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Controlled Synthesis of *O*-Glycopolypeptide Polymers and Their Molecular Recognition by Lectins

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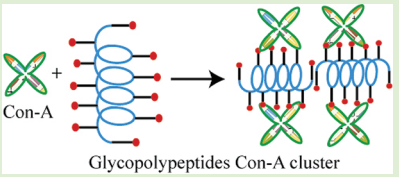
Supporting Information

ABSTRACT: The facile synthesis of high molecular weight water-soluble *O*-glycopolypeptide polymers by the ring-opening polymerization of their corresponding *N*-carboxyanhydride (NCA) in very high yield (overall yield > 70%) is reported. The per-acetylated-*O*-glycosylated lysine-NCA monomers, synthesized using stable glycosyl donors and a commercially available protected amino acid in very high yield, was polymerized using commercially available amine initiators. The synthesized water-soluble glycopolypeptides were found to be α-helical in aqueous solution. However, we were able to control the secondary conformation of the glycopolypeptides (α-helix vs nonhelical structures) by polymerizing racemic amino acid glyco NCAs. We have also investigated the binding of the glycopolypeptide poly(α-manno-*O*-lys) with the lectin Con-A using precipitation and hemagglutination assays as well as by isothermal titration calorimetry (ITC). The ITC results clearly show that the binding process is enthalpy driven for both α-helical and nonhelical structures, with negative entropic contribution. Binding stoichiometry for the glycopolypeptide poly(α-manno-*O*-lys) having a nonhelical structure was slightly higher as compared to the corresponding polypeptide which adopted an α-helical structure.



INTRODUCTION

Glycopolymers, synthetic polymers featuring pendant carbohydrate moieties, have been of particular interest to the field of tissue engineering and drug delivery.[1−12] This interest is derived from the complex roles that carbohydrates play in vivo, particularly in biomolecular recognition events such as extracellular recognition, adhesion, cell growth regulation, cancer cell metastasis, and inflammation.[13,14] The key to the recognition process is their interactions with carbohydrate-binding protein receptors known as lectins.[15,16] The interaction between lectins and carbohydrates is weak; dissociation constants, K_d, are typically 10^{-3} to 10^{-6} M, but may be greatly enhanced through polyvalency. Because glycopolymers are typically polyvalent, as they have several pendant carbohydrate groups; they present a platform for which multiple copies of a carbohydrate can be presented simultaneously, thus, enhancing their affinity and selectivity for lectins many fold. Carbohydrate recognizing receptors are found on many cell surfaces. An excellent example is the asialoglycoprotein receptor (ASGP-R) displayed on the hepatocyte cell surface that interacts uniquely with galactose/N-acetyl-β-galactosamine containing carbohydrate ligands.[17−20] Galactose containing synthetic linear glycopolymers can therefore be used to guide hepatocyte adhesion through this unique ASGP-R−carbohydrate interaction. This strategy has been used to design extracellular matrices using galactose containing synthetic polymers for liver tissue engineering.[21] Similarly, the use of glycopolymers as vehicles for therapeutics has also shown a lot of promise.[8−12] However, a majority of these synthetic glycopolymers are acrylate/acrylamide based and controlled radical polymerization is used to synthesize polymers with controlled molecular weight, glycosylation density, and position attributes that are necessary for biological recognition processes. However, the lack of biocompatibility of some of these polymers can render it difficult for application in medicine such as drug delivery or tissue engineering. On the other hand, glycopolypeptides (glycopolymers with pendant carbohydrates on a polypeptide backbone) not only mimic the molecular composition of proteoglycans but also has the ability to fold into well-defined secondary structures (e.g., helix).[22,23] Therefore, it is desirable to develop methodologies that afford easy and well-defined synthetic glycopolypeptides.

Although well-defined polypeptides based on natural and unnatural amino acids have been very successfully synthesized by the ring-opening polymerization of their corresponding *N*-carboxyanhydrides (NCA),[24−29] the synthesis of glycopolypeptide still remains a major challenge.[30,31] Synthesis of glycopolypeptides by post polymerization modification of synthetic polypeptides on the contrary has been more successful and several methods have been reported recently.[32−36] We have recently reported the synthesis of the per-*O*-benzoylated-D-glyco-L-lysine carbamate NCA from a

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stable glycosyl donor and a commercially available protected amino acid in very high yield (overall yield > 70%).[37] These monomers underwent ring-opening polymerization using simple primary amine initiators to form well-defined, high molecular weight homo glycopolypeptides and diblock coglycopolypeptides. However, attempts to synthesize the fully deprotected water-soluble glycopolypeptide from these polypeptides failed as we were unable to efficiently deprotect the bulky benzoate groups. We hereby report a very efficient synthesis fully water-soluble glycopolypeptides that were synthesized from the ring-opening polymerization of per-*O*-acetylated-D-glyco-L-lysine carbamate NCA monomers. Although these glycopolypeptides are predominantly α-helical in solution, we demonstrate that our synthetic methodology allows us to alter their secondary structures from an α-helix to a nonhelical structure. We have also investigated the binding of these glycopolypeptides with the lectin Con A with the objective of understanding the role of the secondary structure of these polypeptides on Con A binding.

EXPERIMENTAL SECTION

Materials and Methods. Propargyl 1,2-orthoesters of the corresponding carbohydrates were prepared according to literature procedure.[38−40] CbzLys(Boc)OBn was synthesized using standard literature procedure.[41] $HAuCl_4$, triphosgene, and azido-PEG-amine (n = 11) were obtained from Aldrich and Polypure Inc. All other chemicals used were obtained from Merck, India. Diethyl ether, petroleum ether (60−80 °C), ethylacetate, dichloromethane, tetrahydrofuran, and dioxane were brought from Merck and dried by conventional methods and stored in the glovebox. FT-IR spectra were recorded on Perkin-Elmer FT-IR spectrum GX instrument by making KBr pellets. Pellets were prepared by mixing 3 mg of sample with 97 mg of KBr. ^{1}H NMR spectra were recorded on Bruker Spectrometers (200 MHz, 400 or 500 MHz). ^{13}C NMR and DEPT spectra were recorded on Bruker Spectrometer (50, 100, or 125 MHz) and reported relative signals according to deuterated solvent used. HRMS data was recorded on MALDI-TOF using 2,5-dihydroxybenzoic acid as solid matrix. Size exclusion chromatography of the glycopolypeptides was performed using an instrument equipped with Waters 590 pump with a Spectra System RI-150 RI detector. Separations were effected by 10^5, 10^3, and 500 Å Phenomenex 5 μ columns using 0.1 M LiBr in DMF eluent at 60 °C at the samples concentrations of 5 mg/mL. A constant flow rate of 1 mL/min was maintained, and the instrument was calibrated using polystyrene standards.

General Procedure for the Synthesis of Amino Acid Glycosyl Carbamates (2a, 2b, 2b′, and 2c). To a solution of propargyl 1,2-orthoester (0.1 mmol), CbzL\DLys(Boc)Obn and activated 4 Å molecular sieves powder (50 mg) in anhydrous CH_2Cl_2 (5 mL) was added $HAuCl_4$ (10 mol %) under an argon atmosphere at room temperature. The reaction mixture was stirred at room temperature for the specified time and the reaction mixture was filtered and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel column chromatography using ethyl acetate-petroleum ether as the mobile phase to afford the compounds **2a**, **2b**, **2b′**, and **2c**.

Compound ***2a***. $[\alpha]_D^{25}$ = +4.4 (c 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.15−1.91(m, 6H), 1.98, 2.03, 2.04, 2.12 (4s, 12H), 3.12 (td, 2H, J = 2.1, 6.5, 13.5 Hz), 3.98 (t, 1H, J = 6.5 Hz), 4.06 (dd, 1H, J = 7.7, 11.4 Hz), 4.11 (d, 1H, J = 1.0 Hz), 4.15 (d, 1H, J = 3.1 Hz), 4.39 (m, 1H), 5.11 (s, 2H), 5.05 (dd, 1H, J = 3.5, 10.4 Hz), 5.17 (d, 1H, J = 3.8 Hz), 5.29 (dd, 1H, J = 8.1, 10.4 Hz), 5.39 (d, 2H, J = 3.4 Hz), 5.62 (d, 1H, J = 8.2 Hz), 7.32−7.38 (m, 10H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 20.4(3C), 20.5, 22.0, 28.7, 31.8, 40.4, 53.5, 60.8, 66.7, 66.8, 66.9, 67.7, 70.6, 71.1, 93.0, 127.9−128.5, 135.1, 136.1, 153.7, 155.9, 169.4, 169.7, 170.0, 170.2, 172.0; MALDI-TOF (m/z): Calcd for $C_{36}H_{44}KN_2O_{15}$, 783.2379; found, 783.2333.

Compound ***2b***. $[\alpha]_D^{25}$ = +22.1 (c 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.10−1.92 (m, 6H), 1.99, 2.02, 2.08, 2.17 (4s, 12H), 3.13 (q, 2H, J = 6.1, 12.3 Hz), 3.98−4.16 (m, 2H), 4.30 (dd, 1H, J = 4.7, 12.5 Hz), 4.41 (m, 1H), 4.98 (t, 1H, J = 5.7 Hz), 5.10 (d, 2H, J = 1.5 Hz), 5.17 (d, 2H, J = 3.4 Hz), 5.23−5.43 (m, 4H), 6.01 (d, 1H, J = 1.4 Hz), 7.32−7.38 (m, 10H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 20.5(3C), 20.6, 22.2, 28.8, 32.0, 40.6, 53.5, 61.9, 65.4, 66.8, 67.0, 68.4, 68.8, 70.0, 91.1, 127.9−128.5, 135.1, 136.1, 153.0, 155.9, 169.4, 169.6, 170.0, 170.1, 172.1; MALDI-TOF(m/z): Calcd for $C_{36}H_{44}KN_2O_{15}$, 783.2379; found, 783.2373.

Compound ***2b′***. $[\alpha]_D^{25}$ = +30.7 (c 1.0, $CHCl_3$); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.16−1.92 (m, 6H), 1.92, 1.94, 2.08, 2.09 (4s, 12H), 3.13 (m, 2H), 3.94−4.00 (m, 1H), 4.03 (dd, 1H, J = 2.4, 12.3 Hz), 4.21 (dd, 1H, J = 4.5, 12.3 Hz), 4.33 (m, 2H), 4.99 (t, 1H, J = 5.7 Hz), 5.04 (d, 2H, J = 3.3 Hz), 5.10 (dd, 1H, J = 12.3, 23.8 Hz), 5.18−5.35 (m, 3H), 5.43 (d, 1H, J = 8.1 Hz), 6.01 (d, 1H, J = 1.8 Hz), 7.32−7.38 (m, 10H); ^{13}C NMR (100.63 MHz, $CDCl_3$): δ 20.5(2C), 20.6, 20.7, 22.2, 28.9, 32.1, 40.7, 53.6, 62.0, 65.5, 66.9, 67.1, 68.4, 68.8, 70.0, 91.2, 128.0−128.5, 135.2, 136.1, 153.0, 155.9, 169.4, 169.7, 170.0, 170.6, 172.1; MALDI-TOF(m/z): Calcd for $C_{36}H_{44}KN_2O_{15}$, 783.2379; found, 783.2376.

Compound ***2c***. $[\alpha]_D^{25}$ = −7.7 (c 1.0, $CHCl_3$); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.30−1.89 (m, 6H), 1.95, 2.00, 2.03, 2.03, 2.05, 2.08, 2.14 (7s, 21H), 3.09 (q, 2H, J = 5.7, 12.4 Hz), 3.64−3.93 (m, 3H), 4.02−4.19 (m, 4H), 4.31−4.44 (m, 2H), 4.47 (s, 1H), 4.93 (dd, 2H, J = 3.3, 10.3 Hz), 5.07 (dd, 1H, J = 3.7, 10.3 Hz), 5.09 (s, 2H), 5.12−5.28 (m, 3H), 5.34 (d, 1H, J = 3.3 Hz), 5.41 (d, 1H, J = 8.3 Hz), 5.59 (d, 1H, J = 8.3 Hz), 7.32−7.39 (m, 10H); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 20.4, 20.5(4C), 20.7(2C), 22.1, 28.8, 32.0, 40.4, 53.4, 60.7, 61.7, 66.5, 66.9, 67.1, 68.9, 70.4, 70.6, 70.9, 72.5, 73.1, 75.6, 92.5, 100.8, 127.9−128.6, 135.1, 136.1, 153.6, 155.9, 169.0, 169.5, 169.8, 170.0, 170.1, 170.3(2C), 172.1; MALDI-TOF (m/z): Calcd for $C_{48}H_{60}KN_2O_{23}$, 1071.3224; found, 1071.3218.

General Procedure for the Glyco *N*-Carboxyanhydrides. Hydrogenolysis of compounds **2a**, **2b**, **2b′**, and **2c** was carried out using 10% Pd/C in MeOH/EtOAc (9:1) at 400 psi for 12 h. After completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to afford per-*O*-acetylated-D-galactose-L-lysine carbamate, per-*O*-acetylated-D-mannose-D\L-lysine carbamate and per-*O*-acetylated-D-lactose-L-lysine carbamate in almost quantitative yield. The resulting compounds were directly used for NCA synthesis without any further purification.

Compounds ***3a, 3b, and 3b′***. To a solution of per-*O*-acetylated-D-galactose-L-lysine carbamate or per-*O*-acetylated-D-mannose-L/D-lysine carbamate (500 mg, 0.96 mmol) in freshly distilled out tetrahydrofuran (10 mL) was added accordingly a solution of triphosgene (142 mg, 0.480 mmol) in anhydrous tetrahydrofuran (2 mL) under argon and the reaction mixture was heated to 50−55 °C. α-Pienene (0.228 mL, 1.44 mmol) was then added and the reaction mixture was allowed to stir for an additional 1 h. The reaction mixture was then cooled to room temperature and then poured into dry hexane (300 mL) to afford a white precipitate; which was filtered off quickly and crystallized two more times using a mixture of ethyl acetate and petroleum ether. Finally, the white precipitate of glyco *N*-carboxyanhydride **3a**, **3b**, and **3b′** obtained was dried under vacuum and transferred into the glovebox. Final yield: 425 mg, 80%.

Compound ***3c***. To a solution per-*O*-acetylated-D-lactose-L-lysine carbamate (500 mg, 0.618 mmol) in freshly distilled out tetrahydrofuran (10 mL) was added a solution of triphosgene (91.70 mg, 0.309 mmol) in anhydrous tetrahydrofuran (2 mL) under argon and the reaction mixture was heated to 50°-55 °C. α-Pienene (0.147 mL, 0.927 mmol) was then added and the reaction mixture was allowed to stir for an additional 1 h. The reaction mixture was then cooled to room temperature and then poured into dry hexane (300 mL) to afford a white precipitate; which was filtered off quickly and crystallized two more times using a mixture of ethyl acetate and petroleum ether. Finally, the white precipitate of glyco *N*-carboxyanhydride (**3c**) was dried under vacuum and transferred into the glovebox. Final yield: 410 mg, 80%.

Compound ***3a***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.35−1.82 (m, 6H), 1.98, 2.03, 2.04, 2.14 (4s, 12H), 3.12 (td, 2H, J = 2.1, 6.5, 13.7 Hz), 4.04−4.20 (m, 3H), 4.33 (bs, 1H), 5.09 (dd, 1H, J = 3.3, 10.5

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Hz), 4.25 (dd, 1H, J = 8.5, 10.3 Hz), 5.30 (m, 1H), 5.42 (d, 1H, J = 3.1 Hz), 5.62 (d, 1H, J = 8.3 Hz), 7.14 (bs, 1H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 20.5, 20.6(2C), 20.7, 21.6, 28.8, 31.0, 40.3, 57.4, 60.9, 66.8, 68.0, 70.7, 71.4, 93.3, 152.4, 154.1, 169.8, 170.0(2C), 170.1, 170.5; FT-IR (dioxane) 1785 and 1858 cm^{-1} $^{\nu}$co (unsymmetrical stretching).

Compound ***3b***. ^{1}H NMR (500.13 MHz, CD_2Cl_2): δ 1.35−1.88 (m, 6H), 1.99, 2.03, 2.07, 2.16 (4s, 12 Hz), 3.23 (q, 2H, J = 6.7, 12.6 Hz), 3.68 (t, 1H, J = 6.5 Hz), 4.08 (m, 1H), 4.13 (dd, 1H, J = 2.8, 12.2 Hz), 4.23 (dd, 1H, J = 4.6, 12.2 Hz), 4.35 (dd, 1H, J = 4.7, 6.9 Hz), 5.20−5.35 (m, 3H), 5.94 (d, 1H, J = 1.7 Hz), 6.72 (bs, 1H); ^{13}C NMR (125.76 MHz, CD_2Cl_2): δ 20.8(2C), 20.9(2C), 22.2, 29.3, 31.6, 40.7, 57.9, 62.7, 66.1, 68.9, 69.3, 70.5, 91.7, 152.3, 153.8, 170.0, 170.3, 170.4, 170.6, 171.2; FT-IR (dioxane) 1785 and 1858 cm^{-1} $^{\nu}$co (unsymmetrical stretching).

Compound ***3b′***. ^{1}H NMR (400.13 MHz, CD_2Cl_2): δ 1.35−1.88 (m, 6H), 1.99, 2.03, 2.07, 2.16 (4s, 12 Hz), 3.23 (q, 2H, J = 6.7, 12.6 Hz), 3.68 (t, 1H, J = 6.5 Hz), 4.08 (m, 1H), 4.13 (m 1H), 4.23 (dd, 1H, J = 4.6, 12.2 Hz), 4.35 (dd, 1H, J = 4.7, 6.9 Hz), 5.20−5.35 (m, 3H), 5.96 (m, 1H), 7.2 (bs, 1H); ^{13}C NMR (100.61 MHz, CD_2Cl_2): δ 20.8(2C), 20.9(2C), 22.2, 29.3, 31.6, 40.7, 57.9, 62.7, 66.1, 68.9, 69.3, 70.5, 91.7, 152.3, 153.8, 170.0, 170.3, 170.4, 170.6, 171.2; FT-IR (dioxane) 1785 and 1858 cm^{-1} $^{\nu}$co (unsymmetrical stretching).

Compound ***3c***. ^{1}H NMR (500.13 MHz, CD_2Cl_2): δ 1.15−1.88 (m, 6H), 1.94, 2.01, 2.03, 2.04, 2.05, 2.10, 2.12 (7s, 21H), 3.18 (m, 2H), 3.75 (d, 1H, J = 7.9 Hz), 3.86 (t, 1H, J = 9.1 Hz), 3.91 (t, 1H, J = 5.9 Hz), 4.05−4.17 (m, 3H), 4.32 (t, 1H, J = 5.5 Hz), 4.50 (d, 1H, J = 8.0 Hz), 4.56 (d, 1H, J = 12.0 Hz), 4.97 (m, 2H), 5.05 (m, 1H), 5.14 (t, 1H, J = 7.4 Hz), 5.22 (t, 1H, J = 9.0 Hz), 5.35 (d, 1H, J = 2.7 Hz), 5.60 (d, 1H, J = 8.2 Hz), 6.65 (s, 1H); ^{13}C NMR (125.76 MHz, CD_2Cl_2): δ 20.7(2C), 20.8(3C), 20.9, 21.0, 22.0, 29.3, 31.5, 40.5, 57.9, 61.3, 61.7, 67.1, 69.2, 70.7, 71.2, 71.2, 72.6, 73.7, 75.8, 93.1, 101.2, 152.2, 154.3, 169.4, 170.0, 170.1, 170.3, 170.4, 170.4, 170.6, 170.8; FT-IR (dioxane) 1785 and 1858 cm^{-1} $^{\nu}$co (unsymmetrical stretching).

General Procedure for the Synthesis of Glycopolypeptides. To a solution of glyco-L/D-lysine NCA (100 mg/mL) in dry dioxane was added with "proton sponge" *N*,*N*′-tetramethylnapthalene (1.0 equiv to monomer; 1 M) as an additive and azido-PEG-amine (0.5 M) as the initiator inside the glovebox. The progress of the polymerization were monitored by FT-IR spectroscopy by comparing with the intensity of the initial NCA's anhydride stretching at 1785 and 1858 cm^{-1}. The reactions generally completed within 36 h. Aliquotes were removed after completion of polymerization for GPC analysis. Finally the solvent was removed under reduced pressure from the reaction mixture. The resulting residue was redissolved in dichloromethane and then the polymer was precipitated out by addition of methanol. The precipitated polymer was collected by centrifugation and dried to afford white glycopolypeptides **4a**, **4c**, **5a**, **5c**, and **6a** in almost 85−90% yield.

For synthesis of polymers **5e** and **5g**, **3b** (α-manno-*O*-L-lys NCA) and **3b′** (α-manno-*O*-D-lys NCA) were mixed in equal proportions (by weight) and then polymerization was carried out as has been described above.[42−44]

Polymer ***4a***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.30−1.97 (m, 6H), 1.98−2.14 (4s, 12H), 3.07−3.30 (m, 2H), 3.62−3.68 (m, for CH_2CH_2O unit in initiator), 3.55−3.90 (m, 1H), 4.04−4.40 (m, 3H), 5.00−5.55 (m, 3H), 5.50−5.76 (m, 1H), 5.56−5.95 (amide H).

Polymer ***4c***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.30−1.97 (m, 6H), 1.98−2.14 (4s, 12H), 3.07−3.30 (m, 2H), 3.62−3.68 (m, for CH_2CH_2O unit in initiator), 3.55−3.90 (m, 1H), 4.04−4.40 (m, 3H), 5.00−5.55 (m, 3H), 5.50−5.76 (m, 1H), 5.56−5.95 (amide H).

Polymer ***5a***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.98 (m, 6H), 1.99−2.16 (4s, 12 Hz), 3.10−3.40 (m, 2H), 3.62−3.68 (m, for CH_2CH_2O unit in initiator), 4.0−4.32 (m, 4H), 5.10−5.60 (m, 3H), 5.80−5.98 (m, 1H).

Polymer ***5c***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.98 (m, 6H), 1.99−2.16 (4s, 12 Hz), 3.10−3.40 (m, 2H), 3.62−3.68 (m, for CH_2CH_2O unit in initiator), 4.0−4.32 (m, 4H), 5.10−5.60 (m, 3H), 5.80−5.98 (m, 1H).

Polymer ***5e***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.98 (m, 6H), 1.99−2.16 (4s, 12 Hz), 3.10−3.40 (m, 2H), 3.62−3.68 (m, for CH_2CH_2O unit in initiator), 4.0−4.32 (m, 4H), 5.10−5.60 (m, 3H), 5.80−5.98 (m, 1H).

Polymer ***5g***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.98(m, 6H), 1.99−2.16 (4s, 12 Hz), 3.10−3.40 (m, 2H), 3.62−3.68 (m, for CH_2CH_2O unit in initiator), 4.0−4.32 (m, 4H), 5.10−5.60 (m, 3H), 5.80−5.98 (m, 1H).

Polymer ***6a***. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.15−1.92 (m, 6H), 1.94−2.12 (br, m, 21 Hz), 3.10−3.18 (m, 2H), 3.62−3.68 (m, for CH_2CH_2O unit in initiator), 3.70−3.90 (m, 2H), 4.0−4.20 (m, 4H), 4.40−4.58 (m, 3H), 4.90−5.09 (m, 3H), 5.15−5.35 (m, 3H), 5.50−5.60 (m, 1H).

Deprotection Procedure for the Glycopolypeptides. Hydrazine monohydrate (25 equiv) was added to the solutions of all the acetyl-protected glycopolypeptides in methanol (10 mg/mL), and the reactions were stirred for 7−8 h at room temperature. Reactions were quenched by addition of acetone and then solvent was removed almost completely under reduced pressure. The solid residues were dissolved in deionized water and transferred to dialysis tubing (3.5 and 12 KDa molecular weight cutoff according to polymer molecular weight). The samples were dialyzed against deionized water for 3 days, with water changes once every 2 h for the first day, and then thrice per day. Dialyzed polymers were lyophilized to yield glycopolypeptides (**4b**, **4d**, **5b**, **5d**, **5f**, **5h**, and **6b**) as white fluffy solids (around 90% yield).

Polymer ***4b***. ^{1}H NMR (400.13 MHz, D_2O): δ 1.10−2.01 (m, 6H), 3.12 (m, 2H), 3.65−3.70 (m, for CH_2CH_2O unit in initiator), 3.58−3.82 (m, 7H), 3.85−3.95 (m, 1H), 3.98−4.32 (m, 1H), 5.20−5.45 (m, 1H).

Polymer ***4d***. ^{1}H NMR (400.13 MHz, D_2O): δ 1.10−2.01 (m, 6H), 3.12(m, 2H), 3.65−3.70(m, for CH_2CH_2O unit in initiator), 3.58−3.82 (m, 7H), 3.85−3.95 (m, 1H), 3.98−4.32 (m, 1H), 5.20−5.45 (m, 1H).

Polymer ***5b***. ^{1}H NMR (400.13 MHz, D_2O): δ 1.08−2.01 (m, 6H), 3.10−3.322 (m, 2H), 3.65−3.70 (m, for CH_2CH_2O unit in initiator), 3.70−3.93 (m, 7H), 3.85−3.95 (m, 1H), 3.98−4.32 (m, 1H), 5.70−5.89 (m, 1H).

Polymer ***5d***. ^{1}H NMR (400.13 MHz, D_2O): δ 1.08−2.01 (m, 6H), 3.10−3.322 (m, 2H), 3.65−3.70 (m, for CH_2CH_2O unit in initiator), 3.70−3.93 (m, 7H), 3.85−3.95 (m, 1H), 3.98−4.32 (m, 1H), 5.70−5.89 (m, 1H).

Polymer ***5h***. ^{1}H NMR (400.13 MHz, D_2O): δ 1.08−2.01 (m, 6H), 3.10−3.322 (m, 2H), 3.65−3.70 (m, for CH_2CH_2O unit in initiator), 3.70−3.93 (m, 7H), 3.85−3.95 (m, 1H), 3.98−4.32 (m, 1H), 5.70−5.89 (m, 1H).

Polymer ***6b***. ^{1}H NMR (400.13 MHz, D_2O): δ 1.15−1.88 (m, 6H), 3.10−3.18 (m, 2H), 3.46−3.54 (m, 2H), 3.65−3.70 (m, for CH_2CH_2O unit in initiator), 3.55−4.0 (m, 11H), 4.35−4.47 (m, 1H), 5.35−5.48 (m, 1H).

Circular Dichroism Measurements. Aqueous solution of glycopeptides **4b**, **4d**, **5b**, **5d**, **5f**, **5h**, and **6b** were filtered through 0.22 μm syringe filters. CD (190−250 nm) spectra of the glycopolypeptides (0.50 mg/mL in deionized water) were recorded (JASCO CD SPECTROPOLARIMETER, Model Name J-815) in a cuvette with a 1 mm path length. All the spectra were recorded for an average of three scans and the spectra were reported as a function of molar ellipticity $[\theta]$ versus wavelength. The molar ellipticity was calculated using the standard formula, $[\theta] = (\theta \times 100 \times M_w)/(C \times l)$, where θ = experimental ellipticity in millidegrees, M_w = average molecular weight, C = concentration in mg/mL, and l = path length in cm. The % α helicity was calculated by using the formula % α helicity = $(-[\theta]_{222\ nm} + 3000)/ 39000$.[45]

Precipitation Assay. Quantitative precipitations and analysis were carried out by a method modified of Brewer,[46] as adapted by Keissling,[47] Cloninger, and co-workers (Figure 2 SI).[48]

Hemagglutination Assay (HA assay). HA assays were performed as has been described by Finn et al. (Figure 3 SI).[49]

Isothermal Titration Calorimetry. Calorimetric titrations were performed on a VP-ITC isothermal titration calorimeter from MicroCal (Northampton, MA), essentially as described earlier.[50,51] Briefly, 5 μL aliquots of a 200−300 μM glycopolymer solution were added at 7 min intervals via a rotating stirrer syringe to a 70 μM

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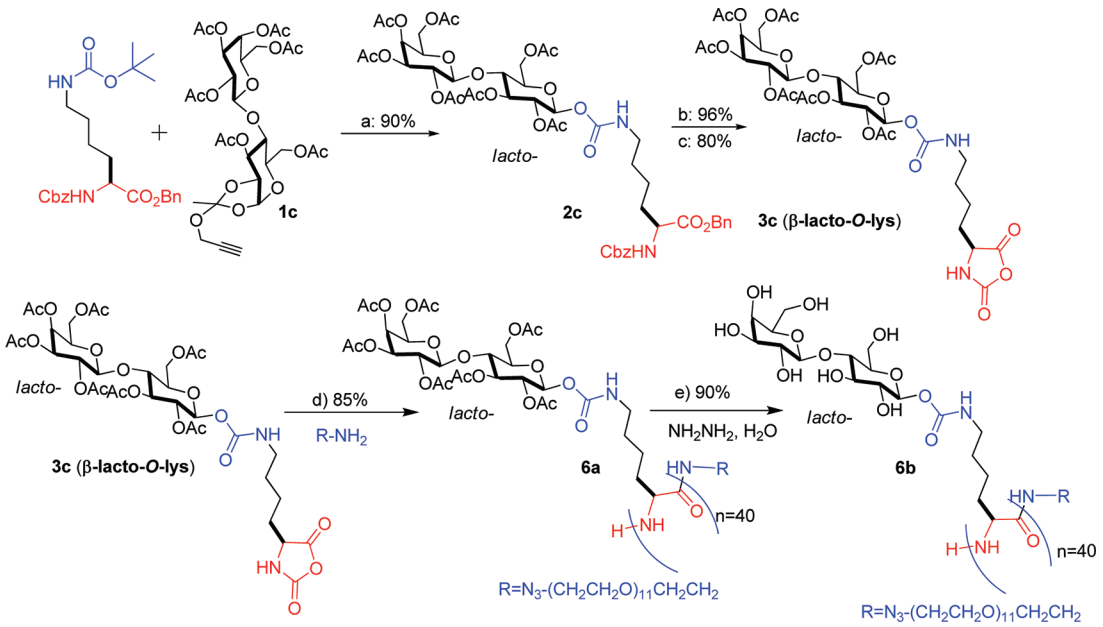
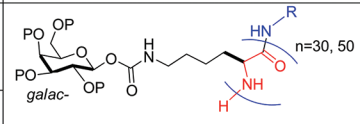
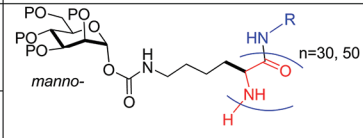
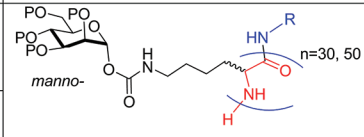
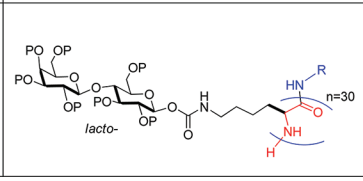
Scheme 1. General Synthesis of Glycopolypeptides by the Ring-Opening Polymerization of Their Glycosylated Amino Acid NCAs[a]



[a](a) $HAuCl_4$, CH_2Cl_2, rt, 0.5 h, 4Å MS Powder, Yield (90%); (b) Pd/C, H_2, 400 psi, CH_3OH, 12 h, Yield (>95%); (c) Triphosgene, THF, α-pienene, 70 °C, Yield (80%); (d) Dioxane, Proton Sponge (1.0 equiv), 24 h, RT; (e) Methanol, Hydrazine hydrate (25 equiv), 6 h.

Table 1. Different Glycopolypeptides Synthesized by Our Methodology

Polymer No	Polymer Name	Structure of Polymer	Polymer No	Polymer Name
4a	30-β-galac-*O*-*L*-lys, P=Ac		**4b**	30-β-galac-*O*-*L*-lys(OH), P=H
4c	50-β-galac-*O*-*L*-lys, P=Ac		**4d**	50-β-galac-*O*-*L*-lys(OH), P=H
5a	30-α-manno-*O*-*L*-lys, P=Ac		**5b**	30-α-manno-*O*-*L*-lys(OH), P=H
5c	50-α-manno-*O*-L-lys, P=Ac		**5d**	50-α-manno-*O*-*L*-lys(OH), P=H
5e	Rac-30-α-manno-*O*-*LD*-lys, P=Ac		**5f**	Rac-30-α-manno-*O*-*LD*-lys(OH), P=H
5g	Rac-50-α-manno-*O*-*LD*-lys, P=Ac		**5h**	Rac-50-α-manno-*O*-*LD*-lys(OH), P=H
6a	30-β-lacto-*O*-*L*-lys, P=Ac		**6b**	30-β-lacto-*O*-*L*-lys(OH), P=H

solution of Con A (subunits) contained in a 1.445 mL sample cell. Samples were dialyzed extensively against 50 mM Hepes buffer, pH 7.4 (containing 0.9 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 0.02% of sodium azide) and degassed prior to loading into the cell. Because the first injection was often found to be inaccurate, a 2 μL injection was added first and the resultant point was deleted before the remaining data were analyzed using the "one set of sites" binding model in MicroCal Origin ITC analysis software, as described earlier.[50,51] The analysis yielded values of the following parameters: number of binding sites (n), binding constant for the interaction (K_b), and enthalpy of binding (ΔH_b). From these values, free energy of binding ($\Delta G°_b$) and entropy of binding (ΔS_b) were calculated according to the following basic thermodynamic equations:

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Table 2. Synthesis of Glycopolypeptides at RT

		protected polymer						deprotected polymer	
run No.	monomer (M)	M/I^a	M^b_{exp}	polymer	$M_n{}^c$	$M_w/M_n{}^d$	DP[e]	polymer	conformation[f]
1	β-galac-*O*-L-lys	30	15630	**4a**	21654	1.12	42	**4b**	α-helix
2	β-galac-*O*-L-lys	50	25670	**4c**	32698	1.14	64	**4d**	α-helix
3	α-manno-*O*-L-lys	30	15630	**5a**	19646	1.08	38	**5b**	α-helix
4	α-manno-*O*-L-lys	50	25670	**5c**	30690	1.10	60	**5d**	α-helix
5	Rac-α-manno-*O*-LD-lys	30	15630	**5e**	18642	1.08	36	**5f**	nonhelical
6	Rac-α-manno-*O*-LD-lys	50	25670	**5g**	28180	1.10	55	**5h**	nonhelical
7	β-lacto-*O*-L-lys	30	24330	**6a**	28220	1.07	35	**6b**	α-helix

[a]M/I indicates monomer to initiator ratio. [b]Expected molecular weight calculated from monomer/initiator. [c]Number average molecular weight calculated from NMR. [d]Polydispersity index was estimated from GPC (DMF/0.1 M LiBr, 60 °C, RI) and calibrated with polystyrene standards. [e]Degree of polymerization from ^{1}H NMR (DP). [f]Secondary conformation was determined by CD spectroscopy in water.

$$\Delta G^\circ_b = -RT \ln K_b \quad (1)$$

$$\Delta G^\circ_b = \Delta H_b - T\Delta S_b \quad (2)$$

RESULTS

Synthesis of *O*-Glycopolypeptides. We have recently reported the synthesis of per-*O*-benzoylated-D-glyco-L-lysine carbamate by reaction of ε-Boc-protected CbzLysOBn and propargyl 1,2-orthoester of per-*O*-benzoylated-glucose/mannose in the presence of $HAuCl_4/CH_2Cl_2$/4 Å MS powder/rt.[38−40] The highlight of this reaction is the near quantitative yield of the glycosidation step (>80%), which allows the synthesis of their corresponding NCAs with an overall yield of 70%. The same methodology was used to synthesize per-*O*-acetylated-D-glyco-L-lysine carbamate from their corresponding propargyl 1,2-orthoester of per-*O*-acetylated carbohydrates. Accordingly, glycosylation reaction between ε-Boc-protected CbzLysOBn and propargyl 1,2-orthoester of galactose, mannose, and lactose was conducted in the presence of $HAuCl_4$ and 4 Å molecular sieves powder in CH_2Cl_2 at room temperature to afford the carbamates **2a**, **2b**, **2b′** (D-lysine), and **2c** in around 80−90% yield (Scheme 1). Furthermore, we continued our journey toward the glyco-NCA in two steps: first, we subjected the glycoconjugates **2a**, **2b**, **2b′**, and **2c** to hydrogenolysis using 10% Pd/C at 400 psi to obtain per-*O*-acetylated-D-galactose-L-lysine carbamate, per-*O*-acetylated-D-mannose-L-lysine carbamate, and per-*O*-acetylated-D-lactose-L-lysine carbamate. They were then subsequently converted to their corresponding NCAs **3a** (β-galacto-*O*-L-lys), **3b** (α-manno-*O*-L-lys), **3b′** (α-manno-*O*-D-lys), and **3c** (β-lactose-*O*-L-lys) using triphosgene and α-pienene in 80% yield after three crystallizations (Scheme 1). The purified NCAs **3a**, **3b**, **3b′**, and **3c** were thoroughly characterized by NMR and FT-IR spectroscopic studies.

Polymerization of **3a**(β-galac-*O*-lys NCA) was carried out in the presence of 1.0 equiv of *N*,*N*′-tetramethylnapthalene "proton sponge", using azido-PEG-NH_2 (n = 11) as the initiator (M/I = 30, 50) in dry dioxane, as has been described before (Scheme 1). The progress of the polymerization was followed by monitoring the disappearance of the anhydride stretch of the NCA ring at 1785 and 1858 cm^{-1}, as was observed by FT-IR. The resulting polymers **4a** and **4c** were purified by reprecipitation and the structure was identified by ^{1}H and ^{13}C NMR (Figures 10−53 SI). The molecular weight distribution observed from GPC was monomodal and found to be reasonably narrow (Figure 6 SI). Its molar mass was estimated from the relative intensity of the peak at 3.62−3.68 ppm due to characteristic protons present in the initiator (O-CH_2-CH_2), with the proton peaks of the acetate group (CH_3CO-) present in the carbohydrate moiety (1.98−2.14 ppm). The M_n was estimated to be 21654 and 32698, while the PDI was calculated to be 1.12 and 1.14 for **4a** and **4c**, respectively. The slightly higher molecular weight that is observed is probably due to incomplete initiation by azido-PEG-NH_2. In the same way, **3b** (α-manno-*O*-L-lys NCA) was polymerized using azido-PEG-NH_2 (n = 11) as the initiator (M/I = 30, 50) in dioxane (Table 1, runs 3 and 4). The molecular weight distribution was again found to be reasonably narrow (PDI 1.08 and 1.10, respectively), and the molecular weights of the resulting polymers **5a** and **5c** were estimated to be 19646 and 30690, respectively. Polymerization of **3c** (β-lacto-*O*-L-lys NCA), using azido-PEG-NH2 (n = 11) as the initiator (M/I = 30) in dry dioxane (Scheme 1) afforded polymer **6a**. The molecular weight distribution observed from GPC was monomodal, while the molecular weight and PDI were calculated to be 28220 and 1.07, respectively. Although per-*O*-acetate-D-lactose-*O*-L-serine NCA has been synthesized before, their polymerization to the corresponding glycopolypeptide has not been reported.[52] It should be noted that all the molecular weights obtained were reasonably close to their expected molecular weights. This is in contrast to transition metal catalyzed polymerization of the *C*-linked glycopolypeptides reported recently, where the molecular weights obtained were nearly three times that of the expected molecular weights.[30,31] All the glycopolypeptides obtained were then deprotected by using $NH_2NH_2{\cdot}H_2O$ in MeOH to remove the acetyl groups present in the carbohydrate moiety. The deprotected polymers were then purified by extensive dialysis against deionized water to afford fully water-soluble glycopolypeptides **4b**, **4d**, **5b**, **5d**, **5f**, **5h**, and **6b** (Table 2). The complete removal of the acetyl groups was confirmed by the absence of the acetyl protons in the ^{1}H NMR of the water-soluble glycopolypepitdes (Figure 10−53 SI).

All the glycopolypeptides above, were synthesized by the polymerization of enantiomerically pure glyco-*O*-L-lysine NCA's to afford glycopeptides having a backbone composed only of L-lysine. We were interested in synthesizing glycopolypeptides which would have a backbone composed of racemic DL-lysine. This would allow us to study the properties of glycopolypeptides with a racemic peptide backbone.[42−44] To accomplish this, we synthesized α-manno-*O*-L-lysine NCA (**3b**) and α-manno-*O*-D-lysine NCA (**3b′**) from their corresponding L- or D-lysine amino acid glycol conjugates. These NCAs (**3b** and **3b′**) were mixed in equal proportions and polymerized to afford polymers **5e** and **5g**. The acetate groups

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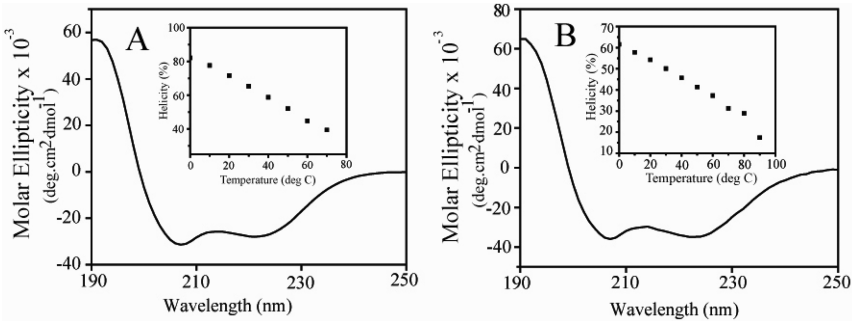

Figure 1. (A) Circular dichroism spectra of poly(β-gal-*O*-L-lys) **4d** at 20 °C (0.5 mg/mL); (B) Circular dichroism spectra of poly(β-lacto-*O*-L-lys) **6b** at 20 °C (0.5 mg/mL); Inset: Percentage of α-helicity content (% helicity calculated using molar ellipticity at 222 nm) as a function of temperature.

of **5e** and **5g** were then deprotected to afford fully water-soluble glycopolypeptide **5f** and **5h**.

To prove that the initiator was incorporated into the polymer, the resultant polymers **4a** and **5a** were purified by multiple reprecipitations and then characterized by NMR and FT-IR. The FT-IR of **4a** and **5a** show sharp peak at 2110 cm^{-1} that is characteristic for the organo azide stretch (Figure 1 SI). Usage of this bifunctional azido-PEG-amine initiator allows the synthesis of end functionalized polymer **4a** and **5a** which was further manipulated using Cu(I) catalyzed azide−alkyne "click chemistry" with fluorecein alkyne (Scheme 1 SI).[53] After click reactions the azide intensity decreased by >80%. The fluorecein labeled glycopolypeptides were deprotected by hydrazine monohydrate in methanol to obtained water-soluble fluorecein-labeled glycopolypeptides, **7** and **8**, respectively, the polymers were characterized by ^{1}H NMR (Figures 10−53 SI). These fluorecein-labeled glycopolypeptides can be used to study their cellular internalization and trafficking as has been shown before.[54,55]

Conformation of Glycopolypeptides in Solution. The conformation of the glycopolypeptides in solution was investigated by circular dichroism (CD). The fully deprotected polymer poly(glyco-*O*-lys) **4b**, **4d**, **5b**, **5d**, and **6b** all of which have a polypeptide backbone compose of enantiomerically pure L-lysine, were found to be α-helical in water with a characteristic minima at 208 and 222 nm (Figure 2; Figure 4 SI). For example, poly(β-galacto-*O*-L-lys) polypeptide was found to be 70% helical in water at RT. The percentage helicity of **4d** as a function of temperature was also studied using CD. It was found that the α-helical conformation got disrupted as the temperature was increased from 0 to 70 °C. The polymer regained its helicity completely upon cooling it back to 0 °C. The percentage of helicity of these glycopolypeptides was found to be dependent on the length of the glycopolypeptide. For example, the percentage helicity of poly(α-manno-*O*-L-lys) **5d** (60 mer) was 62%, while the % helicity of its corresponding 38 mer (**5b**) decreased to 30% at room temperature. The conformation of the deprotected polypeptide poly(β-lacto-*O*-L-lys) **6b** was also studied as a function of temperature. This polymer was also observed to be α-helical in water and the helicity content was determined to be 55% at room temperature (Figure 1).

Glycopolypeptides **5f** and **5h**, where the polypeptide backbone consisted of racemic DL-lysine, showed no helicity (helicity <2% in water at RT, Figure 4 SI). This was expected because a backbone having a racemic amino acid is not expected to fold into an α-helix, as has been shown before.[42−44] However, it must be noted that if stereoblock polymers

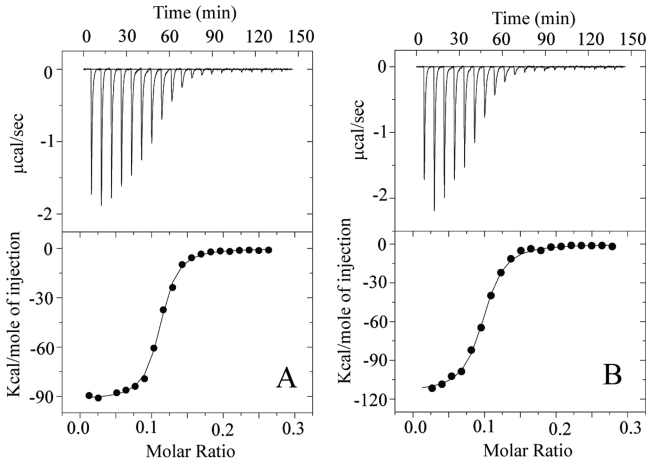

Figure 2. Calorimetric titration of Con A with A [50-α-manno-*O*-L-lys(OH)] **5d**] and B [Rac-50-α-manno-*O*-LD-lys(OH) **5h**] at 298 K. Upper panels show the ITC raw data obtained from 20 automatic injections of 5 μL aliquots of the glycopeptide (in the syringe) into 1.445 mL of Con A in the ITC cell. Lower panels show the integrated heat of binding obtained from the raw data. See text for more details.

containing blocks of L- and D-glyco amino acids would also give no CD signal. To ascertain that no stereoselection took place during our polymerization, we initiated polymerization of racemic manno NCAs with a preincubated solution of 1:1 mixture of α-manno-*O*-L-lys NCA and N_3-PEG-amine. The CD of the reaction mixture was recorded at 50 and 100% completion of the reaction and they were found to be identical. This shows that the D- and L-glyco-NCAs are randomly incorporated and no stereoselection occurred during the polymerization (Figure 9 SI).

Interaction of Glycopolypeptides with Con A. *Precipitation and Hemagglutination Assay.* Preliminary studies on the binding of the mannose containing glycopolypeptides (**5b**, **5d**, **5f**, and **5h**) with Con A was evaluated by performing precipitation and hemagglutination assays. The precipitation assay was performed to determine the number of mannose units available in the glycopolypeptides (**5b**, **5d**, **5f**, and **5h**) for binding to each Con A lectin. In all experiments the ratio of the mannose functionalized polypeptide to Con A increased with increasing amount of the glycopolypeptide and then remained fairly constant after a maximum had been reached (Figure 2 SI). The stoichiometry of binding (number of Con A tetramer per glycopolypeptide) was determined to be 4, 5, 3.5, and 3.5 for **5d**, **5h**, **5b**, and **5f**, respectively. The data from the experiments suggest (Table 1 SI) that the number of

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Table 3. Thermodynamic Parameters for the Binding of Glycopolypeptides to Con A[a]

polymer	conformation[b] (units)[c]	n[d]	K_a (M^{-1}) × 10^{-6}	ΔH (kcal/mol)	ΔS (cal/mol/K)	polyvalency (sugar)
α-methyl mannopyranoside		1	0.0051 (±0.0001)	−10.20 (±0.07)	−17.15 (±0.25)	1
5b [30-α-manno-*O*-L-lys(OH)]	α-helix (38 units)	7.0 (±0.5)	2.44 (±0.44)	−81.20 (±3.3)	−243 (±1.4)	12
5f [Rac-30-α-manno-*O*-LD-lys(OH)]	nonhelical (36 units)	8.1 (±0.7)	4.13 (±0.29)	−88.40 (±2.7)	−266 (±5.7)	22
5d [50-α-manno-*O*-L-lys(OH)]	α-helix (60 units)	9.2 (±0.1)	10.21 (±3.51)	−95.50 (±2.1)	−288.5 (±17.7)	33
5h [Rac-50-α-manno-*O*-LD-lys(OH)]	nonhelical (55 units)	10.2 (±0.6)	9.11 (±2.52)	−115.0 (±1.4)	−354.5 (±3.5)	32

[a]Values shown are the average of two independent titrations. [b]Determined from CD spectra in water. [c]Degree of polymerization was determined by ^{1}H NMR [d]Number of Con-A subunit per glycopolypeptide

mannopyranoside units in the polypeptide bound per Con A tetramer for a glycopolypeptide having a helical structure is slightly higher than the corresponding glycopolypeptide with no secondary structure (**5d** vs **5h** and **5b** vs **5f**).

Hemagglutination assays (HA) were performed with glycopolypeptides (**5b**, **5d**, **5f**, and **5h**) to get preliminary information regarding binding affinity,[49] the use of HA to measure inhibition of protein carbohydrate interactions is well documented and gives us an essential entry-level comparison of the different mannose containing glycopolypeptides synthesized by us. When compared to the control monomer methyl mannose, poly(α-manno-*O*-lys) glycopolypeptides **5b**, **5d**, **5f**, and **5h**, showed an increase in binding affinity ranging from 15- to 36-fold per mannose unit. This indicates an increase in activity toward Con A of 1 order of magnitude per mannose unit, which is suggestive of a glycoside clustering motif of all the synthesized glycopolypeptides. Because the error associated with the dose determination is a factor of 2, as dictated by the 2-fold dilutions of the assay, the polyvalencies of **5b**, **5d**, **5f**, and **5h** are the same within experimental error.

Isothermal Titration Calorimetry. Results of representative calorimetric titrations obtained for the binding of glycopolymers to Con A at 25 °C are shown in Figure 2. Figure 2A,B correspond to the titration of Con A with glycopolypeptides **5d** and **5h**, respectively. While the upper panels in these two figures show the exothermic heat released upon binding at each injection, the lower panels show plots of incremental heat released as a function of the ligand/Con A subunit ratio. Nonlinear least-squares fits of the data to one set of sites model (shown as solid lines) indicate that the experimental data could be described well by this model as judged by the high quality of the fits. Similar high quality data were obtained for the titration of Con A with glycopolypeptides **5b** and **5f** (Figure 4 SI). In each case very similar data were obtained in duplicate experiments and the average values of binding constants (K_b), stoichiometry of binding (n), enthalpy of binding (ΔH_b), and entropy of binding (ΔS_b) obtained from the calorimetric titrations for the interaction of all the glycopolymers with Con A are listed in Table 3.

A comparison of the binding constants, stoichiometry, and thermodynamic parameters obtained from the ITC studies yielded a number of interesting features. First, the stoichiometry of binding is higher for the longer glycopolymers (**5d** and **5h**), but it does not increase in proportion to the increase in chain length, which can be attributed to steric factors. More interestingly, the binding constant increases by 2−4-fold for the longer glycopeptides. Because even the shorter glycopeptides (**5b** and **5f**) already have a large number of covalently attached mannose residues in close proximity, this additional increase in binding affinity may be attributed to the increased statistical probability of the binding event due to the increase in the number of accessible sugar residues for binding. Finally, the racemic glycopolypeptides were found to exhibit a slightly higher binding stoichiometry as compared to the α-helical counterparts, which may be due to their slightly longer lengths.

DISCUSSION

Design of Glycopolypeptides for Lectin Binding Study. We chose a set of four glycopolypeptides to probe their binding to the lectin Con A and also probe the difference between helical and nonhelical polypeptides. While glycopolypeptide **5h** and **5d** have the same structure and similar molecular weights, their secondary structures are different. CD measurements show **5d** to be α-helical (62% helicity), whereas the **5h** shows no secondary structure (<2% helicity) at RT. Similar glycopolypeptides **5b** (30% α-helical) and **5f** (nonhelical, <2% helicity) have similar molecular weights but differ in their secondary structures. It is expected that a predominantly α-helical polypeptide will be stiff, and good binding is likely to be observed only if the distance between two sugar units matches exactly to the distance between two binding sites. This is unlikely in most cases as have been observed by Kobayashi et al.[56] where a rigid helical poly(glycosyl phenyl isocyanate) was observed to have very little specific interactions with lectins, while the equivalent polymer with a flexible phenylacrylamide showed good binding. On the contrary, Kiick et al.[57] have showed that a helical backbone in polypeptides can be superior to coiled structures for binding to lectin CT B_5.[57] Hence, conformational factors that determine binding are complex. Because our synthetic methodology allows the synthesis of polypeptides with the same structure and molecular weight but with different secondary conformation (for example **5h** and **5d**), we can probe the effect of secondary conformation of glycopolypeptides on lectin binding.

Lectin Binding of the Glycopolypeptides. ITC studies show that there is very little difference in the number of Con A monomer units bound per polypeptide for glycopolypeptides having an α-helical conformation (**5b**, **5d**) and with no secondary structure (**5f**, **5h**) of similar molecular weight. A similar trend is also observed for the precipitation assay (Figure 2 SI), although the stoichiometry determined from the precipitation assay is higher for all the glycopolypeptides used in this study. Precipitation assays are semiquantitative and are known to overestimate stoichiometry of binding, because a macroscopic precipitation event could result from even partially saturated binding events. To understand the stoichiometry for Con A binding with the different glycopolypeptides, a qualitative estimation of the length of the different glycopolypeptides in solution would be useful. The three limiting conditions for the length of the glycopolypeptides can be assumed as (a) purely α-helical, (b) a coiled structure with the freely jointed chain, and (c) a coiled structure with an

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extended chain. The length of the glycopolypeptide (n = 60) under these limiting cases can be estimated to be 8.6, 7.9, and 21.7 nm (Figure 8 SI). This indicates that polypeptides with purely α-helical and the freely jointed conformation can have a similar length. Because racemic glycopolypeptides **5f** and **5h** have a slightly higher binding stoichiometry than their helical counterparts, they have a conformation that is probably in between a freely jointed chain and an extended chain (more toward a freely jointed chain). However, more structural studies are required to justify this.

The polyvalent effect observed for all the polypeptides were low and only 1 order of magnitude higher (per mannose) than their corresponding monomer α-methyl mannopyranoside. Polyvalency arises from mainly two related but distinct terms: (i) multivalent binding (the ability of one glycopolypeptide to bind to multiple lectin binding sites) and (ii) glycoside clustering (a ligand concentration effect). While the former shows mild binding enhancements (typically 1 order of magnitude), the later shows very large enhancements (two orders and higher). Because our synthetic glycopolypeptides only show small binding enhancements, we believe that this effect is due to glycoside clustering and not due to multivalent binding. It must be noted that both the ITC and HA assay shows similar affinity data and polyvalency for all the glycopolypeptides used in this study (Table 2 SI, Figure 3 SI). A closer look at the ITC data gives us an idea why two glycopolypeptides with differing secondary structures have similar binding constants. It is observed that the ΔH_b values are higher for the nonhelical polymers (**5f** and **5h**) as compared to the helical polymers (**5b** and **5d**) for the same (comparable) length, whereas the ΔS_b values are higher for the helical polymers. This can be rationalized in the following way. The nonhelical polymers are more flexible than their helical counterparts and hence bind better to the lectin, which results in a larger enthalpy change. On the other hand, the higher ΔS_b values for the helical polymers are consistent with the entropy penalty being less for binding to a rigid structure. However, it should be noted that the differences in the enthalpy of binding and entropy of binding for the helical versus nonhelical polymers are relatively small and also partially compensate each other, resulting in nearly comparable values of binding constant.

A careful examination of the ΔH_b and ΔS_b values for the different glycopolypeptides (both helical and nonhelical) suggested that the changes in enthalpy and entropy are compensatory in nature. This is clearly seen in a plot of $-\Delta H_b$ versus $-T\Delta S_b$ shown in Figure 3. A linear least-squares fit of the data yielded a slope of 0.99 indicating that the enthalpy of binding and entropy of binding are exactly compensated in the binding of the glycopolypeptides to Con A. Such close compensation of enthalpy and entropy of binding has been observed for a large number of protein−ligand interactions including lectin−carbohydrate interactions and this phenomenon has been attributed to the reorganization of water structure around the binding site on the protein and the ligand. A number of studies suggest that water molecules play an important role in lectin−carbohydrate interaction.[50,51,58−60] Indeed, ITC studies provided evidence indicating the involvement of water molecules in the binding of manno-oligosaccharides to Con A.[61] In view of this, the enthalpy−entropy compensation observed here can be explained in terms of changes in water structure during the Con A/glycopolypeptide association.

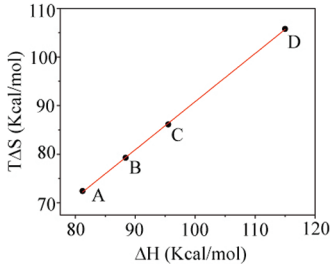

Figure 3. Enthalpy−entropy compensation plot for the Con A−glycopolypeptide interaction (A) [30-α-manno-*O*-L-lys(OH) **5b**], (B) [Rac-30-α-manno-*O*-LD-lys(OH) **5f**], (C) [50-α-manno-*O*-L-lys(OH) **5d**], and (D) [Rac-50-α-manno-*O*-LD-lys(OH) **5h**].

CONCLUSIONS

We have reported a very easy three step synthesis of per-acetylated-*O*-glycosylated lysine-NCA using a stable glycosyl donor and a commercially available protected amino acid. The highlight of the synthesis is that the key glycosylation step and the subsequent deprotection reaction proceeds to completion in near quantitative yield. The glycosylated NCAs were then polymerized using commercially available simple amine initiators to yield well-defined high molecular weight glycopolypeptides in very high yields. We have also reported the synthesis of poly(β-lacto-*O*-lys) glycopolypeptides having a disaccharide lactose as the pendant side group which demonstrates that this methodology can be extended to synthesize glycopolypeptides having complex carbohydrates on its side chains. Poly(β-galacto-*O*-lys) glycopolypeptide was also synthesized. Because certain cancer cells like HepG2 cells have receptors that bind specifically to β-galactose, we believe these polymers can be used for drug delivery. The fully water-soluble glycopolypeptides were found to be α-helical in aqueous solution. However, we were able to control the secondary conformation of the glycopolypeptides by polymerizing racemic amino acid glyco NCAs. The poly(α-manno-*O*-lys) polypeptides synthesized by us also bind specifically to the lectin Con A, and the binding affinity was found to be nearly the same between polypeptides having enantiomerically pure L-lysine and the corresponding DL-lysine backbone.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data for all NCA monomers, glycopolypeptides, and fluorescently-labeled polypeptides. FT-IR, NMR spectra of polypeptides, precipitation assay, hemagglutination assay, and additional ITC isotherms. 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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