Electronic Supplementary Information

Macrocyclization efficiency for Poly(2-oxazoline)s and Poly(2-oxazine)s

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1. Materials and Methods

Materials

All chemicals were used as received unless noted otherwise. 2-*n*-propyl-2-oxazoline and 2-*n*-propyl-2-oxazoline were synthesized using a previously published procedure by Witte and Seeliger¹. Magnesium sulphate (\geq 99.5%), *N*,*N*,*N'*,*N''*,*Pu*-pentamethyldiethylenetriamine (PMDTA, 99%), copper(I) bromide (98%), lithium bromide (99%), sodium azide (99%), propargyl benzenesulfonate (96%), dimethylacetamide (DMAc, >99.9%), calcium hydride and deuterated chloroform (\geq 99%) were purchased from Sigma Aldrich. Dichloromethane (DCM, \geq 99.8%) was purchased from Thermo Fisher Scientific. Dimethyl sulfoxide (>99%), and ethanol (absolute, >99.5%), acetic acid (glacial, >99.7%), diethyl ether (anhydrous, >99.5%), and ethanol (absolute, >99.5%) were purchased from Ajax Finechem. Propargyl benzenesulfonate was distilled over calcium hydride and stored under vacuum at -20 °C until used. Copper(I) bromide was stirred overnight with an excess of glacial acetic acid, filtered, and washed with generous amounts of absolute ethanol and finally anhydrous diethyl ether. The resulting off-white powder was stored under vacuum until used. Dry acetonitrile was obtained using a solvent purification system (Innovation Technology).

Size Exclusion Chromatography (SEC)

The SEC measurements were conducted on a PSS SECurity² system consisting of a PSS SECurity Degasser, PSS SECurity TCC6000 Column Oven (60 °C), PSS GRAM Column Set (8x150 mm 10 μ m Precolumn, 8x300 mm 10 μ m Analytical Columns, 1000 Å, 1000 Å and 30 Å) and an Agilent 1260 Infinity Isocratic Pump, Agilent 1260 Infinity Standard Autosampler, Agilent 1260 Infinity Diode Array and Multiple Wavelength Detector (A: 254 nm, B: 360 nm), Agilent 1260 Infinity Refractive Index Detector (35 °C). HPLC grade DMAc + 0.08% LiBr, is used as eluent at a flow rate of 1 mL·min⁻¹. Narrow disperse linear poly(styrene) (M_n : 266 g mol⁻¹ to 2.52 × 10⁶ g mol⁻¹) and poly(methyl methacrylate) (M_n : 202 g mol⁻¹ to 2.2 × 10⁶ g mol⁻¹) standards (PSS ReadyCal) were used as calibrants. All samples were passed over 0.22 μ m PTFE membrane filters. Molecular weight and dispersity analysis was performed in PSS WinGPC UniChrom software (version 8.2).

Nuclear Magnetic Resonance (NMR) Spectroscopy

¹H NMR spectra were recorded on a Bruker System 600 Ascend LH, equipped with a BBO-Probe (5 mm) with z-gradient (¹H: 600.13 MHz, ¹³C 150.90 MHz,¹⁹F 564.63 MHz, ³¹P 242.92 MHz). Resonances are reported in parts per million (ppm) relative to tetramethyl silane (TMS). The δ -scale was calibrated to the respective solvent signal of CHCl₃ in ¹H spectra.

Infrared (IR) Spectroscopy

Infrared spectra were recorded on a Bruker Alpha-P Fourier Transform Infrared (FTIR) spectrometer equipped with a diamond crystal attenuated total reflectance (ATR) accessory. Spectra were obtained between 4000 and 400 cm⁻¹, as an average of 32 scans per sample.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry curves were obtained using a NETZSCH DSC 204 F1 Phoenix instrument. The sample mass for each measurement was between 11 and 13 mg, prepared in concave aluminium crucibles with a pierced lid. Each sample was exposed to three heating and cooling cycles under nitrogen atmosphere between -90 and 200 °C at a temperature gradient of 20 K min⁻¹. Data was processed using the NETZSCH Proteus Thermal Analysis software.

Turbidimetry

Turbidimetry measurements were conducted using a Varian Cary 4000 UV-Vis spectrometer equipped with Cary Temperature Controller unit. Polymer samples were prepared at a concentration of 5 mg mL⁻¹ in deionized water. Transmittance of each sample was measured at 600 nm in an appropriate temperature range between 2 and 35 °C, employing a temperature gradient of 1 °C min⁻¹. The cloud point temperature (T_{CP}) was determined as the temperature at 50% transmittance. The measurement chamber was purged with a constant nitrogen flow to avoid condensation on the cuvettes.

SEC Elugram Deconvolution

SEC elugrams were deconvoluted using OriginLab®'s OriginPro® 9.0 software. To achieve the best possible fit of the elugram curve, few assumptions were implemented. Firstly, as no knowledge of the relationship between molecular weight and the hydrodynamic radius was present, the bands of the high molecular weight species (cyclic and/or linear) were combined and approximated using gaussian distributions without identifying each species. It was assumed that the present species in each elugram were (1) monocyclic and (2) unreacted linear polymers, as well as (3) side-products formed through intermolecular chain-chain coupling. The unreacted linear and monocyclic polymers were approximated through gaussian curves with values for signal centre and full-width-half-maximum (FWHM) obtained from the elugram of the linear polymer and the elugram showing the strongest shift toward higher elution volumes, respectively. In case of the elugrams with the largest shift toward higher elution volumes, full conversion was assumed supported by the ¹H NMR and IR spectra. Samples that showed no shift towards higher elution volumes were regarded as 0% yield. High molecular weight species were fitted using the minimum number of curves that was needed to yield convergence. In case of strong tailing of the elugram towards higher elution volumes, a gaussian distribution was added to accommodate and give a better fit. Minimum amplitude for gaussian curves was chosen as 10-8 to avoid fitting using negative amplitudes. Signal centres and FWHM values that were used for the fitting of linear and monocyclic polymers are summarized below.

Species	Signal Centre [mL]	FWHM [mL]
Linear	28.9058	1.1049
Monocyclic	29.3611	1.0356
Linear	31.0925	1.1902
Monocyclic	31.4436	1.1517
Linear	28.9276	1.0108
Monocyclic	29.3904	1.0943
Linear	30.6988	1.2177
Monocyclic	31.1008	1.1477
	Species Linear Monocyclic Linear Monocyclic Linear Monocyclic Linear Monocyclic	SpeciesSignal Centre [mL]Linear28.9058Monocyclic29.3611Linear31.0925Monocyclic31.4436Linear28.9276Monocyclic29.3904Linear30.6988Monocyclic31.1008

Table S1 Overview over parameters used for the deconvolution of SEC elugrams.

 $\overline{FWHM} = full-width-half-maximum$

Synthesis of poly(2-n-propyl-2-oxazoline)₅₀ (PnPropOx₅₀)

2-*n*-propyl-2-oxazoline (1.50 g, 1.60 mL, 13.26 mmol, 50 eq.), acetonitrile (1.72 mL) and propargyl benzenesulfonate (0.05 g, 0.04 mL, 0.27 mmol, 1 eq.) were added under argon flow to a flame-dried 10 mL Schlenk tube. The reaction mixture was heated to 70 °C and vigorously stirred for 8 h. The reaction was cooled to room temperature before adding acetonitrile (1.72 mL) and sodium azide (0.17 g, 2.65 mmol, 10 eq.). After stirring overnight, residual sodium azide was removed via centrifugation and the polymer was precipitated in cold anhydrous diethyl ether. Finally, the polymer was redissolved in DI water, and lyophilized. The final product (0.91 g, 0.16 mmol, 60.4%) could be obtained as a white powder.

¹H NMR (600 MHz, CDCl₃) δ = 4.33 – 4.00 (m, 2H), 3.45 (br, 210H), 2.69 (m, 1H), 2.51 – 2.04 (m, 108H), 1.64 (br, 107H), 0.94 (m, J = 5.0 Hz, 161H) ppm; SEC (DMAc, PMMA cal.): $M_{\rm n} = 8,900 \text{ g mol}^{-1}, D = 1.14;$ FTIR: $v = 2098 \text{ cm}^{-1}$ (N₃).

Synthesis of poly(2-n-propyl-2-oxazoline)₂₀ (PnPropOx₂₀)

2-*n*-propyl-2-oxazoline (1.50 g, 1.60 mL, 13.26 mmol, 20 eq.), acetonitrile (1.72 mL) and propargyl benzenesulfonate (0.13 g, 0.11 mL, 0.66 mmol, 1 eq.) were added under argon flow to a flame-dried 10 mL Schlenk tube. The reaction mixture was heated to 70 °C and vigorously stirred for 4.5 h. The reaction was cooled to room temperature before adding acetonitrile (1.72 mL) and sodium azide (0.43 g, 6.63 mmol, 10 eq.). After stirring overnight, residual sodium azide was removed via centrifugation. The solvent was removed, and the crude polymer was redissolved in chloroform (25 mL), before extracting the organic phase using brine (3x 25 mL) and DI water (1x 25 mL). The organic phase was dried over magnesium sulphate, the solvent removed *in vacuo* and the polymer redissolved in DI water. After lyophilization, the final product (1.06 g, 0.47 mmol, 70.4%) could be obtained as a white powder.

¹H NMR (600 MHz, CDCl₃) δ = 4.32 – 4.04 (m, 2H), 3.45 (m, 86H), 2.34 (m, 44H), 1.64 (m, 43H), 0.94 (m, 65H) ppm; SEC (DMAc, PMMA cal.): M_n = 3,200 g mol⁻¹, D = 1.12; FTIR: v = 2098 cm⁻¹ (N₃).

Synthesis of poly(2-n-propyl-2-oxazine)₅₀ (PnPropOzi₅₀)

2-*n*-propyl-2-oxazine (1.50 g, 1.60 mL, 11.79 mmol, 50 eq.), acetonitrile (1.35 mL) and propargyl benzenesulfonate (0.05 g, 0.04 mL, 0.24 mmol, 1 eq.) were added under argon flow to a flame-dried 10 mL Schlenk tube. The reaction mixture was heated to 70 °C and vigorously stirred for 4 days. The reaction was cooled to room temperature before adding acetonitrile (1.35 mL) and sodium azide (0.15 g, 2.36 mmol, 10 eq.). After stirring overnight, residual sodium azide was removed via centrifugation and

the polymer was precipitated in cold anhydrous diethyl ether. Finally, the polymer was redissolved in DI water, and lyophilized. The final product (1.18 g, 0.19 mmol, 78.7%) could be obtained as a yellow viscous oil.

¹H NMR (600 MHz, CDCl₃) δ = 4.27 – 4.01 (m, 2H), 3.51 – 3.10 (m, 159H), 2.25 (m, 83H), 1.93 – 1.71 (m, 111H), 1.71 – 1.51 (m, 87H), 0.95 (t, J = 7.6 Hz, 122H) ppm; SEC (DMAc, PMMA cal.): M_n = 8,600 g mol⁻¹, D = 1.14; FTIR: v = 2098 cm⁻¹ (N₃).

Synthesis of poly(2-n-propyl-2-oxazine)20 (PnPropOzi20)

2-*n*-propyl-2-oxazine (1.80 g, 1.92 mL, 14.15 mmol, 20 eq.), acetonitrile (1.62 mL) and propargyl benzenesulfonate (0.14 g, 0.11 mL, 0.71 mmol, 1 eq.) were added under argon flow to a flame-dried 10 mL Schlenk tube. The reaction mixture was heated to 70 °C and vigorously stirred for 45 h. The reaction was cooled to room temperature before adding acetonitrile (1.62 mL) sodium azide (0.46 g, 7.08 mmol, 10 eq.). After stirring overnight, residual sodium azide was removed via centrifugation. The solvent was removed, and the crude polymer was redissolved in chloroform (25 mL), before extracting the organic phase using brine (3x 25 mL) and DI water (1x 25 mL). The organic phase was dried over magnesium sulphate, the solvent removed *in vacuo* and the polymer redissolved in a DI water / dimethyl sulfoxide mixture (1:4 v/v) with a few drops of acetonitrile. After lyophilization, the final product (1.19 g, 0.47 mmol, 66.1%) could be obtained as a yellow viscous oil.

¹H NMR (600 MHz, CDCl₃) δ = 4.28 – 4.01 (m, 2H), 3.32 (m, 83H), 2.25 (m, 41H), 1.78 (m, 41H), 1.72 – 1.57 (m, 59H), 1.08 – 0.82 (m, 67H) ppm; SEC (DMAc, PMMA cal.): M_n = 3,700 g mol⁻¹, D = 1.11; FTIR: v = 2097 cm⁻¹ (N₃).

General procedure for CuAAC cyclization of PnPropOx₅₀, PnPropOx₂₀, PnPropOzi₅₀ and PnPropOzi₂₀ using 100 eq. N,N,N',N'', Pentamethyldiethylenetriamine and copper(I) bromide with a syringe pump

In separate flasks, PMDTA (0.17 g, 0.21 mL, 1.00 mmol, 100 eq.) and a corresponding polymer were dissolved in dichloromethane. The PMDTA solution was degassed for 30 min using argon, while the polymer solution was degassed for 15 min. Copper(I) bromide (0.14 g, 1.00 mmol, 100 eq.) was added to the PMDTA containing flask and the mixture was degassed for additional 30 min. The polymer solution was added to the reagent solution using a syringe pump at a rate of 1 mL h⁻¹ (5 mL h⁻¹ for aerosol). After complete polymer addition, the reaction mixture was stirred vigorously overnight. If necessary, the solvent was reduced to approximately 100 mL and the reaction mixture was stirred for 45 min against saturated ammonium chloride solution (150 mL) and afterwards washed with fresh saturated ammonium chloride solution until the organic phase became colourless. The organic phase

was dried over magnesium sulphate, the solvent removed under reduced pressure and the polymer was redissolved in DI water. After lyophilization, the final products could be obtained as either a white powder (PnPropOx) or a yellow viscous oil (PnPropOzi).

General procedure for CuAAC cyclization of PnPropOx₅₀, PnPropOx₂₀, PnPropOzi₅₀ and PnPropOzi₂₀ using 1 and 0.01 eq. *N*,*N*,*N'*,*N''*,*N''*-pentamethyldiethylenetriamine and copper(I) bromide with a syringe pump

In separate flasks, a desired batch volume of dichloromethane and a corresponding polymer dissolved in dichloromethane were degassed using argon. The dichloromethane was degassed for 30 min using argon, while the polymer solution was degassed for 15 min. In another flask, PMDTA was added to dichloromethane to result in a total concentration of 10 mmol L⁻¹. The solution was degassed for 30 min before copper(I) bromide was added in equimolar amounts to PMDTA. After further 30 minutes of degassing, 1 mL of the required Cu(I)Br/PMDTA stock solution was added to the degassed vessel with dichloromethane to yield the required catalyst concentration of 1 eq. per azide on the polymer backbone. For 0.01 eq. experiments, the stock solution used for 1 eq. experiments was diluted 1:100 with degassed dichloromethane. The polymer solution was added dropwise to the reaction vessel using a syringe pump at a rate of 1 mL h^{-1} . After complete polymer addition, the reaction mixture was stirred vigorously overnight. If necessary, the solvent was reduced to approximately 100 mL and the reaction mixture was stirred for 45 min against saturated ammonium chloride solution (150 mL) and afterwards washed with fresh saturated ammonium chloride solution until the organic phase became colourless. The organic phase was dried over magnesium sulphate, the solvent removed under reduced pressure and the polymer was redissolved in DI water. After lyophilization, the final products could be obtained as either a white powder (PnPropOx) or a yellow viscous oil (PnPropOzi).

2. Supplementary Data



Figure S1 Differential scanning calorimetry (DSC) curves of the second heating cycle of $PnPropOx_{50}$ (black solid), $PnPropOx_{20}$ (black dashed), $PnPropOz_{150}$ (red solid) and $PnPropOz_{20}$ (red dashed). Curves were acquired from 11-13 mg sample using a temperature gradient of 20 K min⁻¹ in three heating and cooling cycles. Inserted black lines indicate the found glass transition temperatures (T_{g} s).



Figure S2 SEC elugram of polymers obtained via CuAAC, using dropwise addition (red), addition through a submerged needle (blue) and through an aerosol (green) of a solution of 0.01 mmol PnPropOzi₅₀ (black) in 2.5 mL dichloromethane. The reaction was conducted in a 100 mL batch volume of solvent with 100 eq. CuBr.



Figure S3 Magnified ¹H NMR spectrum (top), SEC elugram (bottom, left) and IR spectrum (bottom, right) of PnPropOx₅₀ (A) and PnPropOzi₅₀ (B) before (black) and after (red) CuAAC in 1000 mL DCM with 100 eq. CuBr.



Figure S4 Data obtained from turbidimetry measurements for $PnPropOzi_{50}$ (left, black) and $PnPropOzi_{20}$ (right, red) before (squares) and after (circles) CuAAC using 1000 mL DCM with 100 eq. CuBr. Measurements were conducted using a temperature ramping program of 1 °C min⁻¹ and polymer concentrations of 5 mg mL⁻¹ in deionized water.



Figure S5 ^{1}H NMR spectrum of PnPropOx₂₀. The spectrum was recorded at 600 MHz in deuterated chloroform as solvent.



Figure S6 ¹*H NMR spectrum of PnPropOx*₅₀*. The spectrum was recorded at 600 MHz in deuterated chloroform as solvent.*



Figure S7¹H NMR spectrum of PnPropOzi₂₀. The spectrum was recorded at 600 MHz in deuterated chloroform as solvent.



Figure S8 ¹*H NMR spectrum of PnPropOzi*₅₀. *The spectrum was recorded at 600 MHz in deuterated chloroform as solvent.*



Figure S9 IR spectrum of $PnPropOx_{20}$. The band at around 2100 cm⁻¹ indicated presence of azide groups in the polymer. Spectra were obtained as an average of 32 scans using a diamond crystal ATR setup.



Figure S10 IR spectrum $PnPropOx_{50}$. The band at around 2100 cm⁻¹ indicated presence of azide groups in the polymer. Spectra were obtained as an average of 32 scans using a diamond crystal ATR setup.



Figure S11 IR spectrum of $PnPropOzi_{20}$. The band at around 2100 cm⁻¹ indicated presence of azide groups in the polymer. Spectra were obtained as an average of 32 scans using a diamond crystal ATR setup.



Figure S12 IR spectrum of $PnPropOzi_{50}$. The band at around 2100 cm⁻¹ indicated presence of azide groups in the polymer. Spectra were obtained as an average of 32 scans using a diamond crystal ATR setup.

Polymer	Syringe Pump			Reaction Vessel		
	Feed rate	Polymer	DCM	PMDTA	CuBr	DCM
	[mL h ⁻¹]	mass [mg]	[mL]	[µL]	[mg]	[mL]
	1	56.6	2.5	208.8	143.5	10
	1	56.6	2.5	208.8	143.5	100
	1	56.6	2.5	208.8	143.5	1000
	1	56.6	2.5	2.1	1.4	10
PnPropOx ₅₀	1	56.6	2.5	2.1	1.4	100
	1	56.6	2.5	2.1	1.4	1000
	1	56.6	2.5	0.02	0.01	10
	1	56.6	2.5	0.02	0.01	100
	1	56.6	2.5	0.02	0.01	1000
	1	22.6	2.5	208.8	143.5	10
	1	22.6	2.5	208.8	143.5	100
	1	22.6	2.5	208.8	143.5	1000
	1	22.6	2.5	2.1	1.4	10
PnPropOx ₂₀	1	22.6	2.5	2.1	1.4	100
	1	22.6	2.5	2.1	1.4	1000
	1	22.6	2.5	0.02	0.01	10
	1	22.6	2.5	0.02	0.01	100
	1	22.6	2.5	0.02	0.01	1000
	1	63.6	2.5	208.8	143.5	10
	1	63.6	2.5	208.8	143.5	100
	1	63.6	2.5	208.8	143.5	1000
	1	63.6	2.5	2.1	1.4	10
PnPropOzi ₅₀	1	63.6	2.5	2.1	1.4	100
	1	63.6	2.5	2.1	1.4	1000
	1	63.6	2.5	0.02	0.01	10
	1	63.6	2.5	0.02	0.01	100
	1	63.6	2.5	0.02	0.01	1000
	1	25.4	2.5	208.8	143.5	10
	1	25.4	2.5	208.8	143.5	100
	1	25.4	2.5	208.8	143.5	1000
	1	25.4	2.5	2.1	1.4	10
PnPropOzi 20	1	25.4	2.5	2.1	1.4	100
1 20	1	25.4	2.5	2.1	1.4	1000
	1	25.4	2.5	0.02	0.01	10
	1	25.4	2.5	0.02	0.01	100
	1	25.4	2.5	0.02	0.01	1000

Table S2 Overview over experimental conditions for methodological study of the cyclization of PnPropOx and PnPropOzi. The polymer amount in each experiment corresponds to 0.01 mmol.

3. Supporting References

 Witte, H.; Seeliger, W. Cyclische Imidsäureester Aus Nitrilen Und Aminoalkoholen. Justus Liebigs Ann. Chem. 1974, 1974 (6), 996–1009. https://doi.org/https://doi.org/10.1002/jlac.197419740615.