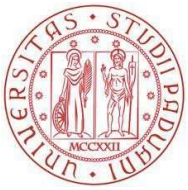

UNIVERSITÀ DEGLI STUDI DI PADOVA DIPARTIMENTO DI SCIENZE CHIMICHE

CORSO DI LAUREA IN CHIMICA INDUSTRIALE

Synthesis of Polyoxazolines with Controlled Topologies

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TABLE OF CONTENT

1 INTRODUCTION

Since they were first reported in 1966 by four independent groups[1-4], numerous 2-oxazolines have been used in cationic ring-opening polymerization (CROP) to obtain the corresponding poly (2-alkyl-2-oxazoline).[5] They are organic polymers that belong to a very exclusive category of compounds, which let them receive much interest in the biomedical field owing to their improved performance over common biomedical polymers such as poly (ethylene glycol).[6] A great deal of interest is indeed focused on cyclic PAOx, especially for biomedical applications, due to their exceptional mechanical, electrical, and thermal properties in terms of biocompatibility. A strategy for their synthesis is the intramolecular copper(I)-catalyzed azide– alkyne cycloaddition (CuAAC) reaction which has come to represent click chemistry for many, although other reactions are also included in this concept.[7]

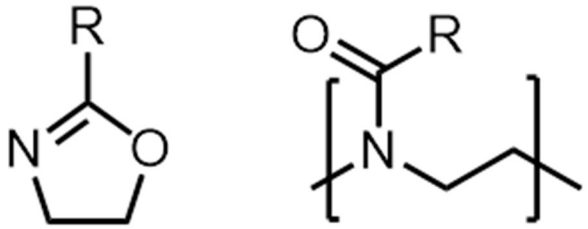
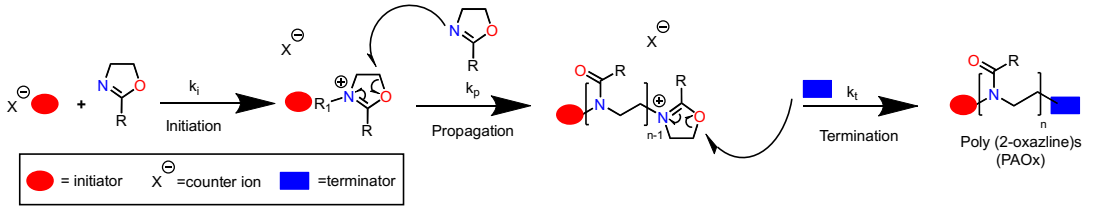


FIGURE 1 STRUCTURE OF A 2-OXAZOLINE (LEFT) AND PAOX WITH R = H, ALKYL, ARYL (RIGHT)

1.1 CATIONIC RING-OPENING POLYMERIZATION OF 2-OXAZOLINES

Cationic Ring-Opening Polymerization is a technique used to synthesize various types of polymers by initiating the opening of cyclic monomers in the presence of a cationic initiator. This process results in the formation of linear or branched polymers. CROP mechanism consists of three steps: initiation, propagation and termination.



SCHEME 1 THE CATIONIC RING-OPENING POLYMERIZATION OF 2-OXAZOLINES

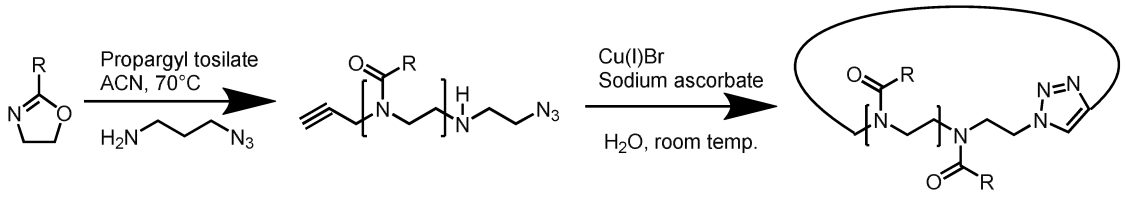
In the first step, a nucleophilic attack is performed by the nitrogen monomer towards the initiator, forming an oxazolinium cation that can further react with a second monomer, promoting the polymerization reaction. The employed initiator systems vary and include, for example, alkyl sulfonates like methyl p-toluenesulfonate. In the successive propagation step, the 2-oxazoline monomer reacts with the cationic oxazolinium intermediate, forming the PAOx backbone by ring-opening and amide formation while the living oxazoline chain-end remains. The CROP of 2-oxazolines finally terminates through nucleophilic attack by an added terminating agent on the living cationic chain end.[5]

The purity of every component of the polymerization system is needed to obtain a living polymerization.[5] No nucleophilic species can be tolerated in the polymerization mixture and extreme purity and dryness of solvent as well as initiator and monomer are required for reaching optimal polymerization conditions, leading to low dispersity.[8]

1.2 SYNTHESIS OF CYCLIC POLY(2-ALKYL-2-OXAZOLINE) THROUGH COPPER(I)-CATALYZED AZIDE– ALKYNE CYCLOADDITION REACTION

Cyclic polymers are an interesting class of polymers, as their physical properties tend to be quite different from their linear counterparts of the same molar mass due to the absence of chain ends.[9] A typical strategy to synthesize cyclic polymers involves copper(I)-catalyzed azide-alkyne cycloaddition of a heterotelechelic precursor polymer for the intramolecular ring closure, promoted by rapid reaction kinetics and high selectivity. This makes PAOx very well suited for this task since both functionalities, alkyne and azide, are

quickly introduced through the initiator and terminator during polymerization.[10] One of the main downsides to use cyclic polymers arises from the need to use very dilute conditions to prevent intermolecular coupling side reactions. Therefore, scale-up limitations exist for using cyclic polymers more widely.[6]



SCHEME 2 CATIONIC RING OPENING POLYMERIZATION OF PAOX AND SUBSEQUENTLY COPPER(I)-CATALYZED AZIDE–ALKYNE CYCLOADDITION REACTION

2 AIM OF THE THESIS

In this thesis, poly(2-ethyl-2-oxazoline) (PEtOx) was synthesized via Cationic Ring Opening Polymerization (CROP), terminated with an azide group. Subsequently, a cyclization was attempted via ring closure, in which the terminal functional groups react with each other through click-chemistry. The polymer was characterized and will be the subject of future studies.

3 EXPERIMENTAL SECTION

3.1 MATERIALS

2-Ethyl-2-oxazoline (EtOx, Sigma-Aldrich, 99%) was distilled from potassium hydroxide (KOH) under Ar. Acetonitrile (ACN, Sigma-Aldrich, 99%) was first stirred overnight with calcium hydride (CaH_2) and distilled under Ar before use. Propargyl p-toluensulfonate was first stirred overnight with CaH_2 under Ar, and later distilled under vacuum. 3-Azido-1-propanamine (synthesized in lab following the procedure in the next paragraph) was distilled from KOH under vacuum using membrane pump connected to the Schlenk line. Copper (I) bromide (CuBr) was purified by stirring with glacial acetic acid for 24 hours at room temperature, followed by rinsing with ethanol and diethyl ether, and then drying overnight under high vacuum. Milli-Q grade water was utilized in all the experiments. All other chemicals were used without additional purification unless stated elsewhere.

3.2 SYNTHESIS OF 3 AZIDO 1 PROPANAMMINE

3-Bromopropylamine hydrobromide (14.0 g, 64.0 mmol, 1 eq) was dissolved in 100 mL water. NaN_3 (13.7 g, 211 mmol, 3.3 eq) was added to the solution, and the mixture was heated to reflux (oil 120 °C) and stirred for 25 hours. Upon completion of the reaction solution was cooled down using an ice/water bath. The pH of the solution was raised to 14 by slowly adding KOH pellets while keeping the temperature below 10 °C. Subsequently, Et_2O (200 mL) was added. The organic layer was separated, and the aqueous phase was extracted with Et_2O (two portion of 100 mL). The combined organic phases were dried with $MgSO_4$ and filtered, and the solvent was carefully removed under vacuum yielding. The crude compound was as a lightly yellow liquid. The compound was stored at -20°C in the dark.

3.3 SYNTHESIS OF LINEAR POLY(2-ETHYL-2-OXAZOLINE)

In a flask previously dried in an oven and equipped with a magnetic stirrer, vacuum-argon cycles were performed. Freshly distilled dry ACN was transferred into the flask, and the freshly distilled monomer was dissolved in it (monomer concentration: 4M). The solution was then cooled to 0°C, and the initiator,

propargyl p-toluenesulfonate, was added to the mixture. The mixture was then heated to 70°C under stirring until quantitative conversion was achieved. After this time, the mixture was allowed to cool to room temperature, and the chosen termination agent was added. The mixture was then stirred at room temperature for another 36 hours. The polymer was then purified by precipitation in diethyl ether, filtered and dried under vacuum. After freeze-dried α-propargyl-ω-azido PEtOx was obtained as a white solid.

3.4 CYCLIZATION OF A-PROPARGYL-Ω-AZIDO PETOX

Cyclic PEtOx was synthesized via Cu-catalyzed Huisgen cycloaddition. In two round-bottom flasks, α-propargyl-ω-azido PEtOx (300 mg, 0.03 mmol) and sodium ascorbate (75 mg, 0.38 mmol) with CuBr (60 mg, 0.42 mmol) were dissolved in 200 mL and 1.2 L of milli-Q grade water, respectively. The two solutions were degassed separately under Ar for one hour. The degassing process was stopped, and the polymer solution was slowly added to the sodium ascorbate/CuBr mixture using a high precision tubing pump (IPC-N4, ISMATEC, Switzerland) at a rate of 45 $\mu L \cdot min^{-1}$ at room temperature under Ar atmosphere. When the addition was completed, the reaction was kept under argon and stirred for additional 24 hours prior to volume reduction, filtration (0.45 um PTFE Chromafil filters) and freeze-dried. The crude product was dissolved in 5 mL of methanol and passed through a column of neutral alumina to remove any Cu salt. The solvent was then removed, and the product was re-dissolved in chloroform, filtered (0.25 um PTFE Chromafil filters). The final product was obtained as a white solid after freeze-drying.[11]

3.5 CHARACTERIZATION TECHNIQUES

Nuclear magnetic resonance (^{1}H-NMR) spectra were performed with a Bruker AV400 spectrometer (400 MHz). Deuterated chloroform ($CDCl_3$) was used as solvent.

Gel permeation chromatography (GPC) analysis were performed using a Viscotek GPCmax VE 2001 over a TriSec Model 302 triple detector array. The

samples were dissolved in a DMF/LiBr solution to a resulting concentration of 2 mg/ml.

FT-IR spectra were recorded on an Agilent Cary 630 FTIR spectrometer.

4 RESULTS AND DISCUSSION

The role of an initiator in polymerizing poly(2-oxazoline) s is vital. An efficient initiator will lead to a well-defined polymer with high functionality of end groups. Herein, propargyl p-toluene sulfonate was chosen because the initiator is efficient for polymerizing 2-ethyl-2-oxazoline and clean polymerization takes place, and the initiator can be consumed entirely. It was also noted that electrophilicity and steric hindrance in initiators could influence the efficiency of cationic ring-opening polymerization.[12]

In the first step, linear PEtOx was synthesized using an efficient initiator, propargyl p-toluene sulfonate, which showed the reaction to be ~100% conversion. In the second step, the living polymer solution was capped with 3-azido-1-propanammine to give azide end groups on the polymer. The polymer was purified by precipitation and washed to remove unreacted azide.

Figure 2 represents the ^{1}H NMR spectra of α-propargyl-ω-azido PEtOx. The three peaks (c, b and d) at 1.12, 2.31-2.41, and 3.45 ppm corresponds to the polymer backbone. The initiator appears at ~4.06-4.19 ppm (a).

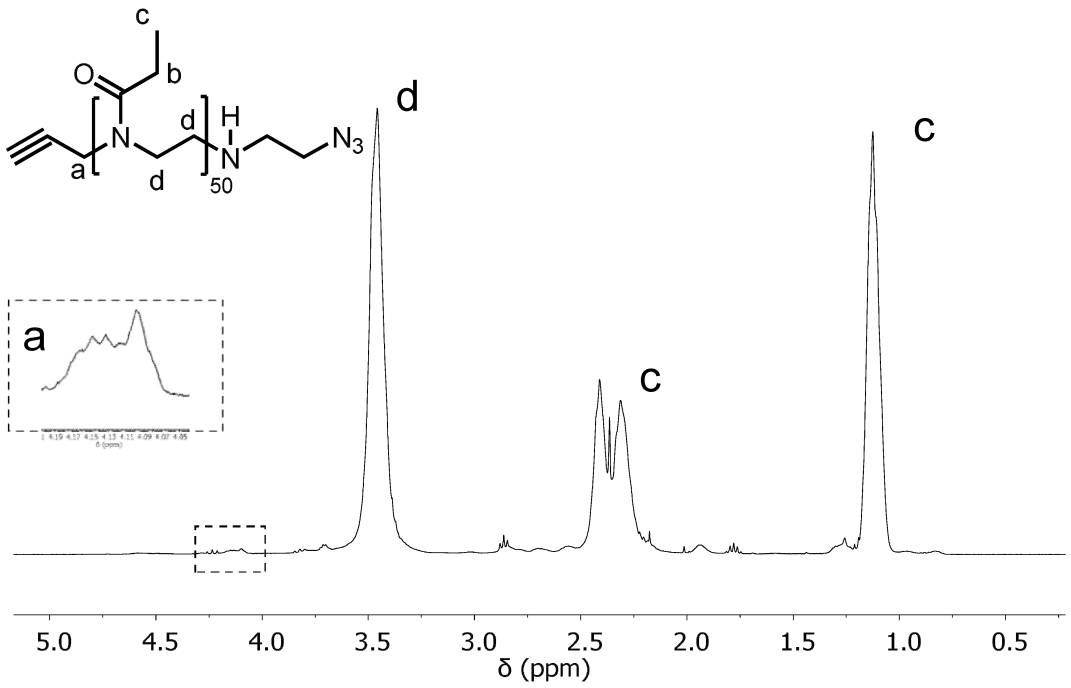


FIGURE 2 ^{1}H NMR SPECTRA OF A-PROPARGYL-Ω-AZIDO PETOX

The azide end group was confirmed by using IR spectroscopy as azide shows a sharp peak at 2100 cm^{-1}, relative to the stretching of the azide group.

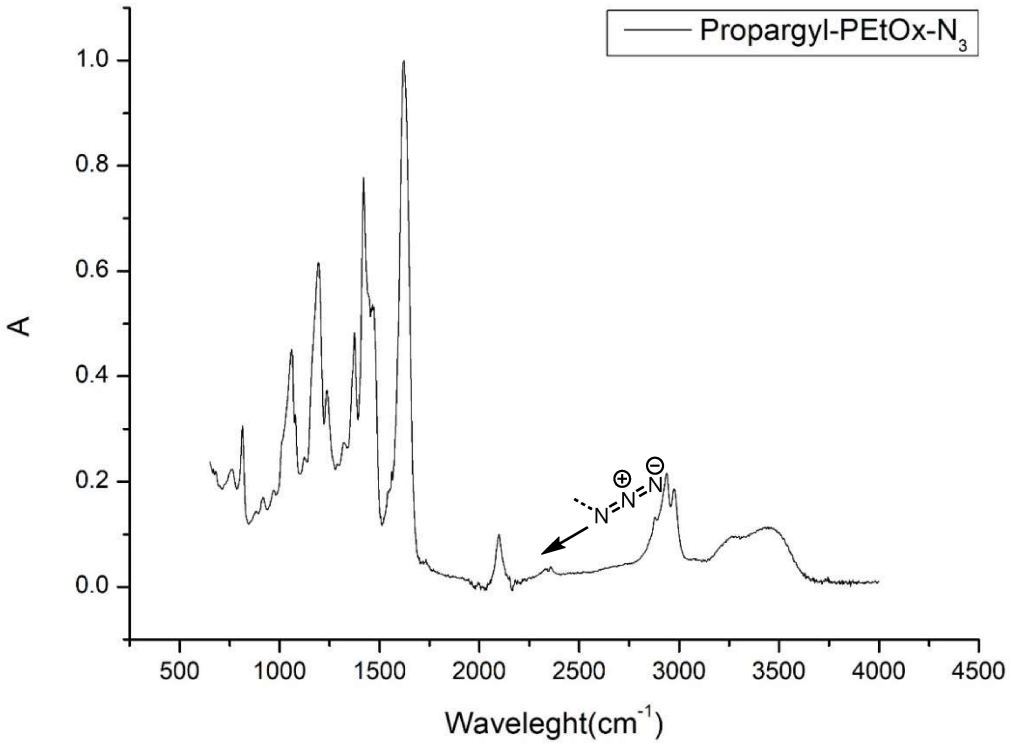


FIGURE 3 FT-IR SPECTRA OF A-PROPARGYL-Ω-AZIDO PETOX

As shown in figure 4, molecular weights and distribution PEtOx polymers were determined by GPC in DMF. The PDI from GPC were recapitulated in Table 1.

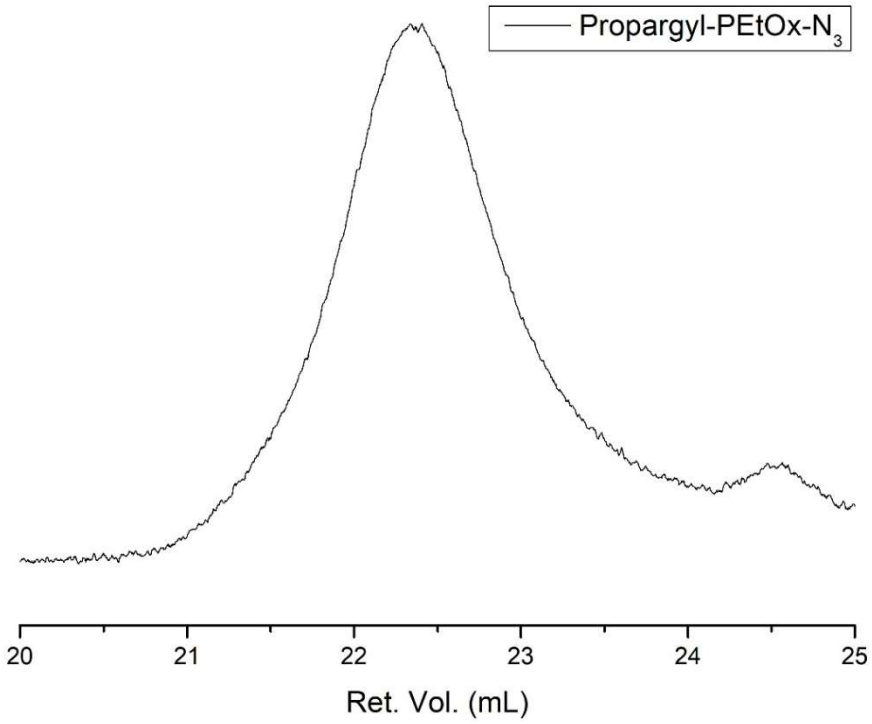


FIGURE 4 GPC EUGRAM OF A-PROPARGYL-Ω-AZIDO PETOX

Despite various attempts, cyclic PEtOx was not synthesized following the CuAAC procedure previously described. To assess the effective cyclization of the polymer precursor during CuAAC, ^{1}H-NMR, IR spectroscopy and GPC analysis have been employed (Figure 5-7).

The disappearance of the signals assigned to the protons adjacent to the alkyne group, between 4.05 and 4.30 ppm, may indicate the complete conversion of the alkyne group for the PEtOx. However, no new signal group appearance above 4.30 ppm indicates the non-formation of the triazole ring. IR spectroscopy showing the azide signal at 2100 cm^{-1} with the same intensity as the precursor indicates the absence of conversion of the reaction. Furthermore, even though there was no significant increase of dispersity, no strong shift in GPC elugram was seen.

A further attempt was made by using an alternative source of Cu for the reaction. In 170 mL of water PEtOx (200 mg) was dissolved. Separately, 40 mg of ascorbate were added to 1 L of Milli-Q water. The two solutions were degassed for 30 minutes. Then Cu (II) SO_4 anhydrous (20 mg) was added to the ascorbate solution and further degassed with Ar for other 30 minutes. Then the polymer solution was poured into the Cu solution using a high precision tubing pump (IPC-N4, ISMATEC, Switzerland) at a rate of 45µL min^{-1} at room temperature while under an Ar atmosphere. At the end of the addition the previous purification procedure followed. Also, this time cyclic PEtOx was not synthesized as shown in Figure 8.

5 CONCLUSIONS

In summary, while cyclic PAOx has found potential in various biomaterial applications, such as protein-repellent surfaces[11] or the construction of cartilage[13], the synthesis of these materials is still challenging. As shown in this work, cyclization efficiency depends on many parameters that go far beyond a simple need for dilution or for using enough catalysts. Special conditions for click chemistry are necessary due to the presence of catalysts sensible to oxidation like Cu(I) for azide-alkyne cycloaddition, and the inert atmosphere is to be maintained for the efficient reaction progress. It may also be present a problem during the polymerization. It has been proven that the CROP of 2-oxazolines suffers from side reactions, such as predominately chain transfer.[14] In this reaction pathway, the living oxazolinium chain end transfers a proton to an adjacent monomer, which on the one hand leads to an unreactive enamine ω-end group. In contrast, on the other, it leads to a reactive oxazoline cation. The polymer chains emerging from the generated oxazolinium, therefore, present a hydrogen α-end group and, in response, the general size distributions of polymeric products are broadened, thus making the synthesis of well-defined PAOx more complex. In addition, impurities such as residual moisture can produce α-hydrogen end groups on the PAOx chains. Nucleophilic impurities with hydrogen atoms in the nucleophile center, like water, can quench active PAOx chains, releasing a proton capable of initiating a new PAOx chain.[15]

6 APPENDIX

6.1 CHARACTERIZATION

Polymer	GPC		
	Mw (Da)	Mn (Da)	PDI
Propargyl-PEtOx-N_3	5500	4550	1.2
Cyclic-PEtOx_A	5750	4650	1.2
Cyclic-PEtOx_B	5900	4800	1.2
Cyclic-PEtOx_C	5700	4750	1.2
Cyclic-PEtOx_$CuSO_4$	5500	4250	1.3

TABLE 1 MOLECULAR WEIGHTS AND POLYDISPERSITY INDEX (PDI) OF PROPARGYL-PETOX-N3 PRECURSOR AND CYCLIC DERIVATES

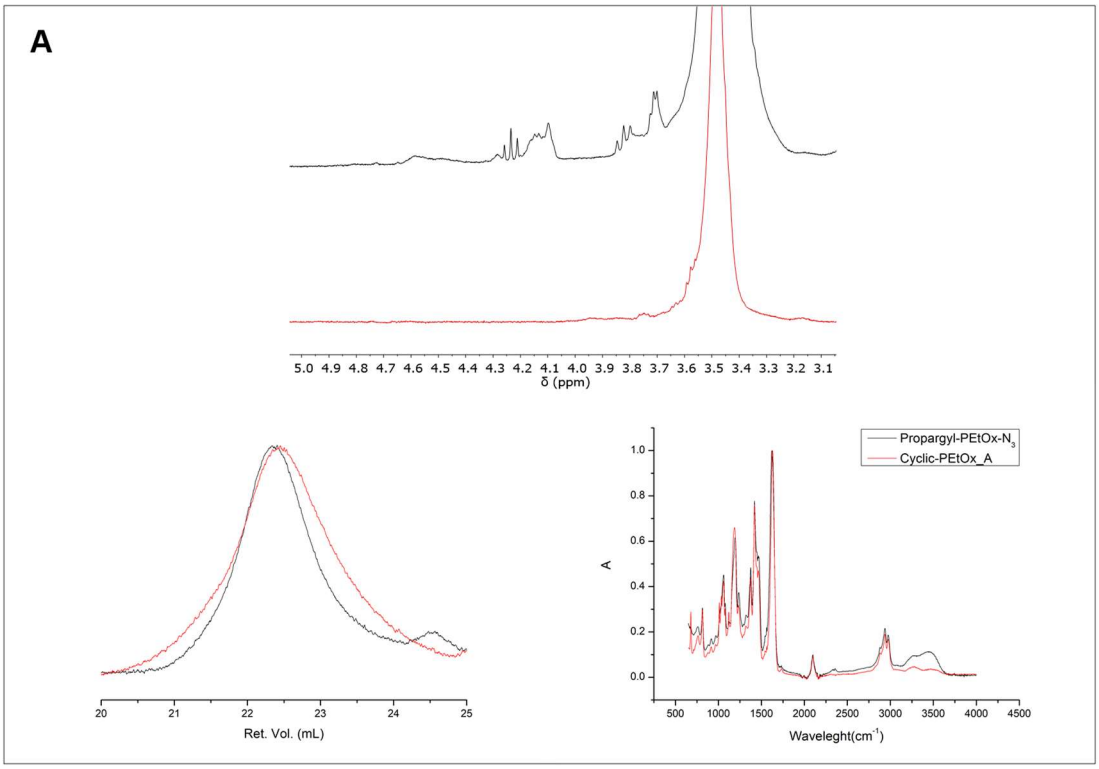


FIGURE 5 ^{1}HNM SPECTRA (TOP), GPC ELUGRAM (BOTTOM, LEFT) AND FT-IR SPECTRA (BOTTOM, RIGHT) OF PETOX BEFORE (BLACK) AND AFTER (RED) CUUAAC

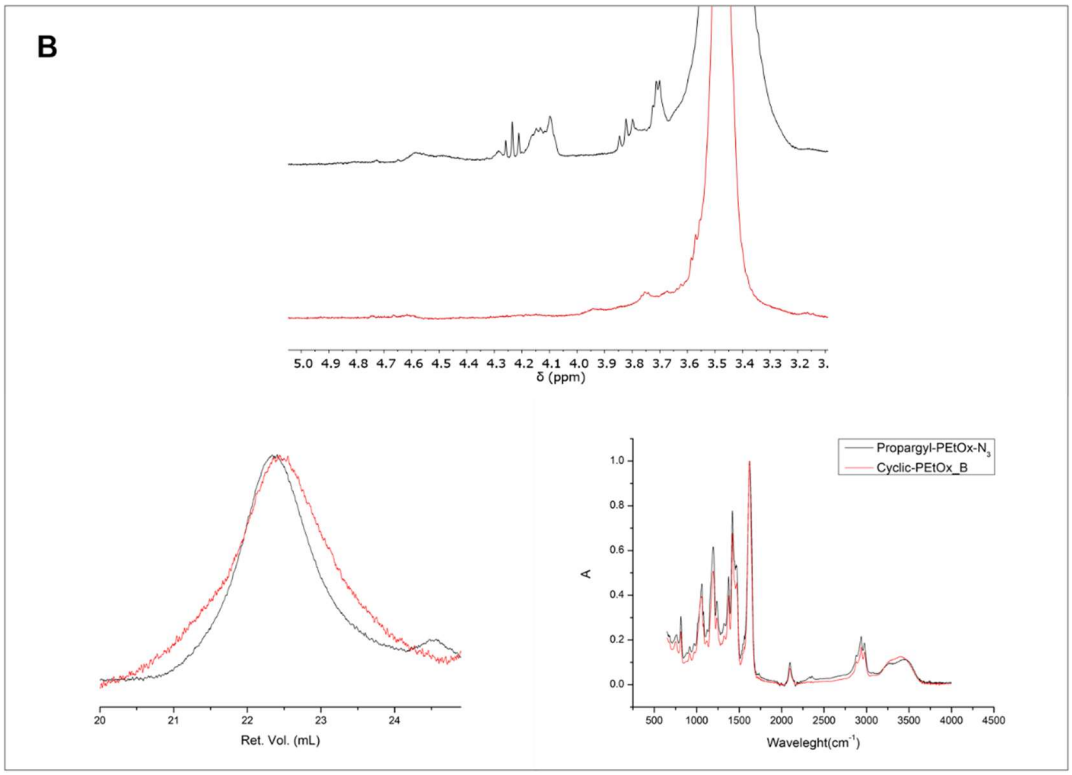


FIGURE 6 ^{1}HNM SPECTRA (TOP), GPC ELUGRAM (BOTTOM, LEFT) AND FT-IR SPECTRA (BOTTOM, RIGHT) OF PETOX BEFORE (BLACK) AND AFTER (RED) CUUAAC

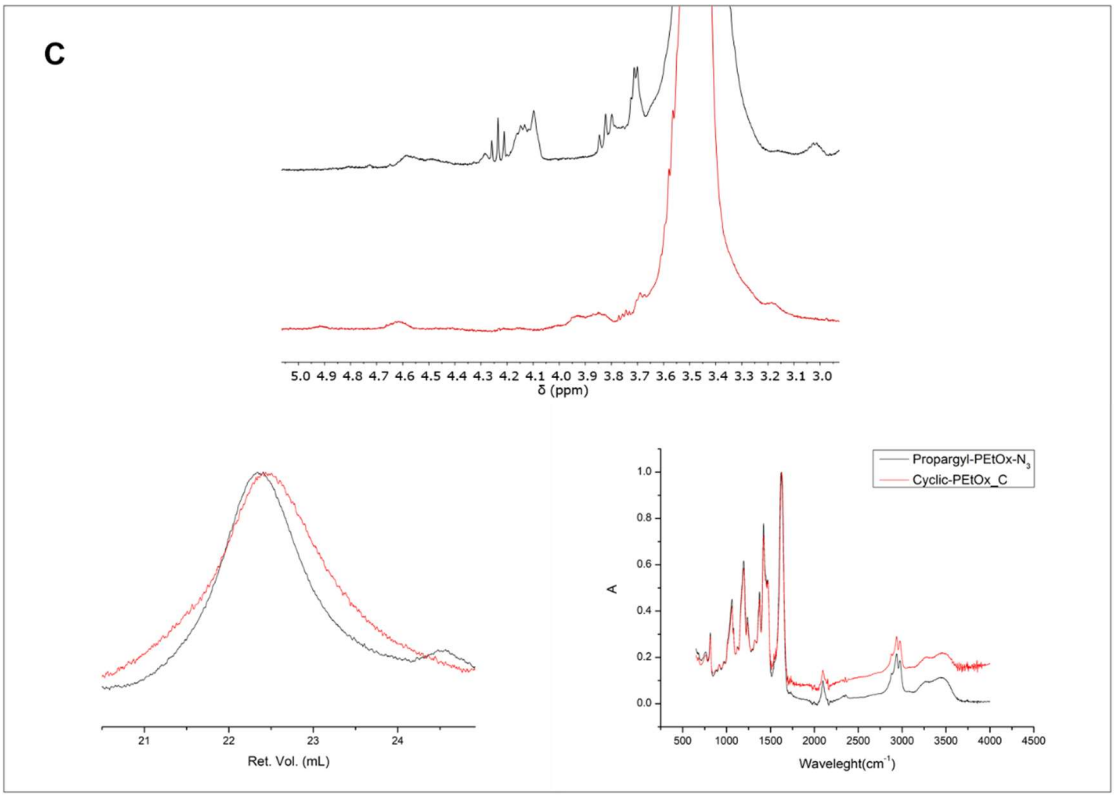


FIGURE 7 ^{1}HNM SPECTRA (TOP), GPC ELUGRAM (BOTTOM, LEFT) AND FT-IR SPECTRA (BOTTOM, RIGHT) OF PETOX BEFORE (BLACK) AND AFTER (RED) CUUAAC

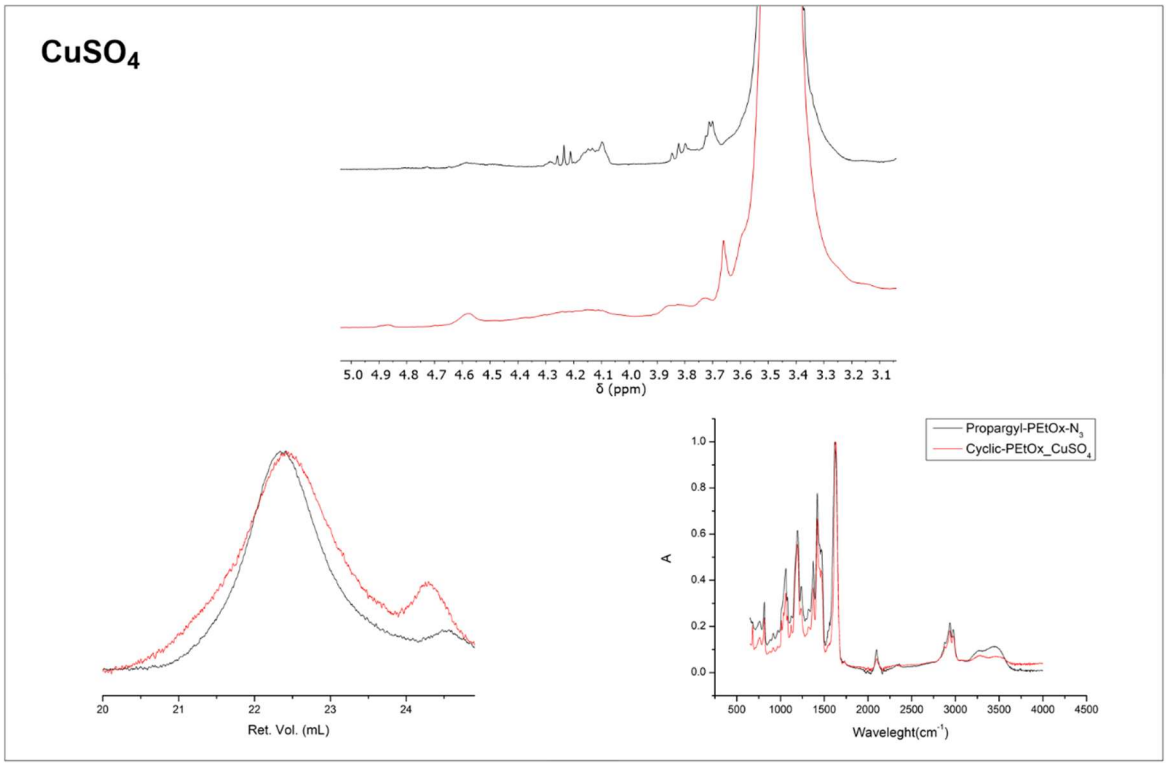


FIGURE 8 ^{1}HNM SPECTRA (TOP), GPC ELUGRAM (BOTTOM, LEFT) AND FT-IR SPECTRA (BOTTOM, RIGHT) OF PETOX BEFORE (BLACK) AND AFTER (RED) CUUAAC

BIBLIOGRAPHY

1. Tomalia, D. A.; Sheetz, D. P.; *J. Polym. Sc.i A-1 Polym. Chem.* **1966**, 4, 2253–2265.
2. Seeliger, W; Aufderhaar, E; Diepers, W; Feinauer, R; Nehring, R; Their, W.; et al.; *Angew. Chem. Int. Ed.* **1966**, 5, 875–888.
3. Bassiri, T. G.; Levy, A.; Litt, M.; *J. Polym. Sci. B. Polym. Lett.* **1967**, 5, 871–879.
4. Kagiya, T.; Narisawa, S.; Maeda, T.; Fukui, K.; *J. Polym. Sci. B. Polym. Lett.* **1966**, 4, 441–445.
5. Jana, S.; Hoogenboom, R.; *Poly(2-oxazoline) s: a comprehensive overview of polymer structures and their physical properties, Polym. Int.* **2022**, 71, 935-949.
6. Drain, B. A.; Beyer, V. P.; Cattoz, B.; Becer, C. R.; *Macromolecules* **2021**, 54 (12), 5549-5556.
7. Presolski, S. I.; Hong, V. P.; Finn, M. G.*; Copper-Catalyzed Azide-Alkyne Click Chemistry for Bioconjugation. Curr. Protoc. Chem. Biol.* **2011**, 3 (4), 153-162.
8. Verbraeken, B.; Monnery, B. D.; Lava, K.; Hoogenboom, R.; *The Chemistry of Poly(2-oxazoline)s. **2024**, In Encyclopedia of Polymer Science and Technology, (Ed.).*
9. Muhammad, W. A.; Zahir, M.; Qun, J.; Lianwei, L.; Muhammad, S.; Muhammad, S.; Yougen, C.; *Polym. Chem*. **2020**,11, 4164-4171.
10. Huettner, N.; Goldmann, A. S.; Hoogenboom, R.; Dargaville, T. R.; *Polym. Chem.* **2022**, 13, 3975-3980.
11. Morgese, G.; Trachsel, L.; Romio, M.; Divandari, M.; Ramakrishna, S. N.; Benetti, E. M.; *Angew. Chem. Int. Ed.* **2016**, *55*, 15583
12. Ali, M.W.; Cheng, S.; Si, J.; Siddiq, M.; Ye, X.; *Synthesis and characterization of degradable hyperbranched poly(2-ethyl-2-oxazoline). J. Polym. Sci. Part A: Polym. Chem.* **2019**, 57, 2030-2037.
13. Morgese G.; Cavalli, E.; Rosenboom, J. G.; Zenobi-Wong, M.; Benetti, E. M.; *Angew. Chem. Int. Ed.* **2018**, 57, 1621– 1626.

14. Litt, M.; Levy, A.; Herz, J; *Polymerization of Cyclic Imino Ethers. X. Kinetics, Chain Transfer, and Repolymerization. Journal of Macromolecular Science: Part A - Chemistry*, **1975**, *9* (5), 703–727.
15. Brunzel, M; Dirauf, M; Sahn, M; Czaplewska, J. A.; Fritz, N.; Weber, C.; Nischang, I.; Schubert, U. S.; *On the identification and quantification of proton-initiated species in the synthesis of poly(2-alkyl-2-oxazoline)s by high resolution liquid chromatography. J. Chromatogr. A.* **2021**, 1653, 462364.