

Supplementary Information

*Highly efficient disulfide bridging polymers for bioconjugates
from radical-compatible dithiophenol maleimides*

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Experimental

N-(Ethyl)-2-pyridylmethanimide¹ was synthesised as described previously. Copper(I) bromide was purified as described by Keller and Wycoff.² Tris(2-carboxyethyl)phosphine hydrochloride (purum, $\geq 98.0\%$) was purchased from Sigma Aldrich and used as received. Membrane dialysis was performed using Spectra/Por 6 dialysis tubing, 1K MWCO, which was supplied by Spectrum Laboratories. Salmon Calcitonin was purchased from Polypeptide Laboratories (Hillerod, Denmark) and stored at 4 °C. Sequence – CYS¹-SER²-ASN³-LEU⁴-SER⁵-THR⁶-CYS⁷-VAL⁸-LEU⁹-GLY¹⁰-LYS¹¹-LEU¹²-SER¹³-GLN¹⁴-GLU¹⁵-LEU¹⁶-HIS¹⁷-LYS¹⁸-LEU¹⁹-GLN²⁰-THR²¹-TYR²²-PRO²³-ARG²⁴-THR²⁵-ASN²⁶-THR²⁷-GLY²⁸-SER²⁹-GLY³⁰-THR³¹-PRO³²-NH₂. Disulphide bridge: CYS¹-CYS⁷.

GPC Analysis – Tetrahydrofuran eluent

GPC was performed on a Varian 390-LC MDS system equipped with a PL-AS RT/MT autosampler, a PL-gel 3 μm (50 \times 7.5 mm) guard column, two PL-gel 5 μm (300 \times 7.5 mm) mixed-D columns equipped with a differential refractive index and a Shimadzu SPD-M20A diode array detector, using THF as the eluent with a flow rate of 1.0 mL min⁻¹. Narrow molecular weight PMMA standards (200 - 1.0 $\times 10^6$ g mol⁻¹) were used for calibration using a second order polynomial fit.

GPC Analysis – Chloroform eluent

GPC was performed on a Varian 390-LC MDS system equipped with a PL-AS RT/MT autosampler, a PL-gel 3 μm (50 \times 7.5 mm) guard column, two PL-gel 5 μm (300 \times 7.5 mm) mixed-D columns equipped with a differential refractive index detector, using CHCl₃ as the eluent with a flow rate of 1.0 mL min⁻¹. Narrow molecular weight PMMA standards (200 - 1.0 $\times 10^6$ g mol⁻¹) were used for calibration using a second order polynomial fit.

RP-HPLC

RP-HPLC was carried out using a Varian PLRP-S 100A (5 μm) 250 \times 4.6 mm columns. The HPLC system comprised of two Gilson 306 pumps a Gilson 811B mixer and a Gilson 805 manometric module; the sample was injected using a SPARK Endurance autosampler. Sample detection was carried out using two UV detectors connected in series, a Jasco-975 and Knauer K-2001 monitored at $\lambda = 280$ nm.

The mobile phases used were:

- mobile phase A: 90 % v/v water, 10 % v/v MeCN (far UV) and 0.05 % v/v TFA;
- mobile phase B: 100 % v/v MeCN (far UV) and 0.04 % v/v TFA.

The column was equilibrated for 10 minutes by washing with mobile phase A before sample injection.

<u>Time (min)</u>	<u>(% A)</u>	<u>(% B)</u>	<u>Flow (ml/min)</u>
0	90	10	1.0
27	40	60	1.0
35	40	60	1.0
36	90	10	1.0
45	90	10	1.0

To ensure that the column was thoroughly washed before each sample injection and to prevent the build up of contaminants, the gradient included a final washing step whereby the concentration of mobile phase B was increased to 60 %. HPLC grade solvents/reagents were used in all experiments.

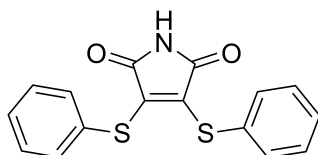
MALDI-ToF-MS Analysis

Mass spectra were acquired by MALDI-ToF-MS (matrix-assisted laser desorption ionisation time-of-flight mass spectrometry) using a Bruker Daltonics Ultraflex II MALDI-ToF mass spectrometer, equipped with a nitrogen laser delivering 2 ns laser pulses at 337 nm with positive ion ToF detection performed using an accelerating voltage of 25 kV. Samples were prepared by layering matrices 2,5-dihydroxybenzoic acid (DHB) (2 μL of a 10 mg mL^{-1} solution), α -cyano-4-hydroxycinnamic acid (CHCA) (2 μL of a 10 mg mL^{-1} solution) or *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) followed by 2 μL of a trifluoroacetic acid (10 mg mL^{-1} solution) or sodium trifluoroacetate (10 mg mL^{-1} solution) and the analyte solution (2 μL of a 10 mg mL^{-1} solution).

Fourier Transform Infra-Red (FTIR) spectrometry and mass spectrometry

Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell. Mass spectra were recorded using a Micromass Autospec apparatus.

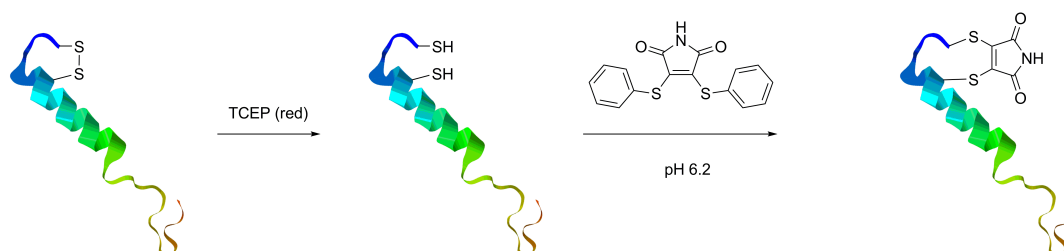
3,4-Bis-phenylsulfanyl-pyrrole-2,5-dione (1)



3,4-Dibromomaleimide (12.00 g, 47.1 mmol) was dissolved in diethyl ether (300 mL) and cooled to 0 °C in an ice bath. To the cooled solution, thiophenol (10.63 g, 96.5 mmol) was added and stirred for 5 minutes. Triethylamine (13.45 mL, 96.5 mmol) was added dropwise to the cooled solution, whereby an immediate yellow colour was observed and a white precipitate. Upon complete addition of triethylamine, the solution was allowed to warm to room temperature and left to stir for 12 hours. The solution was quenched with 200 mL of water and separated. The organic layer was washed with a further 3 portions of water (100 mL) and dried over anhydrous magnesium sulfate. The solution was filtered and concentrated under vacuum and the crude solid obtained as a bright yellow solid. The crude mixture was purified by flash chromatography (SiO₂, 9:1 petroleum ether : diethyl ether followed by 1 : 1 petroleum ether : diethyl ether) to yield the product as a bright yellow powder (12.42 g, 84.1 % yield).

M.p.: 124 –126 °C. ¹H NMR (400.03 MHz, CDCl₃, 298 K) = 7.09–7.22 (m, 10H, CH). ¹³C NMR (100.59 MHz, CDCl₃, 298 K) = 128.6 (2C), 128.9 (2C), 129.2 (2C), 132.0 (2C), 136.8 (2C), 166.4 (2C). IR (neat) = 3229, 1774, 1701, 1685, 1327, 1037 cm⁻¹. HRMS (ES+) calcd for C₁₆H₁₁NO₂S₂ [M+ Na]⁺ 336.0123, observed 336.0128.

Maleimide bridged sCT (3)



sCT (5.0 mg, 1.45 μmol) was dissolved in 1.5 mL of H₂O and TCEP (0.46 mg, 1.60 μmol) (in 0.5 mL H₂O) added. The solution was left to stir at ambient temperature until complete reduction of the disulfide bridge was observed by RP-HPLC. After 30 minutes, 5 mL of phosphate buffer (pH 6.2, 50 mM) was added along with 3 mL of acetonitrile. 3,4-Bis-phenylsulfanyl-pyrrole-2,5-dione (**1**) (0.50 mg, 1.60 μmol) in 0.5 mL DMF was added to the solution and the solution left to stir for 15 minutes before sampling for RP-HPLC analysis. RP-HPLC analysis confirmed the complete consumption of the reduced polypeptide, as well as the appearance of a single new corresponding to the bridged species (Figure S1). MALDI-ToF-MS analysis of the crude reaction mixture is shown in Figure S2.

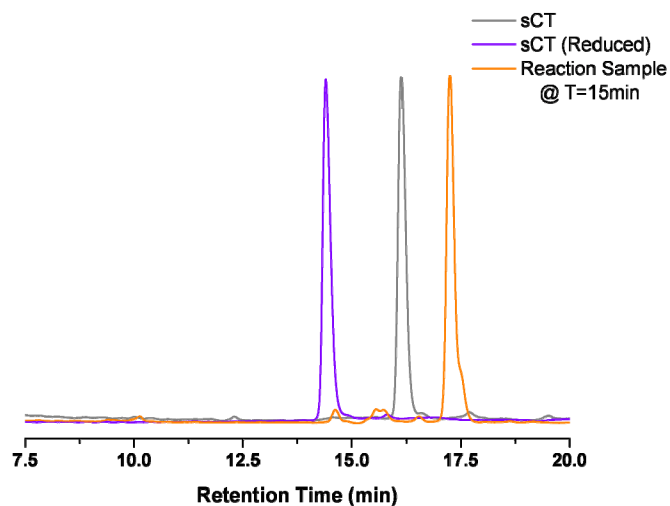


Figure S1. RP-HPLC analysis of the disulfide re-bridging of sCT using dithiophenol maleimide (1).

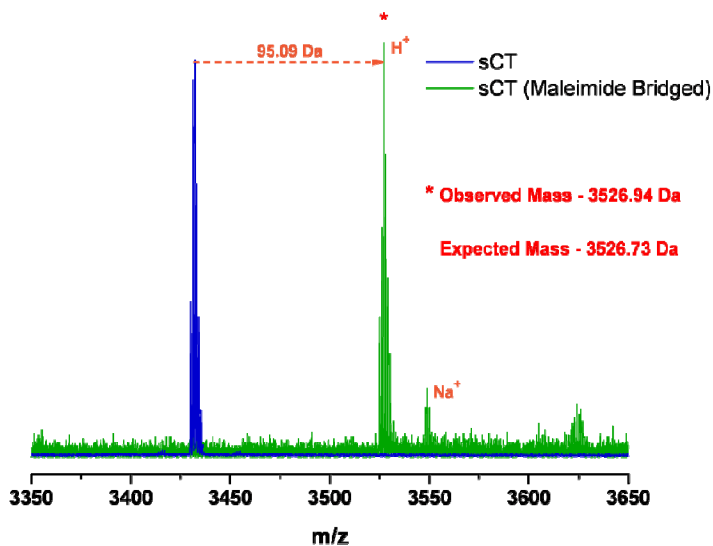


Figure S2. MALDI-ToF-MS analysis of the crude reaction mixture (15 min) following addition of dithiophenol-maleimide to a solution of reduced sCT.

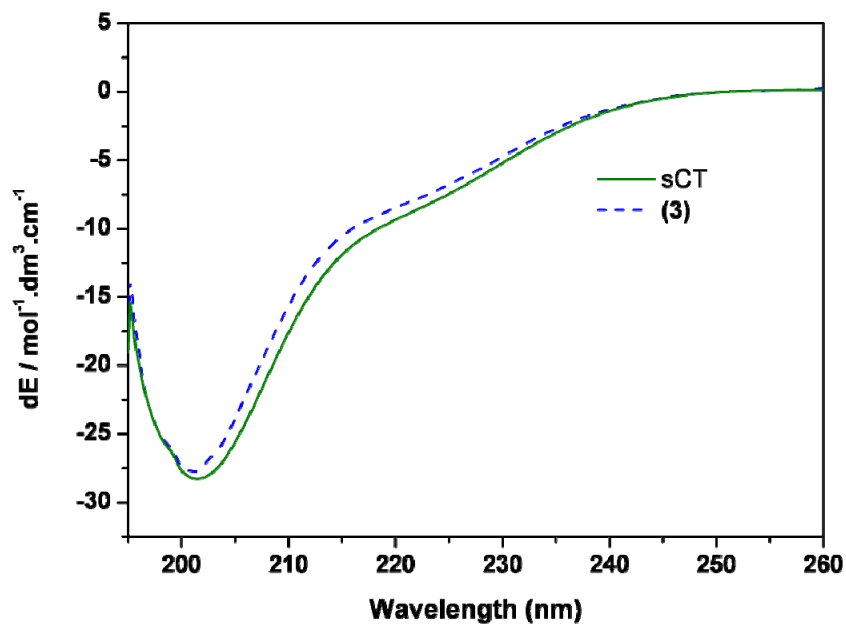


Figure S3. Circular Dichroism Analysis of sCT and (3), showing little if any disruption to the structure of sCT upon re-bridging of the disulfide with dithiophenolmaleimide.

Trypsin Digest of Conjugate (3)

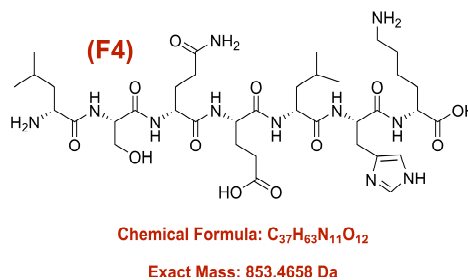
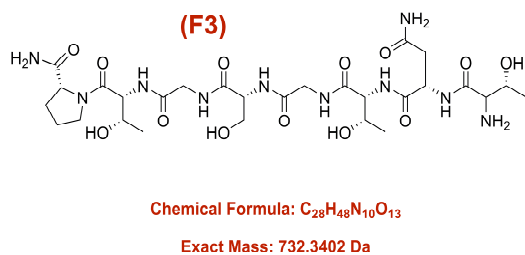
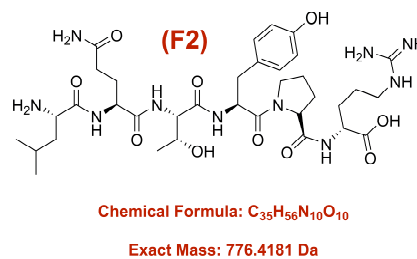
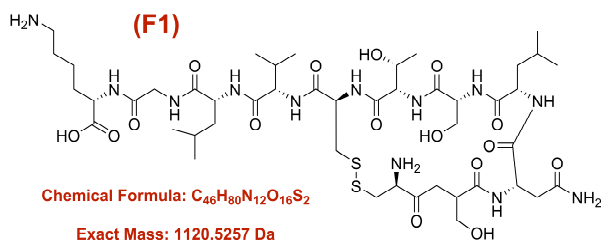


Figure S4. Native salmon calcitonin's trypsin digest fragments and expected masses.

(3) was subjected to trypsin digestion and the obtained fragments analysed. The disulfide containing fragment (F1) was the only fragment observed in its modified state, with F2, F3 and F4 observed as unmodified fragments. No trace of unmodified F1 was observed (suggesting quantitative and selective modification), with the maleimide-bridged species visualized in both its protonated and sodiated forms, in good agreement with the expected mass of the modified fragment (see Figure S5).

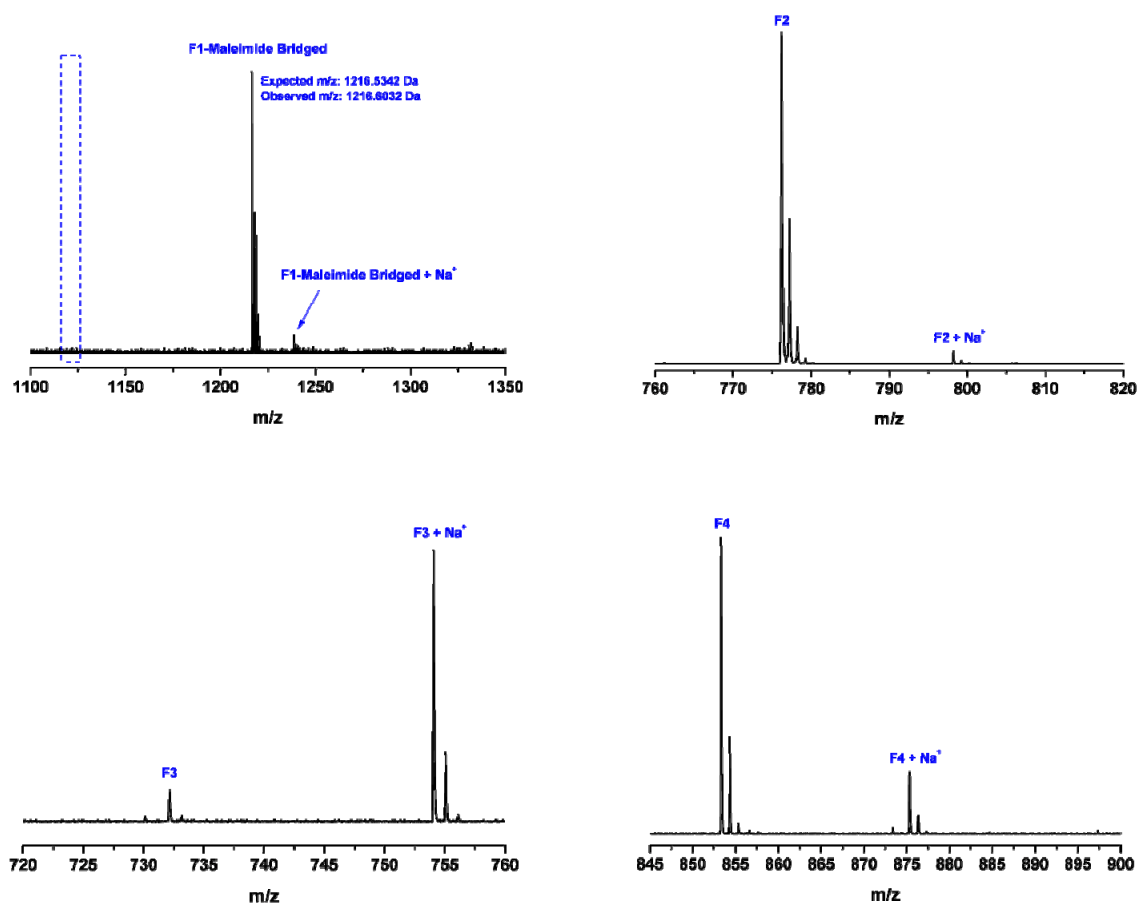
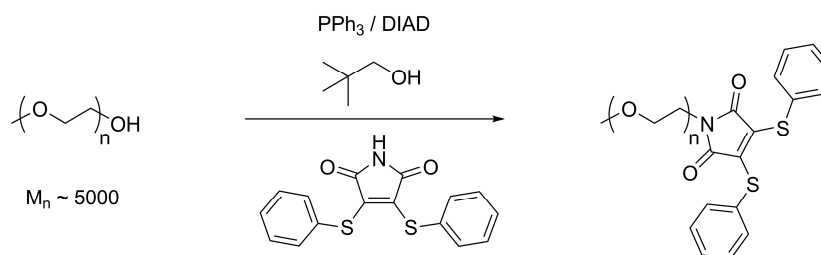


Figure S5. MALDI-ToF-MS analysis of the obtained fragments upon trypsin digestion of (3). F1 – top left. F2 – top right. F3 – bottom left. F4 – bottom right. The blue box depicted for fraction F1 corresponds to the mass of the mass of the unmodified fragment.

Dithiophenol-maleimide functional PEG₅₀₀₀ (2)



Triphenyl phosphine (1.57 g, 6.0 mmol) was dissolved in dry THF (50 mL) and cooled to -78 °C. Diisopropyl azodicarboxylate (1.18 mL, 6.0 mmol) was added dropwise to the solution and was left to stir for 5 minutes. Monomethoxy-PEG₅₀₀₀ (3.00 g, 0.6 mmol) was dissolved in dry DCM (20 mL) and added dropwise to the cooled solution and left to stir for a further 5 minutes. Neopentyl alcohol (0.48 g, 5.4 mmol) was added to the solution and left to stir for a further 10 minutes. 3,4-Bis-phenylsulfanyl-pyrrole-2,5-dione (1.88 g, 6.0 mmol) was added to the solution and left to stir at -78 °C for a further 1 hour. The solution was allowed to warm to ambient temperature and left to stir for 24 hours. The solution was reduced in volume to around 30 mL under reduced pressure and precipitated three times into 600 mL of 1:1 petroleum ether / diethyl ether. The isolated polymer was purified twice by flash chromatography (SiO₂, 0.5 : 100 to 1 : 10 gradient of methanol / dichloromethane) to yield the product as a yellow powder (2.07 g, 69.0 % yield).

M.p.: 57 – 59 °C. ¹H NMR (400.03 MHz, CDCl₃, 298 K) δ = 3.33 (s, 3H), 3.62 (m, 450H), 7.18 – 7.27 (m, 10H). ¹³C NMR (100.59 MHz, CDCl₃, 298 K) δ = 70.6 (225C), 128.4 (2C), 129.0 (4C), 129.1 (2C), 131.9 (4C), 135.7 (2C), 166.7 (2C). IR (neat) ν = 3498, 2881, 1959, 1711, 1578 cm⁻¹.

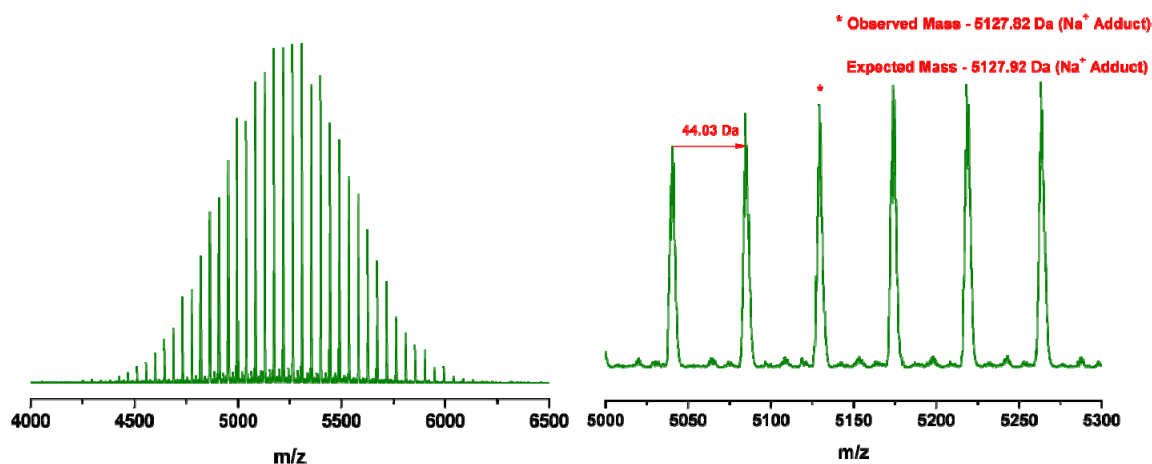
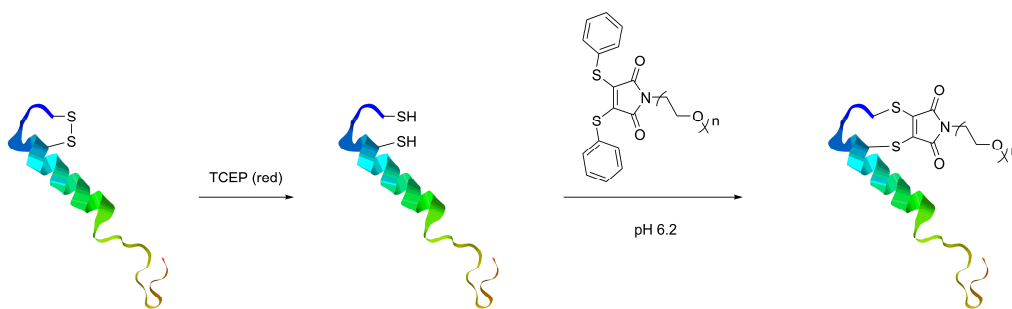


Figure S6. MALDI-ToF-MS analysis of dithiophenol maleimide-functional linear PEG (left) as well as a zoom of the 5000 – 5300 Da region (right).

sCT-PEG₅₀₀₀ Conjugate (4)



sCT (2.0 mg, 0.58 μmol) was dissolved in 500 μL of H_2O , along with TCEP (0.18 mg, 0.64 μmol) and left to stir at ambient temperature for 30 minutes. Upon complete reduction of the disulfide bridge (as observed by RP-HPLC), 2 mL of phosphate buffer (pH 6.2, 100 mmol) was added to the solution, followed by a solution of the synthesised dibromomaleimide-functional PEG₅₀₀₀ (3.2 mg, 0.64 μmol) in 1 mL of the same buffer. Upon addition of the PEG chain, the solution was left to stir at ambient temperature for 10 minutes and a sample was taken for RP-HPLC analysis (Figure S7). Complete consumption of the reduced polypeptide was observed, and the formation of a new peak, which was confirmed by MALDI-ToF as the PEGylated product (Figure S8).

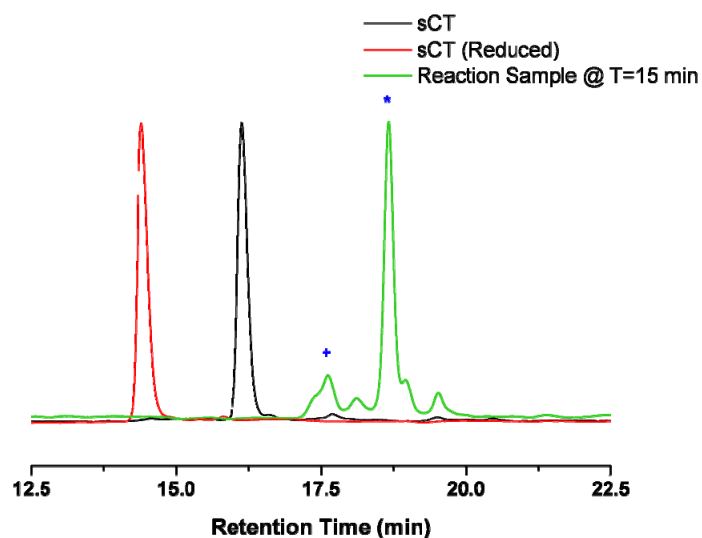


Figure S7. Crude RP-HPLC analysis of a 15 minute sample of the disulfide bridging of sCT using the dithiophenol-maleimide functional PEG chain.

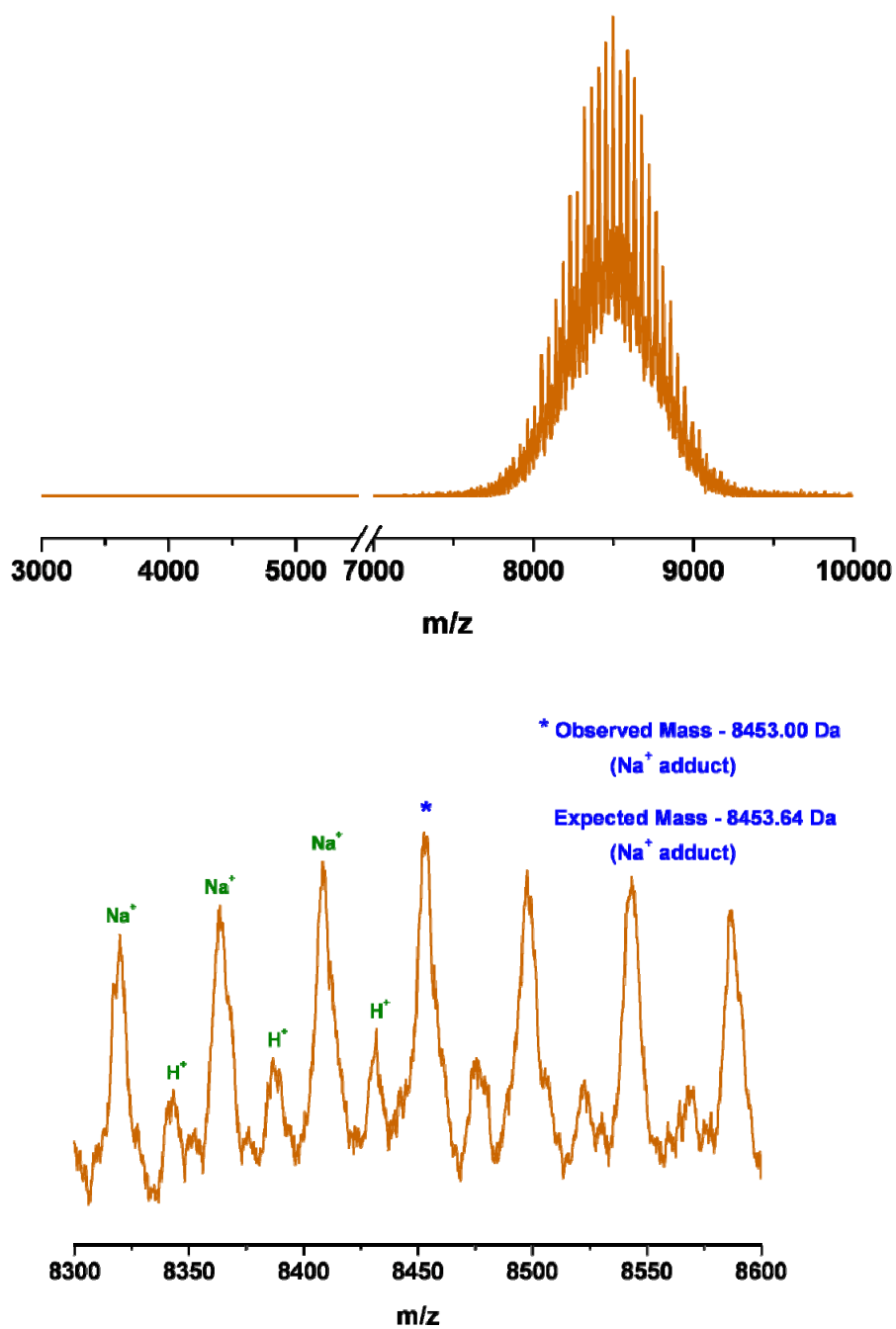
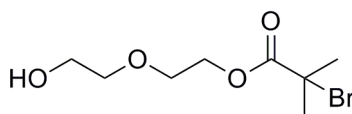


Figure S8. MALDI-ToF-MS of the reaction mixture @ 15 minutes, showing the formation of the monoconjugated sCT-PEG₅₀₀₀ species (top) and a zoomed region showing the different species observed (bottom). N.B. Low mass suppression was employed to suppress the slight excess of unreacted PEG in order to visualise the conjugate distribution.

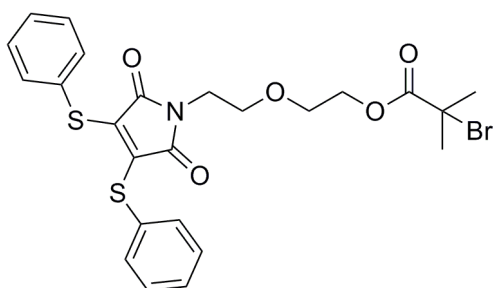
2-Bromo-2-methyl-propionic acid 2-(2-hydroxy-ethoxy)-ethyl ester (5)



Diethylene glycol (8.00 g, 75.4 mmol) was dissolved in 200 mL of THF and cooled to 0 °C in an ice bath. Triethylamine (11.60 mL, 82.9 mmol) was added to the solution and left to stir for 10 minutes. 2-Bromoisobutyryl bromide (9.31 mL, 75.4 mmol) was added dropwise to the solution over 20 minutes, whereby an immediate white precipitate was observed. Following complete addition, the solution was allowed to warm to ambient temperature and left to stir for 12 hours. The solution was filtered and the solvent removed under reduced pressure. The crude mixture was redissolved in dichloromethane (150 mL) and washed with saturated sodium carbonate (2 × 100 mL), water (2 × 100 mL) and brine (100 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum. The crude product was purified by flash chromatography (SiO₂, 4 : 1 petroleum ether / ethyl acetate followed by 1 : 1 petroleum ether : ethyl acetate) to yield the product as a colourless oil (6.40 g, 33.2% yield).

¹H NMR (400.03 MHz, CDCl₃, 298 K) δ = 1.88 (s, 6H, CH₃), 2.52 (br s, 1H, OH), 3.57 (t, *J* = 4.5 Hz, 2H, CH₂), 3.67 (t, *J* = 4.5 Hz, 2H, CH₂), 3.70 (t, *J* = 4.5 Hz, 2H, CH₂), 4.29 (t, *J* = 4.5 Hz, 2H, CH₂). ¹³C NMR (100.59 MHz, CDCl₃, 298 K) δ = 30.7 (2C), 55.7 (1C), 61.7 (1C), 65.0 (1C), 68.7 (1C), 72.5 (1C), 171 (1C). IR (neat) ν = 3331, 2869, 1733, 1463, 1389, 1371, 1273 cm⁻¹. HRMS (ES+) calcd for C₈H₁₅BrO₄ [M + Na]⁺ 277.0046, observed 277.0053.

2-Bromo-2-methyl-propionic acid 2-[2-(2,5-dioxo-3,4-bis-phenylsulfanyl-2,5-dihydro-pyrrol-1-yloxy)-ethoxy]-ethyl ester (6)

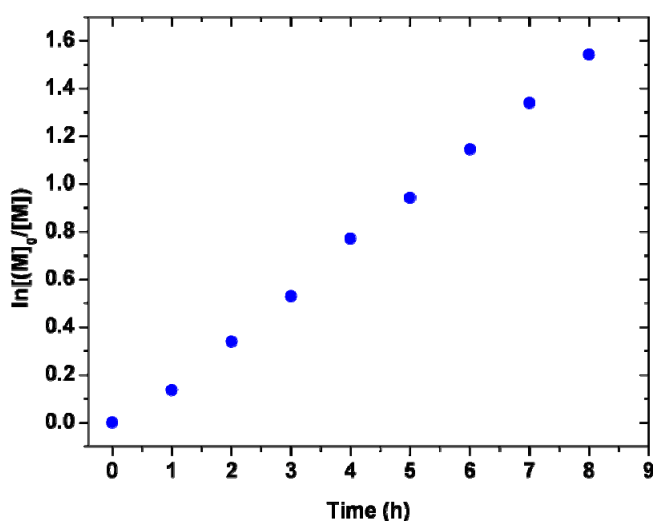
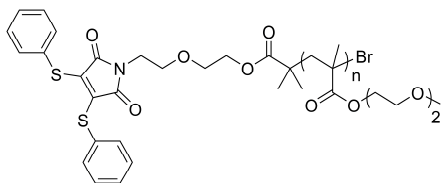


Triphenyl phosphine (2.056 g, 7.84 mmol) was dissolved in 70 mL of dry THF and cooled to -78 °C in a dry ice bath. Diisopropyl azodicarboxylate (1.54 mL, 7.84 mmol) was added dropwise to the solution and left to stir for 5 minutes. 2-Bromo-2-methyl-propionic acid 2-(2-hydroxy-ethoxy)-ethyl

ester (2.000 g, 7.84 mmol) was added to the solution and left to stir for a further 5 minutes. The dummy ligand neopentyl alcohol (0.346 g, 3.92 mmol) was added to the solution and left to stir for a further 10 minutes. 3,4-Bis-phenylsulfanyl-pyrrole-2,5-dione (2.457 g, 7.84 mmol) was added to the solution and stirred for 1 hour at $-78\text{ }^{\circ}\text{C}$. The solution was then allowed to warm to ambient temperature and left to stir for 12 hours. The solvent was removed under reduced pressure and the crude mixture purified by flash chromatography (SiO_2 , 2:1 petroleum ether/diethyl ether) to yield the product as a viscous orange oil (3.51 g, 81.3% yield).

^1H NMR (400.03 MHz, CDCl_3 , 298 K) δ = 1.94 (s, 6H, CH_3), 3.63 – 3.71 (m, 6H, CH_2), 4.26, (t, J = 5.0 Hz, 2H, CH_2), 7.20 – 7.31, (m, 10H, CH). ^{13}C NMR (100.59 MHz, CDCl_3 , 298 K) δ = 30.9 (2C), 38.1 (1C), 55.9 (1C), 65.1 (1C), 67.9 (1C), 68.2 (1C), 128.5 (2C), 129.1 (4C), 129.2 (2C), 132.0 (4C), 135.8 (2C), 166.8 (2C), 171.7 (2C). IR (neat) ν (cm^{-1}) = 2867, 1773, 1734, 1707, 1581, 1441, 1395, 1166, 1108, 1023 cm^{-1} . HRMS (ES $^+$) calcd for $\text{C}_{24}\text{H}_{24}\text{BrNO}_5\text{S}_2$ $[\text{M} + \text{Na}]^+$ 572.0171, observed 572.0175.

Dithiophenol-maleimide functional poly(DEGMEMA) (7)



To an oven dried Schlenk tube, 2-bromo-2-methyl-propionic acid 2-[2-(2,5-dioxo-3,4-bis-phenylsulfanyl-2,5-dihydro-pyrrol-1-yloxy)-ethoxy]-ethyl ester (0.292 g, 0.53 mmol), di(ethylene glycol) methyl ether methacrylate (5.00 g, 26.56 mmol), copper(I) bromide (0.0762 g, 0.53 mmol) and toluene (10 mL) were added. The tube was sealed and subjected to four freeze-pump-thaw cycles and left under a blanket of nitrogen. *N*-Ethyl-2-pyridylmethanimine (0.24 mL, 1.59 mmol) was added to the solution *via* a degassed syringe and the Schlenk tube immersed in an oil bath at $60\text{ }^{\circ}\text{C}$. Samples were taken hourly and analysed by ^1H NMR and GPC. After 8 hours, the reaction was quenched by immersing the flask in liquid nitrogen. The solution was then diluted with toluene (100 mL) and

bubbled with air for 5 hours. The solution was passed through a short column of neutral alumina and the solvent removed under vacuum to yield the crude mixture. The oil was then dissolved in methanol and dialysed (1000 Da MWCO) against the same solvent for 3 days. The solvent was then removed to yield the polymer as a bright yellow oil (2.83 g, 57 % yield).

M_n (GPC, CHCl_3) 6800 g mol^{-1} .

PDi (GPC, CHCl_3) 1.24.

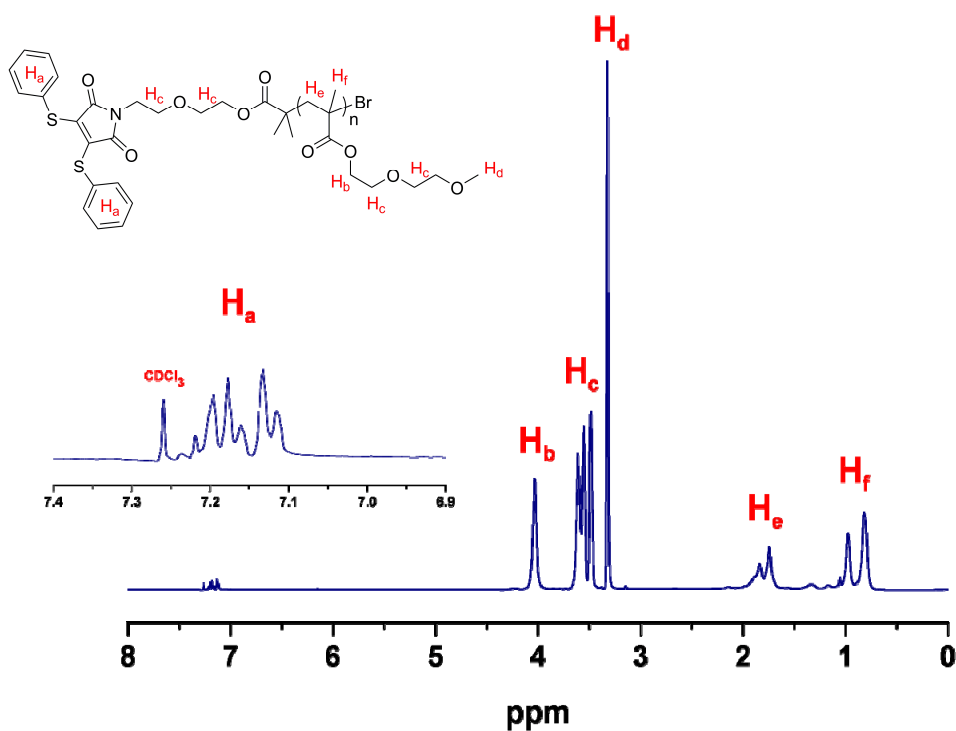


Figure S9. ^1H NMR analysis (400 MHz, CDCl_3) of poly(DEGMEMA) synthesised by ATRP using a dithiomaleimide-functional initiator.

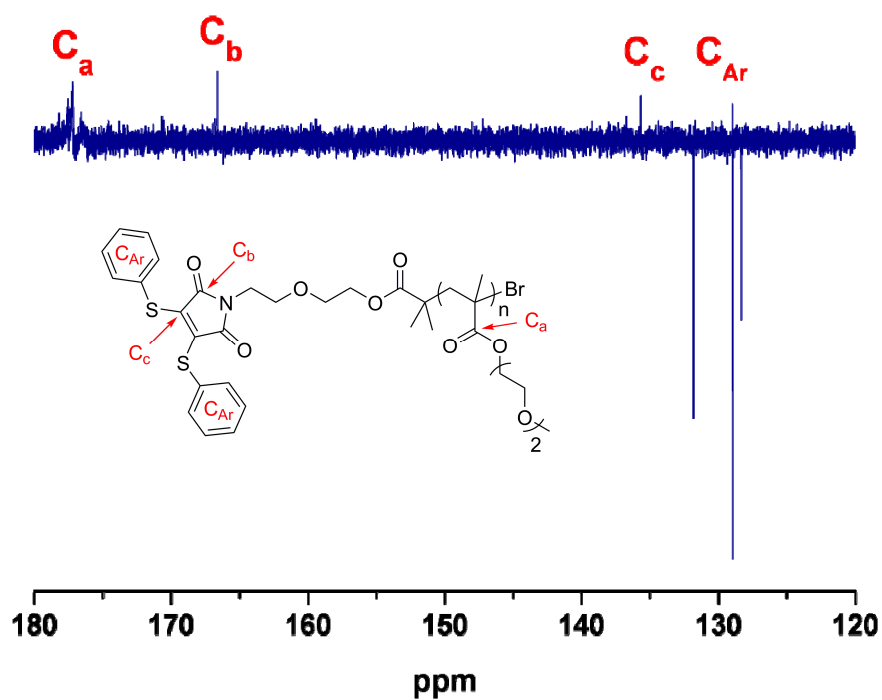


Figure S10. Partial DEPT ^{13}C NMR spectrum (400 MHz, CDCl_3) of the synthesised poly(DEGMEMA) showing the presence of the intact maleimide end-group.

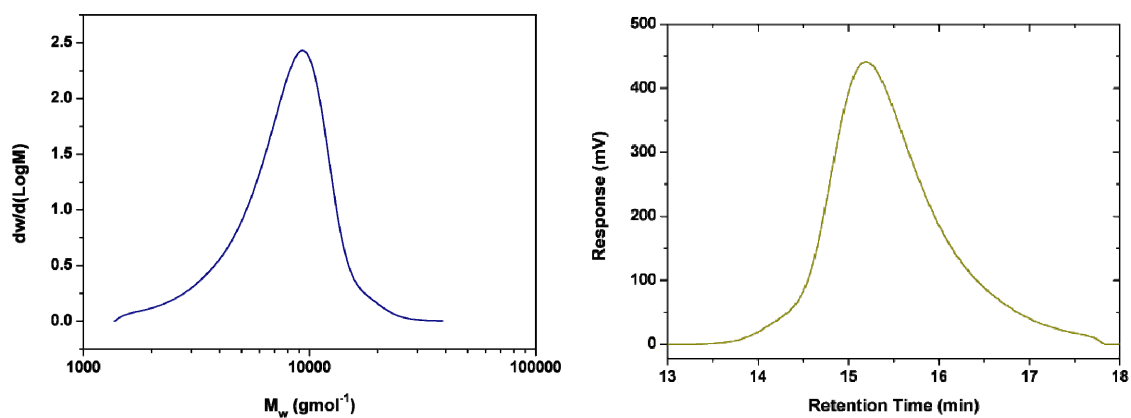


Figure S11. GPC analysis of purified polymer (7).

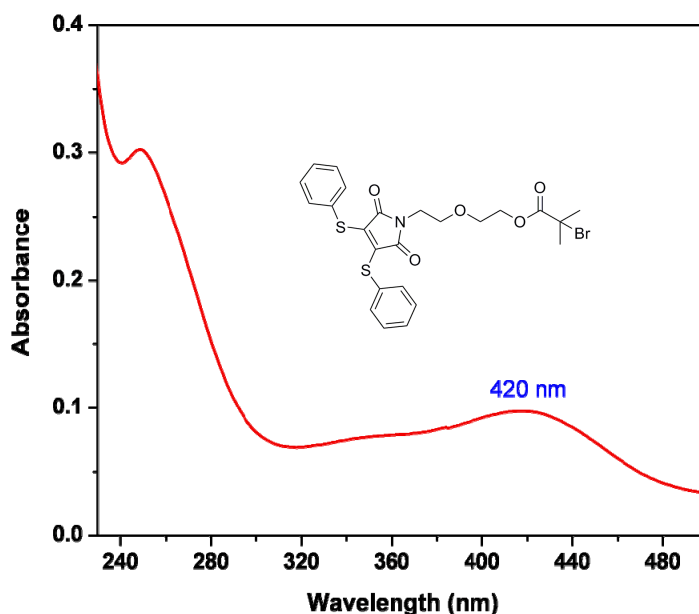
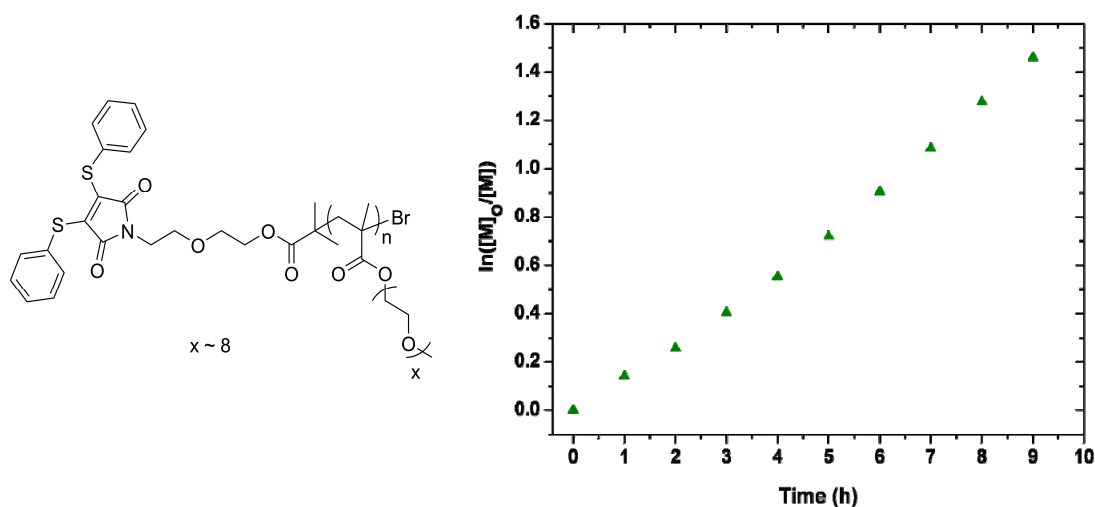


Figure S12. UV Spectrum of dithiophenol maleimide initiator (6) in methanol.

Dithiophenol-malimide functional poly(PEGMA) (8)



To an oven dried Schlenk tube, 2-bromo-2-methyl-propionic acid 2-[2-(2,5-dioxo-3,4-bis-phenylsulfanyl-2,5-dihydro-pyrrol-1-yloxy)-ethoxy]-ethyl ester (0.2897g, 0.52 mmol), poly (ethyleneglycol) methyl ether methacrylate ($M_n \sim 475$) (5.00 g, 10.5 mmol), copper(I) bromide (0.0755 g, 0.52 mmol) and toluene (10 mL) were added. The tube was sealed and subjected to four freeze-pump-thaw cycles and left under a blanket of nitrogen. *N*-ethyl-2-pyridylmethanimine (0.23 mL, 1.57 mmol) was added to the solution *via* a degassed syringe and the Schlenk tube immersed in an oil bath at 60 °C. Samples were taken hourly and analysed by ^1H NMR and GPC. After 9 hours, the reaction was quenched by immersing the flask in liquid nitrogen. The solution was then diluted with toluene (100 mL) and bubbled with air for 5 hours. The solution was passed through a short

column of neutral alumina and the solvent removed under vacuum to yield the crude mixture. The oil was then dissolved in methanol and dialysed (1000 Da MWCO) against the same solvent for 3 days. The solvent was then removed to yield the polymer as a bright yellow oil (2.41 g, 54.5% yield).

M_n (GPC, THF) 8520 g mol⁻¹.

PDI (GPC, THF) 1.16.

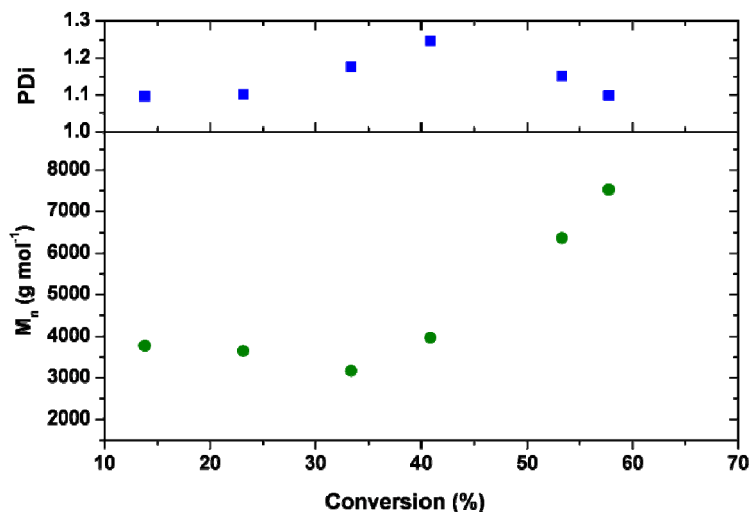


Figure S13. Evolution of M_n and PDI with conversion using [dithiophenol-maleimide initiator]:[Cu(I)Br]:[Lig]:[PEGMA] = 1:1:3:20, toluene/PEGMA 2:1 (v/w), 60 °C.

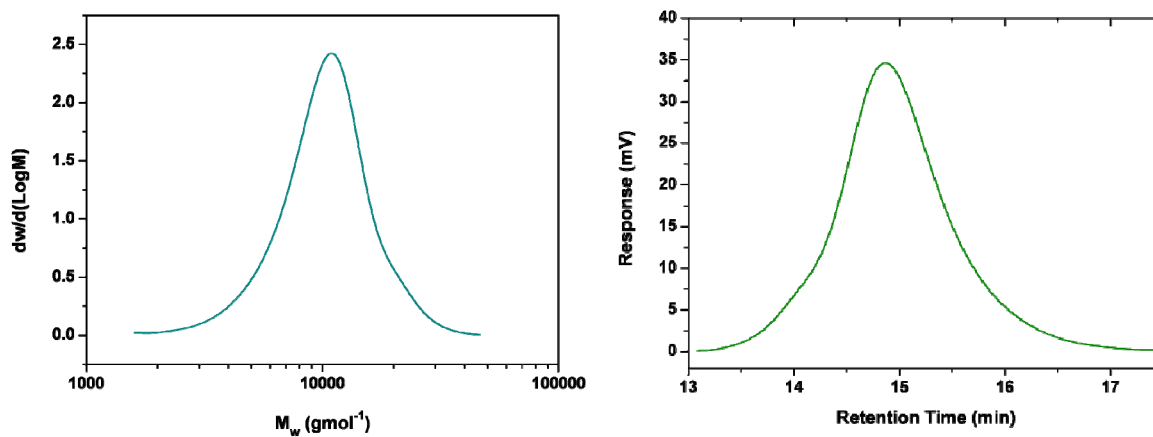


Figure S14. GPC analysis of purified polymer (8).

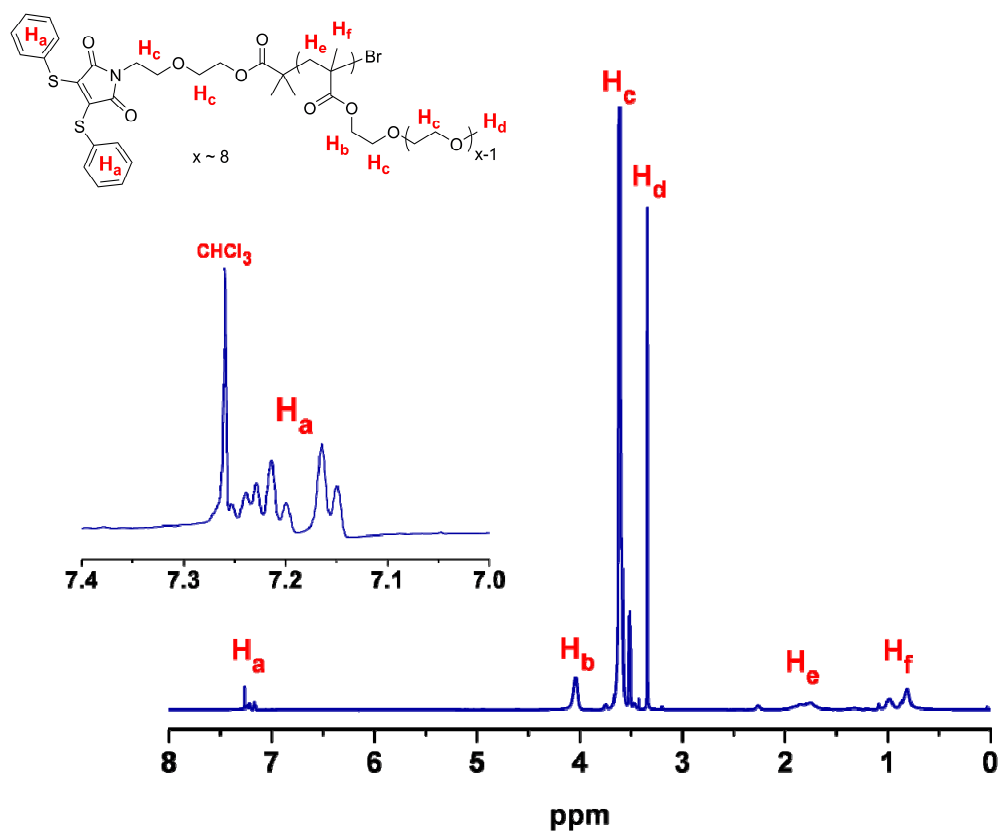


Figure S15. ¹H NMR analysis (400 MHz, CDCl₃) of poly(DEGMEMA)-co-(PEGMA) synthesised by ATRP using a dithiomaleimide-functional initiator.

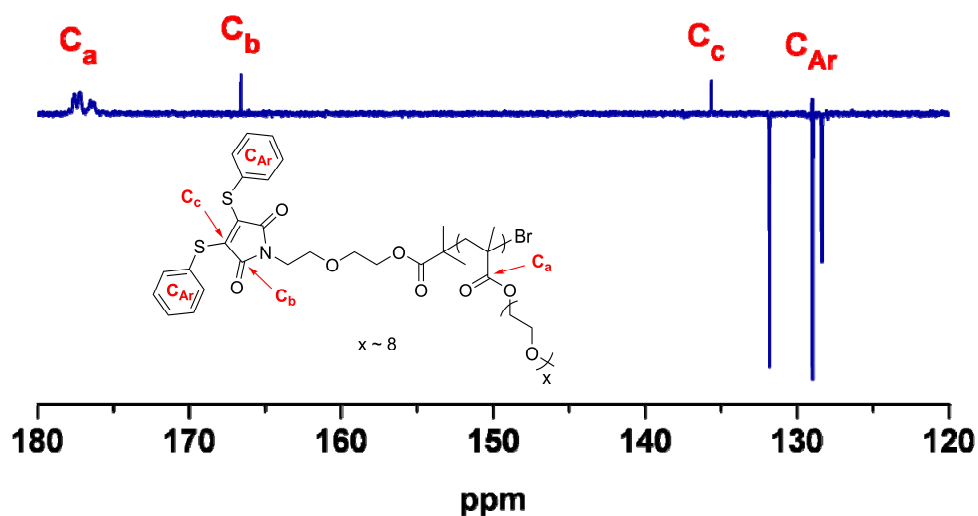
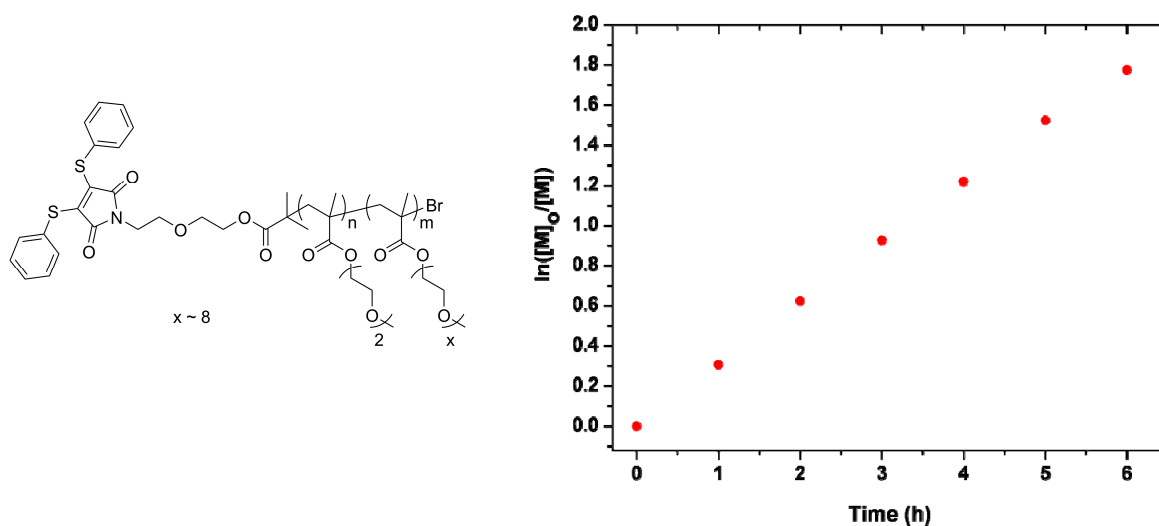


Figure S16. Partial DEPT ^{13}C NMR (400 MHz, CDCl_3) of the synthesised poly(PEGMA) showing the presence of the intact maleimide end-group.

Dithiophenol-maleimide functional poly(DEGMEMA)-*co*-(PEGMA) (Gradient Copolymer) (9)



To an oven dried Schlenk tube, 2-bromo-2-methyl-propionic acid 2-[2-(2,5-dioxo-3,4-bis-phenylsulfanyl-2,5-dihydro-pyrrol-1-yloxy)-ethoxy]-ethyl ester (0.54 g, 0.99 mmol), poly (ethyleneglycol) methyl ether methacrylate ($M_n \sim 475$) (1.40 g, 2.96 mmol), di(ethylene glycol) methyl ether methacrylate (5.0 g, 26.6 mmol), copper(I) bromide (0.140 g, 0.99 mmol) and toluene (12.8 mL) were added. The tube was sealed and subjected to four freeze-pump-thaw cycles and left under a blanket of nitrogen. *N*-Ethyl-2-pyridylmethanimine (0.44 mL, 2.96 mmol) was added to the solution *via* a degassed syringe and the Schlenk tube immersed in an oil bath at 60 °C. Samples were

taken hourly and analysed by ^1H NMR and GPC. Kinetics were analysed by monitoring the disappearance of both monomer peaks combined. After 6 hours, the reaction was quenched by immersing the flask in liquid nitrogen. The solution was then diluted with toluene (100 mL) and bubbled with air for 5 hours. The solution was passed through a short column of neutral alumina and the solvent removed under vacuum to yield the crude mixture. The oil was then dissolved in methanol and dialysed (1000 Da MWCO) against the same solvent for 3 days. The solvent was then removed to yield the polymer as a bright yellow oil (3.47 g, 56.2% yield).

M_n (GPC, THF) 6240 g mol^{-1} .

PDI (GPC, THF) 1.28.

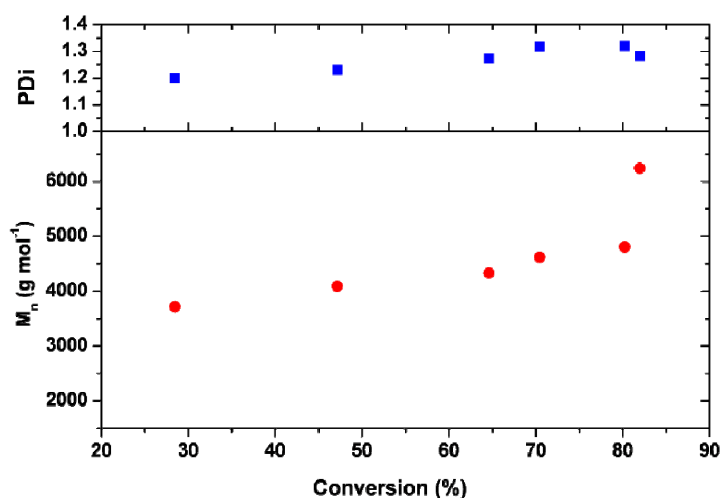


Figure S17. Evolution of M_n and PDI with conversion using [dithiophenol-maleimide initiator]:[Cu(I)Br]:[Lig]:[DEGMEMA]:[PEGMA] = 1:1:3:27:3, toluene/(DEGMEMA and PEGMA) 2:1 (v/w), 60°C .

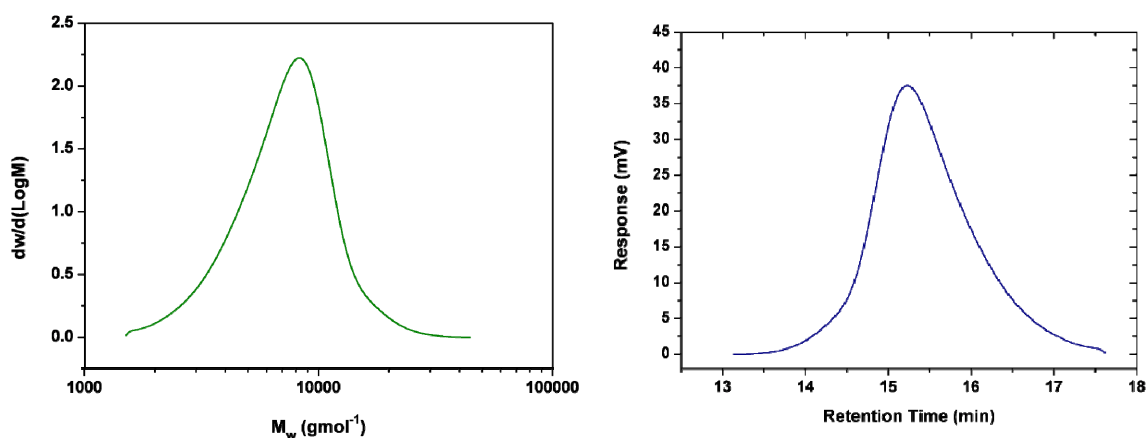


Figure S18. GPC analysis of purified polymer (9).

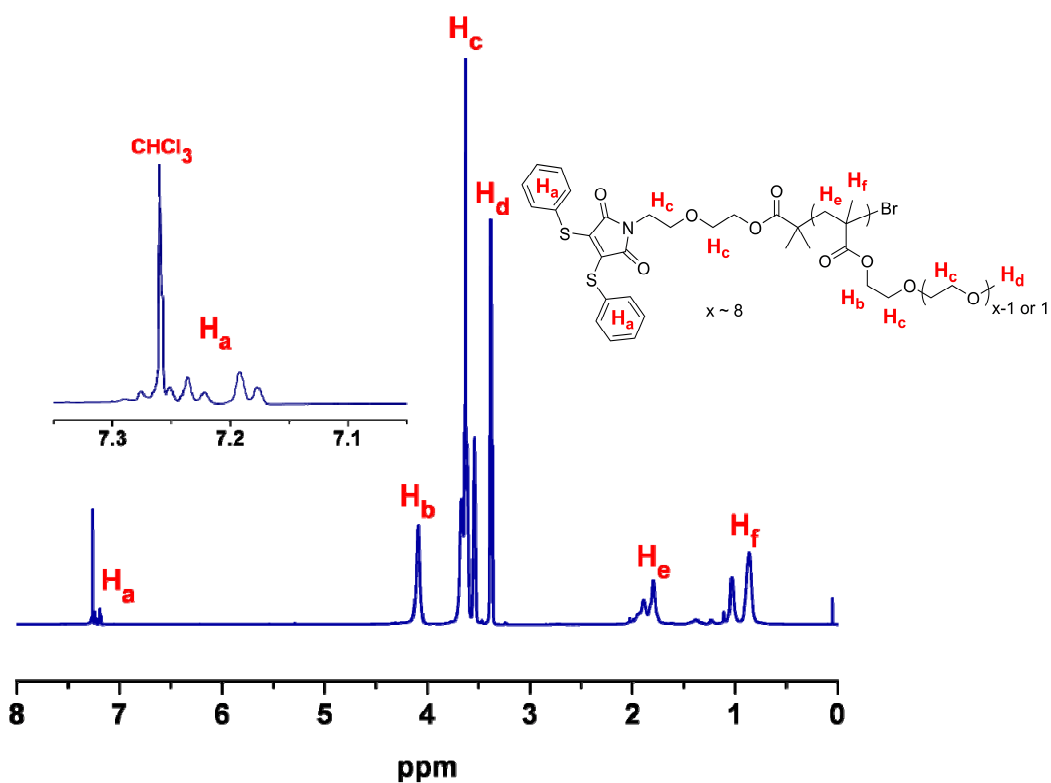


Figure S19. ¹H NMR analysis (400 MHz, CDCl₃) of poly(DEGMEMA)-co-(PEGMA) synthesised by ATRP using a dithiomaleimide-functional initiator.

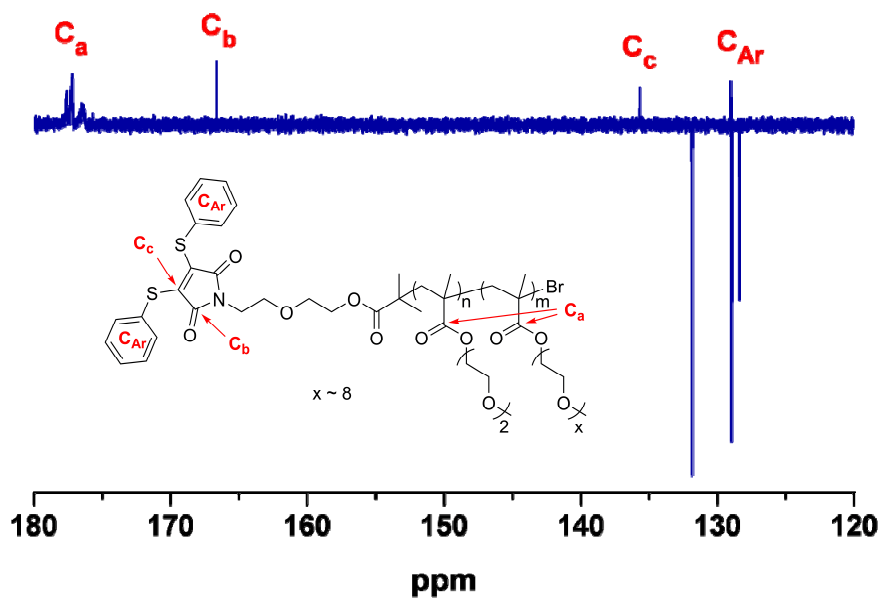
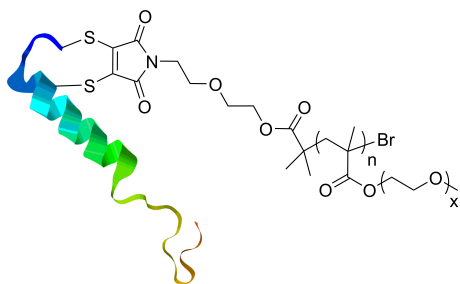


Figure S20. Partial DEPT ¹³C NMR spectrum (400 MHz, CDCl₃) of the synthesised poly(DEGMEMA)-co-(PEGMA) showing the presence of the intact maleimide end-group.

sCT-poly(PEGMA) Conjugate (10)



sCT (5.0 mg, 1.45 μmol) was dissolved in 500 μL of H_2O , along with TCEP (0.46 mg, 1.60 μmol) and left to stir at ambient temperature for 30 minutes. Upon complete reduction of the disulfide bridge (as observed by RP-HPLC), 2 mL of phosphate buffer (pH 6.2, 100 mmol) was added to the solution, followed by a solution of polymer (**8**) (10.9 mg, 1.60 μmol) in 1 mL of the same buffer. Upon addition of the polymer, the solution was left to stir at ambient temperature for 10 minutes and a sample was taken for RP-HPLC analysis (Fig 2). Complete consumption of both reduced sCT and the polymer starting material were observed and the conjugate was passed through a desalting column and lyophilised to yield the conjugate as a yellow oil.

N.B. Despite a small excess of polymer being employed relative to sCT, no free polymer was observed by RP-HPLC following sampling. This was attributed to the inherent inaccuracies of poly(OEGMA) molecular weight determination by GPC against pMMA standards.

References

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2. R. N. Keller and H. D. Wycoff, *Inorg. Synth.*, 1946, 1-4.