Supporting Information

Fluorescent Covalently Crosslinked Cellulose Networks *via* Light-Induced Ligation

Andrea Hufendiek,[a,b,c] *Anna Carlmark,*[d] *Michael A. R. Meier**[a] *and*
*Christopher Barner-Kowollik**[b,c]

[a]Laboratory of Applied Chemistry, Institute of Organic Chemistry (IOC), Karlsruhe Institute of Technology (KIT), Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany. E-mail: m.a.r.meier@kit.edu.

[b]Preparative Macromolecular Chemistry, Institut für Technische Chemie und Polymerchemie, Karlsruhe Institute of Technology (KIT), Engesserstr. 18, 76128 Karlsruhe, Germany. Fax: +49 721 608 45740; E-mail: christopher.barner-kowollik@kit.edu.

[c]Institut für Biologische Grenzflächen, Karlsruhe Institute of Technology (KIT), Herrmann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.

[d]KTH Royal Institute of Technology, Department of Fibre and Polymer Technology, Teknikringen 56, SE-100 44 Stockholm, Sweden.

Materials

Acetic acid (Roth, 100%), acetic anhydride (Acros, > 99%,) *p*-Anisidine (ABCR, 99%), *N*-(dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC; Roth, 99%), 4-(dimethylamino)pyridine (DMAP; Sigma Aldrich, 99%), dimethyl carbonate (Acros, 99%), *N,N*-dimethylformamide (DMF; VWR Analpur), dimethyl sulfoxide (DMSO; Carl Roth, 99.8%), furan (Acros, 99%), 3,3'-dithiopropionic acid(Aldrich, 99%), 4-formylbenzoic acid (TCI, 98%), hydrochloric acid (Roth, 37%), maleic anhydride (Alfa Aesar, 98%), potassium carbonate (technical), pyridine (Sigma Aldrich, 99.8%), sodium acetate (Roth, 99%), sodium nitrite (Alfa Aesar, 98%), DL-dithiothreitol (DTT; AppliChem, ≥ 99.5%) *p*- toluenesulfonyl hydrazide (Alfa Aesar, 98%), 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD; Sigma Aldrich, 98%), of 4,7,10-trioxa-1,13-tridecanediamine (Sigma Aldrich, 97%) and triethylamine (Merck, 99%) were used as received. All other solvents were of analytical grade and used as received. 1-Butyl-3-methylimidazolium chloride (BMIMCl; Aldrich, 95%), lithium chloride (Sigma Aldrich, 99%), and Whatman filter paper no. 5 were stored in a desiccator over silica gel. Ethanolamine (Acros, 99%) was dried over molecular sieve (3 Å) prior to use.

Characterization Methods

NMR measurements were performed on a Bruker Ascend spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei, respectively. All NMR spectra were recorded at ambient temperature. The chemical shifts δ were referenced to the solvent residual signals, respectively ($\delta_H(CHCl_3)=7.26$, $\delta_H(DMSO)=2.50$, $\delta_C(DMSO)=39.52$). *J* values are given in Hz. The degree of substitution (*DS*) of cellulose 4-(2-(4-methoxyphenyl)-*2H*-tetrazol-5-yl) benzoate (cellulose-tetrazole) was determined according to a procedure adopted from the literature.[1, 2] A defined amount of maleic acid as the standard and a defined amount of the cellulose-tetrazole were dissolved in DMSO-d_6. The *DS* was calculated using the integral ratio of the ^{1}H NMR resonance corresponding to the aromatic protons **d**, **e**, **i**, **j** of the cellulose-tetrazole (Figure S1) and the resonance corresponding to the double bond protons of the maleic acid (δ = 6.27 ppm).

Size exclusion chromatography (SEC). SEC on the cellulose-tetrazoles and the cellulose networks after reductive degradation was performed at a sample concentration of 1-2 g $L^{-1}$with DMAc containing 10 g L^{-1} LiCl as eluent at 70 °C on an Agilent Series 1200 HPLC system including an isocratic pump (G1310A), an autosampler (G1329A), a thermostat controlled column compartment (G1316A), a refractive index detector (G1362A) that was kept at 50 °C, and a diode array and a multiple wavelength detector (G1315D) at a flow rate of 0.5 mL min^{-1}. The separation was achieved on a SEC column (PSS, GRAM analytical, 300 × 8.00 mm, 10 μm particle size, 3000 Å porosity) with a precolumn (50 × 8.00 mm). Linear poly(methyl methacrylate) standards with molecular weights ranging from 4300 to 3.73×10^6 g mol^{-1} were used for calibration. The samples were filtered through PTFE membranes with a pore size of 0.45 μm prior to injection. Dissolution of Whatman filter paper no. 5 was achieved in a solvent exchange procedure adopted from the literature.[3-5]

UV-vis measurements. UV-vis spectra were recorded on a Cary 300 Bio UV-vis spectrophotometer (Varian) at ambient temperature in DMSO from 200 nm to 800 nm with a resolution of 1 nm and a slit width of 2 nm.

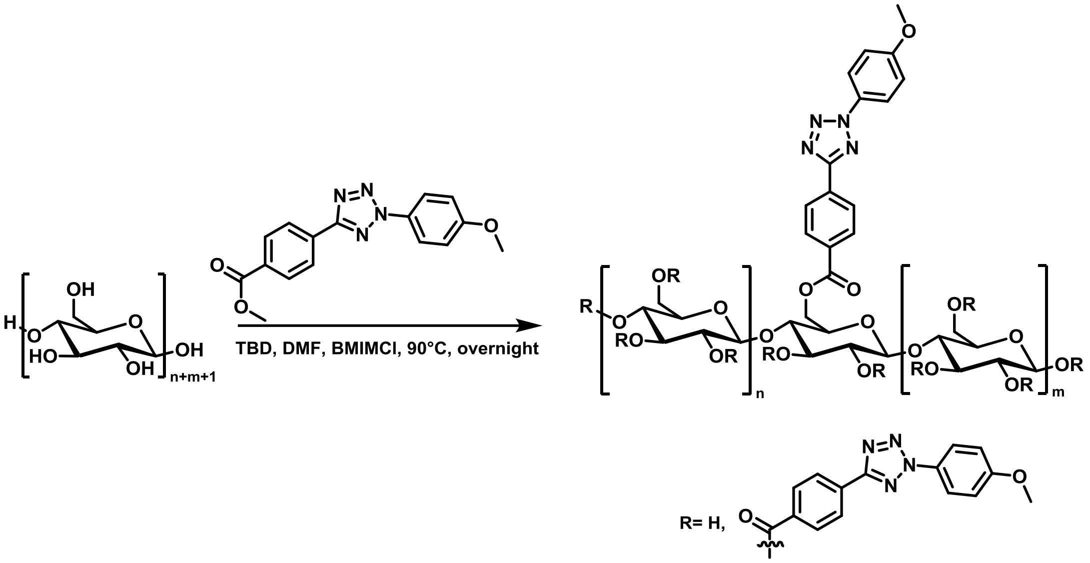
Fluorescence measurements. Fluorescence emission spectra were recorded on a Varian Cary Eclipse spectrometer at an excitation wavelength of 365 nm. Samples of thin films were placed in a quartz cuvette of 2 mm thickness, which was placed in the sample holder in a 45° angle. The spectra were recorded from 400 nm to 800 nm at an excitation slit width of 5 nm, an emission slit width of 5 nm, a resolution of 0.5 nm, and a scan rate of 30 nm min^{-1}. A filter was applied from 430-1100 nm. The reference sample was prepared in the same manner as **network 1** and **network 2** employing **cellulose-tetrazole 2** without the addition of a bismaleimide linker. The reference sample was whashed extensively with methanol and subsequently dried with a stream of compressed air.

Photoluminescence Quantum Yield (PLQY). PLQY was determined for solutions in LiCl/DMAc (10 g L^{-1}) obtained from **network 2** and **network 4** after the reduction of disulfide bonds (see below) on a Hamamatsu Quantaurus QY at an excitation wavelength of λ_{ex} = 400 nm in the emission range in wavelengths from 450 – 800 nm.

Differential scanning calorimetry (DSC). DSC experiments were carried out on a DSC821e (Mettler Toledo) calorimeter, under nitrogen atmosphere, at a heating rate of 10 K min^{-1} from 25 °C up to a temperature of 350 °C, and using a sample mass of approximately 5 mg. Prior to data acquisition the samples were heated at a heating rate of 10 °C min^{-1} to 125 °C and kept at this temperature for 10 min to remove water and solvent residues, and subsequently cooled to 25 °C at a cooling rate of -20 °C min^{-1}.

Thermogravimetric Analysis (TGA). TGA was perfomed on a Du Pont Instruments 951 Thermogravimetric Analyzer in a nitrogen atmosphere (nitrogen flow: 100 mL min^{-1}). The samples were heated to 125 °C at a heating rate of 10 K min^{-1} and kept at 125 °C for 20 min. Subsequently, the samples were heated to 730 °C at a heating rate of 10 K min^{-1}.

Experimental Procedures



Scheme S1: Synthesis of cellulose 4-(2-(4-methoxyphenyl)-*2H*-tetrazol-5-yl) benzoate (**cellulose-tetrazole**).

Cellulose 4-(2-(4-methoxyphenyl)-*2H*-tetrazol-5-yl) benzoate (cellulose-tetrazole)

Cellulose-tetrazole was synthesized from Whatman filter paper no. 5 in solution in BMIMCl/DMF following a previous report employing a transesterification procedure with an isolated product yield of 75%.[2, 6] The experimental conditions for the synthesis of **cellulose-tetrazole 1-3** are summarized in Table 1.

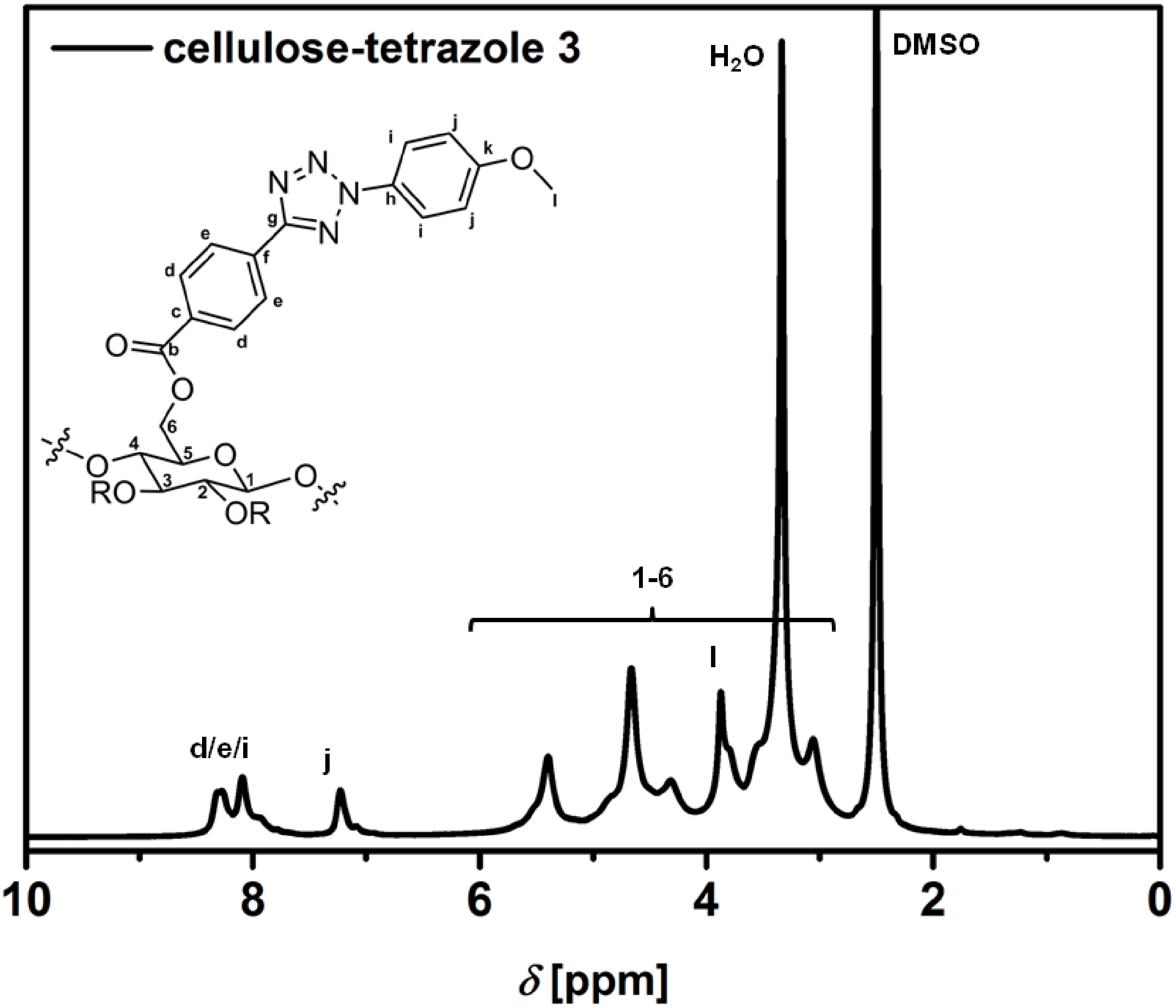


Figure S1: ^{1}H-NMR spectrum of **cellulose-tetrazole 3** in DMSO-d_6 as solvent.

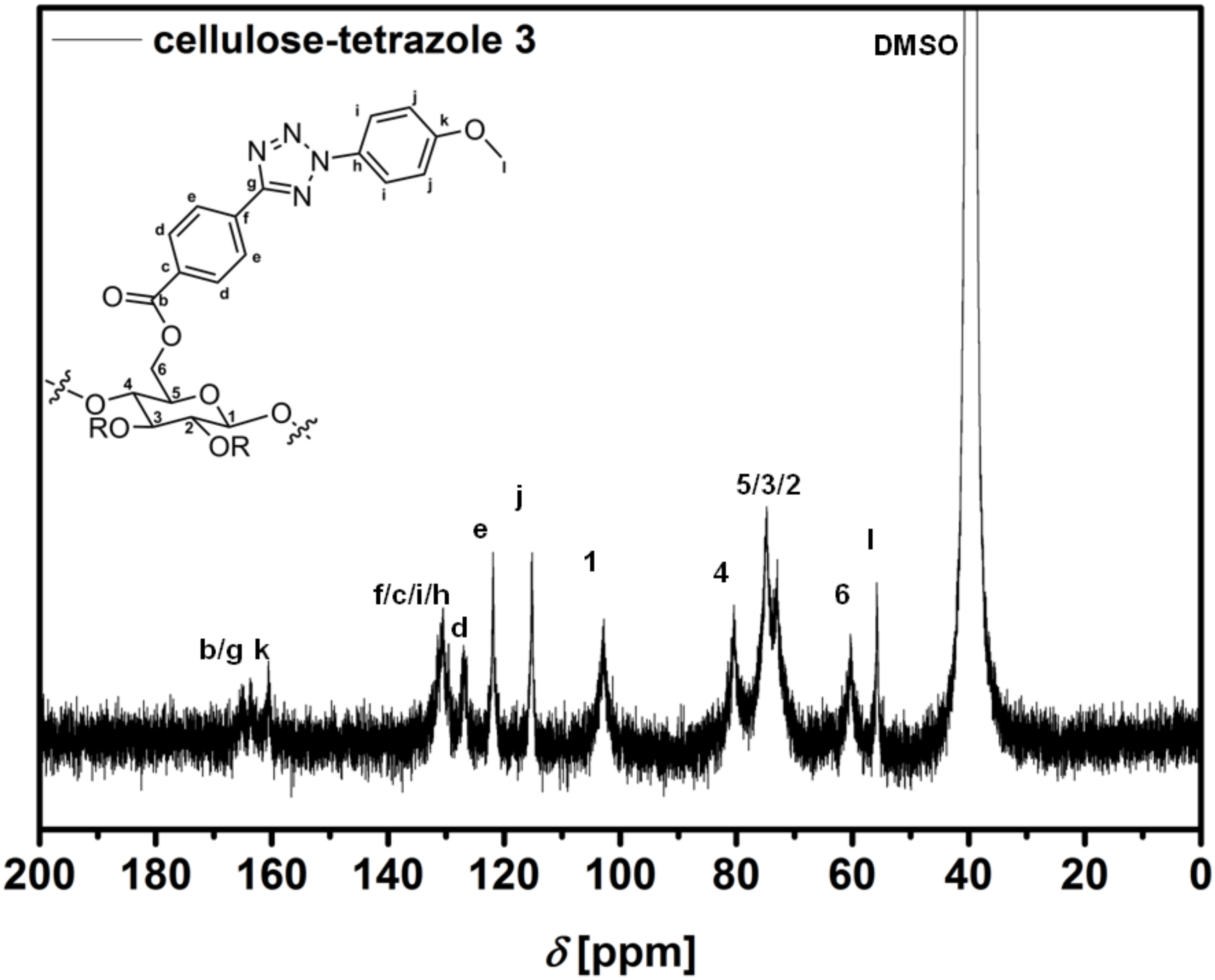


Figure S2: ^{13}C-NMR spectrum of **cellulose-tetrazole 3** in DMSO-*d*$_6$ as solvent.

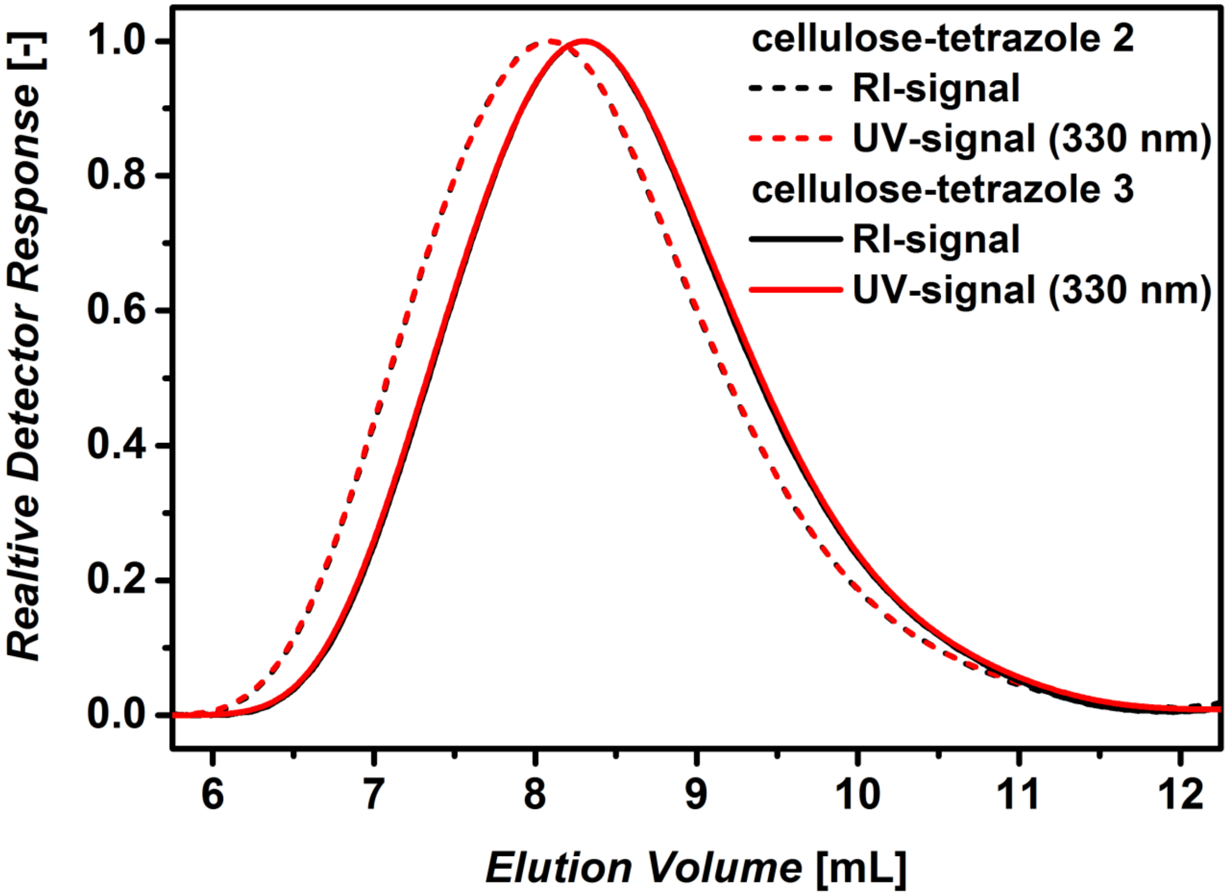
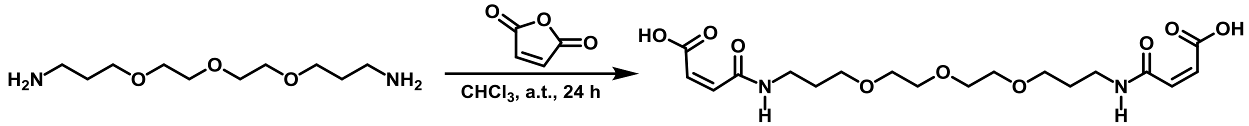


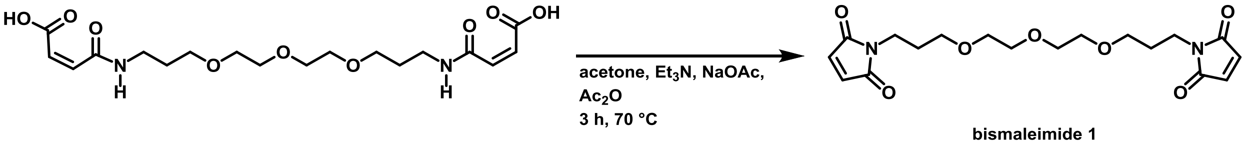
Figure S3: SEC traces of **cellulose-tetrazole 2** (dashed line) and **cellulose-tetrazole 3** (solid line) in DMAc/LiCl as the eluent at 70 °C. $M_{\mathrm{n,SEC,UV}}$(**cellulose-tetrazole 2**) = 112000 g mol^{-1}, $Đ$ = 2.67; $M_{\mathrm{n,SEC,UV}}$(**cellulose-tetrazole 3**) = 96000 g mol^{-1}, $Đ$ = 2.39.



Scheme S2: Synthesis of *N,N'*-[4,7,10-trioxa-tridecane]bismaleamic acid.

N,N'-[4,7,10-Trioxa-tridecane]bismaleamic acid

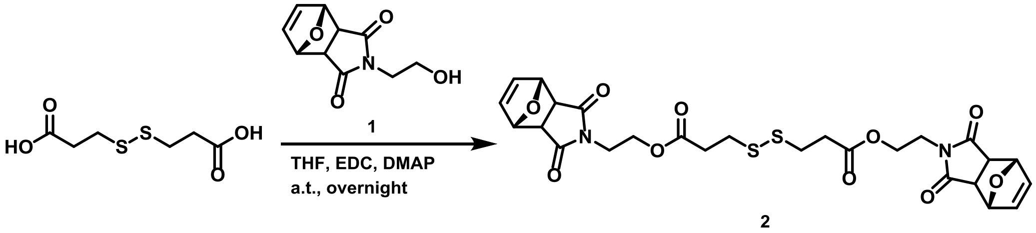
The synthesis was performed according to a previously reported procedure.[7] A solution of maleic anhydride (8.90 g, 90.8 mmol, 2.00 equiv.) in chloroform (50 mL) was cooled to 0 °C. After the dropwise addition of 4,7,10-trioxa-1,13-tridecanediamine (9.95 mL, 45.4 mmol 1.00 equiv.) dissolved in chloroform (5 mL) the reaction mixture was stirred at 0 °C for an additional 1 h and subsequently at ambient temperature overnight. The evaporation of the solvent gave *N,N'*-[4,7,10-trioxa-tridecane]bismaleamic acid as an intermediate product as a slightly yellow oil that was not further purified.



Scheme S3: Synthesis of *N,N'*-[4,7,10-trioxa-tridecane]bismaleimide (**bismaleimide 1**).

N,N'-[4,7,10-Trioxa-tridecane]bismaleimide (bismaleimide 1)

The synthesis was performed according to a previously reported procedure.[7] Triethylamine (4.12 mL, 29.5 mmol, 0.65 equiv.), sodium acetate (2.83 g, 34.5 mmol, 0.78 equiv.) and acetic anhydride (27.25 mL, 288.3 mmol, 6.35 equiv.) were added to a solution of crude *N,N'*-[4,7,10-trioxa-tridecane]bismaleamic acid (18.91 g, 45.4mmol, 1.00 equiv.) in acetone (80 mL). The reaction mixture was heated to reflux for 3 h and was allowed to cool slowly. After filtration, the solvent was removed under reduced pressure to give a brown oil. To remove the acetic acid/acetic anhydride mixture, the crude product was dissolved in water (50 mL) and stirred for additional 50 h. The aqueous phase was extracted with dichloromethane (3 × 50 mL). Purification by flash chromatography (silica gel, diethyl ether/methanol = 98/2 v/v) gave a white crystalline solid (yield: 52 %). ^{1}H-NMR (400 MHz, DMSO-d_6): [δ, ppm] = 6.99 (s, 4H, C*H*=C*H*), 3.49 – 3.41 (m, 12H, 2 × NCH_2, 2 × OCH_2CH_2O), 3.37 (t, *J*=6.1, 4H, 2 × $CH_2CH_2C\boldsymbol{H}_2O$), 1.81 – 1.62 (m, 4H, 2 × $CH_2C\boldsymbol{H}_2CH_2$).

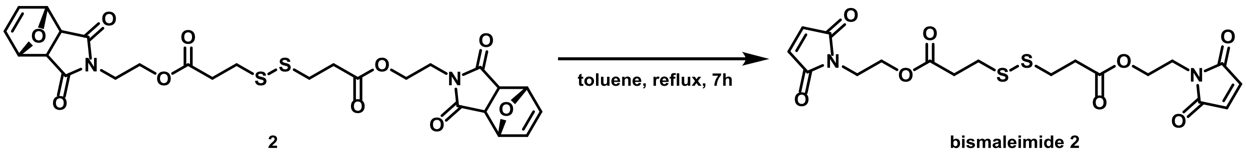


Scheme S4: Synthesis of 3,3’-dithiodipropionic acid di(2-(3,5-dioxo-10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl) ethyl ester) (**2**).

3,3’-Dithiodipropionic acid di(2-(3,5-dioxo-10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl) ethyl ester) (2)

4-(2-Hydroxyethyl)-10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (**1**) was synthesized according to a procedure from the literature.[8] To a solution of dithiodipropionic acid (1.00 g, 4.76 mmol, 1.00 equiv.) in 140 mL dry tetrahydrofuran (THF) alcohol **1** (2.98 g, 14.27 mmol, 3 equiv.) and DMAP (0.23 g, 1.90 mmol, 0.40 equiv.) were added at ambient temperature. The reaction mixture was cooled to 0 °C, and EDC (2.74 g, 28.54 mmol, 3.00 equiv.) was added. After stirring for 1 h at 0 °C the reaction mixture was stirred at ambient temperature overnight.

The solvent was removed under reduced pressure, and the residue was dissolved in 40 mL dichloromethane. The resulting solution was washed with saturated $NaHCO_3$ solution (100 mL), brine (3 × 100 mL), and water (3 × 100 mL). The organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by column chromatography with cyclohexane/ethyl acetate (silica gel, 1/3 v/v) as the eluent. Product **2** was obtained as a white solid (1.65 g, 60%). ^{1}H-NMR (400 MHz, DMSO-d_6): [δ, ppm] = 6.55 (s, 4H, 2 × CH=CH), 5.13 (s, 4H, 2 × CHOCH), 4.12 (t, J = 5.5 Hz, 4H, 2 × CH_2O), 3.62 (t, J = 5.5 Hz, 4H, 2 × CH_2N), 2.94 (s, 4H, 4 × CHCON), 2.85 (t, J = 7.1 Hz, 4H, 2 × CH_2S), 2.61 (t, J = 7.1 Hz, 4H, 2 × CH_2COO).



Scheme S5: Synthesis of 3,3'-dithiodipropionic acid di(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) ethyl ester) (**bismaleimide 2**).

3,3'-Dithiodipropionic acid di(2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl) ethyl ester) (bismaleimide 2)

Product **2** (0.60g, 1.04 mmol) was treated according to a procedure from the literature.[8] A pinch of hydroquinone was added to the reaction mixture. **Bismaleimide 2** was obtained as an off-white solid (0.44 g, 97%). ^{1}H-NMR (400 MHz, DMSO-d_6): [δ, ppm] = 7.04 (s, 4H, 2 × CH=CH), 4.16 (t, J = 5.3 Hz, 4H, 2 × CH_2O), 3.66 (t, J = 5.3 Hz, 4H, 2 × CH_2N), 2.84 (t, J = 6.9 Hz, 4H, 2 × CH_2S), 2.62 (t, J = 6.9 Hz, 4H, 2 × CH_2COO).

Exemplary procedure for network formation of cellulose-tetrazole with bismaleimide 1

Cellulose-tetrazole 1 with *DS*(tetrazole)=0.10 (10.0 mg, 0.053 mmol AGU, 5.26 mmol tetrazole, 1.00 eq) was dissolved in 150 µL LiCl/DMSO (4 g L^{-1}) at 60 °C. 25 µL of a solution of **bismaleimide 1** in LiCl/DMSO (40.0 g L^{-1}, 1.0 mg, 2.63 mmol, 0.50 equiv./tetrazole) were added and the resulting solution was stirred for 1 h at 60 °C. The reaction was performed in a custom-built photoreactor equipped with a single compact low-pressure fluorescent lamp (36 W, Arimed B6, λ_{max} = 320 nm, Figure S4 and S5), and a magnetic stirrer and the samples were irradiated for pre-set time intervals at ambient conditions. In addition, a control sample containing no bismaleimide linker was irradiated for a given amount of time. Subsequently, 500 µL (525 µL for the control sample) of DMSO were added, the samples were placed in a shaker at 60 °C and shaken at 400 min^{-1} for 4.5 h. 25 µL of the solution (that are not incorporated in the gel-like network) were diluted with 1 mL DMSO and subsequently analyzed by UV/vis spectroscopy.

Table S1: Experimental conditions for the synthesis of cellulose networks with **cellulose-tetrazole 1** or **cellulose-tetrazole 2** and **bismaleimide 1.** c_0(cellulose-tetrazole)$_{\mathrm{UV}}$ is the of concentration of cellulose-tetrazole at $t = 0$ h employed for the UV/vis measurements.

	c_0(cellulose-tetrazole) [g L^{-1}]	bismaleimide 1 [equiv./AGU]	bismaleimide 1 [equiv./tetrazole]	solid content [wt%]	c_0(cellulose-tetrazole)$_{\mathrm{UV}}$ [g L^{-1}]
cellulose-tetrazole 1	57	0.05	0.50	5.4	0.36
cellulose-tetrazole 2	53	0.12	0.50	5.4	0.35
cellulose-tetrazole 1, control	67	-	-	5.7	0.36
cellulose-tetrazole 2, control	67	-	-	5.7	0.35

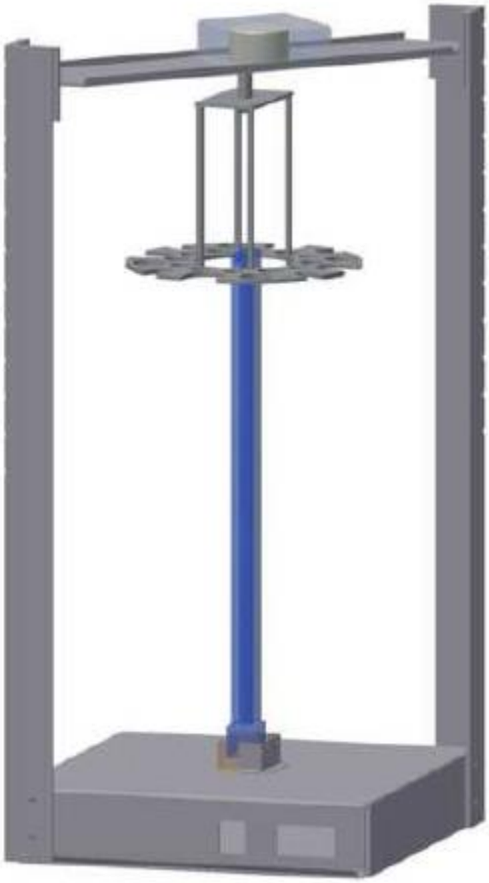


Figure S4: Illustration of the custom-built photoreactor used in the current work.

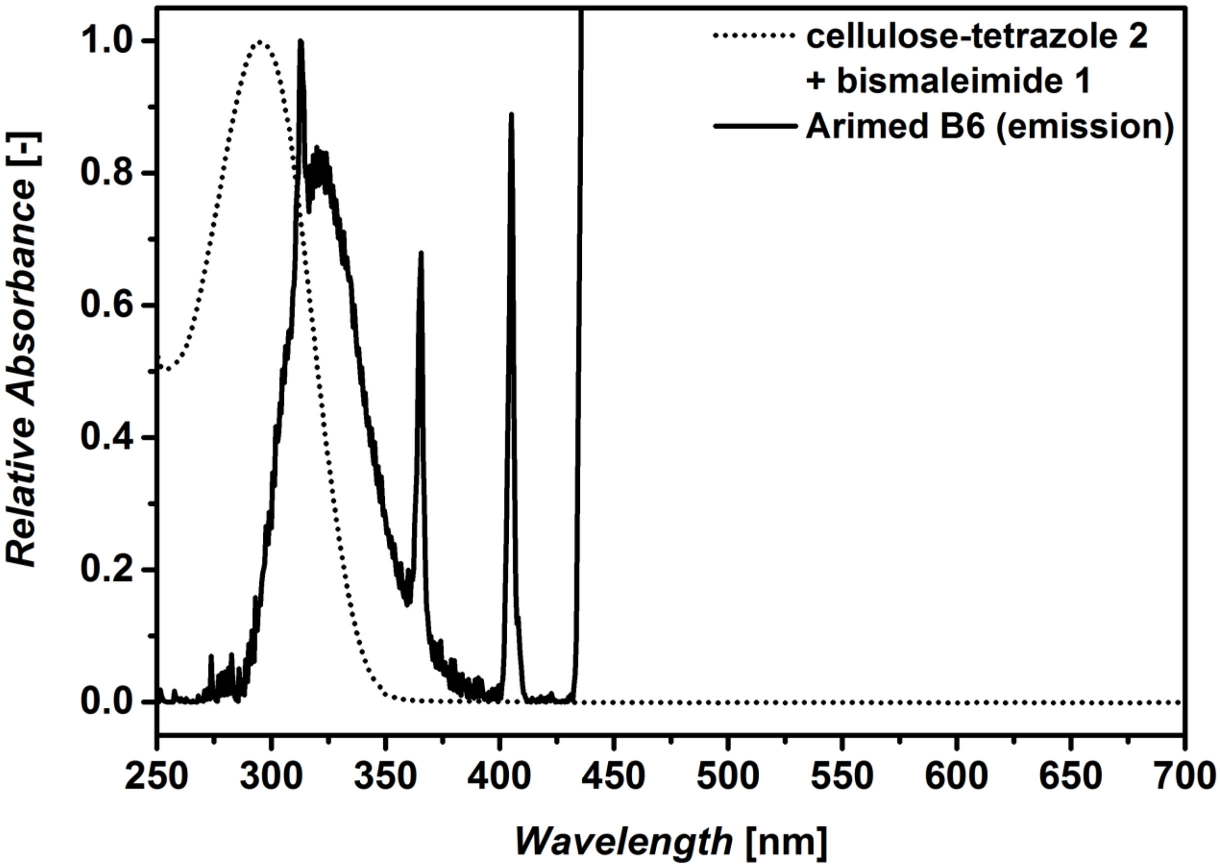


Figure S5: UV-vis absorption spectrum of **cellulose-tetrazole 2** and **bismaleimide 1** in DMSO and emission spectrum of the Arimed B6 lamp used in the current study. The absorbance and the emission are given relative to the peak maximum at 300 nm or 320 nm, respectively. The emission spectrum of the Arimed B6 lamp was measured on a spectroradiometer UVpad E, Opsytec Dr. Gröbel, Ettlingen, Germany, operating in the range of wavelenths from 200 - 440 nm. The power density was determined as 6.72 mW cm^{-2} at a 5 cm distance used in the crosslinking of cellulose films.

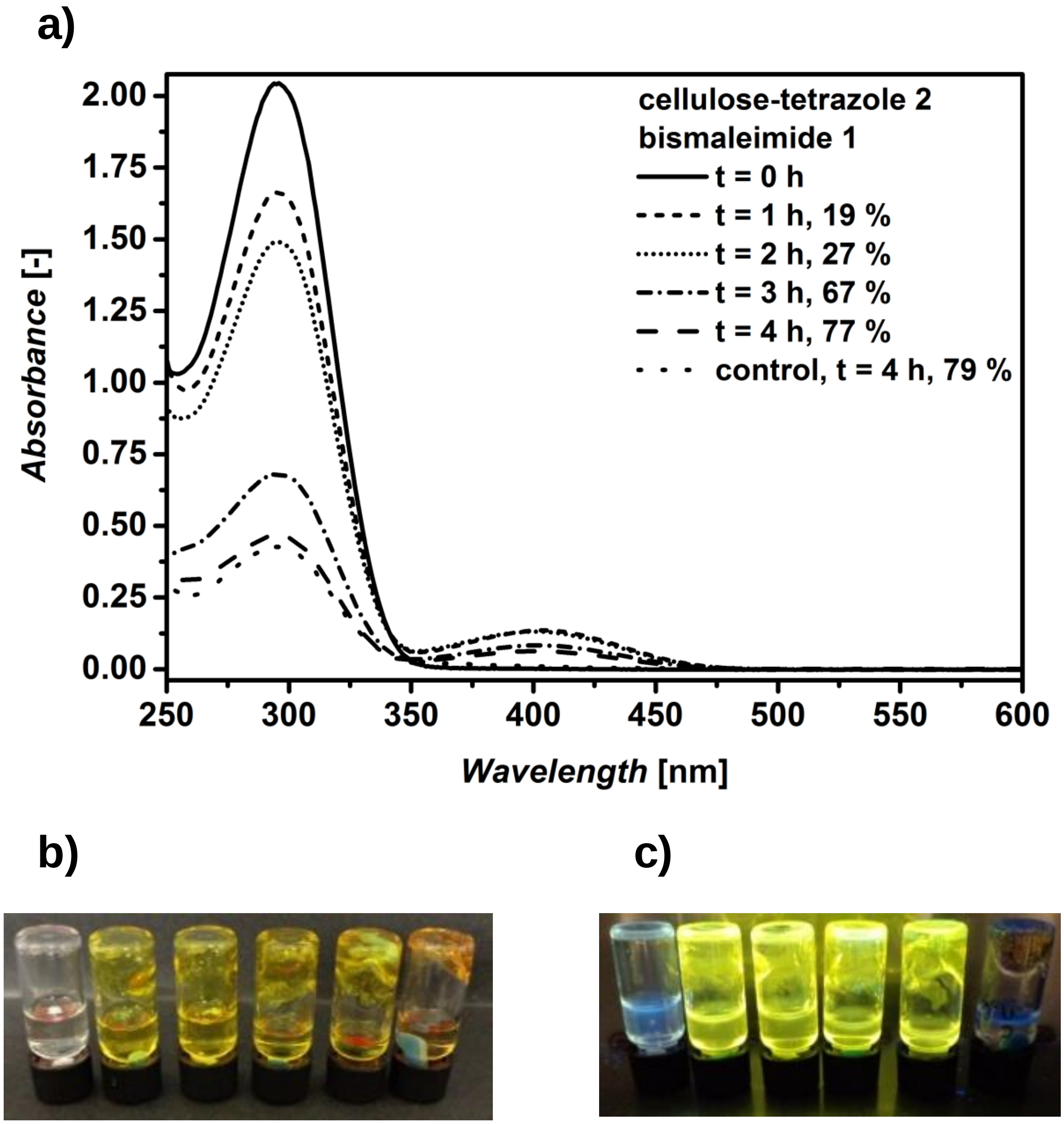


Figure S6: a) UV/vis investigation of the incorporation of **cellulose-tetrazole 2** in networks employing **bismaleimide 1** as crosslinker with increasing irradiation time. The decrease in tetrazole absorbance is given in percent with respect to the peak maximum. **b)** Cellulose network samples corresponding to Figure S6 a after swelling in DMSO for 4 h at daylight (left: t = 0 h; middle:from left to right; t = 1- 4 h; right: control, t = 4 h). **c)** Cellulose network samples from Figure S6 b under irradiation with light at 365 nm.

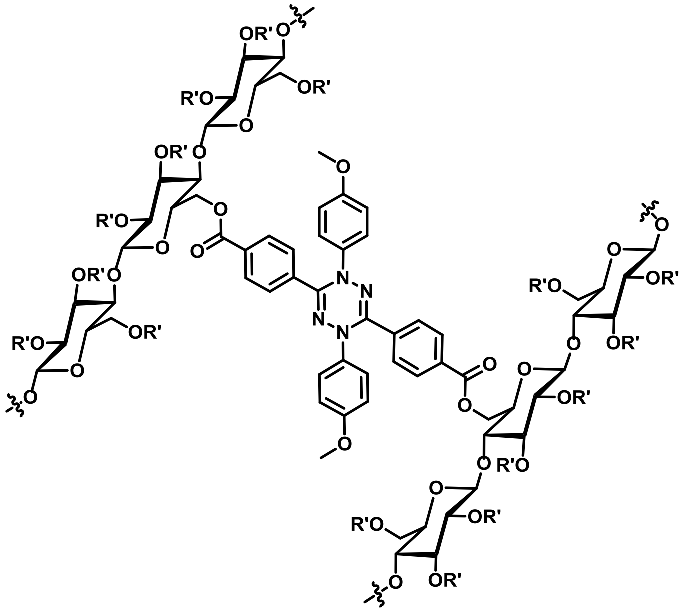


Chart S1: l.4-Dihydro-l.2.4.5-tetrazines forming in the absence of suitable dipolarophiles.[9]

Exemplary procedure for preparation of films of cellulose-tetrazole with bismaleimide linkers. Cellulose-tetrazole 3 (80.0 mg, 398 mmol AGU, 55.7 mmol tetrazole, 1 equiv.) was dissolved in 575.2 µL LiCl/DMSO (4 g L^{-1}) at 60 °C overnight. 74.8 µL of a solution of **bismaleimide 1** in LiCl/DMSO (142 g L^{-1}, 27.8 mmol, 0.50 equiv./tetrazole) or solid **bismaleimide 2** (12.7 mg, 27.8 mmol, 0.50 equiv./tetrazole) and 74.8 µL LiCl/DMSO was added and the resulting solution was stirred at 60 °C for 1 h. The solution was drop cast onto a glass slide and spread into a film with a thickness of 120 µL employing a scraper. A few drops of methanol were placed on the films to induce physical crosslinking. For the spatial resolution experiments, a glass slide with a shadow mask with a hole of approx. 5 mm diameter, consisting of a piece of aluminum foil that was treated with a standard hole puncher, was placed on top of the film. The films were crosslinked in a custom-built photoreactor equipped with a single compact low-pressure fluorescent lamp (36 W, Arimed B6, λ_{max} = 320 nm, Figure S4 and S5) in a 90° angle for 12 h. The samples were removed from the glass slides and shaken in DMSO at 60 °C and 400 min^{-1} for 6 h. Subsequently, the samples were removed from DMSO, rinsed with DMSO and subsequently with ethanol, immersed in ethanol overnight, rinsed with ethanol again and dried at 50 °C under vacuum for 8 h.

Table S2: Experimental conditions for the formation of cellulose thin films using **cellulose-tetrazol 2** or **cellulose-tetrazole 3** and **bismaleimide 1** or **bismaleimide 2**.

	c(cellulose-tetrazole-2) [g L^{-1}]	*c*(cellulose-tetrazole-3) [g L^{-1}]	bismaleimide 1 [equiv./tetrazole]	bismaleimide 2 [equiv./tetrazole]	solid content [wt%]
network 1	123	-	0.50	-	12
network 2	123	-	-	0.50	12
network 3	-	123	0.50	-	11
network 4	-	123	-	0.50	11

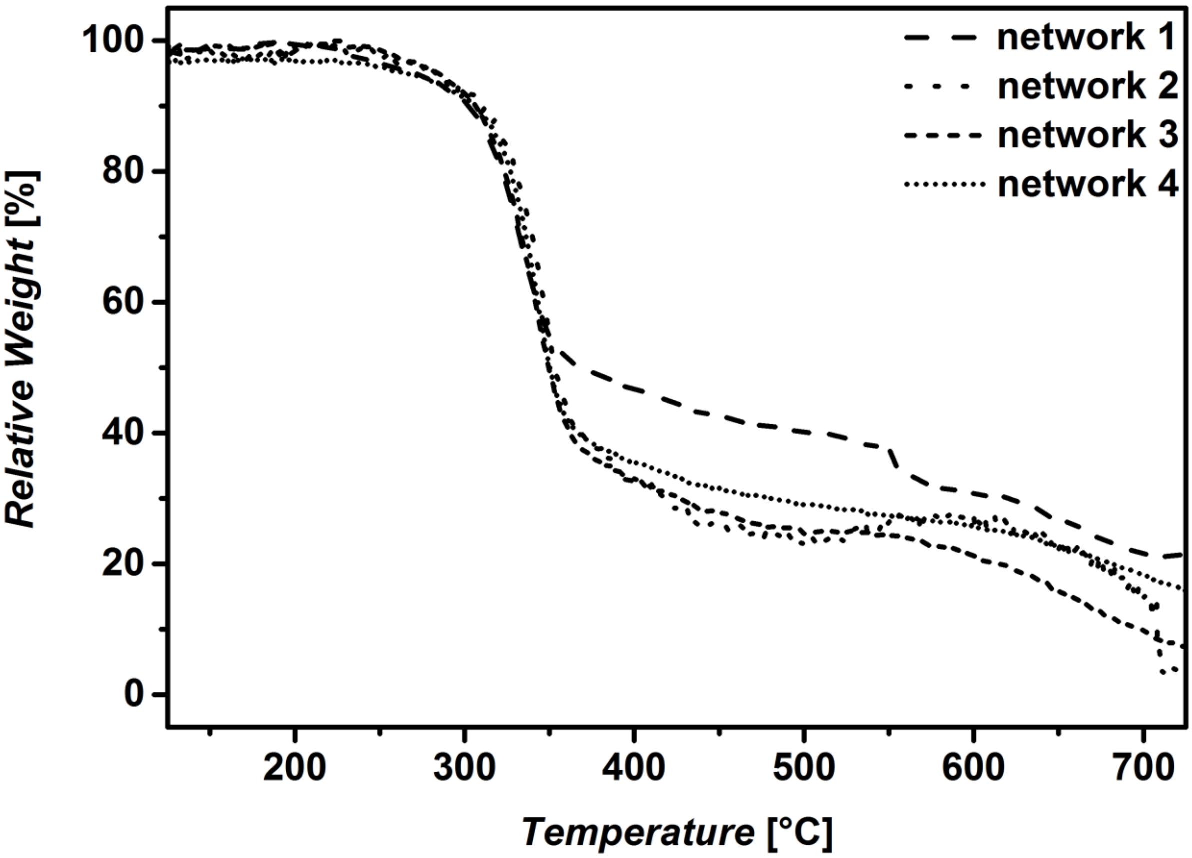
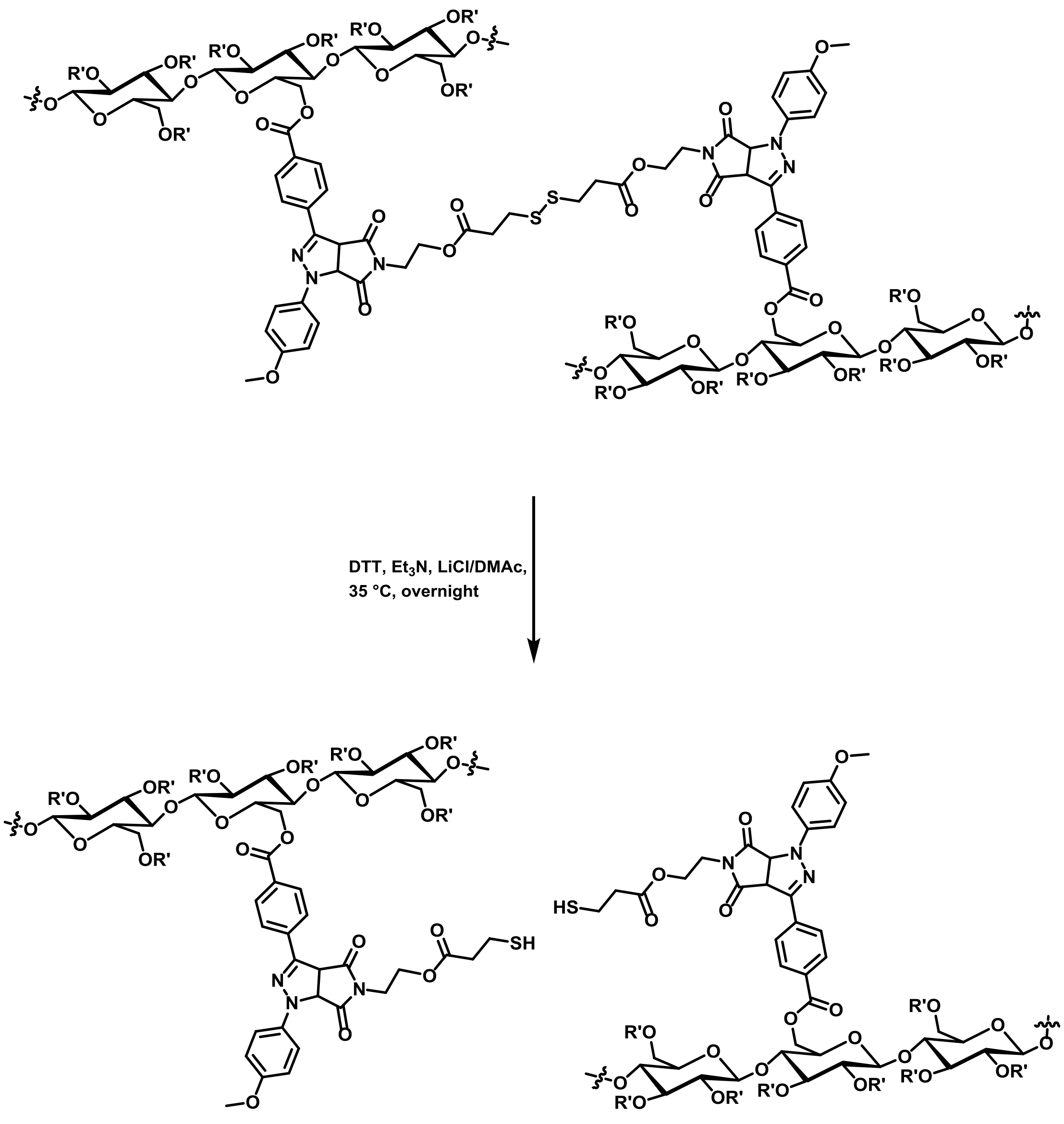


Figure S7: TGA data on **networks 1-4** obtained at a heating rate of 10 K min^{-1}.



Scheme S6: Reduction of disulfide bonds of **network 2** and **network 4** resulting in the cleavage of crosslinks.

Exemplary procedure for reduction of cellulose-tetrazole bismaleimide networks

Network 4 (1.65 mg) was swollen in 1 mL DMSO at 60 °C in a shaker at 200 min^{-1} overnight. Subsequently, the DMSO was decanted and 16.5 mg DTT, 14.9µL triethylamine, and 1.6 mL LiCl/DMAc (10 g L^{-1} for SEC) were added. The sample was degassed with argon for 10 min and placed in a shaker at 35 °C and 200 min^{-1} overnight. Finally, the solution was filtered through a syringe filter and subjected to SEC analysis.

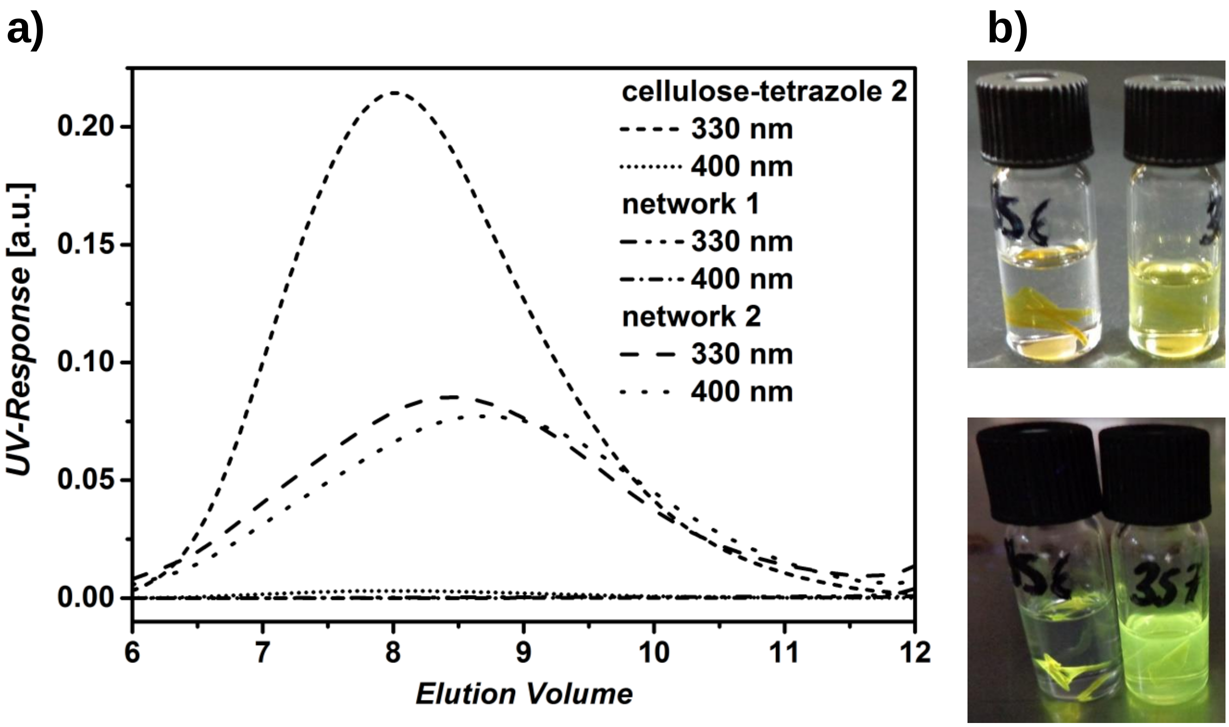


Figure S8: a) SEC traces of **cellulose-tetrazole 2** and **network 1** and **network 2** after treatment with DTT measured with a UV detector at 330 nm and 400 nm. **b) network 1** (left) and **network 2** (right) in DMAc/LiCl after treatment with DTT at daylight (top) and under irradiation at 365 nm (bottom).

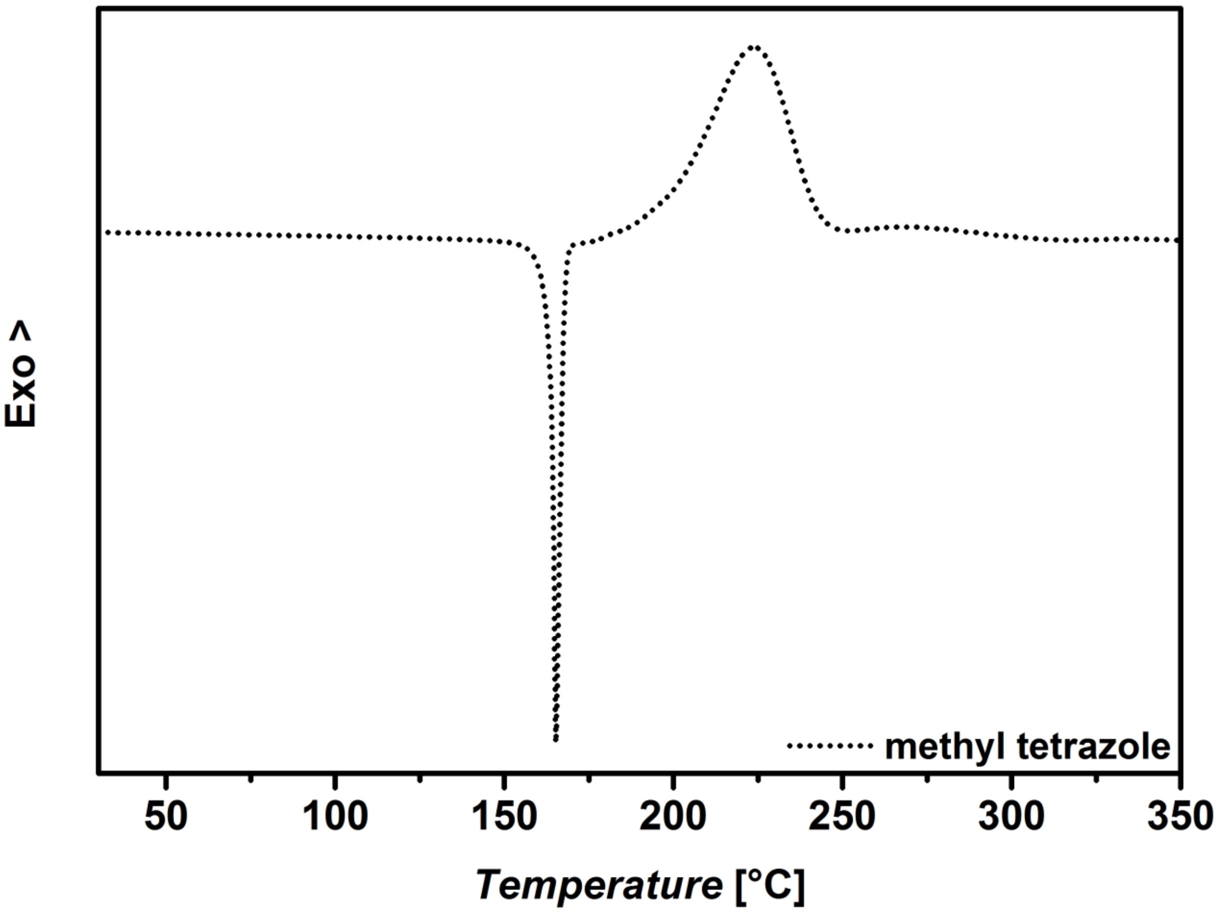


Figure S9: DSC trace of methyl tetrazole.

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