Electronic supporting information for:

Self-immolative Polyplexes for DNA Delivery

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Experimental Procedures

General Materials. Ethyl glyoxylate in toluene solution (50% w/w), *N*,*N*-dimethylethylenediamine, *N*-methylethylenediamine, 4-monomethoxytrityl chloride, AgOTf, and citric acid were obtained from Alfa Aesar. *n*-Butyl lithium in toluene solution (1.4 M), benzyl chloromethyl ether, 3-(dimethylamino)-1-propylamine, trityl chloride, and Tris acetate ethylenediaminetetraacetic acid (EDTA) buffer solution were obtained from Sigma Aldrich. NEt_3 and KH_2PO_4 were obtained from Millipore. NaOH, KOH, and D-luciferin were obtained from Thermo Fisher Scientific. jetPEI solution was obtained from Polyplus-transfection SA. Agarose gel solution (1% w/v), RedSafe electrophoresis stain, and a 1 kb DNA ladder were obtained from FroggaBio. Gel electrophoresis loading dye was obtained from New England Biolabs. Uranyl acetate and 400 mesh Formvar-coated copper grids were obtained from Electron Microscopy Sciences. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum, and penicillin/streptomycin were obtained from VWR. MTT was obtained from Gibco. Ethyl glyoxylate was purified over P_2O_5 as previously reported.[36] Toluene was distilled over sodium and benzophenone under a nitrogen atmosphere before use. NEt_3 was distilled over CaH_2 under a nitrogen atmosphere before use. Purified water was obtained from VWR or from a Barnstead EASYpure II system. All other chemicals were of reagent grade and were used without further purification.

General Methods. ^1H and ^{13}C NMR spectra were obtained using a 400 MHz Bruker AvIII HD instrument, a 400 MHz Varian INOVA instrument, or a 600 MHz Varian INOVA instrument. ^1H NMR chemical shifts were calibrated against the residual solvent signal of $CHCl_3$ (7.26 ppm) or HOD (4.79 ppm) while ^{13}C NMR chemical shifts were calibrated against the solvent signal of

$CDCl_3$ (77.16 ppm). FT-IR spectra were obtained using a PerkinElmer FT-IR Spectrum Two instrument with attenuated total reflectance sampling. Size-exclusion chromatograms were obtained using a DMF chromatograph equipped with a Waters 515 HPLC pump with a Waters In-Line Degasser AF, two PLgel mixed D 5 μm (300 × 1.5 mm) columns connected to a corresponding PLgel guard column, and a Wyatt Optilab Rex RI detector. Samples were dissolved in DMF containing 10 mM LiBr and 1% v/v NEt_3 at a concentration of ~5 mg/mL. Each sample was filtered through a 0.2 μm polytetrafluoroethylene syringe filter prior to injection using a 50 μL loop. Samples were run at a flow rate of 1 mL/min for 30 min at 85 ºC. Molar masses of the samples were calculated relative to PMMA standards. Gel electrophoresis was performed using agarose gel in a tris acetate EDTA running buffer at an applied voltage of 100 V for 1 h. Gels were subsequently imaged using a Gel Doc (Biorad).

Synthesis of PEtGs

All PEtGs were synthesized using the same procedure as ***PEtG-Trit*** *(representative PEtG synthesis)*

Synthesis of PEtG-Trit (representative PEtG synthesis). AgOTf (1.0 g, 3.9 mmol, 9.5 equiv) and trityl chloride (1.1 g, 3.9 mmol, 9.5 equiv) were added to a flask, which was subsequently evacuated and purged with nitrogen. A 10 mL aliquot of dry toluene was then added, and the flask was stirred and heated at 70 °C for 1 h to yield the end-capping mixture. Separately, 40 mL of dry toluene was added to a flame-dried Schlenk flask under a nitrogen atmosphere along with *n*-butyl lithium (0.29 mL, 0.41 mmol, 1.0 equiv) Purified ethyl glyoxylate (10 mL, 100 mmol, 240 equiv) was subsequently added to the flask and the system was stirred and cooled at −20 °C. After 30 min, dry NEt_3 (1.1 mL, 7.9 mmol, 19 equiv) was added to the polymerization flask and the mixture

was allowed to stir for another 30 min. The end-capping mixture was then cooled to −20 °C before being transferred with a wide mouth pipette to the polymerization flask. The polymerization flask was stoppered and stirred at −20 °C for 4 h before being allowed to warm up to room temperature over 16 h. Concentration of the crude polymerization mixture under vacuum followed by filtration and precipitation in 440 mL of MeOH:H_2O (10:1) afforded 6.9 g of pure polymer residue as a clear off-yellow tacky solid, which was collected by decanting off the supernatant and concentrating under vacuum. Yield = 67%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.42–7.49 (m, 7H), 7.09–7.33 (m, 54H), 5.46–5.74 (m, 241H), 4.21 (br s, 485H), 1.27 (br s, 696H), 0.88 (br s, 3H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 166.1–166.5, 127.2–129.8, 90.4–94.3, 62.2, 14.0. FT-IR: 2990, 1750 cm^{-1}. SEC: M_n = 21.4 kg/mol, M_w = 36.4 kg/mol, $Đ$ = 1.7.

Synthesis of PEtG-MMT. 4-monomethoxytrityl chloride (1.2 g, 3.9 mmol, 9.5 equiv) was used to afford 6.0 g of a clear off-yellow tacky solid. Yield = 58%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38–7.51 (m, 6H), 7.08–7.38 (m, 45H), 6.78–6.87 (m, 3H), 5.42–5.78 (m, 210H), 4.22 (br s, 416H), 3.74–3.81 (m, 7H), 1.29 (br s, 623H), 0.88 (br s, 3H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 165.2–166.5, 127.2–129.6, 90.6–94.4, 62.2, 14.0. FT-IR: 2990, 1750 cm^{-1}. SEC: M_n = 18.8 kg/mol, M_w = 33.0 kg/mol, $Đ$ = 1.8.

Synthesis of PEtG-BOM. Dry NEt_3 (2.2 mL, 16 mmol, 39 equiv) and 60% benzyl chloromethyl ether (1.9 mL, 8.2 mmol, 20 equiv) were used at the 30 min and 1 h mark of the reaction respectively. After addition of the benzyl chloromethyl ether, the reaction flask was stirred for 1 h at −20 °C before it was transferred to a −20 °C freezer for 4 days, stirring occasionally. Purification afforded 5.9 g of a clear, colourless, tacky solid. Yield = 57%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30–7.35 (m, 6H), 5.43–5.77 (m, 156H), 4.22 (br s, 307H), 1.29 (br s, 465H), 0.84–0.93 (m, 3H).

$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): δ 165.2–166.7, 127.8–129.3, 90.7–94.2, 62.2, 14.0. FT-IR: 2990, 1750 cm^{-1}. SEC: M_n = 16.5 kg/mol, M_w = 22.7 kg/mol, $Đ$ = 1.4.

Synthesis of PGAms

All PGAms were synthesized using the same procedure as ***PGAm-DMAE-Trit*** *(representative polyglyoxylamide synthesis).*

Synthesis of PGAm-DMAE-Trit (representative PGAm synthesis). PEtG-Trit (0.30 g of polymer, 2.9 mmol of ester, 1.0 equiv) was dissolved in 6.0 mL of 1,4-dioxane in a vial. *N*,*N*-Dimethylethylenediamine (0.96 mL, 8.8 mmol, 3.0 equiv) was added to the solution and the vial was sealed and stirred for 16 h at room temperature. The crude mixture was concentrated and precipitated in 50 mL of *n*-pentane. After decanting, the purified polymer residue was dried to afford 0.40 g of a clear, colourless, brittle solid. Yield = 94%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63–8.92 (m, 230H), 7.45–7.51 (m, 6H), 5.72 (br s, 211H), 3.32 (br s, 451H), 2.43 (br s, 403H), 2.22 (br s, 1160H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): δ 166.0–168.2, 94.6–98.5, 58.0, 45.5, 37.5. FT-IR: 3274, 3085, 2944, 2860, 2820, 2768, 1666, 1539 cm^{-1}. SEC: M_n = 26.7 kg/mol, M_w = 45.7 kg/mol, $Đ$ = 1.7.

Synthesis of PGAm-DMAPr-Trit. 3-(dimethylamino)-1-propylamine (1.1 mL, 8.7 mmol, 3.0 equiv) was used to afford 0.38 g of a clear, colourless, tacky solid. Yield = 82%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12–9.05 (m, 210H), 7.43–7.51 (m, 6H), 5.71 (br s, 216H), 3.25 (br s, 408H), 2.30 (br s, 403H), 2.17 (br s, 1215H), 1.66 (br s, 413H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): δ 166.0–168.0, 94.8–98.5, 57.3, 45.4, 38.2, 26.8. FT-IR: 3270, 3080, 2940, 2860, 2820, 2760, 1670, 1540 cm^{-1}. SEC: M_n = 28.4 kg/mol, M_w = 46.6 kg/mol, $Đ$ = 1.6.

Synthesis of PGAm-MAE-Trit. 0.36 g of **PEtG-Trit** (3.5 mmol of ester, 1.0 equiv) and *N*-methylethylenediamine (0.92 mL, 11 mmol, 3.1 equiv) were used to afford 0.44 g of a white brittle solid. Yield = 96%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.99–9.10 (m, 184H), 7.40–7.50 (m, 6H), 5.70 (br s, 217H), 3.34 (br s, 448H), 2.71 (br s, 472H), 2.37 (br s, 681H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 167.3, 96.5, 50.5, 39.2, 36.0. FT-IR: 3290, 3070, 2930, 2890, 2850, 2800, 1660, 1540 cm^{-1}.

Synthesis of PGAm-DMAE-MMT. PEtG-MMT (0.30 g of polymer, 2.9 mmol of ester, 1.0 equiv) was used to afford 0.36 g of a clear, colourless, brittle solid. Yield = 85%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77–8.92 (m, 198H), 7.40–7.52 (m, 9H), 7.20–7.40 (m, 70H), 6.78–6.85 (m, 4H), 5.73 (br s, 196H), 3.78 (br s, 6H), 3.32 (br s, 335H), 2.43 (br s, 323H), 2.22 (br s, 959H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 166.0–168.3, 94.6–98.7, 58.1, 45.6, 37.6. FT-IR: 3270, 3080, 2940, 2860, 2820, 2770, 1670, 1540 cm^{-1}. SEC: M_n = 24.4 kg/mol, M_w = 44.9 kg/mol, $Đ$ = 1.8.

Synthesis of PGAm-DMAPr-MMT. PEtG-MMT (0.30 g of polymer, 2.9 mmol of ester, 1.0 equiv) and 3-(dimethylamino)-1-propylamine (1.1 mL, 8.7 mmol, 3.0 equiv) were used to afford 0.37 g of a clear, colourless, tacky solid. Yield = 80%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15–9.01 (m, 229 H), 7.42–7.53 (m, 8H), 7.15–7.42 (m, 74H), 6.76–6.86 (m, 4H), 5.72 (br s, 218 H), 3.77 (br s, 6H), 3.25 (br s, 428 H), 2.30 (br s, 434H), 2.18 (br s, 1301H), 1.66 (br s, 439H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 167.1, 96.6, 57.4, 45.5, 38.3, 26.7. FT-IR: 3270, 3090, 2940, 2860, 2820, 2760, 1670, 1540 cm^{-1}. SEC: M_n = 26.4 kg/mol, M_w = 42.9 kg/mol, $Đ$ = 1.6.

Synthesis of PGAm-MAE-MMT. PEtG-MMT (0.30 g of polymer, 2.9 mmol of ester, 1.0 equiv) and *N*-methylethylenediamine (0.77 mL, 8.8 mmol, 3.0 equiv) were used to afford 0.35 g of a white, brittle solid. Yield = 92%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97–9.22 (m, 196H),7.38–7.51

(m, 8H), 7.71–7.38 (m, 62H), 6.78–6.86 (m, 4H), 5.71 (br s, 193H), 3.78 (br s, 6H), 3.36 (br s, 402H), 2.72 (br s, 629H), 2.38 (br s, 613H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): δ 167.3, 96.6, 50.4, 39.1, 36.0. FT-IR: 3290, 3080, 2940, 2850, 2800, 1660, 1540 cm^{-1}.

Synthesis of PGAm-DMAE-BOM. PEtG-BOM (0.30 g of polymer, 2.9 mmol of ester, 1.0 equiv) was used to afford 0.23 g of a clear, pale-yellow, brittle solid. Yield = 54%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74–8.82 (m, 173H), 7.30–7.34 (m, 5H), 5.72 (s, 162H), 3.32 (s, 305H), 2.43 (s, 302H), 2.22 (s, 878H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): δ 167.3, 95.0–97.9, 58.1, 45.5, 37.5. FT-IR: 3280, 3090, 2950, 2360, 2820, 2770, 1670, 1540 cm^{-1}. SEC: M_n = 24.5 kg/mol, M_w = 42.2 kg/mol, $Đ$ = 1.7.

Synthesis of PGAm-DMAPr-BOM. PEtG-BOM (0.30 g of polymer, 2.9 mmol of ester, 1.0 equiv) and 3-(dimethylamino)-1-propylamine (1.1 mL, 8.7 mmol, 3.0 equiv) were used to afford 0.15 g of a clear, colourless, tacky solid. Yield = 32%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.19–9.00 (m, 150H), 7.30–7.34 (m, 5H), 5.72 (s, 156H), 3.25 (s, 265H), 2.29 (s, 397H), 2.71 (s, 821H), 1.66 (s, 287H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): δ 167.2, 96.5, 57.5, 45.5, 38.2, 27.0. FT-IR: 3270, 3090, 2940, 2860, 2820, 2770, 1670, 1540 cm^{-1}. SEC: M_n = 24.0 kg/mol, M_w = 34.2 kg/mol, $Đ$ = 1.4.

Synthesis of PGAm-MAE-BOM. PEtG-BOM (0.30 g of polymer, 2.9 mmol of ester, 1.0 equiv) and *N*-methylethylenediamine (0.77 mL, 8.8 mmol, 3.0 equiv) were used and the crude was precipitated in Et_2O to afford 0.25 g of an off-white, brittle solid. Yield = 65%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08–9.17 (m, 140H), 7.30–7.34 (m, 5H), 5.71 (br s, 139H), 3.37 (br s, 293H), 2.73 (br s, 519 H), 2.39 (br s, 370H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): δ 167.1, 96.4, 50.5, 39.1, 36.0. FT-IR: 3290, 3090, 2940, 2850, 2800, 1660, 1540 cm^{-1}.

Depolymerization of PGAms. Deuterated pH 5.0 and 6.0 buffers were prepared by dissolving citric acid into D_2O followed by correction to the desired pH with NaOH. Deuterated pH 7.4 buffer was prepared by dissolving KH_2PO_4 into D_2O followed by correction to the desired pH with KOH. All buffers were made at a 0.2 M concentration. To examine the depolymerization of the PGAms, each PGAm was dissolved into each buffer solution at 10 mg/mL and placed into an NMR tube. The tubes were promptly sealed, and the solutions were monitored over time via ^{1}H NMR spectroscopy. Percent depolymerization at each time point was determined by comparing the integration value of the polymer backbone methine proton peak at ~5.6 ppm with that of the monomer hydrate methine proton peak at ~5.3 ppm. For PGAm-MAE samples, an additional peak (corresponding to the monomer hemiaminal methine proton peak) was observed at ~4.5 ppm and its integration value was combined with that of the monomer hydrate.

Plasmid DNA. Plasmid DNA used for the complexation and transfection experiments was composed of a human elongation factor 1 alpha (EGF1α) promotor driven plasmid expressing tdTomato and Firefly luciferase (FLuc2) separated by a self-cleaving 2A peptide. The DNA was produced by transfecting ZYCY10P3S2T *E. coli* and viable kanamycin-resistant colonies were selected and cultured at 37 ºC in lysogeny broth overnight. The bacteria were then pelleted and isolated using an endotoxin-free Maxi kit (Qiagen) according to the manufacturer's instructions.

Complexation of PGAms with plasmid DNA. Each of the PGAms was dissolved in purified water to prepare 1.0 mg/mL solutions immediately prior to being used. An appropriate aliquot of each solution was mixed with 300 ng of DNA and purified water was added to the mixture to make up a total volume of 20 μL. Aliquots of the PGAm solutions were determined using Equation 1 and selecting for N/P ratios of 1, 5, 10, 25, and 50:

$$v = \frac{rm(3\ nmol/\mu g)}{c} \qquad (1)$$

where v = volume of aliquot, r = N/P ratio, m = mass of DNA (in μg), and c = molarity of cationic nitrogen in the polymer solution (in mM; for PGAm-DMAE samples, c = 6.9 mM; for PGAm-DMAPr samples, c = 6.3 mM; and for PGAm-MAE samples, c = 7.7 mM). The polyplex mixtures were incubated for 15 min before each being placed in a well and gel electrophoresis was run. Examination of the gels afterwards revealed DNA travel through the gels, with delayed travel of the DNA compared to the lane with DNA only (N/P ratio = 0) indicative of the presence of polyplexes.

Triggered Decomplexation of the Polyplexes. Each of the PGAms was dissolved in purified water to prepare 1.0 mg/mL solutions immediately prior to being used. Aliquots of polymer solution needed to prepare 50 N/P ratio polyplex mixtures were calculated using Equation 1 and mixed with 300 ng of DNA. For each polyplex tested, two different polyplex mixtures were prepared. One mixture was diluted with purified water to a total volume of 20 μL before being loaded into a gel and run through electrophoresis. The other mixture was diluted with concentrated citrate buffer (pH 5) to a total volume of 20 μL and a final buffer concentration of 0.2 M. The buffered polyplexes were stored at room temperature for 1 day before being loaded into a gel and run through electrophoresis. Examination of the gels after electrophoresis revealed DNA travel through the gel, with delayed travel of the DNA compared to the lane with DNA only (N/P ratio = 0) indicative of the presence of polyplexes.

DLS of the Polyplexes. Each of the PGAms was dissolved in purified water to prepare 10 mg/mL solutions immediately prior to use. Appropriate aliquots of each PGAm solution for a N/P ratio of 50 (determined using Equation 1) were added to 10 μg of DNA along with purified water to give

a final volume of 500 µL. The samples were then examined for size before being further diluted with 500 µL of 20 mM saline solution and examined for zeta potential. For samples **PGAm-DMAE-MMT** and **PGAm-DMAE-BOM**, the final dilute saline solutions each were filtered through a 0.45 µm Nylon syringe filter in order to remove aggregates before their sizes and zeta potentials were determined. The DLS measurements were obtained using a Malvern Zetasizer Nano ZS instrument equipped with a 633 nm laser and at a scattering angle of 173°.

TEM of the Polyplexes. Each of the PGAms was dissolved in purified water to prepare 10 mg/mL solutions immediately prior to use. Appropriate aliquots of each PGAm solution for a N/P ratio of 50 (determined using Equation 1) were added to 1.8 µg of DNA and diluted to 9.0 µL using purified water. The samples were incubated for 15 min. For the pH 5 samples, the pH was adjusted to 5 using 30 mM HCl and the samples were incubated for 24 h. Then, a drop of each polyplex suspension (0.20 µg/mL of DNA) was placed on a Formvar-coated copper TEM grid and allowed to sit for 5 min before wicking away the excess liquid. The grids were allowed to dry before being stained with a 1% w/v uranyl acetate solution. After excess stain was removed and the grids allowed to dry, each grid was loaded into the microscope and imaged using a Phillips Electron Optics CM10 transmission electron microscope operating at 80 kV.

Cytotoxicity Assays for Polycations. HEK 293T cells were cultured in DMEM containing 10% v/v of fetal bovine serum and 20 U/mL of penicillin-streptomycin media at 37 °C under a 5% CO_2 atmosphere. The cells were seeded in 96 well plates at a concentration of ~10,000 cells/well and then incubated for 24 h under the culture conditions. Next, the media was aspirated and replaced with either the sodium dodecyl sulfate (SDS) in the cell media at concentrations of 200, 150, 100, or 50 µg/mL (positive controls), just media (negative control), or the serial two-fold dilution of the samples (PGAms and monomers, jetPEI) in media. Monomers were prepared from the PGAm-

MMTs by dissolving them at 10 mg/mL in purified water and letting them sit at room temperature for at least 24 h before use to allow for complete depolymerization. Six replicates of each sample condition were measured. The cells were incubated with the different materials for 48 h and then the media was aspirated and replaced with 110 μL of fresh media containing 0.5 mg/mL of MTT reagent. After another 4 h of incubation, the media was aspirated and replaced with 50 μL of dimethyl sulfoxide (DMSO), and then the absorbance of each well was measured at 540 nm using a Tecan Infinite M1000 Pro plate reader, subtracting the absorbance of wells containing only DMSO. Metabolic activity was calculated by comparing the mean absorbance of the sample with that of the negative control. No activity was observed for cells exposed to the highest concentrations of SDS, confirming the sensitivity of the assay.

Cytotoxicity Assays for Polyplexes. Each of the PGAms was dissolved in purified water to prepare 1.0 mg/mL solutions immediately prior to analysis. Additionally, jetPEI solution (c = 7.5 mM of cationic nitrogen) was used for comparison. An appropriate aliquot of each solution was mixed with 250 ng of DNA and a 150 mM solution of NaCl was added to the mixture to make up a total volume of 20 μL (aliquots of the PGAm and jetPEI solutions were determined by using Equation 1 and selecting for N/P ratios of 0, 5, 10, 25, and 50). Each complex mixture was allowed to sit for 15 min after dilution to the final volume and then the cytotoxicity assays were performed as described above.

Transfection Assays. PGAms and jetPEI were prepared with DNA as polyplexes as described in the section “Complexation of PGAms with plasmid DNA”. HEK 293T cells were cultured as described in the “Cytotoxicity Assays for Polycations” section above. After incubating the cells for 24 h and exchanging the used media with fresh media (150 μL), the polyplex mixtures were added to the wells directly and the cells were incubated for an additional 24 h. For the

bioluminescence assays, D-luciferin (150 μg/mL) was then added to the wells and luminescence readings were performed using an IVIS Lumina XRMS scanner (PerkinElmer, MA, USA). The plates were continually imaged until a peak signal was achieved. Average radiance per well was quantified by placing regions of interest over each well using Living Image 4.5.2 software. Samples were examined in triplicate. For flow cytometry analysis, cells in each well were trypsinized, washed with PBS, then analyzed on the FACSCanto Flow Cytometer (BD Biosciences) for tdTomato positive cells. Prior to analysis, a Sytox Green cell viability dye (Biolegend) was added to the samples to exclude dead cells.

^{1}H NMR Spectra

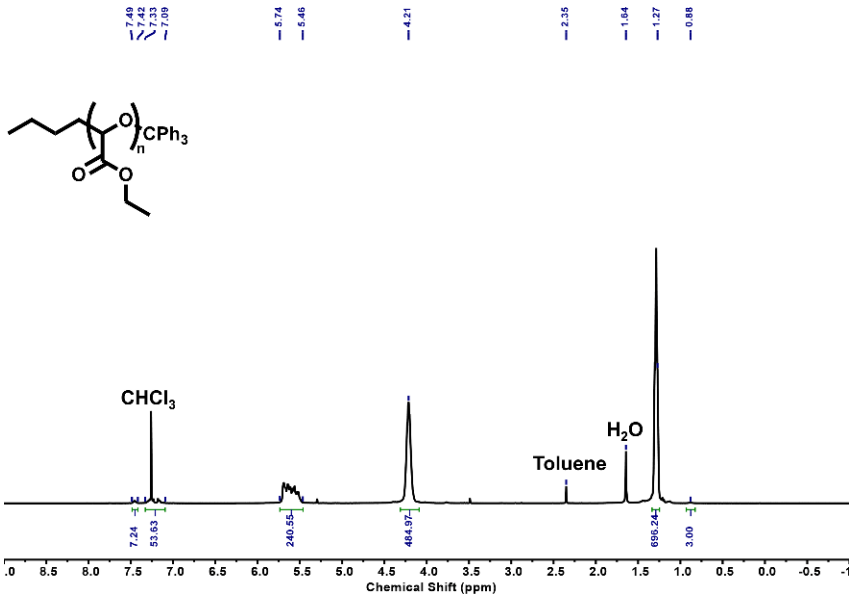

Figure S1. ^{1}H NMR spectrum of **PEtG-Trit** ($CDCl_3$, 400 MHz).

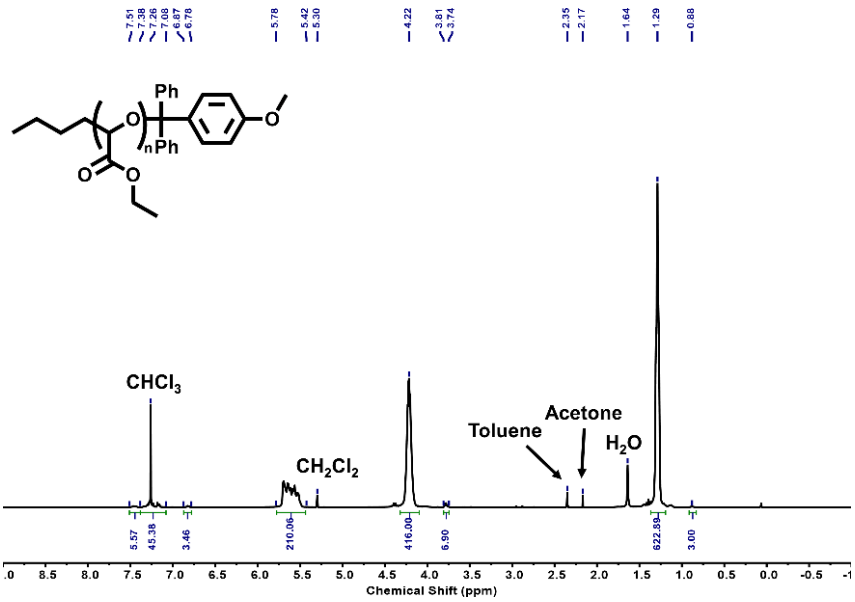

Figure S2. ^{1}H NMR spectrum of **PEtG-MMT** ($CDCl_3$, 400 MHz). End-cap analysis reveals that a large portion of the polymer initiated with residual water and therefore has a 4-monomethoxytrityl end-cap at both ends.

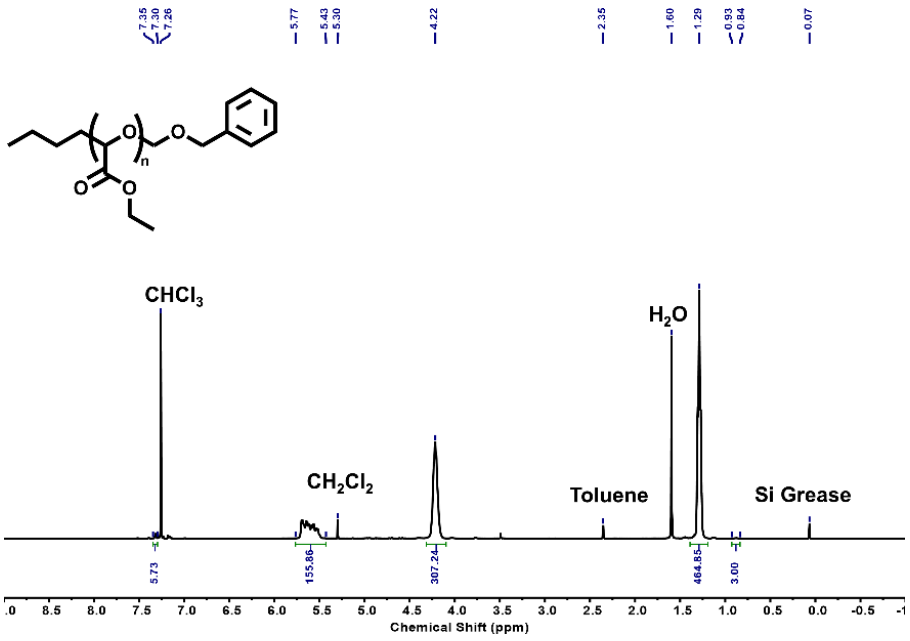

Figure S3. ^{1}H NMR spectrum of **PEtG-BOM** ($CDCl_3$, 400 MHz).

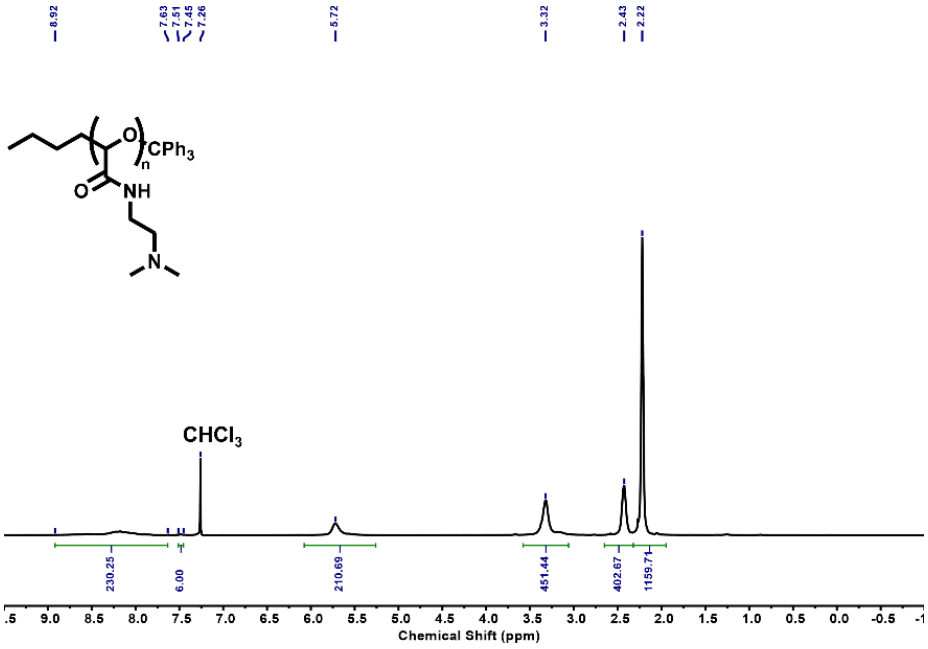

Figure S4. ^{1}H NMR spectrum of **PGAm-DMAE-Trit** ($CDCl_3$, 400 MHz).

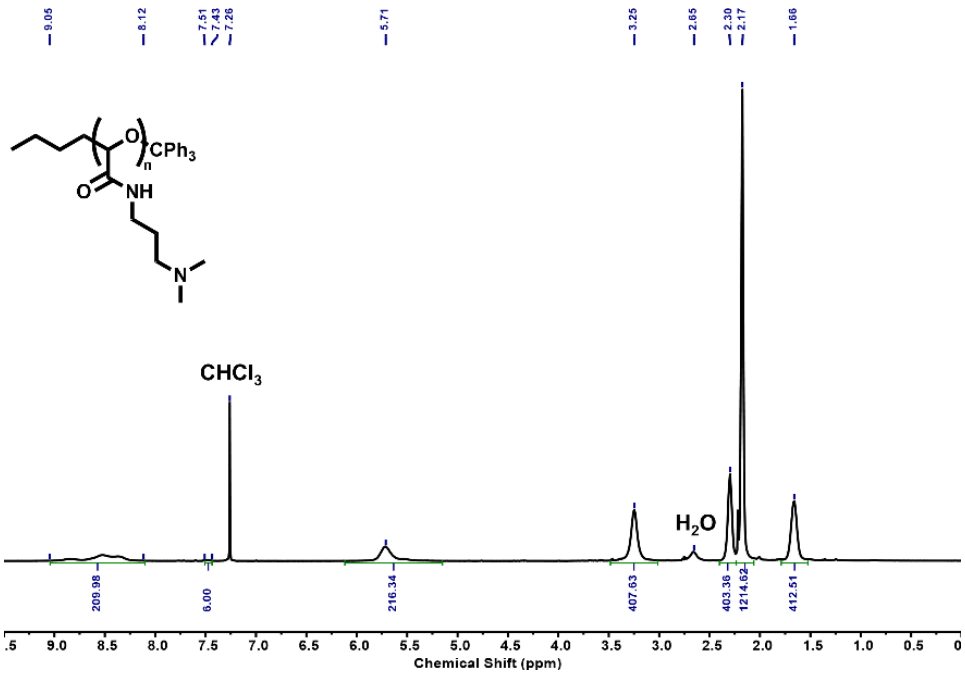

Figure S5. ^{1}H NMR spectrum of **PGAm-DMAPr-Trit** ($CDCl_3$, 400 MHz).

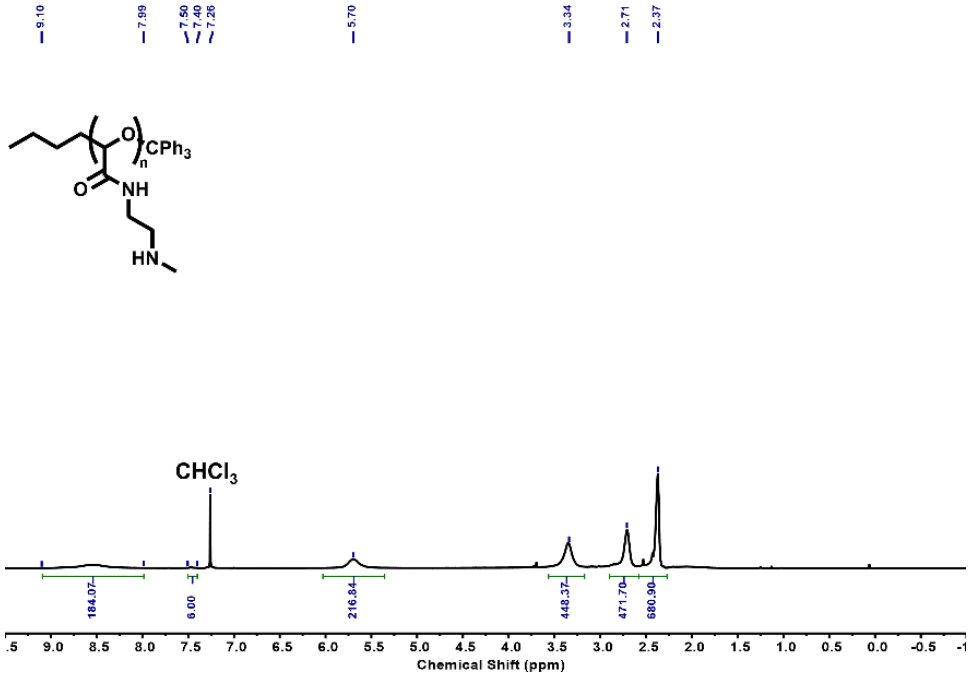

Figure S6. ^{1}H NMR spectrum of **PGAm-MAE-Trit** ($CDCl_3$, 400 MHz).

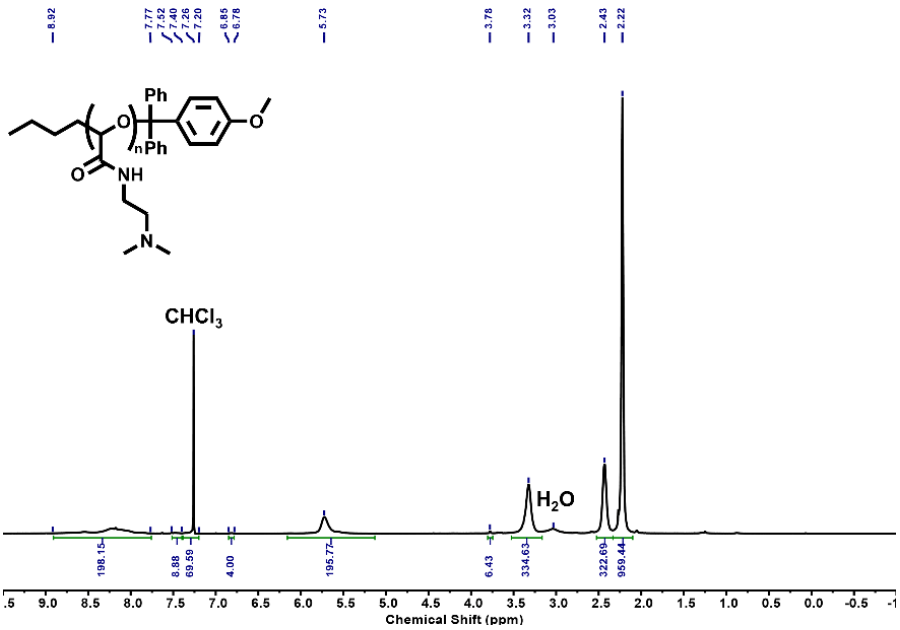

Figure S7. ^{1}H NMR spectrum of **PGAm-DMAE-MMT** ($CDCl_3$, 400 MHz).

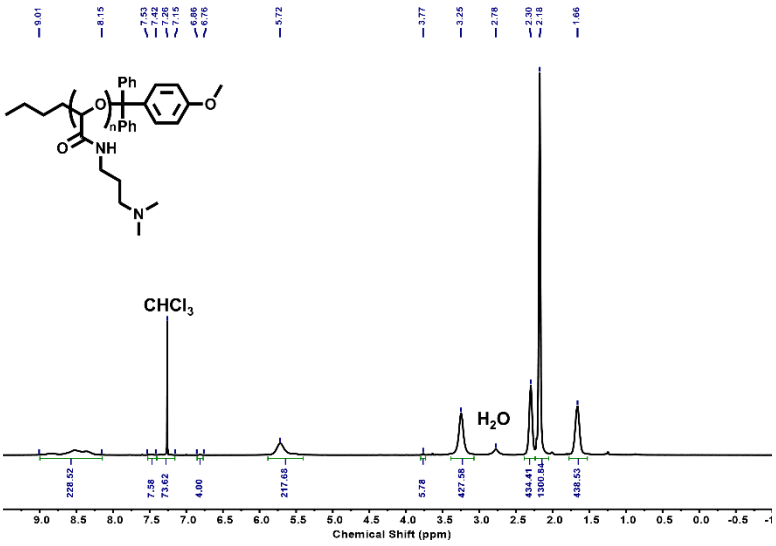

Figure S8. ^{1}H NMR spectrum of **PGAm-DMAPr-MMT** ($CDCl_3$, 400 MHz).

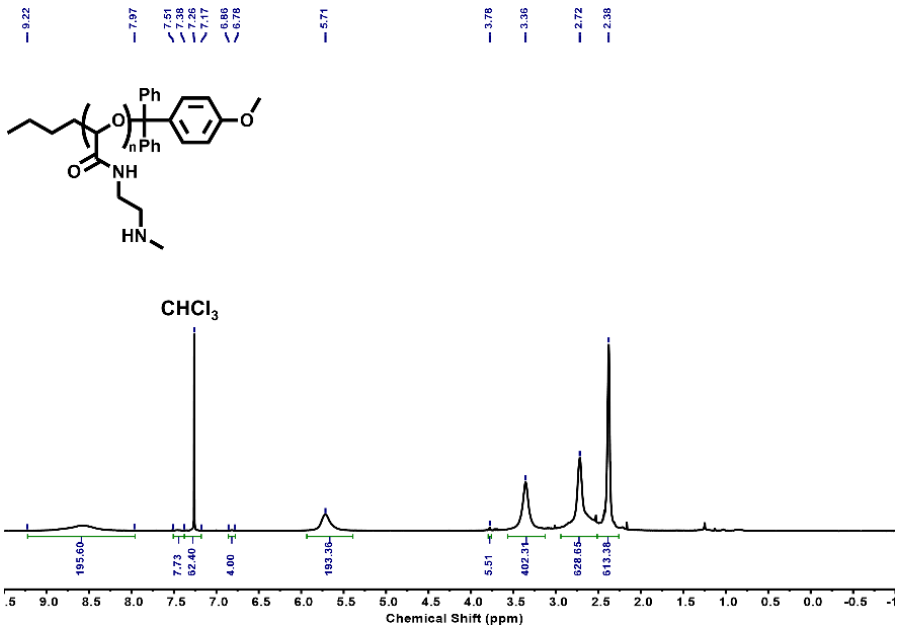

Figure S9. ^{1}H NMR spectrum of **PGAm-MAE-MMT** ($CDCl_3$, 400 MHz). The peak at 2.72 ppm has a higher than expected integration due to an overlapping H_2O peak.

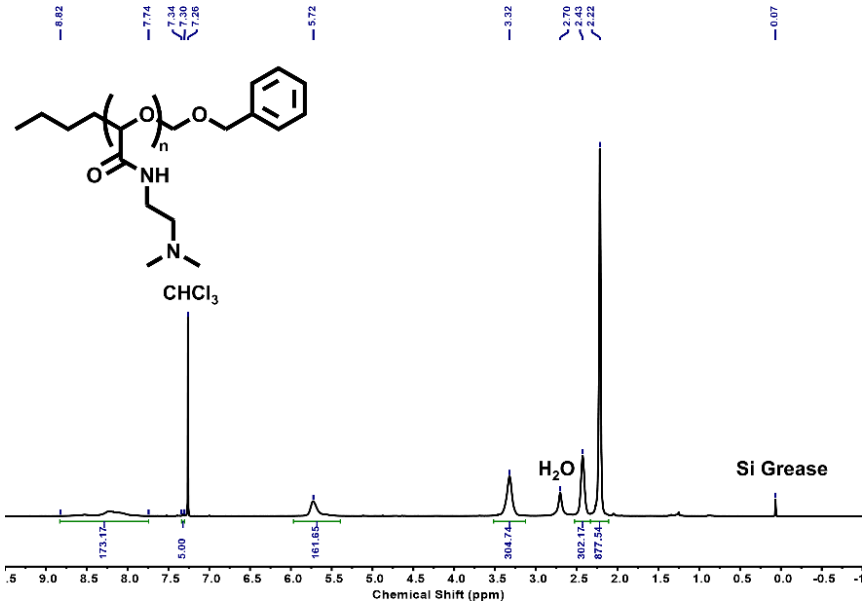

Figure S10. ^{1}H NMR spectrum of **PGAm-DMAE-BOM** ($CDCl_3$, 400 MHz).

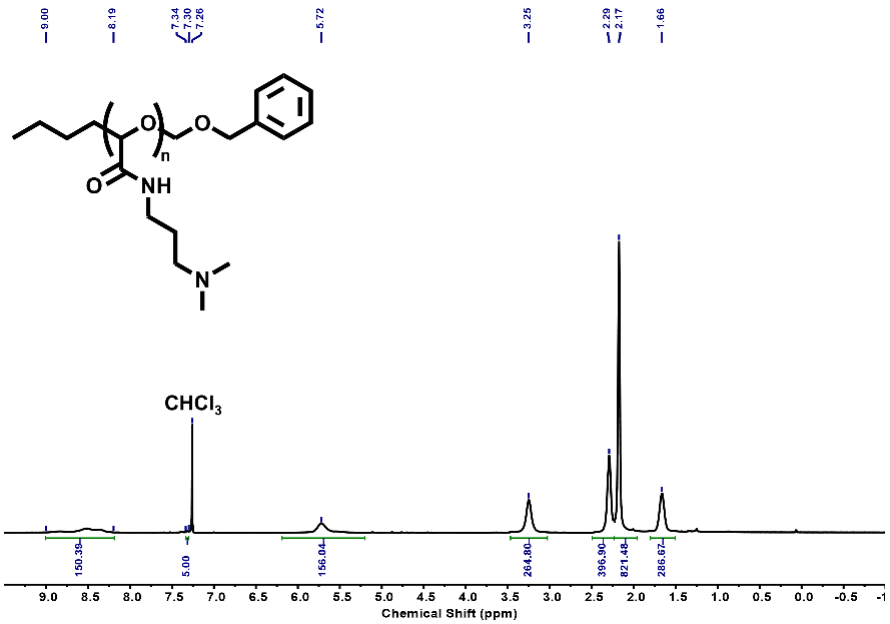

Figure S11. ^{1}H NMR spectrum of **PGAm-DMAPr-BOM** ($CDCl_3$, 400 MHz).

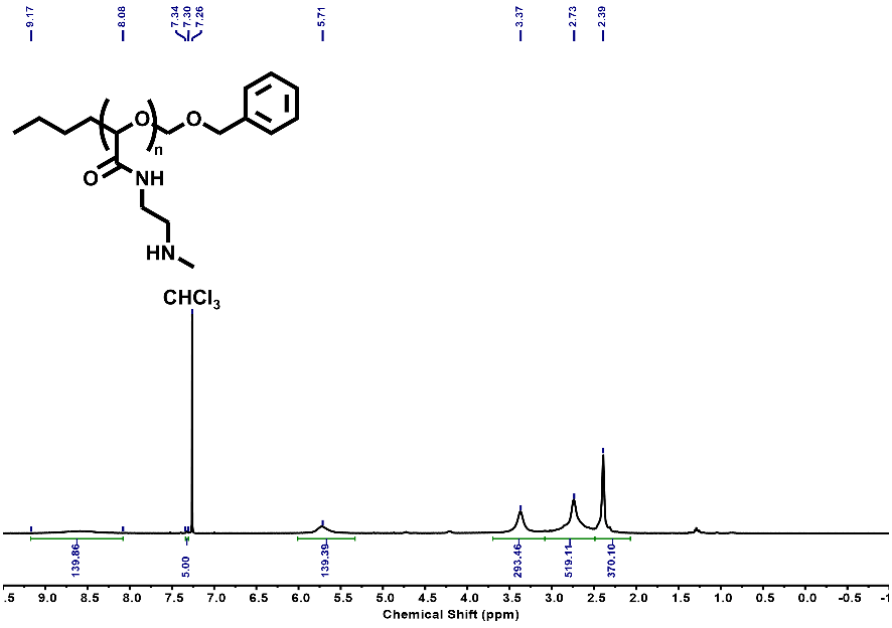

Figure S12. ^{1}H NMR spectrum of **PGAm-MAE-BOM** ($CDCl_3$, 400 MHz). The peak at 2.73 ppm has a higher than expected integration due to an overlapping H_2O peak.

^{13}C NMR Spectra

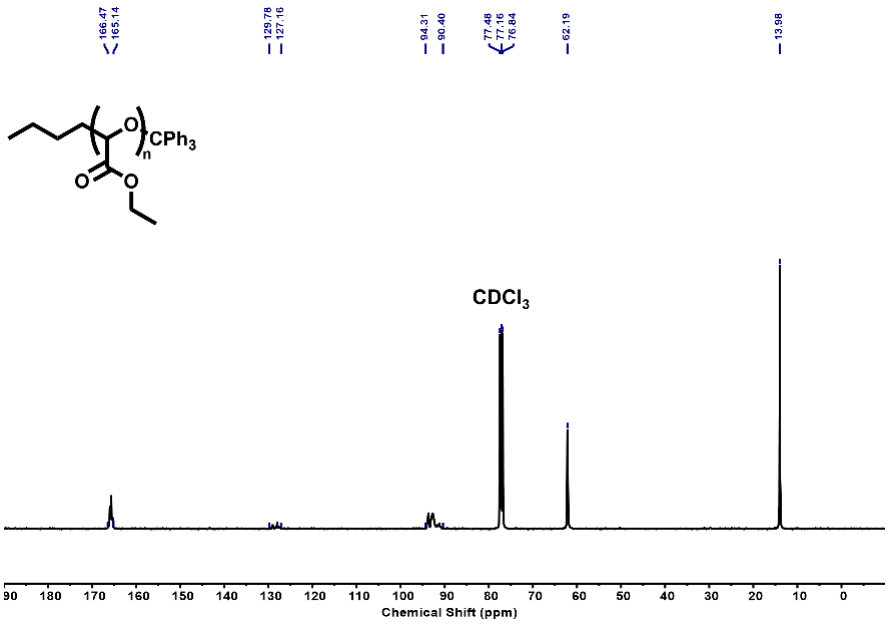

Figure S13. ^{13}C NMR spectrum of **PEtG-Trit** ($CDCl_3$, 100 MHz).

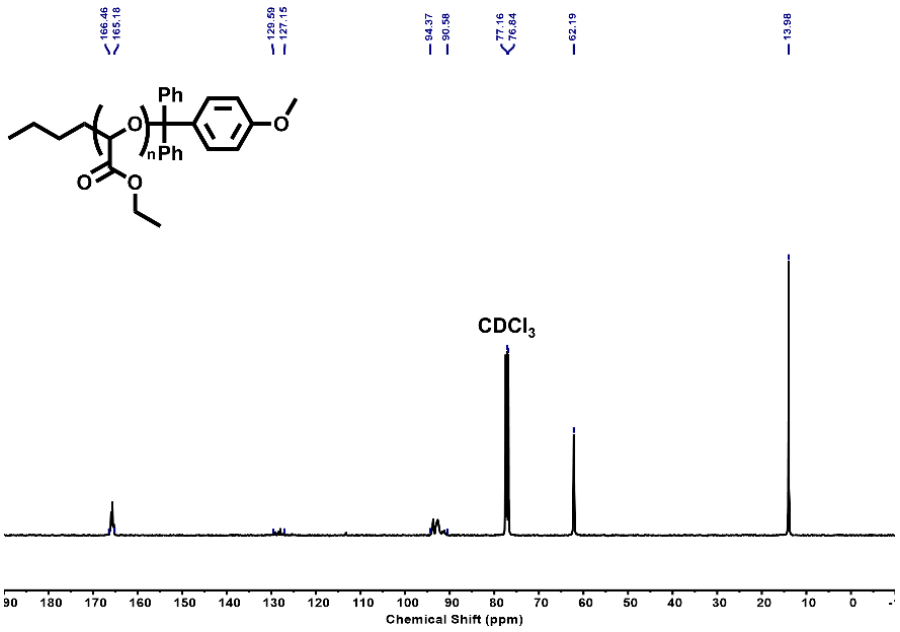

Figure S14. ^{13}C NMR spectrum of **PEtG-MMT** ($CDCl_3$, 100 MHz).

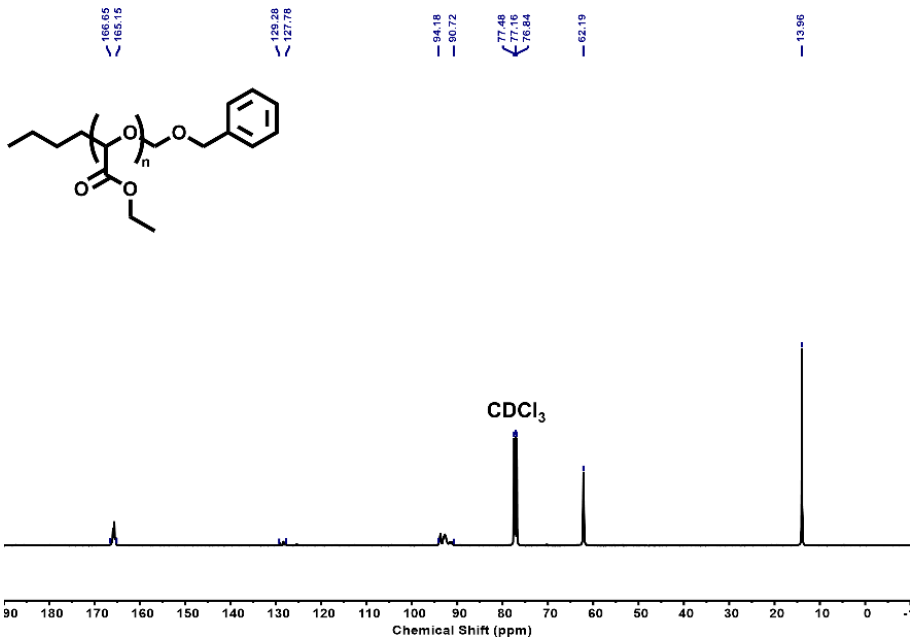

Figure S15. ^{13}C NMR spectrum of **PEtG-BOM** ($CDCl_3$, 100 MHz).

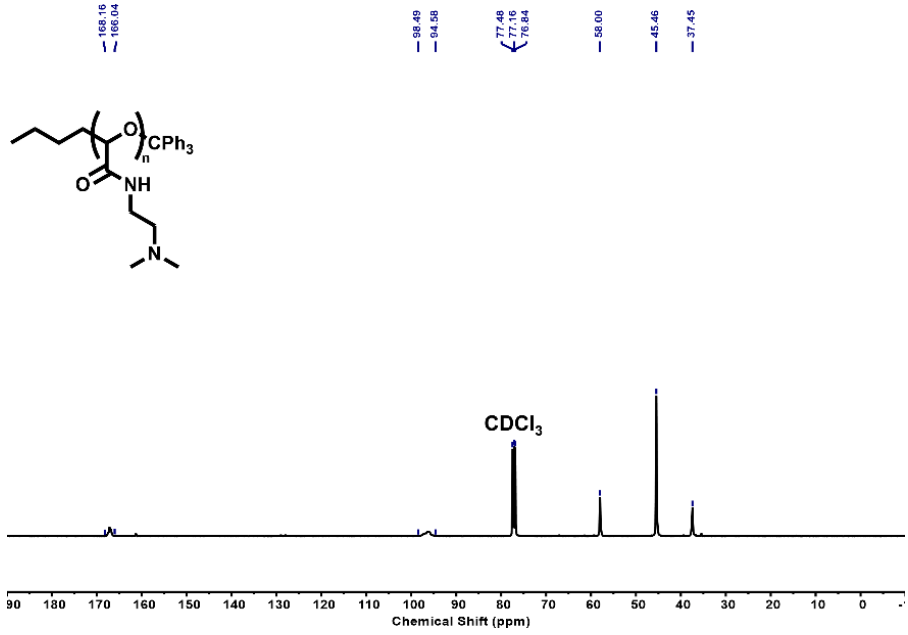

Figure S16. ^{13}C NMR spectrum of **PGAm-DMAE-Trit** ($CDCl_3$, 100 MHz).

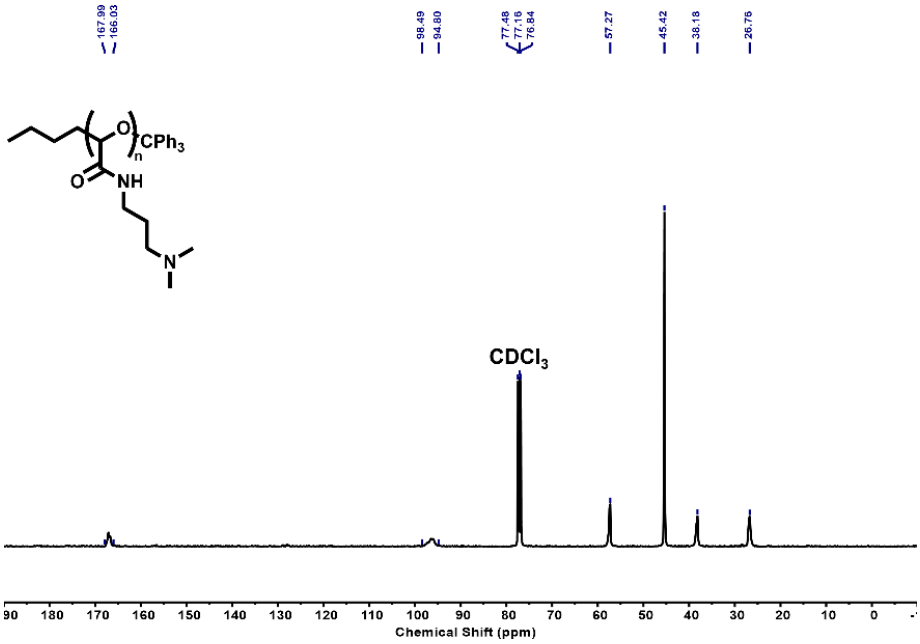

Figure S17. ^{13}C NMR spectrum of **PGAm-DMAPr-Trit** ($CDCl_3$, 100 MHz).

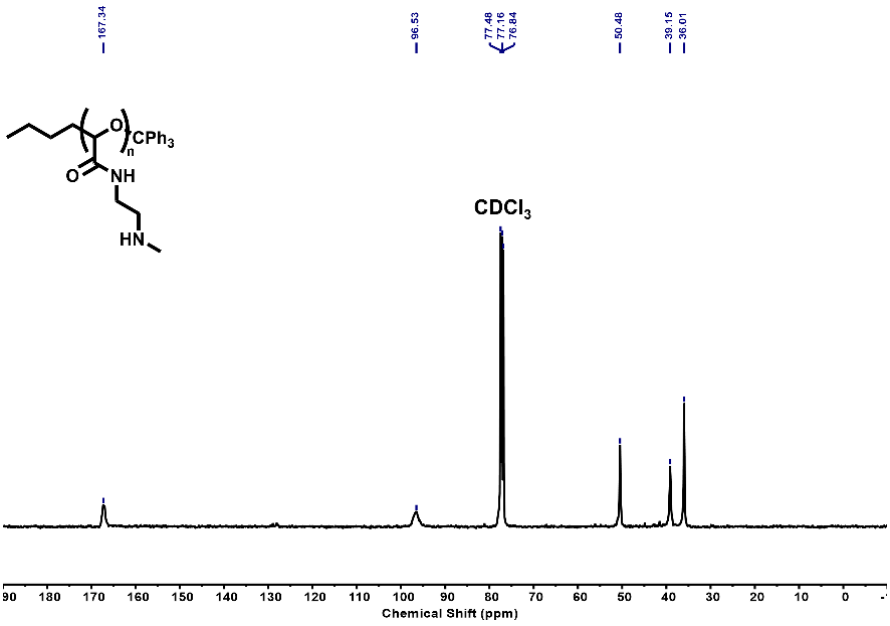

Figure S18. ^{13}C NMR spectrum of **PGAm-MAE-Trit** ($CDCl_3$, 100 MHz).

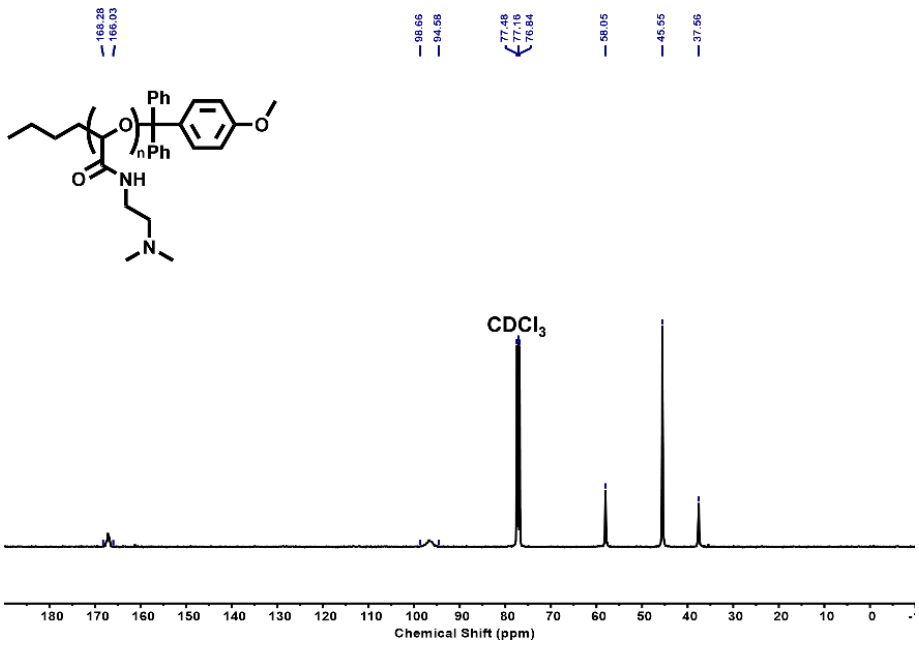

Figure S19. ^{13}C NMR spectrum of **PGAm-DMAE-MMT** ($CDCl_3$, 100 MHz).

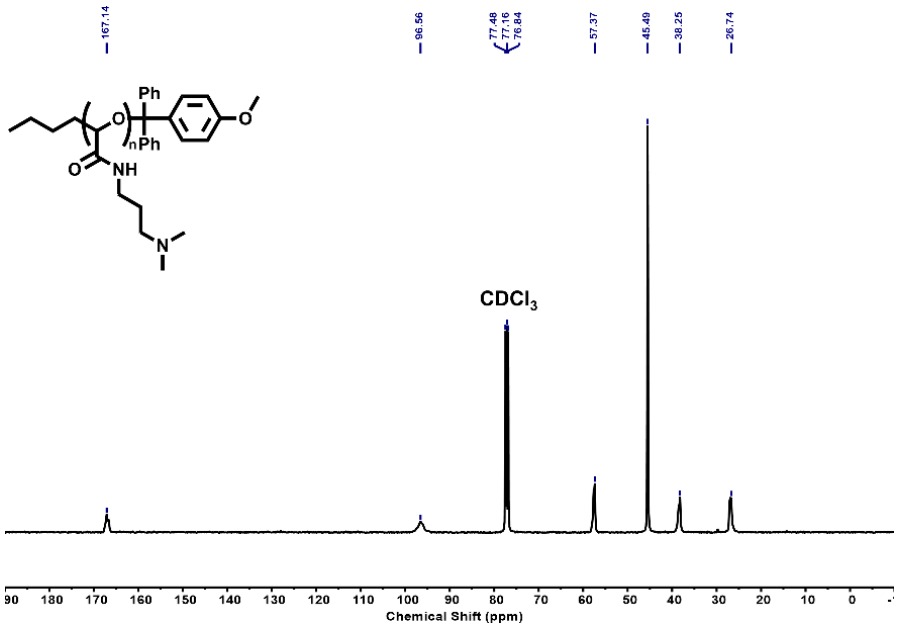

Figure S20. ^{13}C NMR spectrum of **PGAm-DMAPr-MMT** ($CDCl_3$, 100 MHz).

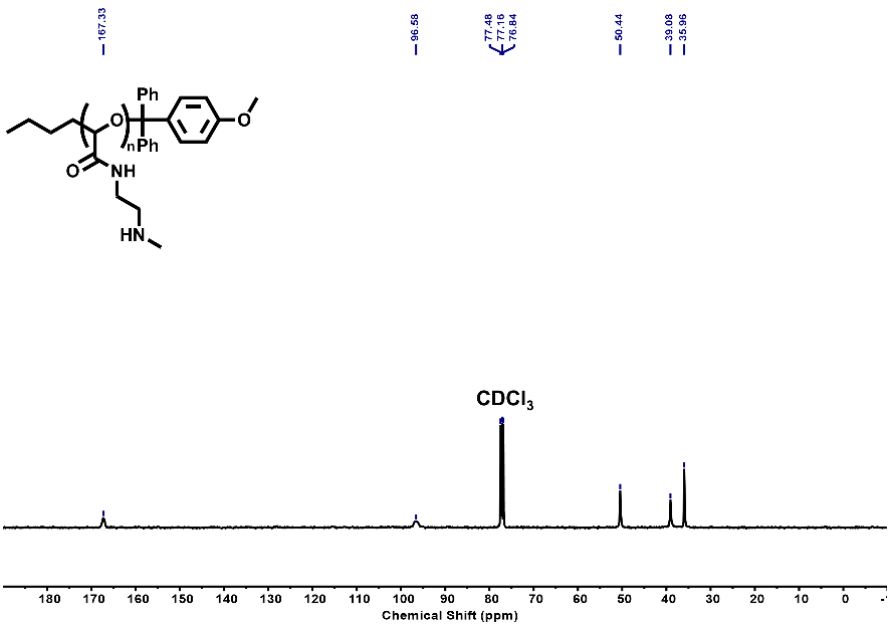

Figure S21. ^{13}C NMR spectrum of **PGAm-MAE-MMT** ($CDCl_3$, 100 MHz).

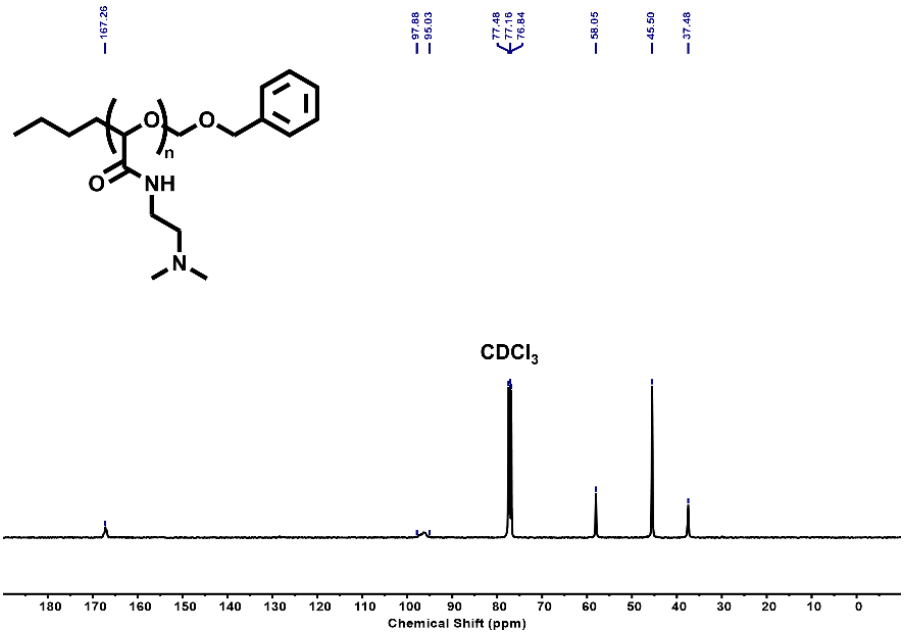

Figure S22. ^{13}C NMR spectrum of **PGAm-DMAE-BOM** ($CDCl_3$, 100 MHz).

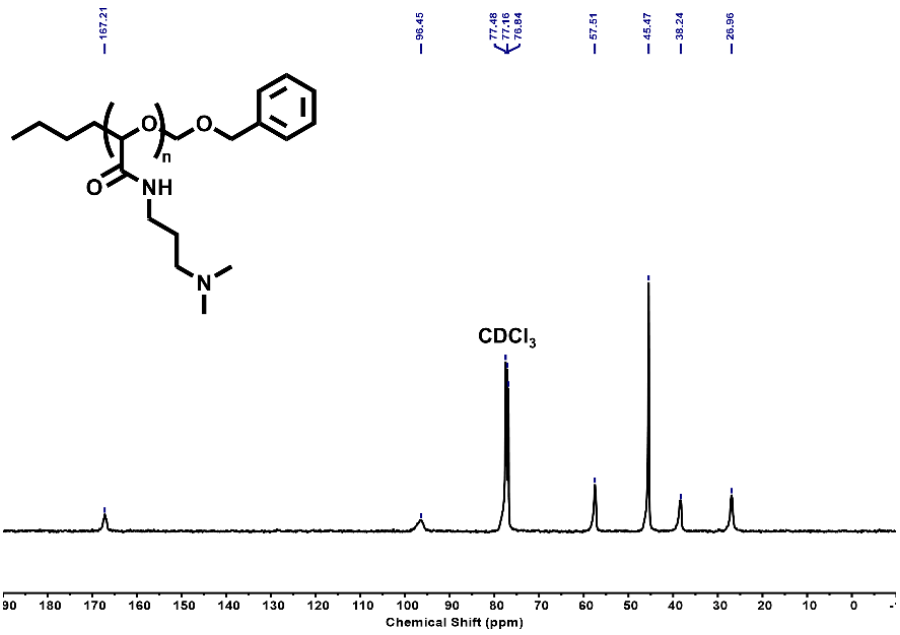

Figure S23. ^{13}C NMR spectrum of **PGAm-DMAPr-BOM** ($CDCl_3$, 100 MHz).

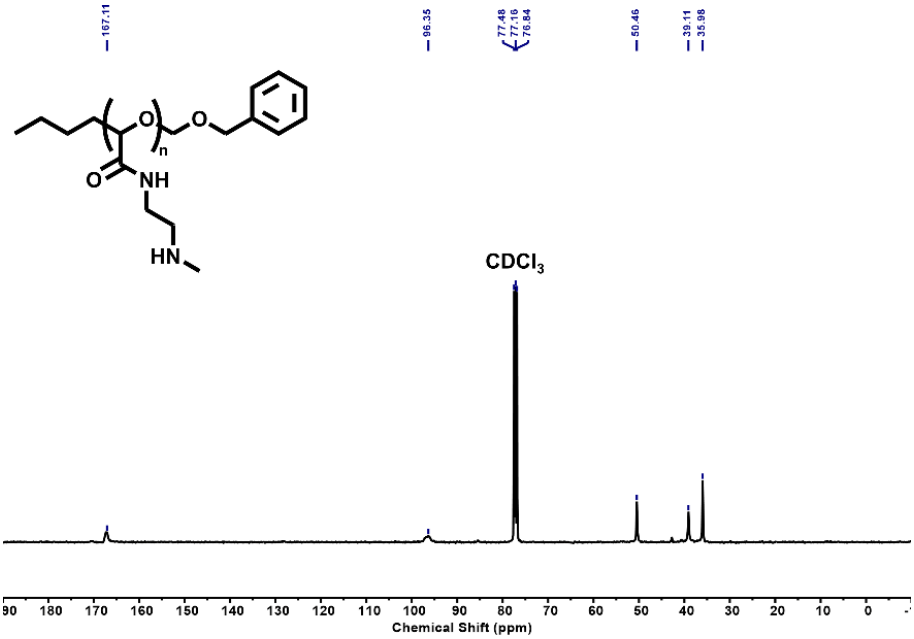

Figure S24. ^{13}C NMR spectrum of **PGAm-MAE-BOM** ($CDCl_3$, 100 MHz).

Size-Exclusion Chromatograms

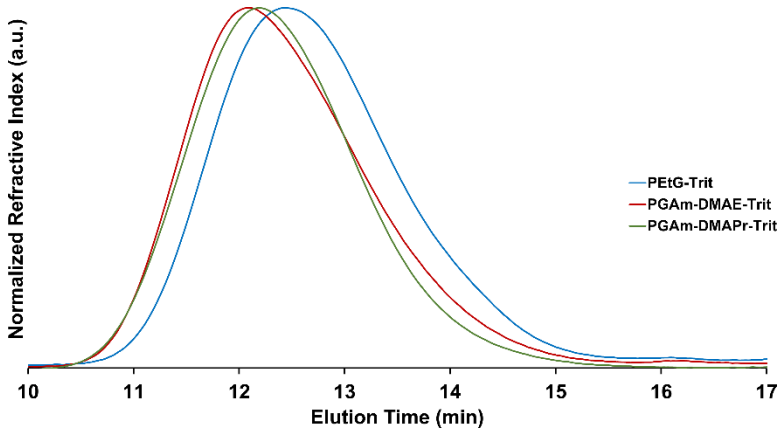

Figure S25. Size-exclusion chromatograms of trityl end-capped polymers in DMF. **PGAm-MAE-Trit** did not elute under these conditions.

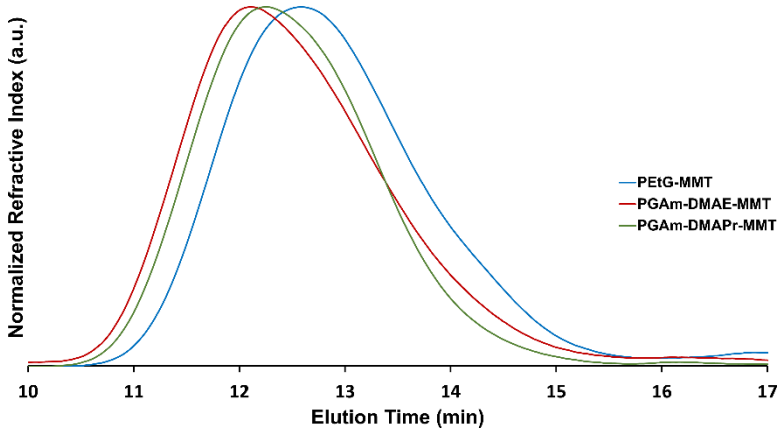

Figure S26. Size-exclusion chromatograms of 4-monomethoxytrityl end-capped polymers in DMF. **PGAm-MAE-MMT** did not elute under these conditions.

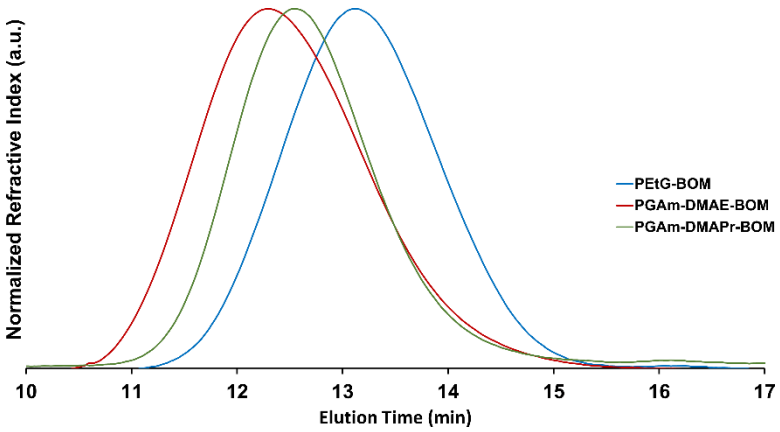

Figure S27. Size-exclusion chromatograms of benzyloxymethyl end-capped polymers in DMF. **PGAm-MAE-BOM** did not elute under these conditions.

Depolymerization Studies

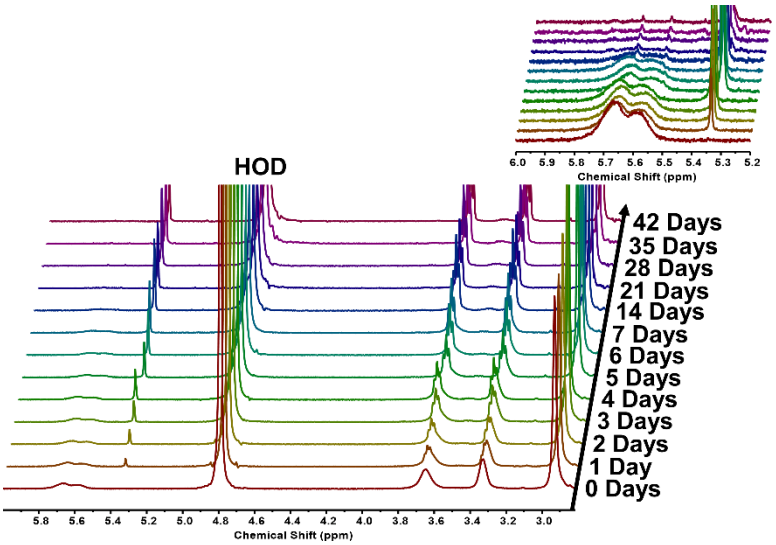

Figure S28. Depolymerization of **PGAm-DMAE-Trit** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (400 MHz). The peaks from the citrate buffer have been cropped off.

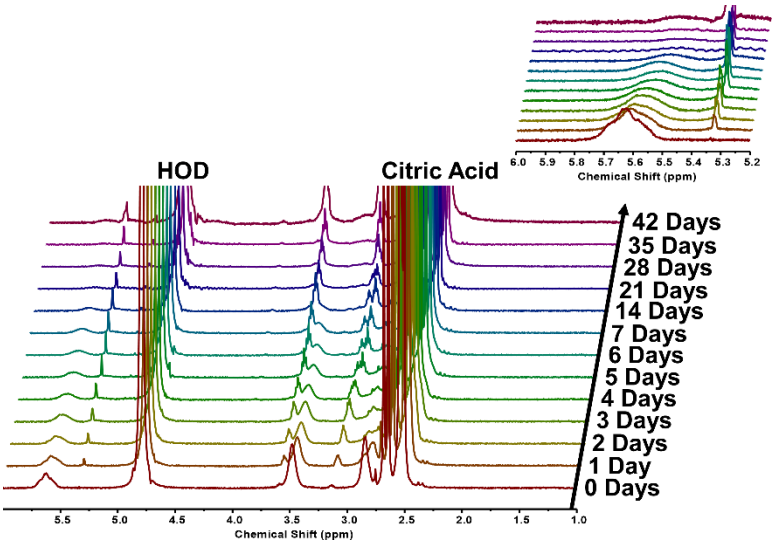

Figure S29. Depolymerization of **PGAm-DMAE-Trit** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (400 MHz).

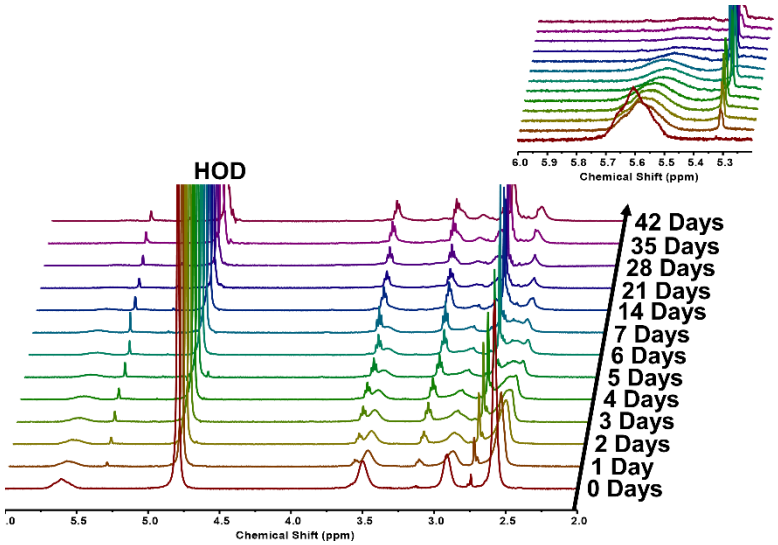

Figure S30. Depolymerization of **PGAm-DMAE-Trit** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (400 MHz).

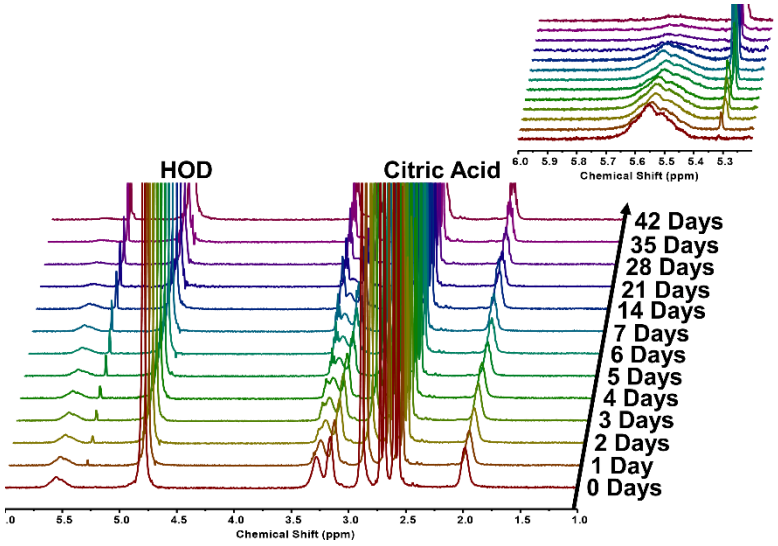

Figure S31. Depolymerization of **PGAm-DMAPr-Trit** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (400 MHz).

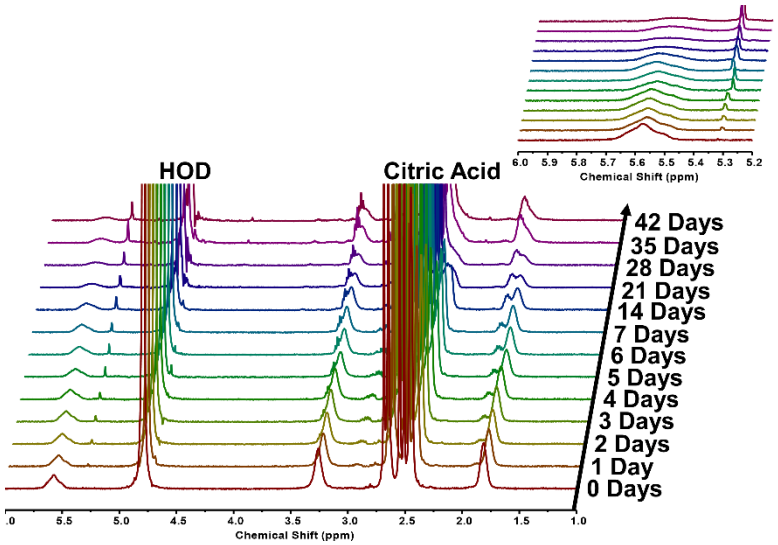

Figure S32. Depolymerization of **PGAm-DMAPr-Trit** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (400 MHz).

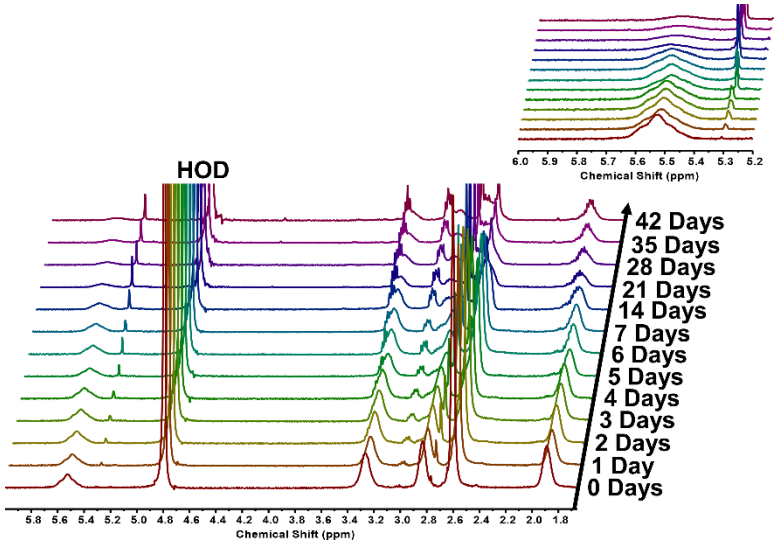

Figure S33. Depolymerization of **PGAm-DMAPr-Trit** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (400 MHz).

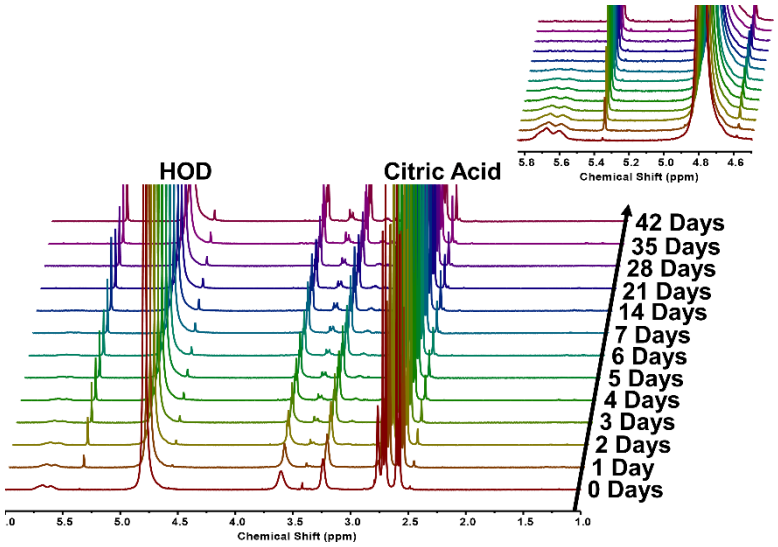

Figure S34. Depolymerization of **PGAm-MAE-Trit** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (600 MHz).

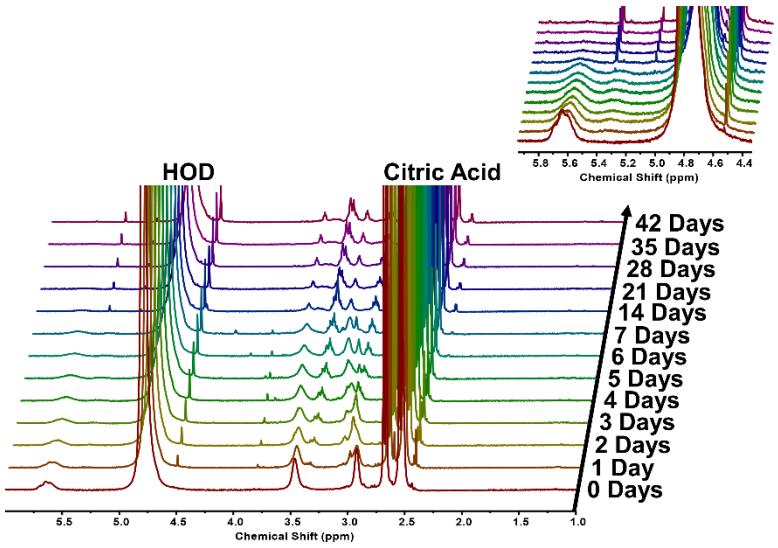

Figure S35. Depolymerization of **PGAm-MAE-Trit** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (600 MHz).

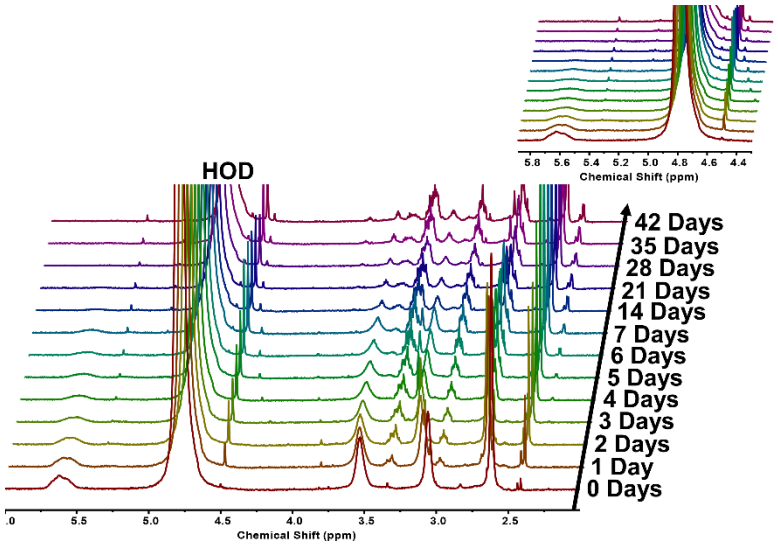

Figure S36. Depolymerization of **PGAm-MAE-Trit** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (600 MHz).

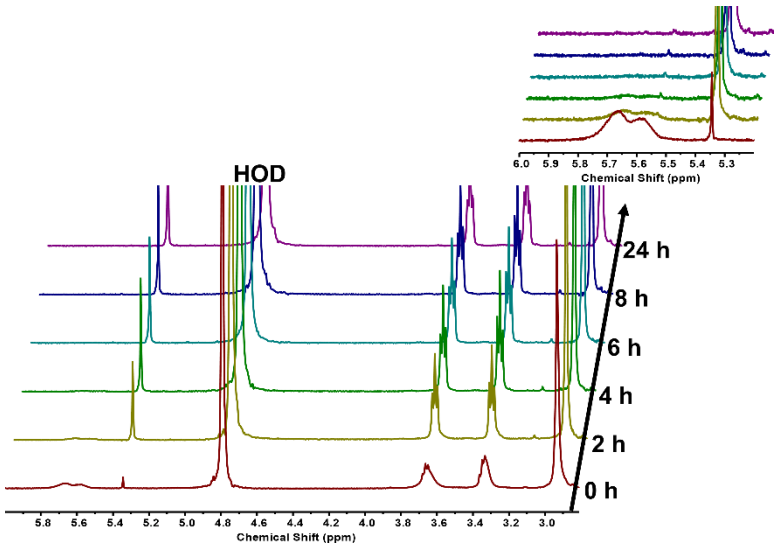

Figure S37. Depolymerization of **PGAm-DMAE-MMT** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (400 MHz). The peaks from the citrate buffer have been cropped off.

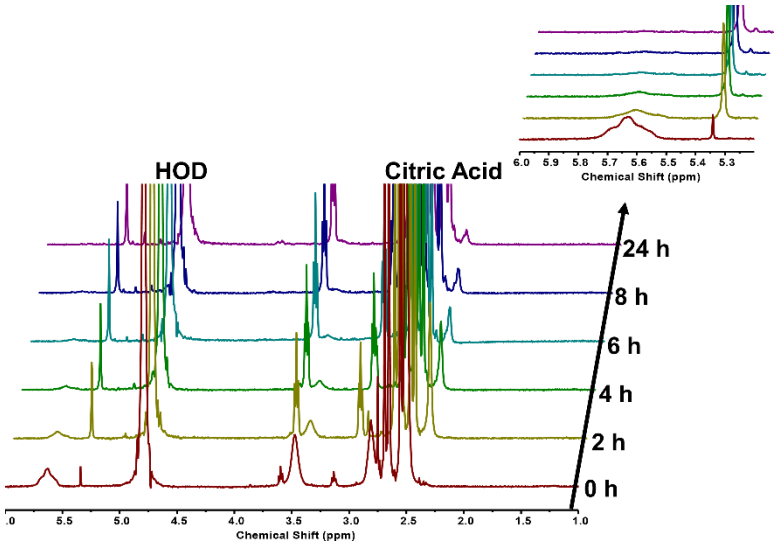

Figure S38. Depolymerization of **PGAm-DMAE-MMT** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (400 MHz).

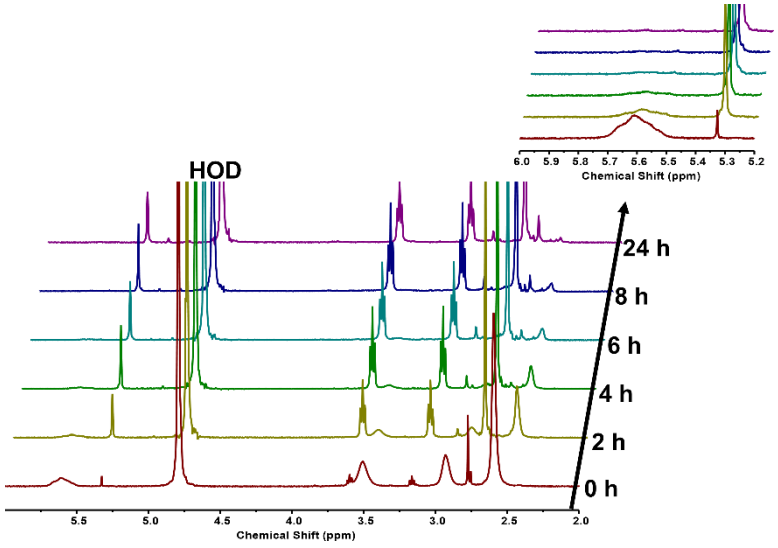

Figure S39. Depolymerization of **PGAm-DMAE-MMT** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (400 MHz).

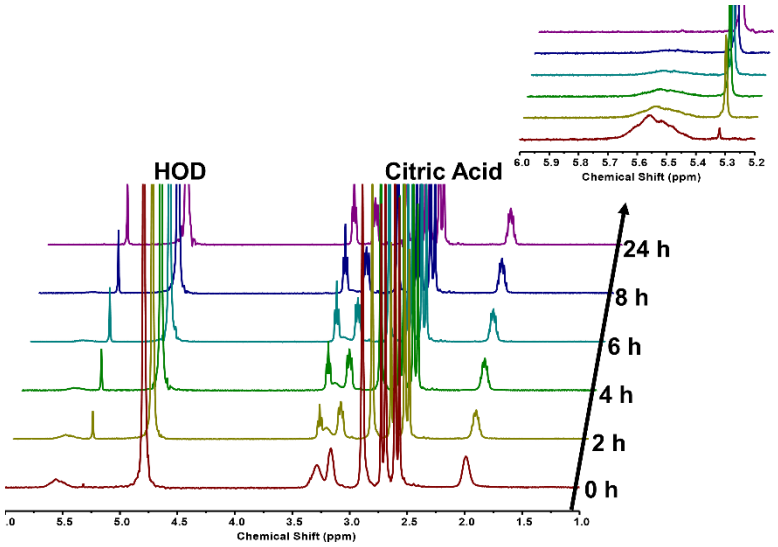

Figure S40. Depolymerization of **PGAm-DMAPr-MMT** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (400 MHz).

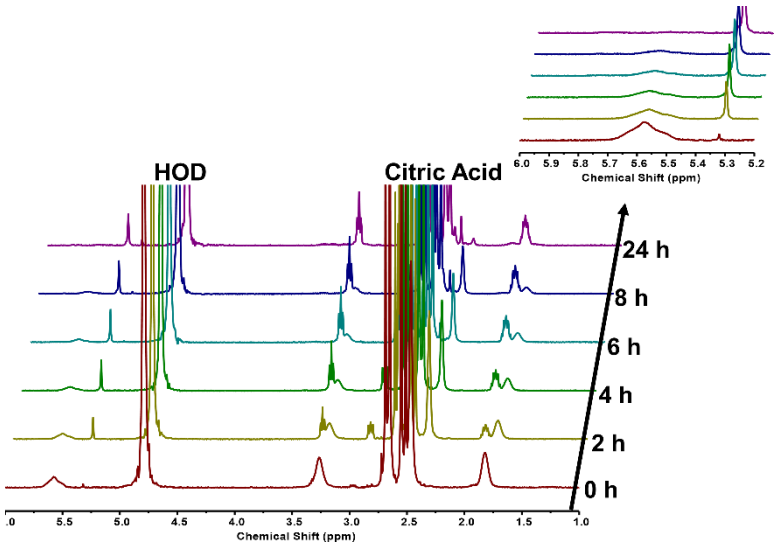

Figure S41. Depolymerization of **PGAm-DMAPr-MMT** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (400 MHz).

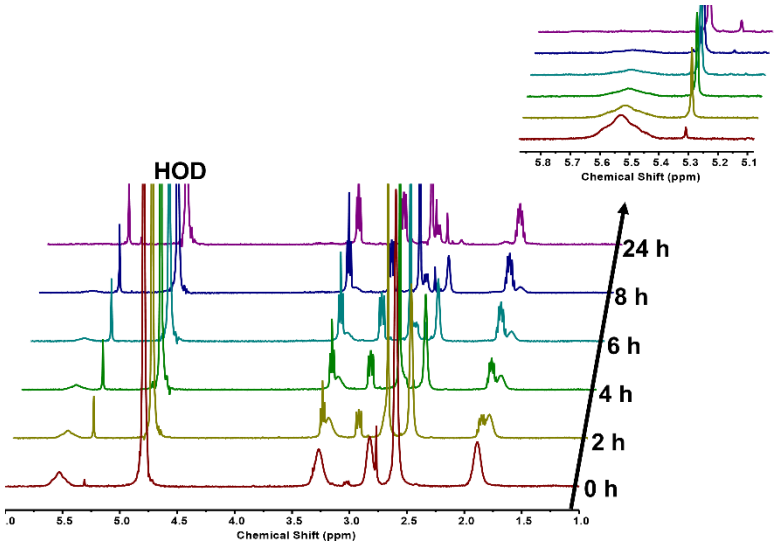

Figure S42. Depolymerization of **PGAm-DMAPr-MMT** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (400 MHz).

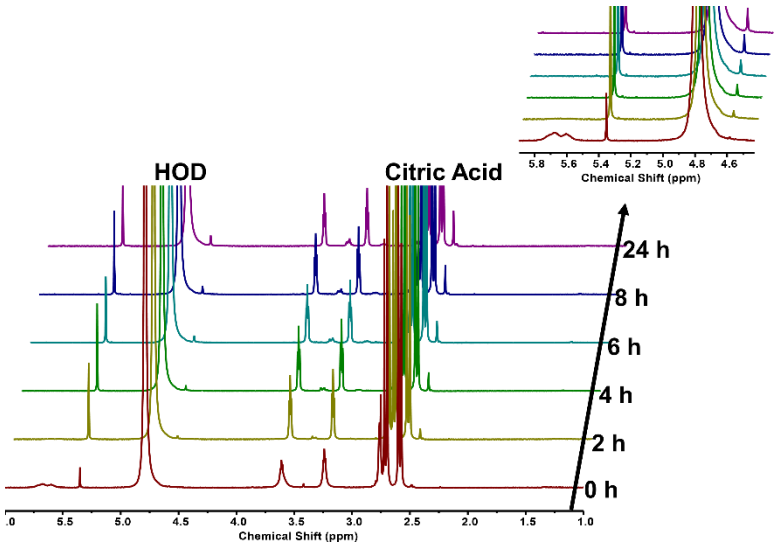

Figure S43. Depolymerization of **PGAm-MAE-MMT** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (600 MHz).

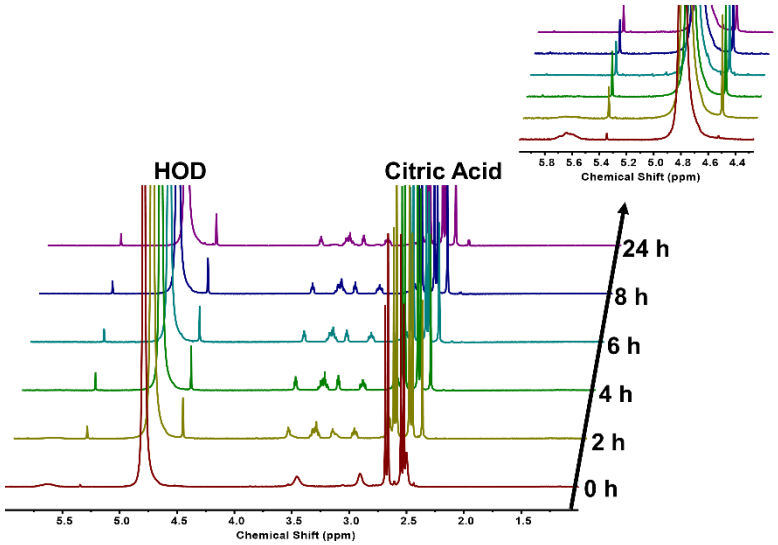

Figure S44. Depolymerization of **PGAm-MAE-MMT** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (600 MHz).

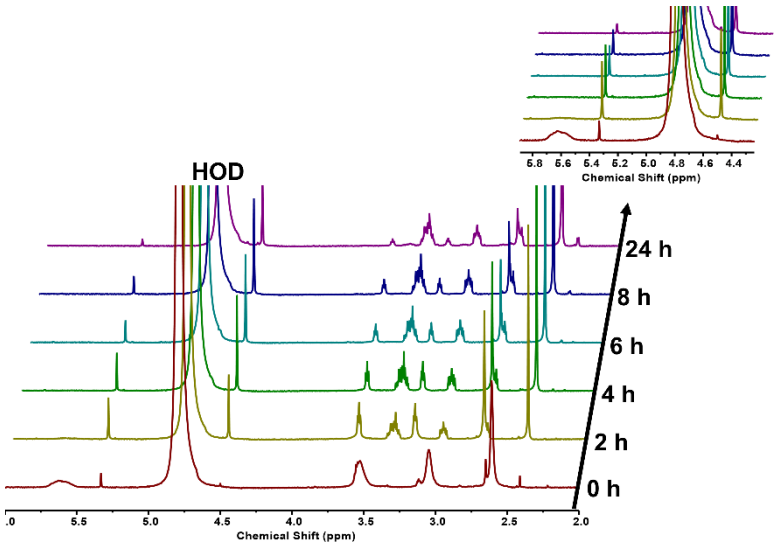

Figure S45. Depolymerization of **PGAm-MAE-MMT** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (600 MHz).

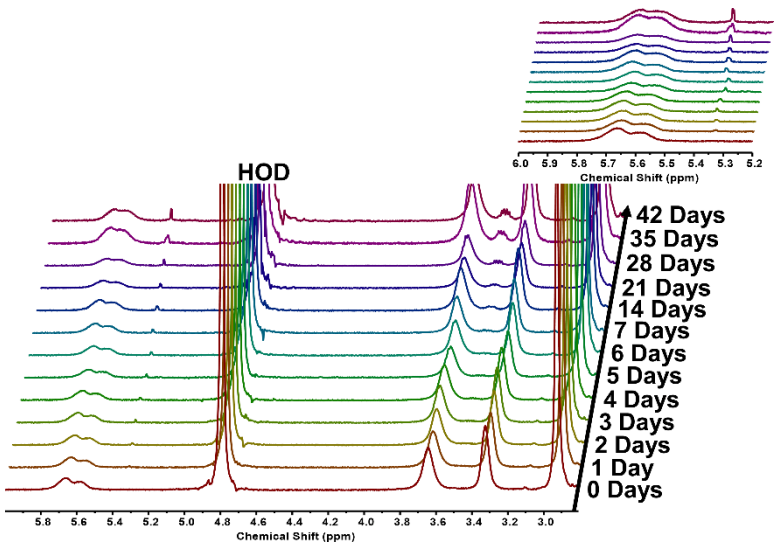

Figure S46. Depolymerization of **PGAm-DMAE-BOM** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (400 MHz). The peaks from the citrate buffer have been cropped off.

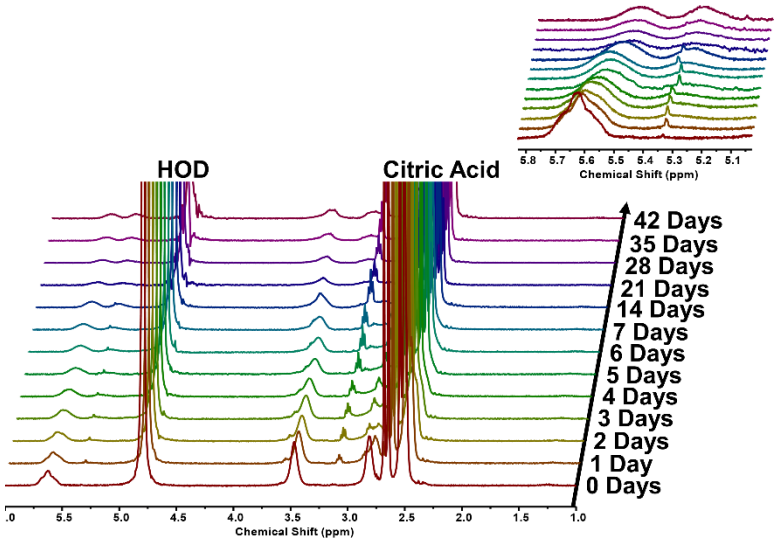

Figure S47. Depolymerization of **PGAm-DMAE-BOM** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (400 MHz).

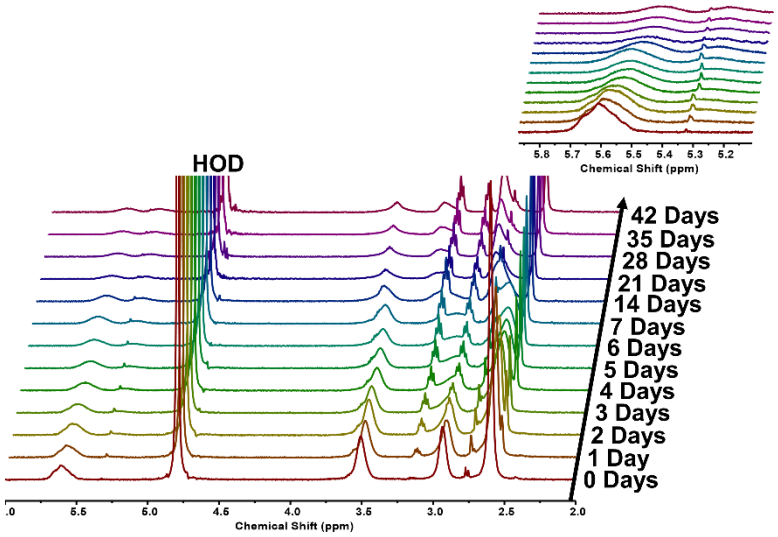

Figure S48. Depolymerization of **PGAm-DMAE-BOM** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (400 MHz).

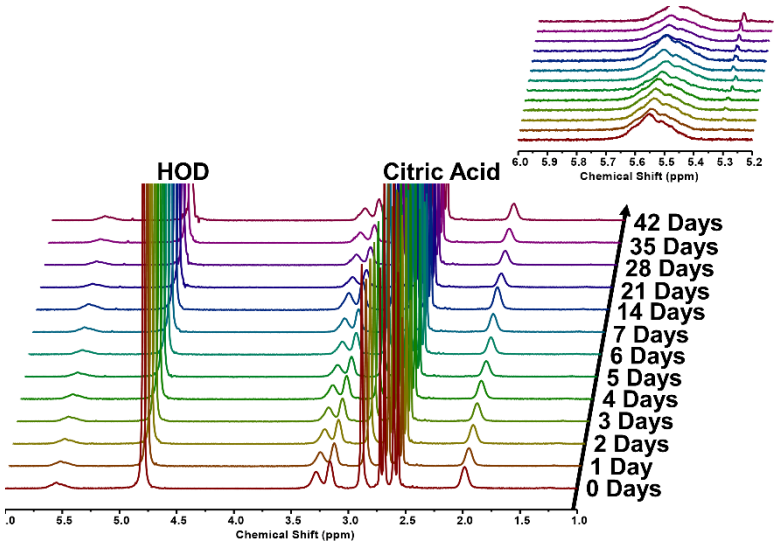

Figure S49. Depolymerization of **PGAm-DMAPr-BOM** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (400 MHz).

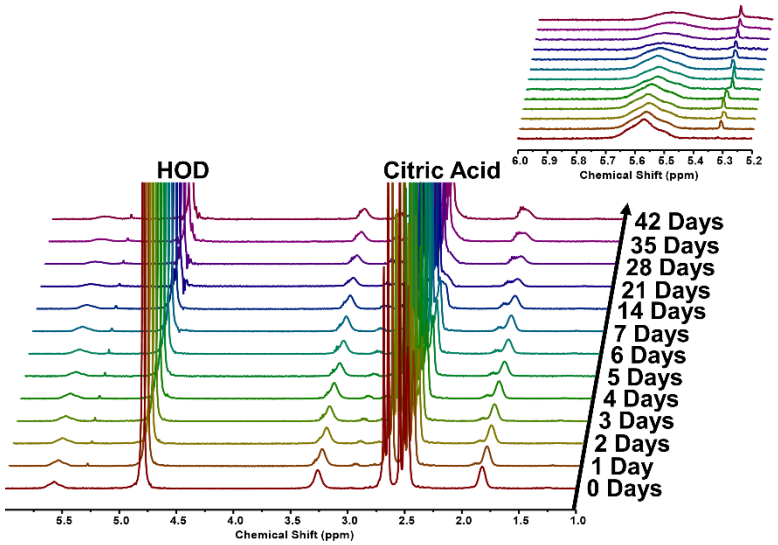

Figure S50. Depolymerization of **PGAm-DMAPr-BOM** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (400 MHz).

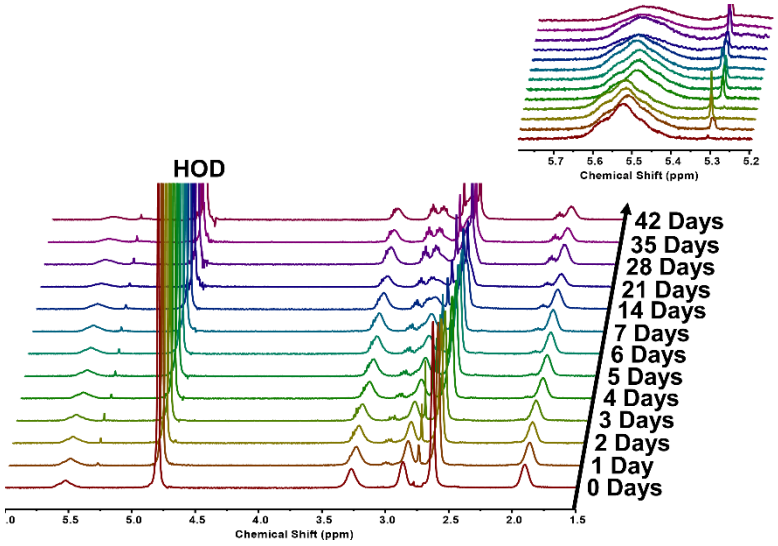

Figure S51. Depolymerization of **PGAm-DMAPr-BOM** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (400 MHz).

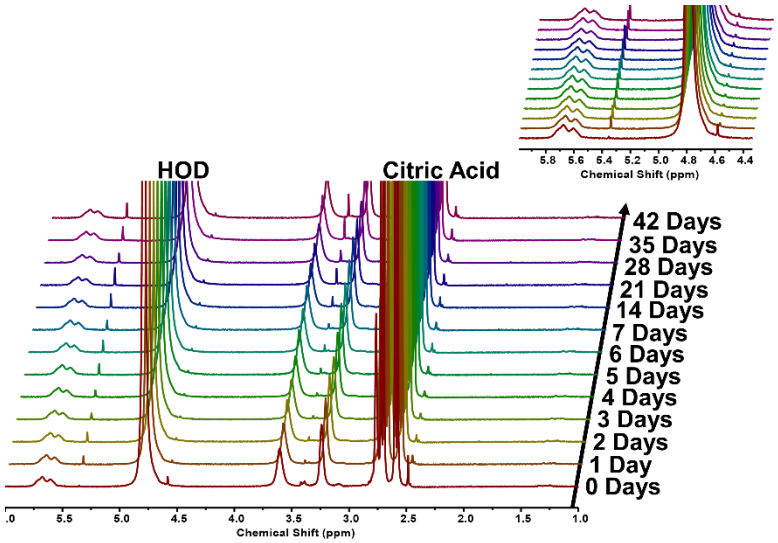

Figure S52. Depolymerization of **PGAm-MAE-BOM** in citrate buffered D_2O (0.2 M, pH = 5.0) monitored by ^1H NMR spectroscopy (600 MHz).

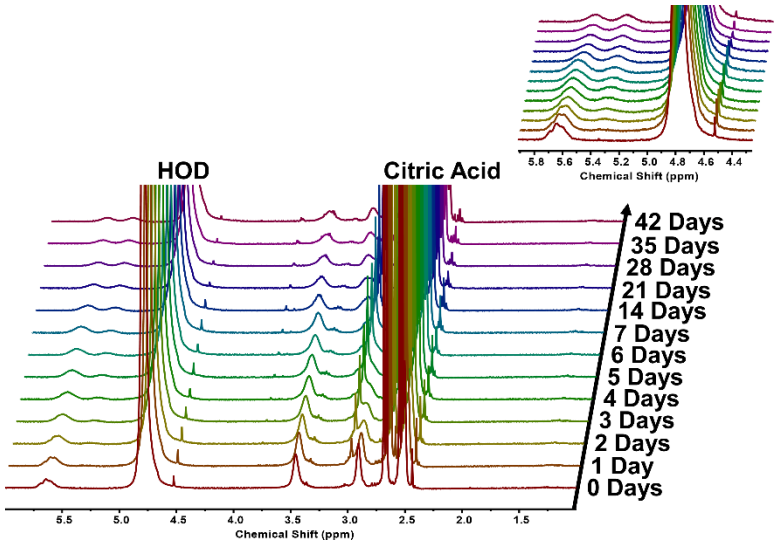

Figure S53. Depolymerization of **PGAm-MAE-BOM** in citrate buffered D_2O (0.2 M, pH = 6.0) monitored by ^1H NMR spectroscopy (600 MHz).

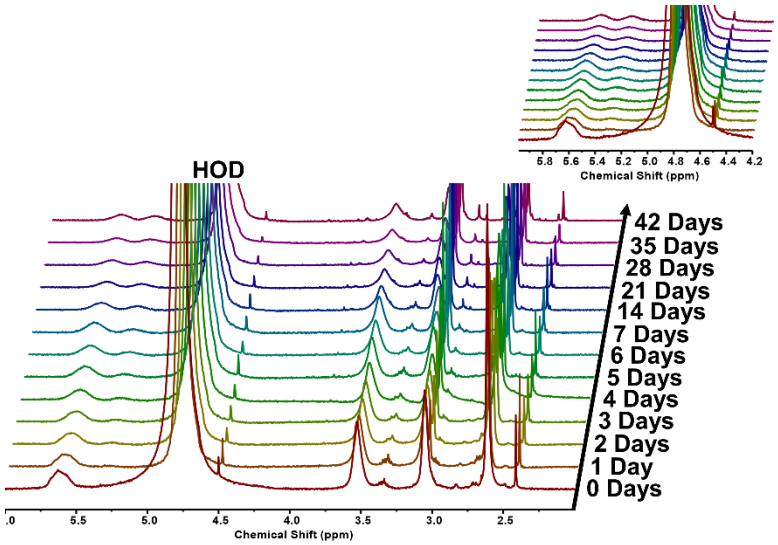

Figure S54. Depolymerization of **PGAm-MAE-BOM** in phosphate buffered D_2O (0.2 M, pH = 7.4) monitored by ^1H NMR spectroscopy (600 MHz).

Complexation Studies

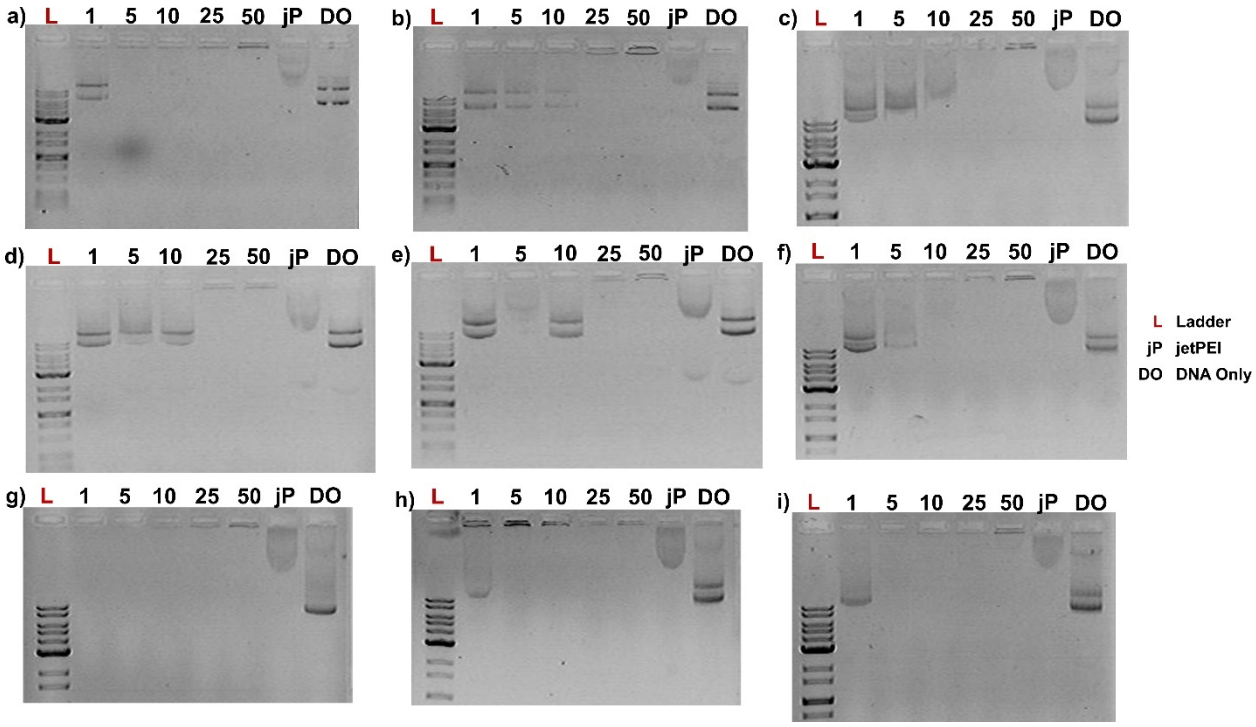

Figure S55. Gel electrophoresis of polyplexes at different N/P ratios (1, 5, 10, 25, 50) along with a commercial transfection agent (jetPEI; N/P = 5) and free pDNA. Polyplexes were prepared in purified water and gel electrophoresis was run after a 15 min incubation time: a) **PGAm-DMAE-Trit**; b) **PGAm-DMAE-MMT**; c) **PGAm-DMAE-BOM**; d) **PGAm-DMAPr-Trit**; e) **PGAm-DMAPr-MMT**; f) **PGAm-DMAPr-BOM**; g) **PGAm-MAE-Trit**; h) **PGAm-MAE-MMT**; i) **PGAm-MAE-BOM**. Images are shown in negative contrast.

Additional TEM images

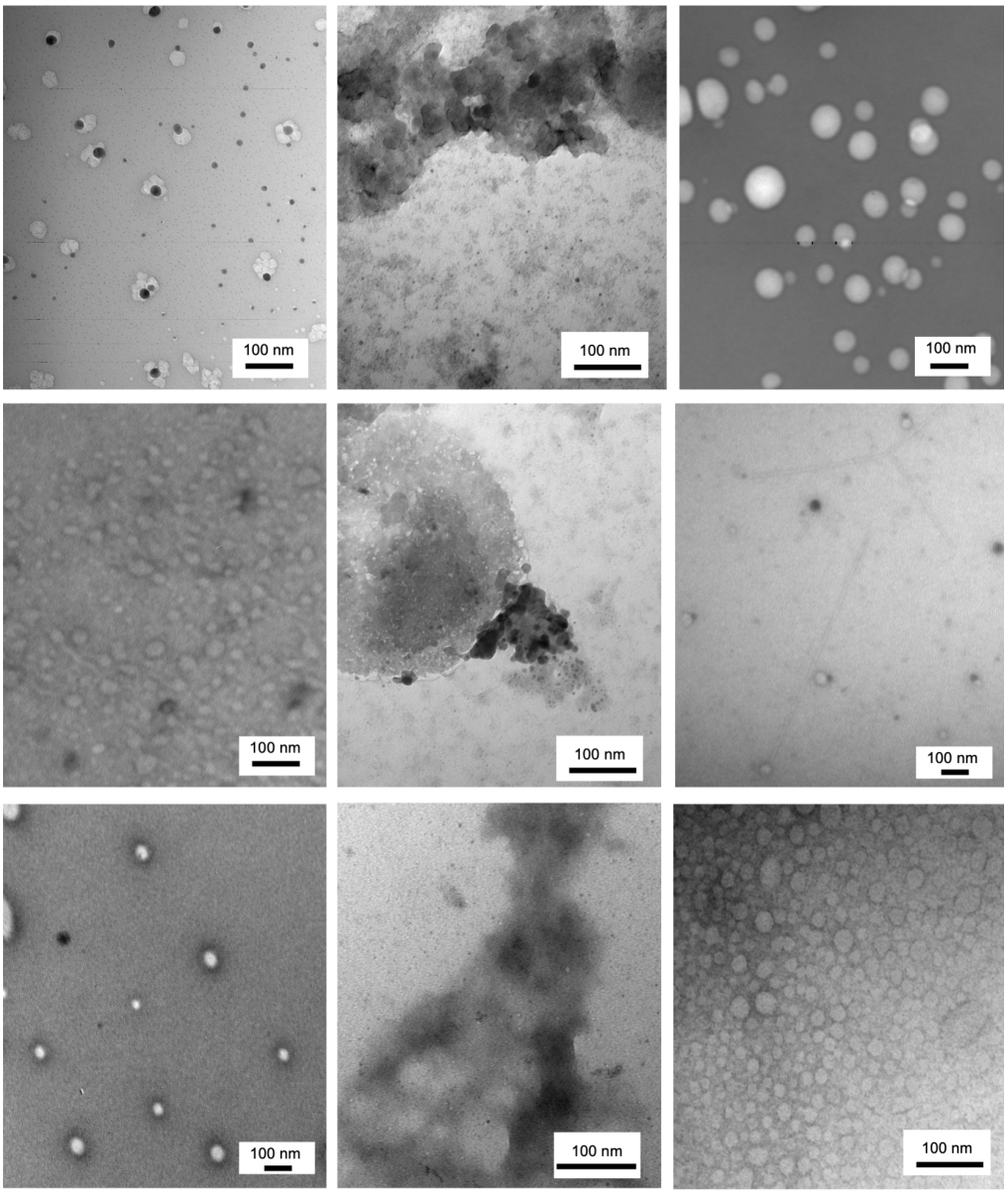

Figure S56. TEM images of plasmid DNA-polycation polyplexes (N/P ratio = 50) after incubation at pH 5 for 24 h. a) **PGAm-DMAE-Trit**; b) **PGAm-DMAE-MMT**; c) **PGAm-DMAE-BOM**; d) **PGAm-DMAPr-Trit**; e) **PGAm-DMAPr-MMT**; f) **PGAm-DMAPr-BOM**; g) **PGAm-MAE-Trit**; h) **PGAm-MAE-MMT**; i) **PGAm-MAE-BOM**. Samples were stained with uranyl acetate.

Additional Cytotoxicity Assays

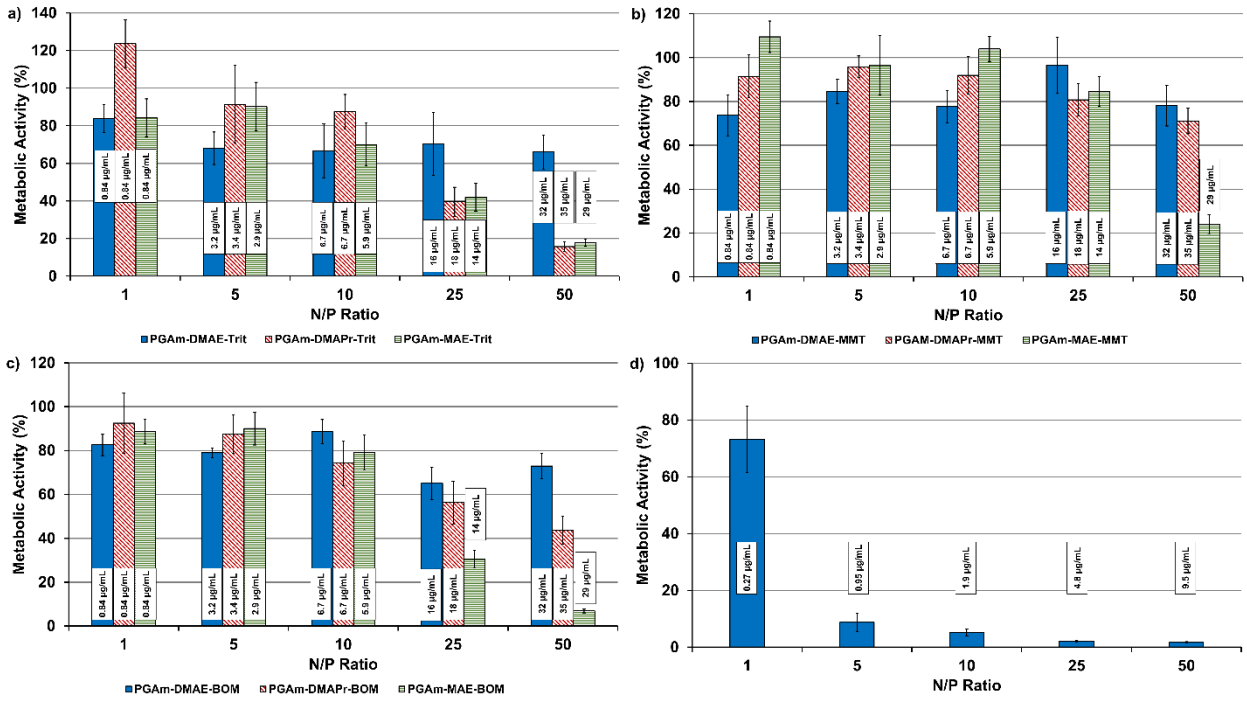

Figure S57. MTT assays of HEK 293T cells treated with varying N/P ratios of DNA-PGAm polyplexes for 48 h (DNA concentration = 1.5 µg/mL): a) Polyplexes composed of PGAms with a trityl end-cap; b) Polyplexes composed of PGAms with a MMT end-cap; c) Polyplexes composed of PGAms with a BOM end-cap; d) Polyplexes composed of jetPEI. The final concentration of the polycation is given for each column. Error bars represent the standard deviation of the replicate (n = 6) measurements.