Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Protein secondary structure mimetics: Crystal conformations of α/γ^4-hybrid peptide12-helices with proteinogenic side chains and their analogy with α-and β-peptide helices

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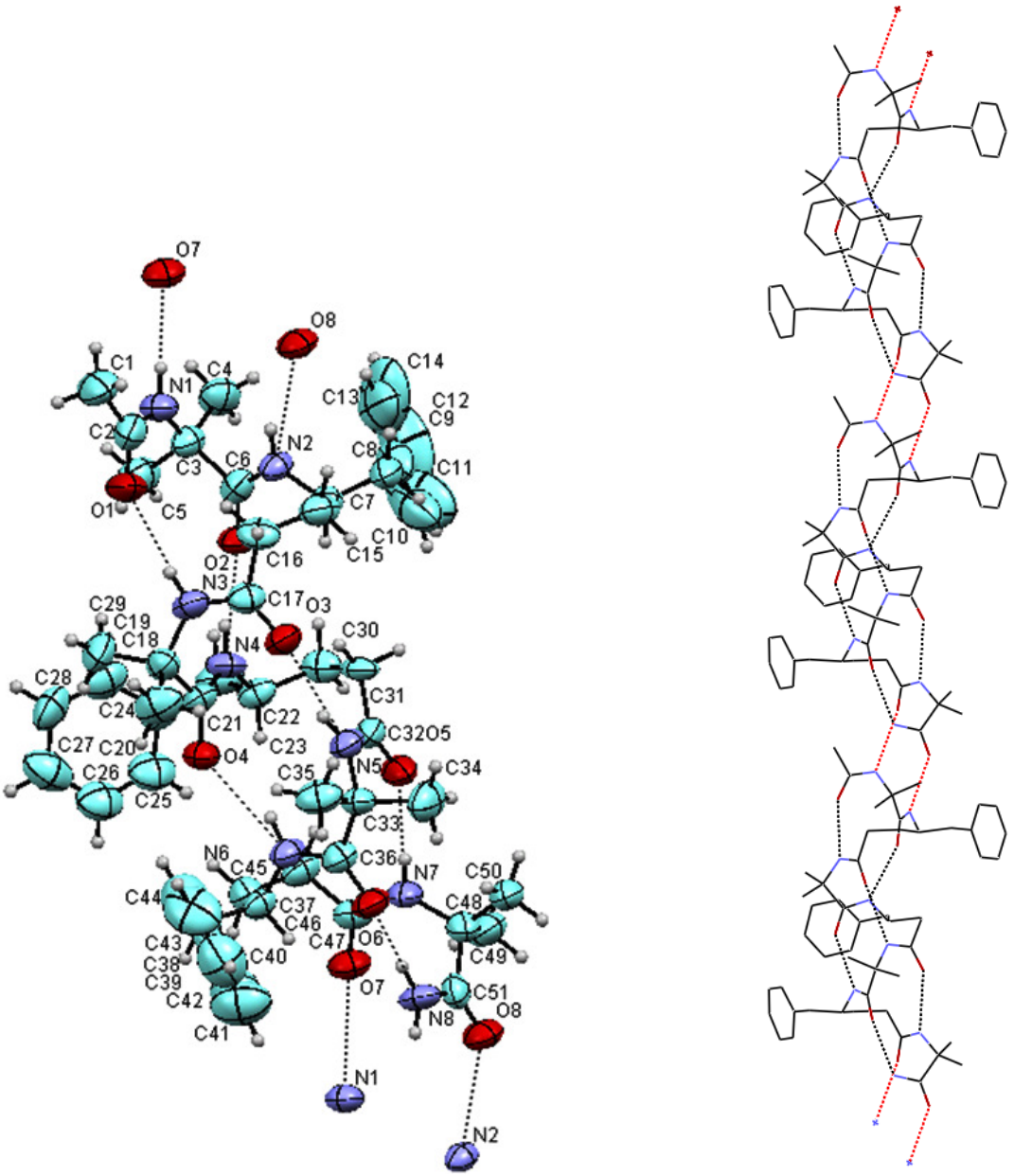
I. Crystal structure of α/γ^4 heptapeptides



A **B**

Figure S1. (A) The ORTEP diagram of α/γ^4 heptapeptide **P1**. Ellipsoids are drawn to the 40 % probability. H-atoms are not labelled for clarity. Six intramolecular and four intermolecular hydrogen bonds are represented in dotted lines. (B) Packing of molecules in crystals of peptide **P1** as viewed down the crystallographic a-axis depicting continuous head to tail intermolecular hydrogen bonding (shown in dotted red lines)

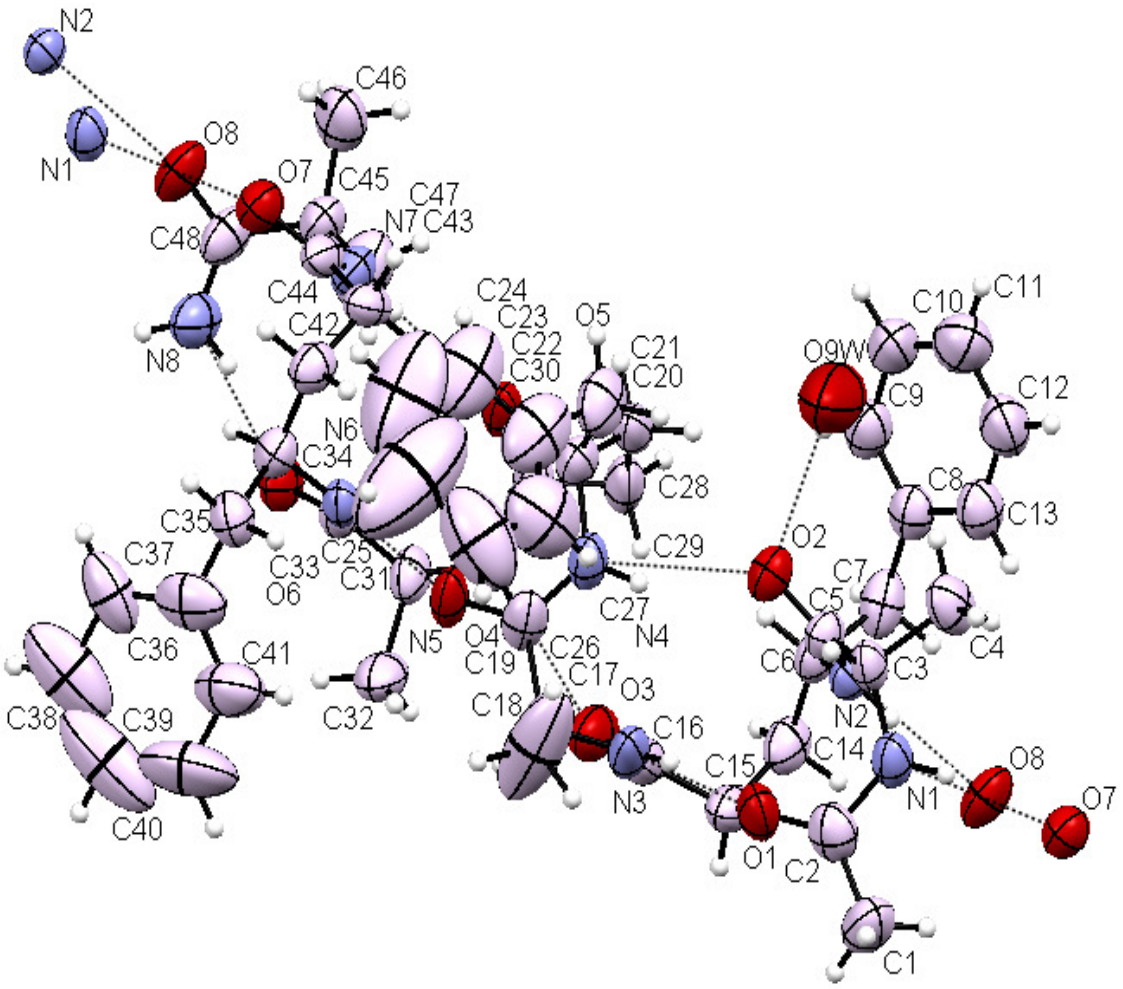


Figure S2. The ORTEP diagram of α/γ^4 heptapeptide **P2** as viewed down along the crystallographic b-axis. Ellipsoids are drawn to the 40 % probability. H-atoms are not labelled for clarity. Six intramolecular and four intermolecular hydrogen bonds are represented in dotted lines. H-bond between O2 and water molecule O9W is also shown.

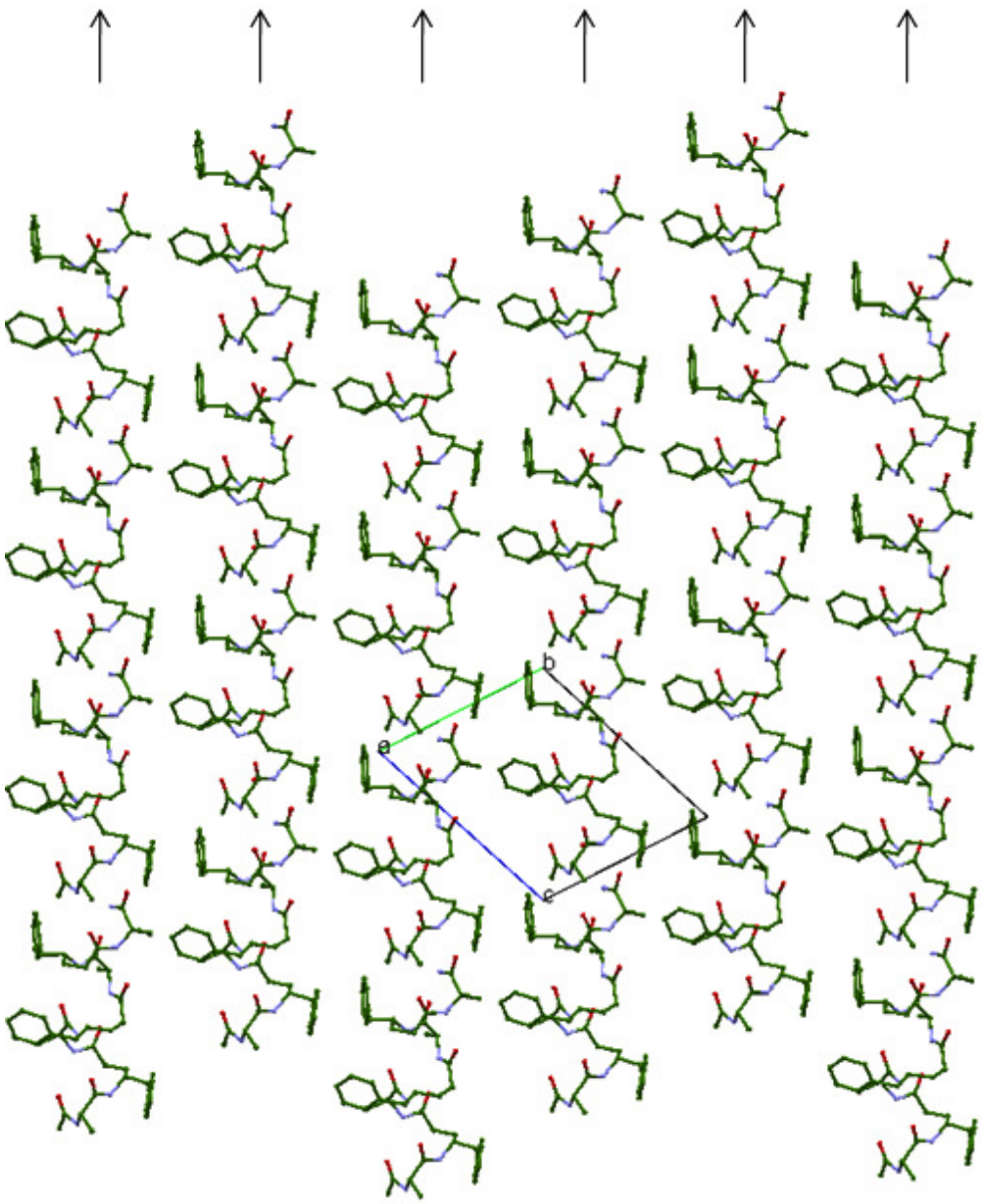


Figure S3. Packing of molecules in single crystals of peptide **P2** as viewed down the crystallographic a-axis. Arrows indicates the direction of helix in lateral packing along crystallographic c-axis which suggests parallel association of **P2**.

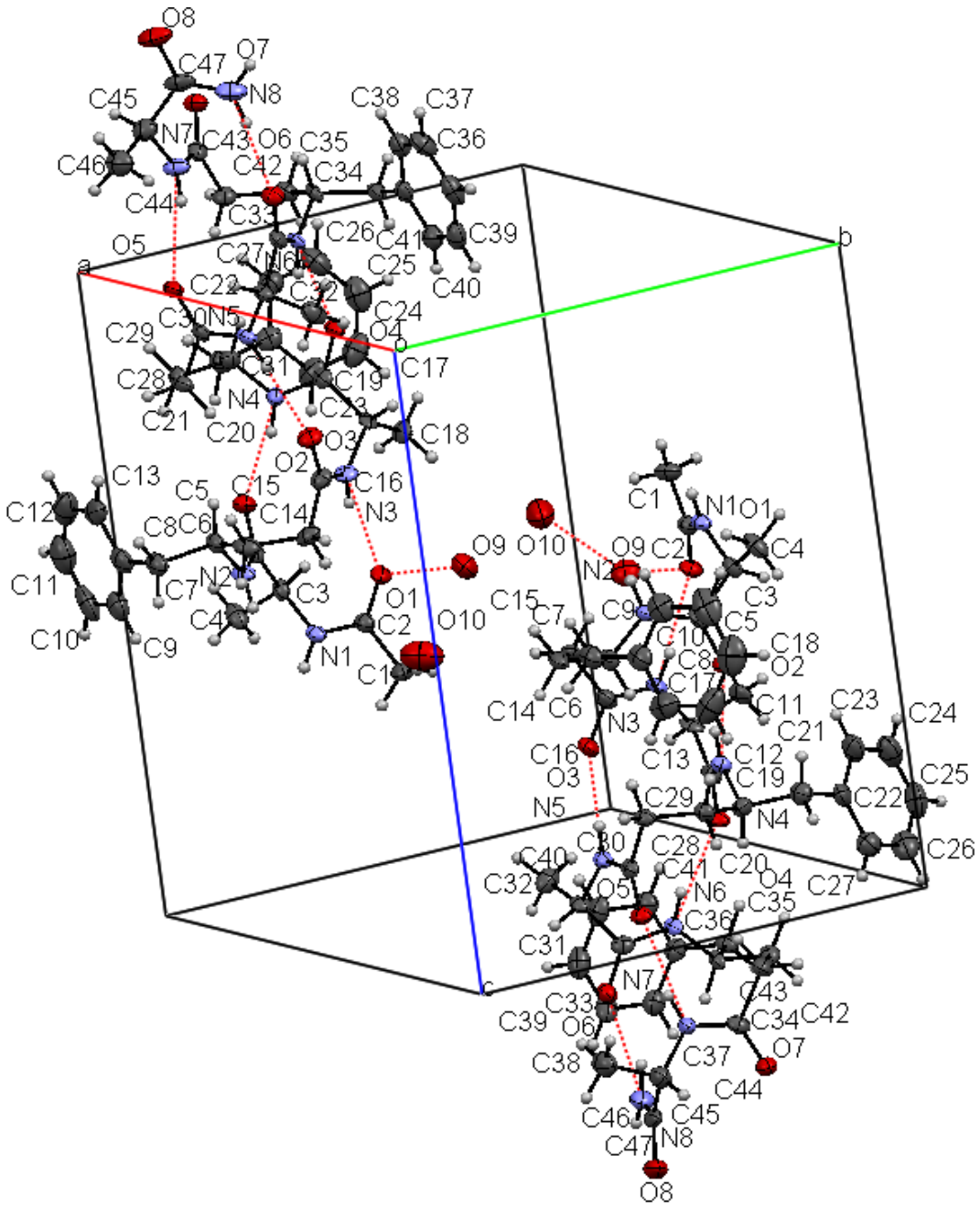


Figure S4. The ORTEP diagram representing the packing of peptide **P3** in single crystals. Hydrogens are not labelled for clarity. The two water molecules observed in unit cell are shown as O9 and O10. H-bonds are shown in dotted red lines.

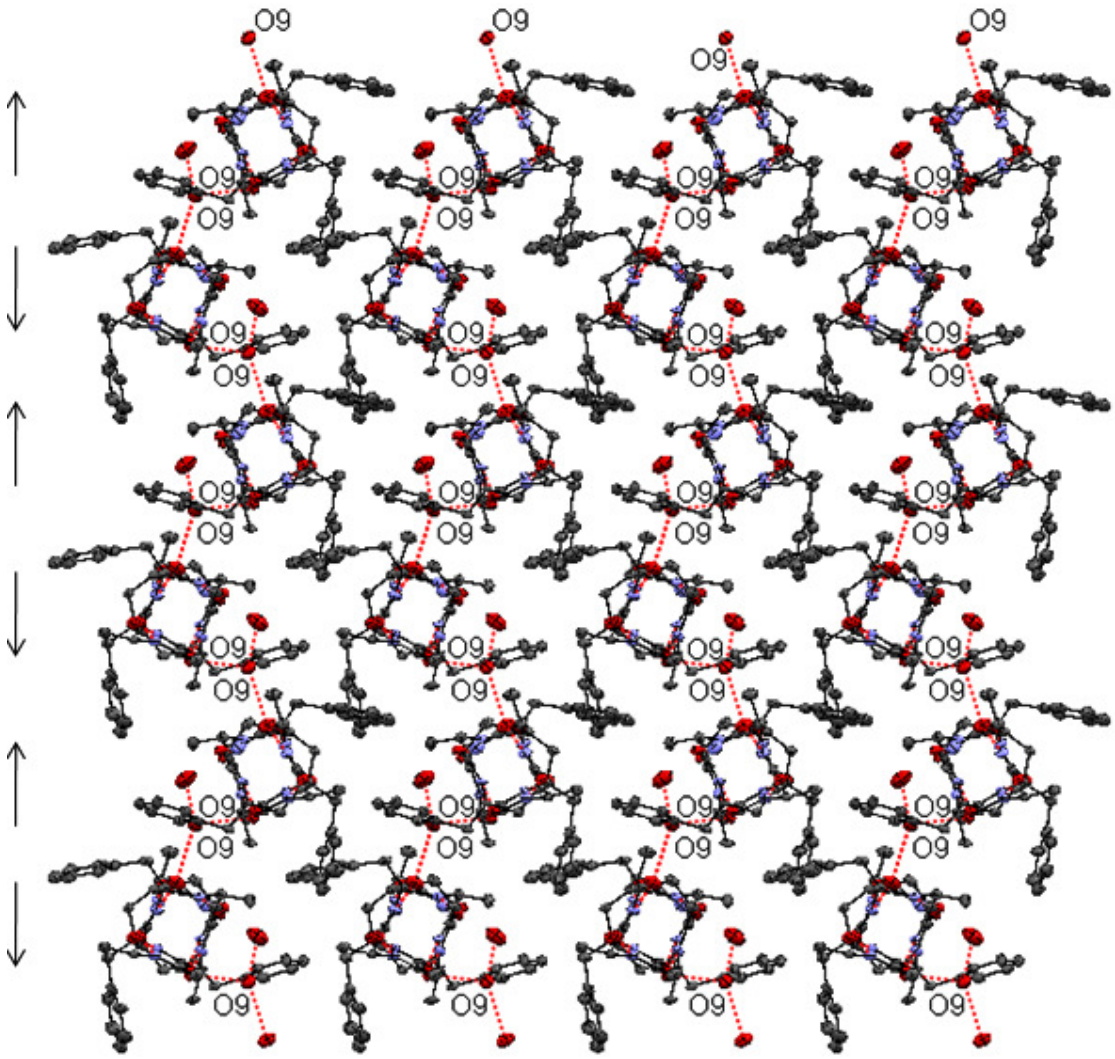


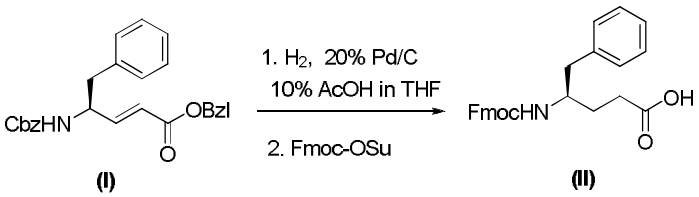
Figure S5. A view of crystal packing of peptide **P3** along the c-axis. The water molecule O9 participated in molecular packing through H-bonding (dotted red lines) is also shown. Arrows indicates the direction of helix in corresponding row suggesting the antiparallel packing of helix.

II. Materials and instrumentation

Phenylalanine,*N*-hydroxysuccinimide, $NaBH_4$, oxone, DCC, HOBt, benzyl bromoacetate, PPh_3, Cbz-Cl, HBTU, HOBt, triisopropyl silane, Fmoc-OSu, Pd/C (10%), were purchased from Sigma-Aldrich. Solvents THF, EtOAc, AcOH were obtained from Merck. The reagents 2-iodobenzoic acid and piperidine was purchased from Spectrochem. Knorr amide MBHA resin was purchased from Novabiochem. Acetic anhydride was obtained from Fisher Scientific. THF was distilled over sodium prior to use. Column chromatography was performed on Merck silica gel (120-200 mesh). Reactions were monitored by analytical thin layer chromatography using aluminium-backed plates coated with Merck Kieselgel 60 F_{254}; Visualization was accomplished with UV light and $KMnO_4$ or ninhydrin stain. Yields refer to chromatographically pure compounds unless otherwise stated. Proton nuclear magnetic resonances (^{1}H NMR) were recorded in deuterated solvent on JEOL 400 MHz (for ^{13}C, 100 MHz) and Brucker 500 MHz (for ^{13}C, 125 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00) or residual protio solvent ($CHCl_3$, δ 7.27 for ^{1}H and δ 77.0 for ^{13}C) unless otherwise mentioned. ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), broad doublet (bd), triplet (t), or doublet of doublets (dd), quintet (qn). Coupling constants (*J*) are reported in Hertz (Hz). The Matrix Assisted LASER Desorption Ionization mass spectrometer (MALDI-TOF/TOF, Applied Biosciences) was used to obtain accurate mass. Circular dichroism was recorded on JASCO (J-815) spectropolarimeter, at ambient temperature. Data for X-ray structure determination were obtained from Bruker APEX II DUO diffractometer using Mo-Kα (λ= 0.71073 Å) graphite monochromated radiation. Helical parameters were obtained using HELANAL *plus* software

III. General procedures

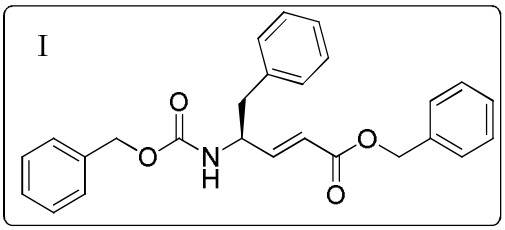
a) Synthesis of γ^4 -Phe



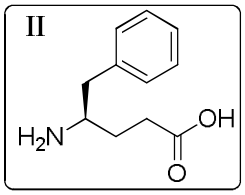
Scheme S1: Synthesis of Fmoc γ^4-Phe

The suspension of activated Pd/C (20 % by weight) and benzyl esters of N-Cbz-protected vinylogous phelylalanine (1.66 g, 4 mmol) which was synthesized using reported method,[25] in 10% acetic acid in THF (20 mL) was stirred overnight at room temperature in the presence of hydrogen. After completion of the reaction, Pd/C was filtered through the bed of celite and the filtrate was evaporated to dryness under *vacuum* to get gummy free γ^4-phenylalanine (γ^4-Phe). The pure γ^4-Phe was isolated as white powder after trituration with cold diethyl ether in excellent yield (0.687 g, 90%).

Further, to the solution of free γ^4-Phe (0.579 g, 3 mmol) in 20% Na_2CO_3 (15 mL) was added Fmoc-OSu (1.11 g, 3.3 mmol, dissolved in 10 mL of THF) and the reaction mixture was stirred overnight. After completion of the reaction, the reaction mixture was acidified with 10% HCl and the precipitated Fmoc protected γ^4-Phe was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with 10% HCl (3 × 15 mL), brine solution (2 x 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give gummy Fmoc-γ^4-Phe. The gummy Fmoc-γ^4-Phe was precipitated using ethyl acetate/pet ether (60-80 °C) to give 1.06 g (85%) as white powder and used directly in the solid phase peptide synthesis.

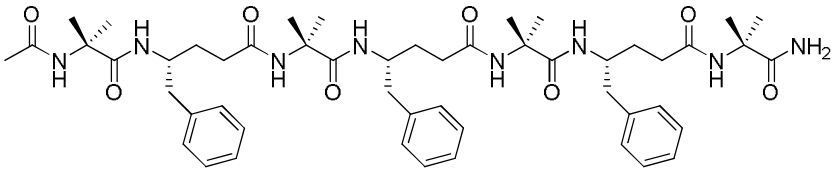


(*S, E*)-benzyl 4-(benzyloxycarbonylamino)-5-phenylpent-2-enoate (I): white solid, mp 57.5 °C; **[α]** $_D^{25}$ = - 5.1(c = 1, MeOH); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 (m, 10H), 6.96 (dd, *J* = 4 Hz, *J* = 16 Hz, 1H), 5.91 (d, *J* = 16 Hz, 1H), 5.15 (s, 2H), 5.04 (s, 2H), 4.66 (m, 1H), 2.87 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 166.09, 155.80, 148.05, 136.03, 129.57, 128.45, 128.30, 127.22, 121.21, 67.15, 66.58, 60.65, 40.78; **MALDI TOF/TOF**- *m/z* calcd. for $C_{26}H_{25}NO_4$ $[M+Na]^+$ 438.1681, obsrvd. 438.1632.



(*R*)-4-amino-5-phenylpentanoic acid (II): white solid (0.687 g, 90 %), mp 135 °C; **[α]** $_D^{25}$ = - 11.2 (c = 1, H_2O); **^{1}H NMR** (400 MHz, D_2O) δ 7.33-7.24 (m, 5H), 3.47 (qn, *J* = 4 Hz, 1H), 3.00-2.82 (m, 2H), 2.30 (t, *J* = 8 Hz, 2H), 1.86 (dd, *J* = 8 Hz, *J* = 12 Hz, 2H) ; **^{13}C NMR** (100 MHz, D_2O) δ 181.04, 135.76, 129.43, 129.12, 127.51, 115.01, 53.12, 38.12, 33.28, 28.50; **MALDI TOF/TOF**- *m/z* calcd. for $C_{11}H_{15}NO_2$ $[M+Na]^+$ 216.1000, obsrvd. 216.1060.

b) Solid phase synthesis of peptide Ac-(Aib-γ^4Phe)$_3$-Aib-NH$_2$ (P1)

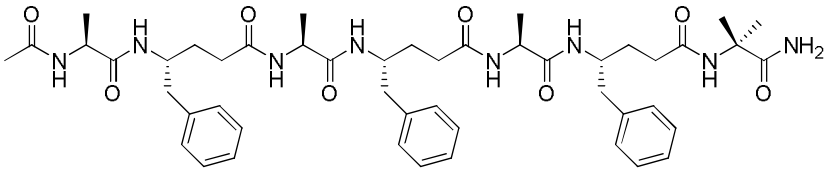


Peptide **P1** was synthesized by manual solid phase peptide synthesis on Knorr Amide MBHA resin (0.3 mmol) using Fmoc-chemistry. Coupling reactions were performed using HBTU/HOBt activation protocol. All Fmoc deprotections were performed using 20 % piperidine in DMF. The *N*-terminal of peptide was capped with acetyl group using acetic anhydride and pyridine. The final peptide was cleaved from resin by using cocktail mixture of 95 % trifluoroacetic acid, 2.5 % water and 2.5 % triisopropyl silane. The peptide was purified using reverse-phase-HPLC (detector: 254 nm and 220 nm) on C18 column with methanol/water gradients at a flow rate of 1.5 mL/min.

^{1}H NMR (500 MHz, DMSO d_6) δ 8.29 (s, 1H), 8.08 (m, 2H), 7.97 (s, 1H), 7.72 (m, 3H), 7.40 (s, 1H), 7.07 (m, 15H, aromatic), 6.64 (s, 1H), 3.88 (m, 3H), 2.63 (m, 6H), 2.24 (m, 4H), 1.92 (m, 4H), 1.85 (s, 3H), 1.52 (m, 4H), 1.27-1.16(m, 18H, -CH_3 of Aib), 0.88 (s, 3H, -CH_3 of Aib), 0.81 (s, 3H, -CH_3 of Aib); **MALDI TOF/TOF**- *m/z* calcd. for $C_{51}H_{72}N_8O_8$ $[M+Na]^+$ 947.5371, obsrvd. 947.5378.

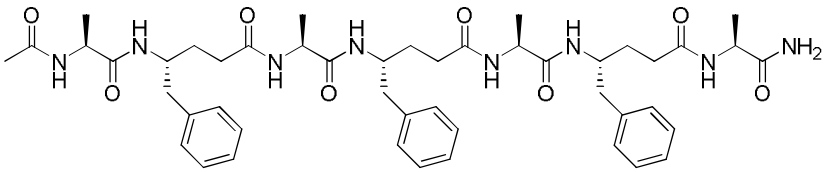
Similar protocol was used for the synthesis of peptides **P2** and **P3**

c) Solid phase synthesis of peptide Ac-(Ala- γ^4Phe)$_3$-Aib-NH$_2$ (P2)



Similar protocol as described in case of P1 was used for the synthesis of peptide **P2**. The peptide was synthesized on Knorr Amide MBHA resin (0.3 mmol) using standard Fmoc-chemistry. Coupling reactions were performed using HBTU/HOBt activation protocols. The final peptide was cleaved from resin using the cocktail mixture of 95 % trifluoroacetic acid, 2.5 % water and 2.5 % triisopropyl silane and purified using reverse-phase-HPLC on a C18 column. **^{1}H NMR** (500 MHz, DMSO d_6) δ 7.93 (d, J = 7 Hz, 1H), 7.87 (d, J = 6.5 Hz, 1H), 7.77 (d, J = 6 Hz, 1H), 7.65 (m, 3H), 7.44 (s, 1H), 7.10 (m, 15H, aromatic), 6.63 (s, 1H), 4.10 (m, 1H), 3.97 (m, 1H), 3.89 (m, 1H), 3.89 (m, 3H), 2.61 (m, 6H), 2.04 (m, 4H), 1.78 (s, 3H), 1.69 (m, 4H), 1.45 (m, 4H), 1.21 (s, 3H, -CH_3 of Aib), 1.19 (s, 3H, -CH_3 of Aib), 1.09 (d, J = 7 Hz, 3H), 1.03 (d, J = 7 Hz, 3H), 0.98 (d, J = 7 Hz, 3H); **MALDI TOF/TOF**- *m/z* calcd. for $C_{48}H_{66}N_8O_8$ $[M+Na]^+$ 905.4901, obsrvd. 905.4950

d) Solid phase synthesis of peptide Ac-(Ala- γ^4Phe)$_3$-Ala-NH$_2$ (P3)



Similar protocol as described in case of P1 was used for the synthesis of peptide **P3**. The peptide was synthesized on Knorr Amide MBHA resin (0.3 mmol) using standard Fmoc-chemistry. Coupling reactions were performed using HBTU/HOBt activation protocols. The final peptide was cleaved from resin using the cocktail mixture of 95 % trifluoroacetic acid, 2.5 % water and 2.5 % triisopropyl silane and purified using reverse-phase-HPLC on a C18 column. **^{1}H NMR** (500 MHz, Methanol d_3) δ 8.21 (m, 4H), 7.97 (d, J = 6.5 Hz, 1H), 7.91 (s, 1H), 7.54 (d, J = 9.5 Hz, 1H), 7.20 (m, 15H, aromatic), 6.92 (s, 1H), 4.21 (m, 1H), 4.04 (m, 5H), 3.69 (m, 1H), 2.73 (m, 6H), 2.25 (m, 9H), 2.02 (s, 3H), 1.50 (m, 3H),1.37 (d, J = 7.5 Hz, 3H), 1.27 (d, J = 7 Hz, 3H), 1.12 (d, J = 7.5 Hz, 3H), 0.98 (d, J = 7 Hz, 3H); **MALDI TOF/TOF**- *m/z* calcd. for $C_{47}H_{64}N_8O_8$ $[M+Na]^+$ 891.4750, obsrvd. 891.5158.

IV. HPLC traces for α/γ^4 heptapeptides

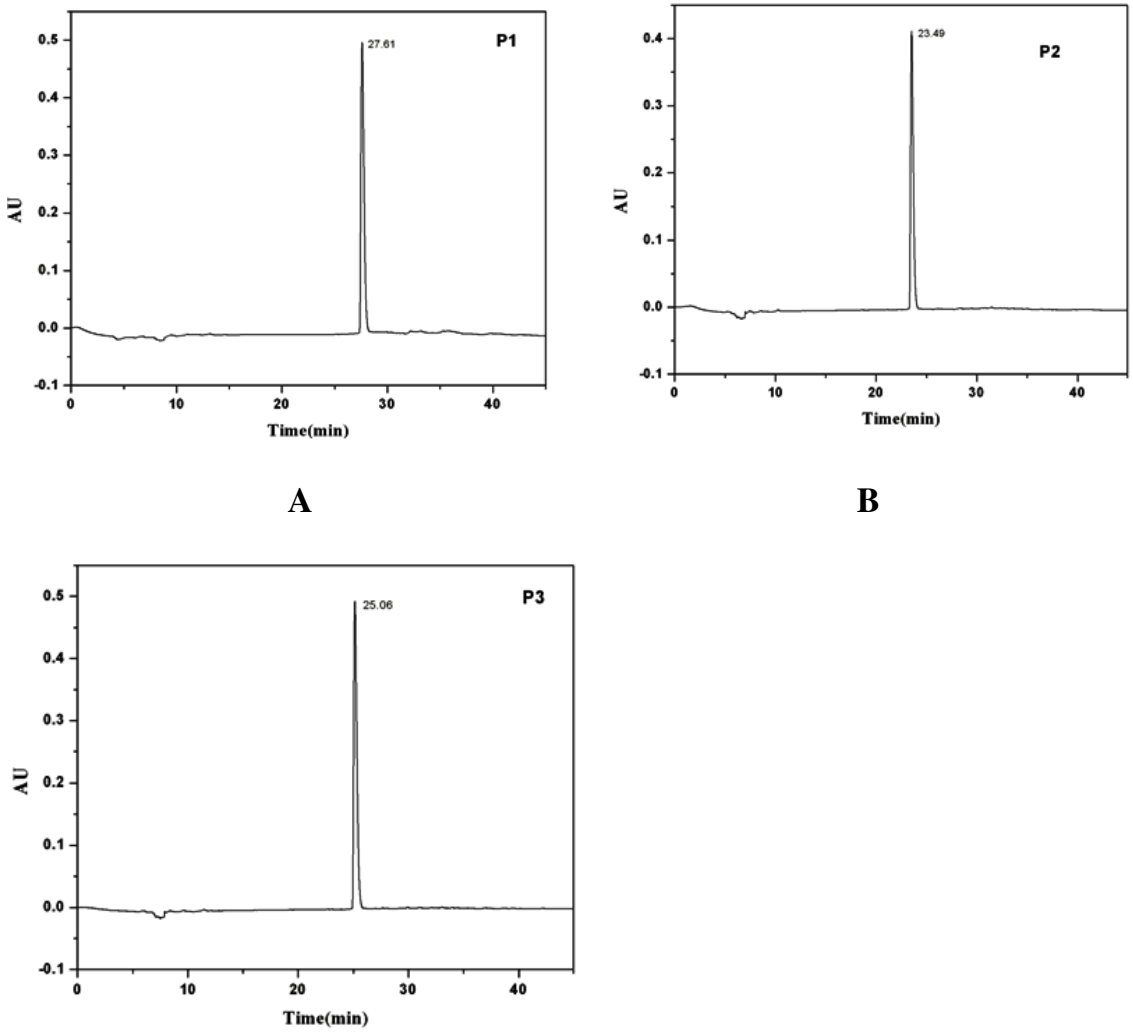


Figure S10. Reverse-phase-HPLC profiles of peptides **P1** (A), **P2** (B) and **P3** (C) on C18 column C18 column using methanol/water gradients at a flow rate of 1.5 mL/min. Eluted compounds were detected by the UV absorbance at 220 nm.

V. CD analysis for α/γ^4 heptapeptides

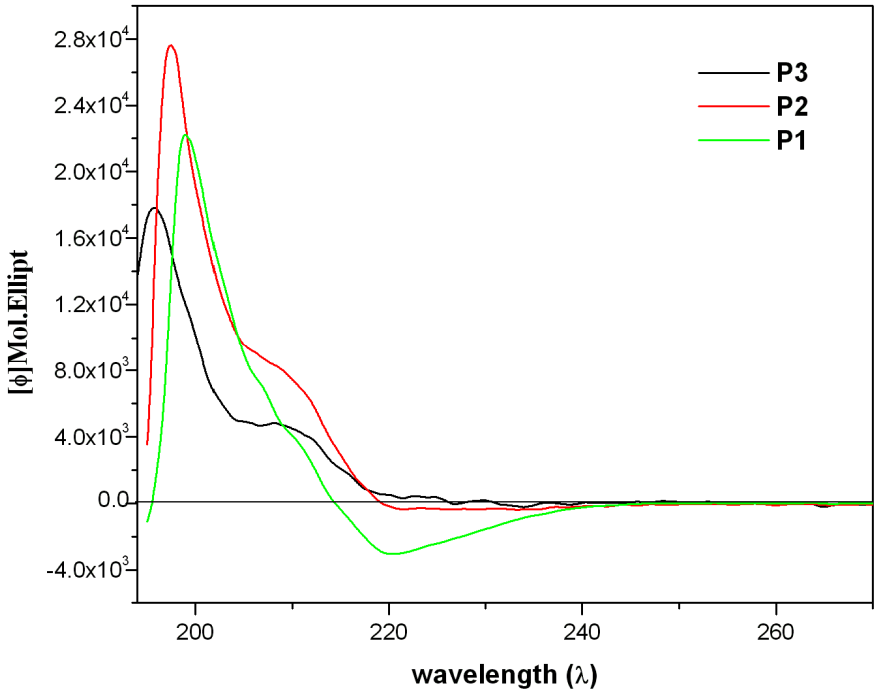


Figure S11. Circular dichroism spectra of α/γ^4-heptapeptides **P1**, **P2** and **P3** (0.2 mM) in Methanol at 20 ^{o}C.

VI. Crystal structure information

Crystallization and X-ray analysis

X-ray quality crystals were grown from various solvent mixtures by slow evaporation as shown in the Table 3.

Table 1.Crystallization conditions

α/γ^4-peptide	crystallization condition (solvent mixture)
P1	Solvent Evaporation ($MeOH/H_2O/CF_3CH_2OH$)
P2	Solvent Evaporation ($MeOH/H_2O/CF_3CH_2OH$)
P3	Solvent Evaporation ($MeOH/H_2O$)

General procedure for crystallization of peptides

All crystallization attempts were conducted at room temperature. All α/γ^4-peptides were purified carefully by HPLC before keeping for crystallization. Glass sample vials (2 mL) were washed with acetone and dried under a nitrogen gas stream before use. PARAFILM "M" was used to close the vials. HPLC-grade solvents were used for crystallization.

Slow evaporation of a methanol/water/2,2,2-Trifluoroethanol mixture (P1)

α/γ^4-Peptide **P1** (10 mg) was dissolved in methanol (1 mL). The solution was transferred through a syringe filter into a glass vial. Few drops (~100 µL) of water were added to the peptide solution in methanol. To this turbid solution, 2,2,2-trifluoroethanol (TFE, 50 µL) was added to get clear solution. The vial was closed with a PARAFILM and then pricked gently with a clean and sharp needle to introduce 3 to 4 pores on PARAFILM to let the solvent mixture evaporate slowly. High-quality crystals were obtained after two days.

Slow evaporation of a methanol/water/2,2,2-Trifluoroethanol mixture (P2)

α/γ^4-Peptide **P2** (10 mg) was dissolved in methanol (1 mL). The solution was transferred through a syringe filter into a glass vial. Few drops of water ((~100 µL) were added the peptide solution in methanol. To this turbid solution, 2,2,2-trifluoroethanol (TFE, 50 µL) was added to get a clear solution. The vial was closed with a PARAFILM and then pricked gently with clean and sharp needle to introduce 3 to 4 pores on PARAFILM to let the solvent mixture evaporate slowly. High-quality crystals were obtained after two days.

Slow evaporation of a methanol/water mixture (P3)

α/γ^4-Peptide **P3** (10 mg) was dissolved in methanol (1 mL). The solution was transferred through a syringe filter into a glass vial. Few drops of water were added until solution starts becoming turbid (~200 µL). Then again few drops of methanol were added to get clear solution. The vial was closed with a PARAFILM and then pricked gently with clean and sharp needle to introduce 2 to 3 pores on PARAFILM to let the solvent mixture evaporate slowly. High-quality crystals were obtained after five days.

Crystal Structure Report of 12-helical Peptides

i) Ac-Aib- γ^4Phe- Aib- γ^4Phe- Aib- γ^4Phe-Aib-NH_2 (P1)

Data Collection

A colorless crystal with approximate dimensions 0.4 x 0.2 x 0.1 mm^3 was selected under oil under ambient conditions and attached on nylon CryoLoops with Paraton-N

(Hampton Research). The crystal was mounted in a stream of cold nitrogen at 100(2) K and centered in the X-ray beam by using a video camera.

The crystal evaluation and data collection were performed on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) with Mo Kα (λ = 0.71073 Å) radiation and the diffractometer to crystal distance of 6.0 cm. The initial cell constants were obtained from three series of ω scans at different starting angles. Each series consisted of 12 frames collected ω with the exposure time of 10 seconds per frame. Obtained reflections were successfully indexed by an automated indexing routine built in the SMART program. The final cell constants were calculated from a set of 26870 strong reflections from the actual data collection.

The data were collected to a resolution of 0.75 Å, with an exposure time 10 sec per frame. The data integration and reduction were processed with SAINT[1] software. A multi-scan absorption correction was applied to the collected reflections.

Structure Solution and Refinement

The systematic absences in the diffraction data were uniquely consistent for the space group *P*21 that yielded chemically reasonable and computationally stable results of refinement.[2]

A successful solution by the direct methods provided most non-hydrogen atoms from the *E*-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighbouring atoms with relative isotropic displacement coefficients. Each foldamer participated in six intramolecular N-H···O hydrogen bonds and four intermolecular N-H···O hydrogen bonds.

There were partially occupied molecules of some solvent 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[3] 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 604.4 $Å^3$, or 20.2 % of the unit cell volume. The program calculated 200 electrons in the unit cell for the diffuse species. No data are given for the diffusely scattering species.

The final least-squares refinement of 596 parameters against 13302 data resulted in residuals *R* (based on F^2 for $I \geq 2\sigma$) and *wR* (based on F^2 for all data) of 0.0813 and 0.1855, respectively.

Table 2. Crystal data and structure refinement for α/γ^4-peptide P1

Compound Identity	**P1**	
Empirical formula	$C_{51}\ H_{72}\ N_8\ O_8$	
Formula weight	925.17	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P21	
Unit cell dimensions	a = 12.866(15)Å	α = 90°.
	b = 17.403(19)Å	β = 107.22(3) °.
	c = 13.962(16)Å	γ= 90°.
Volume	2986(6) $Å^3$	
Z	2	
Density (calculated)	1.029 Mg/m^3	
Absorption coefficient (μ)	0.07 mm^{-1}	
F (000)	996	
Crystal size	0.40 x 0.20 x 0.12 mm^3	
Theta range for data collection	1.53 to 28.25°	
Index ranges	-17<=h<=11, -20<=k<=23, -18<=l<=16	
Reflections collected	26870	
Independent reflections	13302 [R(int) = 0.1294]	
Completeness to theta = 28.25°	98.6 %	
Absorption correction	Empirical with SADABS	
Max. and min. Transmission	0.992 and 0.983	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	13302 / 1 / 596	
Goodness-of-fit on F^2	0.733	
Final R indices [I>2sigma(I)]	R1 = 0.0813, wR2 = 0.1855	
R indices (all data)	R1 = 0.2345, wR2 = 0.2369	

Absolute structure parameter	1(2)
Largest diff. peak and hole	0.213 and -0.239 e.Å^{-3}

ii) Ac-Ala- γ^APhe- Ala- γ^APhe- Ala- γ^APhe-Aib-NH$_2$ (P2)

Data Collection

A colorless crystal with approximate dimensions 0.4 x 0.3 x 0.2 mm^3 was selected under oil under ambient conditions and attached on nylon CryoLoops with Paraton-N (Hampton Research). The crystal was mounted in a stream of cold nitrogen at 100(2) K and centered in the X-ray beam by using a video camera.

The crystal evaluation and data collection were performed on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) with Mo Kα (λ = 0.71073 Å) radiation and the diffractometer to crystal distance of 6.0 cm. The initial cell constants were obtained from three series of ω scans at different starting angles. Each series consisted of 12 frames collected ω with the exposure time of 10 seconds per frame. Obtained reflections were successfully indexed by an automated indexing routine built in the SMART program. The final cell constants were calculated from a set of 24197 strong reflections from the actual data collection.

The data were to a resolution of 0.75 Å, with an exposure time 10 sec per frame. The data integration and reduction were processed with SAINT[1]software. A multi-scan absorption correction was applied to the collected reflections.

Structure Solution and Refinement

The systematic absences in the diffraction data were uniquely consistent for the space group *P*1 that yielded chemically reasonable and computationally stable results of refinement.[2]

A successful solution by the direct methods provided most non-hydrogen atoms from the *E*-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms except the C25 and C26 in phenyl ring were refined with anisotropic displacement coefficients. The C25 and C26 were disordered over two positions and refined isotropically and in idealized geometries. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighbouring atoms with relative isotropic displacement coefficients. Each foldamer participated in six intramolecular N-H···O hydrogen bonds and four intermolecular N-H···O hydrogen bonds.

There were partially occupied molecules of some solvent 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[3] 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 242.4 Å^3, or 17.2 % of the unit cell volume. The program calculated 59 electrons in the unit cell for the diffuse species. No data are given for the diffusely scattering species.

The final least-squares refinement of 569 parameters against 10395 data resulted in residuals *R* (based on F^2 for $I \geq 2\sigma$) and *wR* (based on F^2 for all data) of 0.0823 and 0.2342, respectively.

Table 3. Crystal data and structure refinement for *α*/ *γ*^{4}-peptide P2

Compound Identity	**P2**	
Empirical formula	C_{48} H_{66} N_8 O_8,(O)	
Formula weight	899.09	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P1	
Unit cell dimensions	a = 9.354(10)Å	α = 68.89(3)°.
	b = 12.179(13)Å	β = 85.74(3°) .
	c = 13.950(15)Å	γ= 71.36(3)°.
Volume	1403(3) Å^3	
Z	1	
Density (calculated)	1.064Mg/m^3	
Absorption coefficient (μ)	0.074 mm^{-1}	
F (000)	482	
Crystal size	0.40 x 0.30 x 0.20 mm^3	
Theta range for data collection	1.57 to 28.28°	
Index ranges	-12<=h<=12, -16<=k<=15, -18<=l<=17	
Reflections collected	24197	

Independent reflections	10395 [R(int) = 0.0501]
Completeness to theta = 28.28°	99.1 %
Absorption correction	Empirical with SADABS
Max. and min. Transmission	0.985 and 0.974
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	110395 / 327 / 569
Goodness-of-fit on F^2	0.771
Final R indices [I>2sigma(I)]	R1 = 0.0833, wR2 = 0.2342
R indices (all data)	R1 = 0.1364, wR2 = 0.2723
Absolute structure parameter	0.6(18)
Largest diff. peak and hole	0.056 and -0.257 e.$Å^{-3}$

iii) Ac-Ala- γ^4Phe- Ala- γ^4Phe- Ala- γ^4Phe-Ala-NH_2 (P3)

Data Collection

A colorless crystal with approximate dimensions 0.4 x 0.29 x 0.16 mm^3 was selected under oil under ambient conditions and attached on nylon CryoLoops with Paraton-N (Hampton Research). The crystal was mounted in a stream of cold nitrogen at 100(2) K and centered in the X-ray beam by using a video camera.

The crystal evaluation and data collection were performed on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) with Mo Kα (λ = 0.71073 Å) radiation and the diffractometer to crystal distance of 6.0 cm.

The initial cell constants were obtained from three series of ω scans at different starting angles. Each series consisted of 12 frames collected ω with the exposure time of 10 seconds per frame. Obtained reflections were successfully indexed by an automated indexing routine built in the SMART program. The final cell constants were calculated from a set of 16669 strong reflections from the actual data collection.

The data were collected to a resolution of 0.75 Å, with an exposure time 10 sec per frame. The data integration and reduction were processed with SAINT [1]software. A multi-scan absorption correction was applied to the collected reflections.

Structure Solution and Refinement

The systematic absences in the diffraction data were uniquely consistent for the space group *P*21 that yielded chemically reasonable and computationally stable results of refinement.[2]

A successful solution by the direct methods provided most non-hydrogen atoms from the *E*-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighbouring atoms with relative isotropic displacement coefficients. Each foldamer participated in six intramolecular N-H····O hydrogen bonds, four intermolecular N-H····O hydrogen bonds and two C=O····H(H_2O) hydrogen bond with solvent water.

The final least-squares refinement of 591 parameters against 10827 data resulted in residuals *R* (based on F^2 for $I \geq 2\sigma$) and *wR* (based on F^2 for all data) of 0.0824 and 0.1713, respectively.

Table 4. Crystal data and structure refinement for α/γ^4-peptide P3

Compound Identity	**P3**	
Empirical formula	C_{47} H_{64} N_8 O_8, 2(O)	
Formula weight	901.06	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P21	
Unit cell dimensions	a = 11.218(17)Å	α = 90°.
	b = 14.58(2)Å	β = 100.06(3)°.
	c = 15.75(2)Å	γ= 90°.
Volume	2536(7)Å^3	
Z	2	
Density (calculated)	1.140Mg/m^3	
Absorption coefficient (μ)	0.079mm^{-1}	
F (000)	924	
Crystal size	0.40 x 0.29 x 0.16 mm^3	
Theta range for data collection	1.84 to 28.28°	
Index ranges	-14<=h<=14, -16<=k<=19, -20<=l<=20	
Reflections collected	16669	
Independent reflections	10827 [R(int) = 0.0952]	

Completeness to theta = 28.28°	98.1 %
Absorption correction	Empirical with SADABS
Max. and min. Transmission	0.987 and 0.973
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	10827 / 1 / 591
Goodness-of-fit on F^2	0.909
Final R indices [I>2sigma(I)]	R1 = 0.0824, wR2 = 0.1713
R indices (all data)	R1 = 0.2023, wR2 = 0.2352
Absolute structure parameter	-2.1(19)
Largest diff. peak and hole	0.428 and -0.366 e.$Å^{-3}$

Crystal Structure Report of γ^4Phe

Table 5. Crystal data and structure refinement for γ^4-Phe

Compound Identity	amino acid	
Empirical formula	C_{11} H_{15} N_1 O_2	
Formula weight	193.24	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P21	
Unit cell dimensions	a = 7.757(5)Å	α = 90°.
	b = 6.483(4)Å	β = 92.808(12) (3)°.
	c = 9.907(7)Å	γ = 90°.
Volume	497.6(6)$Å^3$	
Z	2	
Density (calculated)	1.290Mg/m^3	
Absorption coefficient (μ)	0.088mm^{-1}	
F (000)	208	
Crystal size	0.50 x 0.40 x 0.20 mm^3	
Theta range for data collection	2.06 to 27.44°	
Index ranges	-10<=h<=10, -8<=k<=7, -12<=l<=12	

Reflections collected	8120
Independent reflections	2169 [R(int) = 0.0978]
Completeness to theta = 27.44°	99.4 %
Absorption correction	Empirical with SADABS
Max. and min. Transmission	0.983 and 0.959
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2169 / 1 / 128
Goodness-of-fit on F^2	0.947
Final R indices [I>2sigma(I)]	R1 = 0.0704, wR2 = 0.1626
R indices (all data)	R1 = 0.1153, wR2 = 0.1890
Absolute structure parameter	-2(3)
Largest diff. peak and hole	0.424 and -0.314 e.$Å^{-3}$

VII. Helical parameter analysis of Peptides

Table 6. Helical parameters calculated from sets of four consecutive *α*-carbons.

$\alpha/\ \gamma^4$ Peptide	res/turn *n*	rise/turn *p* (Å)	rise/res *d* (Å)	radius *r* (Å)
P1	2.7	5.3	2.0	**2.1**
	2.7	5.4	2.0	2.2
	2.7	5.3	2.0	2.1
	2.7	5.2	2.0	2.1
	2.7	5.3	2.0	2.2
P2	2.7	5.3	2.0	2.1
	2.7	5.4	2.0	2.1
	2.7	5.3	2.0	2.1
	2.6	5.2	2.0	2.1
	2.7	5.3	2.0	2.1
P3	2.7	5.2	2.0	2.1
	2.7	5.3	2.0	2.1
	2.7	5.2	2.0	2.1
	2.7	5.1	2.0	2.1
	2.7	5.2	2.0	2.1
average	**2.7**	**5.3**	**2.0**	**2.1**

VIII. Hydrogen bonding parameters and Torsion angles for peptides P1, P2 and P3

Table 7. Intra and intermolecular Hydrogen bonds of P1 [dist in Å and bond angle in °]. Intramolecular H-bonds are 1←4 backward type.

D-H---A	Dist. H---A(Å)	Dist D---A(Å)	∠D-H---A(°)
N(3)-H(3)...O(1)	2.13	2.99	174.2
N(4)-H(4)...O(2	1.97	2.79	158.4
N(5)-H(5)...O(3)	2.09	2.93	167.9
N(6)-H(6)...O(4)	2.04	2.85	157.0
N(7)-H(7)...O(5)	2.13	2.99	175.3
N(8)-H(8)...O(6)	1.99	2.82	162.7
N(1)-H(1)...O(7)$^{\#}$	2.07	2.93	175.2
N(2)-H(1)...O(8)$^{\#}$	2.02	2.82	154.3
N(1)-H(1)*...O(7)	2.06	2.93	175.2
N(2)-H(1)*...O(8)	2.02	2.82	154.3

Symmetry transformations used to generate equivalent atoms:

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

Table 8. Torsion angles [°] of P1

residue	ϕ	θ_1	θ_2	Ψ	ω
Aib 1	-57.36	---	**---**	-44.84	-175.8
γ^4Phe 2	-118.58	**50.17**	**65.79**	-132.87	-168.17
Aib 3	-62.57	---	---	-34.64	-174.56
γ^4Phe 4	-128.58	**52.62**	**59.61**	-116.56	-173.08
Aib 5	-58.22	---	---	-39.10	-171.56
γ^4Phe 6	-128.38	**51.05**	**62.35**	-118.54	-173.19
Aib 7	-55.37	---	---	-38.23	---

Table 9. Intra and intermolecular Hydrogen bonds of P2 [dist in Å and bond angle in °]. Intramolecular H-bonds are 1←4 backward type.

D-H---A	Dist. H---A(Å)	Dist D---A(Å)	∠D-H---A(°)
N(3)-H(3)...O(1)	2.15	2.99	168.5
N(4)-H(4)...O(2)	2.10	2.91	155.9
N(5)-H(5)...O(3)	2.17	3.00	163.0
N(6)-H(6)...O(4)	1.98	2.81	161.4
N(7)-H(7)...O(5)	2.19	3.05	173.3
N(8)-H(8)...O(6)	1.96	2.80	167.0
N(1)-H(1)...O(7)$^{\#}$	2.05	2.90	167.5
N(2)-H(1)...O(8)$^{\#}$	2.00	2.80	154.0
N(1)-H(1)*...O(7)	2.05	2.90	167.5
N(2)-H(1)*...O(8)	2.00	2.80	154.0

Symmetry transformations used to generate equivalent atoms:

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

Table 10. Torsion angles [°] of P2

residue	ϕ	θ_1	θ_2	Ψ	ω
Ala 1	-70.13	---	**---**	-34.61	179.86
γ^4Phe 2	-126.99	**50.26**	**64.09**	-120.09	-167.98
Ala 3	-73.05	---	---	-29.92	-179.84
γ^4Phe 4	-126.38	**52.92**	**59.86**	-113.02	-172.72
Ala 5	-65.01	---	---	-36.46	-176.51
γ^4Phe 6	-119.02	**49.25**	**65.42**	-123.77	-174.36
Aib 7	-55.32	---	---	-45.29	---

Table 11. Intramolecular and intermolecular Hydrogen bonds for P3 [Å and °]. Intramolecular H-bonds are 1←4 backward type.

D-H---A	Dist. H---A(Å)	Dist D---A(Å)	∠D-H---A(°)
N(3)-H(3)...O(1)	2.12	2.95	162.6
N(4)-H(4)...O(2)	2.00	2.82	159.8
N(5)-H(5)...O(3)	2.01	2.87	178.1
N(6)-H(6)...O(4)	2.02	2.81	152.5
N(7)-H(7)...O(5)	2.13	2.95	160.3
N(8)-H(8)...O(6)	1.93	2.74	156.7
N(1)-H(1)...O(7)$^{\#}$	1.98	2.84	172.3
N(2)-H(1)...O(8)$^{\#}$	2.07	2.85	150.2
N(1)-H(1)*...O(7)	1.98	2.84	172.3
N(2)-H(1)*...O(8)	2.01	2.86	150.2

Symmetry transformations used to generate equivalent atoms:

x,y,1+z, * x.y,-1+z

Table 12. Torsion angles [°] for P3

residue	ϕ	θ_1	θ_2	Ψ	ω
Ala 1	-73.11	---	**---**	-30.65	-176.25
γ^4Phe 2	-128.64	**47.65**	**60.82**	-113.74	-170.49
Ala 3	-67.49	---	---	-34.37	-177.51
γ^4Phe 4	-125.45	**49.82**	**61.39**	-123.42	-171.85
Ala 5	-62.53	---	---	-29.26	-176.09
γ^4Phe 6	-130.18	**53.00**	**57.36**	-108.33	-174.62
Ala 7	-65.30	---	---	-35.50	---

Table 13. Torsion angles [°] for γ^4Phe present in α/γ^4Peptide

α/γ^4-Peptide	ϕ	θ_1	θ_2	Ψ
P1	-118.58	**50.17**	**65.79**	-132.87
	-128.58	**52.62**	**59.61**	-116.56
	-128.38	**51.05**	**62.35**	-118.54
P2	-126.99	**50.26**	**64.09**	-120.09
	-126.38	**52.92**	**59.86**	-113.02
	-119.02	**49.25**	**65.42**	-123.77
P3	-128.64	**47.65**	**60.82**	-113.74
	-125.45	**49.82**	**61.39**	-123.42
	-130.18	**53.00**	**57.36**	-108.33
average	**-125.8**	**50.75**	**61.85**	**-118.93**

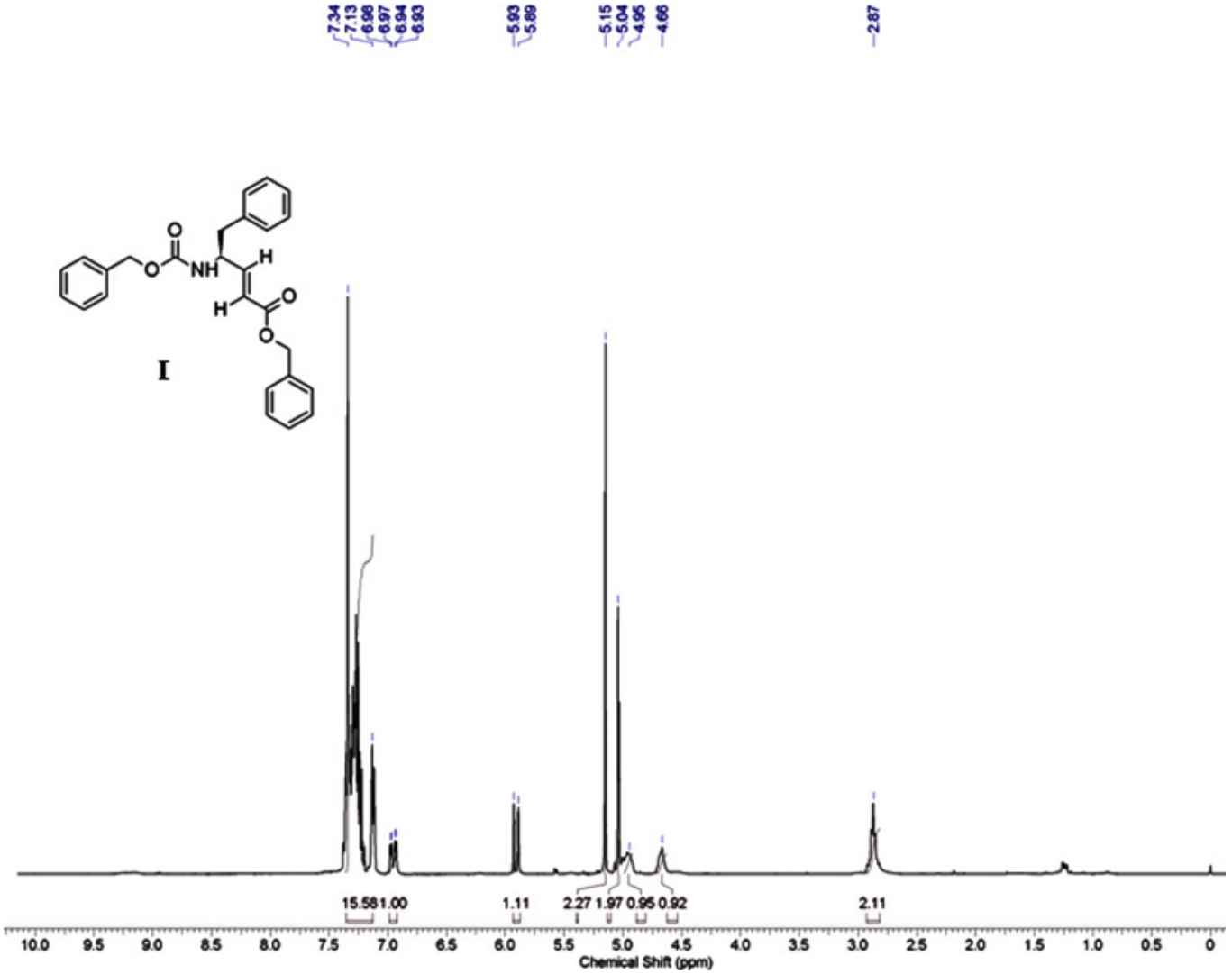
IX. References

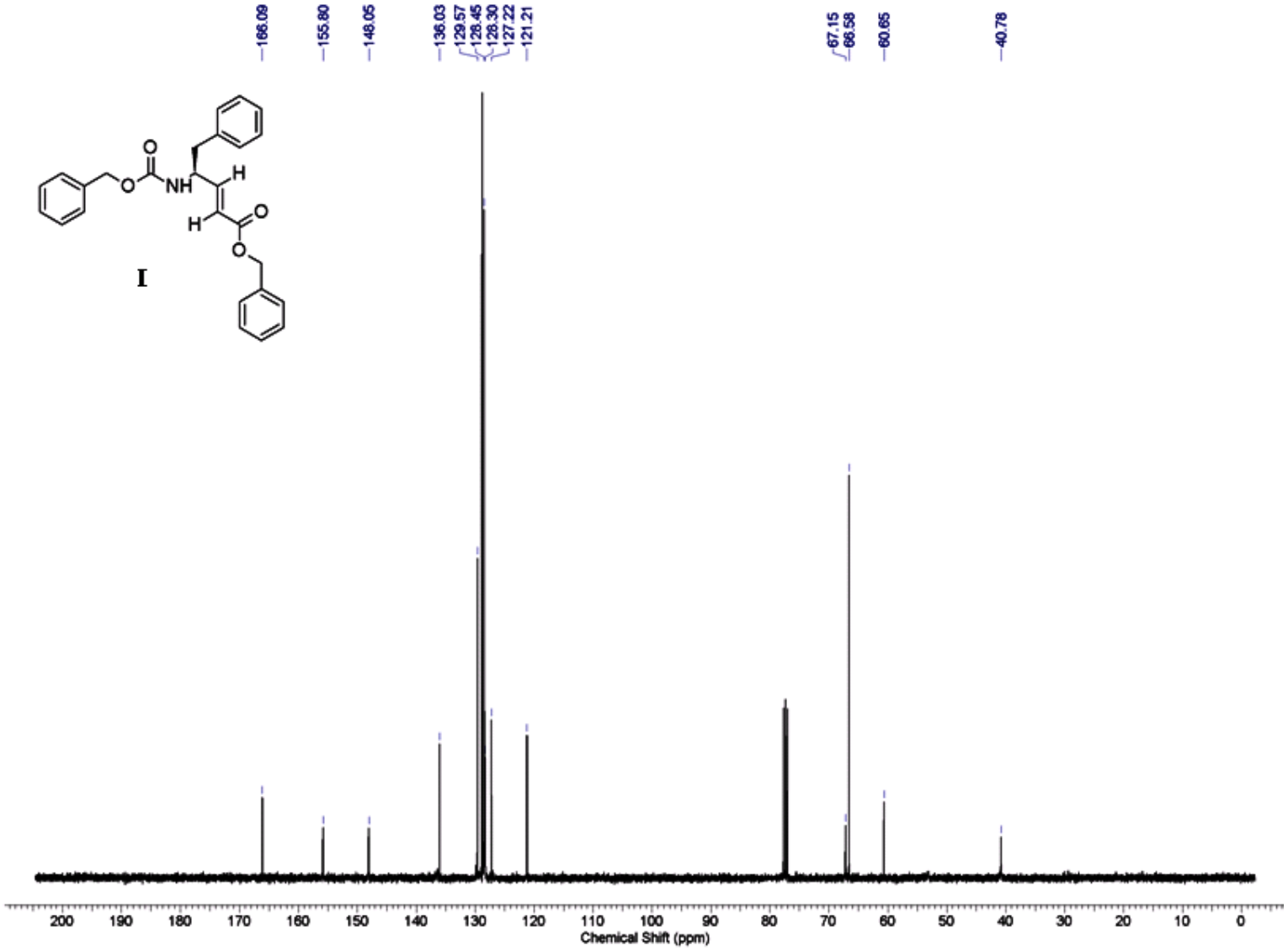
1. *SAINT Plus*, (Version 7.03); Bruker AXS Inc.: Madison, WI, 2004.

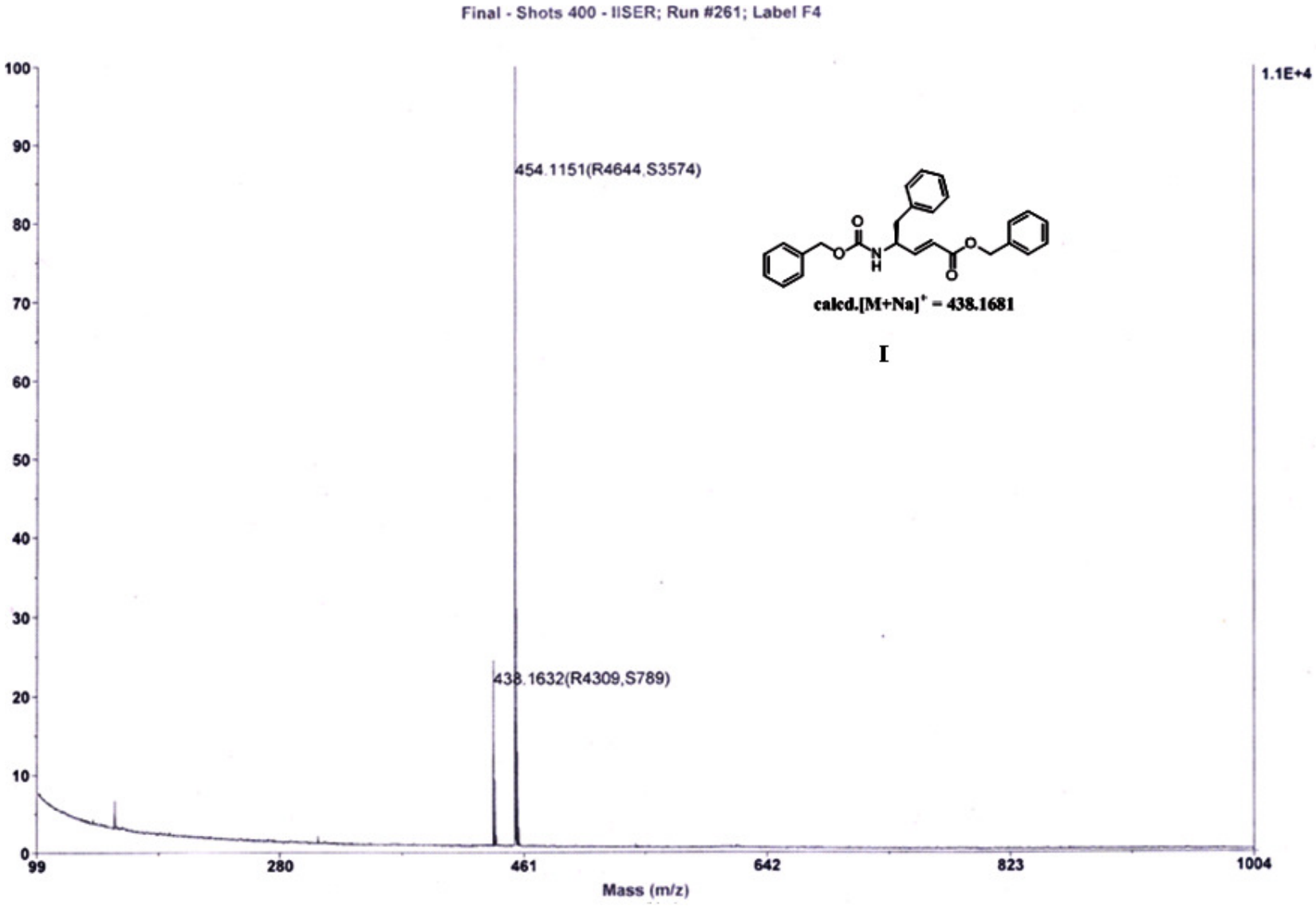
2. Sheldrick, G. M. (2008) SHELXL. Acta Cryst. A64, 112-122.

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

X. ^{1}H, ^{13}C and Mass Spectra of compounds







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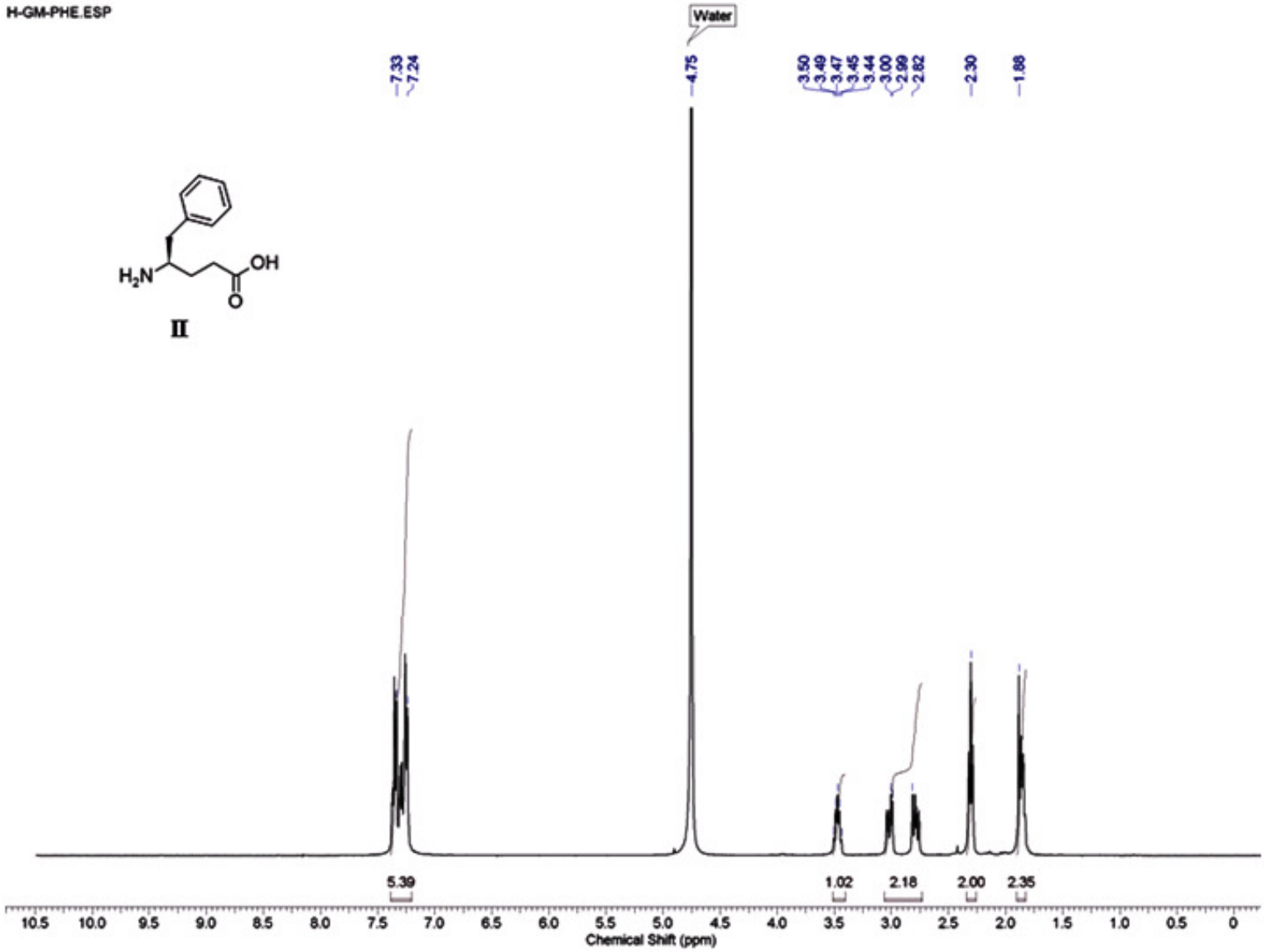

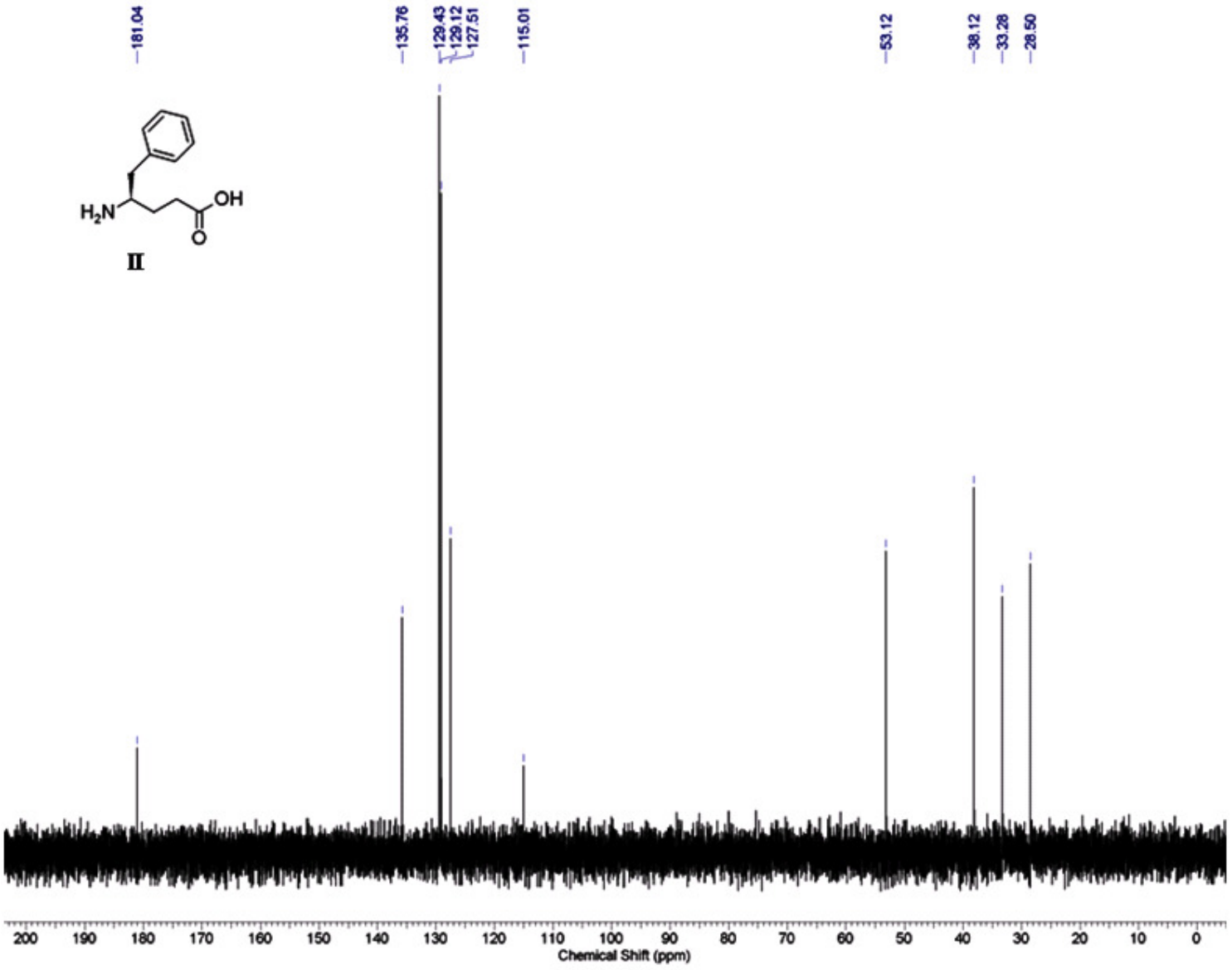

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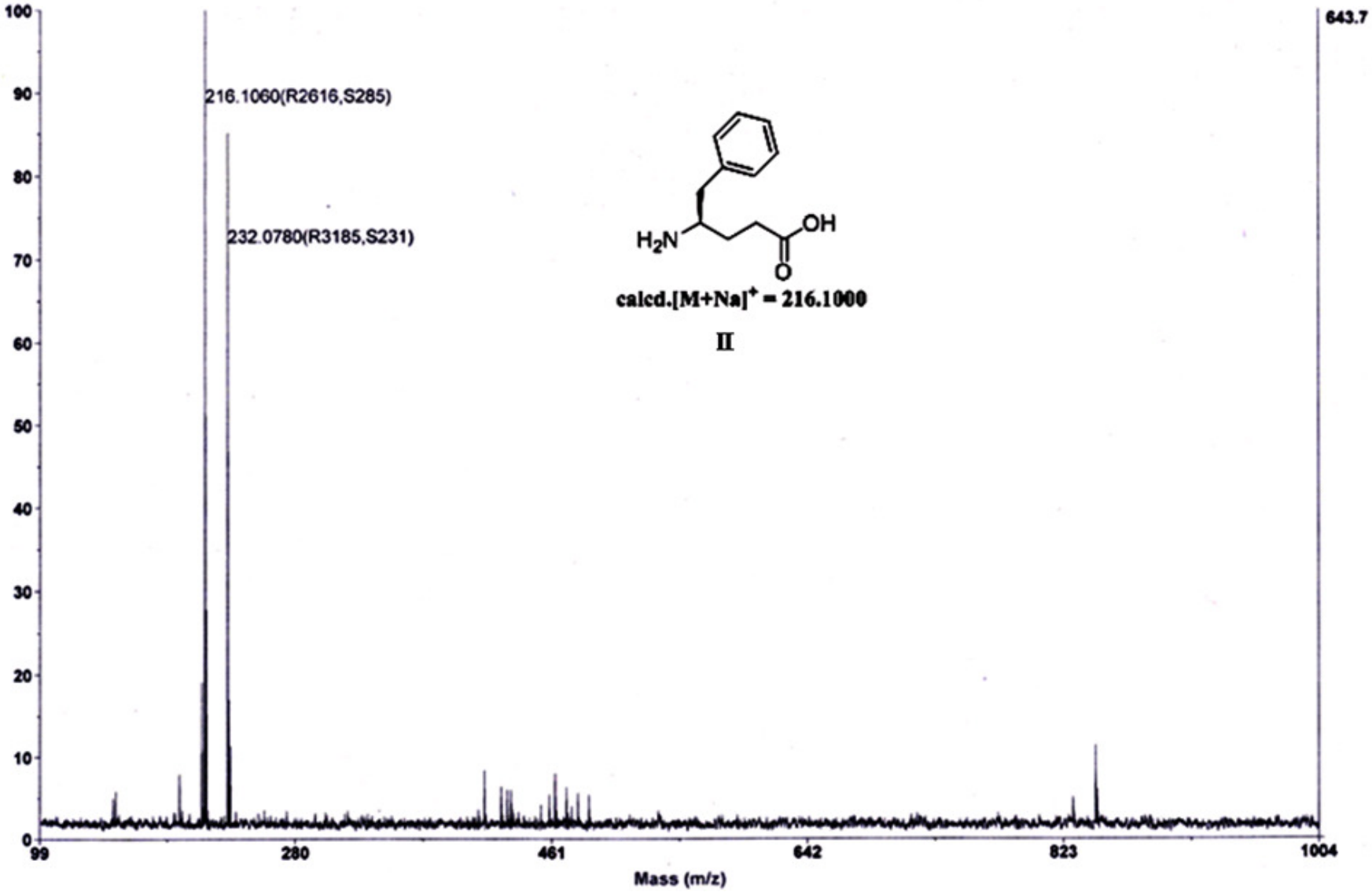

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