

Supplementary Information for *Chemical Communications*

Robust scalable synthesis of a bis-urea derivative forming thixotropic and cytocompatible supramolecular hydrogels

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Supplementary Methods

Materials. 1,3-bis(isocyanatomethyl)benzene, > 98.0 % (**B**), was purchased from TCI. 3-(aminomethyl)pyridine, 99+ % (**A1**); 2-(aminomethyl)pyridine, 99 % (**A0**); 4-(aminomethyl)pyridine, 98 % (**A2**); and dichloromethane (DCM), 99.8 %, Extra Dry stored over Molecular Sieves, Stabilized, AcroSeal™ were purchased from ACROS Organics. Deuterium oxide (D₂O) and dimethyl sulfoxide-*d*₆ (DMSO-*d*₆) were purchased from Sigma Aldrich.

Instrumentation for synthesis and chemical characterization. Ball milling experiments were performed using a Planetary Micro Mill, PULVERISETTE 7 premium line (Fritsch), equipped with a set of zirconium oxide grinding bowls (80 mL) filled with zirconium oxide grinding balls (10 mm). ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz and 100 MHz for the respective nuclei or on a Bruker Avance 600 spectrometer operating at 600 MHz and 150 MHz for the respective nuclei. Chemical shifts are given in ppm (δ) values relative to tetramethylsilane (TMS) or the residual solvent peak. The spectra were analyzed using ACD/Spectrus Processor 2016.1.1 (Advanced Chemistry Development, Inc.). Fourier transform infrared (FTIR) spectra were recorded on a Bruker alpha-P FTIR spectrometer using OPUS 7.5: Build 7,5,18 (20140810) software (Bruker Optik GmbH). The spectra were analyzed using ACD/Spectrus Processor 2016.1.1 (Advanced Chemistry Development, Inc.). Differential Scanning Calorimetry (DSC) measurements were recorded using a Mettler-Toledo DSC 822e under helium atmosphere from 25 °C to 250 °C with a heating rate of 10 °C/min. Mass spectrometry (MS) was performed using a Thermo Electron LCQ Advantage apparatus (Thermo Scientific) equipped with an Agilent 1100 pump and injection system (Agilent Technologies). Spectra were acquired using a quadrupole orthogonal acceleration time-of-flight mass spectrometer Synapt G2 HDMS (Waters). Samples were infused at 3 μL/min, and spectra

were obtained in positive ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Data were analyzed using Xcalibur software (Thermo Fisher Scientific Inc.).

Synthesis of bis-urea compound *meta*-C (1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-3-ylmethyl)urea)) in solution. A flame dried two-neck flask was charged with dry dichloromethane (DCM) and 1,3-bis(isocyanatomethyl)benzene (*m*-xylylene diisocyanate, **B** in text) (1 g; 5.31 mmol) under nitrogen atmosphere. Pyridin-3-ylmethanamine (3-picolylamine, **A**₁ in text) (1.149 g; 10.63 mmol) was added dropwise while stirring. The mixture was allowed to stir for 1 h at room temperature. The product was filtered off as a white precipitate, washed with DCM, and dried under vacuum. 1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-3-ylmethyl)urea) (*meta*-C in text) was obtained in quantitative yield. The compound was characterized with ¹H NMR (Fig. S1a), ¹³C NMR (Fig. S1b), FTIR (Fig. S1c), DSC (melting point, T_m), and MS (ESI).

¹H NMR (400 MHz, DMSO-*d*₆) δ = 4.20 (d, J = 6.02 Hz, 4H), 4.25 (d, J = 6.02 Hz, 4H), 6.45 - 6.58 (m, 4 H), 7.08 - 7.14 (m, 3 H), 7.21 - 7.27 (m, 1 H), 7.34 (dd, J = 7.63, 4.88 Hz, 2 H), 7.65 (br d, J = 7.78 Hz, 2 H), 8.41 - 8.49 (m, 4 H)

¹³C NMR (100 MHz, DMSO-*d*₆) δ = 40.7, 43.0, 123.4, 125.3, 125.7, 128.2, 134.8, 136.4, 140.8, 147.9, 148.6, 158.1

FTIR (cm⁻¹): ν = 3319 (N-H stretch); 1609 (C=O stretch); 1565 (N-H bend)

T_m: 198 °C

MS (ESI) m/z: Calculated mass for C₂₂H₂₄N₆O₂: 404.20; Found mass: 405.1 [M+H]⁺

Solvent-free synthesis of *meta*-C using a planetary ball mill. Two grinding bowls (volume: 80 mL, zirconium oxide) were each charged with 25 balls (diameter: 10 mm, zirconium oxide) to obtain a 32 % filling ratio. 1,3-bis(isocyanatomethyl)benzene (*m*-xylylene diisocyanate, **B** in text) (0.500 g; 2.66 mmol) and pyridin-3-ylmethanamine (3-picolylamine, **A**₁ in text) (0.575 g; 5.31 mmol) were added to each bowl. Both bowls were closed and inserted in the planetary ball mill, which was operated at a rotational speed of 600 revolutions per minute (rpm) for the main disk (1200 rpm as the relative rotational speed for each grinding bowl) for 2 min. The product (1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-3-ylmethyl)urea) (*meta*-C in text) was recovered from the grinding bowls as a solid. Quantitative conversion was confirmed *via* FTIR (Fig. S2).

Synthesis of bis-urea compound *para*-C (1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-4-ylmethyl)urea)). A flame dried two-neck flask was charged with dry DCM and 1,3-bis(isocyanatomethyl)benzene (*m*-xylylene diisocyanate, **B** in text) (1 g; 5.31 mmol) under nitrogen atmosphere. Pyridin-4-ylmethanamine (4-picolylamine, **A**₂ in text) (1.149 g; 10.63 mmol) was added dropwise while stirring. The mixture was allowed to stir for 1 h at room temperature. The product was filtered off as a precipitate, washed with DCM, and dried under vacuum. 1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-4-ylmethyl)urea) (*para*-C in text) was obtained at quantitative yield. The compound was characterized with ¹H NMR (Fig. S3a), ¹³C NMR (Fig. S3b), FTIR (Fig. S3c), DSC (T_m), and MS (ESI).

¹H NMR (400 MHz, DMSO-*d*₆) δ = 4.22 (d, J = 6.02 Hz, 4H), 4.25 (d, J = 6.02 Hz), 6.51 - 6.64 (m, 4 H), 7.09 - 7.18 (m, 3 H), 7.18 - 7.30 (m, 5 H), 8.48 (d, J = 5.80 Hz, 4 H)

¹³C NMR (100 MHz, DMSO-*d*₆) δ = 42.2, 43.2, 122.1, 125.5, 125.9, 128.4, 141.0, 149.6, 150.3, 158.2

FTIR (cm⁻¹): ν = 3315 (s, N-H stretch); 1623 (C=O stretch); 1577 (N-H bend)

T_m: 191 °C

MS (ESI) m/z: Calculated mass for C₂₂H₂₄N₆O₂: 404.20; Found mass: 405.1 [M+H]⁺

Synthesis of bis-urea compound *ortho*-C (1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-2-ylmethyl)urea)). A flame dried two-neck flask was charged with dry DCM and 1,3-bis(isocyanatomethyl)benzene (*m*-xylylene diisocyanate, **B** in text) (1 g; 5.31 mmol) under nitrogen atmosphere. Pyridin-2-ylmethanamine (2-picolylamine, **A0** in text) (1.149 g; 10.63 mmol) was added dropwise while stirring. The mixture was allowed to stir for 1 h at room temperature. The product was filtered off as a precipitate, washed with DCM, and dried under vacuum. 1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-2-ylmethyl)urea) (*ortho*-C in text) was obtained at quantitative yield. The compound was characterized with ¹H NMR (Fig. S4a), ¹³C NMR (Fig. S4b), FTIR (Fig. S4c), DSC (T_m), and MS (ESI).

¹H NMR (600 MHz, DMSO-*d*₆) δ = 4.23 (d, J=6.05 Hz, 4 H), 4.33 (d, J=5.87 Hz, 4 H), 6.43 (br t, J=5.50 Hz, 2 H), 6.48 (br t, J=5.59 Hz, 2 H), 7.13 (d, J=7.52 Hz, 3 H), 7.13 - 7.26 (m, 5 H), 7.72 (td, J=7.61, 1.65 Hz, 2 H), 8.48 (br d, J=4.59 Hz, 2 H)

¹³C NMR (150 MHz, DMSO-*d*₆) δ = 42.9, 44.8, 120.7, 121.5, 125.1, 125.6, 127.8, 136.2, 140.5, 148.4, 157.8, 159.4

FTIR (cm⁻¹): ν = 3308 (N-H stretch); 1621 (C=O stretch); 1568 (N-H bend)

T_m: 117 °C

MS (ESI) m/z: Calculated mass for C₂₂H₂₄N₆O₂: 404.20; Found mass: 405.0 [M+H]⁺

Gelation procedures. To form 0.8 wt.% or 1 wt.% hydrogels, a screw capped vial was filled with *meta-C* (8 mg or 10 mg, respectively) and 1 mL of deionized water, phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), or growth medium. The solid was dispersed by sonicating the vial in a CPX2800H-E ultrasonic bath (Branson) and then heated in a copper block at 100 °C until complete dissolution. The solution was sonicated using a UP50H Ultrasonic processor with MS1 tip (Hielscher) operating at 30 KHz (P = 0.075 W) while cooling down to room temperature to form the hydrogels (Supplementary Movie 1, ESI[†]).

Scanning Electron Microscopy (SEM) measurements. A screw capped vial was charged with 8 mg of compound and 1 mL of H₂O (MilliQ). The solid was dispersed by sonicating the vial in a CPX2800H-E ultrasonic bath, and the vial was heated to 100 °C until the compound dissolved. Next, the solution was allowed to cool to room temperature under sonication using a Hielscher UP50H Ultrasonic processor with MS1 tip operating at P = 0.075 W. When at room temperature, the vial was submerged into liquid nitrogen to freeze the sample completely. The frozen sample was lyophilized to obtain either a xerogel from *meta-C* or a dried precipitate from *ortho-C* and *para-C*. This solid was placed on carbon tape attached to a SEM sample holder and coated with Pd/Pt or Pt using a high-resolution Quorum Sputter coater (Q150T S). The coated sample was placed under high vacuum overnight prior to imaging. The SEM images were acquired using a SEM XL 30 FEG (Philips) with the following imaging parameters: Acc.V = 10.0kV; Spot = 3.0; Magn. = 5000x/20000x/5000x (for Fig. 3a, b, c, respectively); Det. = SE; WD = 7.2/7.3/7.2 (for Fig. 3a, b, c, respectively); Exp. = 1.

Atomic Force Microscopy (AFM) measurements. Hydrogels of *meta-C* were prepared at 0.8 wt.% in deionized water or growth medium as described above. 100 μ L of the hydrogels were further diluted in 1 mL of deionized water, and the suspension was sonicated using a Hielscher

UP50H Ultrasonic processor with MS1 tip to disperse the hydrogel in the water. The samples (20 μL) were deposited on freshly cleaved mica plates, dried, and imaged using a JPK Nanowizard® III Bioscope in contact mode (Probe: NSC35/Al BS; spring constant = 16 N/m; tip radius ~ 8 nm; rectangular geometry with a length of 90 μm , width of 35 μm , and thickness of 2 μm). All of the recorded AFM images consisted of either 512 x 512 or 1024 x 1024 pixels with XY scan size varying from 1 x 1 μm^2 to 10 x 10 μm^2 . AFM images were obtained at multiple locations across the surface of 3 sample preparations to ensure the reproducibility of the results. Bundled or crossing nanofibrils as well as nanofibrils that appeared fragmented were excluded from the width and height analysis. Line profile analysis was performed on the nanofibrils/nanofibers randomly, and the height and width values were calculated as shown in Fig. S5. Width determination of the individual nanofibrils might not reflect the actual width of the nanofibrils because the width measurement is dependent on the size and shape of the AFM tip,¹ and therefore the reported width values of the nanofibrils should only be compared relative to each other. For the height and width correlation, nanofibrils/nanofibers were binned based on height measurements, in increments of 2 nm. Nanofibrils with 0 - 1.9 nm height were the first group, nanofibrils with 2 - 3.9 nm height were the second group, nanofibrils with 4 - 5.9 nm were the third group, and so on. A linear fit through the measured data was made.

Variable-Temperature (VT) NMR measurements. A screw capped vial was charged with 8 mg of *meta*-C, 0.9 mL of H_2O , and 0.1 mL of D_2O , and the solid was dispersed by sonicating the vial in an ultrasound bath. The vial was heated in a copper block at 100 $^\circ\text{C}$ until a clear solution was obtained. The solution was pipetted from the vial using a preheated Pasteur pipet and transferred to a preheated NMR tube. 3-(Trimethylsilyl)propionic-2,2,3,3- d_4 acid sodium salt dissolved in D_2O (3 mM), as an external standard, was placed in an insert tube inside the NMR tube, after

which the tubes were sonicated in an ultrasonic bath while cooling to induce gelation. VT ^1H NMR spectra were recorded on a Bruker Avance 600 spectrometer operating at 600 MHz. Spectra were acquired at 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C.

Rheology measurements. Cylindrical hydrogels were prepared using a plastic syringe and measured on a MCR 501 Rheometer (Anton-Paar). Samples were prepared *via* the following procedure. A screw capped vial was charged with 8 mg of *meta*-C and 1 mL of growth medium. The vial was sonicated until the gelator was dispersed over the liquid and subsequently heated at 100 °C until the gelator dissolved. The supersaturated solution was poured into a syringe with a cut off tip (Fig. S6). The solution was allowed to cool to room temperature under sonication using a Hielscher UP50H Ultrasonic processor with MS1 tip operating at $P = 0.075$ W. The hydrogels were stored overnight at 6.7 °C. A slice of the hydrogel was placed on the rheometer surface. The rheometer probe (8 mm diameter) was lowered to 1.5 mm, and the excess hydrogel was trimmed. The final sample volume was 75 mm³.

Strain sweep measurements were performed by varying the applied strain between 0.01 and 10 % at a constant angular frequency of 6.28 rad/s and a constant temperature of 37 °C. Frequency sweep measurements were performed by varying the angular frequency between 10 and 0.1 rad/s at a constant strain of 0.08 % and a constant temperature of 37 °C. A first dynamic strain amplitude test was performed by systematically applying a strain of 10 % on the sample for 30 s followed by a strain of 0.08 % for period of 5 min, to let the hydrogel recover. During the measurement, the temperature was kept constant at 37 °C, and the angular frequency was kept constant at 6.28 rad/s. A second dynamic strain amplitude test was performed by systematically applying a strain of 100 % on the sample for 60 s followed by a strain of 0.08 % for period of 100 s, to let the hydrogel recover. During the measurement, the temperature was kept constant at 37 °C, and the angular

frequency was kept constant at 6.28 rad/s. Data processing of the rheological measurements was performed using GraphPad Prism 7.05 to obtain the average and standard deviation of three sets of data.

Cell culture. L929 fibroblasts (mouse C3H/AN connective tissue, Sigma Aldrich) at passage 7 were cultured in standard tissue culture flasks at 37 °C in humidified air mixed with 5 % CO₂ in a growth medium consisting of DMEM (Invitrogen) mixed with 10 % fetal bovine serum (FBS; Invitrogen) and 1 % antibiotics (Invitrogen). Cells were subcultured when they reached about 75 % confluence, and the medium was changed every 3 d.

Cell encapsulation. Since the gelation procedure requires heating the sample to 100 °C to dissolve *meta-C* followed by sonicating the solution while cooling, hydrogels were prepared first and then mixed with a cell suspension to encapsulate the cells for culture in 3D in the hydrogels. This was possible because of the shear thinning and self-healing properties of the hydrogel. In this case, *meta-C* was first introduced into a glass vial and sterilized under UV light for 20 min. Then, sterile growth medium (DMEM with 10 % FBS and antibiotics) was added, and hydrogels at 1 wt.% were prepared as previously described. L929 fibroblasts at passage 8 were trypsinized using trypsin-EDTA (0.25 % with phenol red, Thermo Fisher Scientific) and resuspended in growth medium. Cells were stained with trypan blue, and the viable cell concentration was determined using a hemocytometer (Bruker). A concentrated cell suspension (200 µL), to achieve a final seeding density of 1.2×10^6 cells/mL, was then mixed with the hydrogels (800 µL), to achieve a final concentration of 0.8 wt.% *meta-C*, using a syringe. The hydrogels containing cells, each 1 mL in volume, were transferred to standard tissue culture-treated well plates for the proliferation and viability assays or to glass bottom well plates for imaging of cell morphology. The well plates were then incubated for 48 h in an incubator at 37 °C with humidified air mixed with 5 % CO₂.

Evaluation of cell morphology. At selected time points (2 h, 24 h, and 48 h), the hydrogels (in duplicate) were washed with PBS, and the cells were fixed and permeabilized with a paraformaldehyde (PFA)/Triton X-100 solution (4 % PFA + 0.2 % Triton X-100 in PBS) for 40 min at room temperature. The PFA solution was then removed, and glycine (0.1 M) was added for 15 min at room temperature. The glycine solution was removed, and the hydrogels containing cells were washed 2 times with PBS and stained with AlexaFluor 488 phalloidin (2 U/mL in PBS) and DAPI (2.5 μ g/mL) for 2 h. The hydrogels were then washed 2 times with PBS. Z-stacked confocal images of the stained cells were acquired using a Zeiss LSM 780 confocal microscope with 488 nm and 405 nm laser lines for AlexaFluor 488 phalloidin and DAPI excitation, respectively. For the widefield images, a 25 \times water immersion lens (0.8 N.A.) was used for scanning a depth of 100 μ m with a 5 μ m step size. The magnified inset images were acquired using a 63 \times water immersion lens (1.15 N.A.) and step size of \sim 0.8 μ m. Images were analyzed using ImageJ (NIH), and the circularity was calculated as the ratio between minor and major axes within the perimeter of the F-actin (or nuclear) signal for each cell, with 1 indicating a perfect circle.

Cell proliferation and viability assays. At each time point (2 h, 24 h, and 48 h), 1 mL of additional growth medium was added into the wells (4 replicates) and gently mixed with the hydrogels to disrupt the hydrogel and disperse the cells. The harvested cells were then stained with trypan blue and counted using a hemocytometer. The number of live and dead cells was recorded, and the data were processed using GraphPad Prism 5 software.

Statistical analysis. Data for cell proliferation, viability, and circularity were analyzed by calculating the mean and standard deviation of $n = 4$ or 5 , as indicated in the figure captions. A one-way ANOVA with Bonferroni's multiple comparisons test was used to assess the effect of time with $p < 0.05$ considered to indicate statistical significance (GraphPad Prism 5).

Supplementary Movies

Supplementary Movie 1: Gel preparation procedure. First, 1 mL of growth medium is added to a vial containing 8 mg of *meta*-C. Second, the vial is heated in a copper block at 100 °C until the gelator is fully dissolved. Next, the vial is opened, and the solution is sonicated using a UP50H Ultrasonic processor with MS1 tip operating at 30 KHz ($P = 0.075$ W). While cooling down to room temperature, the gel forms. Finally, the vial is capped and inverted to confirm gelation.

Supplementary Movie 2: Illustration of the injectability of the hydrogel. A 0.8 wt.% hydrogel made with growth medium is pushed through a syringe equipped with a needle (0.90 mm in diameter). The gel flows when stress is applied and restores at the surface of the container, after which the container is inverted to demonstrate the gel state.

Supplementary Figures

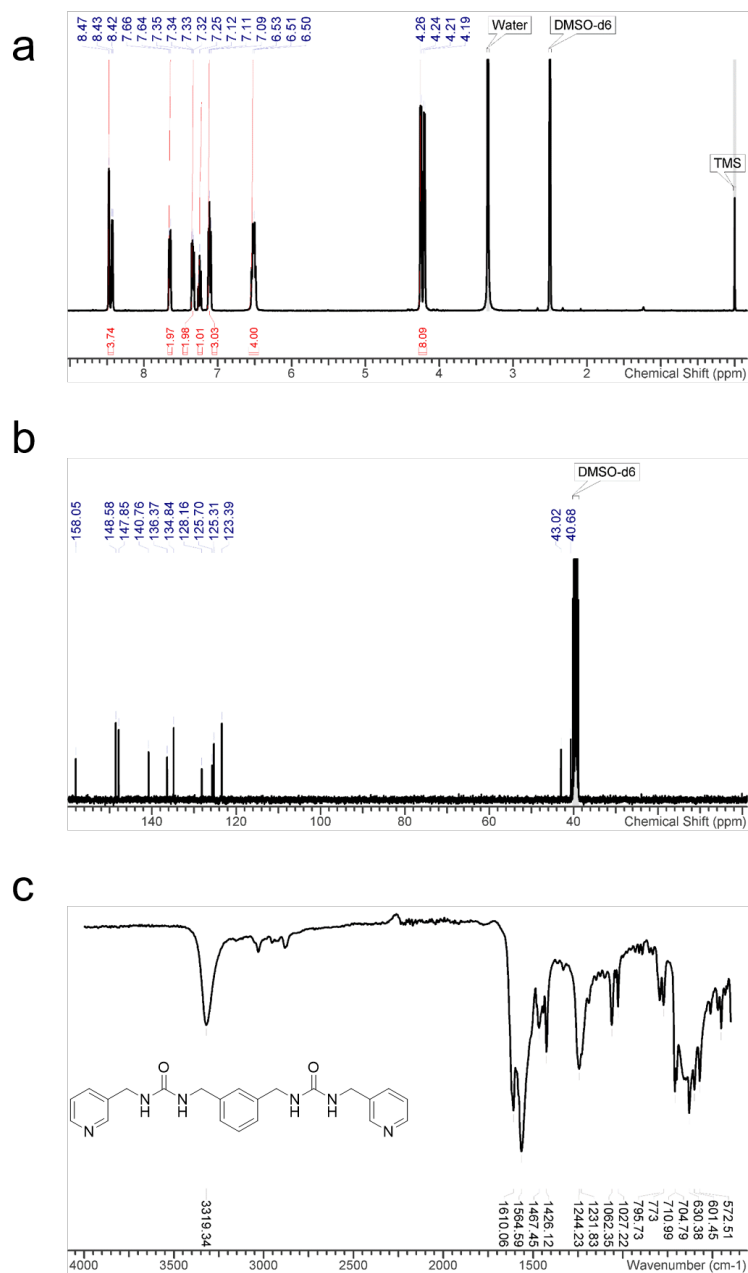


Fig. S1 Chemical characterization of 1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-3-ylmethyl)urea) (*meta-C*) prepared in solution. (a) ^1H NMR spectrum, with the peaks of the CH_2 between 4.26 and 4.19 ppm, peaks of the urea NH between 6.53 and 6.50 ppm, and peaks of the aromatic protons between 8.47 and 7.09 ppm; (b) ^{13}C NMR spectrum, with the carbonyl peak of the urea group at 158.1 ppm; and (c) FTIR spectrum, with main peaks at 3319 cm^{-1} (N-H stretch), 1610 cm^{-1} (C=O stretch), and 1565 cm^{-1} (N-H bend). Inset shows the chemical structure of *meta-C*.

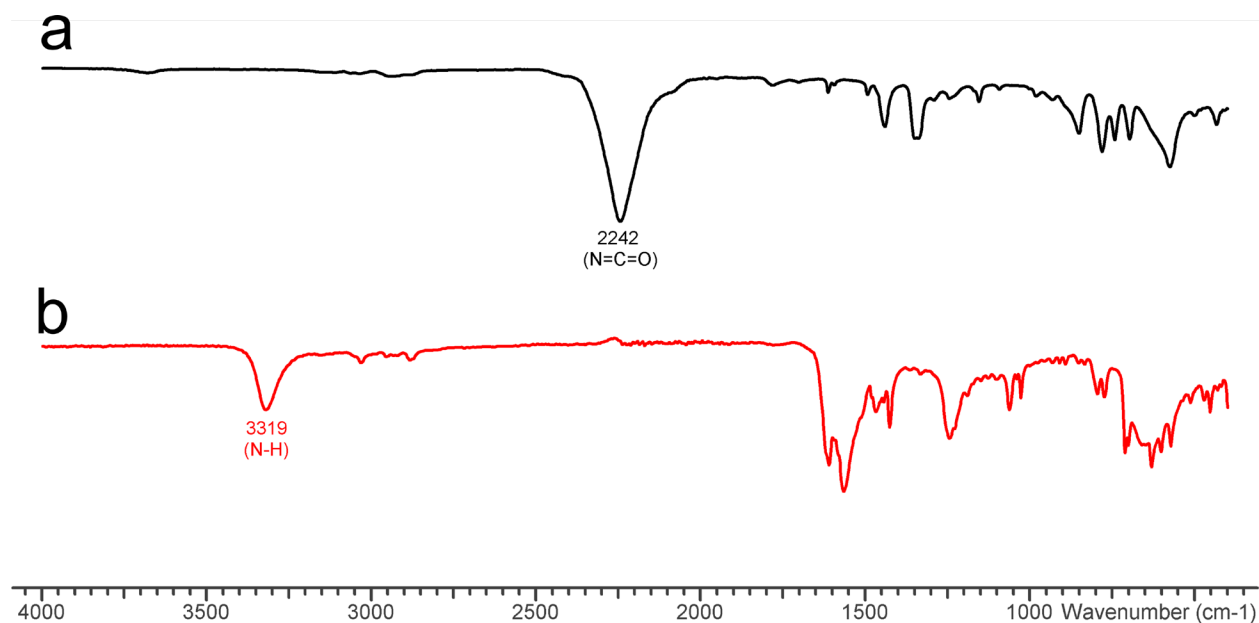


Fig. S2 FTIR spectra showing the conversion of the starting material into *meta-C* via the ball milling method. (a) FTIR spectrum of 1,3-bis(isocyanatomethyl)benzene (*m*-xylylene diisocyanate, **B** in text) with a peak at 2242 cm⁻¹, indicating the presence of an isocyanate functional group. (b) FTIR spectrum of 1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-3-ylmethyl)urea) (*meta-C*) where the peak at 2242 cm⁻¹ has disappeared, indicating full conversion of the starting material.

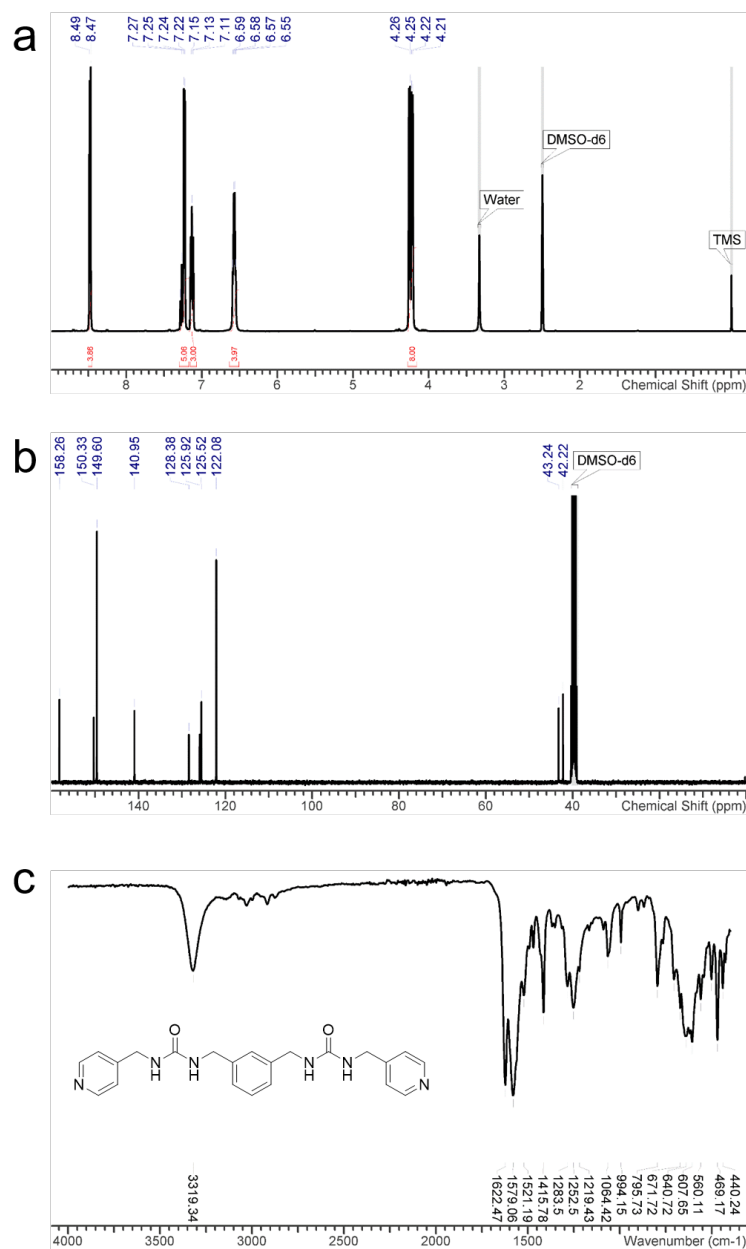


Fig. S3 Chemical characterization of 1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-4-ylmethyl)urea) (*para-C*). (a) ^1H NMR spectrum, with the peaks of the CH_2 between 4.26 and 4.21 ppm, peaks of the urea NH between 6.59 and 6.55 ppm, and peaks of the aromatic protons between 8.49 and 7.11 ppm; (b) ^{13}C NMR spectrum, with the carbonyl peak of the urea group at 158.3 ppm; and (c) FTIR spectrum, with main peaks at 3319 cm^{-1} (N-H stretch), 1622 cm^{-1} (C=O stretch), and 1579 cm^{-1} (N-H bend). Inset shows the chemical structure of *para-C*.

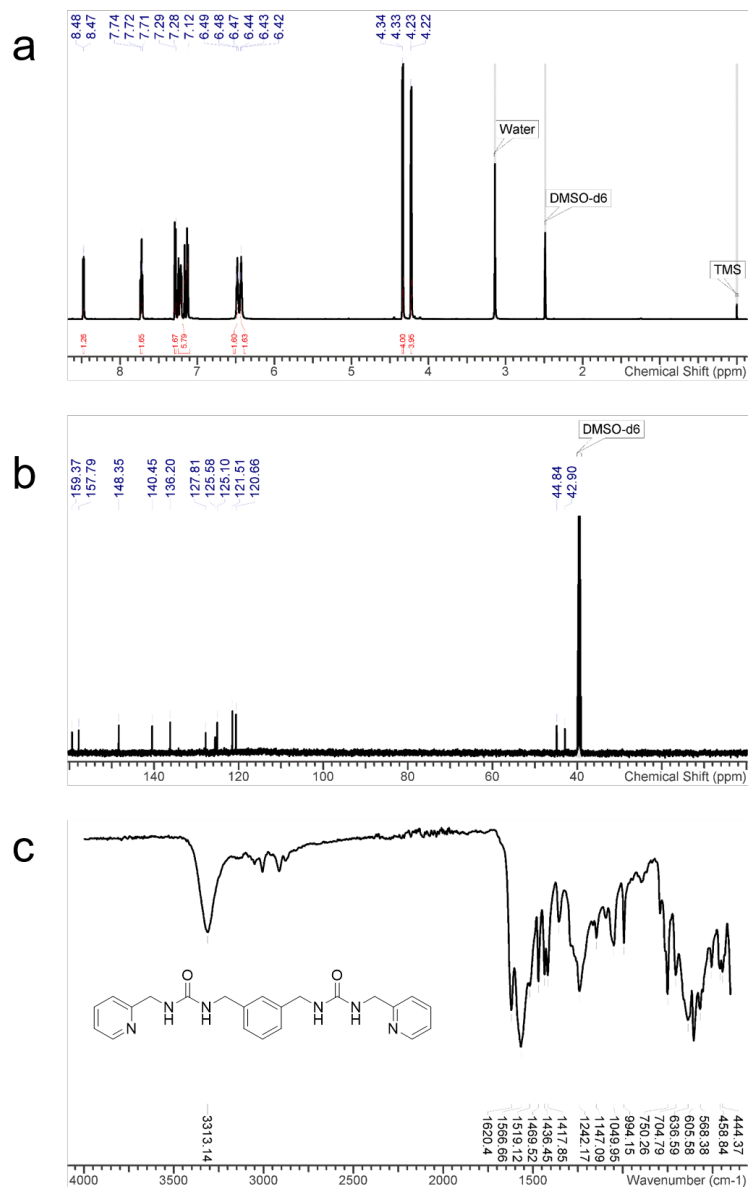


Fig. S4 Chemical characterization of 1,1'-(1,3-phenylenebis(methylene))bis(3-(pyridin-2-ylmethyl)urea) (*ortho-C*). (a) ^1H NMR spectrum, with the peaks of the CH_2 between 4.34 and 4.22 ppm, peaks of the urea NH between 6.49 and 6.42 ppm, and peaks of the aromatic protons between 8.48 and 7.12 ppm; (b) ^{13}C NMR spectrum, with the carbonyl peak of the urea group at 159.4 ppm; and (c) FTIR spectrum, with main peaks at 3313 cm^{-1} (N-H stretch), 1620 cm^{-1} (C=O stretch), and 1567 cm^{-1} (N-H bend). Inset shows the chemical structure of *ortho-C*.

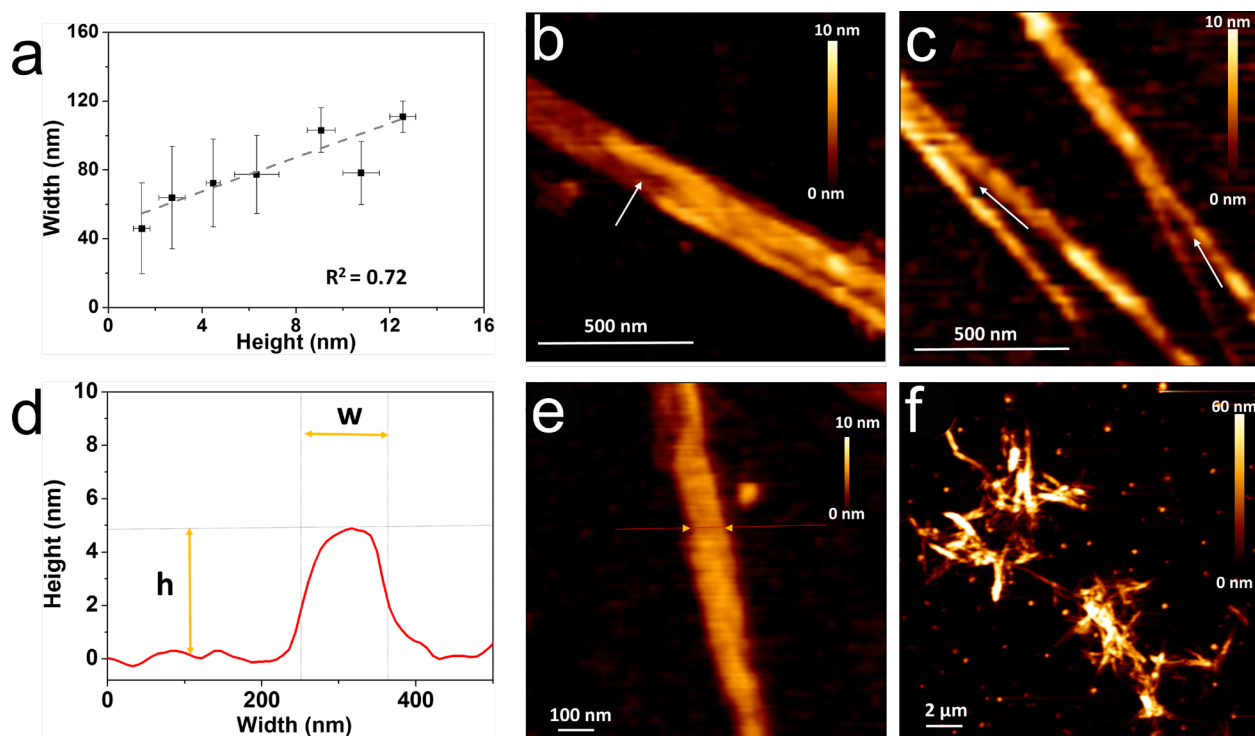


Fig. S5 Detailed AFM structural analysis. (a) Height and width correlation of measured individual nanofibrils of *meta-C* ($n = 169$). Nanofibrils were grouped based on height measurements with a bin size of 2 nm. Average height and width of the corresponding groups are shown (whiskers indicate standard deviation). The dotted line indicates a linear fit through the measured data, $R^2 > 0.7$. (b) An example AFM image of *meta-C* showing nanofibril stacking/thinning on top of other nanofibrils (indicated by white arrow mark). Scale bar is 500 nm, and Z-scale bar is 0-10 nm. (c) An example AFM image of *meta-C* showing nanofibril splitting (indicated by white arrow marks). Scale bar is 500 nm, and Z-scale bar is 0-10 nm. (d) Line profile measurement of AFM width and height analysis of the nanofibril of *meta-C* shown in (e) (measured location indicated by red line with yellow arrow marks to indicate the measured width). Scale bar is 100 nm, and Z-scale bar is 0-10 nm. (f) AFM image showing the formation of nanofibrils/nanofibers of *meta-C* in growth medium. Scale bar is 2 μm , and Z-scale bar is 0-60 nm.

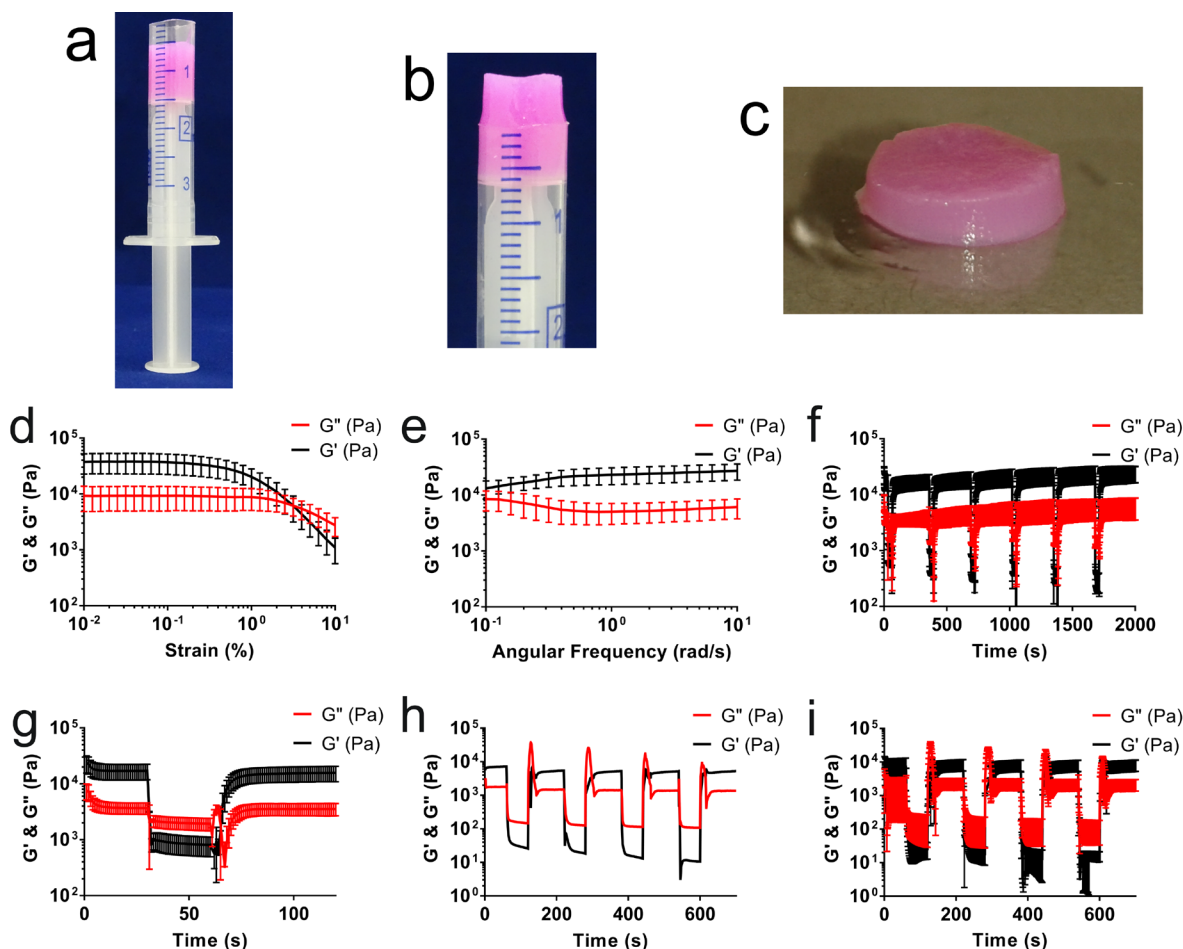


Fig. S6 Sample preparation for rheology, rheology graphs with error bars, and additional rheology graphs. (a) The hydrogel (0.8 wt.% *meta-C* in growth medium) was prepared in a syringe with the tip cut off; (b) the cylindrical hydrogel was pushed out of the syringe; and (c) a disk-shaped part of the hydrogel was cut off using a spatula and placed on the rheometer. (d-g) Graphs of rheology data shown in main text including error bars (average and standard deviation of three independent measurements) with (d) strain sweep (at an angular frequency of 6.28 rad/s); (e) frequency sweep (at a strain of 0.08 %); and (f, g) dynamic strain amplitude test (alternating between 0.08 % and 10 % strain at an angular frequency of 6.28 rad/s). (h, i) Dynamic strain amplitude test performed on 0.8 wt.% hydrogels of *meta-C* made with growth medium. Rheology was performed at an angular frequency of 6.28 rad/s and temperature of 37 °C. The strain was alternated between 0.08 % (100 s) and 100 % (60 s). When the strain changes from 0.08 % to 100 %, the values of the storage modulus (G') fall below the values of the loss modulus (G''), indicating the disruption of the gel network. When the strain changes back to 0.08 %, the values of the storage modulus increase again to their original value, indicating the recovery of the gel properties. (h) average of three independent measurements and (i) average and standard deviation of the same three independent measurements.

References for Supplementary Information

1. Y.-C. Lin, H. Komatsu, J. Ma, P. H. Axelsen and Z. Fakhraai, *RSC Adv.*, 2016, **6**, 114286.