Supporting Information for

Poly(2-deoxy-2-methacrylamido glucopyranose)-*b*-Poly(methacrylate amine)s: Optimization of Diblock Glycopolycations for Nucleic Acid Delivery

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Experimental Procedures

Materials. Monomeric aminoethylmethacrylate (AEMT) was purchased from Polysciences (Warrington, PA). *N*-methyl-aminoethylmethacrylate (MAEMT), *N,N*-dimethyl aminoethylmethacrylate (DMAEMT) and *N,N,N*-trimethylammoniumethylmethacrylate (TMAEMT) was prepared with procedures shown in later chapters. 4-cyano-4-(propylsulfanylthiocarbonyl) sulfanylpentanoic acid (CPP) was synthesized according to a previous procedure.[1] 4,4'-Azobis(4-cyanopentanoic acid) (V-501) was purchased from Aldrich and was crystalized from MeOH prior to use. Methacryloyl chloride was distilled to remove the free radical stabilizer. 2-Deoxy-2-methacrylamido glucopyranose (MAG) was prepared according to a previous publication.[2] All other chemicals were purchased from Aldrich and used

without further purific3ation. Human liver hepatocellular carcinoma (Hep G2) cells were purchased from ATCC (Manassas, VA). Cell culture media and supplements were purchased from Gibco (Carlsbad, CA). JetPEI was purchased from either Invitrogen (Grand Island, NY) or Polyplus-Transfection Inc. (New York, NY). Glycofect was provided by Techulon (Blacksburg, VA). MTT assays were purchased from Molecular Probes, Life Technologies Corp. (Carlsbad, CA). Luciferase assay was purchased from Promega, Corp. (Madison, WI). Protein assay kits were purchased from Bio-Rad Laboratories (Hercules, CA).

Instrumentation. All NMR spectra were recorded from a Varian INOVA-300 or Varian INOVA-500 NMR Spectrometer with D_2O or $CHCl_3$-d as solvents. Number-average molecular weight (M_n) and Đ of the polymeric materials were measured using an Agilent 1260 Infinity gel permeation chromatography (GPC) system consisting of Eprogen (Downers Grove, IL) columns [CATSEC1000 (7 μm, 50 × 4.6), CATSEC100 (5 μm, 250 × 4.6), CATSEC300 (5 μm, 250 × 4.6), and CATSEC1000 (7 μm, 250 × 4.6)], a Wyatt Dawn Heleos-II light scattering module and an Optilab T-rEX refractometer. Millipore water containing 0.1 M Na2SO4 and 1% acetic acid has been used as the mobile phase and delivered at a flow rate of 0.4 mL/min (a mobile phase of Millipore water containing 0.5 M Na_2SO4 and 1% acetic acid was used as mobile phase for characterization of PTMAEMT polymers). Zeta potential and hydrodynamic diameter of the polyplexes were recorded with a Malvern Instrument Zatasizer Nano ZA. Cy5-uptake and Green Fluorescence Protein transfection were measured on a FACSVerse (Becton Dickenson, San Jose, CA) flow cytometer. Luciferase, protein, and MTT assays were measured with a Tecan GENios Pro plate reader (TECAN US). TEM images were obtained with a FEI Tecnai G2 Spirit BioTWIN transmission electron microscope, operated at 120 kV. To prepare the TEM specimen, 3.0μL aliquot of the polyplexes solution was applied onto a 300-mesh carbon coated copper grid

(Ted Pella, Inc), and the excess solution was removed with filter paper after 60s, followed by negative stain with 1% uranyl acetate solution for three times. Images were recorded using Eagle^{TM} 2k CCD camera (up to 4 mega pixels), and phase contrast was enhanced at 6-12 μm underfocus.

Preparation of monomers.

Preparation of N,N-dimethylaminoethyl methacrylate hydrochloride (DMAEMA). N,N-dimethylaminoethyl methacrylate hydrochloride (DMAEMA) was prepared by treatment of the neutral monomer (11.52 g, 73.28 mmol) with hydrochloride solution (9.5%, 27.40 mL) at 0 ^{o}C, followed by removal of the solvent *in vacuo* and washed by mixed solvent of ethyl acetate and methanol (80:20 v:v) to give 12.07 g product. Yield: 85%. ^{1}H NMR (D_2O, 300 MHz) 6.00 (s, 1H), 5.59 (s, 1H), 4.34 (t, 2H, J=6Hz), 3.39 (t, 2H, J=6 Hz), 2.79 (s, 6H), 1.75 (s, 3H).

Preparation of N,N,N-trimethylammonium ethylmethacrylate iodide. *N,N,N*-trimethylammonium ethylmethacrylate iodide was prepared by treatment of the *N,N*-dimethylaminoethyl methacrylate (12.39 g, 78.81 mmol) with MeI (5.90 mL, 94.57 mmol) in chloroform (60 mL), followed by removal of the solvent *in vacuo* to give 23.38 g product. Yield: 99%. ^{1}H NMR (D_2O, 300 MHz) 6.00 (s, 1H), 5.61 (m, 1H), 4.48 (m, 2H), 3.65 (t, 2H, J=6Hz), 3.09 (s, 9H), 1.78 (s, 3H).

Preparation of the BOC protected 2-N-methyl ethanol.[3] A solution of methyl-2-aminoethanol (7.50 g, 100.12 mmol) in chloroform (50 mL) was cooled in an ice bath for 10 minutes. A solution of di-*tert*-butyl dicarbonate (BOC_2O) (21.87 g, 100.12 mmol) in chloroform (50 mL) was added dropwise to the mixture over 30 minutes. The reaction was further stirred at 0 ^{o}C for 1 hour, after which, it was allowed to warm up to room temperature. The solvent was then

removed *in vacuo*. After vacuum distillation, 16.29 g of product (93% yield) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) 3.72 (m, 2H), 3.38 (m, 2H), 2.90 (s, 3H), 1.44 (s, 9H).

Preparation of the BOC protected N-methylaminoethylmethacrylate. A solution of the product obtained from the previous step (BOC protected 2-N-methyl ethanol, 15.73 g, 89.77 mmol) in chloroform (150 mL) was cooled in an ice bath for 10 minutes. Triethyl amine (16.00 mL, 115.07 mmol) was then added to the solution in one portion. After which, methacryloyl chloride (8.92 mL, 92.01 mmol) was added dropwise over 10 minutes. The reaction was allowed to warm up to room temperature and was further stirred overnight. The reaction solution was then washed with water (150 mL) and dried over $MgSO_4$. The desiccant was filtered off and the solution was concentrated *in vacuo*. The final product, BOC protected N-methylaminoethylmethacrylate, was obtained after purification with column chromatography (with a mobile phase containing 25% ethyl acetate and 75% hexane as the mobile phase (Yield: 16.74 g 74%). ^{1}H NMR (300 MHz, $CDCl_3$) 6.12 (s, 1H), 5.88 (s, 1H), 4.25 (m, 2H), 3.51 (m, 2H), 2.92 (s, 3H), 1.94 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) 167.12, 155.53, 136.03, 125.76, 79.61, 62.66, 47.56, 35.16, 28.33, 18.25.

Next, a solution of the product obtained from the previous step (16.16 g, 64.38 mmol) in HCl solution in MeOH (1.25 M, 180 mL) was cooled in ice bath for 1 hour and stirred at room temperature overnight. The solution was then concentrated *in vacuo*. 150 mL ethyl acetate was added to the residue to wash. The ethyl acetate was filtered off and the product was obtained as a white solid, which dried under vacuum to yield a white solid (Yield: 10.86 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) 6.23 (m, 1H), 5.72 (m, 1H), 4.44 (m, 2H), 3.38 (t, 2H, J = 6Hz), 3.30 (m, 1H), 2.77 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), 168.89, 135.14, 127.65, 60.23, 47.66, 33.03, 17.29.

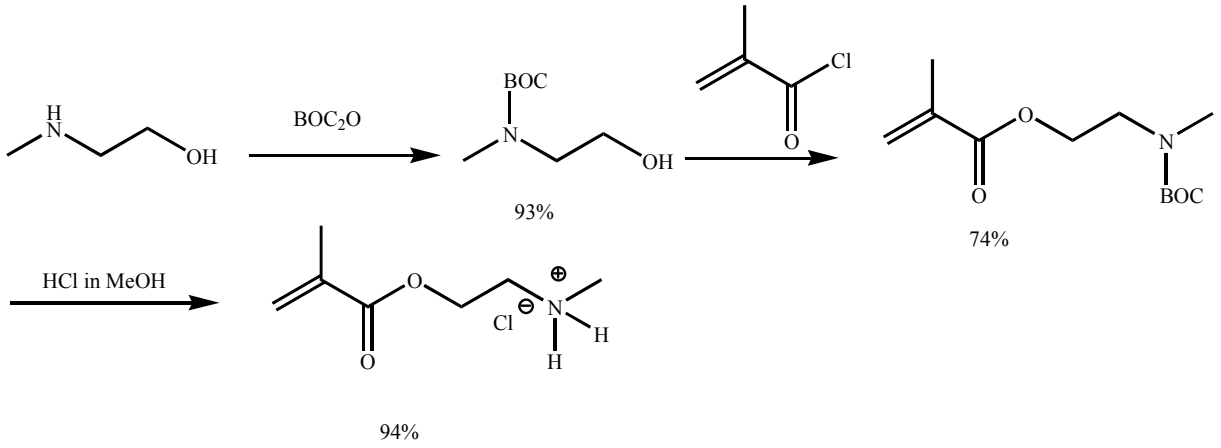


Figure S1. Preparation of methylaminoethylmethacrylate (MAEMT).

Polymerization kinetics. Polymethacrylate block lengths for all of the glycopolycations created in this study were controlled by following the kinetics (via polymerization time) of the monomers. To a 3.0 mL vial charged with a magnetic stir bar under nitrogen gas, AEMT (mg, 0.26 mmol), V501 initiator (0.15 mg, 0.00054 mmol), the polyMAG macroCTA (35 mg, 0.0027 mmol), and an acetate buffer solution in D_2O (pH 5.2, 1.0 M, 0.83 mL) was added. The mixture was stirred to dissolve the reactants and the resulted solution was allowed to stir and further purged with nitrogen for 1 hour before it was cannulated to an NMR tube (that was also purged with nitrogen gas). The NMR tube charged with the reaction solution was further purged for another 5 minutes after which it was sealed quickly with an NMR tube cap. Kinetic analysis was then performed via NMR at 70 ^{o}C by monitoring the change of CH_2=C proton peaks against time. This procedure was also used for kinetic study of the rest monomers

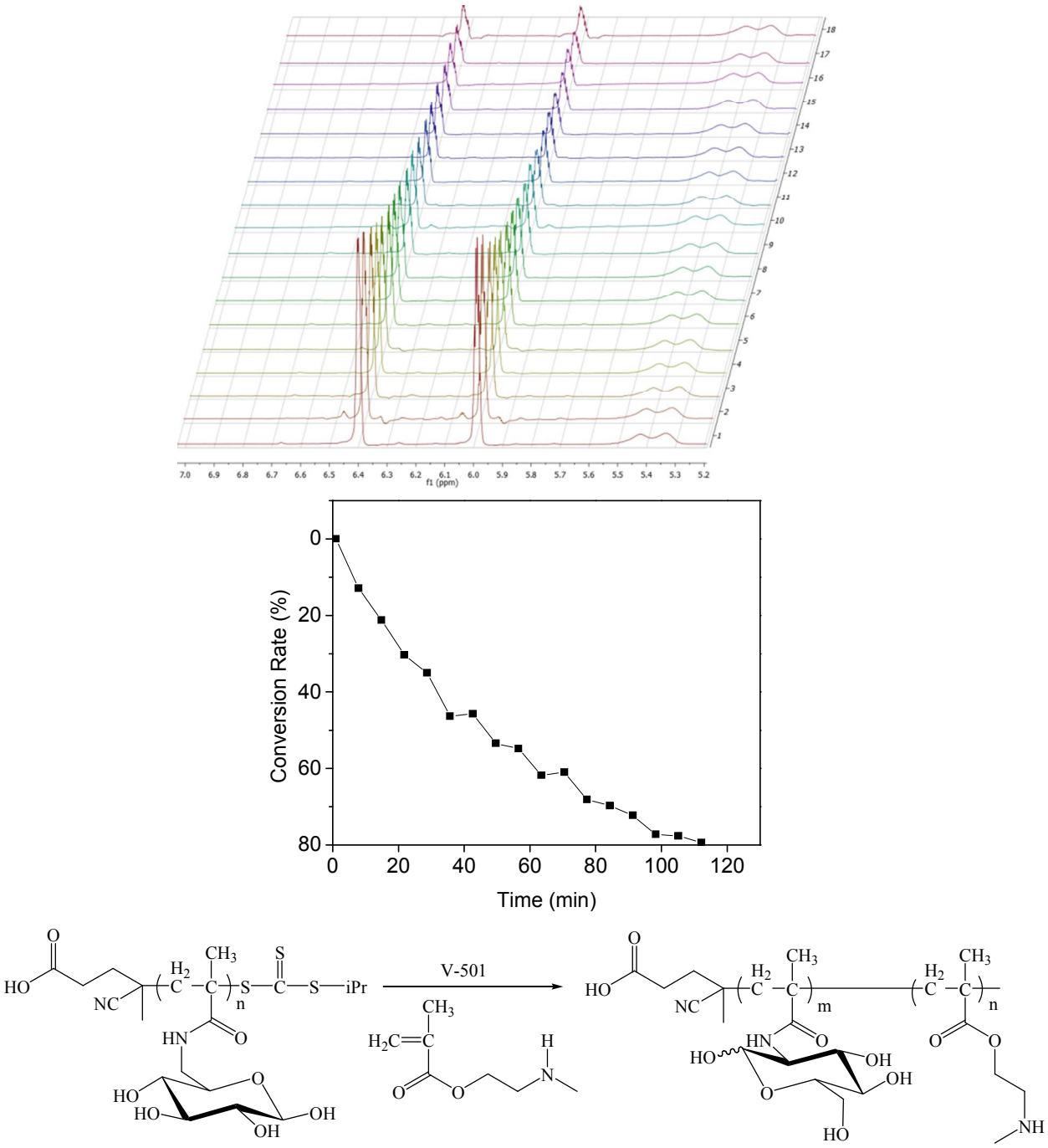
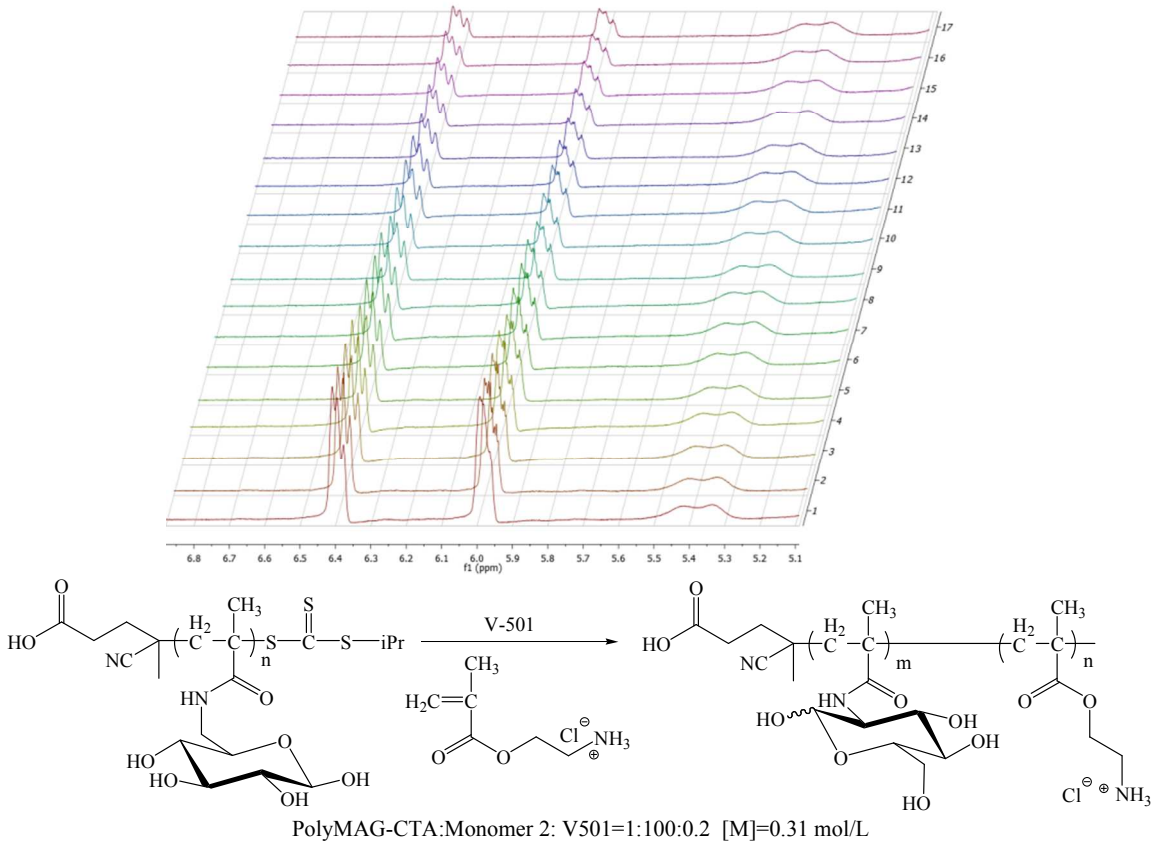


Figure S2. Polymerization kinetics of the MAEMT block by monitoring change of CH_2=C proton peaks against reaction time. (Top: real time NMR spectra taken at 70 ^{o}C; middle: plot of monomer conversion vs time based on the NMR spectra).



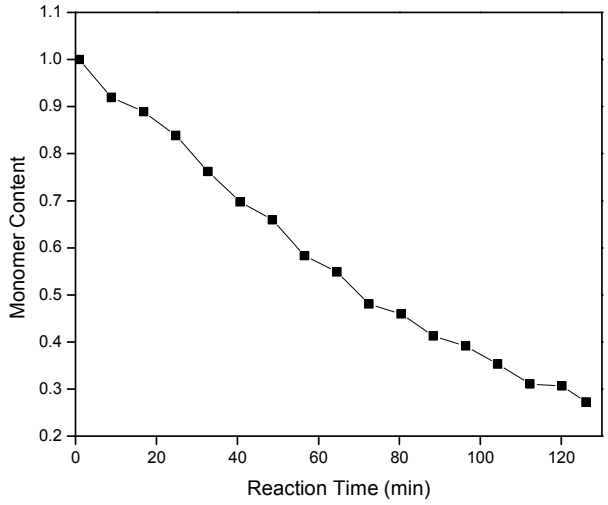
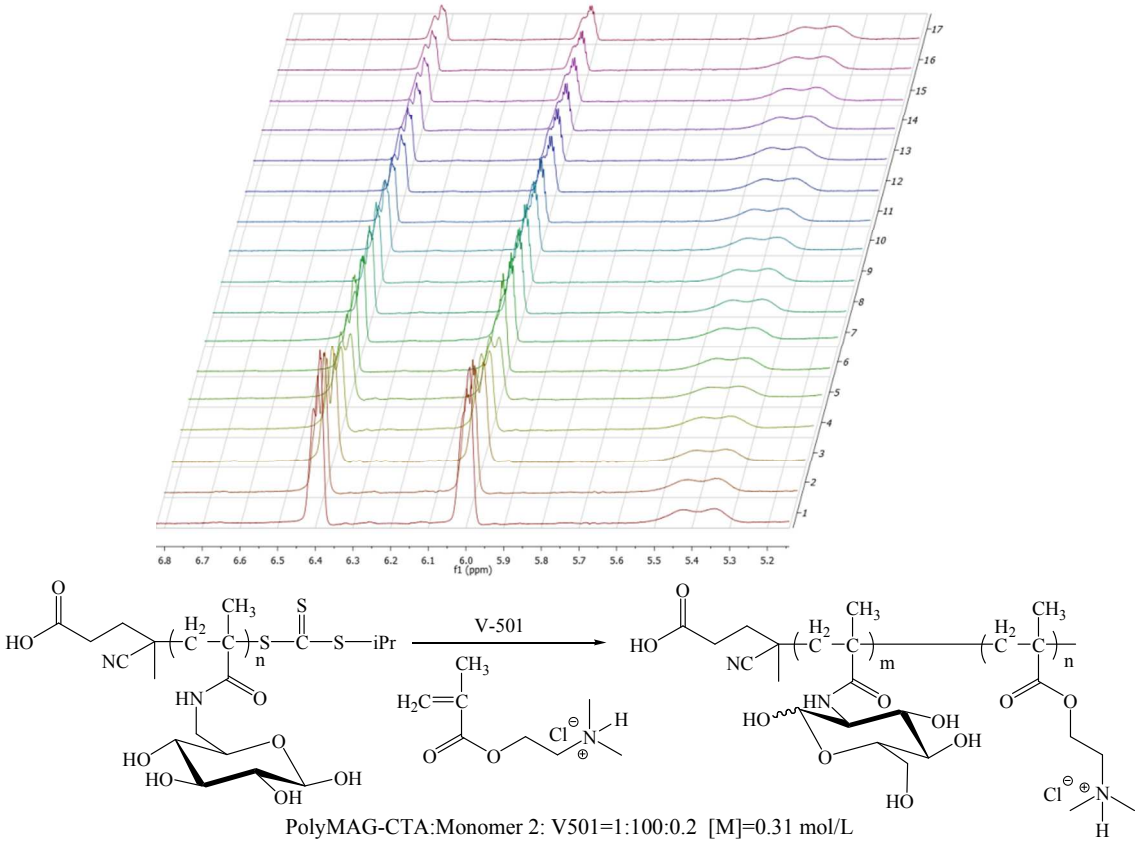


Figure S3. Polymerization kinetics of the AEMT block by monitoring change of CH_2=C proton peaks against reaction time. (Top: real time NMR spectra taken at 70 ^{o}C; middle: plot of monomer conversion vs time based on the NMR spectra).



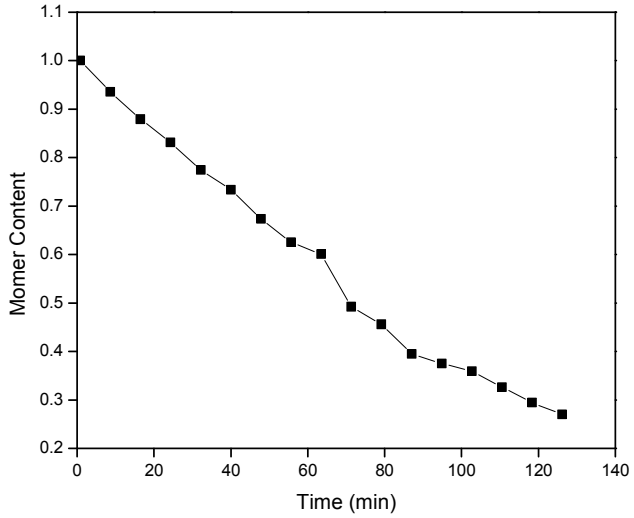


Figure S4. Polymerization kinetics of the DMAEMT block by monitoring change of CH_2=C proton peaks against reaction time. (Top: real time NMR spectra taken at 70 ^{o}C; bottom: plot of monomer conversion vs time based on the NMR spectra).

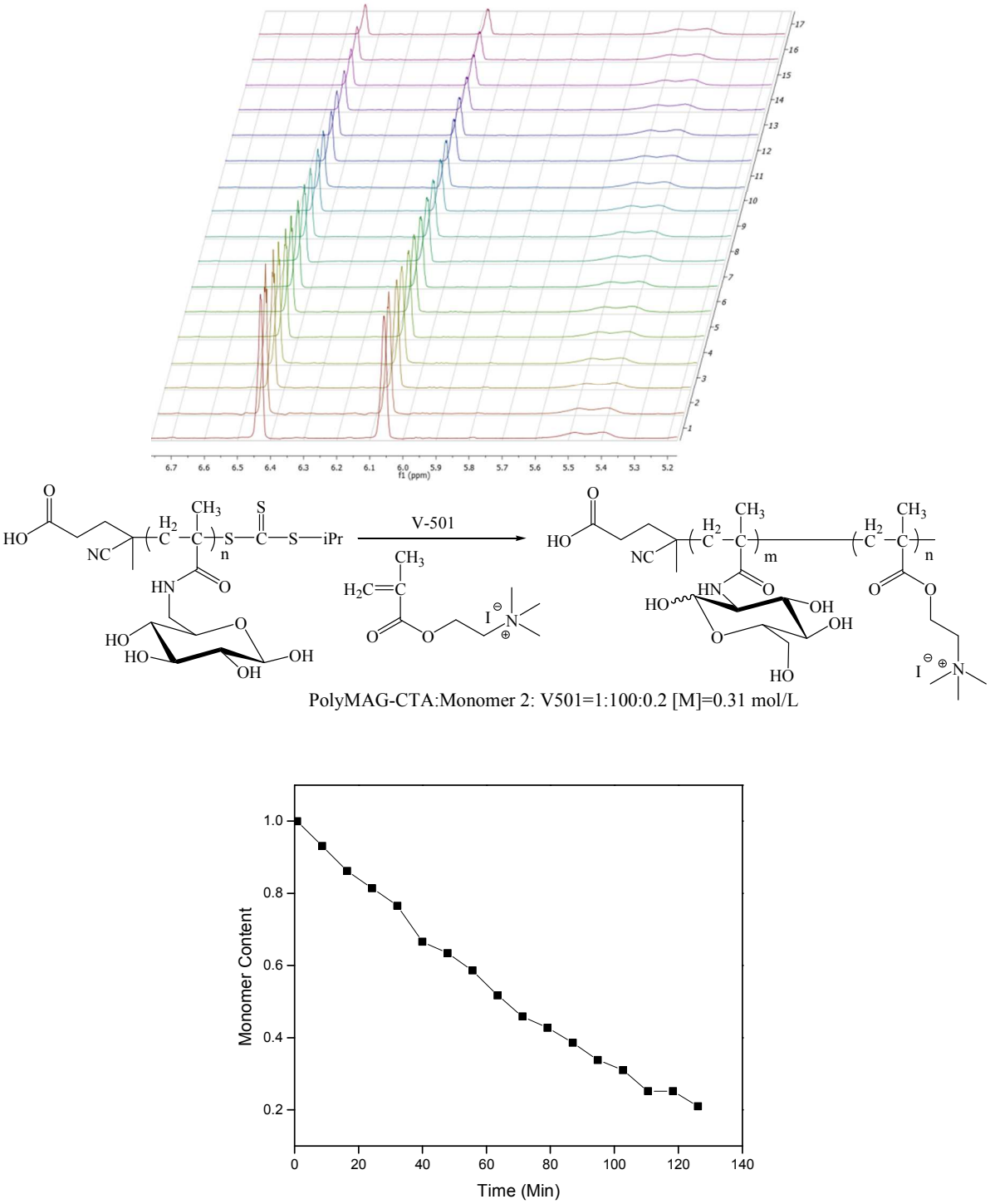


Figure S5. Polymerization kinetics with the TMAEMT block by monitoring change of *CH2*=C proton peaks against reaction time. (Top: real time NMR spectra taken at 70 ^{o}C; bottom: plot of monomer conversion vs time based on the NMR spectra).

Synthesis and characterization of the P(MAG-b-Methacrylate) glycopolycations**.**

Synthesis of the PMAG macroCTA. The PMAG macroCTA was prepared according to a procedure in one of our recent publications.[2] To a 250 mL round bottom flask charged with nitrogen gas, MAG (6.00 g, 24.27 mmol), V501 (5.8 mg, 0.02023 mmol), CPP (56 mg, 0.2023 mmol), acetate buffer (0.1 M, pH =5.2) (72 mL) and ethanol (18 mL) were added and stirred. Nitrogen gas was allowed to purge the solution for 2.5 hours at room temperature. After which, the solution (still under a nitrogen atmosphere) was heated to 70 ^{o}C and stirred for an additional 280 minutes. The reaction solution was quenched by via rapidly freezing the solution with liquid nitrogen and then exposing the contents to air. The solution was then transferred to dialysis tubing (MWCO 3500) and allowed to dialyze against ultra high purity water for three days (water was changed twice a day. The final solution was then lyophilized to dryness yielding 1.68 g of product. Yield: 28%.

Synthesis of the P(MAG-b-Methacrylate) glycopolycations. The following is a typical procedure used to prepare the diblock glycopolymers with the various cationic monomers. The PolyMAG macroCTA (0.20 g), MAEMAT chloride (0.278 g, 1.5479 mmol), V501 (0.87 mg, 0.003096 mmol), and acetate buffer (1.0 M, pH=5.2) (2.40 mL) were added to a 5 mL vial charged with nitrogen gas and a mechanical stirring bar. The mixture was stirred to dissolve the reactants and the solution was purged with nitrogen gas for 30 minutes at room temperature. Next, the reaction mixture was heated to 70 ^{o}C, and the reaction was stirred for 26 minutes. The solution was then dialyzed against water for three days and lyophilized to yielding 0.23 g of the final glycopolycation final product.

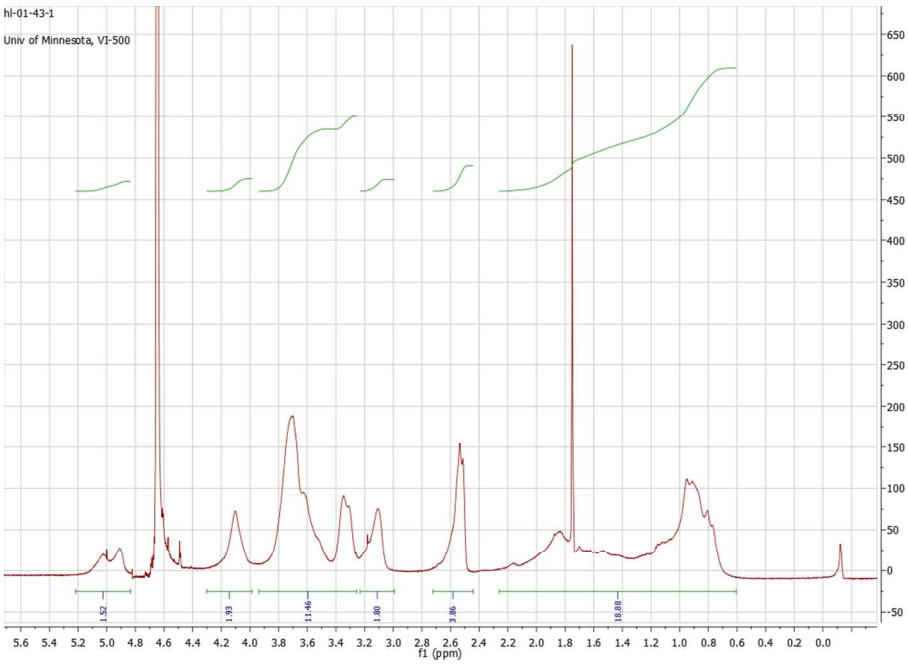


Figure S6a. NMR spectrum of P(MAG_{51}-b-$PMAEMT_{30}$) (PMAEMT-1).

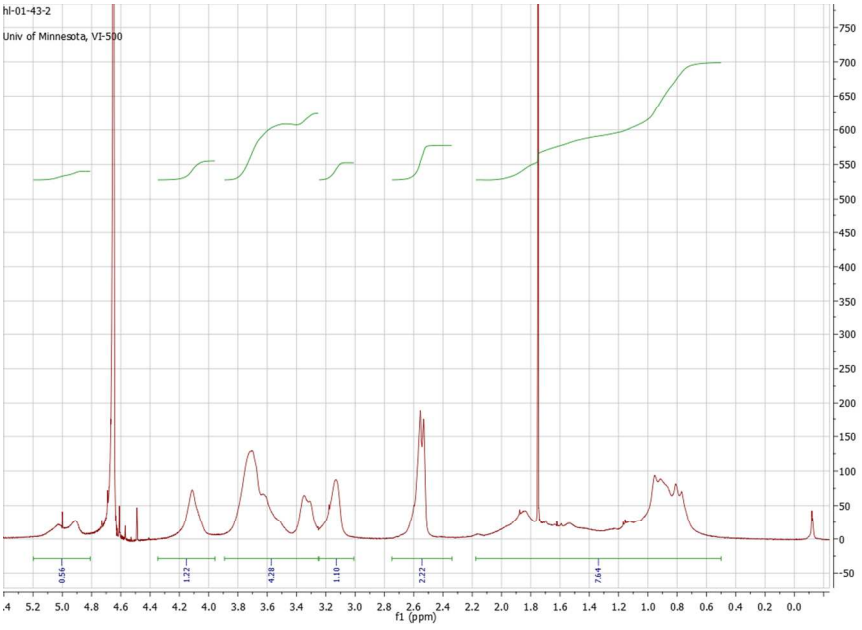


Figure S6b. NMR spectrum of P(MAG_{51}-b-$PMAEMT_{42}$) (PMAEMT-2).

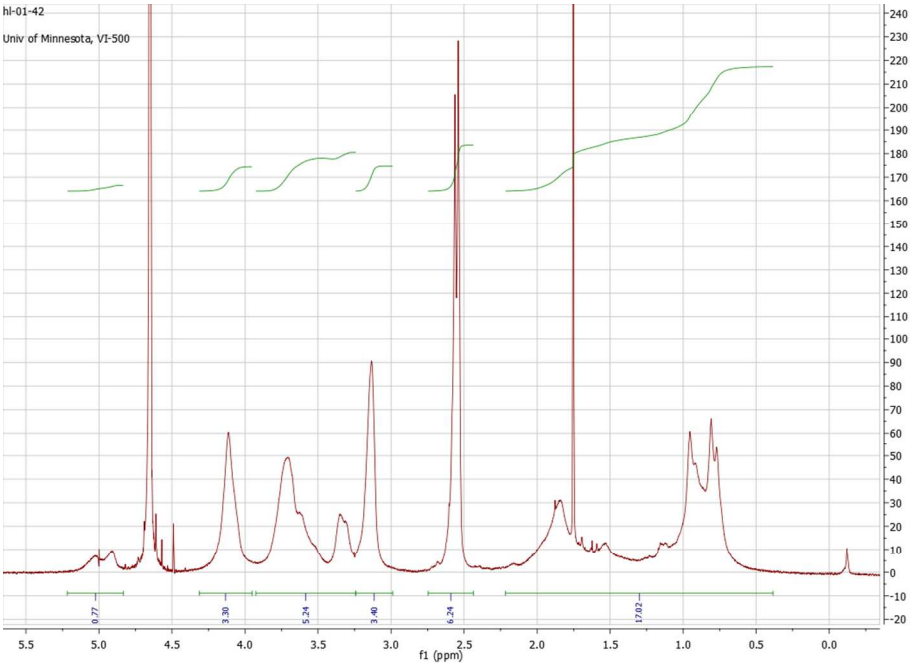


Figure S6c. NMR spectrum of P(MAG_{51}-b-$PMAEMT_{76}$) (PMAEMT-3).

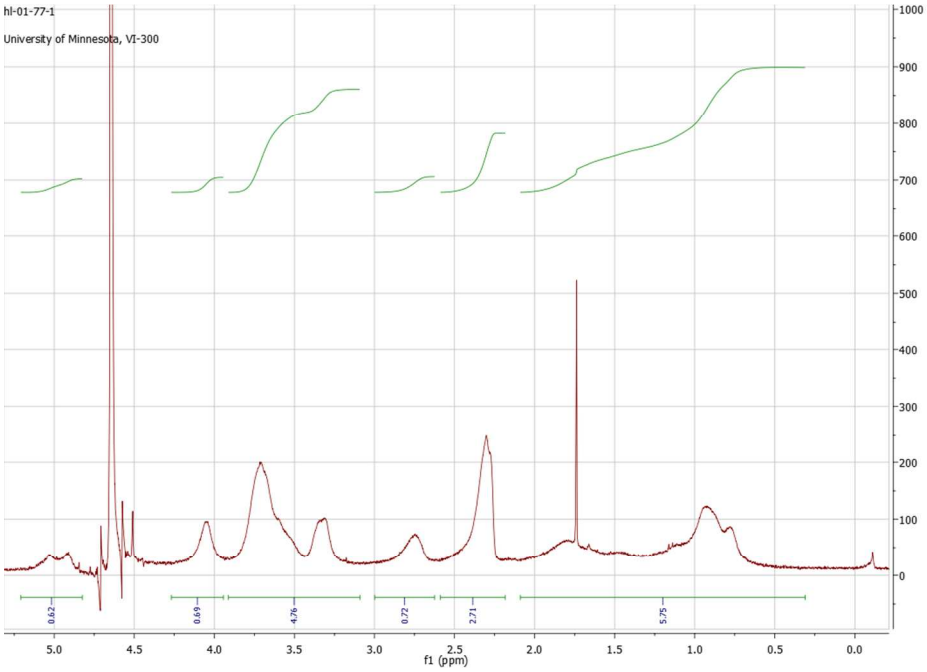


Figure S6d. NMR spectrum of P(MAG_{56}-b-$PDMAEMT_{32}$) (PDMAEMT-1).

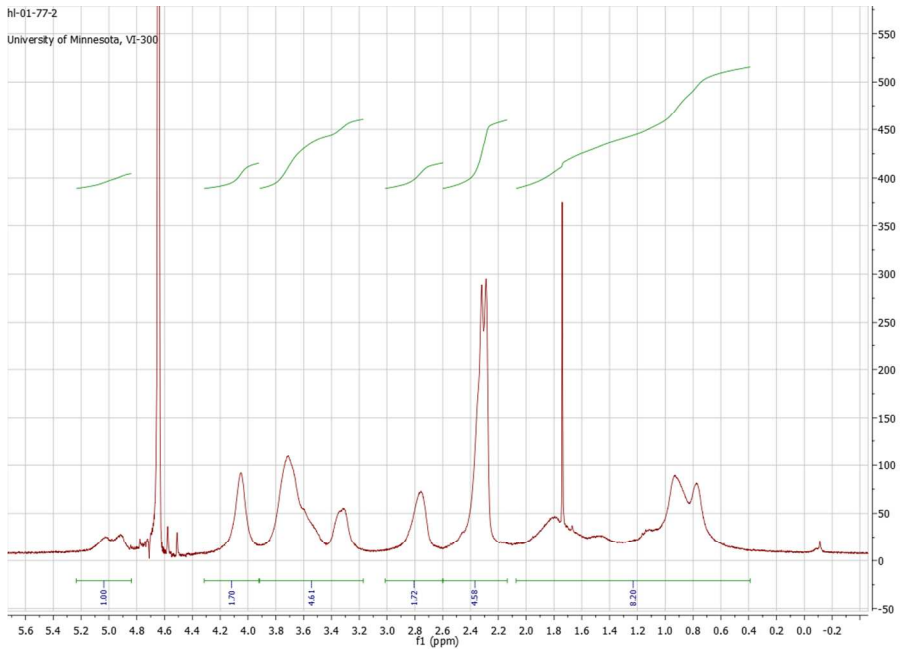


Figure S6e. NMR spectrum of P(MAG_{56}-b-$PDMAEMT_{53}$) (PDMAEMT-2).

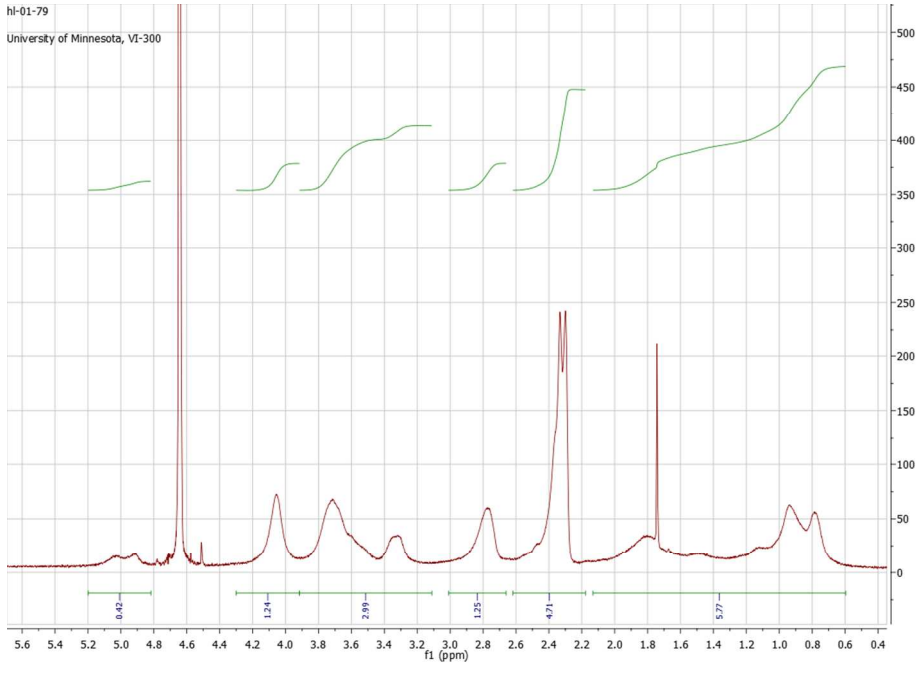


Figure S6f. NMR spectrum of P(MAG_{56}-b-$PDMAEMT_{71}$) (PDMAEMT-3).

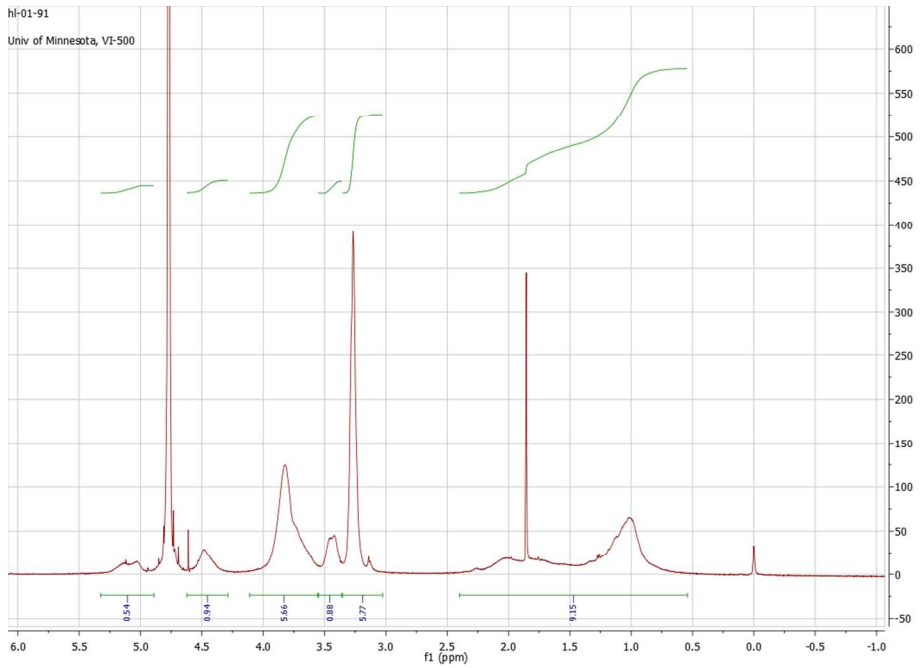


Figure S6g. NMR spectrum of P(MAG_{57}-b-$PDMAEMT_{33}$) (PTMAEMT-1).

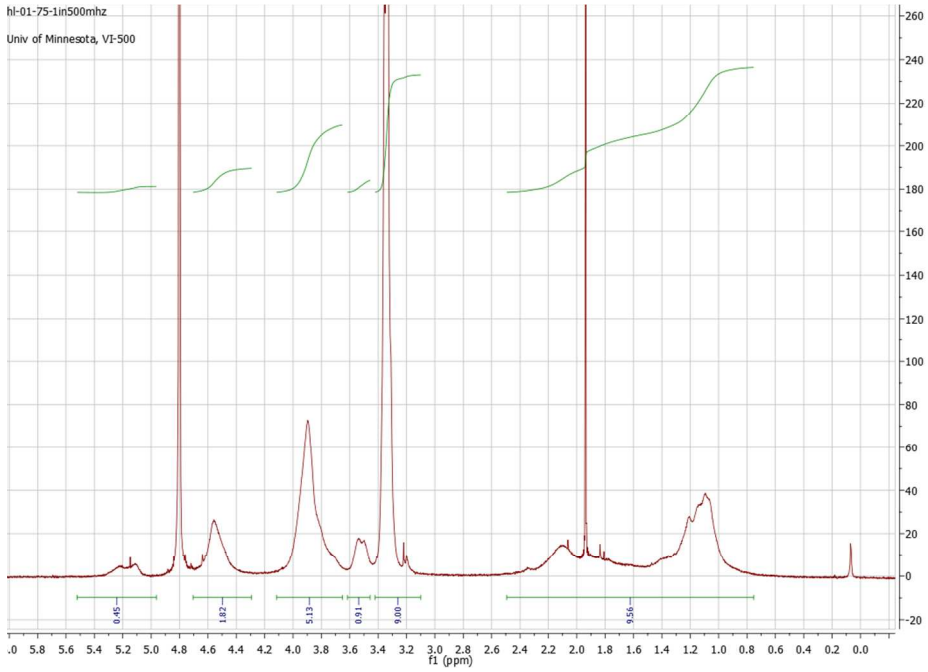


Figure S6h. NMR spectrum of P(MAG_{57}-b-$PDMAEMT_{48}$) (PTMAEMT-2).

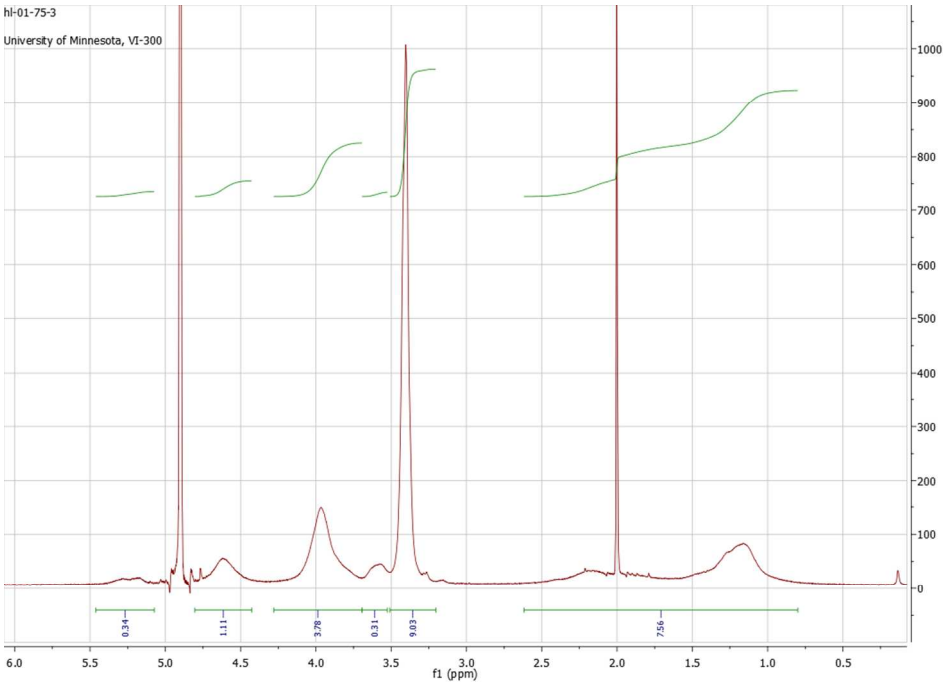


Figure S6i. NMR spectrum of P(MAG_{57}-b-$PDMAEMT_{72}$) (PTMAEMT-3).

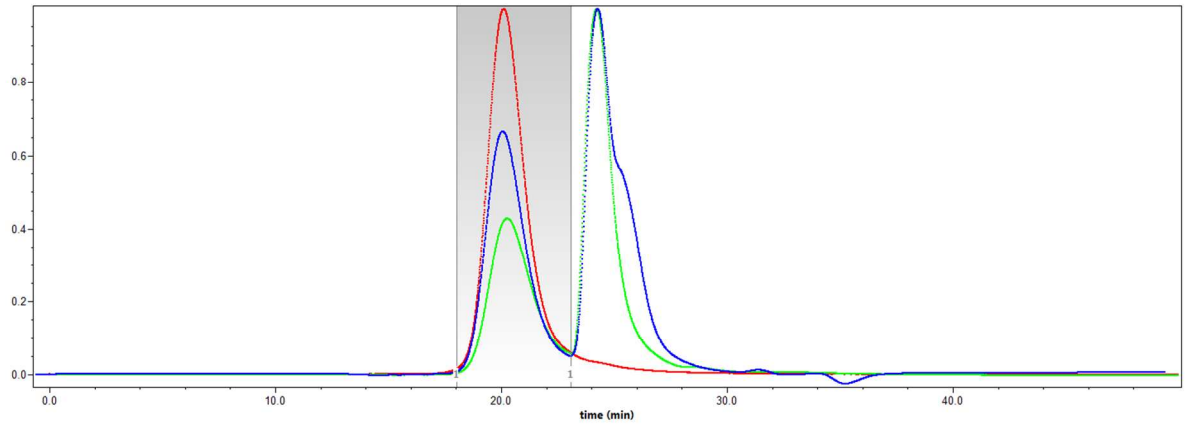


Figure S7a. GPC traces for the crude P(MAG-b-PAEMT)s. Peaks at longer retention times are for the unreacted monomers. (Red trace: light scattering; blue trace: refractive index; green trace: UV). Crude PAEMT-1, M_n=20,200 M_w/M_n=1.01.

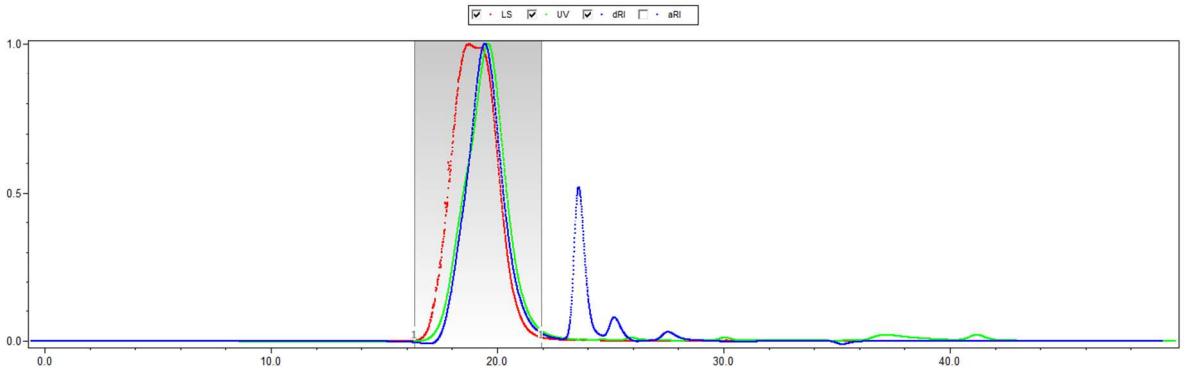


Figure S7b. GPC traces for the crude P(MAG-b-PAEMT)s. (Red trace: light scattering; blue trace: refractive index; green trace: UV). Purified PAEMT-1, M_n=33,820 M_w/M_n=1.28.

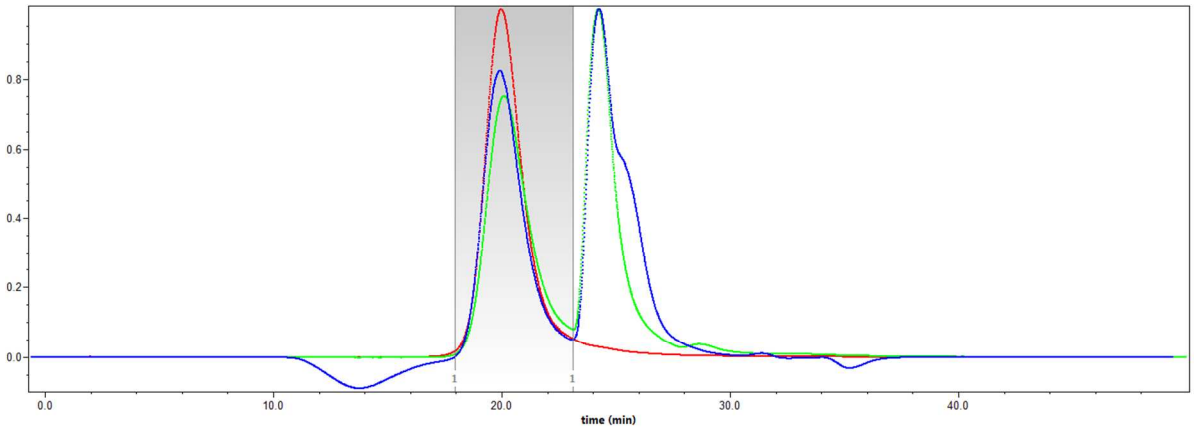


Figure S7c. GPC traces for the crude P(MAG-b-PAEMT)s. Peaks at longer retention times are for the unreacted monomers. (Red trace: light scattering; blue trace: refractive index; green trace: UV). Crude PAEMT-2, M_n=22,960 M_w/M_n=1.01.

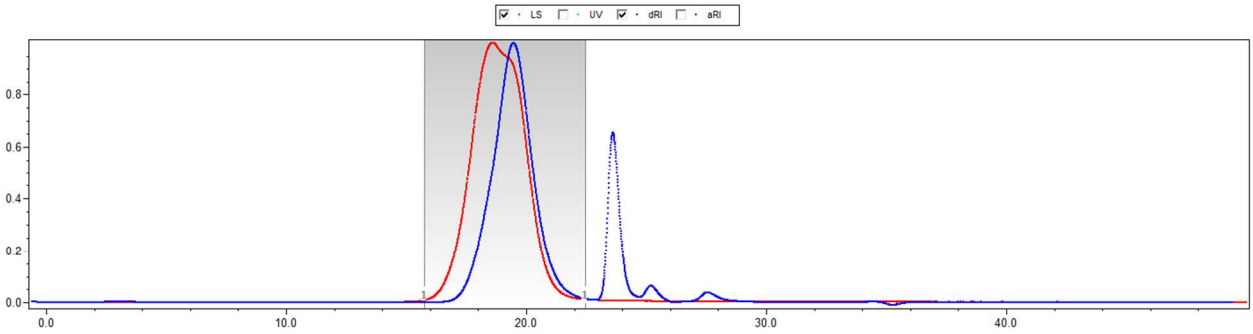


Figure S7d. GPC traces for the crude P(MAG-b-PAEMT)s. (Red trace: light scattering; blue trace: refractive index; green trace: UV). Purified PAEMT-2, M_n=35,300 M_w/M_n=1.36.

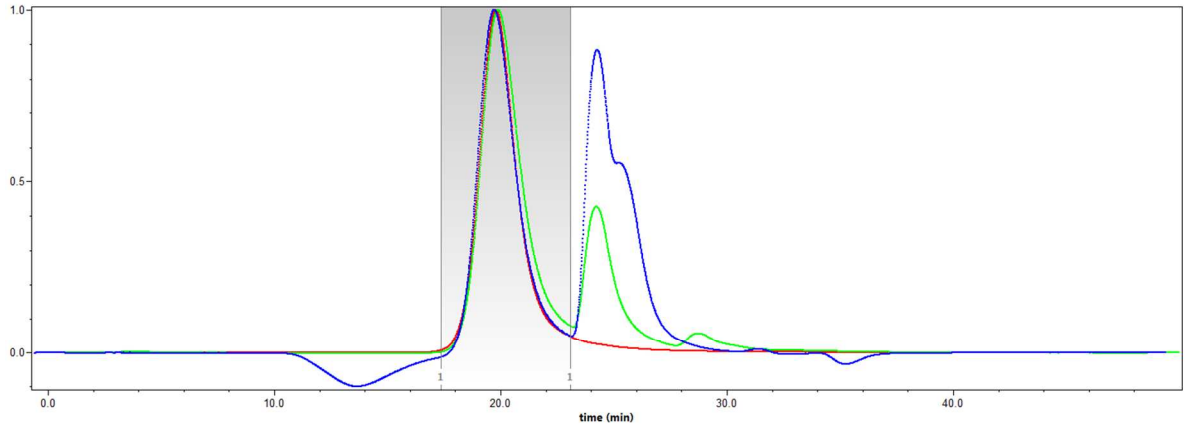


Figure S7e. GPC traces for the crude P(MAG-b- PAEMT)s. Peaks at longer retention times are for the unreacted monomers. (Red trace: light scattering; blue trace: refractive index; green trace: UV). Crude PAEMT-3, M_n=26,300 M_w/M_n=1.01.

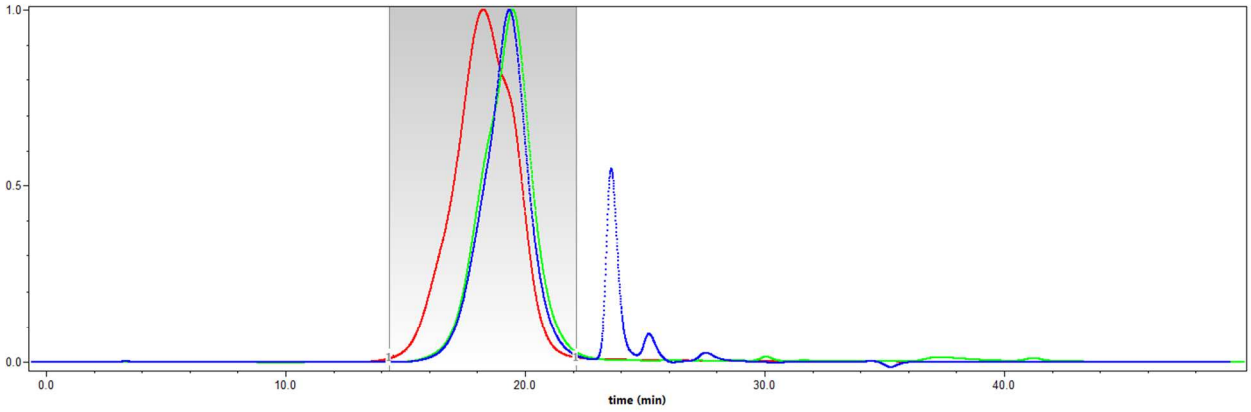


Figure S7f. GPC traces for the crude P(MAG-b-PAEMT)s. (Red trace: light scattering; blue trace: refractive index; green trace: UV). Purified PAEMT-3, M_n=42,100 M_w/M_n=1.57.

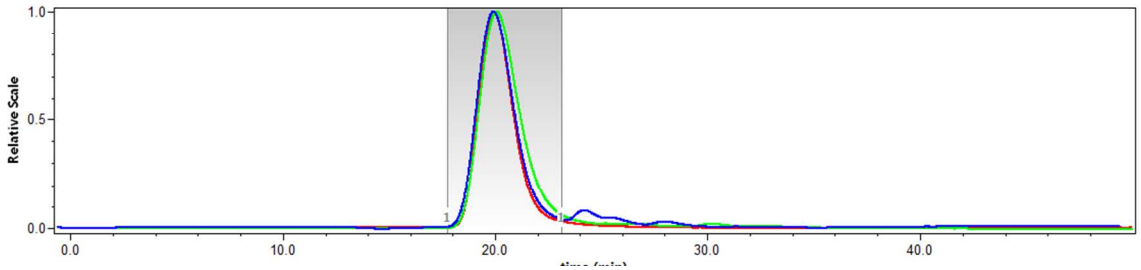


Figure S8a. GPC traces for P(MAG_{51}-b-$PMAEMT_{30}$) (PMAEMT-1). (Red trace: light scattering; blue trace: refractive index; green trace: UV). M_n=18,300 M_w/M_n=1.02.

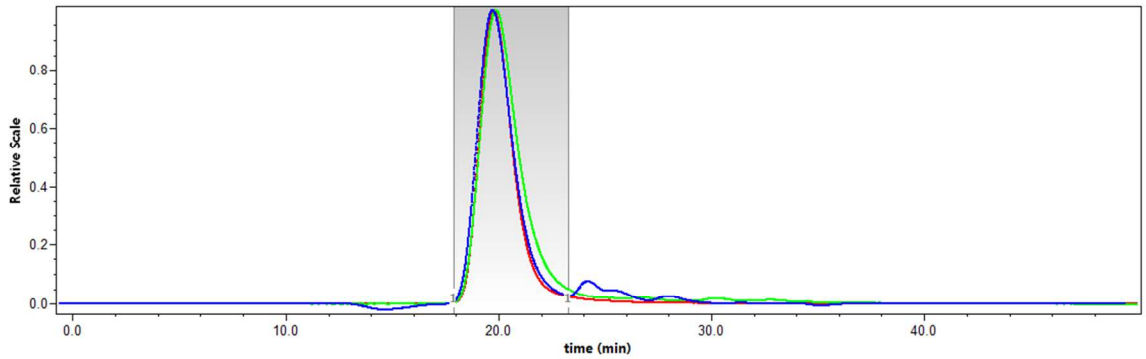


Figure S8b. GPC traces for P(MAG_{51}-b-$PMAEMT_{42}$) (PMAEMT-2). (Red trace: light scattering; blue trace: refractive index; green trace: UV). M_n=20,400, M_w/M_n=1.02.

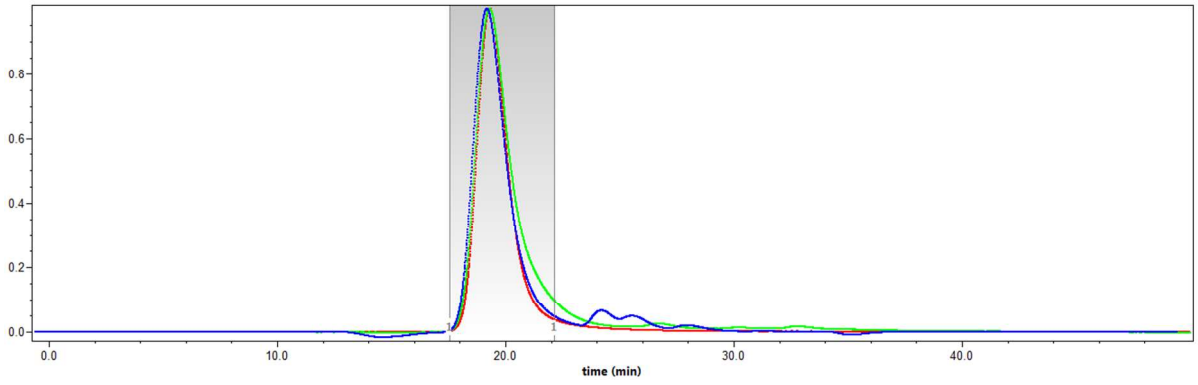


Figure S8c. GPC traces for P(MAG_{51}-b-$PMAEMT_{76}$) (PMAEMT-3). (Red trace: light scattering; blue trace: refractive index; Green trace: UV). Mn=26,600 M_w/M_n=1.05.

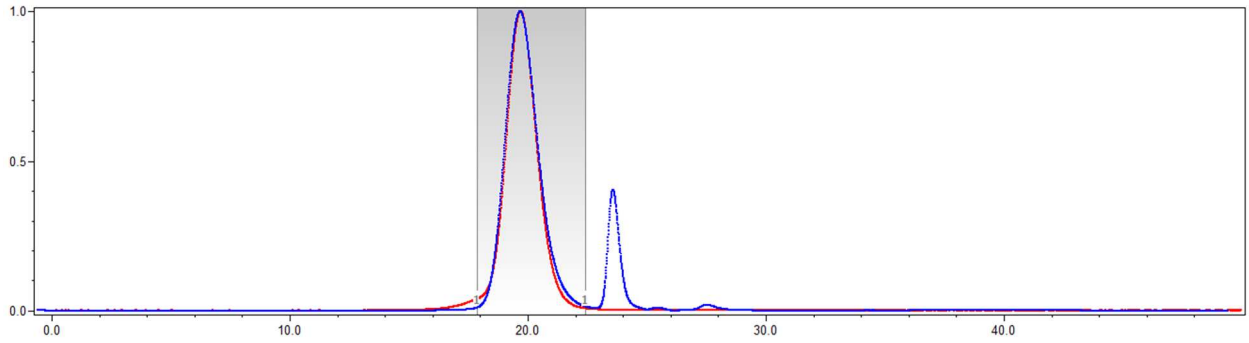


Figure S8d. GPC traces for P(MAG_{56}-b-$PDMAEMT_{32}$) (PDMAEMT-1). (Red trace: light scattering; blue trace: refractive index). M_n=20,400 M_w/M_n=1.02.

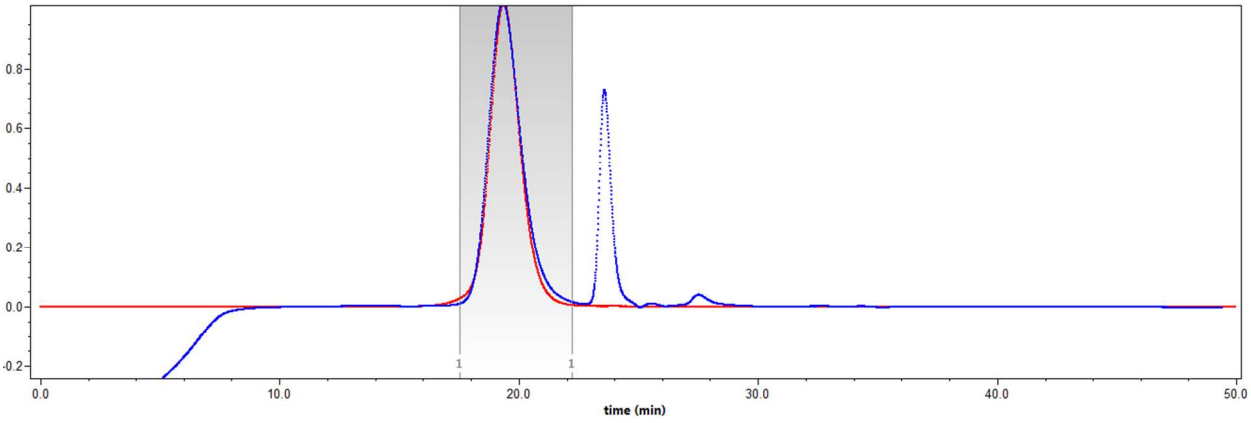


Figure S8e. GPC traces for P(MAG_{56}-b-$PDMAEMT_{53}$) (PDMAEMT-2). (Red trace: light scattering; blue trace: refractive index). M_n=24,400 M_w/M_n=1.02.

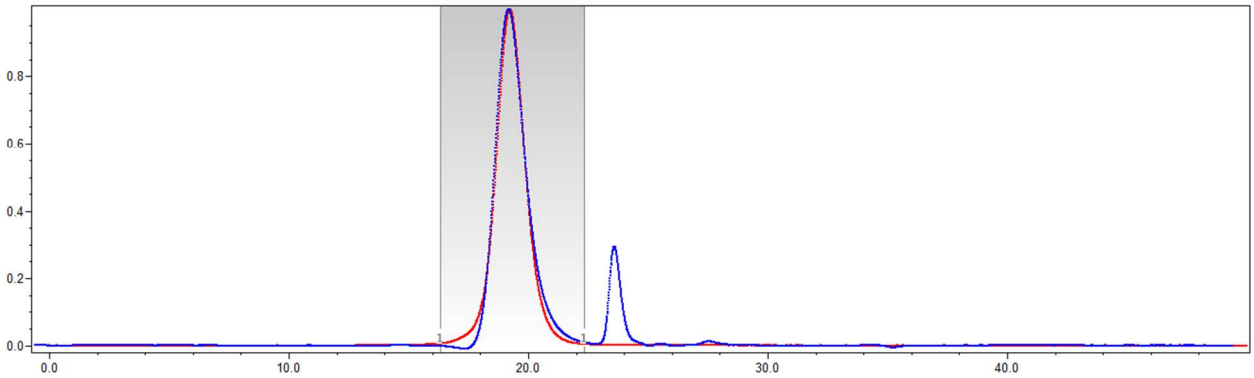


Figure S8f. GPC traces for P(MAG_{56}-b-$PDMAEMT_{71}$) (PDMAEMT-3). (Red trace: light scattering; blue trace: refractive index). M_n=27,900 M_w/M_n=1.03.

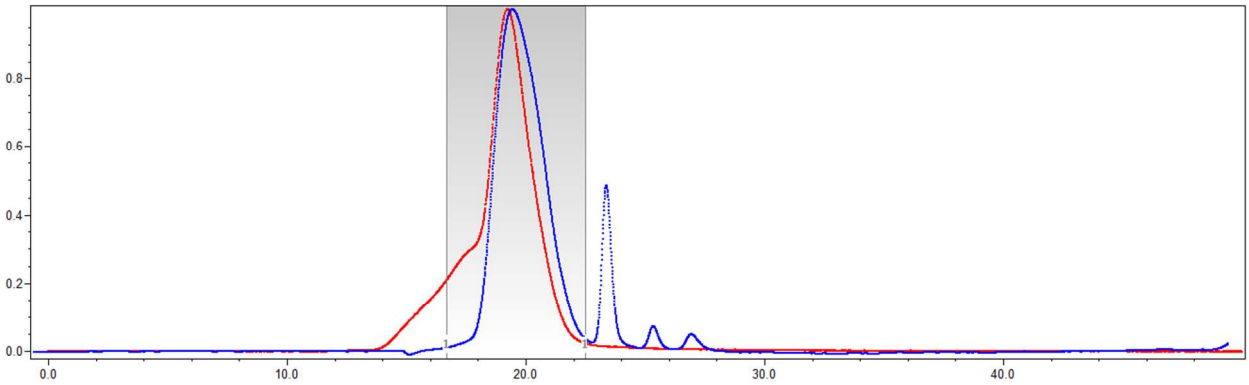


Figure S8g. GPC traces for P(MAG_{57}-b-$PDMAEMT_{33}$) (PTMAEMT-1). (Red trace: light scattering; blue trace: refractive index). PTMAEMT-1, M_n=24,500 M_w/M_n=1.29.

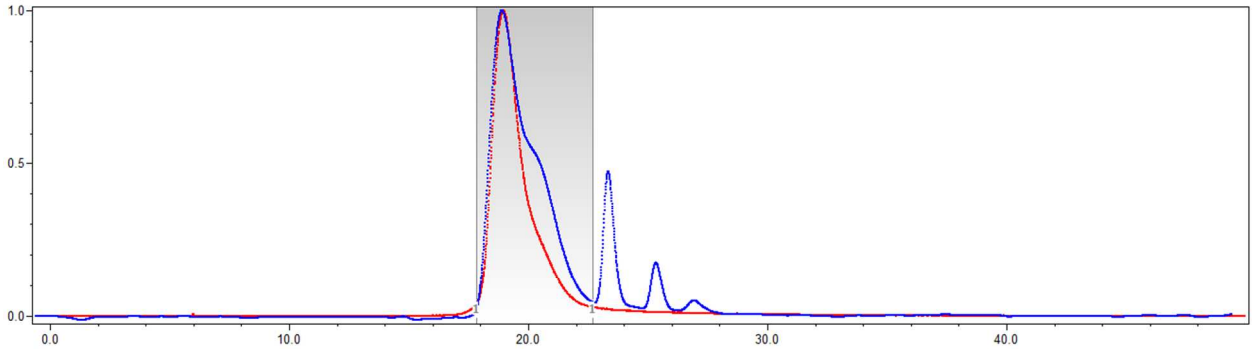


Figure S8h. GPC traces for P(MAG_{57}-b-$PDMAEMT_{48}$) (PTMAEMT-2). (Red trace: light scattering; blue trace: refractive index). PTMAEMT-2, M_n=28,900 M_w/M_n=1.06.

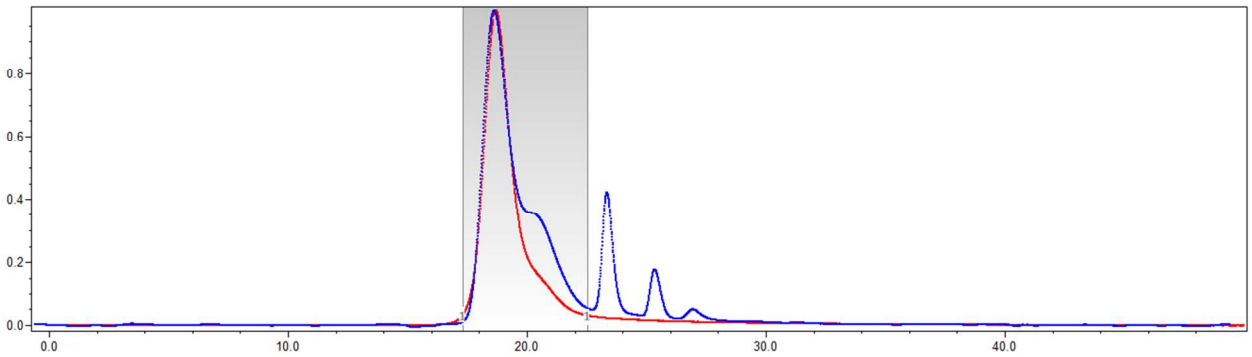
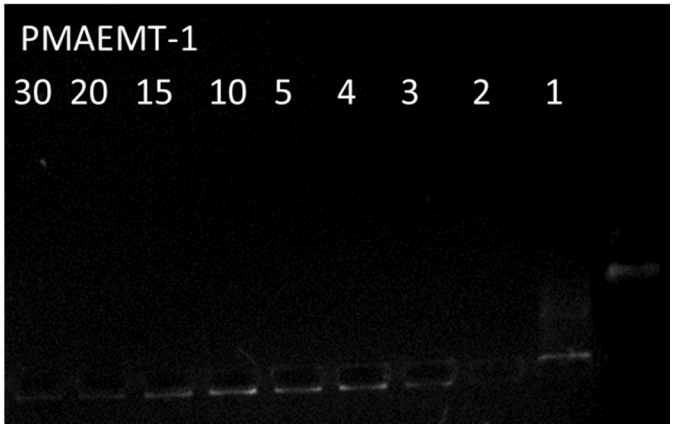


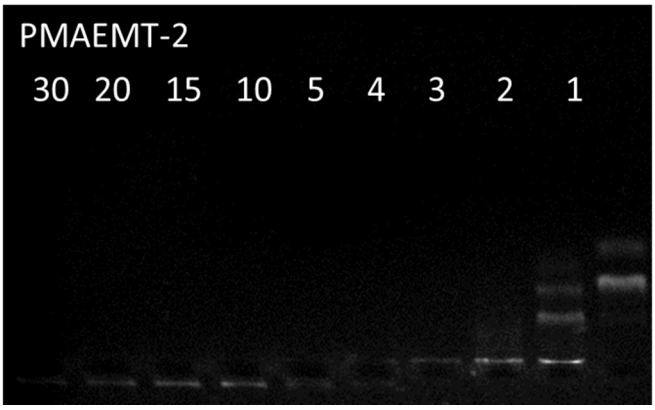
Figure S8i. GPC traces for P(MAG_{57}-b-$PDMAEMT_{72}$) (PTMAEMT-3). (Red trace: light scattering; blue trace: refractive index). M_n=36,100 M_w/M_n=1.12.

Gel Electrophoresis. Binding stability between pDNA and all of the polymers has been examined by gel electrophoresis shift assays. Equal volumes of polymers and pDNA (1 µg/µL) in H2O at different concentrations to generate different N/P ratios were prepared to give a total volume of 20 µL. They were incubated at 25 ^{o}C for 1 hour, then the polyplex suspensions were run on gels made from 0.6 g agarose, 6 µg ethidium bromide and 100 mL TAE buffer (40 mM Tris-acetate, 1mM ethylenediaminetetraacetic acid (EDTA)) at 60 volts for 80 minutes. Binding

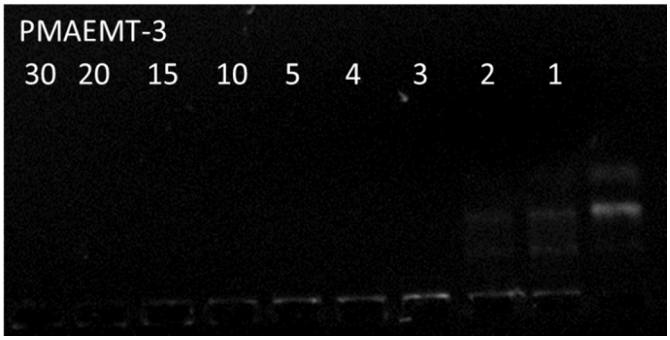
of the polymer to pDNA (and thus polyplex formation) was displayed in a lack of pDNA migration in the gel.



Polymer and pDNA bound at N/P 2.



Polymer and pDNA bound at N/P 3.



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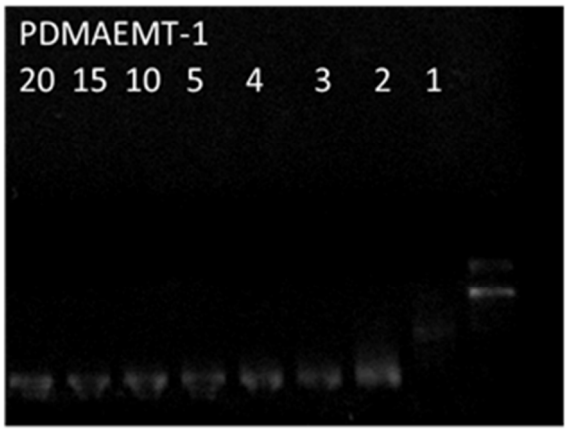

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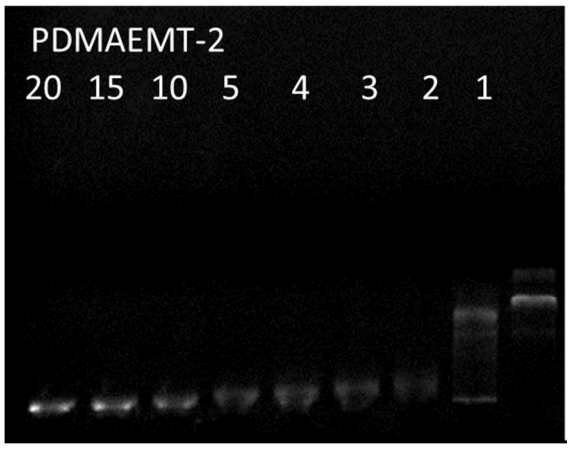
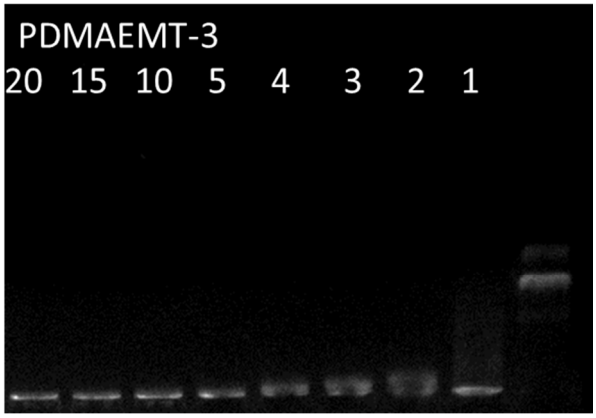
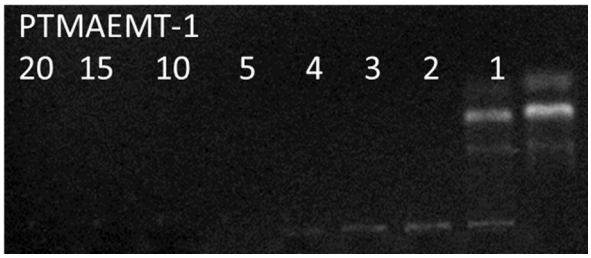


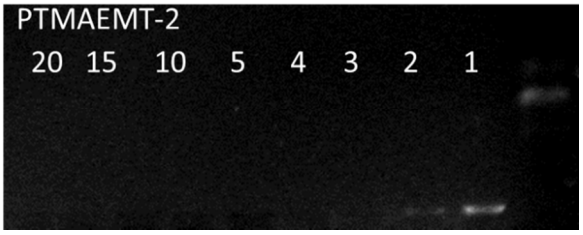
Polymer and pDNA bound at N/P 5.



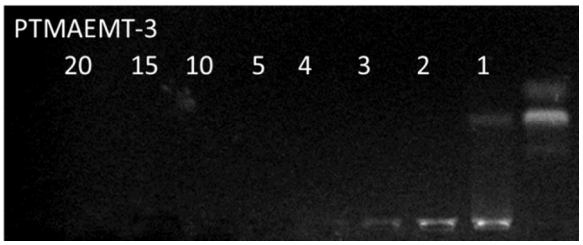
Polymer and pDNA bound at N/P 5.



Polymer and pDNA bound at N/P 2.



Polymer and pDNA bound at N/P 1.



Polymer and pDNA bound at N/P 2.

Figure S9. Gel electrophoresis shift assay images for polyplexes made from pDNA and all polymers P(MAG-b-Methacrylate)s containing the PMAEMT, PDMAEMT, PTMAEMT blocks.

Stability of polyplexes against aggregation in Opti-MEM and determination of the Zeta potential for the polyplexes. 25 μL of a 0.02 μg/ μL pDNA solution was combined with a polymer solution of equal volume to formulate polyplexes at N/P values of 5 and 15. The solutions were mixed and allowed to sit at room temperature for 1 hour to facilitate binding. Each sample was diluted to 150 μL with water, Opti-MEM, and DMEM and the polyplex sizes were measured using a Malvern Zatasizer at time intervals of 0, 2 and 4 hours with a detection angle of 173^{o}. To determine the zeta potential of the polyplexes, 150 μL of a 0.02 μg/ μL pDNA solution was combined in a similar manner above to a polymer solution of equal volume with N/P values of 5 and 15. Again, the solutions were mixed and incubated for 1 hour to allow polyplex formation. Water was then added to dilute the solution to 900 μL. The zeta potential was then measured using the same instrument with a detection angle of 17 ^{o}.

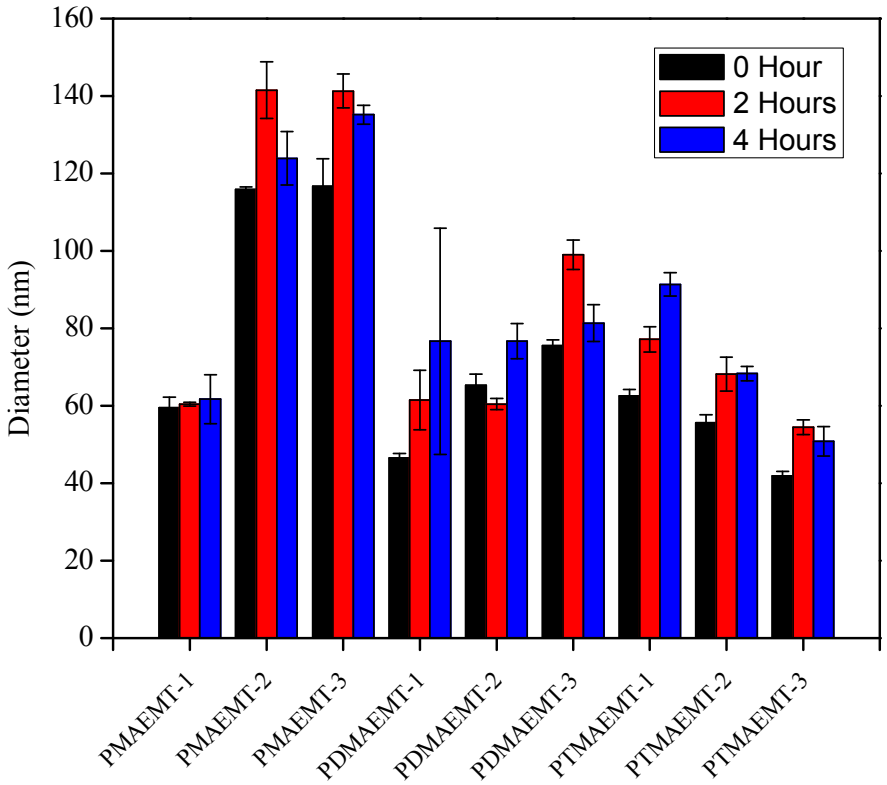


Figure S10. Hydrodynamic diameters for polyplexes made from pDNA and all P(MAG-b-Methacrylate)s polymers containing the PMAEMT, PDMAEMT, and PTMAEMT blocks at an N/P ratio of 5 measured in serum-free Opti-MEM.

Table S1. Polydispersity indexes in dynamic light scattering (DLS) of polyplexes made from pDNA and all P(MAG-b-Methacrylate)s polymers at N/P ratio of 15.

Samples	0 Hour	2 Hours	4 Hours
PMAEMT-1	0.28±0.050	0.28±0.029	0.26±0.017
PMAEMT-2	0.28±0.020	0.26±0.033	0.27±0.005
PMAEMT-3	0.47±0.107	0.38±0.020	0.46±0.010
PDMAEMT-1	0.28±0.042	0.25±0.021	0.41±0.075
PDMAEMT-2	0.19±0.035	0.19±0.053	0.38±0.057
PDMAEMT-3	0.18±0.037	0.20±0.017	0.39±0.057
PTMAEMT-1	0.26±0.014	0.25±0.021	0.25±0.029
PTMAEMT-2	0.26±0.023	0.31±0.036	0.30±0.061
PTMAEMT-3	0.27±0.042	0.28±0.129	0.21±0.026

Table S2. Polydispersity indexes in dynamic light scattering (DLS) of polyplexes made from pDNA and all P(MAG-b-Methacrylate)s polymers at N/P ratio of 5.

Samples	0 Hour	2 Hours	4 Hours
PMAEMT-1	0.27±0.027	0.31±0.033	0.35±0.038
PMAEMT-2	0.24±0.008	0.36±0.049	0.33±0.034
PMAEMT-3	0.45±0.065	0.46±0.044	0.40±0.020
PDMAEMT-1	0.24±0.008	0.50±0.050	0.48±0.082
PDMAEMT-2	0.30±0.022	0.43±0.016	0.56±0.035
PDMAEMT-3	0.35±0.006	0.56±0.036	0.45±0.061
PTMAEMT-1	0.45±0.024	0.44±0.023	0.39±0.016
PTMAEMT-2	0.35±0.033	0.38±0.048	0.44±0.004
PTMAEMT-3	0.24±0.019	0.33±0.046	0.32±0.034

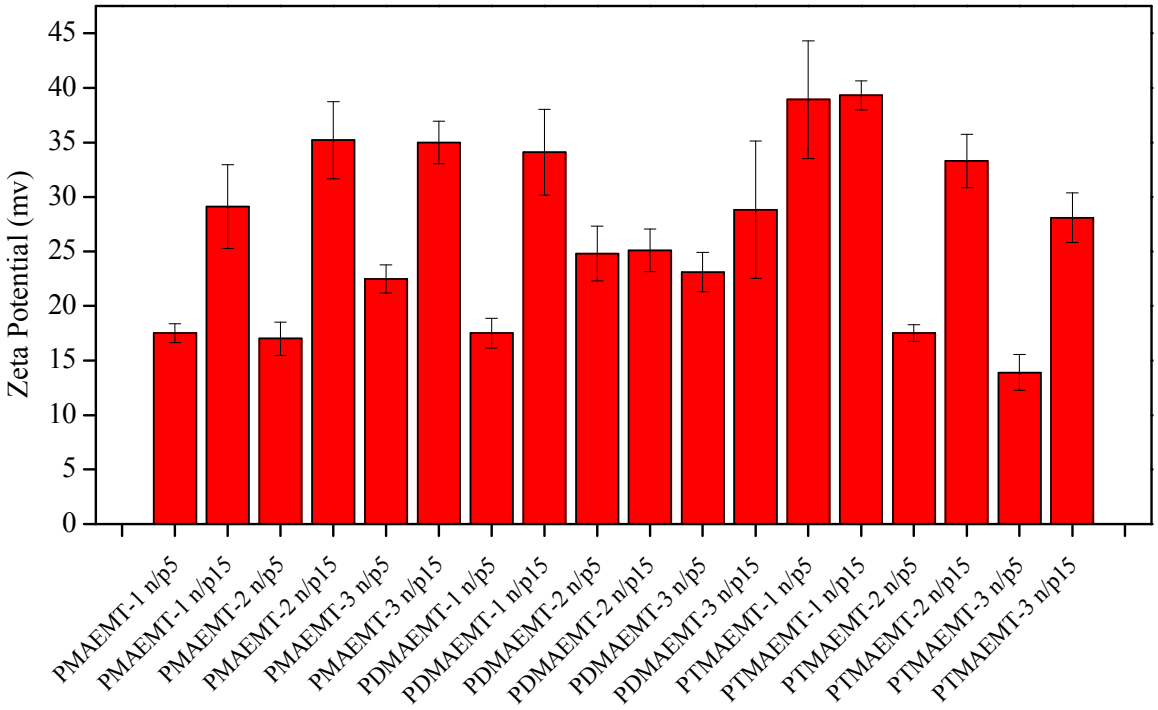
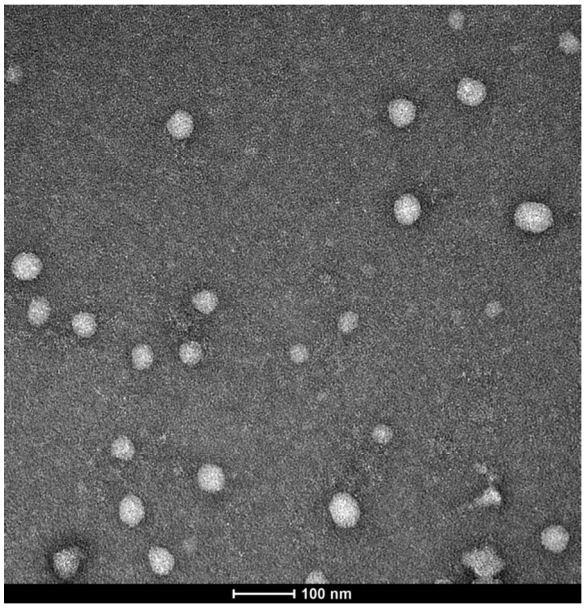


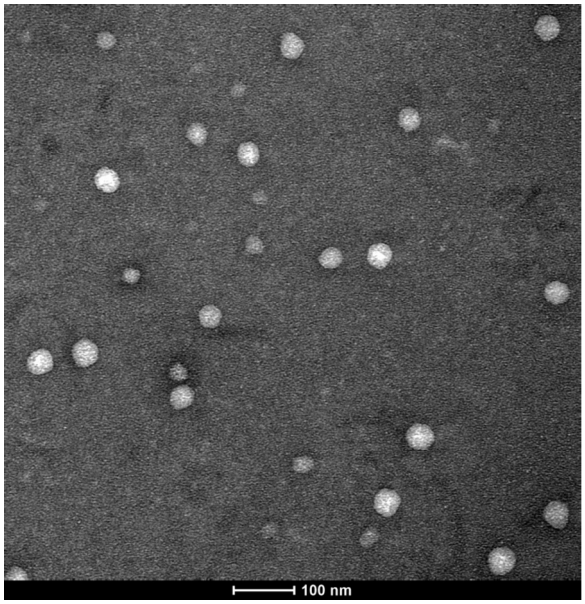
Figure S11. Zeta potential for polyplexes made from pDNA and all of the P(MAG-b-Methacrylate)s polymers containing the PMAEMT, PDMAEMT, PTMAEMT blocks at an N/P ratio of 5 and 15.

Transmission Electron Microscopy (TEM) Imaging. Polyplexes were formulated according to a previously described procedure at an N/P value of 5. A 3.0 μL aliquot of the polyplex solution was applied onto a 300-mesh carbon coated copper grid (Ted Pella, Inc). The excess

solution was removed with filter paper after 60s, followed by negative stain with 1% uranyl acetate solution (three times). Imaging was done with an FEI Tecnai G2 Spirit BioTWIN transmission electron microscope, operated at 120 kV. Images were recorded using an EagleTM 2k CCD camera (up to 4 mega pixels), and phase contrast was enhanced with a 6-12 μm underfocus.



PMAEMT-3



PDMAEMT-3

Figure S12. TEM images for polyplexes made from pDNA and the P(MAG-b-Methacrylate)s containing the PMAEMT-3 andPDMAEMT-3 blocks at an N/P ratio of 15.

Polyplex formation for *in vitro* work. All polyplexes were formed by first weighing dry, lyophilized polymer into a vial and adding DNase and RNase free water to dilute the polymers to 2 mg/ml. The polymers were then further diluted to the concentrations corresponding to the desired N/P ratios. Polymers at the desired N/P ratios were then added to an equal volume of the relevant plasmid with the concentration of plasmid dependent on the assay. The polyplex solutions were then allowed to incubate at room temperature for an hour. Polyplex solutions were then diluted with either Opti-MEM or DEMEM, with volumes dependent on the experiment.

Transfection Studies. Transfection was measured using both luciferase as a reporter gene and Green Fluorescent Protein (eGFP) as a reporter gene. HepG2 (human liver hepatocellular

carcinoma) cells were cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic (Ab/Am). Cells were not used until passaged greater than 4 times. Cells were seeded on 24-well plates at 50,000 cells/well and allowed to incubate in supplemented DMEM at 37°C and 5% CO_2 for 24 hours. The polyplexes were prepared by combining 165 µl of gWiz-luc (1µg) with 165 µl of P(MAG-b-Methacrylate)s containing the blocks: PMAEMT-1,-2, or -3, PDMAEMT-1,-2,or -3, or PTMAEMT-1,-2, or-3 at N/Ps of 2, 5, 10, 15, 20, or 30 or with 165 µl of Jet PEI (N/P=5) or Glycofect (N/P=20) as positive controls, or 165 µl water for pDNA as a negative control. A cell only control was also prepared with 330 µl of water. After an hour of incubation, 660 µl of either serum-free Opti-MEM or DMEM containing 10% serum was added to the polyplex solutions. Each well was then transfected with 300 µl of the polyplex solution. The cells were incubated with each solution for 4 hours to allow the polyplexes to be internalized. After 4 hours, 1 ml of DMEM was added, and the cells were incubated for an additional 20 hours. DMEM was replaced with supplemented DMEM 24 hours after transfection, and the cells were incubated for an additional 24 hours. After 48 hours, the cells were washed with PBS buffer followed by a treatment of 0.1 ml of lysis buffer (Promega, Madison, WI) for 20 minutes at room temperature. Aliquots of cell lysates were measured on 96-well plates with a luminometer (GENios Pro, TECAN US, Research Triangle Park, NC) for luciferase activity. The amount of protein in the lysed cells was measured by a standard curve of bovine serum albumin following the protocol provided by Bio-Rad (Hercules, CA) DC protein assay kit for viability to normalize luciferase results.

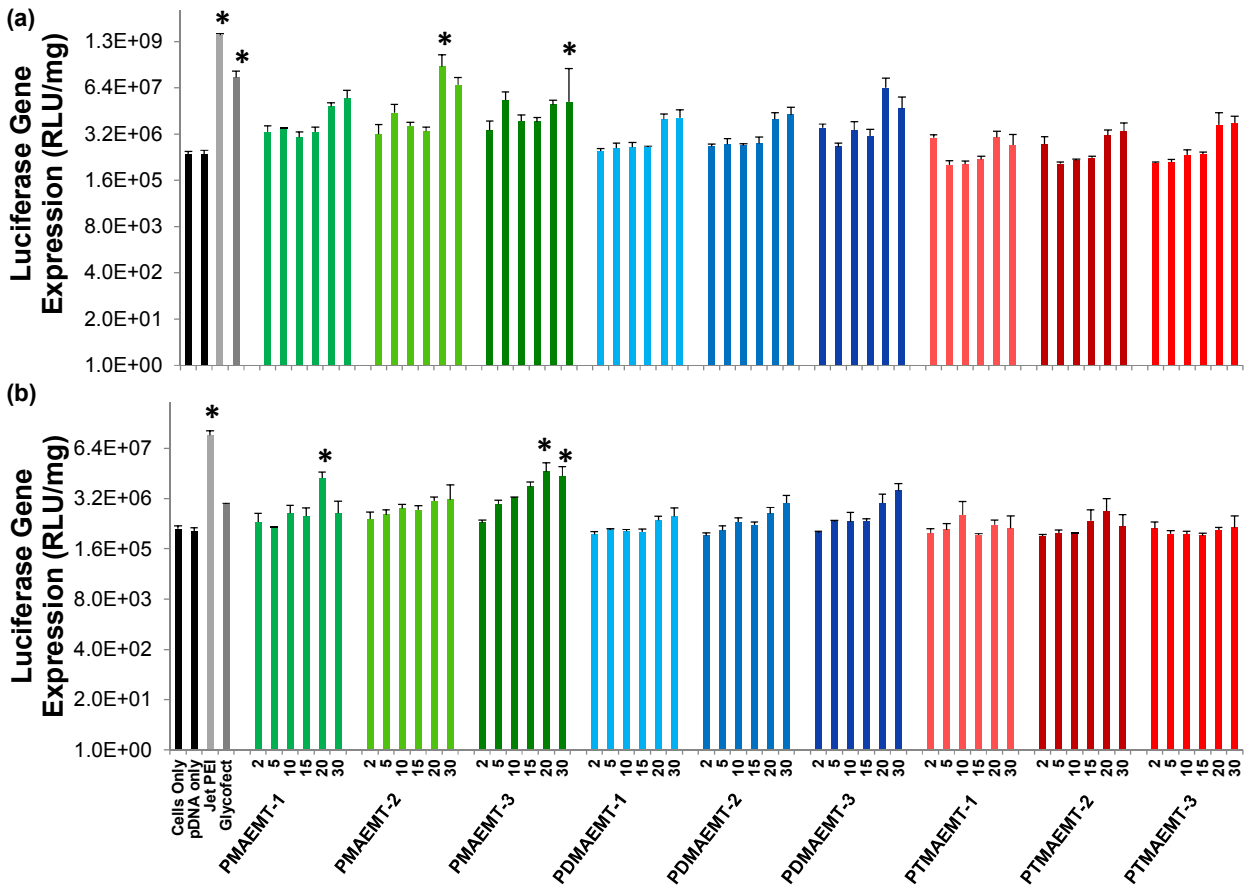


Figure S13. Luciferase gene expression as observed in HepG2 cells transfected with polyplexes formed at N/P ratios of 2, 5, 10, 15, 20, and 30 with gWiz-luc pDNA and all P(MAG-b-Methacrylate)s containing the blocks: PMAEMT-1, 2, 3, PDMAEMT-1, 2, 3, and PTMAEMT-1, 2, 3 polymers in (a) Opti-MEM and (b) DMEM. Error bars are representative of the standard deviation of analyzed data from three replicates. All measurements found to be statistically significant ($p<0.05$) as compared to cells only are marked with an asterisk.

For expression with GFP as a reporter gene, HepG2 cells were plated into 12-well plates at a concentration of 100,000 cells/well and allowed to incubate in supplemented DMEM at 37°C and 5% CO_2 for 24 hours. The polyplexes were prepared by combining 330 µl of pZs-

Green (2μg) with 330 μl of the P(MAG-b-Methacrylate)s containing the following charged blocks: PMAEMT-1,-2, or -3, PDMAEMT -3, or PTMAEMT -3 at an N/P of 15 or with 330 μl of Jet PEI (N/P=5) as a positive control, or 330 μl water for pDNA as a negative control. A cell only control was also prepared with 660 μl of water. After an hour of incubation, 1320 μl of either Opti-MEM or DMEM was added to the polyplex solutions. The cells were then transfected with 600 μl of the polyplex solution and allowed to incubate for 4 hours to allow the polyplexes to be internalized. After 4 hours, 2 ml of DMEM was added, and the cells were incubated for an additional 20 hours. DMEM was replaced with 2 ml of supplemented DMEM 24 hours after transfection, and the cells were incubated for an additional 24 hours. After 48 hours, the cells were washed with PBS buffer, trypsinized with 500 μl trypsin/well, collected with 500 μl DMEM/well, centrifuged, washed with PBS buffer three times, and resuspended in PBS buffer. A FACSVerse (Becton Dickenson, San Jose, CA) equipped with a 488nm 20mw Solid State laser, a 640nm 40mw Red Solid State laser, and a 405nm 40mw Solid State laser to excite GFP (488 nm) was used. A total of 10,000 events were collected for each sample. The positive fluorescence level was ascertained by insuring that <1% of negative control cells (cells only) appeared in the positive region of the histogram.

Cell viability studies. Cell viabilities after treatment with polyplexes of PMAEMT-1,-2, or -3, PDMAEMT-1,-2,or -3, or PTMAEMT-1,-2, or-3 at N/Ps of 2, 5, 10, 15, 20, or 30 were measured by an MTT assay. For this assay, cells were seeded in 24-well plates at a density of 50,000 cells/well and were allowed to incubate in supplemented DMEM at 37°C and 5% CO_2 for 24 hours. The polyplexes were prepared by combining 165 μl of gWiz-luc (1μg) with 165 μl of desired polymer or with 165 μl of Jet PEI (N/P=5) or Glycofect (N/P=20) as a positive control. After an hour of incubation, 660 μl of either Opti-MEM or DMEM was added to the polyplex

solutions. The cells were then transfected with 300µl of the polyplex solution and incubated for 4 hours to allow the polyplexes to be internalized. After 4 hours, 1 ml of DMEM was added, and the cells were incubated for an additional 20 hours. DMEM was replaced with 1 ml supplemented DMEM 24 hours after transfection, and the cells were incubated for an additional 24 hours. After 48 hours, the cells were washed with PBS buffer followed by the addition of supplemented DMEM containing 0.25 mg/ml of 3-[4,5-dimethylthiazol-2-yl]2, 5-diphenyltetrazolium bromide (MTT) (Molecular Probes, Eugene Oregon) to the cells, after which, the cells were incubated for 1 hour at 37°C. The media was then aspirated, the cells were washed with PBS, and then they were lysed with 0.600 ml of DMSO. Cells were then incubated on a shaker for 15 minutes to ensure distribution of the purple formazan. After 15 minutes, 0.200 ml of cell lysate was pipetted from each well into a clear 96-well plate and analyzed for absorbance at 570 nm using a Tecan GENios Pro plate reader (TECAN US).

Cellular uptake profile. For the cellular uptake profile, HepG2 cells were plated into 6-well plates at a concentration of 250,000 cells/well and allowed to incubate in supplemented DMEM at 37°C and 5% CO_2 for 24 hours. Cy5-labeled pDNA was prepared with a Label-IT Cy5 labeling kit (Mirus, Madison, WI) according to the manufacture's protocol. The polyplexes were prepared by combining 825 µl of the Cy5-labeled pDNA (5µg) with 825 µl of the P(MAG-b-Methacrylate)s containing the following blocks: PMAEMT-1,-2, or -3, PDMAEMT -3, or PTMAEMT -3 at an N/P of 15. Also the controls were created with 825 µl of Jet PEI (N/P=5) as a positive control, or 825 µl water for pDNA as a negative control. A cell only control was also prepared with 1650 µl of water. After an hour of incubation, 3300 µl of either Opti-MEM or DMEM was added to the polyplex solutions. The cells were then transfected with 1500 µl of the polyplex solution and allowed to incubate for 4 hours to allow the polyplexes to be internalized.

After 4 hours, cells were washed with PBS buffer, trypsinized with 500 µl trypsin/well, collected with 500 µl DMEM/well, centrifuged and washed with PBS buffer three times, and resuspended in PBS buffer. A FACSVerse (Becton Dickenson, San Jose, CA) equipped with a 488nm 20mw Solid State laser, a 640nm 40mw Red Solid State laser, and a 405nm 40mw Solid State laser to excite Cy5 (633 nm) was used. A total of 10,000 events were collected for each sample. The positive fluorescence level was ascertained by insuring that <1% of negative control cells (cells only) appeared in the positive region of the histogram.

Reference

1. Xu, X.; Smith, A. E.; Kirkland, S. E.; McCormick, C. L. *Macromolecules* **2008,** *41* , 8429-8435.
2. Smith, A. E.; Sizovs, A.; Grandinetti, G.; Xue, L.; Reineke, T. M. *Biomacromolecules* **2011,** *12* , 3015-3022.
3. Kato, S.; Harada, H.; Morie, T. *J. Chem. Soc., Perkin Trans. 1* **1997,** , 3219-3226.