

Additional materials and methods

FT-IR was performed on a Thermo Nicolet Nexus 870 equipped with a KBr beamsplitter, DTGS-KBr detector, and DuraSamplIR II ATR accessory (SENSIR).

Attempts at PPLQ synthesis by direct polymerization

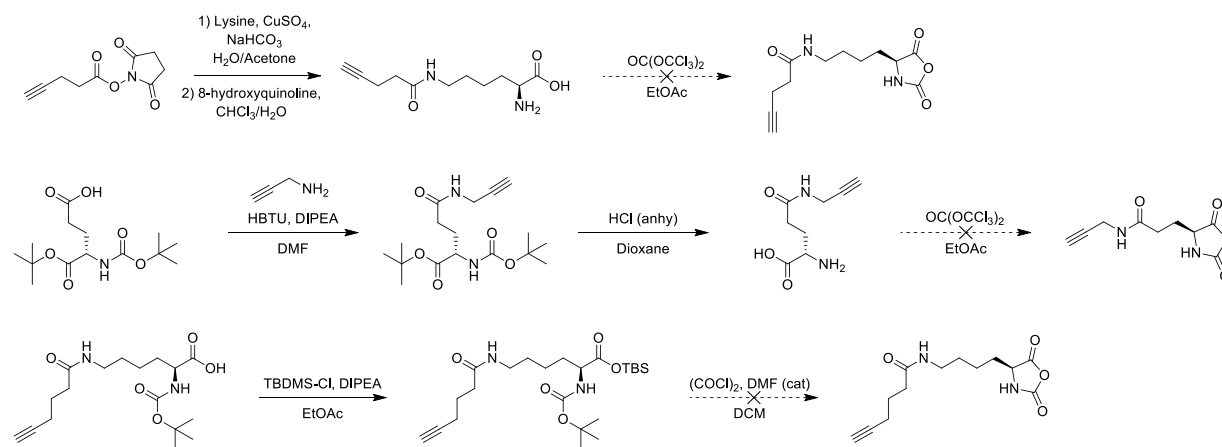


Figure S1. Strategies for synthesis of NCA monomer with an amide bond on the side chain. Formation of the amino acid was not an issue, but subsequent reactions proved unsuccessful. Synthesis of NCA by phosgene or triphosgene (Fuchs-Farthing method) begins by a dispersion of amino acid in organic solvent. Normally, the dispersion becomes a homogenous solution as the insoluble amino acid is converted to soluble NCA over the course of the reaction. For all conditions tested, the amino acid remained insoluble, indicating no conversion of amino acid to NCA. Various solvents (ethyl acetate, dichloroethane, dioxane, dichloromethane, tetrahydrofuran, acetonitrile) and additives (3 eq. pinene to amino acid, added prior to triphosgene) were screened by the same general procedure for NCA synthesis. A different amino acid structure resembling γ -propargyl-L-glutamine was also tested. None of the conditions described changed the outcome of the reaction. Changing the method of NCA formation by protection of the α -carboxylic acid by TBDMS followed by treatment with oxalyl chloride/DMF was also tested. TLC of the reaction did not show formation of product but rather reversion of the starting material to the unprotected carboxylic acid.

4-Pentynoic acid N-hydroxysuccinimide ester

To a stirring solution of 4-pentynoic acid (4.49 g, 45.7 mmol) and N-hydroxysuccinimide (5.54 g, 48.2 mmol) in DCM (185 mL) was added EDC HCl (9.24 g, 48.2 mmol) in one portion. The reaction was stirred overnight. Afterwards the solution was washed thrice with water (150 mL), rinsed with brine, and dried over MgSO_4 . The solvent was removed *in vacuo* to yield a yellow-white solid (5.51 g, 28.2 mmol, 61%). ^1H NMR (400 MHz, CDCl_3) δ 2.94 – 2.86 (m, 2H), 2.84 (s, 4H), 2.67 – 2.57 (m, 2H), 2.06 (t, J = 2.7 Hz, 1H).

ϵ -6-Pentynoic-L-lysine¹

To a stirring solution of L-lysine (0.47 g, 2.56 mmol) and sodium bicarbonate (0.90 g, 10.24 mmol) in water (7 mL) was added a solution of Cu(II) sulfate (0.32 g, 1.28 mmol) in water (3.5 mL) dropwise over 1 minute. The solution was stirred for 10 minutes after which a solution of 4-Pentynoic acid N-hydroxysuccinimide ester (0.50 g, 2.56 mmol) in acetone (3.5 mL) was added dropwise over 2 minutes. The reaction was stirred for 17 hours after which the precipitate was removed by filtration and washed with water followed by acetone. The isolated solid (0.66 g) was dissolved in a 1:1 mixture of chloroform and water (30 mL). 8-Hydroxyquinoline (0.55 g, 3.84 mmol) was added in one portion and the solution was stirred for 10 minutes. The precipitate was removed by filtration and the aqueous solution was washed four times with chloroform (30 mL). Residual chloroform was removed by sparging with N₂ and the aqueous solution was lyophilized to give a white powder (0.46 g, 2.01 mmol, 79%). ¹H NMR (400 MHz, D₂O) δ 3.73 (t, *J* = 6.1 Hz, 1H), 3.23 (t, *J* = 6.8 Hz, 2H), 2.56 – 2.39 (m, 4H), 1.98 – 1.79 (m, 2H), 1.65 – 1.50 (m, 2H), 1.50 – 1.30 (m, 2H).

γ -Propargyl-Boc-L-Gln-OtBu

To a stirring solution of Boc-L-Glu-OtBu (3.00 g, 9.89 mmol) and DMF (90 mL) was added HBTU (4.50 g, 11.7 mmol) in one portion. Upon complete dissolution of the solids, propargylamine (0.665 mL, 10.4 mmol) followed by DIPEA (4.31 mL, 24.7 mmol) were added. The reaction was stirred for 3 hours after which EtOAc (360 mL) was added and the solution was washed with water (3 x 100 mL), rinsed with brine, and dried over MgSO₄. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (10% followed by 30% EtOAc:DCM) yielding a white solid (2.47 g, 7.26 mmol, 73%). ¹H NMR (400 MHz, CDCl₃) δ 6.53 (s, 1H), 5.25 (d, *J* = 7.5 Hz, 1H), 4.06 (dd, *J* = 5.2, 2.6 Hz, 2H), 2.35 – 2.25 (m, 2H), 2.23 (t, *J* = 2.6 Hz, 1H), 2.22 – 2.11 (m, 1H), 1.93 – 1.80 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H). R_f 0.67 (20% EtOAc:DCM, visualized with CAM).

γ -Propargyl-L-glutamine

To a stirring solution of 4M HCl in dioxane (3.8 mL, 15.2 mmol) was added γ -propargyl-Boc-L-Gln-OtBu (0.496 g, 1.38 mmol) in one portion, and the reaction was stirred for 3 hours. EtOAc was added and the precipitate was collected by centrifugation and dried *in vacuo* yielding an off white powder (291 mg, 1.32 mmol, 96%). ¹H NMR (400 MHz, D₂O) δ 4.05 (t, *J* = 6.4 Hz, 1H), 3.97 (d, *J* = 2.2 Hz, 2H), 2.61 (s, 1H), 2.59 – 2.35 (m, 2H), 2.35 – 2.13 (m, 2H).

N α -Boc-N ϵ -5-hexynoic-L-lysine

To a stirring solution of NHS (2.22 g, 19.0 mmol) and 5-hexynoic acid (2.03 g, 18.1 mmol) in DCM (76 mL) was added solid EDC (3.74 g, 19.5 mmol) over 30 seconds. The reaction was stirred overnight, after which consumption of the starting material was confirmed by TLC. The solution was washed three times with water, rinsed with brine, dried over MgSO₄, and the solvent was removed *in vacuo*. The resulting oil was used without further purification. R_f 0.3 (30% EtOAc:Hexane, visualized with KMnO₄).

To a chilled, stirred solution of Boc-Lys-OH (4.46 g, 18.1 mmol) in saturated sodium bicarbonate (45 mL) was added the 5-hexynoic acid NHS ester in a solution of THF (45

mL) dropwise over 5 minutes. The reaction was stirred at room temperature for 24 hours. The THF was removed *in vacuo* and the resulting aqueous solution was cooled to 0°C by an external ice bath. The pH was adjusted to 2 by dropwise addition of 1N HCl with stirring. Afterwards, the aqueous solution was extracted by EtOAc (75 mL) and the organic extract was rinsed with brine and dried over MgSO₄. Removal of solvent *in vacuo* yielded a white solid (5.12 g, 15.0 mmol, 83% over 2 steps) ¹H NMR (400 MHz, Deuterium Oxide) δ 3.74 (t, *J* = 6.1 Hz, 1H), 3.21 (t, *J* = 6.9 Hz, 2H), 2.42 – 2.32 (m, 3H), 2.25 (td, *J* = 7.0, 2.7 Hz, 2H), 1.98 – 1.74 (m, 4H), 1.68 – 1.51 (m, 2H), 1.51 – 1.31 (m, 2H).

Attempted formation of N_ε-5-hexynoic-L-lysine NCA by oxalyl chloride²

To a chilled, stirring solution of N_α-Boc-N_ε-5-hexynoic-L-lysine (228 mg, 0.674 mmol) in EtOAc (4 mL) was added TBDMS-Cl (114 mg, 0.756 mmol) in one portion. The solution was stirred for two minutes and DIPEA (120 μL, 0.67 mmol) was added dropwise over 30 seconds. The reaction was stirred at 0°C for 1 hour, after which the starting material was completely consumed (TLC). R_f 0.69 (10% MeOH:DCM, visualized with KMnO₄). The salt was removed by filtration with a 0.45 μm PTFE syringe filter after which the solvent was removed *in vacuo*. DCM (2 mL) was added to dissolve the intermediate and the solution was cooled by an ice water bath. Oxalyl chloride (72 μL, 0.84 mmol) followed by DMF (1 drop) was added while stirring resulting in evolution of gas. TLC (10% MeOH:DCM, visualized with KMnO₄) showed presence of the starting amino acid. The procedure was also performed with isolation of the intermediate TBDMS protected amino acid by flash chromatography (5% MeOH:DCM) prior to treatment with oxalyl chloride. The outcome of the reaction was the same.

Screening of deprotection conditions

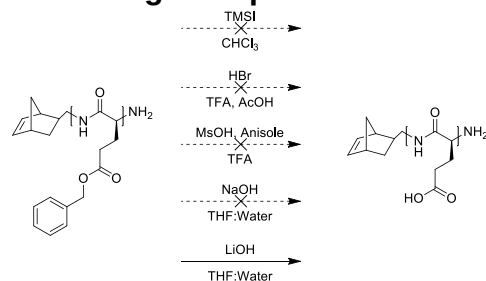


Figure S2. Screening of conditions for benzyl ester deprotection and preservation of norbornene end groups. All tested methods were adequate for the conversion of benzyl esters to the free carboxylic acid. However, only deprotection with LiOH demonstrated preservation of the norbornene end group as determined by NMR.

TMSI deprotection^{3,4}

To a stirring solution of PBLG (0.50 g, 2.28 mmol repeat units) in chloroform (5 mL) was added TMS-I (0.39 mL, 2.73 mmol) dropwise over 1 minute. The reaction was stirred at 50°C for 2 hours after which diethyl ether was added and the precipitate was collected by centrifugation, washed with water, and dried *in vacuo* to yield an off white powder (256 mg, 1.98 mmol repeat units, 87%).

HBr deprotection

To a stirring solution of PBLG (0.52 g, 2.37 mmol repeat units) in trifluoroacetic acid (3 mL) was added 33 wt% HBr in AcOH (1.1 mL). The reaction was stirred for 1 hour after which diethyl ether was added and the precipitate was collected by filtration, washed with diethyl ether, and dried *in vacuo* to yield an off white powder (0.27 g, 2.06 mmol repeat units, 87%).

MsOH, Anisole deprotection⁵

To a chilled stirring solution of PBLG (1.0 g, 4.56 mmol repeat units) in trifluoroacetic acid (10 mL) was added methanesulfonic acid (9.5 mL) dropwise over 1 minute followed by anisole (1.6 mL) dropwise over 1 minute. The reaction was stirred at 0°C for 20 minutes after which the cooling bath was removed and the reaction was stirred for an additional 20 minutes. Diethyl ether was added and the precipitate was collected by centrifugation, washed with diethyl ether, and dissolved in sat. NaHCO₃. The solution was exhaustively dialyzed against deionized water across a 2 kDa regenerated cellulose membrane. Afterwards the pH of the solution was adjusted to 2 by dropwise addition of 1M HCl and the precipitate was collected by centrifugation and dried *in vacuo* to yield a white powder (0.41 g, 3.19 mmol repeat units, 70%).

NaOH deprotection

To a chilled stirring solution of PBLG (1.0 g, 4.56 mmol repeat units) in THF (8 mL) was added a chilled solution of NaOH (0.37 mg, 9.1 mmol) in water (1 mL) dropwise over 2 minutes. The reaction was stirred for 20 hours at room temperature after which the solution was cooled to 0°C and the pH adjusted to 2 by dropwise addition of 1M HCl. The precipitate was collected by centrifugation and dried *in vacuo* yielding a white powder (88 mg, 0.68 mmol repeat units, 15%).

Screening of amide bond coupling conditions

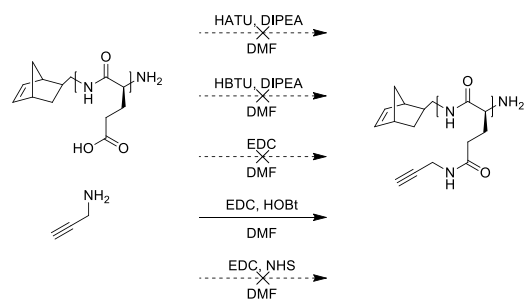


Figure S3a. Screening of amide bond coupling conditions. Reactions were determined to be unsuccessful by spectroscopy or inability to isolate product by precipitation.

Representative procedure for uronium/aminium coupling of propargylamine

To a stirring solution of poly(glutamate) (395 mg, 3.06 mmol repeat units) in DMF (10 mL) was added HBTU (1.53 g, 3.98 mmol) in one portion. Upon complete dissolution of

the solid, DIPEA (1.6 mL, 9.2 mmol) was added and the solution stirred for 15 minutes. Afterwards, propargylamine (274 μ L, 4.28 mmol) was added and the solution stirred 12 hours. The product was precipitated by addition of isopropanol, collected by centrifugation, and dried *in vacuo* to give a white powder (0.338 g) (see Fig. S3b).

Coupling by EDC

Coupling of poly(L-glutamate) and propargylamine by only EDC with no additives followed the same procedure as outlined in the main manuscript with exclusion of HOBt (see Fig. S3c). Coupling of poly(L-glutamate) and propargylamine by EDC and NHS followed the same procedure as outlined in the main manuscript with substitution of HOBt with NHS. No product was isolated from precipitation and the reaction was determined to be incomplete.

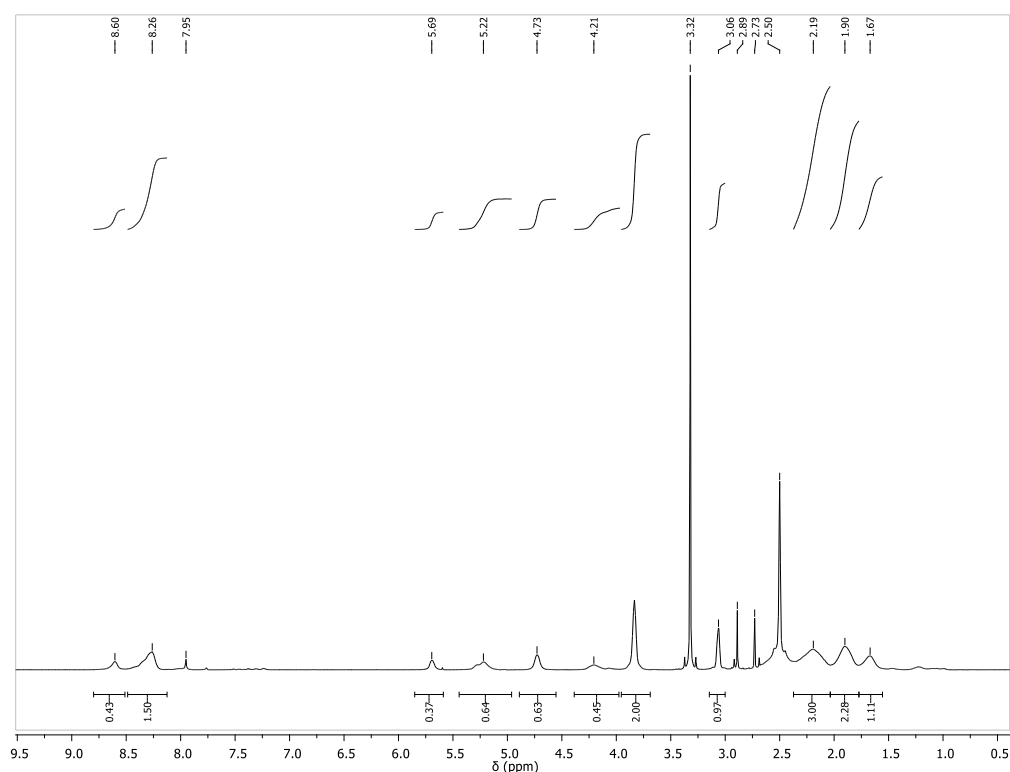


Figure S3b. The NMR of the product by HATU coupling in DMSO- d_6 exhibited extra peaks and incomplete integrations. Known solvents and impurities are located at δ 7.95, 2.89, 2.73 (DMF); δ 2.5 (DMSO); δ 3.32 (water)

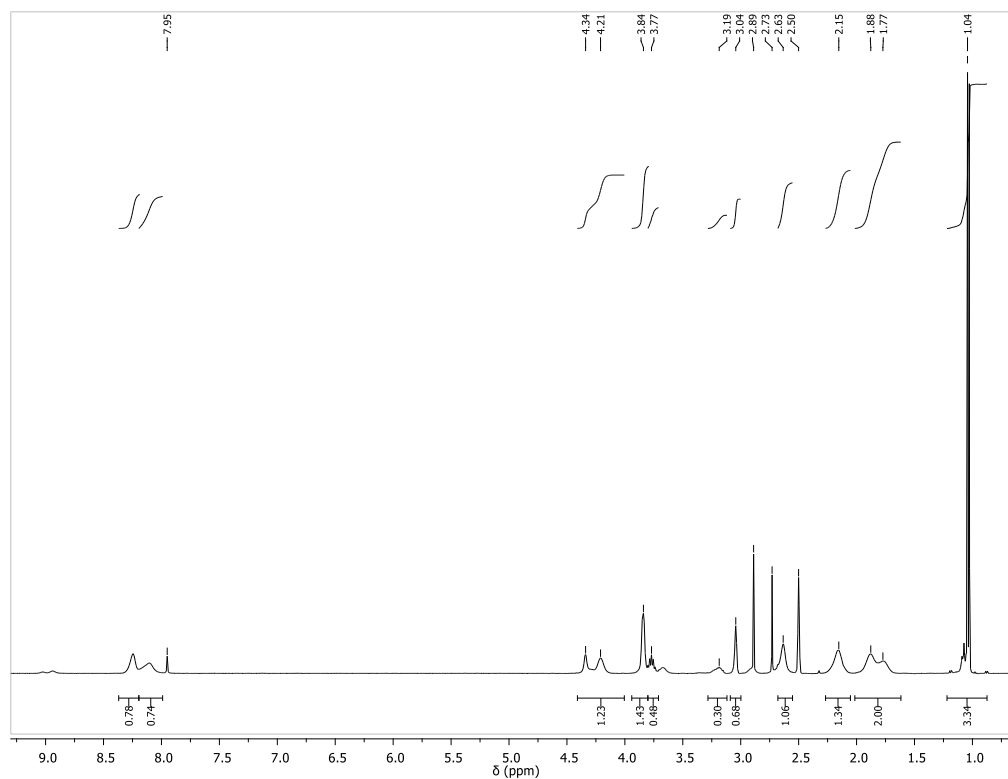


Figure S3c. The NMR of the product by EDC coupling with no additives in DMSO-d₆ exhibited extra peaks and incomplete integrations. Known solvents and impurities are located at δ 7.95, 2.89, 2.73 (DMF) and δ 2.5 (DMSO).

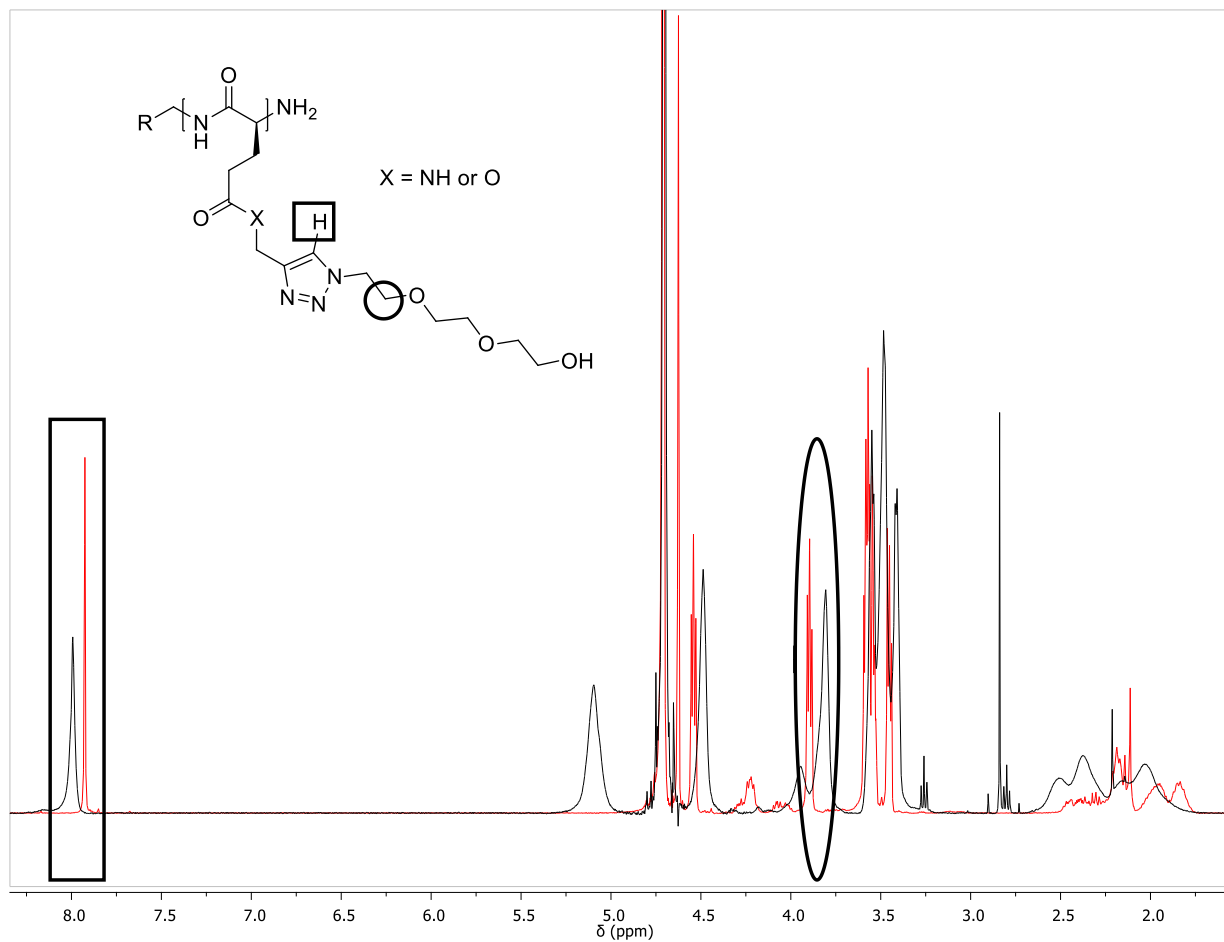


Figure S4. Protons monitored by ^1H -NMR to evaluate hydrolysis. The black trace represents PPLG grafted with an oligomeric ethylene glycol. The red trace represents the polymer sample after complete hydrolysis. The protons are highlighted on the structure and spectra by corresponding shapes.

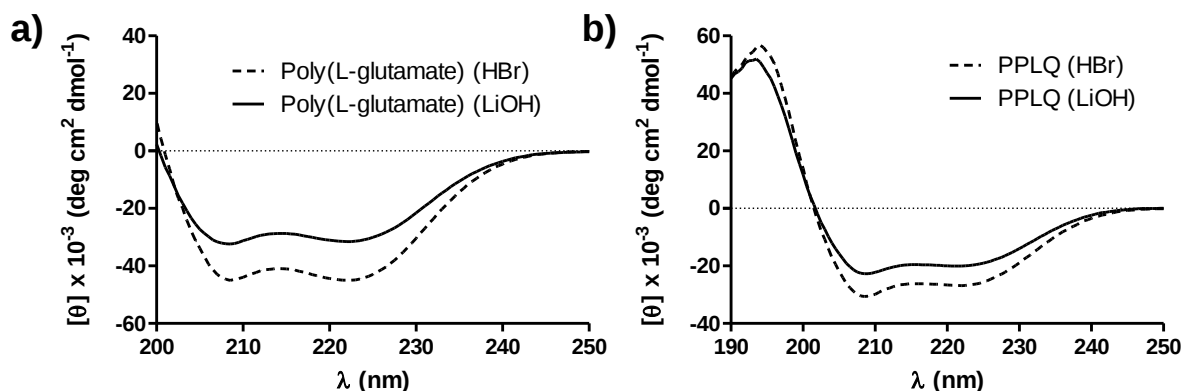


Figure S5. (a) Circular dichroism of poly(L-glutamate) obtained through deprotection by either HBr or LiOH as denoted in legend. Spectra was taken at 0.5 mg/mL in 100 mM sodium acetate pH 4.5. (b) Circular dichroism of PPLQ obtained through deprotection by either HBr or LiOH as denoted in legend. Spectra was taken at 0.5 mg/mL in hexafluoroisopropanol.

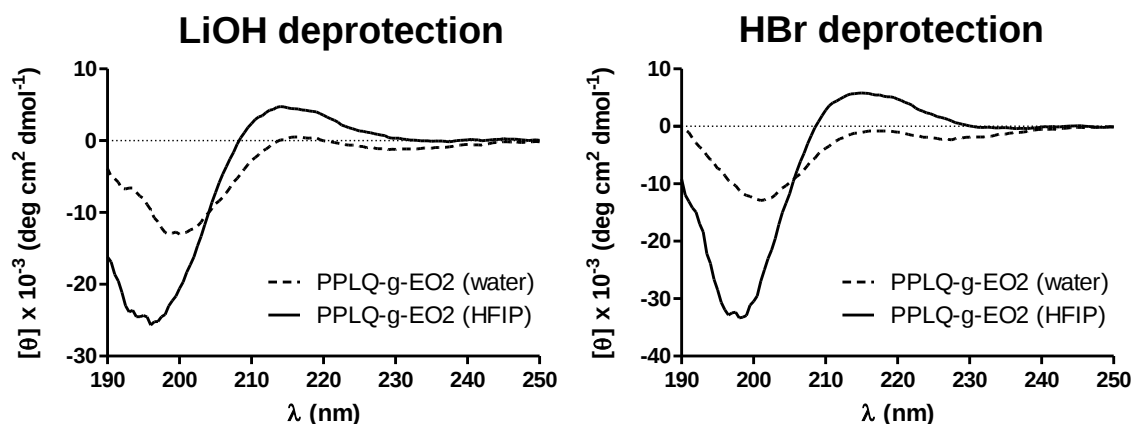


Figure S6. Circular dichroism of PPLQ-g-EO2 obtained through deprotection by LiOH or HBr. Spectra was taken at 0.5 mg/mL in either water or hexafluoroisopropanol as denoted in legend.

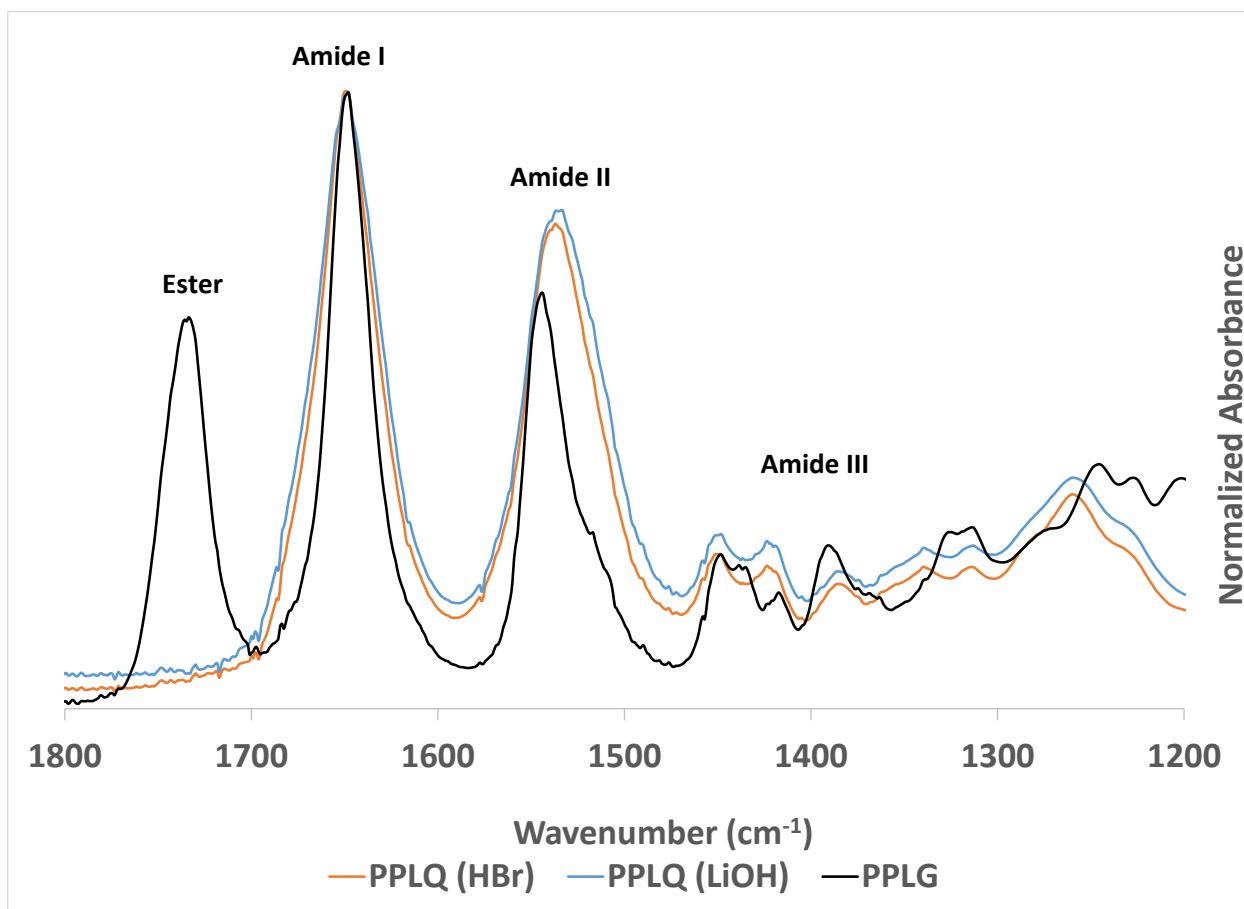


Figure S7. Overlaid FTIR spectra of ester PPLG as well as amide PPLQ synthesized through PBLG deprotection by either HBr or LiOH. Though the spectra of PPLQ and PPLG differ, likely due to the differences of the side chain ester or amide, PPLQ synthesized through acidic and basic deprotection are similar. Spectra are normalized to peak amide I absorbance.

References

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