ACS Macro Letters

pubs.acs.org/macroletters Letter

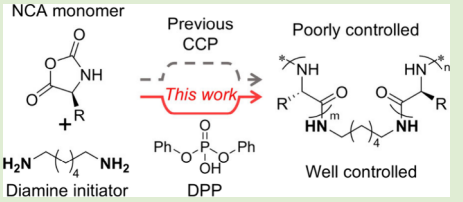
Controlled Synthesis of Telechelic Polypeptides via Polymerization of *N*-Carboxyanhydrides Mediated by Phosphate Kinetic Modulators

Tingting Cao, Chenlin Ji, Qianyi Qin, Hui Wang, Yu Zhao,* Ziyuan Song,* and Jianjun Cheng*

Cite This: *ACS Macro Lett.* 2025, 14, 858−864 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Telechelic polypeptides add structural diversity to their homo- and block-analogues and have potentially expanded applications in supramolecular chemistry, drug delivery, and tissue engineering. Their synthesis via the ring-opening polymerization of amino acid *N*-carboxyanhydride (NCA), however, is limited by slow polymerization rates or poor control over molecular weights (MWs). Here, we report a diamine-initiated, diphenyl phosphate (DPP)-regulated cooperative covalent polymerization to achieve a controlled synthesis of telechelic polypeptides with predictable MWs and narrow dispersity ($Đ$ < 1.1). Mechanistic studies reveal that DPP mediates noncovalent interactions with propagating amino chain-ends, suppressing side reactions, and facilitating controlled chain propagation. Remarkably, this robust method is compatible with crude NCAs, avoiding tedious purification steps that enable the scalable synthesis of telechelic polypeptides with tailored architectures and unprecedented simplicity.



Downloaded via WESTLAKE UNIV on September 23, 2025 at 02:30:34 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

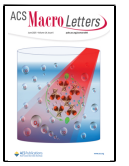
Polypeptides have gained significant attention in biomedical applications owing to their distinctive structures, versatile functions, and inherent biocompatibility,[1−7] which frequently serve as building blocks for hierarchical architectures and advanced biomaterials.[8−17] Among various polypeptide materials being developed, telechelic polypeptides, which feature functional end groups at both termini, have emerged as structurally unique building blocks relative to their homo- and block-polypeptide analogues for further expanded opportunities in supramolecular chemistry and biomedical applications.[18−22] In theory, telechelic polypeptides can be synthesized through two primary approaches: diamine-initiated ring-opening polymerization (ROP) of *N*-carboxyanhydrides (NCAs) that generate polymers with identical terminal groups at both ends, or alternatively, through ROP processes employing functionalized initiators in combination with end-capping reagents to produce telechelic architectures with either identical or differentiated end-group structures.[23−25] Of these synthetic strategies, the diamine-initiated NCA polymerization approach demonstrates particularly advantageous operational convenience.

However, a few previously reported telechelic polypeptide syntheses via diamine-initiated ROP of NCA in DMF undergo a poorly controlled process, depicting a slow polymerization rate, competing side reactions, and the requirement of ultrapure NCA monomers.[9,26−28] In our previous studies, we observed that diamine could initiate NCA polymerization in solvent with a low dielectric constant at a rate a few orders of magnitude higher than that of monoamine, indicating the added complexity of controlling the polymerization.[29] The surprising acceleration of polymerization is presumably due to the covalent cooperative polymerization (CCP) in which the polymerization rates can be greatly affected by the neighboring polypeptide chains in proximity.[30] However, the intrinsic kinetic disparity between the two stages of CCP, namely, the slow first stage and the fast second stage, led to significantly elevated MWs and broad molecular weight distributions (MWDs) in the synthesis of telechelic polypeptides.[31,32] This limitation inevitably hinders the full potential of CPP in constructing well-defined polypeptide structures in diamine-initiated polymerization.

Organic acids have emerged as promising organocatalysts for achieving well-controlled ROPs by regulating the activity of both chain-propagating centers and monomers. Several research groups, including ours, demonstrated the rapid and controlled ROP of NCAs and their analogues catalyzed by acetic acid or benzoic acid.[33−36] To improve the MW control during the preparation of telechelic polypeptides, it is crucial to have a balanced growth of polypeptides on both chain ends instead of just on one end. We thus attempted to use organic acids to coregulate the α-helix-induced (macrodipole-induced) auto-acceleration of polypeptide chain propagation, by slowing down the second stage of CPP.

In this Letter, we report the controlled synthesis of telechelic polypeptides from diamine initiators through the introduction of diphenyl phosphate (DPP) (Figure 1a), a kinetic modulator, which balances the growth of two propagating sites. Compared to the reported diamine-initiated CCP,[29] the incorporation of

Received: May 13, 2025
Revised: May 30, 2025
Accepted: June 2, 2025
Published: June 6, 2025



© 2025 American Chemical Society

https://doi.org/10.1021/acsmacrolett.5c00319
ACS Macro Lett. 2025, 14, 858−864

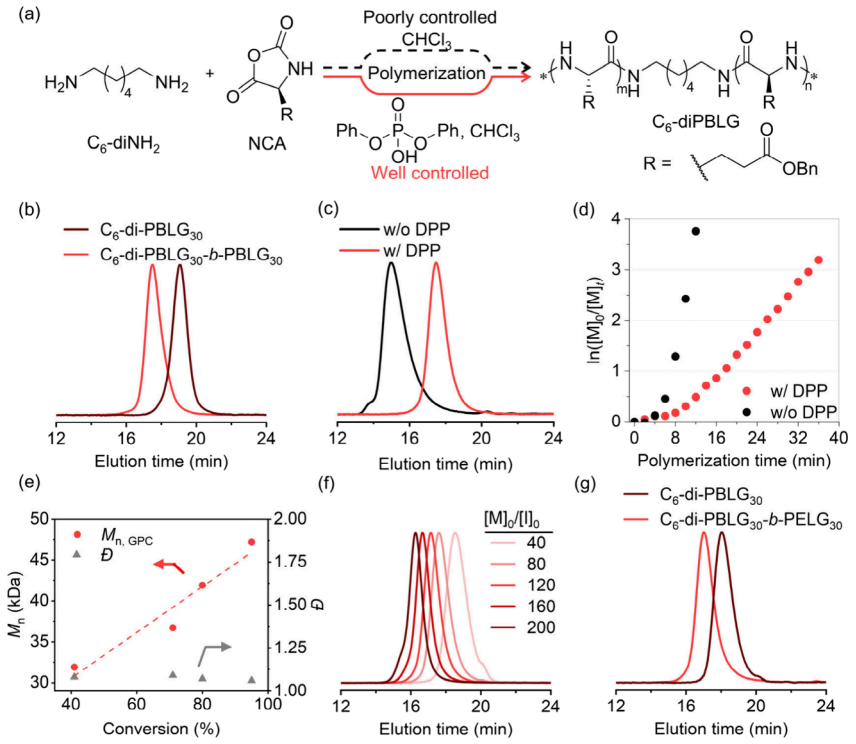


Figure 1. Living polymerization of BLG-NCA in $CHCl_3$ with DPP. (a) Schematic overview of the ROP of NCA in the presence and absence of DPP. (b) Normalized GPC-LS traces of the diamine macroinitiators C_6-di-$PBLG_{30}$ and the resulting telechelic PBLG after CCP in the absence of DPP ($[M]_0/[I]_0$ = 60). (c) Normalized GPC-LS traces of telechelic PBLG from the C_6-diNH_2-initiated polymerization of BLG-NCA in the presence and absence of DPP ($[M]_0/[I]_0$ = 100). (d) Semilogarithmic kinetic plot illustrating the C_6-diNH_2-initiated polymerization of BLG-NCA, as monitored by FT-IR, in the presence and absence of DPP ($[M]_0/[I]_0$ = 100). (e) Changes in MWs and dispersity of C_6-di-$PBLG_{50}$ through the polymerization of BLG-NCA in the presence of DPP at various monomer conversions ($[M]_0/[I]_0$ = 100). (f) Normalized GPC-LS traces of telechelic PBLG from C_6-diNH_2-initiated polymerization of BLG-NCA at various $[M]_0/[I]_0$ ratios. (g) Normalized GPC-LS traces of diblock copolypeptide C_6-di-$PBLG_{30}$-*b*-$PELG_{30}$ and its first-block intermediate C_6-di-$PBLG_{30}$ synthesized from sequential polymerization of NCA in the presence of DPP ($[M]_0/[I]_0$ = 60). All polymerizations were conducted at a $[M]_0$ of 0.1 M and a $[DPP]_0/[I]_0$ of 1.

DPP mediates effective noncovalent interactions with partially protonated, propagating amino chain-ends, thus guaranteeing the simultaneous growth of all polypeptide chains ($Đ$ < 1.1). A series of telechelic polypeptides with different chain lengths, side-chain structures, and block compositions can be efficiently prepared within hours. Notably, the DPP strategy is compatible with the direct polymerization of crude NCAs, circumventing tedious NCA purifications. This robust method offers the scalable synthesis of telechelic polypeptides with tailored architectures, expanding their potential in the construction of complex structures.

In order to validate that the poor control over MWs for diamine-initiated CCP originates from the two-stage polymerization kinetics, we first prepared a diamine macroinitiator by polymerizing γ-benzyl-$_L$-glutamate NCA (BLG-NCA) initiated by 1,6-diaminohexane (C_6-diNH_2) in DMF (Scheme S1).[37] The macroinitiator was then used to initiate the CCP of BLG-NCA in $CHCl_3$, resulting in well-defined telechelic polypeptides with predictable MWs ($Đ$ < 1.1) (Figures 1b and S1, Table S1), demonstrating the concurrent growth of all propagating chains is crucial to regulate the MW in diamine-initiated CCP. To address the challenge of uncontrolled reaction kinetics in chlorinated solvents, we evaluated the impact of a range of weak organic acids on the polymerization of BLG-NCA ($[M]_0$ = 0.1 M, $[M]_0/[I]_0/[Acid]_0$ = 100/1/1, [I] refers to the concentration of initiation molecule) (Table S2). Among various acids, the presence of DPP resulted in well-controlled polymerization initiated by C_6-diNH_2 in $CHCl_3$, yielding well-defined telechelic polypeptides ($Đ$ = 1.07) with a molecular weight (M_n) of 25.8 kDa that agreed well with the expected value (M_n = 22.0 kDa) (Figure 1c, Table 1). In fact, DPP has been reported to enable the living and controlled ROP of δ-valerolactone and ε-caprolactone.[38−40] In contrast, other acids failed to suppress the

Table 1. Polymerization of BLG-NCA in the Presence of DPP[a]

entry	$[M]_0/[I]_0$	t (min)	$M_{n,\,theo.}$ (kDa)	$M_{n,\,GPC}$[b] (kDa)	$Đ$[b]
1[c]	100	15	22.0	65.7	1.14
2	100	35	22.0	25.8	1.07
3	40	20	8.9	10.3	1.31
4	80	30	17.6	22.9	1.07
5	120	45	26.4	30.2	1.05
6	160	60	35.2	39.7	1.05
7	200	90	43.9	49.6	1.05
8[d]	60 + 60	30 + 20	13.3/22.5	16.7/22.8	1.18/1.07
9[c,e]	100	40	22.0	23.5	1.05

[a]All polymerizations were initiated by C_6-diNH_2 in $CHCl_3$ ($[M]_0$ = 0.1 M and $[DPP]_0/[I]_0$ = 1). The monomer conversions were >95% for all polymerizations, as determined by FT-IR. [b]Determined by GPC; dn/dc = 0.095. [c]Polymerization in the absence of DPP. [d]Diblock copolypeptide synthesis through sequential addition of BLG-NCA and ELG-NCA monomers. [e]Polymerization of racemic $_{DL}$-NCA monomers.

https://doi.org/10.1021/acsmacrolett.5c00319
ACS Macro Lett. 2025, 14, 858−864

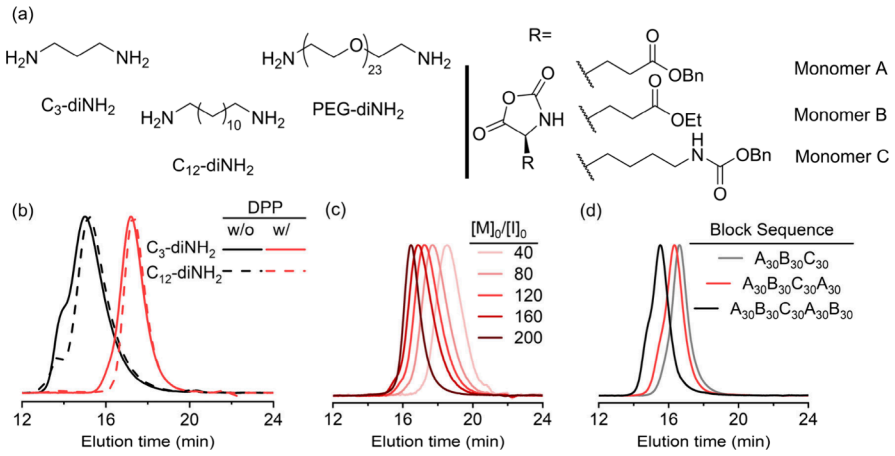


Figure 2. DPP-mediated polymerization of various monomers with different diamine initiators. (a) Chemical structures of different diamine initiators and NCA monomers. (b) Normalized GPC-LS traces of telechelic polypeptides with different aliphatic diamine initiators in the presence and absence of DPP ($[M]_0$ = 0.1 M, $[M]_0/[I]_0/[DPP]_0$ = 100/1/1). (c) Normalized GPC-LS traces of telechelic polypeptides initiated with PEG-diNH$_2$ at various $[M]_0/[I]_0$ ratios ($[M]_0$ = 0.1 M, $[DPP]_0/[I]_0$ = 2). (d) Normalized GPC-LS traces showing the synthesis of block copolypeptides from various NCA monomers ($[M]_0$ = 0.1 M, $[M]_0/[I]_0/[DPP]_0$ = 60/1/1).

uneven growth of polypeptide chains from different propagating chain-ends, leading to much higher MWs (48.3−59.2 kDa) with broad MWD ($Đ$ > 1.2) (Figure S2, Table S2). Indeed, the gel permeation chromatography (GPC) characterization revealed a shoulder peak at the low-MW region, suggesting the existence of oligomeric species.[41] The polymerization kinetics in the presence and absence of DPP were then monitored by FT-IR, which also revealed that DPP preserves the characteristic two-stage profile but crucially attenuates the polymerization rates compared with that without DPP (Figure 1d). The slightly slower polymerization rate contributed to the concurrent growth of polypeptide chains while remaining significantly faster than the conventional polymerization in DMF or the monoamine-initiated CCP. Indeed, the ratio between the reaction rate constants for the first and second stages (k_1/k_2) became larger in the presence of DPP, suggesting a more balanced polymerization at both kinetic stages (Figure S3).

To further optimize the reaction conditions, the molar ratio between DPP and initiators ($[DPP]_0/[I]_0$) was tuned from 0.1 to 10 while maintaining $[M]_0/[I]_0$ = 100. At substoichiometric DPP (i.e., $[DPP]_0/[I]_0$ = 0.1), poor control over MWs was observed (M_n = 46.4 kDa, $Đ$ = 1.17), which was presumably attributed to the insufficient suppression of unbalanced propagation. The increase in $[DPP]_0/[I]_0$ led to a monotonous decrease in MWs as well as elongated polymerization time (Figure S4, Table S3). The addition of equimolar DPP resulted in the overall best control over polypeptide MW and narrow MWD ($Đ$ = 1.07), whose obtained M_n matched the theoretical value. Further increases in $[DPP]_0/[I]_0$, nevertheless, yielded telechelic polypeptides with significantly smaller MWs and broad dispersity ($Đ$ > 1.3) (Table S3). The existence of excessive DPP protonated most propagating chain-ends, leading to elongated polymerization time. Since the polymerization was conducted under open-air conditions, the hydrolysis of NCA monomers is inevitable with the slow polymerization rate.[9] These findings highlight the importance of accelerated polymerization in inhibiting side reactions, thus simplifying the preparation of polypeptide materials. In addition, the monomer concentration was also varied, which revealed insignificant impact on the MW control, with all obtained telechelic polypeptides showed predictable MWs and low dispersity ($Đ$ < 1.1) (Figure S5). Nevertheless, the polymerization at lower $[M]_0$ (0.01 M) required prolonged polymerization time to finish (>4 h), in contrast to the fast polymerization at higher $[M]_0$, which reached >95% conversion within 30 min (Figure S5, Table S4).

With the optimized conditions, we moved on to explore the preparation of various telechelic polypeptides in the presence of DPP. The MWs and monomer conversion exhibited a linear correlation throughout the polymerization (Figures 1e and S6), suggesting a living polymerization process. As a result, the MWs of telechelic polypeptides were easily modulated by changing the $[M]_0/[I]_0$. GPC traces showed monomodal peaks correlating linearly with $[M]_0/[I]_0$ (Figures 1f and S7). Moreover, the living nature of DPP-mediated polymerization enabled an efficient chain extension. Sequential addition of BLG-NCA ($[M]_0/[I]_0$ = 60) and γ-ethyl-$_L$-glutamate NCA (ELG-NCA) ($[M]_0/[I]_0$ = 60) yielded diblock copolymers with narrow dispersity ($Đ$ = 1.07) (Figure 1g, Table 1). The clear MW shift on GPC traces substantiated the high end-group fidelity for chain extension.

The DPP-mediated, accelerated and controlled synthesis of telechelic polypeptides encouraged us to further evaluate the polymerization with other diamine initiators or NCA monomers. Diamines with varied alkyl spacers (i.e., C_3- and C_{12}-diNH$_2$) were first used as the initiators to polymerize BLG-NCA in $CHCl_3$ in the absence of DPP, yielding polypeptides with bimodal GPC distributions ($Đ$ > 1.27) that agreed well with the previous reports (Figure 2a,b, Table S5).[29] In contrast, the polymerization in the presence of DPP produced polypeptides with monomodal GPC traces ($Đ$ < 1.1), regardless of the spacer lengths (Table S5). Moreover, DPP was also incorporated in the polymerization initiated by PEGylated diamines (M_n = 1.0 kDa), which balanced the chain growth that led to reduced obtained MWs compared to that in the absence of DPP (Figure S8). It should be noted that due to the catalytic role of PEG segments in $CHCl_3$,[42] higher $[DPP]_0/[I]_0$ at 2.0 was necessary to suppress the unbalanced chain growth (Figure S9, Table S6), forming polypeptides with monomodal distributions and predictable M_n through the tuning of $[M]_0/[I]_0$ (Figure 2c, Table S7). Additionally, DPP-

https://doi.org/10.1021/acsmacrolett.5c00319
ACS Macro Lett. 2025, 14, 858−864

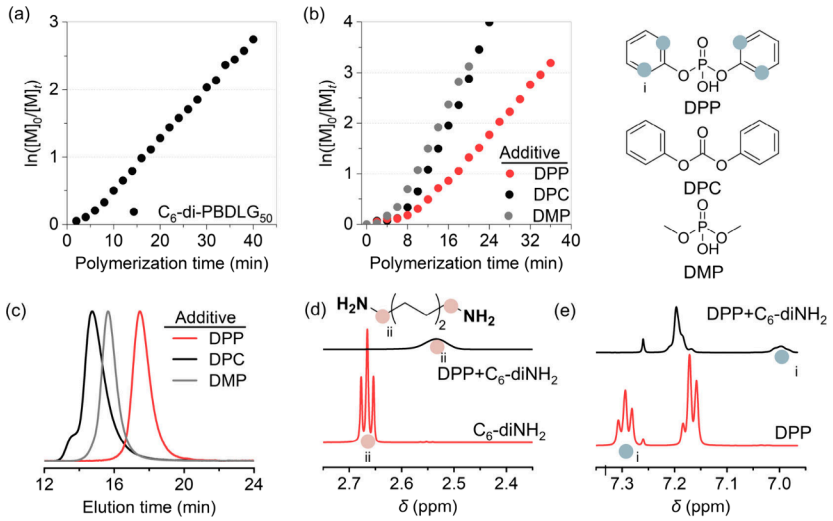


Figure 3. Mechanism study of DPP-mediated polymerization of NCA. (a) Semilogarithmic kinetic plot illustrating the polymerization of BDLG-NCA initiated by C_6-diNH_2 in $CHCl_3$. (b) Semilogarithmic kinetic plot of C_6-diNH_2-initiated BLG-NCA polymerization in $CHCl_3$ in the presence of different small molecules ($[M]_0$ = 0.1 M, $[M]_0/[I]_0$ = 100). (c) Normalized GPC-LS traces of telechelic polypeptides obtained from the polymerization in the presence of various small molecules ($[M]_0$ = 0.1 M, $[M]_0/[I]_0$ = 100). (d, e) Overlaid ^{1}H NMR spectra of DPP/C_6-diNH_2 complexes with DPP or C_6-diNH_2 as control ($[DPP]_0/[C_6\text{-di}NH_2]_0$ = 1).

mediated polymerization was extended to other NCA monomers for the preparation of sequence-defined block copolypeptides via the sequential addition of NCAs. BLG-NCA, ELG-NCA, and N^{ε}-benzyloxycabonyl-$_L$-lysine NCA (ZLL-NCA) were employed as A, B, and C, respectively (Figure 2a). All block polymerizations proceeded as expected with monomodal GPC peaks and negligible low-MW tailing ($Đ$ < 1.1) (Figure 2d, Table S8), demonstrating the successful synthesis of well-defined block copolymers.

With excellent control over MWs, we moved on to study the mechanism for the balanced growth of telechelic polypeptides in the presence of DPP. Since the uncontrolled polymerization initiated by diamine in the absence of DPP was attributed to the two-stage kinetics, we first checked the polymerization of racemic $_{DL}$-NCA monomers that do not have conformational transition processes. As expected, the polymerization of BDLG-NCA proceeded in a one-stage manner, yielding well-defined telechelic polypeptides with predictable MWs (Figures 3a and S10, Table 1). The macrodipole-induced acceleration of chain propagation, evidenced by the coil-to-helix transition, is clearly better regulated in DPP-mediated system (Figure S11),[43] substantiating the importance of DPP for balanced growth of polypeptide chains. Considering the controlled preparation of telechelic polypeptides with helical macro-initiators, all of these results collectively suggested that the DPP improved the MW control of diamine-initiated polymerization by reducing the kinetic differences in the coiled and helical stage among all polypeptide chains. Meanwhile, the use of low-polarity solvent led to accelerated polymerization that is still much faster than that in conventional DMF or THF solvent,[29] even in the presence of DPP. Adding DPP into the polymerization in DMF or THF, on the other hand, resulted in slow kinetics (i.e., >12 h) that generated telechelic polypeptides with much higher MWs than expected values, underscoring the necessity of low-polarity solvent for DPP-mediated, balanced polymerization (Figure S12, Table S9).

The polymerization mechanism was further explored by designing two molecules with similar structures to DPP and checking their impact on polymerization behaviors. Both dimethyl phosphate (DMP) lacking aromatics and diphenyl carbonate (DPC) without phosphoric acid exhibited a fast polymerization that was comparable with that in the absence of any added molecules (Figure 3b). While both DPP and DMP showed strong acidity (pK_a = 1.6 and 2.5 for DPP and DMP, respectively), their impacts on the polymerization kinetics are completely different from that of organic carboxylic acids, which will completely inhibit the polymerization with strong acidity (Figure S13). The unbalanced polymerization generated polypeptides with much larger MWs than the theoretical values, which were 63.8 and 83.6 kDa in the presence of DMP and DPC, respectively (Figure 3c, Table S10). The DPP-mediated balanced polymerization was thus correlated with both the phosphoric acid group and the benzene rings. Specifically, we hypothesize that DPP partially protonates the propagating chain-end that reversibly converts NH_2 into NH_3^+, whose cationic form further complexes with DPP through noncovalent interactions, thereby modulating the balanced growth of polypeptides from different initiation sites.

In order to validate our hypothesis, Nuclear Overhauser Effect Spectroscopy Nuclear Magnetic Resonance (NOESY NMR) was used to probe the noncovalent interactions between DPP and C_6-diNH_2, the latter being a model compound of propagating chain-ends, as evidenced by the NOESY NMR result that revealed obvious spatial correlation between DPP aromatic protons (δ 6.8−7.1 ppm) and NH_3^+ protons (δ 1.3 ppm) (Figure S14). The NH_3^+ species likely sandwiches between two DPP aromatic rings, which is consistent with the simulation results in the literature.[44] Additionally, the sandwich-like complex structure resulted in the abnormal upfield shift of the methylene protons adjacent to the amino groups in C_6-diNH_2 (Figure 3d), which was attributed to the shielding effect of π electrons.[45] Interestingly, the aromatic protons in DPP also exhibited an upfield shift (Figure 3e). The reason for the shift is currently not clear and will be included in our future studies. In contrast, the protonation of amines was negligible in the presence of nonacidic DPC, with unsubstantial interactions observed in the NMR spectrum (Figure S15). Moreover, DPP also exhibited

https://doi.org/10.1021/acsmacrolett.5c00319
ACS Macro Lett. 2025, 14, 858−864

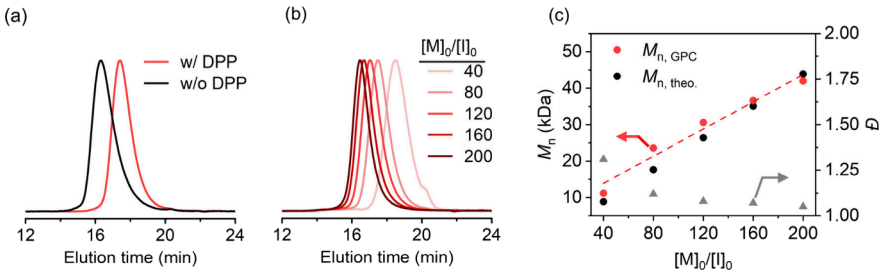


Figure 4. DPP-mediated polymerization of crude NCAs. (a) Normalized GPC-LS traces of the resulting telechelic polypeptides from C_6-diNH_2-initiated polymerization of crude BLG-NCA in the presence and absence of DPP ($[M]_0$ = 0.1 M, $[M]_0/[I]_0$ = 100). (b) Normalized GPC-LS traces of the resulting telechelic polypeptides from DPP-mediated, C_6-diNH_2-initiated polymerization of the crude BLG-NCA at various $[M]_0/[I]_0$ ratios ($[M]_0$ = 0.1 M). (c) MWs and dispersity of polypeptides obtained from polymerization of the crude BLG-NCA at various $[M]_0/[I]_0$ ratios ($[M]_0$ = 0.1 M).

weak interactions with the NCA monomer, as evidenced by the upfield shift of the phosphoric acid proton in ^{1}H NMR (Figure S16) as well as the P atom in ^{31}P NMR (Figure S17).

Based on our findings, we propose a tentative mechanism for the polymerization of BLG-NCA in the presence of DPP, which primarily involves noncovalent complexation between DPP and partially protonated chain-ends that modulates the chain propagation kinetics. Compared to previously reported systems employing primary amine hydrochlorides as initiators,[46] the phosphate ammonium salts generated in this system exhibit enhanced dissociation properties, allowing the release of free primary amines.[47] In this system, C_6-diNH_2 or the terminal amine of the propagating chain is likely protonated by the phosphate group of DPP, leading to the conversion of NH_2 to NH_3^+ and the formation of ammonium salts. The resulting NH_3^+ species interacts with the benzene rings of DPP, forming a complex intermediate that stabilizes the ammonium species that mediated concurrent growth of polypeptide chains, thus achieving a controlled synthesis of telechelic polypeptides (Scheme S2).

Conventional NCA polymerization requires stringent anhydrous purification to remove acidic or electrophilic impurities (e.g., HCl, residual phosgene), often sacrificing the yield of NCA (e.g., 60−80% for BLG-NCA) and scalability.[7] It is therefore of great interest to prepare polypeptide materials from crude NCA monomers. To evaluate the feasibility of DPP-mediated polymerization with crude NCAs, we prepared the monomers by skipping the moisture-free setups and tedious recrystallization procedures.[48] Due to the circumvention of recrystallization purification steps, the crude BLG NCAs were obtained with isolation yield (>95%) in much shorter time (~5 h). Elemental analysis confirmed the higher contents of impurities with 0.24 wt % Cl (vs 0.02 wt % in purified NCAs, Table S11). As anticipated, rapid consumption of the crude NCA monomers was observed after a facile purification step with hexane precipitation, yielding telechelic polypeptides with the desired MWs (Figure 4a, Table S12). These results highlight the robustness of this method, which accommodates crude monomers while enabling the synthesis of well-defined polypeptides and block copolymers (Figure 4b,c, Table S12).

In summary, we developed a robust method for the controlled synthesis of telechelic polypeptides via NCA ROP. By leveraging the synergy of α-helix-induced CCP and a noncovalent interaction mediated by DPP, this approach enables rapid and controlled living polymerization of NCAs initiated by widely available diamine. This enhanced controllability allows for the efficient synthesis of telechelic polypeptides with reduced polydispersity and well-defined amino-terminal functionality at both chain ends. This method also demonstrates its tolerance to the use of crude NCA monomers, enabling the robust synthesis of telechelic polypeptide building blocks without requiring tedious NCA purification. In addition to facilitating the design of high-performance telechelic hybrid biomaterials incorporating polypeptide segments, this strategy also provides new insights of NCA ROP with diamine initiators.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmacrolett.5c00319.

Materials and methods, proposed mechanism, additional polymerization kinetics and GPC characterizations for optimization and mechanism studies, and NMR spectra for molecular interactions (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Jianjun Cheng − *Research Center for Industries of the Future, Westlake University, Hangzhou 310030, China; Department of Materials Science and Engineering, Westlake University, Hangzhou 310024, China; Institute of Advanced Technology, Westlake Institute for Advanced Study, Hangzhou 310024, China;* orcid.org/0000-0003-2561-9291; Email: chengjianjun@westlake.edu.cn

Ziyuan Song − *Institute of Functional Nano and Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Soochow University, Suzhou 215123, China;* orcid.org/0000-0002-3165-3712; Email: zysong@suda.edu.cn

Yu Zhao − *Research Center for Industries of the Future, Westlake University, Hangzhou 310030, China; Department of Materials Science and Engineering, Westlake University, Hangzhou 310024, China; Institute of Advanced Technology, Westlake Institute for Advanced Study, Hangzhou 310024, China*; Email: zhaoyu51@westlake.edu.cn

Authors

Tingting Cao − *School of Materials Science and Engineering, Zhejiang University, Hangzhou 310027, China; Research Center for Industries of the Future, Westlake University, Hangzhou 310030, China; Department of Materials Science and Engineering, Westlake University, Hangzhou 310024,*

https://doi.org/10.1021/acsmacrolett.5c00319
ACS Macro Lett. 2025, 14, 858−864

China; Institute of Advanced Technology, Westlake Institute for Advanced Study, Hangzhou 310024, China

Chenlin Ji − *Research Center for Industries of the Future, Westlake University, Hangzhou 310030, China; Department of Materials Science and Engineering, Westlake University, Hangzhou 310024, China; Institute of Advanced Technology, Westlake Institute for Advanced Study, Hangzhou 310024, China*

Qianyi Qin − *Research Center for Industries of the Future, Westlake University, Hangzhou 310030, China; Department of Materials Science and Engineering, Westlake University, Hangzhou 310024, China; Institute of Advanced Technology, Westlake Institute for Advanced Study, Hangzhou 310024, China;* orcid.org/0009-0004-7912-6833

Hui Wang − *Research Center for Industries of the Future, Westlake University, Hangzhou 310030, China; Department of Materials Science and Engineering, Westlake University, Hangzhou 310024, China; Institute of Advanced Technology, Westlake Institute for Advanced Study, Hangzhou 310024, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmacrolett.5c00319

Author Contributions

T.C. conceived the concept, designed this research and drafted the manuscript. T.C., C.J., Q.Q., and H.W. performed experiments and analyzed the data. Y.Z., Z.S., and J.C. supervised this research and revised the manuscript. All authors approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Jianbing Lingyan Program of Zhejiang Province (2024SDXHDX0004), National Natural Science Foundation of China (52233015 for J.C., 22101194 for Z.S., and U24A2076 for Y.Z.). We acknowledge the support of the New Cornerstone Investigator Program, New Cornerstone Science Foundation. We thank the Service Center for Molecular Sciences (ISCMS) of Westlake University for technical support.

■ ABBREVIATIONS

NCA, *N*-carboxyanhydride; ROP, ring-opening polymerization; DPP, diphenyl phosphate; CCP, cooperative covalent polymerization; MWs, molecular weights; MWDs, molecular weight distributions; BLG-NCA, γ-benzyl-$_L$-glutamate NCA; C_6-diNH$_2$, 1,6-diaminohexane; DMF, *N*,*N*-dimethylformamide; ZLL-NCA, N^{ε}-benzyloxycarbonyl-$_L$-lysine NCA; ELG-NCA, γ-ethyl-$_L$-glutamate NCA; DMP, dimethyl phosphate; DPC, diphenyl carbonate; NOESY, Nuclear Overhauser Effect Spectroscopy; NMR, Nuclear Magnetic Resonance

■ REFERENCES

(1) Deming, T. J. Synthesis of Side-Chain Modified Polypeptides. *Chem. Rev.* **2016**, *116*, 786−808.

(2) Cai, C.; Lin, J.; Lu, Y.; Zhang, Q.; Wang, L. Polypeptide Self-Assemblies: Nanostructures and Bioapplications. *Chem. Soc. Rev.* **2016**, *45*, 5985−6012.

(3) Song, Z.; Han, Z.; Lv, S.; Chen, C.; Chen, L.; Yin, L.; Cheng, J. Synthetic Polypeptides: from Polymer Design to Supramolecular Assembly and Biomedical Application. *Chem. Soc. Rev.* **2017**, *46*, 6570−6599.

(4) Zhou, X.; Li, Z. Advances and Biomedical Applications of Polypeptide Hydrogels Derived from α-Amino Acid *N*-Carboxyanhydride (NCA) Polymerizations. *Adv. Healthcare Mater.* **2018**, *7*, 1800020.

(5) Liu, Y.; Yin, L. α-Amino Acid *N*-Carboxyanhydride (NCA)-Derived Synthetic Polypeptides for Nucleic Acids Delivery. *Adv. Drug Delivery Rev.* **2021**, *171*, 139−163.

(6) Mocny, P.; Klok, H.-A. Complex Polymer Topologies and Polymer—Nanoparticle Hybrid Films Prepared via Surface-Initiated Controlled Radical Polymerization. *Prog. Polym. Sci.* **2020**, *100*, 101185.

(7) Lv, W.; Wang, Y.; Li, M.; Wang, X.; Tao, Y. Precision Synthesis of Polypeptides via Living Anionic Ring-Opening Polymerization of *N*-Carboxyanhydrides by Tri-thiourea Catalysts. *J. Am. Chem. Soc.* **2022**, *144*, 23622−23632.

(8) Kricheldorf, H. R. Polypeptides and 100 Years of Chemistry of α-Amino Acid *N*-Carboxyanhydrides. *Angew. Chem. Int. Ed.* **2006**, *45*, 5752−5784.

(9) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. Synthesis of Well-Defined Polypeptide-Based Materials via the Ring-Opening Polymerization of α-Amino Acid *N*-Carboxyanhydrides. *Chem. Rev.* **2009**, *109*, 5528−5578.

(10) Itaka, K.; Ishii, T.; Hasegawa, Y.; Kataoka, K. Biodegradable Polyamino Acid-Based Polycations as Safe and Effective Gene Carrier Minimizing Cumulative Toxicity. *Biomaterials* **2010**, *31*, 3707−3714.

(11) He, C.; Zhuang, X.; Tang, Z.; Tian, H.; Chen, X. Stimuli-Sensitive Synthetic Polypeptide-Based Materials for Drug and Gene Delivery. *Adv. Healthcare Mater.* **2012**, *1*, 48−78.

(12) Deng, C.; Wu, J.; Cheng, R.; Meng, F.; Klok, H.-A.; Zhong, Z. Functional Polypeptide and Hybrid Materials: Precision Synthesis via α-Amino Acid *N*-Carboxyanhydride Polymerization and Emerging Biomedical Applications. *Prog. Polym. Sci.* **2014**, *39*, 330−364.

(13) Rasines Mazo, A.; Allison-Logan, S.; Karimi, F.; Chan, N. J.; Qiu, W.; Duan, W.; O'Brien-Simpson, N. M.; Qiao, G. G. Ring Opening Polymerization of α-Amino Acids: Advances in Synthesis, Architecture and Applications of Polypeptides and Their Hybrids. *Chem. Soc. Rev.* **2020**, *49*, 4737−4834.

(14) Huang, J.; Heise, A. Stimuli Responsive Synthetic Polypeptides Derived from *N*-Carboxyanhydride (NCA) Polymerization. *Chem. Soc. Rev.* **2013**, *42*, 7373−7390.

(15) Wu, Y.; Chen, K.; Wang, J.; Chen, M.; Dai, W.; Liu, R. Recent Advances and Future Developments in the Preparation of Polypeptides via *N*-Carboxyanhydride (NCA) Ring-Opening Polymerization. *J. Am. Chem. Soc.* **2024**, *146*, 24189−24208.

(16) Yuan, H.; Jiang, M.; Fang, H.; Tian, H. Recent Advances in Poly(Amino Acids), Polypeptides, and Their Derivatives in Drug Delivery. *Nanoscale* **2025**, *17*, 3549−3584.

(17) Palivan, C. G.; Heuberger, L.; Gaitzsch, J.; Voit, B.; Appelhans, D.; Borges Fernandes, B.; Battaglia, G.; Du, J.; Abdelmohsen, L.; van Hest, J. C. M.; Hu, J.; Liu, S.; Zhong, Z.; Sun, H.; Mutschler, A.; Lecommandoux, S. Advancing Artificial Cells with Functional Compartmentalized Polymeric Systems - In Honor of Wolfgang Meier. *Biomacromolecules* **2024**, *25*, 5454−5467.

(18) Tsuchiya, K.; Numata, K. Papain-Catalyzed Chemoenzymatic Synthesis of Telechelic Polypeptides Using Bis(Leucine Ethyl Ester) Initiator. *Macromol. Biosci.* **2016**, *16*, 1001−1008.

(19) Zhou, P.; Li, Z.; Lu, Y.; Kong, J.; Ling, J. Telechelic Triblock Poly(α-Amino Acid)-Poly(Tetrahydrofuran)-Poly(α-Amino Acid) Copolymers: Chain-End Transformation, Polymerization and pH-Responsive Hydrolysis. *Chin. J. Chem.* **2021**, *39*, 2852−2856.

(20) Tsuchiya, K.; Masunaga, H.; Numata, K. Tensile Reinforcement of Silk Films by the Addition of Telechelic-Type Polyalanine. *Biomacromolecules* **2017**, *18*, 1002−1009.

(21) Ishii, S.; Kaneko, J.; Nagasaki, Y. Development of A Long-Acting, Protein-Loaded, Redox-Active, Injectable Gel Formed by A Polyion Complex for Local Protein Therapeutics. *Biomaterials* **2016**, *84*, 210−218.

(22) Vong, L. B.; Bui, T. Q.; Tomita, T.; Sakamoto, H.; Hiramatsu, Y.; Nagasaki, Y. Novel Angiogenesis Therapeutics by Redox Injectable

Hydrogel-Regulation of Local Nitric Oxide Generation for Effective Cardiovascular Therapy. *Biomaterials* **2018**, *167*, 143−152.
(23) Kirkham, S.; Castelletto, V.; Hamley, I. W.; Reza, M.; Ruokolainen, J.; Hermida-Merino, D.; Bilalis, P.; Iatrou, H. Self-Assembly of Telechelic Tyrosine End-Capped PEO and Poly (Alanine) Polymers in Aqueous Solution. *Biomacromolecules* **2016**, *17*, 1186−1197.
(24) Brzezinska, K. R.; Curtin, S. A.; Deming, T. J. Polypeptide End-Capping Using Functionalized Isocyanates: Preparation of Pentablock Copolymers. *Macromolecules* **2002**, *35*, 2970−2976.
(25) Edwards-Gayle, C. J.; Greco, F.; Hamley, I. W.; Rambo, R. P.; Reza, M.; Ruokolainen, J.; Skoulas, D.; Iatrou, H. Self-Assembly of Telechelic Tyrosine End-Capped PEO Star Polymers in Aqueous Solution. *Biomacromolecules* **2018**, *19*, 167−177.
(26) Dong, C.-M.; Sun, X.-L.; Faucher, K. M.; Apkarian, R. P.; Chaikof, E. L. Synthesis and Characterization of Glycopolymer-Polypeptide Triblock Copolymers. *Biomacromolecules* **2004**, *5*, 224−231.
(27) Karatzas, A.; Iatrou, H.; Hadjichristidis, N.; Inoue, K.; Sugiyama, K.; Hirao, A. Complex Macromolecular Chimeras. *Biomacromolecules* **2008**, *9*, 2072−2080.
(28) Koda, Y.; Nagasaki, Y. Poly($_L$-Lysine)-Block-Poly(Ethylene Glycol)-Block-Poly($_L$-Lysine) Triblock Copolymers for the Preparation of Flower Micelles and Their Irreversible Hydrogel Formation. *Sci. Technol. Adv. Mater.* **2025**, *26*, 2432856.
(29) Chen, C.; Fu, H.; Baumgartner, R.; Song, Z.; Lin, Y.; Cheng, J. Proximity-Induced Cooperative Polymerization in ″Hinged″ Helical Polypeptides. *J. Am. Chem. Soc.* **2019**, *141*, 8680−8683.
(30) Baumgartner, R.; Fu, H.; Song, Z.; Lin, Y.; Cheng, J. Cooperative Polymerization of α-Helices Induced by Macromolecular Architecture. *Nat. Chem.* **2017**, *9*, 614−622.
(31) Song, Z.; Fu, H.; Baumgartner, R.; Zhu, L.; Shih, K.-C.; Xia, Y.; Zheng, X.; Yin, L.; Chipot, C.; Lin, Y.; Cheng, J. Enzyme-Mimetic Self-Catalyzed Polymerization of Polypeptide Helices. *Nat. Commun.* **2019**, *10*, 5470−5476.
(32) Wang, W.; Fu, H.; Lin, Y.; Cheng, J.; Song, Z. Cooperative Covalent Polymerization of *N*-Carboxyanhydrides: from Kinetic Studies to Efficient Synthesis of Polypeptide Materials. *Acc. Mater. Res.* **2023**, *4*, 604−615.
(33) Siefker, D.; Williams, A. Z.; Stanley, G. G.; Zhang, D. Organic Acid Promoted Controlled Ring-Opening Polymerization of α-Amino Acid-Derived *N*-Thiocarboxyanhydrides (NTAs) toward Well-Defined Polypeptides. *ACS Macro Lett.* **2018**, *7*, 1272−1277.
(34) Zheng, B.; Xu, S.; Ni, X.; Ling, J. Understanding Acid-Promoted Polymerization of the *N*-Substituted Glycine *N*-Thiocarboxyanhydride in Polar Solvents. *Biomacromolecules* **2021**, *22*, 1579−1589.
(35) Wang, S.; Lu, M. Y.; Wan, S. K.; Lyu, C. Y.; Tian, Z. Y.; Liu, K.; Lu, H. Precision Synthesis of Polysarcosine via Controlled Ring-Opening Polymerization of *N*-Carboxyanhydride: Fast Kinetics, Ultrahigh Molecular Weight, and Mechanistic Insights. *J. Am. Chem. Soc.* **2024**, *146*, 5678−5692.
(36) Liu, X.; Huang, J.; Wang, J.; Sheng, H.; Yuan, Z.; Wang, W.; Li, W.; Song, Z.; Cheng, J. Accelerated and Controlled Polymerization of *N*-Carboxyanhydrides Assisted by Acids. *CCS Chem.* **2024**, 1.
(37) Song, Z.; Fu, H.; Wang, J.; Hui, J.; Xue, T.; Pacheco, L. A.; Yan, H.; Baumgartner, R.; Wang, Z.; Xia, Y.; et al. Synthesis of Polypeptides via Bioinspired Polymerization of in situ Purified *N*-Carboxyanhydrides. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 10658−10663.
(38) Wang, X.; List, B. Asymmetric Counteranion-Directed Catalysis for the Epoxidation of Enals. *Angew. Chem. Int. Ed.* **2008**, *47*, 1119−1122.
(39) Penczek, S.; Pretula, J. Activated Monomer Mechanism (AMM) in Cationic Ring-Opening Polymerization. The Origin of the AMM and Further Development in Polymerization of Cyclic Esters. *ACS Macro Lett.* **2021**, *10*, 1377−1397.
(40) Fan, H.; Hu, C.; Niu, M.; Zhang, Q.; Li, B.; Pang, X.; Chen, X. Modular Access from Acrylate to a Sustainable Polyester Platform with Large-Span Tunability and Chemical Circularity under Mild Conditions. *J. Am. Chem. Soc.* **2025**, *147*, 9836−9843.
(41) De Greef, T. F.; Smulders, M. M.; Wolffs, M.; Schenning, A. P.; Sijbesma, R. P.; Meijer, E. W. Supramolecular Polymerization. *Chem. Rev.* **2009**, *109*, 5687−5754.
(42) Xia, Y.; Song, Z.; Tan, Z.; Xue, T.; Wei, S.; Zhu, L.; Yang, Y.; Fu, H.; Jiang, Y.; Lin, Y.; Lu, Y.; Ferguson, A. L.; Cheng, J. Accelerated Polymerization of *N*-Carboxyanhydrides Catalyzed by Crown Ether. *Nat. Commun.* **2021**, *12*, 732−739.
(43) Blout, E. R.; Asadourian, A. Polypeptides. V. The Infrared Spectra of Polypeptides Derived from γ-Benzyl-$_L$-Glutamate. *J. Am. Chem. Soc.* **1956**, *78*, 955−961.
(44) Zhu, W.; Jiang, H.; Tan, X.; Chen, J.; DI, Y.; Gu, J.; Lin, M.; Chen, K.; Ji, R.; Cao, Y. Theoretical Studies on Cation-π Interactions 2: Density -Funtional Theory Investigation on the Configurations of and Interactions in Ammonium Cation-Bibenzene Complexes. *Acta Chim. Sin.* **1999**, *57*, 852−859.
(45) Islas, R.; Heine, T.; Merino, G. The Induced Magnetic Field. *Acc. Chem. Res.* **2012**, *45*, 215−228.
(46) Dimitrov, I.; Schlaad, H. Synthesis of Nearly Monodisperse Polystyrene-Polypeptide Block Copolymers via Polymerisation of *N*-carboxyanhydrides. *Chem. Commun.* **2003**, 2944−2945.
(47) Liang, J.; Zhi, X.; Zhou, Q.; Yang, J. Binaphthol-Derived Phosphoric Acids as Efficient Organocatalysts for the Controlled Ring-Opening Polymerization of γ-Benzyl-$_L$-Glutamate *N*-Carboxyanhydrides. *Polymer* **2019**, *165*, 83−90.
(48) Sun, X.; Li, A.; Li, N.; Ji, G.; Song, Z. Facile Preparation of Heteropolypeptides from Crude Mixtures of α-Amino Acid *N*-Carboxyanhydrides. *Biomacromolecules* **2024**, *25*, 6093−6102.

https://doi.org/10.1021/acsmacrolett.5c00319
ACS Macro Lett. 2025, 14, 858−864