Chirality-directed hydrogel assembly and interactions with enantiomers of an active pharmaceutical ingredient

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Contents

Materials and Methods 2
Synthesis and Characterisation of New Gelator Molecules 2
Synthesis of L-DBS-CO_2Me 2
Synthesis of L-DBS-$CONHNH_2$ 3
Preparation of Gels 3
Assay Methods 3
Gelation Screening and Thermal Stability 5
Rheology 6
Scanning and Transmission Electron Microscopy (SEM and TEM) 7
Gelation Screening and Thermal Stability of Mixtures of Enantiomers 8
Rheology for Mixtures of Enantiomers 9
NMR Study of Mixtures of Enantiomers 10
Scanning and Transmission Electron Microscopy (SEM and TEM) on Mixtures of Enantiomers 11
UV-Vis Spectroscopy of DBS-$CONHNH_2$ 12
IR Spectroscopy of DBS-$CONHNH_2$ and Mixtures of Enantiomers 13
Gelation of Enantiomeric Gels in the Presence of Enantiomers of Naproxen 16
Rheology of Enantiomeric Gels in the Presence of Enantiomers of Naproxen 16
NMR Studies of Enantiomeric Gels in the Presence of Enantiomers of Naproxen 17

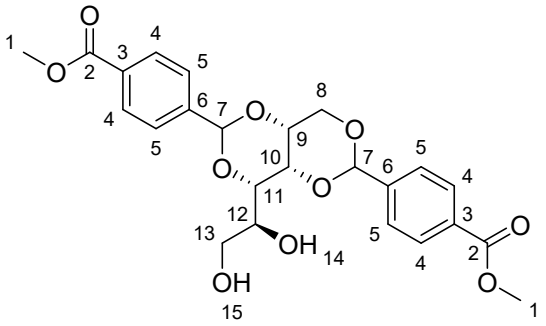
Materials and Methods

General Experimental Methods: All compounds required for synthesis and analysis were purchased from standard chemical suppliers and used without further purification. D-DBS-$CONHNH_2$ was synthesised as described previously.[1] ^1H and ^{13}C NMR were recorded on a Jeol 400 spectrometer (^1H 400 MHz, ^{13}C 100 MHz), with the exception of the variable temperature NMR, which were recorded on a Bruker 500 (^1H 500 MHz). Coupling constants (*J*) are recorded in Hz. Mass spectrometry was performed by the University of York Mass Spectrometry Service. IR were recorded on a ThermoNicolet Avatar 370 FT-IR spectrometer. Melting points were recorded using a Stuart SMP3 apparatus. All rheological measurements were carried out using a Malvern Instruments Kinexus Pro+ rheometer. T_{gel} values were recorded using a high precision thermoregulated oil bath. Circular dichroism (CD) measurements were carried out using a Jasco J810 CD Spectrophotometer. UV-vis absorbance was measured on a Shimadzu UV-2401 PC spectrophotometer.

Synthesis and Characterisation of New Gelator Molecules

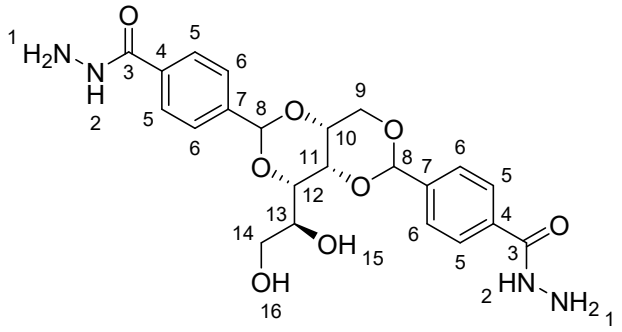
The synthesis of the L-version of the gelator was performed in the same way as the D-version reported previously,[1] however, for completeness, we present the full synthesis and characterisation here.

Synthesis of L-DBS-CO_2Me



L-sorbitol (1.49 g, 2.69 mmol) was added to a three-necked flask, fitted with Dean-Stark equipment. Cyclohexane (30 ml) and methanol (20 ml) were added, and the suspension stirred, at 50 °C and under N_2, for 20 minutes. 4-methylcarboxybenzaldehyde (0.90 g, 5.48 mmol) was dissolved in methanol (20 ml) along with *p*-TsOH (0.8 g, 4.21 mmol), and this solution added dropwise to the L-sorbitol suspension. The temperature was then increased to 70 °C. The reaction was allowed to continue for two hours, with additional cyclohexane added as required. The resulting white powder was removed by filtration, then washed with cold methanol (150 ml). The white solid was then washed with hot water (3x50 ml) followed by hot DCM (3x50 ml). The white powder was then dried under a high vacuum. Yield: 725 mg (1.528 mmol, 57%); M.p: 186.6-87.9 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 8.00-7.97 (m, 4H, H_4), 7.63-7.58 (m, 4H, H_5), 5.76 (s, 2H, H_7), 4.93 (d, *J*=8 Hz, 1H, H_{14}), 4.47 (dd, *J*=6 Hz, 6 Hz, 1H, H_{15}), 4.26-4.17 (m, 3H, H_8, H_9), 4.01 (ap. s, 1H, H_{10}), 3.91-3.88 (m, 1H, H_{11}), 3.85 (s, 6H, H_1), 3.81-3.75 (m, 1H, H_{12}), 3.64-3.59 (m, 1H, H_{13}), 3.49-3.43 (m, 1H, H_{13}); ^{13}C NMR (100 MHz, DMSO-d_6), 166.00 (C_2), 165.98, (C_2), 143.32 (C_6), 143.06 (C_6), 129.77 (C_3), 129.72 (C_3), 129.02 (C_4), 128.93 (C_4), 126.49 (C_5), 98.53 (C_7), 98.45 (C_7), 77.59 (C_{11}), 70.17 (C_{10}), 69.31 (C_8), 68.52 (C_9), 67.59 (C_{12}), 62.55 (C_{13}), 52.19 (C_1); $[\alpha]_D^{25}$ (deg cm^3 g^{-1} dm^{-1}) -31.8 ± 0.2 (c=1, DMSO); ESI-MS (m/z) calc. for $[M+Na]^+$, $C_{24}H_{27}O_{10}Na^+$, 497.1424; found 497.1431 (100% $[M+Na]^+$); ν_{max} (cm^{-1}) (solid): 3246*w*, 2955*w*, 1723*s*, 1615*w*, 1579*w*, 1435*w*, 1414*w*, 1399*m*, 1368*w*, 1342*w*, 1277*s*, 1220*w*, 1193*w*, 1167*w*, 1090*s*, 1065*m*, 1051*m*, 1018*s*, 982*m*, 964*m*, 906*w*, 883*w*, 856*m*, 836*m*, 816*w*, 764*m*, 750*s*, 708*s*, 657*m*, 603*w*, 588*m*, 551*m*, 525*w*.

Synthesis of L-DBS-CONHNH$_2$



L-DBS-CONHNH$_2$ (498 mg, 1.05 mmol) was suspended in THF (50 ml), and $N_2H_2.H_2O$ (5 ml, 103 mmol) added slowly. This was heated to reflux and left overnight. On cooling, a white precipitate was formed. This was removed by filtration, and washed with water (3x50 ml). The resulting white paste was dried first under high vacuum, then to a constant mass in a vacuum oven. Yield: 449.5 mg (0.947 mmol, 90%); 294.9-296.6 °C; ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 9.81 (s, 2H, H$_2$) 7.84-7.81 (m, 4H, H$_5$), 7.54-7.49 (m, 4H, H$_6$), 5.71 (s, 2H, H$_8$), 4.93 (d, *J*=6, 1H, H$_{15}$), 4.50-4.46 (m, 5H, H$_1$, H$_{16}$), 4.24-4.15 (m, 3H, H$_9$, H$_{10}$), 3.98 (br. s, 1H, H$_{11}$), 3.88-3.86 (m, 1H, H$_{12}$), 3.80-3.74 (m, 1H, H$_{13}$), 3.63-3.58 (m, 1H, H$_{14}$), 3.48-3.45 (m ,1H, H$_{14}$); ^{13}C NMR (100 MHz, DMSO-*d$_6$*): 165.71 (C$_3$), 141.33 (C$_4$), 141.06 (C$_4$) 133.58 (C$_7$), 133.50 (C$_7$), 126.84 (C$_5$), 126.76 (C$_5$), 126.15 (C$_6$), 126.13 (C$_6$) 98.83 (C$_8$), 98.76 (C$_8$), 77.62 (C$_{12}$), 70.18 (C$_{11}$), 69.39 (C$_9$), 68.53 (C$_{10}$), 67.72 (C$_{13}$), 62.66 (C$_{14}$); [α]D^{25} (deg cm^3 g^{-1} dm^{-1}) -55.5 ± 0.4 (c=1, DMSO); ESI-MS (m/z) calc for [M+Na]$^+$, $C_{22}H_{26}N_4O_8Na^+$, 497.1649; found 497.1643 (100% [M+Na]$^+$); ν_{max} (cm^{-1}) (solid): 3290*m*, 1631*m*, 1594*m*, 1568*m*, 1541*m*, 1506*w*, 1399*m*, 1369*w*, 1338*m*, 1164*w*, 1091*s*, 1039*m*, 1006*m*, 977*m*, 904*w*, 847*m*, 829*m*, 753*w*, 683*m*, 644*m*, 622*m*, 545*m*.

Preparation of Gels

Preparation of hydrogels. A known mass of gelator(s) was weighed into a sample vial, and 0.5 ml of deionised water added. This was sonicated for 15 minutes, followed by a heat-cool cycle. On cooling, transparent gels were formed rapidly. The same procedure was followed for the preparation of gels containing NPX, with NPX (1 eq.) added to the sample vial with the gelator.

Assay Methods

Thermal Characterisation. To determine T_{gel} values, hydrogels were first prepared as described above, with the only difference being that the volume of water used was reduced to 0.5 ml. Once the gels had been formed, they were placed into a thermoregulated oil bath, and the temperature increased at a rate of 1 °C min^{-1}. The vials were removed every minute, gelation tested by the tube inversion test, and the vial replaced. This was continued until a gel-sol transition occurred.

Rheology. A known mass of DBS-CONHNH$_2$ was added to a sample vial, and H_2O added (with naproxen if being used). This was sonicated for 15 minutes, to give a suspension. The solid was then dissolved by heating, and while the solution was hot, transferred to a bottomless vial. On cooling, a translucent gel was formed. The bottomless vial was then removed, and the gel disc transferred to the rheometer. The temperature was maintained at 25 °C. For amplitude sweeps, frequency was constant at 1 Hz. For frequency sweeps, amplitude was constant at a value determined by the previously carried out amplitude sweep.

SEM. All SEM images were obtained using the following method: A small portion of gel was transferred to a copper support, then freeze-dried by plunging into liquid nitrogen. The samples were then lyophilised for 12 hours, and any excess material removed. The dried sample was then sputter coated with a thin layer of gold/palladium, to prevent sample charging, and imaging carried out. Sample preparation and imaging was carried out by Meg Stark at the at the Biology Technology Facility, Department of Biology, University of York.

TEM. All TEM imaging was carried out using the following method: A small portion of gel was transferred, by drop-casting, to a heat-treated copper support. Excess material was removed using a filter paper, and the samples air-dried for 20 minutes. Sample preparation and imaging was carried out by Meg Stark at the at the Biology Technology Facility, Department of Biology, University of York.

UV-Vis. UV-Vis spectrometry was performed on a solution of DBS-$CONHNH_2$ at a loading of 0.01% wt/vol (0.21 mM) in water in a quartz cuvette.

CD Studies. Known masses of both D-DBS-$CONHNH_2$ (or/and L-DBS-$CONHNH_2$) were added to a sample vial, and H_2O (0.5 ml) added. This was sonicated for 15 minutes to give a suspension. The solids were then dissolved by heating, and while the solution was hot, transferred to a warn quartz cuvette (pathlength=1 mm). On cooling within the cuvette the sample was analysed. CD experiments were carried out using the following settings: Data Pitch = 0.5 nm, Scanning Mode = continuous, Scan Speed = 100 nm min^{-1}, Response = 1 s, Bandwidth = 2 nm, Accumulation = 5. Quartz cuvettes (pathlength 1 mm) were used. For variable temperature experiments, measurements were carried out at 5 °C intervals, from 20-90 °C.

IR Spectroscopy. Infra-red was performed on both solvated gels and dried xerogels. Solvated gels were made at loadings of 0.3% wt/vol in water using the standard method. Xerogels were prepared by drying in vacuo under ambient conditions of temperature. Analysis was carried out directly using ATR methods.

NMR Studies. Known masses of both D-DBS-$CONHNH_2$ and L-DBS-$CONHNH_2$ (with a known mass of either (*R*)-NPX or (*S*)-NPX if being used) were added to a sample vial, and D_2O (0.7 ml) added, along with a DMSO standard (2 µl). This was sonicated for 15 minutes to give a suspension. The solids were dissolved by heating, and while the solution was hot, transferred to a warm NMR tube. This was then allowed to cool, and a translucent gel was formed within the NMR tube.

Gelation Screening and Thermal Stability

Table S1. Gelation screen for L-DBS-$CONHNH_2$ and D-DBS-$CONHNH_2$ in water at a range of loadings.

Concentration / % wt/vol	L-DBS-$CONHNH_2$	D-DBS-$CONHNH_2$
0.16	S	S
0.20	G	G
0.25	G	G
0.28	G	G
0.30	G	G
0.37	G	G

Table S2. Thermal stability of gels formed by L-DBS-$CONHNH_2$ and D-DBS-$CONHNH_2$ in water N.B. T_{gel} values are only monitored up to 100 °C for hydrogels - above this, it would not be possible to determine if the loss of the gel is due to the breakdown of the network or the evaporation of the solvent.

L-DBS-$CONHNH_2$ / % wt/vol	T_{gel} / °C	D-DBS-$CONHNH_2$ / % wt/vol	T_{gel} / °C
0.20	72	**0.20**	66
0.25	97	**0.25**	96
0.28	100+	**0.28**	99
0.30	100+	**0.30**	100+
0.37	100+	**0.37**	100+

Rheology

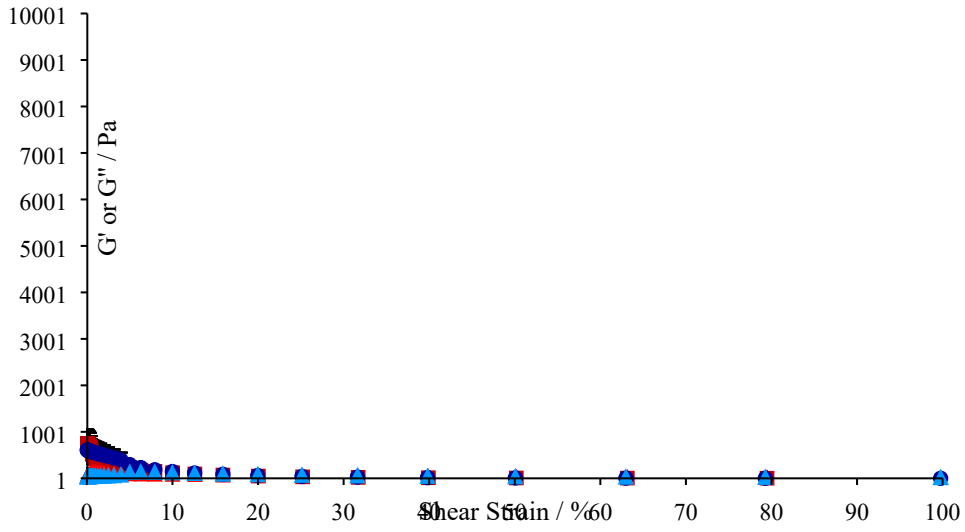


Figure S1. Amplitude sweep for D-DBS-CONHNH$_2$ and L-DBS-CONHNH$_2$ hydrogels, gelator concentration 0.28% wt/vol. Dark blue circles: D-DBS-CONHNH$_2$ G', Light blue diamonds: D-DBS-CONHNH$_2$ G'', Dark red squares: L-DBS-CONHNH$_2$ G', Light red triangles: L-DBS-CONHNH$_2$ G''.

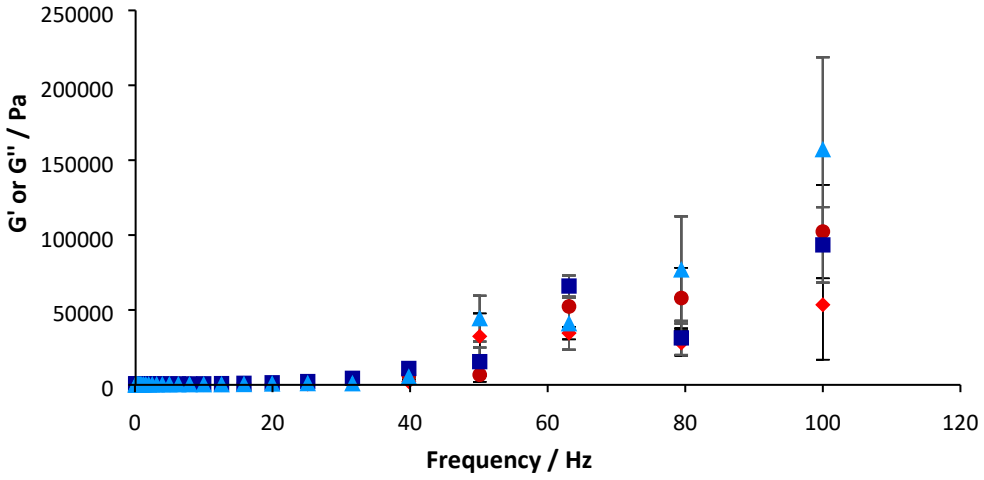


Figure S2. Frequency sweep for D-DBS-CONHNH$_2$ and L-DBS-CONHNH$_2$ hydrogels, gelator loading 0.28% wt/vol. Dark red circles: D-DBS-CONHNH$_2$ G', Light red diamonds: D-DBS-CONHNH$_2$ G'', Dark blue squares: L-DBS-CONHNH$_2$ G', Light blue triangles: L-DBS-CONHNH$_2$ G''.

Scanning and Transmission Electron Microscopy (SEM and TEM)

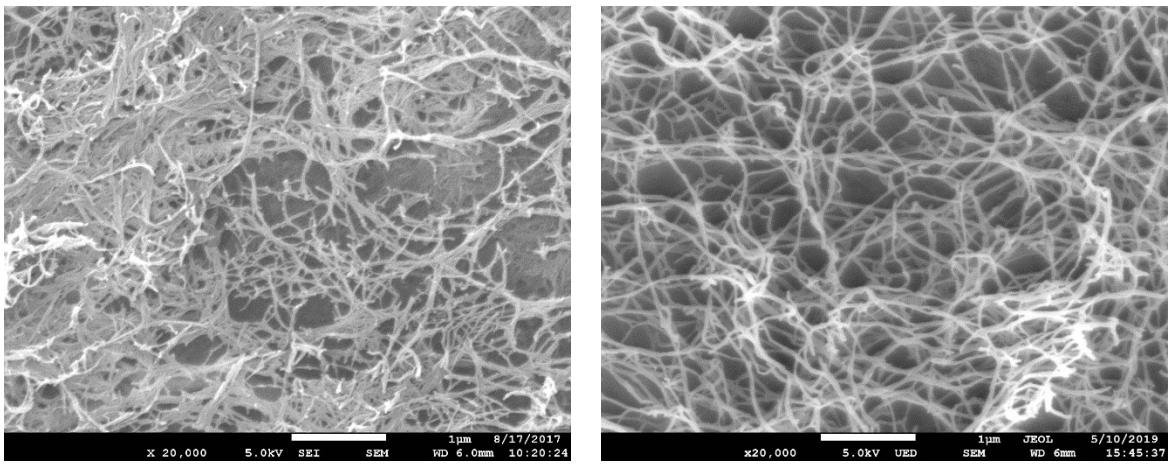


Figure S3. SEM images, scale bar 1 µm (left: L-DBS-$CONHNH_2$, right: D-DBS-$CONHNH_2$).

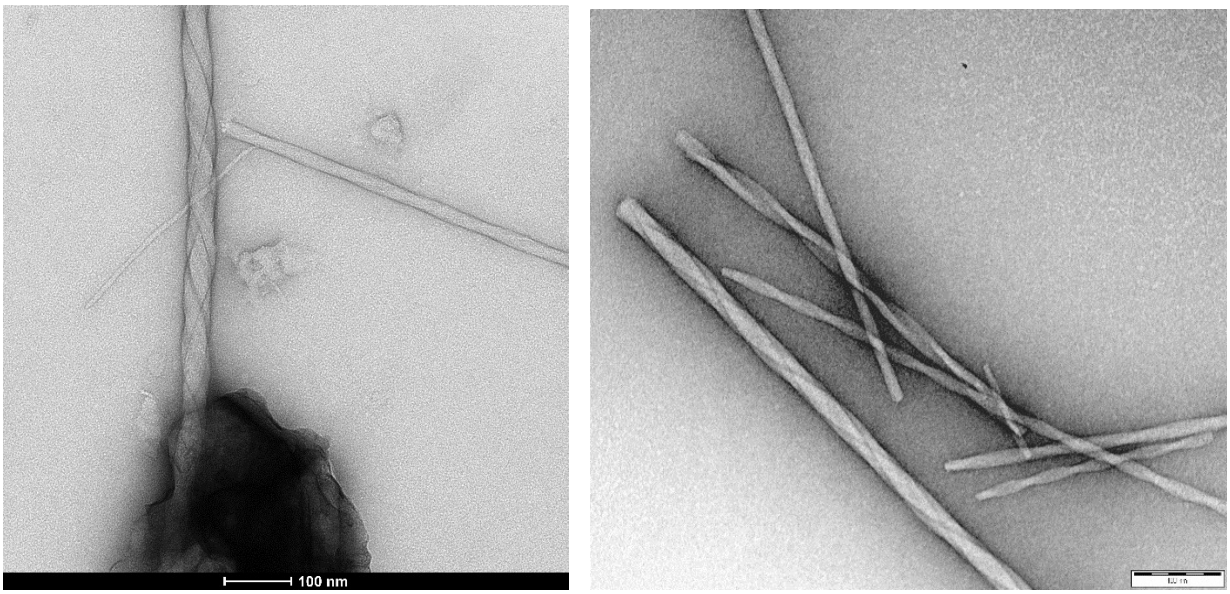


Figure S4. TEM images, scale bar 100 nm (left: L-DBS-$CONHNH_2$, right: D-DBS-$CONHNH_2$).

Gelation Screening and Thermal Stability of Mixtures of Enantiomers

Table S3. Gelation screen for mixtures of D-DBS-$CONHNH_2$ and L-DBS-$CONHNH_2$. The percentage of each gelator shown is the proportion of the total gelator, at a loading of 0.28% wt/vol. G=Gel.

D-DBS-$CONHNH_2$ / %	**L-DBS-$CONHNH_2$ / %**	**Gel?**
90	**10**	G
80	**20**	G
70	**30**	G
60	**40**	G
50	**50**	G
40	**60**	G
30	**70**	G
20	**80**	G
10	**90**	G

Table S4. T_{gel} values for hydrogels based on mixtures of D-DBS-$CONHNH_2$ and L-DBS-$CONHNH_2$. Total gelator loading 0.28% wt/vol.

L-DBS-$CONHNH_2$ / %	**T_{gel} / °C**
0	99
10	50
20	35
30	43
40	31
50	47
60	52
70	38
80	34
90	48
100	100+

Rheology for Mixtures of Enantiomers

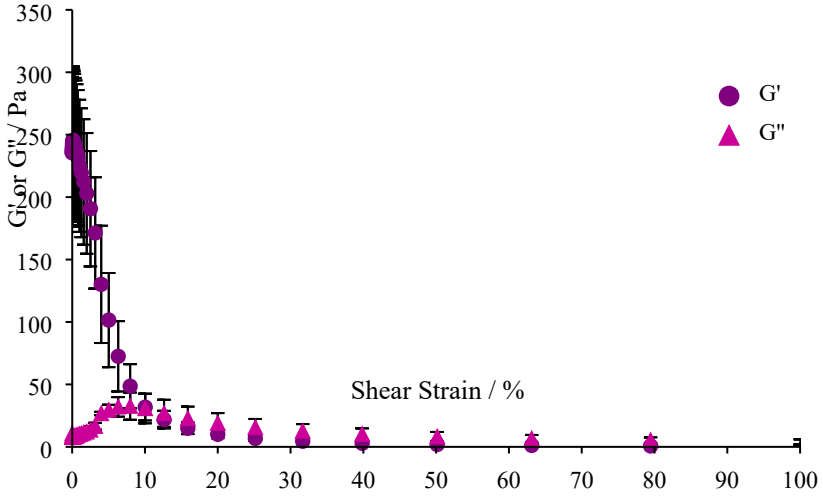


Figure S5. Amplitude sweep for racemic DBS-CONHNH$_2$ hydrogels. Total gelator loading 0.28% wt/vol.

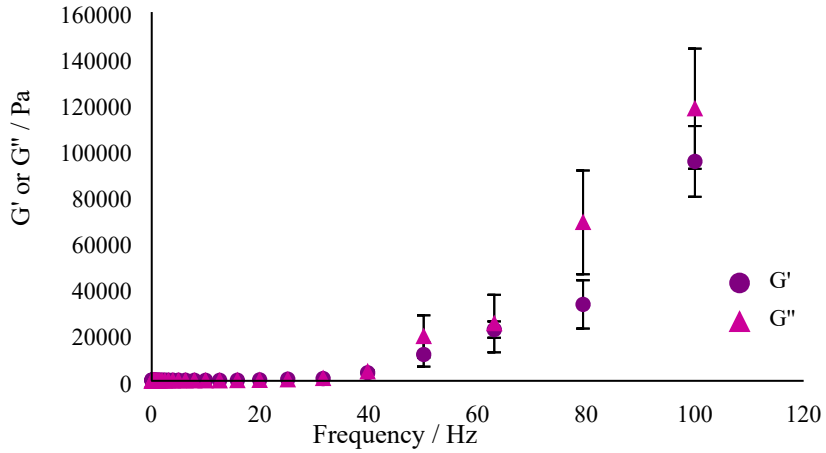


Figure S6. Frequency sweep for racemic DBS-CONHNH$_2$ hydrogels. Total gelator loading 0.28% wt/vol.

Table S5. G' values for hydrogels based on mixtures of D-DBS-CONHNH$_2$ and L-DBS-CONHNH$_2$ (total concentration 0.28% wt/vol).

Ratio D-DBS-CONHNH$_2$:L-DBS-CONHNH$_2$	**G' / Pa**
100:0	610 ± 280
75:25	420 ± 70
50:50	240 ± 60
25:75	240 ± 50
0:100	740 ± 300

NMR Study of Mixtures of Enantiomers

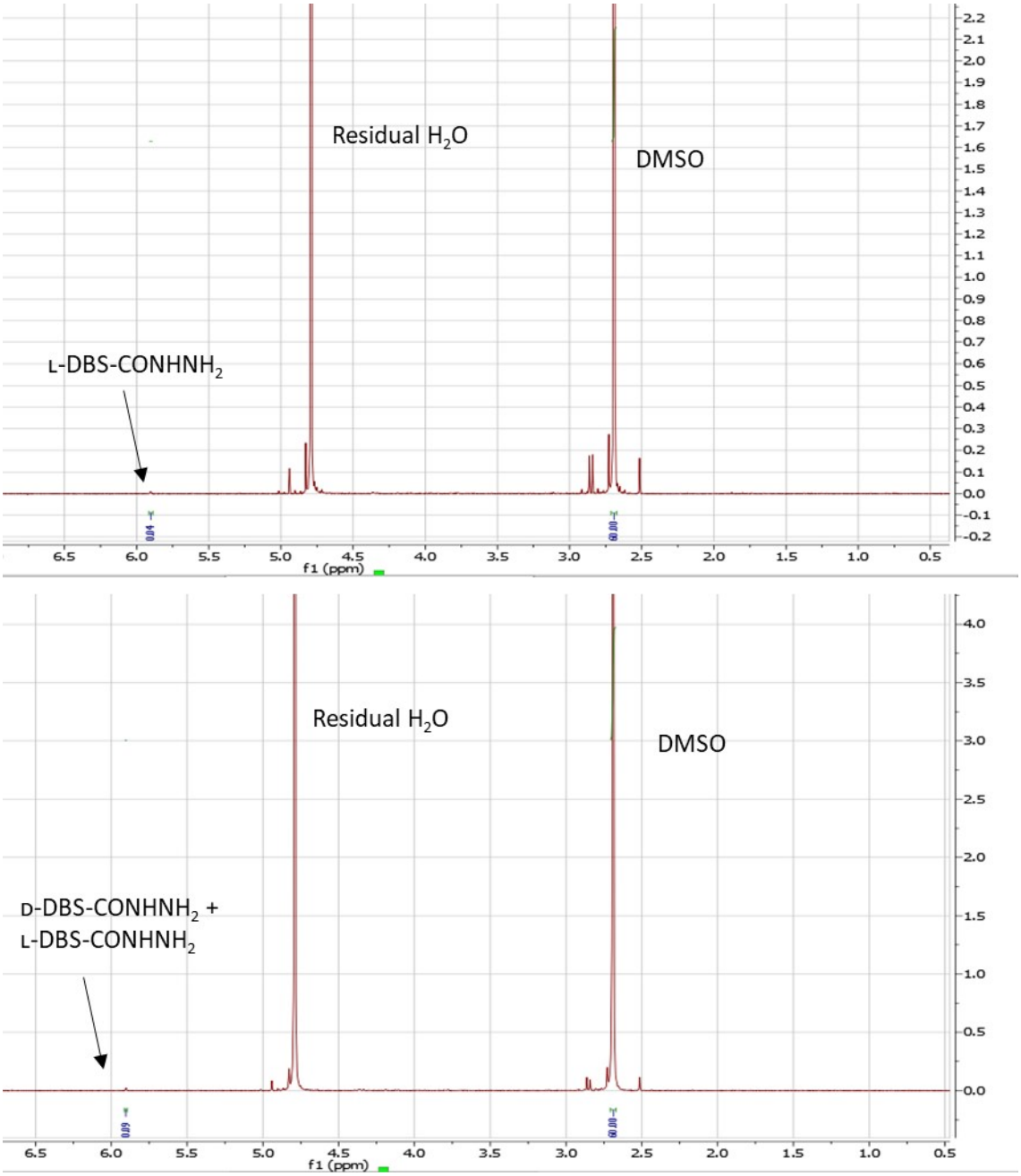


Figure S7. ^{1}H NMR spectra for L-DBS-CONHNH$_2$ (top) and D-DBS-CONHNH$_2$ and L-DBS-CONHNH$_2$ (bottom) hydrogels. Total loading for both hydrogels is 0.24% wt/vol.

Scanning and Transmission Electron Microscopy (SEM and TEM) on Mixtures of Enantiomers

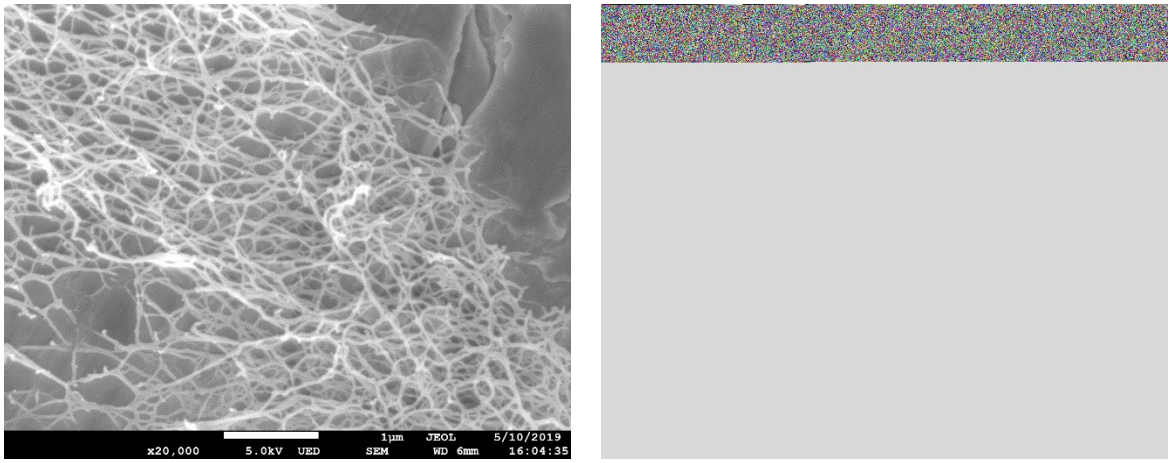


Figure S8. SEM images (scale bar = 1 μm) for racemic DBS-$CONHNH_2$ hydrogel (left) and L-DBS-$CONHNH_2$ hydrogel (right). Total gelator loading for both samples: 0.28% wt/vol.

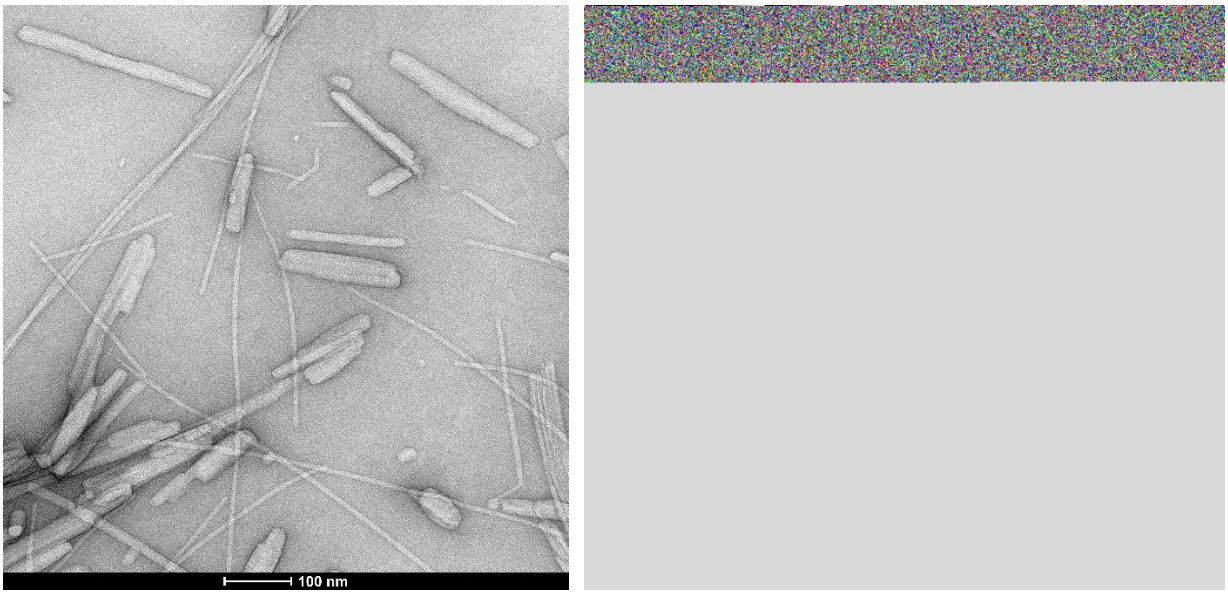


Figure S9. TEM images (scale bar = 100 nm) for racemic DBS-$CONHNH_2$ hydrogel (left) and L-DBS-$CONHNH_2$ hydrogel (right). Total gelator loading for both samples: 0.28% wt/vol.

UV-Vis Spectroscopy of DBS-$CONHNH_2$

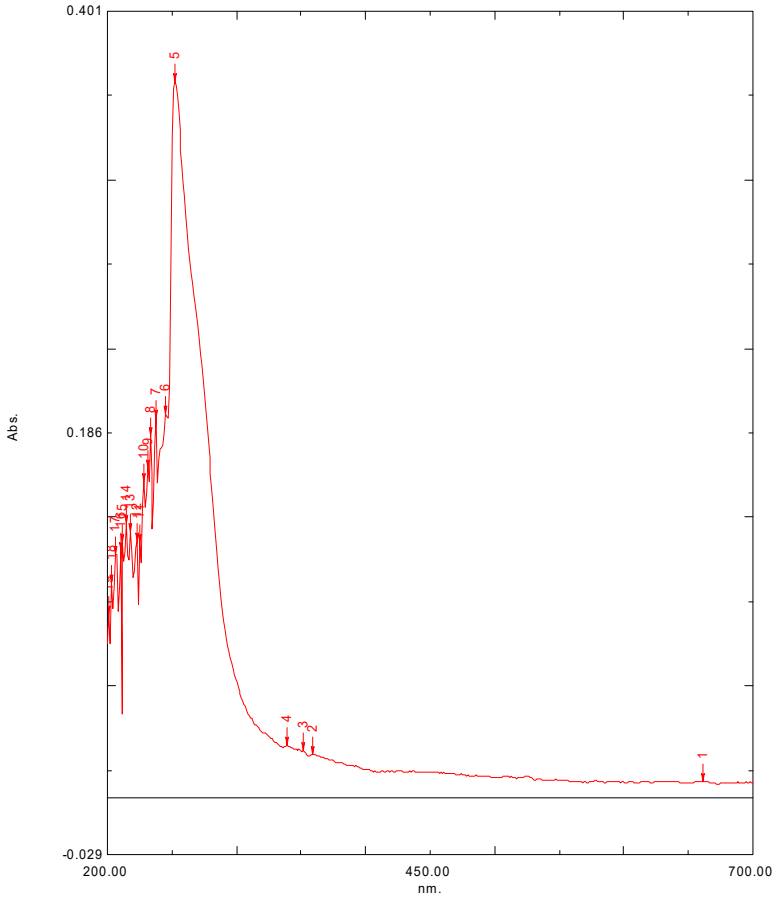


Figure S10. UV-Vis spectrum of D-DBS-$CONHNH_2$ in water at 0.01% wt/vol (0.21 mM), λ_{max} = 252 nm.

IR Spectroscopy of DBS-CONHNH$_2$ and Mixtures of Enantiomers

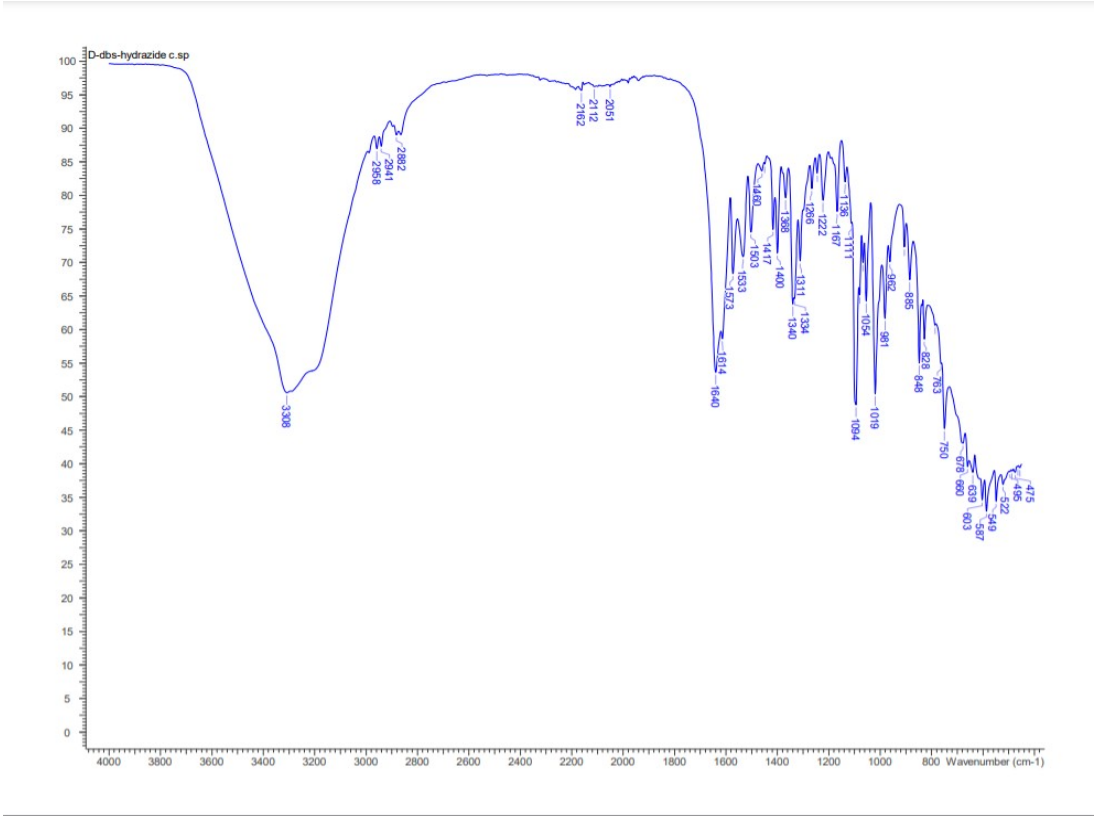


Figure S11. IR spectrum of solvated gel of D-DBS-CONHNH$_2$.

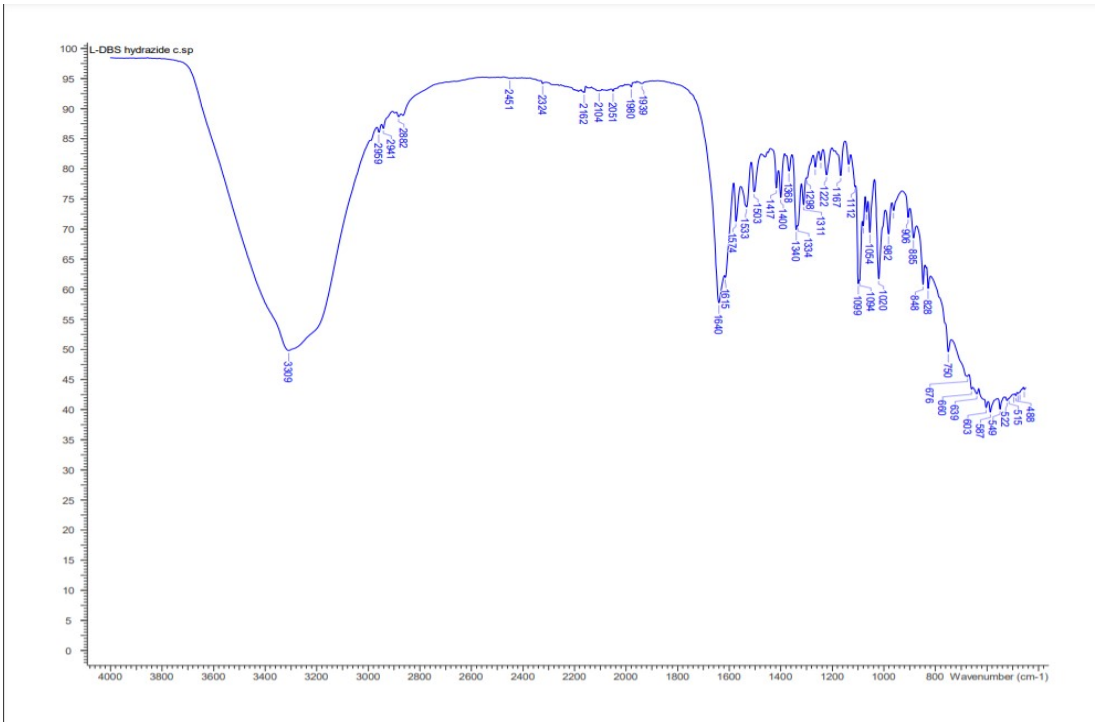


Figure S12. IR spectrum of solvated gel of L-DBS-CONHNH$_2$.

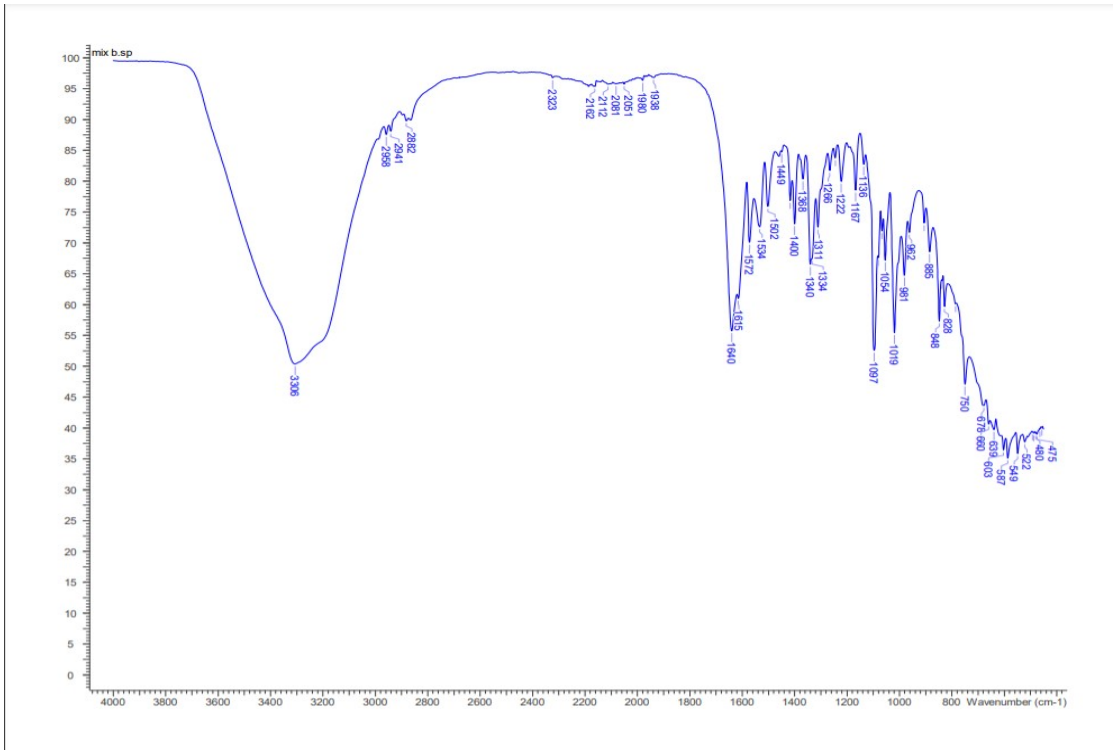


Figure S13. IR spectrum of solvated gel of 50:50 enantiomeric mixture of D-DBS-CONHNH$_2$ and L-DBS-CONHNH$_2$.

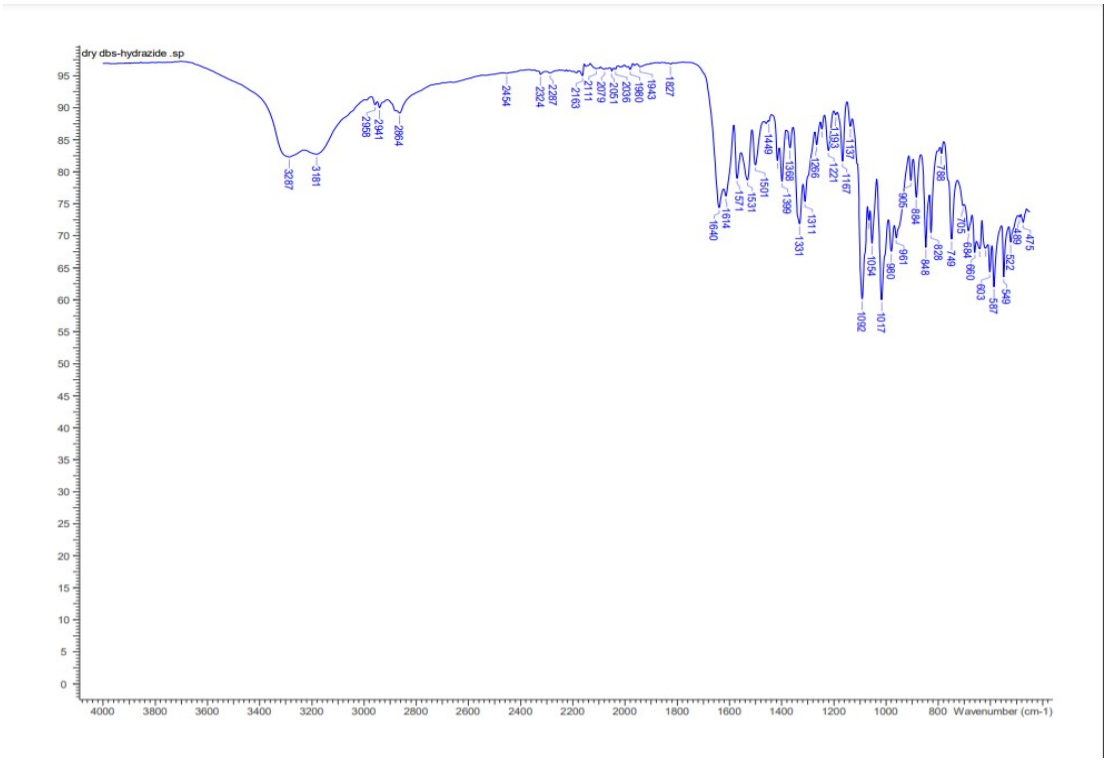


Figure S14. IR spectrum of xerogel of D-DBS-CONHNH$_2$.

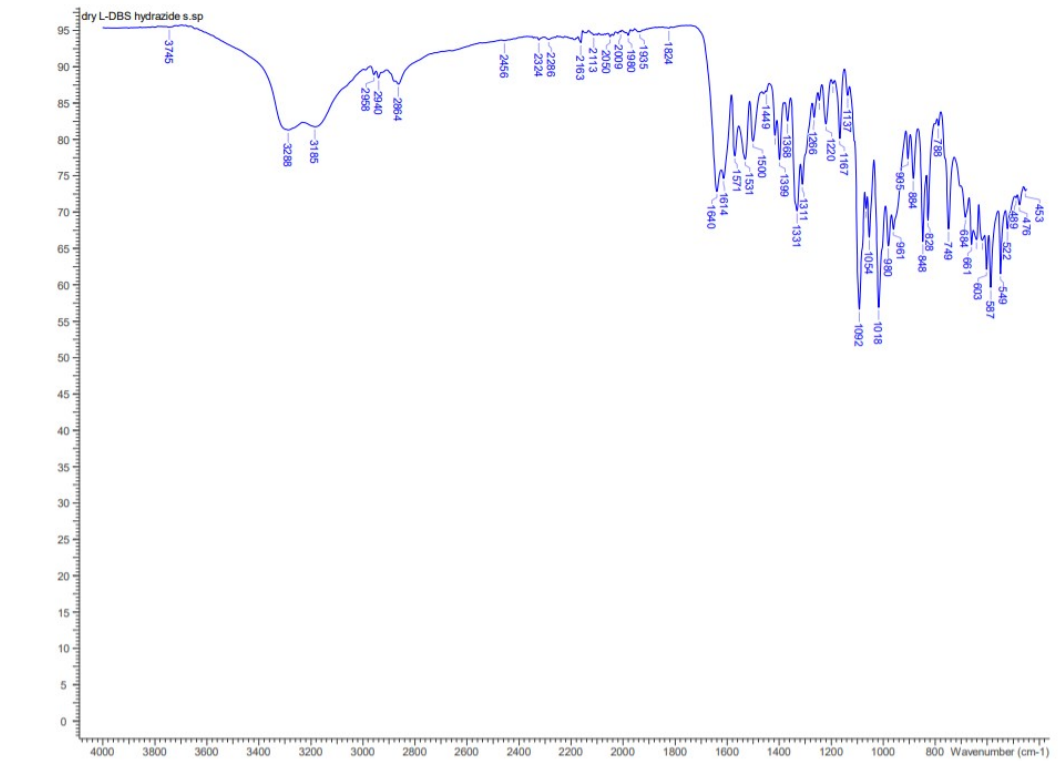


Figure S15. IR spectrum of solvated gel of L-DBS-CONHNH$_2$.

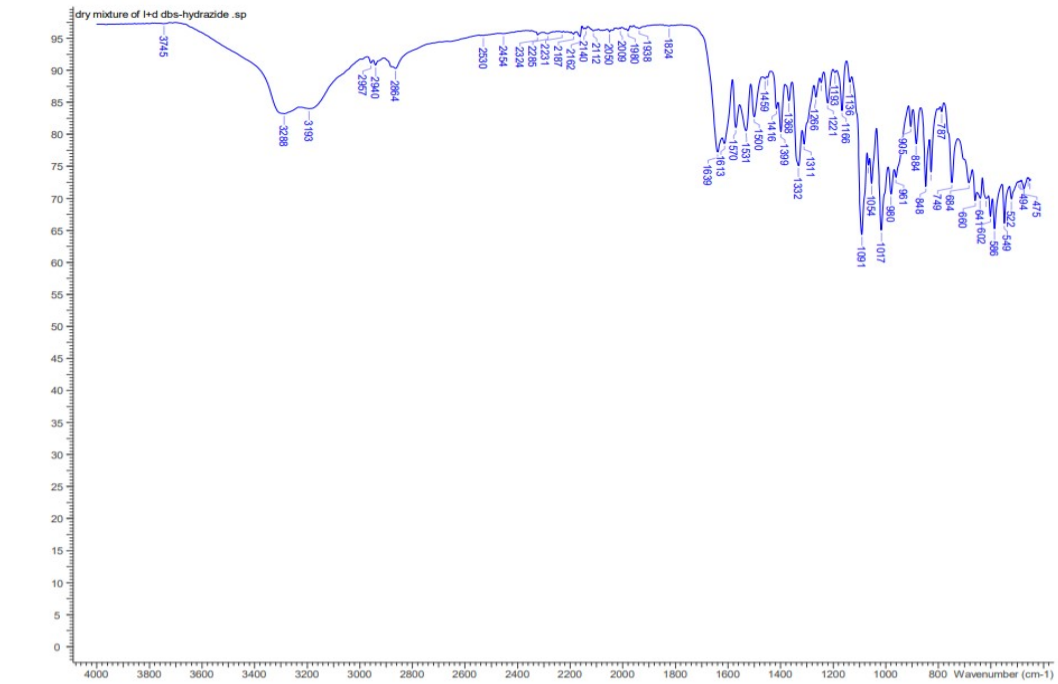


Figure S16. IR spectrum of xerogel of 50:50 enantiomeric mixture of D-DBS-CONHNH$_2$ and L-DBS-CONHNH$_2$.

Gelation of Enantiomeric Gels in the Presence of Enantiomers of Naproxen

Table S6. Gelation testing for different combinations of DBS-$CONHNH_2$ and naproxen enantiomers. Gelator loading is 0.28% wt/vol, with one equivalent of NPX.

	(*S*)-naproxen	**(*R*)-naproxen**
D-DBS-$CONHNH_2$	G	G
L-DBS-$CONHNH_2$	G	G

Table S7. T_{gel} values for D-DBS-$CONHNH_2$ and L-DBS-$CONHNH_2$ hydrogels, with (*S*)-NPX or (*R*)-NPX. Hydrogels have gelator loading of 0.28% wt/vol, with 1 equivalent of NPX.

Gelator	**(*S*)-naproxen**	**(*R*)-naproxen**	**None**
D-DBS-$CONHNH_2$	98	100+	100+
L-DBS-$CONHNH_2$	100+	81	100+

Rheology of Enantiomeric Gels in the Presence of Enantiomers of Naproxen

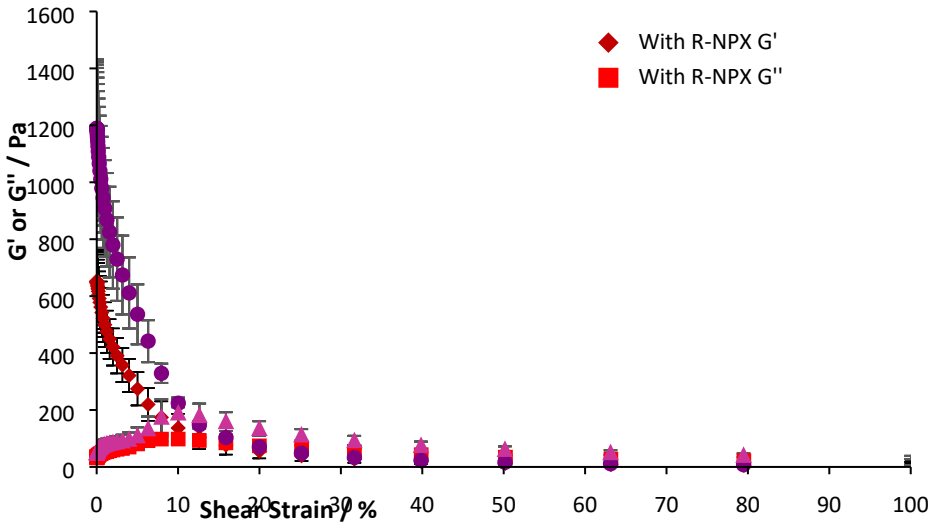


Figure S17. Amplitude sweep for D-DBS-$CONHNH_2$ hydrogels with (*R*)- or (*S*)-NPX. Gelator loading 0.28% wt/vol, one equivalent NPX.

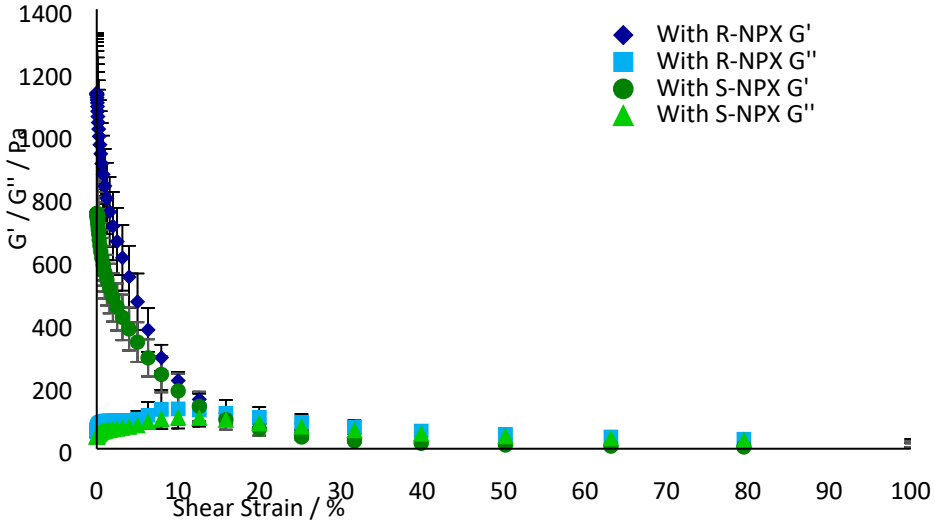


Figure S18. Amplitude sweep for L-DBS-$CONHNH_2$ hydrogels with (*R*)- or (*S*)-NPX. Gelator loading 0.28% wt/vol, one equivalent NPX.

Table S8. G' values of hydrogel gels formed on the addition of NPX enantiomers to DBS-$CONHNH_2$. Gelator concentration 0.28% wt/vol, with one equivalent of NPX.

Gel	Additive	G' / Pa
D-DBS-$CONHNH_2$	None	740 ± 300
D-DBS-$CONHNH_2$	(R)-NPX	630 ± 110
D-DBS-$CONHNH_2$	(S)-NPX	1190 ± 240
L-DBS-$CONHNH_2$	None	610 ± 280
L-DBS-$CONHNH_2$	(R)-NPX	1130 ± 190
L-DBS-$CONHNH_2$	(S)-NPX	750 ± 120

NMR Studies of Enantiomeric Gels in the Presence of Enantiomers of Naproxen

Table S9. The percentage of (*R*)-NPX and (*S*)-NPX not bound to the gel network, as determined by ^{1}H NMR in the presence of a DMSO internal standard. Gelator loading 0.28% wt/vol, one equivalent of NPX.

Gelator	(*S*)-NPX Unbound / %	(*R*)-NPX Unbound / %
D-DBS-$CONHNH_2$	32 ± 0.4	26 ± 1.5
L-DBS-$CONHNH_2$	27 ± 0.18	28 ± 0.6

References

1. B. O. Okesola and D. K. Smith, *Chem. Commun.*, 2013, **49**, 11164-11166