Supporting Information

α/γ^4–Hybrid Peptide Helices: Synthesis, Crystal Conformations and Analogy with α-Helix

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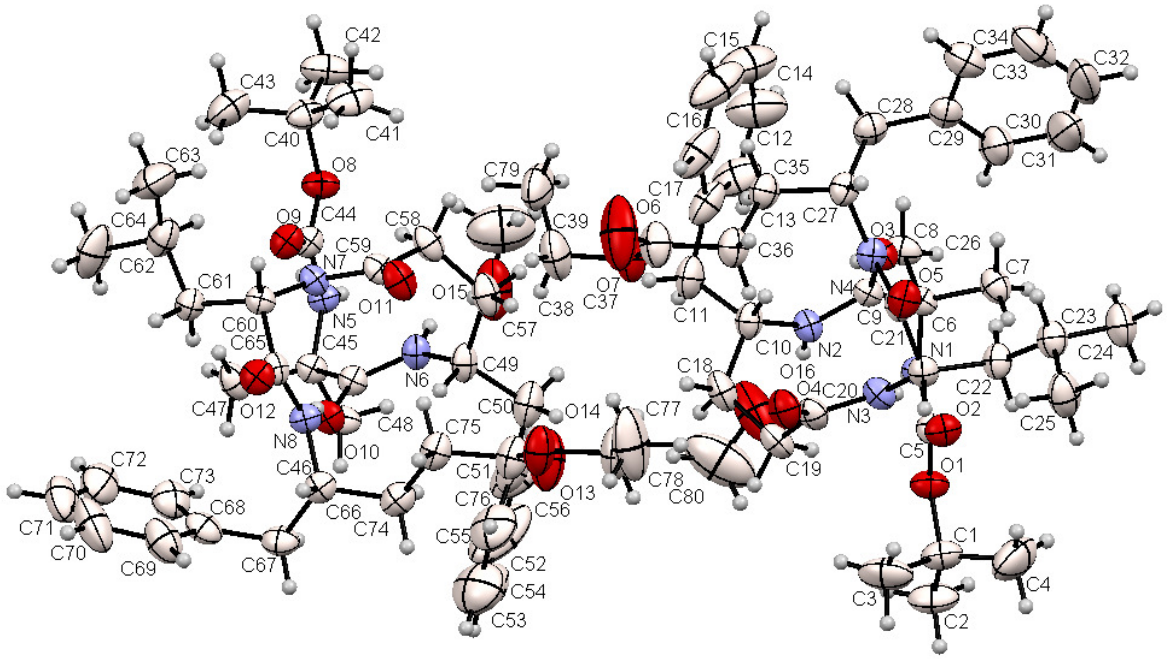
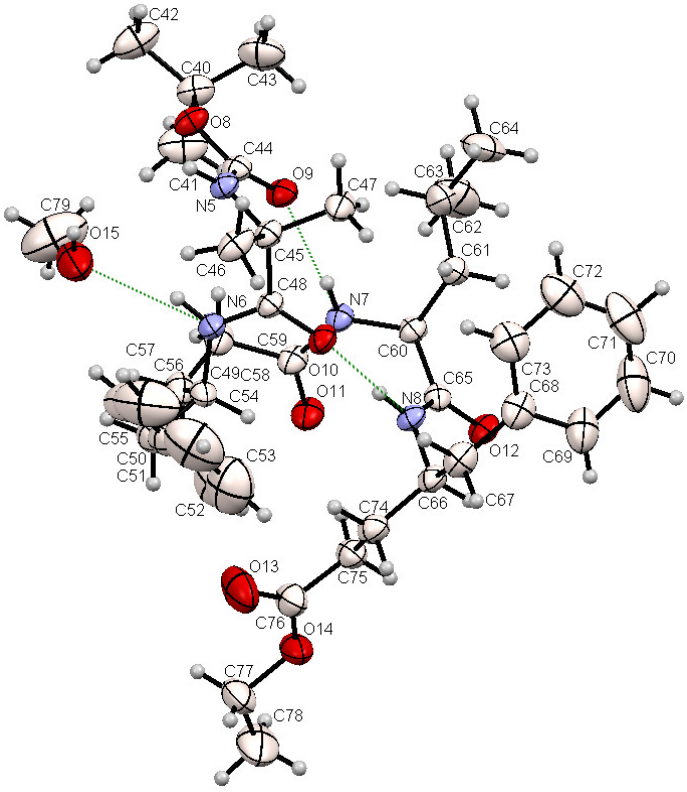


A



B

Figure 1. The ORTEP diagram of α/γ^4-hybrid tetrapeptide Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-OEt (**P1**). Two independent peptide molecules (**a** and **b**) along with two methanol molecules present in asymmetric unit is shown in **A**. H-atoms are not labeled for clarity. Molecule **a** is shown separately in **B**. Two intramolecular hydrogen bonds and one intermolecular H-bond with MeOH are represented in dotted lines.

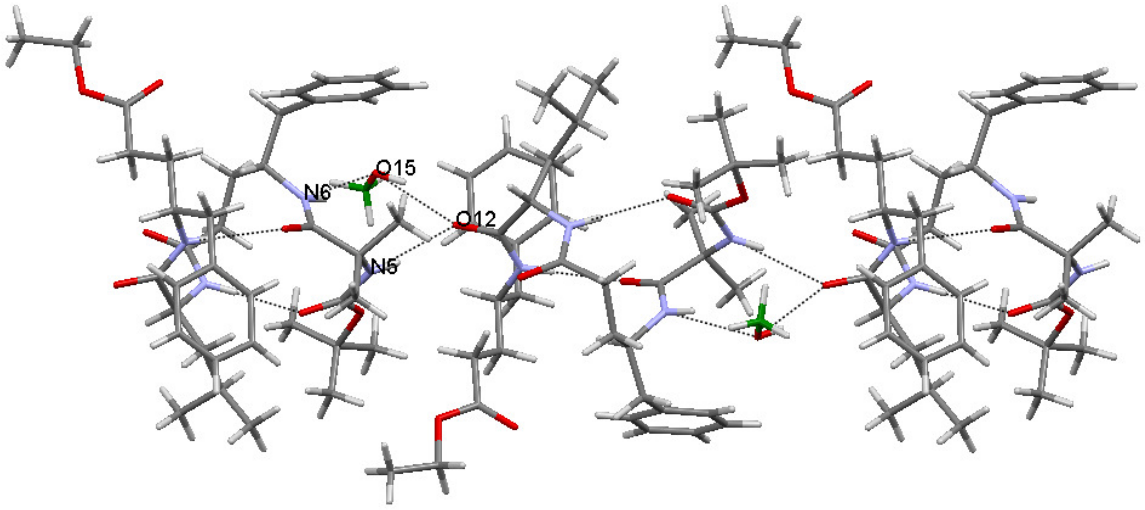
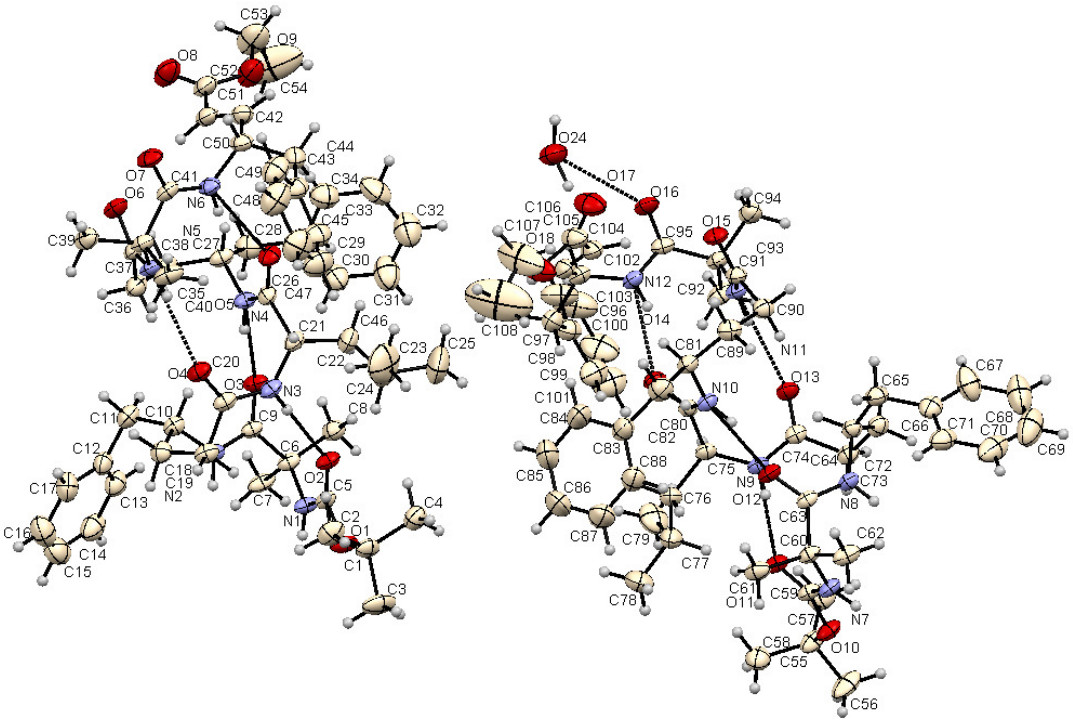


Figure2 : The two independent helices (**a** and **b**) in assymetric unit are connected to the other helices through intermolecular H-bonding in a head-to-tail fashion. The solvent methanol is playing a crucial role in interconnection the two helices through intermolecular H-bonding. Donar atom N5 of **a** is interacting directly with acceptor O12 atom of another helix (**a**) through intermolecular H-bonding [C=O(12)···NH(5); NH···O dist. 2.11 Å, N···O dist 2.96 Å, ∠N-H···O = 167^{o}]. In addition, the donor atom N6 is involved in the intermolecular H-bonding with O15 of the methanol [C-O(15)···NH(6); NH···O dist. 2.17 Å, N···O dist 2.99 Å, ∠N-H···O = 159^{o}] and the acceptor O12 is interacted with donar OH group of methanol [C=O(15)···OH(15); OH···O dist. 1.96 Å, N···O dist 2.94 Å]. The carbon atom of methanol molecule is highlighted in green color. Similar pattren of intermolecualr H-bonding is observed in the molecule **b**.



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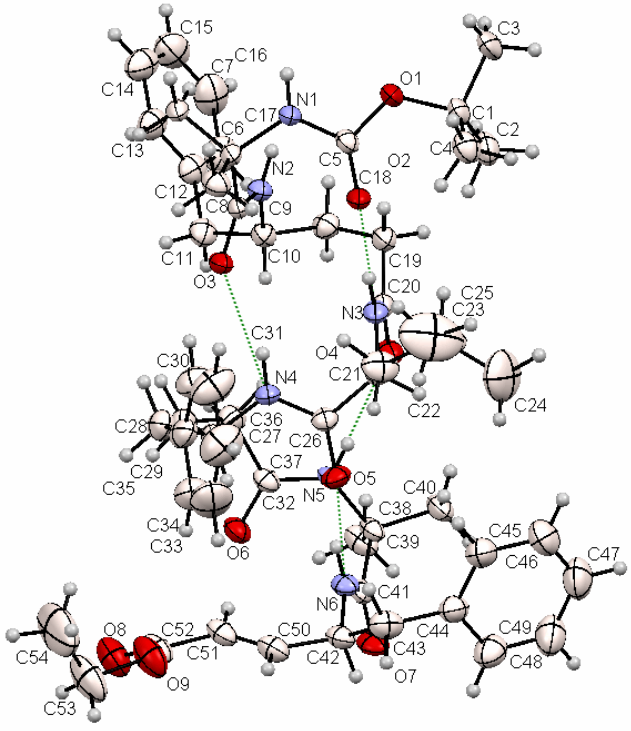
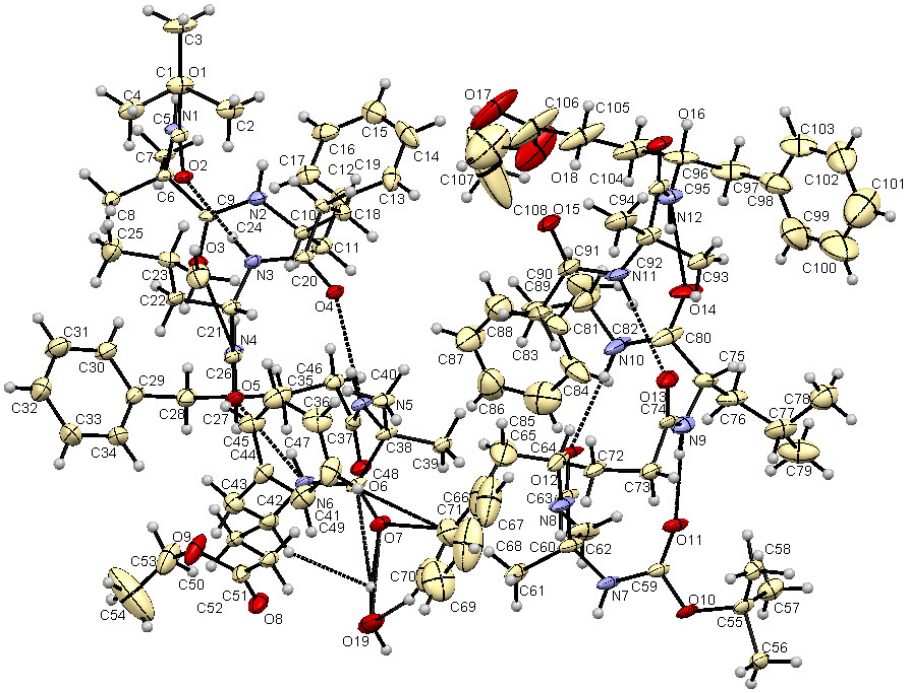


B

Figure 3. The ORTEP diagram of α/γ^4-hybrid hexapeptide Boc-Aib-γ^4Phe-Leu-γ^4Phe-Aib-dgF-OEt (**P2**). **A**. Two independent peptide molecules (**a** and **b**) (along with a solvent water molecule) are present in the asymmetric unit with slight variation in the torsional values. Molecule **a** is shown separately in **B**. H-atoms are not labeled for clarity. Four intramolecular H-bonds are represented in dotted lines. The two helices are independently interconnected with the other helices in a head-to-tail fashion through four

intermolecular H-bonds, NH(7)---O(6) [NH---O dist. 2.132Å, N---O dist. 2.927 Å and ∠N-H···O = 154^{o}], NH(8)---O(8) [NH---O dist. 2.235 Å, N---O dist. 3.062 Å and ∠N-H···O = 161^{o}], NH(1)---O15 [NH---O dist. 2.210 Å, N---O dist. 2.921 Å and ∠N-H···O = 155^{o}], and NH(2)---O17[NH---O dist. 2.225 Å, N---O dist. 3.061 Å and ∠N-H···O = 164^{o}].



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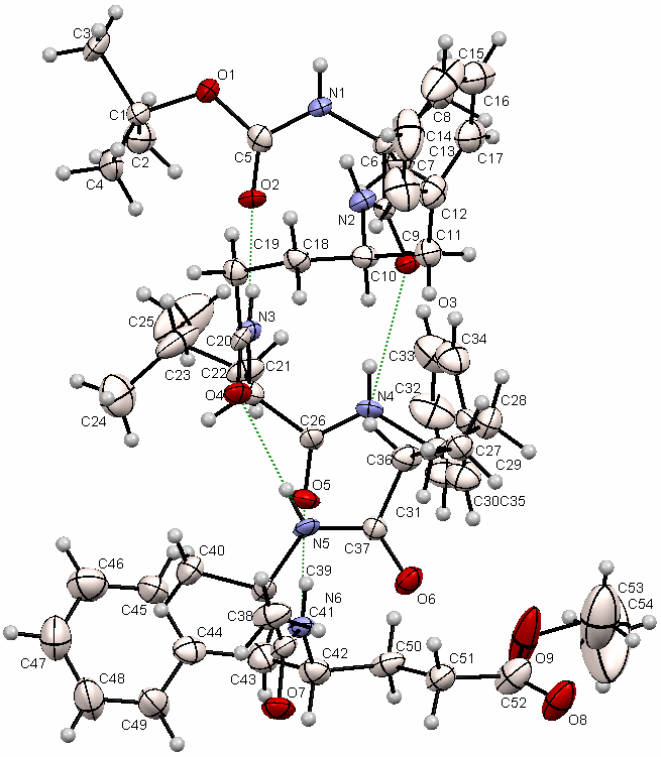


B

Figure 3. The ORTEP diagram of α/γ^4-hybrid hexapeptide Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-Aib-γ^{4}Phe-OEt (**P3**). **A**. Two peptide molecules (**a** and **b**)(along with a solvent water molecule) are present in the asymmetric unit with slight variation in the torsional values. Peptide molecules displayed antiparallel orientation in the asymmetric unit. In contrast to **P1** and **P2**, the two peptide molecules in **P3** are interconnected through the intermolecular C-H---π interaction between the aromatic side-chains of γPhe2 (**a**) and γPhe4 (**b**) [C-H---π dist. 2.898 Å] along with water mediated CH(70)---O(19) [CH---O dist. 2.457 Å, C---O dist. 3.264 Å and ∠C-H···O = 145^o] and O(7)---H-O(19) [O---OH dist. 1.764 Å, O---O dist.

2.807 Å and ∠O-H···O = 170°] H-bonds. Molecule **a** is shown separately in **B**. H-atoms are not labeled for clarity. Four intramolecular hydrogen bonds are represented in dotted lines. The two peptides (**a** and **b**) in the asymmetric unit are independently interacting with other helices in a head-to-tail fashion through two intermolecular H-bonds. In the molecule **a,** NH(1)---O(6) [NH---O dist. 2.114 Å, N---O dist. 2.889 Å and ∠N-H···O = 150°] and NH(2)---O(8) [NH---O dist. 2.151 Å, N---O dist. 2.959 Å and ∠N-H···O = 156°] are involved in the intermolecular H-bonding, while in the molecule **b,** NH(7)---O(15) [NH---O dist. 2.226 Å, N---O dist. 3.050 Å and ∠N-H···O = 160°] and NH(8)---O(17) [NH---O dist. 2.069 Å, N---O dist. 2.882 Å and ∠N-H···O = 157°] are involved in the intermolecular H-bonding.

Table1: Backbone torsional variables of α/γ^4-hybrid peptide Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-OEt (P1).

(Two molecules are present in asymmetric unit)

Peptide (a)

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib(1)	-59.48	-	-	-37.51	-175.07
γ^{4}Phe(2)	-122.77	47.54	66.41	-115.34	-175.76
Leu(3)	-61.15	-	-	-36.99	-176.52
γ^{4}Phe(4)	-115.35	63.22	-176.60	-142.78	-

Peptide (b)

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib(1)	-60.70	-	-	-35.07	-175.64
γ^{4}Phe(2)	-129.83	52.39	60.28	-107.50	-174.99
Leu(3)	-64.65	-	-	-38.58	178.48
γ^{4}Phe(4)	-102.04	61.43	179.75	65.05	-

Mean torsional values of two peptides are tabulated with e.s.d. value

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib(1)	-60.09(44)	-	-	-36.29(1.72)	-175.35(0.40)
γ^{4}Phe(2)	-126.3(4.99)	49.96(3.42)	63.345(4.33)	-111.42(5.54)	-175.37(0.54)
Leu(3)	-62.9(2.47)	-	-	-37.78(1.12)	177.5(1.38)
γ^{4}Phe(4)	-102.04(41)	62.23(1.26)	-	-	-

Table 2: Backbone torsional variables of α/γ^4-hybrid peptide Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-Aib-dgF-OEt (P2).

(Two molecules are present in the asymmetric unit)

Peptide (a)

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib(1)	-55.25	-	-	-49.24	-174.84
γ^{4}Phe(2)	-137.64	61.29	59.28	-114.27	-175.38
Leu(3)	-65.44	-	-	-33.59	-175.55
γ^{4}Phe(4)	-121.41	48.11	64.10	-122.71	-168.96
Aib(5)	-62.78	-	-	-32.64	-171.81
dgPhe(6)	-113.38	23.23	174.13	27.93	-

Peptide (b)

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib(1)	-56.79	-	-	-49.70	-175.26
γ^{4}Phe(2)	-138.09	61.71	58.58	-115.88	-175.85
Leu(3)	-64.09	-	-	-33.93	-173.55
γ^{4}Phe(4)	-125.46	49.59	62.03	-117.27	-171.25
Aib(5)	-60.20	-	-	-33.37	-173.88
dgPhe(6)	-112.91	26.05	172.21	31.11	-

Mean torsional values of two peptides are tabulated with e.s.d. values

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib(1)	-55.52(1.79)	-	-	-49.47(0.32)	-175.05(0.29)
γ^{4}Phe(2)	-137.86(0.31)	61.5(0.29)	58.93(0.49)	-115.07(1.13)	-175.61(0.33)
Leu(3)	-64.76(0.95)	-	-	-33.76(0.24)	-174.55(1.41)
γ^{4}Phe(4)	-123.43(2.86)	48.85(1.04)	63.06(1.46)	-119.99(3.84)	-170.10(1.61)
Aib(5)	-61.49(1.82)	-	-	-33(0.51)	-172.84(1.46)
dgPhe(6)	-113.14(0.33)	24.64(1.99)	173.17(1.35)	29.52(2.24)	-

Table 3: Backbone torsional variables of α/γ^4-hybrid peptide Boc-Aib-γ^4Phe-Leu-γ^4Phe-Aib-γ^4Phe-OEt (P3).

(Two molecules are present in asymmetric unit)

Peptide (a)

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib	-59.11	-	-	-42.85	-175.51
γ^4Phe	-137.25	56.85	61.07	-110.55	-175.76
Leu	-72.41	-	-	-24.81	-179.62
γ^4Phe	-122.42	44.87	65.90	-122.94	-170.73
Aib	-58.51	-	-	-35.63	-170.85
γPhe	-121.42	59.01	176.48	23.89	-

Peptide (b)

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib	-62.21	-	-	-47.58	-179.35
γ4Phe	-132.14	62.15	60.26	-119.69	-173.50
Leu	-66.80	-	-	-33.58	-173.54
γ4Phe	-141.38	49.42	61.97	-103.58	-173.86
Aib	-62.31	-	-	-39.91	-179.29
γ4Phe	-104.91	56.04	179.34	20.37	-

Mean torsional values of two peptides are tabulated with e.s.d. values

Resd.	ϕ	θ_1	θ_2	ψ	ω
Aib(1)	-60.66(2.19)	-	-	-45.21(3.34)	-177.43(2.71)
γ^4Phe(2)	-134.69(3.61)	59.17(3.28)	60.66(0.57)	-115.12(6.46)	-174.63(1.59)
Leu(3)	-69.60(3.96)	-	-	-29.19(6.20)	-176.58(4.29)
γ^4Phe(4)	-131.9(13.40)	47.14(3.21)	63.93(2.77)	-113.26(13.68)	-172.29(2.21)
Aib(5)	-60.41(2.68)	-	-	-37.77(3.02)	-175.07(5.96)
dgPhe(6)	-113.16(11.67)	57.52(2.1)	177.91(2.02)	22.13(2.48)	-

Table 4: Intramolecular Hydrogen-Bond Parameters in Peptides P1, P2 and P3

Type of H-bonds	Donar (D)	Acceptor (A)	D····A (Å)	D-H····A (Å)	∠N-H···O (deg)
Boc-Aib-γPhe-Leu-γPhe-OEt (G3)					
1←4	N3	O0(Boc)	2.95(0.007)	2.11(0.02)	163.63(4.69)
1←4	N4	O1	2.89(0.01)	2.05(0.02)	163.48(3.62)
Boc-Aib-γPhe-Leu-γPhe-Aib-dgPhe-OEt (D4)					
1←4	N3	O0(Boc)	2.89(0.003)	2.03(0.003)	175.95(0.57)
1←4	N4	O1	2.96(0.01)	2.15(0.01)	155.76(0.86)
1←4	N5	O2	3.04(0.01)	2.19(0.02)	171.71(0.72)
1←4	N6	O3	2.8(0.007)	2.00(0.007)	153.42(0.84)
Boc-Aib-γPhe-Leu-γPhe-Aib-γPhe-OEt (G5)					
1←4	N3	O0(Boc)	2.86(0.001)	2.00(0.007)	172.25(2.3)
1←4	N4	O1	2.98(0.06)	2.17(0.01)	158.85(12.92)
1←4	N5	O2	2.98(0.004)	2.13(0.04)	170.17(3.09)
1←4	N6	O3	2.85(0.06)	2.05(0.04)	154.4(4.06)

General Experimental Details

All amino acids, DIPEA, TFA, triphenylphosphine were purchased from Aldrich. THF, DCM, DMF, NaOH were purchased from Merck. Ethyl bromoacetate, $MeNH_2$ solution in water, HOSu, HBTU, HOBT, EtOAc, Pet-ether (60-80 ^oC) 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). The ^1H and ^{13}C spectra were recorded on Jeol 400 MHz (or 100 MHz for ^{13}C) and Bruker 500 MHz using residual solvents signals as an internal reference ($CDCl_3$ δ_H, 7.26 ppm and $\delta_{C,}$ 77.00). 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 a Bruker AXS APEX II CCD Duo diffractometer (operated at 1500 W power:50 kV, 30 mA) with Mo K_α (λ = 0.71073 Å) radiation.

Crystal structure analysis

Crystal structure analysis of (Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-OEt) P1: Crystals of peptide were grown by slow evaporation from a solution of methanol. A single crystal (0.42 × 0.35 × 0.32 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$_\alpha$ radiation (λ = 0.71073 Å), ω-scans (2θ = 56.56^o), for a total number of 14908 independent reflections. Space group *P2(1)*, *a* = 10.132(2), *b* = 20.141(4), *c* = 21.010(4) Å, α = 90.00 , β = 99.384(5), γ = 90.00, V= 4230.1(14) Å^3, Monoclinic P, Z=4 for chemical formula ($C_{39}H_{58}N_4O_7$).CH_4O, with two molecule in asymmetric unit; ρ_{calcd} = 1.141 g cm^{-3}, μ = 0.079 mm^{-1}, *F*(000) = 1576, R_{int} = 0.0699. The structure was obtained by direct methods using SHELXS-97.[1] 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 0.0574 (*wR2*= 0.1331) for 6991 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 958 variables, *S* = 0.929. The largest difference peak and hole were 0.256 and -0.212 e Å^3, respectively. Strange C-O-H Geometry (C-O .LT. 1.25 Ang…….O15) is reported in check cif. This is due to the disordered over two sites occupancy of oxygen atom (O15) in methanol solvent molecule.

Crystal structure analysis of (Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-Aib-dgF-OEt) P2: Crystals of peptide were grown by slow evaporation from a solution of ethyl acetate and hexane. A single crystal (0.35 × 0.28 × 0.16 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$_\alpha$ radiation (λ = 0.71073 Å), ω-scans (2θ = 56.56^o), for a total number of 28846 independent reflections. . Space group *P2(1)*, *a* = 14.850(6), *b* = 18.173(7), *c* = 24.075(9) Å, α = 90.00 , β = 107.254(7), γ = 90.00, V = 6204(4) Å^3, Monoclinic *P*, Z = 4 for chemical formula 2($C_{54}H_{76}N_6O_9$).H_2O, with two molecule in asymmetric unit; ρ_{calcd} = 1.029 g cm^{-3}, μ= 0.071 mm^{-1}, *F*(000) = 2072, R_{int}= 0.0660. The structure was obtained by direct methods using SHELXS-97.[1] 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 0.0576 (*wR2*= 0.1400) for 13821 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 1272 variables, *S* = 0.788. The largest difference peak and hole were 0.215 and -0.197 e Å^3, respectively.

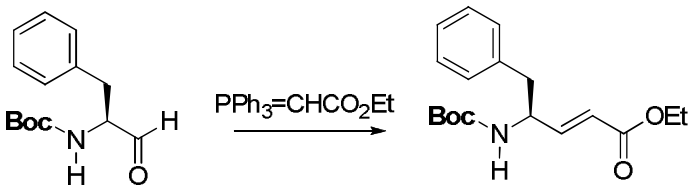
There were some partially occupied solvent molecules also present in the asymmetric unit. A significant amount of time was invested in identifying and refining the disordered molecules. Bond length restraints were applied to model the molecules but the resulting isotropic displacement coefficients suggested the molecules were highly mobile. Option SQUEEZE of program PLATON[2] was used to correct the diffraction data for diffuse scattering effects and to identify the solvent molecule. PLATON calculated the upper limit of volume that can be occupied by the solvent to be 1149.4Å^3, or 18.5% of the unit cell volume. The program calculated 60 electrons in the unit cell for the diffuse species. No data are given for the diffusely scattering species. Output of SQUEEZE reports are apended in cif file P2.

Crystal structure analysis of (Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-γ⁴Phe-OEt) P3: Crystals of peptide were grown by slow evaporation from a solution of acetone. A single crystal (0.42 × 0.32 × 0.18 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°), for a total number of 27803 independent reflections. Space group *P 2(1), 2(1), 2(1)*; *a* = 17.910(3), *b* = 24.339(4), *c* = 26.446(4) Å, α = 90.00 , β = 90.00, γ = 90.00, V = 11528(3) Å^3, Orthorhombic *P*, Z = 4 for chemical formula $2(C_{54}H_{78}N_6O_9).H_2O$, with two molecule in asymmetric unit; ρ_{calcd} = 1.111 g cm^{-3}, μ = 0.076 mm^{-1}, *F*(000) = 4168, R_{int}= 0.0661. The structure was obtained by direct methods using SHELXS-97.[1] 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 0.0929 (*wR2*= 0.2465) for 11730 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 1257 variables, *S* = 1.080. The largest difference peak and hole were 0.713 and -0.363 e Å^3, respectively.

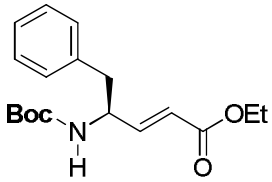
There were some partially occupied solvent molecules also present in the asymmetric unit. A significant amount of time was invested in identifying and refining the disordered molecules. Bond length restraints were applied to model the molecules but the resulting isotropic displacement coefficients suggested the molecules were highly mobile. Option SQUEEZE of program PLATON[2] was used to correct the diffraction data for diffuse scattering effects and to identify the solvent molecule. PLATON calculated the upper limit of volume that can be occupied by the solvent to be 1151.4Å^3, or 10% of the unit cell volume. The program calculated 208 electrons in the unit cell for the diffuse species. No data are given for the diffusely scattering species. Output of SQUEEZE reports are included in cif file P3.

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

Boc-(*S*)-Phenylalanal [(*S*)-*tert*-butyl 1-oxo-3-phenylpropan-2-ylcarbamate][3] (5 g, 20 mmol) was dissolved in 60 mL of dry THF. Then ylide (PPh_3=$CHCO_2Et$) (30 mmol, 10.45 g) was added to this solution at RT. Reaction mixture was stirred for about 5 hrs at RT. Completion of the reaction was monitored by TLC. After completion, the reaction mixture was quenched with 2(N) ammonium chloride solution in water (100 mL). Then the product was extracted with EtOAc (3× 100 mL). Combined organic layer was washed with brine (100 mL) and dried over anhydrous Na_2SO_4. Organic layer was concentrated under reduced pressure to give the crude product, which was further purified on silica gel column chromatography using EtOAc/Pet-ether (60-80 °C) to get pure ethyl ester of *N*-Boc- (*S, E*)- α, β-unsaturated γ-phenylalanine.

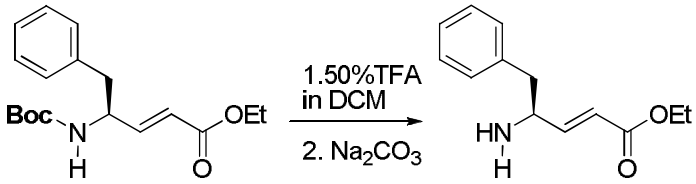


(*S*, *E*)-ethyl 4-(*tert*-butoxycarbonylamino)-5-phenylpent-2-enoate (Boc-dgF-OEt) : White powder (8.04 g, 84%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.3006-7.1423(m, 5H, -Ph), 6.9188-6.8670(dd, J = 5.04, J = 11, 1H, C*H*=CHCO$_2$Et), 5.8586-5.8195 (d, J = 17.4, 1H, CH=C*H*CO$_2$Et), 4.5956 (b, 1H, NH), 4.5244 (b, 1H, C*H*-CH=CH), 4.1893-4.1358 (q, J = 6.88, 2H, -OC*H*$_2$), 2.922-2.8555 (m, 2H, C*H*$_2$-Ph), 1.3763 (s, 9H, -(CH$_3$)$_3$ Boc), 1.2739-1.2381 (t, J = 7.3, 3H, -OCH$_2$C*H*$_3$) ; **^{13}C NMR** (100MHz, $CDCl_3$) δ 166.1454, 154.9128, 147.5676, 136.3376, 129.3685, 128.5495, 126.8338, 121.0495, 79.8302, 60.4434, 52.1647, 40.8203, 28.2629, 14.1884; **MALDI TOF/TOF** m/z Calcd. For $C_{18}H_{25}NO_4$ $[M+Na]^+$ 342.1681, observed 342.1657.



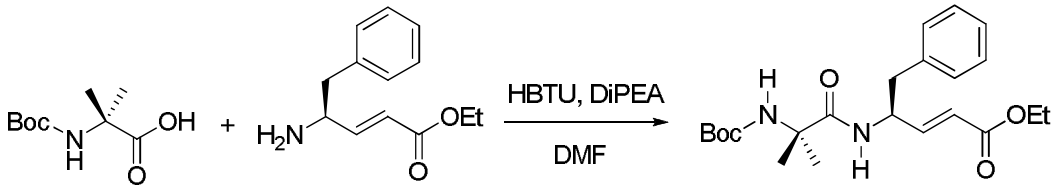
Synthesis of Dipeptide

Boc-Aib-dgF-OEt: (*S*, *E*)-ethyl 4-(*tert*-butoxycarbonylamino)-5-phenylpent-2-enoate (Boc-dgF-OEt) (11 mmol, 3.15 g) was dissolved in DCM (10 mL) and cooled the solution in ice bath. Then, 10 mL of neat TFA was added slowly to this solution. After completion of the reaction (~ 30 min), TFA was removed from the reaction mixture under *vacuum*. The residue was dissolved in water and the pH was adjusted to ~10 by the slow addition of solid Na_2CO_3 in ice cold conditions. Then Boc deprotected free amine was extracted with ethyl acetate (3 × 40 mL). The combined organic layer was washed with brine (60 mL), dried over Na_2SO_4, concentrated under *vacuum* to *ca*. 2 mL and directly used for the coupling reaction in the next step.

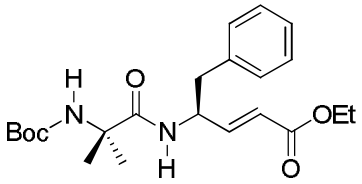


Boc-Aib-OH (10 mmol, 2 g) and NH_2-dgF-OEt were dissolved together in DMF (6 mL), followed by HBTU (11 mmol, 4.2 g) was added to the reaction mixture and cooled to 0 ^{o}C for 5 min. Then, DiPEA (12 mmol, 2.15 mL) was added to the reaction mixture with stirring and the reaction mixture was allowed

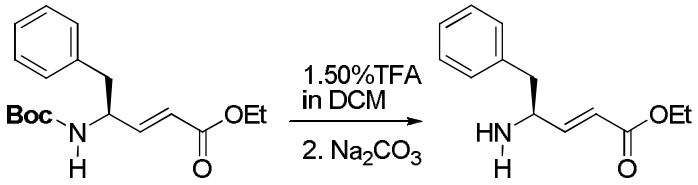
to come to room temperature. The progress of the reaction was monitored by TLC. After completion (roughly 6 hrs), the reaction mixture was diluted with 400 mL of ethyl acetate and washed with 5% HCl (2 ×150 mL), 10 % sodium carbonate solution in water (2 ×150 mL) and followed by brine (130 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give gummy yellowish product, which was purified on silica gel column chromatography using EtOAc/Pet-ether (60-80 ^oC) to get white crystalline product. Overall yield 75% (3.25 g, 7.5 mmol).



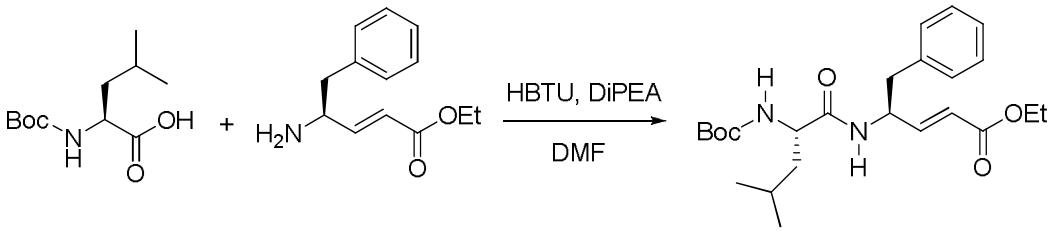
Boc-Aib-dgF-OEt: White crystalline powder (3.25 g, 75%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31-7.17(m, 5H, -Ph), 6.96-6.91(dd, J = 5.04, J = 11.3, 1H, C*H*=CHCO$_2$Et), 6.69 (b, 1H, NH), 5.94-5.90 (d, J = 16.8, 1H, CH=C*H*CO$_2$Et), 4.98-4.91 (m, 1H, C*H*-CH=CH), 4.81 (s, 1H, NH), 4.19-4.13 (q, J = 6.88, 2H, -OC*H*$_2$), 2.99-2.84 (m, 2H, C*H*$_2$-Ph), 1.44 (s, 15H, -(CH$_3$)$_3$ Boc, -(CH$_3$)$_2$ Aib), 1.27-1.24 (t, J = 7.3, 3H, -OCH$_2$C*H*$_3$); **^{13}C NMR** (100MHz, $CDCl_3$) δ 173.37, 166.30, 154.98, 147.18, 136.55, 129.37, 128.63, 126.94, 121.21, 80.95, 60.44, 56.93, 50.76, 40.64, 28.36, 25.22, 14.27; **MALDI TOF/TOF** m/z Calcd. For $C_{22}H_{32}N_2O_5$ $[M+Na]^+$ 427.2209, observed 427.2511.



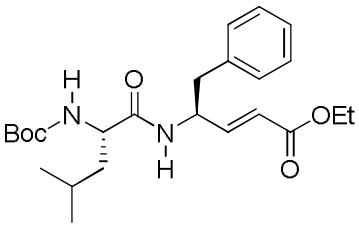
Boc-Leu-dgF-OEt: (*S*, *E*)-ethyl 4-(tert-butoxycarbonylamino)-5-phenylpent-2-enoate (Boc-dgF-OEt) (5.5 mmol, 1.57 g) was dissolved in DCM (5 mL) and the solution was cooled in ice bath. Then, 5 mL of neat TFA was added slowly to the solution. After completion of the reaction (30 min.), the TFA was removed from reaction mixture under reduced pressure. The residue was dissolved in water and the pH was adjusted to ~10 by the slow addition of solid Na_2CO_3 in ice cold conditions. The Boc deprotected free amine was extracted with ethyl acetate (3 × 40 mL). The combined organic layer was washed with brine (60 mL), dried over anhydrous Na_2SO_4 and concentrated under *vacuum* to *ca.* 2 mL and directly used for coupling reaction.



Boc-Leu-OH (5 mmol, 1.15 g) and NH_2-dgF-OEt were dissolved together in DMF (4 mL) and HBTU (5.5 mmol, 2.1 g) was added into the reaction mixture. The reaction mixture was cooled to 0 ^oC for 5 min. Then DiPEA (6 mmol, 1.05 mL) was added to the reaction mixture and allowed to come to room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction (roughly 6 hrs), the reaction mixture was diluted with 300 mL of ethyl acetate and washed with 5% HCl (2 × 100 mL), 10 % sodium carbonate solution in water (2 × 100 mL) followed by brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get gummy yellowish product, which was purified via silica gel column chromatography using EtOAc/Pet-ether (60-80 ^oC) to get white solid product. Overall yield 78% (1.68 g, 3.9 mmol).

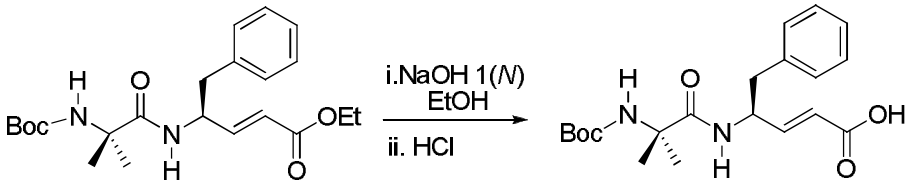


Boc-Leu-dgF-OEt: White powder (1.68 g, 78%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31-7.16 (m, 5H, -Ph), 6.94-6.88 (dd, J = 4.9, J = 10.9, 1H, C*H*=CHCO$_2$Et), 6.34-6.31 (d, J = 8.8, 1H, NH), 5.86-5.82 (d, J = 16.9, 1H, CH=C*H*CO$_2$Et), 4.08-4.90 (m, 1H, α-H of Leu), 4.74-4.73 (d, J = 5.8, 1H, NH), 4.20-4.14 (q, J = 7.1, 2H, -OC*H*$_2$), 4.08-3.99 (m, 1H, C*H*-CH=CH), 2.97-2.85 (m, 2H, C*H*$_2$-Ph), 2.08-2.05 (m, 2H, β-H of Leu), 1.63-1.54 (m, 1H, γ-H of Leu), 1.44 (s, 9H, -(CH$_3$)$_3$ Boc), 1.28-1.25 (t, J = 7.1, 3H, -OCH$_2$C*H*$_3$), 0.92-0.90 (d, J = 6.9, 3H); **^{13}C NMR** (100MHz, $CDCl_3$) δ 171.95, 166.05, 155.74, 146.57, 136.18,129.29, 128.58, 126.94, 121.35, 80.42, 60.47, 50.62, 40.43, 31.57, 28.24, 24.69, 22.88, 14.17; **MALDI TOF/TOF** m/z Calcd. For $C_{24}H_{36}N_2O_5$ $[M+Na]^+$ 455.2522, observed 455.3341

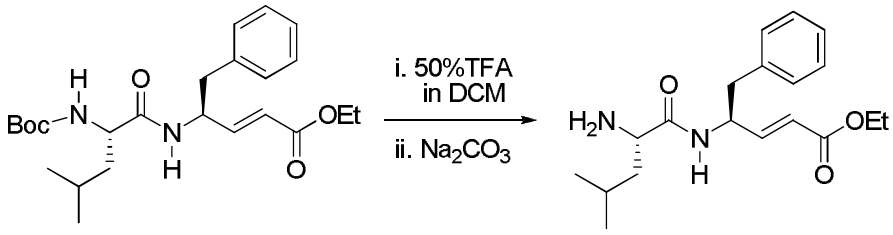


Synthesis of Tetrapeptide Boc-Aib-dgF-Leu-dgF-OEt (P1)

Boc-Aib-dgF-OH: Boc-Aib-dgF-OEt (2 mmol, 0.810 g) was dissolved in ethanol (5 mL). Then 5 mL of 1(N) NaOH was added slowly to this solution. After completion of the reaction (~ 2 hrs), ethanol was evaporated from reaction mixture and the residue was acidified to *p*H ~ 3 using 5% HCl (5% volume in water) at cold conditions after diluting with 50 mL of cold water. Product was extracted with ethyl acetate (3 × 30 mL). Combined organic layer was washed with brine (40 mL) and dried over anhydrous Na_2SO_4. Organic layer was concentrated under reduced pressure to give gummy product with quantitative yield 98% (1.83 mmol, 0.742 g).

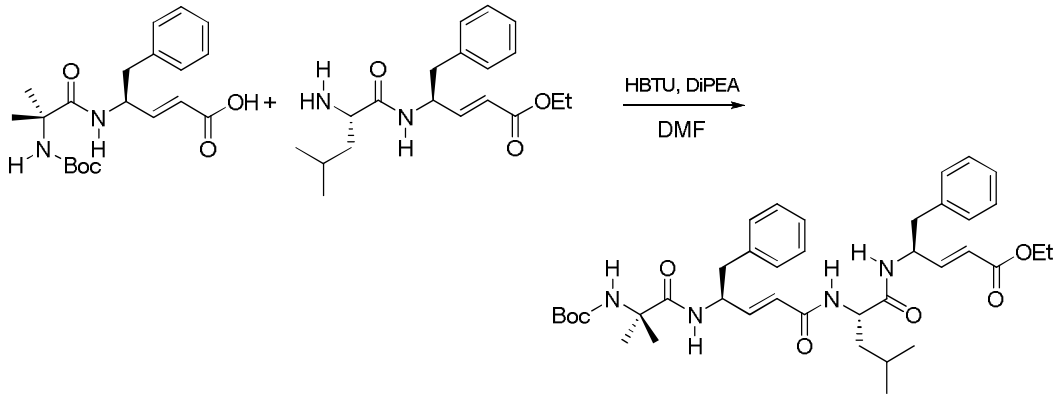


NH_2-Leu-dgF-OEt: Boc-Leu-dgF-OEt (2.2 mmol, 0.870 g) was dissolved in DCM (5 mL) and cooled the solution in ice bath. Then 5 mL of neat TFA was added to the solution. After completion of the reaction (~ 30 min), TFA was removed from reaction mixture under *vacuum*. The residue was dissolved in water and the pH was adjusted to ~10 by the slow addition of solid Na_2CO_3 in ice cold conditions. Then Boc deprotected dipeptide was extracted with ethyl acetate (3 × 40 mL). Combined organic layer was washed with brine (60 mL), dried over anhydrous Na_2SO_4, concentrated under *vacuum* to *ca.* 2 mL and directly used for coupling reaction in the next step.

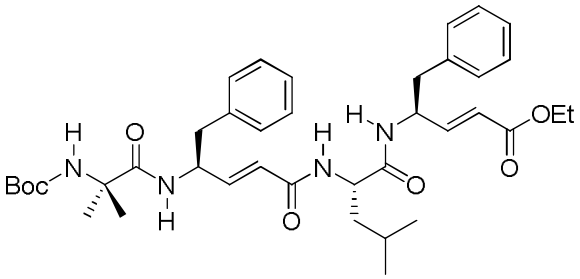


Boc-Aib-dgF-OH (1.83 mmol, 0.742 g) and NH_2-Leu-dgF-OEt were dissolved together in DMF (5 mL) followed by HBTU (2.2 mmol, 0.820 g) was added. The reaction mixture was cooled to 0 ^oC for 5 min. Then DiPEA (2.5 mmol, 0.45 mL) was added to the reaction mixture and it was allowed to come to room temperature at constant stirring. The progress of the reaction was monitored by TLC. After completion of

the reaction (roughly 4hrs), the reaction mixture was diluted with 200 mL of ethyl acetate and washed with 5% HCl (2 × 80 mL), 10 % sodium carbonate solution in water (2 × 80 mL) and followed by brine (80 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give gummy yellowish product, which was purified on silica gel column chromatography using DCM/MeOH solvent system to get gummy product, which was further crystallized using EtOAc/Hexane. Overall yield 68% (1.36 mmol, 0.938 g).

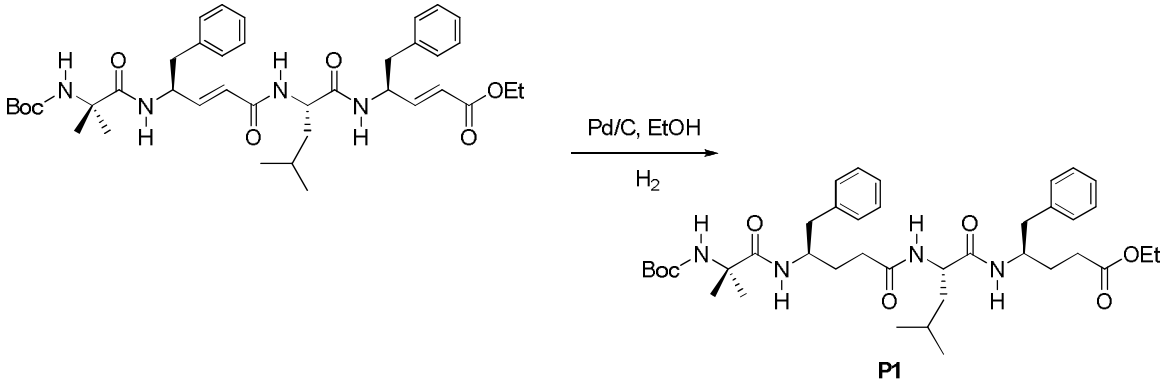


Boc-Aib-dgF-Leu-dgF-OEt: White crystalline powder (0.938 g, 68%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31-7.10 (m, 10H, -Ph), 6.92-6.84 (m, 2H, C*H*=CH), 6.66-6.84 (d, *J* = 8.5, 1H, NH), 6.44-6.42 (d, *J* = 8.5, 1H, NH), 6.06-6.02 (d, *J* = 15.5, 1H, CH=C*H*), 5.88-5.83 (d, *J* = 17.4, 1H, CH=C*H*), 5.80-5.78 (d, *J* = 7.8, 1H, NH), 5.00-4.95 (m, 2H), 4.91-4.84 (m, 1H, -CH-), 4.81 (s, 1H, NH), 4.46-4.40 (m, 1H, -CH-), 4.20-4.15 (q, *J* = 7, 2H, -OC*H*$_2$), 3.03-2.77 (m, 4H, C*H*$_2$-Ph), 1.71-1.52 (m, 3H, α and β-H of Leu), 1.47 (s, 3H, -$(CH_3)_2$ Aib), 1.39 (s, 9H, -$(CH_3)_3$ Boc), 1.29-1.26 (t, *J* = 7.1, 3H, -OCH_2CH_3), 1.27 (s, 3H, -$(CH_3)_2$ Aib), 0.90-0.88 (d, *J* = 6.8, 3H, -C*H*$_3$ of Leu), 0.87-0.86 (d, *J* = 6.8, 3H, -C*H*$_3$ of Leu); **MALDI TOF/TOF** m/z Calcd. For $C_{39}H_{54}N_4O_7$ $[M+Na]^+$ 713.3890, observed 713.4538.



Transformation of α/Vinylogous hybrid peptide (Boc-Aib-dgPhe-Leu-dgPhe-OEt) to saturated α/γ^4-hybrid peptide (Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-OEt) (P1) [4]

Vinylogous hybrid tetra peptide (0.5 mmol) was dissolved in EtOH (5 mL), and was treated with 60 mg of 20% Pd/C. The hydrogen gas was supplied through balloon. The schematic representation is shown below. The reaction mixture was stirred under hydrogen atmosphere for about 6 hrs. The completion of the reaction was monitored by MALDI-TOF/TOF and HPLC. After the completion of reaction, the reaction mixture was diluted with EtOH (30 mL) and it was filtered through sintered funnel using celite bed and celite bed was washed with EtOH (3 × 25mL). The filtrate was evaporated under *vacuum* to get white crystalline pure product. Overall, the α/γ^4-hybrid peptide was isolated with quantitative yield.

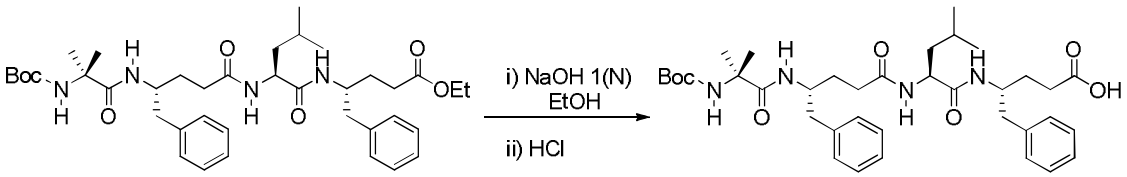


P1

Boc-Aib-γ^4Phe-Leu-γ^4Phe-OEt:(P1): White crystalline powder (332 mg, 96%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.49-7.47 (d, J = 8.7, 1H, NH), 7.29-7.13 (m, 10H, -Ph), 6.76 (s, 1H, NH), 5.85-5.83 (d, J = 9.28, 1H, NH), 4.93 (s, 1H, NH), 4.30-4.18 (m, 2H, -C*H*-), 4.12-4.07 (m, 3H, -OCH_2, -C*H*-), 3.00-2.80 (m, 4H, CH_2-Ph), 2.65-2.60 (m, 1H, CH_2-Ph), 2.57-2.36 (m, 2H, -CH_2-), 2.30-2.10 (m, 3H, -CH_2-), 1.97-1.72 (m, 14H, -CH_2-), 1.60-1.53 (m, 1H, γ-H of Leu), 1.48 (s, 3H, -$(CH_3)_2$ Aib), 1.41 (s, 9H, -$(CH_3)_3$ Boc), 1.26-1.22 (t, J = 7, 3H, -OCH_2CH_3), 1.22 (s, 3H, -$(CH_3)_2$ Aib), 0.84-0.82 (d, J = 6.6, 3H, -CH_3 of Leu), 0.80-0.79 (d, J = 6.9, 3H, -CH_3 of Leu); **MALDI TOF/TOF** m/z Calcd. For $C_{39}H_{58}N_4O_7$ $[M+Na]^+$ 717.4203, observed 717.5516.

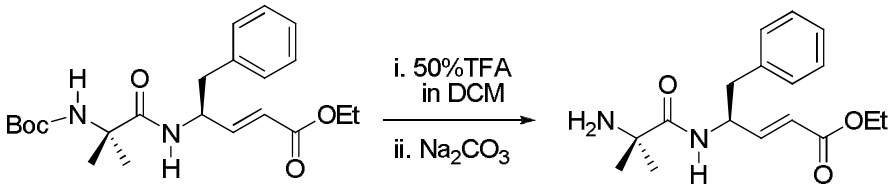
Synthesis of Hexapeptide (Boc-Aib-γ^4Phe-Leu-γ^4Phe-Aib-dgF-OEt) (P2)

Boc-Aib-γ^4Phe-Leu-γ^4Phe-OH: Boc-Aib-γ^4Phe-Leu-γ^4Phe-OEt (0.4 mmol, 0.286 g) was dissolved in ethanol (4 mL). Then 5 mL of 1(N) NaOH was added slowly to this solution. After completion of the reaction (~ 2 hrs), ethanol was evaporated from reaction mixture and residue was acidified to *p*H ~ 3 using 5% HCl (5% volume in water) at cold conditions after diluting with 50 mL cold water. Product was extracted with ethyl acetate (3 × 25 mL). Combined organic layer was washed with brine (40 mL) and dried over anhydrous Na_2SO_4. Organic layer was concentrated under reduced pressure to give gummy product with quantitative yield 98% (0.392 mmol, 0.261 g).

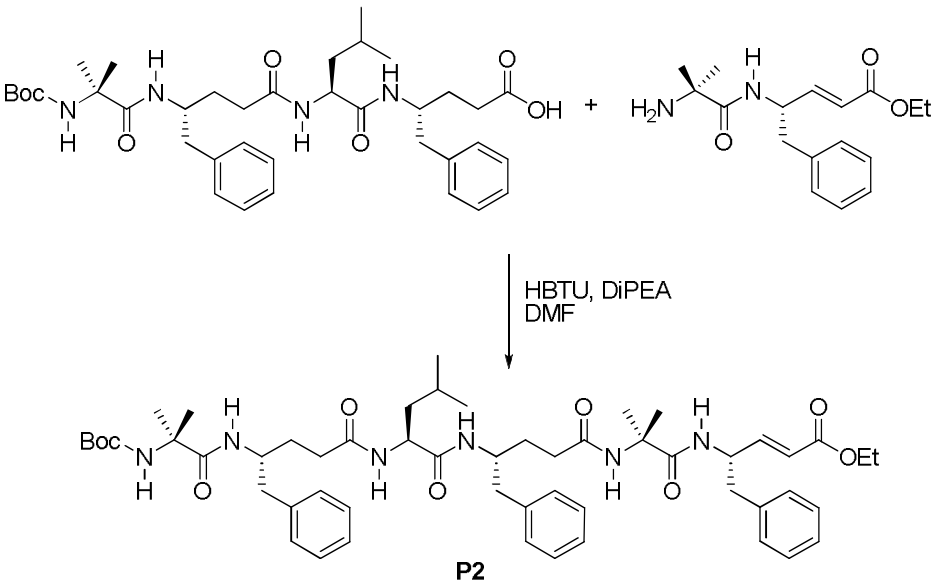


P1 **P1 acid**

NH_2-Aib-dgF-OEt: Boc-Aib-dgF-OEt (0.5 mmol, 0.210 g) was dissolved in DCM (3 mL) and cooled the solution in ice bath. Then 3 mL of neat TFA was added to the solution. After completion of the reaction (~ 30 min), TFA was removed from reaction mixture under vacuum. The residue was dissolved in water and the pH was adjusted to ~10 by the slow addition of solid Na_2CO_3 at ice cold conditions Then Boc deprotected dipeptide was extracted with ethyl acetate (3 × 25 mL). Combined organic layer was washed with brine (40 mL), dried over Na_2SO_4 and concentrated under *vacuum* to *ca*. 2 mL and directly used for the coupling reaction in the next step.

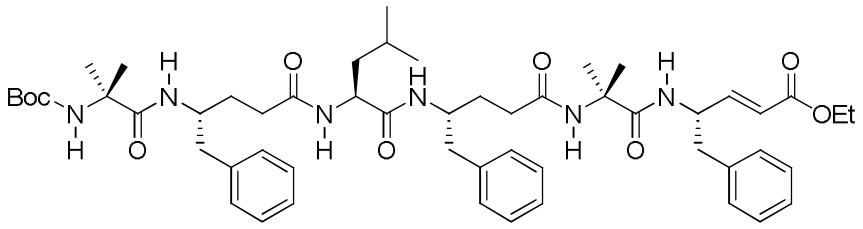


Boc-Aib-γ^4Phe-Leu-γ^4Phe-OH (0.392 mmol, 0.261 g) and NH_2-Aib-dgF-OEt were dissolved together in DMF (2 mL) followed by HBTU (0.5 mmol, 0.190 g) was added. The reaction mixture was cooled to 0 ^oC for 5 min. Then DiPEA (0.7 mmol, 0.12 mL) was added to the reaction mixture and it was allowed to come to room temperature at constant stirring. The progress of the reaction was monitored by TLC. After completion of the reaction (roughly 8 hrs), the reaction mixture was diluted with 100 mL of ethyl acetate. The organic layer was washed with 5% HCl (2 × 40 mL), 10 % sodium carbonate solution in water (2 × 40 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give gummy yellowish product, which was purified on silica gel column chromatography using EtOAc/Pet-ether (product was eluted at 90% EtOAc in pet-ether) solvent system to get gummy product, which was further crystallized using EtOAc/Hexane. Overall yield 60% (0.231 mmol, 0.220 g).



P2

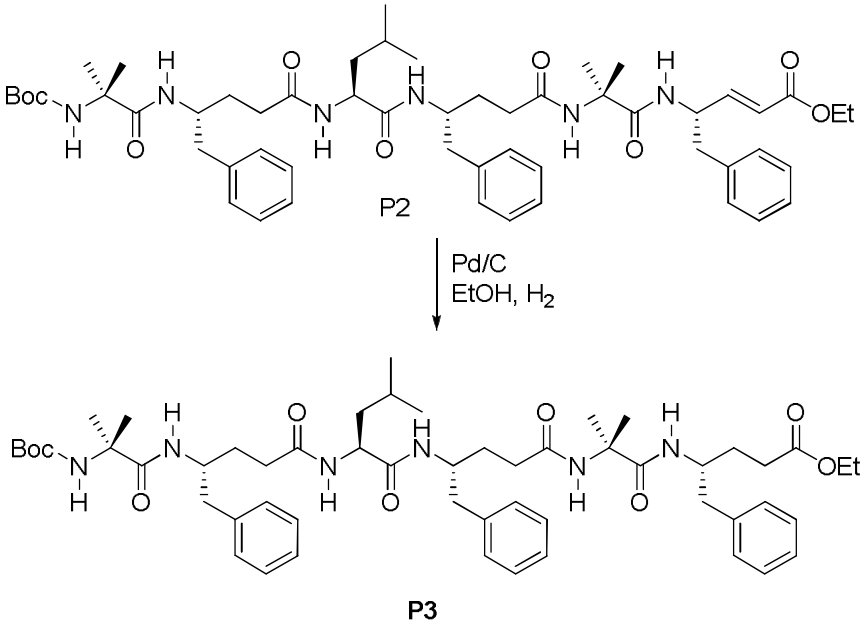
Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-dgF-OEt (P2): White crystalline powder (0.22 g, 60%); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.59-8.57 (d, J = 8.3, 1H, NH), 7.70-7.68 (d, J = 10.2, 1H, NH), 7.48-7.47 (d, J = 3.85, 1H, NH), 7.48-7.07 (m, 17H, -Ph, NH, -C*H*-(β) of dgF), 6.53-6.49 (dd, J = 17.7, J = 2.1, 1H, -CH= -C*H* α of dgF), 6.07-6.05 (d, J = 10.3, 1H, NH), 5.23 (s, 1H, NH), 5.13-4.97 (m, 1H, γ-C*H*- of dgF), 4.26-4.13 (m, 4H γ-C*H*- of γPhe, -OCH_2), 3.80-3.76 (m, 1H, α-C*H*- of Leu), 2.98-2.60 (m, 6H, -CH_2-Ph), 2.29-2.10 (m, 4H, α-CH_2- of γPhe), 1.76 (s, 3H, -$(CH_3)_2$ for Aib),1.71-1.65 (m, 2H, β-CH_2- of γPhe), 1.59 (s, 3H, -$(CH_3)_2$ for Aib), 1.55-1.48 (m, 2H, β-CH_2- of γPhe), 1.45 (s, 3H, -$(CH_3)_2$ for Aib), 1.43 (s, 9H, -$(CH_3)_3$ Boc), 1.30 (s, 3H, -$(CH_3)_2$ for Aib), 1.29-1.26 (t, J = 7, 3H, -OCH_2CH_3), 1.20-1.14(m, 2H, β-CH_2- of Leu), 0.99 (s, 3H, -$(CH_3)_2$ for Aib), 0.94-0.88 (m, 2H, γ-CH_2- of Leu), 0.83-0.82 (d, J = 6.6, 3H, -CH_3 of Leu), 0.78-0.77 (d, J = 6.7, 3H, -CH_3 of Leu); **MALDI TOF/TOF** m/z Calcd. For $C_{54}H_{76}N_6O_9$ $[M+Na]^+$ 975.5571, observed 975.9615



P2

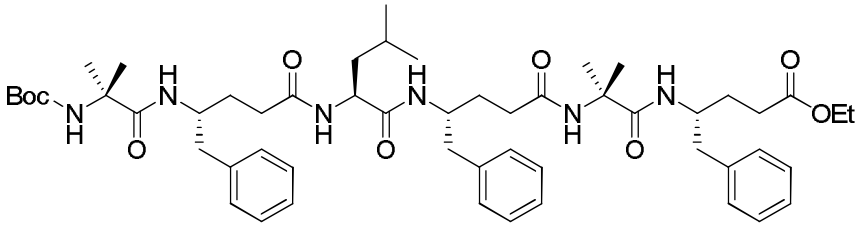
Transformation of Alpha-Gamma-Vinylogous hybrid hexapeptide Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-dgF-OEt (P2) to its saturated hybrid γ⁴-peptide analogue Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-γ⁴Phe-OEt (P3)

Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-dgF-OEt (P2) (0.10 mmol, 100 mg) was dissolved in EtOH (4 mL), and was treated with 20 mg of 20% Pd/C. The hydrogen gas was supplied through balloon. The schematic representation is shown below. The reaction mixture was stirred under hydrogen atmosphere for about 6 hrs. The completion of the reaction was monitored by MALDI-TOF/TOF (m/z Calcd. For $C_{54}H_{78}N_6O_9$ $[M+Na]^+$ 977.5728, observed 977.8813) and HPLC. After the completion of the reaction, the reaction mixture was diluted with EtOH (10 mL) and it was filtered through sintered funnel using celite bed and celite bed was washed with EtOH (3 × 15 mL). The filtrate was evaporated under *vacuum* to get white crystalline pure product. Overall, the α/γ⁴-hybrid peptide was isolated in 97% yield (97 mg, 0.097 mmol).



Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-γ⁴Phe-OEt (P3): White crystalline powder (0.097 g, 97%); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.08-8.06 (d, J = 9.25, 1H, NH), 7.66-7.64 (d, J = 10.2, 1H, NH), 7.39-7.38 (d, J = 4, 1H, NH), 7.39-7.09 (m, 15H, -Ph), 7.01 (s, 1H, NH), 5.96-5.94 (d, J = 10.3, 1H, NH), 5.05 (s, 1H, NH), 4.26-4.13 (m, 5H, -OCH_2, -CH-(γ) of γPhe), 3.85-3.82 (m, 1H, α -CH- of Leu), 2.99-2.59 (m, 6H, CH_2-Ph), 2.49-2.02 (m, 6H, -CH_2-(α) of γPhe), 1.99-1.83(m, 6H, -CH_2-(β) of γPhe), 1.72-1.58(m, 6H, -CH_2-

(β) of γPhe), 1.56-1.48(m, 6H, -C*H*$_2$-(β) of γPhe), 1.60 (s, 3H, -(CH$_3$)$_2$ for Aib), 1.44 (s, 9H, -(CH$_3$)$_3$ Boc), 1.43 (s, 3H, -(CH$_3$)$_2$ for Aib), 1.29 (s, 3H, -(CH$_3$)$_2$ for Aib), 1.27-1.22 (m, 5H, -OCH$_2$C*H*$_3$, C*H*$_2$-, β-H of Leu), 1.13-1.05 (m, 1H, γ-H of Leu), 1.00 (s, 3H, -(CH$_3$)$_2$ for Aib), 0.86-0.85 (d, *J* = 6.6, 3H, -C*H*$_3$ of Leu), 0.81-0.80 (d, *J* = 6.7, 3H, -C*H*$_3$ of Leu); **MALDI TOF/TOF** m/z Calcd. For $C_{54}H_{78}N_6O_9$ $[M+Na]^+$ 977.5728, observed 977.8813



P3

Reference

1. a) SHELXS-97: G. M. Sheldrick, *Acta Crystallogr. Sect A*, **1990**, *46*, 467. b) G. M. Sheldrick, SHELXL-97, Universität Göttingen (Germany) **1997**.

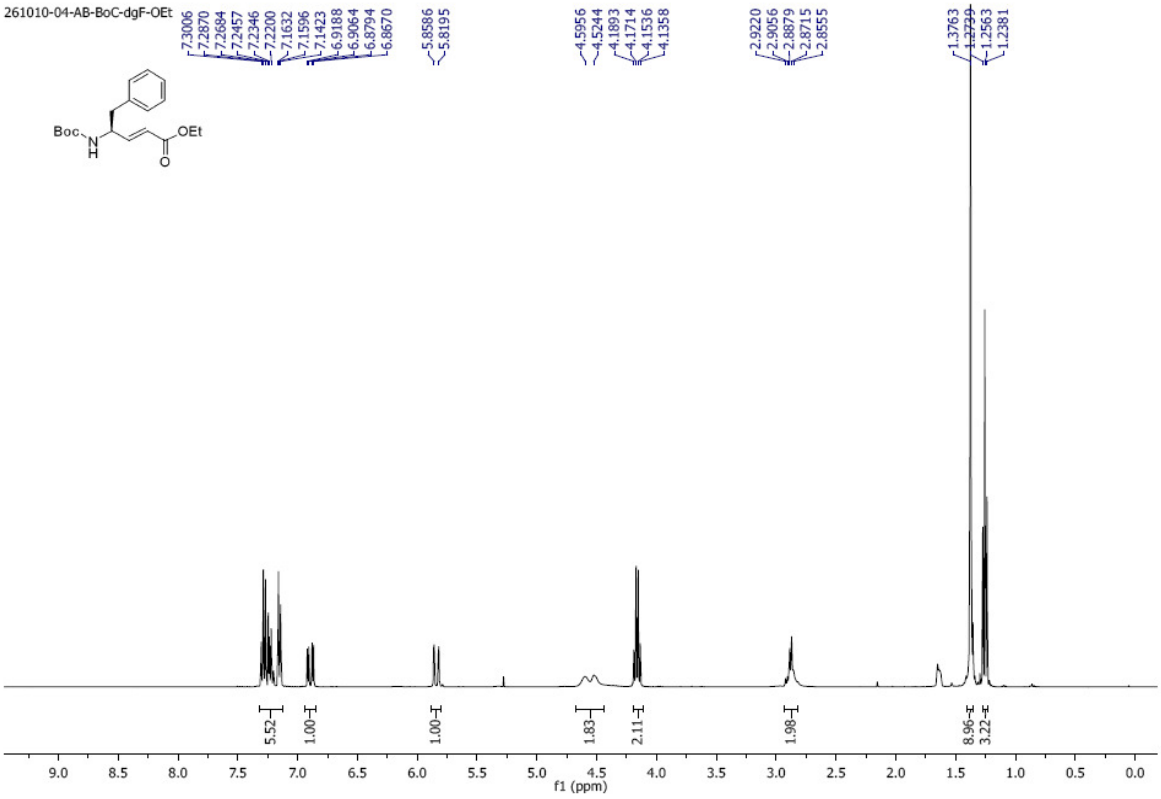
2. A. L. Spek (1990) Acta Cryst. A46, C34.

3. S. M. Mali, A. Bandyopadhyay, S. V. Jadhav, M. G. Kumar, H. N. Gopi, *Org. Biomol. Chem.* **2011**, *9,* 6566.

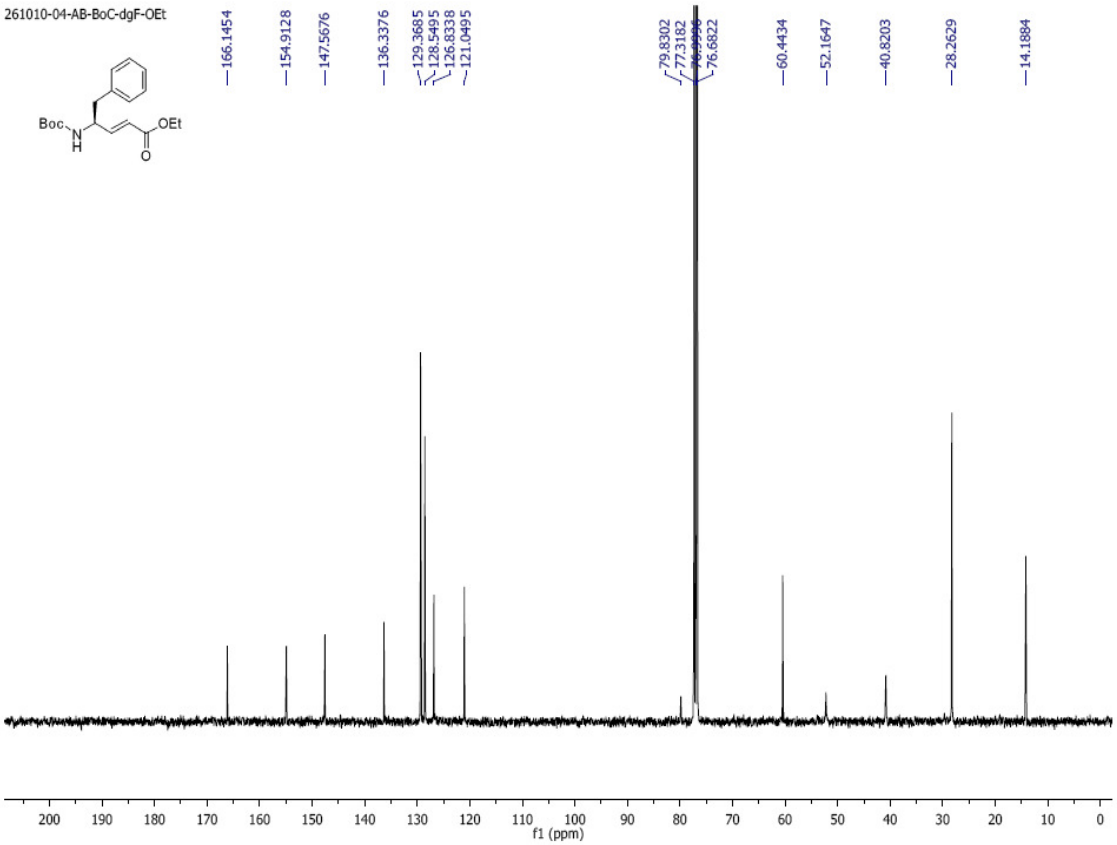
4. A. Bandyopadhyay, S. M. Mali, P. Lunawat, K. M. P. Raja, H. N. Gopi, *Org. Lett.* **2011**, *13*, 4482.

^{1}H, ^{13}C and MALDI-TOF Mass Data

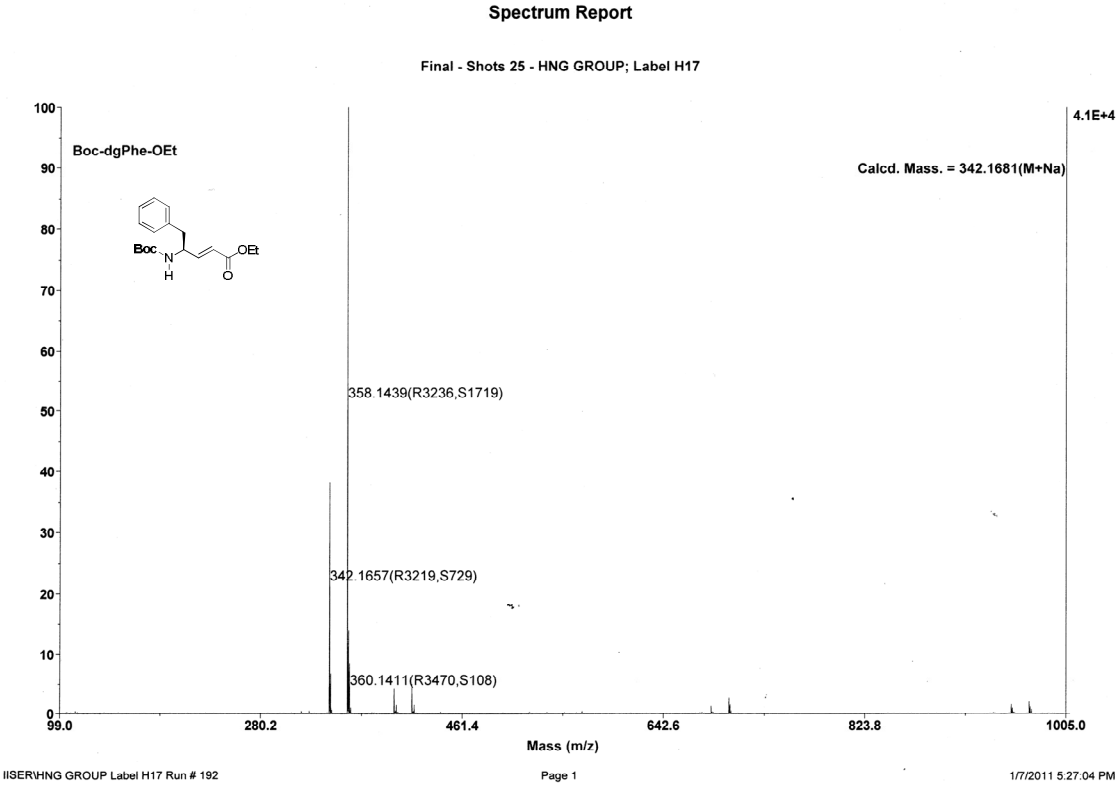
^{1}H NMR spectrum of **Boc-dgF-OEt** acquired in a 400 MHz spectrometer ($CDCl_3$)



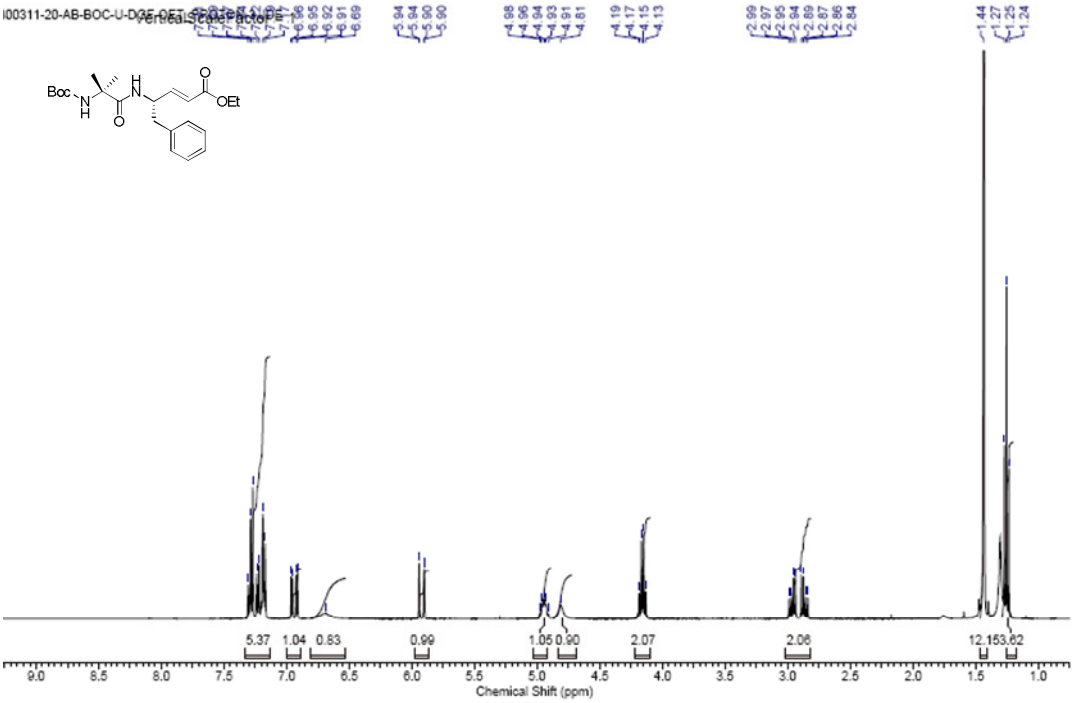
^{13}C NMR spectrum of **Boc-dgF-OEt** acquired in a 100 MHz spectrometer



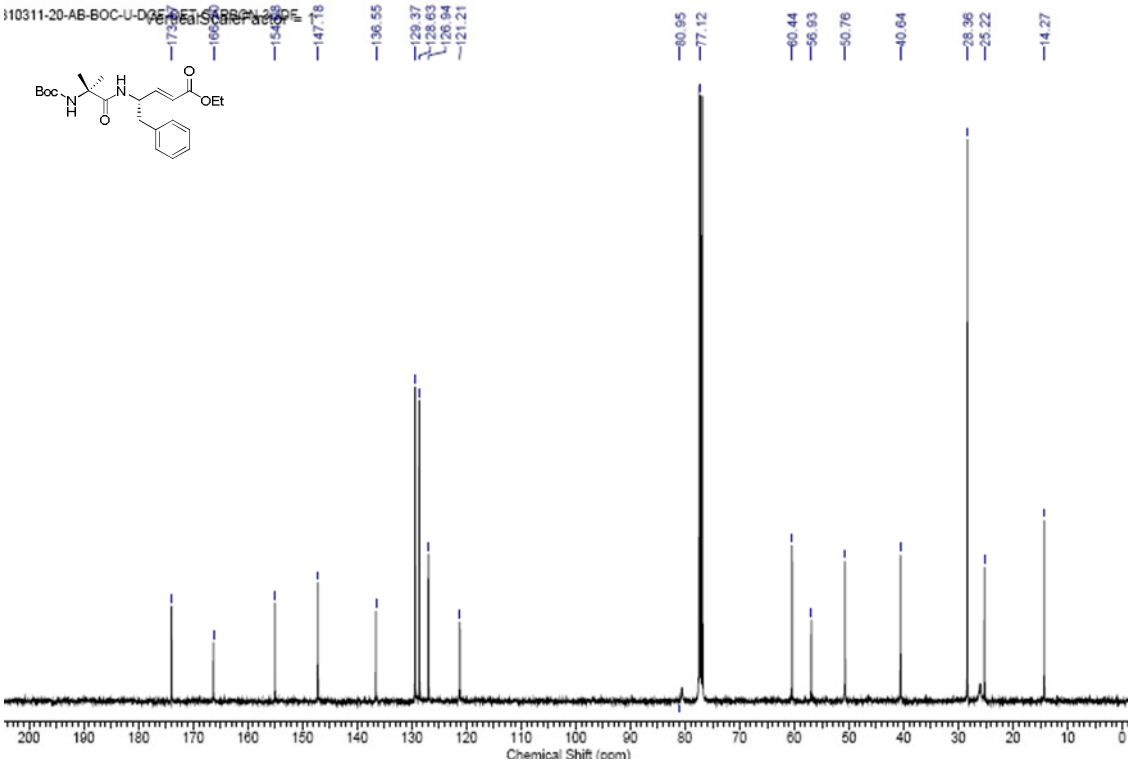
Mass spectrum of **Boc-dgF-OEt** acquired in a MALDI TOF/TOF spectrometer



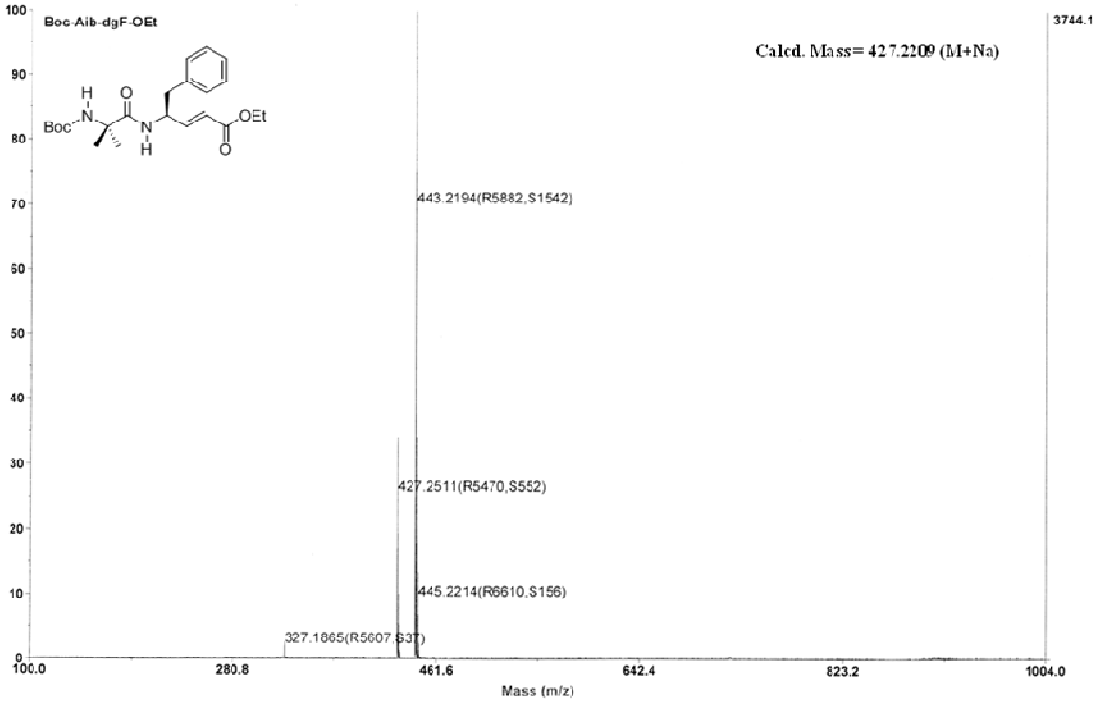
^{1}H NMR spectrum of **Boc-Aib-dgF-OEt** acquired in a 400 MHz spectrometer ($CDCl_3$)



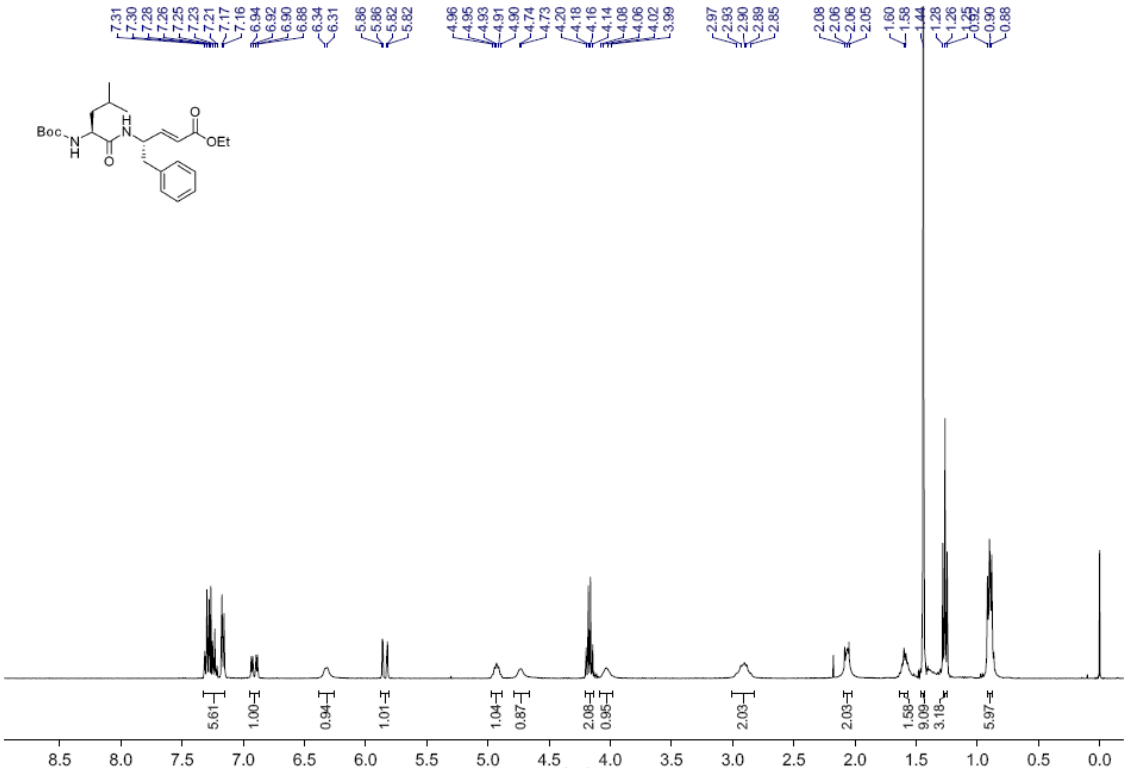
^{13}C NMR spectrum of **Boc-Aib-dgF-OEt** acquired in a 100 MHz spectrometer



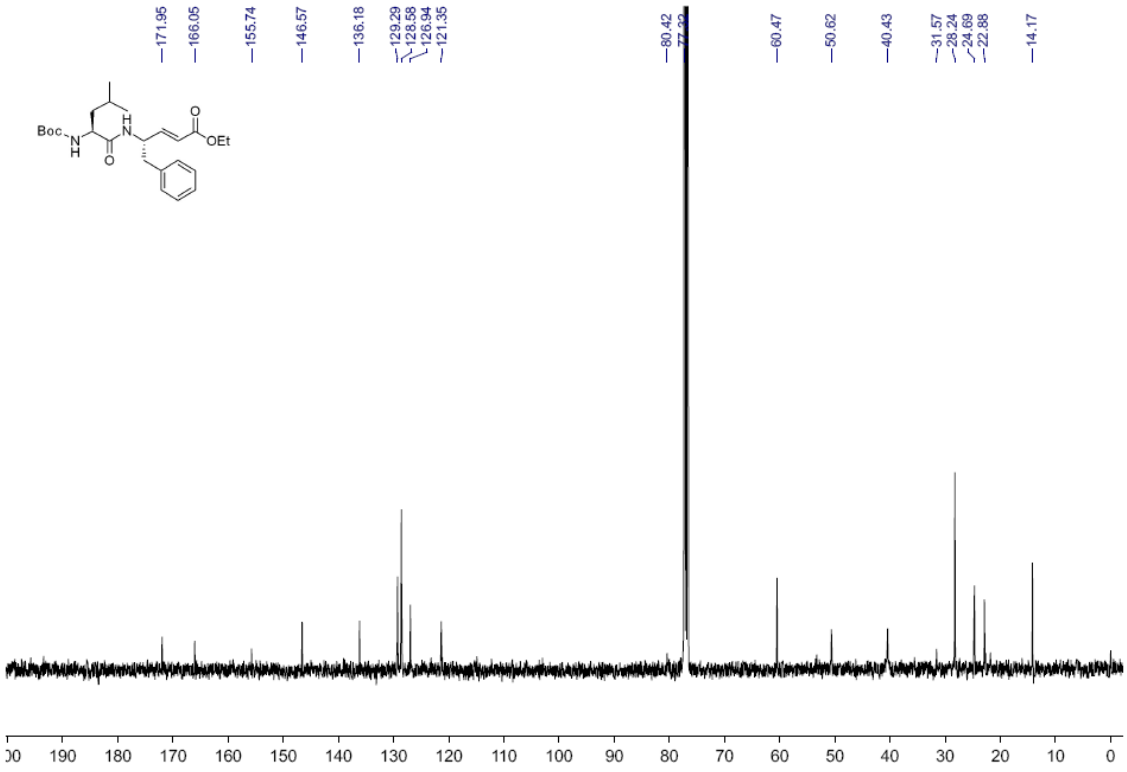
Mass spectrum of **Boc-Aib-dgF-OEt** acquired in a MALDI TOF/TOF spectrometer



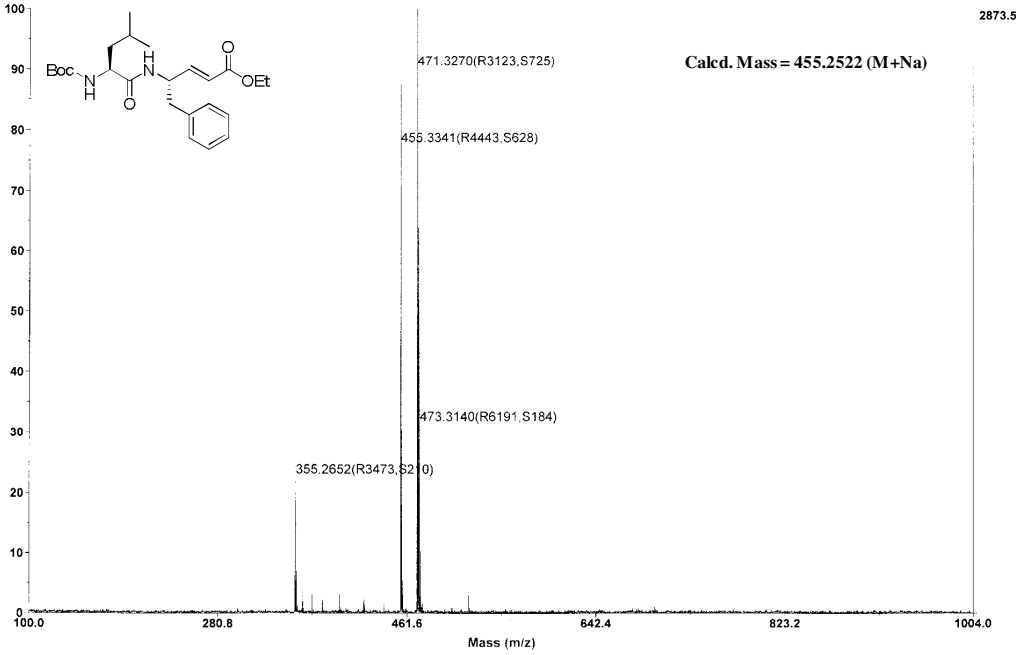
^{1}H NMR spectrum of **Boc-Leu-dgF-OEt** acquired in a 400 MHz spectrometer ($CDCl_3$)



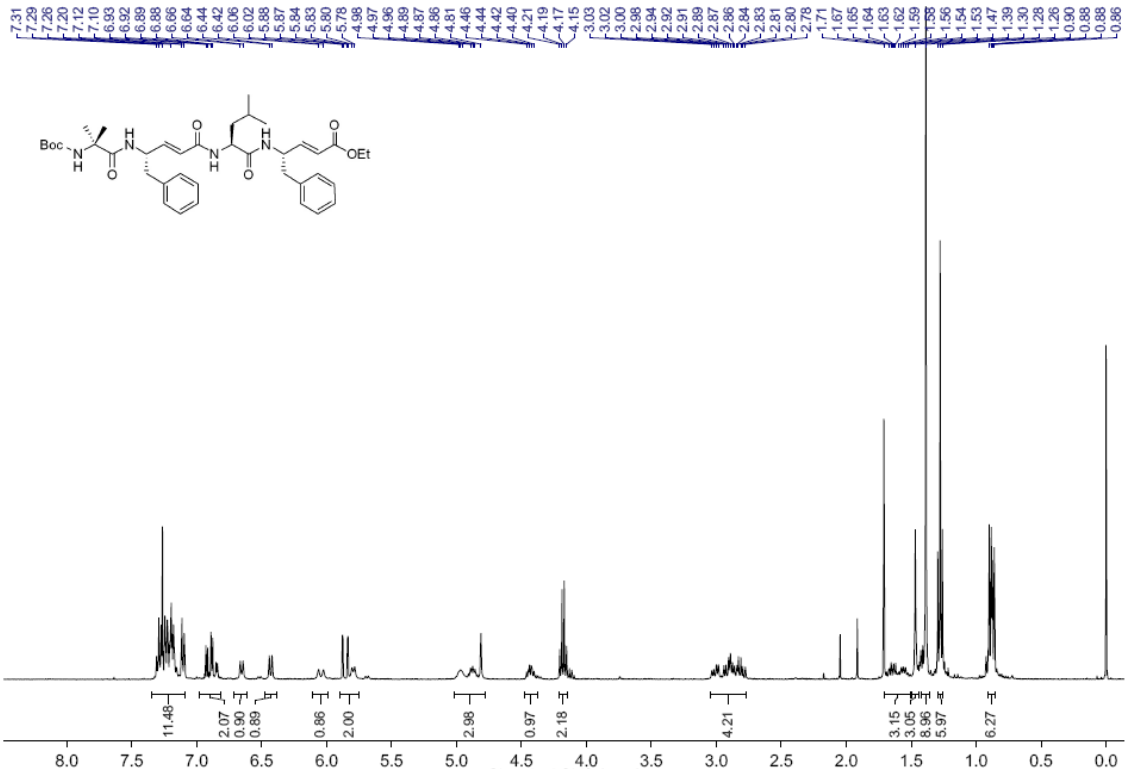
^{13}C NMR spectrum of **Boc-Leu-dgF-OEt** acquired in a 100 MHz spectrometer



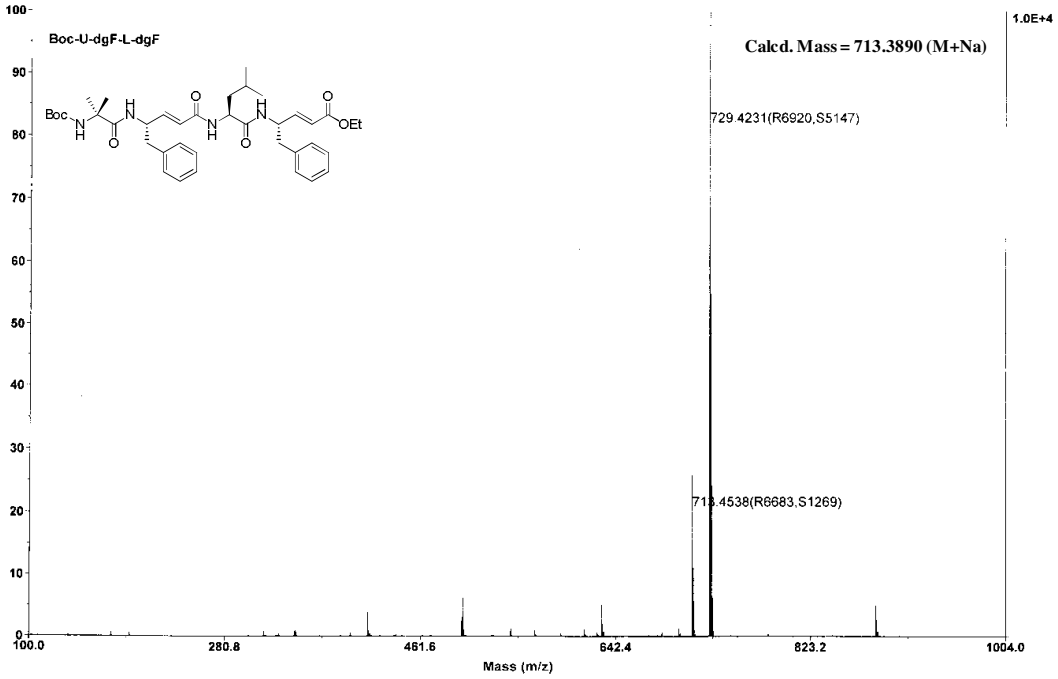
Mass spectrum of **Boc-Leu-dgF-OEt** acquired in a MALDI TOF/TOF spectrometer



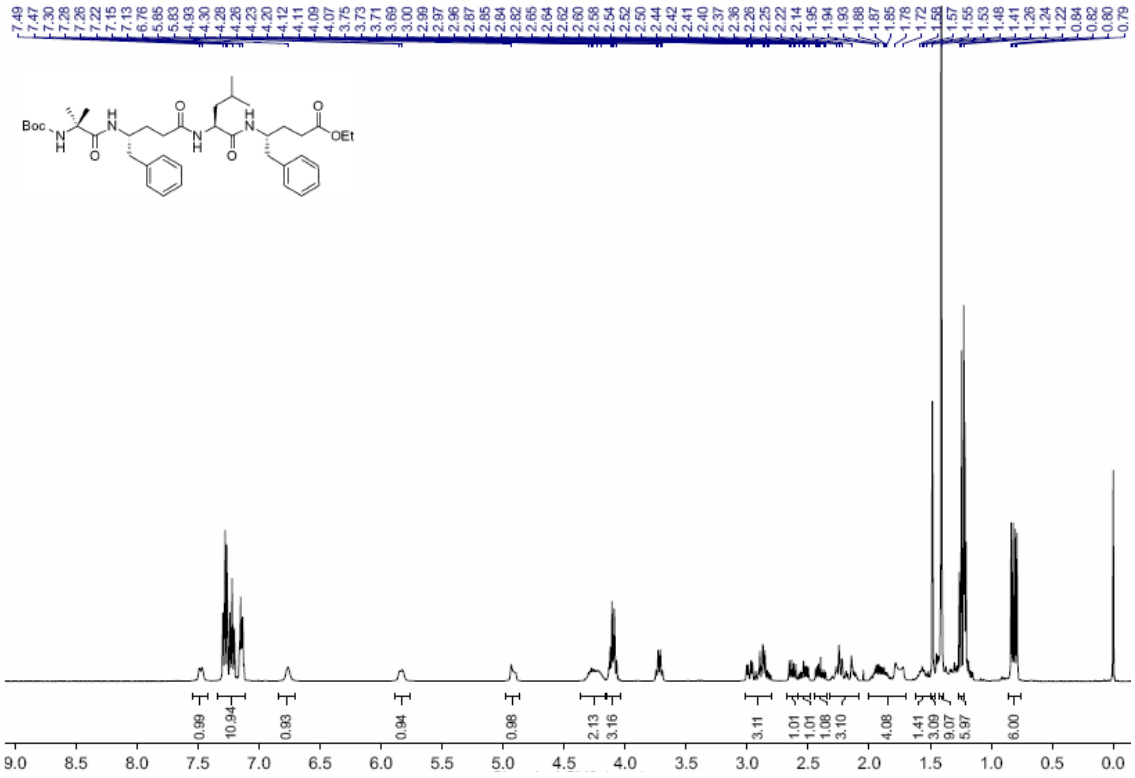
^{1}H NMR spectrum of **Boc-Aib-dgF-Leu-dgF-OEt** acquired in a 400 MHz spectrometer ($CDCl_3$)



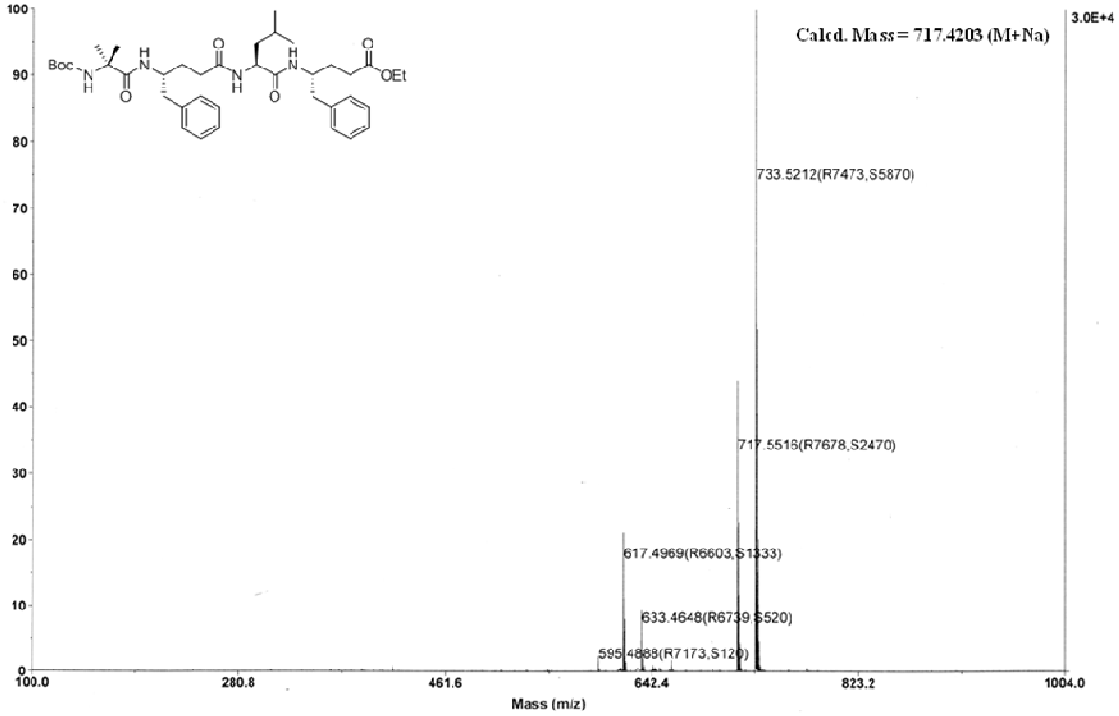
Mass spectrum of **Boc-Aib-dgF-Leu-dgF-OEt** acquired in a MALDI TOF/TOF spectrometer



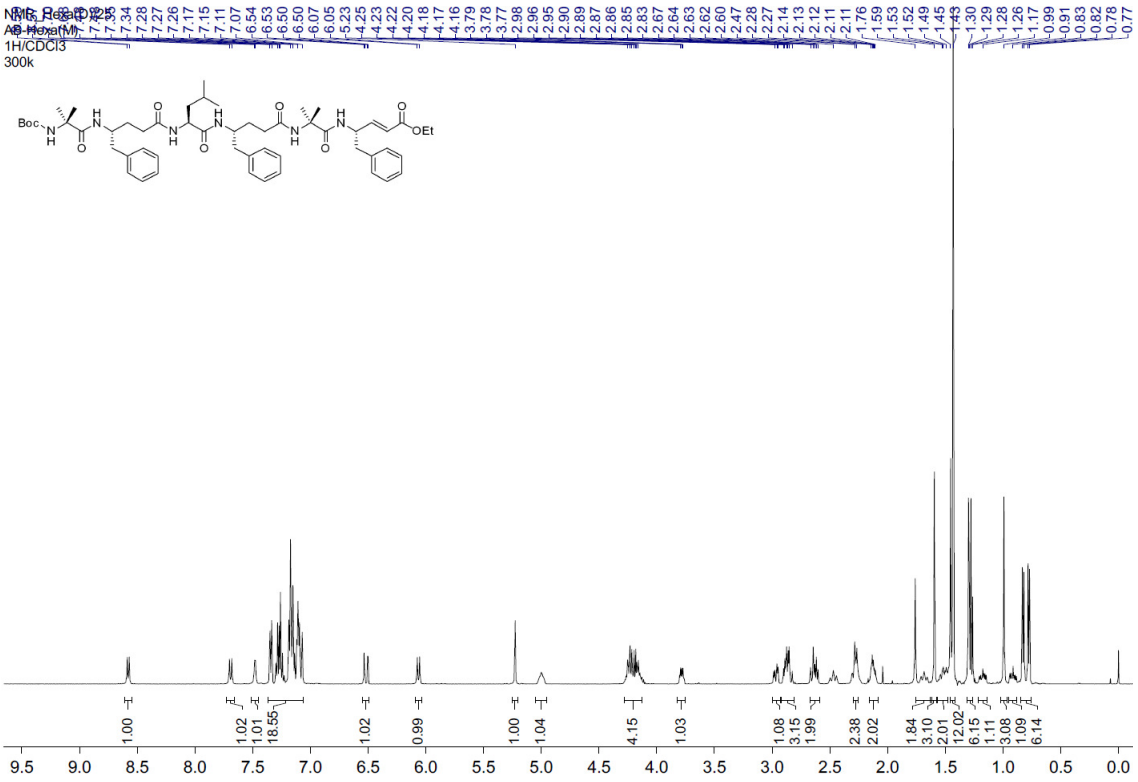
^{1}H NMR spectrum of **Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-OEt (P1)** acquired in a 400 MHz spectrometer ($CDCl_3$)



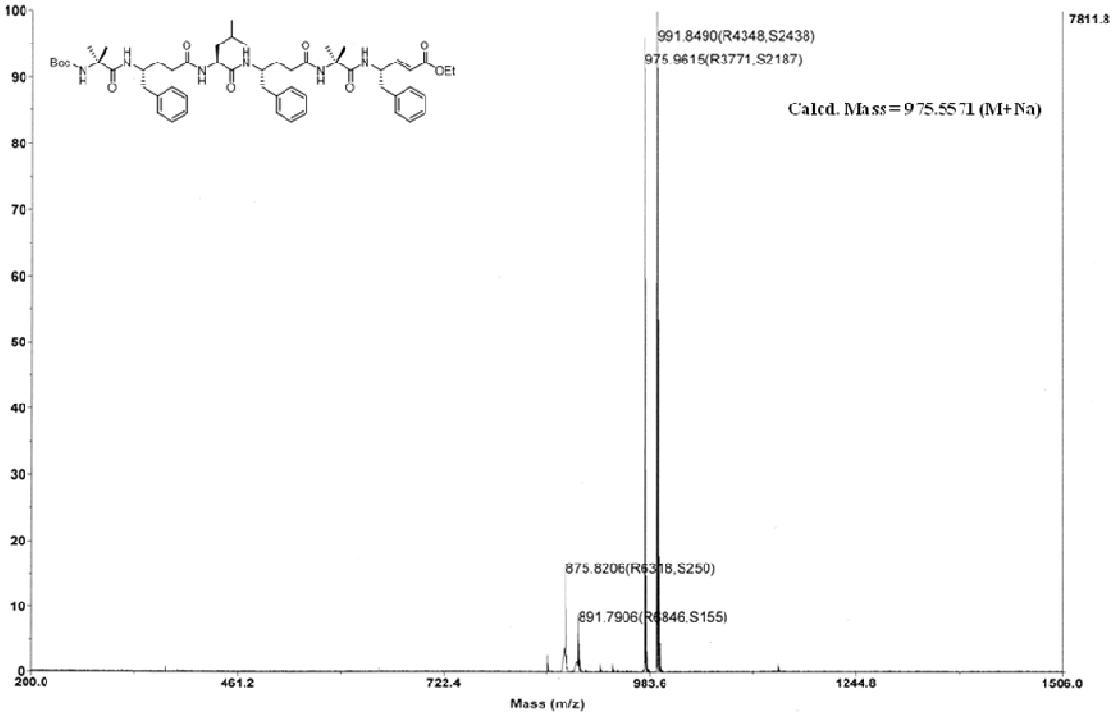
Mass spectrum of **Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-OEt (P1)** acquired in a MALDI TOF/TOF spectrometer



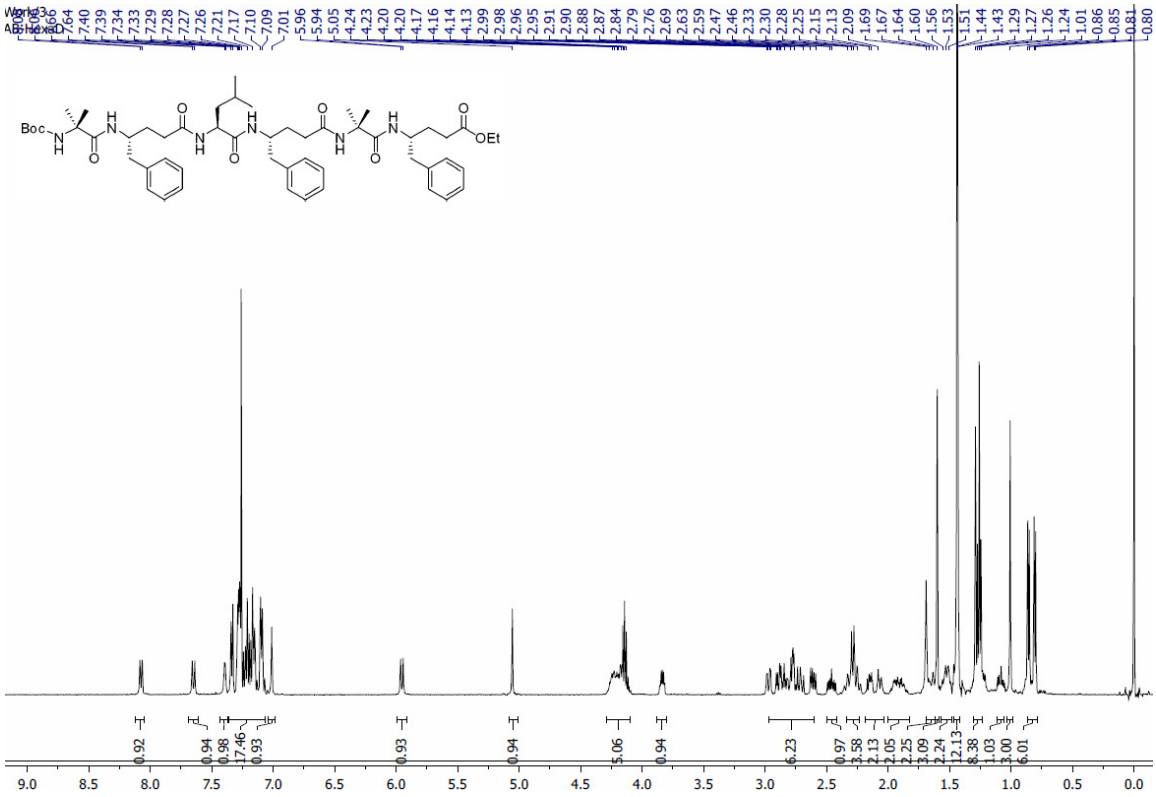
^{1}H NMR spectrum of **Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-Aib-dgF-OEt (P2)** acquired in a 500 MHz spectrometer ($CDCl_3$)



Mass spectrum of **Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-dgF-OEt (P2)** acquired in a MALDI TOF/TOF spectrometer



^{1}H NMR spectrum of **Boc-Aib-γ^{4}Phe-Leu-γ^{4}Phe-Aib-γ^{4}Phe-OEt (P3)** acquired in a 500 MHz spectrometer ($CDCl_3$)



Mass spectrum of **Boc-Aib-γ⁴Phe-Leu-γ⁴Phe-Aib-γ⁴Phe-OEt (P3)** acquired in a MALDI TOF/TOF spectrometer