

1 Functional poly(2-oxazoline)s by direct amidation of
2 methyl ester side chains

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11 KEYWORDS: post polymerization modification, poly(2-oxazoline)s, hydrolysis, poly(ethylene
12 imine), coupling reaction, uncatalyzed amidation, biocompatible polymers

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ABSTRACT Poly(2-alkyl/aryl-2-oxazoline)s (PAOx) are biocompatible pseudo-polypeptides that 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 to the gold standard in this field, poly(ethylene glycol) (PEG), while having similar beneficial properties, such as Stealth behaviour and biocompatibility. In this article, we further expand the PAOx chemical toolbox by demonstrating a novel straightforward and highly versatile post-polymerization modification platform for the introduction of side-chain functionalities. PAOx having side chain methyl ester functionalities are 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 either prepared by partial hydrolysis of a PAOx followed by introduction of the methyl ester via modification of the secondary amine groups with methyl succinylchloride or by direct copolymerization of a non-functional 2-oxazoline monomer with a 2-methoxycarbonylethyl-2-oxazoline. Thus, this novel synthetic platform enable direct access to a wide range of side-chain functionalities from the same methyl ester functionalized poly(2-oxazoline) scaffold.

1 Introduction

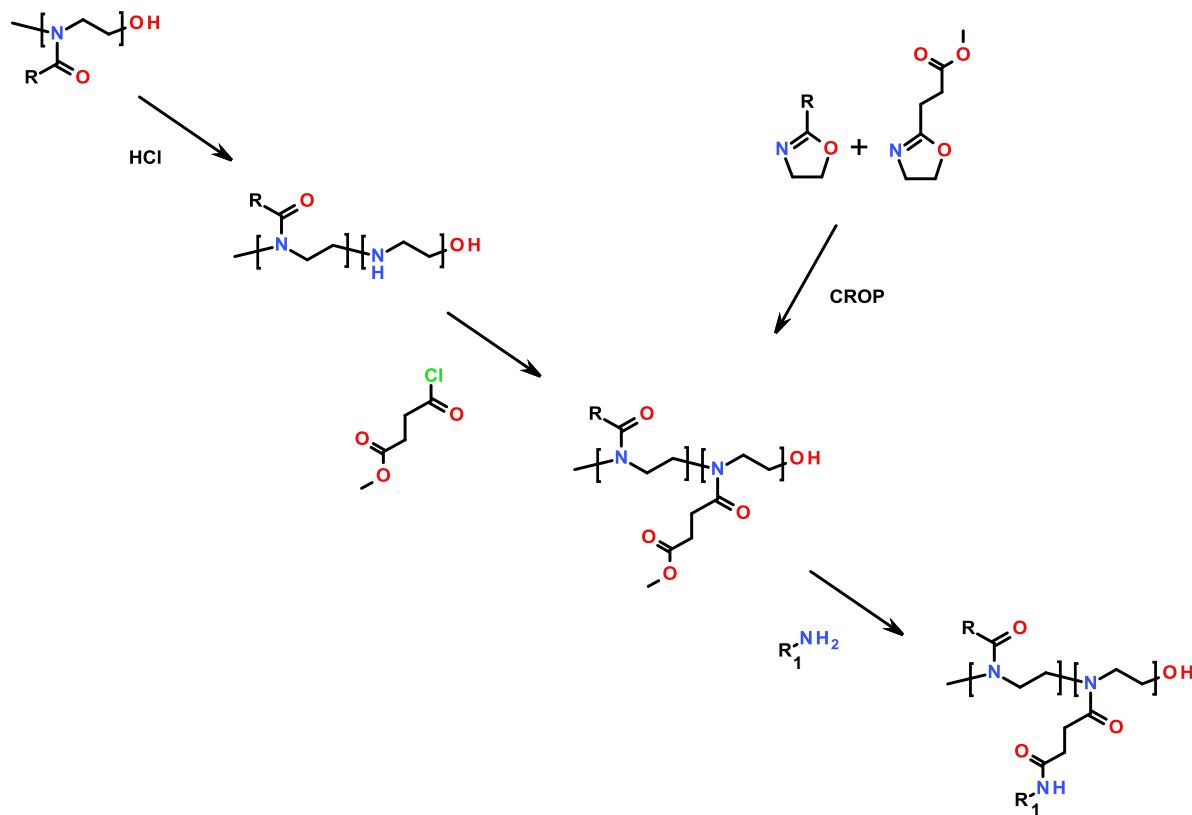
2 The story of poly(2-oxazoline)s started in 1966 when 4 independent research groups reported
3 the cationic ring opening polymerization (CROP) of 2-aryl-2-oxazolines and 2-alkyl-2-
4 oxazolines, such as 2-phenyl-2-oxazoline and 2-methyl-2-oxazoline.¹⁻⁴ After this discovery, the
5 CROP of 2-oxazolines has gained significant interest. Well-defined poly(2-alkyl/aryl-2-
6 oxazoline)s (PAOx) can be obtained if the polymerizations are done under nucleophile free
7 conditions to avoid side reactions such as chain transfer and termination.^{5,6} After the flourishing
8 of PAOx in the seventies and eighties, research interest was retracting in the nineties. Since the
9 dawn of the new millennium, PAOx are quickly gaining interest again, as researchers have
10 discovered their high potential for biomedical applications.⁷⁻¹⁰ In addition, a microwave assisted
11 polymerization protocol was introduced about ten years ago to drastically reduce polymerization
12 times from days and hours to minutes.^{11,12} The livingness of the CROP allows the preparation
13 of defined PAOx with full control over polymer architectures including blocks^{13,14}, gradients¹⁵
14 and star-shaped structure¹⁶. Furthermore the properties of PAOx are highly tunable by variation
15 of the side-chain R group as well as by copolymerization of different monomers.¹⁷⁻¹⁹

16 The current interest in PAOx is more and more shifting towards biomedical applications
17 stimulated by the high biocompatibility and stealth behaviour, which is especially documented
18 for poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx).^{7,20} In fact,
19 they can arguably compete or even outperform the gold standards of the field, namely
20 poly(ethylene glycol) and poly(*N*-hydroxypropylmethacrylamide).²¹⁻²⁴ For use of PAOx in
21 biomedical applications, it is important to have easy and straightforward functionalization
22 methodologies, either for conjugation of the polymers to biological media or substrates or to
23 attach drug molecules, labels or targeting moieties. Up to three orthogonal functionalities can be

1 (simultaneously) introduced in PAOx through initiation, termination and incorporation of
2 functional monomers.^{18,25} The choice of functional monomers is however limited to groups that
3 are non-nucleophilic as any nucleophile will interfere with the polymerization process.

4 A major advantage of PAOx over PEG is the possibility to introduce functionalities in the side
5 chains by simple copolymerization of a functional monomer with a non-functional monomer.
6 However, many functional groups need to be protected during the CROP to avoid interference
7 with the polymerization conditions, as has been reported for monomers with acid^{12,19,26},
8 alcohol¹⁹, amine¹², thiol¹⁹ and aldehyde^{19,27,28} functionalities. An important exception are
9 monomers containing double and triple bonds that are not affected by the living CROP. The
10 direct introduction of internal and terminal double bonds as well as terminal triple bonds into
11 PAOx has been demonstrated^{14,27,29,30}. These polymers could further be utilized for efficient
12 post-polymerization modification by thiol-ene^{25,31,32}, thiol-yne³² and copper(I) catalysed azide-
13 alkyne cycloaddition (CuAAC)^{31,32}, so called click reactions³³.

14 In this work, we report a novel straightforward post-polymerization modification strategy for
15 PAOx, which allows the incorporation of a wide range of side-chain functionalities by direct
16 amidation of the side chain methyl ester groups with different amines as shown in Scheme 1. The
17 PAOx building blocks containing side-chain methyl ester groups can either be prepared by partial
18 hydrolysis of PAOx followed by introduction of the methyl ester functionality by reaction with
19 methyl succinyl chloride or by copolymerization of a non-functional 2-oxazoline monomer with
20 2-methoxycarbonylethyl-2-oxazoline^{4,34}. Within this manuscript we will demonstrate the
21 versatility of this new synthetic methodology.



1
 2 **Scheme 1.** Schematic representation of the partial hydrolysis of a poly(2-oxazoline) followed by
 3 modification with methyl succinyl chloride and finally side-chain modification by direct amidation
 4 with an amine. The direct synthesis of the methyl ester containing PAOx by copolymerization is
 5 also included in the scheme as alternative route³⁴.

6
 7 **Experimental Section**

8 ***Materials and Instrumentation.***

9 Poly(2-ethyl-2-oxazoline) (PEtOx; DP = 100, M_n 9700, PDI 1.13; measured on SEC with DMA
 10 as eluent) was synthesized following a literature protocol.¹¹ Hydrogen chloride, sodium hydroxide,
 11 dichloromethane, aminomethane, aminoethane, 1-aminopropane, dimethylamine, hydrazine,
 12 glycine, Leucine methyl ester, 1,3 aminopropanol, allylamine, 1-amino-3-butyn, allylamine, N,N'-
 13 dimethyl-1,2-ethylene diamine, 1,2 ethylene diamine, methyl succinyl chloride and amino

1 ethanol were obtained from Sigma Aldrich and used as received. ^1H NMR and ^{13}C NMR spectra
2 were recorded in CDCl_3 or in DMSO on a Bruker Avance 300 MHz or 500 MHz spectrometer.
3 Size exclusion chromatography (SEC) was performed on a Agilent HPLC with a 1260 refractive
4 index detector (RID) using dimethylacetamide containing 50 mM LiCl as eluent at a flow rate of
5 0.6 ml/min. Polymethylmethacrylate (PMMA) standards were used to calculate the molar mass
6 values and the column set consisted of two PLgel 5 μm mixed D columns at 40°C and a similar
7 guard column (Agilent) in series. Chromatograms were analyzed using Agilent Chemstation
8 software with the GPC add on. The polymers obtained after amidation were analyzed by size
9 exclusion chromatography (HFIP-SEC) using a Agilent HPLC with a 1260 refractive index
10 detector (RID) with eluent of hexafluoro-2-propanol(HFIP) containing 20 mM Sodium
11 trifluoroacetate at a flow rate of 0.3 ml/min. Polymethylmethacrylate (PMMA) standards were
12 used to calculate the molar mass values and the column set consisted of two PSS PFG 100 \AA gel 5
13 μm mixed D columns and a similar guard column (Agilent) at 35°C in series Chromatograms were
14 analyzed using Agilent Chemstation software with the GPC add on. The FT-IR was performed on
15 a Perkin Elmer 1000 FTIR spectrum meter with PIKE HATR module. All of the polymerization
16 where done in a Vigor glovebox. Matrix assisted laser desorption/ionization time of flight mass
17 spectroscopy (MALDI-TOF MS) was performed on an Applied Biosystems Voyager De STR
18 MALDI-TOF mass spectrometer equipped with 2 m linear and 3 m reflector flight tubes, and a
19 355 nm Blue Lion Biotech Marathon solid state laser (3.5 ns pulse). All mass spectra were obtained
20 with an accelerating potential of 20 kV in positive ion mode and in reflectron mode. 2-(4'-
21 Hydroxybenzeneazo)benzoic acid (HABA) (20 mg/ml in acetone) was used as matrix. Polymer
22 samples were dissolved in acetone (2 mg/ml). Analyte solutions were prepared by mixing 10 μl
23 matrix and 5 μl polymer samples. Samples were applied using the dried droplet method.

1
2 **Partial hydrolysis of PEtOx.** The poly-(2-ethyl-2-oxazoline) ((10 g) was dissolved in 50 ml of
3 water with heating and stirring until everything was dissolved. Then 50 ml of 36% solution of HCl
4 was added and the mixture was heated to 73°C (internal temperature) for 200 min. After the
5 volatiles were removed on a rotary evaporator, the polymer was redissolved in water and the
6 solution was basified to pH 10-11 using a NaOH solution. The solution was then lyophilized and
7 the solid was dissolved in dichloromethane. The organic phase was washed with brine and then
8 concentrated in vacuum to yield the PEtOx-PEI as a solid white product (7 g; 77% yield). ¹H-NMR
9 (300 MHz, δ in ppm, CDCl₃): 1-1.2 (3 H, CH₂-CH₃); 2.3-2.5 (4H, CH₂-CH₃); 2.6-2.8 (4 H, CH₂-
10 NH); 3.2-3.5 (4 H, CH₂NCH₂). The integral ratios between the peaks at 2.6-2.8 ppm and 3.2-3.5
11 ppm were utilized to calculate the degree of hydrolysis to be 18%.

12
13 **Modification of PEtOx-PEI with methyl succinyl chloride.** 7 g of the PEtOX-PEI (18%) was
14 several times co-evaporated with toluene to remove any water. Subsequently, the polymer was
15 dissolved in dry dichloromethane (6 ml/g of polymer) and the mixture was cooled to 0°C. Methyl
16 succinyl chloride (4.5 ml, 3.65 mmol, 2eq.) was added to this solution of PEtOx-PEI followed by
17 drop wise addition of triethylamine (2 eq, 0.3 ml). The mixture was stirred for 24 h after which the
18 reaction mixture was directly precipitated in cold ether. The precipitated polymer was isolated by
19 filtration and dried under vacuum to yield the PEtOx copolymer with methyl ester side chains
20 (PEtOx-MestOx) in 84% yield (7.5 g). ¹H-NMR (300 MHz, δ in ppm, CDCl₃): 1-1.2 (3 H, CH₂-
21 CH₃); 2.3-2.5 (4H, CH₂-CH₃); 2.6-2.8 (4 H, CH₂-NH); 3.2-3.5 (4 H, CH₂N-CH₂); 3.7 (3 H, O-CH₃).
22 SEC-data M_w 1.76 10⁴; M_n 1.67 10⁴ and Đ 1.1. The IR spectrum showed following peaks (cm⁻¹):
23 1731 (CH₃-O, ester) 1630 (all amides).

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Amine coupling method 1: (aminomethane, aminoethane, 1-aminopropane, hydrazine). 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: (1-amino-2-ethanol, 1-aminopropanol, allylamine, amino-3-butyne, N,N-dimethylethylene diamine). 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 equivalents 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.

Polymerisation of the methyl ester functionalized monomer(MestOx) DP 20. The MestOx monomer was synthesised as described earlier using methyl tosylate as an initiator.³⁴ The polymerisation 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 polymerisation was performed by heating

1 the mixture for 2.35 minutes to 140 °C in the microwave. After polymerisation the reaction was
2 terminated by addition of 1 ml of a 1 M solution KOH in methanol. The acetonitrile was then
3 removed via reduced pressure and the polymer was dissolved in dichloromethane and precipitated
4 in cold ether(10-fold excess). The polymer was then dissolved in water and freeze dried to obtain
5 a white powder (3.311g; yield 65%). ¹H-NMR (500 MHz, δ in ppm, D₂O): ;2.5-2.7(4H, CO-CH₂-
6 CH₂-CO-OCH); 3.2-3.5 (4 H, CH₂N-CH₂); 3.7 (3 H, O-CH₃). SEC-data M_w 6600; M_n 5800 and Đ
7 1.12.

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9 **Results and discussions**

10 *Synthesis of PEtOx-MestOx via hydrolysis and modification of PEtOx*

11 To evaluate the newly proposed post-polymerization modification platform of PAOx copolymers
12 with methyl ester side chains with amines (Scheme 1), a well-defined PEtOx was first prepared as
13 starting material, whereby the narrow dispersity (Đ) will facilitate the evaluation of the products
14 after each reaction step by SEC. Therefore, a large batch (10 g) of PEtOx with a DP of 100 was
15 prepared using the previously optimized microwave assisted polymerization protocol yielding
16 well-defined PEtOx with a Đ of 1.1 determined by SEC with DMA as eluent.¹¹

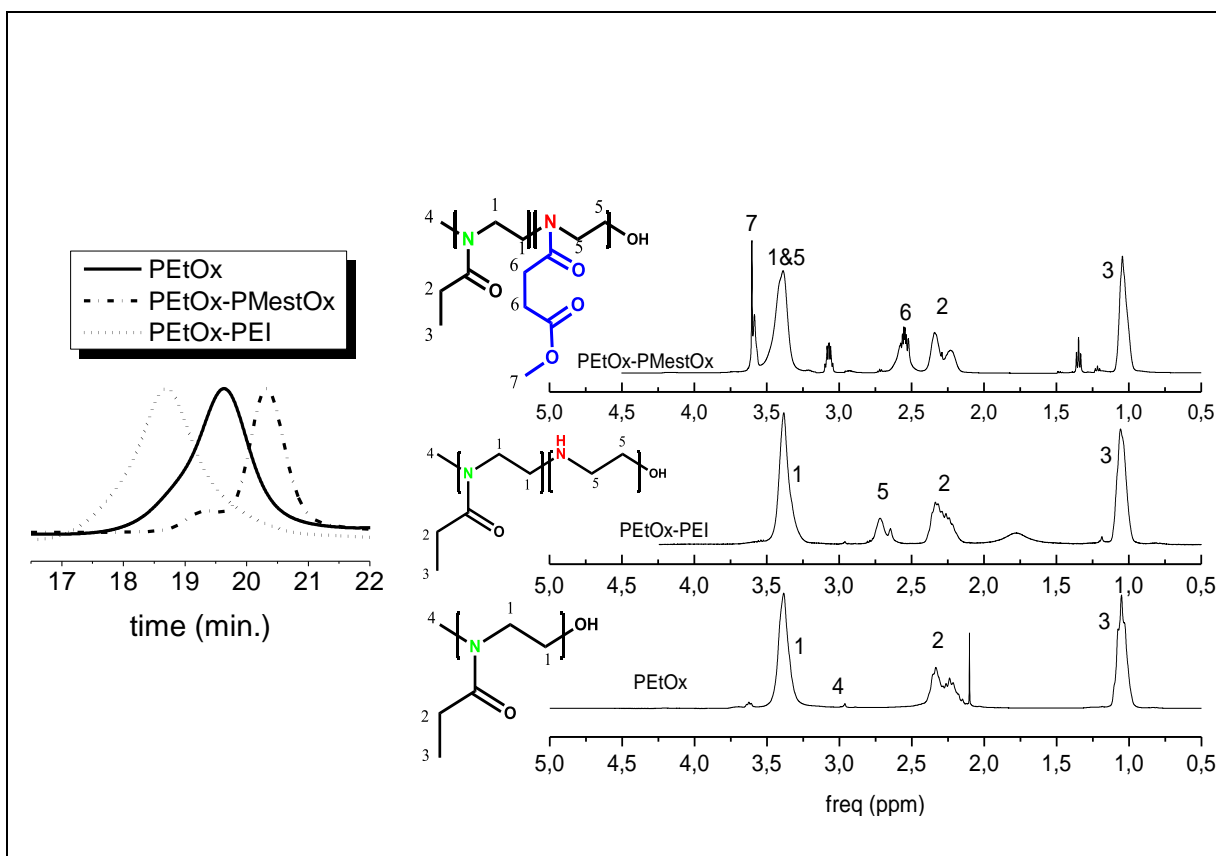
17 Next, the well-defined PEtOx was hydrolyzed in controlled acidic conditions and based on the
18 previously established hydrolysis kinetics, a good control can be achieved over the hydrolysis
19 rate.³⁵ This allows for an easy control over the amount of secondary amines in PEtOx-PEI and,
20 thus, provides control over the amount of methyl ester functional groups that can be attached to
21 the main chain. To aim for a hydrolysis degree around 20% the hydrolysis was performed in ~6.8
22 N hydrochloric acid at 73°C for 200 min. The degree of hydrolysis was determined by ¹H-NMR
23 spectroscopy. The integral ratio of signals corresponding to the backbone of PEtOx at δ 3.5 ppm

1 and the backbone of PEI at δ 2.8 ppm was used to calculate the degree of hydrolysis, as shown in
2 $^1\text{H-NMR}$. It is shown that 18% of the PEtOx units were hydrolysed yielding a PEtOx₈₂-PEI₁₈
3 copolymer. Purification of this PEtOx-PEI was done by first drying the reaction mixture on a rotary
4 evaporator to remove the water, propionic acid and excess of hydrochloric acid. Subsequently the
5 polymer is dissolved in a small amount of water and the pH is adjusted to pH 10-11 with NaOH,
6 to deprotonate the PEI units, followed by lyophilization. The resulting white powder is
7 subsequently dissolved in dichloromethane and extracted with brine and water to remove the traces
8 of propionate as well as the NaOH. The final purified polymer is collected by evaporation of the
9 dichloromethane. This partially hydrolyzed copolymer can no longer be analysed by SEC in DMA
10 due to too strong interactions between the amine groups and the column material leading to tailing
11 of the signal and with higher hydrolysis degrees even to complete sticking of the polymer to the
12 column. Therefore, SEC of PEtOx-PEI was measured with hexafluoroisopropanol (HFIP) as
13 eluent³⁶, revealing a M_n of $1.5 \cdot 10^4$ Da and Đ of 1.3. The broadening of size distribution can be
14 ascribed to the SEC system and column set as the PEtOx starting polymer revealed a M_n of $7.6 \cdot 10^3$
15 Da and a Đ of 1.4 on this SEC system with HFIP. The counterintuitive increase in M_n upon removal
16 of 18% of the side chains is in line with previous observations and can be ascribed to the larger
17 hydrodynamic volume of the partially hydrolyzed copolymer in HFIP, presumably due to better
18 hydrogen bonding of the HFIP to the polymer chains.

19 The prepared PEtOx-PEI represents a well-defined reaction platform containing the
20 biocompatible PEtOx as main structure together with the nucleophilic secondary amine units from
21 the PEI. A wide variety of modification reactions of such partially hydrolysed polymers have
22 already been reported in literature³⁷. In this work, the PEtOx-PEI copolymer was reacted with
23 methyl succinylchloride to reintroduce a side chain amide group similar to the native PAOx

1 structure containing a latent methyl ester functionality yielding PEtOx₈₂MestOx₁₈. The ¹H-NMR
2 spectrum of PEtOx-MestOx revealed the disappearance of the PEI backbone resonances at 2.6
3 ppm as well as the appearance of the methyl ester peak at 3.7 ppm demonstrating the success of
4 the modification reaction. Furthermore, the IR spectrum also revealed the representative methyl
5 ester carbonyl vibration at 1730 cm⁻¹. The SEC trace of PEtOx-PMestOx in HFIP also revealed a
6 decrease in M_n caused by the change in hydrodynamic volume while the Đ remained the same (Đ
7 = 1.3). Overlays of the SEC traces and ¹H NMR spectra of PEtOx, PEtOx-PEI and PEtOx-MestOx
8 are shown in Figure 1 to illustrate the successful modifications.

9 As mentioned in the introduction PEtOx-MestOx can also be obtained by direct
10 copolymerization of 2-ethyl-2-oxazoline (EtOx) and 2-methoxycarboxyethyl-2-oxazoline
11 (MestOx). Although the direct polymerization seems to be a more straightforward method
12 compared to the here presented two-step hydrolysis-modification method, it should be noted that
13 EtOx is available in bulk quantities while MestOx has to be custom synthesized in a two-step
14 synthetic protocol when starting from methyl succinyl chloride with 57% overall yield, including
15 double purification by distillation³⁴. In this perspective, the demonstrated hydrolysis-modification
16 method may actually be more cost-effective and time-efficient than the direct polymerization
17 method. Moreover, the hydrolysis-modification route is also more versatile as different reactive
18 linkers, such as methyl bromopropionate, methyl acrylate or methyl iso(thio)cyanatopropionate
19 may be used to install similar methyl ester side chains.³⁸



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 2 **Figure 1.** Overlay of the SEC traces in HFIP (Left) and ^1H NMR spectra of PEtOx, PEtOx-PEI
 3 and PEtOx-MestOx in CDCl_3

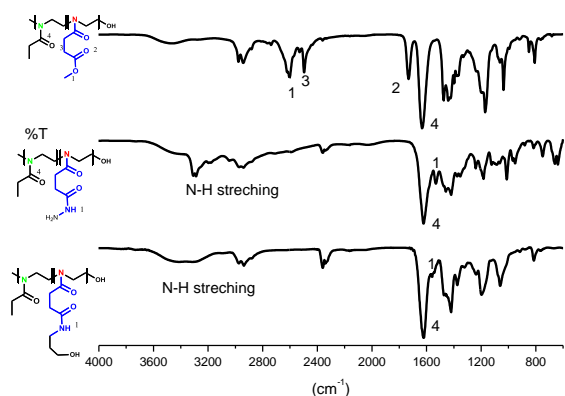
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 6 ***Post-polymerization modification of PEtOx-MestOx by direct amidation***

7 After establishing a robust procedure for the preparation of PEtOx-MestOx, we focused our
 8 attention to the amidation of the methyl ester side chains. Even though it is commonly accepted
 9 that a direct amidation is not feasible under mild conditions, we did try this out by stirring the
 10 PEtOx-MestOx copolymer for two days in neat *n*-propylamine at 70 °C. ^1H NMR spectroscopic
 11 analysis of the resulting polymer after precipitation revealed that the methyl ester resonance at 3.7
 12 ppm disappeared and a new amide signal appeared at 7.9 ppm indicating successful amidation.

1 The disappearance of the methylester peaks gives the prove that there is a full conversion from an
2 ester to an amide. In addition, FT-IR also confirmed the successful amidation by disappearance of
3 the methyl ester band at 1730 cm^{-1} and appearance of a new band at 1531 cm^{-1} representative for
4 the NH bending in a secondary amide group. Furthermore, SEC analysis in DMA revealed an
5 increase in M_n from $9.7 \cdot 10^3$ Da for PEtOx to $11 \cdot 10^3$ for PEtOx-MestOx to $17.8 \cdot 10^3$ Da for the *n*-
6 propyl modified polymer. Importantly, the \bar{D} remained constant at 1.1 indicating that no side
7 reactions take place. Even though it is not fully clear why the direct mild amidation of PEtOx-
8 MestOx is possible, it may be speculated that this is due to the very high concentration and large
9 excess of amine groups, possibly in combination with a neighbouring group effect in which the
10 amide groups attached to the PAOx backbone participate in the reaction mechanism by
11 stabilization of the transition state via the formation of H-bonds.

12 Inspired by the success of this first direct amidation reaction, we continued to explore the scope
13 of this post-polymerization modification reaction for PEtOx-MestOx. Several other readily
14 available and cheap amines, namely aminomethane, aminoethane and hydrazine-hydrate, were
15 utilized using the same Method 1, whereby the reaction is performed in a solution of the amine.
16 ^1H NMR spectroscopy, FT-IR and SEC again revealed the success of these direct amidation
17 reactions (Table 1). The hydrazine modified polymer is especially interesting as it contains
18 hydrazide side-chains that may be further utilized for conjugation of ketone containing molecules
19 via a pH-degradable hydrazone linker³⁹. In a next step, a series of more complex functional amines
20 was explored for direct amidation of PEtOx-MestOx. As these amines are more expensive and
21 often appear as viscous liquids, the reaction conditions were modified resulting in Method 2. In
22 this second method, the polymer is dissolved in acetonitrile and three equivalents of the amine are
23 added. The resulting solution is stirred at $40\text{ }^\circ\text{C}$ overnight. Only if the amine is not soluble in

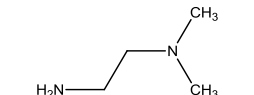
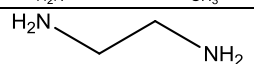
1 acetonitrile, the reaction was performed in a similar manner in DMF, which was the case for
2 allylamine and 1-amino-3-butyne. In the case of amino acids such as Leucine ester and Glycine a
3 deprotonation was needed. Therefore basic water was used as a reaction medium. The different
4 tested amines are listed in Table 1 and successful direct amidation was found based on ^1H NMR
5 and FT-IR spectroscopy as well as SEC for dimethylamine, Leucine methyl ester, glycine, 1,2-
6 aminoethanol and 1,3-aminopropanol, allylamine, 1-amino-3-butyne, *N,N*-dimethyl-1,2-
7 ethylenediamine and 1,2-ethylenediamine. As such, we could include a wide variety of functional
8 groups in the side chains of PAOx based on the same PEtOx-MestOx reactive platform. It is
9 important to note, that the reaction with 1,2-ethylenediamin led to a minor increase of \bar{D} and a
10 decrease in M_n . This observation can be ascribed to tailing of the SEC trace due to interaction of
11 the free amine group with the column materials. There is no evidence of double molar mass
12 shoulders indicating that no cross-linking took place. A series of representative ^1H NMR spectra,
13 FT-IR spectra and SEC traces of the PEtOx-MestOx and some products after amidation are shown
14 in Figure 2, 3 and 4, respectively. The ^1H -NMR spectra clearly show that the peak at 3.7 ppm of
15 the methyl ester group in PEtOx-PMestOx disappears upon amidation. There is also a notable
16 change of signal 6, which after reaction with the amine moves closer to, and partially overlap with
17 the peak around 2.2 ppm. These peaks merge together because of the similarity between the side
18 chain of the PEtOX and the side chain of the succinyl. FT-IR clearly shows the disappearance of
19 the band at 1730 cm^{-1} of the methyl ester carbonyl and reveals the appearance of a new band in
20 the region between $1530\text{-}1560\text{ cm}^{-1}$ a finger print of the NH bending.. Finally, SEC analysis clearly
21 shows that the polymer molar mass distributions have very similar shape before and after
22 amidation demonstrating that no polymer coupling occurred.



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

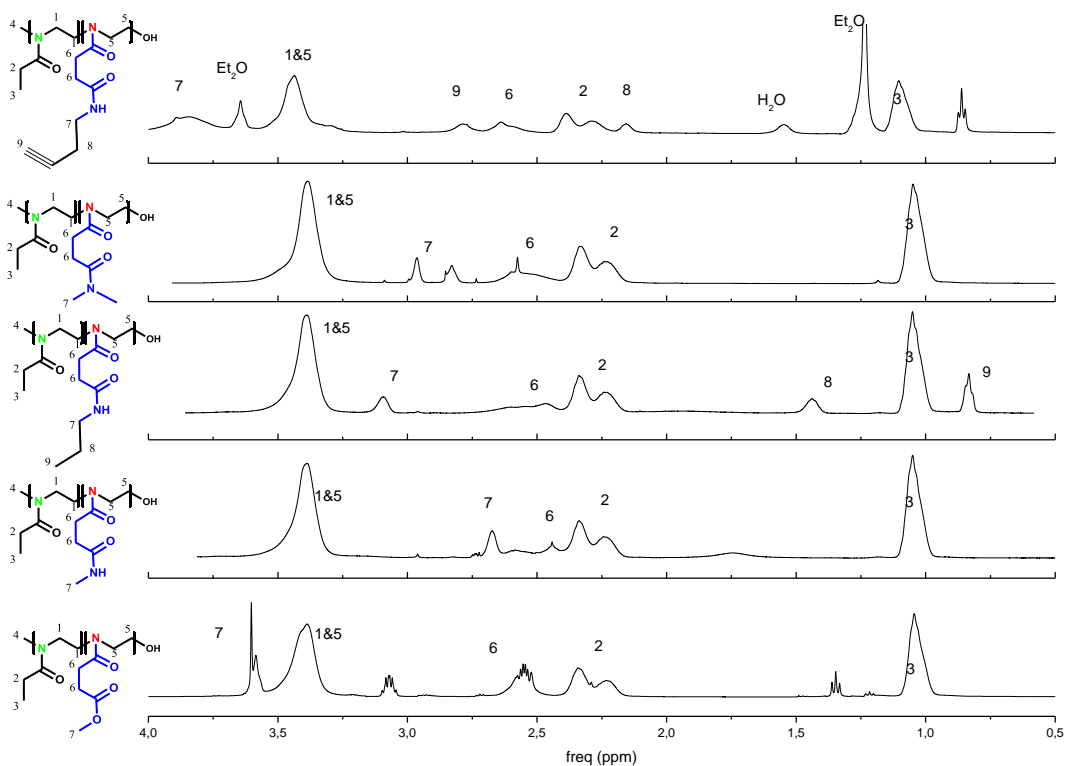
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 5 **Table 1:** Overview of the direct amidation of PEtOx-MestOx with various amines.

	R₁-group	Chemical shift amide proton(ppm)¹	M_n (kDa)²	D²	Method³
aminomethane	<chem>CN</chem>	6.4	17.7	1.1	1
aminoethane	<chem>CCN</chem>	6.5	17.7	1.1	1
1-Amino propane	<chem>CCCN</chem>	7.9	17.8	1.1	1
dimethylamine	<chem>CN(C)C</chem>	6.3	17.8	1.1	2(water)
hydrazine monohydrate	<chem>NN</chem>	9	17.7	1.1	1
leucine methylester	<chem>CC(C)C(N)C(=O)OC</chem>	nd ⁴	18	1.1	2(basic water)
glycine	<chem>NCC(=O)O</chem>	nd ⁴	18	1.1	2(basic water)
2-amino-1-ethanol	<chem>NCCO</chem>	nd ⁴	22	1.1	2(acetonitrile)
3-amino-1-propene	<chem>NCC=C</chem>	nd ⁴	16.5	1.1	2(DMF)
1-amino-3-butyne	<chem>NCCC#C</chem>	7.8	16.7	1.1	2(DMF)

<i>N,N</i>-dimethyl-1,2-ethylenediamine		8.2	16.6	1.1	2(DMF)
1,2-ethylenediamine		nd ⁴	14	1.3	1

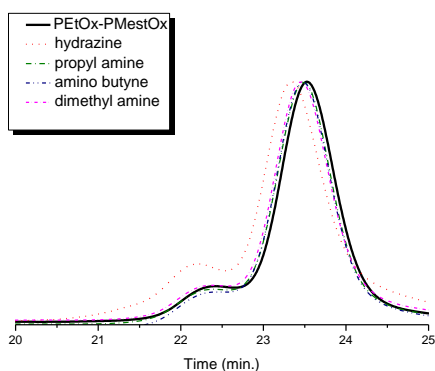
- 1
- 2 ¹ Chemical shift in ¹H-NMR spectra recorded in CDCl₃.
- 3 ² Calculated from SEC analysis with DMA as eluent and PMMA as calibration.
- 4 ³ Method 1: amine is used as reaction solution; method 2: The amidation reaction is done in another
- 5 solvent specified between brackets.
- 6 ⁴ Not distinguishable as the ¹H-NMR-spectra were recorded in DMSO and the NH peak could not
- 7 be seen.

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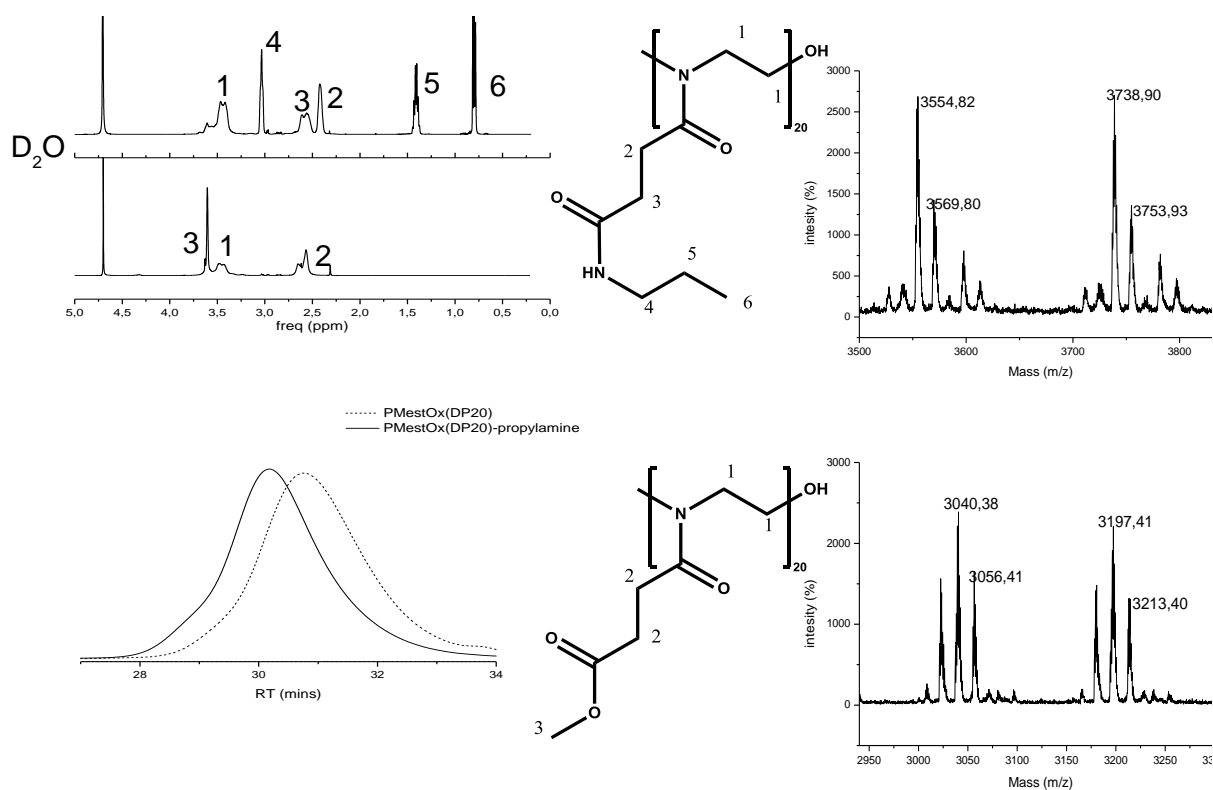
1 **Figure 3:** Overlay of the $^1\text{H-NMR}$ spectra of PEtOx-MestOx and four products after direct
2 amidation with different amines.



3
4 **Figure 4:** Overlay of normalized SEC (HFIP) traces of PEtOx-PMestOx and four examples of
5 amidated polymers functionalized with hydrazine, allyl amine, 1-amino-3-butyne, cystamine

6 To show that the direct amidation is also possible on homopolymers, PMestOx with DP 20 was
7 synthesized by CROP of MestOx using methyl tosylate as initiator. This resulting polymer was
8 analyzed by $^1\text{H-NMR}$ spectroscopy, MALDI-TOF MS and SEC revealing the formation of a
9 defined polymer with a low polydispersity ($\text{Đ}=1.13$; Figure 5). This polymer was subsequently
10 modified with 1-aminopropane via method 1 (bulk method) by dissolving the polymer in 1-
11 aminopropane and stirring overnight at 37°C . The MALDI-TOF mass spectrum of the resulting
12 polymer clearly shows that there is a shift in mass in comparison to the begin product (Figure 5).
13 MALDI-TOF MS of the PMestOx revealed the expected spacing of 157 m/z corresponding to the
14 MestOx monomer while after reaction the spacing increased to 184.24 m/z corresponding to the
15 amidopropane side chain. Furthermore, the exact mass of 3197 m/z for PMestOx corresponds to a
16 polymer with 20 repeat units and methyl and OH end-groups charged with a sodium cation. After
17 the amidation reaction, the exact mass of 3738 is found corresponding to the amido-propane

1 functionalized polymer with 20 repeat units and methyl and OH end-groups and charged with a
 2 sodium cation. These results confirm a clean amidation of all methyl ester side chains into. The
 3 $^1\text{H-NMR}$ spectra also demonstrate that the methyl ester groups disappeared after amidation while
 4 3 new peaks appeared corresponding to the propyl side chains (signals 4-6 in Figure 5). Finally,
 5 SEC confirmed an increase in hydrodynamic volume after the amidation reaction while the \bar{M}_w
 6 mildly increased from 1.13 to 1.17, albeit no clear shoulders are observable (Figure 5).



7
 8 **Figure 5:** Overview of the $^1\text{H-NMR}$ data in D_2O (top left), MALDI-TOF MS (right) and SEC
 9 (DMA as eluent; bottom left) analysis of the pure PMestOx with 20 repeating units before and
 10 after amidation with 1-aminopropane. The bottom $^1\text{H NMR}$ and MALDI TOF mass spectra show
 11 PMestOx and the top spectra show the polymer after amidation with propylamine.

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Conclusion 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 amount 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 proceeds without a catalyst, proposedly due to activation by neighbouring amide groups. Furthermore, we also demonstrated the synthesis of a PMestOx 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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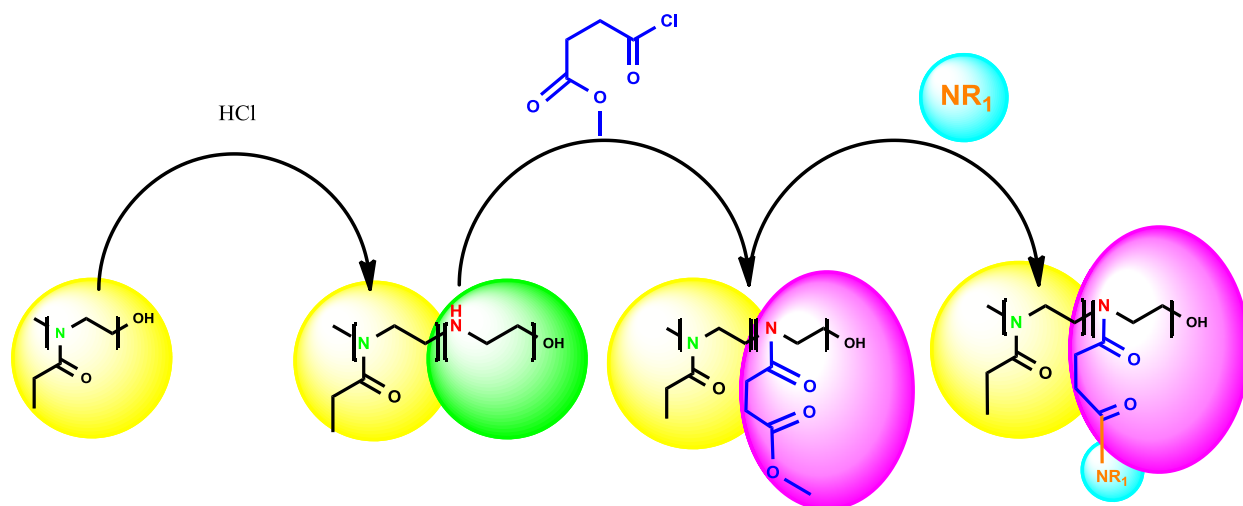
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