Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Dynamic Covalent Single Chain Nanoparticles based on Hetero Diels-Alder Chemistry

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Characterization Methods and Equipment

^{1}H NMR Spectroscopy was performed using a Bruker Ascend 400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz). All samples were dissolved in chloroform-d^{1}. The δ-scale is referenced to the internal standard tetramethylsilane (TMS, δ = 0.00 ppm).

ESI-MS (Electrospray Ionization Mass Spectrometry) was performed on a Q Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a HESI II probe. The instrument calibration was carried out in the *m/z* range 74 – 1822 using calibration solutions from Thermo Scientific. A constant spray voltage of 4.7 kV and a dimensionless sheath gas of 5 were applied. The capillary temperature and the S-lens RF level were set to 320 °C and 62.0, respectively. The samples were dissolved on a THF:MeOH micture (3:2) containing 100 μmol of sodium triflate and injected with a flow of 5 $\mu L \cdot min^{-1}$.

SEC (Size Exclusion Chromatography) measurements were performed on a Polymer Laboratories PL-GPC 50 Plus Integrated System, comprising an autosampler, a PLgel 5 μm bead-size guard column (50 × 7.5 mm) followed by three PLgel 5 μm Mixed-C and one PLgel 3 μm Mixed-E columns (300 × 7.5 mm) and a differential refractive index detector using DMAc as the eluent at 50 °C with a flow rate of 1 $mL \cdot min^{-1}$. Calibration was carried out employing linear poly(styrene) standards ranging from 476 to 2.5×10^{6} $g \cdot mol^{-1}$.The injected polymers were dissolved in DMAc (HPLC-grade) with a concentration of 2 $mg \cdot mL^{-1}$.

***DLS* (*Dynamic Light Scattering*)** was performed on a Zetasizer Nano ZS light scattering apparatus (Malvern Instruments, UK) equipped with He-Ne laser (at a wavelength of 633 nm, 4 mW). The Nano ZS instrument incorporates a non-invasive backscattering (NIBS) optic with a detection angle of 173°. The polymer solutions were prepared in DMSO ($c_{Polymer}$ = 2 $mg \cdot mL^{-1}$) and were subsequently filtered into quartz cuvettes. The prepared samples were stabilized prior to DLS analysis at ambient temperature (20 °C) for 120 seconds. All values of the apparent hydrodynamic diameter for each polymer mixture were averaged over six measurements (average of 20 runs/measurement), and were automatically provided by the instrument using a cumulative analysis.

Experimental Data

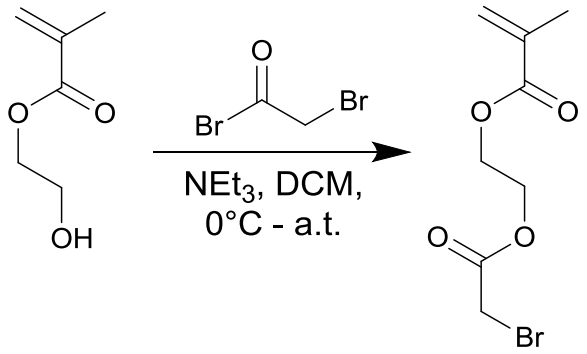
Materials

Acetonitrile (ACN, Normapur, VWR), Azobis(isobutyronitril) (AIBN, Sigma-Aldrich), anhydrous dichloromethane (DCM, 99.8 %, AcroSeal®, Acros), basic Aluminiumoxide (Alox, Acros Organics), Bromoacetyl bromide (98 %, Alfa Aesar), Carbon disulfide (CS_2, 99.9 %, Sigma Aldrich), Chloroform-d1 (99.8 %, EURISO-TOP), 2-cyano-2-propyl benzodithioate (CPBD, 97 %, Sigma-Aldrich), Cyclohexane (cyHex, Normapur, VWR), Dichloromethane (99.5%, VWR), Dicyclopentadiene (stabilized with BHT, Sigma-Aldrich), Diethyl ether (laboratory reagent grade, Fisher), 4-Dimethylaminopyridine (DMAP, Fisher), Dimethylformamide (DMF, Normapur, VWR), Dioxane (99%, Roth), Ethylacetate (EE, Normapur, VWR), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC•HCl, >99%, Roth), Hydrochloric acid ($HCl_{aq.}$, analytic reagent grade, Fisher), (Hydroxyethyl)methacrylate (HEMA, 97 %, Sigma-Aldrich), Isopropyl alcohol (99%, Roth), Malonic acid (99 %, Alfa Aesar), Magnesium sulfate ($MgSO_4$, 99 %, Roth), Methyl methacrylate (MMA, 99%, Sigma-Aldrich), Sodium bicarbonate ($NaHCO_3$, 99%, Roth), Sodium cyanide (NaCN, 97%, Sigma-Aldrich), Sorbic alcohol (SorbOH, 98 %, Alfa Aesar), Toluene (Normapur, VWR), Triethylamine (TEA, NEt_3, 99 %, Acros).

Cyclopentadiene (Cp) was freshly distilled from dicyclopentadiene. Toluene was evaporated once before used. HEMA was destabilized with basic Alox before polymerization. AIBN was recrystallized from ethanol before used. Dioxane was destabilized with basic Alox and stored at 4 °C until used. If not stated otherwise, all chemicals were used as received.

Small Molecule Synthesis

2-(2-bromoacetoxy)ethyl methacrylate (**1**):



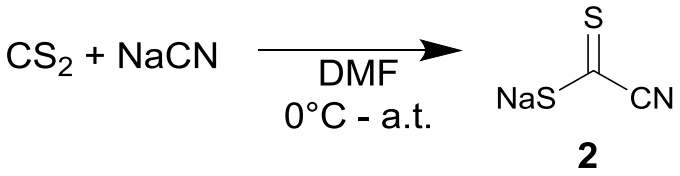
1

HEMA (2.34 mL, 2.50 g, 19.2 mmol, 1 eq.) and TEA (3.99 mL, 2.91 g, 28.8 mmol, 1.5 eq.) were dissolved in dry DCM (25 mL) under inert gas and cooled to 0 °C. Bromoacetyl bromide (2.01 mL, 4.66 g, 23.1 mmol, 1.2 eq) diluted in dry DCM (10 mL) was added carefully over a period of 30 minutes. The reaction was allowed to warm to ambient temperature and stirred over night. The reaction mixture was extracted twice with saturated $NaHCO_{3(aq.)}$, once with 1M $HCl_{(aq.)}$ and dried over $MgSO_4$. The crude product was isolated by removal of the solvent under reduced pressure and purified *via* column chromatography with cyHex/ EE (5/1) as eluent, yielding a slightly yellowish liquid (2.10 g, 8.36 mmol, 44 %).

^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm) = 6.1 (1 H, m, =C**H**H), 5.5 (1 H, m, =C**H**H), 4.3 (4 H, m, -C**H**$_2$-), 3.8 (2), 1.9 (3H, dd, J = 1.6, 1.0 Hz, =CC**H**$_3$)

^{13}C NMR ($CDCl_3$, 400 MHz), δ (ppm) = 167.1, 167.0, 135.8, 126.3, 63.8, 62.0, 25.5, 18.2.

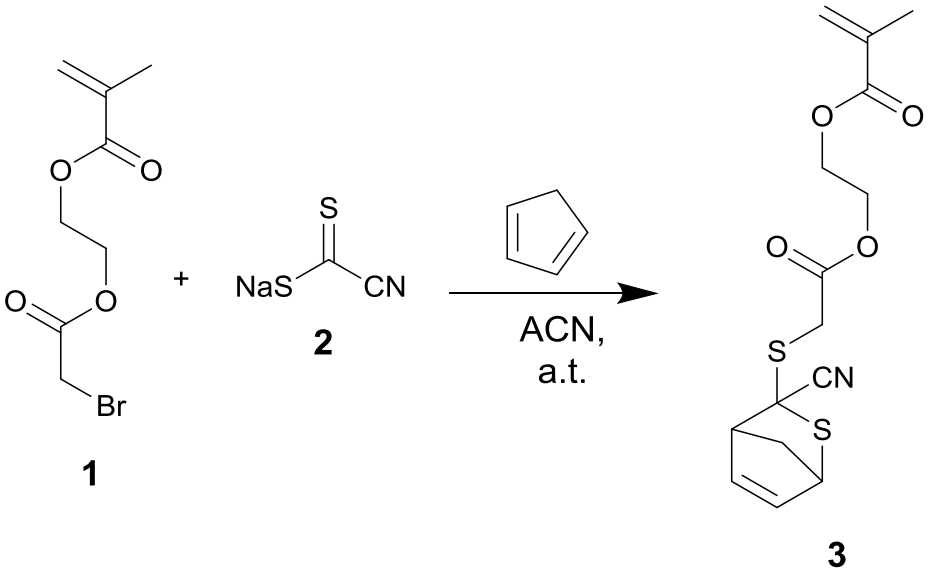
Sodium carbonocyanidodithioate **2**:



2

2 was synthesized according to a modified literature protocol.[1] Sodium cyanide (5,46 g, 111 mmol, 1.1eq) dissolved in dimethylformamide (DMF, 20 mL) and cooled to 0 °C was combined dropwise with carbon disulfide (7.75 g, 102 mmol, 1 eq) dissolved in DMF (13 mL). The reaction was allowed to warm to ambient temperature and was stirred until complete solidification. Isopropyl alcohol (150 mL) was added and the mixture heated to 90 °C. The residual NaCN was filtered off the warm solution, which was subsequently cooled in liquid nitrogen until precipitation of the product. The product was filtered off, washed with diethyl ether and recrystallized from a mixture of isopropyl alcohol and diethyl ether (1:1). The product was obtained as mustard colored brown needles (7.38 g, 59.0 mmol, 58 %) and was used without further characterization.

CDTE Monomer **3**:



Bromide **1** (1.80 g, 7.17 mmol, 1 eq.) and Cp (1.60 mL, 1.28 g, 19.4 mmol, 2.7 eq.) were dissolved in ACN (25 mL) and combined at ambient temperature with a brown solution of salt **2** (2.02 g, 16.1 mmol, 2.25 eq.) in ACN (25 mL). After 5 h the brown color of the reaction mixture disappeared and NaBr had precipitated, resulting in a turbid, yellow solution. ACN was removed *in vacuo*, the residue was dispersed in EE and filtered over a short column with silica to remove solids. Subsequently, the crude product was isolated again under reduced pressure and purified *via* column chromatography in cyHex / EE (5/1). The product was obtained as colorless liquid (1.01 g, 2.98 mmol, 42 %). The ^{1}H-NMR spectrum is provided in Figure S1.

^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm) = 6.7-6.3 (1 H, m, C=C**H**$_{CP}$), 6.1-6.0 (2 H, m, C=C**H**$_{CP}$, (CH_3)C=C**H**$_{Acryl}^{trans}$), 5.6-5.5 (1 H, m, (CH_3)C=C**H**$_{Acryl}^{cis}$), 4.4-4,2 (6 H, m, CO-O-C**H$_2$**-C**H$_2$**-O-CO, CH_2^{bridge}), 3.9-3.4 (3 H, m, O-CO-C**H$_2$**-S-, -C**H**-(CH_2^{bridge})-CH=CH), 2.2-1.8 (5 H, m, C**H$_2^{bridge}$**, C**H$_3$**).

^{13}C NMR ($CDCl_3$, 400 MHz), δ (ppm) = 169.0, 167.1, 141.7, 138.5, 135.8, 131.3, 130.2, 126.3, 119.6, 63.6, 62.1, 56.5, 55.4, 51.6, 48.9, 35.8, 18.3.

ESI-MS: $[M+Na]^+$ experimental value: 362.05 *m/z*, theoretical value: 362.05 *m/z*

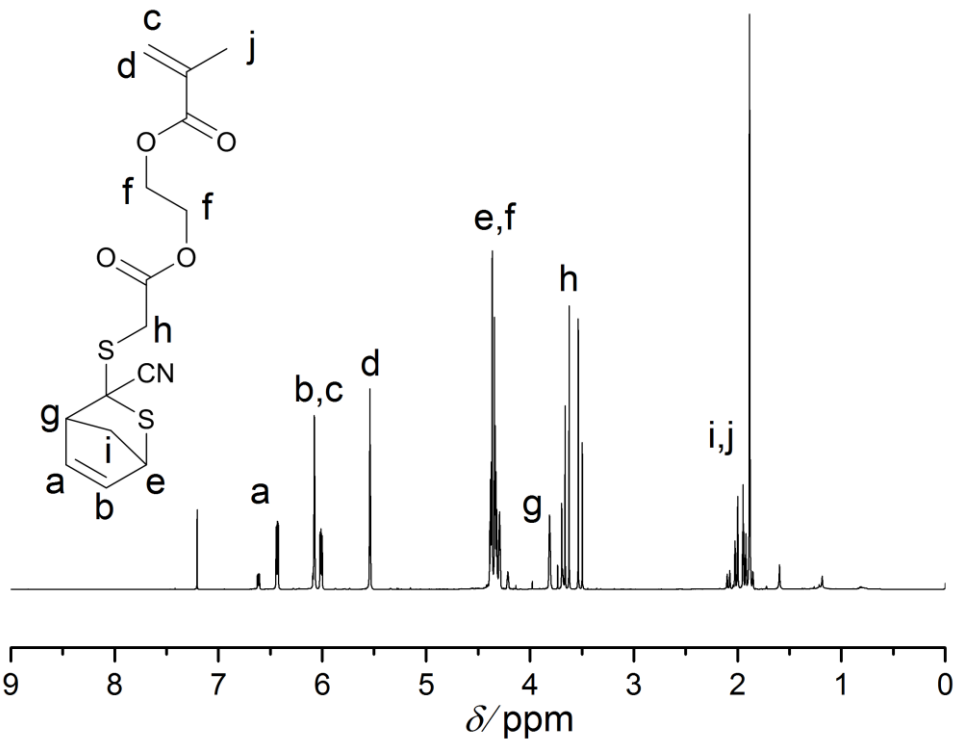
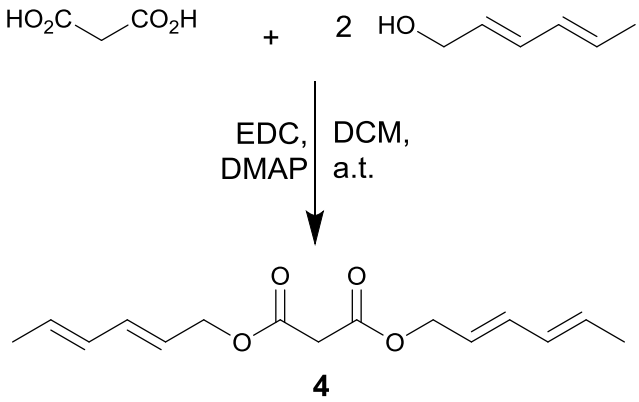


Figure S1. ^{1}H-NMR spectrum of **3** in $CDCl_3$.

Sorbic-Bilinker **4**:



4

Malonic acid (1.17 g, 11.2 mmol, 1.0 eq), sorbic alcohol (2.20 g, 22.4 mmol, 2.0 eq.), DMAP (6.23 mg, 5.10 mmol, 0.5 eq) and EDC·HCl (6.84 g, 35.7 mmol, 3.0 eq) were dissolved in dry DCM (90 mL) and stirred for 24 h at ambient temperature. The solution was extracted with 1M HCl, (3 x 80 mL), 1M KOH (80 mL) and deionized water (80 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified *via* column chromatography with cyHex / EE (20/1) and obtained as pale yellow liquid (1.41 g, 5.32 mmol, 48 %), which solidifies in the fridge. The ^{1}H-NMR spectrum is provided in Figure S2.

^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm) = 6.24-6.14 (2H, m, 2 × OCH_2CH=C**H**), 6.03-5.92 (2H, m, 2 × CH_3CH=C**H**), 5.76-5.63 (2H, m, 2 × OCH_2C**H**=CH), 5.58-5.49 (2H, m, 2 × CH_3C**H**=), 4.57 (4H, m, 2 × O**CH_2**), 3.32 (2H, s, $(CO_2)_2C$***H***$_2$), 1.70 (6H, m, 2 × C***H***$_3$).

^{13}C NMR ($CDCl_3$, 400 MHz), δ (ppm) = 166.27, 136.46, 131.46, 130.33, 122.92, 66.00, 41.60, 18.15.

ESI-MS: $[M+Na]^+$-Ion found; experimental value: 287.12 *m/z*, theoretical value: 287.12 *m/z*. $[M_2+Na]^+$ experimental value: 551.26*m/z*, theoretical value: 551.26 *m/z*

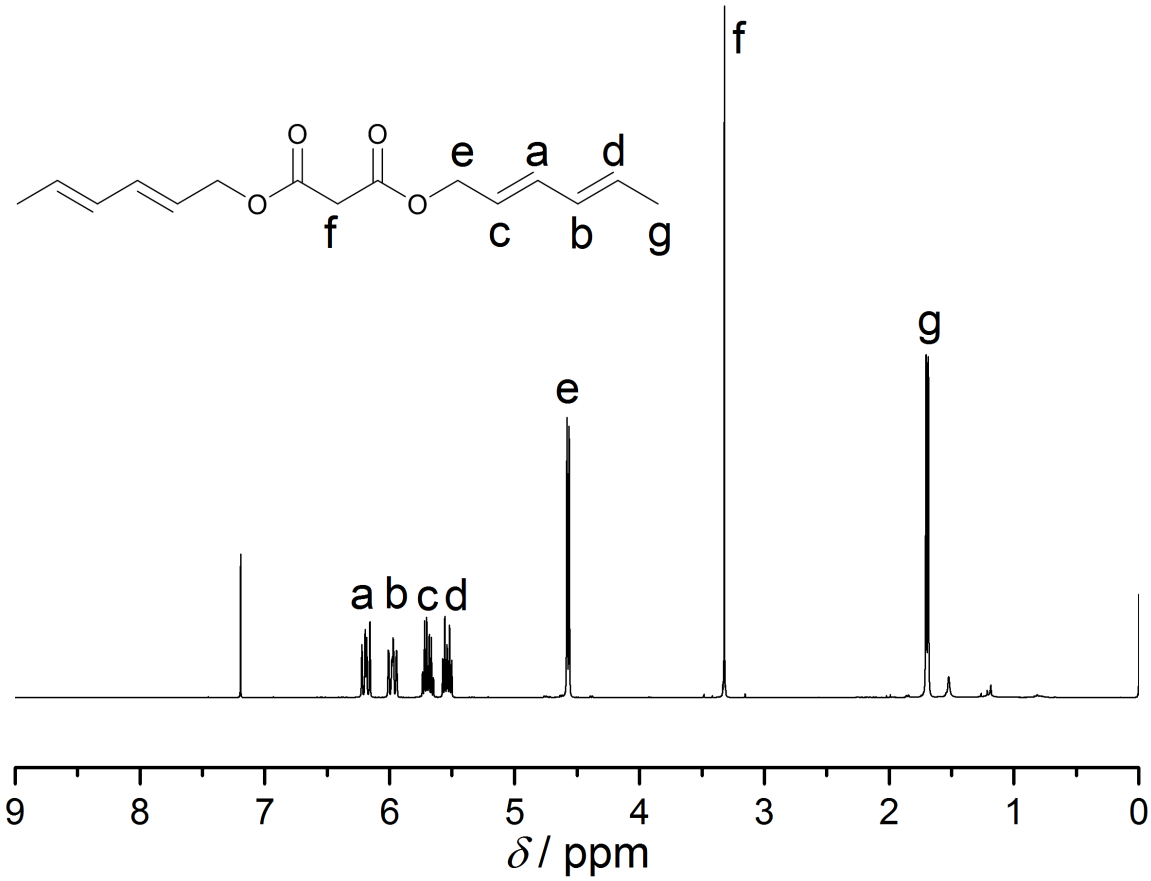
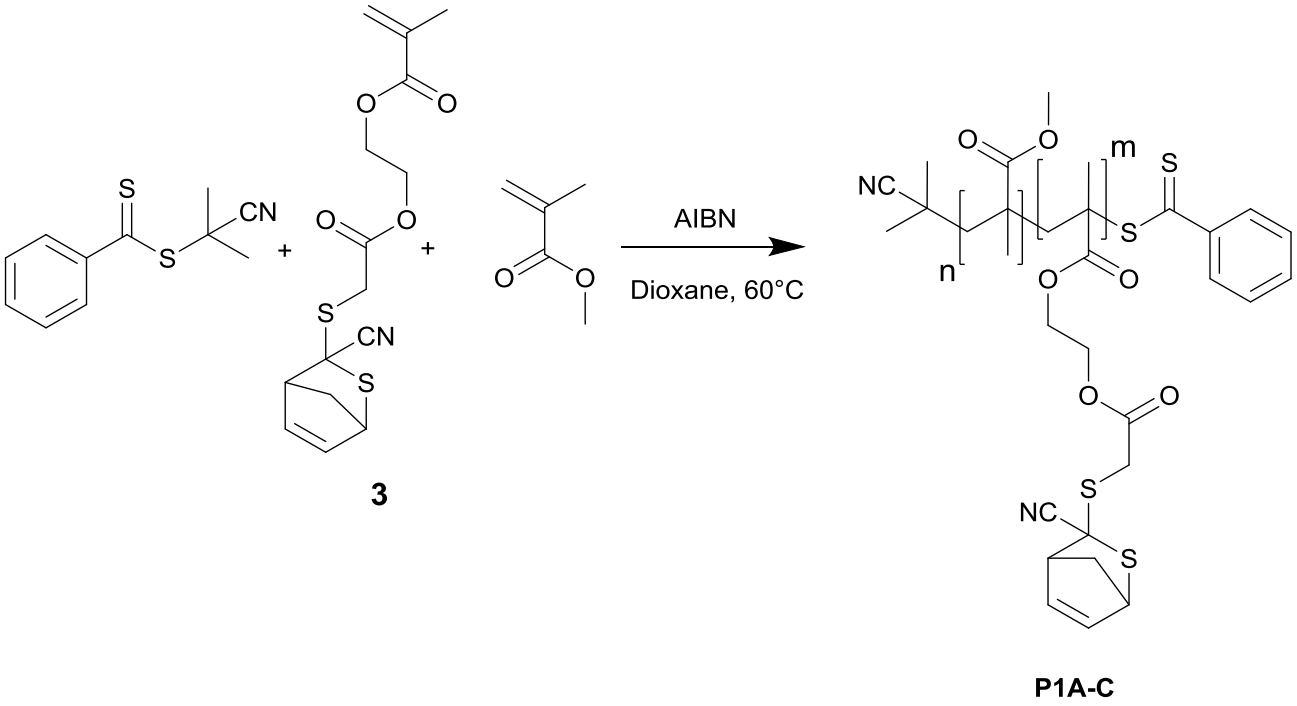


Figure S2. ^{1}H-NMR spectrum of **4** in $CDCl_3$.

Polymer and SCNP Synthesis

Synthesis of open chain precursors **P1A-C**:



3

P1A-C

In a typical experiment, MMA (Table S1), Monomer **3** (Table S1), CPDB (11.0 mg, 0.5 mmol, 1 eq) AIBN (1.64 mg, 0.1 mmol, 0.2 eq) and dioxane (Table S1) were combined in schlenk tube and degassed *via* three freeze-pump-thaw cycles. The polymerization was carried out under inert gas at 60 °C for a defined period of time (Table S1). After polymerization the mixture was cooled to ambient temperature and precipitated in cold cyHex (20 mL). The pink solid was dissolved in DCM (1 mL) and precipitation was repeated twice. The final polymer was characterized *via* SEC and ^{1}H-NMR. The ^{1}H-NMR spectra are provided below (Figure S3,10,11), while GPC elugrams are provided in comparison with the data of the corresponding SCNPs (Figure 2b, 3).

Table S1. Masses, moles and equivalents of MMA and **3** in the RAFT processes towards **P1A-C**.

Polymer	*MMA*			*CDTE Monomer **3***			*t* [h]	$V_{Dioxane}$ [mL]
	m [mg]	*n* [mmol]	*eq.*	*m* [mg]	*n* [mmol]	eq.		
P1A	626	62.5	125	424	12.5	25	40	0.600
P1B	901	90	180	339	10	20	16	0.600
P1C	500	50	100	339	10	20	16	0.500

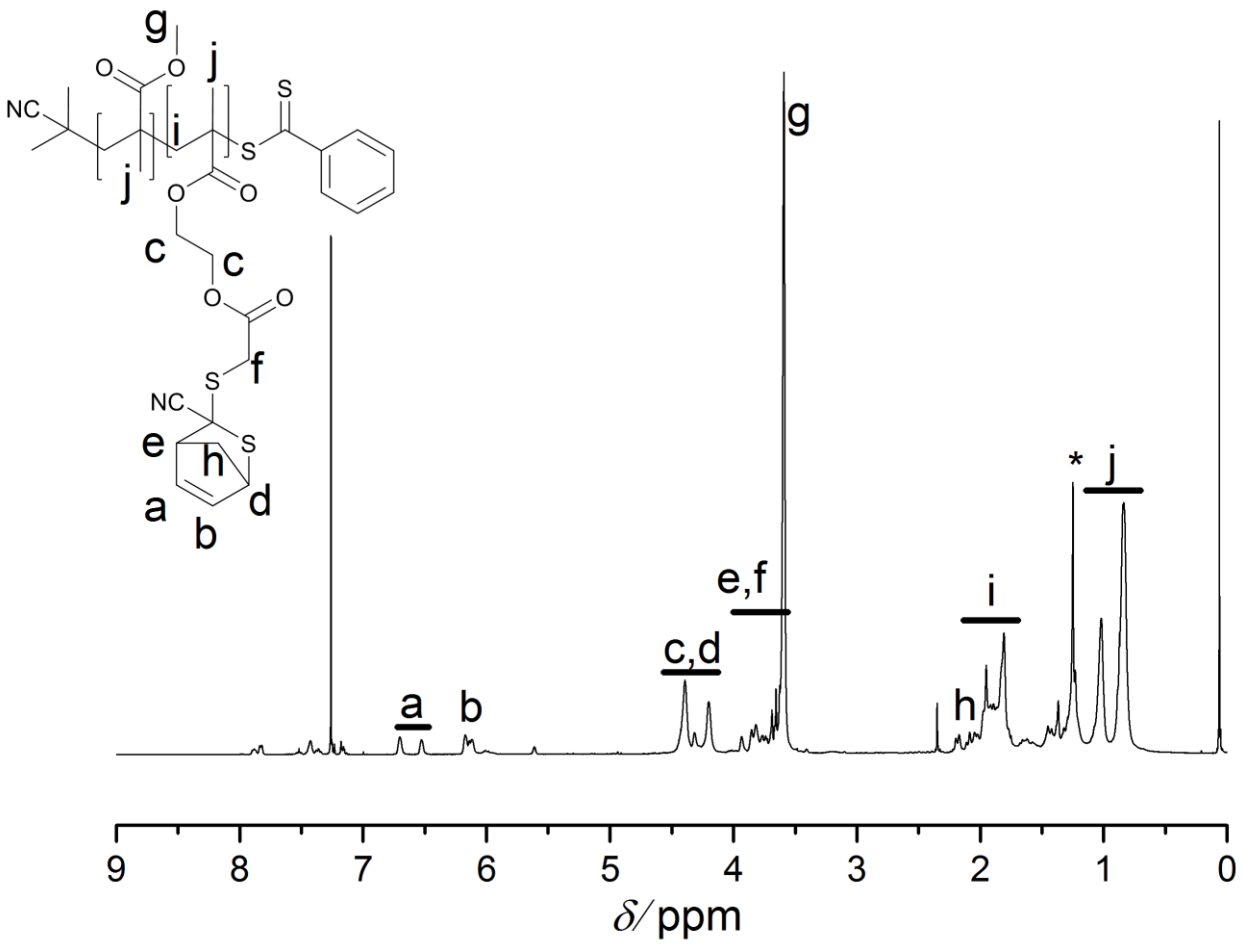
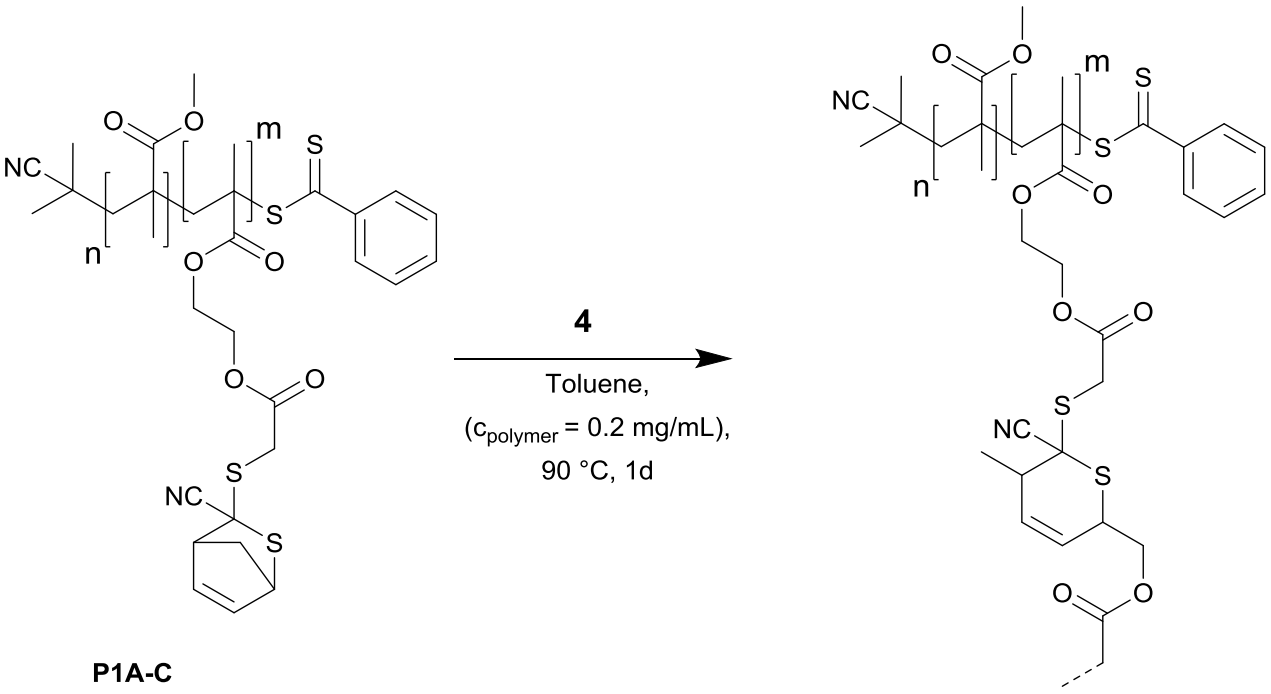


Figure S3. Representative ^{1}H-NMR spectrum of **P1A** in $CDCl_3$. * = cyHex

Collapse towards SCNPs **P2A-C**:



60 mg Polymer **P1A-C** was dissolved in toluene (300 mL) and bilinker (**4**) (Table S2) was added. The solution was stirred for 1 d at 90 °C. To isolate the SCNP, the reaction volume was decreased under reduced pressure (2~3 mL) and the SCNP was precipitated in cold cyHex (20 mL) and received as grayish powder after drying. The SCNPs (**P2A-C**) were characterized by comparative SEC measurement and ^{1}H-NMR. Comparative SEC elugrams (Figure 2b, 3) and the ^{1}H-NMR spectra (Figure S4,12,13) are provided. The kinetic of the folding process is shown in Figure S6.

Table S2. Calculation of masses and moles of **4** based on relative equivalents towards CDTEs in the polymer backbone.

	Polymer				***Bilinker (4)***		
	M_N	mol% CDTE	CDTEs per Chain	mmol CDTE (per 60 mg polymer)	*m* [mg]	*n* [mmol]	eq. (per CDTE)
P1A	10.0k	17	11.9	0.071	9.4	0.036	0.5
P1B	7.9k	10	6.4	0.048	6.4	0.024	0.5
P1C	8.1k	17	9.6	0.071	9.4	0.036	0.5

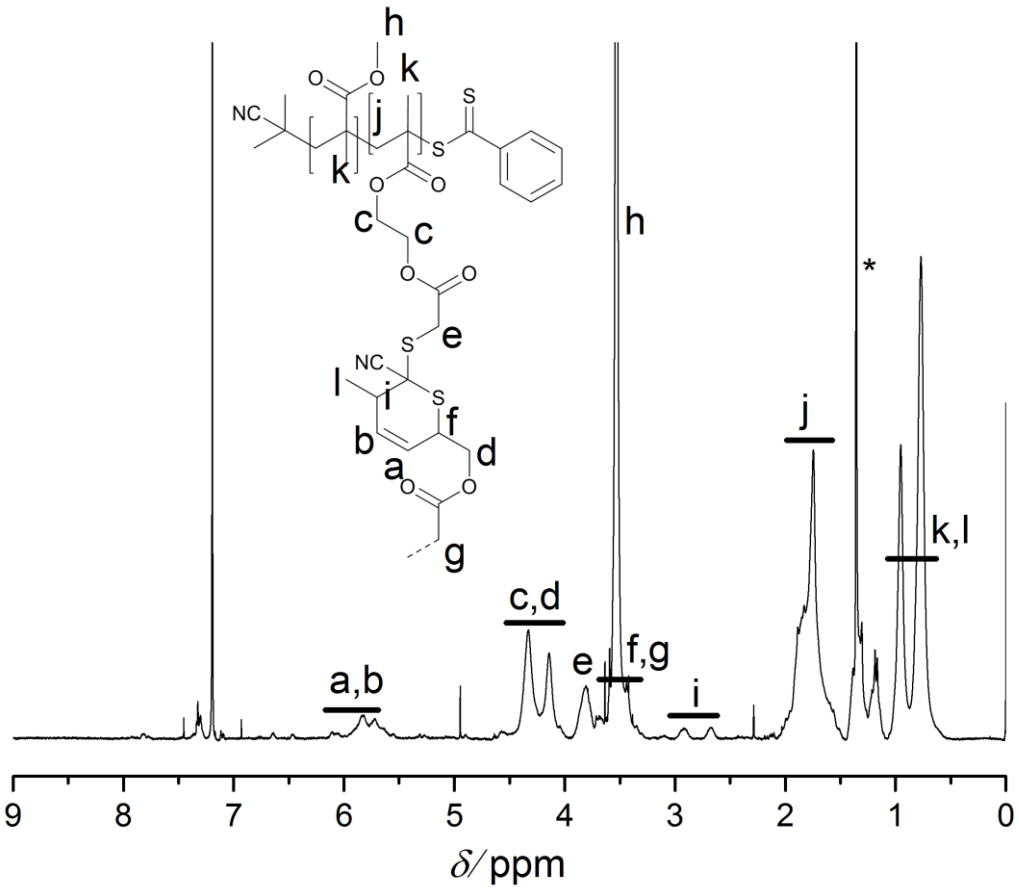
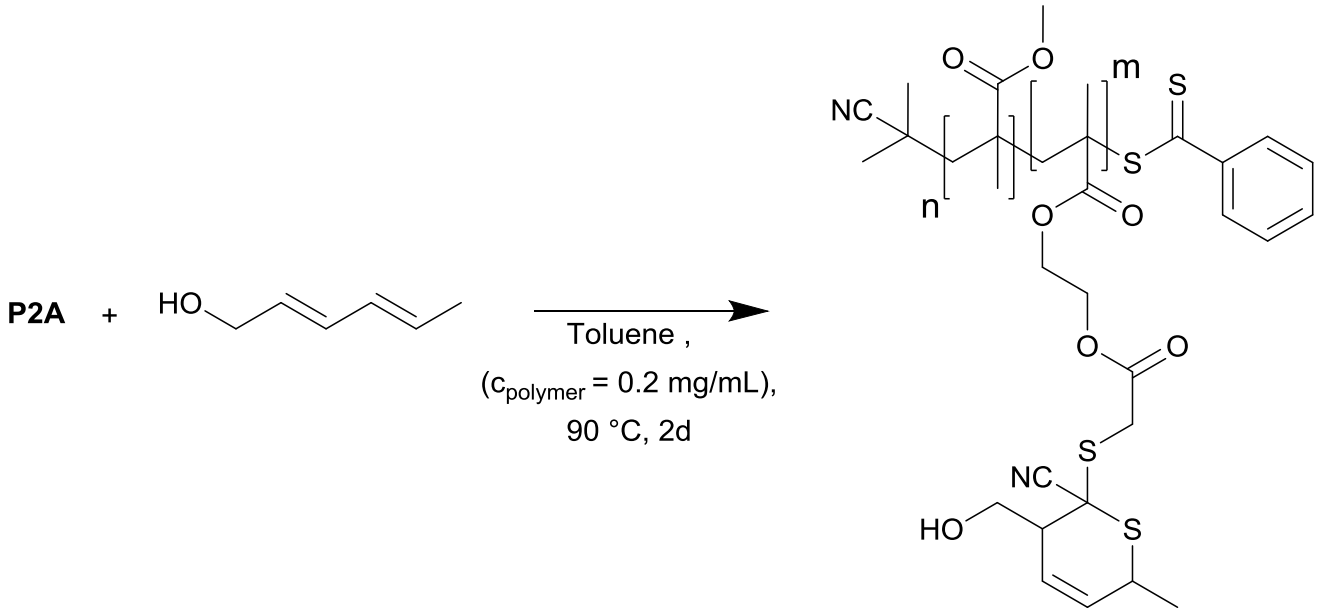


Figure S4. Representative ^{1}H-NMR spectrum of **P2A** in $CDCl_3$. * = cyHex

Chain unfolding towards **P3**:



P3

SCNP **P2A** (30 mg, 0.003 mmol, 0.036 mmol CDTE) was dissolved in toluene (150 mL) and sorbic alcohol was added (17.5 mg, 0.18 mmol). The reaction mixture was heated to 90 °C and stirred for 2 d. For isolation the reaction volume was reduced to 2~3 mL under reduced pressure and precipitated in cold cyHex (20 mL). The grayish powder **P3** was characterized by comparative SEC measurement with **P1A** and **P2A** (Figure 2b), ^{1}H-NMR spectroscopy (Figure S5) and comparative DLS measurement with **P1A** and **P2A** (Figure 2c). The kinetic of the unfolding process is provided in Figure S6.

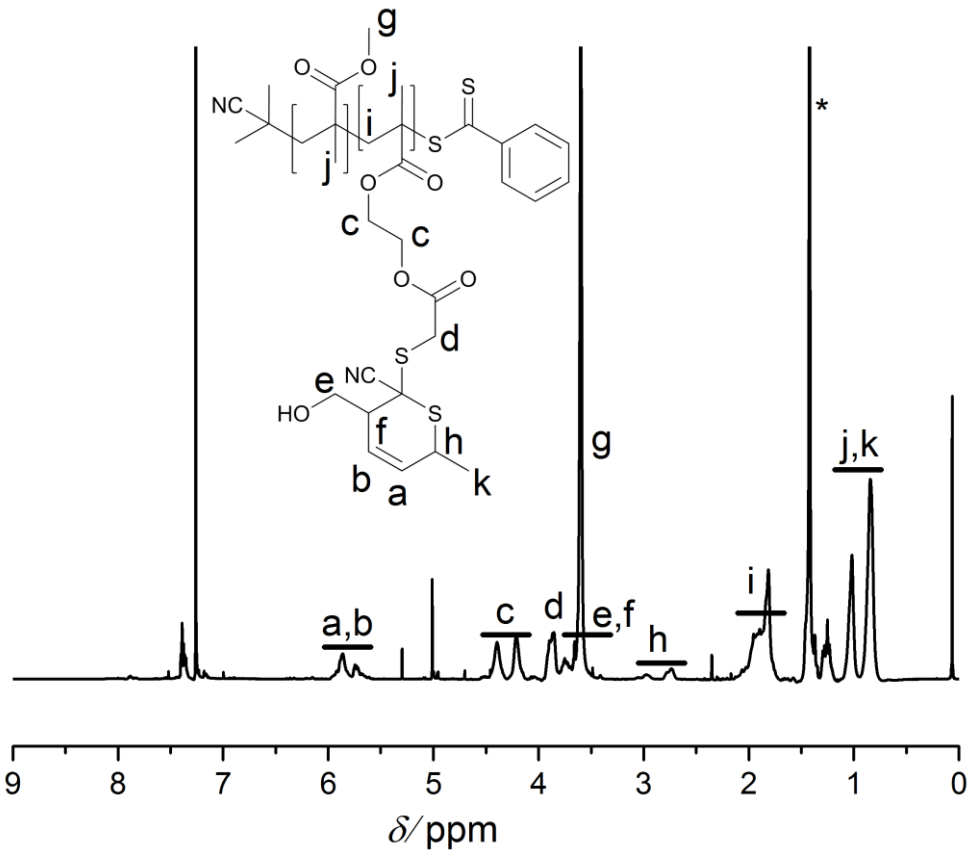


Figure S5. ^{1}H-NMR spectrum of **P3** in $CDCl_3$. * = cyHex

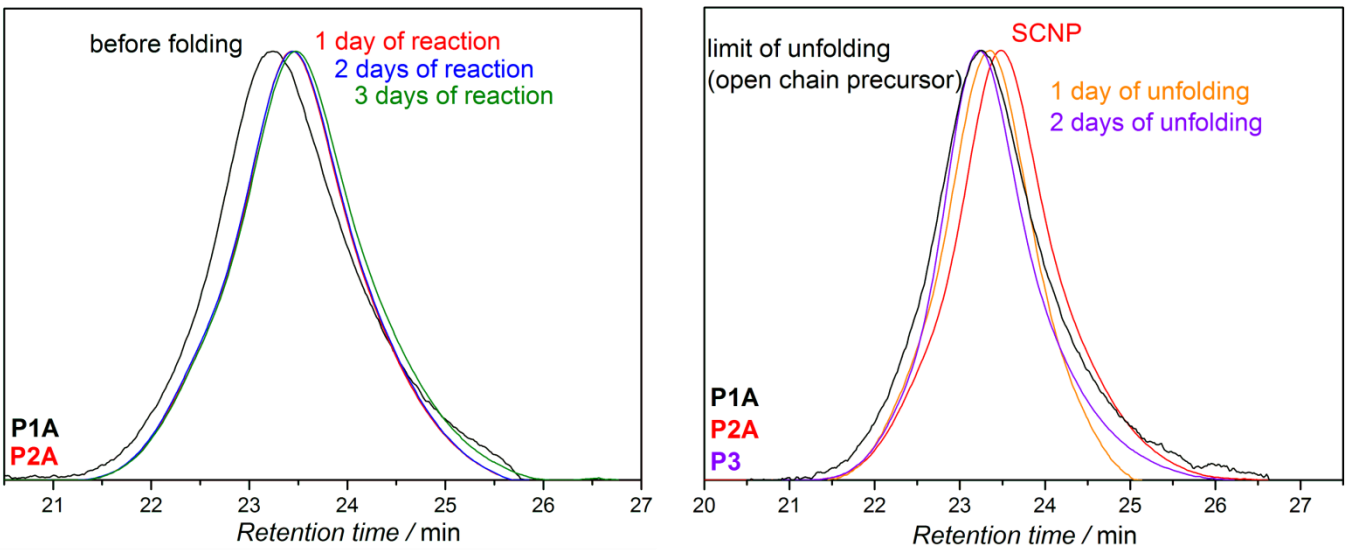
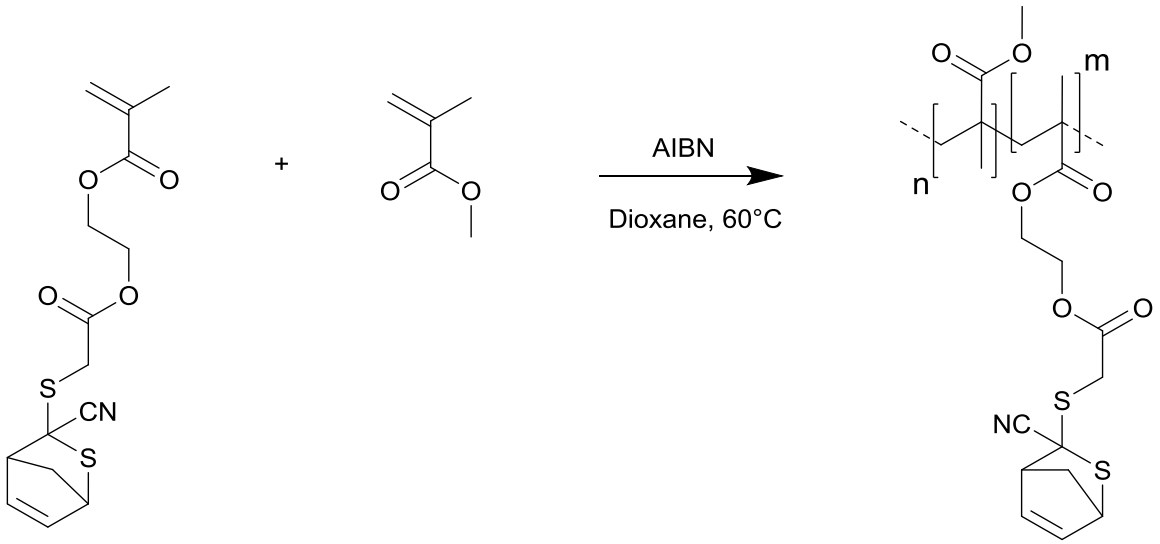


Figure S6: SEC kinetics of SCNP (**P2A**) formation starting from **P1A** (left) and kinetics of unfolding of SCNP **P2A** towards **P3** (right). The folding is complete after one day reaction while the unfolding is final after two days of reaction.

Synthesis of open chain precursor **P4**.



3 **P4**

MMA (668.6 mg, 6.7 mmol, 170 eq), Monomer **3** (400 mg, 1.2 mmol, 30 eq), AIBN (6.5 mg, 0.04 mmol, 1 eq) and dioxane (3.5 mL) were combined in a Schlenk tube and degassed *via* three freeze-pump-thaw cycles. The polymerization was carried out under inert gas at 60 °C for 5 h. After polymerization, the mixture was cooled to ambient temperature and precipitated in cold cyHex (20 mL). The white solid was dissolved in DCM (1 mL) and precipitation was repeated twice. The final polymer was characterized *via* SEC and ^{1}H-NMR. The ^{1}H-NMR spectrum is provided below (Figure S7), while the GPC elugram is provided in comparison with the data of the corresponding SCNP (Figure S9).

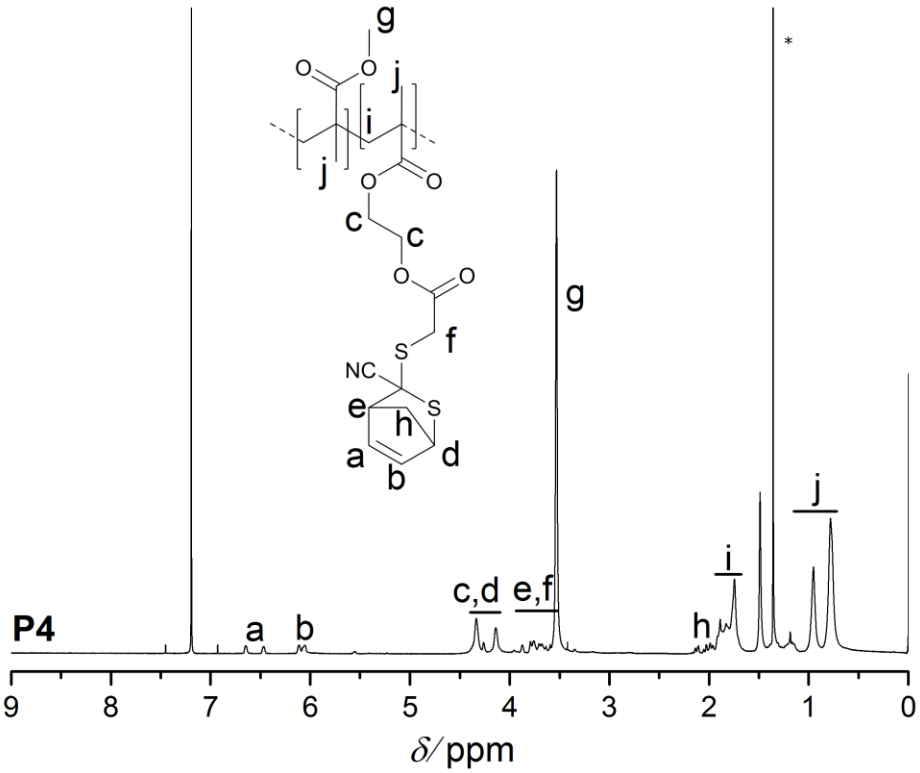
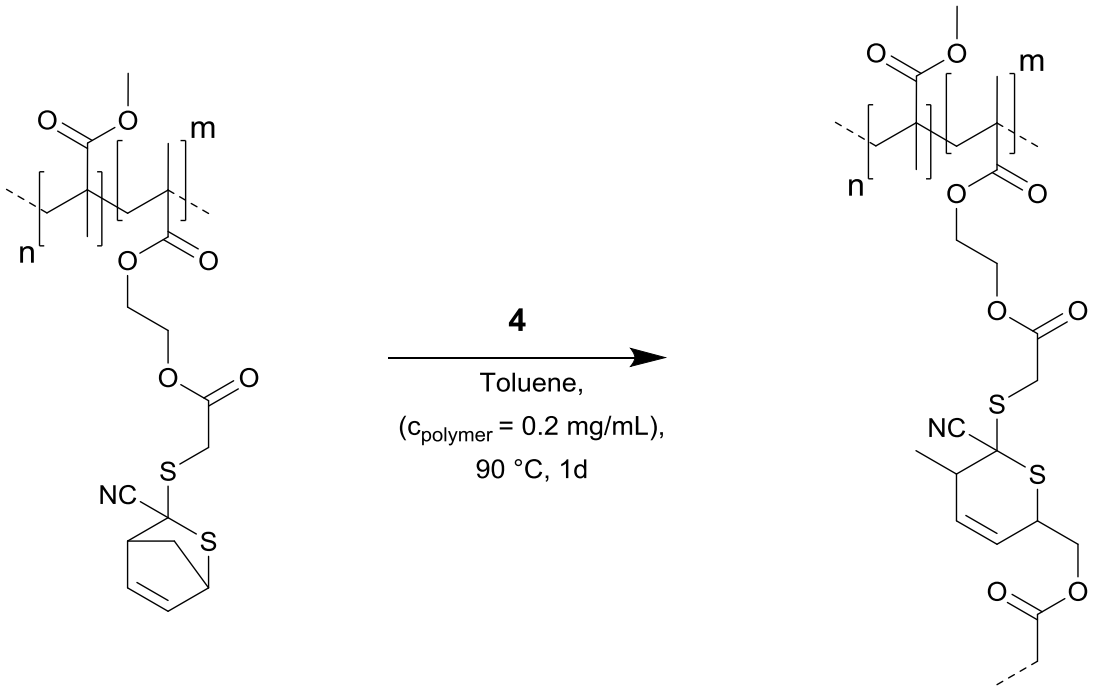


Figure S7: ^{1}H-NMR spectrum of **P4** in $CDCl_3$. * = cyHex

Collapse of **P4** towards **P5.**



P4 **P5**

Polymer **P4** (15 mg, 0.0165 mmol of CDTE) was dissolved in toluene (75 mL) and bilinker (**4**) (2.2 mg, 0.008 mmol) was added. The solution was stirred for 1 d at 90 °C. To isolate the SCNPs, the reaction volume was decreased under reduced pressure (2~3 mL) and the SCNP was precipitated in cold cyHex (20 mL) and obtained as a white powder after drying. The SCNP **P5** was characterized by comparative SEC measurement and ^{1}H-NMR. Comparative SEC elugrams (Figure S9) and the ^{1}H-NMR spectrum (Figure S8) are provided.

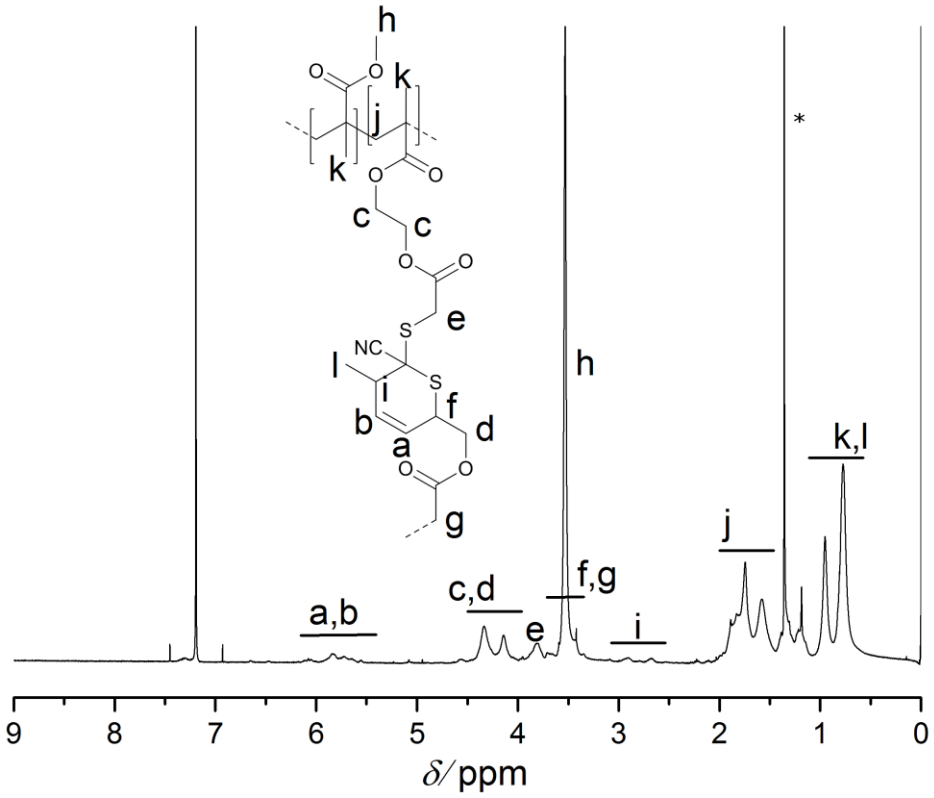


Figure S8: ^{1}H-NMR spectrum of **P5** in $CDCl_3$. * = cyHex

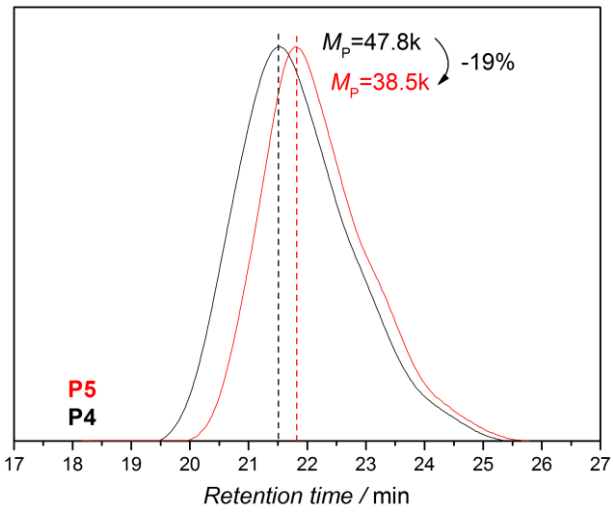


Figure S9: SEC traces of open chain precursor **P4** and the corresponding SCNP **P5**.

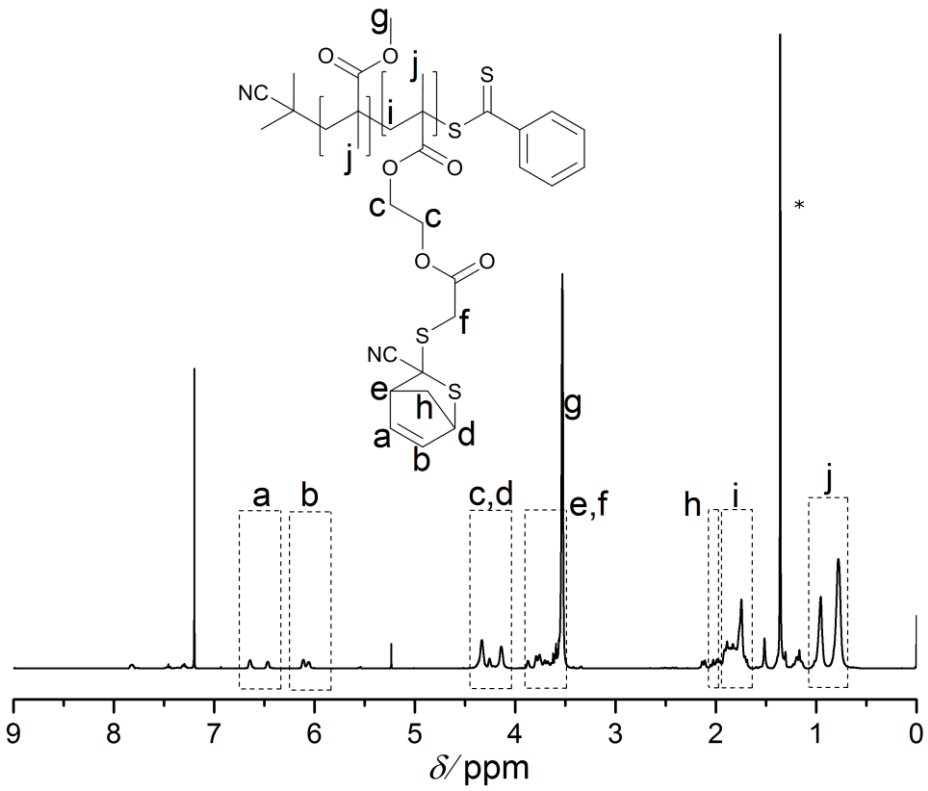


Figure S10: ^{1}H-NMR spectra of **P1B** in $CDCl_3$. * = cyHex

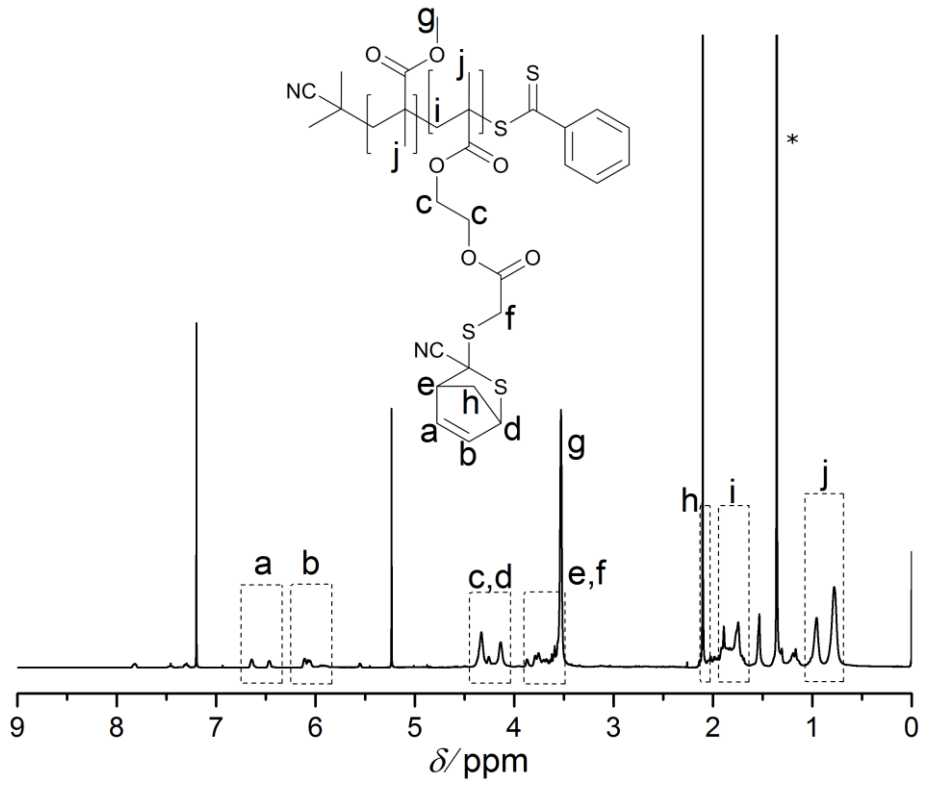


Figure S11: ^{1}H-NMR spectra of **P1C** in $CDCl_3$. * = cyHex

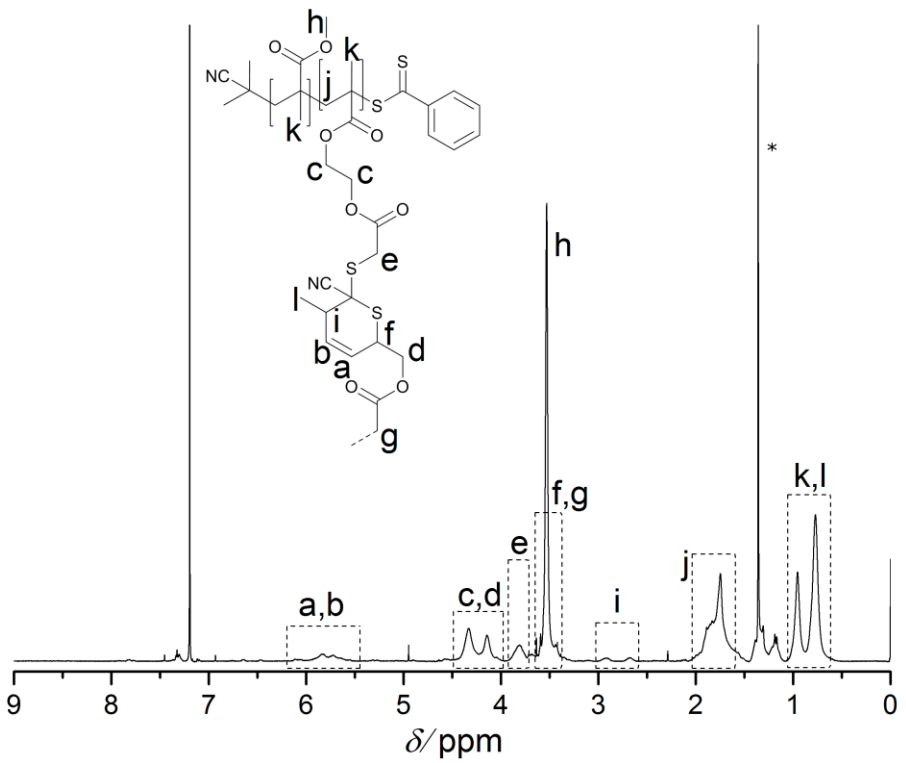


Figure S12: ^{1}H-NMR spectra of **P2B** in $CDCl_3$. * = cyHex

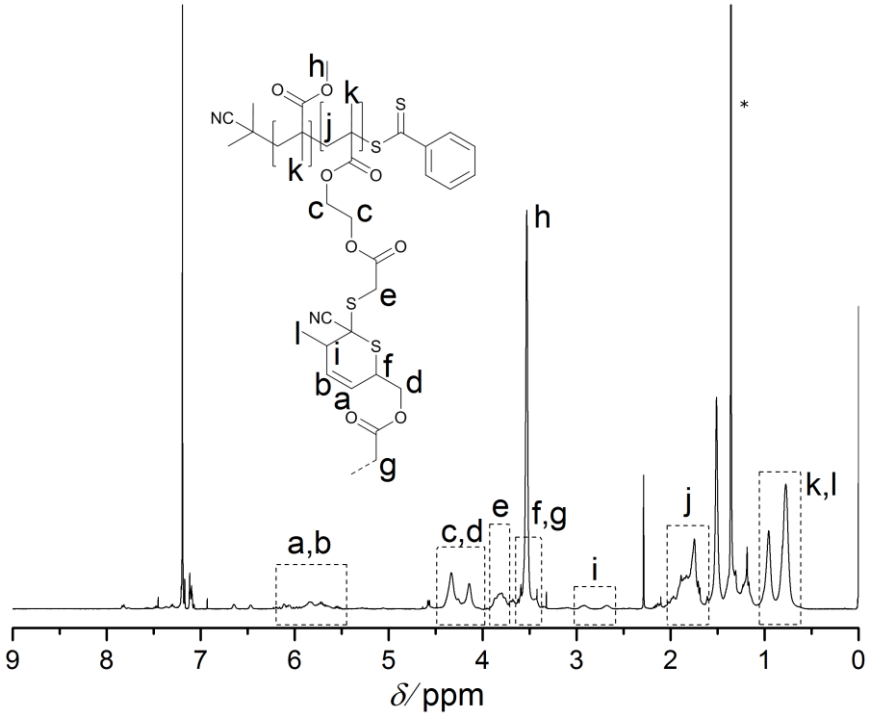


Figure S13: ^{1}H-NMR spectra of **P2C** in $CDCl_3$. * = cyHex

References

1. K. K. Oehlenschlaeger, J. O. Mueller, J. Brandt, S. Hilf, A. Lederer, M. Wilhelm, R. Graf, M. L. Coote, F. G. Schmidt and C. Barner-Kowollik, *Adv. Mater.*, 2014, **26**, 3561-3566.