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Streamlined Synthesis of PEG-Polypeptides Directly from Amino Acids

Tianrui Xue, Ziyuan Song, Yizhuo Wang, Boya Zhu, Zhihao Zhao, Zhengzhong Tan, Xuefang Wang, Yingchun Xia, and Jianjun Cheng*

ABSTRACT: The application of synthetic polypeptides is greatly limited by the difficulty of the purification and polymerization of N-carboxyanhydrides (NCAs). Here, we report a streamlined, controlled synthesis of polypeptides directly from amino acids, avoiding the NCA purification, by adding small-molecular amine scavengers (AS) in situ to efficiently eliminate the remaining organic impurities in the emulsion polymerization system. Such a process enables controlled synthesis of PEG-containing, homo-, block, and random polypeptides in a highly consistent manner under open-air condition, directly from amino acid derivatives in various formats and independent of NCA preparation methods.

INTRODUCTION

Poly(ethylene glycol) (PEG)-polypeptides block copolymers are of great interest in self-assembly and nanobiotechnology, and are widely used in various biomedical applications, such as drug delivery and tissue engineering. While it is possible to conjugate PEG to the terminal of polypeptides, this method is less desirable because of the low efficiency of conjugating two polymer chain ends and the difficulties of synthesizing PEGylated multiblock polypeptides. Most of the PEG-polypeptide syntheses are through the ring-opening polymerization of amino acid N-carboxyanhydrides (NCA) by a PEG-initiator. Controlled polymerization of NCAs by preformed PEG-amido amidate/Ni complexes was reported by Deming and co-workers, but this method requires multistep synthesis of the specific PEG amido amidate initiator and therefore is challenging to handle to nonexperts. PEG-polypeptides are almost exclusively synthesized by PEG amine-initiated NCA polymerization, following the normal amine polymerization mechanism.

Controlled NCA polymerizations by PEG-amine or amine derivatives have been reported with special techniques, such as under high vacuum, under nitrogen flowing at low temperature, using ammonium salts, amine-borane Lewis pairs, or PEG-amine-trimethylsilylecarbonate. Besides the drawbacks of stringent polymerization setup and slow polymerization rates, all of these methods require the use of ultrapure NCA monomers obtained through tedious, multistep crystallization, or column purification under inert gas protection. Otherwise, the acidic and electrophilic impurities generated during NCA synthesis would inhibit the polymerization by protonating or reacting with the amine initiators. Despite the recent development of simplified strategies of NCA synthesis and purification, its handling and subsequent polymerization still impose significant challenges to nonexperts, limiting the broad applications of polypeptide materials. In addition, synthesis, storage, and polymerization of NCAs all require water-free conditions due to their instability to water, further complicating the handling of NCA monomers. It would be of great interest and profound impact to synthesize PEG-polypeptides directly from amino acids with in situ formed NCA through a robust, simple, and reproducible process, rendering the easy access to polypeptides by nonexperts.

Here, we report the synthesis of PEG-polypeptides with homo-, random-, and block polypeptide segments directly from amino acids. NCAs are used as unpurified intermediates in this polymerization process and still yield polypeptides in controlled molecular weight (MW) and low molecular weight distribution (MWD, D < 1.3) from the unpurified NCAs. This polymerization process tolerates impurities from at least two most popular NCA synthesis methods: the Fuchs-Farthing method and the Leuchs method. The strategy is simple, robust, and reproducible for the synthesis of PEG-polypeptides, with polypeptide segment bearing a variety of side chain functionalities and predesigned compositions and structures.

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RESULTS AND DISCUSSION

We recently reported the preparation of polypeptides from nonpurified NCAs in a water-in-oil (w/o) emulsion system following a polymerization method namely SIMPLE (Segregation-Induced Monomer-Purification and Initiator-Localization promoted rate-Enhancement).45 In the emulsion system of SIMPLE polymerization, impurities of the nonpurified NCA were largely extracted from the oil phase (usually CH₂Cl₂ or CHCl₃) to the water phase, which leaves in situ purified NCAs in the organic phase for fast, cooperative polymerization initiated by PEG-block-poly(γ-benzyl-l-glutamate) (PEG−PBLG) that outpaces water-induced side reactions. However, batch-to-batch variations were observed in polypeptide syntheses from nonpurified NCAs with higher phosgene feedings (>1.5 equiv). For example, polymerization of nonpurified γ-benzyl-l-glutamate NCA (BLG-NCA) and N₂-carboxybenzyl-l-lysine NCA (ZLL-NCA) sometimes resulted in multimodal GPC peaks (Figure S2a−c and entries 1−3, Table S1). In addition, nonpurified NCAs with multistep amino acid synthesis exhibited relatively poor polymerization results, limiting the scope of the emulsion polymerization strategy. For example, GPC analysis of the polypeptides from the polymerization of nonpurified, alkyne-functionalized NCA, γ-(4-propargyloxybenzyl)-l-glutamate (POB-NCA), showed a shoulder peak at the low MW region (Figure S2d) with a fairly broad dispersity (D = 1.73; entry 4, Table S1).

Analyzing the Impurities of the Nonpurified NCAs in SIMPLE Polymerization. For all these poorly controlled polymerizations, one of the shoulder peaks has a similar elution time as the PEG−PBLG macroinitiators, suggesting the termination of macroinitiator at the beginning of the polymerization (Figure S2). While we previously hypothesized that most impurities were removed by petition of water-soluble impurities into the water phase by aqueous extraction prior-cleanup and in SIMPLE emulsion polymerization due to their good water-solubility and high hydrolytic tendency and therefore controlled polymerizations under most conditions were realized,45 the GPC analyses suggested presumably incomplete removal of the impurities in the nonpurified NCAs and the existence of impurities in the oil phase that impairs polymerization by deactivating the initiators or terminating the propagating polypeptide chains in SIMPLE polymerization (Figure S2 and Table S1). Here, we classify impurities with great water-solubility and high hydrolytic tendency as hydrophilic impurities and those that stay in oil phase as hydrophobic impurities.

We first attempted to identify the reactive impurities after the aqueous extraction steps that may remain in organic phase and quench nucleophilic amine initiators. Nonpurified ε-leucine NCA (Leu-NCA) was selected as the model NCA, which was obtained by treating ε-leucine with phosgene. After the removal of excessive phosgene and the solvent in vacuo, the nonpurified Leu-NCA was dissolved in CHCl₃, which was subsequently extracted with an aqueous buffer (pH 3) to remove hydrophilic impurities. Benzylamine (10 equiv. to Leu-NCA) was then used as a probe to react with the Leu-NCA and other possible amine-reactive, electrophilic organic impurities that would otherwise deactivate amine initiators and amine-terminal polypeptide chain ends in an NCA polymerization. The molecular structures of the products were analyzed by LC-MS, which revealed three substrates with presumable structures of 1a, 2a, and 3a (as shown in Figure 1b).

Figure 1. Analysis of impurities from the reaction of benzylamine and the nonpurified Leu-NCA derived from phosgenation. Chemical structure determination of the quenched impurities 1a, 2a, and 3a by LC/MS (a) and their corresponding precursors 1b, 2b, and 3b as the reactive impurities that would terminate the initiation and the chain propagation of NCA polymerization by an amine initiator (b).

derived from the reactions between the remaining impurities from the phosgene/THF reaction and benzylamine, corresponding to residual reactive impurities phosgene (1b) and phosgene derivatives 2b and 3b as the reactive impurities that would terminate the initiation and the chain propagation of NCA polymerization by an amine initiator (b).

Hypothesis of Using Small Molecule Amine As the Scavenger of the Organic Impurities for SIMPLE Polymerization. Our previous studies showed that when NCA polymerization was initiated by a macroinitiator with pendant, multiple amine groups in close proximity, the polymerization can be finished in 30−40 min at [M]₀ = 50 mM.46,47 However, if a similar polymerization was initiated by a single amine, the polymerization required much longer time to complete (>20 h). Their polymerization kinetics differ by at least hundreds of times because of the accelerated, cooperative polymerization induced by the added dipole effect due to aligned helical polypeptides in proximity and significantly enhanced reactive polymer chain−NCA binding in branched polymer macroinitiator.48 We previously found that in the presence of both a macroinitiator (a polynorbornene with pendant amine) and n-butylamine, NCA polymerization was initiated almost exclusively by the macroinitiator (not reported). As the rate of macroinitiator initiated NCA polymerization is comparable to that of SIMPLE polymerization initiated at the water/oil interfaces45 and because of the impurities in the organic phase resulted from the NCA synthesis are typically electrophiles that are reactive to nucleophiles, such as amine, we seek the possibility of using small molecule amine as the scavengers (amine scavenger (AS)) of the hydrophobic impurities prior to the SIMPLE polymerization, which would presumably enable the stream-
linded synthesis of polypeptides directly from amino acids (Schemes 1 and 2).

**Introduction of Amine Scavenger (AS) in SIMPLE Polymerization.** We first tested if the addition of AS would lead to uncontrolled, competing polymerization of purified NCA monomers in SIMPLE polymerization. *n*-Hexylamine was added as the AS and PEG−PBLG was used as the macroinitiators to polymerize the purified BLG-NCA in a w/o emulsion. After the aqueous extraction step, various amounts of AS ([AS]:[I]0 from 0.05 to 1) was added into the biphasic mixture, vortexed for 30 s, and mixed with the w/o emulsion containing PEG−PBLG macroinitiators. The addition of ∼1 equiv. AS (relative to PEG−PBLG macroinitiator) showed negligible impact on NCA polymerization (Figures 2a and S4 and Table S2), validating our hypothesis that the small molecular AS does not interfere with the NCA polymerization by PEG−PBLG at the w/o interfaces in the SIMPLE polymerization. For polymerization at monomer-to-initiator ([M]0/[I]0) ratio = 200, the MW of the resulting polymer was 54.6 and 52.7 kDa in the absence and presence of AS, respectively, with nearly identical dispersity (Figure 2a and Table S2). These results agree well with our previous finding that the polymerization initiated by PEG−PBLG in the biphasic SIMPLE polymerization is much faster than that initiated by *n*-hexylamine in a conventional NCA polymerization in a single phasic organic solution.45

We then tested the efficiency of impurity removal with the addition of *n*-hexylamine as the AS prior to the SIMPLE polymerization of the nonpurified BLG-NCA that showed poorly controlled polymerization by SIMPLE after aqueous extraction. The water/CHCl3 emulsion with nonpurified NCA was first prepared. After the removal of aqueous phase by centrifugation, the AS was added in the emulsion for the intended removal of the hydrophobic impurities, prior to the addition of PEG−PBLG in CHCl3, the polymerization of nonpurified NCAs as above-mentioned. The introduction of AS to SIMPLE polymerization indeed significantly improved the polymerization, resulting in polypeptides with monomodal GPC peak, expected MW, and sharply decreased dispersity (D = 1.16 for polymerization with AS addition vs D = 1.66 without AS addition; Figures 2b and S5 and Table S3). The polymerizations also showed excellent reproducibility with the addition of AS. For polymerizations with the AS added, the obtained MWs of the resulting PEG-polypeptide copolymers synthesized from different batches of nonpurified BLG-NCA were in excellent agreement with the MWs obtained from the polymerization of purified BLG-NCA (Figure S6 and Table S4).

**Streamlined Synthesis of PEG-Polypeptides in the Presence of AS.** The successful removal of impurities by emulsion and the addition of AS that enabled controlled SIMPLE polymerization of NCA encouraged us to further simplify the preparation of polypeptides. With the high conversion of amino acids in the NCA synthesis step,43 we reasoned that we were able to skip not only the NCA purification but also the NCA isolation and storage, leading to a streamlined synthesis of polypeptides directly from amino acids. Specifically, after the phosgenation of γ-benzyl-L-glutamate (BLG) and the removal of solvent, the obtained nonpurified BLG-NCA was dissolved in CHCl3 and directly subjected to aqueous extraction and AS treatment, followed by the addition of the w/o emulsion containing PEG−PBLG macroinitiator to start the polymerization. This streamlined strategy ensures the synthesis of polypeptides directly from amino acid within 3−4 h, which circumvents the tedious and
time-consuming NCA purification and storage that requires moisture-free apparatus (i.e., glovebox).

We first carried out the SIMPLE polymerization in the presence of AS from BLG, the starting amino acid, with various designed MWs, which were calculated based on the feeding amount of the amino acid BLG (rather than the isolated and quantified nonpurified BLG-NCA) to the PEG−PBLG macro-initiator. The obtained MWs of the resulting polypeptides were in perfect agreement with the calculated MWs, and the polypeptides have very narrow polydispersities (Đ < 1.1; Figure 3a,b, and entries 1−4, Table 1). These results suggest the almost quantitative conversion of BLG to BLG-NCA and validate our streamlined SIMPLE polymerization design by adding AS to remove the remaining impurities. The polymerizations were extremely fast as monitored by NMR with the use of PEG−PBLG initiator, reaching >98% NCA conversion within 15 min at [M]0/[I]0 (BLG/PEG−PBLG) = 150 (Figure S7). The fast NCA polymerization is crucial to outpace the competing reaction of the NCA with water that otherwise would take hours to complete, as reported in our previous studies that NCA hydrolysis by water contributes to less than 0.03% of NCA consumption in SIMPLE polymerizations with similar polymerization kinetics.45 In addition, the fast NCA polymerization would also outpace the NCA ring-opening reaction with AS that has a reaction rate 10^3−10^4 slower than the cooperative chain propagation rate in the SIMPLE polymerization.45,47 When the polymerization was stopped at several selected time intervals, the MWs of the obtained polymers were found to increase linearly with the conversion of BLG-NCA (Figures 3c and S8 and Table S5), indicating the livingness of SIMPLE polymerization in the presence of AS for the nonpurified BLG-NCA.

The streamlined strategy can also be applied to many other types of amino acids. We tested the synthesis of various polypeptides from several other amino acids, including Nε-carboxybenzyl-L-lysine (ZLL), l-leucine (Leu), and γ-(4-propargyloxybenzyl)-l-glutamate (POB; structures shown in Scheme 1), with the streamlined strategy in the presence of AS. The MWs of all obtained polypeptides agreed very well with the expected MWs with low dispersity (Đ < 1.2; entries 5−7, Table 1 and Figure S9a,b), demonstrating the robustness of the streamlined SIMPLE polymerization strategy for the synthesis of polypeptides directly from amino acids.

We also explored if the streamlined process can be used to prepare polypeptides in a much larger scale (gram-scale instead of tens to sub-100 mg scale). Polypeptides can be easily obtained with great control over MWs, narrow dispersity (<1.1) and high isolated yield (89%) with gram-scale amino acid starting materials (Figure 3e,f and Table S6).
Streamlined Synthesis of PEG-Polypeptides with Complex Chemical Compositions.

After demonstrating that the streamlined synthesis of polypeptides directly from amino acids is very robust and highly reproducible via the living polymerization of in situ synthesized NCAs from a broad range of amino acids, we next explored the synthesis of random or block copolypeptides using this streamlined synthesis strategy.

The chemical composition of random copolypeptides is typically tuned by changing the NCAs ratio in a mixed NCA solution, one such example composed of four amino acids (glutamic acid, lysine, alanine, and tyrosine), known as glatiramer acetate, is an FDA-approved immunomodulator medication currently used to treat clinical multiple sclerosis. Instead of undertaking purification of each NCA monomer, the streamlined strategy was tested if it would enable the preparation of random copolypeptides with desired MWs and chemical composition directly from the mixture of various amino acids. BLG, ZLL, and Leu amino acids were mixed at certain ratio and treated with phosgene to in situ form BLG-, ZLL-, and Leu-NCA, which were applied to the streamlined polymerization method above-mentioned. The chemical composition of the obtained random copolypeptides agreed perfectly with the feeding ratio of the amino acids based on the feeding ratio of the amino acids based on the feeding ratio of the amino acids based on the feeding ratio of the amino acids based on the feeding ratio of the amino acids based on the feeding ratio of the amino acids based on the feeding ratio of the amino acids.

![Figure 3](image_url)

**Figure 3.** (a) GPC analysis (light scattering (LS) traces) of PEG–PBLG prepared via streamlined strategy at various [M]₀/[I]₀ (BLG/PEG–PBLG) ratios in the presence of n-hexylamine as AS. [I]₀ = [AS]₀ = 0.5 mM; (b) comparison of the expected MWs and the obtained MWs, and the polydispersity of the obtained polypeptides in panel a; (c) comparison of the expected MWs and the obtained MWs, and the polydispersity of the obtained polymers at various calculated NCA conversion in the presence of n-hexylamine as AS. [M]₀ = 0.075 M, [AS]₀ = 0.375 mM, [M]₀/[I]₀ (BLG/PEG–PBLG) = 150; (d) GPC analysis (LS traces) of triblock copolypeptides, [M]₀/[I]₀ (corresponding amino acid/PEG–PBLG) = 50 for each block; (e) photo for the open-air streamlined polymerization for the synthesis of PEG–PBLG₂₀₀ starting from 1 g of BLG. (f) GPC analysis (LS traces) of PEG–PBLG₂₀₀ starting from 1 g of BLG compared with PEG–PBLG₂₀₀ starting from 10 mg of purified BLG-NCA. [M]₀ = 0.1 M, [M]₀/[I]₀ = 200.

<table>
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<th>entry</th>
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<th>cyclization method</th>
<th>cyclization reagent</th>
<th>[M]₀/[I]₀</th>
<th>Mₘ/ Mₘ*</th>
<th>Đ</th>
<th>yield (%)</th>
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<td>37.6/37.0</td>
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<td>40.9/40.7</td>
<td>1.14</td>
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<td>Fuchs-Farthing</td>
<td>COCl₂</td>
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<td>26.2/25.8</td>
<td>1.09</td>
<td>86</td>
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<td>COCl₂</td>
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<td>29.5/26.0</td>
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Polypeptides were synthesized at room temperature using PEG–PBLG initiator in the presence of n-hexylamine as AS. [I]₀ = [AS]₀ = 0.5 mM. The conversion of NCA intermediate was above 99% as confirmed by FTIR. BLG: γ-benzyl-L-glutamate amino acid; ZLL: N-ε-carboxybenzyl-L-lysine; Leu: L-leucine; POB: γ-(4-propargyloxybenzyl)-L-glutamate; Z-BLG: α-carboxybenzyl-γ-benzyl-L-glutamate; Z-α-glC-C: N-Carbobenzyloxy-L-cysteine-1-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside). COCl₂: phosgene; Cl₂CHOCH₃: dichloromethyl methyl ether. Obtained by GPC. Obtained by NMR. Poly(α-glC-C) was synthesized from crude Z-α-glC-C.
polypeptides containing PBLG-
2, Table S7). Random copolymerization of NCAs allows the synthesis of block copolypeptides by GPC.

Table 2. Preparation of Random and Block Co-Polypeptides via Streamlined Synthesis in the Presence of ASa

<table>
<thead>
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<th>entry</th>
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<th>ratioa</th>
<th>ratioab</th>
<th>M/Mn,c (kDa)</th>
<th>Df</th>
<th>yield</th>
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<td>37:41:22</td>
<td>36.2/36.5</td>
<td>1.10</td>
<td>87</td>
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<tr>
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<td>POB50-PLeu20</td>
<td>80:20</td>
<td>82:18</td>
<td>40.7/39.1</td>
<td>1.14</td>
<td>81</td>
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<tr>
<td>3</td>
<td>PBLG40-PBLS50</td>
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<td>73:27</td>
<td>35.1/35.3</td>
<td>1.21</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>PBLG40-PPOB50-PZLL50</td>
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<td>37:28:35</td>
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<td>1.20</td>
<td>86</td>
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<tr>
<td>5</td>
<td>(PBLG40-r-PZLL50)-PPOB50</td>
<td>33:33:33</td>
<td>33:34:32</td>
<td>52.6/52.7</td>
<td>1.15</td>
<td>87</td>
</tr>
</tbody>
</table>

aAll polymerizations were carried out at room temperature initiated by PEG−PBLG in the presence of n-hexylamine as AS. [I]o = [AS]o = 0.5 mM. The conversion of NCA intermediate was above 99% as confirmed by FTIR. Random copolypeptides were synthesized from a mixture of amino acids. Block copolypeptides were synthesized by sequential addition of the corresponding nonpurified NCAs. All polypeptides contain a PEG−PBLG segment from the macroinitiator. The expected composition. The obtained composition of final polypeptides by NMR analysis. Obtained by GPC. The obtained MWs/the expected MWs.

Figure 4. (a) Synthesis of NCAs from N-benzoylcarbonyl-l-amino acid and the formation of benzyl chloride as the presumable hydrophobic impurity. (b) LC-MS analysis of the products of benzylamine (as the AS) reaction with nonpurified Leu-NCA derived from the Leuchs method. (c) GPC analysis (dRI traces) for PEG−PBLG from SIMPLE polymerization of nonpurified BLG-NCA derived from Leuchs method with or without n-hexylamine as AS. [M]o = 0.1 M, [AS]o = 0.5 mM, [M]o/[I]o = 200. (d and e) GPC analysis (LS traces) (d) and comparison of the expected MWs, the obtained MWs and the obtained dispersity (e) for PBLG prepared via streamlined strategy using nonpurified BLG-NCA derived from Leuchs method at various [M]o/[I]o ratios in the presence of n-hexylamine as AS. [I]o = [AS]o = 0.5 mM.

NMR analysis; the obtained MWs (Mn = 36.2 kDa) of the random copolypeptides were also in perfect agreement with the expected MWs (Mn = 36.5 kDa; entry 1, Table 2, Figure S9c and entry 1, Table S7). PEG-polypeptides containing PPOR-P-Leu segment can be easily obtained with narrow dispersity and great control over MWs and chemical composition as well (entry 2, Table 2, Figure S9d, and entry 2, Table S7). Random copolymerization of NCAs allows the incorporation of other NCA monomers such as β-sheet-forming NCAs, whose resulting polypeptides usually exhibit poor solubility. We further investigated if the streamlined method could be used for the incorporation of β-sheet-forming amino acids into polypeptides in a random manner. PEG-polypeptides containing PBLG-r-poly(O-benzyl-1-serine) (PBLG-r-PBLS) segments with 25% of O-benzyl-L-serine (BLS) residues were easily obtained, with great control over the MWs and chemical composition from BLS and BGL mixture (entry 3, Table 2 and Figure S9e). These results validate the robustness of our designed streamlined polymerization strategy for the preparation of random copolypeptides.

Conventional preparation of block copolypeptides typically requires very high monomer purity due to the requirement for the high fidelity of the terminal amines on the propagating chain end during polymerization.56,57 We next tested if block copolypeptides can be prepared in the streamlined manner in the presence of AS. Nonpurified BLC-NCA, POB-NCA, and ZLL-NCA were prepared separately and then added sequentially with the w/o emulsion containing PEG−PBLG to start the polymerization after aqueous extraction and AS treatment. The resulting polymers with the desired block copolymer characteristics were demonstrated by GPC and NMR analysis (Figure 3d and S10). All obtained block copolypeptides presented predictable MWs and chemical compositions with narrow dispersity (D < 1.2; entries 3−4, Tables 2 and S8), validating the compatibility of this streamlined process for the synthesis of block copolypeptides directly from amino acids.

Streamlined Synthesis of PEG-Polypeptides Using Nonpurified NCA from the Leuchs Method. We have demonstrated that well-defined polypeptides with controlled MWs and low dispersity can be synthesized in a streamlined manner in the presence of AS via nonpurified NCA intermediate prepared from amino acid phoshgenation, the so-called Fuchs-Farthing method.52,53 Leuchs method,54 using N-alkoxycarbonyl α-amino acids with halogenating reagents, is also widely utilized for the preparation of NCAs.11,52,55,56 During the synthesis, however, various hydrophobic impurities (e.g., alkyl halide, Figure 4a) are formed and therefore the resulting NCAs in poor purities are nonusable for controlled polymerization synthesis;37,40 the clean NCAs suitable for polymerization after recrystallization or other purification steps are often in suboptimal yields. For amino acids with complex side-chain functionalities, they are often in its nonpurified NCA form that can only be subject to the Leuchs method for NCA preparation. We next tested if the AS-present, streamlined SIMPLE polymerization process above-mentioned can be started with Nα-alkoxycarbonyl α-amino acids via the nonpurified NCAs synthesized from the Leuchs method.

Similar to the polypeptides from the SIMPLE polymerization of nonpurified POB-NCA, the polypeptides from the SIMPLE polymerization of nonpurified BLC-NCA derived...
from Leuchs method also showed a shoulder peak in its GPC trace, (Figure 4c) and has a MW higher than the expected value (entry 1, Table S9). Since this shoulder peak has similar elution time as the PEG−PBLG, we hypothesized that the hydrophobic impurities in nonpurified BLG-NCA from the Leuchs method after aqueous extraction also led to the deactivation of the propagating polypeptide chain ends, resulting in poor control over the polymerization. We then attempted to characterize the major organic impurities of the Leuchs method. Nonpurified Leu-NCA was obtained by treating N-benzyloxycarbonyl-L-leucine with dichloromethyl methyl ether. The molecular structures of the major organic impurities were then investigated by LC-MS. Benzyl chloride was found to be one of the major amine-reactive impurity in the organic phase (Figure 4a,b), which is consistent with previous reports.37 A various amount of AS ([AS]₀/[I]₀ from 0.2:1 to 10:1) was added into the biphasic mixture of the nonpurified BLG-NCA obtained from the Leuchs method by reacting α-carboxybenzyl-γ-benzyl-L-glutamate (Z-BLG) with dichloromethyl methyl ether followed by aqueous extraction, the w/o emulsion containing PEG−PBLG initiator was then added to start the polymerization. The introduction of 2 equiv. AS (relative to initiator) significantly eliminated the shoulder peak in the GPC trace of the obtained polymers, yielding polypeptides with the expected MW (Figure 4c and S11 and Table S9). All obtained polymers have predictable MWs and narrow dispersity (D < 1.1; Figure 4d,e and entries 8−11, Table 1). In addition, crude N-carbobenzyloxy-L-cysteine-1-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside) (Z-α-glC) was prepared following the reported method45 and used as the starting materials for the preparation of PEG-polypeptides. The narrow dispersity and the well-defined GPC-LS trace (entry 12, Table 1 and Figure S9f) of the obtained glyco-polypeptides demonstrated the great compatibility of streamlined method with glycosylated amino acids. The suboptimal yield and lower MWs presumably originated from the impurities in Z-α-glC-C (Figure S13) that complicated the calculation. The results above demonstrated the robustness of streamlined synthesis of polypeptides from α-amino derivatized amino acids.

**CONCLUSION**

We previously reported the SIMPLE polymerization method for the open-air polymerization of nonpurified NCAs. However, the SIMPLE method sometimes shows suboptimal polymerization due to uncontrollable amounts of organic impurities with different conditions of starting materials. Helical PEG−PBLG with an amine chain end aligned at the o/w interfaces polymerizes NCA in a very fast manner (typically <20 min), following the second-stage kinetics of the cooperative NCA polymerization that is further enhanced by the added macrodipole effects of aligned polypeptide chains in close proximity, as demonstrated in our previous studies.45−47 In contrast, a small molecule amine (e.g., n-hexylamine used as the scavenger in this study) initiated NCA polymerization has to go through a much slower first-stage of the cooperative polymerization before reaching a faster second-stage. In general, PEG−PBLG in the SIMPLE polymerization process with second-stage chain propagation rates are hundreds to thousands of times faster than the small molecule amine induced NCA reaction or first stage oligomerizations, as explained in our previous detailed kinetic studies.45−47 Thus, though primary amines (e.g., n-hexylamine) are widely used as the initiator of NCA polymerization in many conventional single phasic systems, our studies show that they are very safe to be used as the scavenger of organic impurities and induce negligible NCA polymerization in the presence of another polymerization mechanism with a polymerization kinetics hundreds to thousands of times faster. The addition of AS greatly improves the SIMPLE polymerization and together contributes to the design of the streamlined, controlled synthesis of polypeptides from various amino acid starting derivatives. This method is highly reproducible and robust to various NCA preparation methods, presumably yielding polypeptides with any complex structures. As the current polymerization is initiated by a PEG−PBLG macroinitiator (PBLG can be changed to any other helical polypeptides), the current streamlined polymerization is very useful for the preparation of PEG-containing homo-, block, and random polypeptides in controlled MWs and low polydispersities in open-air condition at any scale.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.macromol.0c00470.

Materials and methods, experimental procedures, and additional figures and tables (PDF)

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