Supplementary information

Tunable Solvent-Induced Gelation of Dipeptide-based Gelators: Exploring the Role of Solvent and Acid Concentration

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Table of contents

S1 Materials and methods

Chemicals

All chemicals were used as supplied and used without further purification.

N-(*tert*-butoxycarbonyl)-L-phenylalanine, L-phenylalanyl-L-phenylalanine, L-phenylalanine methyl ester-HCl, *tert*-butyl acetoacetate (*t*BuAcAc), and *tert*-butyl methyl ether (*t*BuOMe) were purchased from TCI, (*S*)-3-phenylalanine-*tert*-butyl ester-HCl from Carbosynth (UK), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU), $NaHCO_3$ and L-phenylalanine-L-phenylalanine methyl ester-HCl from FluoroChem, *N*-(*tert*-butoxycarbonyl)-L-leucine, L-leucine-L-phenylalanine, *tert*-butyl formate (*t*BuOCHO) and $MgSO_4 \cdot H_2O$ from Sigma Aldrich, *tert*-butyl chloroacetate (*t*BuClOAc) from Thermo Scientific, dichloromethane, toluene, and ethyl acetate from VWR Chemicals, concentrated sulfuric acid and hydrochloric acid from Fluka, d_6-dimethylsulfoxide from Eurisotop and *N*,*N*-dimethylformamide from Acros Organics.

NMR spectroscopy

NMR spectra were recorded on a Bruker Advance III HD 300 MHz and 500 MHz spectrometers using d_6-DMSO deuterated solvent. Chemical shifts are given in ppm and the coupling constant *J* in Hz. The spectra were referenced to the solvent signals 2.50 ppm (^{1}H NMR) and 39.52 ppm (^{13}C NMR).

Sample preparation: The gels were dried under vacuum for about 8 hours, after which the xerogel was dissolved in deuterated solvent for NMR measurement.

ATR-FTIR spectroscopy

The spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer in Attenuated Total Reflection (ATR) mode in the spectral range 400-4000 cm^{-1}, spectral resolution of 4 cm^{-1} and 124 scans. All spectra are baseline corrected.

High resolution mass spectrometry (HR-MS)

Experiments were performed with an Agilent 6560 Ion mobility Q-TOF mass spectrometer equipped with a dualESI ion source. The samples were first dissolved in MeOH (2 mM), diluted in MeOH (10 µM) and measured on positive mode using direct infusion (5 µL/min flowrate).

Atomic force microscopy (AFM)

Images were captured on a Bruker Dimension Icon atomic force microscope using PeakForce tapping mode. AFM images were processed with Gwyddion software.

Standard protocol: The diluted gels with original solvent (x5) were drop casted (1 µL) on top of Si-chips and allowed to dry in open air at R.T. overnight.

Transmission electron microscopy (TEM)

Images were captured on a JEOL JEM-1400HC microscope. The samples were prepared on carbon films (400 mesh copper grids) obtained from Agar Scientific.

Standard protocol: The diluted gels with original solvent (x5) were drop casted (1 µL) on top of the carbon films and allowed to dry in open air at R.T. overnight.

Sample preparation for gel system I 0.5 eq in tBuClOAc: A blob of gel was placed on top of the carbon films and allowed to dry in the open air at R.T. overnight.

Scanning electron microscopy (SEM)

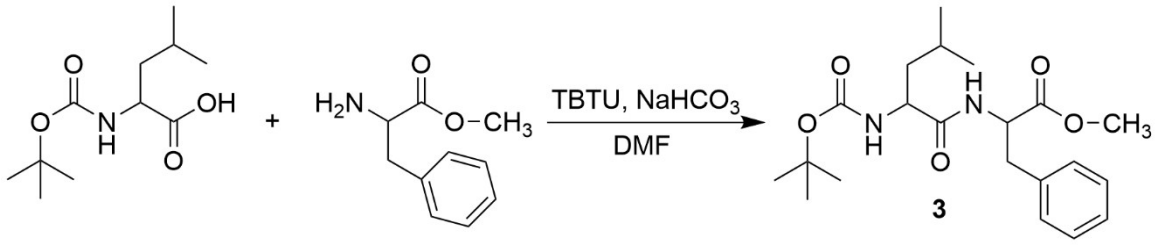
Images were captured with a Zeiss EVO-50XVP microscope. The samples were prepared on carbon films (400 mesh copper grids) obtained from Agar Scientific.

Sample preparation: A carbon film was dipped into gel, and this was left to dried overnight under vacuum.

S2 Synthesis of precursor gelators and the corresponding activated gelators

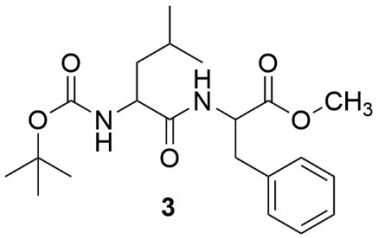
Compounds **1-2** and **1a-2a** were synthesised according to previous protocols.[1]

S2.1 Synthesis of compounds 3 and 2c



General protocol for the synthesis of **3**: Boc-protected leucine (1.0 eq), TBTU (1.0 eq) and $NaHCO_3$ (1.0 eq) were suspended in anhydrous DMF under N_2 atmosphere. Phenylalanine methyl ester (1.1 eq) and $NaHCO_3$ (1.1 eq) were suspended in anhydrous DMF in another flask. The two solutions were left to stir at R.T. for 1 hour. The solutions were mixed and left to stir under N_2 atmosphere at R.T. overnight. The solvent was evaporated under vacuum, co-evaporated with toluene (x3) and the yielded residue was dissolved in DCM. The organic phase was extracted with water (x2), HCl (1.0 M), water (x2), saturated solution of $NaHCO_3$ and water (x2). NaCl was added to the aqueous phases to avoid the formation of a suspension. The obtained organic phase was dried with $MgSO_4$ and evaporated under vacuum.

Boc-Leu-Phe-OMe 3



White powder, yield 74 %. **Melting point:** 83-86 °C

^{1}H NMR (500 MHz, DMSO) δ 8.10 (d, J = 7.7 Hz, 1H), 7.30 – 7.17 (m, 5H), 6.77 (d, J = 8.6 Hz, 1H), 4.49 (q, J = 7.9 Hz, 1H), 3.95 (q, J = 8.7 Hz, 1H), 3.57 (s, 3H), 3.02 (dd, J = 13.9, 5.8 Hz, 1H), 2.94 (dd, J = 13.9, 8.8 Hz, 1H), 1.56 – 1.49 (m, 1H), 1.37 – 1.29 (m, 11H), 0.83 (dd, J = 15.7, 6.6 Hz, 6H). Solvent impurities signals: chloroform at 8.3 ppm and toluene 2.7 ppm.

^{13}C NMR (125 MHz, DMSO) δ 172.50, 171.79, 155.08, 137.02, 129.04, 128.14, 126.46, 77.96, 53.21, 52.62, 51.76, 40.76, 36.59, 28.14, 24.09, 22.80, 21.59.

HR-MS: *m/z* for $C_{21}H_{32}N_2O_5$ $[M+Na]^+$ calculated 415.2203, found 415.2185, mass accuracy 1.8 mDa.

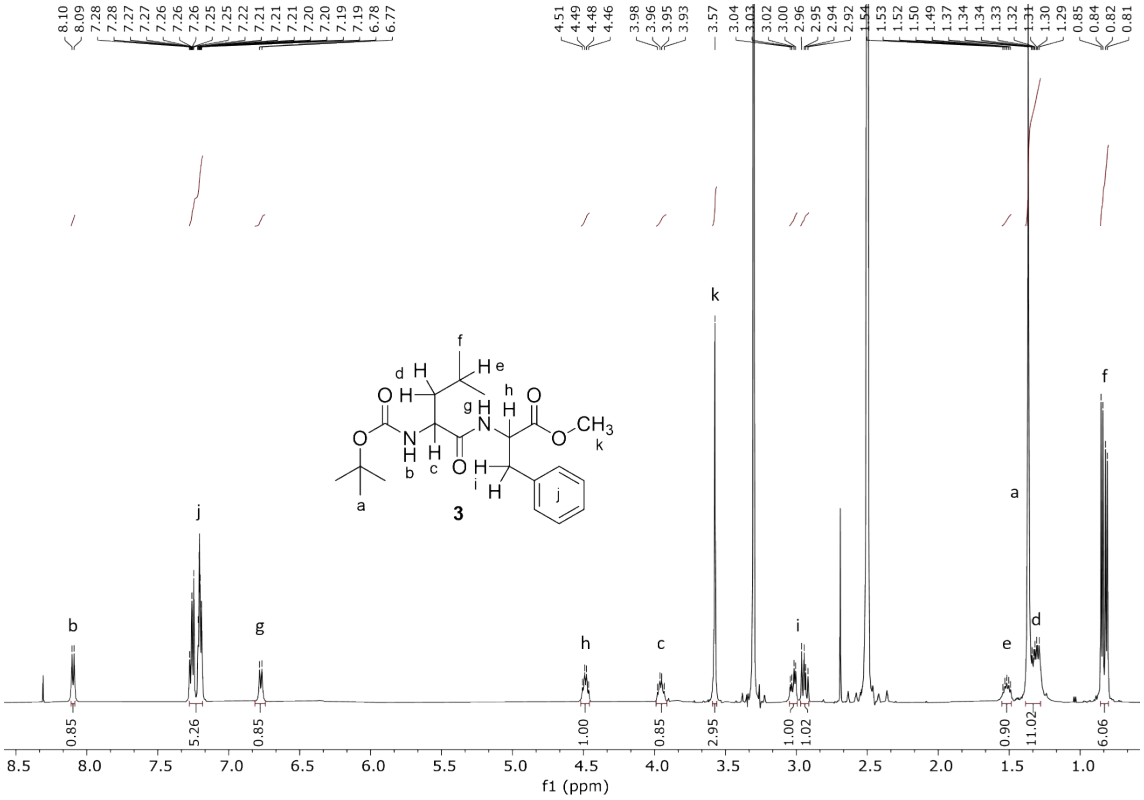


Figure S1. ^{1}H NMR (500 MHz, d_6-DMSO) spectrum of Boc-Leu-Phe-OMe **3**.

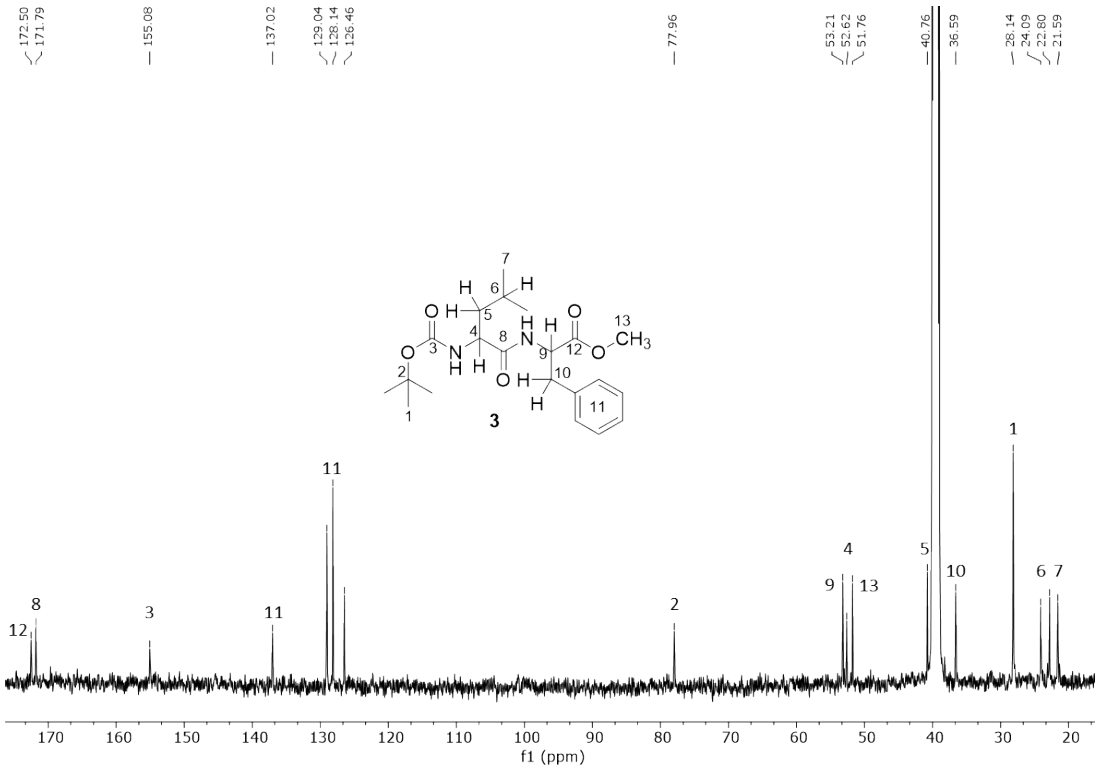
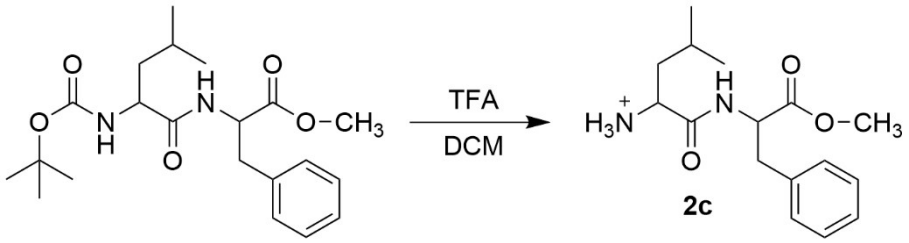


Figure S2. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of Boc-Leu-Phe-OMe **3**.

Leu-Phe-OMe 2c



General protocol for the synthesis of **2c**: The Boc-protected dipeptide methyl ester (1.0 eq) was suspended in DCM at a concentration of 0.2 M. TFA (10 eq) was added dropwise in the solution at R.T., and the reaction mixture was left to stir under N_2 atmosphere overnight. DCM was added to dilute the reaction mixture before evaporating the solvents under vacuum. DCM was added to the obtained residue (x3) and evaporated further.

Yellowish powder, yield 87 %. **Melting point:** 166-168 ℃

^{1}H NMR (500 MHz, DMSO) δ 8.93 (d, *J* = 7.4 Hz, 1H), 8.11 (s, 3H), 7.32 – 7.29 (m, 2H), 7.25 – 7.23 (m, 3H), 4.57 – 4.53 (m, 1H), 3.77 (q, *J* = 6.6 Hz, 1H), 3.60 (s, 3H), 3.08 (dd, *J* = 14.0, 5.9 Hz, 1H), 2.99 (dd, *J* = 14.1, 8.6 Hz, 1H), 1.69 – 1.61 (m, *J* = 6.6 Hz, 1H), 1.55 – 1.51 (m, 2H), 0.89 (t, *J* = 6.2 Hz, 6H).

^{13}C NMR (125 MHz, DMSO) δ 171.24, 169.29, 136.79, 128.98, 128.33, 126.68, 53.82, 51.95, 50.57, 36.30, 23.32, 22.71, 21.65. The CH_2 peak of the Leu unit is under the DMSO solvent peak.

HR-MS: *m/z* for $C_{16}H_{25}N_2O_3^+$ $[M+H]^+$ calculated 293.1860, found 293.1863, mass accuracy 0.3 mDa.

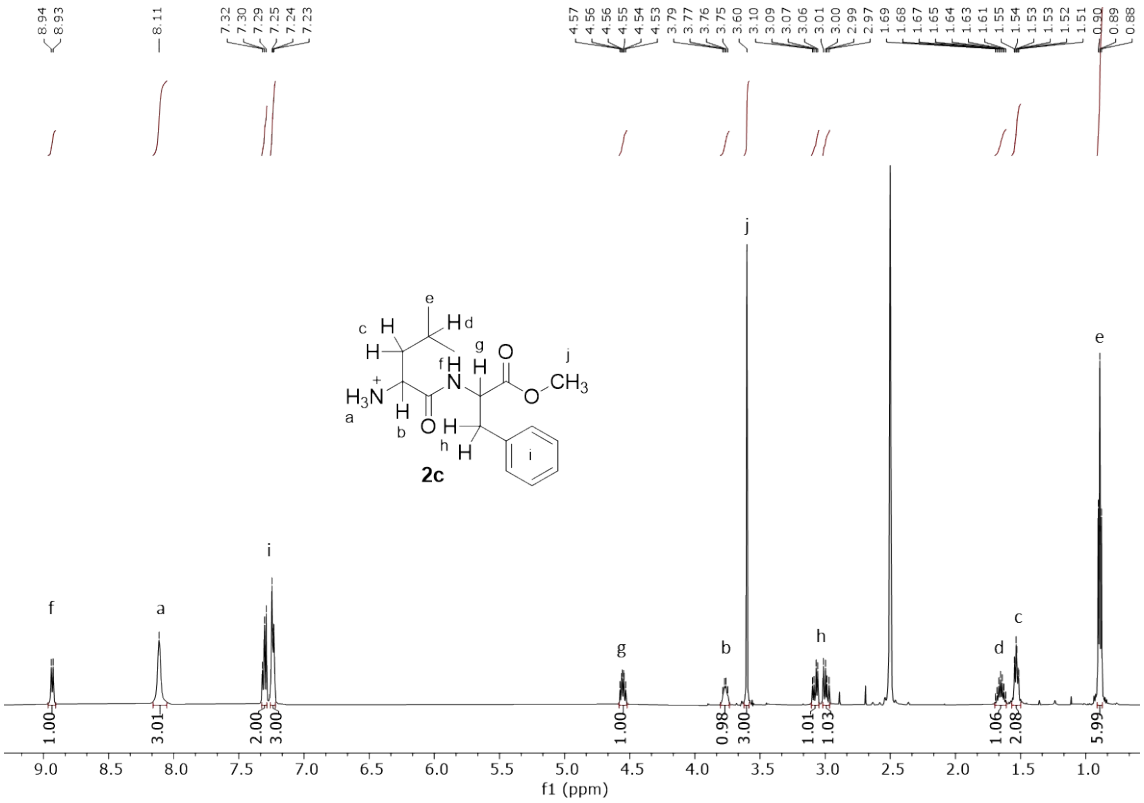


Figure S3. ^{1}H NMR (500 MHz, d_6-DMSO) spectrum of Leu-Phe-OMe **2c**.

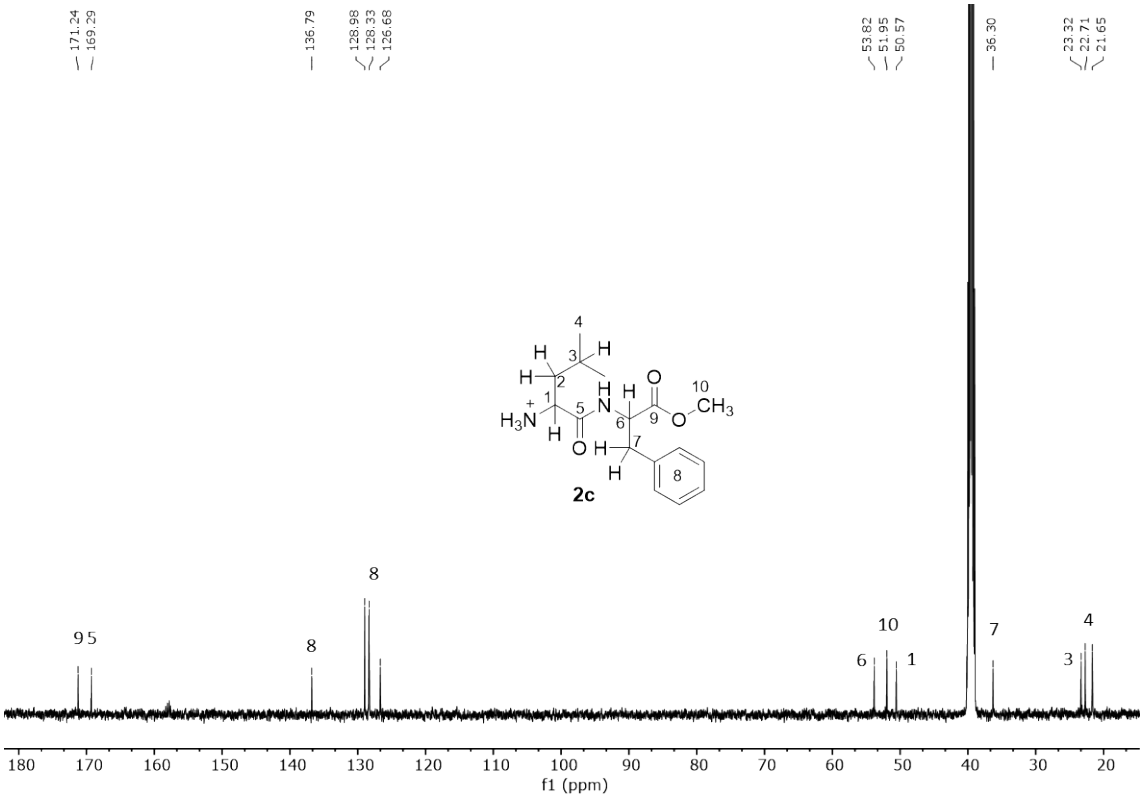


Figure S4. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of Leu-Phe-OMe **2c**.

S3 Gelation, control experiments, and determination of $T_{gel-sol}$

S3.1 Gelation trials

General protocol: The precursor gelator (**1**: 23.5 mg, 0.05 M or **2**: 21.7 mg, 0.05 M) was suspended in the corresponding organic solvent (1 mL) and sonicated until dissolved or a fine suspension was formed, before adding concentrated H_2SO_4 (1.0, 0.5 or 0.18 eq). The mixture was gently swirled and left at R.T. to allow gelation to occur. Lack of free gravitational flow was verified by the vial inversion test. The gelation outcome is presented in Table S1, and images of the gels' inversed vials in Figure S5.

During the gelation studies, it was observed that the gels were sensitive to external vibrations. This can potentially affect the gelation outcome, especially in gels prepared at low acid concentrations. Adjusting the amount of acid was also tested for gel system **II** in *t*BuOMe and gel system **I** in *t*BuClOAc. Higher acid concentrations (1.5 eq and 2.0 eq) were tested for gel system **II** in *t*BuOMe (see main text). The acid amount of 0.75 eq was tested for system **I** in *t*BuClOAc , since addition of 1.0 eq of acid did not yield a gel, while 0.5 eq was successful. Notably, the system remained in the sol state, and the gel was formed only when 0.5 eq of acid or less was used.

Table S1. Gelation trials' outcome for precursor gelators **1** and **2** in different solvents and different concentrations of acid (H_2SO_4).

Precursor gelator	Acid (eq)	*t*BuClOAc	*t*BuOMe	*t*BuAcAc	*t*BuOCHO
BocPhePheO*t*Bu **1**	1.0	Sol	SSG, transparent / opaque, day 5–8	Sol	Sol
	0.5	SSG, opaque, day 1	SSG, transparent / opaque, day 14	Sol	Sol
	0.18	SSG, opaque, day 1	SSG, transparent / opaque, day 14	Sol	Sol
BocLeuPheO*t*Bu **2**	1.0	SSG, opaque, day 1˙	SSG, transparent, day 8–13	Sol	Sol
	0.5	SSG, transparent, day 1	SSG / PG, transparent, day 12–14*	PG, opaque, day 1–2	Sol
	0.18	SSG, transparent, day 1	PG, transparent, day 13–14*	Sol	Sol

*Gelation outcome (*e.g.,* amount of gel) varies; ˙lifespan 2 days

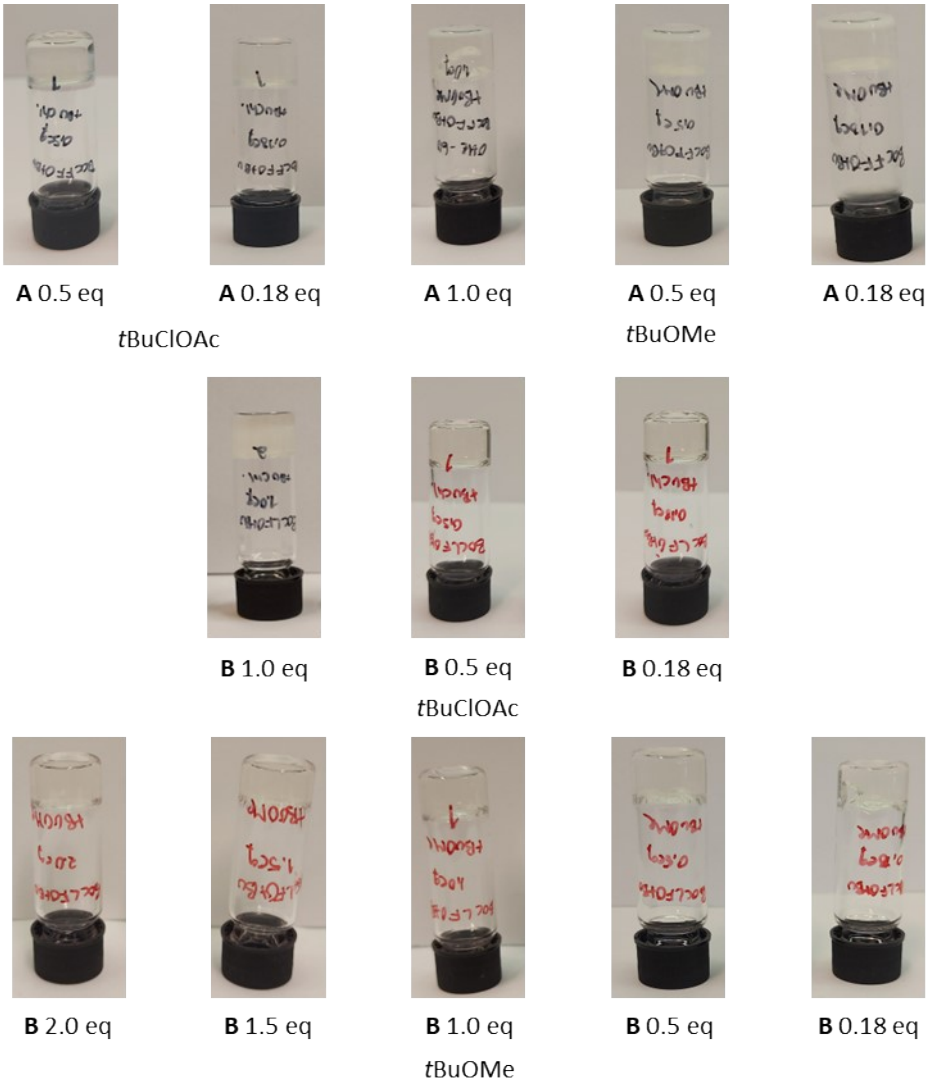


Figure S5. Vial inversion test. A) Gel system **I**, precursor gelator is Boc-Phe-Phe-O*t*Bu **1** and B): Gel system **II**, precursor gelator is Boc-Leu-Phe-O*t*Bu **2**.

S3.2 Gel material solubility test in *t*BuOH

The solubility of the gel systems in tBuOH was examined due to the observed transient property in gel system **II** in tBuClOAc, 1.0 eq of acid. The highest-equivalency gel systems were prepared by the general gelation protocol (S3.1) in triplicate. 500 µL of tBuOH was added on top of the material after formation to determine whether it dissolved / broke. All tested systems eventually dissolved. Gel system **II** dissolved within a day in both solvents. Gel system **I** dissolved in both solvents after the addition of extra tBuOH (500 µL) within a day.

S3.3 Gelation controls

Gelation control trials were performed in *t*BuClOAc and *t*BuOMe using the corresponding gelators, synthesised from the gelator precursors. These trials allowed us to determine the gelation efficacy of the activated gelators and assess their actual gelation behaviour. The obtained results are given in Table S2. No control trials have been performed for the systems marked with an asterisk as the formation of the corresponding methyl-protected gelators (**1c** and **2c**) does not occur in *t*BuClOAc.

Table S2. Gelation control trials and gelation outcome of individual gelators.

Gelator	**Acid (eq)**	***t*BuClOAc**	**Acid (eq)**	***t*BuOMe**
Phe-Phe-O*t*Bu **1a**	0.18	Sol	0.18	SSG
Phe-Phe **1b**	0.18	SSG	0.18	SSG
Phe-Phe-OMe **1c**	*	*	1.0	SSG
Leu-Phe-O*t*Bu **2a**	0.18	PG	1.0	SSG
Leu-Phe **2b**	0.5	Sol	0.5	Sol
Leu-Phe-OMe **2c**	*	*	1.0	SSG

* Gelation control trials cannot be performed.

S3.4 Phase transition temperature ($T_{gel\text{-}sol}$) measurements

The phase transition temperature ($T_{gel\text{-}sol}$) and the reformation of the organogels after heating were assessed for SSGs and PGs obtained during the gelation trials. The organogels were heated using a block heater. The temperature was raised, starting from 30 ℃, by 5 ℃ intervals every 10 min until the gels transitioned to the sol state. The lack of free gravitational flow of the gels was assessed by the vial inversion method before each temperature increment. All gels reformed after cooling at R.T. suggesting that the materials are thermoreversible by nature.

S3.5 pH measurements of the precursor gelator solutions

Table S3. pH values of precursor gelators 1 and 2 in solution prior acid addition. pH values are given for both samples in triplicate and their corresponding average value.

Precursor gelator	*t*BuClOAc		*t*BuOMe	
Boc-Phe-Phe-O*t*Bu **1**	2.30	2.46	4.25	4.38
	2.52		4.72	
	2.58		4.16	
Boc-Leu-Phe-O*t*Bu **2**	3.44	3.38	5.21	5.22
	3.39		5.22	
	3.30		5.23	

S4 NMR and MS spectra of gels

Gel samples were prepared in vials and dried under vacuum overnight. No further purification was performed prior NMR analysis.

S4.1 Boc-Phe-Phe-O*t*Bu in *t*BuClOAc

S4.1.1 Sample gel prepared with 0.5 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.89 (d, *J* = 7.5 Hz, 1H), 8.82 (d, *J* = 7.8 Hz, 0.2H), 7.32 – 7.23 (m, 12H), 4.48 – 4.41 (m, 2H), 4.04 (dd, *J* = 8.1, 5.1 Hz, 1H), 3.14 (dd, *J* = 14.2, 5.0 Hz, 1H), 2.96 (dq, *J* = 14.2, 7.0 Hz, 4H), 1.32 (s, 9H). Solvent peak signals appear at 4.25 and 1.43 ppm. Solvent impurities are marked with an asterisk.

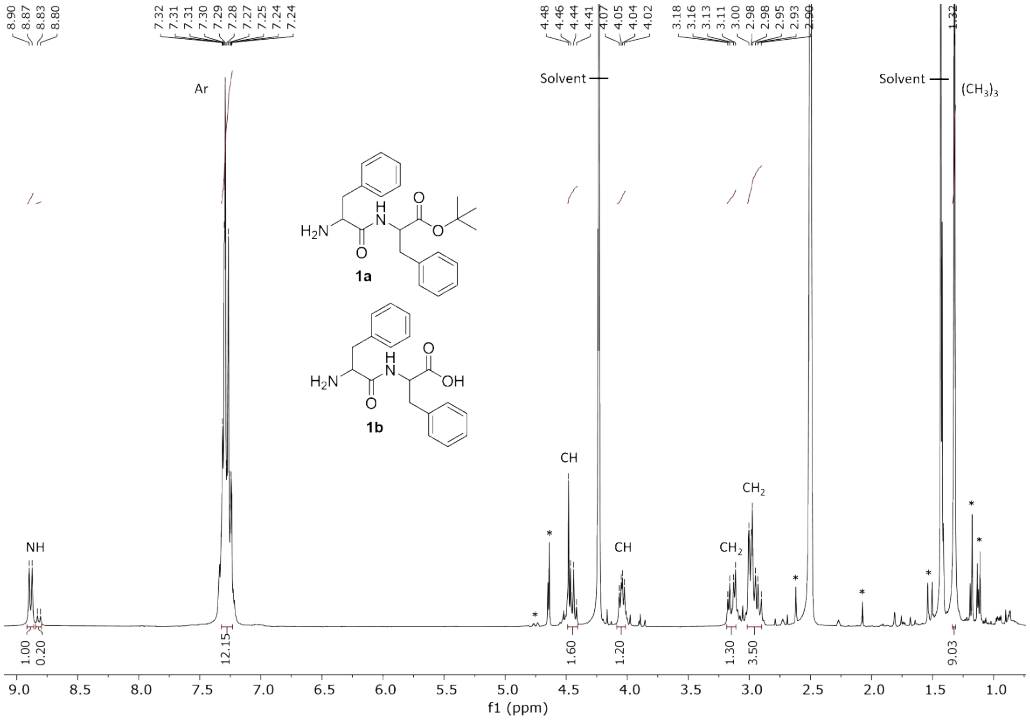


Figure S6. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 174.12, 172.22, 169.95, 168.58, 168.53, 168.15, 167.09, 166.30, 166.20, 137.19, 136.77, 134.71, 129.66, 129.27, 129.21, 128.52, 128.35, 128.32, 127.21, 126.72, 126.64, 82.17, 81.94, 81.17, 67.69, 62.08, 61.73, 60.13, 59.90, 59.55, 57.25, 54.46, 53.87, 53.17, 41.99, 41.50, 40.78, 40.69, 37.03, 36.91, 29.67, 27.66, 27.59, 27.51, 26.61. Solvent peaks are marked with an asterisk.

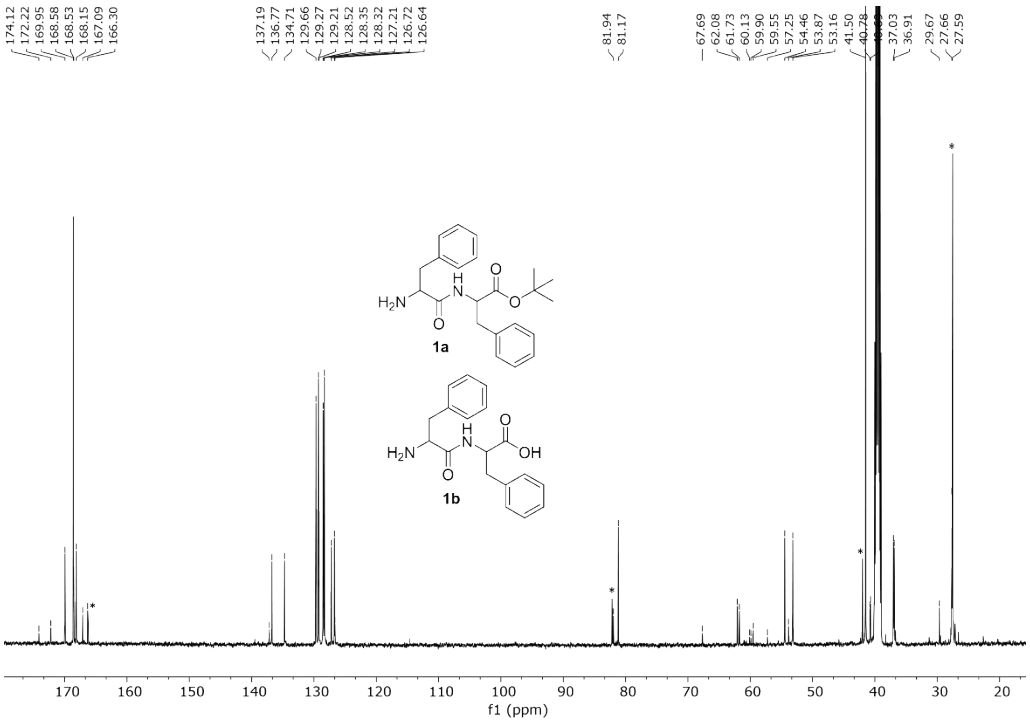


Figure S7. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5eq of acid.

HR-MS: *m/z* for $C_{27}H_{36}N_2O_5$ $[M + Na]^+$ calculated 491.2561, found 491.2519, mass accuracy -0.3 mDa. *m/z* for $C_{22}H_{28}N_2O_3$ $[M + H]^+$ calculated 369.2173, found 369.2172, mass accuracy 0.1 mDa. *m/z* for $C_{18}H_{20}N_2O_3$ $[M + H]^+$ calculated 313.1547, found 313.1570, mass accuracy -2.3 mDa.

S4.1.2 Sample gel prepared with 0.18 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.90 (d, *J* = 7.5 Hz, 1H), 8.85 – 8.82 (m, 0.09H), 8.19 (d, *J* = 7.6 Hz, 0.47H), 7.38 – 7.16 (m, 16H), 6.82 (d, *J* = 8.8 Hz, 0.47H), 4.52 – 4.32 (m, 2H), 4.06 (dd, *J* = 8.1, 5.1 Hz, 1H), 3.22 – 3.08 (m, 1H), 3.06 – 2.87 (m, 5H), 2.75 – 2.65 (m, 1H), 1.32 (s, 13H), 1.28 (s, 4H). Solvent peaks signals appear at 4.25 and 1.43 ppm. Solvent impurity peaks are marked with an asterisk, and the precursor peaks are marked with a square.

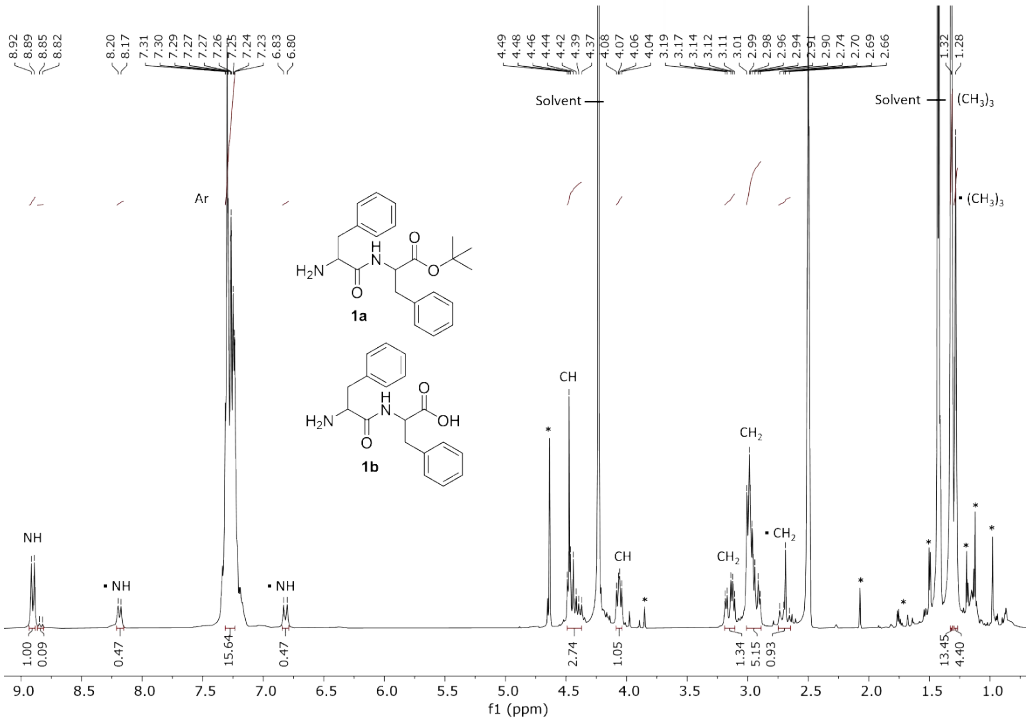


Figure S8. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 174.09, 171.70, 170.39, 169.92, 168.55, 168.12, 167.06, 166.27, 166.17, 155.14, 138.10, 137.10, 136.75, 134.71, 129.63, 129.25, 128.48, 128.28, 128.16, 127.97, 127.18, 126.69, 126.50, 126.16, 82.14, 81.91, 81.14, 80.72, 78.00, 62.05, 61.70, 60.10, 59.52, 55.53, 54.44, 54.09, 53.14, 41.96, 41.47, 40.75, 40.67, 37.47, 36.99, 36.92, 36.87, 31.29, 31.15, 29.64, 29.43, 28.11, 27.63, 27.56, 27.49. Solvent peaks are marked with an asterisk.

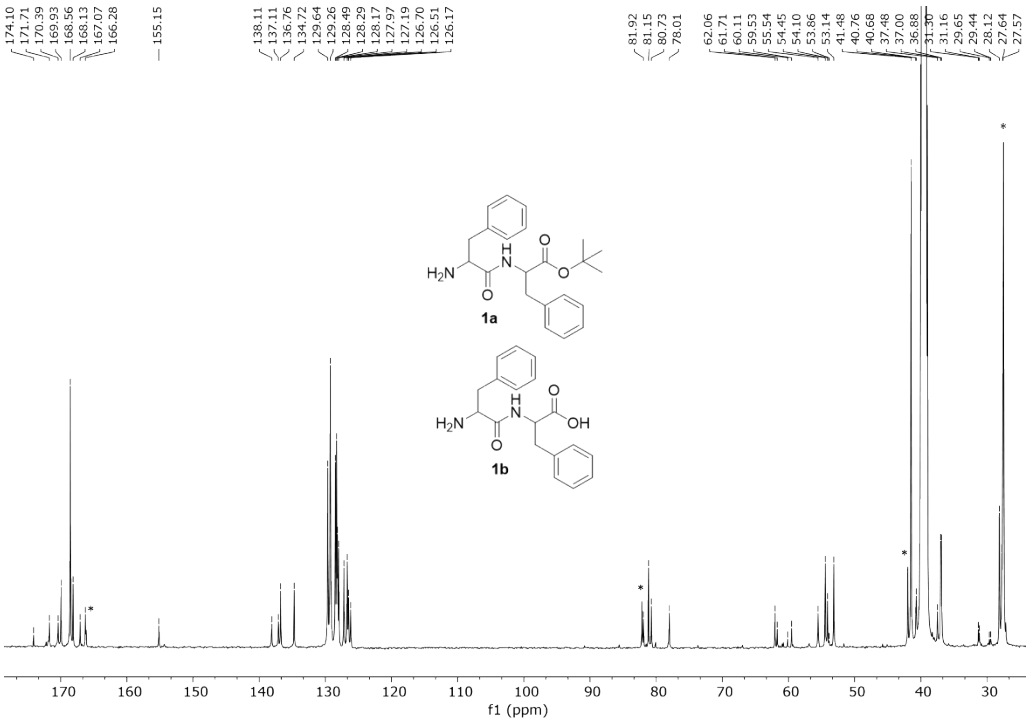


Figure S9. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq of acid.

HR-MS: *m/z* for $C_{27}H_{36}N_2O_5$ $[M + Na]^+$ calculated 491.2516, found 491.2520, mass accuracy -0.4 mDa. *m/z* for $C_{22}H_{28}N_2O_3$ $[M + H]^+$ calculated 369.2173, found 369.2167, mass accuracy 0.6 mDa. *m/z* for $C_{18}H_{20}N_2O_3$ $[M + H]^+$ calculated 313.1547, found 313.1575, mass accuracy -2.8 mDa.

S4.2 Boc-Leu-Phe-O*t*Bu in *t*BuClOAc

S4.2.1 Sample gel prepared with 1.0 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.83 (d, *J* = 7.3 Hz, 0.5H), 8.76 (d, *J* = 7.7 Hz, 1H), 8.05 (s, 4H), 7.31 – 7.21 (m, 7H), 4.52 – 4.43 (m, 2H), 3.76 (s, 1H), 3.09 (dd, *J* = 14.5, 4.9 Hz, 1H), 2.98 (dd, *J* = 10.7, 3.7 Hz, 2H), 1.72 – 1.62 (m, 1H), 1.57 – 1.51 (m, 3H), 1.31 (s, 4H), 0.95 – 0.83 (m, 9H). Solvent peak signals appear at 4.25 and 1.43 ppm. Solvent impurities are marked with an asterisk.

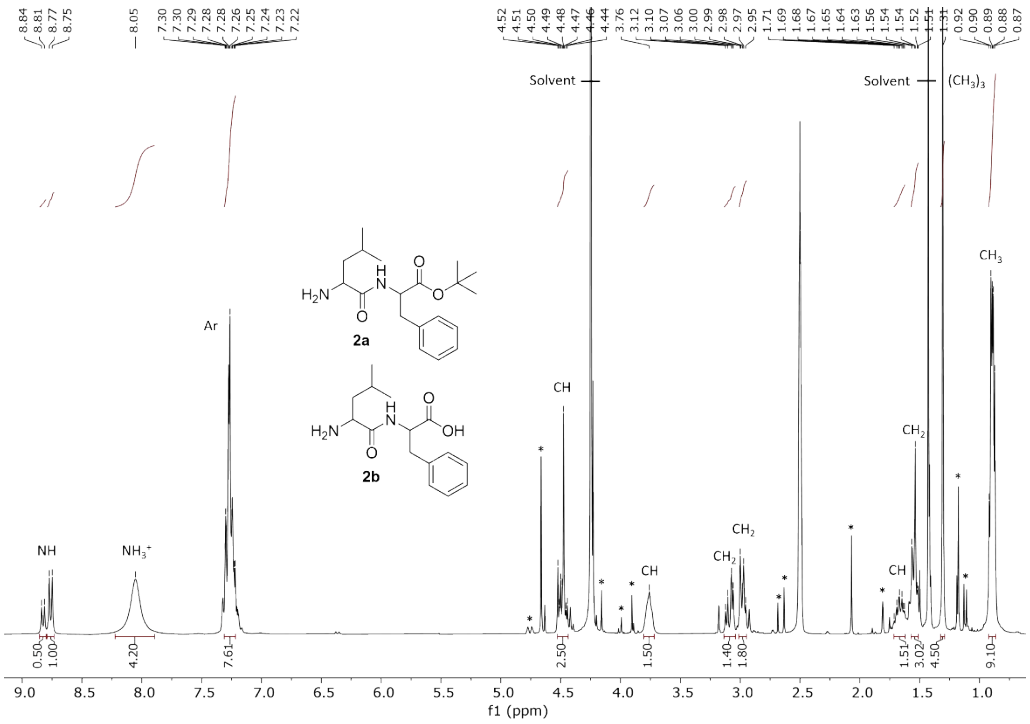


Figure S10. ^{1}H NMR (300 MHz, d6-DMSO) spectrum of the xerogel prepared with 1.0 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 172.12, 169.77, 169.06, 169.02, 168.44, 168.38, 166.98, 166.20, 137.23, 136.84, 129.10, 129.04, 128.24, 128.22, 126.59, 126.51, 82.10, 80.96, 67.61, 61.66, 60.49, 59.52, 54.46, 53.78, 50.73, 50.69, 41.90, 41.38, 40.67, 40.27, 40.19, 36.70, 36.47, 29.61, 27.61, 27.53, 27.44, 26.52, 23.37, 23.32, 22.75, 22.70, 21.75, 21.72. Solvent peaks are marked with an asterisk.

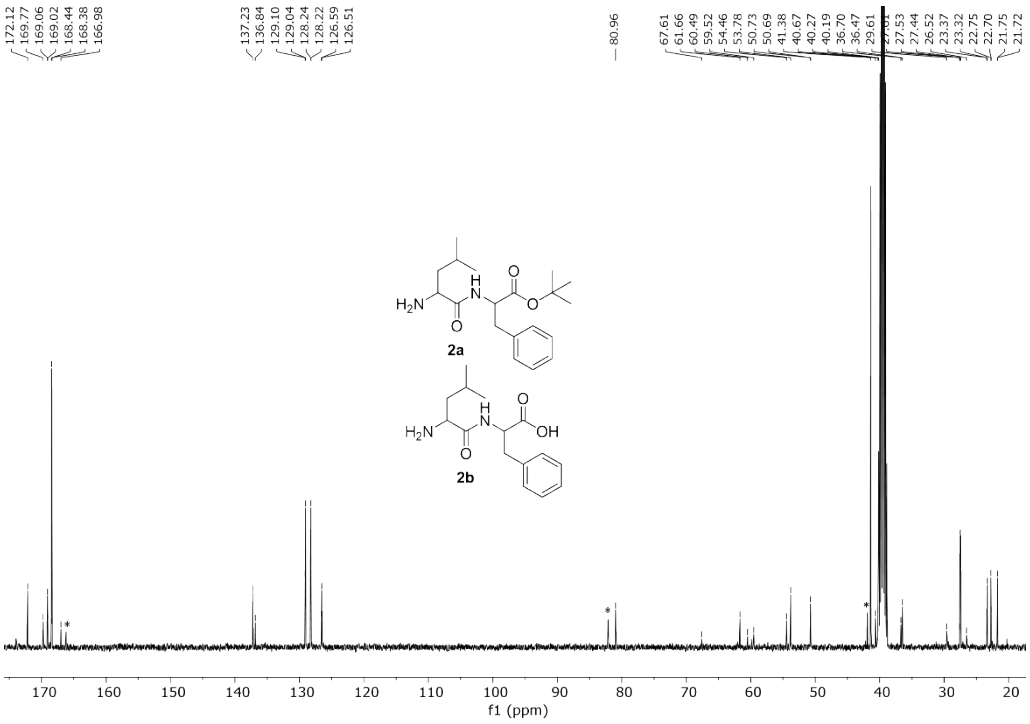


Figure S11. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 1.0 eq of acid.

HR-MS: *m/z* for $C_{19}H_{30}N_2O_3$ $[M + H]^+$ calculated 335.2329, found 335.2323, mass accuracy 0.6 mDa. *m/z* for $C_{15}H_{22}N_2O_3$ $[M + H]^+$ calculated 279.1705, found 279.1700, mass accuracy 0.5 mDa.

S4.2.2 Sample gel prepared with 0.5 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.83 (d, *J* = 7.4 Hz, 1H), 8.77 (d, *J* = 7.7 Hz, 0.17H), 7.33 – 7.21 (m, 6H), 4.49 – 4.40 (m, 2H), 3.79 – 3.75 (m, 1H), 2.99 (dd, *J* = 7.3, 3.1 Hz, 2H), 1.67 (dq, *J* = 12.6, 6.4 Hz, 1H), 1.59 – 1.53 (m, 2H), 1.31 (s, 9H), 0.90 (dd, *J* = 6.3, 3.9 Hz, 7H). Solvent peaks appear at 4.25 and 1.43 ppm. Solvent impurity peaks are marked with an asterisk, and precursor peaks are marked with a square.

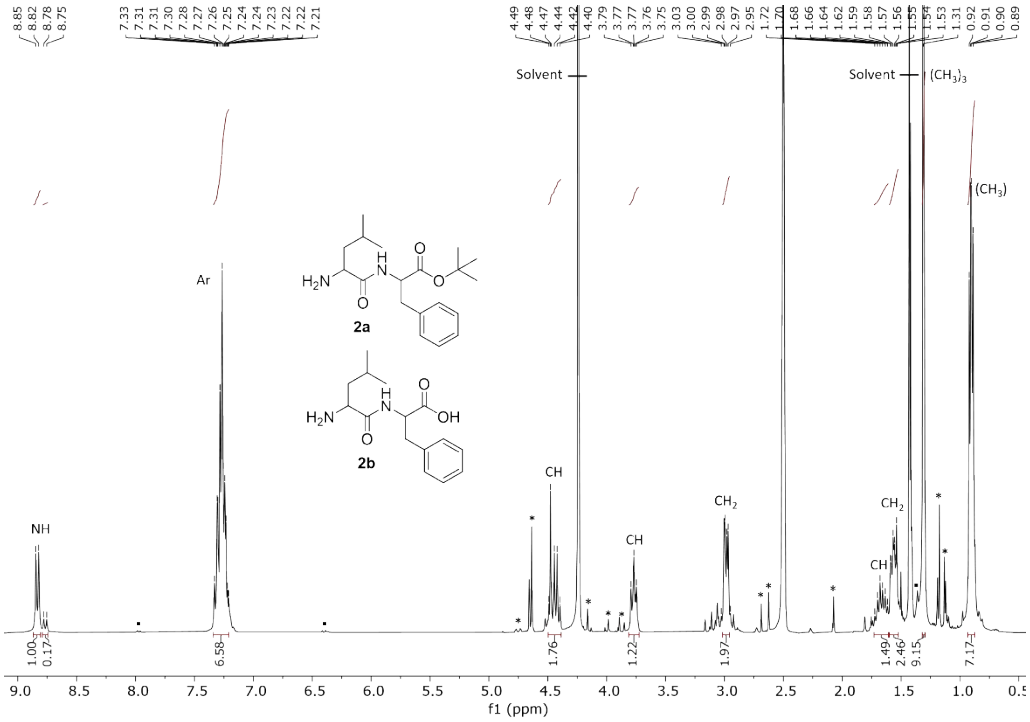


Figure S12. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 174.09, 172.23, 169.85, 169.12, 168.56, 167.07, 166.28, 166.18, 137.27, 136.87, 129.17, 129.12, 128.31, 128.28, 126.65, 126.57, 82.14, 81.92, 80.98, 62.06, 61.71, 60.11, 59.52, 54.51, 53.83, 50.63, 41.97, 41.48, 40.31, 40.23, 36.69, 36.46, 31.30, 29.66, 27.64, 27.57, 27.47, 26.59, 23.40, 23.36, 22.83, 22.79, 21.78, 21.75. Solvent peaks are marked with an asterisk.

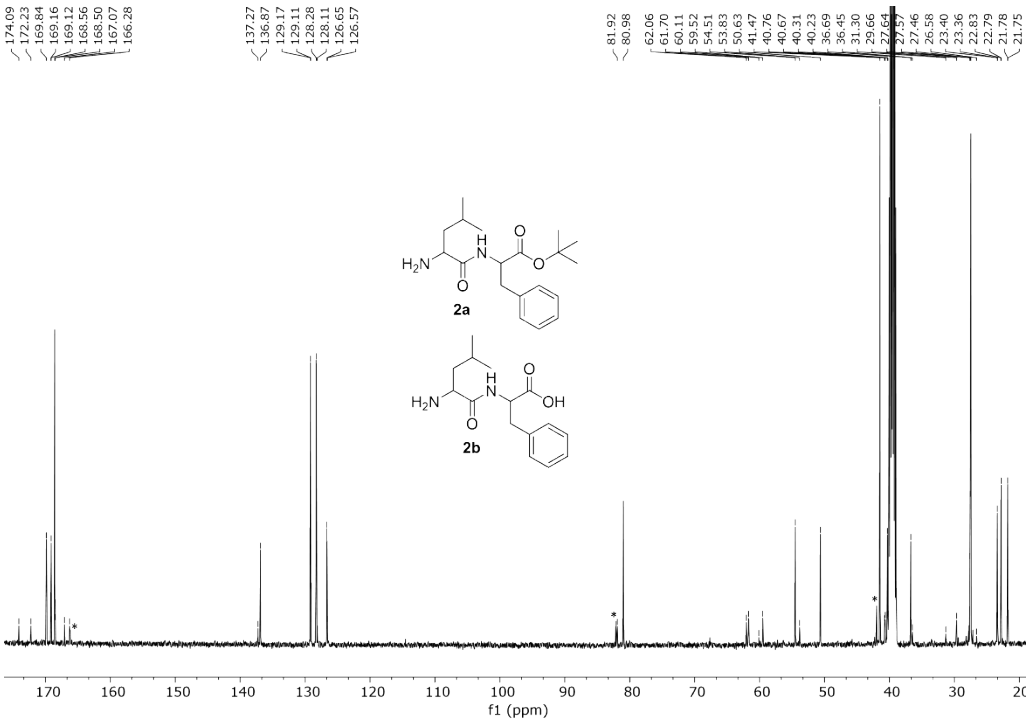


Figure S13. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5 eq of acid.

HR-MS: *m/z* for $C_{24}H_{38}N_2O_5$ $[M + H]^+$ calculated 435.2854, found 435.2852, mass accuracy 0.2 mDa. *m/z* for $C_{19}H_{30}N_2O_3$ $[M + H]^+$ calculated 335.2329, found 335.2326, mass accuracy 0.3 mDa. *m/z* for $C_{15}H_{22}N_2O_3$ $[M + H]^+$ calculated 279.1703, found 279.1704, mass accuracy -0.1 mDa.

S4.2.3 Sample gel prepared with 0.18 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.83 (d, *J* = 7.4 Hz, 0.65H), 8.78 – 8.75 (m, 0.04H), 7.97 (d, *J* = 7.6 Hz, 1H), 7.31 – 7.18 (m, 9H), 6.78 (d, *J* = 8.6 Hz, 1H), 4.50 – 4.42 (m, 1H), 4.35 (q, *J* = 7.3 Hz, 1H), 4.02 – 3.90 (m, 1H), 3.78 (d, *J* = 8.1 Hz, 1H), 3.01 – 2.92 (m, 3H), 1.73 – 1.60 (m, 2H), 1.61 – 1.51 (m, 3H), 1.36 (s, 9H), 1.31 (s, 15H), 0.91 (dd, *J* = 6.3, 3.8 Hz, 4H), 0.84 (dd, *J* = 8.4, 6.5 Hz, 6H). Solvent peaks appear at 4.25 and 1.43 ppm. Solvent impurity peaks are marked with an asterisk, and precursor peaks are marked with a square.

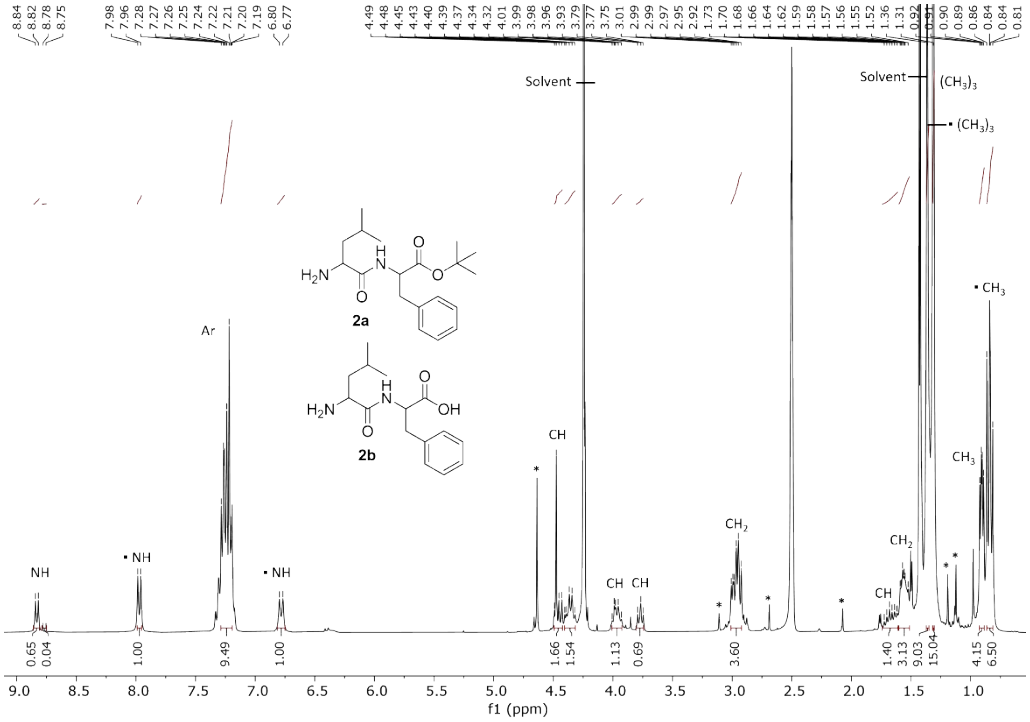


Figure S14. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 174.08, 172.41, 170.36, 169.84, 169.12, 168.55, 167.05, 166.26, 166.16, 155.16, 137.15, 136.87, 129.22, 129.17, 128.27, 128.09, 126.64, 126.42, 82.13, 81.90, 80.98, 80.63, 77.96, 62.05, 61.70, 60.10, 59.52, 54.49, 53.86, 52.65, 50.62, 41.46, 36.83, 36.69, 31.29, 31.15, 28.17, 27.63, 27.56, 27.48, 24.19, 23.40, 22.89, 22.78, 21.77, 21.55. Solvent peaks are marked with an asterisk.

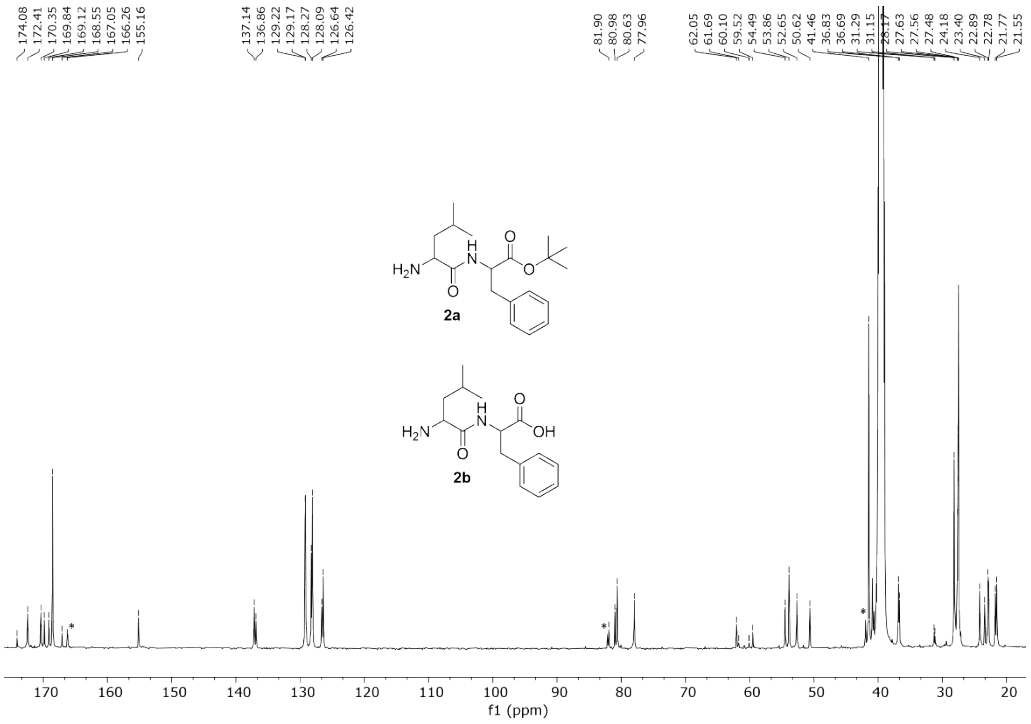


Figure S15. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq of acid.

HR-MS: *m/z* for $C_{24}H_{38}N_2O_5$ $[M + Na]^+$ calculated 457.2673, found 457.2659, mass accuracy 1.4 mDa. *m/z* for $C_{19}H_{30}N_2O_3$ $[M + H]^+$ calculated 335.2329, found 335.2305, mass accuracy 2.4 mDa. *m/z* for $C_{15}H_{22}N_2O_3$ $[M + H]^+$ calculated 279.1703, found 279.1685, mass accuracy 1.8 mDa.

S4.3 Boc-Phe-Phe-O*t*Bu in *t*BuOMe

S4.3.1 Sample gel prepared with 1.0 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.90 (d, *J* = 7.5 Hz, 1H), 8.84 (d, *J* = 7.8 Hz, 0.26H), 8.07 (s, 4H), 7.32 – 7.24 (m, 13H), 4.54 – 4.40 (m, 1H), 4.06 (s, 1H), 3.19 – 3.11 (m, 1H), 3.01 – 2.91 (m, 3H), 1.32 (s, 9H). Solvent peaks appear at 3.07 and 1.10 ppm. Solvent impurity peaks are marked with an asterisk, and precursor peaks are marked with a square.

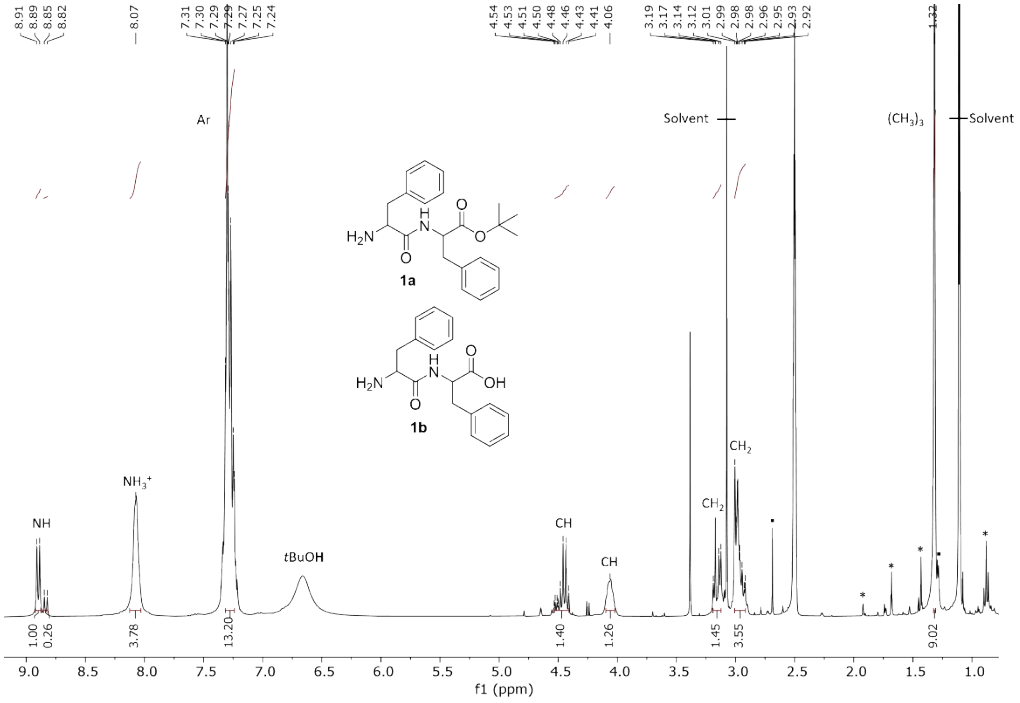


Figure S16. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 1.0 eq of acid.

^{13}C NMR (100 MHz, DMSO) δ 169.71, 167.91, 136.62, 134.52, 129.46, 129.05, 128.99, 128.34, 128.14, 127.04, 126.52, 126.45, 81.03, 54.29, 53.69, 53.04, 36.94, 36.77, 31.17, 27.38. Solvent peaks are marked with an asterisk.

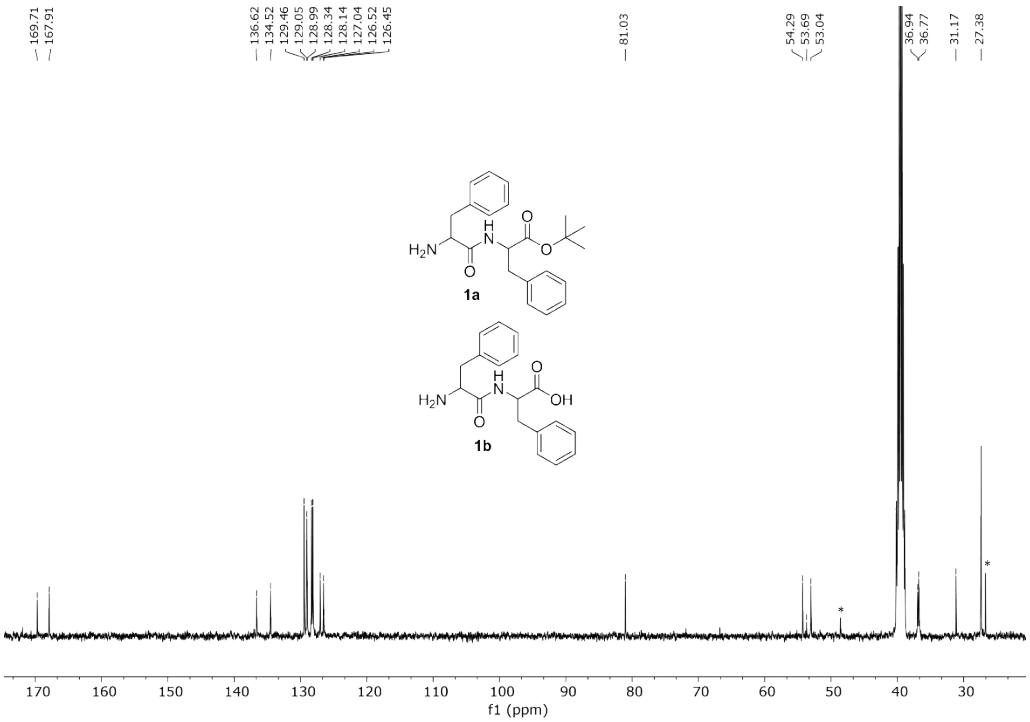


Figure S17. ^{13}C NMR (100 MHz, d_6-DMSO) spectrum of the xerogel prepared with 1.0 eq of acid.

HR-MS: *m/z* for $C_{27}H_{36}N_2O_5$ $[M + H]^+$ calculated 469.2697, found 469.2683, mass accuracy 1.4 mDa. *m/z* for $C_{22}H_{28}N_2O_3$ $[M + H]^+$ calculated 369.2173, found 369.2159, mass accuracy 1.4 mDa. *m/z* for $C_{18}H_{20}N_2O_3$ $[M + H]^+$ calculated 313.1547, found 313.1541, mass accuracy 0.6 mDa. *m/z* for $C_{19}H_{22}N_2O_3$ $[M + H]^+$ calculated 327.1703, found 327.1698, mass accuracy 0.5 mDa.

S4.3.2 Sample gel prepared with 0.5 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.90 (d, *J* = 7.5 Hz, 1H), 8.84 (d, *J* = 7.8 Hz, 0.22H), 8.19 (d, *J* = 7.5 Hz, 0.35H), 8.07 (s, 4H), 7.32 – 7.23 (m, 15H), 6.82 (d, *J* = 8.8 Hz, 0.35H), 4.54 – 4.31 (m, 1H), 4.24 – 4.14 (m, 0.35H), 4.06 (s, 1H), 3.16 (dd, *J* = 14.2, 5.0 Hz, 2H), 3.05 – 2.87 (m, 4H), 2.75 – 2.66 (m, 0.55H), 1.32 (s, 12H), 1.28 (s, 3H). Solvent peaks appear at 3.07 and 1.10 ppm. Solvent impurity peaks are marked with an asterisk, and precursor peaks are marked with a square.

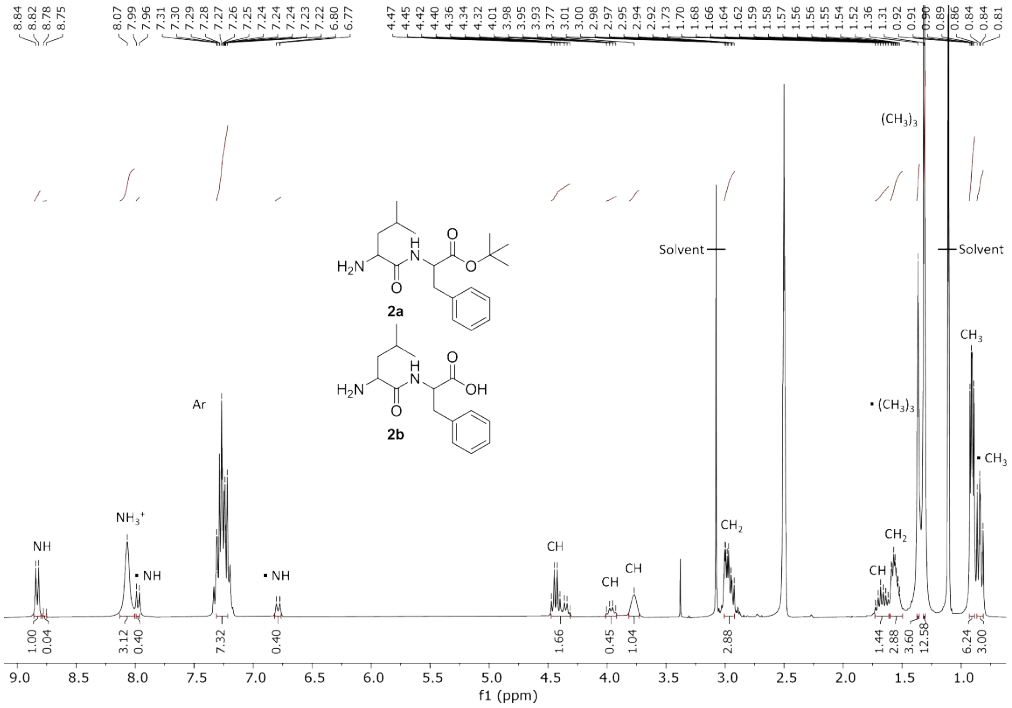


Figure S18. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 172.19, 171.70, 170.38, 169.92, 168.14, 168.12, 155.14, 138.10, 137.14, 137.09, 136.71, 134.67, 134.65, 129.62, 129.53, 129.23, 129.17, 128.51, 128.33, 128.30, 128.17, 127.98, 127.21, 127.19, 126.71, 126.63, 126.51, 126.16, 81.16, 80.73, 78.00, 66.94, 55.52, 54.40, 54.09, 53.82, 53.16, 53.13, 52.84, 37.00, 36.91, 36.69, 31.30, 28.12, 27.49. Solvent peaks are marked with an asterisk.

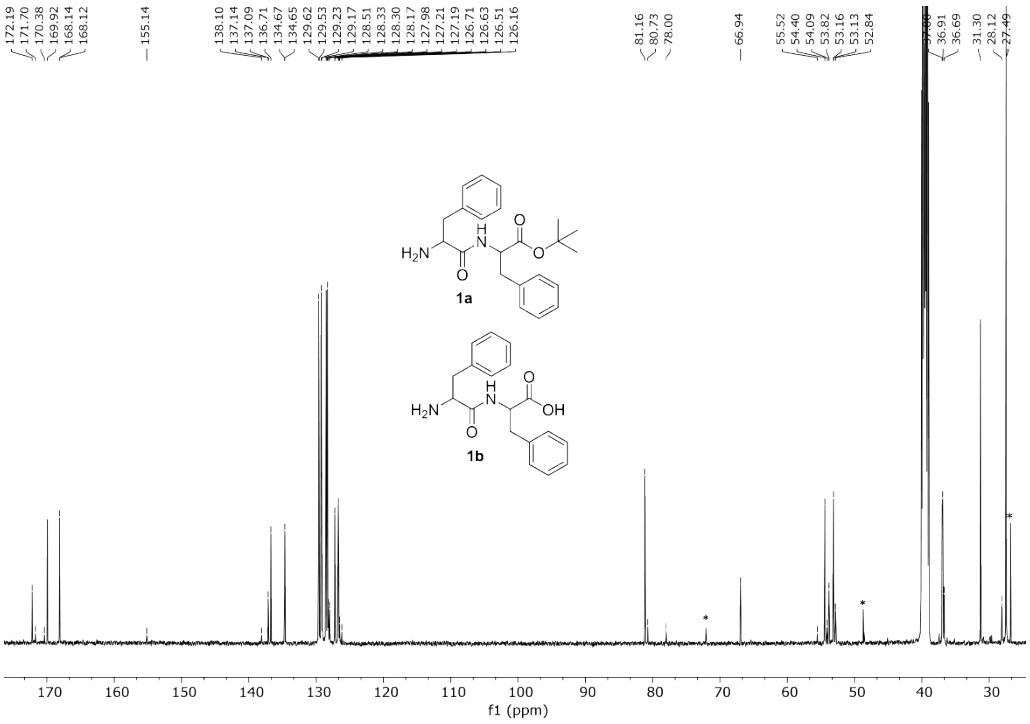


Figure S19. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5 eq of acid.

HR-MS: *m/z* for $C_{27}H_{36}N_2O_5$ $[M + H]^+$ calculated 469.2697, found 469.2685, mass accuracy 1.2 mDa. *m/z* for $C_{22}H_{28}N_2O_3$ $[M + H]^+$ calculated 369.2173, found 369.2161, mass accuracy 1.2 mDa. *m/z* for $C_{18}H_{20}N_2O_3$ $[M + H]^+$ calculated 313.1547, found 313.1537, mass accuracy 1.0 mDa. *m/z* for $C_{19}H_{22}N_2O_3$ $[M + H]^+$ calculated 327.1703, found 327.1692, mass accuracy 1.1 mDa.

S4.3.3 Sample gel prepared with 0.18 eq of acid

^{1}H NMR (500 MHz, DMSO) δ 8.95 (d, *J* = 7.6 Hz, 0.15H), 8.90 (d, *J* = 7.5 Hz, 0.6H), 8.84 (d, *J* = 7.9 Hz, 0.3H), 8.20 (d, *J* = 7.5 Hz, 1H), 8.11 – 8.04 (m, 3H), 7.32 – 7.24 (m, 21H), 6.84 (d, *J* = 8.8 Hz, 1H), 4.60 – 4.52 (m, 2H), 4.45 (q, *J* = 7.3 Hz, 1H), 4.38 (q, *J* = 7.3 Hz, 1H), 4.19 (td, *J* = 9.4, 3.8 Hz, 1H), 4.05 (dt, *J* = 9.9, 5.1 Hz, 1H), 3.61 (s, 0.44H), 3.17 – 3.09 (m, 1H), 2.96 (dddd, *J* = 24.2, 14.0, 9.8, 5.1 Hz, 6H), 2.69 (s, 2H), 1.32 (d, *J* = 3.7 Hz, 14H), 1.28 (s, 9H). Solvent peaks appear at 3.07 and 1.10 ppm. Solvent impurity peaks are marked with an asterisk, and precursor peaks are marked with a square.

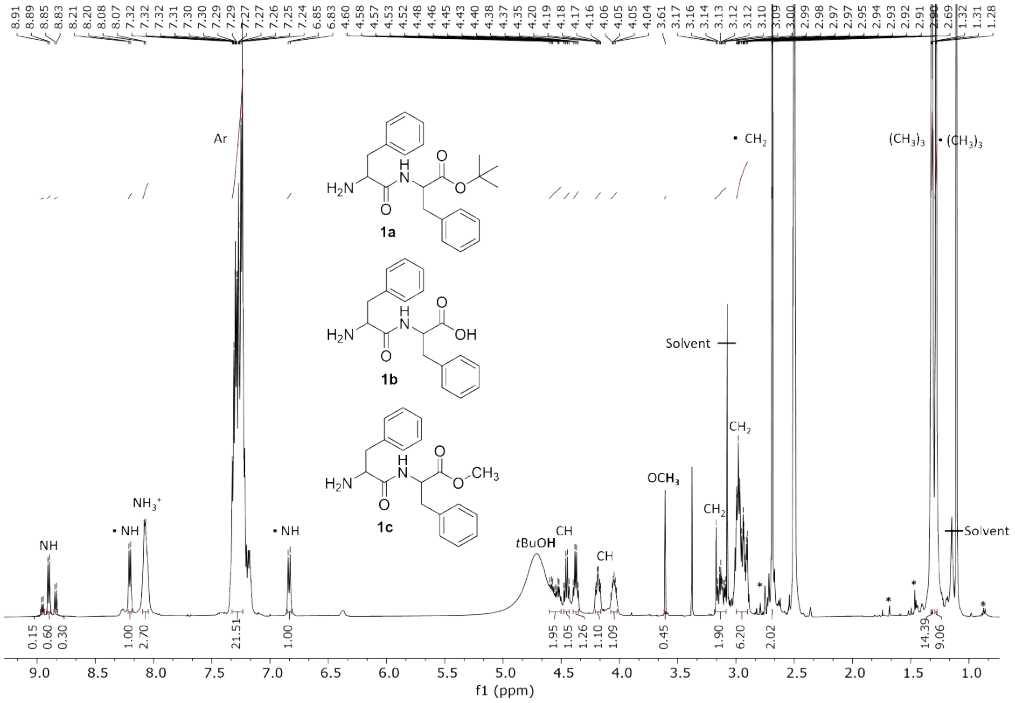


Figure S20. ^{1}H NMR (500 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq. of acid.

^{13}C NMR (125 MHz, DMSO) δ 172.18, 171.70, 170.38, 169.92, 168.14, 168.12, 155.14, 138.10, 137.13, 136.70, 134.67, 134.64, 129.61, 129.26, 129.22, 129.16, 128.51, 128.33, 128.30, 128.16, 127.97, 127.21, 127.18, 126.70, 126.62, 126.50, 126.16, 81.16, 80.71, 77.99, 66.93, 55.52, 54.39, 54.08, 53.81, 53.15, 53.13, 52.82, 37.45, 37.00, 36.90, 36.68, 31.30, 28.11, 27.48. Solvent peaks are marked with an asterisk.

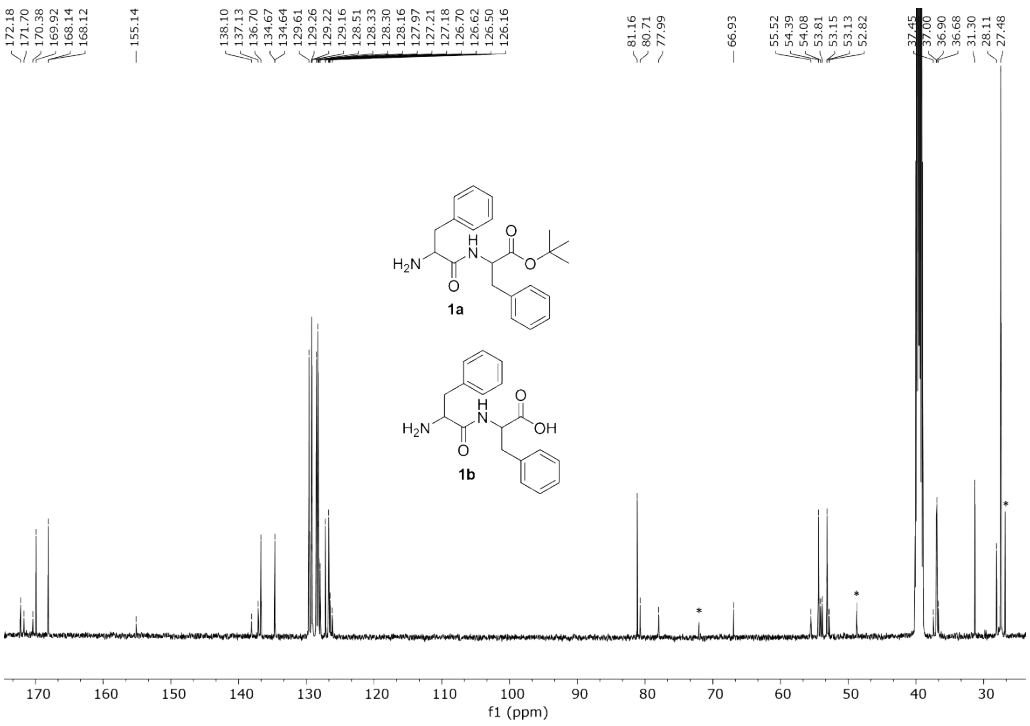


Figure S21. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq of acid.

HR-MS: *m/z* for $C_{27}H_{36}N_2O_5$ $[M + H]^+$ calculated 469.2697, found 469.2683, mass accuracy 1.4 mDa. *m/z* for $C_{22}H_{28}N_2O_3$ $[M + H]^+$ calculated 369.2173, found 369.2163, mass accuracy 1.0 mDa. *m/z* for $C_{18}H_{20}N_2O_3$ $[M + H]^+$ calculated 313.1547, found 313.1541, mass accuracy 0.6 mDa. No Phe-Phe-OMe was observed in HR-MS.

S4.4 Boc-Leu-Phe-O*t*Bu in *t*BuOMe

S4.4.1 Sample gel prepared with 1.0 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.91 – 8.88 (m, 0.04H), 8.83 (d, *J* = 7.3 Hz, 0.75H), 8.76 (d, *J* = 7.7 Hz, 1H), 8.05 (s, 5H), 7.31 – 7.23 (m, 9H), 4.48 (ddd, *J* = 18.9, 9.4, 6.3 Hz, 2H), 3.76 (s, 2H), 3.61 (d, *J* = 5.3 Hz, 0.12H), 3.13 – 3.05 (m, 1H), 3.01 – 2.94 (m, 2H), 1.74 – 1.50 (m, 7H), 1.31 (s, 6H), 0.89 (dt, *J* = 4.7, 1.8 Hz, 11H). Solvent impurity peaks are marked with an asterisk.

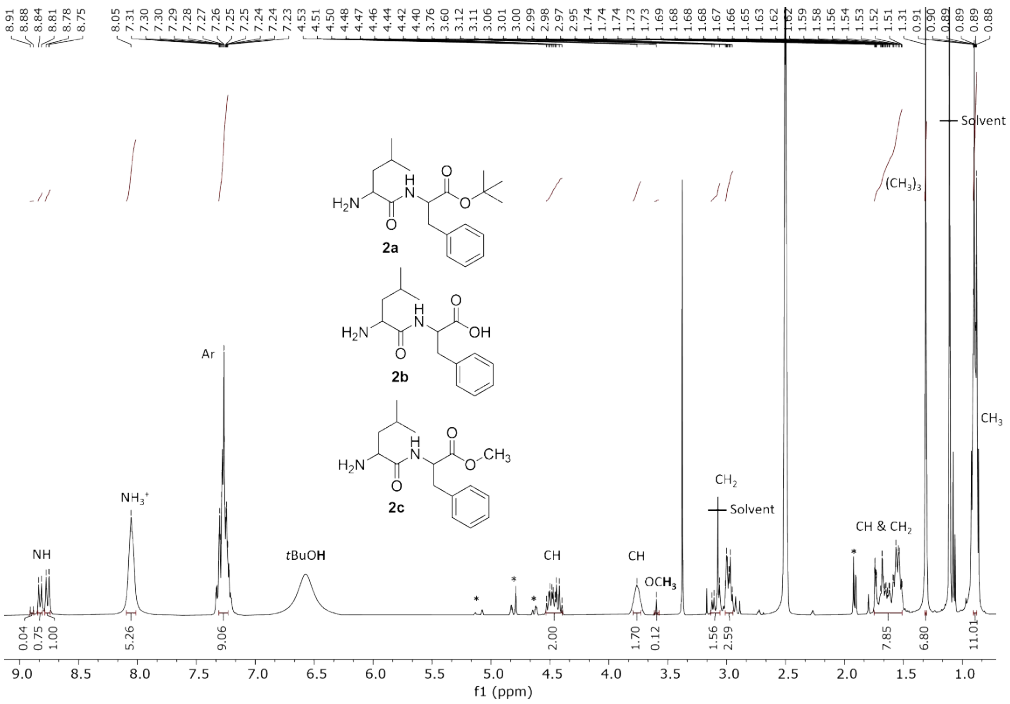


Figure S22. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 1.0 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 172.23, 169.85, 169.17, 169.13, 137.25, 136.85, 129.16, 129.10, 128.32, 128.29, 126.67, 126.59, 81.01, 66.95, 54.50, 53.83, 52.83, 50.68, 50.65, 40.30, 40.22, 36.69, 36.45, 31.31, 29.85, 29.64, 27.47, 23.39, 23.35, 22.85, 22.81, 21.73, 21.70. Solvent peaks are marked with an asterisk.

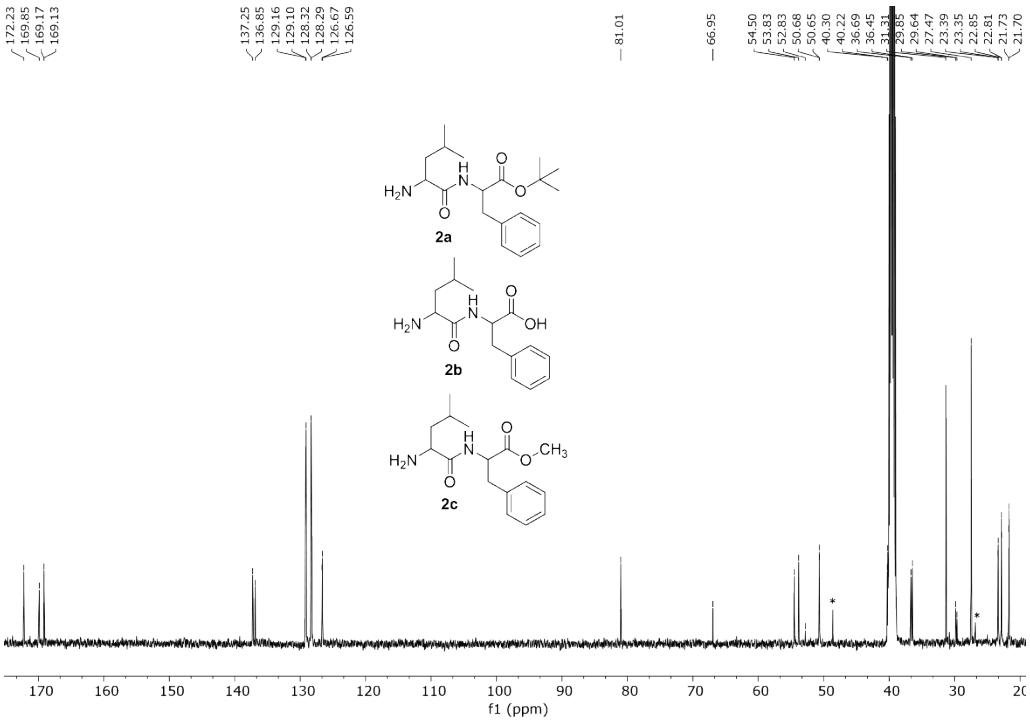


Figure S23. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 1.0 eq of acid.

HR-MS: *m/z* for $C_{19}H_{30}N_2O_3$ $[M + H]^+$ calculated 335.2329, found 335.2308, mass accuracy 2.1 mDa. *m/z* for $C_{15}H_{22}N_2O_3$ $[M + H]^+$ calculated 279.1703, found 279.1693, mass accuracy 1.0 mDa. *m/z* for $C_{16}H_{24}N_2O_3$ $[M + H]^+$ calculated 293.1860, found 293.1834, mass accuracy 2.6 mDa.

S4.4.2 Sample gel prepared with 0.5 eq of acid

1H NMR (300 MHz, DMSO) δ 8.83 (d, J = 7.3 Hz, 1H), 8.78 – 8.75 (m, 0.04H), 8.07 (s, 3H), 7.98 (d, *J* = 7.6 Hz, 0.4H), 7.31 – 7.22 (m, 7H), 6.79 (d, *J* = 8.6 Hz, 0.4H), 4.47 – 4.32 (m, 2H), 3.97 (q, *J* = 8.1 Hz, 1H), 3.77 (s, 1H), 3.02 – 2.91 (m, 3H), 1.67 (dq, *J* = 12.7, 6.4 Hz, 1H), 1.56 (ddd, *J* = 11.2, 7.1, 3.3 Hz, 3H), 1.36 (s, 4H), 1.31 (s, 13H), 0.91 (dd, *J* = 6.3, 3.8 Hz, 6H), 0.84 (dd, *J* = 8.4, 6.5 Hz, 3H).

Figure S24. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 172.41, 170.35, 169.84, 169.12, 155.15, 137.13, 136.84, 129.21, 129.15, 129.09, 128.28, 128.09, 126.65, 126.42, 80.99, 80.63, 77.96, 72.06, 66.93, 54.48, 53.86, 52.80, 52.63, 50.64, 40.88, 40.29, 36.82, 36.68, 31.29, 28.17, 27.49, 27.46, 24.18, 23.38, 22.89, 22.79, 21.71, 21.55. Solvent peaks are marked with an asterisk.

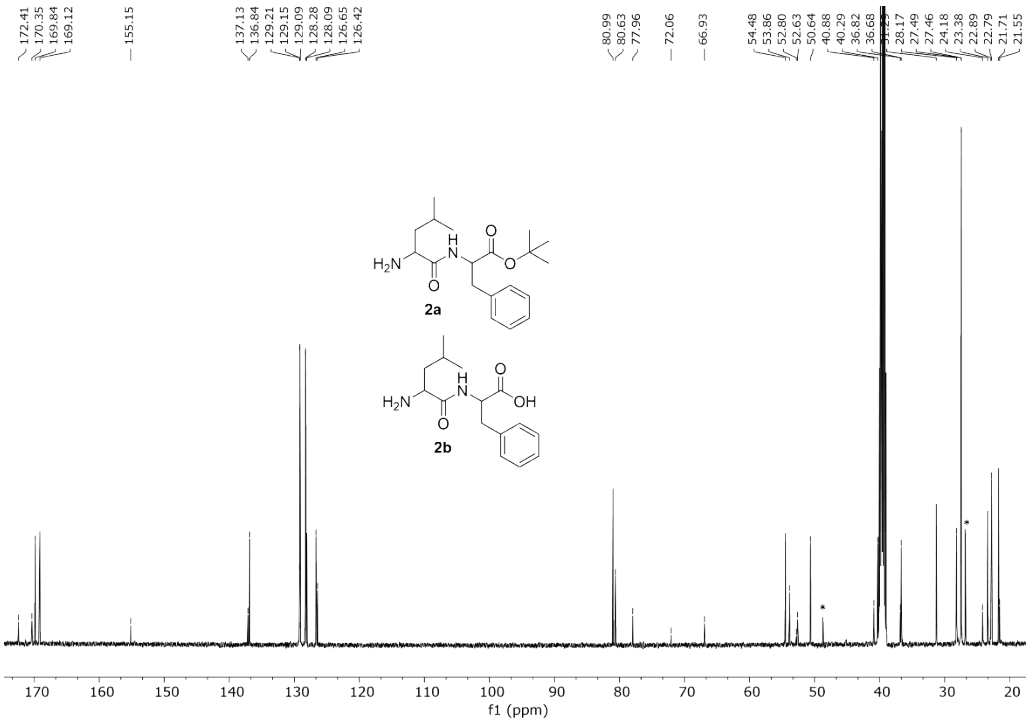


Figure S25. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.5 eq of acid.

HR-MS: *m/z* for $C_{15}H_{22}N_2O_3$ $[M + H]^+$ calculated 279.1703, found 279.1700, mass accuracy 0.3 mDa. *m/z* for $C_{16}H_{24}N_2O_3$ $[M + Na]^+$ calculated 315.1679, found 315.1665, mass accuracy 1.4 mDa. No Boc-Leu-Phe-O*t*Bu or Leu-Phe-O*t*Bu was observed in HR-MS.

S4.4.3 Sample gel prepared with 0.18 eq of acid

^{1}H NMR (300 MHz, DMSO) δ 8.83 (d, *J* = 7.3 Hz, 1H), 8.78 – 8.75 (m, 0.06H), 8.06 (s, 3H), 7.97 (d, *J* = 7.6 Hz, 0.55H), 7.37 – 7.14 (m, 8H), 6.79 (d, *J* = 8.6 Hz, 0.55H), 4.48 – 4.32 (m, 1H), 3.97 (q, *J* = 8.0 Hz, 1H), 3.77 (d, *J* = 6.4 Hz, 1H), 2.97 (ddd, *J* = 14.0, 7.4, 4.4 Hz, 3H), 1.73 – 1.61 (m, 2H), 1.56 (ddd, *J* = 10.9, 7.0, 3.2 Hz, 3H), 1.36 (s, 5H), 1.31 (s, 14H), 0.91 (dd, *J* = 6.3, 3.8 Hz, 6H), 0.84 (dd, *J* = 8.4, 6.5 Hz, 3H).

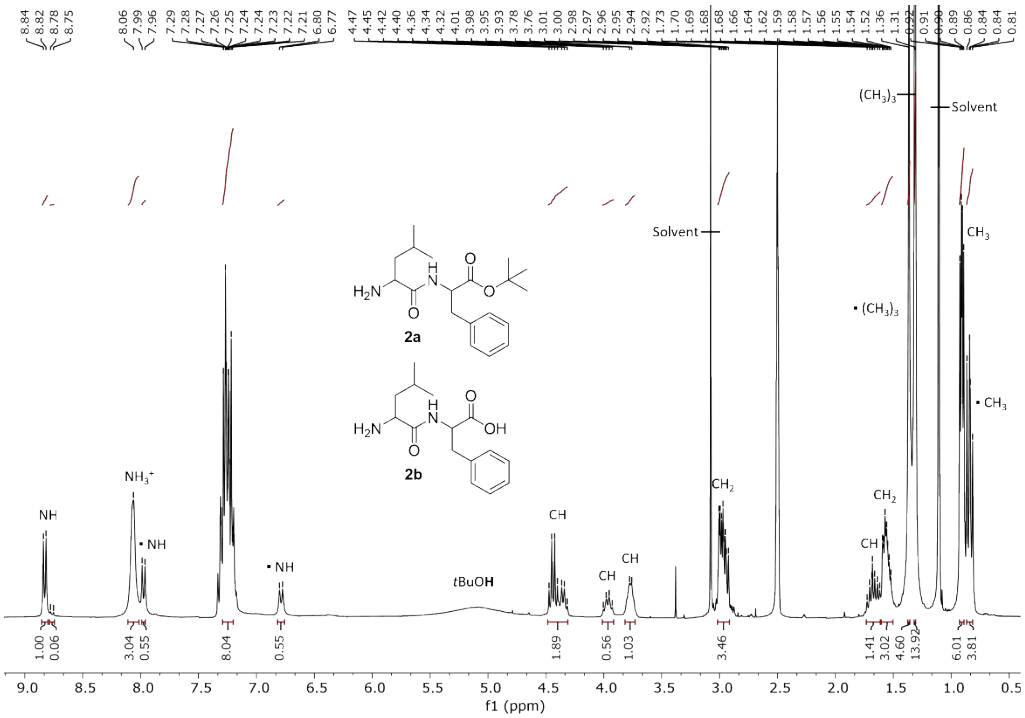


Figure S26. ^{1}H NMR (300 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq of acid.

^{13}C NMR (125 MHz, DMSO) δ 172.41, 170.35, 169.83, 169.12, 155.15, 137.13, 136.84, 129.15, 128.28, 128.09, 126.65, 126.42, 80.99, 80.63, 77.96, 66.93, 54.48, 53.86, 52.81, 52.63, 50.64, 40.88, 40.29, 36.82, 36.68, 31.29, 28.17, 27.46, 24.18, 23.38, 22.89, 22.79, 21.72, 21.55. Note! Solvent peaks are marked with an asterisk.

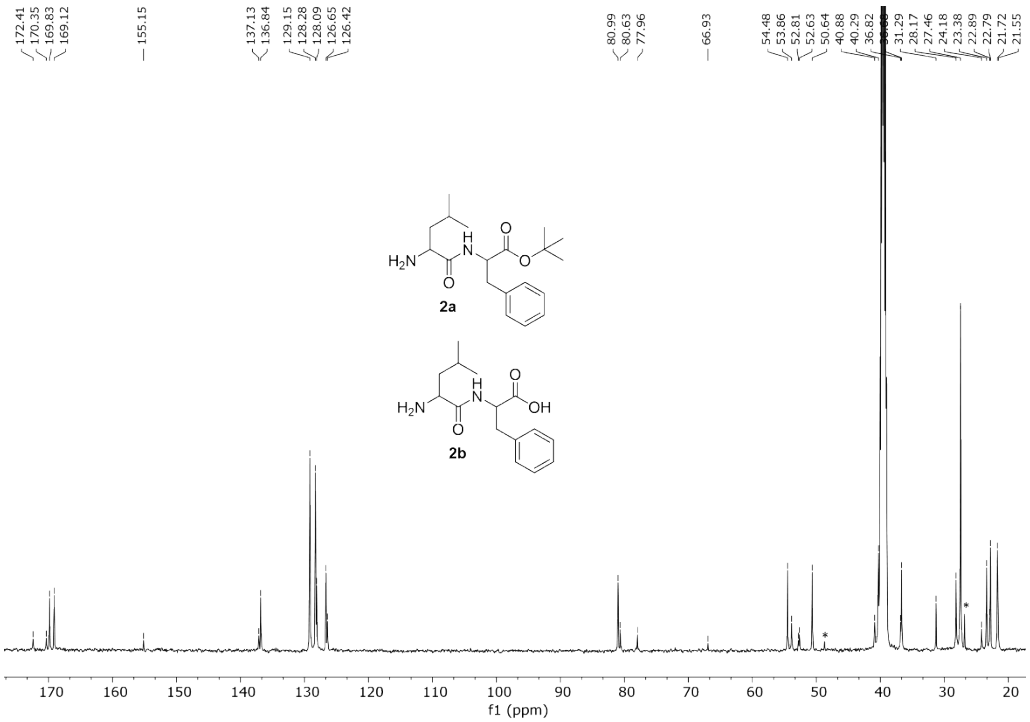


Figure S27. ^{13}C NMR (125 MHz, d_6-DMSO) spectrum of the xerogel prepared with 0.18 eq of acid.

HR-MS: *m/z* for $C_{24}H_{38}N_2O_5$ $[M + H]^+$ calculated 435.2854, found 435.2852, mass accuracy 0.2 mDa. *m/z* for $C_{19}H_{30}N_2O_3$ $[M + H]^+$ calculated 335.2329, found 335.2326, mass accuracy 0.3 mDa. *m/z* for $C_{15}H_{22}N_2O_3$ $[M + H]^+$ calculated 279.1703, found 279.1703, mass accuracy 0 mDa. No Leu-Phe-OMe was observed in HR-MS.

S4.5 Ration of gelators

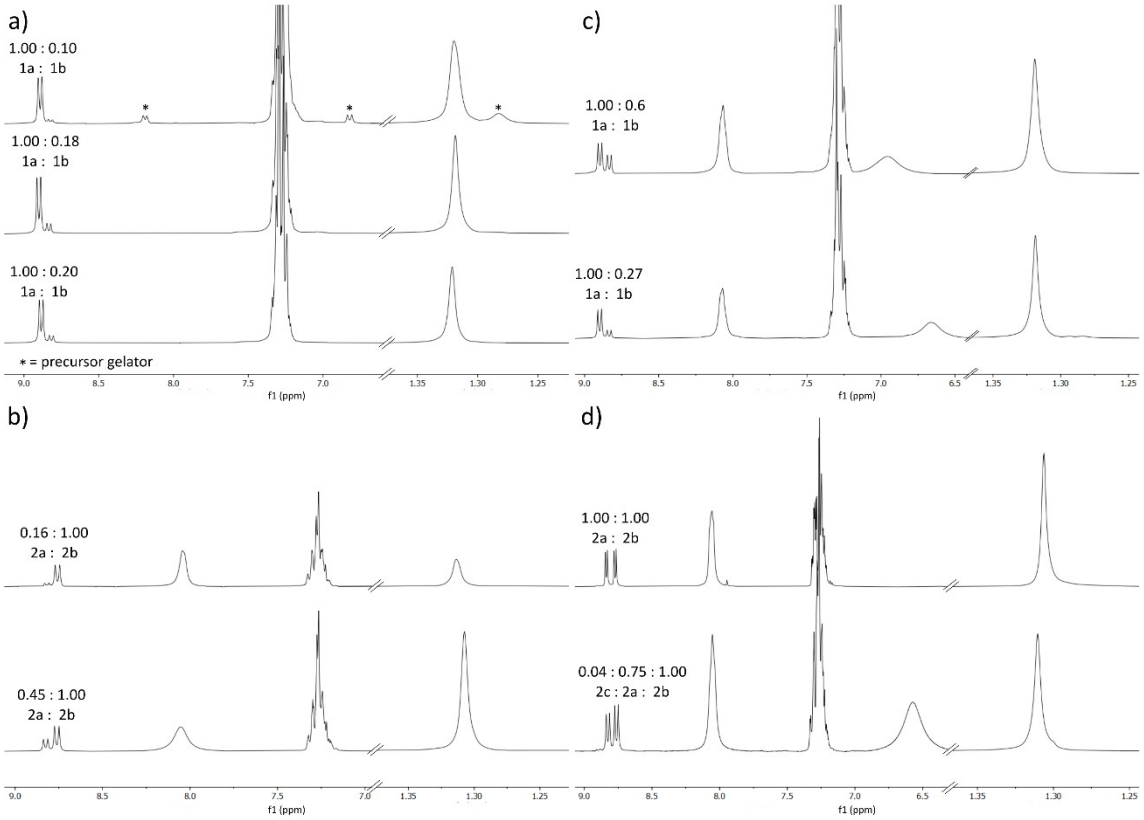


Figure S28. Collection of ^{1}H NMR (300 MHz, d_6-DMSO) spectra of gel systems from different batches on the day material was formed showing ration differences between gelators. a) 0.5 eq gel system **I** in tBuClOAc, b) 1.0 eq gel system **II** in tBuClOAc, c) 1.0 eq gel system **I** in tBuOMe and d) 1.0 eq gel system II in tBuOMe. Note! Spectrum (d) with 1:1 ration is measured with 500 MHz.

S5 ATR-FTIR spectra of xerogels

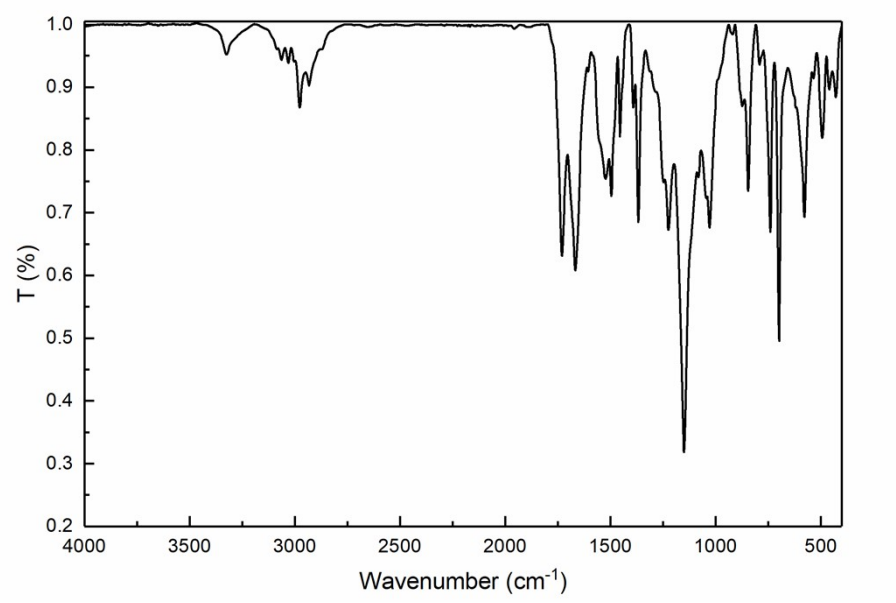


Figure S29. . ATR-FTIR spectrum of xerogel Boc-Phe-Phe-O*t*Bu **1** in *t*BuClOAc and 0.5 eq of acid.

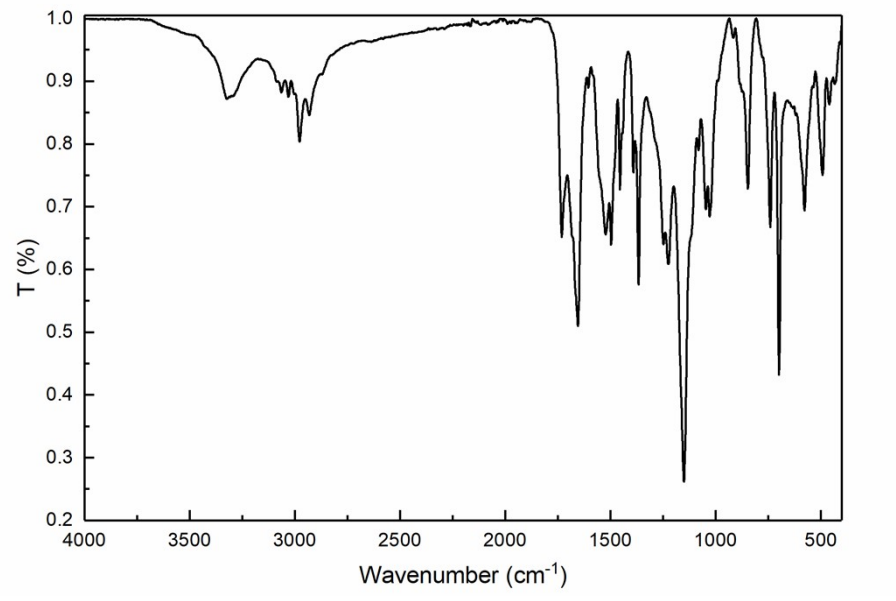


Figure S30. ATR-FTIR spectrum of xerogel Boc-Phe-Phe-O*t*Bu **1** in *t*BuClOAc and 0.18 eq of acid.

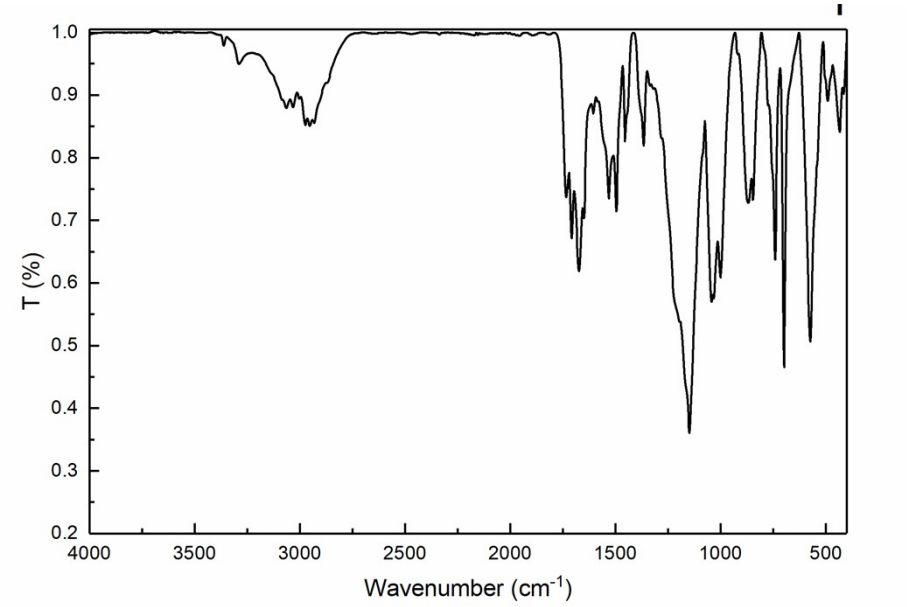


Figure S31. ATR-FTIR spectrum of xerogel Boc-Leu-Phe-O*t*Bu **2** in *t*BuClOAc and 1.0 eq of acid.

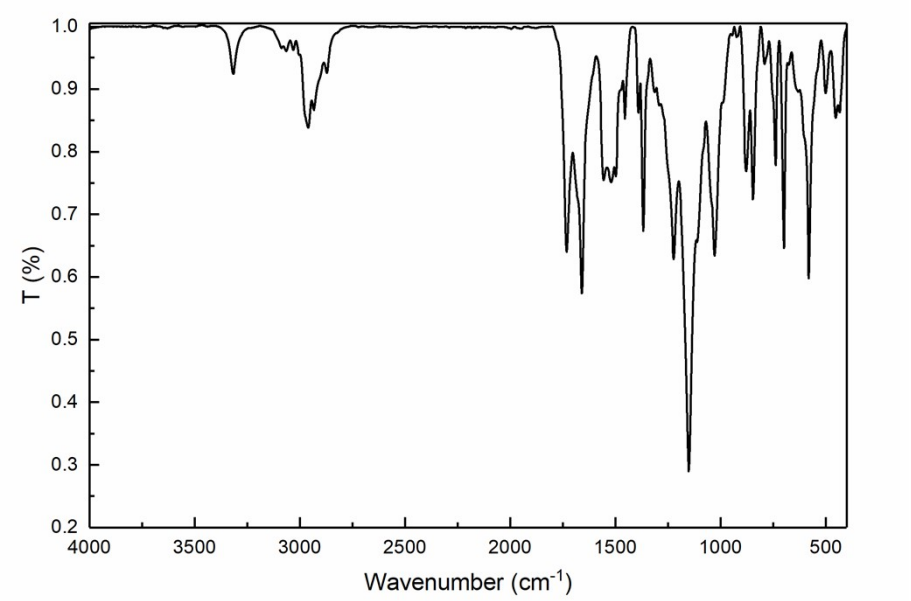


Figure S32. ATR-FTIR spectrum of xerogel Boc-Leu-Phe-O*t*Bu **2** in *t*BuClOAc and 0.5 eq of acid.

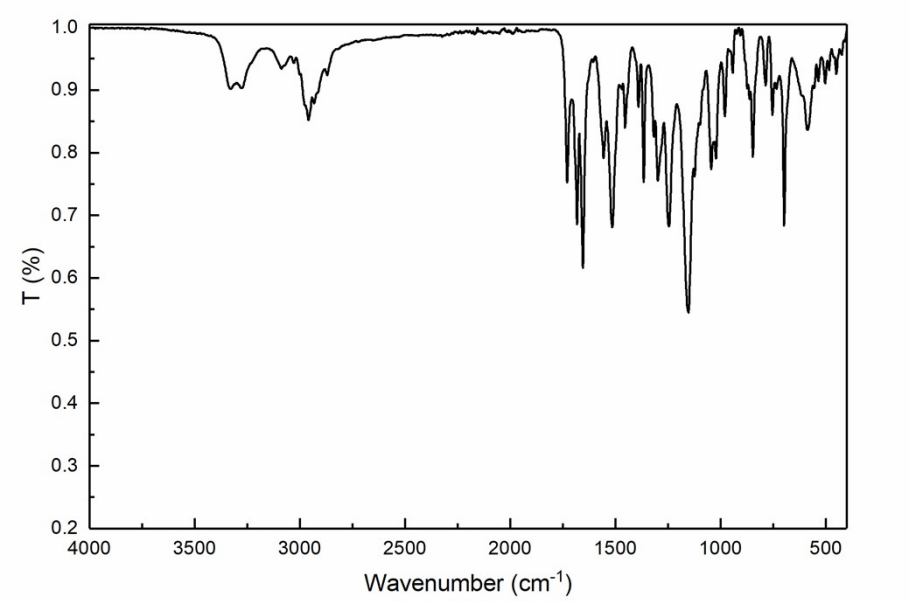


Figure S33, ATR-FTIR spectrum of xerogel Boc-Leu-Phe-O*t*Bu **2** in *t*BuClOAc and 0.18 eq of acid.

Figure S34. ATR-FTIR spectrum of xerogel Boc-Phe-Phe-O*t*Bu **1** in *t*BuOMe and 1.0 eq of acid.

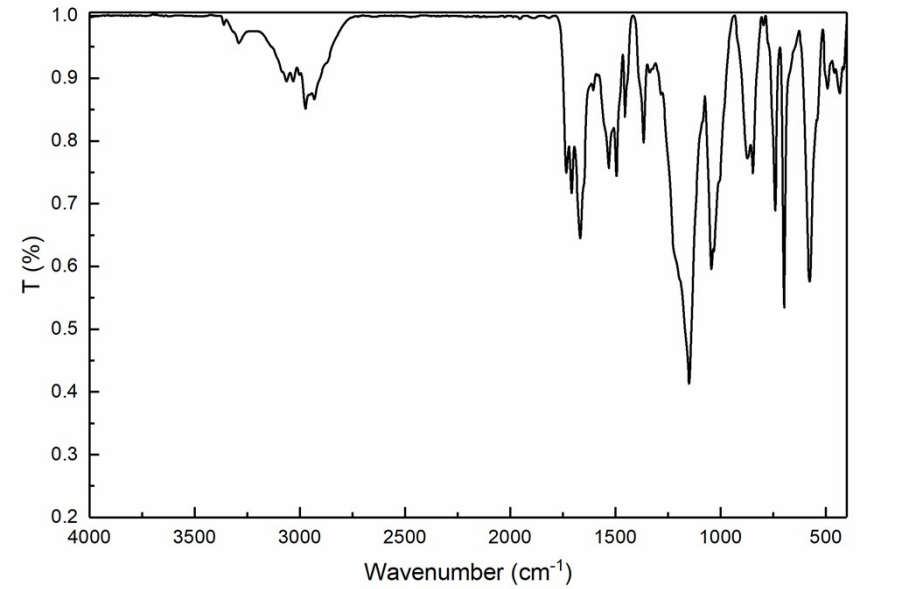


Figure S35. ATR-FTIR spectrum of xerogel Boc-Phe-Phe-O*t*Bu **1** in *t*BuOMe and 0.5 eq of acid.

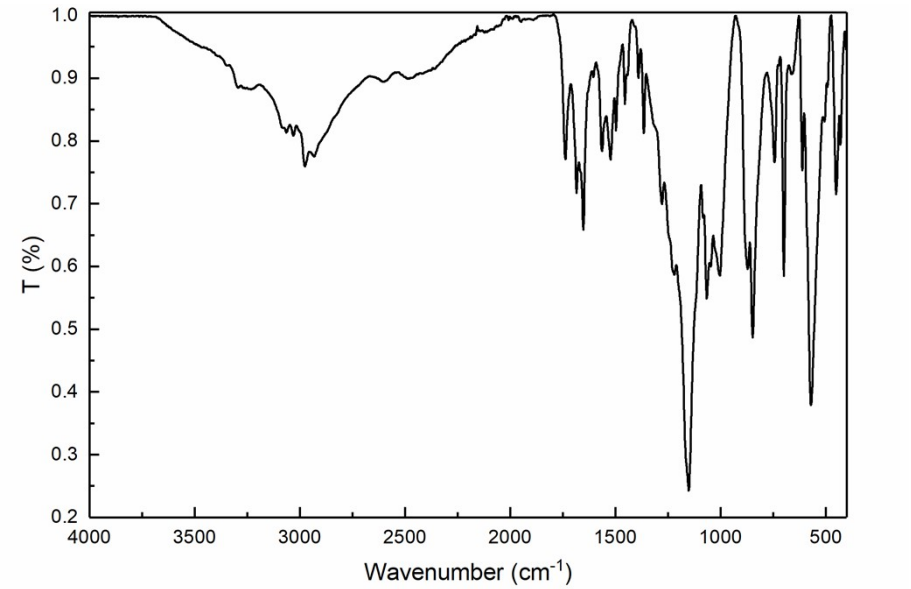


Figure S36. ATR-FTIR spectrum of xerogel Boc-Phe-Phe-O*t*Bu **1** in *t*BuOMe and 0.18. eq of acid.

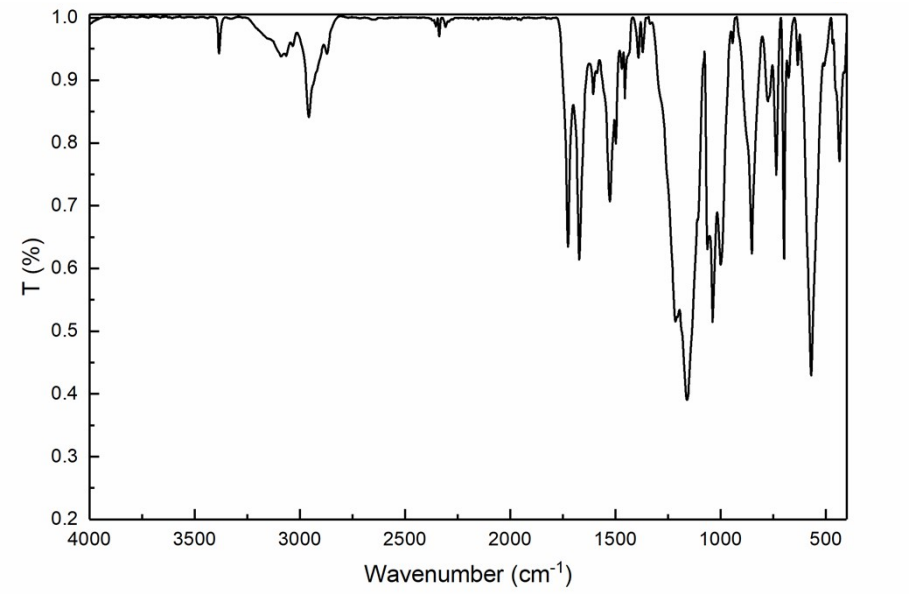


Figure S37. ATR-FTIR spectrum of xerogel Boc-Leu-Phe-O*t*Bu **2** in *t*BuOMe and 1.0 eq of acid.

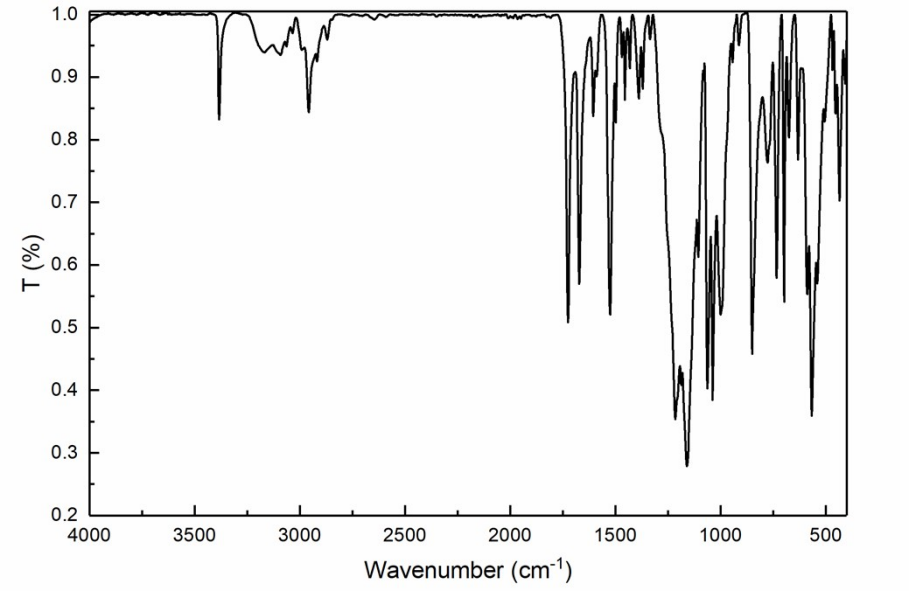


Figure S38. ATR-FTIR spectrum of xerogel Boc-Leu-Phe-O*t*Bu **2** in *t*BuOMe and 0.5 eq of acid.

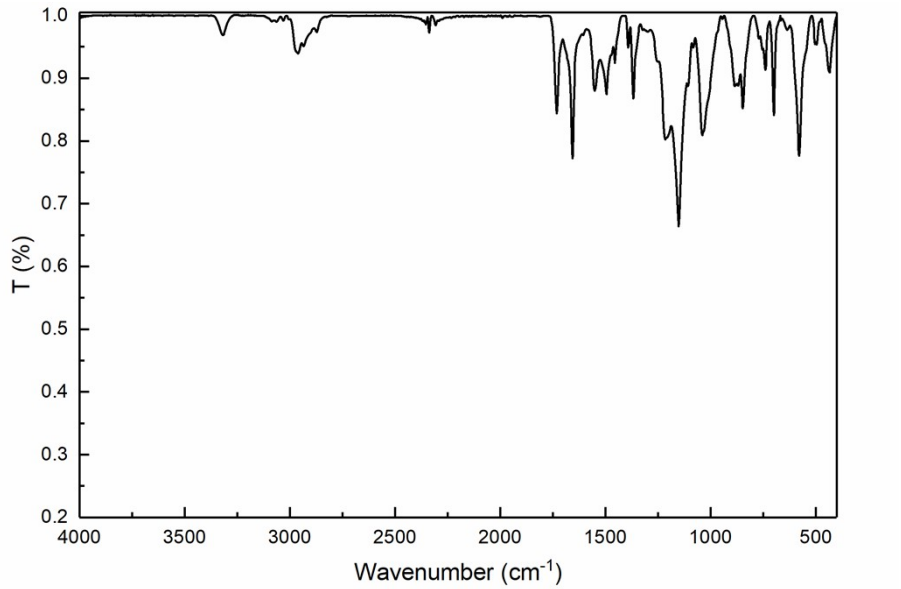


Figure S39. ATR-FTIR spectrum of xerogel Boc-Leu-Phe-O*t*Bu **2** in *t*BuOMe and 0.18 eq of acid.

S6 AFM, TEM and SEM Imaging

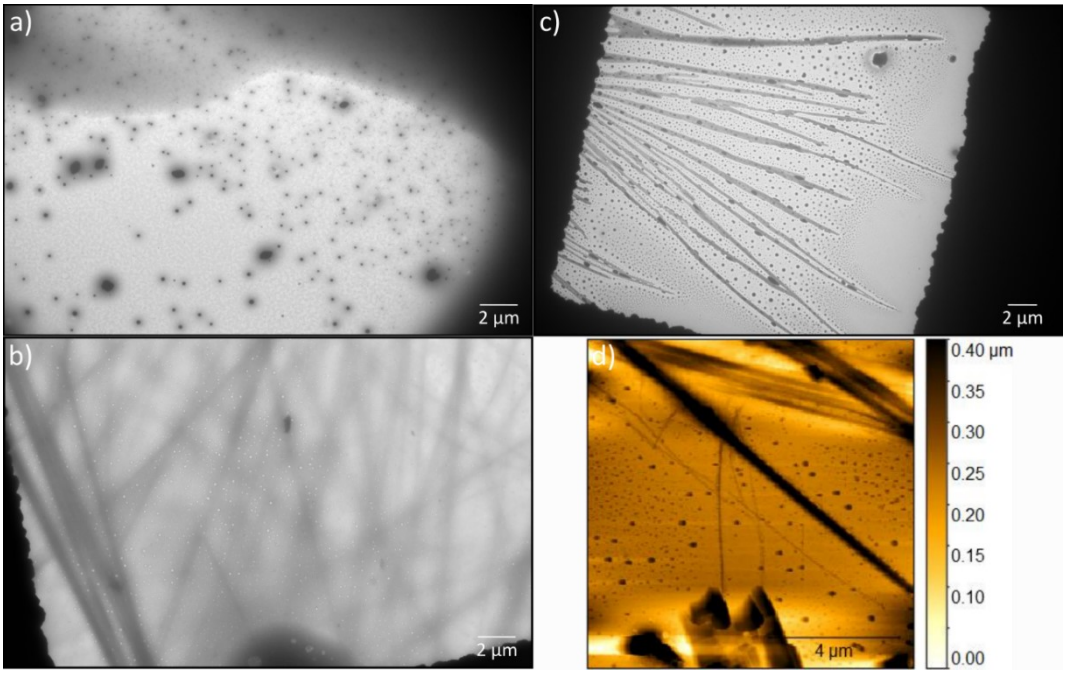


Figure S40. Images of xerogels prepared in *t*BuClOAc with 0.5 eq of acid (a: gel system **I**, TEM and b: gel system **II**, TEM) and in *t*BuOMe (c: gel system **I**, TEM and d: gel system **II**, AFM).

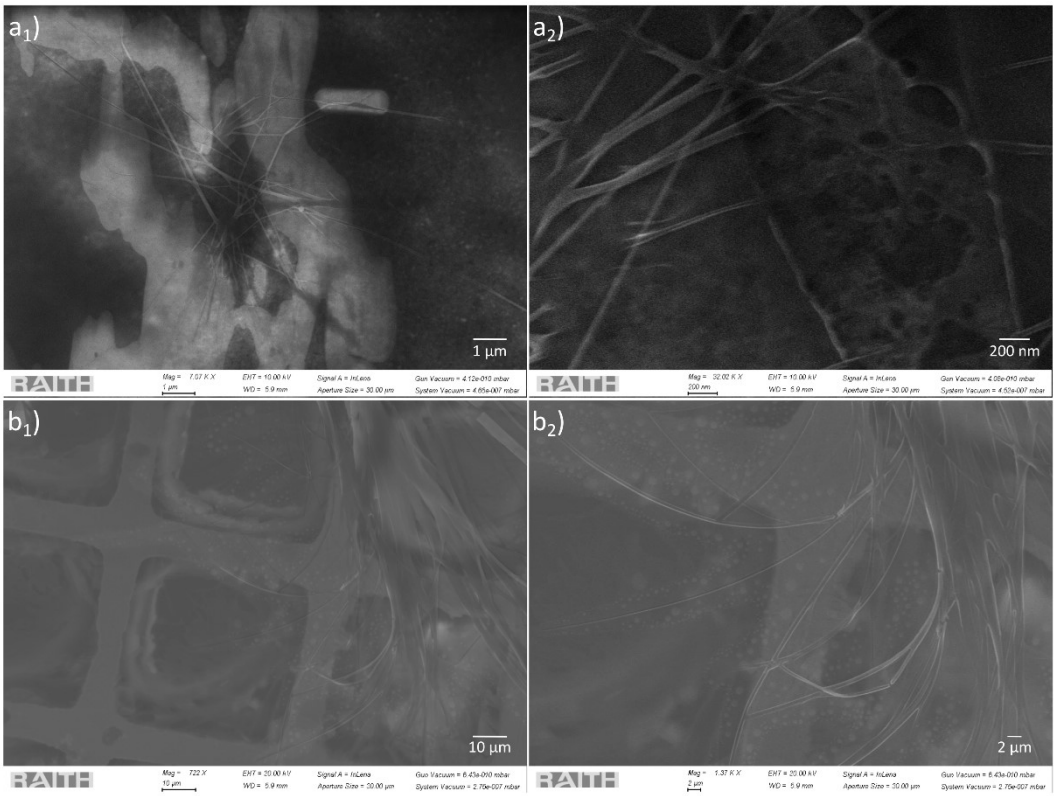


Figure S41. SEM images of gel system **I** xerogels prepared a_1-a2) in *t*BuClOAc with 0.18 eq of acid and b_1-b_2) in *t*BuOMe with 0.5 eq of acid.

S7 NMR spectra of degraded solvents

The degradation of the primary solvent in the presence of H_2SO_4 was assessed to investigate the formation of the secondary solvents (*t*BuOH or MeOH) during the gelation cycle. The chosen conditions correspond to the preparation of gels using 1.0 eq of acid. The NMR spectra of the solvents were measured prior acid addition, immediately after addition, after 1 and 5 hours.

Solvent decomposition was observed in both solvents. A new peak forms in *t*BuClOAc at 9 ppm and in *t*BuOMe at 8 ppm, corresponding to the products bearing a hydroxyl group. Additionally, the peaks' shifting durring the solvents' decomposition is due to the changes in the electron micro-environment adjucent the observed functional groups.

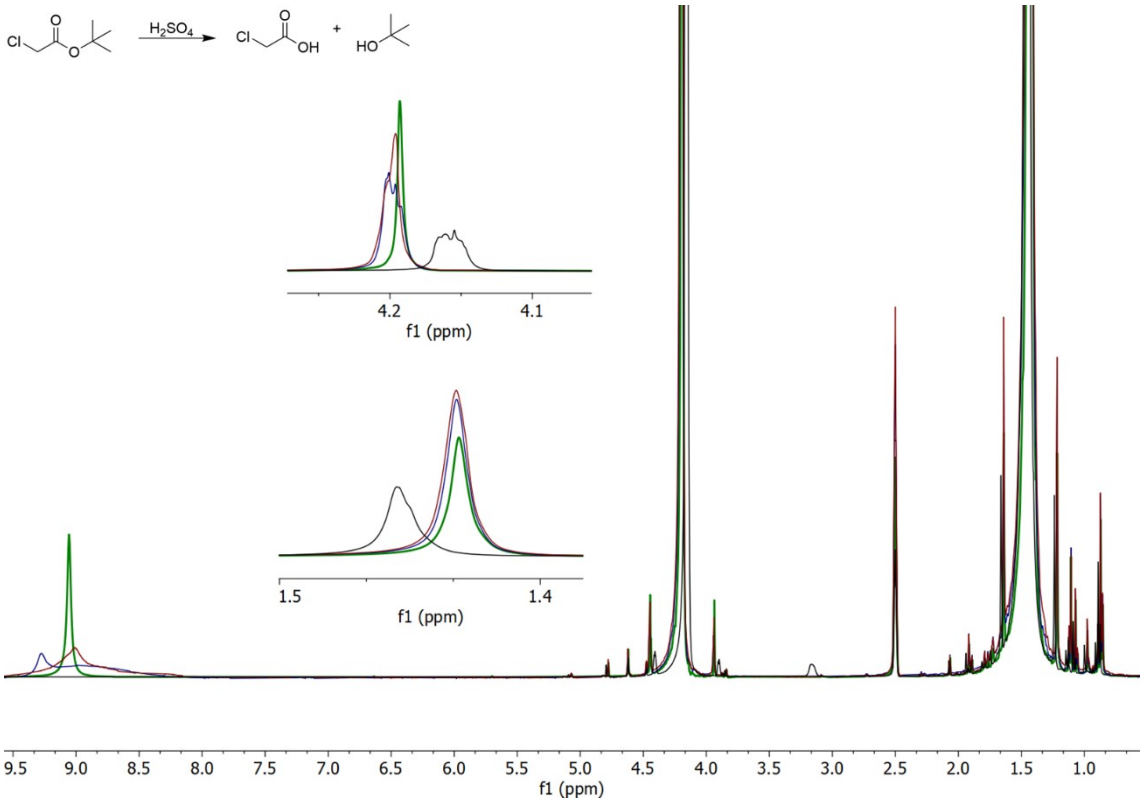


Figure S42. ^{1}H NMR (300 MHz, d_6-DMSO) spectra of *t*BuClOAc solvent. Black: neat solvent prior acid addition, red: immediately after acid addition, blue: 1 h after acid addition and green: 5 h after acid addition.

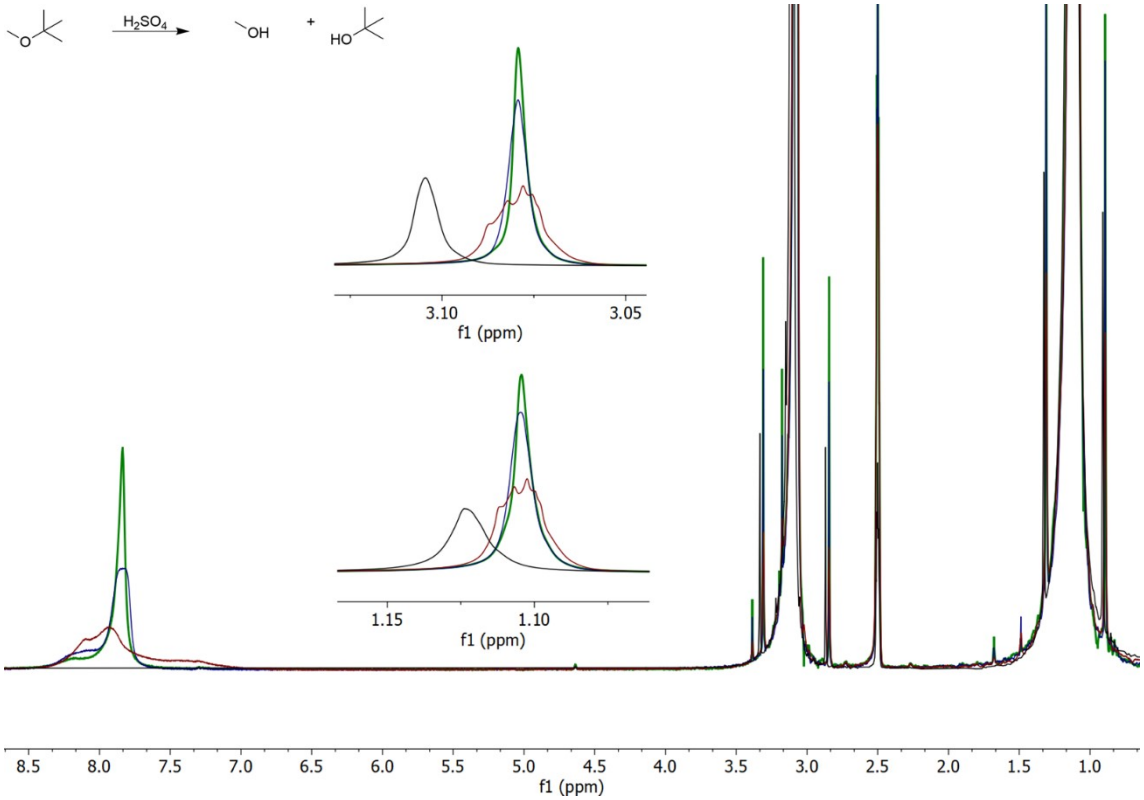


Figure S43. ^{1}H NMR (300 MHz, d_6-DMSO) spectra of *t*BuOMe solvent. Black: neat solvent prior acid addition, red: immediately after acid addition, blue: 1 h after acid addition and green: 5 h after acid addition.

References

1 R. Chevigny, H. Rahkola, E. D. Sitsanidis, E. Korhonen, J. R. Hiscock, M. Pettersson and M. Nissinen, *Chemistry of Materials*, 2024, **36**, 407–416.