SUPPORTING INFORMATION

Heterochirality Restricts the Self-Assembly of Phenylalanine Dipeptides Capped with Highly Aromatic Groups

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Experimental Methods

Peptide Synthesis and Characterization

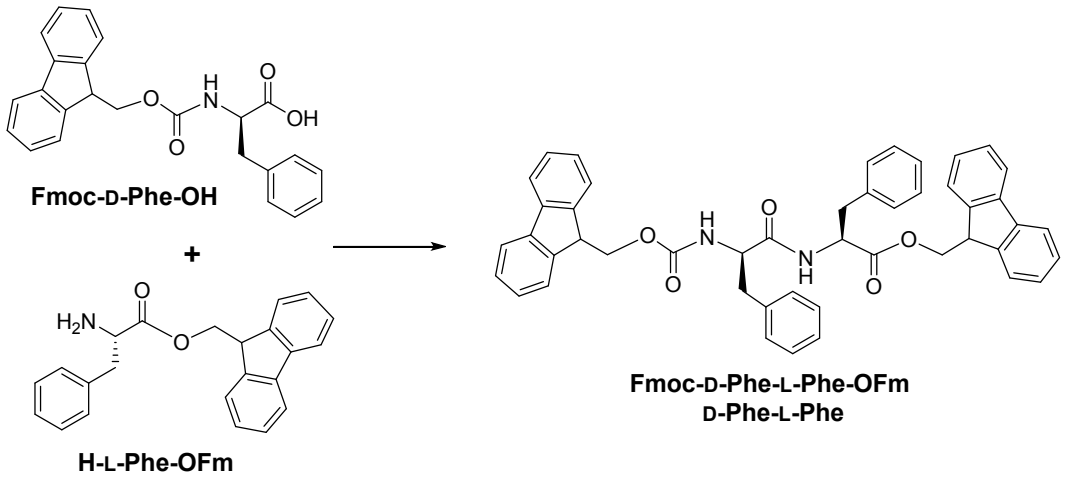
Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were registered on a Spectrum 100 Perkin Elmer FTIR spectrophotometer with an ATR accessory; ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 instrument at room temperature, using the residual solvent signal as the internal standard, chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hertz. High-resolution mass spectra were obtained on a Bruker Microtof-Q spectrometer. Column chromatography was performed using 60 Å (0.04-0.063 mm) silica gel from Macherey-Nagel. The dipeptide Fmoc-L-Phe-L-Phe-OFm was synthesized as reported in our previous work.[S1]

Fmoc-D-Phe-L-Phe-OFm (abbreviated as **D-Phe-L-Phe**). A solution of Boc-L-Phe-OFm[S2] (Boc-L-phenylalanine 9-fluorenylmethyl ester) (1.00 g, 2.25 mmol) in dichloromethane (10 mL) was treated with trifluoroacetic acid (TFA) (2 mL) at room temperature for 1 h. After evaporation to dryness, the residue was taken up in water and lyophilized. The trifluoroacetate salt thus obtained, TFA·H-L-Phe-OFm, was suspended in dichloromethane (5 mL) and neutralized by addition of *N,N*-diisopropylethylamine (DIPEA) (0.39 mL, 2.25 mmol). The resulting solution, containing H-L-Phe-OFm, was immediately used in the next step as described in the following.

An ice-cooled solution of Fmoc-D-phenylalanine (Fmoc-D-Phe-OH) (1.05 g, 2.70 mmol) in dichloromethane (15 mL) was treated with 1-hydroxybenzotriazole (HOBt) (365 mg, 2.70 mmol) and *N*-[3-(dimethylamino)-propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) (518 mg, 2.70 mmol). After 15 min, *N,N*-diisopropylethylamine (DIPEA) (0.47 mL, 2.70 mmol) was added followed by the solution of H-L-Phe-OFm obtained in the previous step (2.25 mmol). Stirring was

continued for 30 min at 0ºC and then at room temperature for 24 h. The mixture was washed with 5% aqueous $NaHCO_3$ (3 x 15 mL) and next with 5% aqueous $KHSO_4$ (3 x 15 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. The residue obtained was purified by column chromatography on silica gel eluting with dichloromethane/isopropanol 8:2 to provide Fmoc-D-Phe-L-Phe-OFm as a white solid (1.28 g, 1.80 mmol, 80% yield).

Mp: 225-226ºC. IR (neat): ν 3297, 1736, 1694, 1654 cm^{-1}. ^1H NMR (DMSO-d_6, 100 MHz): δ 2.52–2.59 (m, 1H), 2.63 (dd, 1H, J = 13.7 Hz, J = 3.8 Hz), 2.75 (dd, 1H, J = 13.8 Hz, J = 9.8 Hz), 2.88 (dd, 1H, J = 13.7 Hz, J = 4.9 Hz), 3.95–4.13 (m, 3H), 4.20–4.35 (m, 2H), 4.35–4.51 (m, 2H), 4.55 (ddd, 1H, J = 9.8 Hz, J = 8.2 Hz, J = 4.9 Hz), 7.09–7.46 (m, 18H), 7.49–7.72 (m, 5H), 7.80–7.93 (m 4H), 8.66 (d, 1H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): 36.76, 37.60, 46.28, 46.47, 53.41, 55.97, 65.68, 66.23, 120.06, 120.10, 125.27, 125.36, 126.23, 126.62, 127.02, 127.20, 127.24, 127.61, 127.73, 127.75, 127.99, 128.24, 129.17, 129.21, 137.10, 138.05, 140.72, 140.80, 143.39, 143.63, 143.67, 143.75, 155.70, 171.44, 171.71. HRMS (ESI) $C_{47}H_{40}N_2NaO_5$ $[M+Na]^+$: calcd. 735.2829, found 735.2806. ^1H NMR, ^{13}C NMR and FTIR spectra of Fmoc-D-Phe-L-Phe-OFm are shown in Figures S1-S3.



Samples preparation

Initial stock solutions of the dipeptides Fmoc-D-Phe-L-Phe-OFm (abbreviated as D-Phe-L-Phe) and Fmoc-L-Phe-L-Phe-OFm (abbreviated as L-Phe-L-Phe) were prepared at 5 mg/mL concentration using HFIP (hexafluoroisopropanol) as solvent. The peptide concentration was reduced by adding milli-Q water, metanol (MeOH), isopropanol (^{i}PrOH) or acetone as a co-solvent to the starting 5 mg/mL stock solutions in HFIP. More specifically, peptide concentrations of 4.0, 2.0, 0.5, 0.25, 0.1, 0.05 and 0.01 mg/mL were obtained using 4:1, 4:6, 1:9, 1:19, 1:49, 1:99 and 1:499 HFIP:co-solvent ratios, respectively. Finally, 20 μL aliquots of the peptide solutions thus prepared were placed on microscope coverslips and kept inside a cold chamber (5ºC) until dryness. All organic solvents were purchased from Sigma-Aldrich, Fisher Scientific and Scharlab.

Optical microscopy

Morphological observations were performed using a Zeiss Axioskop 40 microscope. Micrographs were taken with a Zeiss AxiosCam MRC5 digital camera.

Scanning electron microscopy (SEM)

SEM studies were performed in a Focussed Ion Beam Zeiss Neon 40 scanning electron microscope operating at 5 kV and equipped with an EDX spectroscopy system. Samples were mounted on a double-side adhesive carbon disc and sputter-coated with a thin layer of carbon to prevent sample charging problems.

Atomic force microscopy (AFM)

Topographic AFM images were obtained using either a Dimension 3100 Nanoman AFM or a Multimode, both from Veeco (NanoScope IV controller), under ambient

conditions in tapping mode. AFM measurements were performed on various parts of the morphologies, which produced reproducible images similar to those displayed in this work. Scan window sizes ranged from 5×5 μm^2 to 20×20 μm^2.

Theoretical calculations

Density Functional Theory (DFT) calculations were performed using the Gaussian 09 computer package.[S3] The geometries of the different investigated systems were fully optimized using the M06L[S4,S5] functional, which was developed by Zhao and Truhlar to account for dispersion, combined with the 6-31G(d,p) basis set. No symmetry constraints were used in the geometry optimizations.

References

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Table S1. Summary of the self-assembled structures obtained at 5ºC from Fmoc-D-Phe-L-Phe-OFm and Fmoc-L-Phe-L-Phe-OFm (abbreviated as D-Phe-L-Phe and L-Phe-L-Phe, respectively) solutions in different HFIP:co-solvent mixtures.

HFIP:co-solvent	Peptide concentration	Structure
	D-Phe-L-Phe	
HFIP:water	4 mg/mL	Nanofibers with nanoplates
	< 4 mg/mL and ≥ 0.5 mg/mL	Nanofibers
HFIP:MeOH	< 2 mg/mL	Microfibers made of nanofibers
HFIP:^{i}PrOH	< 2 mg/mL	Smooth microfibers
HFIP:acetone	≤ 4 mg/mL and ≥ 2 mg/mL	Random distribution of fibers
	≤ 1 mg/mL and ≥ 0.5 mg/mL	Nucleated branched-like structures
	≤ 0.1 mg/mL and ≥ 0.05 mg/mL	Branched structures
	L-Phe-L-Phe	
HFIP:water	> 2 mg/mL	Doughnut-like microstructures
	≤ 2 mg/mL and ≥ 0.5 mg/mL	Stacked braid-like microstructures
HFIP:MeOH	2 mg/mL	Upon sonication for 27 min, “desert rose” on a nanofibers bed
	< 0.1 mg/mL and > 0.05 mg/mL	Stacked braid-like microstructures
HFIP:^{i}PrOH	4 mg/mL	Microfibers
	< 4 mg/mL and ≥ 2 mg/mL	Stacked braids
	< 2 mg/mL	Poorly defined fibrous-like clusters
HFIP:acetone	4 mg/mL	Bundled arrays of nanofibers forming microfibers

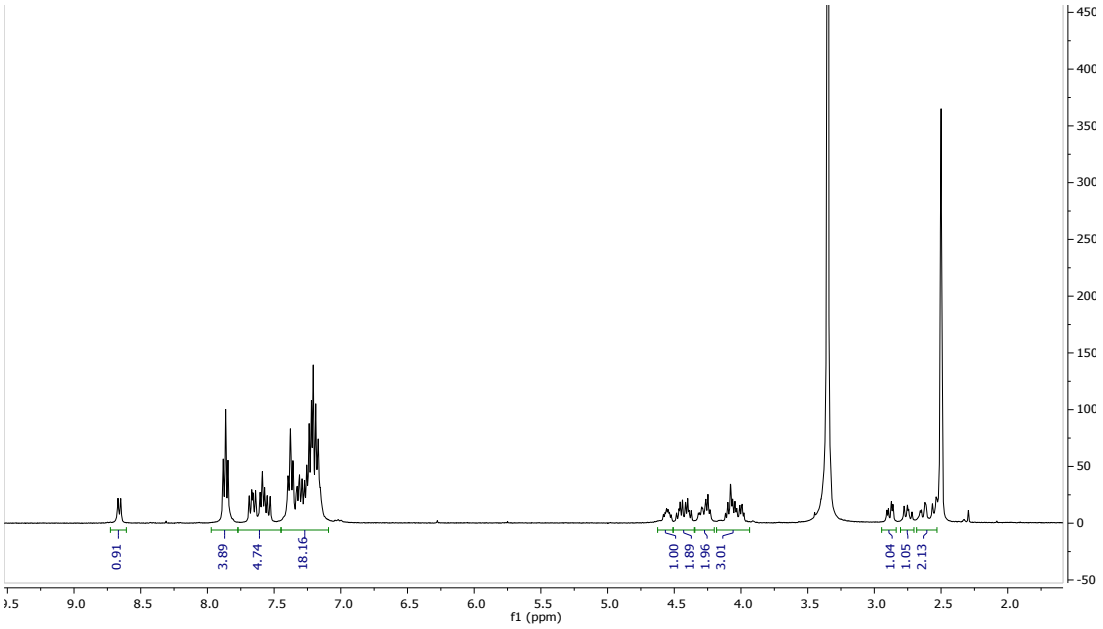

Figure S1. ^{1}H-NMR spectrum (DMSO-d_6, 400 MHz) of Fmoc-D-Phe-L-Phe-OFm.

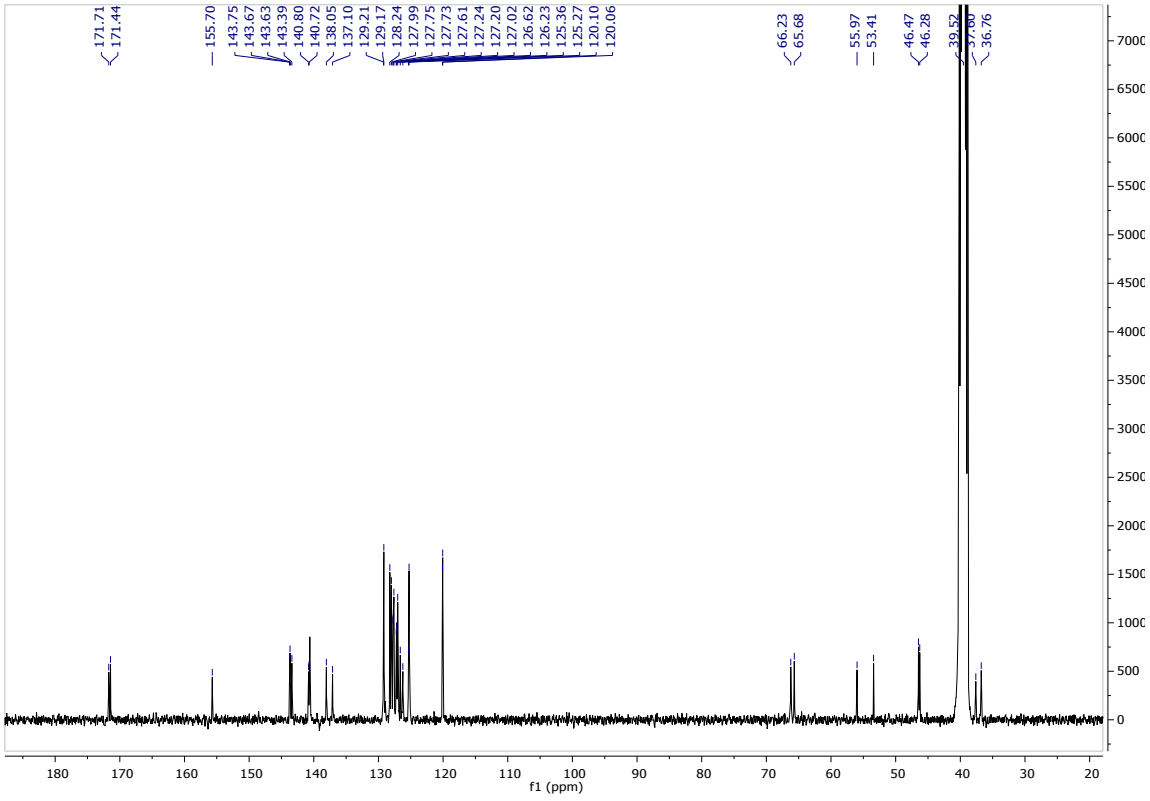

Figure S2. ^{13}C-NMR spectrum (DMSO-d_6, 100 MHz) of Fmoc-D-Phe-L-Phe-OFm.

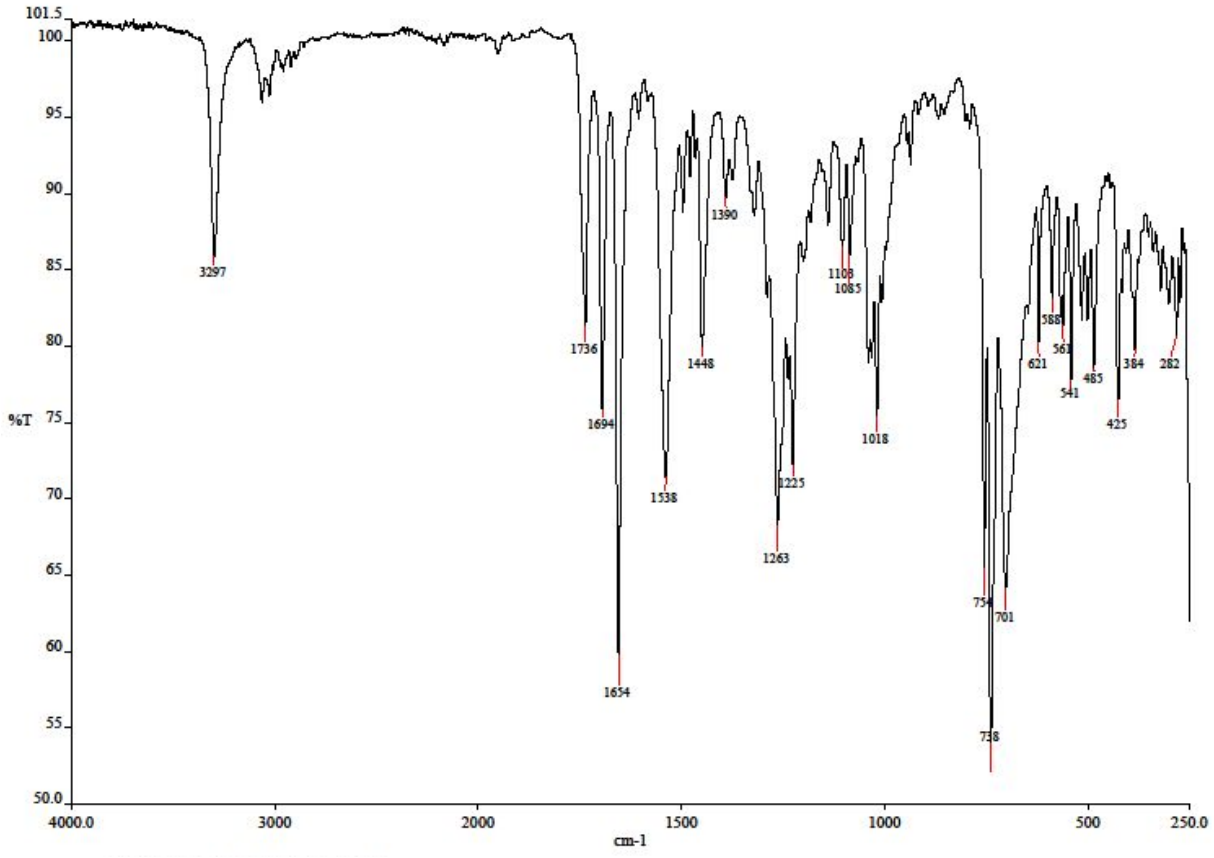

Figure S3. IR spectrum of Fmoc-D-Phe-L-Phe-OFm.

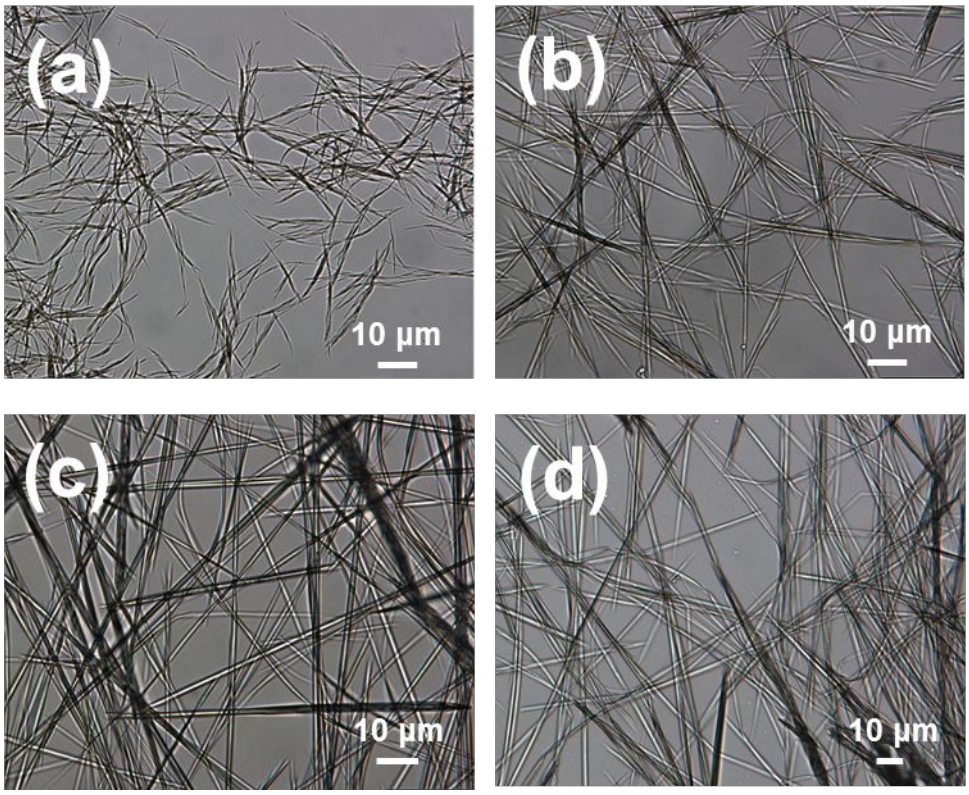

Figure S4. Representative optical micrographs obtained at 5ºC for 2 mg/mL D-Phe-L-Phe (Fmoc-D-Phe-L-Phe-OFm) solutions in different 4:6 HFIP:co-solvent mixtures: (a) HFIP:water; (b) HFIP:MeOH; (c) HFIP:^{i}PrOH; (d) HFIP:acetone.

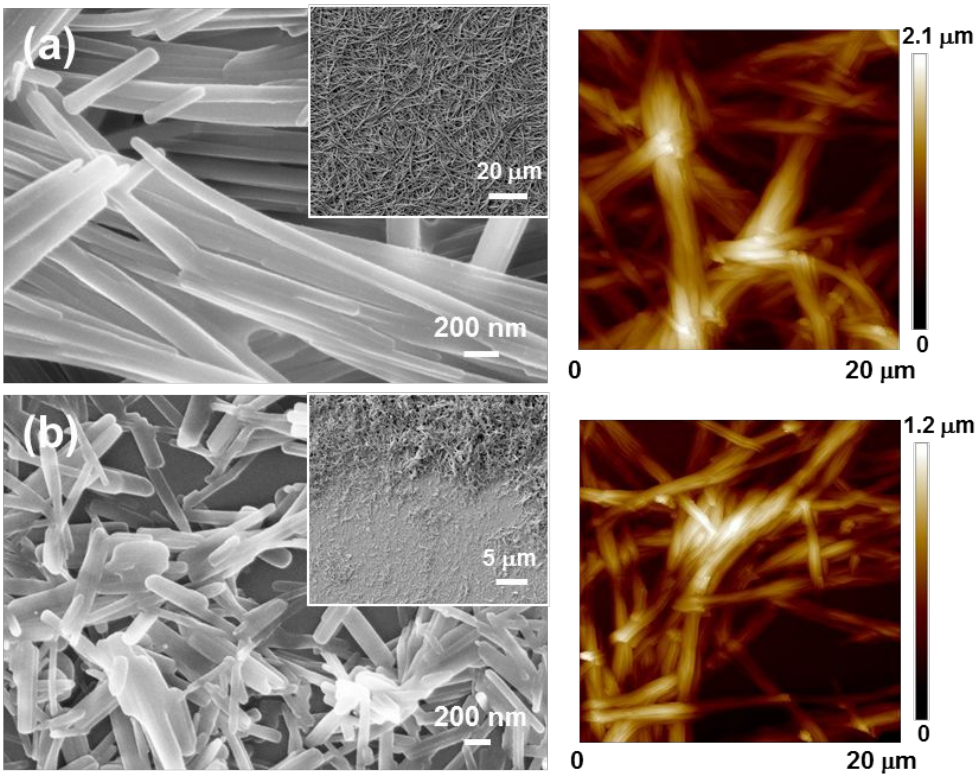

Figure S5. Representative SEM (left) and AFM images (right) of the D-Phe-L-Phe (Fmoc-D-Phe-L-Phe-OFm) fibers obtained at 5ºC from (a) 2 mg/mL and (b) 0.5 mg/mL peptide solutions in 4:6 and 1:9 HFIP:water, respectively.

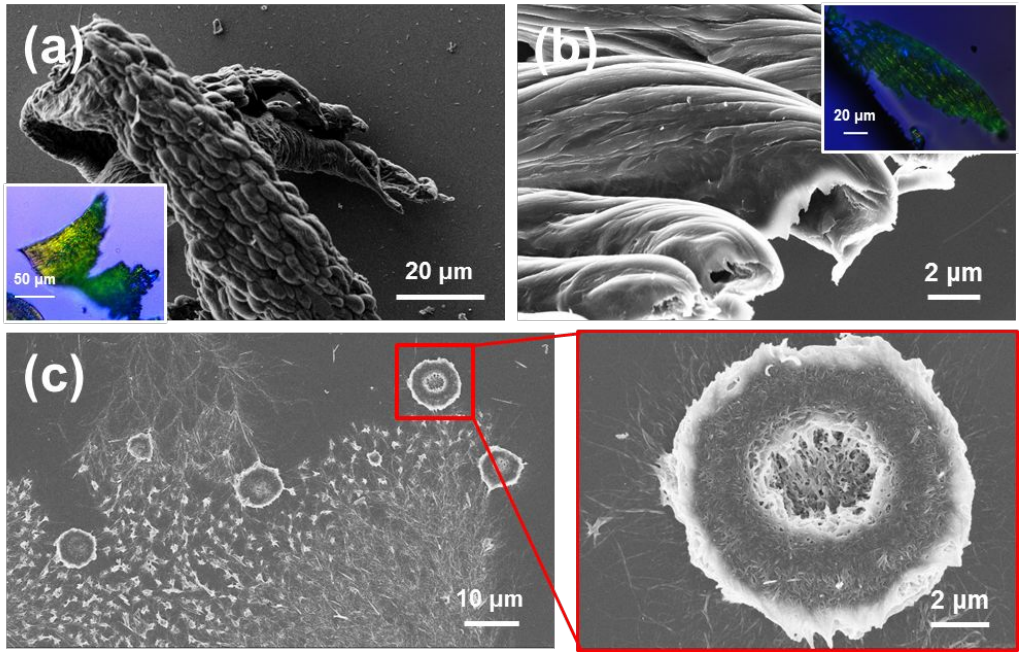

Figure S6. Representative SEM images of the L-Phe-L-Phe (Fmoc-L-Phe-L-Phe-OFm) assemblies obtained at 5ºC from (a) 0.5 mg/mL, (b) 2 mg/mL and (c) 4 mg/mL peptide solutions in 1:9, 4:6 and 4:1 HFIP:water, respectively. Insets in (a) and (b) correspond to optical micrographs.

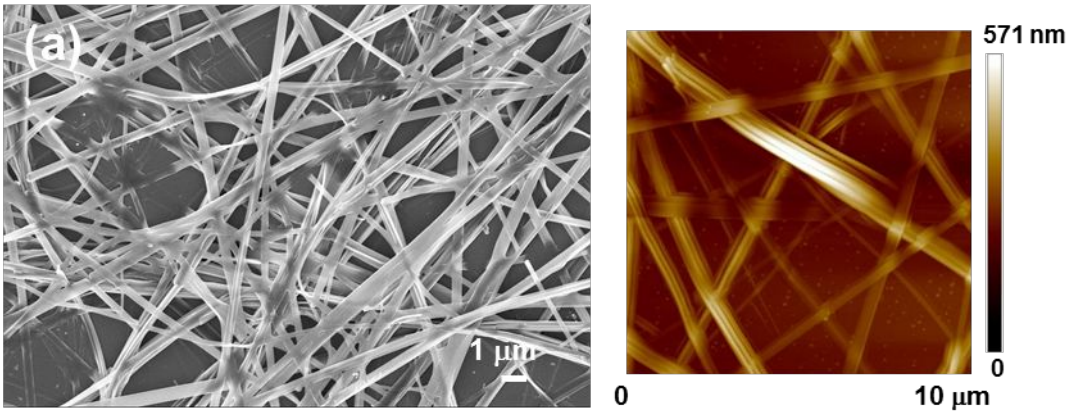

Figure S7. Representative SEM (left) and AFM images (right) of the D-Phe-L-Phe (Fmoc-D-Phe-L-Phe-OFm) fibers obtained at 5ºC from a 0.25 mg/mL peptide solution in 1:19 HFIP:^{i}PrOH.

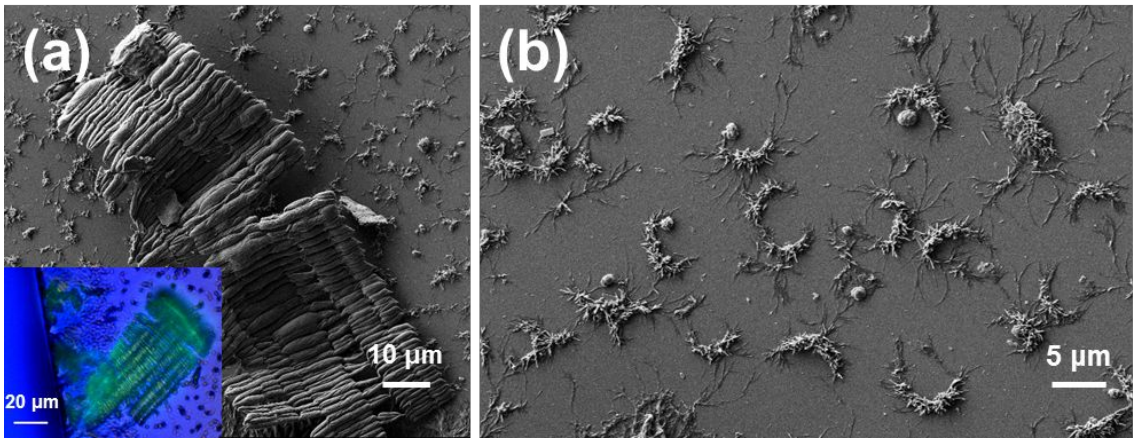

Figure S8. Representative SEM images of L-Phe-L-Phe (Fmoc-L-Phe-L-Phe-OFm) assemblies obtained at 5ºC from (a) 2 mg/mL and (b) 0.5 mg/mL peptide solutions in 4:6 and 1:9 HFIP:^{i}PrOH, respectively. The inset in (a) corresponds to an optical micrograph.

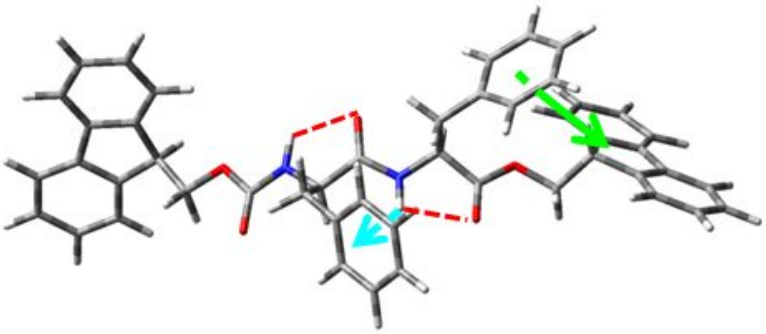

Figure S9. First local minimum of D-Phe-L-Phe (Fmoc-D-Phe-L-Phe-OFm), which is disfavoured with respect to the global minimum by 1.7 kcal/mol. This fully-extended conformation (known as C_5) is stabilized by two intramolecular N–H···O=C hydrogen bonds (red dashed lines) that close 5-membered cycles, one π-π stacking interaction (green arrow) and one N–H···π interaction (blue arrow).