

RAFT synthesis of poly(vinylpyrrolidone) amine and preparation of a water-soluble C₆₀-PVP conjugate

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Supporting Information

Table of Content

| | |
|--|----|
| I. Kinetic Study of the polymerization | 2 |
| I-1. Preparation of the reaction mixture | 2 |
| I-2. Determination of the conversion by ¹ H-NMR | 2 |
| I-3. Determination of M _n by ¹ H-NMR | 4 |
| I-4. Determination of M _n and M _w by GPC | 7 |
| II. Synthesis | 8 |
| II-1. Synthesis of the RAFT agent | 8 |
| II-2. Synthesis of PVP polymer | 15 |

General. NMR spectra were recorded on Varian 300 spectrometer, Bruker 400 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). FT-IR spectra were recorded on JASCO FT-IR-4100 (JASCO Co., Tokyo, Japan) and PerkinElmer Spectrum One FT-IR Spectrometer with Universal ATR Sampling Accessory (PerkinElmer Inc., Waltham, MA, USA). UV-vis-NIR were recorded on JASCO V-570 spectrophotometer (JASCO Co.). All the solvents used are HPLC grade and were purchased from Acros Organic (Thermo Fischer Scientific, Inc., Geel, Belgium). DLS were recorded on Zetasized Nano S (Malvern) using a fresh disposable cuvette (half-mikro PS, 1.6 mL, VWR Int.). All the reagents were purchased from corresponding suppliers and purified as described when needed. GPC analysis was carried out on JASCO PU-2080 Plus HPLC pump, JASCO MD-2018 Plus UV detector, JASCO RI02031 Plus RI detector, ChromNAV Chromatography Data System (JASCO Co.). Four Phenogel columns are linearly connected: linear (2), 104 Å, 103 Å, 500 Å columns (5µm, 300 mm x 7.8 mm) (Phenomenex, Torrance CA, USA) using PMMA for calibration and DMF with 0.1% LiBr as mobile phase at 60°C inside JASCO CO-2065 Plus column oven (JASCO Co.). ~1mM specimen was vitrified using a plunge-freezer (Gatan, USA). CryoTEM images were recorded by JEM2200FS transmission electron microscope (JEOL, Japan) with a F416 CMOS camera (TVIPS, Germany) at the nominal magnification of 20,000 at the accelerating voltage of 200kV and the 3-5 µm underfocus.

I. Kinetic Study of the polymerization

I-1. Preparation of the reaction mixture

A solution of NVP (13.9 g, 13.3 mL, 120.0 mmol, conc. = 9.38 mol/L) containing AIBN (7.9 mg, 48.1 μ mol) and **5** (84.8 mg, 217.7 μ mol) was placed in a schlenk flask and degassed with four freeze-evacuate-thaw cycles and heated under nitrogen at 60 °C. An aliquot was taken after 0.5, 1.0, 1.5, 2.0 hrs and few drops were transferred to a CDCl₃ solution to measure the conversion. The remaining aliquot was diluted with CHCl₃ and precipitated twice in Et₂O (2 x 15 mL). The obtained white amorphous solid was dried under vacuum and used for ¹H-NMR and GPC measurements.

I-2. Determination of the conversion by ¹H-NMR

Conversion was determined by using the vinyl protons (δ = 4.33 ppm, 2H) as a standard reference. Estimation of monomer / polymer ratio was calculated from the integration between δ 1.0 - 4.0 ppm, which correspond to the protons of both the NVP monomer (pyrrolidone backbone protons, 6 protons) and PVP polymer (9 protons per unit).

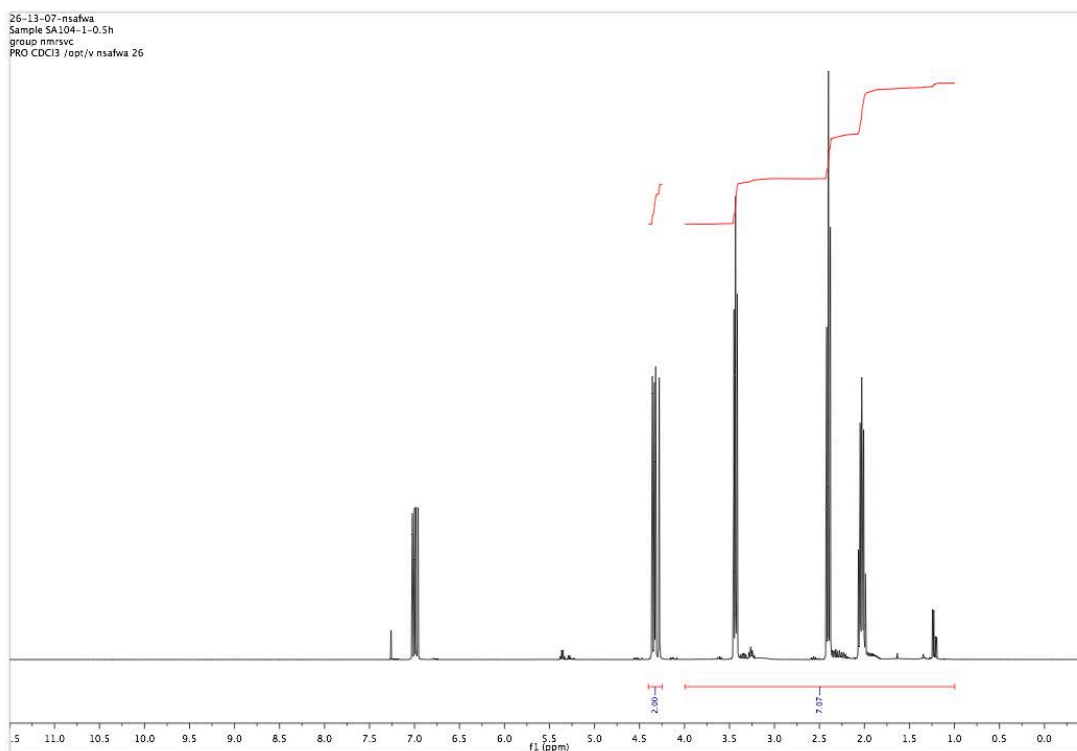


Figure S1. ¹H-NMR of the reaction mixture after 30 min of reaction time (CDCl₃, 300 MHz).

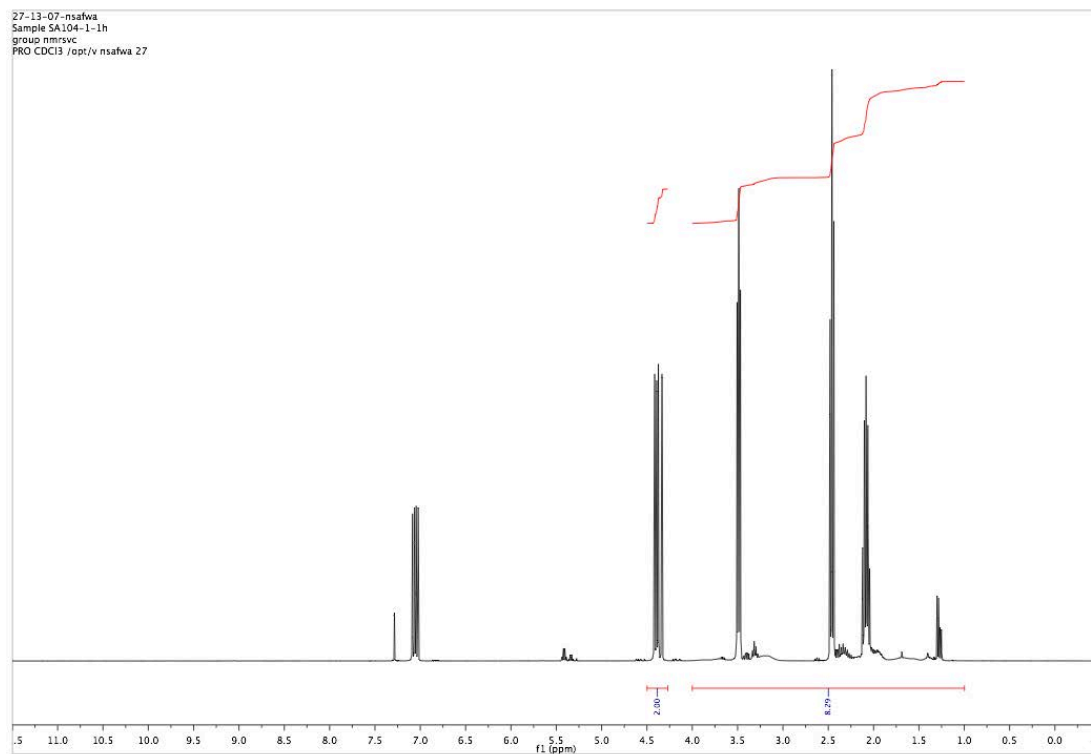


Figure S2 ^1H -NMR of the reaction mixture after 1 h of reaction time (CDCl_3 , 300 MHz).

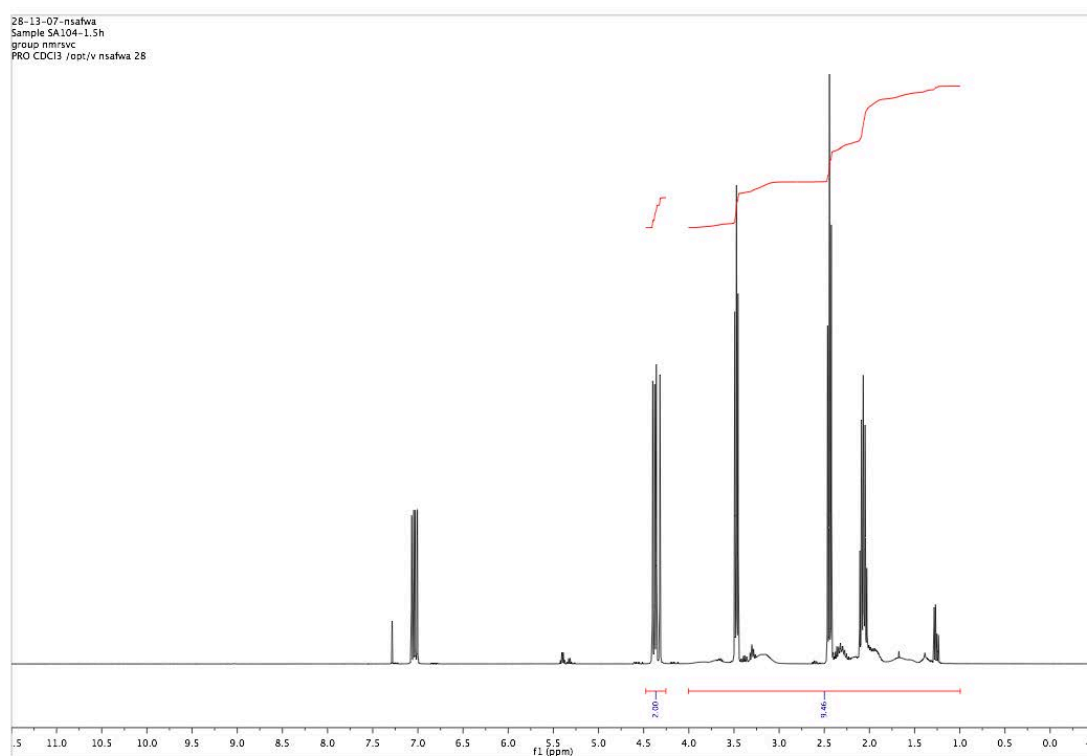


Figure S3. ^1H -NMR of the reaction mixture after 1.5 h of reaction time (CDCl_3 , 300 MHz).

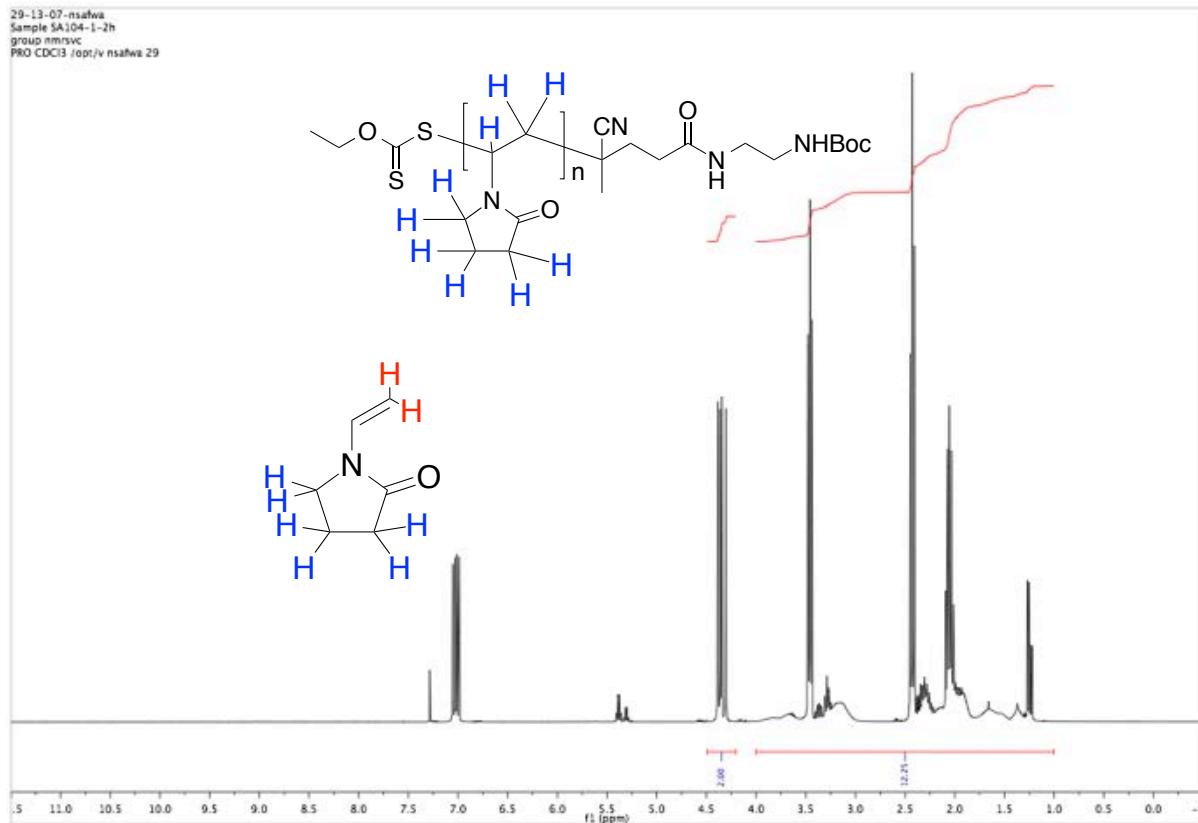


Figure S4. $^1\text{H-NMR}$ of the reaction mixture after 2 h of reaction time (CDCl_3 , 300 MHz).

I-3. Determination of M_n by $^1\text{H-NMR}$

Molecular weight M_n was estimated using a methylene in xanthate end-group ($\text{CH}_3\text{-CH}_2\text{-O-(C=S)-S}$, $\delta 4.60$ ppm, 2H) as a standard. Protons corresponding to the PVP polymer (3H, in red, **Figure S5**) $\delta 3.0 - 4.1$ ppm were compared relative to a CH_2 in xanthate end-group. Degree of polymerization was calculated as follows:

$$DP_n = \left[\frac{\text{Integration}_{3.0-4.1 \text{ ppm}}}{3} - \frac{\text{Integration}_{4.55-4.70 \text{ ppm}}}{2} \right]$$

Molecular weight M_n was calculated as follows:

$$M_n = DP_n \cdot MW_{NVP}$$

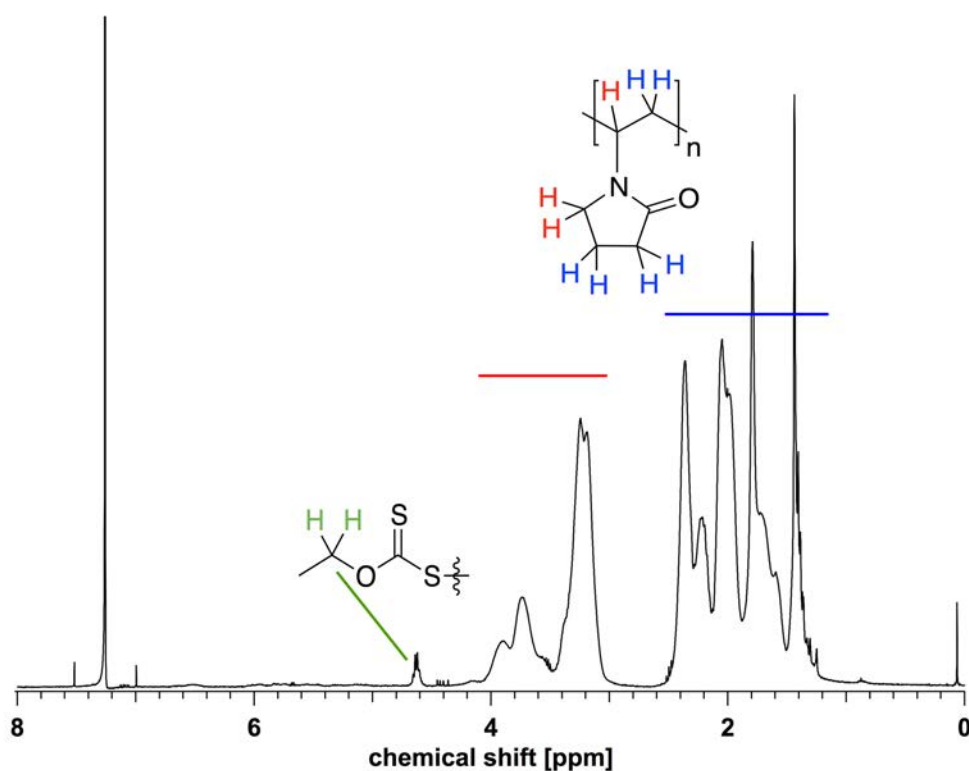


Figure S5. Typical ^1H NMR spectrum used for the calculation of the molecular weight M_n (xanthate end group used as a reference).

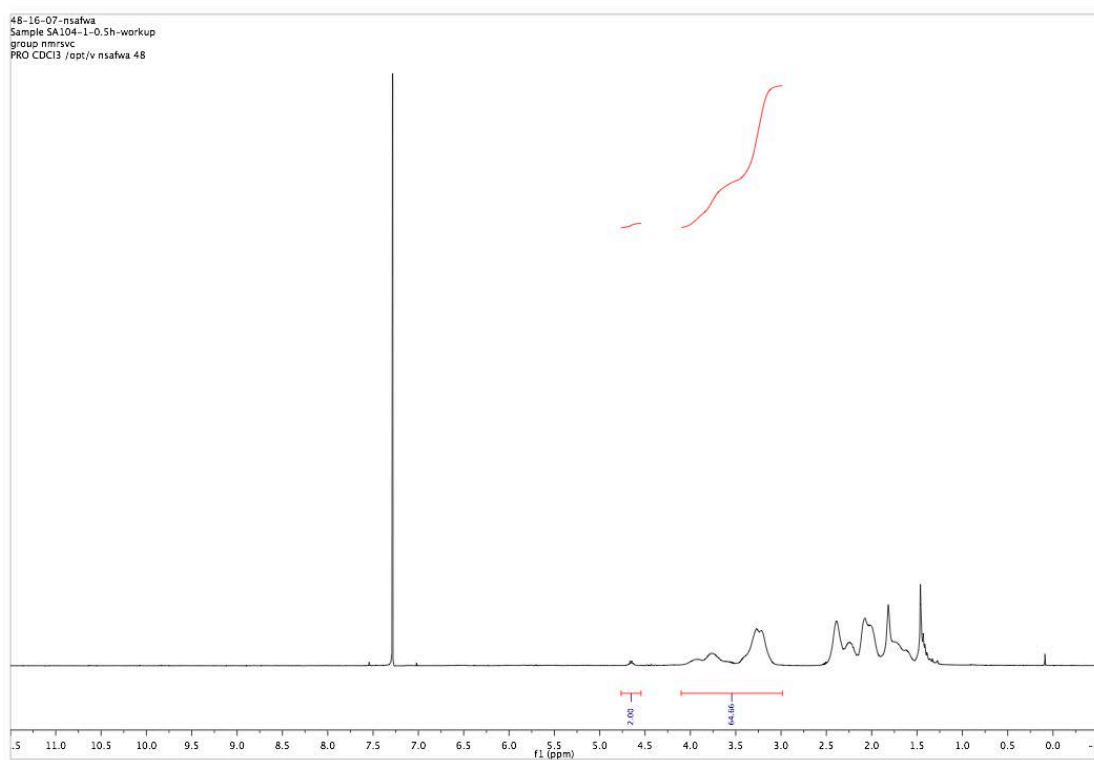


Figure S6. ^1H -NMR of the precipitated mixture after 30 min of reaction time (CDCl_3 , 300 MHz).

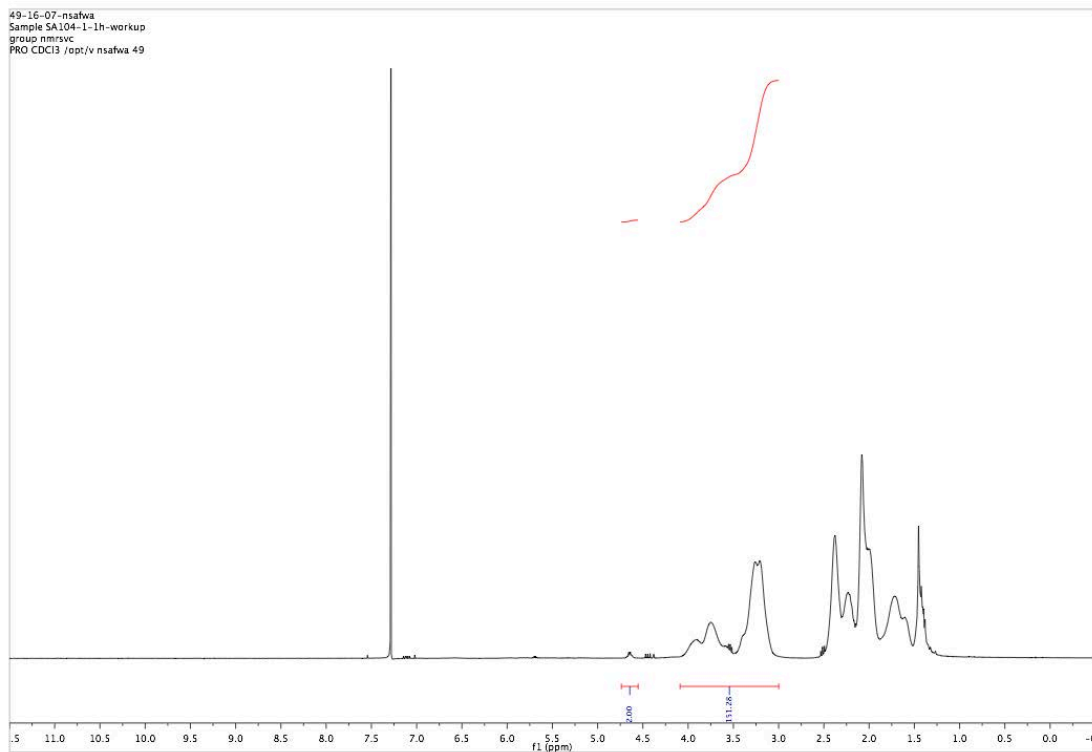


Figure S7. ^1H -NMR of the precipitated mixture after 1 h of reaction time (CDCl_3 , 300 MHz).

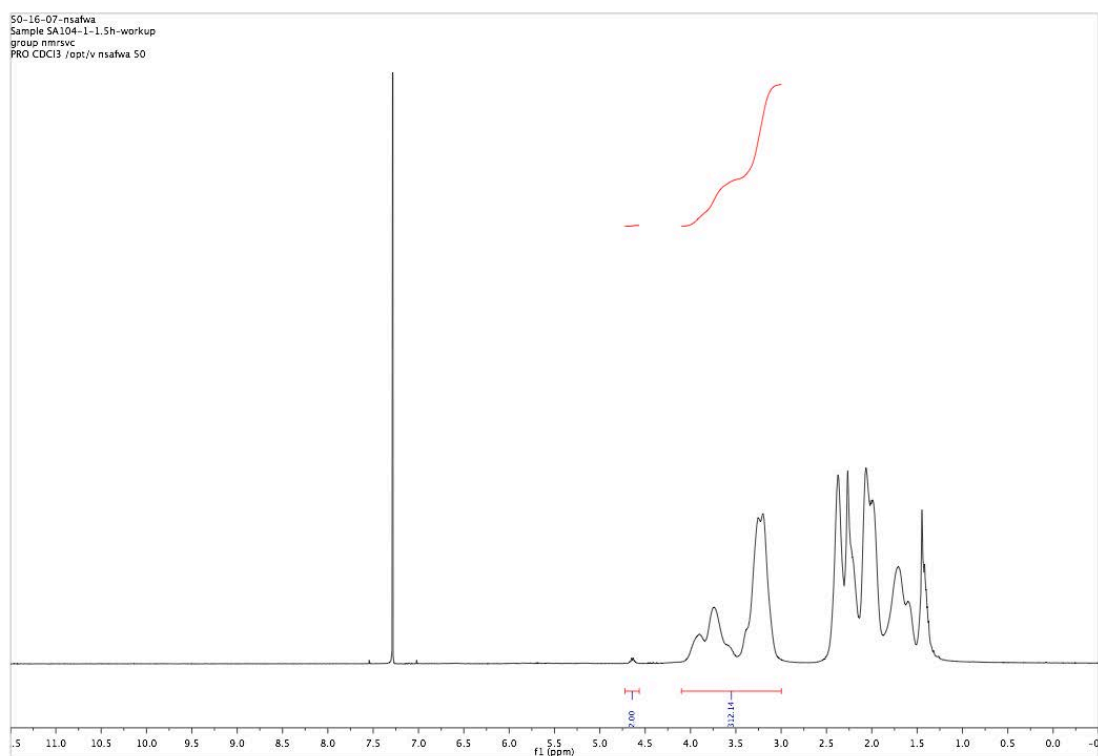


Figure S8. ^1H -NMR of the precipitated mixture after 1.5 h of reaction time (CDCl_3 , 300 MHz).

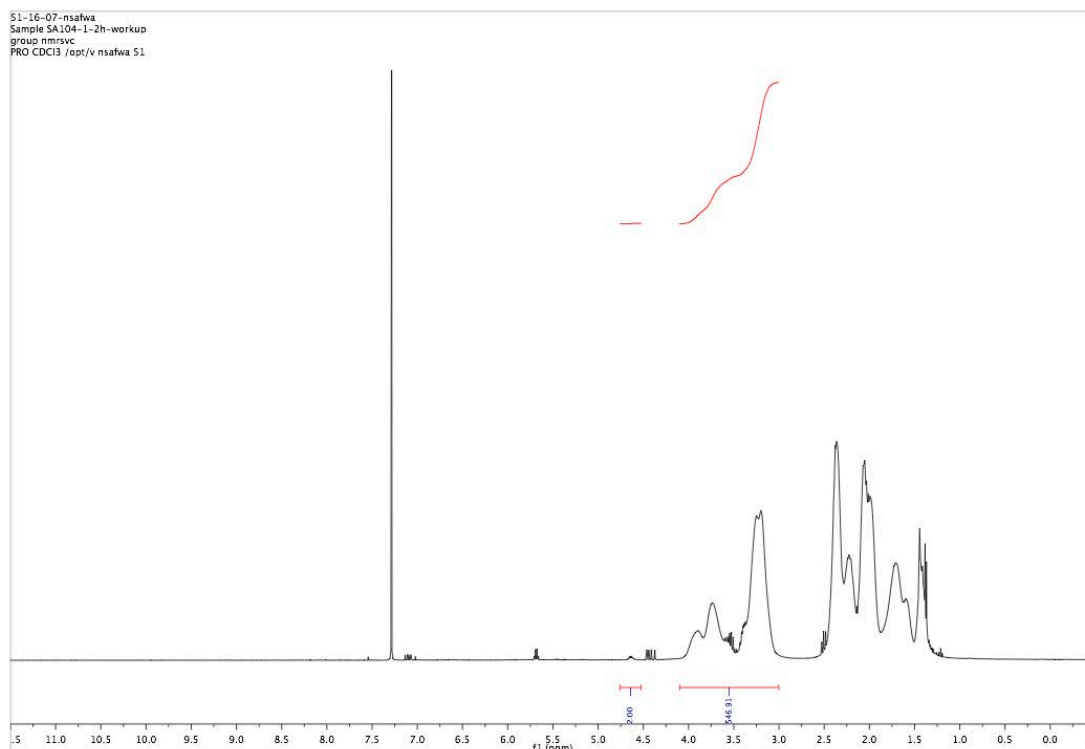


Figure S9. $^1\text{H-NMR}$ of the precipitated mixture after 2 h of reaction time (CDCl_3 , 300 MHz).

Table S1. Evolution of the molecular weight M_n with the reaction time by $^1\text{H-NMR}$.

| Reaction time (hours) | M_n |
|--------------------------|-------|
| 0.5 | 2410 |
| 1.0 | 5630 |
| 1.5 | 11550 |
| 2.0 | 20260 |

I-4. Determination of M_n and M_w by GPC

Samples were prepared with ca. 5 mg of each polymer in 1 mL of DMF (GPC grade, Fischer) containing 0.1% LiBr and filtered prior to the analysis with 0.22 μm PTFE hydrophilic syringe filters (Simplepure). GPC analysis was carried out using JASCO PU-2080 Plus HPLC pump, JASCO MD-2018 Plus UV detector, JASCO RI02031 Plus RI detector, ChromNAV Chromatography Data System (JASCO Co.), and Phenogel columns (Phenomenex, Torrance, CA, USA) in a linear combination : linear (2), 10^4 \AA , 10^3 \AA , 500 \AA columns ($5\mu\text{m}$, 300 mm x 7.8 mm, Phenomenex) using PMMA as standards and dimethylformamide (DMF) with 0.1% LiBr as mobile phase at 60°C inside JASCO CO-2065 Plus column oven.

Table S2. Evolution of the molecular weight M_n and M_w with the reaction time by GPC.

| Reaction time (hours) | M_n | M_w | PDI |
|-----------------------|-------|-------|------|
| 0.5 | 3310 | 4940 | 1.49 |
| 1 | 8250 | 10800 | 1.31 |
| 1.5 | 12200 | 15300 | 1.25 |
| 2 | 16900 | 20700 | 1.22 |

II. Synthesis

II-1. Synthesis of the RAFT agent

Compound 2. To a solution of potassium *O*-ethyl xanthate (10.80 g, 67.4 mmol) in distilled water (50 mL), a solution of iodine (3.30 g, 13.0 mmol) and potassium iodide (2.15 g, 13.6 mmol) in distilled water (50 mL) was added dropwise. The mixture was left to stir overnight at room temperature. An orange oil was separated by extraction with diethylether (3 x 50 mL) and the combined organic layers were washed with water (3 x 50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a yellow-orange oil **2** (5.80 g, 23.9 mmol, 71%); 1H -NMR (300 MHz, $CDCl_3$) 1.43 (t, $J = 7.1$ Hz, CH_3-CH_2-O , 6H), 4.69 (q, $J = 7.1$ Hz, CH_3-CH_2-O , 4H).

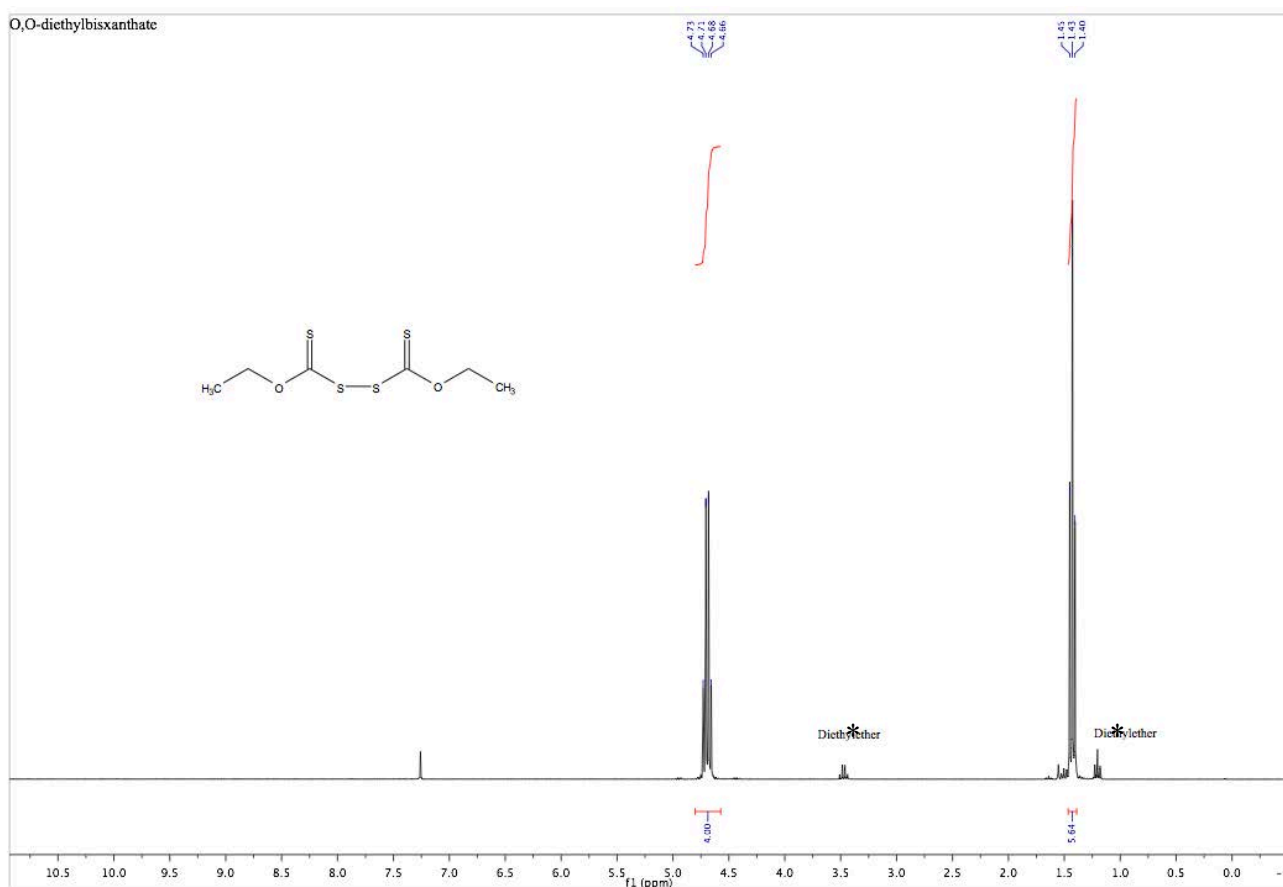


Figure S10. 1H -NMR of **2** ($CDCl_3$, 300 MHz). Asterisks denoted the presence of diethyl ether as an impurity.

Compound 3. Compound **2** (2.65 g, 10.93 mmol) and ACVA (3.67 g, 3.12 mmol, 1.2 equiv) in dioxane (25 mL) was degassed by N₂ bubbling for 30 min and then heated to 95°C. After 4 hours, 0.8 equiv of ACVA was added portionwise and the reaction was left to stir overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in DCM and a white precipitate was filtered off. The crude product was purified twice by SiO₂ gel column chromatography (20% EtOAc in Hexane with 0.5% AcOH) to give a yellow oil **3** (3.71 g, 15.00 mmol, 69%); ¹H-NMR (300 MHz, CDCl₃) 1.53 (t, *J* = 7.1 Hz, CH₃–CH₂–O, 3H), 1.78 (s, CH₃–C_q, 3H), 2.46 – 2.20 (m, CH₂–CO₂H, 2H), 2.67 (t, *J* = 8.2 Hz, CH₂–C_q, 2H), 4.76 (q, *J* = 7.1 Hz, CH₃–CH₂–O, 2H); LR-MS (ESI+) *m/z*: 270.1 ([*M*+Na]⁺).

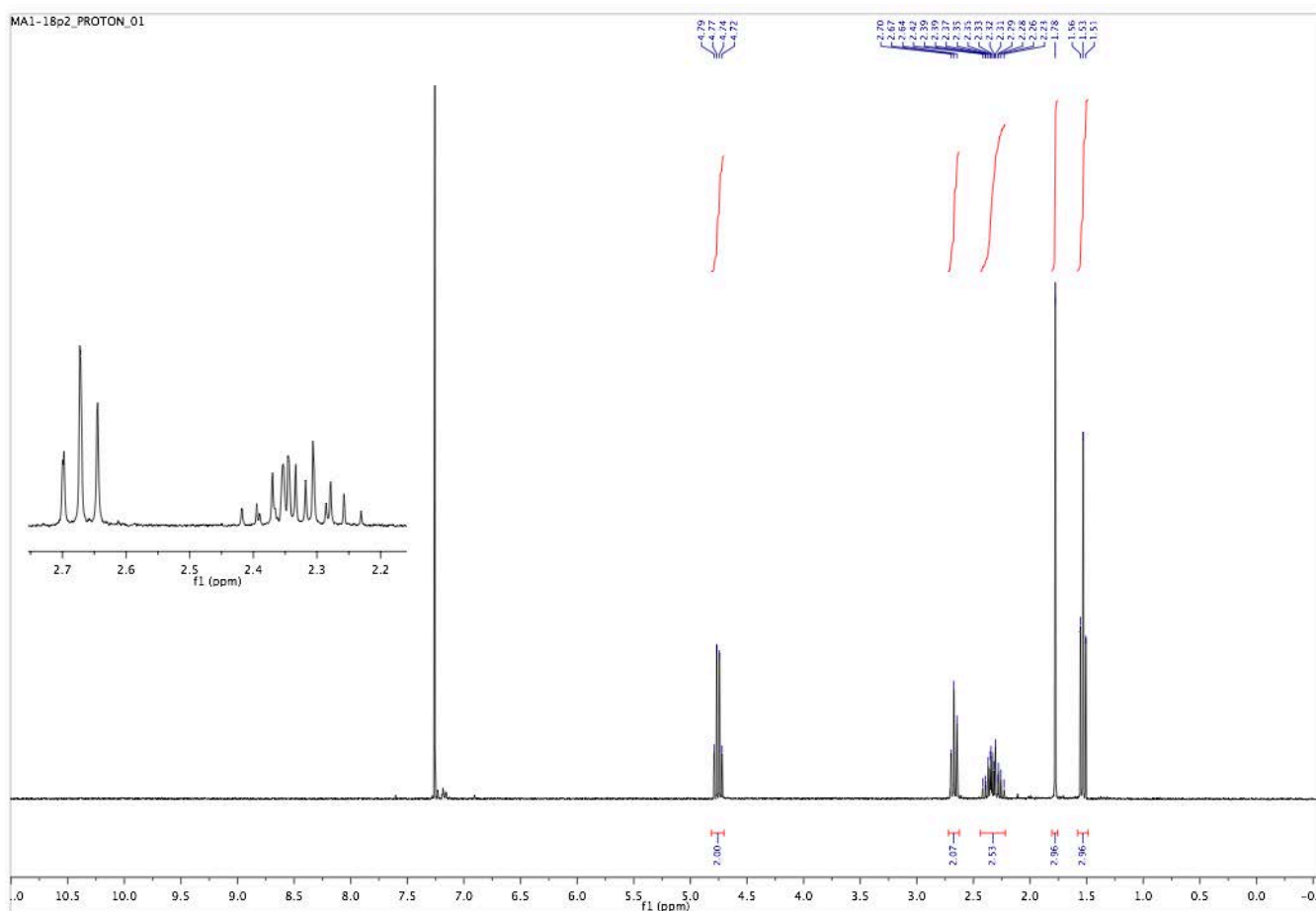


Figure S11. ¹H-NMR of **3** (CDCl₃, 300 MHz).

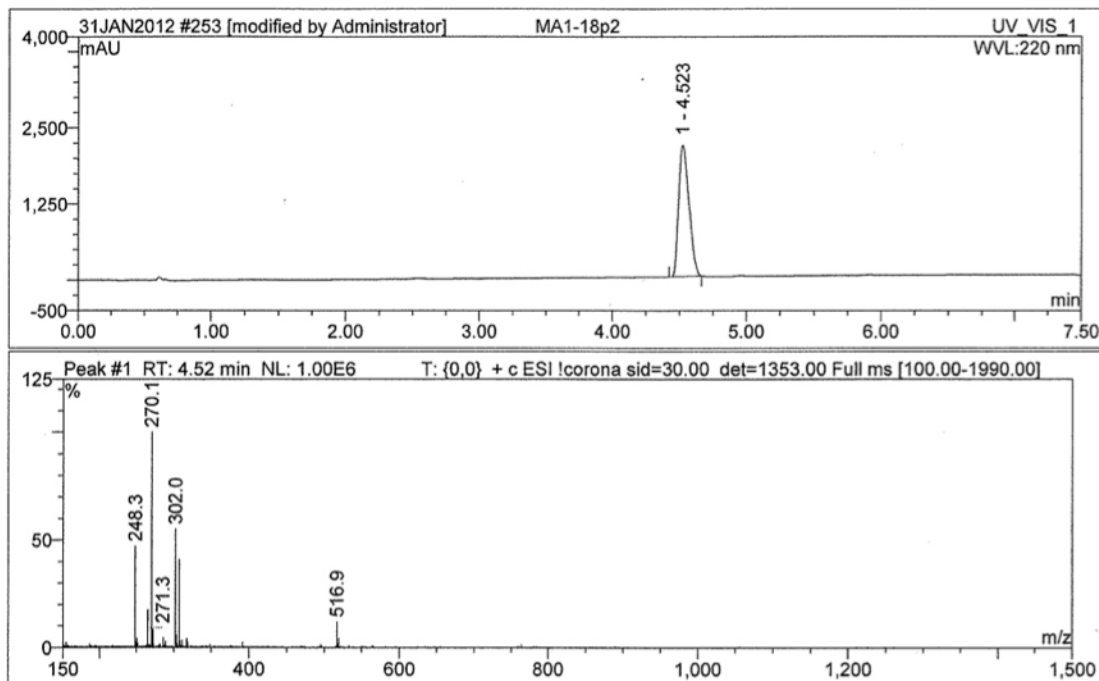


Figure S12. LC-MS trace of **3** (ESI+).

Compound 4.¹ To a solution of compound **3** (1.00 g, 4.04 mmol) and NHS (558.4 mg, 4.85 mmol, 1.2 equiv) in dry DCM (150 mL), DCC (1.00 g, 4.85 mmol, 1.2 equiv) was added at $-15\text{ }^{\circ}\text{C}$. The reaction was monitored by TLC and after 10 min there was almost no starting material left. The reaction mixture was slowly warmed to RT and stirred for additional 3 hours. The solvent was evaporated under reduced pressure to give a residue that was dissolved in EtOAc. Obtained white precipitates were filtered off. This procedure was repeated one more time. The crude product was purified by SiO_2 gel column chromatography (0% \rightarrow 50% EtOAc in hexane) to give a pale yellow solid **4** (1.20 g, 3.48 mmol, 85%); m.p. = $72 - 73\text{ }^{\circ}\text{C}$; IR (neat) $\nu_{\text{max}}(\text{cm}^{-1})$: 2985 (w), 1939 (w), 2235 (w), 1814 (w), 1784 (w), 1737 (s), 1251 (w), 1205 (m), 1152 (w), 1039 (m); $^1\text{H-NMR}$ (400 MHz, CDCl_3) 1.53 (t, $J = 7.1\text{ Hz}$, $\text{CH}_3\text{-CH}_2\text{-O}$, 3H), 1.78 (s, $\text{CH}_3\text{-C}_q$, 3H), 2.56 - 2.32 (m, $\text{CH}_2\text{-CO}_2\text{Su}$, 2H), 2.85 (s, $\text{CH}_2\text{-CH}_2$, 4H), 2.92 (t, $J = 8.2\text{ Hz}$, $\text{CH}_2\text{-C}_q$, 1H), 4.76 (q, $J = 7.1\text{ Hz}$, $\text{CH}_3\text{-CH}_2\text{-O}$, 2H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) 13.46, 24.86, 25.59, 26.98, 33.49, 44.48, 71.00, 119.33, 166.99, 168.77, 206.23.

¹ X. Zhang, J. Li, W. Li and A. Zhang, *Biomacromol.*, 2007, **8**, 3557-3567.

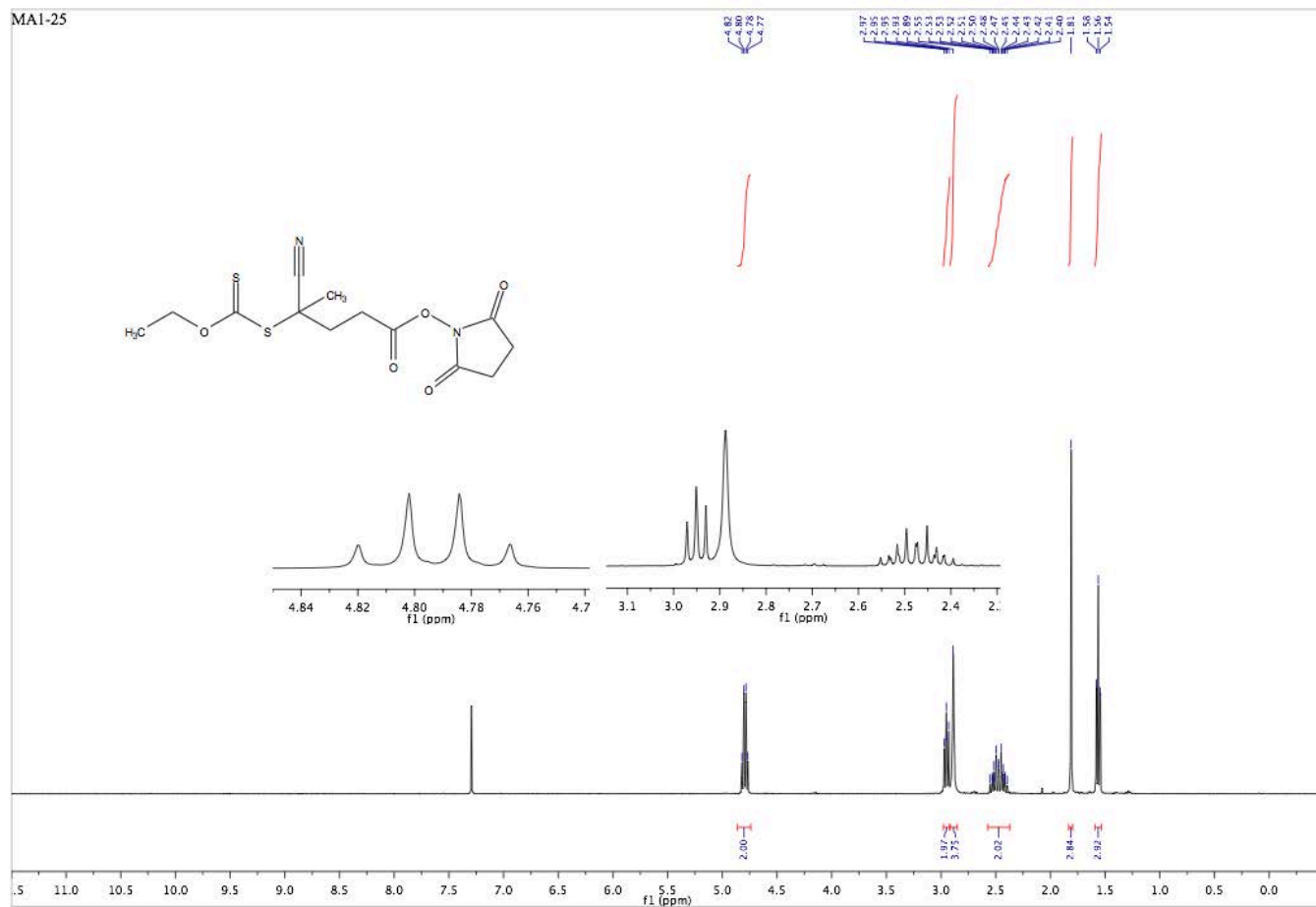
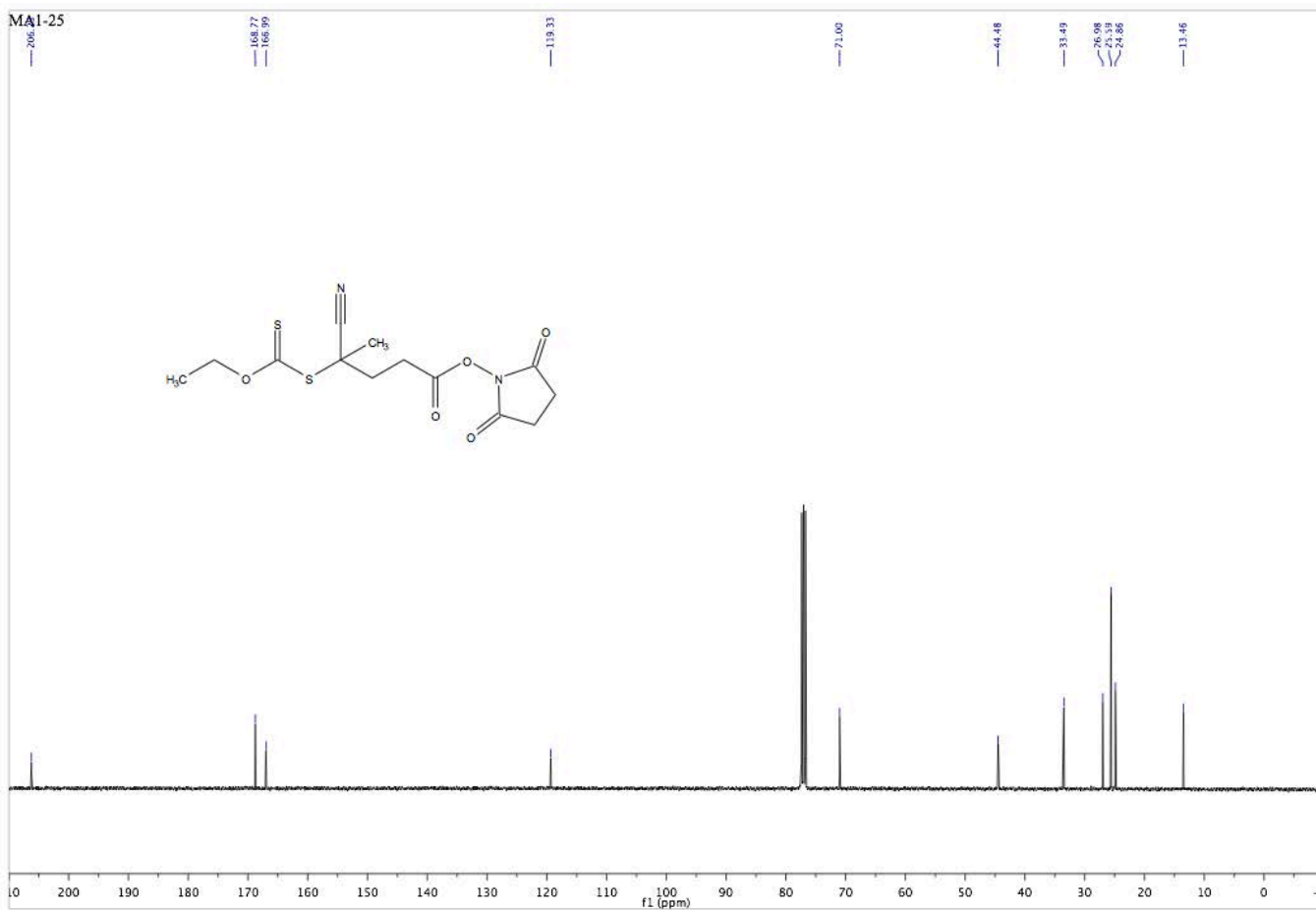
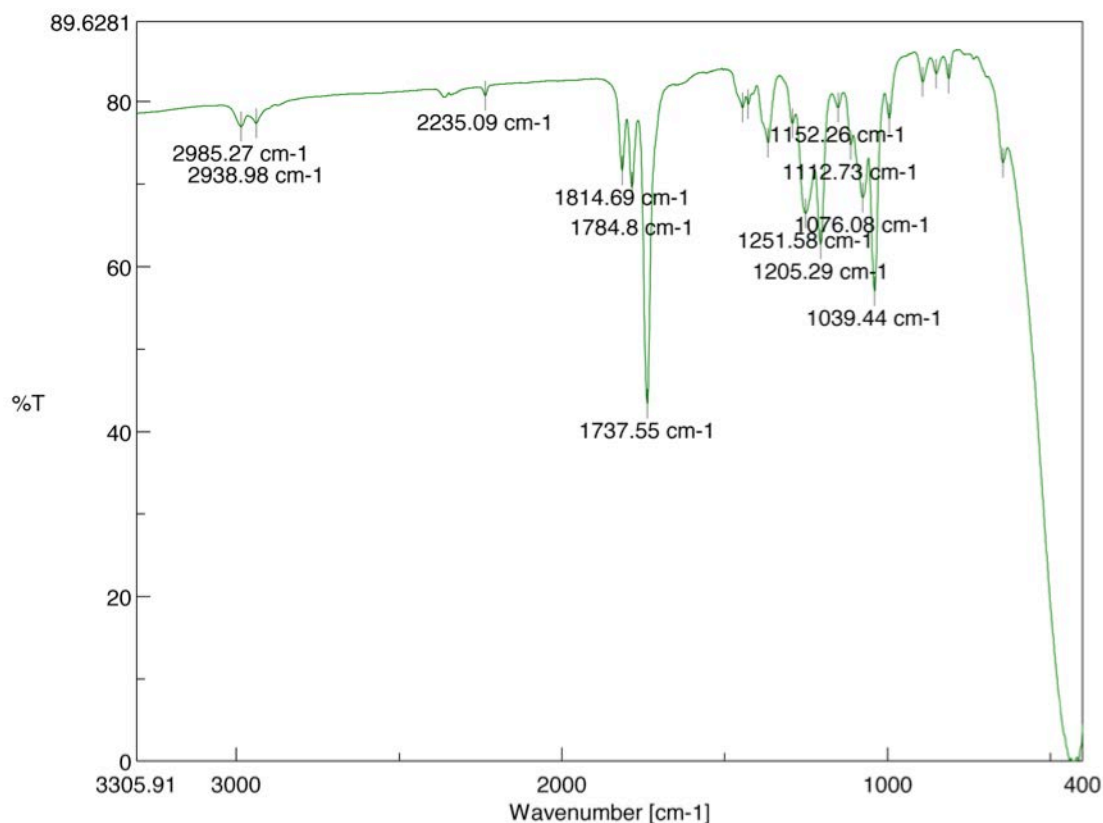
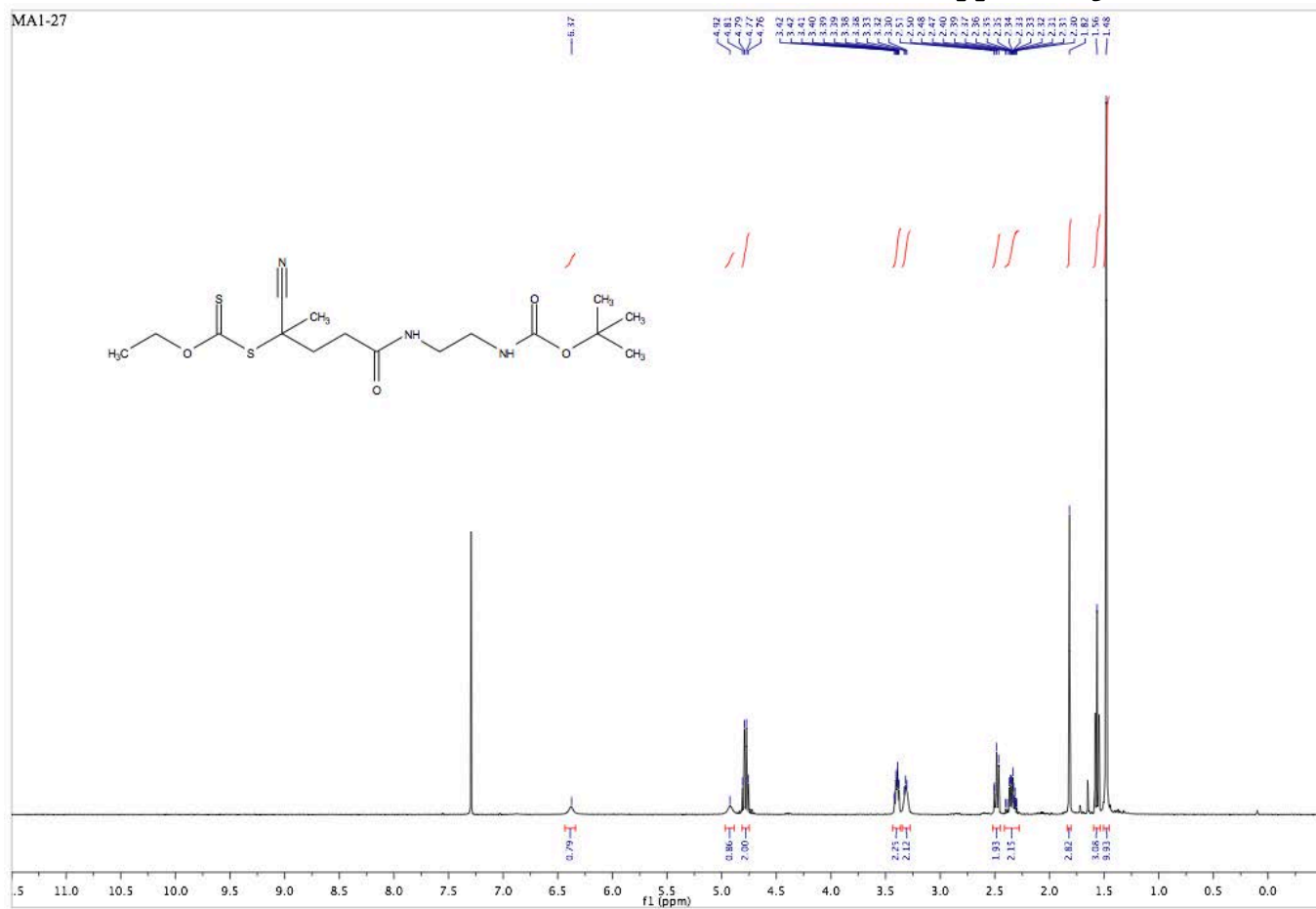
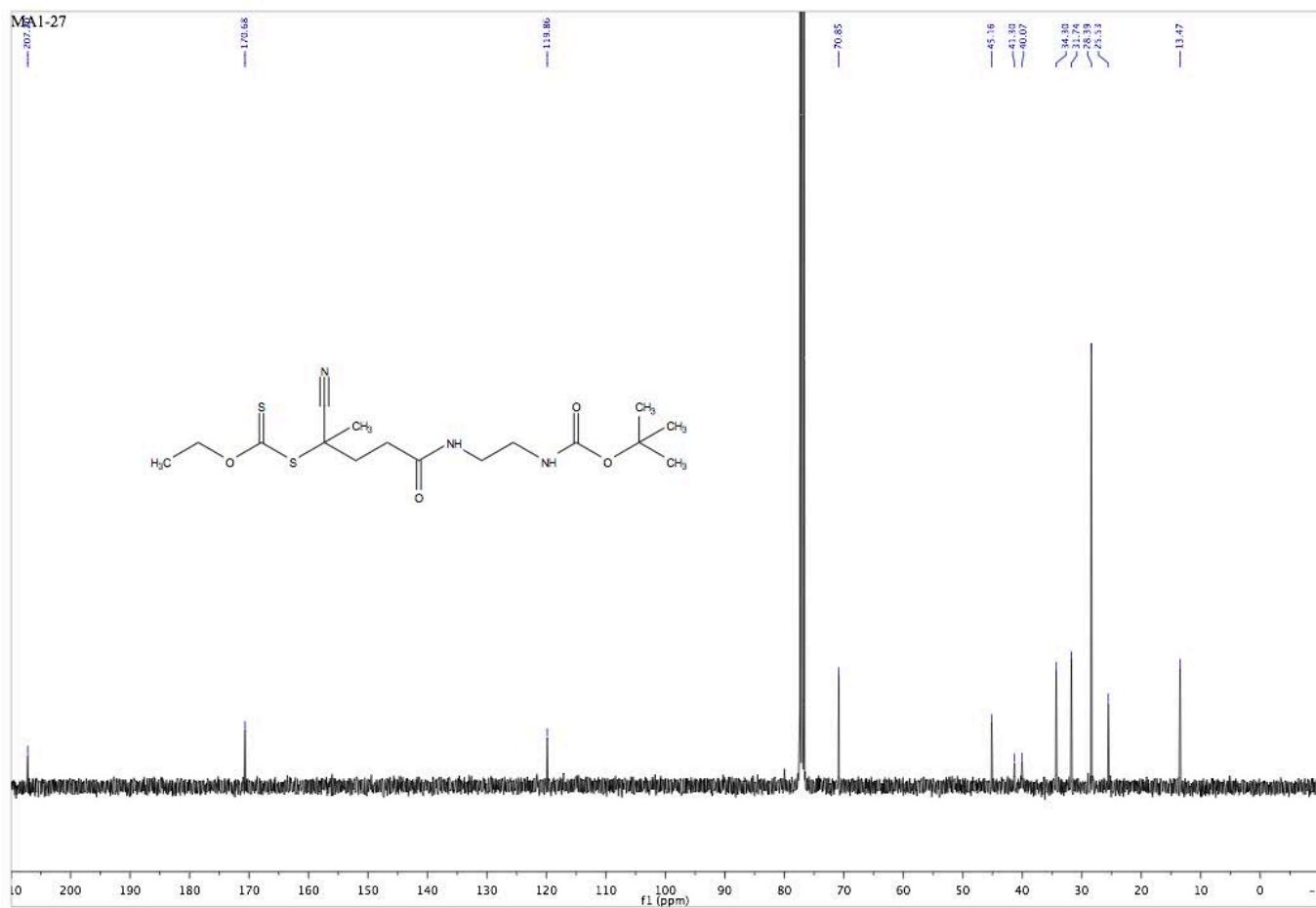
Figure S13. ¹H-NMR of 4 (CDCl₃, 400 MHz).

Figure S14. ^{13}C -NMR of **4** (CDCl_3 , 100 MHz).Figure S15. FT-IR spectra of **4**.

Synthesis of 5.² To a solution of *N*-Boc-1,2-diaminoethane³ (256 mg, 1.6 mmol, 1.1 eq) and TEA (222.4 μL , 1.6 mmol, 1.1 eq) in dry DCM (12 mL), a solution of **4** (500 mg, 1.45 mmol) in dry DCM (8 mL) was added dropwise at -15°C . The reaction was completed after 30 min. The solution was washed with NaHCO_3 solution (25 mL) and brine (25 mL) then dried over MgSO_4 . The crude product was purified by a SiO_2 gel column chromatography (70 \rightarrow 80% EtOAc in hexane) to give a yellow sticky solid **5** (445.3 mg, 80%). IR (neat) $\nu_{\text{max}}(\text{cm}^{-1})$: 3325 (m), 2978 (w), 2934 (w), 2235 (w), 1693 (m), 1656 (m), 1249 (m), 1169 (m), 1041 (m); ^1H -NMR (400 MHz, CDCl_3) 1.48 (s, $\text{C}(\text{CH}_3)_3$, 9H), 1.56 (t, 7.1 Hz, $\text{CH}_3\text{-CH}_2\text{-O}$, 3H), 1.82 (s, $\text{CH}_3\text{-C}_q$, 3H), 2.41 – 2.27 (m, $\text{CH}_2\text{-CONH}$, 2H), 2.48 (m, $\text{CH}_2\text{-C}_q$, 2H), 3.36 – 3.28 (m, $\text{CH}_2\text{-NHCO}_2^t\text{Bu}$, 2H), 3.43 – 3.36 (m, $\text{CH}_2\text{-NHCO}$, 2H), 4.78 (q, $J = 7.1$ Hz, $\text{CH}_3\text{-CH}_2\text{-O}$, 2H), 4.92 (s, NHCO , 1H), 6.37 (s, NHCO , 1H); ^{13}C -NMR (100 MHz, CDCl_3) 13.47, 25.53, 28.39, 31.74, 34.30, 40.07, 41.30, 45.16, 70.85, 119.86, 170.68, 207.20; HR-MS (ESI+) m/z calc. for $\text{C}_{16}\text{H}_{28}\text{N}_3\text{O}_4\text{S}_2^+$: 390.1516, found: 390.1508.

2. X. Zhang, J. Li, W. Li and A. Zhang, *Biomacromol.*, 2007, **8**, 3557-3567.

3. D. Muller, I. Zeltser, G. Bitan and C. Gilon, *J. Org. Chem.*, 1997, **62**, 411-416.

Figure S16. $^1\text{H-NMR}$ of **5** (CDCl_3 , 400 MHz).

Aroua et al. supporting information
Figure S17. ^{13}C -NMR of **5** (CDCl_3 , 100 MHz).

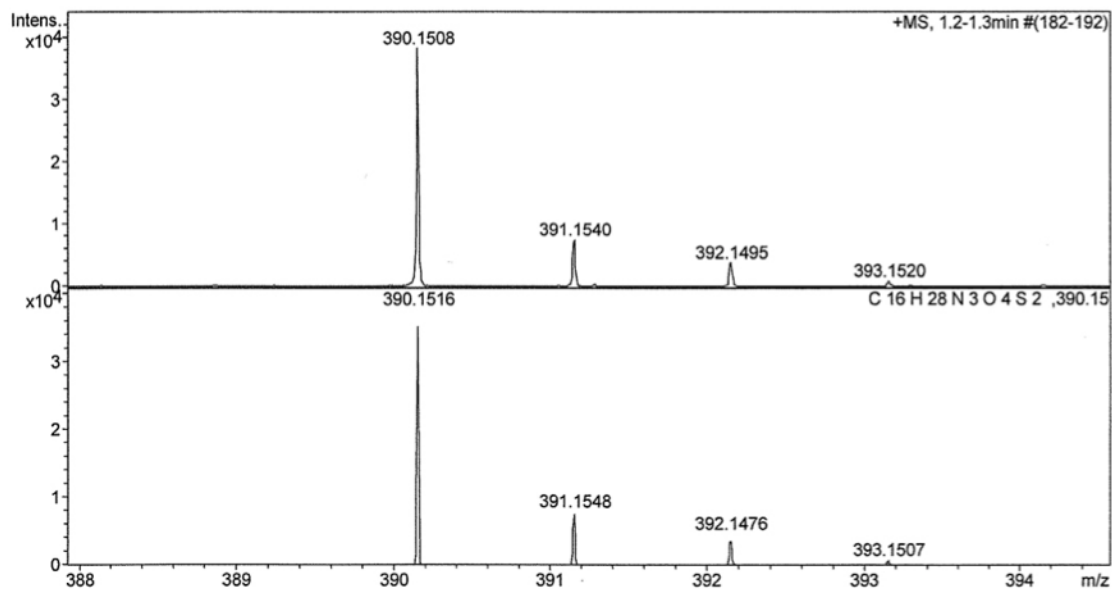


Figure S18. HR-MS spectrum of **5** (ESI+).

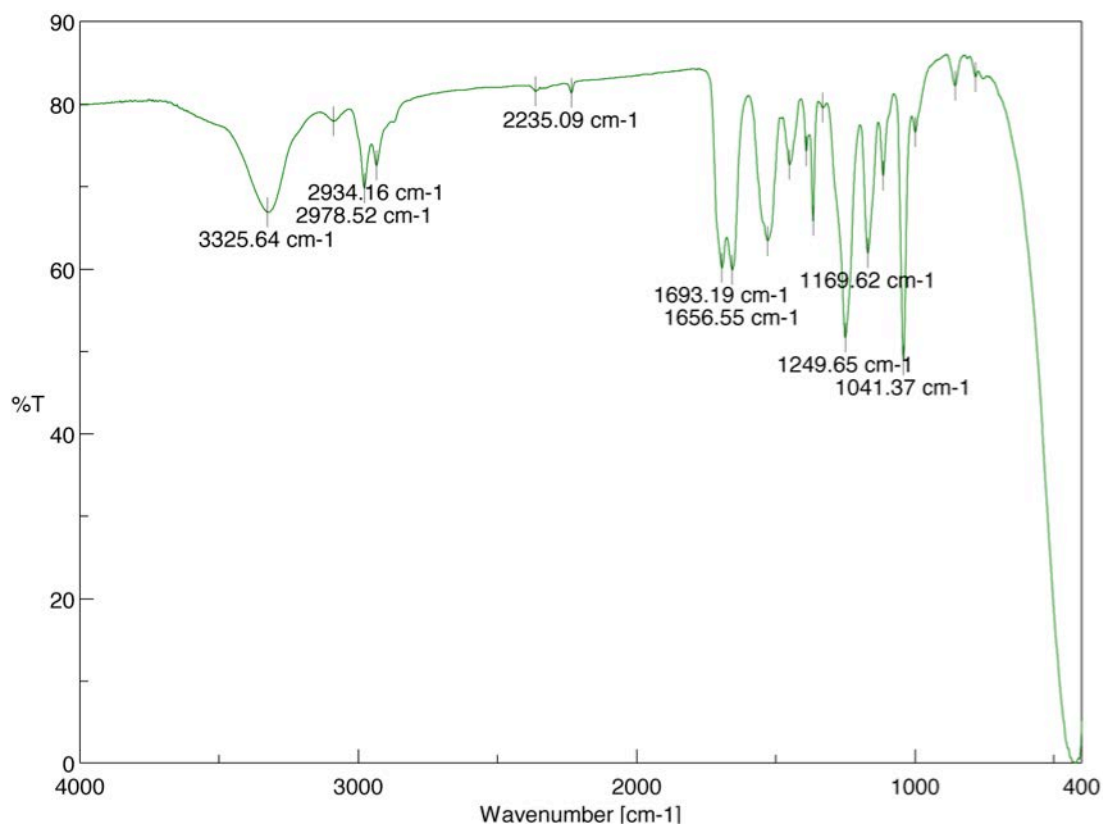


Figure S19. FT-IR spectra of **5**.

II-2. Synthesis of PVP polymer

Polymer 6. A solution of NVP (15.7 g, 15.05 mL, 141.3 mmol, conc. = 9.38 mol/L) containing AIBN (8.9 mg, 54.2 μmol) and **5** (96.0 mg, 246.5 μmol) was placed in a schlenk flask and degassed with four freeze-evacuate-thaw cycles and heated under nitrogen at 60 °C for 3 hours. The product was dissolved in CHCl_3 and precipitated in Et_2O (repeated 2 times). The product was collected by filtration and dried under vacuum to get **6** as a white powder (5.7 g, 36%). $M_n = 19500$, $M_w = 22900$, PDI = 1.18.

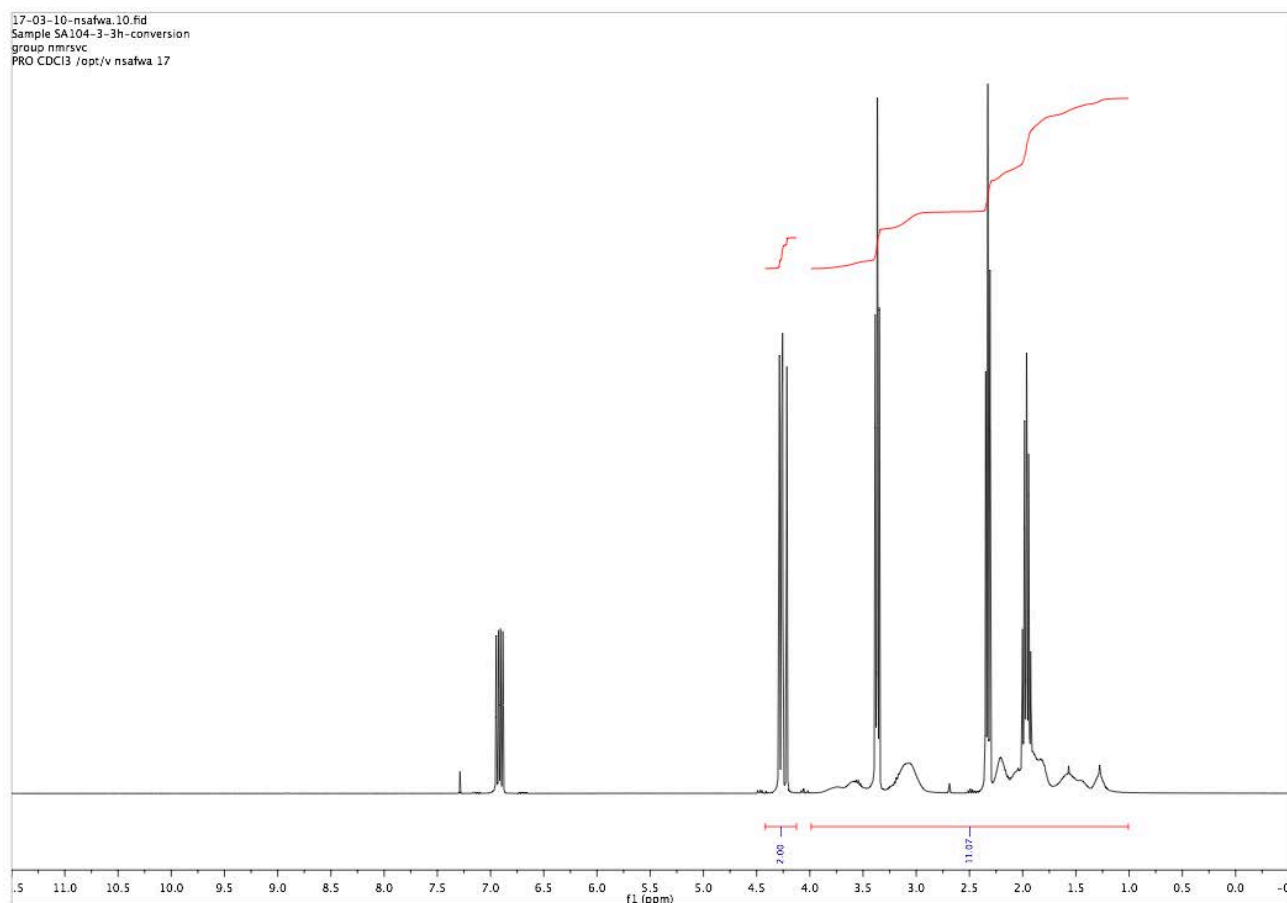


Figure S20. $^1\text{H-NMR}$ of the reaction mixture (CDCl_3 , 300 MHz).

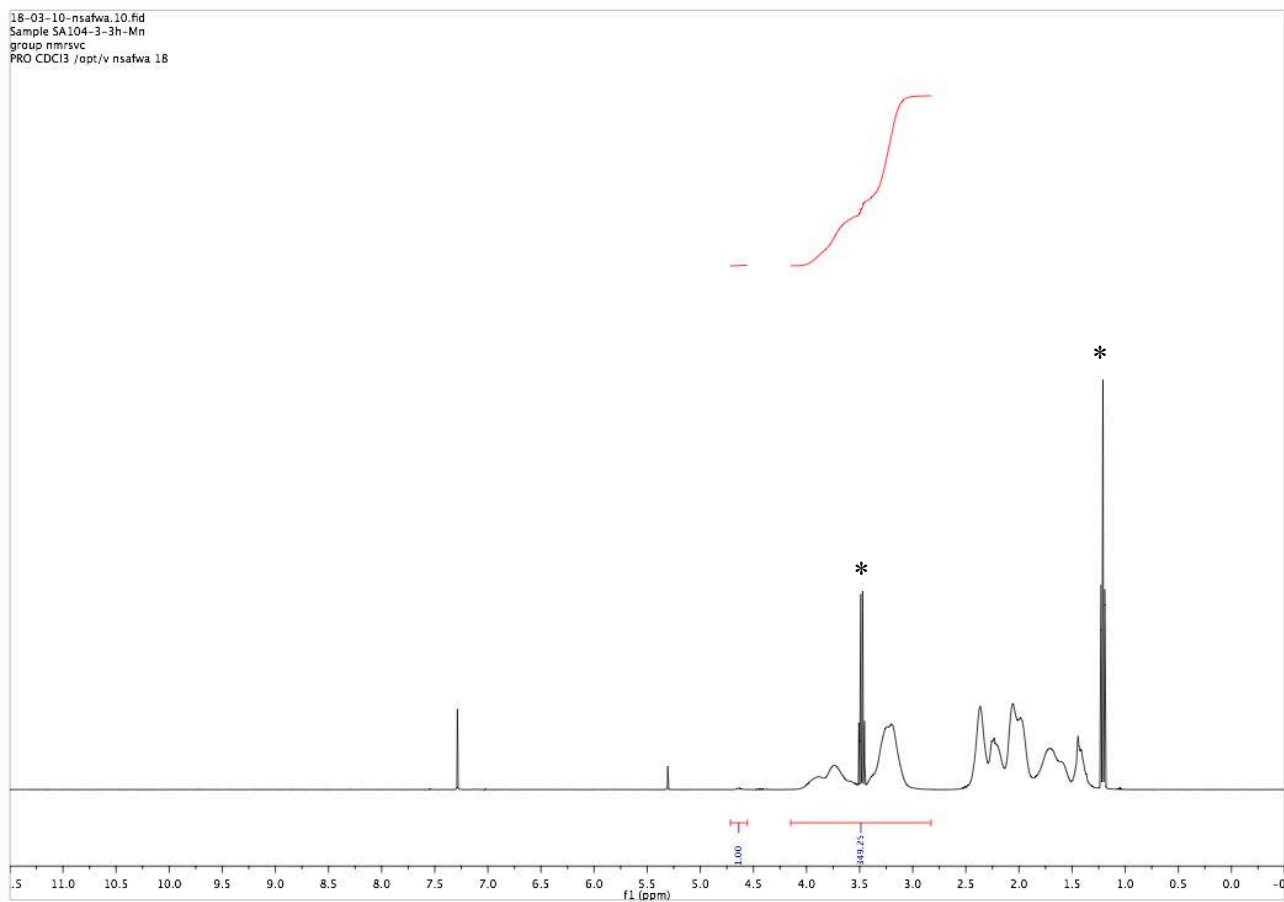


Figure S21. $^1\text{H-NMR}$ of the precipitated mixture **6** (CDCl_3 , 300 MHz). Asterisks denoted the presence of diethyl ether as an impurity.

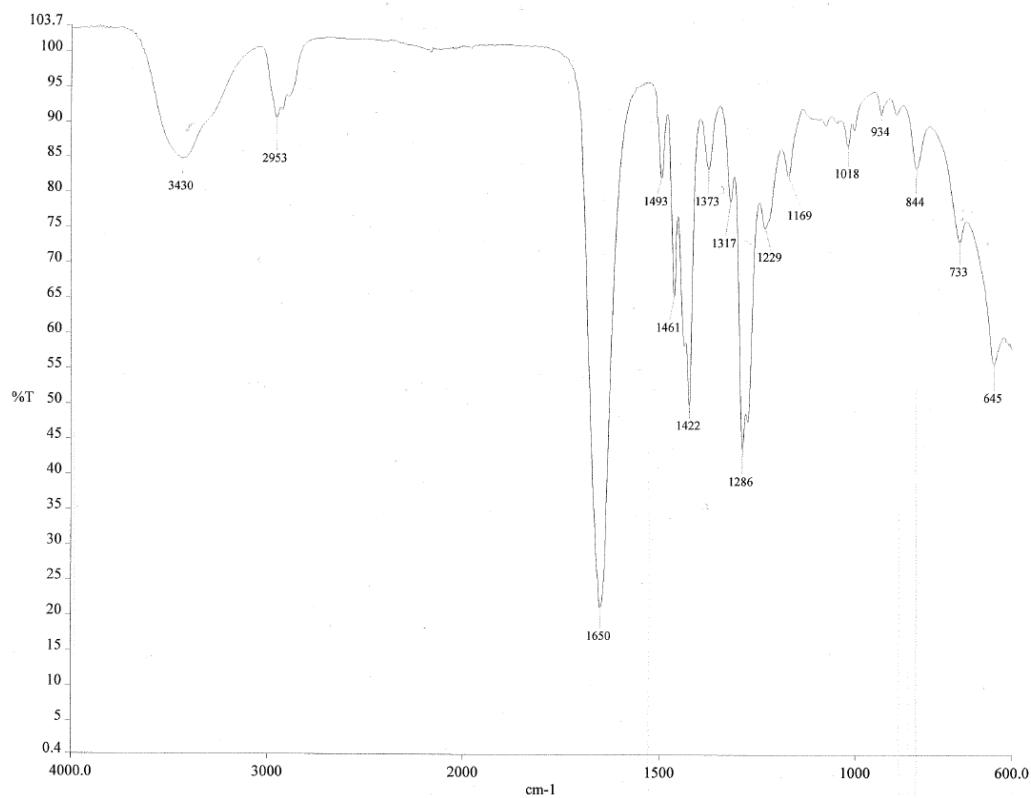


Figure S22. FT-IR spectra of **6**.

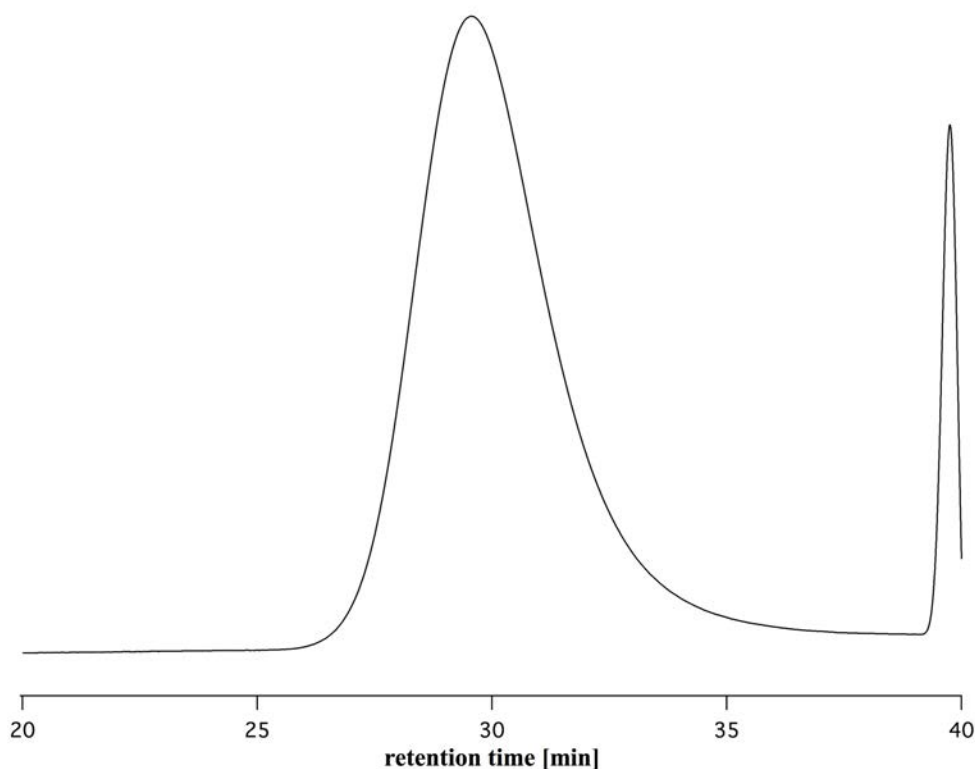


Figure S23. GPC trace of **6**. $M_n = 19500$, $M_w = 22900$, PDI = 1.18.

Polymer 7.⁴ To a solution of polymer **6** (5.7 g, 292 μmol) in *o*-DCB / DMF (190 / 10 mL) AIBN (82.6 mg, 503 μmol) and Bu_3SnH (1.91 g, 1.77 mL, 6.58 mmol) were added. The solution was degassed with four freeze-evacuate-thaw cycles and warmed at 70 $^\circ\text{C}$ for 4 hours. The solvent was evaporated under reduced pressure, reconstituted into CHCl_3 , and precipitated in Et_2O (2 times). The product was collected, dried under reduced pressure, dissolved in water and then dialyzed (MWCO 3500) for 24 hours. The solution was then freeze-dried to obtain a white solid **7** (4.96 g, 87 %).

4. Y. K. Chong, G. Moad, E. Rizzardo and S. H. Thang, *Macromol.*, 2007, **40**, 4446-4455.

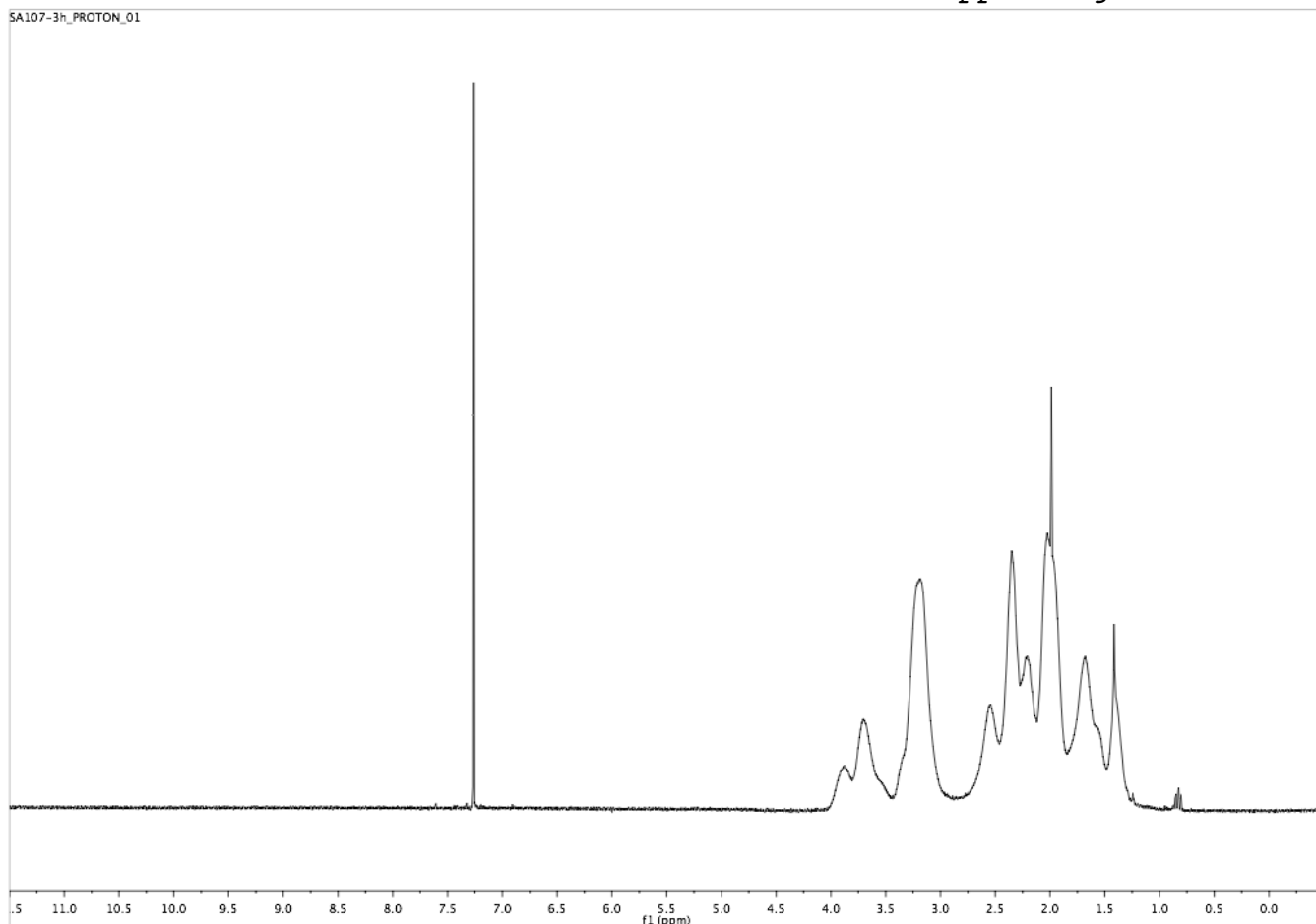


Figure S24. $^1\text{H-NMR}$ of 7 (CDCl_3 , 300 MHz).

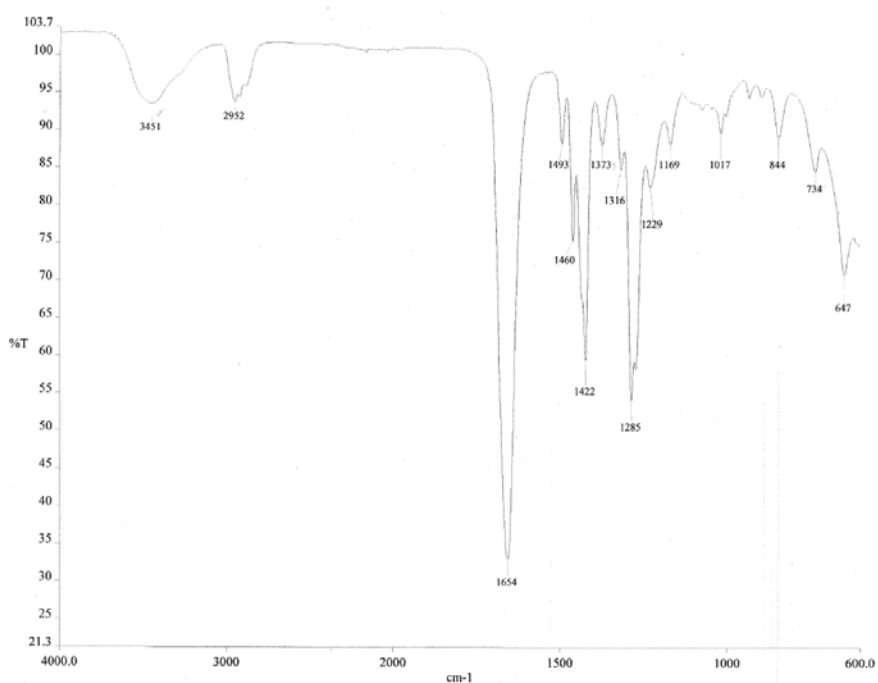


Figure S25. FT-IR spectra of 7.

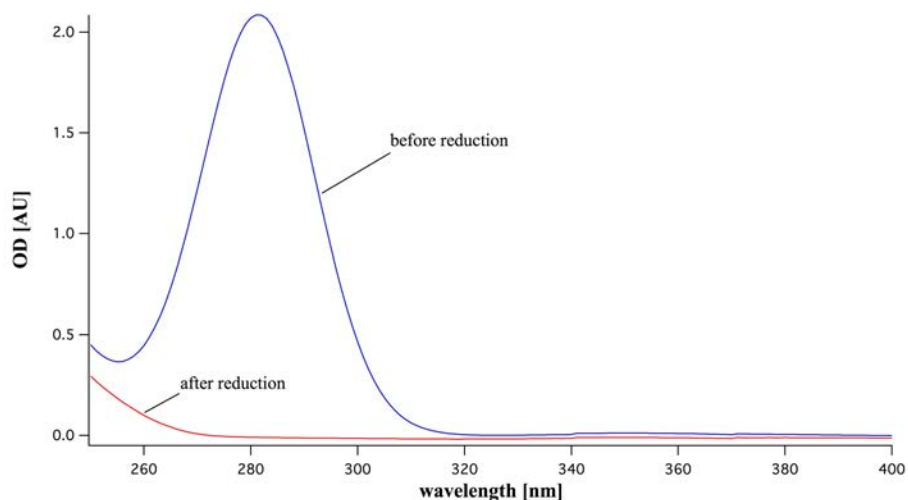


Figure S26. Evidence of PVP polymer xanthate end-group reduction ($M_n = 16900$, $M_w = 20700$); (before reduction, 4.0 mg/mL in CHCl_3 , blue line, after reduction, 4.0 mg/mL in CHCl_3 , red line).

Polymer 8. A solution of polymer **7** (4.96 g) in TFA (50 mL) was stirred overnight at room temperature. The solvent was removed by nitrogen flushing, the resulting gel was reconstituted into CHCl_3 , and precipitated in Et_2O (2 times). The product was collected, dried under vacuum, dissolved in water, and then dialyzed (MWCO 3500) for 24 hours. The solution was freeze-dried to obtain a white solid **8** (4.71 g, 95 %), $M_n = 19900$, $M_w = 25800$, PDI = 1.29.

In order to confirm the Boc group deprotection, the same reaction was performed on the polymer with a smaller molecular weight ($M_n = 12200$, $M_w = 15300$, **Figure S27**).

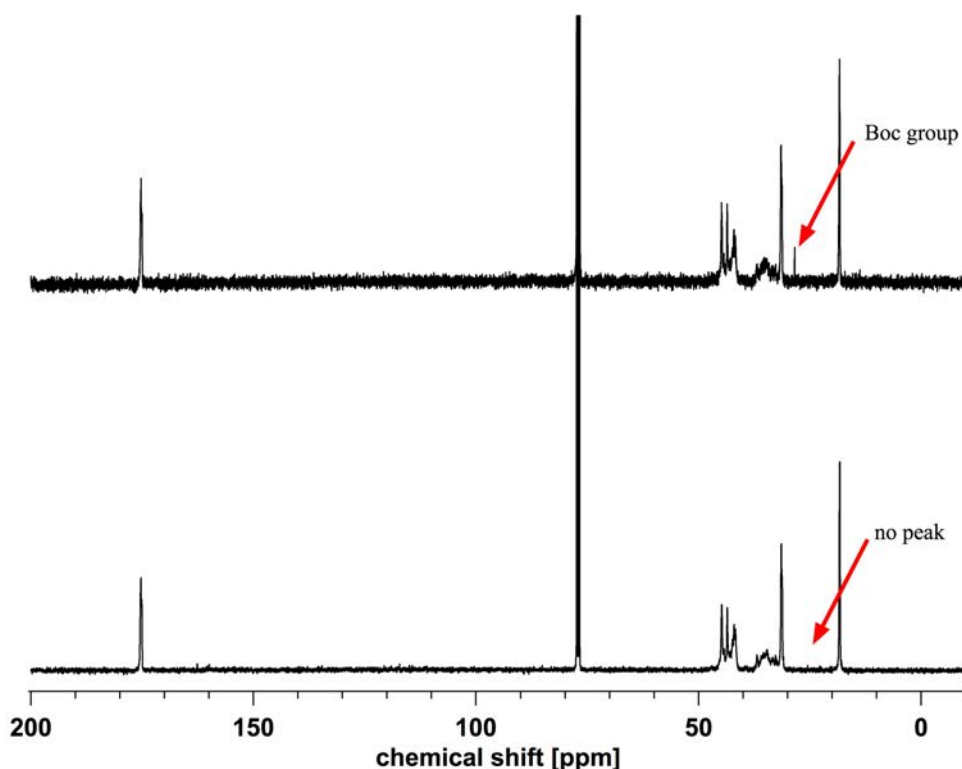


Figure S27. Evidence of Boc group deprotection by ^{13}C -NMR (CDCl_3 , 400 MHz), ($M_n = 12200$, $M_w = 15300$) (top) before TFA deprotection; (bottom) after TFA deprotection.

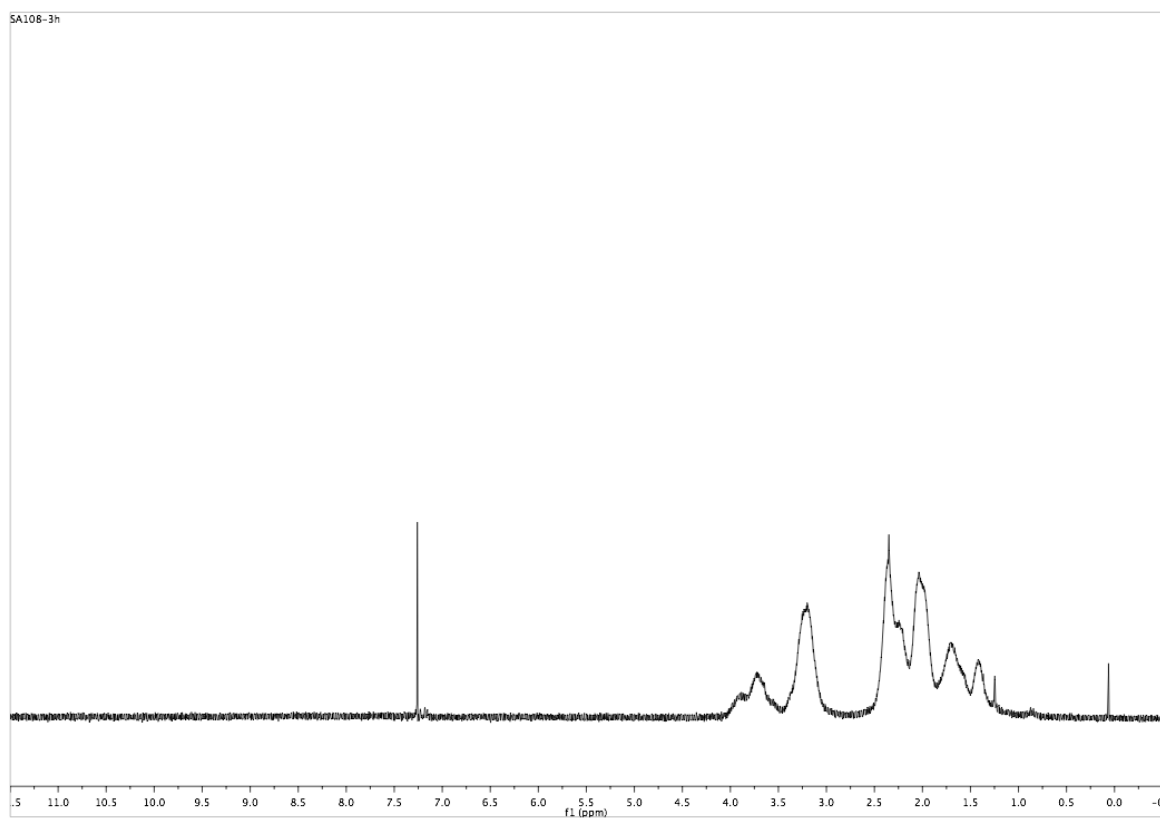


Figure S28. ^1H -NMR of **8** (CDCl_3 , 600 MHz).

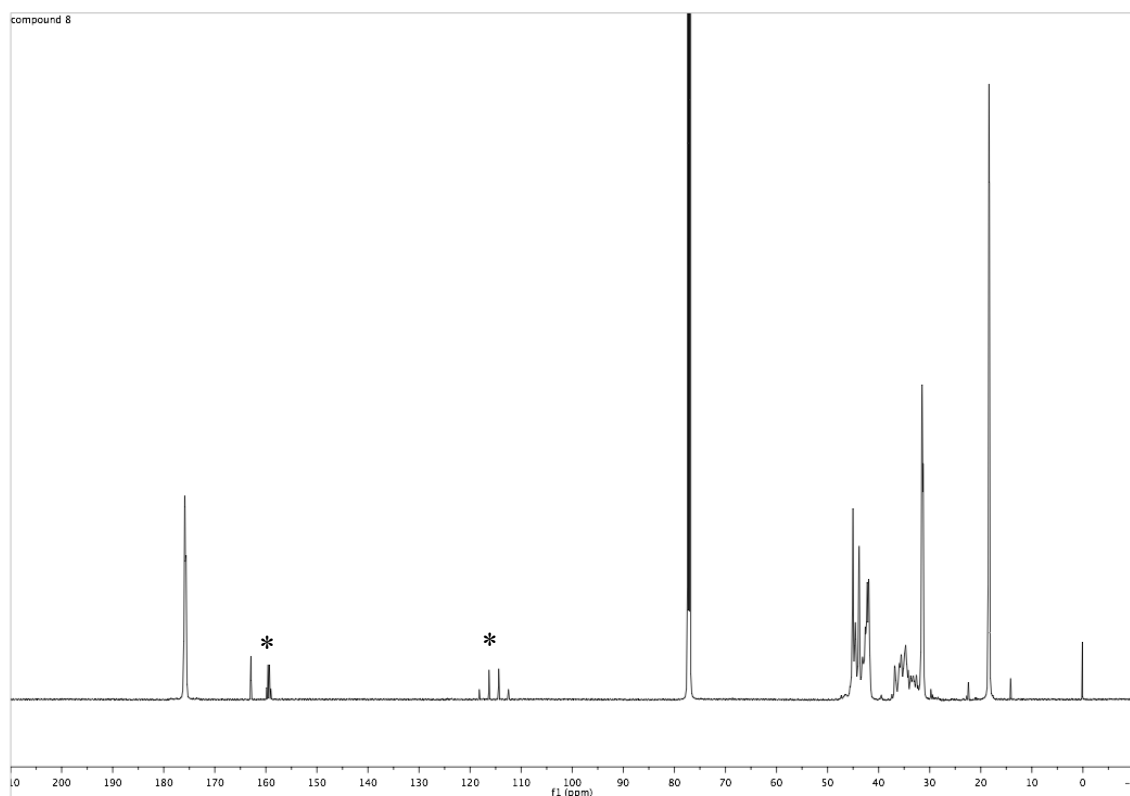


Figure S29. ^{13}C NMR of **8** (CDCl_3 , 150 MHz). Asterisks denoted the presence of trifluoroacetic acid as an impurity.

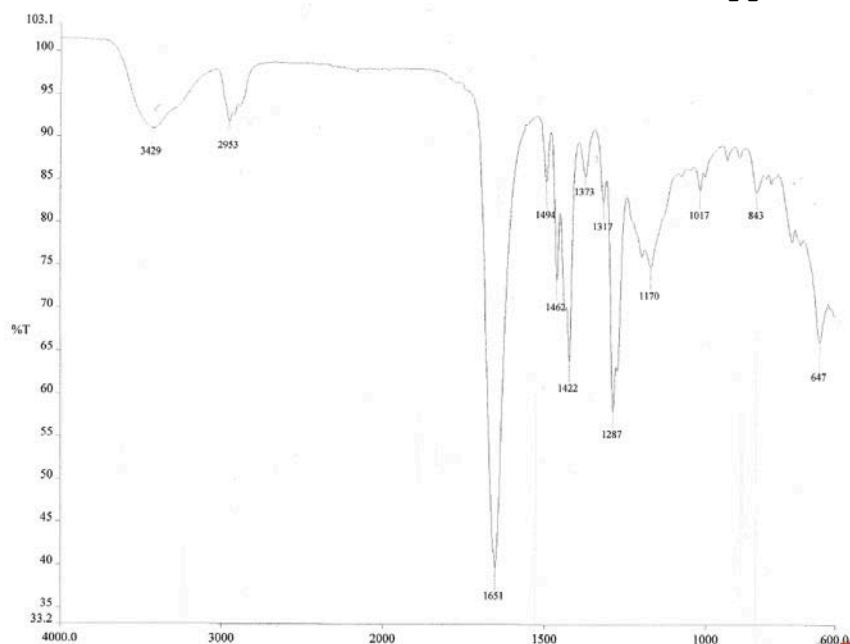


Figure S30. FT-IR spectra of **8**.

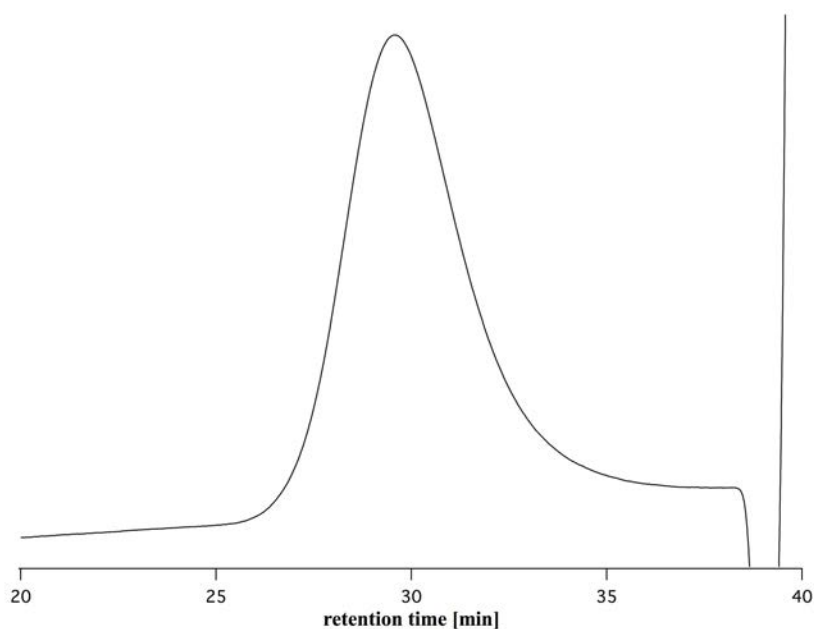


Figure S31 GPC trace of **8**

Polymer 10. In a Schlenk flask, to a solution of **9** (73.0 mg, 83.6 μmol , 7.5 equiv) in 1-methylnaphthalene : dry DMF (1:1, 0.84 mL) was added dropwise a solution of **8** (223 mg, 11.2 μmol) in 1-methylnaphthalene : dry DMF (1:1, 1.12 mL) containing *N,N*-diisopropylethylamine (19.4 μL , 111 μmol , 10 equiv). The reaction mixture was stirred overnight at room temperature under nitrogen. The solvents were removed under reduced pressure. A minimum amount of DCM was added to reconstitute the residue and precipitated in Et₂O (repeated 3 times). After centrifugation, the collected powder was dried and dissolved in water (30 mL). The reaction mixture was filtered over cellulose acetate microfilter (0.45 μm) and dialyzed for 24 hours (Fischer, MWCO 3500). The solution was freeze-dried and the product was

Aroua et al. supporting information
collected as a brown amorphous solid **10** (172 mg, isolated yield 74 %). $M_n = 18325$, $M_w = 23965$, PDI = 1.31.

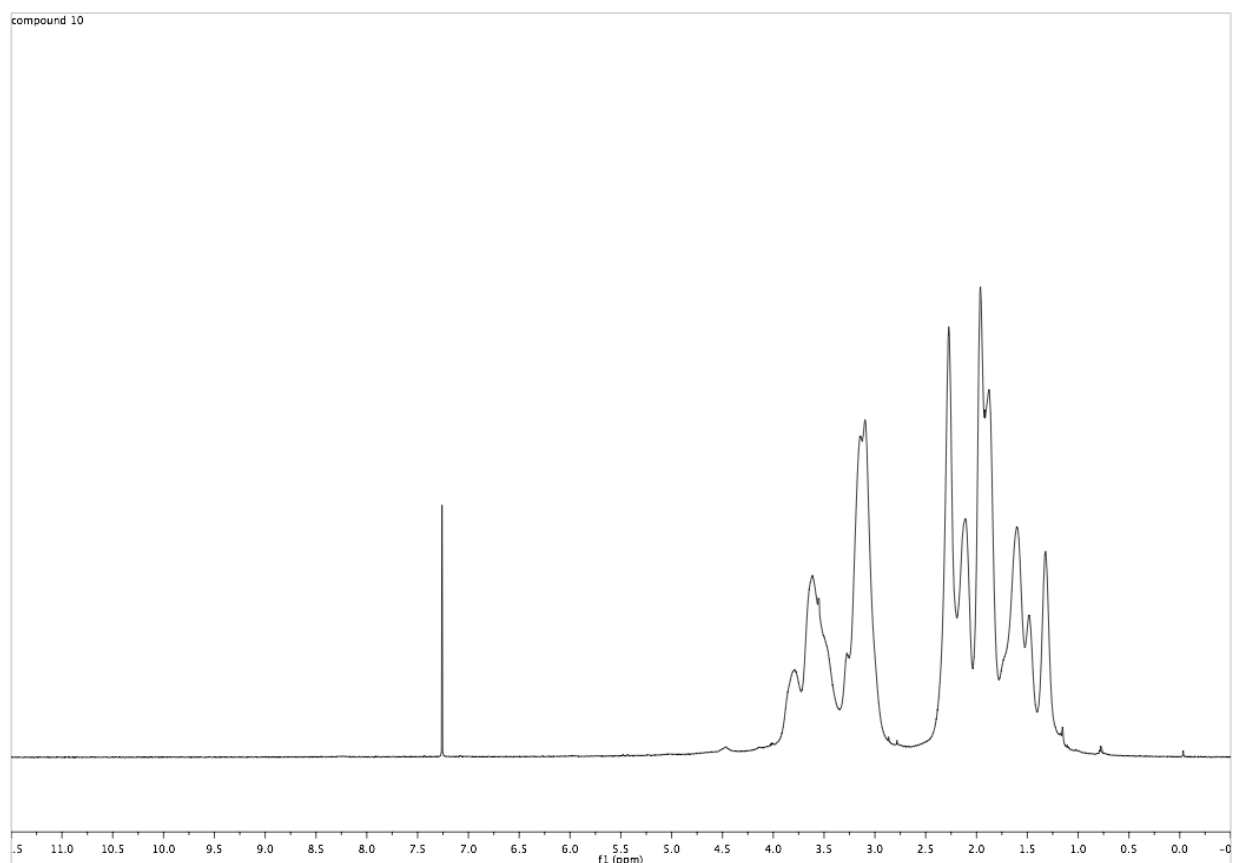


Figure S32. $^1\text{H-NMR}$ of **10** (CDCl_3 , 600 MHz).

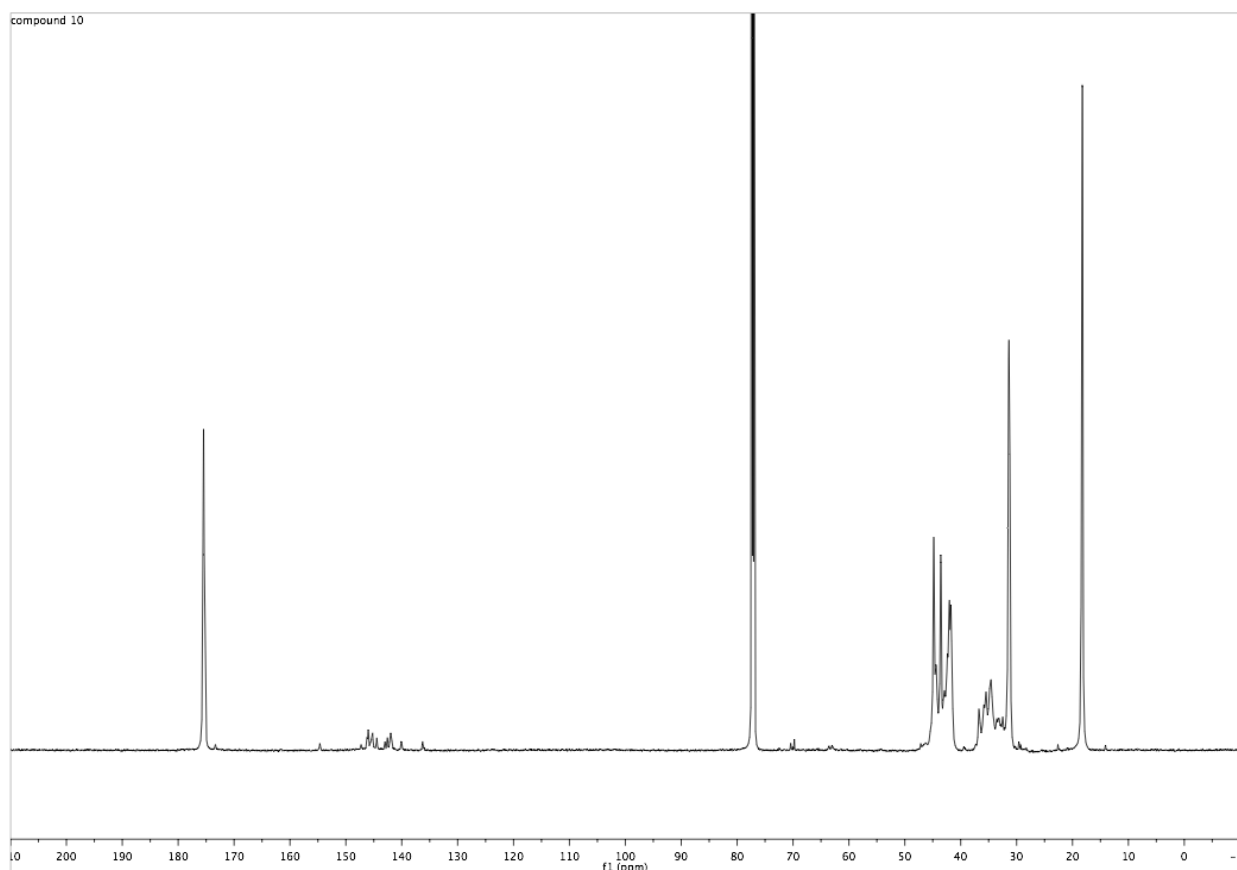


Figure S33. $^{13}\text{C NMR}$ of **10**. (CDCl_3 , 150 MHz)

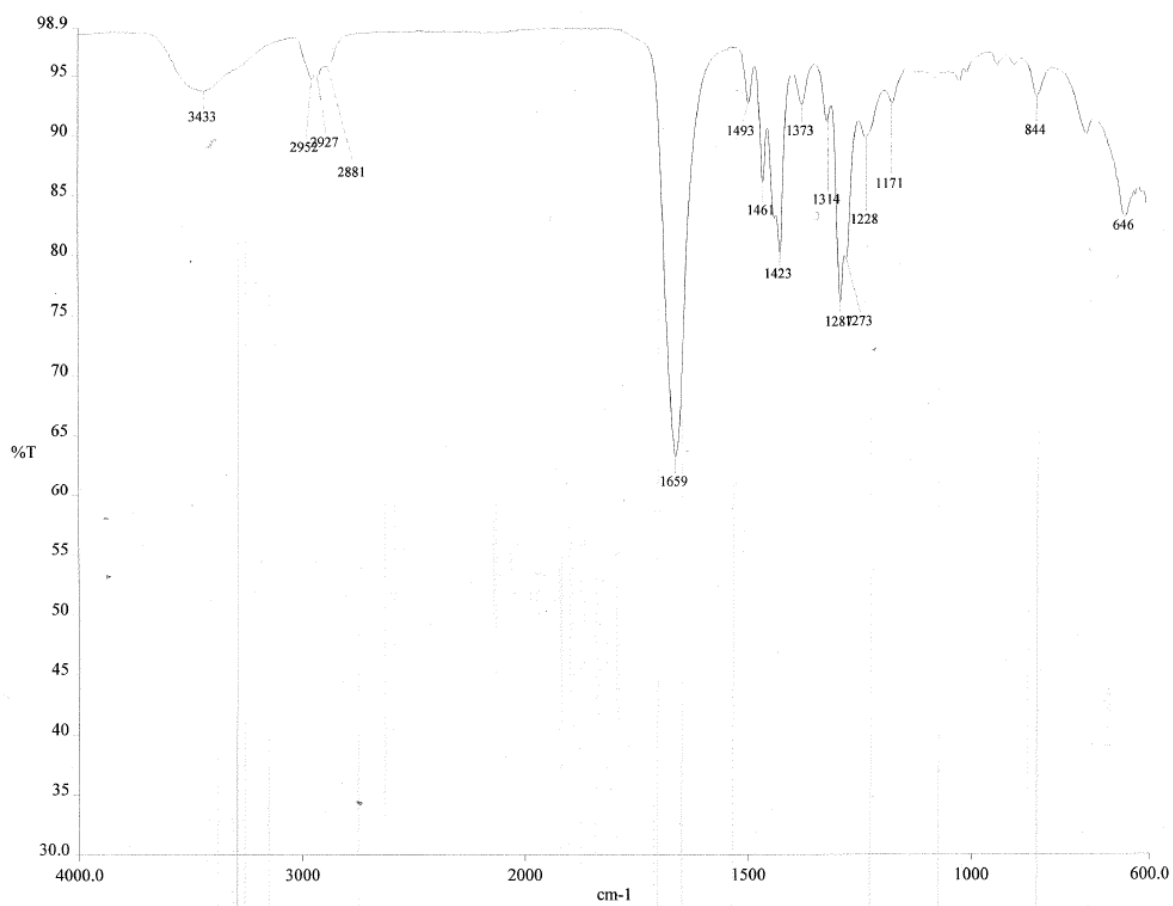


Figure S34. FT-IR spectra of 10.

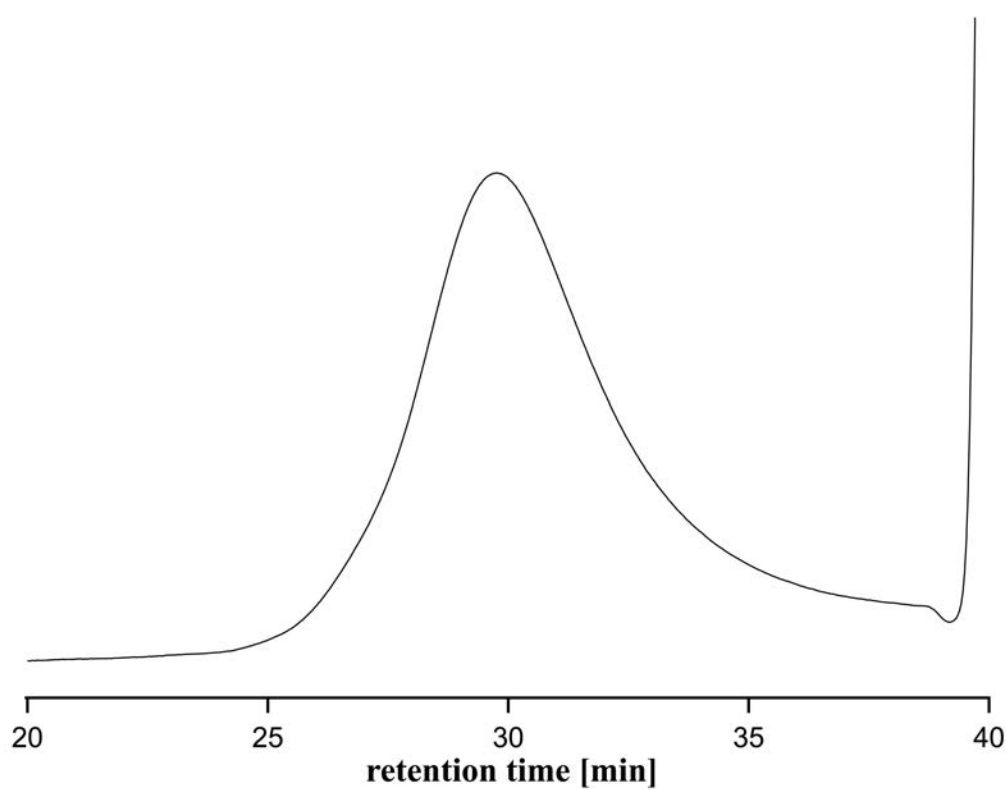


Figure S35. GPC trace of 10.

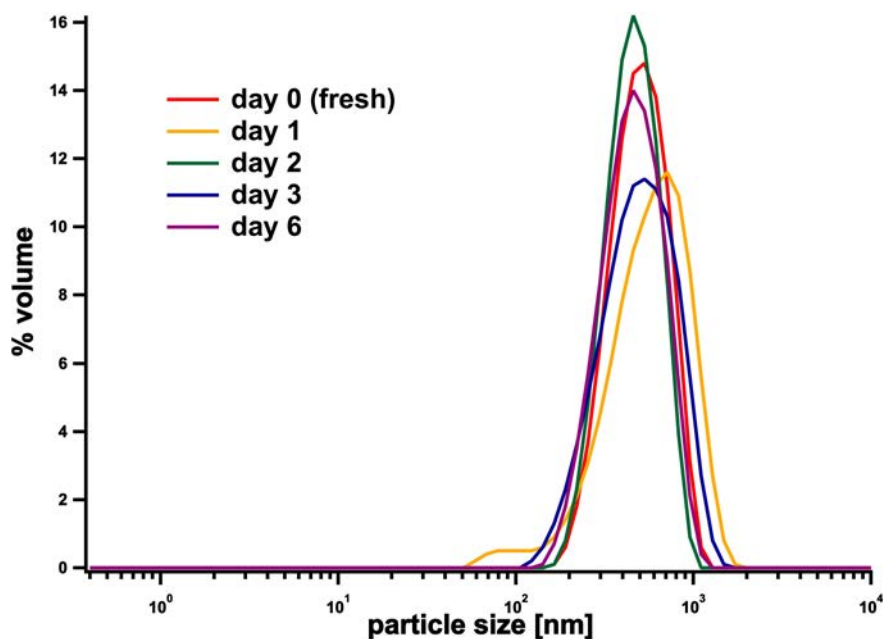


Figure S36. DLS data of **10**. Day 0 (mean = 561 nm, width = 268.4 nm), Day 1 (mean = 720.5 nm, width = 406.0 nm), Day 2 (mean = 504.4 nm, width = 227.3 nm), Day 3 (mean = 605.3 nm, width = 342.4 nm), Day 6 (mean = 520.7 nm, width = 262.1 nm)

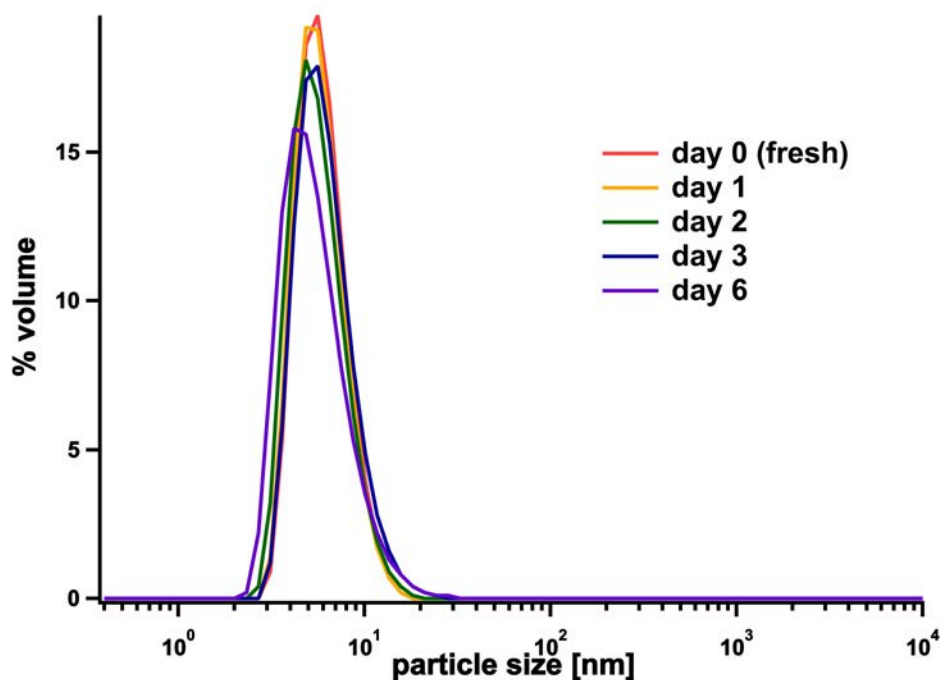


Figure S37. DLS data of **8**. Day 0 (mean = 6.0 nm, width = 2.2 nm), Day 1 (mean = 5.8 nm, width = 2.2 nm), Day 2 (mean = 5.6 nm, width = 2.2 nm), Day 3 (mean = 6.0 nm, width = 2.4 nm), Day 6 (mean = 5.2 nm, width = 2.2 nm).

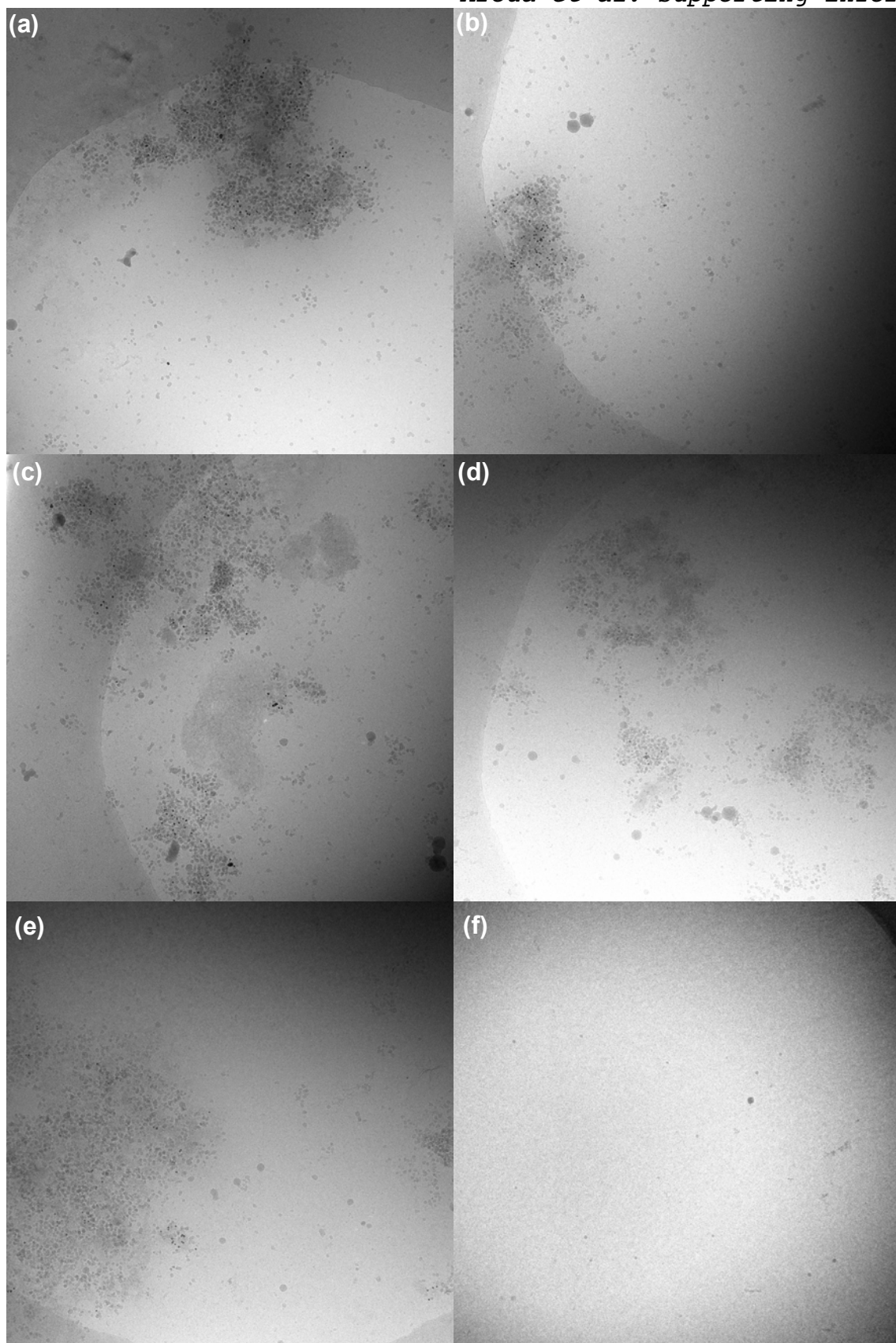


Figure S38. CryoTEM images of C₆₀-PVP **10** (a-e) and PVP-NH₂ **8** (f) (1 mM in water for both). Image size: 2450 nm x 2450 nm. Small black dots are 10nm gold markers.