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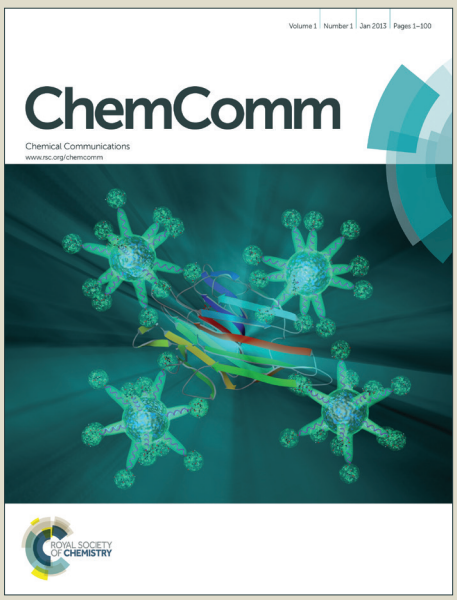

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From competition to cooperation: a highly efficient strategy towards well-defined (co)polypeptides

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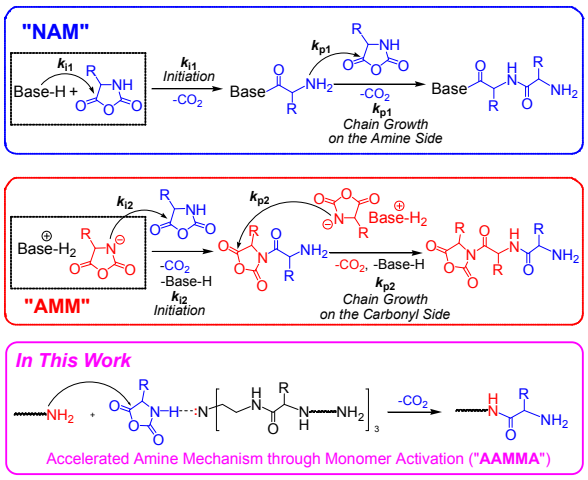
By associating primary (slow but controlled ring-opening polymerization; ROP) and tertiary (fast but uncontrolled ROP) amines in the same molecule, a novel highly active organocatalytic system proceeding by accelerated amine mechanism through monomer activation (AAMMA) and leading to living ROP of *α*-amino acid *N*-carboxyanhydrides at room temperature was successfully developed.

Synthetic polypeptides as a highly valuable class of biopolymers have found applications in the fields of drug delivery, tissue engineering, sensing and catalysis.[1] Among different methods to synthesize polypeptides, the ring-opening polymerization (ROP) of *α*-amino acid *N*-carboxyanhydrides (NCA) stands out as the most efficient in producing long polypeptide chains. The ROP of NCAs, which is highly electrophilic, can be triggered not only by strong bases such as alkyllithium but also by weak bases such as amines, alcohols and water. In the ROP of NCAs, two mechanisms, the so-called "normal amine mechanism" (NAM) and the "activated monomer" mechanism (AMM) (Scheme 1) occur and often compete, complicating the polymerization process, and making the synthesis of well-defined polypeptides challenging.[2] This competition was the main reason for the failure to control the polymerization of NCA monomers in the 100-year history of NCA until Deming unveiled in his seminal work of 1997 the utilization of transition metal complexes.[3] In the following years, a few controlled NCA polymerizations were reported by employing primary amine hydrochloride,[4] or an organosilicon reagent derivative as the initiator,[5] or by using conventional amine initiators under high vacuum, low temperature or nitrogen flow.[6]

It is indeed well known that primary amines are excellent initiators leading to polypeptides with predetermined molecular weights and low polydispersity indices (PDI); these primary amines being more nucleophilic than the *ω*-amino group of the propagating chain, initiation is faster than propagation (low PDI). As the latter occurs via NAM, chain breaking reactions are absent (control of molecular weight). However, the propagation is slow under normal conditions and long reaction times are needed to complete the polymerization.[2, 6e-f] On the other hand, tertiary amines, which are more basic than nucleophilic, can easily abstract the acidic N-H proton of NCA and generate negatively charged NCAs that are sufficiently nucleophilic to attack another NCA molecule and initiate polymerization via AMM. Because AMM involves anions, it induces a higher propagation rate than that observed with NAM yielding samples of higher molecular weights than expected,; but because initiation is slower than propagation and both AMM and NAM occur simultaneously, polypeptides with very large PDI values and uncontrolled molecular weights are generally produced.[7]

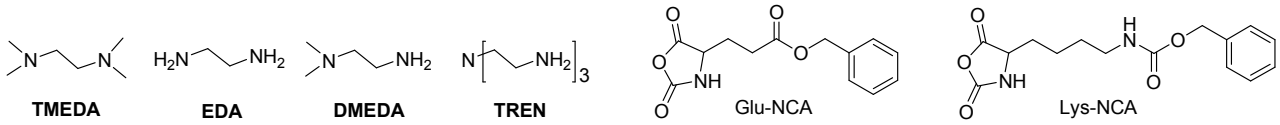
Confronted to the difficulty in achieving control over MWs and simultaneously bringing about fast ROP of NCAs when using metal-free initiators, we searched for an initiating system that would afford both features: fast ROP of NCA at room temperature and formation of well-defined homo- and co- polypeptides by "living" ROP.



Scheme 1. The three mechanisms of NCA ring-opening polymerization: "normal amine mechanism" (NAM), "activated monomer mechanism" (AMM) and accelerated amine mechanism by monomer activation (AAMMA).

We first investigated the potential of initiators that would include both primary amines and tertiary amines. A priori such initiating systems can only produce fast and uncontrolled polymerization due to the simultaneity of AMM and NAM. Actually we observed that triethylaminetriamine (**TREN**), a compound that contains both a sterically hindered tertiary amine and three primary amines does bring about a fast and yet perfectly controlled polymerization of NCAs (Figure 1). For comparison purposes, we also polymerized Glu-NCA in the presence of ethylenediamine (**EDA**) and tetramethylethylenediamine (**TMEDA**), initiators that possess two primary amines and two tertiary amines, respectively. As shown in Table 1 and Figure S1A, under identical conditions, **EDA**, **TMEDA**, and **TREN** exhibit different behaviors in the ROP of NCA. **TMEDA** obviously polymerizes NCA faster with 79% monomeric units incorporated into the polymer chains after one hour. **TREN** affords 70% monomer conversion, which is slightly slower than **TMEDA** (79%) but faster than **EDA** (50%).

Table 1. Homo- and co- polymerization and of Glu-NCA and Lys-NCA initiated by Various Amines.



entry[a]	initiator	monomer	$[M]_0/[I]_0$	Time (min)	conv.(%)[b]	$M_{n,calcd}\times10^{-4}$ [c]	$M_{n,SEC\text{-}LS}\times10^{-4}$ [d]	PDI [d]
1[e]	**TMEDA**	Glu-NCA	100/1	60	79	1.73	5.46	1.65
2	**EDA**	Glu-NCA	100/1	60	50	1.10	0.99	1.09
3[e]	**DMEDA**	Glu-NCA	100/1	60	75	1.64	2.05	1.38
4	**TREN**	Glu-NCA	50/1	60	85	0.93	1.00	1.13
5	**TREN**	Glu-NCA	50/1	75	100	1.09	1.19	1.14
6	**TREN**	Glu-NCA	75/1	60	75	1.23	1.36	1.15
7	**TREN**	Glu-NCA	75/1	90	100	1.64	1.75	1.14
8	**TREN**	Glu-NCA	100/1	60	70	1.53	1.55	1.14
9	**TREN**	Glu-NCA	100/1	96	100	2.19	2.22	1.15
10	**TREN**	Glu-NCA	150/1	60	60	1.97	2.07	1.16
11	**TREN**	Glu-NCA	150/1	108	100	3.29	3.45	1.15
12	**TREN**	Glu-NCA	200/1	60	54	2.37	2.50	1.16
13	**TREN**	Glu-NCA	200/1	120	100	4.38	4.52	1.17
14	**TREN**	Lys-NCA	50/1	120	100	1.31	1.22	1.19
15	**TREN**	Lys-NCA	75/1	120	100	1.97	1.87	1.18
16	**TREN**	Lys-NCA	100/1	120	100	2.62	2.50	1.17
17[f]	**TREN**	Glu-NCA+Lys-NCA (random)	(37.5+37.5)/1	180	100	1.80	1.71	1.18
18[f]	**TREN**	Glu-NCA+Lys-NCA (block)	(37.5+37.5)/1	180	100	1.80	1.72	1.18
19[f]	**TREN**	Lys-NCA+Glu-NCA (block)	(37.5+37.5)/1	180	100	1.80	1.82	1.19

[a] Polymerization was performed in DMF at 25 °C with $[NCA]_0$ = 0.19M and >99% conversion could be obtained in 2 to 3h. [b] FT-IR is used to determine the conversion of NCA by analyzing the intensity of the NCA anhydride absorption band at 1787 cm^{-1}. [c] Calculated by [NCA]/[I] × (M_{NCA}-44) × X (X = Conv.). [d] Determined by size-exclusion chromatography (SEC) combined with multi-angle light scattering (MALS), viscometry (VISC), and differential refractive index (DRI) triple detection in 0.1 M LiBr in DMF at 60 °C. [e] The SEC curves are bimodal. [f] $[Glu\text{-}NCA]_0$ = 0.19M and $[Lys\text{-}NCA]_0$ = 0.19M.

The technique used to characterize the PBLG samples produced and the structures generated, namely a size exclusion chromatography (SEC) equipped with multi-angle light scattering (MALS), viscometry (VISC), and differential refractive index (DRI) triple detection, clearly indicates that **TREN**-initiated samples all exhibit a unimodal distribution and the expected MWs. Obviously only one mechanism, namely NAM, occurred during those polymerizations but the rates observed remind of those typically exhibited by AMM-type propagation (Figure S1B). In contrast the **TMEDA**-initiated polymerization of Glu-NCA at a monomer/initiator ratio of 100/1, resulted in bimodal peaks with a high PDI (1.65, Table 1, entry 1) and a much higher value of M_n (5.46×10^4) than the theoretical one (1.73×10^4), indicative of the coexistence of both NA and AM mechanisms. As expected the rate of polymerization was fast in the latter case but only marginally higher compared to **TREN**-initiated polymerization. Unsurprisingly the polymerization run in the presence of **EDA** was slow and took 12 h to reach full monomer conversion; a very narrow and symmetric SEC peak was obtained as a result, with a low PDI value (1.09).

Pursuing with the idea of combining both primary and tertiary amines in a same initiating system we then investigated the behavior of *N, N*-dimethylethylenediamine (**DMEDA**). The samples produced in the presence of the latter initiator exhibited a broad, bimodal distribution and uncontrolled MWs, featuring the unabated prevalence of AM mechanism. This shows the very peculiar nature of **TREN** that allows simultaneously fast and yet perfectly controlled polymerization, a feature that could not be generalized to other initiators containing both primary and tertiary amines.

With its "core" tertiary amine and three outer primary amines **TREN** is a star-like molecule that exhibits potentially three initiating sites for NAM and a fourth one for AMM. The very fact that only one narrowly distributed population of chains is formed and perfect three-armed polypeptides are obtained in a living mode following NAM suggests that its tertiary amine is unable to abstract protons from NCA, preventing AMM from occurring.[8] Yet this tertiary amine is not inert towards NCA and "silent" throughout

polymerization as indicates the fast polymerization observed in the presence of **TREN**. The ^{15}N NMR characterization of **TREN**, **EDA**, **TMEDA/EDA** (1:1) and **TMEDA** sheds an interesting light on the peculiar behavior of **TREN**: it shows that the tertiary amine of **TREN** exhibits a much higher chemical shift (27.03ppm) than that of **TMEDA** (22.76ppm), meaning that the tertiary amine carried by **TREN** is relatively "electron-poor" (vs.**TMEDA**) (Figure S2). This explains why proton abstraction from NCA is prevented but not the activation of its amido proton which in turn may well be responsible for promoting the fast propagation observed. In addition the ^{15}N NMR and ^{1}H NMR of Glu-NCA and **TREN** show that the nitrogen of NCA is "electron-poorer" than that of **TREN** and that the amido proton is rather acidic (Figure S3 and S4). So, we propose that the acidic monomer proton get activated by the tertiary amine of **TREN** through the formation of hydrogen bonding without being abstracted (for detailed explanations see supporting information). As a result, **TREN** is almost as efficient as **DMEDA** at activating the propagation, affording 70% monomer conversion in one hour without any detrimental side reactions. This rate of polymerization is slightly slower than in the case of **TMEDA** (79%) but much faster than with **EDA** (50%). In particular, when the monomer/initiator ratio [Glu-NCA]/[**TREN**] is increased to 200 by reducing [**TREN**] to half and keeping [Glu-NCA] unchanged, **TREN** still exhibits a higher rate of polymerization than does **EDA** ([Glu-NCA]/[EDA]=100) and yields 54% monomer conversion in one hour.[9] The resulting polypeptide from **TREN**-mediated polymerization shows a very low PDI and a symmetric SEC peak, dramatically different from those of the polymers prepared by **TMEDA** or **DMEDA** (Figure S1B). The polymerization of NCA in the presence of **TREN** thus proceeds via accelerated amine mechanism by monomer activation (AAMMA).

We further varied the [Glu-NCA]/[**TREN**] ratios and performed a systematic investigation of the ROP. As shown in Table 1, under a broad range of NCA/**TREN** molar ratios, the polymerizations proceeded rapidly, yielding PBLGs with variable MWs (M_n= 1.00×10^4–4.52×10^4) and narrow molecular weight distributions (PDI = 1.13−1.17). In addition, the MWs of the resulting PBLGs are very close to the targeted values, indicating 100% efficiency of the three primary amine initiating sites. Furthermore, a linear relationship between the number-average molecular weight (M_n) and the monomer conversion is observed (Figure S1C), which indicates the living character of the polymerization.

To fully characterize the initiation step, the kinetics of **TREN**-mediated NCA-ROP was investigated in DMF at 25 °C using three concentrations of [**TREN**] (3.80, 2.53, and 1.90 mM). The plots of $\ln([NCA]_0/[NCA]_t)$ vs. polymerization time follow straight lines with zero intercepts for each $[\mathbf{TREN}]_0$ (Figure S5), which suggests that the polymerization proceeds with a first-order dependence on [NCA] and without termination. This observation is further strengthened by using *operando* IR spectroscopy which shows that the polymerization has a first-order dependence on [NCA] from the very beginning of reaction and the absence of any induction period (Figure S6). This first-order dependence on [NCA] further indicates that the polymerization exclusively follows NAM.[7] SEC-MALS-VISC analysis of the PBLG resulting from **TREN**-mediated ROP of Glu-NCA revealed that these polymers exhibited lower intrinsic viscosities than their linear analogues (produced by EDA) but with identical MWs (Figure S7). The $[\eta]_{TREN}/[\eta]_{EDA}$ ratio (0.948) suggests that the three primary amine groups in **TREN** can simultaneously initiate NCA-ROP and yield PBLGs with a perfect three-armed structure.[10]

In an attempt to generalize the previous observations made about Glu-NCA, we used **TREN** to initiate the ROP of Lys-NCA. In this case, **TREN** again initiated a fast ROP of Lys-NCA and yielded 100% monomer conversion in 3 hours. Furthermore, in the presence of **TREN** remarkable control over the polymerization of ε-Cbz-L-lysine NCA (Lys-NCA) could be obtained, along with poly(ε-Cbz-L-lysine) (PZLL) with expected MWs and very low PDIs (Table 1, entries 14-16). In addition, random and block copolypeptides, such as PBLG-*random*-PZLL, PBLG-*block*-PZLL and PZLL-*block*-PBLG could also be readily prepared with predetermined MWs and narrow MWDs by using **TREN** as the initiator for random or sequential copolymerization (Table 1, entries 17-19).

In summary, a novel, metal-free strategy supported by an original mechanism which allows activation of the monomer without abstracting its proton and cooperation between a primary and a tertiary amine is unveiled; this paves the way for the successful synthesis of well-defined homo- and copolypeptides of *α*-amino acid under fast conditions by association of a primary amine with a tertiary amine in the same molecule of TREN. Future work in our laboratory will seek to further the study of this polymerization mechanism (Accelerated Amine Mechanism by Monomer Activation, AAMMA) and extend its scope to the synthesis of well-defined homo- and copolypeptides with complex macromolecular architectures.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details as well as supporting data including kinetic experiment data, SEC profiles of obtained polypeptides are presented in the supporting inforamtion. See DOI: 10.1039/c000000x/

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8 The Mark-Houwink-Sakurada plot of resulted PBLGs in Figure S7 suggested the resultant polypeptides had a perfect three-armed structure. A linear relationship between the molecular weight and the monomer conversion was observed in Figure S1C, indicating the polymerization proceeded in a living mode. Kinetic study by using *operando* IR showed that the polymerization had a first-order dependence on [NCA] from the very beginning of reaction and exclusively follows NAM (Figure S6).
9 A comparison by using same [-NH_2]/[NCA] ratio was also provided in Table 1(entries 2 and10) and Figure S1A. Polymerizations of entry 2 and entry 10 have same [-NH_2], but entry10 went faster than entry 2. Even the [-NH_2] of polymerization initiated by **TREN** is lower than that of **EDA**-mediated polymerization (entry 2 vs. entry 12), **TREN** still showed higher activity than **EDA**. (The calculations of [NH_2]: entry 2: [NH_2]=([NCA]/100)×2=3.8Mm; entry 7: [NH_2]=([NCA]/150)×3=3.8mM; entry 8: [NH_2]=([NCA]/200)×3 =2.8mM).
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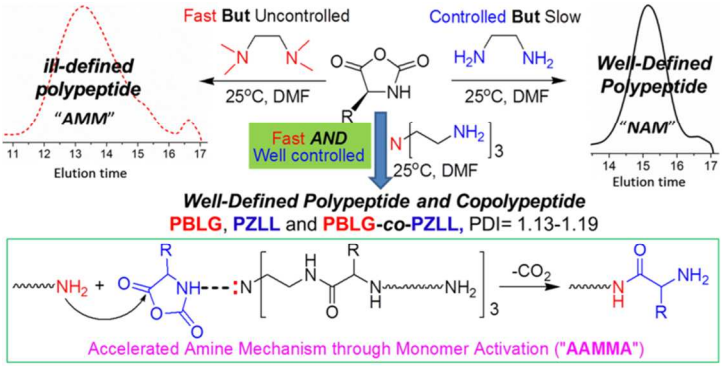
Table of Content use only

From competition to cooperation: a highly efficient strategy towards well-defined (co)polypeptides

Wei Zhao,[a] Yves Gnanou[b] and Nikos Hadjichristidis*[a]



A highly efficient strategy towards well-defined (co)polypeptides