Supporting Information

Self-Reporting Refoldable Fluorescent Single-Chain Nanoparticles

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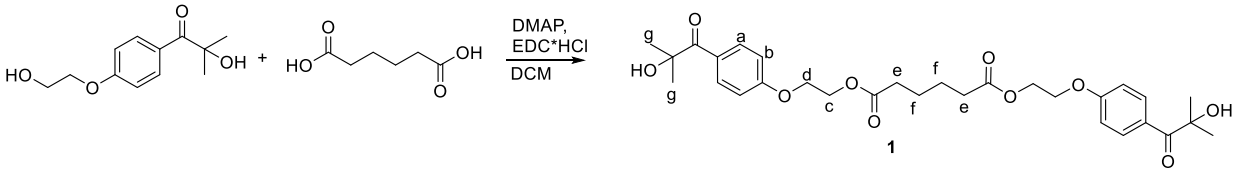
Content

A. Experimental Procedures

A.1 Chemicals

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid-hydrochloride (EDC-HCl; ≥99%, Carl Roth), 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (>98%, TCI), 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO; 98%, Sigma), 3-Chloroperoxybenzoic acid (70-75%, Acros) 4-Carboxy-2,2,6,6-tetramethylpiperidine 1-oxyl (4-carboxy-TEMPO, >97%, TCI), adipic acid (99%, Sigma), anisole (99%, Acros), chloroform-d_1 ($CDCl_3$, 99.8%, EURISO-TOP), dichloromethane (dry) (DCM, 99.8%, extra dry, over molecular sieve, stabilized, Acros), dimethylaminopyridine (DMAP, ≥99%, Sigma-Aldrich), dimethylsulfoxide-d_6 (DMSO-d_6, 99.8%, EURISO-TOP), potassium carbonate (≥99%, Alfa Aesar), sodium hydrogen carbonate (≥, Carl Roth), sodium sulfate (≥99%, Roth). Styrene (>99.5%, Sigma-Aldrich) was passed over a column of basic alumina (Acros) and 4-(chloromethyl)styrene (>90%, TCI) was distilled to remove inhibitor and subsequently stored at -20 °C. Tetrahydrofuran (THF), dichloromethane (DCM), cyclohexane and methanol were purchased as analytical grade solvents (VWR) and used as received.

A.2 Synthesis of bis(2-(4-(2-hydroxy-2-methylpropanoyl)phenoxy)ethyl) adipate (1)

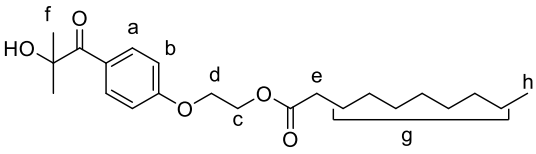


Adipic acid (1.0 g, 6.8 mmol, 1 equiv.), DMAP (125 mg, 1.03 mmol, 0.15 equiv.) and 2-hydroxy-1-(4-(2-hydroxyethoxy)phenyl)-2-methylpropan-1-one (Irgacure2959 (4.60 g, 20.5 mmol, 3 equiv.) were dissolved in dry DCM (100 mL), and the mixture was cooled to 0 °C. Subsequently, EDC-HCl (5.25 g, 27.3 mmol, 4 eq.) was dissolved in 20 mL dry DCM and added in a dropwise manner. The solution was allowed to come to ambient temperature and was stirred for another 2 d. The solution was washed with distilled water (3×). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product was isolated after column chromatography on silica gel (cyclohexane/ethyl acetate 1:1) as a white solid (3.07g, 5.5 mmol, 81%).

^{1}H NMR (400 MHz, DMSO) δ / ppm = 8.23 (d, ^{3}J = 9.0 Hz, 4H, CH^{a}), 7.01 (d, ^{3}J = 9.0 Hz, 4H, CH^{b}), 5.65 (s, 2H, C-OH), 4.38 (m, 4H, CH_2^{c}), 4.31 – 4.22 (m, 2H, CH_2^{d}), 2.34 (s, 4H, CH_2^{e}), 1.55 (s, 4H, CH_2^{f}), 1.40 (s, 12H, CH_3^{g}).

^{13}C NMR (101 MHz, DMSO) δ / ppm = 202.0, 172.7, 161.4, 132.5, 127.8, 113.8, 76.7, 66.0, 62.2, 33.0, 28.2, 23.8.

A.3 Synthesis of bis(2-(4-(2-hydroxy-2-methylpropanoyl)phenoxy)ethyl) adipate (2)

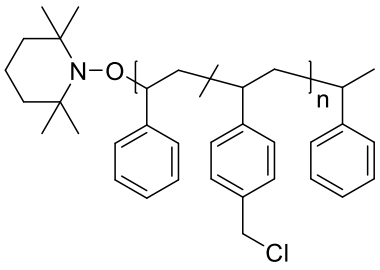


Decanoic acid (384 mg, 2.23 mmol, 1 eq), DMAP (40.8 mg, 0.33 mmol, 0.15 eq.) and 2-hydroxy-1-(4-(2-hydroxyethoxy)phenyl)-2-methylpropan-1-one (Irgacure2959 (500 mg, 2.23 mmol, 1 eq.) were dissolved in dry DCM (50 mL) and cooled to 0 °C. Subsequently, EDC-HCl (1.71 g, 8.9 mmol, 4 eq.) was dissolved in 10 mL dry DCM and added dropwise. The solution was allowed to come to ambient temperature and was stirred for another 24 h. The solution was washed with water (3x). The organic phase was dried over Na_2SO_4 and the solvent was remover under reduced pressure. The product was obtained as a colourless oil (0.27 g, 0.7 mmol, 32%) after column chromatography (cyclohexane/ethyl acetate 7:3).

^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm = 8.06 (d, ^{3}J = 9.1 Hz, 4H, CH^{a}), 6.96 (d, ^{3}J = 9.1 Hz, 4H, CH^{b}), 4.45 (m, 4H, CH_2^{c}), 4.24 (m, 4H, CH_2^{d}), 2.34 (t, J = 7.5 Hz, 4H, CH_2^{e}), 1.63 (s, 6H, CH_3^{f}), 1.35 – 1.18 (m, 14H, CH_2^{g}), 0.87 (t, ^{3}J = 6.9 Hz, 6H, CH_3^{h}).

^{13}C NMR (101 MHz, $CDCl_3$) δ / ppm = 202.7, 173.9, 162.5, 132.5, 126.4, 114.3, 75.9, 66.3, 62.3, 34.3, 32.0, 29.4, 28.8, 25.0, 22.8, 14.2.

A.4 Synthesis of PS-*stat*-CMS P1



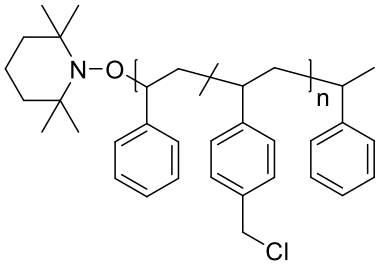
Styrene (2.5 mL, 21.8 mmol, 190 equiv.), 4-(chloromethyl)styrene (0.16 mL, 1.15 mmol, 10 equiv.) and 2,2,6,6-tetramethyl-1-(1-phenylethoxy)piperidine (30 mg, 0.218 mmol, 1 equiv.) and 2.66 mL of dry toluene were placed into a flame-dried Schlenk flask and deoxygenated by four consecutive freeze–pump–thaw cycles. Subsequently, the reaction mixture was placed into an

oil bath tempered at 125 °C. After 18 h, the polymerization was stopped by cooling the flask with liquid nitrogen and exposing the reaction mixture to the ambient atmosphere. The crude product was diluted with THF (10 mL) and precipitated twice into ice cold methanol (200 mL). The polymer was yielded as a white powder by filtration and dried under high vacuum.

M_n = 10100 g mol^{-1}, $Đ$ = 1.22.

^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm = 7.09 – 6.50 (aromatic protons of PS and initiator), 4.52 (bs, 2H, CH_2Cl), 2.33 – 0.93 (m, aliphatic protons of PS and initiator).

A.5 Synthesis of PS-*stat*-CMS P2

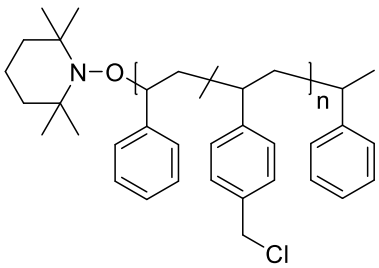


Styrene (2.3 mL, 20.1 mmol, 175 equiv.), 4-(chloromethyl)styrene (0.57 mL, 4.02 mmol, 35 equiv.) and 2,2,6,6-tetramethyl-1-(1-phenylethoxy)piperidine (30 mg, 0.218 mmol, 1 equiv.) and 2.8 mL dry toluene were placed into a flame-dried Schlenk flask and deoxygenated by four consecutive freeze–pump–thaw cycles. Subsequently, the reaction mixture was placed into an oil bath tempered at 125 °C. After 20 h, the polymerization was stopped by cooling the flask with liquid nitrogen and exposing the reaction mixture to the ambient atmosphere. The crude product was diluted with THF (10 mL) and precipitated twice into ice cold methanol (200 mL). The polymer was afforded as a white powder by filtration and dried under high vacuum.

M_n = 10900 g mol^{-1}, $Đ$ = 1.31.

^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm = 7.09 – 6.50 (aromatic protons of PS and initiator), 4.52 (bs, 2H, CH_2Cl), 2.33 – 0.93 (m, aliphatic protons of PS and initiator).

A.6 Synthesis of PS-*stat*-CMS P3



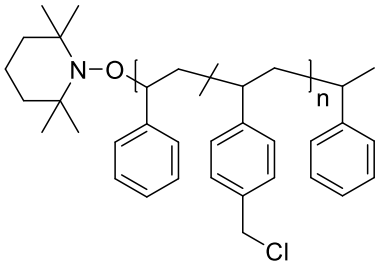
Styrene (2.0 mL, 17.2 mmol, 150 equiv.), 4-(chloromethyl)styrene (0.81 mL, 5.74 mmol, 50 equiv.) and 2,2,6,6-tetramethyl-1-(1-phenylethoxy)piperidine (30 mg, 0.218 mmol, 1 equiv.) and 2.7 mL dry toluene were placed into a flame-dried Schlenk flask and deoxygenated by four

consecutive freeze–pump–thaw cycles. Subsequently, the reaction mixture was placed into an oil bath tempered at 125 °C. After 20 h the polymerization was stopped by cooling the flask with liquid nitrogen and opening it to the atmosphere. The crude product mixture was diluted with THF (10 mL) and precipitated twice into cold methanol (200 mL). The polymer was afforded as a white powder by filtration and dried under high vacuum.

M_n = 8600 g mol^{-1}, $Đ$ = 1.42.

^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm = 7.09 – 6.50 (aromatic protons of PS and initiator), 4.52 (bs, 2H, CH_2Cl), 2.33 – 0.93 (m, aliphatic protons of PS and initiator).

A.7 Synthesis of PS-*stat*-CMS P4

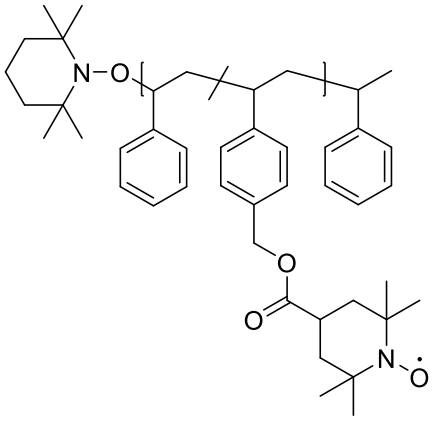


Styrene (11.8 mL, 104 mmol, 750 equiv.), 4-(chloromethyl)styrene (0.58 mL, 4.13 mmol, 30 equiv.) and initiator (36 mg, 0.14 mmol, 1.0 equiv.) was dissolved in 8 mL dry toluene in a flame-dried Schlenk flask and deoxygenated by four consecutive freeze–pump–thaw cycles. Subsequently, the reaction mixture was placed into an oil bath tempered at 125 °C. After 20 h the polymerization was stopped by cooling the flask with liquid nitrogen and opening it to the atmosphere. The crude product was diluted with THF (20 mL) and precipitated twice into cold methanol (200 mL). The polymer was afforded as a white powder by filtration and dried under high vacuum.

M_n = 31600 g mol^{-1}, $Đ$ = 1.35.

^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm = 7.09 – 6.50 (aromatic protons of PS and initiator), 4.52 (bs, 2H, CH_2Cl), 2.33 – 0.93 (m, aliphatic protons of PS and initiator).

A.8 General Procedure for the Synthesis of PS-*stat*-TEMPO P1'-P4'

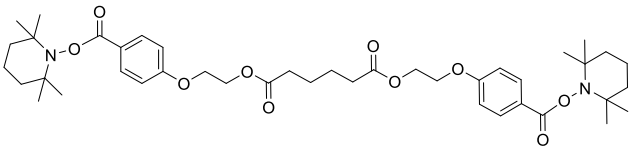


The PS-*stat*-CMS polymer (300 mg), K_2CO_3 (5 eq. per CMS) and 4-carboxy-TEMPO (2 eq. per CMS) were dissolved in DMF (10 mL). The mixture was stirred at 50 °C for 3 d. Subsequently, the mixture was diluted with EA and extracted three times with brine. The organic phase was dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude product was diluted with THF (10 mL) and precipitated twice into cold methanol (200 mL).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ / ppm = 7.12 – 6.63 (aromatic protons of PS and initiator), 5.15 (bs, 2H, C*H*$_2$-OCO), 2.33 – 0.96 (m, aliphatic protons of PS, initiator and carboxy TEMPO).

A.9 Model reaction for the UV light induced crosslinking reaction

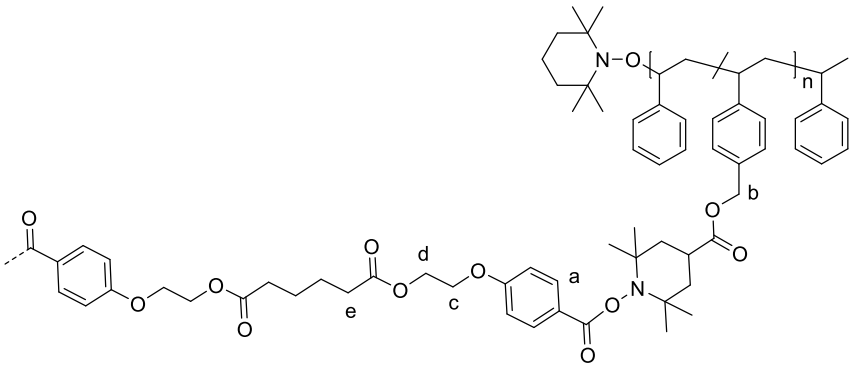
bis(2-(4-(((2,2,6,6-tetramethylpiperidin-1-yl)oxy)carbonyl)phenoxy)ethyl) adipate (3)



TEMPO (50 mg, 0.32 mmol, 2.1 eq) and **1** (71.7 mg 0.152 mmol) were dissolved in 20 mL anisole. The solution was purged for 10 min with argon and the flask was placed in a photo reactor equipped with an Arimed B6 lamp (λ_{max} = 320 nm). The mixture was irradiated overnight under stirring. Subsequently, the solvent was removed.

m/*z* (theo) [M+Na$^+$] = 775.4140; *m*/*z* (found) [M+Na$^+$] = 775.4153.

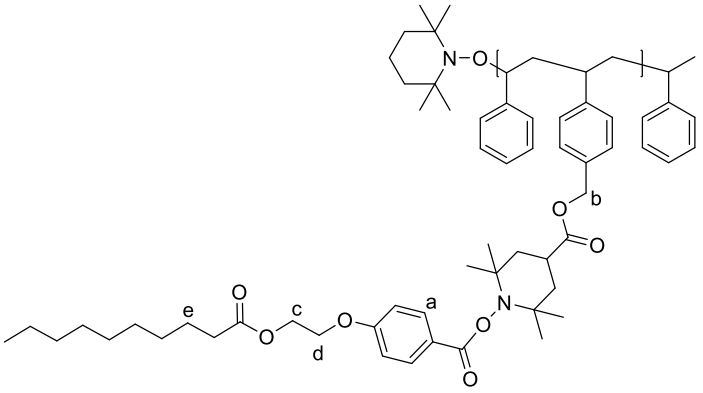
A.10 General Procedure for the Folding of the liner Precursors



The linear parent precursor (20 mg) and **1** (0.5 eq. per TEMPO) were dissolved in a flame dried Schlenk-flask in 500 mL anisole. The solution was purged for 30 min with argon and subsequently put in a photo reactor equipped with an Arimed B6 lamp (λ_{max} = 320 nm). The mixture was irradiated for 24 h under stirring. The solvent was removed under reduced pressure, the remainder dissolved in THF and precipitated into cold MeOH (20 mL). The polymer was dried under high vacuum and obtained as a yellow-brownish solid.

^{1}H NMR (400 MHz, DMSO) δ / ppm = 8.03 (m, 2H, CH^a), 7.12 – 6.63 (aromatic protons of PS and initiator), 5.02 (bs, 2H, CH_2^b), 4.44 (m, 2H, CH_2^c), 4.21 (2H, CH_2^c), 2.81 – 2.71 (m, 4H, CH_2^e), 2.33 – 0.96 (m, aliphatic protons of PS, initiator and carboxy TEMPO).

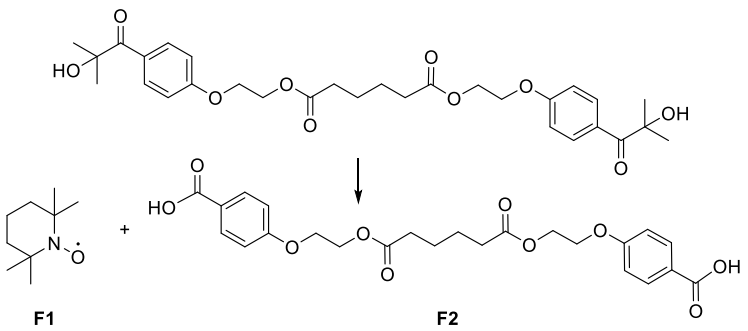
A.11 Photoreaction of P4' with 2



P4' (20 mg, 0.0031 mmol TEMPO, 1.0 eq. TEMPO) and **2** (2.4 mg 0.0063 mmol, 2 eq.) were dissolved in 500 mL anisole. The solution was purged for 30 min with argon and the flask was put in a photo reactor equipped with an Arimed B6 lamp (max. 320 nm). The mixture was irradiated for overnight under stirring. Subsequently, the solvent was removed, the remainder dissolved in THF and precipitated into cold MeOH (20 mL).

^{1}H NMR (400 MHz, DMSO) δ / ppm = 8.03 (m, 2H, CH^a), 7.12 – 6.63 (aromatic protons of PS and initiator), 5.02 (bs, 2H, CH_2^b), 4.45 (m, 2H, CH_2^c), 4.24 (2H, CH_2^c), 2.81 – 2.71 (m, 4H, CH_2^e), 2.33 – 0.96 (m, aliphatic protons of PS and **2**, initiator and carboxy TEMPO).

A.12 Model reaction for Unfolding/Deprotection



bis(2-(4-(((2,2,6,6-tetra-methy-lpiperidin-1-yl)-oxy)carbonyl)-phenoxy)ethyl) adipate **1** (50 mg, 0.066 mmol) was dissolved in 4 mL DCM and heated to 35 °C. *m*CPBA (57 mg 0.33 mmol, 5 eq.) dissolved in 1 mL DCM was added dropwise and the solution was stirred at 35 °C for 0.5 h. $NaHCO_3$ was added to quench the reaction. Subsequently, the solution was diluted with DCM and extracted with sat. $NaHCO_3$ solution. The solvent was removed under reduced pressure.

m/*z* (theo) [F1+H2$^+$] = 158.1545; *m*/*z* (found) [M+Na$^+$] = 158.1543.

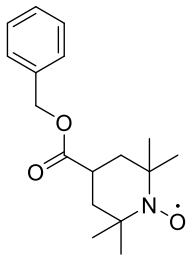
m/*z* (theo) [F2+Na$^+$] = 497.1418; *m*/*z* (found) [F2+Na$^+$] = 497.1428.

A.13 General Procedure for the Unfolding of the SCNPs

The SCNPs were dissolved in 4 mL dry DCM and heated to 35 °C. *m*CPBA (10 eq. per TEMPO) dissolved in 1 mL DCM was added dropwise. The mixture was stirred at 35 °C for 4 h. The reaction was quenched by adding $NaHCO_3$. Subsequently, the solution was diluted with DCM and extracted three times with sat. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The polymer was precipitated in cold MeOH. The polymer was dried under high vacuum to afford a white solid.

^{1}H NMR (400 MHz, DMSO) δ / ppm = 8.05 (m, 2H, C*H*^{a}), 7.12 – 6.63 (aromatic protons of PS and initiator), 5.02 (bs, 2H, CH_2^b), 4.45 (m, 2H, CH_2^c), 4.23 (2H, CH_2^c), 2.33 – 0.96 (m, aliphatic protons of PS, initiator and carboxy TEMPO).

A.14. Synthesis of benzyl carboxy-TEMPO (4)

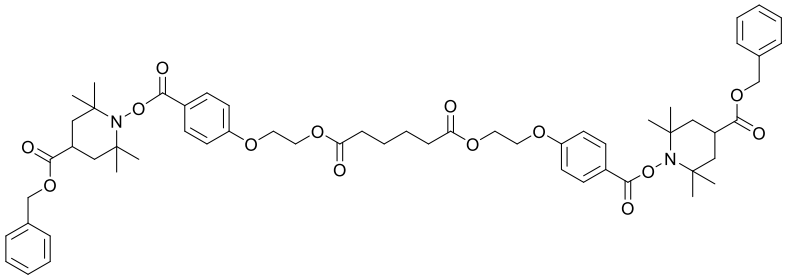


Benzylchloride (50 mg, 0.395 mmol, 1 eq.), K_2CO_3 (273 mg, 1.97 mmol, 5 eq.) and 4-carboxy-TEMPO (158 mg, 0.79 mmol, 2 eq.) were dissolved in DMF (10 mL). The mixture was stirred at 50 °C for 2 d. Subsequently, the mixture was diluted with EA and extracted three times with brine. The organic phase was dried over Na_2SO_4, filtered and the solvent removed under reduced

pressure. The crude product was diluted with THF (10 mL) and precipitated twice into cold methanol (200 mL).

m/z (theo): [M+Na$^+$] = 313.1648; *m/z* (found): [M+Na$^+$] = 313.1647.

A.15. Synthesis of bis(2-(4-(((4-((benzyloxy)carbonyl)-2,2,6,6-tetramethylpiperidin-1-yl)oxy)carbonyl)phenoxy)ethyl) adipate (5)



3 (25 mg, 0.086 mmol, 1 eq.) and **1** (96 mg, 0.17 mmol, 2 eq.) were dissolved in anisole (20 mL). The solution was purged for 30 min with argon and the flask was put in a photo reactor equipped with an Arimed B6 lamp (max. 320 nm). The mixture was irradiated for overnight under stirring. Subsequently the solvent was removed prior to fluorescence analysis.

The mixture of the structures 1':5 (given in Scheme S1) according to NMR analysis is 2.8:1.

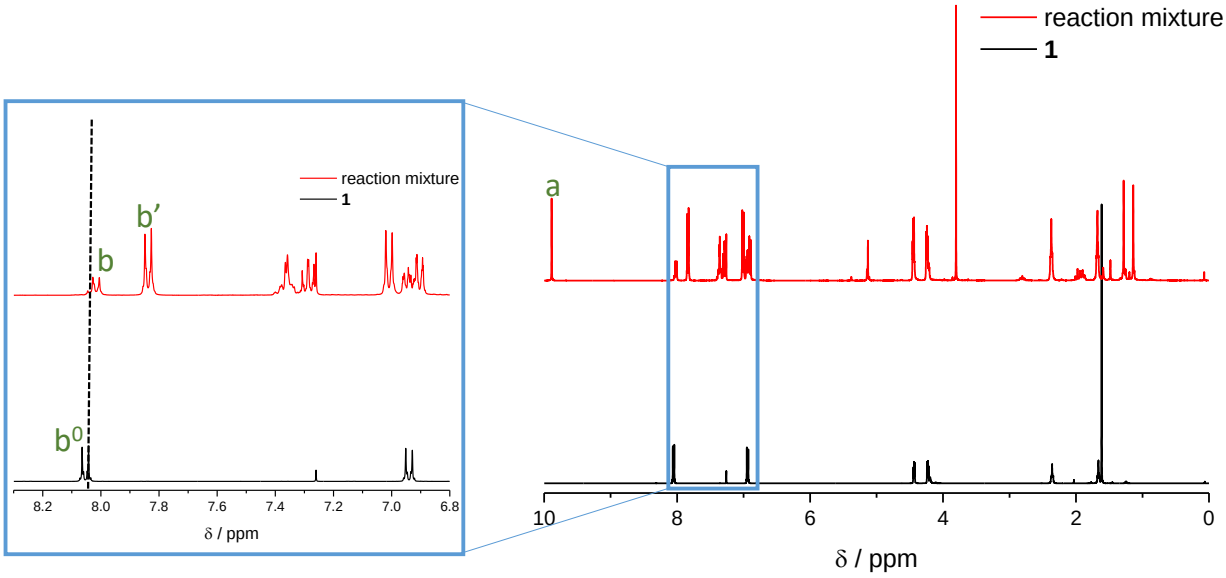
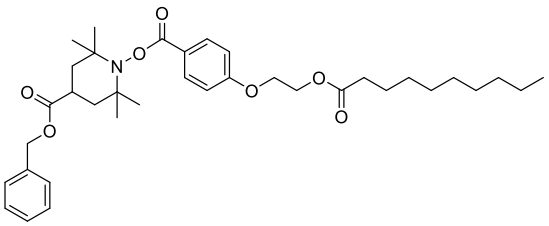


Fig. S1. ^{1}H NMR spectrum of the crude reaction mixture of **5** with **3** after irradiation at 320 nm.

A.16 Synthesis of benzyl 1-((4-(2-(decanoyloxy)ethoxy)benzoyl)oxy)-2,2,6,6-tetramethylpiperidine-4-carboxylate (6)



3 (25 mg, 0.086 mmol, 1 eq) and **2** (32.5 mg, 0.086 mmol, 1 eq.) were dissolved in anisole (20 mL). The solution was purged for 30 min with argon and the flask was put in a photo reactor equipped with an Arimed B6 lamp (max. 320 nm). The mixture was irradiated for overnight under stirring. Subsequently the solvent was removed prior to fluorescence analysis.

The mixture of the structures 2':4':6 (given in Scheme S1) according to NMR analysis is 1.2:1.2:1.

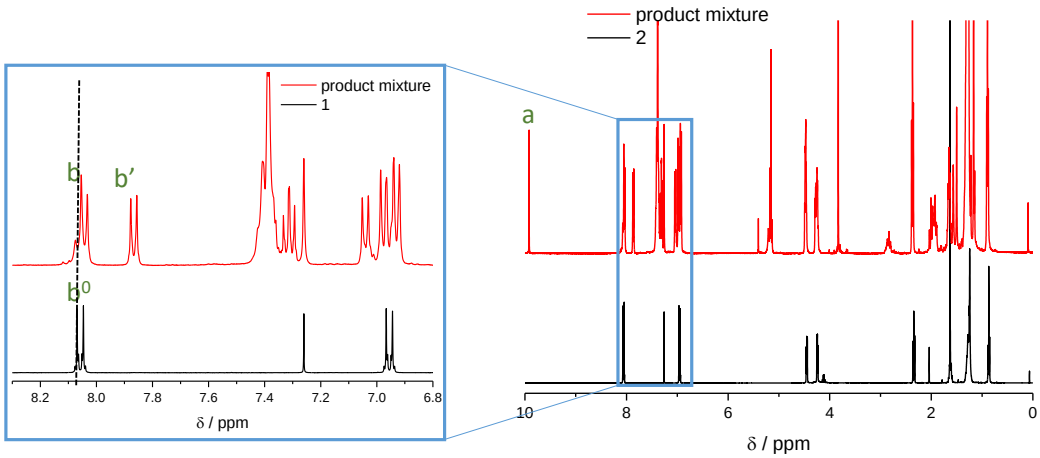
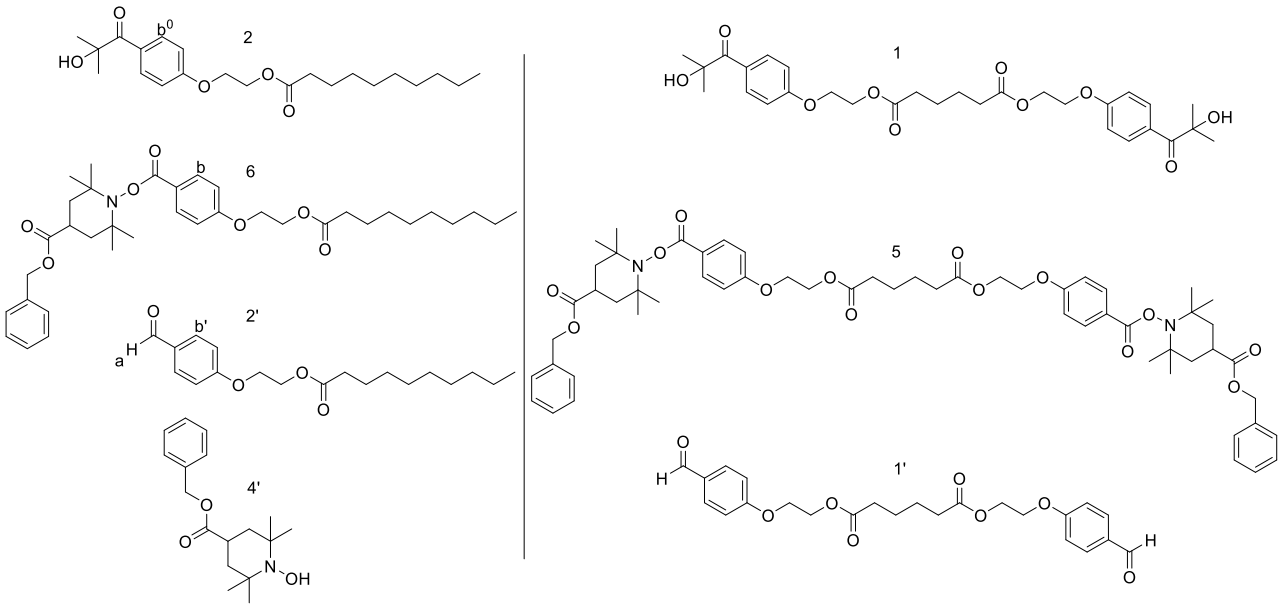


Fig. S2. ^{1}H NMR spectrum of the crude reaction mixture of **5** with **3** after irradiation at 320 nm.



Scheme S1. Side products in the photoreaction of bisfunctional crosslinker **1** and the monofunctional Irgacure 2959 derivative **2** with the benzyl carboxy-TEMPO **4**.

A.17 Table S1. Molecular characteristics of the parent polymers **P1'-P3'** and the resulting SCNPs **P5-P7**. The molecular weights, dispersities, DLS derived hydrodynamic diameter and incorporated mol% of TEMPO are listed.

ID	M_n[a] / g mol^{-1}	$Đ$[a]	D_h,[b]	mol/%TEMPO[c]	1 (Equiv.)[d]
P1'	11100	1.2	1.7	7.8	-
P2'	13200	1.3	1.9	19.6	-
P3'	11500	1.4	1.9	30.5	-
P5	10100	1.2	1.4	7.8	0.5
P6	11700	1.3	1.4	19.6	0.5
P7	7800	1.4	1.1	30.5	0.5
P8	27400	1.4	2.4	5.0	0.5

[a] Determined via SEC in THF (35 °C, 1 mL min^{-1}) as eluent, calibrated with PS standards. [b] Determined *via* DLS in DMAc + LiBr (0.3%) as solvent [c] Assumption based on complete shift of CH_2-Cl resonance from the CMS group after modification. Determined *via* ^{1}H NMR. [d] Equivalents were calculated to the corresponding amount of TEMPO.

B Measurements and Analysis

B.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H NMR measurements were performed on a Bruker Ascend 400 MHz spectrometer. ^{1}H NMR chemical shifts are reported in ppm relative to the solvent's residual ^{1}H signal of $CDCl_3$ (7.26 ppm). The ^{13}C NMR spectra were referenced to $CDCl_3$ at δ = 77.16 ppm. All NMR data were reported as follows: s (singlet), bs (broad singlet). d (duplet), t (triplet), m (multiplet). The coupling constants (*J*) are reported in Hertz [Hz]. NMR spectra of synthesized compounds are enclosed in the appendix. The mole fraction of CMS and styrene was calculated according to literature.[1]

B.2 Size Exclusion Chromatography (SEC)

SEC was performed to obtain the molecular weight distribution of synthesized polymers. The employed system was a PL-SEC 50 Plus (Polymer Laboratories, Varian) running on tetrahydrofuran (THF) (HPLC-grade) featuring an autosampler, a PLgel Mixed C guard column (50 × 7.5 mm), followed by three PLgel Mixed C linear columns (300 × 7.5 mm, 5µm bead-size) and a differential refractive index (RI) detector. The device was operated at 35 °C column temperature with a flow rate of 1 mL·min^{-1}. The calibration was carried out with narrow linear polystyrene standards ranging from 476 to 2.5·10^{6}g mol^{-1}. The injected samples were dissolved in THF (2 mg·mL^{-1}) and filtered through a 0.2 µm filter.

B.3 Attenuated Total Reflectance Infrared Spectroscopy (ATR-IR)

All IR measurements were performed on a Bruker Alpha ATR-IR Spectrometer with a range of 500 to 4000 cm^{-1} at ambient temperature.

B.4 Dynamic Light Scattering (DLS)

The apparent hydrodynamic diameters ($D_{h,app}$) were determined at 25 °C by means of a dynamic light scattering (DLS) analysis using 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 samples were dissolved at a concentration of 2 mg mL^{-1} in DMAc (+0.3% LiBr) and filtered into quartz cuvettes over a 0.2 µL filter prior to the measurement. The prepared samples were stabilized for 30 min prior to DLS analysis at ambient temperature. All values of the apparent hydrodynamic diameter for each polymer mixture were averaged over

three measurements (60 runs/measurement), and were automatically provided by the instrument using a cumulative analysis.

B.5 High-Resolution Electrospray Ionization Mass Spectrometry (ESI-MS)

ESI-MS spectra were recorded on a Q Exactive (Orbitrap) mass spectrometer (ThermoFisher Scientific, San Jose, CA, USA) equipped with a HESI II probe. The instrument was calibrated in the *m*/*z* range of 74-1822 by using a S2 premixed standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA) and a mixture of fluorinated phosphazenes (Ultramark 1621). A constant spray voltage of 4.6 kV and a dimensionless sweep gas flow rate 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 in a mixture of DCM and MeOH (3:1) containing 100 μmol sodium trifluoracetate (NaTFA). Finally, the samples were infused with a flow rate of 5 μL min^{-1}.

B.6 Fluorescence Spectroscopy

Fluorescence emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer, using quartz cuvettes loaded with 400 μL of sample. An excitation wavelength of 240 nm (slit 2.5 nm) was used and the emission was recorded from 250 to 800 nm (slit 10nm).

B.7 Ultraviolet/Visible light (UV/Vis) Spectroscopy

The UV / Vis spectra were recorded on a Cary 100 UV-Visible Spectrophotometer (Agilent Technologies, USA) equipped with a tungsten halogen light source (190 to 900 nm, accuracy +/-2 nm) and a R928 PMT detector. The analysis was performed at ambient temperature.

B.8 Electron Paramagnetic Resonance (EPR) Spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy was performed on a Bruker EMXNano spectrometer. All samples were recorded in toluene at 23 °C. The following parameters were used for the measurement: Centre field: 3434 G; sweep width: 100 G; sweep time: 180 s; sample *g*-factor: 2.00; receiver gain: 40 dB; modulation amplitude: 0.452 G; number of scans: 1; microwave attenuation: 60 dB; number of points: 2212; modulation frequency: 100 kHz, modulation phase: 0; conversion time: 81.38 ms; time constant: 1.28 ms; points/modulation amplitude: 10.

The *g*-factors were calculated according to:

$$g = \frac{71{,}4484 \cdot \upsilon}{B} \quad (2)$$

B.9 Diffusion Ordered Spectroscopy (DOSY)

DOSY experiments were performed on a 600 MHz Bruker Avance II+ HD spectrometer equipped with a BBI dual channel ambient temperature probe using an Eddy current compensated bipolar gradient pulse sequence (BPLED) at a temperature of 298 K. Proton pulse lengths were determined to be 8.45 µs on the 600 MHz spectrometer. Bipolar sine-shaped gradients δ between 2.4 ms and 3.0 ms length (depending on the diffusion behaviour of the measured sample) were incremented from G = 0.96 G/cm to 47.19 G/cm in 32 steps. 16 scans with 16k complex data points were recorded for each increment with 4 dummy scans per experiment, leading to an overall experiment time of 36 minutes and 58 seconds per sample. The d1 time was set to 3 s with an acquisition time of 1.139 s. The diffusion delay Δ was set between 80 and 110 ms (depending on the diffusion behaviour of the measured sample). Processing was achieved using Topspin 3.2 with the Dynamics Center 2.4 program. After apodisation using an exponential window function with an additional linewidth of 40 Hz (due to the paramagnetic TEMPO radical), 1D increment spectra were Fourier transformed and the signal-decay due to gradients was fitted using

$$f(G) = I_0 \cdot e^{(-{\gamma_H}^2 \cdot G^2 \cdot \delta^2 \cdot (\Delta - \frac{\delta}{3})) \cdot D}$$

with the proton gyromagnetic ratio γ_H and the full signal intensity I_0. The resulting diffusion coefficients D of the polymer signals and the solvent are the result of the fitting procedure. As only relative effective hydrodynamic radii are needed for monitoring the folding behaviour of the polymers, we used the best matching mono-exponential fits for comparison.

The hydrodynamic diameter were calculated with the Stokes-Einstein equation:

$$R_h = \frac{kT}{6\pi\eta D} \quad (1)$$

where R_h is the hydrodynamic radius of the polymer coil in meters, k is the Boltzmann constant (1.380×10^{-23} J K^{-1}), T is the temperature in Kelvin (298 K), η is the viscosity of the solvent in Pascal seconds (0.42 mPa s) and D is the diffusion coefficient.

C Additional Data and Figures

C.1 NMR Data

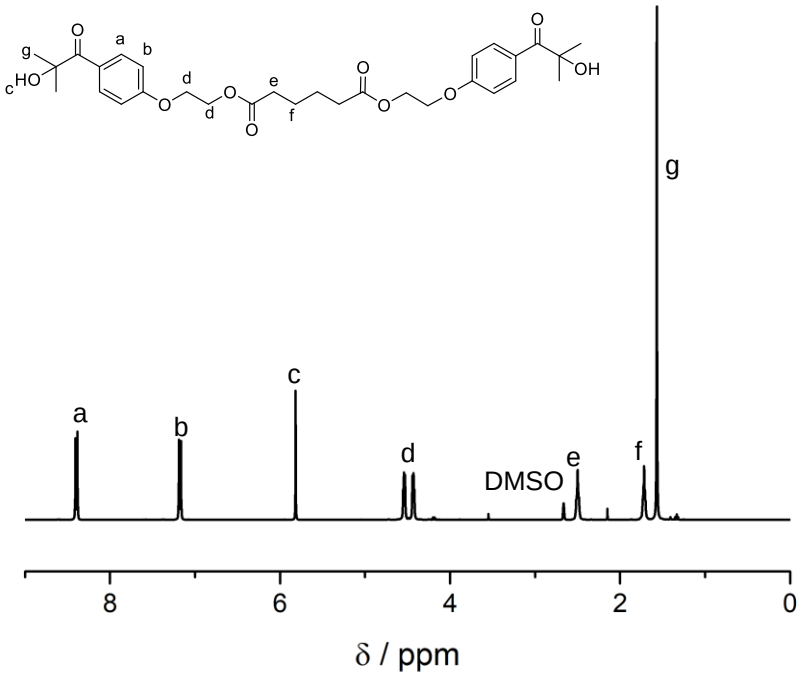


Fig. S3. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) spectra of **1**.

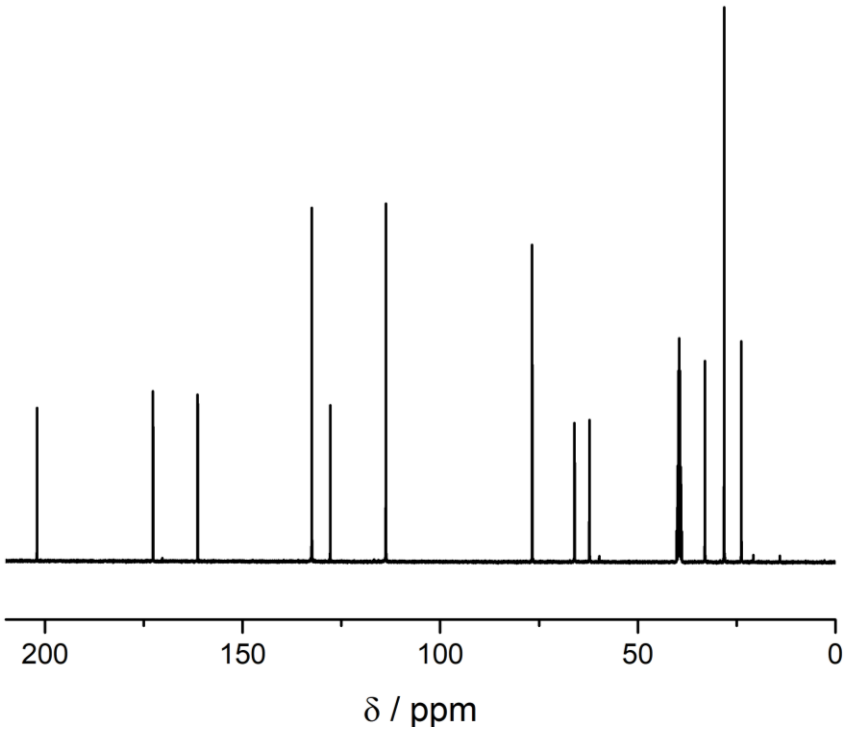


Fig. S4. ^{13}C NMR (101 MHz, DMSO-d_6, 298 K) spectra of **1**.

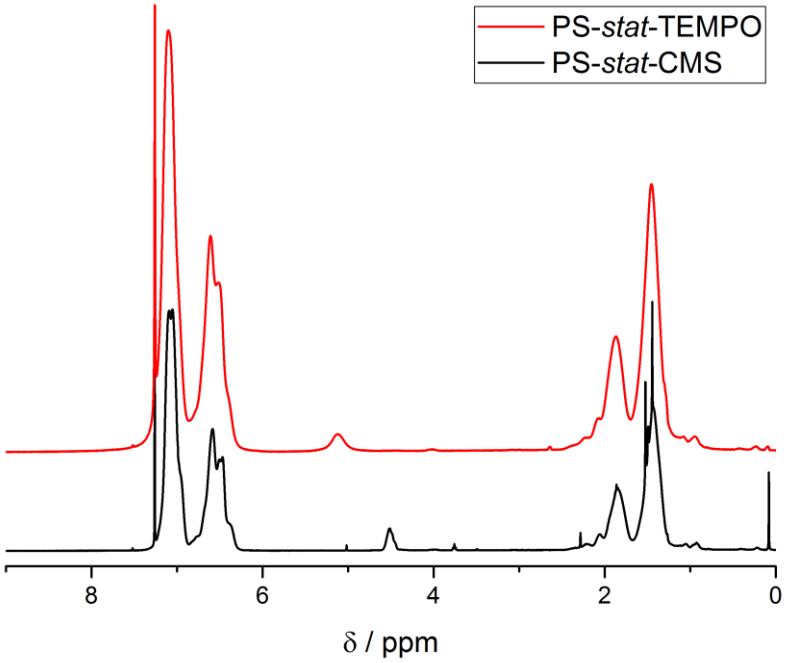


Fig. S5. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectra of **P1** and the TEMPO functionalized polymer **P1'**.

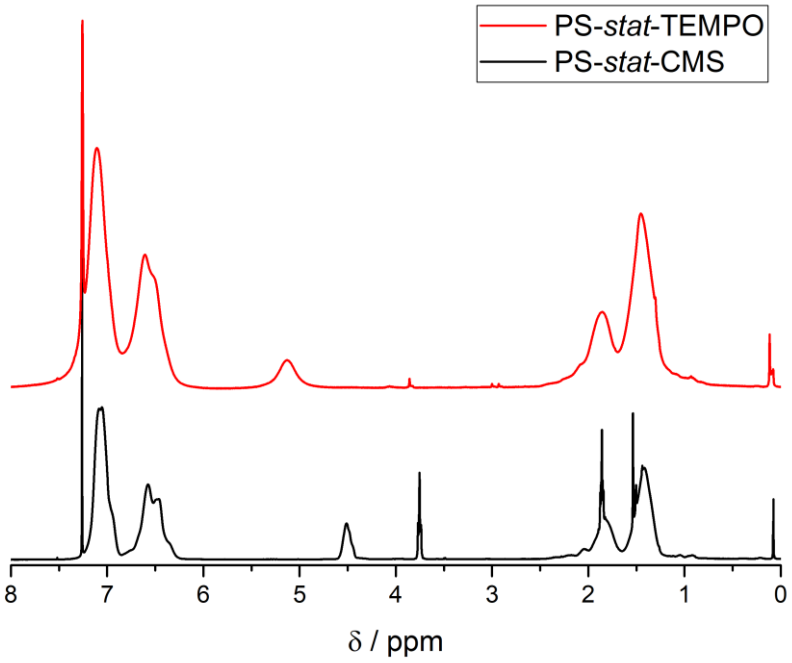


Fig. S6. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectra of **P2** and the TEMPO functionalized polymer **P2'**.

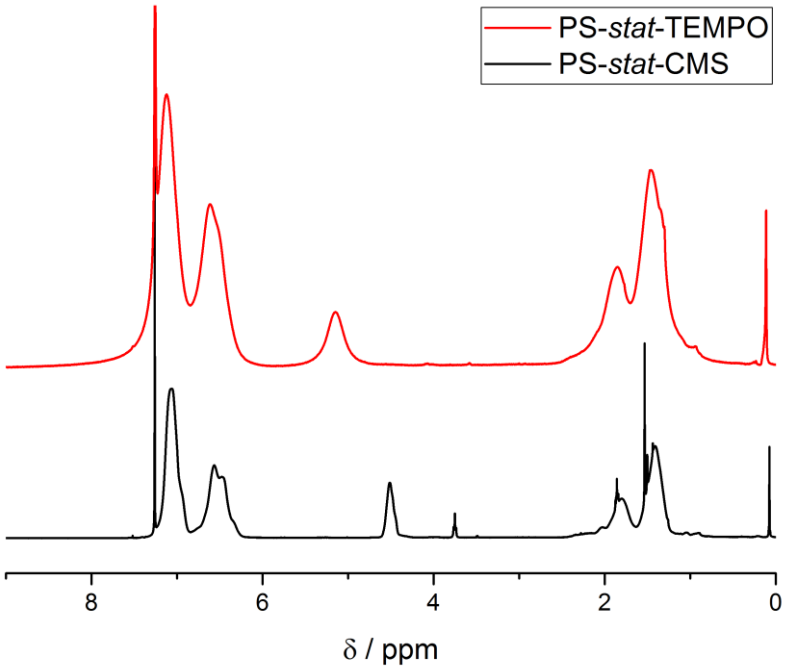


Fig. S7. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectra of **P3** and the TEMPO functionalized polymer **P3'**.

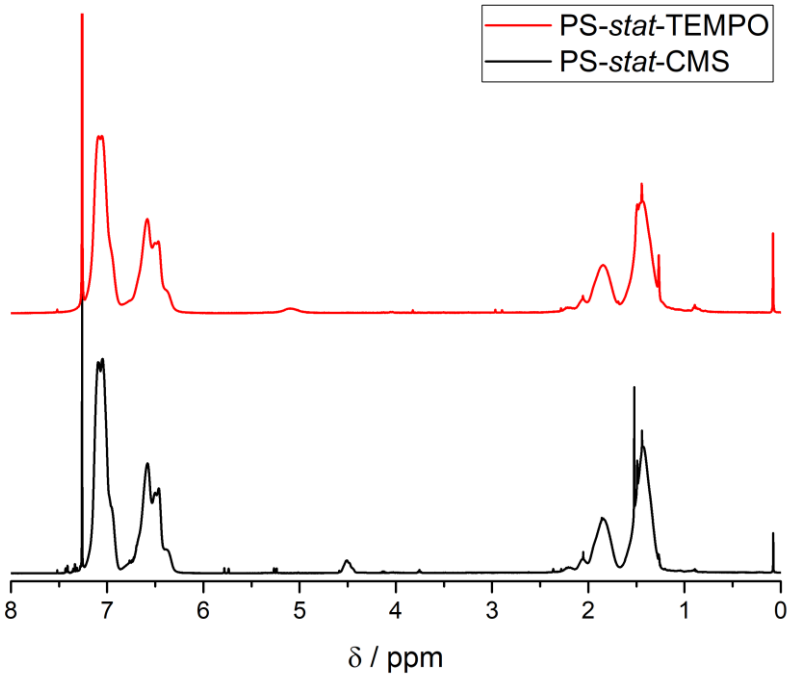


Fig. S8. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectra of **P4** and the TEMPO functionalized polymer **P4'**.

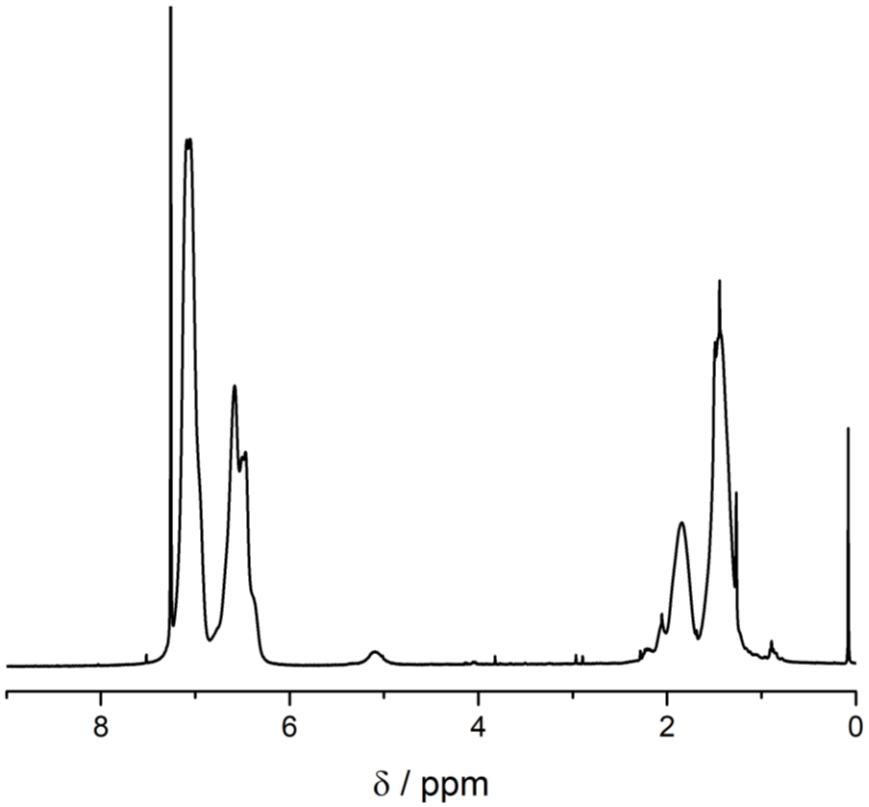


Fig. S9. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of the linear precursor polymer **P4'**.

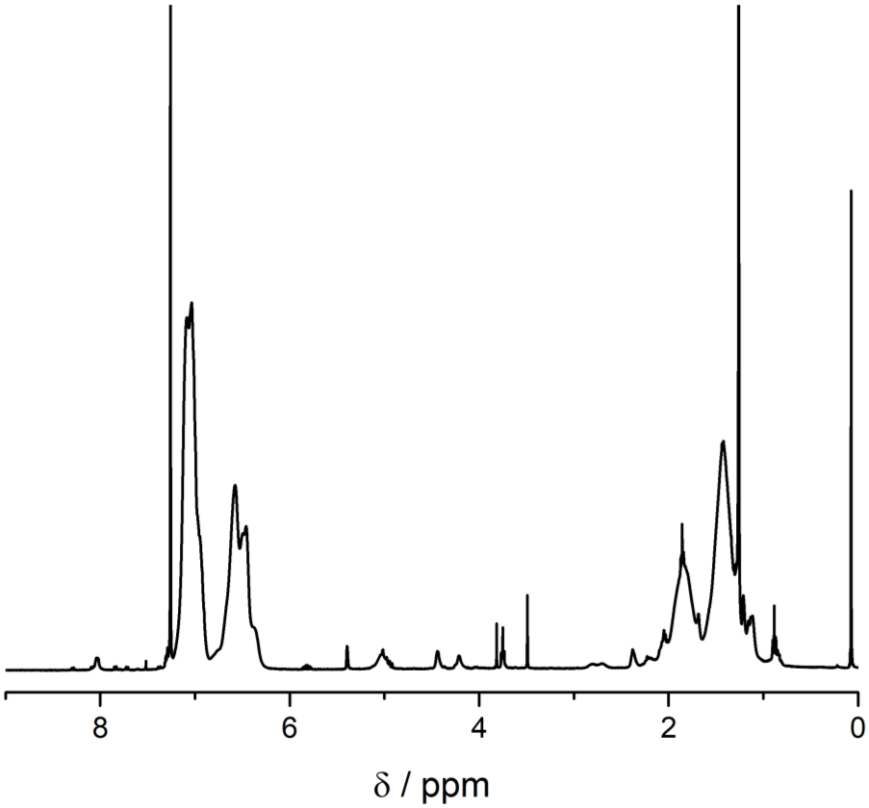


Fig. S10. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of the SCNP **P9**.

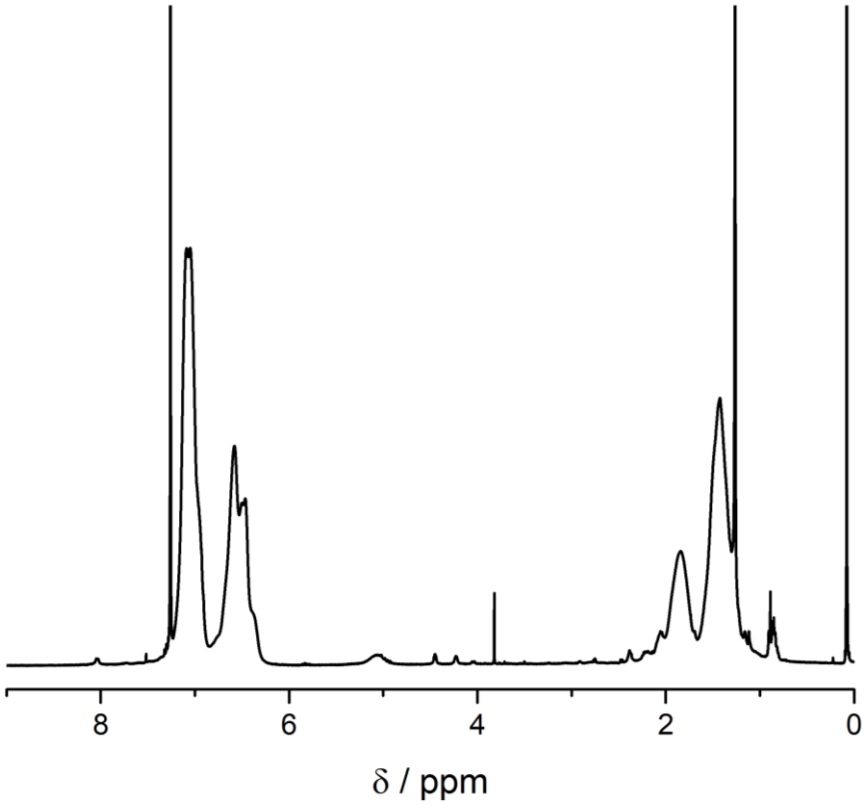


Fig. S11. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of the unfolded SCNP **P10**.

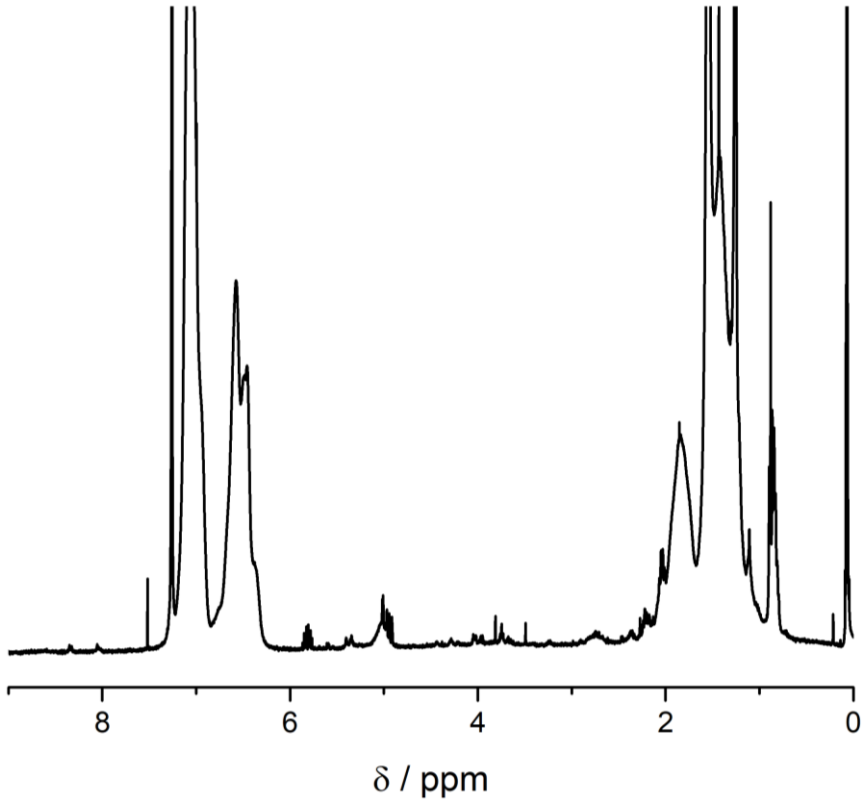


Fig. S12. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of the refolded SCNP **P11**.

C.2 SEC Data

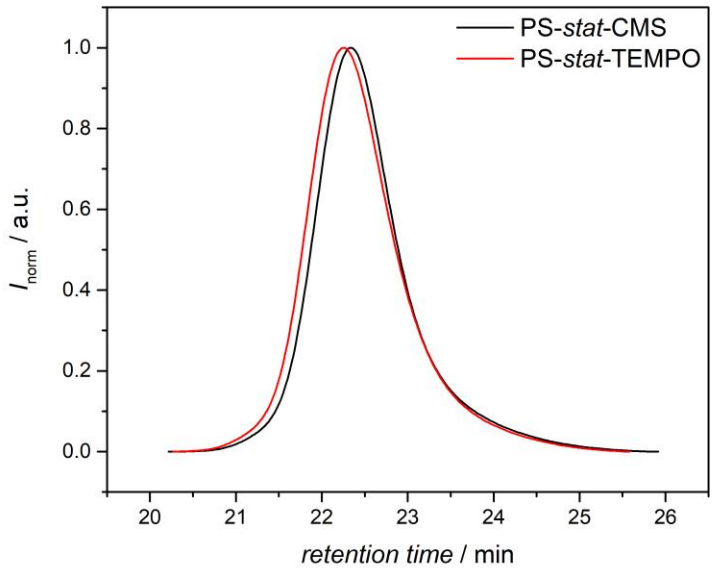


Fig. S13. SEC chromatogram of the PS-*stat*-CMS polymer **P1** and the TEMPO functionalized polymer **P1'** (THF, 35 °C, 1 mL min^{-1}).

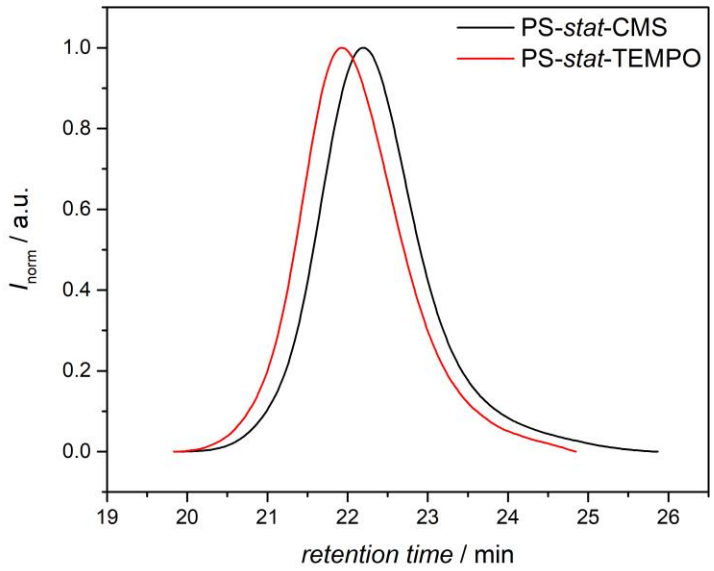


Fig. S14. SEC chromatogram of the PS-*stat*-CMS polymer **P2** and the TEMPO functionalized polymer **P2'** (THF, 35 °C, 1 mL min^{-1}).

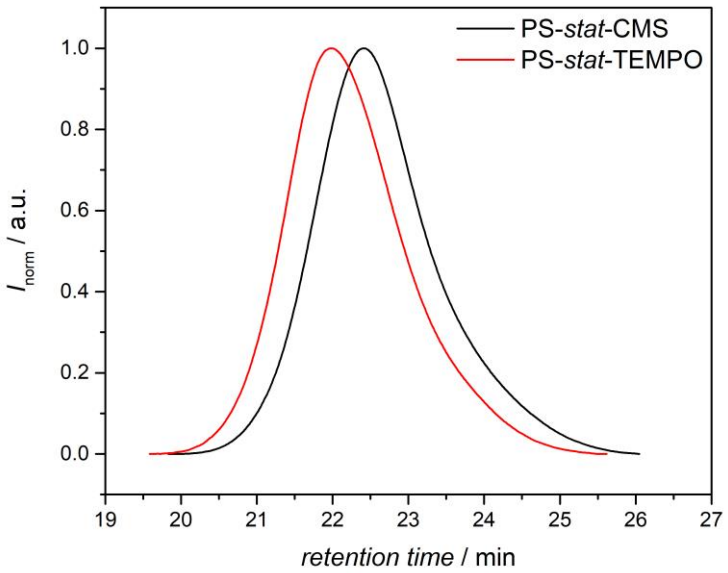


Fig. S15. SEC chromatogram of the PS-*stat*-CMS polymer **P3** and the TEMPO functionalized polymer **P3'** (THF, 35 °C, 1 mL min^{-1}).

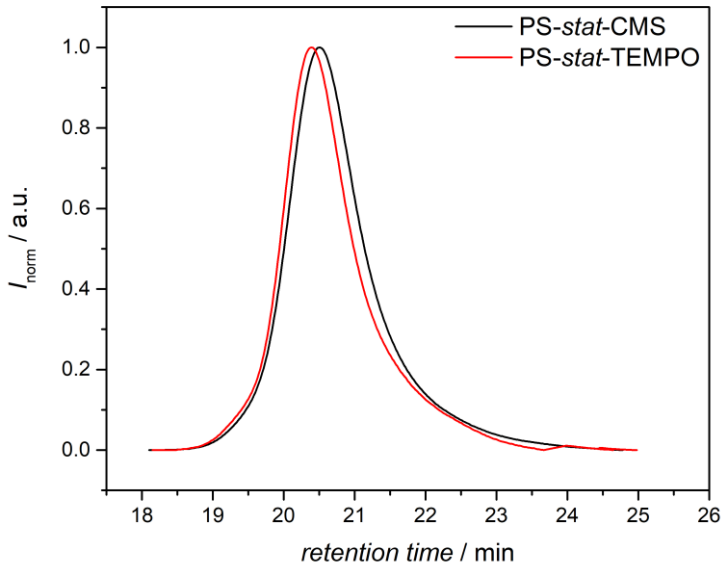


Fig. S16. SEC chromatogram of the PS-*stat*-CMS polymer **P4** and the TEMPO functionalized polymer **P4'** (THF, 35 °C, 1 mL min^{-1}).

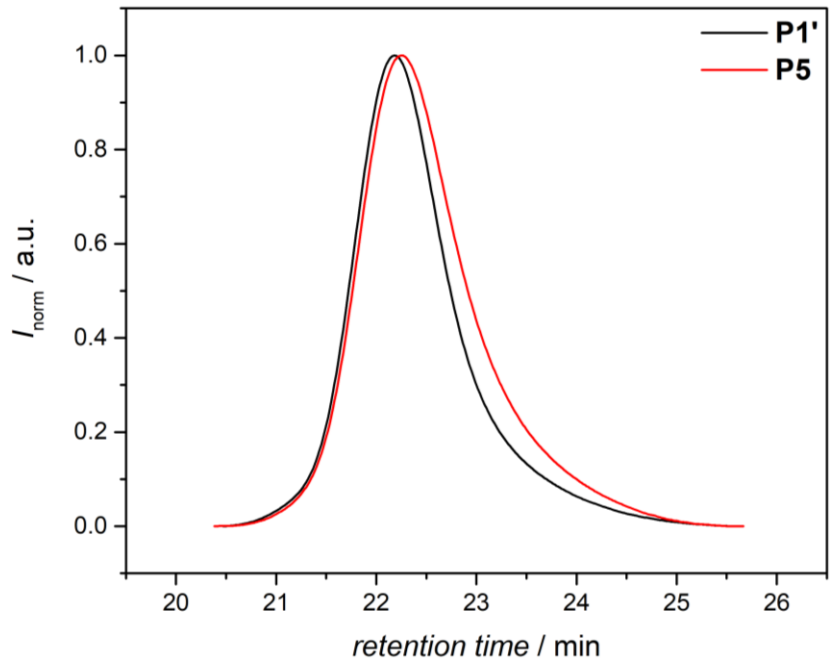


Fig. S17. SEC chromatogram of parent polymer **P1'** and the single-chain nanoparticle **P5** (THF, 35 °C, 1 mL min^{-1}).

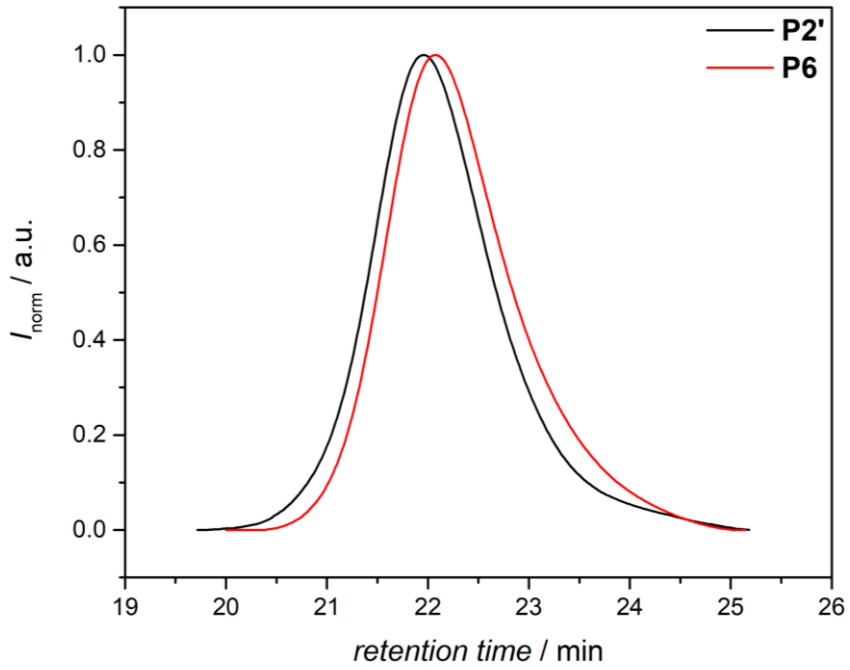


Fig. S18. SEC chromatogram of parent polymer **P2'** and the single-chain nanoparticle **P6** (THF, 35 °C, 1 mL min^{-1}).

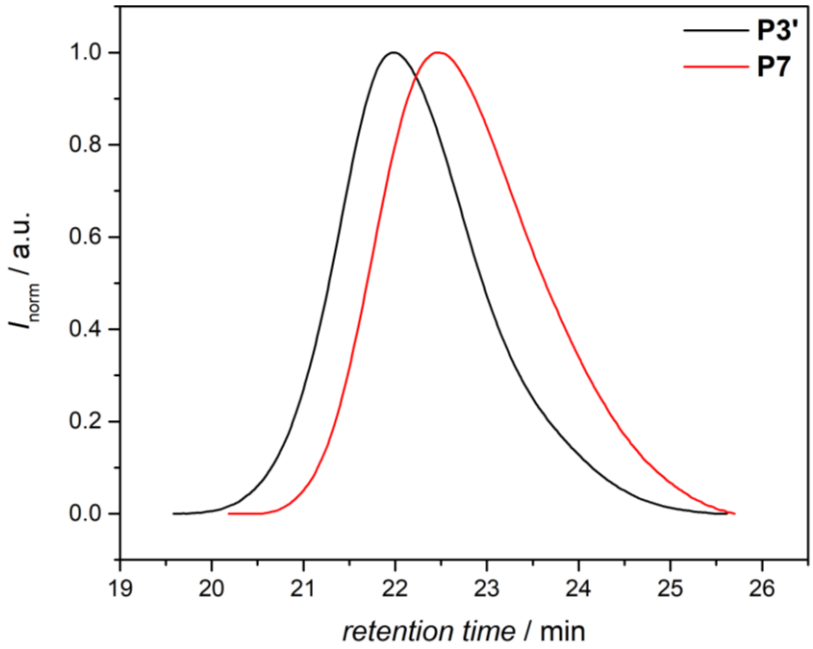


Fig. S19. SEC chromatogram of parent polymer **P3'** and the single-chain nanoparticle **P7** (THF, 35 °C, 1 mL min^{-1}).

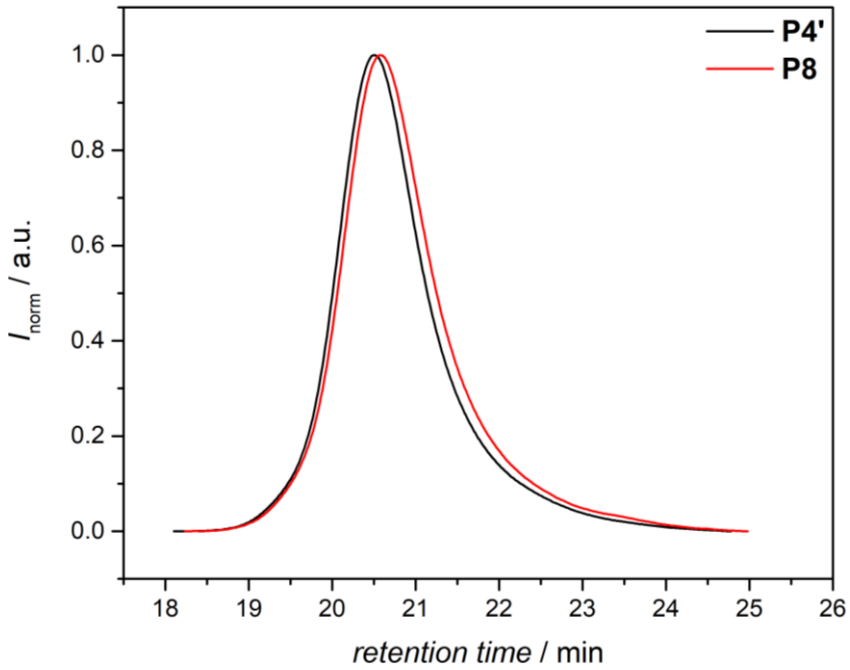


Fig. S20. SEC chromatogram of parent polymer **P4'** and the single-chain nanoparticle **P8** (THF, 35 °C, 1 mL min^{-1}).

C.3 UV/VIS Data

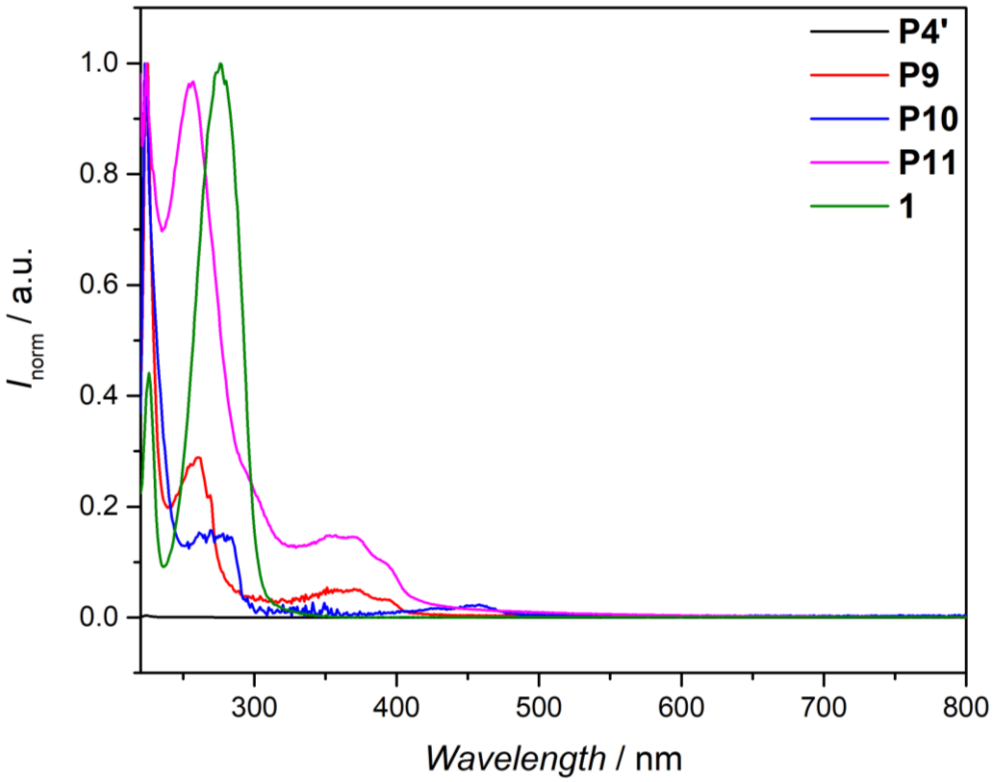


Fig. S21. UV/Vis spectra of the liner polymer precursor **P4'**, the folded SCNP **P9** the unfolded SCNP **P10** and the refolded polymer **P11** and the crosslinker **1**(c = 0.2 mg mL^{-1}).

C.4 Fluorescence Data

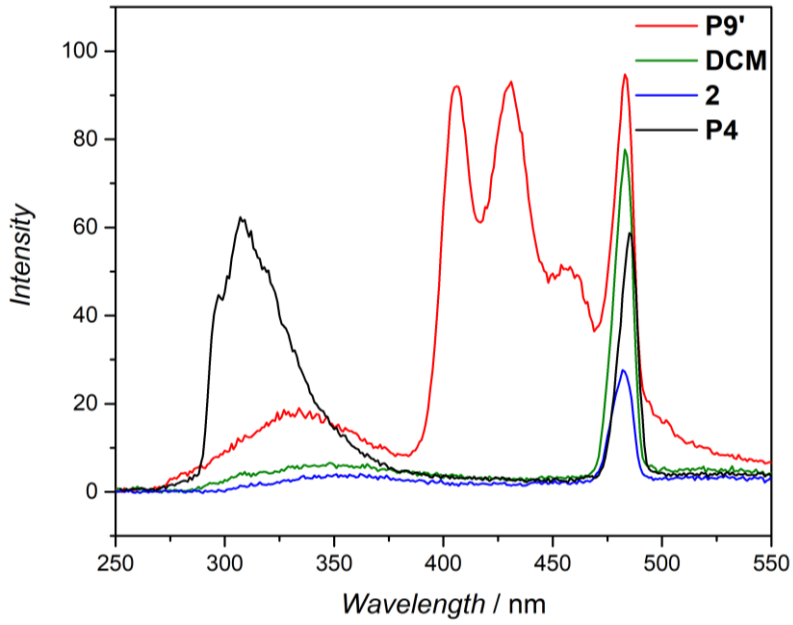


Fig. S22. Fluorescence spectra of DCM, the monofunctional Irgacure **2** and the precursor polymer **P4** and the non-folded polymer **P9'** (c = 0.2 mg mL^{-1}).

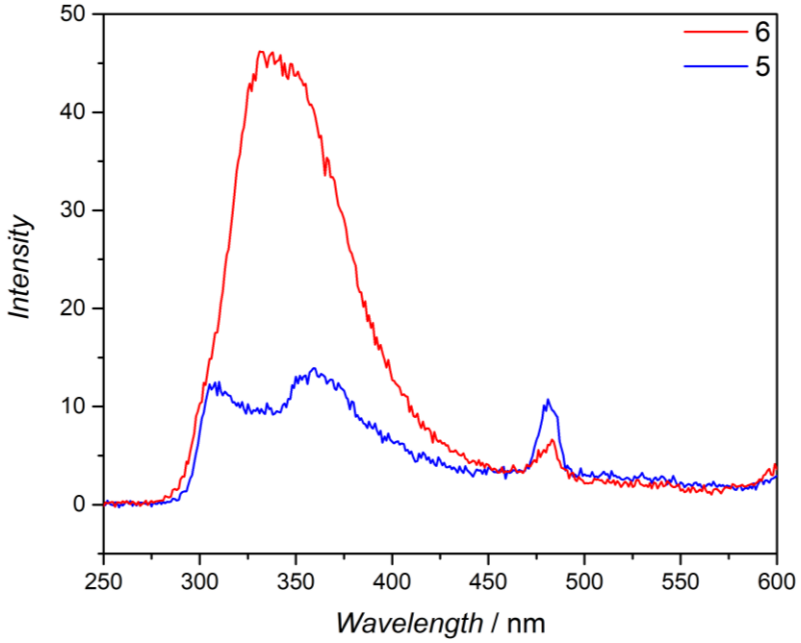


Fig. S23. Fluorescence spectra of the small test molecules **5**, and **6** (c = 0.2 mg mL^{-1}), showing no significant luminescence in the range of the SCNPs **P9** and **P11** (between 400-500 nm).

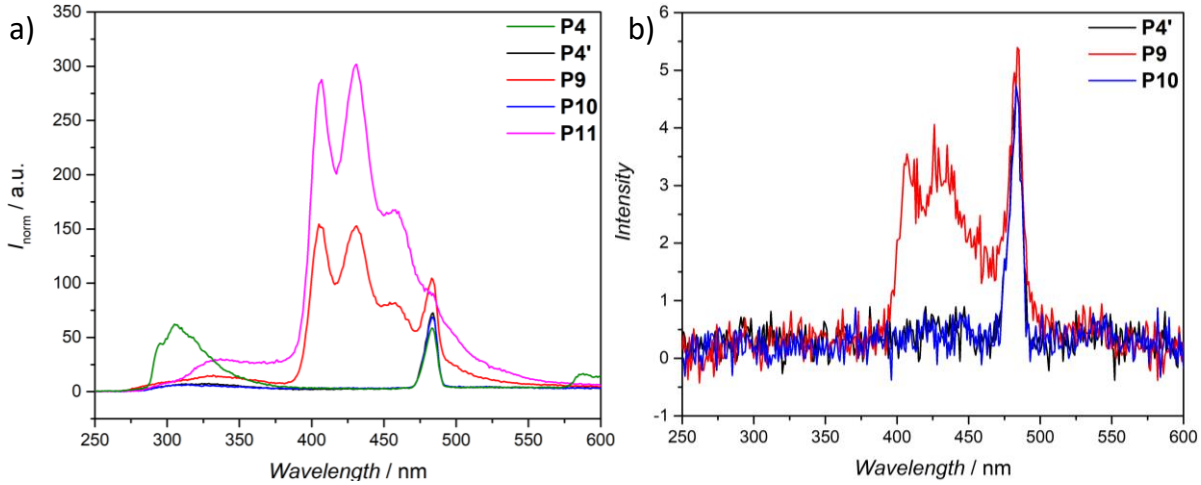


Fig. S24. Fluorescence spectra of: a) the PS-*stat*-CMS polymer **P4**, the parent polymer **P4'**, the initially folded SCNP **P9**, the unfolded SCNP **P10** and the refolded SCNP **P11** (c = 0.2 mg mL^{-1}) in DCM; b) the precursor polymer **P4'**, the folded SCNP **P9** and the unfolded **P10** in DMAc (c = 0.2 mg mL^{-1}).

C.5 ATR-IR Data

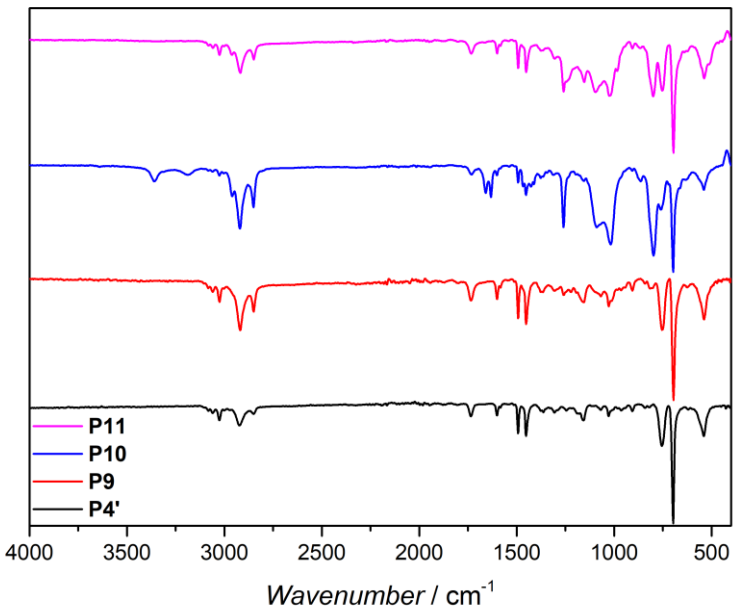


Fig. S25. ATR-IR spectra of the liner polymer precursor **P4'**, the folded SCNPs **P9**, the unfolded SCNPs **P10** and the refolded polymer **P11**.

C.6 GC-MS Data

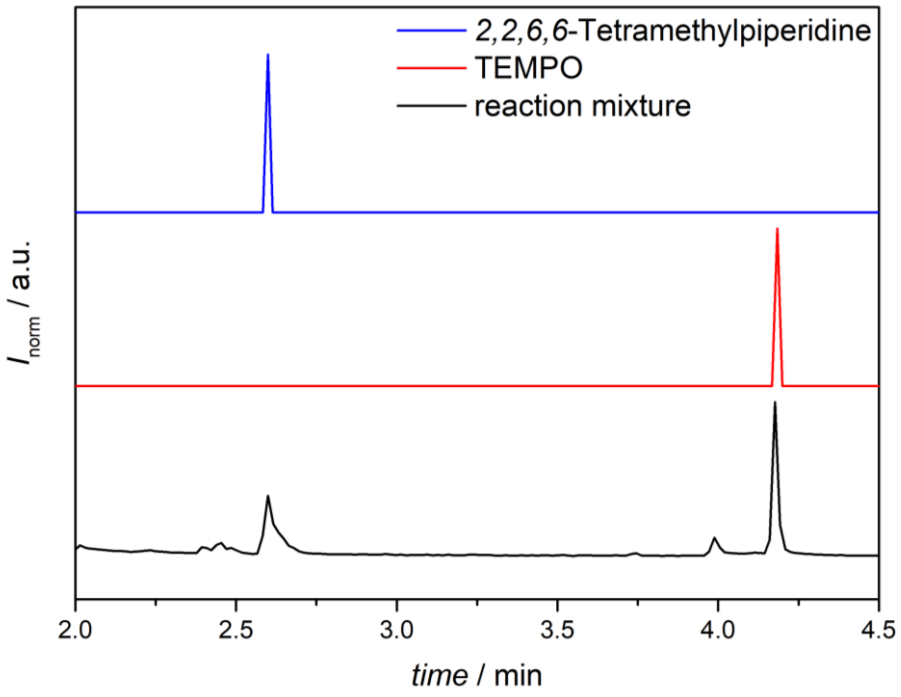


Fig. S26. GC-MS chromatogram of the unfolding/deprotection test reaction mixture.

C.7 Quantum Yield Determination

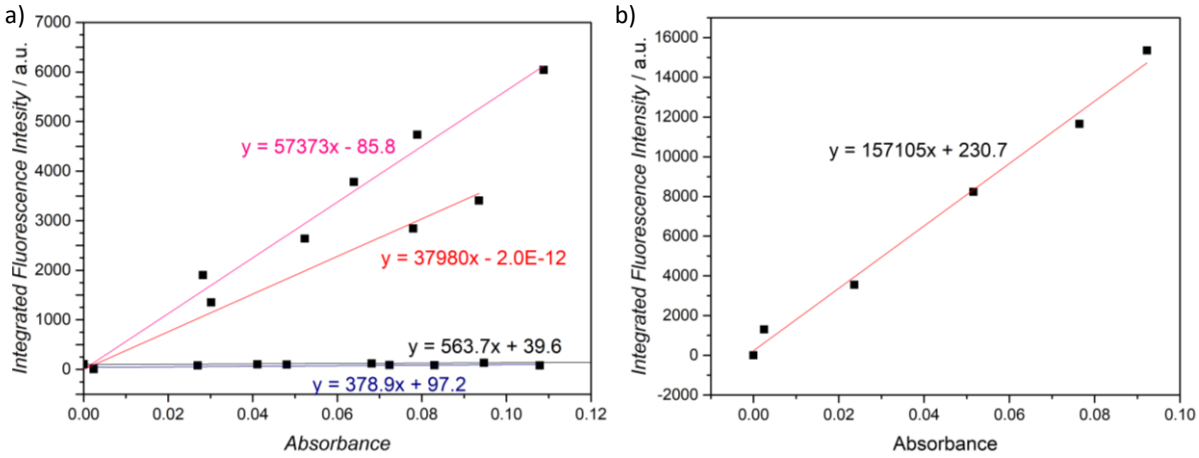


Fig. S27. (a) Fluorescence quantum yield estimation in DCM for **P4'**, **P9**, **P10** and **P11**. The fluorescence quantum yields for the folded polymer **P9** and **P11** were determined to be close to 0.069 and 0.102, respectively, while the unfolded linear polymers **P4'** and **P9** displayed fluorescence quantum yields of approximately 1.0×10^{-3} and 6.9×10^{-4} utilizing (b) anthracene (in EtOH) as reference system, as reported in literature.[2] The procedure for the calculation of the quantum yields was adopted from refs. [3] and [4]. The respective refractive indices of DCM and ethanol at 25 °C read 1.4125 and 1.361.

C.8 EPR Data

The TEMPO density per polymer was calculated according to:

$$\frac{Spin\ concentration}{Polymer\ concentration}$$

Table S.2 Material information used for the preparation of the EPR experiments and resulting concentrations.

	m /g	M /g/mol	n /mmol	V /mL	$c_{Polymer}$ /mol/L
P4'	0.0027	33300	8.10811E-05	0.5	0.000162
P9	0.0034	18500	0.000183784	1.13	0.000162
P10	0.0015	33400	4.49102E-05	0.27	0.000166
P11	0.005	16200	0.000308642	1.9	0.000162

Table S.3. *g*-factors and hyperfine coupling constants (a_N) for the parent polymer **P4'**, the folded **P9**, the unfolded **P10** and the refolded **P11** SCNPs.

	P4'	**P9**	**P10**	**P11**
g	2.00725	2.00707	2.00727	2.00697
a_N **/ G**	15.24	15.51	15.24	15.48
***Spin* /mm^3**	3.79E+14	4.21E+13	2.16E+14	3.16E+12
c_{spin} **/ mol L^{-1}**	3.79E-4	7.00E-5	3.59E-4	5.25E-6
Spin Count	2.69E+16	2.98E+15	1.53E+16	2.23E-14
***Microwave frequency* / GHz**	9.644	9.644	9.646	9.645
***Magnetic field B* / mT**	343.34	343.34	343.34	343.34

C.9 Autocorrelation Data

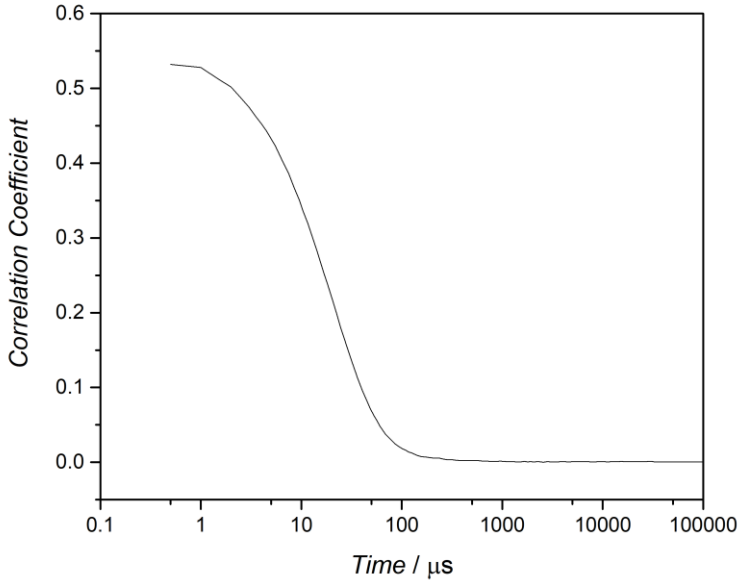


Fig. S28. Autocorrelation curve for **P4'** measured in DMAc +0.3% LiBr.

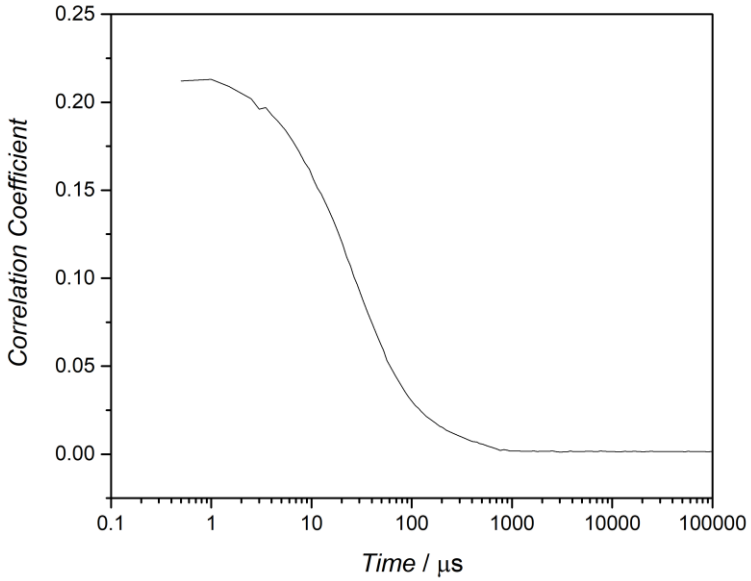


Fig. S29. Autocorrelation curve for **P9** measured in DMAc +0.3% LiBr.

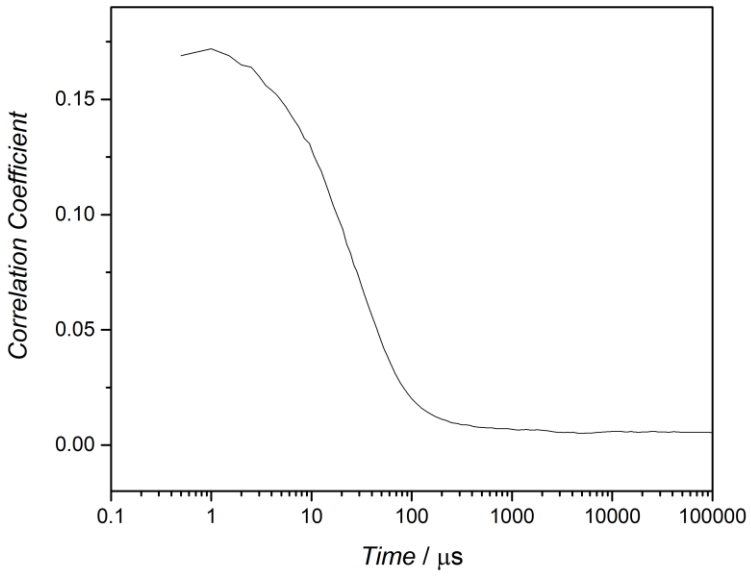


Fig. S30. Autocorrelation curve for **P10** measured in DMAc +0.3% LiBr.

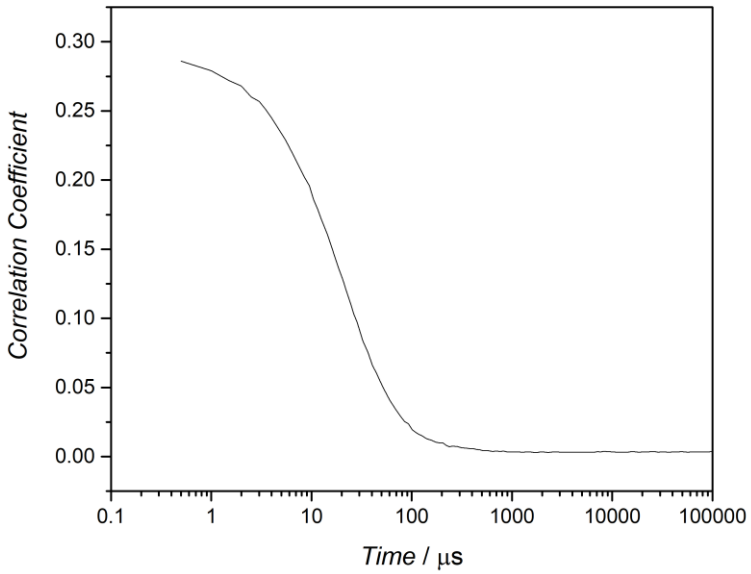


Fig. S31. Autocorrelation curve for **P11** measured in DMAc +0.3% LiBr.

C.10 Primary DOSY Data

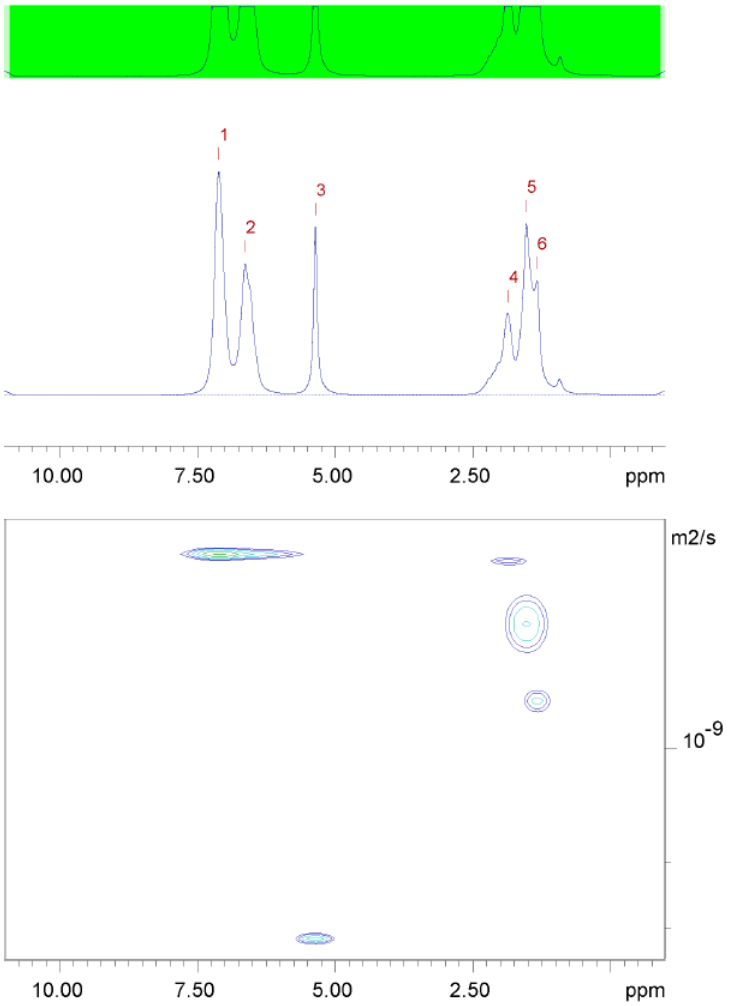


Fig. S32. Original Figure of DOSY measurements of **P4'**, recorded in DCM at 298 K.

Fitted function:	f (x) = Io * exp (-D * x^2 * gamma^2 * littleDelta^2 (bigDelta-littleDelta/3)* 10^4
used gamma:	26752 rad/(s*Gauss)
used little delta:	0.0030000 s
used big delta:	0.10990 s
used gradient strength:	variable
Random error estimation of data:	RMS per spectrum (or trace/plane)
Systematic error estimation of data:	worst case per peak scenario
Fit parameter Error estimation method:	from fit using arbitray y uncertainties
Confidence level:	95%
Used peaks:	
Used integrals:	peak intensities
Used Gradient strength:	all values (including replicates) used

Peak name	F2 [ppm]	D [m2/s]	error
1	7.114	3.09e-10	3.462e-12
2	6.633	3.08e-10	3.559e-12
3	5.357	3.17e-09	2.134e-11
4	1.854	3.15e-10	2.824e-12
5	1.531	4.72e-10	7.317e-11
6	1.339	7.56e-10	4.722e-11

Fig. S33. Original Data of DOSY measurements of **P4'**, recorded in DCM at 298 K.

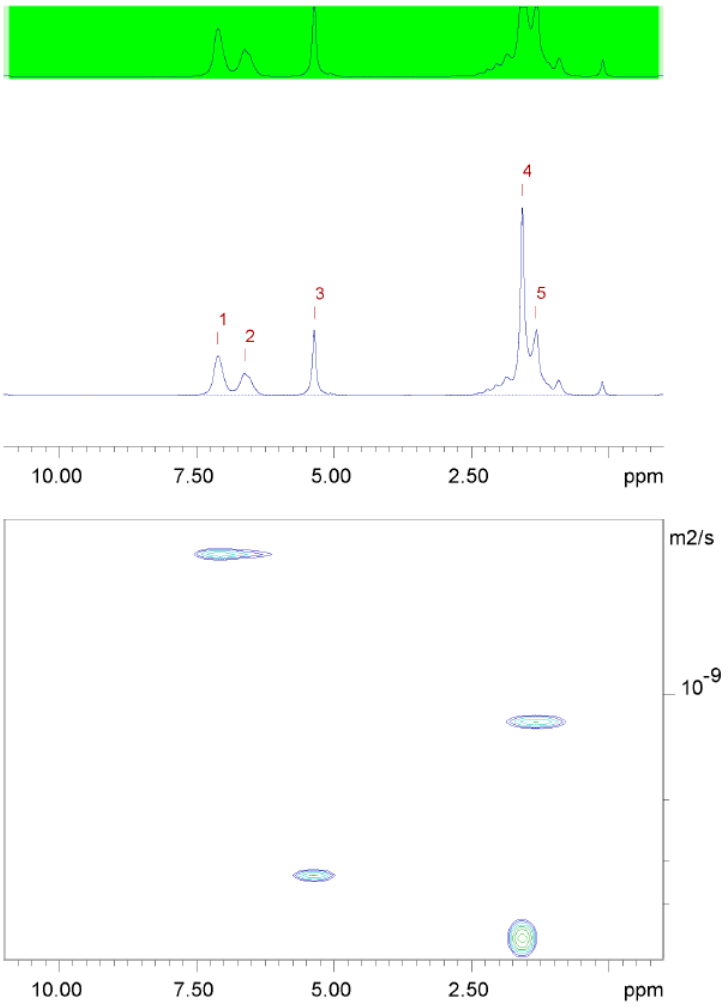


Fig. S34. Original Figure of DOSY measurements of **P9**, recorded in DCM at 298 K.

Fitted function:	f (x) = Io * exp (-D * x^2 * gamma^2 * littleDelta^2 (bigDelta-littleDelta/3)* 10^4
used gamma:	26752 rad/(s*Gauss)
used little delta:	0.0024000 s
used big delta:	0.079900 s
used gradient strength:	variable
Random error estimation of data:	RMS per spectrum (or trace/plane)
Systematic error estimation of data:	worst case per peak scenario
Fit parameter Error estimation method:	from fit using arbitray y uncertainties
Confidence level:	95%
Used peaks:	
Used integrals:	peak intensities
Used Gradient strength:	all values (including replicates) used

Peak name	F2 [ppm]	D [m2/s]	error
1	7.114	3.93e-10	6.139e-12
2	6.624	3.97e-10	6.366e-12
3	5.357	3.25e-09	2.563e-11
4	1.583	5.00e-09	3.588e-10
5	1.330	1.19e-09	4.093e-11

Fig. S35. Original Data of DOSY measurements of **P9**, recorded in DCM at 298 K.

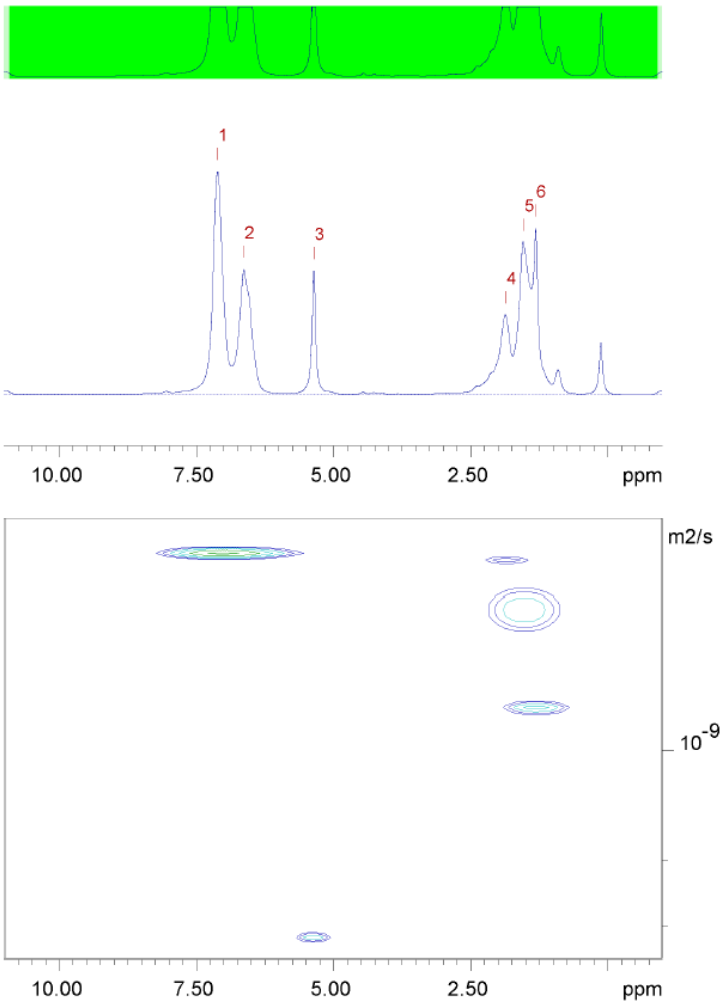


Fig. S36. Original Figure of DOSY measurements of **P10**, recorded in DCM at 298 K.

Fitted function:	f (x) = Io * exp (-D * x^2 * gamma^2 * littleDelta^2 (bigDelta-littleDelta/3)* 10^4
used gamma:	26752 rad/(s*Gauss)
used little delta:	0.0030000 s
used big delta:	0.10990 s
used gradient strength:	variable
Random error estimation of data:	RMS per spectrum (or trace/plane)
Systematic error estimation of data:	worst case per peak scenario
Fit parameter Error estimation method:	from fit using arbitray y uncertainties
Confidence level:	95%
Used peaks:	
Used integrals:	peak intensities
Used Gradient strength:	all values (including replicates) used

Peak name	F2 [ppm]	D [m2/s]	error
1	7.114	2.96e-10	2.027e-12
2	6.633	2.95e-10	1.988e-12
3	5.357	3.20e-09	7.985e-12
4	1.854	3.12e-10	4.236e-12
5	1.531	4.12e-10	4.888e-11
6	1.312	7.58e-10	2.753e-11

Fig. S37. Original Data of DOSY measurements of **P10**, recorded in DCM at 298 K.

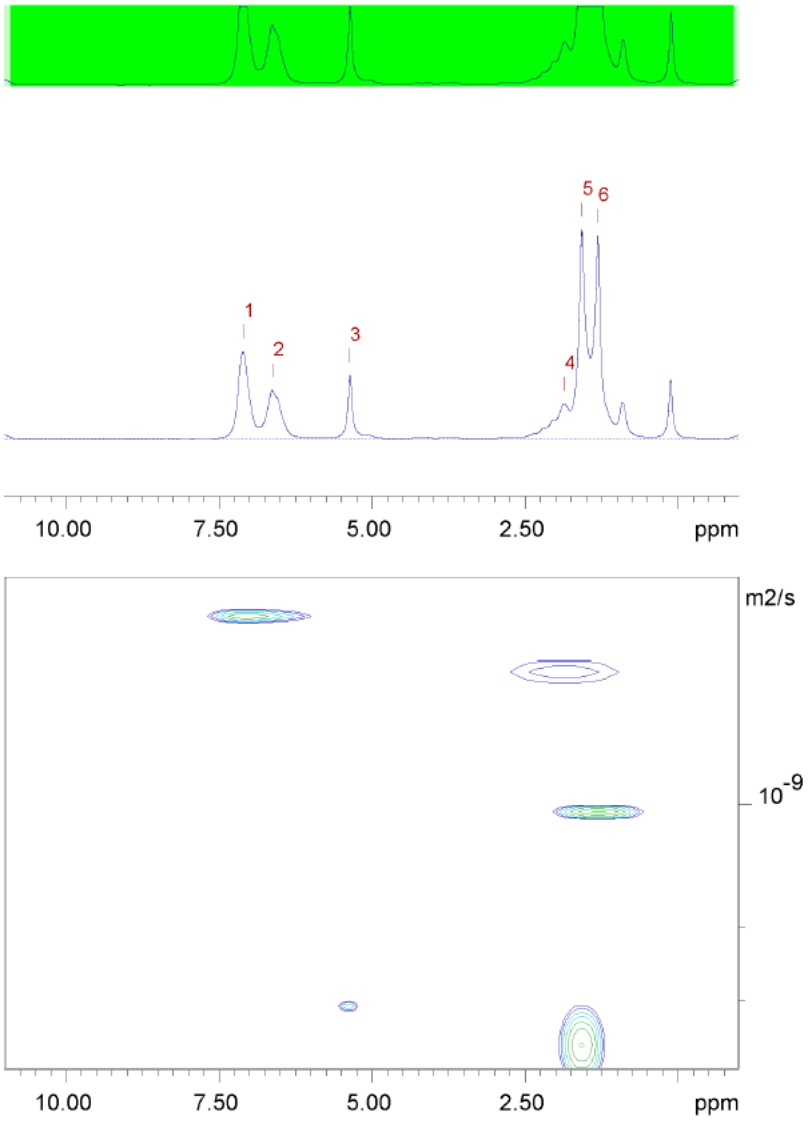


Fig. S38. Original Figure of DOSY measurements of **P11**, recorded in DCM at 298 K.

Fitted function:	f (x) = Io * exp (-D * x^2 * gamma^2 * littleDelta^2 (bigDelta-littleDelta/3)* 10^4
used gamma:	26752 rad/(s*Gauss)
used little delta:	0.0024000 s
used big delta:	0.079900 s
used gradient strength:	variable
Random error estimation of data:	RMS per spectrum (or trace/plane)
Systematic error estimation of data:	worst case per peak scenario
Fit parameter Error estimation method:	from fit using arbitray y uncertainties
Confidence level:	95%
Used peaks:	
Used integrals:	peak intensities
Used Gradient strength:	all values (including replicates) used

Peak name	F2 [ppm]	D [m2/s]	error
1	7.105	3.54e-10	4.073e-12
2	6.624	3.55e-10	3.911e-12
3	5.375	3.08e-09	4.530e-11
4	1.863	4.75e-10	3.392e-11
5	1.575	3.83e-09	5.387e-10
6	1.312	1.04e-09	2.106e-11

Fig. S39. Original Data of DOSY measurements of **P11**, recorded in DCM at 298 K.

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