Supporting Information

N-Trimethylsilyl Amines for Controlled Ring-Opening Polymerization of Amino Acid *N*-Carboxyanhydrides and Facile End Group Functionalization of Polypeptides

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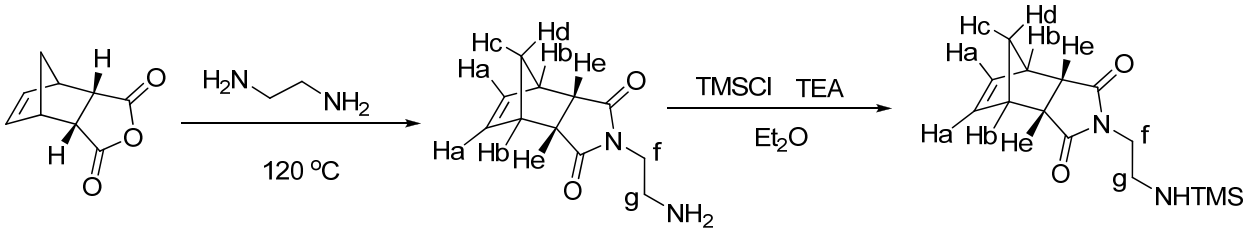
General:

Anhydrous dimethylformamide (DMF) was prepared by passing regular DMF (Sigma-Aldrich, St. Louis, Mo) through dry aluminum column. Anhydrous THF, anhydrous hexane, *N*-trimethylsilyl (*N*-TMS) allylamine (**1-TMS**), *N*-TMS morpholine (**3-TMS**), redistilled chlorotrimethylsilane, hexamethyldisilazane, 5-norbornene-2,3-dicarboxylic anhydride, propargylamine, *N*-TMS *tert*-butylamine, N,O-bis(trimethylsilyl) acetamide (BSA), ethylenediamine and triphosgene were purchased from Sigma-Aldrich and used as received. Anhydrous triethylamine (TEA) was prepared by treating regular TEA (Sigma-Aldrich) with calcium hydride at 40^{o}C under nitrogen overnight followed by distillation under nitrogen. Anhydrous DMSO-d_6 was prepared by treating regular DMSO-d_6 (Cambridge Isotope Laboratories, Andover, MA) with calcium hydride at 70^{o}C under nitrogen overnight followed by distillation under reduced pressure.[1] Anhydrous $CDCl_3$ was prepared by treating regular $CDCl_3$ (Sigma-Aldrich) with P_2O_5 overnight followed by distillation under nitrogen. Anhydrous allylamine (**1**) and benzylamine (**2**) were prepared by treating the corresponding amine (Sigma-Aldrich) overnight with KOH followed by distillation. Anhydrous propargylamine (**4**) was obtained by treating regular propargylamine with 4Å molecular sieves for 6 h under nitrogen. Silylation of amines (**2**, **4**, **5** and **6)** was performed by following the literature reported procedures.[2,3] All purified anhydrous reagents were stored in the presence of 4Å molecular sieves in a glove box. H-Glu(OBn)-OH and H-Lys(Z)-OH were purchased from Chem-Impex International (Des Plaines, IL) and used as received. Glu-NCA and Lys-NCA were prepared and recrystalized by following the published procedures.[4] NMR spectra were recorded on a Varian UI500NB MHz or on a VXR-500 MHz spectrometer. Tandem gel permeation chromatography (GPC) was performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA), a DAWN HELEOS 18 angle MALLS light scattering detector (Wyatt Technology, Santa Barbara, CA) and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA). The detection wavelength of HELEOS was set at 658 nm. Separations of polypeptides were achieved by using four series-connected columns (50Å, 500Å, 10^3Å and 10^4Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA) at 60°C using DMF (containing 0.1 M LiBr) as mobile phase. ESI-MS was collected on a Waters Quattro II Mass Spectrometer. Matrix Assisted Laser Desorption/Ionization-Time Of Flight mass spectrometer (MALDI-TOF MS) spectra were collected on a Applied Biosystems Voyager-DE^{TM} STR system.

Synthesis of *N*-TMS Amines:

Synthesis of *N*-TMS benzylamine (2-TMS)[2] Anhydrous benzylamine (2.14 g, 20 mmol) and HMDS (1.81 g, 11 mmol) were mixed in a 100-mL, round-bottom flask under nitrogen. One drop of concentrated H_2SO_4 was added to the mixture followed by refluxing for 3 h. A colorless liquid (**2-TMS**) was distilled under reduced pressure (2.5 g, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.30-7.40 (m, 5H, ArH), 4.02 (s, 2H, $C_6H_5C\underline{H}_2$), 0.91 (broad, 1H, NH), 0.10 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 144.3, 128.2, 126.9, 126.3, 45.9, 0.00. The ^{13}C NMR and ^{1}H NMR spectra of this material are identical to the authentic *N*-TMS benzylamine.[2]

Synthesis of *N*-TMS propargylamine (4-TMS) **4** (110 mg, 2.0 mmol) was syringed into a dry, 50-mL, two-neck round-bottom flask equipped with a stir bar, and was cooled in an ice bath. BSA (0.5 mL, 2.0 mmol) was dropwise added. The reaction mixture was then stirred in the ice bath for 30 min under nitrogen followed by vacuum distillation to give the desired **4-TMS** as colorless oil **(**165 mg, 65%). Note: **4-TMS** is volatile and should be collected in a receiving flask cooled by liquid nitrogen during the vacuum distillation. **4-TMS** was stored at –30°C in a glove box. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.39 (dd, 2H, CH_2, J_1 = 2.5 Hz, J_2 = 8.0 Hz), 2.95 (t, 1H, $\underline{H}C$, J = 2.5 Hz), 1.86 (broad, 1H, NH), 0.02 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 86.9, 72.3, 31.0, 0.8. Anal. Calcd. for $C_6H_{13}NSi$: 56.63% C, 10.30% H, 11.01% N; found 54.98% C, 10.47% H, 10.50% N.



5 **5-TMS**

Synthesis of *N*-(*N*-trimethylsilylaminoethylene)-5-norbornene-endo-2,3-dicarboximide (5-TMS)

Step 1**:** 5-Norbornene-2,3-dicarboxylic anhydride (1.57 g, 10 mmol) was added dropwise to a flask containing vigorously stirred ethylenediamine (30 mL, 0.45 mol) at room temperature. The reaction temperature was then raised to 120°C. After the solution was stirred for 12 h at 120°C, it was cooled to room temperature. DI water (30 mL) and ethyl acetate (30 mL) were added subsequently. The organic phase (ethyl acetate) that contained **5** was collected. The aqueous phase was extracted with ethyl acetate (2 × 20 mL). The combined organic phase was washed with DI water (10 mL) to remove residual ethylenediamine and then dried with $MgSO_4$. The solvent was removed under vacuum to give **5** in white solid form (1.4 g, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.12 (s, 2H, Ha), 3.42-3.40 (m, 4H, Hb and Hf), 3.28 (s, 2H, He), 2.76-2.74 (m, 2H, Hg), 1.75 (d, 1H, Hd, J = 9.0 Hz), 1.54 (d, 1H, Hc, J = 9.0 Hz), 1.02 (broad, 2H, NH_2). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 178.2, 134.8, 52.5, 46.0, 45.2, 42.0, 40.4.

Step 2: In a glove box, **5** (206 mg, 1.0 mmol) was dissolved in 5 mL anhydrous THF. This solution was mixed with an ether solution (2 mL) of TEA (120 mg, 1.2 mmol). After TMSCl (120 mg, 1.1 mmol) was added, the mixture was stirred overnight at room temperature under argon. The precipitate formed was removed by filtration. The solvent of the filtrate was removed under reduced pressure to give the crude **5-TMS** (250 mg, 92%). The crude material was recrystallized with ether at −30°C to give **5-TMS** in needle crystalline form. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.09 (s, 2H, Ha), 3.38 (s, 2H, Hb), 3.32 (t, 2H, Hf, J = 7 Hz), 3.25 (s, 2H, He), 2.76-2.72 (m, 2H, Hg), 1.73 (d, 1H, Hd, J = 8.5 Hz), 1.54 (d, 1H, Hc, J = 8.5 Hz), 0.43 (broad, 1H, NH), 0.01 (9H, $Si(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 178.1, 134.7, 52.4, 46.0, 45.1, 42.1, 39.8, 0.17. Anal. Calcd. for $C_{12}H_{22}N_2O_2Si$: 60.39% C, 7.96% H, 10.06% N; found: 59.94% C, 7.40% H, 9.95% N. M.P.: 69-70°C. MS (ESI): calcd. for $C_{12}H_{22}N_2O_2Si$ $[M + H]^+$ 279.4, found 279.2.

***N*-TMS mPEG$_{2000}$-amine (6-TMS)** In a glove box, mPEG-NH_2 (**6**, 100 mg, 0.05 mmol) was dissolved in anhydrous THF (2 mL) in a 20-mL vial. BSA (1 mL, 40 mmol) was added. The reaction mixture was stirred for 2 days. Anhydrous hexane (10 mL) was added to the reaction mixture to precipitate a white solid. The white solid was washed with anhydrous hexane (4 × 10 mL) and dried under vacuum to give **6-TMS** in quantitative yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.53 (s, 200 H), 0.01 (s, 9H).

General procedure for the polymerization of Glu-NCA

In a glove box, Glu-NCA (26 mg, 0.1 mmol) was dissolved in DMF (0.5 mL). The Glu-NCA solution was added to a DMF solution containing **1-TMS** (10 μL, 0.1 mmol/mL). The reaction mixture was stirred for 15 h at room temperature. The concentration of NCA was monitored by measuring the intensity of NCA's anhydride peak at 1789 cm^{-1} using FT-IR. The conversion of NCA was determined by comparing the NCA concentration in the polymerization solution with the initial NCA concentration. An aliquot of the polymerization solution was diluted to 10 mg PBLG/mL DMF (containing 0.1 M LiBr), and then analyzed by GPC. The remaining PBLG was precipitated with 8 mL methanol. The obtained PBLG was sonicated for 5 min in ether and centrifuged to remove the solvent. After the sonication-centrifugation procedure was repeated two more times, PBLG was collected and dried under vacuum (17 mg, 78%).

Evaluation of equal molar mixture of 1-TMS and Glu-NCA with ESI-MS

Glu-NCA (26 mg, 0.1 mmol) was dissolved in anhydrous DMF (500 μL). The solution was added dropwise to a DMF solution (100 μL) of **1-TMS** (14 mg, 0.1 mmol). The reaction mixture was stirred overnight at room temperature and analyzed with ESI-MS under anhydrous conditions.

Supplementary Figure 1

Objective: To determine whether **1-TMS** can mediate controlled polymerization of Lys-NCA

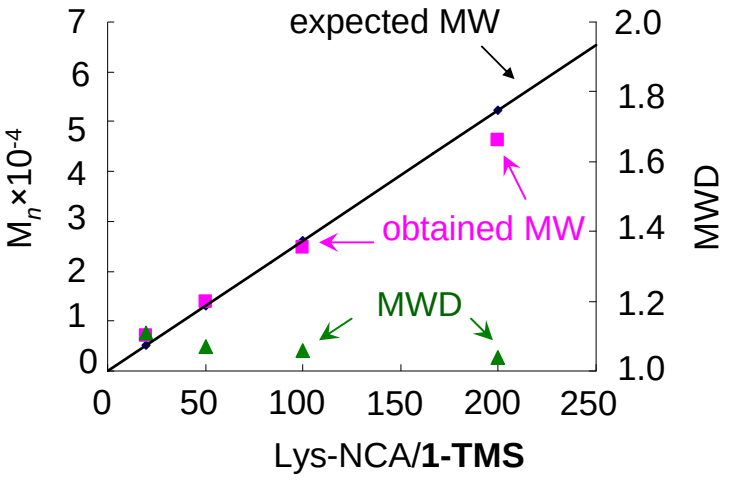


Figure S1. **1-TMS** initiated Lys-NCA polymerization in DMF. The obtained MWs of the resulting PZLL, denoted by the magenta squares, were in nearly perfect agreement with the expected MWs (the straight line). The MWDs of the obtained PZLLs are denoted by the green triangles.

Experimental:

In a dry box, ε-Cbz-L-lysine NCA (Lys-NCA) (30 mg, 0.1 mmol) was dissolved in DMF (0.5 mL). The Lys-NCA solution was then added to a DMF solution containing **1-TMS** (10 μL, 0.1 mmol/mL). The reaction mixture was stirred for 24 h at room temperature. After Lys-NCA was completely consumed (monitored by checking the NCA anhydride band at 1790 cm^{-1} using FT-IR), poly(ε-cbz-L-lysine) (PZLL) was precipitated with methanol and analyzed with GPC.

Discussion:

1-TMS-mediated polymerizations of Lys-NCA gave PZLLs with the expected MWs and narrow MWDs (less than 1.10). The control of MWs observed in Lys-NCA polymerization was comparable to **1-TMS**-mediated Glu-NCA polymerization (Fig. 1a).

Supplementary Table 1 and Figure 2

Objective: To demonstrate **1-TMS**-mediated Glu- and Lys-NCA copolymerization for the syntheses of block co-polypeptides

Table S1. **1-TMS** Mediated Copolymerization of Lys- and Glu-NCA for the Synthesis of Block Copolypeptides

entry	NCA (NCA/initiator ratio)[a]	M_n expected (g/mol)[b]	M_n obtained (g/mol)[b]	M_w/M_n	conv. of NCA (%)[c]
1	Lys(50)/Glu(60)	13,100/26,240	14,100/31,300	1.07/1.04	>99/>99
2	Lys(100)/Glu(120)	26,200/52,480	24,800/52,800	1.06/1.07	>99/>99

[a] Synthesis of PZLL-*b*-PBLG block copolypeptide via sequential addition of Lys-NCA and Glu-NCA to **1-TMS**. [b]M_n of PZLL/M_n of PZLL-*b*-PBLG.. [c]determined by measuring the intensity of NCA anhydride band at 1789 cm^{-1} using FT-IR.

Experimental:

In a dry box, Lys-NCA (30 mg, 0.1 mmol) was dissolved in DMF (0.5 mL). The Lys-NCA solution was then added to a DMF solution containing **1-TMS** (10 μL, 0.1 mmol/mL). The reaction mixture was stirred for 24 h at room temperature. After the Lys-NCA was completely consumed (monitored by checking the NCA anhydride band at 1790 cm^{-1} using FT-IR), Glu-NCA (26 mg, 0.12 mmol) in 0.5 mL DMF was added to the polymerization solution. The polymerization of Glu-NCA was complete in 24 h, which was indicated by FT-IR analysis of the polymerization solution. The molecular weights of first block (PZLL) and the block co-polypeptide (PZLL-*b*-PBLG) were analyzed by GPC (Figure S2).

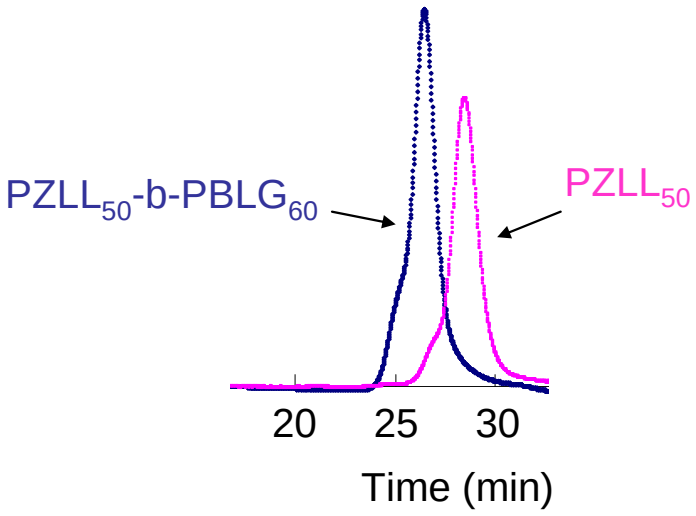


Figure S2. Analysis of $PZLL_{50}$ and $PZLL_{50}$-*b*-$PBLG_{60}$ with GPC

Supplementary Figure 3

Objective: To verify the formation of TMS-CBM propagating group when equal molar Glu-NCA and *N*-TMS *tert*-butyl amine were mixed

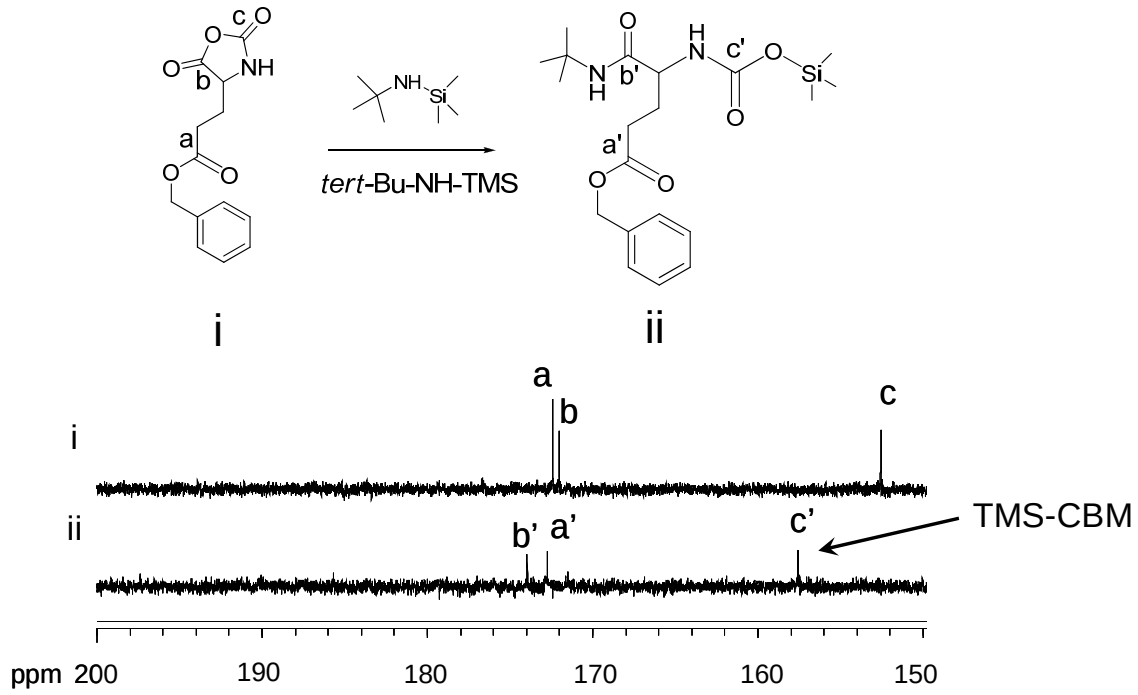


Figure S3. ^{13}C NMR spectrum of equal molar mixture of *N*-TMS *tert*-butylamine and Glu-NCA

Experimental:

In a glove box, an anhydrous DMSO-d_6 solution (600 μL) containing 26 mg Glu-NCA (0.1 mmol) was added dropwise to an anhydrous DMSO-d_6 solution (400 μL) containing 15 mg *N*-TMS *tert*-butylamine (0.1 mmol). The mixture was stirred in the glove box for 30 min at room temperature. The reaction solution was then transferred to a NMR tube in the glove box. The NMR tube was capped, sealed with parafilm to avoid the reaction mixture being exposed to moisture, and analyzed on a Varian VXR-500 MHz NMR spectrometer.

Supplementary Table 2

Objective: To demonstrate controlled polymerization of Glu- and Lys-NCA using various *N*-TMS amine initiators

Table S2. *N*-TMS Amines Mediated Glu- and Lys-NCA Polymerization at Various M/I Ratios.*

entry	NCA (NCA/initiator)	initiator	M_n obtained (g/mol)	M_n expected (g/mol)	M_w/M_n
1	Glu (100)	**2-TMS**	23,500	21,900	1.27
2	Glu (300)	**2-TMS**	82,000	65,700	1.29
3	Glu (500)	**2-TMS**	107,000	109,500	1.29
4	Glu (50)	**3-TMS**	11,500	10,950	1.25
5	Glu (100)	**3-TMS**	21,800	21,900	1.21
6	Glu (200)	**3-TMS**	39,700	43,800	1.17
7	Glu (300)	**3-TMS**	59,100	65,700	1.19
8	Glu (50)	**4-TMS**	11,800	10,950	1.16
9	Glu (100)	**4-TMS**	21,900	21,900	1.18
10	Glu (200)	**4-TMS**	41,900	43,800	1.12
11	Glu (300)	**4-TMS**	65,700	65,700	1.21
12	Glu (500)	**4-TMS**	83,400	109,500	1.22
13	Lys (50)	**5-TMS**	13,300	13,100	1.09
14	Lys (100)	**5-TMS**	22,700	26,200	1.04
15	Lys (200)	**5-TMS**	41,200	52,400	1.05

*Conversions of monomers, determined by FT-IR, were over 95% in all experiments.

Experimental:

The polymerizations were performed and the resulting polypeptides were analyzed in the same manner as described for **1-TMS** mediated Glu-NCA polymerization (see the section of "General procedure for the polymerization of Glu-NCA" on page S3).

References:

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(3) Rajeswari, S.; Jones, R. J.; Cava, M. P. *Tetrahedron Lett.* **1987**, *28*, 5099-5102.

(4) Lu, H.; Cheng, J. *J. Am. Chem. Soc.* **2007**, *129*, 14114-14115.