Supporting Information

Highly Branched Polydimethylacrylamide Copolymers as Functional Biomaterials

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Contents

1 Synthesis of TA-CTA S2

2 Polymer Synthesis and Characterization S2
  2.1 DP_{PC} 25 S2
  2.1.1 Polymer Synthesis S2
  2.1.2 NMR Spectroscopy S3
  2.1.3 Polymer Fractional Precipitation S3
  2.2 DP_{PC} 50 S3
  2.2.1 Polymer Synthesis S3
  2.2.2 Polymer Fractional Precipitation S4
  2.2.3 NMR Spectroscopy S5
  2.2.4 Fractional Precipitation SEC Traces S6
  2.3 DP_{PC} 100 S7
  2.3.1 Polymer Synthesis S7
  2.3.2 Polymer Fractional Precipitation S7
  2.3.3 Fractional Precipitation SEC Traces S7
  2.4 Exploration of Laboratory Scale Ultrafiltration S8
  2.5 Z group removal S9

3 Amine Conjugations to Polymer S10
  3.1 Maintain CTA reactivity through Synthesis and Workup S10
  3.2 Conjugation of Benzylamine to Highly Branched Polymer S11
  3.3 Conjugation of $\alpha$-CD to Highly Branched Polymer S11
  3.4 SEC traces after conjugation S12

4 Degradation Assays S13
  4.1 Basic Degradation S13
  4.2 Acidic Degradation S14
1 Synthesis of TA-CTA

The synthesis was adapted from literature. 4-Cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (10 g, 24.7 mmol, 1 eq), 2-thiazoline-2-thiol (3.807 g, 31.9 mmol, 1.29 eq), and 4-dimethylaminopyridine (0.435 g, 3.55 mmol, 0.143 eq) were dissolved in 200 mL of dry DCM. N,N’-Diisopropylcarbodiimide (3.59 g, 28.4 mmol, 1.15 eq) in 30 mL dry DCM was added at 0°C. The solution was brought to room temperature while stirring for 24 hours. The solution was washed with 30 mL of water twice. Product was extracted from an emulsion that formed at the interface via repeated washes with DCM. The organic phase was dried with sodium sulfate, reduced, and purified on a Biotage, yielding an orange oil which crystalized overnight into an orange solid with poppy-like brilliance (8.214 grams, 16.3 mmol, 66 % yield).

![Figure S1: 1H NMR spectrum of TA-CTA in DMSO.](image)

2 Polymer Synthesis and Characterization

2.1 DP<sub>PC</sub> 25

2.1.1 Polymer Synthesis

DMA-co-NPGDA highly branched polymer targeting a CTA:MVM ratio of 1.45:1 and a CTA:VM of 25 is as follows. DMA (4 g, 40.4 mmol, 25 eq, filtered through basic alumina), NPGDA (497 mg, 2.34 mmol, 1.45 eq), TA-CTA (0.815 g, 1.61 mmol, 1 eq), and AIBN (353 mg, 0.32 mmol, 0.2 eq) were diluted with DMF to a total volume of 10 mL ([DMA] = 4M). The solution was added to a 20 ml scintillation vial equipped with a PTFE septa. The reaction mixture was sparged with N₂ for 15 minutes and heated to 24 hours at 65 °C. AIBN (53 mg, 0.32 mmol, 0.2 eq) was then added to the scintillation vial, which was sparged with N₂ for 15 minutes and heated for 12 hours at 65 °C. Monomer conversion was determined by 1H NMR spectroscopy and the $M_n$ and $Ð$ were obtained by SEC-MALLS.
2.1.2 NMR Spectroscopy

Figure S2: $^1$H NMR spectra of DMA-co-NPGDA targeting a NPGDA:CTA ratio of 1.45:1 and a DP$_{PC}$ 25 (DMA:CTA ratio of 1). Traces show pre-polymerization (red), after 24 hours (green), and after another 12 hours (blue) after the addition of extra AIBN initiator (0.2 eq compared to the CTA). Conversion determined to be 93.4% (green, 24 hours) and 99.9%+ (blue, extra AIBN and 12 hours) Inlet shows disappearance of monomer vinyl peaks (6.6 - 5.5 ppm) while preserving activity of the TA group on the CTA (4.5 ppm)

2.1.3 Polymer Fractional Precipitation

3 grams of highly branched copolymer was dissolved in 30 mL of dioxane in a 1 L round bottom flask. To the solution, with turbulent stirring, a 65:35 (by volume) mixture of ether:dioxane was added until the solution turned cloudy and a viscous yellow phase separated on the bottom of the flask (350 mL). The supernatant was decanted. The deep yellow, viscous, high molecular weight fraction was diluted in dioxane and precipitated into ether to generate the first fraction. This recovered 1 g of the first fraction (33% yield).

2.2 DP$_{PC}$ 50

2.2.1 Polymer Synthesis

DMA-co-NPGDA highly branched polymer targeting a CTA:MVM ratio of 1.45:1 and a CTA:VM of 50 is as follows. DMA (10 g, 101 mmol, 50 eq, filtered through basic alumina), NPGDA (621 mg, 2.92 mmol, 1.45 eq), TA-CTA (1.02 g, 2.01 mmol, 1 eq), and AIBN (66 mg, 0.4 mmol, 0.2 eq) were diluted with DMF to a
total volume of 25 mL ([DMA] = 4M). The solution was divided into two 20 ml scintillation vials equipped with a PTFE septa. The reaction mixture was purged with N₂ for 15 minutes and heated for 36 hours at 65 °C. AIBN (33 mg, 0.2 mmol, 0.1 eq) was added to each scintillation vial, which was purged with N₂ for 15 minutes and heated for 24 hours at 65 °C. Monomer conversion was determined by ¹H NMR spectroscopy through disappearance of vinyl proton (1H, δ = 5.6 ppm) with DMF (δ = 8.0) as an internal standard. Mₙ and D were obtained by SEC-MALLS.

2.2.2 Polymer Fractional Precipitation

8.5 grams of highly branched copolymer was dissolved in 90 mL of dioxane in a 1 L round bottom flask. To the solution, with turbulent stirring, a 60:40 (by volume) mixture of ether:dioxane was added until the solution turned cloudy (560 mL). The resulting solution was centrifuged. The pale yellow and transparent supernatant was set aside for a second fractional precipitation. The deep yellow, viscous, high molecular weight fraction was diluted in dioxane and precipitated into ether to generate the first fraction. The previously collected supernatant was stirred vigorously. 300 mL of a 60:40 (by volume) mixture of ether:dioxane was added to the supernatant (150 mL is when the solution first turned cloudy again). The solution settled overnight into two phases. The upper, pale yellow phase was decanted. The bottom, high-molecular weight, viscous, dark-yellow phase was diluted in dioxane and precipitated into ether. This yielded 600 mg of the first fraction (7% yield) and 2.5 grams for the second fraction (30% yield).
2.2.3 NMR Spectroscopy

Figure S3: $^1$H NMR spectra of DMA-co-NPGDA targeting a NPGDA:CTA ratio of 1.45:1 and a $D_{PC}^{50}$ (DMA:CTA ratio of 50). Traces show pre-polymerization (red), after 36 hours (green), and after another 12 hours (blue) after the addition of extra AIBN initiator (0.2 eq compared to the CTA). Conversion determined to be 90% (green, 36 hours) and 99.9%+ (blue, extra AIBN and 12 hours). Inlet shows disappearance of monomer vinyl peaks (6.6 - 5.5 ppm) while preserving activity of the TA group on the CTA (4.5 ppm).
2.2.4 Fractional Precipitation SEC Traces

![DP<sub>PC</sub> 50 SEC](image)

**Figure S4:** DMF SEC traces of DMA-co-NPGDA targeting a NPGDA:CTA ratio of 1.45:1 and a DP<sub>PC</sub> 50 (DMA:CTA ratio of 50). Traces show post polymerization SEC (black), first fractional precipitation collection (green), and second fractional precipitation collection (blue).
2.3 DP<sub>PC</sub> 100

2.3.1 Polymer Synthesis

DMA-co-NPGDA highly branched polymer targeting a CTA:MVM ratio of 1.45:1 and a CTA:VM of 100 is as follows. DMA (10 g, 101 mmol, 100 eq, filtered through basic alumina), NPGDA (310 mg, 1.46 mmol, 1.45 eq), TA-CTA (0.509 g, 1.01 mmol, 1 eq), and AIBN (33 mg, 0.2 mmol, 0.2 eq) were diluted with DMF to a total volume of 25 mL ([DMA] = 4M). The solution was divided into two 20 ml scintillation vials equipped with a PTFE septa. The reaction mixture was sparged with N<sub>2</sub> for 15 minutes and heated for 24 hours at 65 °C. Monomer conversion was determined by <sup>1</sup>H NMR spectroscopy and the <i>M</i><sub>n</sub> and <i>D</i> were obtained by SEC-MALLS.

2.3.2 Polymer Fractional Precipitation

7 grams of highly branched copolymer was dissolved in 70 mL of dioxane in a 1 L round bottom flask. To the solution, with turbulent stirring, a 60:40 (by volume) mixture of ether:dioxane was added until the solution turned cloudy (450 mL). The resulting solution was centrifuged. The pale yellow and transparent supernatant was set aside for a second fractional precipitation. The deep yellow, viscous, high molecular weight fraction was diluted in dioxane and precipitated into ether to generate the first fraction. The previously collected supernatant was stirred vigorously. 300 mL of a 60:40 (by volume) mixture of ether:dioxane was added to the supernatant (150 mL is when the solution first turned cloudy again). The solution settled overnight into two phases. The upper, pale yellow phase was decanted. The bottom, high-molecular weight, viscous, dark-yellow phase was diluted in dioxane and precipitated into ether. This recovered 300 mg of the first fraction (4% yield) and 3.2 grams for the second fraction (45% yield).

2.3.3 Fractional Precipitation SEC Traces

![SEC traces](https://example.com)

**Figure S5:** DMF SEC traces of DMA-co-NPGDA targeting a NPGDA:CTA ratio of 1.45:1 and a DP<sub>PC</sub> 100 (DMA:CTA ratio of 100). Traces show post polymerization SEC (black), first fractional precipitation collection (green), and second fractional precipitation collection (blue).
2.4 Exploration of Laboratory Scale Ultrafiltration

DMA-co-MBAM highly branched polymer targeting a CTA:MVM ratio of 1.45:1 and a CTA:VM of 100 is as follows.[^30] DMA (1 g, 10.1 mmol, 100 eq, filtered through basic alumina), methylenebisacrylamide (MBAM) (18.69 mg, 0.12 mmol, 1.2 eq), 2-cyano-2-propyl dodecyl trithiocarbonate (34.9 mg, 0.101 mmol, 1 eq), and AIBN (3.31 mg, 0.02 mmol, 0.2 eq) were diluted with DMF to a total volume of 2.9 mL ([DMA] = 3.5M) in an 8 ml scintillation vial equipped with a PTFE septa. The reaction mixture was sparged with N\textsubscript{2} for 15 minutes and heated for 24 hours at 65 °C. The resulting polymer was precipitated into ether and the precipitate was dissolved in water (10 wt%). Attempted ultrafiltration of the solution of highly branched polymer in water was carried out using 30000 MWCO spin filter (Amicon Ultra Centrifugal Filters). SEC traces of the highly branched polymer, the filtered product (below spin filter membrane) and collected product (above spin filter membrane) were determined after passing through two size exclusion chromatography columns (Resolve Mixed Bed Low DVB, ID 7.8 mm, \(M_w\) range 200-600,000 g mol\(^{-1}\) (Jordi Labs)) in a mobile phase of N,N-dimethylformamide (DMF) with 0.1 M LiBr at 35 °C and a flow rate of 1.0 ml min\(^{-1}\) (Dionex Ultimate 3000 pump, degasser, and autosampler (Thermo Fisher Scientific). We found this process to be ineffective at removing unreacted primary chains, even when the molecular weight cutoff was significantly larger than the primary chain molecular weight. We believe this did not work because the loaded polymeric solution to be filtered would concentrate as water passed through the filter. We hypothesized that the viscosity of the polymeric solution prevented the transport of unreacted primary chains through the filter. For the laboratory scale, fractional precipitation was significantly more effective at removing unreacted primary chains and lowering dispersity.

![Synthetic scheme](image)

**Figure S6:** a. Synthetic scheme. b. SEC trace of highly branched polymer, the filtered product (below spin filter membrane) and collected product (above spin filter membrane).
2.5 Z group removal

Figure S7: a. SEC-UV absorbance ($\lambda = 300$ nm) normalized by concentration (refractive index area under the curve) demonstrating removal of trithiocarbonate Z group for DP$_{PC}$ 100 FP$_2$. b. $^1$H NMR spectra of DP$_{PC}$ 25 FP$_1$ before and after Z group removal, which is sufficiently nucleophilic to remove amine-reactive R group.
3 Amine Conjugations to Polymer

3.1 Maintain CTA reactivity through Synthesis and Workup

Figure S8: $^1$H NMR spectra demonstrating the preservation of the TA group ($\delta = 4.5$ ppm, blue square) on the polymer ($\delta = 2.6$ and 1.65 ppm, blue circle) after synthesis (light blue trace) and after fractional precipitation (maroon trace).
3.2 Conjugation of Benzylamine to Highly Branched Polymer

The protocol for conjugation of benzylamine to a primary chain free (DP_{PC} 25) highly branched polymer is as follows. The protocol for other DP_{PC} only varies in stoichiometry. 75 mg (0.03 mmol end group, 1 eq) of the highly branched polymer isolated from fractional precipitation was dissolved in 1 mL of dioxane. 2.8 µL of benzylamine (0.026 mmol, 0.85 eq) was added to the solution, which was agitated, and left at room temperature for 10 minutes. The polymer was precipitated 3 times into ether. Conjugation was confirmed through $^1$H NMR spectra in deuterated DMSO by the presence of aromatic protons ($\delta = 7.3$ ppm).

![NMR spectra](image)

Figure S9: $^1$H NMR spectra demonstrating the conjugation of benzylamine ($\delta = 7.3$ ppm) to the highly branched copolymer ($\delta = 2.8$ ppm) for DP_{PC} 25 (light blue), DP_{PC} 50 (blue), and DP_{PC} 100 (navy). Molar ratio of the phenyl ($\delta = 7.3$ ppm) to dimethyl ($\delta = 2.8$ ppm) group for DP_{PC} 25, 50, and 100 are approximately 26:1, 64:1, and 110:1, respectively.

3.3 Conjugation of $\alpha$-CD to Highly Branched Polymer

The protocol for conjugation of $\alpha$-CD to a primary chain free (DP_{PC} 25) highly branched polymer is as follows. The protocol for other DP_{PC} only varies in stoichiometry. 75 mg (0.03 mmol end group, 1 eq) of the highly branched polymer isolated from fractional precipitation was dissolved in 0.5 mL of DMSO. 29 mg of $\alpha$-CD (0.026 mmol, 0.85 eq) was dissolved in 0.5 mL of DMSO, added to the highly branched polymer solution, agitated, and left at room temperature for 10 minutes. The solution was washed with ether 3 times, extracting residual DMSO. The resulting polymer was dissolved in water, dialyzed (Slide-A-Lyzer 3500 MWCO), and lyopholized. Conjugation was confirmed through $^1$H NMR in deuterated DMSO by the presence of carbohydrate protons ($\delta = 4.4-6.0$ ppm).
3.4 SEC traces after conjugation

Figure S10: SEC traces of highly branched polymers before synthesis (black line) and after conjugation with benzylamine (dotted line) and \(\alpha\)-CD (dashed line) for DP\(_{P_C} 25\) (light blue, left), DP\(_{P_C} 50\) (blue, middle), and DP\(_{P_C} 100\) (navy, right).

Figure S11: Expanded SEC traces of highly branched polymers before synthesis (black line) and after conjugation with benzylamine (dotted line) and \(\alpha\)-CD (dashed line) for DP\(_{P_C} 50\) (blue, middle), indicating a greater shift to higher molecular weights for \(\alpha\)-CD than benzylamine.
4 Degradation Assays

4.1 Basic Degradation

The protocol for basic degradation for a primary chain free (DP$_{PC}$ 100) highly branched polymer is as follows. To remove the trithiocarbonate CTA, 100 mg of highly branched polymer (0.01 mmol CTA, 1 eq), 8 mg of lauroyl peroxide (0.02 mmol, 2 eq) and 33 mg of AIBN (0.2 mmol, 20 eq) were dissolved in 1 mL of DMF and heated to 90 °C for 12 h, and precipitated into ether. 25 mg of the resulting polymer was dissolved in 2 mL of 0.1M NaOH for 24 hours, neutralized with acetic acid, and lyophilized. Degradation into primary chains was analyzed by SEC in DMF.

![Degradation SEC traces](image)

Figure S12: The basic degradation assay. DMF SEC traces of DMA-co-NPGDA targeting a NPGDA:CTA ratio of 1.45:1 and a DP$_{PC}$ 25(light blue), 50 (blue) and 100 (navy). Traces show second fractional precipitation collection (dotted), degradation into the primary chains after 24 hours (bold line).
4.2 Acidic Degradation

The protocol for acidic degradation for a primary chain free (DP$_{PC}$ 100) highly branched polymer is as follows. To remove the trithiocarbonate CTA, 100 mg of highly branched polymer (0.01 mmol CTA, 1 eq), 8 mg of lauroyl peroxide (0.02 mmol, 2 eq) and 33 mg of AIBN (0.2 mmol, 20 eq) were dissolved in 1 mL of DMF and heated to 90 °C for 12 h, and precipitated into ether. 25 mg of the resulting polymer was dissolved in 2 mL of 12 M HCl for 48 hours (degradation into primary chains was also successful in 1M HCl). Aliquots were taken at 24 and 48 hours, neutralized with sodium carbonate, filtered, and analyzed by aqueous SEC.

**Figure S13:** The acidic degradation assay. Aqueous SEC traces of DMA-co-NPGDA targeting a NPGDA:CTA ratio of 1.45:1 and a DP$_{PC}$ 50 (left) and 100 (right). Traces show second fractional precipitation collection (dotted), degradation into the primary chains after 24 hours (thin line) and 48 hours (bold line).
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