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The Effect of Fluorine on Supramolecular Hydrogelation of 4-Fluorobenzyl-capped Diphenylalanine

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Supporting Information

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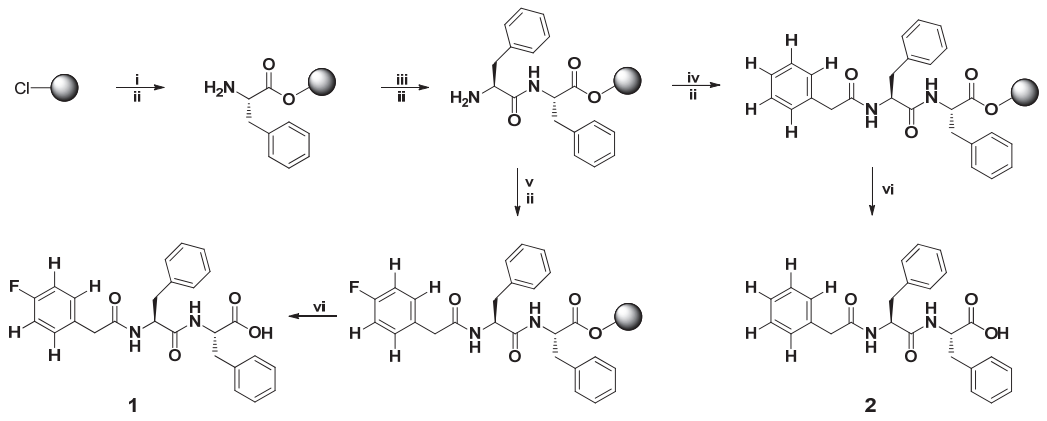

Scheme S1. The synthetic routes of **1** and **2**.

Synthesis of 4-fluorobenzyl-diphenylalanine (**1**): ^{1}H NMR (300 MHz, [D_6]DMSO): δ=2.65-3.15(m, 4H; CH_2), 3.55-3.65 (m, 2H; CH_2), 4.30-4.40 (m, 1H; CH), 4.45-4.60 (m, 1H; CH), 6.95-7.35 (m, 14H; CH), 8.10-8.20 (br, 1H; NH), 8.32(d, J=9.00 Hz 1H; NH); ^{13}C NMR (75 MHz, [D_6]DMSO): δ=37.7, 38.5, 42.1, 54.7, 58.5, 115.5, 115.8, 127.1, 127.2, 128.9, 129.0, 130.2, 131.6, 131.7, 133.3, 138.7, 138.8, 170.6, 172.0, 173.9; MS [ESI$^-$]: calcd. m/z 448.18., obsvd. 447.2 $[M - H]^-$.

Synthesis of benzyl-diphenylalanine (**2**): The peptide derivative of **2** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine and phenylacetic acid (Scheme 1)**.** The resin (0.6 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.290 g, 0.750 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-diisopropylethylamine

(DIEA; 0.309 mL, 1.875 mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc-L-phenylalanine (0.387 g, 1.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.379 g, 1.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.413 mL, 2.500 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, Phenylacetic acid (0.204 g, 1.500 mmol) was coupled to the free amino group using HBTU (0.568 g, 1.500 mmol) and DIEA (0.620 mL, 3.750 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (white solid: 0.077 g). ^{1}H NMR (300 MHz, [D_6]DMSO): δ=2.70-3.15 (m, 4H; CH_2), 3.25-3.45 (m, 2H; CH_2), 4.40-4.50 (m, 1H; CH), 4.50-4.65 (m, 1H; CH), 7.00-7.35(m, 15H; CH), 8.26(d, J=9.00 Hz, 1H; NH), 8.30-8.40(br, J=6.30 Hz, 1H; NH); ^{13}C NMR (75 MHz, [D_6]DMSO): δ=37.6, 38.5, 43.0, 54.37, 54.44, 127.06, 127.09, 127.4, 128.86, 128.94, 129.1, 129.8, 130.0, 130.2, 137.1, 138.3, 138.7, 170.7, 172.2, 173.6; MS [ESI$^-$]: calcd.

m/z 430.19, obsvd. 429.3 $[M - H]^-$.

Inverted tube method: Gelation tests were performed by weighing a compound (2.0 mg) in a screw-capped 2-mL vial (diameter: 10 mm), adding a solvent (0.20 mL), sealing the vial tightly, sonicating it until the compound had dissolved, and then cooling the vial to room temperature. Gelation was considered to have occurred when a solid-like material was obtained that did not exhibit gravitational flow (inverted test tube method) during a period of 5 min.

Rheological tests: Rheological tests were conducted using an Anton Paar rheometer and a 25-mm parallel plate. The hydrogel sample (200μL, 1 wt %) was placed on the parallel plate for the angular frequency sweep test (test range: 0.1 to 100 $rads^{-1}$; strain, 0.8%; 13 points per decade; sweep mode, "log"; temperature,25 °C).

Cell viability tests: The biocompatibilities of different peptides were measured by the MTT cell viability test. The PC-3 (MCF-7) cells were seeded in 24-well plates at a density of 50000 cells per well with 0.5 mL medium (DMEM) contained 10 % FBS and 1 % Penicillin-streptomycin solution and incubated for 24 h. Compounds at different concentrations (10, 50, 100, 200, 500 μM) were added when cells were

plated. 24 and 48 h later, replaced the medium with fresh medium supplemented with 0.5 mL of MTT reagent (4 mg mL^{-1}) per well. After another 4 h, the medium containing MTT was removed and DMSO (0.5 mL per well) was added to dissolve the formazan crystals. Each 24-well was transferred to 96 well plate. The optical density of the result solution was measured at 595 nm, using an absorbance microplate reader (Infinite F50, TECAN). Cells without the treatment of the compounds were used as the control. The cell viability percentage was calculated by the following formula: The cell viability percentage (%) = OD_{sample} / $OD_{control}$.

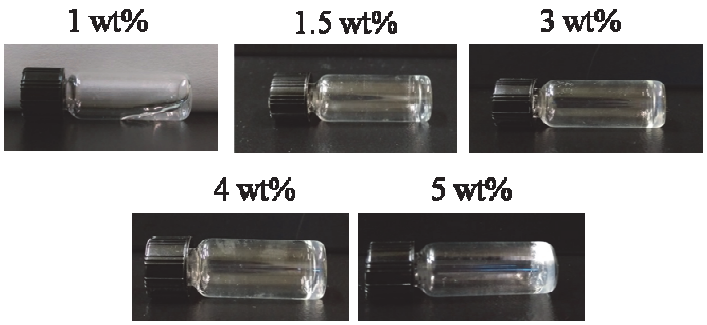

Fig. S1. Optical images of **1** at the concentrations of 1, 1.5, 3, 4 and 5 wt% in water at pH 7.89.

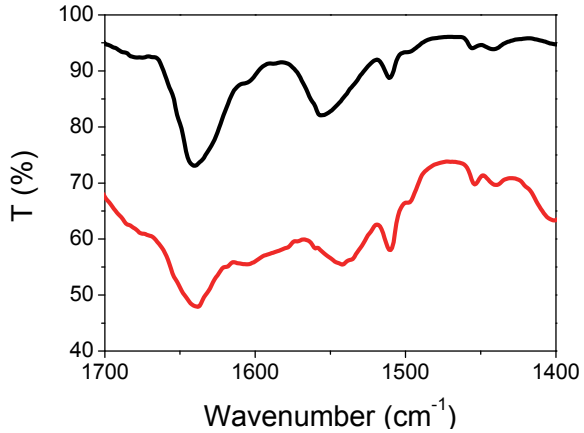

Fig. S2. FT-IR spectra of **1** at 2 wt% in water (red) and in TFE (black).

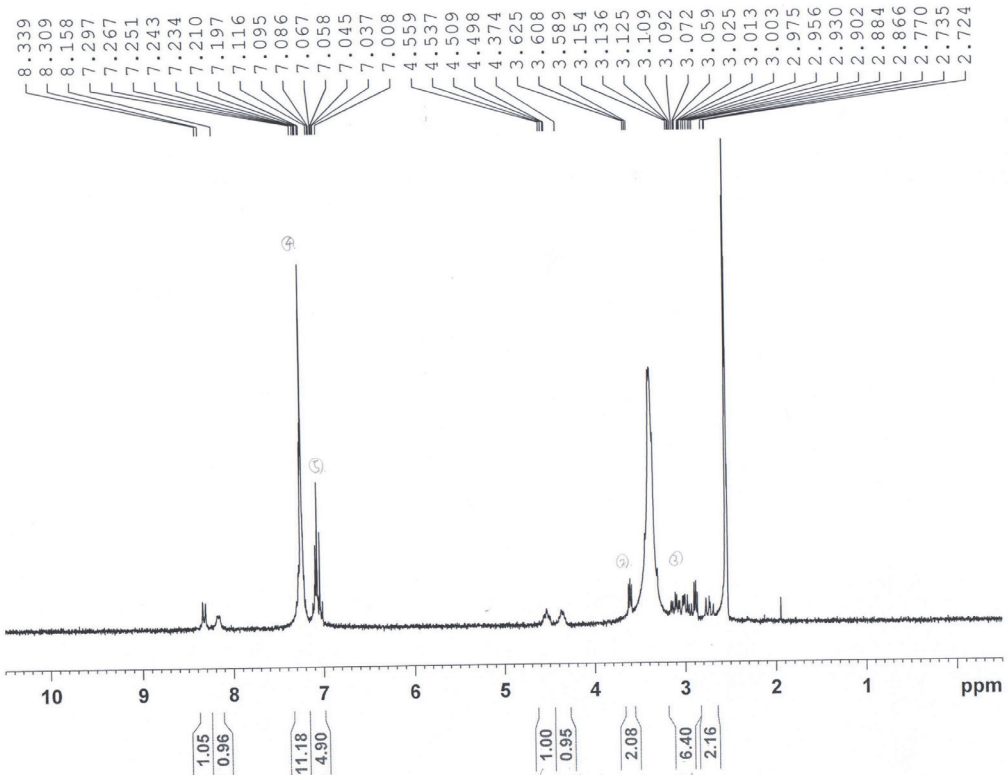

Fig. S3. ^{1}H NMR spectrum of **1** in [D_6]DMSO.

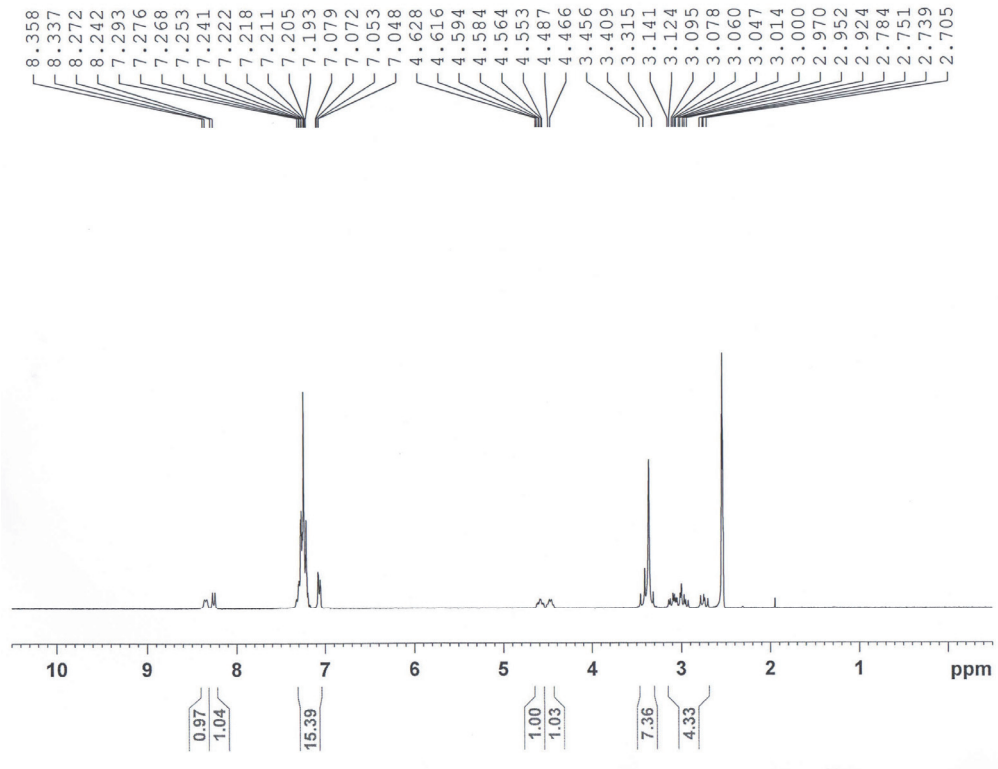

Fig. S4. ^{1}H NMR spectrum of **2** in [D_6]DMSO.