Supporting Information to

Metathesis Polymerization of Cystine-Based Macrocycles

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Analytical instrumentation and methods

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 300 MHz, 500 MHz, or Bruker Avance III 600 MHz spectrometers. Samples were prepared in $CDCl_3$, DMSO-d_6, or CD_3CN. Signals were referenced to the respective solvent peaks; $CDCl_3$ δ (^{1}H) 7.26 ppm, DMSO-d_6 δ (^{1}H) 2.50 ppm, δ (^{13}C) 39.52 ppm, CD_3CN δ (^{1}H) 1.94 ppm.

Electronspray ionization time-of-flight (ESI-ToF) mass spectrometry was measured in positive ionization mode on a Micromass Q-TOF Micro (Waters Inc.). Samples were prepared in acetonitrile acidified with 0.2% formic acid. Ortho-phosphoric acid was used as the standard.

Elemental analysis (C/H/N/S) was performed on an Vario EL III (Elementar Analysensysteme GmbH), operating with helium as carrier gas.

Melting points were obtained by a MEL-TEMP II device (Laboratory Devices Inc., USA); measurements were conducted in open capillaries.

Size exclusion chromatography (SEC) with simultaneous UV and RI detection was performed with THF as the eluent (flow rate: 0.5 mL·min^{-1}) at room temperature. The stationary phase used was a 300 x 8 mm^2 PSS SDV linear M column (3 µm particle size, molar mass range 10^2-10^6 Da). Solutions containing ~0.15 wt% polymer were filtered through 0.45 µm filters; the injected volume was 100 µL. Polystyrene standards (PSS, Mainz, Germany) were used for calibration.

Static light scattering (SLS) was performed at RT using an ALV-7004 multiple-tau digital correlator in combination with a CGS-3 compact goniometer and a He-Ne laser (Polytec, 34 mW, λ = 633 nm). Stock solutions of polymers were prepared in ethyl acetate or water and filtered with 0.45 µm PTFE or PVDF syringe filters. Solutions with different polymer concentrations were measured at scattering angles from 70°-150° in 10° steps. Data evaluation was carried out using Zimm plot models. Refractive index increments (dn/dc) were measured with a PSS DnDc2010 device operating at λ = 620 nm.

Thermogravimetric analysis (TGA) was done on a Mettler Toledo TGA/SDTA851 from 25-900 °C at a heating rate of 10 °C min^{-1} under a nitrogen flow of 20 mL min^{-1}. Differential scanning calorimetry (DSC) was carried out on a Mettler Toledo DSC822e or Netzsch DSC 214 Polyma at -80-140 °C under a nitrogen flow. The glass transition temperature was determined from the third heating curve at a heating rate of 10 °C min^{-1} (cooling rate: 20 °C min^{-1}).

Chemicals

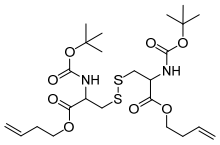
Potassium carbonate (99+%), triethylamine (TEA) (99%, pure), hydrogen chloride (1M solution in ethyl acetate), and ethyl vinyl ether (99%, stabilized) were received from Acros Organics. *para*-Benzoquinone (≥98%, reagent grade), acetic anhydride (≥99%, puriss), succinic anhydride (96%), *N,N*-dimethyl-formamide (DMF) (≥99.8% puriss, absolute), DMSO-d_6 (99.9% atom D), 4-bromo-1-butene (97%), 11-bromo-1-undecene (95%), (1,3-Bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(*o*-isopropoxy-phenylmethylene)-ruthenium (Hoveyda-Grubbs catalyst 2nd generation (HG2)) (97%), and [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)bis(3-bromopyridine) ruthenium(II) (Grubbs catalyst 3rd generation (G3)) (purity n/a, batch no. MKBS6084V) were purchased from Sigma-Aldrich. Heptanes (isomers, ≥99%, p.a.), tetrahydrofuran (THF) (99.5%), and 1,4-dithioerythritol (DTE) (≥99%, p.a.) were received from Carl Roth. Ethyl acetate (technical 99%), n-pentane (99.0%, rectapur), toluene (99.0%, rectapur), acetonitrile-d_3 (99.8% atom D), and chloroform (HPLC) were received from VWR. *N,N'*-Di-(*tert*-butyloxycarbonyl)-L-cystine, (Boc-L-Cys-OH)$_2$, **1**, was purchased from Iris Biotech. Chloroform-d_1 (99.8% atom D) was received from Deutero GmbH. Silica gel (60M, 0.04–0.063 mm) was purchased from Macherey-Nagel.

Chloroform used for polymerizations was stored for at least one day over molecular sieves (3 Å), ethyl acetate, heptanes, and toluene were distilled in a rotatory evaporator prior to use.

Experimental procedures

Unless otherwise noted all reactions were carried out under nitrogen atmosphere.

N,N'-Di-Boc-L-cystine-di(but-3-enyl)-ester **2a**



To a suspension of 8.0 g of *N,N*'-di-Boc-L-cystine and 7.6 g potassium carbonate in 75 mL of DMF, 5.6 mL of 4-bromobut-1-ene was slowly added. The resulting mixture was stirred for 4 days at room temperature. The suspension was poured into diethyl ether and the remaining solids were filtered out and washed with additional diethyl ether. The clear yellowish solution was washed with saturated sodium bicarbonate solution, saturated sodium chloride solution and water. The organic phase was dried with magnesium sulfate. After the filtration the solution was concentrated on a rotatory evaporator and again solubilized in diethyl ether and filtrated over a silica pad. Removing the solvent in vacuum resulted in 9.0 g of a white solid (yield: 90%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.45-6.94 (m, 2H), 5.79 (ddt, 2H), 5.19-4.99 (m, 4H), 4.24 (td, J = 9.0, 4.9 Hz, 2H), 4.19-4.01 (m, 4H), 3.12-2.83 (m, 4H), 2.34 (q, J = 6.5 Hz, 4H), 1.38 (s, 18H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.80, 155.23, 134.22, 117.18, 78.44, 63.73, 52.78, 38.90, 32.46, 28.07. Anal. calculated for $C_{24}H_{40}N_2O_8S_2$: C 52.5, H 7.4, N 5.1, O 23.3, S 11.7; found: C 52.6, H 7.7, N 5.1, S 11.9.

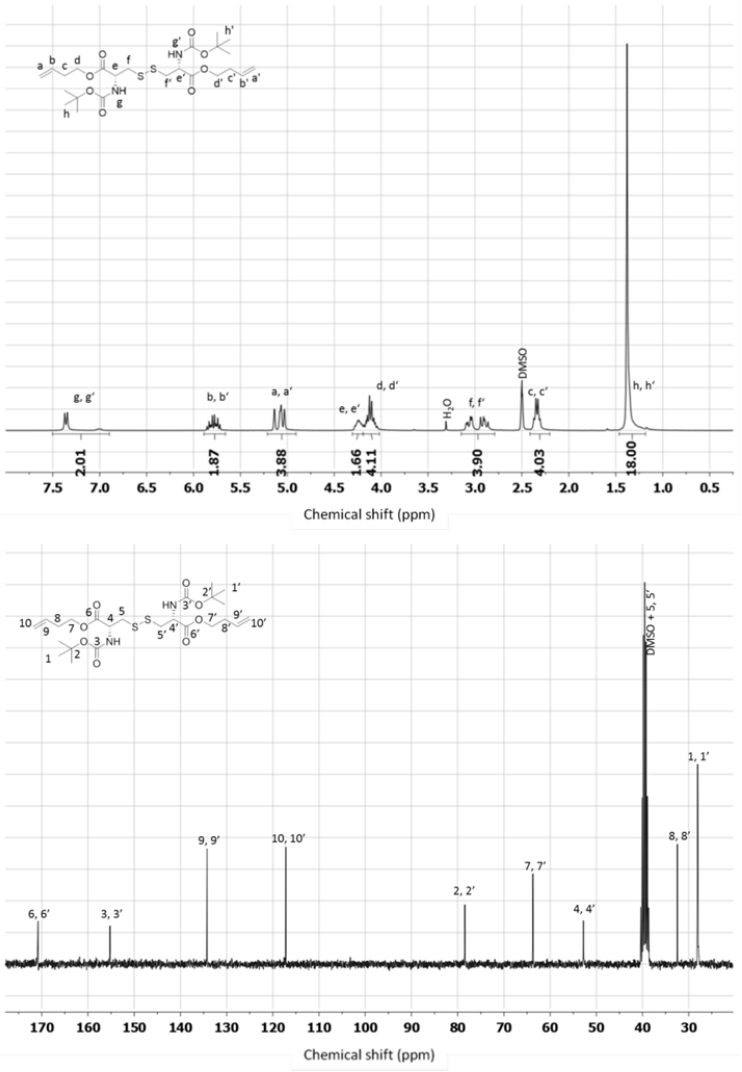
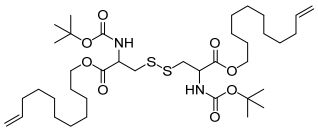


Figure S1. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) (top) and ^{13}C NMR spectrum (75 MHz, DMSO-d_6) (bottom) of **2a**.

<u>*N,N*'-Di-Boc-L-cystine-di(undec-10-en-1-yl)-ester **2b**</u>



To a suspension of 2.5 g of *N,N*'-di-Boc-L-cystine and 2.35 g potassium carbonate in 23 mL of DMF, 3.75 mL of 11-bromoundec-1-ene was slowly added. The resulting mixture was stirred for 6 days at room temperature. The suspension was poured on diethyl ether and the remaining solids were filtered out and washed with additional diethyl ether. The clear solution was washed with saturated sodium bicarbonate solution, saturated sodium chloride solution and water. The organic phase was dried with magnesium sulfate. After filtration the solution was concentrated in a rotatory evaporator to yield a yellow oil, which was dissolved in 50 mL of n-pentane and put in a freezer at -20 °C overnight. The resulting gel was partially redissolved by warming it slightly and subsequently placed back in the freezer. After two hours, a precipitate was formed, which was filtered and redissolved in 50 mL of n-pentane. The gelation

precipitation procedure was repeated to afford 2.6 g of a white solid (yield: 61%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.54-6.79 (m, 2H), 5.78 (ddt, J = 16.9, 10.2, 6.6 Hz, 2H), 5.13-4.77 (m, 4H), 4.32-4.16 (m, 2H), 4.13-3.92 (m, 4H), 3.12-2.83 (m, 4H), 2.00 (dd, J = 13.8, 6.8 Hz, 4H), 1.65-1.49 (m, 4H), 1.38 (s, 18H), 1.25 (s, 24H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.85, 155.20, 138.72, 114.51, 78.37, 64.68, 52.84, 33.15, 28.86, 28.76, 28.60, 28.48, 28.24, 28.05, 25.24. Anal. calculated for $C_{38}H_{68}N_2O_8S_2$: C 61.3, H 9.2, N 3.8, O 17.2, S 8.6; found: C 61.3, H 10.5, N 3.8, S 8.8.

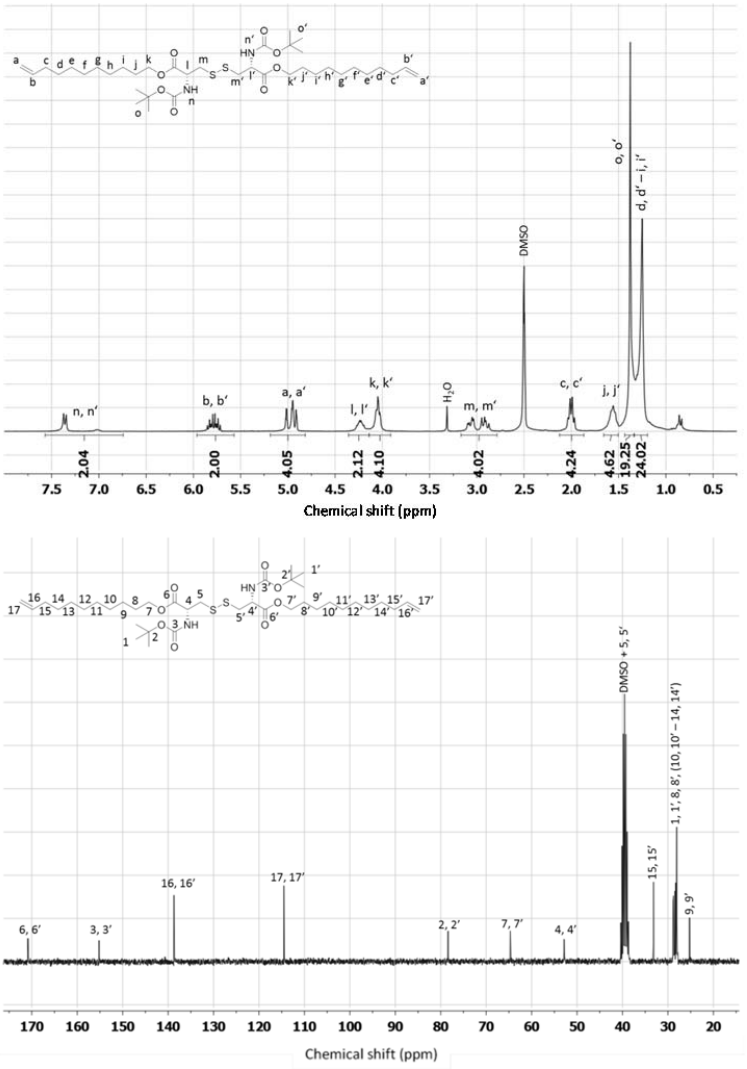
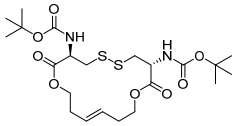


Figure S2. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) (top) and ^{13}C NMR spectrum (75 MHz, DMSO-d_6) (bottom) of **2b**.

(3*R*,8*R*)-3,8-bis(Boc-amino)-1,10-dioxa-5,6-dithiacyclohexadec-13-ene-2,9-dione **3a**



5.2 g of **2a**, 63 mg of HG2, and 22 mg of *p*-benzoquinone were dissolved in 1 L of DCM and refluxed for 6h. The reaction was monitored by TLC (heptanes/ethyl acetate 4:1) The reaction was quenched by the addition of 5 mL of ethyl vinyl ether and refluxed for additional 15 minutes, followed by the addition of 5 mL of DMSO and refluxing for

another 15 minutes. The reaction mixture was stirred overnight at room temperature and afterwards concentrated in vacuum. The brown oil was purified by column chromatography over silica (heptanes/ethyl acetate 5:1). The resulting off-white solid was suspended in warm heptanes. After cooling to room temperature the solvent was decanted, resulting in a white solid (4.4 g ,yield: 85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.44-6.91 (m, 2H), 5.65-5.33 (m, 2H), 4.45-4.35 (m, 2H), 4.34-4.21 (m, 4.3 Hz, 2H), 4.09-3.93 (m, 2H), 3.07-2.72 (m, 4H), 2.43-2.20 (m, 4H), 1.38 (s, 18H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.18, 155.42, 128.83, 78.45, 63.90, 51.00, 35.12, 31.00, 28.10, 26.62. ESI-ToF *m/z* calculated for $[C_{22}H_{37}N_2O_8S_2]^+$ $[M+H]^+$:521.1991, found: 521.1996. Anal. calculated for $C_{22}H_{36}N_2O_8S_2$: C 50.8, H 7.0, N 5.4, O 24.6, S 12.3; found: C 50.8, H 7.3, N 5.3, S 12.5.

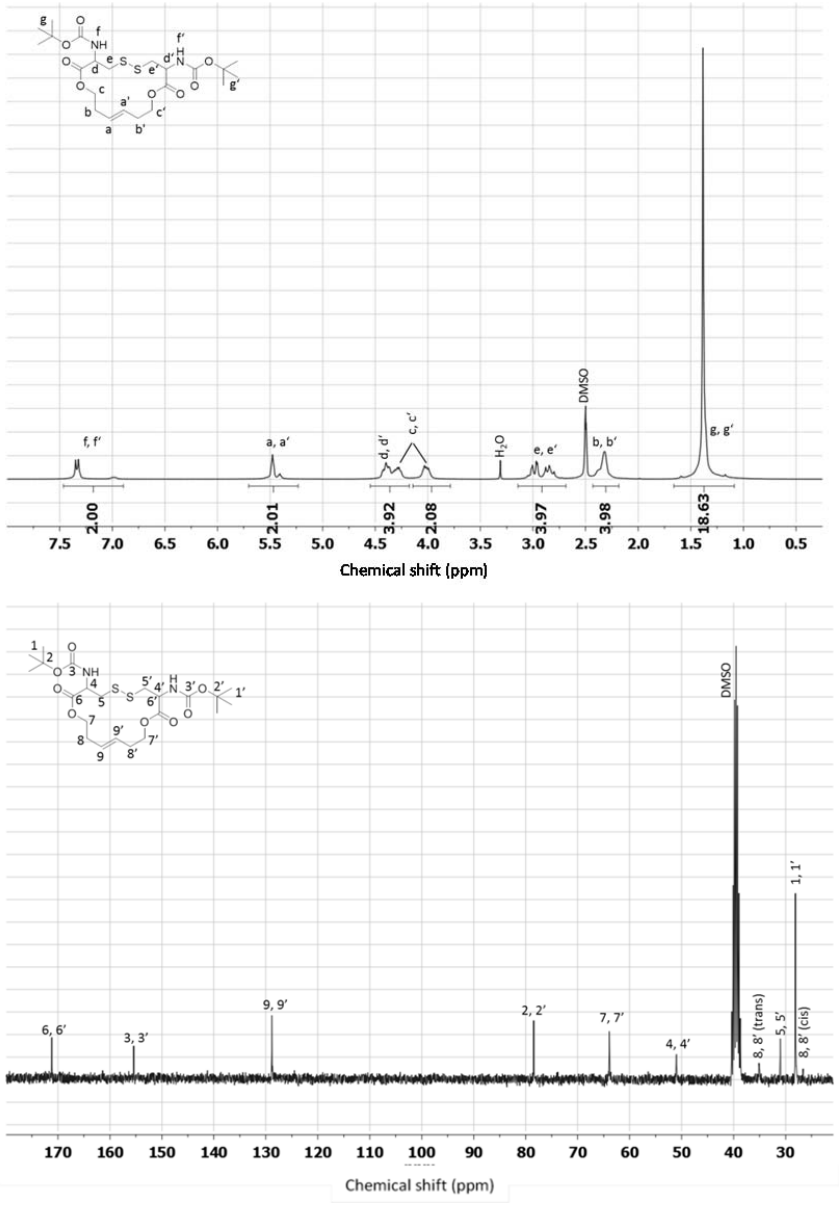


Figure S3. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) (top) and ^{13}C NMR spectrum (75 MHz, DMSO-d_6) (bottom) of **3a**.

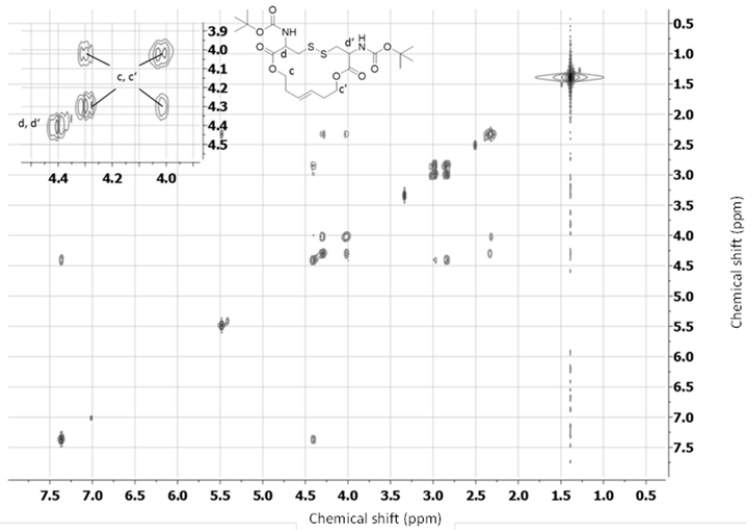


Figure S4. 2D [^{1}H,^{1}H] COSY NMR spectrum (300 MHz, DMSO-d_6) of **3a**. Inset shows the expanded region between 3.8 and 4.6 ppm revealing the geminal coupling of -OCH_2- groups.

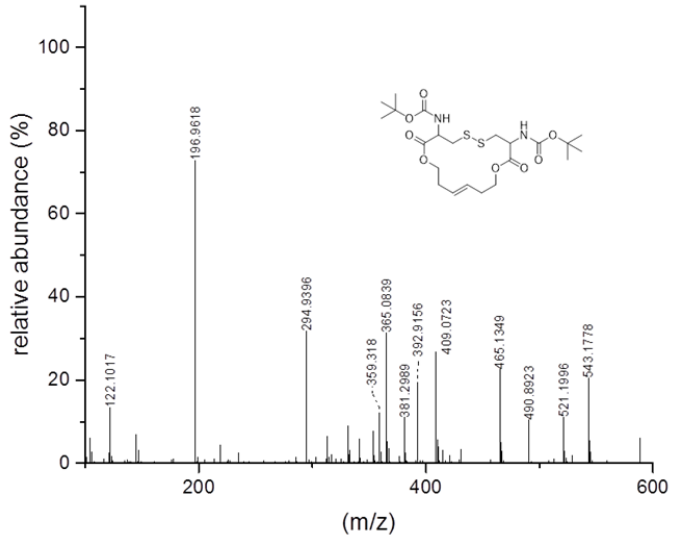
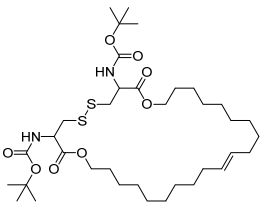


Figure S5. ESI-ToF mass spectrum of **3a**.

(3*R*,8*R*)-3,8-Bis(Boc-amino)-1,10-dioxa-5,6-dithiacyclotriacont-20-ene-2,9-dione **3b**



200 mg of **2b**, 8.4 mg HG2, and 2.8 mg of *p*-benzoquinone were dissolved in 27 mL of $CHCl_3$ and heated to 50 °C for 16 h. The reaction was monitored by TLC (toluene/ethyl acetate 9:1). The reaction was quenched by the addition of 200 µL of ethyl vinyl ether and stirred at 50 °C for additional 15 minutes, followed by the addition of 200 µL of DMSO and another 15 minutes of stirring at 50 °C. The reaction mixture was stirred overnight at room temperature and afterwards concentrated in vacuum. The brown oil was dissolved in DCM and adsorbed on silica gel and purified by column chromatography over silica (toluene/ethyl acetate 9:1) to give 60 mg of a white solid (yield: 31%). ^{1}H NMR (600 MHz,

DMSO-d_6) δ 7.55-6.75 (m, 2H), 5.38-5.25 (m, 2H), 4.30-4.23 (m, 2H), 4.11-3.98 (m, 4H), 3.10-2.85 (m, 4H), 1.98-1.93 (m, 4H), 1.60-1.53 (m, 4H), 1.40-1.33 (m, 18H), 1.33-1.28 (m, 8H), 1.24 (s, 16H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.93, 155.27, 130.30, 78.47, 64.77, 52.81, 38.93, 31.57, 28.80, 28.48, 28.39, 28.11, 28.03, 27.66, 26.27, 25.31. ESI-ToF *m/z* calculated for $[C_{36}H_{65}N_2O_8S_2]^+$ $[M+H]^+$: 717.4182, found: 717.4180. Anal. calculated for $C_{36}H_{64}N_2O_8S_2$: C 60.3, H 9.0, N 3.9, O 17.9, S 8.9; found: C 60.4, H 9.6, N 3.8, S 9.4.

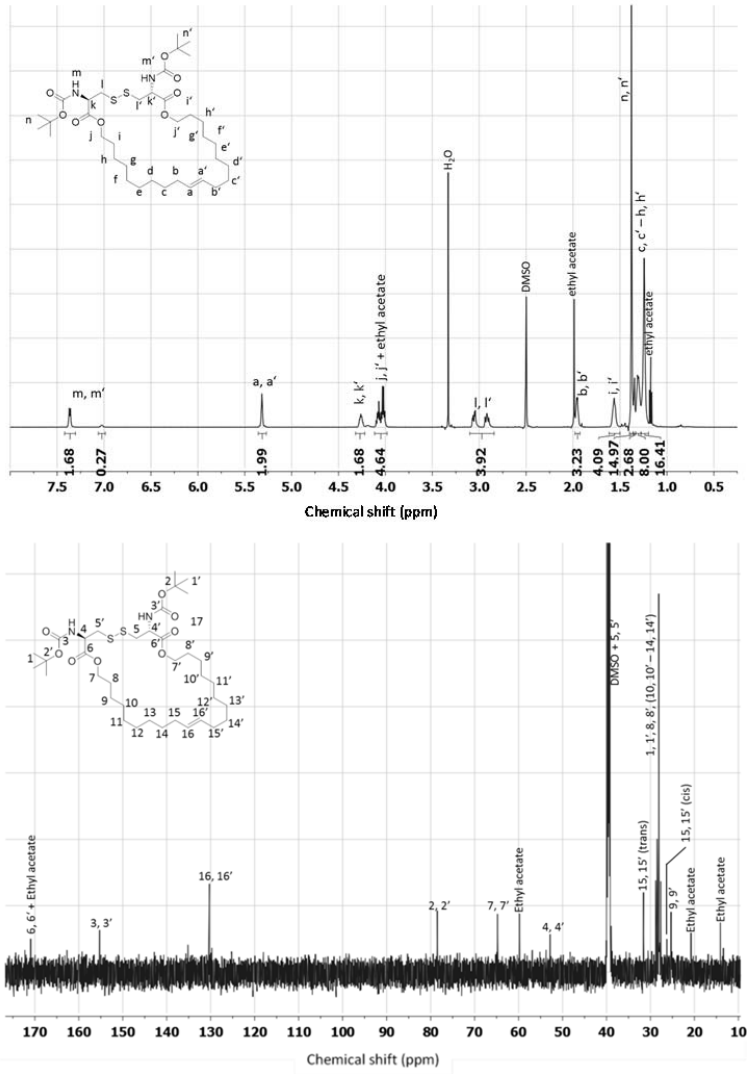


Figure S6. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) (top) and ^{13}C NMR spectrum (151 MHz, DMSO-d_6) (bottom) of **3b**.

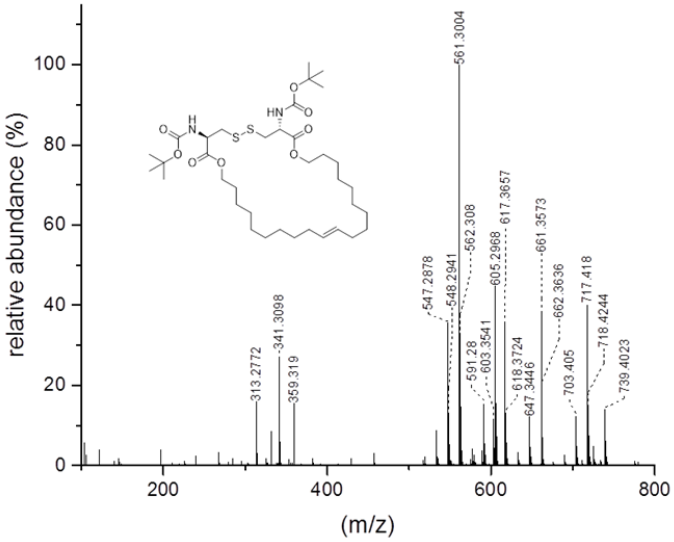


Figure S7. ESI-ToF mass spectrum of **3b**.

ADMET polymerization of **2a**

A) 550 mg of **2a** were placed in a 10 mL round-bottom flask equipped with a condenser and dissolved in 5 mL of DCM (0.2 M). To this 31.3 mg HG-2 and 2.2 mg of para-benzoquinone were added and the resulting solution was heated to 40 °C for 42 h. The headspace of the condenser was constantly flushed with nitrogen during the course of the reaction. The reaction was quenched by the addition of an excess of ethyl vinyl ether.

B) 200 mg of **2a** were placed in a 1 mL of conical flask equipped with a condenser and dissolved in 0.3 mL of $CHCl_3$ (1.2 M). To this 11.4 mg HG-2 and a pinch of para-benzoquinone were added and the resulting solution was heated to 40 °C for 24 h. The headspace of the condenser was constantly flushed with nitrogen during the course of the reaction. The reaction was quenched by the addition of an excess of ethyl vinyl ether.

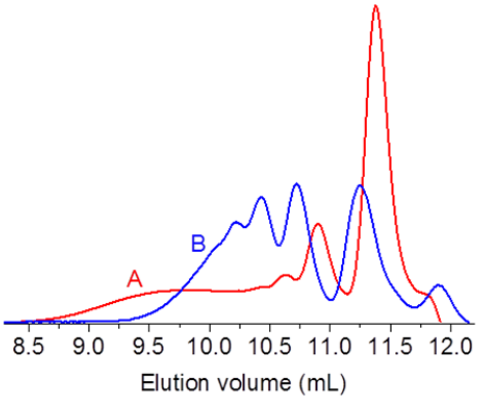


Figure S8. SEC-RI traces (eluent: THF) of the crude products obtained by ADMET polymerizations **A** and **B**, revealing the presence of just low molar mass and cyclic products.

Metathesis polymerization (ED-ROMP) of **3a** (➞ polymer **4a**) and **3b** (➞ polymer **4b**)

The macrocyclic monomer (**3a** or **3b**) was placed in a 2.5 mL vial equipped with a stir bar and a septum cap and purged with nitrogen. One half of the total solvent amount was added via a syringe and the dispersion was tempered at 40-45 °C. To this dispersion a stock solution containing the G3 catalyst in chloroform was added. The reaction was quenched by the addition of ethyl vinyl ether and stirred for additional 15 minutes. Purification of the polymer (**4a** or **4b**) was done by dialysis (Spectra/Por® dialysis tubing, molecular weight cutoff: 3500 Da) against THF. **4a** ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.43-6.96 (m, 2H), 5.50 (s, 2H), 4.30-4.13 (m, 2H), 4.12-3.98 (m, 4H), 3.13-2.84 (m, 4H), 2.41-2.23 (m, 4H), 1.42-1.32 (m, 18H). **4b** ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.45-5.30 (m, 4H), 4.63-4.51 (m, J = 5.7 Hz, 2H), 4.14 (qt, J = 10.7, 6.8 Hz, 4H), 3.25-2.99 (m, 4H), 2.05-1.91 (m, J = 29.3, 13.7, 8.2 Hz, 4H), 1.70-1.60 (m, 4H), 1.45 (s, 18H), 1.38-1.21 (m, 24H).

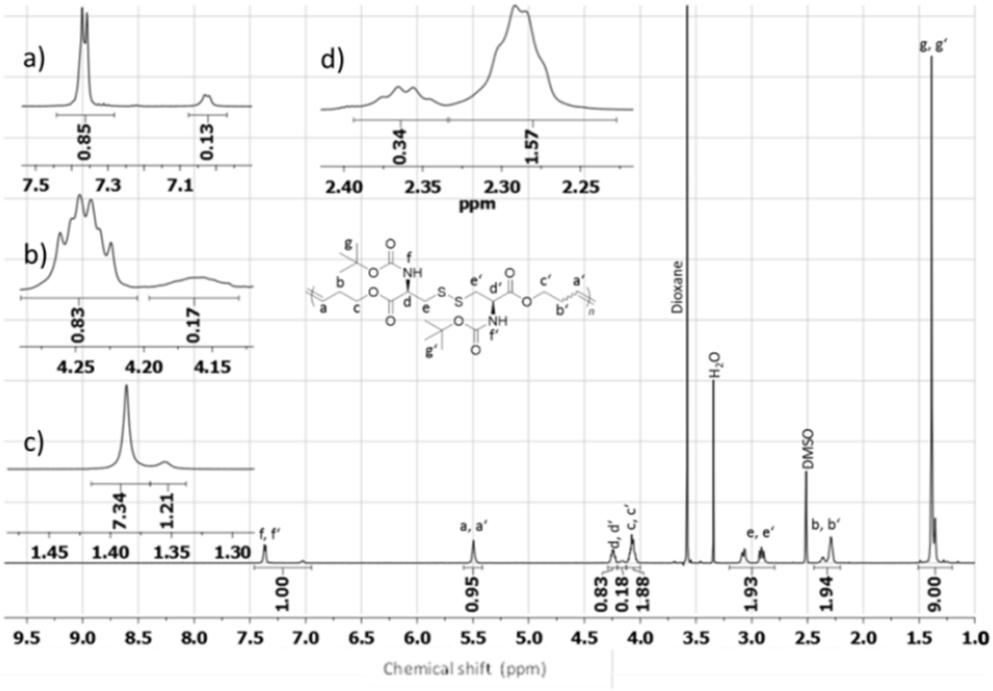


Figure S9. ^{1}H NMR (600 MHz, DMSO-d_6) of polymer **4a**. Expanded regions show two signals for the a) NH urethane (*f*, *f'*) b) α-CH (*d*, *d'*) and c) *tert*-butyl group (*g*, *g'*) due to the hindered rotation of the Boc group; d) two signals of -CH_2-C= (*b, b'*) arise from the *cis* and *trans* isomers of the double bond.

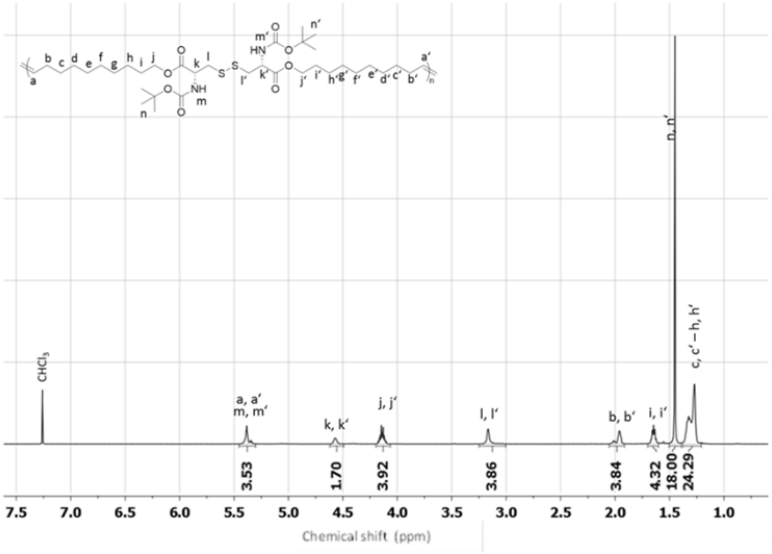


Figure S10. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of polymer **4b**.

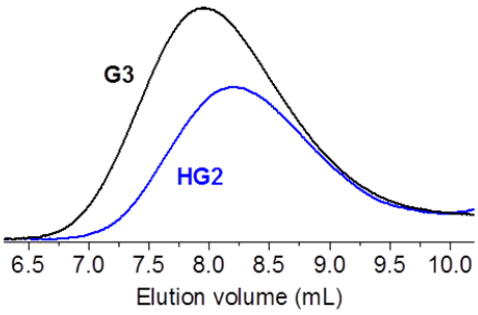


Figure S11. SEC-RI traces (eluent: THF) of the polymer products obtained by polymerization of **3a** with catalysts G3 (→ **4a**, monomer conversion: 80%) and HG2 (70%). Polymerizations were performed applying the same reaction conditions (see above).

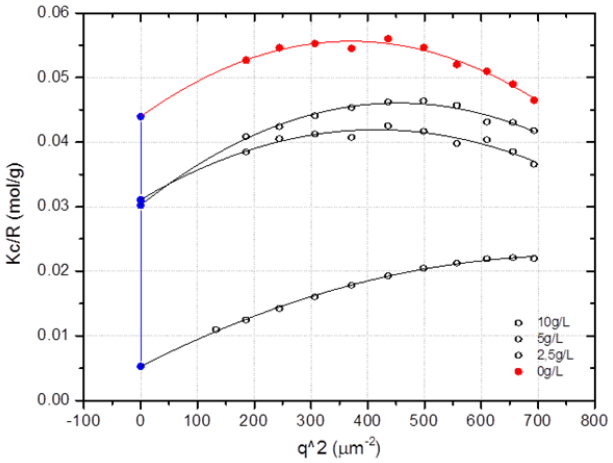


Figure S12. Concentration- and angle-dependent SLS data for polymer **4a** in ethyl acetate at room temperature (d*n*/d*c* = 0.128 mL/g).

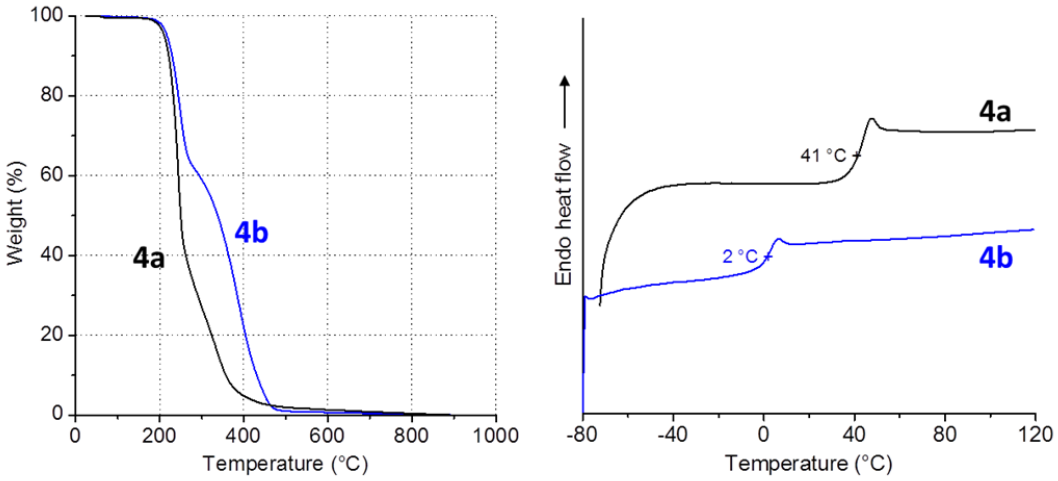


Figure S13. TGA (left) and DSC (right) curves of polymers **4a** and **4b**. Thermal decomposition proceeds in two steps at 160-170 °C (decomposition of Boc protecting group) and >260 °C (decomposition of polymer backbone). Glass transitions occurred at 41 °C (**4a**) and 2 °C (**4b**).

Deprotection of polymer **4a** (→ **5a**) and **4b** (→ **5b**)

20 mg of **4a** (or **4b**) were placed in a dried 2.5 mL vial equipped with a stir bar and a septum cap and purged with nitrogen. To this 420 µL of 1 M hydrochloric acid in ethyl acetate was added via a syringe. The reaction was stirred at room temperature for 2 days. The solvent was evaporated in vacuum and the resulting solid was dispersed in diethyl ether. The solvent was again evaporated to give the deprotected polymer **5a** (or **5b**). **5a** ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.97 (bs, 6H), 5.59 (bs, 2H), 4.31 (bs, J = 18.5, 4.9 Hz, 2H), 4.18 (bs, 4H), 3.53-3.12 (bs, 4H), 2.37 (bs, 4H). **5b** ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (s, 6H), 5.46-5.23 (m, 2H), 4.33 (t, J = 5.5 Hz, 2H), 4.14 (t, J = 6.4 Hz, 4H), 3.46-3.19 (m, 4H), 2.01-1.86 (m, J = 33.6, 9.4 Hz, 4H), 1.68-1.52 (m, 4H), 1.38-1.16 (m, 24H).

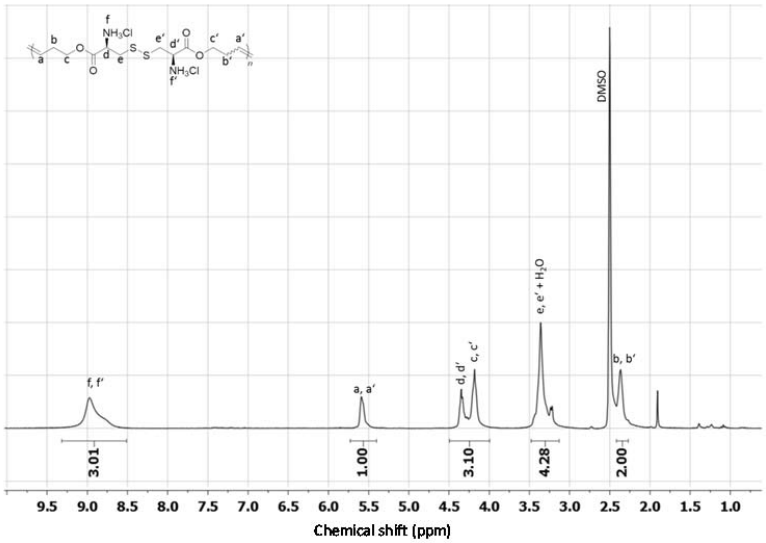


Figure S14. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of deprotected polymer **5a**.

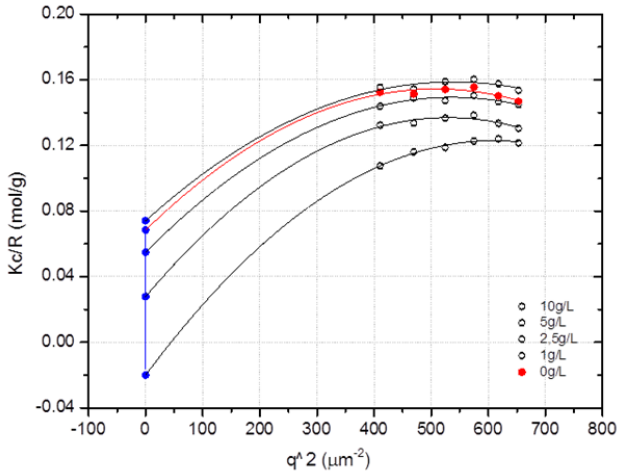


Figure S15. Concentration- and angle-dependent SLS data for polymer **5a** in water at room temperature (d*n*/d*c* = 0.184 mL/g).

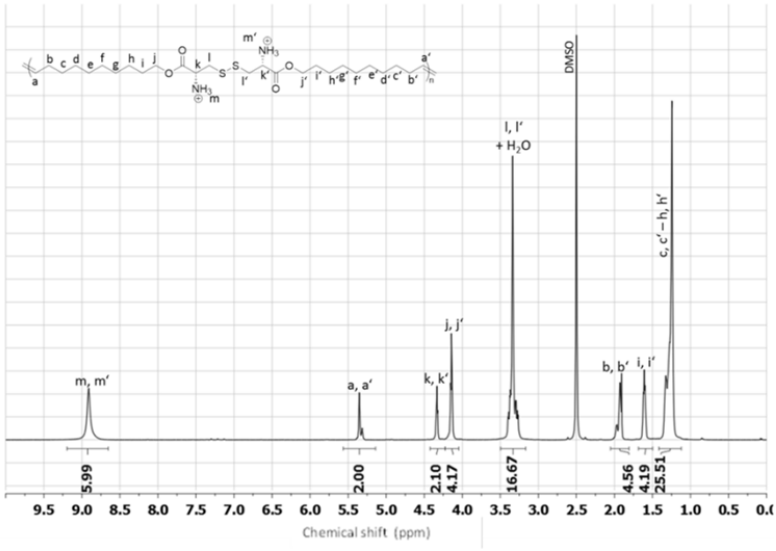


Figure S16. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of deprotected polymer **5b.**

Deprotection of **3a** and **3b** macrocycles

20 mg of **3a** (**3b**) were placed in a dried 2.5 mL vial equipped with a stir bar and a septum cap and purged with nitrogen. To this 420 µL of 1 M hydrochloric acid in ethyl acetate was added via a syringe. The reaction was stirred at room temperature overnight. The solvent was evaporated in vacuum and the resulting solid was dispersed in diethyl ether. The solvent was again evaporated. ^{1}H NMR of the product revealed a conversion of 98%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (s, 6H), 5.62-5.45 (m, J = 37.6 Hz, 2H), 4.34-4.23 (m, 4H), 4.22-4.13 (m, 2H), 3.27 (ddd, J = 18.3, 14.3, 6.0 Hz, 4H), 2.46-2.29 (m, J = 56.6 Hz, 4H).

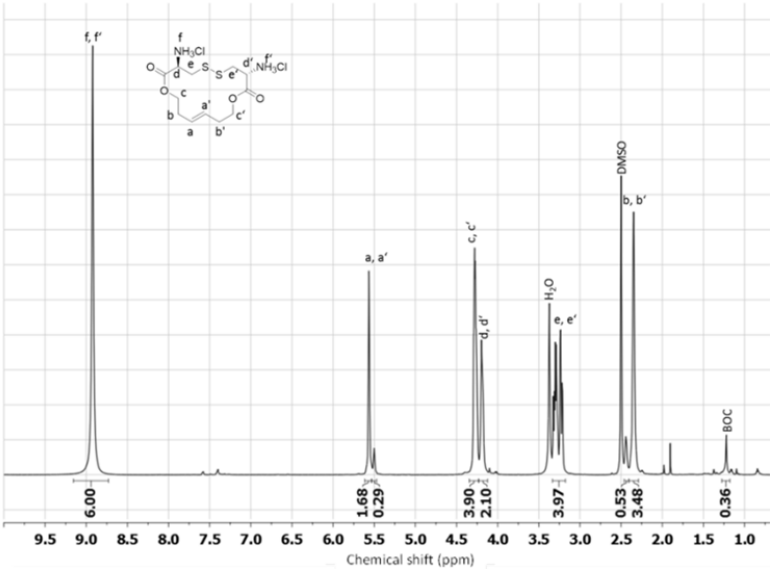


Figure S17. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of the deprotected macrocycle **3a**.

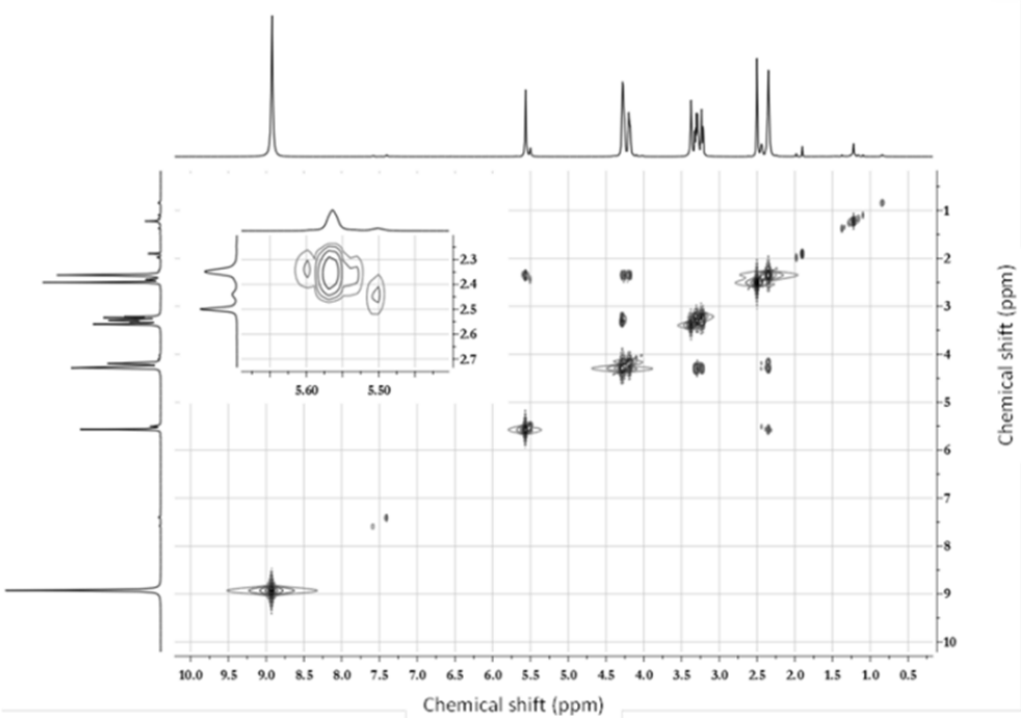


Figure S18. 2D [^{1}H,^{1}H] COSY NMR spectrum (600 MHz, DMSO-d_6) of the deprotected macrocycle **3a**. The expanded region shows the coupling between the double bond and the adjacent methylene group. Moreover the *cis* double bond at 5.5 ppm and the peak at 2.45 ppm are coupling, while the *trans* double bond at 5.58 ppm only couples with the peak 2.35 ppm. This indicates that the two peaks at ~2.3 ppm for **4a** (see Figure S6) are due to the *cis* and *trans* isomers.

Post-polymerization modification of the deprotected polymer **5a** with acid anhydrides

50 mg of **5a** were placed in a dried 4 mL vial equipped with a stir bar and a septum cap and purged with nitrogen. To this 1.1 mL of 1 M hydrochloric acid in ethyl acetate was added via a syringe. The reaction was stirred at room temperature for 2 days. The solvent was evaporated in vacuum. The resulting solid was dispersed in 2 ml of DMF. A solution of 670 µL (50 eq.) TEA in 3.2 mL of DMF was prepared in a second 4 mL vial. To this solution acetic anhydride or succinic anhydride was slowly added. An aliquote of 440 µL (correlating to 5 eq. of TEA and 3 eq. of acid anhydride) was taken and added to the deprotected polymer **5a** dispersion. After 5 hours the reaction mixture was diluted with THF and water and dialyzed against THF/water 1:1. **6a** (**5a** + acetic anhydride) ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (d, J = 7.7 Hz, 2H), 5.47 (s, 2H), 4.51 (dd, J = 13.2, 7.9 Hz, 2H), 4.06 (t, J = 6.2 Hz, 4H), 3.14-2.82 (m, 4H), 2.39-2.16 (m, 4H), 1.85 (s, 6H). **7a** (**5a** + succinic anhydride) ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63-8.30 (m, 1H), 5.47 (s, 1H), 4.67-4.44 (m, J = 34.9, 6.8 Hz, 1H), 4.29-3.94 (m, 2H), 3.20-2.81 (m, 2H), 2.43-2.12 (m, J = 24.0 Hz, 6H).

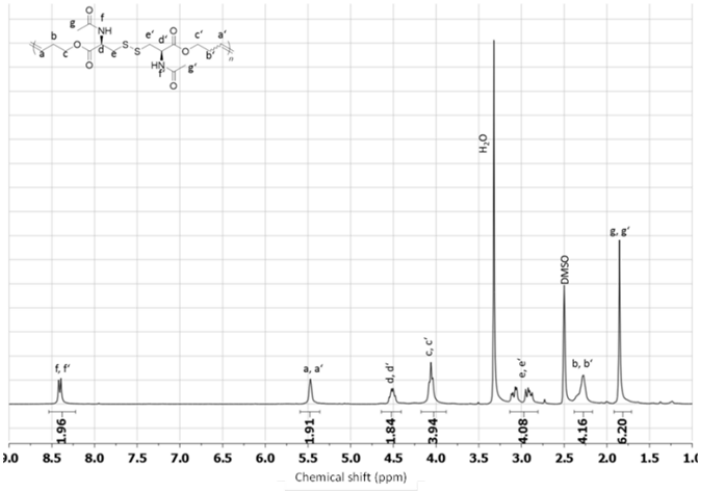


Figure S19. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of functionalized polymer **6a**.

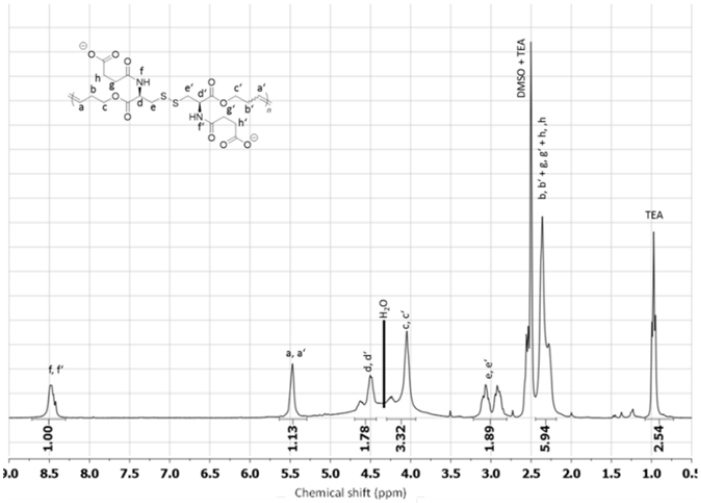


Figure S20. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of functionalized polymer **7a**.

Reduction of main-chain disulfide

15 mg of **4a** (or macrocycle **3a**) were placed in a 2.5 mL vial equipped with a stir bar and a septum cap. 44 mg of DTE and one drop of TEA were dissolved in 200 µL of THF. The resulting solution was added to the polymer (or macrocycle). After 10 and 30 minutes an aliquot was taken and diluted to 1.5 mg mL^{-1} and analyzed by SEC. After 30 minutes the reaction was quenched by the addition of an aqueous saturated ammonium chloride solution. The resulting mixture was extracted with DCM. The aqueous phase was discarded and the organic layer was washed with brine and water. The solvent was evaporated in vacuum. **5a** ^{1}H NMR (600 MHz, CD_3CN) δ 5.78-5.60 (m, 2H), 5.61-5.47 (m, 2H), 5.36-5.28 (m, 1H), 4.42-4.21 (m, 2H), 4.20-4.06 (m, 4H), 2.95-2.79 (m, 4H), 2.45-2.28 (m, 4H), 1.74 (t, J = 8.7 Hz, 2H), 1.42 (s, 18H).

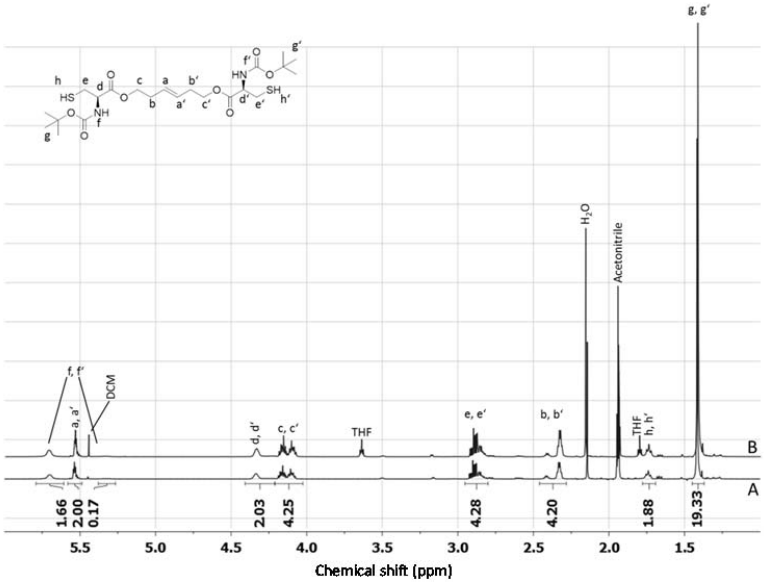


Figure S21. ^{1}H NMR spectra (600 MHz, CD_3CN) of the products obtained by disulfide reduction of polymer **4a** (A) and macrocycle **3a** (B).

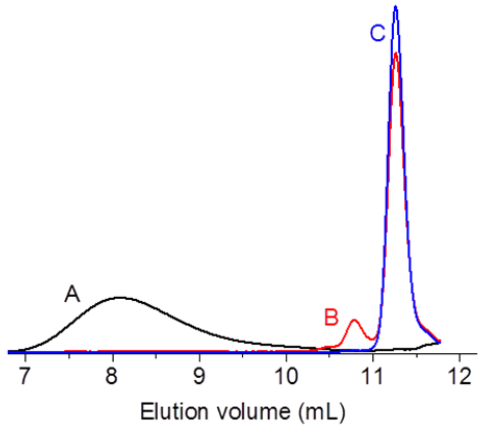


Figure S22. SEC-RI traces (eluent: THF) of the polymer **4a** (**A**) and the degradation products obtained by reduction of main-chain disulfide after reaction time of 10 minutes (**B**) and 30 minutes (**C**).