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Soluble, Clickable Polypeptides from Azide-Containing *N*-Carboxyanhydride Monomers

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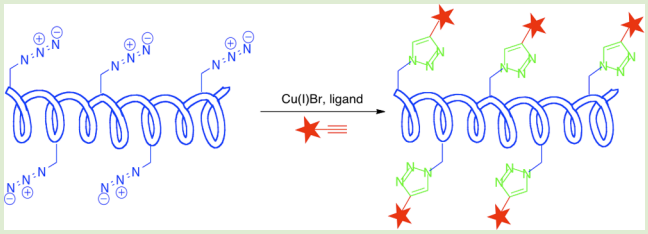
Supporting Information

ABSTRACT: We report a method for the synthesis of soluble, well-defined, azide-functionalized polypeptides via living polymerization of new azide-containing amino acid *N*-carboxyanhydride (NCA) monomers. Homo and diblock azidopolypeptides were prepared with controlled segment lengths using $(PMe_3)_4Co$ initiator and were subsequently modified by reaction with functional alkyne reagents. The azide groups were found to be quantitatively converted to the corresponding triazole derivatives, and the functionalized polymers were obtained in high yield. This methodology provides a facile and straightforward method for preparation of functional and side-chain reactive, high molecular weight polypeptides.



Since its introduction in 2001 by Sharpless and Meldal,[1,2] the copper-catalyzed azide−alkyne cycloaddition (CuAAC) coupling reaction has gained popularity in polymer science for its selectivity, efficiency, and broad scope.[3,4] In the polypeptide field, this method is particularly attractive since it provides chemoselective conjugation and bioorthogonal conjugation, in the case of strain-promoted cycloaddition,[5] in the presence of a wide variety of natural amino acid functional groups. Consequently, there has been much recent activity in the preparation of polypeptides capable of utilizing CuAAC, as well as thiol−ene and thiol−yne coupling reactions.[6−16] These reactive precursor polypeptides have been used to create side-chain functional materials,[17−19] hybrid polypeptide−synthetic block copolymers,[20] as well as polypeptide-containing polymer brushes via a grafting to approach.[21−23]

Although proven useful when incorporated into proteins, peptides, and other biomolecules,[24−31] the azide functionality has only seen limited use in synthetic polypeptides,[14,15,32] in contrast to the more widespread incorporation of alkyne and alkene functionality.[6−9,16−21] This difference may be due to the availability of alkyne- and alkene-bearing amino acid *N*-carboxyanhydride (NCA) monomers that can be directly polymerized to the functional polypeptides,[6] while the only reported preparations of azidopolypeptides require the derivatization of polypeptide precursors.[14,15,32] A facile route to azide-containing NCA monomers would be advantageous since these would streamline synthesis of azidopolypeptides, avoiding reactions on polymers and allowing precise placement of azide functionality in copolypeptide sequences. Here, we describe the synthesis of new azide-containing NCA monomers that allow the direct preparation of soluble, high molar mass, α-helical azidopolypeptides. Homopolypeptides and diblock copolypeptides were prepared with controlled segment lengths via living polymerization using $(PMe_3)_4Co$ initiator. To highlight their potential as reactive polymers, the azidopolypeptides were quantitatively functionalized with carboxylic acid, amino acid, and sugar groups using CuAAC.

The conversion of primary amines to azides in biomolecules has been the subject of much attention following the recent development of improved diazotransfer reagents,[33] such as imidazole-1-sulfonyl-azide·HCl reported initially by Goddard-Borger and co-workers in 2007.[34,35] These reagents have been used to selectively introduce azide groups in amino acids, peptides, proteins, and other biomolecules.[24,26,29−31,33] For synthesis of azide-containing NCAs, we used inexpensive L-lysine and L-ornithine as starting materials since their side-chain amine groups can be readily converted to azides in a single step.[33] Azido amino acids were prepared from the N_α-carboxybenzyl (Cbz) protected amino acids using modified literature procedures.[34,35] These derivatives were then directly converted to NCAs, via the acid chloride using Ghosez's reagent, with no complications arising from the azide functionality. Although the resulting NCA monomers were oils, they were readily purified using our flash column chromatography methodology and obtained in reasonable yields (Scheme 1).[36]

Polymerizations of Anl NCA and Anv NCA using $(PMe_3)_4Co$ in THF proceeded readily at ambient temperature to give the corresponding homopolypeptides,[37] poly(Anl) and poly(Anv), with complete monomer conversions and no reactions at the side-chain azido groups. Residual Co salts were readily removed by precipitation of the hydrophobic polypeptides in 0.1 M aqueous HCl. Although both monomers

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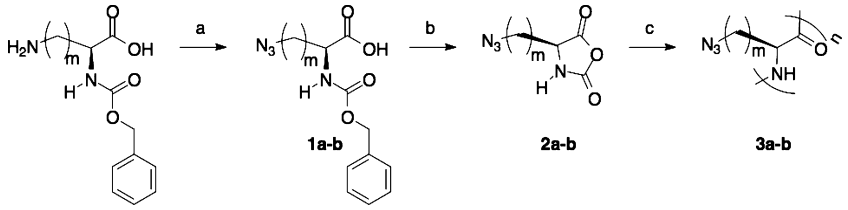
Scheme 1. Three-Step Synthesis of Azidopolypeptides from N_α-Carboxybenzyl Amino Acids[a]



[a](a) Imidazole-1-sulfonyl-azide·HCl, $CuSO_4{\cdot}5H_2O$, K_2CO_3, 1:1 THF:H_2O, 16 h (88% yield, **1b**). (b) Ghosez's reagent, THF, 21 °C, 48 h (67% yield, **2b**). (c) $(PMe_3)_4Co$, THF, 21 °C, 1 h (96% yield, **3b**). **2a** = L-azidonorvaline-*N*-carboxyanhydride (m = 3, Anv NCA), **2b** = L-azidonorleucine-*N*-carboxyanhydride (m = 4, Anl NCA), **3a** = poly(L-azidonorvaline), poly(Anv), **3b** = poly(L-azidonorleucine), poly(Anl).

polymerized well (see Supporting Information (SI)), we focused our studies on polymerizations of Anl NCA since poly(Anl) was found to have better solubility in THF. To see if chain length could be controlled, Anl NCA was polymerized to complete monomer conversion at different monomer to initiator (M:I) ratios, and the active chains were then end-capped with isocyanate-terminated PEG (M_n = 2000 Da).[38] Compositional analysis of purified, end-capped polymers by ^{1}H NMR gave number average poly(Anl) chain lengths that increased linearly with M:I stoichiometry (Figure 1). Chain

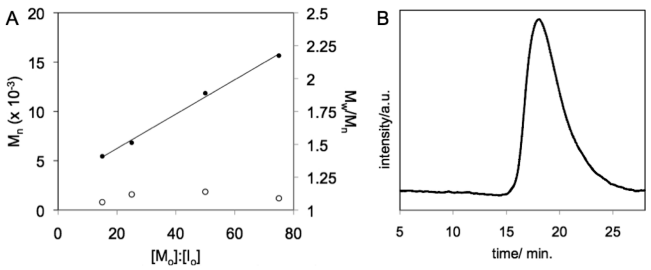


Figure 1. Synthesis of azidopolypeptides. (a) Molecular weight (M_n, filled circles) and polydispersity index (M_w/M_n, open circles) of poly(Anl) as a function of monomer to initiator ratio ([M]:[I]) after 100% monomer conversion. M_n and M_w/M_n were determined by ^{1}H NMR and gel permeation chromatography (GPC/LS), respectively. (b) GPC chromatogram (RI intensity in arbitrary units (au) versus elution time) of a poly(Anl) sample (see SI, Table S1, entry 3).

length distributions of these poly(Anl) samples were obtained by GPC/LS analysis, and polydispersity indices (M_w/M_n) were found to be between 1.06 and 1.12, indicating well-defined polypeptides were formed (Figure 1). Poly(Anl) was obtained in high yield with precisely controlled chain lengths up to over 100 residues long and could also be incorporated into diblock copolymers with controlled segment lengths (Table 1). These chain lengths, e.g., 25 to 100 residues, cover a desirable range for many polypeptide materials applications.[39] Overall, these data show that Anl NCA, similar to other NCAs,[37] is able to undergo living polymerization when initiated with $(PMe_3)_4Co$.

After preparation of Anl NCA in high purity and synthesis of poly(Anl) with controlled chain lengths and low polydispersity, we explored the reaction of poly(Anl) with model functional alkynes. To study the versatility of this reaction, carboxylic acid (**4**), amino acid (**5**), and monosaccharide (**6**) functionalities were separately added to poly(K)$_{60}$-*b*-poly(Anl)$_{54}$ via CuAAC in DMSO (Figure 2). A poly(K)-*b*-poly(Anl) diblock

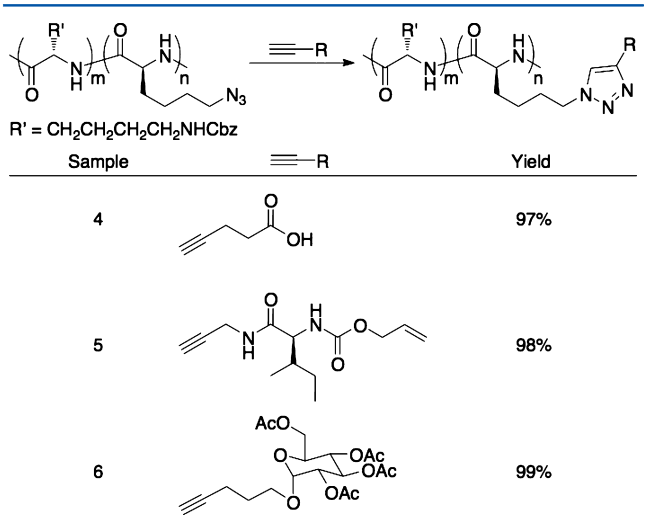


Figure 2. Reaction of poly(K)$_m$-*b*-poly(Anl)$_n$ (m = 60, n = 54) with model functional alkynes using CuAAC. Reagents and conditions: 1.8 equiv of alkyne per azide group, Cu(I)Br, PMDETA, DMSO, 21 °C, 48 h. Cu salts were readily removed by precipitation of the products in 0.1 M aqueous HCl. Yield is total isolated yield of completely functionalized copolypeptide.

Table 1. Synthesis of Diblock Copolypeptides Using $(PMe_3)_4Co$ in THF at 21 °C

		first segment[b]			diblock copolymer[c]			
1st monomer[a]	2nd monomer[a]	M_n	M_w/M_n	DP	M_n	M_w/M_n	DP	yield (%)[d]
15 K NCA	7.5 Anl NCA	14 400	1.17	55	18 700	1.09	83	99
15 K NCA	15 Anl NCA	14 400	1.17	55	22 900	1.17	110	99
15 Anl NCA	7.5 K NCA	5500	1.19	39	13 600	1.12	61	97
15 Anl NCA	15 K NCA	5500	1.19	39	16 200	1.1	78	98

[a]First and second monomers added stepwise to the initiator; number indicates equivalents of monomer per $(PMe_3)_4Co$. K NCA = N_ε-Cbz-L-lysine-*N*-carboxyanhydride. Anl NCA = L-azidonorleucine-*N*-carboxyanhydride. [b]Molecular weight and polydispersity index after polymerization of the first monomer (determined by GPC/LS for poly(K); determined by GPC/LS and ^{1}H NMR for poly(Anl)). [c]Molecular weight and polydispersity index after polymerization of the second monomer (as determined by GPC/LS and ^{1}H NMR). [d]Total isolated yield of diblock copolypeptide. DP = degree of polymerization.

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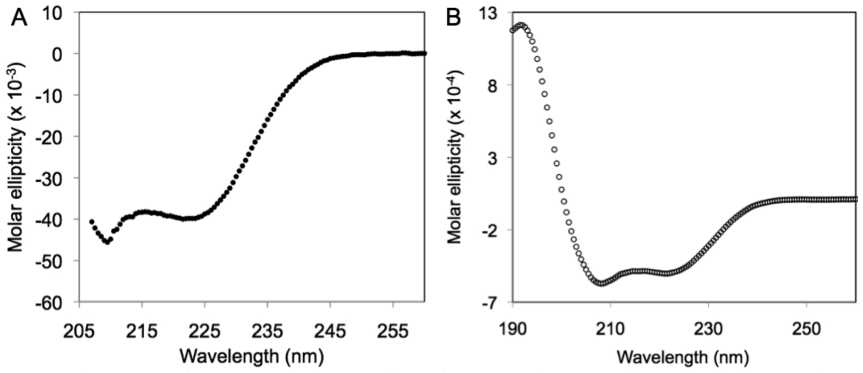

Figure 3. Circular dichroism spectra of poly(Anl) and its glycosylated derivative. (a) poly(Anl)$_{52}$ in THF (50 μg/mL); spectrum was cutoff at 205 nm due to solvent absorbance below this wavelength. (b) poly(Anl)$_{52}$ after CuAAC with **6** and removal of acetate groups; in H_2O, pH = 7.0 (5 μg/mL).

copolypeptide was used for these studies to provide a poly(K) reference segment in ^{1}H NMR analysis. The functionality of **5** is potentially useful for controlled preparation of polypeptide cylindrical brushes,[40] and the functionality of **6** provides a facile method for synthesis of glycopolypeptides. The modified polymers were obtained in >97% isolated yields, and ^{1}H NMR analysis showed complete conversions to the corresponding 1,4-substituted triazole-derivatized copolypeptides (see SI, Figure S3).

Circular dichroism spectroscopy of homopolypeptide poly-(Anl)$_{52}$ in THF revealed that its conformation is predominantly α-helical (Figure 3), which imparts poly(Anl) with good solubility in organic solvents and may provide an exposed presentation of the side-chain azido groups.[6] CuAAC functionalization of poly(Anl)$_{52}$ with glycoside (**6**) followed by removal of the acetyl protecting groups (see SI) gave a water-soluble, fully glycosylated polypeptide. This glycopolypeptide was also found by circular dichroism analysis to be predominantly α-helical, showing that poly(Anl) is useful for preparation of α-helical, functionalized polypeptide derivatives (Figure 3B). It is also noteworthy that the derivatized polypeptides are stable to reagents necessary for removal of protecting groups, such as the ester groups in the glycopolypeptide described above.

In summary, we prepared new azide-containing NCAs that undergo living polymerization to give well-defined, high molecular weight homopolypeptides and block copolypeptides that are stable at ambient temperature for months. This is a facile and straightforward method for preparation of functional and side-chain reactive polypeptides via chemoselective CuAAC of the azidopolypeptides with alkyne reagents. The use of azide-containing NCA monomers also provides quantitative azide incorporation into polypeptide side chains and adds to the capability to prepare complex polypeptide sequences containing different combinations of side-chain reactive groups.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data for all new compounds, polymerization data, and M_n vs [M]/[I] plots for poly(Anv). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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