Supporting Information

Double-Modified Glycopolymers from Thiolactones to Modulate Lectin Selectivity and Affinity

Laura E. Wilkins,[a] Nezha Badhi,[c] Filip du Prez[c] and Matthew I. Gibson[a,b]*

[a]Department of Chemistry, University of Warwick Coventry, UK, CV4 7AL,

[b]Warwick Medical School, University of Warwick, Coventry, UK, CV47AL, [c] Department of Organic and Macromolecular Chemistry, University of Ghent, Belgium

Corresponding Author; m.i.gibson@warwick.ac.uk

Experimental Section

Materials

Ultra-pure water with resistance < 18 Ω, was obtained from a Milli-Q© Integral Water Purification System. All chemicals were purchased from Sigma-Aldrich and used as supplied unless otherwise stated. GM1-ganglioside was purchased from Carbosynth. Deuterated solvents used (methanol, chloroform, water) were purchased from Sigma-Aldrich). *n*-Hexane, THF, DMF and ethyl acetate were purchased from Fisher. The toxins used in fluorescence-linked assays were Cholera toxin B subunit, FITC-conjugate, lyophilized powder from Sigma-Aldrich and Fluorescein labelled Ricinus Communis Agglutinin I (RCA I, RCA120) from Vector Laboratories. HEPES buffer stock solution was prepared with the following concentrations, and adjusted to pH 7.5 using the minimum volume required of 0.1 M $HCl_{(aq)}$ and 0.1 M $NaOH_{(aq)}$: 10mM HEPES, 0.15 M NaCl, 0.1 mM $CaCl_2$ and 0.01 mM Mn^{2+}. The toxins using in bilayer interferometry were Cholera toxin B subunit, lyophilized powder from Sigma-Aldrich and Unconjugated Ricinus Communic Agglutinin I (RCA I, RCA120) from Vector Laboratories.

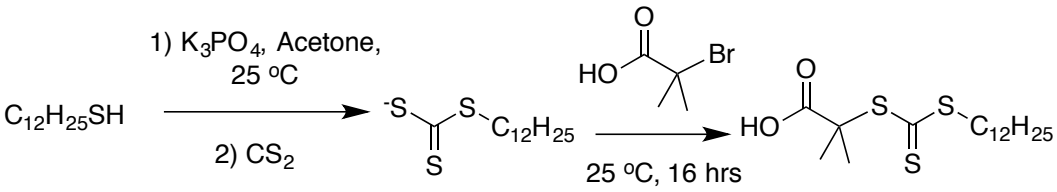
Analytical Methods

^{1}H and ^{13}C-NMR spectra were obtained using a Bruker DPX-400 or Bruker DPX-300 NMR Spectrometer; all chemical shifts are reported in ppm (δ) relative to residual non-deuterated solvent. Mass spectrometry was carried out in methanol or water on the Agilent 6130B ESI-Quad instrument using electrospray in positive mode. FTIR spectroscopy was carried out on a Bruker Vector 22 FTIR spectrometer with a Golden gate diamond attenuated total reflection cell. SEC (GPC) measurements were carried out on a Varian 390-LC MDS system equipped with a PL-AS RT/MT2 autosampler, a PL-gel 3 μm (50 x 7.5 mm) guard column, two PL-gel 5 μm (300 x 7.5 mm) mixed-D columns held at 30°C and the instrument equipped with a differential refractive index and a Shimadzu SPD- M20A diode array detector. SEC samples were filtered through PTFE syringe filters 0.22 microM, 13 mm, from Gilson Scientific. Tetrahydrofuran (with 2% Triethylamine) or Dimethylformamide eluent was used at 1mL.min^{-1} flow rate. Data were analysed using Agilent GPC software and molecular weight determined relative to narrow molecular weight PMMA standards (200 - 1.0 x 106 g.mol-1). Fluorescence plate readings were performed on a BioTek Synergy HT Microplate Reader. Bilayer interferometry was performed on a ForteBio Octet Red 96 Bilayer Interferometer using Dip and Read™

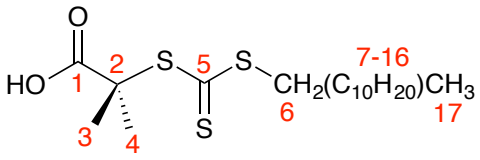
Amine Reaction Second-Generation (AR2G) Biosensors. Kinetic parameters were extracted using a heterogeneous sites model in the Fortebio software.

Synthetic Methods

Synthesis of 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DMP/DDMAT)



Dodecane thiol (4.75 mL, 19.8 mmol) was added dropwise to a stirred suspension of K_3PO_4 (4.02g, 18.9 mmol) in acetone (60 mL). The reaction vessel was placed in an ice bath. Carbon disulfide (3.20 mL, 53.0 mmol) was added and the solution turned bright yellow, but was still cloudy. After stirring for ten minutes, 2-bromo-2- methylpropionic acid (3.00 g, 18.0 mmol) was added and a precipitation of KBr was noted. The ice bath was removed after 10 minutes and the reaction was left stirring at room temperature for 16 hours. Solvent was removed *in vacuo* and the residue was extracted into DCM (2 x 50 mL) from 1 M HCl (100 mL). The organic extracts were further washed with water (100 mL) and brine (100 mL) and dried over $MgSO_4$. Recrystallisation from n-hexane yielded a bright yellow solid (1.80 g, 27.5%).



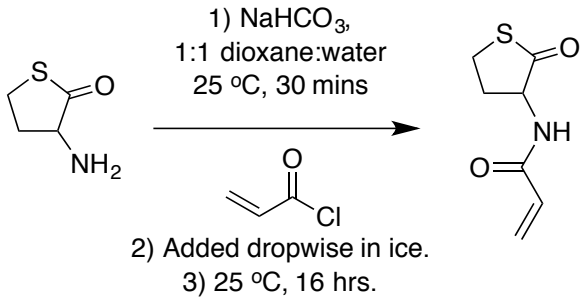
^{1}H NMR (400 MHz, $CDCl_3$) δ_{ppm}: 3.28 (2H, t, J_{HH}=7.5, H6); 1.66 (6H, s, H3/4); 1.10-1.25 (20H, alkyl, H7-16); 0.79 (3H, m, H17).

^{13}C NMR (400 MHz, $CDCl_3$) δ_{ppm}: 220.86 (C5); 178.04 (C1); 55.51 (C2); 37.08 (C7); 31.92 (C6); 29.64, 29.57, 29.46, 29.35, 29.12, 28.98, 27.82 (C8-15); 25.23 (C3/4); 22.70 (C16); 14.13 (C17).

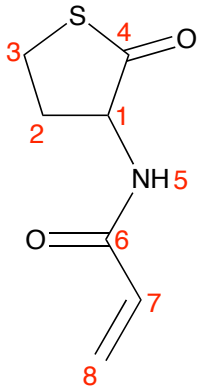
FTIR (solid, ν_{max}/cm^{-1}): 2910 (CH_2); 1710 (C=O); 1440 (C-C); 1305 (C-O); 1070 (S-(C=S)-S).

ESI-MS, positive mode (m/z): 365.2 ($M+H^+$, expected 365.63), 387.1 ($M+Na^+$, expected 387.61).

Synthesis of *N*-Thiolactone Acrylamide



D,L-homocysteine thiolactone hydrochloride (7.04 g, 45.6 mmol) was dissolved in 1:1 dioxane: water (100 mL). The reaction vessel was transferred to an ice bath, and sodium hydrogen carbonate (19.2 g, 228 mmol) was added whilst stirring. After 30 mins stirring, acryloyl chloride (7.45 mL, 91.2 mmol) was added dropwise. The reaction was stirred for 16 hours at room temperature. The reaction was extracted into ethyl acetate (200 mL) from brine (200 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield a white solid (5.57 g, 71.3%).



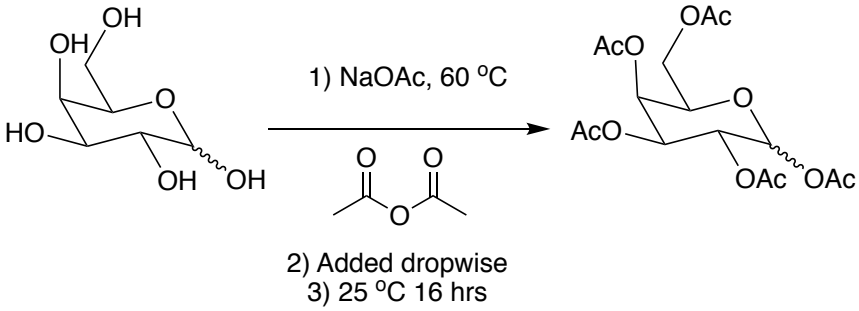
^{1}H NMR (400 MHz, $CDCl_3$) δ_{ppm}: 6.35 (1H, m, H7); 6.17 (1H, m, H8, trans); 5.73 (1H, m, H8, cis); 4.61 (1H, s, H1); 3.41 (1H, m, H3); 3.3 (1H, m, H3); 3.02 (1H, m, H2); 1.99 (1H, qd, J_{HH}=12.5(x3), 6.9, H2).

^{13}C NMR (400 MHz, $CDCl_3$) δ_{ppm}: 165.83 (C), 129.93 (CH, C1), 127.73 (CH_2, C3), 59.59 (CH, C6), 32.07 (CH_2, C7), 27.70 (CH_2, C3).

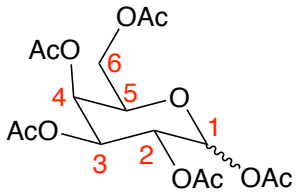
FTIR (solid, ν_{max}/cm^{-1}): 3290 (N-H); 2900 (CH_2); 1680 (C=O); 1560 (N-H); 1405 (C-C stretch in ring); 1210 (C-N aliphatic); 1005 (=C-H bend); 930 (alkene).

ESI-MS, positive mode (m/z): 194.0 ($M+Na^+$, expected 194.20), 365.0 ($2M+Na^+$, expected 365.41)

Synthesis of β-D-galactose pentaacetate



Sodium acetate trihydride (2.06 g, 20.2 mmol) was ground by mortar and pestle and stirred in a 60 °C oil bath. D(+)-galactose (2.00 g, 11.1 mmol) was added, and a condenser was attached to the reaction vessel. Acetic anhydride (10 mL, 106 mmol) was added dropwise. The reaction was refluxed for 10 minutes, after which it was cooled to room temperature and left stirring overnight. Ethanol (22 mL) was added to evolve acetic acid. The mixture was concentrated *in vacuo*, extracted into DCM (40 mL) and washed with warm water (2 x 40 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Ethanol (25 mL) was added, as was a few spatulas of activated charcoal, and the reaction was refluxed for 20 mins, cooled, filtered and recrystallized in ethanol to yield white crystals (1.30 g, 30%).[76]



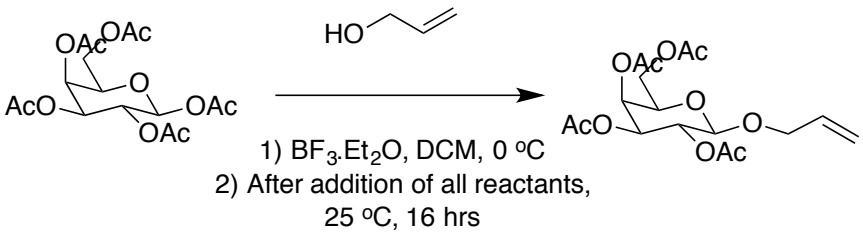
^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm}: 5.72 (1H, d, J_{HH}=8.2, H1); 5.45 (1H, br s, H4); 5.36 (1H, t, J_{HH}=9.3 x 2, H2); 5.11 (1H, m, H3); 4.17 (2H, m, H6); 4.07 (1H, m, H5); 2.19, 2.15, 2.07, 2.02 (15H, 4 x s, acetyl groups).

^{13}C NMR (300 MHz, $CDCl_3$) δ_{ppm}: 92.17 (CH, C1), 71.12 (CH, C4), 70.88 (CH, C2), 67.82 (CH, C3), 66.79 (CH, C5), 61.03 (CH_2, C6), 20-21 (CH_3, Acetyls).

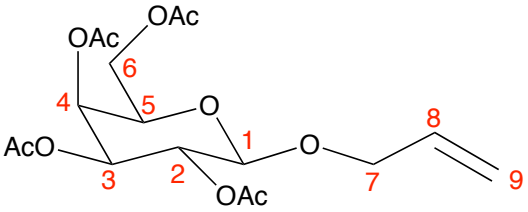
FTIR (solid, ν_{max}/cm^{-1}): 2973 (CH_2); 1765 (C=O); 1374 (C-H); 1210 (C-O stretch); 957 (=C-H bend); 900 (C-H "oop").

ESI-MS, positive mode (m/z): 413.1 ($M+Na^+$, expected 413.33)

Synthesis of 1-β-allyl-D-galactose pentaacetate



B-D-galactose pentaacetate (2.00 g, 5.13 mmol) was dissolved in DCM (40 mL). Allyl alcohol (0.425 mL, 6.25 mmol) was added whilst stirring in an ice bath. Boron trifluoride diethetate (1.35 mL, 7.11 mmol) was added dropwise. 20 minutes later, the reaction was taken out of the ice bath and stirred for 16 hours. Anhydrous potassium carbonate (1.08 g, 7.24 mmol) was added whilst stirring. 30 minutes later, the reaction was filtered and washed with water (2 x 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica using an eluent comprising 2:3 ethyl acetate: 40 – 60 °C petroleum ether to yield a yellow oil (1.02 g, 51.2%)



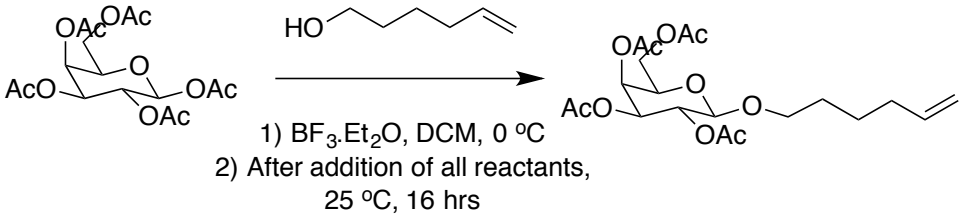
^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm}: 5.88 (1H, m, H8); 5.41 (1H, d, J_{HH}=3.2, H1); 5.34 (1H, m, H4); 5.28 (1H, m, H2); 5.17 (1H, m, H9); 5.05 (1H, m, H3); 4.5 (2H, m, H6); 4.15 (2H, m, H7); 4.13 (1H, m, H5); 2.15, 2.06, 2.05, 1.99 (15H, 4 x s, acetyl groups)

^{13}C NMR (300 MHz, $CDCl_3$) δ_{ppm}: 117.61 (CH_2, C9), 100.12 (CH, C8), 70.96 (CH, C1), 70.67 (CH, C4), 70.04 (CH_2, C7), 69.01 (CH, C2), 67.07 (CH, C3), 61.30 (CH_2, C6), 30.94 (CH, C5), 20-21 (CH_3, Acetyls).

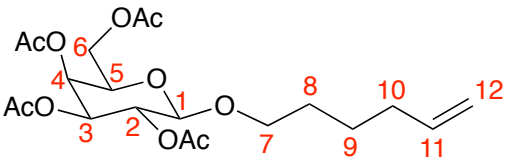
FTIR (solid, ν_{max}/cm^{-1}): 3027 (O-H); 2920 (CH_2); 1754 (C=O); 1495 (C-H); 1220 (C-O stretch); 705 (C-H).

ESI-MS, positive mode (m/z): 411.1 ($M+Na^+$, expected 411.36)

Synthesis of 1-β-hexyl-D-galactose pentaacetate



Beta-D-galactose pentaacetate (4.00 g, 10.26 mmol) was dissolved in DCM (60 mL). 5-hexen-1-ol (1.25 mL, 12.5 mmol) was added whilst stirring in an ice bath. Boron trifluoride diethyerate (2.70 mL, 14.2 mmol) was added dropwise. 20 minutes later, the reaction was taken out of the ice bath and stirred for 16 hours. Anhydrous potassium carbonate (2.0 g, 14.5 mmol) was added whilst stirring. 30 minutes later, the reaction was filtered and washed with water (2 x 100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica using an eluent comprising 2:3 ethyl acetate: 40 – 60 °C petroleum ether to yield a clear oil (1.45 g, 32.7%)



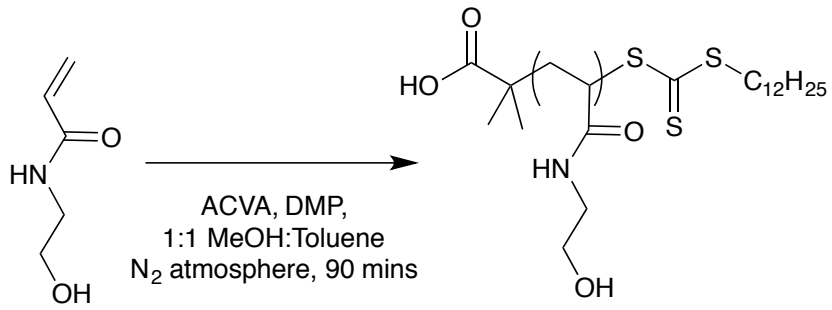
^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm}: 5.83 (1H, m, H11); 5.41 (1H, m, H1); 5.23 (1H, m, H4); 5.12 (1H, m, H2); 5.00 (3H, m, H12/3); 4.49 (1H, m, H5); 3.92 (2H, m, H6); 3.68 (2H, m, H7); 2.07 (15H, m, acetyl groups); 1.5 (4H, m, H8/9).

^{13}C NMR (300 MHz, $CDCl_3$) δ_{ppm}: 114.69 (CH_2, C12), 101.36 (CH, C11), 70.98 (CH, C1), 70.60 (CH, C4), 70.00 (CH_2,, 7), 68.94 (CH, C2), 67.69 (CH, C3), 67.09 (CH, C5), 61.30 (CH_2, C6), 33.31 (CH_2, C10), 28.82 (CH_2, C8), 25.07 (CH_2, C9), 20.69 (CH_3, Acetyls).

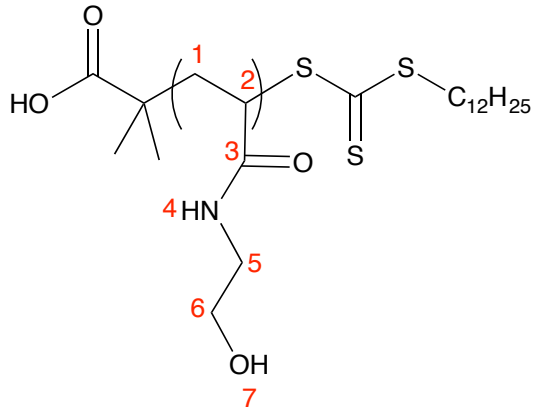
FTIR (solid, ν_{max}/cm^{-1}): 3014-2823 (alkyl/alkenyl CH_2); 1743 (C=O); 1430 (weak, alkyl C-H); 1369 (C-O); 1216 (C-H stretch); 1045 (C-O); 906 (monosubstituted alkene).

ESI-MS, positive mode (m/z): 453.0 ($M+Na^+$, expected 453.4), 883.3 ($2M+Na^+$, expected 883.9).

General Procedure for Polymerisation of *N*-hydroxyethyl acrylamide (HEA)



The following procedure describes a reaction with a theoretical degree of polymerisation (DP) of 100 repeat units. 4,4-azobis(4-cyanovaleric acid) (5 mg, 0.018 mmol), 2- (dodecylthiocarbonothioylthio)-2-methylpropanoic acid (CTA, 32 mg, 0.088 mmol) and *N*-hydroxyethyl acrylamide (1 g, 8.8 mmol) were dissolved in 1:1 methanol: toluene (4 mL) in a glass vial with a stirrer bar. Mesitylene (200 µL) was added and a sample was removed for ^{1}H-NMR analysis in $CDCl_3$. The reaction mixture was degassed by N_2 for 30 minutes, sealed and placed in a 70 °C oil bath. After 90 minutes, the solution was opened to air and quenched in $N_{2(l)}$. The polymer (pHEA) was precipitated three times from methanol into diethyl ether to give a light yellow solid.

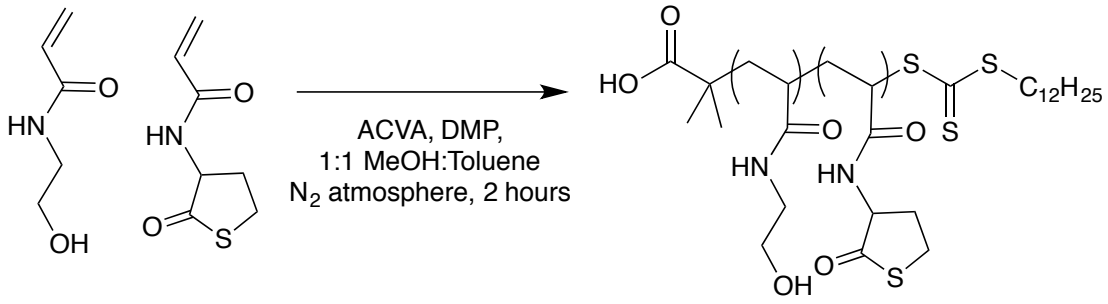


Conversion (NMR): 83.6%; M_n (theoretical): 7051 g.mol^{-1}; M_n (SEC) 13904 g.mol^{-1}; M_w (SEC) 15000 g.mol^{-1}; M_w/M_n (SEC): 1.08.

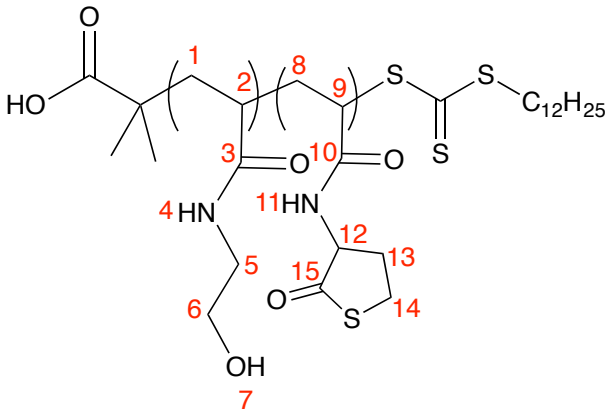
^{1}H NMR (300 MHz, D_4-MeOH) δ_{ppm}: 4.60-4.90 (br s, H6), 3.55-3.75 and 3.05-3.20 (2 x br s, H5), 2.00-2.20 and 1.50-1.80 (2 x br s, H1/2).

FTIR (solid, ν_{max}/cm^{-1}) = 3300 (N-H and O-H stretch), 2854 (alkyl C-H stretch), 1641 (amide C=O stretch), 1555 (N-H bend), 1443 (alkane), 1225 (C-O stretch), 1060 (C-O stretch).

General Procedure for Copolymerisation of *N*-hydroxyethyl acrylamide (HEA) and *N*-thiolactone acrylamide (TLA)



The following procedure describes a reaction with a theoretical degree of polymerisation (DP) of 50 repeat units, with 0.9 equivalents HEA and 0.1 equivalents TLA. 4,4-azobis(4-cyanovaleric acid) (2.5 mg, 0.0089 mmol), 2- (dodecylthiocarbonothioylthio)-2-methylpropanoic acid (CTA, 32 mg, 0.088 mmol), *N*-hydroxyethyl acrylamide (0.458 g, 3.98 mmol) and *N*-thiolactone acrylamide (69 mg, 0.44 mmol) were dissolved in 1:1 methanol: toluene (3 mL) in a glass vial with a stirrer bar. Mesitylene (100 µL) was added and a sample was removed for ^{1}H-NMR analysis in $CDCl_3$. The reaction mixture was degassed by N_2 for 30 minutes, sealed and placed in a 70 °C oil bath. After 2 hours, the solution was opened to air and quenched in $N_{2(l)}$. The polymer (pHEA-co-pTLA) was precipitated three times from methanol into diethyl ether to give a light yellow solid. The characterization below is from a theoretical 20%TLAm copolymer.

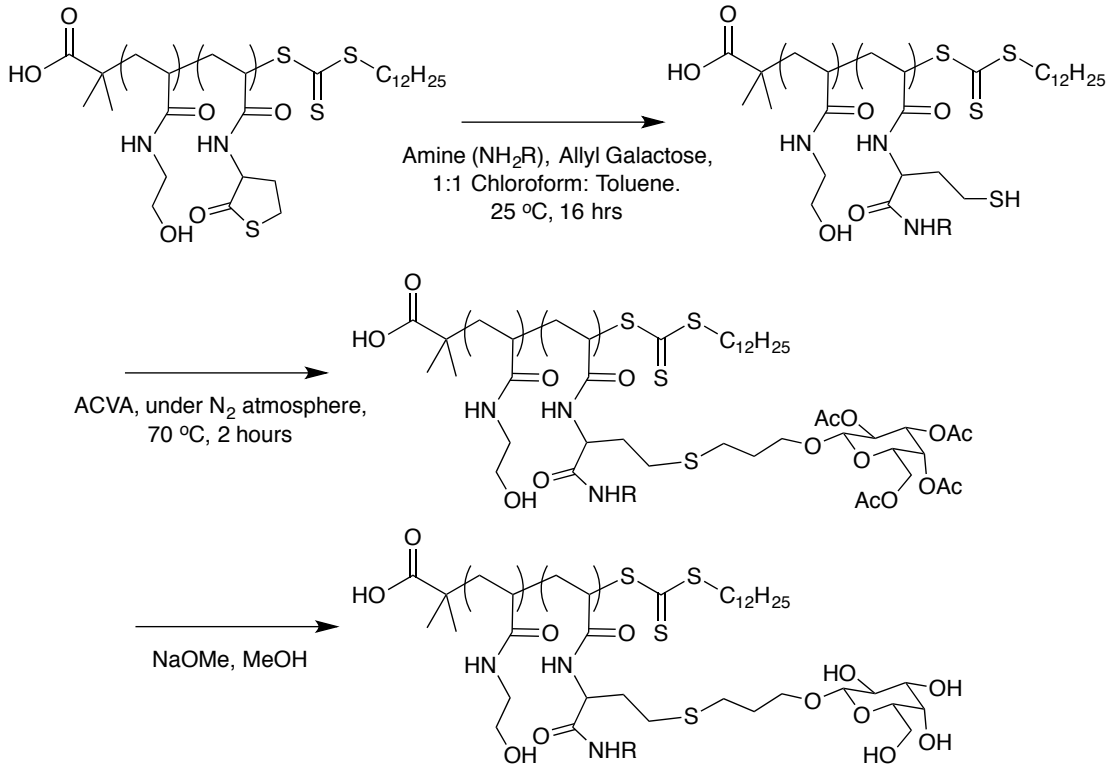


For 5% TLAm: Conversion (NMR): 95.2%; Mn (theoretical): 4400 g.mol^{-1}; Mn (SEC) 8600 g.mol^{-1}; Mw (SEC) 9958 g.mol^{-1}; Mw/Mn (SEC): 1.16. For 10% TLAm: Conversion (NMR): 93.2%; Mn (theoretical): 4500 g.mol^{-1}; Mn (SEC) 8900 g.mol^{-1}; Mw (SEC) 10100 g.mol^{-1}; Mw/Mn (SEC): 1.13.

^{1}H NMR (300 MHz, D4-MeOH) δppm: 7.9-8.15 (br s, N-H (H4/11)); 4.75-4.90 (br s, H6/14); 3.6-3.8 and 3.05-3.20 (2 x br s, H5/13); 2.55-2.7 (br s, H12 (this peak is less visible in 5 or 10% TLAm copolymer)), 2.00-2.40 (the left should of this peak is less visible in 5 or 10% TLAm copolymer) and 1.50-1.85 (2 x br m, H1/2/8/9).

FTIR (solid, νmax/cm^{-1}) = 3300 (N-H and O-H stretch); 2854 (alkyl C-H stretch); 1641 (amide C=O stretch); 1555 (N-H bend); 1443 (alkane); 1225 (C-O stretch); 1060 (C-O stretch, peak has a shoulder).

Representative Double Modification of TLAm-containing copolymer: Aminolysis and Thiol-ene "click"



Polymer (100 mg of 5% pTLAm copolymer) and amine (eg. 56 mg of glucosamine, 5 equivalents per TLAm moiety) was dissolved in 1:1 methanol: water (5 mL) and stirred overnight at room temperature. Alkene-galactose pentaacetate (eg. 67.4 mg of 1-b-allyl-D-galactose pentaacetate, 5 equivalents per TLAm moiety) and 4,4-azobis(4-cyanovaleric acid) (1.2 mg, 0.0045 mmol) were added. The reaction mixture was degassed by N_2 for 30 minutes, sealed and placed in a 70 °C oil bath. After 2 hours, the solution was opened to air and quenched in $N_{2(l)}$. The reaction mixture was concentrated *in vacuo*, redissolved in methanol and filtered. The polymer was then precipitated three times from methanol into diethyl ether to give a light yellow solid. The

different precipitation behaviour (all polymers were very difficult to dissolve in solution) was an indication of successful modification. The polymer was subsequently dissolved in methanol (5 mL) and deprotected with sodium methoxide (0.5 μL of 5.4 M solution in methanol) and precipitated three times from methanol into diethyl ether to give a light yellow solid.[40,77]

Fluorescence-linked sorbent assay for polymer inhibitory activity

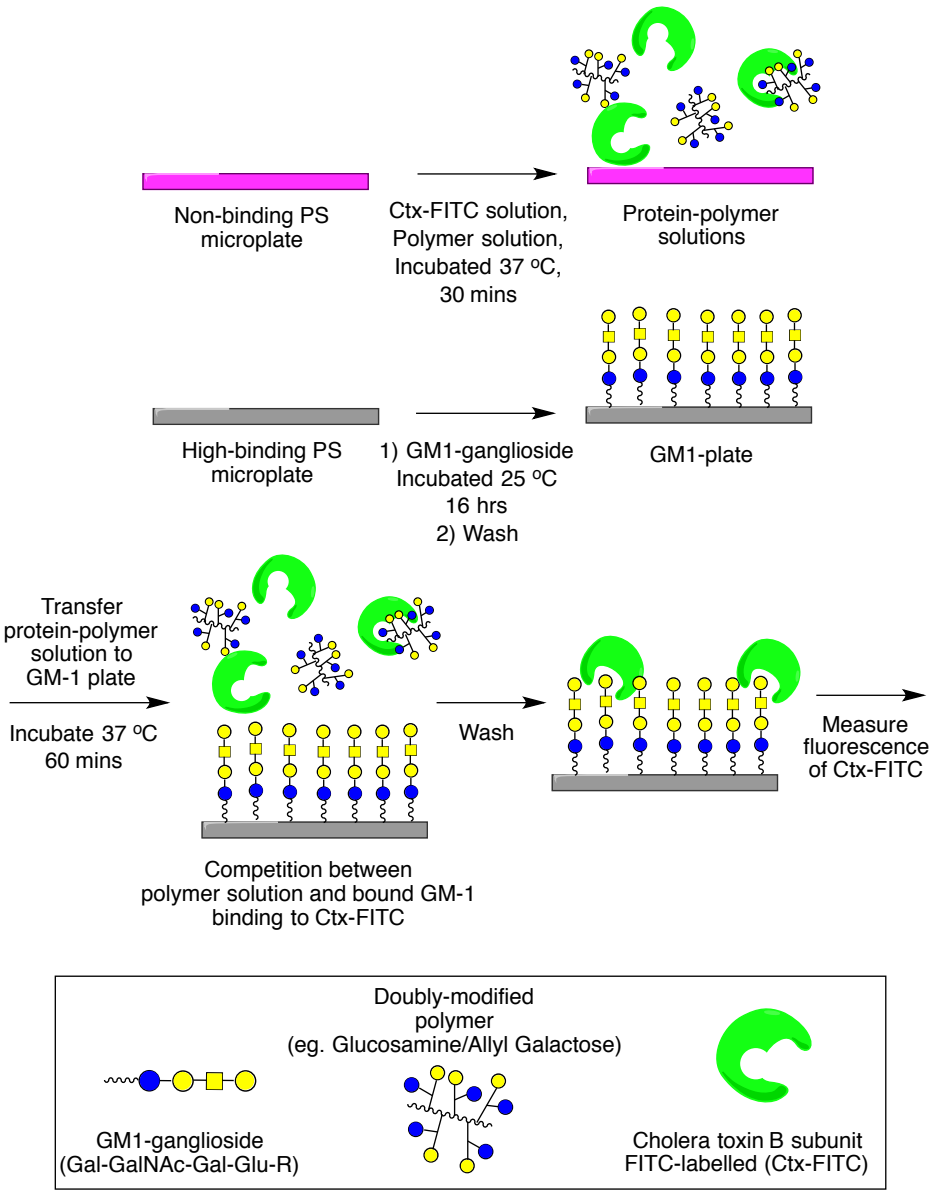


Figure S1. Inhibitory Assay procedure.

384-well high-binding PS plates were incubated for 16 hours with 50 µL of 100 µg.mL^{-1} GM-1 glycolipid (in PBS). Unbound glycolipid was removed by washing with water (x3). Polymer solutions were made up as serial dilutions (up to 12 dilutions by 2 from 1 mg.mL^{-1} in HEPES). CTx-FITC (4 µL of 100 µg.mL^{-1} in 10mM HEPES buffer with 0.15 M NaCl, 0.1 mM $CaCl_2$ and 0.01 mM Mn^{2+} (pH 7.5)) was added to 36 µL of polymer solution. The CTx/polymer solutions were then transferred to the GM-1-coated plates and incubated at 37 °C for 60 minutes. The wells were then washed (x3) with HEPES buffer. Fluorescence of wells was measured at excitation/emission wavelengths of 485/528 nm respectively. All experiments were carried out in

triplicate, using pure CTx-FITC wells (with no polymer) as controls. The above protocol is repeated for RCA-FITC to compare polymer inhibitory activity.

Bilayer interferometry assay for polymer inhibitory activity

The tips of a row of 8 AR2G sensors were soaked in water for 30 minutes before running the assay. Each dipping solution was prepared in a 8-well row of a 96-well plate with 200 μL in each well, as follows. During the assay, these sensor tips were then dipped in water for baseline, dipped in EDC/NHS solution for activation, dipped in toxin (25 μg.mL^{-1} in HEPES buffer) for loading and ethanolamine (1 M solution in water) for deactivation before being exposed to polymer sample solutions. Polymer solutions were made up as serial dilutions (7 dilutions by 10 from 1 mg.mL^{-1} in HEPES). Association was run for 15 minutes in polymer solution, followed by dissociation in HEPES for 30 minutes. Data was fitted using a heterogeneous sites (2:1) model.

Additional Data

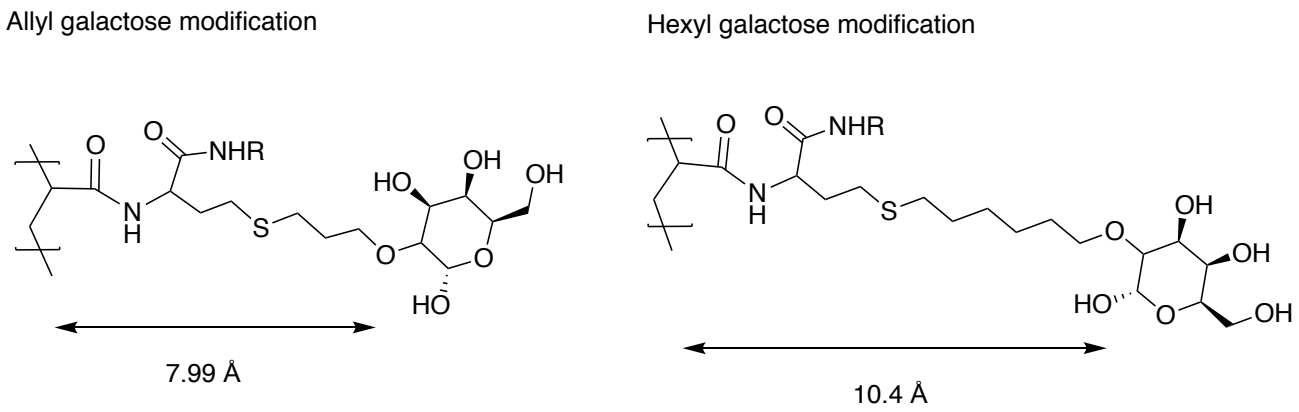


Figure S2: Polymer side-chain linker length estimation. Distance is from terminal b-Gal residue to the polymer backbone.

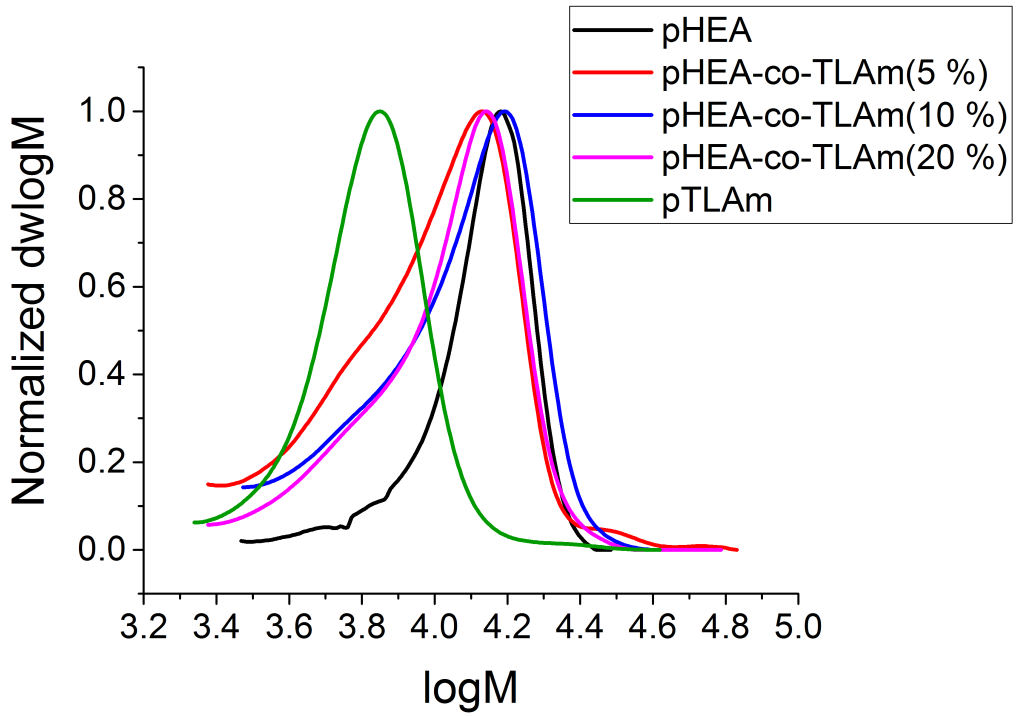


Figure S3: SEC analysis of polymers with different thiolactone acrylamine proportions.

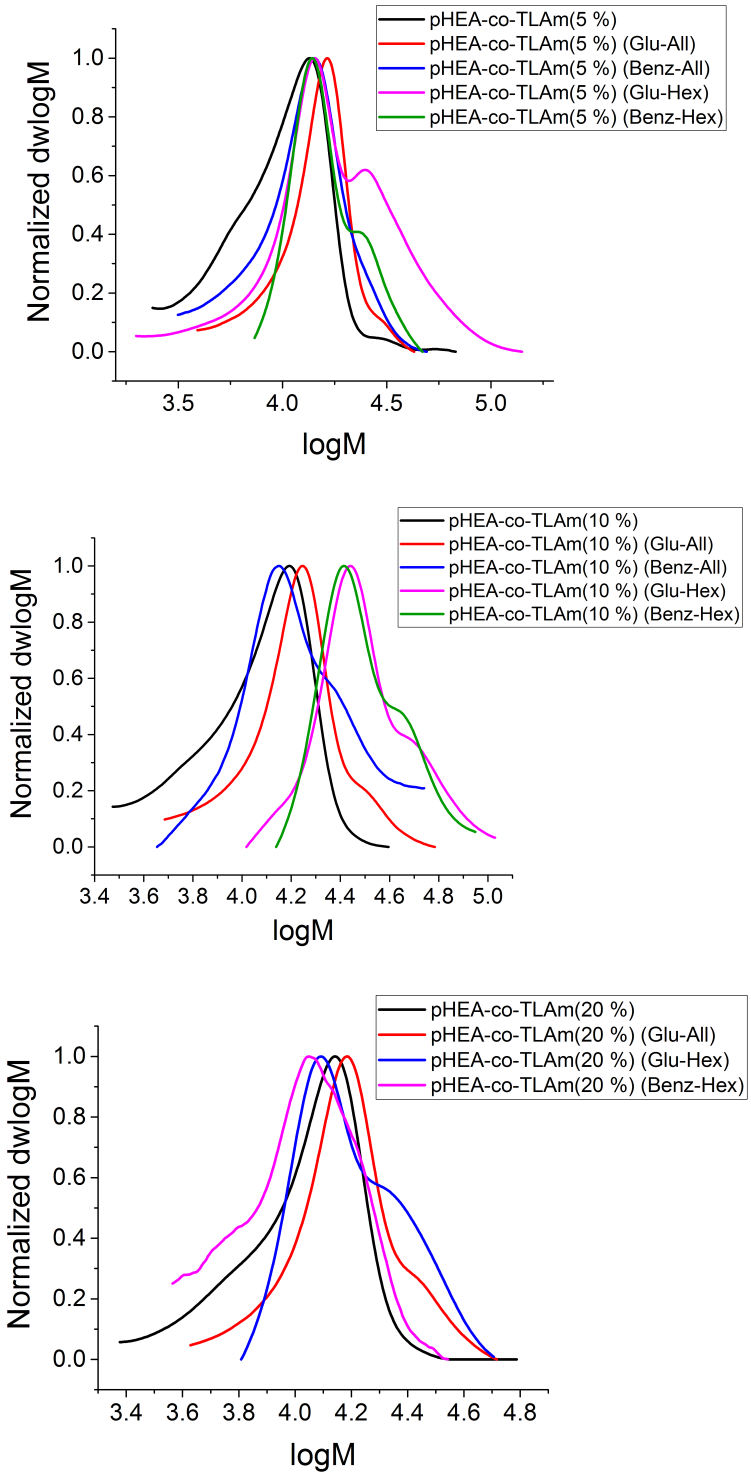


Figure S4: SEC analysis of pHEA-co-TLAm copolymers doubly modified at the thiolactone acrylamine residue.

All SECs showed a shift upon modification, which was consistent with the change in solution properties as expected for adding bulky functional groups onto the side chain.

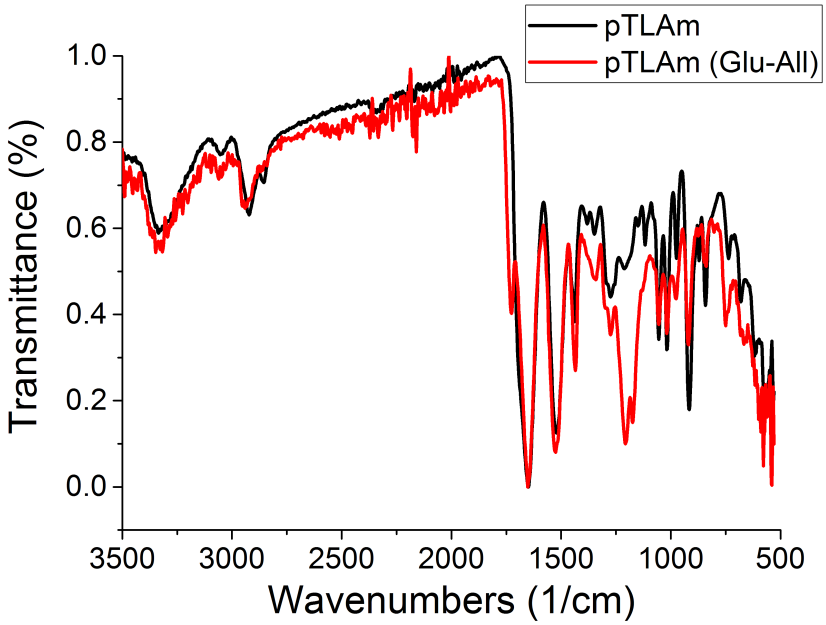


Figure S5: Representative TIR analysis of TLAm homopolymers doubly modified with glucosamine and allyl galactose.

The presence of a second carbonyl peak (as a shoulder on the ~1600 cm^{-1} peak) suggests a shift in the thiolactone's carbonyl upon ring opening of this residue. The significant enrichment of the C-O peaks at ~1200 cm^{-1} demonstrates the presence of many more C-O moieties (present on the sugar residues).

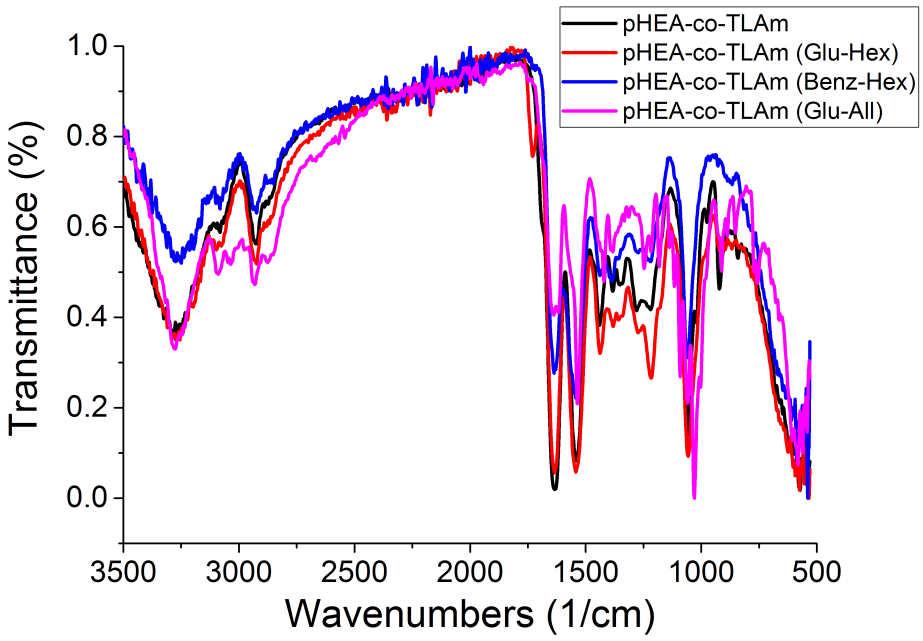


Figure S6: FTIR analysis of pHEA-co-TLAm(20%) copolymers doubly modified at the thiolactone acrylamine residue.

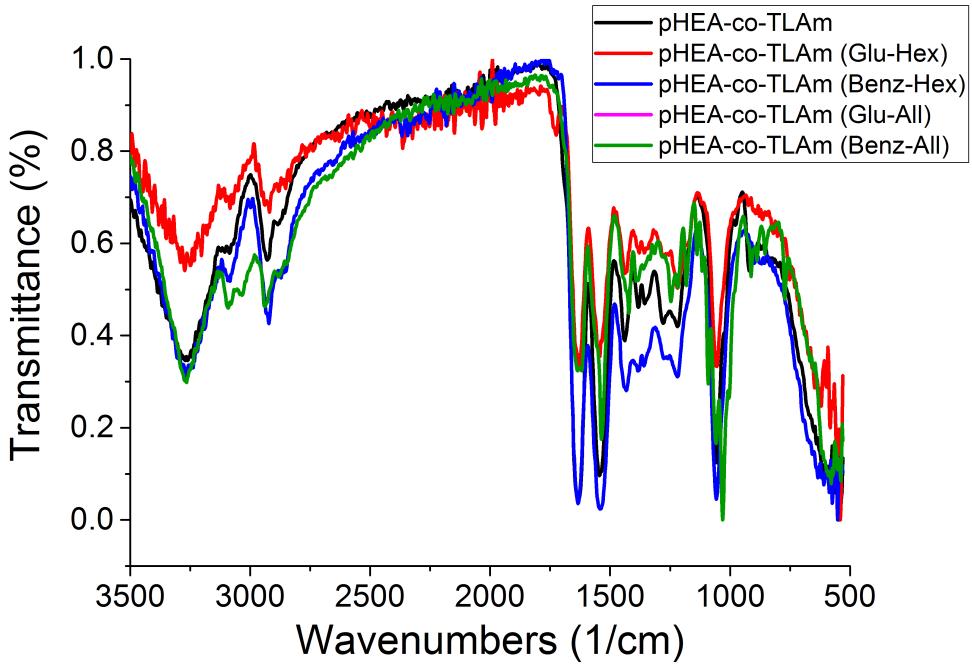


Figure S7: FTIR analysis of pHEA-co-TLAm(10%) copolymers doubly modified at the thiolactone acrylamine residue.

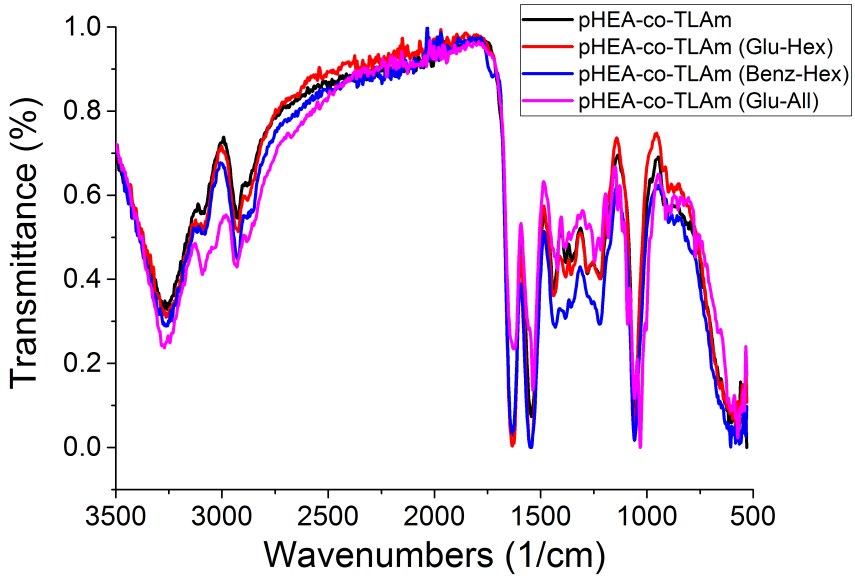


Figure S8: FTIR analysis of pHEA-co-TLAm(5%) copolymers doubly modified at the thiolactone acrylamine residue.

As the polymers are still comprised of predominantly HEA repeat units, there is very little difference in the FTIR spectra. The presence of further peaks at 1750 and 1000-1100 suggests successful modification.

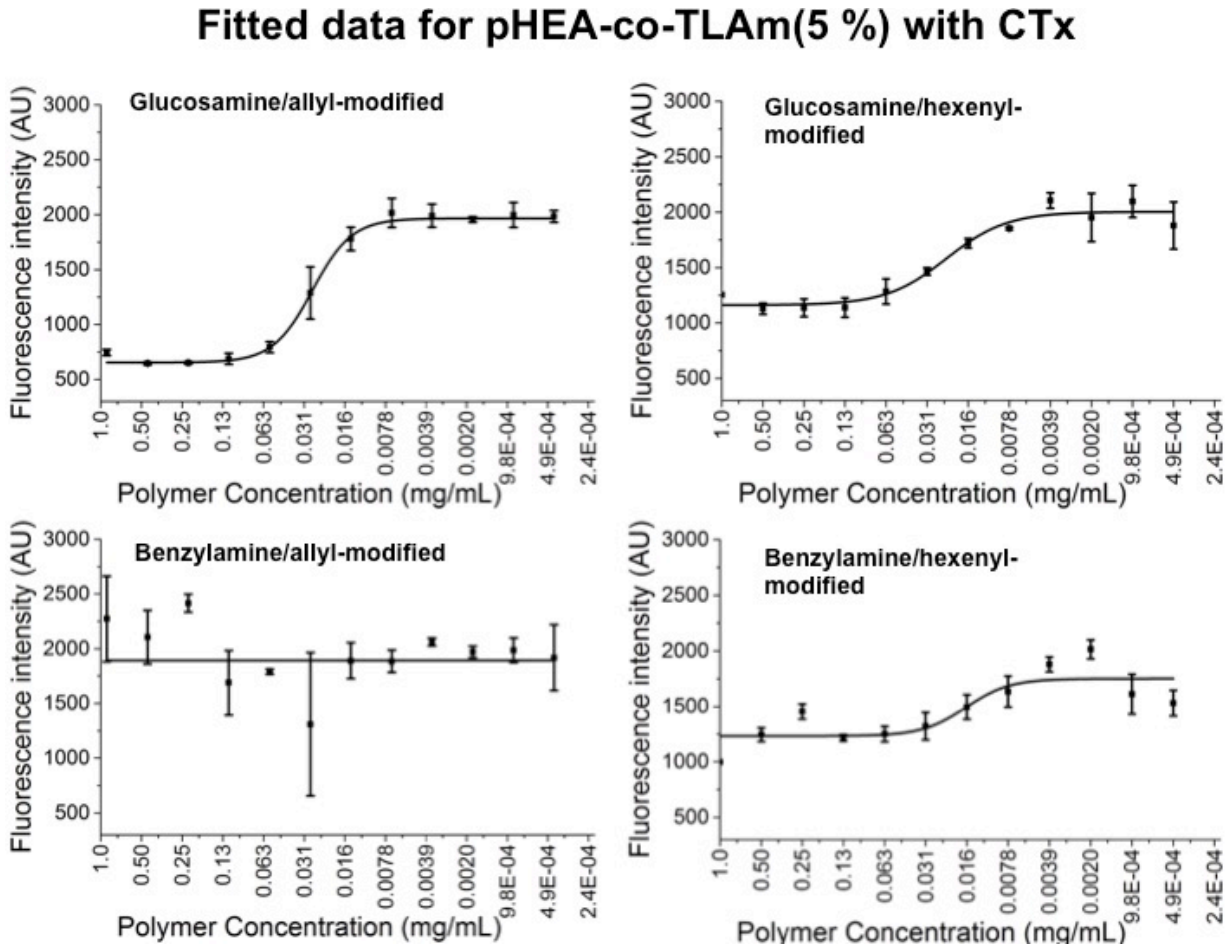


Figure S9: Fluorescence-linked sorbent assay binding curves for CTx binding to modified pHEA-co-TLAm(5%) copolymers.

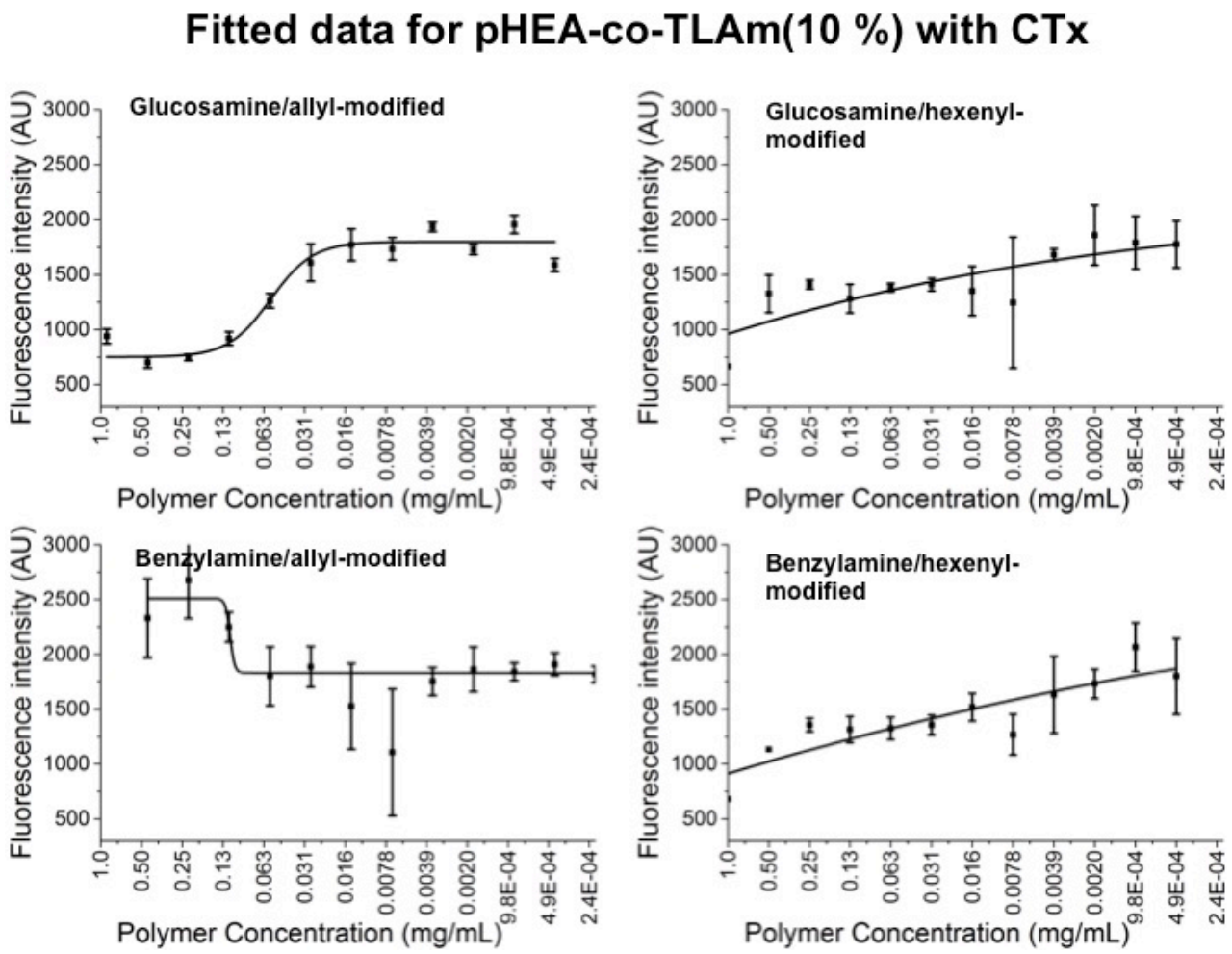


Figure S10: Fluorescence-linked sorbent assay binding curves for CTx binding to modified pHEA-co-TLAm(10%) copolymers.

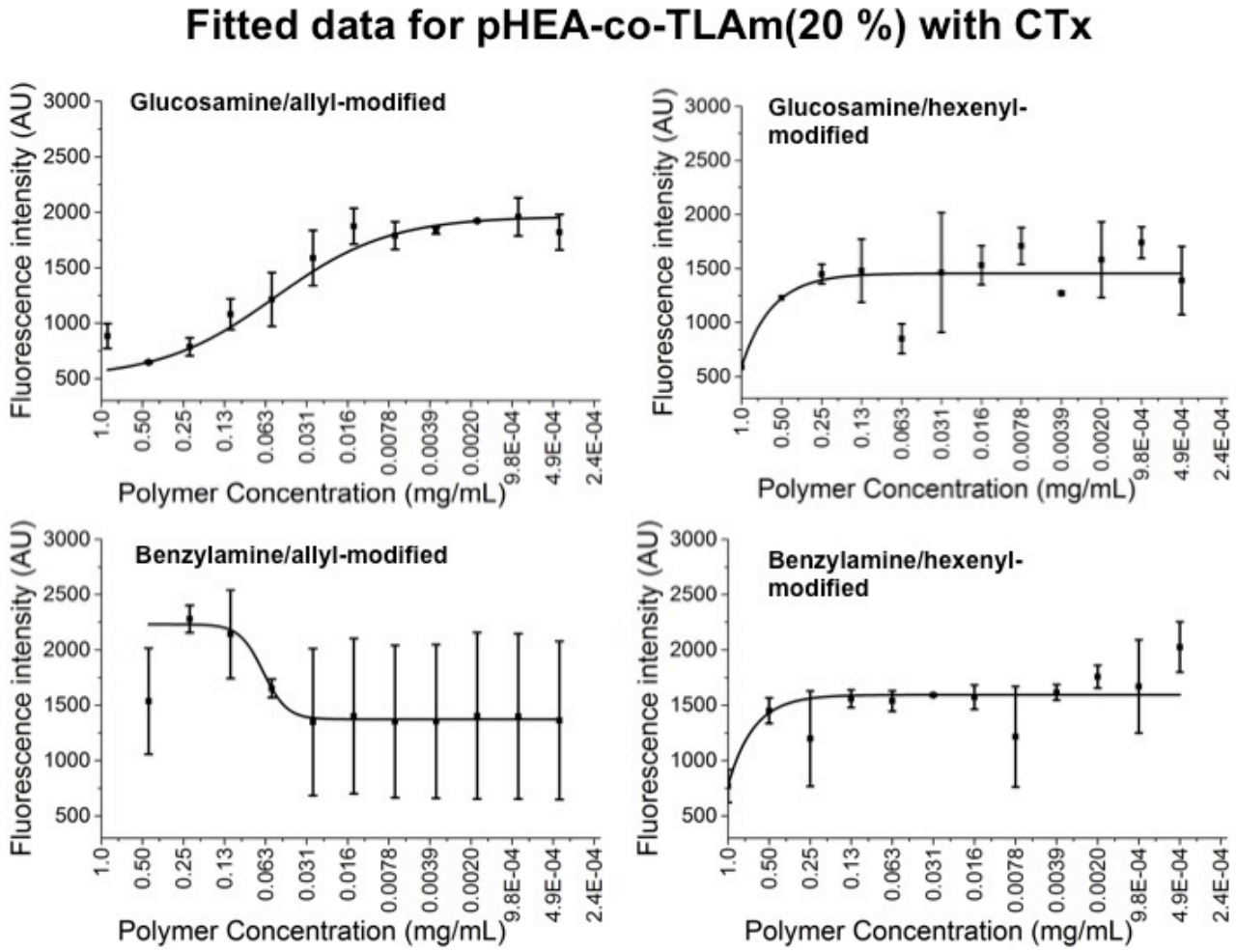


Figure S11: Fluorescence-linked sorbent assay binding curves for CTx binding to modified pHEA-co-TLAm(20%) copolymers.

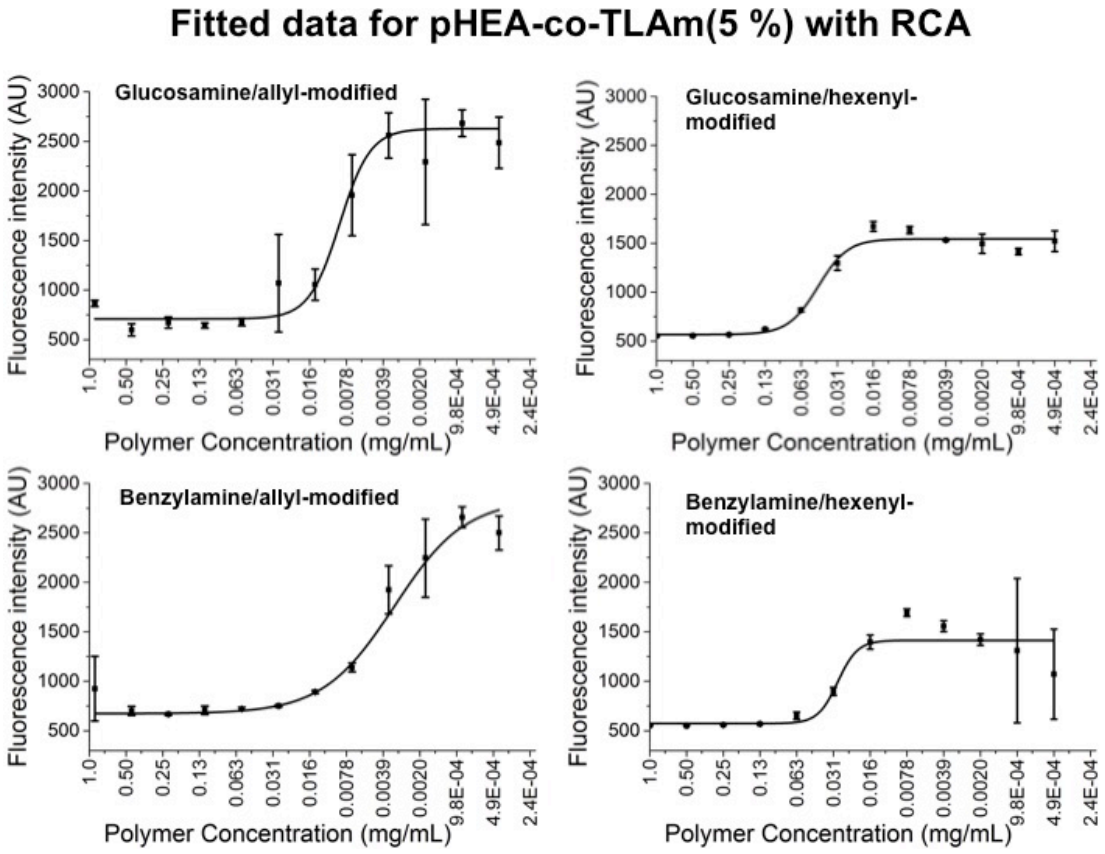


Figure S12: Fluorescence-linked sorbent assay binding curves for RCA binding to modified pHEA-co-TLAm(5%) copolymers.

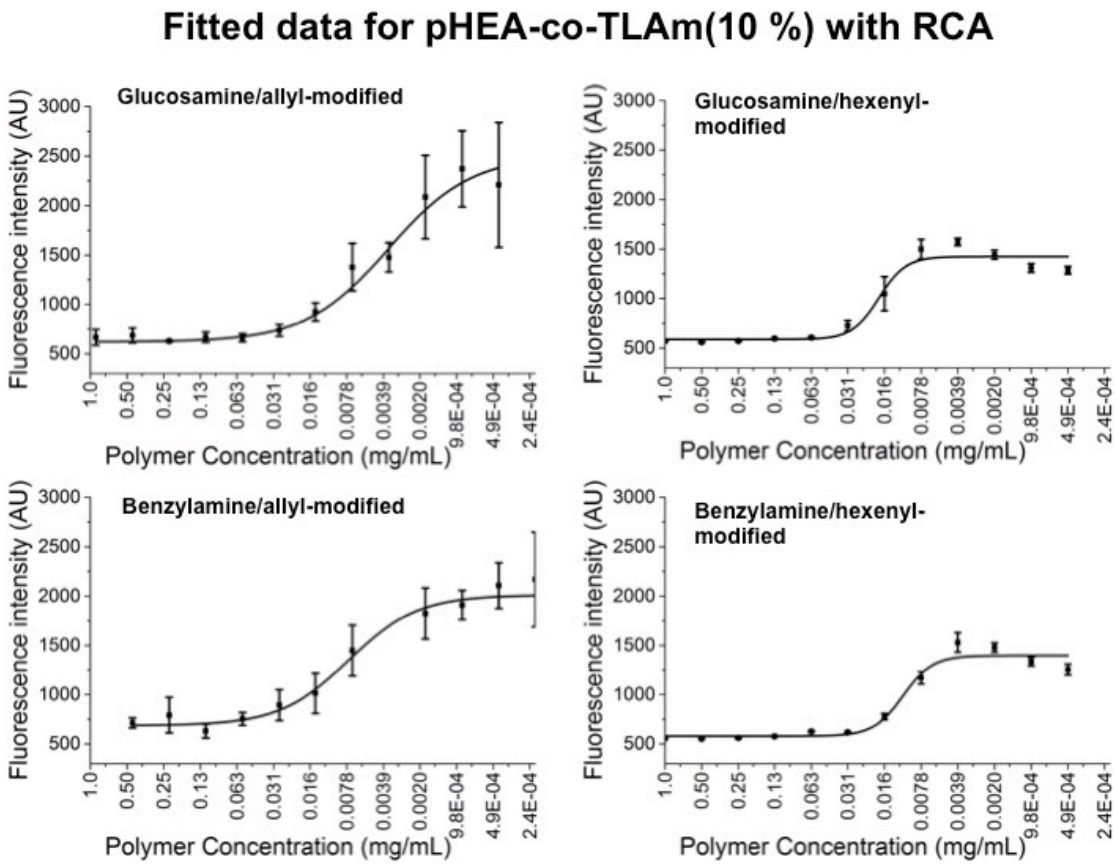


Figure S13: Fluorescence-linked sorbent assay binding curves for RCA binding to modified pHEA-co-TLAm(10%) copolymers.

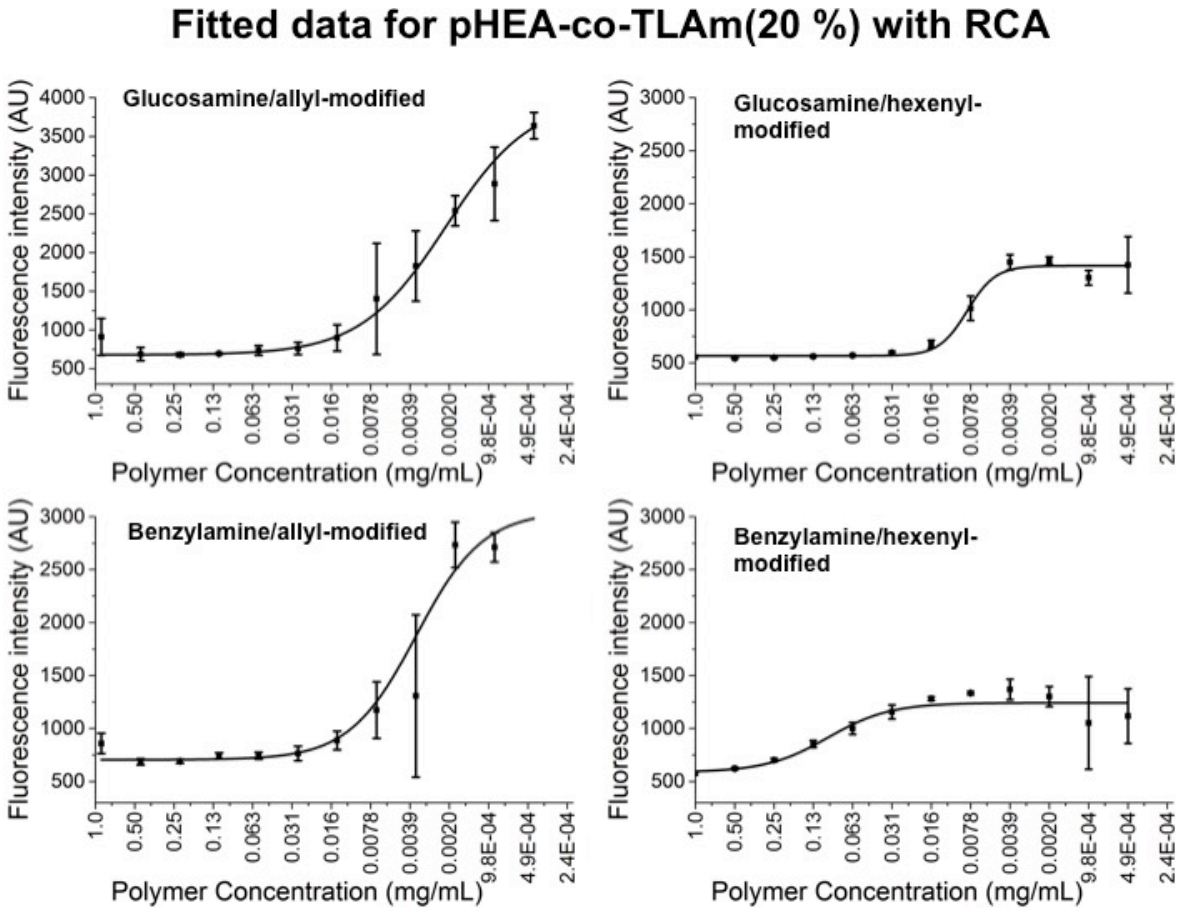


Figure S14: Fluorescence-linked sorbent assay binding curves for RCA binding to modified pHEA-co-TLAm(20%) copolymers..

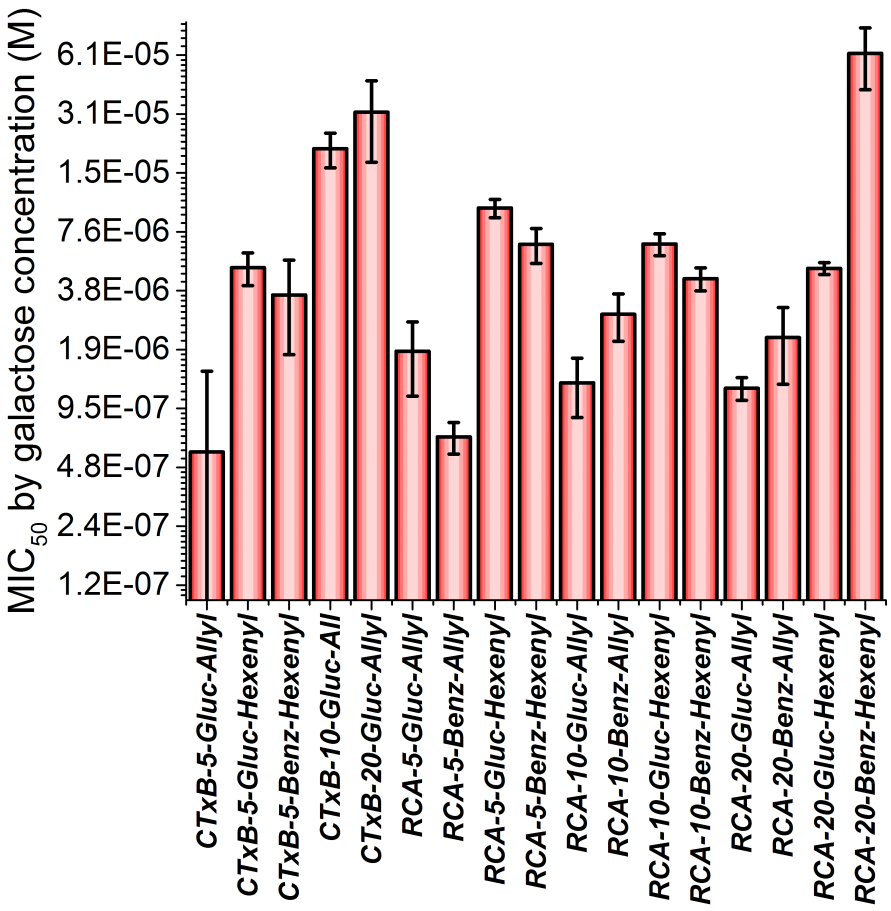


Figure S15: MIC_{50} values from fluorescence-linked sorbent assays.

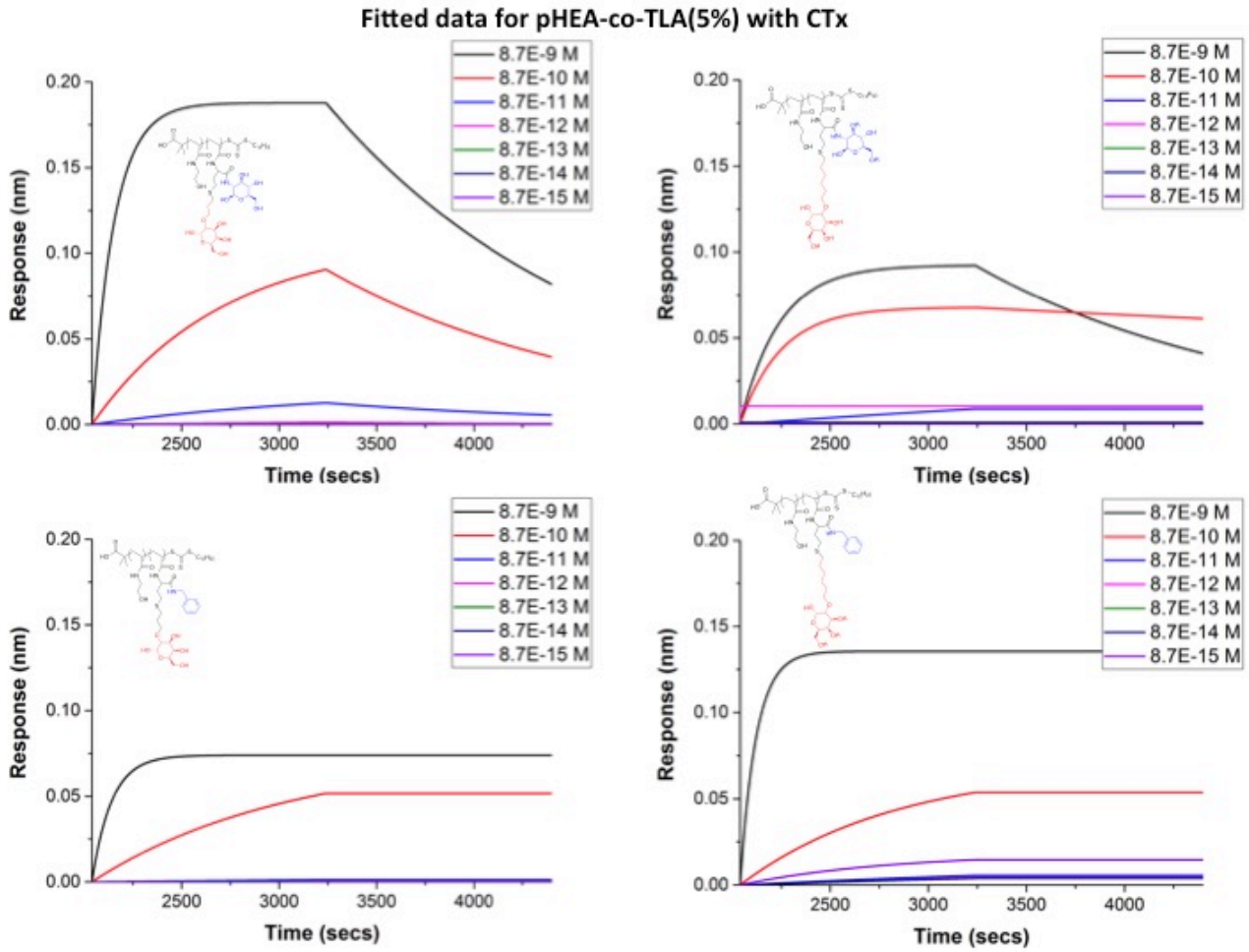


Figure S16: Bilayer interferometry dissociation curves for CTx binding to modified pHEA-co-TLAm(5%) copolymers.

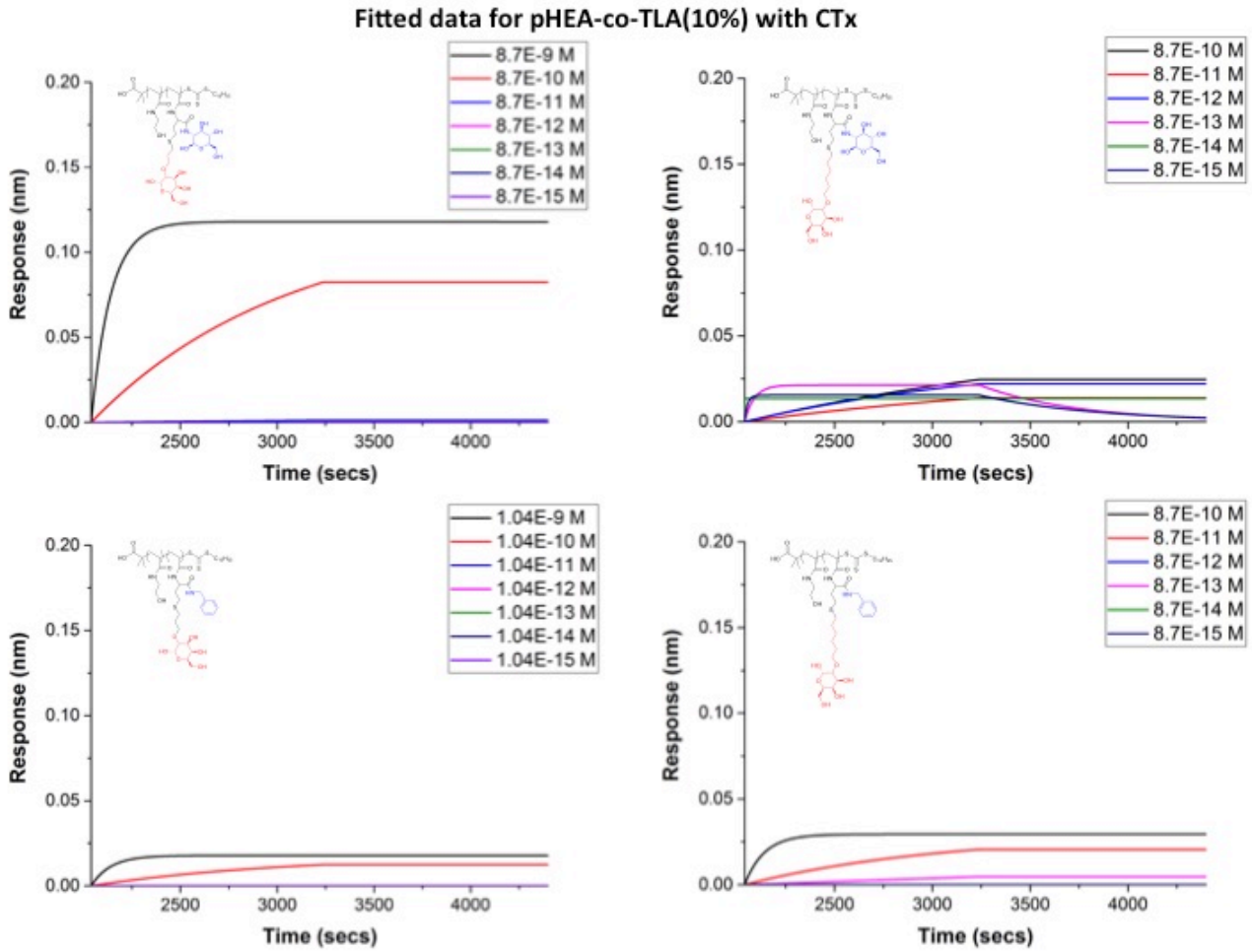


Figure S17: Bilayer interferometry dissociation curves for CTx binding to modified pHEA-co-TLAm(5%) copolymers.

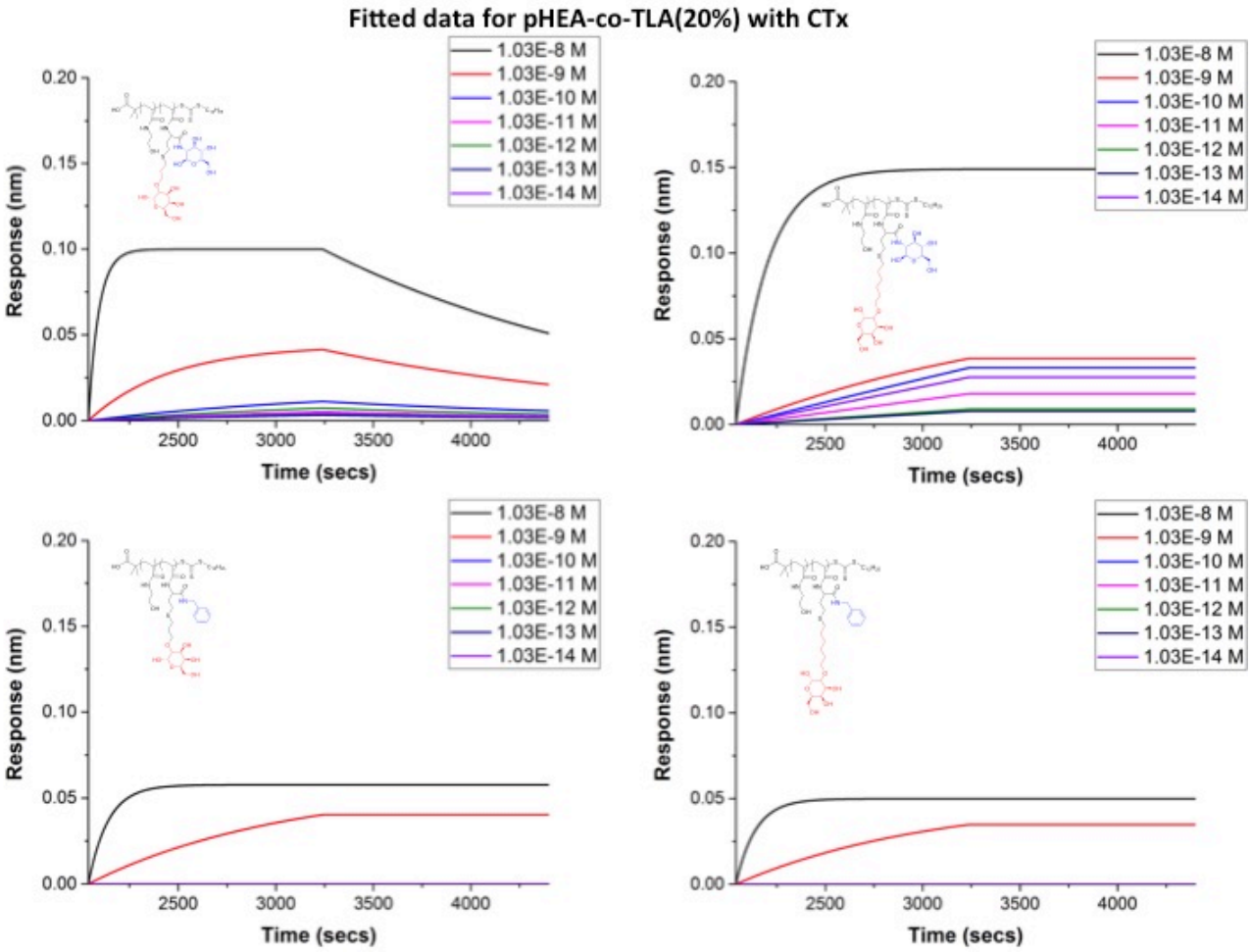


Figure S18: Bilayer interferometry dissociation curves for CTx binding to modified pHEA-co-TLAm(10%) copolymers.

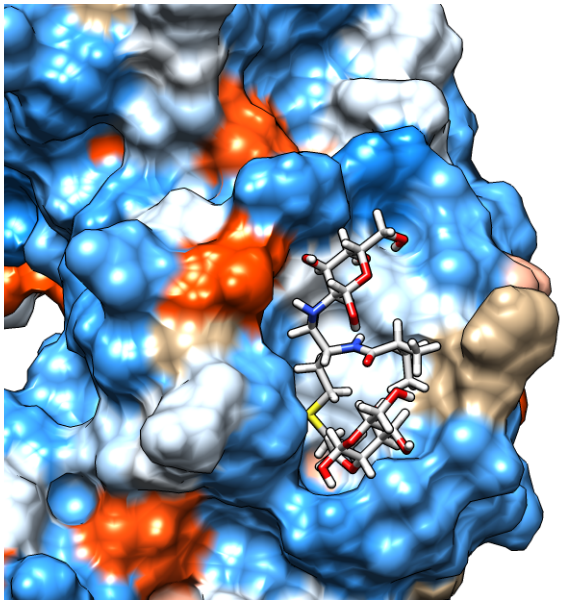


Figure S19: Image of thiolactone polymer repeat unit modified with glucosamine and allyl galactose, bound in CTx active site taken from Swiss Dock simulation.

TLA m %	Linker to gal	Amine	Lectin	KD (M)	KD Error	Kon (1/Ms)	kon Error	Kdis (1/s)	kdis Error	RMax	RMax Error
5	Allyl	Glucosamine	CTx	7.79×10^{-10}	8.12×10^{-12}	9.20×10^{5}	6.78×10^{3}	7.16×10^{-4}	5.28×10^{-6}	0.205	0.0005
5	Allyl	Benzylamine	CTx	$<1.0 \times 10^{-12}$	7.37×10^{-12}	1.15×10^{6}	4.22×10^{4}	$<1.0 \times 10^{-7}$		0.0740	0.0004
10	Allyl	Glucosamine	CTx	$<1.0 \times 10^{-12}$	7.54×10^{-12}	1.15×10^{6}	4.32×10^{4}	9.90×10^{-7}	8.67×10^{-6}	0.118	0.0007
10	Allyl	Benzylamine	CTx	$<1.0 \times 10^{-12}$	1.56×10^{-12}	9.59×10^{6}	6.20×10^{5}	$<1.0 \times 10^{-7}$		0.0178	0.0002
20	Allyl	Glucosamine	CTx	3.29×10^{-10}	4.34×10^{-12}	1.77×10^{6}	2.11×10^{4}	5.82×10^{-4}	3.27×10^{-6}	0.103	0.0002
20	Allyl	Benzylamine	CTx	$<1.0 \times 10^{-12}$	1.17×10^{-11}	9.63×10^{5}	4.31×10^{3}	$<1.0 \times 10^{-7}$		0.0576	0.0004
5	Hexyl	Glucosamine	CTx	1.40×10^{-9}	2.87×10^{-11}	5.01×10^{5}	8.80×10^{3}	6.99×10^{-4}	7.46×10^{-6}	0.107	0.0006
5	Hexyl	Benzylamine	CTx	$<1.0 \times 10^{-12}$	2.75×10^{-12}	1.40×10^{6}	2.33×10^{4}	$<1.0 \times 10^{-7}$		0.136	0.0003
10	Hexyl	Glucosamine	CTx	$<1.0 \times 10^{-12}$	4.20×10^{-11}	5.74×10^{5}	1.49×10^{5}	$<1.0 \times 10^{-7}$		0.0544	0.0114
10	Hexyl	Benzylamine	CTx	$<1.0 \times 10^{-12}$	2.22×10^{-11}	1.15×10^{7}	1.28×10^{7}	$<1.0 \times 10^{-7}$		0.029	0.005
20	Hexyl	Glucosamine	CTx	$<1.0 \times 10^{-12}$	5.34×10^{-12}	5.97×10^{5}	6.60×10^{3}	$<1.0 \times 10^{-7}$		0.149	0.0003
20	Hexyl	Benzylamine	CTx	$<1.0 \times 10^{-12}$	5.39×10^{-11}	9.70×10^{5}	2.20×10^{5}	$<1.0 \times 10^{-7}$		0.0498	0.0017
5	Allyl	Glucosamine	RCA	2.83×10^{-10}	4.68×10^{-12}	3.68×10^{6}	5.84×10^{4}	1.04×10^{-3}	4.92×10^{-6}	0.201	0.0003
5	Allyl	Benzylamine	RCA	7.66×10^{-10}	1.46×10^{-11}	3.33×10^{5}	3.98×10^{3}	2.55×10^{-4}	3.81×10^{-6}	0.0403	0.0002
10	Allyl	Glucosamine	RCA	1.15×10^{-12}	7.16×10^{-12}	1.15×10^{6}	4.10×10^{4}	1.32×10^{-6}	8.23×10^{-6}	0.168	0.0009
10	Allyl	Benzylamine	RCA	$<1.0 \times 10^{-12}$	1.68×10^{-12}	9.59×10^{6}	19.7	$<1.0 \times 10^{-7}$		0.010	0.0001
20	Allyl	Glucosamine	RCA	$<1.0 \times 10^{-12}$	8.96×10^{-12}	9.70×10^{5}	3.65×10^{4}	9.44×10^{-7}	8.69×10^{-6}	0.147	0.0008

20	Allyl	Benzylamine	RCA	$<1.0 \times 10^{-12}$	1.28×10^{-11}	9.70×10^{5}	5.21×10^{4}	$<1.0 \times 10^{-7}$		0.0151	0.0001
5	Hexyl	Glucosamine	RCA	9.71×10^{-10}	1.34×10^{-11}	4.87×10^{5}	5.17×10^{3}	4.73×10^{-4}	4.14×10^{-6}	0.101	0.0003
5	Hexyl	Benzylamine	RCA	$<1.0 \times 10^{-12}$	2.67×10^{-9}	2.22×10^{3}	3.49×10^{3}	$<1.0 \times 10^{-7}$		1.52	2.3723
10	Hexyl	Glucosamine	RCA	4.19×10^{-9}	5.75×10^{-11}	2.61×10^{5}	3.35×10^{3}	1.09×10^{-3}	5.31×10^{-6}	0.139	0.001
10	Hexyl	Benzylamine	RCA	6.47×10^{-12}	3.96×10^{-11}	1.15×10^{6}	2.33×10^{5}	7.44×10^{-6}	4.55×10^{-5}	0.182	0.0056
20	Hexyl	Glucosamine	RCA	$<1.0 \times 10^{-12}$	2.89×10^{-11}	9.70×10^{5}	6.63×10^{3}	1.70×10^{-7}	2.80×10^{-5}	0.0455	0.0008
20	Hexyl	Benzylamine	RCA	4.45×10^{-12}	$<1.0 \times 10^{-12}$	1.08×10^{8}	2.38×10^{6}	4.79×10^{-4}	4.07×10^{-6}	0.176	0.0004

Table S1: Extracted parameters from BLI analysis. Included for completeness.