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# 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(*t*Bu) and L-Ser(*t*Bu) (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 distillated under reduced pressure over BaO. Polysarcosine ($PSar_{17}$, $M_n$ = 5300, $Đ$ = 1.11 as determined by SEC) was synthesized according to our previous work.[1]

### Measurements

Molecular weights (MWs) and polydispersity indices (Đ) 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(*t*Bu)-UD obtained by mixing of D- and L-isomers (Chiralpak, AD-H, 1 mL/min, 35 °C, isopropanol/*n*-hexane =

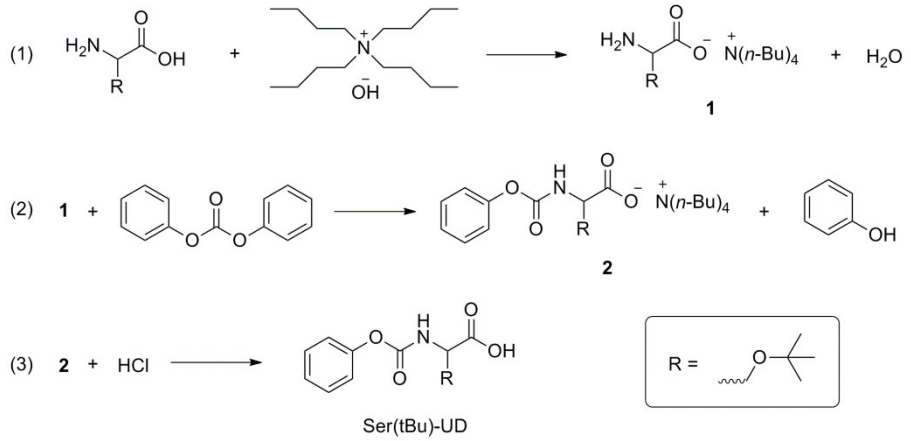
10/90). Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF MS) were collected on a Bruker UltraFLEX MALDI-ToF mass spectrometer in the reflector mode. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix and $K^+TFA^-$ was added as cationic agent. HFIP and water are used as solvents for PSer(*t*Bu) and PSer samples, respectively.

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

Tertrabutylammonium hydroxide (37 wt% in methanol) (28.05 g, 40.0 mmol) was slowly added into a suspension of L-Ser(*t*Bu) (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 $Na_2SO_4$, 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%). $^1$H NMR (400 MHz, $CDCl_3$, $\delta$ 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(*t*Bu)-UD was synthesized in the same way with the yield of 59%. $^1$H NMR(400 MHz, $CDCl_3$, $\delta$ 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(*t*Bu)-UD was obtained as a 50/50 mixture of L-Ser(*t*Bu)-UD and D-Ser(*t*Bu)-UD and was confirmed to be racemic by HPLC with chiral column.




**Scheme S1.** Synthesis of Ser(*t*Bu)-UD.

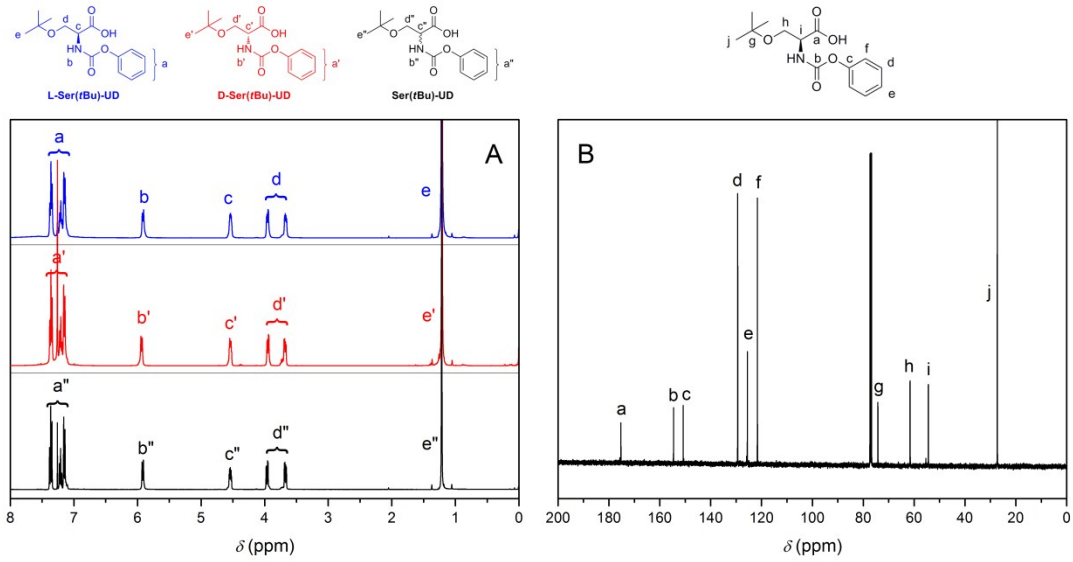



**Figure S1.** (A) $^{1}$H NMR spectra of L-Ser(*t*Bu)-UD, D-Ser(*t*Bu)-UD and Ser(*t*Bu)-UD. (B) $^{13}$C NMR spectrum of L-Ser(*t*Bu)-UD.

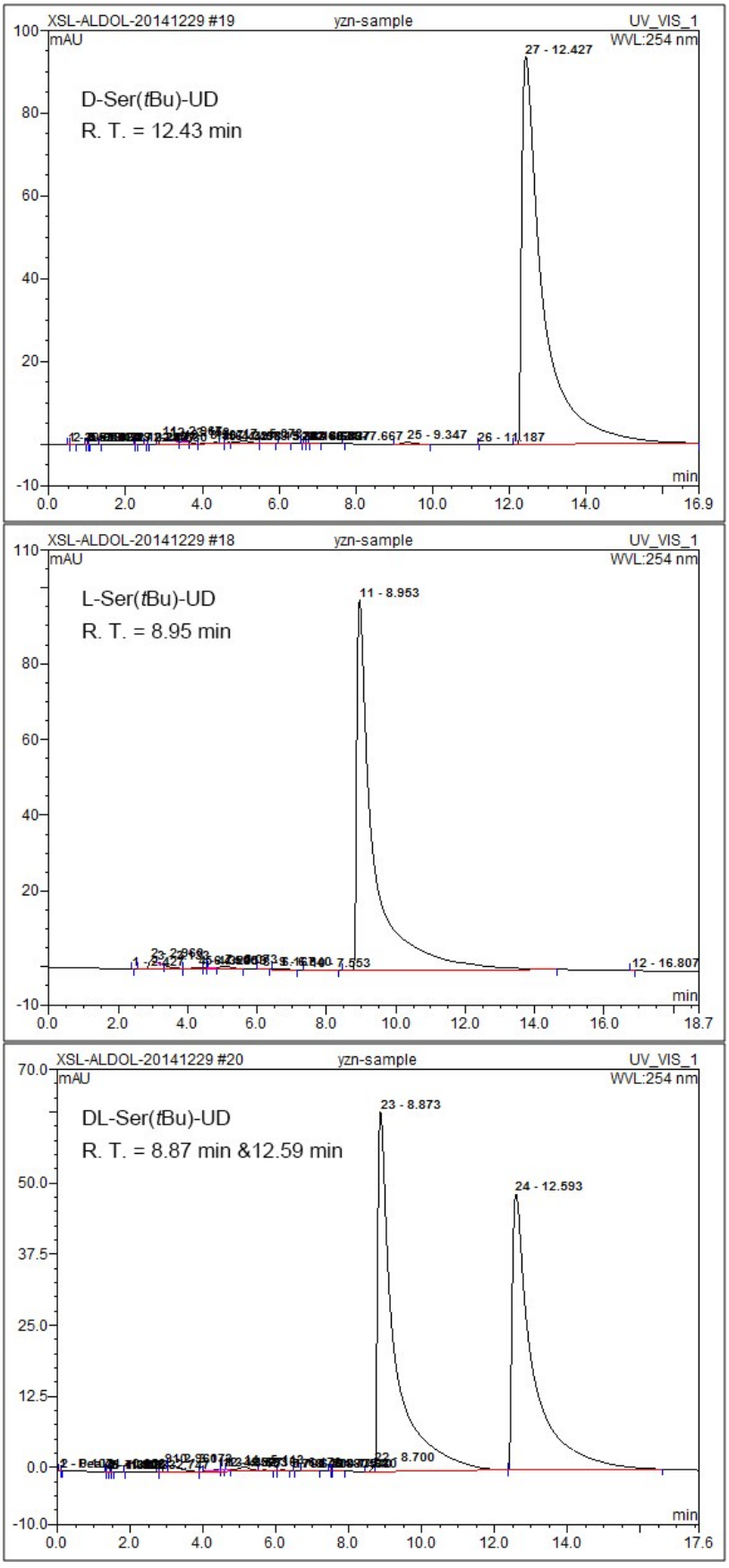



**Figure S2.** HPLC spectra of L-Ser(*t*Bu)-UD, D-Ser(*t*Bu)-UD and Ser(*t*Bu)-UD.

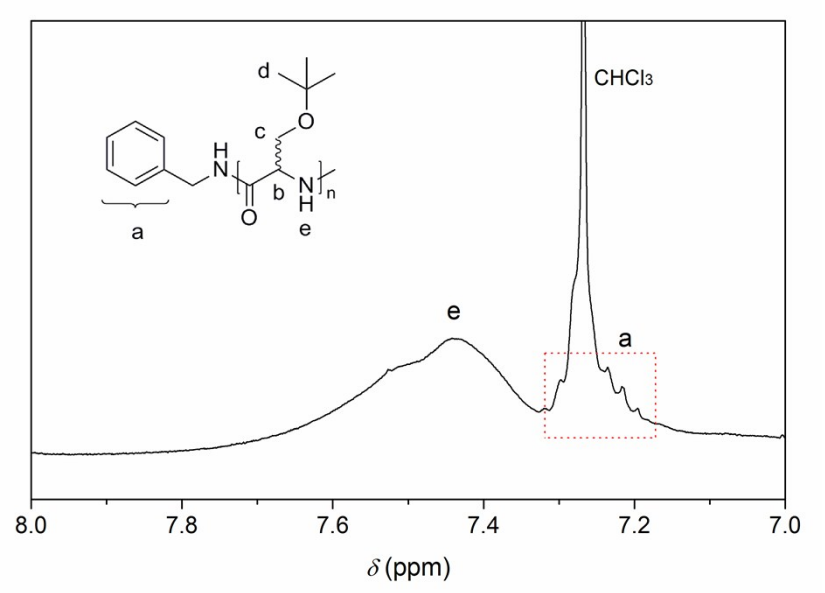



**Figure S3.** The aromatic protons of PSer(*t*Bu) in $^{1}$H NMR as a zoomed-in picture of Figure 2A.

**Polymerization of Ser(*t*Bu)-UD**

As a typical procedure, Ser(*t*Bu)-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(*t*Bu)**

A typical procedure is as follows. Poly-DL-serine(*t*Bu) (0.1 g) was dissolved in $CHCl_3$ (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**

(1) Tao, X.; Deng, C.; Ling, J. *Macromol. Rapid Comm.* **2014**, *35*, 875-881.