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Supporting Information

Supramolecular hydrogels based on antimycobacterial amphiphiles

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Materials and methods

Chemicals

All chemicals were used as received without further purification. Solvents were distilled prior to usage. Water was desalted by a standard ion exchange setup.

NMR experiments

NMR experiments were carried out on a Bruker Avance AC 300 spectrometer at 298 K (300 MHz). The compounds were dissolved in DMSO-d_6, which also acted as internal reference.

Mass spectroscopy

Mass spectra were recorded on a Finnigan MAT 8500 apparatus (EI, 70 eV) using direct injection mode.

Elemental analysis

Elemental analysis (C, H, N) was carried out with an EA 3000 instrument (HEKAtech). The theoretical amount of all elements was calculated using Isis Draw.

Melting point (m.p.):

The melting point was measured using a MP90 Melting Point System (Mettler Toledo) at a heating rate of 10 °C/min (onset of transmittance increase, average of three measurements).

Size exlusion chromatography (SEC)

For evaluation of the purity of the synthesized target molecules **1**-**6** SEC was carried out. The oligomeric SEC was performed utilizing a Waters 515 HPLC pump and stabilized THF as eluent at a flow rate of 0.5 mL min^{-1}. 20 µl of a solution with a concentration of approx. 1 mg mL^{-1} was injected into a column setup comprising a guard column (Varian, 5 × 0.8 cm, Meospore gel, particle size 3 µm,) and two analytical columns (Varian, 30 × 0.8 cm, Mesopore gel, particle size 3 µm). Oligomeric compounds were monitored with a WATERS 486 tunable UV detector at 254 nm and a Waters 410 differential RI detector. Molecular weights were given with respect to an oligo-styrene calibration.

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Preparation of the NaOH solutions:

The sodium hydroxide solutions were prepared by adding 1 M or 5 M aqueous NaOH solutions to desalted water under stirring while monitoring the pH value with a digital pH meter (Piccolo Plus, HEKAtech).

Gelation tests ("inverted test tube method")

A mixture of the amphiphile and the corresponding aqueous media (approx. 1.0 mL of solvent, 1-10 g L^{-1} of amphiphile) was put in a screw cap vial (inner diameter: 12 mm; filling height: 9 mm) containing a small stirring bar. After the vial was sealed, the mixture was heated to approx. 95 °C under stirring. Samples were allowed to stand at 18 °C for 24 hours. Gel state was defined if upon inversion of the test tube no gravitational flow was observed.

Determination method for the gel-sol transition temperature (T_{gel})

The gel-sol transition temperature T_{gel} was determined using the following "falling steel ball method". For the measurement 1 mL of a hot, homogeneous fluid gelator solution was filled into a screw cap vial (inner diameter: 12 mm; filling height: 9 mm), sealed and stored at 18 °C overnight. A steel ball (diameter: 2 mm; weight: 33 mg) was put on top of the gel, and the vial was heated slowly in an oil bath (heating rate: approx. 1 °C min^{-1}). T_{gel} was defined as the temperature at which the steel ball reached the bottom of the vial (average of two measurements). The temperature was measured in a reference vial filled with 1 mL of pure water.

Scanning electron microscopic (SEM) study

Small fragments of hydrogels were dried on a piece of paper pulp under ambient conditions. The dried samples were mounted on a standard sample holder by a conductive adhesion pad and examined with a Zeiss LEO 1530 (FE-SEM with Schottky-field-emission cathode; in-lens detector) using an accelerating voltage of 2-4 kV. The samples were sputtered with platinum prior to SEM imaging.

XRD measurements

XRD analysis of hydrogels and bulk material was performed on a Huber Guinier-Diffraktometer 6000, equipped with a Huber Quarz-Monochromator 611, a Cu-anode ($CuK_{\alpha 1}$-beam, λ = 154.051 pm, X-ray generator from Seifert Company (Germany)), a Huber SMC 9000 stepping motor controller and a self-developed gate system, primary beam stopper and

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sample oven. XRD analysis of the hydrogel was carried out by filling pre-heated Mark tubes (outer diameter: 2 mm, wall thickness: 0.01 mm) with a hot solution (10 g L^{-1} of **3** in aqueous NaOH solution). The samples were sealed and stored at room temperature for 24 h prior to the XRD measurements.

Molecular modelling

Molecular dimensions were calculated using Chem3D Ultra 7.0 (MOPAC energy minimization).

IR measurements

IR spectra of bulk material from synthesis and of dried hydrogel samples were recorded with a Perkin-Elmer Spectrum 100 FT-IR spectrometer in ATR mode. Hydrogel samples were put on paper pulp and dried under ambient conditions. All samples were powdered prior to the measurements.

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Synthesis of compounds 1-6

General procedure

Diglycolic acid anhydride is dissolved in dichloromethane (approx. 50 mL per gram of anhydride). An equimolar amount of the corresponding 4-alkoxyaniline is added under cooling (water bath, 15 °C). After the addition of the amine, the mixture is stirred at room temperature for 2 h. Twice the initial volume of cyclohexane is added, the precipitate is filtered off, washed with a mixture of dichloromethane and cyclohexane (1:3) and dried under reduced pressure (high vacuum, room temperature) overnight. All compounds were obtained as white crystalline solids. Yield range 58-95 %, 82 % average.

1 ([(4-butyloxy-phenylcarbamoyl)-methoxy]-acetic acid):

Yield: 94 %. ^{1}H-NMR (300 MHz, DMSO-d_6, 20 °C): δ 12.87 (br s, 1 H, -CO_2**H**), δ 9.72 (s, 1 H, -CO-N**H**-), δ 7.52 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 6.88 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 4.20 (s, 2 H, HO_2C-C**H$_2$**-O-), δ 4.13 (s, 2 H, -O-C**H$_2$**-CO-NH-), δ 3.32 (t, ^3J(H,H) = 6.5 Hz, 2 H, -O-C**H$_2$**-CH_2-), δ 1.68 (m, 2 H, -O-CH_2-C**H$_2$**-), 1.42 (m, 2 H, -O-CH_2-CH_2-C**H$_2$**-), δ 0.93 (t, ^3J(H,H) = 7.4 Hz, 3 H, -C**H$_3$**). Anal. Calcd. for $C_{14}H_{19}NO_5$ (281.31): C, 59.78; H, 6.81; N, 4.98 %. Found C, 59.72; H, 6.96; N, 5.19 %. MS (EI) m/z: 281 (M^+), 225, 151, 122, 121, 109, 108. mp: 112.8 °C.

2 ([(4-pentyloxy-phenylcarbamoyl)-methoxy]-acetic acid):

Yield: 95 %. ^{1}H-NMR (300 MHz, DMSO-d_6, 20 °C): δ 12.80 (br s, 1 H, -CO_2**H**), δ 9.71 (s, 1 H, -CO-N**H**-), δ 7.52 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 6.87 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 4.20 (s, 2 H, HO_2C-C**H$_2$**-O-), δ 4.14 (s, 2 H, -O-C**H$_2$**-CO-NH-), δ 3.92 (t, ^3J(H,H) = 6.5 Hz, 2 H, -O-C**H$_2$**-CH_2-), δ 1.69 (m, 2 H, -O-CH_2-C**H$_2$**-), δ 1.36 (m, 4 H, -O-CH_2-CH_2-(C**H$_2$**)$_2$-), δ 0.89 (t, ^3J(H,H) = 7.1 Hz, 3 H, -C**H$_3$**). Anal. Calcd. for $C_{15}H_{21}NO_5$ (295.34): C, 61.00; H, 7.17; N, 4.74 %. Found C, 60.98; H, 7.32; N, 5.03 %. MS (EI): m/z 295 (M^+), 225, 122, 109, 108, 43. mp: 93.1 °C.

3 ([(4-hexyloxy-phenylcarbamoyl)-methoxy]-acetic acid):

Yield: 94 %. ^{1}H-NMR (300 MHz, DMSO-d_6, 20 °C): δ 12.86 (br s, 1 H, -CO_2**H**), δ 9.71 (s, 1 H, -CO-N**H**-), δ 7.52 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 6.87 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 4.20 (s, 2 H, HO_2C-C**H$_2$**-O-), δ 4.14 (s, 2 H, -O-C**H$_2$**-CO-NH-), δ 3.92 (t,

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^3J(H,H) = 6.5 Hz, 2 H, -O-C**H$_2$**-CH$_2$-), δ 1.69 (m, 2 H, -O-CH$_2$-C**H$_2$**-), δ 1.40 (m, 2 H, -O-CH$_2$-CH$_2$-C**H$_2$**-), δ 1.31 (m, 4 H, -(C**H$_2$**)$_2$-CH$_3$), δ 0.88 (t, ^3J(H,H) = 7.0 Hz, 3 H, -C**H$_3$**). Anal. Calcd. for $C_{16}H_{23}NO_5$ (309.37): C, 62.12; H, 7.49; N, 4.53 %. Found C, 61.84; H, 7.58; N, 4.74 %. MS (EI) m/z: 309 (M^+), 225, 122, 109, 108, 43. mp: 89.8 °C.

4 ([(4-heptyloxy-phenylcarbamoyl)-methoxy]-acetic acid):

Yield: 58 %. ^{1}H-NMR (300 MHz, DMSO-d$_6$, 20 °C): δ 12.86 (br s, 1 H, -CO$_2$**H**), δ 9.70 (s, 1 H, -CO-N**H**-), δ 7.52 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 6.87 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 4.20 (s, 2 H, HO$_2$C-C**H$_2$**-O-), δ 4.14 (s, 2 H, -O-C**H$_2$**-CO-NH-), δ 3.92 (t, ^3J(H,H) = 6.5 Hz, 2 H, -O-C**H$_2$**-CH$_2$-), δ 1.69 (m, 2 H, -O-CH$_2$-C**H$_2$**-), δ 1.40 (m, 2 H, -O-CH$_2$-CH$_2$-C**H$_2$**-), δ 1.28 (m, 6 H, -(C**H$_2$**)$_3$-CH$_3$), δ 0.87 (t, ^3J(H,H) = 6.8 Hz, 3 H, -C**H$_3$**). Anal. Calcd. for $C_{17}H_{25}NO_5$ (323.39): C, 63.14; H, 7.79; N, 4.33 %. Found C, 62.82; H, 7.99; N, 4.94 %. MS (EI) m/z: 323 (M^+), 225, 207, 109, 108. mp: 85.8 °C.

5 ([(4-octyloxy-phenylcarbamoyl)-methoxy]-acetic acid):

Yield: 67 %. ^{1}H-NMR (300 MHz, DMSO-d$_6$, 20 °C): δ 12.86 (br s, 1 H, -CO$_2$**H**), δ 9.71 (s, 1 H, -CO-N**H**-), δ 7.52 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 6.87 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 4.20 (s, 2 H, HO$_2$C-C**H$_2$**-O-), δ 4.13 (s, 2 H, -O-C**H$_2$**-CO-NH-), δ 3.91 (t, ^3J(H,H) = 6.5 Hz, 2 H, -O-C**H$_2$**-CH$_2$-), δ 1.69 (m, 2 H, -O-CH$_2$-C**H$_2$**-), δ 1.40 (m, 2 H, -O-CH$_2$-CH$_2$-C**H$_2$**-), δ 1.26 (m, 8 H, -(C**H$_2$**)$_4$-CH$_3$), δ 0.86 (t, ^3J(H,H) = 6.7 Hz, 3 H, -C**H$_3$**). Anal. Calcd. for $C_{18}H_{27}NO_5$ (337.42): C, 64.07; H, 8.07; N, 4.15 %. Found C, 64.03; H, 8.27; N, 4.06 %. MS (EI) m/z: 337 (M^+), 225, 221, 109, 108. mp: 94.0 °C.

6 ([(4-nonyloxy-phenylcarbamoyl)-methoxy]-acetic acid):

Yield: 86 %. ^{1}H-NMR (300 MHz, DMSO-d$_6$, 20 °C): δ 12.86 (br s, 1 H, -CO$_2$**H**), δ 9.71 (s, 1 H, -CO-N**H**-), δ 7.52 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 6.87 (d, ^3J(H,H) = 9.0 Hz, 2 H, phenylene-**H**), δ 4.20 (s, 2 H, HO$_2$C-C**H$_2$**-O-), δ 4.13 (s, 2 H, -O-C**H$_2$**-CO-NH-), δ 3.91 (t, ^3J(H,H) = 6.5 Hz, 2 H, -O-C**H$_2$**-CH$_2$-), δ 1.69 (m, 2 H, -O-CH$_2$-C**H$_2$**-), δ 1.40 (m, 2 H, -O-CH$_2$-CH$_2$-C**H$_2$**-), δ.26 (m, 10 H, -(C**H$_2$**)$_5$-CH$_3$), δ 0.86 (t, ^3J(H,H) = 6.7 Hz, 3 H, -C**H$_3$**). Anal. Calcd. for $C_{19}H_{29}NO_5$ (351.45): C, 64.93; H, 8.32; N, 3.99 %. Found C, 65.01; H, 8.55; N, 4.55 %. MS (EI) m/z: 351 (M^+), 235, 225, 109, 108, 43. mp: 98.3 °C.

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Antimicrobial activity of compounds 1-6

The antimicrobial activity of compounds **1-6** was determined at the Hans-Knöll Institute Jena, Germany by agar diffusion assays. 34 mL of nutrient agar were liquefied and inoculated with a suspension of the corresponding microorganisms so that a final concentration of 10^7 cells per 34 mL of nutrient agar was reached. The inoculated nutrient medium was put immediately into the test plates resulting in an even layer with a thickness of about 3 mm. 12 holes per test plate were punched out using an appropriate device. 50 µL of a solution of the test substances in DMSO (1000 mg L^{-1} of test substance) were put in each hole, respectively. For comparison purposes, pure solvent (DMSO) was put in one of the holes. The test plates were cultivated for 18 h at 37 °C. Evaluation of the antimicrobial activity was done by detection of the zones of growth inhibition by the naked eye (diameter in mm) according to the chart below.

Inhibition zone (diameter / mm)	*Description*
0	no effect
15-21	moderate activity
21-25	good activity
> 25	very good activity

The substances were tested for their influence on the growth of the following microorganisms:

Bacillus subtillis
Staphylococcus aureus
Micrococcus luteus
Vancomycin-resistant Enterococcus faecalis (VRE)
Escherichia coli
Proteus vulgaris
Pseudomonas aeruginosa
Multidrug-resistant Staphylococcus aureus (MRSA)
Sporobolomyces salmonicolor
Candida albicans
Penicillium notatum
Glomerella

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Table S1. Antimicrobial activity of compounds **1-6** determined by agar diffusion assay. Values: zone of growth inhibition (diameter in mm). p: few colonies in the zone of growth inhibition. P: many colonies in the zone of growth inhibition. A: indication for growth inhibition. F: promotion of growth.

Compound	c [µg mL^{-1}]	*Bacillus subtilis* ATCC 6633	*Staphylococcus aureus* SG511	*Micrococcus luteus* ATCC 10240	*Enterococcus faecalis* (VRE) 1528	*Escherichia coli* SG458	*Proteus vulgaris* Ox19	*Pseudomonas aeruginosa* SG137	*Pseudomonas aeruginosa* K799/61	*Staphylococcus aureus* (MRSA) 134/93	*Sporobolomyces salmonicolor* 549	*Candida albicans* C.A.	*Penicillium notatum* JP36	*Glomerella*
1	1000	0	0	0	0	0	0	0	0	0	0	0	0	20p
2	1000	0	0	0	0	0	0	0	0	0	0	0	0	16p
3	1000	0	0	0	0	0	0	0	0	0	0	11P/A	0	20p
4	1000	0	0	0	0	0	0	0	0	0	0	12P/A	0	20p
5	1000	16P	20p-P	0	13/23p/F	0	0	0	0	15p-P	0	12P/A	0	16p-P
6	1000	11/16P	23p-P	0	15/27p/F	0	0	0	0	12/14p-P	0	11P/A	0	0

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Antiproliferative effects and cytotoxicity of compounds 1-6

Antiproliferative effects and cytotoxicity of the compounds **1-6** were evaluated at the Hans-Knöll Institute Jena, Germany according to methods described elsewhere.[1] Cells were incubated with dilutions of the test substances for 72 h at 37 °C.

Table S2: Antiproliferative effects and cytotoxicity of compounds **1-6**. HUVEC: human umbilical vein endothelial cells. K-562: human immortalised myelogenous leukaemia cells. HeLa: human cervix carcinoma cells. GI_{50}: concentration for 50 % inhibition of cell growth. CC_{50}: concentration for 50 % cell death.

> 50 µg mL^{-1}: no or marginally cytotoxic/antiproliferative

10-50 µg mL^{-1}: moderately cytotoxic / antiproliferative

1-10 µg mL^{-1}: highly cytotoxic / antiproliferative

< 1 µg mL^{-1}: extremely cytotoxic / antiproliferative

Compound	Antiproliferative effect		Cytotoxicity
	HUVEC	K-562	HeLa
	GI_{50} [µg mL^{-1}]	GI_{50} [µg mL^{-1}]	CC_{50} [µg mL^{-1}]
1	> 50	> 50	> 50
2	> 50	> 50	> 50
3	> 50	> 50	> 50
4	> 50	> 50	41.2
5	> 50	> 50	37.6
6	48	> 50	40.3

[1] F. Krauth, H.-M. Dahse, H.-H. Rüttinger and P. Frohberg, *Bioorg. Med. Chem.*, 2010, **18**, 1816.

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pH-Dependent hydrogel formation of 3 in aqueous NaOH solution

Mixtures of 10 g L^{-1} of **3** and aqueous NaOH solutions at different pH values were heated under stirring up to approx. 95 °C. After standing at room temperature over night, the macroscopic appearance of the sample was investigated. The degree of deprotonation (dod) was roughly estimated by Formula S1 (c(**3**) = 10 g L^{-1}; **3Na**: sodium salt of **3**).

$$
\begin{aligned}
&\textit{with } pH = 14 - pOH \textit{ and } pOH = -\log[c(OH^-) \times L / mol] \\
&\rightarrow pH = 14 + \log[c(OH^-) \times L / mol] \\
&\rightarrow c(OH^-) = 10^{pH-14}\, mol / L \\
&\textit{assumption}: c(3Na) = c(OH^-) \rightarrow c(3Na) = 10^{pH-14}\, mol / L \\
&dod / \% = c(3Na) / c(3) \times 100 \\
&\textit{with } c(3) = 10\ g / L \textit{ and } M(3) = 309.37 g / mol \rightarrow c(3) = 0.0323\ mol / L \\
&\rightarrow dod / \% = (10^{pH-14}\, mol / L) / (0.0323\ mol / L) \times 100 \\
&\rightarrow dod / \% = 10^{pH-14} \times 3093
\end{aligned}
$$

Formula S1. Estimation of the degree of deprotonation (dod) of **3** in aqueous NaOH solutions.

Table S3. Estimated degree of deprotonation (dod) and macroscopic appearance of samples of **3/3Na** in aqueous NaOH solutions at different initial pH values.

pH	***dod [%]***	***Appearance***
11.5	*10*	*gel*
11.7	*16*	*gel*
11.9	*25*	*gel*
12.0	*31*	*inhomogeneous gel + solution*
12.2	*49*	*inhomogeneous gel + solution*
12.4	*78*	*clear solution*
12.5	*98*	*clear solution*

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FT-IR spectra of 3 as bulk and as dried hydrogel from different pH values

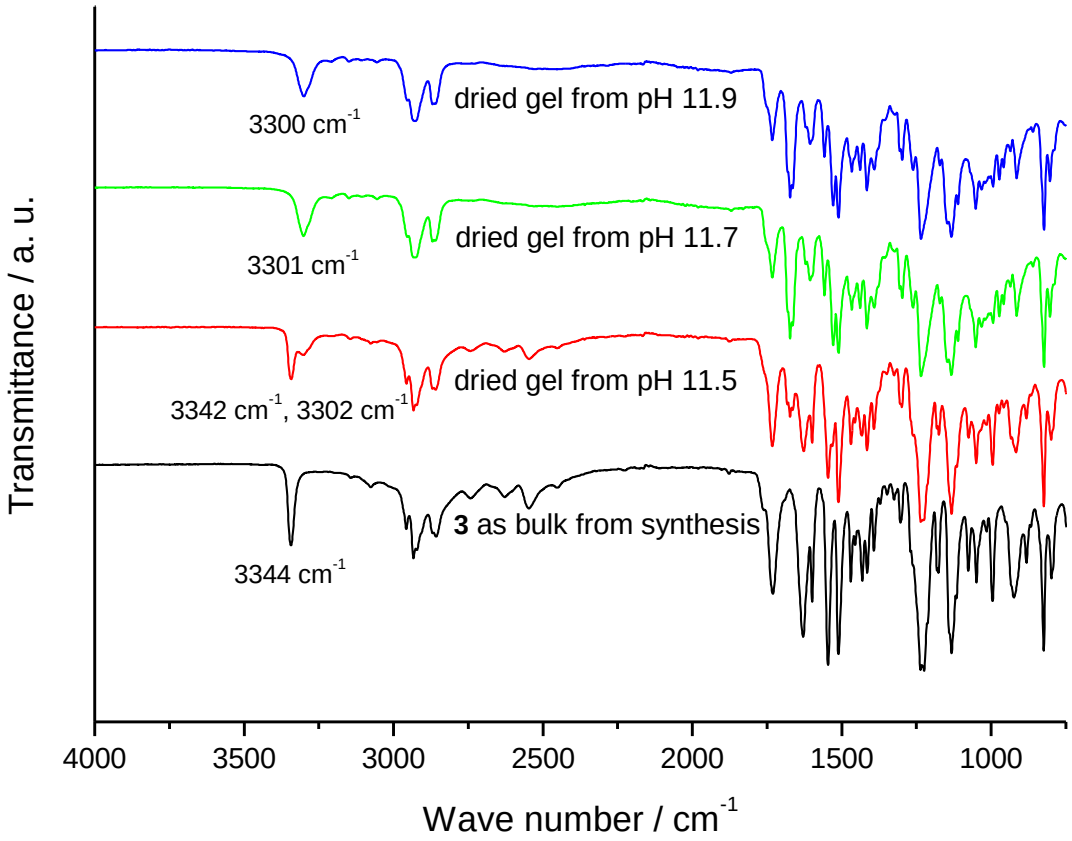

Figure S1. FT-IR spectra of **3** as bulk from synthesis and as dried hydrogel samples obtained from 10 g L^{-1} of **3** in aqueous NaOH solution at different initial pH values. Note: in order to ease visualization, plots have been offset in the y-axis.

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XRD analysis of 3 as bulk and as hydrogels at different pH values

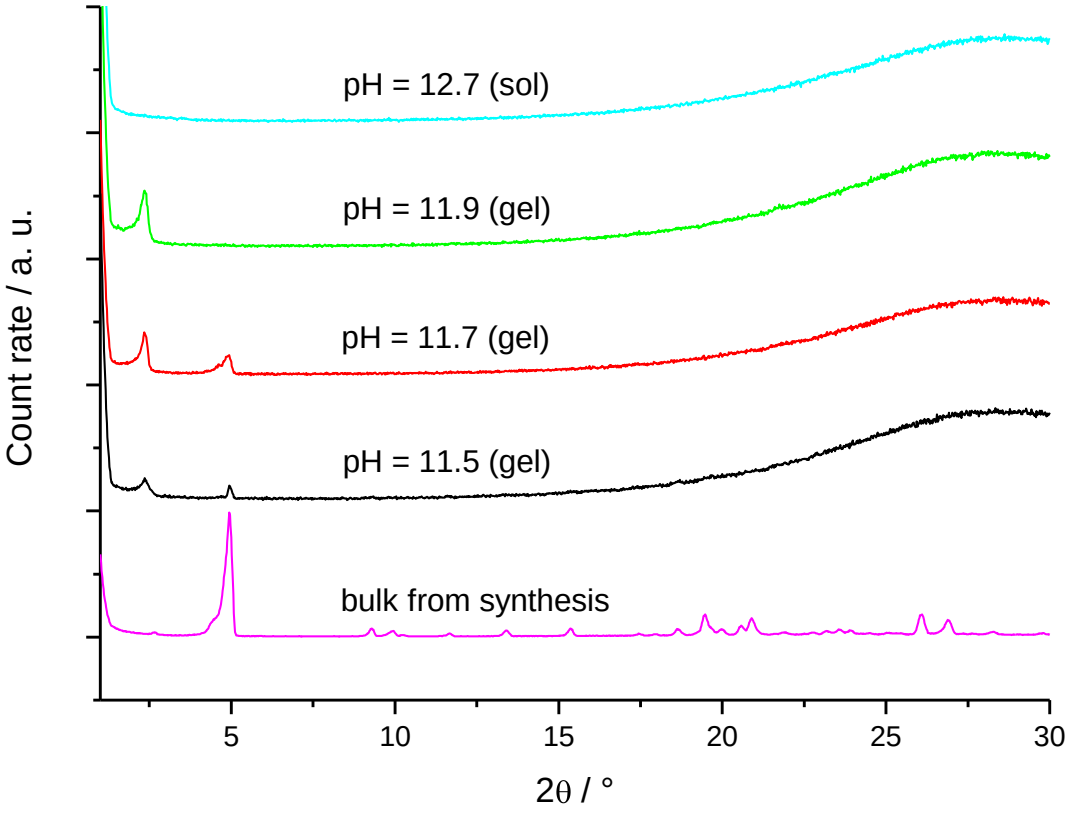

Figure S2. X-Ray diffraction patterns of samples obtained from 10 g L^{-1} of **3** in aqueous NaOH solution at different initial pH values and of **3** as bulk as obtained from synthesis. Note: in order to to ease visualization, plots have been offset in the y-axis.

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Cryo-TEM investigations of samples of 3 in aqueous NaOH solutions

(cryo-)Transmission electron microscopy

3 was dissolved in aqueous NaOH solutions at different pH values under stirring at room temperature. For cryogenic transmission electron microscopy studies, a 2µL droplet of the sample was put on a hydrophilized lacey carbon filmed copper grid (200 mesh, Science Services, Muenchen, Germany). Most of the liquid was removed with blotting paper leaving a thin film stretched over the carbon net holes. The specimens were instantly shock frozen by rapid immersion into liquid ethane and cooled to approximately 90 K by liquid nitrogen in a temperature-controlled freezing unit (Zeiss Cryobox, Zeiss NTS GmbH, Oberkochen, Germany). The temperature was monitored and kept constant in the chamber during all the sample preparation steps. After freezing the specimens, the remaining ethane was removed using blotting paper. The specimen was inserted into a cryotransfer holder (CT3500, Gatan, Muenchen, Germany) and transferred to a Zeiss EM922 EFTEM (Zeiss NTS GmbH, Oberkochen, Germany). Examinations were carried out at temperatures around 90 K. The TEM was operated at an acceleration voltage of 200 kV. Zero-loss filtered images ($\Delta E = 0$ eV) were taken under reduced dose conditions (ca. 100 – 1000 e nm^{-2}). All images were registered digitally by a bottom mounted CCD camera system (Ultrascan 1000, Gatan, Muenchen, Germany) combined and processed with a digital imaging processing system (Digital Micrograph 3.10 for GMS 1.8, Gatan, Muenchen, Germany).

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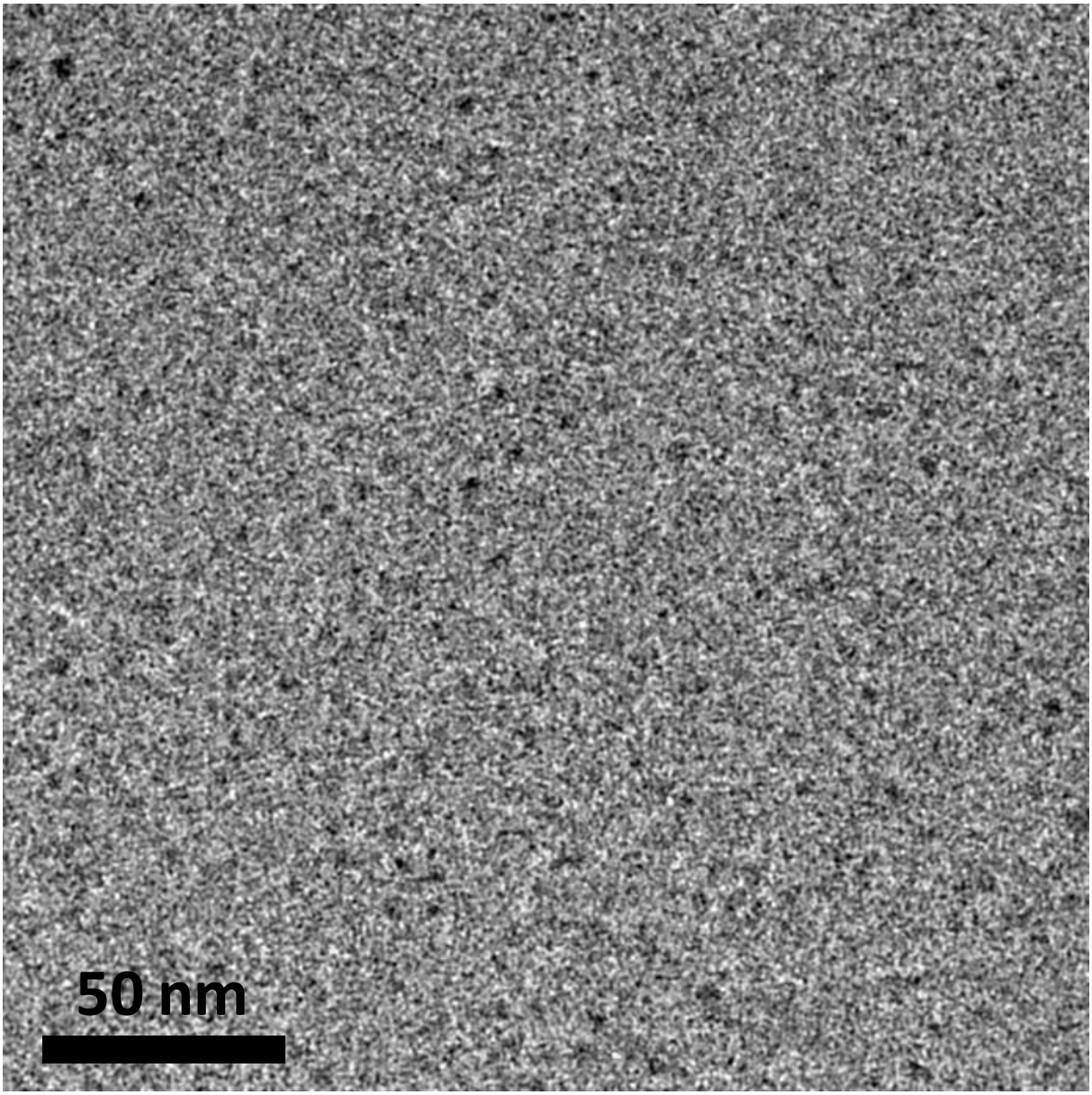

Figure S3. cryo-TEM image of 10 g L^{-1} of **3** in aqueous NaOH solution (initial pH 12.7) featuring small spherical micelles.

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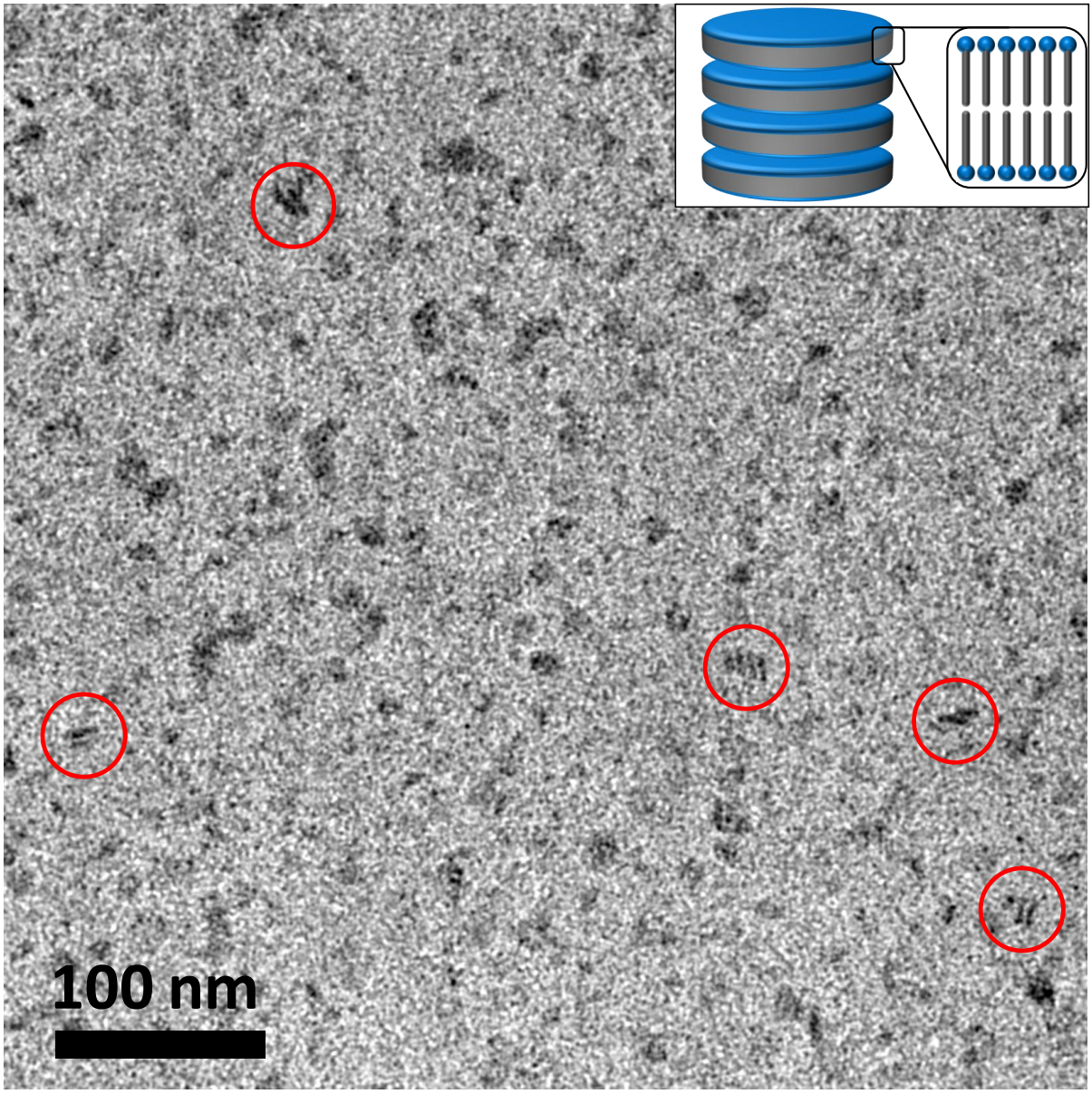

Figure S4. cryo-TEM image of 10 g L^{-1} of **3** in aqueous NaOH solution (initial pH 14.0) featuring micellar superstructures reminiscent of stacked disk-like micelles. Some of the aggregates have been marked by circles for better clarity (side view of stacks). The inset shows the proposed molecular arrangement of the amphiphiles within these superstructures.

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Micro-differential scanning calorimetry (µ-DSC) of hydrogels of 3

µ-DSC measurements were performed on a Setaram MikroDSC III. The samples were prepared by mixing a weighted amount of amphiphile **3** and aqueous NaOH solution (initial pH 11.9) at elevated temperatures under stirring until a homogeneous solution with a concentration of 10 g L^{-1} of **3** was obtained. Approximately 0.7 mL of this hot solution was filled into a Hastelloy C276 sample cell. The reference cell was filled with an equal amount of pure solvent. Prior to measurement, the sealed cells were allowed to stand at room temperature for at least 20 minutes. The measurements were carried out at a heating and cooling rate of 1 °C min^{-1}.

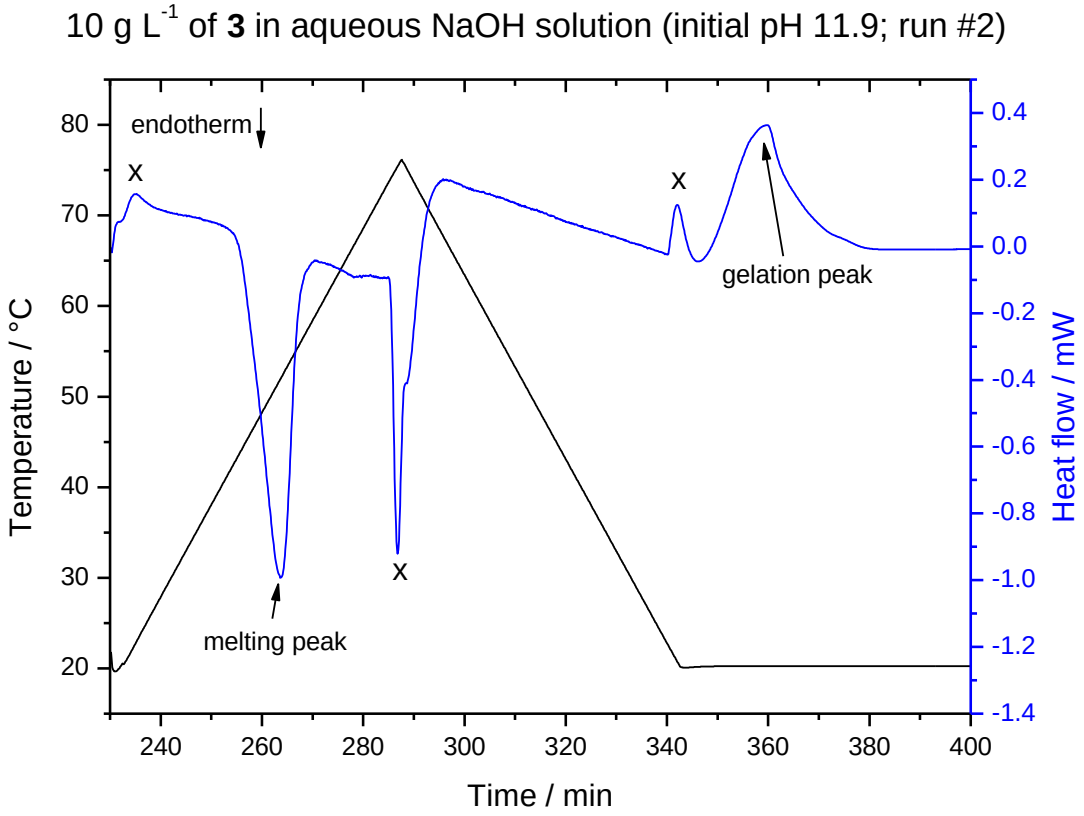

Figure S5. µ-DSC curve of run #2 of a hydrogel sample obtained from 10 g L^{-1} of **3** in aqueous NaOH solution (initial pH 11.9). Temperature program: 20-75 °C with 1 °C min^{-1}, 75-20 °C with 1 °C min^{-1}, 60 min at 20 °C. Melting and gelation peak are clearly visible. Note: the peaks marked with "x" are caused by the µ-DSC apparatus (change of the temperature slope).

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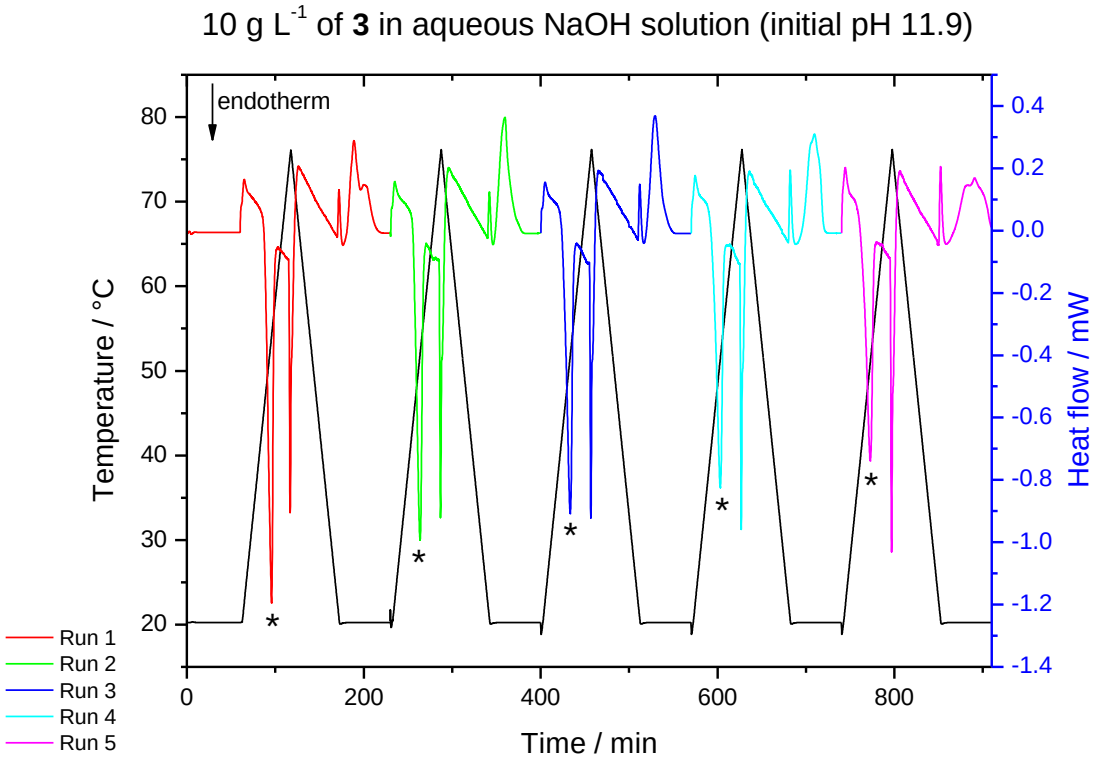

Figure S6. μ-DSC curves of a hydrogel sample obtained from 10 g L^{-1} of **3** in aqueous NaOH solution (initial pH 11.9). Temperature program per run: 60 min at 20 °C, 20-75 °C with 1 °C min^{-1}, 75-20 °C with 1 °C min^{-1}. Note: for better visualization, the thermograms of every run were colored differently. "*" indicates the gel melting peak for each run.

Table S4. Melting enthalpies ΔH_m of the hydrogel sample investigated by μ-DSC experiments (10 g L^{-1} of **3** in aqueous NaOH solution, initial pH = 11.9).

Number of runs #	**ΔH_m [J g^{-1}]**
1	47.8
2	47.5
3	47.0
4	45.4
5	44.4
6	41.9

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HPLC investigations of thermally treated hydrogel sample of 3

Hydrogel samples with a volume of approx. 1 mL with an initial concentration of 10 g L^{-1} of **3** in aqueous NaOH solution (initial pH 11.9) were prepared as described above. The yielding hydrogel samples were tempered at 85 °C for 5 h under stirring and allowed to cool to room temperature over night. The samples were extracted three times with 1 mL of methyl tert-butyl ether successively. The organic extracts were combined, and the solvent and traces of water were removed using a rotary evaporator (40 °C; $p < 6$ mbar). The resulting residue (white solid) was dissolved in a mixture of 75 vol.-% of THF and 25 vol.-% of H_2O, filtrated using a PTFE syringe filter with a pore size of 0.20 µm and submitted to the HPLC analysis.

An Agilent 1100 series HPLC with a ZORBAX Bonus-RP column (column dimensions 4.6 x 150 mm; particle size 5 µm) at a flow rate of 1 mL min^{-1} with an eluent composition of 75 vol.-% of THF and 25 vol.-% of H_2O was used. For UV detection, a wavelength of 300 nm was chosen, as this allowed monitoring of both the hydrogelator **3** and the drug 4-hexyloxyaniline.

Between every run, the whole setup was rinsed with an eluent composition of 90 vol.-% of THF and 10 vol.-% of H_2O and with 20 vol.-% of THF and 80 vol.-% of H_2O, respectively. Subsequently, the setup was equilibrated back to the initial eluent composition of 75 vol.-% of THF and 25 vol.-% of H_2O to assure identical column conditions for each run.

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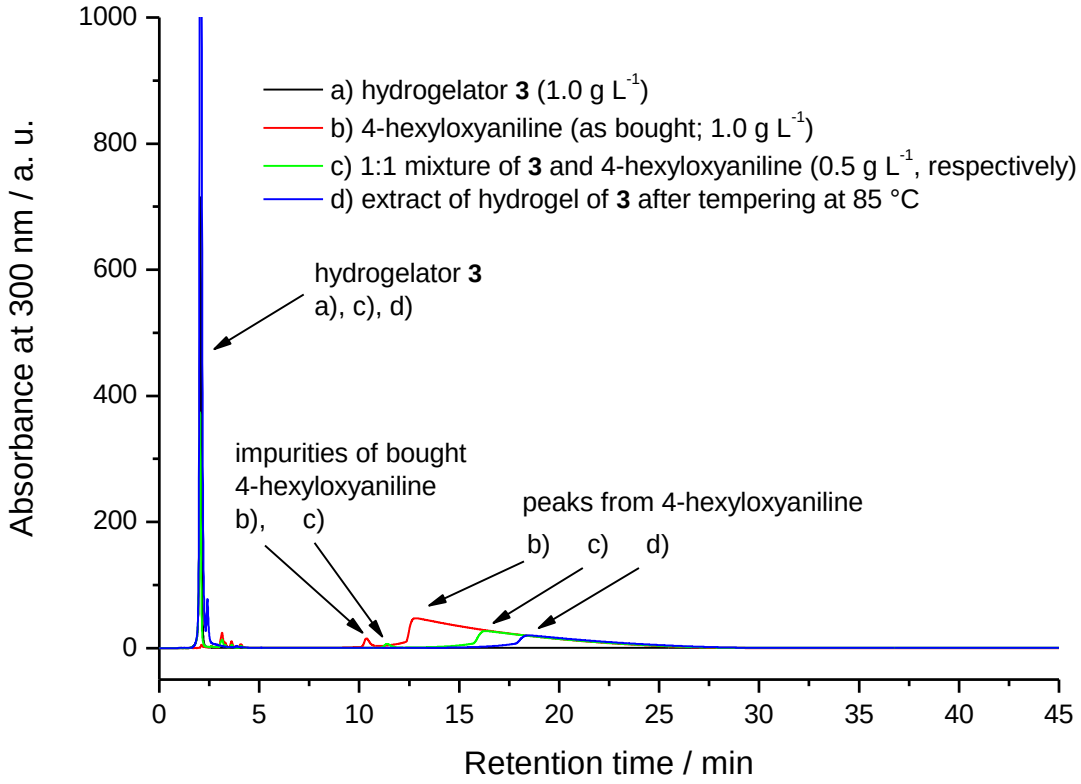

Figure S7. HPLC traces of a) hydrogelator **3**, b) 4-hexyloxyaniline, c) a 1:1 mixture of **3** and 4-hexyloxyaniline and d) the extract obtained from a thermally treated hydrogel sample (10 g L^{-1} of **3**, aqueous NaOH solution, initial pH 11.9). Peaks for both the remaining hydrogelator **3** and the hydrolysis product 4-hexyloxyaniline in the extract from the partially hydrolyzed hydrogel sample d) are clearly visible. As can be seen, the retention time of 4-hexyloxyaniline in the used solvent system is concentration-dependent, probably due to aggregation effects of this amphiphilic compound. Although lower concentrations lead to higher retention times (compare trace b) at 1.0 g L^{-1} with trace c) at 0.5 g L^{-1}), the corresponding signal decay is practically identical for all samples containing 4-hexyloxyaniline (b), c), d)). Note: Although several eluent compositions were tested, it was not possible to avoid the smearing of 4-hexyloxyaniline on the used reversed-phase column setup.