Polymeric dibromomaleimides as extremely efficient disulfide bridging bioconjugation and pegylation agents

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Electronic Supporting Information

Experimental

N-(Ethyl)-2-pyridylmethanimide,[1] azide-initiator (5)[2] and tri(ethylene glycol) methacrylate were synthesised as described previously.[3] Copper(I) bromide was purified as described by Keller and Wycoff.[4] Tris(2-carboxyethyl)phosphine hydrochloride (purum, ≥98.0%) was purchased from Sigma Aldrich and used as received. Salmon Calcitonin was purchased from Polypeptide Laboratories (Hillerod, Denmark) and stored at 4 ^{o}C. Sequence – CYS^{1}-SER^{2}-ASN^{3}-LEU^{4}-SER^{5}-THR^{6}-CYS^{7}-VAL^{8}-LEU^{9}-GLY^{10}-LYS^{11}-LEU^{12}-SER^{13}-GLN^{14}-GLU^{15}-LEU^{16}-HIS^{17}-LYS^{18}-LEU^{19}-GLN^{20}-THR^{21}-TYR^{22}-PRO^{23}-ARG^{24}-THR^{25}-ASN^{26}-THR^{27}-GLY^{28}-SER^{29}-GLY^{30}-THR^{31}-PRO^{32}-NH_2. Disulphide bridge: CYS^{1}-CYS^{7}.

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 × 7.5 mm) guard column, two PL-gel 5 µm (300 × 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 × 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 × 7.5 mm) guard column, two PL-gel 5 µm (300 × 7.5 mm) mixed-D columns equipped with a differential refractive index detector, using $CHCl_3$ as the eluent with a flow rate of 1.0 mL min^{-1}. Narrow molecular weight PMMA standards (200 - 1.0 ×10^{6} g mol^{-1}) were used for calibration using a second order polynomial fit.

RP-HPLC

RP-HPLC was carried out using Jupiter C 18 (5 μm) 250 x 4.6 mm and Varian PLRP-S 100A (5 μm) 250 x 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 λ = 280 nm.

The mobile phases used were:

a) mobile phase A: 90 % v/v water, 10 % v/v MeCN (far UV) and 0.05 % v/v TFA;

b) 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.

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 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 matiricies 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

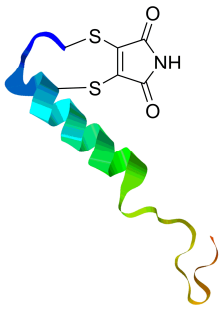
Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell. Mass spectra were recorded using a Micromass Autospec apparatus.

Cloud point measurements

Cloud-point analysis was performed on an OptiMelt MPA100 system by Stanford Research Systems. Conjugates were dissolved in 18 MΩ water at a concentration of 1 mg mL^{-1} and heated at a rate of 0.5 °C min^{-1}. Two runs were performed simultaneously and an average of both runs plotted to determine the cloud point of the respective polymer/conjugate.

Synthetic Protocols

sCT-dibromomaleimide Conjugate (2)



sCT (5.0 mg, 1.45 µmol) was dissolved in 2 mL 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), 5 mL of phosphate buffer (pH 6.2, 100 mmol) was added to the solution, along with 2.5 mL of acetonitrile followed by a solution of the dibromomaleimide (0.82 mg, 1.60 µmol) in 0.5 mL of DMF. Upon addition of dibromomaleimide, the solution turned pale yellow in colour and after 2 minutes, a sample was taken for RP-HPLC analysis. Complete consumption of the reduced polypeptide was observed, and the formation of a new peak, which was confirmed by MALDI-ToF as the re-bridged product.

Trypsin Digest of Conjugate (2)

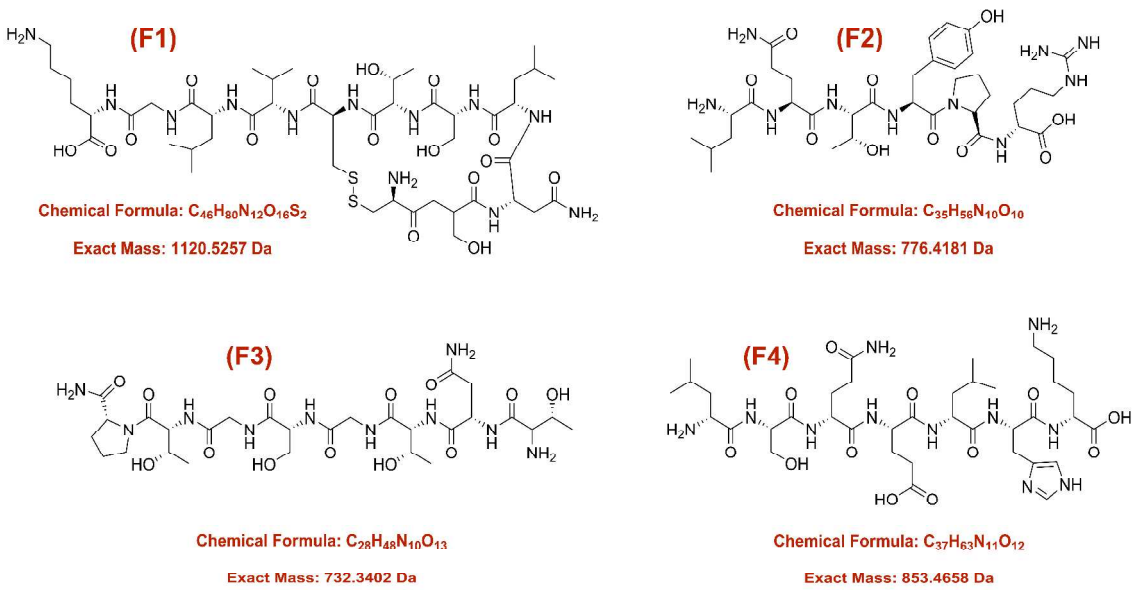


Figure S1. Trypsin digest fragments of salmon calcitonin.

(**2**) was subjected to trypsin digestion and the obtained fragments analyzed. 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.

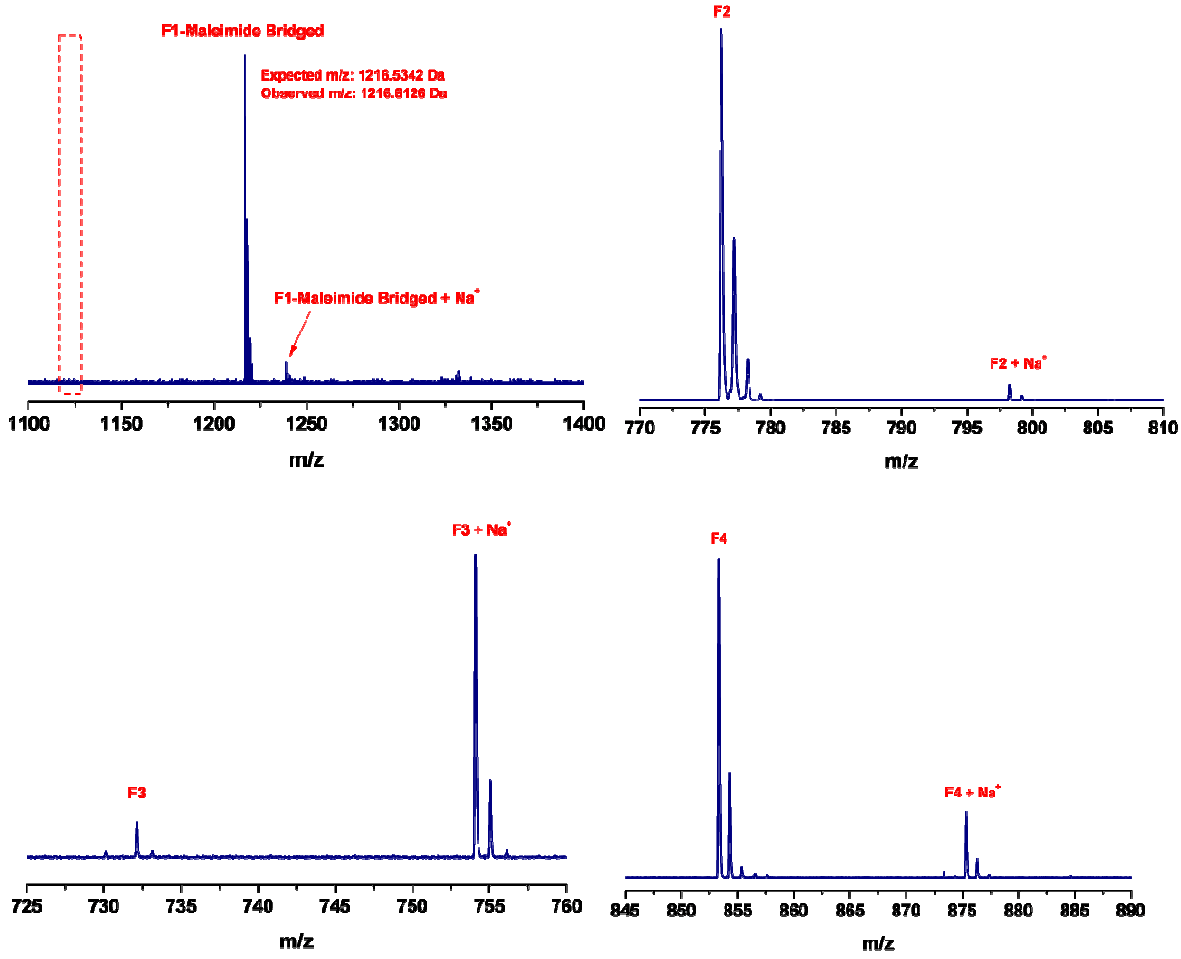


Figure S2. MALDI-ToF-MS analysis of the obtained fragments upon trypsin digestion of (2). F1 – top left. F2 – top right. F3 – bottom left. F4 – bottom right. The red box depicted for fraction F1 corresponds to the mass of the mass of the unmodified fragment.

Circular Dichroism Analysis of sCT and (2)

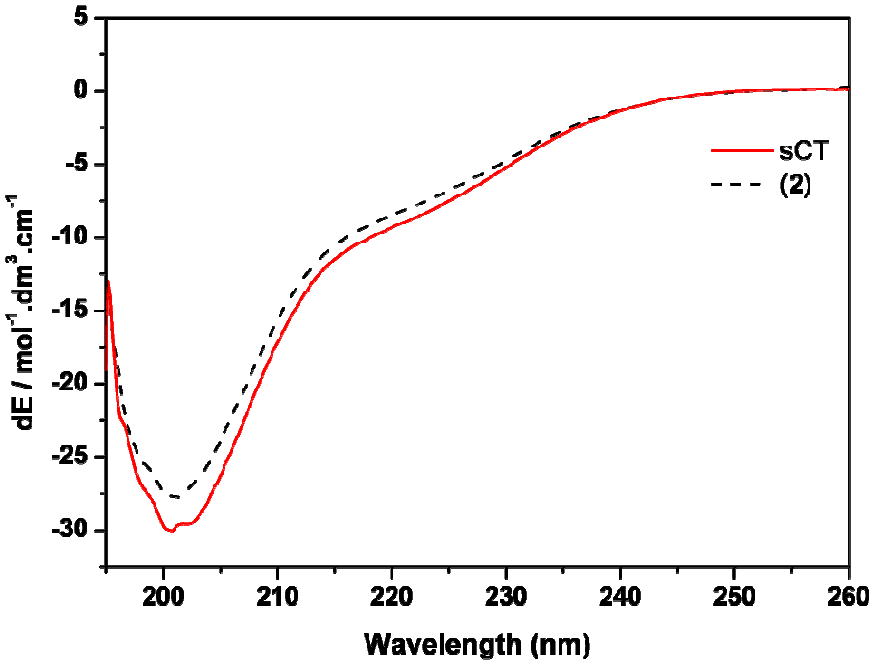
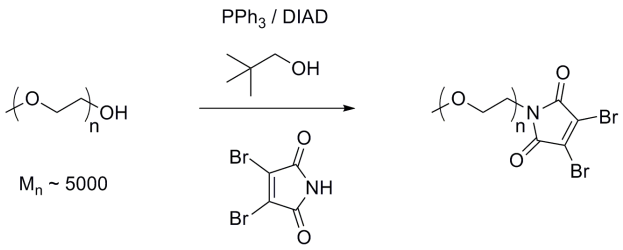


Figure S3. Circular Dichroism Analysis of sCT and (2), showing little if any disruption to the structure of sCT upon re-bridging of the disulfide with dibromomaleimide.

3,4-Dibromomaleimide-functional PEG$_{5000}$ (3)



Triphenyl phosphine (2.62 g, 10.0 mmol) was dissolved in dry THF (100 mL) and cooled to -78 °C. Diisopropyl azodicarboxylate (1.97 mL, 10.0 mmol) was added dropwise the solution and was left to stir for 5 minutes. Monomethoxy-PEG$_{5000}$ (5.00 g, 1.0 mmol) was dissolved in dry DCM (40 mL) and added dropwise to the cooled solution and left to stir for a further 5 minutes. Neopentyl alcohol (0.79 g, 9.0 mmol) was added to the solution and left to stir for a further 10 minutes. 3,4-dibromomaleimide (2.55 g, 10.0 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 (SiO_2, 0.5 : 100 to 1 : 10 gradient of methanol / dichloromethane) to yield the product as a cream coloured powder (1.05 g, 21.0 % yield).

M.p.: 53 – 57 °C. ^{1}H NMR (400.03 MHz, CDCl3, 298 K) δ = 3.58 (m, 450H). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 163.8 (2C), 129.6 (2C), 70.6 (57C). IR (neat) ν = 3517, 2872, 1977, 1727, 1641 cm^{-1}.

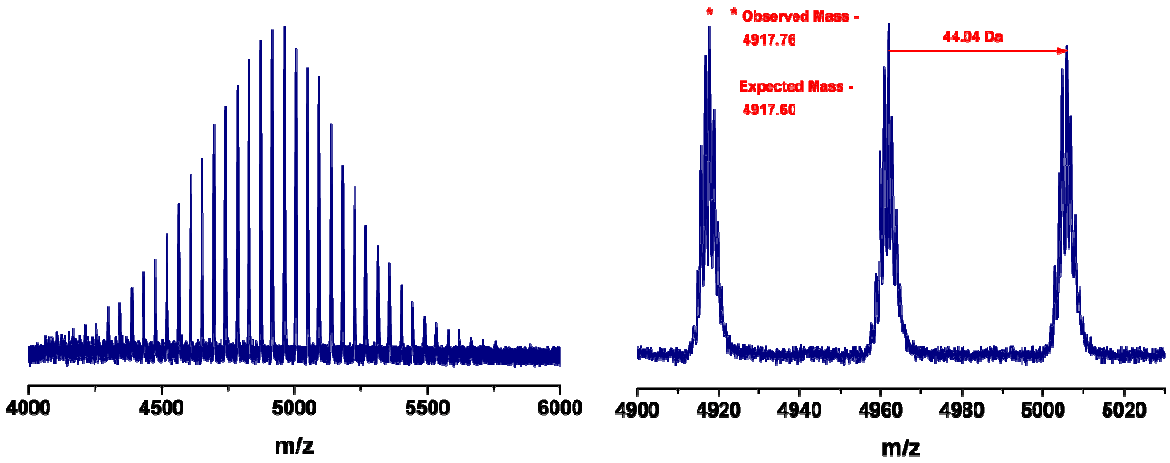
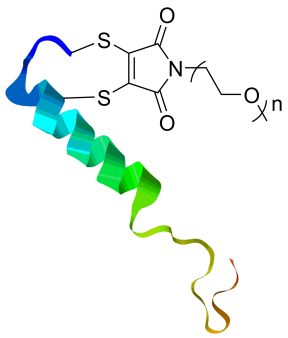


Figure S4. MALDI-ToF-MS analysis of the synthesised dibromomaleimide-functional PEG chain (3).

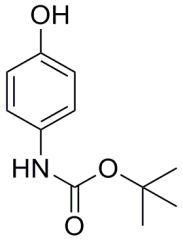
Synthesis of sCT-PEG$_{5000}$ conjugate (4)



sCT (2.0 mg, 0.58 mmol) was dissolved in 500 µL of H_2O, along with TCEP (0.18 mg, 0.64 mmol) 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 PEG$_{5000}$ (3.2 mg, 0.64 mmol) in 1 mL of the same buffer. Upon addition of the PEG chain, the solution turned pale yellow in colour

and after 10 minutes, a sample was taken for RP-HPLC analysis. 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.

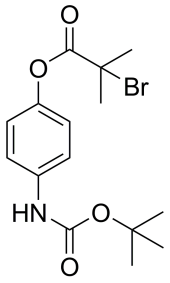
Tert-butyl 4-hydroxyphenylcarbamate



4-Amino phenol (5.45 g, 49.9 mmol) was dissolved in DMF along with triethylamine (6.96 mL, 49.9 mmol) and cooled to 0 °C. Di-tertiary butyl carbonate (10.90 g, 49.9 mmol) was added in one portion to the solution and was left to stir and warm to room temperature overnight. The solvent was removed under vacuum and the residue redissolved in DCM (150 mL). The organic layer was washed with 15% NH_4Cl solution (2 x 150 mL), water (2 x 150 mL) and brine (150 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent removed under vacuum. The resultant solid was recrystalised from hot toluene, filtered and isolated as white crystals (8.36 g, 80.1% yield).

M.p.: 143 – 144 °C. ^{1}H NMR (400.03 MHz, $CDCl_3$, 298 K) δ = 1.50 (s, 9H, 3 x C*H*$_3$), 6.73 (d, 2H, *J* = 8.8 Hz, C*H*), 7.14 (d, 2H, *J* = 8.6 Hz, C*H*). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 28.51 (3C), 80.71 (C), 115.95 (2C), 121.94 (2C), 130.64 (C), 152.54 (C), 154.02 (C). IR (neat) ν = 3357, 1732, 1695, 1607, 1511, 1437, 1367, 1224, 1162 cm^{-1}. HRMS (ES+) calcd for $C_{11}H_{15}NO_3$ [M + Na]$^+$ 209.1052, observed 209.1054.

4-(Tert-butoxycarbonylamino)phenyl 2-bromo-2-methylpropanoate (6)

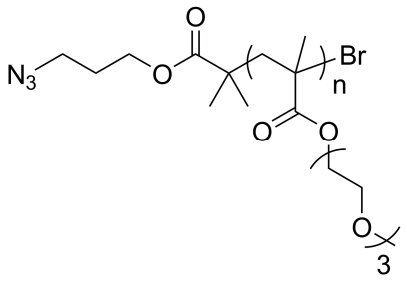


Tert-butyl 4-hydroxyphenylcarbamate (5.00 g, 23.9 mmol) was dissolved in anhydrous THF (100 mL) and cooled to 0 °C in an ice bath. Triethylamine (3.66 mL, 26.3 mmol) was added to the solution and left to stir for 10 minutes. α-Bromoisobutyryl bromide (3.24 mL, 26.3 mmol) was dissolved in THF

(20 mL) and added dropwise to the cooled solution over a period of 15 minutes. Following complete addition, the solution was allowed to warm to ambient temperature and left to stir for a further 12 hours. The solution was then filtered and the solvent removed under reduced pressure. The obtained residue was redissolved in dichloromethane (150 mL) and washed with water (2 x 100 mL), saturated $NaHCO_3$ solution (2 x 100 mL) and brine (100 mL). The organic layer was dried over anhydrous magnesium sulphate, filtered and the solvent removed. The crude mixture was purified by flash chromatography (SiO_2, 4:1 petroleum ether/ethyl acetate) to yield the product as a white crystalline powder (7.02 g, 82.0 % yield).

^{1}H NMR (400.03 MHz, $CDCl_3$, 298 K) δ = 1.44 (s, 9H, 3 x CH_3), 1.98 (s, 6H, 2 x CH_3), 6.97 (d, 2H, *J* = 8.8 Hz, 2 x CH_{Ar}), 7.31 (d, 2H, *J* = 8.8 Hz,2 x CH_{Ar}). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 28.46 (3C), 30.78 (2C), 55.52 (2C), 119.48 (2C), 121.57 (2C), 136.50 (1C), 146.14 (1C), 152.81 (1C), 170.58 (1C). IR (neat) ν = 1741, 1693, 1608, 1526, 1509, 1154, 1102 cm^{-1}. HRMS (ES+) calcd for $C_{15}H_{20}BrNO_4$ $[M + Na]^+$ 380.0468, observed 380.0461.

Azide-functional poly(TEGMEMA) (7)



Copper(I) bromide (0.0772 g, 0.54 mmol), azide-initiator (**5**) (0.1346 g, 0.54 mmol), tri(ethylene glycol) methyl ether methacrylate (5.00 g, 21.5 mmol), mesitylene (0.97 g, 8.07 mmol) and toluene (10 ml) were added to a Schlenk tube, sealed and subjected to four freeze-pump-thaw cycles. *N*-(Ethyl)-2-pyridylmethanimide (0.24 mL, 1.61 mmol) was added to the reaction mixture *via* a degassed syringe and the solution lowered into an oil bath at 70 °C. The polymerisation was sampled periodically and analysed by ^{1}H NMR and GPC. The reaction was quenched after 3 hours by exposing the solution to air. The reaction mixture was diluted with toluene (50 mL) and bubbled with air for 10 hours. The solution was then filtered through a short pad of neutral alumina, concentrated under vacuum and then purified by membrane dialysis (1 kDa cut-off) in methanol for 48 hours. The solvent was then removed under reduced pressure to yield the polymer as colourless oil (2.58 g, 51.6 % yield).

M_n (GPC, THF) = 7300 g mol^{-1}. PDi (GPC, THF) = 1.18. IR (neat) ν = 2100 cm^{-1} (azide).

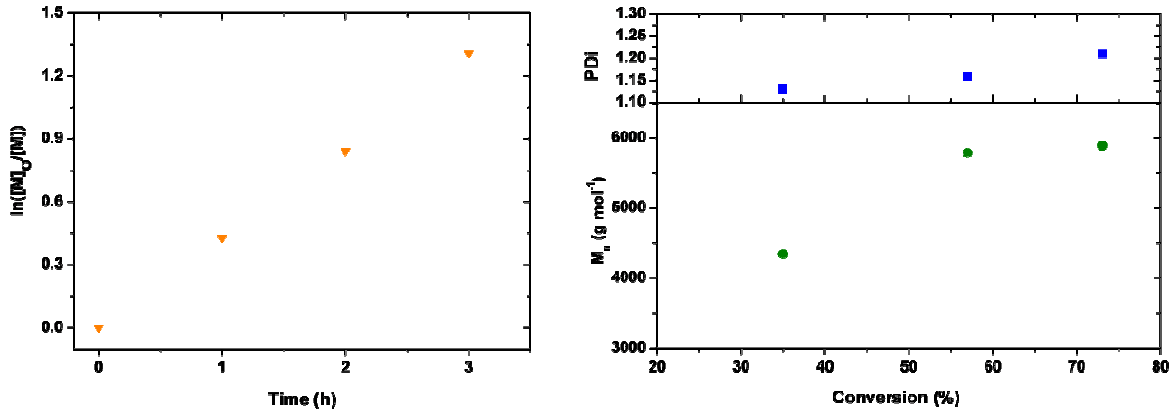
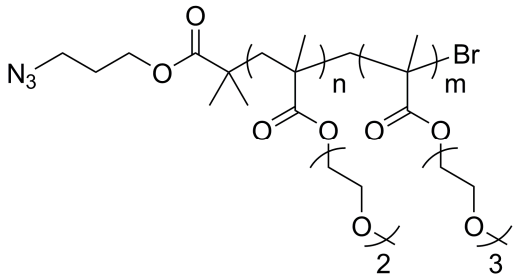


Figure S5. Kinetic analysis (left hand side) and evolution of M_n and PDi with conversion (right hand side) using [azide initiator]:[Cu(I)Br]:[Lig]:[TEGMEMA] = 1:1:3:40, toluene/TEGMEMA 2:1 (v/w), 70 °C.

Azide-functional poly(DEGMEMA-*co*-TEGMEMA) (8)



Copper (I) bromide (0.0953 g, 0.66 mmol), azide-initiator (**5**) (0.166 g, 0.66 mmol), di(ethylene glycol) methyl ether methacrylate (2.50 g, 13.3 mmol), tri(ethylene glycol) methyl ether methacrylate (3.09 g, 13.3 mmol), mesitylene (1.19 g, 9.96 mmol) and toluene (11.2 ml) were added to a Schlenk tube, sealed and subjected to four freeze-pump-thaw cycles. *N*-(Ethyl)-2-pyridylmethanimide (0.31 mL, 1.99 mmol) was added to the reaction mixture *via* a degassed syringe and the solution lowered into an oil bath at 70 °C. The polymerisation was sampled periodically and analysed by ^{1}H NMR and GPC. The reaction was quenched after 3 hours by exposing the solution to air. The reaction mixture was diluted with toluene (50 mL) and bubbled with air for 10 hours. The solution was then filtered through a short pad of neutral alumina, concentrated under vacuum and then purified by membrane dialysis (1 kDa cut-off) in methanol for 48 hours. The solvent was then removed under reduced pressure to yield the polymer as a colourless oil (2.89 g, 57.8 % yield).

M_n (GPC, THF) = 8000 g mol^{-1}. PDi (GPC, THF) = 1.23. IR (neat) ν = 2100 cm^{-1} (azide).

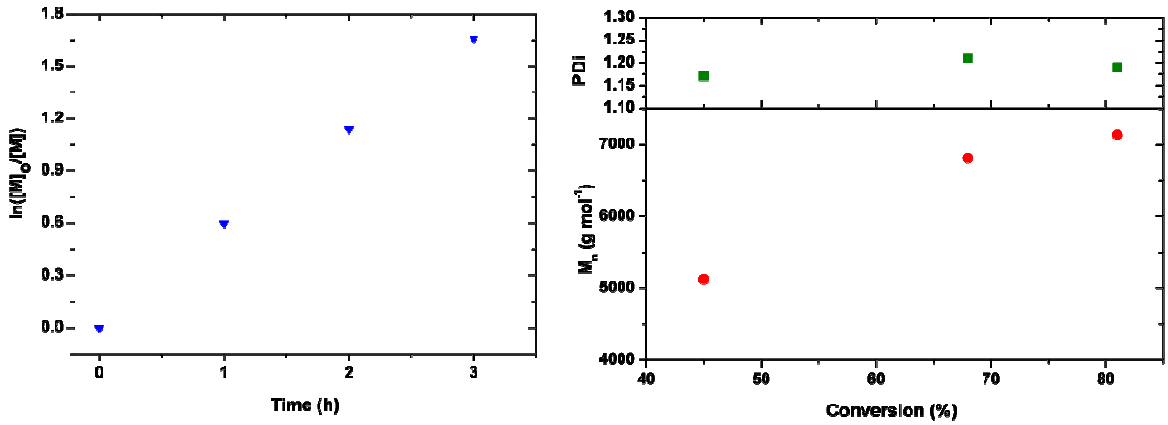
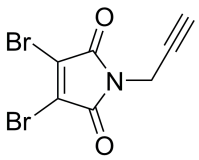


Figure S6. Kinetic analysis (left hand side) and evolution of M_n and PDi with conversion (right hand side) using [azide initiator]:[Cu(I)Br]:[Lig]:[DEGMEMA]:[TEGMEMA] = 1:1:3:20:20, toluene/TEGMEMA 2:1 (v/w), 70 °C.

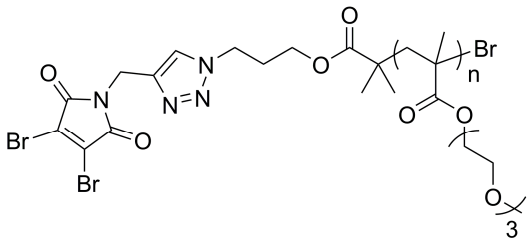
3,4-Dibromo-1-(prop-2-ynyl)-1H-pyrrole-2,5-dione (9)



2,3-Dibromomaleimide (3.00 g, 11.8 mmol) and anhydrous potassium carbonate (1.79 g, 12.9 mmol) were dissolved in acetone (40 mL) and stirred at room temperature for 5 minutes. Propargyl bromide (80% in toluene) (1.44 mL, 12.9 mmol) was added dropwise to the solution and following complete addition, left to stir for 24 hours. The solvent was removed and the crude mixture redissolved in DCM and filtered to remove salts. The solvent was removed and the mixture purified by flash chromatography (SiO_2, 19:1 petroleum ether : diethyl ether) to yield the product as a white crystalline powder (1.26 g, 36.5 % yield).

M.p.: 118 – 120 °C. ^{1}H NMR (400.03 MHz, $CDCl_3$, 298 K) δ = 2.27 (t, *J* = 2.5 Hz, 1H), 4.38 (d, *J* = 2.5 Hz, 2H). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 28.7 (1C), 72.7 (1C), 76.2 (1C), 129.9 (2C), 162.8 (2C). IR (neat) = 3241, 1720, 1569, 1386, 1314, 1138, 894 cm^{-1}. HRMS (ES+) calcd for $C_7H_3Br_2NO_2$ $[M + Na]^+$ 313.8423, observed 313.8429.

Synthesis of polymer (10)



Polymer **(7)** (1.50 g, 0.20 mmol) was dissolved in 15 mL of methanol and added to an oven dried Schlenk tube. Cu(I)Br (14.1 mg, 0.098 mmol) and 3,4-dibromo-1-(prop-2-ynyl)-1H-pyrrole-2,5-dione (172.1 mg, 0.58 mmol) were added to the Schlenk tube and the solution subjected to four freeze-pump thaw cycles. 2,2'-Bipyridine (30.6 mg, 0.20 mmol) was added to the frozen solution and the flask subjected to further degassing prior to addition to the Schlenk tube to a water bath at 25 °C. The solution was allowed to stir at ambient temperature for 24 hours, bubbled with air for 2 hours and passed through a short column of neutral alumina. The solution was then dialysed against methanol for 3 days in order to remove small molecules and isolated as viscous yellow oil following removal of the solvent (1.23 g, 82 % yield).

End group analysis - ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 123.2, 129.4, 141.6, 163.2.

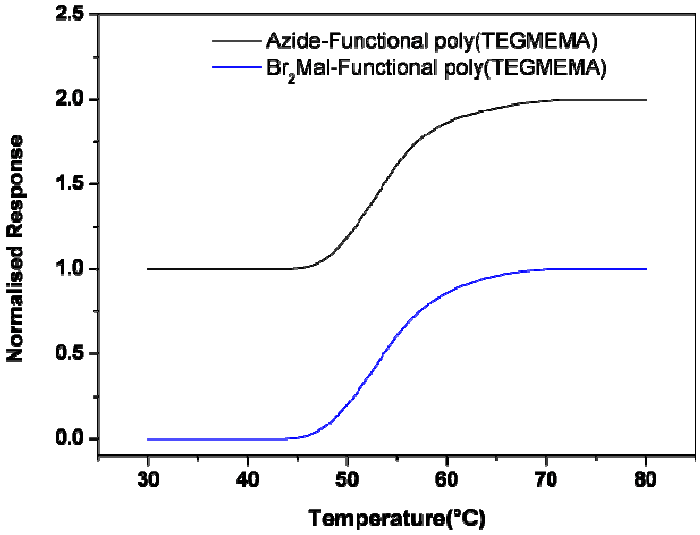


Figure S7. Cloud point analysis of the synthesised poly(TEGMEMA) prior to (black line) and following modification (blue line) with dibromomaleimide using CuAAC.

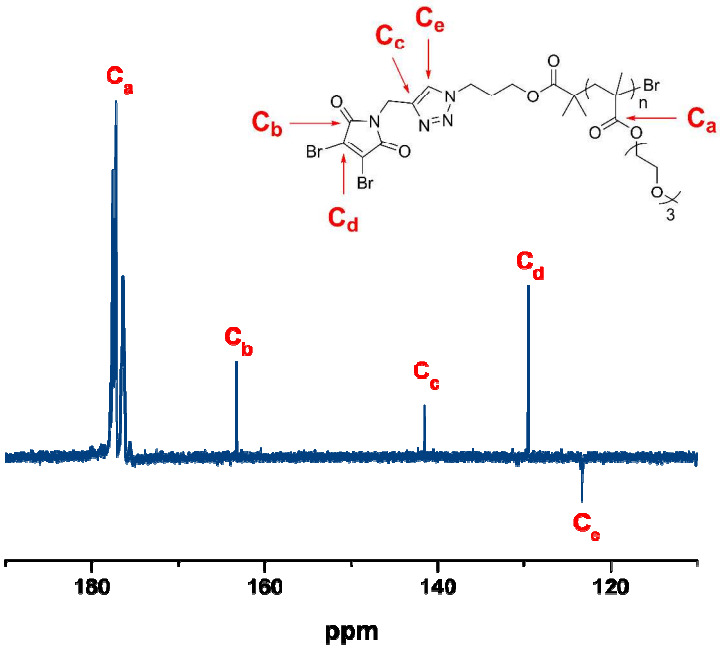


Figure S8. Partial DEPT ^{13}C NMR spectrum and assignment of the synthesised poly(TEGMEMA) showing the introduction of the dibromomaleimide end-group following modification by CuAAC.

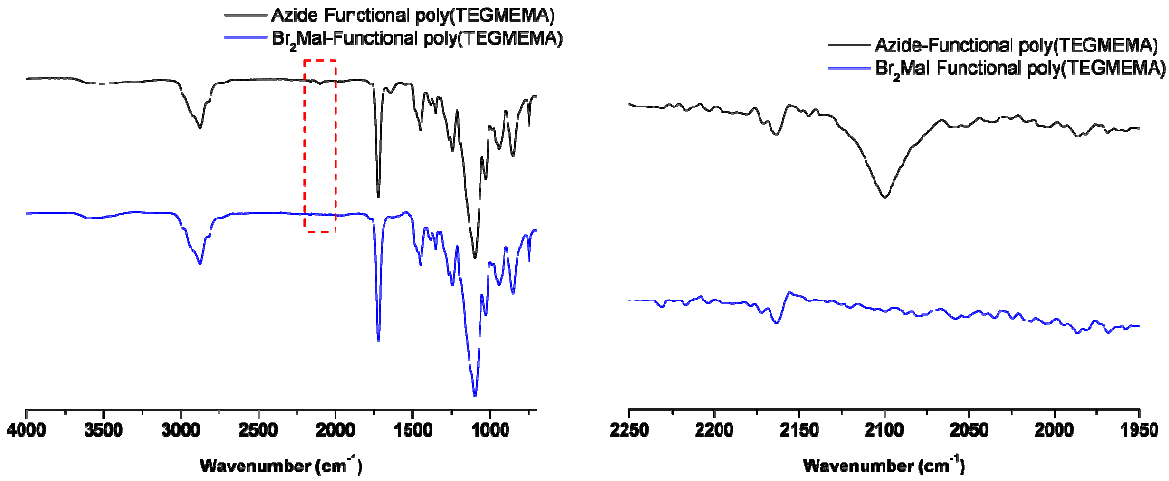
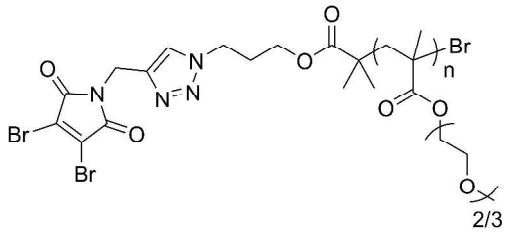


Figure S9. FT-IR spectra of the azide-functional polymer (black trace) and following introduction of the dibromomaleimide functionality by CuAAC to yield the dibromomaleimide functional polymer (blue trace). The observed loss of the characteristic azide stretch at 2100 cm^{-1} confirms polymer modification.

Synthesis of polymer (11)



Polymer **(8)** (1.50 g, 0.17 mmol) was dissolved in 15 mL of methanol and added to an oven dried Schlenk tube. Cu(I)Br (12.7 mg, 0.083 mmol) and 3,4-dibromo-1-(prop-2-ynyl)-1H-pyrrole-2,5-dione (153.3 mg, 0.53 mmol) were added to the Schlenk tube and the solution subjected to four freeze-pump thaw cycles. 2,2'-Bipyridine (27.6 mg, 0.17 mmol) was added to the frozen solution and the flask subjected to further degassing prior to addition to the Schlenk tube to a water bath at 25 °C. The solution was allowed to stir at ambient temperature for 24 hours, bubbled with air for 2 hours and passed through a short column of neutral alumina. The solution was then dialysed against methanol for 3 days in order to remove small molecules and isolated as viscous yellow oil following removal of the solvent (1.17 g, 78 % yield).

End group analysis - ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 123.2, 129.4, 141.6, 163.2.

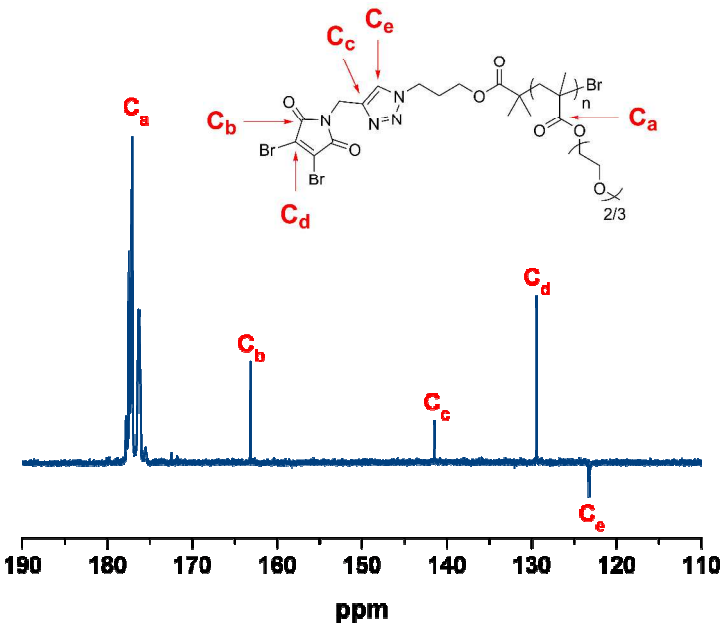


Figure S10. Partial DEPT ^{13}C NMR spectrum and assignment of the synthesised poly(DEGMEMA-*co*-TEGMEMA) showing the introduction of the dibromomaleimide end-group following modification by CuAAC.

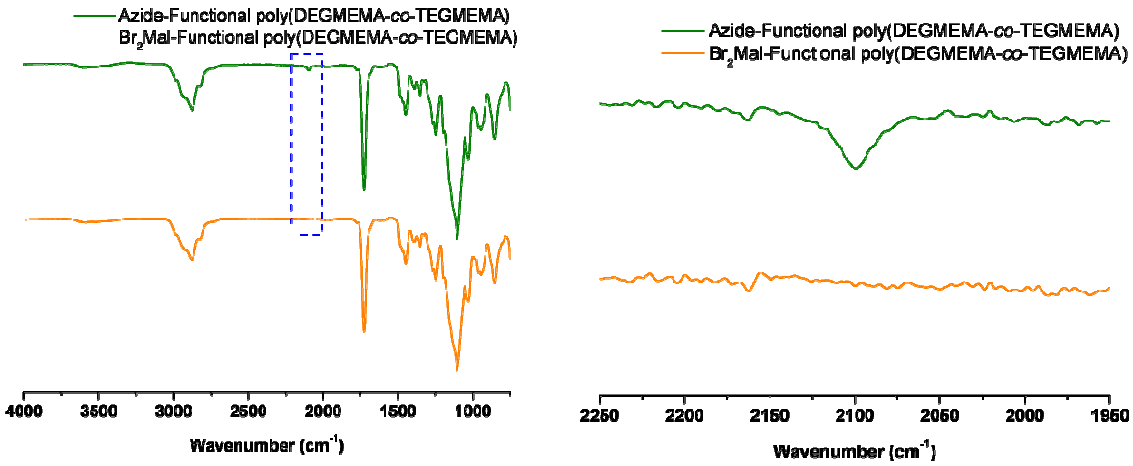


Figure S11. FT-IR spectra of the azide-functional polymer (green trace) and following introduction of the dibromomaleimide functionality by CuAAC to yield the dibromomaleimide functional polymer (orange trace). The observed loss of the characteristic azide stretch at 2100 cm^{-1} confirms polymer modification.

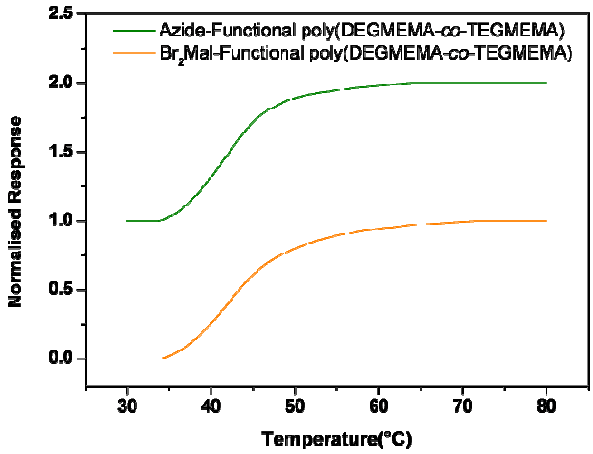
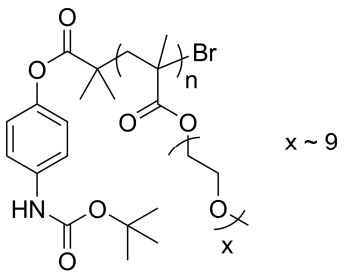


Figure S12. Cloud point analysis of the synthesised poly(DEGMEMA-*co*-TEGMEMA) prior to (green line) and following modification (orange line) with dibromomaleimide using CuAAC.

Synthesis of polymer (12)



Copper(I) bromide (0.0755 g, 0.52 mmol), 4-(tert-butoxycarbonylamino)phenyl 2-bromo-2-methylpropanoate (0.180 g, 0.52 mmol), poly(ethylene glycol) methyl ether methacrylate (M_n ~ 475) (5.00 g, 10.5 mmol) and toluene (10 ml) were added to a Schlenk tube, sealed and subjected to four freeze-pump-thaw cycles. *N*-(Ethyl)-2-pyridylmethanimide (0.23 mL, 1.57 mmol) was added to the reaction mixture *via* a degassed syringe and the solution lowered into an oil bath at 60 °C. The polymerisation was sampled periodically and analysed by ^{1}H NMR and GPC. The reaction was quenched after 7 hours by exposing the solution to air. The reaction mixture was diluted with toluene (50 mL) and bubbled with air for 10 hours. The solution was then filtered through a short pad of neutral alumina, concentrated under vacuum and then purified by membrane dialysis (1 kDa cut-off) in methanol for 48 hours. The solvent was then removed under reduced pressure to yield the polymer as colourless oil (3.09 g, 61.8 % yield).

M_n (GPC, THF) = 8900 g mol^{-1}. PDi (GPC, THF) = 1.11. End group analysis - ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 119.5, 121.6, 136.5, 146.1, 152.8, 170.6.

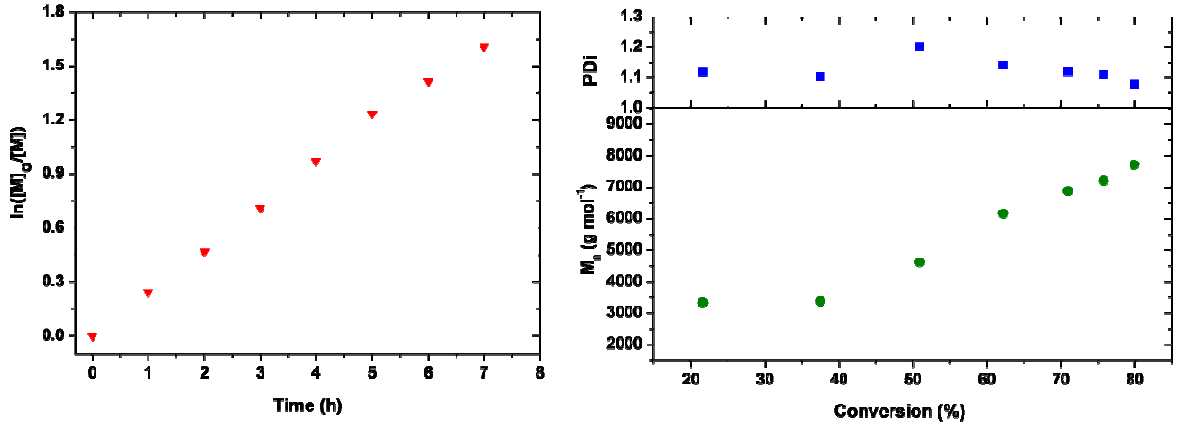
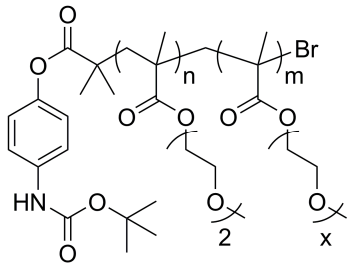


Figure S13. Kinetic analysis (left hand side) and evolution of M_n and PDi with conversion (right hand side) using [boc-protected aniline initiator]:[Cu(I)Br]:[Lig]:[PEGMA] = 1:1:3:20, toluene/PEGMA 2:1 (v/w), 60 °C.

Synthesis of polymer (13)



x ~ 9

Copper (I) bromide (0.140 g, 0.99 mmol), 4-(tert-butoxycarbonylamino)phenyl 2-bromo-2-methylpropanoate (0.336g, 0.99 mmol), di(ethylene glycol) methyl ether methacrylate (5.00 g, 22.6 mmol), poly(ethylene glycol) methyl ether methacrylate (M_n ~ 475) (1.40 g, 2.96 mmol) and toluene (12.8 ml) were added to a Schlenk tube, sealed and subjected to four freeze-pump-thaw cycles. *N*-(Ethyl)-2-pyridylmethanimide (0.44 mL, 2.96 mmol) was added to the reaction mixture *via* a degassed syringe and the solution immersed in an oil bath at 60 °C. The polymerisation was sampled periodically and analysed by ^{1}H NMR and GPC. The reaction was quenched after 7 hours by exposing the solution to air. The reaction mixture was diluted with toluene (50 mL) and bubbled with air for 10 hours. The solution was then filtered through a short pad of neutral alumina, concentrated under vacuum and then purified by membrane dialysis (1 kDa cut-off) in methanol for 48 hours. The solvent was then removed under reduced pressure to yield the polymer as a colourless oil (3.31 g, 66.2 % yield).

M_n (GPC, THF) = 7800 g mol^{-1}. PDi (GPC, THF) = 1.10. End group analysis - 6.97 (d, 2H, *J* = 8.8 Hz, 7.31 (d, 2H, *J* = 8.8 Hz). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 119.5, 121.6, 136.5, 146.1, 152.8, 170.6.

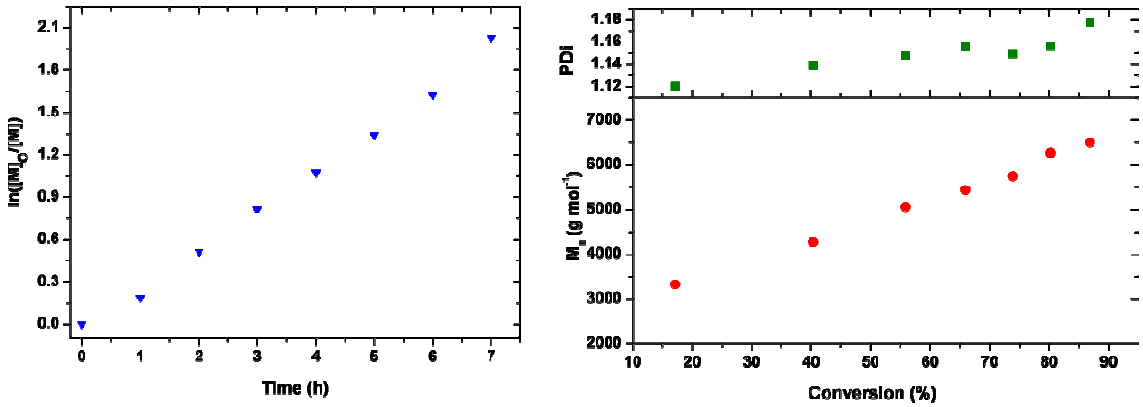
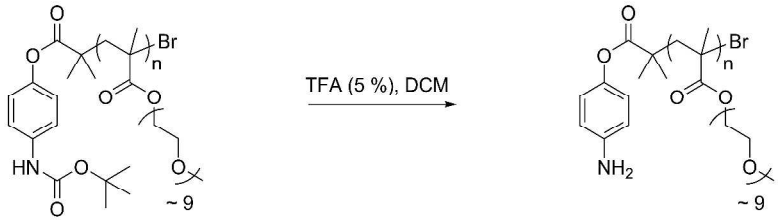


Figure S14. Kinetic analysis (left hand side) and evolution of M_n and PDi with conversion (right hand side) using [boc-protected aniline initiator]:[Cu(I)Br]:[Lig]:[PEGMA]:[DEGMEMA] = 1:1:3:3:23, toluene/(PEGMA/DEGMEMA) 2:1 (v/w), 60 °C.

***Boc*-deprotection to yield aniline-functional polymer (14)**



Boc-protected poly(PEGMA) (**12**) (2.00 g) was dissolved in dichloromethane (50 mL) and trifluoroacetic acid (2.5 mL) added and the solution left to stir for 12 hours at ambient temperature. The solvent was then removed under vacuum. Residual trifluoroacetic acid was removed by precipitation into 200 mL of a 1:1 diethyl ether : hexane solution containing 10 mL of triethylamine. The precipitate was dried under vacuum to yield the final polymer as viscous oil (1.63 g, 81.5% yield).

M_n (GPC, THF) 8950 g mol^{-1}. PDi (GPC, THF) 1.10.

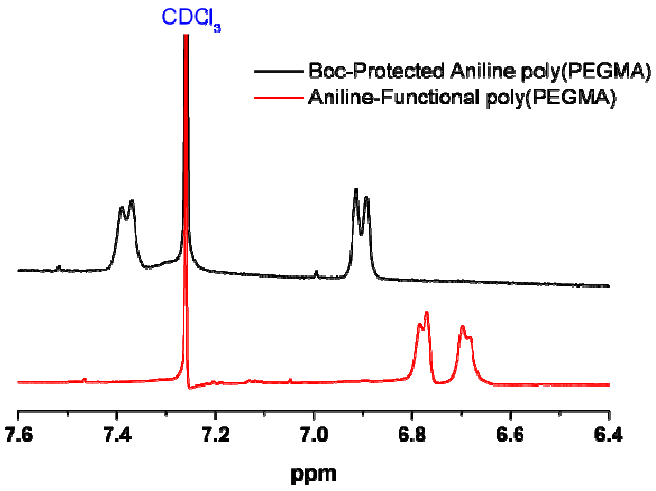
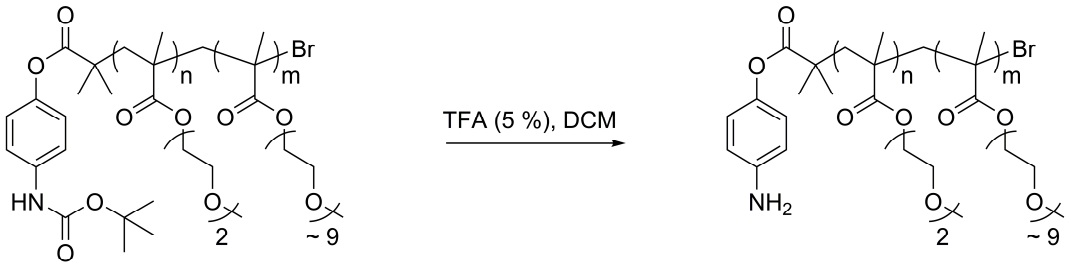


Figure S15. Partial ^{1}H NMR of the α-chain end region of the synthesised polymer prior to (black trace) and following deprotection (red trace) of the boc-group to yield the aniline end-group.

Boc-deprotection to yield aniline-functional polymer (15)



Boc-protected poly(PEGMA) (**13**) (2.00 g) was dissolved in dichloromethane (50 mL) and trifluoroacetic acid (2.5 mL) added and the solution left to stir for 12 hours at ambient temperature. The solvent was then removed under vacuum. Residual trifluoroacetic acid was removed by precipitation into 200 mL of a 1:1 diethyl ether : hexane solution containing 10 mL of triethylamine. The precipitate was dried under vacuum to yield the final polymer as viscous oil (1.65 g, 82.6% yield).

M_n (GPC, THF) 7660 g mol^{-1}. PDi (GPC, THF) 1.09.

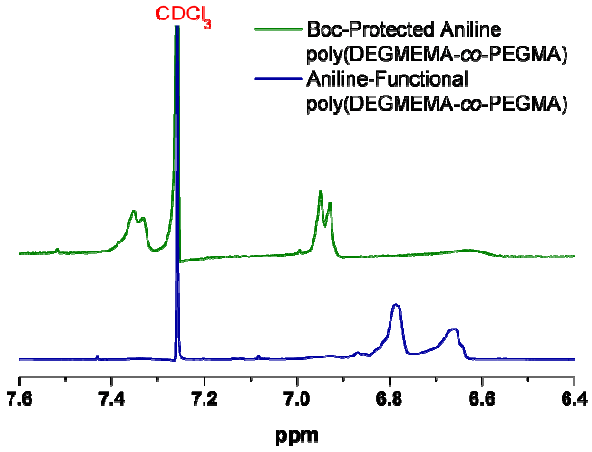
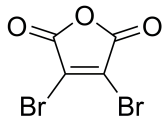


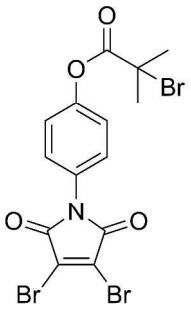
Figure S16. Partial ^{1}H NMR of the α-chain end region of the synthesised polymer prior to (green trace) and following deprotection (blue trace) of the boc-group to yield the aniline end-group.

3,4-Dibromofuran-2,5-dione[5]



To a large oven-dried ampoule was added maleic anhydride (4.00 g, 40.8 mmol), aluminium (III) chloride (0.0816 g, 0.612 mmol) and bromine (4.21 mL, 81.5 mmol). The ampoule was sealed and heated to 120 °C for 16 hours. The ampoule was allowed to cool and ethyl acetate added (200 mL). The solution was filtered and the solvents removed under reduced pressure. The crude solution was redissolved in chloroform (200 mL) and sonicated for 30 minutes, the solution was filtered and washed with water (2 x 100 mL), dried over anhydrous magnesium sulphate, filtered and the solvents removed to yield the product as an off-white crystalline solid (6.91 g , 64.2 % yield).
M.p.: 114 –115 °C. ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) = 131.4 (2C), 158.7 (2C). IR (neat) = 1857, 1823, 1778, 1592, 1279, 1237, 1180, 1158, 971, 924 cm^{-1}.

***N*-phenyl dibromomaleimide functional ATRP initiator Synthesis**



Initiator **(6)** (3.58 g, 9.99 mmol) was dissolved in DCM (60 mL) along with 5 mL of TFA. The solution was allowed to stir for 12 hours at room temperature and the solvent removed under reduced pressure. The obtained oil was redissolved in acetic acid (80 mL) and dibromomaleic anhydride (2.55 g, 9.99 mmol) added. The solution was heated to reflux for 48 hours, upon which the solvent was removed and the crude mixture redissolved in dichloromethane (100 mL) and washed with saturated $NaHCO_3$ solution (3 x 100 mL), water (3 x 100 mL) and brine (100 mL). The organic phase was dried over anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure. The final product was isolated following flash chromatography (SiO_2, 1:1 petroleum ether / diethyl ether) to yield the product as an off-white crystalline solid (4.20 g, 84.7 % yield).

^{1}H NMR (400.03 MHz, $CDCl_3$, 298 K) δ = 2.08 (s, 6H, 2 x CH_3), 7.26 (d, 2H, J = 9.0 Hz, 2 x CH_{Ar}), 7.40 (d, 2H, J = 8.8 Hz, 2 x CH_{Ar}). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 30.68, (2C), 55.24 (1C), 122.16 (2C), 127.20 (2C), 128.83 (1C), 130.03 (1C), 150.48 (1C), 162.84 (1C), 169.99 (1C). IR (neat) ν = 1784, 1745, 1720, 1599, 1508, 1383, 1267, 1210 cm^{-1}. HRMS (ES+) calcd for $C_{14}H_{10}Br_3NO_4$ $[M + Na]^+$ 515.8052, observed 515.8058.

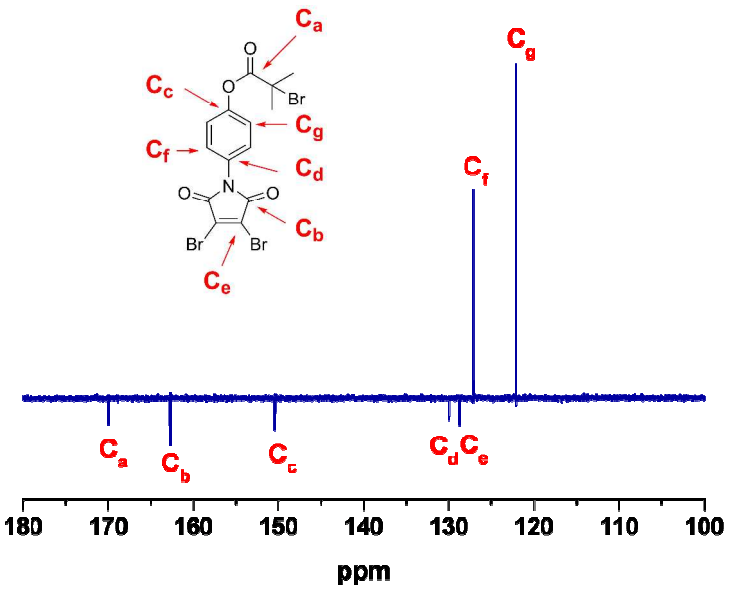
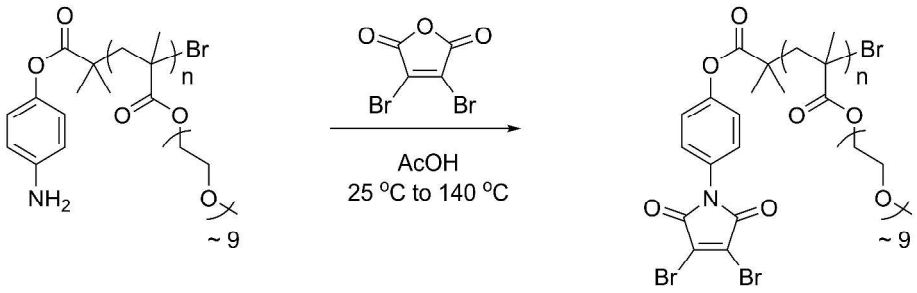


Figure S17. Partial DEPT ^{13}C NMR spectrum and assignment of the ATRP initiator synthesised as a model compound for characterisation of polymer dibromomaleimide end groups.

End group modification to yield polymer (16)



Dibromomaleic anhydride (0.255 g, 1.13 mmol) was added to aniline-functional polymer (**14**) (1.00 g, 0.11 mmol) in acetic acid (100 mL) and the reaction mixture was stirred for 6 hours at ambient temperature. The solution was then heated at reflux for 3 days. Upon cooling, the solvent was removed under vacuum and dissolved in toluene to azeotropically remove residual acetic acid. Water was added to the solution and residual removed by centrifugation. The aqueous solution was dialysed against water for 2 days and lyophilised to yield the product as pale yellow oil (0.834 g, 83.4 % yield).

End Group Analysis - ^{1}H NMR (400.03 MHz, $CDCl_3$, 298 K) δ = 7.14 (d, 2H, J = 9.0 Hz,), 7.33 (d, 2H, J = 8.8 Hz). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 122.4 (2C), 127.0 (2C), 128.3 (1C), 129.8 (1C), 150.6 (1C), 162.7 (1C).

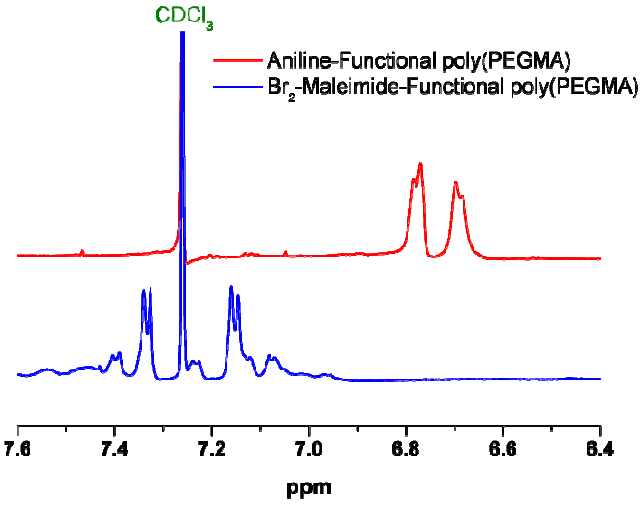


Figure S18. Partial ^{1}H NMR of the α-chain end region of the synthesised polymer prior to (red trace) and following modification (blue trace) of the end-group with dibromomaleic anhydride to yield the dibromomaleimide functionality.

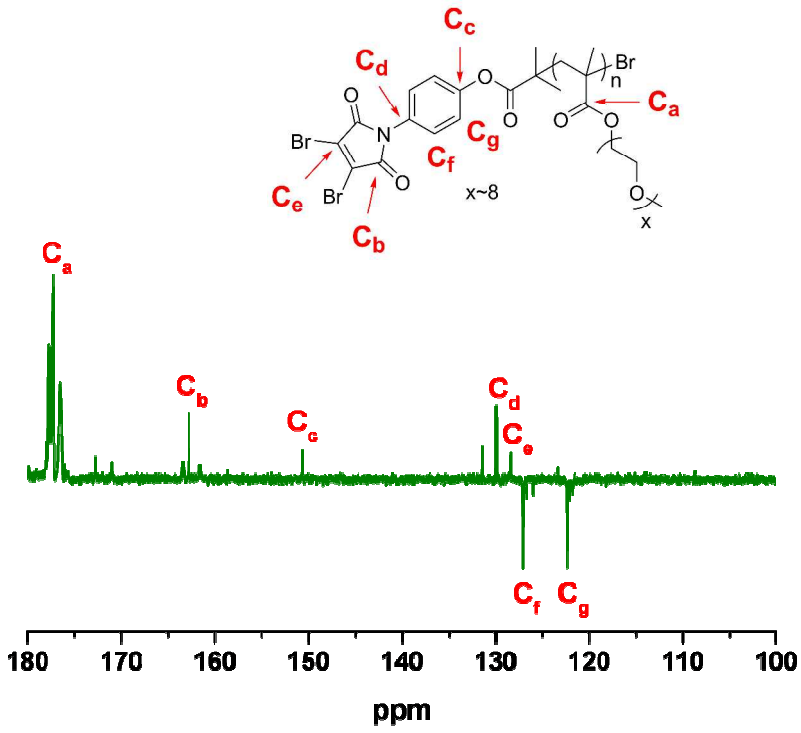
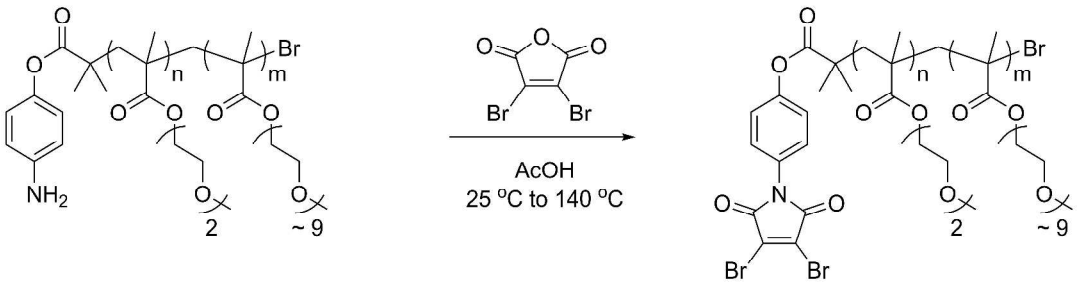


Figure S19. Partial DEPT ^{13}C NMR spectrum and assignment of the synthesised poly(PEGMA) displaying the introduction of the dibromomaleimide end-group following modification of aniline functional polymer (14) to furnish the dibromomaleimide end-group.

End group modification to yield polymer (17)



Dibromomaleic anhydride (0.326 g, 1.276 mmol) was added to aniline-functional polymer (**15**) (1.00 g, 0.11mmol) in acetic acid (100 mL) and the reaction mixture was stirred for 6 hours at room temperature. The solution was then heated at reflux for 3 days. Upon cooling, the solvent was removed under vacuum and dissolved in toluene to azeotropically remove residual acetic acid. Water was added to the solution and residual removed by centrifugation. The aqueous solution was dialysed against water for 2 days and lyophilised to yield the product as a yellow oil (0.842 g, 84.2% yield).

End Group Analysis - ^{1}H NMR (400.03 MHz, $CDCl_3$, 298 K) δ = 7.14 (d, 2H, *J* = 9.0 Hz,), 7.33 (d, 2H, *J* = 8.8 Hz). ^{13}C NMR (100.59 MHz, $CDCl_3$, 298 K) δ = 122.4 (2C), 127.0 (2C), 128.3 (1C), 129.8 (1C), 150.6 (1C), 162.7 (1C).

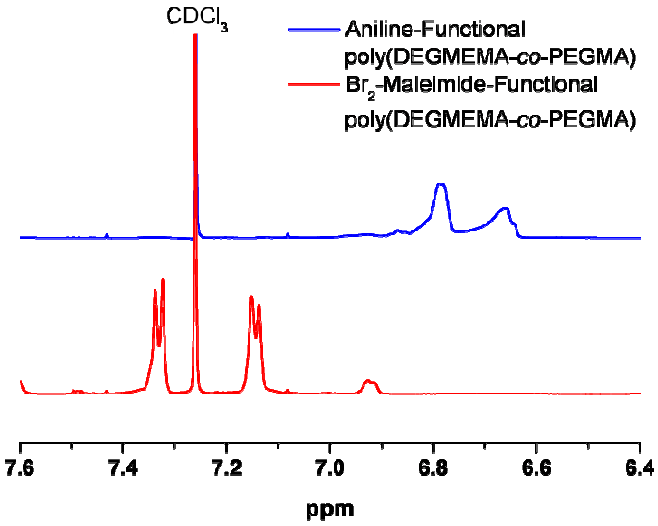


Figure S20. Partial ^{1}H NMR of the α-chain end region of the synthesised polymer prior to (blue trace) and following modification (red trace) of the end-group with dibromomaleic anhydride to yield the dibromomaleimide functionality.

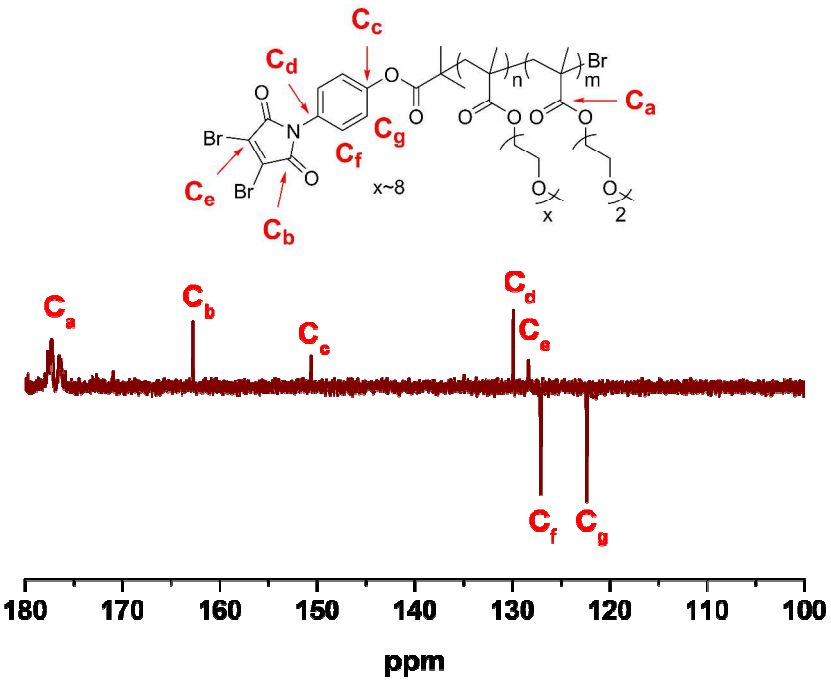
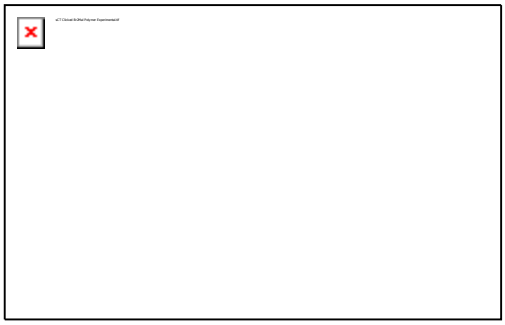


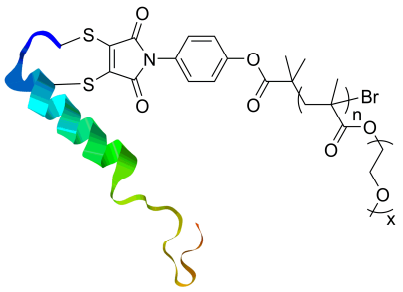
Figure S21. Partial DEPT ^{13}C NMR spectrum and assignment of the synthesised poly(DEGMEMA-*co*-PEGMA) displaying the introduction of the dibromomaleimide end-group following modification of aniline functional polymer (15) to furnish the dibromomaleimide end-group.

Synthesis of sCT-polymer (10) conjugate



sCT (5.0 mg, 1.45 µmol) was dissolved in 1.5 mL of H_2O and TCEP (0.46 mg, 1.60 µmol) (in 0.5 mL H_2O) added. The solution was left to stir at ambient temperature until complete reduction of the disulfide birdge was observed by RP-HPLC. After 30 minutes, 6 mL of phosphate buffer (pH 6.2, 50 mM) was added. Polymer (**10**) 11.21 mg, µmol) in 1 mL H_2O was added to the solution and the solution left to stir for 10 minutes before sampling for RP-HPLC analysis. HPLC analysis confirmed the complete consumption of the reduced polypeptide, as well as the appearance of a broad new peak corresponding to the conjugate peak.

Synthesis of sCT-polymer (16) conjugate



sCT (5.0 mg, 1.45 µmol) was dissolved in 1.5 mL of H_2O and TCEP (0.46 mg, 1.60 µmol) (in 0.5 mL H_2O) added. The solution was left to stir at ambient temperature until complete reduction of the disulfide birdge was observed by RP-HPLC. After 30 minutes, 6 mL of phosphate buffer (pH 6.2, 50 mM) was added. Polymer (**16**) 11.65 mg, µmol) in 1 mL H_2O was added to the solution and the solution left to stir for 10 minutes before sampling for RP-HPLC analysis. HPLC analysis confirmed the complete consumption of the reduced polypeptide, as well as the appearance of a broad new peak corresponding to the conjugate peak (Figure S19).

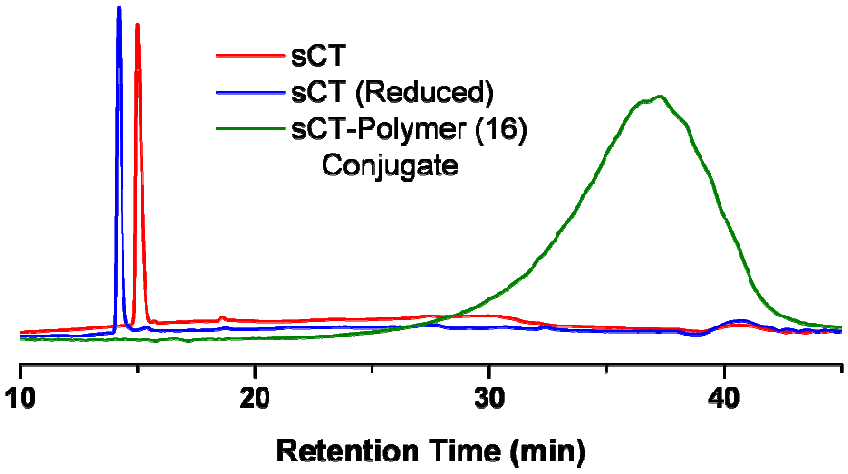


Figure S22. RP-HPLC analysis of the disulfide-bridging of sCT using polymer (16) following reduction of the disulfide bridge with TCEP.

References

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(5) Dubernet, M.; Caubert, V.; Guillard, J.; Viaud-Massuard, M. C. *Tetrahedron* **2005**, *61*, 4585.