Supplementary Information

Poly(3-methylene-2-pyrrolidone). Synthesis, characterization and evaluation of cytotoxicity

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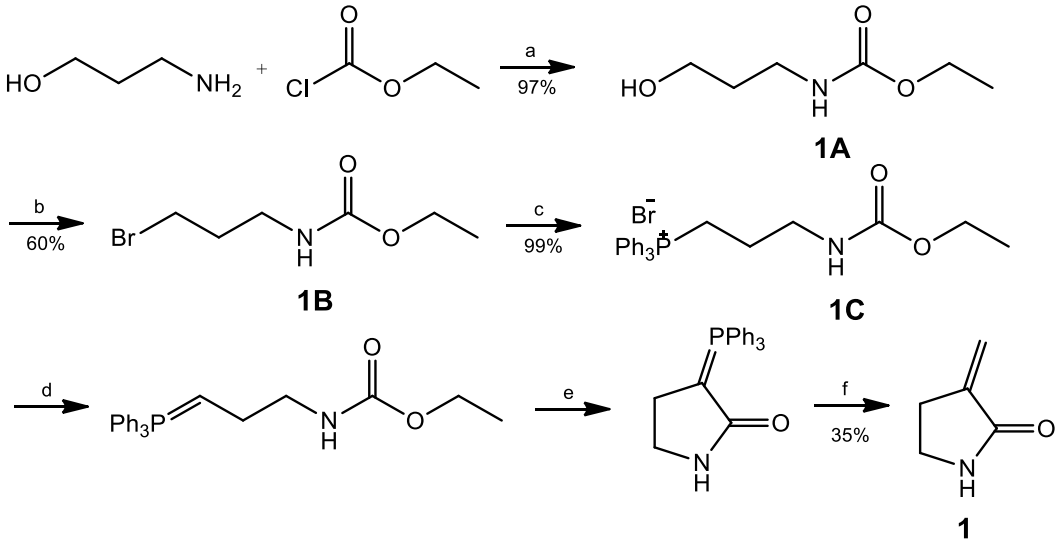
Experimental

Synthesis of 3M2P (Approach 1) monomer:



Scheme S1: a) K_2CO_3, water, 0 °C, b) PBr_3, toluene, r.t. to reflux, c) PPh_3, acetonitrile, reflux, d) t-BuOK, toluene, r.t. e) 50 °C, f) paraformaldehyde (1 equivalent), 50 °C to r.t.

Preparation of Ethyl *N*-(3-hydroxypropyl) carbamate (1A):[1]

This compound was prepared from an adapted literature procedure. 3-Aminopropanol (9.0 g, 119.8 mmol) was dissolved in 30 mL water. Potassium carbonate (26.55 g, 192.11 mmol) in 30 mL water was added to the reaction mixture. Ethyl chloroformate (19.56 g, 180.24 mmol) was added dropwise over 2 h, while the reaction was kept at 0 °C. The reaction mixture was allowed to stir for an additional 2 h, where after it was filtered and extracted with three portions of DCM (3 × 40 mL). The organic layer extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to yield a colourless oil (17.1 g, 97 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.11 (bs, 1H, -NH), 4.08 (q, J = 7.1 Hz, 2H, -O-CH_2), 3.64 (t, J = 5.7 Hz, 2H, -CH_2-OH), 3.29 (q, J = 6.1 Hz, 2H, -CH_2-NH), 2.79 (bs, 1H, -OH), 1.70 (p, 6.0 Hz, 2H, -CH_2-CH_2-CH_2), 1.21 (t, J = 7.1 Hz, 3H, -CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 156.92, 61.09, 39.49, 32.75, 30.91, 14.83. MS (ESI): *m/z* = 148.1 (calculated: 148.17 for $[M + H]^+$), 170.1 (calculated: 170.16 for $[M + Na]^+$).

Preparation of Ethyl *N*-(3-bromopropyl) carbamate (1B):[2]

A solution of PBr_3 (11.3 mL, 119.1 mmol) in anhydrous toluene (40 mL) was added to a solution of ethyl *N*-(3-hydroxypropyl) carbamate (13.5 g, 104.8 mmol) in anhydrous toluene (140 mL) over 2 h at room temperature. Precautions were taken for the highly air and moisture sensitive PBr_3. The reaction mixture was allowed to stir for a further 2 h,

subsequently it was refluxed for 25 min. The mixture was washed with water (3 × 80 mL) and was saturated with NaCl. The water phase was extracted with DCM (3 × 50 mL). The DCM and toluene phases were dried over anhydrous $MgSO_4$ and solvents were removed under reduced pressure. Majority of the product was in the toluene phase as a yellow liquid (11.5 g, 60 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.94 (bs, 1H, -NH), 4.14 (q, J = 7.1 Hz, 2H, -O-CH_2), 3.47 (t, J = 6.5 Hz, 2H, -Br-CH_2), 3.36 (q, J = 6.4 Hz, 2H, -CH_2-NH), 2.10 (p, J = 6.5 Hz, 2H, -CH_2-CH_2-CH_2), 1.27 (t, J = 7.1 Hz, 3H, -CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 156.68, 60.87, 39.28, 32.56, 30.71, 14.62. MS (ESI): *m/z* = 210.0 (calculated: 210.07 for $[M]^+$), 212.0 (calculated: 212.07 for $[M]^+$).

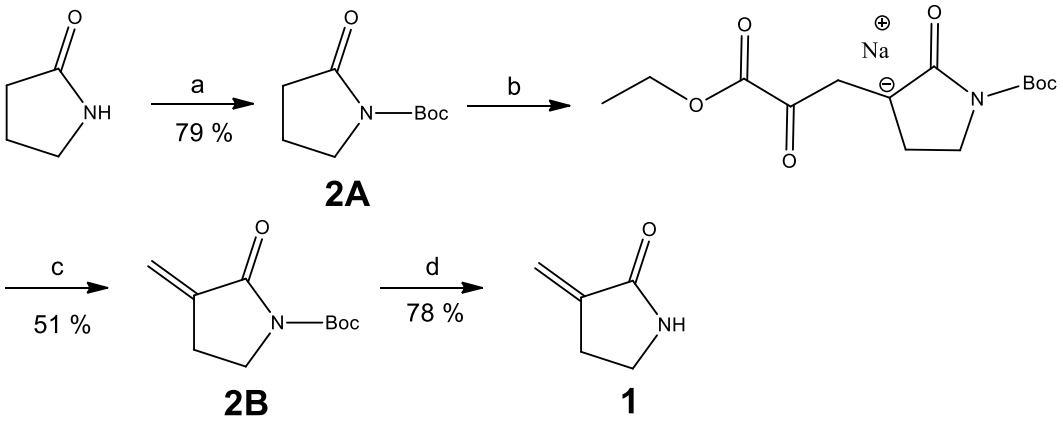
Preparation of Ethyl *N*-(3-bromo triphenylphosphoniumpropyl) carbamate (1C):[2]

To a solution of ethyl *N*-(3-bromopropyl) carbamate (13.5 g, 64.1 mmol) in acetonitrile (80 mL), recrystallized PPh_3 (17.6 g, 67.2 mmol) was added and refluxed at 100 °C for 24 h. After cooling, the mixture was precipitated in ethyl acetate (200 mL), filtered and dried, to afford the product as a white solid (29.9 g, 99 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.79-7.62 (m, 15H, -P-Ar-H), 6.97 (bs, 1H, -CH_2-NH), 4.11 (q, J = 7.1 Hz, 2H, -O-CH_2), 3.44 (t, J = 6.4 Hz 2H, -CH_2-NH), 1.89-1.77 (m, 2H, -CH_2-CH_2-CH_2), 1.23 (t, J = 7.2 Hz, 3H, -CH_3), 1.17 (t, J = 7.1 Hz, 2H, -P-CH_2). ^{31}P NMR (300 MHz, $CDCl_3$) δ: 25.18. MS (ESI): *m/z* = 392.0 (calculated: 392.27 for $[M]^+$).

Preparation of 3-Methylene-2-pyrrolidone(1):[2]

A solution of ethyl *N*-(3-bromo triphenylphosphoniumpropyl) carbamate (3.2 g, 6.9 mmol) in anhydrous toluene (30 mL) was dried by an azeotropic distillation for 3 h. The solution was cooled to room temperature, whereafter a base, *t*-BuOK (0.8 g, 7.2 mmol), was added, under an argon atmosphere. The solution turned bright orange, and was stirred for a further 1 h at 50 °C. Paraformaldehyde (0.05 g, 1.7 mmol) was added to the reaction mixture at this temperature, under an argon atmosphere. Afterwards the reaction was stirred for a further 30 min. The crude reaction mixture was filtered, concentrated and recrystallized from ethyl acetate. The recrystallized compound was dissolved in warm chloroform and extracted into water. The water was removed under reduced pressure to yield a colourless crystalline compound (0.23 g, 35 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.05 (bs, 1H, -NH), 5.67 (td, J = 2.9, 1.3 Hz, 1H, =CH_b), 5.26-5.24 (m, 1H, =CH_a), 3.25 (t, J = 6.6 Hz, 2H, -CH_2-NH), 2.75-2.71 (m, 2H, -CH_2-C=). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 175.45, 141.62, 114.47, 38.90, 26.32. MS (ESI): *m/z* = 98.06 (calculated: 98.11 for $[M + H]^+$), 115.96 (calculated: 115.15 for $[M + NH_4]^+$). R_f in ethyl acetate = 0.423.

Synthesis of 3M2P (Approach 2):



Scheme S2: a) Di-tert-butyl dicarbonate, acetonitrile, 0 °C. b) Diethyl oxalate, sodium hydride, diisopropyl ether, 35 °C. c) Paraformaldehyde, dimethylformamide, 100 °C. d) Trifluoroacetic acid, dichloromethane.

Preparation of *N*-boc-2-pyrrolidone (2A):[3]

To a cooled solution of 2-pyrrolidone (8.0 g, 94.0 mmol) in acetonitrile (40 mL) at 0 °C, a solution of di-tert-butyl dicarbonate (21.7 g, 99.2 mmol) in acetonitrile (40 mL) was added drop-wise over 20 min. After the addition, 4-dimethylaminopyridine (1.2 g, 9.4 mmol) was added. The reaction mixture was allowed to stir for 3h at room temperature, where after the reaction mixture was concentrated under reduced pressure and poured to water (50 mL). Diluted HCl (1 M) was added to achieve a pH ≈ 7, then extracted with ethyl acetate. The organic layer was washed multiple times with brine (5 × 60 mL) and dried over anhydrous Na_2SO_4. The dried organic layer was concentrated and further purification followed by column chromatography on silica gel, to yield a yellow oil (13.7 g, 79 %). ^{1}H NMR (300 MHz, $CDCl_3$-d_1) δ: 3.73 (t, 2H, 8.0 Hz -C$\underline{H}_2$-N), 2.49 (t, J = 8.2 Hz, 2H, -C$\underline{H}_2$-C=O), 1.96 (m, 2H, -CH$_2$-C$\underline{H}_2$-CH$_2$), 1.51 (s, 9H, (C$\underline{H}_3$)$_3$). ^{13}C NMR (300 MHz, $CDCl_3$-d_1) δ: 174.18, 150.22, 82.83, 46.16, 32.96, 28.06, 17.40. MS (ESI): m/z = 208.1 (calculated: 208.22 for $[M + Na]^+$).

Preparation of *N*-boc-3-methylene-2-pyrrolidone (2B):

This procedure was adapted from literature.[4,5] Sodium hydride (1.0 g, 42.1 mmol) and diethyl oxalate (9.5 g, 64.8 mmol) was added to diisopropyl ether (30 mL) and stirred at 35 °C. A solution of *N*-boc-2-pyrrolidone (6.0 g, 32.4 mmol) in 30 mL diisopropyl ether was added, drop-wise over 1h. The reaction mixture was allowed to stir at 35 °C for 2 days. Subsequently, the residue was washed with diisopropyl ether and re-dissolved in DMF. Paraformaldehyde (1.9 g, 64.8 mmol) was added and the reaction mixture was refluxed at 100 °C for 1.5 h. After the reaction mixture was allowed to cool, it was filtered. Purification by silica gel column chromatography then yielded a yellow oil (3.3 g, 51 %). ^{13}C NMR (300 MHz, $CDCl_3$-d_1) δ: 167.44, 162.30, 140.40, 115.55, 66.69, 42.08, 30.64, 23.84. MS (ESI): m/z = 220.1 (calculated: 220.22 for $[M + Na]^+$).

Preparation of 3-methylene-2-pyrrolidone (1):[6]

N-boc-3-methylene-2-pyrrolidone (3.3 g, 16.7 mmol) was added to 20 mL of DCM and stirred at room temperature. Trifluoroacetic acid (1 mL) was added to the solution and allowed to stir for 1.5 h. Subsequently, the reaction mixture was concentrated, DCM (20 mL) added and concentrated (× 8). Purification was followed by column chromatography on silica gel, yielding the product (1.3 g, 78 %). ^{1}H NMR (300 MHz, cdcl$_3$) δ 6.06 (td, *J* = 2.8, 0.5 Hz, 1H, =C$\underline{H}_b$), 5.43 (td, *J* = 2.5, 0.5 Hz, 1H, =C$\underline{H}_a$), 3.52 – 3.46 (m, 2H, -C$\underline{H}_2$-NH), 2.84 – 2.77 (m, 2H, -C$\underline{H}_2$-C=). MS (ESI): m/z = 98.1 (calculated: 98.12 for $[M + H]^+$).

RAFT agent synthesis

The RAFT agent utilized was 2-hydroxyethyl 2-(butylthiocarbonothioylthio)-2-methylpropanoate and was prepared in a two-step reaction, adapted from literature:[7]

To a 500 mL round bottom flask was added potassium phosphate tribasic (16.60 g, 78.20 mmol) in 130 mL acetone and the reaction mixture was stirred for 5 h, yielding a yellow suspension. Subsequently, 1-butanethiol (8.0 mL, 74.2 mmol) was added to the reaction mixture and stirred for an hour. Carbon disulfide (9.10 mL, 151 mmol) was added dropwise and the reaction mixture was stirred for 2 h, while immersed in an ice bath. Where after 2-bromo-2-methylpropionic acid (11.70 g, 70.0 mmol) was added to the reaction mixture at room temperature whilst stirring and left stirring overnight. The solid was filtered and the filtrate concentrated. Cold 10 % HCl solution (100 mL) was added to the residue at room temperature and stirred overnight. The reaction mixture was extracted with two portions of hexane (50 mL) where after the organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure giving a bright yellow solid. The solid was purified by column chromatography using ethyl acetate:pentane (1:4) on silica gel. Solvents were removed *in vacuo* to yield the product, the carboxylic acid terminated trithiocarbonate (72 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.29 (t, J = 7.5 Hz, 2H, -S-C$\underline{H}_2$), 1.71 (s, 6H, -S-CH-

($C\underline{H}_3)_2$), 1.64 (p, J = 8.9 Hz, 2H, $-CH_2-C\underline{H}_2-CH_2$), 1.39 (m, 2H, $-CH_3-C\underline{H}_2-CH_2$), 0.90 (t, J = 7.3 Hz, 3H, $-C\underline{H}_3-CH_2$).

The second step was carried out to esterify the carboxylic acid terminated trithiocarbonate. The purified carboxylic acid terminated trithiocarbonate (1.50 g, 5.94 mmol) and ethylene glycol (3.60 g, 59.0 mmol) was dissolved in 75 mL THF. The reaction mixture was stirred and DCC (1.23 g, 5.94 mmol) was added to the reaction flask, which was immersed in an ice bath 5 minutes prior to the addition of DMAP (0.07 g, 0.59 mmol) whilst stirring. The ice bath was removed after 1 h and the reaction was allowed to run overnight at room temperature. Subsequently, the reaction mixture was dispersed in diethyl ether and filtered. The filtrate was washed with a 5 % sodium hydrogen carbonate solution (2 × 30 mL) and water (1 × 30 mL). Whereafter the organic phase was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a dark orange oil. The product was purified by silica gel chromatography, using ethyl acetate: pentane (1:4), and the product was obtained in 62 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.18 (m, 2H, $-O-C\underline{H}_2$), 3.74 (m, 2H, $-C\underline{H}_2-OH$), 3.29 (t, J = 7.4 Hz, 2H, $-S-C\underline{H}_2$), 1.65 (s, 6H, $-CH(C\underline{H}_3)_2$), 1.62 (m, 2H, $-S-CH_2-C\underline{H}_2$), 1.37 (m, 2H, $-CH_3-C\underline{H}_2$), 0.86 (t, J = 7.3 Hz, 3H, $-C\underline{H}_3-CH_2$).

FRP polymerizations:

General FRP of poly(3M2P):

Homopolymerizations were carried out in a 5 mL pear flask, where 3M2P (100 mg, 1.03 mmol) and AIBN, in a ratio of 100:1 were added. Homopolymerizations were carried out in DMSO as solvent and under an inert atmosphere. When the polymerization had reached completion, the mixture was precipitated slowly into acetone, filtered and yielded a white powder. For specified analyses, poly(3M2P) was dialyzed against water for ~5 days using SnakeSkin®, replacing the water every 12 h.

Statistical copolymerization of *N,N*-dimethylaminoethyl methacrylate and 3M2P:

A reaction vessel was charged with 3M2P (50 mg, 0.51 mmol) and *N,N*-dimethylaminoethyl methacrylate (DMAEMA) (81 mg, 0.52 mmol) as a co-monomer. AIBN (1.7 mg, 0.010 mmol) was added as an initiator in 1.0 mL DMF and the mixture was then purged with an inert gas. The vessel was then immersed in an oil bath, preheated to 75 °C, for 24 h. The reaction was stopped by opening the flask to air, and cooling. The polymer was isolated by precipitating from cooled diethyl ether. From SEC; M_n = 15 800 g/mol, M_w = 28 100 and $Đ$ = 1.78. Calculated mol ratio of the copolymer from ^{1}H NMR spectroscopy, P(DMAEMA): P(3M2P), (61 % : 39 %).

RDRP polymerizations:

General RAFT homopolymerization procedure of 3M2P:

To a 5 mL pear flask was added 3M2P (110 mg, 1.13 mmol), RAFT agent (1.65 mg, 0.01 mmol) and AIBN (0.18 mg, 0.001 mmol), in 0.8-1.0 mL DMSO. After the reaction mixture was dissolved and degassed with argon gas for 45 min, the polymerization vessel was placed in the heated oil bath at 75 °C. The reaction was stopped by opening the flask to air, and cooling. The polymer was isolated by precipitation from acetone, to yield a yellowish powder.

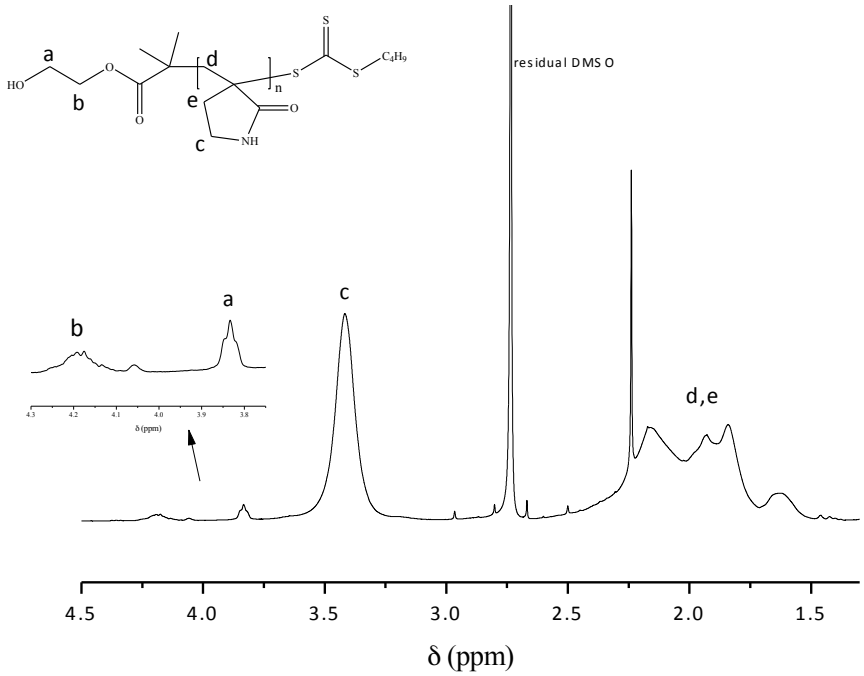


Figure 1S: ^{1}H NMR spectrum of poly(3M2P), in D_2O, prepared via RAFT polymerization.

General SET-LRP homopolymerization procedure of 3M2P at 50 °C:

The procedure was adapted from literature.[8] To a 25 mL Schlenk flask was added 3M2P (100 mg, 1.03 mmol), Cu(0) wire wrapped around the stirring bar and 1,2-dihydroxypropane-3-oxy-(2-bromo-2-methylpropionyl) (2.47 mg, 0.10 mmol) as initiator, in 1.0 mL DMSO. 1,2-dihydroxypropane-3-oxy-(2-bromo-2-methylpropionyl)[9] and Me_6TREN[10] were prepared as described in literature. The reaction mixture was freeze-pump-thawed for five cycles where after the flask was filled with argon. A micro-syringe, flushed with argon thrice, was used to transfer the ligand, Me_6TREN (1.19 mg, 1.4 µL, 0.01 mmol), to the already deoxygenized reaction mixture. The reaction mixture was filtered through a short basic aluminium oxide column to remove copper, where after the polymer was precipitated from acetone to yield a white powder.

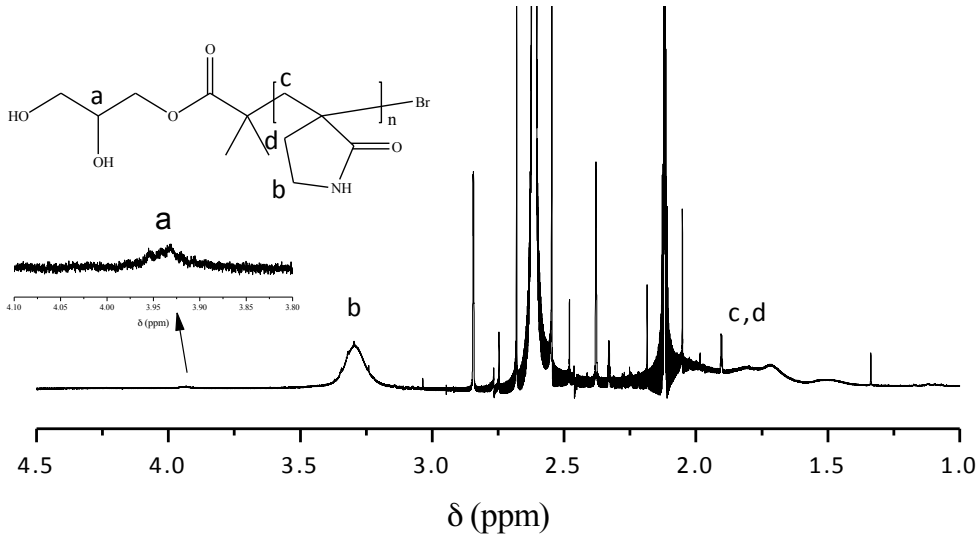


Figure 2S: ^{1}H NMR spectrum of poly(3M2P), in D_2O, prepared via SET-LRP.

Block copolymer consisting of DMAEMA and 3M2P, with the process mediated by RAFT, is given below:

A reaction vessel was charged with DMAEMA (1.0 g, 6.36 mmol), AIBN (3.0 mg, 0.02 mmol) as an initiator, and 2-hydroxyethyl 2-(butylthiocarbonothioylthio)-2-methylpropanoate (31 mg, 0.10 mmol) as the RAFT agent, in 2.0 mL 1,4-dioxane. The RAFT agent was prepared as described previously. The mixture was purged with an inert gas, whereafter the vessel was placed in a heated oil bath at 75 °C for 24h. The reaction was stopped by opening the flask to air, and cooling. The polymer was isolated by precipitation from cooled petroleum ether. From SEC, M_n = 17 000 g/mol, M_w = 23 200 g/mol and $Đ$ = 1.37. Calculated number average molecular weight from chain-ends on ^{1}H NMR spectroscopy: M_n = 13 900 g/mol.

Secondly, the resulting PDMAEMA macro-RAFT agent was coupled with 3M2P. A reaction vessel was charged with PDMAEMA macro-RAFT agent (0.1 g, 0.01 mmol), 3M2P (35 mg, 0.36 mmol), AIBN (0.2 mg, 0.001 mmol) were dissolved in a mixture of 1.0 mL 1,4-dioxane and 0.2 mL DMF. The mixture was purged with an inert gas. The mixture was then immersed in a heated oil bath at 75 °C for 24h. The reaction was stopped by opening the flask to air, and cooling. The polymer was isolated by precipitation from cooled diethyl ether. From SEC: M_n = 23 300 g/mol, M_w = 29 400 g/mol and Đ = 1.26. Calculated mol ratio of the block copolymer from ^{1}H NMR spectroscopy, P(DMAEMA) block : P(3M2P) block, (34 % : 66 %).

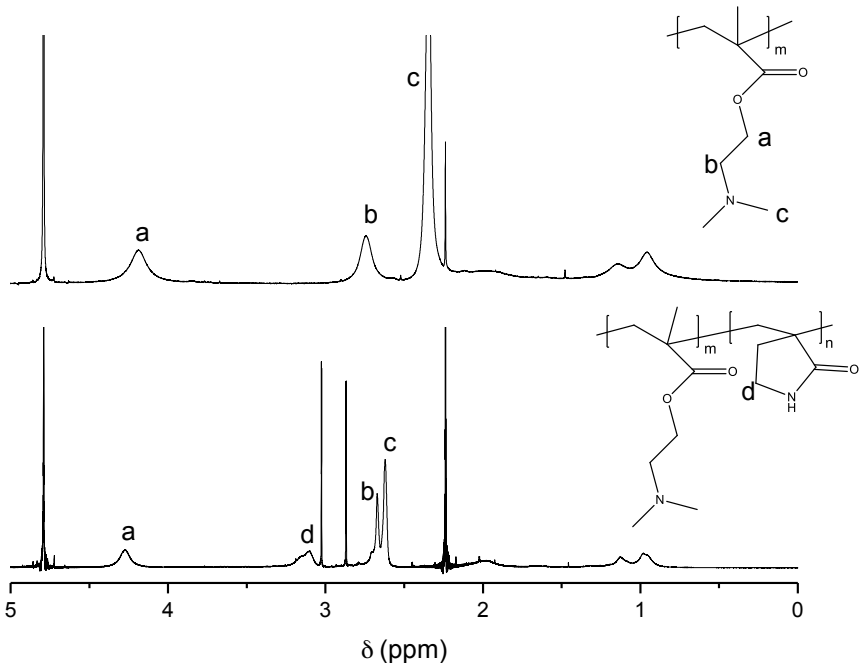


Figure 3S. ^{1}H NMR spectrum of poly(DMAEMA) (top) and poly(DMAEMA)-*b*-poly(3M2P) (bottom), in D_2O.

Cytotoxicity assay

Poly(3M2P) was dialyzed against water for ~5 days using SnakeSkin®, replacing the water every 12 h. Subsequently the polymer was freeze-dried for 24 h.

To determine the cytotoxity of poly(3M2P), different concentrations of dialyzed and freeze-dried poly(3M2P) (1 mg/mL, 1 µg/mL and 1 ng/mL) were dissolved Dulbecco's modified eagle medium (DMEM) containing 1 % penicillin/streptomycin and 10 % fetal calf serum and added to GT1-7 cell (hypothalamic mouse cells) cultures. A control (media and cells) and a positive control (media and cells treated with ethanol) were used as references.

Subsequently, the samples were incubated at 37 °C for 4 h, where after the cells were stained with fluorescent markers, Hoechst 33342 dye and propidium iodide, respectively. Fluorescence and light transmission micrographs were obtained and cell viability was determined.

Analysis of poly(3M2P)

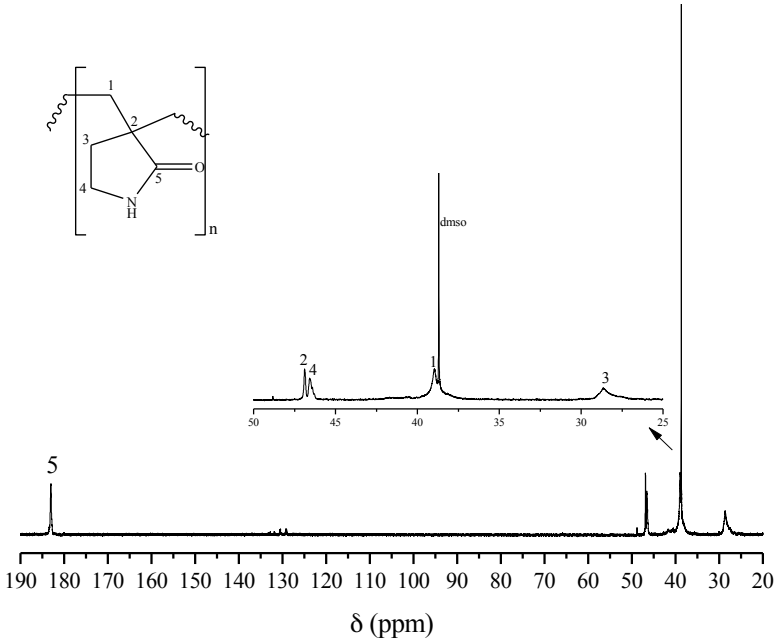


Figure 4S: ^{13}C NMR spectrum of poly(3M2P) in D_2O.

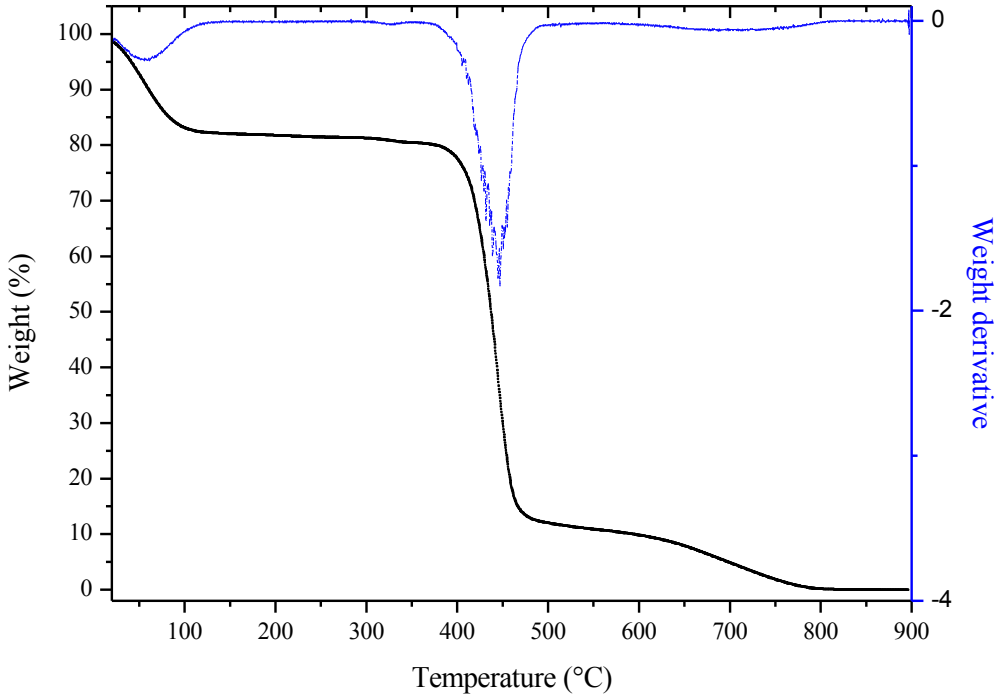


Figure 5S: TGA thermogram and corresponding weight derivative of the decomposition of poly(3M2P).

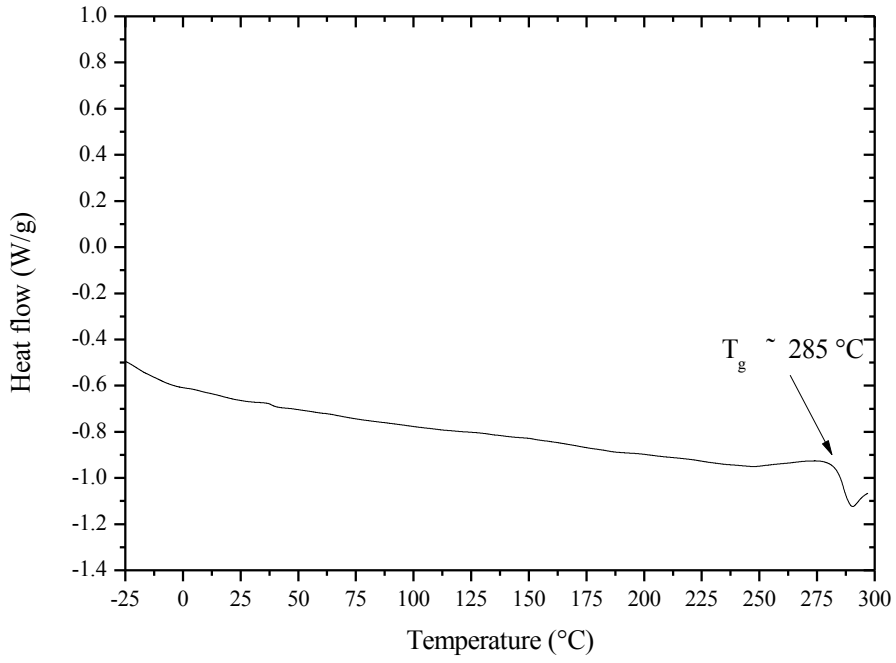


Figure 6S: DSC thermogram of second heating cycle of poly(3M2P).

References

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