Supplemental Material

Methylated Mono and Diethyleneglycol Functionalized Polylysines: Non-ionic, α-Helical, Water Soluble Polypeptides

Miaoer Yu, Andrew P. Nowak and Timothy J. Deming*

Departments of Materials and Chemistry

University of California, Santa Barbara

Santa Barbara, CA 93106

Darrin J. Pochan

Department of Materials Science and Engineering

University of Delaware

Newark, DE 19716

Experimental

General Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on a SSI Accuflow Series III liquid chromatograph pump equipped with a Wyatt DAWN DSP light scattering detector and Wyatt Optilab DSP. Separations were effected by 10^5Å, 10^4Å and 10^3Å Phenomenex 5μm columns using 0.10 M LiBr in DMF as eluent at 60 °C. MALDI mass spectra were collected using a Thermo BioAnalysis DYNAMO mass spectrometer running in positive ion mode with samples prepared by

mixing solutions of analyte in THF with solutions of 6-aza-2-thiothymine in THF and allowing the mixtures to dry under N_2. NMR spectra were recorded on a Bruker AVANCE 200 MHz spectrometer. Circular dichroism spectra were recorded on an OLIS RSM CD spectrophotometer running in conventional scanning mode. X-ray scattering experiments were performed at beamline X10 at the National Synchrotron Light Source, Brookhaven National Laboratory in Upton, NY. All IR samples were prepared as thin films on NaCl plates and spectra were recorded on a Perkin Elmer model 1605 FTIR. Chemicals were obtained from commercial sources and used without further purification unless otherwise stated. Deionized water (18 MΩ-cm) was obtaining by passing in-house deionized water through a Barnstead E-pure purification system. Hexanes and THF were purified by first purging with dry nitrogen, followed by passage through columns of activated alumina.[1] γ-Benzyl-L-glutamate-N-carboxyanhydride (Glu NCA)[2], ε-Z-L-lysine-N-carboxyanhydride (Lys NCA)[3], $Co(PMe_3)_4$[4], and poly(hydroxybutyl-L-glutamine) (PHBG; $\overline{M}_n$ = 105,000, $\overline{M}_w/\overline{M}_n$ = 1.45)[5] were prepared according to literature procedures.

N-Hydroxysuccinimidyl 2-[2-(2-Methoxyethoxy)ethoxy]acetate A mixture of 2-[2-(2-methoxyethoxy)ethoxy]acetic acid (10 g, 58 mmol) and N-hydroxysuccinimide (7.5 g, 64 mmol) dissolved in THF (ca. 300 mL) in a round bottom flask was cooled using an ice water bath. Dicyclohexylcarbodiimide (12 g, 58 mmol) was then added with stirring. A white precipitate was observed to form after 5 min and the reaction mixture was then let stand in a refrigerator (4 °C) for 16 h. The white precipitate, dicyclohexylurea, was removed by filtration and the filtrate was concentrated under vacuum to give an oil. This crude product was then dissolved in a small amount of

THF (ca. 10 mL) and the resulting suspension was filtered to remove the precipitate. This procedure was repeated until a clear solution was obtained upon dissolution in THF. Removal of the residual THF under vacuum gave the product as an oil (9.0 g, 59%). ^{1}H NMR($CDCl_3$): δ 4.49 (s, -OC(O)C$\underline{H}_2$O-, 2H), 3.77 (m, -OC(O)CH_2OC$\underline{H}_2$$CH_2$O-, 2H), 3.65 (m, -O$CH_2C\underline{H}_2OC\underline{H}_2$$CH_2$-, 4H), 3.52 (m, -O$CH_2C\underline{H}_2OCH_3$, 2H), 3.34 (s, -$CH_2OC\underline{H}_3$, 3H), 2.82 (s, -C(O)C$\underline{H}_2C\underline{H}_2$C(O)-, 4H).

N_ε-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-N_α-Z-L-Lysine To a mixture of N_α-Z-L-lysine (4.9 g, 17 mmol) and $NaHCO_3$ (2.0 g, 23 mmol) in THF:H_2O (75 mL:75 mL) was added N-hydroxysuccinimidyl 2-[2-(2-methoxyethoxy)ethoxy]acetate (3.2 g, 12 mmol) in THF (10 mL). After stirring for 1 h at 20 °C, the THF was removed under vacuum. The product was extracted with ethyl acetate (2 x 50 mL), the organic fractions were combined, and the solvent was removed under vacuum to leave a white solid. This crude product was recrystallized from MeOH and diethyl ether to give the product as white crystals (3.0 g, 59%). MP = 115-117 °C. ^{1}H NMR($CDCl_3$): δ 7.25 (m, -$CH_2C_6\underline{H}_5$, 5H), 5.18 (s, -C$\underline{H}_2C_6H_5$, 2H), 4.65 (t, -NHC$\underline{H}$(R)C(O)OH, 1H), 3.72 (m, -NHCH((CH_2)$_3$C$\underline{H}_2$NHC(O)R)C(O)- + -C$\underline{H}_2$O(C$\underline{H}_2$C$\underline{H}_2$O)$_2$C$\underline{H}_3$, 15H), 1.70 (m, -NHCH(($C\underline{H}_2$)$_3$$CH_2$NHC(O)R)C(O)-, 6H).

N_ε-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-L-Lysine-N-Carboxyanhydride, EG_2-Lys NCA To a solution of N_ε-2-[2-(2-methoxyethoxy)ethoxy]acetyl-N_α-Z-L-lysine (4.9 g, 11 mmol) in anhydrous CH_2Cl_2 (125 mL) under nitrogen was added 1,1-dichlorodimethylether (1.5 mL, 17 mmol). The solution was then heated to reflux for 20 h, after which the solvent was removed under vacuum. The crude oil was crystallized from THF and hexanes to give the product as white crystals (2.8 g, 75%). ^{1}H

NMR($CDCl_3$): δ 7.68 (br s, -NH, 1H), 7.35 (br s, -NH, 1H), 4.30 (t, -NHCH(R)C(O)O-, 1H), 3.15 (m, -NHCH($(CH_2)_3CH_2$NHC(O)R)C(O)- + -$CH_2O(CH_2CH_2O)_2CH_3$, 15H), 1.70 (m, -NHCH($(CH_2)_3CH_2$NHC(O)R)C(O)-, 6H). FTIR(THF): 1856 cm^{-1} (νCO, anhydride, s), 1789 cm^{-1} (νCO, anhydride, vs), 1677 cm^{-1} (νCO, amide, s). MALDI MS: calc'd: 333.33 (MH^+); found: 332.37 (MH^+).

N_ε-2-(2-Methoxyethoxy)acetyl-L-Lysine-N-Carboxyanhydride, EG-Lys NCA This compound, and its precursors, were prepared in a manner analogous to that for the longer-chain version. The product was isolated as white crystals from THF and hexanes (80%). ^{1}H NMR($CDCl_3$): δ 7.70 (br s, -NH, 1H), 7.36 (br s, -NH, 1H), 4.28 (t, -NHCH(R)C(O)O-, 1H), 3.14 (m, -NHCH($(CH_2)_3CH_2$NHC(O)R)C(O)- + -$CH_2OCH_2CH_2OCH_3$, 11H), 1.70 (m, -NHCH($(CH_2)_3CH_2$NHC(O)R)C(O)-, 6H). FTIR(THF): 1855 cm^{-1} (νCO, anhydride, s), 1789 cm^{-1} (νCO, anhydride, vs), 1678 cm^{-1} (νCO, amide, s). MALDI MS: calc'd: 289.27 (MH^+); found: 288.41 (MH^+).

Poly(N_ε-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-L-Lysine), 1 In the dry box, EG_2-Lys NCA (730 mg, 2.2 mmol) was dissolved in THF (15 mL) and placed in a 75 mL reaction tube which could be sealed with a Teflon stopper. An aliquot of $Co(PMe_3)_4$ (600 μL of a 36 mM solution in THF) was then added *via* syringe to the flask. A stirbar was added and the flask was sealed, removed from the dry box, and stirred in a thermostated 25 °C bath for 24 h. Polymer was isolated by addition of the reaction mixture to diethyl ether causing precipitation of the polymer. The polymer was then dissolved in THF and reprecipitated by addition to diethyl ether. The polymer was dried *in vacuo* to give **1** as a white fibrous solid (596 mg, 87 % yield). GPC of the polymer in 0.1M LiBr in DMF at 60 °C: $\overline{M}_n$ = 141,000; $\overline{M}_w/\overline{M}_n$ = 1.14. ^{1}H NMR($CDCl_3$): δ 7.40 (br, -NH, 2H), 4.25 (br t,

-NHC<u>H</u>(R)C(O)O-, 1H), 3.10 (br m, -NHCH((CH$_2$)$_3$C<u>H$_2$</u>NHC(O)R)C(O)- + -C<u>H$_2$</u>O(C<u>H$_2$</u>C<u>H$_2$</u>O)$_2$C<u>H$_3$</u>, 15H), 1.63 (br m, -NHCH((C<u>H$_2$</u>)$_3$CH$_2$NHC(O)R)C(O)-, 6H). FTIR(THF): 1672 cm^{-1} (νCO, amide, s), 1650 cm^{-1} (νCO, Amide I, br vs), 1538 cm^{-1} (νCO, Amide II, br s).

Poly(N$_\varepsilon$-2-(2-Methoxyethoxy)acetyl-L-Lysine), 2 In the dry box, EG-Lys NCA (630 mg, 2.2 mmol) was dissolved in THF (15 mL) and placed in a 75 mL reaction tube which could be sealed with a Teflon stopper. An aliquot of Co(PMe$_3$)$_4$ (600 μL of a 36 mM solution in THF) was then added *via* syringe to the flask. A stirbar was added and the flask was sealed, removed from the dry box, and stirred in a thermostated 25 °C bath for 24 h. Polymer was isolated by addition of the reaction mixture to diethyl ether causing precipitation of the polymer. The polymer was then dissolved in THF and reprecipitated by addition to diethyl ether. The polymer was dried *in vacuo* to give **2** as a white fibrous solid (505 mg, 94 % yield). GPC of the polymer in 0.1M LiBr in DMF at 60 °C: $\widetilde{M}_n$ = 121,000; $\widetilde{M}_w/\widetilde{M}_n$ = 1.14. ^{1}H NMR(CDCl$_3$): δ 7.35 (br, -N<u>H</u>, 2H), 4.29 (br t, -NHC<u>H</u>(R)C(O)O-, 1H), 3.16 (br m, -NHCH((CH$_2$)$_3$C<u>H$_2$</u>NHC(O)R)C(O)- + -C<u>H$_2$</u>OC<u>H$_2$</u>C<u>H$_2$</u>OC<u>H$_3$</u>, 11H), 1.70 (br m, -NHCH((C<u>H$_2$</u>)$_3$CH$_2$NHC(O)R)C(O)-, 6H). FTIR(THF): 1670 cm^{-1} (νCO, amide, s), 1650 cm^{-1} (νCO, Amide I, br vs), 1540 cm^{-1} (νCO, Amide II, br s).

Poly(N$_\varepsilon$-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-L-Lysine-*block*-γ-Benzyl-L-Glutamate), 1-*b*-PBLG, Diblock Copolymer. In the dry box, EG$_2$-Lys NCA (15 mg, 0.045 mmol) was dissolved in THF (0.5 mL) and placed in a 15 mL reaction tube which could be sealed with a Teflon stopper. An aliquot of (PMe$_3$)$_4$)Co (30 μL of a 46 mM solution in THF) was then added *via* syringe to the flask. A stirbar was added, the

flask was sealed and then stirred for 16 h. An aliquot (50 μL) was removed from the polymerization for GPC analysis ($\widetilde{M}_n$ = 45,200; $\widetilde{M}_w/\widetilde{M}_n$ = 1.15). Glu NCA, (45 mg, 0.17 mmol) dissolved in THF (1.0 mL) was then added to the reaction mixture. After stiring for an additional 16h, polymer was isolated by addition of the reaction mixture to diethyl ether containing HCl (1 mM) causing precipitation of the copolymer. The copolymer was then dissolved in THF and reprecipitated by addition to diethyl ether. The polymer was dried *in vacuo* to give a white solid, **1**-*b*-PBLG (45 mg, 90 % yield). ^{13}C {^1H} NMR, ^1H NMR, and FTIR spectra of this material were identical to a combination of data found for authentic individual samples of PBLG and **1**.[2] GPC of the block copolymer in 0.1M LiBr in DMF at 60 °C: $\widetilde{M}_n$ = 166,250; $\widetilde{M}_w/\widetilde{M}_n$ = 1.17.

Poly(N_ε-2-[2-(2-Methoxyethoxy)ethoxy]acetyl-L-Lysine-*block*-ε-CBZ-L-Lysine), 1-*b*-PZLL, Diblock Copolymer. In the dry box, EG_2-Lys NCA (15 mg, 0.045 mmol) was dissolved in THF (0.5 mL) and placed in a 15 mL reaction tube which could be sealed with a Teflon stopper. An aliquot of $(PMe_3)_4)Co$ (30 μL of a 46 mM solution in THF) was then added *via* syringe to the flask. A stirbar was added, the flask was sealed and then stirred for 16 h. An aliquot (50 μL) was removed from the polymerization for GPC analysis ($\widetilde{M}_n$ = 44,600; $\widetilde{M}_w/\widetilde{M}_n$ = 1.19). Lys NCA, (45 mg, 0.15 mmol) dissolved in THF (1.0 mL) was then added to the reaction mixture. After stiring for an additional 16h, polymer was isolated by addition of the reaction mixture to diethyl ether containing HCl (1 mM) causing precipitation of the copolymer. The copolymer was then dissolved in THF and reprecipitated by addition to diethyl ether. The polymer was dried *in vacuo* to give a white solid, **1**-*b*-PZLL (47 mg, 91 % yield). ^{13}C {^1H} NMR, ^1H NMR, and FTIR spectra of this material were identical to a combination of data found

for authentic individual samples of PZLL and **1**.[3] GPC of the block copolymer in 0.1M LiBr in DMF at 60 °C: $\widetilde{M}_n$ = 126,300; $\widetilde{M}_w/\widetilde{M}_n$ = 1.20.

Attempted Digestion of 1 with Trypsin. A sample of Polymer **1** (20 mg, $\widetilde{M}_n$ = 93,100; $\widetilde{M}_w/\widetilde{M}_n$ = 1.10) was dissolved in 10mM TRIS buffer (1.0 mL, pH 7.4). Trypsin (3000 units, 100 μL) was then added and the resulting solution was incubated for 8 h at 30 °C (sample of poly-L-lysine-HBr was found to be completely degraded under these conditions). The crude solution was then lyophilized and the resulting white solid was extracted with THF (1.0 mL). After removal of solids by filtration, **1** was recovered by addition of diethyl ether (4 mL) to the THF solution. The polymer was washed with excess ether and then dried(yield, 19 mg). GPC of the recovered polymer in 0.1M LiBr in DMF at 60 °C: $\widetilde{M}_n$ = 94,000; $\widetilde{M}_w/\widetilde{M}_n$ = 1.11.

References

1) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

2) Blout, E. R.; Karlson, R. H. *J. Am. Chem Soc.*, **1956**, *78*, 941-950.

3) Yamamoto, H.; Hayakawa, T. *Biopolymers*, **1977**, *16*, 1593-1607.

4) Klein, H-F.; Karsh, H. H. *Chem. Ber.*, **1975**, *108*, 944-955.

5) Lupu-Lotan, N.; Yaron, A.; Berger, A. *Biopolymers*, **1966**, *4*, 365-368.

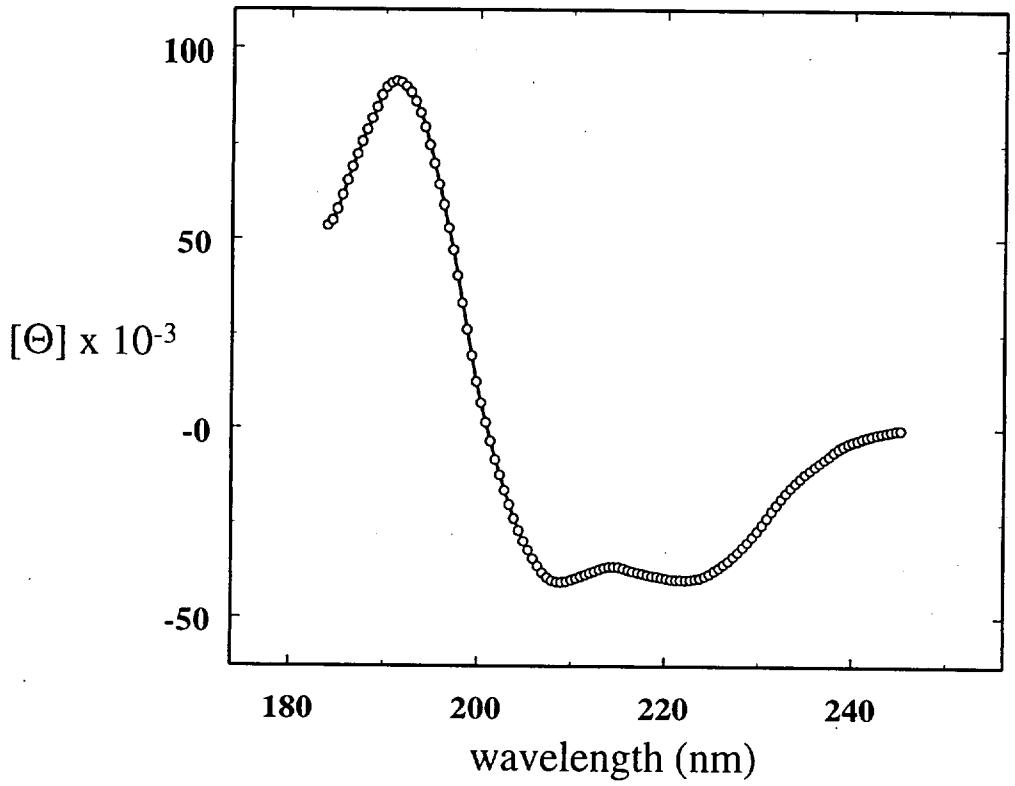

Figure 1. CD spectrum of polymer **1** ($\bar{M}_n$ = 93100) in H_2O ([**1**] = 0.5 mg/mL, pH 7).