0 *ppm*, $CDCl_3$ *δH* 7.26 *ppm*, *δC* 77.3 *ppm*). The chemical shifts (*δ*) and coupling constants were reported in ppm and Hz respectively. The mass of all pure peptides was confirmed by Matrix Assisted LASER Desorption Ionization mass spectrometer (MALDI-TOF/TOF). Single crystal XRD data for peptide structure determination were collected from an X-ray diffractometer using Mo-Kα (λ = 0.71073 Å) graphite monochromated radiation.

6) Procedures for Building Blocks Synthesis and its Characterization

A) Synthesis of Fmoc-NH-(*E*)-α, β-Unsaturated γ-amino acids (4):

Fmoc-NH-(*E*)-α, β-Unsaturated γ-amino acids were synthesized by following three steps (I – III) :

I) Synthesis of Boc-NH-(*E*)-α, β-Unsaturated γ-amino ethyl esters (2): Boc-NH-(*E*)-α, β-Unsaturated γ-amino ethyl esters (2) were synthesized using our reported protocol.**[1]** briefly; boc-amino aldehyde (1) (10 mmol) was dissolved in dry THF (180 ml) under N_2 atmosphere. Then Wittig ylide (PPh_3=$CHCO_2Et$) (36 mmol) was added to the above solution. The reaction mixture was stirred for 6 h at room temperature. The progress of the reaction was monitored by using TLC. Upon completion of the reaction, THF was evaporated from the reaction mixture, and the product Boc-NH-(*E*)-α, β-unsaturated γ-amino ethyl ester (2) was purified by column chromatography using ethyl acetate in pet ether (5%) solvent system.

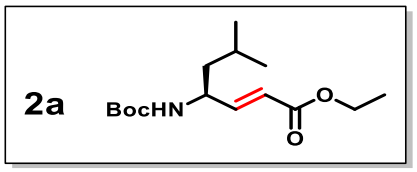


1a) Boc-Leu-CHO = R = *i*-Bu
1b) Boc-Val-CHO = R = *i*-Pr
1c) Boc-Phe-CHO = R = Phenyl

2a) Boc-(E)-dγ-Leu-OEt = R = *i*-Bu
2b) Boc-(E)-dγ-Val-OEt = R = *i*-Pr
2c) Boc-(E)-dγ-Phe-OEt = R = Phenyl

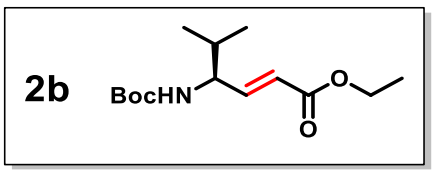
Scheme S2: Synthesis of Boc-NH-(*E*)-α, β-Unsaturated γ-amino ethyl esters (2).[1]

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



^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.81 (dd, *J* = 15.6, 5.4 Hz, 1H, βC*H*), 5.90 (d, *J* = 15.5 Hz, 1H, αC*H*), 4.48 (d, *J* = 8.8 Hz, 1H, N*H*), 4.36 – 4.28 (m, 1H, γC*H*), 4.18 (q, *J* = 7.1 Hz, 2H, -O-CH_2-), 1.71 – 1.64 (m, 1H, -εC*H*-$(CH_3)_2$), 1.43 (s, 9H, *Boc*), 1.37 (t, *J* = 7.4 Hz, 2H, -δCH_2-CH-$(CH_3)_2$), 1.27 (t, *J* = 7.1 Hz, 3H,-O-CH_2-CH_3), 0.92 (d, 6H, -CH-(ζCH_3)$_2$). **^{13}C NMR** (101 MHz, CHLOROFORM-*D*) δ 166.56, 155.22, 149.04, 120.53, 79.81, 77.47, 77.15, 76.83, 60.55, 49.92, 43.95, 28.47, 24.83, 22.83, 14.34. **HRMS** (ESI-QTOF) m/z calculated value for $C_{15}H_{27}NO_4$ is [M+Na] 308.1837 and observed 308.1845.

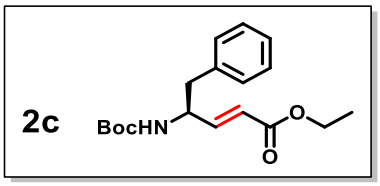
ethyl (*S*,*E*)-4-((tert-butoxycarbonyl)amino)-5-methylhex-2-enoate (2b):



^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.85 (dd, *J* = 15.6, 5.5 Hz, 1H, βC*H*), 5.91 (dd, *J* = 15.7, 1.7 Hz, 1H, αC*H*), 4.56 (d, *J* = 9.2 Hz, 1H, N*H*), 4.24 – 4.12 (m, 3H, γC*H* & -O-CH_2-), 1.91 – 1.81 (m, 1H, -δC*H*-$(CH_3)_2$), 1.44 (s, 9H, *Boc*), 1.28 (t, *J* = 7.1 Hz, 3H, -O-CH_2-CH_3), 0.94 (d, *J* = 6.7 Hz, 3H, -CH-(εCH_3)$_2$, 0.91 (d, *J* = 6.9 Hz, 3H, -CH-(εCH_3)$_2$). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 166.43, 155.51, 147.51, 121.64, 79.85, 77.48, 77.16, 76.84, 60.58, 56.82, 32.40,

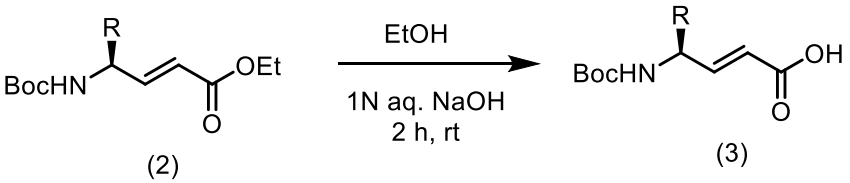
28.50, 19.00, 18.13, 14.38. **HRMS** (ESI-QTOF) m/z calculated value for $C_{14}H_{25}NO_4$ is [M+Na] 294.1681 and observed 294.1663.

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



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.31 – 7.27 (m, 2H, Aromatic ortho), 7.25 – 7.15 (m, 3H, Aromatic meta & para), 6.90 (dd, *J* = 15.7, 4.8 Hz, 1H, βC*H*), 5.85 (dd, *J* = 15.7, 1.6 Hz, 1H, αC*H*), 4.61 (s, 1H, N*H*), 4.55 (s, 1H, γC*H*), 4.17 (d, *J* = 7.1 Hz, 2H, -O-C*H*$_2$-CH$_3$), 2.93 – 2.84 (m, 2H, *Benzylic*), 1.39 (s, 9H, *Boc*), 1.27 (t, *J* = 7.1 Hz, 3H, -O-CH$_2$-C*H*$_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.17, 154.96, 147.59, 136.40, 129.40, 128.57, 126.86, 121.10, 79.86, 77.36, 77.04, 76.73, 60.46, 52.28, 40.88, 28.30, 14.22. **HRMS** (ESI-QTOF) m/z calculated value for $C_{18}H_{25}NO_4$ is [M+Na] 342.1681 and observed 342.1688.

II) Ethyl ester hydrolysis reaction: Purified Boc-NH-(*E*)-α, β-Unsaturated γ-amino ethyl ester (2) (8 mmol) was dissolved in 10 mL of EtOH and then 10 ml of 1N aq. NaOH solution was added to the reaction mixture. The reaction mixture was stirred at that temperature for 3 h. The progress of the reaction was monitored by TLC. After completion of the ethyl ester hydrolysis reaction, EtOH was removed under a *vacuum*. The residue was acidified with 5 % aq. HCl solution (25 ml). The hydrolyzed acid product (3) was extracted with ethyl acetate (40 x 3). The extracted ethyl acetate solution was washed with brine (150 ml) solution. The organic layer was then dried over anhydrous Na_2SO_4 and the product was concentrated under reduced pressure.



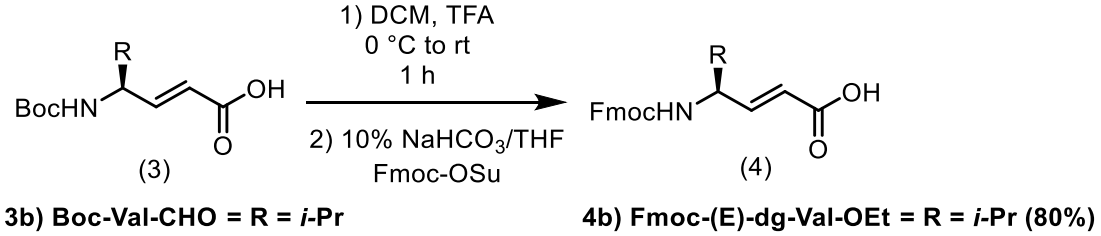
(2) (3)

2a) Boc-(E)-dg-Leu-OEt = R = *i*-Bu
2b) Boc-(E)-dg-Val-OEt = R = *i*-Pr
2c) Boc-(E)-dγ-Phe-OEt = R = Phenyl

3a) Boc-(E)-dg-Leu-OH = R = *i*-Bu
3b) Boc-(E)-dg-Val-OH = R = *i*-Pr
3c) Boc-(E)-dγ-Phe-OH = R = Phenyl

Scheme S3: Synthesis of Boc-NH-(*E*)-α, β-Unsaturated γ-amino acids (3).[1]

III) Boc deprotection and Fmoc protection reaction: The Boc-NH-(*E*)-α, β-Unsaturated γ-amino acid (3) (8 mmol) was dissolved in 5 mL of DCM and then 12 mL of TFA was added to the reaction mixture at 0 °C. After 1 h, TFA was removed under a *vacuum*. The residue was dissolved in 22 mL of 10% $NaHCO_3$ (adjusts pH to ~8). The solution of Fmoc-OSu (8 mmol) in 30 mL of THF was added slowly to the reaction mixture and stirred overnight at room temperature. After completion of the reaction, the reaction mixture was acidified with 5% aq. HCl solution and extracted with ethyl acetate (40 x 3). The extracted ethyl acetate solution was washed with brine (150 ml) solution. The organic layer was then dried over anhydrous Na_2SO_4 and the product was concentrated under reduced pressure to give the white solid product of Fmoc-(*E*)-α, β- Unsaturated γ-amino acids (4) in 78-80% yields, which were directly used for solid-phase peptide synthesis without further purifications.



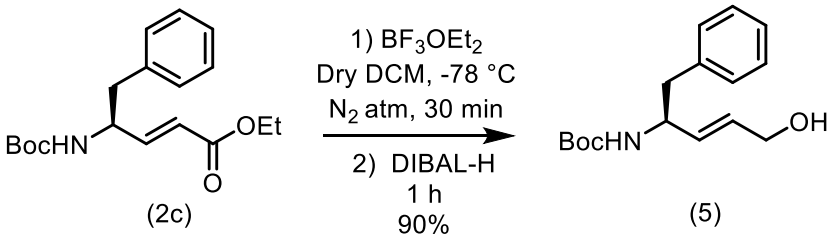
Scheme S4: Synthesis of Fmoc-NH-(*E*)-α, β-Unsaturated γ-amino acids (4).

B) Synthesis of Fmoc-NH-(*E,E*)-αβ, γδ-unsaturated ε-Phenylalanine[2] (9):

Fmoc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-Phenylalanine amino acid (9) were synthesized using our reported protocol[2]. Briefly, synthesis is shown in following five steps (I – V), starting from the above synthesized Boc-(*E*)-dγ-Phe-OEt (2c).

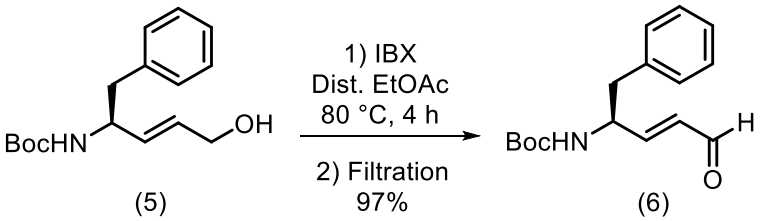
I) Procedure for the Synthesis of Boc-Amino-Phenylalanine-Allylic Alcohol[2] (5): Boc-NH-(*E*)-α,β-Unsaturated γ-phenylalanine ethyl ester (2c) (10 mmol) was dissolved in dry DCM (50 ml) under N_2 atmosphere, cooled to -78 °C then BF_3.OEt_2 (11.5 mmol) was added to the solution. The reaction mixture was stirred at that temperature for 30 min. Then, DIBAL-H of 1 M Toluene solution (35 mmol) was added to the reaction mixture and the resultant reaction mixture was stirred at -78 °C for 1 h. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was quenched with 5 % aqueous HCl solution (80 ml). The resultant reaction mixture was allowed to warm up to room temperature. Then DCM was evaporated and the product was extracted with ethyl acetate (70 ml X 3). The extracted ethyl acetate solution was washed with 10 % Na_2CO_3 (100 ml) followed by brine (100 ml) solution.

The organic layer was then dried over anhydrous Na_2SO_4 and the product was concentrated under reduced pressure. The pure product Boc-amino-phenylalanine-allylic alcohol (5) was isolated after the column chromatography using EtOAc / pet ether (1:3) solvent system.



Scheme S5: Synthesis of Boc -Amino Phenylalanine Allylic Alcohol (5).

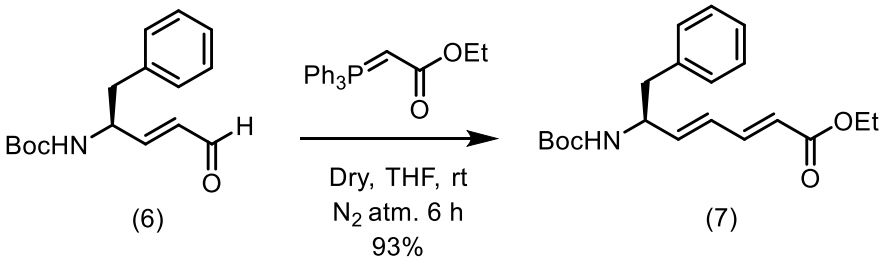
II) Procedure for the Synthesis of Boc-Amino-Phenylalanine-vinylogous Aldehyde[2] (6): The oxidation of Boc-amino phenylalanine allylic alcohol (5) using *o*-Iodoxybenzoic acid (IBX) resulted in the formation of Boc-amino phenylalanine vinylogous aldehyde (6). In the event, to a solution of Boc-amino phenylalanine allylic alcohol (5) (8 mmol) in dist. Ethyl acetate (80 ml) was added *o*-Iodoxybenzoic acid (IBX) (9.5 mmol). The reaction mixture was stirred for 4 h at 80 °C using a reflux condenser. The progress of the reaction was monitored using TLC in 30% (ethyl acetate in pet ether) solvent system. After completion, the reaction mixture was filtered using a sintered funnel, the filtrate is our product (6) which was concentrated under reduced pressure. The IBX and IBX-derived by-products are insoluble in ethyl acetate at room temperature i.e. no further purification is required beyond simple filtration. The IBX permits clean oxidation of phenylalanine allylic alcohol (5) to Phenylalanine-vinylogous Aldehyde (6) in ethyl acetate solvent. Due to the less stability of α, β-unsaturated aldehyde, we further immediately used it for the next Wittig reaction.



Scheme S6: Synthesis of Boc-Amino-Phenylalanine-vinylogous Aldehyde (6).

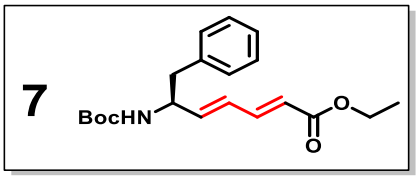
III) Procedure for the Synthesis of Boc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-Phenylalanine Ester[2] (7): Boc-amino phenylalanine vinylogous aldehyde (6) (6 mmol) was dissolved in dry THF (80 ml) under N_2 atmosphere. Then Wittig ylide (PPh_3=$CHCO_2Et$) (7.2 mmol) was added

to the above solution. The reaction mixture was stirred for 6 h at room temperature. The progress of the reaction was monitored by using TLC. Upon completion of the reaction, THF was evaporated from the reaction mixture, and the product 'Boc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-phenylalanine ester (7)' was purified by column chromatography using ethyl acetate in pet ether (6%) solvent system.



Scheme S7: Synthesis of Boc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-Phenylalanine Ester (7).

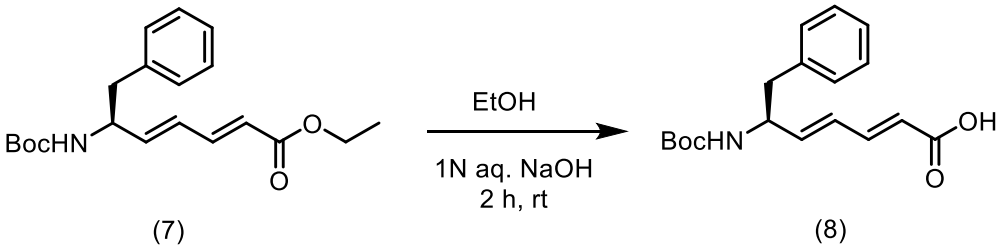
(*S*, 2*E*, 4*E*)-ethyl 6-((*tert*-butoxycarbonyl)amino)-7-phenylhepta-2,4-dienoate (7):



White solid, yield: 4.49g (93%), mp: 81 °C, $[\alpha]_D^{25}$ = -12.48 (c = 1 $CHCl_3$). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 7.32 – 7.29 (m, 2H, aromatic), 7.26 – 7.23 (m, 1H, aromatic), 7.23 – 7.19 (m, 1H, aromatic), 7.17 – 7.14 (m, 2H, aromatic1H & βC*H* merged), 6.20 (dd, *J* = 15.3, 10.9 Hz, 1H, γC*H*), 6.03 (dd, *J* = 15.4, 5.1 Hz, 1H, δC*H*), 5.82 (d, *J* = 15.3 Hz, 1H, αC*H*), 4.54 (s, 2H, N*H* & εC*H* merged), 4.19 (q, *J* = 7.1 Hz, 2H, -O-C*H*$_2$-CH$_3$), 2.87 (t, *J* = 5.1 Hz, 2H, *Benzylic*), 1.41 (s, 9H, *Boc*), 1.29 (t, *J* = 7.1 Hz, 3H, -O-CH$_2$-C*H*$_3$). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 166.95, 155.04, 143.63, 142.14, 136.72, 129.47, 128.51, 128.00, 126.75, 121.48, 79.78, 77.36, 77.04, 76.72, 60.36, 52.90, 41.36, 28.33, 14.29. **HRMS** (ESI-QTOF) m/z calculated value for $C_{20}H_{27}NO_4$ is [M+Na] 368.1837 and observed 368.1830.

IV) Ethyl ester hydrolysis reaction[2]: Purified Boc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-Phenylalanine Ester (7) (5 mmol) was dissolved in 10 mL of EtOH and then 10 ml of 1N aq. NaOH solution was added to the reaction mixture. The reaction mixture was stirred at that temperature for 3 h. The progress of the reaction was monitored by TLC. After completion of

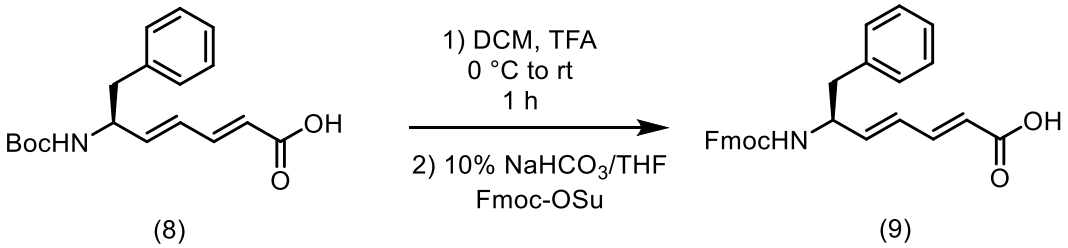
the ethyl ester hydrolysis reaction, EtOH was removed under a *vacuum.* The residue was acidified with 5 % aq. HCl solution (25 ml). The hydrolyzed acid product (8) was extracted with ethyl acetate (40 x 3). The extracted ethyl acetate solution was washed with brine (100 ml) solution. The organic layer was then dried over anhydrous Na_2SO_4 and the product was concentrated under reduced pressure.



(7) (8)

Scheme S8: Synthesis of Boc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-Phenylalanine Acid (8).

V) Boc deprotection and Fmoc protection reaction: The Boc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-Phenylalanine acid (8) (5 mmol) was dissolved in 4 mL of DCM and then 10 mL of TFA was added to the reaction mixture at 0 °C. After 1 h, TFA was removed under a *vacuum*. The residue was dissolved in 15 mL of 10% $NaHCO_3$ (adjusts pH to ~8). The solution of Fmoc-OSu (5 mmol) in 22 mL of THF was added slowly to the reaction mixture and stirred overnight at room temperature. After completion of the reaction, the reaction mixture was acidified with 5% aq. HCl solution and extracted with ethyl acetate (30 x 3). The extracted ethyl acetate solution was washed with brine (100 ml) solution. The organic layer was then dried over anhydrous Na_2SO_4 and the product was concentrated under reduced pressure to give the white solid product of Fmoc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε -Phenylalanine acid (9) in 79% yields, which were directly used for solid-phase peptide synthesis without further purifications.



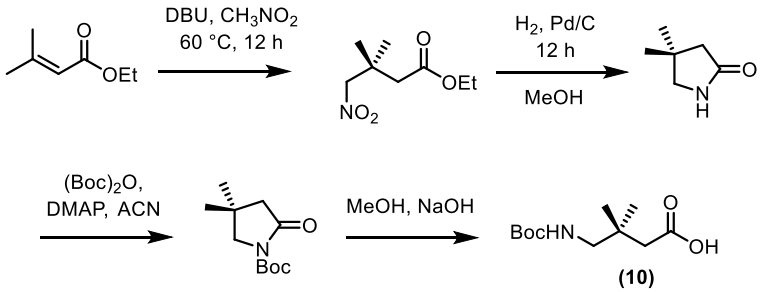
(8) (9)

Scheme S9: Synthesis of Fmoc-NH-(*E*, *E*)-αβ, γδ-unsaturated ε-Phenylalanine Acid (9).

C) Synthesis of Fmoc-NH-3,3-Dimethyl-Butanoic Acid (Fmoc-Adb)[3] (11):

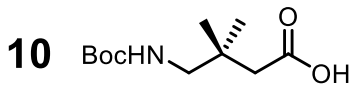
Boc-NH-3,3-Dimethyl-Butanoic Acid (Boc-Adb) (10) was synthesized using our reported protocol.[3] Briefly, Ethyl 3,3-dimethyl acrylate (10 mmol) was dissolved in neat nitromethane (50 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) base (15 mmol) were added. The reaction mixture was heated at 60 °C overnight. After completion of the reaction, nitromethane was evaporated under reduced pressure. The residue is acidified with 20 % aq. HCl solution. The acidic aqueous layer was extracted with ethyl acetate (40 x 3). The extracted ethyl acetate solution was washed with brine (100 ml) solution, and then dried over anhydrous Na_2SO_4 and the product was concentrated under reduced pressure.

The product, 3,3-dimethyl-4-nitro-butyric acid ethyl ester (1.5 g, 8 mmol) and activated Pd/C (20% by weight) in MeOH (18 mL) and acetic acid (3 mL) was stirred at room temperature in the presence of hydrogen. After completion of the reaction (24 h), Pd/C was filtered through the pad of celite and the filtrate was concentrated under reduced pressure to get 4,4-dimethyl-2-pyrrolidinone as oil. The amide NH group of 4,4-dimethyl-2-pyrrolidinone was further protected with the Boc group and then hydrolyzed using NaOH in MeOH to get the final product Boc-NH-3,3-Dimethyl-Butanoic Acid (Boc-Adb) (10) (1.38 g, 75%).



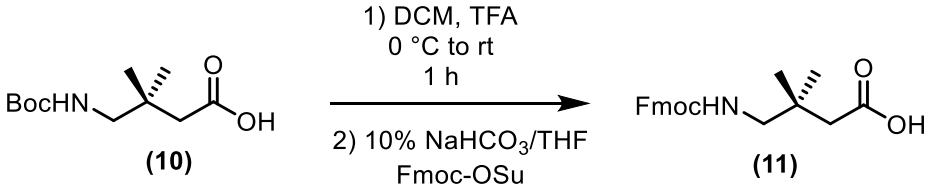
Scheme S10: Synthesis of Boc-NH-3,3-Dimethyl-Butanoic Acid (Boc-Adb) (10)[3].

4-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoic acid (10):



^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.99 (t, *J* = 7.0 Hz, 1H, N*H*), 3.06 (d, *J* = 6.9 Hz, 2H, -NH-γCH_2-), 2.23 (s, 2H, -αCH_2-COOH), 1.44 (s, 9H, *Boc*), 1.00 (s, 6H, -C(CH_3)$_2$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 175.91, 157.13, 80.04, 77.35, 77.04, 76.72, 49.71, 44.05, 34.99, 28.34, 25.61. **HRMS** (ESI-QTOF) m/z calculated value for $C_{11}H_{21}NO_4$ is [M+Na] 254.1368 and observed 254.1359.

Boc deprotection and Fmoc protection reaction: Boc-NH-3,3-Dimethyl-Butanoic Acid (Boc-Adb) (10) (5 mmol) was dissolved in 4 mL of DCM and then 10 mL of TFA was added to the reaction mixture at 0 °C. After 1 h, TFA was removed under a *vacuum*. The residue was dissolved in 15 mL of 10% $NaHCO_3$ (adjusts pH to ~8). The solution of Fmoc-OSu (5 mmol) in 22 mL of THF was added slowly to the reaction mixture and stirred overnight at room temperature. After completion of the reaction, the reaction mixture was acidified with 5% aq. HCl solution and extracted with ethyl acetate (30 x 3). The extracted ethyl acetate solution was washed with brine (100 ml) solution. The organic layer was then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the white solid product, Fmoc-NH-3,3-Dimethyl-Butanoic Acid (Fmoc-Adb) (11). which were directly used for solid-phase peptide synthesis without further purifications.



Scheme S11: Synthesis of Fmoc-NH-3,3-Dimethyl-Butanoic Acid (Fmoc-Adb) (11).

7) General procedure for the solid phase synthesis of peptides P1-P3:

Peptides were synthesized at 0.2 mmol scales on Knorr-Amide resin using standard Fmoc-chemistry. HBTU/HOBT was used as a standard coupling reagent. To avoid Michael addition of HOBT, we carried out-coupling reactions of *E*-vinylogous γ-amino acids only with HBTU.[7] Reaction time for Fmoc-deprotections and couplings of amino acids were 45 min and 1.5 h, respectively. The final Fmoc-deprotected amine was acylated using Acetic anhydride and pyridine base (Ac_2O/Py). After completion of the synthesis, peptides were

cleaved from the resin using 20 mL of TFA/TIPS/Phenol/H_2O (88:2:5:5). After peptide cleavage, the resin was filtered out using the sintered funnel. The filtrate (which contains peptide) was evaporated under reduced pressure to give the crude peptide products. Peptides were further precipitated out using cold diethyl ether and purified through reverse phase HPLC on the C-18 column using MeOH/H_2O gradient. The purity of peptides was further confirmed using an analytical C-18 column in the same MeOH/H_2O gradient system. The mass of peptides was confirmed by MALDI TOF/TOF.

8) 2D-NMR (TOCSY & ROESY) analysis and NOE correlations with solid state structure of peptides P1-P3.

(The 2D-NMR analysis of Peptides P1 and P2 was conducted in both CD_3OH and $CDCl_3$. However, due to insolubility of peptide P3 in $CDCl_3$, it was analysed only in CD_3OH. The NMR analysis of Peptides P1, P2 and P3 in CD_3OH are provided first, followed by the NMR data acquired in $CDCl_3$.)

8.1) Peptide P1 2D-NMR (TOCSY & ROESY) analysis in CD_3OH:

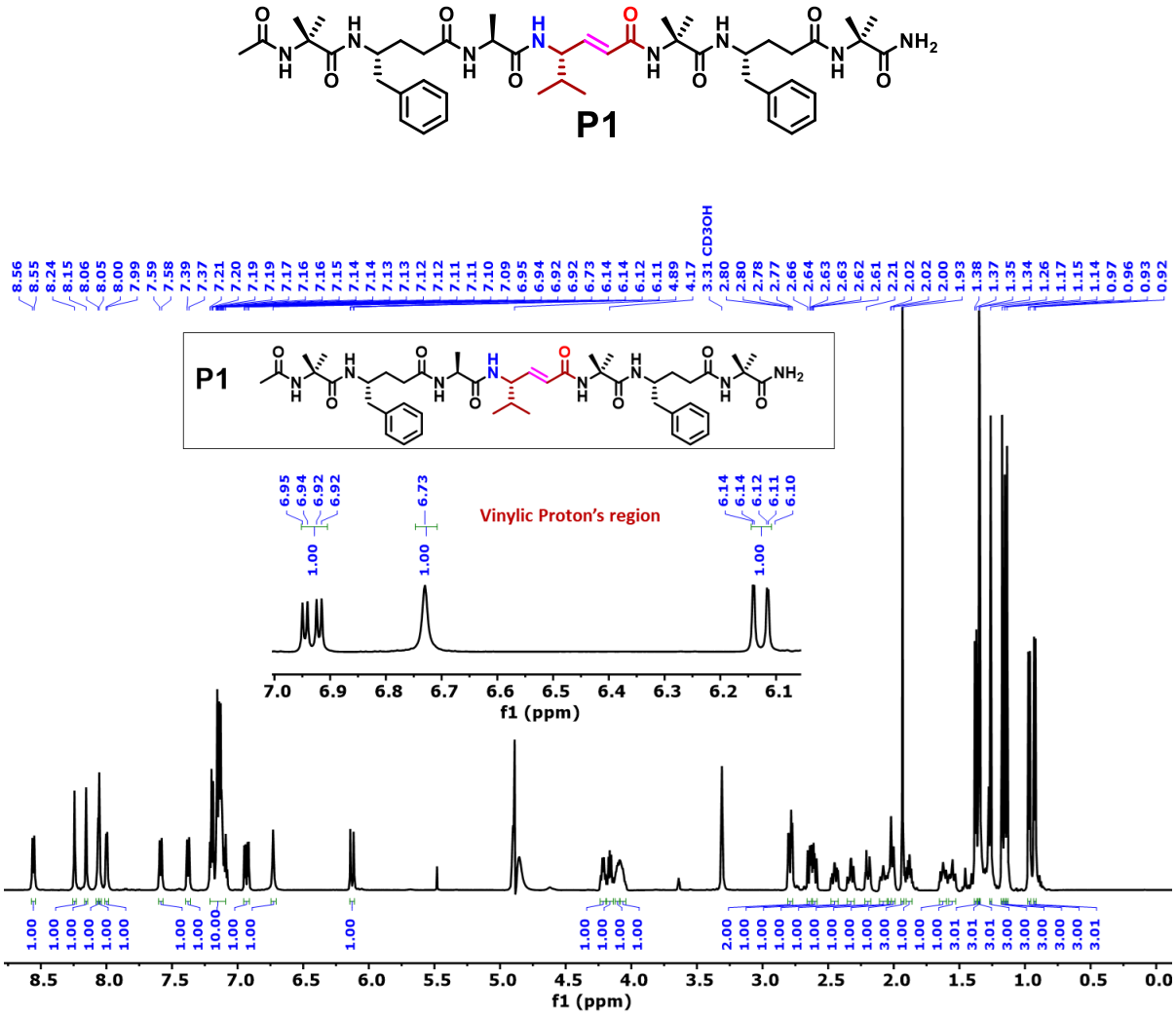


Figure S4: ^{1}H NMR spectrum of peptide **P1** in **CD_3OH**.

^{1}H NMR (600 MHz, CD_3OH) δ 8.56 (d, *J* = 8.5 Hz, 1H), 8.24 (s, 1H), 8.15 (s, 1H), 8.06 (s, 1H), 8.05 (s, 1H), 8.00 (d, *J* = 5.7 Hz, 1H), 7.59 (d, *J* = 9.7 Hz, 1H), 7.38 (d, *J* = 9.6 Hz, 1H), 7.21 – 7.09 (m, 10$H_{Aromatic}$), 6.93 (dd, *J* = 15.3, 5.4 Hz, 1H), 6.73 (s, 1H), 6.13 (dd, *J* = 15.3,

1.8 Hz, 1H), 4.21 (m, 1H), 4.16 (m, 1H), 4.10 (m, 1H), 4.07 (m, 1H), 2.79 (dd, *J* = 13.7, 5.2 Hz, 2H), 2.64 (dd, *J* = 13.7, 9.9 Hz, 1H), 2.61 (dd, *J* = 13.7, 9.4 Hz, 1H), 2.45 (ddd, *J* = 14.3, 11.8, 4.7 Hz, 1H), 2.33 (m, 1H), 2.20 (dt, *J* = 14.3, 4.7 Hz, 1H), 2.08 (m, 1H), 2.03 (m, 1H), 2.00 (m, 1H), 1.93 (s, 3H), 1.88 (m, 1H), 1.62 (m, 1H), 1.55 (m, 1H), 1.38 (d, *J* = 7.3 Hz, 3H), 1.35 (s, 3H), 1.34 (s, 3H), 1.26 (s, 3H), 1.17 (s, 3H), 1.15 (s, 3H), 1.14 (s, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H). **MALDI (TOF/TOF)** *m/z* calculated value for $C_{46}H_{68}N_8O_8$ [M+Na^+] is 883.5052 and observed at 883.5010.

Table S7. ^{1}H NMR Chemical Shifts (ppm) of **P1** in **CD_3OH** at 298 K.

Residues	NH	$_\alpha$CH	$_\beta$CH	$_\gamma$CH	$_\delta$CH	$_\varepsilon$CH	CH_{term}
(N-Terminus)-$COCH_3$	—	—	—	—	—	—	1.93
Aib(1)	8.15	—	1.17 (3H) 1.14 (3H)	—	—	—	—
γPhe(2)	7.38	2.45 (1H) 2.20 (1H)	2.08 (1H) 1.62 (1H)	4.07	Benzylic 2.79(1H) 2.64 (1H)	Aromatic 7.21-7.09 (5H)	—
Ala(3)	8.00	4.16	1.38 (3H)	—	—	—	—
dγVal(4)	8.56	6.13	6.93	4.21	1.88	0.97 (3H) 0.93 (3H)	
Aib(5)	8.24	—	1.26 (3H) 1.15 (3H)	—	—	—	—
γPhe(6)	7.59	2.33 (1H) 2.03 (1H)	2.00 (1H) 1.55 (1H)	4.10	Benzylic 2.79 (1H) 2.61 (1H)	Aromatic 7.21-7.09 (5H)	—
Aib(7)	8.05	—	1.35 (3H) 1.34 (3H)	—	—	—	—
-NH_2 (C-Terminus)	8.06[a] 6.73[b]	—	—	—	—	—	—

[a] $CONH_2$ Proton involved in Hydrogen bonding. [b] $CONH_2$ Proton is not involved in Hydrogen bonding. Aromatic protons are shown in $_\varepsilon$CH column. Chemical shift assigned by using TOCSY and ROESY spectra.

Assignment of chemical shifts using TOCSY and ROESY spectra

The amino acid residues and their positions in the peptide sequence were identified using TOCSY and ROESY spectra, respectively.

The TOCSY spectrum was used to identify amino acids in the sequence of the peptides.

A) Partial TOCSY spectrum analysis of peptide P1 in CD_3OH

Below partial TOCSY spectrum showing the amino acid residues in peptide **P1**

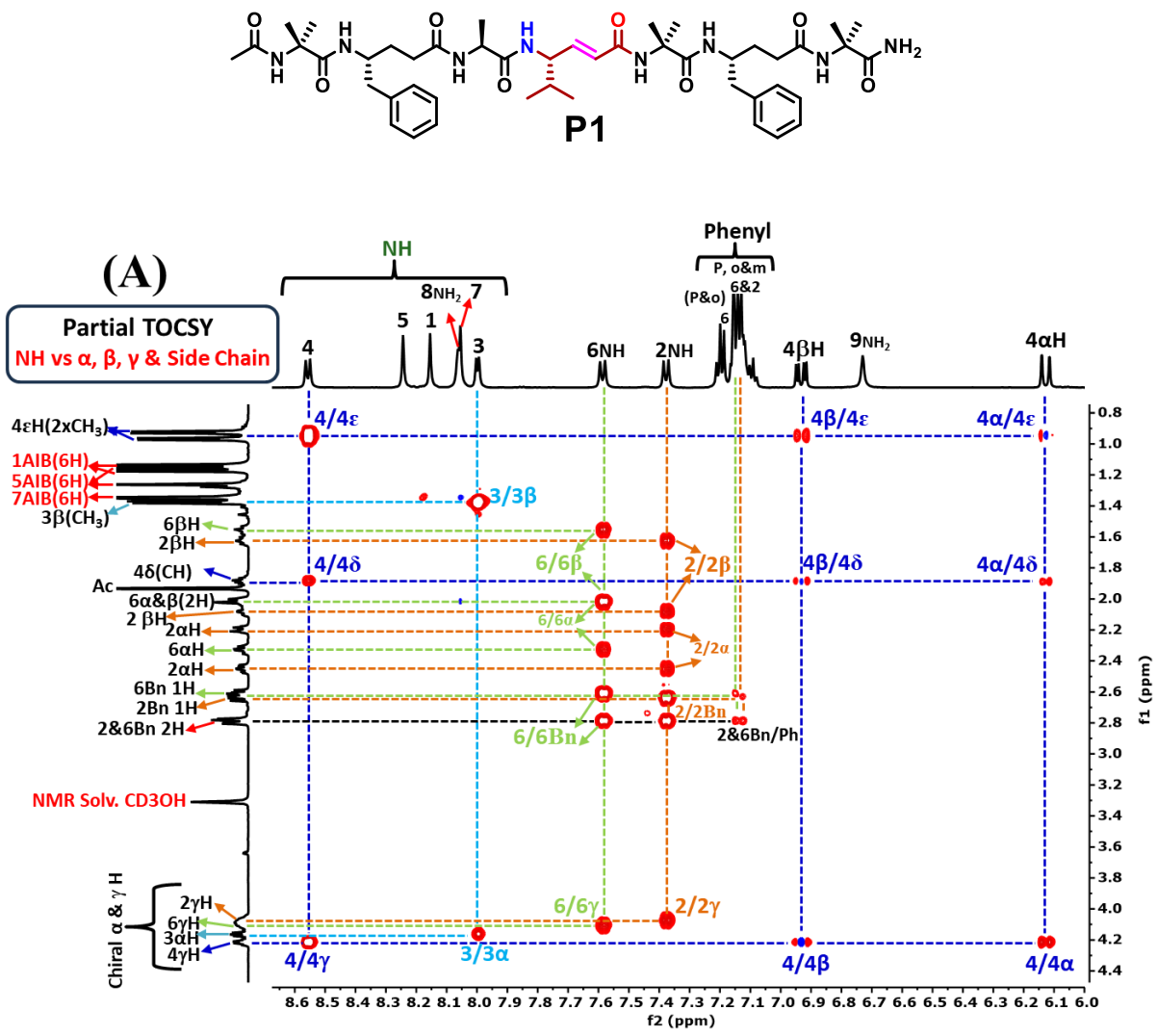
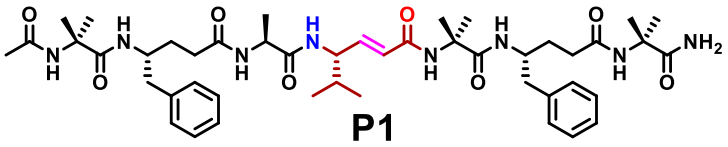
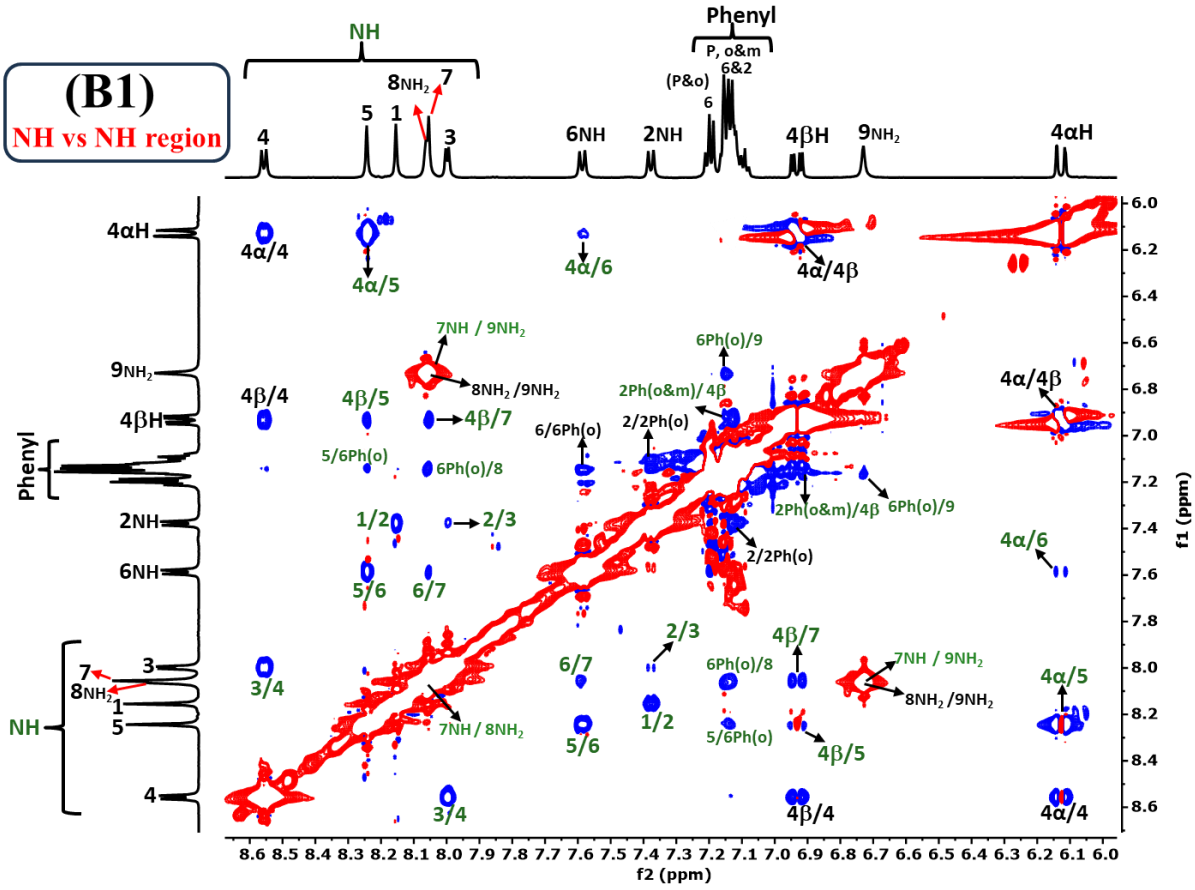


Figure S5: (**A**) Partial TOCSY spectrum (NH vs α, β, γ, Backbone & Side Chain protons) of peptide **P1** showing correlation between intra residue protons.

B) Partial ROESY spectrum analysis of Peptide P1 in CD_3OH.

Below ROESY spectrums (**B1**) and (**B2**) depicting the **NH↔NH, NH↔chiral $C_\alpha H$, $C_\gamma H$** interactions respectively. The inter and intra-residue NOEs are marked with green and black labels respectively.





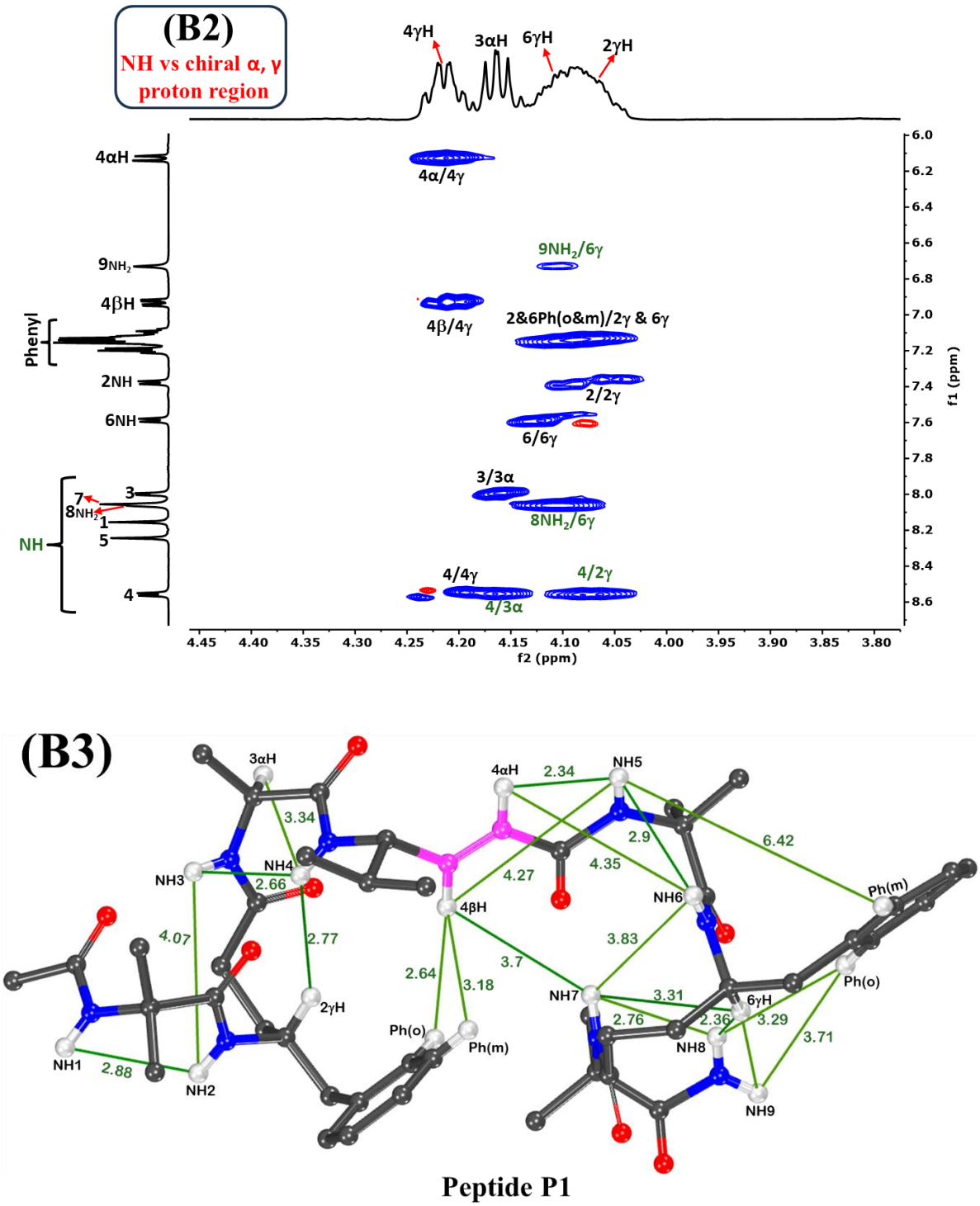


Figure S6: (**B1**) NH vs NH region of peptide **P1** ROESY spectrum in **CD_3OH**. (**B2**) NH vs Chiral α, and γ protons region in ROESY spectrum of peptide **P1** in **CD_3OH**. (**B3**) Crystal structure of peptide **P1** depicting the distance between inter residue NOE corresponding protons in the ROESY spectrum (**B1**) and (**B2**). (inter-residue NOEs marked with green labels in the ROESY spectrum (**B1**) and (**B2**)).

Table S8: NOEs Observed in **NH↔NH** and **NH↔C_αH/ C_βH /C_γH** Region of Peptide **P1** in **CD_3OH** and the distance observed between respective protons in its crystal structure.

C) List of Inter-residue NOEs (marked with green labels) in partial ROESY spectrum (**B1**) and (**B2**) of peptide **P1** in CD_3OH.							
Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**	**Type of NOE**	**Protons Region**	**Distance in crystal structure (Å)**
Aib (1)	NH	γPhe (2)	NH	Strong	NH/NH (1/2)	**(Inter-Residue NOE) NH vs NH region (Fig. S5: B1)**	2.88
γPhe (2)	NH	Ala (3)	NH	Very Weak	NH/NH (2/3)		4.07
Ala (3)	NH	(*E*)-dγVal (4)	NH	Strong	NH/NH (3/4)		2.66
Aib (5)	NH	γPhe (6)	NH	Strong	NH/NH (5/6)		2.9
γPhe (6)	NH	Aib (7)	NH	Weak	NH/NH (6/7)		3.83
Aib (7)	NH	$CONH_2$ (8)	NH (Intra H-Bonded)	Strong (at Diagonal)	NH/NH (7/8)		2.76
Aib (7)	NH	$CONH_2$ (9)	NH	Medium (Merged in Tocsy Signal)	NH/NH (7/9)		4.09
(*E*)-dγVal (4)	α CH (Backbone)	Aib (5)	NH	Very Strong	*α*CH/NH (4/5)		2.34
(*E*)-dγVal (4)	α CH (Backbone)	Aib (6)	NH	Very Weak	*α*CH/NH (4/6)		4.35
γPhe (2)	Phenyl (o & m) CH (side-chain)	(*E*)-dγVal (4)	β CH (Backbone)	Strong	Ph(o & m)/*β*CH (2/4)		2.64 (ortho) 3.18 (meta)
(*E*)-dγVal (4)	β CH (Backbone)	Aib (5)	NH	Medium	*β*CH/NH (4/5)		4.27
(*E*)-dγVal (4)	β CH (Backbone)	Aib (7)	NH	Medium	*β*CH/NH (4/7)		3.7
γPhe (6)	Phenyl (ortho) CH	$CONH_2$ (8)	NH (intra H-Bonded)	Medium	Ph(o)/NH (6/8)		3.29 (ortho)

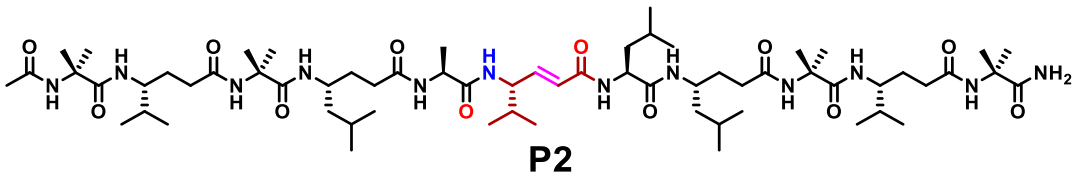
	(side-chain)						
γPhe (6)	Phenyl (ortho) CH (side-chain)	$CONH_2$ (9)	NH	Medium	Ph(o)/NH (6/9)		3.71 (ortho)
Aib (5)	NH	γPhe (6)	Phenyl (ortho) CH (side-chain)	Very Weak	NH/ Ph(o) (5/6)		6.42
$CONH_2$(9)	NH	γPhe (6)	γ CH (Backbone)	Medium	NH /γCH (9/6)	**(Inter-Residue NOE) NH vs γ & α CH region (Fig. S5: B2)**	3.25
$CONH_2$(8)	NH (intra H-Bonded)	γPhe (6)	γ CH (Backbone)	Very Strong	NH /γCH (8/6)		2.36
(*E*)-dγVal (4)	NH	Ala (3)	α CH (Backbone)	Strong	NH /αCH (4/3)		3.34
(*E*)-dγVal (4)	NH	γPhe (2)	γ CH (Backbone)	Strong	NH /γCH (4/2)		2.77

D) Intra-residue NOEs (marked with **black** labels) in partial ROESY spectrum (**B1**) and (**B2**) of peptide **P1** in CD_3OH.

Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**	**Protons Region**	**Distance in crystal structure (Å)**
(*E*)-dγVal (4)	α CH (Backbone)	(*E*)-dγVal (4)	NH	Strong	**(Intra-Residue NOE) NH vs NH region (Fig. S5: B1)**	4.43
(*E*)-dγVal (4)	β CH (Backbone)	(*E*)-dγVal (4)	NH	Strong		2.97
(*E*)-dγVal (4)	α CH (Backbone)	(*E*)-dγVal (4)	β CH (Backbone)	Very Strong		2.69
γPhe (2)	NH	γPhe (2)	Phenyl (ortho) CH (side-chain)	Strong		3.03 & 4.77 (ortho)
γPhe (6)	NH	γPhe (6)	Phenyl (ortho) CH	Medium		3.83 &4.19 (ortho)
$CONH_2$ (8)	NH (Intra H-Bonded)	$CONH_2$ (9)	NH	Strong		1.48
(*E*)-dγVal (4)	α CH	(*E*)-dγVal (4)	γ CH (Backbone)	Strong		2.31
(*E*)-dγVal (4)	β CH (Backbone)	(*E*)-dγVal (4)	γ CH (Backbone)	Strong		2.84

γPhe (2)	Phenyl (o & m) CH (side-chain)	γPhe (2)	γ CH (Backbone)	Very Strong	**(Intra-Residue NOE) NH vs γ & α CH region (Fig. S5: B2)**	3.96 & 2.79 (ortho) 4.54 & 5.38 (meta)
γPhe (6)	Phenyl (o & m) CH (side-chain)	γPhe (6)	γ CH (Backbone)	Very Strong		2.38 & 4.62 (ortho) 4.67 &6.16 (meta)
γPhe (2)	NH	γPhe (2)	γ CH (Backbone)	Medium		2.75
γPhe (6)	NH	γPhe (6)	γ CH (Backbone)	Medium		2.75
Ala (3)	NH	Ala (3)	α CH (side-chain)	Strong		2.56
(*E*)-dγVal (4)	NH	(*E*)-dγVal (4)	γ CH (Backbone)	Strong		2.76

8.2) Peptide P2 2D-NMR (TOCSY & ROESY) analysis in CD_3OH:



P2

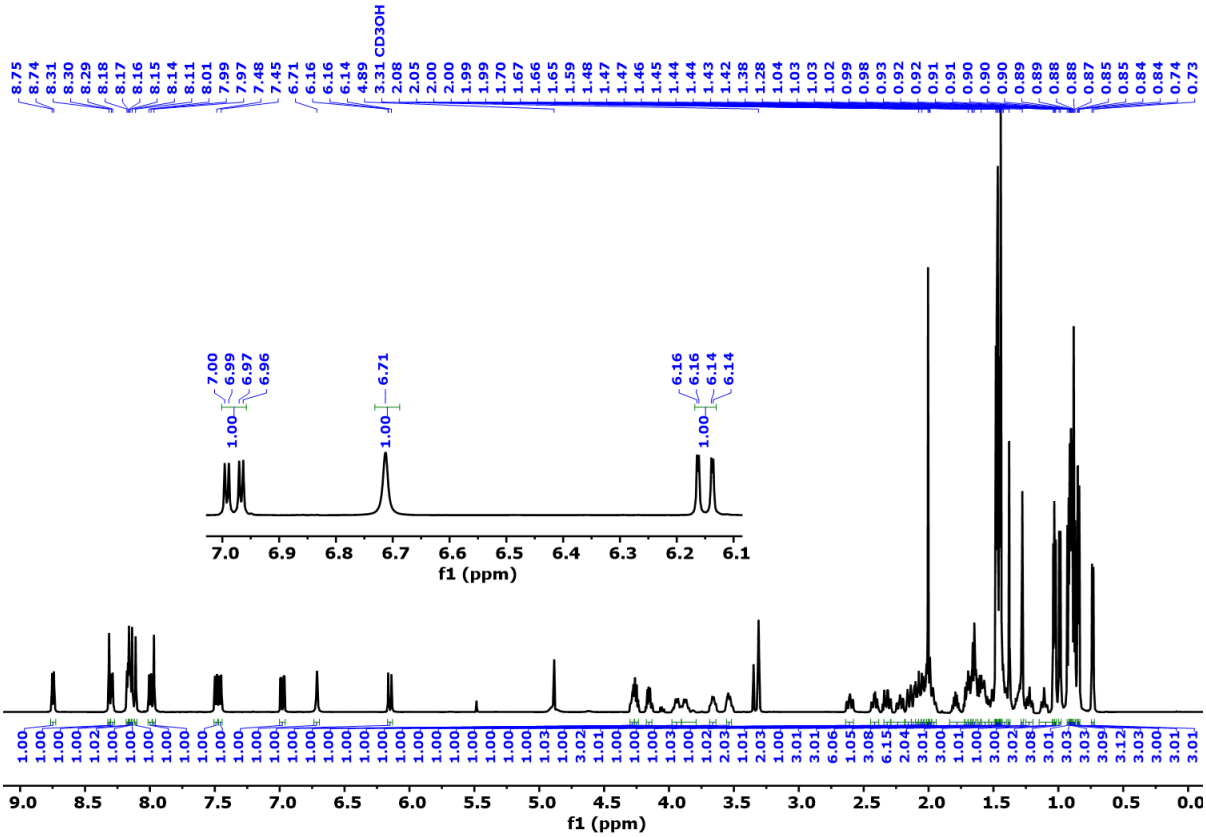


Figure S7: ^{1}H NMR spectrum of peptide **P2** in **CD_3OH**.

^{1}H NMR (600 MHz, CD_3OH) δ 8.75 (d, *J* = 8.5 Hz, 1H), 8.31 (s, 1H), 8.29 (d, *J* = 6.3 Hz, 1H), 8.17 (d, *J* = 5.0 Hz, 1H), 8.16 (s, 1H), 8.15 (s, 1H), 8.14 (s, 1H), 8.11 (s, 1H), 8.00 (d, *J* = 9.8 Hz, 1H), 7.97 (s, 1H), 7.49 (d, *J* = 9.7 Hz, 1H), 7.46 (d, *J* = 9.9 Hz, 1H), 6.98 (dd, *J* = 15.2, 4.3 Hz, 1H), 6.71 (s, 1H), 6.15 (dd, *J* = 15.2, 2.0 Hz, 1H), 4.28 (m, 1H), 4.25 (m, 1H), 4.15 (m, 1H), 3.94 (m, 1H), 3.87 (m, 1H), 3.67 (m, 1H), 3.54 (m, 1H), 2.61 (ddd, *J* = 14.3, 12.3, 4.2 Hz, 1H), 2.42 (m, 1H), 2.32 (m, 1H), 2.22 (m, 1H), 2.15 (dt, *J* = 12.3, 3.4 Hz, 1H), 2.11 (m, 1H), 2.09 (m, 1H), 2.07 (m, 1H), 2.05 (m, 1H), 2.03 (m, 1H), 2.00 (s, 3H), 1.99 (s, 1H), 1.98 (m, 1H), 1.96 (m, 1H), 1.79 (m, 1H), 1.71 (m, 1H), 1.69 (m, 1H), 1.67 (m, 1H), 1.65 (m, 2H), 1.61 (m, 1H), 1.56 (m, 2H), 1.51 (m, 1H), 1.48 (s, 3H), 1.47 (s, 3H), 1.466 (s, 6H), 1.46 (br, 1H), 1.45 (s, 3H), 1.44 (s, 6H), 1.41 (m, 2H), 1.38 (s, 3H), 1.28 (s, 3H), 1.23 (m, 1H), 1.11 (m, 1H), 1.04 (d, *J* = 4.7 Hz, 3H), 1.02 (d, *J* = 4.7 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 4.5 Hz, 3H), 0.91 (d, *J* = 4.5 Hz, 3H), 0.90 (d, *J* = 5 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 5 Hz, 3H), 0.85 (d, *J* = 6.4 Hz, 3H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.74 (d, *J* = 6.4 Hz, 3H). **MALDI (TOF/TOF)** *m/z* calculated value for $C_{64}H_{116}N_{12}O_{12}$ [M+Na^+] is 1267.87 and observed at 1267.94.

Table S9. ^{1}H NMR Chemical Shifts (ppm) of **P2** in **CD_3OH** at 298 K.

Residues	NH	$_{\alpha}$CH	$_{\beta}$CH	$_{\gamma}$CH	$_{\delta}$CH	$_{\varepsilon}$CH	$_{\zeta}$CH	CH_{term}
-$COCH_3$	—	—	—	—	—	—	—	2.00
Aib(1)	8.31	—	1.466(6H)	—	—	—	—	—
γVal(2)	7.46	2.32 (1H)	2.07 (1H)	3.54	1.51	0.90 (3H)	—	—
		2.11 (1H)	1.61 (1H)			0.87 (3H)		
Aib(3)	8.14	—	1.38 (3H)	—	—	—	—	—
			1.28 (3H)					
γLeu(4)	8.17	2.61 (1H)	2.05 (1H)	3.87	1.56 (1H)	1.11 (1H)	0.85(3H)	—
		2.15(1H)	1.67 (1H)				0.74(3H)	
Ala(5)	8.16	4.15 (1H)	1.45 (3H)	—	—	—	—	—
dγVal(6)	8.75	6.15 (1H)	6.98 (1H)	4.28	2.03 (1H)	1.04 (3H)	—	—
						1.02 (3H)		
Leu(7)	8.29	4.25 (1H)	1.65 (2H)	1.79	0.99 (3H)	—	—	—
					0.93 (3H)			
γLeu(8)	7.49	2.22 (1H)	1.98 (1H)	3.94	1.41 (2H)	1.23 (1H)	0.89(3H)	—
		2.09 (1H)	1.46 (1H)				0.84(3H)	
Aib(9)	8.11	—	1.48 (3H)	—	—	—	—	—
			1.47 (3H)					
γVal(10)	8.00	1.99 (1H)	1.96 (1H)	3.67	1.69 (1H)	0.91 (3H)	—	—
		1.99 (1H)	1.71 (1H)			0.91 (3H)		
Aib(11)	7.97	—	1.44 (6H)	—	—	—	—	—
-NH_2	8.15[a]	—	—	—	—	—	—	—
	6.71[b]							

[a] $CONH_2$ Proton involved in Hydrogen bonding. [b] $CONH_2$ Proton is not involved in Hydrogen bonding. Chemical shift assigned by using TOCSY and ROESY spectra.

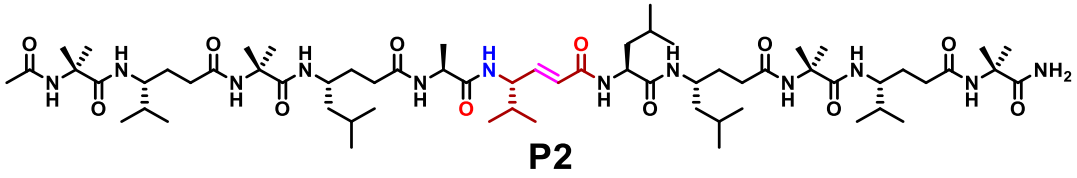
Assignment of chemical shifts using TOCSY and ROESY spectra

The amino acid residues and their positions in the peptide sequence were identified using TOCSY and ROESY spectra, respectively.

The TOCSY spectrum was used to identify amino acids in the sequence of the peptides.

A) Partial TOCSY spectrum analysis of peptide P2 in CD_3OH.

Below partial TOCSY spectrum showing the amino acid residues in peptide **P2**.



P2

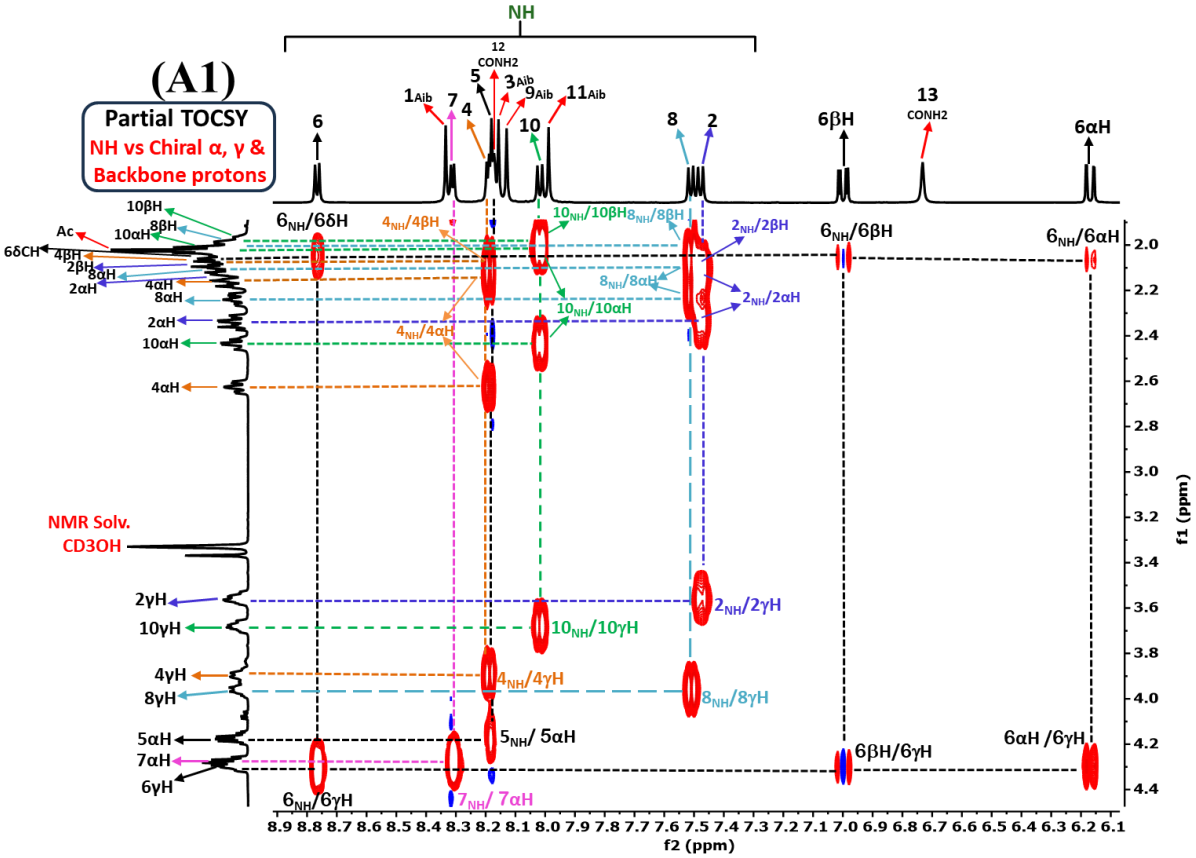
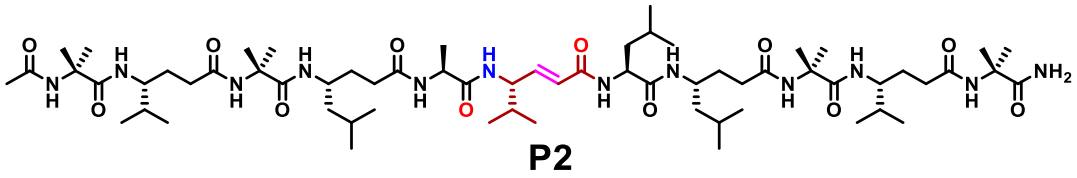


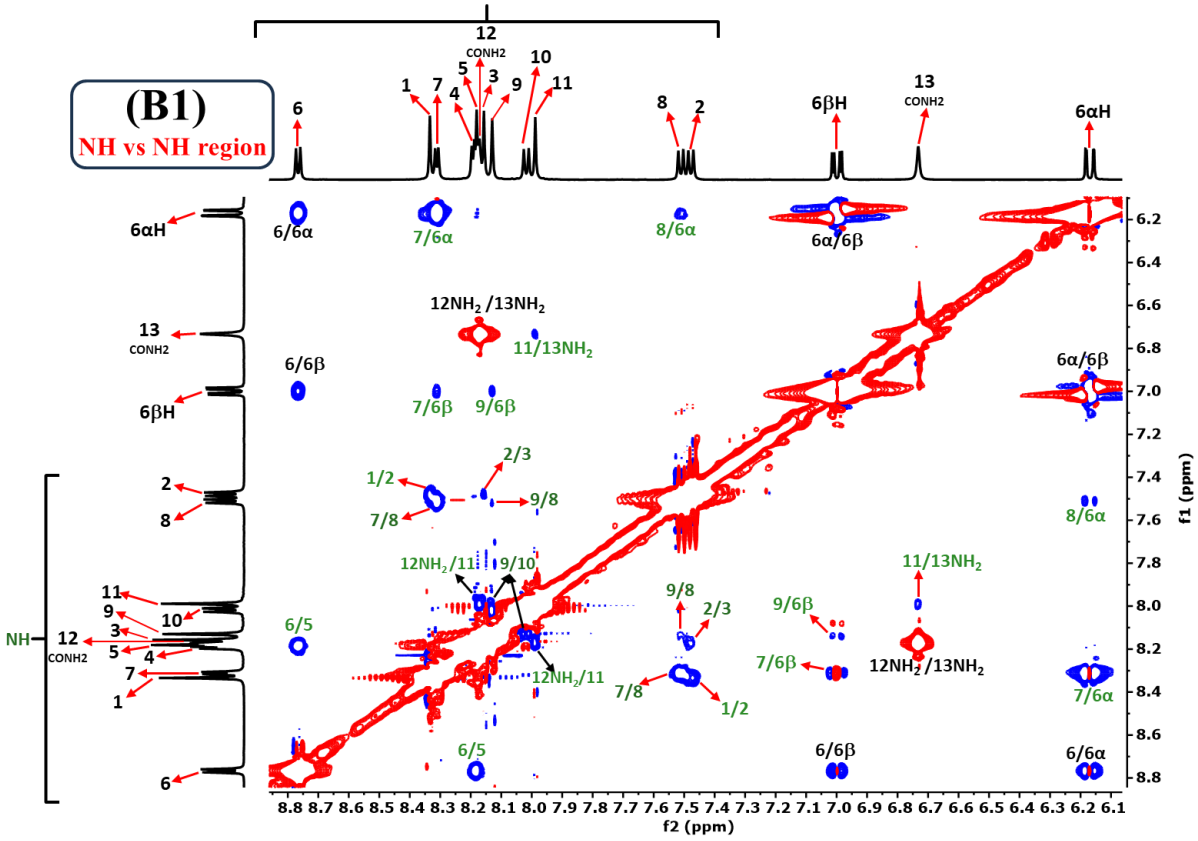
Figure S8: (**A1**) Partial TOCSY spectrum (NH vs α, β, γ & Backbone protons) of peptide **P2** showing correlation between intra residue protons.

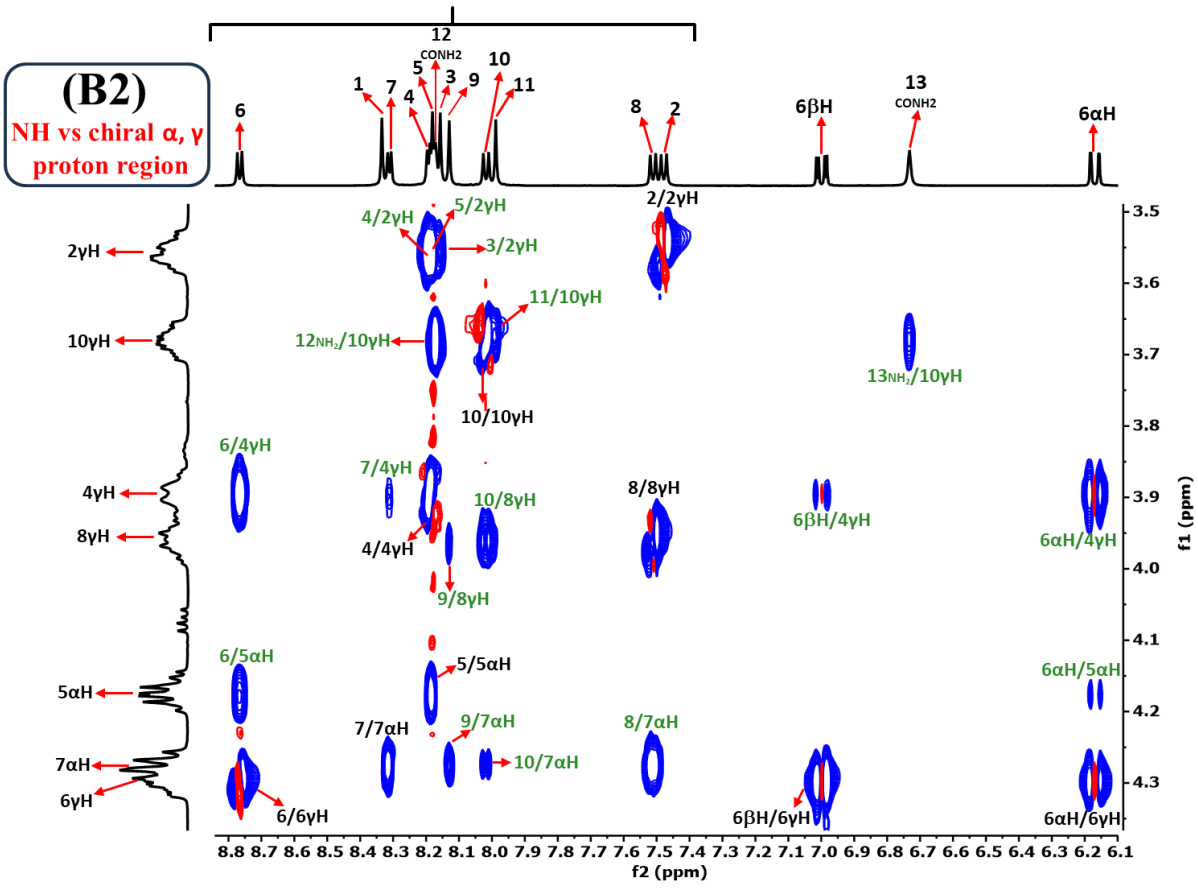
B) Partial ROESY spectrum analysis of Peptide P2 in CD_3OH.

Below ROESY spectrums (**B1**) and (**B2**) depicting the **NH↔NH, NH↔chiral $C_\alpha H$, $C_\gamma H$** interactions respectively. The inter and intra-residue NOEs are marked with green and black labels respectively.



P2


(B1)
NH vs NH region
f2 (ppm)
f1 (ppm)


(B2)
NH vs chiral α, γ proton region
f2 (ppm)
f1 (ppm)

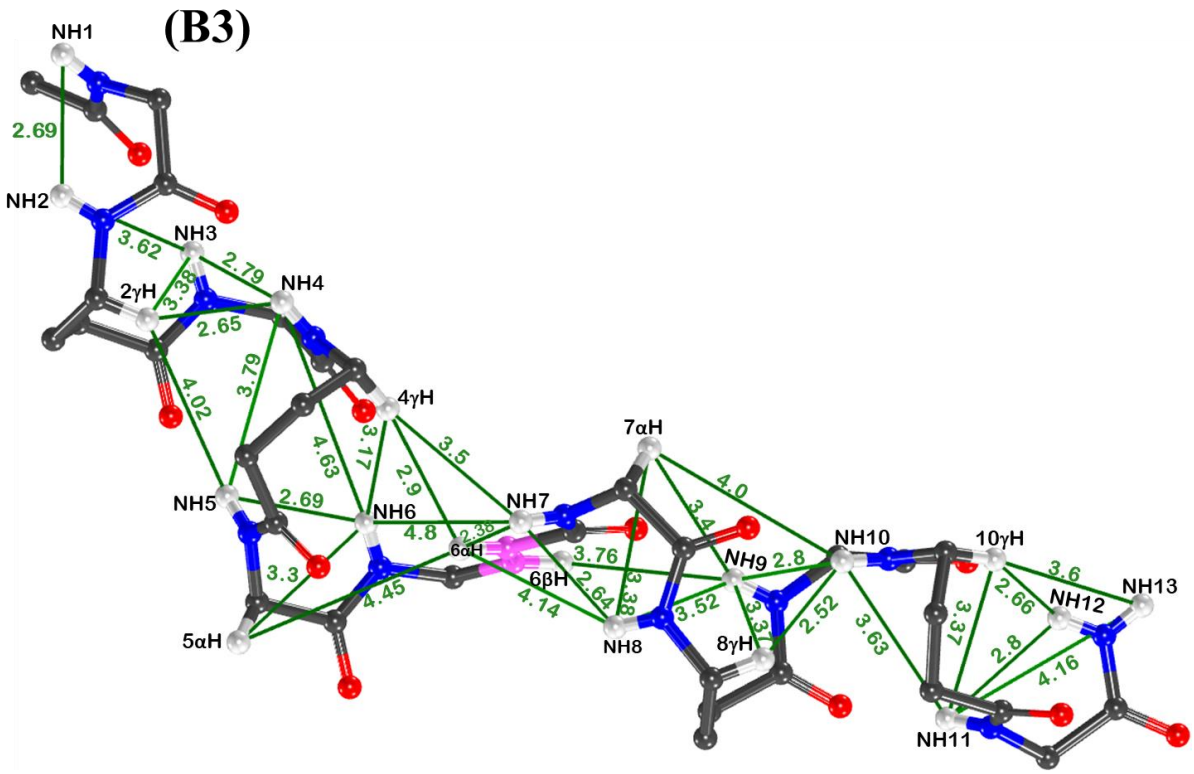


Figure S9: (**B1**) NH vs NH region of peptide **P2** ROESY spectrum in **CD_3OH**. (**B2**) NH vs Chiral α, and γ protons region in ROESY spectrum of peptide **P2** in **CD_3OH**. (**B3**) Crystal structure of peptide **P2** depicting the distance between inter residue NOE corresponding protons in the ROESY spectrum (**B1**) and (**B2**). (inter-residue NOEs marked with green labels in the ROESY spectrum (**B1**) and (**B2**)).

Table S10: NOEs Observed in **NH↔NH** and **NH↔C_αH/ C_βH /C_γH** Region of Peptide **P2** in **CD_3OH** and the distance observed between respective protons in its crystal structure.

C) List of Inter-residue NOEs (marked with green labels) in partial ROESY spectrum (**B1**) and (**B2**) of peptide **P2** in CD_3OH.							
Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**	**Type of NOE**	**Protons Region**	**Distance in crystal structure (Å)**
Aib (1)	NH	γVal (2)	NH	Strong	NH/NH (1/2)		2.69
γVal (2)	NH	Aib (3)	NH	Weak	NH/NH (2/3)		3.62
Aib (3)	NH	γLeu (4)	NH	Strong	NH/NH (3/4)		2.79

				(at Diagonal)			
γLeu (4)	NH	Ala (5)	NH	Weak (at Diagonal)	NH/NH (4/5)		3.79
Ala (5)	NH	(*E*)-dγVal (6)	NH	Strong	NH/NH (5/6)		2.69
Leu (7)	NH	γLeu (8)	NH	Very Strong	NH/NH (7/8)		2.64
γLeu (8)	NH	Aib (9)	NH	Very Weak	NH/NH (8/9)		3.52
Aib (9)	NH	γVal (10)	NH	Strong	NH/NH (9/10)		2.80
γVal (10)	NH	Aib (11)	NH	Weak (at Diagonal)	NH/NH (10/11)	**(Inter-Residue NOE) NH vs NH region (Fig. S7: B1)**	3.63
Aib (11)	NH	$CONH_2$ (12)	NH (intra H-Bonded)	Strong	NH/NH (11/12)		2.80
Aib (11)	NH	$CONH_2$ (13)	NH	Weak	NH/NH (11/13)		4.16
Leu (7)	NH	(*E*)-dγVal (6)	β CH (Backbone)	Weak	NH/ *β*CH (7/6)		4.24
Aib (9)	NH	(*E*)-dγVal (6)	β CH (Backbone)	Very Weak	NH/ *β*CH (9/6)		3.77
Leu (7)	NH	(*E*)-dγVal (6)	α CH (Backbone)	Very Strong	NH/ *α*CH (7/6)		2.38
γLeu (8)	NH	(*E*)-dγVal (6)	α CH (Backbone)	Very Weak	NH/ *α*CH (8/6)		4.14
(*E*)-dγVal (6)	NH	γLeu (4)	γ CH (Backbone)	Strong	NH/ *γ*CH (6/4)	**(Inter-Residue NOE) NH vs γ & α CH region (Fig. S7: B2)**	3.17
(*E*)-dγVal (6)	NH	Ala (5)	α CH (Backbone)	Strong	NH/ *α*CH (6/5)		3.3
Leu (7)	NH	γLeu (4)	γ CH (Backbone)	Weak	NH/ *γ*CH (7/4)		3.5
γLeu (4)	NH	γVal (2)	γ CH (Backbone)	Very Strong	NH/ *γ*CH (4/2)		2.65
Ala (5)	NH	γVal (2)	γ CH (Backbone)	Very Weak	NH/ *γ*CH (5/2)		4.02
$CONH_2$	NH (intra	γVal (10)	γ CH	Very	NH/ *γ*CH		2.66

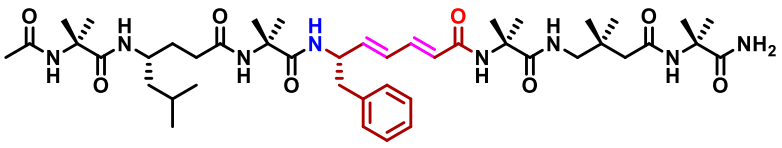
(12)	H-Bonded)		(Backbone)	Strong	(12/10)		
Aib (3)	NH	γVal (2)	γ CH (Backbone)	Medium	NH/ γCH (3/2)		3.38
Aib (9)	NH	γLeu (8)	γ CH (Backbone)	Medium	NH/ γCH (9/8)		3.37
Aib (9)	NH	Leu (7)	α CH (Backbone)	Medium	NH/ αCH (9/7)		3.43
γVal (10)	NH	γLeu (8)	γ CH (Backbone)	Strong	NH/ γCH (10/8)		2.526
γVal (10)	NH	Leu (7)	α CH (Backbone)	Weak	NH/ αCH (10/7)		4.06
Aib (11)	NH	γVal (10)	γ CH (Backbone)	Medium	NH/ γCH (11/10)		3.38
γLeu (8)	NH	Leu (7)	α CH (Backbone)	Strong	NH/ αCH (8/7)		3.38
(*E*)-dγVal (6)	β CH (Backbone)	γLeu (4)	γ CH (Backbone)	Weak	βCH / γCH (6/4)		4.94
$CONH_2$ (13)	NH	γVal (10)	γ CH (Backbone)	Medium	NH/ γCH (13/10)		3.61
(*E*)-dγVal (6)	α CH (Backbone)	γLeu (4)	γ CH (Backbone)	Strong	α CH/ γ CH (6/4)		2.91
(*E*)-dγVal (6)	α CH (Backbone)	Ala (5)	α CH (Backbone)	Weak	α CH/ α CH (6/5)		4.456

D) Intra-residue NOEs (marked with **black** labels) in partial ROESY spectrum (**B1**) and (**B2**) of peptide **P2** in CD_3OH.

Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**	**Protons Region**	**Distance in crystal structure (Å)**
(*E*)-dγVal (6)	NH	(*E*)-dγVal (6)	α CH (Backbone)	Strong	**(Intra-Residue NOE) NH vs NH region**	2.57
(*E*)-dγVal (6)	NH	(*E*)-dγVal (6)	β CH (Backbone)	Medium		3.64
$CONH_2$ (12)	NH	$CONH_2$ (13)	NH	Very Strong		1.52

	(Intra H-Bonded)				**(Fig. S7: B1)**	
(*E*)-dγVal (6)	α CH (Backbone)	(*E*)-dγVal (6)	β CH (Backbone)	Very Strong		2.76
(*E*)-dγVal (6)	NH	(*E*)-dγVal (6)	γ CH (Backbone)	Strong	**(Intra-Residue NOE) NH vs chiral γ & α CH region (Fig. S7: B2)**	2.78
Leu (7)	NH	Leu (7)	α CH (Backbone)	Strong		2.70
γLeu (4)	NH	γLeu (4)	γ CH (Backbone)	Very Strong		2.80
Ala (5)	NH	Ala (5)	α CH (Backbone)	Strong		2.68
γVal (10)	NH	γVal (10)	γ CH (Backbone)	Very Strong		2.79
γVal (2)	NH	γVal (2)	γ CH (Backbone)	Strong		2.78
γLeu (8)	NH	γLeu (8)	γ CH (Backbone)	Very Strong		2.77
(*E*)-dγVal (6)	β CH (Backbone)	(*E*)-dγVal (6)	γ CH (Backbone)	Very Strong		2.44
(*E*)-dγVal (6)	α CH (Backbone)	(*E*)-dγVal (6)	γ CH (Backbone)	Medium		3.29

8.3) Peptide P3 2D-NMR (TOCSY & ROESY) analysis in CD_3OH:



P3

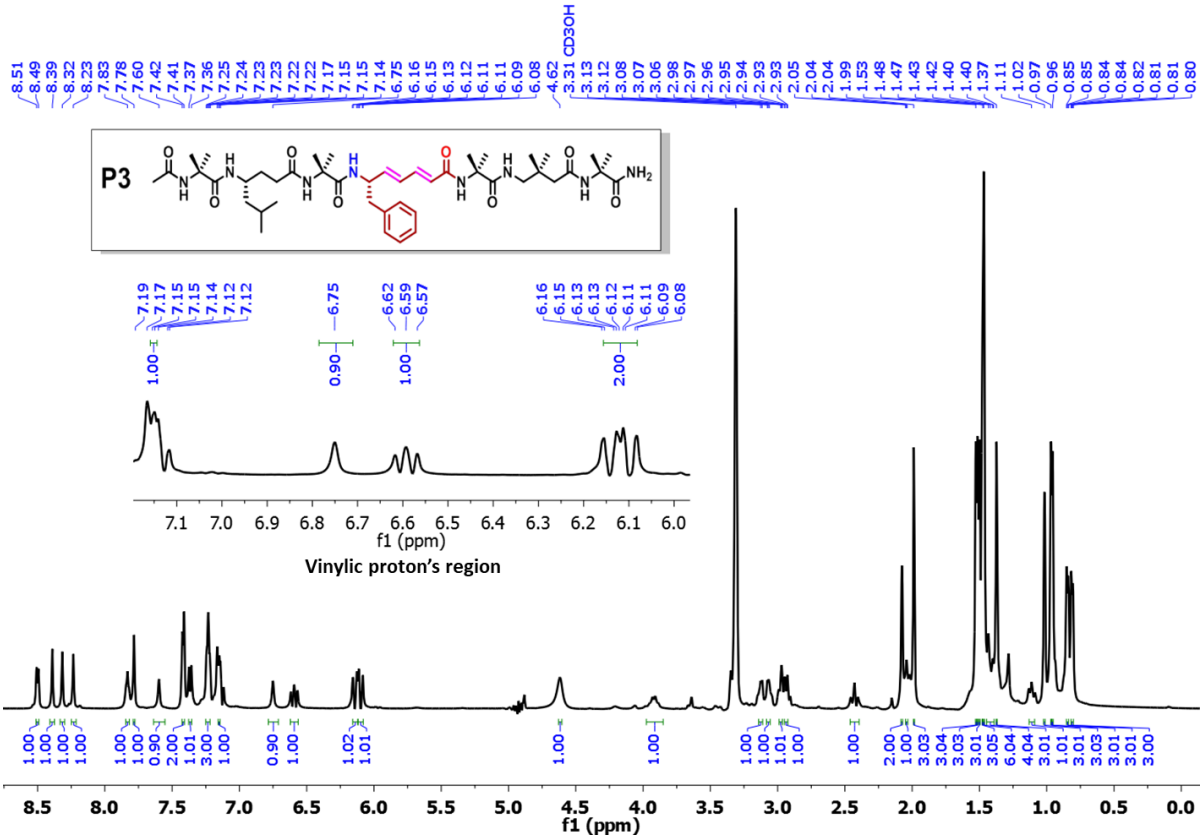


Figure S10: ^{1}H NMR spectrum of peptide **P3** in **CD_3OH**.

1H NMR (600 MHz, CD_3OH) δ 8.50 (d, *J* = 8.5 Hz, 1H), 8.39 (s, 1H), 8.32 (s, 1H), 8.23 (s, 1H), 7.83 (t, *J* = 7.2 Hz, 1H), 7.78 (s, 1H), 7.60 (s, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 11.0 Hz, 1H), 7.23 (dt, *J* = 7.9, 3.7 Hz, 3H), 7.15 (m, 1H), 6.75 (s, 1H), 6.59 (m, 1H), 6.14 (m, 1H), 6.10 (m, 1H), 4.62 (s, 1H), 3.91 (m, 1H), 3.13 (t, *J* = 6.6 Hz, 1H), 3.07 (t, *J* = 6.6 Hz, 1H), 2.99 – 2.96 (m, 1H), 2.95 – 2.93 (m, 1H), 2.46 – 2.39 (m, 1H), 2.08 (s, 2H), 2.04 (br, 1H), 1.99 (s, 3H), 1.53 (s, 3H), 1.51 (s, 3H), 1.50 (s, 3H), 1.48 (s, 3H), 1.47 (s, 6H), 1.43 – 1.42 (m, 4H), 1.37 (s, 3H), 1.11 (m, 1H), 1.02 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.84 (m, 3H), 0.81 (m, 3H). **MALDI (TOF/TOF)** *m/z* calculated value for $C_{45}H_{72}N_8O_8$ [M+Na^+] is 875.60 and observed at 875.68.

Table S11. ^{1}H NMR Chemical Shifts (ppm) of **P3** in **CD_3OH** at 298 K.

Residues	NH	$_{\alpha}$CH	$_{\beta}$CH	$_{\gamma}$CH	$_{\delta}$CH	$_{\varepsilon}$CH	CH_{term}
-$COCH_3$	—	—	—	—	—	—	1.99(3H)
Aib(1)	8.39	—	1.53 (3H) 1.51 (3H)	—	—	—	—

γLeu(2)	7.37	2.43(1H) 2.04(1H)	1.42(2H)	3.91(1H)	1.42(1H) 1.11(1H)	1.42(1H)	Sidechain 0.84(3H) 0.81(3H)
Aib(3)	7.78	—	1.37 (3H) 1.02 (3H)	—	—	—	—
ddεPhe(4)	8.50	6.10(1H)	7.15(1H)	6.59(1H)	6.14(1H)	4.62(1H)	Benzylic 2.98(1H) 2.94(1H) Aromatic 7.42 (O) 7.23(M,P)
Aib(5)	8.32	—	1.50 (3H) 1.48 (3H)	—	—	—	—
γAdp(6)	7.83	2.08(2H)	Sidechain 0.97 (3H) 0.96 (3H)	3.13(1H) 3.07(1H)	—	—	—
Aib(7)	8.23	—	1.47 (6H)	—	—	—	—
-NH_2	7.60[a] 6.75[b]	—	—	—	—	—	—

[a] $CONH_2$ Proton involved in Hydrogen bonding. [b] $CONH_2$ Proton is not involved in Hydrogen bonding. Aromatic protons are shown in CH_{term} column. Chemical shift assigned by using TOCSY and ROESY spectra.

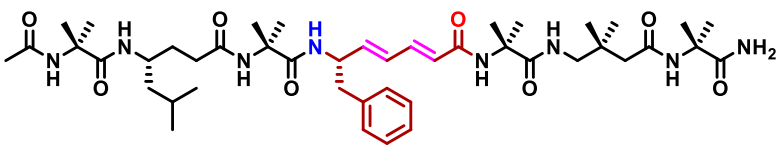
Assignment of chemical shifts using TOCSY and ROESY spectra

The amino acid residues and their positions in the peptide sequence were identified using TOCSY and ROESY spectra, respectively.

The TOCSY spectrum was used to identify amino acids in the sequence of the peptides.

A) Partial TOCSY spectrum analysis of peptide P3 in CD_3OH.

Below partial TOCSY spectrum showing the amino acid residues in peptide **P3**.



P3

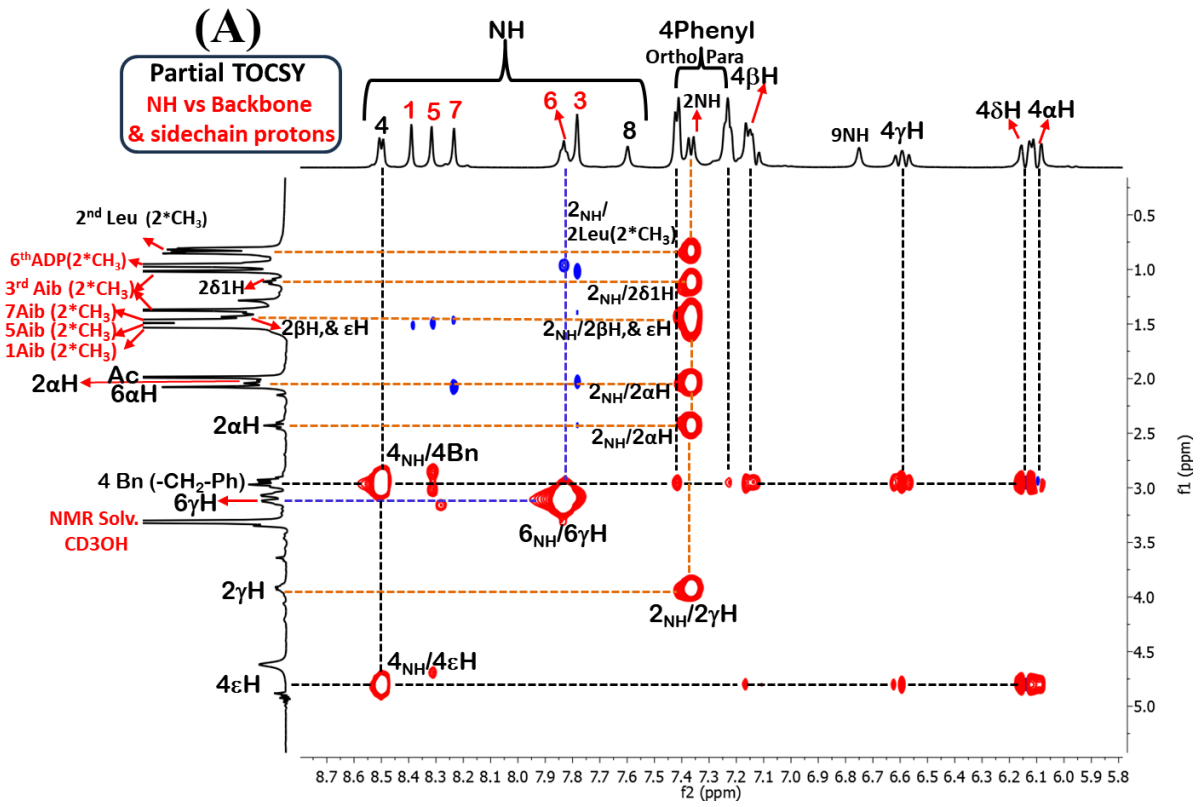
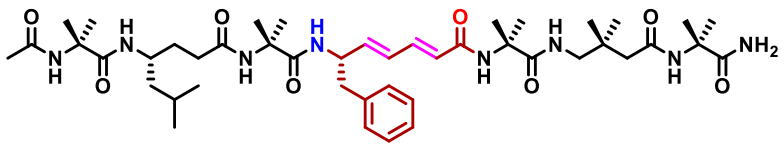


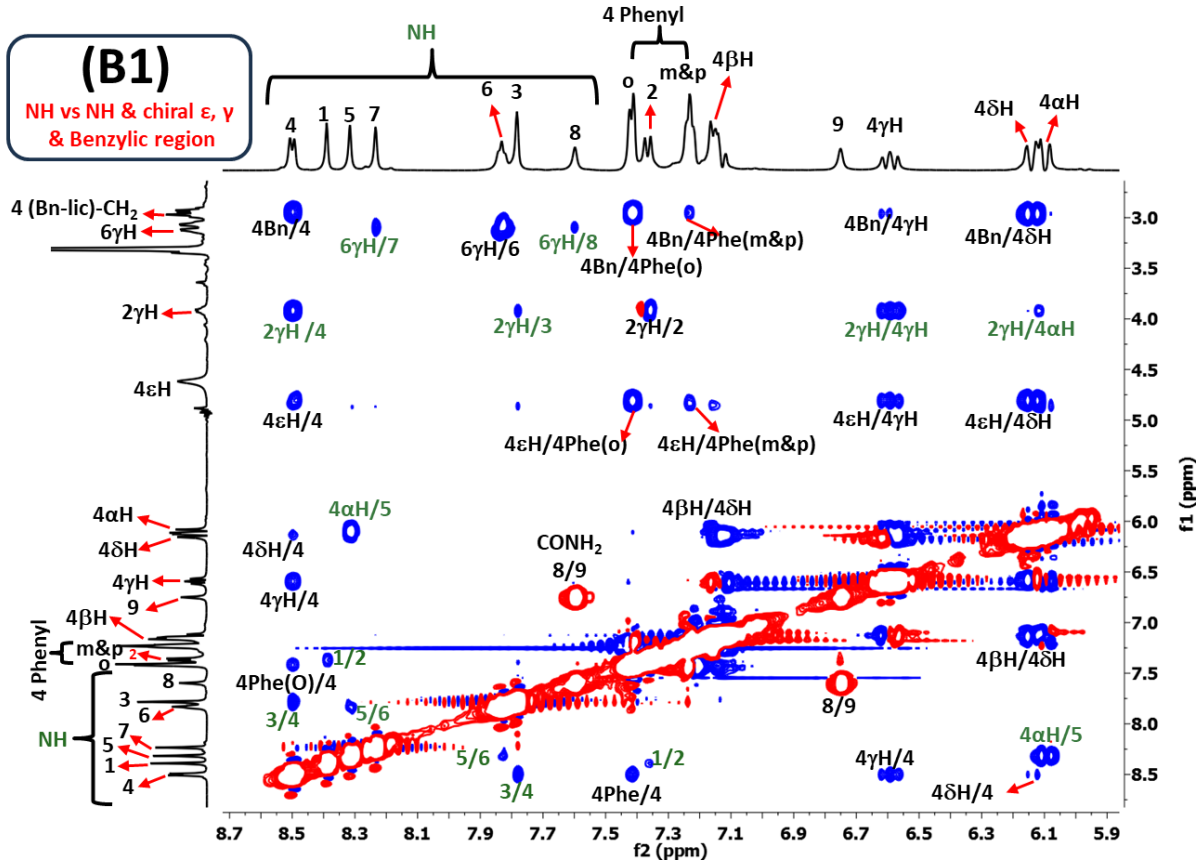
Figure S11: (**A**) Partial TOCSY spectrum (NH vs α, β, γ, Backbone & Side Chain protons) of peptide **P3** showing correlation between intra residue protons.

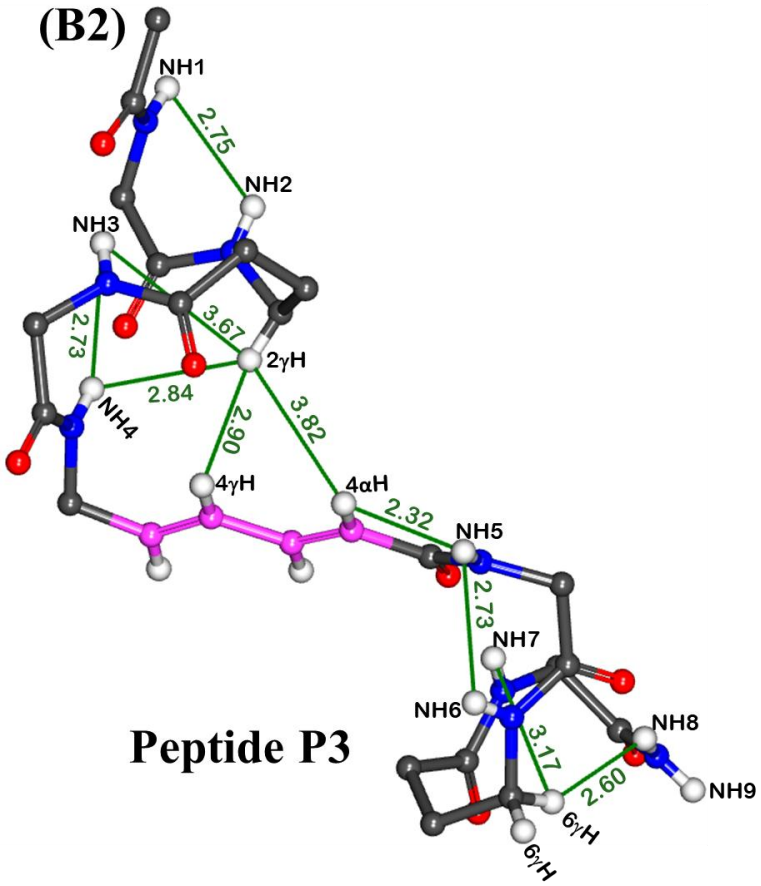
B) Partial ROESY spectrum analysis of Peptide P3 in CD_3OH.

Below partial ROESY spectrum (**B1**) depicting the **NH↔NH, NH↔C_αH/ C_βH /chiral C_γH** interactions respectively. The inter and intra-residue NOEs are marked with green and black labels respectively.



P3


(B1)
NH vs NH & chiral ε, γ
& Benzylic region
NH
4 Phenyl
4 (Bn-lic)-CH2
6γH
2γH
4εH
4αH
4δH
4γH
4βH
m&p
o
NH
4 Phenyl
4Bn/4
6γH/7
6γH/6
6γH/8
4Bn/4Phe(o)
4Bn/4Phe(m&p)
4Bn/4γH
4Bn/4δH
2γH /4
2γH/3
2γH/2
2γH/4γH
2γH/4αH
4εH/4
4εH/4Phe(o)
4εH/4Phe(m&p)
4εH/4γH
4εH/4δH
4αH/5
4βH/4δH
4δH/4
CONH2
8/9
4γH/4
1/2
4Phe(O)/4
3/4
5/6
4βH/4δH
8/9
4γH/4
4αH/5
4δH/4
4Phe/4
f1 (ppm)
f2 (ppm)


(B2)
NH1
2.75
NH2
NH3
2.73
3.67
2γH
2.84
NH4
2.90
3.82
4γH
4αH
2.32
NH5
2.73
NH7
NH6
3.17
NH8
2.60
NH9
6γH
6γH

Peptide P3

Figure S12: (**B1**) is the partial ROESY spectrum of peptide **P3** in **CD_3OH** showing sequential NOEs of NH↔NH and NH↔$C_\alpha H$/ $C_\beta H$ /chiral $C_\gamma H$. (**B2**) Crystal structure of peptide **P3** depicting the distance between inter residue NOE corresponding protons in the ROESY spectrum (B1). (inter-residue NOEs marked with green labels in the ROESY spectrum (B1)).

Table S12: NOEs Observed in **NH↔NH** and **NH↔$C_\alpha H$/ $C_\beta H$ /chiral $C_\gamma H$** region of Peptide **P3** and the distance observed between respective protons in its crystal structure.

C) List of Inter-residue NOEs (marked with green labels) in partial ROESY spectrum (**B1**) of peptide **P3** in CD_3OH.

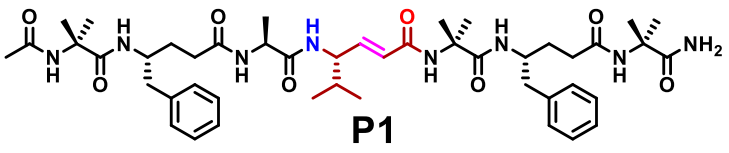
Residue	H-atom	Residue	H-atom	NOE observed	Type of NOE	Protons Region	Distance in crystal structure (Å)
Aib (1)	NH	γLeu (2)	NH	Weak	NH*i*↔NH*i+1*	**(Inter-Residue NOE) NH vs NH/$C_\alpha H$/ $C_\beta H$ /chiral $C_\gamma H$ region (Fig. S9: B1)**	2.75
γLeu (2)	γ CH	Aib (3)	NH	Weak	γCH*i*↔NH*i+1*		3.67
γLeu (2)	γ CH	(*E*,*E*)-ddεPhe (4)	NH	Strong	γCH*i*↔NH*i+2*		2.84
γLeu (2)	γ CH	(*E*,*E*)-ddεPhe (4)	γ CH	Strong	γCH*i*↔γCH*i+2*		2.90
γLeu (2)	γ CH	(*E*,*E*)-ddεPhe (4)	α CH	Weak	γCH*i*↔αCH*i+2*		3.82
Aib (3)	NH	(*E*,*E*)-ddεPhe (4)	NH	Medium	NH*i*↔NH*i+1*		2.73
(*E*,*E*)-ddεPhe (4)	α CH	Aib (5)	NH	Strong	αCH*i*↔NH*i+1*		2.32
Aib (5)	NH	Adb (6)	NH	Weak	NH*i*↔NH*i+1*		2.73
Adb (6)	γ CH	Aib (7)	NH	Medium	γCH*i*↔NH*i+1*		3.17
Adb (6)	γ CH	$CONH_2$ (NH8)	NH	Weak	γCH*i*↔NH*i+2*		2.6

D) Intra-residue NOEs (marked with **black** labels) in partial ROESY spectrum (**B1**) of peptide **P3** in CD_3OH.

Residue	H-atom	Residue	H-atom	NOE observed	Protons Region	Distance in crystal structure (Å)
γLeu (2)	γ CH	γLeu (2)	NH	Strong	**(Intra-Residue NOE)**	2.77
(*E*,*E*)-ddεPhe (4)	Benzyl CH_2	(*E*,*E*)-ddεPhe (4)	NH	Strong		2.66

(*E*,*E*)-ddεPhe (4)	Benzyl CH_2	(*E*,*E*)-ddεPhe (4)	Phenyl (o) H	Very Strong	**NH vs NH/CαH/ CβH /chiral CγH region (Fig. S9: B1)**	2.30
(*E*,*E*)-ddεPhe (4)	Benzyl CH_2	(*E*,*E*)-ddεPhe (4)	Phenyl(m&p) H	Weak		4.51 (m) 5.57 (p)
(*E*,*E*)-ddεPhe (4)	Benzyl CH_2	(*E*,*E*)-ddεPhe (4)	*γ* CH	Weak		2.31
(*E*,*E*)-ddεPhe (4)	Benzyl CH_2	(*E*,*E*)-ddεPhe (4)	*δ* CH	Very Strong		2.55
(*E*,*E*)-ddεPhe (4)	*ε* CH	(*E*,*E*)-ddεPhe (4)	NH	Medium		2.75
(*E*,*E*)-ddεPhe (4)	*ε* CH	(*E*,*E*)-ddεPhe (4)	Phenyl (o) H	Strong		2.46
(*E*,*E*)-ddεPhe (4)	*ε* CH	(*E*,*E*)-ddεPhe (4)	Phenyl(m&p) H	Weak		5.58(m) 5.94(p)
(*E*,*E*)-ddεPhe (4)	*ε* CH	(*E*,*E*)-ddεPhe (4)	*γ* CH	Medium		2.98
(*E*,*E*)-ddεPhe (4)	*ε* CH	(*E*,*E*)-ddεPhe (4)	*δ* CH	Very Strong		2.58
(*E*,*E*)-ddεPhe (4)	Phenyl(o) H	(*E*,*E*)-ddεPhe (4)	NH	Medium		3.60
(*E*,*E*)-ddεPhe (4)	*γ* CH	(*E*,*E*)-ddεPhe (4)	NH	Medium		2.75
(*E*,*E*)-ddεPhe (4)	*δ* CH	(*E*,*E*)-ddεPhe (4)	NH	Weak		3.50
(*E*,*E*)-ddεPhe (4)	*β* CH	(*E*,*E*)-ddεPhe (4)	*δ* CH	Very Strong		2.50
Adb (6)	*γ* CH	Adb (6)	NH	Very Strong		2.75
$CONH_2$ (NH8)	NH	$CONH_2$ (NH9)	NH	Very Strong		1.49

8.4) Peptide P1 2D-NMR (TOCSY & ROESY) analysis in $CDCl_3$:



P1

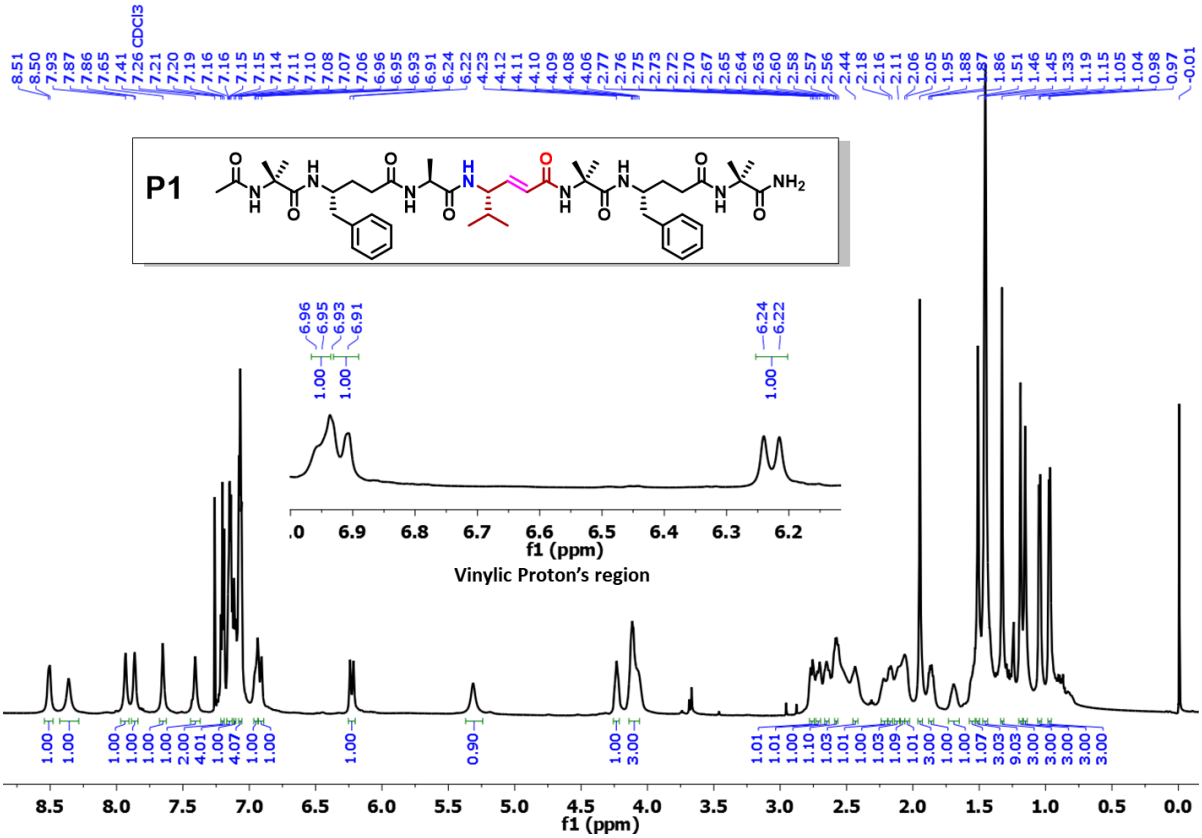


Figure S13: ^{1}H NMR spectrum of peptide **P1** in **$CDCl_3$**.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (d, *J* = 6 Hz, 1H), 8.36 (s, 1H), 7.93 (s, 1H), 7.86 (d, *J* = 6 Hz, 1H), 7.65 (s, 1H), 7.41 (s, 1H), 7.20 (m, $2H_{Aromatic}$), 7.15 (m, $4H_{Aromatic}$), 7.11 (d, *J* = 7 Hz, 1H), 7.07 (m, $4H_{Aromatic}$), 6.96 (d, *J* = 7 Hz, 1H), 6.92 (d, *J* = 15 Hz, 1H), 6.23 (d, *J* = 15 Hz, 1H), 5.31 (s, 1H), 4.23 (m, 1H), 4.12 — 4.07 (m, 3H), 2.75 (t, 1H), 2.70 (t, 1H), 2.65 (t, 1H), 2.57 (t, 1H), 2.43 (m, 1H), 2.22 (br, 1H), 2.17 (br, 1H), 2.11 (br, 1H), 2.08 (br, 1H), 2.05 (br, 1H), 1.95 (s, 3H), 1.86 (m, 1H), 1.69 (br, 1H), 1.54 (s, 1H), 1.51 (s, 3H), 1.45 (s, 9H), 1.33 (s, 3H), 1.19 (s, 3H), 1.15 (s, 3H), 1.04 (d, 3H), 0.97 (d, 3H). **MALDI (TOF/TOF)** *m/z* calculated value for $C_{46}H_{68}N_8O_8$ [M+Na^+] is 883.5052 and observed at 883.5010.

Table S13. ^{1}H NMR Chemical Shifts (ppm) of **P1** in **$CDCl_3$** at 298 K.

Residues	NH	αCH	βCH	γCH	δCH	εCH	CH_{term}
-$COCH_3$ (N-Terminus)	—	—	—	—	—	—	1.95
Aib(1)	7.65	—	1.33 (3H) 1.19 (3H)	—	—	—	—

γPhe(2)	7.11	2.43 (1H) 2.17 (1H)	2.08 (1H) 1.69 (1H)	4.07	Benzylic 2.7(1H) 2.65 (1H)	Aromatic 7.2 (P) 7.15 (O) 7.07 (M)	—
Ala(3)	7.86	4.11	1.45 (3H)	—	—	—	—
dγVal(4)	8.51	6.23	6.92	4.23	1.86	1.04 (3H) 0.97 (3H)	
Aib(5)	7.41	—	1.45 (3H) 1.15 (3H)	—	—	—	—
γPhe(6)	6.96	2.22 (1H) 2.11 (1H)	2.05 (1H) 1.54 (1H)	4.12	Benzylic 2.75 (1H) 2.57 (1H)	Aromatic 7.2 (P) 7.15 (O) 7.07 (M)	—
Aib(7)	7.93	—	1.51 (3H) 1.45 (3H)	—	—	—	—
$-NH_2$ (C-Terminus)	8.36[a] 5.31[b]	—	—	—	—	—	—

[a] Proton involved in Hydrogen bonding. [b] Proton is not involved in Hydrogen bonding. Aromatic proton indicated by (O) ortho, (P) para, and (M) Meta in $_{\varepsilon}$CH column.

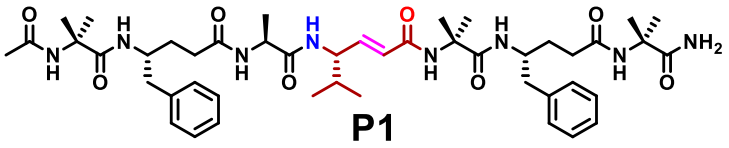
Assignment of chemical shifts using TOCSY and ROESY spectra

The amino acid residues and their positions in the peptide sequence were identified using TOCSY and ROESY spectra, respectively.

The TOCSY spectrum was used to identify amino acids in the sequence of the peptides.

A) Partial TOCSY spectrum analysis of peptide P1 in $CDCl_3$.

Below partial TOCSY spectrum showing the amino acid residues in peptide **P1**.



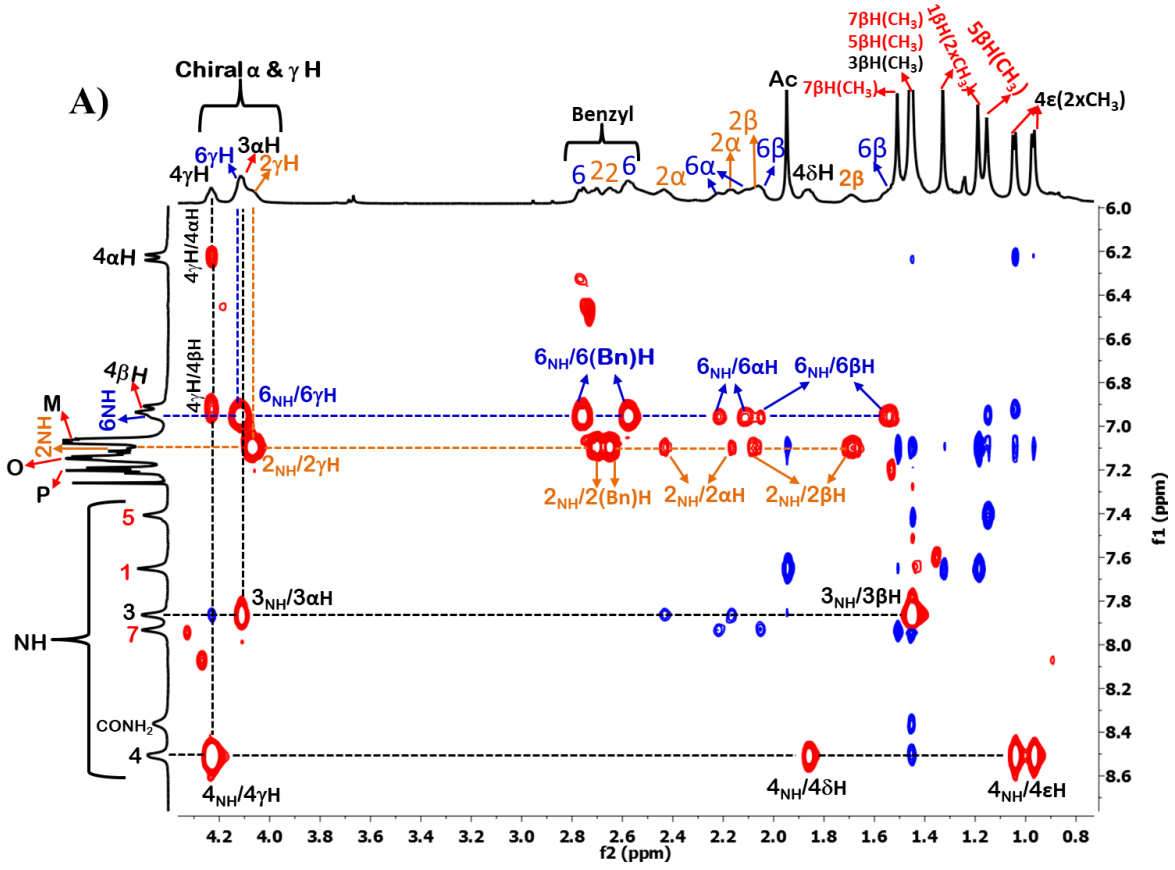
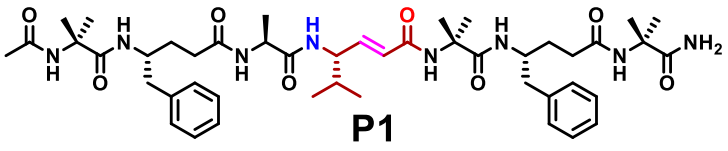
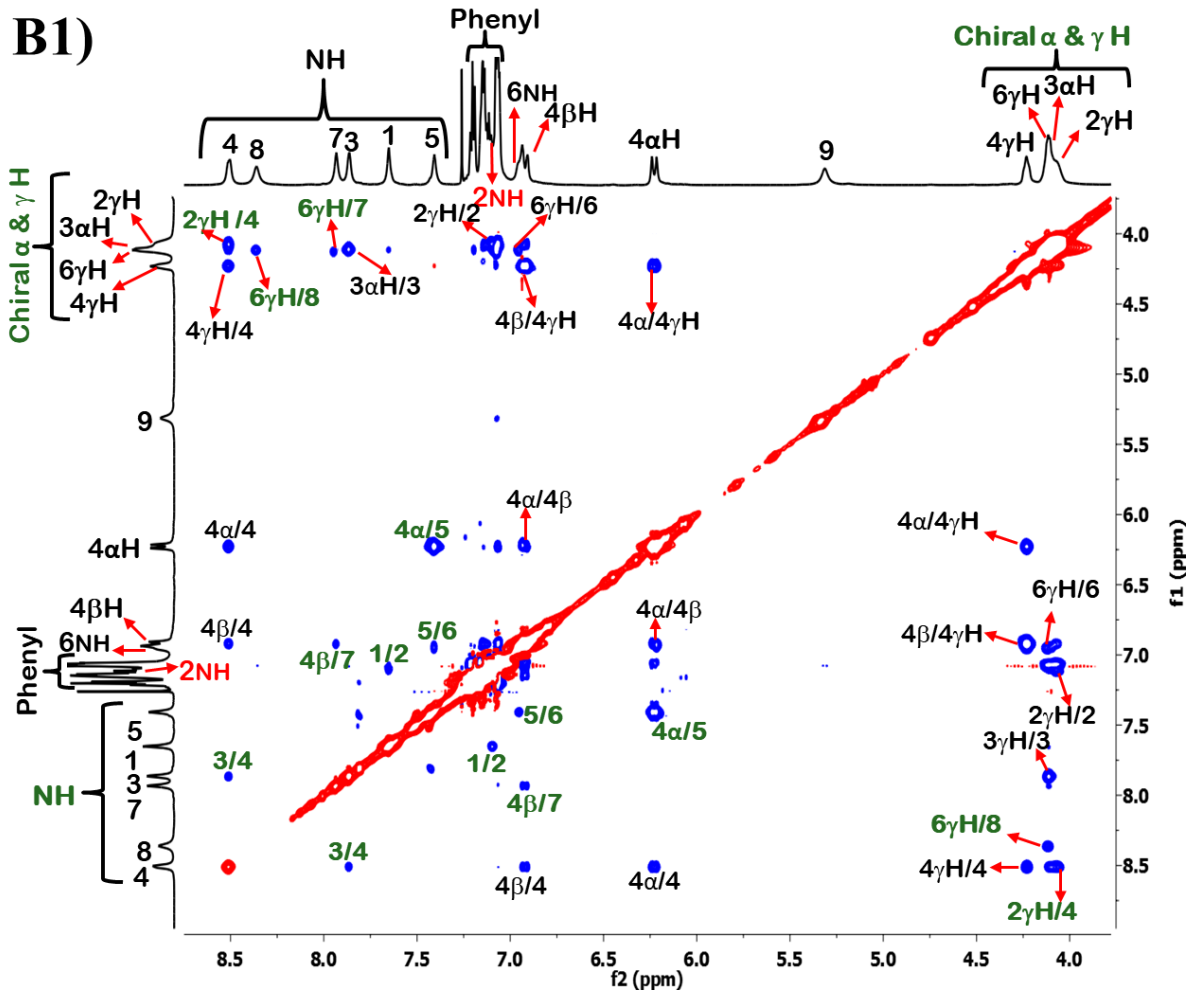


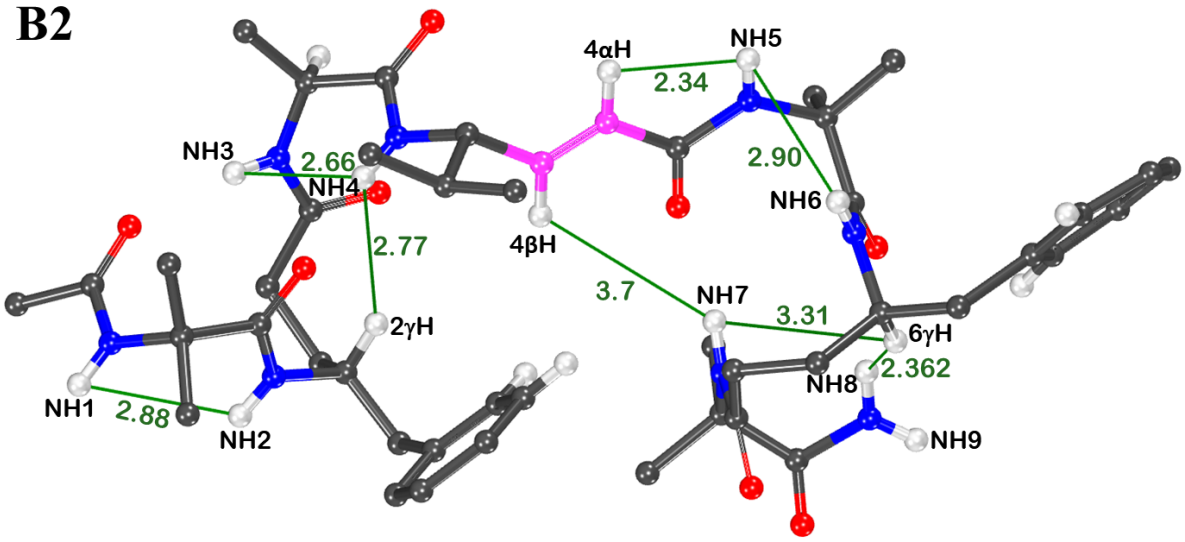
Figure S14: (**A**) Partial TOCSY spectrum (NH vs α, β, γ, Backbone & Side Chain protons) of peptide **P1** showing correlation between intra residue protons.

B) Partial ROESY spectrum analysis of Peptide P1 in $CDCl_3$.

Below ROESY spectrum (**B1**) depicting the **NH↔NH, NH↔chiral $C_\alpha H$, $C_\gamma H$** interactions respectively. The inter and intra-residue NOEs are marked with green and black labels respectively.




B1)
Phenyl
NH
Chiral α & γ H
6NH
4βH
6γH
3αH
2γH
4γH
4 8 7 3 1 5
4αH
9
2NH
2γH /4
6γH/7
2γH/2
6γH/6
6γH/8
3αH/3
4γH/4
4β/4γH
4α/4γH
4α/4β
4α/4
4α/5
5/6
4β/4
4β/7
1/2
3/4
f2 (ppm)
f1 (ppm)


B2
4αH
NH5
2.34
NH3
2.66
NH4
2.90
NH6
2.77
4βH
3.7
NH7
3.31
2γH
6γH
2.362
NH8
NH1
2.88
NH2
NH9

Peptide P1

Figure S15: (**B1**) NH vs NH, NH vs Chiral α, and γ protons region in ROESY spectrum of peptide **P1** in **$CDCl_3$**. (**B2**) Crystal structure of peptide **P1** depicting the distance between inter residue NOE corresponding protons in the ROESY spectrum (**B1**). (inter-residue NOEs marked with green labels in the ROESY spectrum (**B1**)).

Table S14: NOEs Observed in **NH↔NH** and **NH↔C_αH/ C_βH /C_γH** Region of Peptide **P1** in **$CDCl_3$** and the distance observed between respective protons in its crystal structure.

C) List of Inter-residue NOEs (marked with green labels) in partial ROESY spectrum (**B1**) of peptide **P1** in **$CDCl_3$**.						
Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**	**Distance in crystal structure (Å)**	**Type of NOE**
Aib (1)	NH	γPhe (2)	NH	Medium	2.88	NH*i*↔NH*i+1*
γPhe (2)	*γ* CH	(*E*)-dγVal (4)	NH	Strong	2.77	*γ*CH*i*↔NH*i+2*
Ala (3)	NH	(*E*)-dγVal (4)	NH	Medium	2.66	NH*i*↔NH*i+1*
(*E*)-dγVal (4)	*β* CH	Aib (7)	NH	Weak	3.7	*β*CH*i*↔NH*i+3*
(*E*)-dγVal (4)	*α* CH	Aib (5)	NH	Very Strong	2.34	*α*CH*i*↔NH*i+1*
Aib (5)	NH	γPhe (6)	NH	Weak	2.9	NH*i*↔NH*i+1*
γPhe (6)	*γ* CH	Aib (7)	NH	Weak	3.31	*γ*CH*i*↔NH*i+1*
γPhe (6)	*γ* CH	$CONH_2$(NH8)	NH	Medium	2.36	*γ*CH*i*↔NH*i+2*
D) Intra-residue NOEs (marked with **black** labels) in partial ROESY spectrum (**B1**) of peptide **P1** in **$CDCl_3$**.						
Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**		
γPhe (2)	*γ* CH	γPhe (2)	NH	Medium		
Ala (3)	*α* CH	Ala (3)	NH	Strong		
(*E*)-dγVal (4)	*α* CH	(*E*)-dγVal (4)	NH	Strong		
(*E*)-dγVal (4)	*β* CH	(*E*)-dγVal (4)	NH	Medium		

(*E*)-dγVal (4)	γ CH	(*E*)-dγVal (4)	NH	Strong
(*E*)-dγVal (4)	α CH	(*E*)-dγVal (4)	β CH	Strong
(*E*)-dγVal (4)	α CH	(*E*)-dγVal (4)	γ CH	Strong
(*E*)-dγVal (4)	β CH	(*E*)-dγVal (4)	γ CH	Very Strong
γPhe (6)	γ CH	γPhe (6)	NH	Medium

8.5) Peptide P2 2D-NMR (TOCSY & ROESY) analysis in $CDCl_3$:

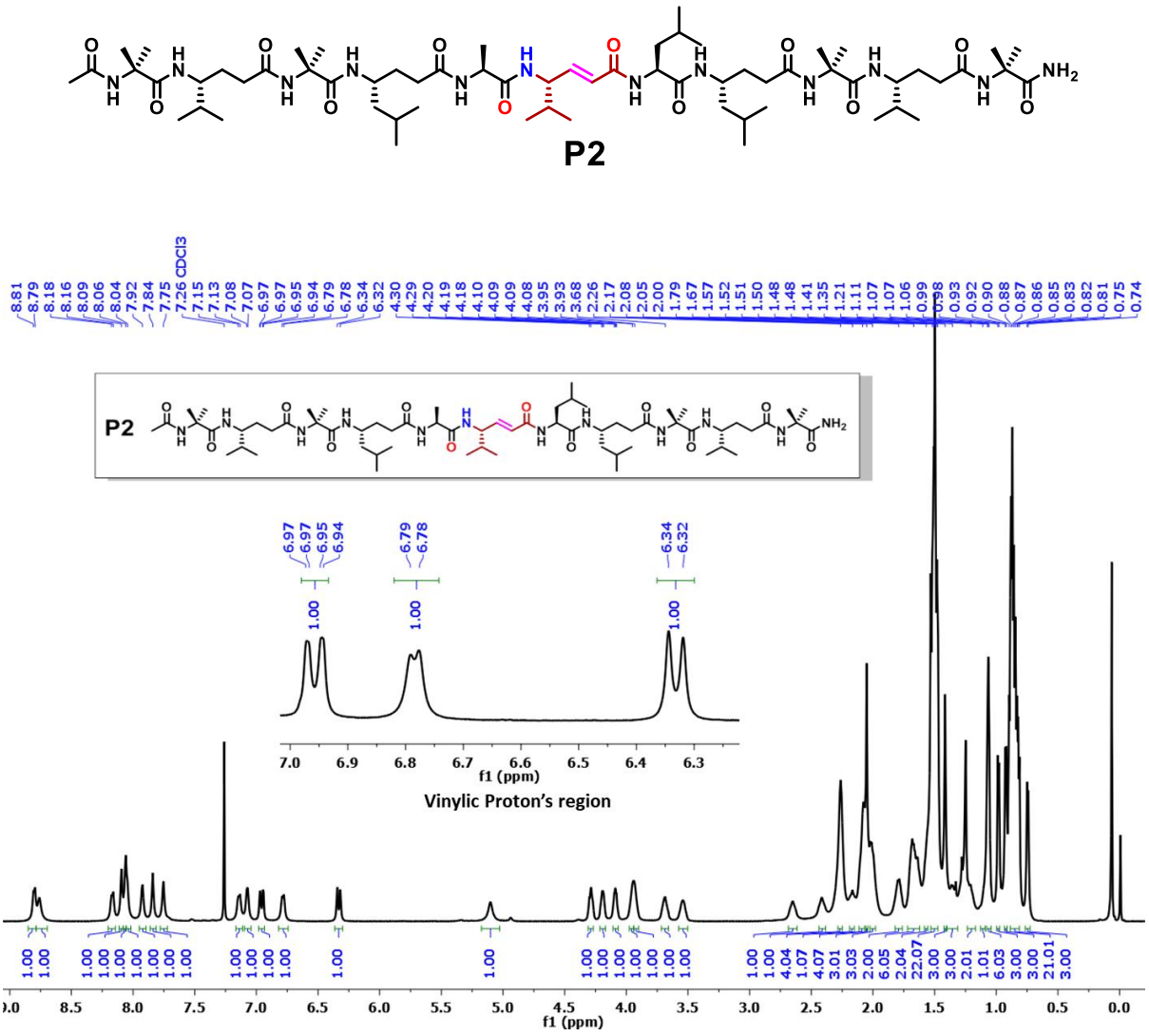


Figure S16: ^{1}H NMR spectrum of peptide **P2** in **$CDCl_3$**.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.80 (d, *J* = 6 Hz, 1H), 8.76 (s, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 8.09 (s, 1H), 8.06 (s, 1H), 8.04 (s, 1H), 7.92 (s, 1H), 7.84 (s, 1H), 7.75 (s, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 4.8 Hz, 1H), , 6.96 (dd, *J* = 15, 2.4 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.33

(d, *J* = 15 Hz, 1H), 5.10 (s, 1H), 4.29 (m, 1H), 4.19 (m, 1H), 4.09 (m, 1H), 3.95 (m, 1H), 3.93 (m, 1H), 3.68 (m, 1H), 3.54 (m, 1H), 2.65 (br, 1H), 2.41 (br, 1H), 2.26 (br, 4H), 2.17 (br, 1H), 2.08 (m, 4H), 2.05 (s, 3H), 2.00 (m, 3H), 1.79 (m, 2H), 1.67 (m, 6H), 1.57 (m, 2H), 1.50 (m, 22H), 1.41 (s, 3H), 1.35 (br, 3H), 1.21 (br, 2H), 1.11 (br, 1H), 1.07 (m, 6H), 0.99 (d, *J* = 6 Hz, 3H), 0.93 (d, *J* = 6 Hz, 3H), 0.85 (m, 21H), 0.75 (d, *J* = 6 Hz, 3H). **MALDI (TOF/TOF)** *m/z* calculated value for $C_{64}H_{116}N_{12}O_{12}$ [M+Na^+] is 1267.87 and observed at 1267.94.

Table S15. ^{1}H NMR Chemical Shifts (ppm) of **P2** in **$CDCl_3$** at 298 K.

Residues	NH	αCH	βCH	γCH	δCH	εCH	ζCH	CH_{term}
-$COCH_3$	—	—	—	—	—	—	—	2.05
Aib(1)	7.841	—	1.492(6H)	—	—	—	—	—
γVal(2)	7.14	2.26 (1H) 2.17 (1H)	1.64 (1H) 1.5 (1H)	3.54	2.10	0.86 (6H)	—	—
Aib(3)	8.09	—	2.27 (3H) 1.42 (3H)	—	—	—	—	—
γLeu(4)	8.17	2.65 (1H) 2.07(1H)	1.65 (2H)	3.95	1.56 (1H) 1.08 (1H)	2.00 (1H)	0.83(3H) 0.74(3H)	—
Ala(5)	7.92	4.09 (1H)	1.50 (3H)	—	—	—	—	—
dγVal(6)	8.80	6.33 (1H)	6.96 (1H)	4.29	2.02 (1H)	1.06 (6H)	—	—
Leu(7)	7.08	4.19 (1H)	1.67 (1H) 1.56 (1H)	1.78	0.98 (3H) 0.92 (3H)	—	—	—
γLeu(8)	6.78	2.07 (2H)	1.36 (2H)	3.93	1.20 (2H)	1.40 (1H)	0.83(6H)	—
Aib(9)	7.75	—	1.51 (6H)	—	—	—	—	—
γVal(10)	8.04	2.41 (1H) 1.99 (1H)	1.68 (2H)	3.68	1.80 (1H)	0.88 (6H)	—	—
Aib(11)	8.06	—	1.49 (6H)	—	—	—	—	—
-NH_2	8.76[a] 5.10[b]	—	—	—	—	—	—	—

[a] Proton involved in Hydrogen bonding. [b] Proton is not involved in Hydrogen bonding.

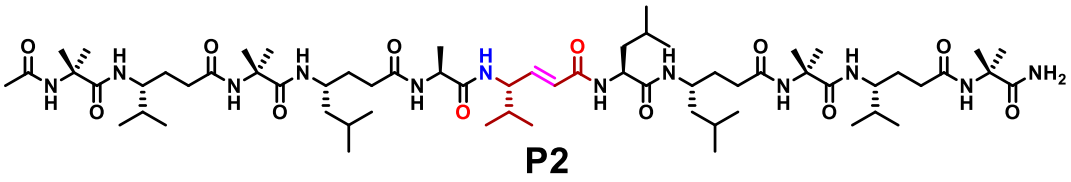
Assignment of chemical shifts using TOCSY and ROESY spectra

The amino acid residues and their positions in the peptide sequence were identified using TOCSY and ROESY spectra, respectively.

The TOCSY spectrum was used to identify amino acids in the sequence of the peptides.

A) Partial TOCSY spectrum analysis of peptide P2 in $CDCl_3$.

Below partial TOCSY spectrum showing the amino acid residues in peptide **P2**.



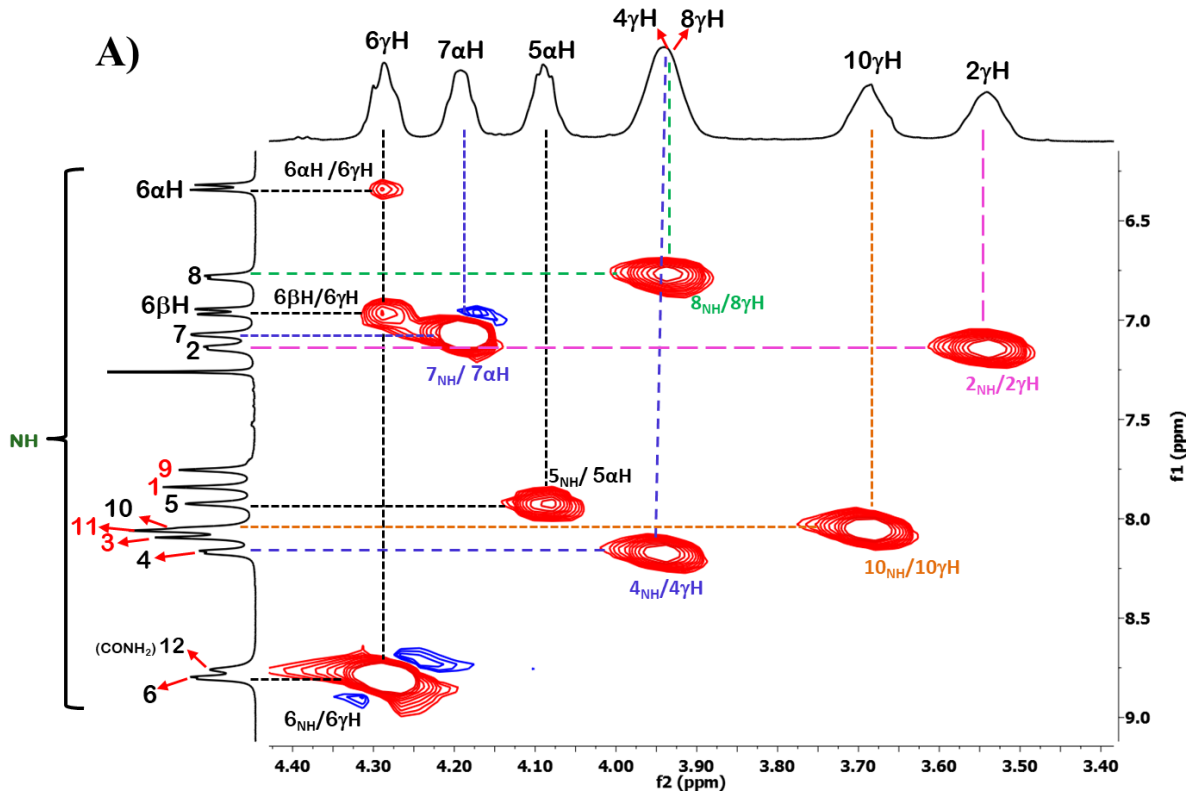


Figure S17: (**A**) Partial TOCSY spectrum (NH vs chiral α, & γ protons) of peptide **P2** showing correlation between intra residue protons.

B) Partial ROESY spectrum analysis of Peptide P2 in $CDCl_3$.

Below ROESY spectrums (**B1**) depicting the **NH↔NH, NH↔chiral $C_\alpha H$, $C_\gamma H$** interactions respectively. The inter and intra-residue NOEs are marked with green and black labels respectively.

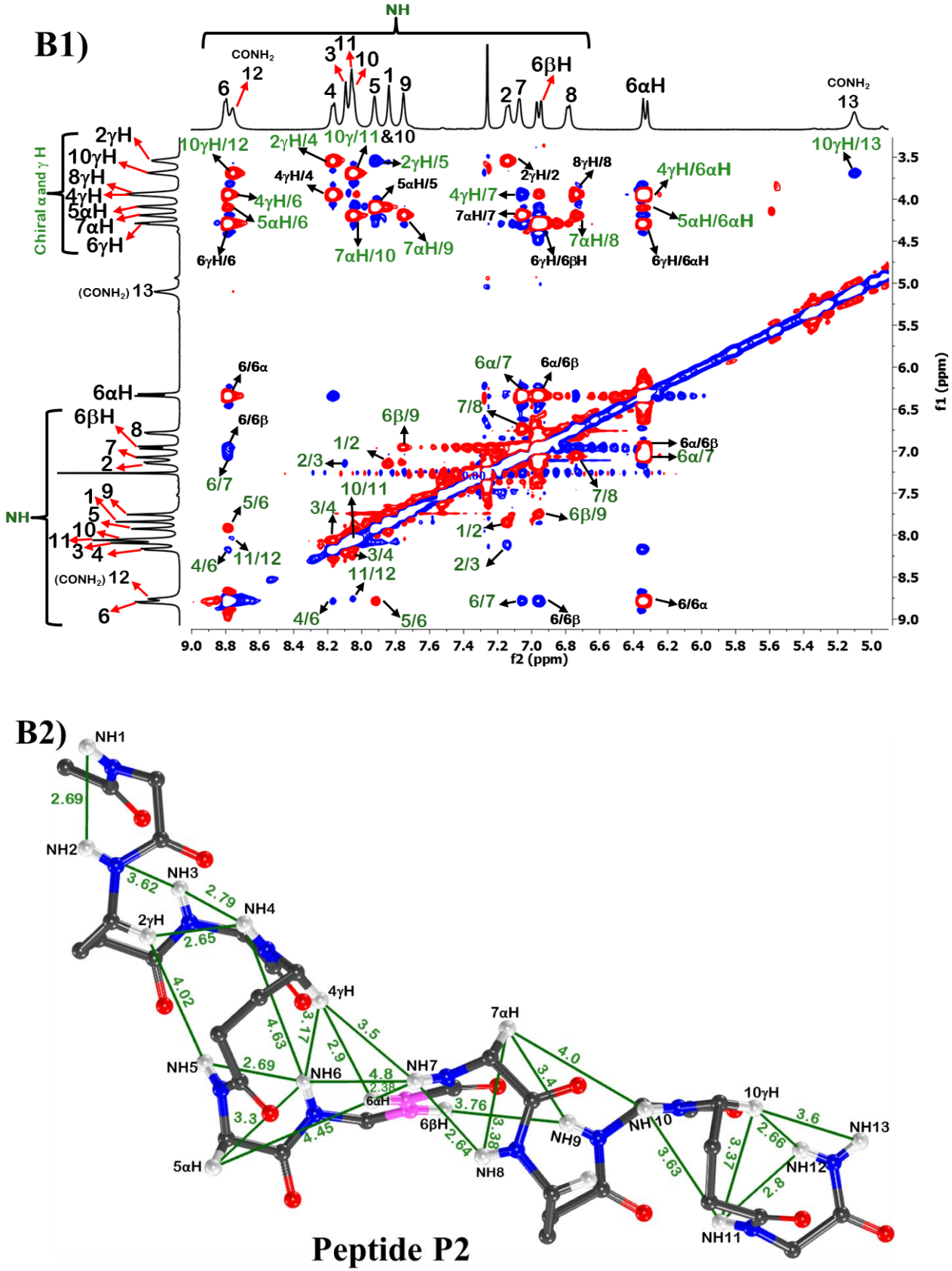


Figure S18: (**B1**) NH vs NH, NH vs Chiral α, and γ protons region in ROESY spectrum of peptide **P2** in **CDCl3**. (**B2**) Crystal structure of peptide **P2** depicting the distance between inter

residue NOE corresponding protons in the ROESY spectrum (**B1**). (inter-residue NOEs marked with green labels in the ROESY spectrum (**B1**)).

Table S16: NOEs Observed in **NH↔NH** and **NH↔C_αH/ C_βH /C_γH** Region of Peptide **P2** in **$CDCl_3$** and the distance observed between respective protons in its crystal structure.

C) List of Inter-residue NOEs (marked with green labels) in partial ROESY spectrum (**B1**) of peptide **P2** in **$CDCl_3$**.						
Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**	**Distance in crystal structure (Å)**	**Type of NOE**
Aib (1)	NH	γVal (2)	NH	Medium	2.69	NH*i*↔NH*i+1*
γVal (2)	NH	Aib (3)	NH	Weak	3.62	NH*i*↔NH*i+1*
γVal (2)	*γ* CH	γLeu (4)	NH	Strong	2.65	*γ*CH*i*↔NH*i+2*
γVal (2)	*γ* CH	Ala (5)	NH	Medium	4.02	*γ*CH*i*↔NH*i+3*
Aib (3)	NH	γLeu (4)	NH	Medium	2.79	NH*i*↔NH*i+1*
γLeu (4)	NH	(*E*)-dγVal (6)	NH	Weak	4.63	NH*i*↔NH*i+2*
γLeu (4)	*γ* CH	(*E*)-dγVal (6)	NH	Strong	3.17	*γ*CH*i*↔NH*i+2*
γLeu (4)	*γ* CH	(*E*)-dγVal (6)	*α* CH	Very Strong	2.9	*γ*CH*i*↔*α*CH*i+2*
γLeu (4)	*γ* CH	Leu (7)	NH	Strong	3.5	*γ*CH*i*↔NH*i+3*
Ala (5)	NH	(*E*)-dγVal (6)	NH	Medium	2.69	NH*i*↔NH*i+1*
Ala (5)	*α* CH	(*E*)-dγVal (6)	NH	Medium	3.3	*α*CH*i*↔NH*i+1*
Ala (5)	*α* CH	(*E*)-dγVal (6)	*α* CH	Weak	4.45	*α*CH*i*↔*α*CH*i+1*
(*E*)-dγVal (6)	NH	Leu (7)	NH	Weak	4.8	NH*i*↔NH*i+1*
(*E*)-dγVal	*β* CH	Aib (9)	NH	Medium	3.76	*β*CH*i*↔NH*i+3*

(6)						
(*E*)-dγVal (6)	*α* CH	Leu (7)	NH	Very Strong	2.38	*α*CH*i*↔NH*i+1*
Leu (7)	NH	γLeu (8)	NH	Medium	2.64	NH*i*↔NH*i+1*
Leu (7)	*α* CH	γLeu (8)	NH	Medium	3.38	*α*CH*i*↔NH*i+1*
Leu (7)	*α* CH	Aib (9)	NH	Strong	3.4	*α*CH*i*↔NH*i+2*
Leu (7)	*α* CH	γVal (10)	NH	Strong	4.0	*α*CH*i*↔NH*i+3*
γVal (10)	NH	Aib (11)	NH	Strong	3.63	NH*i*↔NH*i+1*
γVal (10)	*γ* CH	Aib (11)	NH	Strong	3.37	*γ*CH*i*↔NH*i+1*
γVal (10)	*γ* CH	$CONH_2$ (NH12)	NH	Strong	2.66	*γ*CH*i*↔NH*i+2*
γVal (10)	*γ* CH	$CONH_2$ (NH13)	NH	Medium	3.6	*γ*CH*i*↔NH*i+3*
Aib (11)	NH	$CONH_2$ (NH12)	NH	Weak	2.8	NH*i*↔NH*i+1*

D) Intra-residue NOEs (marked with **black** labels) in partial ROESY spectrum (**B1**) of peptide **P2** in **$CDCl_3$**.

Residue	**H-atom**	**Residue**	**H-atom**	**NOE observed**
γVal (2)	*γ* CH	γVal (2)	NH	Strong
γLeu (4)	*γ* CH	γLeu (4)	NH	Strong
Ala (5)	*α* CH	Ala (5)	NH	Very Strong
(*E*)-dγVal (6)	*γ* CH	(*E*)-dγVal (6)	NH	Strong
(*E*)-dγVal (6)	*γ* CH	(*E*)-dγVal (6)	*β* CH	Very Strong
(*E*)-dγVal (6)	*γ* CH	(*E*)-dγVal (6)	*α* CH	Strong
(*E*)-dγVal (6)	*β* CH	(*E*)-dγVal (6)	NH	Medium
(*E*)-dγVal (6)	*α* CH	(*E*)-dγVal (6)	NH	Strong
(*E*)-dγVal (6)	*α* CH	(*E*)-dγVal (6)	*β* CH	Strong
Leu (7)	*α* CH	Leu (7)	NH	Strong
γLeu (8)	*γ* CH	γLeu (8)	NH	Strong
γVal (10)	*γ* CH	γVal (10)	NH	Strong

9) HPLC Trace of Peptides P1-P3:

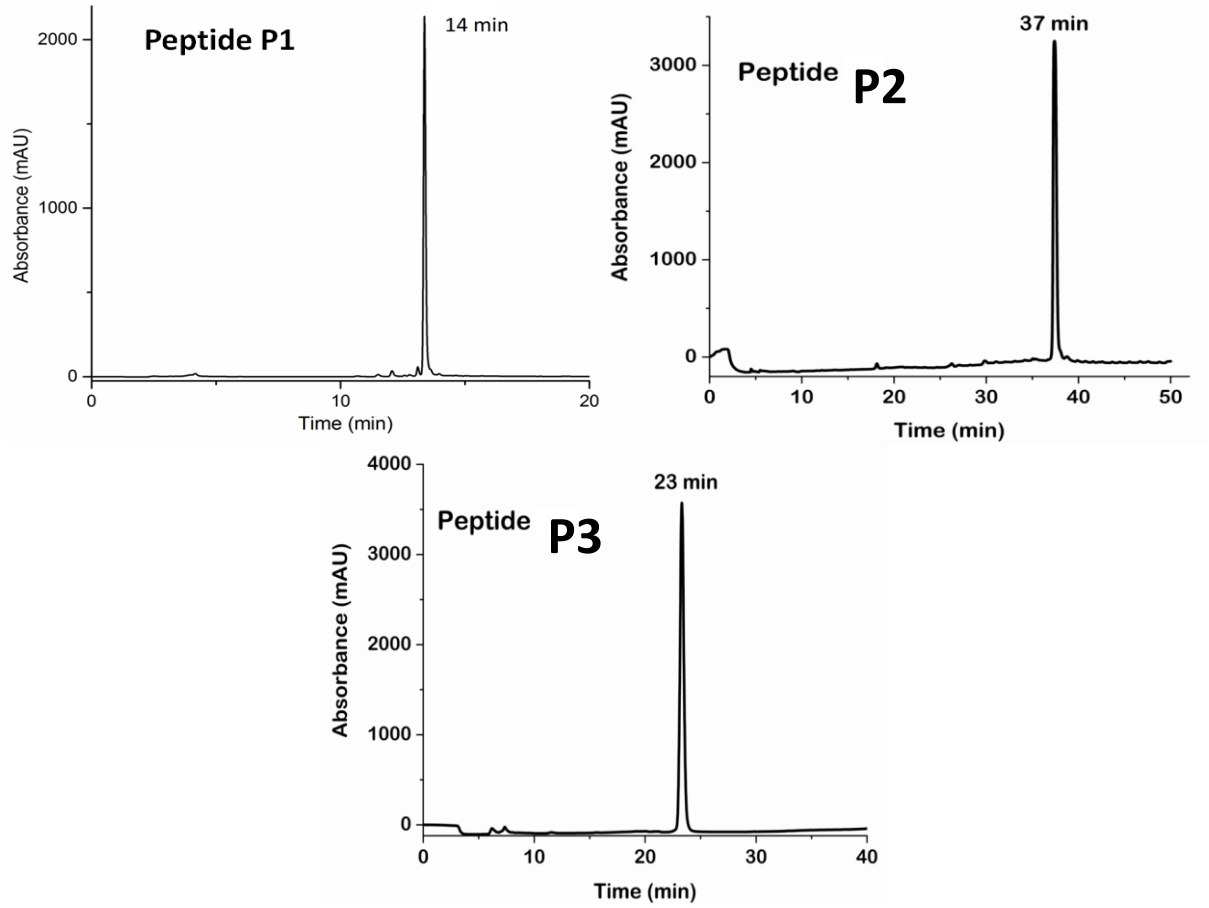


Figure S19: Reverse-phase-HPLC profiles of peptides **P1**, **P2** and **P3** on a C-18 column using methanol/water gradients at a flow rate of 2.0 mL/min. Eluted compounds were detected by the UV absorbance at 220 nm.

10) CD Spectroscopy of peptides P1-P3

10.1) CD Spectroscopy of Peptide P1-P3 in Methanol (CH_3OH):

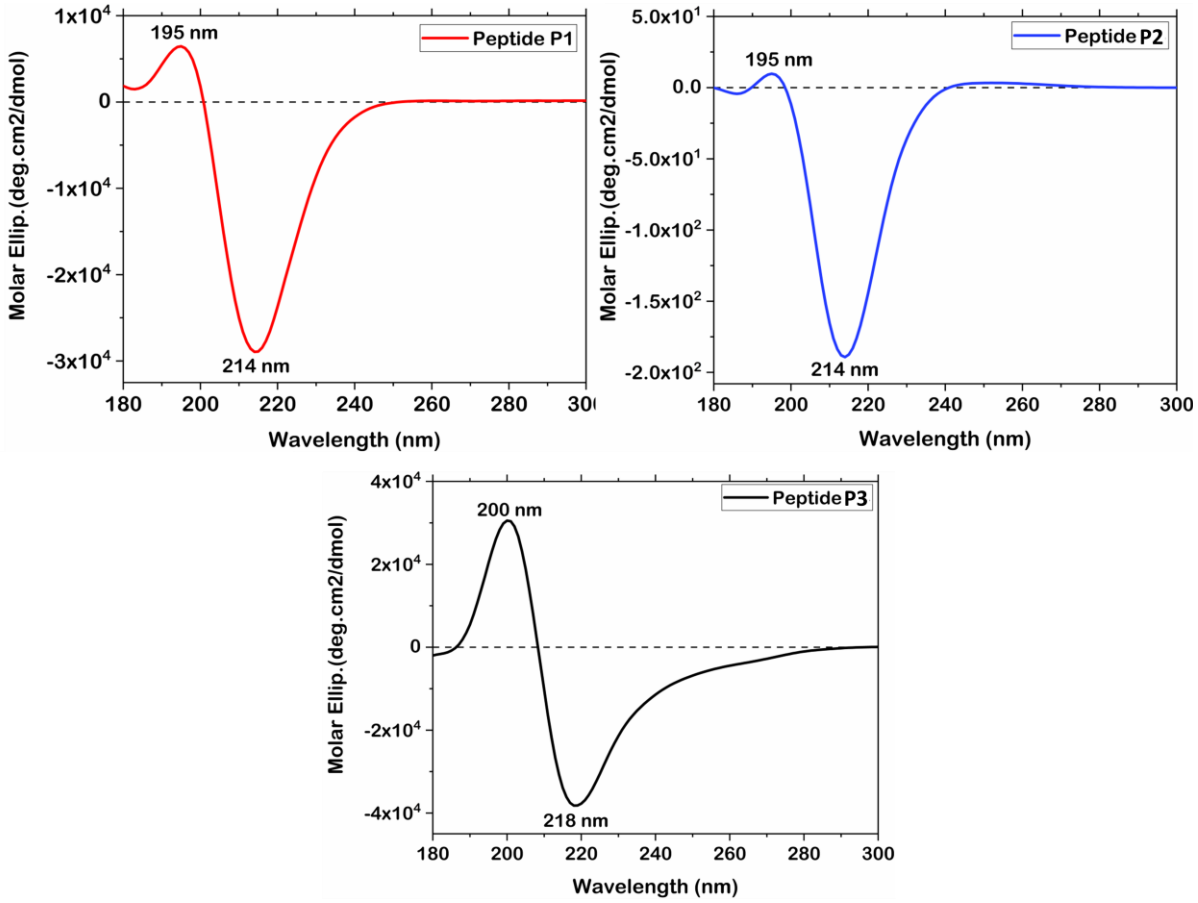


Fig S20: CD Spectroscopy of peptides **P1, P2** and **P3** in Methanol (CH_3OH) at a concentration of 1mg/mL.

10.2) CD Spectroscopy of Peptide P1 and P2 in Chloroform ($CHCl_3$):

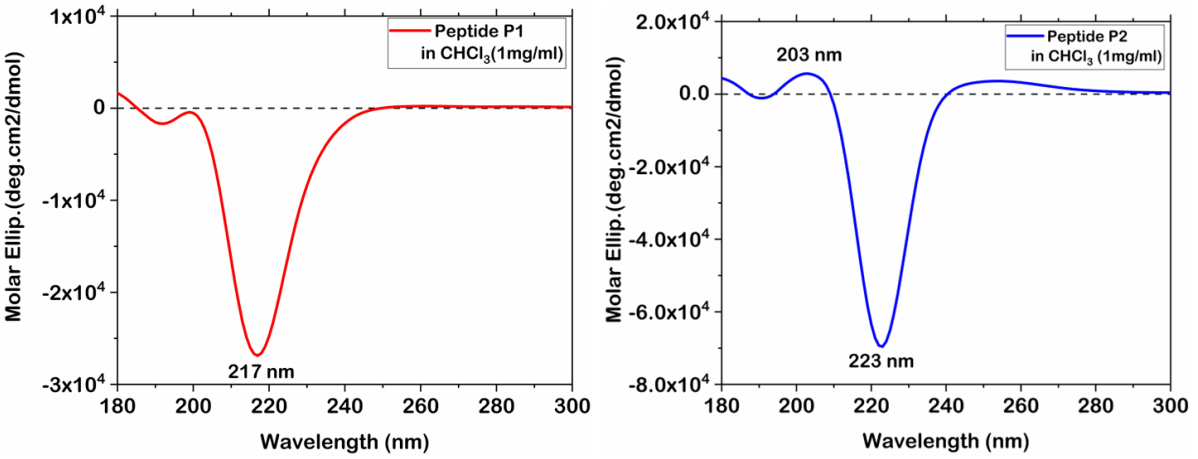


Fig S21: CD Spectroscopy of peptides **P1** and **P2** in Chloroform ($CHCl_3$) at a concentration of 1mg/mL. (Peptide P3 is insoluble in $CHCl_3$)

11) Superimposed structures of Peptide ‘P3’ and '434 repressor DNA-binding domain' protein

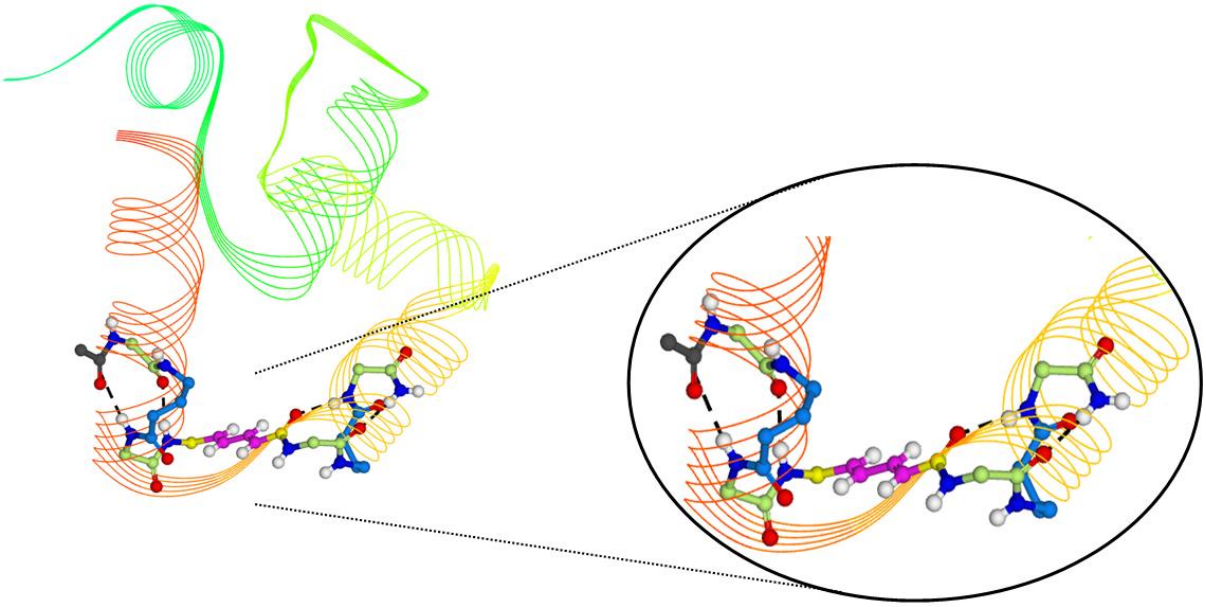
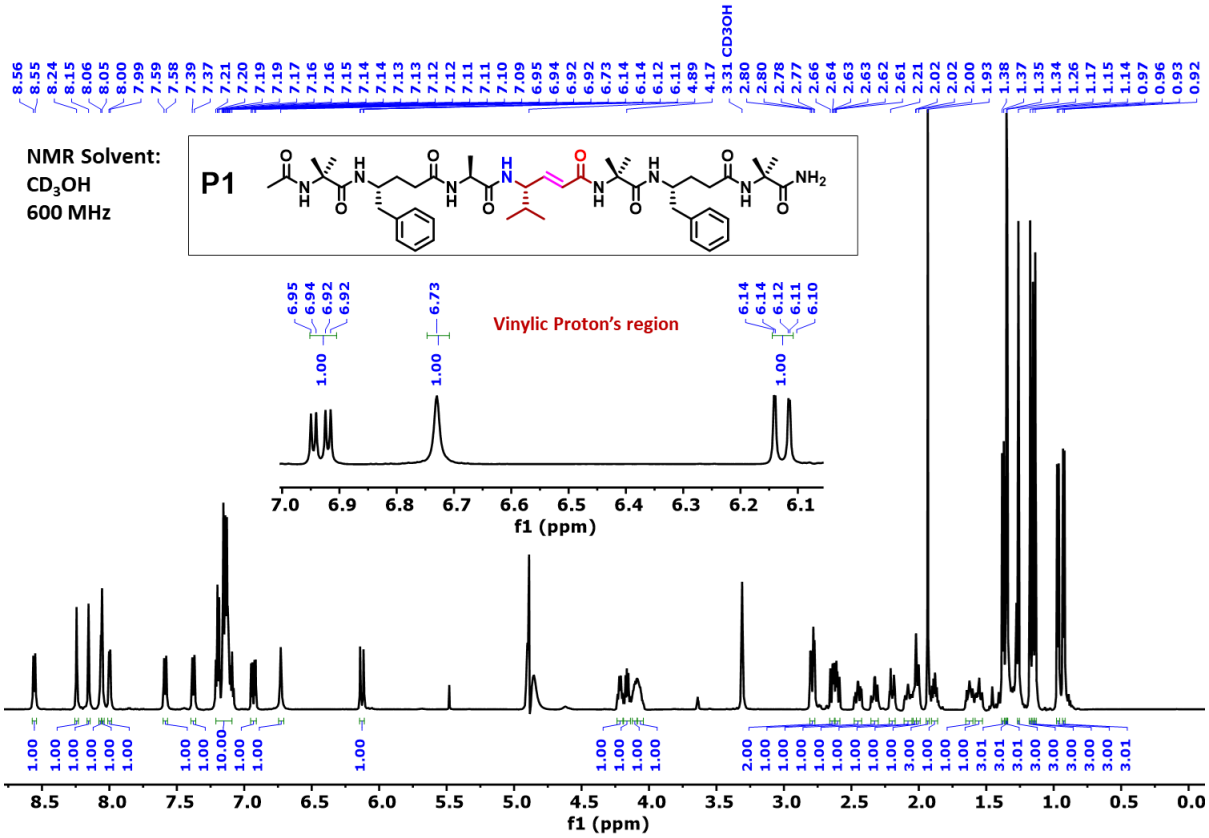


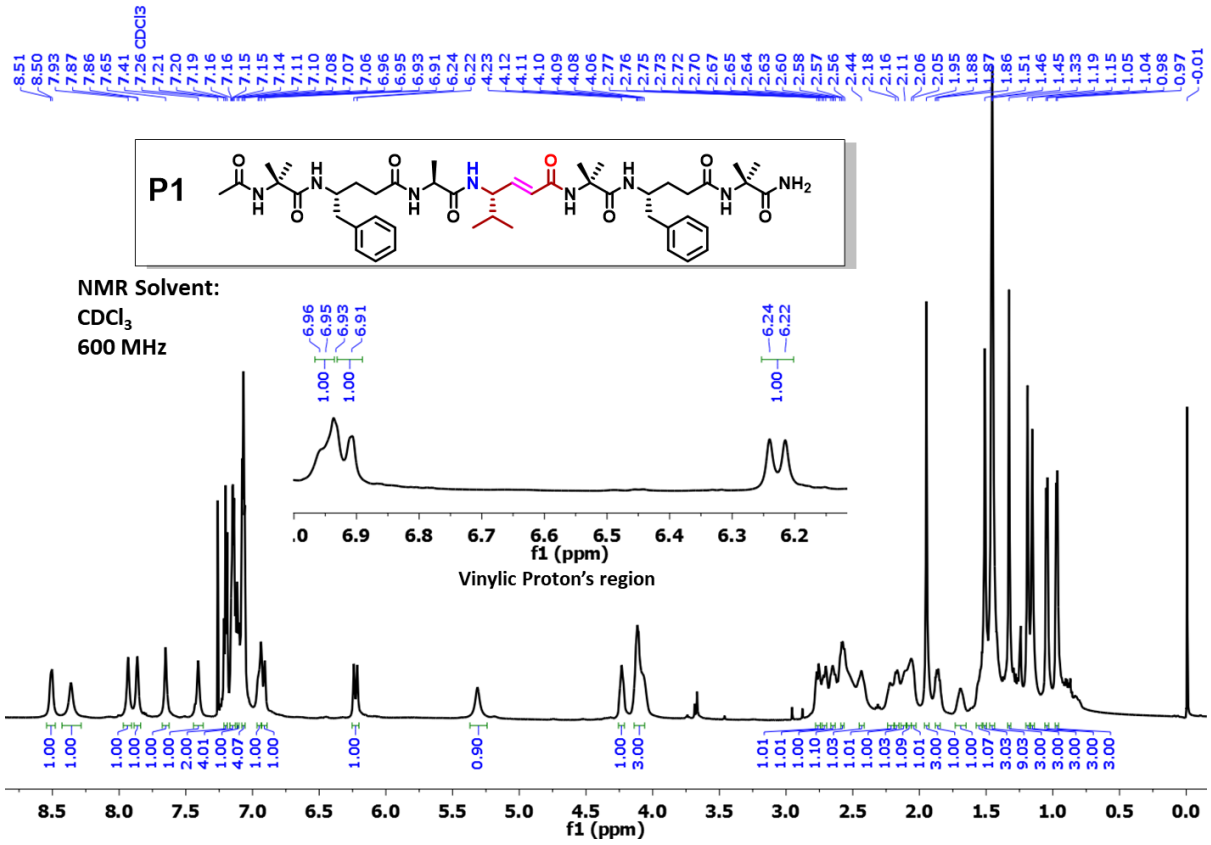
Figure S22: Superimposed crystal structure of peptide P3 (shown in ball and stick model) with the solution NMR structure of '434 repressor DNA-binding domain' (shown in line ribbon) protein in the region of Helix-Turn-Helix motif along residues 8 to 21 (**PDB DOI: https://doi.org/10.2210/pdb2r63/pdb**)

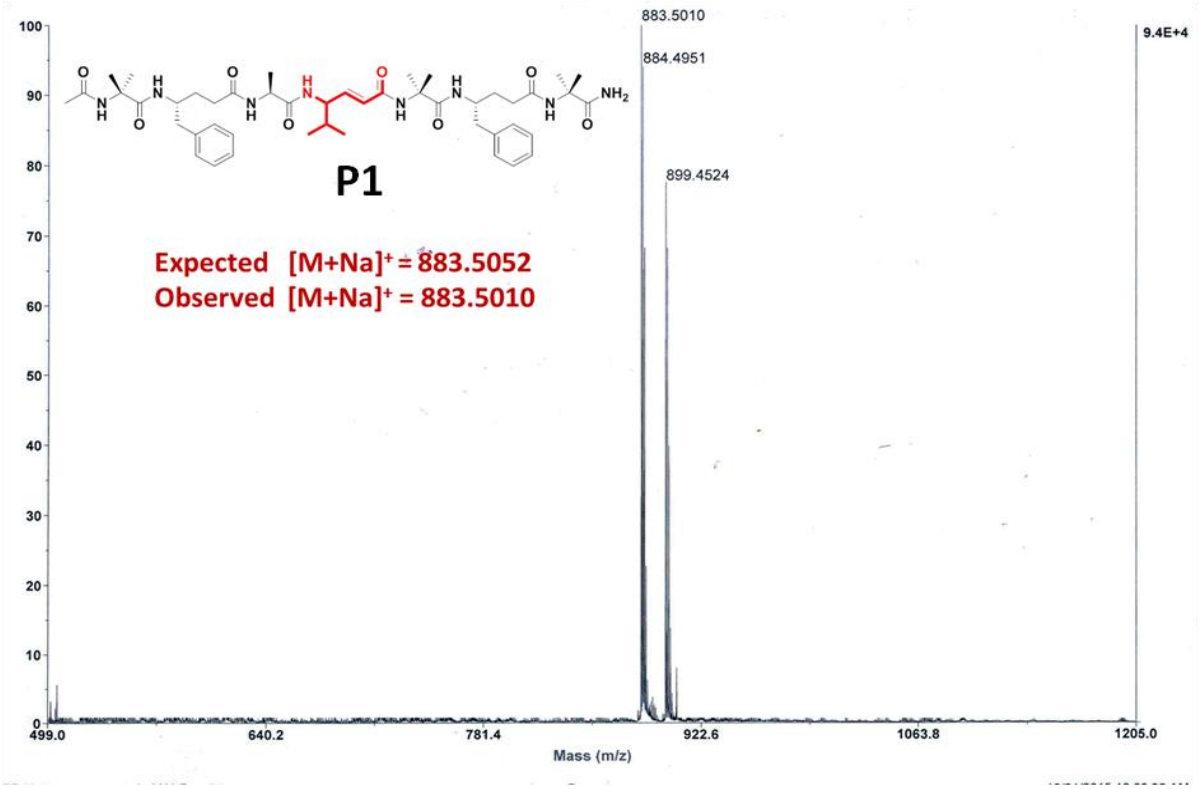
12) 1H NMR, and Mass Spectra of Peptides P1-P3 and monomers:

12.1) Peptide P1 ^{1}HNMR in CD_3OH:

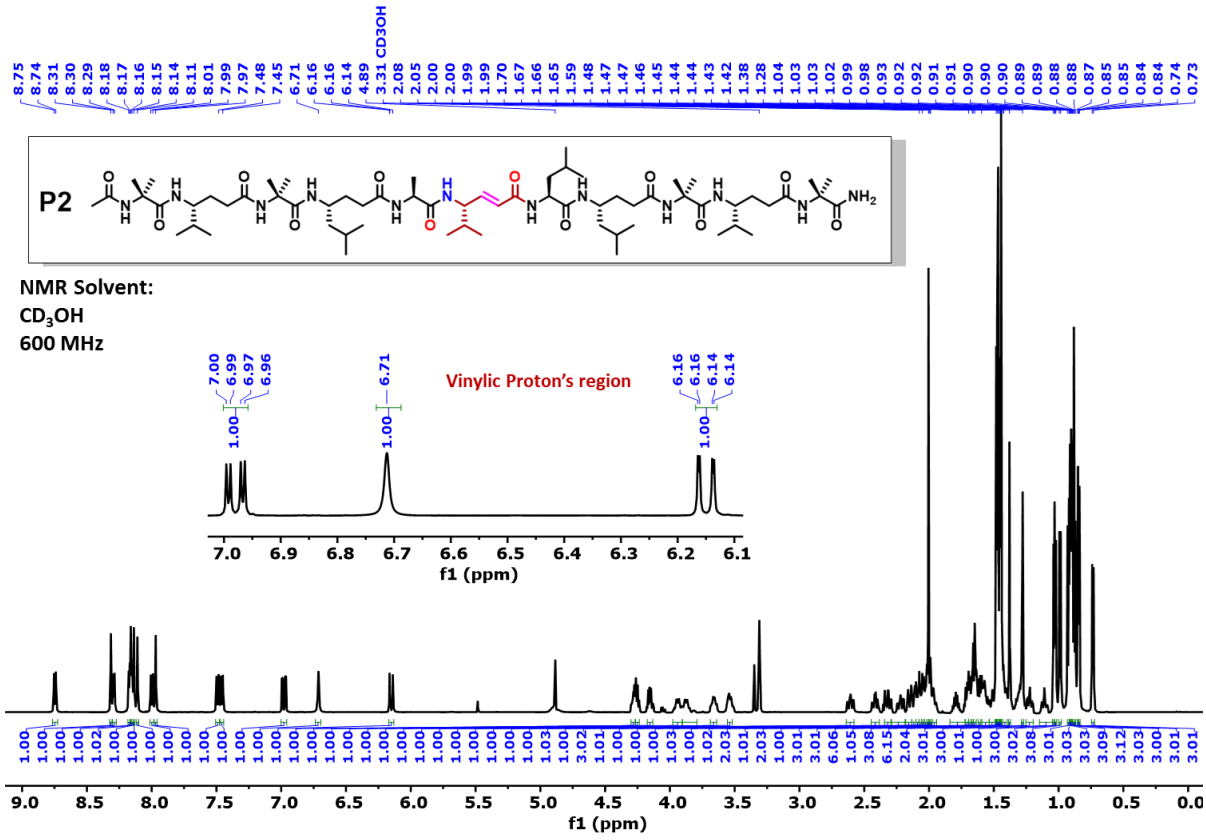


Peptide P1 ^{1}HNMR in $CDCl_3$:

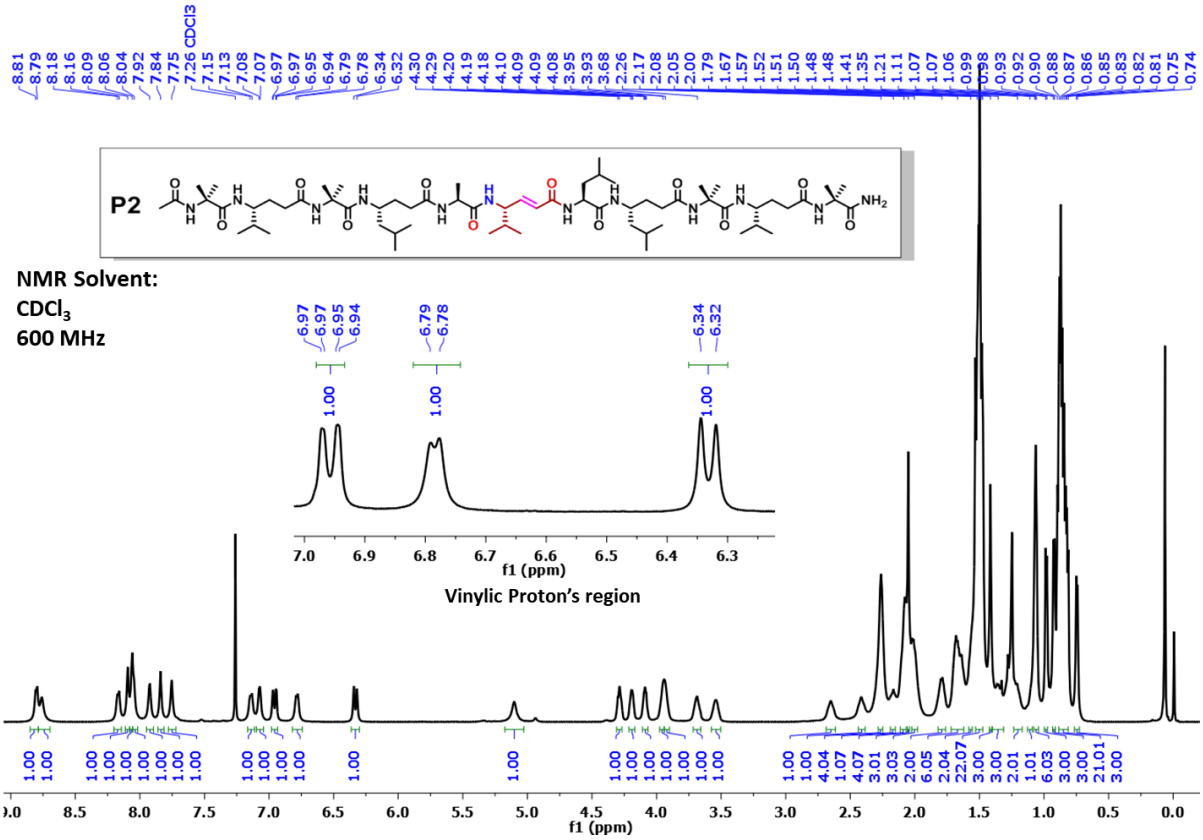


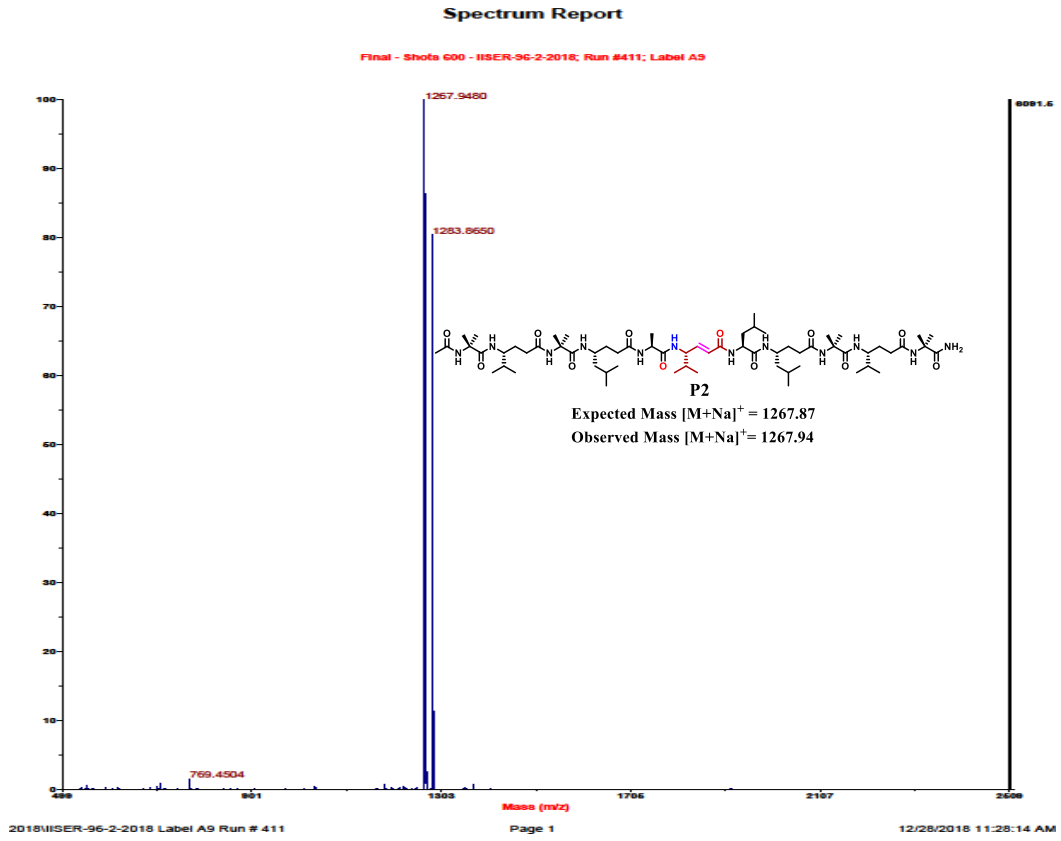


Peptide P2 ^{1}HNMR in CD_3OH:

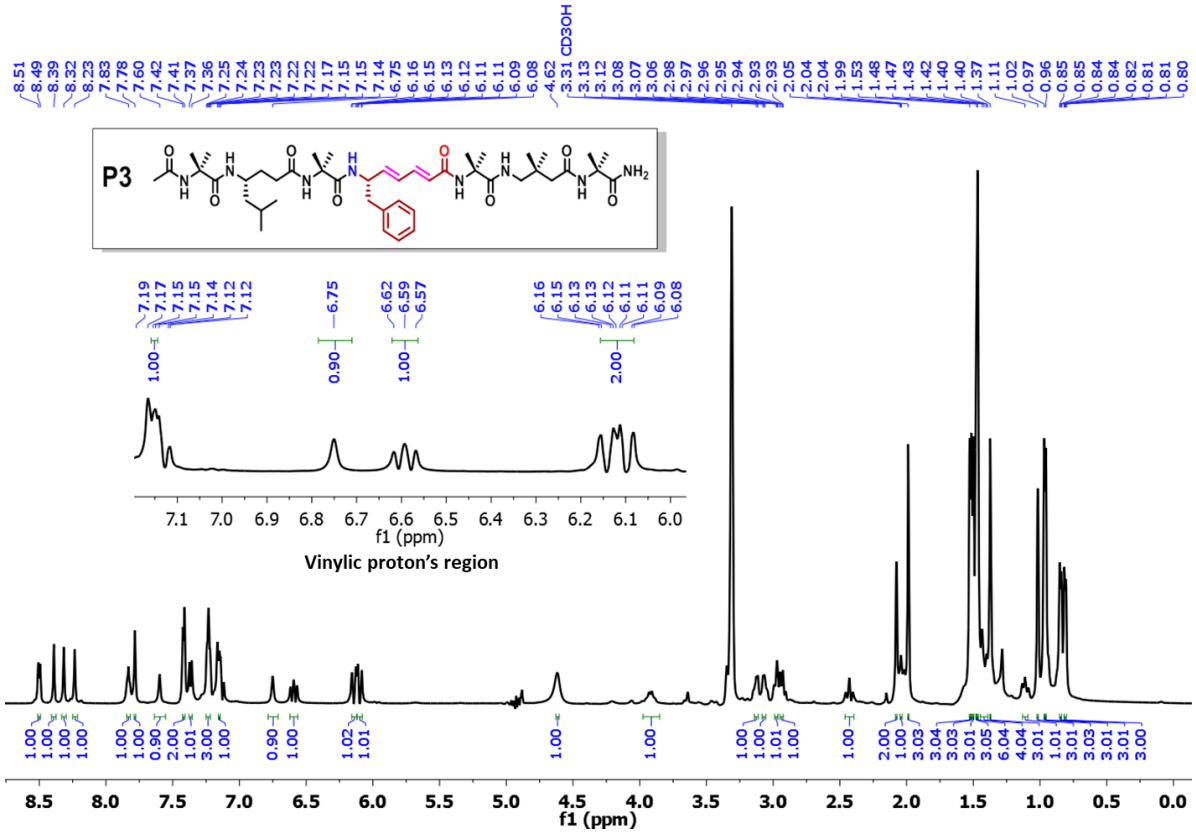


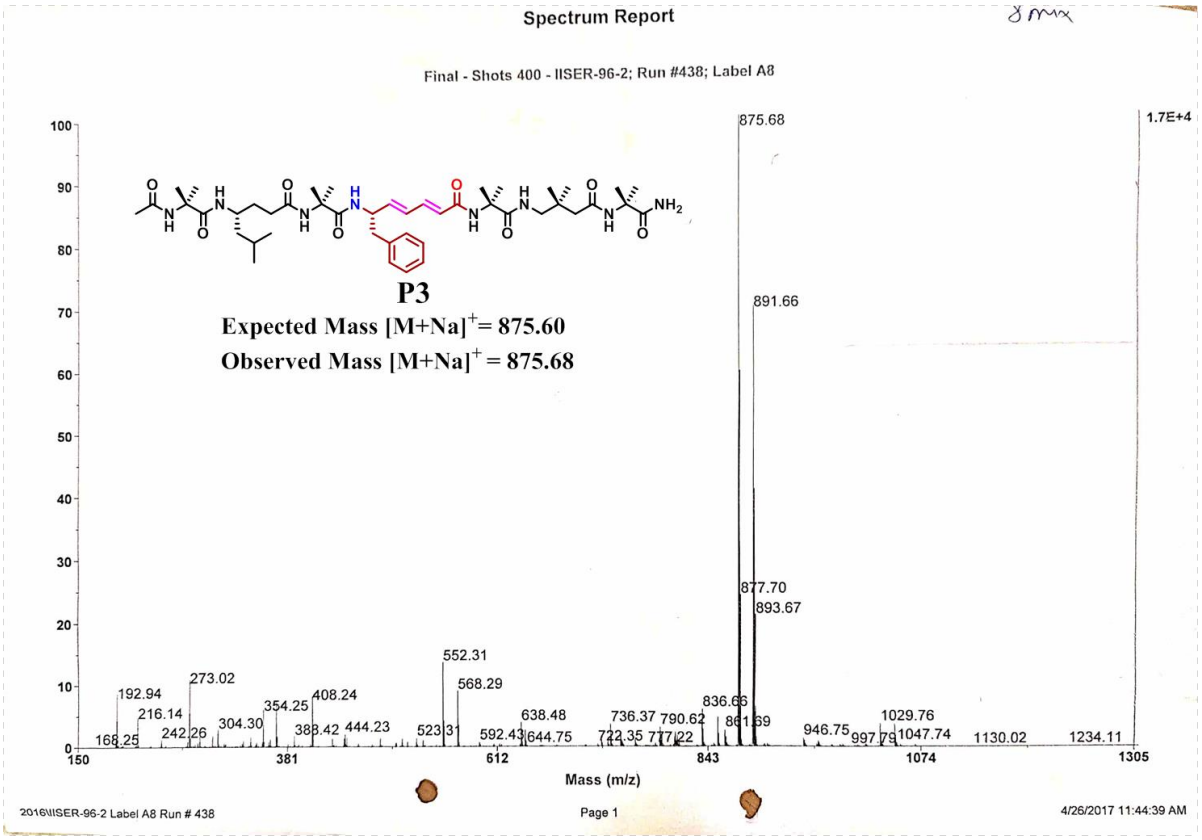
Peptide P2 ^{1}HNMR in $CDCl_3$:



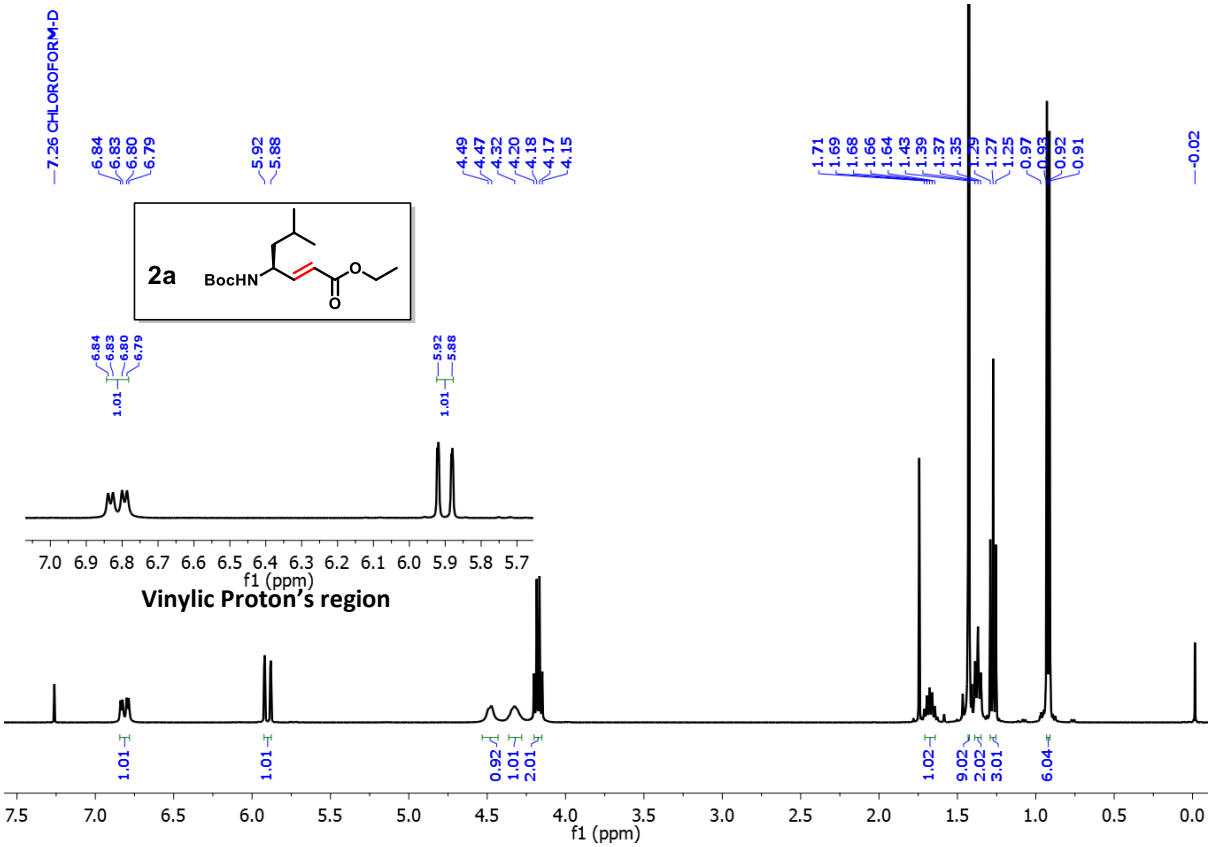


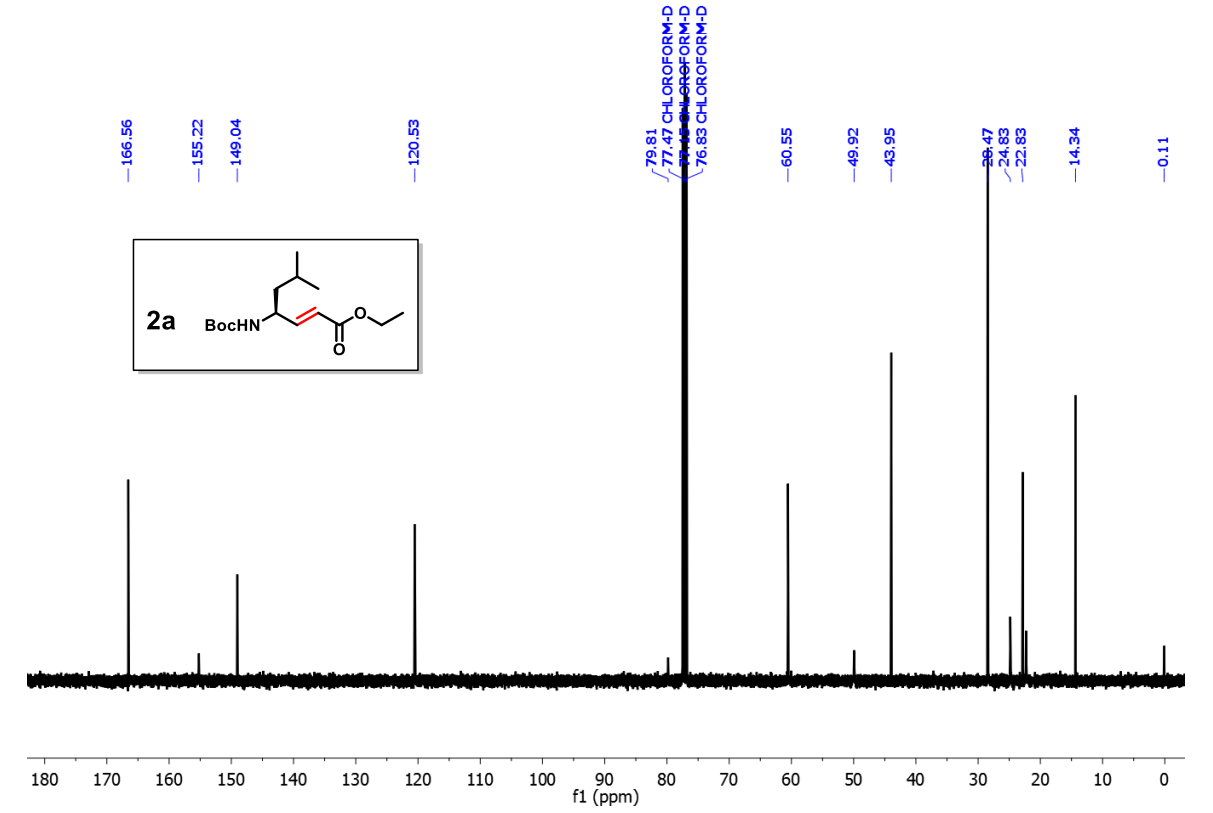
Peptide P3 ^{1}HNMR in CD_3OH:

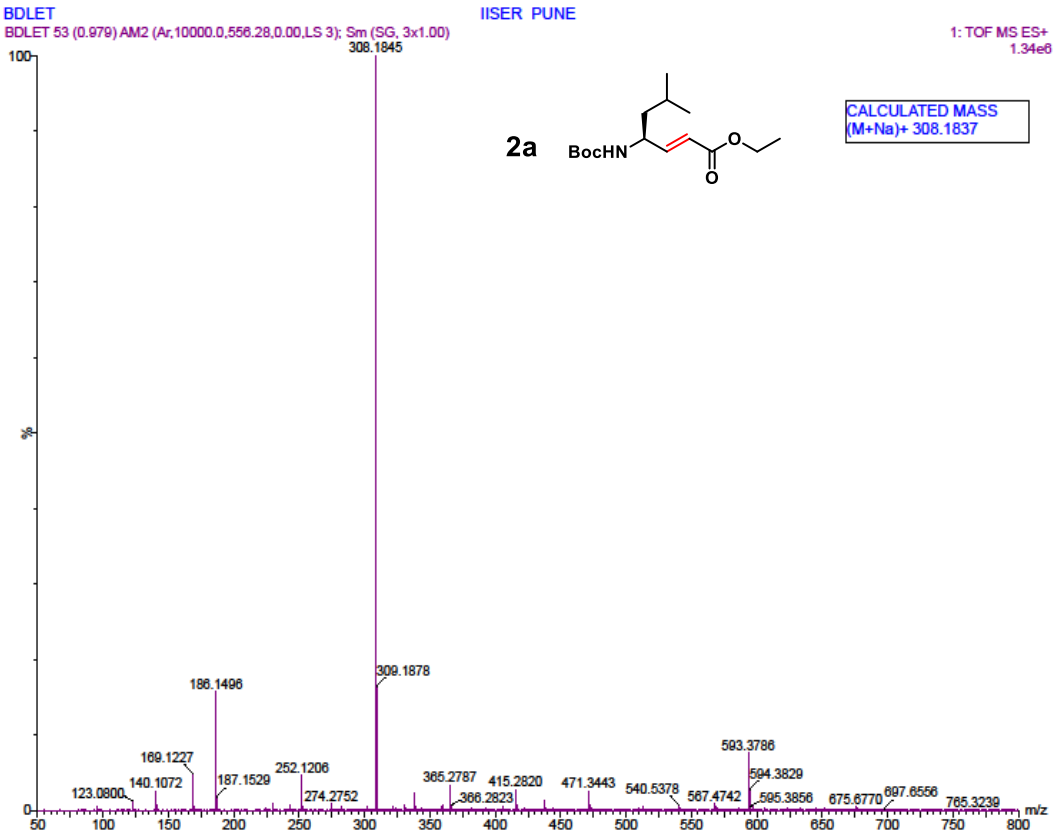


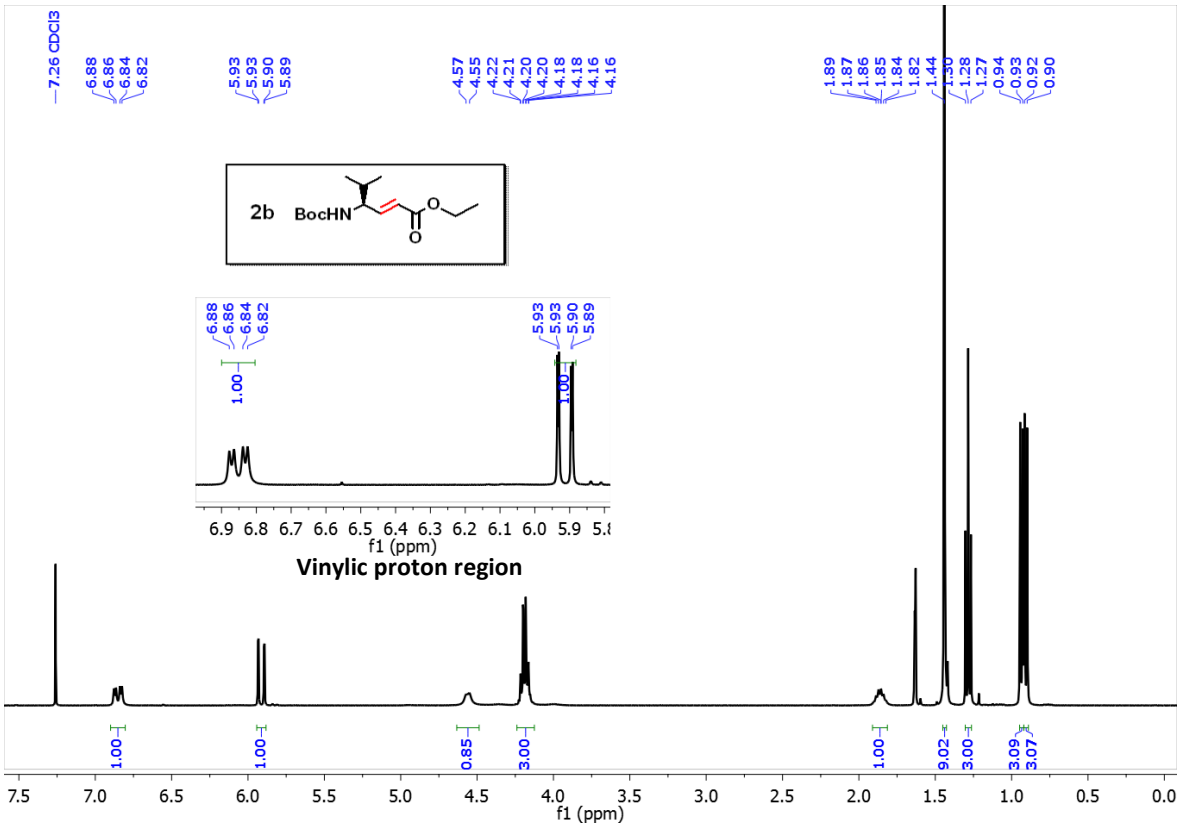


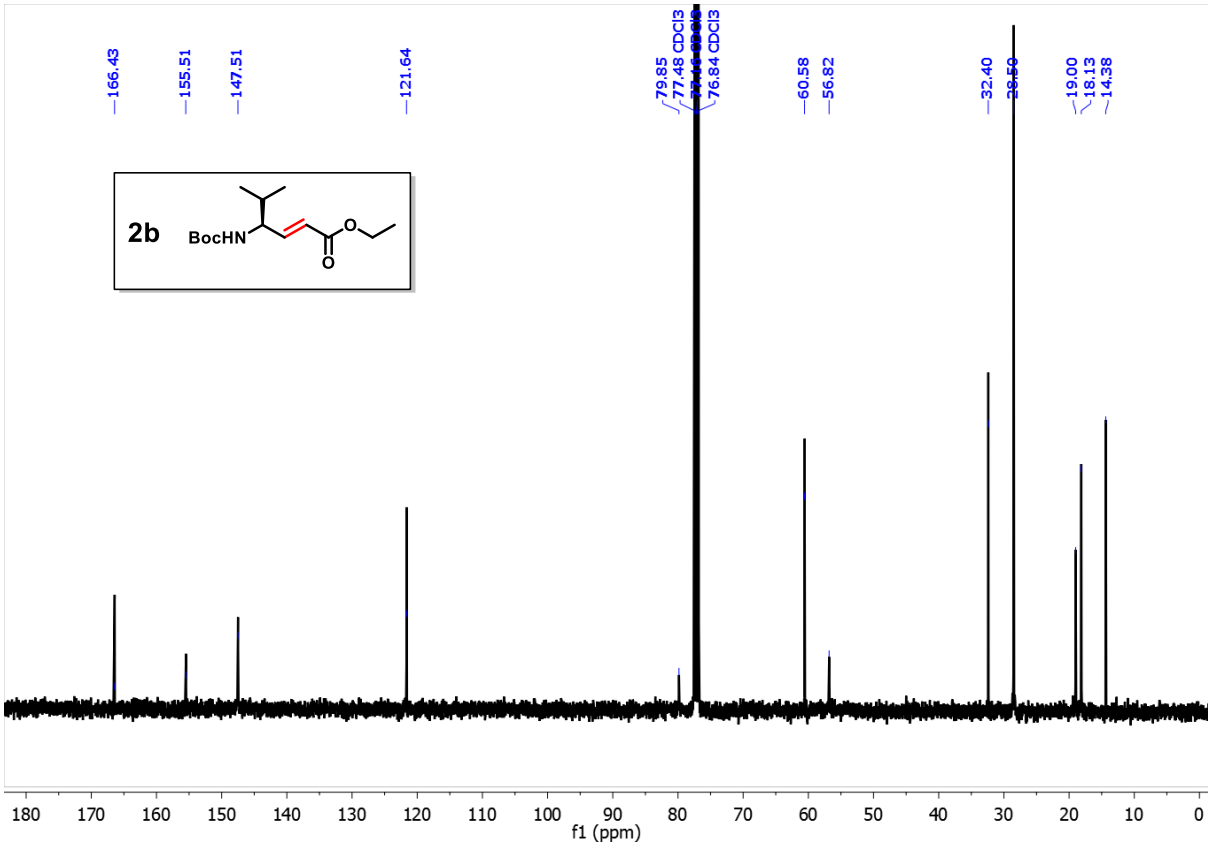
All Building Blocks ^{1}H and ^{13}C NMR and HRMS spectra's:

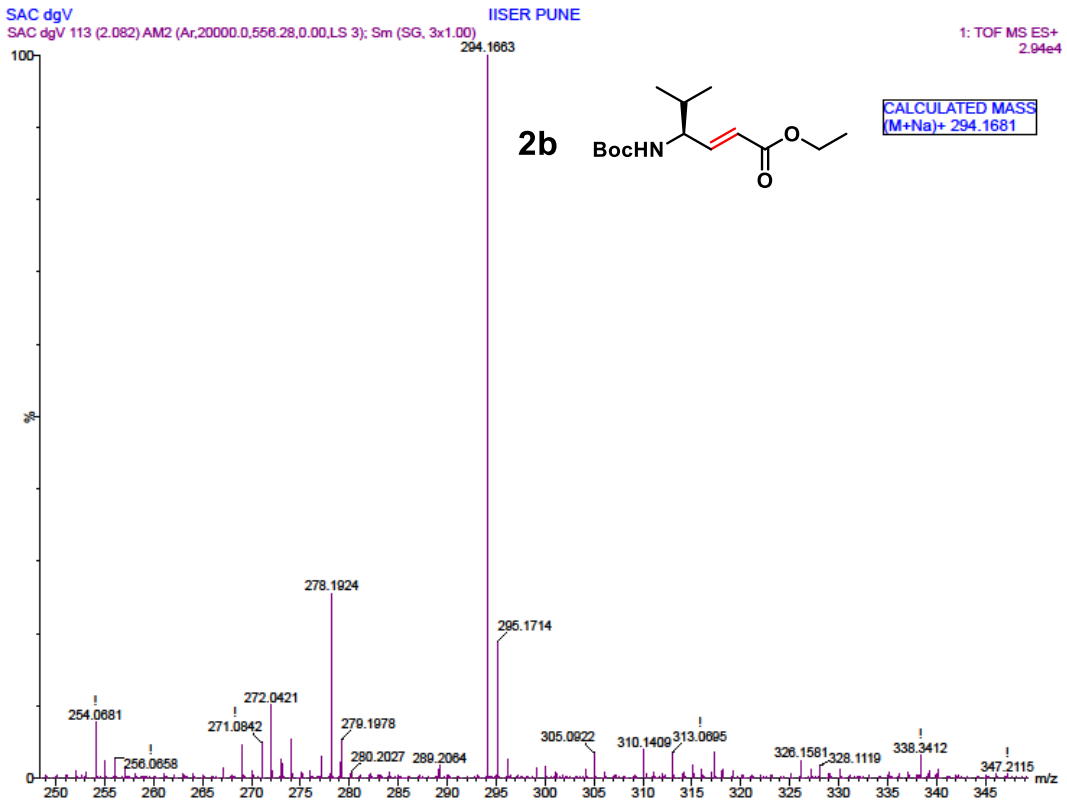

7.26 CHLOROFORM-D
6.84
6.83
6.80
6.79
5.92
5.88
4.49
4.47
4.32
4.20
4.18
4.17
4.15
1.71
1.69
1.68
1.66
1.64
1.43
1.39
1.37
1.35
1.29
1.27
1.25
0.97
0.93
0.92
0.91
-0.02
2a
BocHN
O
O
1.01
1.01
Vinylic Proton's region
f1 (ppm)
1.01
1.01
0.92
1.01
2.01
1.02
9.02
2.02
3.01
6.04
f1 (ppm)

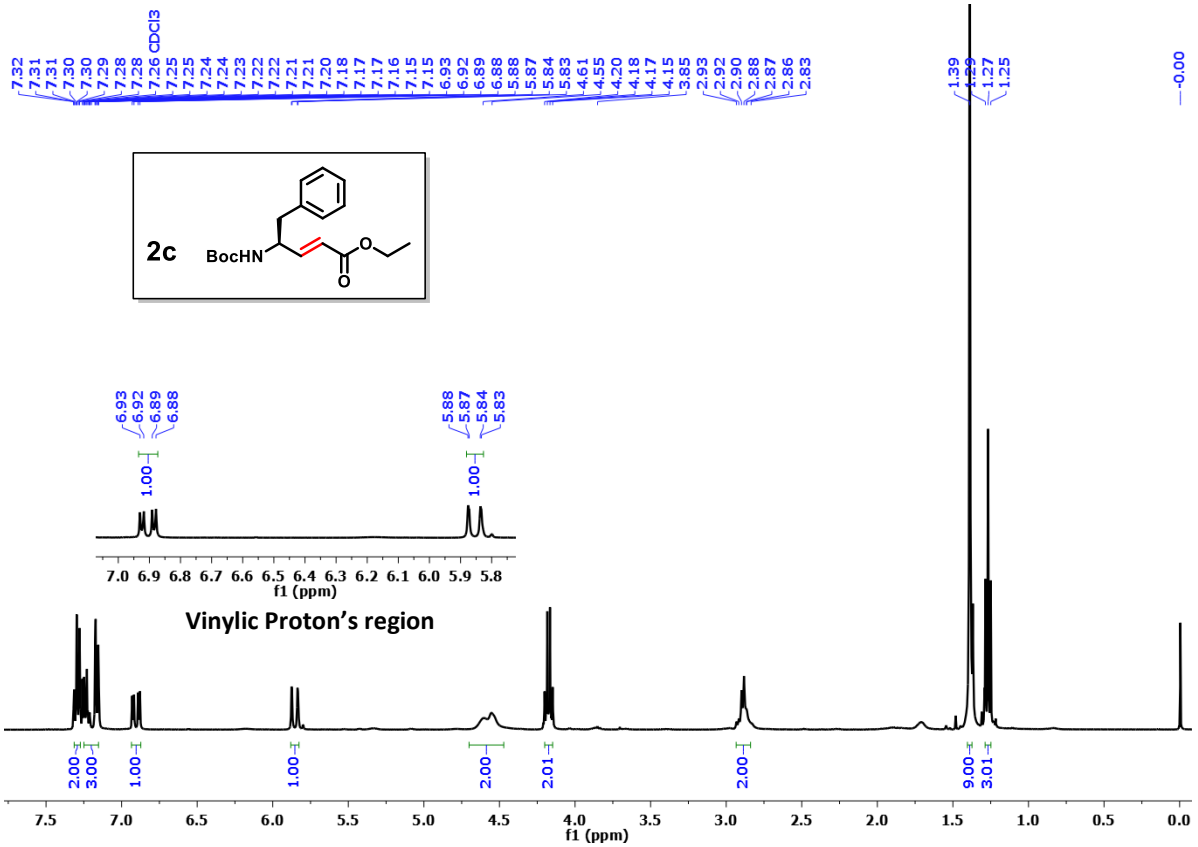

166.56
155.22
149.04
120.53
79.81
77.47 CHLOROFORM-D
76.83 CHLOROFORM-D
60.55
49.92
43.95
28.47
24.83
22.83
14.34
0.11
2a
BocHN
O
O
f1 (ppm)

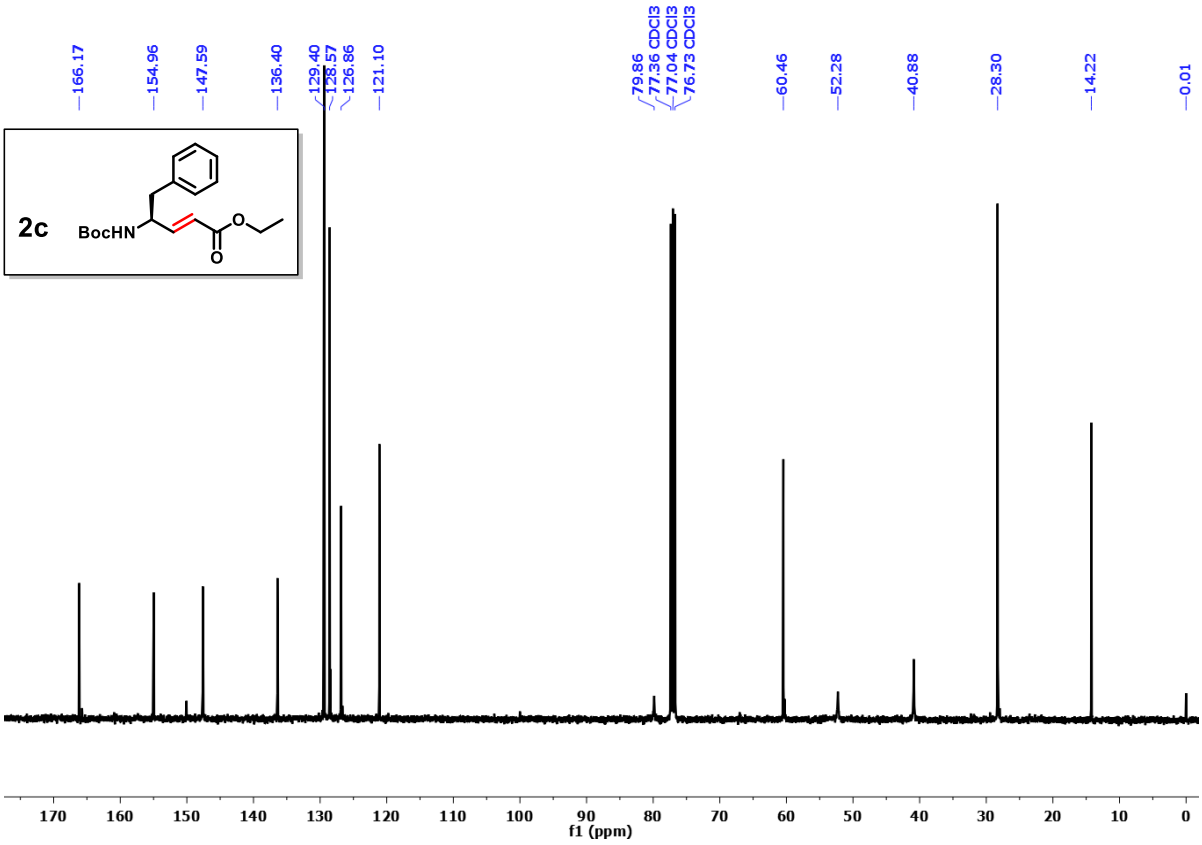

BDLET
BDLET 53 (0.979) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
IISER PUNE
1: TOF MS ES+
1.34e6
2a
BocHN
CALCULATED MASS
(M+Na)+ 308.1837
308.1845
309.1878
186.1496
169.1227
140.1072
123.0800
187.1529
252.1206
274.2752
365.2787
366.2823
415.2820
471.3443
540.5378
567.4742
593.3786
594.3829
595.3856
675.6770
697.6556
765.3239
m/z

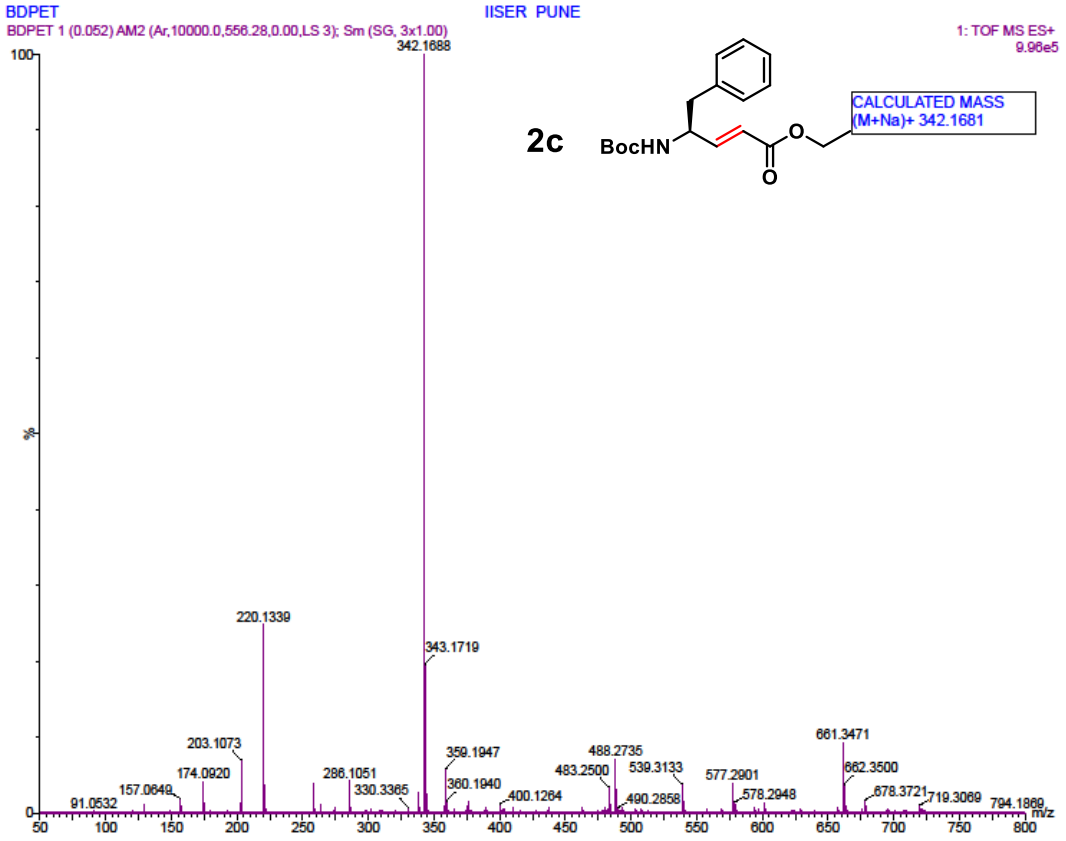

7.26 CDCl3
6.88
6.86
6.84
6.82
5.93
5.93
5.90
5.89
4.57
4.55
4.22
4.21
4.20
4.20
4.18
4.18
4.16
4.16
1.89
1.87
1.86
1.85
1.84
1.82
1.44
1.30
1.28
1.27
0.94
0.93
0.92
0.90
2b
BocHN
1.00
1.00
0.85
3.00
1.00
9.02
3.00
3.09
3.07
f1 (ppm)
Vinylic proton region

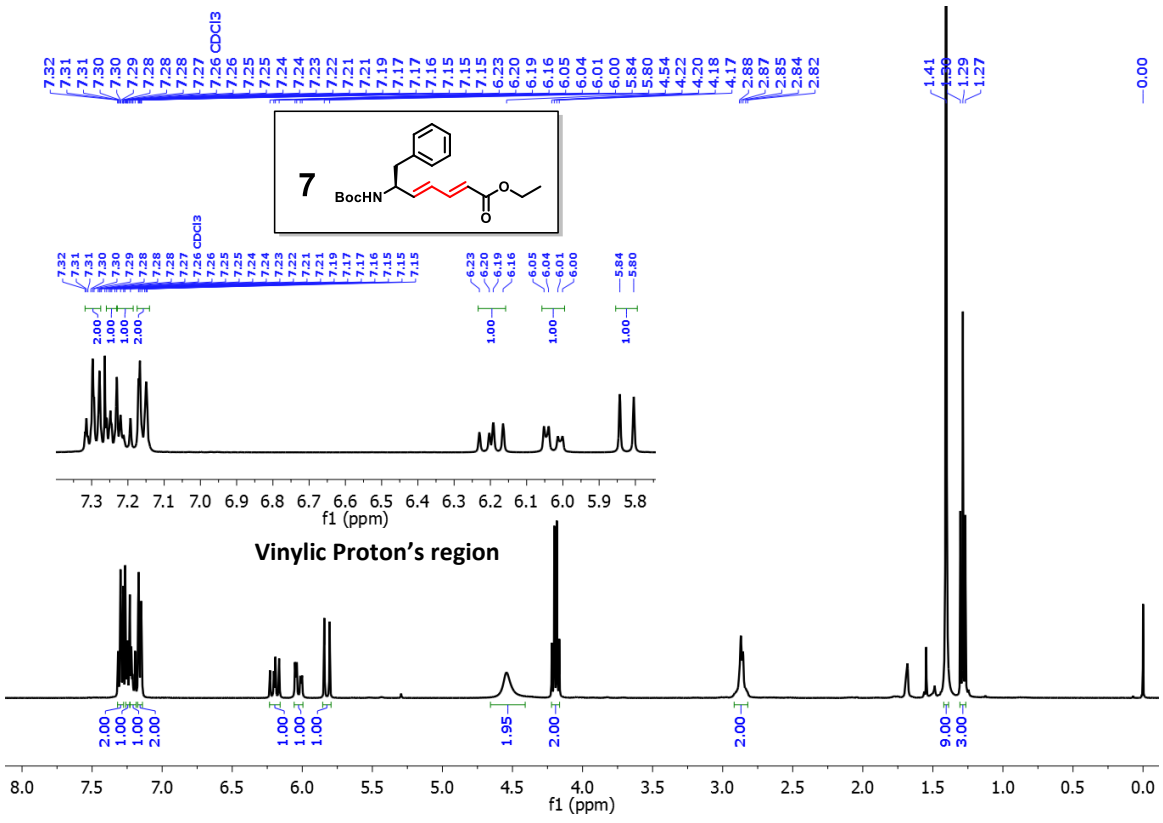

166.43
155.51
147.51
121.64
79.85
77.48 CDCl3
76.84 CDCl3
60.58
56.82
32.40
28.50
19.00
18.13
14.38
2b
BocHN
O
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

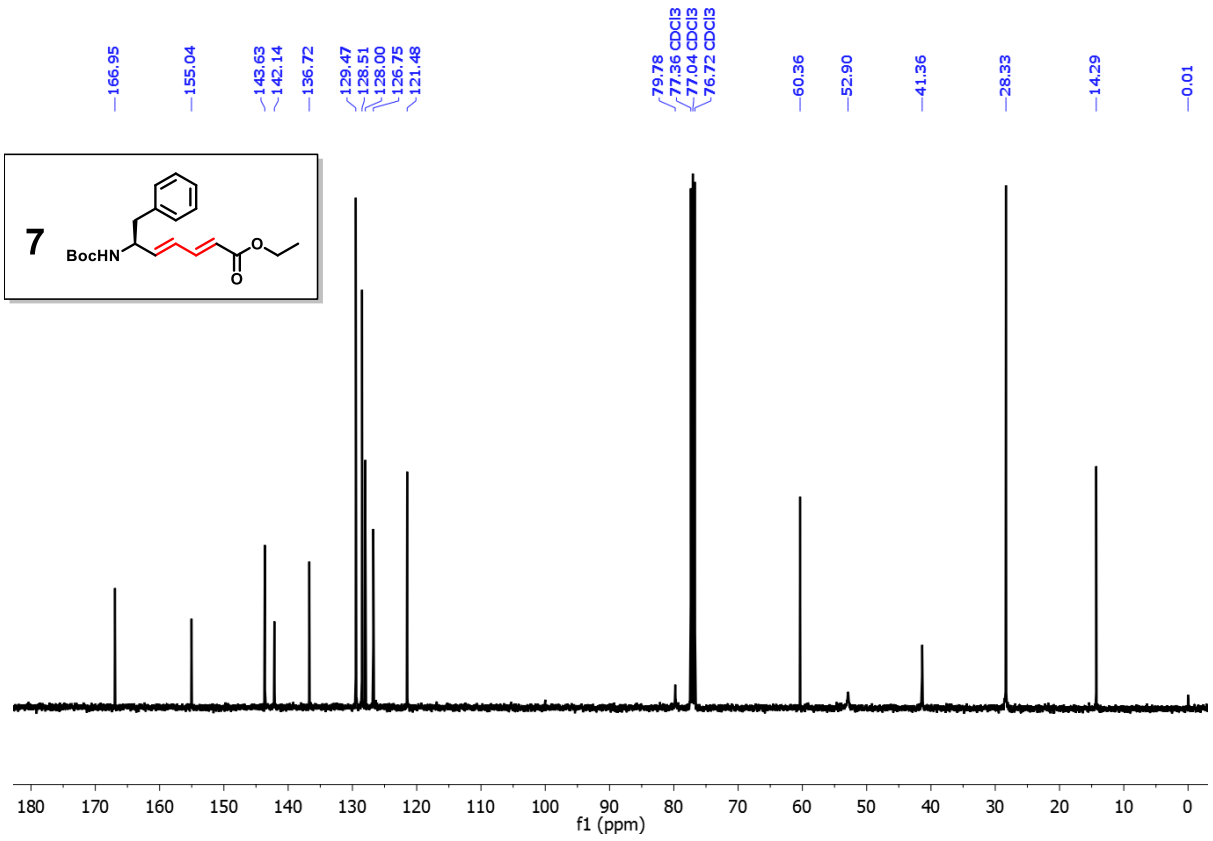

SAC dgV
IISER PUNE
SAC dgV 113 (2.082) AM2 (Ar,20000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
2.94e4
100
%
0
294.1663
2b
BocHN
O
O
CALCULATED MASS
(M+Na)+ 294.1681
278.1924
295.1714
272.0421
254.0681
271.0842
279.1978
256.0658
280.2027
289.2064
305.0922
310.1409
313.0695
326.1581
328.1119
338.3412
347.2115
250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345
m/z

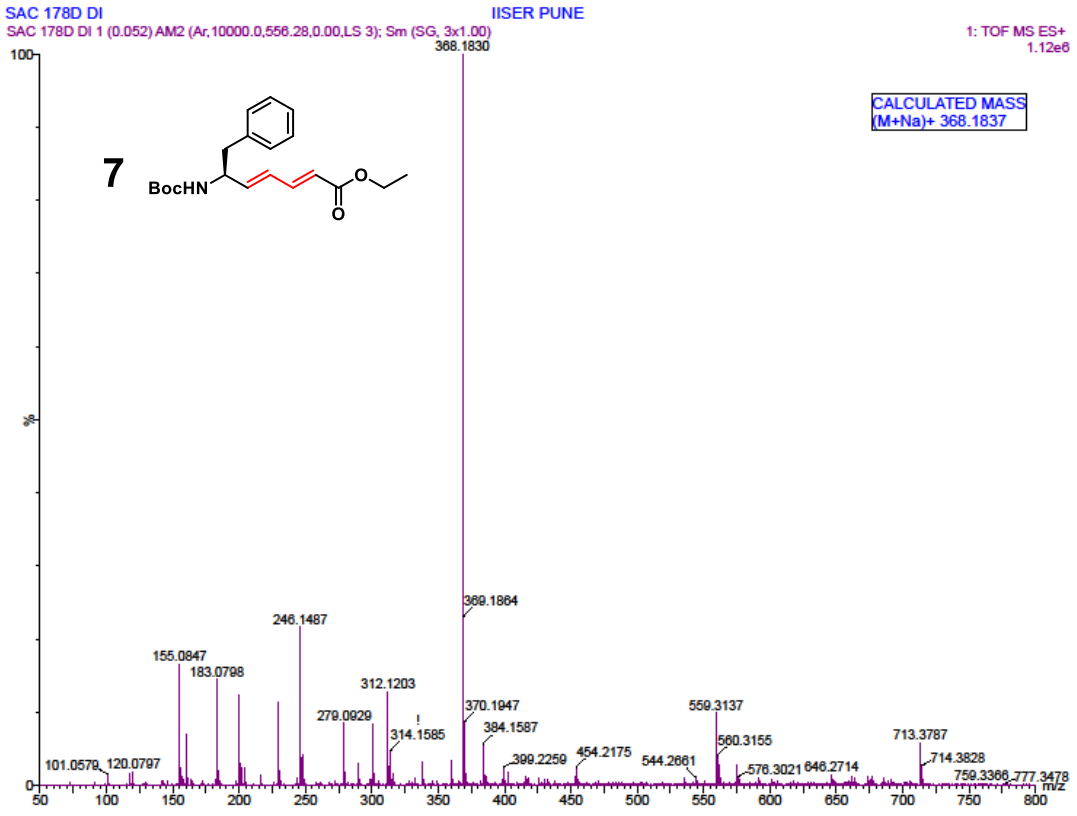

2c
BocHN
Vinylic Proton's region
f1 (ppm)

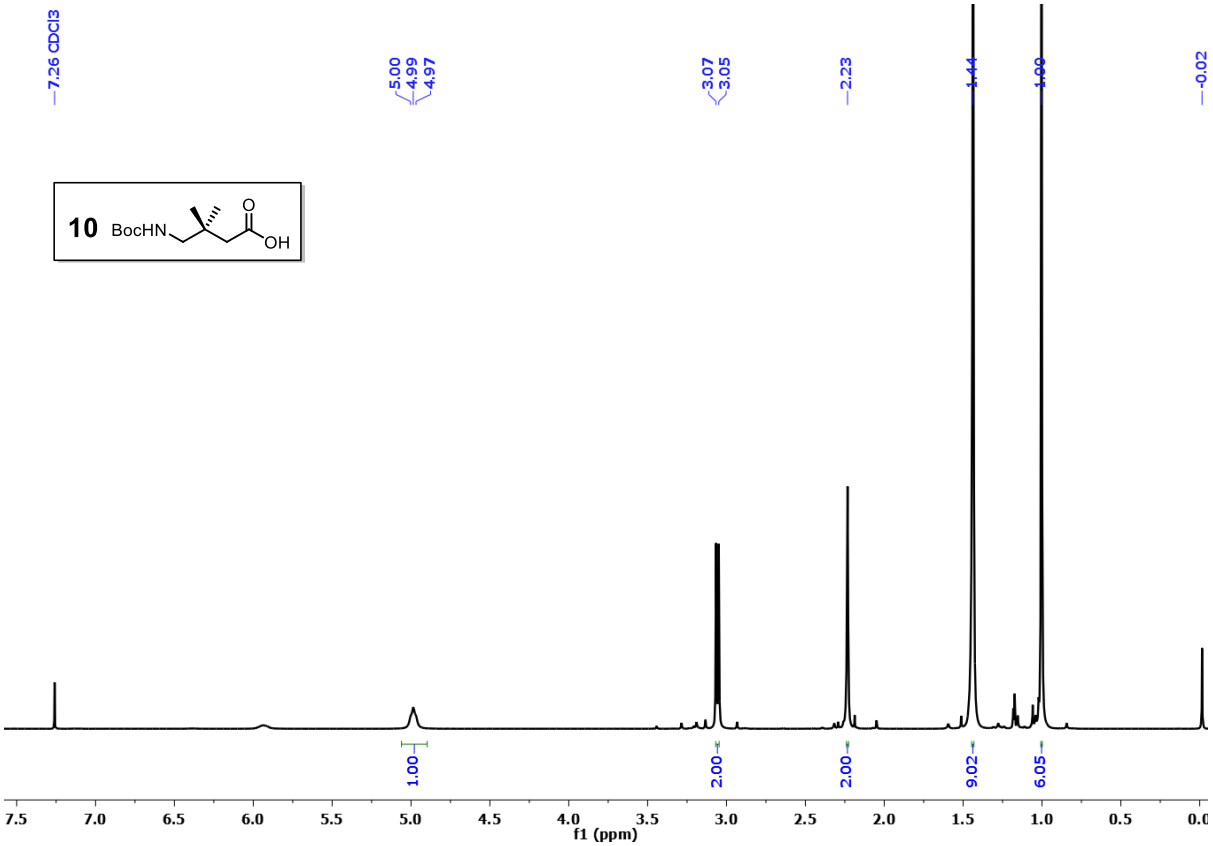

2c
BocHN
f1 (ppm)

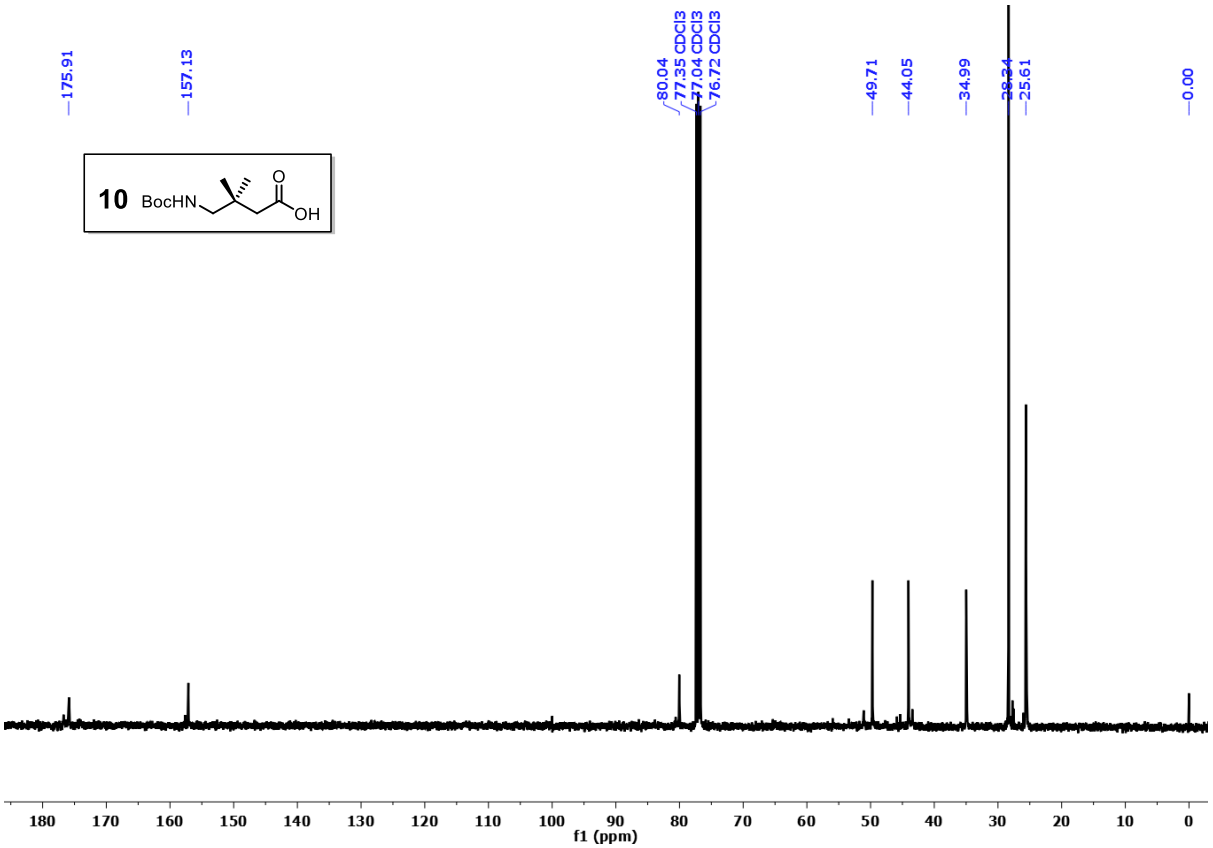

BDPET
IISER PUNE
BDPET 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
9.96e5
2c
BocHN
CALCULATED MASS
(M+Na)+ 342.1681
342.1688
220.1339
343.1719
203.1073
174.0920
157.0649
91.0532
286.1051
330.3365
359.1947
360.1940
400.1264
488.2735
483.2500
539.3133
490.2858
577.2901
578.2948
661.3471
662.3500
678.3721
719.3069
794.1869
m/z

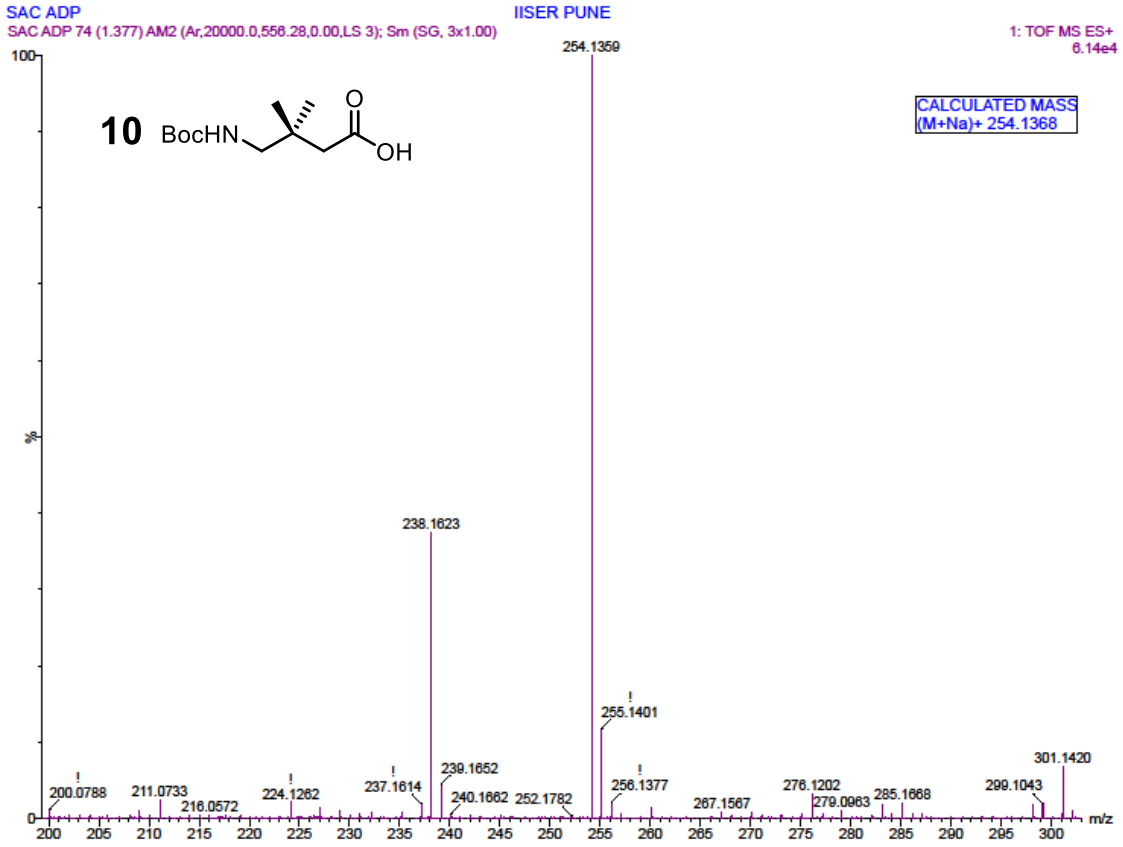

7
BocHN
CDCl3
f1 (ppm)
Vinylic Proton's region


166.95
155.04
143.63
142.14
136.72
129.47
128.51
128.00
126.75
121.48
79.78
77.36 CDCl3
77.04 CDCl3
76.72 CDCl3
60.36
52.90
41.36
28.33
14.29
0.01
7
BocHN
O
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


SAC 178D DI
IISER PUNE
SAC 178D DI 1 (0.052) AM2 (Ar,10000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
1: TOF MS ES+
1.12e6
CALCULATED MASS
(M+Na)+ 368.1837
7
BocHN
O
O
368.1830
369.1864
246.1487
155.0847
183.0798
312.1203
279.0929
314.1585
370.1947
384.1587
399.2259
454.2175
544.2661
559.3137
560.3155
576.3021
646.2714
713.3787
714.3828
759.3366
777.3478
101.0579
120.0797
%
m/z


7.26 CDCl3
5.00
4.99
4.97
3.07
3.05
2.23
1.44
1.00
-0.02
10 BocHN OH
1.00
2.00
2.00
9.02
6.05
f1 (ppm)


175.91
157.13
80.04
77.35 CDCl3
77.04 CDCl3
76.72 CDCl3
49.71
44.05
34.99
28.24
25.61
0.00
10 BocHN OH
f1 (ppm)



13) SI References:

(1) Mali, S. M.; Bandyopadhyay, A.; Jadhav, S. V.; Ganesh Kumar, M.; Gopi, H. N. Synthesis of α, β-unsaturated γ-amino esters with unprecedented high (*E*)-stereoselectivity and their conformational analysis in peptides. *Org.Biomol.Chem.* **2011**, *9*, 6566-6566.

(2) Nalawade, S. A.; Singh, M.; Puneeth Kumar, Drgk. R.; Dey, S.; Gopi, H. N. Stereoselective Synthesis of Backbone Extended π-Conjugated Amino Esters. *Org. Biomol. Chem.* **2023**. https://doi.org/10.1039/d3ob00090g.

(3) Misra, R.; George, G.; Saseendran, A.; Raghothama, S.; Gopi, H. N. Ambidextrous α,γ-Hybrid Peptide Foldamers. *Chem. Asian J.* **2019**, *14*, 4408-4414.

(4) (a) Sheldrick, G. M. Phase annealing in *SHELX*-90: direct methods for larger structures. *Acta Crystallogr.* **1990**, *A46*, 467-473. (b) Sheldrick, G. M. SHELXL-97, Program for Crystal Structure Solution and Refinement, Universität Göttingen (Germany) **1997**.

(5) Spek, A. L. *Acta Crystallogr*. PLATON, an integrated tool for the analysis of the results of a single crystal structure determination. **1990**, *A46*, C34.

(6) The solvent mask (void determination) in OLEX is based on: Jiang, J.- S.; Brünger, A. T. Protein hydration observed by X-ray diffraction. Solvation properties of penicillopepsin and neuraminidase crystal structures. *J. Mol. Biol.* **1994**, *243*, 100-115.

(7) Mali, S. M.; Ganesh Kumar, M.; Katariya, M. M.; Gopi, H. N. HBTU mediated 1-hydroxybenzotriazole (HOBt) conjugate addition: synthesis and stereochemical analysis of β-benzotriazole *N*-oxide substituted γ-amino acids and hybrid peptides. *Org. Biomol. Chem.* **2014**, *2*, 8462-8472.