Electronic Supporting Information

Self-assembly of penta-selenopeptide into amyloid fibrils

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Materials and Methods:

Rink Amide AM Resin (loading 0.62 mmole/g) was purchased from Novabiochem. Hydroxybenzotriazole (HOBt) N,N′-Diisopropylcarbodiimide (DIC) were purchased from Sigma-Aldrich. Fmoc-Sec(Bzl)-OH was prepared as previously describe procedure[1] and used HPLC grade solvents; Dimethylformamide (DMF), Methanol, Dichloromethane (DCM), Diethyl ether were used.

Solid phase peptide synthesis of Peptide 1:

Solid phase peptide synthesis (SPPS) of the selenopeptide was performed manually using Fmoc protocol.[2] In brief, 80 mg Rink Amide AM resin (0.62 mmole/g loading capacity) was swelled for 1 hours in DMF followed by deprotection of amine group. Fmoc-Sec(Bzl)-OH (143.16 mg, 0.2976 mmol) dissolved in DMF and to this solution HOBt (45.57 mg, 0.2976 mmol) and DIC (93.19 µL, 0.5952 mmol) were added. This mixture was added to the resin and stirred for 6 hours. After completion of reaction, the resin was filtered and washed with DMF, methanol, DCM, and diethyl ether consecutively under vacuum. Then, 30% v/v piperidine solution in DMF was used to remove the Fmoc protecting group by shaking for 25 minutes followed by the washing with DMF, methanol, DMF and deprotection procedure repeated two times. The same procedure was applied to introduce other four Sec residues in the peptide chain with Fmoc-Sec(Bzl)-OH (71.58 mg, 0.1488 mmol) HOBt (22.78 mg, 0.1488 mmol) and DIC (46.59 µL, 0.2976 mmol).

Resin cleavage procedure:

Peptide **1** loaded on resin, was stirred in a solution of (1650 µL) TFA and (100 µL) water in the presence of ethane dithiol, thioanisole and phenol scavengers for 3 hours, cleaved peptide was precipitated in dry chilled diethyl ether. The mixture was centrifuged and diethyl ether was then

decanted. This step was repeated twice, resulting in an off white precipitate. The purity of the peptide was confirmed by eluting the crude peptide by reverse phase High Performance Liquid Chromatography (HPLC) with 50-50% acetonitrile:water mixture for 30 minutes. There is no stench issue with this reported peptide. Yield: 31.5% (25.2 mg with respect to resin): White colour solid. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 8.89 (d, J=8.0, 1H), 8.65 (d, J=8.0, 1H), 8.43 (d, J=8.0, 1H), 8.25 (d, J=8.0, 1H), 8.26 (s, 1H, 2H), 7.43 (s, 1H), 7.14-7.27 (m, 25H), 4.62-4.75 (m, 3H), 4.47 (q, J=8.0, 1H), 4.10 (t, J= 8.0, 1H), 3.72-3.85(m, 10H), 2.59-2.91 (m, 10H); ^{13}C NMR (125.75 MHz, DMSO, 25 °C): δ 24.0, 25.2, 25.3, 26.7, 26.7, 26.8, 27.3, 52.0, 52.8, 52.9, 52.9, 53.0, 128.3, 126.4, 126.5, 126.7, 128.3, 128.3, 128.4, 128.8, 128.8, 139.0, 139.4, 139.4, 139.5, 167.3, 169.5, 169.6, 169.8, 171.7; ^{77}Se NMR (100 MHz, DMSO-d_6, 25 °C): 215.17, 232.31, 233.53, 235.17; IR (Acetonitrile): 1634 cm^{-1} ; HRMS (ESI^{+}): calcd. for $C_{50}H_{58}N_6O_5Se_5$ $(M+H)^{+}$ 1219.0412, found 1219.0413.

NMR Spectra and assignments of peptide 1:

The ^{1}H, ^{13}C, ^{77}Se and ^{1}H-^{1}H, COSY and TOCSY NMR spectra of peptide **1** are shown in Fig. S6-S10. The five chiral center protons of Sec units appear as a triplet, quartet and multiplet at 4.10, 4.47 and 4.60-4.77 ppm respectively, which are assigned as type a and e-c protons.. The two singlet signals for primary amide (k l-type) appeared at 7.43 and 8.25 ppm overlapped with terminal amine (j-type) protons (Fig. S6). The e-c type protons showed cross peak correlation with doublets of secondary amides in COSY spectrum in the range of 8.25-8.89 ppm assigned as f i g h- type protons (Fig. S9). We observed four signals in 77Se NMR spectrum at 215.17, 232.31, 233.53 and 235.17 ppm (Fig. S8) although the peptide contains five selenium atoms. However, the presence of the fifth Se was confirmed from the molecular ion peak with characteristic isotopic pattern in the mass spectrum (Fig. S4) along with the ^{1}H NMR peak

integration that established the identity of desired peptide **1**. The spectra clearly reveal the highest level of purity with complete structural justification of selenopeptides.

FT-IR, circular dichroism (CD) and fluorescence Spectroscopy:

Infra-red spectrum of peptide **1** in acetonitrile was recorded at room temperature using PerkinElmer Spectrum One FT-IR spectrometer. Peptide **1** (20 μM-80 μM) in acetonitrile was placed into 1cm path-length quartz cell for CD measurements. Spectra were acquired using JASCO, J-815 Circular dichroism spectrometer. Each spectrum was scanned thrice and the average was taken for final plot. ThT (0.5mM) was prepared in deionized water for ThT fluorescence study. This ThT solution was mixed with peptide **1 (**10 μM- 500μM) in acetonitrile. ThT fluorescence spectrum was collected by using Varian Cary Eclipse Fluorescence spectrophotometer, in standard quartz fluorescence cuvettes of 1 cm path-length for both, i.e. only ThT and peptide **1** mixed ThT solution. All the spectroscopic measurements were done in 25 °C.

TEM, AFM and SEM Measurements: Peptide **1**(0.25mM) in acetonitrile was spotted on 400 mesh carbon coated copper grid and kept for 5-10 minutes till excess solvent evaporated and kept in desiccator for overnight. The sample was prepared without any staining. TEM images were collected on Philips (CM 200) transmission electron microscope at an accelerating voltage of 200 kV. The sample for SEM images was prepared by placing 10 μL (20-80 μM) of same sample on the aluminum foil and the images was collected on JEOL JSM-7600F field emission gun-scanning electron microscopes. For atomic force microscopy (AFM), 20 μL of 250 μM peptide **1** in acetonitrile was spotted on freshly cleaved mica sheet. The sample on mica was dried in vacuum desiccator for overnight. AFM imaging was done in in tapping mode using AFM (Asylum research, USA) with 300 KHz silicon nitride cantilever.

Fluorescence Anisotropy Imaging Measurements:

A home-built epifluorescence/total internal reflection fluorescence (TIRF) microscope set up based on inverted optical microscope (Nikon TE2000U) was used for imaging ThT bonded peptide **1** and for anisotropy imaging. The details of the home built set up are provided elsewhere. [03]In brief, the sample was illuminated by using a circularly polarized (using a λ/4 wave plate) laser line from a 405 nm DPSS laser (Melles Griot, model: LDWL206) via a high numerical aperture (NA) objective lens (Nikon, 1.49 NA, 60X TIRF). The emission from the sample was collected by the same objective and passed through appropriate dichroic and a 445−530 nm long pass filter. The emission was recorded by a CCD camera (DVC 1412AM) as a movie. Anisotropy imaging was carried out by using a using a polarizing beam displacing prism (ThorLabs BD27) before the CCD, which separates the emission signal into two mutually orthogonal components (S- and P-). The movies were collected at 10° consecutive analyzer angle interval. All movies were collected with an exposure of 100 ms and averaged over 100 frames in ImageJ. The anisotropy (r) images from these s and p channel images were computed in MATLAB 9.0 using the following formula.

$$r = \frac{I_s - I_p}{I_s + 2I_p}$$

The maximum projection of anisotropy images and at which angle where maximum of r images is also computed in MATLAB 9.0.All measurements were performed at 295 K.

Supplementary Figures, Table and Images:

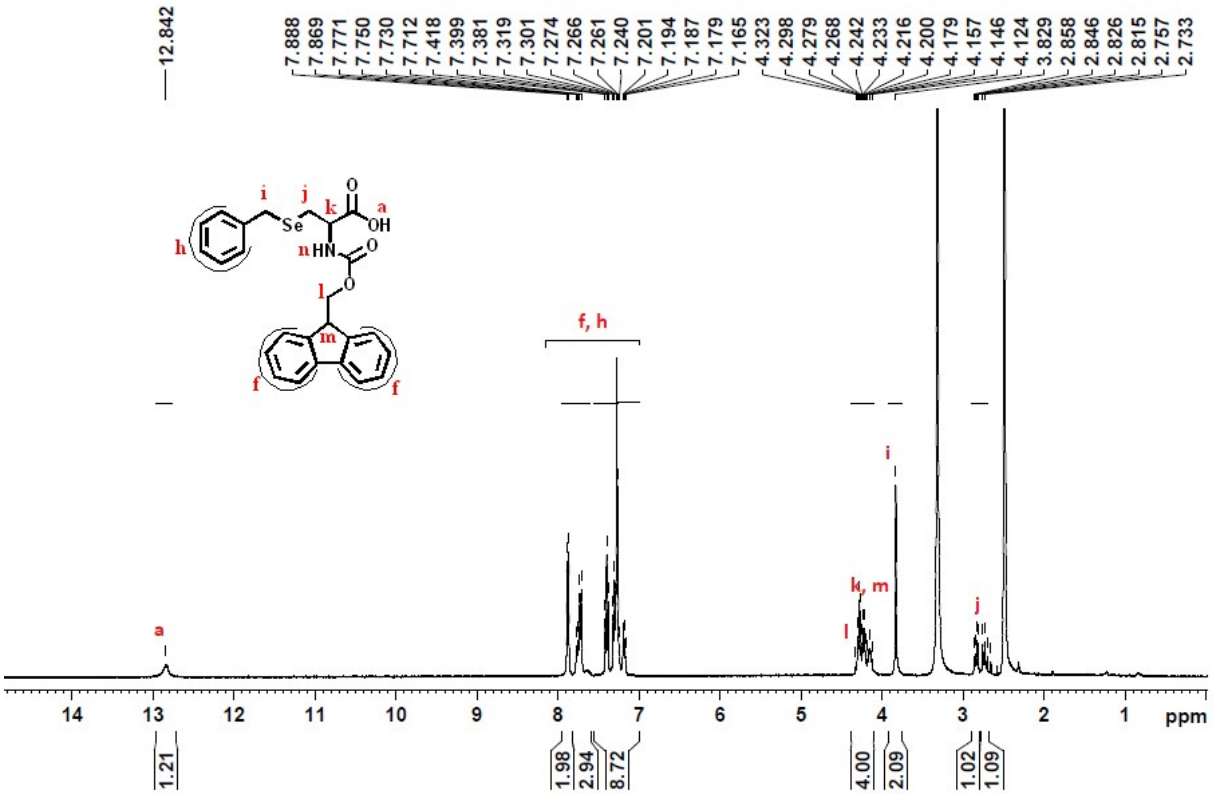

Fig. S1: ^{1}H NMR of Fmoc-Sec(Bzl)-OH

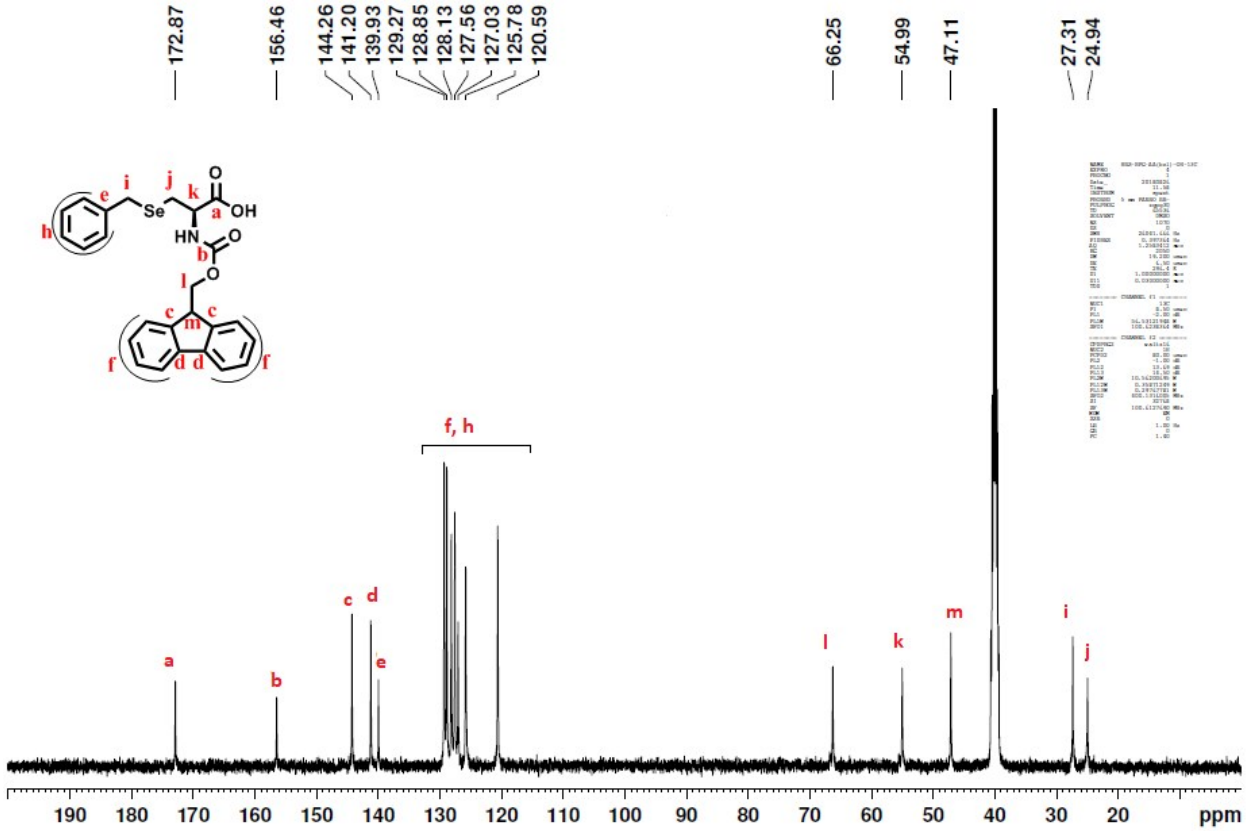

Fig. S2: ^{13}C NMR of Fmoc-Sec(Bzl)-OH

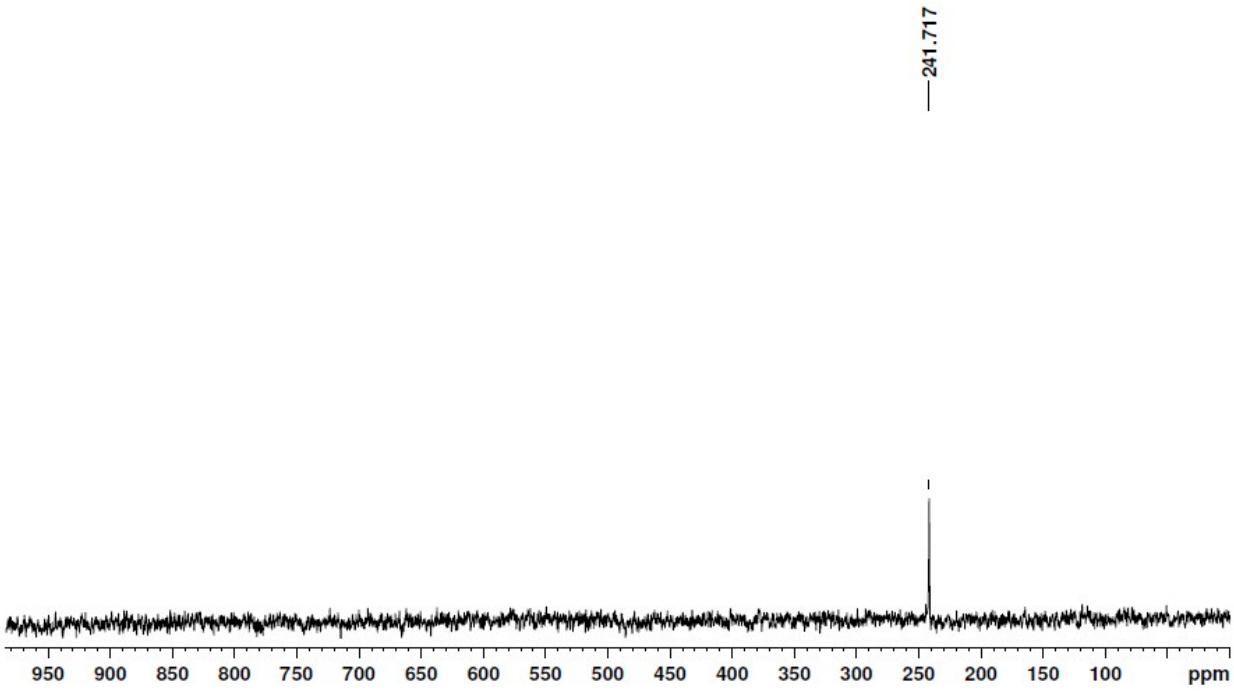

Fig. S3: ^{77}Se NMR of Fmoc-Sec(Bzl)-OH

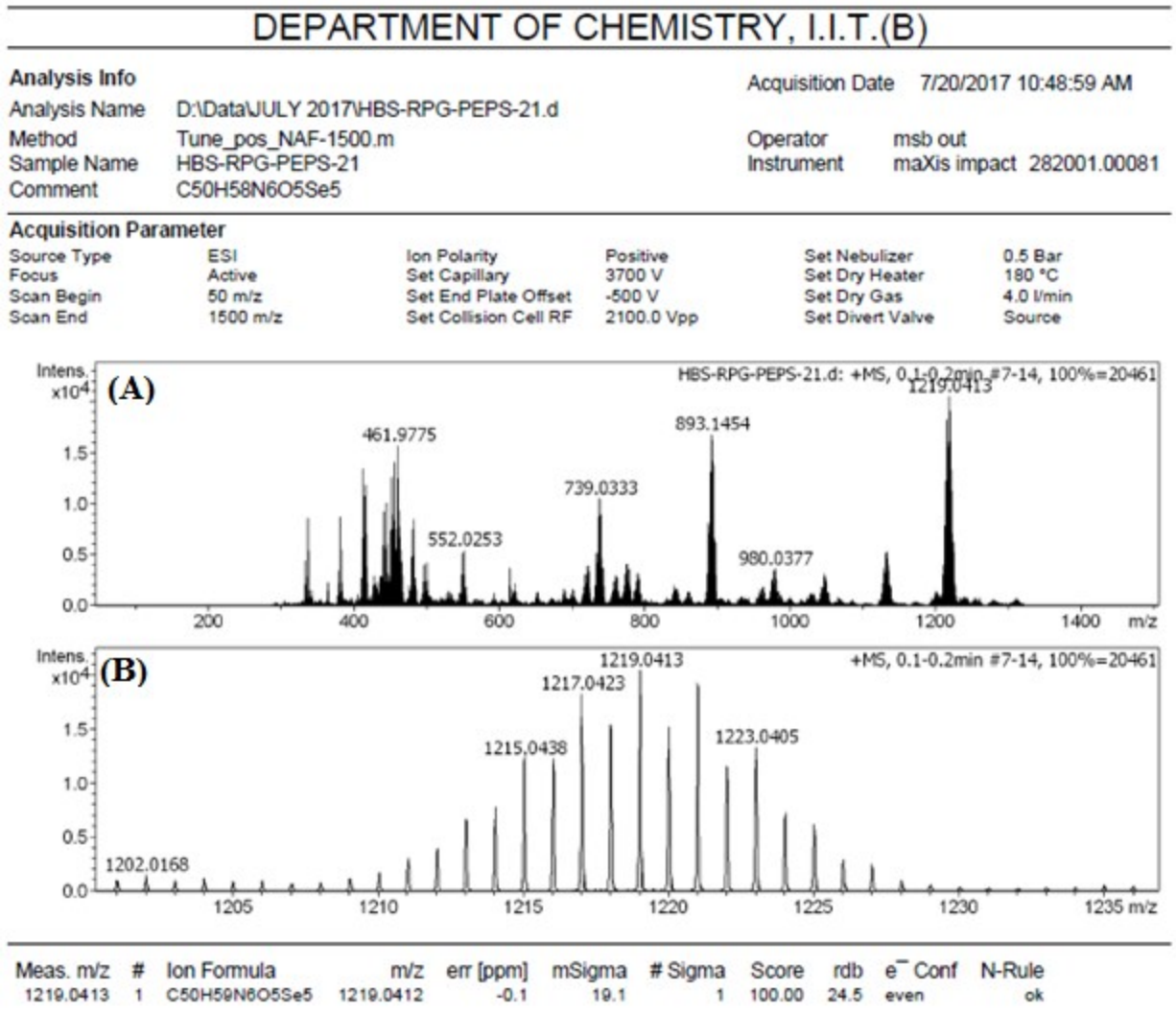

Fig. S4: (A) Mass spectrum of peptide **1**, (B) Expanded view of characteristic isotopic pattern of selenium.

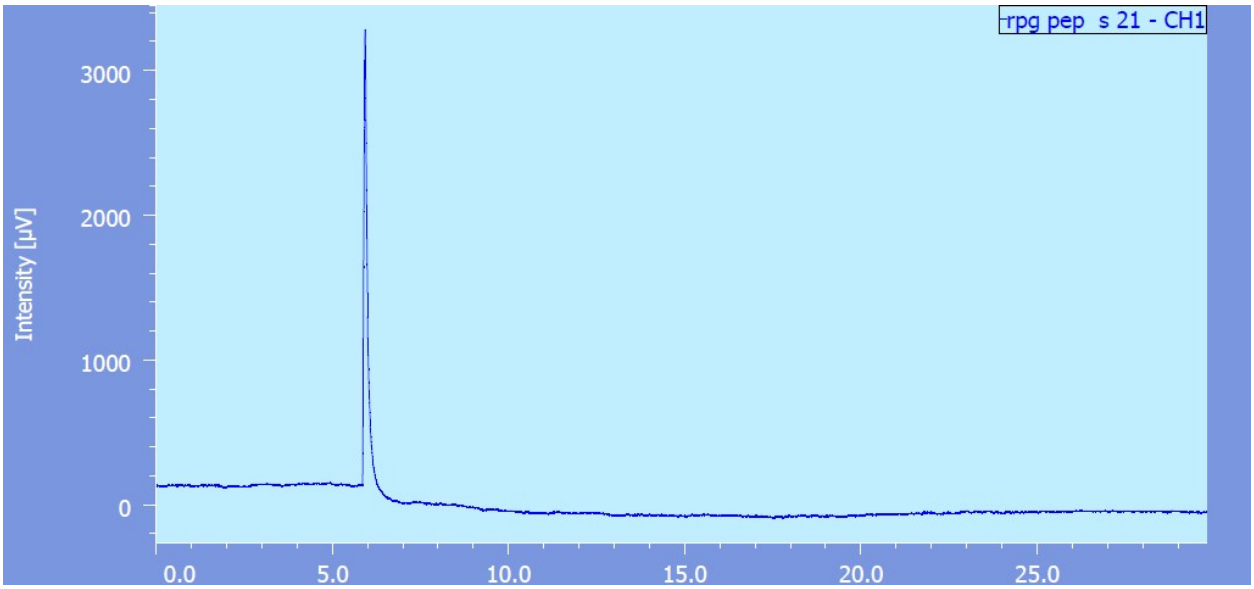

Fig. S5: HPLC Chromatogram of Peptide **1**

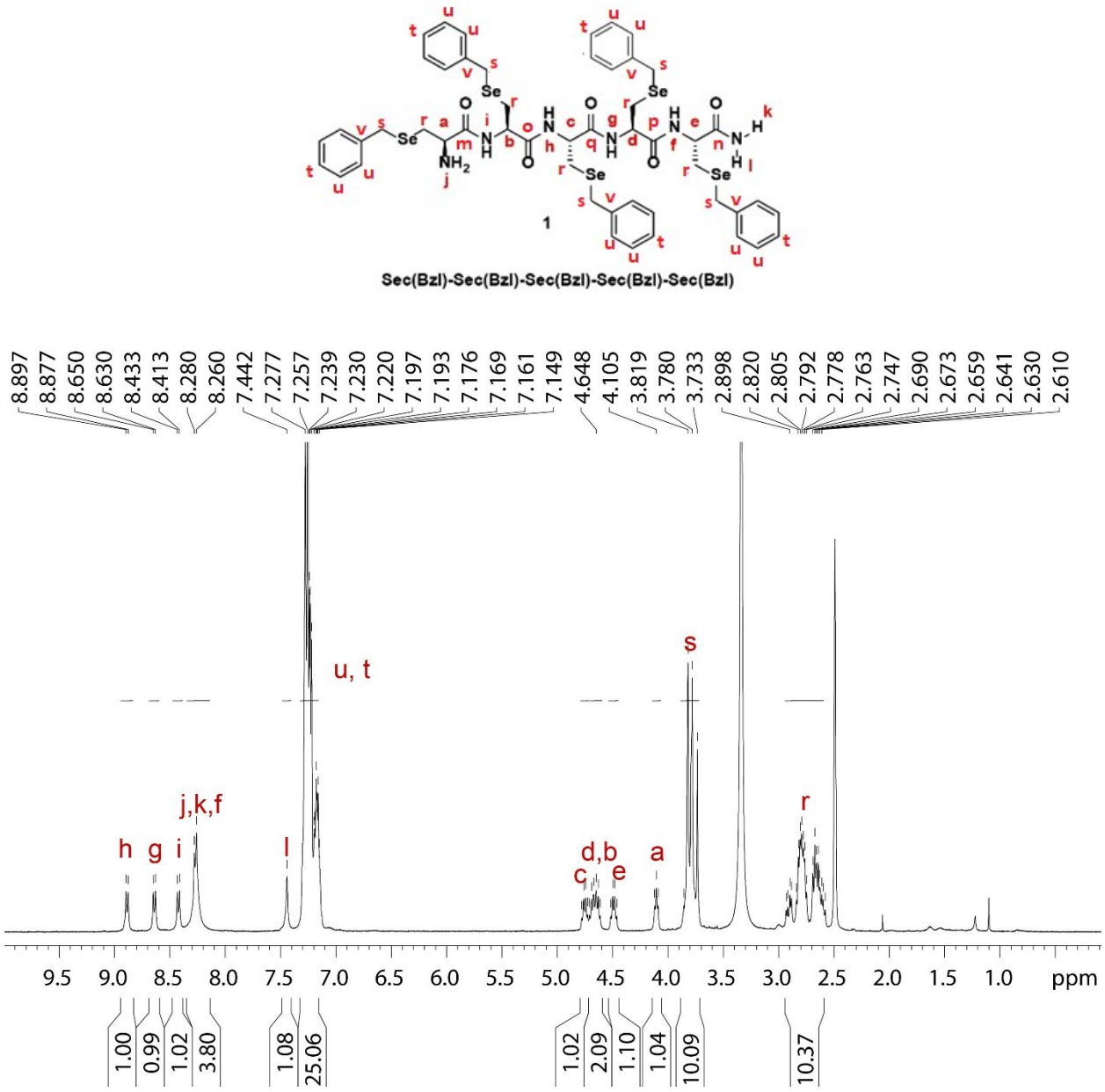

Fig. S6: ^{1}H NMR of Peptide **1**

^{13}C NMR

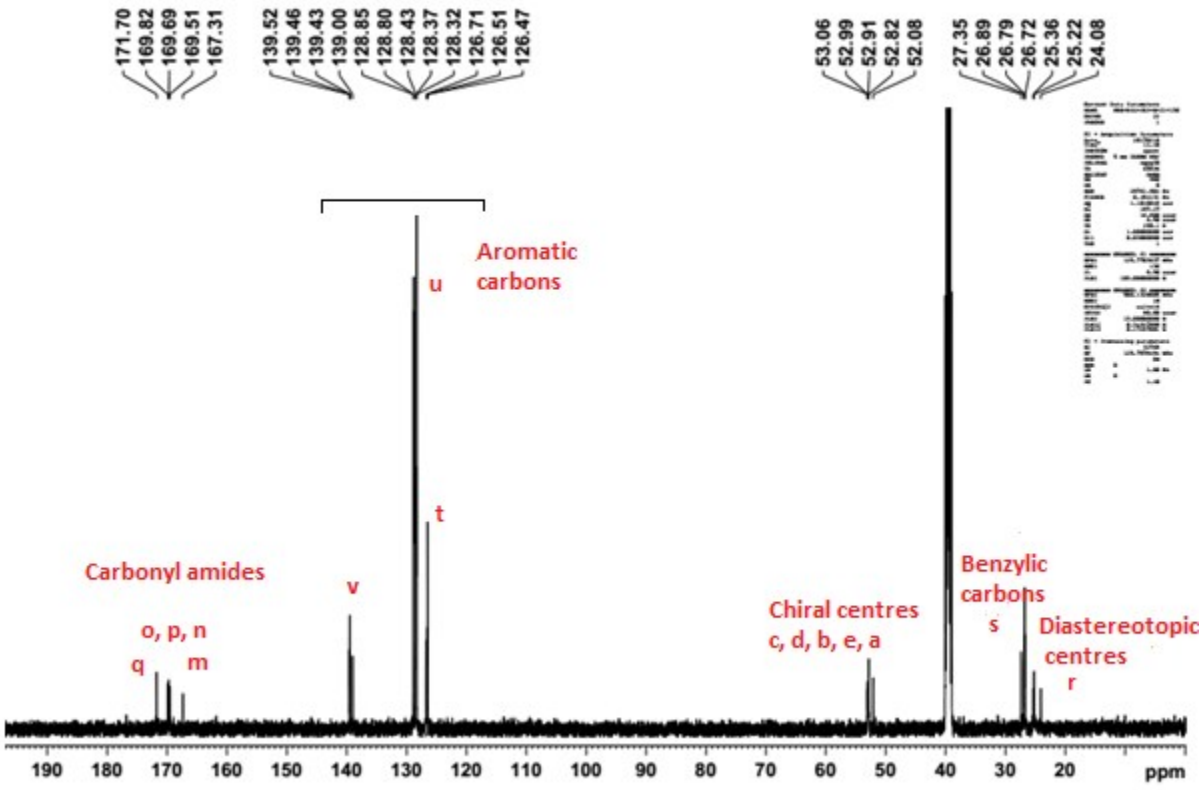

Fig. S7:^{13}C NMR of Peptide **1**

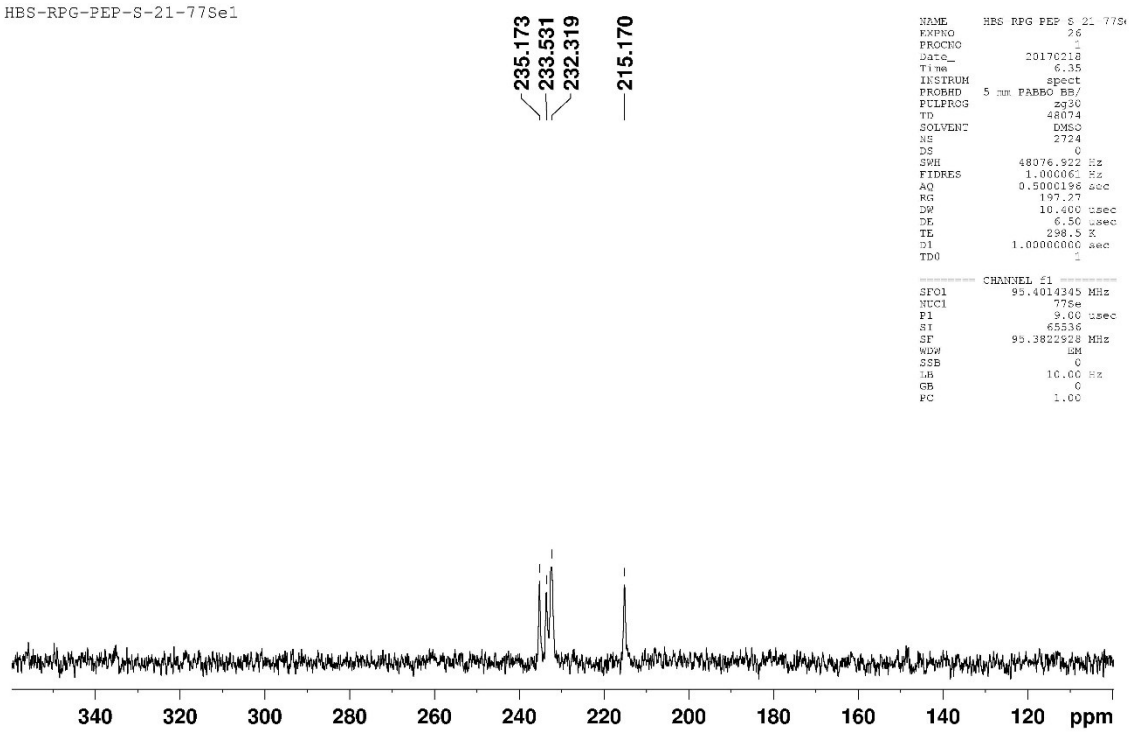

Fig. S8: ^{77}Se NMR of Peptide **1**

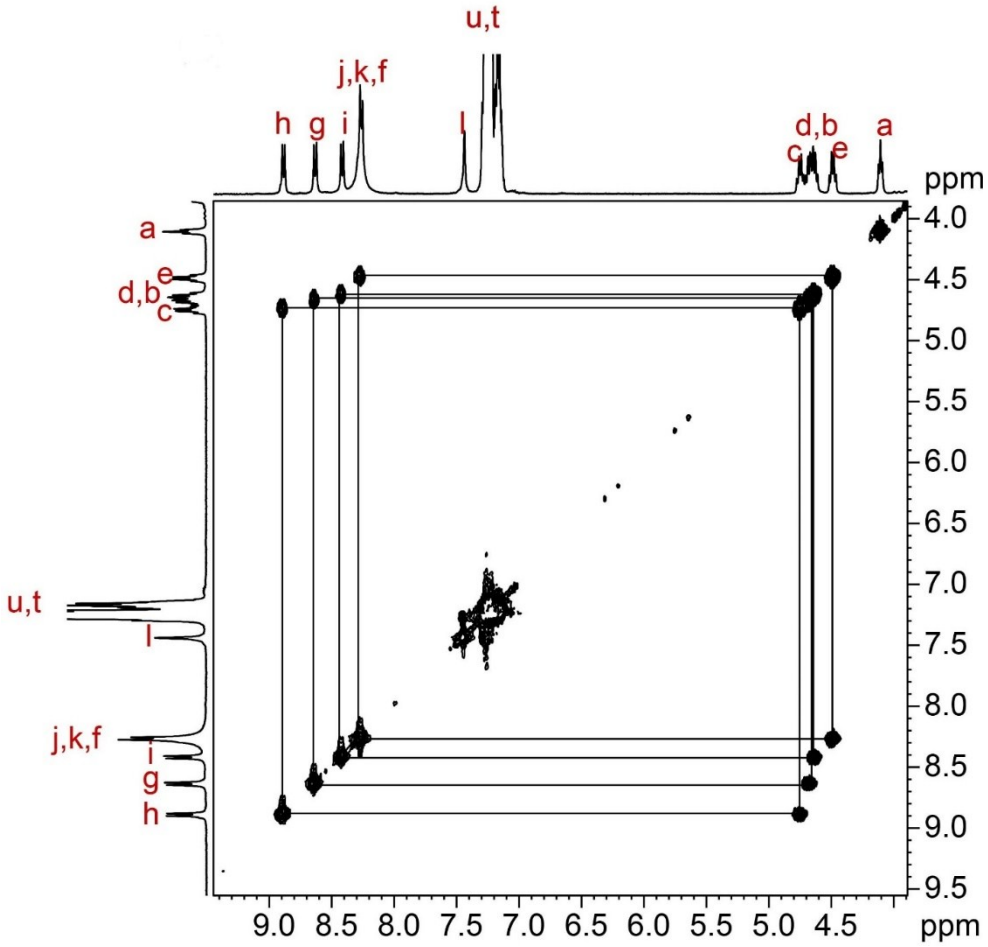

Fig. S9: ^{1}H-^{1}H COSY NMR of Peptide **1**

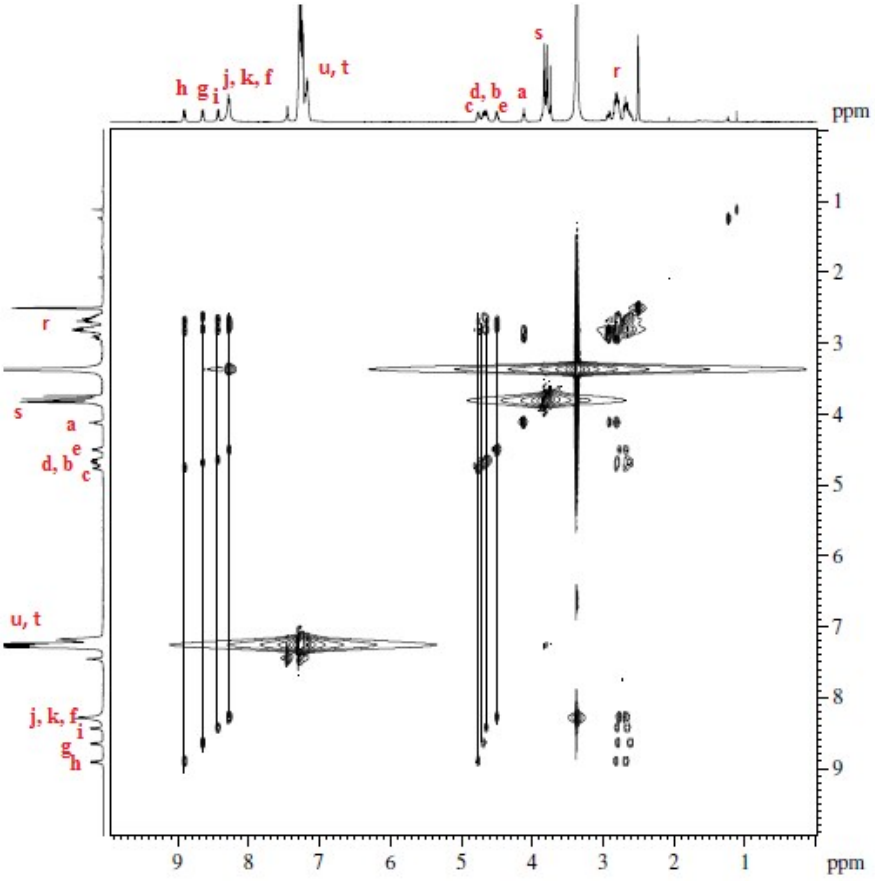

Fig. S10: TOCSY NMR of Peptide **1**

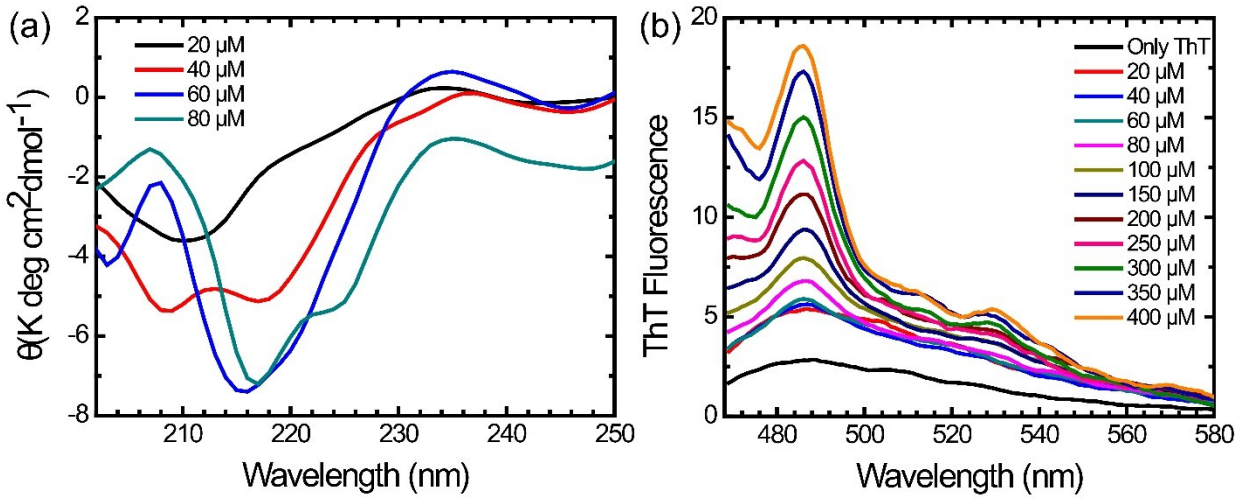

Fig. S11: (a) CD spectrum and (b) ThT fluorescence spectrum with peptide **1** in acetonitrile at different concentration.

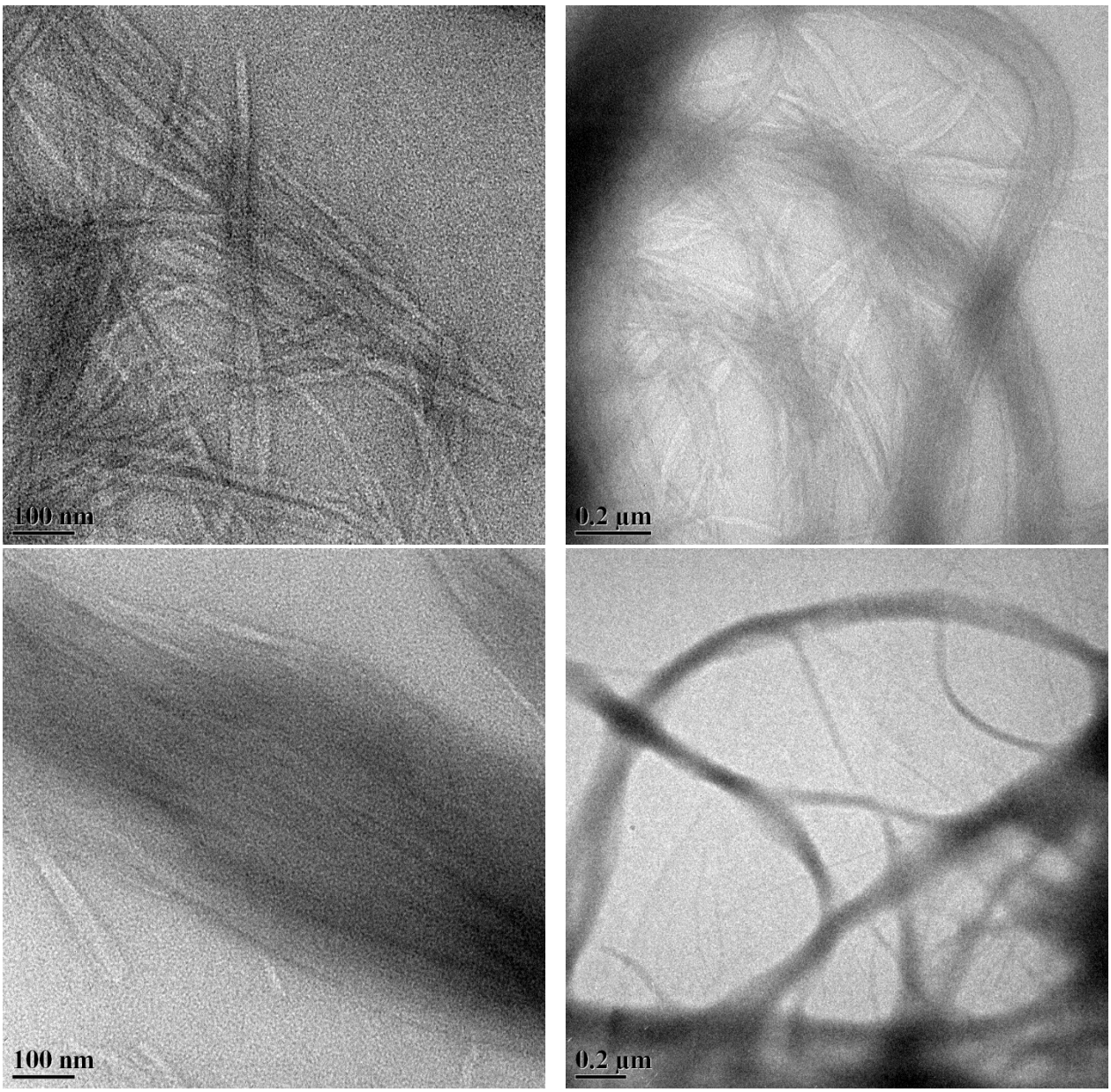

Fig. S12: TEM images of peptide **1** at 250 µM concentration.

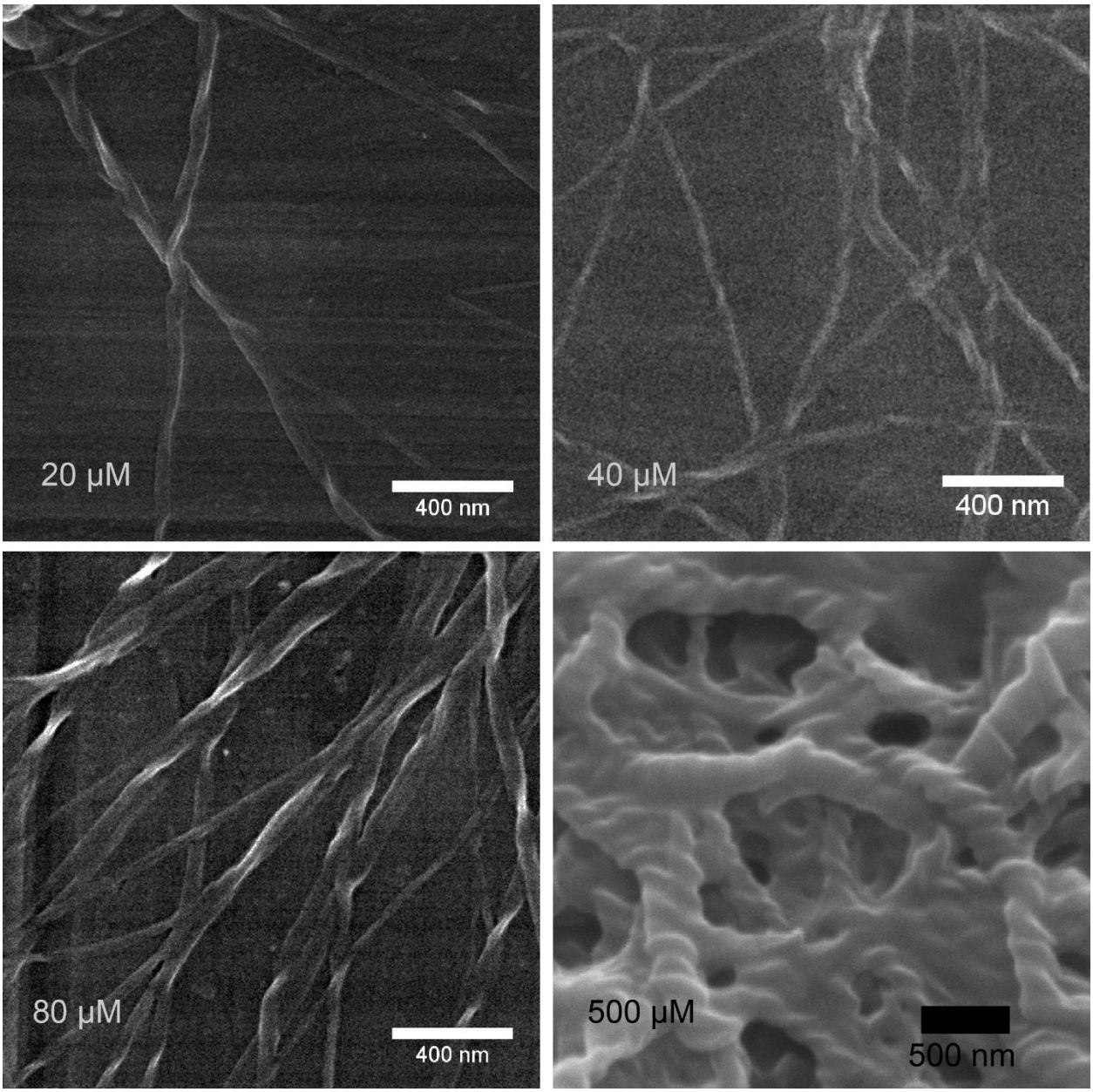

Fig. S13: SEM images of peptide **1** at different concentration

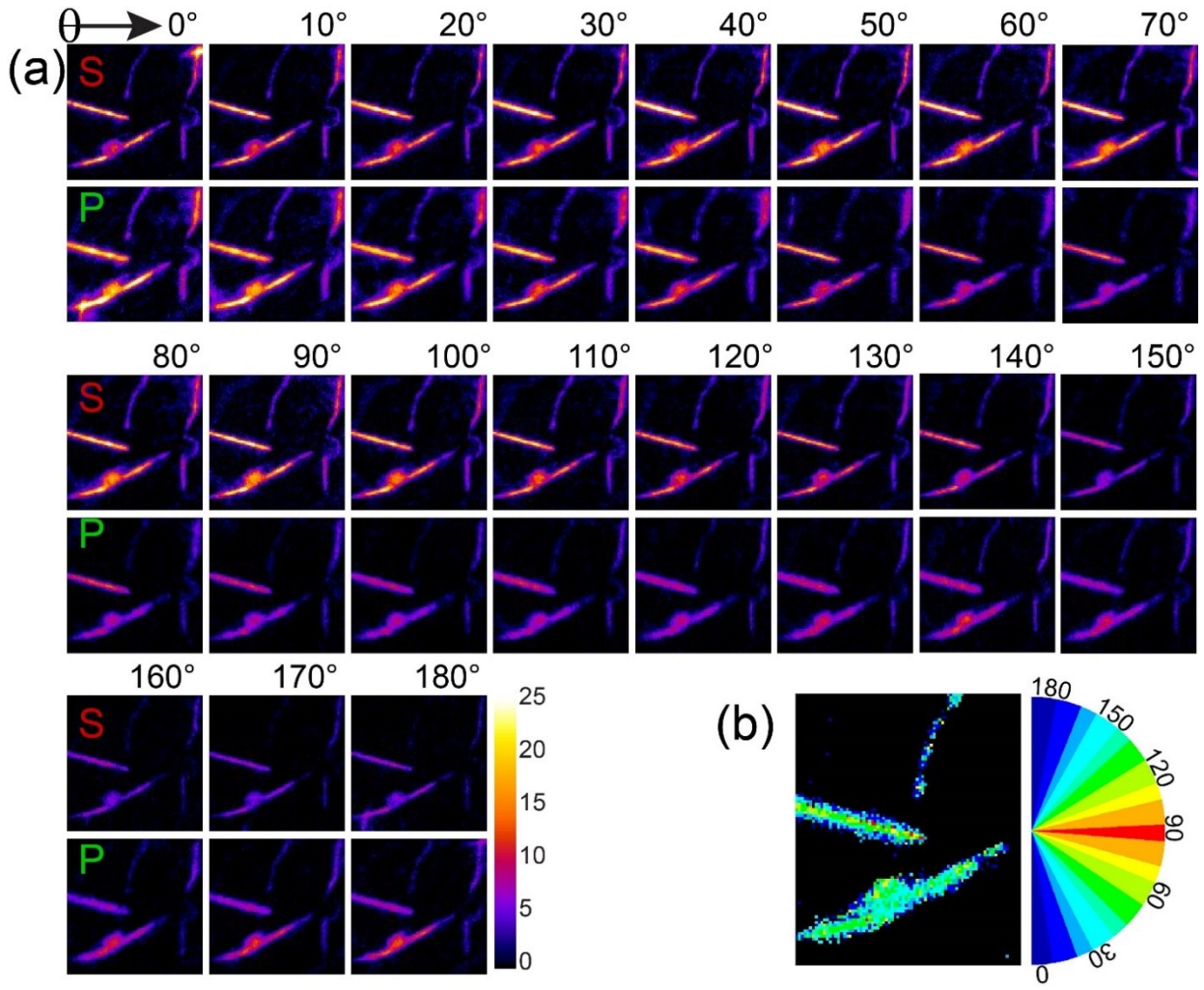

Fig. S14: Polarisation-Resolved fluorescence microscopy images of ThT bonded peptide **1**. (a) Sequence of fluoresce image taken at 10° analyser angle into two orthogonal polarised channel (S- and P-). (b) Mapping of analyser angle where maximum anisotropy observed. Color coded calibration bar represents the orientation of ThT in the peptide **1** fibrils.

Supplementary References:

[1] Jr. S. Flemer, *Protein Peptide Lett.,* **2014**, *21*, 1257-1264

[2] G. B. Fields, R. L. Noble, *Int. J. Peptide Protein Res*, **1990**, *35*, 161-214

[3] S. Bhattacharya, D. K. Sharma, S. De, J. Mahato, and A. Chowdhury, *J. Phys. Chem. B*, **2016**, *120*, 12404-12415