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SUPPORTING INFORMATION

Triggering a transient organo-gelation system in a chemically active solvent†

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S1 MATERIALS AND METHODS

Materials

Boc-phenylalanine (Boc-Phe) and *tert*-butyl acetate (*t*BuOAc) solvent were purchased from Tokyo Chemical Industry (TCI), phenylalanine-*tert*-butyl ester from Carbosynth (UK), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) from Sigma Aldrich, sodium hydrogen carbonate from AnalaR NORMAPUR and sulphuric acid from Fluka. All chemicals were used as supplied without further purification.

NMR spectroscopy

All spectra were recorded on a Bruker Advance III HD 300 MHz spectrometer using d_6-DMSO solvent; chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. The solvent signal was used as reference (d_6-DMSO at 2.5 ppm and 39.52 ppm for ^{1}H and ^{13}C NMR, respectively). The peaks have been assigned as singlet (s), doublet (d), triplet (t), multiplet (m), broad signal (br)). ^{13}C NMR spectra were recorded with broadband ^{1}H decoupling.

FTIR spectroscopy

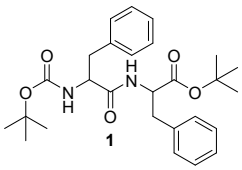
All spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer in Attenuated Total Reflection (ATR) mode in a range from 400 cm^{-1} to 4000 cm^{-1} (step: 2 cm^{-1}; number of scans: 124). All spectra were baseline corrected.

SEM imaging

Images were obtained on a Zeiss EVO-50XVP microscope. Xerogel samples were prepared on carbon films (400 mesh copper grids) obtained from Agar Scientific. The carbon films were dipped into the gels and allowed to dry in the open air overnight.

S2 SYNTHESIS

S2.1 Synthesis of Boc-Phe-Phe-O*t*Bu (1)



Boc-phenylalanine (1000 mg, 1.0 eq) and TBTU (1210.25 mg, 1.0 eq) were added to a solution of $NaHCO_3$ (316.6 mg, 1.0 eq) in anhydrous DMF (10 mL) under N_2 atmosphere. The solution was left to stir for 1 hour at room temperature. Phenylalanine-*tert*-butyl ester (1068 mg, 1.1 eq) and $NaHCO_3$ (348.26 mg, 1.1 eq) were dissolved in anhydrous DMF (10 mL) as above. The two solutions were mixed and stirred overnight at room temperature under N_2 atmosphere. The next day, the starting materials had been consumed, and a new spot appeared, as indicated by TLC (Hex: EA 1:1, Hanessian´s stain). The solvent was evaporated under vacuum (co-evaporation with toluene x3), and the residue was dissolved in DCM. The organic phase was extracted with water (x2) and subsequently washed with aqueous HCl (0.1 M), water (x2), and a saturated aqueous

solution of $NaHCO_3$. The organic phase was then dried with $MgSO_4$ and evaporated under a vacuum, yielding a yellow powder (1.036 g, 60%). The analysis is in agreement with previously published data.[1,2] **^{1}H NMR** (300 MHz, d_6-DMSO) δ 8.19 (d, J = 7.5 Hz, 1H), 7.37 – 7.12 (m, 10H), 6.82 (d, J = 8.8 Hz, 1H), 4.38 (q, J = 7.6 Hz, 1H), 4.19 (q, J = 7.9 Hz, 1H), 3.03 – 2.86 (m, 2H), 2.75 – 2.63 (m, 2H), 1.32 (s, 9H), 1.28 (s, 9H). **^{13}C NMR** (75 MHz, d_6-DMSO) δ 171.7, 170.4, 155.1, 138.1, 137.1, 129.2, 129.1, 128.1, 127.9, 126.5, 126.1, 80.7, 77.9, 55.5, 54.0, 37.4, 36.9, 28.1, 27.5. **HR-MS**: *m/z* for $C_{27}H_{36}N_2O_5$ $[M + H]^+$ calculated 469.27, found 469.2675.

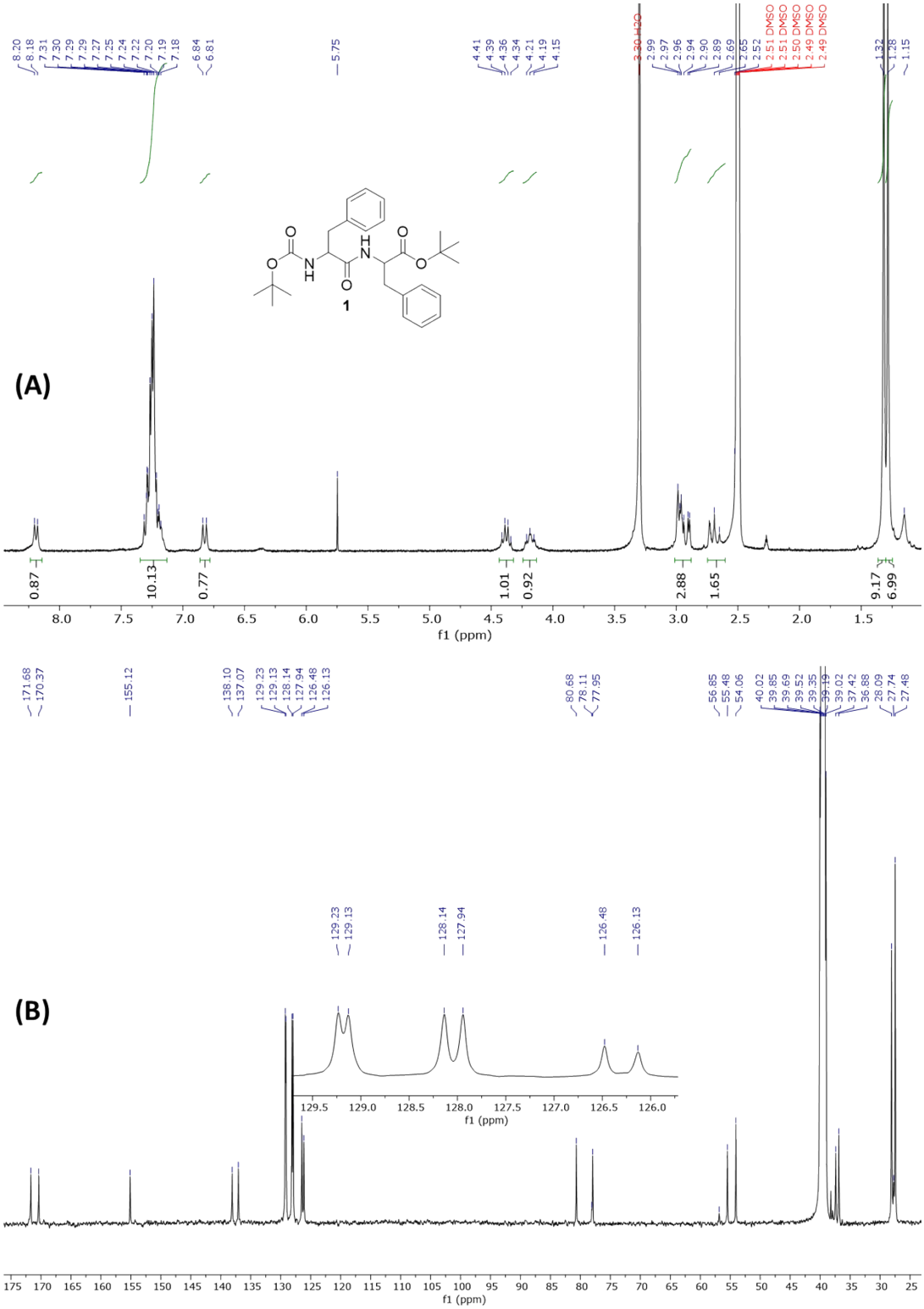
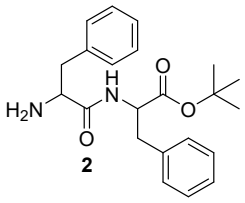


Figure S1. ^{1}H NMR (300 MHz, d_6-DMSO) (A) and ^{13}C NMR (75 MHz, d_6-DMSO) (B) spectra of Boc-Phe-Phe-O*t*Bu **1**.

S2.2 Synthesis of Phe-Phe-O*t*Bu (2)



Boc-Phe-Phe-O*t*Bu **1** (120 mg, 1.0 eq) was suspended in *t*BuOAc (1.28 mL) at a final concentration of 0.2 M. Concentrated H_2SO_4 (0.0411 mL, 3.0 eq) was then added dropwise at room temperature. After 1 h, the formation of the new compound was confirmed by TLC (Hex: EA, 3:1, Hanessian's stain). The reaction mixture was then neutralized by adding a saturated aqueous solution of $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried with $MgSO_4$ and evaporated under a vacuum, yielding a yellow gummy solid (113.5 mg, 94.6%). The analysis is in agreement with previously published data.[3,4] **^{1}H NMR** (300 MHz, d_6-DMSO) δ 8.15 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.09 (m, 10H), 4.44 (q, *J* = 7.0 Hz, 1H), 3.42 (dd, *J* = 8.3, 4.6 Hz, 1H), 2.92 (dd, *J* = 14.8, 5.7 Hz, 2H), 2.57 (dd, *J* = 13.5, 8.4 Hz, 2H), 1.33 (s, 9H). **^{13}C NMR** (75 MHz, d_6 -DMSO) δ 174.5, 170.9, 139.0, 137.4, 129.9, 129.7, 128.63, 128.57, 127.0, 126.6, 81.4, 56.3, 53.9, 41.2, 37.7, 28.0. **HR-MS**: *m/z* for $C_{22}H_{28}N_2O_3$ $[M + H]^+$ calculated 369.21, found 369.2161.

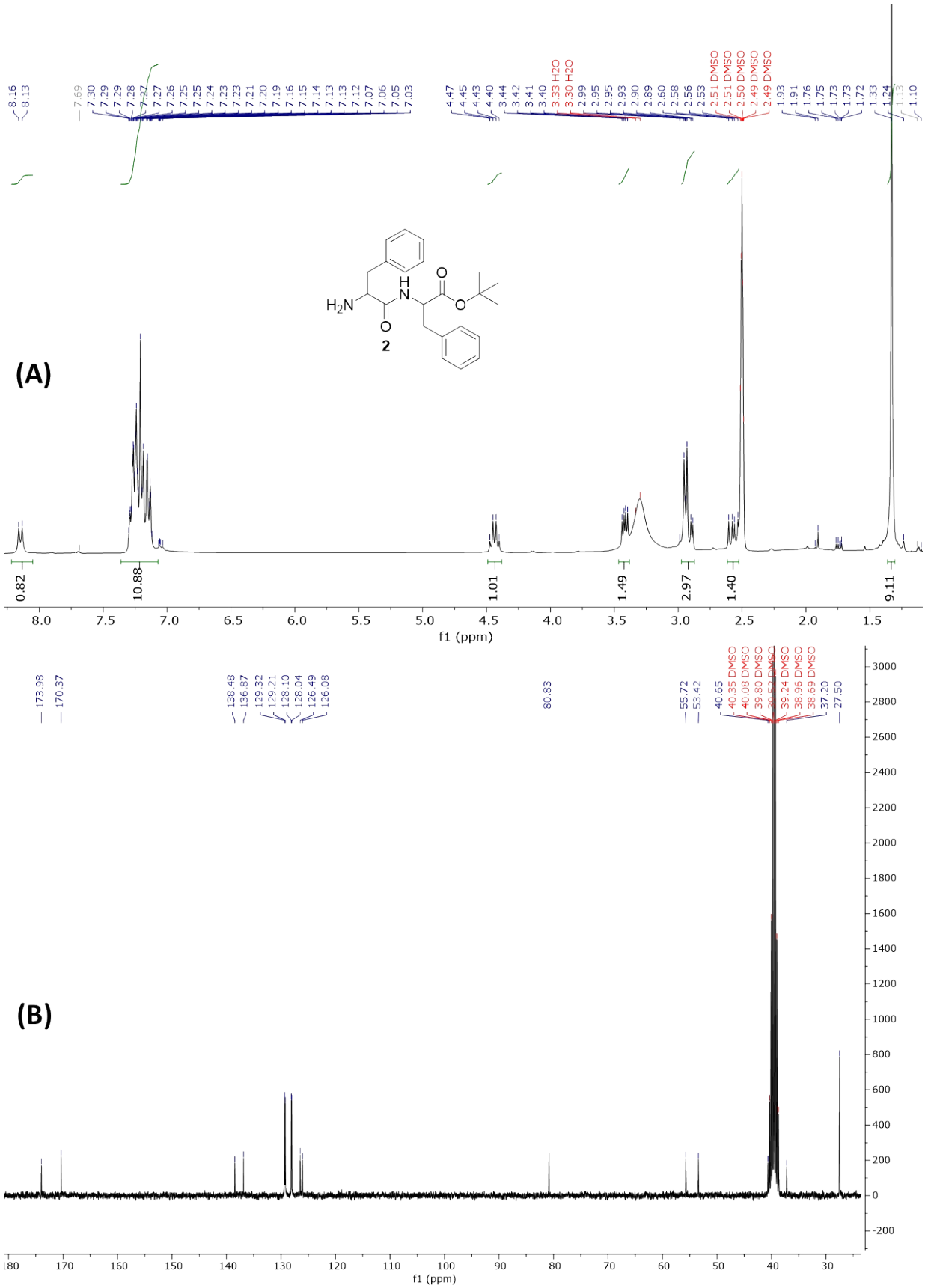


Figure S2. ^{1}H NMR (300 MHz, d_6-DMSO) (A) and ^{13}C NMR (75 MHz, d_6-DMSO) (B) spectra of Phe-Phe-O*t*Bu **2**.

S3 GELATION TRIALS

S3.1 Gelation protocol

Boc-Phe-Phe-O*t*Bu **1** (23.5 mg, 0.05 M) was suspended in *t*BuOAc solvent (1.0 mL) and sonicated until a fine suspension was formed, followed by the addition of concentrated H_2SO_4 (1.0 eq., 2.7 µL). The mixture was gently swirled and left to rest at room temperature for at least 12 h. Gelation was verified by the vial inversion method.

Table S1. Boc-Phe-Phe-O*t*Bu **1** concentration screening trials in *t*BuOAc solvent (1.0 mL) at room temperature.

Boc-Phe-Phe-O*t*Bu (mg)	conc. H_2SO_4(eq)	conc. H_2SO_4(µL)	M (mol/L)	wt%	Gelation outcome	$T_{gel-sol}$ (°C)
94	1.0	11	0.2	9.4	Self-supporting gel	45-50
47	1.0	5.3	0.1	4.7	Self-supporting gel	45-50
23.5*	1.0	2.7	0.05	2.35	Self-supporting gel	45-50
11.8	1.0	1.3	0.025	1.18	Partial gel	45-50
9.4	1.0	1.1	0.02	0.94	Partial gel	45-50
9.4	0.5	0.6	0.02	0.94	Partial gel	45-50

* *Minimum gelation concentration*

Table S2. Acid concentration effects on the organogel´s lifetime.

Boc-Phe-Phe-O*t*Bu (M)	conc. H_2SO_4(eq)	Gelation outcome	gel-to-sol (lifetime)
0.05	1.0	Self-supporting gel	4 days
0.05	0.5	Self-supporting gel	intact gel beyond 4 weeks
0.1	1.0	Self-supporting gel	4 days
0.1	0.5	Self-supporting gel	intact gel beyond 4 weeks

S3.2 Reformation of heated organogels and phase transition temperature ($T_{gel-sol}$) measurements

Organogels prepared at a range of concentrations (0.02 to 0.2 M) were heated in a controlled manner using a block heater. The temperature was raised from room temperature up to 55 °C by 5 °C increments every 10 min. Before moving to a higher temperature, the gels were evaluated by the vial inversion method. All organogels broke at 50 °C (free gravitational flow of the gels was observed upon inversion of the vials) and reformed upon cooling at room temperature within 12 h.

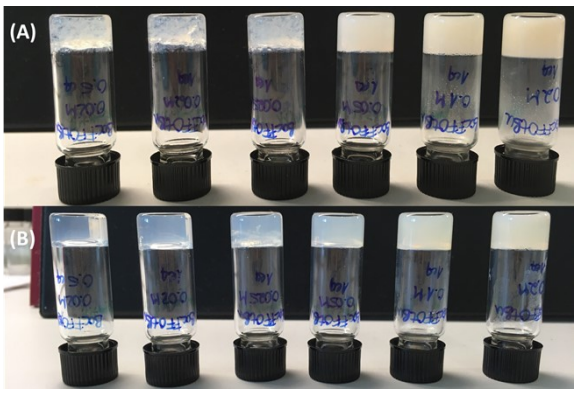


Figure S3. Thermoreversibility evaluation of the organogels. Vial inversion test of the organogels before heating (A); evaluation of gelation after heating/cooling of the gel samples (B). Concentration of the gels: (Molarity, right to left: 0.2, 0.1, 0.05, 0.025, 0.02, 0.02); in all samples 1.0 equivalent of sulphuric acid was used except the far left sample (0.02M-0.5 equivalents of acid).

S3.3 Swelling studies

Swelling studies were performed at room temperature. Gel samples (dry gels) were initially weighted, and *t*BuOAc solvent (500 µL) was subsequently added on top of their surface. After 24 h, the solvent was slowly removed by pipette before weighing the swollen gels (wet gels). The swelling degree (SD) was calculated according to the following equation, where w_t refers to the mass of the wet gel and w_0 refers to the mass of the dry gel: $SD(\%) = \frac{w_t}{w_0} \times 100$

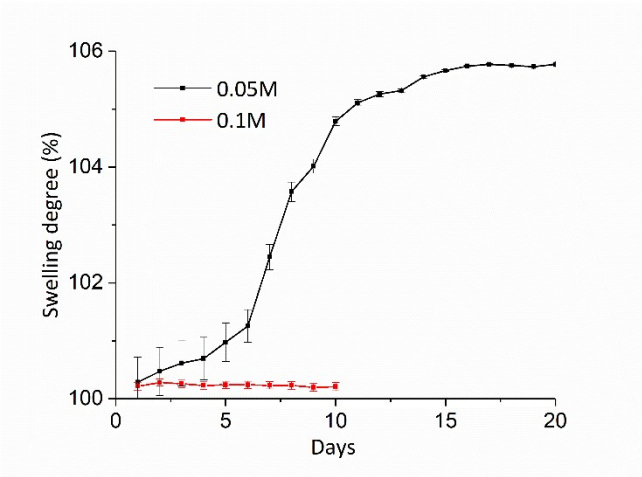
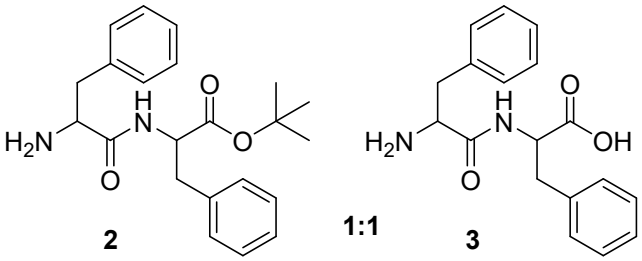


Figure S4. Swelling studies of the organogel in *t*BuOAc solvent at two different concentrations.

S4 NMR ANALYSIS

S4.1 Preparation and analysis of xerogels

Gel samples were prepared in vials as above and transferred on a glass slide. The samples were left to dry in the open air overnight. No further purification was performed before the NMR analysis. The xerogels consist of a mixture of compounds Phe-Phe-O*t*Bu **2** and Phe-Phe **3** in ~1:1 ratio, *tert*-butanol and impurities of the *t*BuOAc solvent. Only signals assigned to compounds **2** and **3** are given. Signals of tertiary CH overlap with the broad peak of the hydroxyl group of *tert*-butanol and water. The amine groups of compounds **2** and **3** are protonated.



Xerogel I: **^{1}H NMR** (300 MHz, d_6-DMSO) δ 8.89 (d, *J* = 7.5 Hz, 1H), 8.83 (d, *J* = 7.8 Hz, 1H), 8.06 (s, 6H), 7.37 – 7.18 (m, 20H), 4.17 – 3.91 (m, 2H), 3.24 – 3.04 (m, 3H), 3.04 – 2.84 (m, 5H), 1.32 (s, 9H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 172.2, 169.9, 168.1, 137.1, 136.7, 134.6, 134.6, 129.6, 129.21, 129.14, 128.53, 128.32, 128.3, 127.2, 126.71, 126.63, 81.2, 54.37, 53.78, 53.14, 36.98, 36.91, 36.68, 27.5.

Xerogel II: **^{1}H NMR** (300 MHz, d_6-DMSO) δ 8.91 (d, *J* = 7.5 Hz, 1H), 8.85 (d, *J* = 7.9 Hz, 1H), 8.06 (s, 6H), 7.40 – 7.18 (m, 20H), 4.58 – 4.38 (m, 2H), 4.04 – 3.98 (m, 2H), 3.21 – 3.00 (m, 4H), 3.06 – 2.84 (m, 4H), 1.32 (s, 9H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 172.2, 169.88, 168.11, 137.1, 136.6, 134.6, 129.53, 129.18, 129.11, 128.52, 128.31, 128.28, 127.22, 126.70, 81.2, 54.33, 53.75, 53.13, 36.91, 36.90, 36.66, 27.46.

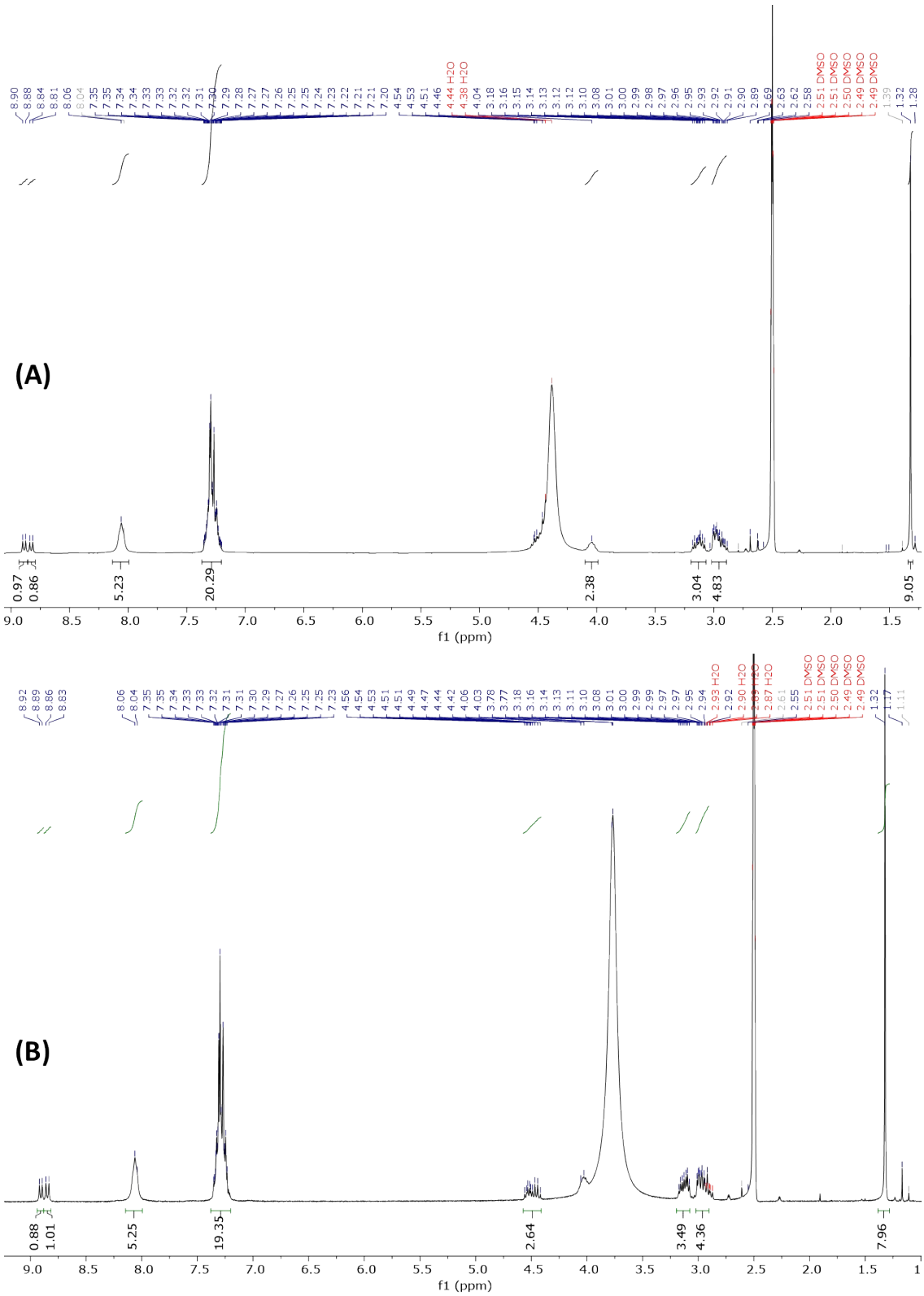


Figure S5. ^{1}H NMR (300 MHz, d_6-DMSO) spectra of xerogel **I** (A) and xerogel **II** (B).

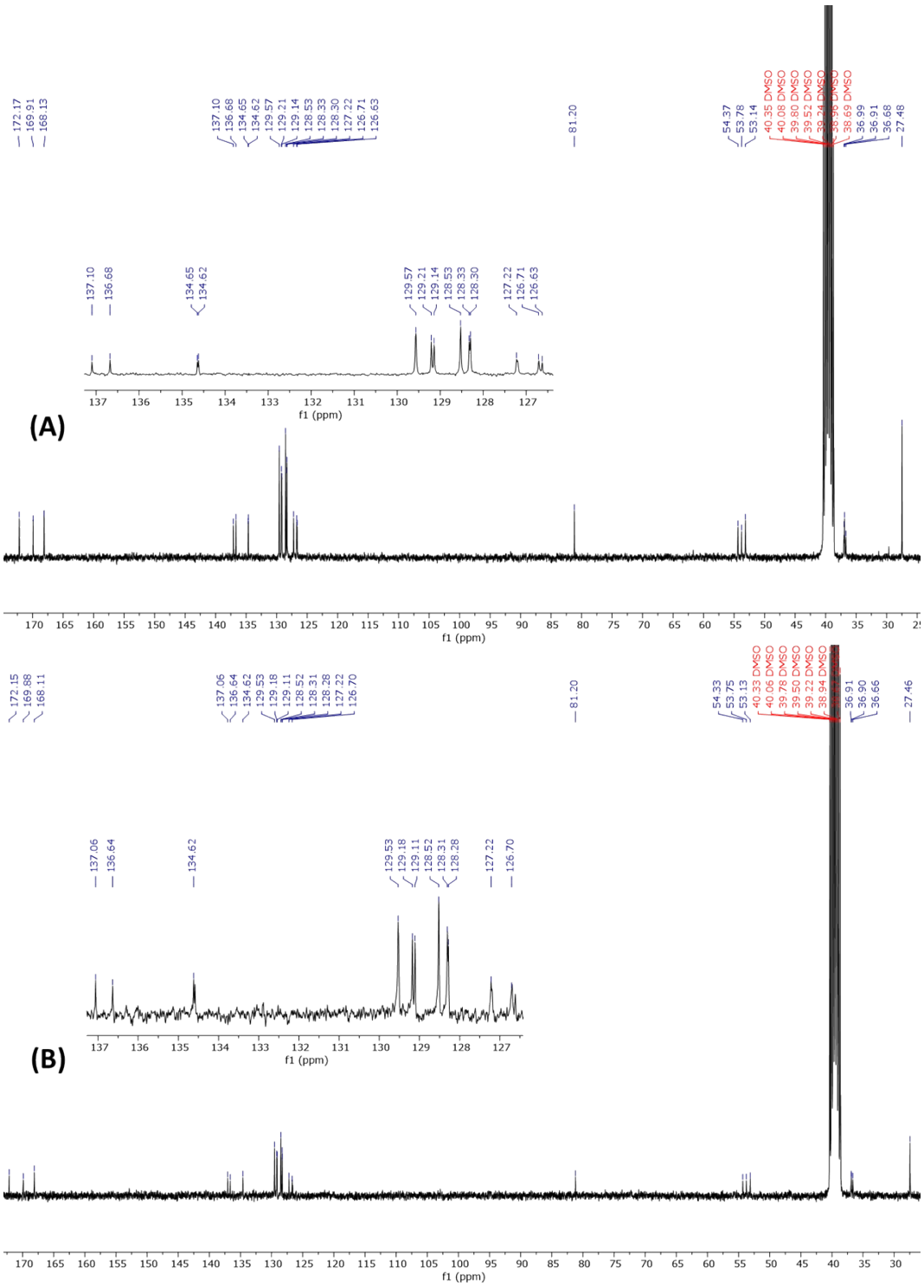
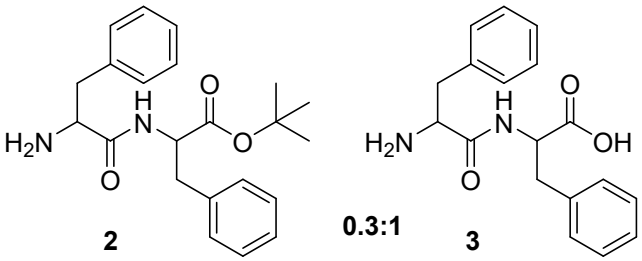


Figure S6. ^{13}C NMR (75 MHz, d_6-DMSO) spectra of xerogel **I** (A) and xerogel **II** (B).

S4.2 NMR analysis of the decayed organogel (after 4 days)

The decayed organogel was dried under vacuum. No further purification was performed on the sample before NMR analysis. The crude sample consists of a mixture of compounds Phe-Phe-O*t*Bu **2** and Phe-Phe **3** in ~0.3:1 ratio, *tert*-butanol and impurities of the *t*BuOAc solvent. Only signals assigned to compounds **2** and **3** are given. The amine groups of compounds **2** and **3** are protonated.



^{1}H NMR (300 MHz, d_6-DMSO) δ 8.90 (d, *J* = 7.5 Hz, 0.3H), 8.84 (d, *J* = 7.8 Hz, 1H), 8.06 (s, 3.7H), 7.39 – 7.19 (m, 15H), 4.57 – 4.43 (m, 1.5H), 4.05 (s, 1.5H), 3.18 – 3.06 (m, 3H), 3.02 – 2.89 (m, 3H), 1.32 (s, 2.7H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 172.7, 170.7, 168.61, 168.59, 137.61, 137.19, 135.15, 135.13, 130.1, 129.7, 129.6, 128.99, 128.80, 128.77, 127.66, 127.18, 127.10, 81.7, 54.9, 54.3, 53.6, 40.84, 40.56, 40.28, 40.00, 39.72, 39.44, 39.17, 37.48, 37.37, 37.17, 27.0.

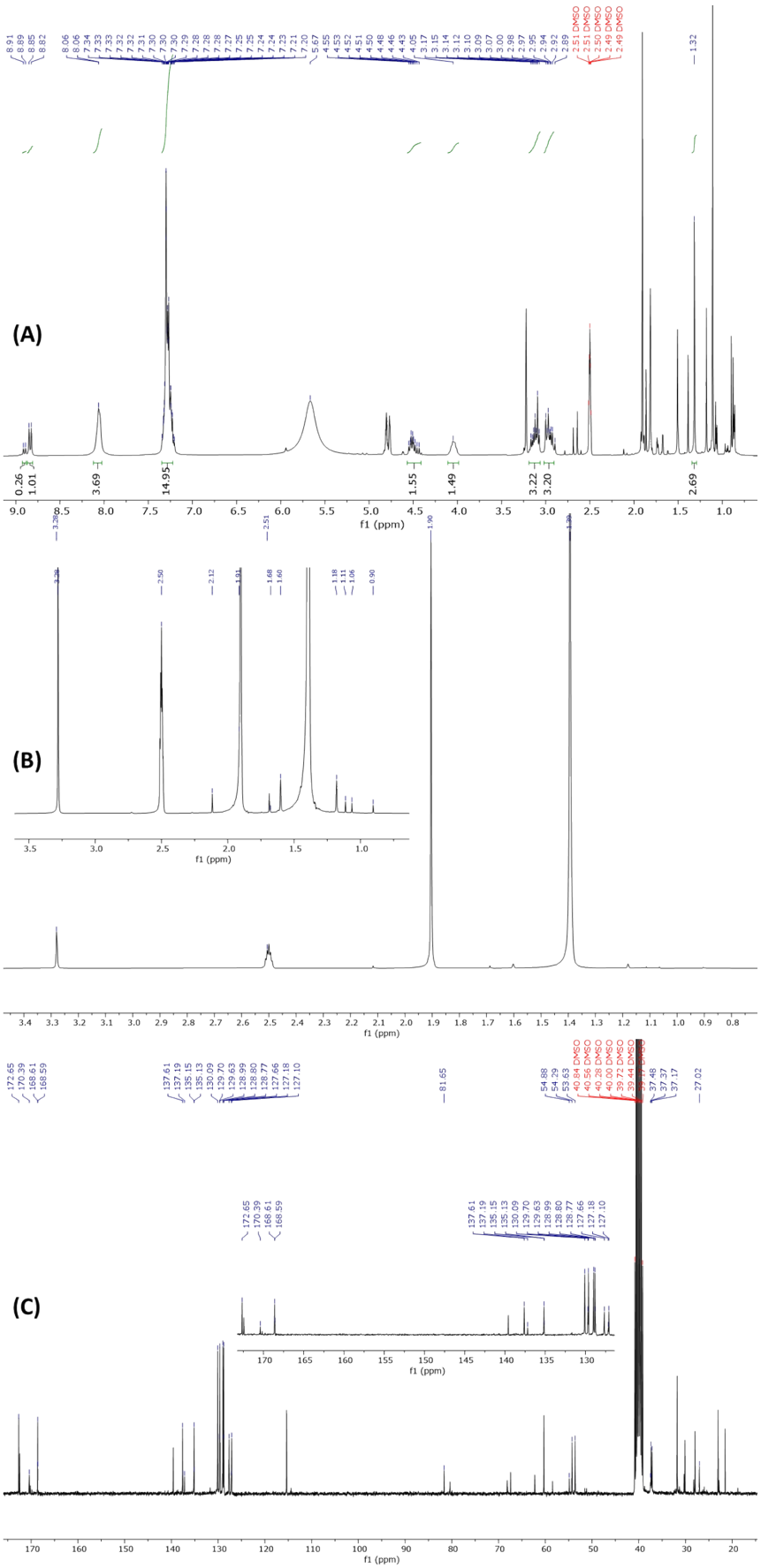
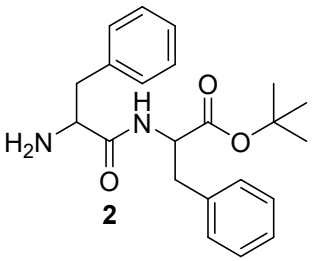


Figure S7. ^{1}H NMR (300 MHz, d_6-DMSO) spectra of the decayed organogel (A) and *t*BuOAc solvent (B); ^{13}C NMR (75 MHz, d_6-DMSO) spectrum of the decayed organogel (C).

S4.3 NMR analysis of the swollen organogels

Gel samples (20 days and 7 months old) were transferred on a glass slide and left to dry in the open air overnight. No further purification was performed on the obtained xerogels before NMR analysis. The xerogels consist only of compound Phe-Phe-O*t*Bu **2**. The amine group of compound **2** is protonated.



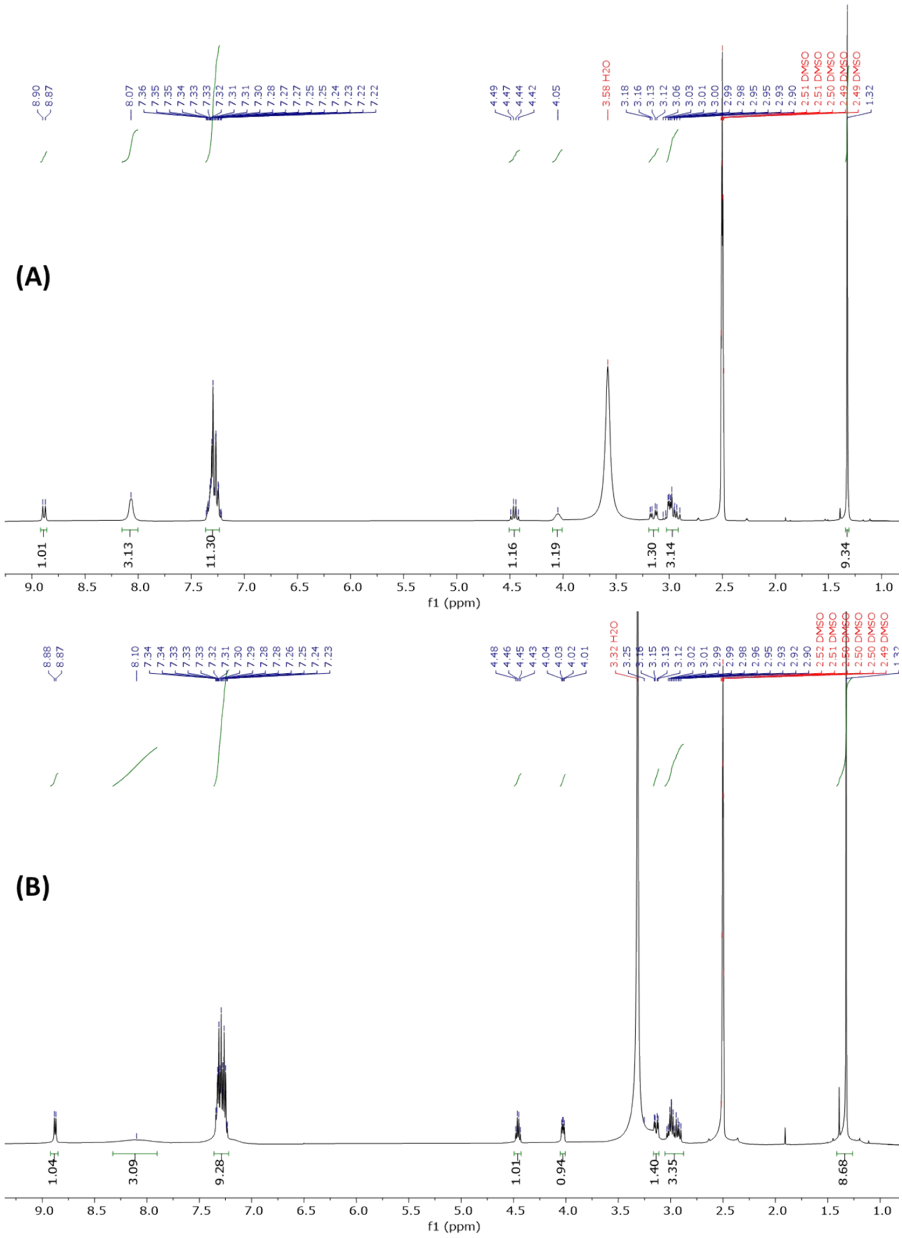


Figure S8. ^{1}H NMR (300 MHz, d_6-DMSO) spectra of swollen organogels after twenty days (A) and after seven months (B).

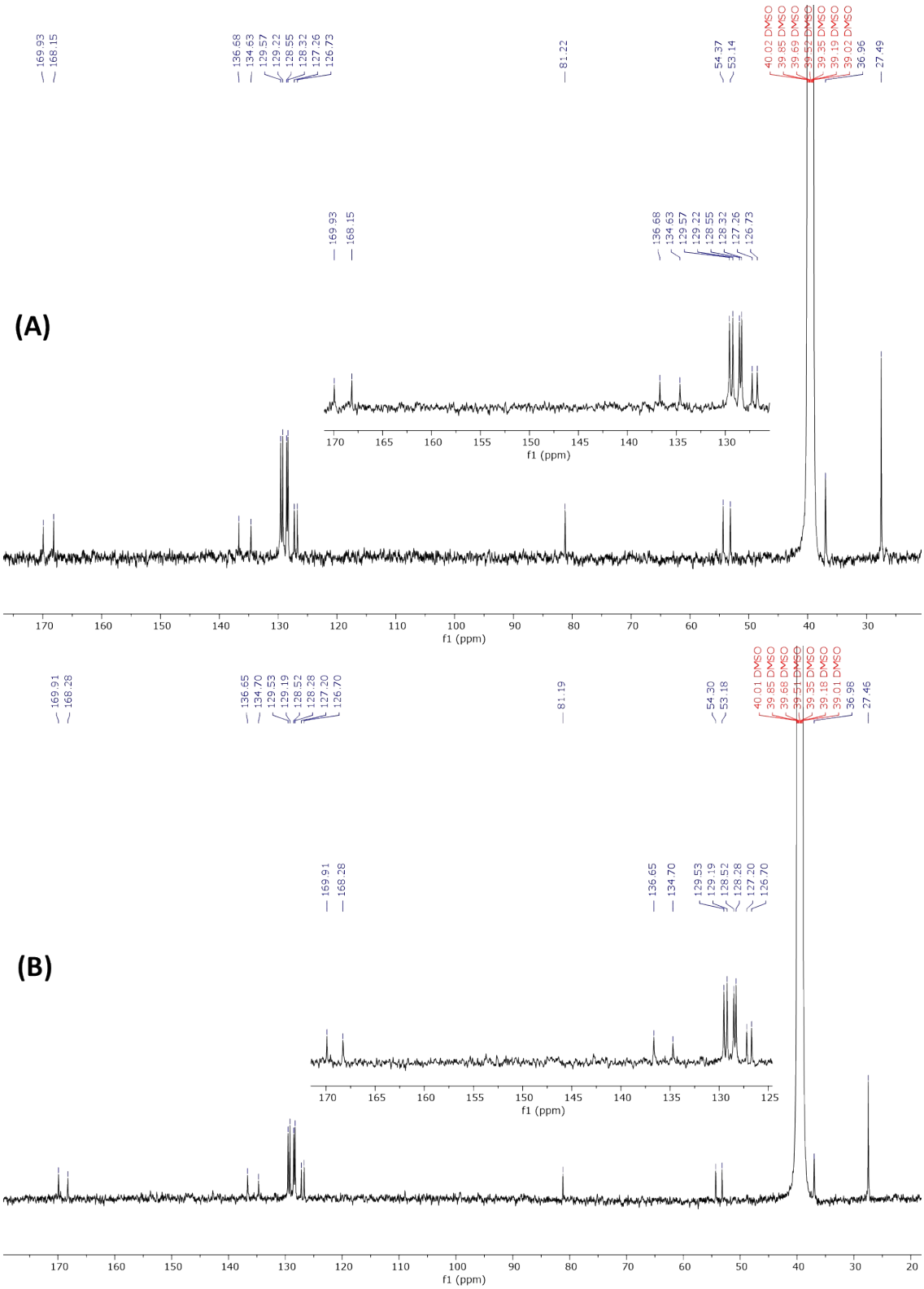


Figure S9. ^{13}C NMR (75 MHz, d_6-DMSO) spectra of swollen organogels after twenty days (A) and after seven months (B).

S5 HIGH-RESOLUTION MASS SPECTROMETRY, HR-MS (ESI-TOF)

High-resolution mass spectrometry experiments were performed to confirm the presence of **2** and **3** in the xerogel sample (**I**). The mass spectrometry experiments were performed with Agilent 6560 Ion mobility Q-TOF mass spectrometer equipped with a dualESI ion source. The samples of **1**, **2** and xerogel **I**, were separately dissolved in DCM (1 mg/ml), diluted in MeOH (5 µl/ml) and measured using direct infusion (5 µl/min flowrate). Data were analyzed using MassHunter B.08.00 software (Agilent Technologies, USA).

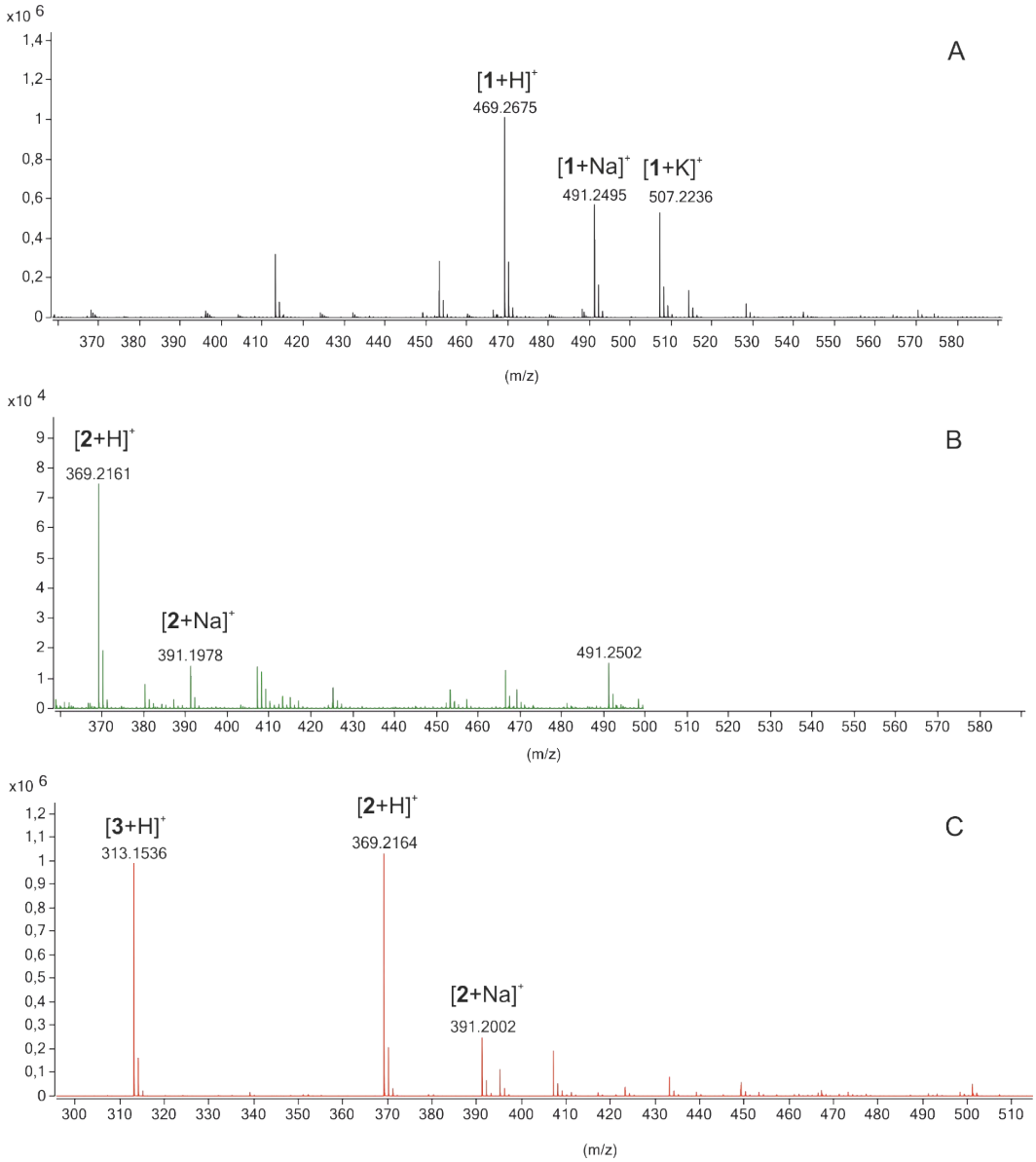


Figure S10. HR-MS analysis of: (A) Boc-Phe-Phe-O*t*Bu **1** (*m/z* for $C_{27}H_{36}N_2O_5$ [**1** + H]$^+$ calculated 469.2697, found 469.2675); (B) Phe-Phe-O*t*Bu **2** (*m/z* for $C_{22}H_{28}N_2O_3$ [**2** + H]$^+$ calculated 369.2173, found 369.2161); (C) xerogel **I** (both compounds Phe-Phe-O*t*Bu **2** and Phe-Phe **3** were found (*m/z* for $C_{22}H_{28}N_2O_3$ [**2** + H]$^+$ calculated 369.2173 found 369.2164; *m/z* for $C_{18}H_{20}N_2O_3$ [**3** + H]$^+$ calculated 313.1547, found 313.1536).

Table S3. *m/z* values, mass accuracies and molecular formulas.

sample	molecular formula	ion	*m/z* (theor)	*m/z* (exp)	mass accuracy (mDa)
1	$C_{27}H_{36}N_2O_5$	[**1**+H]$^+$	469.2697	469.2675	2.2
		[**1**+Na]$^+$	491.2516	491.2495	2.1
		[**1**+K]$^+$	507.2256	507.2236	2.0
2	$C_{22}H_{28}N_2O_3$	[**2**+H]$^+$	369.2173	369.2161	1.2
		[**2**+Na]$^+$	391.1992	391.1978	1.4
Xerogel I	$C_{22}H_{28}N_2O_3$	[**2**+H]$^+$	369.2173	369.2164	0.9
	$C_{22}H_{28}N_2O_3$	[**2**+Na]$^+$	391.1992	391.2002	-1.0
	$C_{18}H_{20}N_2O_3$	[**3**+H]$^+$	313.1547	313.1536	1.1

S6 FT-IR ANALYSIS

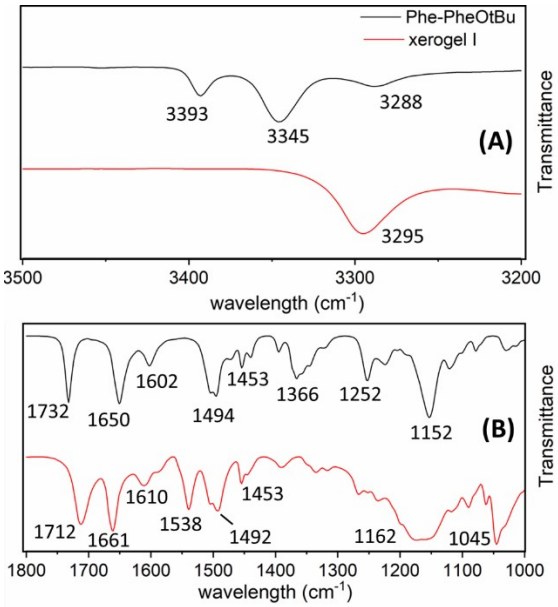


Figure S11. Infrared analysis of organogelator Phe-Phe-O*t*Bu **2** and xerogel **I**. (A) Amide A and B regions; (B) Amide I, II and III regions.

To probe the supramolecular interactions responsible for gelation, we analyzed the Fourier transform infrared (FT-IR) spectra of organogelator **2** and the corresponding xerogel **I**. In the amide A region, the NH stretching vibration gives rise to two bands in the neat Phe-Phe-O*t*Bu **2** spectrum at 3339 and 3345 cm^{-1}, while xerogel **I** shows only one broad peak shifted at a lower frequency (3295 cm^{-1}). Additionally, the NH bending band of xerogel **I** appears upshifted from 1602 to 1610 cm^{-1}. Both observations suggest the involvement of the amine group in H-bond formation.[5] The peak at 1732 cm^{-1} of neat Phe-Phe-O*t*Bu **2** is due to the C=O stretching vibration of the ester group. Xerogel **I**, however, absorbs lower at 1712 cm^{-1} due to the carboxyl group (COOH) of Phe-Phe **3**, which appears to be H-bonded. According to the literature, Phe-Phe **3** and its corresponding analogues adopt *β*-sheet conformations when assembled, as per the amide I band's reported position (1623-1643 cm^{-1} and 1666-1698 cm^{-1}).[6-8] Here, the xerogel shows a peak at 1661 cm^{-1} suggesting the potential formation of 3_{10}-helices[9] rather than a parallel or antiparallel *β*-sheet secondary structure. The amide I band of pure Phe-Phe-O*t*Bu **2** at 1650 cm^{-1} is assigned to aperiodic secondary structures.[10] The amide II (1538 cm^{-1}) peak of the xerogel is due to the combination of the NH out-of-phase and in-plane bends with the C-N stretching vibration. The signal at 1494 cm^{-1} is assigned to the aromatic C=C stretching vibration of the side

chains and is characteristic of the phenylalanine amino acid.[11] Finally, the C-H bend at 1366 cm^{-1} is missing from the xerogel spectrum, while the C-O/ C-N stretching peaks of neat dipeptide **2** at 1252 and 1152 cm^{-1} are merged in a broad peak centered at 1162 cm^{-1}. These spectral differences corroborate the participation of the dipeptide backbone in supramolecular interactions.

S7 RHEOLOGICAL CHARACTERIZATION

Rheology measurements were performed using a Malvern Kinexus Pro+ rheometer, fitted with an 8 mm parallel plate upper geometry. Strain amplitude measurements were performed within the linear viscoelastic region (LVR), where the elastic G′ and loss G″ moduli are independent of the strain amplitude. All measurements were taken at a temperature of 25 °C.

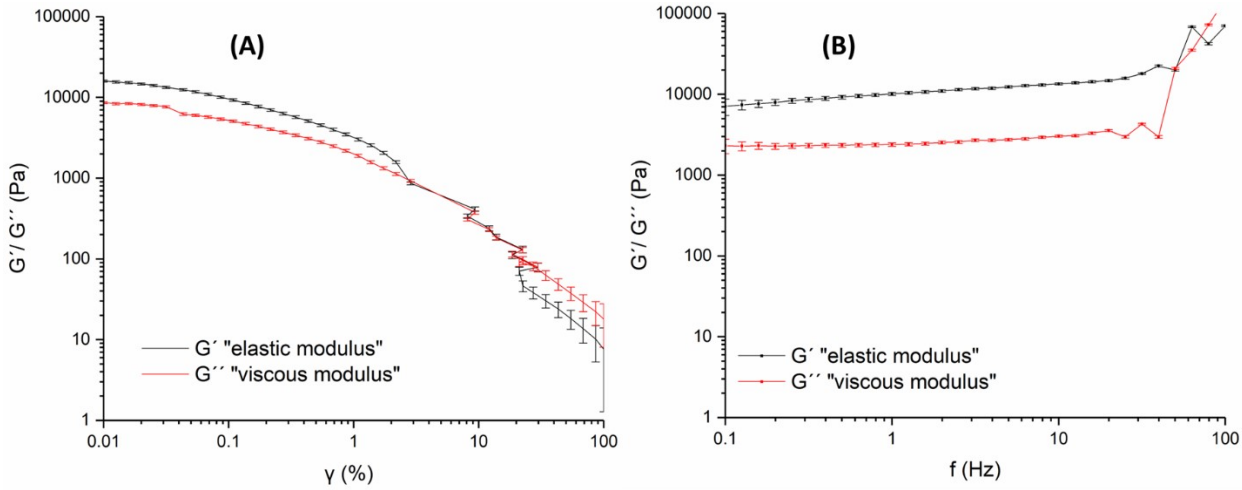


Figure S12. Rheology studies. Amplitude sweep measurements under the constant frequency of 1 Hz (A); Frequency sweep measurements under constant strain (γ%) of 0.01 % (B). The organogel was prepared using 0.05 M of Boc-Phe-Phe-O*t*Bu **1**.

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