Supporting Information for:

Designing Polymers for Cartilage Uptake: Effects of Architecture and Molar Mass

Jue Gong,[†] Jordan Nhan,[§] Jean-Philippe St-Pierre,*[§] and Elizabeth R. Gillies*[†‡]

† Department of Chemistry and the Centre for Advanced Materials and Biomaterials Research, The University of Western Ontario, 1151 Richmond St., London, Ontario, Canada, N6A 5B7.

§ Department of Chemical and Biological Engineering, University of Ottawa, 161 Louis-Pasteur Pvt., Ottawa, Ontario, Canada, K1N 6N5.

‡ Department of Chemical and Biochemical Engineering, The University of Western Ontario, 1151 Richmond St., London, Ontario, Canada, N6A 5B9.

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Experimental Procedures

Materials

The CTP RAFT agent was synthesized as previous reported.[1] The rhodamine B *N*-hydroxysuccinimide (NHS)-carbonate was synthesized as previously reported.[2] 2-mercaptoethanol was purchased from EMD Millipore (Billerica, MA, USA). *N*,*N*-Diisopropylethylamine was purchased from Oakwood Chemical (Estill, SC, USA). Chloroform was purchased from VWR Chemicals. Di-*tert*-butyl dicarbonate (99%) and 3-bromo-1-propanol (95%) were purchased from AK Scientific (Union City, CA, USA). Hexanes was purchased from Innova Chemicals (Brampton, ON, Canada). Potassium carbonate, sodium bicarbonate, dichloromethane (DCM), diethyl ether, acetone, dimethylformamide (DMF) and trifluoroacetic acid (TFA) were purchased from Caledon Laboratory Chemicals (Georgetown, ON, Canada). 1,3-Diaminopropane (98%), triethylamine (TEA) (99%), chloroacetyl chloride (98%), potassium iodide (KI) (99%), oxalyl chloride (98%), pentaerythritol (98%) and piperazine were purchased from Alfa Aesar (Tewksbury, MA, USA). Magnesium sulfate, sodium sulfate, tetrahydrofuran (THF) and ethyl acetate were purchased from Thermo Fisher Scientific (Burlington, ON, Canada). THF, DCM and pyridine were distilled freshly before use. The following reagents were purchased from Sigma-Aldrich (Oakville, ON, Canada): 1-amino-2-propanol (93%), methacryloyl chloride (97%), Amberlyst A21 ion exchange resin (99%), 4-(dimethylamino)pyridine (DMAP), *N*,*N*′-dicyclohexylcarbodiimide (DCC), hydrochloric acid (ACS reagent 37%), 2,2-bis(hydroxymethyl)propionic acid (Bis-MPA), *N*,*N*′-disuccinimidyl carbonate, 4,4’-azobis(4-cyanopentanoic acid) (V-501) (≥ 98%), dioxane (≥ 99%), dimethyl sulfoxide-d_6 (99.9 atom% D), chloroform-*d* (99.8 atom% D) and deuterium oxide (99.9 atom% D). Water used for RAFT polymerization reactions was obtained from a Barnstead Easypure II system and registered a

resistance of 15 MΩ or greater. Phosphate buffered saline (PBS) contained 137 mM NaCl, 2.7 mM KCl, and 11.8 mM phosphate buffer (pH 7.4). 2 kDa MWCO Spectra/Por® 6 dialysis membrane was obtained from Spectrum Labs.

Characterization

^{1}H and ^{13}C NMR spectra for characterization were acquired using a 400 MHz Bruker AvIII HD instrument. NMR chemical shifts (δ) are reported in ppm and were calibrated against residual protonated solvent signals of DMSO-d_6 (2.50 ppm), D_2O (4.70 ppm) and $CDCl_3$ (7.28 ppm). Fourier transform infrared (FT-IR) spectra were obtained using a PerkinElmer FT-IR Spectrum Two instrument with attenuated total reflectance (ATR) sampling. UV–visible spectra was obtained using a Cary 300 UV–visible spectrophotometer. Size-exclusion chromatograms (SEC) were obtained in DMF, using an instrument equipped with a Waters 515 HPLC pump, Waters In-Line Degasser AF, two PLgel mixed D 5 μm (300 ×1.5 mm) columns attached 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 and filtered through a 0.2 μm PTFE 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. SEC molar masses of the samples were calculated based on PMMA standards. Hydrodynamic radii and zeta potential were measured at 1 mg/mL of polymer in 1.5 g/L sodium bicarbonate buffer (pH 7.4, same buffer as in the media used for the cartilage studies) using a Malvern Zetasizer Nano ZS instrument equipped with a 633 nm laser at a scattering angle of 173 °.

Synthesis of Monomers and the 4-Arm and 8-Arm Chain Transfer Agents (CTAs)

Synthesis of *N*-(2-hydroxypropyl) methacrylamide (HPMA)

A mixture of 1-amino-2-propanol (7.25 mL, 95.0 mmol) and potassium carbonate (14.5 g, 105 mmol) in 60 mL of dry CH_2Cl_2 was cooled to -10 °C. A solution of methacryloyl chloride (9.25 mL, 95.0 mmol) in 10 mL of dry CH_2Cl_2 was then added dropwise under vigorous stirring at -10 °C. After the addition was complete, the reaction was stirred at room temperature overnight. Next, potassium carbonate was filtered off and the reaction mixture was dried over magnesium sulfate. The solvent was removed by rotary evaporation, leaving a white solid, which was purified by recrystallization from acetone. Yield: 42%. ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ = 7.80 (s, 1H), 5.66 (s, 1H), 5.32 (s, 1H), 4.68 (s, 1H), 3.65-3.75 (m, 1H), 3.00-3.10 (m, 2H), 1.85 (s, 3H), 1.01 (d, J=6.2 Hz, 3H). Spectral data agreed with those previously reported.[3]

Synthesis of *N*-(3-aminopropyl)carbamic acid *tert*-butyl ester

A solution of di-*tert*-butyl dicarbonate (5.00 g, 22.9 mmol) in $CHCl_3$ (40 mL) was added dropwise to a solution of 1,3-diaminopropane (17.2 mL, 206 mmol) in $CHCl_3$ (100 mL) at 0 °C. Once the addition was complete, the reaction was stirred at room temperature overnight. $CHCl_3$ was then evaporated, and 100 mL of water was added to the oily product. The insoluble bis-substituted diamine was filtered off. The filtrate was then extracted with 100 mL of CH_2Cl_2 three times. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and the filtrate was evaporated to yield a colorless, oily product. Yield: 78%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 4.93 (s, 1H), 3.15-3.26 (m, 2H), 2.74-2.82 (m, 2H), 1.58-1.67 (m, 2H), 1.45 (s, 9H), 1.31 (s, 2H). Spectral data agreed with those previously reported.[4]

Synthesis of *tert*-butyl (3-methacrylamidopropyl)carbamate (Boc-APMA)

N-(3-aminopropyl)carbamic acid *tert*-butyl ester (3.10 g, 17.8 mmol) and triethylamine (7.19 mL, 51.6 mmol) were dissolved in $CHCl_3$ (40 mL). A solution of methacryloyl chloride (1.84 mL, 18.9 mmol) in $CHCl_3$ (20 mL) was then added dropwise to the reaction mixture under vigorous stirring

at 0 °C. Once the addition was complete, the mixture was stirred for another 2 h at room temperature. The organic layer was then extracted with 30 mL of water five times, dried over anhydrous sodium sulfate, filtered, and the solvent was removed using a rotary evaporator. The crude product was recrystallized from 3/5 diethyl ether/hexane (v/v), yielding a white solid. Yield: 56%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 6.70 (s, 1H), 5.78 (s, 1H), 5.36 (s, 1H), 4.89 (s, 1H), 3.36-3.43 (m, 2H), 3.18-3.25 (m, 2H), 2.01 (s, 3H), 1.63-1.72 (m, 2H), 1.47 (s, 9H). Spectral data agreed with those previously reported.[5]

Synthesis of the 4-Arm Chain Transfer Agent (4-arm CTA)

Synthesis of Compound 1

Pentaerythritol (9.99 g, 73.4 mmol) was refluxed with chloroacetyl chloride (34.9 mL, 440 mmol) for 3 h. The excess of acid chloride was removed *in vacuo* in the fume hood after the reaction and then the crude product was purified by washing with diethyl ether 3 times to yield a white solid. Yield: 90%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 4.31 (s, 8H), 4.12 (s, 8H). ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$, ppm): δ = 166.7, 63.3, 42.6, 40.5. FT-IR: 3054-3688, 2886-3043, 1749 cm^{-1}. HRMS (ESI): calcd. for $[C_{13}H_{16}Cl_4NaO_8]^+$: 464.9467; found $[M+Na]^+$: 464.9458.

Synthesis of Compound 2

Compound **1** (0.508 g, 1.15 mmol), 2-mercaptoethanol (0.486 mL, 6.90 mmol), Amberlyst A21 (0.956 g, 3.45 mmol) and potassium iodide (0.0382 g, 0.230 mmol) were combined in THF (30 mL). The reaction mixture was stirred for 24 h at 60 °C. It was then cooled, concentrated *in vacuo*, and precipitated in 2/1 diethyl ether/hexane to yield a colorless oil. Yield: 33%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 4.27 (s, 8H), 3.82 (t, J = 5.8 Hz, 8H), 3.36 (s, 8H), 2.85 (t, J = 5.8 Hz, 8H). ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$, ppm): δ = 170.6, 62.6, 61.1, 42.9, 36.1, 33.8. FT-IR: 3060-

3649, 2764-3044, 1730 cm^{-1}. HRMS (ESI): calcd. for $[C_{21}H_{36}NaO_{12}S_4]^+$: 631.0987; found $[M+Na]^+$: 631.0994.

Synthesis of 4-arm CTA

CTP RAFT agent (275 mg, 0.980 mmol), Compound **2** (100 mg, 0.160 mmol) and DMAP (12.0 mg, 0.0980 mmol) were dissolved in dry CH_2Cl_2 (15 mL). The solution was stirred for 10 min at room temperature and then DCC (203 mg, 0.980 mmol) was added at 0 °C. The reaction mixture was stirred for 24 h at room temperature. The insoluble solids were removed by filtration. The organic layer was extracted with 1 M HCl (30 mL), saturated aqueous Na_2CO_3 (30 mL) and then brine (30 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and then the filtrate was evaporated *in vacuo*. The crude product was purified by silica column using 1/1 hexane/ethyl acetate to yield a red oil. Yield: 57%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 7.90-7.95 (m, 8H), 7.56-7.62 (m, 4H), 7.38-7.46 (m, 8H), 4.32 (t, *J* = 6.5 Hz, 8H), 4.25 (s, 8H), 3.32 (s, 8H), 2.90 (t, *J* = 6.5 Hz, 8H), 2.46-2.72 (m, 16H), 1.96 (s, 12H). ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$, ppm): δ = 222.3, 171.3, 169.6, 144.5, 133.1, 128.6, 126.7, 118.5, 63.2, 62.7, 45.8, 42.7, 33.4, 33.3, 31.1, 29.7, 24.2. FTIR: 2805-3046, 1732 $cm^{-1.}$ HRMS (ESI): calcd. for $[C_{73}H_{80}N_4NaO_{16}S_{12}]^+$: 1675.2116; found $[M+Na]^+$: 1675.1850.

Synthesis of the 8-Arm Chain Transfer Agent (8-arm CTA)

Synthesis of Compound 3

Bis-MPA (2.00 g, 14.9 mmol), NEt_3 (5.20 mL, 37.3 mmol), and DMAP (0.0910 g, 0.745 mmol) were dissolved in dry CH_2Cl_2 (50 mL). Then, chloroacetyl chloride (2.49 mL, 31.3 mmol) was added dropwise to the reaction mixture. Once the addition was complete, the reaction mixture was stirred for another 30 min at room temperature, and then the solvent was evaporated *in vacuo*. The crude mixture was purified by silica gel column using pure hexanes gradually increasing to 2/3 hexanes/ethyl acetate to yield a yellow oil. Yield: 83%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 4.34-4.48 (m, 4H), 4.11 (s, 4H), 1.37 (s, 3H). ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$, ppm): δ = 178.1,

166.8, 66.2, 46.2, 40.6, 17.7. FTIR: 3088-3516, 2904-3113, 1740 cm^{-1}. HRMS (ESI): calcd. for $[C_{18}H_{25}Cl_4O_{12}]^+$: 573.0095; found $[2M+H]^+$: 572.9626.

Synthesis of Compound 4

Oxalyl chloride (0.652 mL, 7.60 mmol) was added dropwise to a solution of Compound **3** (1.09 g, 3.80 mmol) in dry CH_2Cl_2 (20 mL) containing 3 drops of DMF. The reaction mixture was stirred for 2 h at room temperature. The solvent and excess oxalyl chloride were then evaporated *in vacuo* and the light yellow oil was obtained. Yield: 93%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 4.39-4.52 (m, 4H), 4.11 (s, 4H), 1.45 (s, 3H). $^{13}C\{^1H\}$ NMR (400 MHz, $CDCl_3$, ppm): δ = 174.6, 166.5, 66.0, 55.7, 40.4, 17.9. FTIR: 2845-3085, 1750 cm^{-1}.

Synthesis of Compound 5

A solution of Compound **4** (1.79 g, 5.87 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise into a solution of pentaerythritol (159 mg, 1.17 mmol), NEt_3 (816 µL, 5.87 mmol), and DMAP (71.8 mg, 0.587 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C under N_2. The reaction mixture was stirred for another 15 h at room temperature and then the solvent was evaporated *in vacuo*. The crude mixture was purified by silica gel chromatography using pure hexanes gradually increasing to 7/3 hexanes/ethyl acetate to yield a colorless oil. Yield: 65%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 4.34-4.44 (m, 16H), 4.21 (s, 8H), 4.14 (s, 8H), 1.34 (s, 12H). $^{13}C\{^1H\}$ NMR (400 MHz, $CDCl_3$, ppm): δ = 176.3, 166.9, 66.5, 62.0, 46.7, 43.3, 40.6, 17.8. FTIR: 2855-3059, 1733 cm^{-1}. HRMS (ESI): calcd. for $[C_{41}H_{52}Cl_8NaO_{24}]^+$: 1235.0195; found $[M+Na]^+$: 1234.9901.

Synthesis of Compound 6

Amberlyst A21 (349 mg, 1.26 mmol), 2-mercaptoethanol (0.176 mL, 2.50 mmol) and potassium iodide (14.9 mg, 0.0900 mmol) were added to a solution of Compound **5** (218 mg, 0.180 mmol)

in dry THF (25 mL). The reaction mixture was stirred for 24 h at 60 °C. It was then concentrated *in vacuo* and precipitated in 1/2 hexanes/diethyl ether to yield colorless oil. Yield: 83%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 4.35 (s, 16H), 4.27 (s, 8H), 3.74-3.84 (m, 16H), 3.36 (s, 16H), 2.84 (t, *J* = 5.9 Hz, 16H), 1.32 (s, 12H). ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$, ppm): δ = 170.4, 166.9, 67.4, 66.5, 62.0, 43.3, 40.7, 35.9, 25.7, 17.7. FT-IR: 3143-3632, 2808-3060, 1733 cm^{-1}. HRMS (ESI): calcd. for $[C_{57}H_{92}KO_{32}S_8]^+$: 1584.9093; found $[M+K]^+$: 1584.4068.

Synthesis of 8-arm CTA

CTP RAFT agent (508.5 mg, 1.82 mmol), Compound **6** (200mg, 0.13 mmol), and DMAP (22.2 mg, 0.182 mmol) were dissolved dry CH_2Cl_2 (50 mL). The solution was stirred for 10 min at room temperature and then it was cooled to 0 °C and DCC (375.5 mg, 1.82 mmol) was added. The reaction mixture was stirred for 24 h at room temperature. The solids were then removed by filtration, and the filtrate was concentrated *in vacuo*. The crude product was purified by silica gel chromatography using 4/1 hexanes/ethyl acetate gradually increasing to 3/7 hexanes/ethyl acetate. The resulting red oil was dialyzed against water using a 2 kDa MWCO membrane. It precipitated during this process and then was lyophilized to yield a red tacky solid. Yield: 86%. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ = 7.90-7.96 (m, 16H), 7.56-7.62 (m, 8H), 7.38-7.46 (m, 16H), 4.14-4.33 (m, 40H), 3.33 (s, 16H), 2.90 (t, *J* = 5.9 Hz, 16H), 2.48-2.90 (m, 32H), 1.96 (s, 24H), 1.33 (s, 12H). ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$, ppm): δ = 222.3, 171.3, 169.6, 166.8, 144.5, 133.1, 128.6, 126.7, 118.5, 66.5, 63.2, 60.4, 45.7, 40.6, 33.3, 31.0, 29.7, 24.1, 21.1, 17.8, 14.2. FTIR: 2790-3044, 1732, 1446, 1380, 1176, 1127, 1013, 908, 731 cm^{-1}.

Linear Polymer Synthesis

Synthesis of low DP linear poly(HPMA-*ran*-Boc-APMA)

A mixture of HPMA (0.308 g, 2.15 mmol, 61.5 equiv), Boc-APMA (0.174 g, 0.718 mmol, 20.5 equiv), CTP (9.78 mg, 0.0350 mmol, 1 equiv) and V-501 (3.25 mg, 0.0116 mmol, 0.33 equiv) in 4.5 mL of 2/1 water/dioxane was purged with nitrogen for 1 h and then immersed in a preheated oil at 70 °C for 17 h. After 17 h, the crude reaction mixture was analyzed by ^{1}H NMR spectroscopy using D_2O as the solvent to determine the conversion. After confirming that the conversion was 83% (Figure S12), the remaining solution was precipitated into 45 mL of acetone and the product was collected by centrifugation. The polymer was dried *in vacuo* at room temperature, yielding a pink solid. Copolymer composition was determined by ^{1}H NMR spectroscopy using D_2O as the solvent. Yield: 21% (100% theoretical yield is calculated based on the monomer conversion). ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.83 (s, 2H), 7.61 (s, 1H), 7.43 (s, 2H), 3.84 (s, 49H), 2.83-3.28 (m, 129H), 1.49-1.99 (m, 115H), 1.38 (s, 123H), 0.62-1.22 (m, 271H). FTIR: 3333, 2973, 2929, 1635, 1520 cm^{-1}. M_n (NMR) = 10.5 kg/mol, M_n (SEC) = 12.5 kg/mol, M_w (SEC) = 14.9 kg/mol, $Đ$ = 1.19.

Synthesis of medium DP linear poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (0.321 g, 2.24 mmol, 127.5 equiv), Boc-APMA (0.181 g, 0.748 mmol, 42.5 equiv), CTP (4.92 mg, 0.0176 mmol, 1 equiv), V-501 (1.63 mg, 0.00580 mmol, 0.33 equiv) and 2.9 mL of 2/1 water/dioxane were used. The conversion was 65%. Yield: 54%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.83 (s, 2H), 7.61 (s, 1H), 7.43 (s, 2H), 3.84 (s, 120H), 2.77-3.34 (m, 379H), 1.50-1.97 (m, 307H), 1.39 (s, 361H), 0.71-1.22 (m, 810H). FTIR: 3341, 2974, 2932, 1634, 1523 cm^{-1}. M_n (NMR) = 27.2 kg/mol, M_n (SEC) = 22.2 kg/mol, M_w (SEC) = 25.1 kg/mol, $Đ$ = 1.13.

Synthesis of high DP linear poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (0.368 g, 2.57 mmol, 292.5 equiv), Boc-APMA (0.208 g, 0.858 mmol, 97.5 equiv), CTP (2.46 mg, 0.00880 mmol, 1 equiv), V-501 (0.81 mg, 0.00290 mmol, 0.33 equiv) and 5.8 mL of 2/1 water/dioxane were used. The conversion was 73%. Yield: 74%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.83 (s, 2H), 7.61 (s, 1H), 7.44 (s, 2H), 3.84 (s, 216H), 2.76-3.35 (m, 698H), 1.48-2.01 (m, 542H), 1.39 (s, 679H), 0.63-1.23 (m, 1530H). FTIR: 3336, 2971, 2929, 1633, 1521 cm^{-1}. M_n (NMR) = 49.5 kg/mol, M_n (SEC) = 50.5 kg/mol, M_w (SEC) = 62.6 kg/mol, $Đ$ = 1.24.

Synthesis of ultra-high DP linear poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP linear poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (1.44 g, 10.1 mmol, 573 equiv), Boc-APMA (0.813 g, 3.36 mmol, 191 equiv), CTP (4.92 mg, 0.0176 mmol, 1 equiv), V-501 (1.63 mg, 0.00580 mmol, 0.33 equiv) and 5.8 mL of 2/1 water/dioxane were used. The conversion was 70%. Yield: 35%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.83 (s, 2H), 7.61 (s, 1H), 7.44 (s, 2H) 3.84 (s, 352H), 2.86-3.29 (m, 1062H), 1.51-1.94 (m, 899H), 1.39 (s, 1094H), 0.62-1.22 (m, 2247H). FTIR: 3337, 2974, 2930, 1634, 1521 cm^{-1}. M_n (NMR) = 80.0 kg/mol, M_n (SEC) = 111.7 kg/mol, M_w (SEC) = 171 kg/mol, $Đ$ = 1.53.

4-Arm Polymer Synthesis

Synthesis of low DP 4-arm poly(HPMA-*ran*-Boc-APMA)

A mixture of HPMA (0.193 g, 1.35 mmol, 102 equiv), Boc-APMA (0.109 g, 0.449 mmol, 34 equiv), 4-arm RAFT agent (21.8 mg, 0.0132 mmol, 1 equiv) and V-501 (5.10 mg, 0.0182 mmol, 1.38 equiv) in 12 mL of 1/3 water/dioxane was purged with nitrogen for 1 h and then immersed in a preheated oil at 70 °C for 8 h. After 8 h, the crude reaction mixture was analyzed by ^{1}H NMR spectroscopy using D_2O as the solvent to determine the conversion. After confirming that the conversion was 50%, the remaining solution was precipitated into 120 mL of acetone and collected

by centrifugation. The polymer was dried *in vacuo* at room temperature, yielding a pink solid. Yield: 30%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.39-7.85 (m, 20H), 3.85 (s, 35H), 2.84-3.35 (m, 85H), 1.50-2.02 (m, 76H), 1.38 (s, 101H), 0.62-1.20 (m, 192H). FTIR: 3338, 2975, 2927, 1628, 1526 cm^{-1}. M_n (NMR) = 9.4 kg/mol, M_n (SEC) = 9.1 kg/mol, M_w (SEC) = 10.7 kg/mol, $Đ$ = 1.18.

Synthesis of medium DP 4-arm poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP 4-arm poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (0.315 g, 2.20 mmol, 166.5 equiv), Boc-APMA (0.177 g, 0.733 mmol, 55.5 equiv) were used. The polymerization time was 14 h. The conversion was 50%. Yield: 21%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.35-7.90 (m, 20H), 3.84 (s, 110H), 2.78-3.44 (m, 325H), 1.52-2.19 (m, 322H), 1.38 (s, 337H), 0.61-1.23 (m, 594H). FTIR: 3334, 2977, 2929, 1636, 1518 cm^{-1}. M_n (NMR) = 26.4 kg/mol, M_n (SEC) = 18.3 kg/mol, M_w (SEC) = 21.0 kg/mol, $Đ$ = 1.15.

Synthesis of high DP 4-arm poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP 4-arm poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (0.578 g, 4.04 mmol, 306 equiv), Boc-APMA (0.327 g, 1.35 mmol, 102 equiv) were used. The polymerization time was 28 h. The conversion was 70%. Yield: 30%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.35-7.90 (m, 20H), 3.84 (s, 213H), 2.84-3.32 (m, 723H), 1.53-2.10 (m, 681H), 1.38 (s, 669H), 0.70-1.25 (m, 1395H). FTIR: 3340, 2972, 2929, 1634, 1520 cm^{-1}. M_n (NMR) = 50.0 kg/mol, M_n (SEC) = 30.0 kg/mol, M_w (SEC) = 36.9 kg/mol, $Đ$ = 1.23.

Synthesis of ultra-high DP 4-arm poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP 4-arm poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (1.08 g, 7.56 mmol, 573 equiv), Boc-APMA (0.610 g, 2.52 mmol,

191 equiv) were used. The polymerization time was 26 h. The conversion was 70%. Yield: 35%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.35-7.90 (m, 20H), 3.85 (s, 366H), 2.74-3.33 (m, 1613H), 1.51-2.03 (m, 1248H), 1.38 (s, 1173H), 0.61-1.21 (m, 3766H). FTIR: 3340, 2972, 2929, 1634, 1520 cm^{-1}. M_n (NMR) = 85.5 kg/mol, M_n (SEC) = 86.4 kg/mol, M_w (SEC) = 129 kg/mol, $Đ$ = 1.44.

8-Arm Polymer Synthesis

Synthesis of low DP 8-arm poly(HPMA-*ran*-Boc-APMA)

A mixture of HPMA (0.0978 g, 0.684 mmol, 171 equiv), Boc-APMA (0.0552 g, 0.228 mmol, 57 equiv), 8-arm RAFT agent (14.5 mg, 0.00400 mmol, 1 equiv) and V-501 (2.80 mg, 0.0100 mmol, 2.5 equiv) in 4 mL of 1/3 water/dioxane was purged with nitrogen for 1 h and then immersed in a preheated oil at 70 °C for 6 h. After 6 h, the crude reaction mixture was analyzed by ^{1}H NMR spectroscopy using D_2O as the solvent to determine the conversion. After confirming that the conversion was 30%, the polymer was precipitated in 40 mL acetone and collected by centrifugation. The polymer was dried *in vacuo* at room temperature, yielding a pink solid. Yield: 24%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.39-7.88 (m, 40H), 3.84 (s, 39H), 2.81-3.29 (m, 97H), 1.49-2.00 (m, 78H), 1.38 (s, 95H), 0.65-1.22 (m, 241H). FTIR: 3341, 2975, 2928, 1635, 1524 cm^{-1}. M_n (NMR) = 11.8 kg/mol, M_n (SEC) = 15.8 kg/mol, M_w (SEC) = 26.4 kg/mol, $Đ$ = 1.35.

Synthesis of medium DP 8-arm poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP 8-arm poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (0.159 g, 1.11 mmol, 278 equiv), Boc-APMA (0.0895 g, 0.370 mmol, 93 equiv) were used. The polymerization time was 4 h. The conversion was 30%. Yield: 31%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.39-7.86 (m, 40H), 3.84 (s, 78H), 2.81-3.32 (m, 201H), 1.52-2.11 (m, 175H), 1.38 (s, 245H), 0.57-1.19 (m, 475H). FTIR: 3330, 2979, 2930, 1634, 1526 cm^{-1}. M_n (NMR) = 21.3 kg/mol, M_n (SEC) = 21.9 kg/mol, M_w (SEC) = 31.1 kg/mol, $Đ$ = 1.42.

Synthesis of high DP 8-arm poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP 8-arm poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (0.408 g, 2.85 mmol, 713 equiv), Boc-APMA (0.230 g, 0.950 mmol, 238 equiv) were used. The polymerization time was 6 h. The conversion was 30%. Yield: 64%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.36-7.94 (m, 40H), 3.84 (s, 185H), 2.73-3.35 (m, 541H), 1.51-1.97 (m, 452H), 1.38 (s, 512H), 0.57-1.22 (m, 1250H). FTIR: 3332, 2969, 2930, 1634, 1525 cm^{-1}. M_n (NMR) = 43.9 kg/mol, M_n (SEC) = 41.2 kg/mol, M_w (SEC) = 58.9 kg/mol, $Đ$ = 1.43.

Synthesis of ultra-high DP 8-arm poly(HPMA-*ran*-Boc-APMA)

The polymer was synthesized by the same procedure as **low DP 8-arm poly(HPMA-*ran*-Boc-APMA)**, except that HPMA (0.764 g, 5.34 mmol, 1335 equiv), Boc-APMA (0.431 g, 1.78 mmol, 445 equiv) were used. The polymerization time was 10 h. The conversion was 30%. Yield: 46%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 7.39-7.89 (m, 40H), 3.84 (s, 396H), 2.84-3.29 (m, 1641H), 1.54-1.96 (m, 1330H), 1.38 (s, 1193H), 0.62-1.20 (m, 4092H). FTIR: 3334, 2976, 2930, 1635, 1520 cm^{-1}. M_n (NMR) = 92.3 kg/mol, M_n (SEC) = 80.1 kg/mol, M_w (SEC) = 130 kg/mol, $Đ$ = 1.62.

Cleavage of the terminal dithiobenzoate from high DP linear poly(HPMA-*ran*-Boc-APMA) and representative procedure applied to all polymers

High DP linear poly(HPMA-*ran*-Boc-APMA) (100 mg, 0.0021 mmol, 1 equiv) and V-501 (12 mg, 0.042 mmol, 20 equiv) were dissolved in 2/1 water/dioxane (1.5 mL) and then the solution was purged with nitrogen for 1 h. It was then heated at 75 °C for 24 h, cooled to room temperature, and then precipitated into 25 mL of acetone. The polymer was collected by centrifugation and dried under vacuum at room temperature, yielding a white solid. Yield: 86%.

The same procedure was applied to all polymers with typical yields of 70-88%.

General procedure for the cleavage of the pendent Boc protecting groups

The following procedure was used for all polymers, with typical yields of 52-78%. The polymer (50 mg) was dissolved in 1 mL of TFA, and the reaction mixture was stirred for 2 h. The product was then precipitated into 10 mL of diethyl ether, collected by centrifugation, and then dialyzed against de-ionized water using 2 kDa MWCO membrane. The water was removed by lyophilization.

Low DP linear poly(HPMA-*ran*-APMA): Yield: 52%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 1H), 2.89-3.22 (m, 3H), 1.51-1.97 (m, 3H), 0.71-1.19 (m, 7H). FTIR: 3342, 2972, 2929, 1635, 1533 cm^{-1}.

Medium DP linear poly(HPMA-*ran*-APMA): Yield: 78%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 1H), 2.82-3.27 (m, 3H), 1.44-1.99 (m, 3H), 0.70-1.19 (m, 7H). FTIR: 3339, 2978, 2935, 1633, 1533 cm^{-1}.

High DP linear poly(HPMA-*ran*-APMA): Yield: 69%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 1H), 2.78-3.27 (m, 3H), 1.46-1.98 (m, 3H), 0.67-1.21 (m, 7H). FTIR: 3342, 2973, 2934, 1634, 1532 cm^{-1}.

Ultra-high DP linear poly(HPMA-*ran*-APMA): Yield: 60%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 1H), 2.81-3.29 (m, 3H), 1.52-2.05 (m, 3H), 0.71-1.25 (m, 6H). FTIR: 3341, 2973, 2935, 1635, 1533 cm^{-1}.

Low DP 4-arm poly(HPMA-*ran*-APMA): Yield: 57%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 4H), 2.78-3.28 (m, 14H), 1.47-1.96 (m, 12H), 0.67-1.19 (m, 28H). FTIR: 3343, 2974, 2932, 1635, 1533 cm^{-1}.

Medium DP 4-arm poly(HPMA-*ran*-APMA): Yield: 55%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.83 (s, 4H), 2.79-3.29 (m, 13H), 1.50-1.97 (m, 12H), 0.64-1.19 (m, 28H). FTIR: 3343, 2973, 2936, 1635, 1533 cm^{-1}.

High DP 4-arm poly(HPMA-*ran*-APMA): Yield: 68%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.80 (s, 4H), 2.75-3.20 (m, 12H), 1.42-1.90 (m, 9H), 0.73-1.15 (m, 30H). FTIR: 3338, 2973, 2937, 1634, 1533 cm^{-1}.

Ultra-high DP 4-arm poly(HPMA-*ran*-APMA): Yield: 68%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 4H), 2.77-3.28 (m, 13H), 1.46-1.98 (m, 12H), 0.67-1.19 (m, 28H). FTIR: 3338, 2973, 2937, 1635, 1533 cm^{-1}.

Low DP 8-arm poly(HPMA-*ran*-APMA): Yield: 54%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 8H), 2.76-3.27 (m, 26H), 1.43-1.95 (m, 24H), 0.60-1.19 (m, 57H). FTIR: 3333, 2973, 2932, 1635, 1533 cm^{-1}.

Medium DP 8-arm poly(HPMA-*ran*-APMA): Yield: 64%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 8H), 2.85-3.23 (m, 24H), 1.41-1.91 (m, 18H), 0.79-1.16 (m, 66H). FTIR: 3336, 2973, 2932, 1635, 1533 cm^{-1}.

High DP 8-arm poly(HPMA-*ran*-APMA): Yield: 78%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 8H), 2.79-3.33 (m, 27H), 1.46-1.94 (m, 26H), 0.75-1.23 (m, 56H). FTIR: 3342, 2974, 2932, 1636, 1534 cm^{-1}.

Ultra-high DP 8-arm poly(HPMA-*ran*-APMA): Yield: 67%. ^{1}H-NMR (400 MHz, D_2O, ppm): δ = 3.84 (s, 8H), 2.79-3.26 (m, 26H), 1.46-2.03 (m, 24H), 0.70-1.22 (m, 56H). FTIR: 3337, 2974, 2933, 1635, 1539 cm^{-1}.

General procedure for the labeling of the polymers with rhodamine B NHS-carbonate

The following procedure was used for all polymers, with typical yields of 58-91%. The polycation (20.0 mg, 0.535 moles of monomer) and rhodamine B NHS-carbonate (1.25 mg, 0.0135 equiv. per monomer) were dissolved in 1 mL of 0.1 M sodium bicarbonate solution (pH 8.3). The solution was stirred overnight, then dialyzed against de-ionized water using 2 kDa MWCO membrane, and then lyophilized to yield a pink powder. The extent of rhodamine modification was determined by UV-vis spectroscopy in 0.1 M $NaHCO_3$ (pH 8.3) at 565 nm, using an extinction coefficient (ε) of 13169 M^{-1} cm^{-1} measured in the same buffer.

Table S1. Summary of % monomer units modified with rhodamine for all polycations.

Architecture	Target DP	% monomer units modified with rhodamine
Linear	Low	0.66
	Medium	1.00
	High	0.43
	Ultra-high	0.68
4-arm	Low	0.20
	Medium	0.88
	High	0.32
	Ultra-high	0.57
8-arm	Low	0.64
	Medium	0.30
	High	0.40
	Ultra-high	0.38

^{1}H NMR Spectra of Monomers, 4-Arm and 8-Arm CTAs and Precursors

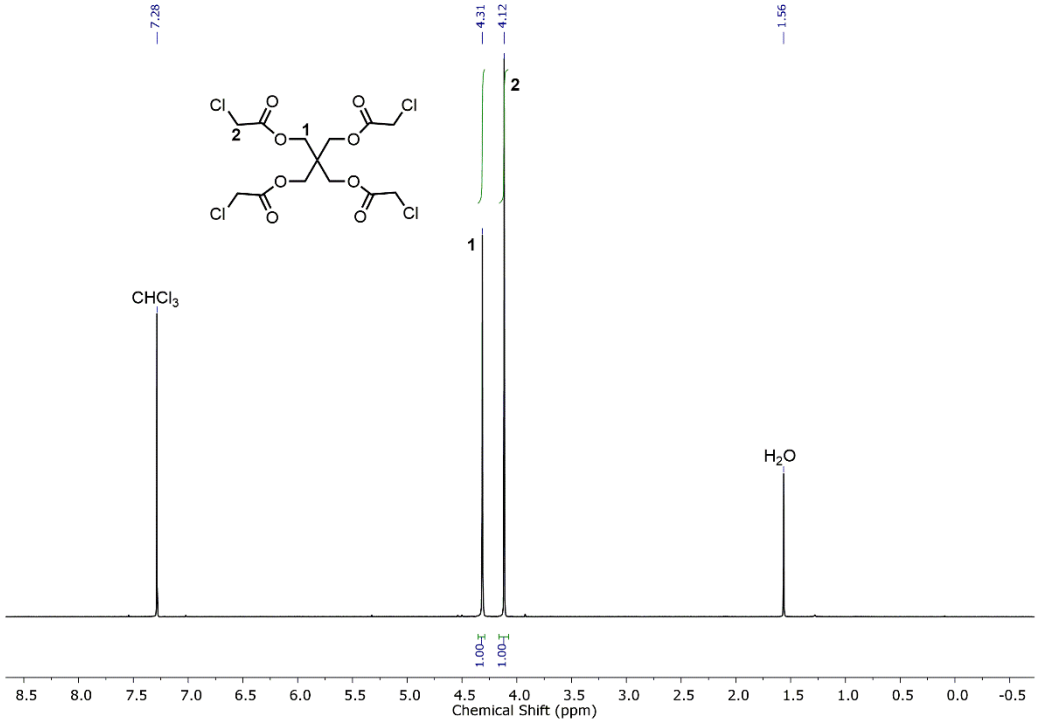

Figure S1. ^{1}H NMR spectrum of Compound **1** (400 MHz, $CDCl_3$).

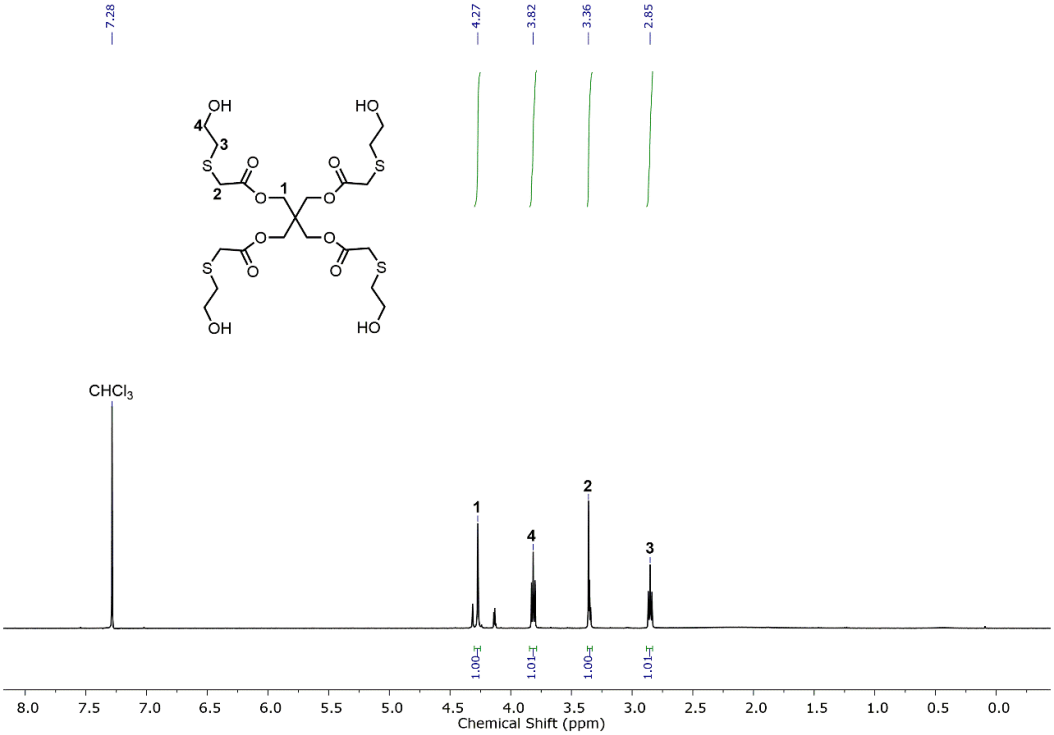

Figure S2. ^{1}H NMR spectrum of Compound **2** (400 MHz, $CDCl_3$).

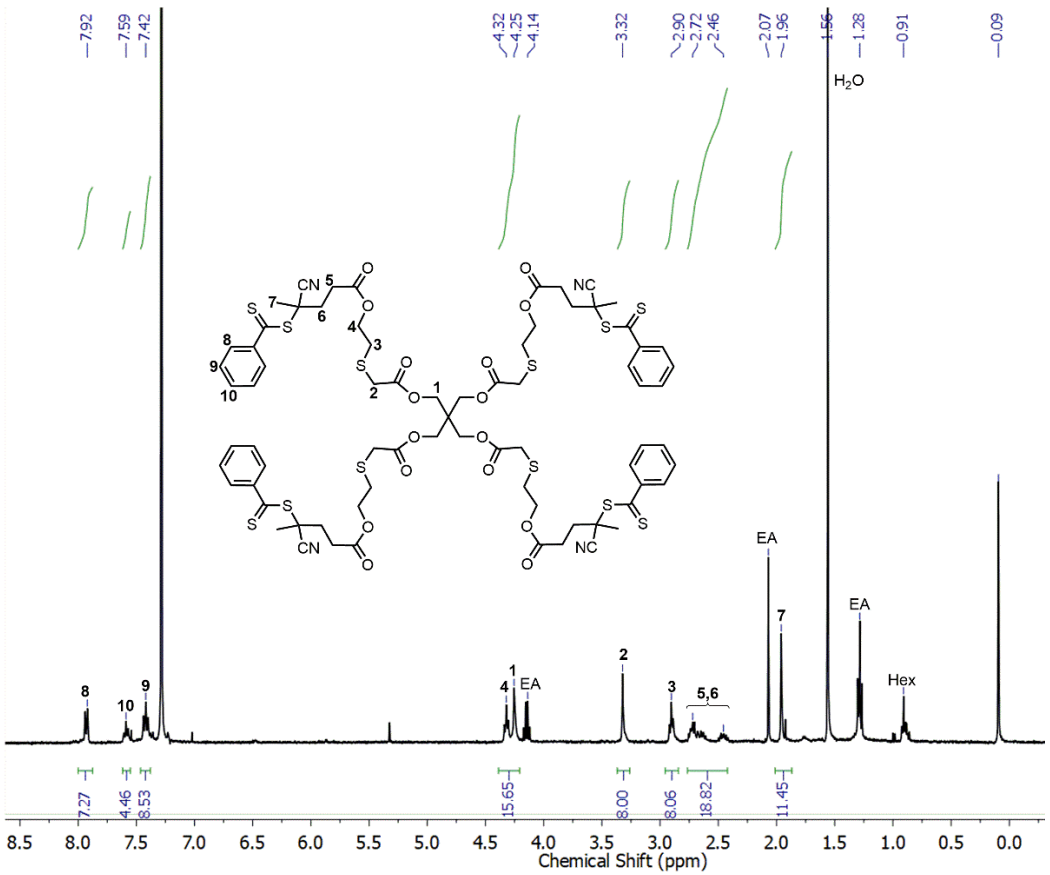

Figure S3. ^{1}H NMR spectrum of the **4-arm CTA** (400 MHz, $CDCl_3$). EA: ethyl acetate. Hex: hexanes. Full functionalization of the tetraol was confirmed based on complete disappearance of the methylene groups adjacent to the OH in compound **2** at 3.8 ppm (Figure S6) and the appearance of a new peak at 4.3 ppm (labeled as 4 above).

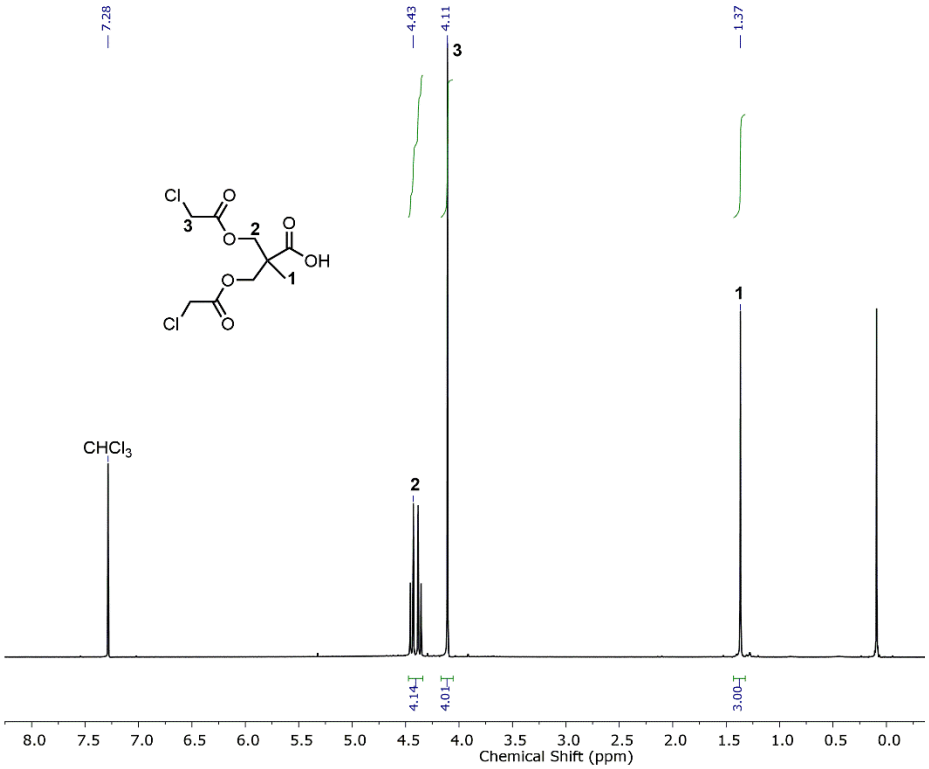

Figure S4. ^{1}H NMR spectrum of Compound **3** (400 MHz, $CDCl_3$).

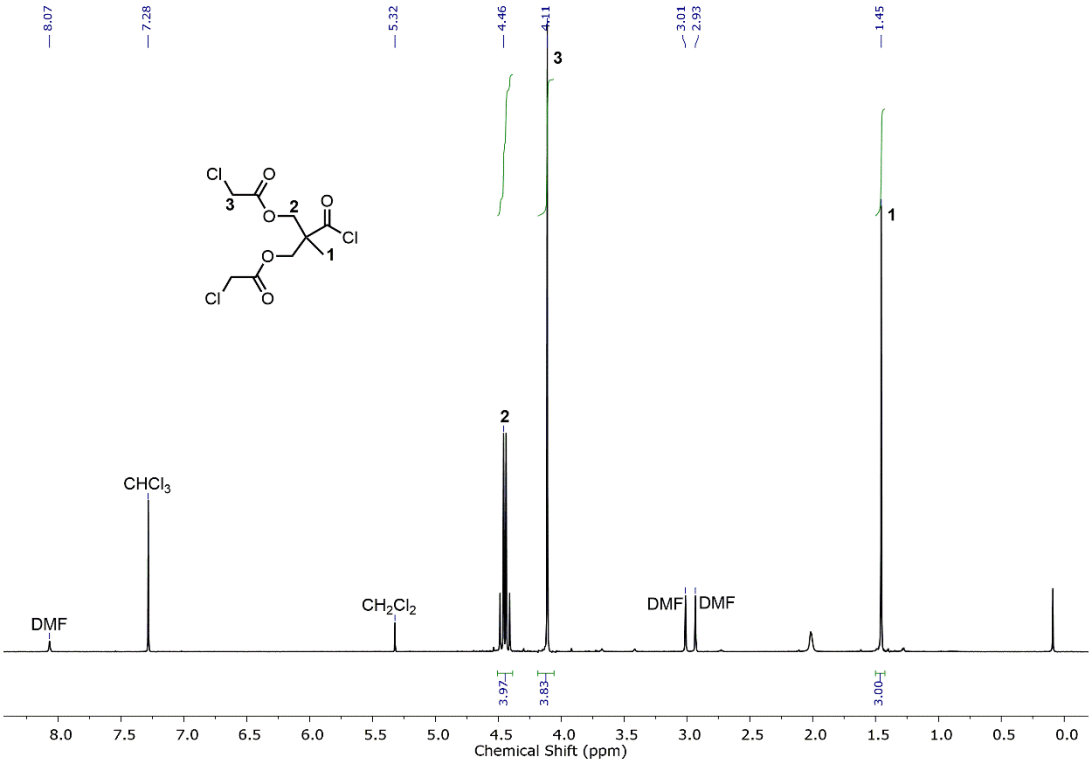

Figure S5. ^{1}H NMR spectrum of Compound **4** (400 MHz, $CDCl_3$).

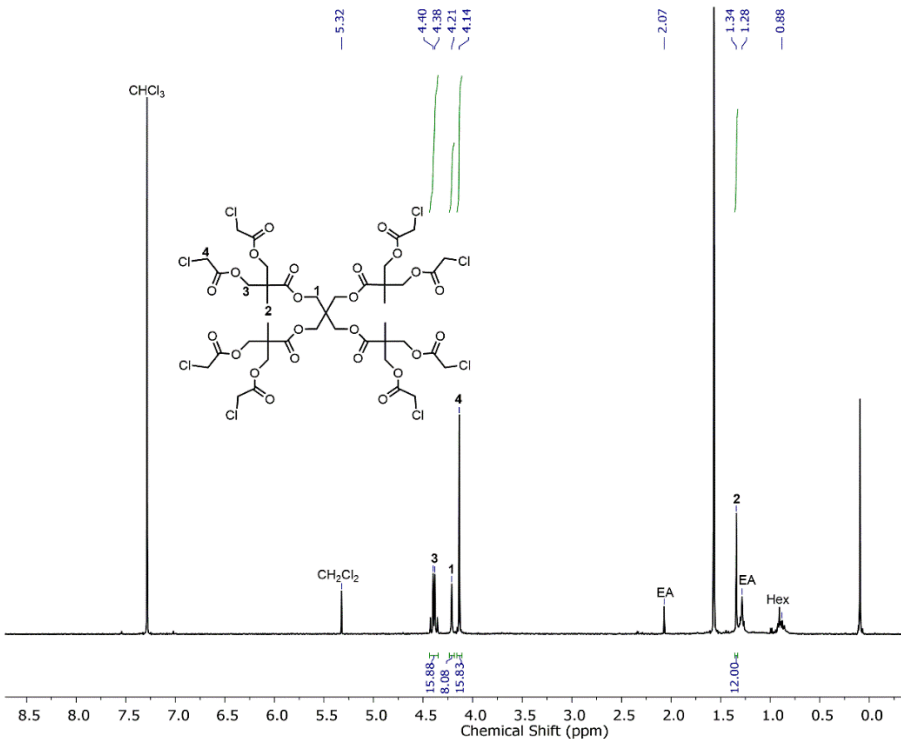

Figure S6. ^{1}H NMR spectrum of Compound **5** (400 MHz, $CDCl_3$). EA: ethyl acetate. Hex: hexanes.

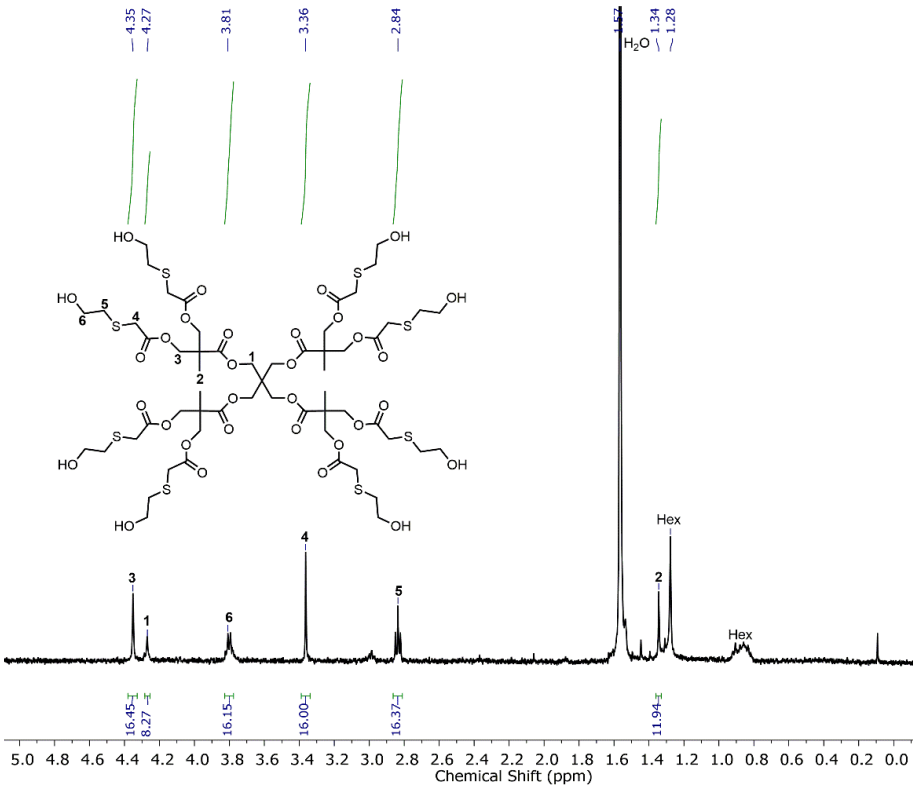

Figure S7. ^{1}H NMR spectrum of Compound **6** (400 MHz, $CDCl_3$). Hex: hexanes.

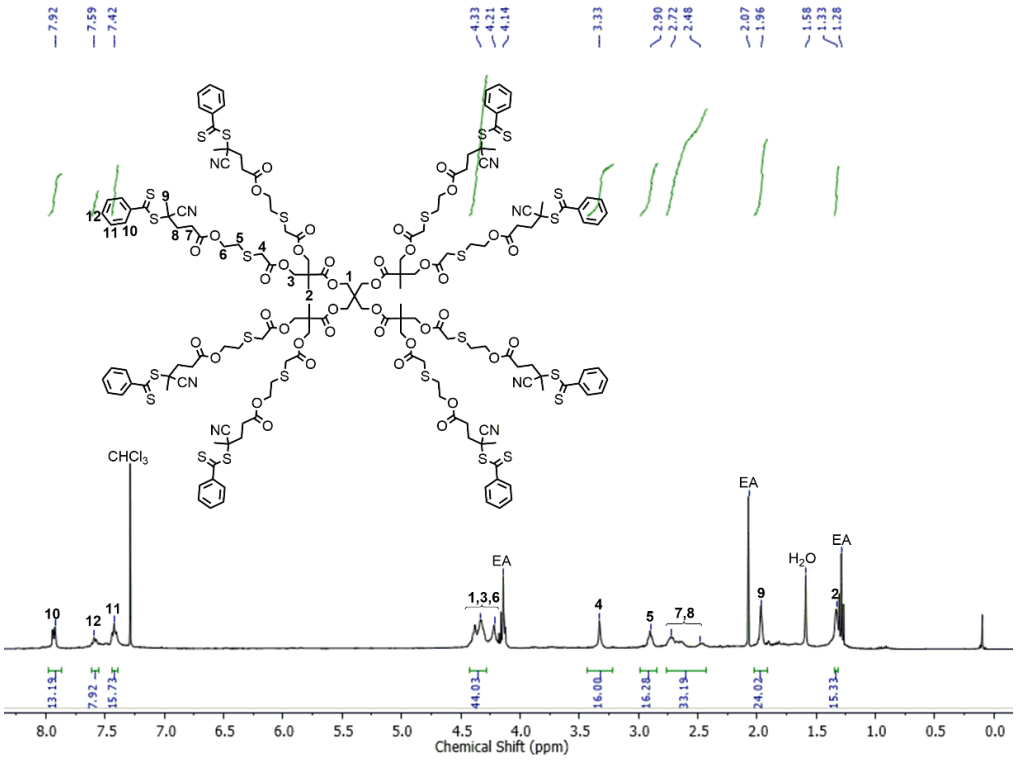

Figure S8. ^{1}H NMR spectrum of **8-arm CTA** (400 MHz, $CDCl_3$). EA: ethyl acetate.

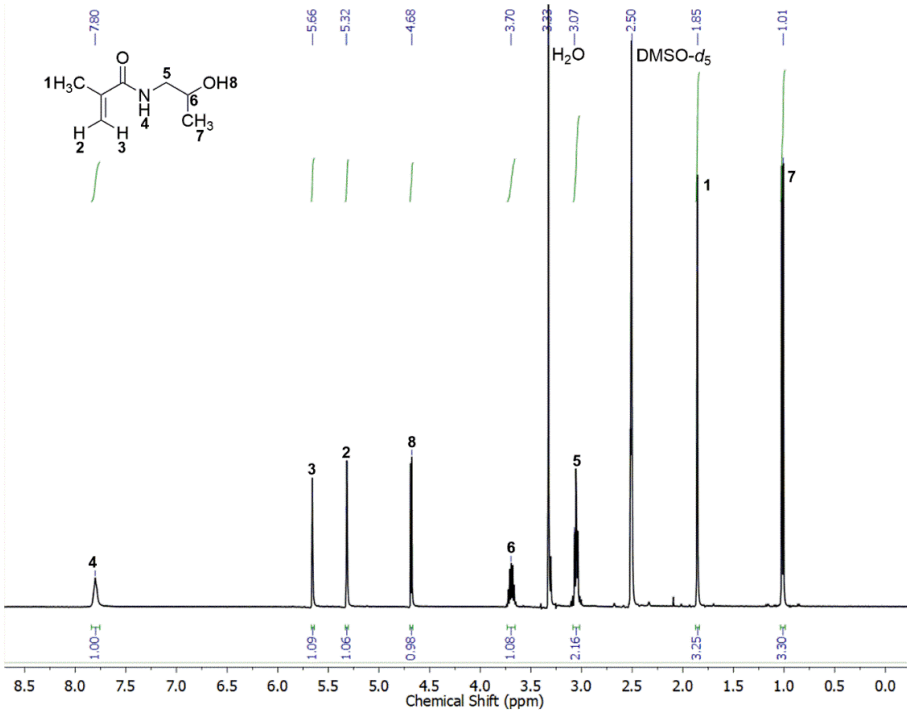

Figure S9. ^{1}H NMR spectrum of HPMA (400 MHz, DMSO-d_6).

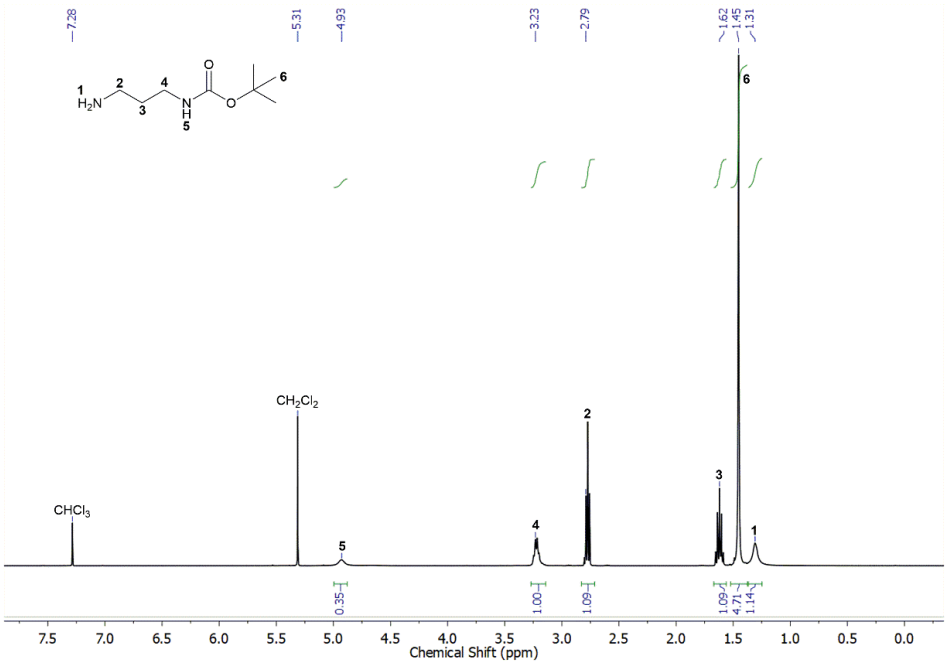

Figure S10. ^{1}H NMR spectrum of *N*-(3-aminopropyl)carbamic acid *tert*-butyl ester (400 MHz, $CDCl_3$).

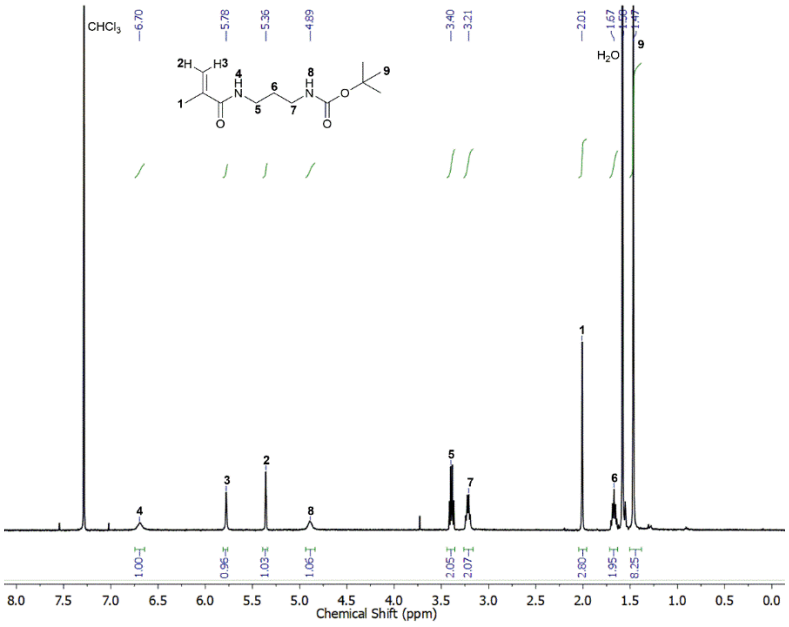

Figure S11. ^{1}H NMR spectrum of *tert*-butyl (3-methacrylamidopropyl)carbamate (Boc-APMA) (400 MHz, $CDCl_3$).

[1]H NMR Spectra of Boc-Protected Polymers

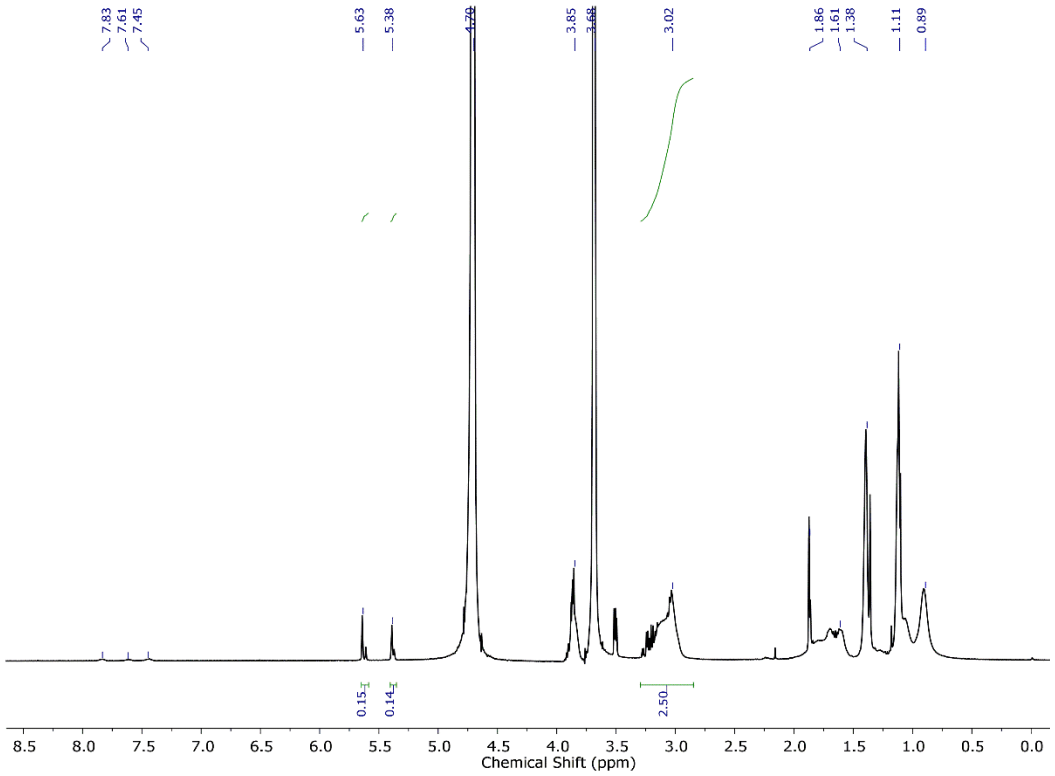

Figure S12. [1]H NMR spectrum of crude **low DP linear poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O). The conversion was determined by comparing the integrations of the vinyl proton signals at 5.6 and 5.4 ppm to the combined CH_2 peak of monomers and polymers appearing between ~2.9 and 3.2 ppm.

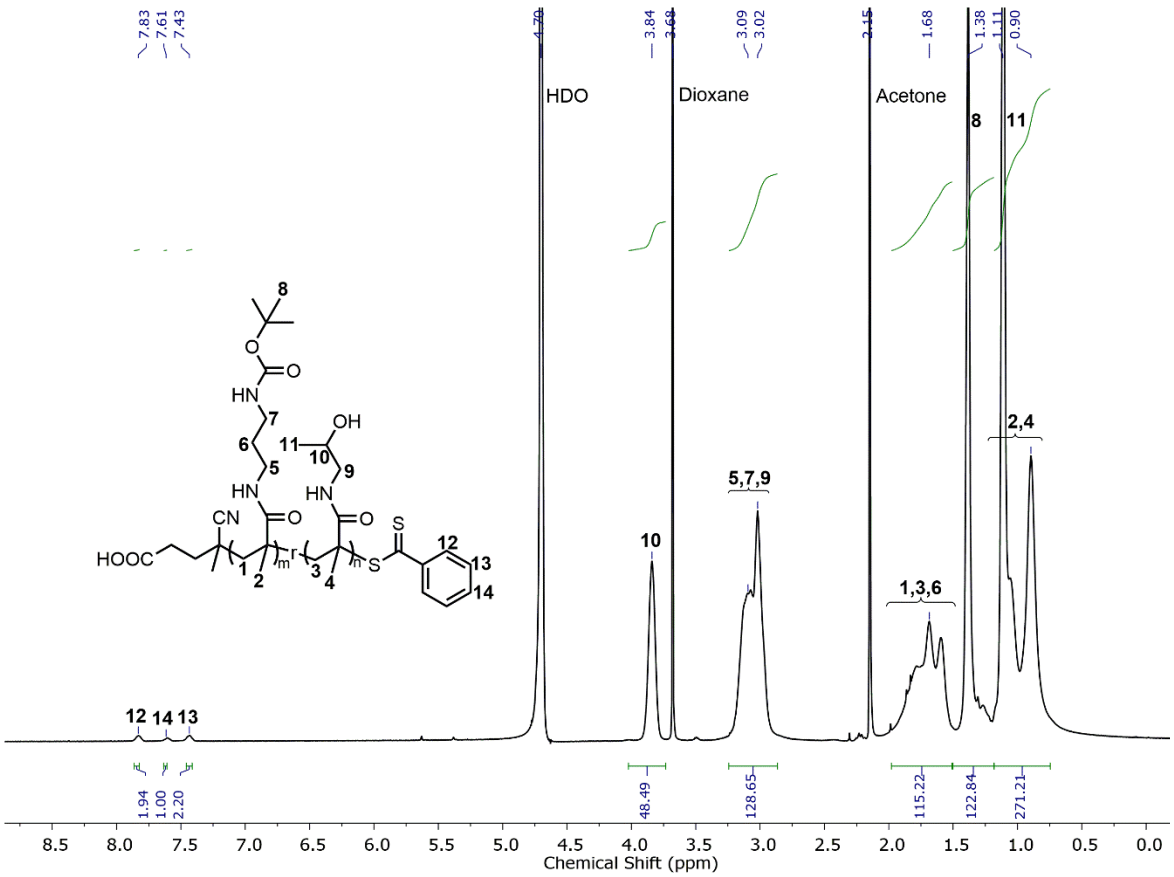

Figure S13. ^{1}H NMR spectrum of **low DP linear poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O). Based on the integration of the peak at 3.8 ppm corresponding to the proton adjacent to the OH group in HPMA moiety (labeled 10) compared to the peak at 1.4 ppm corresponding to the Boc methyl groups (labeled 8) on the Boc-APMA moiety, the ratio of HPMA/Boc-APMA was confirmed to be ~3/1. The molar mass of the polymer was calculated using end-group analysis as follows. The integral of the peak at 7.6 ppm (labeled 14) was set as 1. The integral of the peak at 3.8 ppm corresponding to the proton adjacent to the OH group in the HPMA moiety (labeled 10) was 48. The integration of the peak at 1.4 ppm corresponding to the Boc methyl groups (labeled 8) on the Boc-APMA moiety was 123. Molar mass of the polymer was calculated based on the formulas shown below, where MW = molecular weight for the monomers:

$$DP_{HPMA} = \text{Integral of the peak at 3.8 ppm}$$

$$DP_{Boc\text{-}APMA} = \frac{\text{Integral of the peak at 1.4 ppm}}{9 \text{ Boc protons per monomer repeat unit}}$$

$$M_n \text{ (NMR)} = DP_{HPMA} \times MW_{HPMA} + DP_{Boc\text{-}APMA} \times MW_{Boc\text{-}APMA} + MW_{end\text{-}group}$$

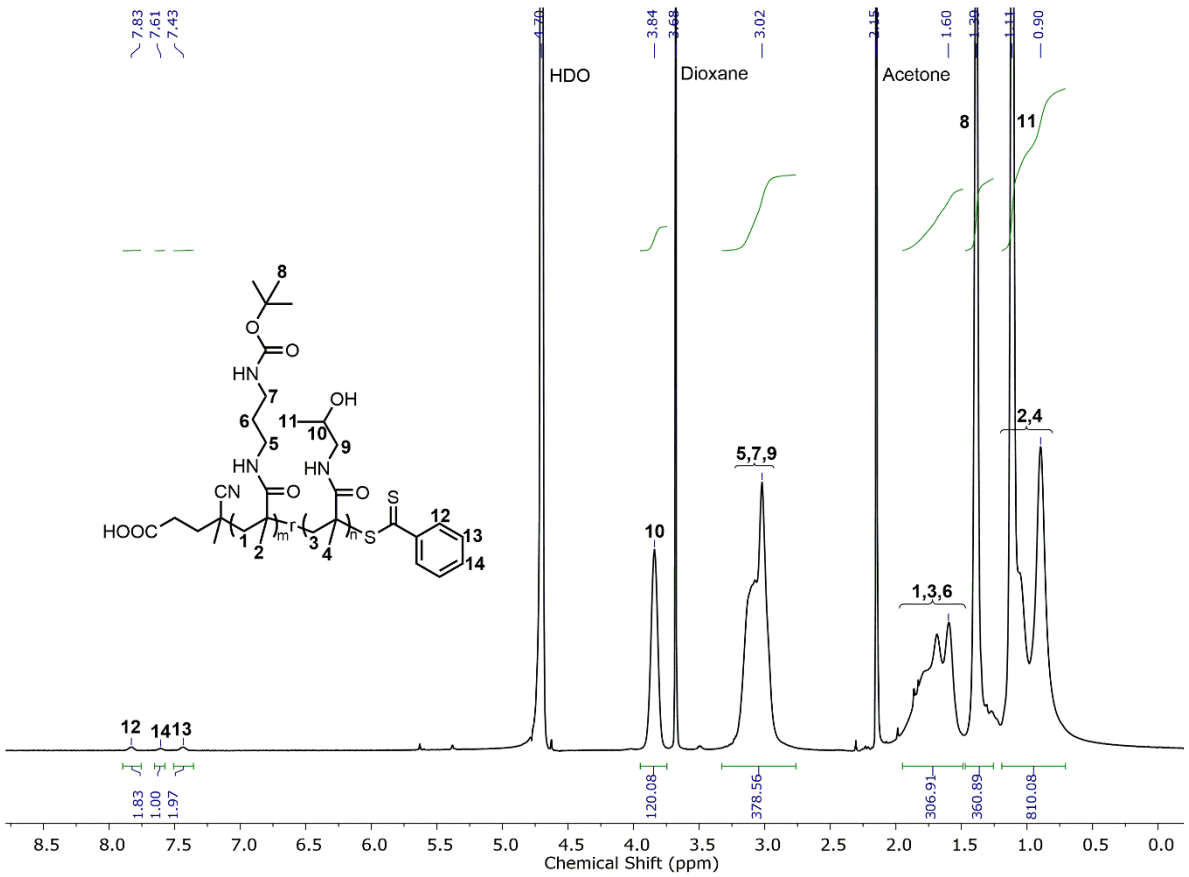

Figure S14. ^{1}H NMR spectrum of **medium DP linear poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

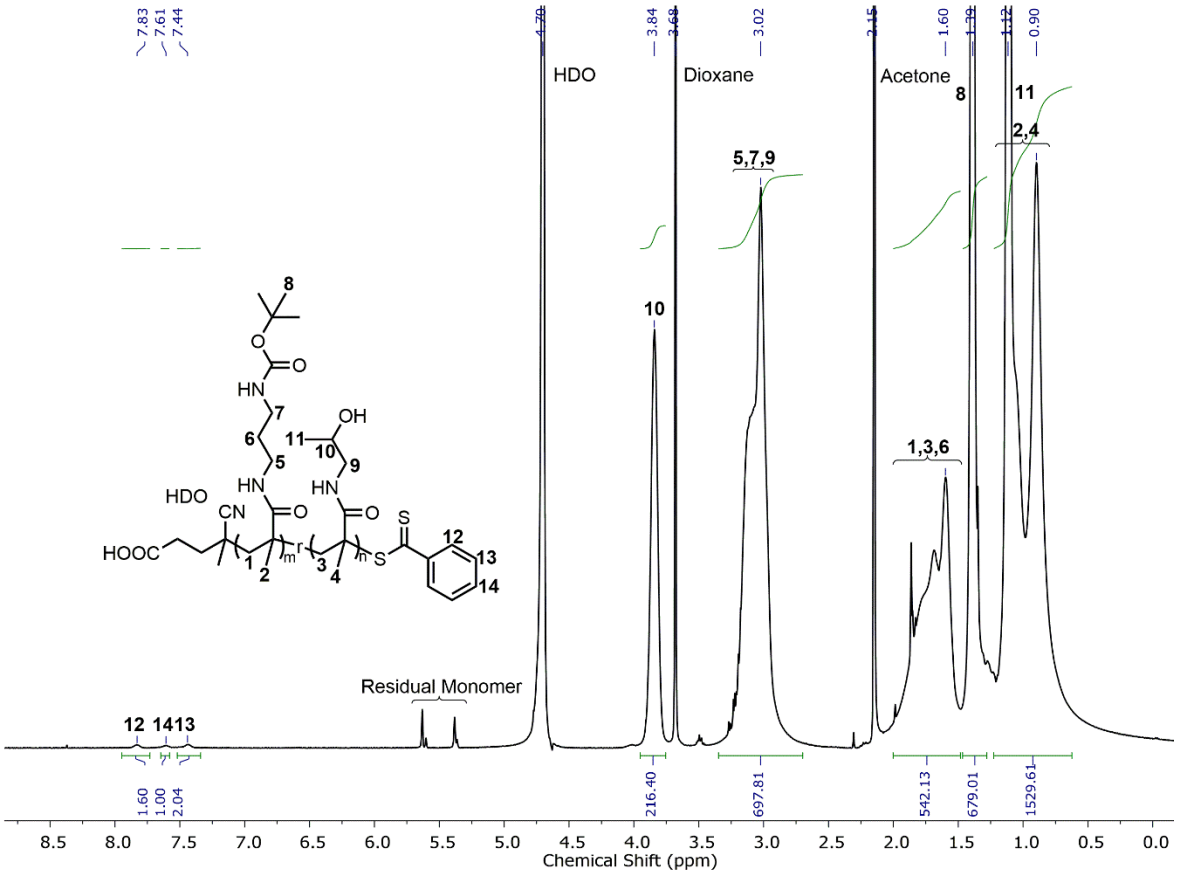

Figure S15. ^{1}H NMR spectrum of **high DP linear poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

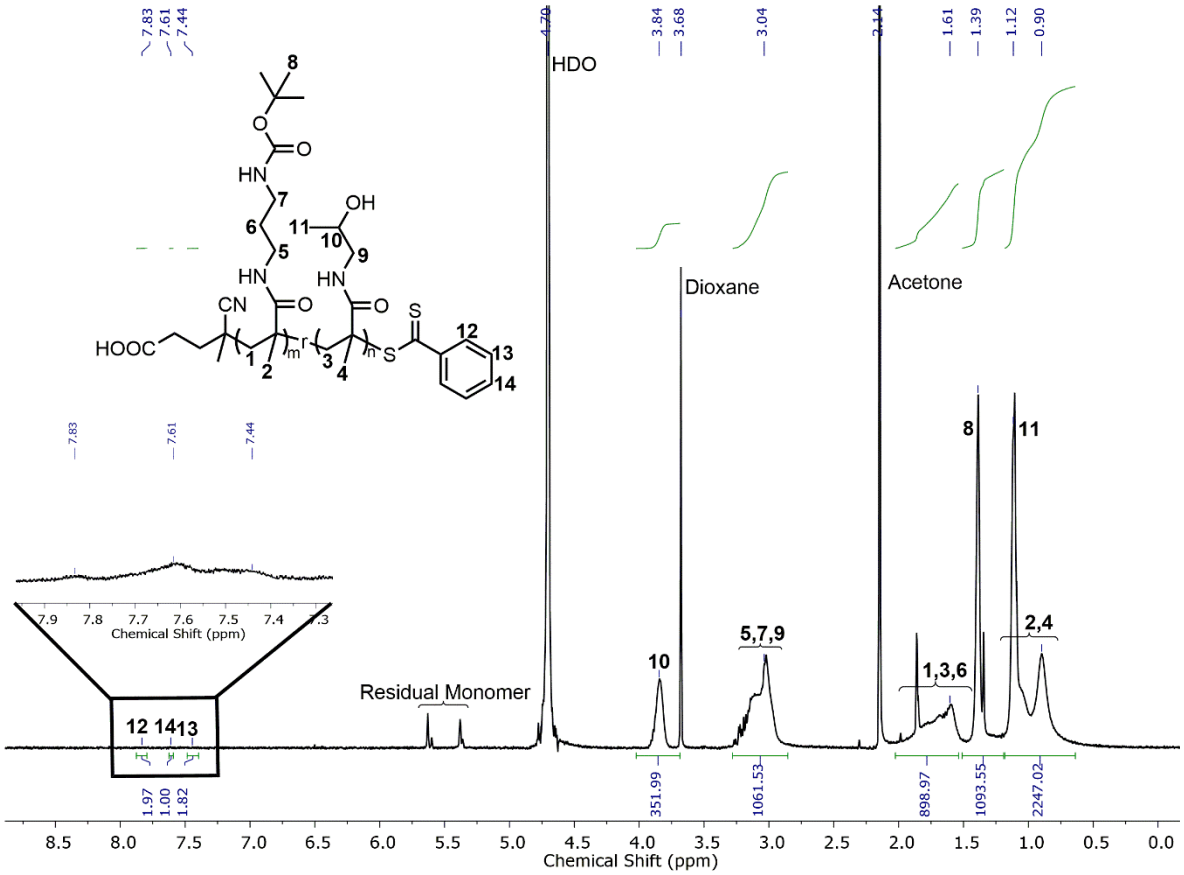

Figure S16. ^{1}H NMR spectrum of **ultra-high DP linear poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

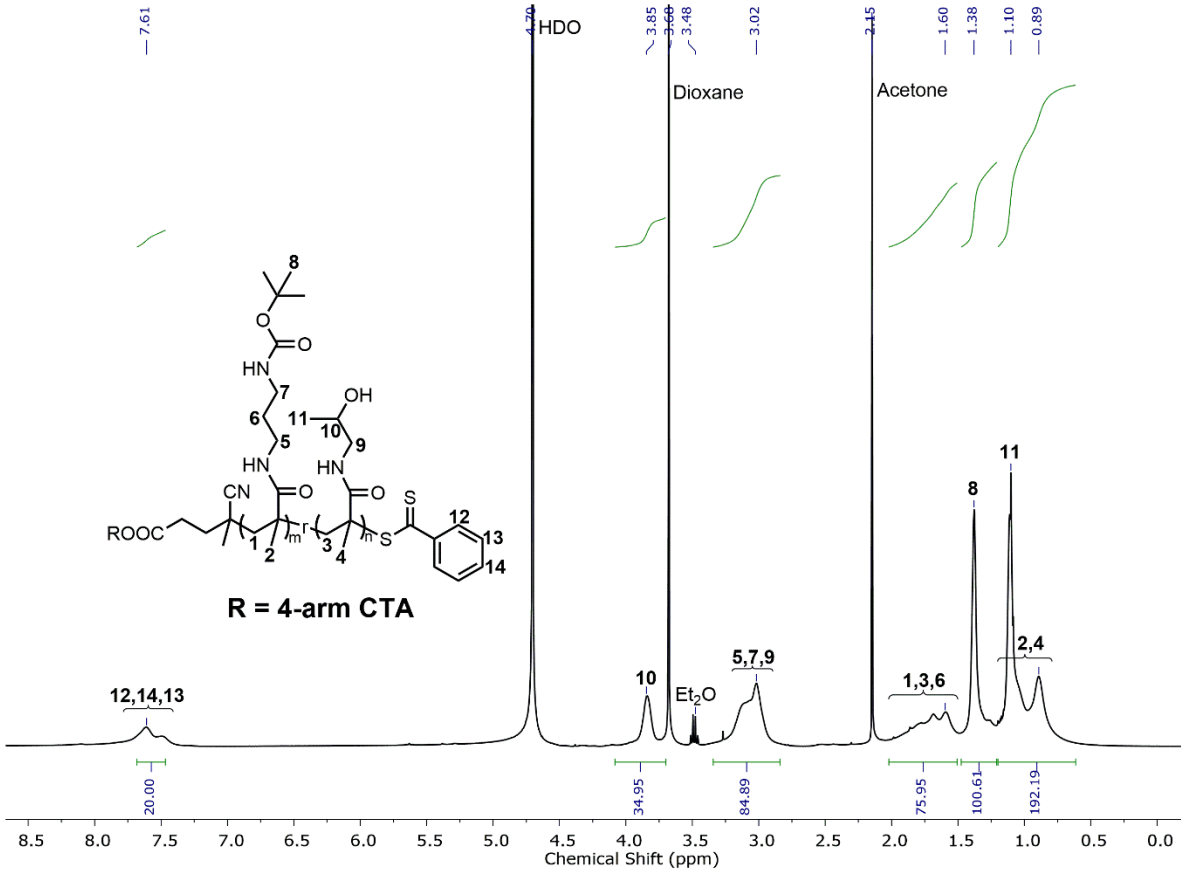

Figure S17. ^{1}H NMR spectrum of **low DP 4-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O). The molar mass of the star-shaped polymer was calculated using end-group analysis as described above for the linear polymers, except that in this case the integral of the aromatic protons from the RAFT agent was set to 20 (due to the 4 arms).

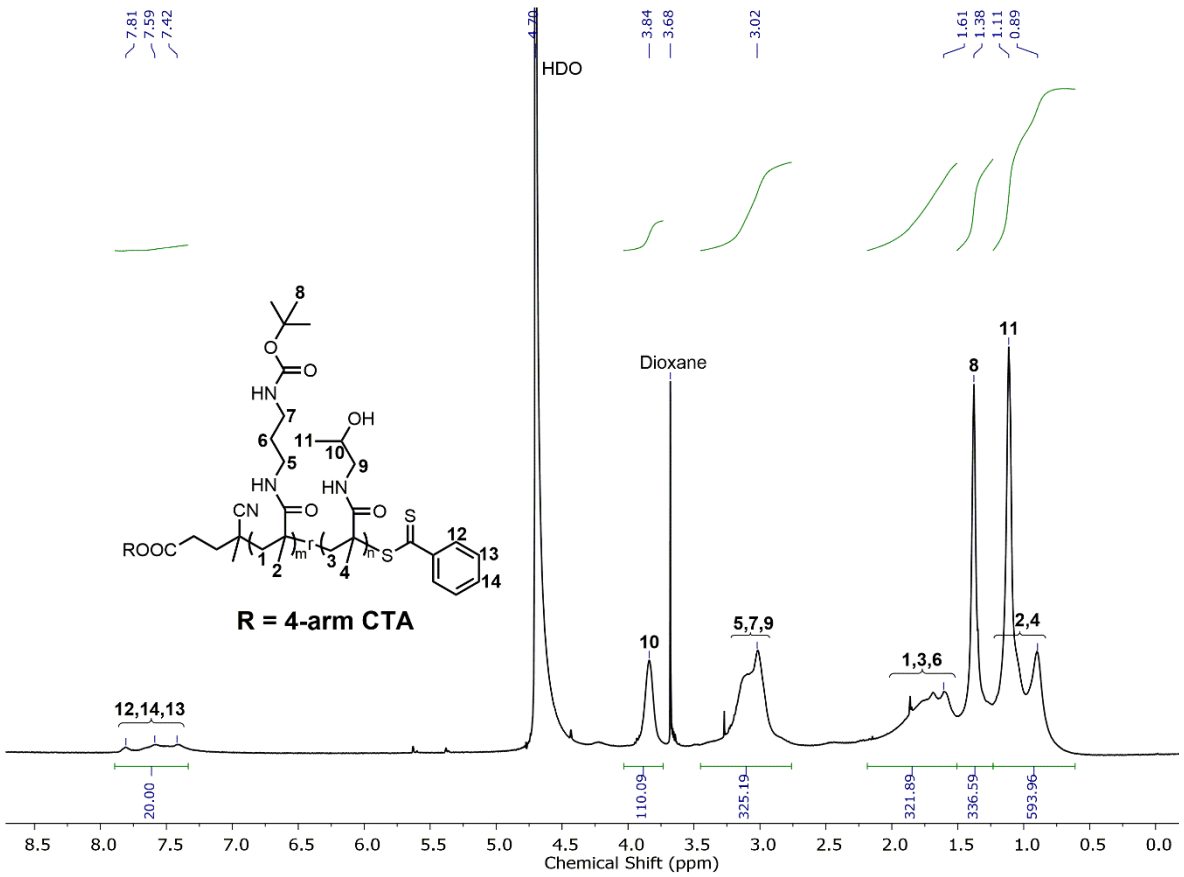

Figure S18. ^{1}H NMR spectrum of **medium DP 4-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

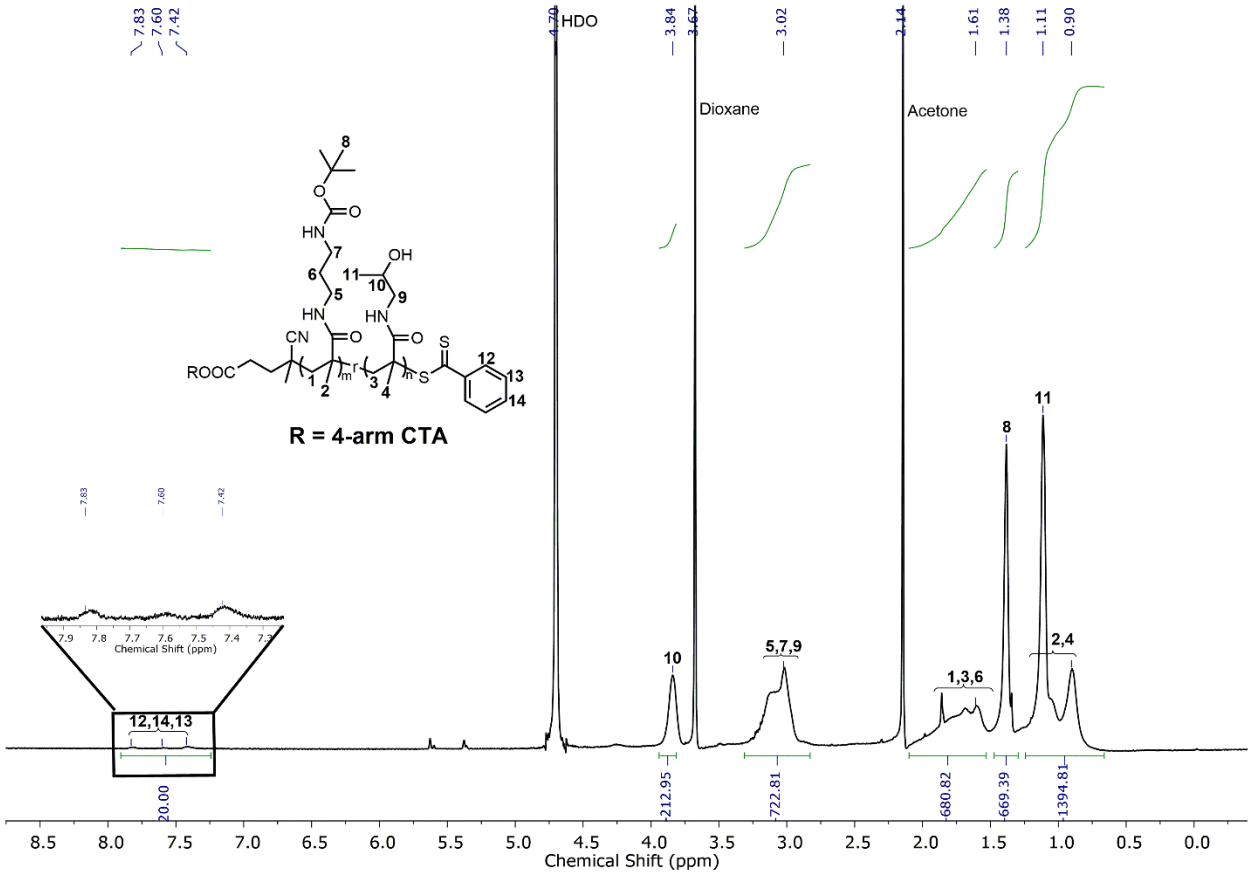

Figure S19. ^{1}H NMR spectrum of **high DP 4-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

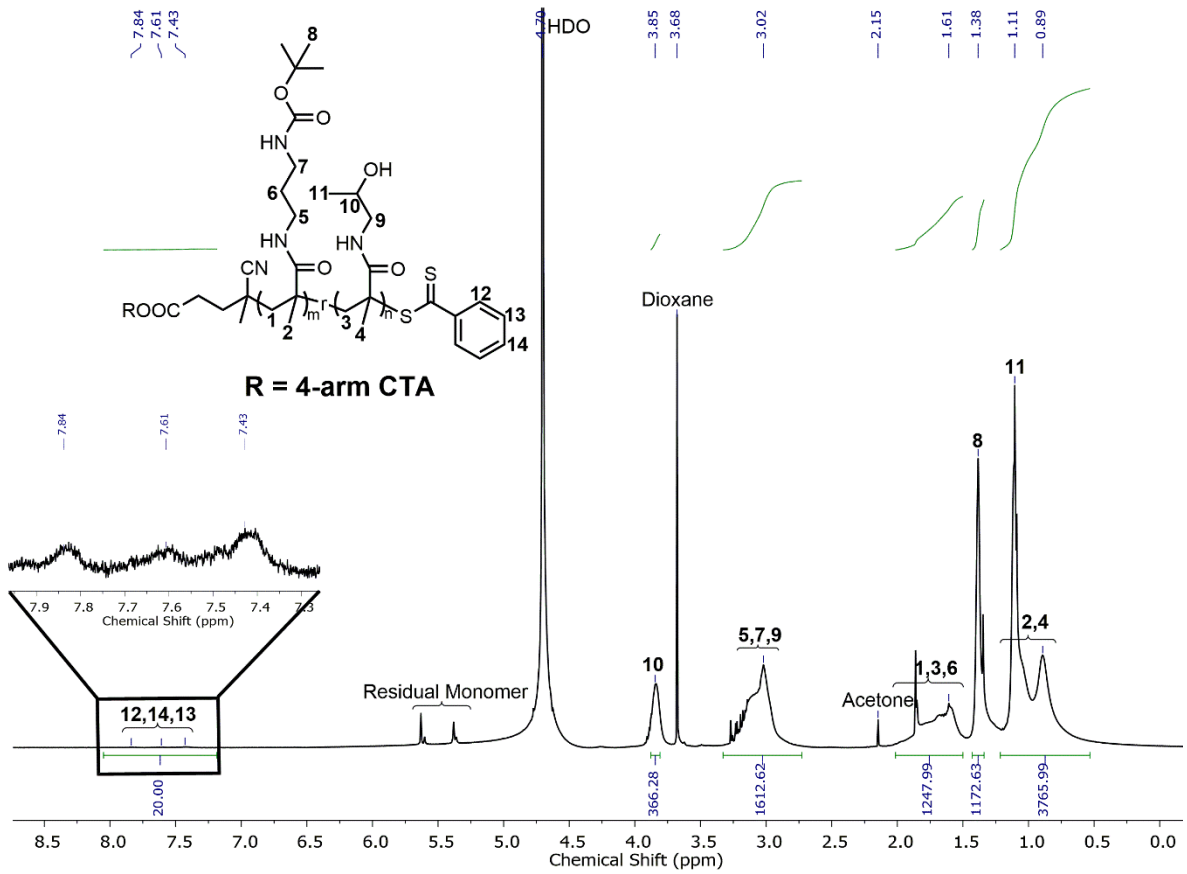

Figure S20. ^{1}H NMR spectrum of **ultra-high DP 4-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

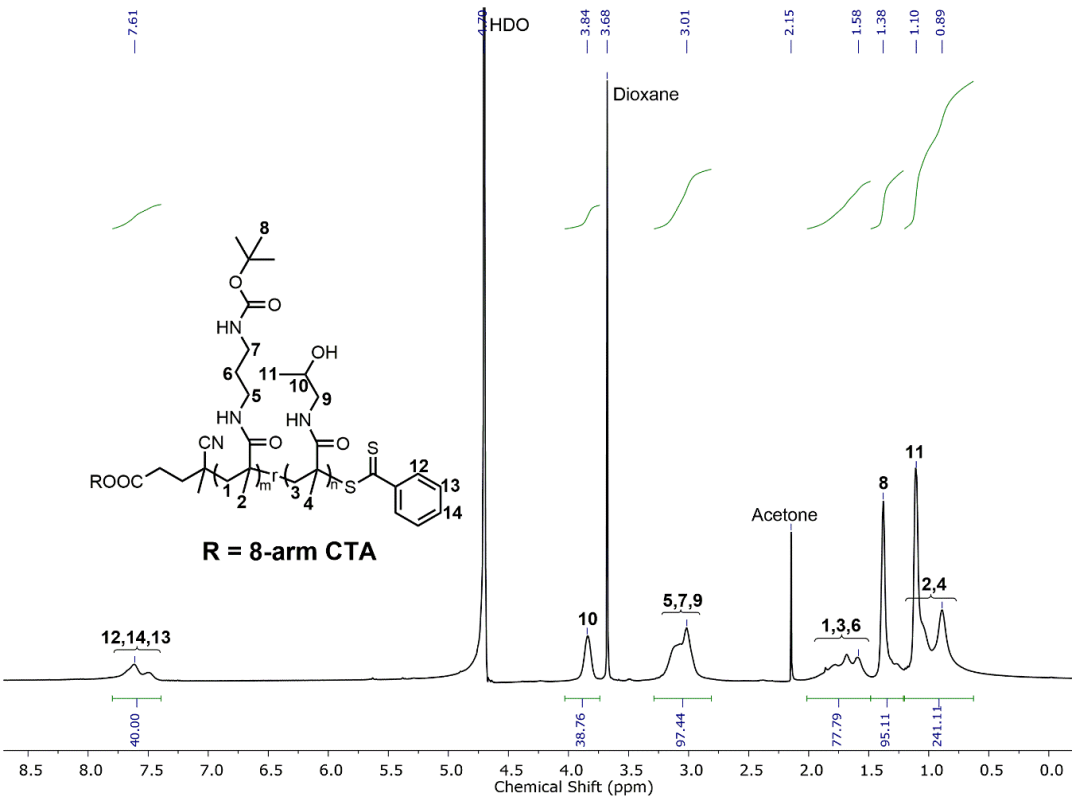

Figure S21. ^{1}H NMR spectrum of **low DP 8-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O). The molar mass was calculated as described above for the linear polymers except that the integral of the peaks corresponding to the aromatic group on the RAFT agent was set to 40.

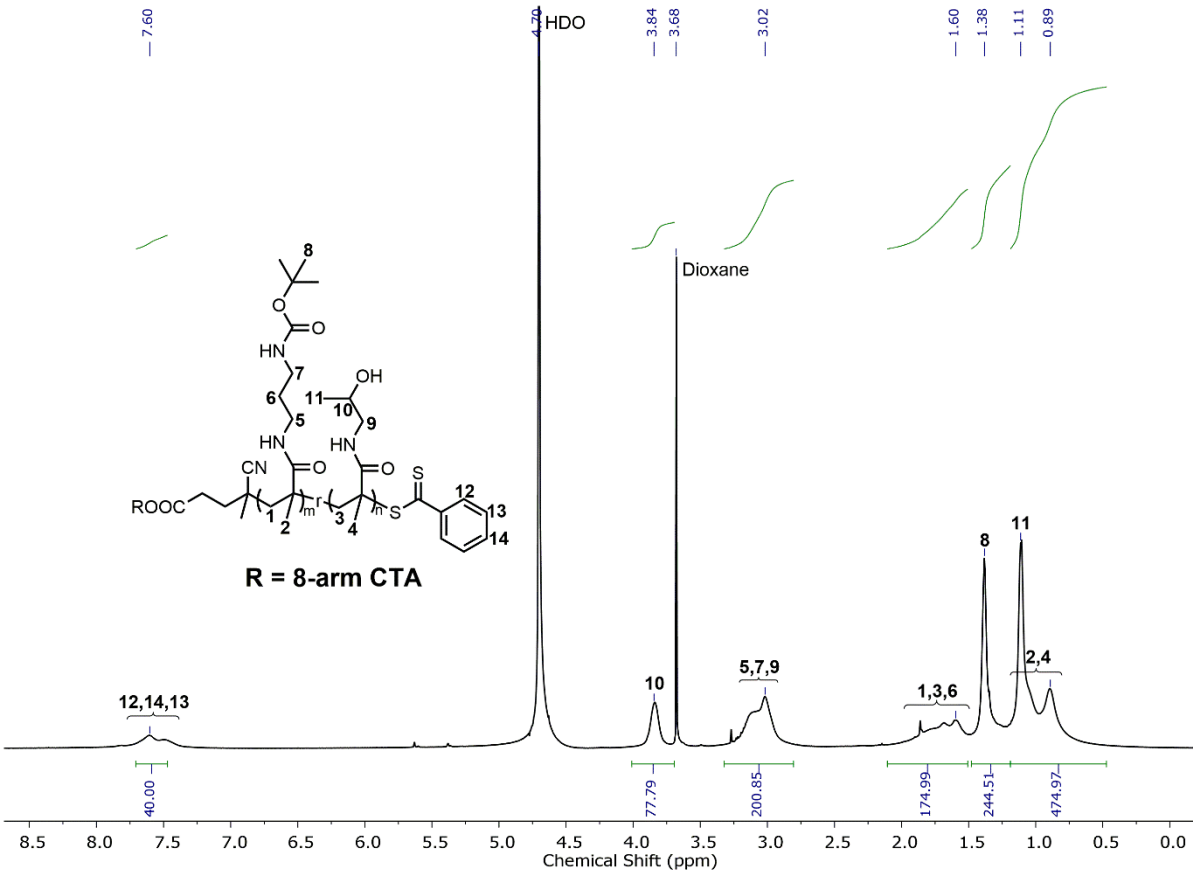

Figure S22. ^{1}H NMR spectrum of **medium DP 8-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

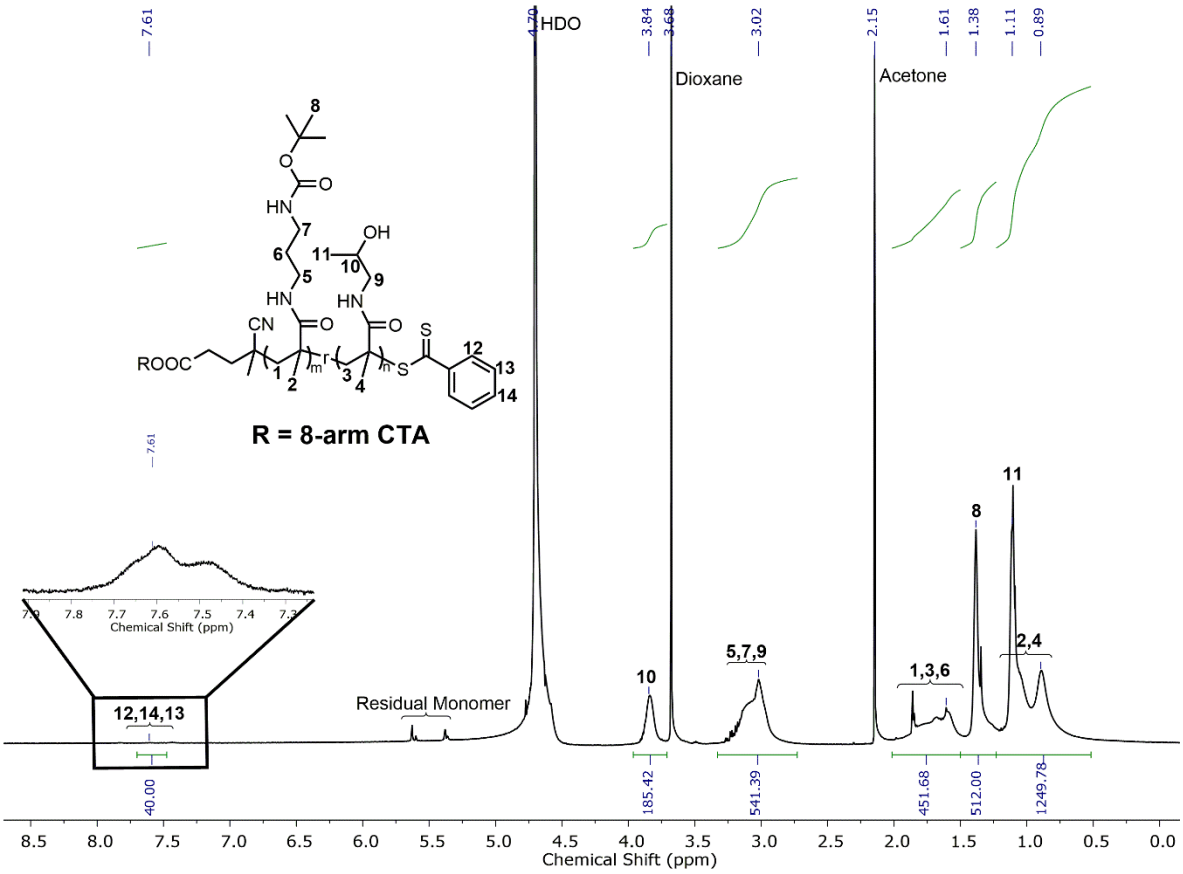

Figure S23. ^{1}H NMR spectrum of **high DP 8-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

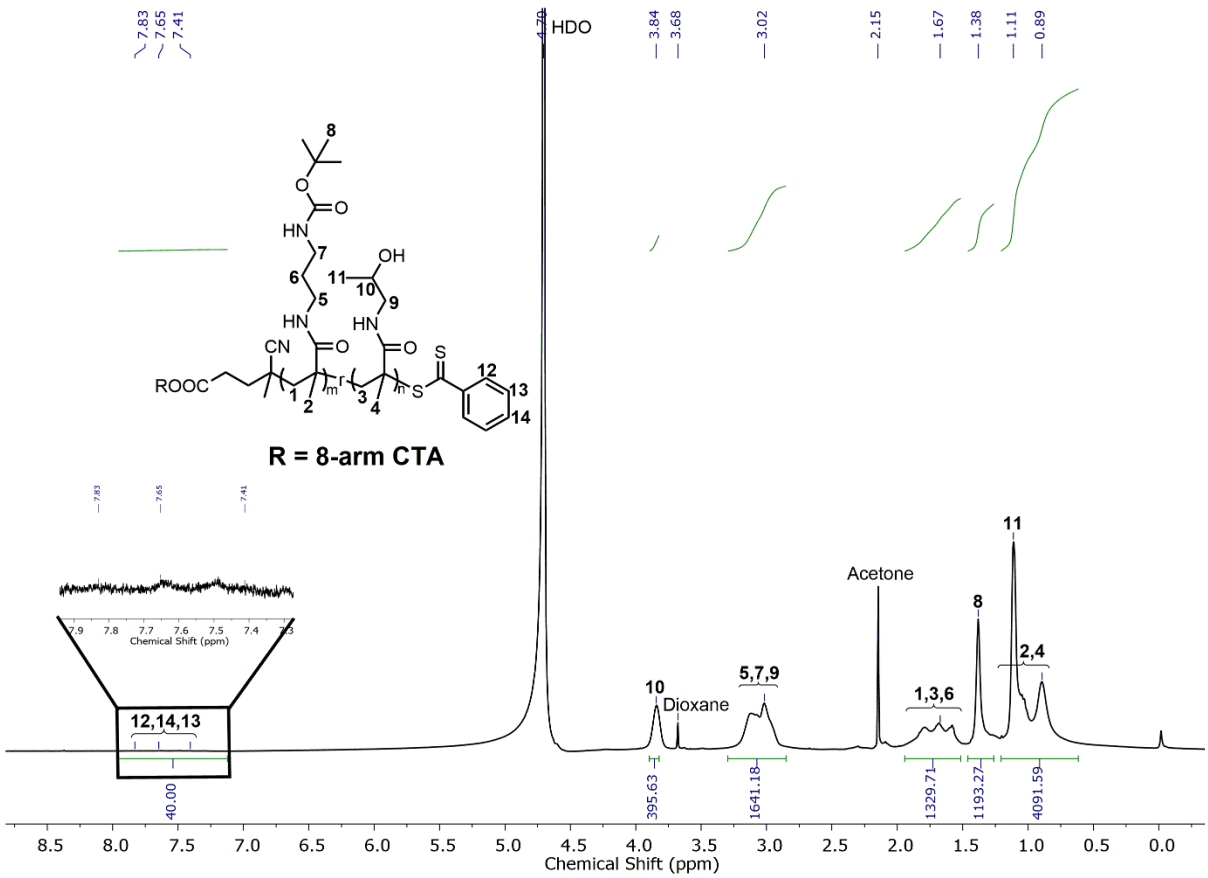

Figure S24. ^{1}H NMR spectrum of **ultra-high DP 8-arm poly(HPMA-*ran*-Boc-APMA)** (400 MHz, D_2O).

Representative ^{1}H NMR and UV-vis Spectra to Show End-group Removal

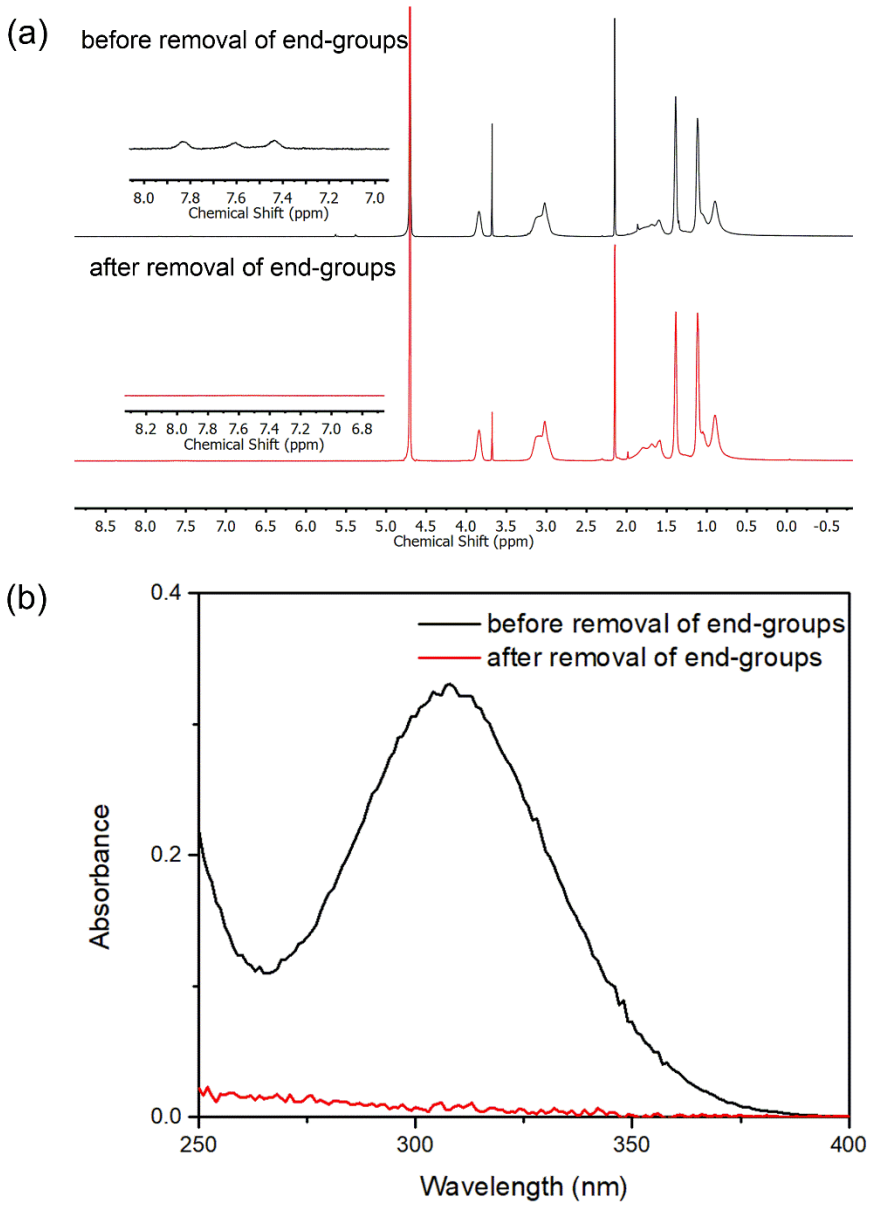

Figure S25**.** Representative example for **high DP linear poly(HPMA-*ran*-Boc-APMA)** showing the removal of the dithiobenzoate end-group: a) ^{1}H NMR spectra showing the disappearance of aromatic peaks from 7.4 – 7.9 ppm (400 MHz, D_2O); b) UV-vis spectra showing the disappearance of the peak at 308 nm corresponding to the absorption of the dithiobenzoate.

^{1}H NMR Spectra of Polycations

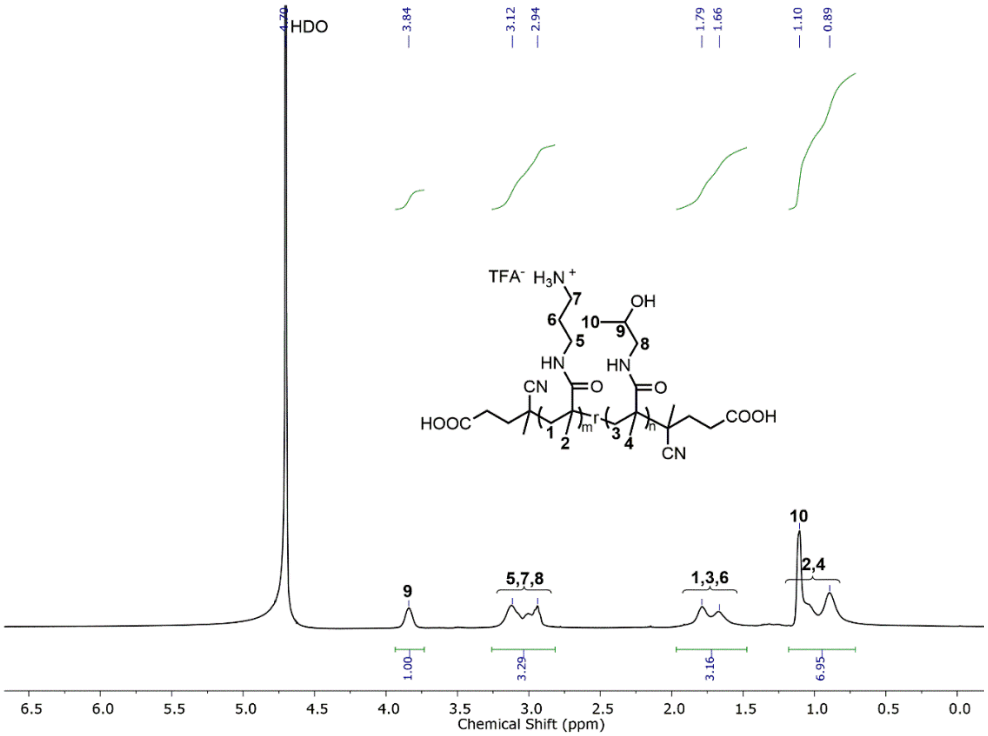

Figure S26. ^{1}H NMR spectrum of **low DP linear poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

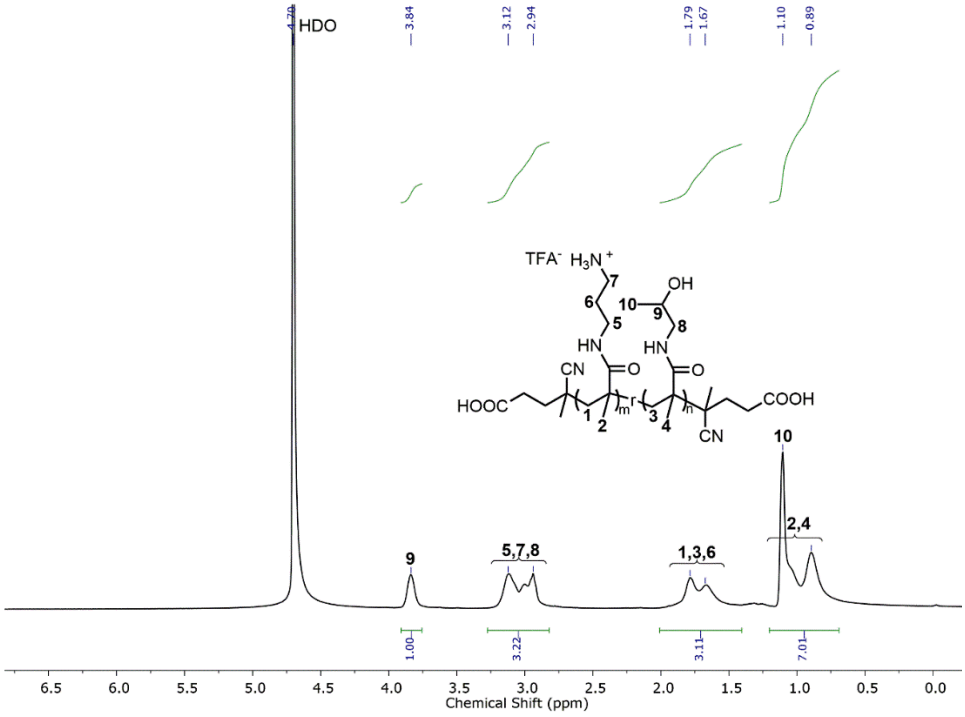

Figure S27. ^{1}H NMR spectrum of **medium DP linear poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

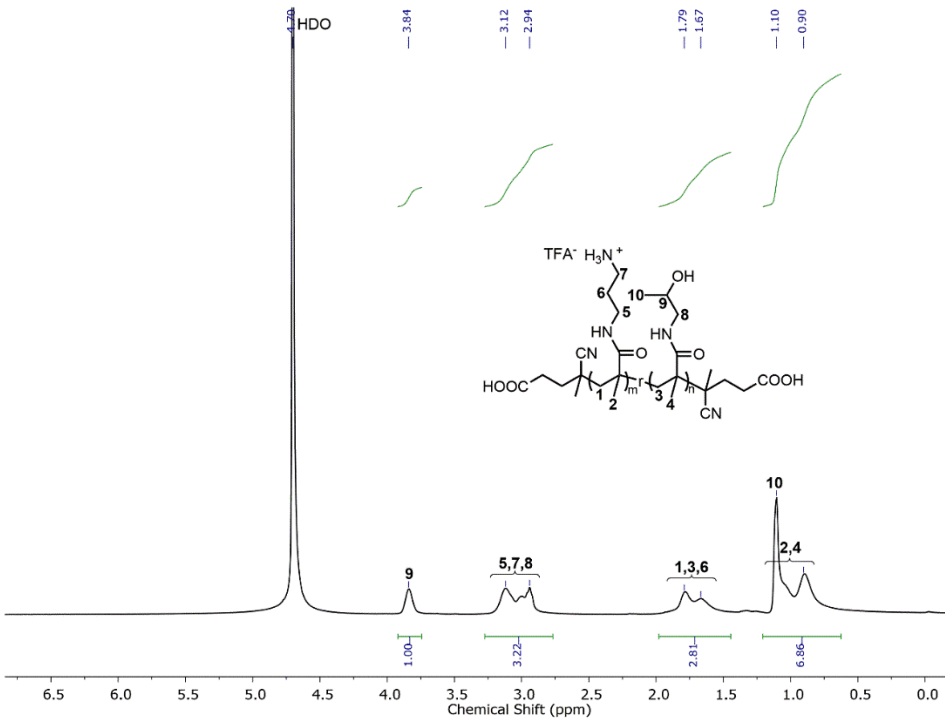

Figure S28. ^{1}H NMR spectrum of **high DP linear poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

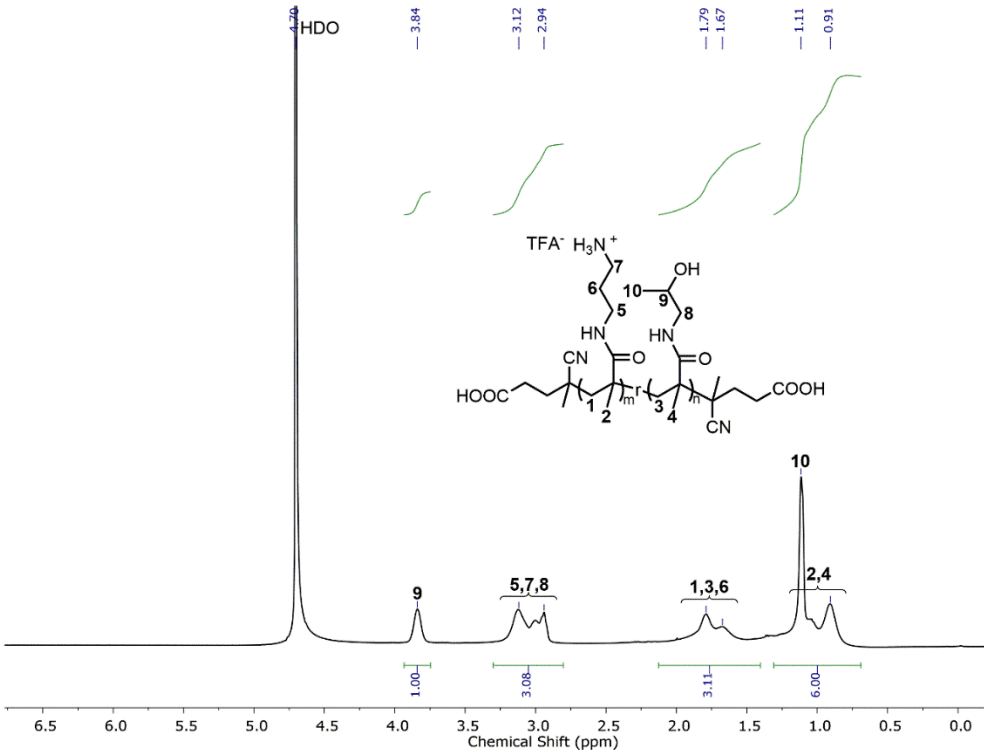

Figure S29. ^{1}H NMR spectrum of **ultra-high DP linear poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

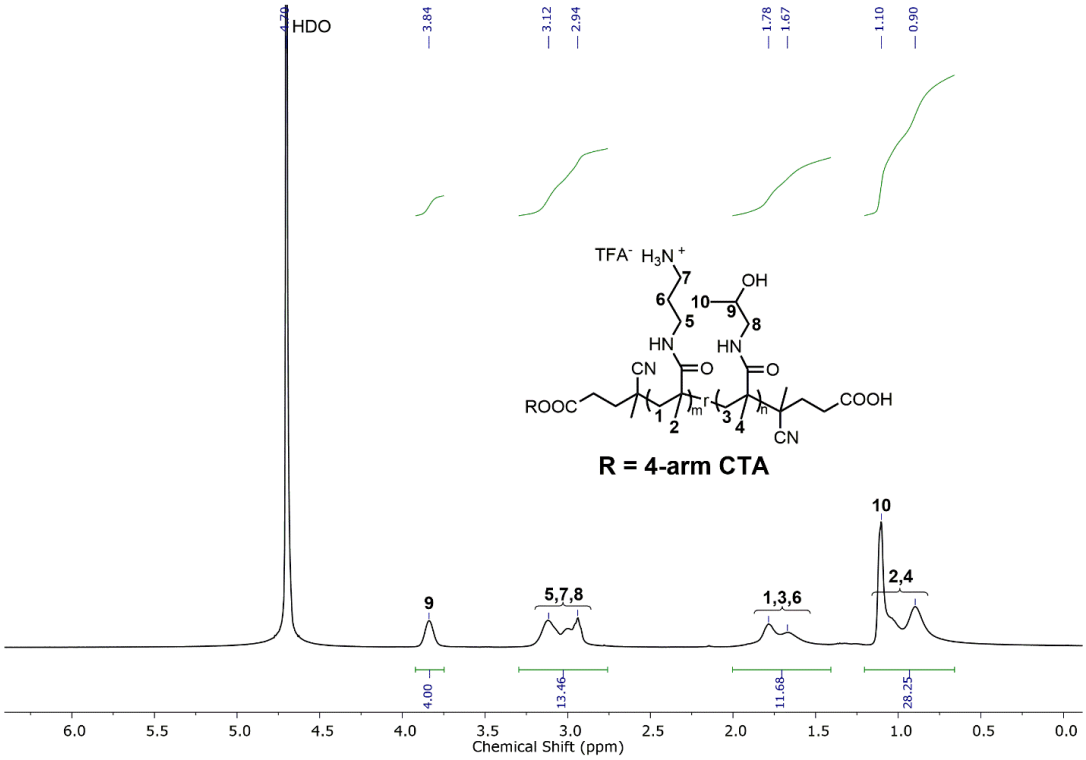

Figure S30. ^{1}H NMR spectrum of **low DP 4-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

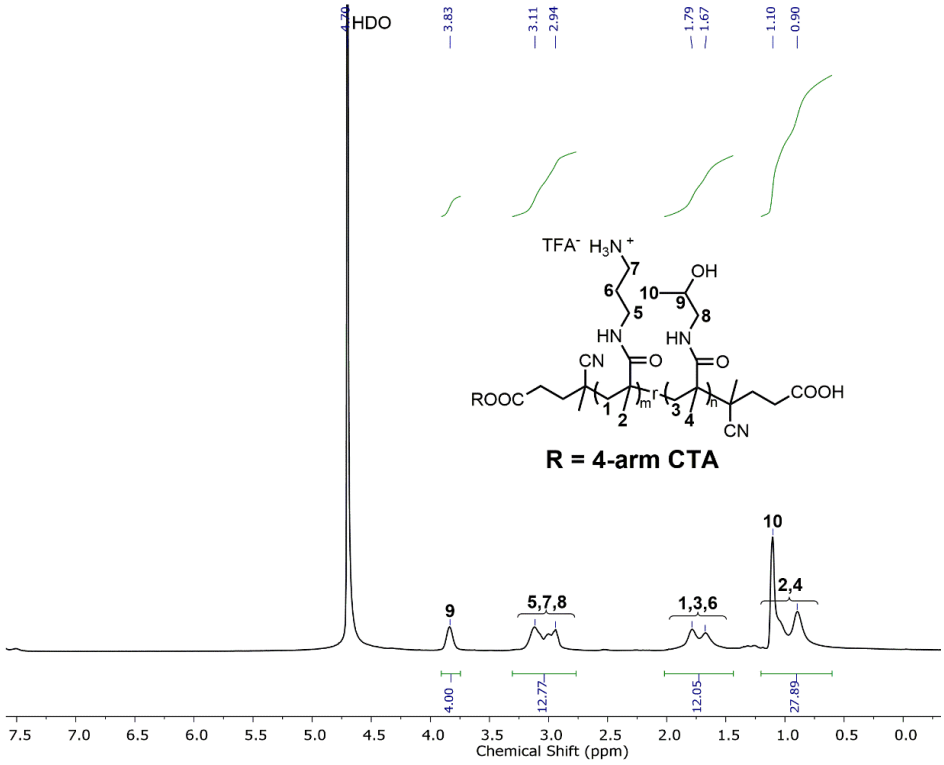

Figure S31. ^{1}H NMR spectrum of **medium DP 4-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

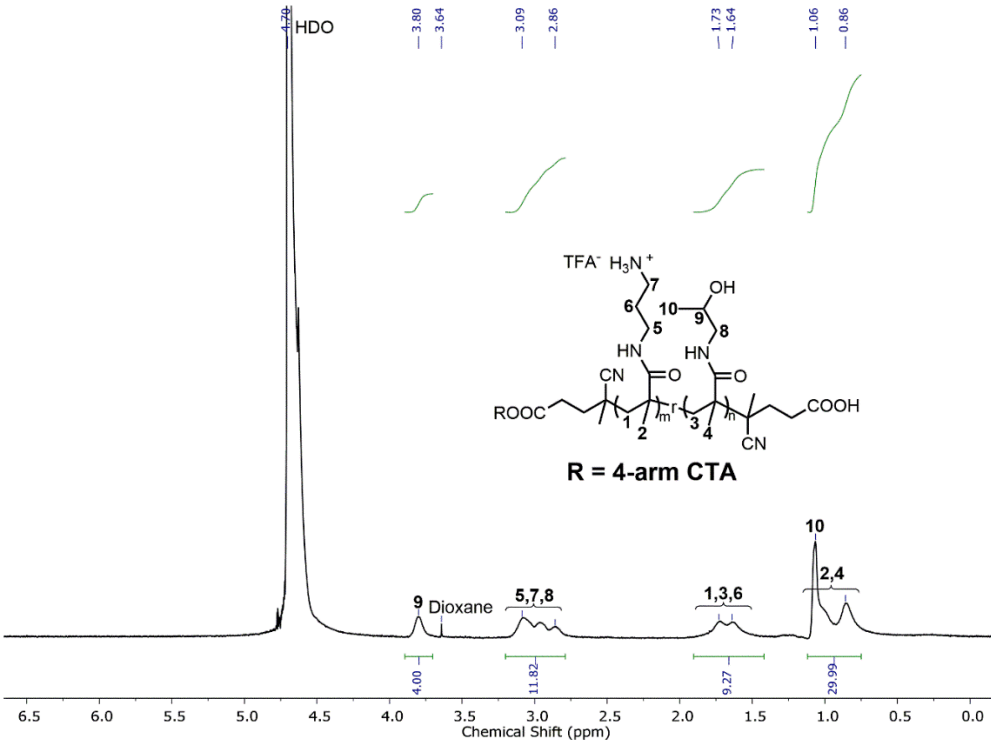

Figure S32. ^{1}H NMR spectrum of **high DP 4-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

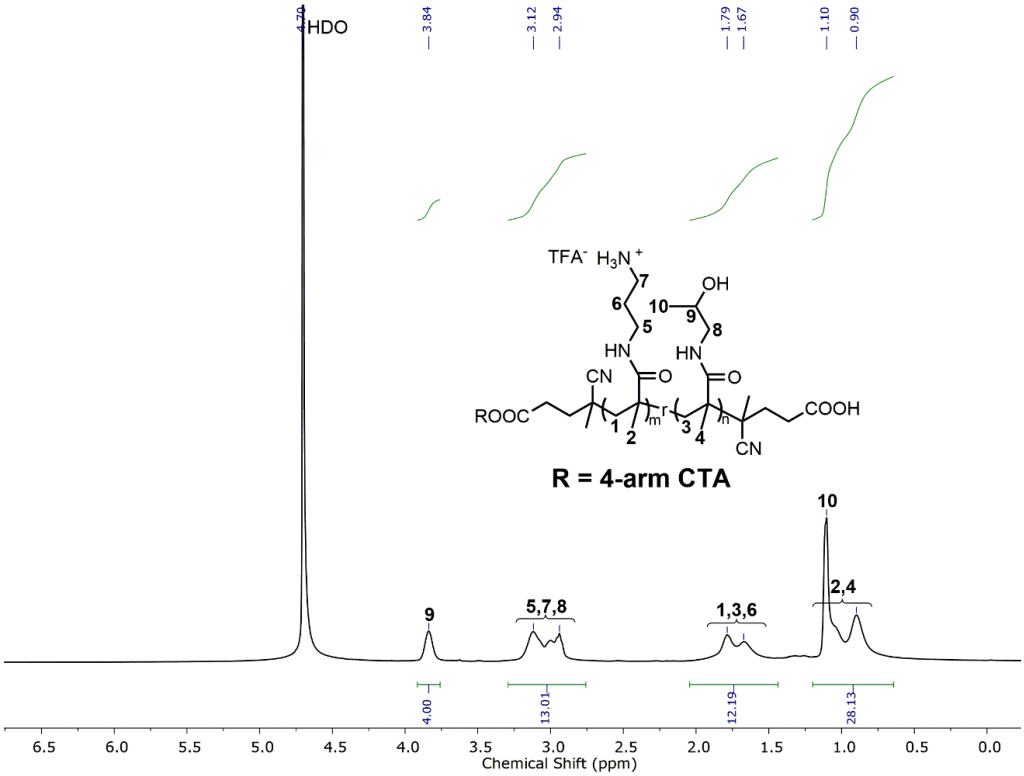

Figure S33. ^{1}H NMR spectrum of **ultra-high DP 4-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

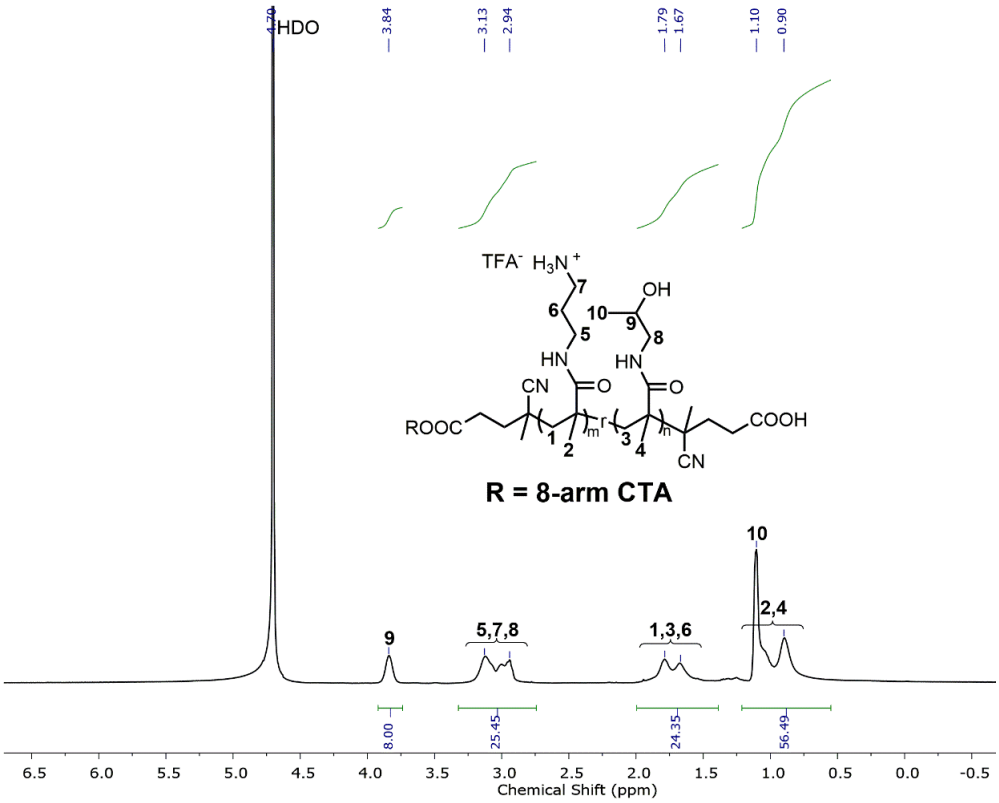

Figure S34. ^{1}H NMR spectrum of **low DP 8-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

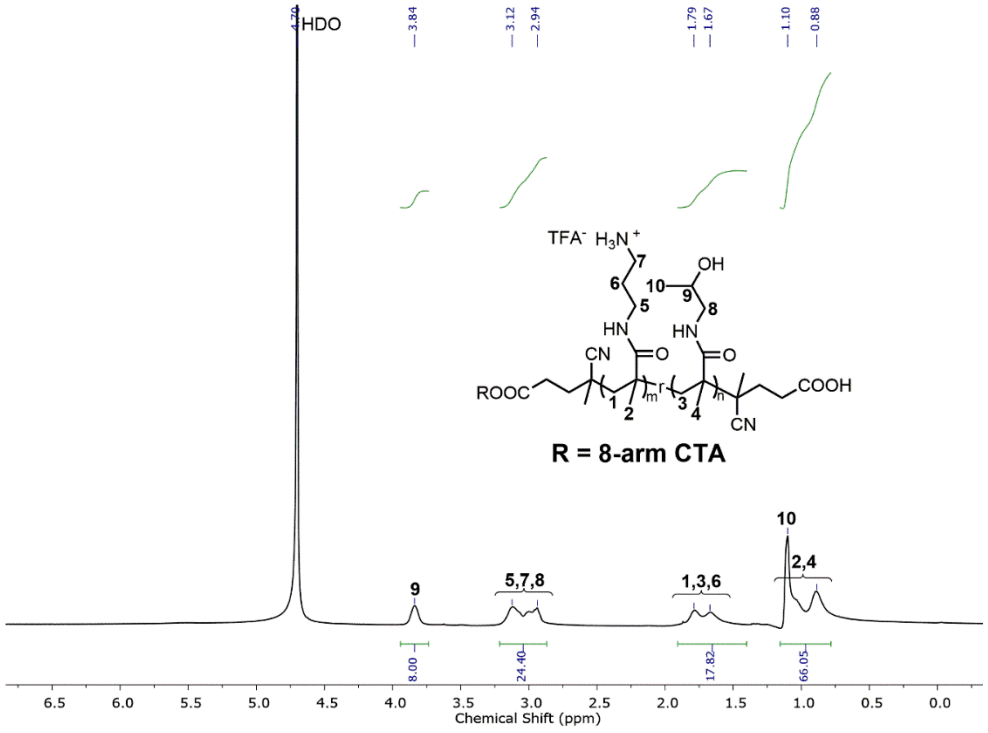

Figure S35. ^{1}H NMR spectrum of **medium DP 8-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

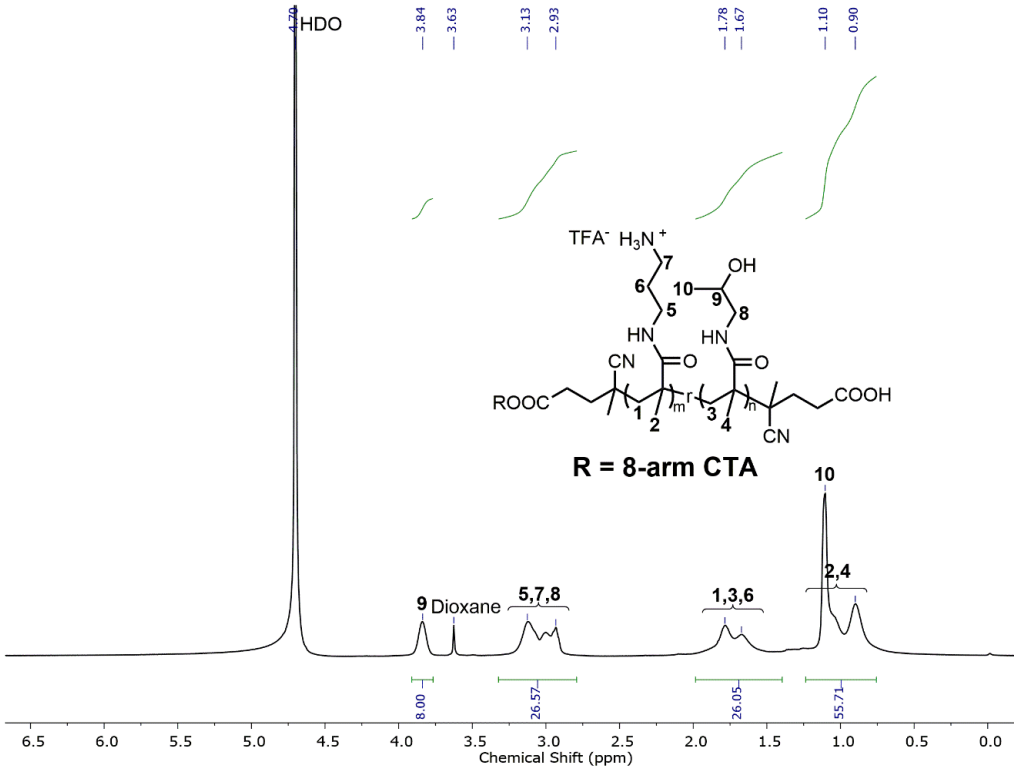

Figure S36. ^{1}H NMR spectrum of **high DP 8-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

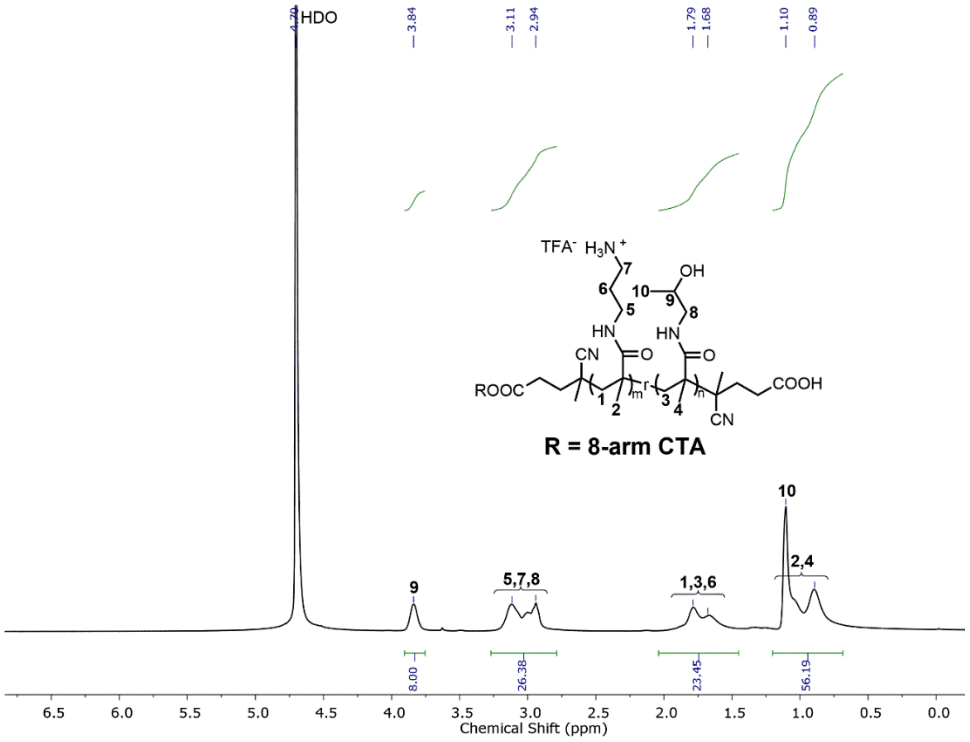

Figure S37. ^{1}H NMR spectrum of **ultra-high DP 8-arm poly(HPMA-*ran*-APMA)** (400 MHz, D_2O).

^{13}C NMR Spectra of Monomers, 4-Arm and 8-Arm CTAs and Precursors

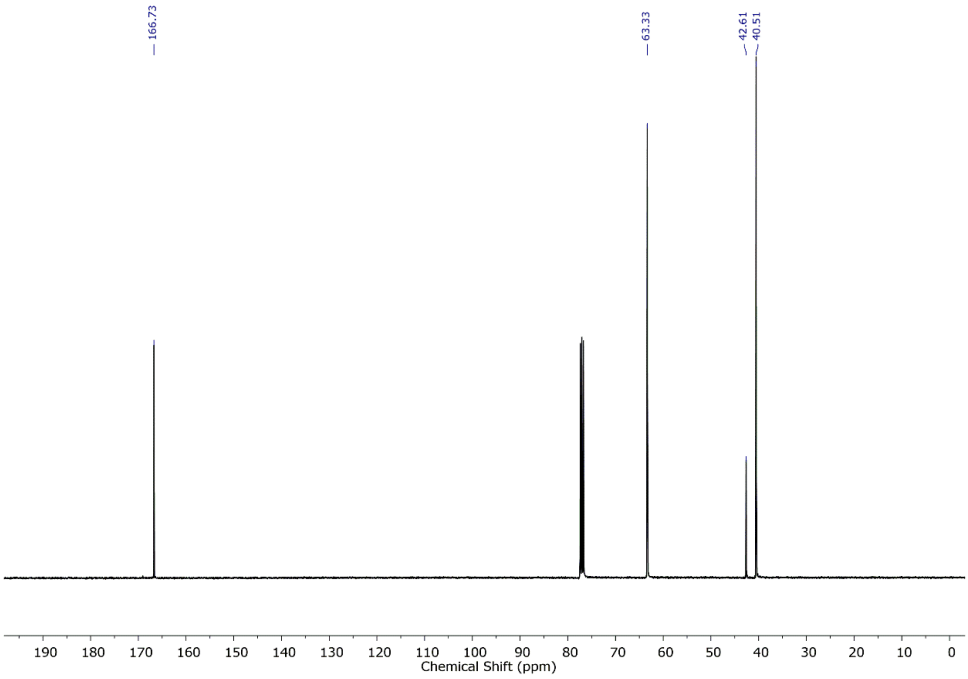

Figure S38. $^{13}C\{^1H\}$ NMR spectrum of Compound **1** (400 MHz, $CDCl_3$).

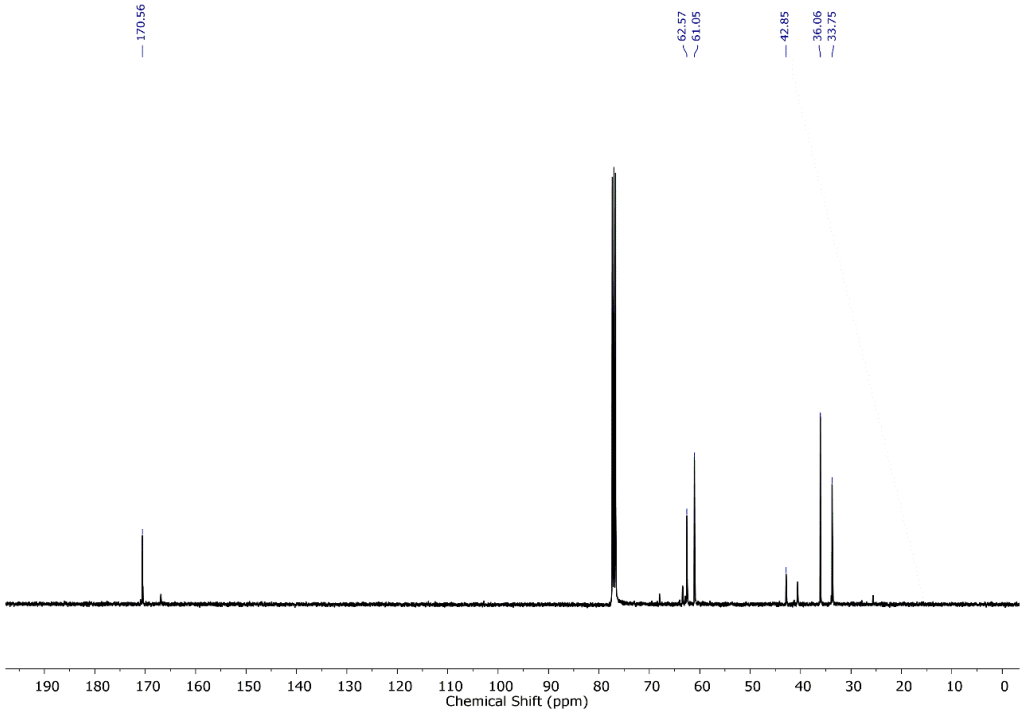

Figure S39. $^{13}C\{^1H\}$ NMR spectrum of Compound **2** (400 MHz, $CDCl_3$).

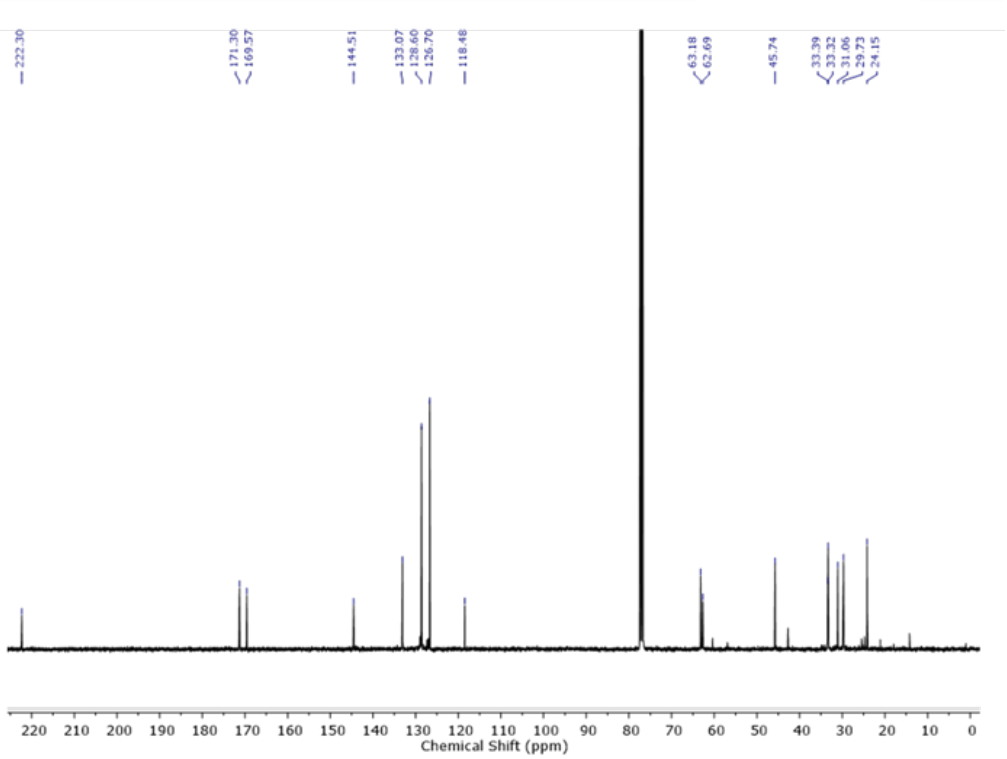

Figure S40. $^{13}C\{^{1}H\}$ NMR spectrum of the **4-arm CTA** (400 MHz, $CDCl_3$).

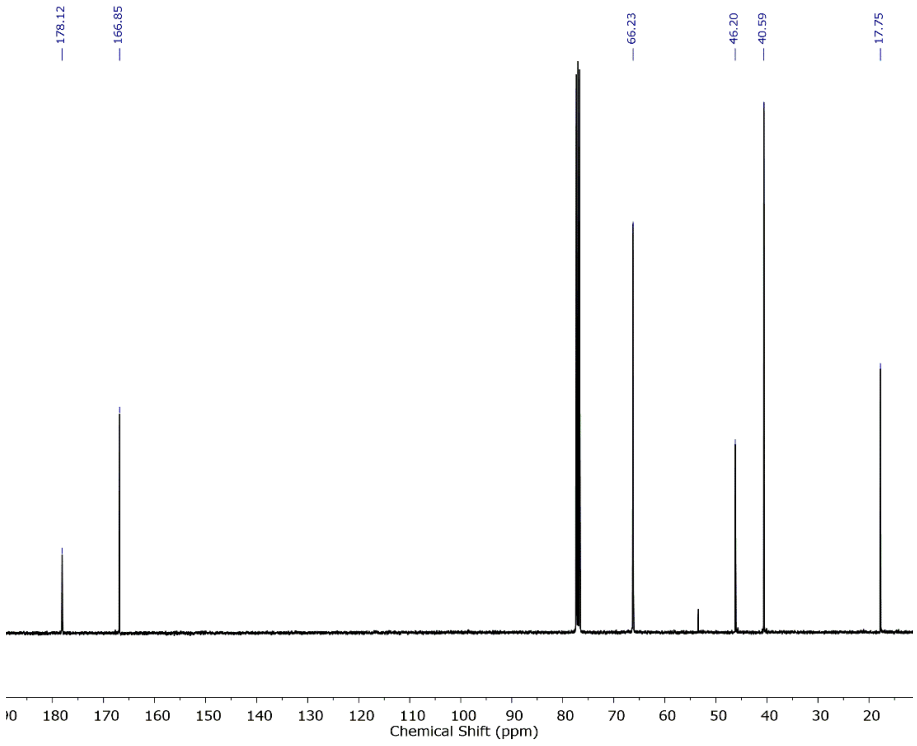

Figure S41. $^{13}C\{^{1}H\}$ NMR spectrum of Compound **3** (400 MHz, $CDCl_3$).

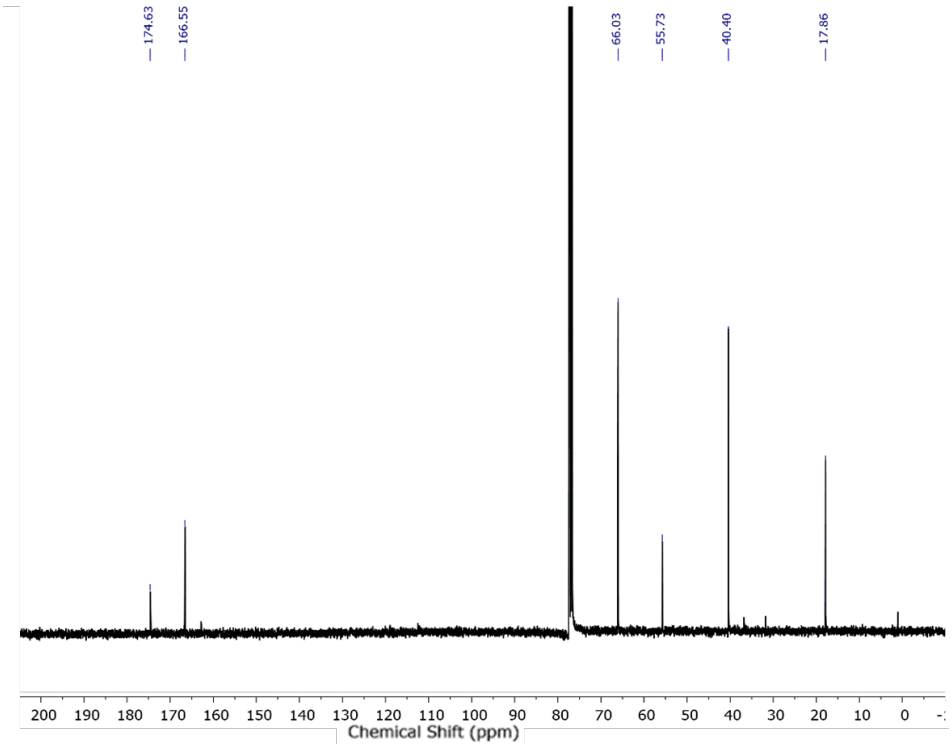

Figure S42. $^{13}C\{^{1}H\}$ NMR spectrum of Compound **4** (400 MHz, $CDCl_3$).

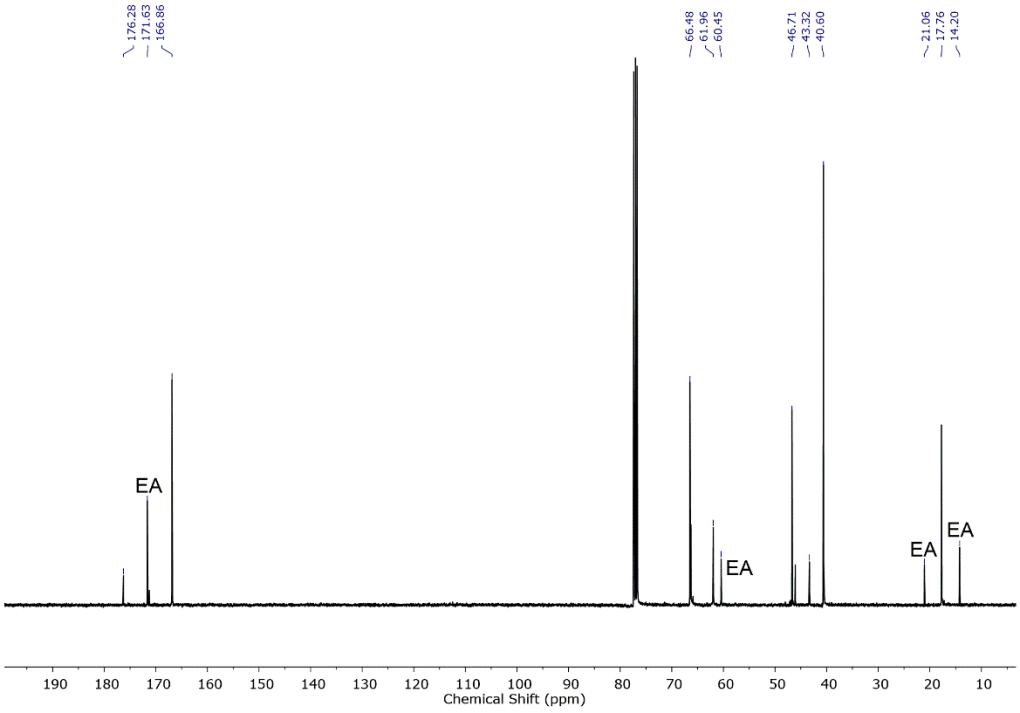

Figure S43. $^{13}C\{^{1}H\}$ NMR spectrum of Compound **5** (400 MHz, $CDCl_3$). Peaks at 171.6, 60.5, 21.1 and 14.2 ppm correspond to ethyl acetate (EA).

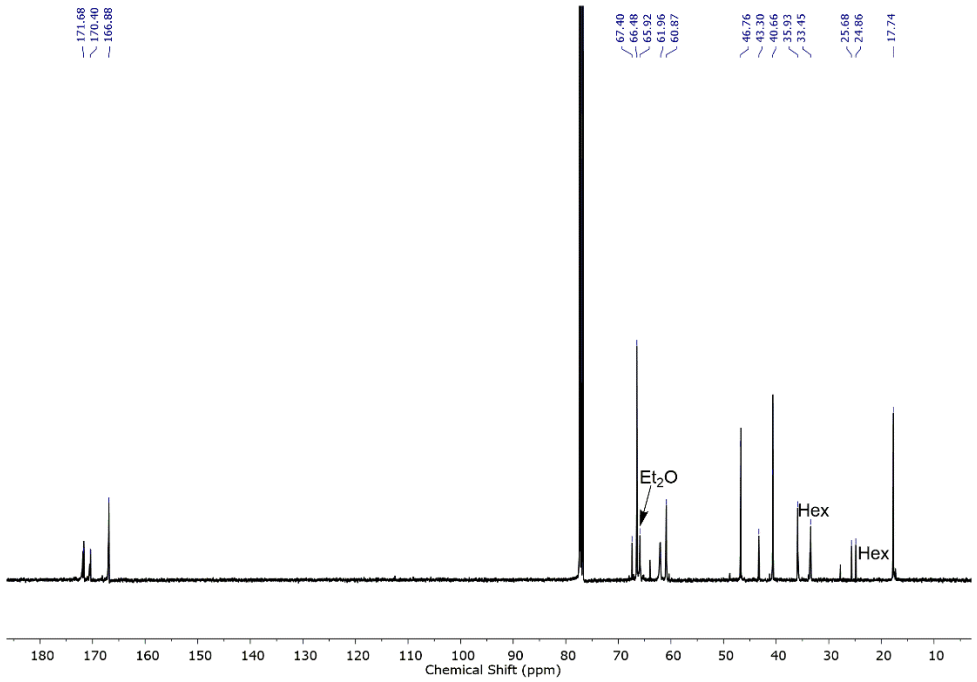

Figure S44. $^{13}C\{^{1}H\}$ NMR spectrum of Compound **6** (400 MHz, $CDCl_3$). Peaks at 24.9 and 33.4 ppm correspond to hexanes (Hex) and the peak at 65.9 ppm corresponds to diethyl ether (Et_2O).

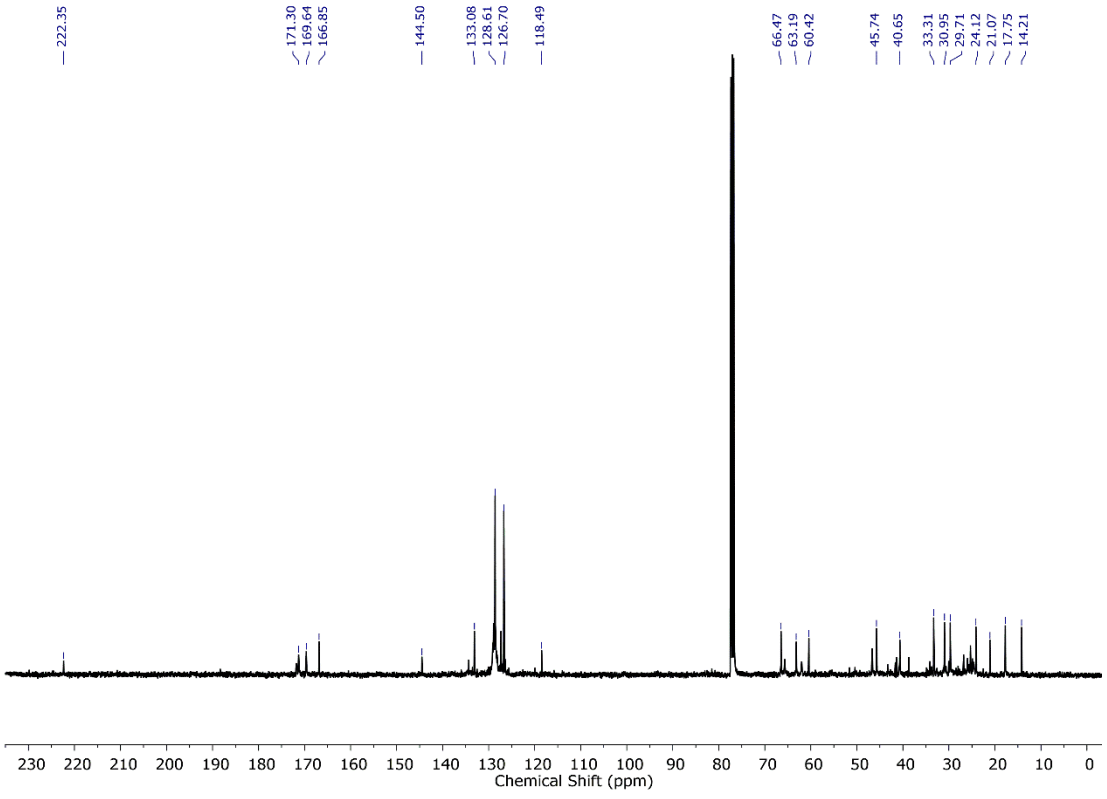

Figure S45. $^{13}C\{^{1}H\}$ NMR spectrum of **8-arm CTA** (400 MHz, $CDCl_3$).

Monomer conversion rates for the 4-arm and 8-arm copolymers

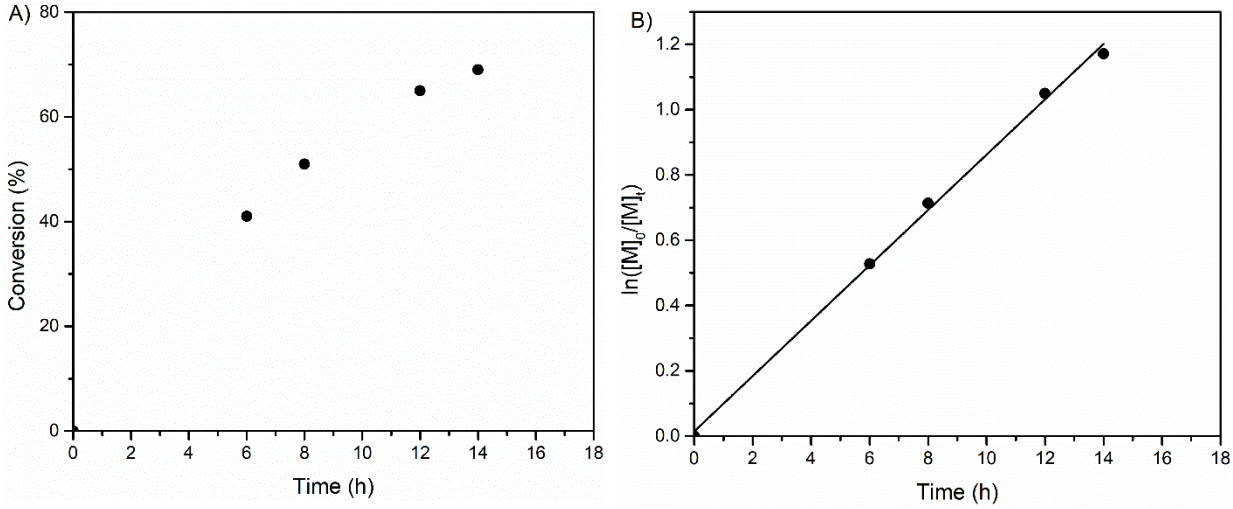

Figure S46. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**4-arm CTA**]/[V-501]=102:34:1:1.38. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

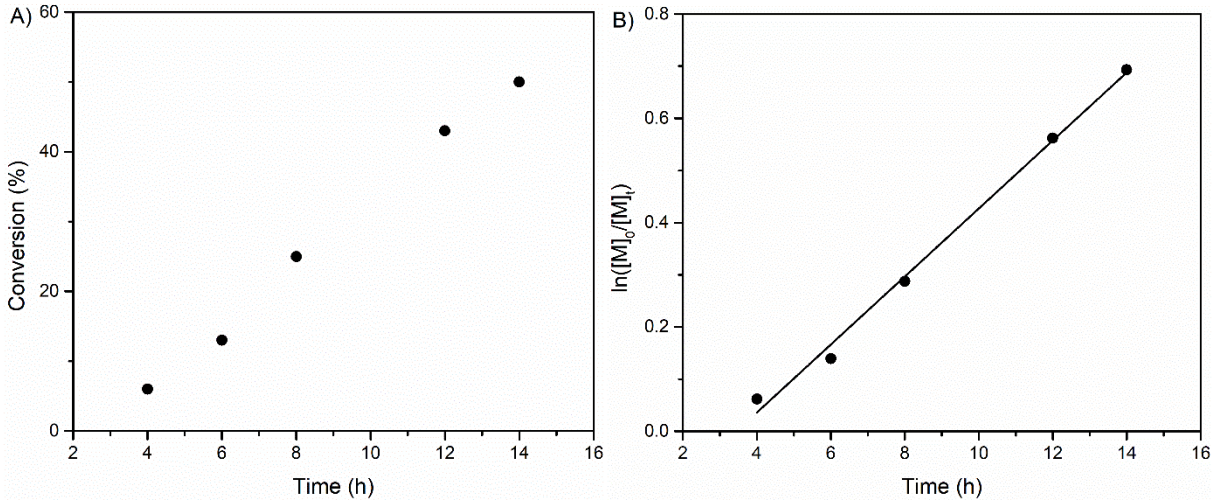

Figure S47. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**4-arm CTA**]/[V-501]=167:56:1:1.38. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

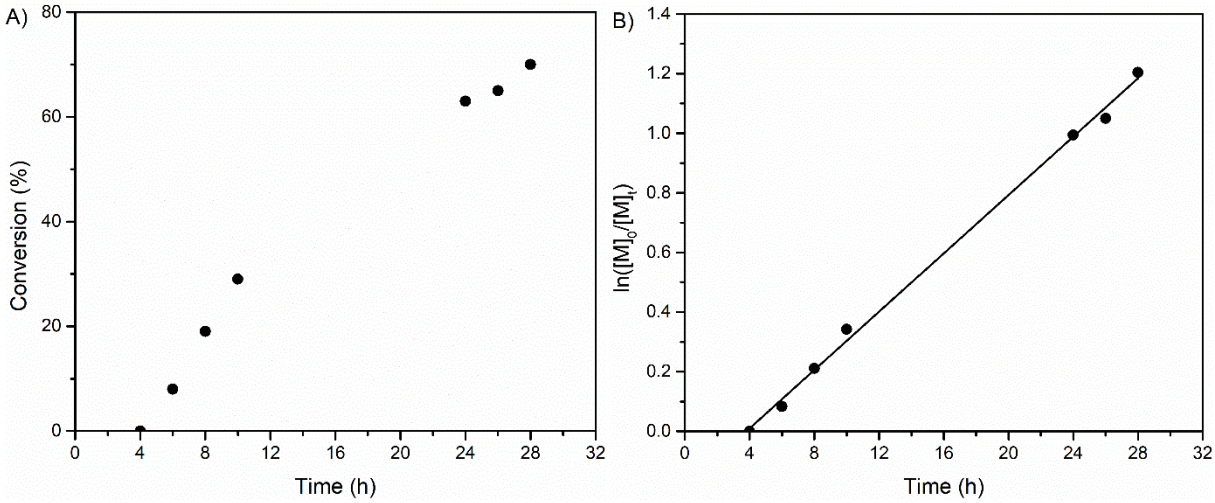

Figure S48. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**4-arm CTA**]/[V-501]=306:102:1:1.38. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

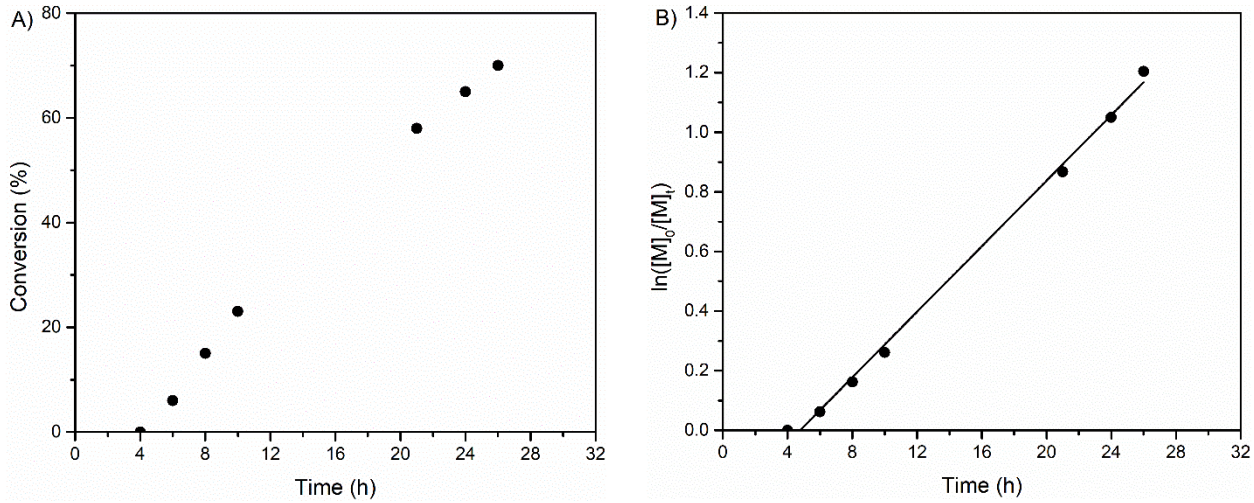

Figure S49. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**4-arm CTA**]/[V-501]=573:191:1:1.38. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

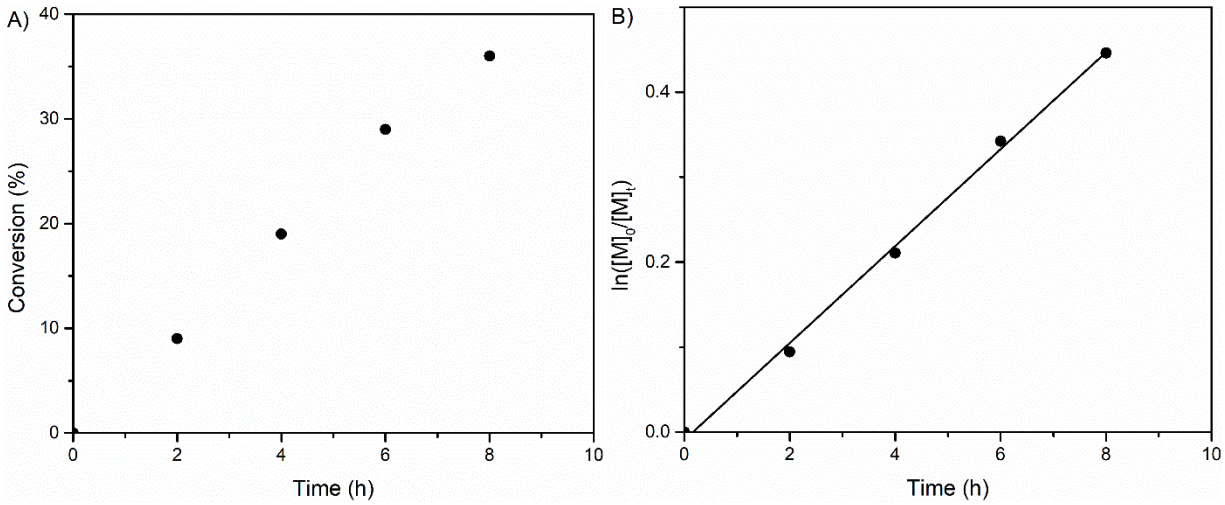

Figure S50. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**8-arm CTA**]/[V-501]=171:57:1:2.5. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

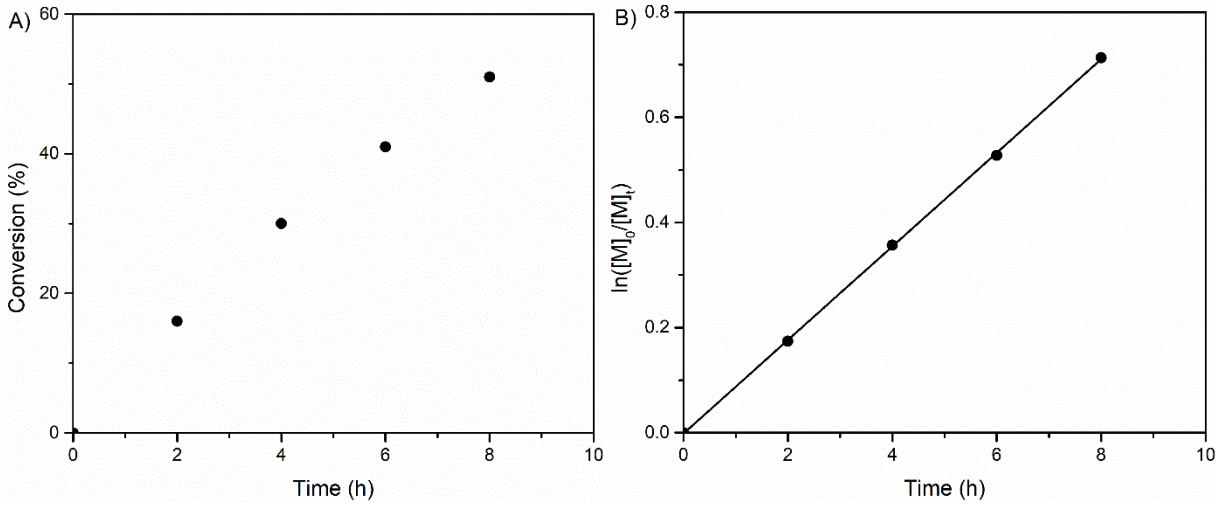

Figure S51. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**8-arm CTA**]/[V-501]=278:93:1:2.5. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

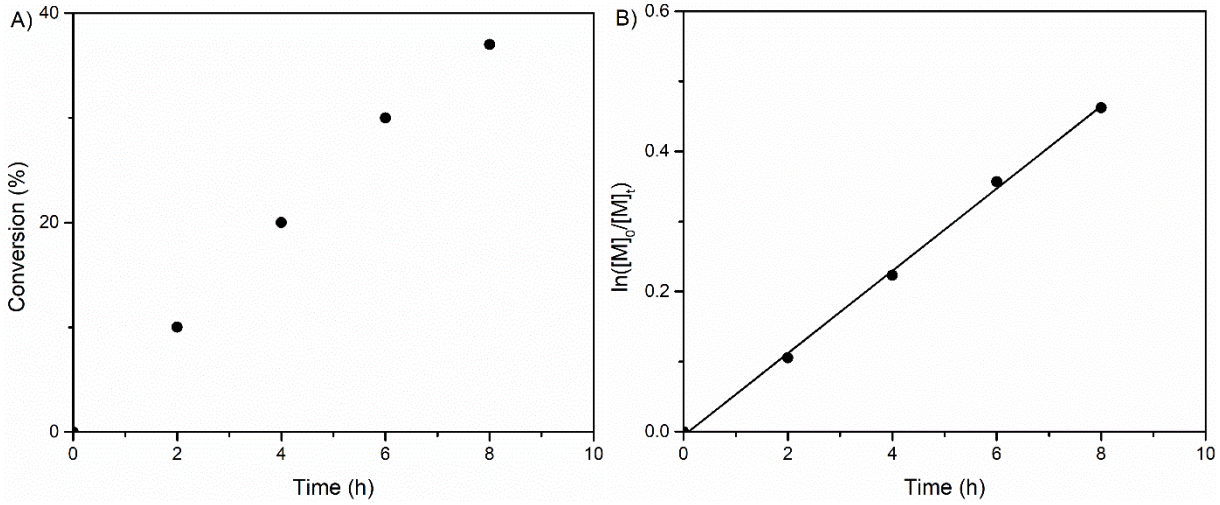

Figure S52. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**8-arm CTA**]/[V-501]=713:238:1:2.5. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

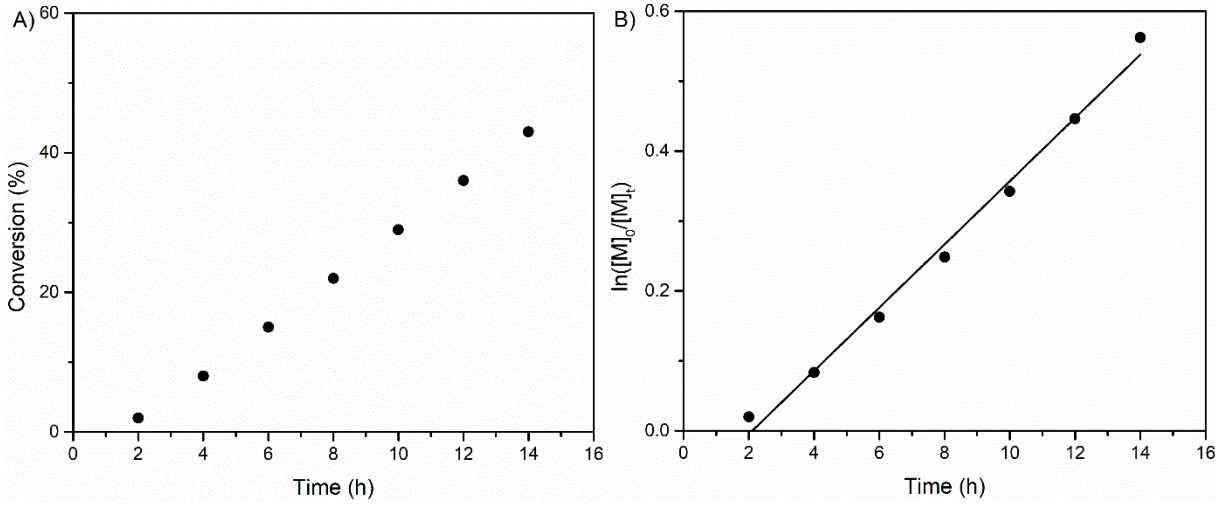

Figure S53. Kinetics of solution RAFT polymerization of HPMA and Boc-APMA using a molar ratio of [HPMA]/[Boc-APMA]/[**8-arm CTA**]/[V-501]=1335:445:1:2.5. (A) Monomer conversion versus reaction time. (B) $\ln([M]_0/[M]_t)$ versus reaction time.

Hydrodynamic radii and zeta potential

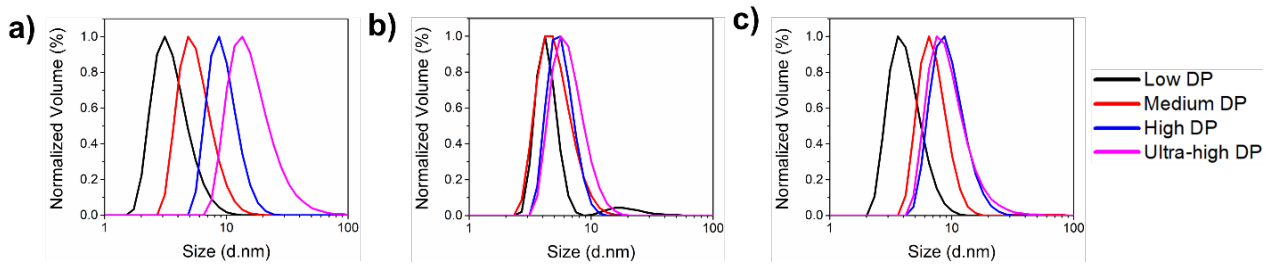

Figure S54. Volume distributions from dynamic light scattering for (A) linear; (B) 4-arm; (C) 8-arm poly(HPMA-r-APMA) copolymers in 1.5 g/L bicarbonate buffer, pH 7.4.

Table S2. Summary of hydrodynamic diameters (peak from the volume distribution) and zeta potential for poly(HPMA-r-APMA) copolymers in 1.5 g/L bicarbonate buffer (pH 7.4). Errors on the measurements correspond to the standard deviations on triplicate measurements.

Architecture	Target DP	Hydrodynamic diameter (nm)	Zeta potential (mV)
Linear	Low	3.7 ± 1.4	0.33 ± 3.9
	Medium	5.8 ± 2.0	0.61 ± 5.5
	High	9.6 ± 2.8	5.8 ± 7.5
	Ultra-high	17 ± 9.2	6.3 ± 5.7
4-arm	Low	4.3 ± 1.0	2.5 ± 10
	Medium	5.2 ± 1.8	0.41 ± 4.8
	High	5.8 ± 1.4	1.4 ± 6.2
	Ultra-high	6.7 ± 2.2	8.6 ± 5.5
8-arm	Low	4.3 ± 1.4	0.54 ± 4.8
	Medium	7.1 ± 1.9	1.5 ± 4.8
	High	9.7 ± 3.3	8.4 ± 5.0
	Ultra-high	10 ± 5.3	10 ± 6.2

Uptake and Release Studies

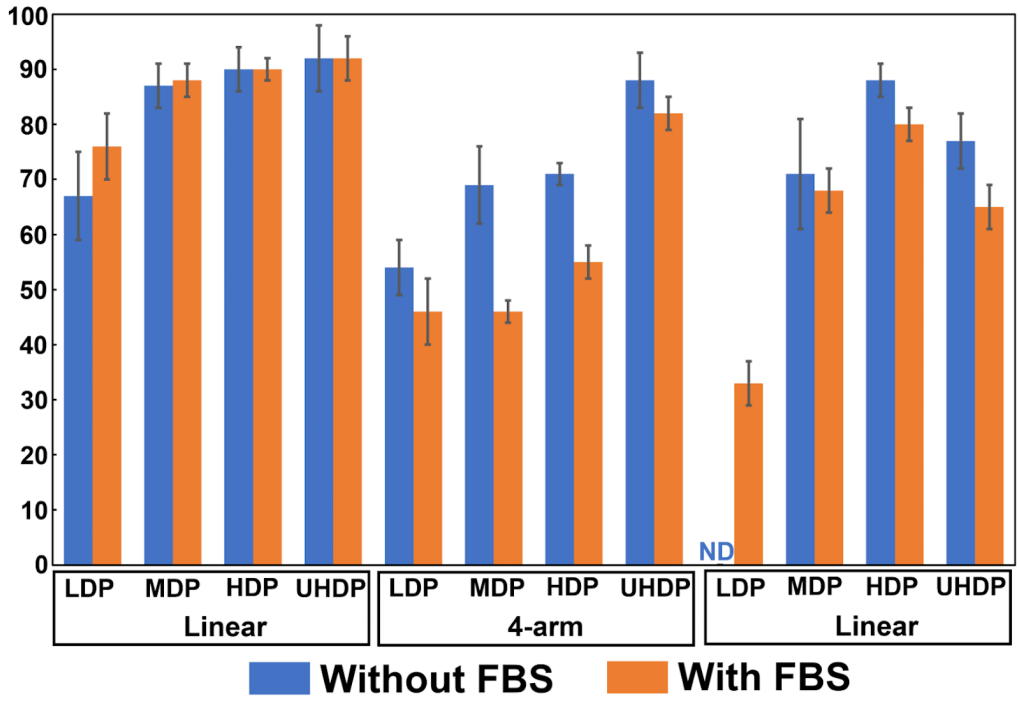

Figure S55. Percent of polycation uptaken that is retained in bovine cartilage explants without (blue) and with FBS (orange). ND = not detected (detectable levels were not taken up initially). Error bars correspond to the standard deviation.

Additional Fluorescent Microscopy Images

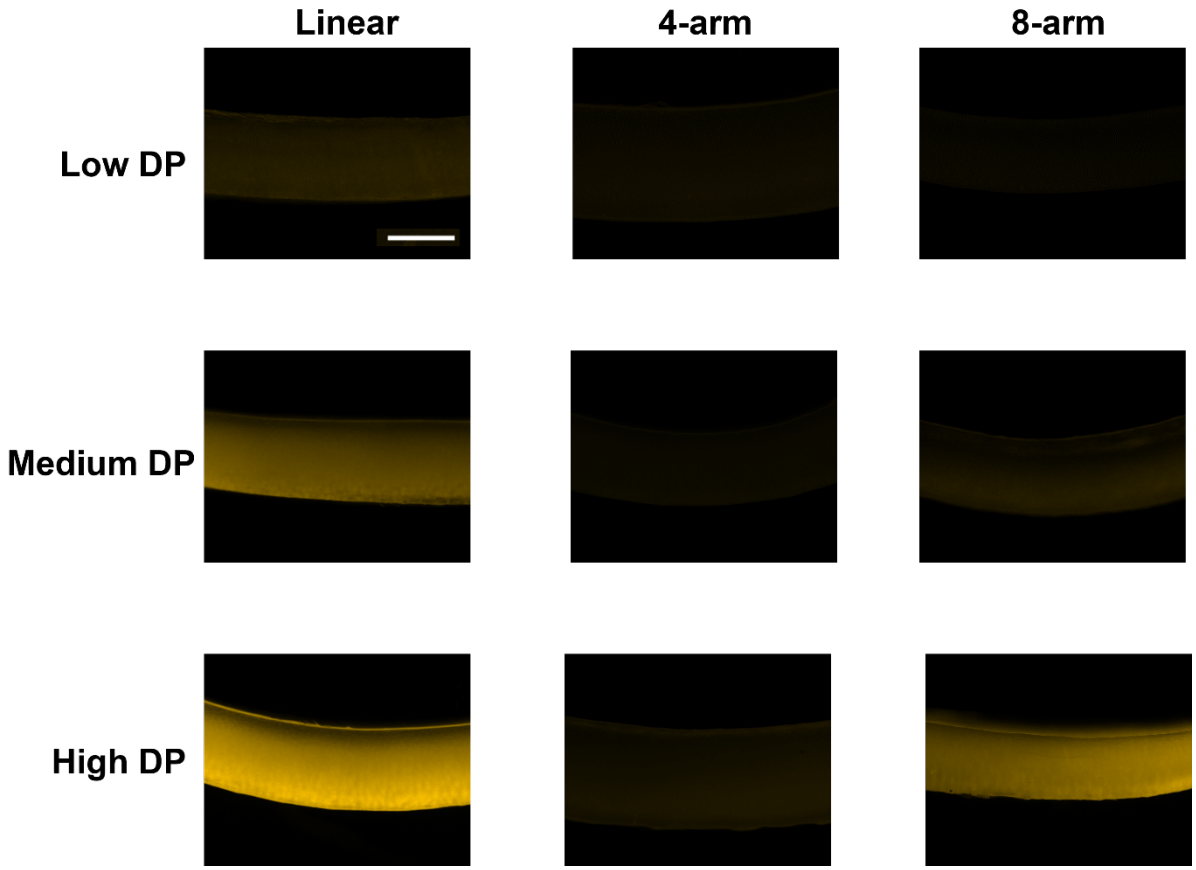

Figure S56. Fluorescent microscopy images showing cartilage uptake for linear, 4-arm, and 8-arm rhodamine-labeled poly(HPMA-*r*-APMA) in the medium without FBS. The superficial zone is at the top and the deep zone is at the bottom in each image. Scale bar = 200 μm.

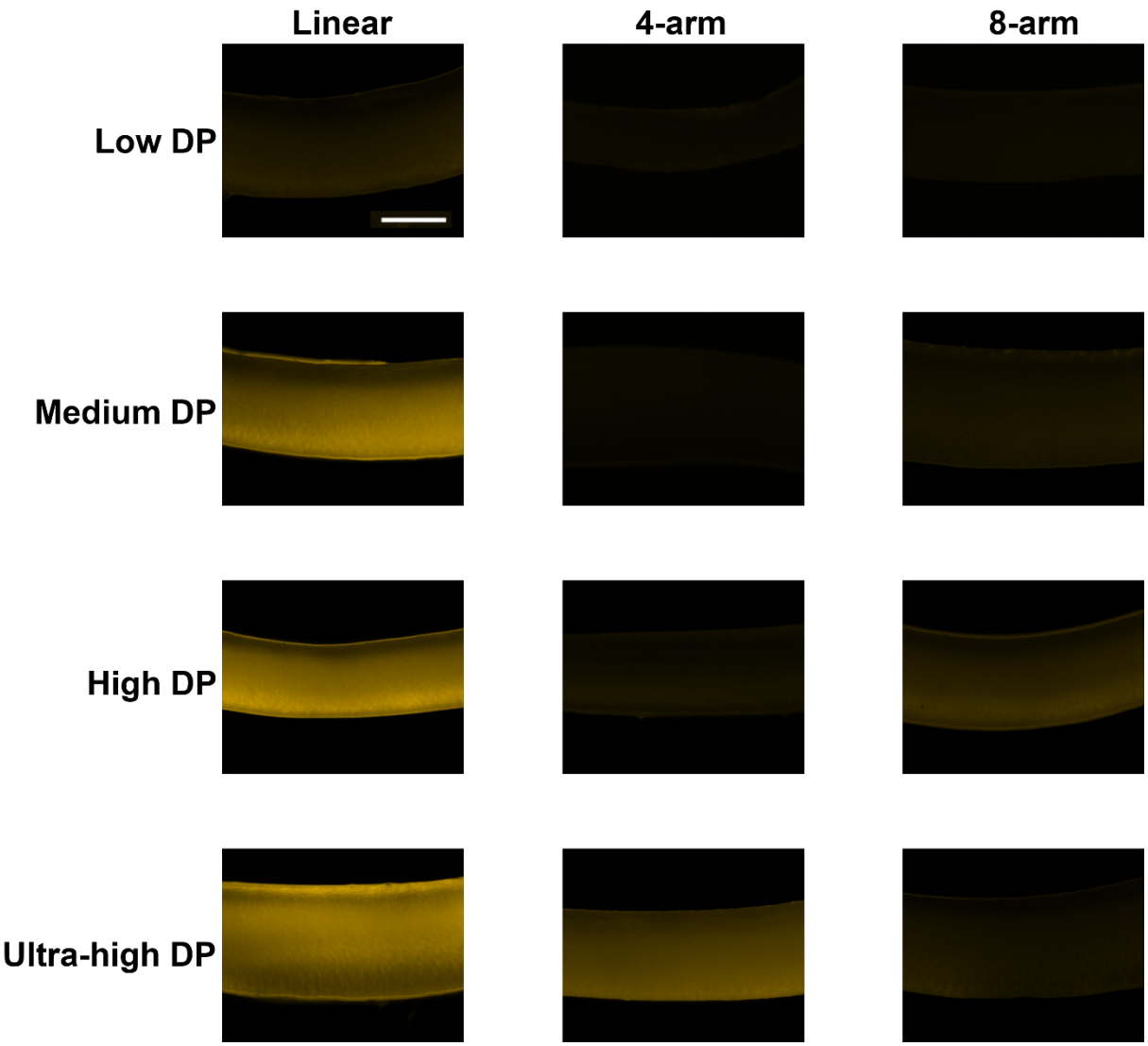

Figure S57. Fluorescent microscopy images showing cartilage uptake for linear, 4-arm, and 8-arm rhodamine-labeled poly(HPMA-*r*-APMA) in the medium with FBS. The superficial zone is at the top and the deep zone is at the bottom in each image. Scale bar = 200 μm.

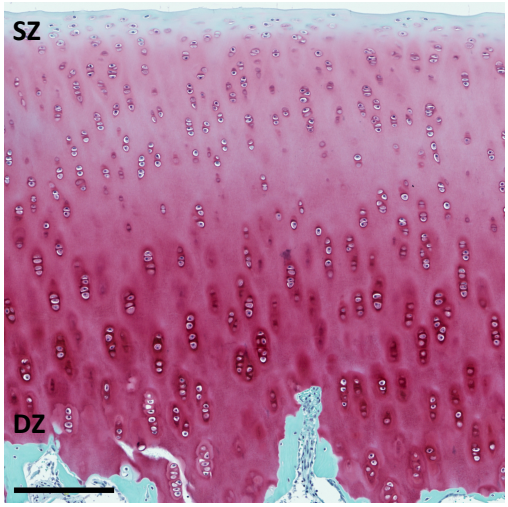

Figure S58. Image of histological section of bovine articular cartilage stained with Safranin-O and counter-stained with Fast Green showing the distribution of glycosaminoglycans through the depth of the tissue. SZ and DZ refer to superficial and deep zone, respectively. Scale bar = 200 μm.

References

1. Ghamkhari, A.; Massoumi, B.; Agbolaghi, S., An in vitro focus on doxorubicin hydrochloride delivery of novel pH-responsive poly(2-succinyloxyethylmethacrylate) and poly[(N-4-vinylbenzyl), N, N-diethylamine] diblock copolymers. *Polym. Int.* **2018,** *67*, 283-291.

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4. Amirbekyan, K. et al. Design, Synthesis, and Binding Affinity Evaluation of Hoechst 33258 Derivatives for the Development of SequenceSpecific DNA-Based Asymmetric Catalysts. *ACS Catal.* **2016**, *6*, 3096-3105.

5. Kitov, P. I. et al. Impact of the Nature and Size of the Polymeric Backbone on the Ability of Heterobifunctional Ligands to Mediate Shiga Toxin and Serum Amyloid P Component Ternary Complex Formation. *Toxins* **2011**, *3*, 1065-1088.