Electronic Supplementary Material (ESI) for Polymer Chemistry.

Biodegradable poly(amidoamine)s with uniform degradation fragments via sequence-controlled macromonomers

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Electronic Supplementary Information

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Materials

2,2′-(ethylenedioxy)bis(ethylamine) was obtained from TCI Europe. Amino acids Fmoc-Gly-OH, Fmoc-L-Phe-OH, Fmoc-L-Leu-OH and Fmoc-L-Lys(Boc)-OH was purchased from Iris Biotech. Trifluoroacetic acid and 9-fluorenylmethyl chloroformate (Fmoc-Cl) was from Fluorochem UK. Papain was obtained from Merck. Copper(II)sulfate, piperidine and trityl chloride was purchased from Acros. Tentagel S RAM resin (loading 0.23 mmol/g) (Fmoc-protected) was from Rapp Polymers. Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) was obtained from Novabiochem. Ethyl trifluoroacetate was purchased from abcr. Sodium azide and diethyl dithiocarbamate was from Applichem. *N,N*-Diisopropylethylamine (DIPEA) and triethyl amine was from Carl Roth and Fischer Scientific UK, respectively. All additional chemicals were purchased from Aldrich. Peptide synthesis grade *N*,*N*-Dimethylformamide was used. All reagents were of analytical reagent grade unless otherwise indicated.

Instrumentation

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR (300 and 600 MHz) and ^{13}C-NMR (75 and 151 MHz) spectra were recorded on a Bruker Avance III – 300 and III - 600 respectively. Chemical shifts of all NMR spectra were reported in delta (δ) expressed in parts per million (ppm) downfield from tetramethylsilane. For ^{1}H-NMR the residual, non-deuterated solvent was used as internal standard (δ 7.26 ppm for $CHCl_3$, δ 2.50 ppm for DMSO, δ 3.31 ppm for MeOD). For ^{13}C-NMR, the chemical shifts are reported relative to the carbon signal of the solvent (δ 77.16 ppm for $CDCl_3$, δ 39.52 ppm for DMSO, δ 49.00 ppm for MeOD). Coupling constants are reported in hertz (Hz). The following abbreviations are used to indicate the multiplicities: s, singlet, d, doublet; t, triplet; m, multiplet.

Mass Spectrometry Coupled Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC/MS)

RP-HPLC/MS was performed on an Agilent Technologies 1260 Infinity system using an analytical Zorbax SB-C18 column (2.1x50 mm) with 1.8 µm particle size at 40 °C and a flow rate of 0.4 mL/min. Eluents: (A) 95% water + 4.9% acetonitrile + 0.1% formic acid; (B) 95% acetonitrile + 4.9% water + 0.1% formic acid. Compound retention time and purity was determined using the Agilent VWD detector by 214 nm UV absorption peak detection and -signal integration. Compound mass detection was performed using the Agilent 6120 Quadrupole LC/MS. UV and MS spectral analysis was done within the OpenLab ChemStation software for LC/MS from Agilent Technologies. Preparative RP-HPLC was performed using a preparative Nucleodur C18 HTec column (21x250 mm) with 5 µm particle size and a flow rate of 20.0 mL/min.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FTIR)

IR spectra were recorded with a Nicolet 6700, attenuated total reflectance Fourier transform infrared spectroscopy (ATR FTIR) spectrometer from Thermo Scientific and spectra analyzed using Omnic software 7.4.

Matrix-Assisted Laser Desorption Ionization (MALDI) Spectroscopy

Compounds were analyzed using a Bruker MALDI-TOF Ultraflex I system with α-Cyano-4-hydroxycinnamic acid (HCCA) matrix. Spectra were acquired in both linear and reflector mode.

Accurate Mass Measurement

The exact mass was measured by electrospray ionization time-of-flight (ESI-TOF) high resolution mass spectrometry on a Bruker Maxis 4G system.

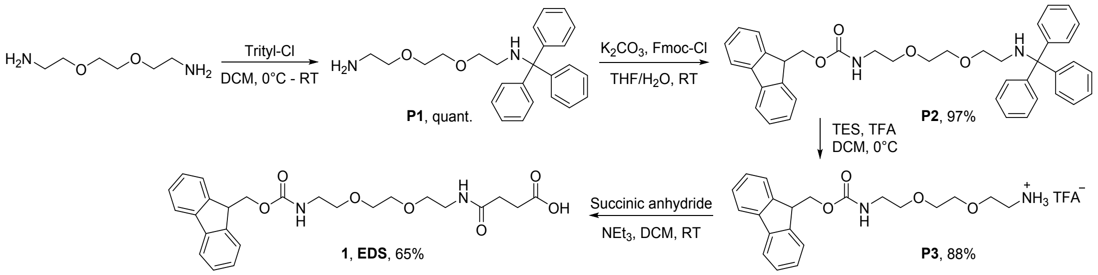
Gel Permeation Chromatography (GPC) Analysis

GPC was performed using an Agilent 1200 series HPLC system equipped with three aqueous GPC columns from Polymer Standards Service (PSS) Mainz, Germany (Suprema Lux analytical 8 mm diameter, 5 µm particle size, precolumn of 50 mm, 2x 100 Å of 300 mm, 1000 Å of 300 mm). MilliQ water with 50 mM NaH_2PO_4, 150 mM NaCl, 250 ppm NaN_3 and of pH7 + 30%MeCN, filtered through an inline 0.1 um membrane filter, was used as GPC eluent with a flow rate of 1 mL/min. UV spectra were recorded on a Waters 486 Tunable Absorbance Detector. Multi-angle light scattering- and differential refractive index spectra were recorded using a miniDAWN TREOS and Optilab rEX, respectively, that were both from Wyatt Technologies EU. Data analysis was performed using the Astra 6 software using a measured dn/dc value of 0.156 mL/g for all the poly/oligo(amidoamine)s.

Synthesis and Characterization of the Building Blocks EDS and TDS and 4-Azidobutanoic Acid

EDS (1)

The compound 1-(9H-fluoren-9-yl)-3,14-dioxo-2,7,10-trioxa-4,13-diazaheptadecan-17-oic acid, EDS (**1**), was prepared following a four-step procedure adapted from a previously published general method for the synthesis of Fmoc-protected building blocks.[1] Trityl monoprotection of 2,2′-(ethylenedioxy)bis(ethylamine) (precursor **P1**) enabled subsequent Fmoc mono-protection (**P2**). After detritylation (**P3**) using 10% TFA in DCM with triethylsilane scavenging, the primary amine was coupled to succinic anhydride to introduce the carboxylic acid and yield the final EDS building block (**1**) in a total yield of over 50% and in high purity (> 99% as determined by RP-HPLC analysis).



*Scheme S1. Building block EDS (**1**) synthesis.*

2-(2-(2-aminoethoxy)ethoxy)-N-tritylethan-1-amine (P1)

2,2′-(ethylenedioxy)bis(ethylamine) (512 mmol, 75.0 mL) was dissolved in DCM (1.0 L) in an oven-dried, nitrogen-flushed, round bottomed flask and the solution was cooled to 0 °C. Then, a solution of trityl chloride (128 mmol, 35.7 g) in DCM (800 mL) was added slowly and dropwise under thorough stirring that was continued overnight while allowing the reaction to reach room temperature. The reaction mixture was concentrated to half the volume, extracted with an aqueous saturated sodium bicarbonate solution (4x 1.0 L) and dried over $MgSO_4$. After filtration and solvent evaporation **P1** (50.0 g, 128 mmol) was obtained in quantitative yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 – 7.44 (m, 6H), 7.32 – 7.23 (m, 6H), 7.23 – 7.14 (m, 3H), 3.65 – 3.56 (m, 4H), 3.56 – 3.45 (m, 4H), 2.83 (td, J = 5.4, 1.6 Hz, 2H), 2.36 (t, J = 5.4 Hz, 2H), 2.08 – 1.54 (m, 3H, $-NH_2$/-NH-) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 146.13, 128.71, 127.81, 126.25, 73.21, 71.28, 70.67, 70.28, 70.11, 43.07, 41.72 ppm. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{25}H_{31}N_2O_2$ 391.2380; Found 391.2384.

(9H-fluoren-9-yl)methyl (2-(2-(2-(tritylamino)ethoxy)ethoxy)ethyl)carbamate (P2)

P1 (128 mmol, 50.0 g) was dissolved in THF (600 mL) and a solution of K_2CO_3 (640 mmol, 88.5 g in 600 mL water) was added. 9-fluorenylmethyl chloroformate (Fmoc-Cl) (128 mmol, 33.1 g) was added and the biphasic mixture was stirred vigorously for 16 h, monitored by TLC (EtOAc:Hex 1:1, *v/v*). After THF evaporation, the organic residue was dissolved in EtOAc (800 mL), washed three times with water and dried over Na_2SO_4. Filtration and solvent evaporation yielded 76.0 g (124 mmol, 97% yield) of a colorless tacky oil of **P2** containing approx. 25% residual Fmoc-OH. ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 – 7.73 (m, 2H), 7.64 – 7.56 (m, 2H), 7.52 – 7.45 (m, 6H), 7.43 – 7.36 (m, 2H), 7.33 – 7.22 (m, 9H), 7.21 – 7.13 (m, 3H), 5.25 (s, 1H), 4.38 (d, *J* = 7.0 Hz, 2H), 4.19 (t, *J* = 7.0 Hz, 1H), 4.12 (t, *J* = 6.5 Hz, ¼H), 4.02 (d, *J* = 6.5 Hz, ¼H), 3.69 – 3.43 (m, 8H), 3.35 – 3.18 (m, 2H), 2.37 (t, *J* = 5.4 Hz, 2H), 2.08 (s, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.48, 146.10, 144.02, 141.33, 128.71, 127.83, 127.68, 127.06, 126.29, 125.10, 119.98, 71.30, 70.68, 70.38, 70.14, 70.03, 66.62, 47.28, 43.03, 40.97 ppm. NB. ^1H NMR peaks in the aromatic region and at 4.11 and 4.02 ppm indicates minor amounts of Fmoc-OH that is eliminated in later purification steps. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{40}H_{41}N_2O_4$ 613.3061; Found 613.3069.

(9H-fluoren-9-yl)methyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (TFA salt) (P3)

A solution of **P2** (124 mmol, 76.0 g) and triethylsilane (248 mmol, 39.6 mL) in DCM (1.0 L) was cooled to 0 °C and TFA (10%, 100 mL) was added slowly to avoid raising the solution temperature**.** TLC monitoring (EtOAc:Hex 1:1 *v/v*, ninhydrin) showed complete trityl deprotection after 30 min. After evaporating half of the solvent, the TFA was co-evaporated with toluene (2x 300 mL). The crude product was precipitated in diethyl ether (1.3 L) from a DCM solution (130 mL) and the fine white precipitate filtered off and washed with diethyl ether using a fine sintered glass filter to give the TFA salt of **P3** (110 mmol, 51.5 g, 88% yield). ^1H NMR (300 MHz, MeOD) δ 7.81 (d, *J* = 7.5, 2H), 7.65 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.30-7.05 (m, trityl residual), 4.54 (s, trityl-OH residual ¼H), 4.38 (d, *J* = 6.8 Hz, 2H), 4.22 (t, *J* = 6.8 Hz, 1H), 3.74 – 3.59 (m, 6H), 3.55 (t, *J*

= 5.6 Hz, 2H), 3.32 – 3.26 (m, CH_2-NH_3^+ merged w. MeOD, 5H), 3.09 (t, *J* = 5.1 Hz, 2H) ppm. ^{13}C NMR (75 MHz, MeOD): δ = 161.5, 157.4, 143.8, 141.1, 127.4, 126.8, 124.8, 119.6, 69.8, 69.7, 66.1, 46.9, 40.2, 39.1 ppm. NB. ^{1}H NMR peaks in the aromatic region and at 4.54 ppm indicates minor impurities from residual Trityl-OH. This was completely removed in later purification steps. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{27}N_2O_4$ 371.1965; Found 371.1973.

1-(9H-fluoren-9-yl)-3,14-dioxo-2,7,10-trioxa-4,13-diazaheptadecan-17-oic acid (1, EDS)

To a solution of **P3** (110 mmol, 51.5 g) in DCM (1.0 L) was added no more than 1 eq. of succinic anhydride (110 mmol, 11.0 g) and 3eq. triethylamine (330 mmol, 45.9 mL). After 30 min stirring, TLC (DCM:MeOH 9:1 + 1 drop acetic acid) showed complete conversion of **P3** and the organic layer was washed with 5% citric acid (4x 600 mL), dried over Na_2SO_4 and evaporation under reduced pressure. A few days crystallization from EtOAc with ~1 vol% hexane gave **1** as white crystals in >99% purity (71.0 mmol, 33.4 g, 65% yield). ^{1}H NMR (600 MHz, MeOD) δ 7.80 (d, *J* = 7.5 Hz, 2H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 4.37 (d, *J* = 6.8 Hz, 2H), 4.21 (t, *J* = 6.9 Hz, 1H), 3.60 (s, 4H), 3.56 – 3.48 (m, 4H), 3.35 (t, *J* = 5.5 Hz, 2H), 3.30 (t, *J* = 5.6 Hz, 2H), 2.58 (t, *J* = 6.9 Hz, 2H), 2.47 (t, *J* = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, MeOD) δ 176.19, 174.61, 158.89, 145.32, 142.60, 128.76, 128.13, 126.14, 120.92, 71.30, 70.95, 70.58, 67.65, 49.00, 41.68, 40.37, 31.53, 30.30.

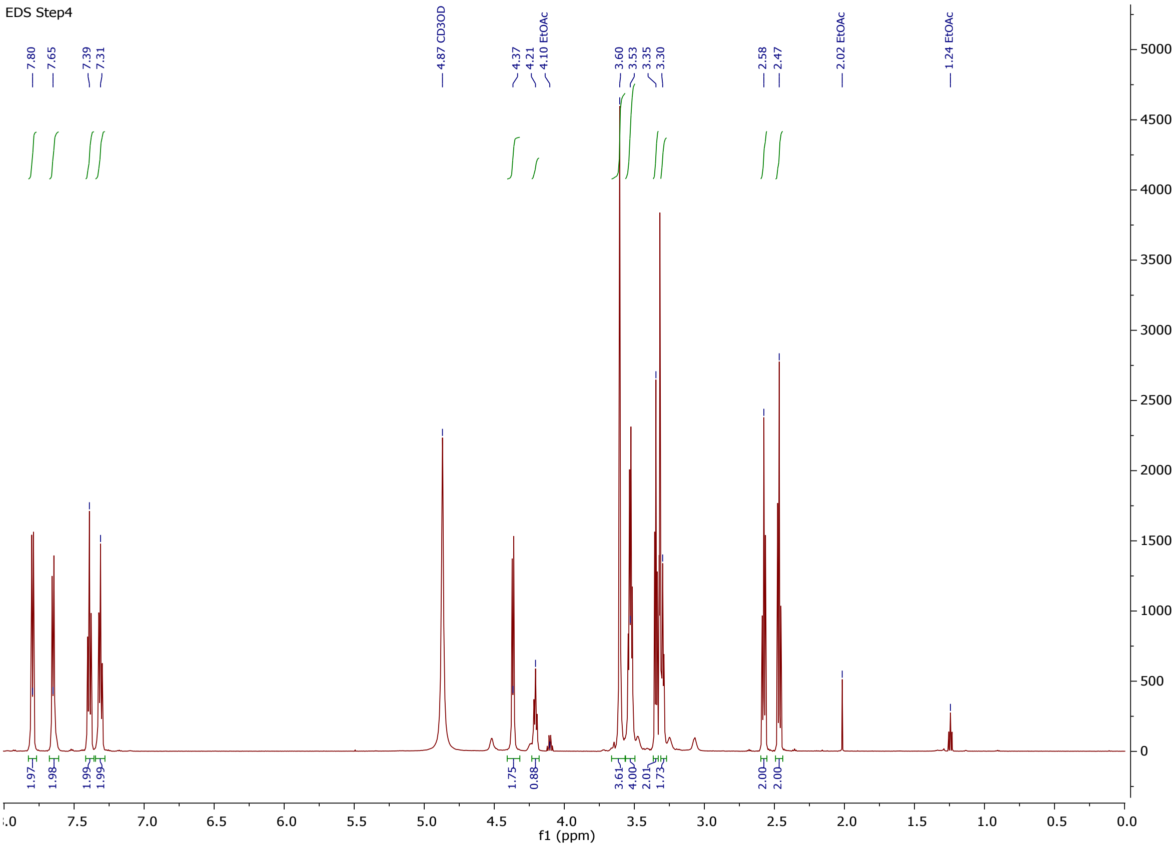


*Figure S1. ^{1}H-NMR of EDS Step 4 (**1**).*

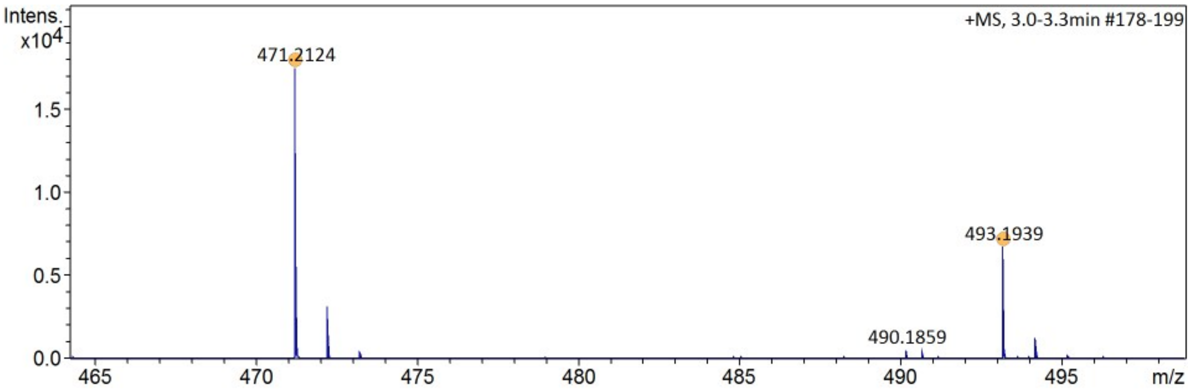


*Figure S2. HRMS (ESI-TOF), EDS step 4 (**1**), m/z: [M + H]$^+$ Calcd for $C_{25}H_{31}N_2O_7$ 471.2126; Found 471.2124.*

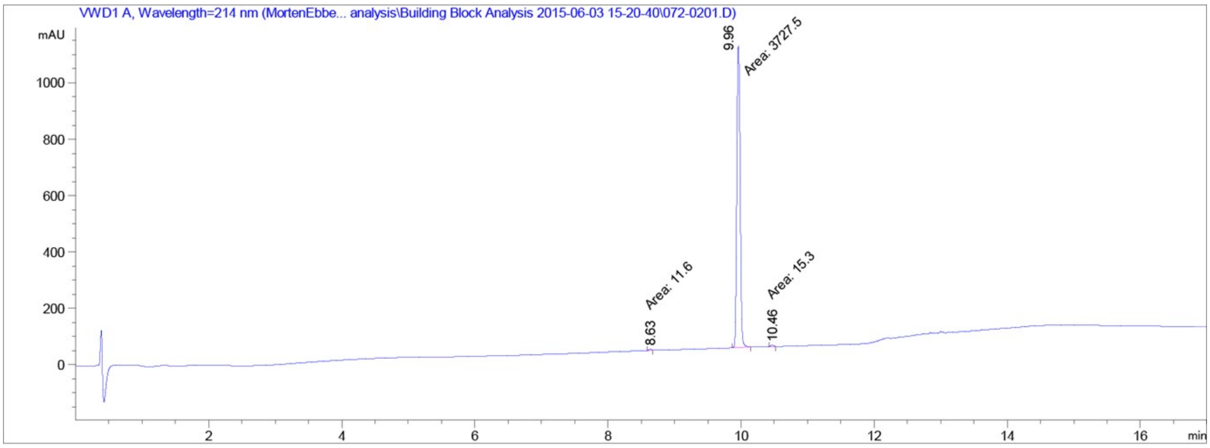
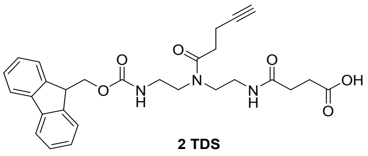


*Figure S3. LC-MS EDS step 4 (**1**) (Eluent A to B, 17 min):* t_r *= 9.96 min, purity = 99.3%.*

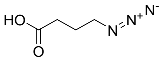
TDS (2)



2 TDS

The compound 1-(Fluorenyl)-3,11-dioxo-7-(pent-4-ynoyl)-2-oxa-4,7,10-triazatetradecan-14-oic acid, TDS (**2**), was prepared according to a previously established six-step procedure in our group.[2] The final compound was synthesized as an amorphous white solid in a total yield of 27%. ^{1}H-NMR (300 MHz, DMSO-*d*$_6$): δ 12.05 (br s, 1H), 8.01 (t, *J* = 5.7 Hz, ½H), 7.87 (d, *J* = 7.4 Hz, 2H+½H), 7.65 (d, *J* = 7.4 Hz, 2H), 7.40 (td, *J* = 7.4, 0.9 Hz, 2H), 7.31 (tt, *J* = 7.4, 1.2 Hz, 2H), 4.29 (dd, *J* = 8.7, 6.6 Hz, 2H), 4.19 (t, *J* = 6.7 Hz, 1H), 3.31 – 3.21 (m, 4H), 3.20 – 3.00 (m, 4H), 2.71 (tt, *J* = 2.6, 1.4 Hz, 1H), 2.51 – 2.45 (m, 2H), 2.44 – 2.36 (m, 2H), 2.36 – 2.20 (m, 4H) ppm. ^{13}C NMR (75 MHz, DMSO) δ 173.78 (1H), 171.23 (1H), 170.54 (1H), 156.17 (1H), 143.90 (2H), 140.75 (2H), 127.62 (2H), 127.07 (2H), 125.06 (2H), 120.13 (2H), 84.08 (1H), 71.17 (1H), 65.45 (1H), 46.75 (2H), 45.04 (1H), 37.32 (2H), 31.28 (1H), 30.02 (1H), 29.01 (1H), 14.02 (1H) ppm. ESI-MS m/z calcd. for $C_{28}H_{31}N_3O_6$ $[M+H]^+$ 506.2; found 506.2. RP-HPLC analysis (0 − 50% eluent B in 17 min) of compound **2** revealed the product peak at t_R = 10.24 min and a purity of 98.9%.

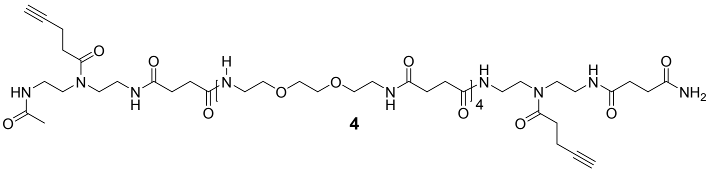
4-Azidobutanoic Acid (3)



This compound (**3**), was prepared according to a previously established two-step procedure.[3] The first step of this procedure involves inorganic azides and precautions should thus be taken e.g. to avoid excessive heating and direct contact with acid, as HN_3 is volatile, explosive, and highly toxic. The final compound was synthesized as a colorless transparent oil in a final amount of 3.6 g (27.9 mmol, 93% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.43 (s, 1H), 3.37 (t, *J* = 6.7 Hz, 2H), 2.47 (t, *J* = 7.2 Hz, 2H), 1.91 (p, *J* = 6.9 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.17, 50.57, 31.03, 24.05 ppm. IR (film) *v* 3032 (COOH), 2941 and 2881 ($-CH_2-$), 2100 ($-N_3$), 1709 (C=O), 1278 (C-O) cm^{-1}.

Solid-Phase Synthesis (SPS)

Short non-degradable dialkynyl oligomer (4)



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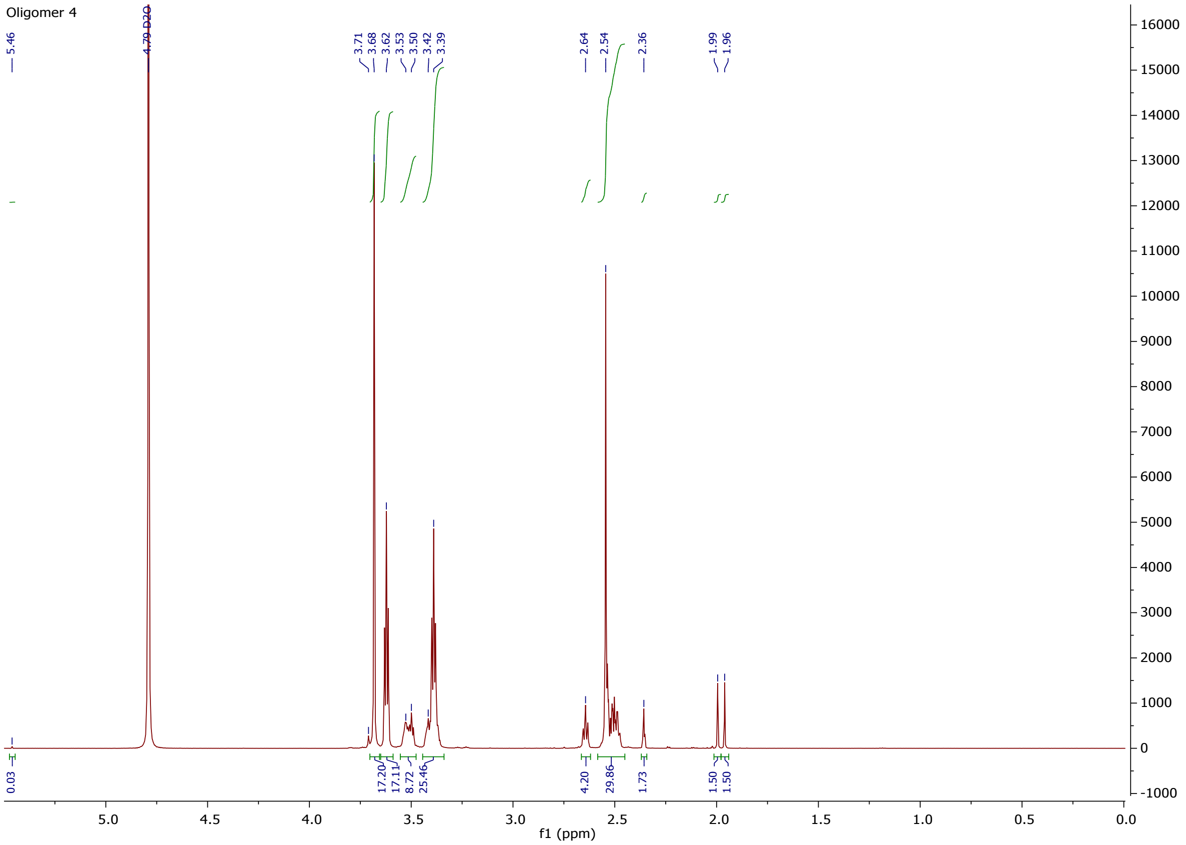


*Figure S4. ^{1}H-NMR of oligomer **4***

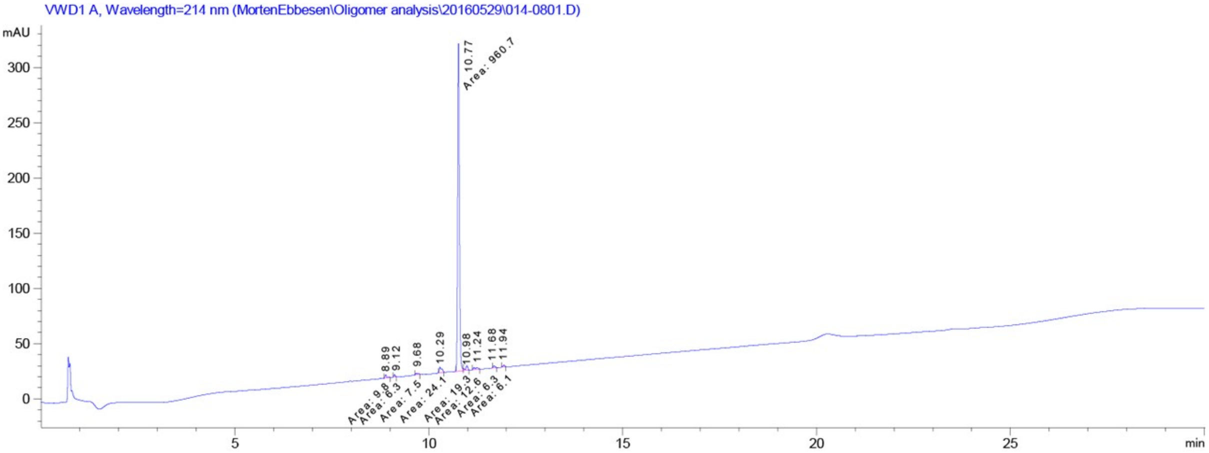


*Figure S5 - RP-HPLC analysis (0 − 50% eluent B in 30 min) of compound **4** revealed the oligomer product peak at t_R = 10.77 min and a purity of 91.3%.*

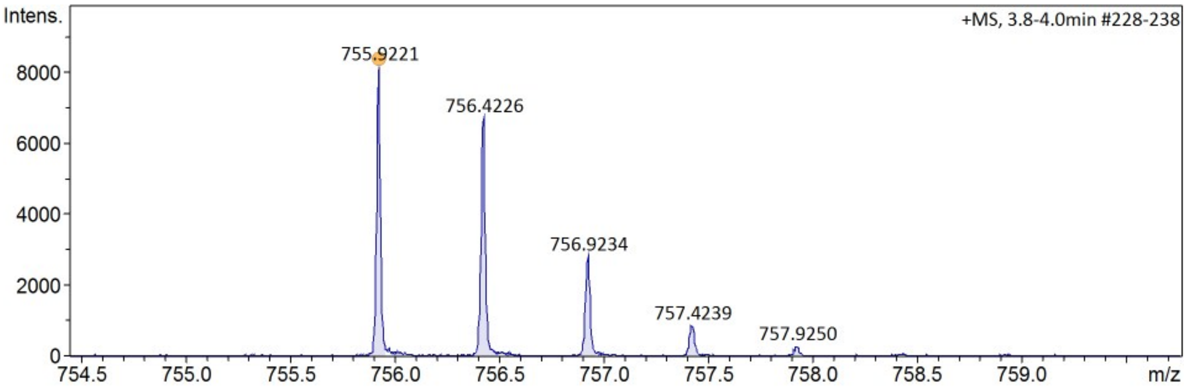
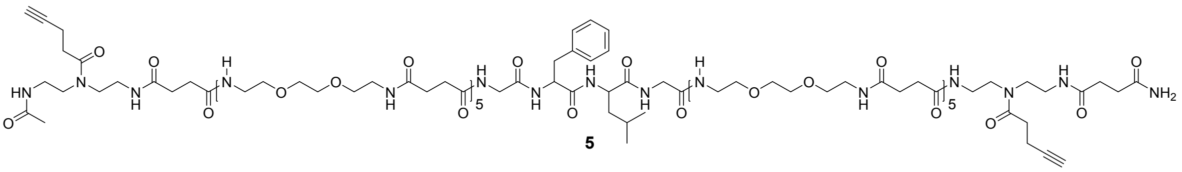


*Figure S6 – HRMS (ESI-TOF), oligomer **4**. m/z: $[M + 2H]^{2+}$ Calcd for $C_{68}H_{117}N_{15}O_{23}$ 755.9218; Found 755.9221.*

Long degradable bis-alkynyl oligomer (5)



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A definitive assignment of the ^{1}H NMR resonance of **5** was made with the assistance of a COSY spectrum. The spectrum of **4** was assigned by analogy with **5**.

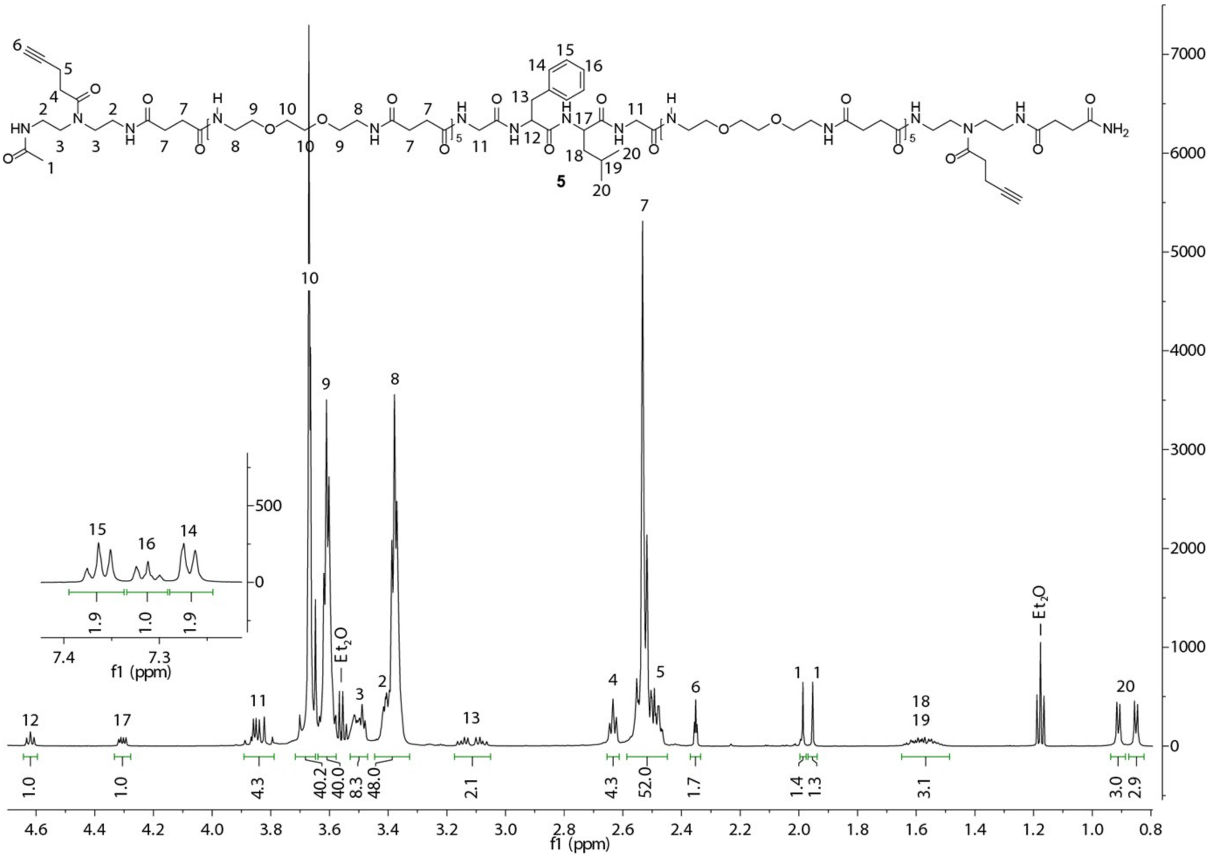

Figure S7. ^{1}H-NMR assignment for bis-alkyne oligomer ***5****.*

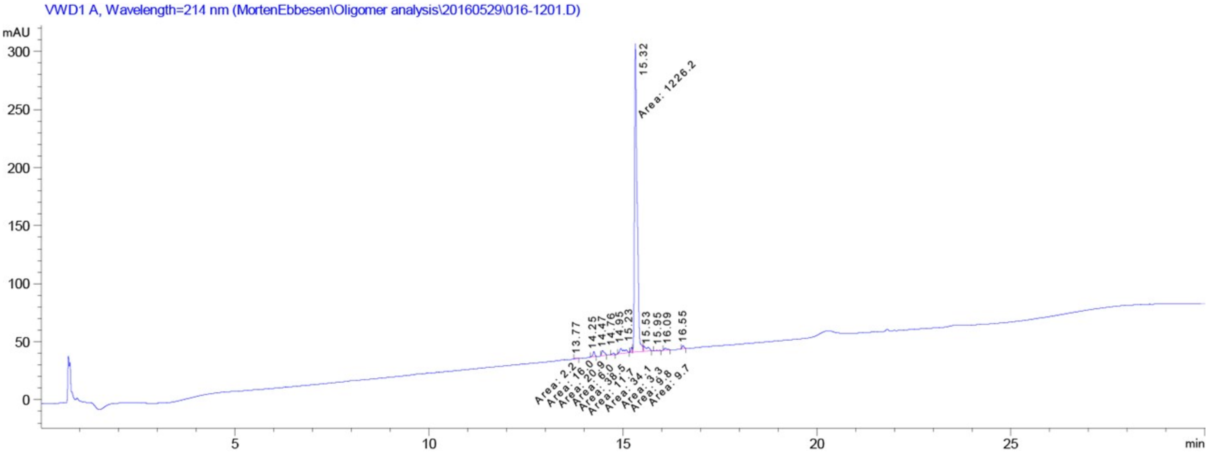

Figure S8 - RP-HPLC analysis (0−50% eluent B in 30 min) of oligomer ***5*** *revealed the oligomer product peak at t_R = 15.32 min and a purity of 88.9%.*

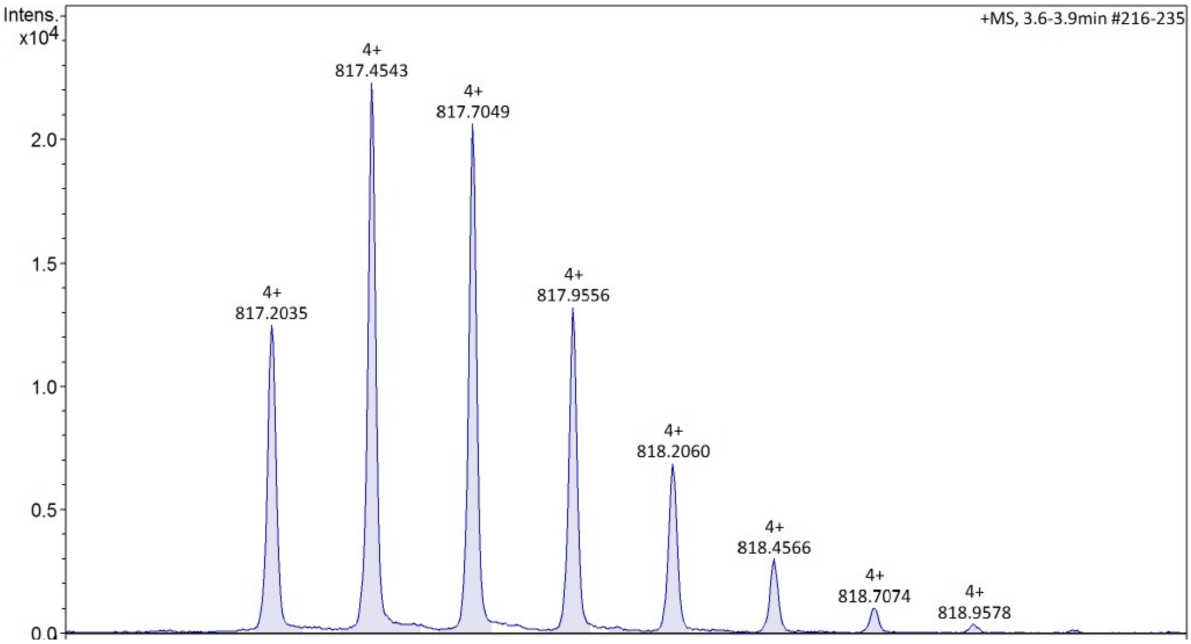

Figure S9. HRMS (ESI-TOF), oligomer ***5****, m/z: [M + 4H]*$^{4+}$ *Calcd for* $C_{147}H_{253}N_{31}O_{51}$ *817.2034; Found 817.2035.*

Short non-degradable bis-azido oligomer (6)

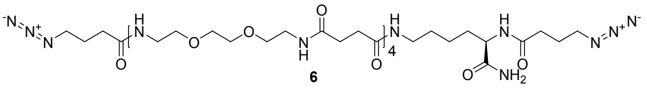
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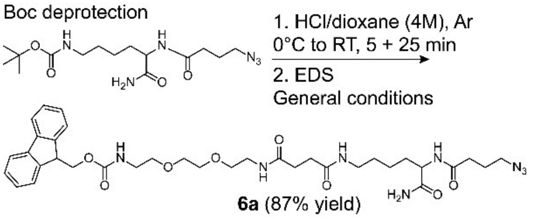
Figure S10. Target structure for oligomer ***6****.*

Lysine ε-amine deprotection

A few strategies were tried in the attempts of azido-modifying the oligomer C-terminal through modification with 4-azidobutanoic acid. This required an orthogonal amine deprotection that was initially pursued via an *N*-ε aloc-protected lysine, however, without achieving full aloc deprotection.

Boc deprotection



Scheme S2 - Boc deprotection strategy before oligomer C-terminal modification with ***3****.*

Another strategy was pursued through the use of Fmoc-L-Lys(Boc)-OH as the initial C-terminal building block. After initial standard Fmoc deprotection and capping of the Lys α-amino group with 4-azidobutanoic acid, various Boc deprotection conditions were tested with the best being a modified procedure from Han *et al.*[4] (Scheme S2) described here for our structure. A solution of HCl (4M) in dioxane was first purged with argon, cooled to 0 °C and then transferred to the resin and agitated for 5 min. The solution was filtered of and the resin was washed once in dioxane before repeating the treatment for 25 min. After washing the resin first with dioxane and alternating with DCM and IPA (three times each), any remaining HCl was neutralized w. a solution of DIPEA in DCM (5%, 2 x 10 min) followed by a final wash with DCM and IPA. After coupling Fmoc-protected EDS to the resin, the outcome of the Boc-deprotection was evaluated by measuring, via UV absorption, the ratio of cleaved Fmoc from the EDS- and initial Fmoc-L-Lys(Boc)-terminated oligomers, respectively. The best Boc deprotection conditions showed 87% EDS-associated Fmoc (Scheme S2) compared to the initial Lys-associated Fmoc with the loss being ascribed to either incomplete deprotection or premature oligomer cleavage. Also, a qualitative analysis was performed via RP-HPLC/MS (Figure S11) on an analytical amount of **6a** cleaved from the resin after Fmoc deprotection that showed the product peak at 0.686 min.

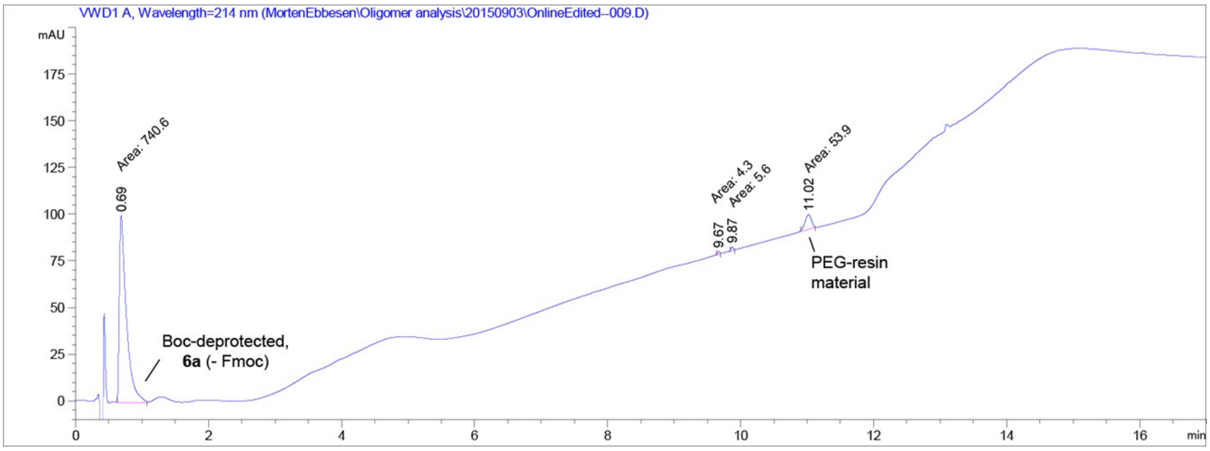


*Figure S11 - RP-HPLC analysis (0−50% eluent B in 12 min + 5 min post time) of compound **6a** (Fmoc deprotected). Residual, non-integrated, events in the elugram are artifacts and also present in blank runs.*

After coupling of the remaining building blocks, the final oligomer product **6** was obtained.

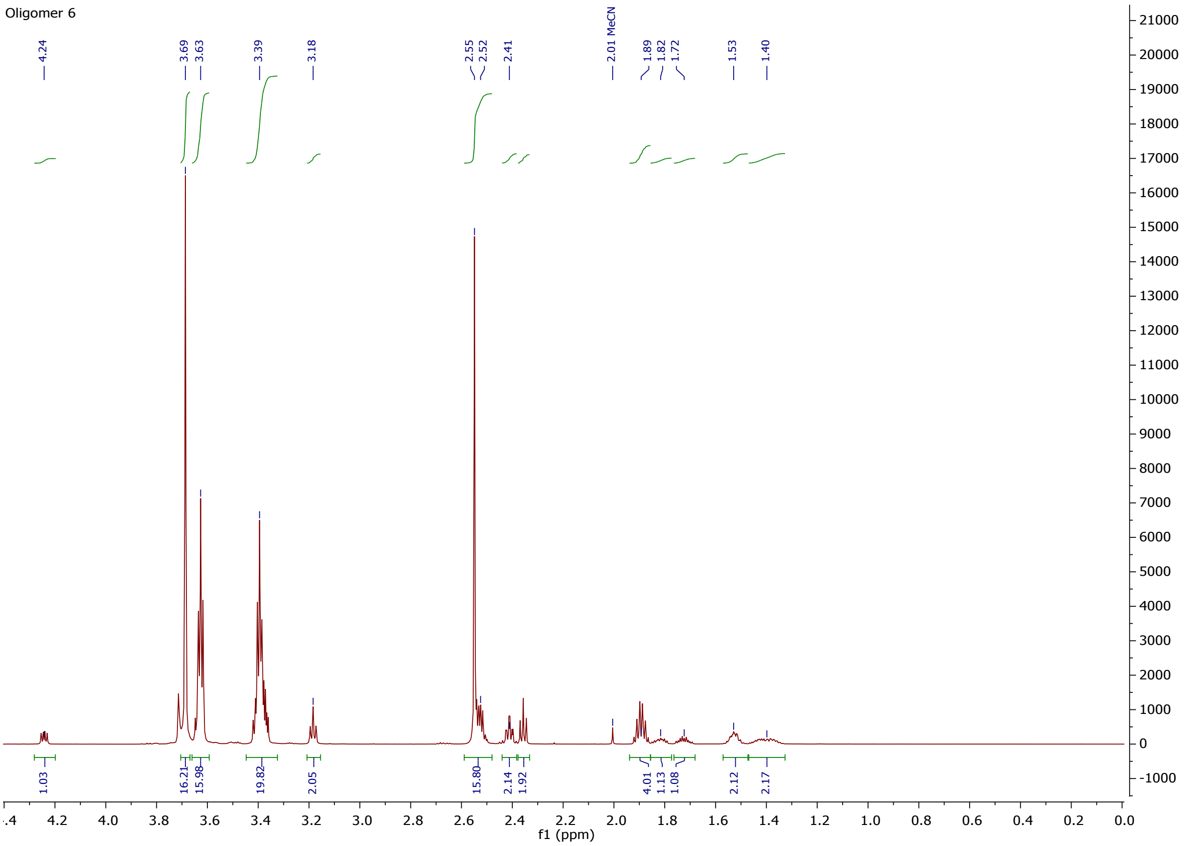


*Figure S12. ^{1}H-NMR of oligomer **6**.*

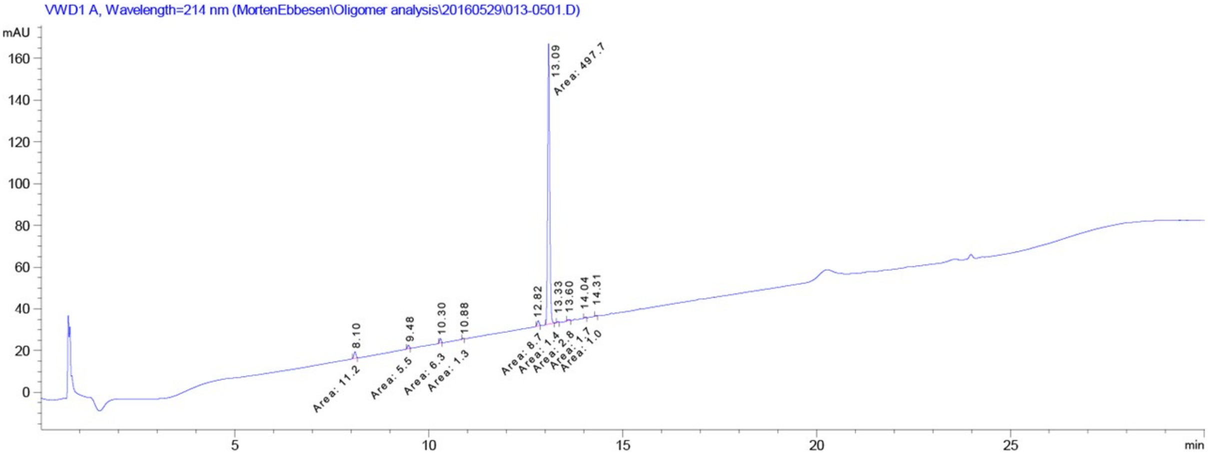


*Figure S13 - RP-HPLC analysis (0−50% eluent B in 30 min) of compound **6** revealed the oligomer product peak at t_R = 13.09 min and a purity of 92.6%.*

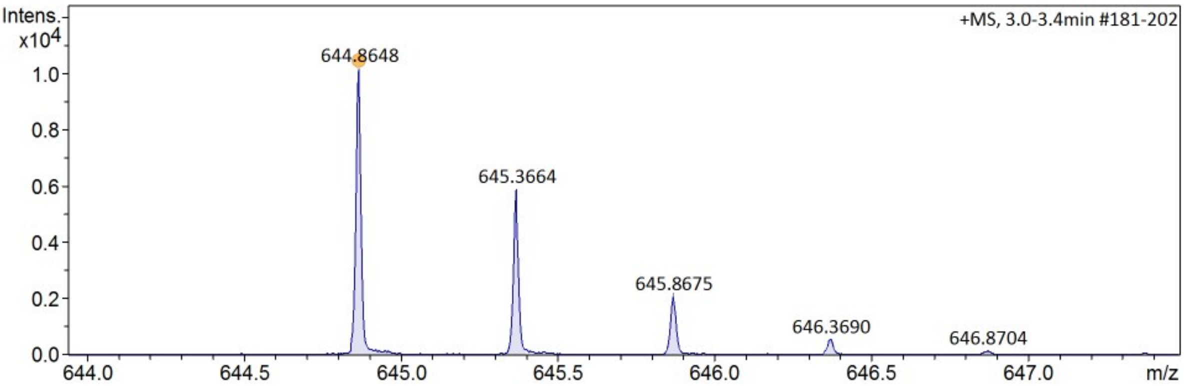
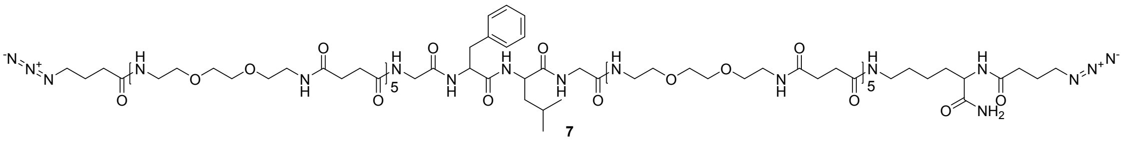


*Figure S14. HRMS (ESI-TOF), oligomer **6**, m/z: $[M + 2H]^{2+}$ Calcd for $C_{54}H_{99}N_{17}O_{19}$ 644.8646; Found 644.8648.*

Long degradable bis-azido oligomer (7)



7

A definitive assignment of the ^{1}H NMR resonance of **7** was made with the assistance of a COSY spectrum. The spectrum of **6** was assigned by analogy with **7**.

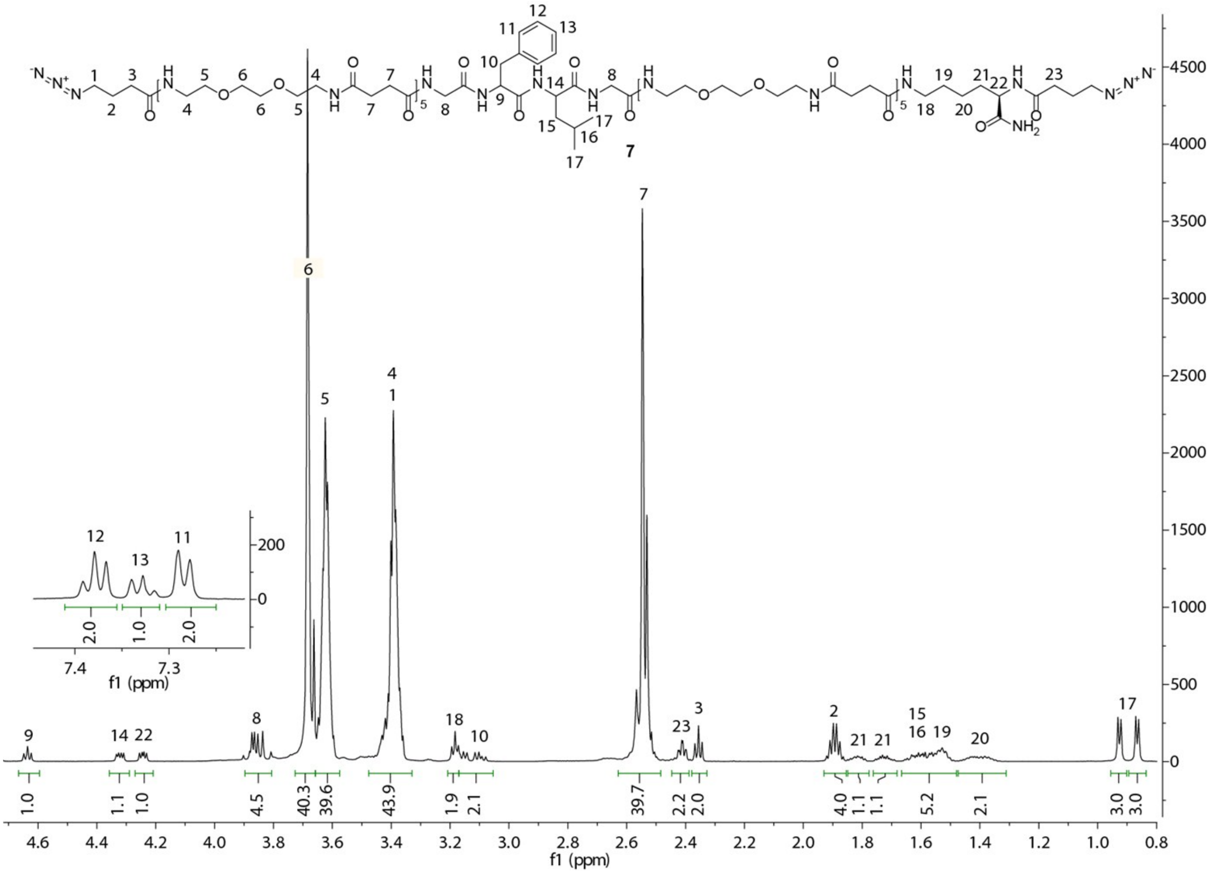


*Figure S15. ^{1}H-NMR assignment for bis-azide oligomers **7**.*

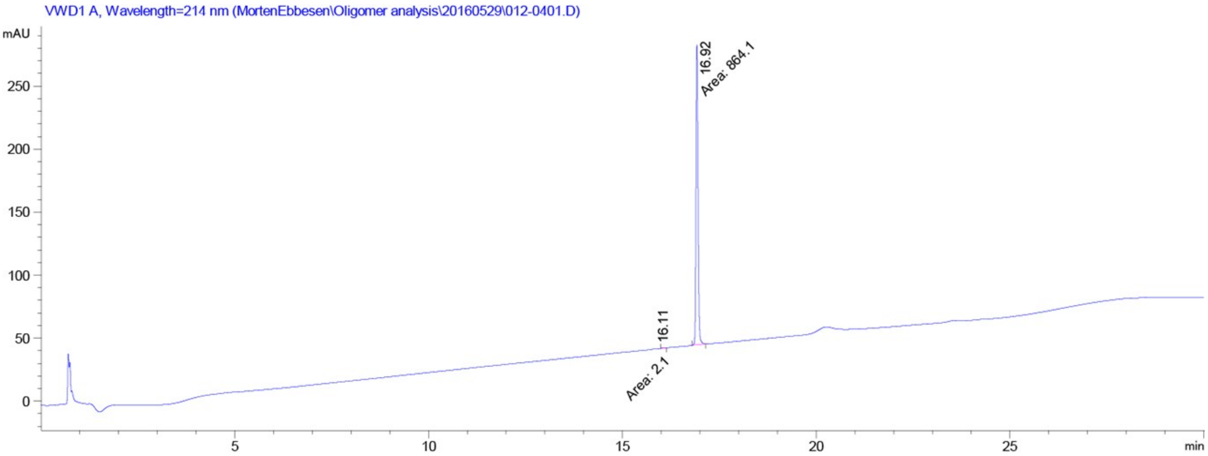


*Figure S16. RP-HPLC/MS analysis of oligomer **7** (0 − 50% eluent B in 30 min), revealed the oligomer product peak a t_R = 16.92 min and a purity of 99.8%.*

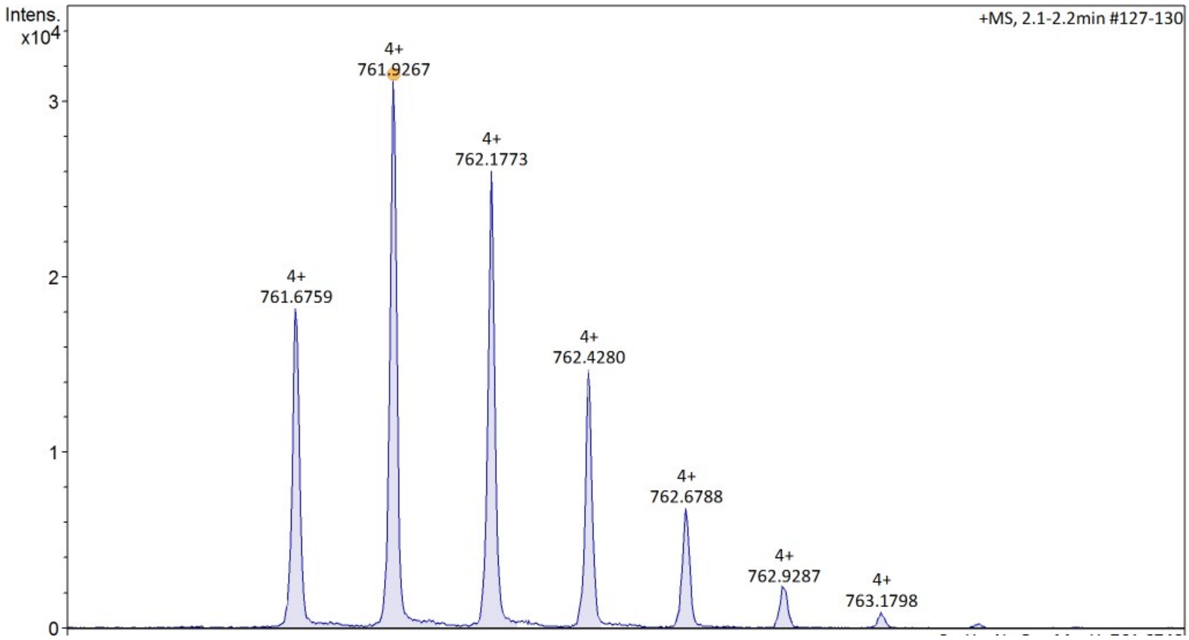
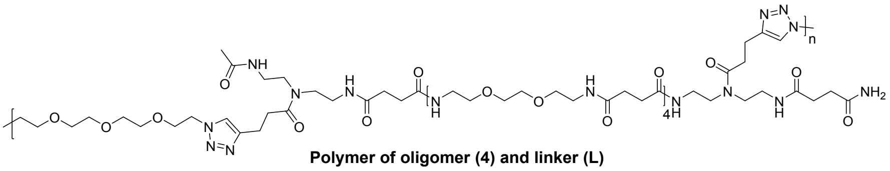


*Figure S17. HRMS (ESI-TOF), oligomer **7**, m/z: $[M + 4H]^{4+}$ Calcd for $C_{133}H_{235}N_{33}O_{47}$ 761.6748; Found 761.6759.*

Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC)-Mediated Oligomer Polymerization

Polymerization of oligomer 4 and linker 1,11-diazido-PEG3 (13 - 23)



Polymer of oligomer (4) and linker (L)

^{1}H NMR (300 MHz, D_2O) δ 7.83 (s, 2H), 4.57 (t, *J* = 4.9 Hz, 4H), 3.93 (t, *J* = 4.8 Hz, 4H), 3.67 (s, 16H), 3.65 – 3.51 (m, 26H), 3.51 – 3.42 (m, 8H), 3.42 – 3.35 (m, 16H), 3.35 – 3.26 (m, 8H), 2.97 (t, *J* = 7.0 Hz, 4H), 2.78 (t, *J* = 6.9 Hz, 4H), 2.58 – 2.40 (m, 24H), 1.92 (d, *J* = 7.2 Hz, 3H). See Table S1 for GPC data.

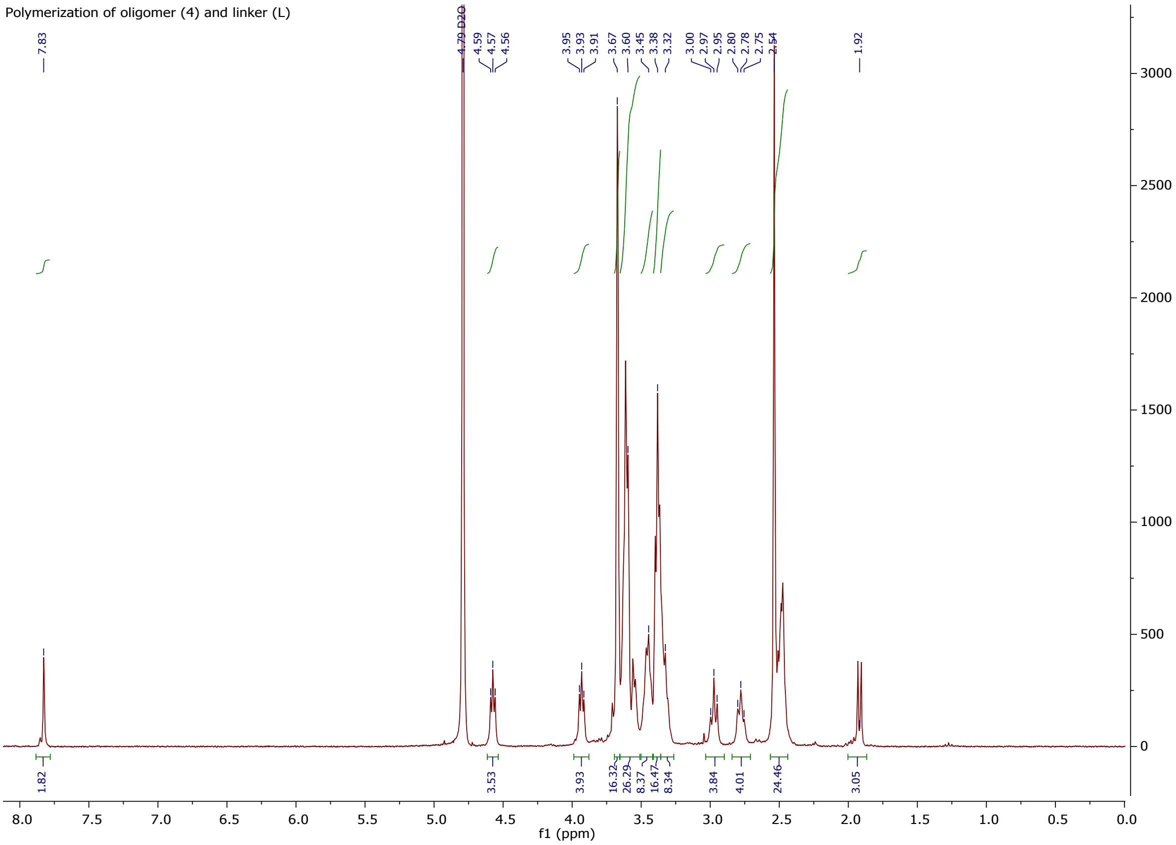
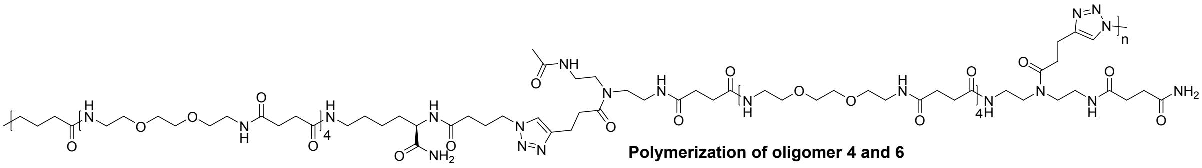


*Figure S18. Example of ^{1}H-NMR spectrum of polymerization of oligomer **4** and linker **L**.*

Polymerization of oligomer 4 + oligomer 6 (8 - 10)



^{1}H NMR (300 MHz, D_2O) δ 7.81 (d, *J* = 2.5 Hz, 2H), 4.42 (t, *J* = 6.5 Hz, 4H), 4.17 (dd, *J* = 8.7, 5.5 Hz, 1H), 3.69 – 3.65 (m, 32H), 3.61 (t, *J* = 5.3 Hz, 32H), 3.45 (t, *J* = 6.0 Hz, 8H), 3.42 – 3.28 (m, 40H), 3.17 (t, *J* = 6.7 Hz, 2H), 2.99 (t, *J* = 7.1 Hz, 4H), 2.79 (t, *J* = 7.3 Hz, 4H), 2.57 – 2.43 (m, 40H), 2.34 – 2.12 (m, 8H), 1.92 (d, *J* = 7.0 Hz, 3H), 1.72 (dt, *J* = 23.7, 8.0 Hz, 2H), 1.51 (p, *J* = 6.8 Hz, 2H), 1.44 – 1.30 (m, 2H). See Table 1 and Figure 2 in main text for GPC data.

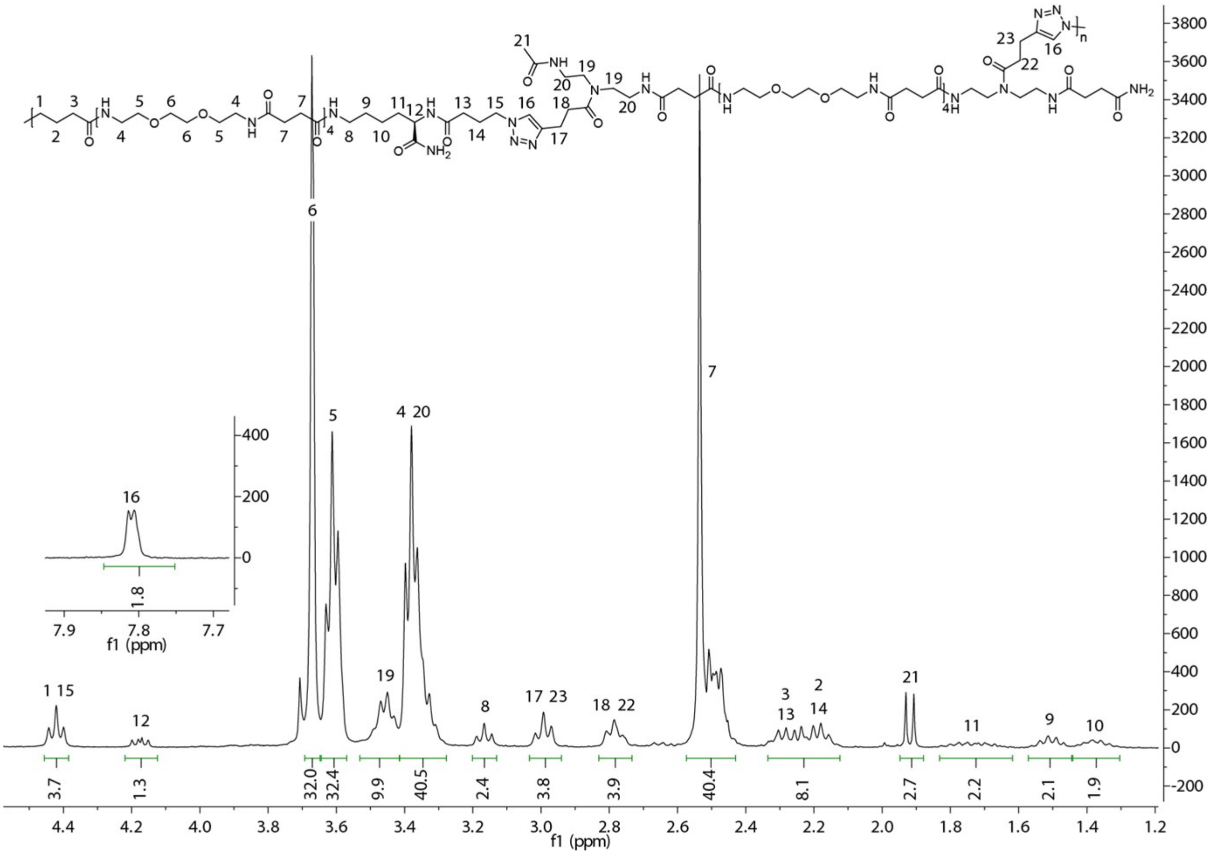


Figure S19. Example of ^{1}H-NMR spectrum of the polymerization of oligomer 4 and 6 (polymer 9).

GPC characterization and optimization of polymerization parameters

Table S1. Optimization of polymerization parameters of the linker 1,11-diazido-PEG3 and the biz-alkyne oligomer 4.

#	Bis-N_3 ligomer	Bis-C≡CH oligomer	DMSO/DMF /H_2O	Catalyst	Rx. [h]	Mn [kDa][a]	$Đ_M$[b]	X_n[c]
13	**L** (0.244 kDa)	**4** (1.51 kDa)	3/0/7	$CuSO_4$	2	6.5	1.4	7.4
14	-	-	-	-	4	6.9	1.7	7.9
15	-	-	-	-	24	6.3	1.7	7.2
16	**L** (0.244 kDa)	**4** (1.51 kDa)	0/10/0	$CuSO_4$	4	1.9	1.1	2.2
17	-	-	0/0/10	-	-	5.9	1.4	6.7
18	-	-	7/0/3	-	-	6.8	1.8	7.8
19	-	-	0/7/3	-	-	7.9	1.8	9.0
20	-	-	0/9/1	-	-	9.3	1.5	10.6
21	**L** (0.244 kDa)	**4** (1.51 kDa)	0/10/0	CuBr	4	1.9	1.3	2.2
22	-	-	0/9/1	-	-	6.5	1.6	7.4
23	-	-	0/7/3	-	-	6.7	1.7	7.7

[a] Measured using GPC as described in the experimental section. [b] Molecular mass distribution (M_w/M_n).
[c] Number-average degree of polymerization.

Enzyme-mediated Polymer Degradation

MALDI analysis of degradation fragments

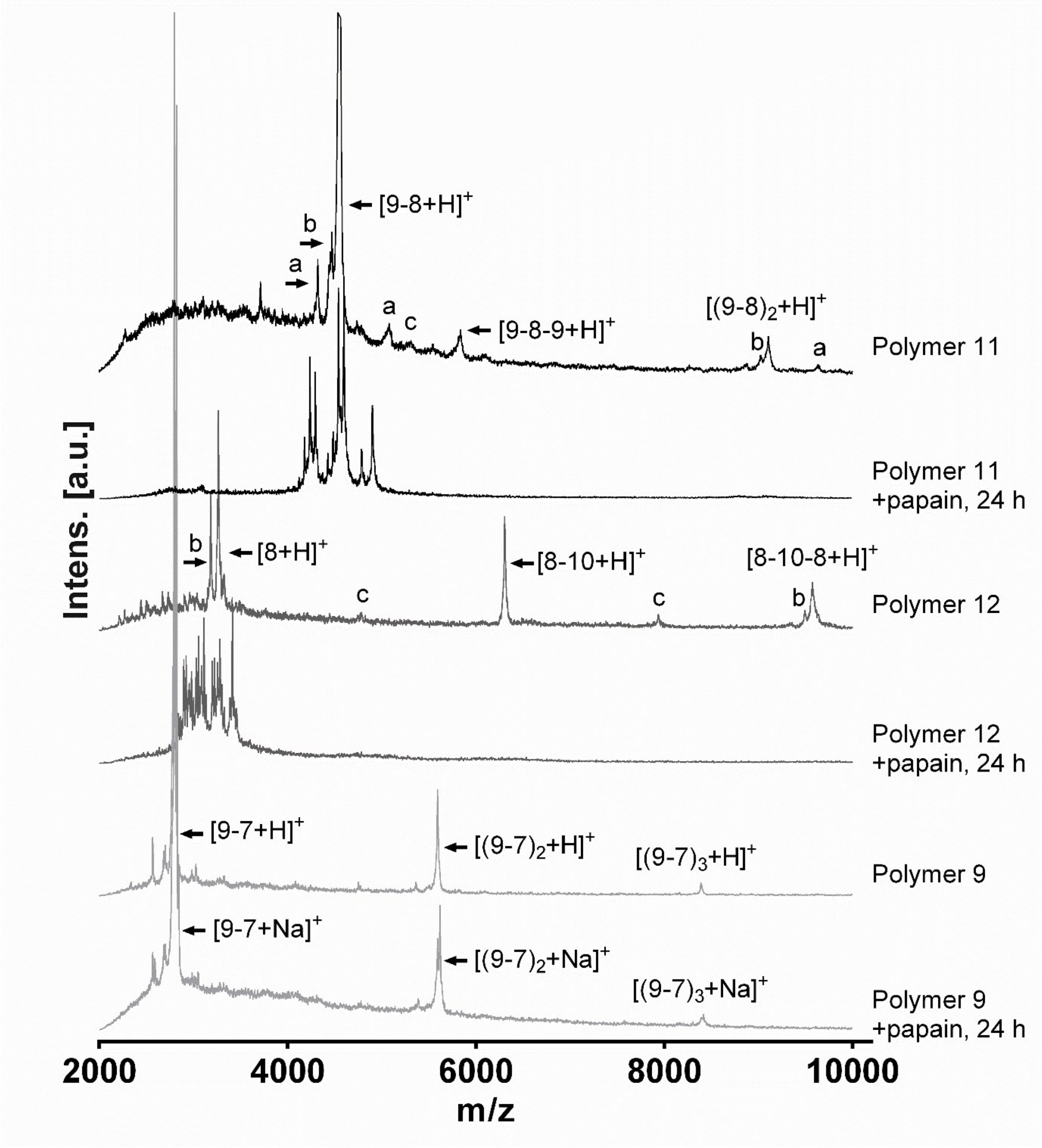


*Figure S20. MALDI-TOF MS spectra recorded in linear mode of degradable polymers **11** – **12** and non-degradable polymer **9**. Peaks corresponding to various degrees of polymerization is observed for all non-treated polymers **9**, **11** and **12**. After 24 h papain treatment, as described in the experimental section, the degradable polymers only show a narrow range of fragments (4.2 – 4.9 kDa and 2.8 – 3.4 kDa for polymer **11** and **12**, respectively) whereas the non-degradable remains essentially unchanged. Residual minor peaks can be assigned to byproducts originating from observed and identified* *oligomer impurities (<1%) (e.g. oligomer termination sequences) (a) and byproducts originating from observed but unidentified oligomer impurity (<1%) (b). Minor peaks from double charged polymer species are also present (c).*

*Table S2. Comparison of oligomer constituents (**4** - 7) of polymers **9, 11** and **12** and observed fragments subsequent to papain treatment via MALDI-MS analysis.*[a]

#	Bis-N_3 oligomer	Bis-C≡CH oligomer	Degradation fragments [kDa][a]
9	**6** (1.29 kDa)	**4** (1.51 kDa)	None
11	**6** (1.29 kDa)	**5** (3.27 kDa)	4.2 – 4.9
12	**7** (3.04 kDa)	**5** (3.27 kDa)	2.8 – 3.4

[a] Observed range of degradation fragments via MALDI-MS analysis excl. end-group derived fragments.

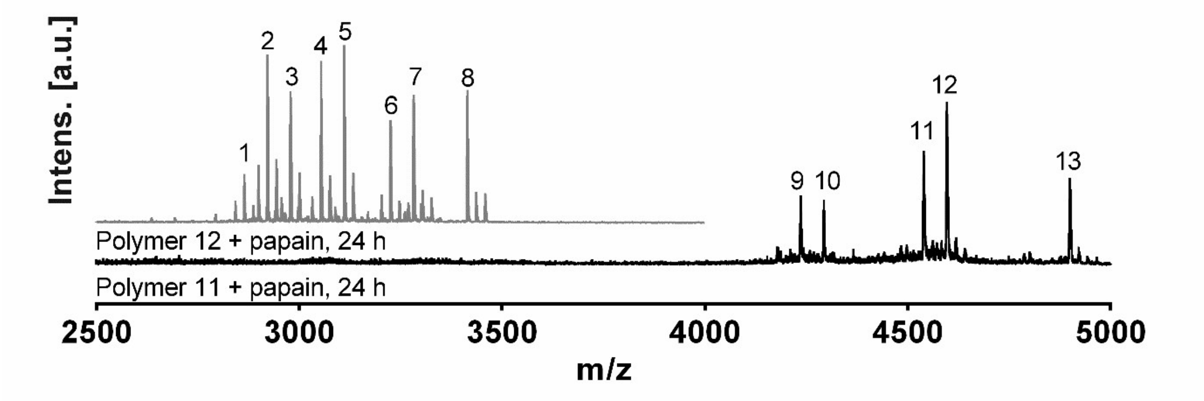


*Figure S21. MALDI-TOF MS spectra recorded in reflector mode of degradable polymers **11** – **12**. Peak numbering corresponds to those tabulated in Table S3. The spectra reveal the narrow range of fragments (4.2 – 4.9 kDa and 2.8 – 3.4 kDa) for polymer **11** and **12**. Peaks are further analyzed - see Figure S22 and Table S3.*

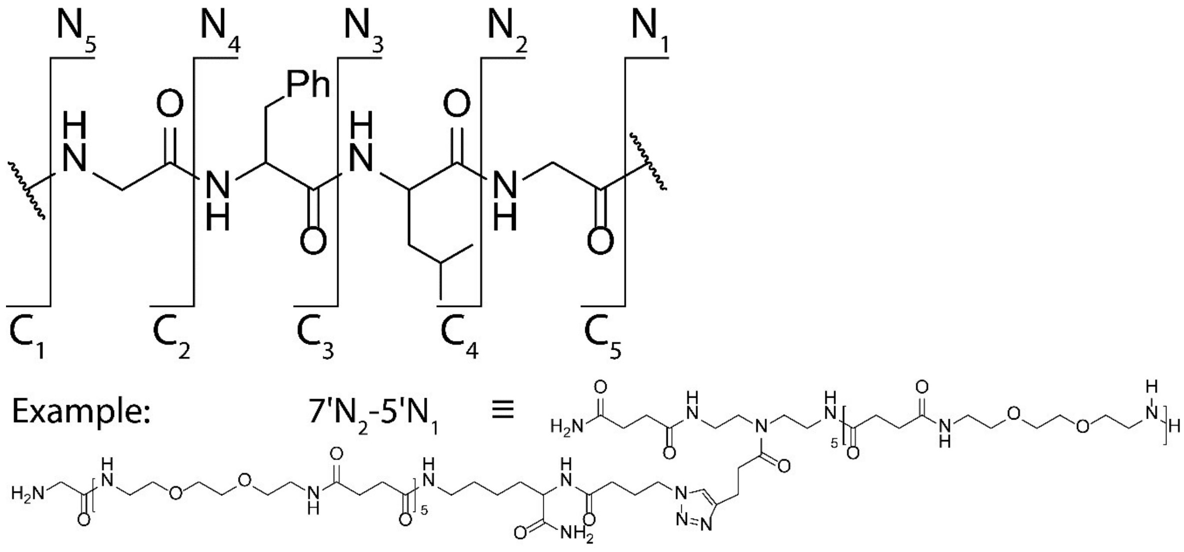


Figure S22. Peptide degradation notation (adapted from mass spectrometry) designating potential enzymatic cleavage sites within the GFLG sequence.

Table S3. Observed major MALDI-MS peaks from spectra in Figure S21 and the potential degradation fragments following the notation in Figure S22. NB: MALDI-MS was operated in positive ion mode and thus the general higher peak intensity with amine- vs. carboxylic acid terminated fragments is reflected in the table with only few carboxylic acid only terminated oligomers observed.

# Peak	m$_{found}$ [m/z]	m$_{calc}$ [m/z]	Degradation fragment ion
Polymer 12 (oligomer 7 + 5)			
1	2862.9	2862.6	[7'N$_1$-5'N$_1$+Na]$^+$
2	2919.9	2919.6	[7'N$_2$-5'N$_1$+Na]$^+$
	-	-	[7'N$_1$-5'N$_2$+Na]$^+$
3	2976.8	2976.6	[7'N$_2$-5'N$_2$+Na]$^+$
4	3052.9	3052.7	[7'C$_1$-5'N$_4$+Na]$^+$
	-	-	[7'C$_4$-5'N$_1$+Na]$^+$
5	3109.9	3109.7	[7'C$_1$-5'N$_5$+Na]$^+$
	-	-	[7'C$_2$-5'N$_4$+Na]$^+$
	-	-	[7'C$_4$-5'N$_2$+Na]$^+$
	-	-	[7'C$_5$-5'N$_1$+Na]$^+$
6	3222.9	3222.8	[7'N$_1$-5'C$_4$+Na]$^+$

	-	-	$[7'N_4\text{-}5'C_1+Na]^+$
7	3280	3279.8	$[7'N_1\text{-}5'C_5+Na]^+$
	-	-	$[7'N_2\text{-}5'C_4+Na]^+$
	-	-	$[7'N_4\text{-}5'C_2+Na]^+$
	-	-	$[7'N_5\text{-}5'C_1+Na]^+$
8	3413.1	3412.8	$[7'C_4\text{-}5'C_4+Na]^+$
Polymer 11 (oligomer 6 + 5)			
9	4233.6	4233.3	$[5'N_2\text{-}6\text{-}5'N_1+Na]^+$
10	4290.4	4290.4	$[5'N_2\text{-}6\text{-}5'N_2+Na]^+$
11	4536.9	4536.5	$[5'C_1\text{-}6\text{-}5'N_4+Na]^+$
	-	-	$[5'N_1\text{-}6\text{-}5'C_4+Na]^+$
12	4593.6	4593.5	$[5'N_1\text{-}6\text{-}5'C_5+Na]^+$
	-	-	$[5'C_1\text{-}6\text{-}5'N_5+Na]^+$
	-	-	$[5'C_2\text{-}6\text{-}5'N_4+Na]^+$
	-	-	$[5'C_4\text{-}6\text{-}5'N_2+Na]^+$
13	4897.0	4896.6	$[5'C_4\text{-}6\text{-}5'C_4+Na]^+$

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