Supporting Information to

pH-Responsive Bioactive Glycopolypeptides with Enhanced Helicity and Solubility in Aqueous Solution

Kai-Steffen Krannig and Helmut Schlaad

Department of Colloid Chemistry, Max Planck Institute of Colloids and Interfaces, Research Campus Golm, 14424 Potsdam, Germany

Materials

All chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. γ-Benzyl-L-glutamate and trifluoroacetic acid (TFA) were supplied from Acros Organics (99+%), DL-allylglycine, L-allylglycine and DL-propargylglycine from BoaoPharma (>98%) and triphosgene from Merck. Tetrahydrofuran (THF) was purchased from VWR and dried over sodium prior to use. Ethylacetate (EtOAc) was purchased from Th. Geyer GmbH and Co KG and dried over CaH_2. Heptane was purchased from Roth (99%). Silica-gel came from Fluka and was dried at 150 °C for 48 h. 1-Thio-β-D-glucopyranose sodium salt was purchased from Carbosynth. 1-Hexylamine was supplied from Aldrich (99.5%) and distilled prior to use. *N*,*N*-Dimethylformamide (DMF, puriss.) and α-pinene were used directly from the bottle under inert atmosphere. Concanavalin A (Con A) for carbohydrate binding studies came from Sigma-Aldrich as lyophilized powder.

Instrumentation

NMR measurements were carried out at room temperature using a Bruker DPX-400 spectrometer operating at 400.1 MHz for ^{1}H NMR at 100.1 MHz for ^{13}C NMR. Deuterated chloroform, acetone, TFA and D_2O were used as solvents (Sigma-Aldrich); signals were referenced to the signal of $CDCl_3$ δ 7.26 ppm, acetone-d_6 δ 2.05 ppm, TFA δ 11.52 ppm and D_2O δ 4.79 ppm, respectively. Melting Points were determined using a MEL-TEMP® apparatus from Lab Devices INC, USA with a Fluke 51 thermometer. Absorbance spectra were recorded on a PG Instruments T70+ spectrometer using UV-Win version 5.1 for data analysis. Circular dichroism (CD) spectra were recorded on a JASCO J-715 spectrometer with 0.01-0.03 wt % solutions of the polymer in Millipore water. Spectra were normalized to the concentration of the respective solution. Size exclusion chromatography (SEC) with simultaneous UV/RI detection was performed with *N*-methyl-2-pyrrolidone (NMP + 5 wt % LiBr) as the eluent at +70 °C using a set of two 300x8 mm^2 PSS-GRAM columns with average particle sizes of 7 µm and porosities of 100 and 1000 Å. SEC in water was performed with 0.1 M aqueous $NaNO_3$ using a combination of two PSS-Suprema columns 300 x 8 mm^2 with particle sizes of 10 µm and porosities of 30 and 3000 Å. Calibration was done with polystyrene standards (PSS,

Mainz, Germany). Analytical ultracentrifugation (AUC) measurements were performed on Beckman Coulter XLI analytical ultracentrifuge at rotational speeds of 60K rpm using Rayleigh interference detection. Sedimentation coefficients were calculated using the software package SEDFIT (version 12.52 beta, P. Schuck 2011). Dynamic light scattering (DLS) experiments were done with 0.02 wt % polymer solutions (dust filtration with 1 µm syringe filter) using an ALV-7004 multiple tau digital correlator equipped with a CGS-3 compact goniometer system, 22 mW He-Ne laser (λ = 632.8 nm), and a pair of avalanche photodiodes operated in a pseudo-cross-correlation mode (ALV, Langen, Germany). The DLS autocorrelation functions were measured at a scattering angle of 90° and evaluated with the CONTIN method.

UV light sources

Photoreactions were performed using either a mercury medium pressure lamp TQ 150 (150W) from Heraeus or a ReptiGlo 5.0 UVB 26W terrarium lamp from ExoTerra.

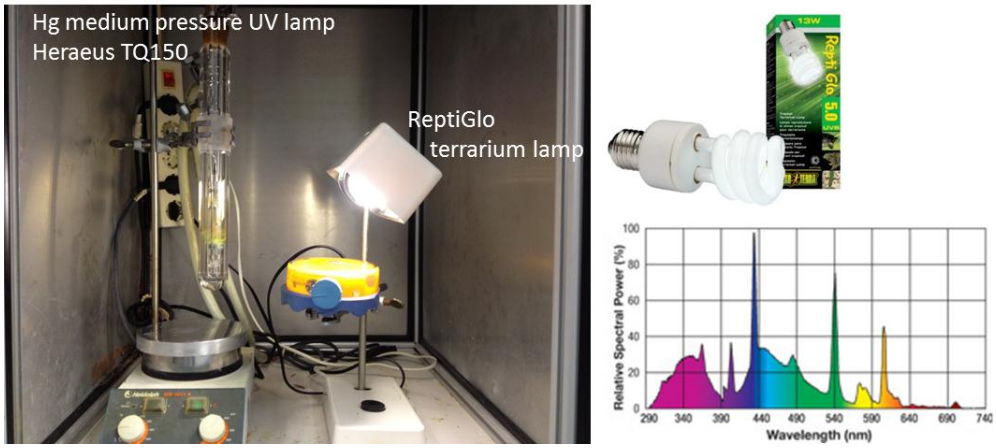

Figure S1. Photographs of the UV light sources used for thiol-ene/yne photochemistry.
Bottom right: UV-visible spectrum of the ReptiGlo terrarium lamp as provided by the manufacturer.

Lectin binding studies

Carbohydrate-protein binding activities were investigated by the increasing absorbance at λ = 450 nm after addition of glucocopolypeptides to a concanavalin A (Con A) solution. 2 mg/mL Con A in HEPES-buffer was stirred for 2 h at room temperature and filtered through a 1 µm glass syringe filter. 1 mg/mL glucocopolypeptides in HEPES-buffer were adjusted at neutral pH. 800 µL Con A solution were transferred into a quartz cuvette (1 cm cell length) and the absorbance was set as zero. Then 80 µL glucocopolypeptide were added and the absorbance was directly measured over a period of 40 min. To prove selective binding two drops of a 10 mg/mL α-methyl mannose solution, which is known to have a higher affinity to Con A than glucose, were added.

Monomer syntheses

γ-Benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA, 1): The NCA was synthesized according to a modified procedure described by Daly and Poché (*Tetrahedron Lett.* **1988**, *29*, 5859-5862): 6.0 g γ-benzyl-L-glutamate (25.3 mmol, 1.0 equiv) were suspended in 200 mL freshly distilled THF and heated to 50 °C. At this temperature 3.0 g triphosgene (10.1 mmol, 0.4 equiv) were added. A clear solution usually formed within 30 min, otherwise additional triphosgene (0.05 equiv/30 min) was added. After 4 h, argon was bubbled through the reaction mixture for 10 min to remove excess phosgene. The solution was concentrated to one third of the volume and precipitated into 10x heptanes. The yellowish precipitate was collected, redissolved in EtOAc and filtered through silica-gel. Upon evaporation of the organic solvent the crude product was recrystallised from EtOAc/Heptane (2:1) to receive 4.5 g (17.2 mmol, 68%) of **1** as colorless needles.

^{1}H NMR (400.1 MHz, $CDCl_3$): δ (ppm) = 7.42-7.34 (m, 5H, Ar-*H*), 6.78 (s, 1H, N*H*), 5.15 (s, 2H, H_2C-Ar), 4.40 (m, 1H, H_2C-C*H*-NH), 2.60 (m, ^3J = 6.96 Hz, 2H, BzO(O)C-CH_2), 2.28 (m, 1H diast., *H2*C-CH-NH), 2.14 (m, 1H diast., H_2C-CH-NH). ^{13}C NMR (100.1 MHz, $CDCl_3$): δ (ppm) = 172.4 (-*C*(=O)O-), 169.4 (-N-*C*(=O)-O-), 152.0 (-CH-*C*(=O)-O-), 135.2 (p-Ar), 128.7 (m/o-Ar), 128.4 (m/o-Ar), 67.1 (Ar-*C*H_2-), 56.8 (*C*(=O)-C*H*-NH-), 29.7 (CH_2-*C*H_2-C*H*-), 26.9 (C(=O)-CH_2-*C*H_2-). Mp: 93-94 °C (rep. 96-97 °C).

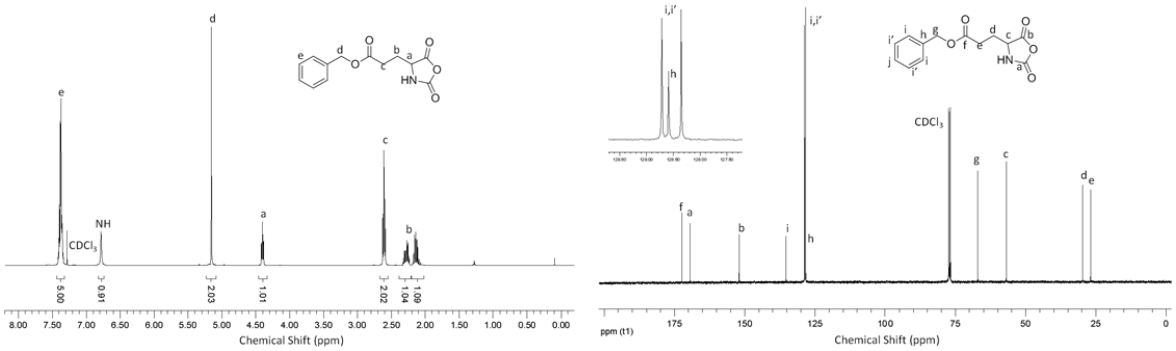

Figure S2. ^{1}H (left) and ^{13}C NMR spectra (right) of γ-benzyl-L-glutamate NCA (**1**) in $CDCl_3$.

L-/DL-Allylglycine *N*-carboxyanhydride (LAG-NCA, 2a/DLAG-NCA, 2b): The NCAs were synthesized according to a method described previously by Sun et al. (*Macromolecules* **2010**, *43*, 4445-4448). 2.5 g allylglycine (21.7 mmol, 1.0 equiv) were suspended in 100 mL freshly distilled THF and heated to 50 °C. At this temperature, 13.75 mL α-pinene (86.8 mmol, 4.0 equiv) and 2.57 g triphosgene (8.7 mmol, 0.4 equiv) were added and a constant stream of argon was flushed through the reaction mixture. A clear solution usually formed within 45 min, otherwise additional triphosgene was added (0.05 equiv/30 min). After 3h, the solution was concentrated to one third of the volume and precipitated in excess heptanes. The white precipitate was collected, redissolved in EtOAc and filtered through silica-gel. Upon removing the organic solvent the crude product was precipitated from EtOAc/Heptane (2:1). Yield: L-allylglycine NCA (**2a**) 2.8 g (12.4 mmol, 57%), DL-allyglycine NCA (**2b**) 1.6g (11.3 mmol, 52%).

^{1}H NMR (400.1 MHz, $CDCl_3$): δ (ppm) = 6.59 (s, 1H, N*H*), 5.74 (m, 1H, H_2C=C*H*), 5.28 (m, 2H, H_2C=CH), 4.40 (dd, ^{3}J = 7.0 Hz, ^{4}J = 4.3 Hz, 1H, H2C-C*H*-NH), 2.53 (td, ^{3}J = 14.6 Hz, ^{3}J = 7.4 Hz, 1H diast., H_2C-CH-NH), 2.10 (td, ^{3}J = 14.6 Hz, ^{3}J = 7.4 Hz, 1H diast., H_2C-CH-NH). ^{13}C NMR (100.1 MHz, $CDCl_3$): δ (ppm) = 168.8 (-N-*C*(=O)-O-), 152.5 (-CH-*C*(=O)-O-), 129.9 (H_2C=CH-), 121.5 (H_2C=CH), 57.2 (*C*(=O)-C*H*-NH-), 35.8 (HC-CH_2-CH). Mp: **2a**: 48 °C (rep. 48 °C), **2b**: 89.5-90.5 °C (rep. 89-91 °C).

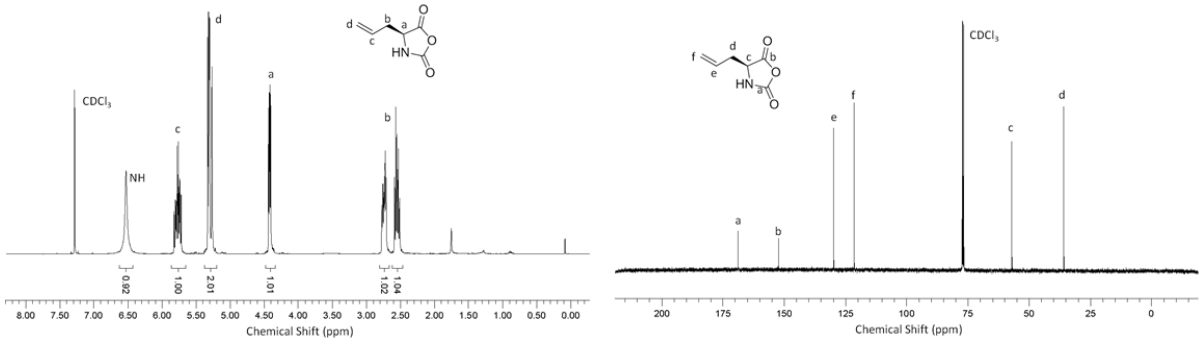

Figure S3. ^{1}H (left) and ^{13}C NMR spectra (right) of L-allylglycine NCA (**2a**) in $CDCl_3$.

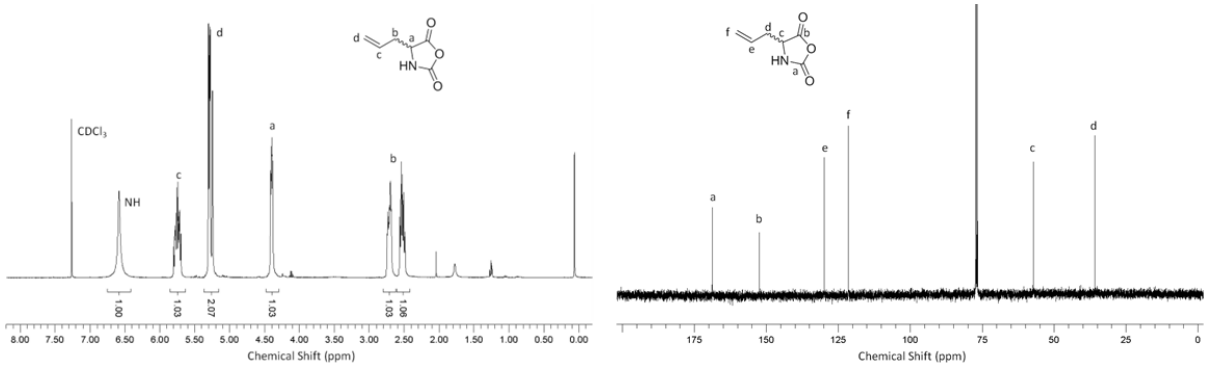

Figure S4. ^{1}H (left) and ^{13}C NMR spectra (right) of DL-allylglycine NCA (**2b**) in $CDCl_3$.

DL-Propargylglycine *N*-carboxyanhydride (DLPG-NCA, 2c): The NCA was synthesized according to a modified procedure described by Huang et al. (*Macromolecules* **2010**, *43*, 6050-6057.). 1.5 g DL-propargylglycine (13.3 mmol, 1.0 equiv) were suspended in 40 mL freshly distilled THF and heated to 50 °C. At this temperature 8.4 mL α-pinene (53.2 mmol, 4.0 equiv) and 2.96 g triphosgene (10.0 mmol, 0.75 equiv) in 10 mL THF were added slowly and a constant stream of Argon was bubbled through the reaction mixture. A clear solution usually formed within 30 min, otherwise additional triphosgene (0.05 equiv/30 min) was added. After 4h, the solution was concentrated to one third of the volume and precipitated in 10x heptanes. The yellowish precipitate was collected, and recrystallised from THF/Heptane (2:1) to receive 0.46 g (3.3 mmol, 25%) of the NCA as white-yellow crystals.

^{1}H NMR (400.1 MHz, acetone-d_6): δ (ppm) = 7.99 (s, 1H, N*H*), 4.73 (td, ^{3}J = 4.5 Hz, ^{4}J = 1.08 Hz, 1H, C*H*), 2.83 (dd, ^{3}J = 4.50 Hz, ^{3}J = 2.63 Hz, 2H, H_2C-C*H*-NH), 2.59 (t, ^{3}J = 2.64 Hz, 1H, H_2C-CH-NH), ^{13}C NMR (100.1 MHz, $CDCl_3$): δ (ppm) = 171.0 (-*C*(=O)O-), 153.1 ((-N-*C*(=O)-O-), 78.6 (HC-*C*-CH_2), 74.0 (H*C*-C-CH2), 57.8 (H*C*-NH), 22.7 (CH_2). Mp: 119-121 °C (rep. 114 °C).

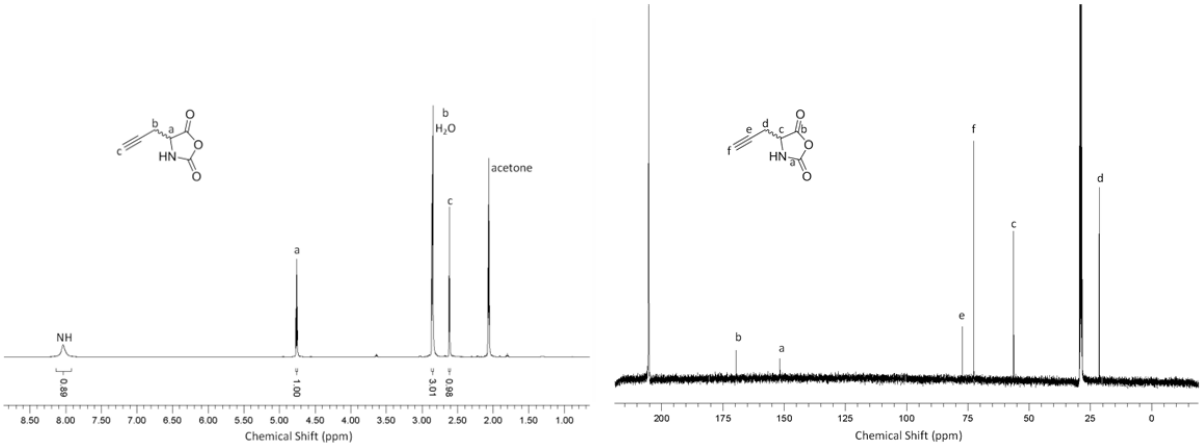

Figure S5. ^{1}H (left) and ^{13}C NMR spectra (right) of DL-propargylglycine NCA (**2c**) in acetone-d_6.

General polymerization procedure

γ-Benzyl-L-glutamate NCA (BLG-NCA) and the respective amount of the co-NCA were dissolved in little THF and transferred into a reactor. Upon removing the solvent by cryo-condensation DMF was added to obtain a solution of 4% by weight. The reaction mixture was degassed by three consecutive freeze-pump-thaw cycles. The polymerization was initiated by addition of a 0.1 M solution of freshly distilled 1-hexylamine in DMF. The vessel was evacuated (0.5 mbar) and the reaction-mixture stirred at room-temperature for 7 d. Each day, the polymerization was degassed twice to remove CO_2. The polymerization was terminated by precipitation into 10x MeOH. The product was collected by centrifugation and dried at 65 °C in high *vacuo*.

Poly[BLG-*co*-LAG] (3a): 4.0 g BLG-NCA (15.2 mmol, 10.0 equiv), 215 mg L-allylglycine NCA (1.52 mmol, 1.0 equiv), 105 mL DMF, 3.04 mL 0.1 1M $HexNH_2$ (0.3 mmol, 0.2 equiv). Yield: 3.05 g (88%). ^{1}H NMR (400.1 MHz, TFA-d_1): δ (ppm) = 7.48-7.02 (240H), 5.70-5.50 (5H), 5.30-4.90 (105H), 4.80-4.40 (53H), 3.30 (2H), 2.80-1.75 (202H), 1.50 (2H), 1.30 (6H), 0.80 (3H, CH_3). SEC (NMP, 70 °C, PS calibration): polydispersity index, PDI = 1.20.

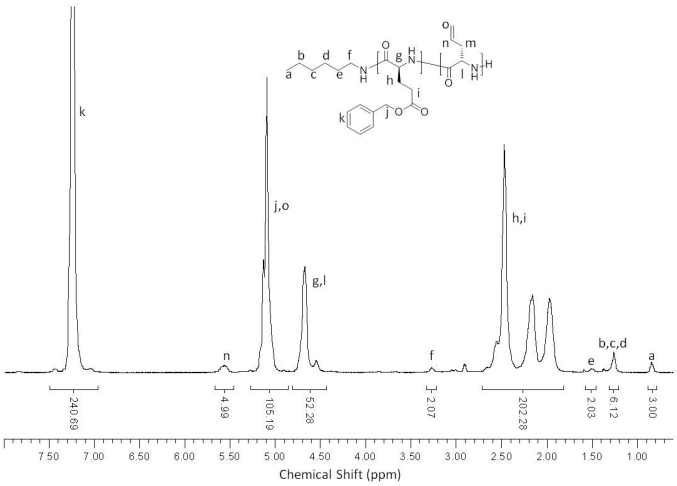

Figure S6. ^{1}H NMR spectrum of poly[BLG-*co*-LAG] (**3a**) in TFA-d_1.

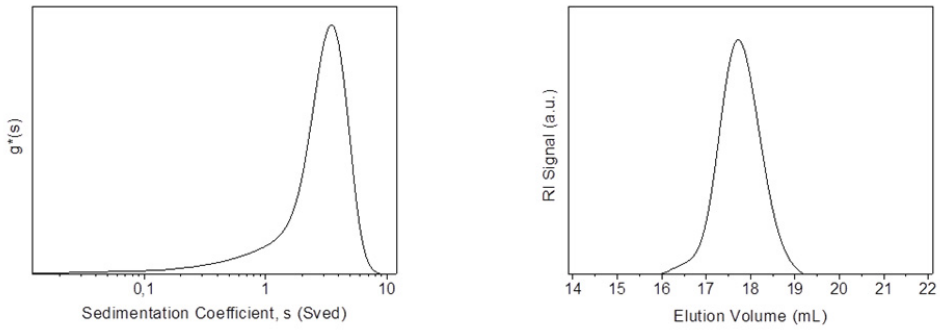

Figure S7. AUC sedimentation coefficient distribution (THF, left) and SEC RI trace (NMP, right) of poly[BLG-*co*-LAG] (**3a**).

Poly[BLG-*co*-DLAG] (3b): 5.0 g BLG-NCA (19.01 mmol, 10.0 equiv), 268 mg DL-allylglycine NCA (1.90 mmol, 1.0 equiv), 133 mL DMF, 3.80 mL 0.1 1M $HexNH_2$ (0.38 mmol, 0.2 equiv). Yield: 3.81 g (87%). ^{1}H NMR (400.1 MHz, TFA): δ (ppm) = 7.48-7.02 (250H), 5.70-5.50 (6H), 5.40-5.00 (110H), 4.60-4.90 (56H), 3.30 (2H), 2.80-1.75 (215H), 1.55 (2H), 1.30 (6H), 0.80 (3H, CH_3). SEC (NMP, 70 °C, PS calibration): PDI = 1.10.

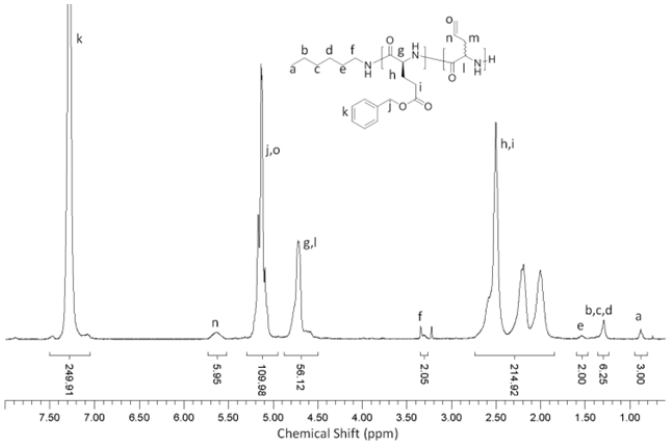

Figure S8. ^{1}H NMR spectrum of poly[BLG-*co*-DLAG] (**3b**) in TFA-d_1.

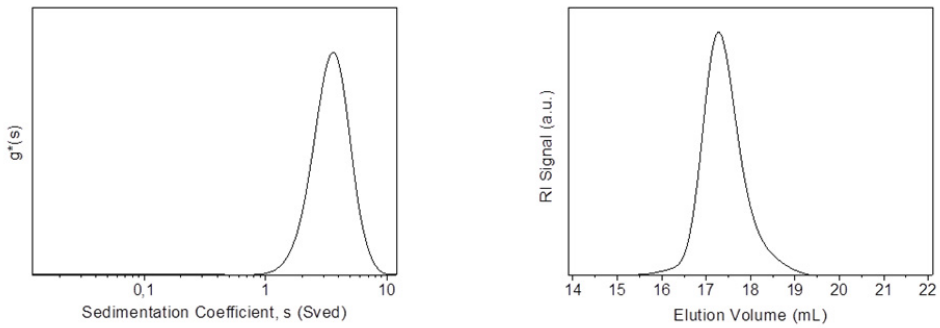

Figure S9. AUC sedimentation coefficient distribution (THF, left) and SEC RI trace (NMP, right) of poly[BLG-*co*-DLAG] (**3b**).

Poly[BLG-*co*-DLPG] (3c): 4.4 g BLG-NCA (16.7 mmol, 5.0 equiv), 462 mg DL-propargylglycine NCA (3.2 mmol, 1.0 equiv), 120 mL DMF, 3.34 mL 0.1M $HexNH_2$ (0.33 mmol, 0.1 equiv). Yield: 3.3 g (83%). ^{1}H NMR (400.1 MHz, TFA-d_1): δ (ppm) = 7.48-7.02 (254H), 4.90-5.20 (100H), 4.50-5.80 (60H), 3.30 (2H), 2.80-1.75 (231H), 1.55 (2H), 1.30 (6H), 0.80 (3H, CH_3). SEC (NMP, 70 °C, PS calibration): PDI = 1.17.

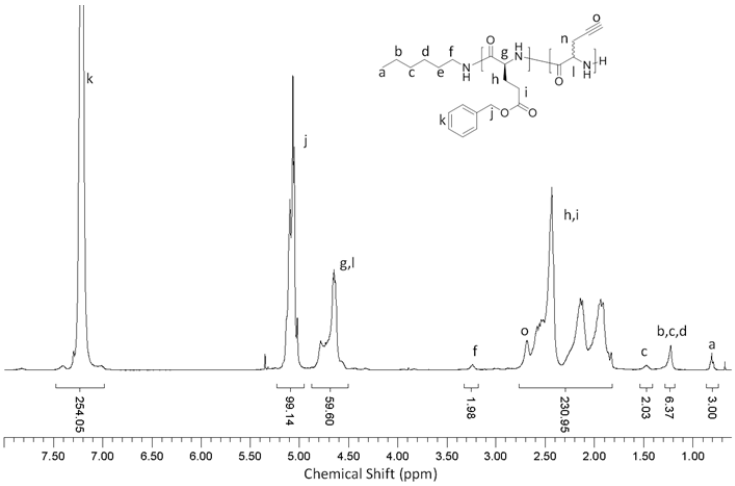

Figure S10. ^{1}H NMR spectrum of poly[BLG-*co*-DLPG] (**3c**) in TFA-d_1.

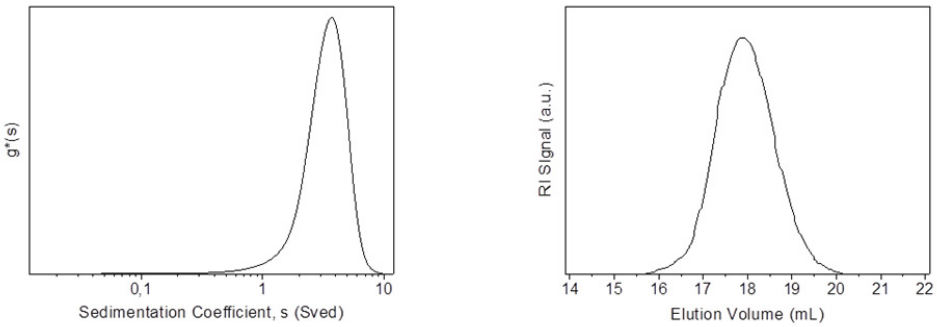

Figure S11. AUC sedimentation coefficient distribution (THF, left) and SEC RI trace (NMP, right) of poly[BLG-*co*-DLPG] (**3c**).

General debenzylation procedure

Polymer (1.0 equiv relative to BLG) was dissolved in TFA (30 equiv) under dry conditions. Upon cooling to 5 °C methanesulfonic acid (MSA) (34 equiv) and anisole (5 equiv) were added under vigorous stirring. The reaction mixture was stirred for 18 min at this temperature and then for further 20 min at room temperature. The polymer was precipitated from 10x Et_2O and collected by centrifugation. The centrifugate was dissolved in 10% $NaHCO_3$, extensively dialyzed (RC 1000) against Millipore water for 2 d and finally freeze-dried from water to obtain the debenzylated polymer as a brittle solid.

Poly[LG-*co*-LAG] (4a): 1.0 g **3a** (1.0 equiv, 4.34 mmol relative to BLG), 10.03 mL TFA (30 equiv, 130 mmol), 9.59 mL MSA (34 equiv, 148 mmol), 1.59 mL anisole (5.0 equiv, 22 mmol). Yield: 505 mg (83%), debenzylation: 98%. ^{1}H NMR (400.1 MHz, D_2O): δ (ppm) = 5.70-5.60 (5H), 5.20-5.00 (10H), 4.40-4.00 (52H), 3.10 (2H), 2.50-2.30 (12H), 2.40-1.70 (199H), 1.55 (2H), 1.30 (6H), 0.80 (3H, C*H*$_3$).

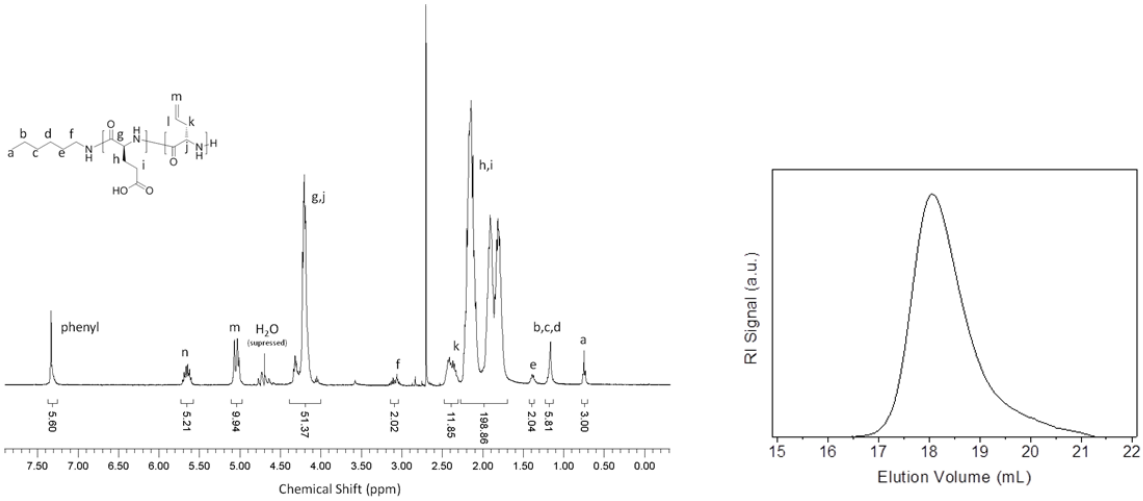

Figure S12. ^{1}H NMR spectrum (D_2O, left) and SEC RI trace (0.1 M aq $NaNO_3$, right) of poly[LG-*co*-LAG] (**4a**).

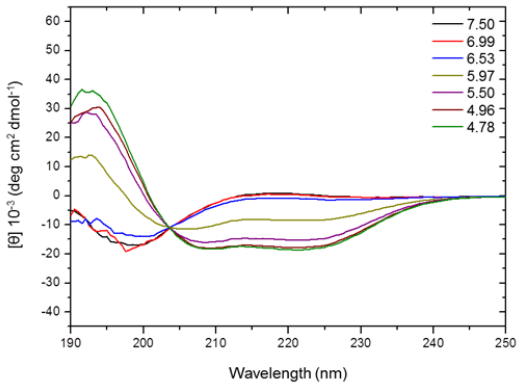

Figure S13. CD spectra of poly[LG-*co*-LAG] (**4a**) in water at different pH.

Poly[LG-*co*-DLAG] (4b): 1.0 g **3b** (1.0 equiv, 4.31 mmol relative to BLG), 9.97 mL TFA (30 equiv, 129 mmol), 9.52 mL MSA (34 equiv, 147 mmol), 1.58 mL anisole (5.0 equiv, 22 mmol). Yield: 510 mg (84%), debenzylation: 98%. ^{1}H NMR (400.1 MHz, D_2O): δ (ppm) = 5.80-5.70 (6H), 5.30-5.10 (12H), 4.50-4.10 (56H), 3.20 (2H) 2.70-2.40 (12H), 2.40-1.70 (200H), 1.55 (2H), 1.30 (6H), 0.80 (3H, C*H*$_3$).

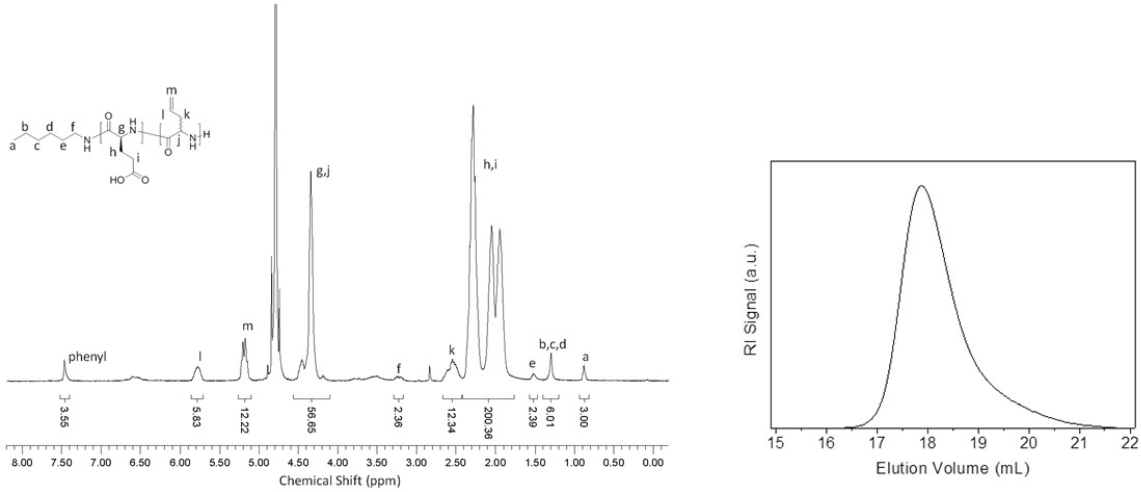

Figure S14. ^{1}H NMR spectrum (D_2O, left) and SEC RI trace (0.1 M aq $NaNO_3$, right) of poly[LG-*co*-DLAG] (**4b**).

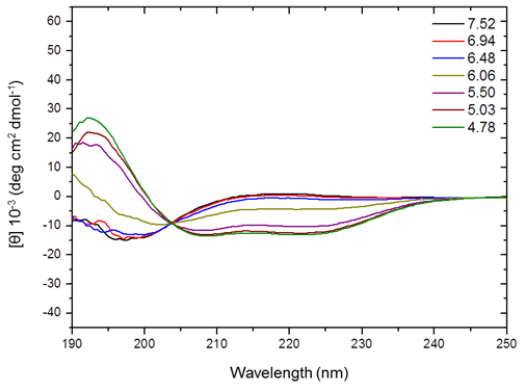

Figure S15. CD spectra of poly[LG-*co*-DLAG] (**4b**) in water at different pH.

Poly[LG-*co*-DLPG] (4c): 0.8 g **3c** (1.0 equiv, 3.34 mmol relative to BLG), 7.72 mL TFA (30 equiv, 100 mmol), 7.37 mL MSA (34 equiv, 113 mmol), 1.22 mL anisole (5.0 equiv, 17 mmol). Yield: 485 mg (87%), debenzylation: 97%. ^{1}H NMR (400.1 MHz, D_2O): δ (ppm) = 4.50-4.40 (11H), 4.40-4.10 (50H), 3.10 (2H), 2.70-2.50 (20H), 2.30-1.60 (208H), 1.55 (2H), 1.30 (6H), 0.80 (3H, C*H*$_3$).

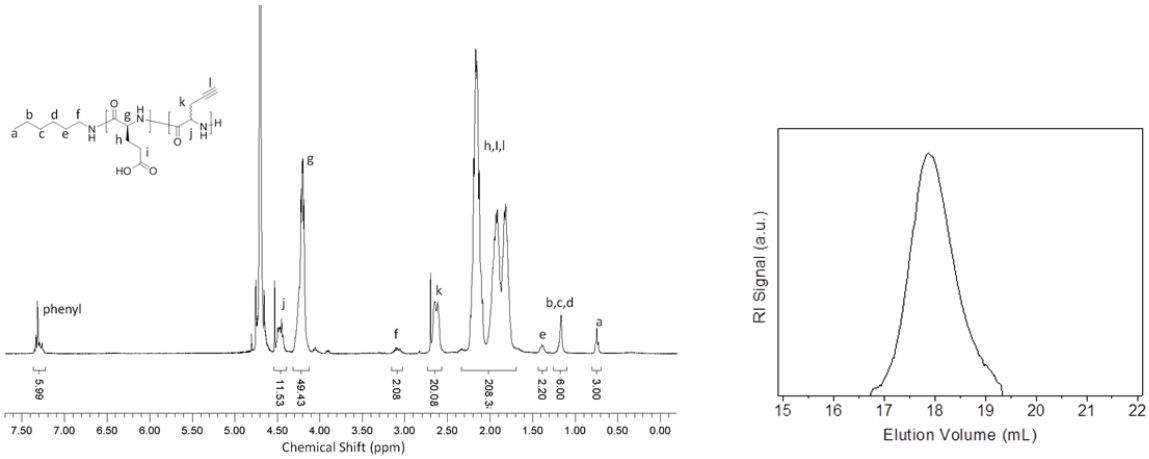

Figure S16. ^{1}H NMR spectrum (D_2O, left) and SEC RI trace (0.1 M aq $NaNO_3$, right) of poly[LG-*co*-DLPG] (**4c**).

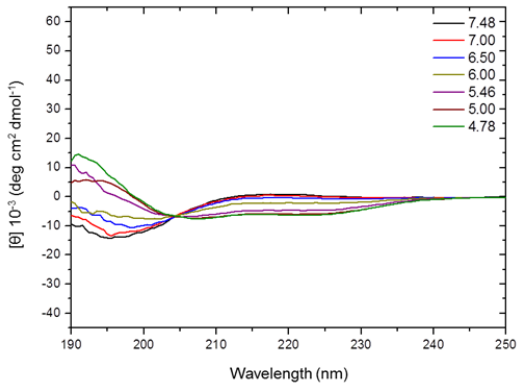

Figure S17. CD spectra of poly[LG-*co*-DLPG] (**4c**) in water at different pH.

General glycosylation procedure: 1-Thio-β-D-glucose (**5**) was dissolved in little water and freshly prepared acidic resin (DOWEX 50) was added. Upon stirring for 20 min the solution was filtered and the filtrate concentrated by freeze-drying under high vacuum over night to yield a hygroscopic solid (storage in the freezer under argon!). Polymer (1.0 equiv relative to allyl-/propargylglycine repeating units) and the relative amount of sugar (1.5 equiv) were dissolved in 0.1M acetate buffer (1.0 wt % relative to allyl-/propargylglycine units) and degassed using Argon. The vessel was sealed and placed under the UV-lamp overnight. The reaction mixture was diluted and extensively dialyzed (RC 1000) against Millipore water for 2d. Freeze drying from water yielded the photoaddition product as a fluffy or brittle solid depending on the amount of water used for freeze-drying.

Gluco-poly[LG-*co*-LAG] (6a): 90 mg **4a** (1.0 equiv, 67 µmol relative to allylglycine), 19.85 mg 1-thio-β-D-glucose (1.5 equiv, 101 mmol), 1.51 mg Irgacure 2959 (0.1 equiv, 7 µmol), 0.64 mL 0.1M acetate-buffer (pH 4.75). Yield: 86 mg (83%). ^{1}H NMR (400.1 MHz, D_2O): δ (ppm) = 4.40 (5H), 4.30-3.90 (55H), 3.90-3.20 (32H), 3.10 (2H), 2.80-1.50 (234H), 1.40 (2H), 1.30 (6H), 0.75 (3H, CH_3).

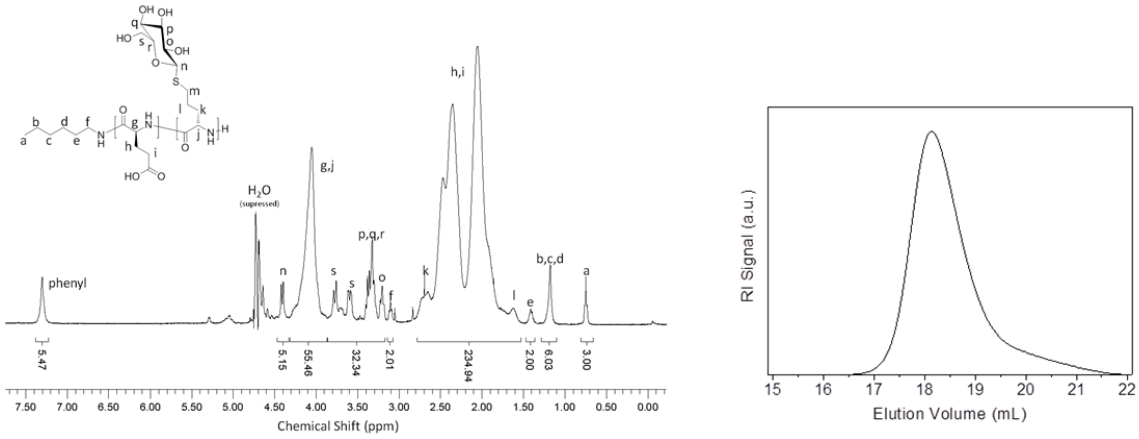

Figure S18. ^{1}H NMR spectrum (D_2O, left) and SEC RI trace (0.1M aq $NaNO_3$, right) of gluco-poly[LG-*co*-LAG] (**6a**).

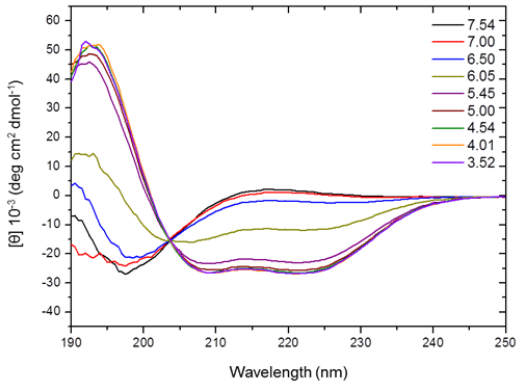

Figure S19. CD spectra of gluco-poly[LG-*co*-LAG] (**6a**) in water at different pH.

Gluco-poly[LG-co-DLAG] (6b): 100 mg **4b** (1.0 equiv, 85 µmol relative to allylglycine), 25.13 mg 1-thio-β-D-glucose (1.5 equiv, 128 mmol), 1.92 mg Irgacure 2959 (0.1 equiv, 9 µmol), 0.81 mL 0.1M acetate-buffer (pH 4.75). Yield 95 mg (81%). ^{1}H NMR (400.1 MHz, D_2O): δ (ppm) = 4.40 (6H), 4.30-3.90 (55H), 3.80 (6H), 3.60 (6H), 3.50-3.20 (23H), 3.10 (2H), 2.80-2.60 (12H), 2.50-1.50 (226H), 1.40 (2H), 1.25 (6H), 0.75 (3H, C*H*$_3$).

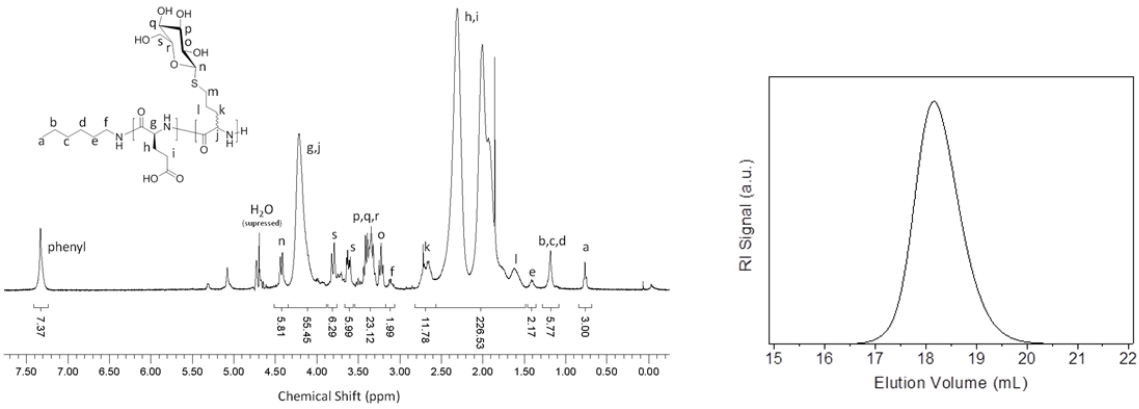

Figure S20. ^{1}H NMR spectrum (D_2O, left) and SEC RI trace (0.1M aq $NaNO_3$, right) of gluco-poly[LG-*co*-DLAG] (**6b**).

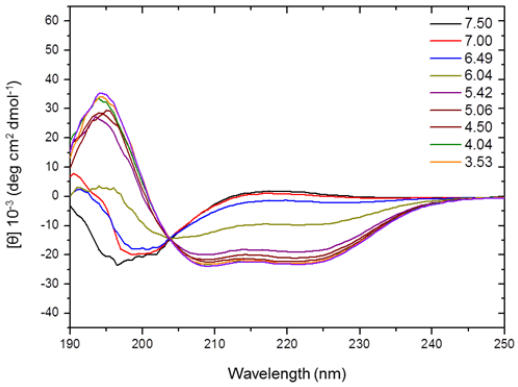

Figure S21. CD spectra of gluco-poly[LG-*co*-DLAG] (**6b**) in water at different pH.

Gluco-poly[LG-*co*-DLPG] (6c): 120 mg **4c** (1.0 equiv, 162 µmol relative to 2x addition onto propargylglycine), 95.3 mg 1-thio-β-D-glucose (1.5 equiv, 486 mmol), 3.63 mg Irgacure 2959 (0.1 equiv, 16 µmol), 1.54 mL 0.1M acetate-buffer (pH 4.75). Yield: 140 mg (92%). ^{1}H NMR (400.1 MHz, D_2O): δ (ppm) = 6.30 (5H), 5.20 (5H), 4.60-4.45 (15H), 4.40-4.10 (60H), 4.00-3.00 (78H), 2.80-2.40 (16H), 2.30-1.60 (232H), 1.40 (2H), 1.25 (6H), 0.75 (3H, CH_3).

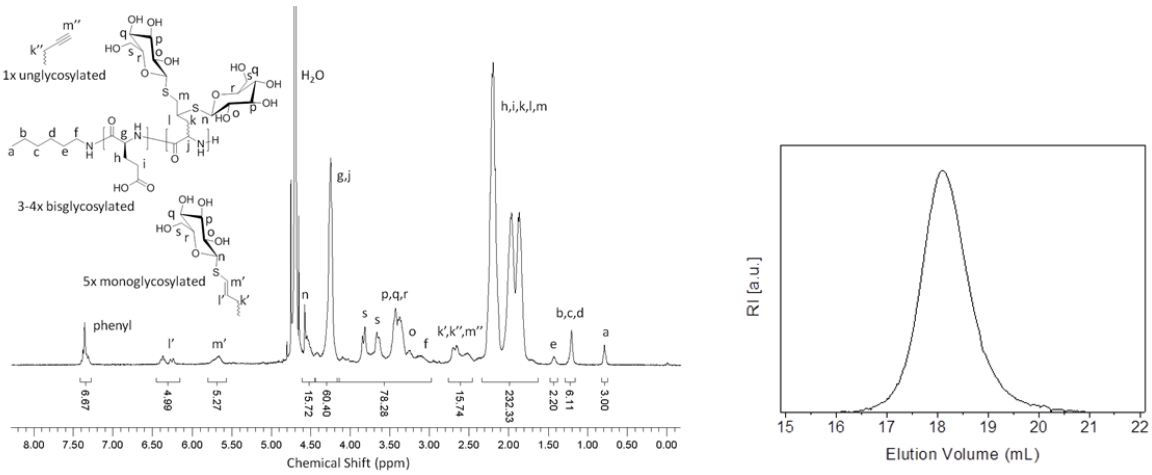

Figure S22. ^{1}H NMR spectrum (D_2O, left) and SEC RI trace (0.1M aq $NaNO_3$, right) of gluco-poly[LG-*co*-DLPG] (**6c**).

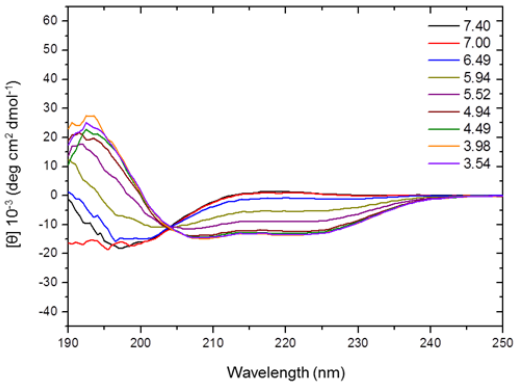

Figure S23. CD spectra of gluco-poly[LG-*co*-DLPG] (6**c**) in water at different pH.

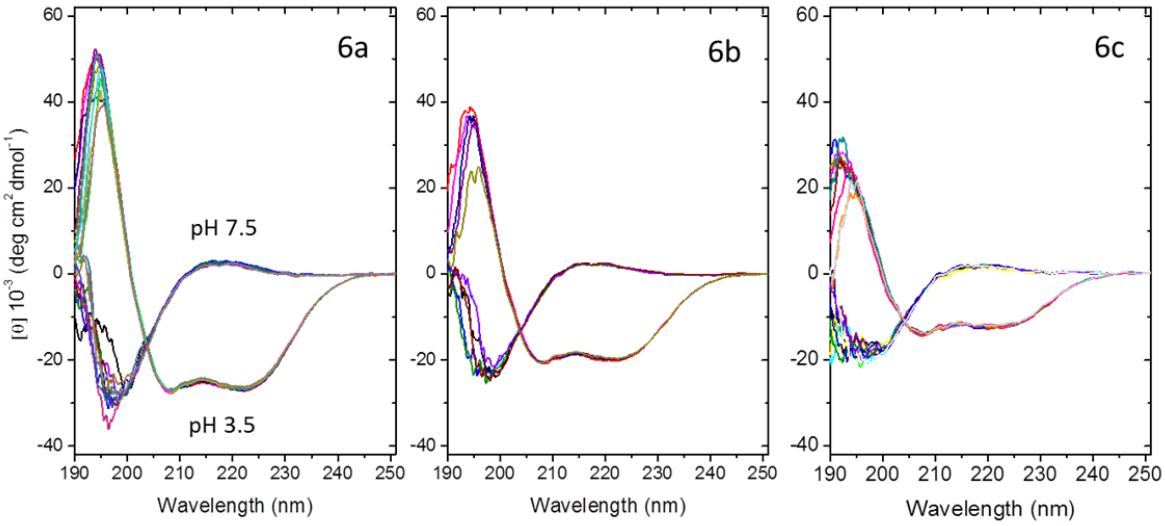

Figure S24. CD spectra of the glucosylated samples **6a-c** at pH 7.5 and pH 3.5, 10 pH cycles, in water.

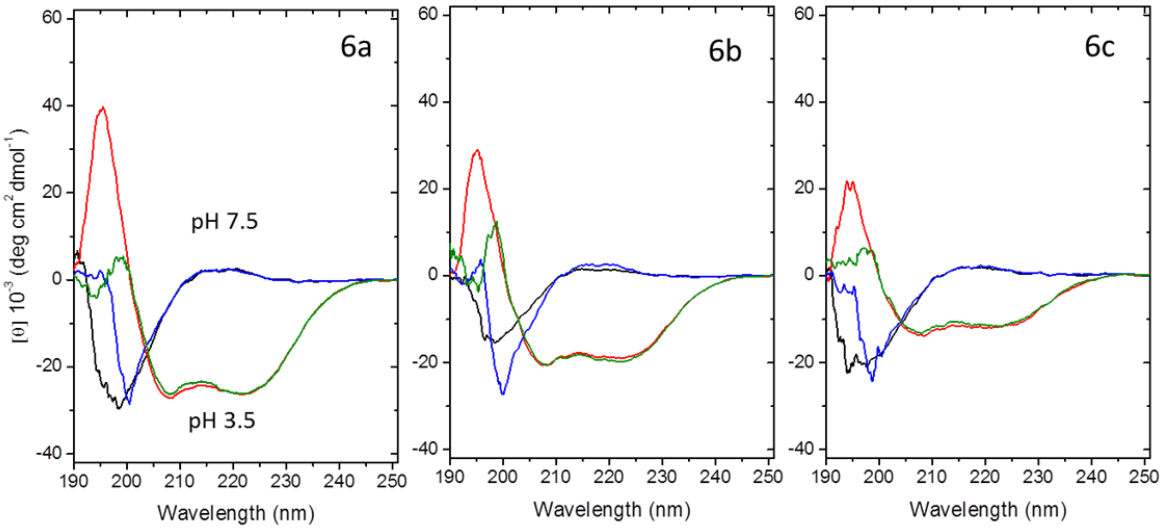

Figure S25. CD spectra of the glucosylated samples **6a-c** at pH 7.5 and pH 3.5 after 10 pH cycles (black and red lines) and further addition of NaCl (0.9 wt %) (blue and green lines).