Supporting Information for

Stimuli-Responsive Thiocarbamate-Based Polymeric Particles for Hydrogen Sulfide Generation

*Daniel A. Paterson, Aggie Lawer, Jared Davidson, Sarah Hook, Allan B. Gamble**

School of Pharmacy,
University of Otago,
18 Frederick St, Dunedin, New Zealand, 9054
E-mail: allan.gamble@otago.ac.nz

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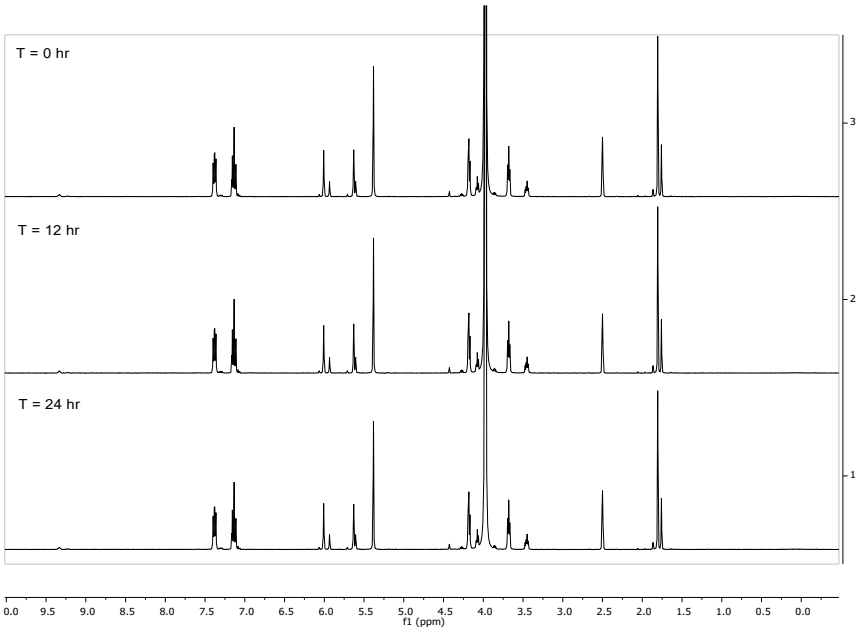


Figure S1. ^{1}H NMR of FBOT monomer (**2**) exposed to H_2O_2 at 20 ± 2 °C in DMSO-d_6/D_2O-PBS mixture (4:1) for up to 24 h.

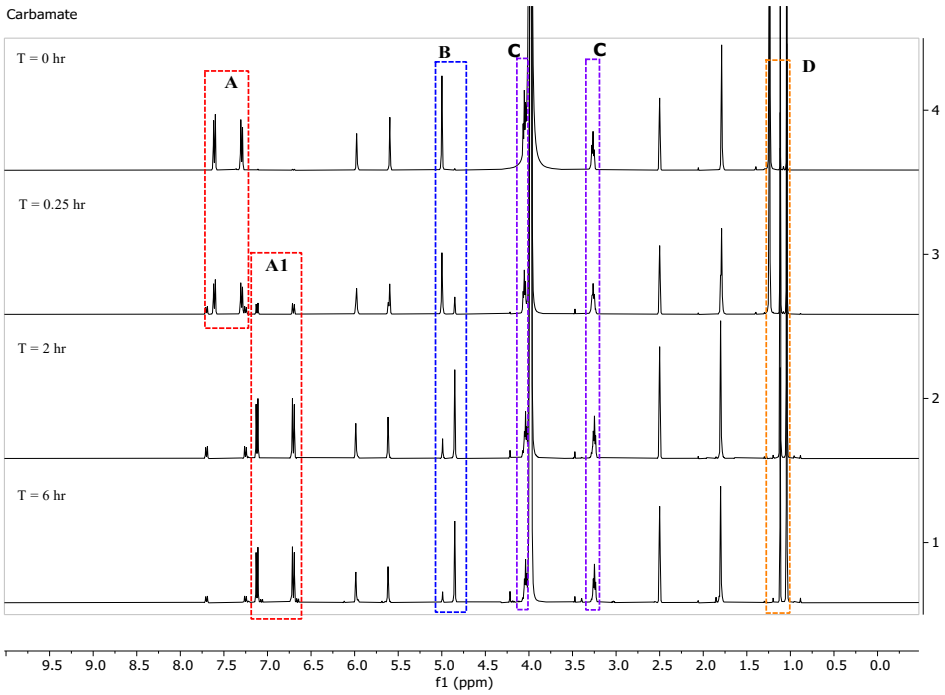


Figure S2. ^{1}H NMR of BBOC monomer (**3**) exposed to H_2O_2 at 20 ± 2 °C in DMSO-d_6/D_2O-PBS mixture (4:1) for up to 6 hours.

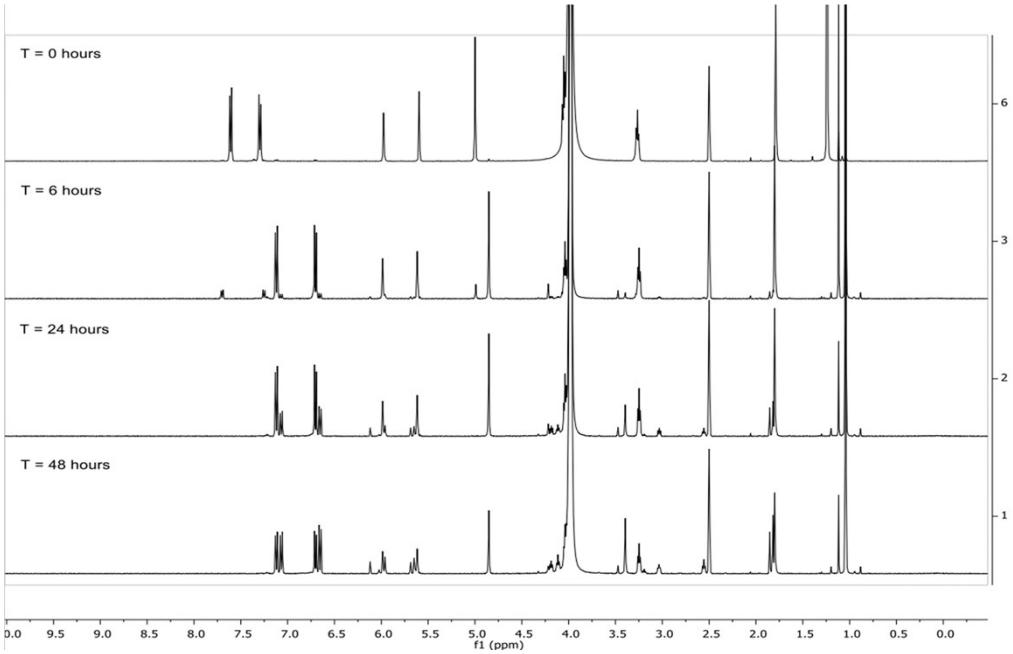


Figure S3. ^{1}H NMR of BBOC monomer (**3**) exposed to H_2O_2 at 20 ± 2 °C in DMSO-d_6/D_2O-PBS mixture (4:1) for up to 48 hours.

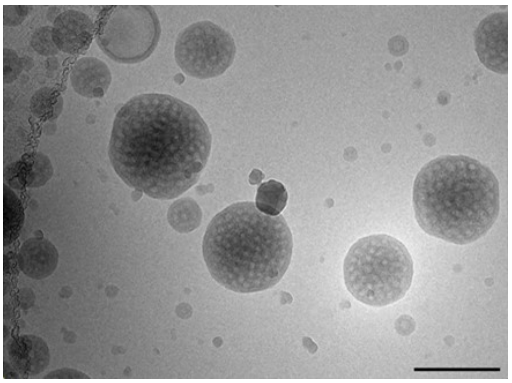


Figure S4. Cryo-TEM image of mPEG$_{49}$-BBOT$_{24}$ (**10**) particles formulated without Pluronic F127. Scale bar is 200 nm.

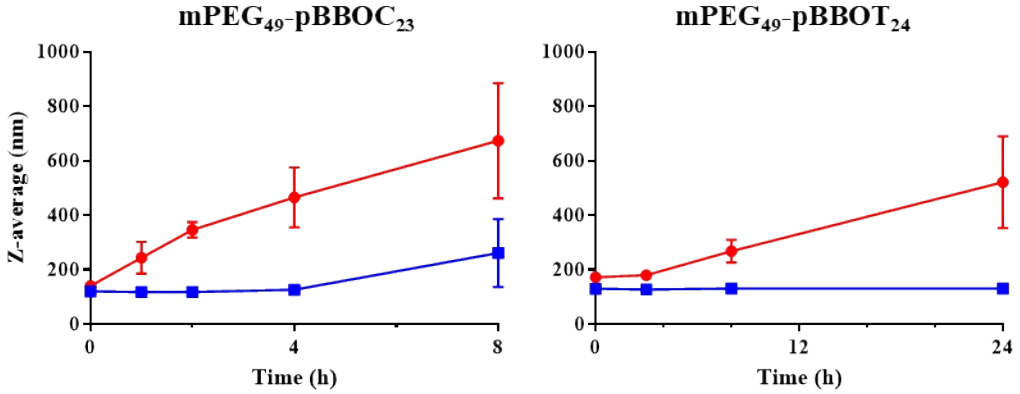


Figure S5. Z-average, as determined by DLS, of particles for mPEG$_{49}$-BBOC$_{23}$ (**12**) and mPEG$_{49}$-BBOT (**10**) incubated at 37 °C in PBS formulated with either block co-polymer (BCP) alone (red) or with 9% *w/w* Pluronic F127 (blue). Mean ± SD, n = 3.

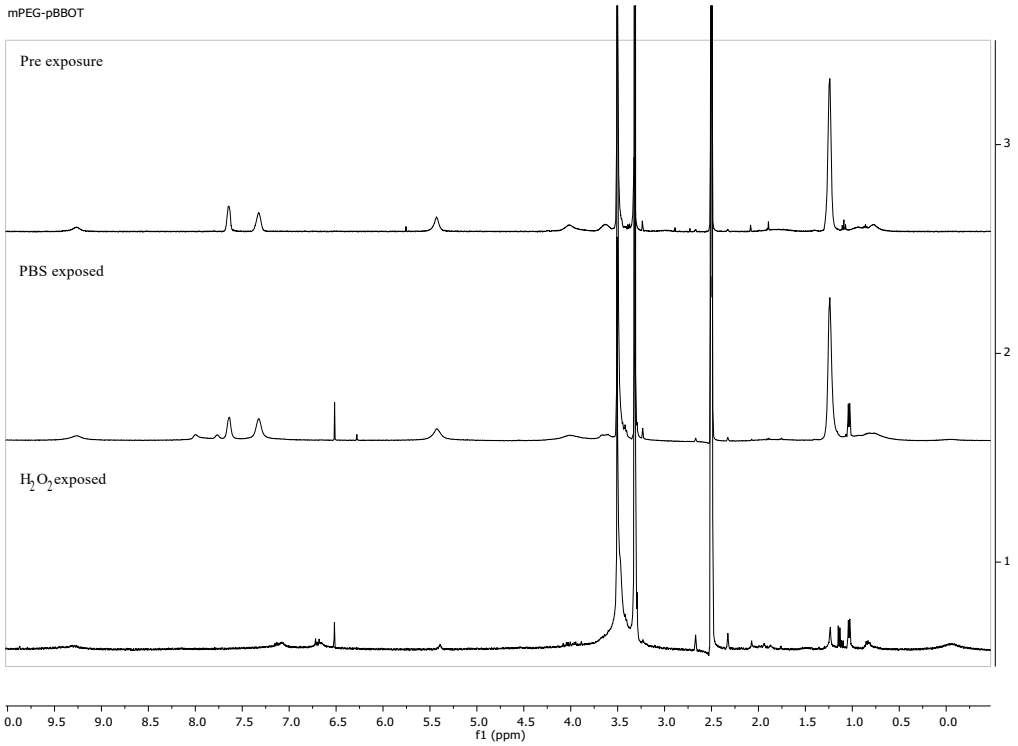


Figure S6. ^{1}H NMR of mPEG$_{49}$-BBOT$_{24}$ (**10**) particles prior to (top) and after recovery from exposure to PBS (middle) or 1 mM H_2O_2 (bottom).

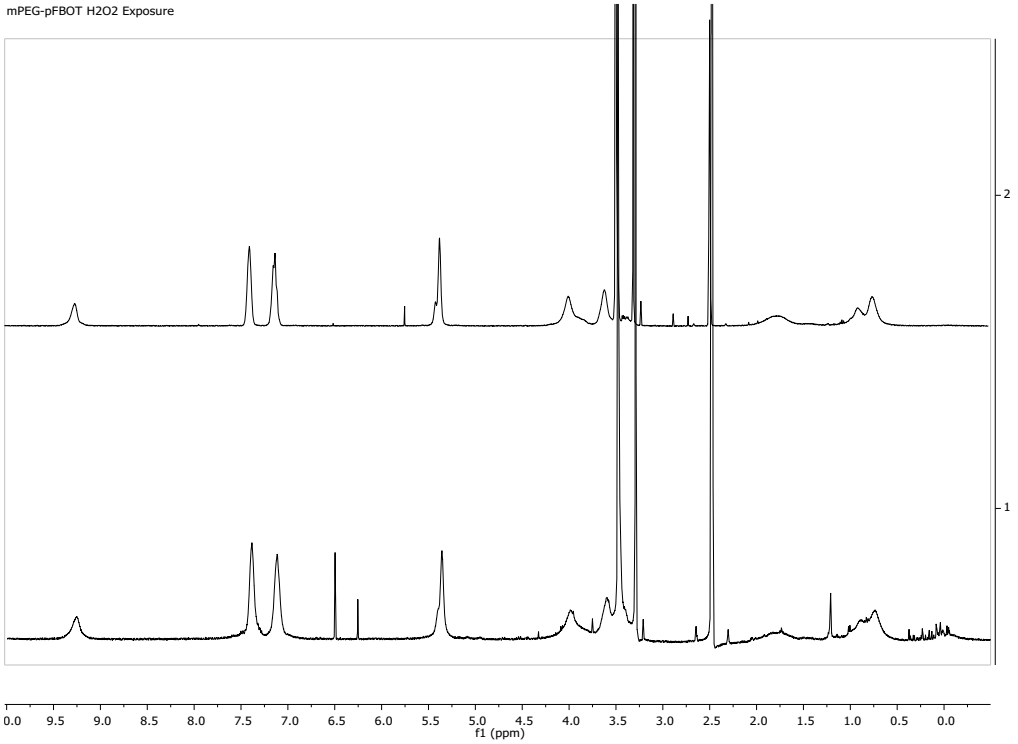


Figure S7. ^{1}H NMR mPEG$_{49}$-FBOT$_{38}$ (**11**) prior to (top) and after recovery from exposure to 1 mM H_2O_2 (bottom).

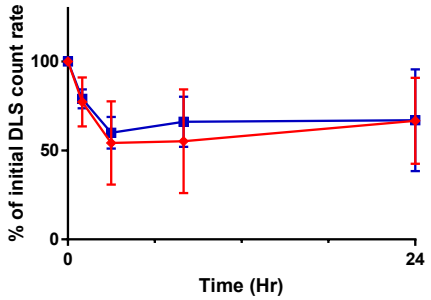


Figure S8. DLS analysis, % of the DLS particle count that is remaining from the original count rate, of $mPEG_{49}$-$FBOT_{38}$ (**11**) particles after exposure to PBS (blue) or 1 mM (red) H_2O_2. Mean ± SD, n = 3.

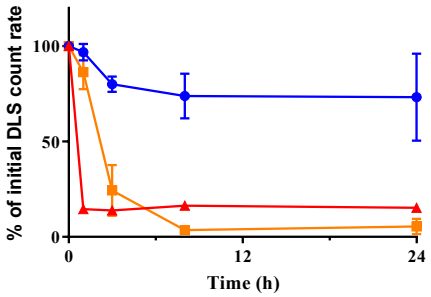


Figure S9. DLS analysis, % of the DLS particle count that is remaining from the original count rate, of $mPEG_{49}$-$BBOC_{23}$ (**12**) particles after exposure to PBS (blue), 0.1 mM (orange) or 1 mM (red) H_2O_2. Mean ± SD, n = 3.

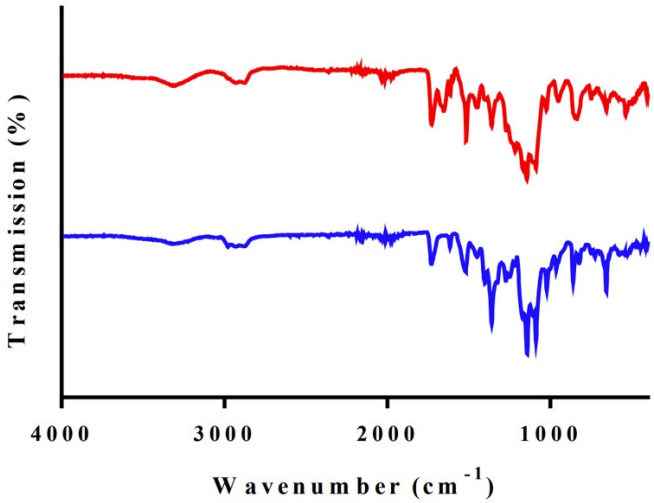


Figure S10. FTIR analysis of dried $mPEG_{49}$-$BBOT_{24}$ (**10**) particles after exposure to PBS (blue) or 0.1 mM H_2O_2 (red). An additional carbonyl stretch can be observed at 1654 cm^{-1} in the top (red) spectrum, indicative of a newly formed amide bond.

Table S1. DLS characterisation of particles formed via nanoprecipitation from polymers at a concentration of 1 mg/mL in the final formulation with 9% *w/w* with Pluronic F127.

Polymer	Z-average (nm)	PDI	Zeta potential (mv)
$mPEG_{49}$-$BBOC_{23}$ (**12**)	137 ± 13	0.193 ± 0.026	-7.3 ± 1.9
$mPEG_{49}$-$BBOT_{24}$ (**10**)	130 ± 4	0.106 ± 0.014	-4.3 ± 0.5
$mPEG_{49}$-$FBOT_{38}$ (**11**)	129 ± 10	0.137 ± 0.003	-2.9 ± 0.5

Table S2. DLS characterisation of particles formed via nanoprecipitation from polymers at a concentration of 2 mg/mL in the final formulation with 9% *w/w* with Pluronic F127.

Polymer	Z-average (nm)	PDI	Zeta potential (mv)
$mPEG_{49}$-$BBOC_{23}$ (**12**)	191 ± 4	0.168 ± 0.023	-9.8 ± 2.5
$mPEG_{49}$-$BBOT_{24}$ (**10**)	164 ± 8	0.170 ± 0.045	-5.3 ± 0.9
$mPEG_{49}$-$FBOT_{38}$ (**11**)	170 ± 7	0.190 ± 0.048	-4.5 ± 0.9

Table S3. Loading of doxorubicin.HCl into particles, and DLS characterisation of loaded particles.

Polymer	Z-average (nm)	PDI	Zeta potential (mv)	Encapsulation Efficiency (%)	Drug Loading Content (%)
$mPEG_{49}$-$BBOC_{23}$ (**12**)	193 ± 20	0.206 ± 0.035	-8.9 ± 1.2	7.6 ± 1.6	1.3 ± 0.3
$mPEG_{49}$-$BBOT_{24}$ (**10**)	217 ± 4	0.205 ± 0.020	-6.0 ± 0.9	13.3 ± 2.5	2.3 ± 0.4
$mPEG_{49}$-$FBOT_{38}$ (**11**)	199 ± 16	0.217 ± 0.045	-3.7 ± 1.0	2.4 ± 0.2	0.4 ± 0.02

Experimental Procedures

Materials

All chemicals were purchased from either Merck/Sigma-Aldrich (USA) or AK Scientific (USA) unless otherwise specified. Carbonic anhydrase from bovine erythrocytes (≥ 3500 W-A units/mg protein) was obtained from Merck/Sigma-Aldrich (USA). Doxorubicin.HCl was obtained from Lancrix (China). Dialysis tubing (MWCO 100 kDa, cellulose ester) was obtained from Repligen (USA). Solvents were purchased from Merck and used without further purification. Thin layer chromatography (TLC) was performed on 0.2 mm aluminium-backed silica gel plates. Flash column chromatography was carried out using 40 – 63 μm silica gel, with AR or liquid chromatography grade solvents. Deuterated solvents were purchased from Cambridge Isotope Laboratories. All reactions using anhydrous solvents were performed under a nitrogen environment unless otherwise specified.

Instrumentation

^{1}H, ^{13}C and ^{19}F NMR spectra were obtained on a 400 MHz Varian MR spectrometer, chemical shifts are reported as δ in parts per million and coupling constants are reported as *J* values in Hz. ATR-FTIR was recorded with a Varian 3100 FT-IR Excalibur series, with spectra recorded over a range of 400 – 4000 cm^{-1}, at a resolution of 4 cm^{-1} displayed as the mean of 64 scans. Data were analyzed via Varian Resolutions Pro software (v4.1.0.101). High resolution mass spectrometry was conducted on a quadrupole ToF Shimadzu LCMS-9030 with electrospray ionization.

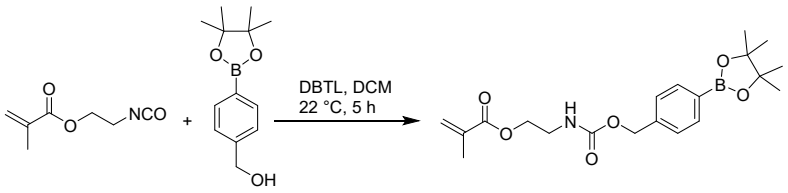
Polymer analysis via GPC was performed using a PL-GPC (Varian, Inc.) integrated GPC system with two PL-gel 5 µm mixed-C 300 × 7.5 mm in series with a PL-gel 5 µm 50 × 7.5 mm guard cartridge using DMF as diluent at 1 mL/min at 35 °C and detection via refractive index, using PEO (poly(ethylene oxide)) standards (MW range 0.194 to 543 kDa).

Particle sizing was performed using Dynamic Light Scattering (DLS), Zetasizer Nano-ZS (Zen3600, 632.8 nm), Malvern Instruments ltd. UK) with 1 in 20 dilution in PBS or 10 mM NaCl for Z-average and zeta potential measurements respectively. Particles for cryo-EM were vitrified onto glow discharge-treated C-Flat CF-2/2 carbon coated grids (Protochips, USA). Specimen vitrification was achieved by plunging the blotted grid into liquid ethane using a vitribot Mk IV (Thermo Fisher Scientific, USA). Zero-loss images of particles were obtained using a JEOL JEM-2200FS transmission electron microscope (JEOL Ltd, Japan) with a Gatan model 914 cryo specimen holder (Gatan Inc, USA). Fluorescence was measured using the POLARstar Omega Microplate Reader, (BMG Labtech, USA) plate reader with 96 well plates (Costar, Corning USA). All data is reported as the mean ± SD unless otherwise stated.

CLogP values were predicted for monomeric structures using ChemDraw Professional v19.1.1.21.

Synthesis

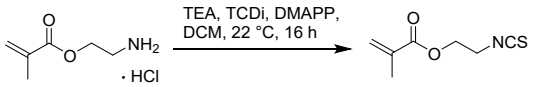
*1.1. Synthesis of methacrylate BBOC (**3**)*



This reaction was modified from Wang *et al*.[1] 4-(Hydroxymethyl)phenylboronic acid pinacol ester (**4**) (0.941 g, 4.02 mmol) was dissolved in 30 mL anhydrous DCM followed by addition of dibutyltin dilaurate (0.05 g, 0.08 mmol). The reaction was cooled on ice prior to dropwise addition of 2-isocyanatoethyl methacrylate (**7**) (0.988 g, 6.37 mmol). The reaction was allowed to warm to room temperature (22 °C) and stirred for 5 hours. The reaction was then diluted to 50 mL with DCM, washed with brine (3 × 50 mL) and dried with magnesium sulfate before passing through a basic alumina plug and drying under vacuum. This gave the pure product **3** as a white solid that was spectroscopically similar to that previously reported.[2]

Yield: 0.782 g, 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 7.9 Hz, 2H), 7.35 (d, *J* = 7.9 Hz, 2H), 6.10 (s, 1H), 5.62 – 5.55 (m, 1H), 5.12 (s, 2H), 5.01 (s, 1H), 4.23 (t, *J* = 5.2 Hz, 2H), 3.52 (q, *J* = 5.4 Hz, 2H), 1.93 (s, 3H), 1.34 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 156.4, 139.5, 136.0, 135.1, 127.3, 126.2, 84.0, 66.8, 63.8, 40.4, 25.0, 18.4. HRMS (ESI+) calculated for $C_{20}H_{28}NNaO_6B$: 412.1906, found: 412.1885.

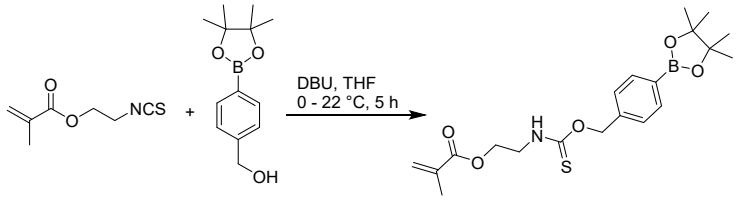
*1.2. Synthesis of 2-isothiocyanatoethyl methacrylate (**6**)*



2-Aminoethyl methacrylate.HCl (2.01 g, 15.6 mmol) was dissolved in 30 mL anhydrous DCM. To this was added triethylamine (3.15 g, 31.1 mmol) and stirring continued for 10 minutes. 1,1′-Thiocarbonyldiimidazole (TDCi) (5.54 g, 31.1 mmol) was added followed by DMAP (0.198 g, 1.62 mmol). The reaction was allowed to stir overnight. The reaction was then diluted with DCM (50 mL) and washed with water (3 × 50 mL), brine (3 × 50 mL) and then dried with magnesium sulfate and concentrated under vacuum. The crude residue was subjected to flash silica gel column chromatography (1:9 ethyl acetate:petroleum benzene) giving a green oil.

Yield: 1.18 g, 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.19 (s, 1H), 5.64 (s, 1H), 4.34 (t, *J* = 5.3 Hz, 2H), 3.78 (t, *J* = 5.3 Hz, 2H), 1.97 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 135.7, 127.0, 62.4, 44.5, 18.4. As previously reported for isothiocyanates,[3] the ^{13}C signal for NCS not observed. HRMS (ESI+) calculated for $C_7H_9NNaO_2S$: 194.0246, found: 194.0247.

*1.3. Synthesis of methacrylate BBOT (**1**)*

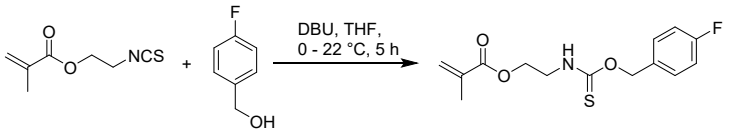


4-(Hydroxymethyl)phenylboronic acid pinacol ester (**4**) (1.08 g, 4.61 mmol) and 2-isothiocyanatoethyl methacrylate (**6**) (0.833 g, 4.87 mmol) were dissolved into 20 mL of anhydrous THF. The solution was cooled on ice prior to addition of DBU (1.02 g, 6.70 mmol). The reaction was allowed to warm to room temperature (22 °C) and stirred for 5 hrs. The crude product was subsequently dried under vacuum, then dissolved in 50 mL DCM, washed with brine (3 × 50 mL), dried with magnesium sulfate and concentrated under vacuum. The crude residue was subjected to flash silica gel column chromatography (1:9 ethyl acetate:petroleum benzene) giving the title compound **1** as a clear oil.

Yield: 1.63 g, 87%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83-7.78 (m, 2H)*, 7.40 – 7.34 (m, 2H)*, 6.98/6.70 (s, 1H)†, 6.12/6.09 (m, 1H)*, 5.61 – 5.56 (m, 1H)*, 5.54/5.48 (s, 2H)†, 4.35/4.20 (t, *J* = 5.5 Hz, 2H)†, 3.90/3.59 (q, *J* = 5.5 Hz, 2H)†, 1.93 (*br s*, 3H)*, 1.34 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.67/190.13‡, 167.49/167.21‡, 138.75/138.31‡, 135.90/135.77‡, 135.17/135.10‡, 127.58/127.50‡, 126.57/126.46‡, 84.0, 73.46/72.06‡, 62.72/62.65‡, 44.7, 42.3, 25.0, 18.41/18.37‡. HRMS (ESI+) calculated for $C_{20}H_{28}NNaO_5BS$: 428.1674, found: 428.1679.

*Broadened due to rotamers. †Due to rotamers peaks are split. Shift ppm is for each peak, integrals are sum of both peaks. ‡Rotamer peak pairs.

*1.4. Synthesis of methacrylate FBOT (**2**)*

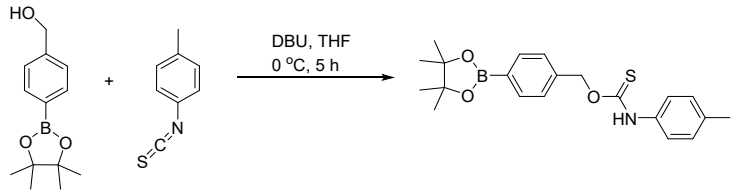


4-Fluorobenzyl alcohol (**5**) (0.810 g, 6.42 mmol) and 2-isothiocyanatoethyl methacrylate (**6**) (0.694 g, 4.06 mmol) were dissolved into 20 mL anhydrous THF. The solution was cooled on ice prior to addition of DBU (0.712 g, 4.68 mmol). The reaction was allowed to warm to room temperature (22 °C) and stirring continued for 5 hrs. The crude product was subsequently dried under vacuum then dissolved in 50 mL of DCM prior to washing with brine (3 × 50 mL), dried with magnesium sulfate and concentrated under vacuum. The crude residue was subjected to flash silica gel column chromatography (1:9 ethyl acetate:petroleum benzene) giving **2** as a white solid.

Yield: 0.410 g, 34%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (*br s*, 1H), 7.51 – 7.41 (m, 2H)*, 7.22 - 7.17 (m, 2H)*, 6.07/6.00 (s, 1H)†, 5.67/5.65 (s, 1H)†, 5.44/5.42 (s, 2H)†, 4.22/4.12 (t, *J* = 5.4 Hz, 2H)†, 3.71/3.47 (t, *J* = 5.4 Hz, 2H)†, 1.87/1.83 (s, 3H)†. ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.89/188.22‡, 166.47/166.34‡, 161.89/161.88‡ ($J_{C\text{-}F}$ = 243 Hz), 135.71/135.65‡, 132.49/132.46‡, 130.14/130.11‡ ($J_{C\text{-}F}$ = 8 Hz), 126.03/125.99‡, 115.25/115.20‡ ($J_{C\text{-}F}$ = 22 Hz), 70.59/69.80‡, 62.51/62.00‡, 43.7/41.3‡, 17.96/17.89‡. ^{19}F NMR (376 MHz, DMSO-d_6) δ -114.0/-116.5‡. HRMS (ESI+) calculated for $C_{14}H_{16}NNaO_3FS$: 320.0727, found: 320.0714.

*Broadened due to rotamers. †Due to rotamers peaks are split. Shift ppm is for each peak, integrals are sum of both peaks. ‡Rotamer peak pairs.

1.5. Synthesis of peroxyTCM-2

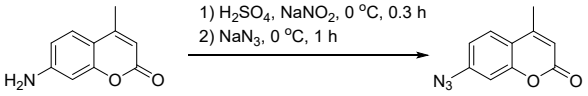


p-Tolyl isothiocyanate (0.420 g, 1.61 mmol) and 4-(hydroxymethyl)phenylboronic acid pinacol ester (0.677 g, 2.89 mmol) was dissolved in 6 mL anhydrous THF and cooled on ice. To this mixture DBU (0.406 g, 2.67 mmol) was added dropwise before being allowed to stir on ice for 4 hours. The reaction was quenched via addition of 20 mL brine and the product was extracted with DCM (3 × 20 mL). The organic layers were combined, dried with magnesium sulfate and

concentrated under vacuum before being subjected to flash silica gel column chromatography (1:9 ethyl acetate: petroleum benzene) to give a white solid. The product was spectroscopically similar to that previously reported.[4]

Yield: 0.479 g, 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.81 (d, *J* = 7.3 Hz, 2H), 7.38 (m, 3H), 7.09 (m, 3H), 5.63 (m, 2H), 2.31 (s, 3H), 1.35 (s, 12H). HRMS (ESI+) calculated for $C_{21}H_{26}NNaO_3BS$: 406.1619, measured: 406.1616.

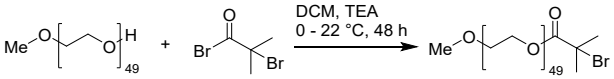
1.6. Synthesis of 7-azido-4-methylcoumarin (7AzMC)



7-Azido-4-methylcoumarin was synthesized as previously reported.[5] 7-Amino-4-methylcoumarin (0.205 g, 1.17 mmol) was dissolved in 5 mL water and cooled on ice. To this, 1.2 mL concentrated sulfuric acid was added dropwise while stirring. Sodium nitrite (0.103 g, 1.58 mmol) was dissolved in 1 mL water and cooled on ice. This was added dropwise to the stirring mixture over 5 minutes before being allowed to stir for 1 hour on ice. Sodium azide (0.109 g, 1.58 mmol) was dissolved in 1 mL water and cooled on ice before being added dropwise to the reaction. A white precipitate formed rapidly and the reaction was allowed to stir for 30 minutes while warming to room temperature (22 °C). The reaction was neutralized with saturated sodium bicarbonate and the solid was collected and washed with water, before being dissolved in chloroform and dried with magnesium sulfate then under vacuum giving a yellow solid. The product was spectroscopically similar to that previously reported.[5]

Yield: 0.161 g, 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.54 (m, 1H), 6.97 – 6.92 (m, 2H), 6.22 (s, *J* = 1.2 Hz, 1H), 2.41 (s, 3H). HRMS (ESI+) calculated for $C_{10}H_7N_3NaO_2$: 224.0431, measured: 224.0426. IR υ_{max}/cm^{-1}: 444, 522, 621, 704, 854, 978, 1066, 1388, 1604, 1718, 2115.

*1.7. Synthesis of ARGET-ATRP macroinitiator mPEG-Br (**9**)*



The ARGET-ATRP macroinitiator mPEG-Br (**9**) was synthesized as previously reported.[6] Methyl-poly(ethylene glycol) (mPEG, Mn 2000) (1.00 g, 0.500 mmol) was dried azeotropically with anhydrous toluene. Anhydrous DCM (15 mL) was added to the dried mPEG, followed by triethylamine (0.106 g, 1.05 mmol). The reaction mixture was cooled on ice before 2-bromo-2-methylpropionyl bromide (1.15 g, 5.00 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature (22 °C) and stirred for 48 hours. The reaction was then diluted into DCM (100 mL), washed with saturated sodium bicarbonate (3 × 50 mL) and dried with magnesium sulfate. The volume was reduced under vacuum and then precipitated in ice-cold diethyl ether twice. The precipitate was filtered and dried under vacuum to give a white solid that was spectroscopically similar to that reported.[6]

Yield: 0.772 g, 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 – 3.59 (m, 196H), 3.38 (s, 3H), 1.94 (s, 6H).

General Polymerization Procedure

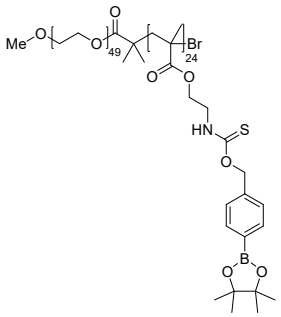
Polymerizations were conducted as previously reported.[6] Briefly the polymerization was conducted as follows; the initiator (mPEG-Br, 100 mg, 0.046 mmol), monomer, $CuBr_2$ (0.25 mg, 0.001 mmol) and tris(2-pyridylmethyl)amine (TPMA) (14 mg, 0.048 mmol) were dissolved in 0.85 mL of DMF. The monomer equivalents are stated below with each synthesized polymer, and where replicates were synthesized a mean value is provided. The reaction mixture was degassed via bubbling nitrogen through the solution in a Schlenk flask. The reaction vessel was then sealed under nitrogen, to which the sodium ascorbate was added dropwise (0.97 mg in 0.15 mL water, 0.005 mmol) and stirred at 22 °C for 5 hours. The reaction was quenched via exposure to air before being dried under vacuum. The resulting crude oil was dissolved in DCM, passed through a neutral alumina plug followed by precipitation into ice-cold diethyl ether:hexane (2:1) twice. The removal of unreacted monomer was confirmed via TLC analysis. The precipitate was dried overnight on high vacuum to give a white powder.

The synthesized BCPs (**10**, **11**, **12**) were characterized via ^{1}H NMR spectroscopy and gel permeation chromatography (GPC). The polymerization was quantified as follows:

- Degree of polymerization = polymerized monomer equivalents per PEG backbone as determined via ^{1}H NMR, as previously described[6]
- Conversion = degree of polymerization / initial equivalents of monomer
- Yield % = mmol polymer (as determined from ^{1}H NMR) × 100 / mmol initiator
- Mass yield % = mass of product × 100 / (mass of initiator + mass of monomer)

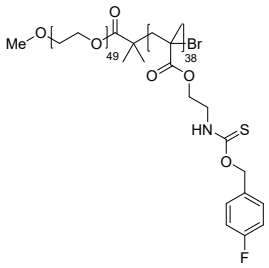
Synthesis of Block Co-Polymers 10, 11 and 12

*1.8. mPEG$_{49}$-BBOT$_{24}$ (**10**)*



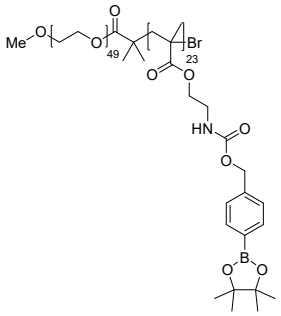
ARGET-ATRP was used to synthesize mPEG$_{49}$-BBOT$_{24}$ (**10**) using BBOT monomer (480 mg, 1.19 mmol). Degree of polymerization: 24, conversion: 93 ± 3%. Yield: 40%, mass yield: 220 ± 38 mg, 38% (*n*=8). ^{1}H NMR (400 MHz, DMSO-d_6) 9.27 (broad singlet from N-H), 7.65 (broad singlet from phenyl ring protons), 7.32 (broad singlet from phenyl ring protons), 5.42 (broad singlet, benzylic proton to thiocarbamate bond), 4.03 (broad singlet from alkyl chain protons in BBOT), 3.64 (broad singlet from alkyl chain protons in BBOT), 3.51 (s, -CH_2CH_2O- in mPEG block), 1.24 (broad singlet from pinacol ester protons). $M_{n(NMR)}$: 11859 ± 906. GPC: M_n 6546 ± 493, M_W 7846 ± 740, Đ 1.19 ± 0.03 (*n*=8).

*1.9. mPEG$_{49}$-FBOT$_{38}$ (**11**)*



ARGET-ATRP was used to synthesize mPEG$_{49}$-FBOT$_{38}$ (**11**) using FBOT monomer (552 mg, 1.86 mmol). Degree of polymerization: 38, conversion: 94%. Yield: 30%, mass yield: 186 mg, 29%. ^{1}H NMR (400 MHz, DMSO-d_6) 9.27 (broad singlet from N-H), 7.41 (broad singlet from phenyl ring protons), 7.14 (broad singlet from phenyl ring protons), 5.38 (broad singlet, benzylic proton to thiocarbamate bond), 4.02 (broad singlet from alkyl chain protons in FBOT), 3.63 (broad singlet from alkyl chain protons in FBOT), 3.50 (s, -CH_2CH_2O- in mPEG block), 1.24 (broad singlet from pinacol ester protons). $M_{n(NMR)}$: 13643. GPC: M_n 8446, M_W 9882, Đ 1.17 (*n*=1).

*1.10. mPEG$_{49}$-BBOC$_{23}$ (**12**)*



ARGET-ATRP was used to synthesize $mPEG_{49}$-$BBOC_{23}$ (**12**) using BBOC monomer (521 mg, 1.34 mmol). Degree of polymerization: 23, conversion: 79 ± 16%. Yield: 50%, mass yield: 261 ± 27 mg, 42%. ^{1}H NMR (400 MHz, $CDCl_3$) 7.75 (broad singlet from phenyl ring protons), 7.28 (broad singlet from phenyl ring protons), 5.05 (broad singlet, benzylic protons to carbamate bond), 3.97 (broad singlet from alkyl chain protons in BBOC), 3.64 (s, -CH_2CH_2O- in mPEG block), 3.38 (singlet, terminal methyl of mPEG), 3.32 (broad singlet from alkyl chain protons in BBOC), 1.30 (broad singlet from pinacol ester protons). $M_{n(NMR)}$: 11439 ± 627. GPC: M_n 6269 ± 457, M_W 8119 ± 1362, Đ 1.30 ± 0.08 (*n*=4).

Formulation of Particles

Block co-polymers (BCP) were self-assembled using nanoprecipitation. In a 25 mL round bottom BCP (3 mg), with or without Pluronic® F127 (4.8, 9, 17% *w/w*), was dissolved in 1.2 mL THF which was stirred at 1250 rpm. To this phosphate buffer saline (PBS) pH 7.4 (3 mL) was added over 3 seconds and stirred overnight. The resulting formulation was subsequently dialyzed against PBS for 48 hours with at least six changes of media (6 × 200 mL) to give a final concentration of 1 mg/mL particles.

Stability measurements of particles was performed via aliquoting formulation into dialysis bags (100 kDa MWCO, cellulose ester, Repligen, USA) which was incubated in 200 mL PBS at 37 °C under agitation. Samples were measured via DLS at timepoints with 1 in 20 dilution in PBS.

Responsiveness to H_2O_2

Triggering of monomers was conducted with a modified procedure using ^{1}H NMR.[7] The monomer, 5 mg, was dissolved in 560 µL DMSO-d_6 to which 125 µL PBS-D_2O was added. H_2O_2 was then added, to a final concentration of 19 mM, as 15 µL of 3% *w/w* H_2O_2. The sample was maintained at 20 ± 2 °C and analyzed via ^{1}H NMR at specified timepoints.

Responsive behavior of particles was performed on particles as formulated above. For DLS analysis the formulated particles were triggered using either a 0.1 or 1 mM H_2O_2 solution. For 0.1 mM exposure, the formulation was diluted 200 µL into 10 mL PBS prior to the addition of H_2O_2 or ultrapure water. These were shaken at 37 °C with samples taken at specified time for DLS measurement without further dilution. For 1 mM triggering the particles were diluted 1:1 in PBS with the addition of H_2O_2 or ultra-pure water. These were shaken at 37 °C with samples taken at specified time for DLS measurement by diluting 1 in 20 in PBS. For ^{1}H NMR, GPC and TEM analysis, prepared particles were aliquoted into dialysis membranes and exposed to 1 mM H_2O_2 in 200 mL PBS for 24 hours. Samples for ^{1}H NMR and GPC were further dialyzed against 200 mL ultrapure water for 48 hours with at least 6 changes of media (6 × 200 mL) and freeze-dried. Samples for cryo-TEM were dialyzed against 200 mL PBS and vitrified onto glow discharge-treated C-Flat CF-2/2 carbon coated grids (Protochips, USA). Specimen vitrification was achieved by plunging the blotted grid into liquid ethane using a vitribot Mk IV (Thermo Fisher Scientific, USA). Zero-loss images of particles were obtained using a JEOL JEM-2200FS transmission electron microscope (JEOL Ltd, Japan) with a Gatan model 914 cryo specimen holder (Gatan Inc, USA).

H_2S Formation from Monomeric Units

Detection of H_2S formation from BBOC (**1**), peroxyTCM-2 and controls (FBOT (**2**), BBOC (**3**)), was performed using 20 μM of monomer (in 1 mL DMSO:PBS (1:9) mixture) in the presence of 30 μg carbonic anhydrase and 0.21 mM (0.042 mg) AzMC. To this, either H_2O_2 (30 μL of 0.03% w/v solution) was added for a final concentration of 0.27 mM H_2O_2, or PBS (pH 7.4) was added as the control and agitated at 37 °C. Samples, 100 μL, were taken at specific timepoints and measured for fluorescence at $\lambda_{ex.}/\lambda_{em.}$ 355/460 nm.

H_2S Formation from Formulated Particles

Detection of COS/H_2S generation from particles was performed in a similar manor to monomers, via measuring the fluorescence of AzMC being switched on ($\lambda_{ex.}/\lambda_{em.}$ 355/460 nm). Particles (1 mg/mL solution; as prepared above) were diluted 1 in 10 with PBS to 1 mL, with 30 μg carbonic anhydrase and 0.21 mM (0.042 mg) AzMC. To this, either H_2O_2 (24 μL of 0.3% w/v solution) was added for a final concentration of 2.1 mM H_2O_2 (~10-fold molar excess to repeating thiocarbamate units in polymer/particles), or PBS (pH 7.4) was added as the control. This was shaken at 37 °C with samples taken at specified time points for fluorescence at $\lambda_{ex.}/\lambda_{em.}$ 355/460 nm.

Payload Loading and Release

Loading of particles with doxorubicin.HCl was achieved via solubilization of the doxorubicin.HCl in the organic solvent phase of the self-assembly. The polymers were self-assembled as described above (Formation of Particles), with the following modification. The polymer was formulated at 6 mg per 3 mL of formulation with 9% *w/w* Pluronic® F127. The doxorubicin.HCl (1.5 mg) was dissolved in DMSO (150 μL), which was added to the THF (1.2 mL) prior to PBS (3 mL) addition. After PBS addition the formulation was stirred overnight then dialyzed against PBS for 48 hours with at least 6 changes of media (6 × 200 mL). Encapsulation was measured via fluorescence of particles diluted in DMSO (1 in 10) at $\lambda_{ex.}/\lambda_{em.}$ 485/560 nm.

Release of doxorubicin from particles was performed using a modified procedure from that previously reported.[8] The formulation (1 mL) was aliquoted into a dialysis membrane which was exposed to either PBS control, 0.1 mM or 1 mM H_2O_2 in 200 mL PBS and shaken at 37 °C in the dark. Samples (100 μL) were taken from within the dialysis bag and diluted in DMSO (1 in 10) prior to fluorescence measurement at $\lambda_{ex.}/\lambda_{em.}$ 485/560 nm.

NMR Spectra for unreported methacrylate monomers 1 and 2

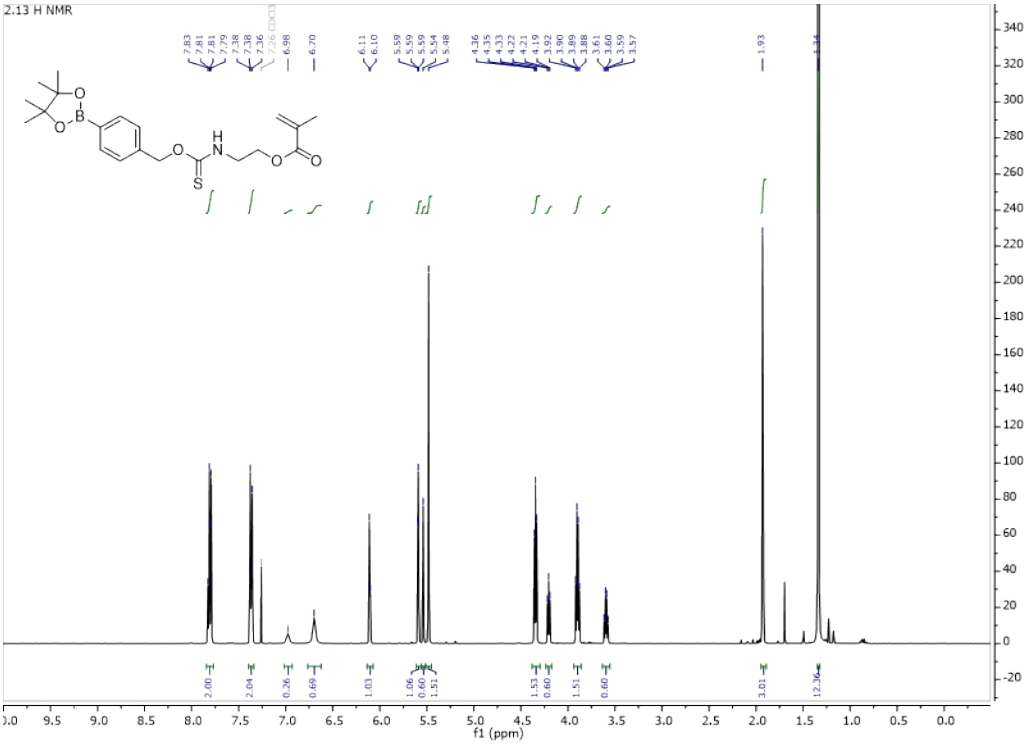


Figure S11. ^{1}H NMR of BBOT monomer (**1**) in $CDCl_3$.

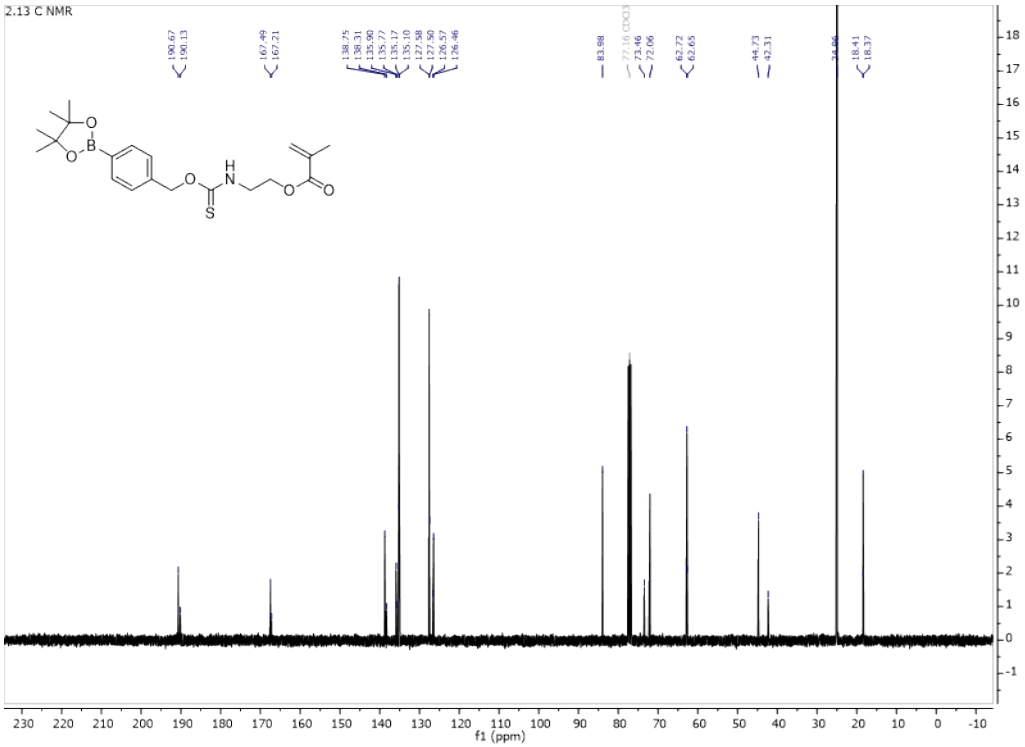


Figure S12. ^{13}C NMR of BBOT monomer (**1**) in $CDCl_3$.

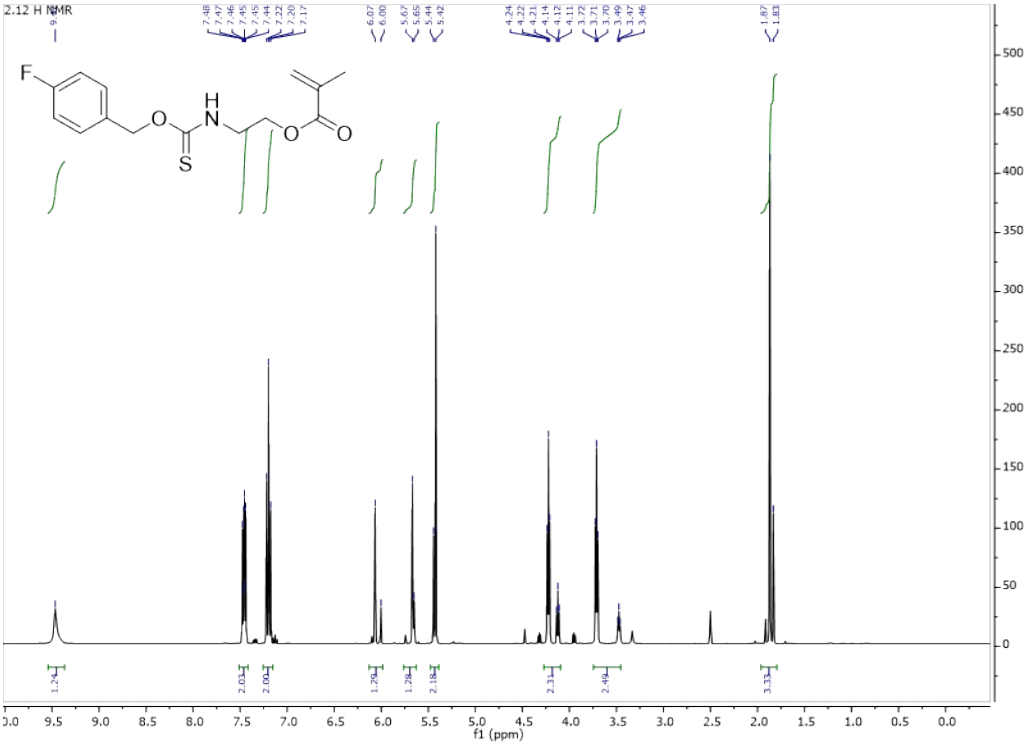


Figure S13. ^{1}H NMR of FBOT monomer (**2**) in DMSO-d_6.

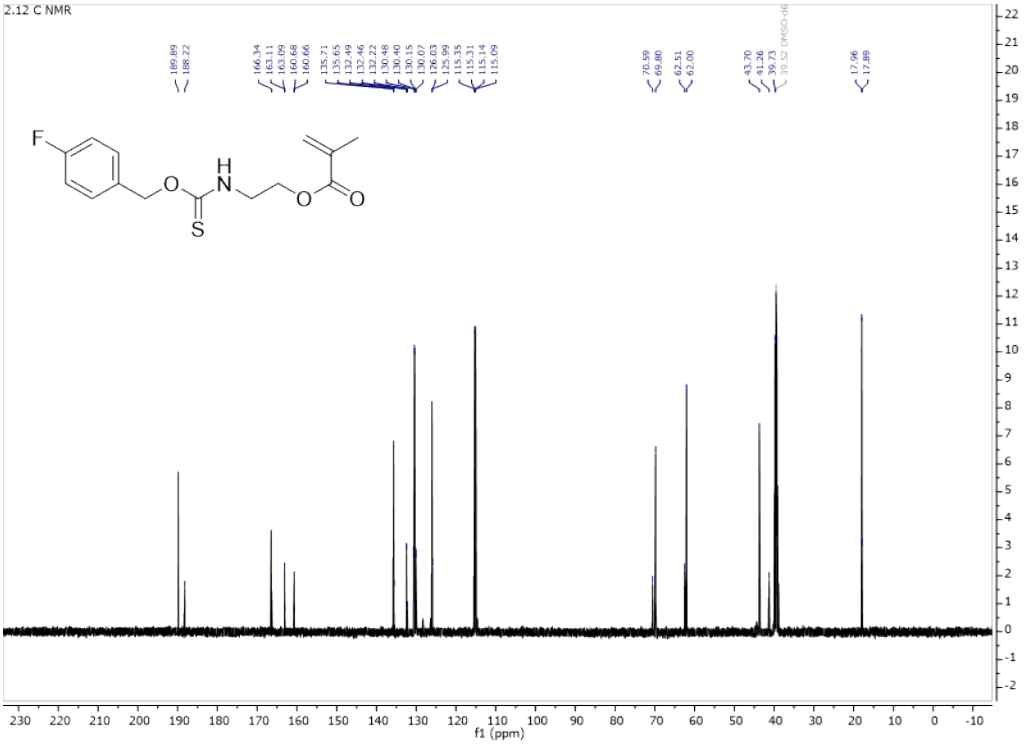


Figure S14. ^{13}C NMR of FBOT monomer (**2**) in DMSO-d_6.

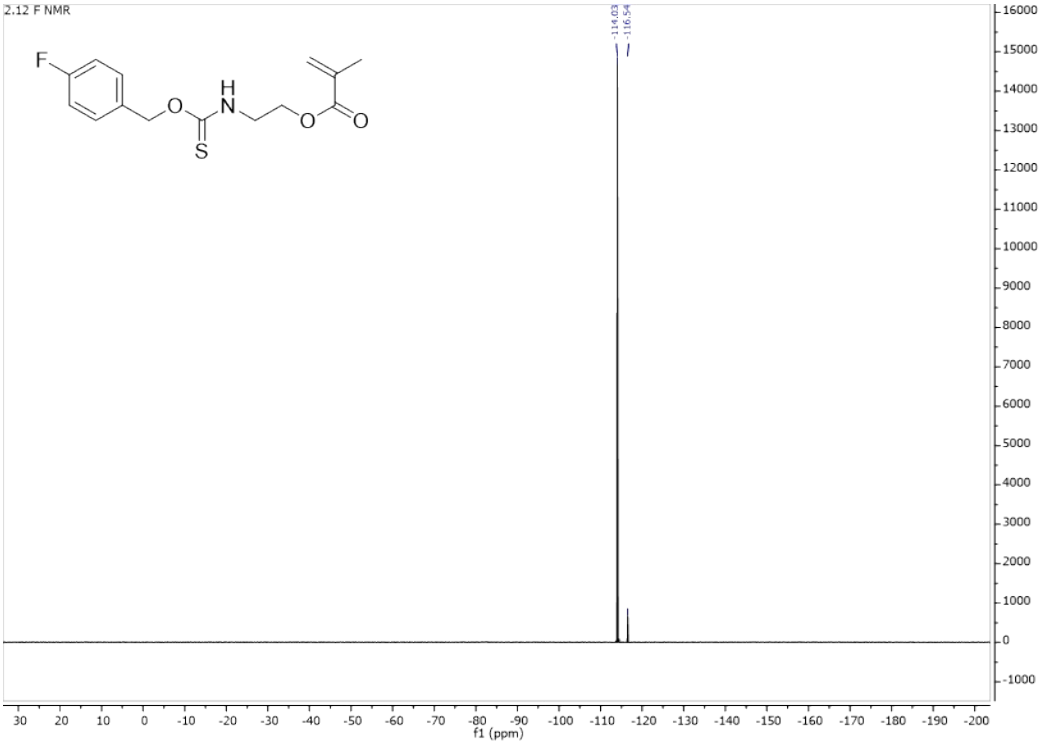


Figure S15. ^{19}F NMR of FBOT monomer (**2**) in DMSO-d_6.

NMR Spectra and GPC Traces for Polymers 10, 11 and 12

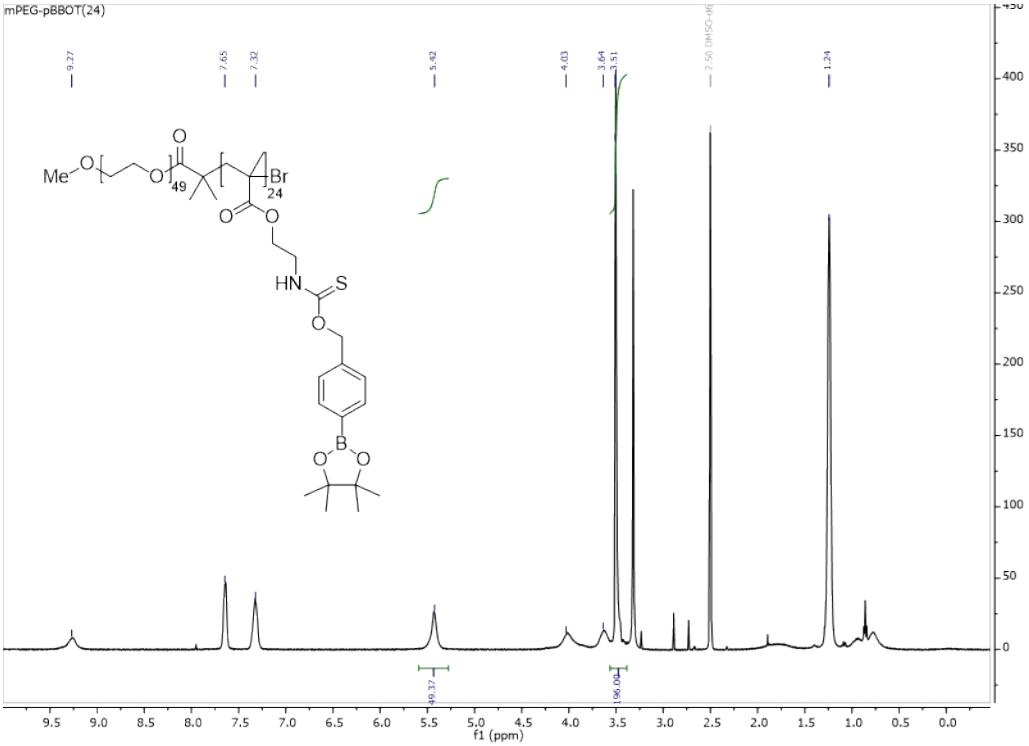


Figure S16. ^{1}H NMR of mPEG$_{49}$-BBOT$_{24}$ (**10**) in DMSO-d_6.

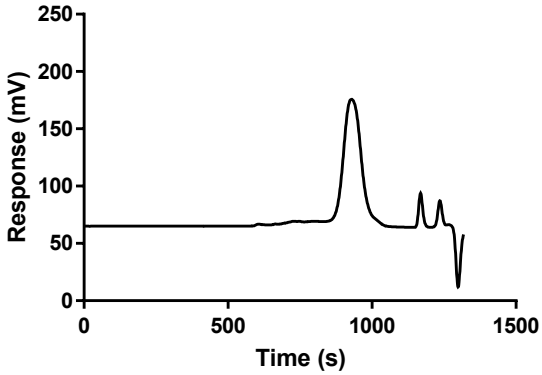


Figure S17. GPC of mPEG$_{49}$-BBOC$_{24}$ (**10**).

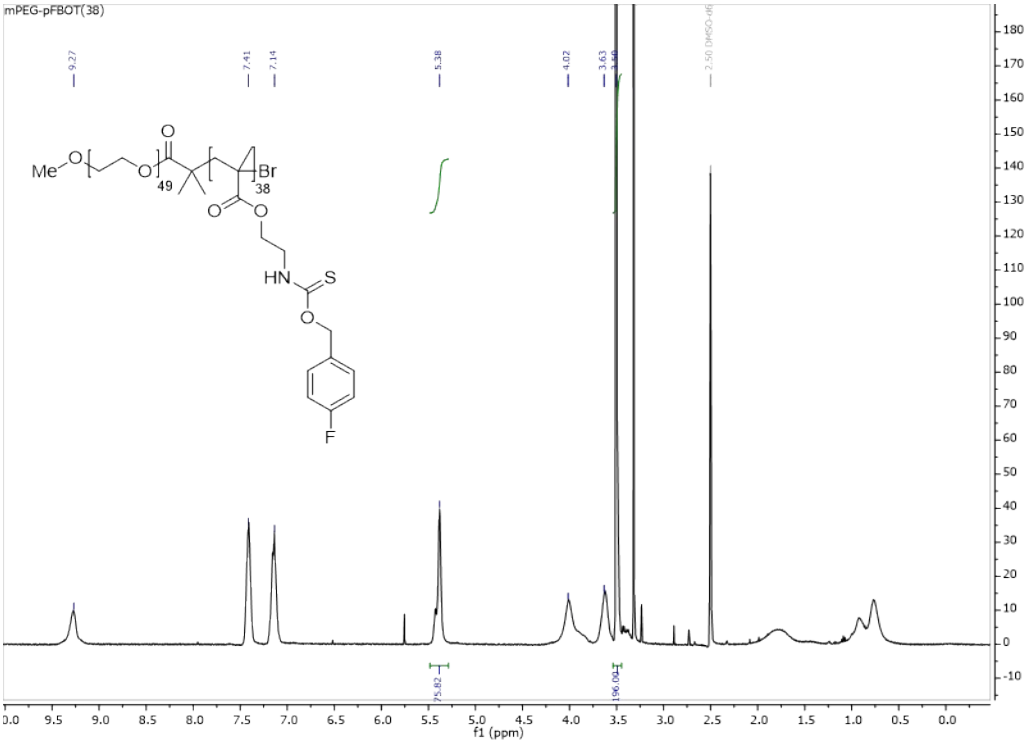


Figure S18. ^{1}H NMR of mPEG$_{49}$-FBOT$_{38}$ (**11**) in DMSO-d_6.

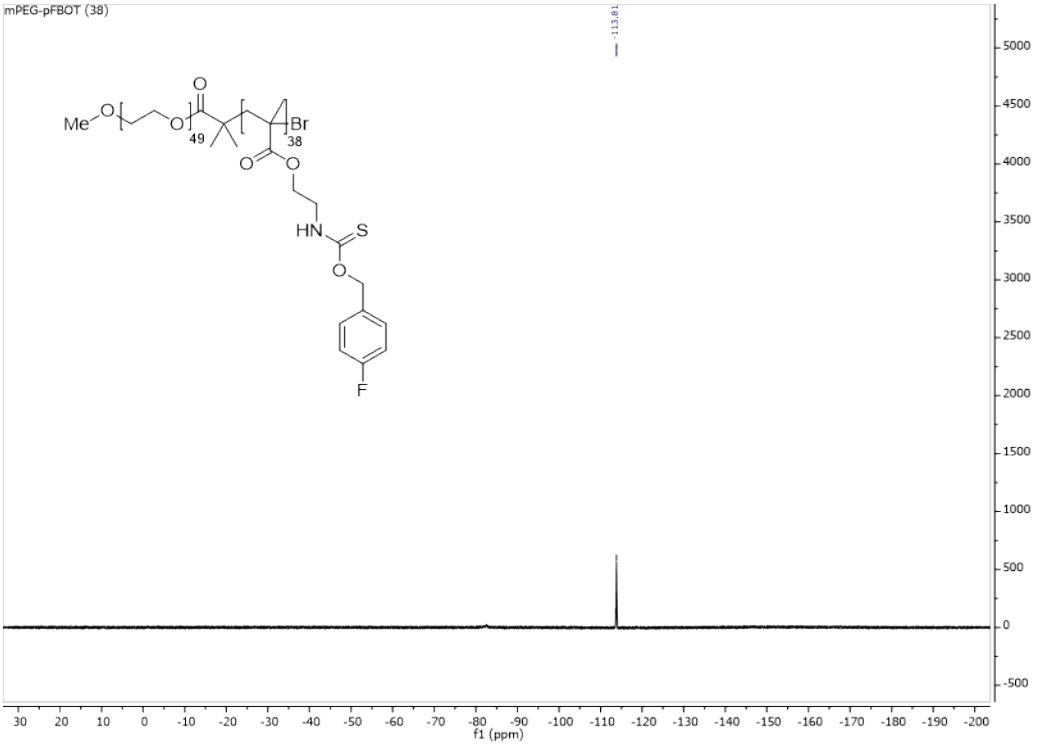


Figure S19. ^{19}F NMR of mPEG$_{49}$-FBOT$_{38}$ (**11**) in DMSO-d_6.

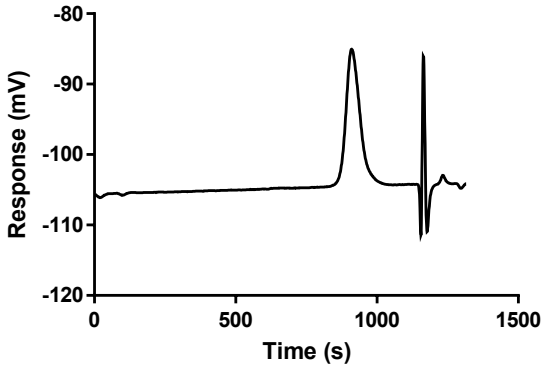


Figure S20. GPC of $mPEG_{49}$-$FBOT_{38}$ (**11**).

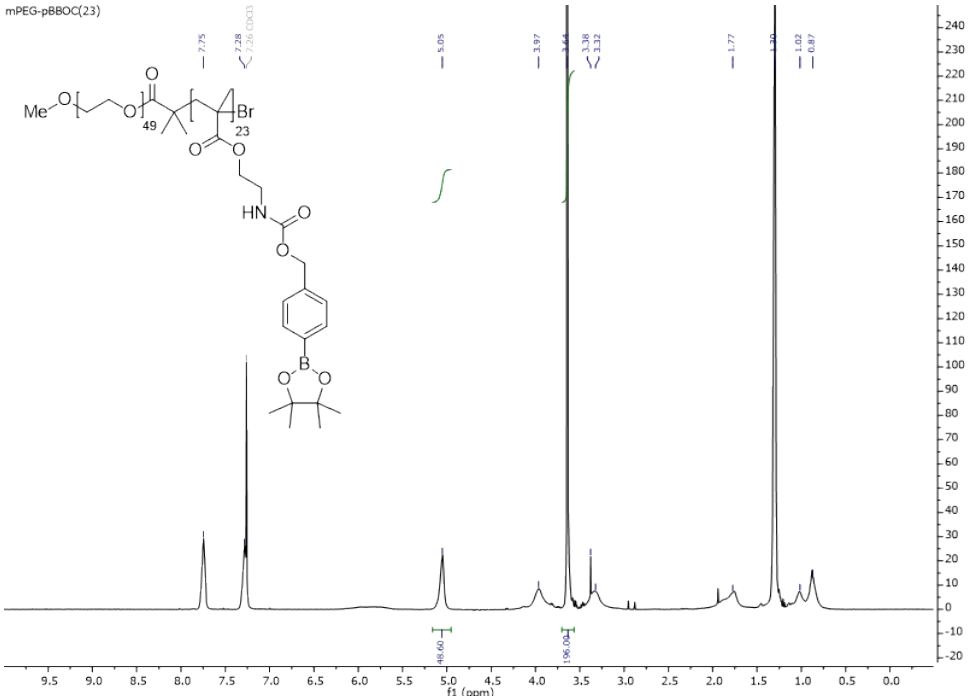


Figure S21. ^1H NMR of $mPEG_{49}$-$BBOC_{23}$ (**12**) in $CDCl_3$.

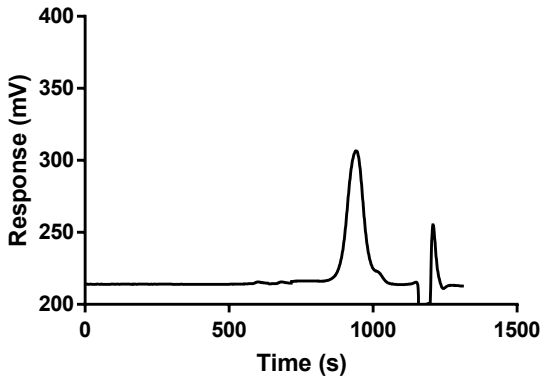


Figure S22. GPC of $mPEG_{49}$-$BBOC_{23}$ (**12**).

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