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Functional Poly(2-oxazoline)s by Direct Amidation of Methyl Ester Side Chains

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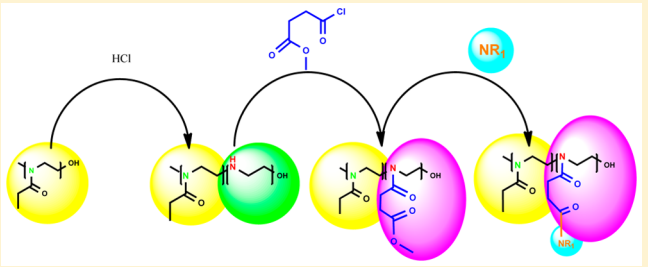
ABSTRACT: Poly(2-alkyl/aryl-2-oxazoline)s (PAOx) are biocompatible pseudopolypeptides that have received significant interest for biomedical applications in recent years. The growing popularity of PAOx in recent years is driven by its much higher chemical versatility compared with the gold standard in this field, poly(ethylene glycol) (PEG), while having similar beneficial properties, such as stealth behavior and biocompatibility. We further expand the PAOx chemical toolbox by demonstrating a novel straightforward and highly versatile postpolymerization modification platform for the introduction of side-chain functionalities. PAOx having side chain methyl ester functionalities is demonstrated to undergo facile uncatalyzed amidation reactions with a wide range of amines, yielding the corresponding PAOx with side-chain secondary amide groups containing short aliphatic linkers as well as a range of side-chain functionalities including acid, amine, alcohol, hydrazide, and propargyl groups. The PAOx with side-chain methyl ester groups can be prepared by either partial hydrolysis of a PAOx followed by the introduction of the methyl ester via modification of the secondary amine groups with methyl succinyl chloride or by the direct copolymerization of a nonfunctional 2-oxazoline monomer with a 2-methoxycarbonylethyl-2-oxazoline. Thus, this novel synthetic platform enables direct access to a wide range of side-chain functionalities from the same methyl-ester-functionalized poly(2-oxazoline) scaffold.



INTRODUCTION

The story of poly(2-oxazoline)s started in 1966 when four independent research groups reported the cationic ring-opening polymerization (CROP) of 2-aryl-2-oxazolines and 2-alkyl-2-oxazolines, such as 2-phenyl-2-oxazoline and 2-methyl-2-oxazoline.[1−4] After this discovery, the CROP of 2-oxazolines gained significant interest. Well-defined poly(2-alkyl/aryl-2-oxazoline)s (PAOx) can be obtained if the polymerizations are done under nucleophile-free conditions to avoid side reactions such as chain transfer and termination.[5,6] After the flourishing of PAOx in the 1970s and 80s, research interest retracted in the 90s. Since the dawn of the new millennium, PAOx are quickly gaining interest again, as researchers have discovered their high potential for biomedical applications.[7−10] In addition, a microwave-assisted polymerization protocol was introduced about 10 years ago to drastically reduce polymerization times from days and hours to minutes.[11,12] The livingness of the CROP allows the preparation of defined PAOx with full control over polymer architectures including blocks,[13,14] gradients,[15] and star-shaped structure.[16] Furthermore, the properties of PAOx are highly tunable by variation of the side-chain R group as well as by copolymerization of different monomers.[17−19]

The current interest in PAOx is more and more shifting toward biomedical applications stimulated by the high biocompatibility and stealth behavior, which is especially documented for poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx).[7,20] In fact, they can arguably compete or even outperform the gold standards of the field, namely, poly(ethylene glycol) and poly(*N*-hydroxypropylmethacrylamide).[21−24] For use of PAOx in biomedical applications, it is important to have easy and straightforward functionalization methodologies, either for conjugation of the polymers to biological media or substrates or to attach drug molecules, labels, or targeting moieties. Up to three orthogonal functionalities can be (simultaneously) introduced in PAOx through initiation, termination, and incorporation of functional monomers.[18,25] The choice of functional monomers is, however, limited to groups that are non-nucleophilic, as any nucleophile will interfere with the polymerization process.

A major advantage of PAOx over PEG is the possibility to introduce functionalities in the side chains by simple copolymerization of a functional monomer with a nonfunctional monomer; however, many functional groups need to be protected during the CROP to avoid interference with the polymerization conditions, as has been reported for monomers with acid,[12,19,26] alcohol,[19] amine,[12] thiol,[19] and aldehyde[19,27,28] functionalities. An important exception are monomers containing double and triple bonds that are not affected by the living CROP. The direct introduction of internal and terminal double bonds as well as terminal triple bonds into PAOx has been demonstrated.[14,27,29,30] These polymers could be further utilized for efficient postpolymerization modification by thiol−

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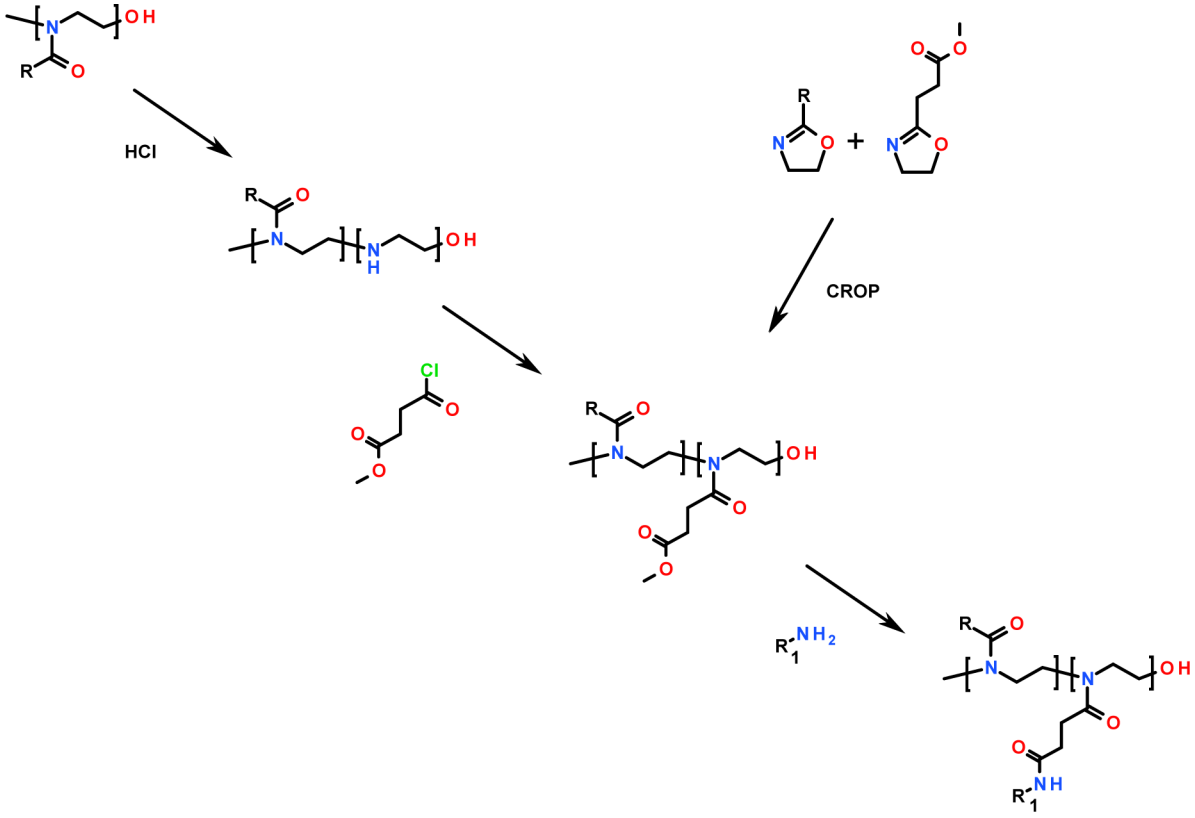
Scheme 1. Schematic Representation of the Partial Hydrolysis of a Poly(2-oxazoline) Followed by Modification with Methyl Succinyl Chloride and Finally Side-Chain Modification by Direct Amidation with an Amine[a]



[a]Direct synthesis of the methyl ester containing PAOx by copolymerization is also included in the scheme as alternative route.[34]

ene,[25,31,32] thiol−yne,[32] and copper(I)-catalyzed azide−alkyne cycloaddion (CuAAC),[31,32] so-called click reactions.[33]

In this work, we report a novel straightforward postpolymerization modification strategy for PAOx, which allows the incorporation of a wide range of side-chain functionalities by direct amidation of the side-chain methyl ester groups with different amines, as shown in Scheme 1. The PAOx building blocks containing side-chain methyl ester groups can either be prepared by partial hydrolysis of PAOx followed by introduction of the methyl ester functionality by reaction with methyl succinyl chloride or by copolymerization of a nonfunctional 2-oxazoline monomer with 2-methoxycarbonylethyl-2-oxazoline.[4,34] Within this manuscript, we will demonstrate the versatility of this new synthetic methodology.

EXPERIMENTAL SECTION

Materials and Instrumentation. Poly(2-ethyl-2-oxazoline) (PEtOx; DP = 100, M_n 9700, PDI 1.13; measured on SEC with DMA as eluent) was synthesized following a literature protocol.[11] Hydrogen chloride, sodium hydroxide, dichloromethane, aminomethane, aminoethane, 1-aminopropane, dimethylamine, hydrazine, glycine, leucine methyl ester, 3-amino-1-propanol, 3-aminopropene, 1-amino-3-butyne, *N*,*N*-dimethyl-1,2-ethylenediamine, dimethylamine, leucine methylester, glycine) 1,2-ethylenediamine, methyl succinyl chloride, and 2-amino-1-ethanol were obtained from Sigma-Aldrich and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or in DMSO on a Bruker Avance 300 or 500 MHz spectrometer. Size exclusion chromatography (SEC) was performed on a Agilent HPLC with a 1260 refractive index detector (RID) using dimethylacetamide containing 50 mM LiCl as eluent at a flow rate of 0.6 mL/min. Poly(methyl methacrylate) (PMMA) standards were used to calculate the molar mass values, and the column set consisted of two PLgel 5 μm mixed D columns at 40 °C and a similar guard column (Agilent) in series. Chromatograms were analyzed using Agilent Chemstation software with the GPC add on. The polymers obtained after amidation were analyzed by size exclusion chromatography (HFIP-SEC) using a Agilent HPLC with a 1260 RID with eluent of hexafluoro-2-propanol (HFIP) containing 20 mM sodium trifluoroacetate at a flow rate of 0.3 mL/min. Poly(methyl methacrylate) (PMMA) standards were used to calculate the molar mass values, and the column set consisted of two PSS PFG 100 Å gel 5 μm mixed D columns and a similar guard column (Agilent) at 35 °C in series. Chromatograms were analyzed using Agilent Chemstation software with the GPC add on. The FT-IR was performed on a PerkinElmer 1000 FTIR spectrum meter with PIKE HATR module. All of the polymerization were done in a Vigor glovebox. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) was performed on an Applied Biosystems Voyager De STR MALDI-TOF mass spectrometer equipped with 2 m linear and 3 m reflector flight tubes and a 355 nm Blue Lion Biotech Marathon solid state laser (3.5 ns pulse). All mass spectra were obtained with an accelerating potential of 20 kV in positive ion mode and in reflectron mode. 2-(4′-Hydroxybenzeneazo)benzoic acid (HABA) (20 mg/mL in acetone) was used as matrix. Polymer samples were dissolved in acetone (2 mg/mL). Analyte solutions were prepared by mixing 10 μL of matrix and 5 μL of polymer samples. Samples were applied using the dried droplet method.

Partial Hydrolysis of PEtOx. The poly(2-ethyl-2-oxazoline) ((10 g) was dissolved in 50 mL of water with heating and stirring until everything was dissolved. Then, 50 mL of 36% solution of HCl was added and the mixture was heated to 73 °C (internal temperature) for 200 min. After the volatiles were removed on a rotary evaporator, the polymer was redissolved in water and the solution was basidified to pH 10 to 11 using a NaOH solution. The solution was then lyophilized and the solid was dissolved in dichloromethane. The organic phase was

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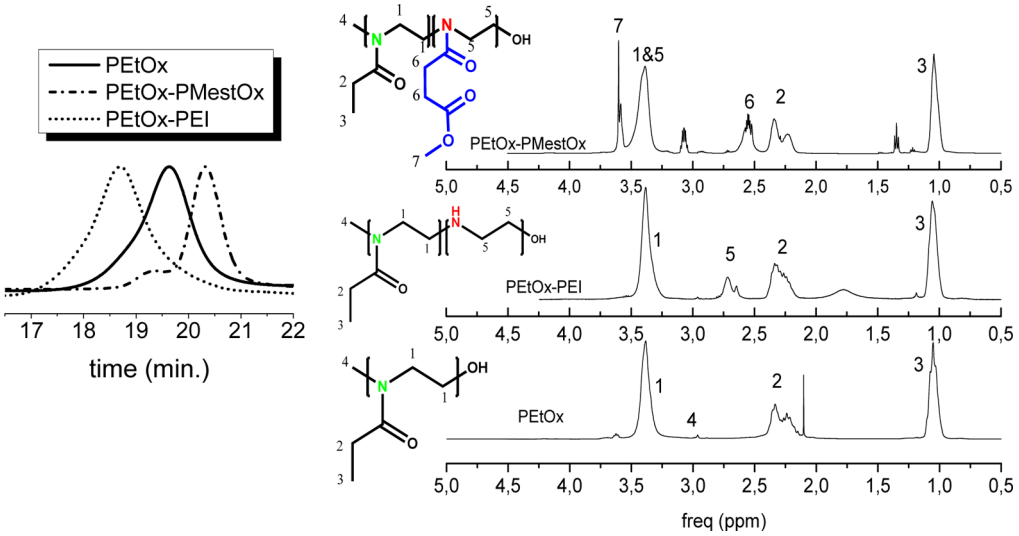

Figure 1. Overlay of the SEC traces in HFIP (left) and ^{1}H NMR spectra of PEtOx, PEtOx-PEI, and PEtOx-MestOx in $CDCl_3$.

washed with brine and then concentrated in vacuum to yield the PEtOx-PEI as a solid white product (7 g; 77% yield). ^{1}H NMR (300 MHz, δ, $CDCl_3$): 1−1.2 (3 H,CH_2-C$\underline{H}_3$); 2.3−2.5 (4H,C$\underline{H}_2$-CH_3); 2.6−2.8 (4 H,C$\underline{H}_2$-NH); 3.2−3.5 (4 H,C$\underline{H}_2$NCH_2). The integral ratios between the peaks at 2.6−2.8 and 3.2−3.5 ppm were utilized to calculate the degree of hydrolysis to be 18%.

Modification of PEtOx-PEI with Methyl Succinyl Chloride. Seven grams of the PEtOX-PEI (18%) was several times coevaporated with toluene to remove any water. Subsequently, the polymer was dissolved in dry dichloromethane (6 mL/g of polymer) and the mixture was cooled to 0 °C. Methyl succinyl chloride (4.5 mL, 3.65 mmol, 2 equiv) was added to this solution of PEtOx-PEI, followed by dropwise addition of triethylamine (2 eq, 0.3 mL). The mixture was stirred for 24 h, after which the reaction mixture was directly precipitated in cold ether. The precipitated polymer was isolated by filtration and dried under vacuum to yield the PEtOx copolymer with methyl ester side chains (PEtOx-MestOx) in 84% yield (7.5 g). ^{1}H NMR (300 MHz, δ, $CDCl_3$): 1−1.2 (3 H,CH_2-C$\underline{H}_3$); 2.3−2.5 (4H,C$\underline{H}_2$-CH_3); 2.6−2.8 (4 H,C$\underline{H}_2$-NH); 3.2−3.5 (4 H,C$\underline{H}_2$N−CH2); 3.7 (3 H,O−C$\underline{H}_3$). SEC-data: M_w 1.76 × 10^4, M_n 1.67 × 10^4, and $Đ$ 1.1. The IR spectrum showed the following peaks (cm^{-1}): 1731 (CH_3−O, ester), 1630 (all amides).

Amine Coupling Method 1: (Aminomethane, Aminoethane, 1-Aminopropane, Hydrazine-hydrate). The PEtOx-PMestOx copolymer (300 mg, 4.6 × 10^{-4} mol) was directly dissolved in an excess of amine (∼6 mL). The reaction was stirred for 2 days at 70 °C, after which the solution was evaporated to dryness. Purification was done using preparative SEC, PD 10 column with water as eluent to yield the modified polymers in near-quantitative yields.

Amine Coupling Method 2: (2-Amino-1-ethanol, 3-amino-1-propanol, 3-aminopropene, 1-Amino-3-butyne, *N*,*N*-Dimethylethylenediamine, Dimethylamine, Leucine Methylester, Glycine). The PEtOx-PMestOx (300 mg, 4.6 × 10^{-4} mol) copolymer was dissolved in 6 mL of acetonitrile; for amines that are not soluble in acetonitrile the solvent was changed to DMF. In the case where the amines were part of an amino acid, such as glycine or leucine, water with a pH of 9 was used to ensure that all amines were deprotonated. A concentrated solution of amine (3 equiv to the methyl ester) was then added, and the reaction mixture was stirred for 1 day at 40 °C. After evaporation of the solution under reduced pressure, the remaining polymer was purified by preparative SEC, PD 10 column with water as eluent, yielding the modified polymers in near-quantitative yields.

Polymerization of the Methyl-Ester-Functionalized Monomer(MestOx) DP 20. The MestOx monomer was synthesized as described earlier using methyl tosylate as an initiator.[34] The polymerization was carried out in a 20 mL microwave vial. The methyl tosylate(0.34 mL; 0.002 mol) was dissolved in a solution containing 7 g of MestOx monomer(7g; 0.04 mol) and 8 mL of acetonitrile obtaining a 3 M polymer solution. The reaction mixture was prepared in the glovebox, ensuring an oxygen- and water-free environment. The polymerization was performed by heating the mixture for 2.35 min to 140 °C in the microwave. After polymerization the reaction was terminated by the addition of 1 mL of a 1 M solution KOH in methanol. The acetonitrile was then removed via reduced pressure, and the polymer was dissolved in dichloromethane and precipitated in cold ether(10-fold excess). The polymer was then dissolved in water and freeze-dried to obtain a white powder (3.311g; yield 65%). ^{1}H NMR (500 MHz, δ, D_2O): 2.5−2.7 (4H, CO-C$\underline{H}_2$-C$\underline{H}_2$-CO-OCH); 3.2−3.5 (4 H,C$\underline{H}_2$N−CH_2); 3.7 (3 H,O−C$\underline{H}_3$). SEC-data: M_w 6600, M_n 5800, and $Đ$ 1.12.

RESULTS AND DISCUSSION

Synthesis of PEtOx-MestOx via Hydrolysis and Modification of PEtOx. To evaluate the newly proposed postpolymerization modification platform of PAOx copolymers with methyl ester side chains with amines (Scheme 1), we first prepared a well-defined PEtOx as starting material, whereby the narrow dispersity ($Đ$) will facilitate the evaluation of the products after each reaction step by SEC. Therefore, a large batch (10 g) of PEtOx with a DP of 100 was prepared using the previously optimized microwave-assisted polymerization protocol, yielding well-defined PEtOx with a $Đ$ of 1.1 determined by SEC with DMA as eluent.[11]

Next, the well-defined PEtOx was hydrolyzed under controlled acidic conditions, and based on the previously established hydrolysis kinetics, a good control can be achieved over the hydrolysis rate.[35] This allows for an easy control over the amount of secondary amines in PEtOx-PEI and thus provides control over the number of methyl ester functional groups that can be attached to the main chain. To aim for a hydrolysis degree around 20%, we performed the hydrolysis in ∼6.8 N hydrochloric acid at 73 °C for 200 min. The degree of hydrolysis was determined by ^{1}H NMR spectroscopy. The integral ratio of signals corresponding to the backbone of PEtOx at δ 3.5 ppm and the backbone of PEI at δ 2.8 ppm was used to calculate the degree of hydrolysis, as shown in ^{1}H NMR. It is shown that 18% of the PEtOx units were hydrolyzed, yielding a $PEtOx_{82}$-PEI_{18} copolymer. Purification of this PEtOx-PEI was done by first drying the reaction mixture on a rotary evaporator to remove the water, propionic acid, and excess of hydrochloric acid. Subsequently the polymer was dissolved in a small amount of water, and the pH was adjusted to pH 10 to 11

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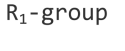
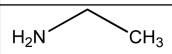
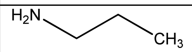
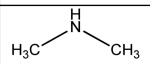
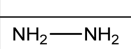
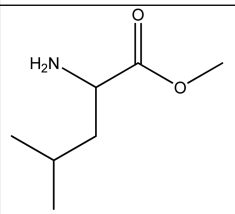
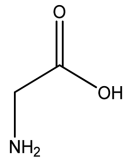
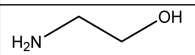
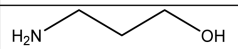
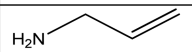
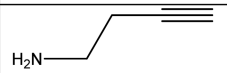
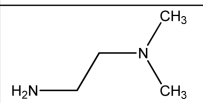
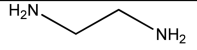
Table 1. Overview of the Direct Amidation of PEtOx-MestOx with Various Amines

	R_1-group	Chemical shift amide proton(ppm)[1]	Mn (kDa)[2]	Đ[2]	Method[3]
aminomethane		6.4	17.7	1.1	1
aminoethane		6.5	17.7	1.1	1
1-aminopropane		7.9	17.8	1.1	1
dimethylamine		6.3	17.8	1.1	2(water)
hydrazine monohydrate		9	17.7	1.1	1
leucine methylester		nd[4]	18	1.1	2(basic water)
Glycine		nd[4]	18	1.1	2(basic water)
2-amino-1-ethanol		nd[4]	22	1.1	2(acetonitrile)
3-amino-1-propanol		nd[4]	24	1.2	2(acetonitrile)
3-aminopropene		nd[4]	16.5	1.1	2(DMF)
1-amino-3-butyne		7.8	16.7	1.1	2(DMF)
N,N'-dimethyl-1,2-ethylenediamine		8.2	16.6	1.1	2(DMF)
1,2-ethylenediamine		nd[4]	14	1.3	1

[1]Chemical shift in ^{1}H NMR spectra recorded in $CDCl_3$. [2]Calculated from SEC analysis with DMA as eluent and PMMA as calibration. [3]Method 1: Amine is used as reaction solution; Method 2: The amidation reaction is done in another solvent specified between brackets. [4]Not distinguishable as the ^{1}H NMR-spectra were recorded in DMSO and the NH peak could not be seen.

with NaOH to deprotonate the PEI units, followed by lyophilization. The resulting white powder was subsequently dissolved in dichloromethane and extracted with brine and water to remove the traces of propionate as well as the NaOH. The final purified polymer was collected by evaporation of the dichloromethane. This partially hydrolyzed copolymer could no longer by analyzed by SEC in DMA due to too strong interactions between the amine groups and the column material, leading to tailing of the signal and with higher hydrolysis degrees even to complete sticking of the polymer to the column. Therefore, SEC of PEtOx-PEI was measured with hexafluoroisopropanol (HFIP) as eluent,[36] revealing a M_n of 1.5×10^4 Da and $Đ$ of 1.3. The broadening of size distribution can be ascribed to the SEC system and column set as the PEtOx starting polymer revealed a M_n of 7.6×10^3 Da and a $Đ$ of 1.4 on this SEC system with HFIP. The counterintuitive increase in M_n upon removal of 18% of the side chains is in line with previous observations and can be ascribed to the larger hydrodynamic volume of the partially hydrolyzed copolymer in HFIP, presumably due to better hydrogen bonding of the HFIP to the polymer chains.

The prepared PEtOx-PEI represents a well-defined reaction platform containing the biocompatible PEtOx as main structure together with the nucleophilic secondary amine units from the PEI. A wide variety of modification reactions of such partially

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hydrolyzed polymers have already been reported in literature.[37] In this work, the PEtOx-PEI copolymer was reacted with methyl succinyl chloride to reintroduce a side-chain amide group similar to the native PAOx structure containing a latent methyl ester functionality, yielding $PEtOx_{82}MestOx_{18}$. The ^{1}H NMR spectrum of PEtOx-MestOx revealed the disappearance of the PEI backbone resonances at 2.6 ppm as well as the appearance of the methyl ester peak at 3.7 ppm, demonstrating the success of the modification reaction. Furthermore, the IR spectrum also revealed the representative methyl ester carbonyl vibration at 1730 cm^{-1}. The SEC trace of PEtOx-PMestOx in HFIP also revealed a decrease in M_n caused by the change in hydrodynamic volume, while the $Đ$ remained the same ($Đ$ = 1.3). Overlays of the SEC traces and ^{1}H NMR spectra of PEtOx, PEtOx-PEI, and PEtOx-MestOx are shown in Figure 1 to illustrate the successful modifications.

As mentioned in the Introduction, PEtOx-MestOx can also be obtained by direct copolymerization of 2-ethyl-2-oxazoline (EtOx) and 2-methoxycarboxyethyl-2-oxazoline (MestOx). Although the direct polymerization seems to be a more straightforward method compared with the here-presented two-step hydrolysis modification method, it should be noted that EtOx is available in bulk quantities, while MestOx has to be custom-synthesized in a two-step synthetic protocol when starting from methyl succinyl chloride with 57% overall yield, including double purification by distillation.[34] In this perspective, the demonstrated hydrolysis modification method may actually be more cost-effective and time-efficient than the direct polymerization method. Moreover, the hydrolysis modification route is also more versatile as different reactive linkers, such as methyl bromopropionate, methyl acrylate, or methyl iso(thio)-cyanatopropionate, may be used to install similar methyl ester side chains.[38]

Postpolymerization Modification of PEtOx-MestOx by Direct Amidation. After establishing a robust procedure for the preparation of PEtOx-MestOx, we focused our attention to the amidation of the methyl ester side chains. Even though it is commonly accepted that a direct amidation is not feasible under mild conditions, we did try this out by stirring the PEtOx-MestOx copolymer for 2 days in neat 1-aminopropane at 70 °C. ^{1}H NMR spectroscopic analysis of the resulting polymer after precipitation revealed that the methyl ester resonance at 3.7 ppm disappeared, and a new amide signal appeared at 7.9 ppm, indicating successful amidation. The disappearance of the methylester peaks gives the prove that there is a full conversion from an ester to an amide. In addition, FT-IR also confirmed the successful amidation by disappearance of the methyl ester band at 1730 cm^{-1} and appearance of a new band at 1531 cm^{-1} representative for the NH bending in a secondary amide group. Furthermore, SEC analysis in DMA revealed an increase in M_n from 9.7 × 10^3 Da for PEtOx to 11 × 10^3 for PEtOx-MestOx to 17.8 × 10^3 Da for the *n*-propyl modified polymer. Importantly, the $Đ$ remained constant at 1.1, indicating that no side reactions take place. Even though it is not fully clear why the direct mild amidation of PEtOx-MestOx is possible, it may be speculated that this is due to the very high concentration and large excess of amine groups, possibly in combination with a neighboring group effect in which the amide groups attached to the PAOx backbone participate in the reaction mechanism by stabilization of the transition state via the formation of H bonds.

Inspired by the success of this first direct amidation reaction, we continued to explore the scope of this postpolymerization modification reaction for PEtOx-MestOx. Several other readily available and cheap amines, namely, aminomethane, aminoethane, and hydrazine-hydrate, were utilized using the same Method 1, whereby the reaction was performed in a solution of the amine. ^{1}H NMR spectroscopy, FT-IR, and SEC again revealed the success of these direct amidation reactions (Table 1). The hydrazine-modified polymer is especially interesting because it contains hydrazide side chains that may be further utilized for the conjugation of ketone-containing molecules via a pH-degradable hydrazone linker.[39] In a next step, a series of more complex functional amines was explored for direct amidation of PEtOx-MestOx. Because these amines are more expensive and often appear as viscous liquids, the reaction conditions were modified resulting in Method 2. In this second method, the polymer was dissolved in acetonitrile, and three equivalents of the amine were added. The resulting solution was stirred at 40 °C overnight. Only if the amine is not soluble in acetonitrile, the reaction was performed in a similar manner in DMF, which was the case for allylamine and 1-amino-3-butyne. In the case of amino acids, such as leucine ester and glycine, a deprotonation was needed. Therefore, basic water was used as a reaction medium. The different tested amines are listed in Table 1, and successful direct amidation was found based on ^{1}H NMR and FT-IR spectroscopy as well as SEC for dimethylamine, leucine methyl ester, glycine, 2-amino-1-ethanol, and 3-amino-1-propanol, 3-aminopropene, 1-amino-3-butyne, *N*,*N*-dimethyl-1,2-ethylenediamine, and 1,2-ethylenediamine. As such, we could include a wide variety of functional groups in the side chains of PAOx based on the same PEtOx-MestOx reactive platform. It is important to note that the reaction with 1,2-ethylenediamine led to a minor increase in $Đ$ and a decrease in M_n. This observation can be ascribed to tailing of the SEC trace due to interaction of the free amine group with the column materials. There is no evidence of double molar mass shoulders, indicating that no cross-linking took place. A series of representative ^{1}H NMR spectra, FT-IR spectra, and SEC traces of the PEtOx-MestOx and some products after amidation are shown in Figures 2, 3, and 4,

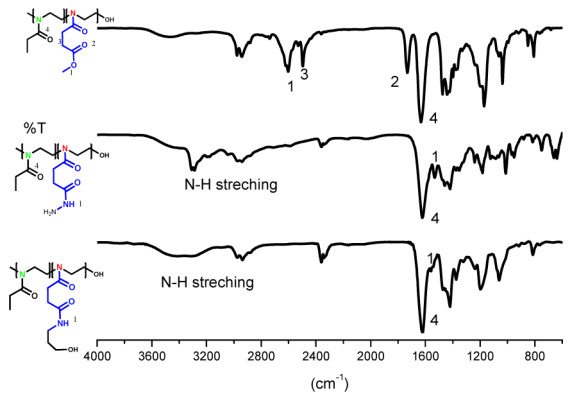

Figure 2. FT-IR data starting from the top with the PEtOx-MestOx and below two examples of polymers after the direct amidation.

respectively. The ^{1}H NMR spectra clearly show that the peak at 3.7 ppm of the methyl ester group in PEtOx-PMestOx disappears upon amidation. There is also a notable change of signal 6, which after reaction with the amine moves closer to and partially overlaps with the peak around 2.2 ppm. These peaks merge together because of the similarity between the side chain of the PEtOx and the side chain of the succinyl. FT-IR clearly shows the disappearance of the band at 1730 cm^{-1} of the methyl ester carbonyl and reveals the appearance of a new band in the region

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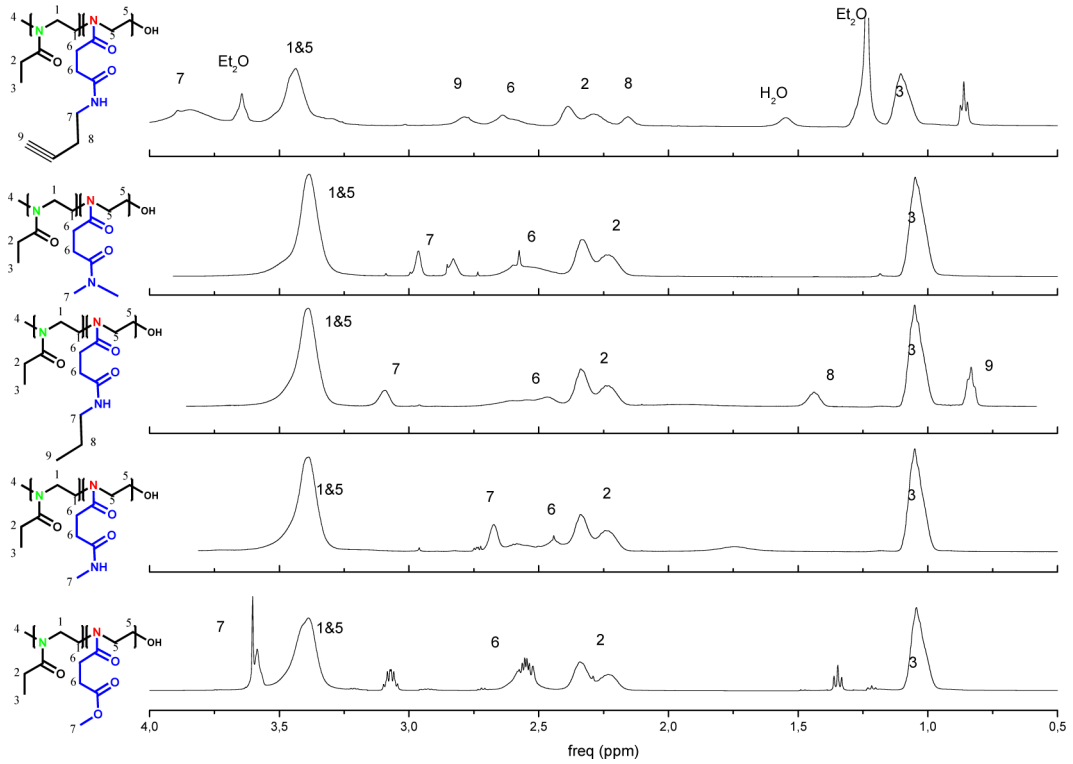

Figure 3. Overlay of the ^{1}H NMR spectra of PEtOx-MestOx and four products after direct amidation with different amines.

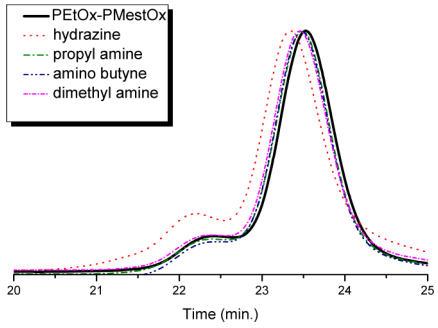

Figure 4. Overlay of normalized SEC (HFIP)traces of PEtOx-PMestOx and four examples of amidated polymers functionalized with hydrazine-hydrate, 3-aminopropene, 1-amino-3-butyne, dimethylamine.

between 1530 and 1560 cm^{-1}, a fingerprint of the NH bending. Finally, SEC analysis clearly shows that the polymer molar mass distributions have very similar shape before and after amidation, demonstrating that no polymer coupling occurred.

To show that the direct amidation is also possible on homopolymers, PMestOx with DP 20 was synthesized by CROP of MestOx using methyl tosylate as initiator. This resulting polymer was analyzed by ^{1}H NMR spectroscopy, MALDI-TOF MS and SEC revealing the formation of a defined polymer with a low polydispersity ($Đ$ = 1.13; Figure 5). This polymer was subsequently modified with 1-aminopropane via method 1 (bulk method) by dissolving the polymer in 1-aminopropane and stirring overnight at 37 °C. The MALDI-TOF mass spectrum of the resulting polymer clearly shows that there is a shift in mass in comparison to the begin product (Figure 5). MALDI-TOF MS of the PMestOx revealed the expected spacing of 157 m/z corresponding to the MestOx monomer, while after reaction the spacing increased to 184.24 m/z, corresponding to the amidopropane side chain. Furthermore, the exact mass of 3197 m/z for PMestOx corresponds to a polymer with 20 repeat units and methyl and OH end groups charged with a sodium cation. After the amidation reaction, the exact mass of 3738 is found corresponding to the amido-propane functionalized polymer with 20 repeat units and methyl and OH end groups and charged with a sodium cation. These results confirm a clean amidation of all methyl ester side chains. The ^{1}H NMR spectra also demonstrate that the methyl ester groups disappeared after amidation, while three new peaks appeared, corresponding to the propyl side chains (signals 4–6 in Figure 5). Finally, SEC confirmed an increase in hydrodynamic volume after the amidation reaction, while the $Đ$ mildly increased from 1.13 to 1.17, albeit no clear shoulders are observable (Figure 5).

CONCLUSIONS AND OUTLOOK

We successfully showed a novel modification procedure to expand the chemical toolbox for poly(2-oxazoline)s PEtOx. Starting from the controlled hydrolysis of well-defined PEtOx a copolymer is obtained with a specific number of amine units to control the number of functional groups that can be incorporated. These amine units are subsequently reacted with methyl succinyl chloride, restoring the poly(2-oxazoline) side-chain structure while installing a methyl ester functionality. Even though such methyl-ester-functionalized copolymers can also be obtained via a direct polymerization method, the post-polymerization modification route is, arguably, more cost-effective and less time-consuming.

The copoly(2-oxazoline)s containing methyl ester side chains methyl ester group is demonstrated to undergo a direct amidation reaction, enabling the installation of a wide-range of side chain amide groups and functionalities. An important feature is that these reactions efficiently proceed without a catalyst, proposedly due to activation by neighboring amide groups. Furthermore, we also demonstrated the synthesis of a PMestOx

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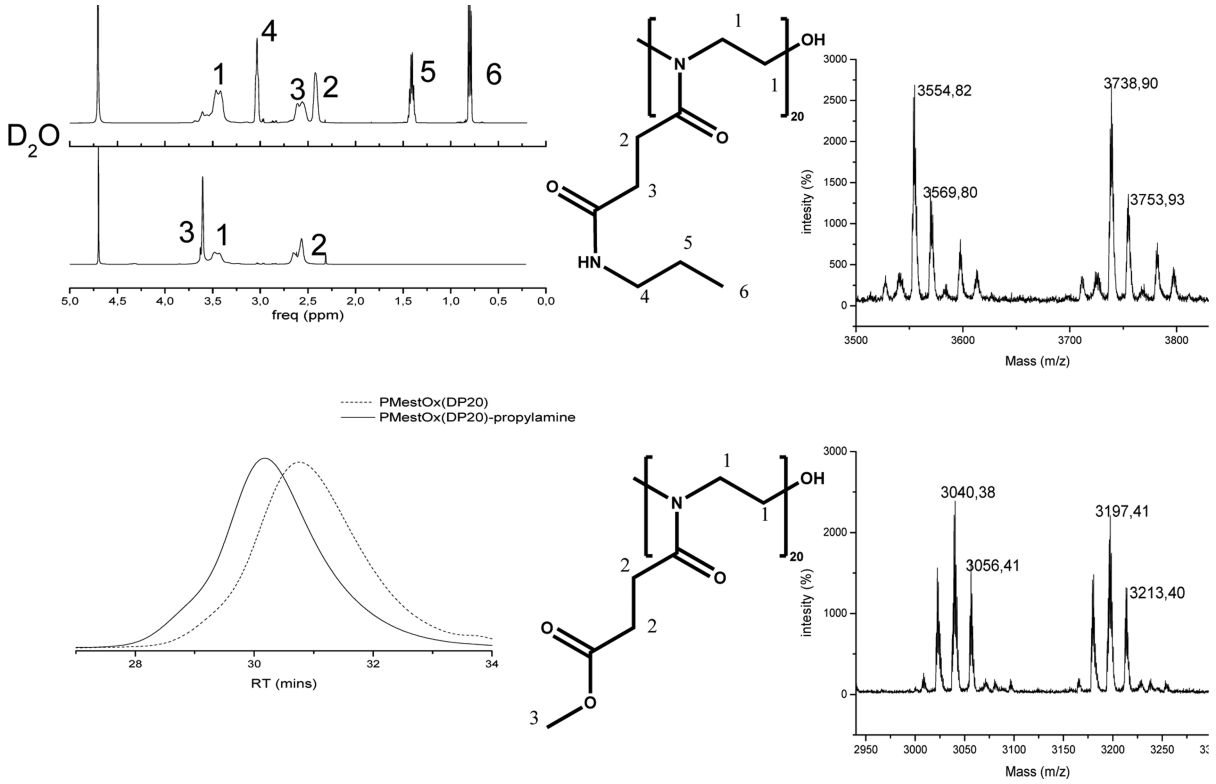

Figure 5. Overview of the ^{1}H NMR data in D_2O (top left), MALDI-TOF MS (right), and SEC (DMA as eluent; bottom left) analysis of the pure PMestOx with 20 repeating units before and after amidation with 1-aminopropane. The bottom ^{1}H NMR and MALDI TOF mass spectra show PMestOx ,and the top spectra show the polymer after amidation with 1-aminopropane.

homopolymer with 20 repeat units by direct CROP of MestOx. The direct amidation of this model polymer with 1-aminopropane clearly revealed that all methyl ester side chains can be modified.

We are confident that this direct amidation side-chain modification approach will be an important extension of the PAOx toolbox that will lead to the development of novel applications of these polymers.

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Notes

The authors declare no competing financial interest.

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