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Glycopolypeptides via Living Polymerization of Glycosylated-L-lysine *N*-Carboxyanhydrides

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Abstract: The preparation of new glycosylated-L-lysine-*N*-carboxyanhydride (glyco-K NCA) monomers is described. These monomers employ C-linked sugars and amide linkages to lysine for improved stability without sacrificing biochemical properties. Three glyco-K NCAs were synthesized, purified, and found to undergo living polymerization using transition metal initiation. These are the first living polymerizations of glycosylated NCAs and were used to prepare well-defined, high molecular weight glycopolypeptides and block and statistical glycocopolypeptides. This methodology solves many long-standing problems in the direct synthesis of glycopolypeptides from *N*-carboxyanhydrides relating to monomer synthesis, purification, and polymerization and gives polypeptides with 100% glycosylation. These long chain glycopolypeptides have potential to be good mimics of natural high molecular weight glycoproteins.

Introduction

Glycosylated peptides and proteins are ubiquitous in nature and display a wide range of biological functions including mediation of recognition events, protection from proteases, and lubrication in eyes and joints.[1] Similarly, synthetic glycopolypeptides are also expected to show great potential as biomedical materials (e.g., scaffolds for tissue repair and drug carriers), as well as serve as valuable tools for probing carbohydrate−protein interactions.[2,3] Although block and hybrid copolypeptides capable of forming vesicles,[4,5] fibrils,[6] and other structures[7,8] are readily prepared by amino acid *N*-carboxyanhydride (NCA) polymerization,[9] the synthesis of well-defined glycopolypeptide materials has been challenging. Even though O-linked glyco-serine NCAs have been known for some time,[10,11] their synthesis is inefficient, and their polymerization gives only short, oligomeric products where chain growth is likely inhibited by steric and H-bonding interactions between the sugar substituents and the NCA rings.[12] An improved synthesis of O- and S-linked glyco-serine as well as glycothreonine NCAs was recently reported; however, these monomers were not sufficiently purified to allow polymerization.[13] To overcome these difficulties in synthesis, purification, and polymerization of glycosylated NCAs, we have prepared new glycosylated-L-lysine NCA (glyco-K NCA) monomers that employ C-linked sugars and amide linkages to lysine[14] for improved stability without sacrificing biochemical properties. Three different glyco-K NCAs were synthesized, purified, and found to undergo living polymerization using transition metal initiation.[15] These are the first living polymerizations of glycosylated NCAs allowing preparation of well-defined, high molecular weight glycopolypeptides and glycopolypeptide containing block copolymers.

Aside from direct polymerization of glycosylated NCAs, other strategies to prepare glycopolypeptides rely primarily on the addition of sugars to existing polypeptides. Glyconamidated polypeptides were prepared by reaction of D-gluconolactone or lactobionolactone with poly(L-lysine),[16,17] or by coupling of β-D-galactosylamine to glutamic acid residues,[18] to attach carbohy-

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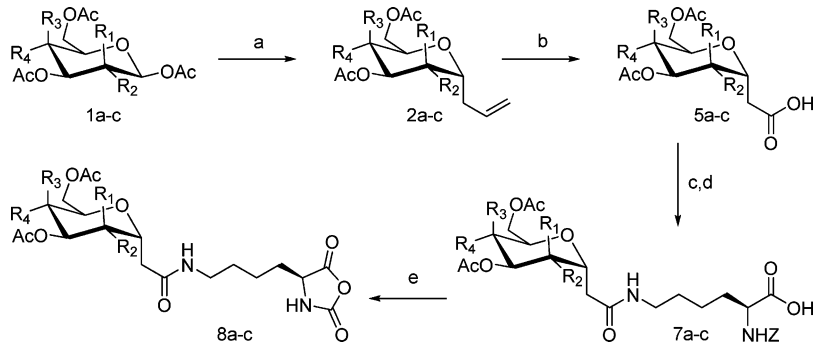

Figure 1. Synthesis of glyco-K NCA monomers. Reagents and conditions: (a) allyltrimethylsilane, BF_3-Et_2O, MeCN (55−75% yield); (b) $NaIO_4$, $RuCl_3 \cdot 3H_2O$, MeCN:CCl_4:H_2O (72−92% yield); (c) NHS, DCC, THF; (d) α-*Z*-L-Lys-OH, $NaHCO_3$ (75−81% yield for (c) and (d)); (e) Cl_2CHOMe, DCM, 50 °C (59−64% yield). **8a** = α-gal-K NCA (R_1, R_4 = H; R_2, R_3 = OAc). **8b** = α-glc-K NCA (R_1, R_3 = H; R_2, R_4 = OAc). **8c** = α-man-K NCA (R_1, R_4 = OAc; R_2, R_3 = H).

drates via amide linkages. Also using polypeptide precursors, glycopolypeptide synthesis has been reported using either copper-catalyzed azide−alkyne[19,20] or thiol−ene[21] click chemistry, respectively. While promising, these methods can suffer from incomplete sugar functionalization,[21] presentation of sugars in non-native forms (i.e., ring-opened),[16,17] or incorporation of triazole groups[19,20] that may limit biological uses. Consequently, we have focused on the glycosylated NCA approach to obtain well-defined glycopolypeptides with 100% glycosylation, as well as the ability to incorporate glycosylated residues either as random or blocky sequences. We designed glycosylated-L-lysine monomers because many derivatives of lysine NCAs are readily synthesized and polymerized to high molecular weights (>100 000 Da),[22] and because acid and base stable linkages can be readily incorporated to prevent deglycosylation. We chose the glycosylated-L-lysine structure shown in Figure 1, which has been used for glycopeptide synthesis[14] and employs C-linked sugars and amide linkages to lysine. Although these are non-native linkages, it is well-known that C-linked glycopeptides can bind targets with nearly equal affinity and conformation as native O-linked analogues[23,24] and are widely utilized when stable glycoprotein mimetics are desired.[25]

Results and Discussion

The sugar−lysine conjugates were prepared using β-D-glucose (glc), β-D-mannose (man), and β-D-galactose (gal). C-Allylation of glc, man, and gal pentaacetates was accomplished according to literature procedures followed by isolation of the pure α anomers (Figure 1 and Supporting Information).[14] Purification of anomers at this stage in the synthesis is crucial for the ultimate preparation of optically pure, glyco-K NCA monomers. Mixtures of anomers would lead to polypeptides containing different sugar configurations, which could make analysis of their properties difficult. Both α- and β-anomers are common in glycopeptides and glycoproteins.[26] Although our methodology allows use of either anomer, we chose to use the α-anomers here because they were easily obtained in high purity and natural glycopeptides utilize both α- and β-anomers.[27] The allyl sugar derivatives were oxidized to carboxylic acids, converted to *N*-hydroxysuccinimide (NHS) esters, and then coupled to N_α-carbobenzyloxy-L-lysine (N_α-*Z*-L-lysine) to give the desired conjugates (Figure 1).[14] The preparation of glyco-K NCAs was accomplished using Cl_2CHOCH_3[22c] followed by purification by precipitation and flash chromatography to give NCAs suitable for polymerization. All three glyco-K NCAs were obtained with acceptable yields (60−95%) in each step. Furthermore, all three monomers were found to polymerize efficiently using $(PMe_3)_4Co$[28] initiator in THF at room temperature (Figure 2),[15] yielding polymers in excellent yields (Table 1, see Supporting Information).

To check for chain-breaking side reactions during polymerizations, experiments to verify molecular weight control and extension of active chains were performed. Variation of monomer to initiator ratios for each glyco-K NCA gave glycopolypeptides whose lengths increased linearly with stoichiometry and which possessed narrow chain length distributions (M_w/M_n). Data for the α-D-mannose-L-lysine monomer (α-man-K NCA) are shown in Figure 3a, and data for the other two monomers are given in the Supporting Information. Good chain length control was obtained, and soluble homoglycopolypeptides could be prepared with degrees of polymerization (DP) greater than 300 residues, significantly larger than chains prepared from other glycosylated NCAs (DP < 50).[12a] As compared to shorter chains, long chain glycopolypeptides are expected to be much better mimics of natural high molecular weight glycoproteins. We found that the polymers had higher than theoretical molecular weights, calculated from monomer

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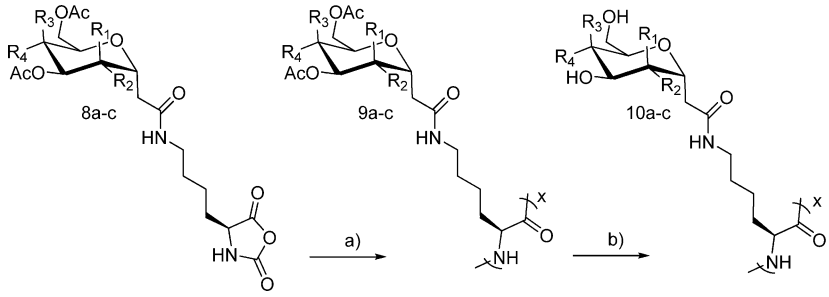

Figure 2. Polymerization of glyco-K NCAs and glycopolypeptide deprotection. Reagents and conditions: (a) $(PMe_3)_4Co$, THF; (b) $(H_2N)_2 \cdot H_2O$, MeOH. **10a** = poly(α-gal-K) (R_1, R_4 = H; R_2, R_3 = OH). **10b** = poly(α-glc-K) (R_1, R_3 = H; R_2, R_4 = OH). **10c** = poly(α-man-K) (R_1, R_4 = OH; R_2, R_3 = H).

Table 1. Synthesis of Glycopolypeptides Using $(PMe_3)_4Co$ in THF at 20 °C

monomer[a]	M_n[b]	M_w/M_n[b]	DP[c]	yield (%)[d]
10 α-man-K NCA	15 870	1.21	32	91
25 α-man-K NCA	36 920	1.14	74	95
50 α-man-K NCA	81 340	1.19	163	89
75 α-man-K NCA	121 300	1.11	242	94
100 α-man-K NCA	158 600	1.14	317	92
25 glyco-K NCAs[e]	47 470	1.23	95	88
50 glyco-K NCAs[e]	84 710	1.07	169	94

[a] Number indicates equivalents of monomer per $(PMe_3)_4Co$. [b] Molecular weight and polydispersity index (as determined by GPC/LS). [c] DP = number average degree of polymerization from GPC/LS. [d] Total isolated yield of glycopolypeptide. [e] Statistical terpolymers prepared from a 1:1:1 mixture of α-man-K:α-gal-K:α-glc-K NCAs.

to initiator stoichiometry, which is due to the known incomplete efficiency of $(PMe_3)_4Co$ initiation in THF.[29] In any case, reproducible and precisely controlled glycopolypeptide lengths were readily prepared. Chain extension experiments of active chains to prepare diblock copolymers (Table 2, see Supporting Information) all proceeded in high conversion to yield predictable compositions, and with no evidence of inactive chains by GPC analysis (Figure 3b). Diblock copolymers were prepared by combining different glyco-K NCAs, as well as by combination of glyco-K NCAs with conventional NCAs regardless of order of monomer addition. In addition to block copolymers, equimolar mixtures of the three glyco-K NCAs were also copolymerized to yield statistical ternary glycocopolypeptides of controllable chain length (Table 1). Overall, these data show that the glyco-K NCAs were able to undergo living polymerization when initiated with $(PMe_3)_4Co$, similar to conventional NCAs,[15] and this chemistry enabled preparation of the first examples of glycosylated diblock copolypeptides.

It is noteworthy that polymerizations of glyco-K NCAs proceeded efficiently at room temperature to yield polymers with DP > 150 in less than 3 h, as compared to 3−6 days for polymerization of O-linked glyco-serine NCAs (DP < 25) using amine initiators.[12a] The sluggish polymerization of O-linked glyco-serine NCAs is thought to be due to either the proximity of the bulky peracetylated sugar to the NCA ring or the H-bonding of acetyl carbonyls with NH groups of NCAs.[12b,c] Although we also see ^{1}H NMR evidence for H-bonding in our glyco-K NCAs (see Supporting Information), the increased tether length between sugar and NCA likely weakens this interaction and removes steric hindrance allowing efficient polymerization. However, the influence of H-bonding is not negligible, because the glyco-K NCAs were found to polymerize 2−5 times more slowly than other lysine NCA derivatives. As all of the acetyl protected poly(glyco-K)s were found to be

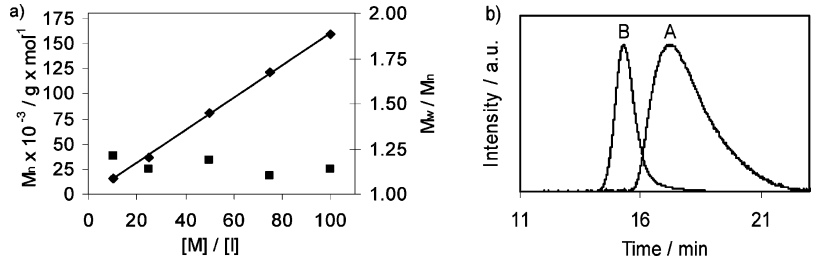

Figure 3. (a) Molecular weight (M_n, ◆) and polydispersity index (M_w/M_n, ■) of poly(α-man-K) as functions of monomer to initiator ratio ([M]/[I]) using $(PMe_3)_4Co$ in THF at 20 °C. (b) GPC chromatograms (normalized LS intensity versus elution time in arbitrary units (au)) of glycopolypeptides after initial polymerization of α-man-K NCA to give a poly(α-man-K)$_{68}$ homoglycopolypeptide (A); and after chain extension by polymerization of α-gal-K NCA to give a poly(α-man-K)$_{68}$-*b*-poly(α-gal-K)$_{200}$ diblock glycopolypeptide (B).

Table 2. Synthesis of Diblock Copolypeptides Using $(PMe_3)_4Co$ in THF at 20 °C

		first segment[b]			diblock copolymer[c]			
first monomer[a]	second monomer[a]	M_n	M_w/M_n	DP	M_n	M_w/M_n	DP[d]	yield (%)[e]
30 Lys NCA	90 α-man-K NCA	18 100	1.15	69	124 000	1.01	281	93
30 α-man-K NCA	90 Lys NCA	34 090	1.23	68	86 820	1.07	269	99
30 α-man-K NCA	90 α-gal-K NCA	34 090	1.23	68	134 200	1.12	268	91

[a] First and second monomers added stepwise to the initiator; number indicates equivalents of monomer per $(PMe_3)_4Co$. Lys NCA = ε-*Z*-L-lysine-*N*-carboxyanhydride.[30] [b] Molecular weight and polydispersity index after polymerization of the first monomer (as determined by GPC/LS). [c] Molecular weight and polydispersity index after polymerization of the second monomer. [d] Total degree of polymerization of diblock glycopolypeptide. [e] Total isolated yield of diblock glycopolypeptide.

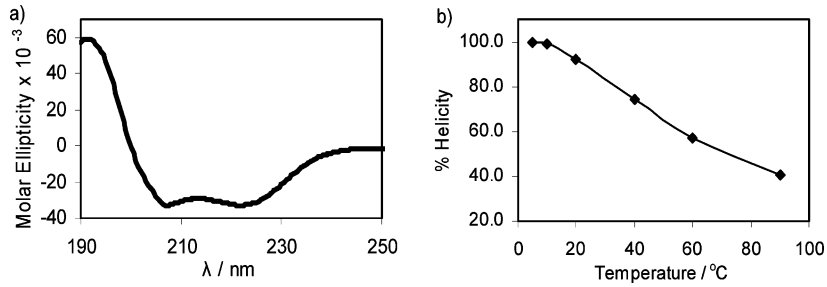

Figure 4. (a) Circular dichroism spectrum of poly(α-gal-K) at 20 °C, 92% α-helical (**10a**). (b) Percent of α-helical content (% helicity, calculated using molar ellipticity at 222 nm) of poly(α-gal-K) as a function of temperature. M_n = 90 040 Da, 0.25 mg/mL in deionized water, pH 7. Molar ellipticity is reported in millideg·cm^2·dmol^{-1}.

soluble in THF, it appears that there are no significant interchain H-bonding interactions in the polymers. Furthermore, all three homoglycopolypeptides were found to have good water solubility after deprotection using $NH_2NH_2 \cdot H_2O$ in MeOH. To investigate the solution conformations of these samples, circular dichroism (CD) spectra of the poly(glyco-K)s were measured in deionized water after purification and removal of all residual cobalt impurities by extensive dialysis against deionized water. All three glycopolypeptides were found to be α-helical in water at room temperature, with characteristic minima at 208 and 222 nm, indicating greater than 88% helicity[31] (Figure 4a, see Supporting Information). Poly-L-lysine inherently prefers to be α-helical when uncharged,[32] and the bulky sugar groups are positioned far enough away from the backbone to avoid perturbing this conformation. Measurement of CD spectra from 4 to 90 °C revealed that the α-helical conformation of poly(α-gal-K) was gradually disrupted as temperature was increased. This behavior is likely due to disruption of amide H-bonding by interactions with water molecules.[33] These disordered polypeptides remained water-soluble, and their α-helical conformations were completely regained upon cooling, showing this process is reversible. Ternary glycocopolypeptides containing randomly placed sugars also were found to be α-helical (see Supporting Information), indicating that the poly-L-lysine backbone is directing the chain conformation rather than any specific interactions between sugars. This result allows the display of a diverse variety of sugars off of ordered α-helical polypeptide scaffolds, potentially useful for diagnostic applications.

Conclusion

We have prepared new glycosylated lysine NCAs that undergo living polymerization to give well-defined, high molecular weight homoglycopolypeptides and block and statistical glycocopolypeptides. This system solves many long-standing problems in the direct synthesis of glycopolypeptides from NCAs relating to monomer synthesis, purification, and polymerization and is advantageous in that polypeptides with 100% glycosylation are easily obtained. These water-soluble glycopolypeptides have potential to impart functionality and improve biocompatibility in copolypeptide materials, such as hydrogels for tissue engineering and vesicles for drug delivery, as well as for the preparation of structurally defined sugar presenting polymers for glycomics research.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds. Polymerization data, M_n versus [M]/[I] plots, and circular dichroism spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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