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Poly(2-ethyl-2-oxazoline)-block-polycarbonate block copolymers: From improved end-group control in poly(2-oxazoline)s to chain extension with aliphatic polycarbonate through a fully metal-free ring-opening polymerisation process

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Block copolymer micelles hold great promise for developing next generation drug delivery vehicles to improve therapeutics. In this work, the biocompatibility of poly(2-alkyl-2-oxazoline)s (PAOx) was combined with the biodegradability and biocompatibility of aliphatic polycarbonates through the preparation of block copolymers. These well-defined blocks were prepared via cationic ring-opening polymerisation (CROP) of 2-oxazolines followed by the organocatalytic ring-opening polymerisation (ROP) of cyclic carbonate monomers. The improved synthesis of hydroxyl terminated poly(2-ethyl-2-oxazoline)s (PEtOx-OH) is reported allowing high end-group fidelity. These polymers were used as macroinitiators in the controlled ROP of various cyclic carbonate monomers (TMC and benzyl, allyl, propargyl, bromide and morpholino functional monomers) resulting in well-defined amphiphilic block copolymers.

Introduction

Supramolecular self-assembly of amphiphilic block copolymers is a widespread strategy in the field of nanoscale drug delivery systems as the resulting nanostructures allow safe transport of the therapeutics compartmentalized in the hydrophobic core, while the hydrophilic corona ensures solubility and stability in the bloodstream, hence increasing its bioavailability and preventing possible premature degradation. High attention has been devoted to the fine tuning of block-copolymer parameters since molecular weight, block compositions, and functionalities will strongly impact particle size, drug loading, and *in vivo* stability. In addition, the ability to precisely tune biodegradability and biocompatibility of materials by the introduction of functional groups (hydrophilic, hydrophobic, responsiveness) onto an otherwise hydrophobic backbone has been crucial in polymer design for biomedical applications. An important contribution to their development is realised through the ring-opening polymerisation (ROP) of functional cyclic monomers.[1-3] The preparation of functional aliphatic polycarbonates (APCs) *via* the ROP of cyclic carbonate monomers has received particular interest over the past decade as a result of their excellent biodegradability and the relative ease to introduce lateral functionality. Indeed, a range of synthetic methods to prepare functional monomers from bio-based and/or biocompatible scaffolds have been reported as well as the development of polymeric scaffolds with pendant functionalities (allyl-, propargyl-, azide-, maleimide,…) serving as building blocks to which a large range of functionalities can be added by post-polymerisation modification.[4-9] Biomedical applications reported for such polymers range from small molecules and gene transfer agents to hydrogels and nanoparticles for drug delivery.[10, 11] Beyond their outstanding features, the ease of tuning their macromolecular parameters through metal-free polymerisation[12] mediated by biocompatible catalysts is a real breakthrough, ensuring well-defined polymer preparation free of toxic agents, a prerequisite for the targeted biomedical field.[13]

Poly(ethylene oxide) (PEO) is the most widely used hydrophilic segment in drug nanocarrier design owing to its high hydrosolubility while being non charged, biocompatibility and its stealth properties.[14-16] However, there are also drawbacks related to the use of PEO in biomedical applications, such as the activation of the immune system based on anti-PEO antibodies.[17, 18]

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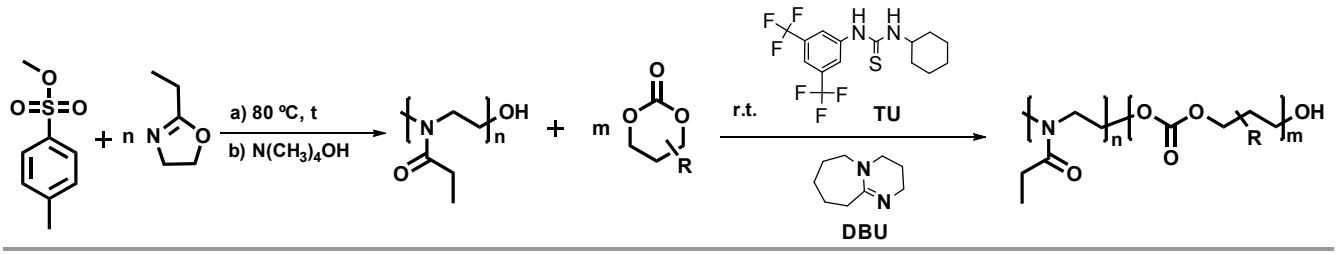


Figure 1. Schematic representation of the synthetic method for the preparation of block copolymers composed of 2-oxazolines and (functional) 6-membered cyclic carbonate.

[22] In recent years, poly(2-alkyl-2-oxazoline)s (PAOx) have attracted increasing attention as a replacement for PEO in reason of their tuneable functionalities, stimuli-responsive nature, defined structure and a biocompatibility analogous to poly(peptide)s. Unsurprisingly, PAOx have been studied as an alternative polymer platform for biomedical applications.[19] Poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) (PEtOx) in particular are interesting as they exhibit low viscosity, high stability, as well as stealth behaviour and protein-antifouling properties. As such, they are a highly interesting alternative to PEO to be used in the development of smart polymer micelles for biomedical applications.[23-25]

In analogy to PEO, the *in vivo* stability of PAOx could hamper effective cargo release. The preparation of amphiphilic block copolymers incorporating a biodegradable block as well as a PAOx block is therefore of high interest in the preparation of the optimal polymeric nanocarriers. The combination of cationic ring-opening polymerisation (CROP) of 2-oxazolines with controlled radical polymerisation techniques (ATRP, NMP, RAFT) has already proved as an excellent synthetic strategy to prepare block copolymers comprising PAOx and poly(meth)acrylates sequences using a range of vinylic monomers.[26-28] A limited number of examples combining a PAOx block with biodegradable polymers are described in literature, that either focus on the use of metal-based catalysts and/or the use of coupling techniques (i.e. DCC, CuAAC) including one example that reports the introduction of an additional benzyl functionality to the biodegradable poly(ester) segment.[29-34]

Considering that the use of toxic metal catalysts is undesired for prospective biomedical applications, we developed a fully metal-free ring-opening polymerisation process to synthesize well-defined PAOx-*b*-polycarbonate copolymers from hydroxyl-functional PEtOx and a range of functional cyclic carbonates.

Experimental

Solvents and reagents were purchased from Sigma-Aldrich, and used as received unless otherwise specified. Methyl tosylate (MeOTs) was distilled twice under vacuum prior to use. 2-Ethyl-2-oxazoline (EtOx, Aldrich), triethylamine (NEt_3; >99%, Aldrich), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; ≥ 99.0%, Fluka) were dried over BaO and distilled under reduced pressure before use. Trimethylene carbonate (TMC) (from Pugh&Co) was dried azeotropically three times with anhydrous toluene and stored under N_2. N-(3,5-Trifluoromethyl)phenyl-N'-cyclohexylthiourea (TU) was prepared as previously reported.[50] 5-methyl-5-allyloxy carbonyl-1,3-dioxan-2-one carbonate (MAC), 5-methyl-5-propargyloxycarbonyl-1,3-dioxan-2-one (MPC), 5-methyl-5-benzoxycarbonyl-propylene carbonate (MBC), 5-methyl-5-propylbromide-1,3-dioxan-2-one (MTC_{Br}),[51-53] 5-methyl-5-(4-ethyl)morpholine-1,3-dioxane-2-one (MTC_{morph}) were synthesized as reported previously.[4,49,54-57] Acetonitrile (CH_3CN, Acros Organics) was dried over molecular sieves (3Å). Toluene and tetrahydrofuran (THF) both (p.a., Chemlab) were dried using a MBRAUN solvent purification system under N_2. Methylene chloride (DCM; CH_2Cl_2, HPLC grade, Fisher) was dried over CaH_2 for 48 h and distilled under reduced pressure. All reagents were stored and handled under a dry argon or nitrogen atmosphere. Oxalyl chloride (98%, Alfa Aesar), 4-dimethylaminopyridine (DMAP, 99% VWR), trifluoroacetic acid (TFA, 99% Aldrich), N,N-dimethylformamide (DMF; 99.8%, Aldrich), ethyl acetate, heptane (p.a., Chemlab), KOH, and tetramethylammonium hydroxide in methanol (25 wt.%) were used as received .

Instrumentation

EtOx polymerisations were performed in a Biotage initiator sixty microwave synthesizer utilizing capped microwave vials. The vials were heated to 120 °C for 24 hours and cooled down to room temperature under vacuum prior use. All polymerisations were performed with temperature control (IR sensor).

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Advance 300 MHz spectrometer. Spectra were all processed using TOPSPIN 3.0.

Gas chromatography was performed on a 7890A from Agilent Technologies with an Agilent J&W Advanced Capillary GC column (30 m, 0.320 mm, and 0.25 µm) equipped with a flame ionization detector (FID) and hydrogen as carrier gas. Injections were performed with an Agilent Technologies 7693 auto sampler.

Size exclusion chromatography (SEC) measurements were performed on two different systems. One was an Agilent 1260-series equipped with a 1260 ISO-pump, a 1260 Diode Array Detector (DAD), a 1260 Refractive Index Detector (RID), and a PSS Gram30 column in series with a PSS Gram1000 column inside a 1260 Thermostated Column Compartment (TCC) at 50°C using dimethylacetamide containing 50 mM of LiCl (flow rate of 0.6 mL min^{-1}) as solvent. Molar masses and dispersities were calculated against poly(methyl methacrylate) standards. The second one was an Agilent 1200 series running in THF + 2 wt % NEt_3 at 35 °C, equipped with a degasser, an isocratic HPLC pump (flow rate = 1 mL·min^{-1}), an autosampler (loop volume = 200 µL, solution concentration = 1 mg·mL^{-1}), refractive-index, and UV–vis detectors, and three columns: a PL gel 10 mm guard column and two PL gel Mixed-B 10 mm columns (linear columns for separation of MW PS ranging from 500 to 106 Da). Poly(styrene) standards were used for calibration.

MALDI-TOF (Matrix-Assisted Laser Desorption and Ionization Time of Flight) mass spectrometry analysis was performed on an Applied Biosystems Voyager-DE STR instrument equipped with nitrogen laser operating at 337 nm, pulsed ion extraction source and reflectron detector. The laser pulse width is 3 ns with a maximum power of 20 Hz. Spectra were recorded in reflector mode with an acceleration voltage of 19 kV and delay of 400 ns. 100 single shot acquisitions were summed to give the spectra and the data were analysed using Data Explorer software. Samples were prepared by dissolving the matrix 2-(4-hydroxyphenylazo) benzoic acid HABA in the solvent (acetone, 20 mg mL^{-1}), mixing with the polymer (1 mg mL^{-1}) and sodium iodide in acetone (15 mg mL^{-1}) that was used as cationising agent.

Poly(2-ethyl-2-oxazoline) synthesis optimisation

A stock solution of 2-ethyl-2-oxazoline (5.65 mL, 5.55 g, 56 mmol) was prepared in acetonitrile (8.35 mL) in the presence of methyl tosylate (283 µL, 0.347 g, 1.87 mmol), yielding a 4 M monomer concentration and a M/I ratio of 30. A sample of the stock solution was taken for GC analysis to calculate the monomer conversion. Aliquots of 2 mL were taken to different microwave reaction vials (10 mL nominal volume) equipped with a stirring bar, and capped. All the process was performed in a glove-box under a dry nitrogen atmosphere. The vials were heated in a microwave synthesizer at different temperatures and times, calculated via the following equation:

$$t = \frac{\ln[M]_0}{[M]_t}\left(k_p\,[I]_0\right) \qquad \text{(Equation 1)}$$

where, for a monomer conversion of 95%, $\ln[M]_0/[M]_t = 3$. For [M] / [I] =30, $[I]_0$ = 4/30 mol l^{-1}. The polymerisation rate constants (k_p) at different temperatures were calculated via the Arrhenius equation using the thermodynamical parameters reported by Wiesbrock, Schubert *et al*.[43]
Upon reaction time completion, a sample was taken under inert atmosphere and dispersed in dichloromethane for GC analysis to calculate monomer conversion. Finally, 108 µL (1 equivalent) of methanolic tetramethylammonium hydroxide (25 wt.%) was injected into the polymerisation mixture, that was let stirring overnight at room temperature. The obtained polymers were analysed without purification by ^{1}H NMR spectroscopy, SEC and MALDI-TOF MS.
The synthesis of PEtOx-OH to be used as macroinitiators for the polymerisation of carbonates was performed in an analogous manner as described. After end-capping of the living polymer (18 hours r.t.), the solvent was evaporated under reduced pressure and the polymer re-dissolved in dichloromethane. The polymer was then purified by precipitation in cold diethyl ether. This process was repeated 3 times to assure complete removal of unreacted EtOx monomer. Polymer purity and structure were evaluated by ^{1}H NMR spectroscopy, SEC and MALDI-TOF MS.

Poly(2-ethyl-2-oxazoline)-polycarbonate block copolymer synthesis

Typical procedure for the organocatalytic ROP of cyclic carbonates from PEtOx-OH macroinitiator.
A glass vial in a glove box, equipped with a magnetic stirrer, was charged with PEt-Ox macroinitiator, the catalyst (DBU, 1 mol% DBU to monomer) and $CHCl_3$ (or $CDCl_3$). Once homogeneous, a solution composed of monomer, TU co-

catalyst (5 mol% to monomer) and $CHCl_3$ were added to give a final monomer concentration of 0.5 M.

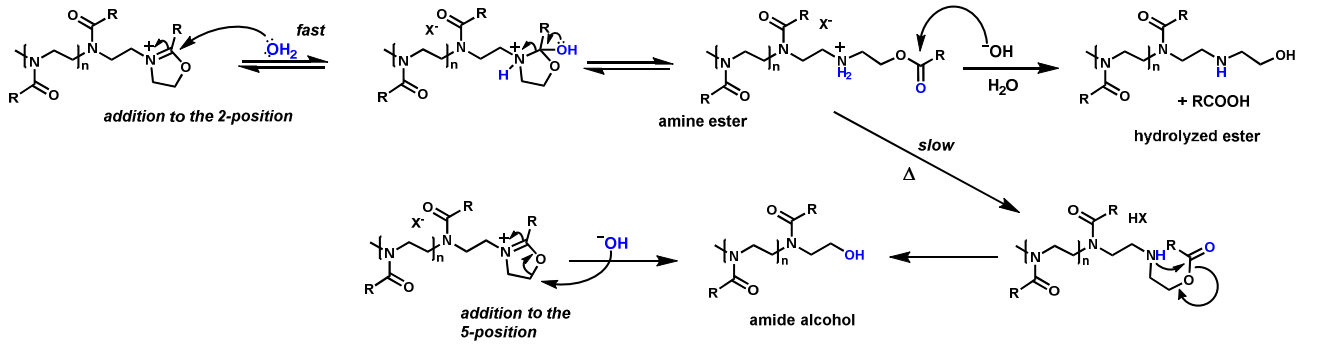


Figure 2. Termination step: the ambident character of oxazolinium ions results in two possible nucleophilic additions, in the 2-position (kinetically controlled and reversible) and in the 5-position (thermodynamically controlled, irreversible).

The vial was sealed and maintained under vigorous stirring until >90% monomer conversion was reached as observed by SEC or ^{1}H NMR. Reactions were quenched with HCl diluted in diethyl ether. The solutions were precipitated in diethyl ether or hexane and the resulting copolymers were dried overnight under reduced pressure.
In case of triblock copolymers PEtOx-b-PMAC-b-P(MTC-morph), the second monomer was added and the polymerisation was continued until >90% monomer conversion was reached as observed by SEC, after which its work-up was carried out.

2. Results and Discussion

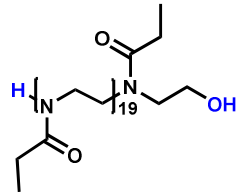
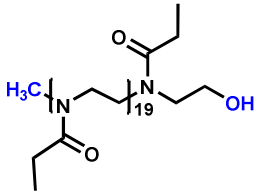
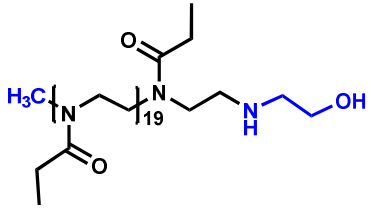
2.1. Poly(2-ethyl-2-oxazoline)-OH synthesis

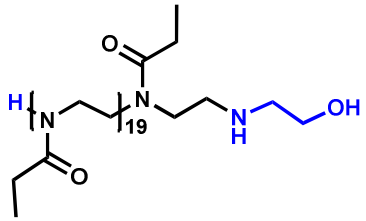
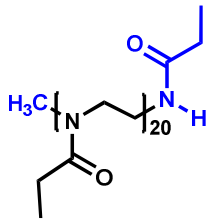
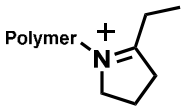
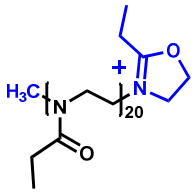
A high level of control on the terminal functionalities of the PAOx macroinitiator is required to obtain the intended PAOx-*b*-polycarbonate architectures. To this end, a study of the influence of the polymerisation temperature and terminating agent on the end-group fidelity of the final polymer composition was performed.

The main side reaction affecting the end-group functionality of poly(2-oxazoline)s is the chain-transfer to monomer, generally ascribed to β-elimination, and produces proton-initiated chains.[35] According to this mechanism, the oxazoline monomer acts as a base extracting a proton in beta position from the oxazolinium species at the terminus of a polymer living chain. This produces a reactive enamine and a protonated oxazolinium unit that further propagates leading to a proton-initiated chain. Proton-initiated chains can also be produced by the protonation of oxazoline units due to traces of water present in the system.[36]
In addition, during the termination step, water molecules may add to the polymer living chain either in position 2 or in position 5 of the oxazolinium ring, producing two different end-group functionalities.[36] The addition to the 2 position is reversible and kinetically controlled, resulting in an easily hydrolysable amine ester functionality. The amine ester is formed as kinetic product, which could be explained by a stereoelectronic theory of the intermediate structures while the amide alcohol is the thermodynamic product.[37, 38] Water termination leads to significant occurrence of the kinetically favored H_2O addition to the 2-position of the oxazolinium species and subsequent formation of the amine ester end-group, therefore the presence of water along with the terminating agent must be avoided to assure quantitative termination in the 5 position of the oxazolinium ring (see **Figure 2**). These initial studies were performed at the optimal polymerization temperature of 80 ^{o}C, *vide infra*.

The amine ester terminated polymer cannot be found by mass spectrometry, as it has the same mass as the target amide alcohol terminated polymer, but can be identified by ^{1}H NMR spectroscopy, due to the characteristic signals of the methylene protons next to the ester oxygen at 4.2-4.3 ppm (see **Figure S1 in Supporting Information**)
Termination with methanolic KOH has been reported to proceed through efficient OH- nucleophilic attack to the 5 position, and as such has been often used for PEtOx termination yielding hydroxyl end-groups quantitatively.[37, 40, 40] Nevertheless, in our hands, PEtOx termination following this protocol sometimes, and mostly for short polymers for which a large amount of KOH has to be added, resulted in the appearance of an extra distribution attributed to the hydrolysed amine ester product, as observed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, see Figure 3a, distribution C). Possibly, the presence of trace amounts of water in the highly alkaline methanolic solution resulted in the fast formation of the amine ester product that was subsequently hydrolysed. In addition, the relatively high amount of KOH added might also induce hydrolysis of the polymer side-chain, in particular on the final unit of the polymer, due to the influence of the -OH group at the chain-end.[42]

Table 1. Detailed peak assignment of the MALDI-TOF spectra of $PEtOx_nOH$

	Polymer Species			Structure	m/z	
Label	**Description**	**Alpha terminus**	**Omega terminus**		**Theoretical**	**MALDI-TOF**
A	Side product 1: Proton initiated polymer due to chain transfer and water present in the polymerisation mixture	H-	-OH		2022.378 (Na^+)	2022.838 (Na^+)
B	Target polymer	CH_3-	-OH		2036.394 (Na^+) 2052.492 (K^+)	2036.867 (Na^+) 2052.849 (K^+)
C	Side product 2: Hydrolysed amine ester. Water-termination in highly basic medium	CH_3-	-$NHCH_2CH_2OH$		2057.436 (H^+) 2079.425 (Na^+)	2057.773 (H^+) 2079.911 (Na^+)

D	Side product 3: H-initiated, hydrolysed amine ester. Water-termination in highly basic medium	H-	-$NHCH_2CH_2OH$		2065.421 (Na^+)	2065.793 (Na^+)
E	Ethene elimination (end-group cleavage)	CH_3-	-$NHCOCH_2CH_3$		2091.436 (Na^+)	2091.837 (Na^+)
F	Living polymer	CH_3-			2110.484	2110.681

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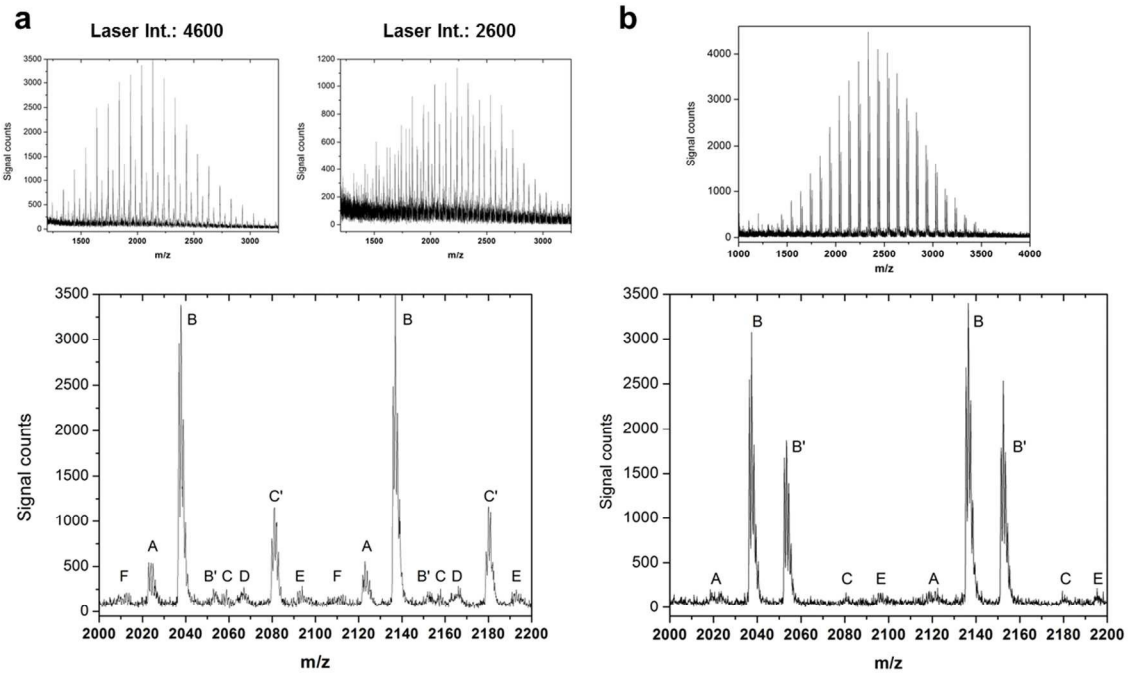

Figure 3. MALDI-TOF MS analysis of two different PEtOx20OH obtained upon polymerisation in acetonitrile at 80 °C for 120 minutes. Initiator: methyl tosylate. [EtOx] = 4 M. [M] / [I] = 20. a) MALDI-TOF mass spectra corresponding to PEtOx20OH terminated with a KOH solution in methanol. Spectra measured with different laser intensities are shown above. b) MALDI-TOF mass spectra corresponding to PEtOx20OH terminated with a 25 wt. % solution of $N(CH_3)_4OH$ in methanol wherein the signals corresponding to the target polymer structure (B) are predominant..

Nevertheless, it should be kept into consideration that MALDI-TOF MS is not a quantitative technique and the relative intensities observed do not necessarily relate to the relative ratios of the different species in the final polymer.

This was highlighted by the relative increase in signal intensity for the hydrolysed amine ester product (C) when low laser intensities were applied (**Figure 3a**), as the hydrolysed species seem to be much easier ionized than the target amide alcohol-terminated polymer. This is possibly a consequence of the basicity of the terminal secondary amine, more prone to form adducts with charged metal ions than the amide alcohol.

In addition, the increased signal for distribution F at low laser intensities confirmed the assignment to living polymer chains (charged species) and ruled out the assignment to neutral coupled species that have the same mass. Distribution E was assigned to end-group degradation, as has been previously reported.[43] However, we could not identify the mechanism of formation of these degradation species, which also may be formed during the MALDI process. The detailed assignment of the MALDI-TOF mass spectra is provided in **Table 1**.

To enhance end-group fidelity by using milder conditions, tetramethylammonium hydroxide was chosen as terminating agent and used in stoichiometric amounts related to living polymer chains in methanol (25 wt. %). This terminating agent yielded reproducible hydroxyl addition to the 5 position, leading to the intended terminal amide alcohol functionality, whatever the molecular weight of the starting PEtOx. The effectiveness of this terminating agent is ascribed to the lower basicity of the $N(CH_3)_4OH$ (pKa = 9.8) solution in comparison to the KOH (pKa = 13.5) solution. In all cases, termination was performed with stoichiometric amounts of terminating agent added at 0 °C into the living polymerisation mixture, and the solution was allowed to warm to room temperature overnight upon stirring. Figure 3b illustrates the impact of the terminating agent on the end-group fidelity of the final polymer, wherein $N(CH_3)_4OH$

termination promotes the near exclusive formation of the desired polymer structure (B).

Though the use of this milder terminating agent decreases significantly the contamination of PEtOx-OH by side products, it was decided to go one step further by optimizing the polymerisation temperature. A stock solution of EtOx and MeOTs ([M] / [I] = 30) was prepared, and a series of polymerisations at different temperatures were carried out under microwave irradiation. The livingness of the polymerisation of 2-oxazolines allows accurate calculation of the required polymerisation times, as the polymerisation follows linear first-order kinetics (Equations 2 and 3).

The polymerisation rate constants for EtOx at different temperatures were calculated based on the reported frequency factor (A) and activation energy (E_A) parameters in the Arrhenius equation (**Equation 4**).[44] The polymerisation time at each temperature was calculated to reach 95 % monomer conversion in each polymerisation,[44] and was verified by gas chromatography.[46, 47]

$$-\frac{d[M]}{dt} = k_p[P^*][M] \quad \text{(Equation 2)}$$

$$ln\frac{[M_0]}{[M_t]} = k_p[I_0]t \quad \text{(Equation 3)}$$

$$k_p = Ae^{-E_A/RT} \quad \text{(Equation 4)}$$

Wherein, for EtOx in acetonitrile and tosylate counter ion, A = 1.99 ± 0.85 10^8 L $mol^{-1}s^{-1}$ and E_A = 73.4 ± 0.5 kJ mol^{-1}.[44]

The polymerisation of EtOx was performed at temperatures ranging from 80 to 140 °C under microwave irradiation, and the obtained polymers were terminated with stoichiometric amounts of $N(CH_3)_4OH$ in methanol. The polymerisation mixtures were analysed by size exclusion chromatography (SEC) to assess the impact of temperature on the polymer molecular weight distribution (see **Table 2**).

Table 2. Overview of the synthesized $PEtOx_{30}OH$ polymers. Higher polymerisation temperatures only slightly increase the extent of chain-transfer, as seen by the low increase in dispersity with temperature. Theoretical degree of polymerization = 30 (3006 Da)

Polymerisation parameters		% Conv. (GC)	SEC		MALDI-ToF	
Temp. (°C)	Time (s)		M_n	Đ	M_n	Đ
140	235	99.6	4850	1.13	2960	1.05
120	660	95.2	4650	1.11	2850	1.04
100	2220	94.0	4800	1.10	2770	1.04
80	8220	94.5	4800	1.08	2730	1.03

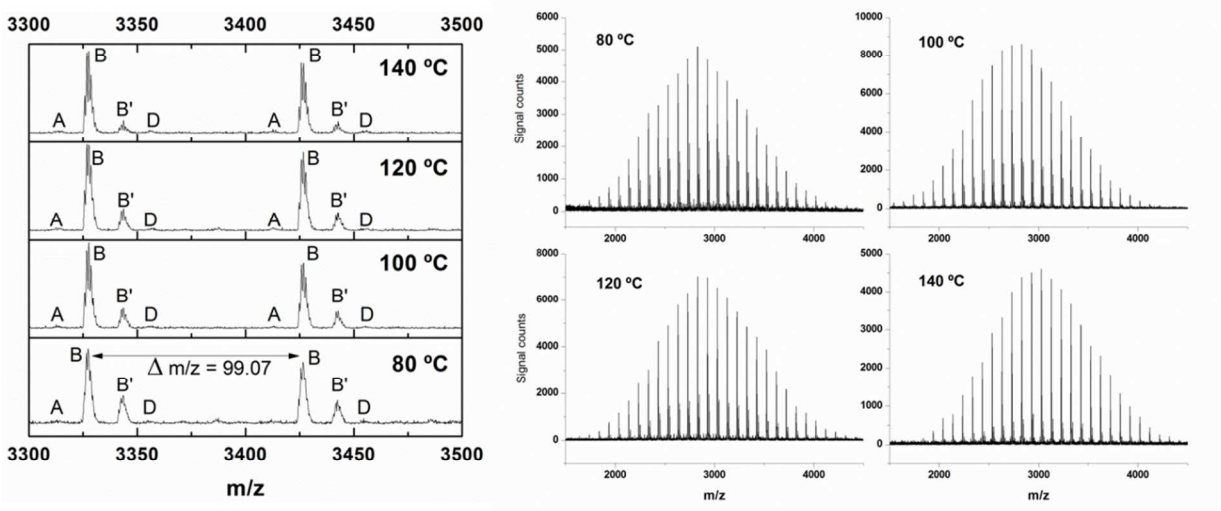

Figure 4. MALDI-TOF mass spectra of $PEtOx_{30}OH$ produced at different temperatures. The overall signal pattern is independent from the polymerisation temperature, as is the occurrence of side-products. Both Na^+ and K^+ adducts of the target polymer species (B and B', respectively) dominate the spectra, and only minor proton-initiated chains (A) and chains bearing a hydrolysed amine ester (D) are detected.

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Higher temperatures consistently resulted in slightly higher dispersities, due to the rise in the high molecular weight distribution ascribed to chain coupling, albeit this effect was minor (SEC traces are shown in **Figure S2**).
Furthermore, although at 140 °C the dispersity was 0.05 units higher than at 80 °C, it should be noted that the conversion was also 5% higher, due to the polymerisation that already occurs during the heating ramp. Therefore, increasing temperature in the investigated range can reduce polymerisation times by a factor of 35 while increasing dispersity by less than 5%.
Nonetheless, for this study end-group fidelity is of highest importance and, therefore, the PEtOx-OH macroinitiators were prepared at 80 °C. These results are in agreement with previous literature that suggested that lower temperatures enhance the control over the living CROP of 2-oxazolines.[36, 48]

Once the effect of increasing temperatures on the polymer molecular weight distribution was assessed, its impact on the end-group fidelity was investigated by MALDI-TOF MS. As seen in **Figure 4**, the overall isotope pattern was not affected by the polymerisation temperature. End-group analysis also showed no differences among the different investigated polymerisation temperatures, all exhibiting the main expected distribution and proton-initiated species (A), together with amine ester hydrolysis product (D) with relative intensities of less than 5%, compared to the main distribution.
It can be thus concluded that increasing the temperature from 80 °C to 140 °C only exerts a minor negative effect on the final PEtOx molecular weight distribution, while not significantly affecting the end-group fidelity of the final polymer.

The optimized protocol for the synthesis of hydroxyl-terminated PAOx was applied to the polymerisation of EtOx to obtain PEtOx-OH macro-initiators for the preparation of PEtOx-*b*-PC copolymers. Two polymers were synthesized, bearing 23 and 43 EtOx repeating units. Full characterization is shown in **Figure 5**, confirming the intended polymer composition.

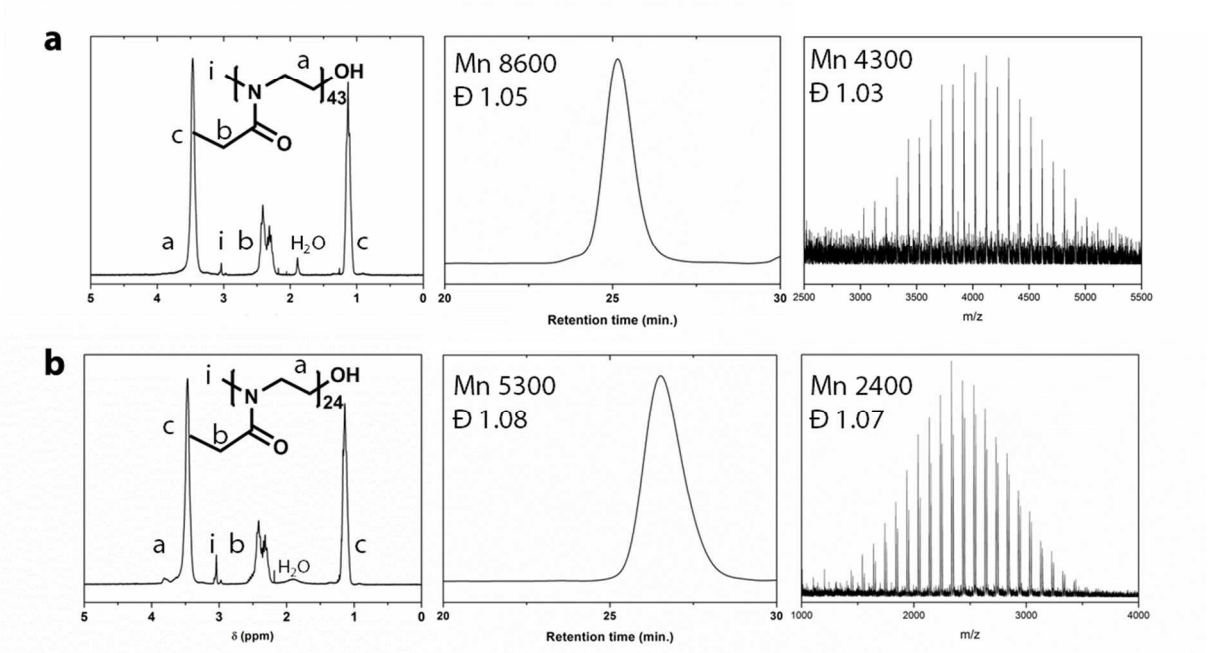

Figure 5. Characterization data of the two $PEtOx_nOH$ homopolymers (a: DP 43, b: DP 24) to be used as macroinitiators for the polymerisation of cyclic carbonates. ^1H NMR spectra (left) confirm the absence of amine ester terminal functionality, whereas MALDI-TOF MS (right) shows quantitative hydroxyl polymer termination. For $PEtOx_{43}OH$, Na^+ adduct is observed, whereas for $PEtOx_{24}OH$, Na^+ and K^+ adducts of the target polymer are observed. SEC traces (center) show monomodal narrow distributions for both polymers, with absence of chain coupling species.

Polymer Chemistry

ARTICLE

Table 3. Poly(2-ethyl-2-oxazoline)-*b*-poly(carbonate) block copolymers characterization data[a]

Polymer	Monomer(s)	DP[b]	M_n (SEC)[c/d] (g.mol^{-1})	M_n (NMR) (g.mol^{-1})[b]	$Đ_M$[c/d]
PEtOx$_{23}$-*OH*	-	23	5400[c]/2400[d]	2300	1.07[c]/1.13[d]
PEtOx$_{43}$-*OH*	-	43	8600[c]/4200[d]	4300	1.05[c]/1.10[d]
PEtOx$_{23}$-*b*-PTMC$_{24}$	TMC	24	10100[c]/7000[d]	4800	1.09[c]/1.14[d]
PEtOx$_{23}$-*b*-PTMC$_{62}$	TMC	62	12400[c]	8600	1.08[d]
PEtOx$_{43}$-*b*-PTMC$_{47}$	TMC	47	14000[c]/13400[d]	9100	1.18[c]/1.08[d]
PEtOx$_{23}$-*b*-PMBC$_{27}$	MBC	27	14200[c]/10500[d]	9100	1.10[c]/1.17[d]
PEtOx$_{23}$-*b*-PMAC$_{23}$	MAC	23	8900[d]	6900	1.23[d]
PEtOx$_{23}$-*b*-PMAC$_{30}$	MAC	30	5800[c]/8900[d]	8300	1.08[c]/1.20[d]
PEtOx$_{23}$-*b*-PMPC$_{28}$	MPC	28	9700[d]	7900	1.29[d]
PEtOx$_{23}$-*b*-PMPC$_{30}$	MPC	30	14500[c]/9400[d]	8300	1.20[c]/1.31[d]
PEtOx$_{23}$-*b*-P(MTC$_{Br}$)$_{11}$	MTC$_{Br}$	11	3800[d]	5400	1.12[d]
PEtOx$_{23}$-*b*-P(MTC$_{Br}$)$_{14}$	MTC$_{Br}$	14	4300[d]	6300	1.12[d]
PEtOx$_{23}$-*b*-P(MTC$_{morph}$)$_{13}$	MTC$_{morph}$	13	3900[d]	5900	1.13[d]
PEtOx$_{23}$-*b*-P(MTC$_{morph}$)$_{26}$	MTC$_{morph}$	26	8000[d]	9400	1.14[d]
PEtOx$_{43}$-*b*-P(MTC$_{morph}$)$_{19}$	MTC$_{morph}$	19	11500[c]/7700[d]	9500	1.13[c]/1.24[d]
PEtOx$_{43}$-*b*-P(MTC$_{morph}$)$_{29}$	MTC$_{morph}$	29	12500[c]/8900[d]	12200	1.15[c]/1.28[d]
PEtOx$_{43}$-*b*-P(MTC$_{morph}$)$_{45}$	MTC$_{morph}$	45	15300[c]/14800[d]	16600	1.17[c]/1.11[d]
PEtOx$_{43}$-*b*-P(MTC$_{morph}$)$_{60}$	MTC$_{morph}$	60	15000[c]/11300[d]	20700	1.20[c]/1.41[d]
PEtOx$_{23}$-*b*-PMAC$_{5.5}$-*b*-(MTC$_{morph}$)$_{26}$	MAC, MTC$_{morph}$	5.5, 26	13500[c]/11000[d]	10500	1.09[c]/1.13[d]
PEtOx$_{43}$-*b*-PMAC$_{5}$-*b*-(MTC$_{morph}$)$_{50}$	MAC, MTC$_{morph}$	5, 50	18000[c]/18300[d]	19000	1.24[c]/1.17[d]

[a]Conditions: [M] = 0.5 M, 25 °C, ROH = PEtOx$_{23}$-*OH* (M_n = 5400 g.mol^{-1}, $Đ_M$ = 1.07 in DMA; M_n = 2400 g.mol^{-1}, $Đ_M$ = 1.13 in THF/3%TEA) or PEtOx$_{43}$-*OH* (M_n = 8600 g.mol^{-1}, $Đ_M$ = 1.05 in DMA; M_n = 4200 g.mol^{-1}, $Đ_M$ = 1.10 in THF/3%TEA). [b]Determined by ^{1}H NMR analysis in $CDCl_3$.[c]Determined by SEC analysis in *N,N*-dimethylacetamide. [d]Determined by SEC analysis in THF/3%TEA).

2.2. Synthesis of poly(2-ethyl-2-oxazoline)-*b*-poly(carbonate) copolymers by metal free ROP

The synthesis of block copolymers was then achieved using PEtOx-OH as macroinitiators for cyclic carbonate ROP whereby the hydroxyl group at the *ω*-chain end acts as the initiating group. Keeping the potential biomedical applications of the block copolymers in mind, polymerisations were carried out using the bifunctional organic catalyst system of DBU and 1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea (TU).[13] ROP was carried out in $CHCl_3$ or $CDCl_3$ at ambient temperature using 5 mol% TU and 1 mol% DBU ([M] = 0.5 M).
Initially, the simple non-functional monomer TMC was used to investigate the suitability of the PEtOx-OH macroinitiators for cyclic carbonate ROP. The ROP of TMC from PEtOx-OH macroinitiators successfully yielded block copolymers with different block sizes (**Table 3**).

More interestingly, the synthesis of PEtOx-initiated polycarbonates was successfully extended to produce block copolymers with a variety of pendant functional groups on each polycarbonate repeating unit. In practice, chain extension of $PEtOX_{23}$ and $PEtOX_{43}$ have been carried out with six membered cyclic carbonate monomers tagged with benzyl (MBC), allyl (MAC), propargyl (MPC), bromide (MTC_{Br}) and morpholino (MTC_{morph}) functionalities to demonstrate the broad scope and versatility of this method (**Figure 6**).

The successful polymerization of these monomers through a metal-free process has previously been reported in the synthesis of biodegradable hydrogels and drug delivery vehicles.[1,4,5,16,49] All block copolymers were analysed by ^{1}H NMR spectroscopy and SEC analysis (**Table 3**). SEC analysis of the block copolymers based on $PEtOx_{23}$-OH revealed that the narrow dispersities of the macroinitiator were mostly retained after copolymerisation, with narrow dispersities for the block copolymers ranging from 1.1 to 1.3, whilst a shift to lower retention time indicated that successful chain extension had taken place (See SEC traces in **Figure S3**).
Block copolymers based on $PEtOx_{43}$-OH revealed the presence of a small amount of unreacted macroinitiator in each case, which could either be due to < 100% PEtOx end group fidelity

or impaired accessibility of the hydroxyl chain end in the reaction medium lowering the initiation efficiency (see **Figure S4**).

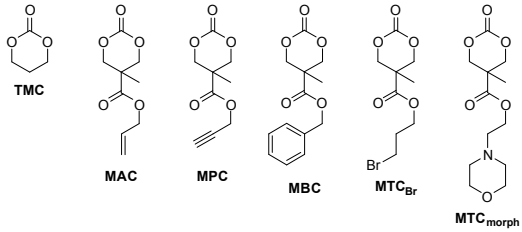

Figure 6. Selected (functional) six-membered cyclic carbonate monomers Trimethylene carbonate (TMC), 5-methyl-5-allyloxy carbonyl-1,3-dioxan-2-one (MAC), 5-methyl-5-propargyloxycarbonyl-1,3-dioxan-2-one (MPC), 5-methyl-5-benzoxycarbonyl-propylene carbonate (MBC), 5-methyl-5-propylbromide-1,3-dioxan-2-one (MTC$_{Br}$), 5-methyl-5-(4-ethyl)morpholine-1,3-dioxane-2-one (MTC$_{morph}$)

^{1}H NMR spectroscopic analysis of the block copolymers revealed clearly the presence of specific resonances of both PEtOx and polycarbonate blocks in all block copolymers, which allowed for the determination of the final copolymer composition and molecular weight. Characteristic signals of PEtOx were observed at 3.45 ppm, 2.23-2.45 ppm and 1.12 ppm, while those corresponding to the poly(carbonate) backbone were observed around 4.3 ppm for the functional blocks and at 4.2 ppm and 2.05 ppm in case of PTMC. Resonances of the pendent groups were observed at 4.73 ppm and 2.54 ppm (propargyl, PMPC), 5.88 ppm, 5.22-5.32 ppm, 4.62 ppm (allyl, PMAC), 3.67 ppm, 2.61 ppm and 2.48 ppm (morpholino, PMTC$_{morph}$), 7.3 ppm and 5.2 ppm (benzyl, PMBC). (see **Figure S5** in Supporting Information).

The living nature of the ROP process was demonstrated through chain extension with a 3rd monomer, leading to well-defined multifunctional triblock copolymers, composed of a middle block carrying pendent allyl functionalities and a final block carrying pendent morpholino functionalities

Figure 7 shows the ^{1}H NMR spectrum of the PEtOx$_{23}$-OH based triblock copolymer wherein the ethyl resonances of the PEtOx block, the allyl resonances of the PMAC block and the resonances of the morpholino groups can be clearly distinguished. Furthermore, the SEC analysis of such triblock copolymers with PEtOx$_{23}$-OH and PEtOx$_{43}$-OH macroinitiators revealed the formation of well-defined triblock copolymers. Although PEtOx$_{43}$-OH macroinitiators led to bimodal SEC distributions due to incomplete initiation, relatively well defined polymers were obtained, with dispersities remaining below 1.20 (**Table 3**).

Conclusions

In conclusion, hydroxyl-terminated poly(2-oxazoline)s with high end-group fidelity can be successfully produced using lower temperature and a milder terminating agent, namely tetramethylammonium hydroxide. The resulting PEtOx-OH were successfully employed as macroinitiators in the ROP of a broad range of six-membered cyclic carbonates.

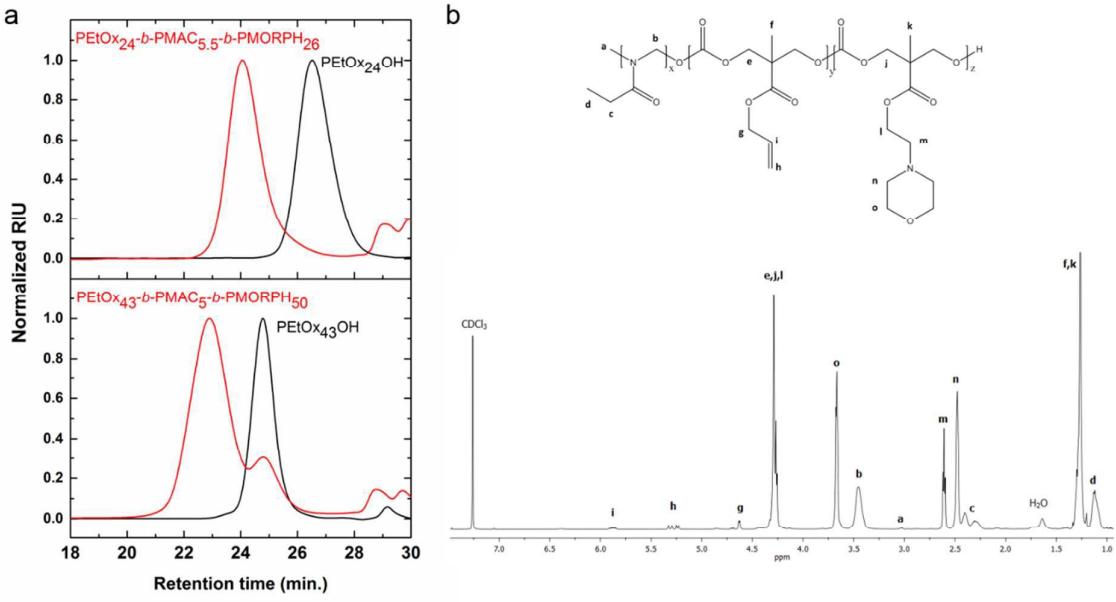

Figure 7. a) SEC traces of PEtOx-*b*-PMAC-*b*-P(MTC$_{morph}$) triblock copolymers with a poly(2-ethyl-2-oxazoline) block and poly(carbonate) blocks with pendant allyl and morpholino groups. The peaks observed at high retention times correspond to residual carbonate monomer. b) ^{1}H NMR spectrum in CDCl$_3$ of a poly(EtOx)-*b*-poly(MAC)-*b*-poly(MTCmorph) triblock copolymer (500 MHz).

The combination of poly(2-oxazoline)s and aliphatic polycarbonates in di- or triblock copolymers associates biocompatibility, biodegradability and physicochemical tunability into one polymer structure, which may lead to the development of interesting novel polymeric nanocarriers with potential use in biomedical applications. Currently, we are investigating the self-assembly behaviour of these copolymers in aqueous medium and research is ongoing to valorise these nanocarriers as useful tools in biomedicine.

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45. The polymerization time was calculated with the following equation: $t = (\ln[M]_0/[M]_t) / (kp\ [I]_0)$ where, for a monomer conversion of 95%, $\ln[M]_0/[M]_t = 3$. For [M] / [I] =30, $[I]_0 = 4/30$ mol l^{-1}.

46. Conversion was calculated from the difference between the t_0 and t_{final} GC samples, whereby acetonitrile was taken as internal standard.

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GRAPHICAL ABSTRACT

TEXT FOR TABLE OF CONTENTS

This work reports on defining optimal conditions to achieve tailored P(EtOx-*co*-PC) copolymers in an efficient and metal-free ring-opening polymerisation processes, utilizing organic catalysts.