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End-group functionalization of poly(2-oxazoline)s using methyl bromoacetate as initiator followed by direct amidation

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ABSTRACT

Poly(2-alkyl/aryl-2-oxazoline)s (PAOx) are an alluring class of polymers for many applications due to the broad chemical diversity that is accessible for these polymers by simply changing the initiator, terminating agent and the monomer(s) used in their synthesis. Additional functionalities (that are not compatible with the cationic ring-opening polymerization) can be introduced to the polymers *via* orthogonal post-polymerization modifications. In this work, we expand this chemical diversity and demonstrate an easy and straightforward way to introduce a wide variety of functional end-groups to the PAOx, by making use of methyl bromoacetate (MeBrAc) as a functional initiator. A kinetic study for the polymerization of 2-ethyl-2-oxazoline (EtOx) in acetonitrile (CH_3CN) at 140 °C revealed relatively slow initiation and slower polymerization than the commonly used initiator, methyl tosylate (MeOTs). Nonetheless, well-defined polymers could be obtained with MeBrAc as initiator, yielding polymers with near-quantitative methyl ester end-group functionality. Next, the post-polymerization modification of the methyl ester end-group with different amines was explored by introducing a range of functionalities, *i.e.* hydroxyl, amino, allyl and propargyl end-groups. The lower critical solution temperature (LCST) behavior of the resulting poly(2-ethyl-2-oxazoline)s was found to vary substantially in function of the end-group introduced, whereby the hydroxyl group resulted in a large reduction of the cloud point transition temperature of poly(2-ethyl-2-oxazoline), ascribed to hydrogen bonding with the polymer amide groups. In conclusion, this paper describes an easy and fast modular approach for the preparation of end-group functionalized PAOx.

1. Introduction

The use of poly(2-alkyl/aryl-2-oxazoline)s (PAOx) in areas such as biomedicine and drug delivery [1–7] has gained an increased interest due to their higher chemical stability as well as their high chemical versatility compared to the commonly utilized poly(ethylene glycol). Moreover, the biocompatibility and non-cytotoxicity of this polymer class, mostly documented for poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx), have been demonstrated in several *in vivo* and *in vitro* studies [6,7]. The synthesis of PAOx *via* living cationic ring-opening polymerization (CROP) provides excellent control over chain-end functionality as well as chain length, and the functionality of the polymers can be tuned in a facile and efficient way through end-group or side chain modification. However, it should be noted that due to the polymerization mechanism, functional groups bearing a nucleophilic character should be avoided or at least protected, as they interfere with the CROP mechanism and lead to premature termination of the polymerization. Introducing additional (nucleophilic) functionalities to the PAOx can however be achieved by using functional initiators, terminators and monomers that are compatible with the CROP of PAOx and converting them in a post-polymerization modification [8,9]. Most modifications have been reported based on thiol-ene/yne coupling [10–12] and copper(I)-catalyzed azide-alkyne cycloaddition [13,14] or require a deprotection step of the competitive reactive site (*e.g.* amines, thiols, alcohols) [15,16]. We hypothesized that using a methyl ester containing functional initiator might overcome these limitations since no protection step is needed for the CROP and direct modification to a large number of functional groups may be possible using a single amidation reaction. Previous reports already demonstrated the successful use of tert-butyl bromoacetate [17] and ethyl 3-bromopropionate [18] as initiators for the CROP of 2-oxazolines and their hydrolysis yielding acid-functionalized PAOx. Furthermore, we reported the modification of PAOx with methyl ester side-chains by amidation with a range of amines [19]. Based on

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these previous studies, it is proposed to use methyl bromoacetate (MeBrAc) as functional initiator as basis for the preparation of a wide range of end-functionalized PAOx.

In this work, we explored the use of MeBrAc as a new functional initiator and evaluated the post-polymerization modification of the resulting methyl ester end-functionalized PAOx towards a variety of other functional handles. Sodium azide (NaN_3) was used as a terminating agent to introduce an azide end-functional group which will remain unaltered during the modifications of the initiator group. Additionally, the azide group has the potential to add extra functionality to the formed PAOx for labelling, targeting or even *in vivo* imaging [20–22]. In a last step, the influence of the end-group functionality on the thermoresponsive properties of the polymer will be examined. The ability of chain-end modification (and tunability of the thermoresponsive properties) of PAOx in a facile way is highly promising for the preparation of a number of PAOx systems such as PAOx-drug conjugates.

2. Experimental section

2.1. Materials

Methyl bromoacetate (MeBrAc, ≥97%), methyl *p*-toluenesulfonate (MeOTs, 98%), sodium azide (NaN_3, 99.5%), ethanolamine (≥98%), allylamine (≥98%), propylamine (≥98%), ethylenediamine (≥98%), 1.5.7-triazabicyclo[4.4.0]dec-5-ene (TBD, 98%), barium oxide (BaO, 90%) and HPLC quality solvents (*i.e.* acetonitrile (CH_3CN) and diethyl ether (Et_2O)) were purchased from Sigma-Aldrich. Dimethyl sulfoxide (DMSO, ≥99.5%) and dry methanol on molecular sieves (< 0.005% water) were purchased from Carl Roth and Acros Organics, respectively. Propargylamine (95%) and tetradecylamine (96%) were purchased from TCI. 2-Methyl-2-oxazoline (MeOx) was purchased from Chemical Point, 2-ethyl-2-oxazoline (EtOx) was kindly donated by Polymer Chemistry Innovations and 2-*n*-propyl-2-oxazoline (*n*PrOx) was synthesized according to a literature procedure [23]. CH_3CN was purified over aluminum oxide by means of a solvent purification system from J.C. Meyer. All 2-oxazolines were distilled over barium oxide and ninhydrin prior to use. MeOTs was distilled over CaH_2 under reduced pressure and MeBrAc was distilled over barium oxide. Deuterated chloroform ($CDCl_3$, ≥99.8%, < 0.01% water) was purchased from Eurisotop.

2.2. Equipment

Monomer conversions for the CROP of the 2-oxazoline were monitored by gas chromatography (GC) based on the ratio of the integrals from the monomer and the polymerization solvent as internal standard. GC was performed on an Agilent Technologies 7890A system equipped with a VWR Carrier-160 hydrogen generator and an Agilent Technologies HP-5 column of 30 m length and 0.320 mm diameter. An FID detector was used and the inlet was set to 250 °C with a split injection of ratio 25:1. Hydrogen was used as carrier gas at a flow rate of 2 mL/min. The oven temperature was increased with 20 °C min^{-1} from 50 °C to 120 °C, followed by a heating ramp of 50 °C min^{-1} from 120 °C to 300 °C.

A Bruker Avance 400 MHz Ultrashield at 25 °C was used to measure ^{1}H-nuclear magnetic resonance (NMR) spectra with the chemical shifts (δ) given in parts per million (ppm) relative to trimethylsilane (TMS) or residual solvent signals.

All stock solutions and polymerisation mixtures were prepared in a VIGOR Sci-Lab SG 1200/750 Glovebox System with a water concentration of ≤0.01 ppm. The polymerizations were performed in a single mode Biotage Initiator EXP Microwave System with Robot Sixty utilizing capped reaction vials. During the polymerizations, the microwave synthesizer operated at a constant set temperature which is monitored by the IR-sensor.

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostatted column compartment (TCC) at 50 °C equipped with two PLgel 5 μm mixed-D columns (7.5 mm × 300 mm) and a precolumn in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent was N,N-dimethylacetamide (DMA) containing 50 mM of LiCl at a flow rate of 0.5 mL min^{-1}. The SEC elugrams were analyzed using the Agilent Chemstation software with the GPC add on. Molar mass values and molar mass distribution, *i.e.* dispersity (Đ), were calculated against polymethylmethacrylate standards from PSS. Multi-angle light scattering (MALS) measurements are performed on a 3-angle static light scaterring detector, *i.e.* miniDAWN TREOS, from Wyatt Technology. The detector is coupled on-line to an Agilent 1260 infinity HPLC system (vide DMA-SEC), and used to determine the absolute molar mass of the analyzed polymer samples. The measurements are performed at ambient temperature, *i.e.* no temperature control unit is supplied/installed with the above mentioned MALS detector. The refractive index (RI) increment (dn/dc) values are either used as reported for the certain polymer in DMA or determined *via* online SEC equipped with an RID, which measures the RI increase for a 1–10 mg/mL concentration series of the mentioned polymers. The MALS results are further analyzed with the provided Astra 7 software from Wyatt Technology.

Lyophilisation was performed on a Martin Christ freeze-dryer, model Alpha 2–4 LSC plus. Preparative SEC was performed on disposable PD-10 desalting columns from GE Healthcare, following the gravity protocol described in the accompanied instructions.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on an Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer, which is the next generation of tandem time-of-flight MS/MS systems. The 4800 MALDI TOF/TOF™ Analyzer uses a diode-pumped, solidstate laser. Under normal operating conditions, the instrument is categorized as a Class 1 laser. All mass spectra were obtained with an accelerating potential of 20 kV in positive ion mode and in reflectron mode. Trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB, 98%, TCI, 20 mg/mL in acetone) was used as matrix. Polymer samples were dissolved in acetone or methanol (2 mg/mL) and applied on the sample plate *via* the dried droplet method (0.5 μL) in between 0.5 μL of matrix solutions. A poly(ethylene oxide) standard (M_n = 2000 Da) was used for calibration. All data was processed using the mMass Mass Spectrometry Tool.

Cloud point temperatures (T_{cp}) were measured on a Crystal16™ parallel crystallizer turbidimeter developed by Avantium Technologies connected to a recirculation chiller and dry compressed air. Aqueous polymer solutions (10 mg/mL) were heated in 3 cycles from 20 °C to 100 °C with heating and cooling rates of 0.5 °C min^{-1} and a stirring rate of 700 rpm. The T_{cp} are given as the 50% transmittance point during heating.

2.3. Kinetic studies for the polymerization of EtOx with MeBrAc and MeOTs as initiator

To perform the kinetic studies, 10 mL stock solutions with a 4 M monomer concentration in CH_3CN as the polymerization solvent and a target degree of polymerization (DP) of 50 were prepared (Table S1). After complete homogenization, the stock solution was devided over 6 different 2 mL microwave vials, each filled with 800 μL of this stock solution. The vials were capped and reacted in a Biotage® microwave reactor at 140 °C for various times. After the reaction, GC samples were made to follow the monomer conversion by sampling 0.1 mL of the solution and adding 0.9 mL chloroform, as non-interfering solvent. Additionally, the M_n and Đ values were determined by SEC by sampling 0.1 mL of the solution, adding 0.9 mL DMA and filtering the mixture over a 0.22 μm syringe filter.

2.4. Synthesis of homopolymers with MeBrAc as functional initiator

PMeOx, PEtOx and P*n*PrOx with a DP of 30, 50 and 100 were synthesized using MeBrAc as functional initiator and CH_3CN as the polymerization solvent in a Biotage® microwave reactor. Polymerization mixtures (10 mL, 4 M monomer concentration) were prepared and reacted at 140 °C, except for PEtOx DP 100, which is reacted at 100 °C since reaction at 140 °C resulted in a relatively high molecular weight shoulder. The polymerization times for all polymers were calculated according to the kinetic data of the polymerization of EtOx with MeBrAc as initiator relative to polymerizations with MeOTs, for which the k_p values are known from literature [24]. After reaction in the microwave, the polymers were terminated overnight by addition of 3.5 equivalents sodium azide (NaN_3), compared to the amount of initiator, and 0.5 mL of dry methanol to aid the solubilization of the terminating agent (Table S2). The polymers were purified by precipitation in a tenfold excess of ice-cold diethyl ether, followed by dialysis against distilled water (500 Da MWCO from SpectrumLabs) and final lyophilization to obtain the polymer as a white powder. All polymers were characterized by ^{1}H NMR spectroscopy, SEC, MALS-SEC and MALDI-TOF MS (Table S3, Figs. 3 and S1–2).

2.5. End-group modification via direct amidation

2.5.1. Kinetic study of the amidation reaction with allylamine

The polymer (200 mg) was dissolved in an excess of allylamine and reacted at refluxing conditions. The specific amounts of reagents for each kinetic study are given in Table S4. During the reaction, ^{1}H NMR samples are taken to follow the conversion by sampling 0.2 mL of the mixture and precipitating this in ice-cold diethyl ether before being centrifuged for 5 min at 10,000 RPM, decanted and dried in the vacuum oven overnight. The ^{1}H NMR spectra were taken in deuterated chloroform.

2.5.2. End-group modification with different functional groups via direct amidation

The polymer (200 mg) was dissolved in an excess of amine and reacted at 70 °C, or refluxing conditions if the boiling point of the amine was lower than 70 °C, for 30 h to reach full conversion. For the polymers that were (partially) insoluble in the amine, a small amount of DMSO was added to the mixture to aid the solubilization and hence to fully convert the methyl ester of the polymer. The specific amounts of reagents for each post-polymerization modification are given in Table S4. The final work-up of the polymers was performed by either precipitation in a tenfold excess of ice-cold diethyl ether, followed by drying in the vacuum oven (*i.e.* allylamine, propylamine and propargylamine) or evaporation of the amine under reduced pressure, followed by dialysis (*i.e.* ethanolamine) (500 Da MWCO) or the use of a PD-10 column (*i.e.* propylamine with DMSO and ethylenediamine) and final lyophilization. The obtained polymers were analyzed by ^{1}H NMR spectroscopy, SEC, MALS-SEC and MALDI-TOF MS (Table S5, Figs. 7 and S3–5).

2.5.3. End-group modification with tetradecylamine using TBD catalyzed amidation

PEtOx DP 50 (200 mg, 0.035 mmol) was mixed with 6 eq. tetradecylamine (44.93 mg, 0.211 mmol) and 0.5 eq. TBD (2.44 mg, 0.018 mmol) in 0.3 mL CH_3CN. The solution was heated to 70 °C and reacted for 47 h, after which the solution was precipitated in ice-cold diethyl ether, followed by dialysis (500 Da MWCO) and lyophilization. The resulting white polymer is fully analyzed by ^{1}H NMR spectroscopy, SEC, MALS-SEC and MALDI-TOF MS (Table S5, Figs. 7 and S6).

3. Results and discussion

A series of polymers of MeOx, EtOx and *n*PrOx were prepared using MeBrAc as an initiator and functionalized in a post-polymerization step by means of direct amidation of the methyl ester. Initially, a kinetic study of the MeBrAc initiator with EtOx was performed to investigate its performance in the CROP of PAOx compared to the more standard used MeOTs initiator. Afterwards, the general applicability of the MeBrAc initiator was tested in the synthesis of 9 well-defined PAOx with varying side chain (*i.e.* PMeOx, PEtOx and P*n*PrOx) and chain length (*i.e.* DP 30, 50 and 100). Finally, the feasibility to introduce different end-groups to the PAOx polymers *via* modification of the initiator group using direct amidation reactions was investigated. Inspired by the direct amidation procedure found in literature [19], a similar procedure was used for the end-group modification of PAOx with amines in bulk. For the amidation part of this research, initially, the effect of the chain length and the side chain (*i.e.* hydrophobicity of the polymer) on the reaction rate of the end-group modification was studied using allylamine. Afterwards, a series of short amines were coupled to different polymers with varying chain length and side chain using direct amidation to illustrate the potential of introducing a wide variety of functional groups. Finally, an amine with a relatively long hydrophobic chain of 14 carbon atoms (*i.e.* tetradecylamine) was coupled to the polymer. Additionally, the influence of the end-group modification on the lower critical solution temperature (LCST) behaviour of the PEtOx DP 50 homopolymer was also studied.

3.1. Kinetic studies of the CROP of EtOx with MeBrAc and MeOTs

In order to obtain PAOx with a methyl ester end-functionality, the MeBrAc functional initiator was used. Although this exact initiator was not used before for the polymerization of PAOx, a similar tert-butyl bromoacetate (*t*BuBrAc) initiator has been reported for the synthesis of well-defined (Đ < 1.1) PMeOx and PEtOx with a theoretical DP of 23 and 20 monomer units, respectively [17]. In this literature reference, the tert-butyl ester was first converted to the methyl ester to allow hydrolysis of the end-group. Therefore, starting directly from the methyl ester functionalized initiator offers an advantage over the already known *t*BuBrAc initiator, as it will allow us to couple the amines without the need for an extra deprotection step, *i.e.* direct amidation. Nonetheless, MeBrAc has not yet been used as an initiator for CROP and the related *t*BuBrAc was only used to make very short oligomers, making it important to investigate the use of MeBrAc as initiator in further detail. Therefore, a kinetic study was performed for the CROP of EtOx with MeBrAc as initiator and a target DP of 50, in CH_3CN at 140 °C under microwave conditions, to compare the results with the widely used MeOTs-initiated polymerization of EtOx. The first order kinetic plot (Fig. 1, left) revealed a decrease of the overall k_p for the MeBrAc-initiated polymerization ($k_p = 45 \times 10^{-3}$ L/mol s) compared to the polymerization with MeOTs ($k_p = 109 \times 10^{-3}$ L/mol s), as determined from the slope of a linear fit of the entire first order kinetic plot. This decrease can be expected since the bromide anion will lead to an equilibrium between cationic and covalent propagating species [19,25], resulting in a lower number of reactive cationic species present during the propagation step with the bromide counterion when compared to the tosylate counterion that leads to near quantitative cationic propagation. Since propagation almost exclusively occurs with the cationic propagating species, it will be slower for MeBrAc and hence a decrease in k_p value is observed. Additionally, conversion rates for the MeBrAc initiator are lower at the initial stages of polymerization indicating slower initiation ($k_{p,1} = 35 \times 10^{-3}$ L/mol s) and faster polymerization after complete initiation ($k_{p,2} = 57 \times 10^{-3}$ L/mol s). This latter value nicely resembles the previously reported k_p for the CROP of EtOx initiated with acyl bromide ($k_p = 54 \times 10^{-3}$ L/mol s), as expected since after initiation the polymerization should be very similar with the bromide counteranion [26].

In the right plot of Fig. 1, the number average molecular weight (M_n) and dispersity (Đ) of the polymers are shown in function of the conversion. During the entire kinetic study, the dispersity remains low

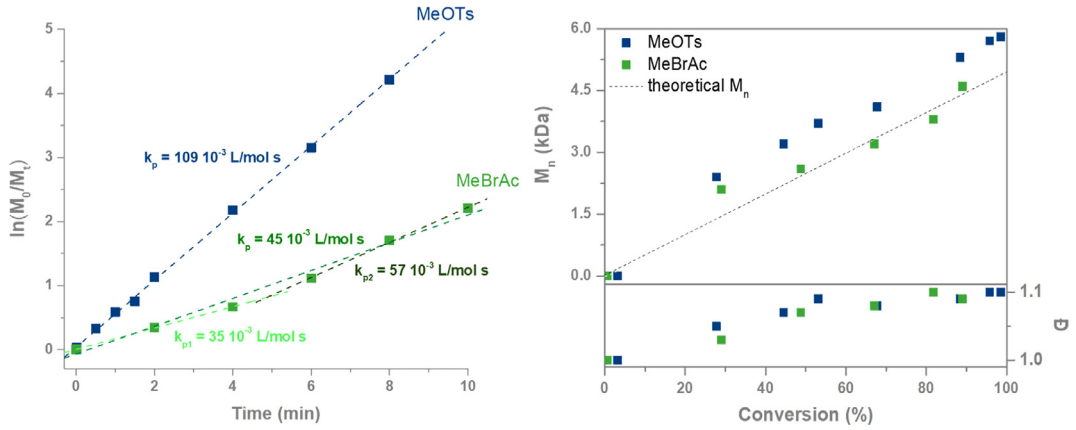


Fig. 1. Kinetic plot (left) and Mn versus conversion plot (right) for the cationic ring-opening polymerization of EtOx with MeBrAc and MeOTs as initiator in CH_3CN at 140 °C, 4 M monomer concentration and a target DP of 50. k_p represents the overall polymerization rate constant obtained from a linear fit of all data points, while $k_{p,1}$ and $k_{p,2}$ represent the rate constants for the initial and final linear parts of the first order kinetic plot with MeBrAc. The polymerization with MeBrAc is about 2.4 times slower than with MeOTs due to the equilibrium between covalent and cationic propagating species while for MeOTs only cationic propagating species are present. The dispersity of both polymers remains low (≤1.1) during the entire kinetic study, indicating well-defined polymers.

for both polymers, indicating the absence of significant chain transfer and termination reactions. Despite the slower initiation with MeBrAc, the resulting polymers have similar Đ values as those obtained with MeOTs.

3.2. Synthesis of homopolymers with MeBrAc as functional initiator

After establishing that well-defined PAOx can be prepared with MeBrAc as initiator, nine different homopolymers were synthesized by CROP with MeBrAc to high conversions (> 98% by GC), with varying side chain (*i.e.* MeOx, EtOx and *n*PrOx) and chain length (*i.e.* DP 30, 50 and 100) (Fig. 2). The data of the kinetic study of the CROP of EtOx with MeBrAc as initiator was used as a first estimate for the reaction times of the different polymerization reactions, by comparison with the commonly used MeOTs initiator [27,28]. The estimation of the reaction time is based on the assumption that the MeOx, EtOx and *n*PrOx monomers have a similar ratio of cationic and covalent species during the propagation reaction and therefore, the same ratio between the k_p values with MeBrAc and MeOTs. This ratio will, however, depend on the nucleophilicity of the monomer, which is not the same for all the used monomers [27] and this assumption is therefore unlikely to be fully accurate. Nonetheless, it can be used as a first approximation to estimate the reaction times for the polymer synthesis and if necessary, the reaction conditions can be further fine-tuned by changing the reaction time and/or temperature. The synthesized polymers were terminated with sodium azide to introduce an azide functional group at the omega-chain-end, which could be used to introduce other functional components to the polymers such as labelling groups, if desired. All polymerization reactions were performed in CH_3CN in a microwave at 140 °C, except for PEtOx DP 100, which was synthesized at 100 °C to reduce the relatively large high molecular weight shoulder that was observed for the polymerization at 140 °C. This larger fraction of high molecular weight chains could be present due to higher conversion of the monomer obtained at 140 °C and/or the possibility that chain transfer and coupling reactions occur less at lower temperature [29]. The reaction time of 285 min for PEtOx DP 100 was based on the comparison of the kinetics of MeBrAc and MeOTs at 100 °C [24]. The SEC traces as well as MALS analysis of the resulting homopolymers indicated the formation of well-defined polymers with a dispersity below 1.16 (Table S3, Fig. 3). All ^{1}H NMR spectra (Figs. 3 and S1) revealed peaks at 3.75 ppm and 4.1 ppm, characteristic for the CH_3 and CH_2 protons of the initiator group. The unanticipated multiplicity of the initiator peaks may be attributed to the conformational change (Fig. 4) of the first amide unit of the polymer to which the CH_3 (3.75 ppm) and CH_2 (4.1 ppm) groups of the initiator are attached. This results in two different chemical environments and might explain the multiplicity of 'two' for the CH_2 signal. Something similar is generally also observed for the methyl end-group when using MeOTs as initiator. A deviating shape was observed for the initiator peaks in the ^{1}H NMR spectra of P*n*PrOx DP 100 and may indicate a lower end-group functionalization of the polymer, which was however difficult to confirm without MALDI-TOF MS data. Further proof of the incorporation of the methyl ester chain-end for the obtained polymers with DP 30 and DP 50 was provided by MALDI-TOF MS (Fig. S2), which showed the expected peak patterns for the unfragmented polymers with Na^+ and/or K^+ as counterions, as well as the frequently observed fragmented patterns of the polymers with the loss of N_2 of the azide group [30]. MALDI-TOF MS spectra could not be obtained for polymers with a DP of 100 since the polymer chain is probably too long to be (efficiently) desorbed from the target plate upon laser irradiation.

3.3. End-group modification via direct amidation

Subsequently, the potential to modify the methyl ester end-group of the abovementioned polymers to a large range of other functional groups *via* direct coupling of amines was explored. This one-step procedure should allow to couple the amine onto the polymer *via* a direct amidation reaction in bulk or in CH_3CN combined with the use of a catalyst, respectively method a and b (Scheme 1). All amidation reactions were carried out at 70 °C or reflux conditions when the boiling

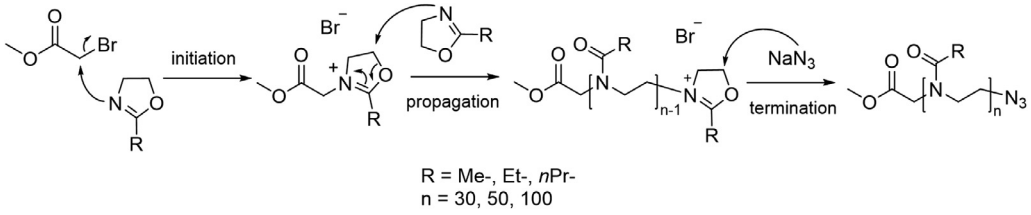


Fig. 2. The CROP mechanism for the polymerizations of MeOx, EtOx and nPrOx with MeBrAc as functional initiator and sodium azide as terminator, with a target DP of 30, 50 or 100 monomer units.

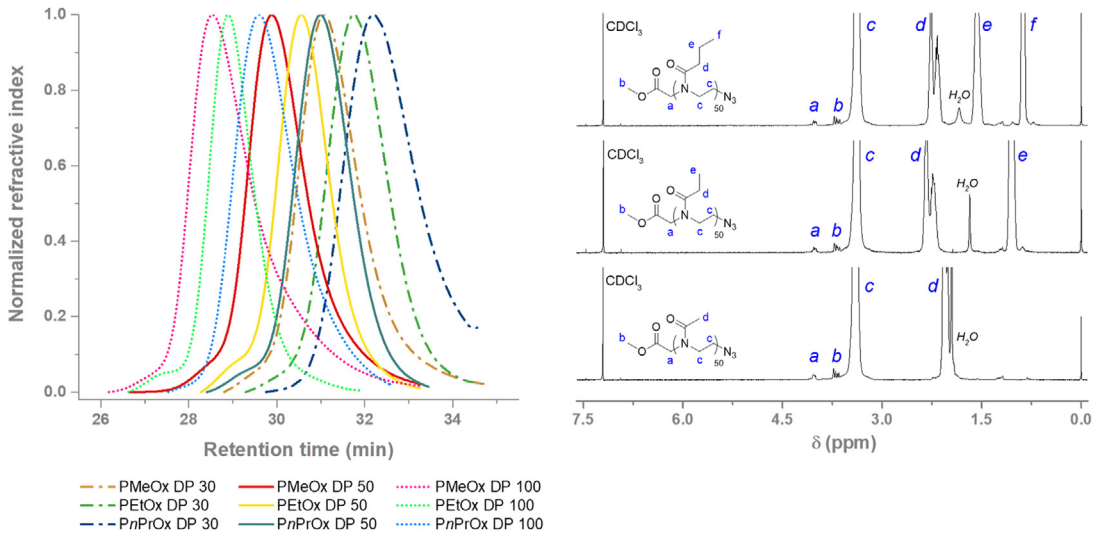


Fig. 3. SEC traces (left) and ^{1}H NMR spectra in $CDCl_3$ (right) of the synthesized polymers with methyl ester and azide end-groups. Narrow SEC traces indicate the formation of well-defined polymers. Generally, more hydrophilic polymers (MeOx > EtOx > nPrOx) elute faster from the column, as well as longer polymer chains (DP 100 > DP 50 > DP 30). Peaks **a** and **b** in the NMR spectra are representative for the CH_2 and CH_3 protons of the methyl ester end-group of the PAOx. Polymers with a DP of 30 and 100 give similar spectra, which are given in the supporting info (Fig. S1).

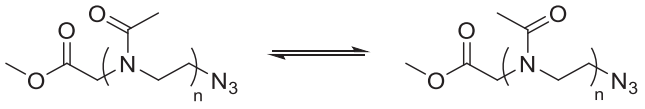


Fig. 4. Conformational change of cisoïd and transoïd of the methyl ester end-group of the PAOx polymers. A different chemical environment of the initiator might explain the multiplicity in the initiator peaks in the ^{1}H NMR spectra of MeBrAc-initiated PAOx.

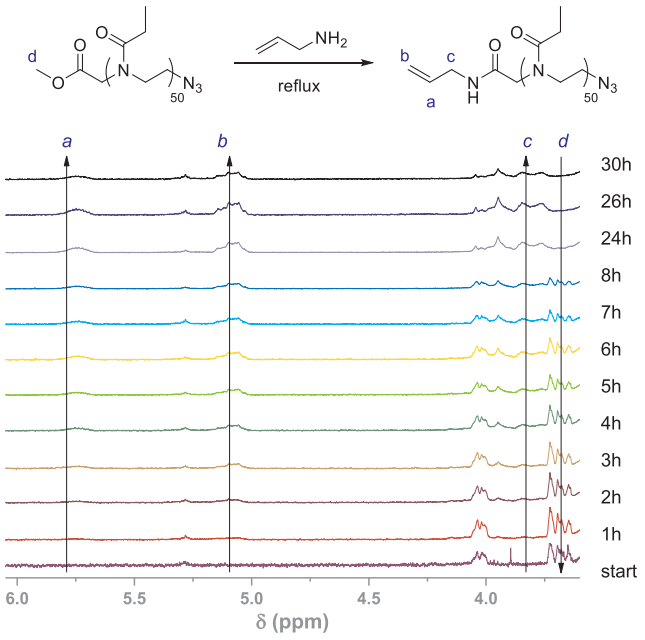
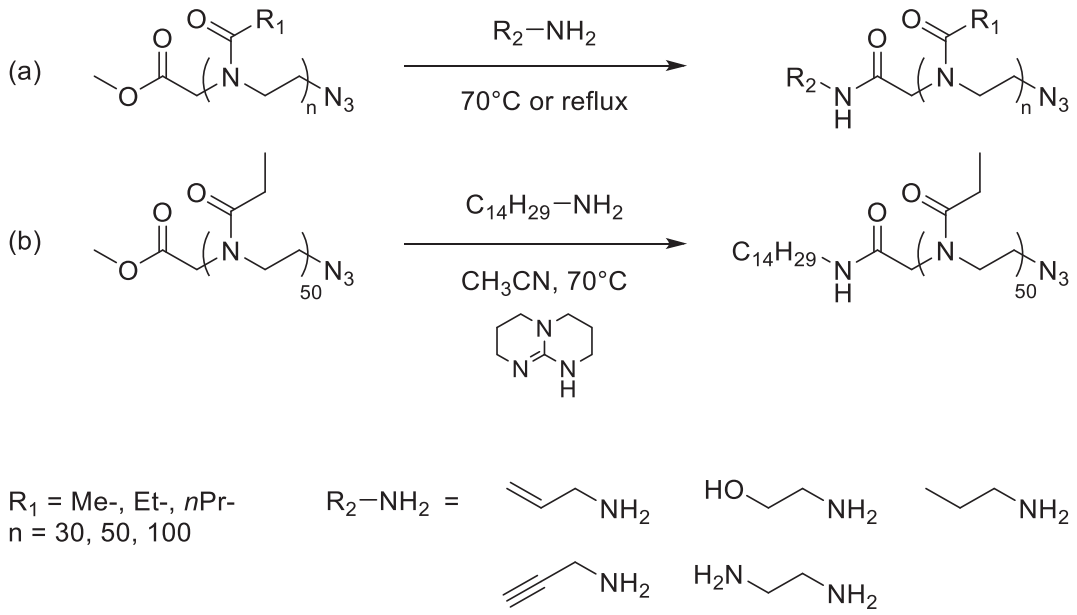


Fig. 5. ^{1}H NMR spectra in $CDCl_3$ for the amidation reaction of PEtOx DP 50 (start) with allylamine after the first 8 h and 24, 26 and 30 h of reacting at refluxing conditions. Based on the integral of the methyl ester peak (d), the kinetic plot in Fig. 6 could be obtained.

point of the amine was lower than 70 °C (method a). In the case when amine was a solid (*i.e.* tetradecylamine), CH_3CN was used as a solvent and 1.5.7-triazabicyclo[4.4.0]dec-5-ene (TBD) as a catalyst, in the presence of an excess of amine (method b). The catalyst was added to compensate for the reduced concentration of the amine compared to the first method a, thereby avoiding a significant extension of the reaction times.

Initially, a series of kinetic studies was performed to study the effect of chain length and side chain on the amidation post-polymerization modification rate with allylamine. The first series contained three different polymers with increasing hydrophobicity of the side chain (*i.e.* PMeOx, PEtOx and P*n*PrOx) and a DP of 30, and in the second series, the influence of the chain length for PEtOx (*i.e.* DP 30, 50 and 100) on the allylamidation modification rate was investigated. For each kinetic study, the polymer was dissolved in an excess of allylamine (1760 equivalents to the methyl ester) and the mixture was heated to refluxing conditions. ^{1}H NMR spectroscopy samples were taken to follow the conversion of the methyl ester group to the allylamide in time and an example of the obtained ^{1}H NMR spectra in time are given in Fig. 5. Note that for the kinetic study of P*n*PrOx DP 30 and PMeOx DP 30, each time one data point was omitted during the fitting as they were regarded as outliers. The kinetic plots were obtained by exponential fitting of the data points and revealed that an increase in the polymer side chain length from methyl to ethyl and finally *n*-propyl did not have a significant influence on the rate of the amidation reaction (Fig. 6, left), while increasing the chain length of the polymer did slightly slow down the reaction (Fig. 6, right). This might be explained by shielding of the end-group by the polymer chain in solution, making the ester less accessible for nucleophilic attack of the amine. However, the kinetic studies should be repeated before drawing any conclusive conclusions. Overall, it could be concluded that all chain-end modifications with allylamine were completed after 30 h. This reaction time will be further used as a starting point to estimate the reaction time required for full conversion of the modification reactions with other small amines.

After determination of the optimized conditions for the amidation reaction with allylamine, the amidation of PAOx with other small amines (*i.e.* ethanolamine, propylamine, propargylamine, ethylenediamine and tetradecylamine) was performed in a similar way to introduce a library of functional groups to the polymers. The amidation reactions were performed in bulk at 70 °C or reflux, where the amine acted as the solvent for the PAOx (Scheme 1a). It should be noted that tetradecylamine is a solid and was therefore coupled to the polymer in the presence of CH_3CN and the use of TBD as a catalyst to enhance the reaction rate after dilution (Scheme 1b). Since during the PMeOx modification with propylamine in bulk a (partial) precipitation was



Scheme 1. Two direct amidation methods that were used for the direct amidation of the methyl ester-initiated PAOx polymers: in bulk (a) or in CH_3CN with TBD as a catalyst (b). The solvent (and catalyst) route was used since the tetradecylamine was a solid and could therefore not be used in bulk as a solvent.

observed, a small amount of DMSO was added to those reaction mixtures to aid the solubilization and therefore fully convert the methyl ester to the propylamide. After isolation, the modified polymers were analyzed by ^{1}H NMR spectroscopy, SEC, MALS-SEC and MALDI-TOF MS (Table S1, Fig. S3–6). As an example, the SEC traces and ^{1}H NMR spectra of PEtOx DP 50 before and after the different amidation reactions are depicted in Fig. 7. All ^{1}H NMR spectra showed full conversion of the methyl ester peak (3.75 ppm) to the corresponding amide after 30 h or 47 h in case of tetradecylamine. The success of the modification reactions was confirmed by the MALDI-TOF MS data which revealed the expected peaks for the unfragmented amidated polymers with Na^+ and/or K^+ as counterions, as well as the frequently observed fragmented patterns of the modified polymer with the loss of N_2 of the azide group [30]. As an example, a simulated spectrum has been prepared and compared to the measured spectrum for the allylamidated PEtOx DP30 polymer (Fig. S7). Polymers that were modified with ethylenediamine and propargylamine exhibited an additional peak pattern contributed to the loss of the entire azide end-group, which might appear due to the occurrence of impurities in the MALDI sample [31]. However, since no peaks of the unmodified polymer were present in the MALDI spectra, full conversion to the amidated polymers could be concluded. Finally, the SEC traces revealed a small increase at high molecular weight for the allylamidated and propargylamidated polymers, possibly due to light-induced homocoupling of the unsaturated end-groups, while the other modifications presented a nearly identical distribution before and after modification, indicating the absence of polymer coupling.

3.4. Influence of the end-group functionality on the LCST behaviour of PEtOx DP 50 homopolymers

Since PEtOx DP 50 was modified with all amines, the thermoresponsive properties of the modified polymers were studied and compared to each other to investigate the influence of the chain-end functionality on the T_{cp}. The LCST behaviour was investigated for each polymer at a concentration of 10 mg/mL between 20 °C and 100 °C and it could be concluded that PEtOx DP 50 modified with propylamine, propargylamine and ethylenediamine did not possess a T_{cp} in the used temperature range. The pH of the ethylenediamine-modified PEtOx DP 50 solution was set to 10–11 to ensure full deprotonation of the amine

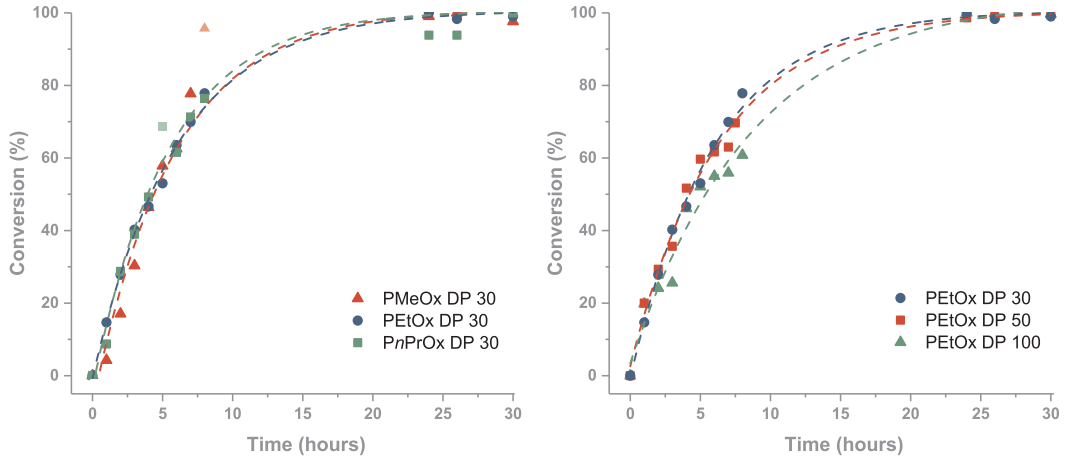


Fig. 6. Kinetic plots for the amidation modification of methyl ester-initiated PAOx with allylamine. The length of the side chain has no significant influence on the reaction rate (left), while an increase in chain length of the polymer does slow down the reaction somewhat (right). The data points are based on the integral of the methyl ester peak (3.75 ppm) in the ^{1}H NMR spectra and an exponential fit is used. All experiments were performed one single time.

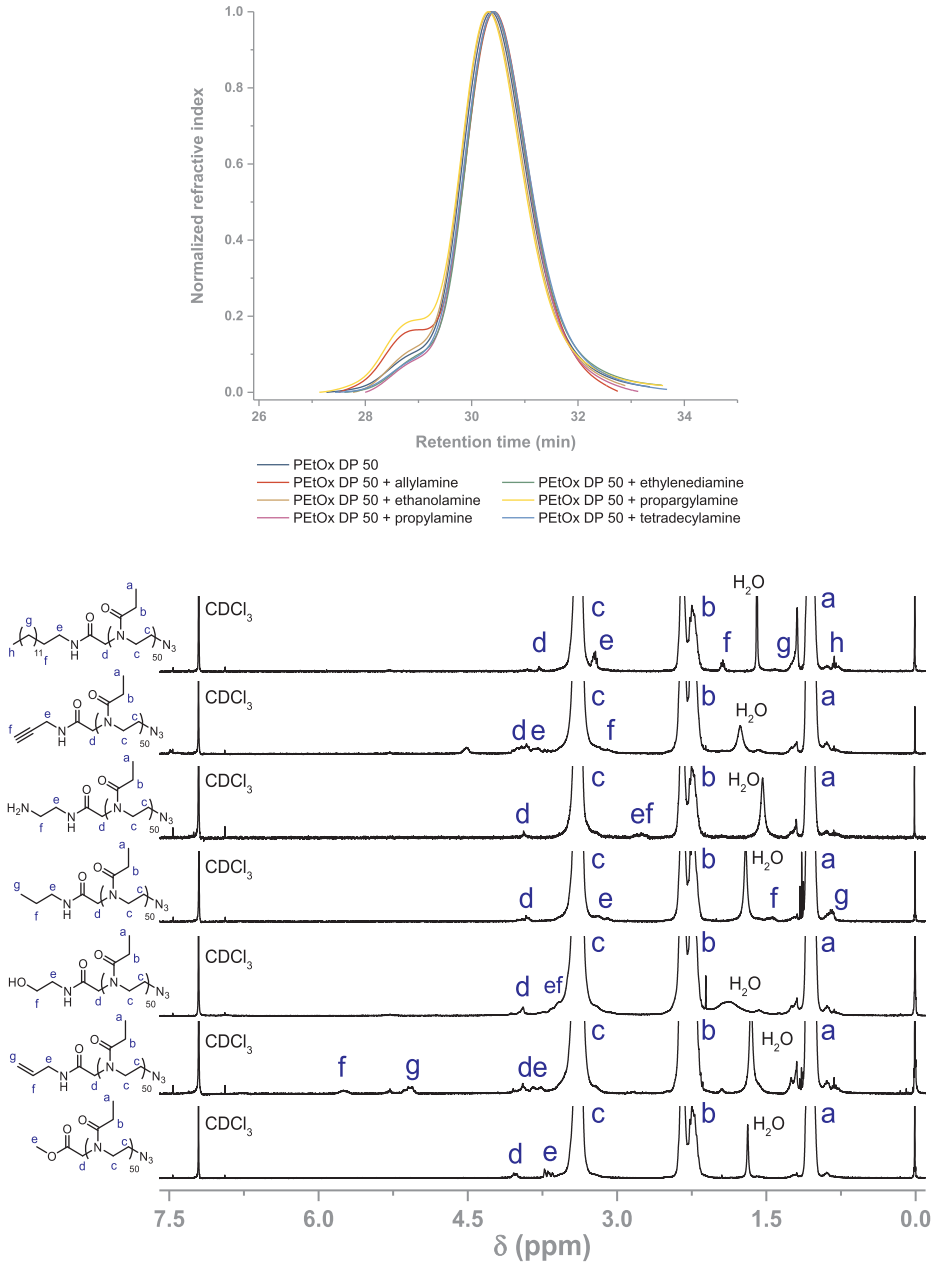


Fig. 7. SEC traces and ^{1}H NMR spectra of PEtOx DP 50 and all amidation reaction performed on this polymer. Full conversion is confirmed by the complete disappearance of the methyl ester peak (3.75 ppm) in the NMR spectra and the appearance of the corresponding amide peaks. SEC traces show the formation of the amidated polymers without any significant crosslinking or side reactions.

group. On the other hand, the methyl-ester functionalized PEtOx DP 50 and the allylamine-modified polymer showed a very similar T_{cp} around 98 °C, while the polymers modified with ethanolamine and tetradecylamine revealed a significant change in LCST behaviour (Fig. 8). The lower T_{cp} value of the tetradecylamine-modified polymer of ~67.6 °C might be explained based on the presence of the hydrophobic C_{14}-chain, which will be less hydrated compared to the more hydrophilic polymer chain resulting in a lower T_{cp} compared to the unmodified PEtOx DP 50. It should be noted that no micellization was observed by DLS and that the decrease in T_{cp} can solely be ascribed to the hydrophobic end-group. Surprisingly, the ethanolamine-modified PEtOx revealed the lowest T_{cp} of 31 °C, which may be ascribed to competition between hydration of the polymer and intramolecular hydrogen bond formation between the polymeric amide groups and the polymer end-group. It is however rather surprising that such a large difference in LCST behaviour results from the incorporation of a single ethanolamine group on the polymer chain end. In previous work, Jordan *et al.* illustrated the influence of the end group of poly(2-*i*-propyl-2-oxazoline) on the T_{cp} of the polymer revealing that hydrophobic end groups decrease the T_{cp}, while hydrophilic groups had the opposite effect, as expected [32]. This is, however, contradictory to our finding that the ethanolamine-modified PEtOx has a lower T_{cp} indicating that not only the hydrophilicity/hydrophobicity of the end group is of importance, but possible interactions with the polymer amide units in the polymer chain that compete for the hydration, thereby lowering the polymer solubility, should also be taken into account. Additionally, no significant hysteresis was observed for the polymer phase transitions as shown in Fig. 8. It should be mentioned that the increase in transmittance after the phase transition for the tetradecylamidated polymer is caused by the sticking of the high polymer concentration phase on the walls of the vial and the stirring bar, as was visually confirmed.

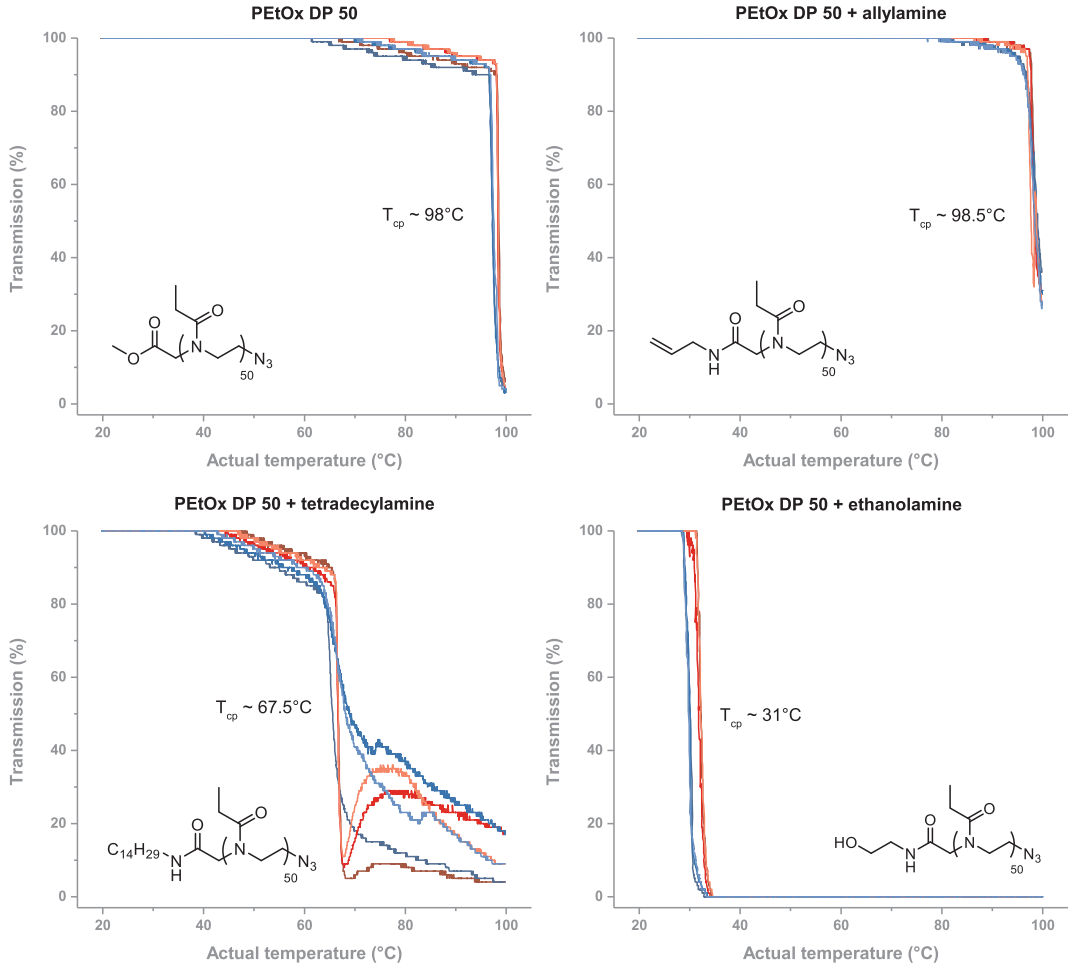


Fig. 8. Cloud point determination of PEtOx DP 50 and the amidation modifications with allylamine, ethanolamine and tetradecylamine at a concentration of 10 mg/mL and 3 heating (red)/ cooling (blue) cycles. PEtOx DP 50 polymers modified with propylamine, ethylenediamine (pH 10–11) and propargylamine did not possess a T_{cp} in the used temperature range and are therefore not shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions and outlook

In this work, MeBrAc was explored as a novel functional initiator for the synthesis of PAOx possessing a methyl ester end-group in the alpha chain-end, and was further used as a functional handle to introduce a variety of different end-functional groups using a direct amidation procedure. A kinetic study of this new initiator revealed a 2.4-fold decrease in the polymerization rate for PEtOx at 140 °C when compared to the state-of-the-art with MeOTs as initiator, as generally observed for bromide containing initiators due to the lower fraction of cationic propagating species resulting from the equilibrium between covalent and cationic propagating species during the polymerization. Further examination of the general applicability of the MeBrAc initiator led to the successful synthesis of methyl ester end-functionalized PMeOx, PEtOx and P*n*PrOx polymers with a chain length of 30, 50 and 100. Those polymers were successfully modified with several small amines bearing different functional groups. Kinetic studies of the modification reaction with allylamine and PEtOx, revealed that the side chain and the chain length of the polymer hardly influences the reaction rate of the end-group modification.

Finally, investigation of the LCST behaviour of the methyl ester functionalized PEtOx DP 50 and all the amidated PEtOx DP 50 polymers revealed a significant decrease in T_{cp} for the ethanolamine-modified and tetradecylamine-modified polymers compared to the T_{cp} of the unmodified ones. Modification with allylamine did not change the T_{cp}, and polymers modified with propylamine, ethylenediamine and propargylamine did not possess a T_{cp} in the temperature range of 20–100 °C. This intriguing influence of the end-groups on the T_{cp} of PEtOx will be the focus of our future studies.

In summary, it can be concluded that MeBrAc can be used as a functional initiator to introduce a wide variety of end-groups to the PAOx in an easy and straightforward approach, although this can influence the thermoresponsive properties of the polymers profoundly, a matter subject to further research. The functional handles introduced provide facile access to functional block copolymers *via* click chemistry, targeting groups for diagnostics or spacers for the coupling of bio-active compounds like proteins. In all, the use of this functional initiator can be regarded as an additional convenient tool for the further exploitation of functional poly(2-oxazoline)s in biomedical applications.

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Data availability statement

All data required to reproduce this work is included in the manuscript and the supporting information.

Declaration of Competing Interest

RH and VdlR are cofounders of Avroxa BVBA that commercializes poly(2-oxazoline)s under the tradename Ultroxa®. The other coauthors have no conflicts to declare.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eurpolymj.2019.109273.

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