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# Design and Synthesis of Biotin Chain-Terminated Glycopolymers for Surface Glycoengineering

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Fundamental studies of glycopolymer properties have provided insight regarding carbohydrate-mediated biomolecular recognition processes that may be attributed, in part, to a multivalent or cluster effect.[1] Significantly, these efforts hold relevance for both pharmaceutical and biomaterial applications. For example, recent investigations have synthesized glycopolymers with surface anchoring groups located along the polymer backbone to generate glycosurfaces with potential utility in bio- and immunochemical assays,[2] as well as biocapture analysis.[3] Nonetheless, glycopolymers bearing a series of pendant anchoring groups characteristically demonstrate reduced bioactivity due to steric hindrance, which is only partially offset through the introduction of a spacer arm between the anchor and the polymer backbone. Avidin (streptavidin)/biotin-based surface engineering has the advantage of being rapidly completed in a mild aqueous environment, with simple washing and purification steps. Thus, potential damage to candidate surface ligand groups due to the conjugation process is limited and any moiety that can be biotinylated can be immobilized onto an avidin/streptavidin surface. As such, polymers with biotinylated end groups have recently been used to generate self-organizing protein-polymer hybrid amphiphiles,[4] as well as molecularly engineered surfaces.[5] Characteristically, these chain-end functionalized polymers are prepared by further modification or conversion in several steps after initial polymer synthesis.[6] In this report, we describe a straightforward approach to synthesize biotin chain-terminated glycopolymers of low polydispersity (Figure 1), via use of a biotin-derivatized arylamine initiator employed in a cyanoxyl-mediated free radical polymerization scheme.

A representative chain end-functionalized glycopolymer was designed as shown in Figure 1, in which multivalent lactose units serve as ligands for lectins and/or antibodies,[7] and a single biotin group provides an anchor by its specific binding activity to avidin or streptavidin.[8] Modulating lactose density as well as polymer solubility was achieved by using acrylamide as a comonomer and a spacer arm (**X**) between biotin and polymer backbone was used for further optimization of polymer-avidin/streptavidin interaction. The synthetic strategy relies on our previously developed cyanoxyl-mediated free-radical polymerization process, in which arylamines have been identified as candidate initiators.[9] Specifically, the biotin derivatized arylamine was used as an initiator for cyanoxyl-mediated free radical polymerization of 2-acrylaminoethyl lactoside and acrylamide so as to provide the desired biotin chain-terminated glycopolymers for subsequent use in glycosurface engineering.

In the current study, 4-aminobenzyl-biotinamide **1** and **2**[10] were designed and investigated as initiators for the polymerization of 2-acrylaminoethyl lactoside **7**[11] with acrylamide. Treatment of **1**

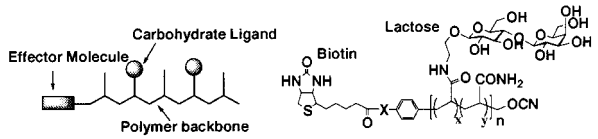
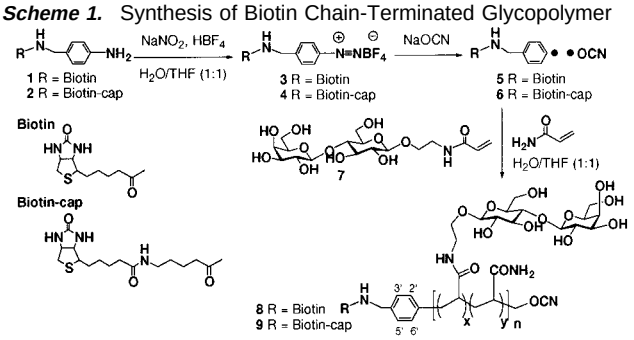



***Figure 1.*** Structure of biotin chain-terminated glycopolymer.

***Scheme 1.*** Synthesis of Biotin Chain-Terminated Glycopolymer




with $HBF_4$ and $NaNO_2$ in deoxygened $H_2O-THF$ (1:1) gave the arenediazonium cation **3**, which upon reaction with NaOCN at 50 °C afforded the biotinyl aryl free radical **5** and the cyanoxyl free radical (•OC≡N) as the initiating system for copolymerization of glycomonomer **7** and acrylamide. The biotin chain-terminated glycopolymer **8** was generated in 75% conversion as a white spongy powder. Similarly, using biotin-cap-arylamide **2** as the initiating species, glycopolymer **9** was generated with a spacer arm between biotin and the polymer chain in 70% conversion.The resultant copolymers were characterized by NMR spectroscopy (Figure 2), as well as by size-exclusion chromatography (SEC) coupled with both refractive index and laser-light scattering (LLS) detectors. Comparison of the integrated signal from the phenyl protons (7.10 ppm, $H_{2',6'}$ and 7.18 ppm, $H_{3',5'}$) with that due to the anomeric protons of lactose (4.36 ppm, $H_{1'-Lact}$ and 4.43 ppm, $H_{1-Lact}$), as well as that of the polymer backbone methine (2.10 ppm, CH) and methylene (1.55 ppm $CH_2$), indicated an average polymer composition of 10 lactose and 70 acrylamide units. Notably, downfield shifts of $H_{2'}$, $H_{6'}$ phenyl protons (7.10 ppm) demonstrated C−C bond formation between the phenyl group and the polymer backbone. The actual molar mass ($M_n$) was 12 kDa with a polydispersity index ($M_w/M_n$) of 1.3 from SEC/RI/LLS for **8**.

A gel shift assay was performed to verify streptavidin-glycopolymer binding in a solution-phase system. Streptavidin-glycopolymer complexes were generated as illustrated in lanes E and F (Figure 3, Gel *I*), while not observed on incubation with a comparable glycopolymer lacking a biotin group [lane D]. Since each streptavidin (60 kDa) contains four identical subunits, a streptavidin band remains present when mixed with only 2 equiv

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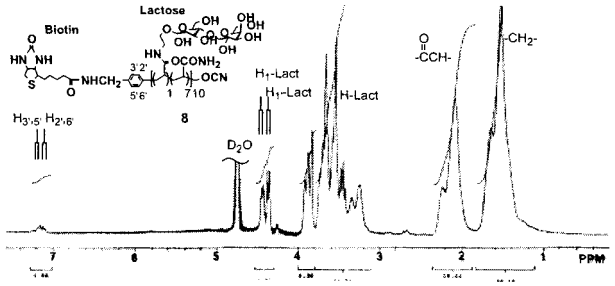


***Figure 2.*** $^1$H NMR spectrum of biotin-terminated glycopolymer (**8**).

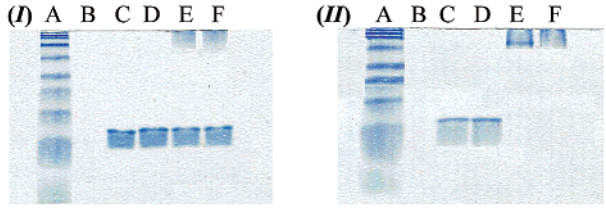


***Figure 3.*** Streptavidin−biotin binding SDS-PAGE gel shift assay: (*I*) 2 equiv of glycopolymer; (*II*) 20 equiv of glycopolymer. A, marker; C, Streptavidin alone; D, streptavidin + *p*-chlorophenyl-glycopolymer (**10**); E, streptavidin + biotin-glycopolymer (**8**); F, streptavidin + biotin-cap-glycopolymer (**9**).

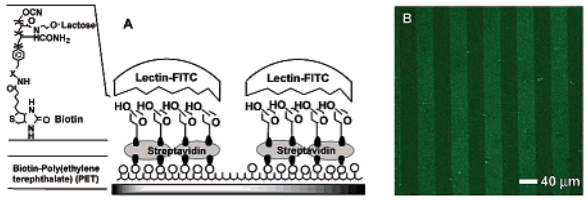


***Figure 4.*** (A) Schematic of lectin binding to a glycopolymer-derivatized surface; (B) fluorescent image of FITC-lectin bound onto a patterned PET membrane.

of biotin-glycopolymer (Gel *I*, Lanes E and F), but disappears in the presence of an excess of glycopolymer (Gel *II*, Lanes E and F). The retarded migration of streptavidin-glycopolymer complexes may be due to both an increase in molecular weight and a reduction in the capacity of the apolar portions of streptavidin to interact with the alkyl moiety of sodium dodecysulfate (SDS).[13] In this system, the presence of a spacer arm did not have a measurable impact on glycopolymer/streptavidin affinity.

Biotin and streptavidin activation techniques have played an important role in the development of biofunctionalized surface for sensor or biomaterial applications.[14,15] Glycopolymer-coated surfaces were produced by incubating streptavidin-derivatized PET membranes[16] in a glycopolymer solution (1 mg/mL in PBS) for 1 h at room temperature. Membranes were subsequently washed with PBS and incubated in a solution of a FITC-labeled galactose binding lectin (1 mg of *psophocarpus tetragonolobus*/mL in PBS). As demonstrated in Figure 4, lectin binding was observed in regions of glycopolymer immobilization, while no such activity was noted in surface regions not derivatized with streptavidin.

In conclusion, cyanoxyl-mediated free-radical polymerization with a biotin-derivatized arylamine initiator provides a straightforward strategy for generating biotin chain-terminated glycopolymers. Streptavidin−biotin binding was verified by a SDS-PAGE gel shift assay and the fabrication of a glycocalyx-mimetic surface achieved. The present approach will facilitate the production of glycosurface arrays of varying carbohydrate species type and density.

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**Supporting Information Available:** Detail synthetic procedures and spectral data for intermediates and final compounds, SDS-PAGE gel shift assay, and glycosurface fabrication and lectin binding procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The biotin-containing arylamine initiators **1** and **2** were prepared by the condensation of commercial available *p*-nitrobenzylamine with *N*-hydroxy-succinimidyl-biotin, and *N*-hydroxy-succinimidyl-biotinamidocaproate followed by hydrogenation with Pd−C in methanol in high yield, respectively.

(11) 2-Acrylaminoethyl lactoside **7** was prepared from lactose *per*-acetate via four steps including glycosylation, hydrogenation, and acrylation with acryloyl chloride in 53% total yield.

(12) 4-Chlorophenyl-glycopolymer **10** was prepared in a similar procedure as described for **8** by using 4-chloroaniline as initiator.

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(16) Biotin-modified poly(etheneterephthate) (PET) surface was patterned with streptavidin by microcontact print (40 μm streptavidin stripes with 40 μm spacing): ref 14.

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