Phosgene-free synthesis of non-ionic hydrophilic polyserine

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Supporting Information

Experimental Section

Materials
Diphenyl carbonate (Tokyo Chemical Industry), tertrabutylammonium hydroxide (37 wt% in methanol, Tokyo Chemical Industry), HBr (33 wt% in acetic acid, Acros Organic), D-Ser(tBu) and L-Ser(tBu) (Xishi Chemical Industry, China) were used as received. PEG$_{22}$-NH$_2$ ($M_n = 1000$) was purchased from Sigma-Aldrich and vacuum-dried at 60 °C for 12 h before use. N,N-Dimethylacetamide (DMAc) was distilled under reduced pressure over BaO. Polysarcosine (PSar$_{17}$, $M_n = 5300$, $D = 1.11$ as determined by SEC) was synthesized according to our previous work.$^{1}$

Measurements
Molecular weights (MWs) and polydispersity indices ($D$) were determined by size-exclusion chromatography (SEC) in HFIP was performed with 3 g/L K$^+$TFA$^-$ at 40 °C. The columns were packed with modified silica (PFG columns, particle size: 7 μm, porosity: 100 and 1000 Å). A refractive index detector (G 1362A RID, Jasco) was used to detect the polymer. Molecular weights were calculated using a calibration performed with PMMA standards (Polymer Standards Services GmbH). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance DMX 400 spectrometer ($^1$H: 400 MHz) with CDCl$_3$ or DMSO-$d_6$ as solvent and tetramethylsilane (TMS) as internal reference. High performance liquid chromatography (HPLC) was used to analyze the Ser(tBu)-UD obtained by mixing of D- and L-isomers (Chiralpak, AD-H, 1 mL/min, 35 °C, isopropanol/$n$-hexane =
Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF MS) were collected on a Bruker UltraFLEX MALDI-ToF mass spectrometer in the reflector mode. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix and K+TFA− was added as cationic agent. HFIP and water are used as solvents for PSer(tBu) and PSer samples, respectively.

**Synthesis of urethane derivatives of O-tert-butyl-L-Serine (L-Ser(tBu)-UD)**

Tertrabutylammonium hydroxide (37 wt% in methanol) (28.05 g, 40.0 mmol) was slowly added into a suspension of L-Ser(tBu) (6.45 g, 40.0 mmol) in methanol (60 mL) while stirring. After 1 h, the reaction mixture became transparent. The solution was concentrated under reduced pressure. The residue was dissolved in acetonitrile (40 mL) and added to a solution of diphenyl carbonate (8.57 g, 40.0 mmol) in acetonitrile (40 mL) dropwise. The reaction was allowed for 2 h. After concentrated, 80 mL of distilled water and HCl aqueous solution (1 mol/L) was added until pH reached 2-3. The mixture was extracted by ethyl acetate for 3 times. The combined organic phase was dried over Na2SO4, filtered, and concentrated under reduced pressure. The crude products were purified by column chromatography (a gradient eluent of 10-50% ethyl acetate in petroleum ether), and then recrystallization from ethyl acetate/n-hexane. Yield: 6.86g (61%). 1H NMR (400 MHz, CDCl3, δ in ppm): 1.21 (s, 9H), 3.66-3.69 (m, 1H), 3.93-3.96 (m, 1H), 4.52-4.55 (m, 1H), 5.94 (d, 1H), 7.14-7.16 (d, 2H), 7.19-7.22 (t, 1H), 7.34-7.38 (t, 2H).

D-Ser(tBu)-UD was synthesized in the same way with the yield of 59%. 1H NMR(400 MHz, CDCl3, δ in ppm): 1.21 (s, 9H), 3.66-3.69 (m, 1H), 3.93-3.96 (m, 1H), 4.52-4.55 (m, 1H), 5.94 (d, 1H), 7.14-7.16 (d, 2H), 7.19-7.22 (t, 1H), 7.34-7.38 (t, 2H).

Ser(tBu)-UD was obtained as a 50/50 mixture of L-Ser(tBu)-UD and D-Ser(tBu)-UD and was confirmed to be racemic by HPLC with chiral column.
Scheme S1. Synthesis of Ser(tBu)-UD.

Figure S1. (A) $^1$H NMR spectra of L-Ser(tBu)-UD, D-Ser(tBu)-UD and Ser(tBu)-UD. (B) $^{13}$C NMR spectrum of L-Ser(tBu)-UD.
Figure S2. HPLC spectra of L-Ser(tBu)-UD, D-Ser(tBu)-UD and Ser(tBu)-UD.
Figure S3. The aromatic protons of PSer(tBu) in $^1$H NMR as a zoomed-in picture of Figure 2A.
Polymerization of Ser(tBu)-UD
As a typical procedure, Ser(tBu)-UD (281 mg, 1 mmol) was dissolved in 1 mL of anhydrous DMAc in a flame-dried Schlenk tube, followed by the addition of a benzyl amine solution in DMAc (0.2 mL, 0.1 mol/L). The tube was placed in an 80 °C oil bath for 48 h. The reaction mixture was cooled to room temperature, and precipitated from diethyl ether. Polymers were isolated by filtered and vacuum dried (0.12 g, yield = 84%).

Deprotection of poly-DL-Ser(tBu)
A typical procedure is as follows. Poly-DL-serine(tBu) (0.1 g) was dissolved in CHCl₃ (5 mL) and a HBr solution in acetic acid (0.5 mL, 33 wt%) was added slowly. The reaction mixture was stirred for 3 h, and precipitated from diethyl ether. Polyserines were isolated by filtered and then dried under vacuum (0.056 g, yield = 92%).

Cytotoxicity assay
All polymers were dissolved in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPEs) buffer and diluted to appropriate concentrations, ranged from 0.05 mg/mL to 0.5 mg/mL, in DMEM culture medium. Human hepatoblastoma cells (HepG2) were seeded in 96-well plates with an initial density of 10 000 cells per well and cultured overnight to 80% cell confluence. The medium was replaced with polymer containing one and the cells were further incubated for 24 h and 72 h, respectively. Cell viability was tested with methyl thiazolyl tetrazolium (MTT) assay. Briefly, the medium was replaced by a free one containing 0.5 mg/mL MTT and the cells were incubated for 4 h. Then, the medium was removed and 200 μL dimethylsulfoxide (DMSO) was added to dissolve the generated formazan. The plates were shaken for 15 min before the detection with a microplate reader (550, Bio-Rad, USA) at 570 nm. A total of 5 replicates were conducted for each sample.

References