Towards An Easy Access To Amphiphilic Rod-Coil Miktoarm Star Copolymers

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Supplementary information

Materials

All reagents were purchased from Aldrich. Styrene (99%) and *tert*-butylacrylate (*t*BA, 98%) were stirred overnight over CaH_2 and distilled prior to use. γ-benzyl-L-glutamate was dried after crystallization from hexane and kept under argon in a glove box. Copper (I) bromide (CuBr, 98%), N,N,N',N',N"-pentamethyldiethylenetriamine (PMDETA, 99%) and ethyl 2-bromoisobutyrate (98%) were used as received. Triethylamine and tetrahydrofuran were distilled over CaH_2 prior to use. Diethanolamine (98%), 1-amino triethylenetriamine (96%), 2-bromoisobutyryl bromide (98%), trifluoroacetic acid (TFA, 99%) and N,N-dimethylformamide (DMF, 99%) were used as received.

Synthesis

Samples referred to as PS(Br), $PS(OH)_2$, $PS(Br)_2$, PS-b-$(PtBA)_2$ and PS-b-$(PAA)_2$ were synthesized as described previously in reference 5 (R. Francis, et al. *Macromolecules* 2002, **35**, 9001).

The $PS(NH_2)_2$ compound was synthesized as follows. Polystyrene (12 g, 4.9×10^{-3} mol, M_n = 2450 g.mol^{-1}, M_w/M_n = 1.08) with bromide end groups was dissolved in dry DMF (100 ml) and 21 ml (0.147 mol) of tris(2-aminoethyl)amine was added. After stirring for 5 days at RT, the excess of unreacted triamine was removed by phase transfer of the polymer in cyclohexane. TLC-analysis (SiO_2: CH_2Cl_2) showed that in addition to the desired compound, the crude product also contained some unfunctionalized polystyrene. These impurities were removed by means of selective precipitation of quaternized amine in apolar solvent. First the polymer was dissolved with cyclohexane and ten drops of concentrated HCl (35%) were added. Then hexane was added slowly to the solution until precipitation of the quaternized amine polystyrene. The polymer was dissolved with dichloromethane and extracted three times with a saturated K_2CO_3 water solution. The organic phase was separated, dried over magnesium sulfate, filtered and evaporated to dryness. Finaly precipitaton in methanol and vacuum drying at 40 °C offerded the ω,ω'-bis(primary amino) polystyrene (yield: 78%). Its ^{1}H NMR spectrum ($CDCl_3$) is given below in Figure 2b and its MALDI-TOF mass spectrum is shown in Figure 1.

Miktoarm star PS-b-$(PGA)_2$ copolymers were synthesized as follows. Bz-L-GluNCA (3.26 g, 1.24×10^{-2} mol) was weighed in a glovebox, introduced in a flame-dried Schlenck, and dissolved with anhydrous CH_2Cl_2 (23 ml). In a separate flask, the $PS(NH_2)_2$ macroinitiator (0.45 g, 1.77×10^{-4} mol) was dissolved with dry CH_2Cl_2 (10 ml) and added via cannula. The solution was degassed and stirred for 24 h at RT. The copolymer was recovered by precipitation in diethylether and dried under hight vacuum. Monomer conversion and overall yield were 97%. SEC (DMF): M_n = 7460 g.mol^{-1}, M_w/M_n = 1.26. The ^{1}H NMR ($CDCl_3$) spectrum of this compound is shown in red in Figure 3. The corresponding PS-b-$(PGA)_2$ sample was obtained as follows. PS-b-$(PBLG)_2$ star polymer (0.8g, M_n = 18500 g.mol^{-1}, 4.32×10^{-5} mol) was dissolved in TFA (8 ml). Then HBr/acetic acid, 33 wt%, (3 ml, 1.55×10^{-2} mol) was added, and the mixture was stirred at room temperature for 1 h. The polymer was preciptated by addition of diethylether, filtered, and washed with distilled water to remove HBr salts. The powder was dried under vacuum for 48 h. The corresponding ^{1}H NMR spectrum run in DMSO-d_6 is shown in blue in Figure C.

Characterization. ^{1}H NMR spectra were recorded on a Bruker AC 400 spectrometer. The molar masses were determined by size exclusion chromatography (SEC) with tetrahydrofurane (THF) as eluent (1 mL/min) at 25 ^{0}C, a refractive index detector (Varian RI-4) and a PSS column (8×300 mm, 5 µm). Comparison was made with a second SEC apparatus fitted with three TSK-gel columns (7.8×30 cm, 5 µm, G 2000, 3000, and 4000 HR with pore sizes of 250, 1500, and 10 000 Å, respectively) and a refractive index (RI) detector (Jasco, RI-1530) with DMF as eluent (0.7 mL/min). Both SECs were calibrated using linear polystyrene samples. MALDI-TOF mass spectrometry was performed using a Micromass TofSpec E spectrometer equipped with a nitrogen laser (337 nm), a delay extraction and a reflector. The MALDI mass spectra represent averages over 100 laser shots. This instrument operated at an accelerating potential of 20kV. The polymer solutions (10 g.L^{-1}) were prepared in THF. The matrix solution (1,8-dithranol-9(10H)-anthracenone, dithranol) was dissolved in THF. The polymer solution (2 µL) was mixed with 20 µL of the matrix solution and 2 µL of a sodium iodide solution (10 g.L^{-1} in methanol) was added to favor ionization by cation attachment. The final solution (1 µL) was deposited onto the sample target and allowed to dry in air at room temperature.

Dynamic (DLS) light scattering experiments

Dynamic light scattering (DLS) experiments were performed using ALV Laser Goniometer, which consists in 22 mW HeNe linear polarized laser with 632.8 nm wavelength and an ALV-5000/EPP Multiple Tau Digital Correlator with 125 ns initial sampling time. The samples were kept at constant temperature (25.0 °C) during all experiments. The accessible scattering angular range varied from 40° up to 150°. The solutions were introduced into 10 mm diameter glass cells. The minimum sample volume required for the experiment was 1 mL. The data acquisition was done with the ALV-Correlator Control Software, and the counting time varied for each sample from 300 s up to 600 s. The hydrodynamic radii (R_H) values of the micelles were obtained by using CONTIN analysis from solutions without added salt or in the presence of 0.5M NaCl, in acidic (pH<5) or basic (pH>12) conditions at a concentration of 1mg/ml. Millipore water was thoroughly filtered through 0.1 µm filters and directly employed for the preparation of the solutions. All the solutions showed a monomodal distribution with a translational diffusive mode. The hydrodynamic radius (R_H) could be calculated from the diffusion coefficient using the Stokes-Einstein relation. The data obtained for two mikto-arm block copolymers PS_{25}-*b*-$(PGA_{18})_2$ and PS_{20}-*b*-$(PAA_{40})_2$ are summarized in Table 1.

Table 1. Hydrodynamic radii (R_H) values of the micelles measured by DLS from solutions of mikto-arm block copolymers PS_{25}-*b*-$(PGA_{18})_2$ and PS_{20}-*b*-$(PAA_{40})_2$ without added salt or in the presence of 0.5M NaCl, in acidic (pH 5) or basic (pH 12) conditions at a concentration of 1mg/ml.

copolymer	NaCl	R_H (pH 5) (nm)	R_H (pH 12) (nm)	ΔR_H (nm)
PS_{20}-*b*-$(PAA_{40})_2$	0 M	18.3	23.7	5.4
PS_{20}-*b*-$(PAA_{40})_2$	0.5 M	18.3	19.3	1.0
PS_{25}-*b*-$(PGA_{18})_2$	0 M	11.1	15.6	4.5
PS_{25}-*b*-$(PGA_{18})_2$	0.5 M	11.2	15.5	4.3

These data show two important features. First, the change in size as a result of pH variations is in the same range for polypeptide-based and poly(acrylic acid) copolymer micelles, as the

ΔR_H measured (0M Nacl) for the two micellar systems are close in spite of the fact that the poly(acrylic acid) block is larger. Secondly, when salt is added to the solution (0.5M NaCl), the micelles formed from poly(acrylic acid) based copolymers show a little variation of their R_H while the polypeptide-based ones retain their behaviour ($\Delta R_H \approx 4.5$ nm with and without salt).These data clearly support that the reversible secondary structure transition of the polypeptides makes these stimuli-responsive nanoparticles efficient whatever the environmental conditions.

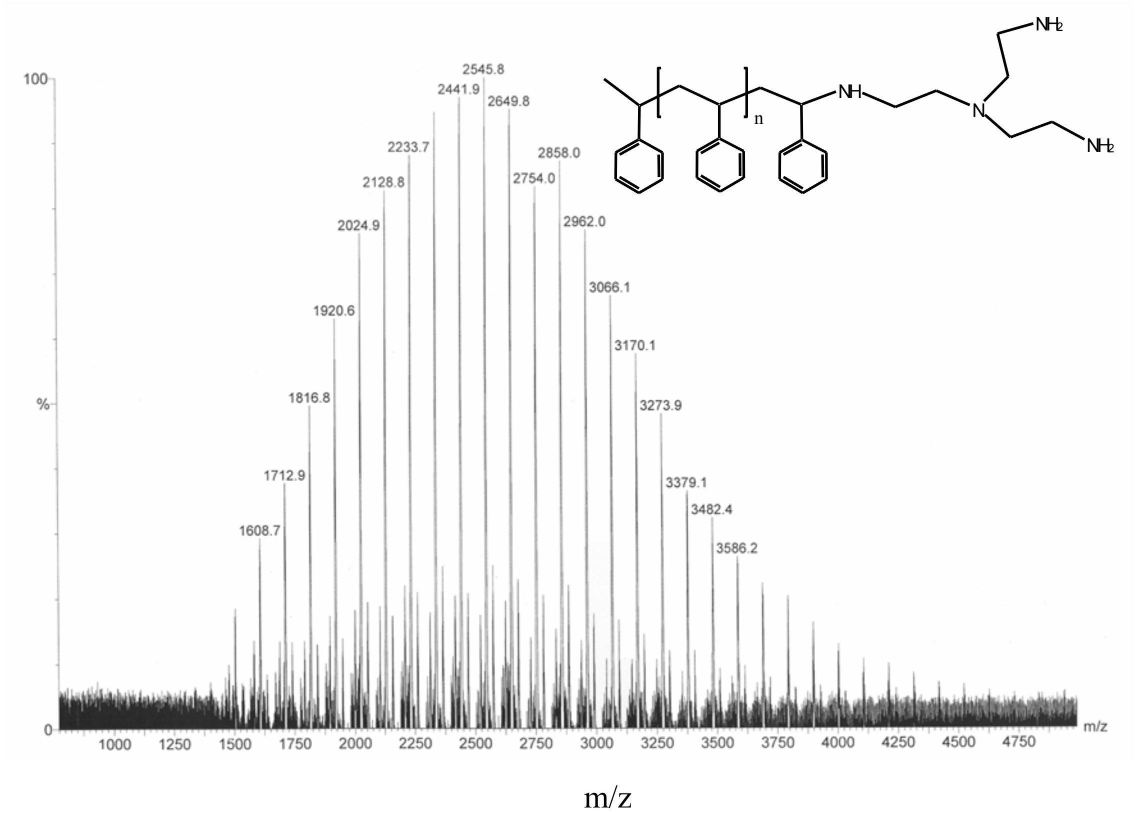

Figure 1. MALDI-TOF mass spectrum of $PS(NH_2)_2$: M_n=2180, M_w/M_n=1.15.

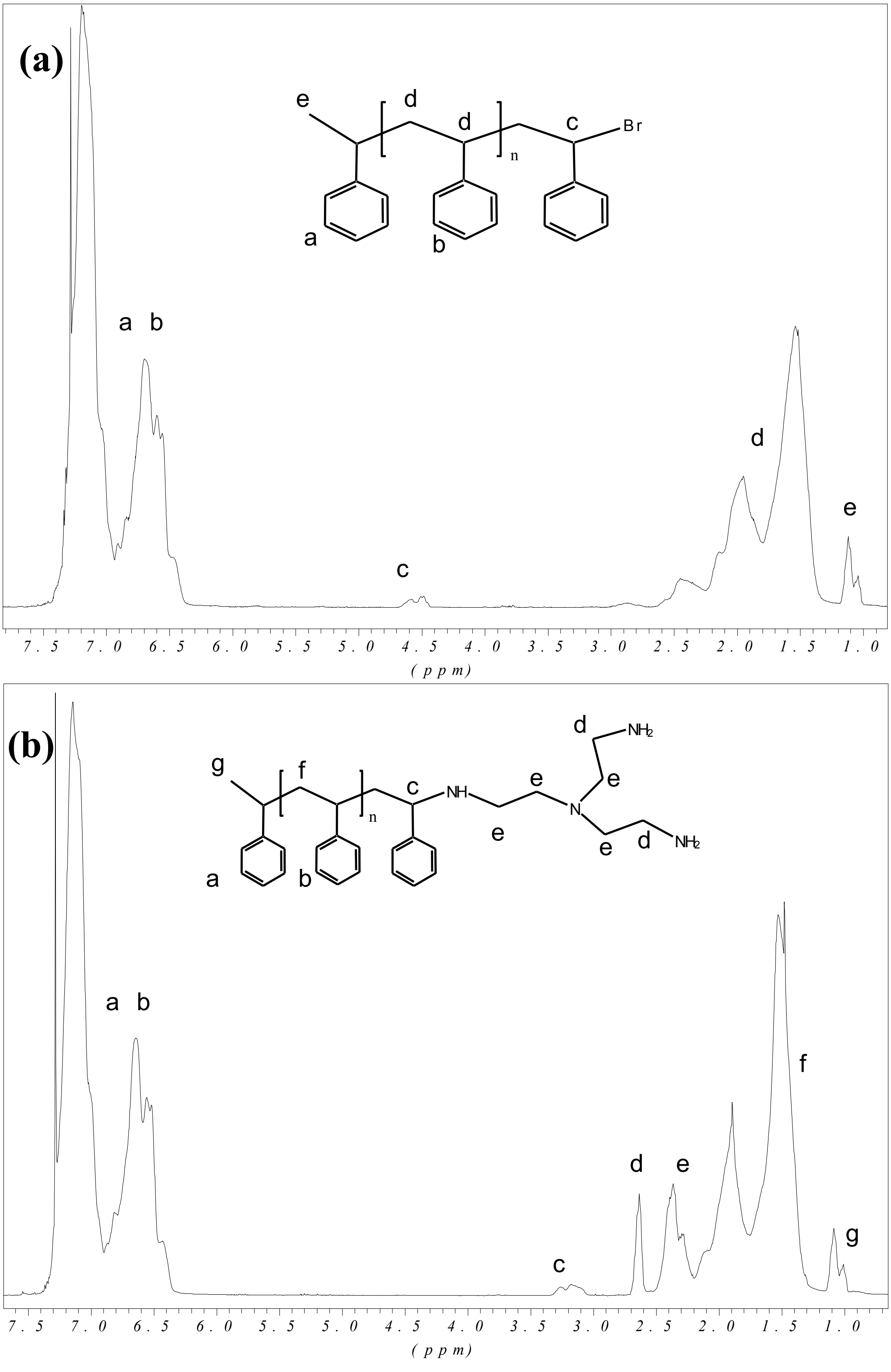

Figure 2. ^1H NMR spectrum of: (a) $PS_{25}(Br)$, (b) $PS_{25}(NH_2)_2$ in $CDCl_3$ (Bruker 400 MHz).

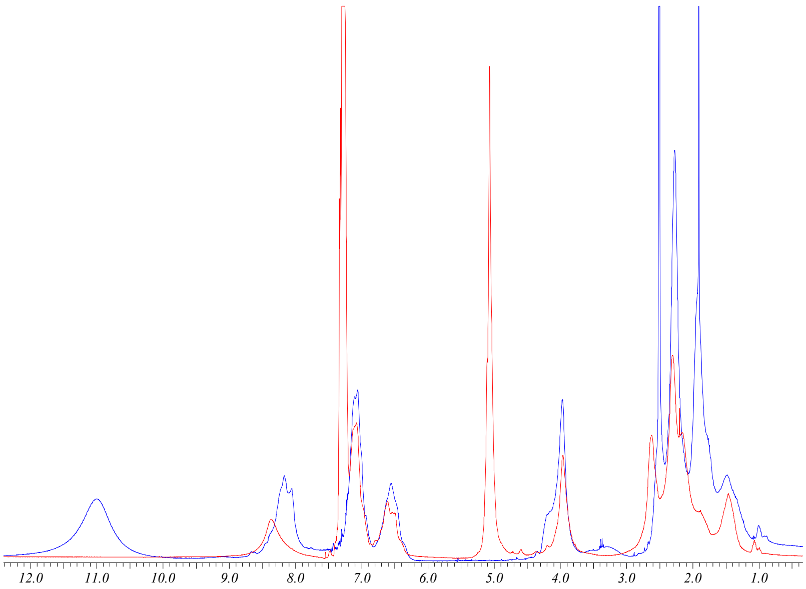

Figure 3. ^{1}H NMR (DMSO-d_6) of PS$_{25}$-b-(PBLG$_{51}$)$_2$ (in red) and PS$_{25}$-b-(PGA$_{51}$)$_2$ (in blue) (Bruker 400 MHz).