Supporting information for:

Synthesis of Heterobifunctional Poly(ethylene glycol)s by an Acetal Protection Method

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Materials and Methods

Materials

All starting compounds were used as received without additional purification except for those specified. Chemicals were purchased from Aldrich unless otherwise indicated. Tetrahydrofuran (THF) (Merck, 99%) was refluxed over sodium wire and distilled from sodium naphthalenide solution. Dimethyl sulfoxide (DMSO) (Merck, 98%) was distilled over CaH_2 under reduced pressure just before use. Ethylene oxide (EO, 99.7%) was purchased from Hong Kong Special Gas Company and used directly. Diphenylmethylpotassium (DPMK) was prepared as described elsewhere (*1*).

Analytical Methods

^{1}H NMR spectra were obtained on a DMX 500 MHz spectrometer with tetramethylsilane (TMS) as the internal standard and $CDCl_3$ as the solvent. Size exclusion chromatography (SEC) was performed in 0.1 M $NaNO_3$ at 40°C with an elution rate of 0.5 mL/min on a Waters HPLC system with a G1310A pump and a G1362A refractive index (RI) detector. Ultrahydrogel 250 (Waters) and Ultrahydrogel 1000 (Waters) columns in series were calibrated by polyethylene glycol standard (Polymer Source, Inc., Canada). MALDI-TOF MS spectra were recorded using Bruker REFLEX III. R-Cyano-4-hydroxycinnamic acid (CHCA) was used as the matrix for the ionization operated in the reflection mode. Elemental analysis was recorded on an Elementar (*Vario EL-III*, Germany).

Experimental Part

Synthesis of 2-(1-ethoxyethoxy) ethanol (EEE) (1)

Scheme 1 shows the synthesis procedure of EEE, which is adapted from Fitton's report (*2*). To a magnetically stirred solution of ethylene glycol (12.4 g, 0.20 mol) and ethyl vinyl ether (23 mL, 0.24 mol) in ethyl ether (150 mL) kept at 0 °C in icy water bath, *p*-toluenesulfonic acid (TsOH) (0.6 g) was added in aliquots. The mixture was stirred for 6 h at the same temperature before saturated $NaHCO_3$ solution (100mL) was added. The organic layer was separated, dried and evaporated under reduced pressure. A colorless liquid (**1**) was obtained after purification by column chromatography (silicon oxide as stationary phase and ethyl ether/hexane = 5/2 as eluent) and distillation (14.7 g , yield=55%). ^1H NMR (ppm) ($CDCl_3$): 1.17 (m, $C\mathbf{H_3}CH_2O$-), 1.30 (d, -O-$CH(C\mathbf{H_3})$-O-), 3.44 (m, $CH_3C\mathbf{H_2}O$-), 3.61(m, -$C\mathbf{H_2}CH_2OH$), 3.79 (m, -$C\mathbf{H_2}CH_2OH$), 4.68 (m, -O-$C\mathbf{H}(CH_3)$-O-) (Fig. 1). Elemental analysis: calculated: C 53.71, H 10.52; found: C 53.65 H 10.56.

Synthesis of ethoxyethoxy-polyethylene glycol-OH (EE-PEG-OH) (3)

A 150 mL stainless steel kettle was vacuumed at 80 °C for 24 h and cooled to room temperature and then to -20 °C. Anhydrous 2-(1-ethoxyethoxy)ethanol (EEE) (1.34 g, 0.01mol) was dissolved in 40 mL of mixed solvents of DMSO and THF (v/v: 3/2). A solution of DPMK in THF (6.7 mL, 0.6 M solution) was slowly added. The orange-red color of DPMK was changed to yellow when alkoxide was formed. The homogeneous initiator solution obtained was introduced into the cooled kettle by a syringe, followed by the addition of EO (40 g, 0.91 mol). After the solution was stirred at 50 °C for 24 h, polymerization was terminated by adding of a few drops of acidified methanol

(0.1 N HCl in methanol). All the solvents were removed by reduced distillation. The crude product was dissolved in CH_2Cl_2, filtered, and dried over anhydrous $MgSO_4$, then precipitated in diethyl ether. EE-PEG-OH was obtained as a white powder (yield= 98-99%).

Synthesis of ethoxyethoxy-PEG-alkyne (EE-PEG-alkyne) (4)

Synthesis of EE-PEG-alkyne was according to a previously reported method (*3*) (Scheme 2). Two grams of EE-PEG-OH ($M_n = 3.81 \times 10^3$ g/mol, 0.52 mmol) with moisture removed by azeotropic distillation with toluene just before use, was mixed with 0.15 g of NaH (0.63 mmol) in 10 mL of anhydrous THF under nitrogen atmosphere at room temperature for 1 h. Then 0.070 g of propargyl bromide (0.59 mmol) was added at room temperature and the reaction was continued for 24 h. After neutralization by 0.1 N HCl, the solvent was removed by rotary evaporation. The crude product was dissolved in water and extracted by CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The polymer was precipitated from an excess volume of diethyl ether twice to afford a white powder (1.90 g, yield = 95%). M_n (^{1}H NMR) = 3.86×10^3 g/mol; SEC: $M_n = 3.83 \times 10^3$ g/mol, M_w/M_n=1.09.

Synthesis of HO-PEG-alkyne (5)

HO-PEG-alkyne was prepared by the hydrolysis of ethoxyethyl group on EE-PEG-alkyne using the method adapted from our previous report (*1*). One gram of EE-PEG-alkyne ($M_n = 3.86 \times 10^3$ g/mol, 0.26 mmol) was mixed with 20 mL of formic acid. The solution was stirred at room temperature for 2 h and formic acid was removed by evaporation. After the residue was dissolved in a mixture of dioxane (20 mL) and methanol (10mL), KOH (1 N) aqueous solution was added until the pH reached 12.0. The mixture was refluxed at 65 ^oC for 24 h and neutralized with 1N HCl aqueous solution. After the solvents were removed under reduced pressure, the residue was dissolved in CH_2Cl_2, dried over anhydrous $MgSO_4$, filtered, and concentrated. The polymer was precipitated from an excess volume of diethyl ether twice to afford a white powder (0.92 g, yield = 92%). M_n (^{1}H NMR) = 3.79×10^3 g/mol; SEC: $M_n = 3.82 \times 10^3$ g/mol, M_w/M_n=1.09.

Synthesis of HS-PEG-alkyne (6)

HS-PEG-alkyne was synthesized by the esterification (*3, 4*) between HO-PEG-alkyne and 3-

mercaptopropionic acid. Half gram of HO-PEG-alkyne (M_n = 3.79 × 10^3 g/mol, 0.13 mmol) was dissolved in 10 mL toluene, then 0.016 g 3-mercaptopropionic acid (MPA) (0.15 mmol) along with $HfCl_4 \cdot 2THF$ (1 mg, 0.002 mmol) were added and stirred at 50 ^oC under nitrogen. The reaction flask was equipped with an azeotropic distillation apparatus and the mixture was refluxed at 110 ^oC under nitrogen for 24 h. Toluene was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2, dried over anhydrous $MgSO_4$, filtered, and concentrated. The polymer was precipitated from an excess volume of diethyl ether twice to afford a white powder (0.44 g, yield = 88%). M_n (^{1}H NMR) = 3.91× 10^3 g/mol; SEC: M_n= 3.86 × 10^3 g/mol, M_w/M_n=1.09.

Reference

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(2) A. O. Fitton, J. Hill, D. E. Jane, and R. Millar, *Synthesi*s, 1987, 1140-1142.

(3) Z. Y. Li and Y. Chau, *Bioconjugate Chem.*, 2009, **20**, 780-789.

(4) D. C. Wan, H. T. Pu, and G. J. Yang, *React. Funct. Polym.,* 2008, ***68***, 431-435.