

Supporting information:

Formation of Molecular Hydrogels from a Bile Acid Derivative and Selected Carboxylic Acids

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Experimental part

Materials

Cholic acid, N-hydroxylsuccinimide (NHS), dicyclohexylcarbodiimide (DCC), diethylenetriamine, and all organic acids were purchased from Sigma-Aldrich (Oakville, ON, Canada) and used without further purification. Anhydrous tetrahydrofuran (THF), acetonitrile (ACN), and dimethylformamide (DMF) were redistilled from a solvent purification system from Glass Contour (Nashua, NH, United States). All the hydrogel samples were prepared with fresh Milli-Q water.

Gelation

For the test of gelation, stock solutions of different carboxylic acids were prepared in Milli-Q water and diluted to the desired concentration. To this solution, a known amount of the cholic acid dimer was added to reach a dimer concentration of 20 mM. The mixture was heated and sonicated and then cooled gradually to room temperature and left to stand for several hours. The samples were periodically verified for signs of gelation by inversion of vials. For the minimal gelation concentration (MGC) tests, the solutions of different carboxylic acids were fixed at 40 mM carboxylic acid groups (i.e., diprotic acids prepared at 20 mM). A series of samples of the dimer with concentrations differing by 0.5 mM were prepared by dissolving the dimer in the acid solutions through heating and sonication. The samples were equilibrated at room temperature and checked for the formation of hydrogels after 48 hours. The lowest concentration of the dimer for the formation of a stable hydrogel by vial-inversion was taken as the MGC.

Characterization

¹H NMR spectroscopy. The hydrogel sample was made in D₂O with 20 mM of the dimer and 40 mM acetic acid, and a trace amount of trioxane was added as an internal standard. The sample was heated first to obtain a homogeneous solution and then transferred to an NMR tube immediately. The hydrogel formed inside the tube at room temperature and was equilibrated for

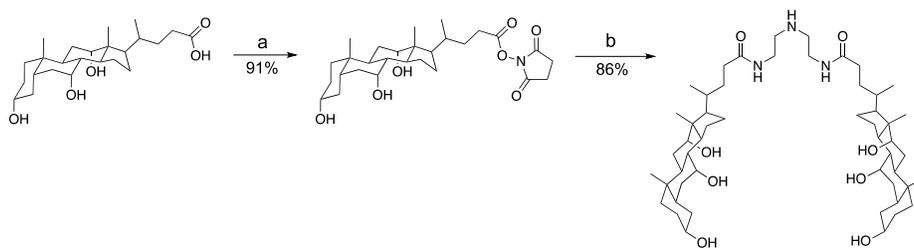
at least 24 hours. The ^1H NMR spectra at different temperatures were recorded on a Bruker AV400 spectrometer operating at 400 MHz.

FT-IR spectroscopy. A Nicolet 6700 FT-IR spectrometer with an attenuated total reflection (ATR) accessory was used to record the FT-IR spectra. The hydrogel samples with the desired concentrations of the dimer and the acids in H_2O were freeze-dried to obtain aerogels for which an FT-IR spectrum was recorded by collecting 128 scans at a resolution of 2 cm^{-1} .

Transmission electron microscopy (TEM). Three μl of the hydrogel samples of the dimer prepared with different carboxylic acids were placed on carbon-coated cooper grids (300 mesh, Carbon Type-B, Ted Pella, Inc.) at room temperature. After equilibration for 1 min., excess sample was removed by a filter paper and the remaining sample was dried under vacuum to obtain a thin film on the grid. Samples were observed on FEI Tecnai 12 TEM at 120 kV, equipped with AMT XR80C CCD camera system.

Rheology. The rheological study of the hydrogel samples was done on a TA-AR 2000 rheometer, using a cone-geometry with 40 mm diameter. All measurements were performed with a fixed gap of $55\ \mu\text{m}$ between the geometry and the plate. The hydrogel samples were melted to obtain a homogeneous solution. Aliquots of the solution were transferred to the center of the rheometer plate and a very small oscillatory stress of 0.1 Pa was applied to record the storage moduli (G') and loss moduli (G'') until a plateau was observed. Oscillatory frequency sweep experiments of the equilibrated hydrogel were performed in the linear viscoelastic region at $25\ ^\circ\text{C}$, with a constant stress of 0.1 Pa, for a scale from 0.01 to 100 Hz. Oscillatory stress sweep experiments were performed at $25\ ^\circ\text{C}$, with a constant frequency of 1 Hz. Oscillatory temperature sweep experiments were performed in the range of $20\text{-}70\ ^\circ\text{C}$ at 1 Hz with a oscillatory stress of 0.1 Pa.

Scheme S1 Synthesis of the dimer ^a



^a Reaction conditions: (a) NHS/DCC in THF/ACN, 18 h, room temperature; (b) Diethylenetriamine in DMF, 2 h, $60\ ^\circ\text{C}$.

Table S1 The acids tested and the minimum gelation concentrations of the cholic acid dimer

Carboxylic acids, [-COOH] = 40 mM	Chemical structure	pK _a	Dimer MGC ^a	
			(mM)	(wt%)
Formic acid	<chem>OC=O</chem>	3.8	5.5	0.49
Acetic acid	<chem>CC(=O)O</chem>	4.9	8.0	0.71
Propionic acid	<chem>CCC(=O)O</chem>	4.9	6.5	0.58
Butyric acid	<chem>CCCC(=O)O</chem>	4.8	5.5	0.49
Succinic acid	<chem>OC(=O)CC(=O)O</chem>	4.2, 5.6	4.0	0.35
Malic acid	<chem>OC(O)C(=O)O</chem>	3.4, 5.2	3.0	0.27
Tartaric acid	<chem>OC(O)(O)C(=O)O</chem>	3.2, 4.8	1.5	0.13

^a Estimated minimum gelation concentration (MGC) of the dimer in the presence of the carboxylic acids (monoacids at 40 mM and diacids at 20 mM).

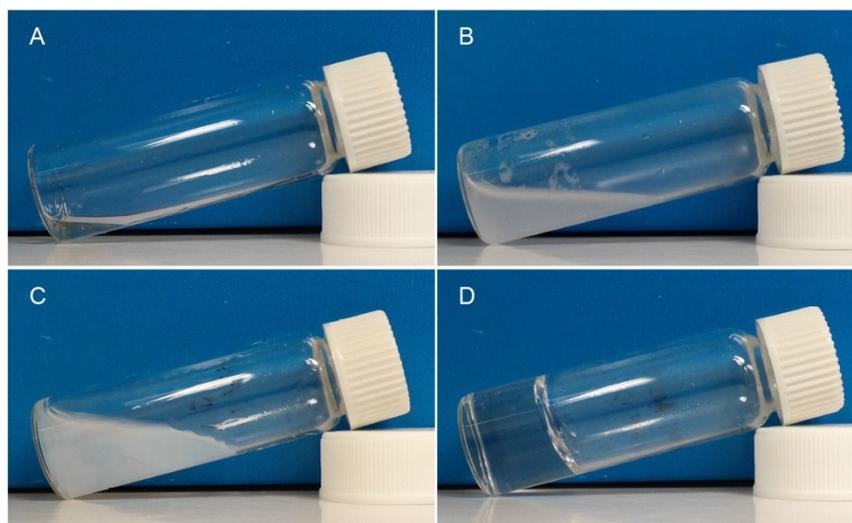


Figure S1 Behavior of the dimer in the presence of certain carboxylic acids: (A) Homogeneous solution obtained from the dimer and trifluoroacetic acid; (B) Precipitation, which occurred after the addition of water into the mixture of the dimer and trifluoroacetic acid; (C) Viscous and cloudy liquid obtained from an aqueous solution of acetic acid and the dimer (both at 20 mM); (D) Transparent and stable hydrogel obtained from an aqueous solution of acetic acid (40 mM) and the dimer (20 mM).

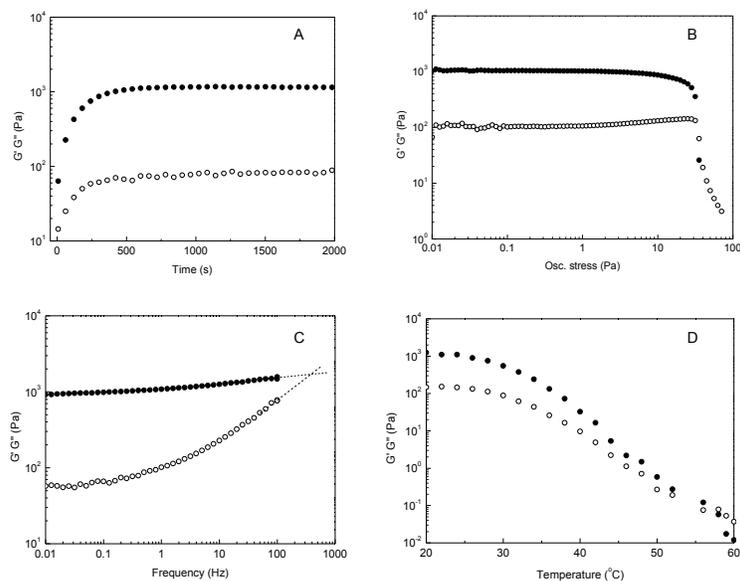


Figure S2 Viscoelastic properties of the dimer-acid hydrogel (20 mM dimer-40 mM acetic acid) in water: (A) Gelation over time; (B) Oscillatory stress sweep; (C) Frequency sweep; (D) Oscillatory temperature sweep. G' (closed circles), G'' (open circles).

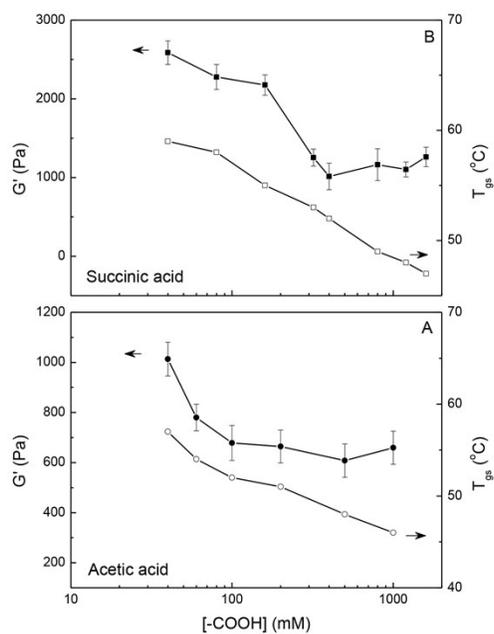


Figure S3 Viscoelastic properties of the hydrogels formed by the cholic acid dimer (20 mM) and various concentration of (A) Acetic acid. (B) Succinic acid. T_{gs} : gel-sol transition temperature.

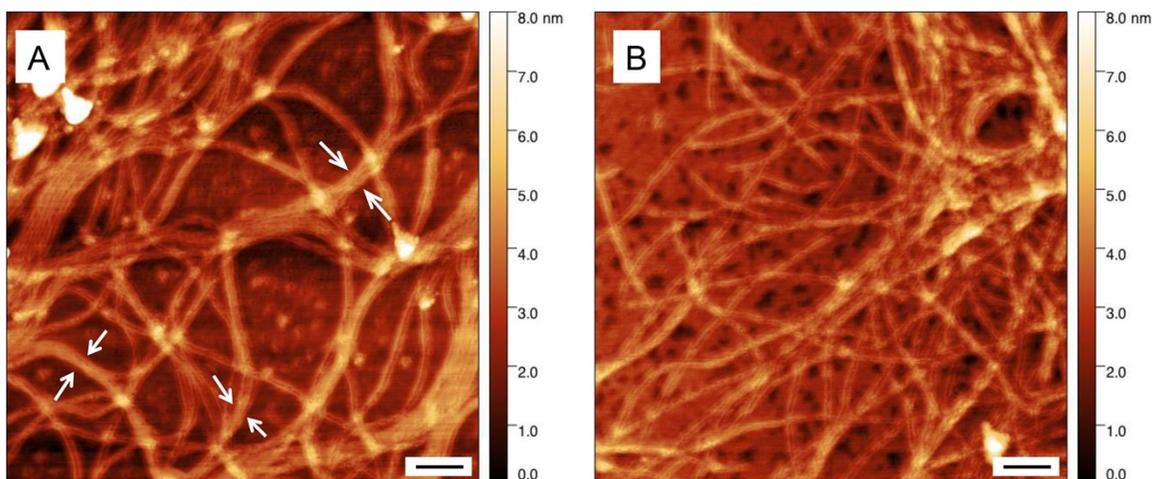


Figure S4 AFM images (black scale bar = 200 nm) of the fibrillar network in hydrogels formed by (A) 6 mM dimer and 12 mM acetic acid (the white arrows indicate entwined nanofibers), and (B) 0.6 mM dimer and 0.6 mM tartaric acid.

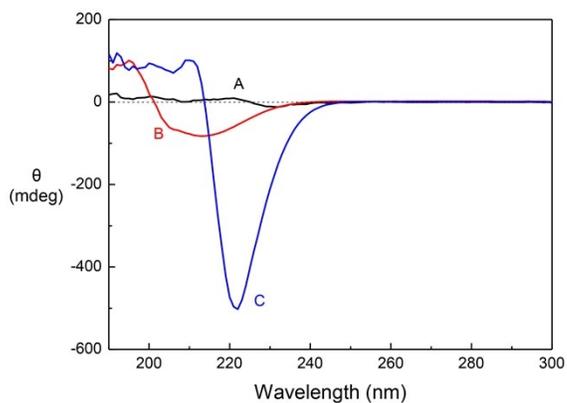


Figure S5 The CD spectra of the hydrogel samples formed with (A) 6 mM dimer and 12 mM acetic acid; (B) 0.6 mM dimer and 0.6 mM tartaric acid; and (C) 6 mM dimer and 6 mM tartaric acid.

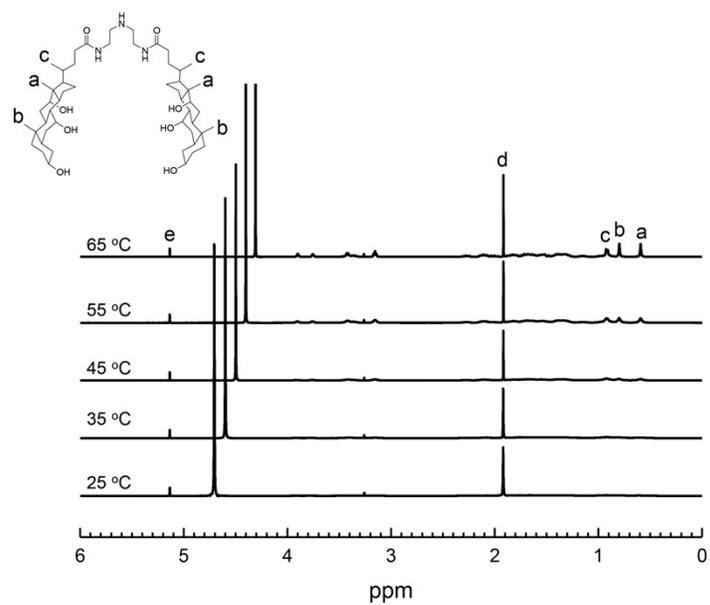


Figure S6 ^1H NMR spectra of hydrogel of acetic acid-dimer (40 mM-20 mM) in D_2O at different temperatures. Peaks a, b, and c indicate the protons at methyl groups on the cholic acid dimer, peak d is from protons of the methyl group on acetic acid, peak e is from protons of trioxane added as an internal standard, the peaks at about 4.7 that varies with temperature are water solvent residual signals.

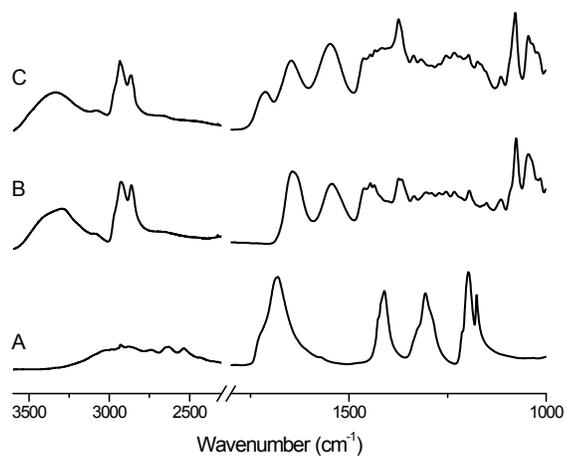


Figure S7 FT-IR spectra of (A) succinic acid; (B) the dimer; (C) aerogel of succinic acid-dimer (20 mM-20 mM).