

Aqueous asymmetric aldol reactions in polymersome membranes

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Supporting Information

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General Information (materials/instrumentation)

Chemicals were purchased from Sigma-Aldrich. Unless stated otherwise, chemicals were used without further purification. Reactions were carried out under an inert atmosphere of dry nitrogen or argon. Standard syringe techniques were applied for the transfer of dry solvents and air- or moisture sensitive reagents. Reactions were followed by thin layer chromatography (TLC) on silica gel-coated plates (Merck 60 F254) with the indicated solvent mixture. Detection was performed with UV-light, and/or by charring at ~ 150 °C after dipping into a solution of aqueous basic KMnO_4 or in a solution of ninhydrin. Column or flash chromatography was carried out using Silicycle Silicaflash P60[®] (40-63 μm). Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on an ATI Matson Genesis Series FT-IR spectrometer fitted with an ATR cell. The vibrations (ν) are given in cm^{-1} .

Dynamic light scattering (DLS) measurements were performed on a Malvern Instrument Zetasizer Nano-S (ZEN 1600), equipped with a He-Ne laser (633 nm, 4 mW) and an Avalanche photodiode detector at an angle of 173° . The DLS data were processed and analyzed with Dispersion Technology Software (Malvern Instruments).

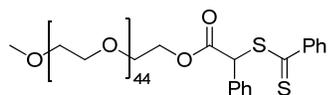
Chiral HPLC measurements were performed on a Shimadzu LC2010C, containing a Chiralpak AD-H (250 \times 4.6 mm) column, using UV detection (220 nm)

NMR spectra were recorded on a Bruker DMX 300 (300 MHz), a Bruker DMX 500 (500 MHz) and a Varian 400 (400 MHz) spectrometer in CDCl_3 solutions (unless reported otherwise). ^1H NMR chemical shifts are given in ppm with respect to tetramethylsilane (TMS, δ 0.00 ppm) as internal standard, ^{13}C NMR shifts are given in ppm with respect to CHCl_3 (δ 77.00 ppm). Coupling constants are reported as *J*-values in Hz. High resolution mass spectra were recorded on a JEOL AccuTOF (ESI).

Transmission electron microscopy (TEM) was performed on a JEOL TEM 1010 microscope with an acceleration voltage of 60 kV equipped with a charge-coupled device (CCD) camera. Sample specimens were prepared by placing a drop (6 μL) of a diluted aqueous vesicle solution on an EM science carbon-coated copper grid (200 mesh). The grid was air dried for at least 2 hours and analyzed without further treatment.

The size exclusion chromatography-multi angle laser light scattering (SEC-MALLS) experiments were conducted at room temperature using a SEC column (Dr. Maisch, GPC-PS, 300 \times 8 mm, particle size 5 μm) in-line with a Wyatt DAWN HELEOS II light scattering detector using a laser operating at 658 nm and a Wyatt Optilab T-Rex refractive index detector. Number-averaged molecular weight calculations were performed using ASTRA 6.0.6.13, using a dn/dc value of 0.185.

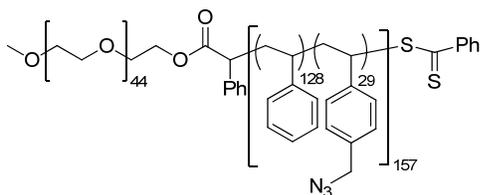
Poly (ethylene glycol)-chain transfer agent PEG₄₄-CTA



Poly(ethylene glycol) ($M_n = 2000 \text{ g mol}^{-1}$) chain transfer agent (PEG₄₄-CTA) was synthesized according to literature procedures. ¹H NMR (CDCl₃, 400 MHz): δ 8.02-7.98 (m, 2H, arom. H), 7.56-7.47 (m, 3H, arom. H), 7.41-7.34 (m, 5H, arom. H), 5.73 (s, 1H, -CH-Ar), 4.42-4.24 (m, 2H, -COOCH₂), 3.87-3.44 (m, 174H, PEG backbone), 3.38 (s, 3H, CH₃OCH₂).

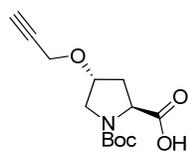
Typical procedure synthesis poly(ethylene glycol)-*b*-poly(styrene-*co*-4-vinylbenzyl azide) polymers

Poly(ethylene glycol)-*b*-poly(styrene-*co*-4-vinylbenzyl azide) PEG₄₄-*b*-P(S₁₂₈-*co*-4-VBA₂₉) (P1)



A flame-dried Schlenk tube equipped with a stirring bar was loaded with styrene (1.66 g, 16.0 mmol, 263 equiv), purified 4-vinylbenzyl chloride (580 mg, 3.42 mmol, 57 equiv), PEG₄₄-CTA (137 mg, 0.06 mmol, 1.0 equiv) and AIBN (2.0 mg, 0.012 mmol, 0.2 equiv). The mixture was degassed by three freeze-pump-thaw cycles. The Schlenk tube was then immersed in a preheated oil bath of 70 °C and the polymerization was monitored by ¹H-NMR spectroscopy. When a 40% conversion was reached, the polymerization was terminated by removing the Schlenk tube from the oil bath. After the reaction mixture had cooled down to room temperature, it was diluted with CHCl₃ and transferred to a round-bottom flask. The product was precipitated by the addition of cold MeOH (250 mL) and subsequently filtered over a glass filter. The latter three steps were repeated three times to remove excess monomer. The resulting pink solid was dried *in vacuo* and added to a flame-dried Schlenk tube. NaN₃ (226 mg, 3.48 mmol, 58 equiv) and DMF (2.0 mL) were added and the mixture was stirred at room temperature for 3 days. The reaction mixture was then diluted with CHCl₃ and transferred to a round-bottom flask. Precipitation was induced upon addition of cold MeOH and the resulting white solid **P1** was filtered and dried *in vacuo*. Yield (397 mg). ¹H NMR (CDCl₃, 500 MHz): δ 7.25-6.25 (m, arom. H), 4.35-4.10 (br. s, -CH₂N₃), 3.72-3.55 (br. s, PEG backbone), 2.40-1.20 (m, P(S-*co*-4-VBA) backbone). M_n (¹H NMR) = 20.0 kDa. M_n (MALLS) = 20.6 kDa. PDI = 1.46 (Table S1).

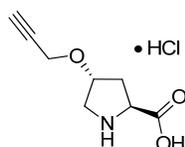
(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(prop-2-yn-1-yloxy)pyrrolidine-2-carboxylic acid (**1**)



N-Boc-*trans*-4-hydroxy-L-proline (2.00 g, 8.66 mmol, 1.0 equiv) was dissolved in anhydrous THF (40 mL) at -78 °C. Sodium hydride (692 mg of a 60 wt. % dispersion in mineral oil, 17.3 mmol, 2.0 equiv) was added in small portions and the solution was stirred for 10 min. Next, an additional amount of THF (20 mL) was added, followed by the addition of propargyl bromide (2.06 g of a 80 wt. % in toluene, 17.3 mmol, 2.0 equiv). After 10 min of stirring, the solution was allowed to warm up to 0 °C and was stirred at this temperature for 2 h. The solution was then warmed up to ambient temperature and was stirred for an additional 18 h. Afterwards, the solution was cooled to -60 °C, quenched with 5 mL water, and allowed to warm up to ambient temperature. Saturated aqueous NH₄Cl (10 mL) was added and the mixture was acidified to pH 2 with 1 M HCl solution. The product was extracted with CH₂Cl₂ (3 × 50 mL), after which the resulting organic fractions were dried (Na₂SO₄) and concentrated *in vacuo*. Column chromatography

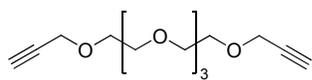
(MeOH/CH₂Cl₂, 2%→4%) yielded compound **1** (1.47 g, 63%) as a yellow solid. *R_f* 0.20 (CH₂Cl₂/MeOH, 9:1). $[\alpha]_D^{20} = -101$ (c 0.1, CH₂Cl₂). FT-IR (ATR): 3263, 2979, 1695, 1407, 1162, 1090 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz, mixture of rotamers): δ 4.45 (t, *J* = 7.5 Hz, 0.5H), 4.38–4.27 (m, 1.5H), 4.23–4.09 (m, 2H), 3.73–3.50 (m, 2H), 2.50–2.37 (m, 2H), 2.36–2.26 (m, 0.5H), 2.21–2.11 (m, 0.5H), 1.49 (s, 4.5H), 1.42 (s, 4.5H). ¹³C NMR (CDCl₃, 75 MHz, mixture of rotamers): δ 178.5 (174.8), 156.6 (153.9), 82.1 (80.9), 79.3, 76.1 (75.9), 75.1, 58.0 (57.9), 56.6, 52.01 (51.2), 36.6 (34.3), 28.5 (28.4). HRMS (ESI) *m/z* calculated for C₈H₁₂NO₃ (M+H)⁺: 270.0817, found: 270.0823.

(2*S*,4*R*)-4-(Prop-2-yn-1-yloxy)pyrrolidine-2-carboxylic acid hydrochloride (**2**)



To a solution of 2 M HCl in EtOAc (44 mL) was added dropwise a solution of **1** (871 mg, 3.24 mmol, 1.0 equiv) in AcOH (2 mL). After stirring for 45 min the reaction mixture was quenched with *tert*-butanol (8.4 mL). The solution was stirred for an additional 15 min before it was concentrated *in vacuo*. Milli-Q (25 mL) was added and the solution was lyophilized. The resulting powder was redissolved in MilliQ (25 mL) and the solution was lyophilized again. Product **2** was isolated as the HCl salt, a yellow solid, in 90% yield (600 mg). $[\alpha]_D^{20} = -26.2$ (c 1.0, H₂O). FT-IR (ATR): 3250, 2912, 1733, 1215, 1080, 672 cm⁻¹. ¹H NMR (D₂O, 400 MHz): δ 4.49 (t, *J* = 3.9 Hz, 1H), 4.43–4.34 (m, 1H), 4.13 (d, *J* = 2.4 Hz, 2H), 3.50–3.35 (m, 2H), 2.78 (t, *J* = 2.4 Hz, 1H), 2.59–2.50 (m, 1H), 2.18–2.08 (m, 1H). ¹³C NMR (D₂O, 75 MHz): δ 171.3, 78.6, 76.4, 75.7, 58.0, 55.7, 50.2, 33.7. HRMS (ESI) *m/z* calculated for C₁₃H₁₉NNaO₅ (M+Na-HCl)⁺: 192.1161, found: 192.1170.

4,7,10,13,16-Pentaoxanonadeca-1,18-diyne (**3**)



To a dispersion of NaH (433 mg of a 60% dispersion in oil, 11.3 mmol, 2.2 equiv) in toluene (40 mL) at 0 °C was added dropwise a solution of tetraethylene glycol (1.00 g, 5.15 mmol, 1.0 equiv) in toluene (10 mL). The reaction mixture was stirred for 1 h at 0 °C after which propargyl bromide (1.68 g of a 80% solution in toluene, 11.3 mmol, 2.2 equiv) was added. After stirring for an additional 2 h at 0 °C the reaction mixture was quenched with saturated aqueous NH₄Cl (50 mL). The product was extracted with CH₂Cl₂ (3 × 50 mL), dried over Na₂SO₄ and concentrated *in vacuo*. Column chromatography (EtOAc/heptane, 1:2) afforded compound **3** as a colorless oil (957 mg, 69%). ¹H NMR (CDCl₃, 400 MHz): δ 4.21 (d, *J* = 2.4 Hz, 4H) 3.73–3.63 (m, 16H), 2.44 (t, *J* = 2.4 Hz, 2H). Spectral data are in accordance with literature.²

Typical procedure catalytic polymersomes preparation

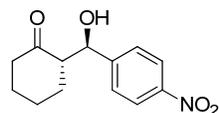
Block copolymer **P2** (21.8 mg, 1.16 μmol, 35.9 μmol of azides, 1.0 equiv) was dissolved in THF (1.5 mL) in a scintillation vial. 1.5 mL of ultrapure water (Milli-Q, 18.2 MΩ) was added dropwise within 1 hour while stirring the solution at 700 rpm. The polymersomes were allowed to self-assemble for 30 min. Then, catalyst **2** (10.0 mg, 48.5 μmol, 1.35 equiv) was added, followed by a solution of cross-linker **3** (1.5 mg, 5.4 μmol, 0.15 equiv) in THF (100 μL), and a solution of CuSO₄·5H₂O (0.9 mg, 3.6 μmol, 0.1 equiv), bathophenanthroline, sulfonated sodium salt (1.9 mg, 3.6 μmol, 0.1 equiv) and sodium ascorbate (71.2 mg, 0.359 mmol, 10.0 equiv) in Milli-Q (200 μL). The dispersion was stirred for 3 days after which the polymersomes were transferred to a dialysis membrane (MWCO 30 kDa). The polymersomes were

subsequently dialyzed against Milli-Q for 24 h to remove THF and the excess of catalyst. The final volume was concentrated to 0.9 mL by centrifugation.

Typical procedure asymmetric aldol reaction

Catalytic polymersomes, prepared as described above, were transferred to a test tube charged with a stirring bar. THF (100 μ L) was added, followed by cyclohexanone (47.1 mg, 0.48 mmol, 4.0 equiv) and 4-nitrobenzaldehyde (18.1 mg, 0.12 mmol, 1.0 equiv). The reaction mixture was stirred at rt for 72 h. Next, the reaction mixture was extracted with CH_2Cl_2 (3 \times 1 mL), dried over Na_2SO_4 , and concentrated *in vacuo*. Alternatively, in case of the recycling experiments, the polymersomes were spun down at 13000 rpm for 25 min. The supernatant was removed and the polymersomes were redispersed in EtOAc. These two steps were repeated three times after which the organic and aqueous fractions were combined and concentrated *in vacuo*. The polymersomes were finally redispersed in 0.9 mL Milli-Q and used in the subsequent reaction cycle.

(S)-2-((R)-Hydroxy(4-nitrophenyl)methyl)cyclohexanone (**4**)



Prepared as described above. Product **4** was recovered as a white solid (29.3 mg, 98%) as a (*anti/syn* = 96/4) mixture of diastereoisomers. ^1H NMR (CDCl_3 , 400 MHz): δ 8.26–8.18 (m, 2H), 7.55–7.47 (m, 2H), 5.49 (m, <1H = *syn*), 4.90 (dd, J = 8.4, 3.1 Hz, 1H = *anti*), 4.07 (d, J = 3.1 Hz, 1H), 2.59 (dddd, J = 14.0, 8.4, 5.5, 1.2 Hz, 1H), 2.50 (dddd, J = 13.8, 4.4, 2.7, 1.8 Hz, 1H), 2.37 (tdd, J = 13.6, 6.2, 1.2 Hz, 1H), 2.12 (ddd, J = 12.1, 6.0, 2.9 Hz, 1H), 1.88–1.79 (m, 1H), 1.75–1.50 (m, 3H), 1.45–1.32 (m, 1H). Ee *anti* diastereoisomer = 98% (HPLC eluent heptane:isopropanol = 95:5, flow 1.0 mL/min). Rt_1 = 45.8 min (*R,S*), Rt_2 = 62.9 min (*S,R*). Spectral data are in accordance with literature.³

Table S1. Molecular characteristics of block copolymers **P1–P6**.

PEG- <i>b</i> -P($\text{S}_{(n-m)}$ - CO-4-VBA_m) _{<i>n</i>}	n^a	m^b	M_n NMR ^c (10^3 g/mol)	M_n MALLS ^d (10^3 g/mol)	PDI (M_w/M_n) ^e
P1	157	29	20.0	20.6	1.46
P2	142	31	18.6	35.9	1.25
P3	150	51	20.4	22.8	1.30
P4	162	68	22.5	32.3	1.27
P5	124	16	16.0	23.9	1.52
P6	141	8	17.4	24.0	1.26

(a) Calculated number average degree of PS determined by ^1H -NMR spectroscopy using PEG as internal standard. (b) Calculated number average degree of P(4-VBA) determined by ^1H -NMR spectroscopy using PEG as internal standard. (c) Number average molecular weight determined by ^1H -NMR spectroscopy. (d) Number average molecular weight determined by Multiangle Laser Light Scattering. (e) Polydispersity (M_w/M_n) determined by Multiangle Laser Light Scattering.

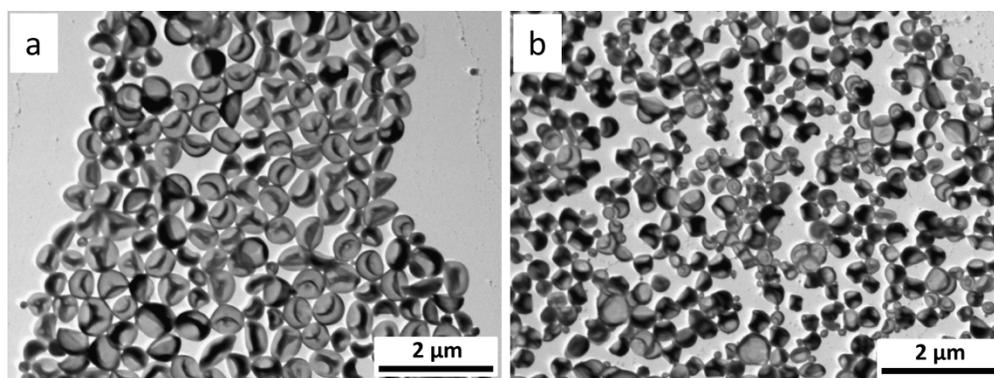


Figure S1. TEM images of polymersomes consisting of block copolymer **P1** before (a) and after (b) functionalization with catalyst **2** and cross-linker **3**.

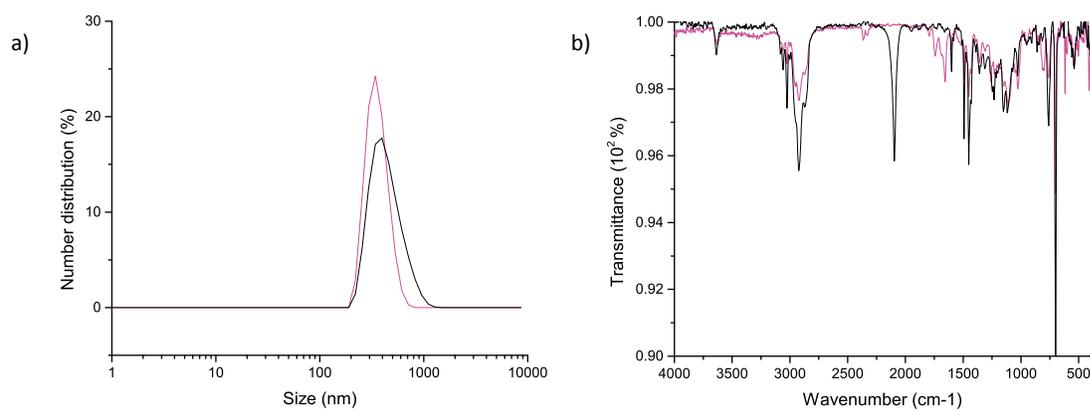


Figure S2. (a) DLS and (b) IR spectra of polymersomes consisting of block copolymer **P1** before (—) and after (—) functionalization with proline catalyst **2** and cross-linker **3**.

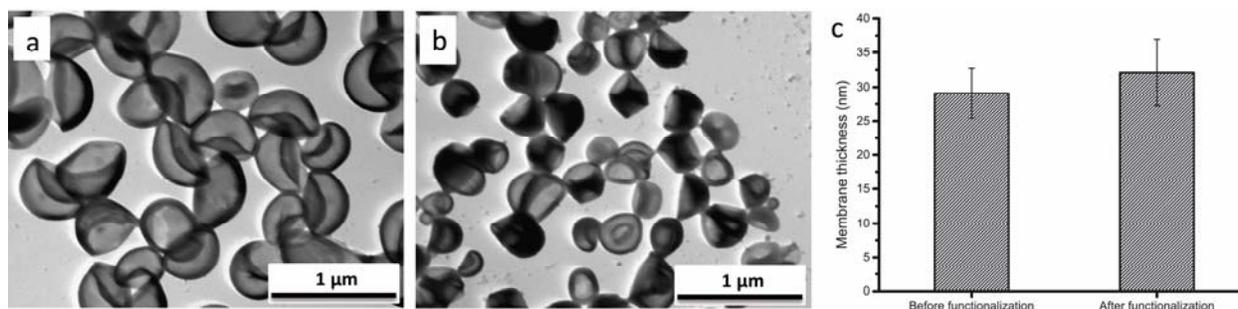


Figure S3. (a, b) Close-up images of polymersomes before (a) and after (b) functionalization. (c) Distribution of polymersome membrane thickness (sample size: 25) before and after functionalization.

Figure S4. ^1H NMR (top) and ^{13}C NMR (bottom) spectra of compound 1.

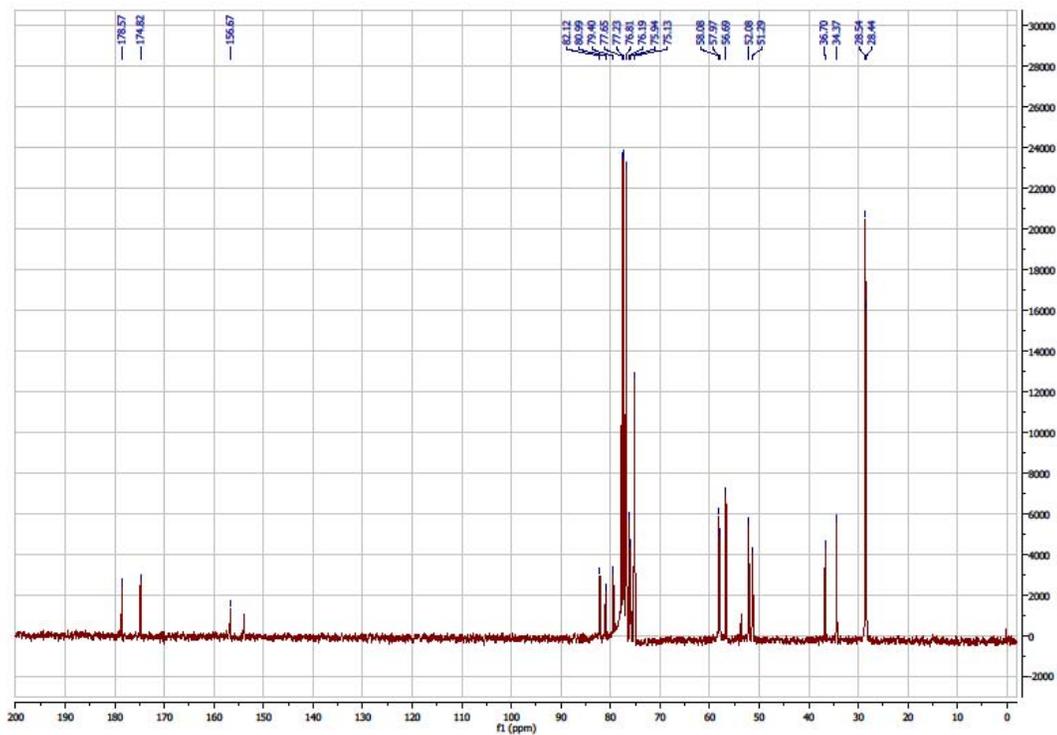
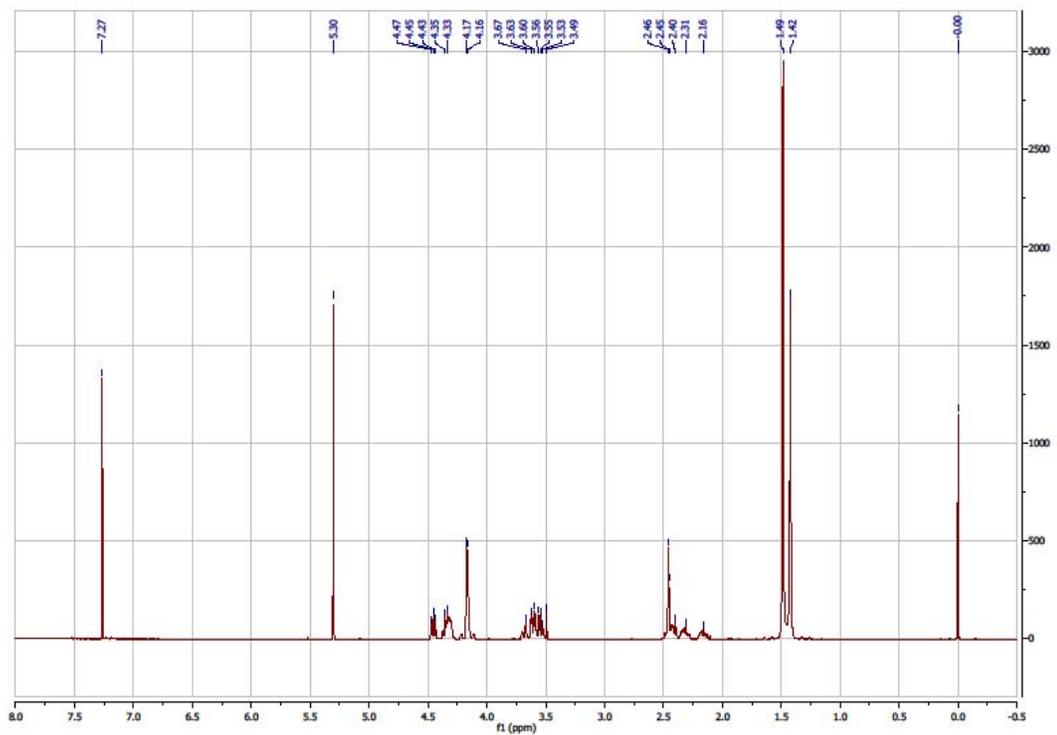


Figure S5. ^1H NMR (top) and ^{13}C NMR (bottom) spectra of compound 2.

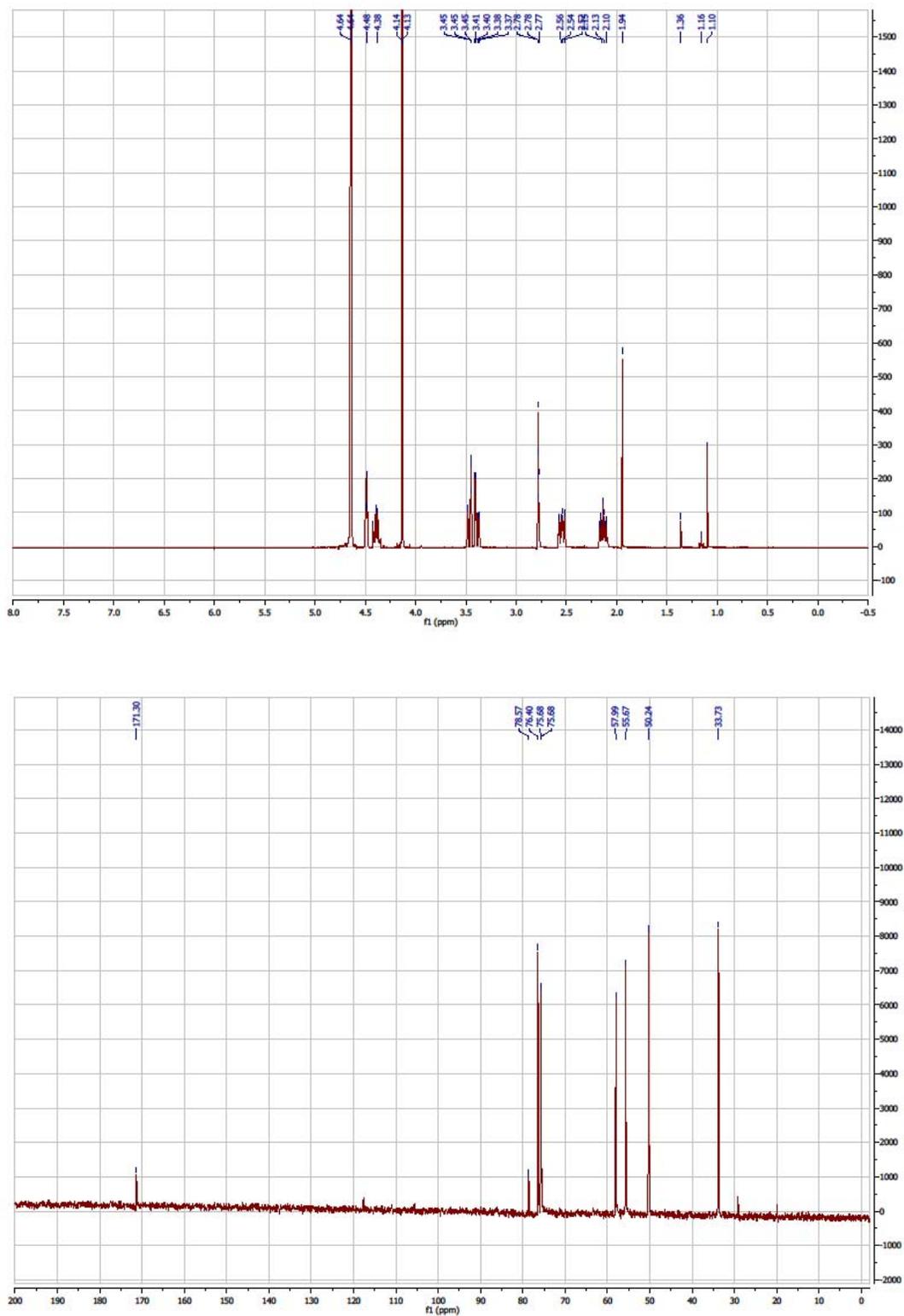
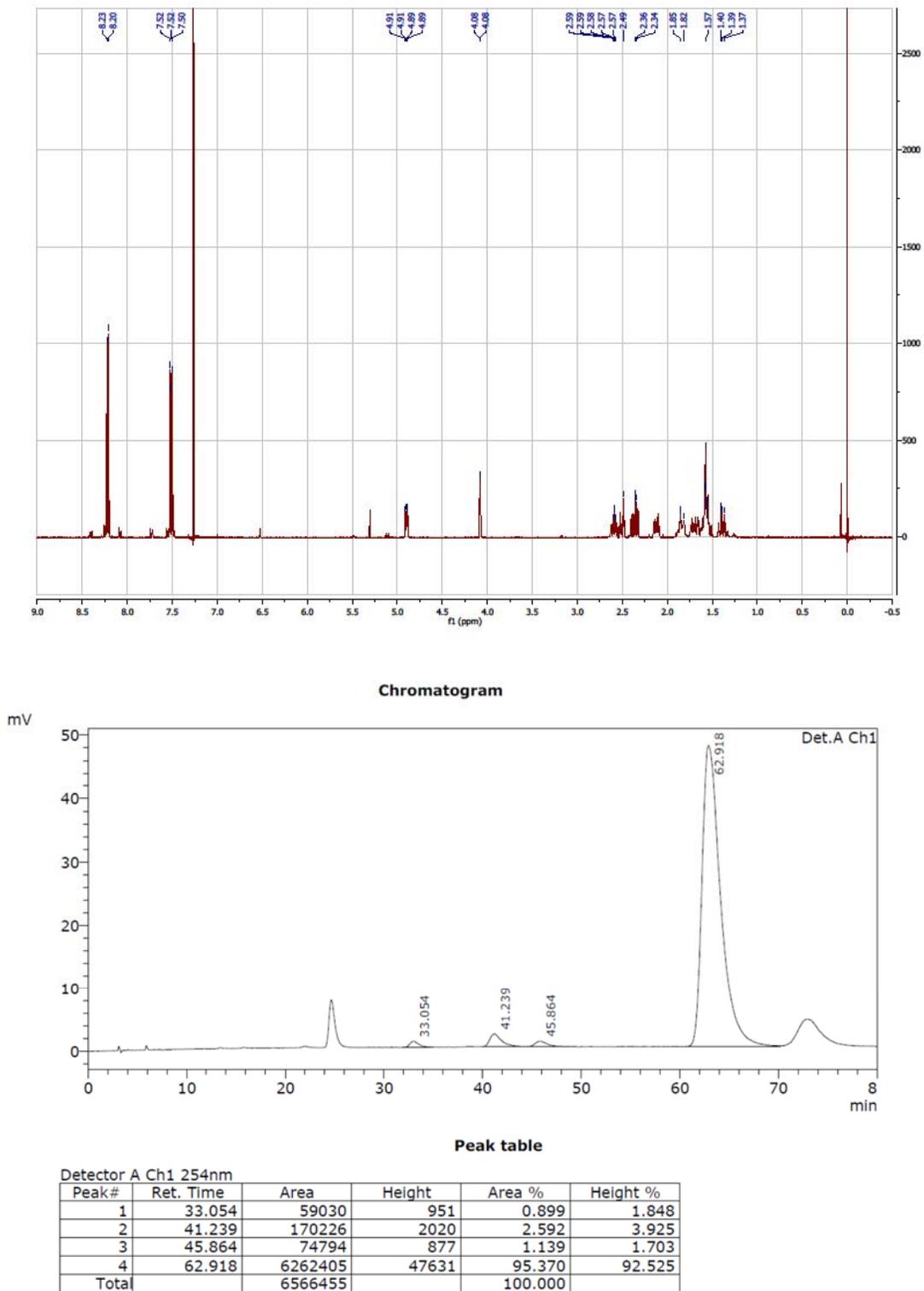


Figure S6. ^1H NMR spectrum (top) and HPLC spectrum (bottom) of compound 4.



References:

- (1) Bang, J.; Kim, S. H.; Drockenmuller, E.; Misner, M. J.; Russell, T. P.; Hawker, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 7622.
- (2) Feng, Y. H.; Li, J. N.; Jiang, L. S.; Gao, Z. H.; Huang, W. C.; Jiang, F.; Luo, N. H.; Han, S. J.; Zeng, R. H.; Yang, D. Q. *Eur. J. Org. Chem.* **2011**, 562.
- (3) Lu, A.; Smart, T. P.; Epps, T. H.; Longbottom, D. A.; O'Reilly, R. K. *Macromolecules* **2011**, *44*, 7233.