Supporting Information

One-pot synthesis of cyclopentadienyl endcapped poly(2-ethyl-2-oxazoline) and subsequent ambient temperature Diels-Alder conjugations

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Materials

4-(2-{[(3-Acetyl-7-oxabicyclo[2.2.1]-hept-5-en-2-yl)carbonyl]aminoethoxy)-4-oxobutanoic acid (**1**)[1] and *N*-(2-hydroxyethyl)maleimide[2] were synthesized according to a literature procedure. Dichloromethane was dried over 4Å molecular sieves. *N*-Phenylmaleimide (ABCR), poly(ethylene glycol) monomethyl ether (MeO-PEG, M_n = 550 g mol^{-1}, ABCR), 4-(dimethylamino)-pyridine (DMAP, ABCR), *N*,*N'*-dicyclohexylcarbodiimid (DCC, Acros), and sodium cyclopentadienide (2.0 M THF, Aldrich) were used as received. 2-Ethyl-2-oxazoline (EtOx, Acros), methyl tosylate (MeOTs, Acros) and acetonitrile (Aldrich) were distilled to dryness over barium oxide (BaO) and stored under argon.

Characterization

The structures of the synthesized compounds were confirmed by ^{1}H-NMR spectroscopy using a Bruker AM 400 spectrometer at 400 MHz for hydrogen nuclei. All samples were dissolved in $CDCl_3$. The δ-scale is referenced to tetramethylsilane as internal standard.

For the determination of molecular weight distributions (MWD), a SEC system (Polymer Laboratories PL-GPC 50 Plus) comprising an auto injector, a guard column (PLgel Mixed C, 50 × 7.5 mm) followed by three linear columns (PLgel Mixed C, 300 × 7.5 mm, 5 µm bead-size) and a differential refractive index detector was employed. THF was used as the eluent at 40 °C with a flow rate of 1 $mL \cdot min^{-1}$. The SEC system was calibrated using linear polystyrene standards ranging from 160 to $6 \cdot 10^6$ g mol^{-1}.

SEC/ESI-MS spectra were recorded on an LXQ mass spectrometer (ThermoFisher Scientific, San Jose, CA, USA) equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electrospray mode. The instrument was calibrated in the *m*/*z* range 195-1822 using a standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA) and a mixture of fluorinated phosphazenes (Ultramark 1621) (all from Aldrich). A constant spray voltage of 4.5 kV and a dimensionless sweep gas flow rate of 2 and a dimensionless sheath gas flow-rate of 12 were applied. The capillary voltage, the tube lens offset voltage and the capillary temperature were set to 60 V, 110 V and 275 °C respectively. The LXQ was coupled to a Series 1200 HPLC-system (Agilent, Santa Clara, CA, USA) consisting of a solvent degasser (G1322A), a binary pump (G1312A), a high-performance autosampler (G1367B), followed by a thermostatted column compartment (G1316A). Separation was performed on two mixed bed size exclusion chromatography columns (Polymer Laboratories, Mesopore 250 × 4.6 mm, particle dia. 3 µm) with a pre-column (Mesopore 50 × 4.6 mm) operating at 30 °C. THF at a flow rate of 0.30 mL min^{-1} was used as eluent. The mass spectrometer was coupled to the column in parallel to an RI-detector (G1362A with SS420x A/D) in a setup described previously.[3] 0.27 $mL \cdot min^{-1}$ of the eluent were directed through the RI-detector and 30 µL min^{-1} infused into the electrospray source after post-column addition of a 100 µM solution of sodium iodide in methanol at 20 µL min^{-1} by a micro-flow HPLC syringe pump (Teledyne ISCO, Model 100DM). 20 µL of a polymer solution with a concentration of approximately 3 mg mL^{-1} were injected onto the HPLC system.

For the MALDI measurements an Ultraflex III TOF/TOF apparatus (Bruker Daltonics, Bremen, Germany) equipped with a Nd:YAG laser and a collision cell was used. All spectra were measured in the positive reflector or linear mode. The instrument was calibrated prior to each measurement with an external PMMA standard from PSS Polymer Standards Services GmbH (Mainz, Germany).

Synthesis of cylopentadienyl functionalized poly(2-ethyl-2-oxazoline) (PEtOx)

A polymerization solution containing methyl tosylate (0.08 g, 0.43 mmol), 2-ethyl-2-oxazoline (0.88 g, 8.9 mmol) and acetonitrile (3.9 g, 95 mmol) was prepared under an argon atmosphere and polymerized under microwave irradiation (140 °C, 9 min). The polymer solution was cooled to 0 °C and 3 equivalents of a sodium cyclopentadienide solution (2.0 M THF) (0.54 mL, 1.1 mmol) were added dropwise under vigorous stirring. After 30 min the solution was allowed to warm to room temperature and stirring was continued overnight. The resulting mixture was then poured into a saturated NH$_4$Cl solution and extracted with dichloromethane. The organic phase was washed twice with cold distilled water, dried over MgSO$_4$ and concentrated in vacuum. Precipitation in ice-cold diethyl ether yielded a light brown solid (550 mg, 70%). $M_{\mathrm{n,SEC}}$ = 1900 g mol^{-1}, *PDI* = 1.06. ^{1}H-NMR (400 MHz, CDCl$_3$, δ/ppm): 6.70–6.17 (m, Cp vinylic), 3.43 (s, N-CH_2-CH_2-N), 3.01 (s, H_3C-N), 2.94 (s, Cp bridge head), 2.53–1.95 (m, CH_2CO), 1.18–1.00 (m, CH_3-CH$_2$CO).

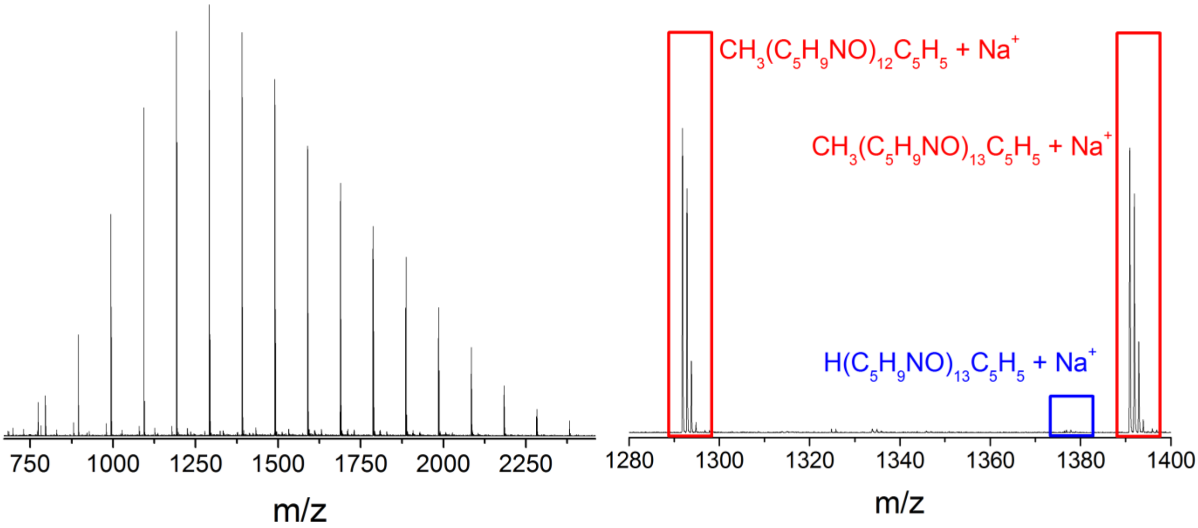
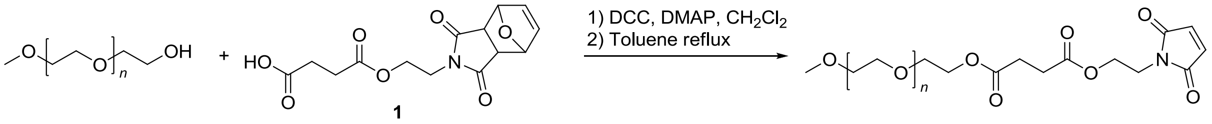


Figure S1 MALDI-TOF MS spectrum of PEtOx-Cp (left) and a zoom-in with assignment of the peaks (right).

Synthesis of maleimide functionalized poly(ethylene glycol) (PEG-Mal)



A solution of DCC (562 mg, 2.72 mmol) in 3 mL dry CH_2Cl_2 was added to a solution of MeO-PEG (M_n = 550 g mol^{-1}) (1.00 g, 1.81 mmol), DMAP (221 mg, 1.81 mmol) and **1** (842 mg, 2.72 mmol) in 5 mL dry CH_2Cl_2. The reaction mixture was stirred overnight at ambient temperature. The mixture was filtered, washed with saturated $NaHCO_3$-solution, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was dissolved in toluene, filtered and refluxed for 6 h. The toluene was removed under reduced pressure and the polymer was dried under high vacuum. $M_{n,SEC}$ = 900 g mol^{-1}, *PDI* = 1.09. ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 6.71 (s, vinylic), 4.24–4.20 (m, N-CH_2CH_2-OCO), 3.78–3.51 (m, PEG backbone), 3.36 (s, PEG-OCH_3), 2.61–2.56 (CO-CH_2CH_2-CO).

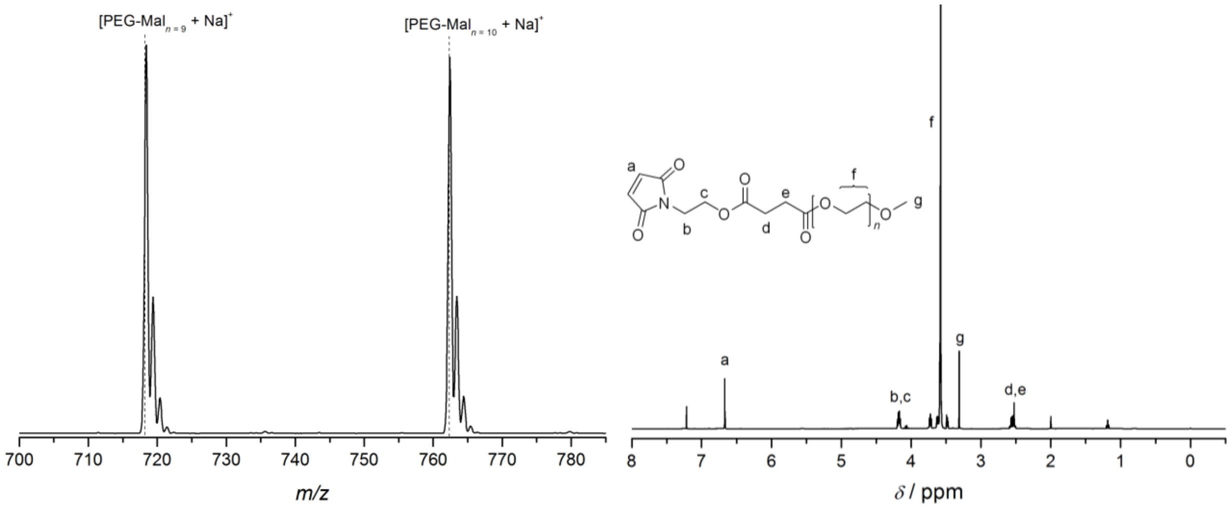


Figure S2 left) SEC/ESI-MS spectrum of PEG-Mal. Right) ^{1}H-NMR spectrum of PEG-Mal in $CDCl_3$

Diels-Alder reactions with small molecule *N*-substituted maleimides

A solution of PEtOx-Cp (2 µmol) and *N*-substituted maleimide (10 µmol) in 0.5 mL CH_2Cl_2 was stirred 24 h at ambient temperature. The solvent was removed under a stream of nitrogen. The residue was re-dissolved in THF and analyzed by SEC/ESI-MS.

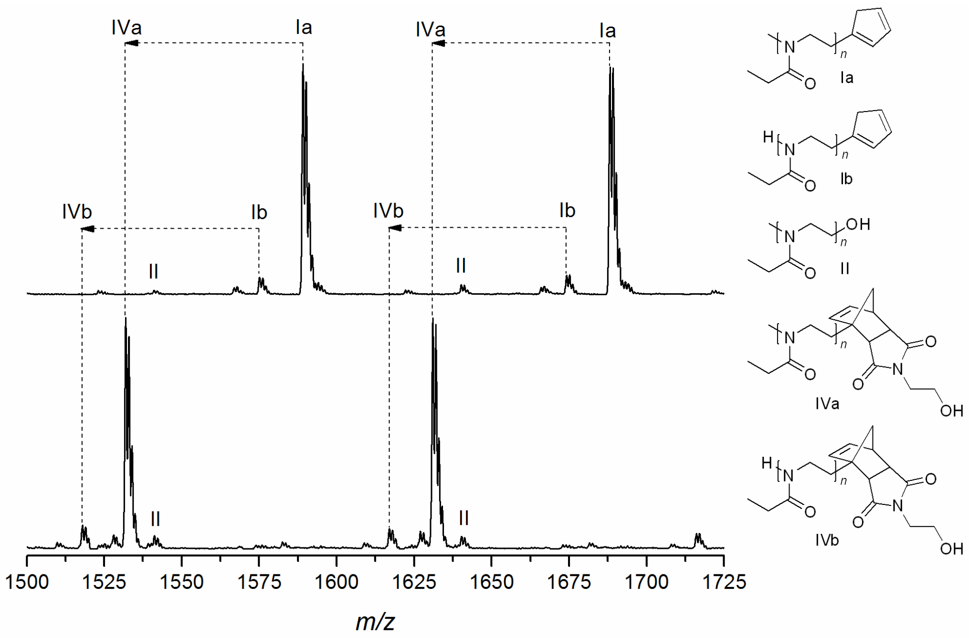


Figure S3 Expanded region of a typical repeat unit in the SEC/ESI-MS spectra of PEtOx-Cp (top) and the Diels-Alder cycloadduct with *N*-(2-hydroxyethyl)maleimide. Within the presented *m*/*z* range, it should be noted that the number of monomer repeat units of Ia, Ib and II differ from that of IVa and IVb.

Table S1 Theoretical and measured *m/z*-ratios of the species involved in the synthesis of PEtOx-Cp and the Diels-Alder reactions with *N*-phenylmaleimide and *N*-(2-hydroxyethyl)maleimide.

	$[M + Na]^+$		
Structure	m/z^{theo}	m/z^{meas}	$\Delta\ m/z$
Ia	1589.08	1589.08	0.00
Ib	1575.06	1575.08	0.02
II	1541.04	1541.08	0.04
IIIa	1563.99	1564.00	0.01
IIIb	1549.97	1549.92	0.05
IVa	1531.98	1531.92	0.06
IVb	1517.97	1518.00	0.03

Synthesis of PEG-*b*-PEtOx

A solution of PEtOx-Cp (0.5 mmol) and PEG-Mal (0.6 mmol) in 5 mL CH_2Cl_2 was stirred 48 h at ambient temperature. PEG-*b*-PEtOx was isolated by two-fold precipitation in diethylether and dried under vacuum. $M_{n,SEC}$ = 3000 g mol^{-1}, *PDI* = 1.07. ^1H-NMR (400 MHz, $CDCl_3$, δ/ppm): 6.17–5.89 (m, cycloadduct vinylic), 4.24–4.20 (m, N-CH_2CH_2-OCO), 3.62 (s, O-CH_2-CH_2-O), 3.43 (s, N-CH_2-CH_2-N), 3.36 (s, PEG-OCH_3), 3.01 (s, H_3C-N), 2.53–1.95 (m, CH_2CO and CO-CH_2CH_2-CO), 1.18–1.00 (m, CH_3-CH_2CO).

Table S2 Characterization of PEG-*b*-PEtOx and the polymeric building blocks PEG-Mal and PEtOx-Cp

Polymer	$M_{n,SEC}$ / g mol^{-1}	$M_{n,NMR}$ / g mol^{-1}	*PDI*
PEG-Mal	800	900	1.09
PEtOx-Cp	1900	1700	1.06
PEG-*b*-PEtOx	3000	2700	1.07

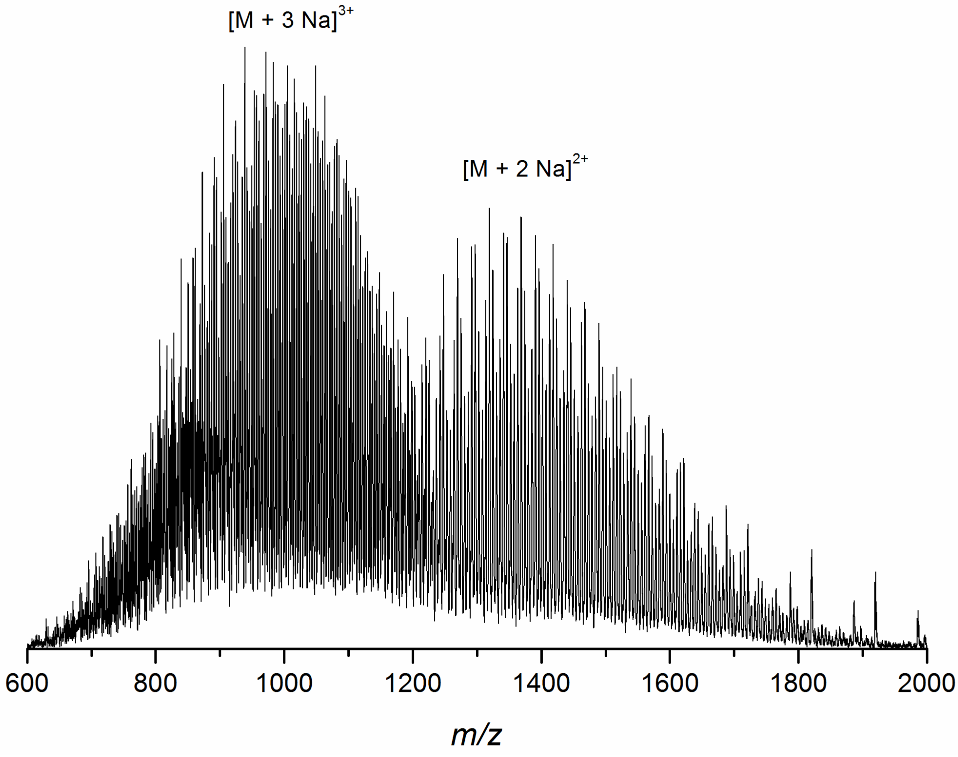
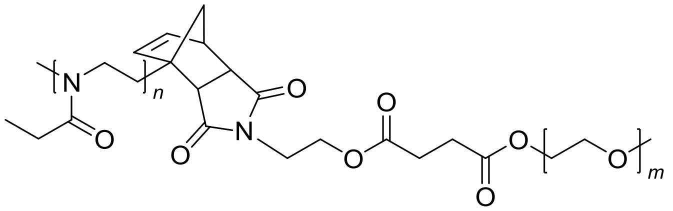


Figure S4 SEC/ESI-MS spectra of PEG-*b*-PEtOx.

Table S3 Theoretical and measured *m/z*-ratios ($[M + 2\,Na]^+$) of possible copolymer compositions. The values refer to the peak-maxima of the isotopic patterns.

		$[M + 2\,Na]^+$		
n (EtOx)	*m* (EG)	m/z^{theo}	m/z^{meas}	$\Delta\, m/z$
20	14	1511.94	1512.00	0.06
20	15	1533.95	1534.00	0.05
20	16	1555.97	1556.00	0.03
20	17	1577.98	1578.00	0.02
21	11	1495.44	1495.58	0.14
21	12	1517.45	1517.42	0.03
21	13	1539.46	1539.42	0.04
21	14	1561.47	1561.58	0.11
21	15	1583.49	1583.58	0.09
22	9	1500.94	1500.92	0.02
22	10	1522.95	1523.00	0.05
22	11	1544.97	1545.08	0.11
22	12	1566.98	1567.00	0.02



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[2] W. H. Heath,F. Palmieri, J. R. Adams, B. K. Long, J. Chute, T. W. Holcombe, S. Zieren, M. J. Truitt, J. L. White, C. G. Willson, *Macromolecules* **2008**, *41*, 719.

[3] T. Gruendling, M. Guilhaus, C. Barner-Kowollik, *Anal. Chem.* **2008**, *80*, 6915.