Homopolymer Bifunctionalization through Sequential Thiol-Epoxy and Esterification Reactions: An Optimization, Quantification, and Structural Elucidation Study

Ikhlas Gadwal, Mihaiela C. Stuparu, Anzar Khan*

*Email: anzar.khan@mat.ethz.ch

General methods and materials

Glycidyl methacrylate, 4,4'-dinonyl-2,2'-dipyridyl, Cu(I)Br, 2-(Boc-amino)ethanethiol, 1-thionaphthalene, hexane thiol, lithium hydroxide monohydrate (LiOH), tetrabutylammonium fluoride (TBAF), triethylamine (TEA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 4-dimethylaminopyridine (DMAP), acetic acid, hexanoyl chloride, trifluoroacetic acid (TFA), dimethyl bromomalonate, 3-bromopropane-1,2-diol, lithium aluminum hydride (LAH) and solvents were purchased from commercial sources and used without further purification. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on AV300 and AV700 (^{1}H: 300 MHz; ^{13}C: 75 MHz) spectrometer, using $CDCl_3$, DMSO-d_6, D_2O as solvents. GPC measurements (against polystyrene standards) were carried out using a PL-GPC 220 instrument with a 2x PL-Gel Mix-B LS column set (1% LiBr in DMF as eluent at 45 °C). Infrared spectroscopic analyses were carried out using attenuated total reflection (ATR)-Fourier Transform infrared (FTIR) spectrometer with OPUS 6 as software (Bruker Optics Alpha system with a built-in diamond ATR).

Synthesis of **3**: ATRP initiator **2** (*Biomacromolecules*, **2014**, 15, 1707) (0.470 g, 1.495 mmol), glycidyl methacrylate **1** (12.75 g, 89.74 mmol), 4,4'-dinonyl-2,2'-bipyridine (dNbpy) (0.55 g, 1.34 mmol), and anisole (22 mL) were taken in a schlenk tube and degassed by two freez-pump-thaw cycles. Cu(I)Br (0.128 g, 0.897 mmol) was added and the resulting mixture was degassed one more time. The polymerization mixture was then stirred under nitrogen at room temperature for 2 h. After this time, the reaction vessel was opened to air and the polymerization mixture was precipitated into 400 mL of isopropanol, filtered, and passed through a small plug of silica gel using DCM as an eluent. The organic solvent was reduced under low pressure and the crude material obtained was precipitated again into isopropanol to give 3.6 g of the product as a white powder. (GPC): M_n = 17600; M_w = 22900; PDI = 1.3; ^{1}H-NMR (δ, ppm, 300 MHz, $CDCl_3$): 7.21 (t, 2H, end group), 6.71 (m, 3H, end group), 4.29 (br s, 1H, COO**CH_2**), 3.81 (br s, 1H, COO**CH_2**), 3.54 (t, 2H, end group), 3.41 (q, 2H, end group), 3.23 (br s, 1H, CH_2**C**HCH_2O), 2.84 (br s, 1H, $COOCH_2CH$**CH_2**O), 2.65 (br s, 1H,

$COOCH_2CH\mathbf{CH_2}O$), 2.05-1.94 (br m, 2H,$\mathbf{CH_2}CCH_3$), 1.08-0.94 (br m, 3H, $C\mathbf{CH_3}$); IR (cm^{-1}): 3002, 2934, 1720, 1484, 1443, 1383, 1256, 1138, 988, 901, 833, 751.

First functionalization through thiol-epoxy reaction:

Using TBAF (as exemplified in Entry 30, Table 2 in the main manuscript): To a solution of polymer **3** (70 mg, 0.492 mmol of epoxide units) and 2-(Boc-amino)ethanethiol **4** (109 mg, 0.615 mmol) in THF (2.0 mL) was added TBAF (40.78 mg, 0.129 mmol) at 0 °C. The cooling was removed and the reaction mixture was stirred at room temperature for 3 h. After this time, the reaction mixture was diluted with DCM and washed with water. The organic layer was dried over sodium sulfate, reduced under low pressure, and precipitated into diethyl ether to give 134 mg of polymer **5**.

Using LiOH (as exemplified in Entry 24, Table 2 in the main manuscript): To a solution of polymer **3** (70 mg, 0.492 mmol of epoxide units) and 2-(Boc-amino)ethanethiol **4** (109 mg, 0.615 mmol) in THF (1.82 mL) was added LiOH (1.8 mg, 0.043 mmol) in (0.18 mL) water at 0 °C. The cooling was then removed and the reaction mixture was stirred at room temperature for 3 h. After this time, the reaction mixture was diluted with DCM and washed with water. The organic layer was dried over sodium sulfate, reduced under low pressure, and precipitated into diethyl ether to give 131 mg of polymer **5**. (GPC): M_n = 23500; M_w = 32200; PDI = 1.3; ^{1}H-NMR (δ, ppm, 300 MHz, $CDCl_3$): 7.23 (t, 2H, end group), 6.70 (m, 3H, end group), 5.4 (br s, 1H, **NH**), 4.35-3.79 (br m, 3H, $O\mathbf{CH_2CH}(OH)CH_2S$), 3.34 (br s, 2H, $NH\mathbf{CH_2}CH_2S$), 2.72 (br s, 4H, $CH(OH)\mathbf{CH_2}S + NHCH_2\mathbf{CH_2}S$), 1.87-1.85 (br s, 2H, $\mathbf{CH_2}CCH_3$), 1.45 (br s, 9H, $C(\mathbf{CH_3})_3$), 1.08-0.95 (br s, 3H, $C\mathbf{CH_3}$); IR (cm^{-1}): 3362, 2975, 2930, 1688, 1511, 1452, 1397, 1365, 1252, 1151, 922, 947, 865, 788, 742.

Using TEA (as exemplified in Entry 4, Table 3 in the main manuscript): To a solution of polymer **3** (70 mg, 0.492 mmol of epoxide moiety) and 1-thionaphthol **6** (98.6 mg, 0.615 mmol) in THF (2.0 mL) was added TEA (26.1 mg, 0.258 mmol) at 0 °C. The cooling was removed and the reaction mixture was stirred at room temperature for 12 h. After this time the reaction mixture was diluted with DCM and washed with water. The organic layer was dried over sodium sulfate, concentrated, and precipitated into diethyl ether to give 140 mg of polymer **7**. (GPC): M_n = 12000; M_w = 19400; PDI = 1.6; ^{1}H-NMR (δ, ppm, 300 MHz, $CDCl_3$): 8.31 (br, 1H, ArH), 7.80-7.31 (br m, 6H, ArH`s), 6.65 (m, 3H, end group), 4.17-3.41 (br m, 4H, $O\mathbf{CH_2CH}(\mathbf{OH})CH_2S$), 2.97 (br s, 2H, $CH(OH)\mathbf{CH_2}S$), 2.08-0.64 (br m, 5H, $\mathbf{CH_2CCH_3}$).

Using TEA in DMSO (as exemplified in Entry 6, Table 4 in the main manuscript): To a solution of polymer **3** (30 mg, 0.211 mmol of epoxide units) and 2-(Boc-amino)ethanethiol **4** (65.4 mg, 0.369 mmol) in DMSO (0.85 mL) was added TEA (93.4 mg, 0.923 mmol) and the reaction mixture was stirred at 50 °C for 12 h. After this time, the reaction mixture was cooled to room temperature, diluted with DCM (15 mL), and washed with water (2×25 mL). The organic layer was dried over sodium sulfate, concentrated under low pressure, and precipitated into diethyl ether to give 50 mg of polymer.

Second functionalization through esterification reaction: To a solution of polymer **5** (30.0 mg, 0.0939 mmol of hydroxyl group), TEA (38.0 mg, 0.375 mmol), and DMAP (1.14 mg, 0.0093 mmol) in THF (1.42 mL) was added hexanoyl chloride **8** (25.2 mg, 0.187 mmol) at 0 °C and the resulting reaction mixture was stirred at room temperature for 12 h. After this time, the THF was removed under low pressure; the crude material was dissolved in DCM (20 mL) and then washed with saturated solution of $NaHCO_3$ followed by washing with water. The organic layer was dried over sodium sulfate, concentrated, and precipitated into hexane to give 37 mg of polymer. (GPC): M_n = 22000; M_w = 30800; PDI = 1.40; ^{1}H-NMR (δ, ppm, 300 MHz, $CDCl_3$): 7.22 (m, end group), 6.70 (m, end group), 5.30 (br s, 1H, **NH**), 5.15 (br s, 1H, $OCH_2\mathbf{C}\mathbf{H}CH_2S$), 4.33-3.91 (br, 2H, $O\mathbf{C}\mathbf{H_2}CHCH_2S$), 3.32 (br s, 2H, $NH\mathbf{C}\mathbf{H_2}CH_2S$), 2.72 (br s, 4H, $CH\mathbf{C}\mathbf{H_2}S$ + $NHCH_2\mathbf{C}\mathbf{H_2}S$), 2.34 (br s, 2H, $CHOCO\mathbf{C}\mathbf{H_2}(CH_2)_3CH_3$), 1.87-1.83 (br s, 2H, $\mathbf{C}\mathbf{H_2}CCH_3$), 1.68 (br s, 2H, $CHOCOCH_2\mathbf{C}\mathbf{H_2}(CH_2)_2CH_3$), 1.44 (br s, 13H, $C(\mathbf{C}\mathbf{H_3})_3$) + $CHOCO(CH_2)_2(\mathbf{C}\mathbf{H_2})_2CH_3$), 1.07-0.78 (br s, 6H, $CHOCO(CH_2)_4\mathbf{C}\mathbf{H_3}$ + $CH_2C\mathbf{C}\mathbf{H_3}$); IR ($cm^{-1}$): 3381, 2958, 2930, 2866, 1716, 1501, 1456, 1375, 1247, 1151, 1056, 942, 911, 865, 778, 728.

Synthesis of **11**: To a solution of 3-bromopropane-1,2-diol **10** (1.0 g, 6.45 mmol) and 2-(boc-amino)ethanethiol (2.28 g, 12.9 mmol) in CH_3CN (7.0 mL) was added TEA (2.61 g, 25.8 mmol) and the reaction mixture was stirred at 75 °C for 5.5 h. After this time, the reaction mixture was cooled to room temperature. At this time, TEA salts precipitated out upon cooling. The reaction mixture was diluted with acetone and filtered. Acetone was removed under reduced pressure and the resulting crude was purified by silica gel column chromatography using DCM:MeOH (97:3 to 94:6) as a solvent mixture to give 1.3 g of the product as a colorless viscous oil (yield = 80%). ^{1}H-NMR (δ = ppm, 300 MHz, $CDCl_3$): 4.96 (br s, 1H), 3.86-3.67 (m, 2H), 3.58 (m, 1H), 3.34 (m, 2H), 2.79-2.52 (m, 4H), 1.44 (s, 9H); ^{13}C-NMR (δ = ppm, 75 MHz, $CDCl_3$): 156.29, 79.95, 70.55, 65.41, 40.04, 35.73, 33.13, 28.52; ESI (observed: 274.10 M+Na, calc. for $C_{10}H_{21}NO_4SNa$: 274.10).

Synthesis of **12**: A suspension of tert-butyl(2-((2,3-dihydroxypropyl)thio)ethyl)carbamate (0.15 g, 0.516 mmol), acetic acid (0.143 g, 2.38 mmol), EDCI (0.457 g, 2.38 mmol), and DMAP (0.029 g, 0.238 mmol) was stirred at 0 °C in DMF (3.0 mL) until the suspension became optically clear. Then it was stirred at room temperature for 15 h. After this time, the reaction was diluted with DCM (25 mL) and washed with water (2 x 25 mL), 0.2 M HCl solution (10 mL), followed by saturated solution of $NaHCO_3$ (10 mL). The organic layer was dried over sodium sulfate, concentrated, and purified by silica gel column chromatography using DCM:EtOAc (90:10) solvent mixture to give 0.165 g of the product as a colorless viscous liquid (yield = 80%). ^{1}H-NMR (δ = ppm, 300 MHz, $CDCl_3$): 5.13 (m ,1H), 4.90 (br s, 1H), 4.36 (dd , *J* = 3.59, 12.0 Hz, 1H), 4.17 (dd, *J* = 5.91, 12.0 Hz, 1H), 3.32 (q, *J* = 6.39 Hz, 2H), 2.71 (m, 4H), 2.09 (s, 3H), 2.08 (s, 3H), 1.44 (s, 9H); ^{13}C-NMR (δ = ppm, 75 MHz, $CDCl_3$): 170.74, 170.44, 155.87, 79.67, 70.73, 63.87, 39.81, 33.05, 32.14, 28.52, 21.12, 20.89; ESI (observed: 358.12 M+Na, calc. for $C_{14}H_{25}NO_6SNa$: 358.14).

Synthesis of **14**: To a solution of dimethyl bromomalonate **13** (2.0 g, 9.47 mmol) and hexane-thiol (2.24 g, 18.9 mmol) in CH_3CN (14.0 mL) was added TEA (5.26 mL, 37.9 mmol) and the reaction mixture was heated to 75 °C for 2.5 h. After this time, the reaction mixture was cooled to room temperature. TEA salts precipitated out upon cooling. The reaction mixture was diluted with acetone, filtered, and washed with acetone. The filtrate was concentrated and the crude obtained was dissolved in DCM and washed with water. The organic layer was dried over sodium sulfate, concentrated, and purified by silica gel column chromatography using 5% EtOAc in hexane to give 0.24 g of the product as a colorless liquid (yield = 10%). ^{1}H-NMR (δ = ppm, 300 MHz, $CDCl_3$): 4.19 (s, 1H), 3.79 (s, 6H), 2.72 (t, 2H), 1.57 (br, 2H), 1.44-1.20 (br m, 6H), 0.88 (br, 3H); ESI (observed: 249.11 M+H, calc. for $C_{11}H_{21}NO_4S$: 249.11).

Synthesis of **15**: To a solution of dimethyl 2-(hexylthio)malonate **14** (0.22 g, 0.885 mmol) in dry THF (1.0 mL) was added drop wise a 1M solution of LAH (1.85 mL, 1.86 mmol) at 0 °C and reaction was stirred for 1 h and then heated to 40 °C for 23 h. The reaction mixture was cooled using an ice bath and then quenched by adding water in a drop wise manner. The reaction mixture was diluted with EtOAc and stirred for 1 h and then passed through a plug of celite. The filtrate was dried over sodium sulfate, concentrated, and then purified by silica gel column chromatography using 2% MeOH in DCM to give 35 mg of colorless liquid (yield = 20%). ^{1}H-NMR (δ = ppm, 300 MHz, $CDCl_3$): 3.76 (m, 4H), 2.94 (quint, *J* = 5.99 Hz, 1H),

2.56 (t, *J* = 7.28 Hz, 2H), 2.24 (t, *J* = 6.16 Hz, 2H), 1.60 (m, 2H), 1.44-1.22 (m, 6H), 0.89 (t, *J* = 6.78 Hz, 3H); EI (observed: 192.11 M^+, calc. for $C_9H_{20}O_2S$: 192.12).

Synthesis of **16**: A suspension of 2-(hexylthio)propane-1,3-diol **15** (30 mg, 0.155 mmol), acetic acid (37 mg, 0.623 mmol), EDC (119 mg, 0.623 mmol), and DMAP (7.6 mg, 0.0623 mmol) were stirred in DMF (0.6 ml) for 15 h. The reaction mixture was diluted with DCM (20 mL) and washed with water (2 x 20 mL), 0.2 M HCl solution (10 mL), and saturated $NaHCO_3$ solution (10 mL). The organic layer was dried over sodium sulfate and concentrated to give 40 mg of the product as a colorless liquid (yield = 92%). ^{1}H-NMR (δ = ppm, 300 MHz, $CDCl_3$): 4.29 (dd, *J* = 5.34, 11.42 Hz, 2H), 4.17 (dd, *J* = 6.89, 11.42 Hz, 2H), 3.07 (quint, *J* = 6.15 Hz, 1H), 2.61 (t, *J* = 7.34 Hz, 2H, 2.08 (s, 6H), 1.59 (quint, *J* = 7.79 Hz, 2H), 1.44-1.22 (m, 6H), 0.88 (t, *J* = 6.69 Hz, 3H); ESI (observed: 276.14 M+H, calc. for $C_{13}H_{25}O_4S$: 276.14).

Synthesis of polymer **17**: To a solution of polymer **9** (53 mg) in DCM (1.0 mL) was added TFA (0.5 mL) drop wise at 0 °C. The reaction mixture was stirred for 1h and then precipitated into diethyl ether (50 mL). The precipitated solid was isolated by filtration, washed, dissolved in water, and lyophilized to give 35 mg of the cationic polymer **17** as a white solid (yield = 87%). ^{1}H-NMR (δ = ppm, 300 MHz, DMSO-d_6): 8.21 (br s, 3H, NH_3^+), 7.21 (m, end group), 6.80- 6.58 (m, end group), 5.19 (br s, 1H, OCH_2**C**$\mathbf{H}$$CH_2S$), 4.39-3.81 (br, 2H, O**C**$\mathbf{H_2}$$CHCH_2S$), 3.08 (br s, 2H, NH_3**C**$\mathbf{H_2}$$CH_2S$), 2.83 (br s, 4H, CH**C**$\mathbf{H_2}$S + NH_3CH_2**C**$\mathbf{H_2}$S), 2.38 (br s, 2H, CHOCO**C**$\mathbf{H}_2(CH_2)_3CH_3$), 1.99-0.55 (br m, 14H, backbone + aliphatic chain)); IR (cm^{-1}): 2944, 2857, 1725, 1670, 1520, 1460, 1419, 1375, 1138, 960, 892, 837, 801, 751, 715.