Supporting Information

Structural Features and Molecular Aggregations of Designed Triple-Stranded β-Sheets in Single Crystals

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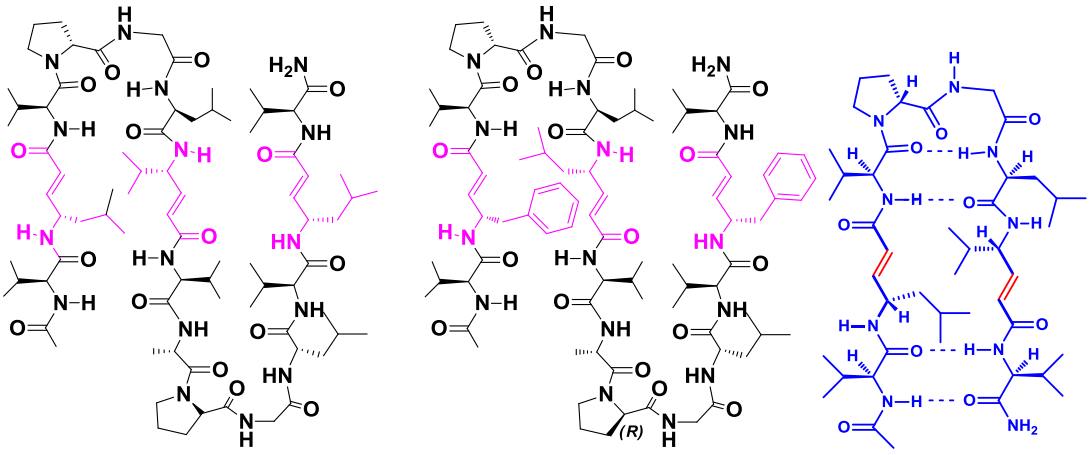
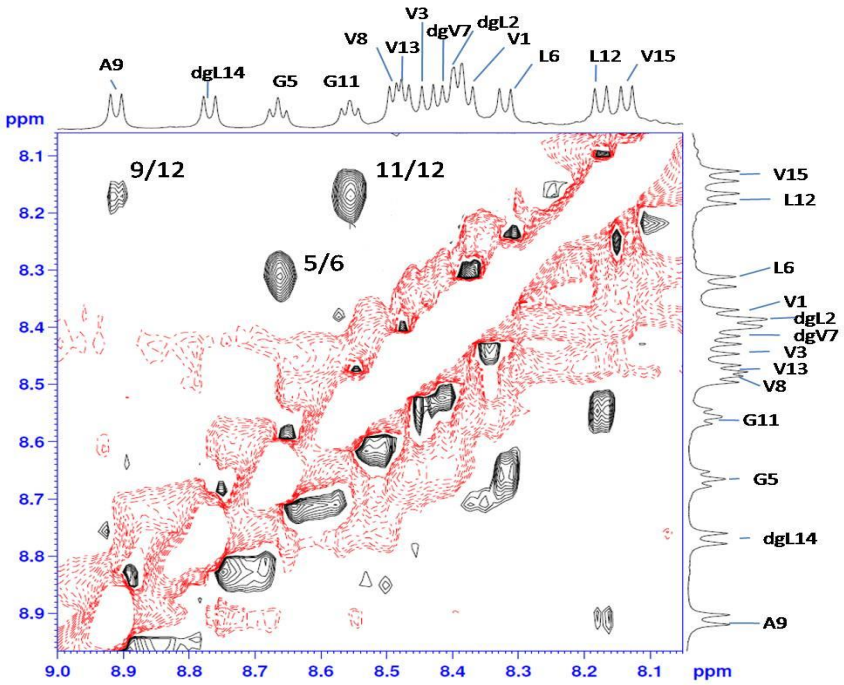


P1 **P2** **P3**

Scheme S1: Schematic representation of three-stranded β-sheet **P1** and **P2** and β-hairpin **P3**

a)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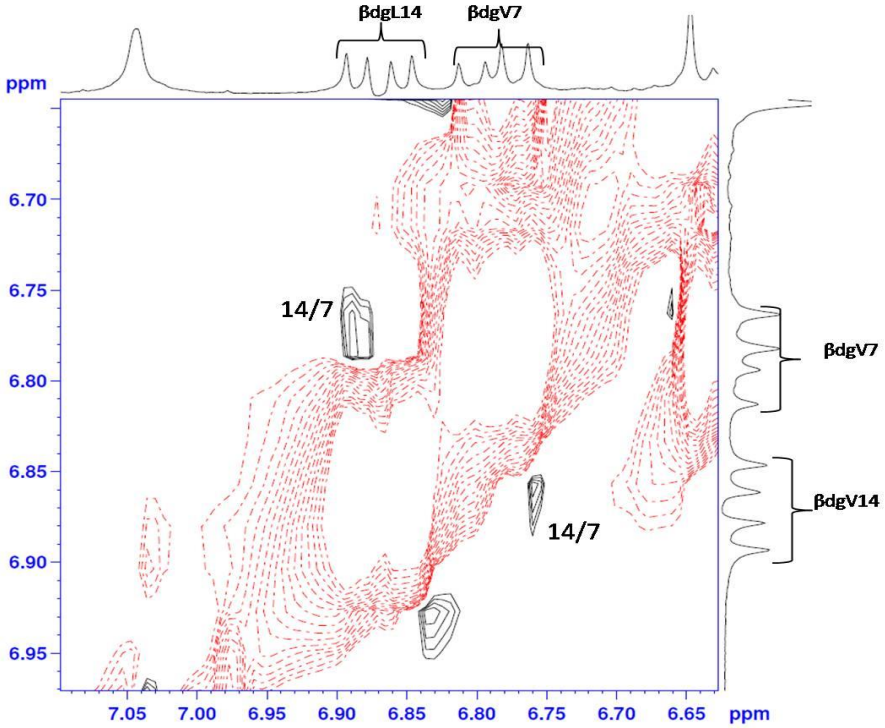
dgL14
G5
G11
V8
V13
V3
dgV7
dgL2
V1
L6
L12
V15
ppm
9/12
11/12
5/6
V15
L12
L6
V1
dgL2
dgV7
V3
V13
V8
G11
G5
dgL14
A9
8.1
8.2
8.3
8.4
8.5
8.6
8.7
8.8
8.9
9.0
ppm

b)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βdgV7
ppm
14/7
14/7
βdgV7
βdgV14
6.70
6.75
6.80
6.85
6.90
6.95
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65
ppm

Figure S1: Partial ROESY spectra of **P1** showing cross-strand NOEs of a) NH ↔NH, and b) vinylogous CβH↔CβH

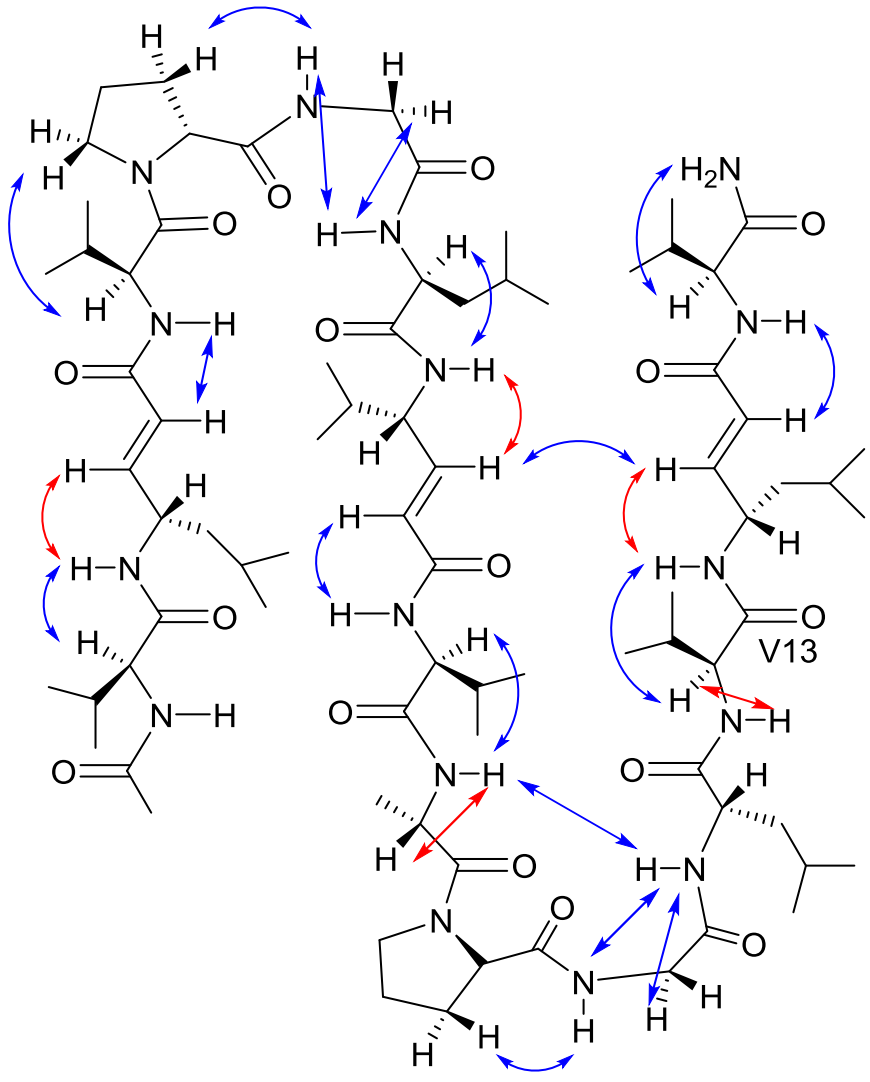

Figure S2: The observed NOEs of the peptide **P1** in the ROESY are schematically shown by double headed arrows, blue for inter-residue and red for intra-residue

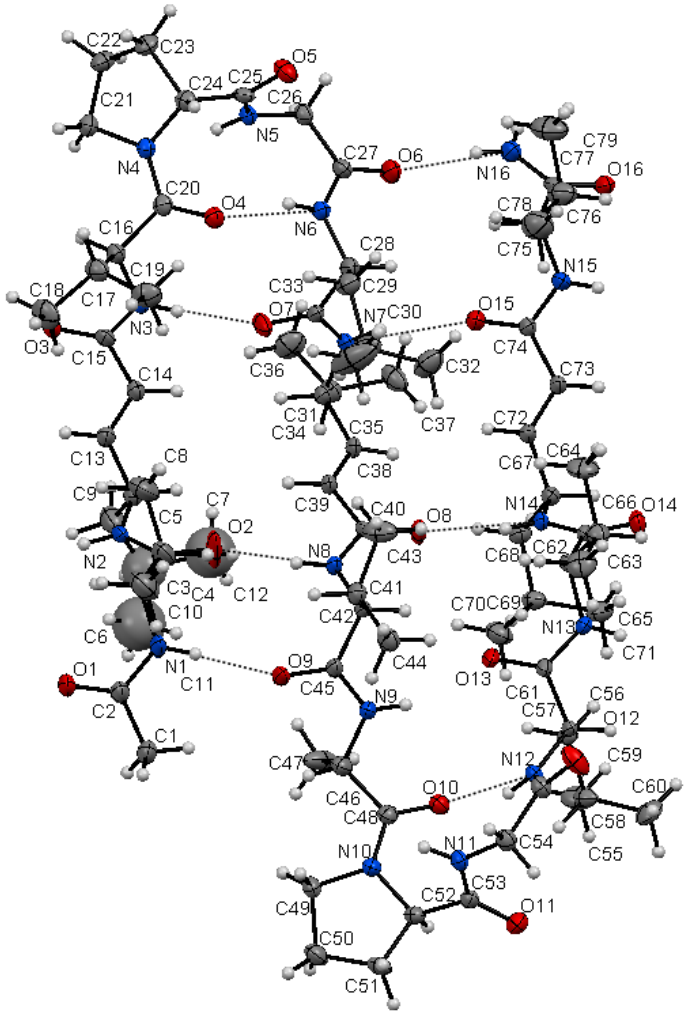

Figure S3. The ORTEP diagram of Ac-Val-dgL-Val-^{D}Pro-Gly-Leu-dgV-Val-Ala-^{D}Pro-Gly-Leu-Val-dgL-Val-NH_2 (**P1**). H-atoms are not labeled for clarity.

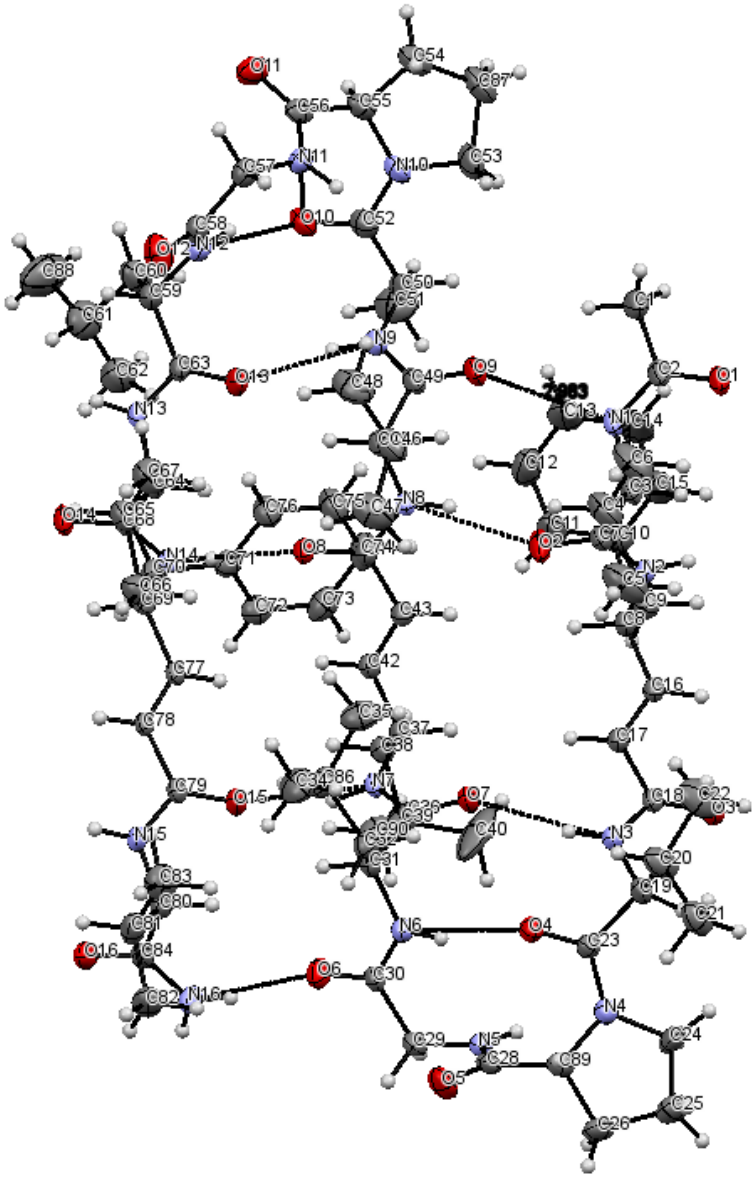

Figure S4. The ORTEP diagram of Ac-Val-dgF-Val-^{D}Pro-Gly-Leu-dgL-Val-Ala-^{D}Pro-Gly-Leu-Val-dgF-Val-NH_2 (**P2**).

Table S1: Torsional variables of three-stranded β-sheet Ac-Val-dgL-Val-^{D}Pro-Gly-Leu-dgV-Val-Ala-^{D}Pro-Gly-Leu-Val-dgL-Val-NH_2 (**P1**).

Resd.	ϕ	θ_1	θ_2	ψ	ω
Val1	-119	-	-	114	170
dgL2	-112	102	168	161	173
Val3	-147	-	-	139	162
^{D}Pro4	66	-	-	25	-177
Gly5	88	-	-	1	174
Leu6	-104	-	-	120	168
dgV7	-102	104	-170	-178	-175
Val8	-127	-	-	116	-173
Ala9	-152	-	-	137	179
^{D}Pro10	57	-	-	30	-178
Gly11	86	-	-	-15	-174
Leu12	-80	-	-	138	171
Val13	-100	-	-	103	-176
dgL14	-111	94	-177	171	176
Val15	-98	-	-	136	-

Table S2: Torsional variables of three-stranded β-sheet Ac-Val-dgF-Val-^{D}Pro-Gly-Leu-dgL-Val-Ala-^{D}Pro-Gly-Leu-Val-dgF-Val-NH_2 (**P2**).

Resd.	ϕ	θ_1	θ_2	ψ	ω
Val1	-129	-	-	112	174
dgF2	-115	108	-171	163	-168
Val3	-152	-	-	132	165
^{D}Pro4	58	-	-	35	178
Gly5	89	-	-	-4	177
Leu6	-105	-	-	114	177
dgL7	-102	115	-174	-178	-173
Val8	-133	-	-	107	-170
Ala9	-127	-	-	119	176
^{D}Pro10	55	-	-	28	-179
Gly11	99	-	-	-19	-173
Leu12	-90	-	-	132	177
Val13	-98	-	-	125	175
dgF14	-131	96	-179	-176	-177
Val15	-98	-	-	138	-

Table S3: Hydrogen bonding parameters of three-stranded β-sheet Ac-Val-dgL-Val-^{D}Pro-Gly-Leu-dgV-Val-Ala-^{D}Pro-Gly-Leu-Val-dgL-Val-NH_2 (**P1**).

Type	Donar [D]	Acceptor [A]	D···A [Å]	H···A [Å]	D-H···A [deg]	D···O=C [deg]
Intramol.	N1	O9	2.85	1.99	176	156
Intermol.	N2	O14[¶]	2.78	1.98	157	169
Intramol.	N3	O7	2.97	2.20	148	157
Pro	N4(Pro)	---	---	---	---	---
Intermol.	N5	O12[*]	2.71	1.97	144	168
Intramol.	N6	O4	2.96	2.13	159	128
Intramol.	N7	O15	2.98	2.17	155	157
Intramol.	N8	O2	2.79	1.96	161	154
Intramol.	N9	O13	3.09	2.32	148	146
Pro	N10(Pro)	---	---	---	---	---
Intermol.	N11	O16[#]	2.92	2.18	156	149
Intramol.	N12	O10	2.87	2.06	157	124
Intermol.	N13	O1	2.88	2.04	164	164
Intramol.	N14	O8	2.77	1.97	157	169
Intermol.	N15	O3[§]	3.00	2.15	172	175
Intramol.	N16	O6	2.91	2.06	167	165
Intermol.	N16	O9[†]	3.01	2.15	175	110

Intermol.; intermolecular, intramol.; intramolecular, #Symmetry equivalent x, y, 1+z, †Symmetry equivalent x, y, -1+z, ¶Symmetry equivalent -1+x, -1+y, z, §Symmetry equivalent 1+x, 1+y,1+z, *Symmetry equivalent -1+x, -1+y, -1+z.

Table S4: Hydrogen bonding parameters of three-stranded β-sheet Ac-Val-dgF-Val-^{D}Pro-Gly-Leu-dgL-Val-Ala-^{D}Pro-Gly-Leu-Val-dgF-Val-NH_2 (**P2**).

Type	Donar [D]	Acceptor [A]	D···A [Å]	H···A [Å]	D-H···A [deg]	D···O=C [deg]
Intramol.	N1	O9	2.98	2.14	170	151
Intermol.	N2	O14*	2.776	1.902	172	159
Intramol.	N3	O7	2.99	2.14	168	152
Pro	N4(Pro)	---	---	---	---	---
Intermol.	N5	O12*	2.78	2.00	147	169
Intramol.	N6	O4	2.98	2.13	164	128
Intramol.	N7	O15	2.84	2.004	160	146
Intramol.	N8	O2	2.79	1.97	154	154
Intramol.	N9	O13	2.89	2.04	164	150
Pro	N10(Pro)	---	---	---	---	---
Intermol.	N11	O16#	2.82	2.04	146	151
Intramol.	N12	O10	2.92	2.07	160	125
Intermol.	N13	O1$	2.89	2.04	164	150
Intramol.	N14	O8	2.82	1.95	171	159
Intermol	N15	O3&	2.98	2.10	173	172
Intramol	N16	O6	2.98	2.07	168	164

*Symmetry equivalent-1+x,y,z, $Symmetry equivalent x, y, -1+z, #Symmetry equivalent -1+x, y, 1+z, &Symmetry equivalent 1+x, y,z,.

Table S5: Comparison of intramolecular H-bond distances (Å) between **P1** and **P3**

	β-hairpin **P3**		three-stranded β-sheet **P1**		Difference (**P3 – P1**)	Difference (**P3 – P1**)
	D-H···A (Å)	D···A (Å)	D-H···A (Å)	D···A (Å)	D-H···A (Å)	D···A (Å)
Val1NH···O=CVal8	2.080	2.933	1.996	2.855	0.084	0.078
Val1C=O···HNVal8	2.213	2.929	1.960	2.791	0.253	0.128
Val3NH···O=CLeu6	2.246	3.069	2.206	2.972	0.040	0.097
Val3C=O···HNLeu6	2.111	2.880	2.139	2.960	-0.028	-0.08

Table S6: Comparison of the atom-to-atom distances (Å) across the anti-parallel β-strands between **P1** and **P3**

Cross-strand backbone Atom↔Atom	β-hairpin **P3** (Å)	three-stranded β-sheet **P1** (Å)	Difference (**P3 – P1**) (Å)
Val1N↔C(O)Val8	4.120	4.026	0.094
Val1C_α↔C_αVal8	5.097	5.072	0.015
Val1C(O)↔NVal8	4.102	3.945	0.157
dgL2N↔C(O)dgV7	5.721	5.523	0.198
dgL2C_γ↔$C_\alpha$dgV7	4.751	4.477	0.274
dgL2C_β ↔$C_\beta$dgV7	5.900	5.608	0.292
dgL2C_α↔$C_\gamma$dgV7	4.937	4.502	0.435
dgL2C(O)↔NdgV7	5.791	5.601	0.190
Val3N↔C(O)Leu6	4.113	4.153	-0.040
Val3C_α↔C_αLeu6	5.024	5.407	-0.383
Val3C(O)↔NLeu6	3.887	3.849	0.038

Table S7: CH…O H-bonds parameters of three-stranded β-sheet Ac-Val-dgL-Val-^{D}Pro-Gly-Leu-dgV-Val-Ala-^{D}Pro-Gly-Leu-Val-dgL-Val-NH_2 (**P1**).

Type	Donar	Acceptor	H…..A (Å)	D-H···A (degree)
Intramol.	C1	O9	2.66	142
Intramol.	C39	O2	2.44	135
Intramol.	C14	O7	2.47	140
Intramol.	C29	O4	2.53	145
Intramol.	C68	O8	2.65	125
Intramol.	C28	O15	2.69	146
Intramol.	C75	O6	2.74	143
Intermol.	C73	O3	2.71	139
Intermol.	C3	O7	2.76	140
Intermol.	C56	O1	2.46	143

Table S8: CH….OH bonds parameters of three-stranded β-sheet Ac-Val-dgF-Val-^{D}Pro-Gly-Leu-dgL-Val-Ala-^{D}Pro-Gly-Leu-Val-dgF-Val-NH_2 (**P1**).

Type	Donar	Acceptor	H…..A (Å)	D-H···A (degree)
Intramol	C1	O9	2.554	147
Intramol.	C43	O2	2.477	136
Intramol.	C17	O7	2.489	141
Intramol.	C32	O4	3.002	125
Intramol.	C60	O10	2.731	133
Intramol.	C45	O13	2.332	150
Intramol.	C64	O10	2.538	145
Intramol.	C38	O15	2.822	124
Intramol.	C80	O6	2.513	144
Intermol.	C78	O3	2.704	138
Intermol.	C3	O14	2.826	138
Intermol.	C61	O1	2.811	154

Table S9: List of type I′ β-hairpin structures derived from the different protein structures to overlay on **P1**.

Proteins	PDB code	Residue numbers	Sequence
Actinidine	1AEC	170-177	GTEGGIDY
Aspartyl Proteinase Penicillopepsin	1APT	21-28	VTIGGTTL
Aspartyl Proteinase Penicillopepsin	1APT	58-65	VSISGYTA
Porcine pancreatic elastase	1QNJ	201-208	CLVNGQYA
Streptomyces griseus protease A	1SGC	48-51	VSVNGVAH
Alpha-lytic protease	1SSX	64-84	ARIGGAVV
Penicillopepsin	3APP	90-97	VTVGGVTA
Penicillopepsin	3APP	258-265	VSISGYTA
Phospholipase A_2	1JIA	76-83	SWKNGTIV
Staphylococcal nuclease	3OSO	92-99	IYADGKMV

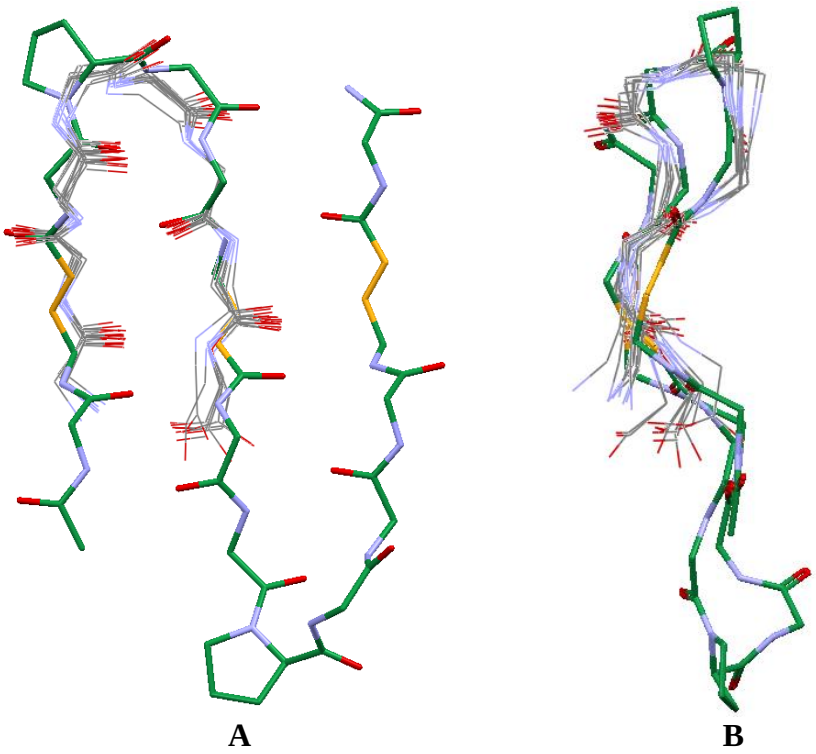

Figure S5: **A**. Superposition of β-hairpins with type I' β-turns form protein structures over three-stranded β-sheet **P1**. **B**. Side-view of the overlay.

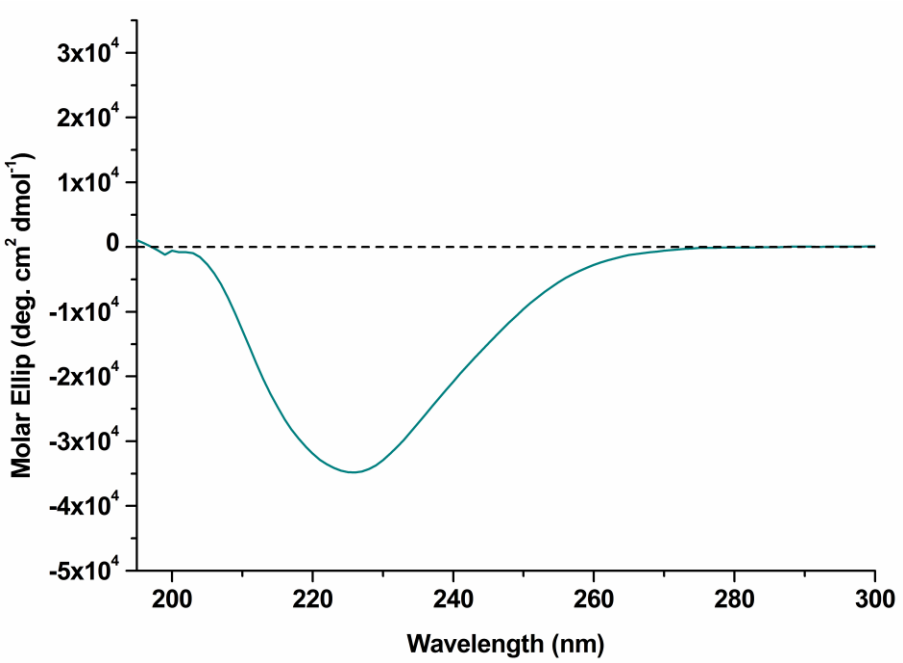

Figure S6: Circular dichroism (CD) spectrum of **P1** (100 μM) in methanol.

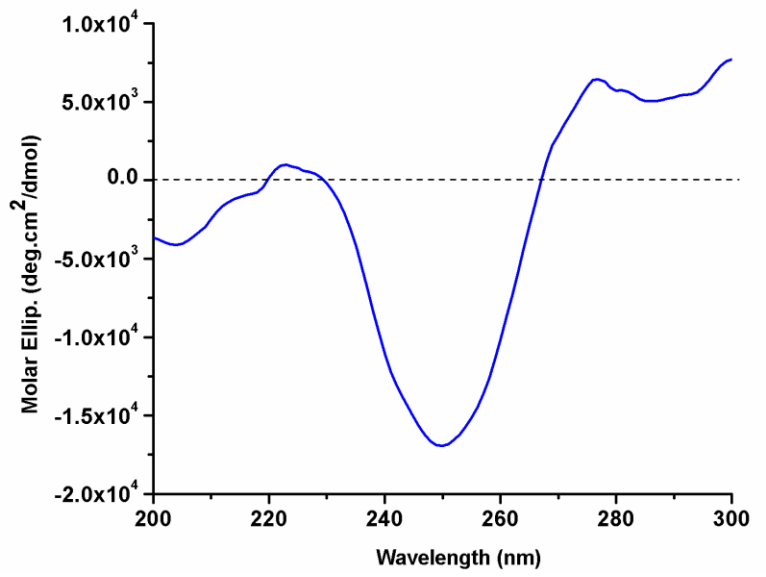

Figure S7: Circular dichroism (CD) spectrum of **P2** (100 μM) in methanol.

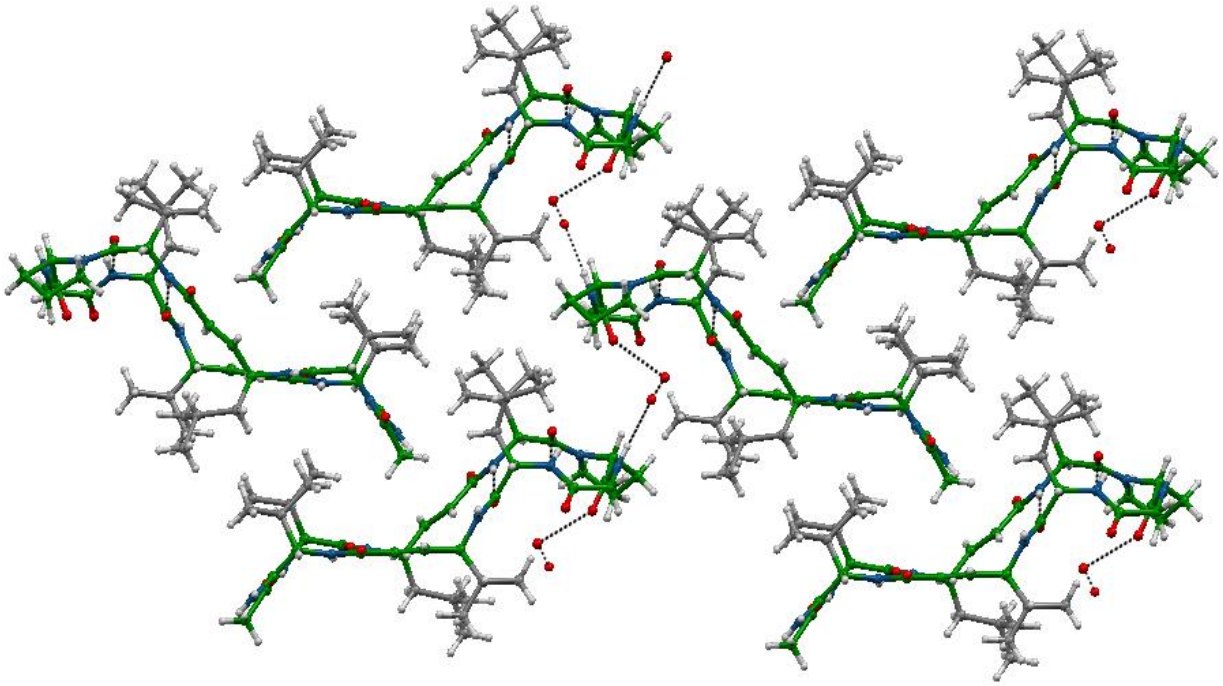

Figure S8: Vertical stacking of **P3**. Water molecules played crucial role in head-to-head interconnection of **P3** molecules

Crystal structure analysis of (Ac-Val-dgLeu-Val-^{D}Pro-Gly-Leu-dgVal-Val-Ala-^{D}Pro-Gly-Leu-Val-dgLeu-Val-NH_2) P1

Crystals of peptide were grown by slow evaporation from a solution of methanol and water. A single crystal (0.18 × 0.14 × 0.11 mm) was mounted in a loop with a small amount of the mother liquor. The X-ray data were collected at 100 K temperature on a Bruker AXS SMART APEX II CCD Duo diffractometer using MoK_α radiation (λ = 0.71073 Å), ω-scans (2θ = 56.56^o), for a total number of 21007 independent reflections. Space group P1, a = 12.367(3), b = 13.818(3), c = 16.091(5) Å, α = 105.224(6) , β = 97.197(6), γ = 113.827(4), V= 2342.2(10) Å^3, Triclinic P, Z = 1 for chemical formula ($C_{79}H_{134}N_{16}O_{16}$), with one molecule in asymmetric unit; ρ_{calcd} = 1.109 g

cm^{-3}, μ = 0.078 mm^{-1}, $F(000)$ = 848, R_{int} = 0.0458. The data integration and reduction were processed with SAINT software.[1] A multi-scan absorption correction was applied to the collected reflections. The structure was solved by the direct method using SHELXTL[2] and was refined on F2 by full-matrix least-squares technique using the SHELXL-2013[2] program package within the WINGX[3] program. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were located in successive difference Fourier maps, and they were treated as riding atoms using SHELXL default parameters. The structures were examined using the Adsym subroutine of PLATON[4] to ensure that no additional symmetry could be applied to the models. During the refinement it was observed that, C10, C11 and C12 atoms are disordered with two site occupancy. A significant amount of time was invested to refine the disordered atoms anisotropically using constrains to fix them. 'dfix', and 'delu' restraint instructions were applied to the moiety for its geometry and atomic displacement parameters, and 'isor' restrain was applied to the big and non-spherical atoms. Furthermore, increasing refinement cycle with no. 80 found to be a better model constructed. In addition, we noticed that the guest molecules (two water molecules and one methanol molecule) in P**1** are highly disordered and could not be modeled properly, so the diffused electron densities resulting from them were removed by the SQUEEZE routine in PLATON [5] and the results were appended in the CIF files. Additionally, the squeeze output result is embedded bellow. After the final CIF file generation, the output *R* value was 0.0640 (*wR2*= 0.1578) for 14462 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 1023 variables, S = 1.041. The largest difference peak and hole were 0.775 and -0.437 e $Å^3$, respectively.

Squeeze output result:

:: TITLE compound -P1

:: CELL 12.3670 13.8180 16.0910 105.224 97.197 113.827 2342.2

:: SPGR P1 Chiral

:: Resd 1, SOF 1.000, Z 1, C79 H134 N16 O16

:: Moiety_Formula = C79 H134 N16 O16

:: Sum_Formula = C79 H134 N16 O16

:: Formula_Weight = 1564.03 [Note: Based on SHELXL97 Atomic Weights]

:: Formula_Z = 1

:: SpaceGroup_Z = 1

:: Formula_Z' = 1.000

:: mu(MoKa) = 0.78 cm-1 = 0.078 mm-1

:: Res.Scat. = 6 x 0.0001

:: Friedif = 6

:: Predicted Vol = 2147.5[2147.5] Ang^3, 100[2]K

:: VOID/SOLV Gridstep (Angstrom) (re)set to 0.20, Percent Memory = 0.9

van der Waals (or ion) Radii used in the Analysis

==========

C H N O

1.70 1.20 1.55 1.52

:: Note: VOID/SOLV/SQUEEZE is relatively compute intense and experimental

:: Total Potential Solvent Area Vol 147.5 Ang^3

per Unit Cell Vol 2342.2 Ang^3 [6.3%]

Note: Expected volumes for solvent molecules are:

A hydrogen bonded H2O-molecule 40 Ang^3

Small molecules (e.g. Toluene) 100-300 Ang^3

Values below for gridpoints and volumes in []
refer to areas where atom centers may reside.

Area #GridPoint VolPerc. Vol(A^3) X(av) Y(av) Z(av) Eigenvector(frac) Sig(Ang)

---

1 10603[818] 3 68[5.3] 0.053 0.831 0.679 1 1.000-0.714 0.333 1.40
2 -0.136 0.340 1.000 1.11
3 -1.000-0.964-0.222 1.04

2 3611[89] 1 23[0.6] 0.427 0.057 1.011 1 0.537 0.076 1.000 0.90
2 1.000 0.622-0.007 0.78
3 -0.234 1.000 0.386 0.73

3 1287[2] 0 8[0.0] 0.450 0.882 0.506 1 -0.006 1.000 0.866 0.60
2 1.000 0.325 0.276 0.55
3 0.335 1.000-0.663 0.54

4 1451[6] 0 9[0.0] 0.576 0.940 0.595 1 0.823-1.000-0.464 0.73
2 0.781 1.000-0.722 0.60
3 1.000 0.670 0.712 0.52

5 1222[2] 0 8[0.0] 0.628 0.098 0.940 1 1.000-0.107 0.300 0.58
2 0.488 1.000 0.549 0.55
3 -0.824-0.626 1.000 0.54

6 4678[61] 1 30[0.4] 0.798 0.550 0.836 1 0.261 1.000 0.160 1.46
2 1.000 0.167-0.247 0.73
3 -0.712-0.287-1.000 0.65

x y z Shortest Contacts within 4.5 Ang. (Excl. H)

==========

1 0.053 0.831 0.679 C47 3.40; C11 3.55; C70 3.72; C40 4.11;

2 0.427 0.057 1.011 O10 3.08; O5 3.08; C6 3.29; C48 3.45;

3 0.450 0.882 0.506 O7 2.93; C30 3.11; C31 3.12; C5 3.14;

```
 4  0.576 0.940 0.595          O2   2.89; N8   3.01; C5   3.05; C39  3.07;
 5  0.628 0.098 0.940          N16  2.87; C77  2.95; C6   2.98; C59  3.02;
 6  0.798 0.550 0.836          C12  2.86; C23  3.06; C22  3.25; C65  3.55;

 :: Note: use CALC VOID (not CALC SOLV) for Packing Index.

    Report the Distance from VOID-CG to Boundary in EV-Directions

 Nr MinEV1 MaxEV1 MinEV2 MaxEV2 MinEV3 MaxEV3 MaxDist (Ang)
=====================================================================
  1  -2.74   3.26  -2.71   1.76  -2.43   2.30   3.26
  2  -1.99   2.15  -1.80   1.84  -1.77   1.71   2.15
  3  -1.28   1.28  -1.19   1.19  -1.21   1.21   1.30
  4  -1.67   1.47  -1.23   1.28  -0.56   1.22   1.73
  5  -1.30   1.25  -1.20   1.21  -1.22   1.25   1.31
  6  -2.70   3.12  -1.67   1.64  -1.43   1.19   3.12
:: ADP  O2        0.014   0.044   0.197 - RATIO(MAX/MIN) =      14.6 prolate

:: TRMX =    1.00   0.00   0.00   0.00   1.00   0.00   0.00   0.00   1.00
:: CELL    12.367    13.818    16.091   105.224    97.197   113.827
:: Reflection Data are READ from File : platon.hkl - ( OBS-Data)

:: # Accepted Reflns     Hmax Kmax Lmax
::            44436      16   18   21

:: A,  B,  C  (Angstrom) =     12.367    13.818    16.091
:: NX, NY, NZ (SOLV-MAP) =         60        72        84
:: NX, NY, NZ (FFT-MAP)  =         64        64        64
:: Resolution (FFT-MAP)  =       0.19      0.22      0.25 Angstrom
```

:: R1 = 0.377 for 30928 Refl. with I > 2 s(I) and

:: wR2 = 1.559 for 44436 reflections

:: S = 4.054

:: Number of Removed Systematic Extinctions = 0

:: Number of Non-extinction Reflections = 44436

:: Number of Missing Low Order Reflections = 12

:: Fo-scale =0.632673E+00 - SinT/L-Min = 0.20 for Fo/Fc-Scaling

:: Cycle = 1, R(F) = 0.10, Nref(Hemi) = 11555, R(F > 4SIGF) = 0.06 Nref = 6124

==========

Unique Density Maxima in Enhanced Difference Map (CutOff level = 0.50 eA-3)

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#	x	y	z	(e/A^3)	Shortest Contacts within 3.2 Ang. (Excl. H)
1	0.839	0.641	0.812	1.37	C12 2.42;
2	0.846	0.717	0.676	1.21	C12 2.25; C11 2.28; C10 2.71; O2 3.07;
3	0.425	0.060	0.032	1.02	void O5 2.90; O10 3.08;
4	0.951	0.792	0.655	0.98	void C11 2.69;
5	0.567	0.445	0.421	0.92	C9 1.59; C10 2.33; C8 2.45; C11 2.66;
6	0.671	0.538	0.407	0.88	C11 2.09; C9 2.55; C10 2.70; C8 2.97;
7	0.738	0.526	0.780	0.84	C12 1.99;
8	0.593	0.348	0.417	0.75	C9 2.35; C10 2.52; C11 3.10;
9	0.719	0.439	0.614	0.69	C10 1.49; C12 1.52; C11 2.64; C9 2.66;

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Unique Density Minima in Enhanced Difference Map (CutOff level = -0.50 eA-3)

#	x	y	z	(e/A^3)	Shortest Contacts within 3.2 Ang. (Excl. H)
1	0.739	0.516	0.569	-0.54	C10 0.61; C11 1.27; C12 1.58; C9 2.07;
2	0.842	0.622	0.546	-0.53	C11 0.65; C10 2.09; C12 2.80; C9 3.16;
3	0.733	0.568	0.533	-0.50	C11 0.57; C10 1.13; C9 1.95; C12 2.23;

Void	X(av)	Y(av)	Z(av)	Volume Ang^3	El-Count (e-)	Vol/Electron	Vol/Atom
1	0.053	0.831	0.679	68	5	13.4	107
2	0.427	0.057	1.011	23	1	20.4	163
3	0.450	0.882	0.506	8	1	15.6	125
4	0.576	0.940	0.595	10	1	14.7	118
5	0.628	0.098	0.940	8	0	40.5	324
6	0.798	0.550	0.836	30	2	14.4	115

Total (Positive) Electron Count in Voids/Cell = 10

Total (Fo-Fc)map Electron Count in Unit Cell = 0

VOID-Fo-Fc-Map: Rho(min) = -0.25, Rho(max) = 1.37, RhoCutOff = 0.00
PeaksCloseToAtoms: Rho(min) = -0.54, Rho(max) = 0.92, RhoCutOff = 0.50

:: Fo-scale =0.633444E+00 - SinT/L-Min = 0.20 for Fo/Fc-Scaling

:: Cycle = 2, R(F) = 0.09, Nref(Hemi) = 11555, R(F > 4SIGF) = 0.05 Nref = 6124

Unique Density Maxima in Enhanced Difference Map (CutOff level = 0.50 eA-3)

#	x	y	z	(e/A^3)	Shortest Contacts within 3.2 Ang. (Excl. H)
1	0.838	0.642	0.813	2.56	C12 2.43;
2	0.856	0.737	0.672	2.28	C11 2.34; C12 2.51; C10 2.90; O2 3.01;
3	0.424	0.060	0.032	1.98	void O5 2.90; O10 3.07;
4	0.951	0.793	0.655	1.90	void C11 2.70;
5	0.739	0.533	0.780	1.43	C12 1.96;
6	0.567	0.446	0.421	0.89	C9 1.59; C10 2.32; C8 2.45; C11 2.66;
7	0.671	0.538	0.406	0.78	C11 2.10; C9 2.56; C10 2.71; C8 2.99;
8	0.595	0.349	0.415	0.77	C9 2.37; C10 2.53; C11 3.09;
9	0.720	0.439	0.613	0.65	C10 1.49; C12 1.53; C11 2.63; C9 2.66;
10	0.012	0.855	0.707	0.62	void C47 2.90;
11	0.065	0.934	0.686	0.55	void C70 3.09; C40 3.12; O8 3.15;
12	0.433	0.935	0.533	0.50	void C31 2.67; C30 3.07; C5 3.11;
13	0.796	0.643	0.616	0.50	C11 1.27; C10 1.72; C12 1.78; C9 2.78;

Unique Density Minima in Enhanced Difference Map (CutOff level = -0.50 eA-3)

#	x	y	z	(e/A^3)	Shortest Contacts within 3.2 Ang. (Excl. H)
1	0.738	0.516	0.566	-0.53	C10 0.61; C11 1.24; C12 1.61; C9 2.05;
2	0.656	0.515	0.559	-0.51	C10 0.45; C9 1.11; C12 1.58; C11 1.67;

Void	X(av)	Y(av)	Z(av)	Volume Ang^3	El-Count (e-)	Vol/Electron	Vol/Atom
1	0.053	0.831	0.679	68	9	7.9	63
2	0.427	0.057	1.011	23	2	12.6	101

3	0.450	0.882	0.506	8	1	7.8	62
4	0.576	0.940	0.595	10	1	7.9	63
5	0.628	0.098	0.940	8	0	26.8	215
6	0.798	0.550	0.836	30	4	7.8	62

Total (Positive) Electron Count in Voids/Cell = 17

Total (Fo-Fc)map Electron Count in Unit Cell = 10

VOID-Fo-Fc-Map: Rho(min) = -0.25, Rho(max) = 2.56, RhoCutOff = 0.00
PeaksCloseToAtoms: Rho(min) = -0.53, Rho(max) = 1.43, RhoCutOff = 0.50

:: Fo-scale =0.633348E+00 - SinT/L-Min = 0.20 for Fo/Fc-Scaling

:: Cycle = 3, R(F) = 0.09, Nref(Hemi) = 11555, R(F > 4SIGF) = 0.05 Nref = 6124

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Unique Density Maxima in Enhanced Difference Map (CutOff level = 0.50 eA-3)

==========

#	x	y	z	(e/A^3)	Shortest Contacts within 3.2 Ang. (Excl. H)
1	0.838	0.642	0.813	2.53	C12 2.43;
2	0.856	0.737	0.673	2.25	C11 2.35; C12 2.50; C10 2.90; O2 3.01;
3	0.424	0.060	0.033	2.00 void	O5 2.90; O10 3.07;
4	0.951	0.793	0.655	1.92 void	C11 2.70;
5	0.739	0.534	0.781	1.39	C12 1.96;
6	0.567	0.446	0.421	0.89	C9 1.59; C10 2.33; C8 2.45; C11 2.66;
7	0.671	0.538	0.406	0.78	C11 2.10; C9 2.56; C10 2.71; C8 2.98;
8	0.595	0.349	0.416	0.78	C9 2.37; C10 2.52; C11 3.09;

9 0.720 0.439 0.613 0.65 C10 1.49; C12 1.53; C11 2.64; C9 2.66;

10 0.012 0.854 0.707 0.60 void C47 2.89;

11 0.066 0.934 0.686 0.54 void C70 3.09; C40 3.13; O8 3.15;

12 0.434 0.935 0.532 0.52 void C31 2.66; C30 3.05; C5 3.14;

==========

Unique Density Minima in Enhanced Difference Map (CutOff level = -0.50 eA-3)

==========

x y z (e/A^3) Shortest Contacts within 3.2 Ang. (Excl. H)

==========

1 0.738 0.516 0.566 -0.54 C10 0.60; C11 1.24; C12 1.61; C9 2.05;

2 0.656 0.515 0.559 -0.51 C10 0.44; C9 1.11; C12 1.57; C11 1.67;

Void X(av) Y(av) Z(av) Volume Ang^3 El-Count (e-) Vol/Electron Vol/Atom

==========

1	0.053	0.831	0.679	68	8	8.0	64
2	0.427	0.057	1.011	23	2	13.0	104
3	0.450	0.882	0.506	8	1	7.6	61
4	0.576	0.940	0.595	10	1	7.8	62
5	0.628	0.098	0.940	8	0	27.5	220
6	0.798	0.550	0.836	30	4	7.8	63

Total (Positive) Electron Count in Voids/Cell = 17

Total (Fo-Fc)map Electron Count in Unit Cell = 17

VOID-Fo-Fc-Map: Rho(min) = -0.24, Rho(max) = 2.53, RhoCutOff = 0.00

PeaksCloseToAtoms: Rho(min) = -0.54, Rho(max) = 1.39, RhoCutOff = 0.50

:: Fo-scale =0.633349E+00 - SinT/L-Min = 0.20 for Fo/Fc-Scaling

:: Cycle = 4, R(F) = 0.09, Nref(Hemi) = 11555, R(F > 4SIGF) = 0.05 Nref = 6124

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Unique Density Maxima in Enhanced Difference Map (CutOff level = 0.50 eA-3)

==========

#	x	y	z	(e/A^3)	Shortest Contacts within 3.2 Ang. (Excl. H)
1	0.838	0.642	0.813	2.53	C12 2.44;
2	0.856	0.737	0.673	2.26	C11 2.35; C12 2.50; C10 2.90; O2 3.01;
3	0.424	0.060	0.033	2.02	void O5 2.90; O10 3.07;
4	0.951	0.793	0.655	1.94	void C11 2.70;
5	0.739	0.534	0.781	1.38	C12 1.96;
6	0.567	0.446	0.421	0.89	C9 1.59; C10 2.33; C8 2.45; C11 2.66;
7	0.671	0.538	0.406	0.78	C11 2.10; C9 2.56; C10 2.71; C8 2.98;
8	0.594	0.349	0.416	0.78	C9 2.36; C10 2.52; C11 3.09;
9	0.720	0.439	0.613	0.65	C10 1.49; C12 1.53; C11 2.64; C9 2.66;
10	0.011	0.854	0.707	0.59	void C47 2.89;
11	0.066	0.933	0.686	0.54	void C70 3.09; C40 3.13; O8 3.16;
12	0.434	0.935	0.531	0.53	void C31 2.65; C30 3.04; C5 3.15;

==========

Unique Density Minima in Enhanced Difference Map (CutOff level = -0.50 eA-3)

==========

#	x	y	z	(e/A^3)	Shortest Contacts within 3.2 Ang. (Excl. H)
1	0.738	0.516	0.566	-0.54	C10 0.60; C11 1.24; C12 1.61; C9 2.05;
2	0.656	0.515	0.559	-0.51	C10 0.44; C9 1.12; C12 1.57; C11 1.67;

Void	X(av)	Y(av)	Z(av)	Volume Ang^3	El-Count (e-)	Vol/Electron	Vol/Atom

==========

1	0.053	0.831	0.679	68	8	8.1	65
2	0.427	0.057	1.011	23	2	13.1	105
3	0.450	0.882	0.506	8	1	7.6	60
4	0.576	0.940	0.595	10	1	7.7	62
5	0.628	0.098	0.940	8	0	27.5	220
6	0.798	0.550	0.836	30	4	7.9	63

Total (Positive) Electron Count in Voids/Cell = 17

Total (Fo-Fc)map Electron Count in Unit Cell = 17

VOID-Fo-Fc-Map: Rho(min) = -0.25, Rho(max) = 2.53, RhoCutOff = 0.00
PeaksCloseToAtoms: Rho(min) = -0.54, Rho(max) = 1.38, RhoCutOff = 0.50

:: Fo-scale =0.633357E+00 - SinT/L-Min = 0.20 for Fo/Fc-Scaling

:: Cycle = 5, R(F) = 0.09, Nref(Hemi) = 11555, R(F > 4SIGF) = 0.05 Nref = 6124

:: TRMX = 1.00 0.00 0.00 0.00 1.00 0.00 0.00 0.00 1.00
:: CELL 12.367 13.818 16.091 105.224 97.197 113.827
:: Reflection Data are READ from File : platon.hkl - (OBS-Data)

:: TRMX = 1.00 0.00 0.00 0.00 1.00 0.00 0.00 0.00 1.00
:: CELL 12.367 13.818 16.091 105.224 97.197 113.827
:: Reflection Data are READ from File : platon.hkl - (OBS-Data)

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Note: SQUEEZE Produces and Analyses a Phase Enhanced

Difference Map. No Model Refinement is done by SQUEEZE.

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Note on how to Proceed after running PLATON/SQUEEZE:

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- The file .hkp now contains solvent free reflection data.
  (Original I(obs), A(solv) and B(solv) are beyond column 80)
- Rename this file from extension .hkp to extension .hkl.
- Use this new .hkl file to continue (SHELXL) refinement with
  a disordered solvent free .ins.
- After L.S.-convergence, run PLATON again with the new .hkl
  and .res files with the instruction 'CALC FCF' in order to
  produce a proper final FoFc-CIF on a file with ext = .hkp.
- Rename this .hkp file to a file with extension .fcf.
- Append the .sqf file produced by PLATON (detailing the
  SQUEEZE results) to the .cif produced by SHELXL.
- Optionally inspect the improved peak list on file .sqz.

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Summary and Remarks : N = NOTE, W = WARNING, E = ERROR

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W: Number of unusual anisotropic displacement parameters 1

N: Total Potential Solvent Accessible Void Vol ..... 147.5 Ang^3

N: Electron Count / Cell = 17 - To be included in D(calc), F000 & Mol.Wght.

Crystal structure analysis of (Ac-Val-dgF-Val-^{D}Pro-Gly-Leu-dgL-Val-Ala-^{D}Pro-Gly-Leu-Val-dgF-Val-NH_2) P2

Crystals of peptide were grown by slow evaporation from a solution of methanol and water. A single crystal (0.20 × 0.16 × 0.14 mm) was mounted in a loop with a small amount of the mother liquor. The X-ray data were collected at 100 K temperature on a Bruker AXS SMART APEX II CCD Duo diffractometer using CuK\a radiation (λ = 1.54178Å), ω-scans (2θ = 67.679^{o}), for a total number of 16610 independent reflections. . Space group P 21, a = 14.1965(4), b = 21.9607(7), c = 16.3804(5) Å, α = 90, β = 111.9040(15), γ = 90(4), V= 4738.2(3)Å^3, monoclinic, Z =2 for chemical formula (C86 H132 N16 O16), with one molecule in asymmetric unit; ρ_{calcd} = 1.154 g cm^{-3}, μ = 0.065 mm^{-1}, F(000) =2090, R_{int} = 0.0457. The structure was obtained by direct methods using SHELXS-97.[2] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. The final R value was 0.0719 ($wR2$= 0.1933) for 14462 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 1081 variables, S =0 .824.

Variable Temperature ^{1}H NMR of P1

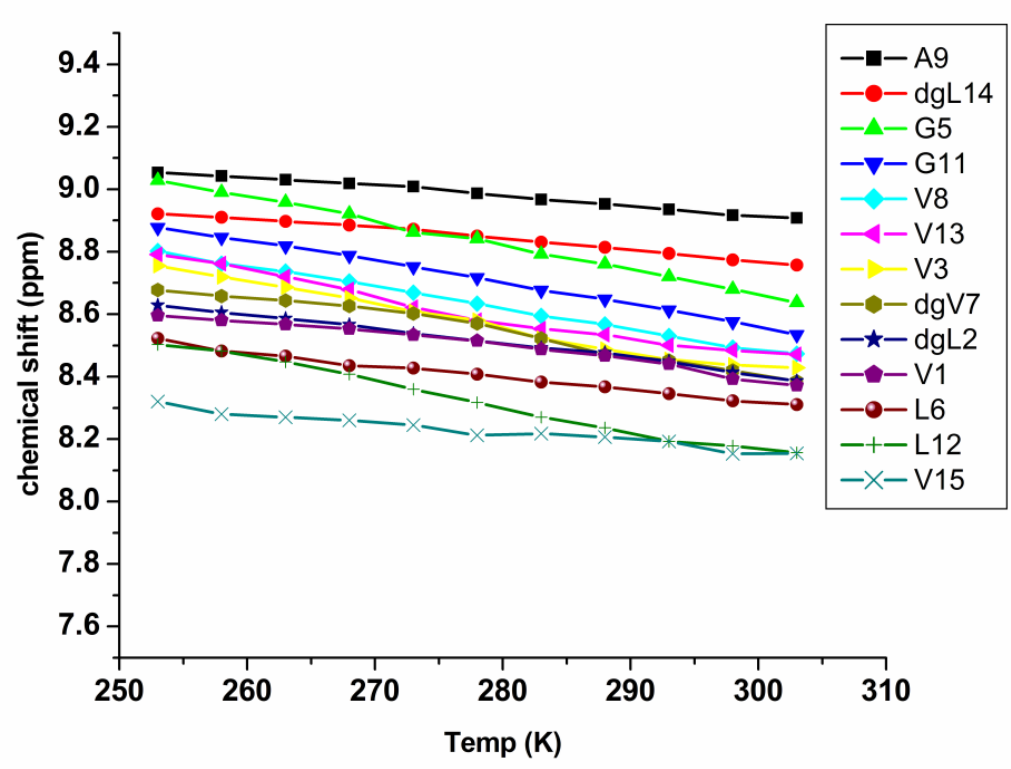


Figure S9: Plot of amides chemical shifts with increasing temperature. The temperature coefficient values (dδ/dt) are given in the Table S10.

Table S10: Chemical Shifts and temperature coefficients of peptide **P1**

Residue	Chemical Shifts (ppm)							$^{3}J_{NHC^{\alpha}H}$	dδ/dT
	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	$C^{\omega}H$	Others	(Hz)	ppb/K
Val1	8.38	4.39	2.03	0.8-0.6	-	-	-	9.15	-4.49
dgL2	8.39	6.61	6.64	4.62	1.36	1.63	0.8-0.6	8.85	-4.83
Val3	8.44	4.74	2.12	0.8-0.6	-	-	-	9.25	-6.48
^{D}Pro4	-	4.33	2.19-1.96	2.17-2.01	3.78	-	-	-	-
Gly5	8.66	3.79-3.98	-	-	-	-	-	6.1	-7.80
Leu6	8.32	4.6	1.88	1.72	0.8-0.6	-	-	8.55	-4.22
dgV7	8.40	6.62	6.80	4.30	1.72	0.8-0.6		8.9	-5.86
Val8	8.48	4.18	2.08	0.8-0.6	-	-	-	9.15	-6.47
Ala9	8.91	4.92	1.32	-	-	-	-	7.95	-2.91
Pro10	-	4.35	2.24-2.02	2.17-2.01	3.76	-	-	-	-
Gly11	8.55	3.69-4.03	-	-	-	-	-	6.4	-6.85
Leu12	8.17	4.61	1.92	1.71	0.8-0.6	-	-	9.15	-6.92
Val13	8.47	5.02	2.03	0.8-0.6	-	-	-	9.15	-6.40
dgL14	8.77	6.22	6.87	4.75	1.58	1.28	0.8-0.6	8.55	-3.28
Val15	8.13	4.52	2.12	0.8-0.6	-	-	-	8.5	-3.31

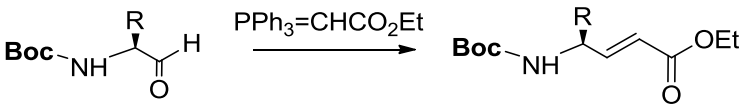
General Experimental Details

All amino acids, DiPEA, TFA, Triphenylphosphine were purchased from Aldrich. THF, DCM, DMF, NaOH were purchased from Merck. Ethyl bromoacetate, HBTU, HOBT, EtOAc, NMP, Pet-ether (60-80 ^{o}C) were obtained from spectrochem and used without further purification. THF and DiPEA were dried over sodium and distilled immediately prior to use. Column chromatography was performed on Merck silica gel (120-200 mesh). Final peptides were purified on reverse phase HPLC (Waters 600) (C18 column, MeOH/H_2O 65:35- 95:5 as gradient, 1.25 mL flow per min). The ^{1}H spectra were recorded on Bruker 500 MHz (or 125 MHz for ^{13}C) and Jeol 400 MHz (or 100 MHz for ^{13}C) using residual solvents signals as an internal reference ($CDCl_3$ δ_H, 7.26 ppm, δ_c 77.3 ppm and CD_3OH δ_H 3.31 ppm, δ_c 49.0 ppm). The chemical shifts (δ) are reported in *ppm* and coupling constants (*J*) in Hz. Mass was recorded on MALDI TOF/TOF (Applied Biosciences) and CD was recorded on JASCO(*J*-815). X-Ray data was collected on Bruker APEX DUO. Self aggregation of peptides were measured using DLS (Malvern: Zetasizer Nano-ZS90). AFM data were cololected on JPK: NanoWizeredII AFM system.

NMR spectroscopy: All NMR studies were carried out by using a Bruker AVANCE^{III}-500 MHz spectrometer at a probe temperature of 300 K. Resonance assignments were obtained by TOCSY and ROESY analysis. All two-dimensional data were collected in phase-sensitive mode, by using the time-proportional phase incrementation (TPPI) method. Sets of 1024 and 512 data points were used in the t_2 and t_1 dimensions, respectively. For TOCSY and ROESY analysis, 32 and 72 transients were collected, respectively. A spectral width of 6007 Hz was used in both dimensions. A spin-lock time of 256 ms was used to obtain ROESY spectra. Zero-filling was carried out to finally yield a data set of 2 K ×1 K. A shifted square-sine-bell window was used before processing.

General procedure for the synthesis of ethyl ester of Boc-Protected α, *β*-unsaturated *γ*-amino acids.

Boc-amino aldehyde (10 mmol) was dissolved in 30 mL of dry THF. The ylide (15 mmol, 5.22g) was added at RT. Reaction mixture was stirred for about 5hrs at RT. Completion of reaction was monitored by TLC. After completion, reaction mixture was quenched with 50 mL of 2*N* ammonium chloride solution. The product was extracted with EtOAc (3× 80 mL). Combined organic layer was washed with brine (60 mL) and dried over anhydrous Na_2SO_4. Organic layer was concentrated under reduced pressure to give crude product, which was further purified on silica gel column chromatography using EtOAc/Pet-ether (60-80 ^oC) to get pure ethyl ester of Boc-protected *α, β*-unsaturated *γ*-amino acid.



R = -^{i}Pr, -^{i}Bu

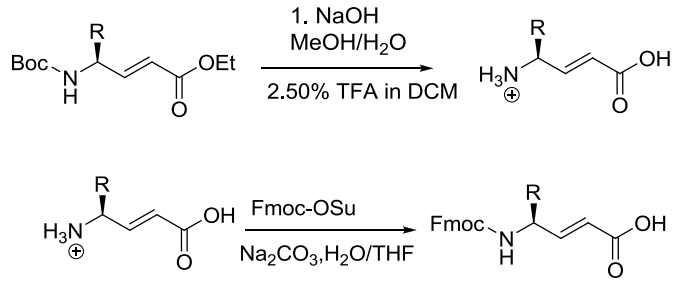
Scheme 1: Synthesis of ethyl ester of Boc-Protected α, *β*-unsaturated *γ*- amino acids

General procedure for the synthesis of Fmoc-*α, β*-unsaturated *γ*- amino acids.

Boc-*α, β*-unsaturated γ-amino acid: Ethyl ester of Boc-*α, β*-unsaturated *γ*- amino acid (2 mmol) was dissolved in 5 mL of ethanol. Then 5 mL of 1*N* NaOH was added slowly to the reaction mixture. After completion of the reaction (~30 min), ethanol was evaporated from the reaction mixture and residue was acidified using 10 mL of 5% HCl (5% volume in water) at cold conditions. The product was extracted with ethyl acetate (3× 40 mL). Combined organic layer was washed with brine (30 mL) and dried over anhydrous Na_2SO_4. The solvent was concentrated under reduced pressure to give Boc-*α, β*-unsaturated γ-amino acid as gummy product in a quantitative yield.

The Boc-*α, β*-unsaturated gamma amino acid (1 mmol) was dissolved in 5 mL of DCM and cooled to 0 ^oC in ice bath followed by 5 mL of neat TFA was added to the reaction mixture. After 30 min, TFA was removed from reaction mixture under *vacuum*. Residue was dissolved in 15 mL of water (15 mL) and the pH was adjusted to ~10 by the slow addition of solid Na_2CO_3. The solution of Fmoc-OSu (1 mmol) in 10 mL of THF was added slowly to the reaction mixture. Reaction mixture was stirred overnight at RT. After completion of the reaction, the reaction mixture was acidified with 20 mL of 20% HCl (20% volume in water) in cold condition. Product was extracted with ethyl acetate (3× 50 mL). Combined organic layer was washed with brine (30 mL) and dried over anhydrous Na_2SO_4. The solvent was concentrated under reduced pressure to give gummy product, which was recrystallized using EtOAc/Pet-ether. The pure white solid of

Fmoc- α, β-unsaturated γ-amino acid was subjected for SPPS. The schematic representation of the synthesis is shown below.



R = -^{i}Pr, -^{i}Bu

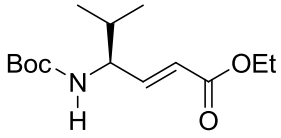
Scheme 2: Synthesis of Fmoc-Protected α, β-unsaturated γ- amino acids

(*S, E*)-ethyl 4-(tert-butoxycarbonylamino)-5-methylhex-2-enoate (Boc-dgV-OEt) Colorless crystalline (2.30g, 85%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88-6.84 (dd, *J* = 15.5, *J* = 4.5, 1H, C*H*=CHCO$_2$Et), 5.93-5.90 (d, *J* = 15.5, 1H, CH=C*H*CO$_2$Et), 4.57-4.56 (d, *J* = 8.5, 1H, NH), 4.21-4.17 (m, 3H, -OC*H*$_2$, C*H*-CH=CH), 1.89-1.85 (m, 1H, C*H*-(CH$_3$)$_2$), 1.45 (s, 9H, -(CH$_3$)$_3$ Boc), 1.30-1.28 (t, *J* = 7, 3H, -OCH$_2$C*H*$_3$), 0.95-0.90 (dd, *J* = 15.5, *J* = 7, 6H, CH-(C*H*$_3$)$_2$);

^{13}C NMR (100MHz, $CDCl_3$) δ 166.24, 155.31, 147.34, 121.40, 79.61, 60.38, 56.61, 32.18, 28.29, 18.81, 17.94, 14.18;

MALDI TOF/TOF mass calcd. for $C_{14}H_{25}NO_4$ $[M+Na]^+$ 294.1681, observed, 294.1606.

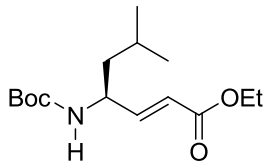


Boc-(*S, E*)-dgV-OEt

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

Colorless crystalline (2.47g, 87%); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.85-6.80 (dd, *J* = 16, *J* = 5.5, 1H, C*H*=CHCO$_2$Et), 5.93-5.89 (d, *J*=16, 1H, CH=C*H*CO$_2$Et), 4.49 (b, 1H, NH), 4.37-4.32 (m, 1H, C*H*-CH=CH), 4.21-4.16 (q, *J* = 7, 2H, -OC*H*$_2$), 1.72-1.67 (m, 1H, C*H*-(CH$_3$)$_2$), 1.44 (s, 9H, -(CH$_3$)$_3$ Boc), 1.40-1.37 (t, *J* = 7, 2H, C*H*$_2$CH-(CH$_3$)$_2$), 1.30-1.27 (t, *J* = 7, 3H, -OCH$_2$C*H*$_3$),

0.94-0.93 (d, *J* = 6.5, 6H, CH-(C*H*$_3$)$_2$); **^{13}C NMR** (100MHz, $CDCl_3$) δ 166.41, 155.05, 148.89, 120.35, 79.65, 60.40, 49.75, 43.78, 28.31, 24.67, 22.68, 22.14, 14.20; **MALDI TOF/TOF** mass calcd. for $C_{15}H_{27}NO_4$ $[M+Na]^+$ 308.1838, observed, 308.1857.

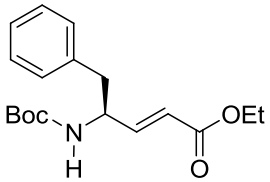


Boc-(*S, E*)-dgL-OEt

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

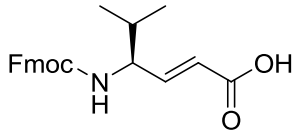
White powder (2.67 g, 84%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.30-7.14 (m, 5H, -Ph), 6.91-6.86 (dd, *J* = 5.04, *J* = 11, 1H, C*H*=CHCO$_2$Et), 5.85-5.81 (d, *J* = 17.4, 1H, CH=C*H*CO$_2$Et), 4.59 (b, 1H, NH), 4.52 (b, 1H, C*H*-CH=CH), 4.18-4.13 (q, *J* = 6.88, 2H, -OC*H*$_2$), 2.92-2.85 (m, 2H, C*H*$_2$-Ph), 1.37 (s, 9H, -(CH$_3$)$_3$ Boc), 1.27-1.23 (t, *J* = 7.3, 3H, -OCH$_2$C*H*$_3$);

^{13}C NMR (100MHz, $CDCl_3$) δ 166.14, 154.91, 147.56, 136.33, 129.36, 128.54, 126.83, 121.04, 79.83, 60.44, 52.16, 40.82, 28.26, 14.18; **MALDI TOF/TOF** m/z Calcd. For $C_{18}H_{25}NO_4$ $[M+Na]^+$ 342.1681, observed 342.1657.



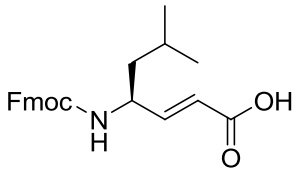
Boc-(*S, E*)-dgF-OEt

(*S, E*)-4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-5-methylhex-2-enoic acid: White powder (0.590g, 90%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.7814-7.3095(m, 8H, -Fmoc aromatic) 6.9853-6.9349(dd, *J*=9.6, *J*=5.34, 1H, C*H*=CHCO$_2$Et), 5.9154-5.8730 (d, *J*=16, 1H, CH=C*H*CO$_2$Et), 4.8123-4.7871 (d, *J*=9.1,1H, NH), 4.4881-4.4732 (d, *J*=6.1, 2H, -OC*H*$_2$-Fmoc), 4.3174-4.2086 (m, 2H, C*H*-CH=CH,-C*H*(of Fmoc)), 1.9360-1.8524(m, 1H, C*H*-(CH$_3$)$_2$), 0.9543-0.9096 (m, 6H, CH-(C*H*$_3$)$_2$); **^{13}C NMR** (100MHz, $CDCl_3$) δ 170.6868, 155.8987, 149.3389, 143.7707, 141.3298, 127.7430, 127.0661, 124.9017, 121.0402, 119.9819, 66.6454, 57.2825, 47.2902, 32.1112, 18.8772, 18.0191; **MALDI TOF/TOF** Mass calcd. for $C_{22}H_{23}NO_4$ [M+Na$^+$] 388.1525, observed, 388.1581

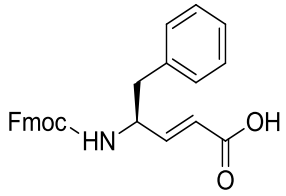


Fmoc-(*S, E*)-dgV-OH

(*S,E*)-4-(((9H-fluoren-9-yl)methoxy)carbonylamino)-6-methylhept-2-enoic acid: White powder (0.60 g, 89%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.7754-7.3033(m, 8H, -Fmoc aromatic) 6.9482-6.8955(dd, *J*=10.5, *J*=5.4, 1H, C*H*=CHCO$_2$Et), 5.9069-5.8680 (d, *J*=15.5, 1H, CH=C*H*CO$_2$Et), 4.6859-4.6652 (d, *J*=8.2,1H, NH), 4.4842-4.4686 (d, *J*=6.2, 2H, -OC*H*$_2$-Fmoc)), 4.4281-4.3926 (t, *J*=4.7, 1H, C*H*-CH=CH), 4.2277-4.1967(t, *J*=4.1, 1H,-C*H*(of Fmoc), 1.6824-1.6033(m, 2H, C*H*$_2$-CH-(CH$_3$)$_2$), 1.4281-1.3926 (t, *J*=4.7, 3H, C*H*-(CH$_3$)$_2$) 0.9372-0.9218 (d, *J* = 6.1, 6H, CH-(C*H*$_3$)$_2$); **^{13}C NMR** (100MHz, $CDCl_3$) δ 170.7535, 155.6031, 150.8358, 143.7325, 141.3298, 127.7335, 127.0661, 124.9017, 119.9724, 66.5501, 50.3032, 47.29.02, 43.4669, 24.6742, 22.7196, 22.0331; **MALDI TOF/TOF** Mass calcd. for $C_{23}H_{25}NO_4$ [M+Na$^+$] 402.1681, observed, 402.1662.



Fmoc-(*S, E*)-dgL-OH **(S,E)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-phenylpent-2-enoic acid(^{1}H** NMR; 400 MHz (DMSO D_6, 25°C,): δ = 2.72-2.80 (m, 1H), 2.87-2.92 (m, 1H), 4.14-4.27 (m,3H), 4.40-4.44 (m, 1H), 5.77-5.81 (d, J=8Hz,1H), 6.80-6.85 (dd, J=4Hz,4Hz, 1H), 7.19-7.34 (m, 8H), 7.39-7.42 (m, 2H), 7.61-7.68 (m, 3H), 7.87-7.89 (m, 2H), 12.32(bs, 1H)ppm ; ^{13}C NMR 100 MHz (DMSO, 25°C,): δ = 47.1, 53.7, 65.9, 120.6, 121.5, 125.6, 126.7, 127.4, 128.0, 128.6, 129.6, 128.6, 129.6 ,138.5, 141.1, 144.1, 144.3, 148.5, 155.9, 167.4ppm; MALDI.TOF/ m/z Calculated value for $C_{26}H_{23}NO_4$ [M+Na]$^+$ 413.16 and Observed 436.13.

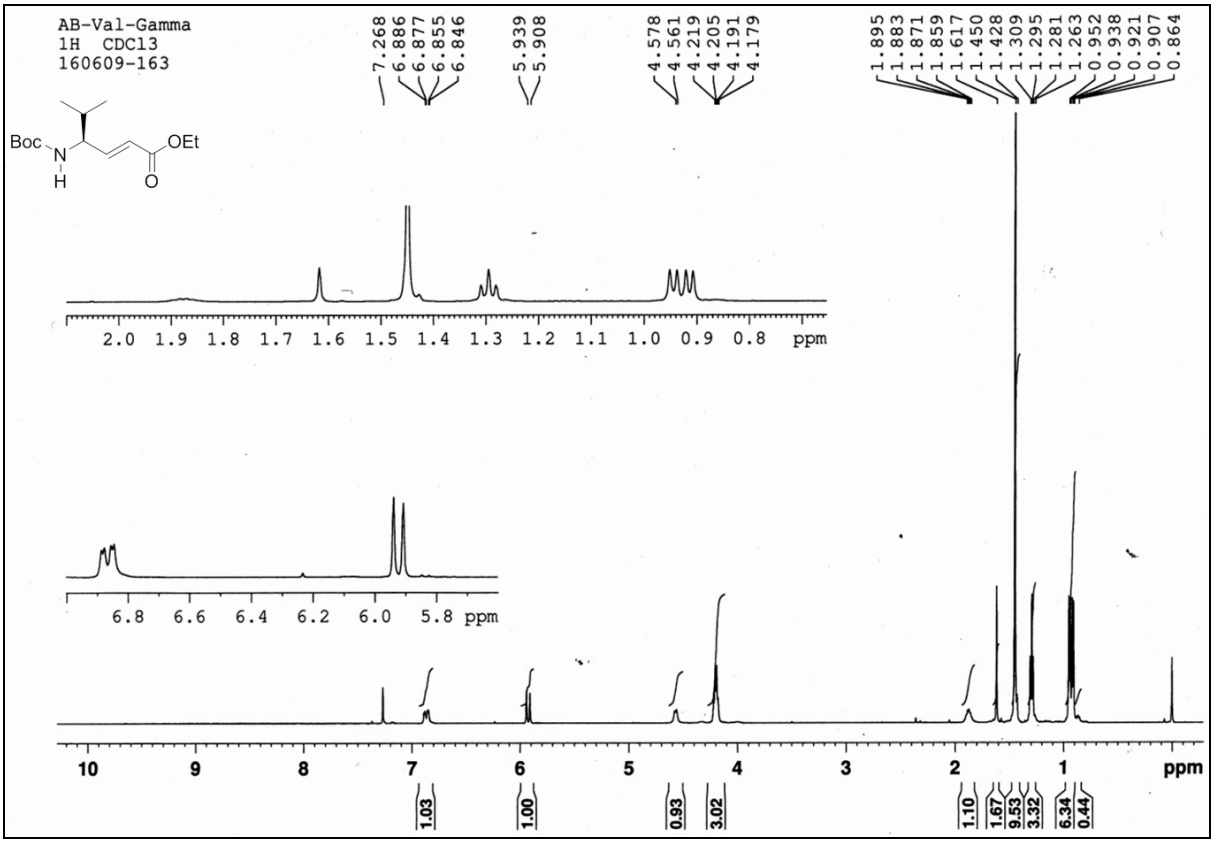


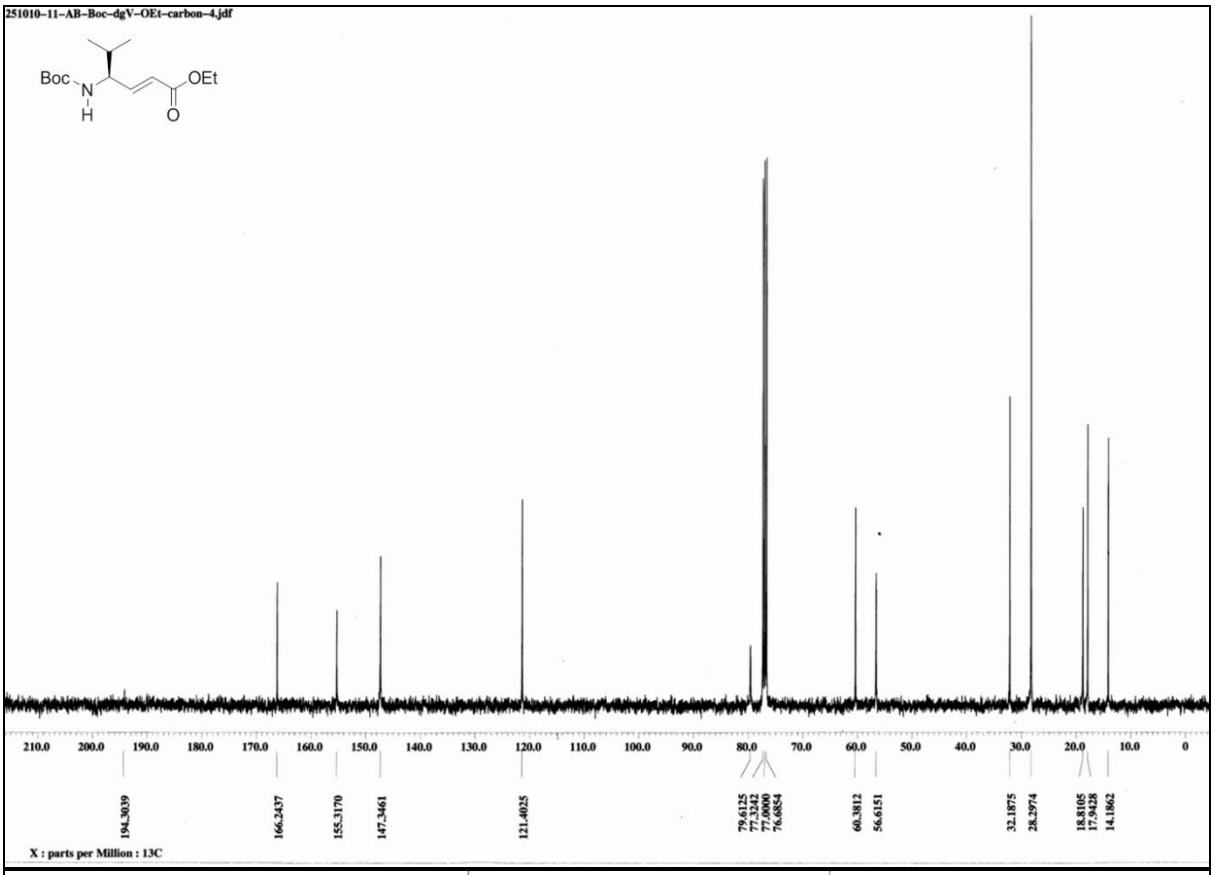
Fmoc-(S,E)-dgF-OH

SPPS peptide synthesis of P1 and P2

Peptides **P5** and **P6** were synthesized at 0.2 mmol scale on Rink Amide resin using standard Fmoc-chemistry. HBTU/HOBT were used as coupling agents for alpha amino acids and only HBTU was used as coupling agent for *E*-vinylogous γ-amino acids. Fmoc deprotections were performed using 20% piperidine in DMF. The coupling reactions were monitored by Kaiser Test. N-terminal of the final peptides was capped with acetyl group using acetic anhydride and pyridine. After completion of the synthesis, peptide was cleaved from the resin using 15 mL of TFA/thioanisole/H_2O (98:1.5:0.5) cocktail mixture. The cleavage mixture was evaporated under reduced pressure to give gummy product. Peptides were precipitated using EtOAc/hexane and purified on reverse phase HPLC, with C_{18} column using MeOH/H_2O (system MeOH/H_2O 65:35-95:5 as gradient, 1.25 mL flow per min) gradient system. Homogeneity of the peptides was further confirmed using analytical C_{18} column in same MeOH/H_2O (system MeOH/H_2O 65:35-95:5 as gradient, 0.75 mL flow per min) gradient system. Homogeneity of the peptides P1was further confirmed using analytical C_{18} column in same MeOH/H_2O (system MeOH/H_2O 70:30-95:5 as gradient, 3 mL flow per min) gradient system.

NMR and Mass Spectral Data

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Boc
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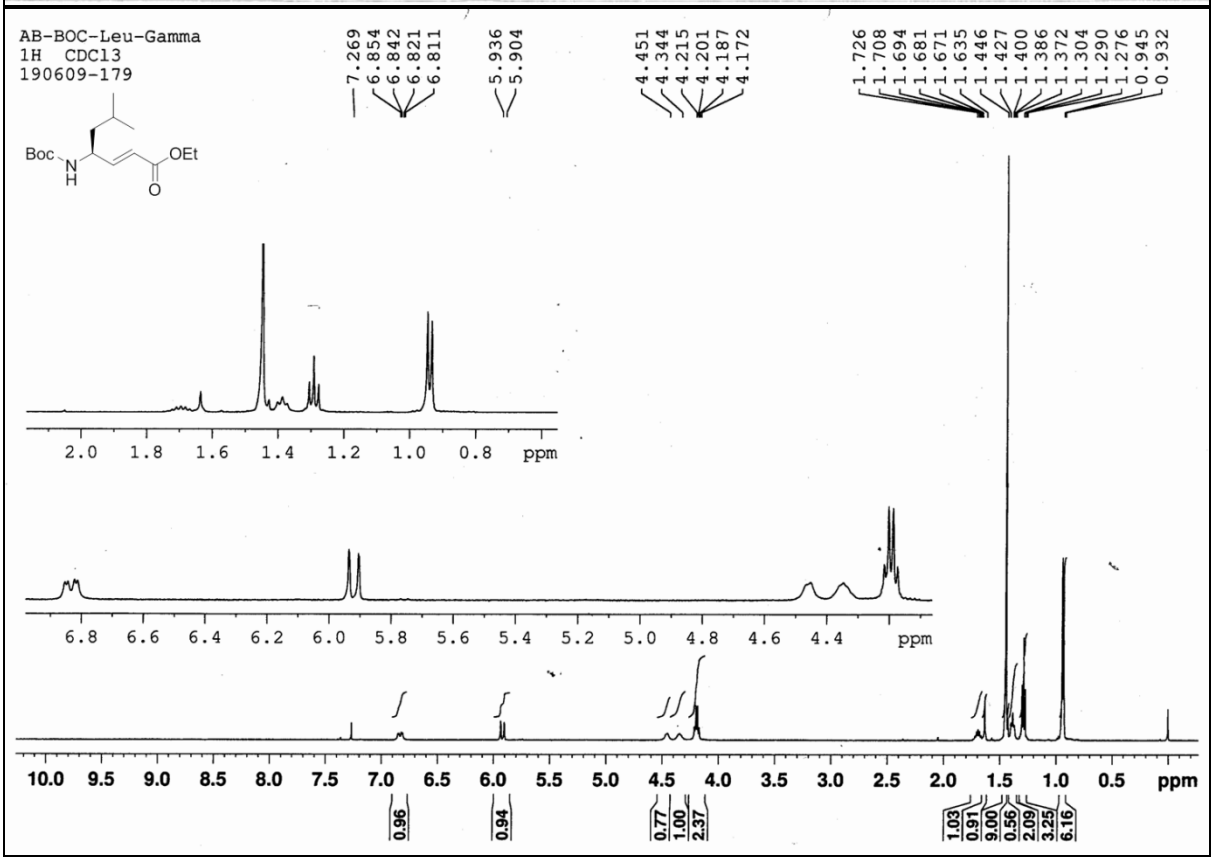
H
OEt
O
194.3039
166.2437
155.3170
147.3461
121.4025
79.6125
77.3242
77.0000
76.6854
60.3812
56.6151
32.1875
28.2974
18.8105
17.9428
14.1862
X : parts per Million : 13C


AB-BOC-Leu-Gamma
1H CDCl3
190609-179
7.269
6.854
6.842
6.821
6.811
5.936
5.904
4.451
4.344
4.215
4.201
4.187
4.172
1.726
1.708
1.694
1.681
1.671
1.635
1.446
1.427
1.400
1.386
1.372
1.304
1.290
1.276
0.945
0.932
Boc
N
H
OEt
O
ppm
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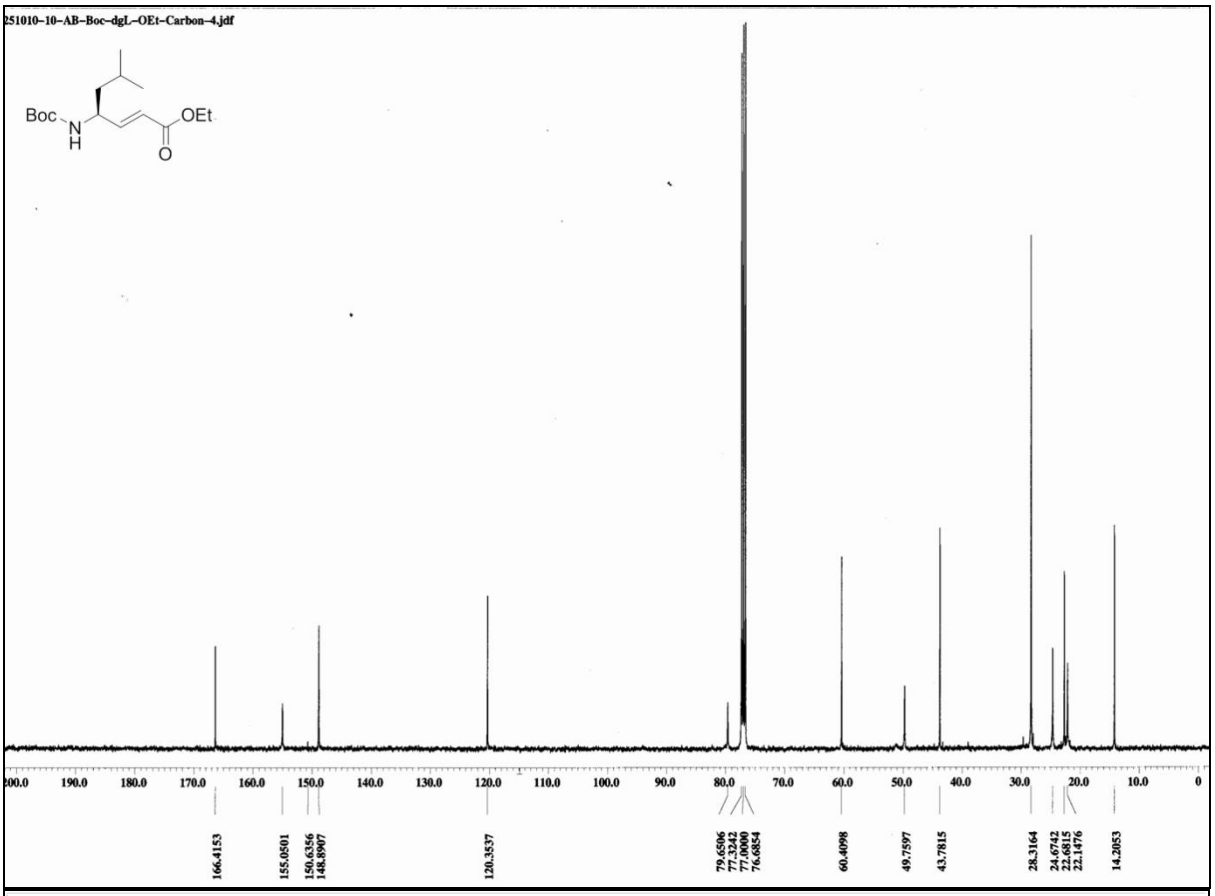
0.94
0.77
1.00
2.37
1.03
0.91
9.00
0.56
2.09
3.25
6.16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Boc
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H
OEt
O
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166.4153
155.0501
150.6356
148.8907
120.3537
79.6506
77.3242
77.0000
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60.4098
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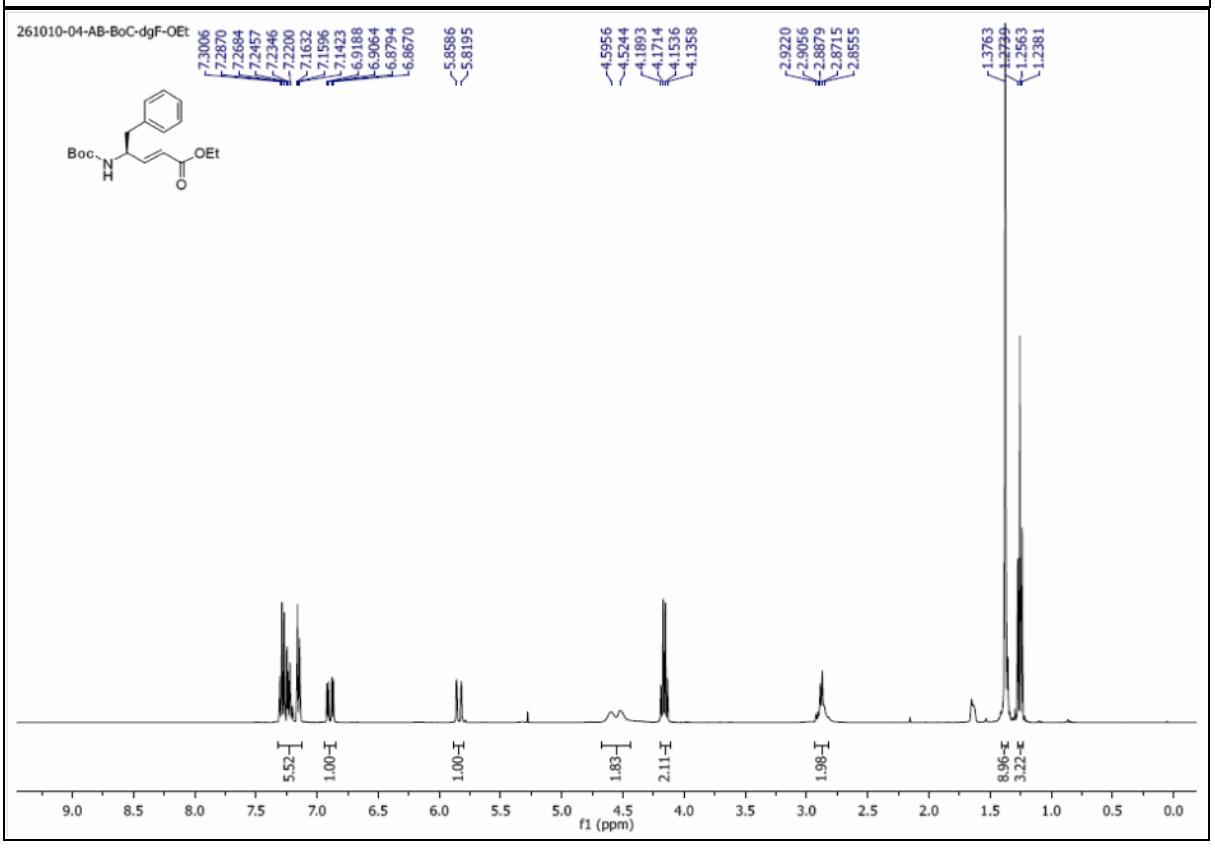
28.3164
24.6742
22.6815
22.1476
14.2053


261010-04-AB-BoC-dgF-OEt
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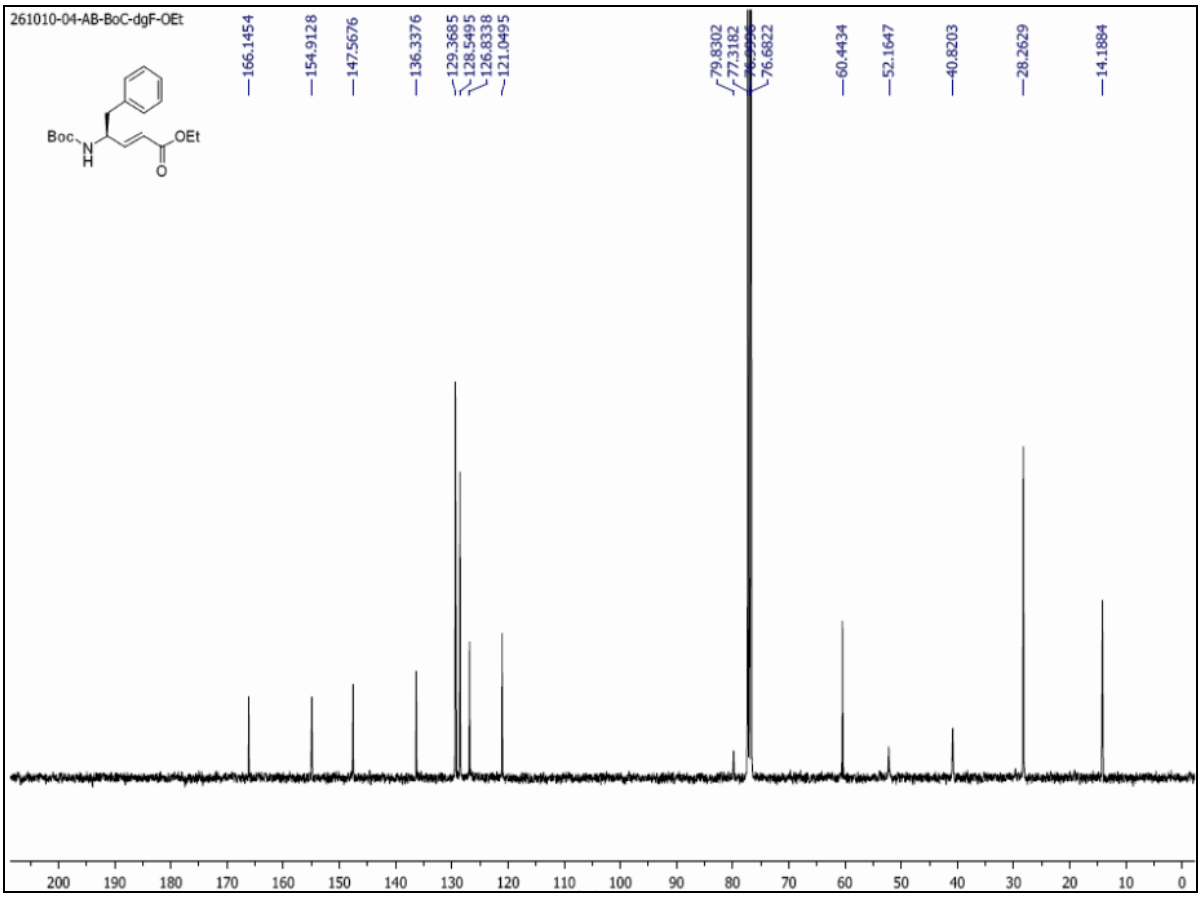
7.2870
7.2684
7.2457
7.2346
7.2200
7.1632
7.1596
7.1423
6.9188
6.9064
6.8794
6.8670
5.8586
5.8195
4.5956
4.5244
4.1893
4.1714
4.1536
4.1358
2.9220
2.9056
2.8879
2.8715
2.8555
1.3763
1.2739
1.2563
1.2381
Boc
N
H
OEt
O
5.52
1.00
1.00
1.83
2.11
1.98
8.96
3.22
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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79.8302
77.3182
76.6822
60.4434
52.1647
40.8203
28.2629
14.1884
Boc
N
H
OEt
O
200
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180
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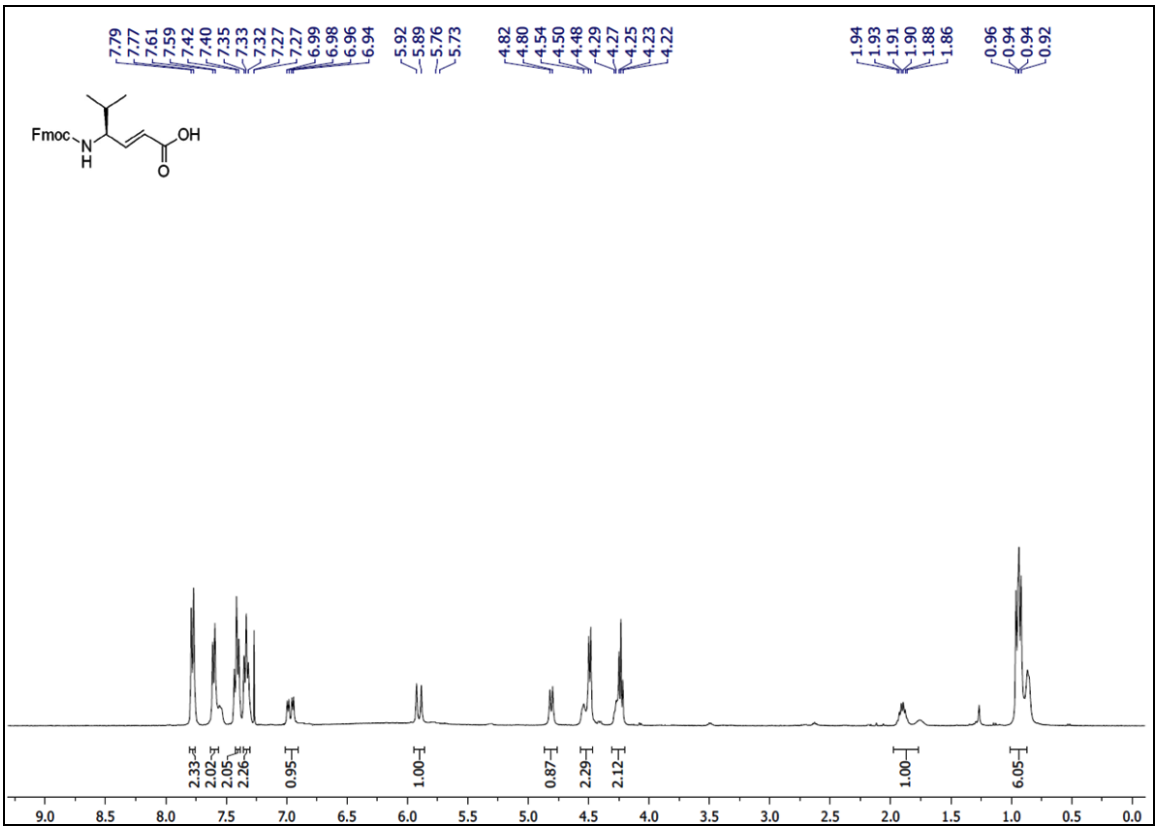
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0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7.42
7.40
7.35
7.33
7.32
7.27
7.27
6.99
6.98
6.96
6.94
5.92
5.89
5.76
5.73
4.82
4.80
4.54
4.50
4.48
4.29
4.27
4.25
4.23
4.22
1.94
1.93
1.91
1.90
1.88
1.86
0.96
0.94
0.94
0.92
Fmoc
N
H
OH
O
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2.02
2.05
2.26
0.95
1.00
0.87
2.29
2.12
1.00
6.05
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.5
4.0
3.5
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2.0
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1.0
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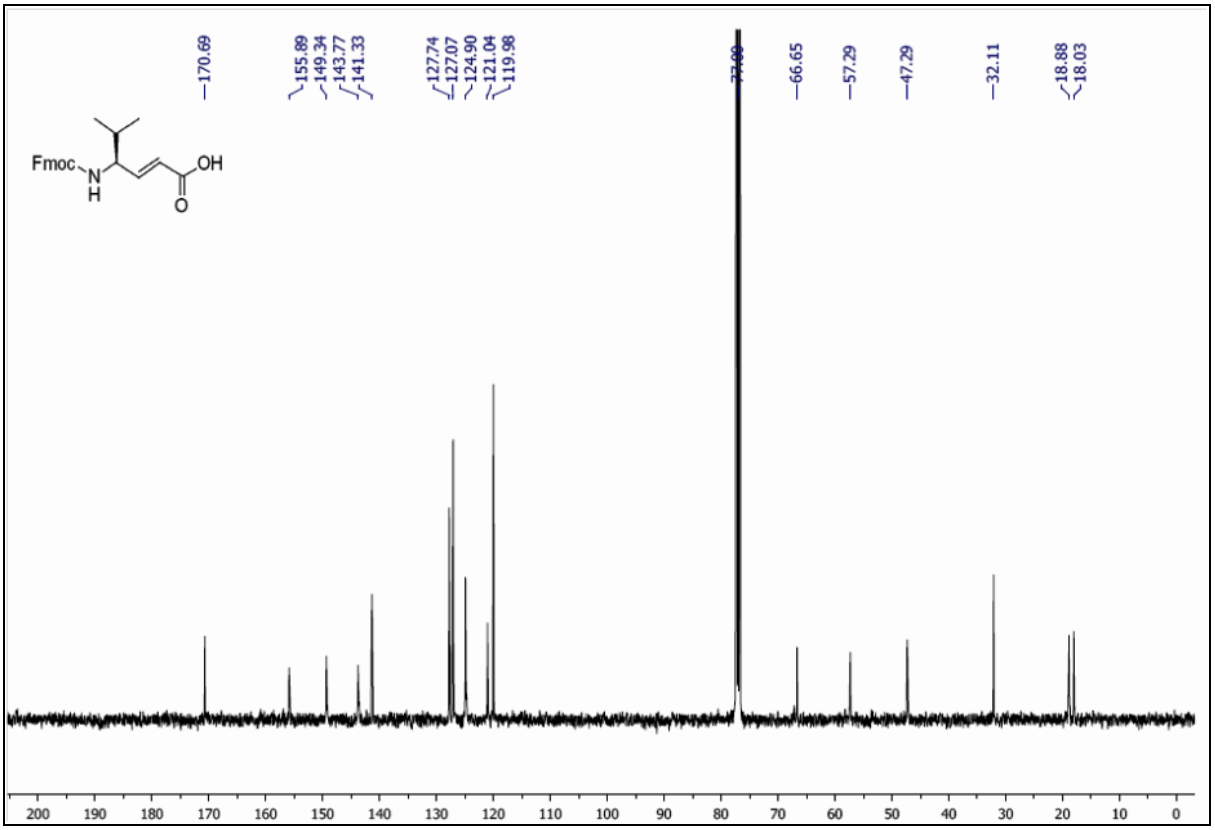
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124.90
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119.98
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66.65
57.29
47.29
32.11
18.88
18.03
Fmoc
N
H
OH
O
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150
140
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120
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10
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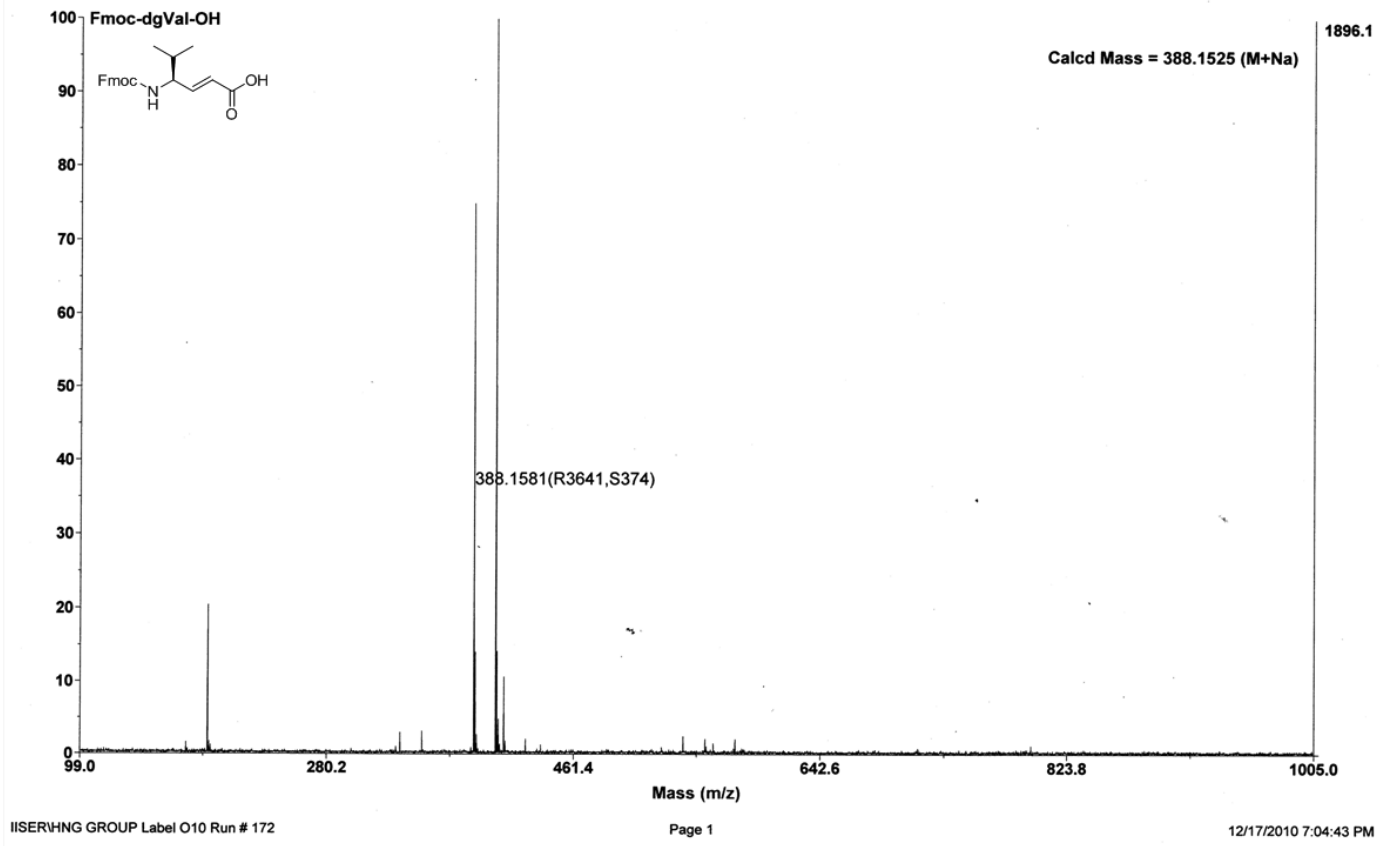
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OH
O
1896.1
Calcd Mass = 388.1525 (M+Na)
388.1581(R3641,S374)
100
90
80
70
60
50
40
30
20
10
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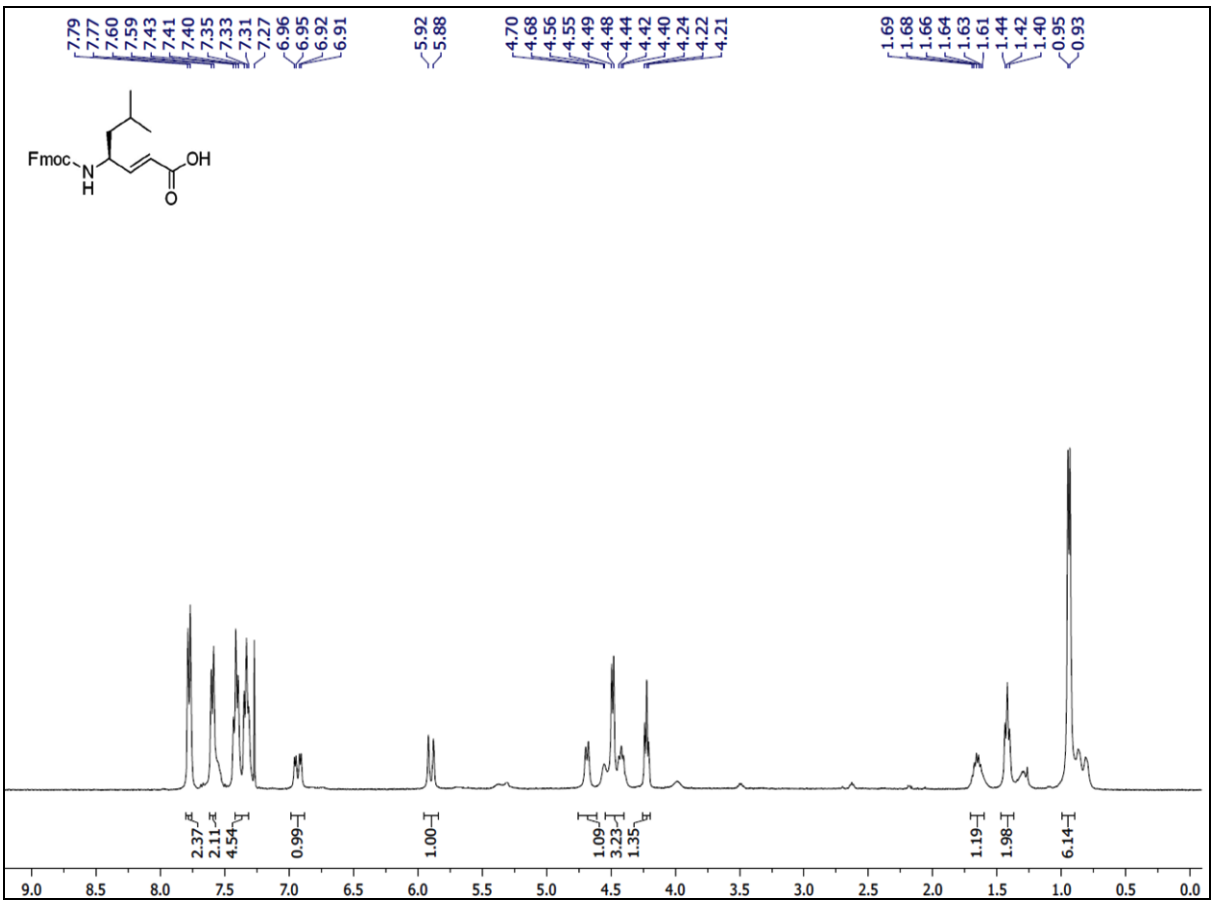
99.0
280.2
461.4
642.6
823.8
1005.0
Mass (m/z)
IISER\HNG GROUP Label O10 Run # 172
Page 1
12/17/2010 7:04:43 PM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4.24
4.22
4.21
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1.68
1.66
1.64
1.63
1.61
1.44
1.42
1.40
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0.93
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H
OH
O
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2.11
4.54
0.99
1.00
1.09
3.23
1.35
1.19
1.98
6.14
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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4.0
3.5
3.0
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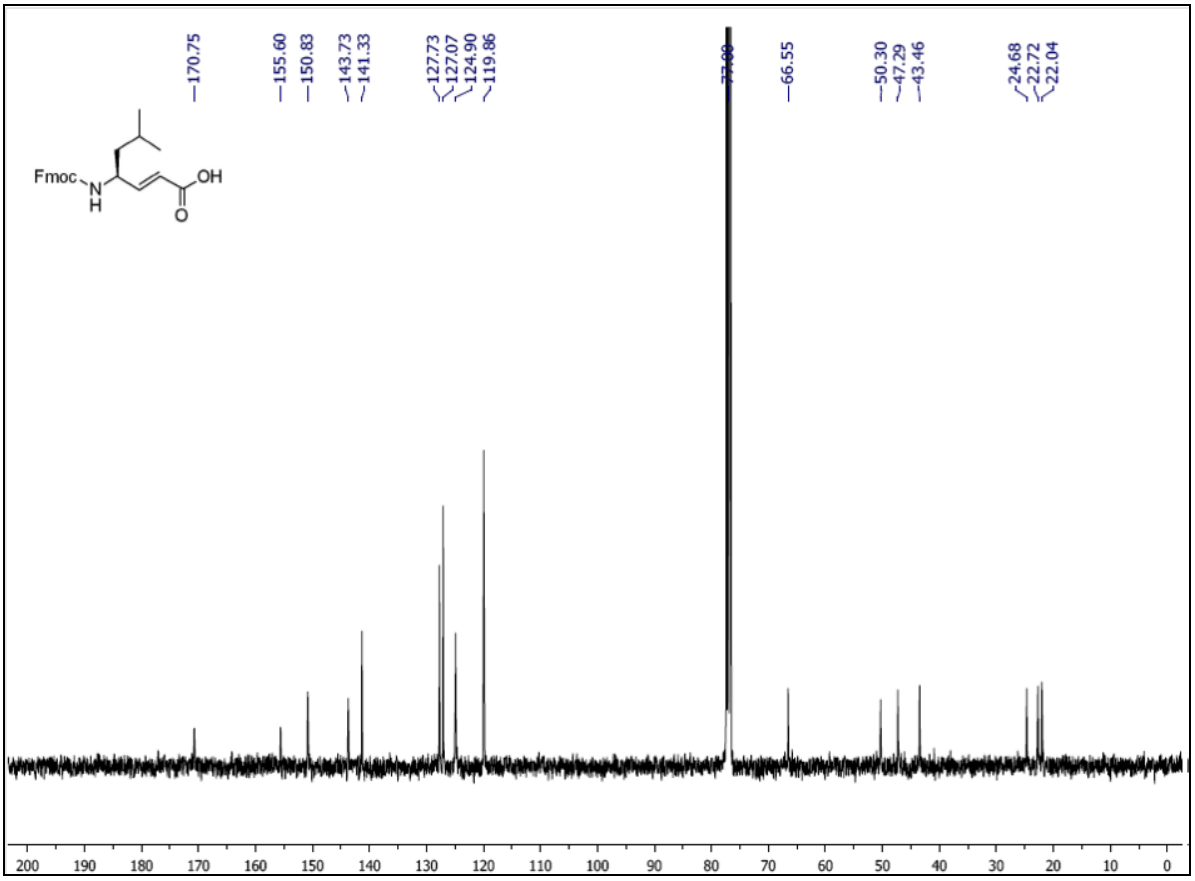
2.0
1.5
1.0
0.5
0.0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143.73
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127.73
127.07
124.90
119.86
77.00
66.55
50.30
47.29
43.46
24.68
22.72
22.04
Fmoc
N
H
OH
O
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180
170
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150
140
130
120
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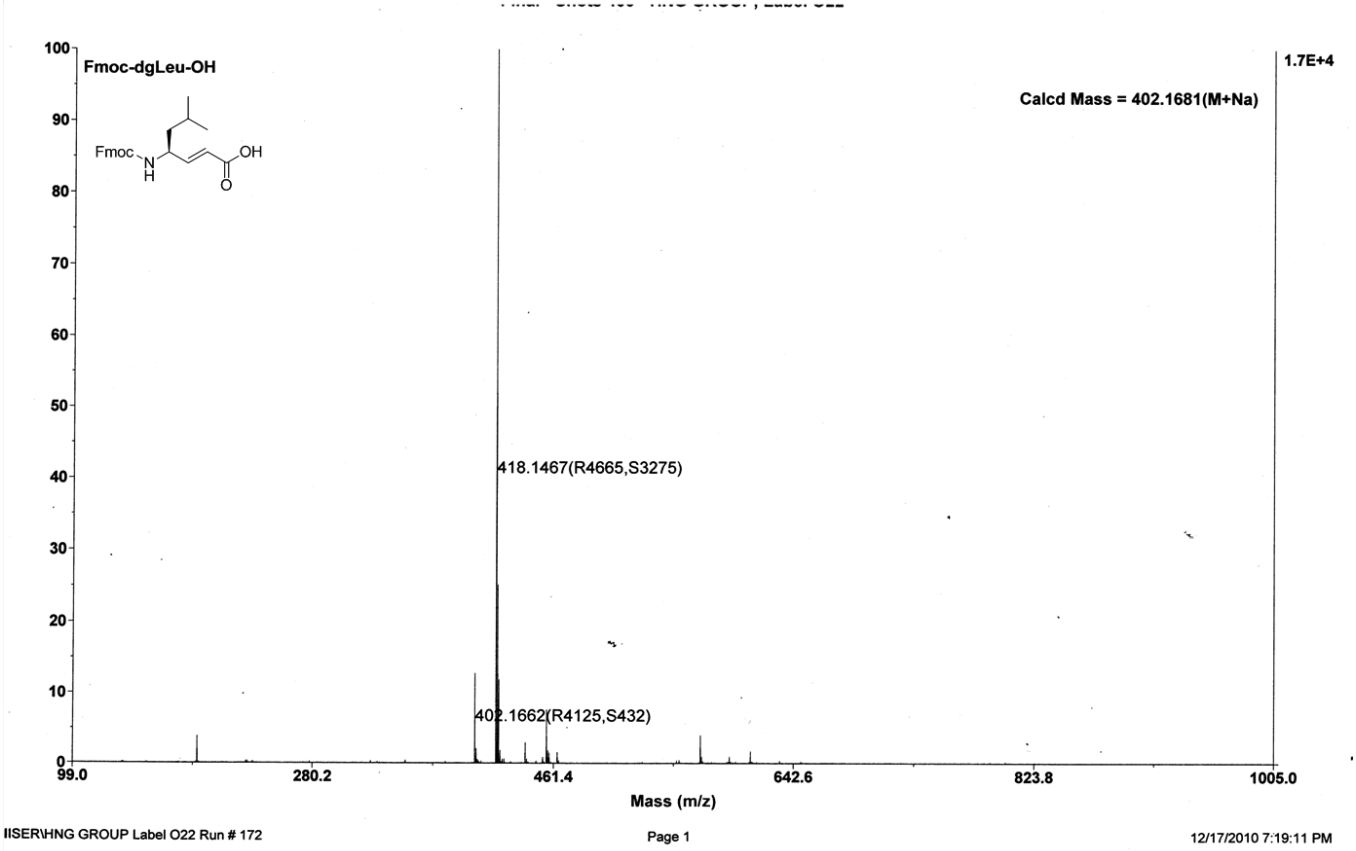
90
80
70
60
50
40
30
20
10
0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Fmoc
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H
OH
O
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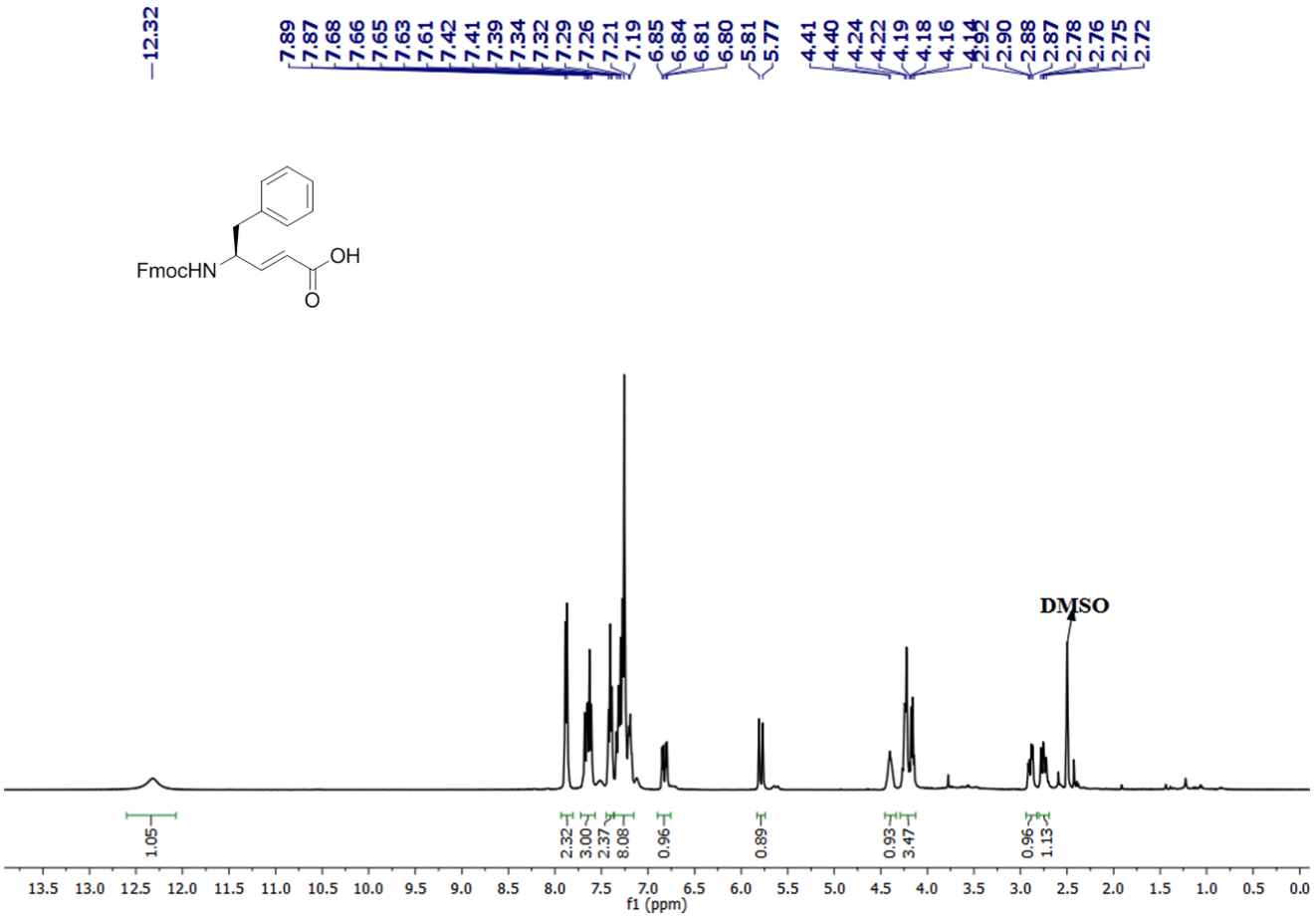
1.7E+4
418.1467(R4665,S3275)
402.1662(R4125,S432)
100
90
80
70
60
50
40
30
20
10
0
99.0
280.2
461.4
642.6
823.8
1005.0
Mass (m/z)
IISER\HNG GROUP Label O22 Run # 172
Page 1
12/17/2010 7:19:11 PM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7.65
7.63
7.61
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7.41
7.39
7.34
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7.29
7.26
7.21
7.19
6.85
6.84
6.81
6.80
5.81
5.77
4.41
4.40
4.24
4.22
4.19
4.18
4.16
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2.76
2.75
2.72
FmocHN
OH
O
DMSO
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2.37
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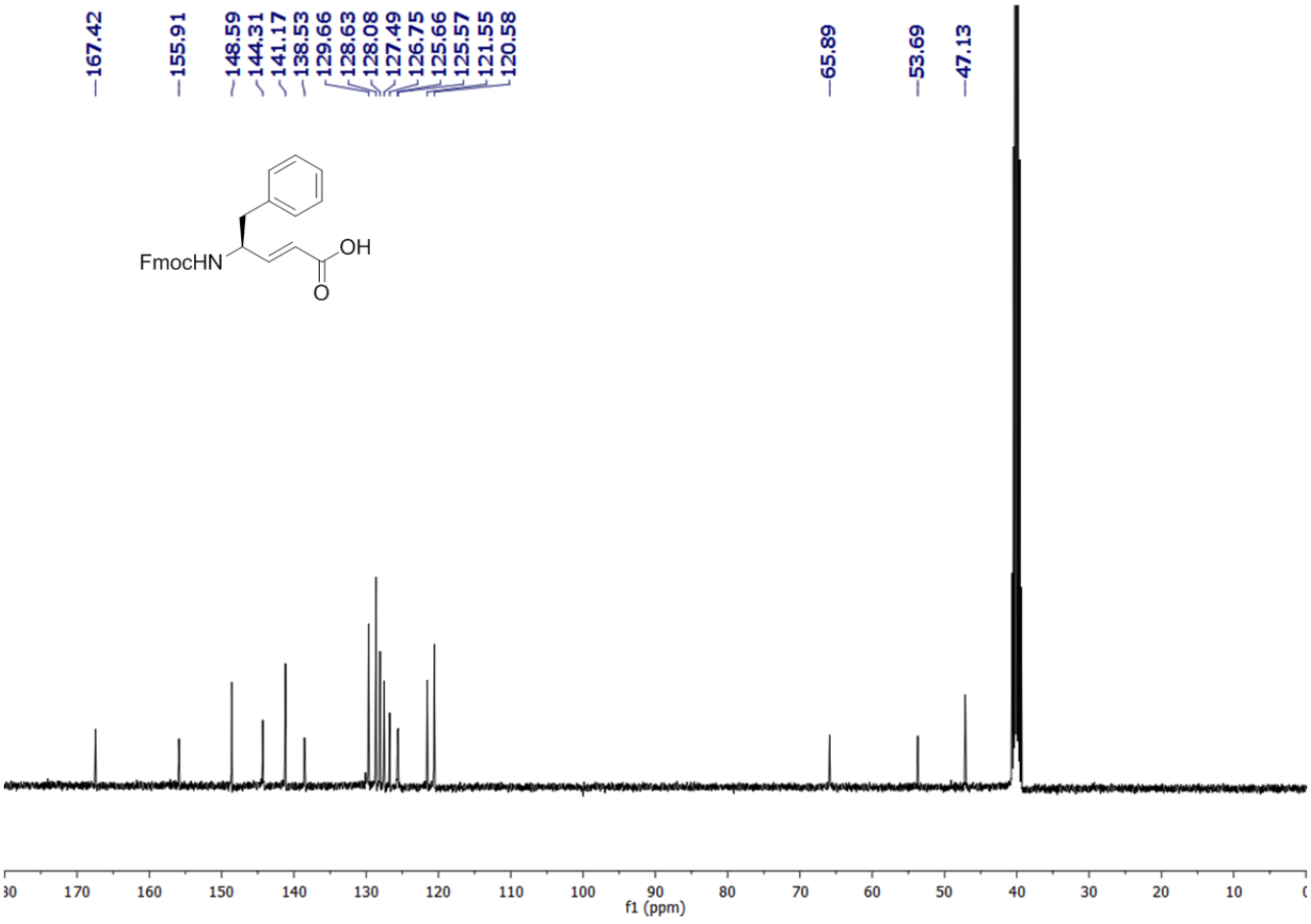
0.89
0.93
3.47
0.96
1.13
f1 (ppm)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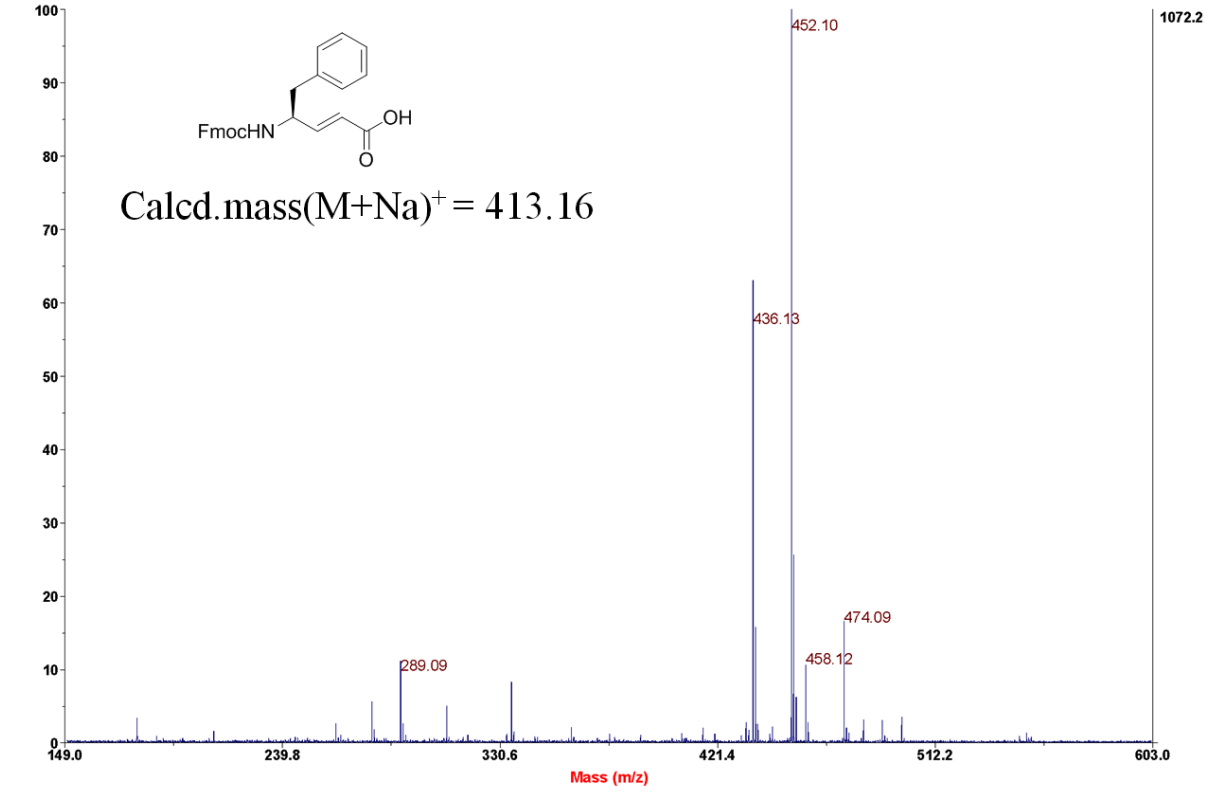
148.59
144.31
141.17
138.53
129.66
128.63
128.08
127.49
126.75
125.66
125.57
121.55
120.58
65.89
53.69
47.13
FmocHN
OH
O
f1 (ppm)

Spectrum Report

Final - Shots 500 - IISER-96-3; Run #101; Label D2



IISER New\IISER-96-3 Label D2 Run # 101 Page 1 2/18/2016 4:27:52 PM

^{1}H NMR spectrum of P1

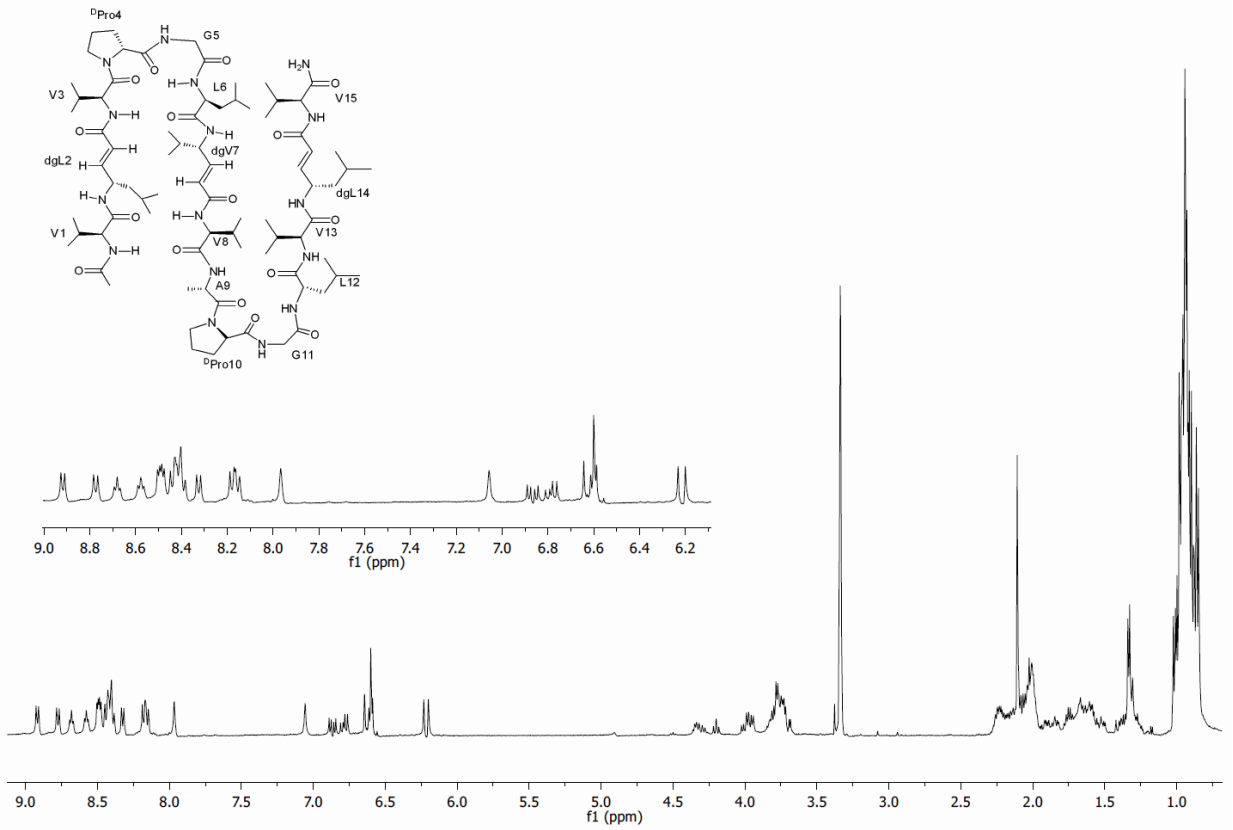


Figure S10: ^{1}H NMR spectrum of **P1** in MeOH.

^{1}H NMR spectrum of **P2**

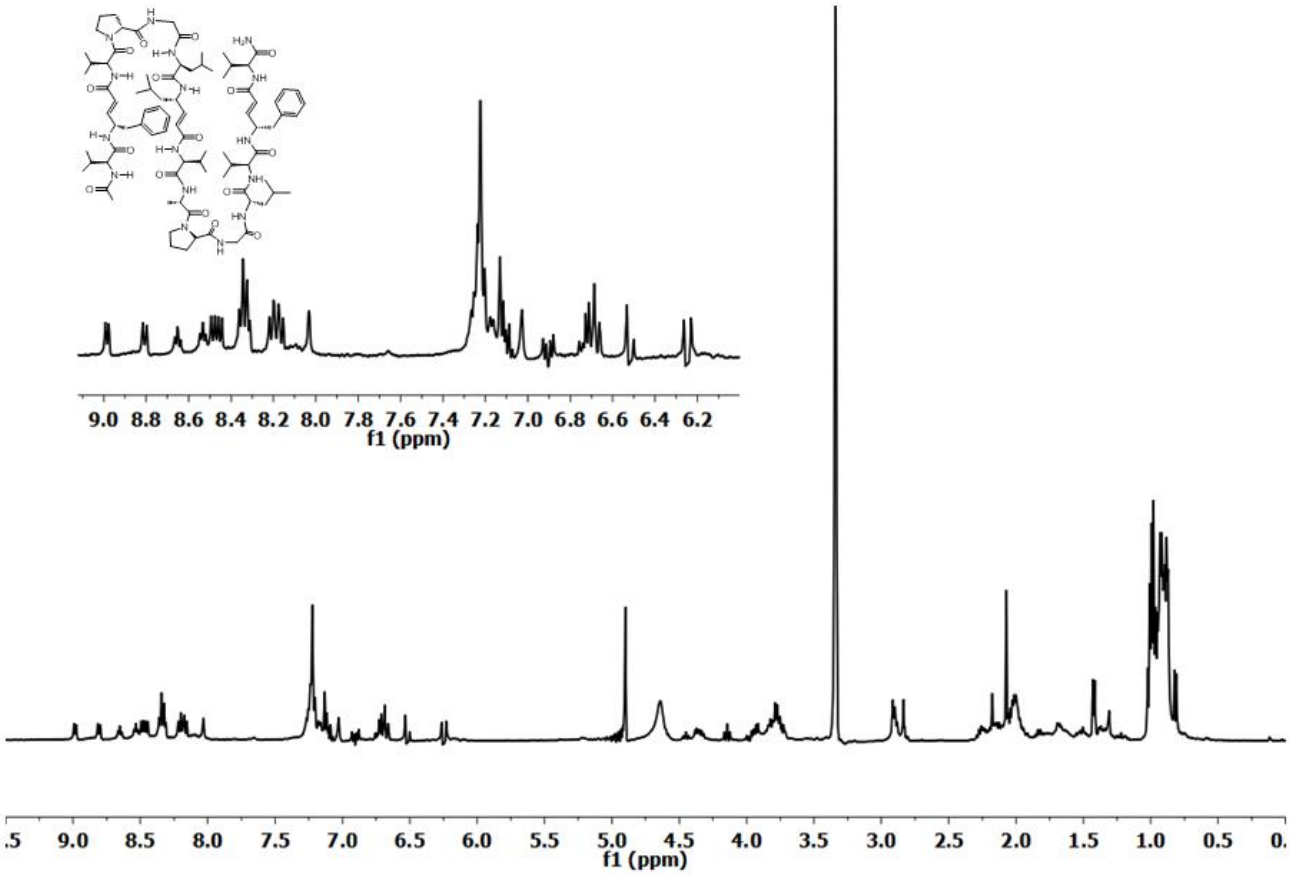


Figure S11: ^{1}H NMR spectrum of **P2** in MeOH.

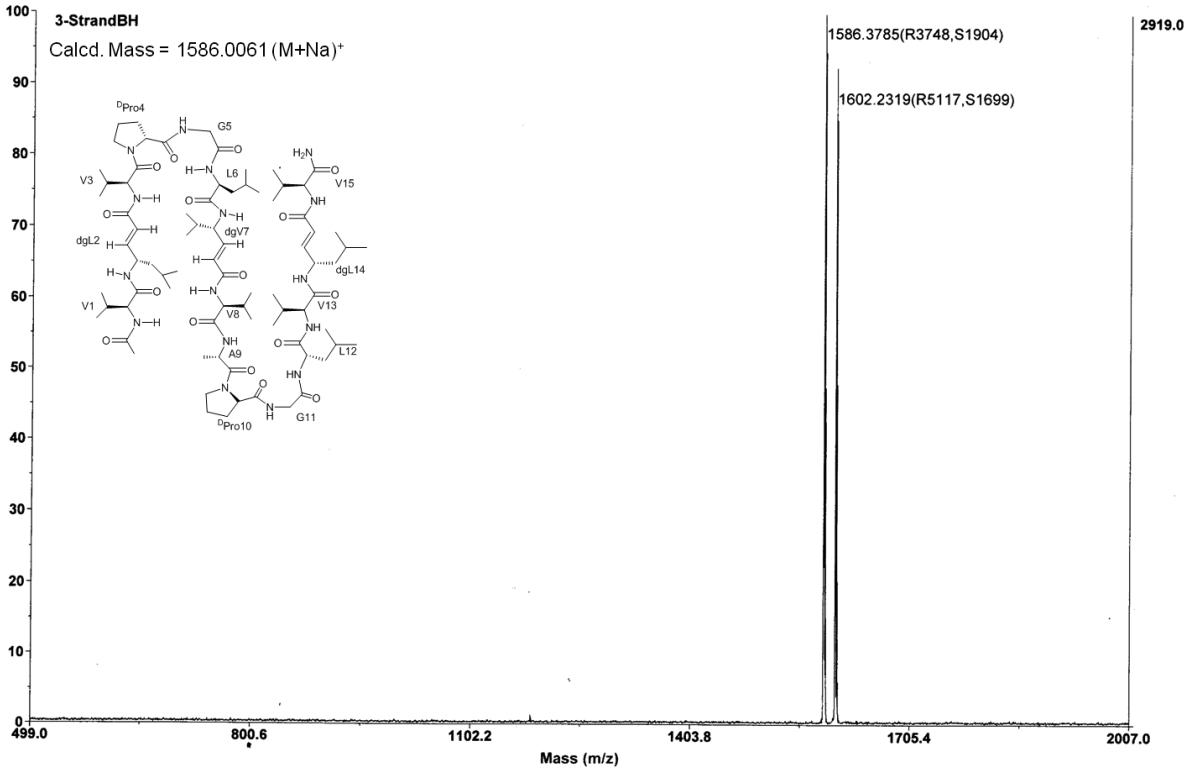


Figure S12: MALDI-TOF/TOF mass spectrum of **P1**

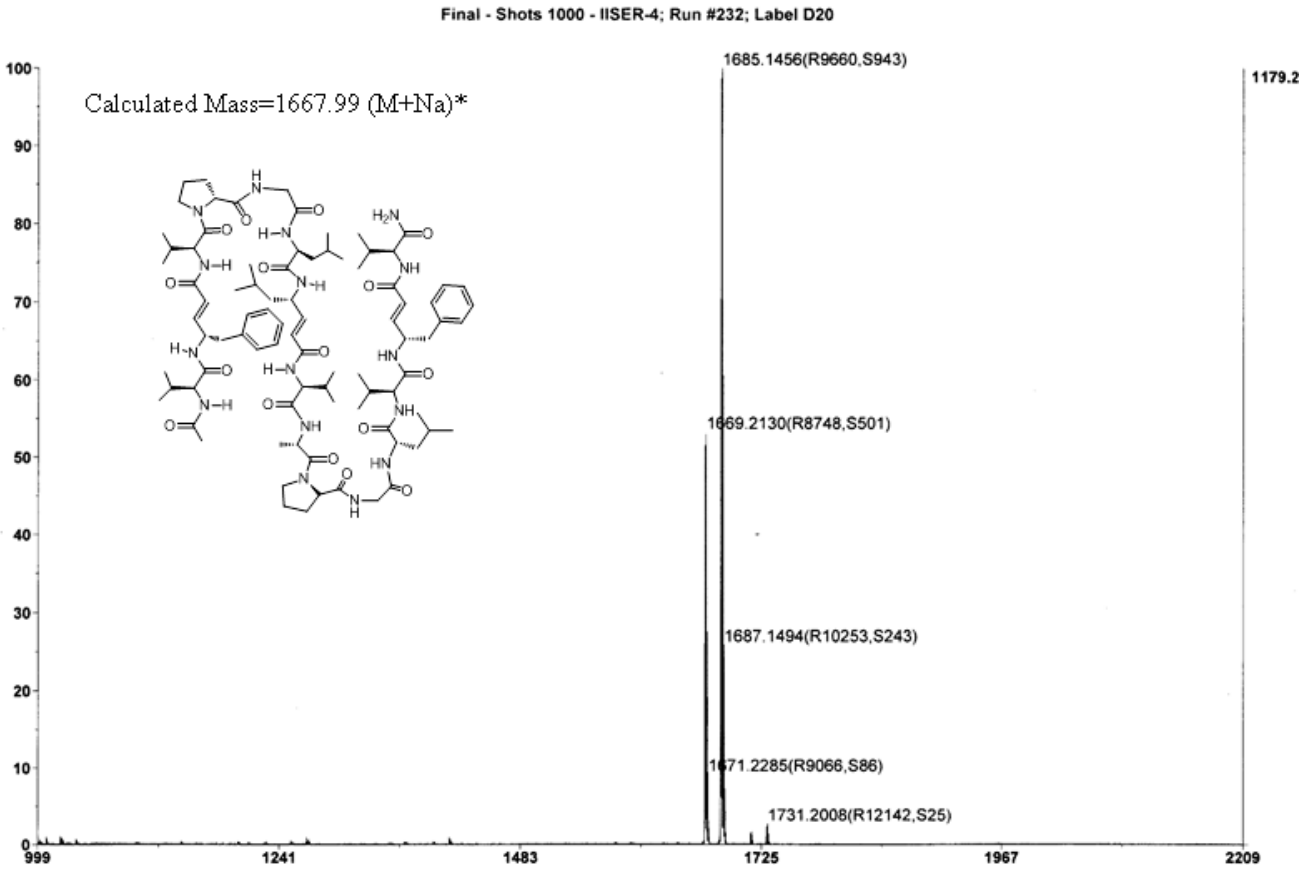


Figure S13: MALDI-TOF/TOF mass spectrum of **P2**

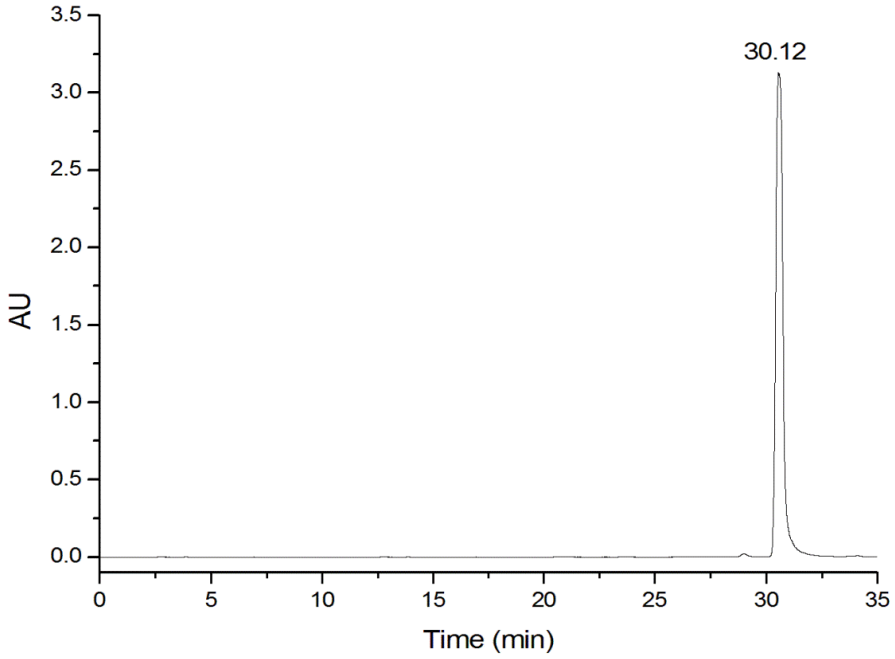


Figure S14: Reverse Phase HPLC profile of 3-stranded β-sheet (**P1**). Methanol/ H_2O were used as a solvent system at a flow rate of 1.25 mL /min.

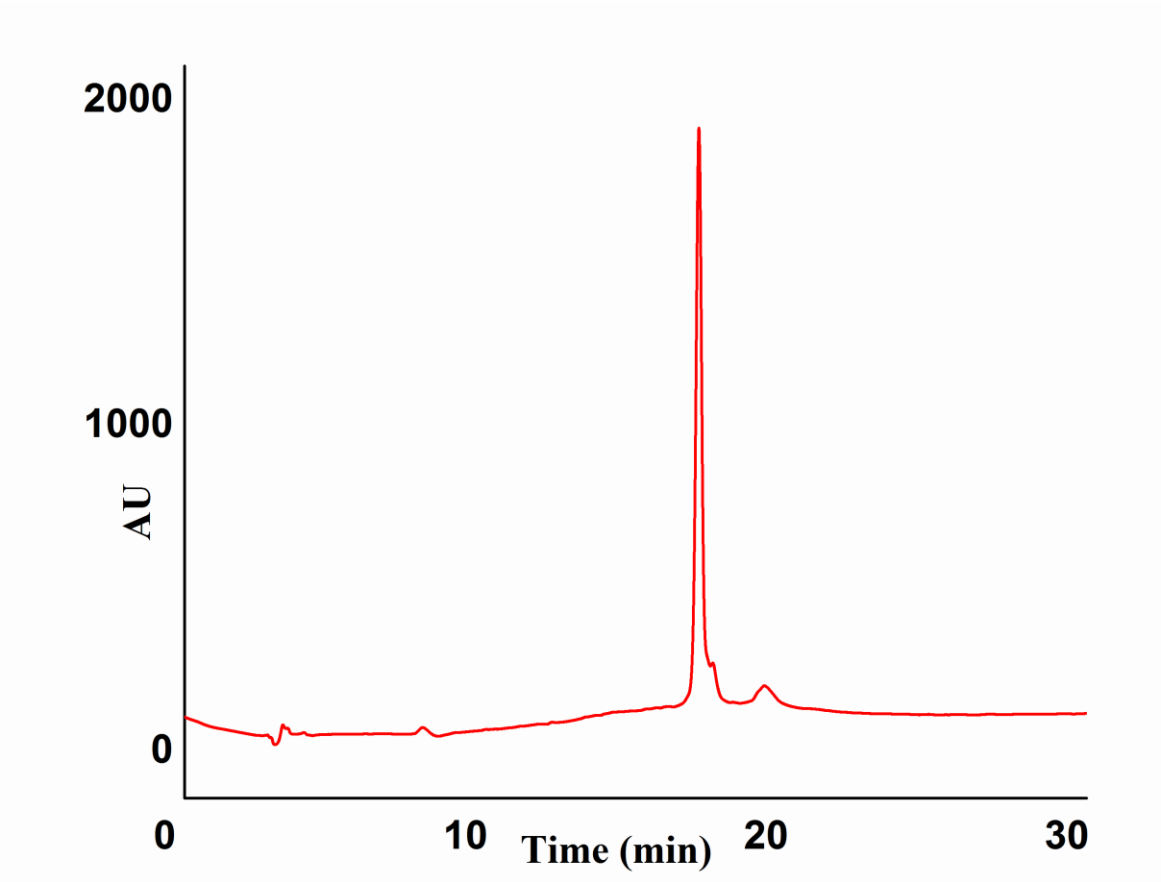


Figure S15: Reverse Phase HPLC profile of 3-stranded β-sheet (**P2**). Methanol/ H_2O were used as a solvent system at a flow rate of 3 mL /min.

Reference

1 **References:**

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