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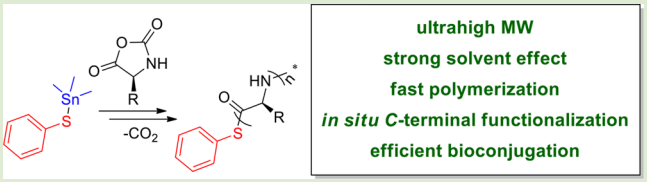
A S-Sn Lewis Pair-Mediated Ring-Opening Polymerization of α-Amino Acid *N*-Carboxyanhydrides: Fast Kinetics, High Molecular Weight, and Facile Bioconjugation

Jingsong Yuan, Yi Zhang, Zezhou Li, Yaoyi Wang, and Hua Lu*

Center for Soft Matter Science and Engineering, Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, People's Republic of China

Supporting Information

ABSTRACT: The rapid and controlled generation of polypeptides with ultrahigh molecular weight (MW) and well-defined chain end functionality has been a great challenge. To tackle this problem, we report here an initiation system based on a S-Sn Lewis pair, trimethylstannyl phenyl sulfide (PhS-$SnMe_3$), for the ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCAs). This initiator displays a strong solvent effect, and can yield polypeptides with high MW (>1.0×10^5 g·mol^{-1}) and low polydispersity index within a few hours. The MWs of the obtained polypeptides are strongly dependent on the THF/DMF ratio. The polymerization follows a typical first-order kinetic character with respect to the monomer concentration in mixed THF and DMF. Moreover, a highly reactive phenyl thioester is *in situ* generated at the C-terminus of the polypeptides, which is readily accessible for native chemical ligation affording high MW and site-specific protein−polypeptide conjugates. Together, this initiator sheds light on regulating the ROP of NCAs via appropriate Lewis pair and solvent selection, and is particularly useful in preparing ultrahigh MW polypeptides within a short period of time.



Synthetic polypeptides, also known as poly(α-amino acid)s, are attractive mimics of proteins with broad biomedical applications such as drug delivery, gene transfection, protein modification, and tissue engineering.[1−9] Currently, the ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCAs) is the most widely used method for up to gram-scale synthesis of polypeptides.[10,11] Over the past two decades, considerable efforts have been devoted to the controlled ROP of NCAs, which have stimulated the development of many interesting initiators/catalysts including nickel- and cobalt-based organometallic catalysts,[12−14] primary amine hydrochloride,[15] *N*-trimethylsilyl (N-TMS) amines and sulfides,[16−18] primary amine tetrafluoroborane,[19] rare earth metal based catalysts,[20,21] secondary amine-assisted primary amine initiators,[22,23] and the thiourea−amine dual initiation system.[24] In addition, primary amine initiated polymerizations have also been optimized under special conditions such as high vacuum,[25,26] low temperature,[27] and nitrogen flow.[28] Despite these progresses, many methods generally afford polypeptides with molecular weight (MW) less than 10^5 g·mol^{-1} at a modest chain propagation reactivity. As such, there is a pressing need for highly reactive initiators/catalysts that can rapidly produce polypeptides with ultrahigh MWs.

One common strategy in NCA ROP is to regulate the chain propagation species (i.e., the amine) by a Brønsted or Lewis acid agent (e.g., proton, trimethylsilane, tetrafluoroborane, and thiourea).[15,19,24] For instance, both hexamethyldisilazane (HMDS) and trimethylsilyl phenylsulfide (PhS-TMS), two initiation systems developed by Cheng and Lu, involve a transferring trimethylsilyl (TMS) at the chain propagation center (Scheme 1A). This proposed mechanism is strongly supported by the identification of the intermediates bearing a trimethylsilyl carbamate end group.[16] Nevertheless, kinetic studies have indicated that both systems have comparable chain propagation rates to common amine initiators.[18] In order to achieve

Scheme 1. (A) PhS-TMS-Mediated ROP of NCA and (B) Synthesis of PhS-$SnMe_3$ and PhS-$SnMe_3$-Mediated ROP of NCA

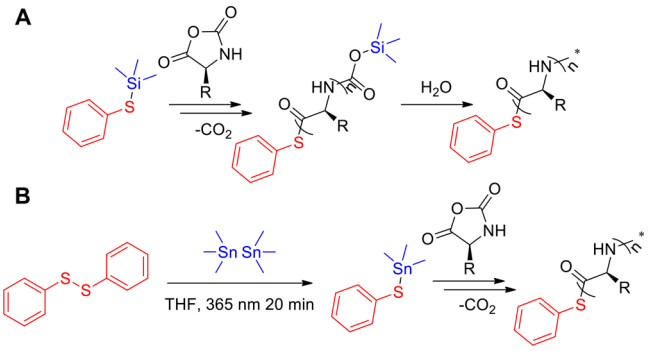


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Table 1. PhS-SnMe$_3$-Mediated ROP of NCA in Mixed THF and DMF[a]

entry	monomer	V_{THF}/V_{DMF} (uL)	time (h)	$MW_{obt.}$ ($\times 10^4$ g mol^{-1})[b]	$Đ$[c]
1	Z-LysNCA	0/400	3	0.75	1.25
2	Z-LysNCA	400/0	8	24.1	1.05
3	Z-LysNCA	340/60	1	3.56	1.08
4	Z-LysNCA	360/40	1	4.98	1.11
5	Z-LysNCA	380/20	1	8.03	1.08
6	Z-LysNCA	390/10	4	15.3	1.14
7	Bn-GluNCA	320/80	2	6.16	1.09
8	Bn-GluNCA	360/40	8	23.6	1.14
9	Bn-GluNCA	380/20	24	48.5	1.17
10	Bn-GluNCA	390/10	24	66.7	1.16
11	Bn-GluNCA	400/0	48	80.6	1.08
12	EG$_3$-GluNCA	320/80	2	6.02	1.08
13	EG$_3$-GluNCA	360/40	12	11.2	1.10
14	EG$_3$-GluNCA	380/20	24	25.9	1.08
15	EG$_3$-GluNCA	400/0	24	27.8	1.16

[a]All polymerizations were performed at 25 °C in a glovebox with $[Z\text{-LysNCA}]_0$ = 0.16 M and quenched with acetic anhydride at ~95% conversion, which was determined by FT-IR spectroscopy. [b]$MW_{obt.}$ = MW obtained. [c]$Đ$ = polydispersity index, determined by SEC.

more rapid polymerization, we reason that a Lewis acid agent "softer" than TMS, for example, the trimethylstannyl group ($SnMe_3$), may give rise to a more reactive end group. Herein, we report our investigation of a S-Sn Lewis pair, trimethylstannyl phenyl sulfide (PhS-SnMe$_3$), for the ROP of NCA.

PhS-SnMe$_3$, a stable compound insensitive to air and moisture, was obtained as the only product in almost quantitative conversion via a simple metathesis reaction between diphenyl disulfide and hexamethylditin under nitrogen and UV light (Scheme 1B, Figure S1).[29] Initial attempts using PhS-SnMe$_3$ for the ROP of ε-carboxybenzyl L-lysine NCA (Z-LysNCA) displayed a strong solvent effect. Namely, the MW of PZLL followed a nonlinear growth up to ~2.2 × 10^4 g·mol^{-1} when the M/I ratio was raised from 25/1 to 200/1 in DMF (Figure S2, Table S1). In contrast, the MW of PZLL obtained at the M/I ratio of 25/1 in THF was ~2.4 × 10^5 g·mol^{-1} (expected MW is 6.6 × 10^3 g·mol^{-1}), which was surprisingly ~32-fold higher than that in DMF at the same M/I ratio (Figure 1A and Table 1, entry 1−2). The results suggested that PhS-SnMe$_3$ had a considerably higher initiation efficiency in DMF than in THF. Next, we examined the kinetics of the polymerizations, which again exhibited interesting solvent-dependent features (Figure 1B). A typical first-order kinetic character with respect to the

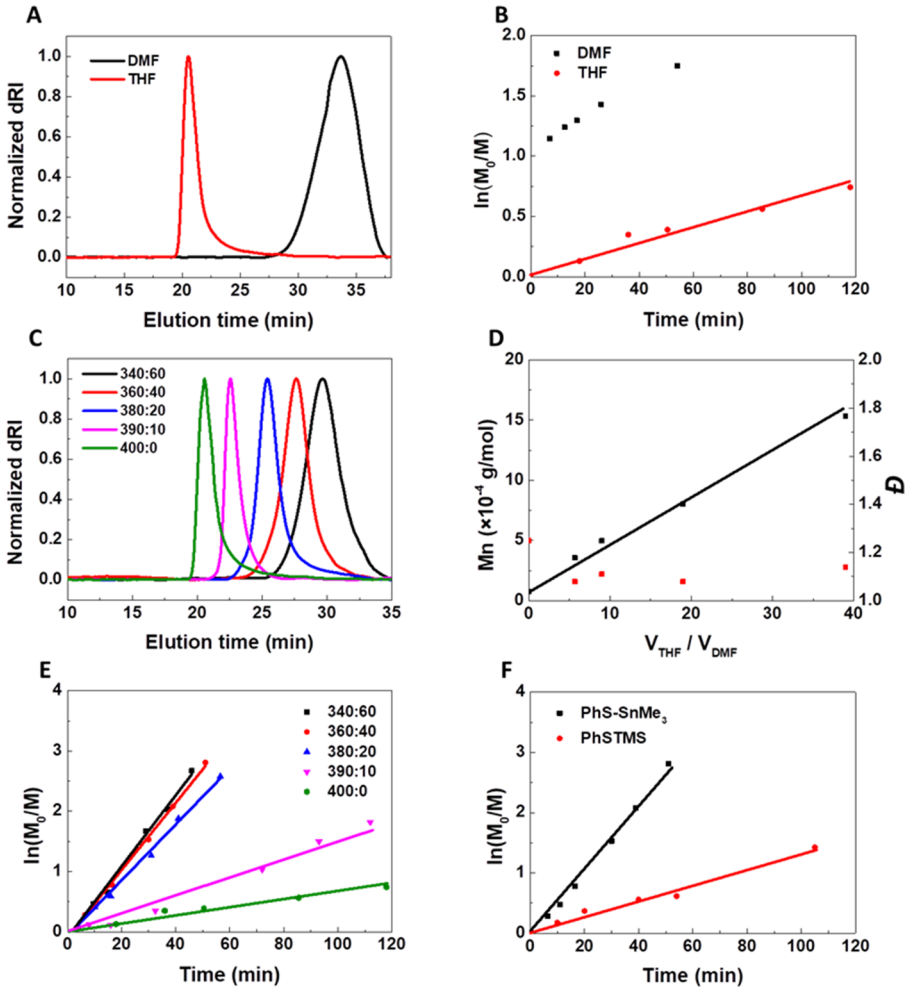

Figure 1. (A,B) PhS-SnMe$_3$-mediated ROP of Z-LysNCA in pure DMF and THF: (A) overlay of the SEC curves and (B) the plot of $\ln([M]_0/[M])$ versus reaction time. (C) Overlay of the SEC curves of the polymerization in mixed THF/DMF. (D) The plots of the MW and $Đ$ of PZLL as a function of the THF/DMF volume ratio. (E) The plots of $\ln([M]_0/[M])$ versus time in mixed THF/DMF. (F) Parallel comparison of the PhS-SnMe$_3$- and PhS-TMS-mediated ROP of Z-LysNCA (V_{THF}/V_{DMF} = 360/40) under the same conditions. All polymerizations were conducted at the same $[M]_0$ (50 mg/mL) and M/I ratio (25/1).

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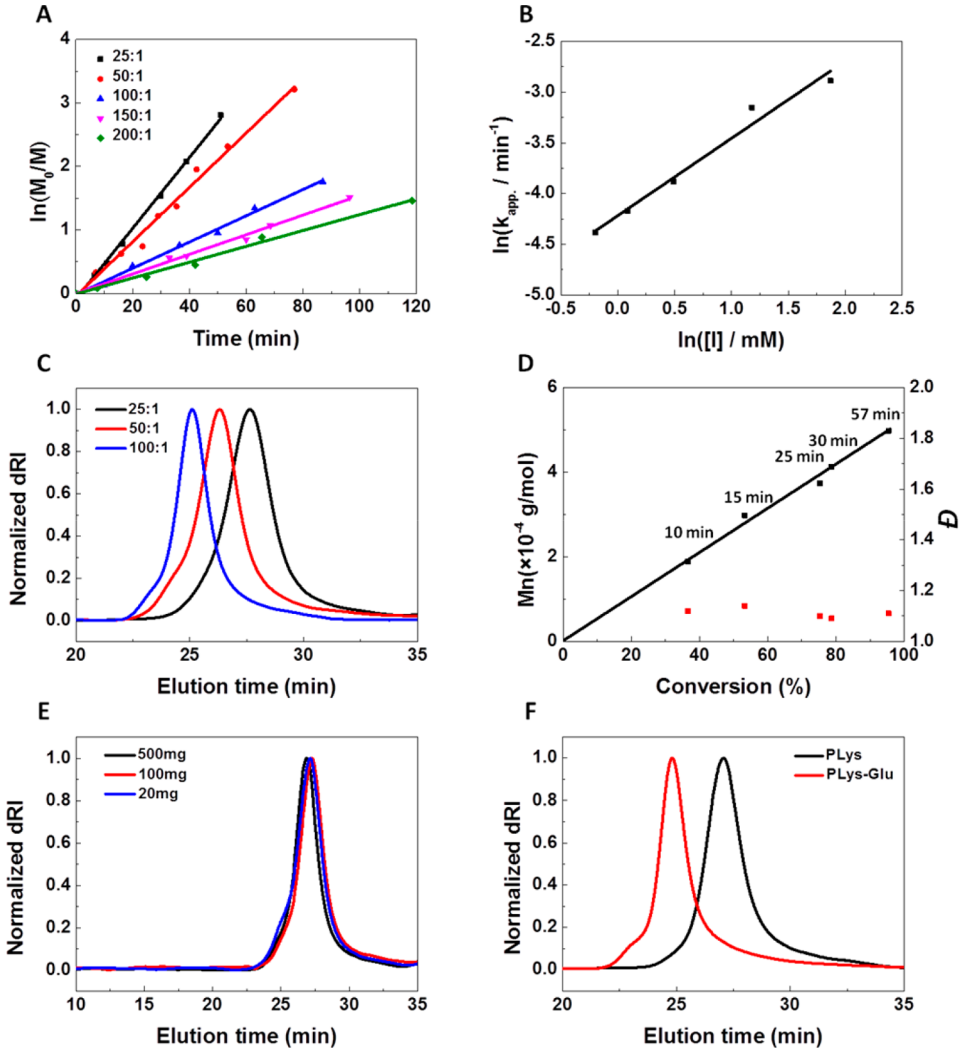

Figure 2. PhS-SnMe$_3$-mediated ROP of Z-LysNCA in mixed THF/DMF (360/40). (A–C) The plot of ln($[M]_0$/$[M]$) versus time (A), the plot of ln k_{app} as a function of ln$[I]_0$ (B), and overlay of the SEC curves (C) for the polymerizations of Z-LysNCA at varied M/I ratios. (D) The plots of MW and $Đ$ of PZLL as a function of monomer conversion. (E) Overlay of the SEC curves of PZLL produced by PhS-SnMe$_3$-mediated ROP at 20, 100, and 500 mg per batch scales. (F) Overlay of the SEC curves of PZLL (MW = 4.76 × 10^4, $Đ$ = 1.11) and block copolymer PZLL-*b*-PBLG (MW = 9.89 × 10^4, $Đ$ = 1.07). All polymerizations were conducted with $[M]_0$ = 50 mg/mL at 25 °C.

monomer concentration $[M]$ was clearly seen for the polymerization in THF, giving an ∼53% monomer conversion in 120 min. In contrast, the same reaction in DMF displayed a two-stage character: ∼67% monomer was consumed within 7 min in the first stage, and the polymerization became much slower in the second stage with only another 18% monomer consumption at 50 min. The monomer conversion stopped at ∼85% in pure DMF, suggesting chain terminations in the late stage. Given the ultrafast kinetics in DMF and the ultrahigh MW obtained in THF, we decided to test the hypothesis of using mixed DMF and THF to achieve both rapid polymerization and ultrahigh MW of the polypeptides.

For this, we conducted the PhS-SnMe$_3$-mediated ROP of Z-LysNCA in a series of mixed THF and DMF. The polymerizations were quenched by acetic anhydride at ∼95% monomer conversion before the *in situ* size exclusion chromatography (SEC) analysis. At a feeding M/I ratio of 25/1 and a fixed initial monomer concentration ($[M]_0$ = 50 mg/mL, total solvent volume = 400 μL), the SEC curves of the resultant PZLLs all showed sharp monomodal peaks readily shifting to the higher MW region (up to ∼1.5 × 10^5 g·mol^{-1}) when the ratio of V_{THF}/V_{DMF} was raised from 340/60 to 390/10 (Figure 1C). Interestingly, the obtained MW of PZLL displayed a linear correlation with the THF/DMF ratio (Figure 1D and Table 1, entries 3–6). Kinetic studies of the polymerizations all exhibited an excellent first-order character against $[M]$. The more DMF yielded the greater apparent rate constant (k_{app}), which could be calculated from the slope of ln($[M]_0$/$[M]$) versus the polymerization time (Figure 1E). Nevertheless, further increase of the DMF ratio (e.g., THF/DMF = 300/100, 200/200, and 100/300) led to shoulder peaks in the SEC curves and significantly smaller MWs of the resultant PZLLs (Figure S3 and Table S2). Notably, PhS-SnMe$_3$ was ∼4.0-fold more rapid than PhS-TMS for the ROP of Z-LysNCA under a given condition, as demonstrated by the comparison of k_{app} (Figure 1F).

Next, we fixed the volume ratio of V_{THF}/V_{DMF} at 360/40 for further investigation because this ratio appeared to offer a reasonable balance of polymerization rate and MW control. We first examined the kinetics of PhS-SnMe$_3$-mediated ROP of Z-LysNCA at different M/I ratios. Again, all polymerizations showed characteristic first-order kinetic dependence with

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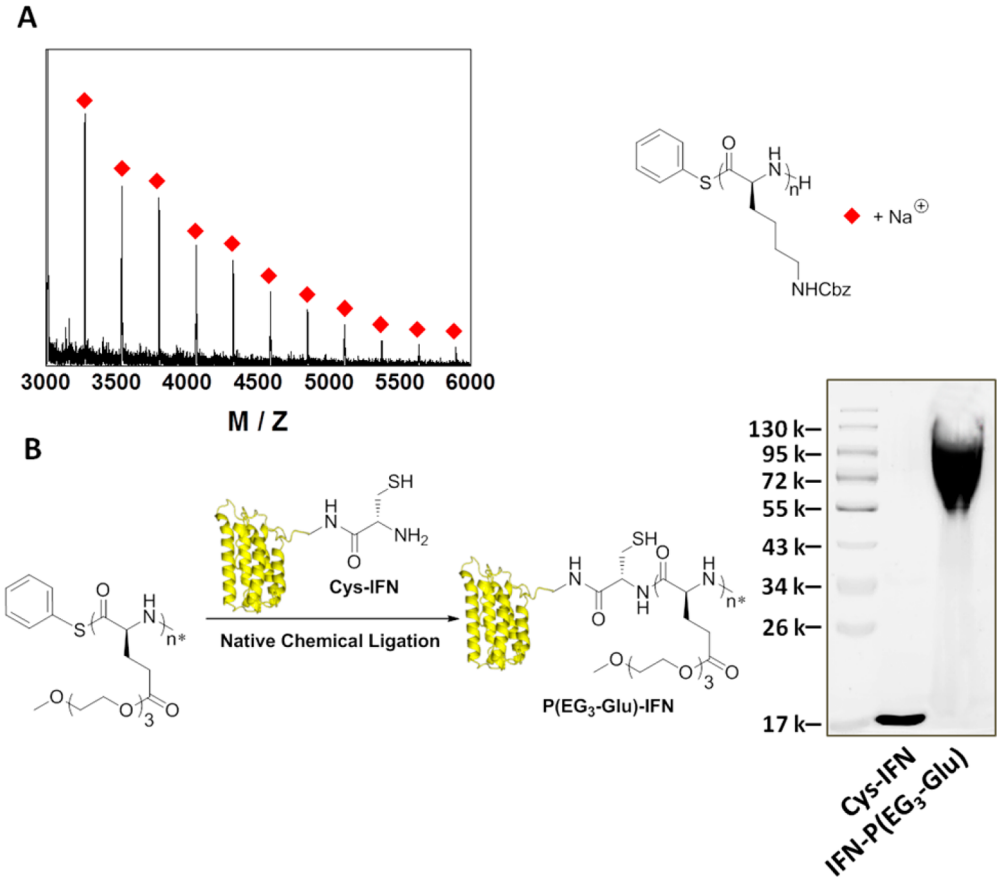

Figure 3. (A) MALDI-TOF mass spectrum of PZLL prepared from PhS-$SnMe_3$-mediated ROP of Z-LysNCAs at a M/I ratio of 10/1 in mixed solvent (V_{THF}/V_{DMF} = 100/300). (B) Synthesis and SDS-PAGE gel of the purified conjugate IFN-P(EG_3-Glu); the NCL reaction was conducted between Cys-IFN and P(EG_3-Glu) (MW ~ 8.2×10^4 g·mol^{-1}) prepared from PhS-$SnMe_3$-mediated ROP of EG_3-GluNCA.

respect to [M] (Figure 2A). A double logarithm plot of the k_{app} as a function of [PhS-$SnMe_3$]$_0$ was fit to a straight line (R^2 = 0.97) with a slope of 0.8 (Figure 2B).[23] SEC analysis of the polymerization solutions showed a clear shift of the polymer peaks to shorter retention time upon the increase of M/I ratio (Figure 2C). Remarkably, PZLL with MW of 1.0×10^5 g·mol^{-1} and $Đ$ ~ 1.07 could be facilely obtained within 3 h (M/I = 100). It was worth pointing out that it would normally require ~48 h to reach merely 4.5×10^4 g·mol^{-1} by using PhS-TMS as the initiator. At a specific M/I ratio, for example 25/1, the MW of the PZLL grew linearly as a function of the monomer conversion, indicating a controlled chain growth of the polymer (Figure 2D and S4). Moreover, the ROPs of Z-LysNCA at 20, 100, and 500 mg per batch gave almost identical results in terms of the GPC curves and the calculated MW and $Đ$, indicating the polymerizations were fully reproducible at various scales (Figure 2E and Table S3).

To examine the scope of the monomer, we tested the PhS-$SnMe_3$-mediated ROP of another two NCA monomers, namely, γ-benzyl L-glutamate NCA (Bn-GluNCA) and γ-2-(2-(2-methoxyethoxy)ethoxy)ethyl L-glutamate NCA (EG_3-GluNCA). A similar solvent effect was clearly seen for the two monomers, which afforded poly(γ-benzyl L-glutamate) (PBLG) and poly(γ-2-(2-(2-methoxyethoxy)ethoxy)ethyl L-glutamate) (P(EG_3-Glu)) with MW up to 6.7×10^5 and 2.8×10^5 g·mol^{-1} in less than 24 h, respectively (Table 1, entry 7−15). All polymers exhibited monomodal peaks and $Đ$ values less than 1.20. Moreover, well-defined two-block copolymers could be facilely produced by sequential addition of different NCA monomers (Figure 2F, Table S4).

We have previously shown that PhS-TMS-mediated ROP of NCAs afforded polypeptides with a C-terminal phenyl thioester and a labile N-terminal TMS-carbamate that decomposed to a free amine upon exposure to moisture.[18] Considering the structural similarity between PhS-$SnMe_3$ and PhS-TMS, we asked the question whether the two followed a similar mechanism of action. Along this direction, we examined the reaction product of PhS-$SnMe_3$ and Z-LysNCA at a M/I ratio of 10/1 in mixed THF/DMF (V_{THF}/V_{DMF} = 100/300). We observed a set of peaks that are attributable to PZLL bearing the C-terminal phenyl thioester and the N-terminal free primary amine in the MALDI-TOF mass spectrometry (Figure 3A). To further validate the presence of the phenyl thioester group on the polypeptides, we examined the native chemical ligation (NCL) reactivity of the polypeptide.[30,31] Specifically, we prepared a highly water-soluble model polypeptide P(EG_3-Glu) via the PhS-$SnMe_3$-mediated ROP of EG_3-GluNCA in mixed THF/DMF, and capped the N-terminal amine by acetic anhydride to avoid chain cyclization. SEC analysis of the polymer gave a MW of 8.2×10^4 g·mol^{-1} and a $Đ$ value of 1.13. An interferon-α mutant bearing the N-Cys (Cys-IFN) was selected as the model protein for NCL. Room-temperature incubation of P(EG_3-Glu) and Cys-IFN for 8 h at a molar ratio of merely 3/1 yielded a modest conversion of the protein (~35%). In the SDS-PAGE gel, the purified conjugate showed a smeared band in the high molecular weight

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region, indicating the successful conjugation (Figure 3B). It should be emphasized that most protein−polymer conjugations require large excesses of polymer (above 10/1 and up to 200/1 molar raito),[32] and the ratio usually increases exponentially with the growth of the MW. Thus, it was remarkable to attach such a high MW polymer to the protein under our experimental conditions. Together, the results unambiguously confirmed the high fidelity of the phenyl thioester end group on the polypeptides. To interrogate the functional group at the *N*-terminus responsible for the chain propagation, we employed mass spectrometry and ^{1}H NMR spectroscopy. Due to the high reactivity and instability, however, all attempts failed to identify the real chain propagation species. In order to use the polypeptide generated from this initiation method for biological applications, the removal of the toxic tin species should be a necessary prerequisite. For this, we measured the remaining tin element in the polypeptides by inductively coupled plasma mass spectrometry (ICP-MS). It was concluded that more than 99.97% elimination efficiency could be easily achieved with less than 8 ppm tin existing in the final polypeptides after routine purification practices (Table S5).

In conclusion, controlled ROP of NCAs was achieved by using a novel Lewis pair PhS-SnMe$_3$. PhS-SnMe$_3$ afforded polypeptides with rapid polymerization rate, ultrahigh MW, and low polydispersity index. An interesting solvent effect was described, which could be utilized to fine-tune the polymerization rate and MW of the polymers. The obtained polypeptides were found to bear the desired C-terminal phenyl thioester readily applicable for site-specific bioconjugation. The unusual solvent effect we observed here is likely due to the different solvent polarity.[33] Indeed, the polymerizations in other low polarity solvents such as dichloromethane gave very similar outcomes (e.g., kinetic profiles and MW control) with those in THF, whereas those conducted in DMSO and DMF resembled each other (data not shown). We hypothesize that the S−Sn bond is stable in low polarity solvents such as THF, which leads to slow and inefficient initiation. This notion is supported by the tailing of the SEC curve at the lower MW region for the ROP in pure THF (Figure 1A), which implies a small portion of late initiation. In high polarity solvents such as DMF, the S−Sn is expected to be easily polarized and activated for highly efficient chain initiation. In the chain propagation step, it is believed that the proposed reactive center, likely the *N*-SnMe$_3$ species, may form relatively looser pairs (more active) in DMF and tighter pairs (less active) in THF, again a result of the polarity effect. This hypothesis is well-supported by our experimental results in mixed THF/DMF (Figure 1C−E). More detailed mechanistic studies, both experimentally and theoretically, will be reported in a separate work for better understanding of this novel initiation system. Overall, this work sheds light on regulating the ROP of NCA via careful solvent selection and is particularly useful in preparing ultrahigh MW polypeptides within a short period of time, which in turn enables the facile generation of high MW protein−polymer conjugates.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.8b00465.

Characterization data (^{1}H NMR, ICP-MS) and SEC curves (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: chemhualu@pku.edu.cn.

ORCID

Hua Lu: 0000-0003-2180-3091

Notes

The authors declare no competing financial interest.

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