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Well-defined (co)polypeptides bearing pendant alkyne group†

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A novel metal-free strategy, using hydrogen-bonding catalytic ring opening polymerization of acetylene-functionalized *N*-carboxy anhydrides of α -amino acids, was developed for the synthesis of well-defined polypeptides bearing pendant alkyne groups. This method provides an efficient way to synthesize novel alkyne-functionalized homopolypeptides (A) and copolypeptides, such as AB diblock (B: non-functionalized), ABA triblock and star-AB diblock, as well as linear and star random copolypeptides, precursors of a plethora complex macromolecular architectures by click chemistry.

Functionalized polypeptides have attracted tremendous interest in recent years and found many stimulating medical applications due to their tunable physico-chemical characteristics including hydrophilicity and stimuli-responsive behavior.¹ Many studies have focused on the synthesis of new side-chain modified (co)polypeptides *via* post-polymerization “click” reactions.^{2–4} These reactions are highly efficient, selective, mild and tolerant to most functional groups, the reason they have been powerful tools for the modification of biopolymers. Polypeptides bearing alkyne groups is one of the important precursors used to create new polypeptide materials since the alkyne groups can directly undergo not only azide–alkyne cycloadditions with a variety of functional azides² but also thiol–yne reactions with various thiols.^{3d, 4} Consequently, the synthesis of well-defined polypeptides bearing alkyne groups remains a challenge in contrast to polypeptides containing protected functional groups, such as poly(γ -benzyl-L-glutamate) and poly(ϵ -Cbz-L-lysine), which can be only used after deprotection.

In 1960, Schlögl *et al.* synthesized (Scheme 1) the first alkyne modified *N*-carboxyanhydride (NCA), D/L-propargylglycine NCA,

from an unnatural amino acid, propargyl glycine and performed ring-opening polymerization (ROP) with ammonia as initiator, resulting in a polypeptide with degree of polymerization 32. However, the high cost of propargyl glycine and the poor solubility of the alkyne-functionalized polypeptide limited its applications.⁵ In 2009, Hammond *et al.* prepared (Scheme 1) a new alkyne-containing monomer, γ -propargyl-L-glutamate NCA (Propargyl-Glu-NCA), from glutamic acid and propargyl alcohol by using a simple two-step process. The ROP of that monomer triggered by heptylamine was carried out in DMF for three days at room temperature leading to a polymer with 54% yield and degree of polymerization (DP) of 40. Unfortunately, the polymer had a bimodal GPC trace with high dispersity ($\mathcal{D}$) 1.45, indicating poor control over polymerization.^{2a} In 2014, Yin and Cheng reported (Scheme 1) the synthesis of γ -(4-propargyloxybenzyl)-L-glutamate NCA. The polymerization of this monomer in DMF with hexamethyldisilazane as initiator was successful leading to DP= 49 and $\mathcal{D}$ = 1.05, but the rate of polymerization was low, as two days were required to get >99% monomer conversion.^{2k} Therefore, it is still challenging to find an efficient way to get well-defined alkyne-functionalized polypeptides.

Scheme 1. Synthetic methodologies leading to alkyne-functionalized polypeptides.

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In our continued efforts towards the synthesis of well-defined polypeptides,⁶ we unveiled two efficient catalytic systems for the ROP of NCA monomers, the allied amine^{6b,c} and the thiourea/aminoalcohol systems.^{6d} We found that the aminoalcohols in the presence of thiourea promote the homo- and copolymerization of Glu-NCA and Lys-NCA at room temperature. The hydroxyl group in aminoalcohol initiates the polymerization and the thiourea provided, through hydrogen bonding, simultaneous activation of NCA monomers/reversible deactivation of polymer chain ends and thus allowed the polymerization to proceed in controllable/living mode with very high activity (the TON for monomer consumption is up to 800 and TOF is up to 600 h⁻¹). Here, we report the successful application of the thiourea/aminoalcohol systems to the homopolymerization of γ -propargyl-L-glutamate NCA (Propargyl-Glu-NCA), and to the (co)polymerization of Propargyl-Glu-NCA and γ -benzyl-L-glutamate NCA (Benzyl-Glu-NCA). This is a very efficient way towards novel well-defined (co)polypeptides bearing alkyne groups such as AB diblock, ABA triblock, star-AB diblock, linear and star-AB random. All (co)polymerizations were controlled/living and possessed high activities (TOF is up to 600 h⁻¹).

By using the method developed by Hammond et al.,^{2a} we synthesized γ -propargyl-L-glutamate NCA (Propargyl-Glu-NCA), from glutamic acid and propargyl alcohol in a simple two-step procedure. We first investigated the polymerization of

propargyl-Glu-NCA initiated by **TU-S** or **DMEA**, respectively. The experimental results showed that **TU-S** could not initiate the NCA polymerization. The monomer conversion was zero after 6 h at 25°C with [Propargyl-Glu-NCA]/[**TU-S**]=10 (Table 1, run 1). Under similar conditions, **DMEA** alone triggered fast polymerization with 100% monomer conversion in 12 minutes, but the molecular weight ($M_n=2.56\times10^4$) was much higher than the targeted one (0.84×10^4).⁷ The $\bar{D}$ of the obtained polypeptide was relatively high ($\bar{D}=1.26$) and the SEC trace was asymmetric, indicating that the polymerization was not well controlled (Table 1, run2 and Figure 1a, black line). We next performed the polymerization of Propargyl-Glu-NCA initiated by **DMEA** in the presence of **TU-S**. In the latter case, the hydrogen-bonding donor **TU-S** played a positive role in improving the performance of Propargyl-Glu-NCA polymerization. With a 4 mol% loading of **TU-S** relative to propargyl-Glu-NCA (**TU-S**/**DMEA**=2, M/I=50) in the system, the polymerization proceeded very fast in DCM at room temperature and all the NCA monomer was converted into polypeptides in 40 minutes. Furthermore, the obtained polymer exhibits a very narrow symmetric SEC trace with a low $\bar{D}$ value of 1.07 and the M_n is close to the targeted one (Table 1, run 3 and Figure 1a, red line). We then increased the M/I from 50 to 100, the M_n of the obtained polypeptide increased from 0.95×10^4 to 1.81×10^4 and the $\bar{D}$ value remained low (from 1.07 to 1.09), suggesting the polymerization was controllable.

Table 1. Homo- and co-polymerization of Benzyl-Glu-NCA and Propargyl-Glu-NCA

run ^a	initiator	[M] ₀ /[I] ₀ /[TU-S]	[TU-S] mM	time (min)	conv. (%) ^b	$M_{n,calcd}$ $\times 10^{-4}$ ^c	$M_{n,SEC-LS}$ $\times 10^{-4}$ ^d	$\bar{D}$ ^d
1	DMEA	50/-/5	9.5	300	0	-	-	-
2 ^e	DMEA	50/1/-	0	12	100	0.84	2.56	1.26
3	DMEA	50/1/2	3.8	40	100	0.84	0.95	1.07
4 ^f	DMEA	100/1/2	3.8	55	100	1.67	1.81	1.09
6 ^g	DMEA	50+50/1/2	3.8	40+60	100	1.67	1.78	1.09
7 ^h	DMEA	50+50/1/2(AB diblock)	3.8	40+60	100	1.93	2.01	1.10
8	DMEA	50+50/1/2(linear AB random)	3.8	110	100	1.93	2.14	1.08
9	MDEA	50/1/2	3.8	35	100	0.84	0.94	1.10
10 ^h	MDEA	50+50/1/2(BAB triblock)	3.8	35+55	100	1.93	2.12	1.08
11 ^f	MDEA	50+50/1/2(linear AB random)	3.8	75	100	1.93	2.09	1.09
12	TEA	50/1/2	3.8	30	100	0.84	0.98	1.08
13 ^h	TEA	50+50/1/2(3-arm AB diblock)	3.8	30+45	100	1.93	2.11	1.07
14 ^f	TEA	50+50/1/2(3-arm AB random)	3.8	55	100	1.93	2.07	1.10
15	THEED	50/1/2	3.8	25	100	0.84	1.01	1.09
16 ^h	THEED	50+50/1/2(4-arm AB diblock)	3.8	25+40	100	1.93	2.15	1.10
17 ^f	THEED	50+50/1/2(4-arm AB random)	3.8	30	100	1.93	2.13	1.13

^a Polymerization was carried out in dichloromethane (DCM) at 25 °C with [NCA]₀ = 0.095M. ^b *In situ* IR was used to determine the conversion of NCA by analysing the intensity of the NCA anhydride absorption band at 1792 cm⁻¹. ^c Calculated by [NCA]/[I] $\times$ (M_{NCA-44}) $\times$ X (X = Conv.). ^d The absolute molecular weights were determined by SEC-MALS, 0.1 M LiBr in DMF at 60 °C. dn/dc(PPLG) = 0.1261 mL g⁻¹ and dn/dc(PPLG-co-PBLG) = 0.1151 mL g⁻¹. ^e The SEC trace was broad and asymmetric. ^f [Propargyl-Glu-NCA]₀ or [Benzyl-Glu-NCA + Propargyl-Glu-NCA]₀ = 0.19M. ^g Sequential polymerization of two portions of Propargyl-Glu-NCA. ^h Sequential polymerization of Benzyl-Glu-NCA and Propargyl-Glu-NCA.

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Figure 1. (a) SEC traces of (co)polypeptides shown in Table 1 (runs 2, 3, 6 and 7). (b) Kinetics of the ROP of propargyl-Glu-NCA promoted by **DMEA/TU-S** ($[M]/[DMEA]/[TU-S] = 100/1/2$, $[M]_0 = 0.19 \text{ M}$) in DCM at 25 °C by *in situ* IR with automatic sampling interval of 10 seconds.

Kinetic experiments of ROP of Glu-NCA, in the presence of **TU-S** at 25 °C in DCM, were carried out to further confirm the controlled/living nature of the polymerization ($[M]_0 = 0.19 \text{ M}$, $[M]_0/[DMEA]_0/[TU-S]_0 = 100 : 1 : 2$). The progress of the polymerization was monitored *in situ* by IR at fixed time intervals (10 seconds) for a minimum of four half-lives. As shown in Figures 1b, the plot of $\ln([NCA]_0/[NCA]_t)$ versus the polymerization time gave two straight lines, confirming that the rate of polymerization $-d[NCA]/dt = k_{\text{app}}[NCA]$ is first-order in monomer concentration. The occurrence of two-stage propagation is a general feature in the formation of polypeptides⁸ since polypeptides with different lengths have different configurations. The change of polypeptide chains from

β to α configurations during chain growth results in two-stage propagation. The β_L -polypeptide (non-helical, $DP < 7$ to 12) corresponds to the lower rate and the α -polypeptide (helical, $DP > 7$ to 12) to the higher rate. Both stages in Figure 1b possess straight lines indicating that the initiation is fast or comparable to chain propagation. The monomer consumption rate was constant meaning that no termination reactions occurred during the polymerization. In addition, the molecular weight of the obtained PPLG increased linearly with the reaction time, with monomer conversion as high as 95%. More importantly, the M_n values of the obtained polymer agreed very well with those calculated by the initial ratio of $[M]_0/[DMEA]_0$ and the monomer conversion. The $\bar{D}$ of the obtained polymer showed low values ranging from 1.06 to 1.10 (Figure 2a) and the SEC traces were narrow and symmetric (Figure 2b).

Figure 2. (a) $M_{n, \text{SEC-LS}}$ and $\bar{D}$ versus conversion (polymerization time in parentheses) for the ROP of propargyl-Glu-NCA initiated by **DMEA/TU-S**; (b) SEC profiles (RI signals) of samples taken at different time during ROP of propargyl-Glu-NCA initiated by

DMEA/TU-S ([M]/[DMEA]/[TU-S]=100/1/2, [M]₀ = 0.19M) in DCM at 25 °C.

A chain extension experiment also supported the controlled/living nature of the polymerization. PPLG with $M_n = 0.95 \times 10^4$ and $\bar{D} = 1.07$ was first obtained {propargyl-Glu-NCA]₀/[DMEA]₀/ [TU-S]₀=50/1/2} with monomer conversion 100%. Subsequent addition of 50 equiv of propargyl-Glu-NCA afforded PPLG with $M_n = 1.78 \times 10^4$ and $\bar{D} = 1.09$, indicating that PPLG chain truly possessed a living nature (Table 1, run 6 and Figure 1a, blue dot line). Furthermore, a well-defined block copolymer of PPLG-*b*-PBL with $M_n = 2.01 \times 10^4$ and $\bar{D} = 1.10$ has been prepared by sequential addition of 50 equiv. propargyl-Glu-NCA followed by addition of 50 equiv. benzyl-Glu-NCA (Table 1, run 7 and Figure 1a, magenta dot line). Therefore, the polymerizations exhibited all characteristic features of “livingness”: (a) complete consumption of the monomer; (b) linearity of M_n with conversion; (c) excellent control of molecular weight; (d) narrow molecular weight distributions; and (e) synthesis of block copolypeptides by sequential monomer addition.⁹

After establishing the “living” character, we then turned towards extending the strategy to other architectures than linear ones. As it is well known, the properties of polymers depend not only on composition but also on their topology. In order to achieve different PPLG architectures and at the same time to show the general character of the aminoalcohol/thiourea catalytic system, we used **MDEA**, **TEA** and **THEED** (bi, tri- and tetra-functional initiators) and investigated the polymerizations in the presence of thiourea. All polymerizations went smoothly and a series of di- and multi-armed alkyne-functionalized polypeptides with well-defined structures were successfully prepared for the first time. We further performed the copolymerization of propargyl-Glu-NCA and benzyl-Glu-NCA using different initiators in DCM, which allow variations in both composition and topology. In this way, a series of novel functionalized copolypeptides with well-defined structures were successfully prepared such as AB diblock, BAB triblock, star-AB diblock, linear and star AB random polypeptides. These materials dramatically increase the variety of polypeptides bearing clickable groups and are valuable precursors for acetylene-functionalized polypeptides with novel well-defined complex structures.

Table 2. Precipitation polymerization of Propargyl-Glu-NCA in toluene.

run ^a	initiator	[M] ₀ /[I] ₀ /[TU-S]	time (min) ^b	$M_{n,SEC-LS}$ $\times 10^{-4}$ ^c	$\bar{D}$ ^c
1 ^d	DMEA	50/1/2	40	1.76	1.18
2 ^e	DMEA	50/1/4	50	1.63	1.20
3 ^d	MDEA	50/1/2	18	1.64	1.23
4 ^e	MDEA	50/1/4	20	1.65	1.25
5 ^d	TEA	50/1/2	10	1.61	1.23
6 ^e	TEA	50/1/4	15	1.59	1.20
7 ^d	THEED	50/1/2	5	1.52	1.38
8 ^e	THEED	50/1/4	8	1.51	1.25

^a Polymerization was carried out in toluene at 25 °C with [NCA]₀ = 0.095M and the calculated M_n is 0.84×10^4 . ^b the time for 100% monomer conversion. ^c SEC-MALS, 0.1 M LiBr in DMF at 60 °C. ^d [TU-S]=3.8mM. ^e [TU-S]=7.6mM

We also performed the polymerization of propargyl-Glu-NCA in toluene with different initiators. Since the monomer is soluble in toluene and the PPLG is not, the polypeptide formed precipitated out of solution. Compared to the polymerizations in DCM, those polymerizations in toluene result in polymers with higher molecular weights and slightly higher $\bar{D}$. However, the polymerization went faster than in DCM and a higher TOF value than 600 h⁻¹ was obtained (Table 2, run 7).

In conclusion, we have demonstrated a new approach towards well-defined (co)polypeptides bearing pendant alkyne groups. For the first time, we report that aminoalcohols in the presence of thiourea (TU-S) can function as excellent initiators for the fast controlled/living ROP of propargyl-Glu-NCA under mild conditions, resulting in a series of well-defined di- and multi-armed alkyne functionalized homo- and copolypeptides. This new method provides a very efficient way to access well-defined alkyne-functionalized (co)polypeptides, which can be decisive for the development of new polypeptide materials by using the powerful click reactions. Further study will be carried out to prepared polypeptide based hybrid materials by using these well-defined alkyne-functionalized (co)polypeptides.

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