Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

Differences in Cytotoxicity of Poly(PEGA)s Synthesized by Reversible Addition–Fragmentation Chain Transfer Polymerization

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Experimental Section

Materials.

All materials were purchased from either Sigma-Aldrich or Fisher Scientific and were used as received unless otherwise indicated. 2,2'-Azobisisobutyronitrile (AIBN) was recrystallized twice from ethanol before use. 4-(3-hydroxy-propyl)-10-oxa-4-aza-tricyclo[5,2,1,$0^{2,6}$] dec-8-ene-3,5-dione,[1] 2-(ethyl trithiocarbonate)propionic acid,[2] ethyl-2-(phenylcarbonothioylthio)propanoate[3], and 1-phenylethyl dithiobenzoate, and furan-protected maleimide-poly(PEGA) (**2**)[4] were synthesized as previously reported. PEGA (M_n ~ 454, ≥ 99%) was purchased from Sigma-Aldrich. Merck 60 (230-400 mesh) silica gel was used for normal phase chromatography. MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was purchased from Research Products International (RPI) Corporation. NIH 3T3 mouse fibroblast cell line was purchased from ATCC. Spectra/Por Dialysis Membrane was purchase from Spectrum Laboratory, Inc. (Rancho Dominguez, CA). DI water was generated in house using a Milli-Q system. Cells were maintained in 10% Calf serum (Colorado Serum Corp., Denver, Colorado) in

DMEM (Invitrogen). Alumina (Al_2O_3) resin, neutral Act I, 50-200 μm was purchased from Sorbent Technologies.

Analytical Techniques.

^1H and ^{13}C NMR spectra were acquired on an ARX 400 MHz or 500 MHz NMR spectrometer, and spectra were processed using Topspin 1.2 NMR software. Infrared absorption spectra were recorded using a PerkinElmer FT-IR equipped with an ATR accessory. TLC plates pre-coated with silica gel 60 F254 were developed in the indicated solvent systems. Size exclusion chromatography (GPC) was conducted on a Shimadzu HPLC system equipped with a refractive index detector RID-10A, one Polymer Laboratories PLgel guard column, and two Polymer Laboratories PLgel 5 μm mixed D columns. Lithium bromide (LiBr) (0.1 M) in dimethylformamide (DMF) at 40 °C was used as the mobile phase (flow rate: 0.80 mL/min). Calibration was performed using near-monodisperse poly(methyl methacrylate) standards from Polymer Laboratories. Because of its size, the M_n of polymer **2** could not be determined accurately by ^1H NMR; thus, the value provided in Table 1 was obtained by GPC with Laser Light Scattering Detection.[4] Chromatograms were processed using the EZStart 7.2 chromatography software. Mass spectra were obtained by GC-MS on an Agilent 6890-5975 GC-MS with Autosampler.

Synthesis of benzyl 2-(ethylthiocarbonothioylthio)propanoate

2-(Ethylthiocarbonothioylthio)propanoic acid (4.2 g, 20 mmol), dicyclohexyl carbodiimide (DCC) (5.15 g, 25 mmol) and dimethylaminopyridine (DMAP) (0.24 g, 2

mmol) were dissolved in anhydrous THF (30 mL), and the solution was cooled to 0 ^{o}C. Benzyl alcohol (2.5 g, 23 mmol) was added to the reaction mixture drop wise. The reaction mixture was allowed to stir for 2 h in an ice bath. The reaction mixture was filtered and then purified via silica column chromatography (1 : 40 ethyl acetate : hexanes). δ ^{1}H NMR (400 MHz, $CDCl_3$) (Figure S1a): 7.40-7.28 (5H, m, C_6H_5), 5.18 (2H, s, OC**H**$_2$C$_6$H$_5$), 4.87 (1H, q, J = 7.3 Hz, SC**H**CH$_3$), 3.36 (2H, q, J = 7.4 Hz, CH$_3$C**H**$_2$S), 1.61 (3H, d, J = 7.3 Hz, SCHC**H**$_3$), 1.35 (3H, t, J = 7.5 Hz, C**H**$_3$CH$_2$S). δ ^{13}C NMR (500 MHz, $CDCl_3$) (Figure S1b): 221.80, 171.21, 135.56, 128.65, 128.44, 128.22, 67.54, 47.98, 31.61, 16.90, 13.06. GC-MS expected (found) ($M + K^+$): 339.11 (338.93). IR: 3032, 2928, 1735, 1497, 1452, 1376, 1303, 1237, 1153, 1080, 877, 718, 748, 697 cm^{-1}.

*Synthesis of trithiomonobenzyl-poly(PEGA) (**1**) by RAFT polymerization*.

PEGA (2.27 g, 4.95 mmol), benzyl 2-(ethylthiocarbonothioylthio)propanoate (30 mg, 0.10 mmol) and AIBN (1.6 mg, 0.0097 mmol) ([Monomer] : [CTA] : [AIBN] = 50 : 1 : 0.1) were loaded into a Schlenk tube along with 5 mL of DMF. The tube was sealed and subjected to three freeze-pump-thaw cycles. The polymerization was then initiated by immersing the Schlenk tube in a 70 °C oil bath. The polymerization was stopped at 68% conversion. The polymer was purified by dialysis against MeOH (molecular weight cut-off, MWCO 6-8,000 Da). GPC (RI) was used to determine the number-average molecular weight (M_n) and the PDI. δ ^{1}H NMR (400 MHz, $CDCl_3$) (Figure S2): 7.37-7.27 (5H, m, C_6H_5, end group), 5.13-5.01 (2H, m, C$_6$H$_5$C**H**$_2$O, end group), 4.15 (CH_2OCO, polymer), 3.84-3.42 (CH_2CH_2O, polymer), 3.36 (OCH_3, polymer), 2.29 (C**H**CH$_2$, polymer), 1.96-

1.37 (CHC**H**$_2$, polymer), 1.33 (3H, t, J = 7.42, SCH$_2$C**H**$_3$, end group), 1.14-1.12 (3H, m, CHC**H**$_3$, end group).

*Synthesis of dithio-monobenzyl-poly(PEGA) (**3**) by RAFT polymerization*

PEGA (2.37 g, 5.17 mmol), ethyl-2-(phenylcarbonothioylthio)propanoate (29.4 mg, 0.116 mmol) and AIBN (3.8 mg, 0.023 mmol) ([Monomer] : [CTA] : [AIBN] = 45 : 1 : 0.2) were loaded into a Schlenk tube along with 5 mL of DMF. The tube was sealed and subjected to three freeze-pump-thaw cycles. The polymerization was then initiated by immersion the Schlenk tube in an 80 °C oil bath. The polymerization was stopped by exposure to air after 20 h (90 % conversion). The polymer was purified by dialysis against MeOH : ethyl acetate (1 : 1) (molecular weight cut-off, MWCO 6-8,000 Da). GPC (RI) was used to determine the number-average molecular weight (M_n) and the PDI. δ ^{1}H NMR (400 MHz, CD_3CN) (Figure S3): 7.98 (2H, d, J = 9.1 Hz, *o*-C_6H_5, end group), 7.65 (1H, t, J = 7.5 Hz *p*-C_6H_5, end group), 7.48 (2H, t, J = 7.3 Hz, *m*-C_6H_5, end group), 4.15 (CH_2OCO, polymer), 3.76-3.34 (CH_2CH_2O, polymer), 3.30 (OCH_3, polymer), 2.32 (C**H**CH_2, polymer), 1.98-1.37 (CHC**H**$_2$, polymer), 1.21 (3H, t, J = 7.1 Hz, OCH$_2$C**H**$_3$, end group), 1.14-1.12 (3H, m, CHC**H**$_3$, end group).

*Synthesis of Dithiodibenzyl-poly(PEGA) (**4**) by RAFT polymerization*

PEGA (1.8 g, 3.97 mmol), 1-phenylethyl benzodithioate CTA (20.5 mg, 0.079 mmol) and AIBN (2.6 mg, 0.016 mmol) ([Monomer] : [CTA] : [AIBN] = 50 : 1 : 0.2) were loaded into a Schlenk tube along with 5 mL of DMF. The tube was sealed and subjected to three freeze-pump-thaw cycles. The polymerization was then initiated by

immersion the Schlenk tube in a 70 °C oil bath. The polymerization was stopped by exposure to air after 20 h (67 % conversion). The polymer was purified by dialysis against MeOH : ethyl acetate (1 : 1) (molecular weight cut-off, MWCO 6-8,000 Da). GPC (RI) was used to determine the number-average molecular weight (M_n) and the PDI. δ ^{1}H NMR (400 MHz, CD_3CN) (Figure S4): 7.98 (2H, d, J = 7.9 Hz, *o*-C_6H_5, end group), 7.65 (1H, t, J = 7.4 Hz *p*-C_6H_5, end group), 7.48 (2H, t, J = 7.7 Hz, *m*-C_6H_5, end group), 7.33-7.19 (5H, m, C_6H_5, end group), 4.15 (CH_2OCO, polymer), 3.76-3.35 (CH_2CH_2O, polymer), 3.29 (OCH_3, polymer), 2.31 ($CHCH_2$, polymer), 1.98-1.37 ($CHCH_2$, polymer), 1.14-1.12 (3H, m, $CHCH_3$, end group).

*Aminolysis of **3** and **4** (**3a** and **4a**)*

Polymer **3** (317 mg, 0.0184 mmol) was dissolved with 1 mL THF in a 5 ml glass vial with a stir bar. Butylamine (27 mg, 0.370 mmol) was added to the polymer solution. The reaction was allowed to proceed for 1.5 h at 24 ^{o}C; **3a** was purified by dialysis against MeOH : ethyl acetate (1 : 1) (molecular weight cut-off, MWCO 6-8,000 Da). The same aminolysis procedure was used for **4** (84 mg, 0.0044 mmol) with butylamine (6.4 mg, 0.088 mmol). The resulting polymers **3a** and **3b** were analyzed by ^{1}H NMR spectroscopy. δ ^{1}H NMR (400 MHz, CD_3CN) of polymer **3a**: 4.15 (CH_2OCO, polymer), 3.77-3.30 (CH_2CH_2O, polymer), 3.30 (OCH_3, polymer), 2.32 ($CHCH_2$, polymer), 1.98-1.37 ($CHCH_2$, polymer), 1.21 (3H, t, J = 7.1 Hz, OCH_2CH_3, end group), 1.13-1.07 (3H, m, $CHCH_3$, end group). δ ^{1}H NMR (400 MHz, CD_3CN) of **polymer 4a**: 7.33-7.19 (5H, m, C_6H_5, end group), 4.15 (CH_2OCO, polymer), 3.76-3.34 (CH_2CH_2O, polymer), 3.29 (OCH_3, polymer), 2.31 ($CHCH_2$, polymer), 1.98-1.37 ($CHCH_2$, polymer), 1.14-1.12 (3H,