

Supporting Information

A versatile supramolecular organogel with outstanding stability towards aqueous interfaces

Evelyn L. Bonifazi,[†] Valeria C. Edelsztejn,[†] Guillermo O. Menéndez,[§] Cecilia Samaniego López,[§] Carla C. Spagnuolo^{,§} and Pablo H. Di Chenna^{*,†}*

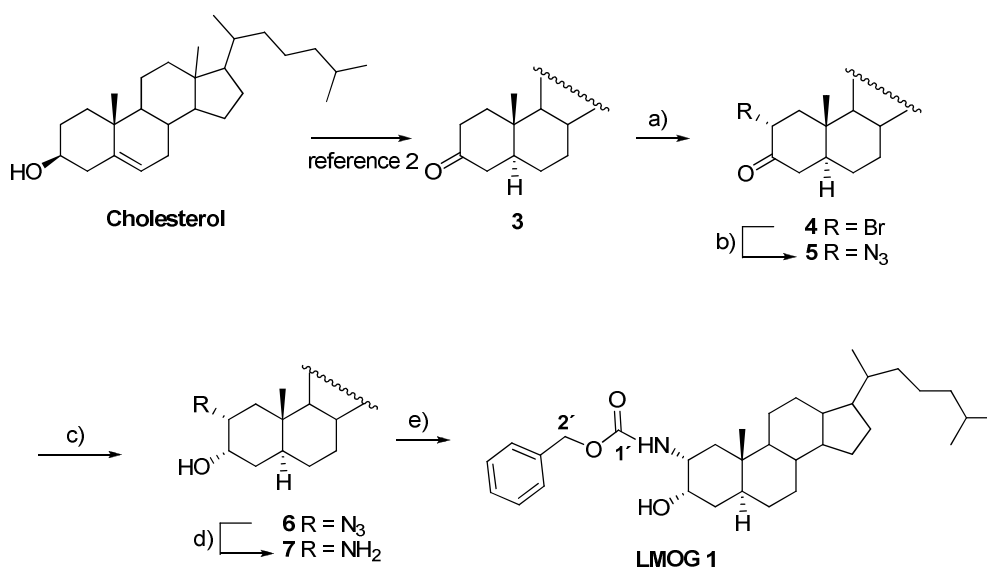
[§] CIHIDECAR-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Dto. Química Orgánica, 3^{er} piso, pabellón II, Cdad. Universitaria, C1428EGA, Buenos Aires, Argentina. *E-mail: carlacs@qo.fcen.uba.ar, Tel/Fax +54 11 45763346

[†] UMYMFOR-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Dto. Química Orgánica, 3^{er} piso, pabellón II, Cdad. Universitaria, C1428EGA, Buenos Aires, Argentina. *E-mail: dichenna@qo.fcen.uba.ar, Tel/Fax +54 11 45763346

Experimental procedures:

Materials: Cholesterol (94%) was purchased from Sigma-Aldrich. All solvents were dried and purified by standard methods prior to use. Cyanine **2** was synthesized according to the literature procedure previously reported by us.¹ The structure and stereochemistry of all compounds were completely characterized by 1D and 2D NMR spectroscopy (HSQC, HMBC, COSY and NOESY). ¹H and ¹³C NMR spectra were recorded on a Bruker Avance II 500 at 500.13 and 125.77MHz in deuteriochloroform. Chemical shifts are given in ppm downfield from TMS as internal standard, *J* values are given in Hz. Multiplicity determinations and 2D spectra were obtained using standard Bruker software. High resolution mass spectra (HRMS) were measured on an Agilent LCTOF, high resolution TOF analyzer with APCI/ESI ionization. Flash Column chromatography was carried out on Kieselgel S 0.040– 0.063 mm (Merck). TLC analysis was performed on Merck aluminium Sheets silica gel 60 F254 (0.2 mm thick). The homogeneity of all compounds was confirmed by thin layer chromatography. Solvents were evaporated at reduced pressure and *ca.* 40–50 °C. IR spectra of solid, gels and liquid samples were recorded on a Nicolet Magna IR 550 FTIR spectrometer using KBr disks.

Experimental procedure for the synthesis of **LMOG 1**: Steroid **3** was prepared from commercial cholesterol following a procedure already described in the literature.²



a) Br₂/HAcO, HBr/HAcO, 0°C; b) NaN₃, NMP, AcOH, r.t.; c) LS-Selectide, THF; d) LiAlH₄, THF; e) PhCH₂OCOCI, Et₃N, THF

Scheme S1: Synthesis of **LMOG 1**

2 α -Bromo-cholestane-3-one (4)

To a solution of compound **3** (1.0 g) in acetic acid (400 ml) and hydrobromic acid (40 drops) at 0°C was added dropwise a solution of bromine (140 μ l) in acetic acid (12.5 ml). Right after addition of bromine the reaction mixture was poured into ice and the resulting precipitate was isolated by filtration and washed with water. The solid was dissolved in dichloromethane and washed with NaHCO₃ (ss) and water, dried with Na₂SO₄ and the solvent evaporated at reduced pressure. Purification of the residue by column chromatography on silica gel (hexane/AcOEt) gave compound **4** (77% yield). ¹H NMR (CDCl₃, 500.13 MHz) δ (ppm): 0.67 (3H, s, CH₃-18), 0.86 (3H, d, J = 6.6 Hz, CH₃-26 or 27), 0.87 (3H, d, J = 6.6 Hz, CH₃-26 or 27), 0.90 (3H, d, J = 6.6 Hz, CH₃-21), 1.08 (3H, s, CH₃-19), 4.75 (1H, dd, J = 6.3, 13.3 Hz, H-2). ¹³C NMR (CDCl₃, 125.77 MHz) δ (ppm): 12.1 (CH₃-18 and 19), 18.6 (CH₃-21), 21.5 (C-11), 22.5 (CH₃-26 or 27), 22.8 (CH₃-26 or 27), 23.8 (C-23), 24.2 (C-15), 28.0 (C-25), 28.2 (C-16), 28.4 (C-6), 31.5 (C-7), 34.9 (C-8), 35.7 (C-20), 36.1 (C-22), 39.0 (C-10), 39.5 (C-24), 39.7 (C-12), 42.6 (C-13), 43.9 (C-4), 47.5 (C-5), 51.7 (C-1), 53.6 (C-9), 54.6 (C-2), 56.1 (C-14 or 17), 56.2 (C-14 or 17), 201.2 (C-3). HRMS-ESI *m/z* [M+Na]⁺ calcd for C₂₇H₄₅BrNaO: 487.25460, found 487.25637.

2 α -Azido-cholestane-3-one (5)

Was prepared from 2 α -bromo-cholestane-3-one (**4**) following the procedure described in the literature³ (66% yield). FT-IR: ν (cm⁻¹) 2931.4; 2107.0, 1718.4. ¹H NMR (CDCl₃, 500.13 MHz) δ (ppm): 0.60 (3H, s, CH₃-18), 0.79 (3H, d, J = 7.0 Hz, CH₃-26 or 27), 0.80 (3H, d, J = 7.0 Hz, CH₃-26 or 27), 0.83 (3H, d, J = 6.5 Hz, CH₃-21), 1.01 (3H, s, CH₃-19), 3.91 (1H, dd, J = 6.4, 13.1 Hz, H-2). ¹³C NMR (CDCl₃, 125.77 MHz) δ (ppm): 12.0 (CH₃-18), 12.4 (CH₃-19), 18.6 (CH₃-21), 21.5 (C-11), 22.5 (CH₃-26 or 27), 22.8 (CH₃-26 or 27), 23.8 (C-23), 24.1 (C-15), 27.9 (C-25), 28.1 (C-16), 28.4 (C-6), 31.5 (C-7), 34.8 (C-8), 35.7 (C-20), 36.1 (C-22), 36.8 (C-10), 39.4 (C-24), 39.6 (C-12), 42.5 (C-13), 43.7 (C-4), 45.4 (C-1), 47.6 (C-5), 53.6 (C-9), 56.0 (C-14 or 17), 56.1 (C-14 or 17), 63.9 (C-2), 205.1 (C-3).

2 α -Azido-cholestane-3 α -ol (6)

Was prepared from 2 α -azido-cholestane-3-one (**5**) following the procedure described in the literature⁴ (64% yield). ¹H NMR (CDCl₃, 500.13 MHz) δ (ppm): 0.65 (3H, s, CH₃-18), 0.82 (3H, s, CH₃-19), 0.86 (3H, d, J = 6.6 Hz, CH₃-26 or 27), 0.87 (3H, d, J = 6.6 Hz, CH₃-26 or 27), 0.90 (3H, d, J = 6.6 Hz, CH₃-21), 3.54 (1H, ddd, J = 3.0, 4.5, 12.5

Hz, H-2), 3.97 (1H, d, $J = 2.5$ Hz, H-3). ^{13}C NMR (CDCl_3 , 125.77 MHz) δ (ppm): 12.1 (CH_3 -18), 12.2 (CH_3 -19), 18.7 (CH_3 -21), 20.9 (C-11), 22.6 (CH_3 -26 or 27), 22.8 (CH_3 -26 or 27), 23.8 (C-23), 24.2 (C-15), 27.7 (C-6), 28.0 (C-25), 28.2 (C-16), 31.7 (C-7), 34.1 (C-4), 35.0 (C-20), 35.8 (C-8), 36.2 (C-22), 36.8 (C-1 and C-10), 38.0 (C-5), 39.5 (C-24), 39.8 (C-12), 42.6 (C-13), 54.1 (C-9), 56.2 (C-17), 56.3 (C-14), 61.0 (C-2), 68.0 (C-3). HRMS-ESI m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{27}\text{H}_{47}\text{N}_3\text{NaO}$: 452.36113, found 452.36153.

2 α -Amino-cholestane-3 α -ol (**7**)

Was prepared from 2 α -azido-cholestane-3 α -ol (**6**) following the procedure described in the literature⁵ (90% yield). ^1H NMR (CDCl_3 , 500.13 MHz) δ (ppm): 0.64 (3H, s, CH_3 -18), 0.80 (3H, s, CH_3 -19), 0.86 (3H, d, $J = 6.6$ Hz, CH_3 -26), 0.86 (3H, d, $J = 6.6$ Hz, CH_3 -27), 0.90 (3H, d, $J = 6.5$ Hz, CH_3 -21), 2.99 (1H, dt, $J = 3.8, 12.1$ Hz, H-2), 3.71 (1H, d, $J = 2.3$ Hz, H-3). ^{13}C NMR (CDCl_3 , 125.77 MHz) δ (ppm): 12.1 (CH_3 -18), 12.5 (CH_3 -19), 18.7 (CH_3 -21), 20.9 (C-11), 22.6 (CH_3 -26 or 27), 22.8 (CH_3 -26 or 27), 23.8 (C-23), 24.2 (C-15), 27.9 (C-6), 28.0 (C-25), 28.2 (C-16), 31.9 (C-7), 34.1 (C-4), 35.1 (C-20), 35.8 (C-8), 36.2 (C-22), 36.5 (C-10), 38.1 (C-5), 39.5 (C-24), 40.0 (C-12), 41.2 (C-1), 42.6 (C-13), 48.7 (C-2), 54.2 (C-9), 56.2 (C-17), 56.4 (C-14), 68.9 (C-3). HRMS-ESI m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{50}\text{NO}$: 404.38869, found 404.38959.

LMOG **1**

To a solution of compound **7** (300 mg) and dry Et_3N (119 ml) in anhydrous THF (8.8 ml), at 0°C and under an argon atmosphere was added dropwise benzyl chloroformate (129 μl). After 1 h water was added and the reaction mixture was extracted with CH_2Cl_2 . The organic layer was dried with Na_2SO_4 and evaporated. Purification of the residue by column chromatography on silica gel (hexane/ AcOEt , gradient) rendered pure LMOG **1** (50% yield). FT-IR: ν (cm^{-1}) 3482.0; 3339.5; 2924.7; 1665.3. ^1H NMR (CDCl_3 , 500.13 MHz) δ (ppm): 0.64 (3H, s, CH_3 -18), 0.85 (CH_3 -19), 0.86 (3H, d, $J = 6.7$ Hz, CH_3 -26 or 27), 0.87 (3H, d, $J = 6.7$ Hz, CH_3 -26 or 27), 0.90 (3H, d, $J = 6.6$ Hz, CH_3 -21), 3.75 (1H, m, H-2), 3.99 (1H, m, H-3), 5.08 (2H, s, H-2'), 5.12 (1H, d, $J = 8.3$ Hz, NH), 7.30-7.33 (5H, H-Ar). ^{13}C NMR (CDCl_3 , 125.77 MHz) δ (ppm): 12.0 (CH_3 -18 or 19), 12.1 (CH_3 -18 or 19), 18.7 (CH_3 -21), 20.9 (C-11), 22.5 (CH_3 -26 or 27), 22.8 (CH_3 -26 or 27), 23.8 (C-23), 24.2 (C-15), 27.8 (C-6), 28.0 (C-25), 28.2 (C-16), 31.9 (C-7), 35.0 (C-20), 35.5 (C-4), 35.8 (C-8), 36.2 (C-22), 36.6 (C-10), 38.3 (C-5), 39.1 (C-1), 39.5 (C-24), 39.9 (C-12), 42.6 (C-13), 49.8 (C-2), 54.2 (C-9), 56.2 (C-17),

56.4 (C-14), 66.6 (C-2'), 68.3 (C-3), 128.1-128.5 (C-Ar), 136.6 (C-3'), 155.8 (C-1'). HRMS-ESI m/z $[M+Na]^+$ calcd for $C_{35}H_{55}NNaO_3$: 560.40742, found 560.40577.

Gelation Studies

The gelation ability of LMOG **1** was investigated by the simple inversion tube method using a set of 35 solvents (table S1). A mixture of a defined amount of gelator (5 mg) and a volume of the solvent (10% wt/v) in a closed flask was heated and shaken until the solid was dissolved and then slowly cooled to room temperature. If a stable gel was observed after inversion of the flask it was considered a gel (G). When gelation was not observed at room temperature, the sample was cooled at 4 °C. The critical concentration for gelation (CCG) was determined from the minimum amount of gelator required for the formation of a gel at room temperature.

T_g and CCG determination

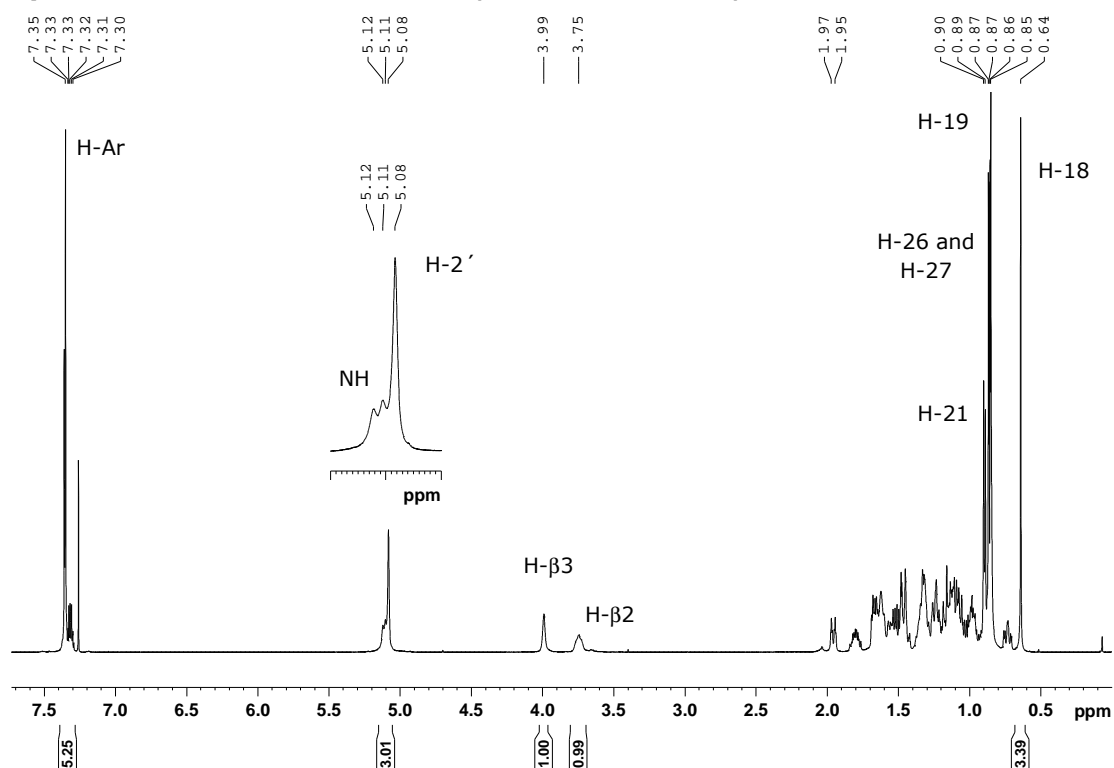
Gel to sol phase transition temperatures (T_g) were determined by the "inverse flow method". A sealed vial containing the gel with different concentration of **1** was immersed upside-down in a thermostated water bath. The temperature of the bath was raised at a rate of 1°C/min. T_g was defined as the temperature at which the gel started to flow.

FTIR measurements

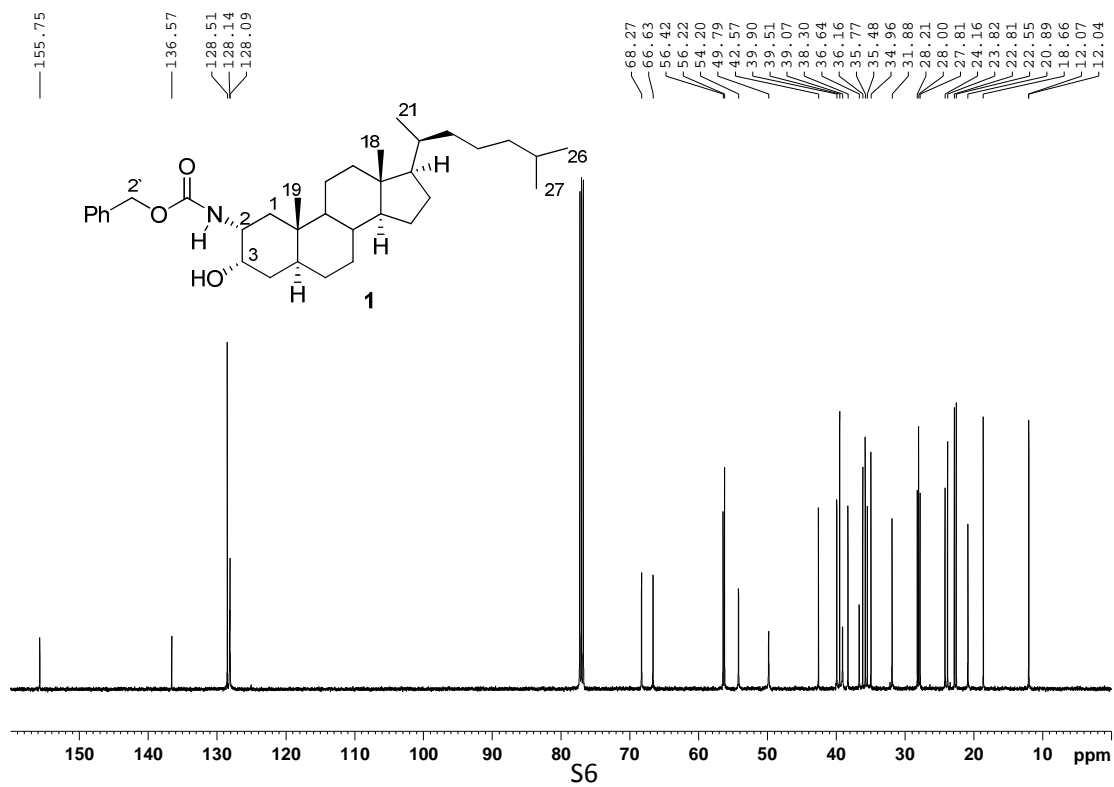
Fourier transform infrared (FTIR) spectra of the solution and the gels of **1** were performed on a Nicolet Magna IR 550 FTIR spectrometer in a demountable liquid cell with two NaBr disks, 32 mm in diameter and a 0.5 mm thick Teflon spacer. For the xylene gel, a warm solution of **1** (4 wt %) was injected into the cell and allowed to cool down to room temperature. Three Spectra were recorded (a) solution at t=0, (b) during sol to gel transition (t=25min) and (b) gel state (19 hours later).

Xerogel preparation: Xerogels of **1** from methanol, hexane, isopropyl ether and acetonitrile were prepared by slowly evaporation of the solvent of the gels in a bath at -60 °C for 1 hour, and then slowly letting the gels get to room temperature under vacuum.

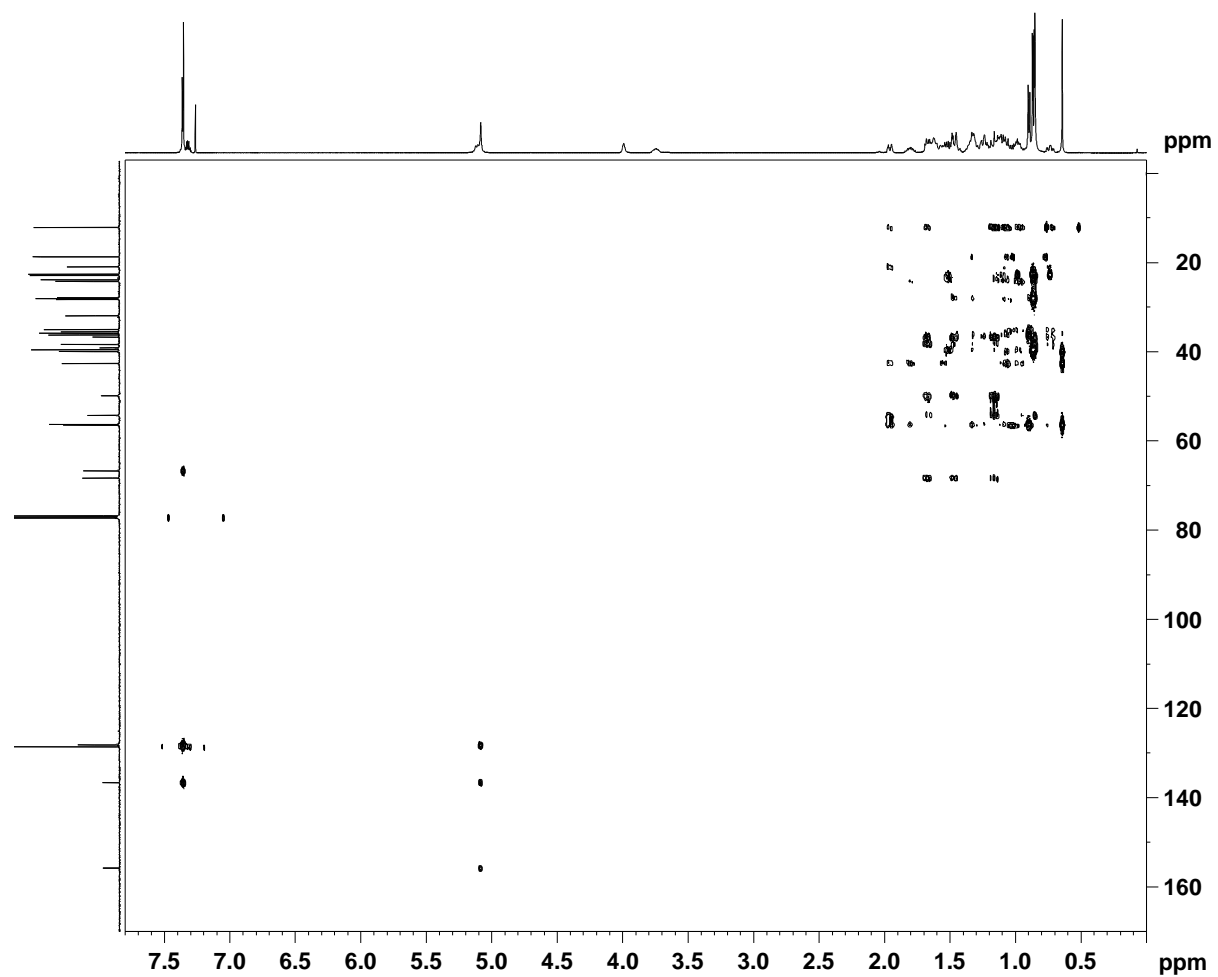
Spectrum S1: ^1H NMR of LMOG **1** (CDCl_3 , 500.13 MHz)



Spectrum S2: ^{13}C NMR of LMOG **1** (CDCl_3 , 125.77 MHz)



Spectrum S3: COSY of LMOG **1** (CDCl₃, 500.13 MHz)



NOESY: stereochemistry of positions 2 and 3 was corroborated by NOESY experiment, NOE correlation between H-19 and H-2 determined the alpha orientation of the carbamate. The α stereochemistry of C-3 was inferred from NOE between H-2 and H-3 at 3.99 ppm. The correlations are marked on spectrum S4. This is in agreement with the stereo controlled synthetic route used.

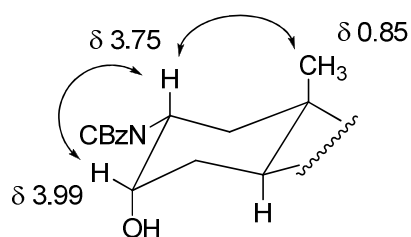


Figure S1: main NOE correlations observed in ring A of LMOG **1**

Spectrum S4: NOESY of LMOG **1** (CDCl₃, 500.13 MHz)

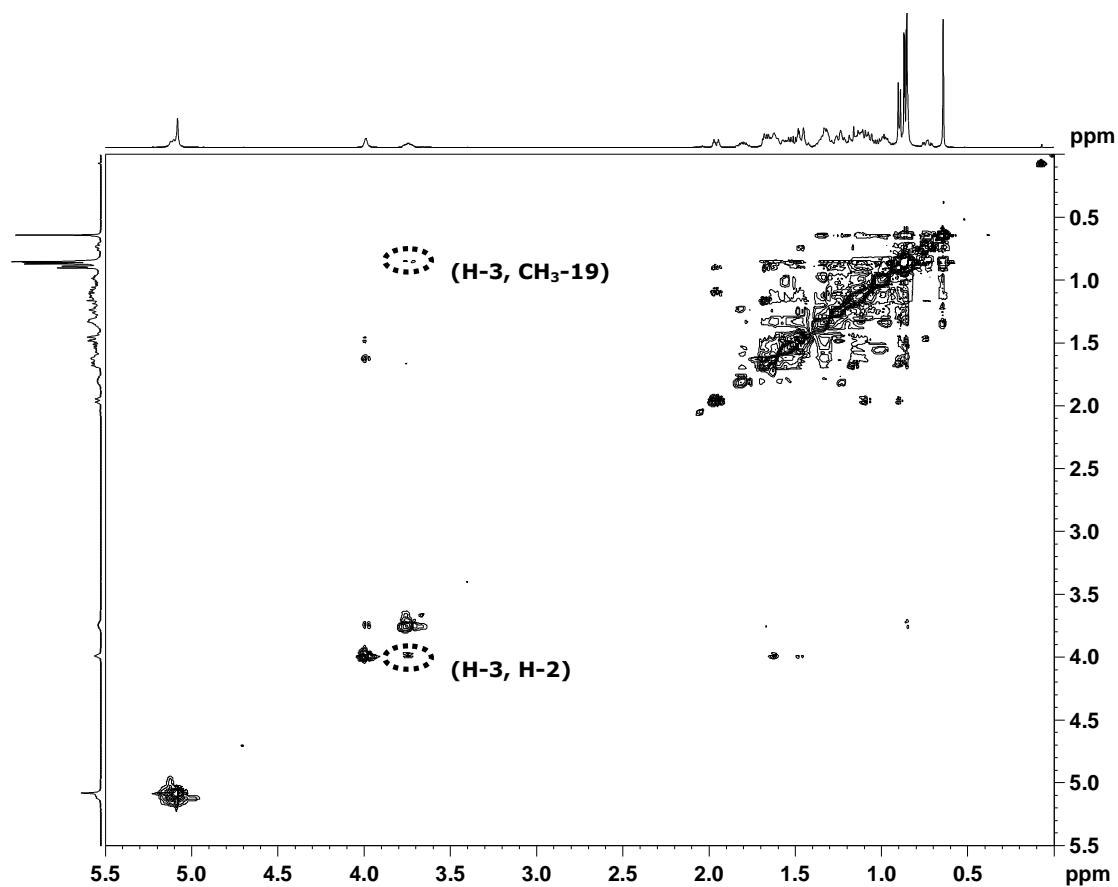
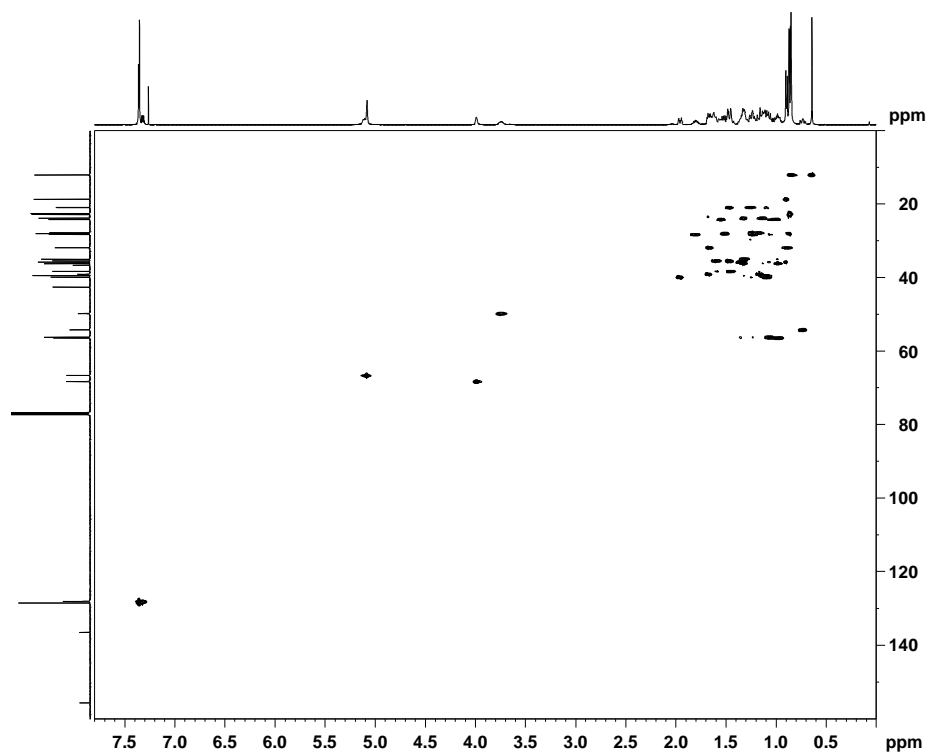


Table S1: assignment of ^{13}C and ^1H NMR for organogelator **1**

Position	^{13}C δ (ppm)	^1H δ (ppm)
1	39.1	1.16 and 1.67
2	49.8	3.75 (m)
3	68.3	3.99 (m)
4	35.5	1.47 and 1.60
5	38.3	1.45
6	27.8	1.16 and 1.24
7	31.9	0.89 and 1.66
8	35.8	1.35
9	54.2	0.73
10	36.6	-
11	20.9	1.25 and 1.47
12	39.9	1.08 and 1.96
13	42.6	-
14	56.4	0.97
15	24.2	1.02 and 1.55
16	28.2	1.23 and 1.80
17	56.2	1.08
18	12.0*	0.64 (s)
19	12.1*	0.85
20	35.0	1.32
21	18.7	0.90 (d, J = 6.6 Hz)
22	36.2	0.98 and 1.32
23	23.8	1.13 and 1.32
24	39.5	1.11
25	28.0	1.51
26	22.8 [†]	0.86 or 0.87 (d, J = 6.7 Hz)
27	22.5 [†]	0.87 or 0.87 (d, J = 6.7 Hz)
1'	155.8	-
2'	66.6	5.08 (s)
3'	136.6	-
Ar	128.1-128.5	7.30-7.33
		5.12 (d, J = 8.3 Hz, NH)

* and [†] are Interchangeable assignments

Spectrum S5: HSQC (CDCl₃, 500.13 MHz)



Spectrum S6: HMBC (CDCl₃, 500.13 MHz)

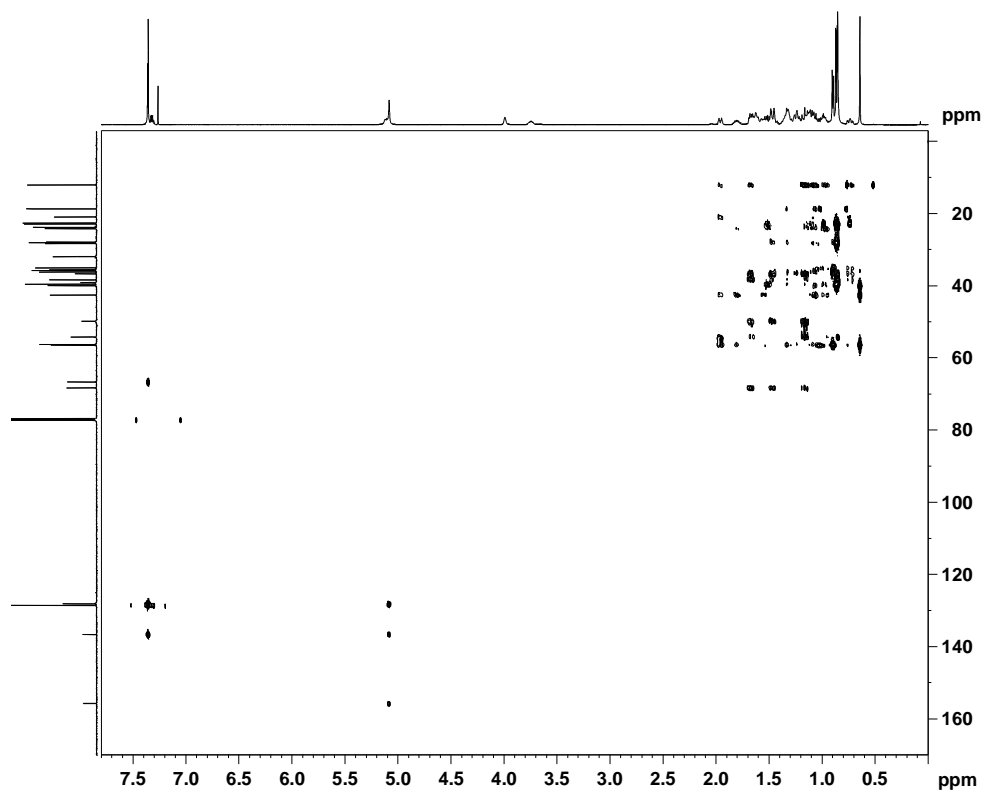


Table S2: Gelation test for **LMOG 1** in 35 common solvents

Entry	Solvent	State ^a	CCG ^b (% w/v)
1	1,2-dichlorobenzene	S	--
2	1,2-dichloroethane	G	5.0
3	1,4-dioxane	S	--
4	1-hexanol	S	--
5	Acetic acid	S	--
6	Acetone	G	2.0 ^c
7	Acetonitrile	G	0.2
8	Aniline	S	--
9	Benzyl alcohol	S	--
10	CCl ₄	I	--
11	Chloroform	S	--
12	Cyclohexane	G	0.2
13	Decane	G	0.06
14	Dichloromethane	S	--
15	DMF	G	2.5
16	DMSO	G	1.7
17	Etanol	G	2.0
18	Ethyl acetate	G	4.3 / 3.0 ^c
19	Glycerol	I	--
20	Isopropyl ether ^d	G	0.6
21	Methanol	G	0.9
22	Methyl acrylate	G	5.1
23	<i>n</i> -Butanol	G	4.2
24	<i>n</i> -Heptane	G	0.12
25	<i>n</i> -Hexane	G	0.04
26	<i>n</i> -Octanol	S	--
27	Nitrobenzene	S	--
28	Pyridine	I	--
29	Styrene	G	10
30	TEA	G	6.0
31	TEOS	G	0.5
32	THF	S	--
33	Toluene	G	3.5
34	Water	I	--
35	Xylene	G	3.0

^a G: gel, S: soluble, I: insoluble; ^b critical concentration for gelation; ^c with sonication; ^d thixotropic

Scanning electron microscopy: SEM pictures of the xerogel were taken on a Carl Zeiss NTS SUPRA 40FEG scanning electron microscope. A small portion of the xerogel previously coated with a thin layer of gold was attached to the holder by using a conductive adhesive carbon tape.

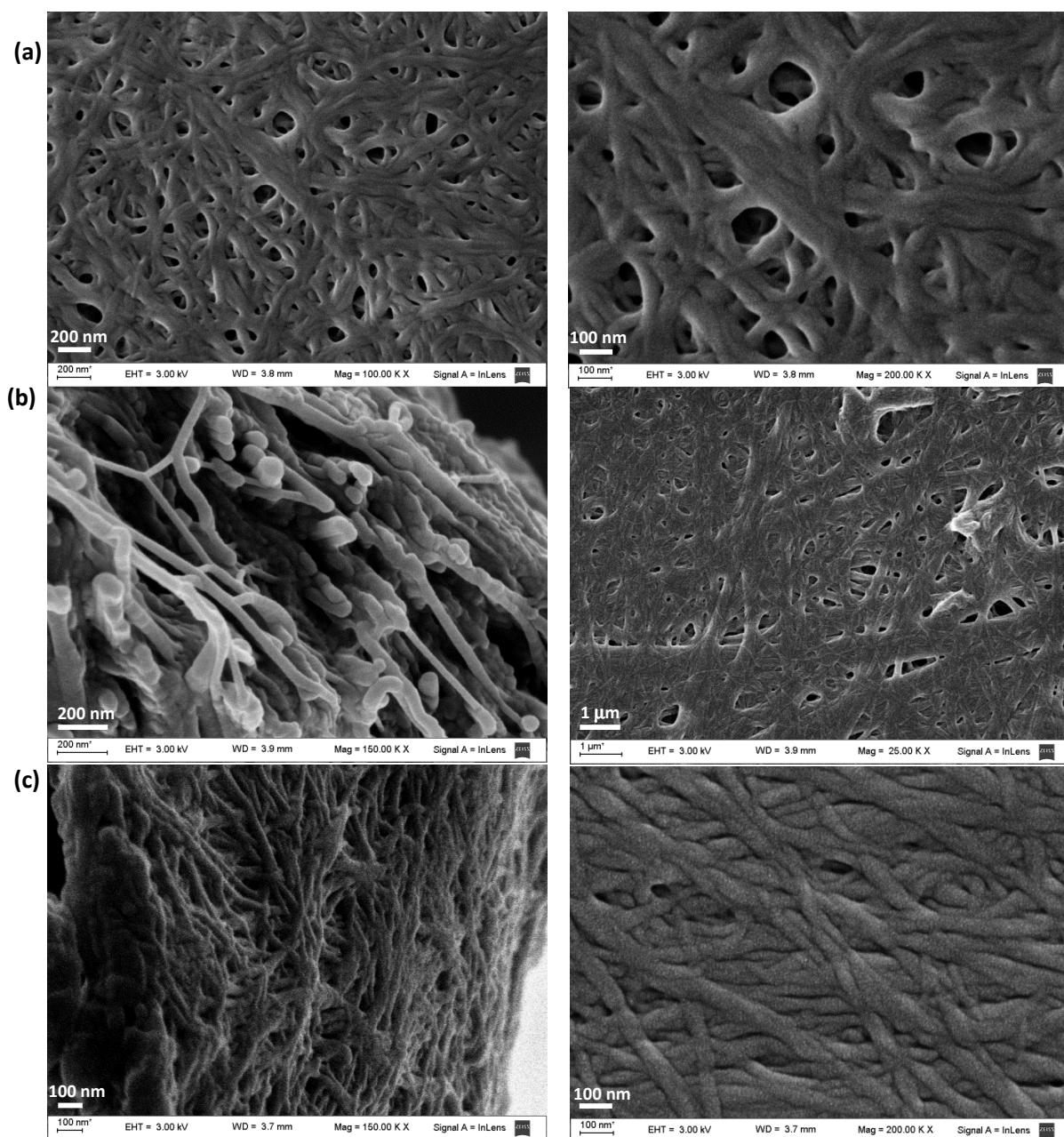


Figure S2: SEM images of xerogel of **LMOG 1** from **(a)** acetonitrile **(b)** *n*-hexane **(c)** *i*-propyl ether

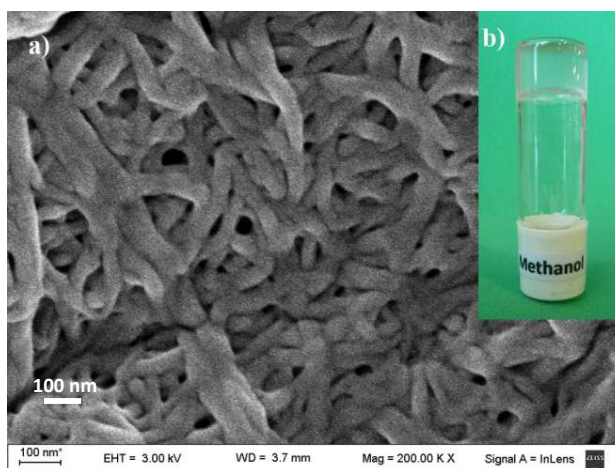


Figure S3: a) SEM image of xerogel of **LMOG 1** from methanol b) picture of a methanolic gel, inverted flask.

FT-IR experiments: FT-IR measurements were carried out on a Nicolet Magna IR 550 FTIR spectrometer in a demountable liquid cell with NaBr disks.

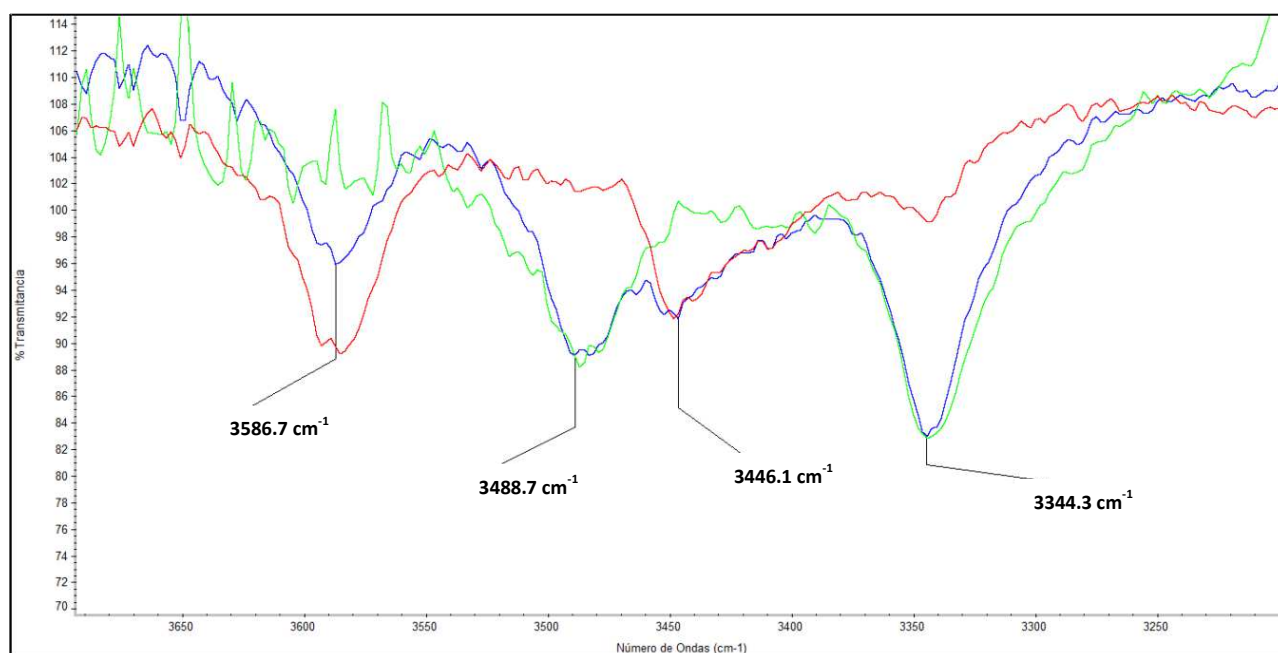


Figure S4: FT-IR spectra for: Red: Hot Solution of **LMOG 1** in xylene; Blue: Partial gel (after 40 min. of slow cooling); Green: Gel of **LMOG 1** in xylene at room temperature (after 1 h).

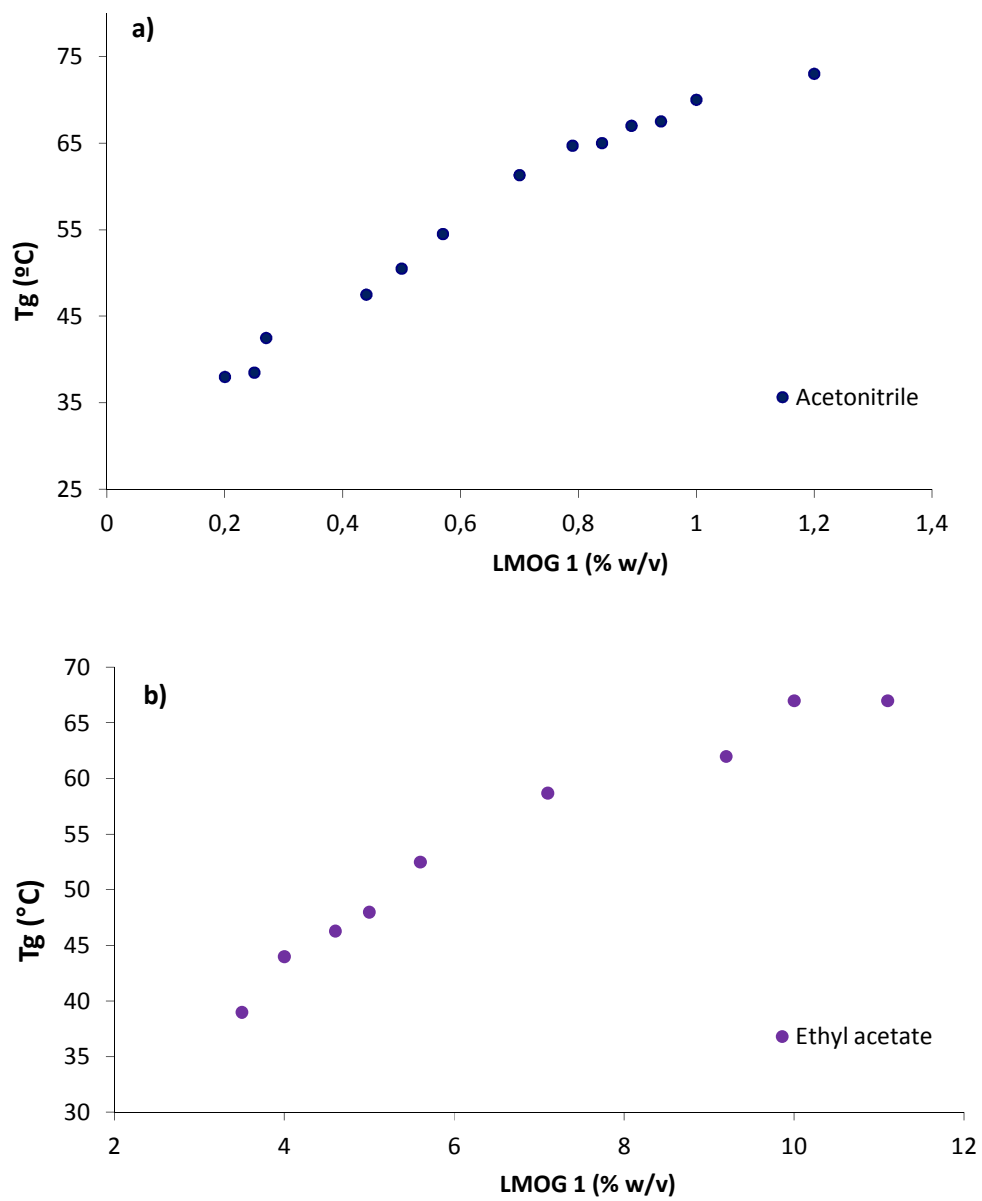


Figure S5: T_g vs. LMOG 1 concentration plots for a) acetonitrile and b) ethyl acetate



Figure S6: Phase selective gelation of a mixture of water and petrol. In the picture, the fluid liquid aqueous phase is retained by the gelled petrol (inverted flask)

Sampling Procedure of Sensing

Corrected fluorescence emission spectra were obtained on a Cary Eclipse spectrophotometer equipped with two Czerny-Turner monochromators and a 15 W Xenon pulse lamp (pulse width: 2-3 μ s, power: 60-75 kW). The excitation wavelength was set at 650 nm.

The fluorescence measurements were conducted using the right angle technique.

For the determination of the emission spectra of the cyanine in solution, 2 μ L from a 0.7mM stock solution of **2** in methanol were diluted to 500 μ L with methanol (final concentration of 2.8 μ M of the cyanine) and then placed in a cuvette.

For the determination of the emission spectra of the cyanine in the organogel, a mixture of the gelator (7.5 mg, 1.5 wt. %) and cyanine (2 μ L from a stock 0.7mM solution) were diluted to 500 μ L with methanol (final concentration: 2.8 μ M). The closed flask was heated (80°C) and shaken until the solid was dissolved. Then, the hot solution was placed in a cuvette and cooled to room temperature to form a stable transparent gel.

The sensing of Zn(II) was carried out by adding 50 μ L of the corresponding solution (ZnCl₂ in water from 5 nM to 45 μ M) in the cuvette on top of the already formed gel and acquiring fluorescence emission spectra every one minute until reaching a plateau of the signal at 782 nm. In every case an exponential quenching of the fluorescence was observed (as shown in Figure 3, main text). The maximum fluorescence quenching percentage for each concentration was calculated as follows:

$$\%Q = 1 - (y_0/y_x)$$

Where y_0 was the intensity signal reached at 45 min and y_x was the maximum emission at the beginning of the experiment.

The %Q reached at 45 min was plotted against Zn(II) concentration (Figure 4). The data was fitted with an exponential curve following a single binding model (1:1) for computation of the dissociation constants (K_D).

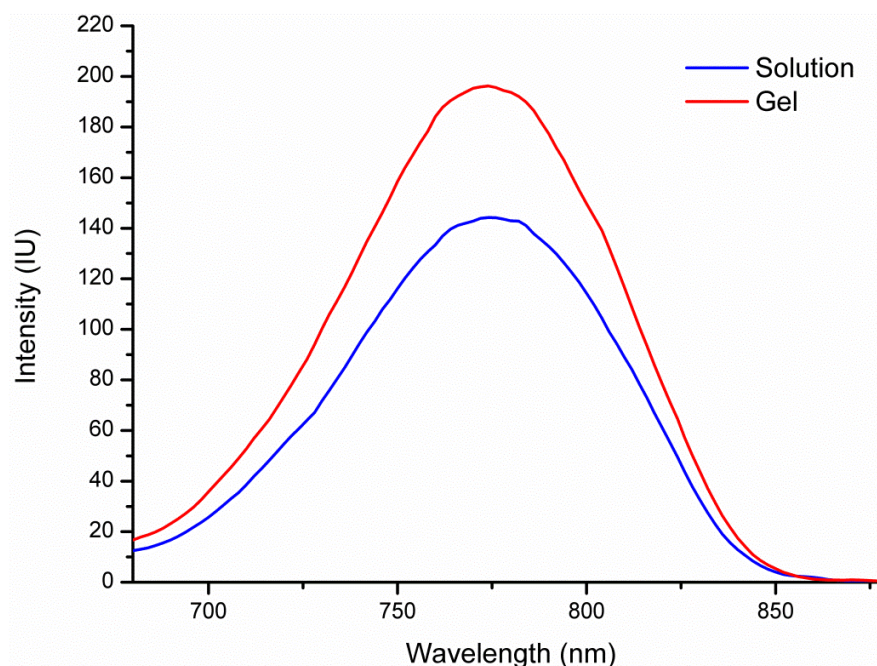


Figure S7: Comparative fluorescence emission spectra of the cyanine **2** (2.8 μ M, λ_{ex} 650 nm) in methanol solution and in the organogel from methanol (1.5% wt)

References:

- (1) Menéndez, G. O.; Samaniego López, C.; Jares-Erijman, E. A.; Spagnuolo, C. C. A Versatile Near-Infrared Asymmetric Tricarbocyanine for Zinc Ion Sensing in Water. *Photochem. Photobiol.* **2013**, 89, 1354-1361.
- (2) Jursic, B. S.; Upadhyay, S. K.; Creech, C. C.; Neumann, D. M. Novel and Efficient Synthesis and Antifungal Evaluation of 2,3-Functionalized Cholestane and Androstane Derivatives. *Bioorg. Med. Chem. Lett.* **2010**, 20(24), 7372-7375.
- (3) Schönecker, B.; Ponsold, K. Steroide. XXX. Synthesen Vicinaler Steroid-Azidoketone. *J. Prakt. Chem.* **1971**, 313, 817-824.
- (4) Gonschior, M.; Kötteritzsch, M.R.; Schönecker, B.; Wyrwa, R. Synthesis of *N,N*-bis[2-(2-pyridyl)ethyl]amino steroids and related compounds intended as chiral ligands for copper ions. *Tetrahedron: Asymmetry* **2000**, 11, 2159-2182.
- (5) Sugandhi, E. W.; Slebodnick, C.; Falkinham III, J.O.; Richard D. Gandour, R.D. Synthesis and Antimicrobial Evaluation of Water-Soluble, Dendritic Derivatives of Epimeric 5 α -Cholestan-3-amines and 5 α -Cholestan-3-yl aminoethanoates. *Steroids* **2007**, 72, 615-626.