Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Atomic Insight into Short Helical Peptide Comprised of Consecutive Multiple Aromatic Residues

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General methods:

All the peptides were purchased from GL Biochem. For self-assembly studies, peptides **P1** and **P2,** 1.5 mg of the peptide was dissolved in 100μL THF and diluted to 500 μL water. Crystalline needles and spherical microstructures appeared after 2 days and these were further characterized by microscopic techniques as follows.

Scanning electron microscopy:

A 20 μL aliquot of the above solution was dried at room temperature on a microscope cover slip and coated with gold. Scanning electron microscopy (SEM) images were taken using a JSM JEOL 6300 SEM operating at 20 kV and (JEOL, Tokyo, Japan) operating at 20 kV.

Transmission electron microscopy:

A 4 μL aliquot of the solution was placed on a 400 mesh copper grids. After 1 min, excess fluid was removed and the sample was left to dry at room temperature. Samples were viewed using a JEOL 1200EX electron microscope operating at 80 kV.

Optical Microscopy:

A 20 μL alquot of the solution was placed on a glass slide and bright-field imaging was performed using an Eclipse Ti-E inverted microscope (Nikon, Japan), equipped with a Zyla SCMOS camera (Andor, UK).

CD spectroscopy:

CD spectra of the peptides **P1** and **P2** (0.5mg/mL) in TFE were recorded using a Chirascan spectrometer (Applied Photophysics) fitted with a Peltier temperature controller set to 25 °C, using quartz cuvettes with an optical path length of 0.1 mm (Hellma Analytics). The scan was performed in steps of 1 nm over a wavelength range of 190-260 nm with a spectral bandwidth of 1.0 nm and an averaging time of 3 s. The full spectrum of the sample was collected three times and averaged. The baseline was similarly recorded for TFE and subtracted from the sample spectra. Data processing was performed using Pro-Data Viewer software (Applied Photophysics).

FTIR spectroscopy:

A 50 μL aliquot of the peptide **P1** (2mg/mL) in isopropanol and a 50 μL aliquot of the peptide **P2** (2mg/mL) in acetonitrile were each deposited on disposable KBr infrared sample cards (Sigma-Aldrich), then dried under vacuum. FTIR spectra were collected using a nitrogen-purged Nicolet Nexus 470 FTIR spectrometer (Nicolet) equipped with a deuterated triglycine sulfate detector. Measurements were performed using a 4 cm-1 resolution, averaging 64 scans. The absorbance maxima values were determined using an OMNIC analysis program (Nicolet). The background was subtracted using a control spectrum. To understand the conformation in the assembled state of the peptide **P1**, it was dissolved in 100μL THF and diluted to 500μL water. 100μL of this solution was then deposited on disposable KBr infrared sample cards (Sigma-Aldrich), dried under vacuum, and analyzed under the similar condition.

Crystallization and Structure Determination:

Single crystals suitable for X-ray diffraction were grown by slow evaporation of isopropanol and acetonitrile for peptide **P1** and **P2** at room temperature. Crystals of diffraction-quality were obtained after 5 days of sample preparation. For data collection, crystals were coated in paratone oil (Hampton Research), mounted on a MiTeGen cryo-loop and flash-frozen in liquid nitrogen. Diffraction data for **P1** were collected at 100 K on a Rigaku XtaLabPro equipped with a Dectris Pilatus 200K detector. Diffraction data for **P2** were collected at 100 K on a Rigaku Synergy R rotating anode system with a HyPix-Arc 150 detector using CuKα radiation at λ = 1.54184 Å. Data were processed and reduced with CrysAlisPro. The structures were solved by direct methods using SHELXT-2018[1] as implemented in Olex2 and refined by full matrix least squares against F2 with SHELXL-2013. All non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were placed in calculated positions and refined in the riding mode. The crystallographic data are given for peptide **P1** in Table **S5** and for peptide **P2** in table **S6**. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC), under deposition number CCDC 2123307, 2132310.

Table S1: Torsion Angles of the Peptide **P1**:

Residue	ϕ (deg)	Ψ (deg)
Aib1	-53	-48
Phe2	-67	-26
Phe3	-52	-45
Phe4	-73	-14
Phe5	-71	-39
Phe6	-97	-2
Aib7	-60	-37

Table S2: Torsion Angles of the Peptide **P2**:

Residue	ϕ (deg)	Ψ (deg)
Aib1	-50	-46
Phe2	-71	-25
Phe3	-65	-48
Phe4	-63	-36
Phe5	-62	-47
Phe6	-80	-37
Phe7	-114	-4

Table S3: Hydrogen Bonding Parameters peptide **P1**

Intramolecular H bond:

Donor (D)	Acceptor (A)	D…. A (Å)	DH…. A (Å)	NH…. O (deg)
N3	O1	2.98	2.40	123
N4	O2	2.99	2.35	129
N5	O2	3.07	2.42	131
N6	O3	3.28	2.49	149
N7	O4	2.86	2.53	103

Intermolecular H bond:

Donor (D)	Acceptor (A)	D…. A (Å)	DH…. A (Å)	NH…. O (deg)
N2	O8	2.81	1.94	168
N1	O7	3.02	2.49	120
N8	O5	2.96	2.08	174

Table S4: Hydrogen Bonding Parameters peptide **P2**

Intramolecular H bond:

Donor (D)	Acceptor (A)	D…. A (Å)	DH…. A (Å)	NH…. O (deg)
N3	O1	2.91	2.32	124
N4	O1	3.16	2.30	165
N5	O2	2.87	2.07	151
N6	O3	3.03	2.17	162
N7	O4	2.87	2.07	150
N8	O5	2.89	2.15	141

Intermolecular H bond:

Donor (D)	Acceptor (A)	D…. A (Å)	DH…. A (Å)	NH…. O (deg)
N2	O7	2.98	2.13	162
N1	O6	2.76	1.89	169

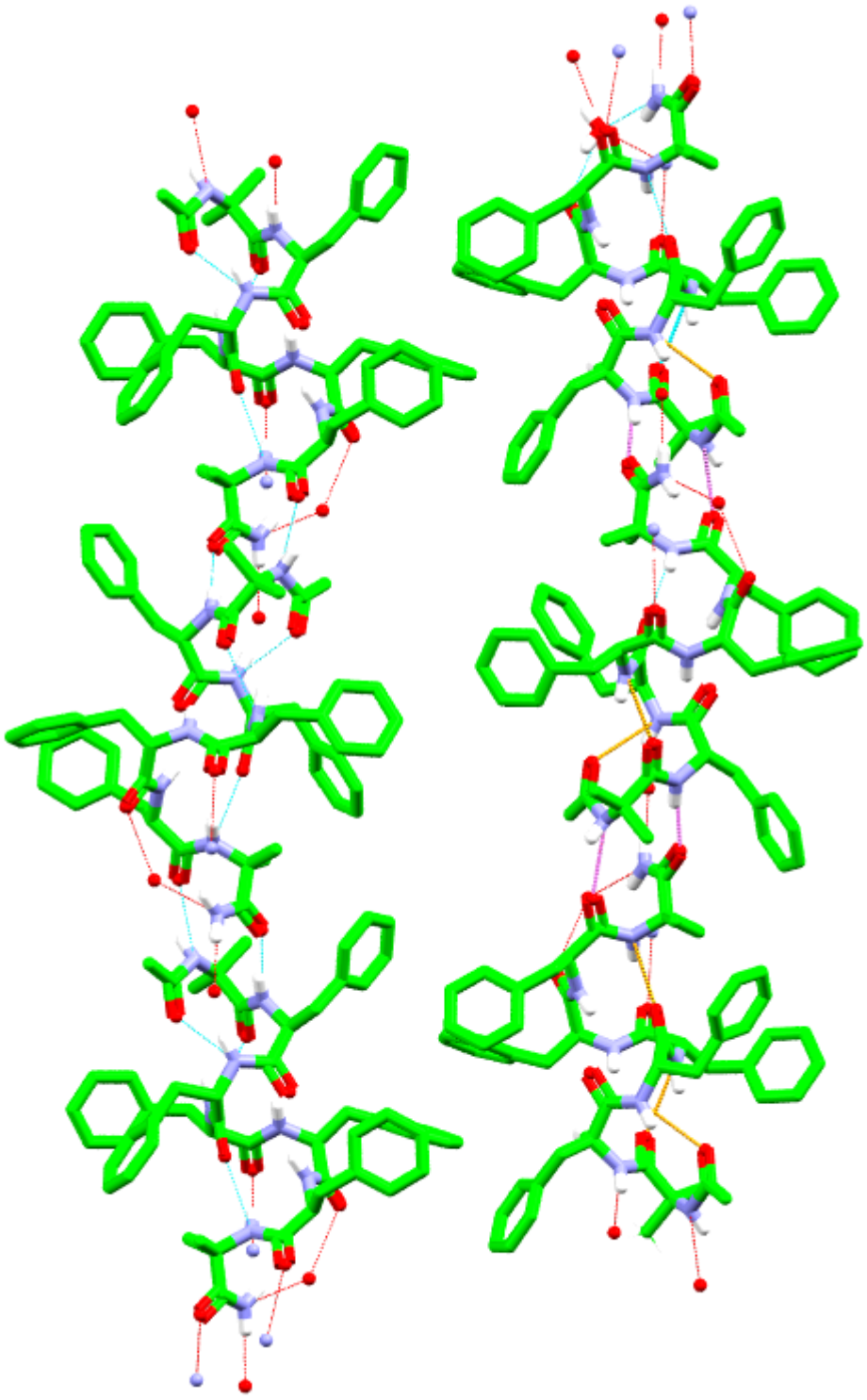


Figure S1: Super helical packing of peptide **P1** through head to tail hydrogen bonding.

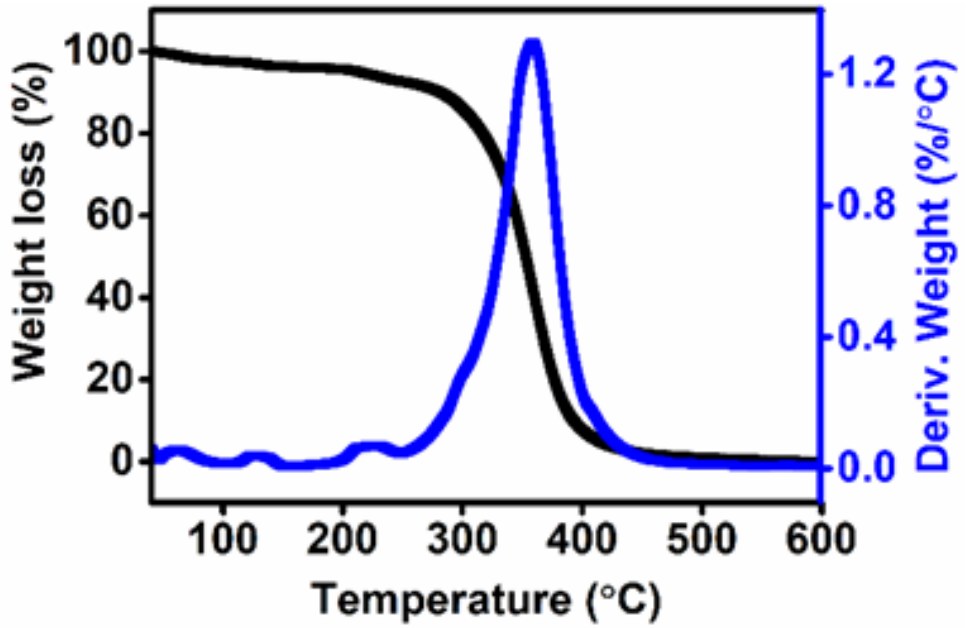


Figure S2: Thermogravimetric analysis of **P1** crystals (black curve) revealing the high thermal stability (≥300 °C). The weight loss is depicted by the first derivative curve (blue).

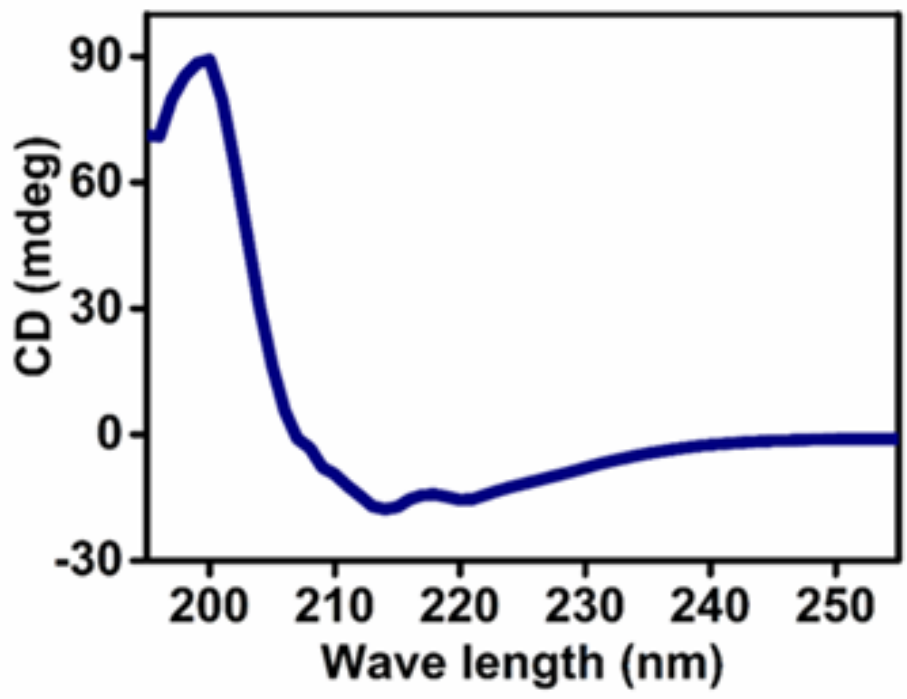


Figure S3: CD spectrum of the **P1** peptide in TFE (0.5mg/mL), indicating helical organization.

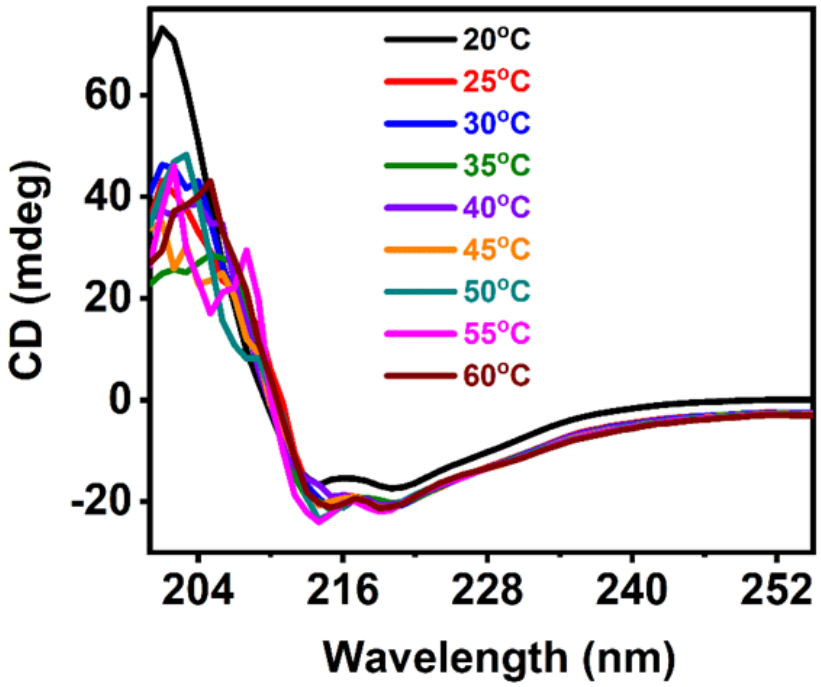


Figure S4: Temperature-dependent CD spectrum of the **P1** peptide in TFE (0.5mg/mL).

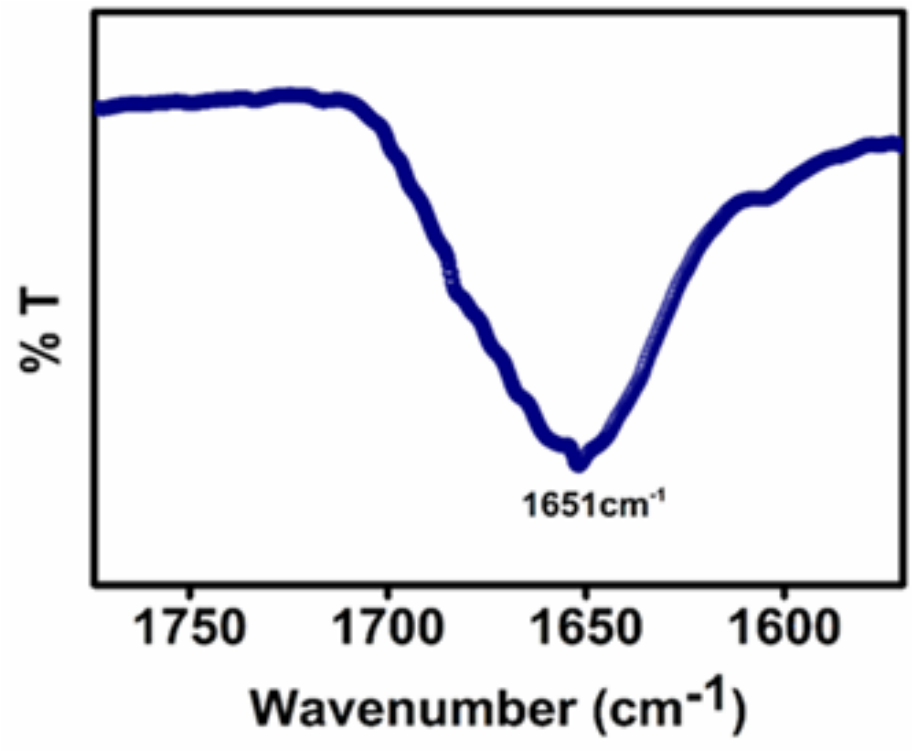


Figure S5: FTIR spectrum of the **P1** peptide showing a characteristic helical peak.

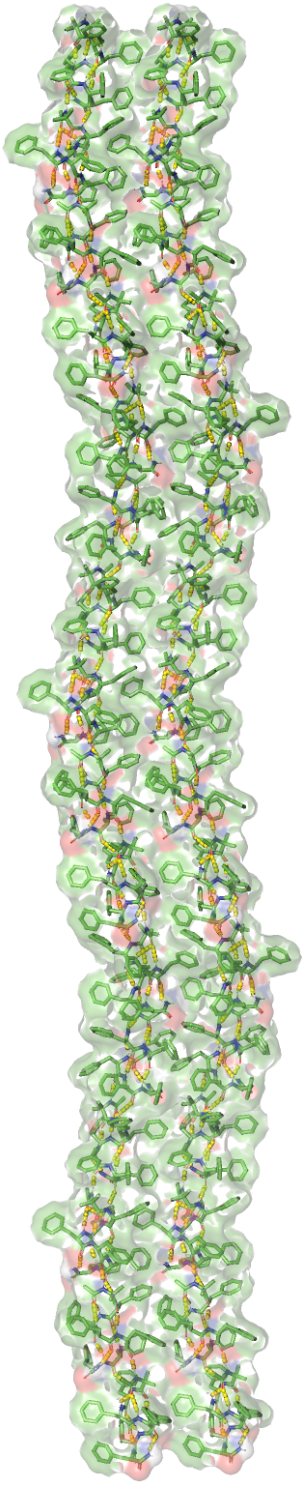


Figure S6: Super helical packing of the **P2** peptide through head to tail hydrogen bonding.

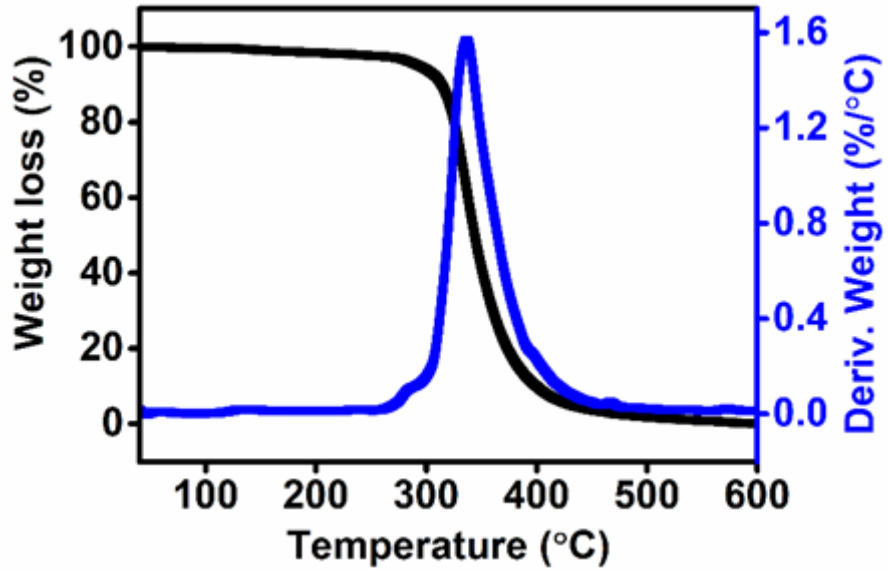


Figure S7: Thermogravimetric analysis of **P2** crystals (black curve) revealing the high thermal stability (≥300 °C). The weight loss is depicted by the first derivative curve (blue).

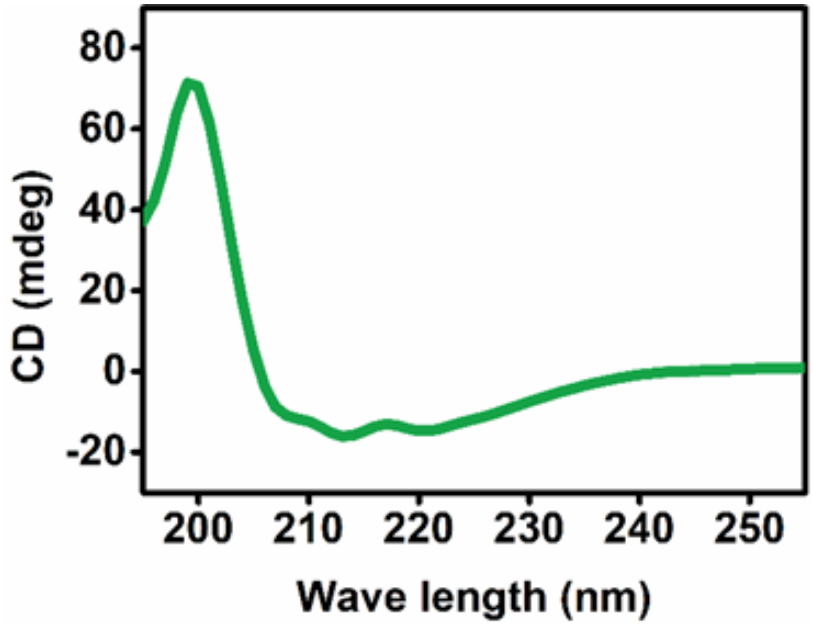


Figure S8: CD spectrum of the **P2** peptide in TFE (0.5mg/mL), indicating helical organization.

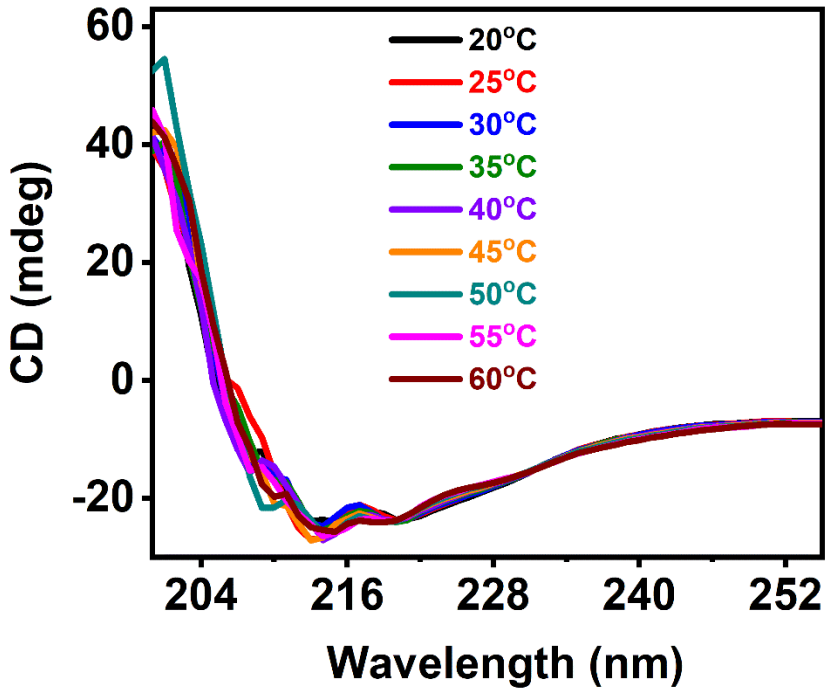


Figure S9: Temperature-dependent CD spectrum of the **P2** peptide in TFE (0.5mg/mL).

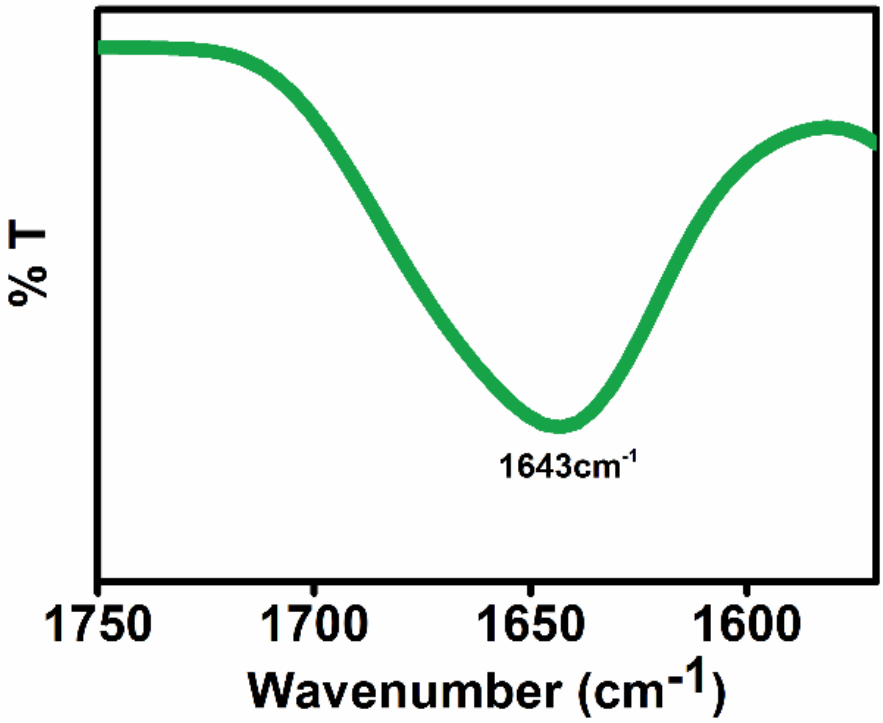


Figure S10: FTIR spectrum of the **P2** peptide showing a characteristic helical peak.

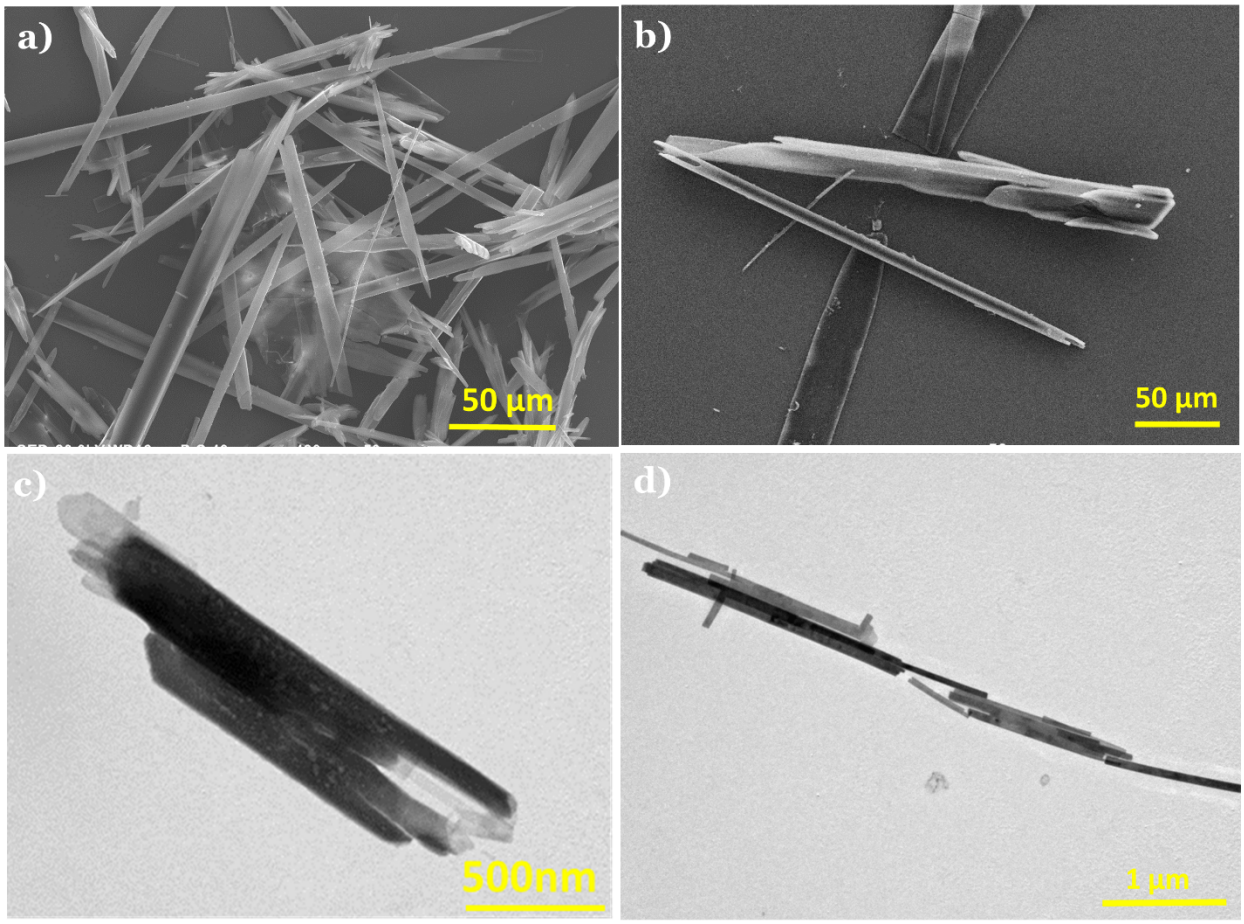


Figure S11: a, b) SEM and c, d) TEM images of the supramolecular assembly needle-like microstructure formed by the **P1** peptide in a 2:8 THF/water combination.

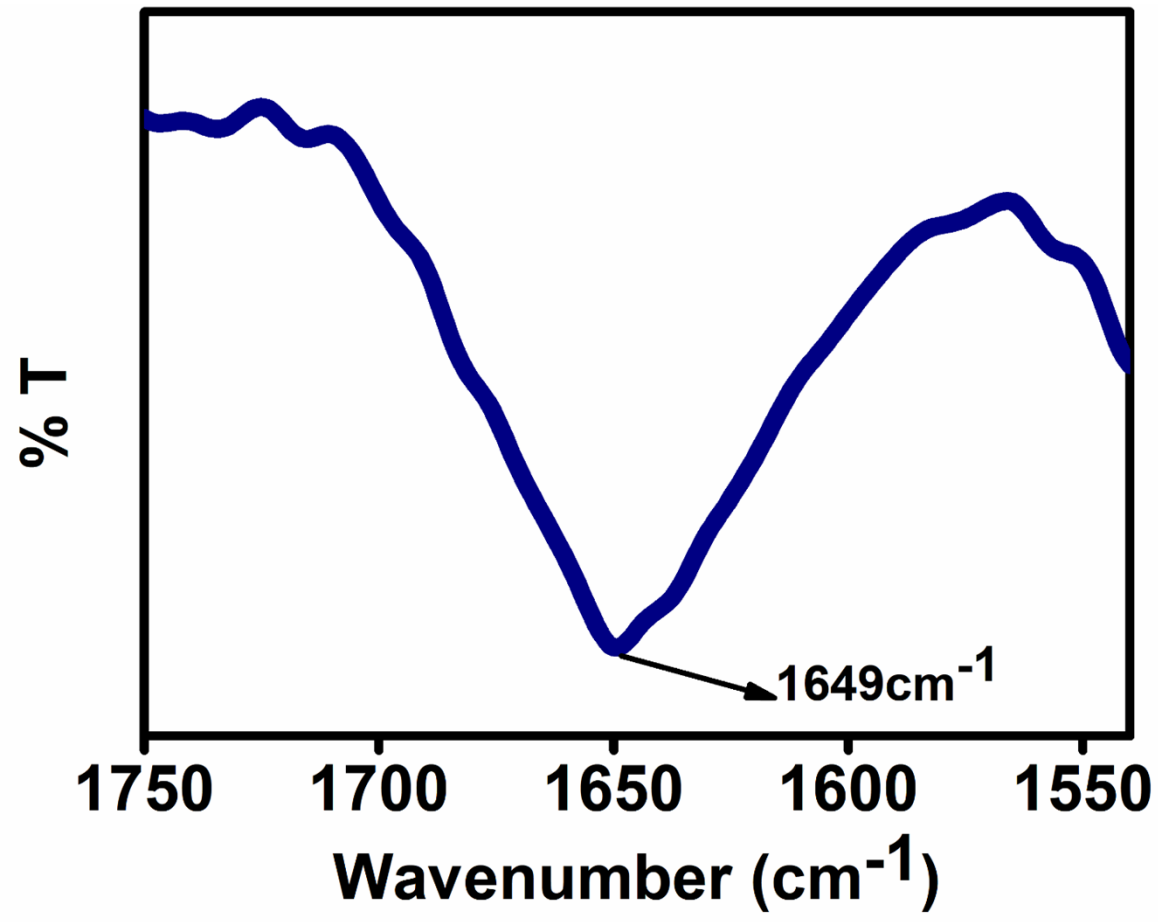


Figure S12: FTIR spectrum of the helical P1 peptide in a 2:8 THF/water combination.

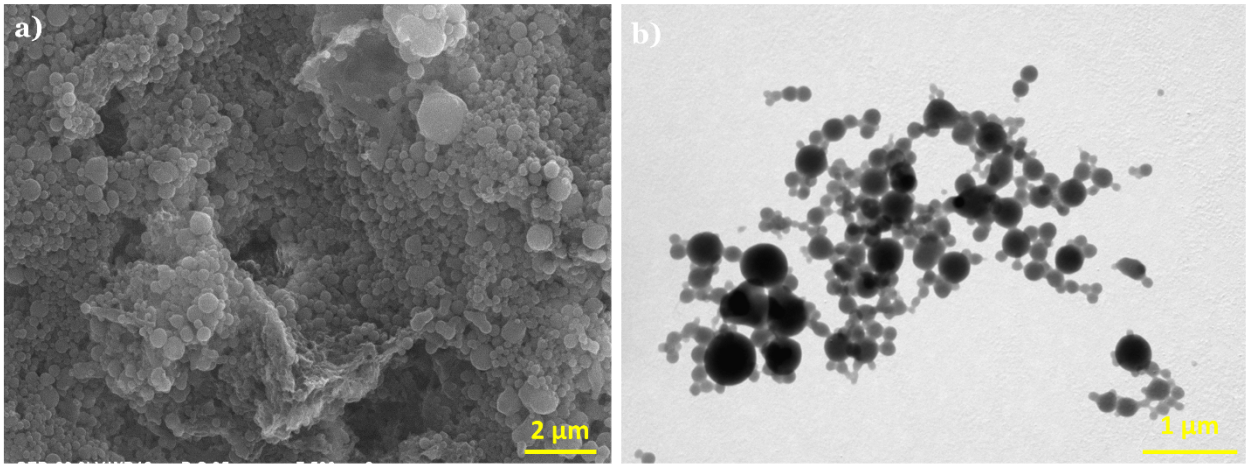


Figure S13: a) SEM and b) TEM images of the supramolecular assembly spherical microstructure formed by the helical **P2** peptide in a 2:8 THF/water combination.

Table S5: Refinement statics data for peptide **P1**

CCDC number	2123307
Crystal description	Colourless plate
Diffractometer	Rigaku XtaLab^{Pro}
Empirical formula	C110 H128 N16 O17
Formula weight (g/mol)	1946.28
Temperature (K)	100 (2)
Wavelength (Å)	1.54184
Crystal system	Orthorhombic
Space group	$C\,222_1$
a, Å	14.9385(1)
b, Å	29.7180(3)
c, Å	27.4444(2)
α°	90
β°	90
γ°	90
Volume (Å3)	12183.71(18)
Z	4
dcalc (mg/cm3)	1.016
μ (mm-1)	0.588
F(000)	4144
Theta range for data collection (°)	3.683 to 80.272
Reflections collected (unique)	52855 (13016)
Rint	0.0213
completeness	99.7
Data/restraints/parameters	13016/60/608
Final R [I > 2 σ (I)]	R1=0.0848 wR2=0.2574
R (all data)	R1=0.0875 wR2=0.2528
Goodness of Fit	1.107
Largest diff. peak and hole (e· Å^{-3})	0.401, -0.271

Table S6: Refinement statics data for peptide **P2**

CCDC number	2132310
Crystal description	Colourless needle
Diffractometer	Rigaku Synergy R
Empirical formula	C60 H66 N8 O8
Formula weight (g/mol)	1027.20
Temperature (K)	100 (2)
Wavelength (Å)	1.54184
Crystal system	hexagonal
Space group	$P\,6_1$
a, Å	11.9093(2)
b, Å	11.9093(2)
c, Å	68.9715(10)
α°	90
β°	90
γ°	120
Volume (Å3)	8471.7(3)
Z	6
dcalc (mg/cm3)	1.208
μ (mm-1)	0.655
F(000)	3276
Theta range for data collection (°)	3.85 to 73.68
Reflections collected (unique)	45992(9676)
Rint	0.0381
completeness	99.8
Data/restraints/parameters	9676/37/637
Final R [I > 2 σ (I)]	R1=0.0626 wR2=0.1627
R (all data)	R1=0.0772 wR2=0.1751
Goodness of Fit	1.068
Largest diff. peak and hole ($e \cdot Å^{-3}$)	0.727, -0.552

HPLC REPORT FOR PEPTIDE P1

Sample:	Ac-Aib $(FF)_5$-Aib-NH_2	Analyzed date:	2021-6-18
Analyst:	HCM	Reconstitution:	1mg/0.25mlACN:0.75mlH2O
Lot. No.:	P210507-JQ873944		
Column:	4.6×250mm, Kromasil 100-5 C18		
Solvent A	A: 0.1% Trifluoroacetic Acid in 100% Acetonitrile		
Solvent B	B: 0.1% Trifluoroacetic Acid in 100% Water		

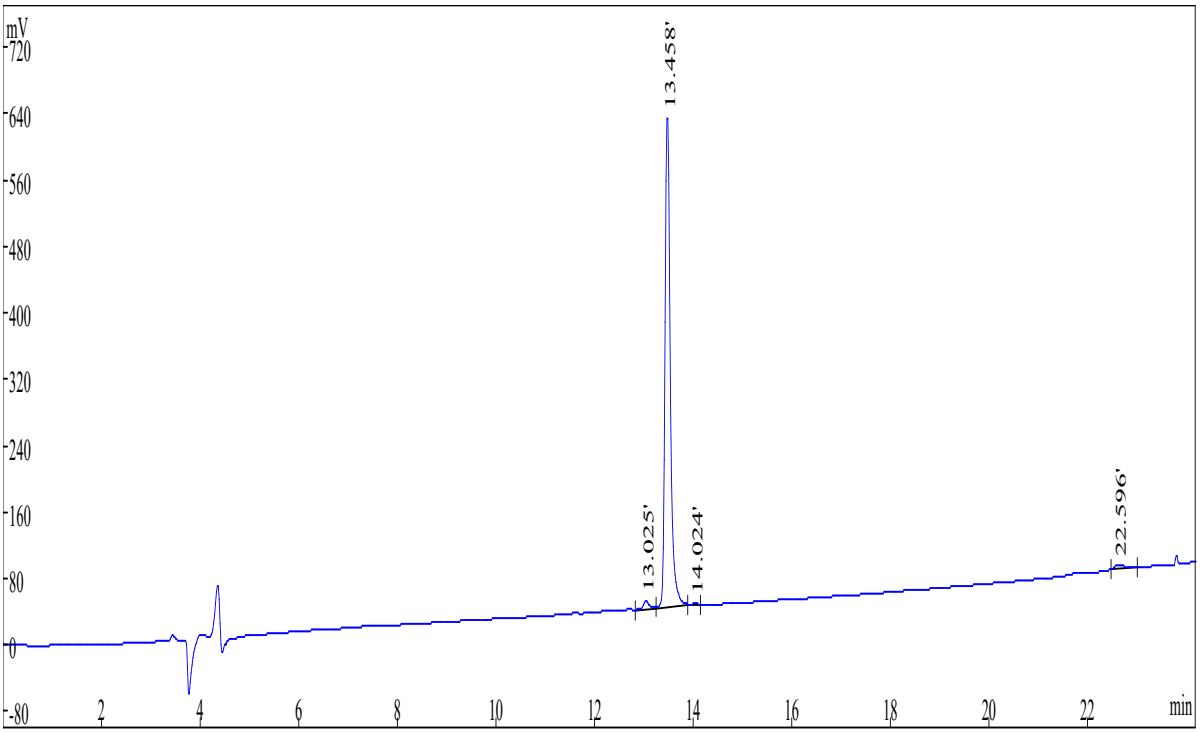
Gradient:

	A	B
0.0min	40%	60%
25.0min	65%	35%
25.1min	100%	0%
30.0min	Stop	

Volume: 5µl

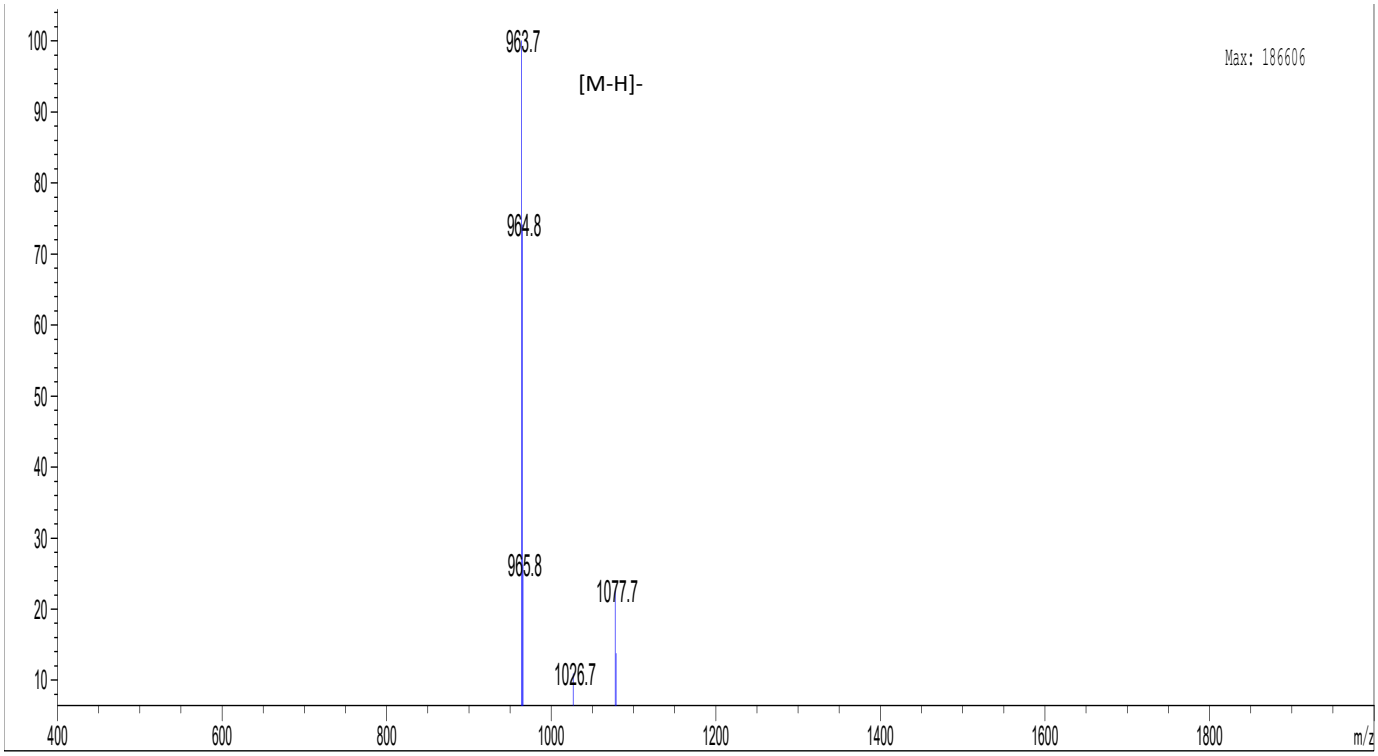
Wavelength: 220nm

Flow rate: 1.0ml/min



Rank	Time	Conc.	Area	Height
1	13.025	1.944	77747	9802
2	13.458	95.95	3836646	589252
3	14.024	0.5283	21125	2325
4	22.596	1.577	63071	5090
Total		100	3998589	606469

MASS SPECTROMETRY REPORT PEPTIDE P1



Sample Description		Instrument	Agilent-6125B
Analyzed date:	2022-04-18	Probe:	ESI
Analyst:	YU	Nebulizer Gas Flow:	1.5L/min
Sample:	FF-5	CDL:	-20.0v
M.W.:	965.17	CDL Temp.:	250 °C
Lot. No.:	P210507-JQ873944	Block Temp.:	200 °C

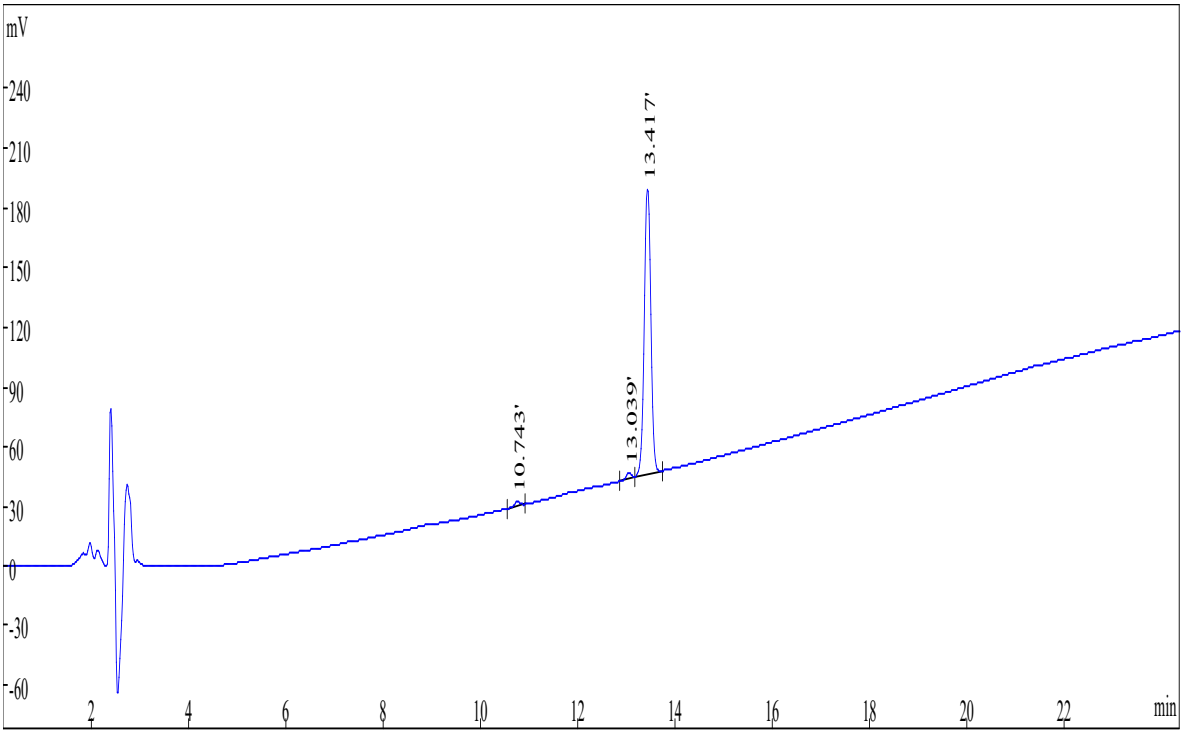
HPLC REPORT FOR PEPTIDE P2

Sample:	AC-Aib(FF)$_6$-NH2	Analyzed date:	2021-5-28
Analyst:	HCM	Reconstitution:	1mg/0.25mlACN:0.75mlH2O
Lot. No.:	P210507-JQ895413		
Column:	4.6×250mm, Kromasil 100-5 C18		
Solvent A	A: 0.1% Trifluoroacetic Acid in 100% Acetonitrile		
Solvent B	B: 0.1% Trifluoroacetic Acid in 100% Water		

Gradient:

	A	B
0.0min	50%	50%
25.0min	100%	0%
25.1min	100%	0%
30.0min	Stop	

Volume:	5µl
Wavelength:	220nm
Flow rate:	1.0ml/min



Rank	Time	Conc.	Area	Height
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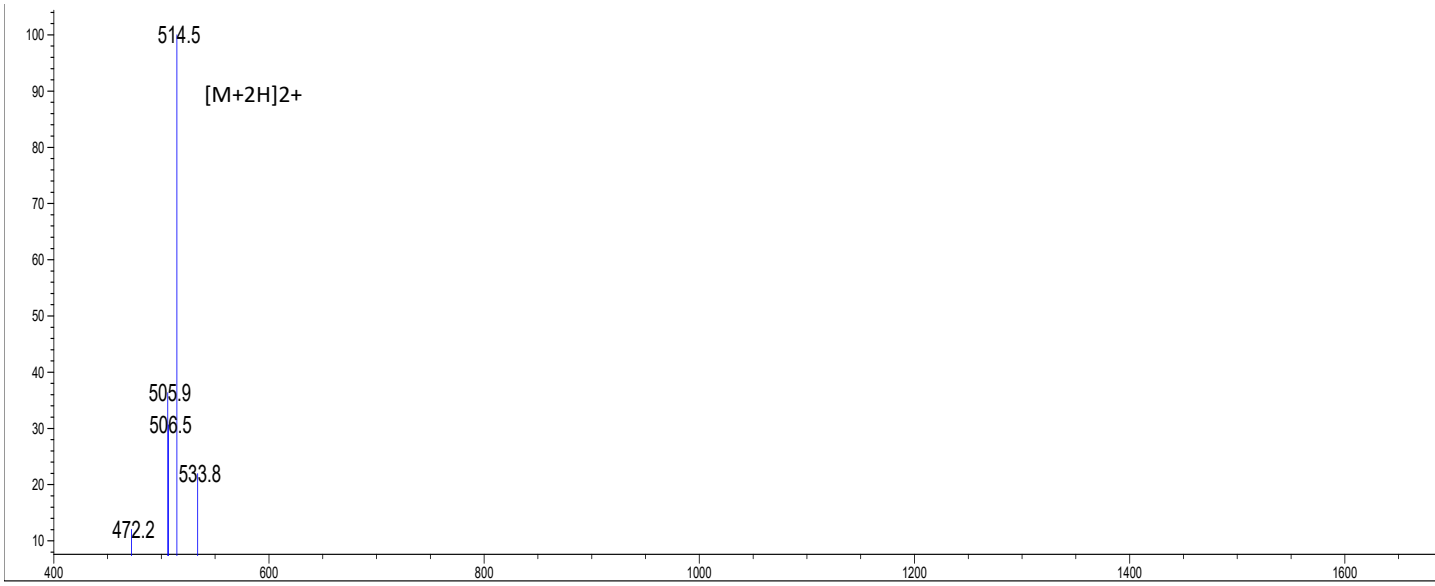
2	13.039	1.69	21117	2794
3	13.417	96.96	1211483	143101
Total		100	1249477	148073

MASS SPECTROMETRY REPORT PEPTIDE P2



Sample Description		Instrument	Agilent-6125B	
Analyzed date:	2021-05-28	Probe:	ESI	Probe Bias:
Analyst:	YU	Nebulizer Gas Flow:	1.5L/min	Detector:
Sample:	AC-AibFF-6-NH2	CDL:	-20.0v	T. Flow:
M.W.:	1027.24	CDL Temp.:	250 °C	B. Conc.:
Lot. No.:	P210507-JQ895413	Block Temp.:	200 °C	

References:

1. G. M. Sheldrick, Acta Cryst. 2015, C71, 3-8