Supplementary Information

Paclitaxel-terminated peptide brush polymers

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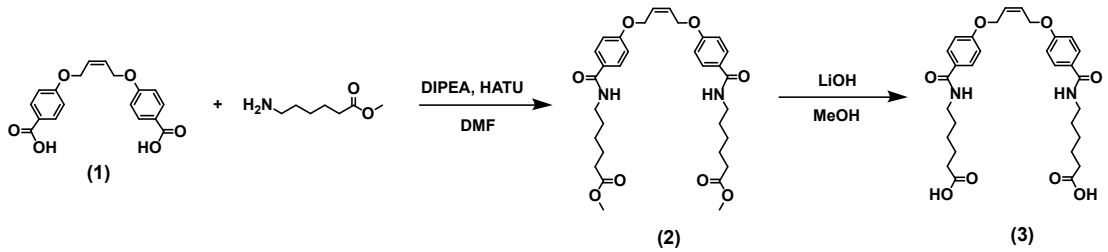
1. General Methods: All reagents were purchased from commercial sources and used without further purification. A549 lung carcinoma cells were obtained from subcultures of cells from ATCC. Sealed ampules of DMF-d_7 (Sigma-Aldrich) were degassed before use. Norbornenyl-glycine was prepared as described[1]. Modified 2nd Generation Grubbs Ruthenium initiator $(IMesH_2)(C_5H_5N)_2(Cl)_2Ru{=}CHPh$ was prepared as described[2]. Polymerizations were performed in a glove box. Peptide monomers were synthesized on a Biotage Alstra peptide synthesizer. Analytical RP-HPLC analysis was performed on a Waters Symmetry column (150 x 4.60 mm) using a Waters 1525 Binary HPLC pump equipped with Waters 2998 Photodiode Array Detector. Peptide monomers were purified on a Semi-Prep RP-HPLC using a Waters SunFire column (250 x 19 mm). The solvent system for both HPLC instruments consists of 0.1% TFA in water (buffer A) and 0.1% TFA in acetonitrile (buffer B). ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. Chemical shifts were reported in ppm relative to the residual solvent peak or TMS peak. ESI-MS spectra were performed on a LCQ-Advantage mass spectrometer. MALDI-TOF-MS spectra were performed on a Bruker Ultraflextreme mass spectrometer. MALDI-TOF-MS spectra of polymers were performed using a matrix solution of 2,5-dihydroxybenzoic acid in 1:3 water: acetonitrile with 0.1% TFA (30 mg/ml), and polymer solutions in water (1 mg/ml). The solutions were mixed in a 10:1 ratio (matrix: polymer). DLS measurements were performed on a Wyatt DynaPro NanoStar. Absorbance at 570 nm was measured in 96 well plates using a TECAN Spark 10M microplate reader. Flow cytometry measurements were performed using a BD Accuri C6 Plus. Confocal images were taken on a Nikon TI-E+A1 microscope (Nikon, Japan).

2. Experimental

2.1. Synthesis of PTX-Containing Chain Transfer Agent



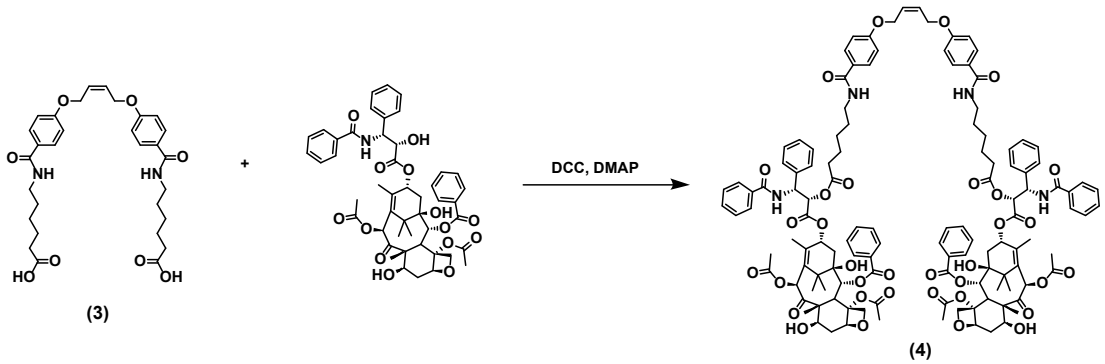
(I). Synthesis of dimethyl 6,6'-((4,4'-(but-2-ene-1,4-diylbis(oxy))bis(benzoyl))bis(azanediyl))(Z)-dihexanoate (2) (Z)-4,4'-(but-2-ene-1,4-diylbis(oxy))dibenzoic acid (1) was prepared as previously described.[3] To a solution of **(1)** (182.4 mg, 1.0 equiv.) in 10 mL DMF, HATU (472 mg, 2.2 equiv.) and DIPEA (450 µL, 4.6 equiv.) were added. The mixture was stirred at room temperature for 30 minutes. To the mixture 6-Aminocaproic acid methyl ester hydrochloride (237 mg, 2.3 equiv.) and DIPEA (240 µL, 2.5 equiv.) were added. The reaction was stirred for another 48 hours and then concentrated to dryness. The residue was resuspended in CH_2Cl_2 and washed by water (x1) and HCl (aq) (1M) (x3). The organic layer was collected and dried by Na_2SO_4, filtered and concentrated. The obtained solid was then purified by flash chromatography (4:1 Ethyl Acetate: Petroleum Ether) to give the product as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.39 (m, 4H, 2×CH_2), 1.63 (m, 8H, 4×CH_2), 2.31 (t, 4H, 2×CH_2), 3.41 (t, 4H, 2×CH_2), 3.65 (s, 6H, 2×CH_3), 4.70 (d, 4H, 2×CH_2), 5.92 (t, 2H, 2×CH), 6.88 (d, 4H, 4×Ar), 7.73 (d, 4H, 4×Ar). ^{13}C NMR (101 MHz, $CDCl_3$): 24.45, 26.41, 29.29, 33.85, 39.59, 51.50, 64.35, 114.39, 127.45, 128.37, 128.77, 160.74, 167.03, 174.09. ESI-MS (+): m/z 605.28 $[M+Na]^+$.

(II). Synthesis of (Z)-6,6'-((4,4'-(but-2-ene-1,4-diylbis(oxy))bis(benzoyl))bis(azanediyl))dihexanoic acid (3)

To a container charged with **(2)** (100.6 mg, 1 equiv.) was added 4:1 MeOH: H_2O (100 mL) containing LiOH (85.4 mg, 20.6 equiv.). The mixture was stirred at 35 °C for 6 h and then MeOH was removed by rotary evaporation until a white precipitate formed. The mixture was diluted by water and acidified with HCl (conc.). The resulting white solid was collected by vacuum filtration, washed with water and dried under vacuum. ^{1}H NMR (400 MHz, MeOD): δ (ppm) 1.42 (m, 4H, 2×CH_2), 1.64 (m, 8H, 4×CH_2), 2.31 (t, 4H, 2×CH_2), 3.36 (m, 4H, 2×CH_2), 4.78 (d, 4H, 2×CH_2), 5.92 (t, 2H, 2×CH), 6.99 (d, 4H, 4×Ar), 7.78 (d, 4H, 4×Ar). ^{13}C NMR (101 MHz, MeOD): 25.79, 27.60,

30.26, 34.85, 40.79, 65.49, 115.46, 128.24, 129.52, 130.10, 162.58, 169.82, 177.55. ESI-MS (-): m/z 553.16 $[M-H]^-$.

(III). Synthesis of PTX-containing chain transfer agent (4)



To a solution of **(3)** (60.1 mg, 1 equiv.), paclitaxel (260.2 mg, 3 equiv.) and DMAP (2.65 mg, 0.2 equiv.) in 4:1 DCM: DMF (10 mL) in a 0ºC ice bath was added DCC (53.8 mg, 2.4 equiv.) slowly. The reaction was stirred for 30 minutes and then the ice bath was removed and the reaction was stirred for another 8 h. The mixture was filtered and washed with water (x1) and HCl (aq) (1M) (x2), then dried (Na_2SO_4), filtered and concentrated to dryness. The white crude material was purified by flash chromatography (3:1 Ethyl Acetate:Petroleum Ether to 100% Ethyl Acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.12 (s, 6H, 2×CH_3), 1.19 (s, 6H, 2×CH_3), 1.37 (m, 4H, 2×CH_2), 1.56-1.67 (m, 14H, 4×CH_2, 2×CH_3), 1.78 (s, 2H, 2×OH), 1.87 (m, 2H, CH_2), 1.92 (s, 6H, 2×CH_3), 2.07-2.12 (dd, 2H, CH_2), 2.21 (s, 6H, 2×CH_3), 2.29-2.34 (dd, 2H, CH_2), 2.42 (m, 10H, 2×CH_3, 2×CH_2), 2.54 (m, 4H, CH_2, 2×OH), 3.29-3.41 (m, 4H, 2×CH_2), 3.80 (d, 2H, 2×CH), 4.18-4.31 (dd, 4H, 2×CH_2), 4.44 (t, 2H, 2×CH), 4.69 (d, 4H, 2×CH_2), 4.96 (d, 2H,2×CH), 5.50 (d, 2H, 2×CH), 5.67 (d, 2H, 2×CH), 5.91 (t, 2H, 2×CH), 5.94-5.97 (dd, 2H, 2×CH), 6.16-6.21 (m, 4H, 4×CH), 6.29 (s, 2H, 2×NH), 6.89 (d, 4H, 4×Ar), 7.15 (t, 2H, 2×NH), 7.32 (m, 2H, 2×Ar), 7.36 (t, 4H, 4×Ar), 7.40 (d, 8H, 8×Ar), 7.46 (t, 2H, 2×Ar), 7.51 (t, 4H, 4×Ar), 7.61 (t, 2H, 2×Ar), 7.67 (d, 4H, 4×Ar), 7.74 (d, 4H, 4×Ar), 8.12 (d, 4H, 4×Ar). ^{13}C NMR (101 MHz, $CDCl_3$): 9.73, 14.92, 21.15, 22.19, 22.77, 24.37, 26.11, 26.85, 29.23, 29.80, 33.70, 35.68, 39.69, 43.27, 45.73, 53.18, 58.56, 64.45, 71.88, 72.17, 74.16, 75.21, 75.72, 76.53, 79.09, 81.13, 84.56, 114.56, 126.89, 127.26, 127.40, 128.26, 128.62, 128.74, 128.81, 128.85, 129.14, 129.38, 130.30, 132.04, 132.89, 133.77, 133.82, 137.08, 142.78, 160.92, 167.02, 167.22, 167.45, 168.46, 169.93, 171.29, 172.83, 203.92. MALDI-TOF-MS (+): m/z 2249.08 $[M+Na]^+$.

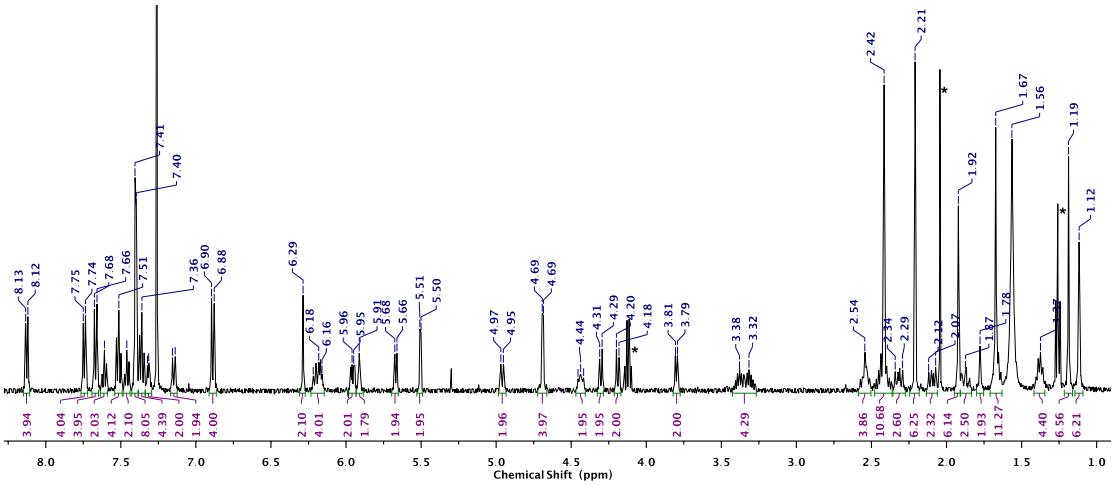


Figure S1: ^{1}H NMR spectrum of PTX-containing chain transfer agent (4) (* remaining Ethyl Acetate).

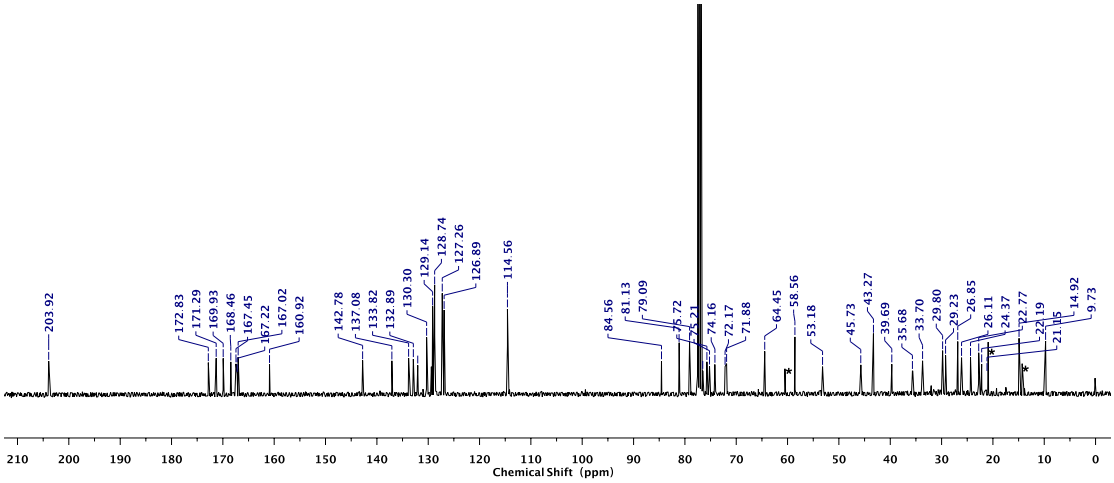


Figure S2: ^{13}C NMR spectrum of PTX-containing chain transfer agent **(4)**.

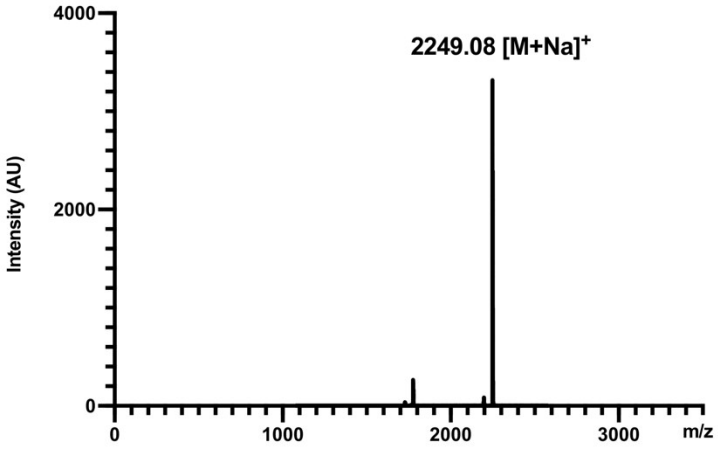
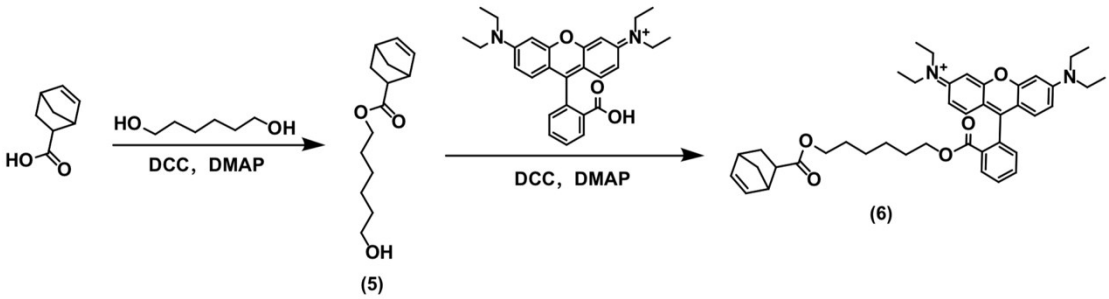


Figure S3: Mass Spectrum of PTX-containing chain transfer agent **(4)**.

2.2. Synthesis of Dye Monomer



(I) Synthesis of 6-hydroxyhexyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (5)

To a solution of 5-Norbornene-2-carboxylic acid (102.1 mg, 1 equiv.), hexane-1,6-diol (360.3 mg, 4.1 equiv.) and DMAP (9.0 mg, 0.1 equiv.) in 1 mL DCM, was slowly added a solution of DCC (198.2 mg, 1.3 equiv.) in 1 mL DCM at 0°C in an ice bath. The reaction was stirred for 30 minutes and then ice bath was removed and the reaction left to stir for another 6 h. The mixture was filtered, washed with HCl (aq) (1M) (x3), then dried (Na_2SO_4), filtered and concentrated. The crude material was further purified by flash column (4:1 Petroleum Ether: Ethyl Acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.34-1.42 (m, 5H, 2×CH_2, CH_2), 1.52 (dd, 1H, CH_2), 1.58 (m, 2H, 1×CH_2), 1.66 (m, 2H, 1×CH_2), 1.89-1.94 (m, 2H, 1×CH_2), 2.20-2.24 (m, 1H, 1×CH), 2.92 (s, 1H, 1×CH), 3.03 (s, 1H, 1×CH), 3.63 (t, 2H, 1×CH_2), 4.09 (t, 2H, 1×CH_2), 6.10-6.15 (m, 2H, 2×CH). ^{13}C NMR (101 MHz, $CDCl_3$): 25.47, 25.82, 28.75, 30.39, 32.65, 41.70, 43.29, 46.42, 46.68, 62.75, 64.49, 135.82, 138.09, 176.46. ESI-MS (+): m/z 261.06 $[M+Na]^+$.

(II)Synthesis of dye monomer (6)

To a solution of **(5)** (50.2 mg, 1 equiv.), rhodamine B (121.6 mg, 1.2 equiv.) and DMAP (2.6 mg, 0.1 equiv.) in 1 mL of DCM, was added slowly a solution of DCC (56.8 mg, 1.3 equiv.) in 0.5 mL of DCM at 0°C in an ice bath. The reaction was stirred for 30 minutes and then ice bath was removed, and the reaction left to stir for another 6 hrs. The mixture was filtered and further purified by flash chromatography (40:1 CH_2Cl_2: MeOH to 20:1 CH_2Cl_2: MeOH) and Semi-Prep RP-HPLC (65-90% buffer B). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.19-1.33 (m, 17H, 2×CH_2, 4×CH_3, CH_2), 1.44-1.57 (m, 6H, 2×CH_2, CH, CH), 1.88 (m, 1H, CH_2), 2.18 (m, 1H, 1×CH), 2.90 (s, 1H, 1×CH), 2.99 (s, 1H, 1×CH), 3.60 (q, 8H, 4×CH_2), 4.02 (s, 4H, 2×CH_2), 6.10 (d, 2H, 2×CH), 6.81 (s, 4H, Ar), 7.06 (d, 2H, Ar), 7.30 (d, 1H, Ar), 7.72-7.81 (m, 2H, Ar), 8.28 (d, 1H, Ar). ^{13}C NMR (101 MHz, $CDCl_3$): 12.32, 25.26, 28.03, 28.30, 30.13, 41.42, 42.99, 45.83, 46.14, 46.42, 63.99, 65.37, 96.20, 113.37, 113.81, 117.26, 129.90, 130.04, 130.20, 131.09, 132.80, 133.25, 135.49, 137.88, 155.36, 157.61, 158.91, 159.68, 160.06, 164.95, 176.11. ESI-MS (+): m/z 663.29 $[M+H]^+$.

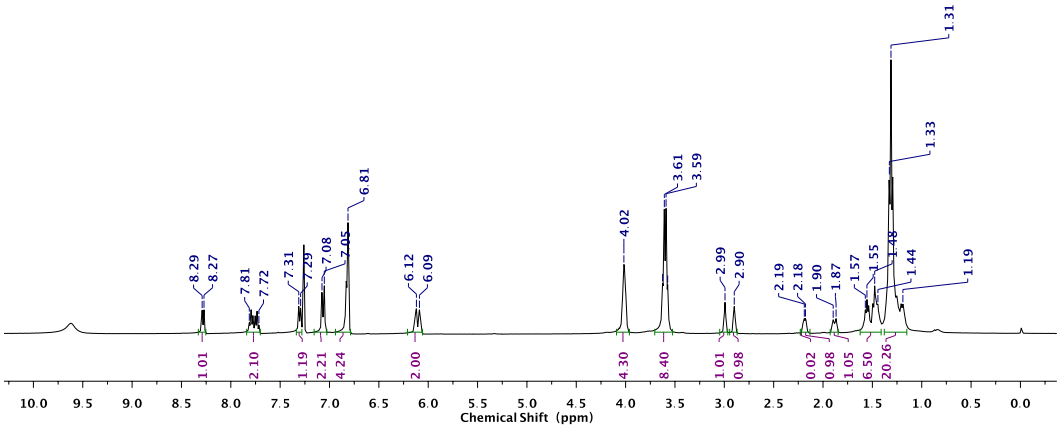


Figure S4: ^{1}H NMR spectrum of dye monomer **(6)**.

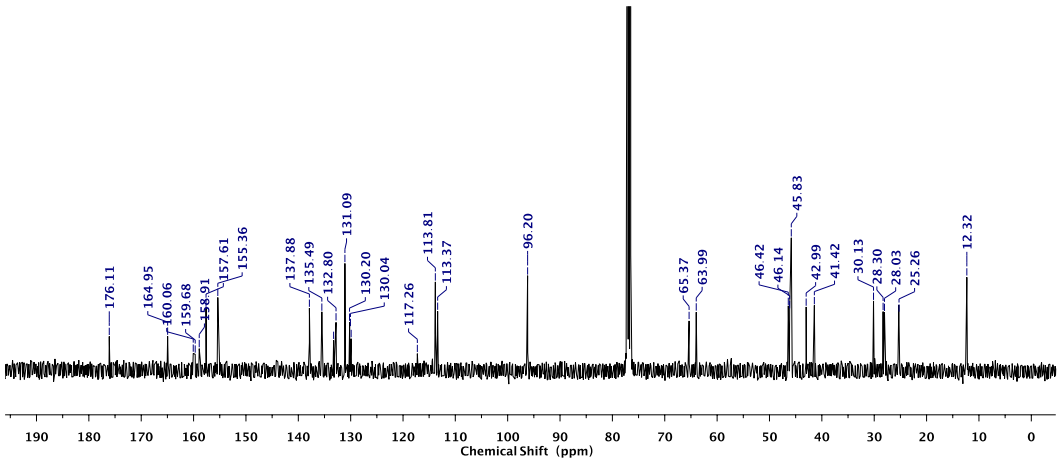


Figure S5: ^{13}C NMR spectrum of dye monomer **(6)**.

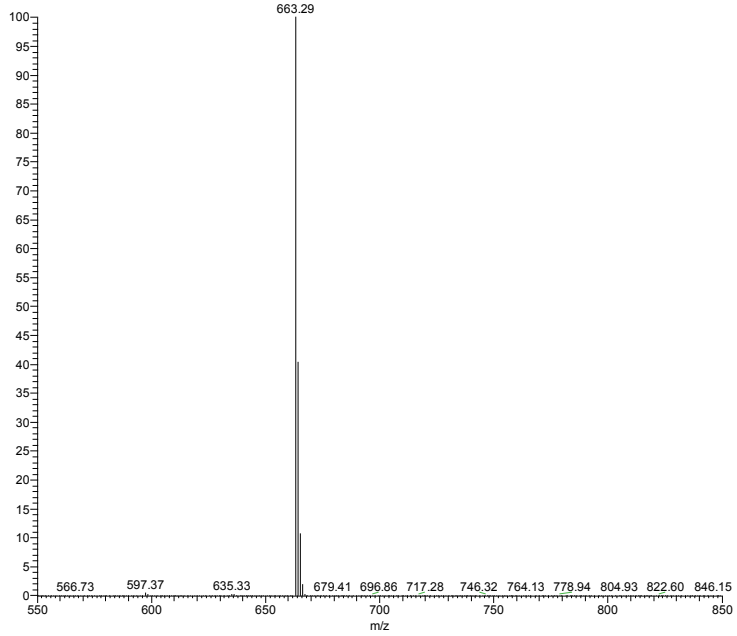
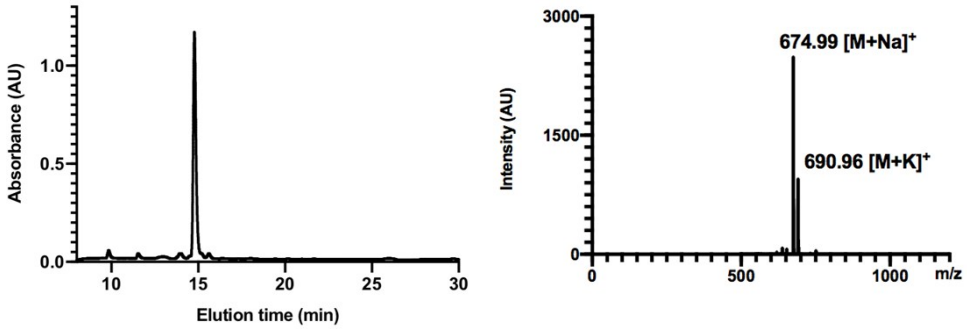


Figure S6: Mass spectrum of dye monomer **(6)**.

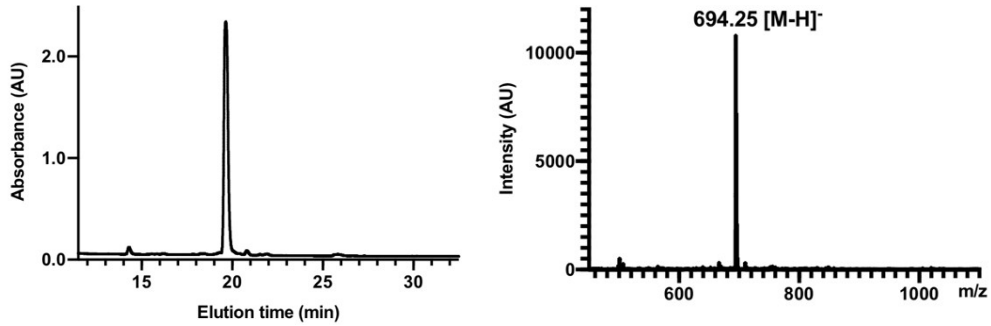
2.3. Synthesis of Peptide Monomers

Peptide monomers were synthesized via standard FMOC-based solid phase peptide synthesis using Rink Amide MBHA resin. FMOC was deprotected using a solution of 20% piperidine in DMF. Amino acid couplings were carried out using HBTU and DIPEA (resin/amino acid/HBTU/DIPEA 1: 3: 2.95: 6) for 45 mins. Norbornenyl-glycine (1.2 equiv.) coupling was carried out using HATU (1.15 equiv.) and DIPEA (2.4 equiv.) for 6 hrs. The final peptide monomers were cleaved from the resin using a mixture of TFA/H2O/TIPS (95:2.5:2.5) for 2 hrs and precipitated from cold ether. The crude products were further purified by semi-prep HPLC using UV-Vis detector at 214 nm.

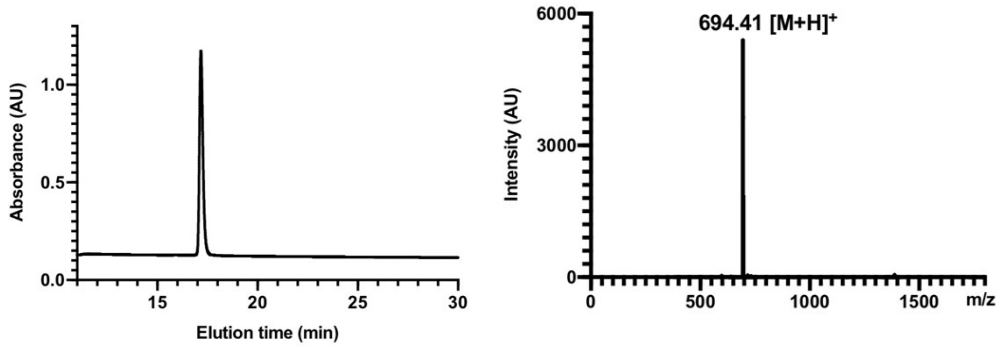
Nor-GGSGSGS



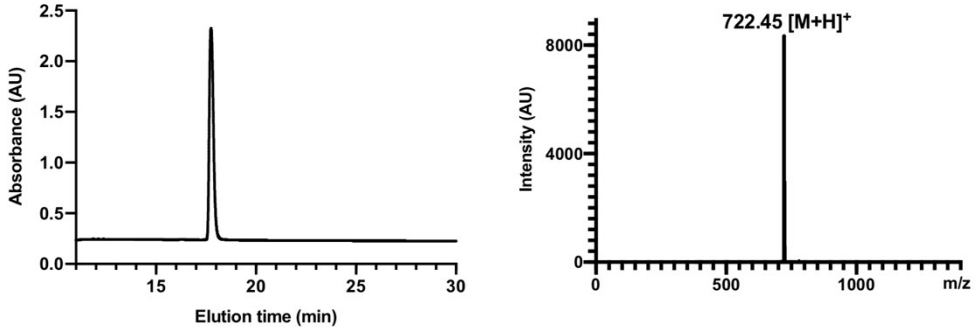
Nor-GGSGSGE



Nor-GGSGSGK



Nor-GGSGSGR



Nor-GGSGSGRR

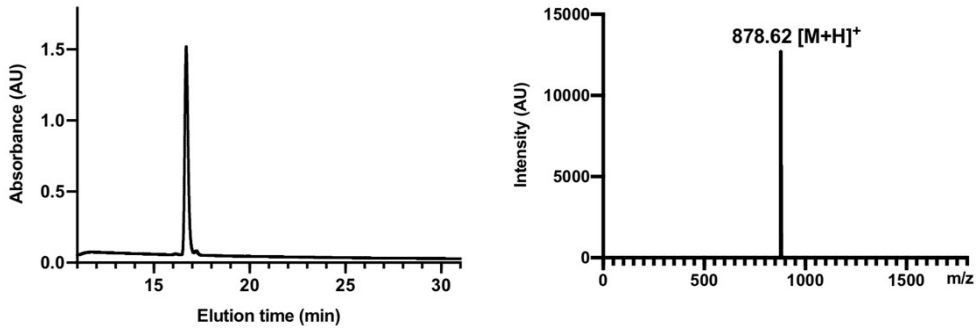


Figure S7: RP-HPLC traces and mass spectra (MALDI-TOF-MS) of all peptide monomers in this study.

2.4. Synthesis of Polymers

To stirred solutions of peptide monomers (10 equiv., 0.03 mmol/L) in DMF-d_7 (0.5 mL) were added modified 2rd Generation Grubbs initiator (1 equiv.) in a small volume of DMF-d_7. The polymerizations were stirred for 4 to 5 h, after which a solution of the dye monomer (0.2 equiv.) in a small volume DMF-d_7 was added to the reactions and left to stir for 1 hour. To each reaction PTX-containing chain transfer agent **(4)** in a small volume of dry DMF (2 equiv.) was added. After 12 h, ethyl vinyl ether was added to ensure full termination. The polymers were precipitated into a cold 1: 2 dichloromethane: ether solution (x3) and then purified by RP-HPLC with the UV-Vis detector monitoring at 214 nm. Each peak obtained from HPLC was characterized by MALDI-TOF-MS.

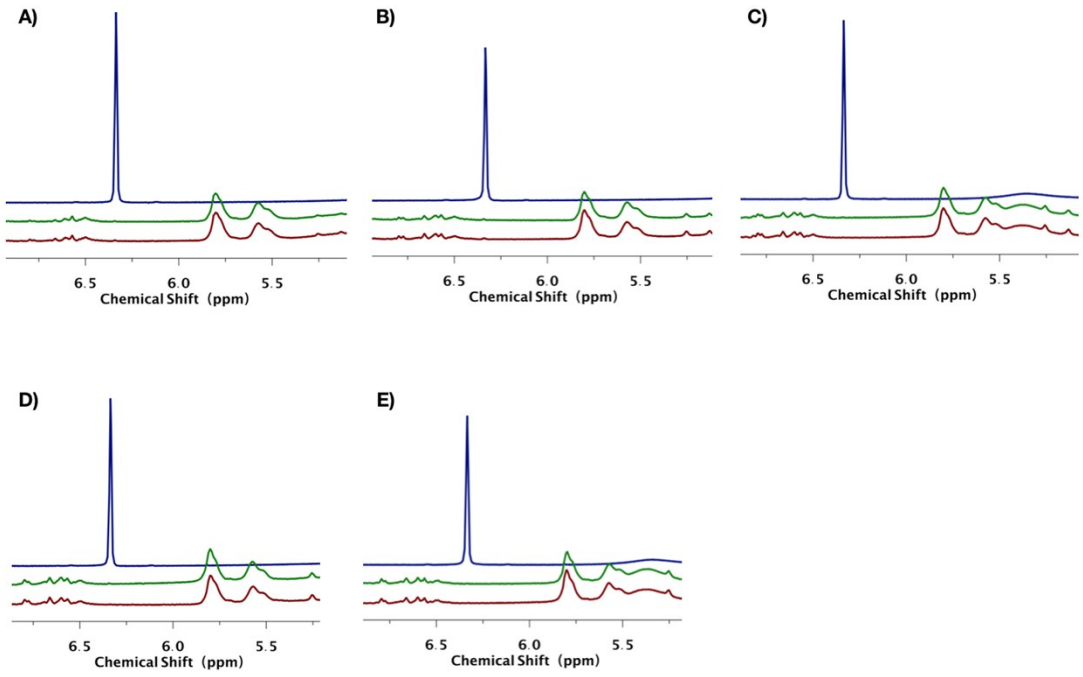


Figure S8: ^{1}H NMR spectra of ROMP polymerization of peptide norbornene monomers. Blue spectra are taken before addition of initiator; Green spectra are recorded after the polymerization of peptide monomers; Red spectra are recorded at the end of polymerization of the dye monomer. Resonance at δ 6.34 ppm corresponds to the norbornene olefin protons of the monomer. The new resonance at ~ δ 5.5-6 ppm corresponds to the cis-trans olefin protons of the polymerized material. A) Nor-GGSGSGS polymerization; B) Nor-GGSGSGE polymerization; C) Nor-GGSGSGK polymerization; D) Nor-GGSGSGR polymerization; and E) Nor-GGSGSGRR polymerization.

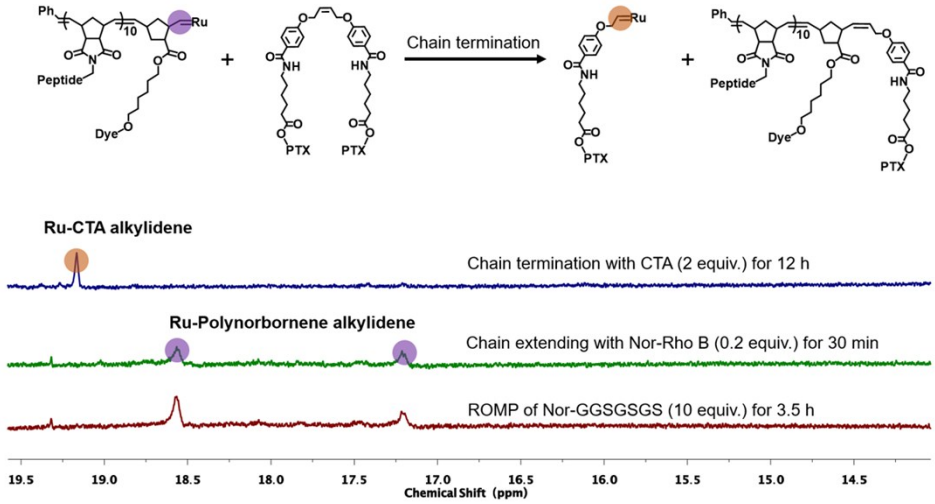


Figure S9. ^{1}H NMR spectra of ROMP of Nor-GGSGSGS, chain extension with dye monomer (0.2 equiv.), and termination with PTX-CTA (2 equiv.).

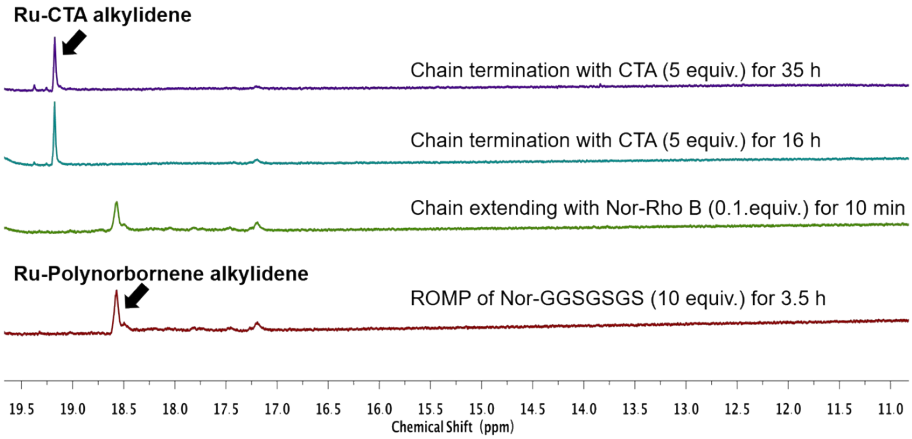


Figure S10. ^{1}H NMR spectra of ROMP of Nor-GGSGSGS, chain extension with dye monomer (0.1 equiv.), and termination with PTX-CTA (5 equiv.).

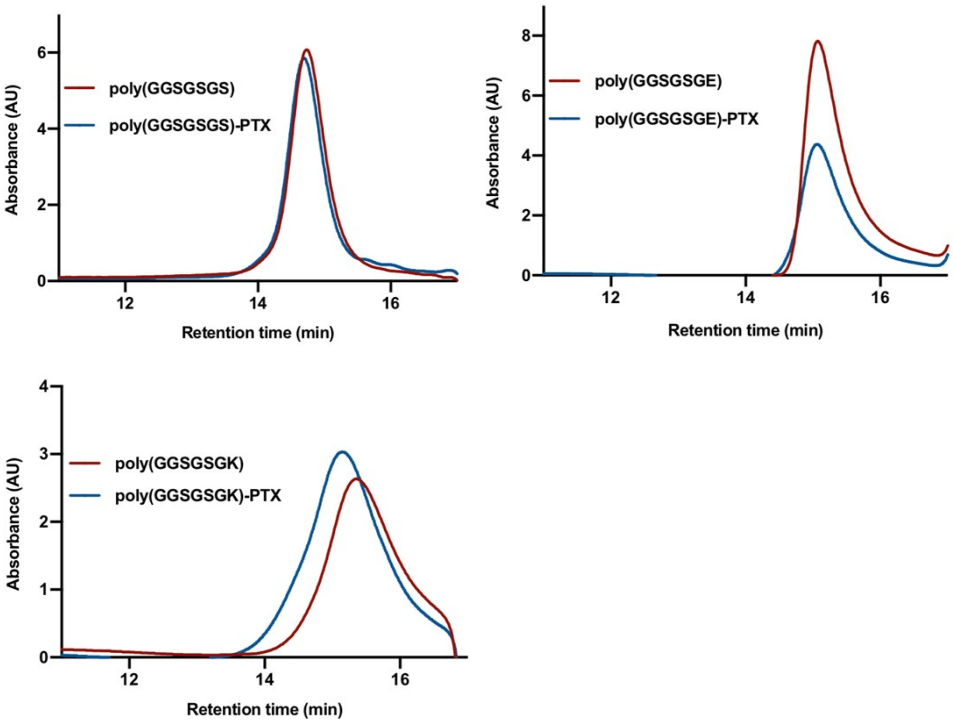


Figure S11: GPC measurements of representative peptide brush polymers. Poly(amino acid sequence) represented the polymer that was only quenched by ethyl vinyl ether. Poly(amino acid sequence)-PTX represented the polymer that was only terminated by PTX without RP-HPLC purification.

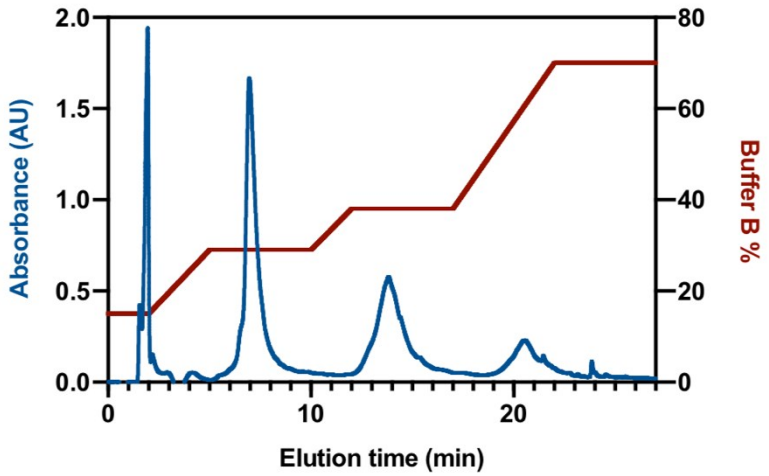


Figure S12: RP-HPLC separation of crude Poly(GGSGSGS) (monomer Nor-GGSGSGS).

Crude Poly(GGSGSGS) (monomer Nor-GGSGSGS)

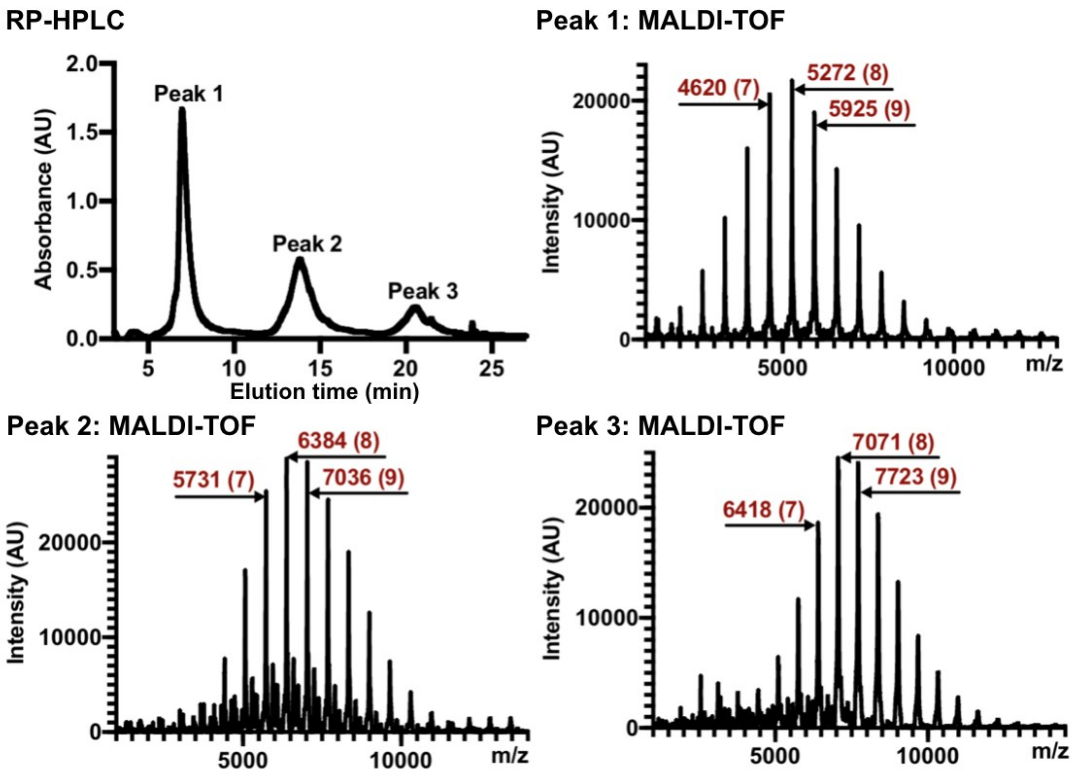


Figure S13: RP-HPLC separation of crude Poly(GGSGSGS) and MALDI-TOF-MS spectra of all the separated peaks. Peak 1, ~51%, was attributed to polymer without dye or drug, denoted as poly(GSGSGS); Peak 2, ~35%, was assigned to polymer with only drug incorporation, denoted as poly(GGSGSGS)-PTX. Peak 3, ~14%, corresponds to the dye-labeled and drug terminated polymer, denoted as poly(GGSGSGS)-Dye-PTX. The numbers in brackets indicates the DP of the polymer.

Crude Poly(GGSGSGE) (monomer Nor-GGSGSGE)

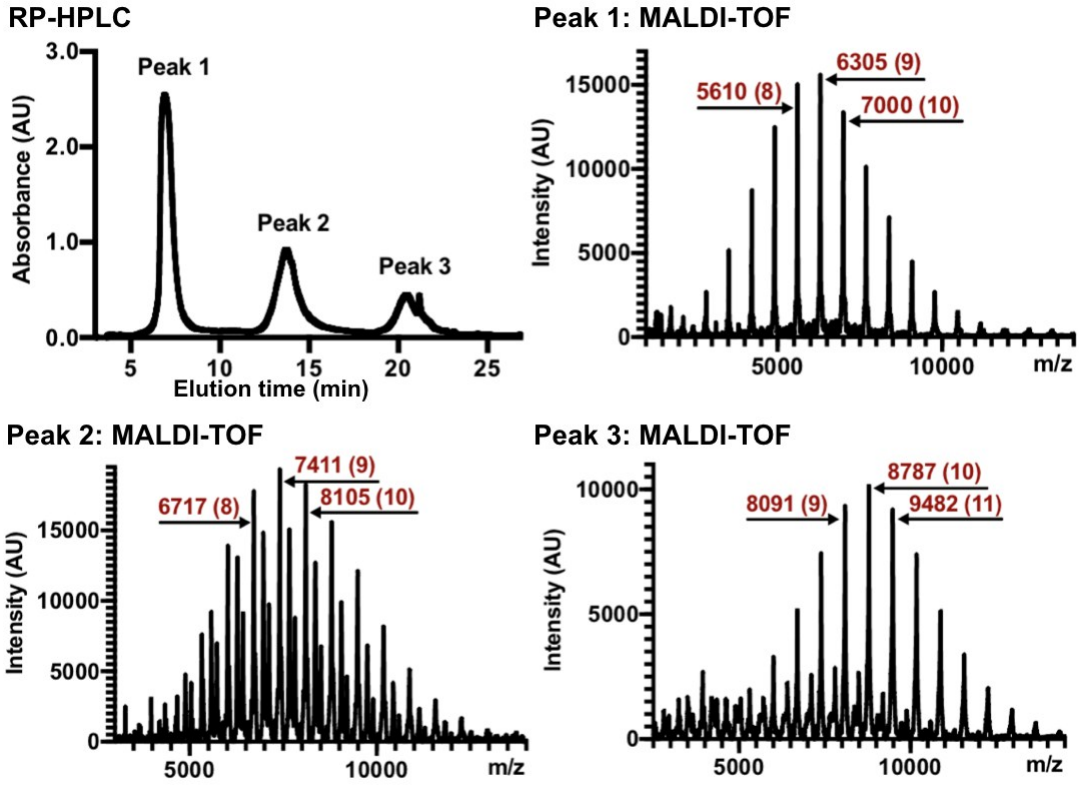


Figure S14: RP-HPLC separation of crude Poly(GGSGSGE) and MALDI-TOF-MS spectra of all the separated peaks. Peak 1, p ~53%, was attributed to polymer without dye or drug, denoted as poly(GGSGSGE); Peak 2, ~31%, was mainly assigned as polymer with drug only, denoted as poly(GGSGSGE)-PTX. Peak 3, ~16%, was the dye-labeled and drug terminated polymer, denoted as poly(GGSGSGE)-Dye-PTX. The numbers in brackets indicates the degree of polymerization.

Crude Poly(GGSGSGK) (monomer Nor-GGSGSGK)

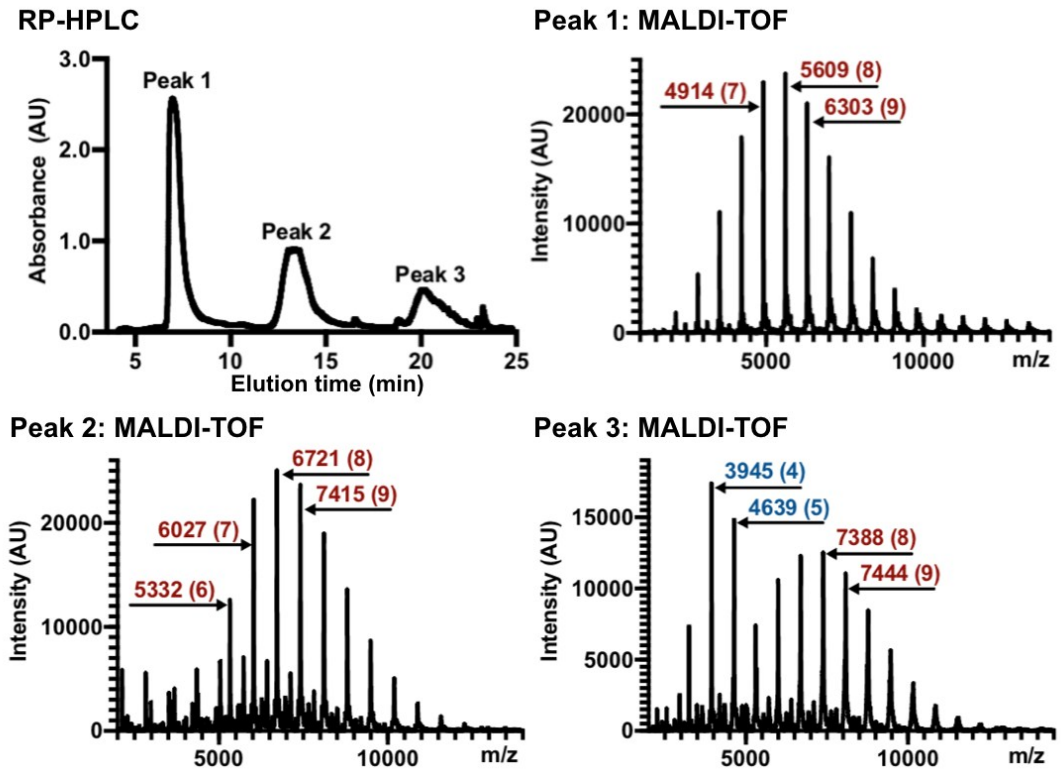


Figure S15: RP-HPLC separation of crude Poly(GGSGSGK) and MALDI-TOF-MS spectra of all the separated peaks. Peak 1, ~52%, was attributed to polymer without dye or drug, denoted as poly(GGSGSGK); Peak 2, ~33%, was mainly assigned as polymer with drug only, denoted as poly(GGSGSGK)-PTX. Peak 3, ~15%, consisted mainly of the dye-labeled and drug terminated polymer (~83%), denoted as poly(GGSGSGK)-Dye-PTX. The numbers in brackets indicates the degree of polymerization. Blue-labelled peaks are assigned as polymers containing drug only.

Crude Poly(GGSGSGR) (monomer Nor-GGSGSGR)

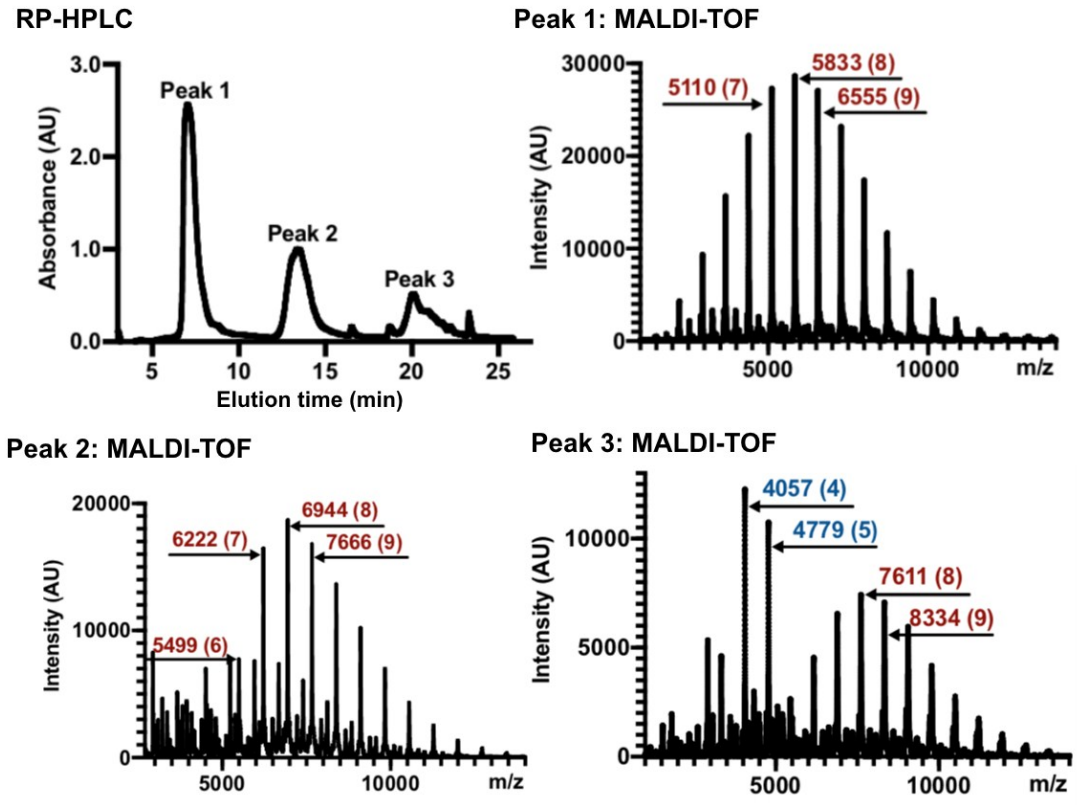


Figure S16: RP-HPLC separation of crude Poly(GGSGSGR) and MALDI-TOF-MS spectra of all the separated peaks. Peak 1, ~53%, was attributed to polymer without dye or drug, denoted as poly(GGSGSGR); Peak 2, ~32%, was mainly assigned to polymer with drug only, still denoted as poly(GGSGSGR)-PTX. Peak 3, ~15%, consisted mainly of the dye-labeled and drug terminated polymer (~81%), still denoted as poly(GGSGSGR)-Dye-PTX. The numbers in brackets indicates degree of polymerization. Blue-labelled peaks are assigned as polymers with drug only.

Crude Poly(GGSGSGRR) (monomer Nor-GGSGSGRR)

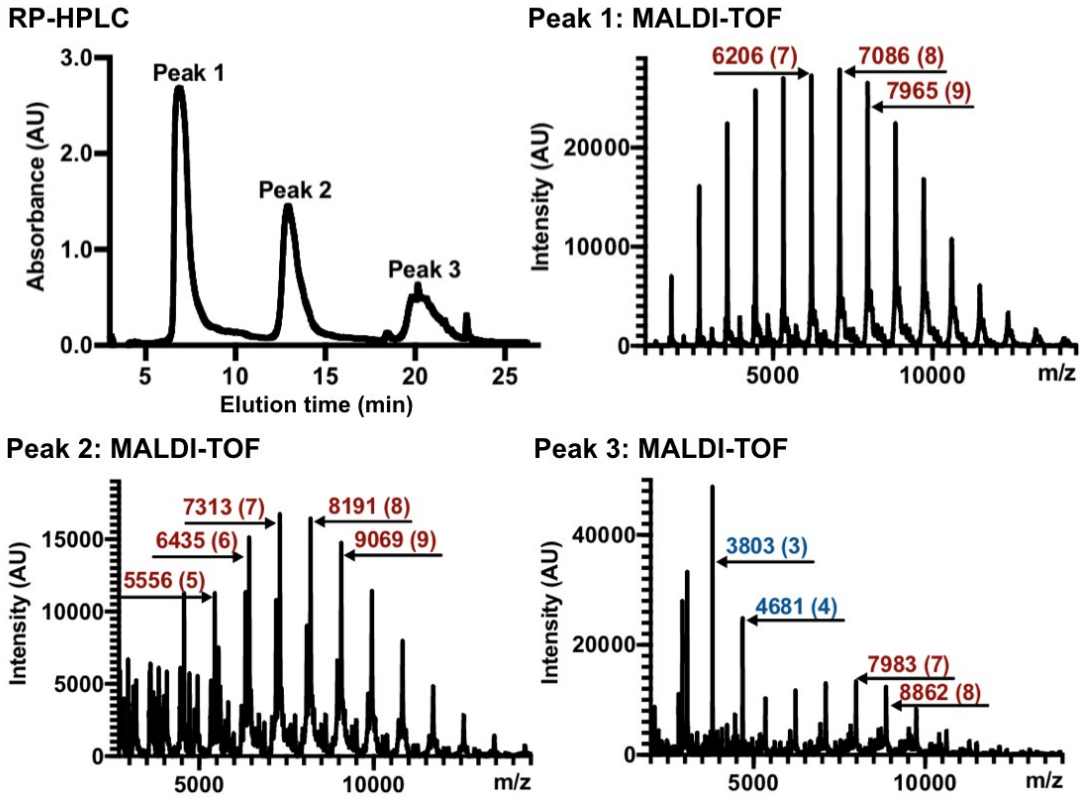


Figure S17: RP-HPLC separation of crude Poly(GGSGSGRR) and MALDI-TOF-MS spectra of all the separated peaks. Peak 1, ~53%, was attributed to polymer without dye or drug, denoted as poly(GGSGSGRR); Peak 2, ~30%, was mainly assigned to polymer with drug only, still denoted as poly(GGSGSGRR)-PTX. Peak 3, ~17%, consisted mainly of dye-labeled and drug terminated polymer (~83%), still denoted as poly(GGSGSGRR)-Dye-PTX. The numbers in brackets indicates the degree of polymerization. Blue-labelled peaks are assigned as polymers with drug only.

Table S1: Summary of theoretical and experimentally determined molecular weights and dispersities of polymers by MALDI-TOF-MS.

	Mn(Theor) (DP=10)	Mn[a]	Mw[b]	Mw/Mn	Mn[c](peak 3)
Poly(GSGSGS)	6612	5696	5999	1.05	
Poly(GSGSGS)-Dye-PTX	8375	7892	8092	1.03	7892
Poly(GSGSGE)	7033	6660	7075	1.06	
Poly(GSGSGE)-Dye-PTX	8796	9253	9481	1.02	9253
Poly(GSGSGK)	7023	6258	6793	1.09	
Poly(GSGSGK)-Dye-PTX	8786	7940	8211	1.03	7281
Poly(GSGSGR)	7303	6589	7130	1.08	
Poly(GSGSGR)-Dye-PTX	9066	8433	8743	1.04	7651
Poly(GSGSGRR)	8864	7410	8163	1.10	
Poly(GSGSGRR)-Dye-PTX	10627	8011	8338	1.04	7310

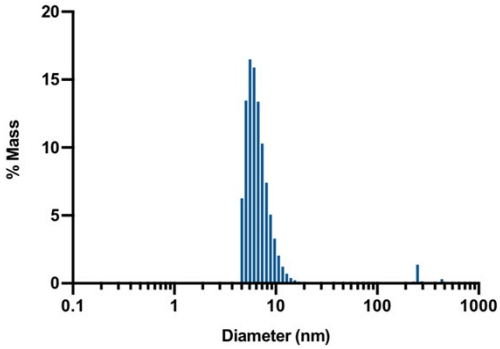
a. $Mn = \sum NiMi / \sum Ni$

b. $Mw = \sum NiMi^2 / \sum NiMi$

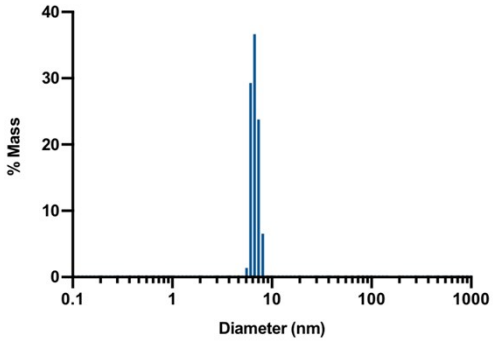
Mi refers to the mass of each peak, Ni refers to the area of each peak. Only the main peaks in the spectra were calculated.

c. The Mn (peak 3) of each polymer was determined by the average molecular weight and portion of each part.

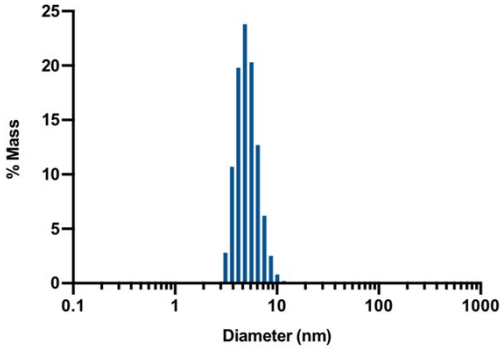
Poly(GGSGSGS)-Dye-PTX



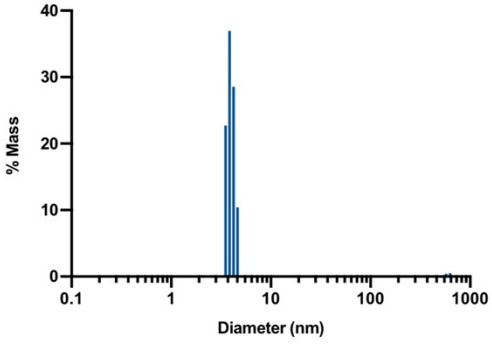
Poly(GGSGSGE)-Dye-PTX



Poly(GGSGSGK)-Dye-PTX



Poly(GGSGSGR)-Dye-PTX



Poly(GGSGSGRR)-Dye-PTX

Figure S18: Representative DLS measurements of drug-terminated peptide brush polymers.

2.5. In Vitro Analyses

2.5.1. Cell culture

A549 lung carcinoma cells were cultured in Ham's F-12k medium, supplemented with 10% fetal bovine serum and 1% antibiotics, and maintained at 37 °C in 5% CO_2.

2.5.2. Cellular uptake studies

By flow cytometry: Cells were plated at a density of 100,000 cells per well in a 24-well plate 16 hrs before treatment. Polymeric materials at 10× the desired concentration (with respect to polymer) were diluted with PBS, added to the appropriate wells, and the plates incubated for 4 hrs at 37 °C. The media was then removed and the cells washed twice with PBS. Cells that were incubated with polymers bearing positive charge (poly(GGSGSGK)-Dye-PTX/ poly(GGSGSGR)-Dye-PTX/ poly(GGSGSGRR)-Dye-PTX) were then incubated with heparin (0.5 mg /mL^{-1} in PBS) for five minutes (×3) and rinsed twice with PBS to remove any un-internalized polymer adhered to the cell surface. The cells were subsequently dissociated from the culture plate by treatment with trypsin for 5 minutes, followed by media addition and PBS. The cells were transferred to centrifuge tubes and centrifuged at 1000 rpm to form a cell pellet. The supernatant was discarded, cells resuspended in PBS, and centrifuged again. The final obtained cell pellets were resuspended in a small volume of PBS containing 1% FBS and analyzed by flow cytometry (10,000 gated events on three separate cultures per condition).

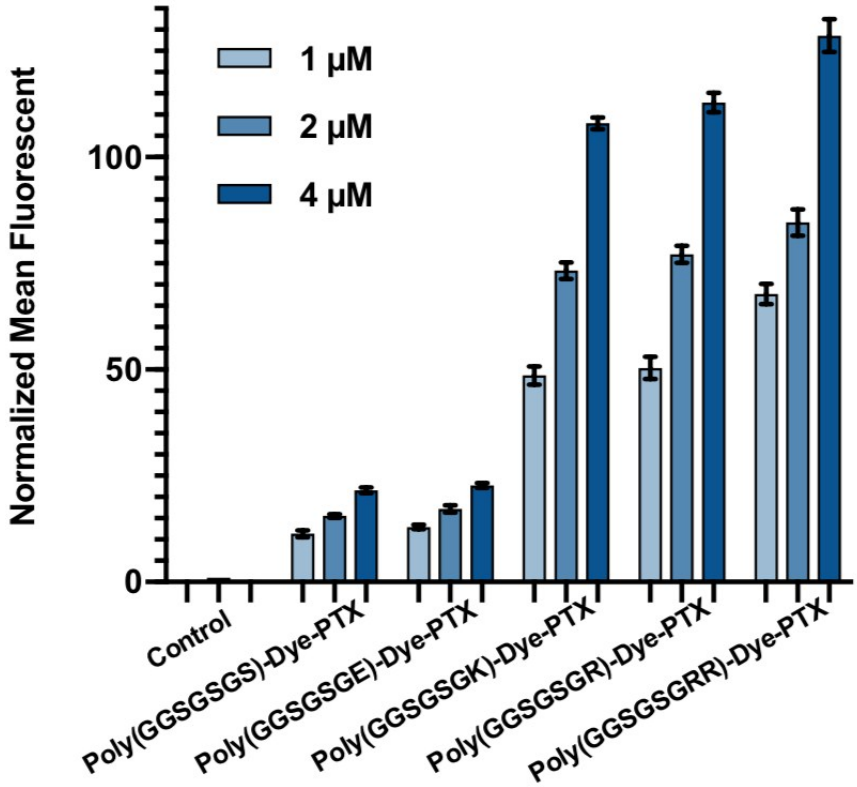


Figure S19. Flow cytometry assay of poly(amino acid sequence)-Dye-PTX with respect to 1, 2 and 4 μM of polymers. Normalized mean fluorescence refers to the mean fluorescence (PE-A) detected for the material divided by the mean fluorescence exhibited by the vehicle control (PBS).

By confocal microscopy: Cells were plated at a density of 50,000 cells in glass bottom plates and incubated for 18 hrs before treatment. 4 μM Poly(GGSGSGRR)-Dye-PTX and poly(GGSGSGS)-Dye-PTX were added to each plate, followed by incubation for 4 hrs. After that, the media was removed and the cells were washed twice with PBS. Cells treated with poly(GGSGSGRR)-Dye-PTX were incubated with heparin (0.5 mg /mL−1 in PBS) for five minutes (×3) and rinsed twice with PBS. Diluted hoechest 33342 staining solution for live cells was then add to the wells for 10 mitunes. The cells were gently washed with PBS for three times and 4% paraformaldehyde was added to fix the cells. After 10 minutes, the fixation solution was removed and cells washed three times with PBS.

2.5.3. Cytotoxicity Assays

The cytotoxicity of all polymers was assessed using the MTT metabolic assay. A549 cells were plated at a density of 2,000 cells/well in a 96-well plate 18 hrs prior to treatment. Materials at 10× the desired concentration (with respect to polymer) were diluted with PBS, added to the appropriate wells, and the plates incubated for 4 hrs at 37°C. Following

incubation, the materials were removed, cells were washed twice with PBS, supplemented with 100 µL Ham's F-12K media, and incubated for an additional 48 or 72 hrs.

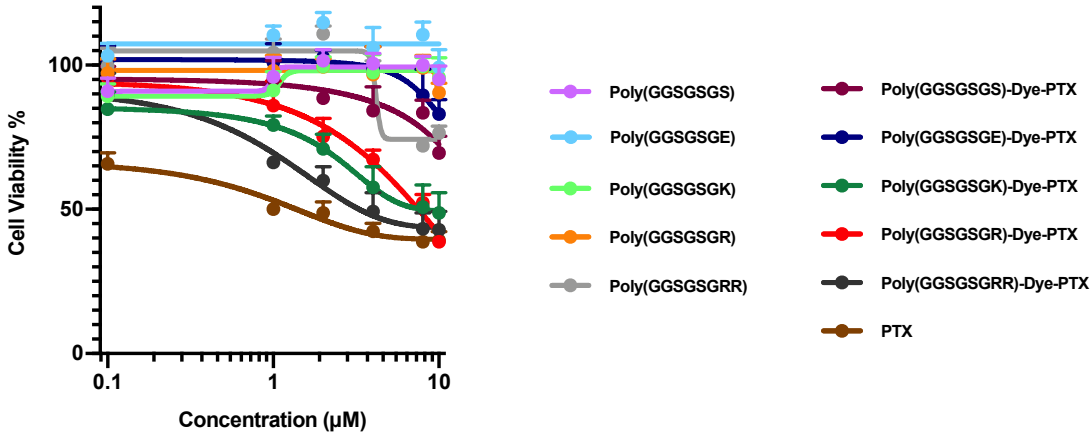


Figure S20. Cytotoxicity of purified polymer-drug conjugates and polymers without drugs. Cell viability was measured relative to vehicle control. Cells were incubated for 72 hrs after treatment with the polymers.

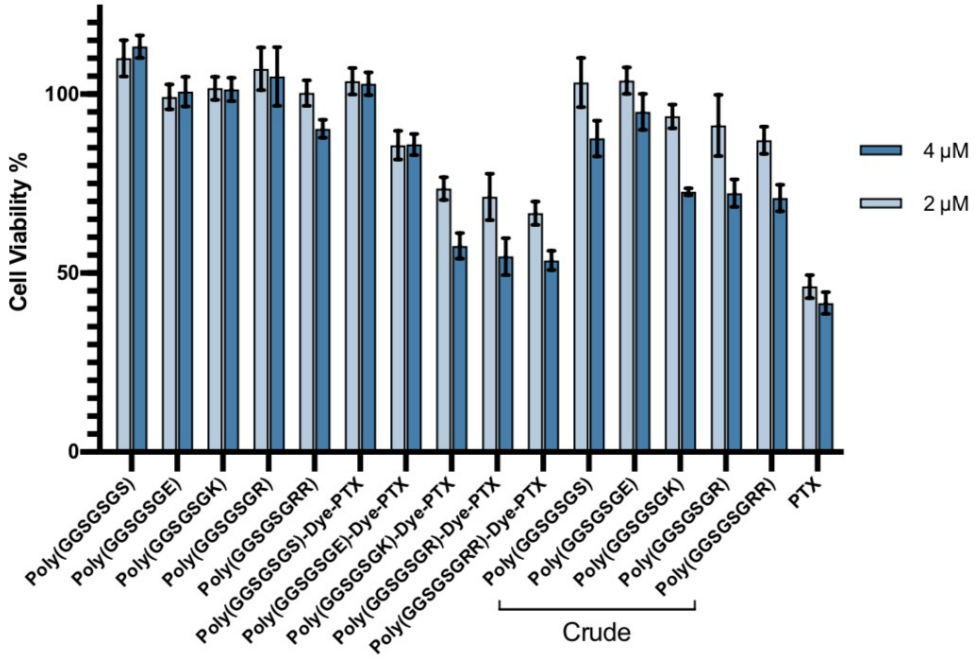


Figure S21. Cytotoxicity of purified polymer-drug conjugates, polymers without drugs, and crude polymers. Cell viability was measured relative to vehicle control. Cells were incubated for 48 hrs after treatment with the polymers.

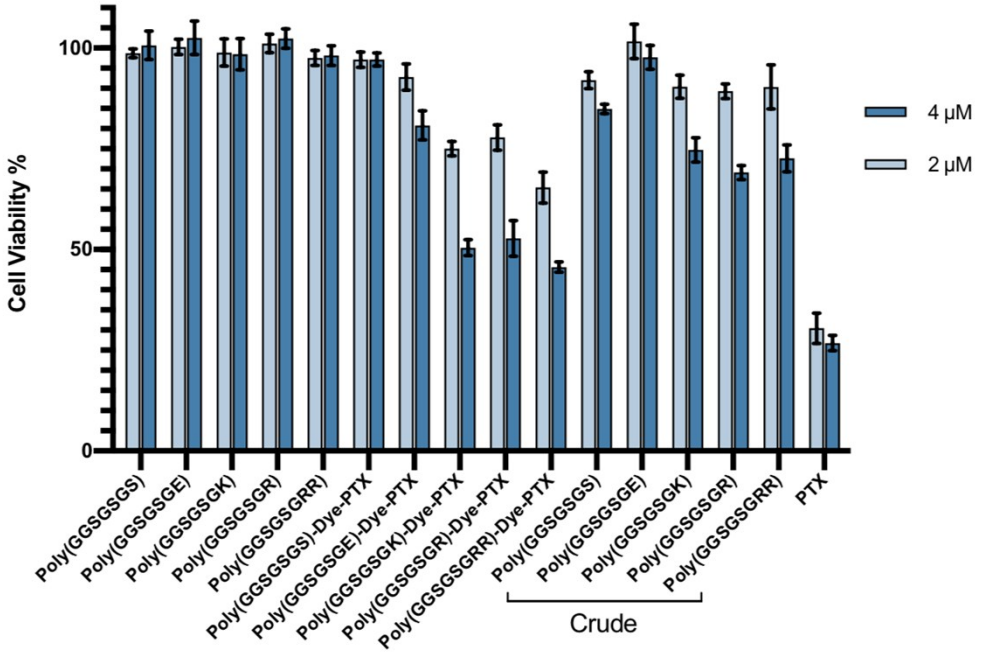


Figure S22. Cytotoxicity of purified polymer-drug conjugates, polymers without drugs, and crude polymers. Cell viability was measured relative to vehicle control. Cells were incubated for 72 hrs after treatment with the polymers.

2.5.4 Proteolytic Degradation Assays

Monomer Nor-GGSGSGK and polymer Poly(GGSGSGK) (50μM, with respect to peptide concentration) were treated with pronase (0.35U/mL) in DPBS at 37 °C. After treatment, the enzyme was heat denatured at 65 °C for 15 min.

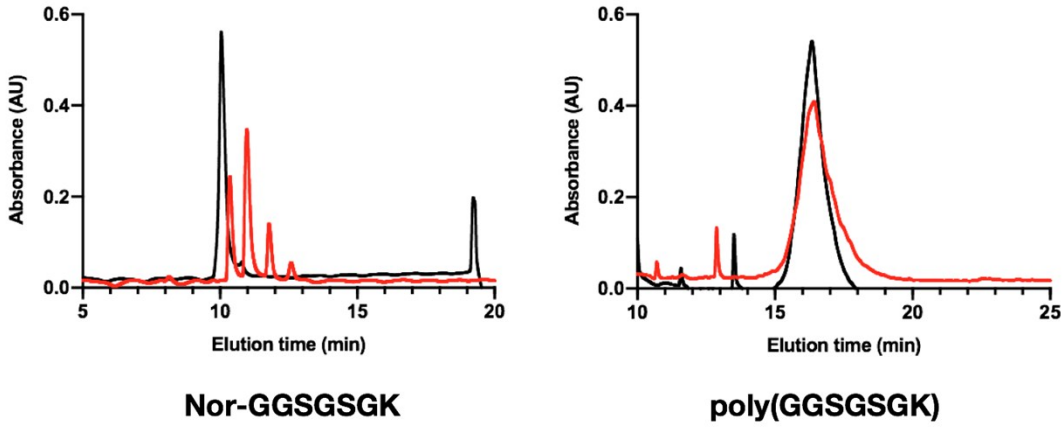


Figure S23. RP-HPLC assay of the proteolytic cleavage of monomer Nor-GGSGSGK and polymer Poly(GGSGSGK). The traces in black are the materials without pronase treatment and the red traces were the same materials after pronase treatment for 3 hrs.

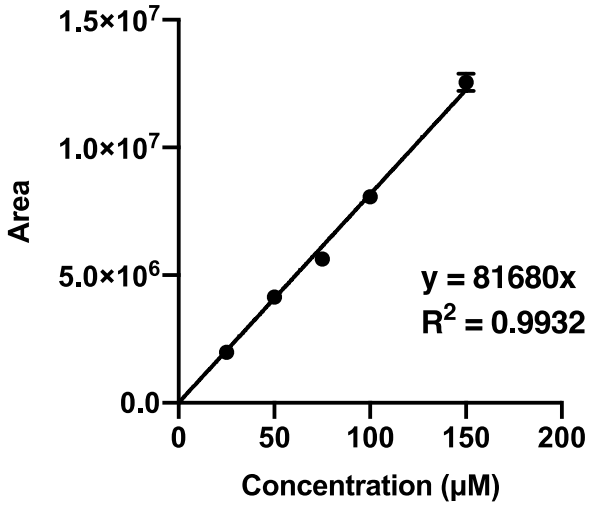


Figure S24. Standard curves, correlating peak area on RP-HPLC chromatograms with concentration on a 50 μL injection, for the determination of the concentration of intact peptide monomer remaining after proteolytic cleavage.

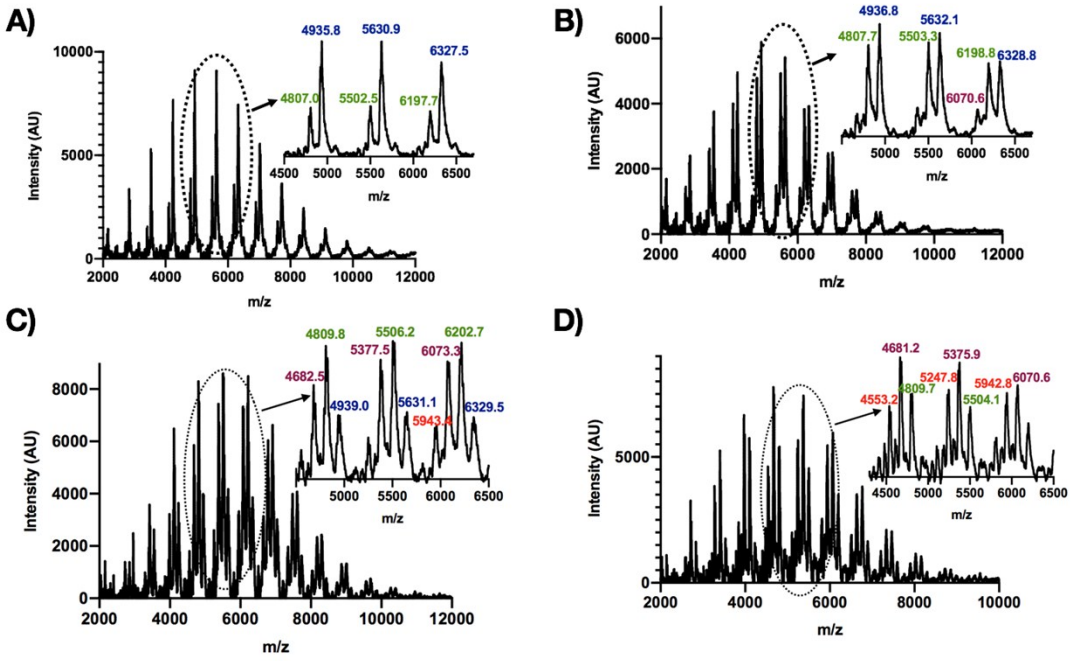


Figure S25. MALDI-TOF-MS spectra of polymer Poly(GGSGSGK) after pronase treatment. Numbers in blue were the mass of intact polymers; Numbers in green were the mass of polymers that lost one lysine (-128.1); Numbers in purple were the mass of polymers that lost two lysine; Numbers in red were the mass of polymers that lost three lysine. A) after 1 hr pronase treatment; B) after 2 hrs pronase treatment; C) after 3 hrs pronase treatment; D) after 4 hrs pronase treatment. Those spectra were used to determine the extent of intact peptide of polymers except spectrum D, whose S/N was too low for quantification.

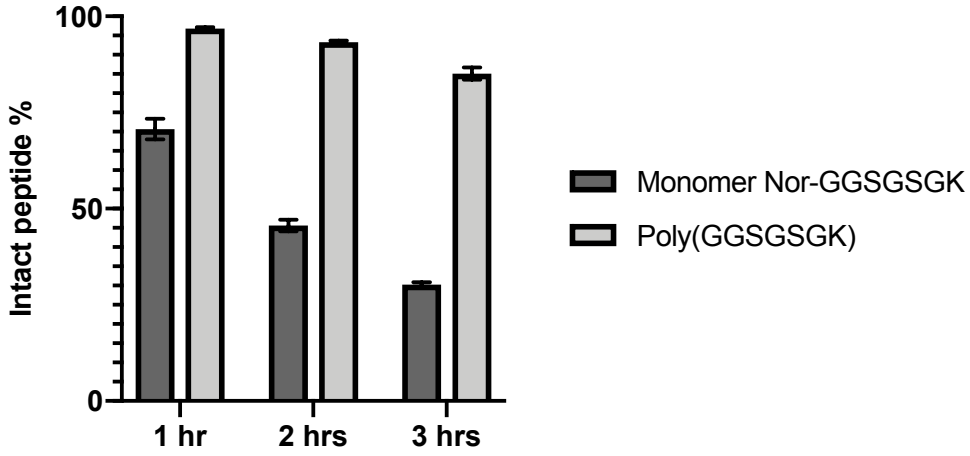


Figure S26. Comparison of intact peptide percentage of Monomer Nor-GGSGSGK and polymer Poly(GGSGSGK) after pronase treatment.

3. References

1. R. M. Conrad and R. H. Grubbs, *Angew. Chem. Int. Ed.*, 2009, **48**, 8328-8330.
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