Supplementary Information

An unexpected route to aldehyde-decorated single-chain nanoparticles from azides

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Materials

Styrene (St) (\geq 99%), 4-(chloromethyl) styrene (CMS) (\geq 90%), sodium azide (NaN₃) (\geq 99%), dansylhydrazine (98%), 4,4'-Azobis(4-cyanovaleric acid) (ACVA) (\geq 98%), N-N-Dimethylformamide (DMF) (\geq 99.9%), *N*-methyl-2-pyrrolidone (NMP) (\geq 99%), benzaldehyde (BA) (purified by redistillation, \geq 99.5%), chloroform (CHCl₃) (\geq 99.8%) and deuterated chloroform (CDCl₃) (99.96 atom % D, containing 0.03 % (v/v) tetramethylsilane, TMS) were purchased from Aldrich and used, unless specified, as received. Methanol (MeOH) (synthesis grade) and tetrahydrofuran (THF) (HPLC grade) were purchased from Scharlab. Benzyl azide (94%) was purchased from Alpha Aesar. Deionized water obtained from a Thermo Scientific apparatus (Barnstead TII Pure Water System) was employed in this work. St and CMS were purified by passing through basic alumina.



Scheme S1. Structures of some compounds used in the current study.

Instrumentation

Microwave-assisted synthesis

Microwave-assisted synthesis was performed in a CEM Discover LabMateTM apparatus. It was equipped with an 80 ml Sealed Vessel Accessory, which has a working volume ranging from 15 ml to 50 ml.

Nuclear Magnetic Resonance Spectroscopy

¹H-NMR and ¹³C-NMR spectra were recorded at room temperature on Bruker spectrometers operating at 500.13 MHz for hydrogen nuclei and 125.06 MHz for carbon nuclei, using CDCl₃ as solvent.

Size Exclusion Chromatography

Size exclusion chromatography (SEC) measurements with triple detection (differential refractive index (DRI), multi-angle laser light scattering (MALLS) and viscosimetric (VI) detectors) were performed at 30 °C on an Agilent 1200 system equipped with PLgel 5 μ m Guard and PLgel 5 μ m MIXED-C columns, a differential refractive index (DRI) detector (Optilab Rex, Wyatt), a multi-angle laser light scattering (MALLS) detector (MiniDawn Treos, Wyatt) and a viscosimetric (VI) detector (ViscoStar-II, Wyatt). THF was used as eluent at a flow rate of 1 ml / min. Data analysis was performed with ASTRA Software (version 6.1) from Wyatt, by using dn / dc = 0.185.

Dynamic Light Scattering

Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano ZS apparatus at 25 °C, using DMF as solvent.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were carried out on ~ 5 mg specimens using a Q2000 TA Instruments in standard mode. A helium flow rate of 25 mL / min was used throughout. Measurements were performed by placing the samples in sealed aluminum pans, heating to 130 °C (NPs to 150 °C) at 10 °C / min, then cooling to 0 °C at 10 °C / min, and heating back to 130 °C (NPs to 150 °C) at 10 °C/ min.

Thermogravimetric analysis

TGA measurements were performed in a Q500-TA Instruments apparatus at a heating rate of 10 $^{\circ}$ C / min under nitrogen atmosphere.

Infrared Spectroscopy

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were recorded at room temperature on a JASCO 3600 FTIR spectrometer.

Small-angle X-ray Scattering

SAXS experiments were conducted on a Rigaku 3-pinhole PSAXS-L equipment operating at 45 kV and 0.88 mA. The MicroMax-002+ X-ray generator system is composed by a microfocus sealed tube S5 source module and an integrated X-ray generator unit which produces Cu K α transition photons of wavelength $\lambda = 1.54$ Å. The flight path and the sample chamber in this equipment are under vacuum. The scattered X-rays are detected on a two-dimensional multiwire X-ray detector (Gabriel design, 2D-200X). This gas-filled proportional type detector offers a 200 mm diameter active area with ca. 200 µm resolution. The azimuthally averaged scattered intensities were obtained as a function of momentum transfer Q = $(4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle. Reciprocal space calibration was done using silver behenate as standard. Measurements were performed at room temperature with a sample to detector distance of 2 m. The solutions (concentration: 4 mg / mL, DMF as solvent) were filling capillaries of 2 mm thickness fixed perpendicular to the beam. The data were background corrected by subtracting the result of a measurement on the capillary filled with DMF and applying the proper transmission corrections.

Dielectric Spectroscopy

A broadband dielectric spectrometer, Novocontrol Alpha, was used to measure the complex dielectric function, $\epsilon^*(\omega) = \epsilon'(\omega) - i\epsilon''(\omega)$, covering a frequency range of $f = (\omega/2\pi) = 10^{-1} - 10^6$ Hz. The sample capacitor preparation for this setup consisted in an upper gold-coated electrode of 15 mm placed on a prepared film of the sample over a 20 mm gold-coated electrode. A separation of 100 µm between both electrodes was maintained by using a Teflon spacer of small area. The sample cell was set in a cryostat, and its temperature was controlled *via* nitrogen gas jet stream coupled with the Novocontrol Quatro controller. The isothermal dielectric experiments of single-chain nanoparticles were performed while cooling the sample from 147 °C to -143 °C in steps of 10 °C. The data reproducibility was checked during subsequent heating from -143 °C to 147 °C. The maximum temperature for the precursor was set at 127 °C to avoid degradation. The films were prepared by solvent casting and dried at 120 °C under

dynamic vacuum for 48 h, in order to remove any trace of moisture and / or solvent residues.

Synthesis

Synthesis of reference P0 (neat polystyrene) and poly(styrene-*co*-chloromethyl styrene) precursors C9, C16, C21 and C30

The synthesis of neat polystyrene (**P0**) was carried out by using St (2 ml, 17.4 mmol) as monomer and ACVA (1.7 mg, 6.1×10^{-3} mmol) as initiator. The reaction mixture was degassed by passing argon for 15 min and then stirred for 3 h at 80 °C. The resulting homopolymer (**P0**) was isolated by precipitation in methanol and further drying at r.t. under dynamic vacuum (Yield: 10 %, M_w (SEC) = 283 kDa, PDI (SEC) = 1.2, R_H (DLS) = 14 nm, T_g (DSC) = 103 °C).

In a typical procedure of synthesis of precursors, the copolymerization of St (2 ml, 17.4 mmol) and CMS (0.434 ml, 3.1 mmol) was carried out using ACVA (1.7 mg, $6.1x10^{-3}$ mmol) as initiator. The reaction mixture was degassed by passing argon for 15 min and then stirred for 3 h at 80 °C. After that, the copolymer was redissolved in a minimal amount of THF and added to a large excess of methanol. The resulting precursor (**C21**) was isolated by filtration and further dried at r.t. under dynamic vacuum (Yield: 30 %, M_w (SEC) = 235 kDa, PDI (SEC) = 1.3). Copolymers **C9**, **C16**, **C21** and **C30** were obtained as light yellow powders with 9-30 mol% of CMS content, as determined by ¹H NMR spectroscopy following reported procedures.^[1]



Figure S1. Synthesis of poly(styrene_x-*co*-chloromethyl styrene_y) copolymers (y = 9-30 mol%).

Synthesis of poly(styrene-*co*-azidomethyl styrene) precursors P9, P16, P21 and P30

In a typical reaction, poly(styrene-*co*-chloromethyl styrene) (C21, 350 mg, 0.64 mmol CMS) was dissolved in DMF (14 ml) at room temperature. Then, NaN₃ (2 eq., 83.2 mg, 1.3 mmol,) was added and the mixture was maintained under stirring for 24 h. After reaction completion, the system was concentrated and precipitated in a mixture of MeOH/H₂O (1:1). Finally, the resulting precursor (P21) was dried in a vacuum oven at room temperature under dynamic vacuum (Yield: 90 %, M_w (SEC) = 242 kDa, PDI (SEC) = 1.3, R_H (DLS) = 13 nm, T_g (DSC) = 95 °C). Precursors P9, P16, P21 and P30 were obtained as powders with 9 - 30% mol of AMS content. The complete transformation of chloromethyl (4.5 ppm) to azidomethyl (4.2 ppm) moieties was confirmed by ¹H NMR spectroscopy (see Figure S2).



Figure S2. ¹H NMR spectra in CDCl₃ of C21 and P21.

Synthesis of network cross-linked materials B9, B16, B21 and B30

In a typical procedure, the precursor (**P21**, 5 mg, 0.0432 mmol) was dissolved in CHCl₃ (5 mL) at room temperature. Then, the mixture was deposited over a gold-coated electrode of 20 mm and dried at 80 °C under dynamic vacuum for 48 h in order to remove any residual solvent. The formed film was covered with a gold-coated electrode of 15 mm, heated in the DS instrument to 200 °C and maintained there for 3 h. The resulting network cross-linked material (**B21**) was found to be insoluble in any solvent.

Synthesis of intra-chain cross-linked nanoparticles NP9, NP16, NP21 and NP30

In a typical reaction, the precursor (**P21**, 50 mg, 0.432 mmol) was dissolved in DMF or NMP (50 mL) at room temperature. Then, the mixture was heated to 200 °C under microwave irradiation (300 W, 150 psi) and maintained there for 30 min. Then, the system was cooled down to room temperature and concentrated in a vacuum line using Schlenk flasks. The resulting nanoparticles (**NP21**) were isolated by precipitation in a mixture of MeOH/H₂O (1:1) and dried in a vacuum oven at 40°C under dynamic vacuum (Yield: 57 %, M_w (SEC) = 242 kDa, PDI (SEC) = 1.3, R_H (DLS) = 10 nm, T_g (DSC) = 123 °C).

Functionalization with dansylhydrazine of nanoparticle NP30

For functionalization of nanoparticle **NP30** with dansylhydrazine, 10 mg of benzaldehyde-decorated single-chain nanoparticles and 4.4 mg of dansylhydrazine were dissolved in 1 mL of chloroform and maintained under agitation for 20 h. After reaction, the mixture was precipitated in a large excess of methanol to remove any amount of unreacted dansylhydrazine and to recover the functionalized nanoparticles which were dried in a vacuum oven at r.t. under dynamic vacuum. Then, 2 mg of the resulting functionalized nanoparticles were dissolved in 1 mL of tetrahydrofuran. The resulting solution was placed under UV light (wavelength: 365 nm) and showed intense fluorescence (Figure 4).

Supplementary data

Characterization data of control P0 and precursors P9, P16, P21 and P30

¹H NMR spectra of control P0 and precursors P9, P16, P21 and P30



Figure S3. ¹H NMR spectra in CDCl₃ of neat polystyrene (**P0**) and poly(styrene-*co*-azidomethyl styrene) precursors (**P9**, **P16**, **P21** and **P30**).

SEC chromatograms of control P0 and precursors P9, P16, P21 and P30





Figure S4. SEC traces (MALLS detector) of neat polystyrene (**P0**) and poly(styrene-*co*-azidomethyl styrene) precursors (**P9**, **P16**, **P21** and **P30**).





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Figure S5. IR sepectra of neat polystyrene (P0) and poly(styrene-*co*-azidomethyl styrene) precursors (P9, P16, P21 and P30).





Figure S6. Size distribution by intensity measured by DLS of poly(styrene-*co*-azidomethyl styrene) precursors (**P9**, **P16**, **P21** and **P30**).





Figure S7. SAXS form factor of poly(styrene-*co*-azidomethyl styrene) precursors (**P9**, **P16**, **P21** and **P30**). Values of the scaling exponent (v) obtained from the intermediate regime, where the intensity scales as I(Q) α Q^{-1/v}, are shown for each sample, which in all cases is the one expected for linear chains in good solvent (Flory exponent, $v = v_F = 0.59$).

DSC thermograms of precursors P0, P9, P16, P21 and P30



Figure S8. Heat capacity normalized to its maximum value of neat polystyrene (P0) and poly(styrene-*co*-azidomethyl styrene) precursors (P9, P16, P21 and P30).

Azide thermal decomposition experiments



Figure S9. Typical TGA curve of a poly(styrene-*co*-azidomethyl styrene) precursor (P30) showing the initial decomposition of azide groups at 200 °C.

Characterization data of nanoparticles NP9, NP16, NP21 and NP30

¹H NMR spectra of nanoparticles NP9, NP16, NP21 and NP30



Figure S10. ¹H NMR spectra in CDCl₃ of intra-chain cross-linked nanoparticles (**NP9**, **NP16**, **NP21**, **NP30**) compared to their corresponding precursors (**P9**, **P16**, **P21**, **P30**).

¹³C NMR spectra of nanoparticles NP9, NP16, NP21 and NP30



Figure S11. ¹³C NMR spectra in CDCl₃ of intra-chain cross-linked nanoparticles (**NP9**, **NP16**, **NP21**, **NP30**).





Figure S12. SEC traces (MALLS detector) of intra-chain cross-linked nanoparticles (NP9, NP16, NP21, NP30) and their corresponding precursors (P9, P16, P21, P30) as reference (dashed lines). All NPs show longer retention times than the corresponding linear precursors, showing consequently a reduction in hydrodynamic volume.

IR spectra of nanoparticles NP9, NP16, NP21 and NP30





Figure S13. IR sepectra of intra-chain cross-linked nanoparticles (NP9, NP16, NP21, NP30) and their corresponding precursors (P9, P16, P21, P30) as reference (dashed lines).





Figure S14. Size distribution by intensity measured by DLS of intra-chain cross-linked nanoparticles (NP9, NP16, NP21, NP30) and their corresponding precursors (P9, P16, P21, P30) as reference (dashed lines). All NPs display smaller hydrodynamic size than the corresponding linear precursors.





Figure S15. SAXS form factor of intra-chain cross-linked nanoparticles (NP9, NP16, NP21, NP30) and their corresponding precursors (P9, P16, P21, P30) as reference (empty circles). All NPs display smaller values of the scaling exponent than the corresponding precursors, which reflect the increased compaction of the chain upon intra-chain cross-linking.

DSC thermograms of nanoparticles NP9, NP16, NP21 and NP30



Figure S16. Heat capacity normalized to its maximum value of intra-chain cross-linked nanoparticles (NP9, NP16, NP21, NP30) and their corresponding precursors (P9, P16, P21, P30) as reference (dashed lines).

Dielectric spectroscopy results

Dielectric spectroscopy results of a mixture of P0 and benzaldehyde



Figure S17. Tan δ normalized to its maximum value at T = -123 °C of a mixture of **P0** and neat benzaldehyde (grey), and **NP21** (green).

Activation energy for nanoparticles NP9, NP16, NP21 and NP30



Figure S18. Arrhenius plot giving the activation energy for the rotation around the phenyl ring of the benzaldehyde pendants in nanoparticles NP9, NP16, NP21 and NP30.

Nanoparticle synthesis in NMP

The results obtained in the characterization of intra-chain cross-linked nanoparticles synthesized in DMF and NMP were very similar. Figure S19 shows IR spectra corresponding to nanoparticles synthesized in DMF (green) and NMP (brown), displaying very similar results. In both cases, the presence of carbonyl groups (intense band at 1700 cm⁻¹) and –NH– groups (1650 cm⁻¹) is observed. Similarly, Figure S20 displays ¹H NMR spectra of both samples having the same characteristic peaks, as indicated.



Figure S19. IR spectra of intra-chain cross-linked nanoparticles NP21 synthesized in DMF (green) and NMP (brown).



Figure S20. ¹H NMR spectra in CDCl₃ of intra-chain cross-linked nanoparticles **NP21** synthesized in DMF (green) and NMP (brown).

Control experiments with benzyl azide

The same experimental procedure followed for nanoparticle synthesis was carried out with benzyl azide, but using a solution of 500 mg of benzyl azide in 50 mL of DMF. After reaction, the crude mixture was concentrated and a portion of the mixture was analyzed by ¹H NMR and ¹³C NMR (Figure S21 and S22, blue color). No sign of aldehyde groups were observed and only signals coming from –NH– groups were present. A portion of the crude mixture was precipitated in water, centrifugated, isolated and redissolved in deuterated chloroform for ¹H NMR and ¹³C NMR analysis (Figure S21 and S22, green color). The presence of signals from aldehyde groups support the mechanism proposed in Scheme 3.



Figure S21. ¹H NMR spectra in CDCl₃ of benzyl azide (red), benzyl azide after m.w. reaction without treatment with water (blue), and benzyl azide after m.w. reaction and treatment with water (green).



Figure S22. ¹³C NMR spectra in CDCl₃ of benzyl azide after m.w. reaction without treatment with water (blue), and benzyl azide after m.w. reaction and treatment with water (green).

References

1. K. W. Huang and S. W. Kuo, *Macromol. Chem. Phys.*, 2012, **213**, 1509.