Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information for

Sequential Nucleophilic "Click" Reactions for Functional Amphiphilic Homopolymers

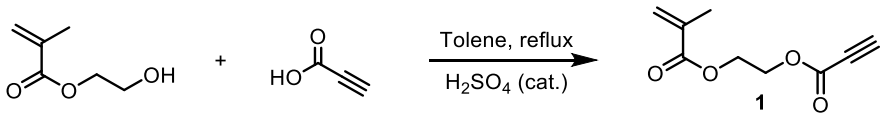
Huan He, Bin Liu, Meizhe Wang, Richard W. Vachet and S. Thayumanavan*

Department of Chemistry, University of Massachusetts, Amherst, MA 01003

Materials and methods:

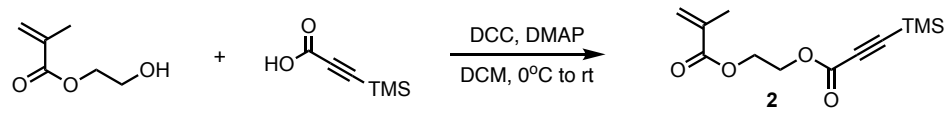
Unless mentioned, all chemicals were used as received from Sigma-Aldrich. ^{1}H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer. Molecular weight of the polymers was measured by gel permeation chromatography (GPC, Waters) using a PMMA standard with a refractive index detector. THF was used as eluent with a flow rate of 1 mL/min. Dynamic light scattering (DLS) measurements were performed using a Malvern Nano zetasizer. The fluorescence spectra were obtained from a JASCO FP-6500 Spectro fluorometer. TEM images were recorded on a JEOL-2000FX machine operating at an accelerating voltage of 100 kV. TGA thermograph were obtained from TA Instrument Q50 machine operating at a heating rate of 10 ^{o}C/min.

Synthesis of monomer 1:

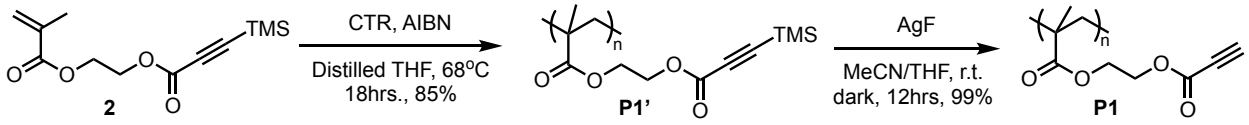


Propiolic acid (1.0 g, 7.04 mmol) and 2-Hydroxyethyl methacrylate (1.008 g, 7.74 mmol) were mixed in 40 mL toluene. After the temperature was increased to 80 ^{o}C, 2 drops of sulfuric acid were added to the above solution. The system was heated to reflux for 36 h with a Dean-Stark apparatus for collecting the condensed water. After the reaction was cooled to room temperature, $NaHCO_3$(Sat.) solution was added to quench the reaction. The crude product was extracted by EtOAc three times. The combined organic phase was washed by brine, dried under sodium sulfate and further condensed under vacuum. The crude was further purified by Combiflash with elute of 0~10% ethyl acetate/hexane to give liquid product. (yield: 42%, 2.66 g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.15 (d, *J* = 1.3 Hz, 1H), 5.61 (d, *J* = 1.6 Hz, 1H), 4.47-4.44 (m, 2H), 4.39 (m, 2H), 2.93 (s, 1H), 1.95 (s, 3H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 167.01, 152.39, 135.74, 126.35, 74.26, 63.69, 61.89, 18.24.

Synthesis of monomer 2:



3-(Trimethylsilyl) propynoic acid (1.00 g, 7.04 mmol), 2-Hydroxyethyl methacrylate (1.01 g, 7.74 mmol) and dicyclohexylcarbodiimide (DCC), (1.45 g, 7.04 mmol) were mixed in cold dichloromethane (50 mL). The solution was kept in ice bath through the time of addition of DMAP (0.43 g, 3.52 mmol) solution (in 2 mL DCM) dropwise. Temperature was brought to r.t. when DMAP solution was finished adding. The system was kept in 40 °C for overnight before checking TLC. The product was purified by precipitating in cold Et_2O to get rid of DHU by product. The crude product was further purified using Combiflash: 0~10% ethyl acetate/hexane to give liquid product. (yield: 52%, 0.94 g). ^{1}H NMR (400 MHz, Chloroform-d) δ 6.15 (d, *J* = 1.3 Hz, 1H), 5.61 (d, *J* = 1.6 Hz, 1H), 4.46-4.43 (m, 4H), 1.96 (s, 3H), 0.25 (s, 9H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 166.84, 152.54, 135.73, 126.12, 94.10, 63.29, 61.99, 18.16, -0.98. ESI-MS m/z calculated for $C_{12}H_{18}O_4Si$+Na+: 277.09; found: 277.13.



Synthesis of polymer P1':

The polymer was prepared through RAFT polymerization[1]. Typical procedure for the polymer P1' with DP=70: in a 10 mL Schlenk flask monomer **2** (770 mg, 2.75 mmol) was added to AIBN (1.29 mg, 0.01 mmol), 4-cyano-4-((thiobenzoyl)-sulfany) pentanoic acid (10.98 mg, 0.04 mmol) and distilled THF (1.4 mL). The solution mixture was then de-gassed using four freeze-pump-thaw cycles. The flask was sealed and immersed in preheated oil bath at 68 °C for 18 hrs. The polymer was precipitated in hexane. The precipitant was dissolved in minimum amount of DCM and

precipitated in hexane. The same procedure was repeated for two more times to afford the pure polymer. The other polymer prepared from monomer **1** was prepared by the same procedure. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.39 (s, 2H), 4.23 (s, 2H), 2.16-1.79 (m, 2H), 1.19-0.84 (m, 3H), 0.29 (s, 8H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 177.88, 176.30, 155.00, 153.39, 100.82, 97.27, 96.06, 94.92, 63.97, 62.89, 54.58, 45.55, 32.56, 31.77, 30.08, 19.93, 17.58, 0.84, 0.00, -0.09.

Synthesis of polymer P1:

Polymer **P1** was prepared through deprotection of **P1'** using modified procedure[2]. Typically, to a degassed solution (acetonitrile/THF) of **P1'** (100 mg, 0.39 mmol), AgF (60 mg, 0.47 mmol) was added in the dark, covering the reaction flask with aluminum foil. The reaction mixture was stirred at room temperature. 1 M HCl (3 equiv.) was added. The mixture was stirred for 10 min. Polymer supernatant was separated by centrifuging out AgCl precipitant. Polymer residue was collected by extensive rinsing precipitant with DCM (twice) and centrifuge. Then polymer was purified by evaporating solvent and precipitating into hexane to get rid of small molecules. Polymer was dried in high vacuum for overnight and gave a yield of 71 mg (99%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.46-4.38 (m, 2H), 4.20 (s, 2H), 3.20 (d, J = 38.9 Hz, 1H), 2.15-1.65 (m, 2H), 1.50-0.71 (m, 3H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 207.81, 178.10, 153.14, 77.52, 75.11, 64.03, 63.07, 55.28, 45.60, 31.77, 19.64, 17.77, 0.83.

Synthesis of polymer P2:

Polymer **P2** was prepared through nucleophilic reaction. Typically, **P1** (63 mg, 0.35 mmol) was dissolved in degassed $CHCl_3$ (2 mL) in a 7 mL vial. Triethylamine (4.82 μL, 0.03 mmol) and 1-thiol octyl (60 μL, 0.35 mmol) was dissolved in a separate vial in $CHCl_3$ and added into the above polymer solution. The reaction was carried out at room temperature for 4 hours. Resulting polymer

was purified by precipitation in methanol (90 mg, 80%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.74 (d, *J* = 14.6 Hz, 1H), 5.79 – 5.69 (m, 1H), 4.34 – 4.29 (m, 4H), 4.20 – 4.14 (m, 2H), 2.79 (d, *J* = 8.2 Hz, 2H), 1.71 – 1.62 (m, 2H), 1.40 (t, *J* = 7.4 Hz, 2H), 1.28 (p, *J* = 6.0, 4.8 Hz, 8H), 1.04 (s, 3H), 0.88 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 177.06, 176.11, 166.21, 164.90, 151.85, 148.42, 112.72, 62.98, 61.31, 54.36, 50.86, 44.64, 36.13, 32.10, 31.80, 30.36, 29.71, 29.16, 28.65, 22.65, 18.62, 16.58, 14.12.

Synthesis of polymer P3-6:

Polymer **P3-6** were prepared through sequential nucleophilic reaction using a stronger base. Typically, **P2** (1.0 equiv.) was dissolved in degassed $CHCl_3$ (30 mg/mL) in a 1 mL vial. TBD (0.1 equiv.) and 2nd thiol reagent (Benzyl mercaptan for **P3**, Cysteamine for **P4**, *tert*-butyl 3-sulfanylpropanoate for **P5'**, TEG-SH for **P6**) (1.1 equiv.) was dissolved in a separate vial in $CHCl_3$ and added into the above polymer solution under argon. The reaction was carried out at room temperature for 4~6 hrs. For polymer **P3** and **P4**, they were designed for monitoring the reaction in NMR tubes, no further purification steps were applied. For polymer **P5'** and **P6**, they were purified by precipitation either in methanol (**P5'**) or hexane (**P6**).

P5': Yield (90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.30-4.16 (m, 5H), 4.20-4.14 (m, 1H), 2.90-2.82 (m, 2H), 2.66-2.53 (m, 3H), 1.82 (m, 2H), 1.67-1.56 (m, 2H), 1.44 (s, 8H), 1.36-1.26 (m, 10H), 1.02 (m, 2H), 0.88-0.85 (m, 3H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 176.79, 170.92, 169.72, 80.74, 61.89, 44.62, 41.39, 35.67, 31.82, 30.52, 29.21, 29.07, 25.82, 22.65, 14.14.

P6: Yield (77%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.34-4.29 (m, 4H), 4.20-4.14 (m, 1H), 3.66-3.64 (m, 11H), 3.56-3.55 (m, 2H), 3.38 (s, 3H), 2.90-2.82 (m, 3H), 2.71-2.60 (m, 2H), 1.83 (m, 2H), 1.69-1.57 (m, 4H), 1.37-1.28 (m, 10H), 1.04 (m, 2H), 0.88 (m, 3H). ^{13}C NMR (500 MHz,

Chloroform-*d*) δ 177.02, 176.07, 169.77, 164.85, 148.24, 112.87, 71.94, 70.61, 70.32, 62.56, 61.82, 61.02, 59.04, 47.36, 44.69, 44.59, 41.19, 31.84, 30.47, 30.01, 29.70, 29.23, 28.85, 22.67, 14.16.

P5 was prepared by deprotecting the *tert*-butyl group using TFA. Typically, **P5'** (41 mg, 0.08 mmol) was dissolved in degassed $CHCl_3$ (1.2 mL) in a 7 mL vial. TFA solution (0.8 mL in 1.2 mL of DCM) was added into the above solution. Argon was purged for 20min. Then the reaction was carried out at room temperature for 4 hrs. The resulting polymer **P5** was purified by evaporating the TFA and byproduct to give a yield of 36.4 mg (92%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.33-4.19 (m, 5H), 2.91-2.59 (m, 5H), 1.88 (m, 2H), 1.68-1.58 (m, 2H), 1.37-1.27 (m, 10H), 1.05 (m, 2H), 0.88-0.86 (m, 3H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 176.74, 62.66, 31.79, 29.17, 22.63, 14.10.

GPC profiles for first additions:

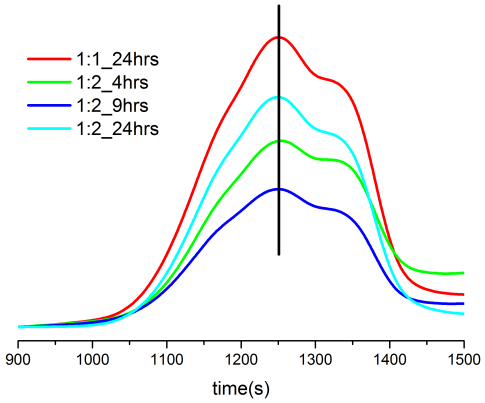


Figure S1. GPC profiles of polymer **P2** with longer reaction time and more thiol reagents under TEA base catalyst.

NMR spectrum and GPC profiles for sequential additions:

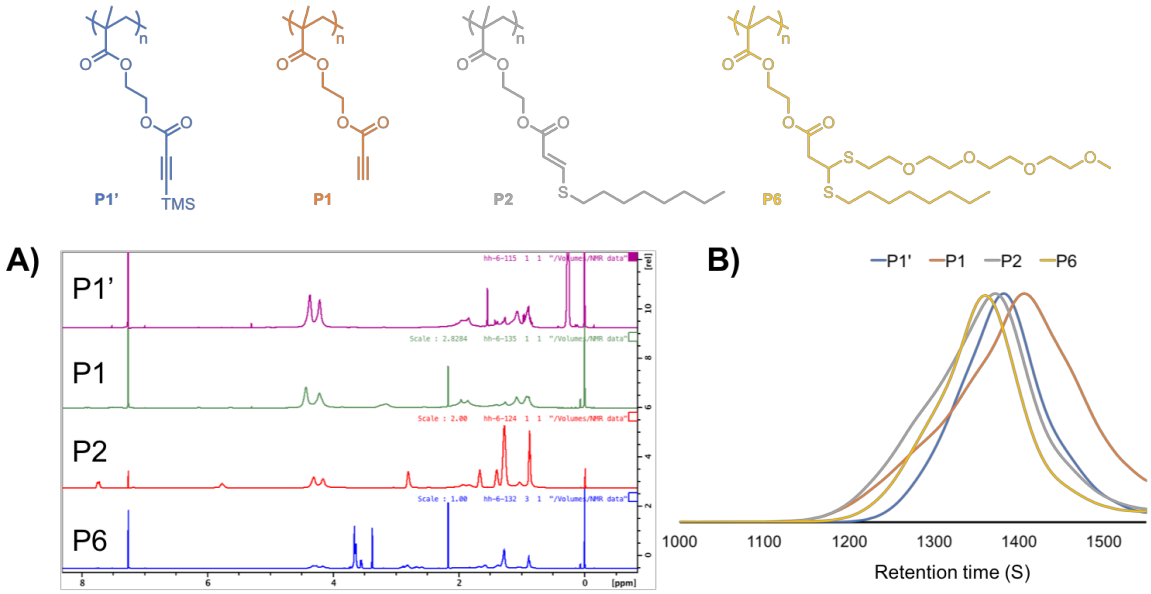


Figure S2. ^{1}H NMR spectrum (A) and GPC profiles (B) of four steps of polymer **P1'**, **P1**, **P2** and **P6**.

Micelle preparation:

Polymer (10 mg) was dissolved in minimum amount of acetone. Distilled water (2 mL) was added to the above solution. The acetone was then dialysis out against water for two days. The exact concentration of micelle solution was determined by lipolyzing certain amount of solution and measure the weight of solid residue. Typically, the final concentration of **P6** micelle solution is 3.5 mg/mL.

Reverse micelle preparation:

Reverse micelle was prepared following the previous literature. 1mg of polymer was dissolved in toluene to make 1 mg/mL concentration solution. Different amount of $NaHCO_3$ (0.1M pH=8.2) solution was added into toluene solution in order to deprotonate COOH and make them more hydrophilic. This helps to make water pool inside reserve micelle. Sonication was applied at least 3 h until a homogenous solution was obtained.

Peptide extraction experiment:

One micromolar of each peptide was dissolved in MOPS buffer of pH 7. Two hundred microliters of the reverse micelle solution made from polymer **P5** was added to 1 mL of the peptide solution. Extraction was done under vigorous vortex for 2 h. Centrifugation at 14 000 rcf for 20 min was followed to separate the two phases. The aqueous phase was removed, and the organic phase was dried by blowing N_2 gas. Ten microliters of aqueous solution was taken and mixed with 10 µL of a DHB matrix solution (25 mg/mL in 70% (v/v) acetonitrile containing 1% (v/v) TFA). The dried organic residue was re-dissolved in 20 µL of THF and mixed with 30 µL of the matrix solution. One microliter of this solution was spotted on the matrix-assisted laser desorption/ionization (MALDI) target for analysis.

MALDI-MS analyses were performed on a Bruker Autoflex III time-of-flight mass spectrometer. All mass spectra were obtained in positive mode and represent an average of 300 shots acquired at 40% laser power with an accelerating voltage of 19kV.

DLS of P5 aggregates:

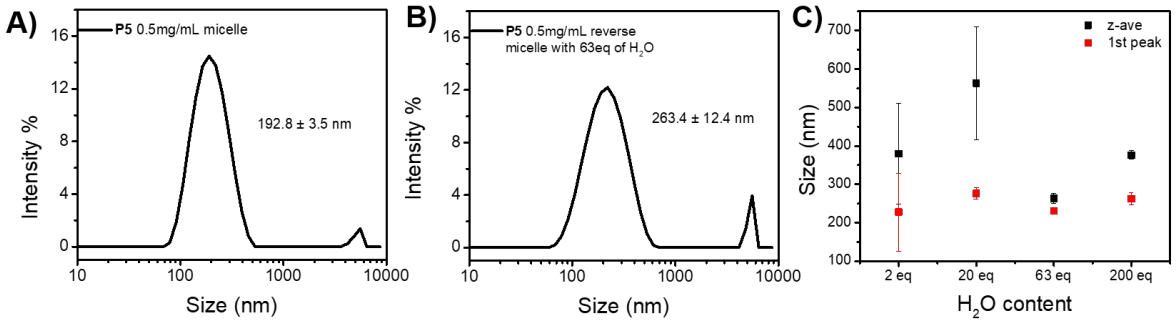


Figure S3. Size distribution of micelle (A) and reverse micelle (B) made from polymer **P5**. (C) Size distribution of reverse micelle with different equivalents of H_2O content addition respect to COOH group.

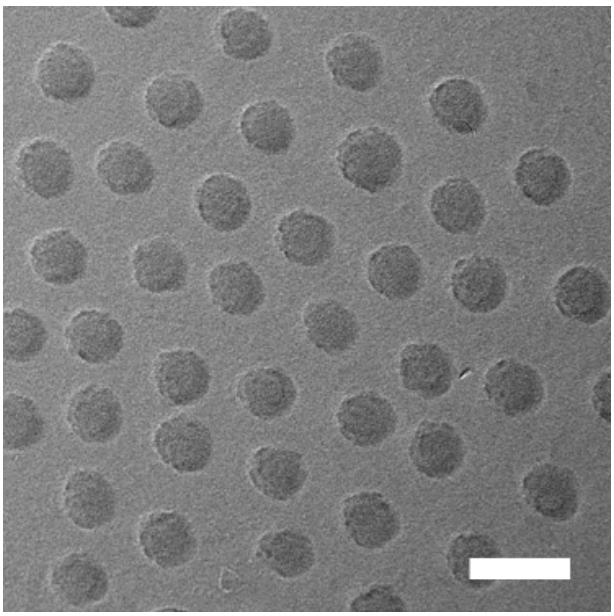


Fig. S4. Enlarged TEM image of the inset in **Fig. 5B.** (Scale bar = 100 nm)

Time dependent DLS and TEM measurements of P6 in response to H_2O_2:

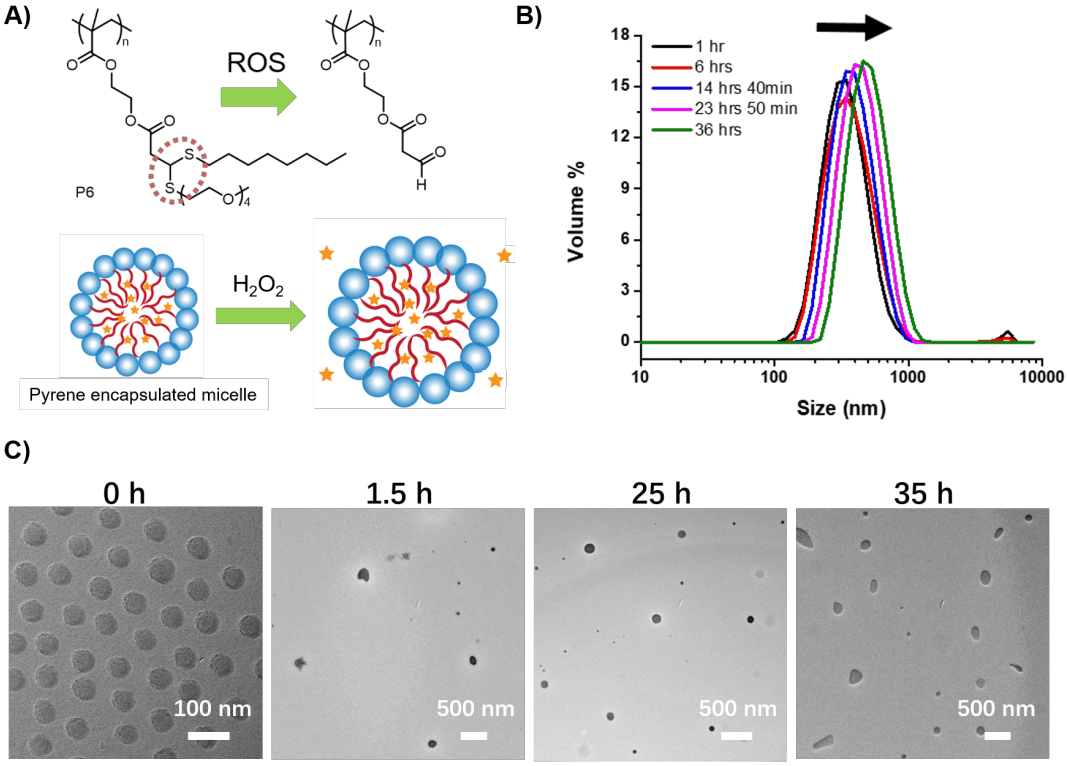


Figure S5. (A) Schematic representative of polymer **P6** degrade in response to ROS. (B) Size distribution changes of micelle made from polymer **P6** in response to H_2O_2. (C) Time dependent TEM images of the nanoaggregate after the treatment of H_2O_2.

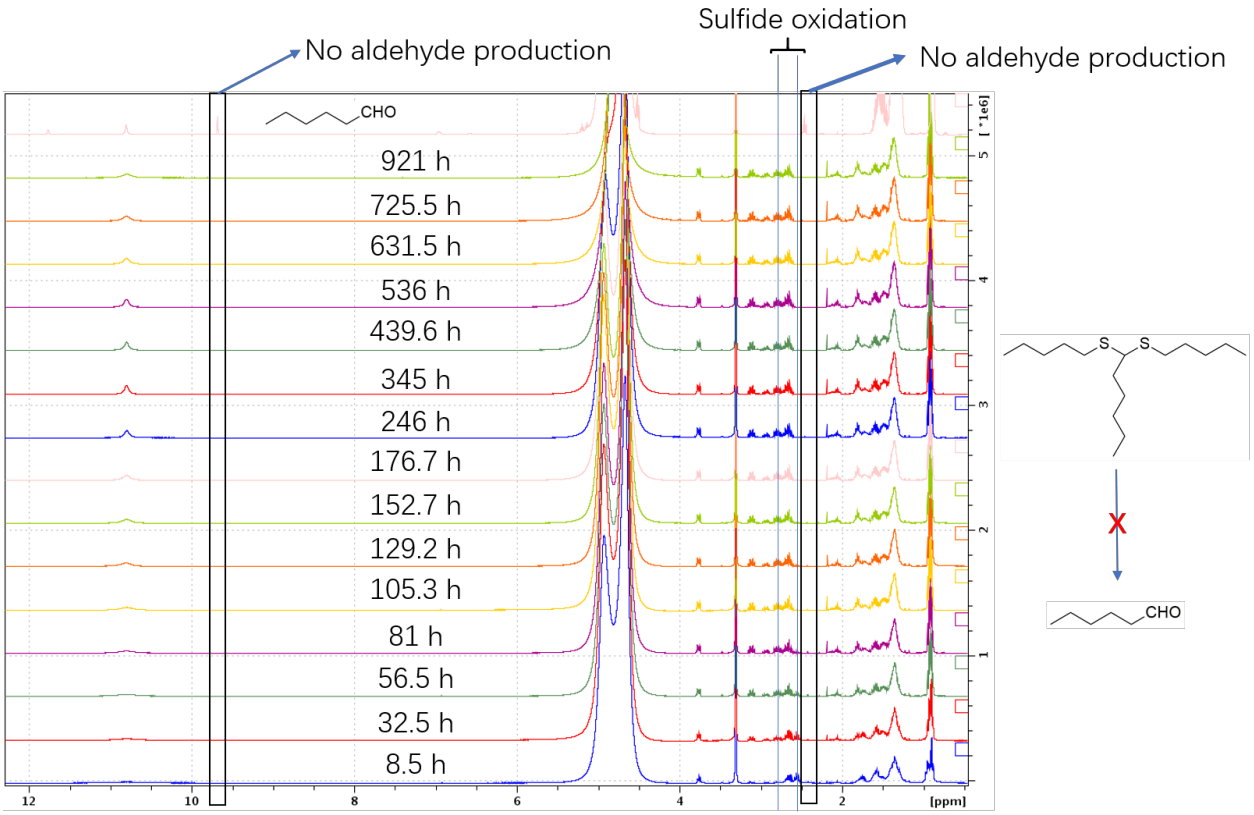


Fig. S6. NMR spectra of thioacetal under H_2O_2 treatment. The thioacetal can be oxidized but cannot be further degraded to produce the aldehyde.

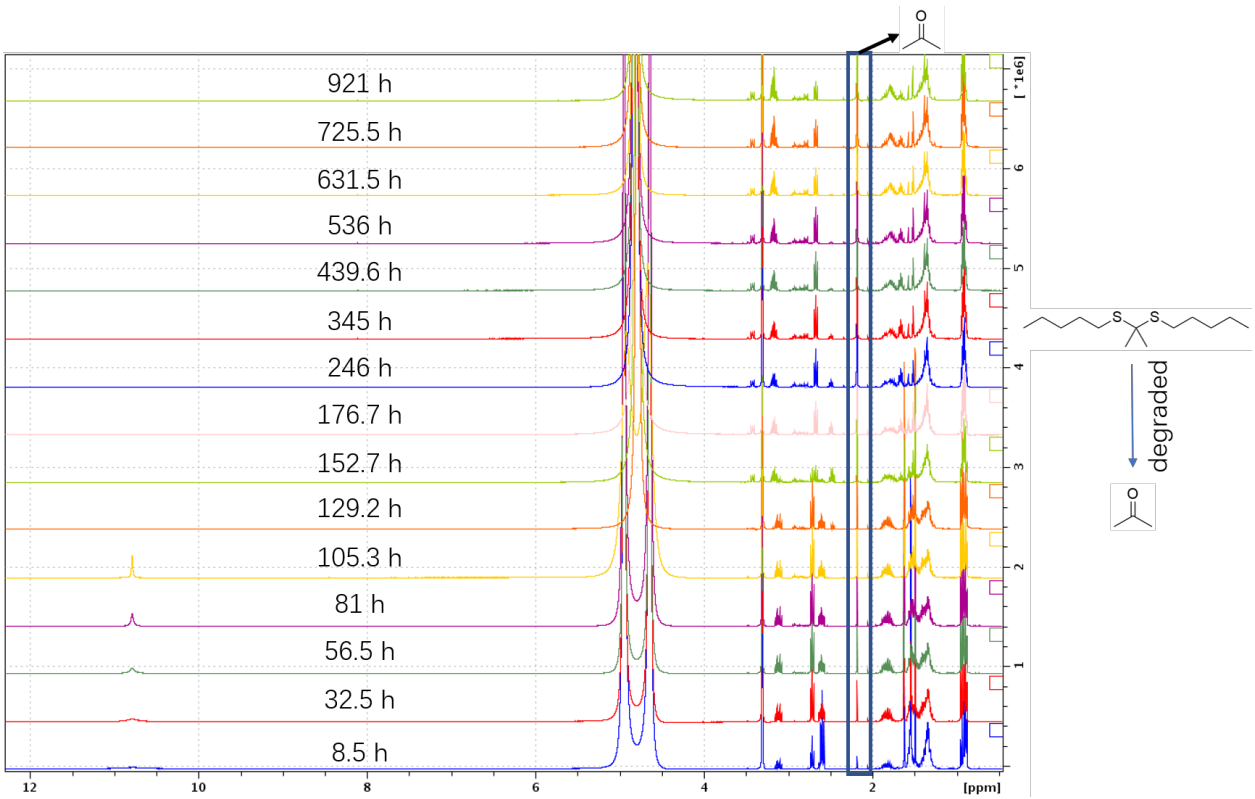


Fig. S7. NMR spectra of thioketal under H_2O_2 treatment. The thioketal can be degraded to produce the ketone.

NMR spectra:

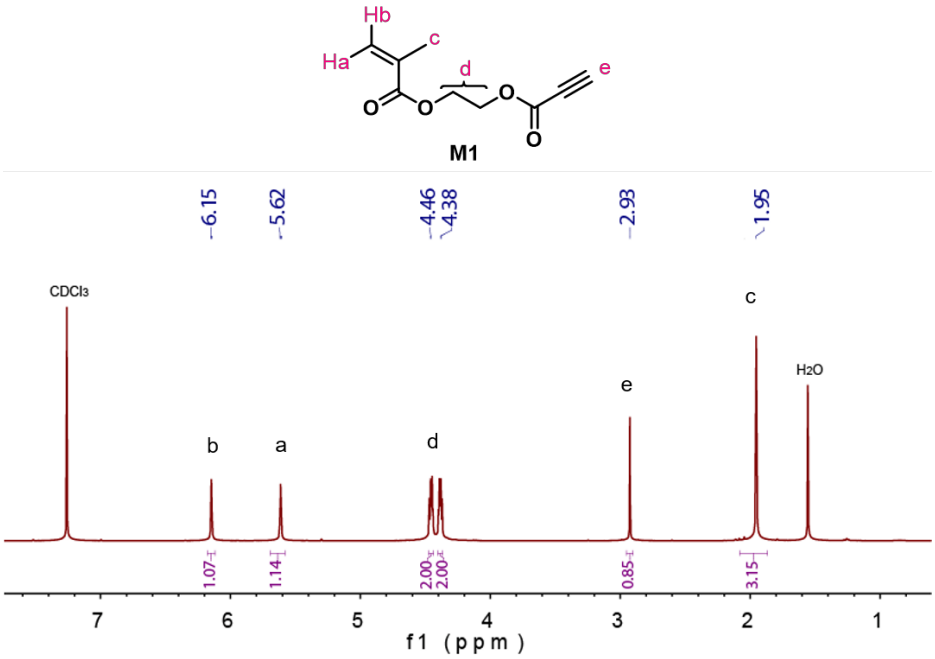


Figure S8. [1]H NMR spectrum of monomer **1**. Solvent: $CDCl_3$.

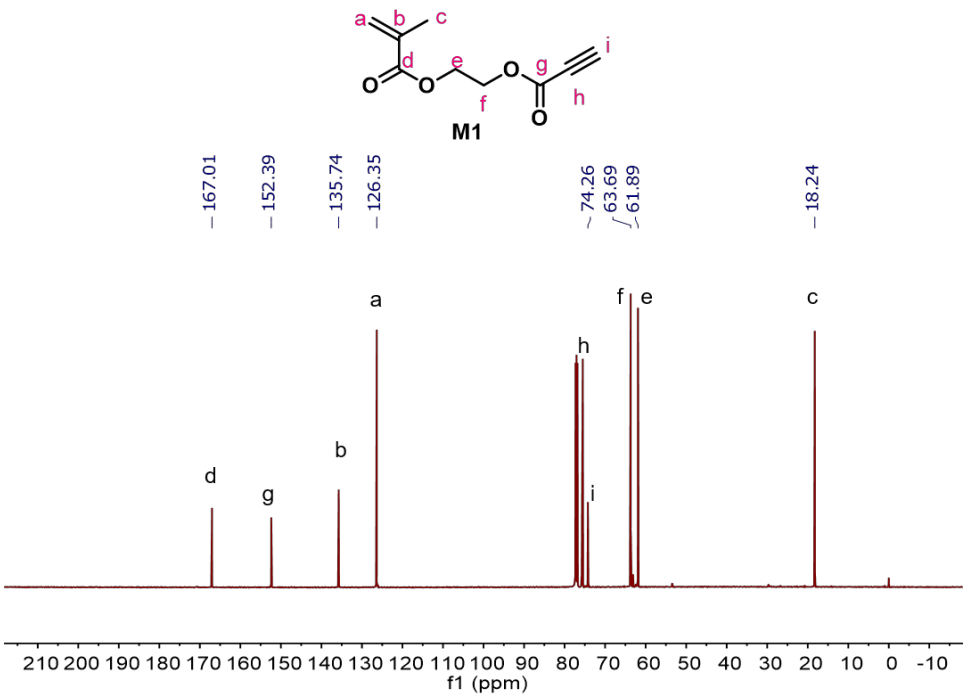


Figure S9. [13]C NMR spectrum of monomer **1**. Solvent: $CDCl_3$

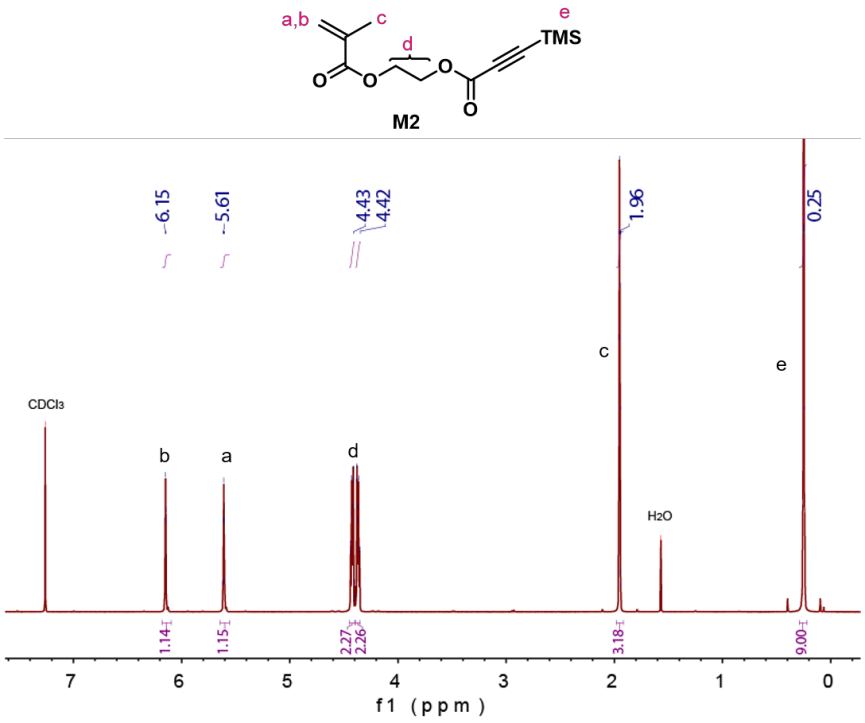


Figure S10. ^{1}H NMR spectrum of monomer **2**. Solvent: $CDCl_3$.

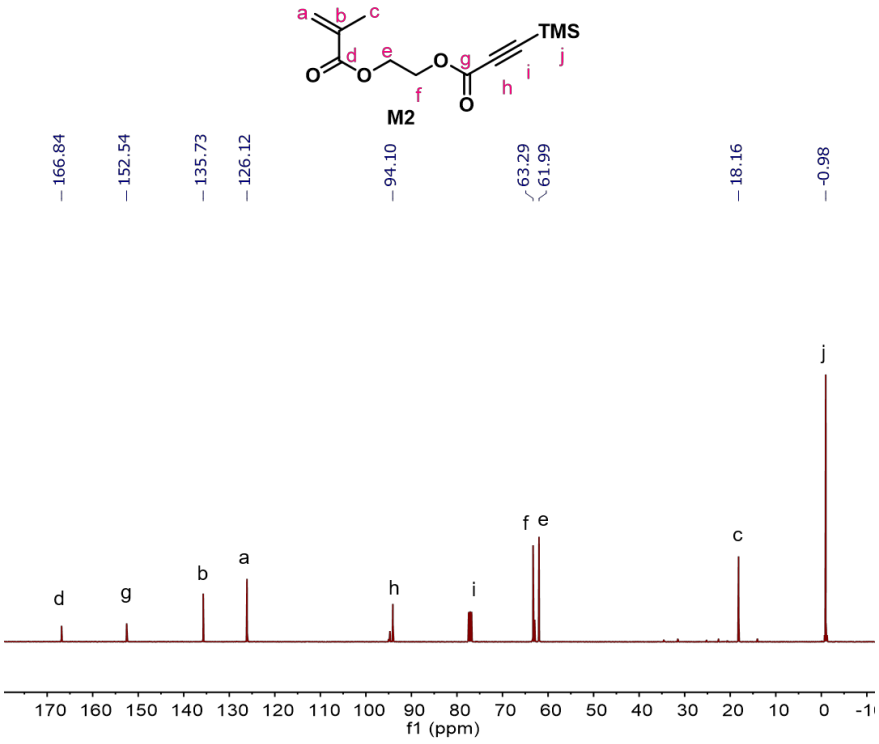


Figure S11. ^{13}C NMR spectrum of monomer **2**. Solvent: $CDCl_3$

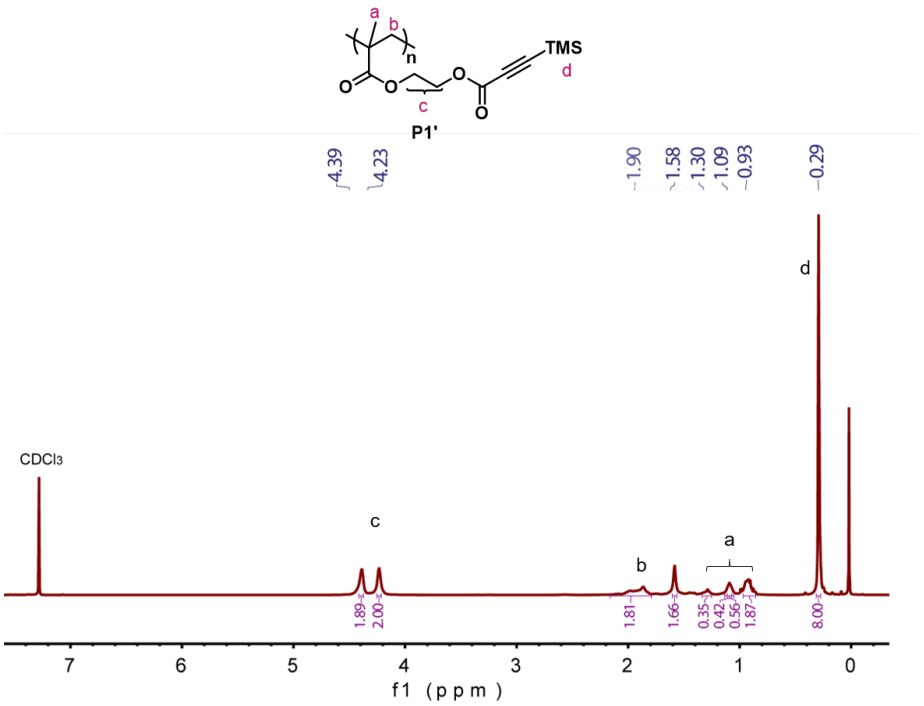


Figure S12. ^{1}H NMR spectrum of polymer **P1'**. Solvent: $CDCl_3$.

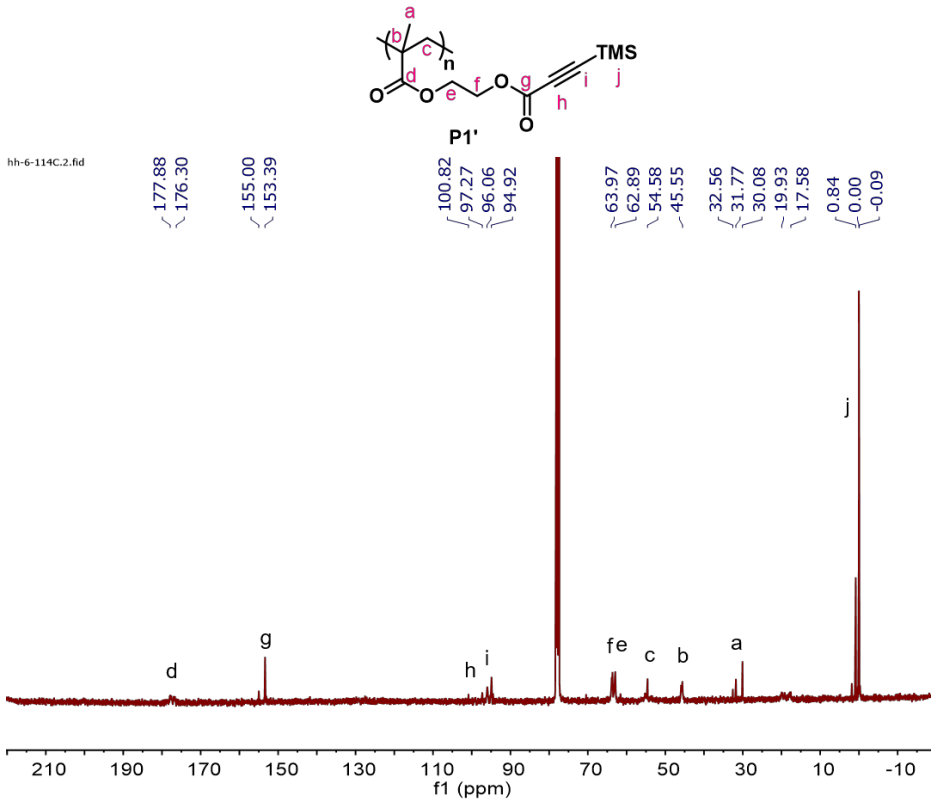


Figure S13. ^{13}C NMR spectrum of polymer **P1'**. Solvent: $CDCl_3$.

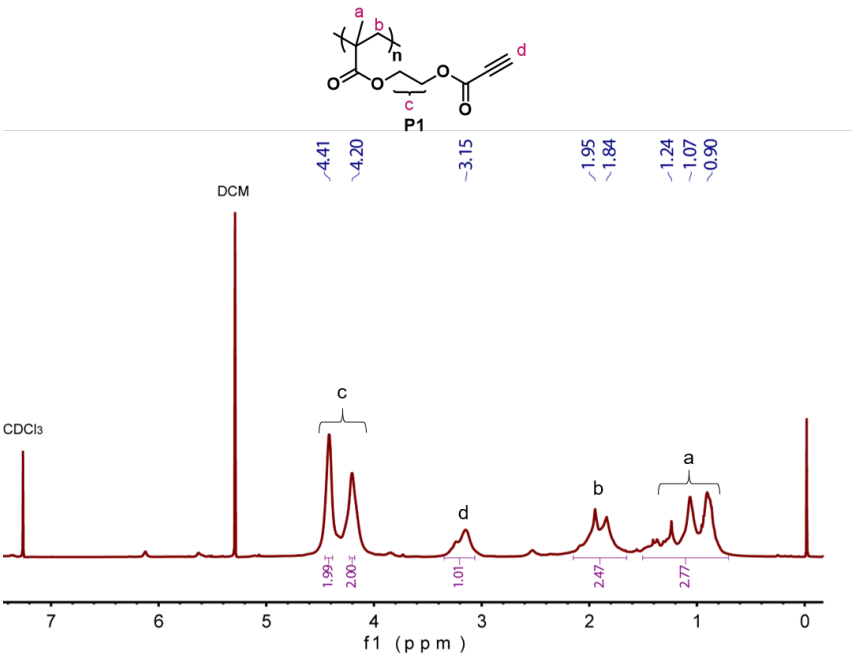


Figure S14. ^{1}H NMR spectrum of polymer **P1**. Solvent: $CDCl_3$.

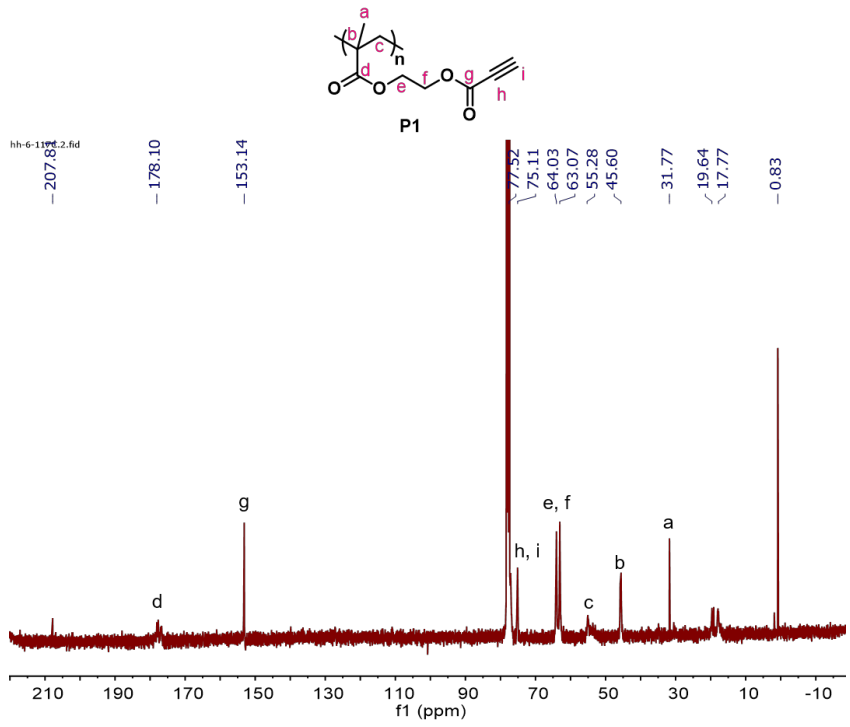


Figure S15. ^{13}C NMR spectrum of polymer **P1**. Solvent: $CDCl_3$.

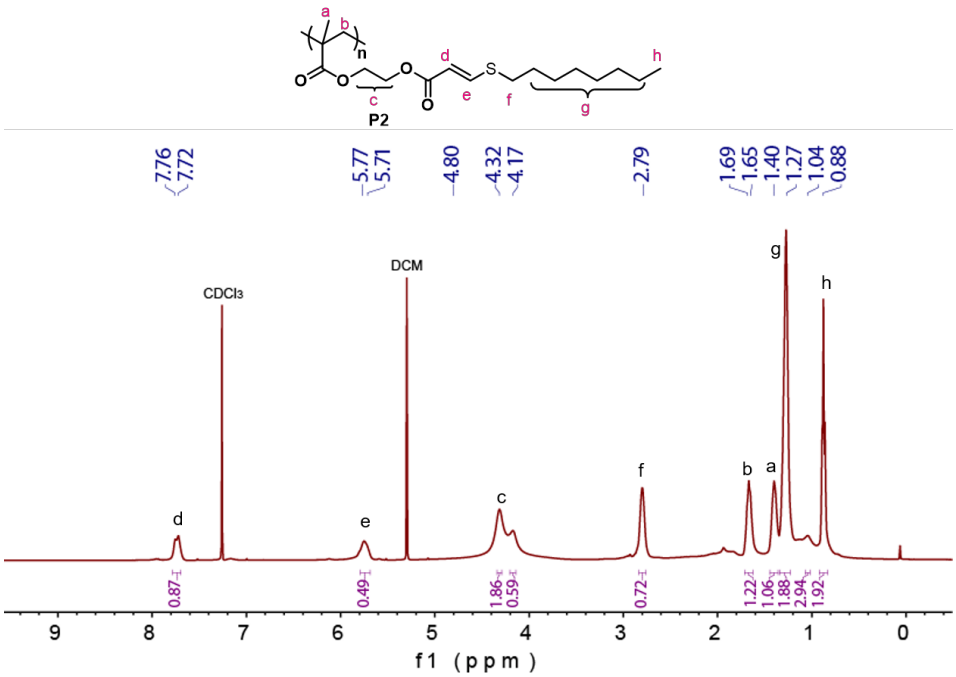


Figure S16. ^{1}H NMR spectrum of polymer **P2**. Solvent: $CDCl_3$.

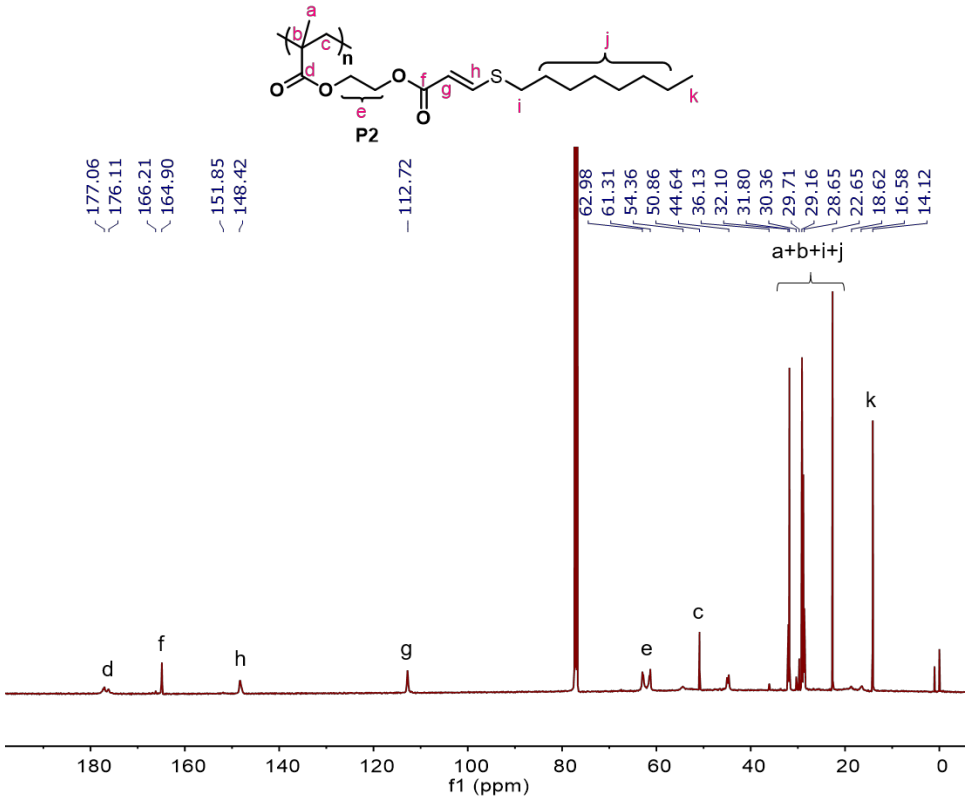


Figure S17. ^{13}C NMR spectrum of polymer **P2**. Solvent: $CDCl_3$.

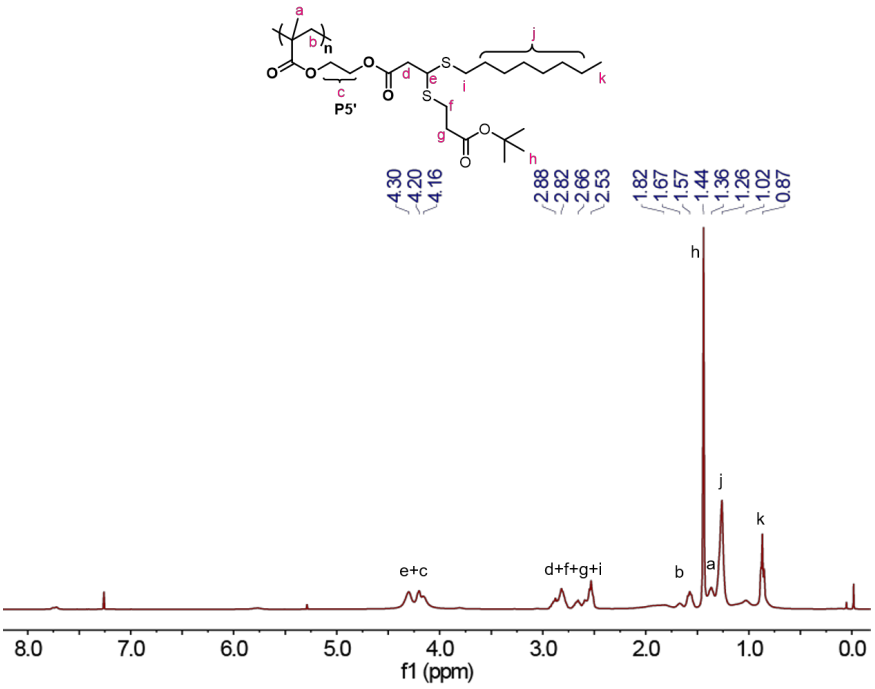


Figure S18. ^{1}H NMR spectrum of polymer **P5'**. Solvent: $CDCl_3$.

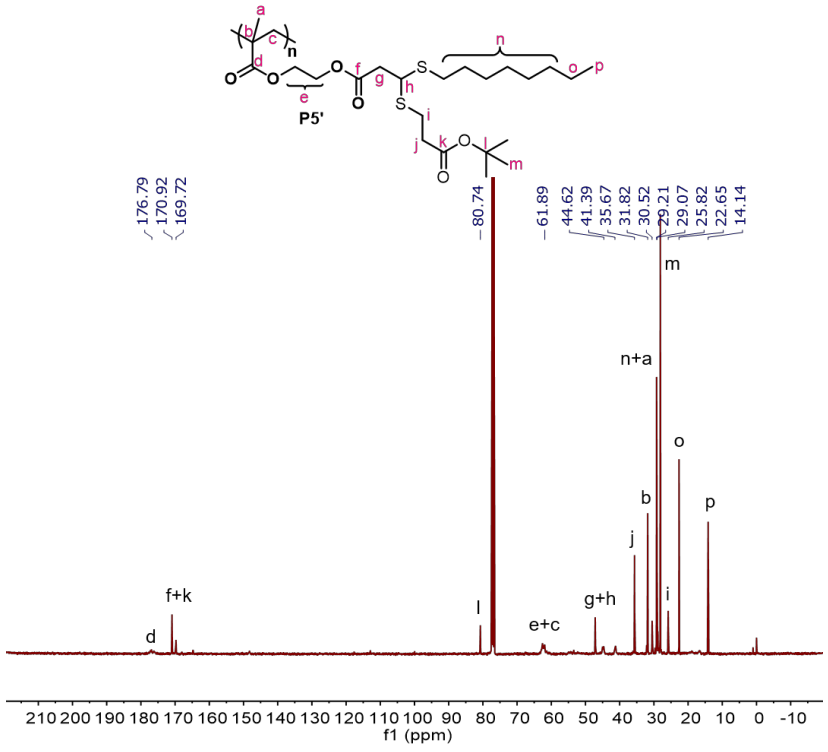


Figure S19. ^{13}C NMR spectrum of polymer **P5'**. Solvent: $CDCl_3$.

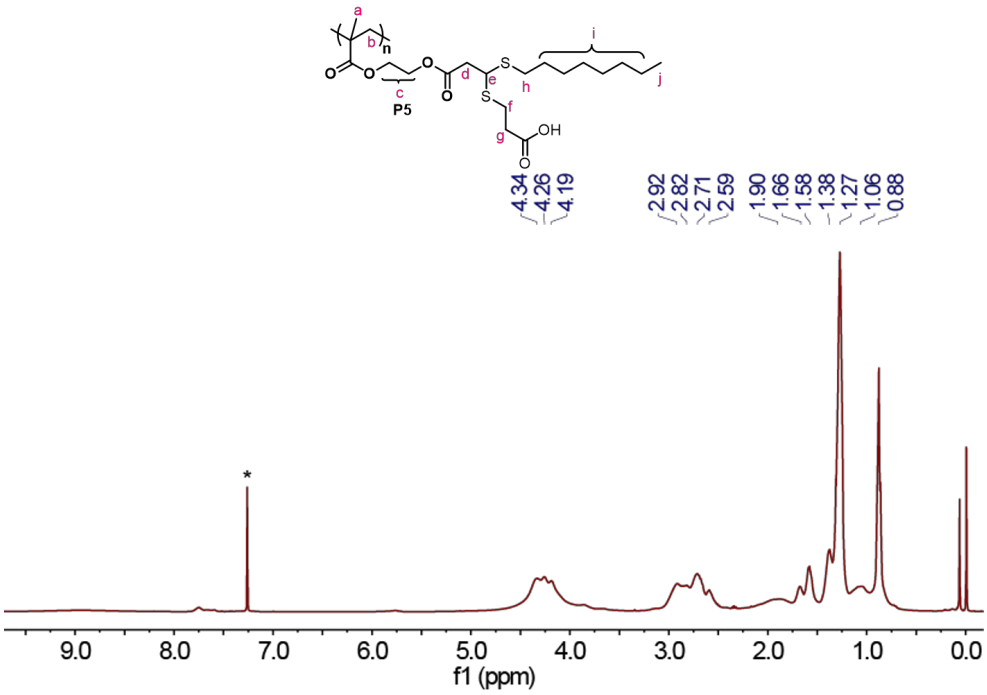


Figure S20. ^{1}H NMR spectrum of polymer **P5**. Solvent: $CDCl_3$.

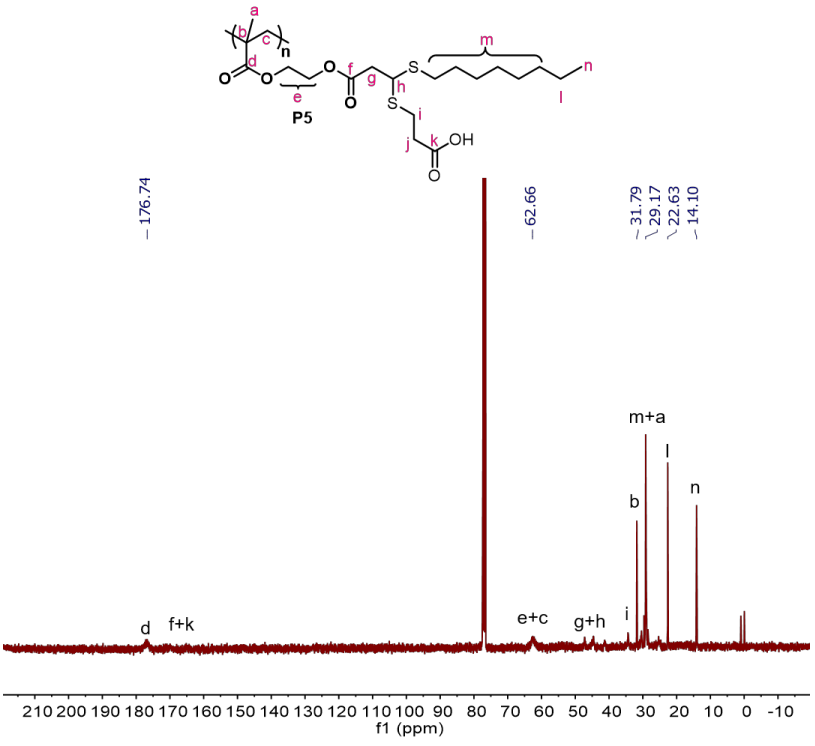


Figure S21. ^{13}C NMR spectrum of polymer **P5**. Solvent: $CDCl_3$

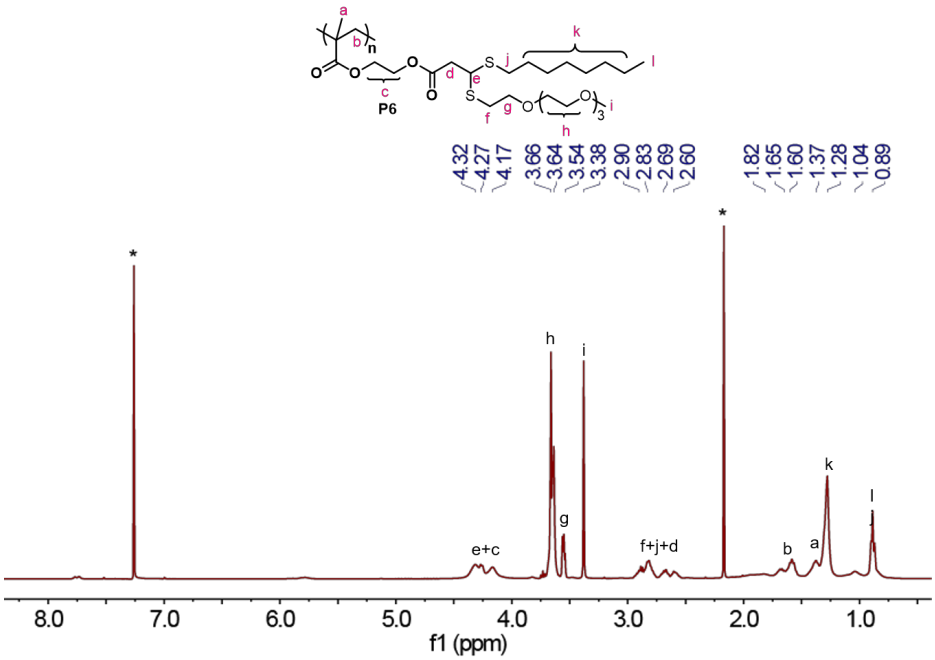


Figure S22. ^{1}H NMR spectrum of polymer **P6**. Solvent: $CDCl_3$. * indicated solvent peak: $CHCl_3$ (7.26ppm), Acetone (2.17ppm).

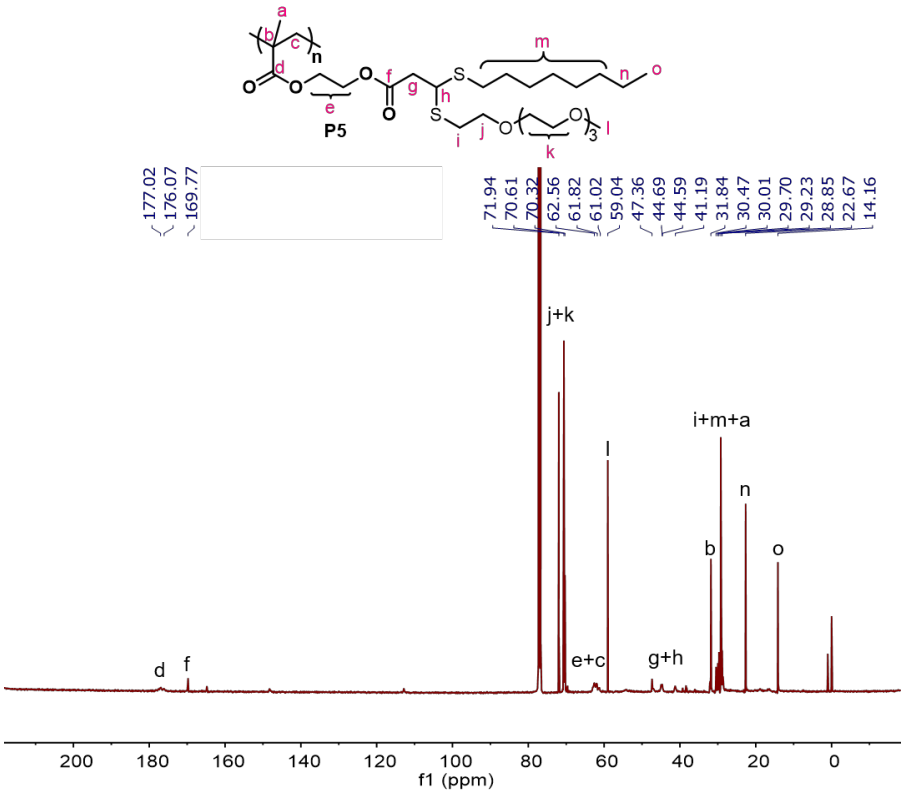


Figure S23. ^{13}C NMR spectrum of polymer **P6**. Solvent: $CDCl_3$

TGA graphs:

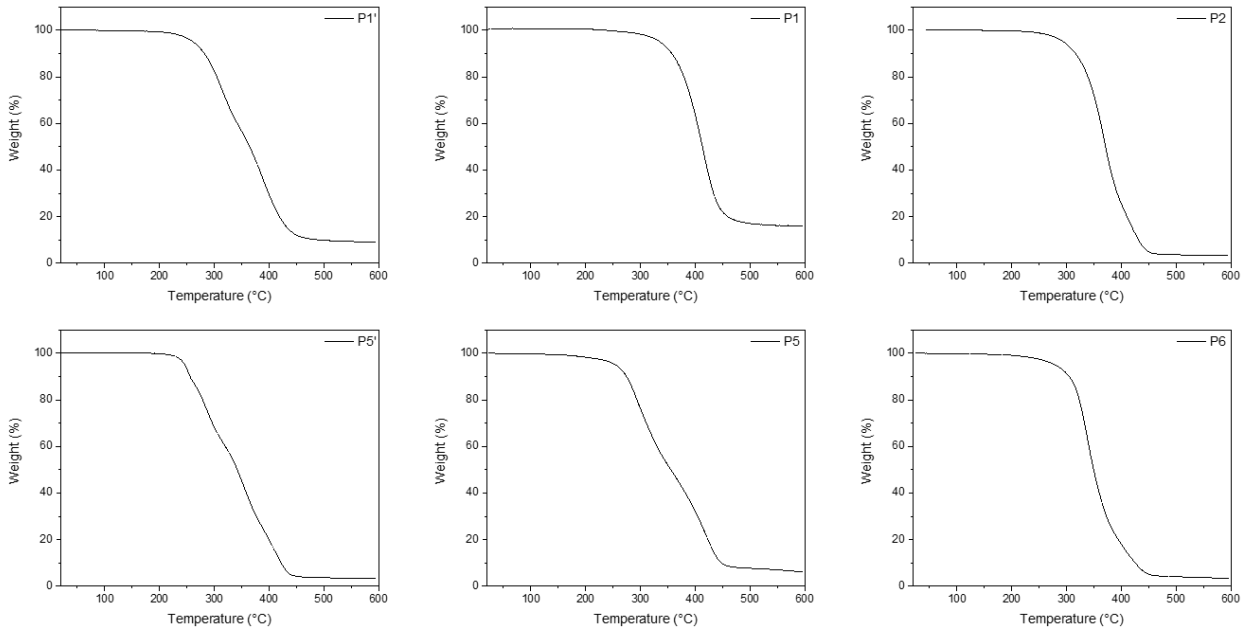


Figure S24. TGA thermographs of six polymers from top to bottom and left to right: **P1'**, **P1**, **P2**, **P5'**, **P5** and **P6**.

Reference:

(1) Liu, B.; Thayumanavan, S. Substituent Effects on the pH Sensitivity of Acetals and Ketals and Their Correlation with Encapsulation Stability in Polymeric Nanogels. *J. Am. Chem. Soc.* **2017**, *139* (6), 2306–2317 DOI: 10.1021/jacs.6b11181.

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