Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

A Tunable One-Pot Three-Component Synthesis of an 125I and Gd-Labelled Star Polymer Nanoparticle for Hybrid Imaging with MRI and Nuclear Medicine

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Methods

Materials

4-Vinylbenzyl chloride (4-VBC, 90%, Sigma-Aldrich) was passed through a column of basic alumina (Sigma-Aldrich) in order to remove the inhibitor before use. 3-Butyn-1-ol (97%), copper(II) chloride (> 99.999%), gadolinium(III) chloride hexahydrate (99%), *N,N'*-methylenebis(acrylamide) (MBAA, 99%), oligo(ethylene glycol) methyl ether acrylate (average M_n 480 g mol^{-1}), propargylamine (98%), sodium iodide (≥ 99.5%), triethylamine (≥ 99%), and trifluoroacetic acid (99%) were used as received from Sigma-Aldrich. *N,N*-dimethylformamide (DMF, ≥ 99.8%) was supplied by Merck Millipore. Potassium hydroxide pellets (≥ 85%) were purchased from Chem-Supply. Butyne-DOTA-tris(t-butyl ester) was obtained from Macrocyclics and Chelex® 100 resin was supplied by Bio-Rad. Azobis(isobutyronitrile) (AIBN, Fluka, 98%) was purified by recrystallization from methanol. Iodine-125 radionuclide (0.1 M sodium hydroxide) was obtained from Perkin Elmer and diluted to 100 MBq/mL in DMSO. Finally, 3-(benzyl sulfanyl thiocarbonyl sulfanyl)-propanoic acid (BSPA) was prepared according to a procedure described in literature.[1]

Physical and analytical methods

^{1}H NMR

^{1}H NMR spectra were recorded using a Bruker UltraShield 400 (400.13 MHz) spectrometer running Bruker Topspin, version 1.3. Deuterated chloroform ($CDCl_3$), dimethyl sulfoxide-d_6 (DMSO-d_6), deuterated methanol (MeOD) or deuterium oxide (D_2O) were used as solvent. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of the residual solvent resonances ($CDCl_3$ = 7.26 ppm, DMSO-d_6 = 2.50, MeOD = 3.31, D_2O = 4.79).

Size exclusion chromatography

DMAc size exclusion chromatography (SEC) was implemented using a Shimadzu modular system comprising a SIL20AD automatic injector, a DGU12A degasser, a CTO10A column oven, a LC10AT pump, a RID10A differential refractive-index detector, and a SPD10A Shimadzu UV/Vis detector. A 50 × 7.8 mm guard column followed by three KF-805L columns in series (300 × 8 mm linear columns, bead size: 10 µm, pore size maximum: 5000 Å) were used for the analyses. *N,N′*-Dimethylacetamide (DMAc, HPLC grade, 0.03% w/v lithium bromide) with a flow rate of 1 mL min^{-1} and a constant temperature of 50 °C was used as the mobile phase. The samples were filtered through 0.45 µm filters prior to injection. The unit was calibrated using commercially available linear poly(styrene) standards (0.5–2000 kDa, Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

For radio-iodine:

Aqueous size exclusion chromatography (SEC) was implemented using a Waters system comprising a Waters 717 autosampler, a Waters In-line degasser, a Waters 515 HPLC pump, a Waters 2414 refractive index detector, a Waters 486 tuneable absorbance detector and an IN/US Systems γ-RAM model 4 radio-HPLC detector (15 mm loop and HV of 190). An Agilent PL aquagel-OH 50 column was used for analysis.

A phosphate buffer (0.10 mM, 0.1 M sodium nitrate, containing 20% DMSO (HPLC grade) with a flow rate of 0.6 mL min^{-1} and a constant temperature of 40 °C was used as the mobile phase. The samples were filtered through 0.45 μm filters (Alltech 13 mm nylon syringe filter polypropylene housing) prior to injection.

Dynamic light scattering

DLS measurements were performed using a Malvern Zetasizer Nano Series running DTS software and using a 4 mW He−Ne laser operating at a wavelength of 633 nm and an avalanche photodiode detector. The scattered light was detected at an angle of 173°.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy

ATR-FTIR measurements were performed using a Shimadzu IRTracer 100 Fourier transform infrared spectrometer with a GladiATR 10 single reflection ATR accessory. Spectra were then obtained in the mid infrared region of 4000–600 cm^{-1} at a resolution of 8 cm^{-1} (512 scans) and analysed using LabSolution IR software.

Ultra-high performance liquid chromatography / mass spectroscopy (UHPLC/MS)

The chromatographic analysis was performed using an Agilent 1260 Infinity system equipped with a binary pump (G1312C), autosampler (G1367E), DAD module (G4212A) and an 1100 MSD mass spectrometer. System control, data acquisition and interpretation were made using LC/MSD Chemstation Rev.B.04.03 coupled with Masshunter Easy Access Software.

For LC: reverse phase HPLC analysis was carried out using a Poroshell 120 EC-C18 column (3.0 x 50 mm, 2.7-micron) at 35°C and an injection volume of 1 μl. Starting with water containing 0.1% formic acid), a gradient was introduced (5-100% acetonitrile containing 0.1% formic acid) over 3.8 minutes with a flow rate of 0.500 mL/min.

For MS: ionization was performed using a quadrupole API-ES source, a capillary voltage of 3000 V and a drying gas temperature of 350°C

Inductively Coupled Plasma−Optical Emission Spectrometry (ICP-OES)

The Gd^{3+} content of the polymer-based nanoparticles was determined by inductively coupled plasma−optical emission spectrometry (ICP-OES) using a Perkin-Elmer OPTIMA 7300 spectrometer. Samples were prepared by dissolving 0.5 mg of polymer in 10 mL of ultrapure water.

Transmission electron microscopy (TEM)

TEM imaging was performed using a Tecnai F20 transmission electron microscope at an accelerating voltage of 200 kV at ambient temperature. An aliquot (10 μL) of a diluted nanoparticle solution in water was deposited on a Formvar coated copper grid (GSCu100F+50, Proscitech) and allowed to dry overnight in air and at ambient temperature.

Magnetic Resonance Imaging

MRI scans were taken from aqueous dilution series in a Costar 96-well assay block (3959, 1 mL well volume, round bottom). To limit artefacts, the backside of the assay block was filled with 3% agarose using the following procedure: 200 mL TAE buffer (40 mM tris base, 40 mM acetic acid, and 1 mM EDTA) was rapidly stirred and 6.0 g agarose (type 1, low eeo) was added slowly to allow thorough dispersion. Next, the dispersion was heated to 95 °C until all agarose was dissolved (15 minutes). Any bubbles were removed by gentle shaking. The solution was then allowed to cool down to 60 °C before carefully decanting it into the backside of the 96-well assay block. The agarose was cooled further for another hour and formed a semi-transparent white hydrogel.

Dilution series of Gd-DOTA conjugated star polymers and butyne-Gd-DOTA were (dilution factor 2, 1.0 mL each, highest concentration ~ 0.25 mM Gd, 5 dilutions), and remaining wells were filled with water as control. Finally, the wells were sealed with parafilm to prevent spillage and well-to-well contamination.

The MRI properties of the gadolinium-labelled polymeric nanoparticles were studied using a state-of-the-art 7-Tesla whole-body MRI scanner (MAGNETON 7T, Siemens) using Nova single channel transmit with 32 receiver channels with an internal diameter of 18.5 cm and a 21 cm field of view along the z-direction). A dilution series of each nanoparticle in water was prepared in a 96 wells plate for high-throughput measurement. The space between the wells was filled with 3% agarose to minimize susceptibility effects caused by water-air interfaces.

For the determination of the r_1 relaxivity, inversion recovery sequences were utilized with different inversion times (22, 250, 500, 1000, 2000, 3000, and 4000 ms), TR = 5000 ms and TE 11.5 ms. All images were acquired with a 2 mm slice thickness, 150 x 112.5 mm FOV, 256 x 192 matrix size, and 1 average. Region of interest (ROI) areas of 5 x 5 voxels (25 voxels) were manually placed over the central part of each well on the images using Matlab (version R2015A). To calculate the R_1 relaxation rate ($R_1 = 1 / T_1$), the signals from each region of interest were averaged and plotted as a function of inversion time. The R_1 values were then calculated (as the decay constants) by numerically fitting the data to the following equation.

$$S = S_0\left(1 - 2e^{-TI \cdot R_1}\right) \tag{1}$$

The longitudinal relaxation rates R_1 were then plotted as a function of the gadolinium concentration and linear least-squares analysis (GraphPad Prism) was used to determine the r_1 and r_2 relaxivities.

$$r_1 = \frac{R_1 - R_{1(0)}}{[Gd]} \tag{2}$$

With R_1 is the relaxation rate in presence of the contrast agent and $R_{1(0)}$ in absence (water only).

Cell viability assay

Cytotoxicity profile of star(N3) and star(Gd-DOTA) at the concentration ranging from 0.06 to 1000 µg mL-1 were carried out using AlamarBlue assay. Human Umbilical Vein Endothelial Cells (HUVECs - Lonza) were seeded onto 96-well plates at a cell density of 5,000 cells per well. After that, the cells were incubated for 24 h in a humidified incubator at 37 °C, 5% CO_2 for allowing the cells to attach. The old media was then removed and replenished with 100 µL of fresh media along with different concentrations of the particles. After 48 h incubation, the cells were washed twice with chilled HEPES buffered saline solution (HBSS – Sigma Aldrich), and then 10% (v/v) AlamarBlue reagent (Life Technologies) in EGM was added. HUVECs were incubated for 4 h further at 37 °C, 5% CO_2 before being analysed by using a ClarioStar microplate reader. Fluorescence were measured at excitation 510 nm and emission 610 nm. The experiments were performed in triplicate, and relative cell viability was calculated as the percentage viable compared to control cells in EGM without the addition of stars.

Syntheses

*Preparation of poly(4-vinylbenzyl chloride-co-oligo(ethylene glycol) methyl ether acrylate) [P(VBC-*co*-OEGA)]*

RAFT agent 3-(benzyl sulfanyl thiocarbonyl sulfanyl)-propanoic acid (BSPA, 70.8 mg, 2.6 x 10^{-4} mol), 4-vinylbenzyl chloride (4-VBC, 189 µL, 1.34 x 10^{-3} mol), oligo(ethylene glycol) methyl ether acrylate (OEGA, 7.0 mL, 1.6 x 10^{-2} mol) and azobis(2-methylpropionitrile) (AIBN, 4.3 mg, 2.6 x 10^{-5} mol) were dissolved in toluene (14.0 mL) in a septa-sealed vial. The vial was placed in an ice bath, and the solution was purged with nitrogen for 45 min. The ratio between the 4-VBC, OEGA, RAFT agent and AIBN in the polymerization medium was 5 : 60 : 1 : 0.1. After deoxygenation, the reaction mixture was placed in an oil bath at 70 °C. The reaction was quenched after 10 h via rapid cooling and exposure to oxygen. The reaction was then directly analysed by ^{1}H NMR to determine the monomer conversion. Subsequently the reaction mixture was concentrated by partial evaporation of solvent, and the polymer was precipitated five times in diethyl ether to petroleum spirit (BR 40 - 60 °C) (2 : 1 v/v) to remove the non-reacted monomers and RAFT-agent. Finally the purified polymer was dried at 30 °C under vacuum overnight to give 1.5 g of a yellow viscous solution.

Preparation of core-cross-linked P(VBC-co-OEGA) stars [P[P(VBC-co-OEGA)-co-MBAA)]]

P(VBC-*co*-OEGA) linear arm (250 mg, 2.91 x 10^{-5} mol, M_n = 8600 g mol-1, Đ = 1.16), *N,N′* - methylenebis(acrylamide) (27.7 mg, 1.80 x 10^{-4} mol) and AIBN (1.6 mg, 9.8 x 10^{-6} mol) were dissolved in 3.0 mL toluene in a septa-sealed vial. The ratio between arm polymer, cross-linker, and AIBN in the polymerization medium was 1 : 6 : 0.33 (for low-molecular-weight star, 10 and 15 for higher molecular-weight stars). The vial was then purged with nitrogen for 30 minutes at 0 °C. The polymerization was carried out at 70 °C for 24 h, before quenching the reaction via rapid cooling and exposure to oxygen. The conversion was determined by ^{1}H NMR and SEC and the star polymer was purified by precipitation in diethyl ether/chloroform (12:1 v/v) and then dried at 30 °C under vacuum. Yield 175 mg. The product was analysed by ^{1}H NMR, SEC, and ATR-FTIR.

Preparation of azide-functional linear polymer P(VBAz-co-OEGA)

P(VBC-co-OEGA) linear arm (500 mg, 5.81 x 10^{-5} mol, Mn = 9,480 g mol^{-1}, Đ = 1.16) was dissolved in 15.0 mL *N,N*-dimethylformamide (DMF) and sodium azide was added (95.9 mg, 1.45 x 10^{-5} mol). The reaction was stirred for 48 h at room temperature in darkness. Afterwards, excess sodium azide was removed by dialysis (molecular weight cut-off 3,500 Da, three times solvent change, followed by three times acetone solvent change, all while protected from light). Finally, the purified polymer was dried under vacuum and analysed by ^{1}H NMR, SEC, and ATR-FTIR.

Preparation of azide-functional core-cross-linked star [P[P(VBAz-co-OEGA)-co-MBAA)]]

Core-cross-linked P(VBC-*co*-OEGA) star (250 mg, 4.46 x 10^{-6} mol, M_n = 55,352 g mol^{-1}, Đ = 1.10) was dissolved in 7.2 mL DMF and sodium azide was added (95.9 mg, 1.45 x 10^{-5} mol). The reaction was stirred for 48 h at room temperature in darkness. Afterwards, excess sodium azide was removed by dialysis (molecular weight cut-off 12,000 – 14,000 Da, three times DMF solvent change, followed by three times acetone solvent change, all while protected from light). Finally, the purified polymer was stored in acetone. A small amount was dried under vacuum and analysed by ^{1}H NMR, SEC, and ATR-FTIR.

Preparation of butyne-DOTA

Butyne-DOTA-tris(t-butyl ester) (180 mg, 2.34 x 10^{-4} mol) was dissolved in 5.0 mL trifluoroacetic acid (TFA) and stirred for 16 h at room temperature. Subsequently, the solvent was removed under reduced pressure at 40 °C and completion of reaction was confirmed by ^{1}H NMR and UHPLC-MS.
MS(ESI): m/z: 456.2 [M+H]+

Preparation of butyne-Gd-DOTA

Butyne-DOTA (100 mg, 2.20 x 10^{-4} mol) was dissolved in 4.0 mL ultrapure water, gadolinium(III) chloride hexahydrate ($GdCl_3$) (117 mg, 3.16 10^{-4} mol) was added and the pH was increased to 6 by dropwise addition of a KOH (2 M) solution. Subsequently, the solution was stirred at 50 °C and the pH was readjusted to 6 after 2 hours. After that, the reaction was continued for a total reaction time of 24 h. The excess of $GdCl_3$ was removed by the addition of Chelex-100 resin. This was followed by filtration and lyophilisation of the filtrate, resulting in an off-white solid.
MS(ESI): m/z: 610.2 $[M+H]^+$

Three component reaction of 3-butyn-1-ol, NaI and linear P(VBAz-co-OEGA)

P(VBAz-*co*-OEGA) (20.0 mg, 2.43 x 10^{-6} mol) and sodium iodide (2.7 mg, 1.8 x 10^{-5} mol) were dissolved in 406 µL DMSO with 74 µL H_2O. Copper(II) chloride (1.6 mg, 1.2 x 10^{-5} mol) was dissolved in 406 µL DMSO in a second vial, and triethylamine (2.6 µL, 1.8 x 10^{-5} mol) was added, followed by 3-butyn-1-ol (0.92 µL, 1.2 x 10^{-5} mol). After five minutes, both vials were combined and the reaction mixture was stirred overnight (16 h) at 60 °C. The ratio between azide group, sodium iodide, copper(II) chloride, triethylamine, and 3-butyn-1-ol was 1 : 1.5 : 1 : 1.5 : 1. Next, the polymer was purified by dialysis (MWCO 3,500 Da) in water with three times solvent change, followed by lyophilisation. The resulting polymer was analysed by ^{1}H NMR, SEC, and ATR-FTIR.

Three component reaction of 3-butyn-1-ol, NaI and P[P(VBAz-co-OEGA)-co-MBAA]

P[P(VBAz-co-OEGA)-co-MBAA] (20.0 mg, 4.11 x 10^{-7} mol) and sodium iodide (2.7 mg, 1.8 x 10^{-5} mol) were dissolved in 406 µL DMSO with 74 µL H_2O. Copper(II) chloride (1.6 mg, 1.2 x 10^{-5} mol) was dissolved in 406 µL DMSO in a second vial, and triethylamine (2.6 µL, 1.8 x 10^{-5} mol) was added, followed by 3-butyn-1-ol (0.92 µL, 1.2 x 10^{-5} mol). After five minutes, both vials were combined and the reaction mixture was stirred overnight (16 h) at 60 °C. The ratio between azide group, sodium iodide, copper(II) chloride, triethylamine, and 3-butyn-1-ol was 1 : 1.5 : 1 : 1.5 : 1. Next, the polymer was purified by dialysis (MWCO 3,500 Da) in water with three times solvent change, followed by lyophilisation. The resulting polymer was analysed by ^{1}H NMR, SEC, and ATR-FTIR.

Three component reaction of butyne-Gd-DOTA, NaI and linear P(VBAz-co-OEGA)

P(VBAz-*co*-OEGA) (2.0 mg, 2.4 x 10^{-7} mol, equals 1.2 x 10^{-6} mol azide groups) was dissolved in 40 µL DMSO and sodium iodide (5 µL of a 345 mM sodium iodide stock solution in DMSO (1.74 x 10^{-6} mol)) was added.

Copper(II) chloride (12.0 µL of a 96.9 mM $CuCl_2$ stock solution in DMSO (1.16 x 10^{-6} mol)) was mixed in a second Eppendorf tube (1.5 mL) with triethylamine (4 µL of a 436 mM triethylamine stock solution in DMSO (3.49 x 10^{-6} mol)), and (4 µL of a 145 mM butyne-Gd-DOTA stock solution in water (1.16 x 10^{-6} mol).

After five minutes, both vials were combined inside the fume hood and the reaction mixture was heated overnight (16 h) at 90 °C, only heating the part of the Eppendorf tube that contains the reaction mixture. Next, the polymer was purified by dialysis (MWCO 3,500 Da) in water with three times solvent change, followed by lyophilisation. The resulting polymer was analysed by ICP-OES, SEC, ATR-FTIR and MRI.

Three component reaction of butyne-Gd-DOTA, NaI and P[P(VBAz-co-OEGA)-co-MBAA]

P[P(VBAz-co-OEGA)-co-MBAA] (1.9 mg, 3.4 x 10^{-8} mol, equals 1.2 x 10^{-6} mol azide groups) was dissolved in 40 µL DMSO and sodium iodide (5 µL of a 345 mM sodium iodide stock solution in DMSO (1.74 x 10^{-6} mol)) was added.

Copper(II) chloride (12.0 µL of a 96.9 mM $CuCl_2$ stock solution in DMSO (1.16 x 10^{-6} mol)) was mixed in a second Eppendorf tube (1.5 mL) with triethylamine (4 µL of a 436 mM triethylamine stock solution in DMSO (3.49 x 10^{-6} mol)), and (4 µL of a 145 mM butyne-Gd-DOTA stock solution in water (1.16 x 10^{-6} mol).

After five minutes, both vials were combined inside the fume hood and the reaction mixture was heated overnight (16 h) at 90 °C, only heating the part of the Eppendorf tube that contains the reaction mixture. Next, the polymer was purified by dialysis (MWCO 3,500 Da) in water with three times solvent change, followed by lyophilisation. The resulting polymer was analysed by ICP-OES, SEC, ATR-FTIR and MRI.

Three component reaction of 3-butyn-1-ol, iodide-125 and linear P(VBAz-co-OEGA)

P(VBAz-*co*-OEGA) (4.0 mg, 4.7 x 10^{-7} mol, equals 2.3 x 10^{-6} mol azide groups) was dissolved in 80 µL DMSO and iodine-125 (40 µL from a 0.1 MBq/ µL iodine-125 stock solution, diluted in DMSO (4.0 MBq)) was added inside a fume hood equipped with a lead brick wall and charcoal filter to limit exposure to iodine-125.

Copper(II) chloride (24.0 µL of a 96.9 mM $CuCl_2$ stock solution in DMSO (2.33 x 10^{-6} mol)) was mixed in a second Eppendorf tube (1.5 mL) with triethylamine (8 µL of a 436 mM triethylamine stock solution in DMSO (3.49 x 10^{-6} mol)), and 3-butyn-1-ol (8 µL of a 291 mM 3-butyn-1-ol stock solution in DMSO (3.49 x 10^{-6} mol).

After five minutes, both vials were combined inside the fume hood and the reaction mixture was heated overnight (16 h) at 60 °C in a heating module (Reacti-Therm I TS18821). Next, 20 µL of the reaction mixture was diluted with SEC buffer and measured by radio-SEC. The remainder was purified by centrifugal filtration (Amicon Ultra-4 centrifugal filter unit with Ultracel-3 membrane and molecular weight cut-off 3,000 Da) for 20 min at 7,000 x g, washing three times with 3.5 mL water. The resulting polymer was again analysed by radio-SEC.

Three component reaction of 3-butyn-1-ol, iodide-125 and P[P(VBAz-co-OEGA)-co-MBAA]

P[P(VBAz-co-OEGA)-co-MBAA] (3.7 mg, 6.6 x 10^{-8} mol, equals 2.3 x 10^{-6} mol azide groups) was dissolved in 80 µL DMSO and iodine-125 (40 µL from a 0.1 MBq/ µL iodine-125 stock solution, diluted in DMSO (2.0 MBq)) was added.

Copper(II) chloride (24.0 µL of a 96.9 mM $CuCl_2$ stock solution in DMSO (2.33 x 10^{-6} mol)) was mixed in a second Eppendorf tube (1.5 mL) with triethylamine (8 µL of a 436 mM triethylamine stock solution in DMSO (3.49×10^{-6} mol)), and 3-butyn-1-ol (8 µL of a 291 mM 3-butyn-1-ol stock solution in DMSO (3.49×10^{-6} mol).

After five minutes, both vials were combined inside the fume hood and the reaction mixture was heated overnight (16 h) at 60 °C in a heating module (Reacti-Therm I TS18821). Next, 20 µL of the reaction mixture was diluted with SEC buffer and measured by radio-SEC. The remainder was purified by centrifugal filtration (Amicon Ultra-4 centrifugal filter unit with Ultracel-3 membrane and molecular weight cut-off 3,000 Da) for 20 min at 7,000 x g, washing three times with 3.5 mL water. The resulting polymer was again analysed by radio-SEC.

Three component reaction of butyne-Gd-DOTA, iodine-125 and linear P(VBAz-co-OEGA)

P(VBAz-*co*-OEGA) (2.0 mg, 2.3×10^{-7} mol, equals 1.2×10^{-6} mol azide groups) was dissolved in 40 µL DMSO and iodine-125 (20 µL from a 0.1 MBq/µL iodine-125 stock solution, diluted in DMSO (2.0 MBq)) was added inside a fume hood equipped with a lead brick wall and charcoal filter to limit exposure to iodine-125.

Copper(II) chloride (12.0 µL of a 96.9 mM $CuCl_2$ stock solution in DMSO (1.16×10^{-6} mol)) was mixed in a second Eppendorf tube with triethylamine (4 µL of a 436 mM triethylamine stock solution in DMSO (1.74×10^{-6} mol)), and butyne-Gd-DOTA (4 µL of a 145 mM butyne-Gd-DOTA stock solution in water (1.16×10^{-6} mol).

After five minutes, both vials were combined inside the fume hood and the reaction mixture was heated overnight (16 h) at 90 °C in a heating module (Reacti-Therm I TS18821), only heating the part of the Eppendorf tube that contains the reaction mixture. Next, 20 µL of the reaction mixture was diluted with SEC buffer and measured by radio-SEC. The remainder was purified by centrifugal filtration (Amicon Ultra-4 centrifugal filter unit with Ultracel-3 membrane and molecular weight cut-off 3,000 Da) for 20 min at 7,000 x g, washing three times with 3.5 mL water. The resulting polymer was again analysed by radio-SEC.

Three component reaction of butyne-Gd-DOTA, iodine-125 and P[P(VBAz-co-OEGA)-co-MBAA]

P[P(VBAz-*co*-OEGA)-*co*-MBAA] (1.9 mg, 3.4×10^{-8} mol, equals 1.2×10^{-6} mol azide groups) was dissolved in 40 µL DMSO and iodine-125 (20 µL from a 0.1 MBq/µL iodine-125 stock solution, diluted in DMSO (2.0 MBq)) was added inside a fume hood equipped with a lead brick wall and chaircoal filter to limit exposure to iodine-125.

Copper(II) chloride (12.0 µL of a 96.9 mM $CuCl_2$ stock solution in DMSO (1.16×10^{-6} mol)) was mixed in a second Eppendorf tube with triethylamine (4 µL of a 436 mM triethylamine stock solution in DMSO (1.74 x 10^{-6} mol)), and butyne-Gd-DOTA (4 µL of a 145 mM butyne-Gd-DOTA stock solution in water (1.16 x 10^{-6} mol).

After five minutes, both vials were combined inside the fume hood and the reaction mixture was heated overnight (16 h) at 90 °C in a heating module (Reacti-Therm I TS18821), only heating the part of the Eppendorf tube that contains the reaction mixture. Next, 20 µL of the reaction mixture was diluted with SEC buffer and measured by radio-SEC. The remainder was purified by centrifugal filtration (Amicon Ultra-4 centrifugal filter unit with Ultracel-3 membrane and molecular weight cut-off 3,000 Da) for 20 min at 7,000 x g, washing three times with 3.5 mL water. The resulting polymer was again analysed by radio-SEC.

Supporting figures, schemes and tables

Table S1: Synthesis of P(VBC-co-OEGA) with different molecular weights by varying molar feed ratio of OEGA, solvent, concentration and reaction time.

	$M_{n,SEC}$ (g mol^{-1})[a]	$M_{n,NMR}$ (g mol^{-1})[b]	$Đ_m$	F_{OEGA}[c]	$F_{4\text{-}VBC}$	f_{OEGA}[d]	$f_{4\text{-}VBC}$[e]	Solvent	Solvent:OEGA	Time (h)
A	5500	4880	1.10	20	5	8	5	MeCN	3 : 1	7
B	7620	6800	1.15	40	5	12	5	Toluene	2 : 1	10
C	9480	8720	1.11	60	5	16	5	Toluene	2 : 1	10

[a] Assessed by size-exclusion chromatography (SEC) in DMAc (0.03% w/v LiBr) using a conventional calibration curve with narrow molecular weight distribution polystyrene standards.
[b] Calculated using the conversion of each monomer.
[c] Initial molar feed ratio of each monomer per chain transfer agent.
[d] molar composition of OEGA per polymer
[e] molar composition of 4-VBC per polymer, ^{1}H NMR of the reaction mixture indicates over 90% conversion for 4-VBC

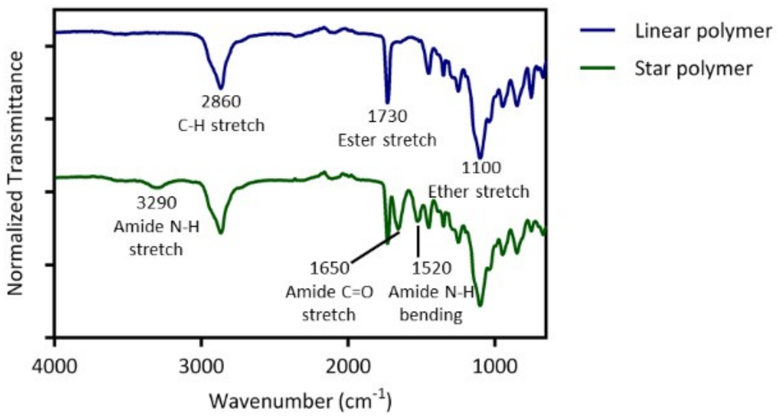


Figure S1: ATR-FTIR spectra before and after core-cross-linking show the appearance of amide signals, indicating successful incorporation of the cross-linker (N,N'-methylenebis(acrylamide)).

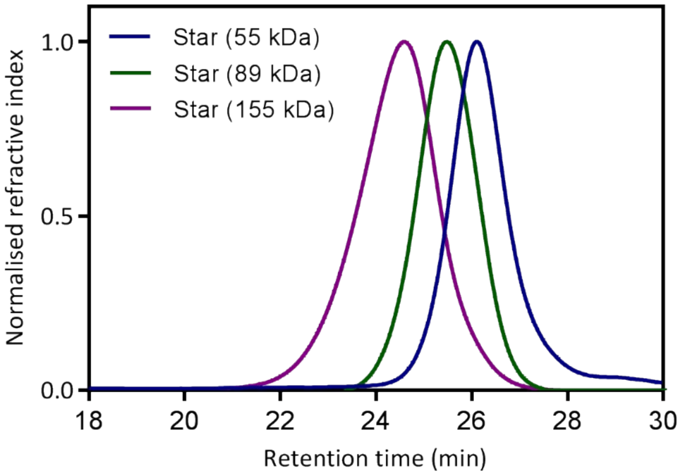


Figure S2: Size-exclusion chromatograms of purified star polymers with different molecular weight.

Table S2: SEC results for core-cross-linked star polymers using P(VBC-*co*-OEGA) copolymers with different molecular weights and the arm incorporation.

	Cross-linker ratio per arm	$M_{n,SEC}$ (g mol^{-1})	Đ	Arm Incorporation (%)[a]	Number of arms[b]	Number of functional groups
POEGA-co-VBC	-	9,480	1.11			
Star 1	6	55,352	1.10	83.5	5.7	29
Star 2	10	89,420	1.15	88.5	9.3	46
Star 3	15	155,070	1.24	90.7	16.1	81

[a] Calculated using size-exclusion chromatogram: arm incorporation = ∫star / (∫star + ∫arm)
[b] Calculated via n = ($M_{n,star} - x \cdot M_{n.cross\text{-}linker}$) / $M_{n,arm}$, with x is the cross-linker ratio per arm

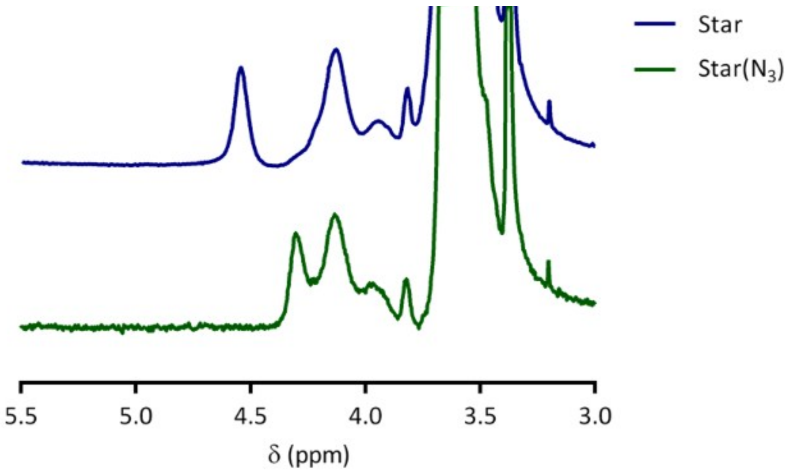


Figure S3: ^{1}H NMR of the star (C) before and after azide substitution as recorded in deuterated DMSO. The spectra showed a clear upfield shift of the methylbenzylic proton signal from δ 4.55 to 4.30 ppm upon azide substitution, which indicated full conversion for the star polymer.

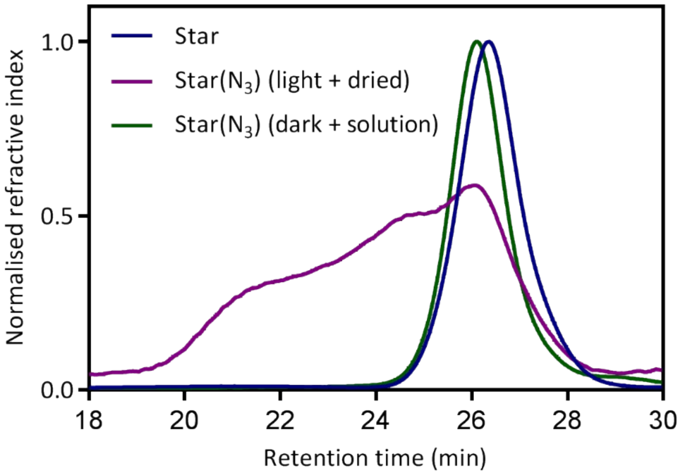


Figure S4: Size-exclusion chromatograms of the azide-substituted star polymers. Star-star coupling was observed upon purification by dialysis and lyophilisation. However, when performing the dialysis in darkness and only drying the compound when needed (acetone as solvent), no star-star coupling was observed.

Table S3: SEC results for core-cross-linked star polymers using P(VBC-*co*-OEGA) copolymers with different molecular weights and effect of azidation.

	Before azidation		After azidation	
	M_n (g mol^{-1})	Đ	M_n (g mol^{-1})	Đ
POEGA-co-VBC	9,480	1.11	9,253	1.10
Star	55,352	1.12	56,670	1.10

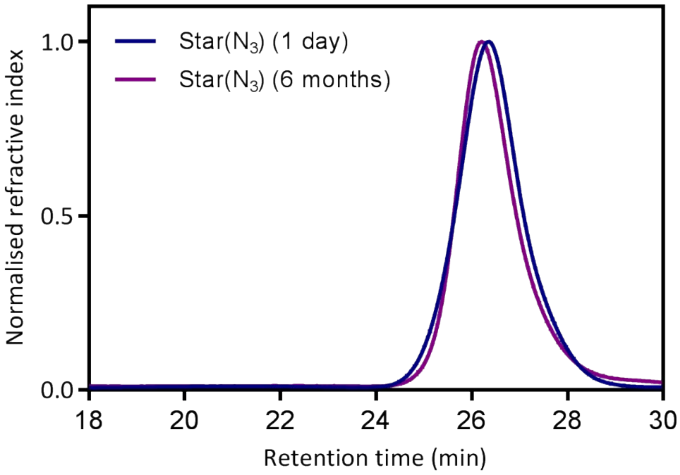
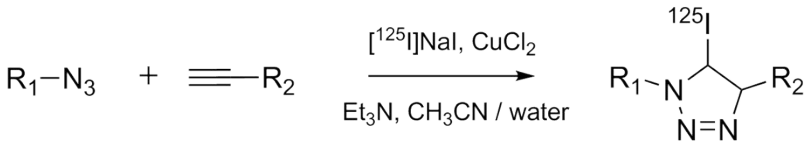


Figure S5: Size-exclusion chromatograms of the azide-substituted star polymers one day after purification and 6 months later when stored in acetone at -20ºC.



Scheme S1: Synthesis of 5-[^{125}I]iodo-1,2,3-triazoles.[2]

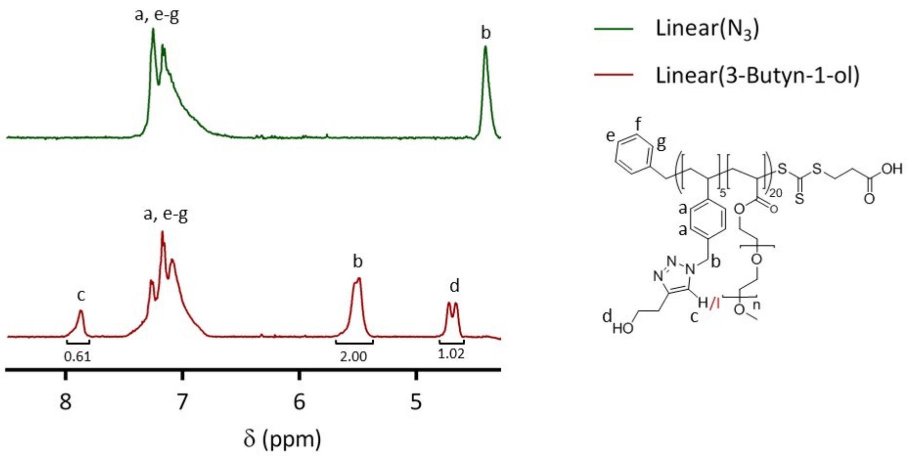


Figure S6: ^{1}H NMR spectra of the linear polymer P(VBAz-*co*-OEGA) (top) and the reaction product of the three-component reaction of P(VBAz-*co*-OEGA) and 3-butyn-1-ol in the presence of sodium iodide (1.1 eq., bottom). The spectra were recorded in deuterated DMSO.

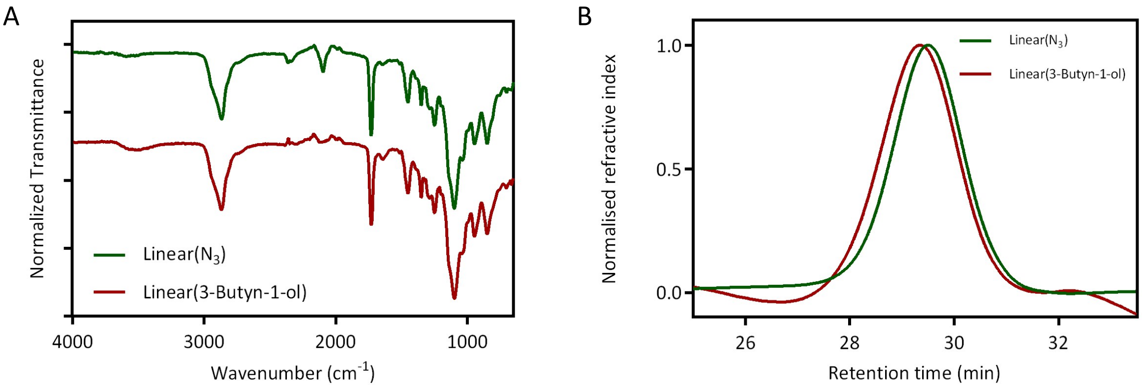
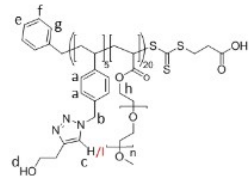


Figure S7: (A) ATR-FTIR spectra of linear polymer before and after three-component reaction show the disappearance of the azide-stretch signal at 2100 cm^{-1}. (B) Size-exclusion chromatograms of linear polymer display a small decrease in retention time after conjugation of 3-butyn-1-ol.

Table S4: Effect of different equivalents of sodium iodide on the formation of iodotriazole when combining linear P(VBAz-*co*-OEGA), 3-butyn-1-ol and sodium iodide. In the table, the signal intensities of the different relevant NMR signals were stated and the percentage of iodotriazole was calculated.

Eq. NaI	∫c	∫a, e-g	∫d	∫b	∫h	Iodotriazole	Triazole
0	1.02	8.42	1.09	2.00	5.10	0%	100%
0.5	0.80	7.23	1.01	2.00	4.85	20%	80%
1.1	0.61	7.02	1.02	2.00	4.70	39%	61%
1.5	0.51	7.13	1.10	2.00	4.69	49%	51%
5	0.52	7.48	1.04	2.00	4.80	51%	49%



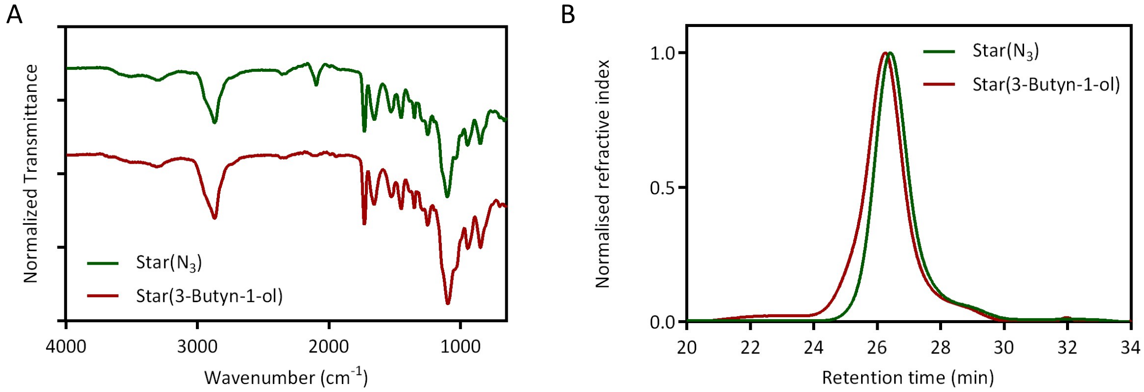
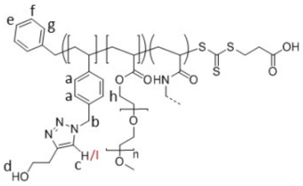


Figure S8: (A) ATR-FTIR spectra of star polymer before and after three-component reaction show the disappearance of the azide-stretch signal at 2100 cm^{-1}. (B) Size-exclusion chromatograms of star polymer display a small decrease in retention time after conjugation of 3-butyn-1-ol.

Table S5: Effect of different equivalents of sodium iodide on the formation of iodotriazole when combining star polymer P[P(VBAz-*co*-OEGA)-*co*-MBAA], 3-butyn-1-ol and sodium iodide. In the table the signal intensities of the relevant NMR signals were stated and the percentage of iodotriazole was calculated.

Eq. NaI	∫c	∫a, e-g	∫d	∫b	Iodo-triazole	Triazole
0	1.04	7.23	1.02	2.00	0%	100%
0.5	0.79	7.31	1.01	2.00	17%	83%
1.1	0.64	7.30	1.04	2.00	36%	64%
1.5	0.55	7.18	0.96	2.00	45%	55%
5	0.54	7.48	0.98	2.00	46%	54%



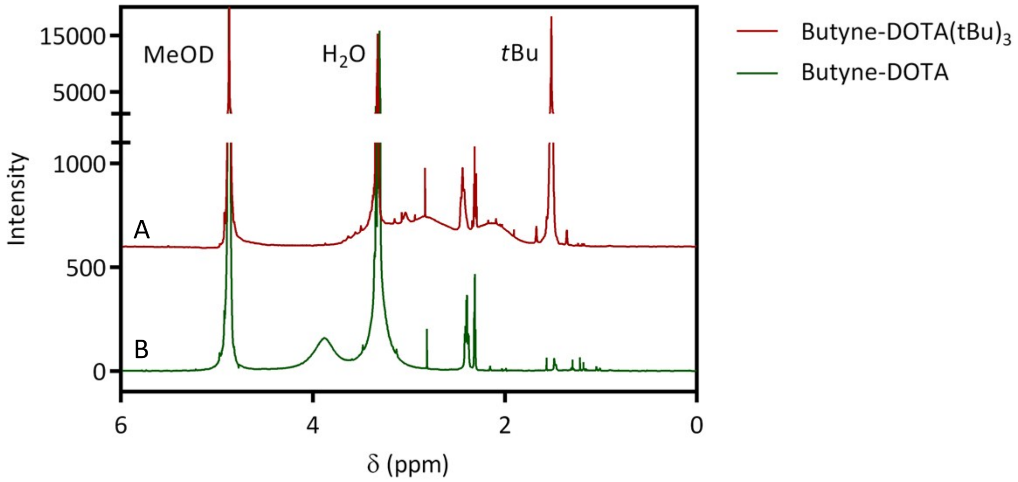


Figure S9: ^{1}H NMR spectra of (A) butyne-DOTA(tBu)$_3$ and (B) deprotected butyne-DOTA as recorded in deuterated methanol (MeOD). The disappearance of the large tert-butyl NMR signal at δ 1.51 ppm confirmed successful deprotection.

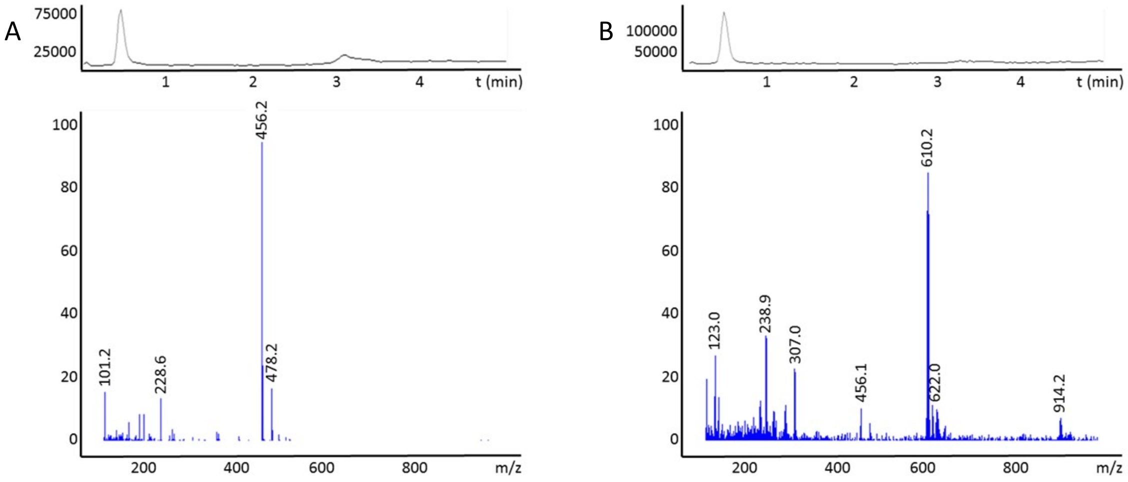
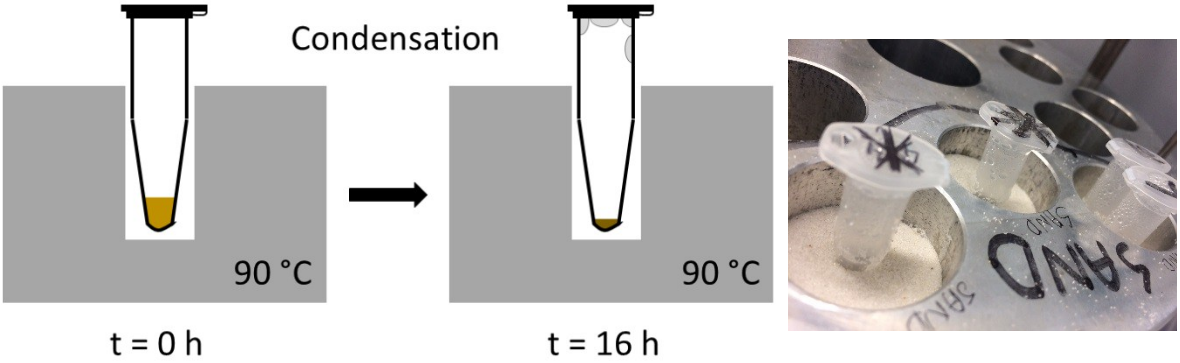


Figure S10: LC-MS spectra before (A) and after (B) gadolinium complexation of the deprotected butyne-DOTA chelate show an increase in m/z from 465.2 to 610.2, indicating successful gadolinium incorporation.



Scheme S2: Proposed mechanism for increased concentration using experimental observations and experimental setup.

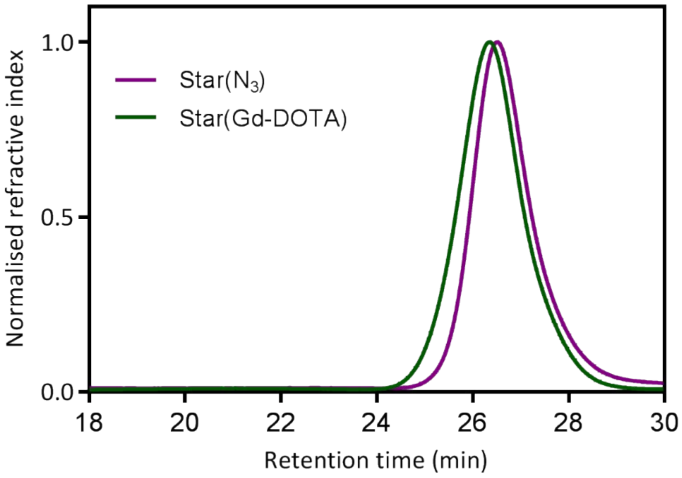


Figure S11: Size-exclusion chromatogram before and after three-component reaction with cold NaI and butyne-Gd-DOTA.

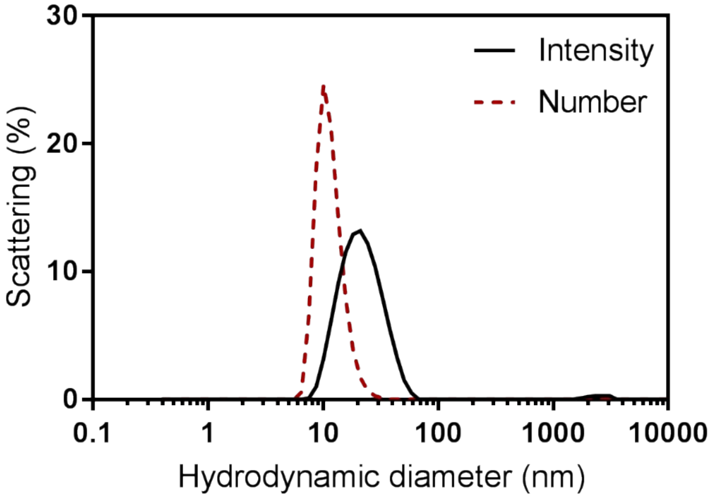


Figure S12: Dynamic light scattering measurement of the Gd-DOTA-I-Star polymer in PBS (pH 7.4).

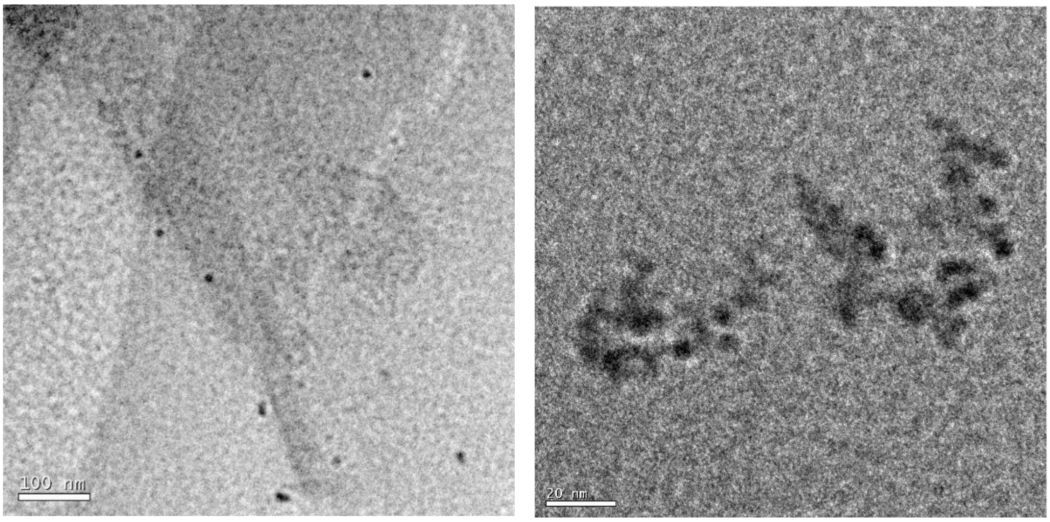


Figure S13: TEM images of Gd-I-star.

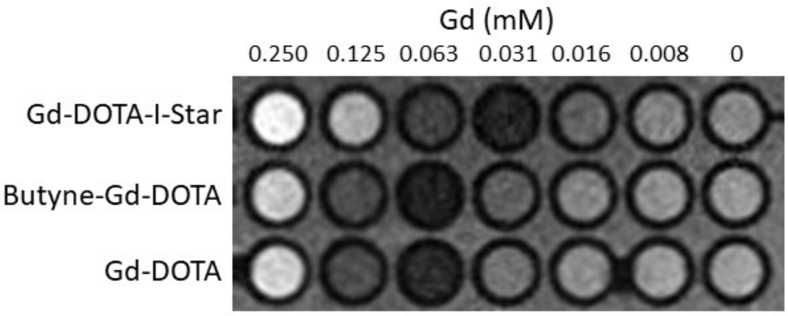


Figure S14: T1-weighed MRI scan at 7 T (inversion recovery) shows the difference in T_1 contrast enhancement between Gd-DOTA-I Star nanoparticle, butyne-Gd-DOTA and Gd-DOTA (Magnevist).

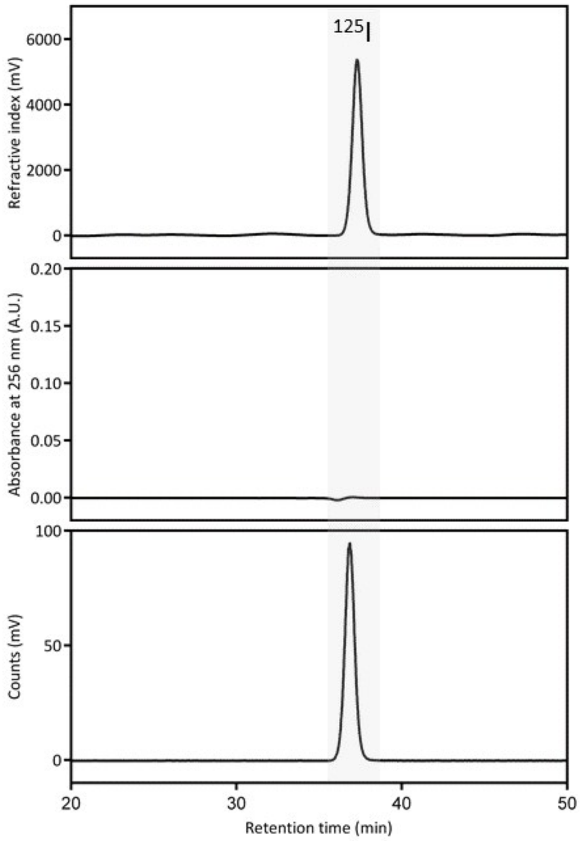


Figure S15: Size-exclusion chromatogram of 1.0 MBq iodine-125: refractive index, absorbance at 256 nm and radioactivity count (optimized for both low and high sensitivity).

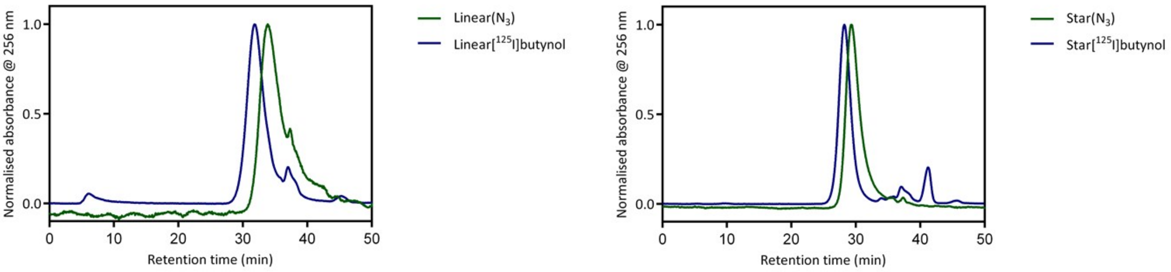


Figure S16: Size-exclusion chromatograms of the linear (left) and star polymer (right) (absorbance at 256 nm) before and after the three-component reaction with 3-butyn-1-ol and iodine-125 (before purification).

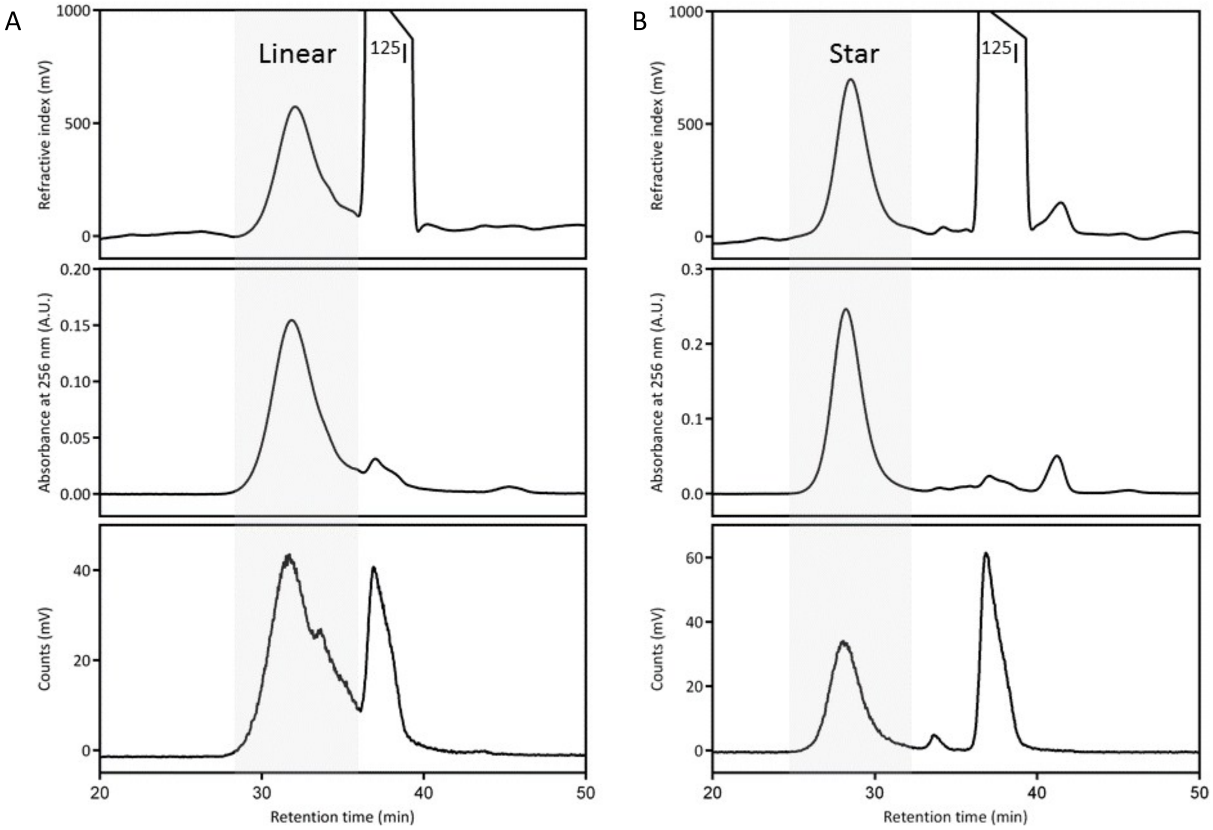


Figure S17: Size-exclusion chromatograms of the reaction mixtures after the three-component reaction of either linear polymer (A) or star polymer (B) with 3-butyn-1-ol and iodine-125. Top row, refractive index detector showed both the polymer and iodine-125; middle row, the absorbance at 256 nm showing the polymer only; and bottom row the radioactivity count rate, showing both iodine-125-labelled polymer and free iodine-125.

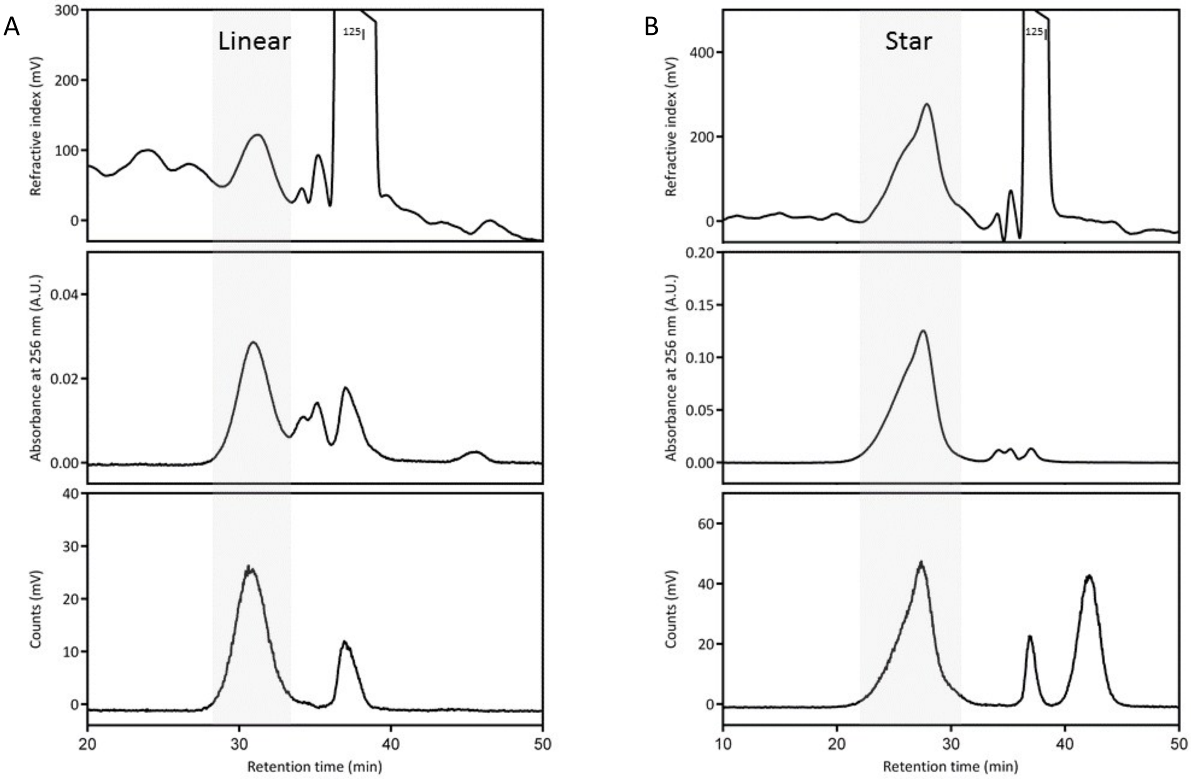


Figure S18: Size-exclusion chromatograms of the reaction mixtures after the three-component reaction of either linear polymer (A) or star polymer (B) with butyne-Gd-DOTA and iodine-125. Top row, refractive index detector showed both the polymer and iodine-125 (the low signal for the linear polymer was caused by using a diluted sample for SEC); middle row, the absorbance at 256 nm showing the polymer only; and bottom row, the radioactivity count rate, showing both iodine-125-labelled polymer and free iodine-125.

References

1. M. H. Stenzel and T. P. Davis, *Journal of Polymer Science Part A: Polymer Chemistry*, 2002, **40**, 4498-4512.
2. R. Yan, E. El-Emir, V. Rajkumar, M. Robson, A. P. Jathoul, R. B. Pedley and E. Årstad, *Angewandte Chemie International Edition*, 2011, **50**, 6793-6795.