Supporting Information for

Synthesis and Noncovalent Protein Conjugation of Linear-Hyperbranched PEG-Poly(glycerol) α,ω_n-Telechelics

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Instrumentation ^{1}H, ^{13}C nuclear magnetic resonance spectra were recorded using a Bruker AC 300 or on a Bruker AMX 400 spectrometer operated at 400 MHz for ^{1}H, employing deuterated chloroform as a solvent. ^{13}C-NMR spectra (referenced internally to solvent signals) at 100.15 MHz. FT-IR spectra were recorded on a Nicolet SDXC FT-IR spectrometer equipped with an ATR unit. SEC measurements in dimethylformamide (DMF) containing 1 g/L of lithium bromide, an Agilent 1100 Series GPC Setup (gel permeation chromatography) was used as an integrated instrument, including a PSS HEMA column ($10^6/10^5/10^4$ g/mol), a UV (254 nm), and RI detector. Calibration was carried out using poly(styrene) or poly(ethylene oxide) standards provided by Polymer Standards Service. The eluent was used at 50 °C and at a flow rate of 1mL/min. Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) measurements were performed on a Shimadzu Axima CFR MALDI-TOF mass spectrometer equipped with a nitrogen laser delivering 3ns laser pulses at 337nm, α-cyano-4-hydroxycinnamic acid was used as a matrix. Samples were prepared by dissolving the polymer in methanol at a concentration of 10g/L. A 10µL aliquot of this solution was

added to 10µL of a 10g/L solution of the matrix and 1µL of a solution of KTFA (0.1M in methanol as cationization agent). A 1µL aliquot of the mixture was applied to a multistage target to evaporate $CHCl_3$ and create a thin matrix/analyte film. The samples were measured in positive ion and in reflection mode of the spectrometer. The RP HPLC analysis was performed on a HP 1090 Liquid Chromatograph (Hewlett Packard) by using a PerfectSil column (MZ Analysentechnik, Mainz, Germany, 250 x 4.0 µm; 120 ODS-2 5 µm). The samples were eluted with an acetonitrile/water gradient that started from 100 % water decreasing to 50 % over a period of 27 min and kept for another 5 min. Both solvents were buffered with 0.05 % TEA. UV-detection was performed at 254 nm.

Reagents: Diglyme (99% Acros), glycidol (99% Acros), ethanolamine (98%, Acros), pyridine (99%, Acros) were purified by distillation from CaH_2 directly prior to use. Ethoxyethyl glycidyl ether (EEGE) was prepared as described in literature,[10] dried over CaH_2 and freshly distilled before used (b.p. 152-154°C, colorless liquid). ^{1}H NMR ($CDCl_3$, δ ppm): 4.76 [-O-CH(CH_3)-O-], 3.35-3.90 (-O-CH_2CH_3 and –O-$CH_2C_2H_3O$), 3.15 (CH of oxirane ring), 2.61-2.91 (CH_2 of oxirane ring), 1.33 -OCH(CH_3)-O-], 1.19 (-OCH3). FTIR (film, ν) 1350, 1254 cm^{-1}. Pentafluorophenol (99%), benzyl bromide (99%) was purchased from Aldrich and used as received. Cesium hydroxide monohydrate, palladium on activated charcoal, potassium carbonate, *N*,*N*′-Dicyclohexylcarbodiimide (DCC) was purchased from Acros and used as received. Biotin was obtained from IRIS Biotech and used as received. Avidin, Streptavidin were purchased from Aldrich and stored at -20°C until used. Deuterated DMSO-d_6 was purchased from Deutero GmbH, dried and stored over molecular sieves. Methanol and other solvents and reagents were purchased from Acros and used as received, if not otherwise mentioned.

N,N-Dibenzyl-2-aminoethanol: A mixture of 2-aminoethanol (30 g, 0,5 mol), benzyl bromide (171,3 g, 1 mol), K_2CO_3 (276 g, 2 mol), and water (900 mL) was refluxed and vigorously stirred for 5 h. The organic phase was diluted with diethyl ether and separated from the aqueous layer. The organic phase was washed two times with water (each 100 mL), dried over Na_2SO), and evaporated to give 105 g of crude product as a pale yellow oil. The product was crystallized from a 9:1 mixture pentane/ethyl acetate and recrystallied two times from to form white crystals mp. 38°C, 60g (50%) ^{1}H-NMR ($CDCl_3$): δ = 7.31-7.32 (m, 10 H, Ar-H), 3.60 **(s,** 4H. CH_2Ph), 3.57 (t, J = 5.7 Hz**,** 2H, CH_2OH), 2.65 (t, J = 5.4 Hz, 2H, CH_2CH_2OH). ^{13}C-NMR ($CDCl_3$): δ = 54.7 (CH_2N), 58.4 ($C_6H_5CH_2N$), 58.5 (CH_2OH), 127.2, 128.4, 129.0, 138.7 (aromatic carbons).

(+)-Biotin pentafluorophenyl ester. (+)-Biotin (0.57 g**,** 2.33 rnmol) was dissolved in dry pyridine (25 mL) at ca. 50°C, cooled to room temperature, and then pentafluorophenol (0.44 g**, 2.37** mmol) was added. The reaction mixture was cooled with an ice bath, and *N*,*N*′-dicyclohexylcarbodiimide (DCC) (0.49 g**, 2**.4 mmol) in dry pyridine (5 mL) was added drop-wise within 30 min. The mixture was allowed to warm up to room temperature over night. The DCU formed was filtered **off** and washed with pyridine (10 mL), the combined solutions were concentrated and kept at room temperature for 5 h. **A** small amount of residual DCU was removed by filtration, the solvent was evaporated, and the resulting oil was crystallized with a $CHCl_3$-hexane mixture (1:l v/v; 20 mL). After filtration and drying the PFP-ester of biotin (0.9 g, 95%) was obtained as a white crystalline powder, mp 188-189°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 6.47 (s, 1H, NH), 6.38 (s, 1H, NH), 4.31 (m, 1H, CHN methin), 4.15 (m, 1H, CHN methin), 3.13 (m, 1H, CHS methin), 2.85-2.77 (m, 4H, CH_2S and CH_2COOR), 1.67-1.43 (br, -$CH_2CH_2CH_2$-).

General procedure for anionic polymerization:

Linear block copolymers N,N-Dibenzyl-poly(ethylene oxide)-block-poly(ethoxyethyl glycidyl ether): N,N-Dibenzyl-2-aminoethanol was dissolved in benzene in a Schlenk flask and a stoichiometric amount of cesium hydroxide monohydrate was added under argon. The mixture was stirred at 60°C for 45 minutes and evacuated at 90°C (10^{-2} mbar) for two hours to remove benzene and water. The dry cesium alkoxide was dissolved in dry DMSO (20 wt%). In a separate setup, THF was cryo-transferred into a Schlenk flask from a dark purple colored sodium/benzophenone THF solution. Subsequently, ethylene oxide was first cryo-transferred to a graduated ampoule and then into the flask containing THF to produce a ca. 50 weight % solution. The solution was tempered at 0°C and the initiator was added via canula. The slightly yellow mixture was allowed to slowly warm up to room temperature and polymerization was performed for 2 days in vacuo. Subsequently the flask was filled with argon, the appropriate amount of ethoxyethyl glycidyl ether was added with a syringe and temperature was raised to 80°C for 12 hrs. The polymerization was terminated by addition of methanol and acidic ion exchange resin. Filtration and precipitation in cold diethyl ether resulted in the pure polymer. For polymers with larger amount of PEEGE, the polymer solution was dried in vacuo after filtration of the resin. Yields: 95%-quantitative.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.33 (m, C_6H_5), 4.69 (br, acetal-H), 3.86-3.37 (polyether backbone), 2.32 (br, NCH_2Ph), 1.28-1.15 (br, CH_3 acetal). DPn of EEGE was determined by comparison of aromatic signals of the initiator and the methyl signals for PEEGE block. Molecular weights were obtained by comparison of the aromatic protons with the polyether signals.

Deprotection to linear block copolymers N,N-Dibenzyl-poly(ethylene oxide)-block-linear-poly(glycerol): The acetal protecting groups of PEEGE were removed by the addition of 1 M

hydrochloric acid to a 20% solution of the polymer in methanol and stirring for 30 min. Purification of the block copolymer was achieved by repetitive precipitation from a concentrated methanolic solution in diethyl ether or by exhaustive dialysis in methanol using a dialysis tube with a MWCO of 1,000 g/mol. Yields: 80-90%. The polymers are usually obtained as the respective ammonium chlorides. Recovery of the free amine is achieved by addition of triethylamine during dialysis.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.14 (br, Bn_2NH^+), 7.62, 7.40 (br, C_6H_5), 4.59 (br, OH), 3.59-3.27 (polyether backbone), 2.46 (br, NCH_2Ph).

***Hypergrafting linear-hyperbranched block copolymers N,N-Dibenzyl-poly(ethylene oxide)-block—hyperbranched-poly(glycerol)*:** The linear macroinitiator was placed in a Schlenk flask and dissolved in benzene (20 weight%). Subsequently the appropriate amount of cesium hydroxide monohydrate was added to achieve 30% of deprotonation of the hydroxyl groups along the backbone. After heating to 60°C for 30 min and evacuation (10^{-2} mbar) at 90°C for 2 hours dry diglyme was added to produce a 20 weight% solution, and the flask was placed in an ultrasonic bath for 15 min to ensure complete suspension of the macroinitiator. The mixture was heated to 100°C and a 20 weight% solution of glycidol in dry diglyme was added slowly with a syringe over a period of ca. 12 hours. The reaction was terminated by addition of an excess of methanol and an acidic cation exchange resin. The products were filtrated, concentrated and precipitated into cold diethyl ether. The resulting material was dried in vacuo for 2 days at 40°C. Yields: quantitative.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 7.62, 7.40 (br, C_6H_5), 4.79-4.42 (br, OH, different signals due to branched PG), 3.59-3.27 (polyether backbone), 2.46 (br, NCH_2Ph).

***Hydrogenation of linear-hyperbranched block copolymers H_2N-poly(ethylene oxide)-block—hyperbranched-poly(glycerol)*:** Under an argon atmosphere, 1 g N,N-Dibenzyl-poly(ethylene oxide)-*block*-hyperbranched-poly(glycerol) was dissolved in methanol and palladium on activated charcoal (10%) was added. The vessel was flushed with hydrogen (8 bar) and the reaction was allowed to stir for 48-72 hrs at room temperature, completion of the reaction was monitored via ^{1}H- NMR spectroscopy. The solution was filtered, concentrated and precipitated into cold diethyl ether. Yields: quantitative.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.79-4.42 (br, OH, different signals due to branched PG), 3.59-3.27 (polyether backbone).

Coupling reaction of α,ω_n-heterotelechelic H_2N-poly(ethylene oxide)-block—hyperbranched-poly(glycerol) with biotin. 0.2 g (M_n=5,200 g/mol, 0.038 mmol) of H_2N-poly(ethylene oxide)-*block*-hyperbranched-poly(glycerol) was dissolved in 3 mL of degassed dimethylformamide (DMF) and 50 µL of freshly distilled triethylamine was added under argon. 32 mg (0.078 mmol) (+)-Biotin pentafluorophenyl ester was added and the reaction was allowed to stir for 24 hrs at room temperature. The excess PFP-ester was removed by repetitive precipitation from DMF into cold acetone or exhaustive dialysis in methanol (MWCO 1,000 g/mol). Yield: 0.2 g (quantitative).

^{1}H-NMR (300 MHz, DMSO-d_6): d = 6.47 (s, 1H, NH), 6.38 (s, 1H, NH), 4.79-4.42 (br, OH, different signals due to branched PG), 4.31 (m, 1H, CHN methin), 4.15 (m, 1H, CHN methin), 3.59-3.27 (polyether backbone), 3.13 (m, 1H, CHS methin), 2.85-2.77 (m, 4H, CH_2S and CH_2COOR), 1.67-1.43 (br, -$CH_2CH_2CH_2$-).

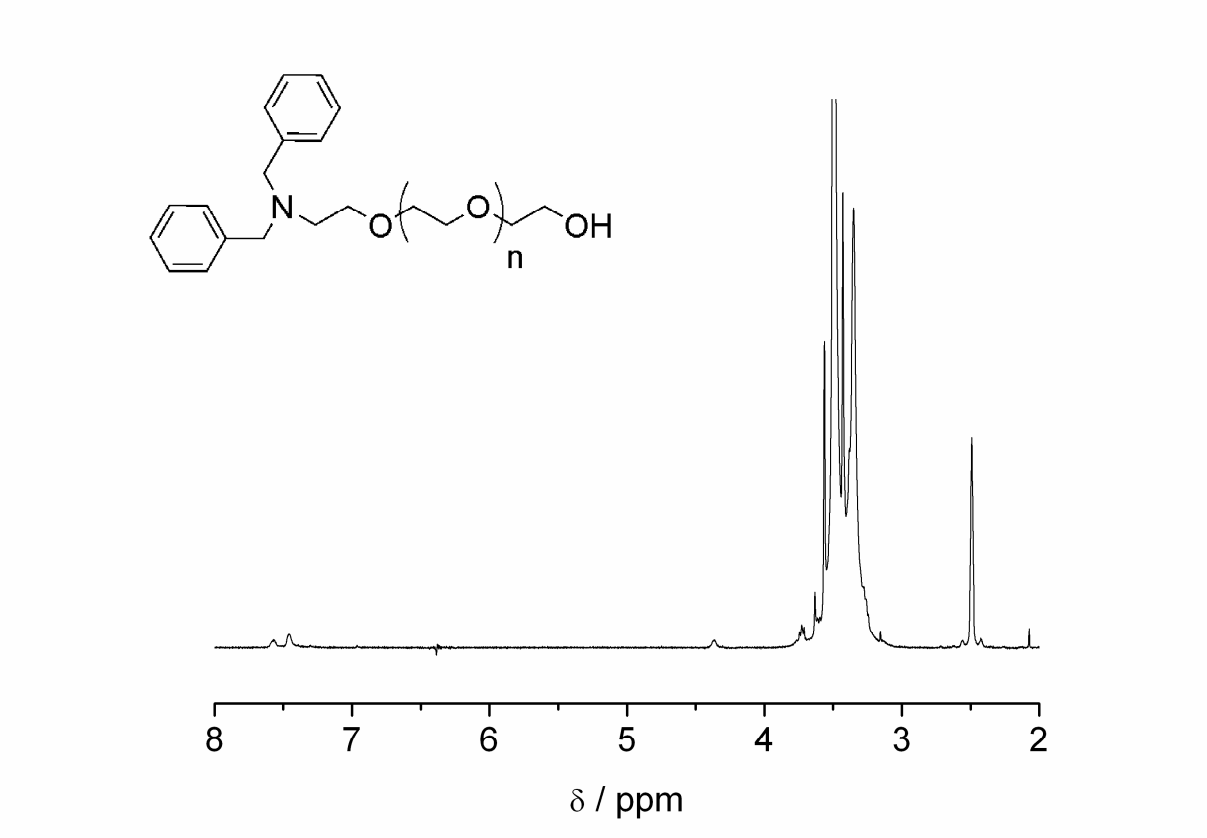


Figure S1: ^{1}H-NMR spectrum (DMSO-d_6) of Bn_2N-$PEG_{120}OH$.

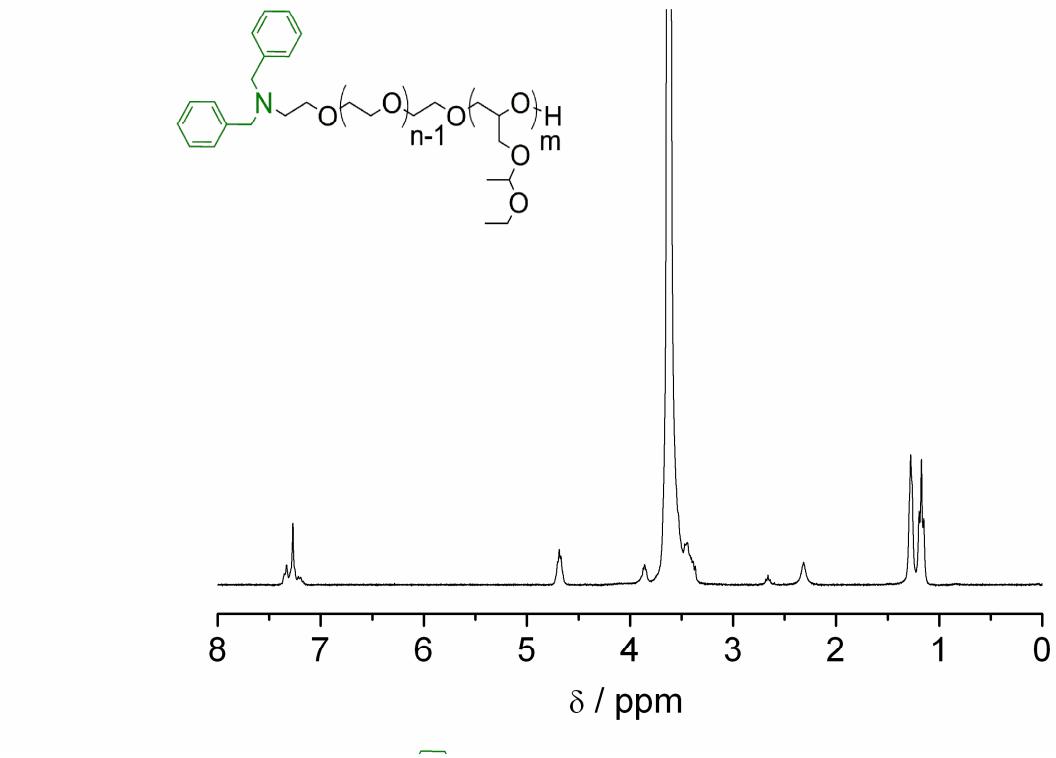


Figure S2: ^{1}H-NMR spectrum ($CDCl_3$) of Bn_2N-PEG_{120}-$PEEGE_{15}$ (entry 2, Table 1).

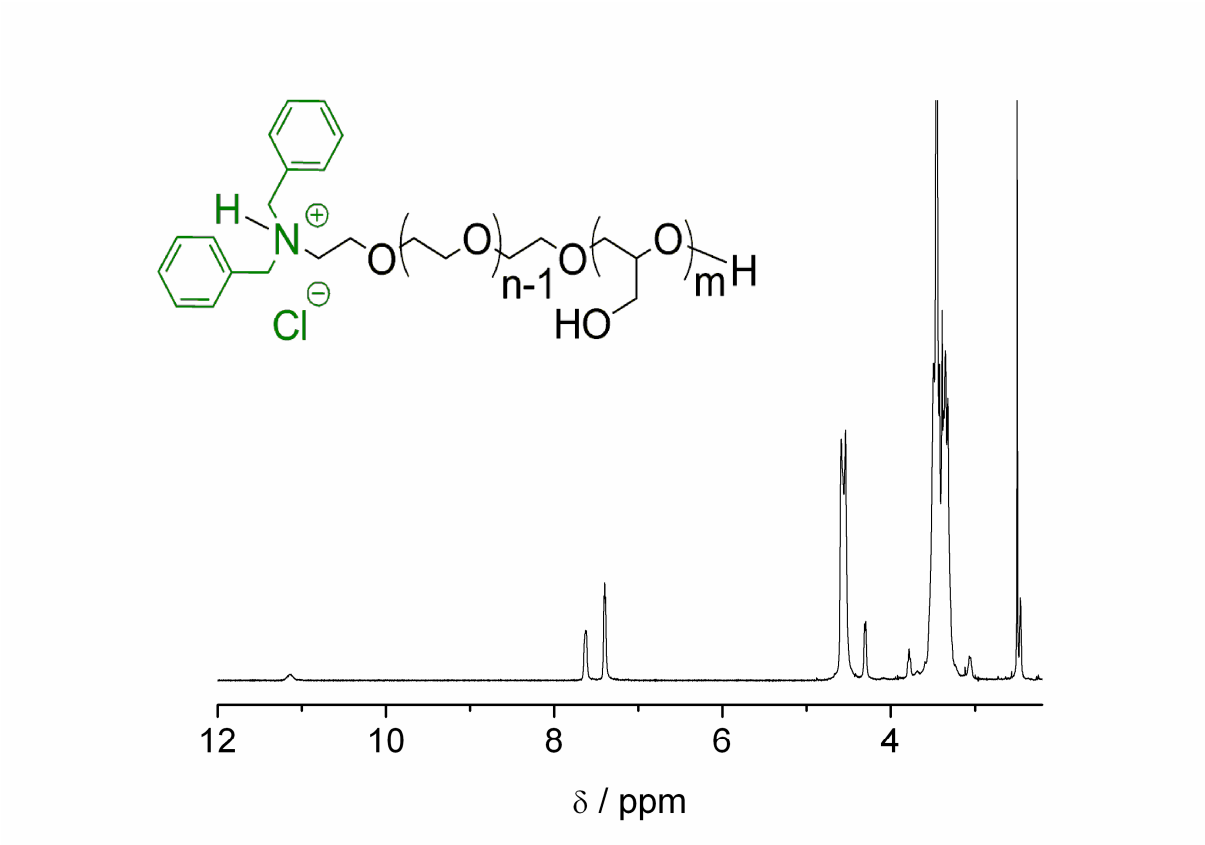


Figure S3: ^{1}H-NMR spectrum (DMSO-d_6) of HCl*Bn_2N-PEG_{20}-linPG_5 (entry 1, Table 1).

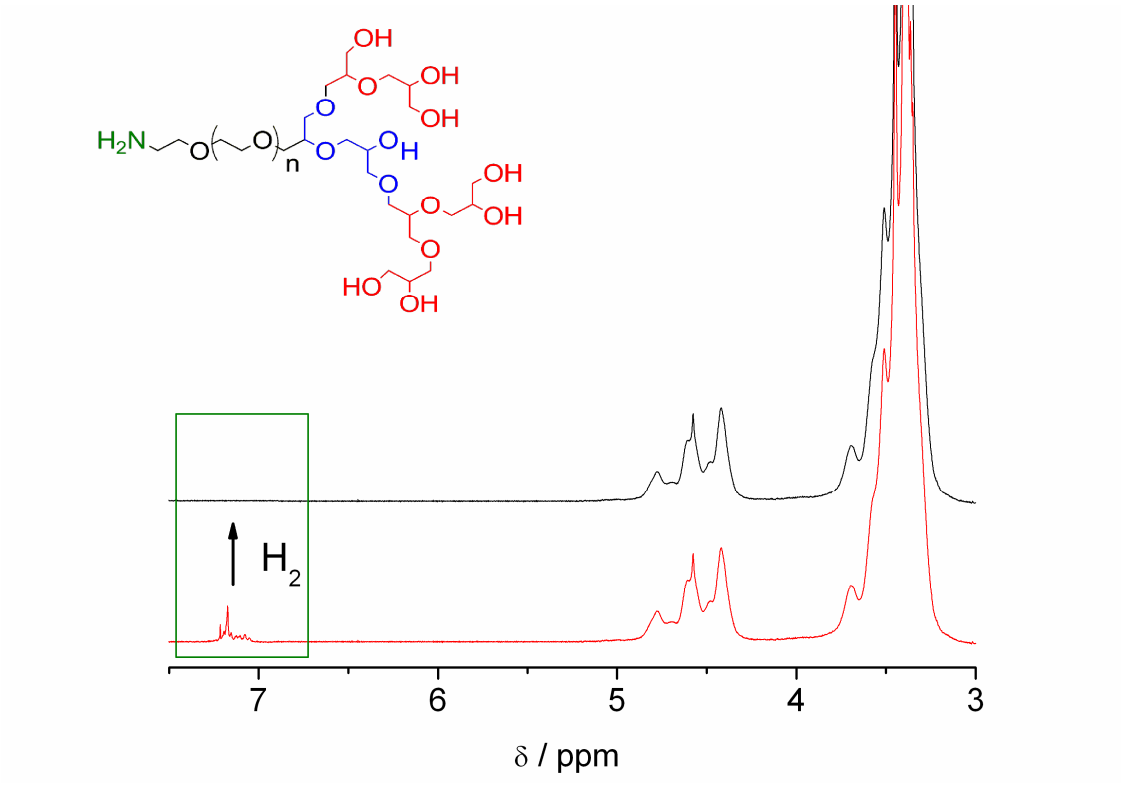


Figure S4: ^{1}H-NMR spectrum (DMSO-d_6) of Bn_2N-PEG_{20}-$hbPG_{50}$ (bottom) and H_2N-PEG_{20}-$hbPG_{50}$ (top) (entry 1b and 1b*, Table 1).

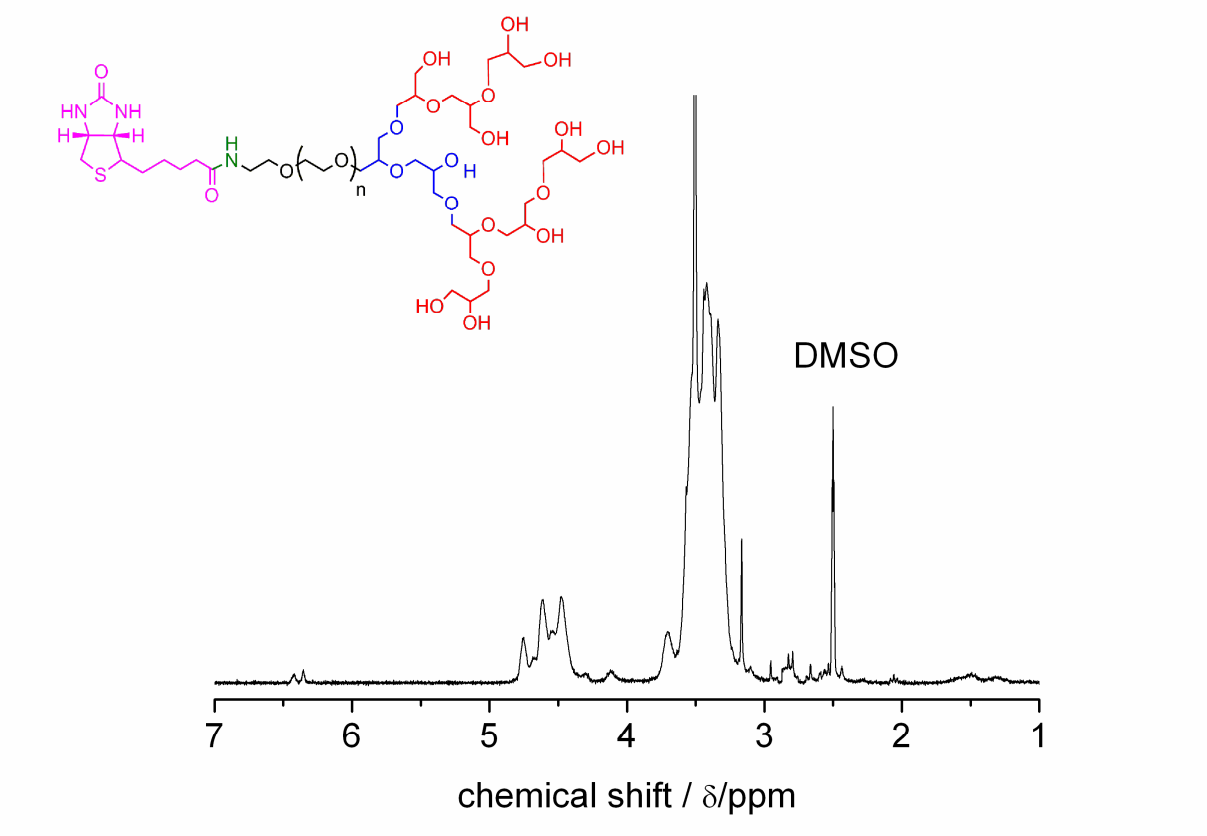


Figure S5: ^{1}H-NMR spectrum (DMSO-d_6) of biotinylated PEG-*hb*PG (from entry 1b*, Table 1).

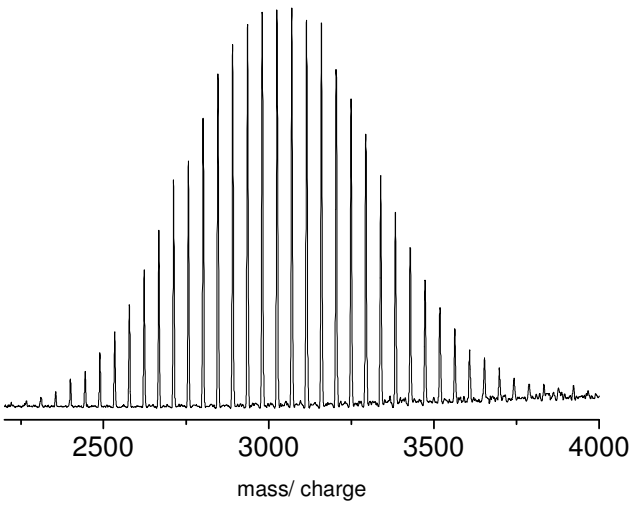


Figure S6: MALDI-ToF of Bn_2N-PEO-OH, evidencing quantitative functionalization.

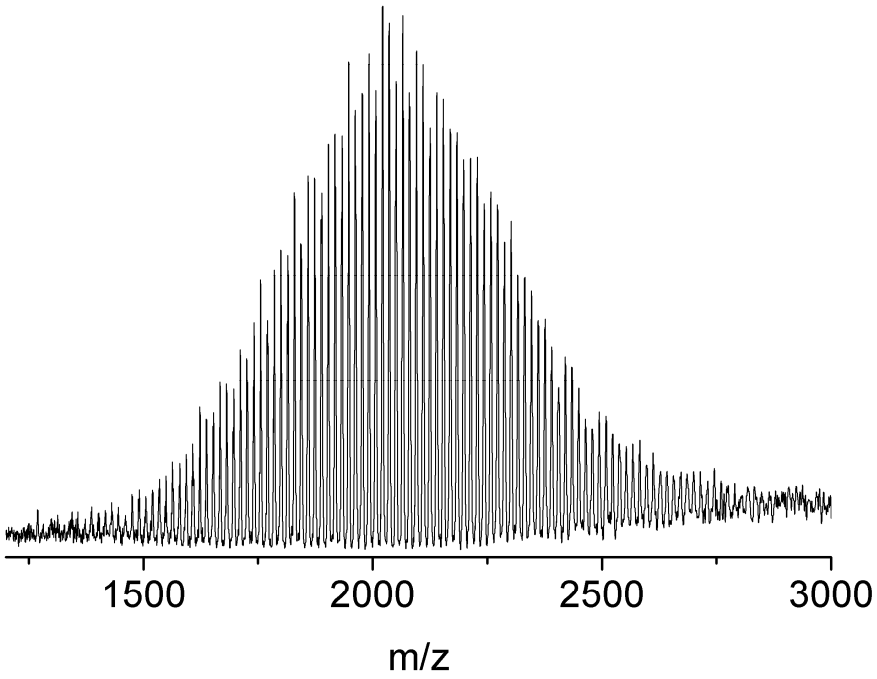


Figure S7: MALDI-ToF of Bn_2N-PEG_{25}-$hbPG_{20}$ demonstrating quantitative functionalization (Table 1, entry 1a).

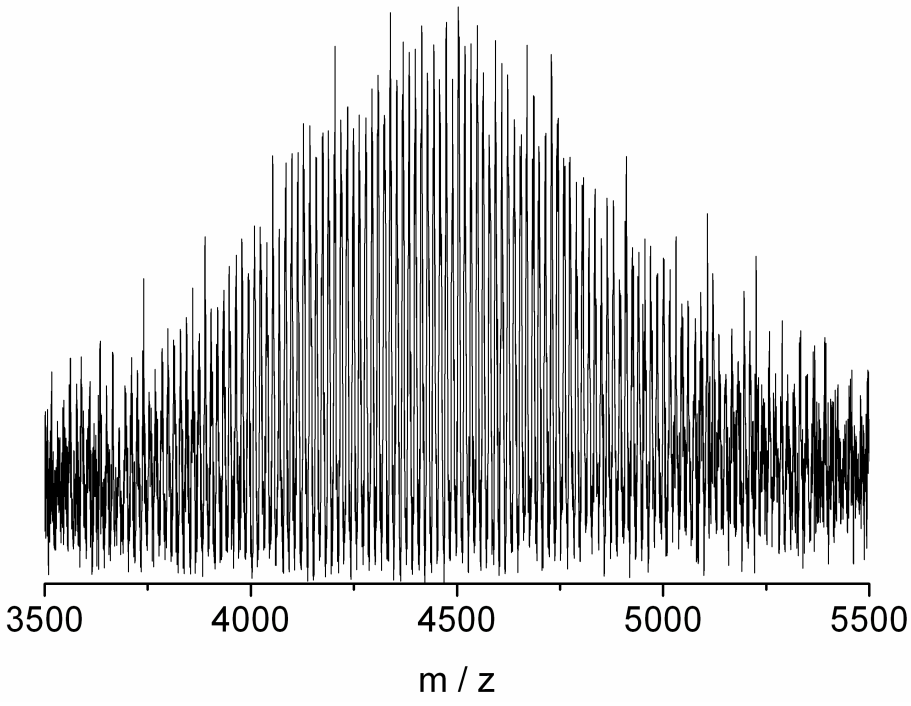
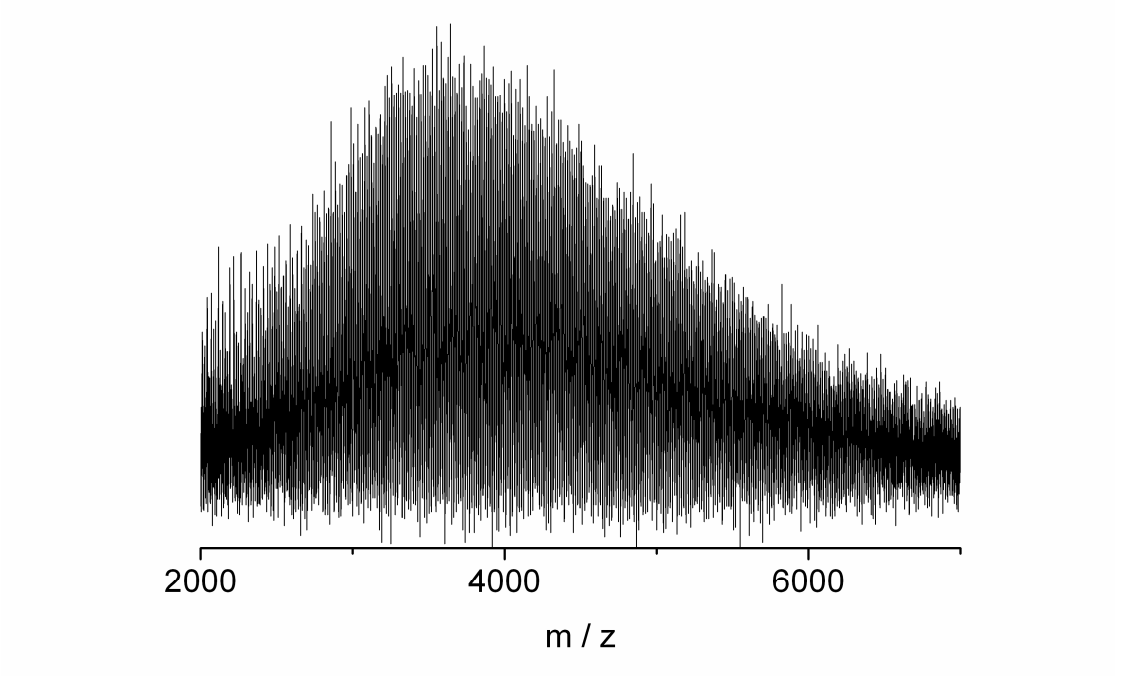


Figure S8: MALDI-ToF of Bn_2N-PEG_{25}-*hb*PG_{20} showing quantitative functionalization (Table 1, entry 1b).



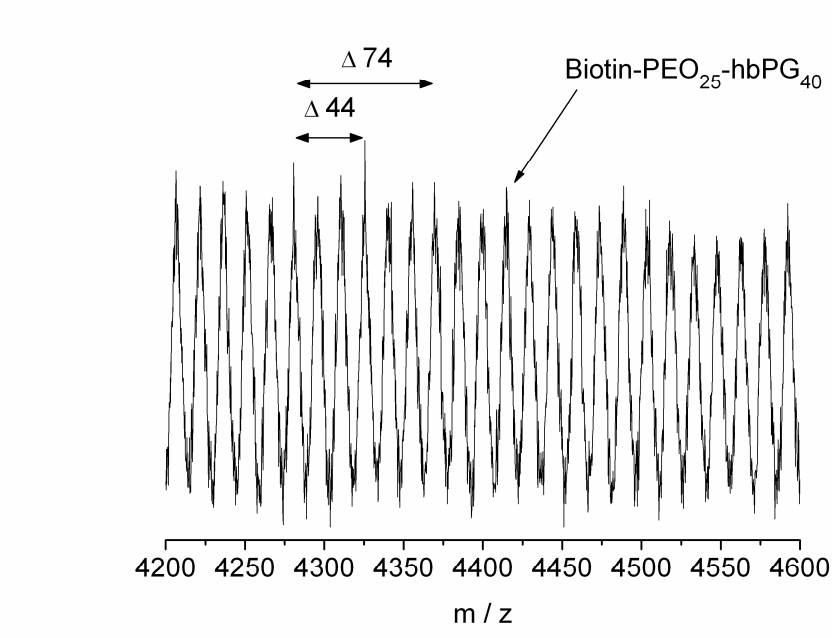


Figure S9: top: MALDI-ToF of biotinylated H_2N-PEO-*hb*PG showing quantitative functionalization (compare zoom-in in Figure 2). Bottom:) Characteristic region of the MALDI-ToF spectrum of BiotinPEG_{25}*hb*PG_{50} (from **1b***).

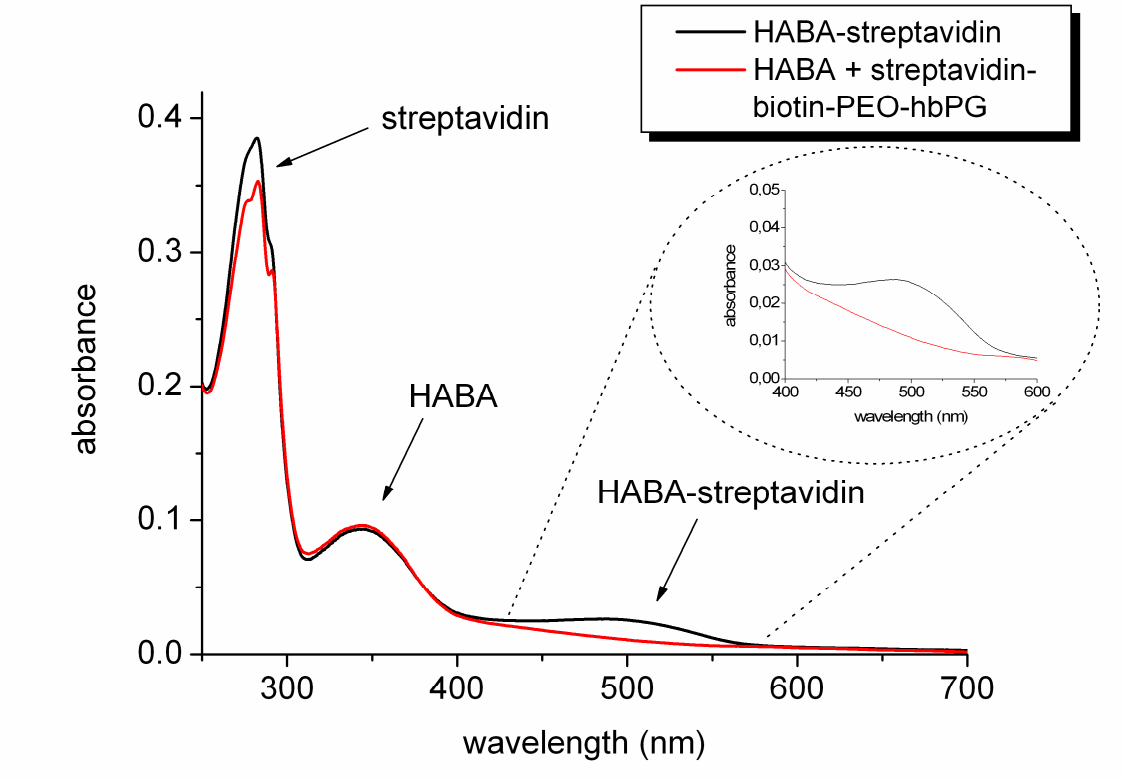


Figure S10: UV/vis spectra of the HABA-streptavidin complex before (red) and after addition of the biotinylated polymer (yellow, characteristic absorption of HABA).

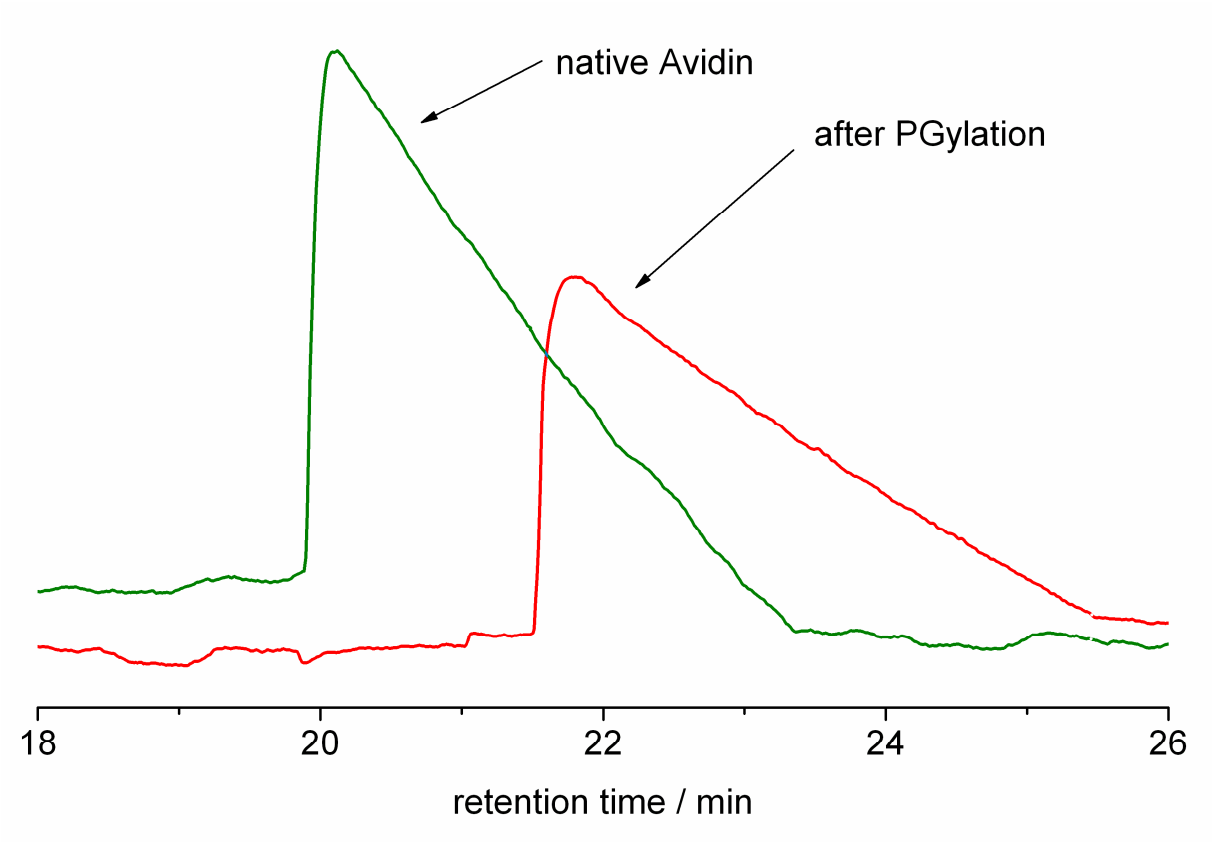


Figure S11: HPLC of native Avidin (green) vs. Avidin-(biotin-PEG-*hb*PG)$_4$ (red).

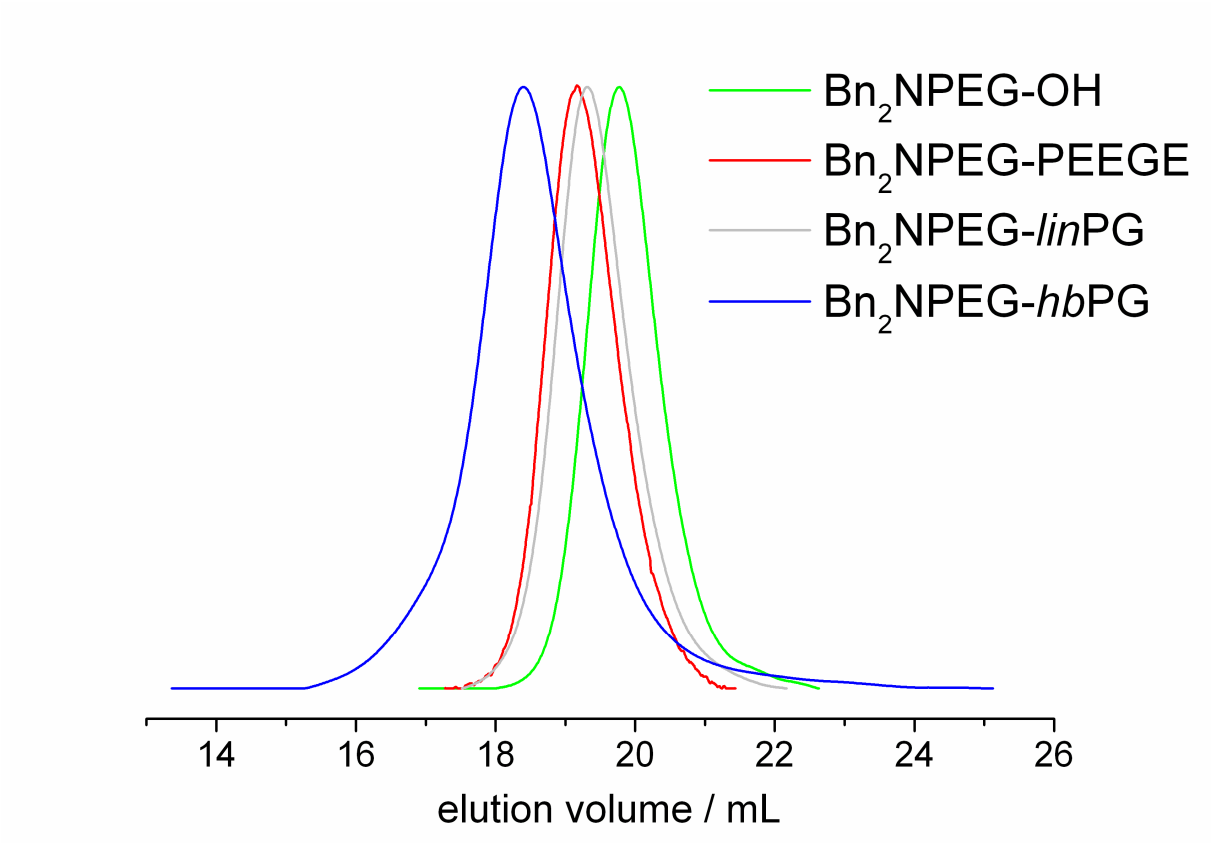


Figure S12: SEC elugrams in DMF of series 2 (compare Table 1, main manuscript).

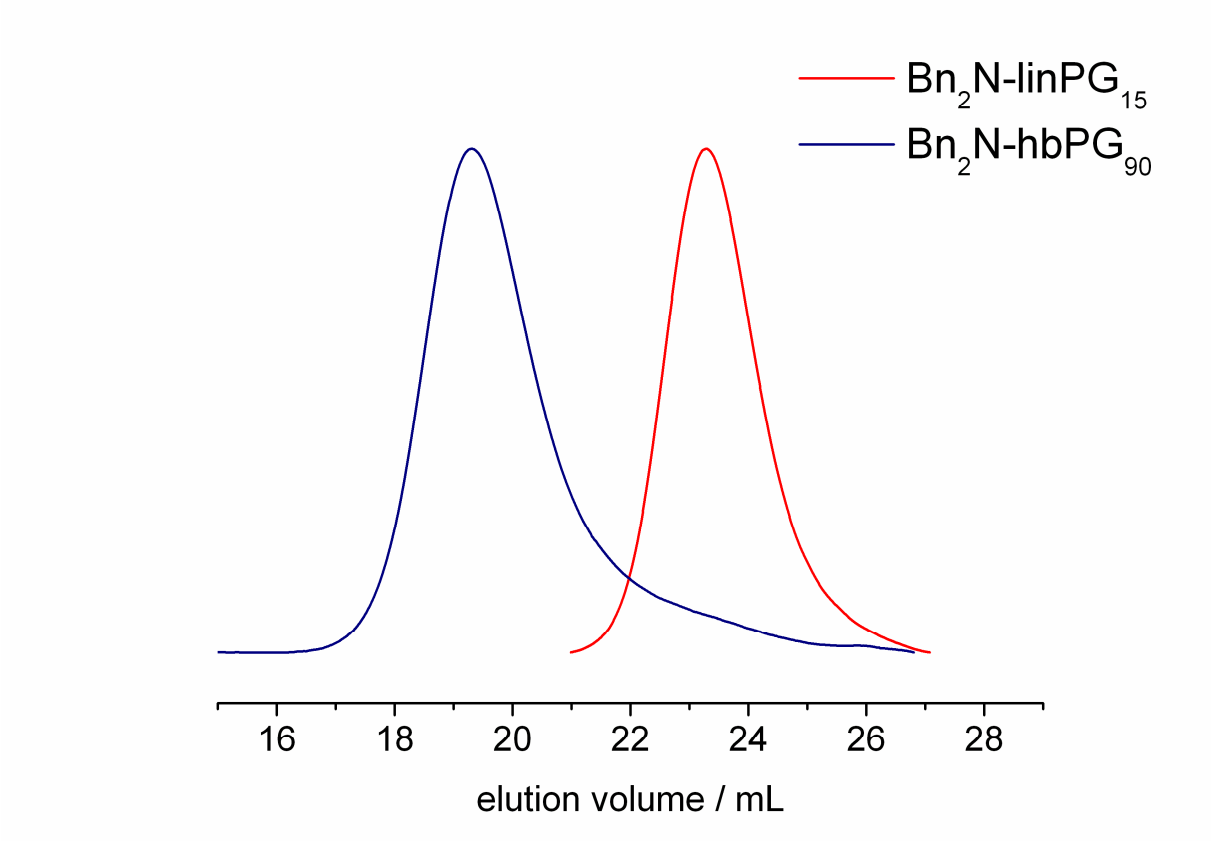


Figure S13: SEC elugrams in DMF of series 3 (compare Table 1, main manuscript).