Click Chemistry with Poly(2-oxazoline)s

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ABSTRACT: A new 2-oxazoline with a pendant alkyne moiety, 2-(pent-4-ynyl)-2-oxazoline, **1** (PynOx), was synthesized from commercial available compounds. Polymerization of PynOx with methyl triflate as initiator and copolymerization with 2-methyl- or 2-ethyl-2-oxazoline (MeOx or EtOx) as comonomers results in well-defined water-soluble polymers of narrow molar mass distributions and predefined degrees of polymerization. Since the alkyne moiety is compatible with the living cationic polymerization, no protection group was needed. The consecutive copper-catalyzed Huisgen 1,3-dipolar cycloadditions of two different azides with the polymer bound alkynes to the 1,2,3-triazoles were quantitative.

Introduction

Our current interest is the development of functional poly(2-substituted-2-oxazoline) copolymers as water-soluble and biocompatible[1] polymer to be used as a carrier system for a new class of polymer therapeutics.[2,3] In this line of research, we recently presented new 2-oxazoline monomers and their corresponding polymers with pendant chemical groups such as aldehyde[4] or amine[5] functions for polymer analogue coupling reactions. The target is to combine the advantages of the living polymerization, such as the good control of composition and structure of the resulting polymer, with the possibility to synthesize a polymer scaffold that can be functionalized with multiple homing devices for improved targeting,[6−9] a higher payload of therapeutically active moieties (e.g., radioactive nuclides for radionuclide therapy or cell toxins for chemotherapy), and the control of the solubility, biodistribution, pharmacokinetics, and immunoresponds of the entire construct by adjusting the polymer composition.

In our first two examples, the functional group allowed quantitative polymer analogue coupling reactions (amine with isothiocyanates and aldehyde with amino-oxy compounds), crucial to obtaining defined polymers for future biomedical applications. However, such groups are not compatible with the living cationic ring-opening polymerization, and the functionalities had to be introduced via monomers bearing the protected functionalities. Hence, an additional polymer analogue deprotection step is needed.

To minimize the necessary reaction steps and, more importantly, to reduce the possibility of the chemical heterogeneity of the final product, a direct route by a defined living copolymerization of a functional monomer that is compatible with the polymerization with a second monomer that ensures the water solubility and biocompatibility of the final polymer are desirable. Only one final, hopefully quantitative, polymer analogue step is then needed to introduce peptidic cell binding motives as homing devices and/or attach the pharmacological active group.

A quantitative polymer analogue conversion is crucial. Click chemistry has drawn much attention in organic chemistry, especially if challenging coupling processes have to be handled. Kolb and Sharpless provide an interesting review of these reactions, i.e., the copper(I)-mediated azide−alkyne 1,3-dipolar cycloaddion.[10] In macromolecular science this method is already in use for highly efficient polymer analogue reactions[11−15] and is reported to have high yields and practically no side reactions. The formed triazole ring has a strong dipolar moment and can form H-bonds giving some hydrophilicity while being stable under biological conditions. All this makes the azide−alkyne 1,3-dipolar cycloaddion very suitable for our purposes.

Here we describe the synthesis of an alkyne bearing 2-substituted 2-oxazoline, its subsequent homo- and copolymerization with 2-methyl- or 2-ethyl-2-oxazoline (MeOx or EtOx), and the results of the polymer analogue click reactions.

Experimental Part

Materials and General Methods. All substances were purchased from Sigma-Aldrich (Steinheim, Germany) and were used as received unless otherwise stated. Methyl triflate (MeOTf), 2-methyl-2-oxazoline (MeOx), acetonitrile (ACN) for polymer preparation, and other solvents were dried by refluxing over CaH_2 under a dry nitrogen atmosphere and subsequent distillation prior to use. Methyl azidoacetate, **4**, was prepared according to a procedure of Allen et al.[16] (Trimethylsilyl)methyl azide, **3**, was synthesized as described by Anderson and Milowsky.[17] Microwave-assisted synthesis was performed using a CEM Discover LabMate system.

NMR spectra were recorded on a Bruker ARX 300 (^{1}H: 300.13 Hz; ^{13}C: 75.47 Hz) at 292 K. The spectra were calibrated using the solvent signals ($CDCl_3$ 7.26 ppm, D_2O 4.67 ppm). FT IR spectra were obtained on a Bruker IFS 55s spectrometer with an ATR sampling accessory from Harrick (single bounce, diamond crystal) and an MCT detector.

MALDI-TOF mass spectrometry was performed on a Bruker Biflex III with a dithranol matrix. The samples were prepared by mixing $CHCl_3$ solutions of the polymer and matrix (10 mg/mL) in a typical ratio of 1:1 (v/v). Gel permeation chromatography (GPC) was carried out using PLgel 5 μm MIXED-C and PLgel 3 μm MIXED-E columns (PLgel 5 μm MIXED-C, 300 × 7.5 mm, PLgel 5 μm MIXED-C, 300 × 7.5 mm, PLgel 3 μm MIXED-E, 300 × 7.5 mm, PLgel 3 μm MIXED-E, 300 × 7.5 mm) using a Waters 410 differential refractometer detector for measurements in DMAc (*N*,*N*-dimethylacetamide). The flow rate was 0.5 mL/min. Prior to each measurement, the samples were filtered through a 0.2 μm

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Teflon filter (Macherey-Nagel) to remove particles. GPC columns were calibrated with poly(methyl methacrylate) standards (Polymer Standards Service (PSS), molar masses: $960-1.64 \times 10^6$ g/mol). Data were processed using Millenium software.

Gas chromatography (GC) was performed on a Finnigan LCQ with a Chrompack CP-8 column and FID detector. The injection temperature was 270 °C. A typical temperature gradient was as follows: 2 min hold at 95 °C after injection, subsequent heating to 320 °C at 30 °C/min, and final hold for 3 min. For dialysis of polymeric products, a Roth ZelluTrans V series dialysis membrane was used (nominal molar mass cutoff: ~1000 g/mol). HPLC was performed on a HP1100 series with water (0.1% TFA) and ACN (0.1% TFA) as the mobile phases (1 mL/min) and Nucleosil 100 RP-18 as the stationary phase. Elemental analysis was measured by the Microanalytical Laboratory of the Inorganic Chemistry Institute of the TU München.

Synthesis. *Hex-5-ynoyl Chloride*. In a flame-dried Schlenk flask and under a dry nitrogen atmosphere 25.485 g (0.227 mol, 1 equiv) of hex-5-ynoic acid (95%) and 32.514 g (0.273 mol, 1.2 equiv) of thionyl chloride (99.0%+) were refluxed at 90 °C for ~1 h. The end of the reaction was indicated as the gas evolution from the reaction mixture ceased. The dark red mixture was distilled under nitrogen, and the product was obtained as a colorless liquid of pungent smell (25.91 g, 92%, bp 104−110 °C). ^{1}H NMR ($CDCl_3$): δ = 2.97 (t, ^3J = 7.3 Hz, 2H, CO−CH_2), 2.21 (td, ^3J_t = 6.6 Hz, ^3J_d = 2.7 Hz, 2H, −CH_2−C≡CH), 1.93 (t, ^3J = 2.7 Hz, 1H, −CH$_2$−C≡CH), 1.82 ppm (ps-quin, ^3J = 7.1 Hz, 2H, −CH$_2$−CH_2−CH$_2$−C≡CH). ^{13}C NMR ($CDCl_3$): δ = 173.4 (−CO−Cl), 82.1 (HC≡C−), 70.0 (HC≡C−), 45.5 (CH$_2$−CO−Cl), 23.6 (≡C−CH$_2$−), 17.2 ppm (≡C−CH$_2$−CH$_2$−CH$_2$−). IR: 3301 (≡C−H str) (m), 2954 (C−H str) (w), 1791 (C═O str) (s), 626 cm^{-1} (≡C−H wag) (s).

Hex-5-ynoic Acid (2-Chloroethyl)amide. Hex-5-ynoyl chloride (24.973 g, 191 mmol, 1 equiv) was dissolved in 220 mL of dry dichloromethane (DCM). 2-Chloroethylamine hydrochloride (22.35 g, 192.7 mmol, 1 equiv) (99%) was added, and the mixture was ice-cooled. Over a period of 1 h, 65 mL (0.47 mol) of triethylamine (TEA) (99.5%) was added dropwise. After additional stirring for 30 min at 0 °C, the mixture was allowed to equilibrate to room temperature (RT) and stirred overnight. The reaction mixture was yellow, and a colorless precipitate was formed. Water (~50 mL) was added until the precipitate dissolved. The organic layer was removed and extracted twice with 30 mL of water and once with 30 mL of brine. The organic layer was separated and dried over Na_2SO_4, and finally, the solvent was removed. The product remained as an orange, highly viscous fluid (24.35 g, 2.4 mmol, 73%) and was used without further purification. ^{1}H NMR ($CDCl_3$): δ = 6.32 (br, 1H, −CO−NH−), 3.55 (m, 4H, −NH−CH_2−CH_2−Cl), 2.29 (t, ^3J = 7.4 Hz, 2H, −CH_2−CO−NH−), 2.21 (td, ^3J_t = 7.1 Hz, ^3J_d = 2.7 Hz, 2H, ≡C−CH_2−), 1.93 (t, ^3J = 2.7 Hz, 1H, HC≡C−), 1.82 ppm (ps-quin, ^3J = 7.1 Hz, 2H, ≡C−CH$_2$−CH_2−CH$_2$−). ^{13}C NMR ($CDCl_3$): δ = 172.5 (-CO−NH−), 83.3 (HC≡C−), 69.2 (HC≡C−), 43.8 (−CH$_2$−Cl), 41.1 (NH-CH$_2$−), 34.8 (−CH$_2$−CO−NH−), 24.0 (≡C−CH$_2$−), 17.7 ppm (≡C−CH$_2$−CH$_2$−CH$_2$−).

2-(Pent-4-ynyl)-2-oxazoline, PynOx (***1***)**.** 2-(Pent-4-ynyl)-2-oxazoline was prepared according to a procedure described by Binder and Gruber.[18] Hex-5-ynoic acid (2-chloroethyl)amide (20 g, 115 mmol, 1 equiv) was dissolved in 70 mL of MeOH and 6 g of freshly grinded NaOH (150 mmol, 1.3 equiv). The product was distilled as a colorless liquid at ~5 mbar at 85 °C to yield 4.29 g (31 mmol, 27%). ^{1}H NMR ($CDCl_3$): δ = 4.23 (t, ^3J = 9.3 Hz, 2H, ═N−CH$_2$−CH_2−O−), 3.82 (t, ^3J = 9.3 Hz, 2H, ═N−CH_2−CH$_2$−O−), 2.41 (t, ^3J = 7.5 Hz, 2 H, −NOC−CH_2−), 2.29 (td, ^3J_t = 7.0 Hz, ^3J_d = 2.7 Hz, 2H, ≡C−CH_2−), 1.97 (t, ^3J = 2.7 Hz, 2 H, HC≡C−), 1.87 ppm (ps-quin, ^3J = 7.2 Hz, 2H, ≡C−CH$_2$−CH_2−CH$_2$−). ^{13}C NMR ($CDCl_3$): δ = 167.8 (−NOC−CH$_2$−), 83.3 (HC≡C−), 68.9 (HC≡C−), 67.1 (═N−CH$_2$−CH$_2$−O−), 54.2 (═N−CH$_2$−CH$_2$−O−), 26.6 (NOC−CH$_2$−), 24.6 (≡C−CH$_2$−), 17.7 ppm (≡C−CH$_2$−CH$_2$−CH$_2$−). IR: 3301 (≡C−H str) (m), 2954 (C−H str) (w), 1666 (C═O str) (s), 1432 (δ(CH$_2$−CO) (m), 630 cm^{-1} (≡C−H wag) (s). $C_8H_{11}NO$ (M = 137.18 g/mol): Calcd C 70.04, H 8.08, N 10.21; Found C 69.91, H 8.29, N 10.30.

N-Methyl-2-(pent-4-ynyl)-2-oxazolinium Triflate, PynOxOTf, (***2***). PynOx (99 mg, 0.72 mmol, 1.0 equiv) and 122 mg (0.743 mmol, 1 equiv) of MeOTf were added to 1 mL of dry acetonitrile at approximately −35 °C. After stirring for 1 h, the mixture was allowed to warm to RT. The solvent was removed, and 213 mg (0.7 mmol, 98%) of a highly viscous colorless liquid was obtained. ^{1}H NMR ($CDCl_3$): δ = 4.91 (t, ^3J = 9.9 Hz, 2H, ═N−CH$_2$−CH_2−O−), 4.21 (t, ^3J = 9.9 Hz, 2H, ═N−CH_2−CH$_2$−O−), 3.32 (s, 3H, CH_3−N), 2.79 (t, ^3J = 7.4 Hz, 2H, −NOC−CH_2−), 2.28 (td, ^3J_t = 6 Hz, ^3J_d = 2.5 Hz, 2H, ≡C−CH_2−), 2.04 (t, ^3J = 2.5 Hz, 1H, HC≡C−), 1.90 ppm (ps-quin, ^3J = 6.6 Hz, 2H, ≡C−CH$_2$−CH_2−CH$_2$−). ^{13}C NMR ($CDCl_3$): δ = 177.8 (−NOC−CH$_2$−), 120.6 (q, $^1J_{C,F}$ = 318 Hz, CF$_3$−), 81.9 (HC≡C−), 71.2 (═N−CH$_2$−CH$_2$−O−), 70.5 (HC≡C−), 52.2 (═N−CH$_2$−CH$_2$−O−), 34.2 (CH$_3$−N), 25.1 (NOC−CH$_2$−), 22.6 (≡C−CH$_2$−), 17.7 (≡C−CH$_2$−CH$_2$−CH$_2$−). $C_{10}H_{14}F_3NOS$ (M = 301.28 g/mol): Calcd C 39.87, H 4.68, F 18.92, N 4.65, S 10.64; Found C 40.60, H 4.99, F 17.1, N 4.63

Polymerizations. The polymerizations and workup procedures were carried out following a general procedure described previously unless otherwise stated.[4,19−22]

Poly(4-pentynyl-2-oxazoline), PPynOx$_{23}$. A typical procedure for polymerizations was as follows (example to prepare PPynOx$_{23}$): MeOTf (27 mg, 0.165 mmol, 1.0 equiv) and 523 mg of **1** (3.81 mmol, 23 equiv) were added to 5 mL of ACN in a flame-dried flask under a dry nitrogen atmosphere and polymerized at 85 °C for 70 h. The mixture was cooled to 0 °C, and 90 mg of *N-tert*-butoxycarbonylpiperazine (*N*-Boc-piperazine) (0.48 mmol, 3 equiv) (98.0%+) dissolved in 350 μL of ACN was added. After stirring the reaction mixture for 4 h at room temperature, an excess of potassium carbonate (~50 mg) was added, and the mixture was stirred overnight. The solvent was removed at reduced pressure, and the residual was collected with 7 mL of MeOH and centrifuged. The centrifugate was collected, and the product was obtained by precipitation into 70 mL of cold diethyl ether as a colorless solid. The product was reprecipitated and freeze-dried (form *tert*-butyl alcohol) to yield 400 mg (0.119 μmol, 72%) of the polymer product. ^{1}H NMR (MeOD): δ = 3.48 (br, 100H, −N−CH_2−CH_2−N−), 3.04/2.90 (m, 3H, CH_3−N), 2.46 (br, 53H, ≡C−CH_2−), 2.21 (br, 55H, −N−CO−CH_2−), 1.76 (br, 54H, ≡C−CH$_2$−CH_2−CH$_2$−), 1.39 ppm (s, 5H, CH_3^{Boc}). IR: 3282 (≡C−H str) (w), 2913(C−H str) (w), 1627 (C═O str, amide I) (vs), 1423 (δ(CH$_2$−CO) (s), 633 cm^{-1} (≡C−H wag) (s). GPC: PDI = 1.09, M_n = 6160 g/mol.

2-(Hex-5-ynoic acid methylamide)ethyl-poly(2-methyl-2-oxazoline), PynOxPMeOx$_{20}$. **2** (23 mg, 76.3 μmol, 1.0 equiv) was dissolved in 5 mL of acetonitrile, and 134 mg of 2-methyl-2-oxazoline (134 mg, 1.57 mmol, 20 equiv) was added. The polymerization was carried out at 80 °C for 20 h and terminated using 58 mg (0.32 mmol, 4 equiv) of *N*-Boc-piperazine. The product was obtained as a colorless solid (155 mg, 76 μmol, 99%). ^{1}H NMR (D_2O): δ = 3.45 (br, 102H, −N−CH_2−CH_2−N−), 3.02/2.86 (m, 3H, CH_3−N), 2.46 (br, 8H, ≡C−CH_2−), 2.17 (br, 2H, −N−CO−CH_2−), 2.00 (br, 72H, −CO−CH_3), 1.65 (br, 2H, ≡C−CH$_2$−CH_2−CH$_2$−), 1.37 ppm (s, 10H, CH_3^{Boc}). GPC: PDI = 1.06, M_n = 3190 g/mol.

Poly[(2-methyl-2-oxazoline)-co-(4-pentynyl-2-oxazoline)], P(MeOx$_{45}$-co-PynOx$_5$)$_{stat}$. MeOTf (50 mg, 0.3 mmol, 1.0 equiv), 208 mg of **1** (1.52 mmol, 5 equiv), and 1.170 g of MeOx (13.7 mmol, 45 equiv) were added to 7.5 mL of ACN. Heating to 135 °C for 20 min was performed by a microwave system (150 W). The reaction mixture was cooled to RT, and 175 mg of *N*-Boc-piperazine (0.94 mmol, 3.2 equiv) was used for termination. The subsequent workup was performed as described in the literature. After freeze-drying, 1.345 g (0.285 mmol, 94%) of a colorless solid was obtained. ^{1}H NMR (D_2O): δ = 3.46 (br, 273H, −N−CH_2−CH_2−N−), 3.02/2.86 (m, 1H, CH_3−N), 2.46 (br, 15H, ≡C−CH_2−), 2.19 (br, 12H, −N−CO−CH_2−), 2.00 (br, 190H, −CO−CH_3), 1.69 (br, 11H, ≡C−CH$_2$−CH_2−CH$_2$−), 1.37 ppm (s, 9H, CH_3^{Boc}). IR: 3282 (≡C−H str) (w), 2938 (C−H str) (m), 1618

(C═O str, amide I) (vs), 1421 (δ(CH_2−CO) (vs),638 cm^{-1} (≡C−H wag) (w). GPC: PDI = 1.06, M_n = 7890 g/mol.

Poly[(2-methyl-2-oxazoline)-co-(4-pentynyl-2-oxazoline)], P(MeOx$_{35}$-co-PynOx$_{12}$)$_{stat}$. MeOTf (13 mg, 79 μmol, 1.0 equiv), 234 mg of MeOx (2.7 mmol, 35 equiv), and 135 mg of **1** (0.98 mmol, 12 equiv) were added to 5 mL of ACN and polymerized using 58 mg of *N*-Boc-piperazine (0.31 mmol, 4 equiv) for termination. The product was obtained as a colorless solid (315 mg, 65 μmol, 83%). ^{1}H NMR (D_2O): δ = 3.46 (br, 240H, −N−CH_2−CH_2−N−), 3.02/2.86 (m, 3H, CH_3−N), 2.41 (br, 35H, ≡C−CH_2−), 2.20 (br, 35H, −N−CO−CH_2−), 2.01 (br, 135H, −CO−CH_3), 1.70 (br, 33H, ≡C−CH_2−CH_2−CH_2−), 1.37 ppm-(s, 9H, CH_3^{Boc}). IR: 3261 (≡C−H str) (w), 2935 (C−H str) (w), 1626 (C═O str, amide I) (vs), 1419 (δ(CH_2−CO) (s),638 cm^{-1} (≡C−H wag) (w). GPC: PDI = 1.17, M_n = 6960 g/mol.

Poly[(2-ethyl-2-oxazoline)-co-(4-pentynyl-2-oxazoline)], P(EtOx$_{20}$-co-PynOx$_2$)$_{stat}$. MeOTf (0.1 g, 0.6 mmol, 1.0 equiv), 1.19 g of EtOx (12 mmol, 20 equiv), and 182 mg of PynOx (1.33 mmol, 2 equiv) were added to 15 mL of ACN and 300 μL of chlorobenzene. An aliquot of 5 mL was taken for parallel GC investigation (see below). The remaining mixture was polymerized using 0.34 g of *N*-Boc-piperazine (1.8 mmol, 3 equiv) for termination. The product was obtained as a colorless solid (935 mg, 0.39 mmol, 92%). ^{1}H NMR ($CDCl_3$): δ = 3.41 (br, 88H, −N−CH_2−CH_2−N−), 2.98/2.92 (m, 3H, CH_3−N), 2.37 (br, 58H, −CO−CH_2−CH_3/−N−CO−CH_2−/≡C−CH_2−), 1.99 (br, 3H, ≡C−*H*), 1.80 (br, 4H, ≡C−CH_2−CH_2−CH_2−), 1.40 (s, 9H, CH_3^{Boc}), 1.07 ppm (br, 57H, −CO−CH_2−CH_3). IR: 3247 (≡C−H str) (w), 2929/2938 (C−H str) (m), 1630 (C═O str, amide I) (vs), 1418 (δ(CH_2−CO) (vs), 638 cm^{-1} (≡C−H wag) (w). GPC: PDI = 1.06, M_n = 3990 g/mol.

Copolymerization Studies of MeOx and EtOx with PynOx by On-Line GC Monitoring. For MeOx/PynOx: Under strict inert conditions (glovebox), 305 mg of PynOx (2.2. mmol, 5 equiv) and 813 mg of MeOx and 80 mg of MeOTf (0.47 mmol, 1 equiv) were dissolved in 8 mL of dry ACN. Chlorobenzene (600 μL) was added as internal standard. The GC test tube was sealed, and the reaction mixture was transferred to a preheated (85 °C) agitator. Samples were automatically taken at t = 0 min and subsequently every 16 or 34 min (see Figure 2). Monomer conversions were calculated from the decay of the peak integrals originating from the respective monomers of the GC traces using the internal standard. The experiment with the EtOx/PynOx copolymer system was performed accordingly. For details (monomer ratio, temperature) refer to the main text and Figure 2.

Click Chemistry on Polymer Side Chains. The Huisgens 1,3-dipolar cycloaddition was carried out according to a slightly modified procedure described previously.[23]

P(MeOx$_{45}$-co-TriazTMS$_5$)$_{stat}$. P(MeOx$_{45}$-*co*-PynOx$_5$)$_{stat}$ (100 mg, 21 μmol, 1 equiv) and 20 mg of (trimethylsilyl)methyl azide, **3** (0.15 mmol, 7.3 equiv), were added to 1 mL of a 1/1 (v/v) mixture of water and *tert*-BuOH. After addition of 2.2 mg of sodium ascorbate (11 μmol, 0.5 equiv) and 1.6 mg of $CuSO_4$·$5H_2O$ (6.4 μmol, 0.3 equiv) the solution was stirred overnight at RT. The solvent was removed and the product collected with 2 mL of 1/1 (v/v) MeOH/$CHCl_3$. After precipitation in 20 mL of cold diethyl ether and freeze-drying from water, 54 mg (48%) of a colorless solid was obtained. ^{1}H NMR (D_2O): δ = 7.63 (br, 4H, H^{Triaz}), 3.96 (br, 12H, CH_2−TMS), 3.45 (br, 310H, −N−CH_2−CH_2−N−), 2.91/2.82 (m, 3H, CH_3−N), 2.64−2.50 (br, 16H, −CH_2−CH_2−C^{Triaz}), 2.23 (br, 17H, −N−CO−CH_2−), 2.00 (br, 240H, −CO−CH_3/−CH_2−CH_2−CH_2−), 1.37 (br, 9H, CH_3^{Boc}), −0.01 ppm (br, 45H, Si(CH_3)$_3$). IR: 2937 (C−H str) (m), 1616 (C═O str, amide I) (vs), 1423 (δ(CH_2−CO) (s), 730 cm^{-1} (r(CH_2) (w). GPC: PDI = 1.06, M_n = 8430 g/mol.

P(MeOx$_{35}$-co-TriazAc$_{12}$)$_{stat}$. P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$ (101 mg, 20.9 μmol, 1 equiv) and 33 mg of **4** (0.29 mmol, 13.7 equiv) were dissolved in 3 mL of water. Sodium ascorbate (6 mg, 30 μmol, 1.45 equiv) and 4.1 mg of $CuSO_4$·$5H_2O$ (16.4 μmol, 0.8 equiv) were added, and the mixture was stirred overnight at RT. ^{1}H NMR (D_2O): δ = 7.78 (br, 4H, $H^{1,4Triaz}$), 7.68 (br, 0.6H, $H^{1,2Triaz}$) 5.26 (br, 9H, −N−CH_2−CO−), 4.89 (br, 0.9H, −$N^{1,2Triaz}$−CH_2−CO−), 3.71 (br, 15H, CH_3−O−), 3.43 (br, 277H, −N−CH_2−CH_2−N−), 2.92/2.82 (m, 3H, CH_3−N), 2.68 (br, 9H, −CH_2−CH_2−C^{Triaz}), 2.27 (br, 14H, N−CO−CH_2−), 2.01 (br, 206H, −CO−CH_3/-CH_2−CH_2−CH_2−), 1.37 (br, 9H, CH_3^{Boc}). IR: 2916 (C−H str) (m), 1739 (C═O str, ester) (m), 1630 (C═O str, amide I) (vs), 1423 (δ(CH_2−CO) (s), 721 cm^{-1} (r(CH_2) (w). GPC: PDI = 1.18, M_n = 8590 g/mol.

P(MeOx$_{45}$-co-TriazAc$_5$)$_{stat}$. ^{1}H NMR (D_2O): δ = 7.78 (br, 13H, H^{Triaz}), 5.27 (br, 27H, N−CH_2−CO−), 3.71 (br, 41H, CH_3−O−), 3.43 (br, 240H, −N−CH_2−CH_2−N−), 2.92/2.82 (m, 3H, CH_3−N), 2.67 (br, 26H, −CH_2−CH_2−C^{Triaz}), 2.27 (br, 34H, N−CO−CH_2−), 2.01 (br, 161H, −CO−CH_3/−CH_2−CH_2−CH_2−), 1.37 (br, 7H, CH_3^{Boc}). IR: 2915 (C−H str) (s), 1737 (C═O str, ester) (m), 1630 (C═O str, amide I) (vs), 1423 (δ(CH_2−CO) (vs), 720 cm^{-1} (r(CH_2) (w). GPC: PDI = 1.07, M_n = 7740 g/mol.

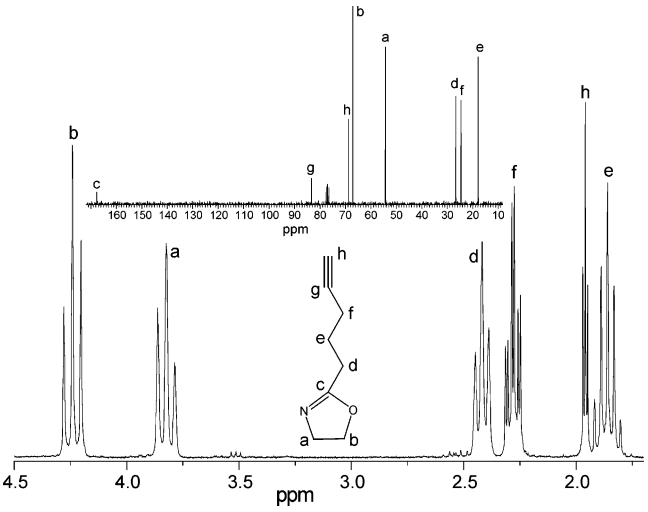
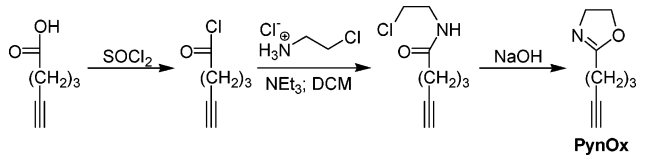


Figure 1. ^{1}H and ^{13}C (inset) NMR spectra of PynOx.

Scheme 1. Synthesis of 2-(Pent-4-ynyl)-2-oxazoline (PynOx)



Results and Discussion

Poly(2-oxazoline)s bearing alkyne or azide functions in the side chain have been described.[18] However, the described compounds were not used for a Huisgens 1,3-dipolar cycloaddition so far. Moreover, both monomers are hydrophobic and thus are not suitable for the synthesis of hydrophilic polymers of our interest. Since aliphatic azides, being pseudo-halogens, are likely to interfere with the cationic ring-opening polymerization of 2-oxazolines, we introduced a terminal alkyne into the 2-oxazoline side chain. Hence, the azide will have to be used in excess in the subsequent Huisgen cycloaddition, although this contrasts with the reported advantage using an excess alkyne in order to increase the reaction yield.[24]

Monomer Synthesis. The synthesis of the new monomer, 2-(pent-4-ynyl)-2-oxazoline (PynOx), is displayed in Scheme 1. Starting from the commercially available 5-hexynoic acid, the formation of hex-5-ynoyl chloride was straightforward and confirmed by ^{1}H NMR and IR spectroscopy. After product isolation, only minor traces of remaining thionyl chloride could be detected by IR spectroscopy ($\nu^{S=O}$ at 1220 cm^{-1}). The acid chloride reacted with 2-chloroethylamine in the presence of triethylamine to give hex-5-ynoic acid (2-chloroethyl)amide in good yields. The ring closure was first attempted in the presence

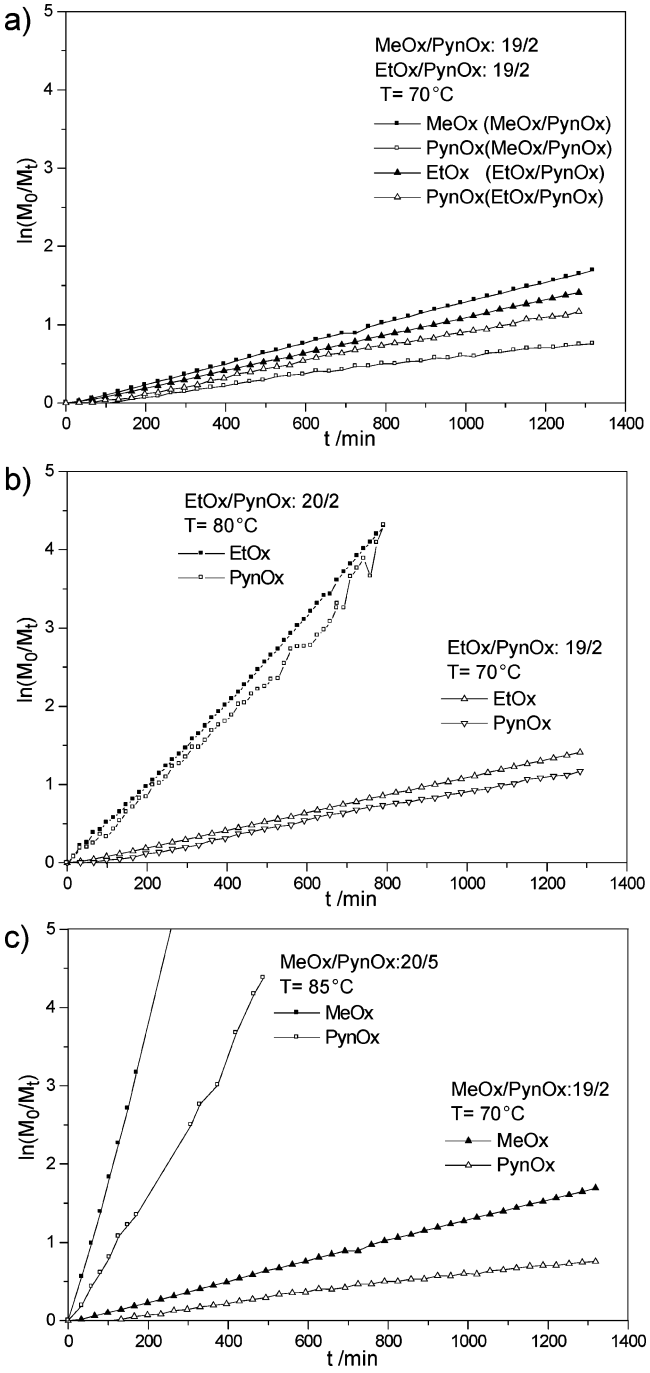


Figure 2. First-order kinetic plots of the copolymerization studies of 2-methyl-2-oxazoline (MeOx**)** or 2-ethyl-2-oxazoline (EtOx**)** with 2-(pent-4-ynyl)-2-oxazoline **(**PynOx**)** as monitored by on-line GC sampling. (a) Comparison of the MeOx/PynOx and EtOx/PynOx copolymerization behavior at $T = 70$ °C and the same monomer ratio (19/2). (b) EtOx/PynOx copolymerization at 70 and 80 °C and EtOx/PynOx ratio of 19/2 and 20/2, respectively. (c) MeOx/PynOx copolymerization at 70 and 85 °C (MeOx/PynOx: 19/2 and 20/5). Please note that the linearity in all plots confirms the highly living character of the copolymerizations.

of anhydrous soda after Zarka et al.,[19] but no product was obtained. The reaction was successful using sodium hydroxide as a base as reported by Binder.[18] The same reaction was not successful with the bromide analogue.

Figure 1 shows the NMR spectra of PynOx along with the peak assignments. All signals can be assigned, and the integrals as well as the coupling constants are in excellent agreement with the expected values. The narrow triplet, **h**, at 1.97 ppm originates from the terminal alkyne proton. In the IR spectrum

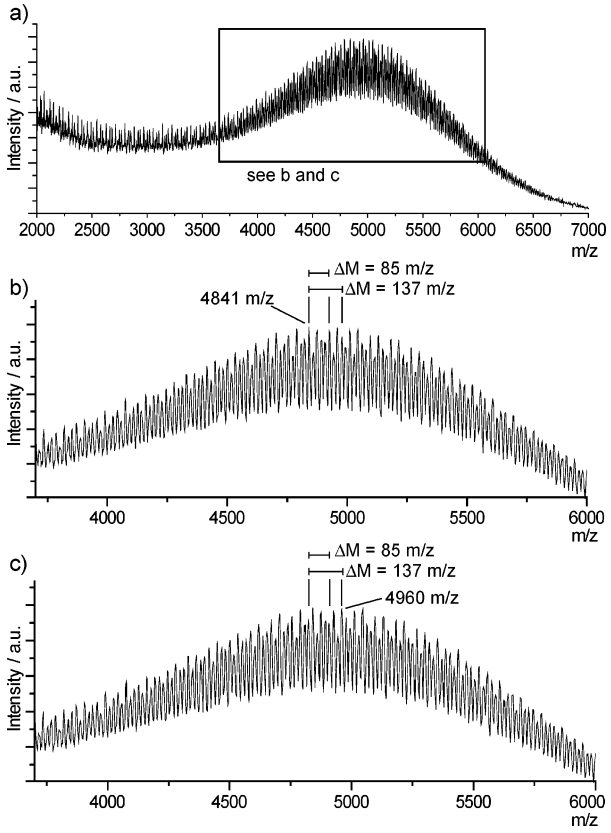


Figure 3. (a) MALDI TOF spectrum of $P(MeOx_{45}\text{-}co\text{-}PynOx_5)_{stat}$. Details of the mass spectrum are displayed in (b) and (c) along with the possible assignments as discussed in the main text.

of PynOx (not shown) the characteristic $\nu^{C=N}$ mode at 1666 cm^{-1} and the $\nu^{\equiv C-H}$ at 3301 and δ_{wag} at 638 cm^{-1} were observed.

Polymerization. A homopolymer of PynOx ($PPynOx_{23}$) and random copolymers ($P(MeOx_{45}\text{-}co\text{-}PynOx_5)_{stat}$, $P(MeOx_{35}\text{-}co\text{-}PynOx_{12})_{stat}$, $P(EtOx_{20}\text{-}co\text{-}PynOx_2)_{stat}$) of PynOx with 2-methyl- or 2-ethyl-2-oxazoline (MeOx**,** EtOx) could be directly obtained with methyl triflate (MeOTf) as the initiator. Additionally, the initiator salt system, PynOx-OTf, was prepared from MeOTf and PynOx and was found to be an excellent initiator for the cationic polymerization. This initiator salt enables a defined positioning of the alkyne function at the polymer chain terminus ($PynOxPMeOx_{20}$) when used in homo- or copolymerizations. In contrast to our previous accounts on functionalized poly(2-oxazoline)s, *N*-Boc-piperazine was used as the terminating reagent. The protected secondary amino group allows later polymer analogue conversions of the pendant side functions such as aldehyde or primary amines and, after deprotection, a final functionalization of the distal chain end. Moreover, the terminal group was helpful for end-group analysis on the basis of the ^1H NMR spectroscopy data.

The polymerization reactions are summarized in Scheme 2. $P(MeOx_{45}\text{-}co\text{-}PynOx_5)_{stat}$ was synthesized with the assistance of microwaves.[25] After only 20 min reaction time at 135 °C (150 W), the final polymer product was obtained in excellent yield (94%). GPC analysis confirmed a monomodal and narrow molar mass distribution of $M_w/M_n = 1.06$.

All homo- and copolymers were characterized by NMR and FT IR spectroscopy as well as MALDI TOF spectrometry and GPC to determine their degree of polymerization and composition. Additionally, the copolymerization behavior of PynOx with MeOx and EtOx were investigated by in situ gas chromatography measurements in order to clarify the randomness of the

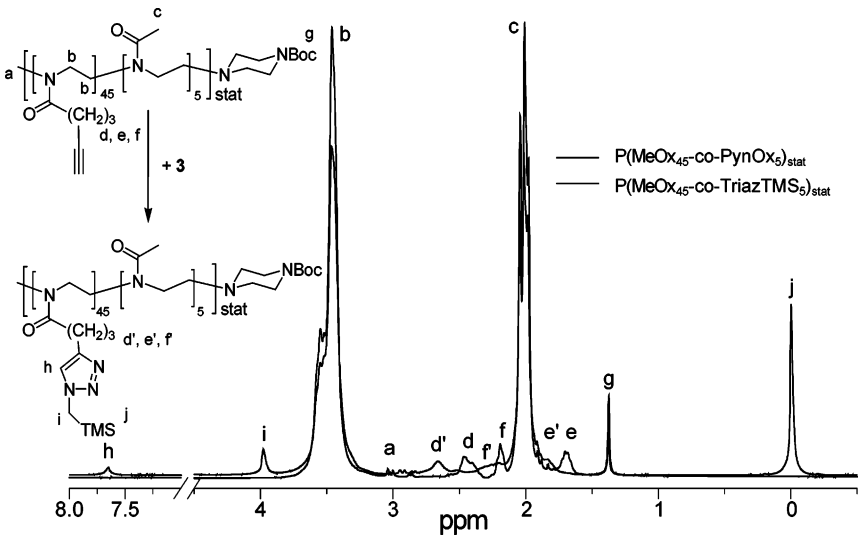
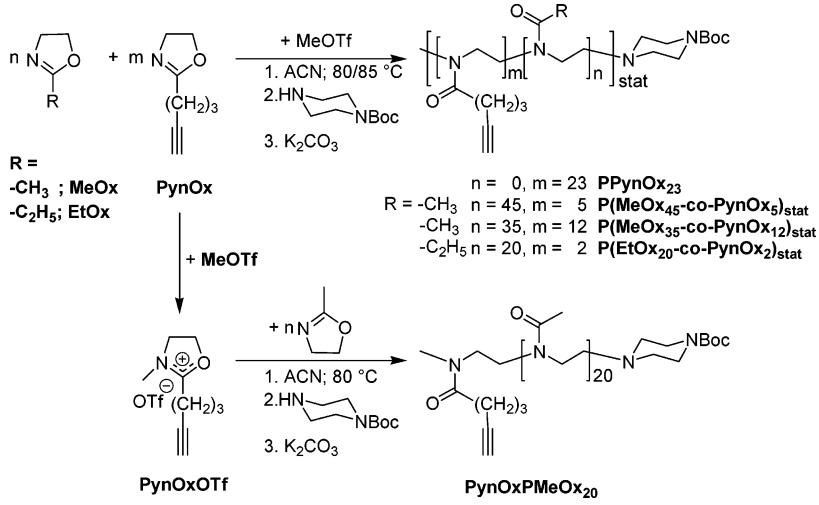


Figure 4. Comparison of the ^{1}H NMR spectra of P(MeOx$_{45}$-*co*-PynOx$_5$)$_{stat}$ and of P(MeOx$_{45}$-*co*-TriazTMS$_5$)$_{stat}$ after the polymer analogue cycloaddition reaction with **3** (recorded in D_2O).

Scheme 2. Homo- and Copolymerization of 2-Methyl-2-oxazoline (MeOx), 2-Ethyl-2-oxazoline (EtOx), and 2-(Pent-4-ynyl)-2-oxazoline (PynOx) at Given Monomer Ratios and Temperatures (See Experimental Part for Details)



copolymerization. Especially for the projected application of the functionalized polymers as therapeutics, it should be known if the chemical function—and later a cell membrane binding site—is distributed randomly or gradual along the polymer chain. First, a polymer analogue coupling of larger ligands might be obstructed by an accumulation of reaction sites. Second, a gradual distribution of peptidic cell binding motifs would result in a different cell attachment behavior of the polymer due to the accumulation of recognition sites and the steric situation. Both random and gradient copolymers are interesting for the application as future polymer therapeutics. Figure 2 displays the results of the copolymerization study. In these studies we found that in copolymerization reactions the consumption of the new monomer, PynOx, is lower than that of MeOx but similar to the EtOx consumption. However, the determined differences in the copolymerization behavior are too small to result in the formation of AB block copolymers. Copolymerization of PynOx with MeOx should lead to a copolymer with a higher PynOx content toward the end (gradient copolymer) (see Figure 2a,c). Figure 2a compares the monomer consumption of PynOx with MeOx or EtOx. It can be seen that the difference of the consumption of monomers is pronounced in the case of MeOx/PynOx polymerization, leading to a gradient copolymer (top and lower plot in Figure 2a). The PynOx/EtOx copolymerization will result in a random distribution of both monomer units along the polymer chain as the monomer consumption rate is very similar at both 70 and 80 °C polymerization temperature (see Figure 2a,b). These findings can be explained by the similarity of the EtOx and PynOx monomer structure, i.e., steric demands of the 2-substitution, and were also previously discussed in the context of other 2-oxazoline copolymerization studies.[4,5,26] An increase of the copolymerization temperature has no significant effect upon the relative consumption in the EtOx/PynOx monomers, while the differences in the MeOx/PynOx system are augmented (Figure 2b,c). Hence, the steepness of the copolymer gradient can be directly controlled by the polymerization temperature.

For all copolymers, the ratio of the integrals in the ^{1}H NMR (e.g., Figure 4 for P(MeOx$_{45}$-*co*-PynOx$_5$)$_{stat}$ and Figure 5 for P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$) that can be attributed to the different monomers fits very well with the values expected from the initial feeds ([MeOx or EtOx]$_0$/[PynOx]$_0$). The determination of the degree of polymerization using the integrals of either the terminal methyl or the Boc group in relation to the monomer unit signals (end group analysis) results in very high degrees of polymerization of $n + m$ up to 150. In light of the initial ratio of $[M]_0/[I]_0$ feeds and polydispersity indices obtained by GPC as low as 1.06, this seems unrealistic. Typically the

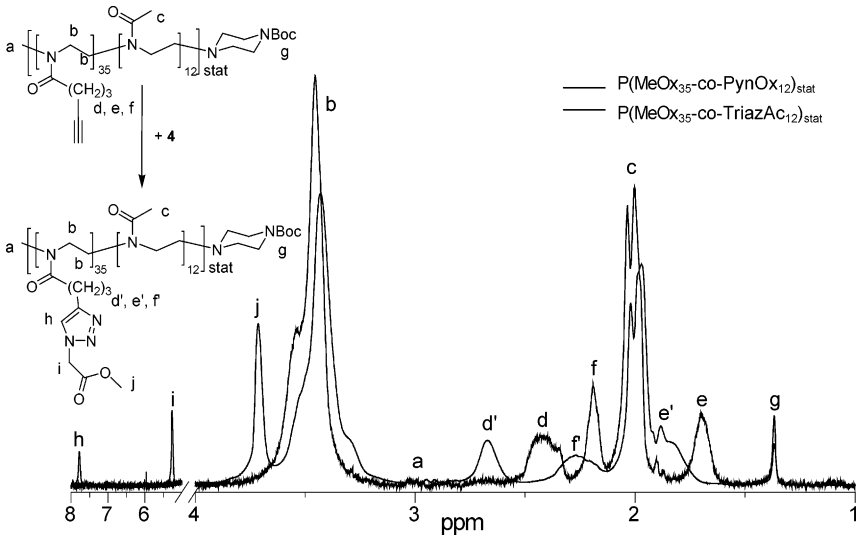


Figure 5. Comparison of the ^{1}H NMR spectra of P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$ and P(MeOx$_{35}$-*co*-TriazAc$_{12}$)$_{stat}$.

Table 1. Selected Analytical Data of Synthesized Polymers before and after Huisgen 1,3-Dipolar Cycloaddition of Azides with the Polymer Bond Alkynes

polymer	$M_{n,calcd}$[a] [$\times 10^{-3}$ g/mol]	$M_{n,NMR}$[b] [$\times 10^{-3}$ g/mol]	$M_{n,GPC}$[c] [$\times 10^{-3}$ g/mol]	PDI[d]	yield [%]
PPynOx$_{23}$	3.49	3.63	6.16	1.09	70
PynOxPMeOx$_{20}$	2.04	2.22	3.19	1.06	99
P(MeOx$_{45}$-*co*-PynOx$_5$)$_{stat}$	4.71	5.82	7.89	1.06	94
P(EtOx$_{20}$-*co*-PynOx$_2$)$_{stat}$	2.46	2.45	4.00	1.04	92
P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$	4.83	6.65	6.96	1.17	83
P(MeOx$_{45}$-*co*-TriazTMS$_5$)$_{stat}$	5.36	7.83	8.43	1.06	47
P(MeOx$_{45}$-*co*-TriazAc$_5$)$_{stat}$	5.29	6.71	7.74	1.07	85
P(MeOx$_{35}$-*co*-TriazAc$_{12}$)$_{stat}$	6.20	7.49	8.59	1.18	77

[a] M_n as calculated from $[M]_0/[I]_0$ and for 100% conversion for modified polymers. [b] As calculated by end-group analysis from ^{1}H NMR spectra, comparing the integrals of the end groups, backbone, and side-chain intensities. [c] As determined by GPC analysis. [d] PDI = M_w/M_n calculated from GPC analysis.

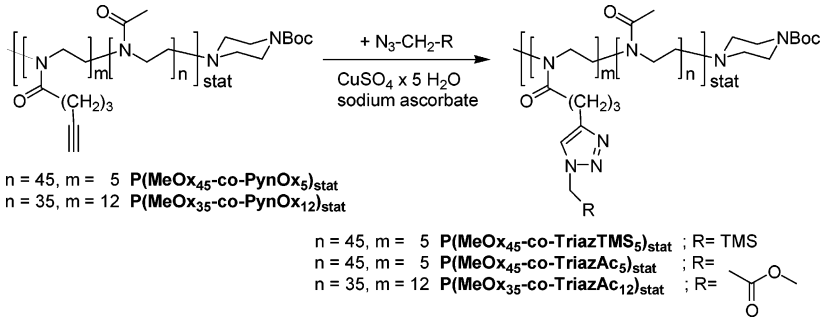
polydispersity indices rise significantly at high degrees of polymerizations in the case of poly(2-oxazoline)s. Moreover, significantly different results were obtained using the methyl or Boc group signals in relation to signals originating from the polymer side chain or methylene backbone. This might be explained by the relative small proton signals of the termini in relation to the strong signals from the monomer units as well as by the different relaxation times for end and side-chain group protons during the measurement. Hence, to verify that the obtained degree of of polymerization is in agreement of the initial feeds ($[M]_0/[I]_0$), additional characterization by MALDI TOF mass spectrometry was performed.

The analysis of the MALDI TOF spectrum of e.g. P(MeOx$_{45}$-*co*-PynOx$_5$)$_{stat}$ gives a different picture. Here, the maximum of the measured mass distribution is located at around $m/z = 4900$ (Figure 3a), which agrees well with the expected value for the $[MeOx]_0/[PynOx]_0/[I]_0$ ratio, and the relative as well as the overall degree of polymerization is as expected. Since all mass signals can be correctly assigned to different copolymer compositions (MeOx to PynOx) because 5 M (MeOx) equals 8 M (PynOx), the two most intense signals can be assigned to either [P(MeOx$_{43}$-*co*-PynOx$_8$)$_{stat}$ + H]$^+$ [P(MeOx$_{49}$-*co*-PynOx$_4$)$_{stat}$ + K]$^+$ or [P(MeOx$_{41}$-*co*-PynOx$_9$)$_{stat}$ + K]$^+$ (with m/z^{calc} = 4958.1, 4957.0, or 4962.0; m/z^{exp} = 4960) (Figure 3b) and [P(MeOx$_{40}$-*co*-PynOx$_9$)$_{stat}$ + H]$^+$, [P(MeOx$_{46}$-*co*-PynOx$_5$)$_{stat}$ + K]$^+$, or [P(MeOx$_{38}$-*co*-PynOx$_{10}$)$_{stat}$ + K]$^+$ (m/z^{calc} = 4839.0, 4840.0, or 4845.0; m/z^{exp} = 4841) (Figure 3c), respectively. The assignment for all other signals is accordingly and fits nicely to this calculation. The mass differences between all signals are found to be either $m/z = 85$ or 137 (Figure 3b,c) and thus equals the monomer unit masses for MeOx (M = 85.11) or PynOx (M = 137.18). As in this example, other selected MALDI TOF experiments corroborated the polymer compositions as adjusted by the initial monomer to initiator feed. It can be concluded that the homo- and copolymerization of PynOx do not result in unexpected side reactions (termination, chain transfer) but follows a defined living cationic polymerization route.

1,3-Dipolar Cycloaddition onto Polymer Bond Alkyne Side Functions. For selected copolymers, the copper-mediated azide−alkyne coupling reaction was performed according to a recently described method,[23] in water or aqueous mixtures with ~0.1 equiv of sodium ascorbate and 0.06 equiv of copper(II) sulfate (Scheme 3). As outlined in the Introduction, we used a slight excess of the azides **3** or **4** (1.1−1.6 equiv azide per alkyne), since in our case the alkyne function is polymer bond.[24] The reaction mixture stays colorless upon addition of the copper salt but turns turquoise after ~6 h. This might be explained by an immediate coordination of the reduced copper ions by the polymer bound alkyne functions and later release upon conversion of the alkynes and oxidation by air. We found this an convenient marker of the reaction progress. After completion of the reaction, the mixture was dissolved in a NH_4OH/citrate buffer and dialyzed, first against this buffer solution and subsequently against water.[23] However, the solution retained its color, indicating that the copper could not be completely removed although after freeze-drying all samples were obtained as colorless powders.

The conversion of the alkyne side chains to the triazole was confirmed by NMR spectroscopy (Figures 4 and 5). The signals (d, e, f) originating from the three methylene groups are shifted quantitatively upon the triazole formation, as shown in Figure 4 for the reaction of P(MeOx$_{45}$-*co*-PynOx$_5$)$_{stat}$ with **3** to P(MeOx$_{45}$-*co*-TriazTMS$_5$)$_{stat}$ and in Figure 5 for the conversion

Scheme 3. Copper-Catalyzed Coupling of Azides to Alkyne Containing Side Chains of Poly(2-methyloxazoline)s



of P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$ with **4** to P(MeOx$_{35}$-*co*-TriazAc$_{12}$)$_{stat}$. The signal, e, at 1.70 ppm is shifted to e′, very close into the signal of the acyl group (c). The strong shift of f from 2.43 to 2.67 ppm (f′) of the methylene group adjacent to the new formed triazole ring can be observed. This signal shift is complete with no observable remaining signal at 2.43 ppm and indicates a quantitative conversion. The proton signal (h) of the formed triazole ring is located at 7.78 ppm. The signal integral is approximately half of the integrals of the two methylene groups of the spacer. Comparing this signal with the one of the methyl ester and the new signals of the TMS group (j) gives, as expected, the ratio of 1/3 and 1/9. Also, the polymer analyses by ^{13}C NMR spectroscopy corroborate the NMR data of a quantitative polymer analogue cycloaddition reaction. For example, the alkynyl signal at 85 ppm of P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$ disappears while a new, broad signal is found at 125 ppm (not shown).

Further confirmation can be taken from IR spectroscopy data. In Figure 6 the IR spectra of P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$ and P(MeOx$_{35}$-*co*-TriazAc$_{12}$)$_{stat}$ are compared. Under the broad water signal (the samples are highly hygroscopic), a strong shoulder at ~3261 cm^{-1} assignable to the ν(C$_{sp}$−H) mode of P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$ is observed. The weak band at 638 cm^{-1} originates from the δ(C$_{sp}$−H) wagging mode. After the 1,3-cycloaddition with **4** to the triazole, both bands disappeared completely, and the new ν(C═O) mode around 1751 cm^{-1} confirms the successful reaction to P(MeOx$_{35}$-*co*-TriazAc$_{12}$)$_{stat}$.

The polymer analytical data that can be obtained by GPC is limited since the functionalized poly(2-oxazoline)s interact quite differently with the chromatographic stationary phase.[4,5,21,22] However, the symmetry of the GPC curves allows a reliable determination of the polydispersity index (PDI). For all samples the molar mass distribution is narrow and do not vary significantly upon the polymer analogue conversions. HPLC elugrams of the polymer samples (not shown) additionally confirmed a quantitative polymer analogue reaction by a quantitative shift of the P(MeOx$_{45}$-*co*-PynOx$_5$)$_{stat}$ signal to the signal position of the elugram of P(MeOx$_{45}$-*co*-TriazAc$_5$)$_{stat}$. In all HPLC traces only one peak was found.

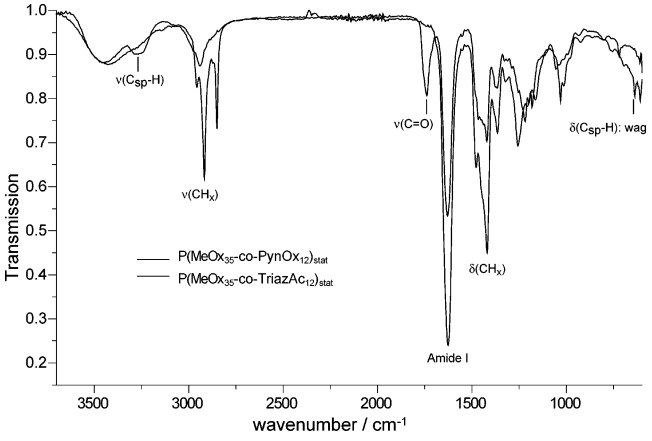


Figure 6. IR spectra of P(MeOx$_{35}$-*co*-PynOx$_{12}$)$_{stat}$ and P(MeOx$_{35}$-*co*-TriazAc$_{12}$)$_{stat}$.

Conclusion

With the synthesis of an alkyne bearing 2-oxazoline (PynOx) and its subsequent living copolymerization with 2-methyl- and 2-ethyl-2-oxazoline, well-defined hydrophilic polymers with pendant alkyne groups can be obtained. Kinetic studies of the copolymerization behavior of PynOx with the hydrophipilc MeOx and EtOx monomers revealed that either random or gradient water-soluble copolymers can be synthesized. MALDI TOF mass spectrometry showed that the molar mass is in excellent accordance with the monomer-to-initiator ratio. Quantitative side-chain conversion of the alkyne moieties to triazoles was confirmed by various analytical techniques. In ongoing studies we intend to utilize this new protection chemistry free coupling method to attach peptidic cell recognition sites for targeted drug delivery in cancer therapy.

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