**AROMA – Anionic Ring-Opening Monomer Addition of Allyl Glycidyl Ether to Methoxy Poly(ethylene glycol) for the Synthesis of Sequence-controlled Polymers**

Sven Schneider1, Benedikt L. Schwalm1, Patrick Theato1,2\*

1Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces 3 (IBG-3), Engesserstr. 18, 76131 Karlsruhe, Germany

2Karlsruhe Institute of Technology (KIT), Institute for Chemical Technology and Polymer Chemistry (ITCP), Engesserstr. 18, 76131 Karlsruhe, Germany

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# Materials & Methods

## Chemicals

Acetic acid (100 %,Carl Roth), Allyl glycidyl ether (AGE, 99+ %, Acros Organics,), 2,2’ Azobis(2-methylpropionitrile) (AIBN, 98 %, Sigma-Aldrich), Benzylthiol (99%, Alfa Aesar), Chloroform-*d*1 (99.8 %, Eurisotop), Diethyl ether (Et2O, 100 %, VWR Chemicals), 1-Dodecanthiol (≥98%, Sigma-Aldrich), Methyl-3-mercaptopropionat (98 % Alfa Aesar), 1-*tert*-Butyl-4,4,4-tris(dimethylamino)-2,2-bis[tris(dimethylamino)-phosphoranylidenamino]-2λ5,4λ5-catenadi(phosphazene) (P4-*t*-Bu, 0.8 M in hexane, Sigma-Aldrich), Tetrahydrofuran (THF, 99.5 % extra dry, Acros Organics) were used as received. If mentioned, methoxy poly(ethylene glycol) 1900 (mPEG-1900, Alfa Aesar) was dried at 40 °C at reduced pressure.

## Analytic Devices

### Nuclear Magnetic Resonance (NMR) spectroscopy

1H and 13C NMR spectra were recorded on a *Bruker Ascend 400* NMR spectrometer (400 or 101 MHz) using chloroform-*d*1 as deuterated solvent. The calculation of the mPEG backbone signals in the 1H NMR spectra are based on the theoretical value of mPEG with a *M*n of 1900 g mol­-1.

### Size Exclusion Chromatography (SEC)

Size Exclusion Chromatography was carried out in THF and an *Agilent Technologies 1260 Infinity II* system with a 5 µm *PSS SDV Lux 1000 Å* column (8 x 300 mm) and a 5 µm *PSS SDV Lux 100.000 Å* column (8 x 300 mm) was used. The operation temperature was set to 40 °C with a flow rate of 1 mL min-1. The system was calibrated *via* polystyrene standards ranging from 370 to 2.52 × 106 g mol-1.

### Attenuated Total Reflection Fourier-Transform Infrared (ATR FT-IR) Spectroscopy

ATR FT-IR measurements were recorded on a *Bruker Alpha II* equipped with an ATR unit. The spectra were measured at a range from 4000 cm-1 to 400 cm-1.

### Differential Scanning Calorimetry (DSC)

All DSC thermograms were recorded on a *Netzsch DSC 214* ranging from -50 °C to 100 °C with a scanning rate of 10 K min-1. For all experiments the second heat cycle was used.

# Synthesis

## Kinetic Studies of the Polymerization of AGE



0.200 g of mPEG-1900 (0.105 mmol, 1.00 eq.) and a stirring bar were given into a 5 mL round-bottom flask and the atmosphere was changed to argon. Afterwards, 2.00 mL of dry THF and 0.248 mL of AGE (0.240 g, 2.11 mmol, 20.0 eq.) were added to the flask, followed by 0.132 mL of a 0.8 M P4-*t*-Bu (0.105 mmol of pure P4-*t*-Bu, 1.00 eq.). Over the course of time, the reaction turned from a yellow to an amber colored mixture. Samples for 1H NMR measurements were taken directly from the flask, quenched with a small amount of acetic acid and taken up with chloroform-*d1.* After the addition of the acid, the mixture turned to a pale yellow.



*Figure 1: Size exclusion chromatogram of the polymerization (P1) of AGE using mPEG-1900 as initiator.*



*Figure 2: 1H NMR spectrum of poly(AGE). Solvent: CDCl3.*

1H NMR (400 MHz, CDCl3) δ in ppm: 5.96 – 5.80 (m, 20H, A), 5.30 – 5.09 (m, 40H, B), 4.03 – 3.95 (m, 40H, C), 3.83 – 3.42 (m, 272H, D), 3.37 (s, 3H, E).

**Impurities**:

* 2.65 ppm: P4-*t*-Bu
* 1.88 ppm: H2O
* 1.27 ppm: P4-*t*-Bu/*n*-Hexane

## 1. Chain Extension of mPEG-1900 with AGE (1. AROMA reaction)



0.200 g of mPEG-1900 (0.105 mmol, 1.00 eq.) and a stirring bar were given into a 5 mL round-bottom flask and the atmosphere was changed to argon. Afterwards, 2.00 mL of dry THF and 0.0154 mL of AGE (0.0150 g, 0.132 mmol, 1.25 eq.) were added to the flask, followed by 0.132 mL of a 0.8 M P4-*t*-Bu (0.105 mmol of pure P4-*t*-Bu, 1.00 eq.). After 5 hours the reaction was stopped by addition of acetic acid. The solvent was removed under reduced pressure and the residue was taken up in THF. After precipitation in cold Et2O (three times), the polymer was dried at 40 °C under reduced pressure over night. A white solid was obtained (yield: 89 %).



*Figure 3: Size exclusion chromatogram after the first chain extension (CE-1) of mPEG-1900 with AGE.*



*Figure 4: 1H NMR spectrum of the AGE chain extended mPEG-1900. The new signals between 5.00 – 6.00 ppm confirm a successful chain extension. Solvent: CDCl3.*

1H NMR (400 MHz, CDCl3) δ in ppm: 5.98 – 5.81 (m, 1H, A), 5.32 – 5.10 (m, 2H, B), 4.09 – 3.90 (m, 2H, C), 3.88 – 3.42 (m, 178H, D), 3.36 (s, 3H, E).

**Impurities:**

* H2O: 2.08 ppm
* P4-*t*-Bu, *n*-Hexane: 1.26 ppm



*Figure 5: 13C NMR spectrum of the AGE chain extended mPEG-1900. Solvent: CDCl3.*

13C NMR (101 MHz, CDCl3) δ in ppm: 134.69 (A), 117.29 (B), 77.36 (C), 72.47 (E), 72.06 (F), 70.69 (G), 59.16 (I).

**Impurities:**

* mPEG: 72.77 (D), 61.85 (H)



*Figure 6: DSC thermogram (heating curve; 2. cycle) of AGE chain extended mPEG-1900 with a visible Tm at 54.2 °C.*



*Figure 7: ATR FT-IR spectrum of mPEG-1900 (black) and CE-1 (blue). After the chain extension a new signal at 1645 cm-1 appears, which could be assigned to the C=C double bond of the AGE.*

**IR Assignment (based on (Vrandečić, Erceg, Jakić, & Klarić, 2010), (Carrillo-Castillo, Castro-Carmona, Luna-Velasco, & Zaragoza-Contreras, 2020) and (Gorin, Skripova, Charskaya, & Menligaziev, 1971)):**

* C–O, C–C stretching, CH2 rocking at 842 cm−1
* CH2 rocking, CH2 twisting at 947 cm−1
* C–O, C–C stretching, CH2 rocking at 1059 cm−1
* C–O, C–C stretching at 1100 cm−1
* C–O stretching, CH2 rocking at 1145 cm−1
* CH2 twisting at 1240 and 1279 cm−1
* CH2 wagging at 1340 cm−1
* CH2 scissoring at 1467 cm−1
* C=C double bond’s stretching vibration at 1645 cm­-1­
* Symmetric and asymmetric stretching vibration bands of the methylene group C–H bonds at 2875 and 2940 cm-1

## Thiol-ene Reaction of mPEG-b-oligo(AGE) with 1-Dodecanthiol



150 mg of mPEG-*b*-oligo(AGE) (0.0745 mmol, 1.00 eq.) was given into a 5 mL round-bottom flask, followed by 6.11 mg of AIBN (0.0372 mmol, 0.500 eq.) and 2 mL of dry THF. Afterwards, 70.9 µL of 1-dodecanthiol (60.3 mg, 29.8 mmol, 4.00 eq.) was added and the atmosphere was changed to argon. The flask was placed into a preheated oil bath (60 °C) and stirred for 24 hours. The next day the system was quenched by contact to air and cooling *via* ice bath. The solvent was removed under reduced pressure and the residue was taken up in THF. After the precipitation in cold Et2O (three times), the product was dried under reduced pressure at 40 °C overnight. A white solid was obtained (yield: 66.9 %).



*Figure 8: Size exclusion chromatogram after the first modification of chain extended mPEG-1900 (PPM-1, violet). After the modification, a shift to higher molar masses is visible in comparison to the previous polymer (CE-1; blue).*



*Figure 9: 1H NMR spectrum of chain extended mPEG-1900 after the thiol-ene reaction with 1-dodecanthiol. After the reaction the signals of the double bond between 5.00 – 6.00 ppm disappeared and the thiol signals (e.g. around 0.8 ppm) appeared, confirming a successful modification reaction. Solvent: CDCl3.*

1H NMR (400 MHz, CDCl3) δ in ppm: 3.86 – 3.41 (m, 179H, A), 3.37 (s, 3H, B), 2.64 – 2.43 (m, 4H, C), 1.91 – 1.78 (m, 2H, D), 1.63 – 1.49 (m, 2H, E), 1.44 – 1.13 (m, 18H, F), 0.86 (t, 3H, G).

**Impurities:**

* H2O: 2.03 ppm



*Figure 10: DSC thermogram (heating curve; 2. cycle) of 1-dodecanthiol modified chain extended mPEG-1900 with a visible Tm at 55.2 °C.*



*Figure 11: ATR FT-IR spectrum of once chain extended mPEG-1900 (CE-1; blue) and the 1-dedcanthiol modified polymer (PPM-1; violet). After the modification the signal at 1645 cm-1 disappeared, which confirms a successful modification.*

**IR Assignment (based on (Vrandečić et al., 2010), (Carrillo-Castillo et al., 2020)):**

* C–O, C–C stretching, CH2 rocking at 842 cm−1
* CH2 rocking, CH2 twisting at 947 cm−1
* C–O, C–C stretching, CH2 rocking at 1059 cm−1
* C–O, C–C stretching at 1100 cm−1
* C–O stretching, CH2 rocking at 1145 cm−1
* CH2 twisting at 1240 and 1279 cm−1
* CH2 wagging at 1340 cm−1
* CH2 scissoring at 1467 cm−1
* Symmetric and asymmetric stretching vibration bands of the methylene group C–H bonds at 2875 and 2940 cm-1

## 2. Chain Extension of mPEG-1900 with AGE (2. AROMA reaction)



57.0 mg of 1-dodecanthiol modified mPEG-1900-*b*-oligo(AGE) (0.0257 mmol, 1.00 eq.) and a stirring bar were given into a 5 mL round-bottom flask and the atmosphere was changed to argon. Afterwards, 2.00 mL of dry THF and 3.78 µL of AGE (3.67 mg, 0.0321 mmol, 1.25 eq.) were added to the flask, followed by 32.2 µL of a 0.8 M P4-*t*-Bu (0.0258 mmol of pure P4-*t*-Bu, 1.00 eq.). The flask was placed in a preheated oil bath (50 °C). After 5.5 hours the reaction was stopped by addition of acetic acid. The solvent was removed under reduced pressure and the residue was taken up in THF. After precipitation in cold Et2O (three times), the polymer was dried at 40 °C under reduced pressure over night. A white solid was obtained (yield: 77 %).



*Figure 12: Size exclusion chromatogram after the second chain extension (CE-2). After the extension, a shift to higher molar masses is visible*



*Figure 13: 1H NMR spectrum of the twice chain extended mPEG-1900. Again, the signals of the double bond between 5.00 – 6.00 ppm are visible, confirming a successful chain extension. Solvent: CDCl3.*

1H NMR (400 MHz, CDCl3) δ in ppm: 5.96 – 5.80 (m, 1H, A), 5.30 – 5.11 (m, 2H, B), 4.06 – 3.86 (m, 2H, C), 3.85 – 3.41 (m, 184H, D), 3.37 (s, 3H, E), 2.62 – 2.40 (m, 4H, F), 1.89 – 1.77 (m, 2H, G), 1.60 – 1.51 (m, 2H, H), 1.40 – 1.16 (m, 18H, I), 0.87 (t, 3H, J).



*Figure 14: DSC thermogram (heating curve; 2. cycle) of twice chain extended mPEG-1900 with a visible Tm at 53 °C.*



*Figure 15: ATR FT-IR spectrum of once modified mPEG-1900 (PPM-1; violet) and twice chain extended mPEG-1900 (CE-2; red). After the chain extension a new signal at 1645 cm-1 appears, which could be assigned to the C=C double bond of the AGE.*

**IR Assignment (based on (Vrandečić et al., 2010), (Carrillo-Castillo et al., 2020) and (Gorin et al., 1971)):**

* C–O, C–C stretching, CH2 rocking at 842 cm−1
* CH2 rocking, CH2 twisting at 947 cm−1
* C–O, C–C stretching, CH2 rocking at 1059 cm−1
* C–O, C–C stretching at 1100 cm−1
* C–O stretching, CH2 rocking at 1145 cm−1
* CH2 twisting at 1240 and 1279 cm−1
* CH2 wagging at 1340 cm−1
* CH2 scissoring at 1467 cm−1
* C=C double bond’s stretching vibration at 1645 cm­-1­
* Symmetric and asymmetric stretching vibration bands of the methylene group C–H bonds at 2885 and 2947 cm-1

## Thiol-ene Reaction of Chain Extended 1-Dodecanthiol modified mPEG-b-oligo(AGE) with Benzylthiol



21.1 mg of chain extended 1-dodecanthiol modified mPEG-*b*-oligo(AGE) (0.00905 mmol, 1.00 eq.) was given into a round-bottom flask, followed by 0.743 mg of AIBN (0.00453 mmol, 0.500 eq.) and 0.250 mL of dry THF. Afterwards, 4.24 µL of benzylthiol (4.50 mg, 0.0362 mmol, 4.00 eq.) was added and the atmosphere was changed to argon. The flask was placed into a preheated oil bath (60 °C) and stirred for 20 hours. The next day the system was quenched by opening to air and cooling *via* ice bath. The solvent was removed under reduced pressure and the residue taken up in THF. After the precipitation in cold Et2O (three times), the product was dried under reduced pressure at 40 °C overnight. A white solid was obtained.



*Figure 16: Size exclusion chromatogram after the second modification of chain extended mPEG-1900 (PPM-2, green). After the modification, a shift to higher molar masses is visible in comparison to the previous polymer (CE-2; red).*



*Figure 17: 1H NMR spectrum of twice chain extended mPEG-1900 after the thiol-ene reaction with benzylthiol. After the reaction the signals of the double bond between 5.00 – 6.00 ppm disappeared and the thiol signals (e.g. around 7.30 ppm) appeared, confirming a successful modification reaction. Solvent: CDCl3.*

1H NMR (400 MHz, CDCl3) δ in ppm: 7.34 – 7.18 (m, 5H, A), 3.84 – 3.44 (m, 186H, B), 3.44 – 3.39 (m, 2H, C), 3.37 (s, 3H, D), 2.60 – 2.43 (m, 6H, E), 1.84 – 1.73 (m, 4H, F), 1.61 – 1.50 (m, 2H, G), 1.39 – 1.16 (m, 18H, H), 0.87 (t, 3H, I).

## 3. Chain Extension of mPEG-1900 with AGE (3. AROMA reaction)



75.0 mg of twice modified mPEG-*b*-oligo(AGE) (0.0306 mmol, 1.00 eq.) and a stirring bar were given into a 5 mL round-bottom flask and the atmosphere was changed to argon. Afterwards, 2.40 mL of dry THF and 4.50 µL of AGE (4.36 mg, 0.0382 mmol, 1.25 eq.) were added to the flask, followed by 38.2 µL of a 0.8 M P4-*t*-Bu (0.0306 mmol of pure P4-*t*-Bu, 1.00 eq.). The flask was placed in a preheated oil bath (50 °C). After 5.5 hours the reaction was stopped by addition of acetic acid. The solvent was removed under reduced pressure and the residue was taken up in THF. After precipitation in cold Et2O (three times), the polymer was dried at 40 °C under reduced pressure over night. A white solid was obtained (yield: 73 %).



*Figure 18: Size exclusion chromatogram after the third chain extension (CE-2). After the extension, a small shift to higher molar masses is visible.*



*Figure 19: 1H NMR spectrum of the three times chain extended mPEG-1900. Again, the signals of the double bond between 5.00 – 6.00 ppm are visible, confirming a successful chain extension. Solvent: CDCl3.*

1H NMR (400 MHz, CDCl3) δ in ppm: 7.34 – 7.18 (m, 5H, A), 5.95 – 5.81 (m, 1H, B), 5.30 – 5.11 (m, 2H, C), 4.04 – 3.86 (m, 2H, D), 3.83 – 3.42 (m, 191H, E), 3.42 – 3.39 (m, 2H, F), 3.37 (s, 3H, G), 2.59 – 2.42 (m, 6H, H), 1.89 – 1.73 (m, 4H, I), 1.61 – 1.50 (m, 2H, J), 1.42 – 1.16 (m, 18H, K), 0.87 (t, 3H, L).

## Thiol-ene Reaction of Chain Extended Benzylthiol modified mPEG-b-oligo(AGE) with Methyl-3-mercaptopropionat



57.4 mg of chain extended benzylthiol modified mPEG-*b*-oligo(AGE) (0.0224 mmol, 1.00 eq.) was given into a round-bottom flask, followed by 1.84 mg of AIBN (0.0112 mmol, 0.500 eq.) and 0.670 mL of dry THF. Afterwards, 9.90 µL of methyl-3-mercaptopropionat (10.7 mg, 0.0894 mmol, 4.00 eq.) was added and the atmosphere was changed to argon. The flask was placed into a preheated oil bath (60 °C) and stirred for 20 hours. The next day the system was quenched by opening to air and cooling *via* ice bath. The solvent was removed under reduced pressure and the residue taken up in THF. After the precipitation in cold Et2O (three times), the product was dried under reduced pressure at 40 °C overnight. A white solid was obtained (yield: 78%).



*Figure 20: Size exclusion chromatogram after the third modification of chain extended mPEG-1900 (PPM-3, black). After the modification, a shift to higher molar masses is visible in comparison to the previous polymer (CE-3; orange).*



*Figure 21: 1H NMR spectrum of three times chain extended mPEG-1900 after the thiol-ene reaction with methyl-3-mercaptopropionat. After the reaction the signals of the double bond between 5.00 – 6.00 ppm disappeared and the thiol signals (e.g. around 2.75 ppm) appeared, confirming a successful modification reaction. Solvent: CDCl3.*

1H NMR (400 MHz, CDCl3) δ in ppm: 7.34 – 7.18 (m, 5H, A), 3.83 – 3.42 (m, 193H, B), 3.42 – 3.39 (m, 2H, C), 3.37 (s, 3H, D), 2.80 – 2.73 (m, 2H, E), 2.66 – 2.43 (m, 10H, F), 1.89 – 1.73 (m, 6H, G), 1.61 – 1.50 (m, 2H, H), 1.42 – 1.16 (m, 18H, I), 0.87 (t, 3H, J).

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