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COMMUNICATION

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

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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.

Functionalyzed 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 ³ 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. ³, 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-carboxy anhydride (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.³ 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 (D) 1.45,
indicating poor control over polymerization.² 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 D = 1.05, but the rate
of polymerization was low, as two days were required to get >99%
monomer conversion.²⁶ Therefore, it is still challenging to find
an efficient way to get well-defined alkyne-functionalyzed polypeptides.

Scheme 1. Synthetic methodologies leading to alkyne-
functionalized polypeptides.
In our continued efforts towards the synthesis of well-defined polypeptides,
we unveiled two efficient synthetic catalyst systems for the ROP of NCA monomers, the allied amine \(6^{,}\) and
the thiourea/aminomethanol systems. 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/aminomethanol systems to the
homopolymerization of \(\gamma\)-propargyl-L-glutamate NCA
(Propargyl-Glu-NCA), and to the (co)polymerization of
Propargyl-Glu-NCA and \(\gamma\)-benzyl-L-glutamate NCA (Benzyl-Glu-
NCA). This is a very efficient way towards novel well-defined
(co)polypeptides bearing alkylene 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., we
synthesized \(\gamma\)-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 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 (\(M_n=2.56×10^4\)) was much higher than
the targeted one (0.84×10^4). The \(D\) of the obtained polypeptide
was relatively high (\(D=1.26\)) and the SEC trace was asymmetric,
indicating that the polymerization was not well controlled
(Table 1, run 2 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 DMAC
with room temperature and all the NCA monomer was converted
into polypeptides in 40 minutes. Furthermore, the obtained
polypeptide exhibits a very narrow symmetric SEC trace with a low
\(D\) 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 \(D\) 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**

<table>
<thead>
<tr>
<th>run</th>
<th>initiator</th>
<th>([M_0]/[I_0]/[TU-S])</th>
<th>[TU-S] (\text{mM})</th>
<th>time ((\text{min}))</th>
<th>conv. ((%))</th>
<th>(M_n,\text{calcd} \times 10^4)</th>
<th>(M_n,\text{SEC-LS} \times 10^4)</th>
<th>(D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMEA</td>
<td>50/1/5</td>
<td>9.5</td>
<td>300</td>
<td>0</td>
<td>0.84</td>
<td>2.56</td>
<td>1.26</td>
</tr>
<tr>
<td>2(^a)</td>
<td>DMEA</td>
<td>50/1/1-</td>
<td>0</td>
<td>12</td>
<td>100</td>
<td>0.84</td>
<td>0.95</td>
<td>1.07</td>
</tr>
<tr>
<td>3</td>
<td>DMEA</td>
<td>50/1/2</td>
<td>3.8</td>
<td>40</td>
<td>100</td>
<td>0.84</td>
<td>1.67</td>
<td>1.09</td>
</tr>
<tr>
<td>4(^d)</td>
<td>DMEA</td>
<td>100/1/2</td>
<td>3.8</td>
<td>55</td>
<td>100</td>
<td>1.67</td>
<td>1.78</td>
<td>1.09</td>
</tr>
<tr>
<td>6(^d)</td>
<td>DMEA</td>
<td>50+50/1/2</td>
<td>3.8</td>
<td>40+60</td>
<td>100</td>
<td>1.93</td>
<td>2.01</td>
<td>1.10</td>
</tr>
<tr>
<td>7(^d)</td>
<td>DMEA</td>
<td>50+50/1/2(AB diblock)</td>
<td>3.8</td>
<td>40+60</td>
<td>100</td>
<td>1.93</td>
<td>2.14</td>
<td>1.08</td>
</tr>
<tr>
<td>8</td>
<td>DMEA</td>
<td>50+50/1/2(linear AB random)</td>
<td>3.8</td>
<td>110</td>
<td>100</td>
<td>1.93</td>
<td>2.14</td>
<td>1.08</td>
</tr>
<tr>
<td>9</td>
<td>MDEA</td>
<td>50/1/1</td>
<td>3.8</td>
<td>35</td>
<td>100</td>
<td>0.84</td>
<td>0.94</td>
<td>1.10</td>
</tr>
<tr>
<td>10(^d)</td>
<td>MDEA</td>
<td>50+50/1/2(BAB triblock)</td>
<td>3.8</td>
<td>35+55</td>
<td>100</td>
<td>1.93</td>
<td>2.12</td>
<td>1.08</td>
</tr>
<tr>
<td>11(^d)</td>
<td>MDEA</td>
<td>50+50/1/2(linear AB random)</td>
<td>3.8</td>
<td>75</td>
<td>100</td>
<td>1.93</td>
<td>2.09</td>
<td>1.09</td>
</tr>
<tr>
<td>12</td>
<td>TEA</td>
<td>50/1/2</td>
<td>3.8</td>
<td>75</td>
<td>100</td>
<td>0.84</td>
<td>0.98</td>
<td>1.08</td>
</tr>
<tr>
<td>13(^d)</td>
<td>TEA</td>
<td>50+50/1/2(3-arm AB diblock)</td>
<td>3.8</td>
<td>30+45</td>
<td>100</td>
<td>1.93</td>
<td>2.11</td>
<td>1.07</td>
</tr>
<tr>
<td>14(^d)</td>
<td>TEA</td>
<td>50+50/1/2(3-arm AB random)</td>
<td>3.8</td>
<td>30</td>
<td>100</td>
<td>1.93</td>
<td>2.07</td>
<td>1.10</td>
</tr>
<tr>
<td>15</td>
<td>THEED</td>
<td>50/1/2</td>
<td>3.8</td>
<td>25</td>
<td>100</td>
<td>0.84</td>
<td>1.01</td>
<td>1.09</td>
</tr>
<tr>
<td>16(^d)</td>
<td>THEED</td>
<td>50+50/1/2(4-arm AB diblock)</td>
<td>3.8</td>
<td>25+40</td>
<td>100</td>
<td>1.93</td>
<td>2.15</td>
<td>1.10</td>
</tr>
<tr>
<td>17(^d)</td>
<td>THEED</td>
<td>50+50/1/2(4-arm AB random)</td>
<td>3.8</td>
<td>30</td>
<td>100</td>
<td>1.93</td>
<td>2.13</td>
<td>1.13</td>
</tr>
</tbody>
</table>

\(^a\) Polymerization was carried out in dichloromethane (DCM) at 25°C with [NCA] = 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
\((M_{n,\text{NCA}}−44)\times X (X = \text{Conv.})\). \(^d\) The absolute molecular weights were determined by SEC-MALS, 0.1 M LiBr in DMF at 60°C. \(^e\) The SEC trace was broad and asymmetric. \(^f\) [Propargyl-Glu-NCA] or
[Benzyl-Glu-NCA+ Propargyl-Glu-NCA] = 0.19M. \(^g\) Sequential polymerization of two portions of Propargyl-Glu-NCA. \(^h\) Sequential polymerization of Benzyl-Glu-NCA and Propargyl-Glu-NCA.)
Please do not adjust margins

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 = 0.19M) 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 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-live times. As shown in Figures 1b, the plot of ln([NCA]₀/[NCA]) versus the polymerization time gave two straight lines, confirming that the rate of polymerization −d[NCA]/dt = k_{app}[NCA] is first-order in monomer concentration. The occurrence of two-stage propagation is a general feature in the formation of polypeptides 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-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 Mₙ 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 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) Mₙ,SEC-LS and 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
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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 Mₙ= 0.95×10⁴ and D = 1.07 was first obtained (propargyl-Glu-NCA)/[DMEA]/[TU-S]=50/1/2 with monomer conversion 100%. Subsequent addition of 50 equiv of propargyl-Glu-NCA afforded PPLG with Mₙ = 1.78×10⁴ and D = 1.09, indicating that PPLG chain truly possessed a living nature (Table 1, run 7 and Figure 1a, magenta dot line). Furthermore, a well-defined block copolymer of PPLG-b-PBLL with Mₙ =2.01×10⁴ and 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, blue dot line). Therefore, the polymerizations exhibited all characteristic features of “livingness”: (a) complete consumption of the monomer; (b) linearity of Mn 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 aminocarbox/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.

<table>
<thead>
<tr>
<th>run</th>
<th>initiator</th>
<th>[M]/[DMEA]/[TU-S]</th>
<th>time (min)</th>
<th>Mₙ,SEC-LS x10⁴</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>DMEA</td>
<td>50/1/2</td>
<td>40</td>
<td>1.76</td>
<td>1.18</td>
</tr>
<tr>
<td>2a</td>
<td>DMEA</td>
<td>50/1/4</td>
<td>50</td>
<td>1.63</td>
<td>1.20</td>
</tr>
<tr>
<td>3a</td>
<td>MDEA</td>
<td>50/1/2</td>
<td>18</td>
<td>1.64</td>
<td>1.23</td>
</tr>
<tr>
<td>4a</td>
<td>MDEA</td>
<td>50/1/2</td>
<td>20</td>
<td>1.65</td>
<td>1.25</td>
</tr>
<tr>
<td>5a</td>
<td>TEA</td>
<td>50/1/2</td>
<td>10</td>
<td>1.61</td>
<td>1.23</td>
</tr>
<tr>
<td>6a</td>
<td>TEA</td>
<td>50/1/4</td>
<td>15</td>
<td>1.59</td>
<td>1.20</td>
</tr>
<tr>
<td>7a</td>
<td>THEED</td>
<td>50/1/2</td>
<td>5</td>
<td>1.52</td>
<td>1.28</td>
</tr>
<tr>
<td>8a</td>
<td>THEED</td>
<td>50/1/4</td>
<td>8</td>
<td>1.51</td>
<td>1.25</td>
</tr>
</tbody>
</table>

a) Polymerization was carried out in toluene at 25 °C with [NCA] = 0.095M and the calculated Mₙ is 0.84×10⁴. b) the time for 100% monomer conversion. c) SEC-MALS, 0.1 M LiBr in DMF at 60 °C. d) [TU-S]=3.8 mM. e) [TU-S]=7.6 mM

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 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 aminocarboxazoles 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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