Electronic Supplementary Material (ESI) for Chemical Communications

Complex Single-chain Polymer Topologies locked by Positionable Twin Disulfide Cyclic Bridges.

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CONTENT:

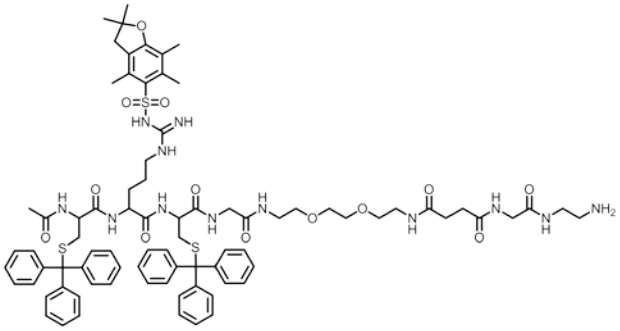
A. Materials and experimental procedures

A.1. Chemicals.

Pentafluorophenyl 4-maleimidobenzoate (PFP-MI **1**), 1,2-bis(bromopropionyloxy)ethane, diethyl 2,2-dimethyl-1-(1,1-dimethylethylamino)propylphosphonate and *N*-*tert*-Butyl-*N*-[1-diethylphosphono(2,2-dimethylpropyl)]nitroxide were synthesized according to literature procedures.[1-4] Styrene (S, Sigma Aldrich, 99 %) was distilled over CaH_2 (90-95%) under reduced pressure and stored under argon atmosphere at -15 ºC. Copper (I) bromide (CuBr, Sigma-Aldrich, 98%) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried. Dry toluene was distilled over sodium with deep red 1,1-diphenylethylene /*sec*-butyllithium. The copper powder (Cu, Alfa Aesar, 99.5%), *N,N,N′,N″,N‴*-pentamethyldiethylenetriamine (Sigma-Aldrich, 99%), *N*-(*tert*-butyl)-*N*-(1-diethylphosphono-2,2-dimethylpropyl)-*O*-(2-carboxylprop-2-yl)-hydroxylamine (BlocBuilder MA® **2**, Arkema, 97 %), anisole (Sigma-Aldrich, 99%), trifluoroacetic acid (TFA, Sigma-Aldrich, 99%), triisopropylsilane (TIS, Sigma-Aldrich, 99%), *N*-diisopropylethylamine (DIPEA, Alfa Aesar, 99%), 1-methyl-2-pyrrolidinone (NMP, Sigma-Aldrich, 99%), dimethyl sulfoxide (DMSO, Sigma Aldrich, >99.6%), 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent, Alfa Aesar, 99%) were used as received.

Chemicals for peptide synthesis: N-α-Fmoc protected amino acids (Fmoc-Gly-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Cys(Trt)-OH), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronoium hexafluorophosphate (HBTU) were used as received from IRIS Biotech GmbH (Marktredwitz, Germany). 2-Chlortrityl chloride resin preloaded with 1,2-diaminoethane, 0,79mmol/g-1 loading, have been used as described. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, Acros, 99+%,pure), acetic anhydride (Roth,≥99%, for synthesis), 1-hydroxybenzotriazole hydrate (HOBt, Sigma-Aldrich,purum) have been used as received. Piperidine (Alfa Aesar, 99%) and *N*,*N*,*N*-diisopropylethylamine (DIPEA; IRIS Biotech, 99,96%) were distilled from KOH prior to use. *N*-methyl-2-pyrrolidone (NMP, 99.9+ %, peptide synthesis grade, IRIS Biotech) was purified by filtration through a column (10×60 cm filled with aluminum oxide and silica gel) at a rate of 1 $mL \cdot min^{-1}$. Dichloromethane (DCM, IRIS Biotech, peptide grade) was distilled from CaH_2 prior to use. Fmoc-4-(2-(2-(2-aminoethoxy)ethoxy)ethylamino)-4-oxobutanoicacid) was prepared as described previously.[5]

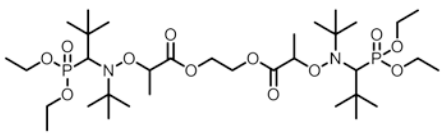
A.2. Synthesis of oligomer 3.



Peptide synthesis was performed on an Applied Biosystems 433a peptide synthesizer in a 0.25 mmol scale, using a 2-chlorotrityl chloride solid support that has been preloaded with 1,2-diaminoethane (0.35 mmol/g). Fmoc-amino acid derivatives were coupled following standard ABI-Fastmoc protocols (no capping, with single coupling except first glycine and Fmoc-4-(2-(2-(2-aminoethoxy)ethoxy)ethylamino)-4-oxobutanoic acid which were coupled via double coupling). Coupling was performed in NMP facilitated by HBTU/DIPEA. After final Fmoc removal the N-terminus was acetylated with a solution of acetic anhydride, DIPEA, HOBt in NMP. The support was washed finally with DCM and dried overnight under vacuum at 25°C. The peptide was liberated from the resin by treatment with 1,1,1,3,3,3-Hexafluoro-2-propanol in DCM (20/80 v/v%) for 0.5 h to obtain the fully protected peptide. The peptide was isolated by diethyl ether precipitation, washed once with diethyl ether, centrifugation and lyophilization from acetonitrile/water. The chemical identity of the product was confirmed by MALDI-TOF-MS.

MALDI-TOF-MS: Theo. mass 1546.9 Da; exp. m/z 1548.1 assignable to $[M+H]^+$. Further ion adducts can be observed with m/z 1569.7 and 1585.7 assignable to $[M+Na]^+$ and $[M+K]^+$ respectively. Additionally mass signals could be found with low intensity that correspond to removed protective groups (-Trt, -Pbf). These are probably caused by in source fragmentation as occasionally observable due to high laser intensity required to desorb fully protected peptides.

A.3. Synthesis of bifunctional initiator 4



The synthesis of bifunctional initiator **4** was adapted from a literature procedure[4] using 1,2-bis(bromopropionyloxy)ethane as a precursor. To Cu(0) (0.81 g, 0.0127 mol, 2 Eq.) and CuBr (1.82 g, 0.0127 mol, 2 Eq.) mixed in 44 mL dry toluene under argon atmosphere was added degazed *N,N,N′,N″,N‴*-pentamethyldiethylenetriamine (5.30 mL, 0.0254 mol, 4 Eq.). In a second flask, *N*-*tert*-Butyl-*N*-[1-diethylphosphono(2,2-dimethylpropyl)]nitroxide (4.40 g, 0.0127

mol, 2 Eq.), 1,2-bis(bromopropionyloxy)ethane (2.11 g, 0.0063 mol, 1 Eq.) were dissolved in 8 mL dry toluene under argon atmosphere. This solution was added dropwise to the first mixture. The dark green mixture was stirred 3 hours at RT. The crude product was filtered through celite to remove the copper salts. The celite was well washed with 500 mL toluene. The filtrate was washed with 3 x 250 mL water until the aqueous phase was no more colored. The organic phase was dried over Na_2SO_4 and the solvent was evaporated. The crude compound was purified by chromatography on silica gel (eluent: diethyl ether/pentane 1/1 to diethyl ether/methanol 95/5, v/v) to give the **4** as a pale yellow oil (3.86 g, 80 %). ^{1}H NMR ($CDCl_3$), δ (ppm): 1.05 - 1.12 (m, 36H, $(CH_3)_3$-C-), 1.24 (m, 12H, CH_3-CH_2-), 1.42 - 1.48 (dd, 6H, CH_3-CH-), 3.19 - 3.40 (m, 2H, $(CH_3)_3$-C-CH-), 3.87 - 4.37 (m, 12H, CH_3-CH_2-, -CH_2-CH_2-), 4.59 (m, 2H, CH_3-CH-). ^{13}C NMR ($CDCl_3$), δ (ppm): 16.12, 16.44, 17.91, 19.16, 27.88, 29.53, 30.13, 35.20, 35.49, 58.71, 58.96, 61.41, 62.11, 68.50, 68.89, 88.18, 172.21, 173.59. ESI-MS (calculated for $C_{34}H_{71}N_2O_{12}P_2$): 761.448 g/mol; m/z found: $[M+H]^+$ = 761.769 g/mol.

A.4. Example of sequence-controlled NMP of styrene and 1 using 2.

Copolymers of styrene with successive additions of **1** were synthesized by nitroxide mediated polymerization. The following example corresponds to polymer **P1** in Table 1. 103 mg of **2** (0.26 mmol, 1 Eq.) and 102 mg of **1** (0.27 mmol, 1 Eq.) were dissolved in 1.5 mL of anisole, the flask was sealed with a septum and purged with dry argon for 30 minutes. Then 3 mL of degassed styrene (26 mmol, 100 Eq.) was added with a degassed syringe. Afterwards, the mixture was immersed in a preheated oil bath at 120°C. During the copolymerization, aliquots were taken from the mixture with a degassed syringe in order to monitor the incorporation of **1** in the growing copolymer chains. After the conversion of styrene reached approximately 50% (i.e. 46 minutes of reaction), a degassed solution of 106 mg of **1** (0.29 mmol, 1 Eq.) in degassed anisole was added with a degassed syringe. Due to the poor solubility of **1** in anisole at RT, the solution was preheated at 60-80 °C using an electronic heat gun. The reaction mixture was exposed to air after the conversion of styrene reached 70% (110 minutes of reaction). ~3 mL of THF were added, and the polymer was precipitated in cold methanol. The precipitate was filtered and dried *in vacuo* at RT. The purified polymer was characterized by FT-IR, ^{1}H NMR, ^{19}F NMR and SEC in THF (M_n 9070 g·mol^{-1}, M_w/M_n 1.16).

A.5. Example of sequence-controlled NMP of styrene and 1 using 4.

A copolymer of styrene with symmetrically inserted **1** at the end of polymer chain was prepared by NMP in the presence of **4** (polymer **P5** in Table 1). 200 mg of **4** (0.26 mmol, 1 Eq.) were put in a flask, dissolved in 1.5 mL of anisole. The flask was sealed with a septum and purged with dry argon for 30 minutes. 3 mL of degassed styrene (26 mmol, 100 Eq.) were added with a degassed syringe. The mixture was then immersed in a preheated oil bath at 120°C. During the copolymerization, aliquots were taken from the mixture with a degassed syringe in order to monitor the incorporation of **1** in the growing copolymer chains. After the conversion of styrene reached approximately 50% (i.e. 60 minutes of reaction), a degassed solution containing 218 mg of **1** (0.57 mmol, 2 Eq.) in degassed anisole was added with a degassed syringe. Due to the poor solubility of **1** in anisole at RT, the solution was preheated at 60-80 °C using an electronic heat gun. The reaction mixture was exposed to air after the conversion of styrene reached 60% (90 minutes of the reaction). ~3 mL of THF were added, and the polymer was precipitated in cold methanol. The precipitate was filtered and dried *in vacuo* at RT. The purified polymer was characterized by FT-IR, ^{1}H NMR, ^{19}F NMR and SEC in THF (M_n 8520 g·mol^{-1}, M_w/M_n 1.18).

A.6. Post-modification of polymer precursors with 3.

Linear polymers with protected CRC-motifs were synthesized by modification of the linear polymer precursors with **3**. An example is given for **P5**. In a flask, a solution of **3** (50 mg, 0.03 mmol) in *N*-methylpyrrolidone (1 mL) was added to a solution of **P5** (110 mg, ~0.01 mmol) in *N*-methylpyrrolidone. The flask was sealed with a septum and purged with argon for 20 minutes. The reaction mixture was left for 2 days at RT. Then the solution was precipitated in cold methanol (150 mL). The polymer was isolated by centrifugation and dried *in vacuo* (102 mg, ~ 70% yield). The purified polymer was characterized by SEC, FT-IR, ^{1}H and ^{19}F NMR spectroscopies.

A.7. Deprotection of CRC-polymer conjugates.

100 mg of CRC-polymer conjugate was dissolved in 0.6 mL of THF, added into a flask, sealed with a septum and purged with dry argon for 15 minutes. TFA solution was added (1.6 mL: 80% TFA, 10% THF, 6% H_2O, 4% TIS) in a flow of argon. The general composition of the reaction mixture: 59% TFA, 34% THF, 4% H_2O, 3% TIS. After 5-10 minutes the reaction mixture was precipitated in cold methanol and isolated by centrifugation. 69 mg, ~69% yield.

A.8. Folding of linear polymers containing CRC-motif

A typical procedure for the CRC-polymer conjugates was the following: the polymer was dissolved in NMP (0.6-0.7 mg/mL) and DIPEA (~0.2% vol.) was added to the solution. After strong air bubbling for 30 hours, the solution was concentrated, precipitated in a large volume of cold methanol, isolated by centrifugation, and dried *in vacuo*. In some cases, the conditions were slightly modified: DMSO (1-2% vol.) was added to the solution of the polymer and time was increased until two days.

B. Measurements and analysis

B.1. Nuclear Magnetic Resonance Spectroscopy (NMR)

The ^{1}H NMR and ^{19}F NMR spectra were recorded using a 400 MHz Bruker Avance spectrometer equipped with Ultrashield magnet, at 25 °C. ^{1}H NMR spectra were calibrated with respect to the solvent signal. Styrene conversion ($Conv._S$) and the consumption of monomer **1** ($Conv._1$) were calculated from the ^{1}H NMR spectra of the crude reaction samples by comparing the integration of unreacted monomers peaks to the integration of proton signals of the formed copolymer. The measurements were performed in deuterated chloroform ($CDCl_3$) or in deuterated dicholoromethane (CD_2Cl_2).

B.2. Size Exclusion Chromatography (SEC)

Characterizations of the sequence-controlled copolymers were performed at 30°C in HPLC quality THF at a flow rate of 1 mL·min^{-1} using a Shimadzu LC20AD pump. The number-average molar mass (M_n) and the polydispersity index (M_w/M_n = PDI) were estimated using a calibration based on 16 linear polystyrene standards from Polymer Laboratories. The SEC (THF) set-up was equipped with four PLgel mixed C columns (particle size 5 µm, separation range 10^3-10^6 g/mol). The detection was performed with a refractometer (Optilab T-rEX), viscometer (Viscostar-II), light scattering detector (TREOS) and a diode array UV detector (Shimadzu SPD M20A). Toluene was used as internal reference.

Characterizations of the CRC-polymer conjugates were performed at 60°C in HPLC quality DMF, 0.1M LiBr and characterizations of the folded polymers – at 28°C in HPLC quality DCM at a flow rate of 1 mL·min^{-1} using a Shimadzu LC20AD pump. The SEC (DMF and DCM) set-up was equipped with three PLgel mixed B columns (particle size 10 µm,

separation range 10^3-10^7 g/mol). The detection was performed with a Viscotek TDA 302 (Light scattering, refractometer and viscometer).

B.3. Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared spectra (IR) were recorded on a Bruker Vertex 70 spectrophotometer in ATR mode.

B.4. Ellman's test

The test is used to detect the presence of thiol groups. Ellman's reagent solution was prepared: 3 mg of 5,5'-dithiobis(2-nitrobenzoic acid) was dissolved in 5mL of the mixture of solvents MeOH (UV) /THF (UV) 1/1, then 15 µL of DIPEA was added. 1-2 mg of polymer was dissolved in 1 mL of the mixture of solvents MeOH (UV) /THF (UV) 1/1, 3-4 drops of Ellman's reagent solution were added. The change of colour to yellow in some minutes indicated the presence of thiol groups.

B. Additional data and figures

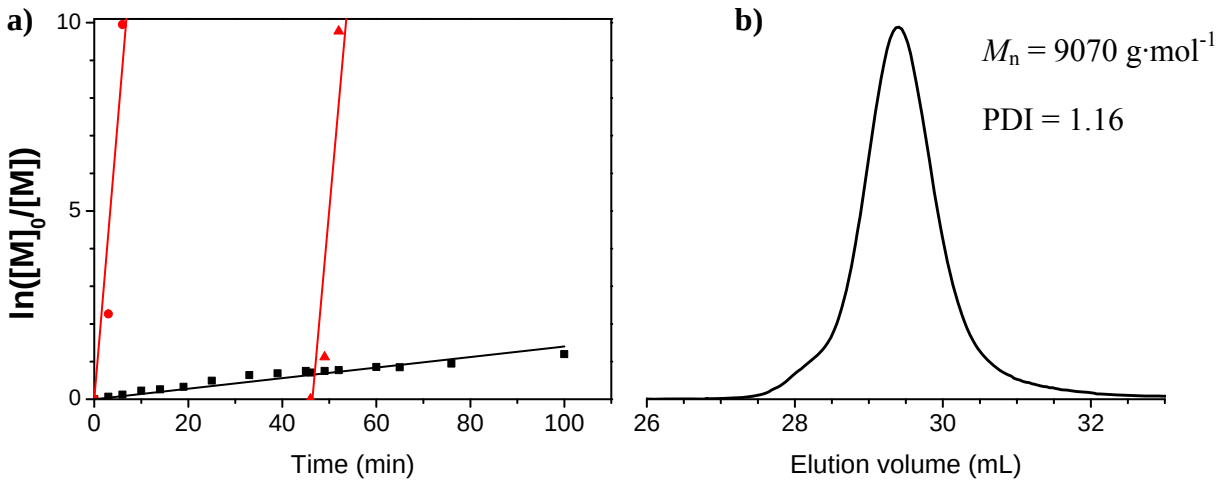


Figure S1. Characterization of precursor **P1**: a) Semi-logarithmic plot of monomer conversion versus time for NMP of styrene (squares) with **1** added in the beginning (dots) and at 3/4 of the chain (triangles); b) Size exclusion chromatogram of the copolymer (THF).

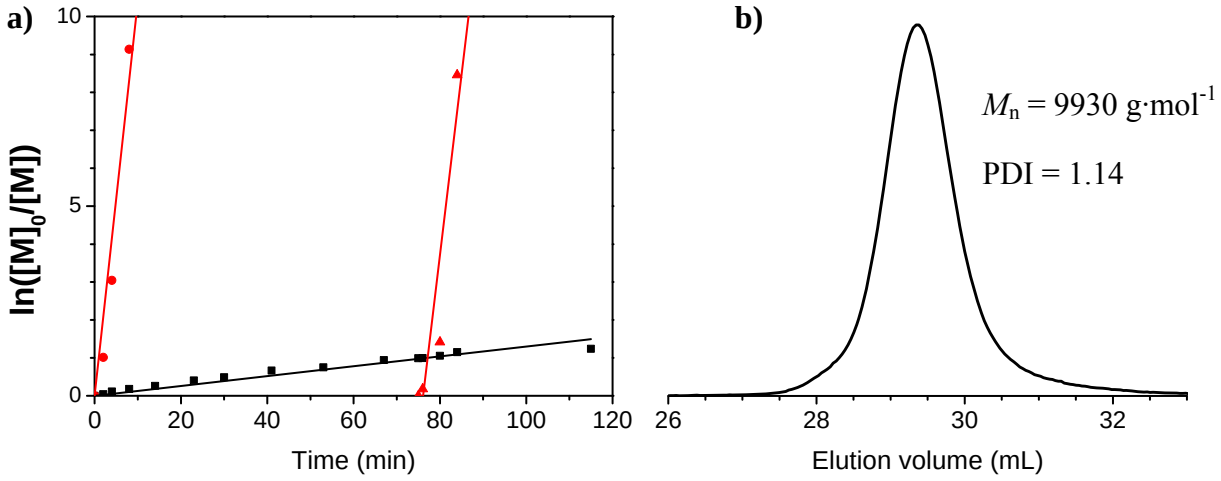


Figure S2. Characterization of precursor **P2**: a) Semi-logarithmic plot of monomer conversion versus time for NMP of styrene (squares) with **1** added in the beginning (dots) and in the end of polymer chain (triangles); b) Size exclusion chromatogram of the copolymer (THF).

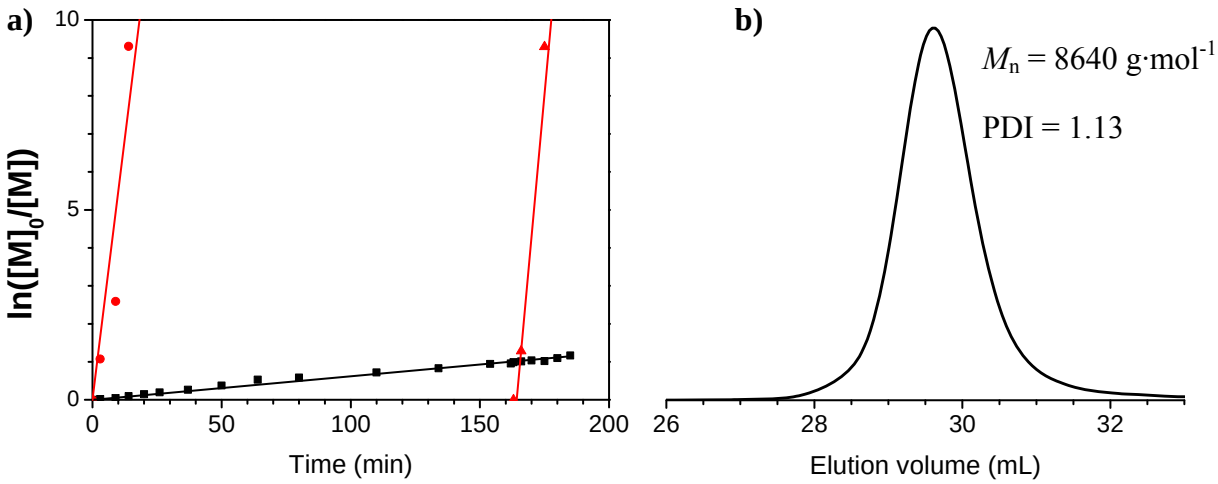


Figure S3. Characterization of precursor **P3**: a) Semi-logarithmic plot of monomer conversion versus time for NMP of styrene (squares) with **1** added in the beginning (dots) and in the end of polymer chain (triangles); b) Size exclusion chromatogram of the copolymer (THF).

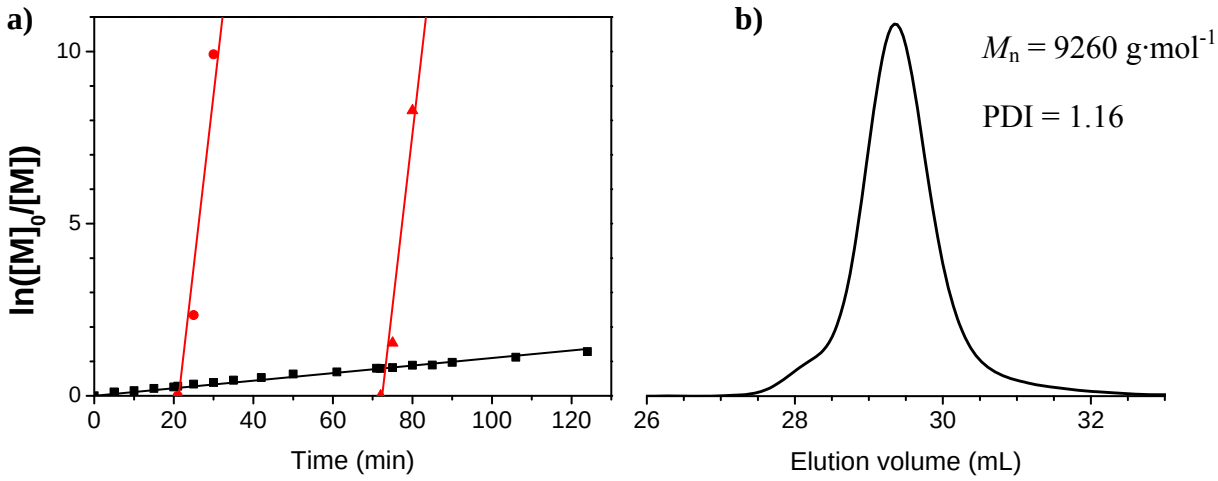


Figure S4. Characterization of precursor **P4**: a) Semi-logarithmic plot of monomer conversion versus time for NMP of styrene (squares) with **1** added at ~ 1/4 (dots) and ~3/4 of polymer chain (triangles); b) Size exclusion chromatogram of the copolymer (THF).

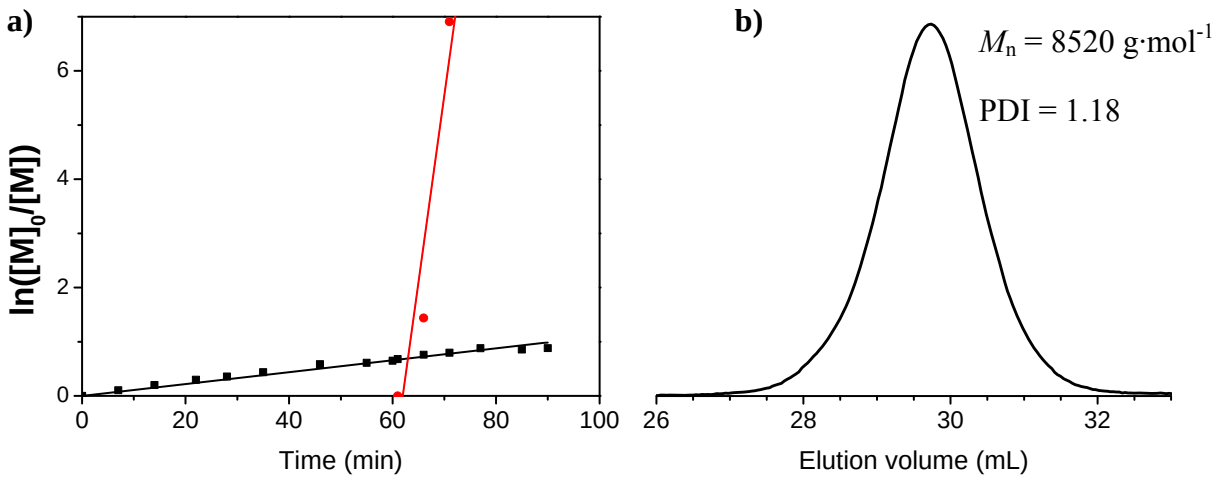


Figure S5. Characterization of precursor **P5**: a) Semi-logarithmic plot of monomer conversion versus time for NMP of styrene (squares) with **1** added close to the ends of polymer chain (dots); b) Size exclusion chromatogram of the copolymer (THF).

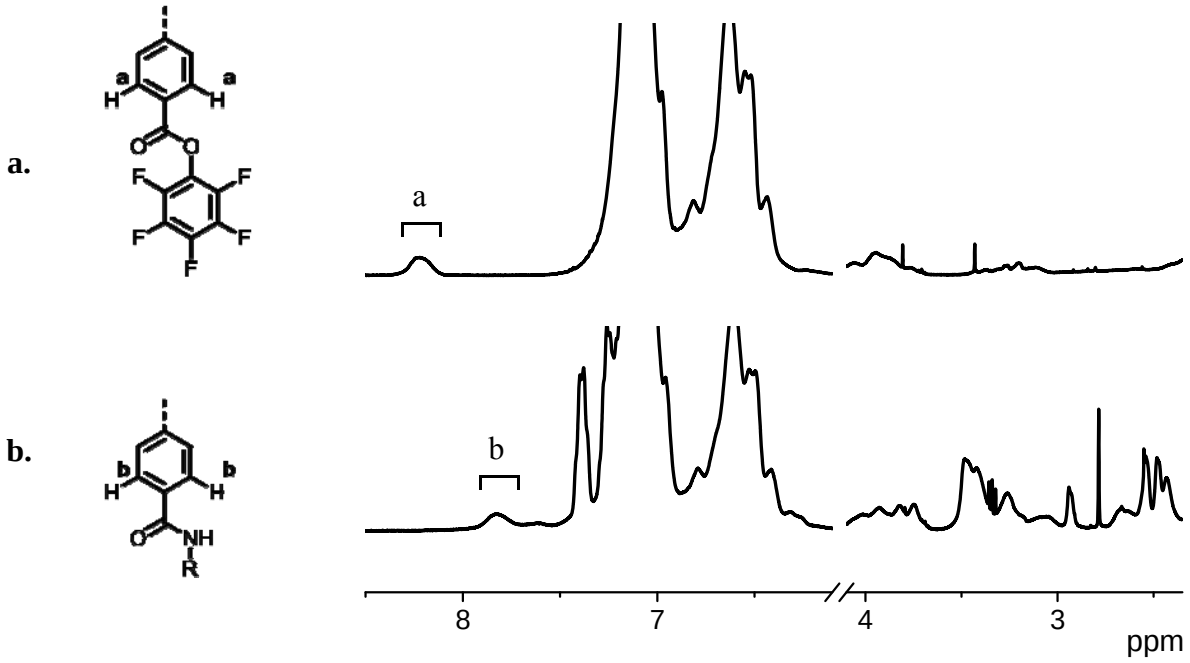


Figure S6. ^{1}H NMR spectra (CD_2Cl_2) of the copolymer **P5** (**a**) and the resulting polymer after reaction with **3** (**b**).

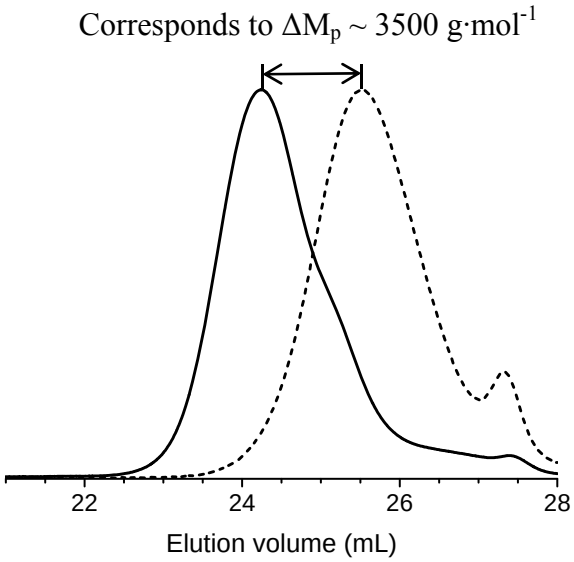


Figure S7. Size exclusion chromatograms of the copolymer **P5** (dashed line) and the resulting polymer after reaction with oligomer **3** (solid line), DMF with 0.1 M LiBr.

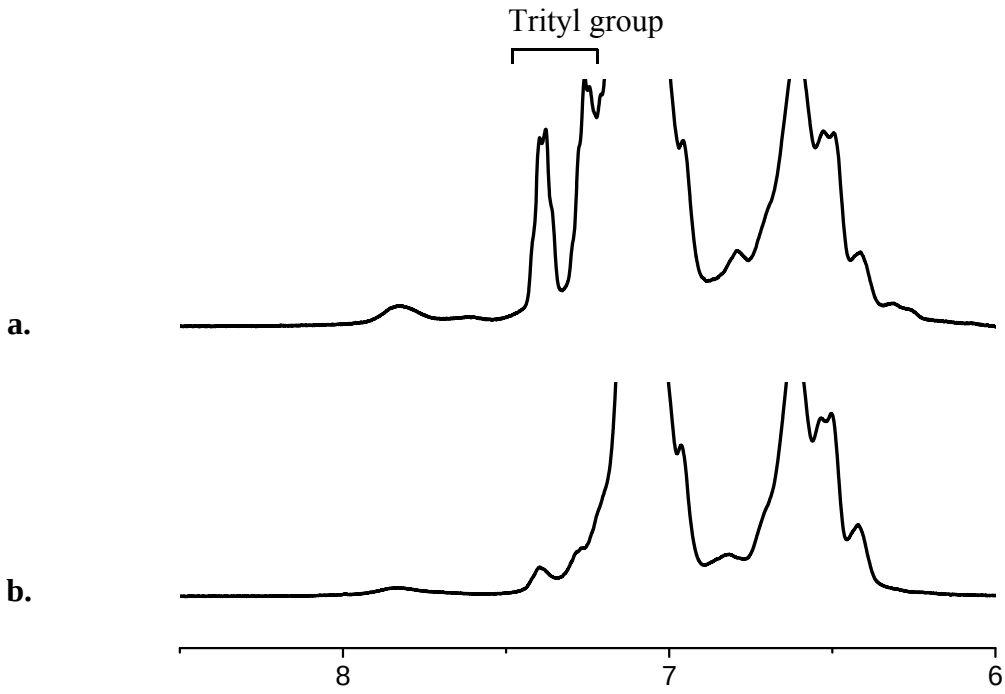


Figure S8. ^{1}H NMR spectra (CD_2Cl_2) of the CRC-polymer conjugate before (**a**) and after deprotection (**b**).

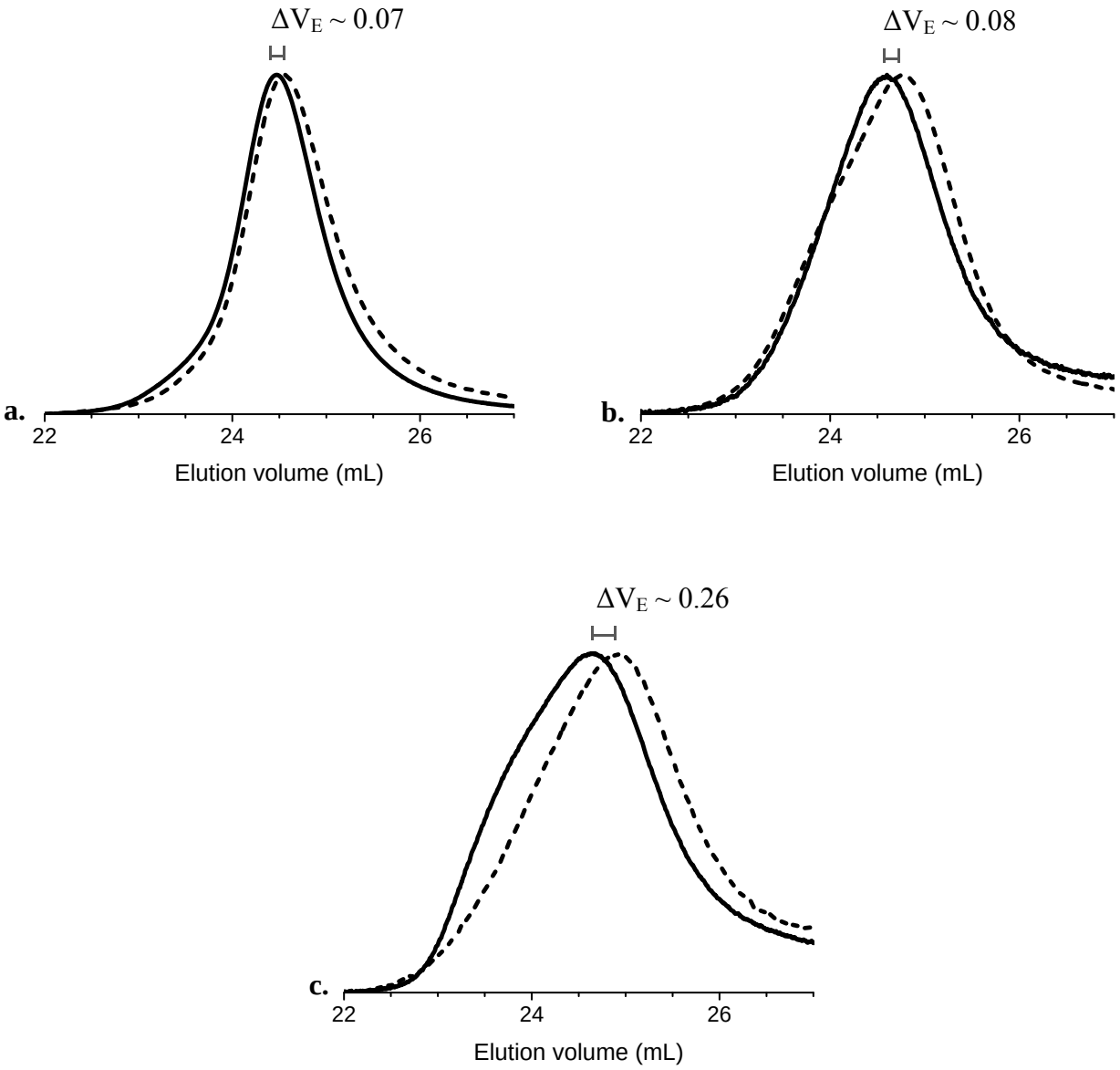


Figure S9. Size exclusion chromatograms of the linear CRC-polymer conjugates (dashed line) and folded CRC-polymer conjugates (solid line) corresponding to the precursors **P2** (**a**), **P5** (**b**; **c** – slightly modified conditions of oxidation), DCM.

D. References

[1] Kakuchi, R.; Zamfir, M.; Lutz, J.-F., Theato, P. *Macromol. Rapid Commun*., **2012**, *33*, 54-60

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[4] Guerret, O.; Couturier, J-L.; Lutz, J-F.; Le Mercier, Ch.; Robin, S.; Vuillemin, B. *Patent N° US 6657043,* **2003**.

[5] Soriano, A.; Ventura, R.; Molero, A.; Hoen, R.; Casadó, V.; Cortés, A.; Fanelli, F.; Albericio, F.; Lluís, C.; Franco, R.; Royo, M. *J. Med. Chem.*, **2009**, *52*, 5590-5602.