Supporting Information

A S-Sn Lewis Pair-Mediated Ring-Opening Polymerization of α-Amino Acid *N*-Carboxyanhydrides: Fast Kinetics, High Molecular Weight, and Facile Bioconjugation

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MATERIALS AND METHODS

Materials. All chemicals were purchased from commercial sources and used as received unless otherwise specified. PhS-TMS was purchased from Sigma-Aldrich (St. Louis, USA). Diphenyl disulfide and hexamethylditin were purchased from TCI (Tokyo, Japan). Anhydrous dichloromethane (DCM), hexane, and tetrahydrofuran (THF) were obtained by passing HPLC grade solvents through columns packed with activated 4Å molecular sieves. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from Sigma-Aldrich. Amino acid derivatives were purchased from GL Biochem Ltd (Shanghai, China). ε-carboxybenzyl L-lysine NCA (Z-LysNCA), γ-benzyl L-glutamate NCA (Bn-GluNCA), and γ-(2-(2-(2-methoxyethoxy)ethoxy)ethyl L-glutamate NCA (EG_3-GluNCA) were synthesized following previously reported procedures.[1] Cys-IFN was expressed as previously reported.[2]

Characterizations. NMR spectra were recorded on a 400 MHz Bruker ARX400 FT-NMR spectrometer. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker Vector FT-IR spectrometer and the quantification was realized by using a KBr cell with fixed pathlength of 0.2 mm. Tandem gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA), a DAWN HELEOS 9-angle laser light scattering detector (also known as multi-angle laser light scattering (MALLS) detector, Wyatt Technology, Santa Barbara, CA) and an Optilab rEX refractive index detector

(Wyatt Technology, Santa Barbara, CA). The detection wavelength of MALLS was set at 658 nm. Separations were performed using serially connected size exclusion columns (500Å, 10^3Å, 10^4Å and 10^5Å Phenogel columns, 5 μm, 7.8 × 300 mm, Phenomenex, Torrance, CA) at 50 °C using DMF containing 0.1 M LiBr as the mobile phase. The molecular weights (MW) of all polymers were determined based on the d*n*/d*c* values of all samples calculated offline by using the internal calibration system processed by the ASTRA V software version 5.1.7.3 provided by Wyatt Technology. d*n*/d*c* values of PZLL and PBLG are 0.093 mL/g and 0.063 mL/g for P(EG_3-Glu), respectively. MALDI-TOF MS spectra were acquired by a Bruker Daltonics ultraflex TOF mass spectrometer. The SDS-PAGE gel was recorded on a typhoon FLA 9500 laser scanner (GE Healthcare Corp.).

Synthesis of PhS-$SnMe_3$. Diphenyl disulfide (100 mg, 0.458 mmol, 1.0 equiv) and hexamethylditin (150 mg, 0.458 mmol, 1.0 equiv) were dissolved in THF (100 μL) respectively in a glove box. Then the solutions were mixed in a vial and kept under 365 nm UV light for 10 min, after which the solvent was removed by evaporation and the pure PhS-$SnMe_3$ was obtained as a colorless oil (247 mg, yield 99%). PhS-$SnMe_3$ was used directly as the initiator without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.33 (m, 2H), 7.28 – 7.08 (m, 3H), 0.55 – 0.37 (m, 9H).

General procedure for PhS-$SnMe_3$ mediated NCA polymerization. In a glovebox, Z-LysNCA (20.0 mg, 0.0653 mmol, 25 equiv) was dissolved in the mixed solvent of

anhydrous THF (340 μL) and DMF (40 μL), to which was added to a PhS-$SnMe_3$ stock solution in THF (26.1 μL× 0.1 M, 1.0 equiv). The reaction was stirred for 70 min at room temperature and the conversion of NCA was monitored by FT-IR spectroscopy. At ~ 95% conversion, the reaction mixture was quenched by acetic anhydride. An aliquot of the solution was diluted to 5 mg/mL in DMF containing 0.1 M LiBr for GPC analysis. To obtain purified polypeptide for further analysis, the reaction solution was poured into diethyl ether (40 mL), and the precipitate was separated by centrifugation, washed extensively with diethyl ether (40 mL × 2), and dried under vacuum to afford PZLL with ~ 65-85% yield. PhS-$SnMe_3$ mediated polymerization of other NCAs were similarly carried out.

PhS-$SnMe_3$ mediated block copolymerization of Z-LysNCA and Bn-GluNCA. In a glovebox, Z-LysNCA (20.0 mg, 0.0653 mmol, 25 equiv) was dissolved in mixed dry THF (340 μL) and DMF (40 μL), to which was added a PhS-$SnMe_3$ stock solution in THF (26.1 μL× 0.1 M, 1.0 equiv) in one portion. The reaction was stirred for 70 min and half of the solution was transferred to a new vial and quenched by acetic anhydride (5 μL) for GPC analysis. The rest solution was added to a solution of Bn-GluNCA (17.1 mg, 0.0653 mmol, 25 equiv) in mixed dry THF (360 μL) and DMF (40 μL). The reaction was allowed to stir for another 4 h and quenched by acetic anhydride for GPC analysis.

General procedure for the kinetic studies. In a glovebox, a solution of Z-LysNCA (50.0 mg, 0.163 mmol, 25 equiv) in mixed dry THF (835 μL) and DMF (100 μL) was added to a PhS-$SnMe_3$ stock solution in THF (65.3 μL× 0.1 M, 1.0 equiv) at room temperature under stirring. The conversion of monomer was monitored by FT-IR spectroscopy by injecting an aliquot of reaction solution (100 μL, which was quenched by 5 μL acetic acid) into a KBr cell with a fixed path length of 0.2 mm at various time intervals. Quantification was achieved by calculating the peak area at 1858 cm^{-1} and fitting to a standard working curve.

Sample preparation for MALDI-TOF analysis. In a glovebox, to the solution of Z-LysNCA (20.0 mg, 0.0653 mmol, 10.0 equiv) in mixed dry THF (100 μL) and DMF (300 μL) was added PhS-$SnMe_3$ (1.8 mg, 0.00653 mmol, 1.0 equiv). The reaction was stirred for 20 min at room temperature and then poured into diethyl ether (40 mL) and the precipitate was separated by centrifugation and used for MALDI-TOF analysis.

Native chemical ligation (NCL) of P(EG_3-Glu) to Cys-IFN. Cys-IFN (4.7 mg, 1.0 equiv) and the N-acetylated P(EG_3-Glu) (MW ~82 kDa, 55.4 mg, 3.0 equiv) were added to a Tris·HCl buffer (50 mM, pH 7.0, 0.6 mL). The mixture was incubated at room temperature for 8 h and analyzed by SDS-PAGE gel to calculate the protein conversion using the Typhoon laser scanner. The pure conjugate was purified by

successive SEC (superdex 200 increase 10/300 GL, PBS with 2 mM DTT) and Ni-NTA columns.

Removal of tin residual. For water insoluble polypeptides (e.g. PZLL and PBLG), the remaining tin was removed by repeating the precipitation-dissolution cycle for three times. For water soluble polypeptides (e.g. P(EG_3-Glu)), the tin was removed by simply passing a size-exclusion PD-10 column and freeze dried.

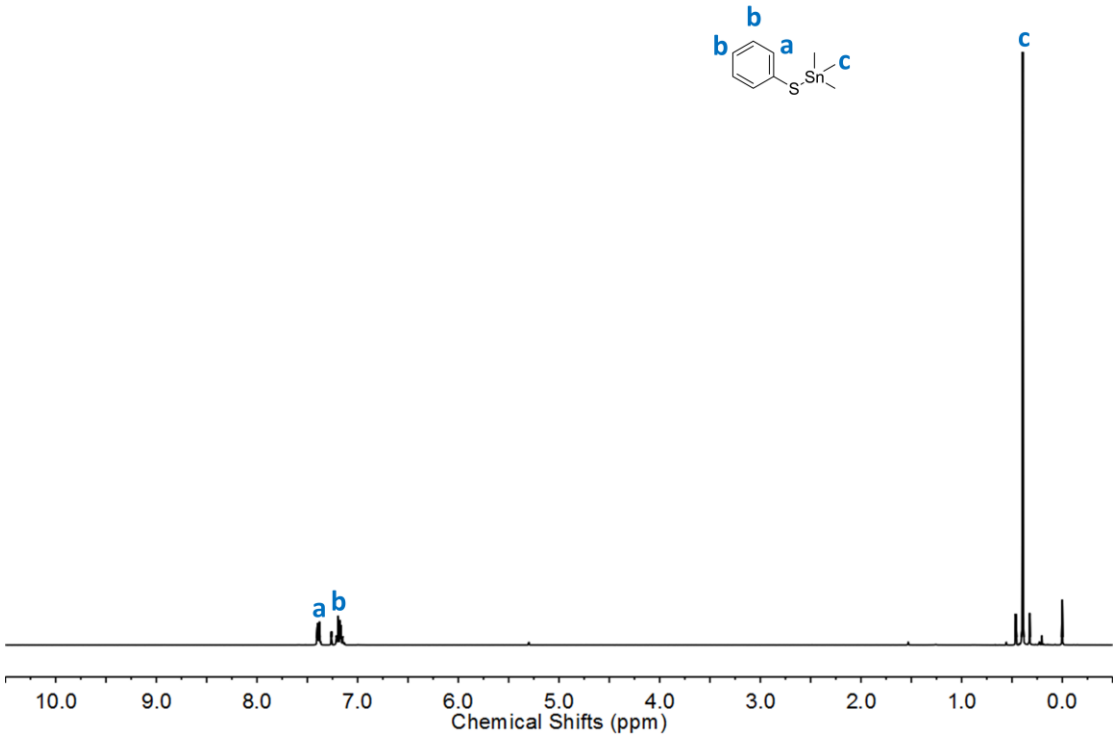

Figure S1. ^{1}H NMR spectrum of PhS-$SnMe_3$ in $CDCl_3$.

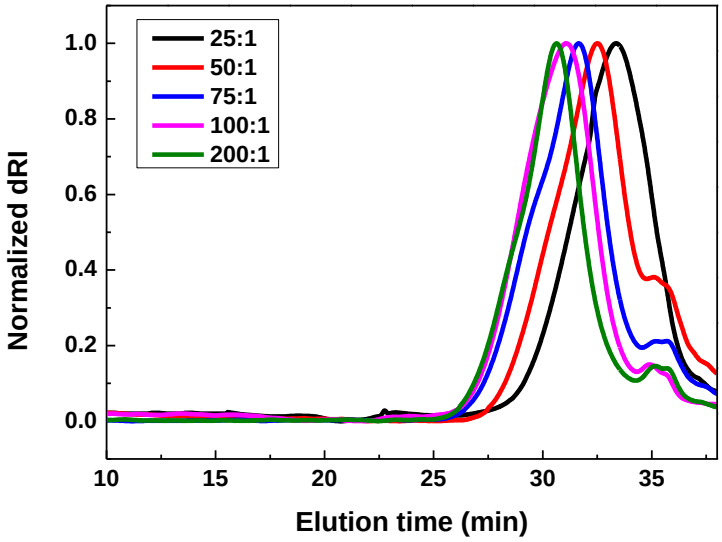

Figure S2. Overlay of the GPC curves for PhS-$SnMe_3$ mediated ROP of Z-LysNCA in DMF with different M/I ratio. All polymerizations were quenched by acetic anhydride at about 85% conversion.

Table S1. PhS-$SnMe_3$−mediated ROP of Z-LysNCA in DMF.

Entry	$[M]_0/[I]_0$	Time (min)	$MW_{cal.}$ ($\times 10^4$ g mol^{-1})	$MW_{obt.}$ ($\times 10^4$ g mol^{-1})	*Đ*
1	25:1	70	0.56	0.75	1.25
2	50:1	100	1.11	1.23	1.16
3	75:1	150	1.67	1.52	1.14
4	100:1	180	2.23	1.77	1.17
5	200:1	480	4.45	2.17	1.15

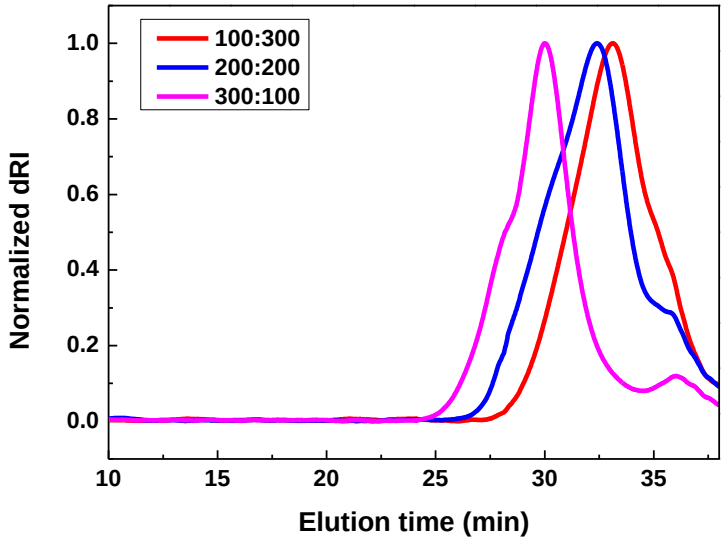

Figure S3. Overlay of the GPC curves for PhS-$SnMe_3$–mediated ROP of Z-LysNCA in mixed THF/DMF. M/I ratio = 25/1.

Table S2. PhS-$SnMe_3$−mediated ROP of Z-LysNCA in mixed THF/DMF, M/I ratio = 25/1.

Entry	V_{THF}/V_{DMF} (uL)	Time (min)	$MW_{obt.}$ ($\times 10^4$ g mol^{-1})	*Đ*
1	100/300	180	0.96	1.22
2	200/200	180	1.42	1.17
3	300/100	180	2.77	1.19

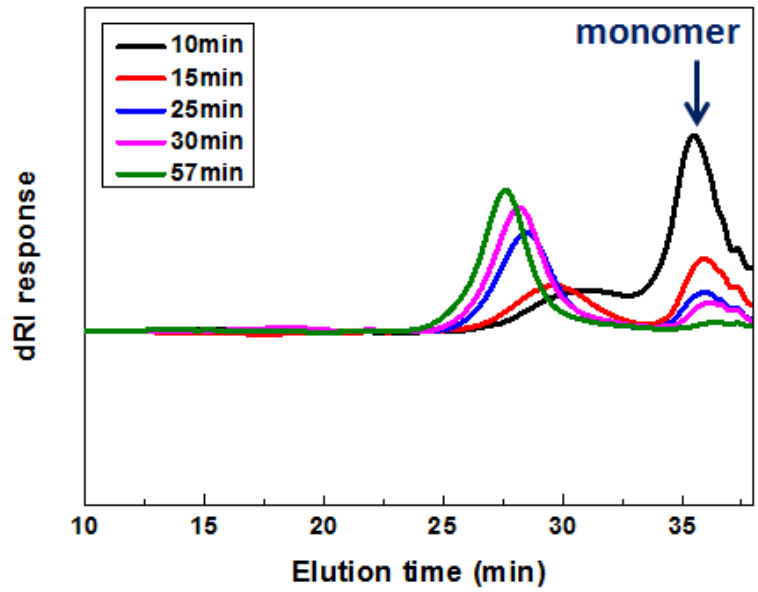

Figure S4. Overlay of the GPC curves for PhS-$SnMe_3$–mediated ROP of Z-LysNCA in V_{THF}/V_{DMF} = 360/40 with M/I ratio of 25/1 quenched at different reaction time.

Table S3. PhS-$SnMe_3$−mediated ROP of Z-LysNCA in V_{THF}/V_{DMF} = 360/40 with M/I ratio of 25/1 at different monomer scales.

Entry	M_{NCA} (mg)	V_{THF}/V_{DMF} (uL)	Time (min)	$MW_{obt.}$ ($\times 10^4$ g mol^{-1})	*Đ*
1	20	360/40	60	5.26	1.05
2	100	1800/200	60	5.52	1.06
3	500	9000/1000	60	5.50	1.05

Table S4. PhS-$SnMe_3$−mediated copolymerization of Z-LysNCA and Bn-GluNCA in V_{THF}/V_{DMF} = 360/40.

Entry	Monomer	$[M]_0/[I]_0$	Time (min)	$Mw_{obt.}$ ($\times 10^4$ g mol^{-1})	*Đ*
1	ZLys	25/1	120	4.76	1.11
2	ZLys-BnGlu	25/25/1	360	9.89	1.07

Table S5. Residual tin measured by ICP-MS.

Entry	[a]Polypeptide	$MW_{obt.}$ ($\times 10^4$ g mol^{-1})	$M_{polymer}$ (mg)[b]	M_{tin} (ng)[c]	C_{tin} (ppm)[d]	Elimination efficiency (%)[e]
1	PZLL	4.98	1.51	11.7	7.8	99.97
2	P(EG_3-Glu)	6.02	20.1	116	5.8	99.98

[a]The polypeptides were both prepared in V_{THF}/V_{DMF} = 360/40 with M/I ratio of 25/1. [b]The mass of the polypeptides used for the ICP-MS analysis. [c]The mass of residual tin determined by ICP-MS. [d]The relative tin content in the product = $M_{tin}/M_{polypeptide}$. [e]elimination efficiency = mass of tin remained in the polypeptides/mass of tin added to the polymerization solution × 100%.

References

(1) Chen, C.; Wang, Z.; Li, Z. *Biomacromolecules* **2011**, *12*, 2859-2863.
(2) Hou, Y.; Yuan, J.; Zhou, Y.; Yu, J.; Lu, H. *J. Am. Chem. Soc.* **2016**, *138*, 10995-11000.