Electronic Supplementary Material (ESI) for ChemComm.

Nanofiber Assembly Directed by Non-classical Antiparallel β-Structure from 4S-(OH) Proline Polypeptide

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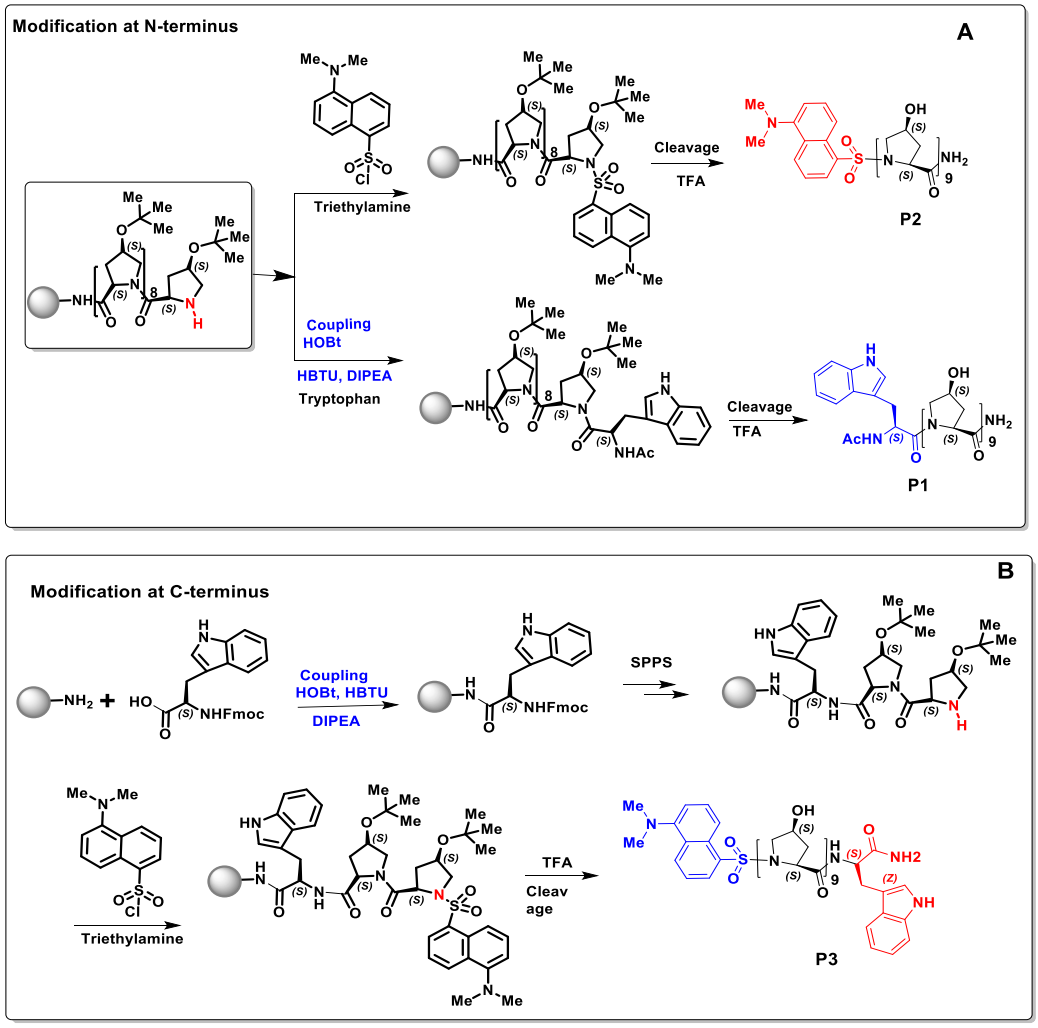
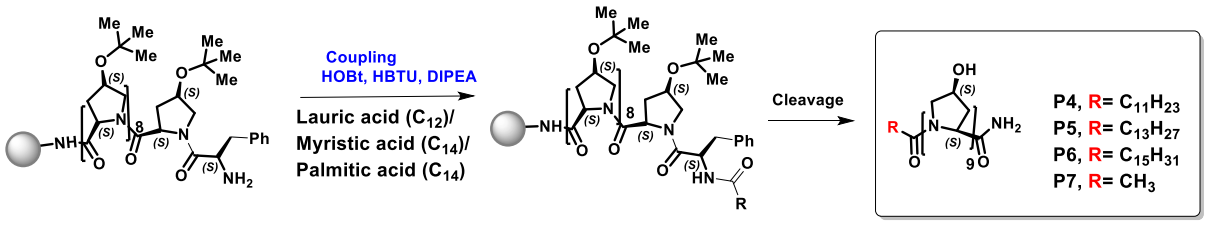
General schematic representation of solid phase peptide synthesis

Scheme 1. Solid phase synthesis for **P1-P3**



Scheme 2. Solid phase synthesis for **P4-P6**

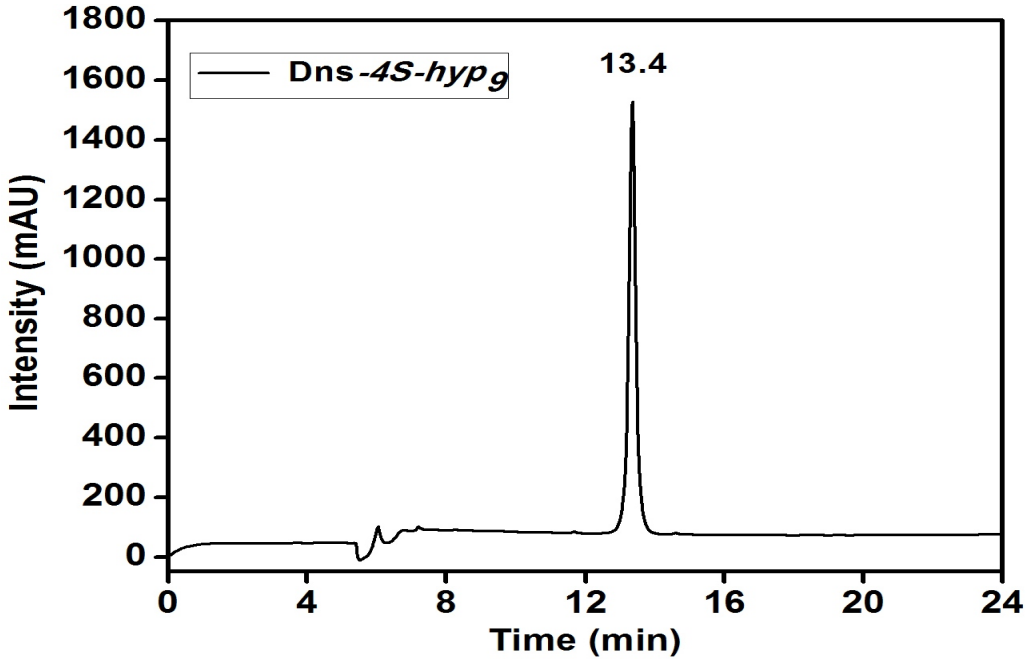


HPLC purification and analytical HPLC of peptides P1-P7

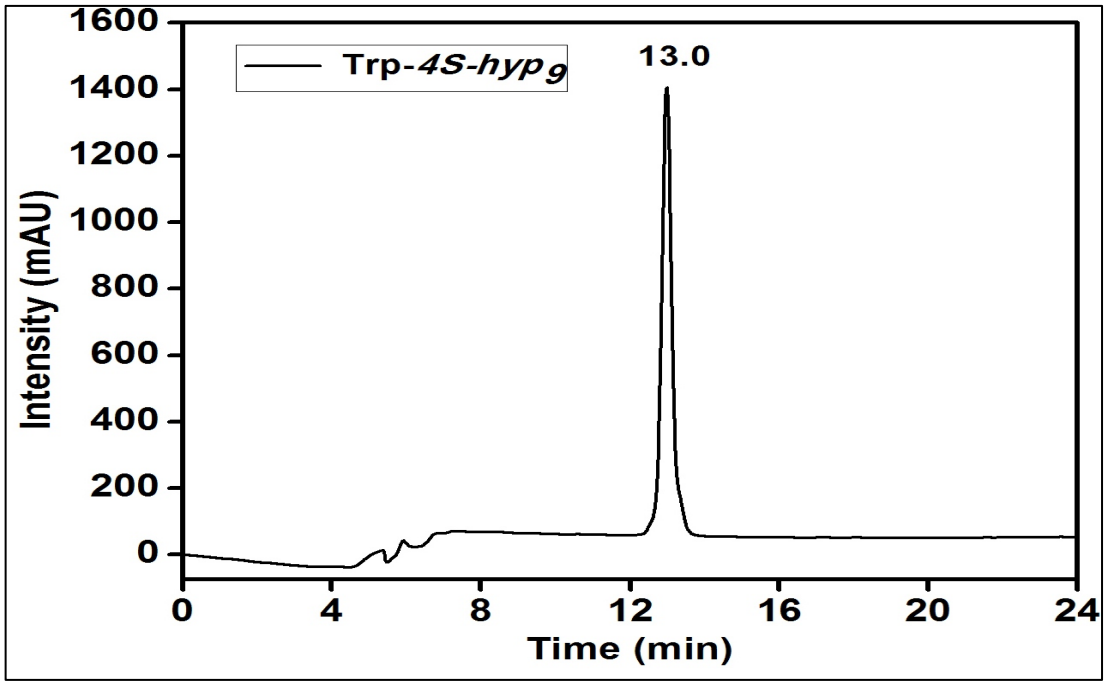
Peptides **P1-P3** were purified by RP-HPLC on RP-4 (15 μM) column connected to HPLC system equipped with UV-970 variable wavelength detector and integrator. Peptides **P1-P3** was purified by reverse phase-HPLC on semi-preparative RP-C18 columns. The solvent system comprised of H_2O:ACN (95:5) with 0.1% TFA for solution A and for solution B H_2O:ACN (50:50), 0.1% TFA. A gradient of 0-100% at a flow rate of 3 mL/min was used to elute the peptide and the eluant was monitored at 220 nm and 254 nm.

Peptides **P4-P7** was purified by reverse phase-HPLC on semi-preparative RP-C18 columns. The solvent system comprised of H_2O:ACN (50:50) with 0.1% TFA for solution A and for solution B H_2O:ACN (5:95), 0.1% TFA. A gradient of 0-100% at a flow rate of 3 mL/min was used to elute the peptide and the eluant was monitored at 220 nm and 254 nm.

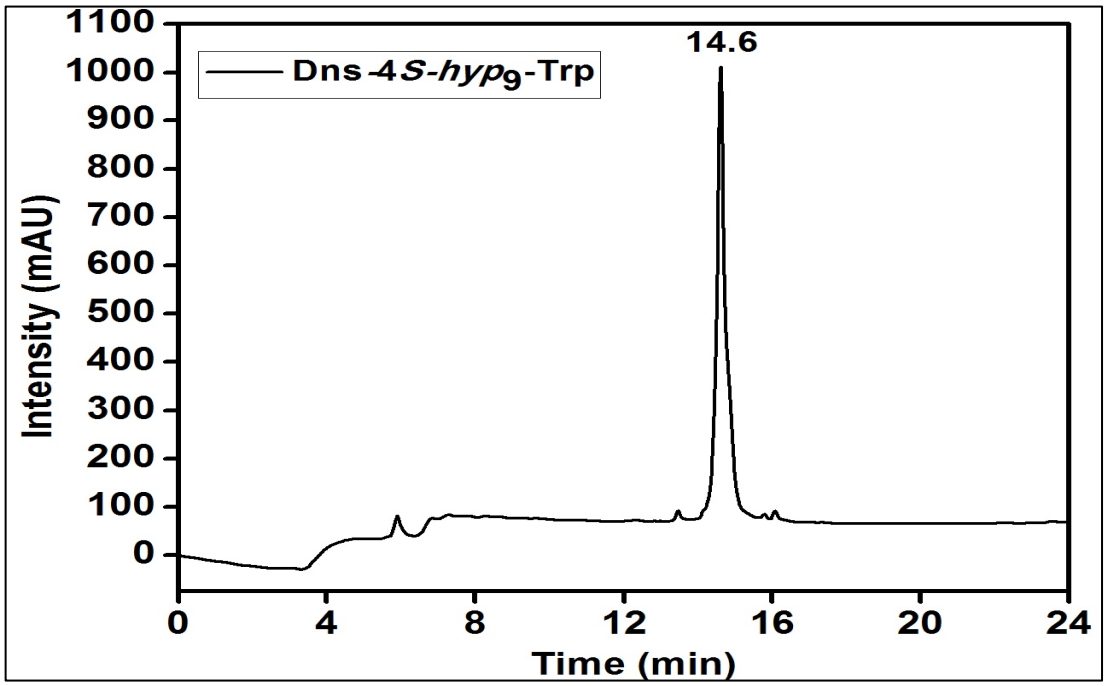
HPLC of peptide 1 (Dns-4*S*-*hyp*$_9$)



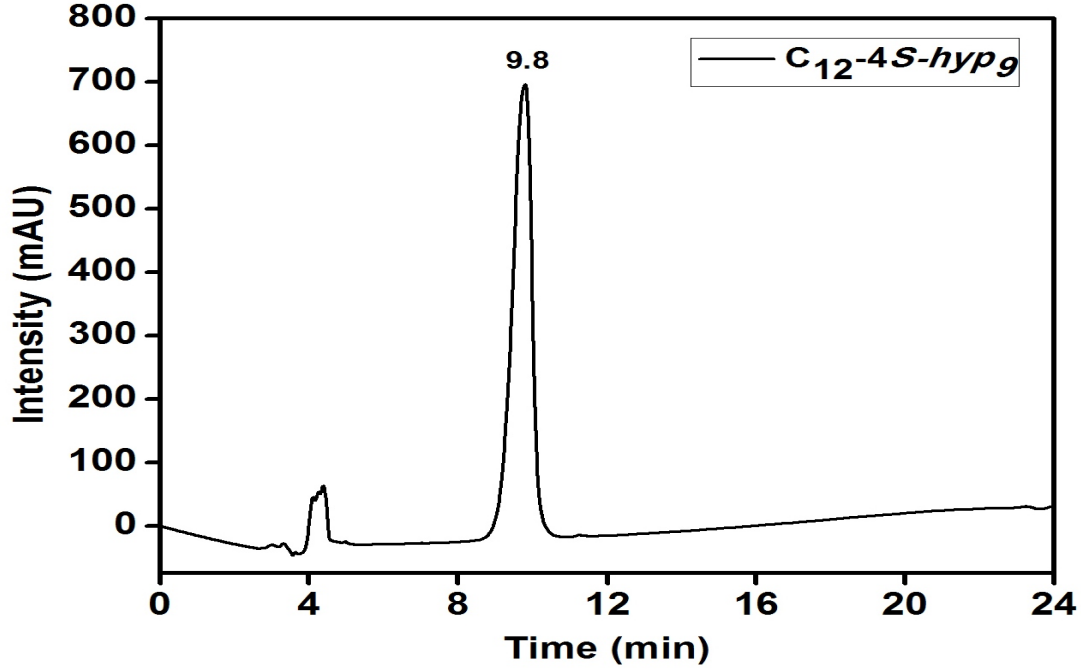
HPLC of peptide 2 (Trp-4*S*-*hyp*$_9$)



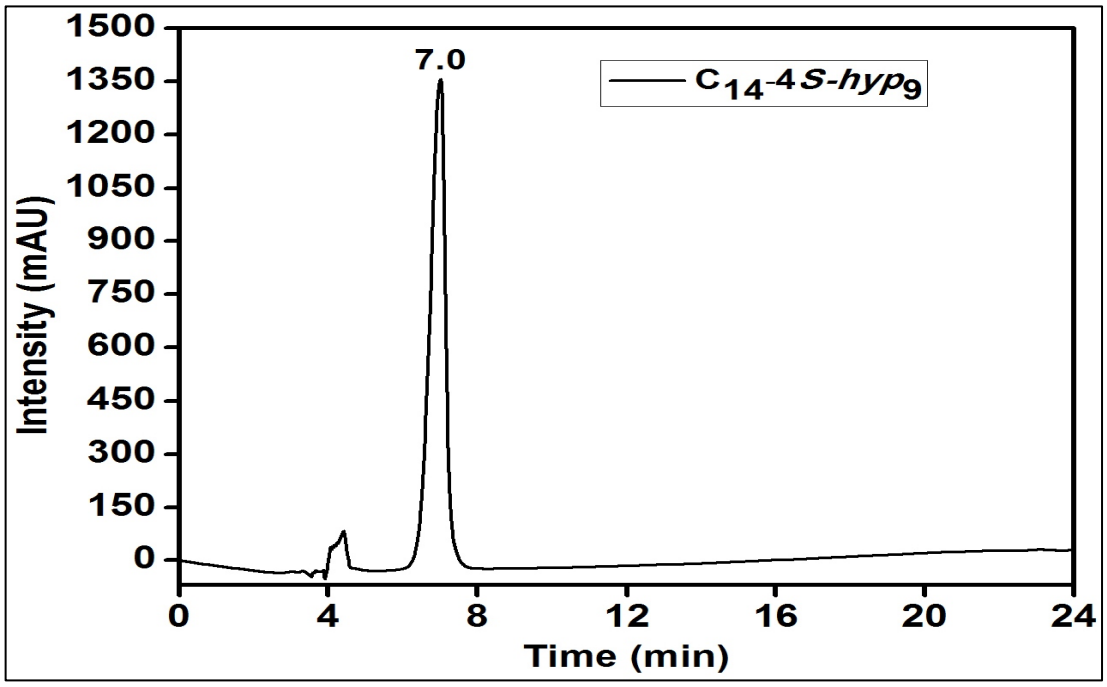
HPLC of peptide 3 (Dns-4*S*-*hyp*$_9$-Trp)



HPLC of peptide 4 (C_{12}-4*S*-*hyp*$_9$)



HPLC of peptide 5 (C_{14}-4*S*-*hyp*$_9$)



HPLC of peptide 6 (C_{16}-4*S*-*hyp*$_9$)

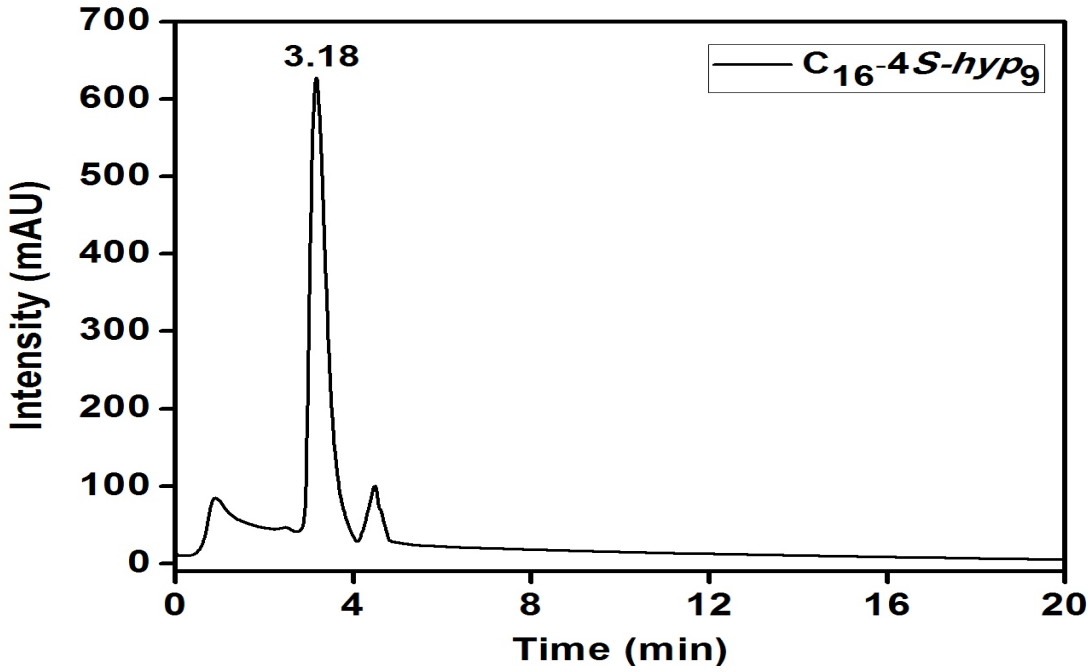
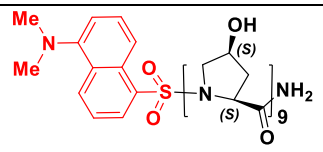
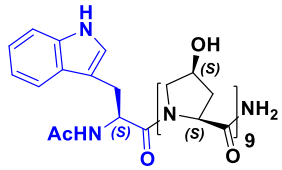
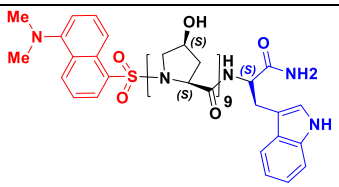
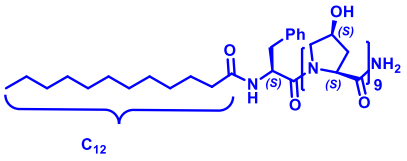
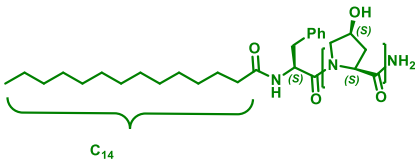
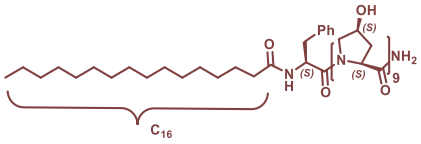


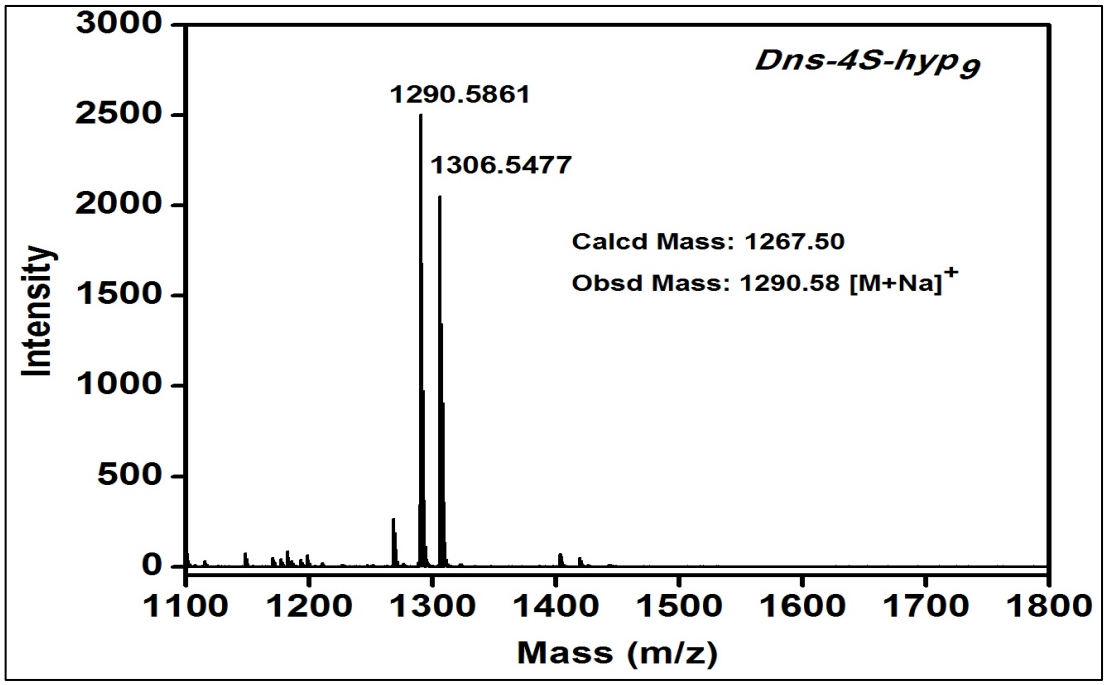
Table 1. Characterization of peptides **P1-P7.**

Peptides		Mol. Formula	Mass (cal) (Peptide+Na^+)	Mass (obs)
P1		$C_{57}H_{77}N_{11}O_{20}S$	1290.49	1290.58 $[M+Na]^+$
P2		$C_{58}H_{78}N_{12}O_{20}$	1285.53	1285.50 $[M+Na]^+$
P3		$C_{68}H_{87}N_{13}O_{21}S$	1476.56	1476.58 $[M+Na]^+$
P4		$C_{66}H_{97}N_{11}O_{20}$	1386.73 $[M+Na]^+$	

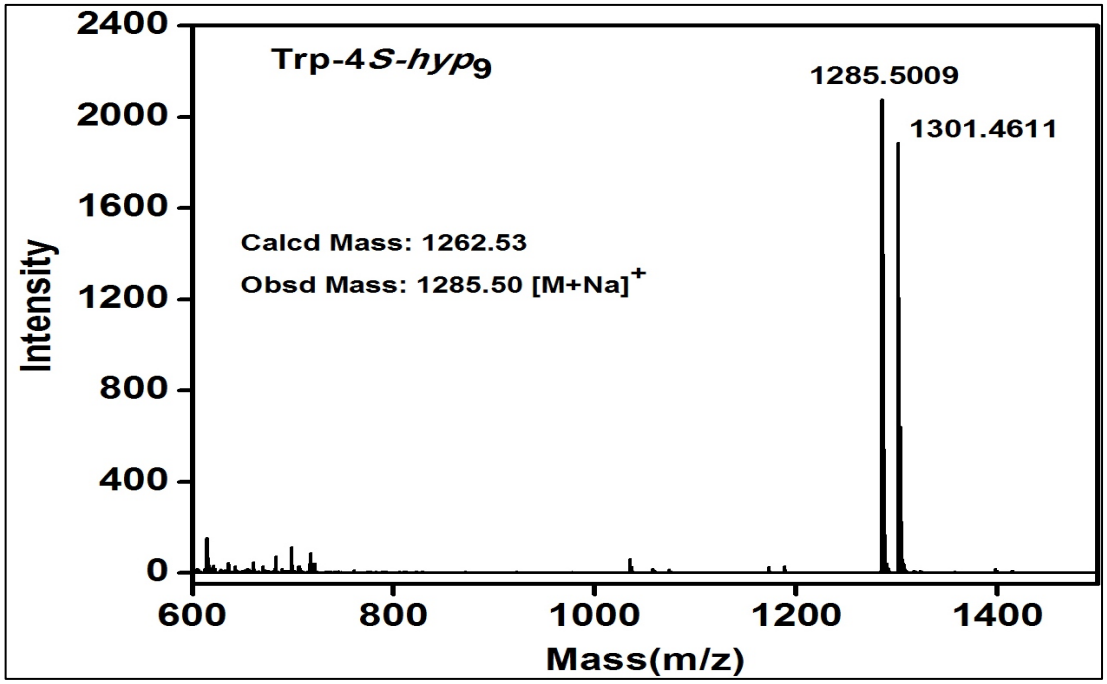
P5		$C_{68}H_{101}N_{11}O_{20}$	1414.70	1414.63 $[M+Na]^+$
P6		$C_{70}H_{106}N_{11}O_{20}$	1443.58	1443.18 $[M+Na]^+$

MALDI-TOF of peptides P1-P6

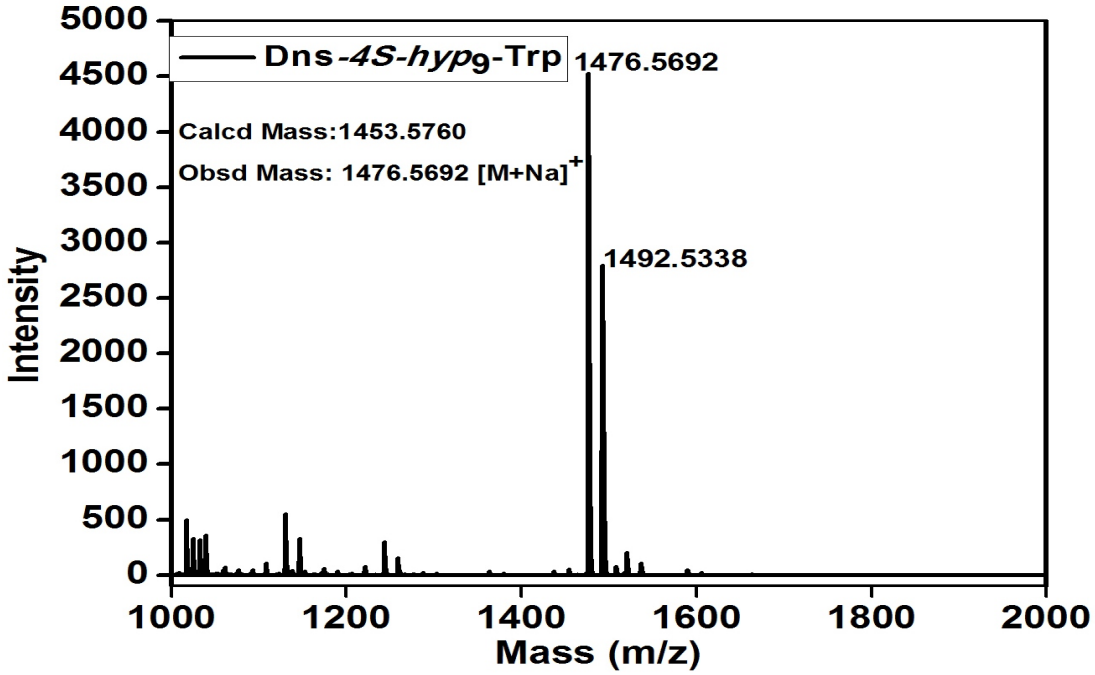
MALDI-TOF. of peptide 1 (Dns-4*S*-*hyp*$_9$)



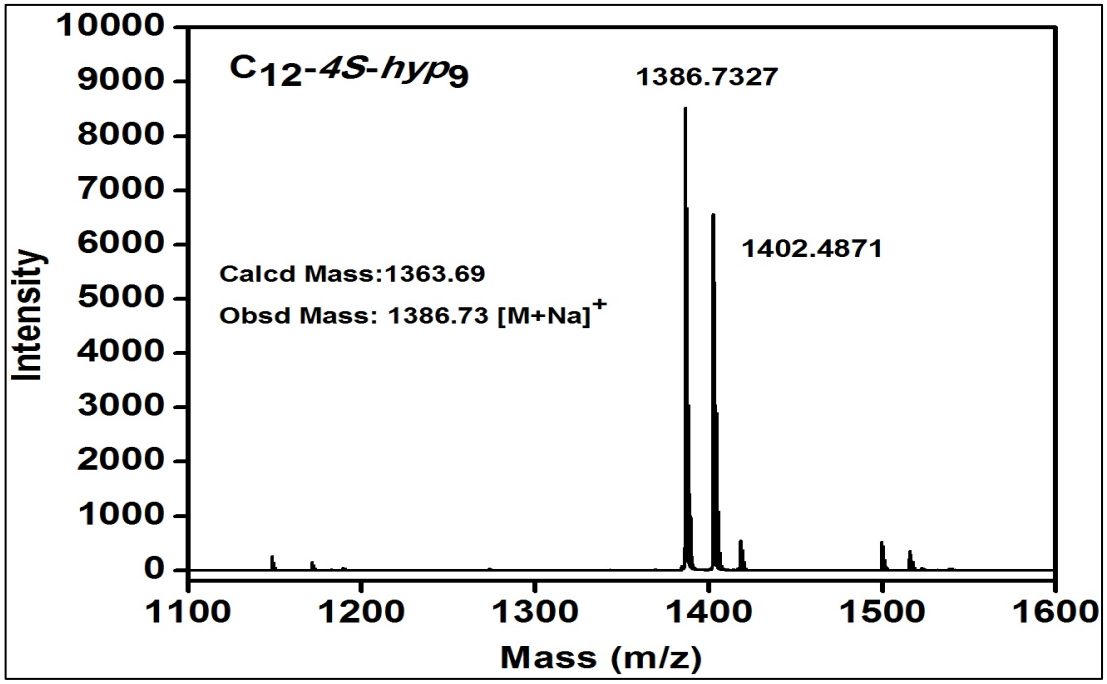
MALDI-TOF of peptide 2 (Trp-4*S*-*hyp*$_9$)



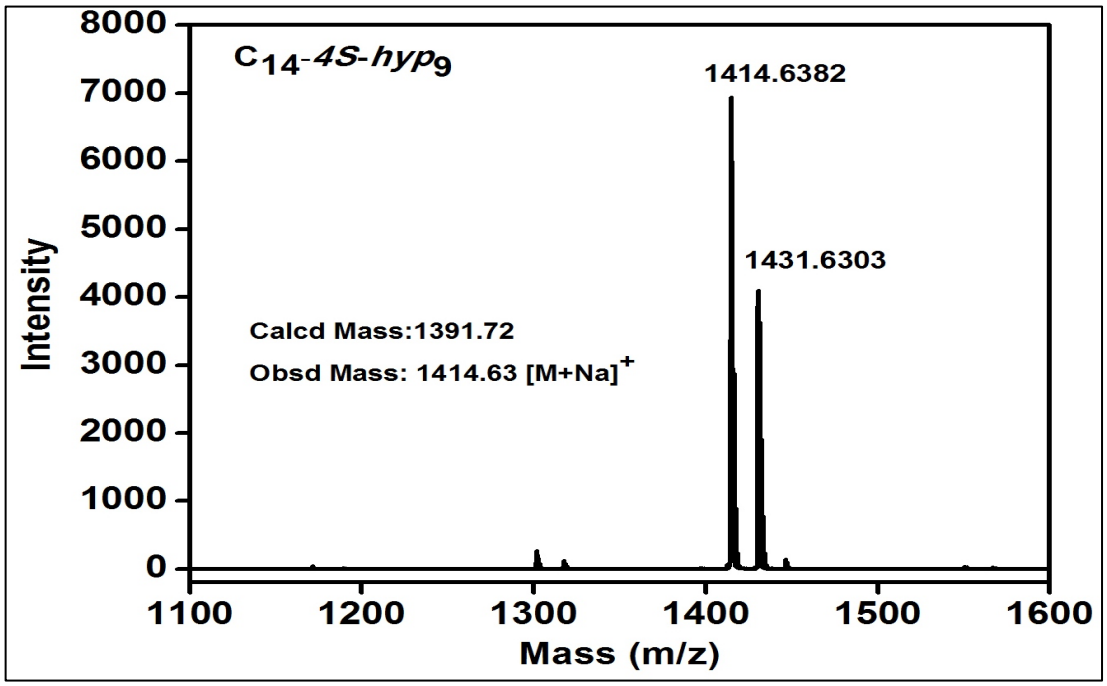
MALDI-TOF. of peptide 3 (Dns-4S-*hyp*$_9$-*Trp*)



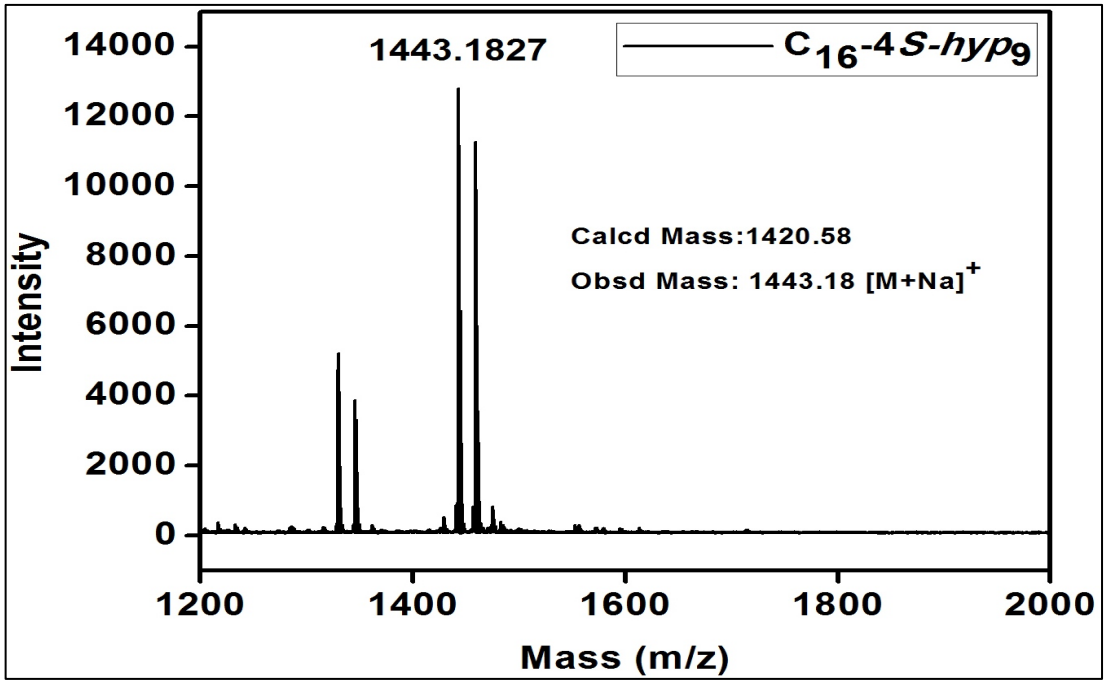
MALDI-TOF. of peptide 4 (C_{12}-4*S*-hyp_9)



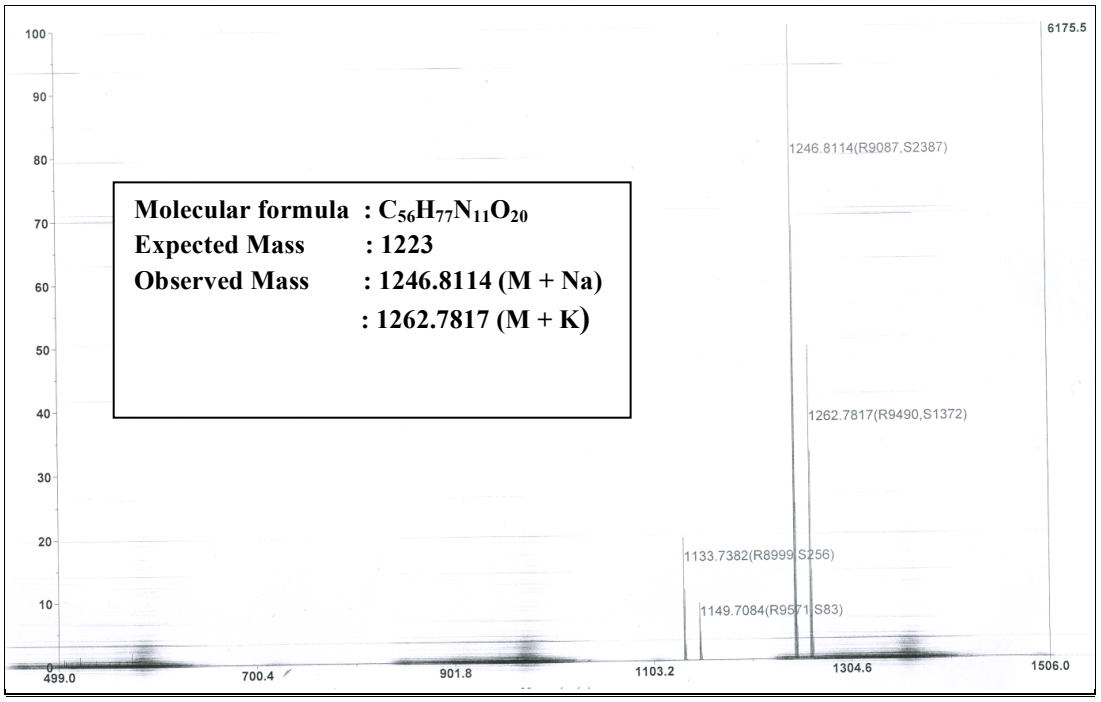
MALDI-TOF. of peptide 5 (C_{14}-4*S*-hyp_9)



MALDI-TOF. of peptide 6 (C_{16}-4*S*-*hyp*$_9$)



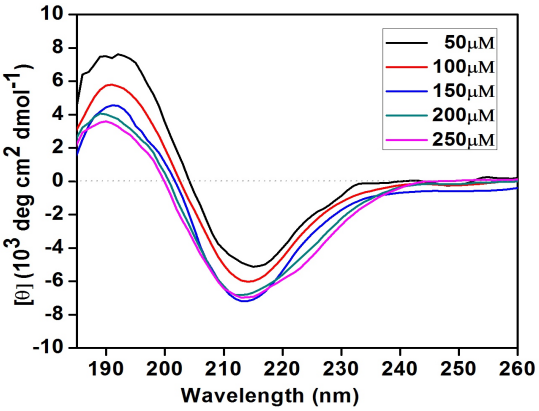
MALDI-TOF. of peptide 7 (4*S*-*hyp*$_9$)



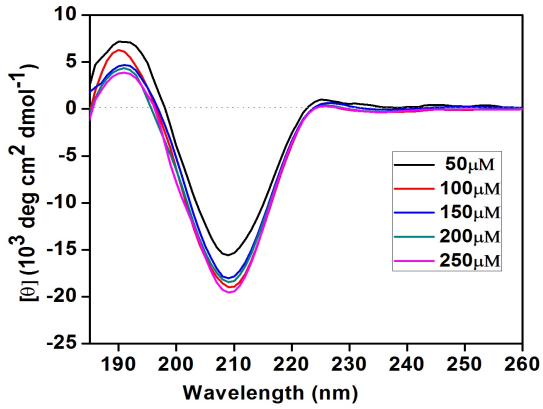
Circular Dichroism studies

Increasing peptide concentration in TFE of peptides 1-7

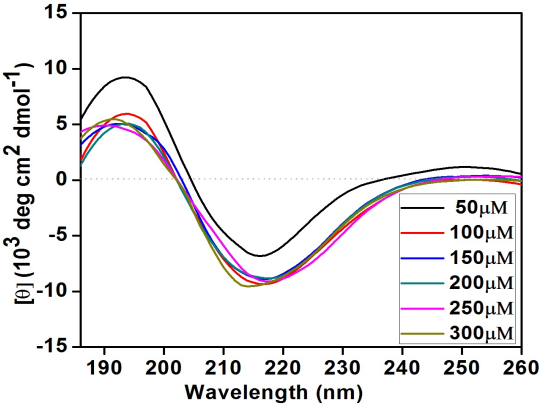
Dns-4*S*-*hyp*$_9$ (Peptide 1)



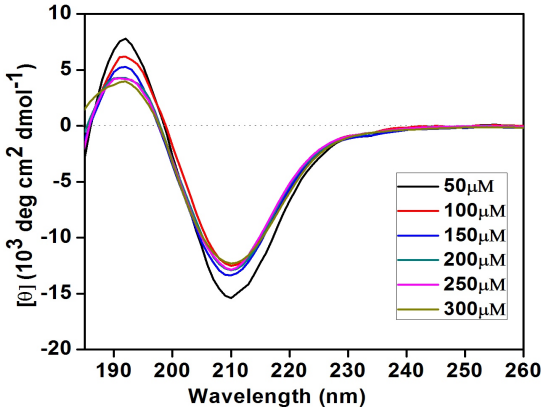
Trp-4*S*-*hyp*$_9$ (Peptide 2)



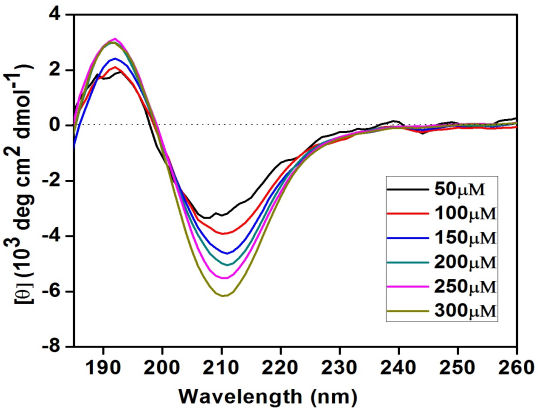
Dns-4*S*-*hyp*$_9$-Trp (Peptide 3)



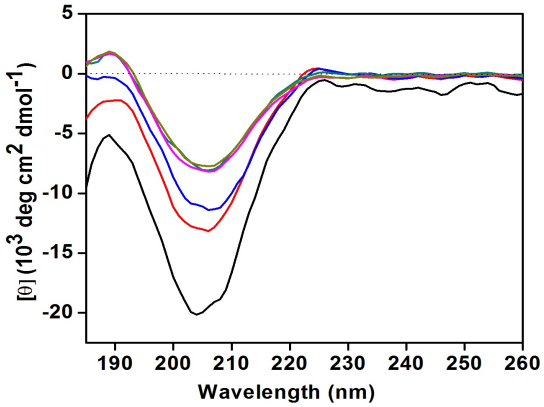
C$_{12}$-4*S*-*hyp*$_9$ (Peptide 4)



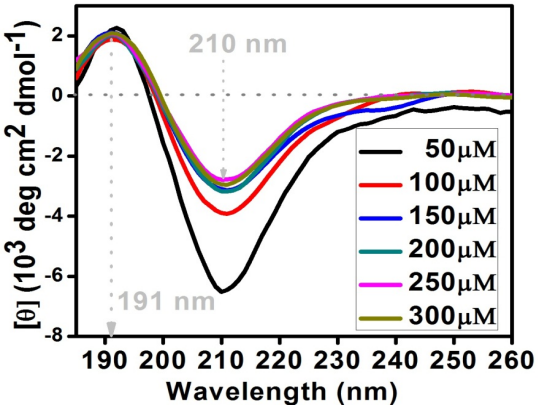
C_{14}-4S-hyp$_9$ (Peptide 5)



C_{16}-4S-hyp$_9$ (Peptide 6)

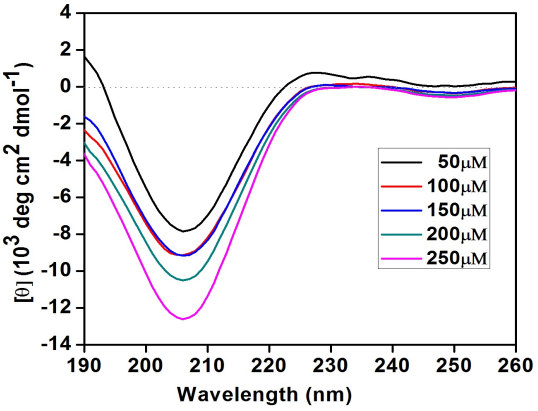


4*S*-hyp$_9$ (Peptide 7)

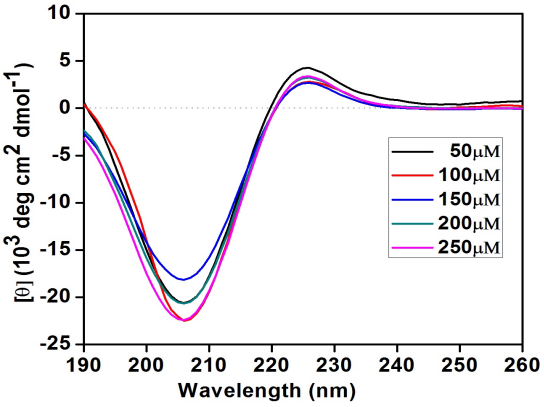


Increasing peptide concentration in buffer of peptides 1-6

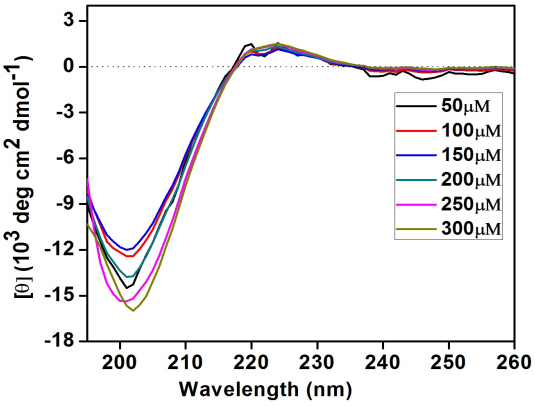
Dns-4S-*hyp*$_9$ (Peptide 1)



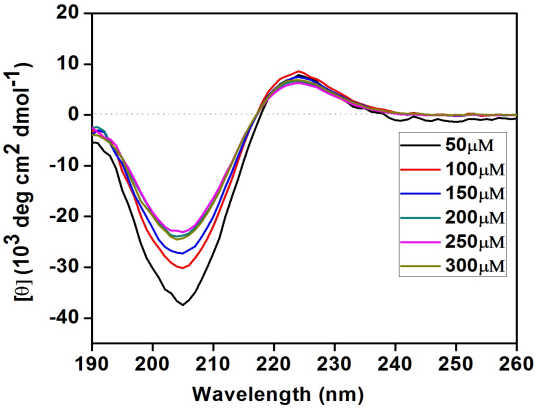
Trp-4S-*hyp*$_9$ (Peptide 2)



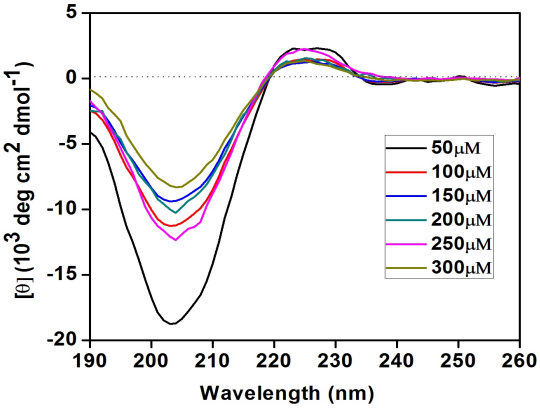
C_{12}-4S-hyp$_9$ (Peptide 4)



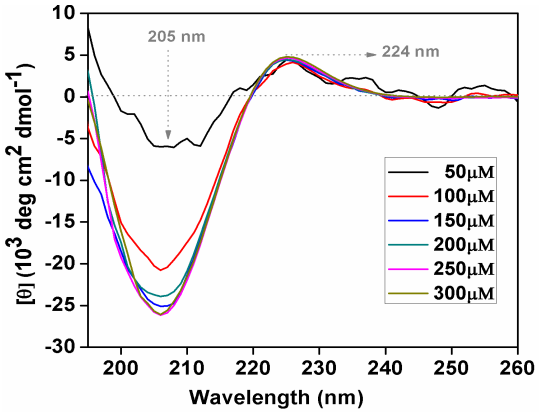
C_{14}-4S-hyp$_9$ (Peptide 5)



C_{16}-4S-hyp$_9$ (Peptide 6)



4S-hyp$_9$ (Peptide 7)



Spectral overlap of donor P1 (Trp-4*S*-*hyp*$_9$), emission and acceptor P2 (Dns-4*S*-*hyp*$_9$) organic dye absorption

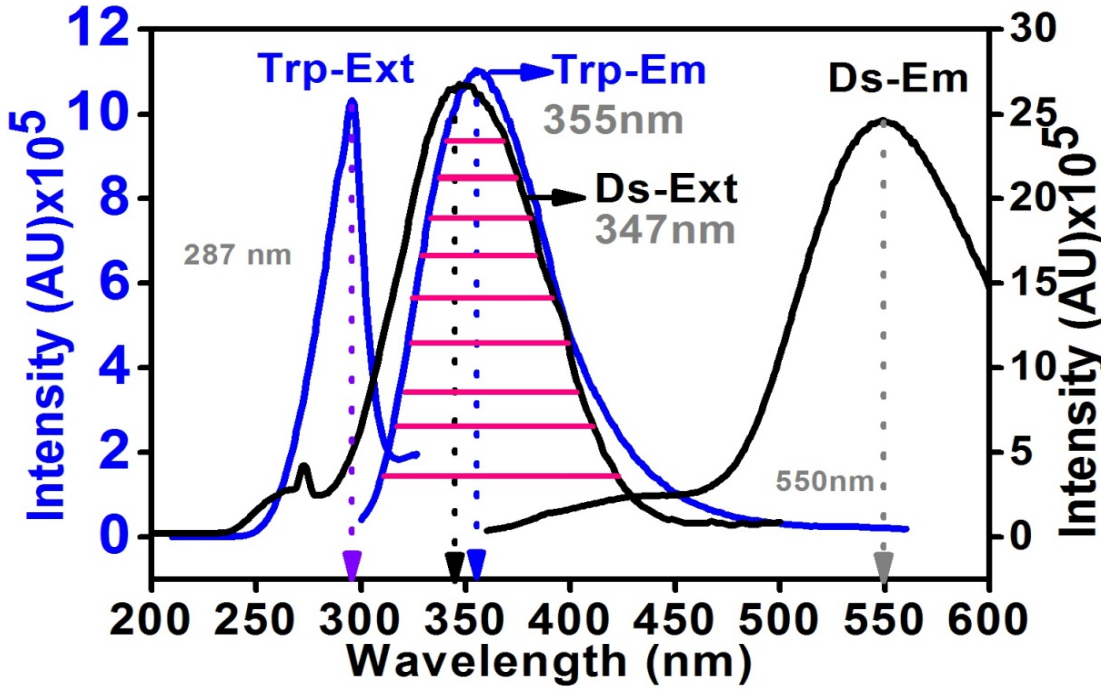
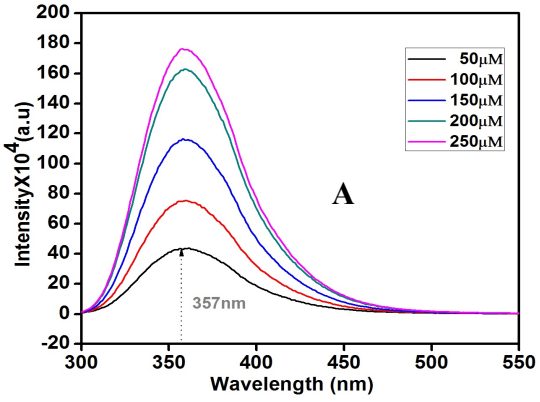


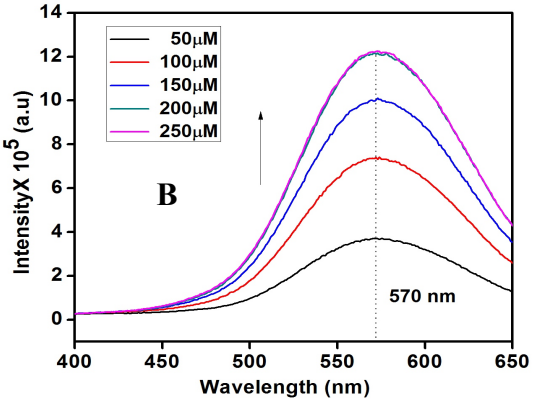
Fig. S1 Spectral overlap of donor **P1** (Trp-4*S*-*hyp*$_9$-Trp, Blue), emission and acceptor **P2** (Dns-4*S*-*hyp*$_9$) organic dye absorption; All fluorescence spectra are recorded in TFE at 100µM.

Fluorescence emission of peptides P1 and P2 in Buffer

Peptide P1 (Trp-4*S*-*hyp*$_9$)



Peptide P2 (Dns-4*S*-*hyp*$_9$)



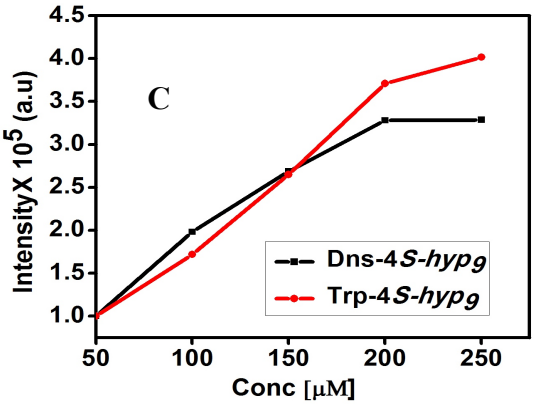
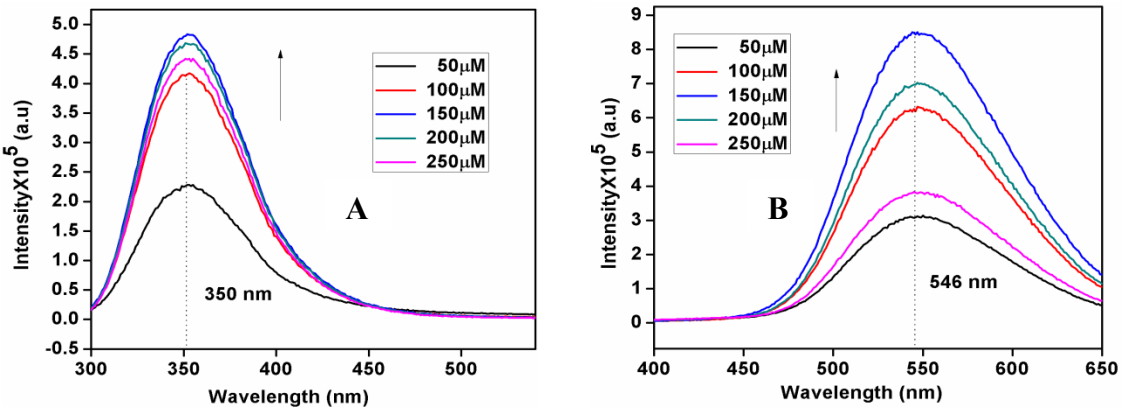


Fig. S2. Fluorescence spectra of peptides (A) **P1** (Trp-4*S*-*hyp*$_9$-Ds), (B) **P2** (Dns-4*S*-*hyp*$_9$), (C) Intensity of fluorescence spectra of as a function of concentration of peptide; All fluorescence spectra are recorded in TFE at 50-250 μM.

Fluorescence emission of peptides P1 and P2 in TFE

Peptide P1 (Trp-4*S*-*hyp*$_9$) **Peptide P2 (Dns-4*S*-*hyp*$_9$)**



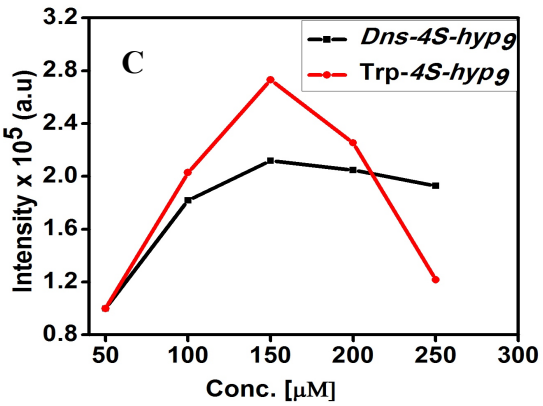


Fig. S3 Fluorescence spectra of peptides (A) **P1** (Trp-4*S*-*hyp*$_9$), (B) **P2** (Dns-4*S*-*hyp*$_9$), (C) Intensity of fluorescence spectra of as a function of concentration of peptide; All fluorescence spectra are recorded in TFE at 50-250 μM.

FESEM images for higher concentration (300μM)

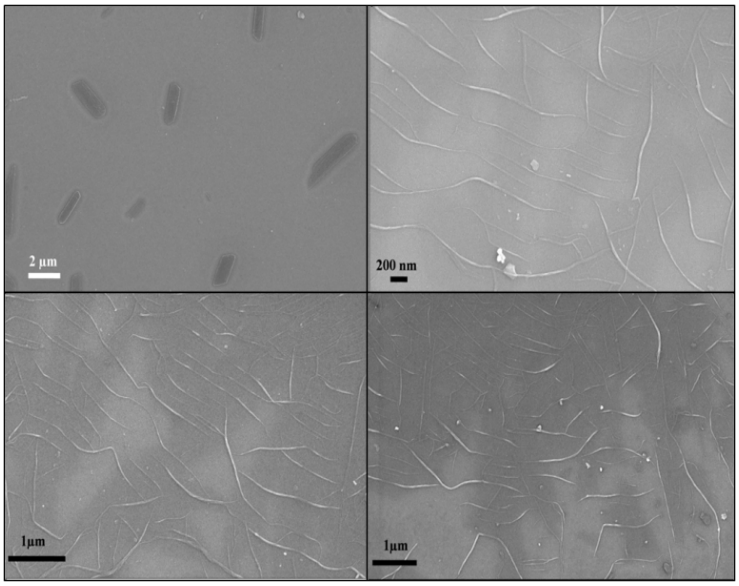


Fig. S4. FESEM images for peptides (A) **P7** (4*S*-*hyp*$_9$), (B) **P4** (C_{12}-4*S*-*hyp*$_9$-), (C) **P5** (C_{14}-4*S*-*hyp*$_9$) (D), **P6** (C_{16}-4*S*-*hyp*$_9$-C_{16}) in TFE at 300μM.

Fluorescence measurement of peptide in various solvents

Fluorescence measurements of peptide conjugated with fluorescent dye were performed on Horiba Jobin Yvon Fluorolog 3 spectrophotometer. The samples for fluorescence spectra were prepared by mixing calculated amounts of

polyproline peptides in sodium phosphate buffer (pH 7.2, 5 mM) and TFE. The prepared samples were used to record fluorescence spectra in a quartz cell (Hellma, path length 1.0 cm) at ambient temperature. The λ_{exc} 287 for tryptophan and 330 nm for dansylwith scan range from 300 nm to 580 nm; The excitation slit width of 3 nm and emission slit width of 7 nm were used.

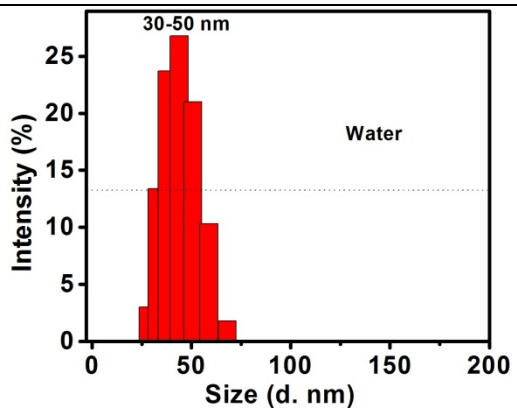
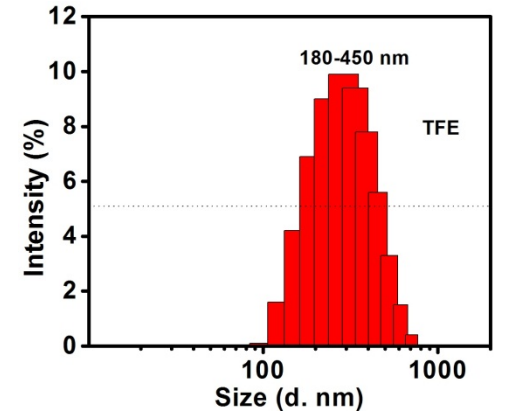
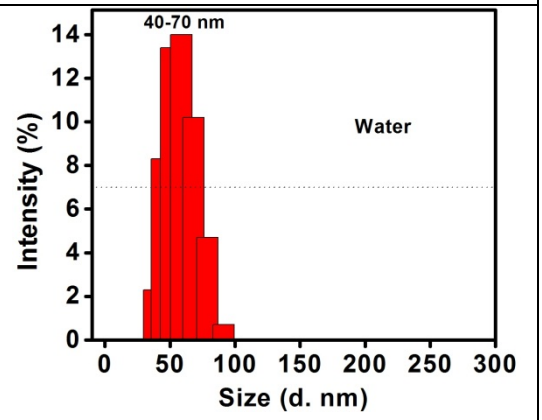
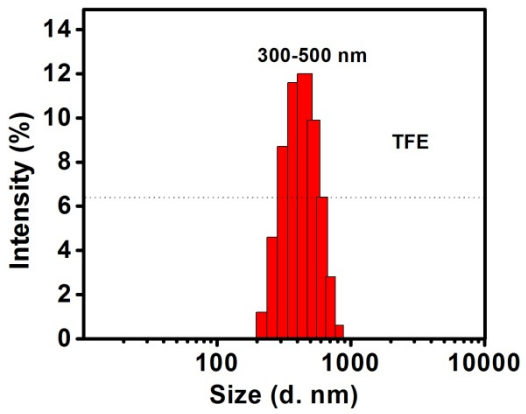
Fluorescence resonance energy transfer (FRET) studies in TFE

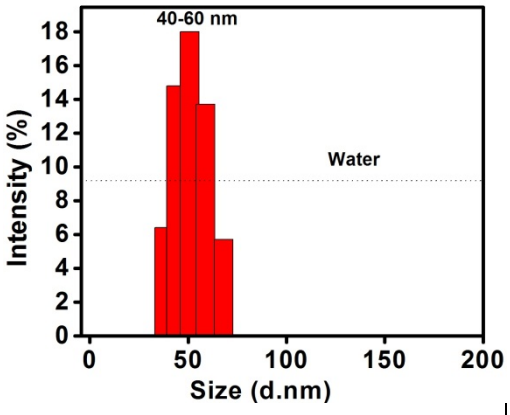
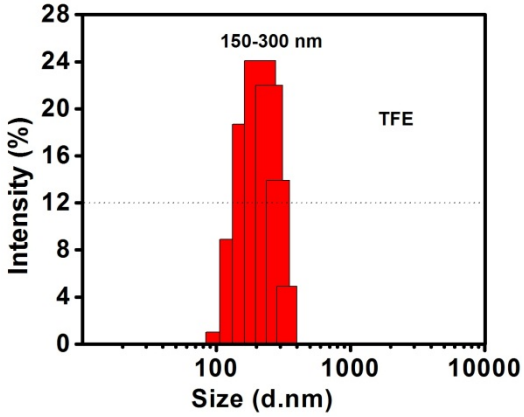
Horiba Jobin Yvon Fluorolog 3 spectrophotometer was used to measure the fluorescence intensity of FRET. The samples for FRET studies were prepared by mixing calculated amounts of polyproline peptides in Trifluoroethanol (TFE). To avoid the dilution effect, calculated amount of peptides were taken into eppendorf from stock solution and water was evaporated by using speed vacuum and desired solvent was added. Intensity of FRET was measured with the exciting wavelength of 295 nm and scan range from 310 nm to 580 nm. The excitation slit width of 3 nm and emission slit width of 7 nm were used. Each sample was measured three times in parallel to be averaged.

Field Emission Scanning Electron Microscopy (FESEM)

Calculated amounts of polyproline peptides were taken into eppendorf from stock solution and water was evaporated by using speed vac. Desired solvent was added to it and solution was vortexed for 1 min and centrifuged. 5 μL of supernatant solution was then drop casted on silicon vapour before 1hr of recoding for TFE samples and overnight for water samples and dried. Samples were allowed to dry at room temperature in vacuum desiccators and then coated with gold. Scanning electron microscopic imaging was performed using ZEISS ULTRA PLUS electron microscope operating at 30 kV.

Dynamic light scattering (DLS) histogram of **P5** and **P6**

Peptide	50 µM	300µM
P5 **C14-4*S*-*hyp*$_9$**	 	 

Peptide	300µM in Water	300µM in TFE
P6 **C16-4*S*-*hyp*$_9$**		

Size of particles in water and TFE at 300 μM*

Peptide	Size (nm) (300μM, Water)	Size (nm) (300μM, TFE)
(**P5**) C14-4*S*-hyp_9	30-60	200-500
(**P6**) C16-4*S*-hyp_9	35-65	125-300

***Particle size corresponds to range at half intensity height**