Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Multiblock sequence-controlled glycopolymers *via* Cu(0)-LRP following efficient thiol-halogen, thiol-epoxy and CuAAC reactions

Qiang Zhang,[a] *Athina Anastasaki,*[a] *Guang-Zhao Li,*[a, b] *Alice Haddleton,*[a] *Paul Wilson*[a, c] *and David M. Haddleton*[*a,c]

[a] Department of Chemistry, University of Warwick, CV4 7AL, Coventry, UK

[b] Department of Chemistry, Vanderbilt University, 7630 Stevenson Center, Nashville, Tennessee 37235, USA

[c] Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Australia.

Materials 3
Instruments and analysis 3
Synthesis of glycidyl acrylate 5
Synthesis of sugar azide 5
Synthesis of acrylic acid 3-trimethylsilanyl-prop-2-ynyl ester (TMSPA) 6
Synthesis of poly(glycidyl acrylate) (poly(GA)) *via* Cu(0)-LRP 7
Synthesis of multiblock poly(glycidyl acrylate)-*co*-(acrylic acid 3-trimethylsilanyl-prop-2-ynyl ester) (poly(GA)-*co*-(TMSPA)) *via* Cu(0)-LRP 8
Thiol-halogen and thiol-epoxy click reaction of poly(GA) with benzyl mercaptan 9
Thiol-halogen and thiol-epoxy click reaction of poly(GA)-*co*-(TMSPA) with 1-thio-β-D-glucose tetraacetate or benzyl mercaptan. 13
General procedure for deprotection of trimethyl silyl protected polymers. 14
CuAAC click reactions of sugar azide with alkyne-containing polymers 15
References 20

Materials

Tris(2-(dimethylamino)ethyl)amine (Me_6TREN) was synthesized according to literature procedure and stored under a nitrogen atmosphere prior to use.[1,2] 3-azido-propan-1-ol was synthesised as previous report.[3]

Copper(II) bromide ($CuBr_2$, 99%, Sigma-Aldrich) was used as received. Copper wire (diameter=0.25 mm) was pre-treated by washing in hydrochloric acid for 15 min and rinsed thoroughly with MiliQ water, dried under nitrogen and used immediately.

Membrane dialysis (1K MWCO) was obtained from Spectrum Laboratories.

Glycidol (96%, Sigma-Aldrich), Ethyl α-bromoisobutyrate (EBiB, 98%, Sigma-Aldrich), benzyl mercaptan (99 %, Sigma-Aldrich), 1-thio-β-D-glucose tetraacetate (97%, Sigma-Aldrich), triethylamine (TEA, 99%, Sigma-Aldrich), lithium hydroxide (LiOH, 98%, Fisher Chemical) and tetrabutylammonium fluoride solution (1M, Sigma-Aldrich) were used as received.

All other reagents and solvents were obtained at the highest purity available from Sigma-Aldrich and used without further purification unless otherwise stated.

Instruments and analysis

^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-300 and DPX-400 spectrometers using deuterated solvents obtained from Aldrich.

SEC was conducted on Varian 390-LC system in DMF (5 mM NH_4BF_4) at 50 ^{o}C, equipped with refractive index, UV and viscometry detectors, 2 × PLgel 5μm mixed-D columns (300 × 7.5 mm), 1 × PLgel 5μm guard column (50 × 7.5 mm) and autosampler. Narrow linear PMMA standards in range of 200 to 1.0 × 10^6 g·mol^{-1} were used to calibrate the system.

SEC using $CHCl_3$ eluent was performed on an Agilent 390-MDS, comprising of an autosampler and a PLgel 5.0 μm bead-size guard column (50 × 7.5 mm), followed by two 5.0 μm bead-size PLgel Mixed D columns (300 × 7.5 mm) and a differential refractive index detector using $CHCl_3$ as the eluent at 30 ^{o}C with a flow rate of 1 mL min^{-1}. The SEC system was calibrated with linear PMMA EasiVial standards (Agilent Ltd.) ranging from 200 to 10^5 g mol^{-1} and PS EasiVial standards (Agilent Ltd.) ranging from 162 to 10^5 g mol^{-1}.

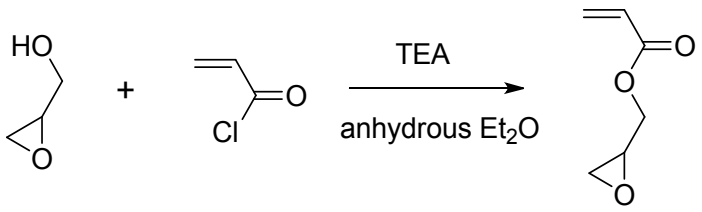
SEC with THF as eluent was performed on an Agilent 390-MDS, comprising of an autosampler and a PLgel 5.0 μm bead-size guard column (50 × 7.5 mm), followed by two linear 5.0 μm bead-size PLgel Mixed D columns (300 × 7.5 mm) and a differential refractive index detector using THF (2% v/v TEA) as the eluent at 30 ^{o}C with a flow rate of 1 mL min^{-1}. The SEC system was calibrated with linear PMMA EasiVial standards (Agilent Ltd.) ranging from 200 to 10^5 g mol^{-1} and PS EasiVial standards (Agilent Ltd.) ranging from 162 to 10^5 g mol^{-1}. All samples were passed through 0.45 μm PTFE filter before SEC analysis.

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

MALDI-ToF MS was recorded in linear or reflex mode on 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. The matrix solution was prepared by dissolving α-cyano-4-hydroxycinnamic acid (CHCA) or *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) in THF (20 mg/mL solution). Sodium iodide was added at a 0.1% overall concentration to improve the ionization. Polymer analytes were dissolved to a concentration of 1 mg/mL. Samples were prepared by layering matrix solution and analyte solution on the stainless steel side. Calibration was performed with different linear poly (ethylene glycol) methyl ether standards.

All reactions were carried out using standard Schlenk techniques under an inert atmosphere of oxygen-free nitrogen, unless otherwise stated. All obtained epoxy-containing products were stored in the fridge (-18 ^{o}C).

Synthesis of glycidyl acrylate



S Scheme 1. Schematic representation of the synthetic approach to glycidyl acrylate.

A solution of glycidol (10.4 mL, 157 mmol), TEA (30.4 mL, 218 mmol), hydroquinone (30 mg, 0.3 mmol) and anhydrous diethyl ether (500 mL) was cooled in an ice-water bath. Acryloyl chloride (15.2 mL, 187 mmol) in 20 mL diethyl ether was then added dropwise into the solution over a period of ca. 30 min. The mixture was stirred in the ice bath for 1 h and then at ambient temperature overnight. The ammonium salts were then removed by filtration and the residue was extracted with water (3×100 mL) and dried over magnesium sulphate. Following that the organic solvent was removed via rotary evaporation. The product was recovered as slightly yellow liquid and then purified *via* flash silica gel column chromatography (petroleum ether: diethyl ether = 8: 1, volume ratio). Final product was obtained as clear liquid after removing the solvent on rotary evaporator and dried under slightly vacuum (10.8 g, 54%).

^{1}H NMR ($CDCl_3$, 298 K, 400 MHz): δ = 6.43 (dd, 1 H, J=1.4, 15.9 Hz, CH_2=), 6.14 (dd, 1 H, J=10.5, 6.9 Hz, =C*H*-), 5.86 (dd, 1 H, J=1.4, 9.1 Hz, CH_2=), 4.47 (dd, 1 H, J=3.1, 9.2 Hz, C=O-O-CH_2-), 4.47 (dd, 1 H, J=6.3, 6.0 Hz, C=O-O-CH_2-), 3.23 (m, 1 H, CH_2-C*H*-O), 2.84 (t, 1 H, O-CH_2), 2.65 (dd, 1 H, J=2.6, 2.2 Hz, O-CH_2) ppm.

^{13}C NMR ($CDCl_3$, 298 K, 400 MHz): δ = 165.1 (*C*=O), 131.5 (*C*H_2=CH-), 127.9 (CH_2=*C*H-), 65.1 (C=O-O-*C*H_2-), 49.3 (CH_2-*C*H-O), 44.7 (CH-*C*H_2-O) ppm.

FT-IR ν: 3009(=C-H), 2953(-C-H), 1741 (C=O), 906 & 845 (epoxy group) cm^{-1}.

ESI-MS *m/z*: calcd for ($M+Na^+$): 151.05; found: 151.05.

Synthesis of sugar azide

Sodium azide (7.26 g, 111 mmol), D-(+)-mannose (2.00 g, 11.1 mmol) and triethylamine (15.5 mL, 111 mmol) were dissolved in water (40 mL) and cooled to 0 ºC. 2-Chloro-1,3-dimethylimidazolinium chloride (5.61 g, 33.3 mmol) was added and

the mixture was stirred for 1 hour at 0 ºC. The solvent was removed under reduced pressure and ethanol was added. The solids were removed by filtration and the solution was purified on Amberlite IR-120 column. The mixture was checked with FTIR to confirm the removal of all sodium azide (ν = 2022 cm^{-1}). The solvent was removed under reduced pressure, water was added and the mixture was washed with dichloromethane. The solvent was removed under reduced pressure, water (10 mL) was added and the solution was freeze-dried overnight to give α-D-mannopyranosyl azide as an off-white solid.

^{1}H NMR (400 MHz, D_2O, 298 K), δ = 3.57 - 3.66 (m, 1H, H^5), 3.68 - 3.77 (m, 3H), 3.84 (dd, J = 3.01, 2.01 Hz, 1H, H^2), 3.88 (d, J = 10.04 Hz, 1H), 5.43 (d, J = 2.01 Hz, 1H, H^1) ppm.

^{13}C NMR (100 MHz, D_2O, 298 K), δ = 60.81 (1C, C^6), 66.38 (1C, C^4), 69.75 (1C, C^3), 69.83 (1C, C^2), 74.62 (1C, C^5), 89.71 (1C, C^1) ppm.

FT-IR ν: 3331 (υ(O-H)), 2933, 2110 ($\upsilon_{as}(-N_3)$), 1407, 1294, 1237, 1092, 1060, 933, 803, 667, 566 cm^{-1}.

ESI-MS (*m/z*): Calcd. for $C_6H_{11}N_3NaO_5^+$ ($M+Na^+$) = 228.06, Found 228.06.

Synthesis of acrylic acid 3-trimethylsilanyl-prop-2-ynyl ester (TMSPA)

A solution of 3-(Trimethylsilyl)propargyl alcohol (5 g, 39 mmol), TEA (7.61 mL, 54.6 mmol), hydroquinone (30 mg, 0.3 mmol) and anhydrous diethyl ether (500 mL) was cooled in an ice-water bath. Acryloyl chloride (3.80 ml) in 20 mL diethyl ether was then added dropwise into the solution over a period of ca. 30 min. The mixture was stirred in the ice bath for 1 h and then at ambient temperature overnight. The ammonium salts were then removed by filtration and the residue was extracted with water (3×100 mL) and dried over magnesium sulphate. Following that the organic solvent was removed via rotary evaporation. The product was recovered as slightly yellow liquid and then purified *via* flash silica gel column chromatography (hexane ether: diethyl ether = 5: 1, volume ratio). Final product was obtained as clear liquid after removing the solvent on rotary evaporator and dried under slightly vacuum (60% yield).

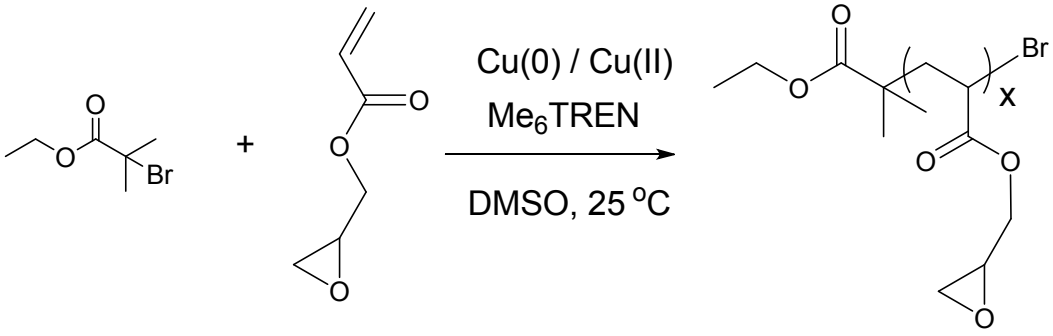
^{1}H NMR ($CDCl_3$, 298 K, 400 MHz): δ = 6.47 (dd, 1 H, J = 17.3, 0.8 Hz, CH_2=), 6.17

(dd, 1 H, J = 17.3, 10.5 Hz, =C*H*-), 5.88 (dd, 1 H, J = 10.5, 0.8 Hz, CH_2=), 4.78 (s 2 H, -O-CH_2-), 0.19 (s, 9 H, Si-(CH_3)$_3$),

^{13}C NMR ($CDCl_3$, 298 K, 400 MHz): δ = 165.62 (*C*=O), 131.98 (*C*H_2=CH-), 128.05 (CH_2=*C*H-), 99.13 (O-CH2-*C*-C), 92.52 (O-CH2-C-*C*)53.14 (C=O-O-*C*H_2-), 0.00 ((*C*H_3)$_3$-Si) ppm.

ESI-MS *m/z*: calcd for (M+Na^+): 205.06; found: 205.06.

Synthesis of poly(glycidyl acrylate) (poly(GA)) *via* Cu(0)-LRP

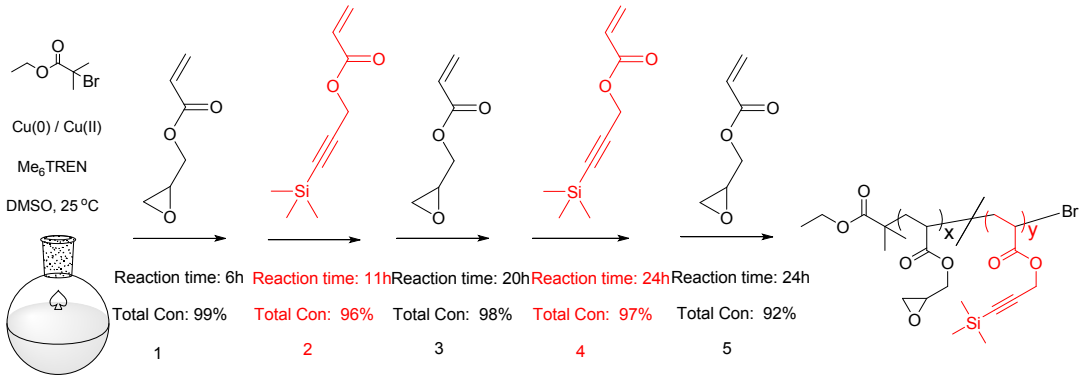


S Scheme 2. Synthesis of poly(GA) via Cu(0)-LRP

To a Schlenk tube fitted with a magnetic stir bar and a rubber stopper, glycidyl acrylate (1537 mg, 12 mmol, 20 eq), $CuBr_2$ (13.4 mg, 0.06 mmol, 0.1 eq), mesitylene (120 mg, 1 mmol, internal standard) and DMSO (3 mL) were charged and the mixture was bubbled with nitrogen for 15 min. Pre-degassed Me_6TREN (25 mg, 0.11 mmol, 0.18 eq) and EBiB (117 mg, 0.6 mmol, 1 eq) were then added via gas tight syringe sequentially. After that, pre-activated copper wire (3 cm, 13 mg, 0.20 mmol) was carefully added under nitrogen protection. The Schlenk tube was sealed and the light green solution was allowed to polymerize at 25 °C. Samples of the reaction mixture were carefully removed at suitable time periods for analysis. The sample for ^{1}H NMR characterization was directly diluted with $CDCl_3$. Catalyst residues were removed by filtering through a column of neutral alumina prior to SEC analysis. After 6 h, the conversion reached 97% according to ^{1}H NMR analysis and reaction was stopped *via* exposure to the air. Following that the mixture was diluted with THF and passed through a short neutral alumina column to remove excess copper catalyst. The obtained solution was concentrated via rotary evaporation and directly transported into one dialysis tubing (MWCO 1000Da) for dialysis against acetone for two days.

Poly(GA) was recovered as white solid after removing the solvent and dried under high vacuum overnight.

Synthesis of multiblock poly(glycidyl acrylate)-*co*-(acrylic acid 3-trimethylsilanyl-prop-2-ynyl ester) (poly(GA)-*co*-(TMSPA)) *via* Cu(0)-LRP

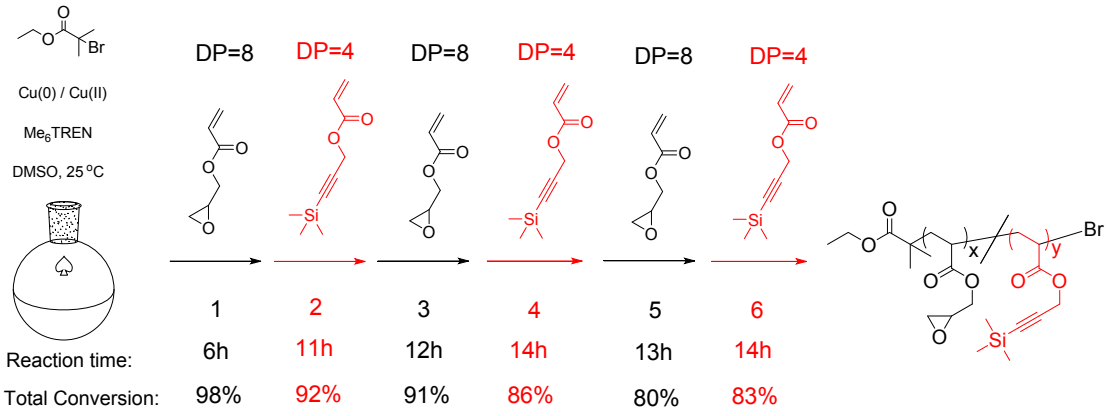


S Scheme 3. Synthesis of multiblock poly(GA)-*co*-(TMSPA) via Cu(0)-LRP

Monomer solutions in DMSO were first made with the same ratio of mesitylene as the internal standard. Glycidyl acrylate (1537 mg, 12 mmol) and mesitylene (60 mg, 0.5 mmol) were soluble in 3 mL DMSO; TMSPA (1092 mg, 6 mmol) and mesitylene (30 mg, 0.25 mmol) were soluble in 4.5 mL DMSO.

To a Schlenk tube fitted with a magnetic stir bar and a rubber stopper, 1/3 volume of the above-mentioned GA solution (4 mmol GA monomer) and $CuBr_2$ (11 mg, 0.05 mmol, 0.1 eq) were charged and the mixture was bubbled with nitrogen for 15 min. Pre-degassed Me_6TREN (21 mg, 0.09 mmol, 0.18 eq) and EBiB (98 mg, 0.5 mmol, 1 eq) were then added via gas tight syringe sequentially. After that, pre-activated copper wire (3 cm, 13 mg, 0.20 mmol) was carefully added under nitrogen protection. The Schlenk tube was sealed and the light green solution was allowed to polymerize at 25 °C. After reaction for 6 h, sample was carefully removed for ^1H NMR and SEC characterization. For chain extension, a further 1/3 volume of the above-mentioned

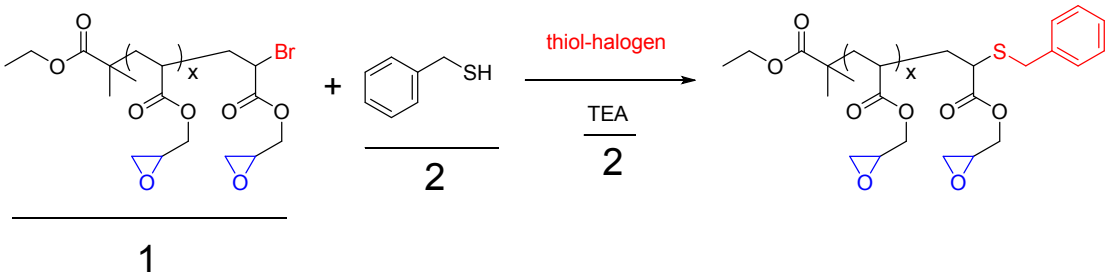
TMSPA solution (2 mmol TMSPA monomer), which has been degassed *via* nitrogen sparging for 15 min, was then transferred *via* cannula under nitrogen into the Schlenk reaction tube. The solution was allowed to polymerize at 25 °C for 11 h with stirring. The above polymerization-sampling-extension procedure was repeated at defined time period as shown in **S Scheme 3**.



S Scheme 4. Synthesis of multiblock poly(GA)-*co*-(TMSPA) *via* Cu(0)-LRP

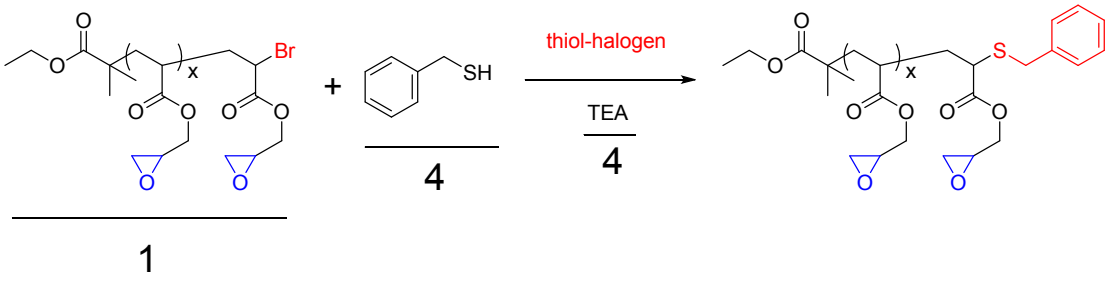
In order to improve the chain end fidelity, the polymerization was repeated and new monomer was added before full conversion of the previous polymerization. The procedure is exactly the same as shown above with only changed reaction time as shown in **S Scheme 4**.

Thiol-halogen and thiol-epoxy click reaction of poly(GA) with benzyl mercaptan



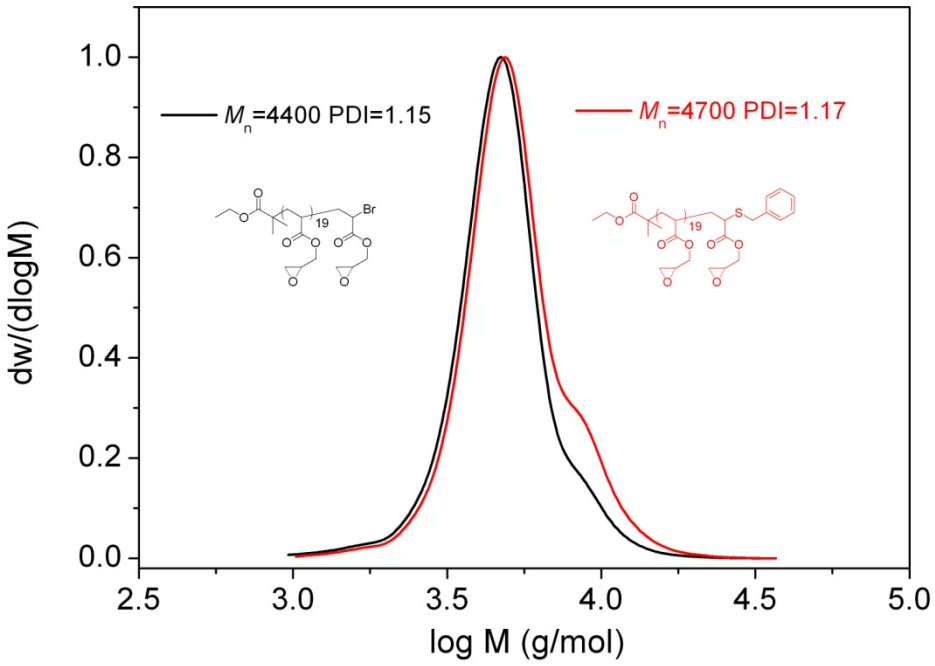
S Scheme 5. Thiol-halogen click reaction of poly(GA) with benzyl mercaptan. $[poly(GA)]_0$: $[benzyl\ mercaptan]_0$ = 1: 2.

To a vial fitted with a magnetic stir bar, DMF (2 mL), poly(GA) (280 mg, ~ 0.1 mmol Br moiety), benzyl mercaptan (25 mg, 0.2 mmol) and TEA (40 mg, 0.4 mmol) were sequentially charged and the mixture was allowed to stir at ambient temperature overnight. Following that, the reaction mixture was directly transferred to one short dialysis tube (MWCO limit, 1000 Da) and dialyzed against acetone for two days during which acetone was changed four times (4 × 300 mL). Solid product (170 mg) could be recovered after removing the solvent under high vacuum overnight.

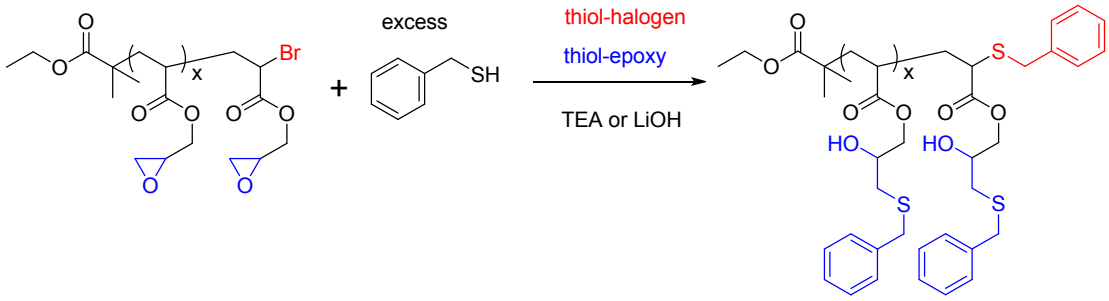


S Scheme 6. Thiol-halogen click reaction of poly(GA) with benzyl mercaptan. $[poly(GA)]_0$: $[benzyl\ mercaptan]_0$ = 1: 4.

To a vial fitted with a magnetic stir bar, DMF (2 mL), poly(GA) (410 mg, ~ 0.15 mmol Br moiety), benzyl mercaptan (74 mg, 0.6 mmol) and TEA (60 mg, 0.6 mmol) were sequentially charged and the mixture was allowed to stir at ambient temperature overnight. Following that, the reaction mixture was directly transferred to one short dialysis tube (MWCO limit, 1000 Da) and dialyzed against acetone for two days during which acetone was changed four times (4 × 300 mL). Solid product (300 mg) could be recovered after removing the solvent under high vacuum overnight.



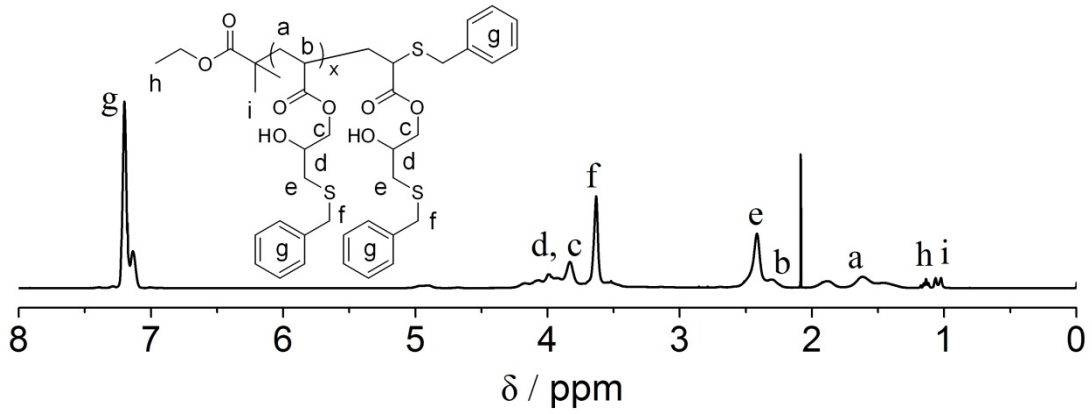
S Figure 1. Molecular weight distributions of bromide-terminated poly(GA) and benzyl mercaptan-terminated poly(GA) via THF SEC.



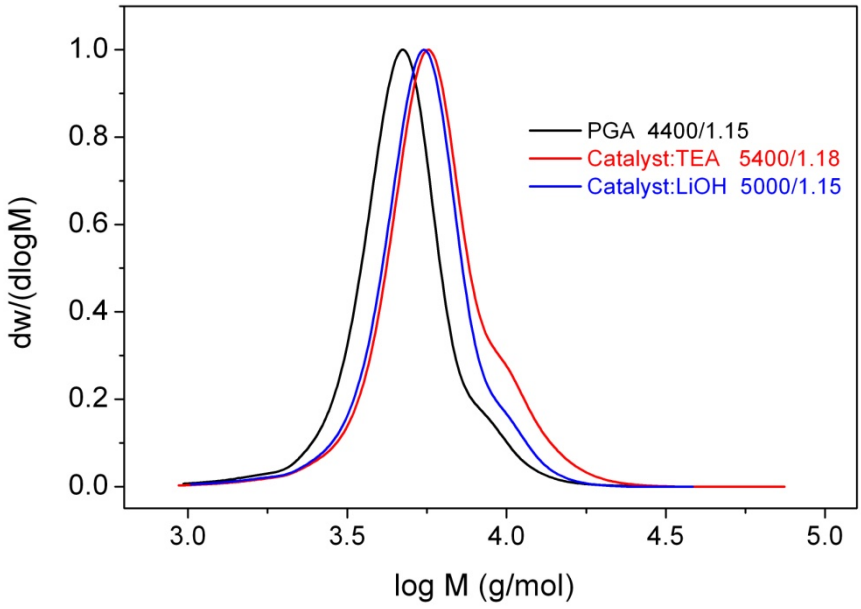
S Scheme 7. Thiol-halogen and thiol-epoxy click reaction of poly(GA) with excess benzyl mercaptan under the catalysis of TEA or LiOH.

To a vial fitted with a magnetic stir bar, DMF (2 mL), poly(GA) (100 mg, ~ 0.7 mmol epoxy moiety), benzyl mercaptan (176 mg, 1.4 mmol) and TEA (57 mg, 0.6 mmol) or LiOH (13 mg, 0.6 mmol) were sequentially charged and the mixture was allowed to stir at ambient temperature overnight. Following that, the reaction mixture was directly transferred to one short dialysis tube (MWCO limit, 1000 Da) and dialyzed

against acetone for two days during which acetone was changed four times (4 × 300 mL). The product could be recovered after removing the solvent under high vacuum overnight.

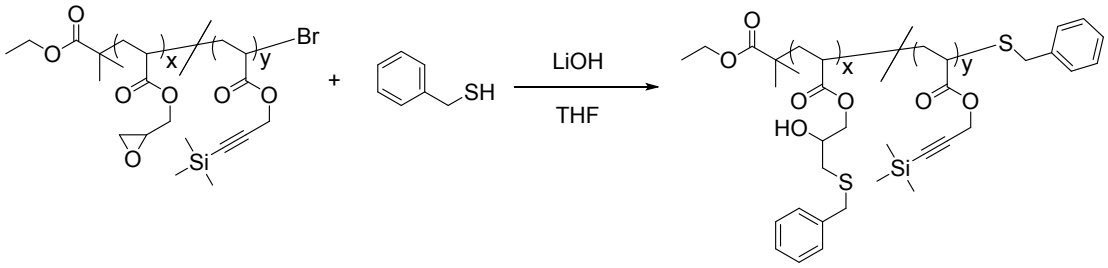


S Figure 2. ^{1}H NMR spectrum ($CDCl_3$) for product from reaction of poly(GA) with excess benzyl mercaptan under the catalysis of LiOH in DMF.



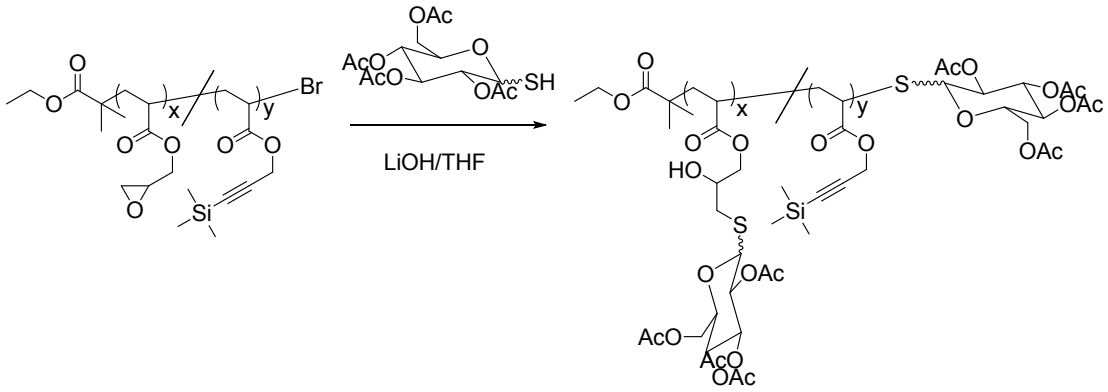
S Figure 3. Molecular weight distributions for the products from reaction of poly(GA) with excess benzyl mercaptan under the catalysis of TEA or LiOH in DMF.

Thiol-halogen and thiol-epoxy click reaction of poly(GA)-*co*-(TMSPA) with 1-thio-β-D-glucose tetraacetate or benzyl mercaptan.



S Scheme 8. Thiol-halogen and thiol-epoxy click reaction of poly(GA)-co-(TMSPA) with benzyl mercaptan.

To a vial fitted with a magnetic stir bar, THF (3 mL), poly(GA)-co-(TMSPA) (260 mg, ~ 1.2 mmol epoxy moiety), benzyl mercaptan (298 mg, 2.4 mmol) and LiOH (14 mg, 0.6 mmol) were sequentially charged and the mixture was allowed to stir at ambient temperature overnight. Following that, the reaction mixture was directly transferred to one short dialysis tube (MWCO limit, 1000 Da) and dialyzed against THF for two days during which acetone was changed four times (4 × 300 mL). The product could be obtained after removing the solvent under high vacuum overnight.

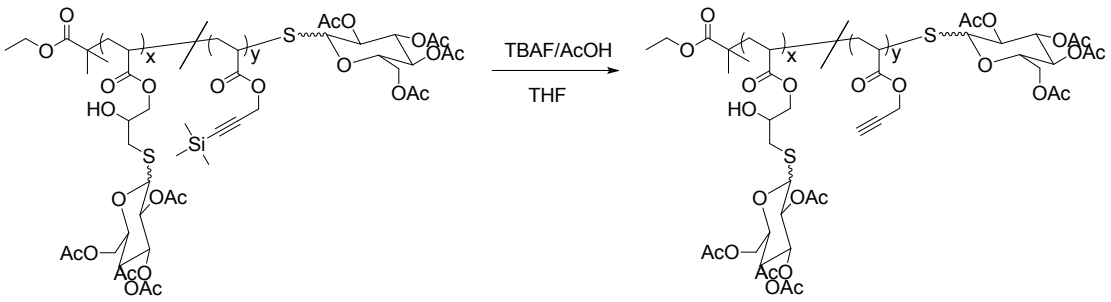


S Scheme 9. Thiol-halogen and thiol-epoxy click reaction of poly(GA)-co-(TMSPA) with 1-thio-β-D-glucose tetraacetate.

To a vial fitted with a magnetic stir bar, THF (5 mL), poly(GA)-co-(TMSPA) (130 mg, ~ 0.6 mmol epoxy moiety), 1-thio-β-D-glucose tetraacetate (364 mg, 1 mmol) and LiOH (6 mg, 0.25 mmol) were sequentially charged and the mixture was allowed to stir at ambient temperature overnight. Following that, the reaction mixture was directly transferred to one short dialysis tube (MWCO limit, 1000 Da) and dialyzed

against THF for two days during which acetone was changed four times (4 × 300 mL). White solid product (0.43 g) could be obtained after removing the solvent under high vacuum overnight.

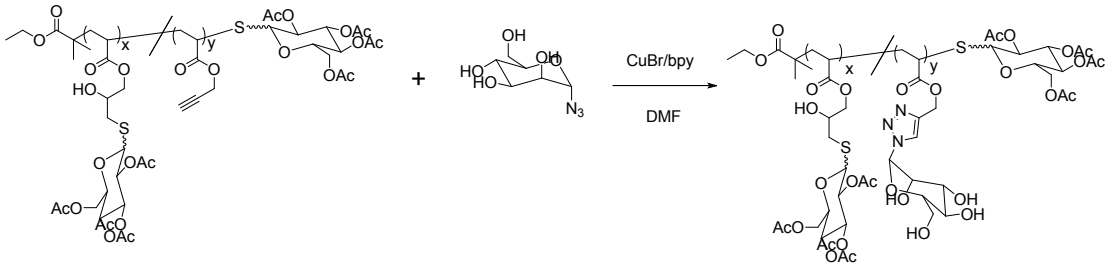
General procedure for deprotection of trimethyl silyl protected polymers.



S Scheme 10. General procedure for deprotection of trimethyl silyl protected polymers.

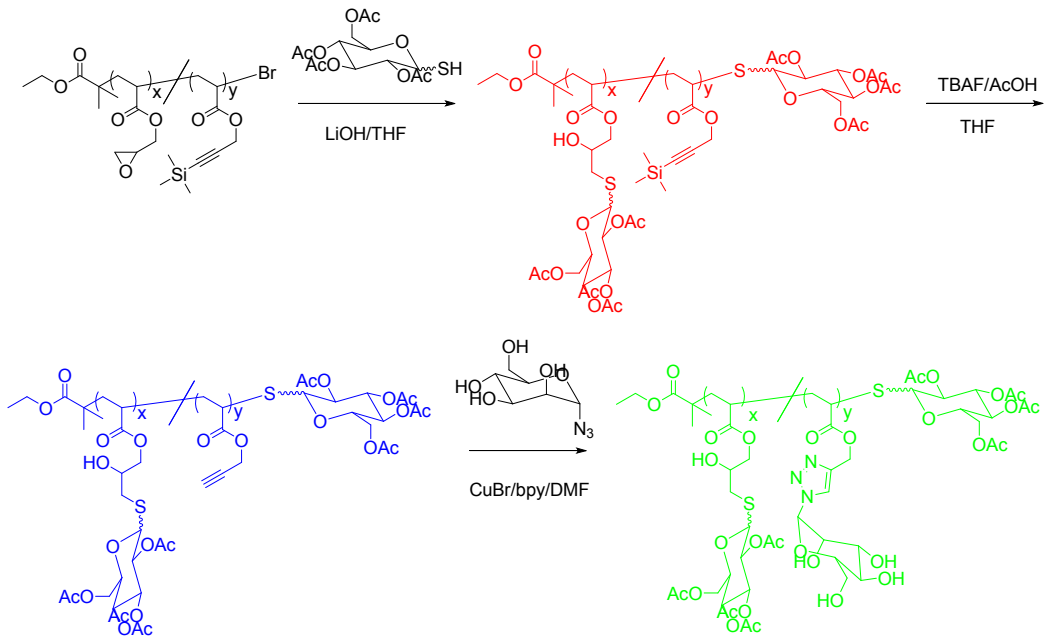
The typical procedure is shown as follow. The trimethyl silyl protected polymer (product after reaction with 1-thio-β-D-glucose tetraacetate, 200 mg, ~0.17 mmol alkyne-trimethylsilyl groups) and acetic acid (204 mg, ~2 equiv. mol/mol with respect to the alkyne-trimethylsilyl groups) were dissolved in THF (2 mL). Nitrogen was bubbled (ca. 10 min) and the solution was cooled to -20 °C. A 1 M solution of TBAF·$3H_2O$ in THF (0.34 mL, ~2 equiv. mol/mol with respect to the alkyne-trimethylsilyl groups) was added slowly via syringe (ca. 2-3 min). The resulting turbid mixture was stirred at this temperature for 30 min and then warmed to ambient temperature. The reaction mixture was allowed to stir under ambient temperature for 24 h. Following that the reaction solution was passed through a short silica pad in order to remove the excess of TBAF and the pad was subsequently washed with additional THF. The resulting solution was then concentrated under reduced pressure and then purified via dialysis against THF for one day. The product (150 mg) could be obtained after removing the solvent under high vacuum overnight.

CuAAC click reactions of sugar azide with alkyne-containing polymers

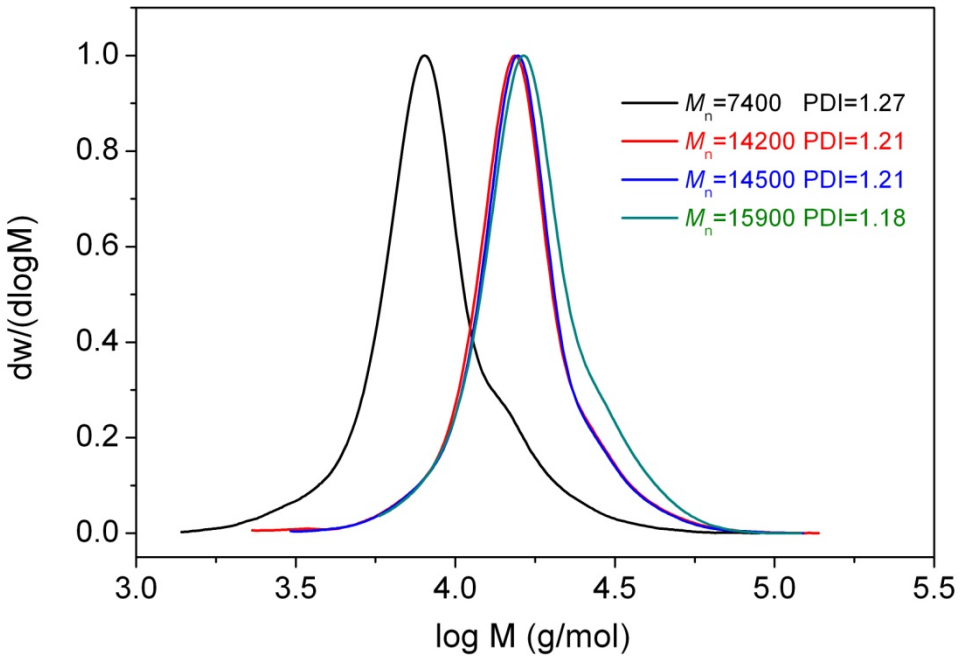


S Scheme 11. CuAAC click reaction of sugar azide with alkyne-containing polymers.

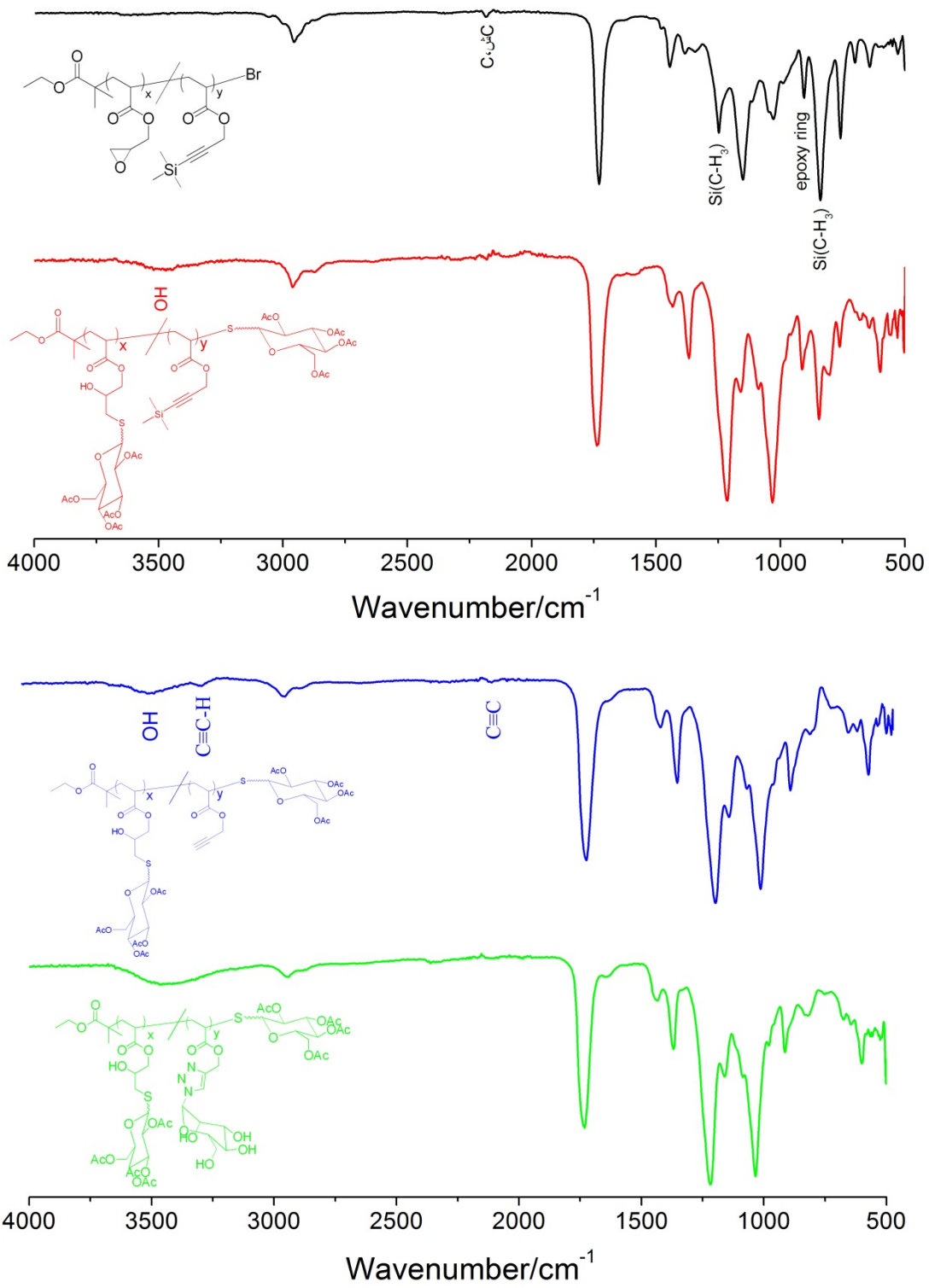
The general procedure is shown as follow. A solution of azide mannose (55 mg, 0.27 mmol), alkyne-containing polymer (100 mg, ~ 0.09 mmol of alkyne groups), bpy (30 mg, 0.20 mmol) in DMSO (5 mL) was deoxygenated by three freeze-pump-thaw cycles. The solution was then transferred via cannula under nitrogen into a Schlenk tube, previously evacuated and filled with nitrogen, containing CuBr (14 mg, 0.10 mmol). The resulting solution was stirred at 25 ^{o}C for 24 h. Upon the reaction was completed, the reaction mixture was diluted with MeOH (20 mL) and purged with air for 1 h and then dialysed against MeOH/H_2O (MeOH: H_2O = 0.8: 0.2, volume ratio) for two days after which the glycopolymer (0.226 g) could be recovered by freeze drying.



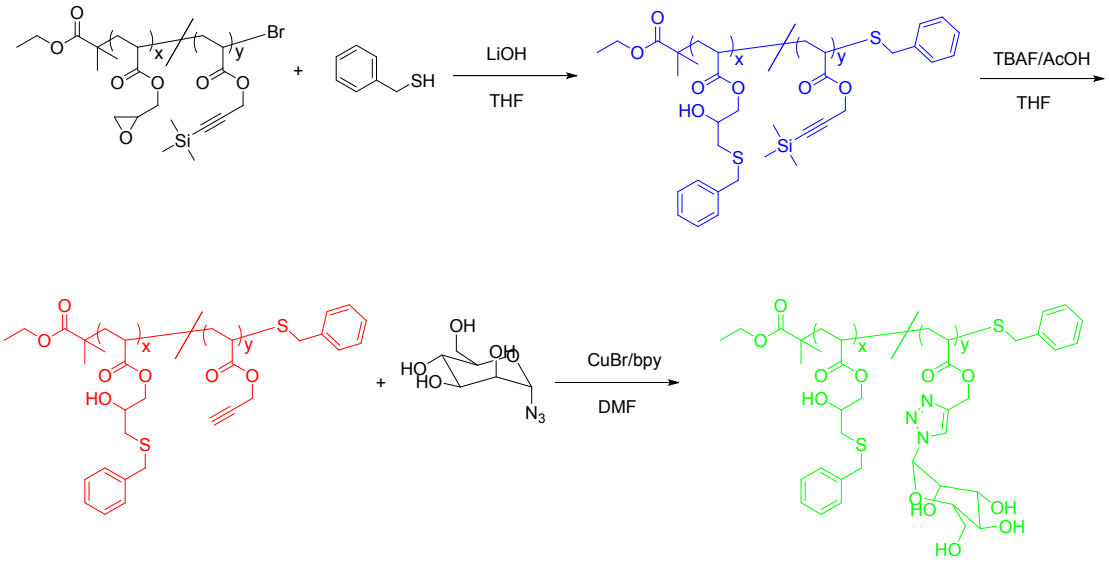
S Scheme 12. Synthesis of multiblock glycopolymers *via* sequential thiol-related and CuAAC click reactions.



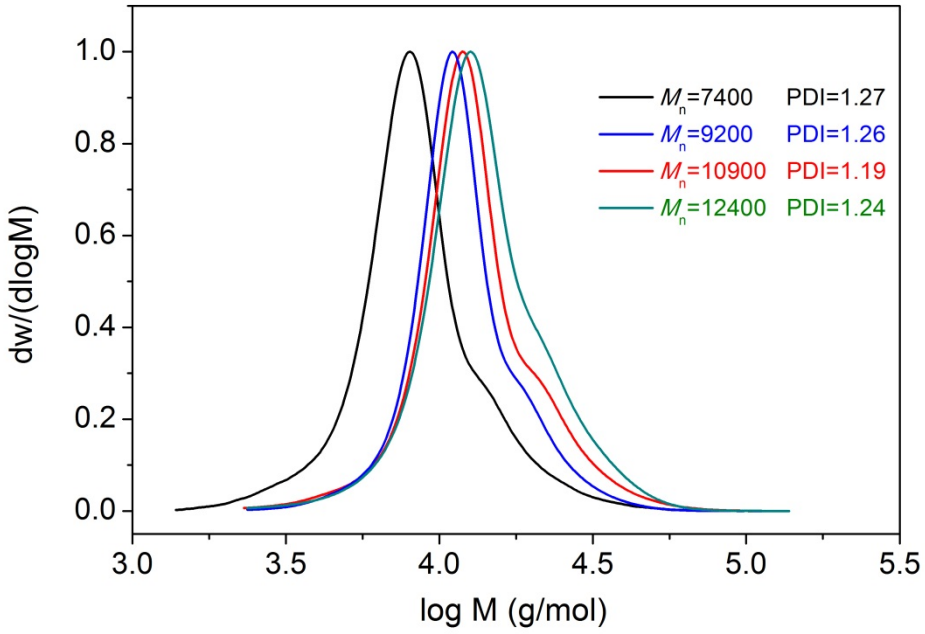
S Figure 4. DMF SEC Molecular weight distributions for the products obtained by sequential thiol-related and CuAAC click reactions. The colour represents relative products as shown in S Scheme 12.



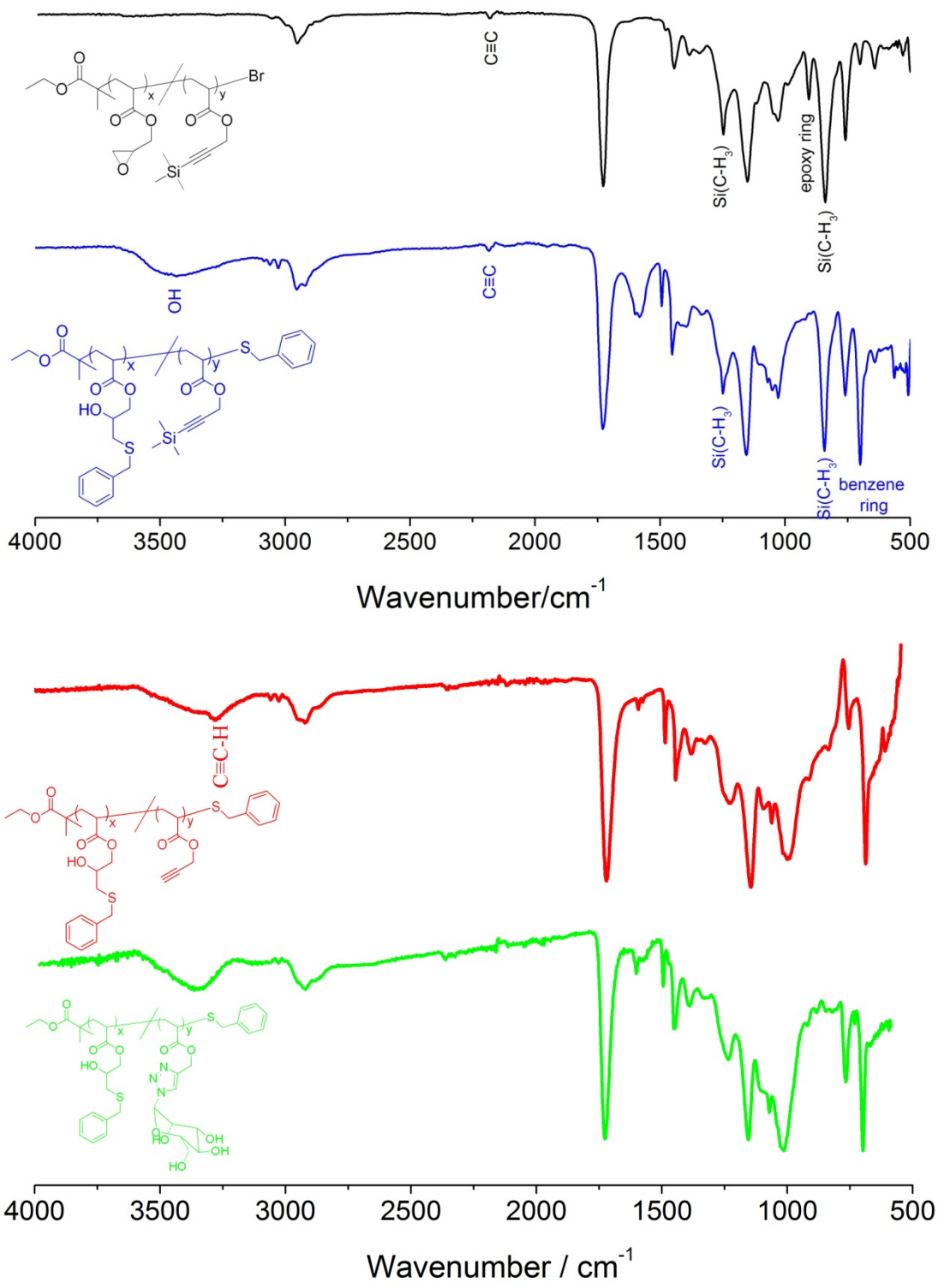
S Figure 5. FTIR spectra for the products obtained by sequential thiol-related and CuAAC click reactions. The colour represents relative products as shown in S Scheme 12.



S Scheme 13. Synthesis of multiblock glycopolymers *via* sequential thiol-related and CuAAC click reactions.



S Figure 6. DMF SEC Molecular weight distributions for the products obtained by sequential thiol-related and CuAAC click reactions. The colour represents relative products as shown in S Scheme 13.



S Figure 7. FTIR spectra for the products obtained by sequential thiol-related and CuAAC click reactions. The colour represents relative products as shown in S Scheme 13.

References

(1) Ciampolini, M.; Nardi, N. *Inorg Chem* **1966**, *5*, 41.

(2) Queffelec, J.; Gaynor, S. G.; Matyjaszewski, K. *Macromolecules* **2000**, *33*, 8629.

(3) Mantovani, G.; Ladmiral, V.; Tao, L.; Haddleton, D. M. *Chem Comm* **2005**, 2089.