Glycosaminoglycan-Mimetic Biomaterials. 1. Nonsulfated and Sulfated Glycopolymers by Cyanoxyl-Mediated Free-Radical Polymerization

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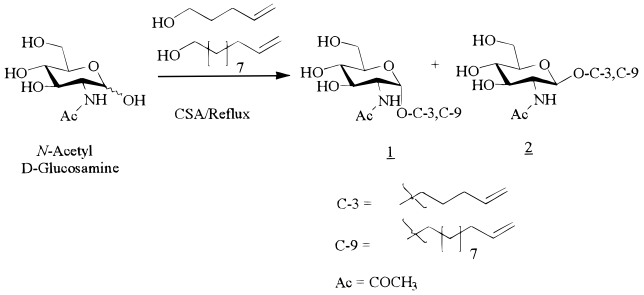
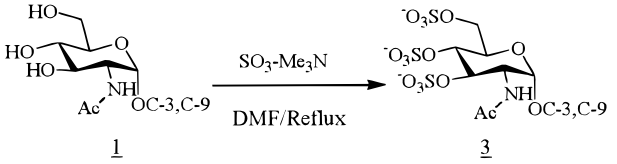
Introduction. Glycosaminoglycans influence a wide range of physiological events including cell proliferation, angiogenesis, and inflammatory processes.[1,2] However, the inherent difficulty associated with the synthesis of these chemically complex polysaccharides has stimulated the development of carbohydrate-mimetic molecules through a variety of approaches.[3,4] The recent identification of minimal oligosaccharide sequences which are often responsible for specific biological activities of the parent polysaccharide carries the promise of enhanced synthetic control and reproducibility.[5,6] Nonetheless, the production of even small oligosaccharides remains a challenging endeavor. In the past 5 years, significant advances in the synthesis of polymers with pendant saccharide residues, also known as "glycopolymers", have offered an alternative biomimetic strategy.[7] For instance, vinyl-containing saccharide derivatives can be polymerized to yield glycopolymers with a chemically stable hydrocarbon backbone and biologically active pendant hydrophilic saccharides.[8] The carbohydrate density, which may affect both ligand binding affinity and other polymer physiochemical properties, can be controlled through the choice of polymerizable saccharide and associated comonomers. Although the use of these polysaccharide-mimetic compounds as biomaterials has been limited, we believe that fundamental studies on the synthesis, properties, and applications of glycopolymers may lead to a better understanding of the molecular recognition processes that occur between carbohydrates and proteins. Furthermore, the potential to stably link a series of bioactive oligosaccharide ligands, which are more easily synthesized than the native polysaccharide, is an important advantage of the glycopolymer approach and presents an opportunity to develop glycosaminoglycan-mimicking structures with improved biological and physiochemical properties. Several synthetic strategies including cationic polymerization,[9,10] ring-opening metathesis polymerization,[11,12] and nitroxyl-mediated free-radical polymerization[13] have been recently proposed to afford glycopolymers that exhibit low fluctuations both in size and in composition. However, a significant limitation of these approaches is the necessity to use protected monomers followed by a final deprotection step(s) of the glycopolymer.

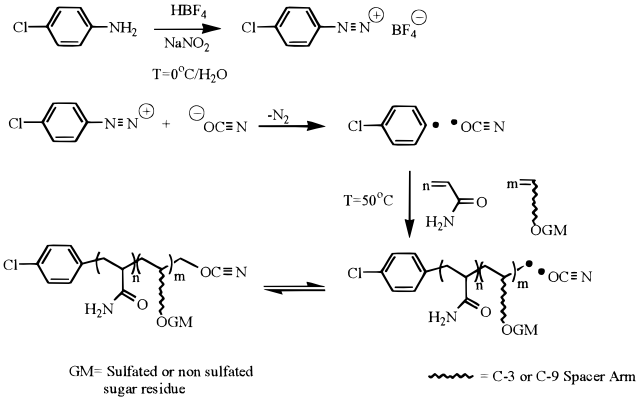
Scheme 1. Synthesis of Nonsulfated Glycomonomers 1 and 2



Scheme 2. Synthesis of Sulfated Glycomonomers 3



Scheme 3. Copolymerization of AM with Glycomonomers in the Presence of Cyanoxyl Radicals



In this communication, we report a straightforward synthetic strategy for obtaining water-soluble glycopolymers by a free-radical process. The methodology utilizes persistent cyanoxyl radicals (•OC≡N) as moderators of the statistical copolymerization of acrylamide (AM) with both nonsulfated and sulfated *N*-acetyl-D-glucosamine-carrying unprotected glycomonomers.

Results and Discussion. a. Synthesis of Nonsulfated and Sulfated Glycomonomers. As a starting point for our synthetic studies, model vinyl-derivatized glycomonomers were synthesized from *N*-acetyl-D-glucosamine. Nishimura et al.[14,15] have previously reported the preparation of similar monomers in two steps from an oxazoline derivative. However, a more efficient strategy was developed that yielded glycomonomers in a single step. Thus, *N*-acetyl-D-glucosamine, on treatment with 4-penten-1-ol or 10-undecen-1-ol in the presence of 10-camphorsulfonic acid (CSA) as catalyst, provided the α,*β*-anomeric mixture of the corresponding spacer arm containing glycomonomer (Scheme 1). Anomers **1** and **2** were successfully separated by column

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Table 1. Experimental Conditions and Results of Free-Radical Copolymerization of AM with Miscellaneous Glycomonomers Using $ClC_6H_4N{\equiv}N^+BF_4^-$/NaOCN as Initiating System[a]

glycomonomer (GM)	monomer ratio GM/AM (mol)	time (h)	yield[b] (%)	polymer composition[c] (mol)	monosaccharide content (wt %)	M_n[d] (g/mol)	M_w/M_n SEC
1(C-3)	1/4	1.5	10	1/7	37.7	24 100	1.46
		16	30	1/5	49.9	43 000	1.47
	1/20	1.5	23	1/90	4.3	94 000	1.17
		16	30	1/70	5.3	112 100	1.20
1(C-9)	1/4	1.5	15	1/16	25.2	43 400	1.25
		16	20	1/6	45.6	99 300	1.45
	1/20	1.5	21	1/166	3.1	25 800	1.14
		16	29	1/100	5.0	28 200	1.24
3(C-3)	1/4	1.5	21	1/10	43.6	16 100	1.13
		16	35	1/6	54.5	57 300	1.37
	1/20	1.5	35	1/93	7.4	25 400	1.10
		16	51	1/58	11.3	47 200	1.29
3(C-9)	1/4	16	26	1/10	45.4	57 200	1.20
	1/20	16	11	1/42	17.0	16 300	1.17

[a] T = 50 °C, $[M]_0 = [GM]_0 + [AM]_0 = 1$ mol/L, $[I]_0 = [ClC_6H_4N{\equiv}N^+BF_4^-]_0 = [NaOCN]_0 = 2 \times 10^{-2}$ mol/L. [b] Total conversion of both comonomers as determined by gravimetry. [c] Molar ratio of monosaccharide to acrylamide monomeric units in the resulting copolymer as determined by ^{1}H NMR. [d] M_n obtained from SEC (eluent, water; column, Waters Ultrahydrogel 250) equipped with a LLS detector (Wyatt Technology).

chromatography (SiO_2, $CHCl_3$/MeOH (9/1)) and were characterized by ^{1}H and ^{13}C NMR spectroscopy. Yields of α- and β-anomers were 31% and 11%, respectively. In the second phase of this investigation, chemoselective sulfation of hydroxy groups on the α-anomer (**1**) was effected using the $SO_3{-}NMe_3$ complex (Scheme 2). The product (**3**) was purified by anion-exchange and size-exclusion chromatography and characterized by ^{1}H and ^{13}C NMR, as well as by mass spectral analysis.

b. Synthesis of Nonsulfated and Sulfated Glycopolymers. To synthesize well-defined glycopolymers, we resorted to a free-radical technique that exhibits some features of a controlled polymerization. In this regard, cyanoxyl (•OC≡N) persistent radicals were used as moderators of the statistical copolymerization of AM with the synthesized glycomonomers. Indeed, in the early 1990s, Druliner[16] claimed a certain degree of control over free-radical polymerization of (meth)acrylic monomers, and particularly acrylamide, in the presence of these oxygen-centered radicals. Moreover, Grande et al.[17,18] have recently shown that these persistent radicals impart some control to the polymerization of methyl methacrylate and acrylic acid by scavenging growing radicals and forming dormant species that can reversibly undergo homolytic bond cleavage, by a manner similar to that reported for nitroxyl-mediated processes. In the presence of cyanoxyl radicals that are unable to initiate chain growth, a low stationary concentration of macroradicals is maintained which prevents bimolecular irreversible termination from occurring to the extent observed in classical free-radical mechanisms. Furthermore, the use of •OC≡N radicals at moderate temperatures (25−70 °C) avoids unintended thermal polymerization of monomers.

Cyanoxyl radicals were readily generated in situ by an electron-transfer reaction between cyanate anions ($^-$OC≡N) and *p*-chlorobenzenediazonium cations ($ClC_6H_4N{\equiv}N^+$). The arenediazonium salts were previously prepared in water through diazotization reaction of *p*-chloroaniline (Scheme 3). The results of copolymerizations performed at 50 °C using $ClC_6H_4N{\equiv}N^+BF_4^-$/NaOCN as the initiating system are shown in Table 1. The statistical copolymers obtained were isolated by precipitation in a 10-fold excess of methanol and characterized by ^{1}H NMR spectroscopy (see Figure 1, as an example), as well as by size-exclusion chromatography (SEC) coupled with a refractive index (RI) detector and a multiangle laser light-scattering (LLS) detector. The monosaccharide content of the copolymers was determined by taking the ratio of the intensities of the resonance signals due to the methyl protons of *N*-acetyl groups from carbohydrate residues (2.0 ppm) and to the methine protons (between 2.1 and 2.4 ppm) of the main chain. Regardless of the glycomonomer used (nonsulfated/sulfated, short/long spacer arm), it is remarkable that the carbohydrate contents as well as the molar masses increased with monomer conversion while the polydispersity indexes remained below 1.5. When an initial ratio of glycomonomer to AM of 1/4 was employed, a copolymer that displayed a monosaccharide content in close agreement with that expected was obtained after 16 h of reaction. Weight proportions of sugar residues as high as 50% were thus reached. Nevertheless, a higher carbohydrate content in the resulting copolymer was associated with an increase in the polydispersity index. Thus, some loss of control over the copolymerization process may occur in the presence of increasing amounts of glycomonomer. This is probably due to the innate low chemical reactivity of the unactivated vinyl group in the saccharide monomer. It is also noteworthy that spacer-arm length of the glycomonomer influenced the polymerization behavior. Indeed, the amount of incorporated carbohydrate was increased with decreasing spacer-arm length.

In a comparative analysis, classical free-radical copolymerizations of glycomonomers and AM were carried out using ammonium peroxodisulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as the initiating system. TMEDA accelerates the homolytic scission of APS yielding sulfate ($SO_4^{\bullet-}$), hemiTMEDA ($(CH_3)_2NCH_2CH_2(CH_3)NCH_2^\bullet$), and hydroxyl (•OH) radical species. It has been previously reported by Nishimura et al.[14,15] that **1**(C-9) glycomonomer cannot undergo a copolymerization reaction in water due to its poor solubility. However, we were able to obtain a copolymer by performing the copolymerization in a dilute solution of water/THF (1/1). The reaction medium was homogeneous, and the polymerization proceeded efficiently at room temperature. Utilizing identical experimental conditions as those used for cyanoxyl-mediated processes ($[M]_0 = 1$ mol/L, $[I]_0 = 2 \times 10^{-2}$ mol/L, GM/AM = 1/4), the resulting glycopolymers exhibited lower monosaccharide contents (up to 30 wt %) and especially higher molar masses and polydispersity

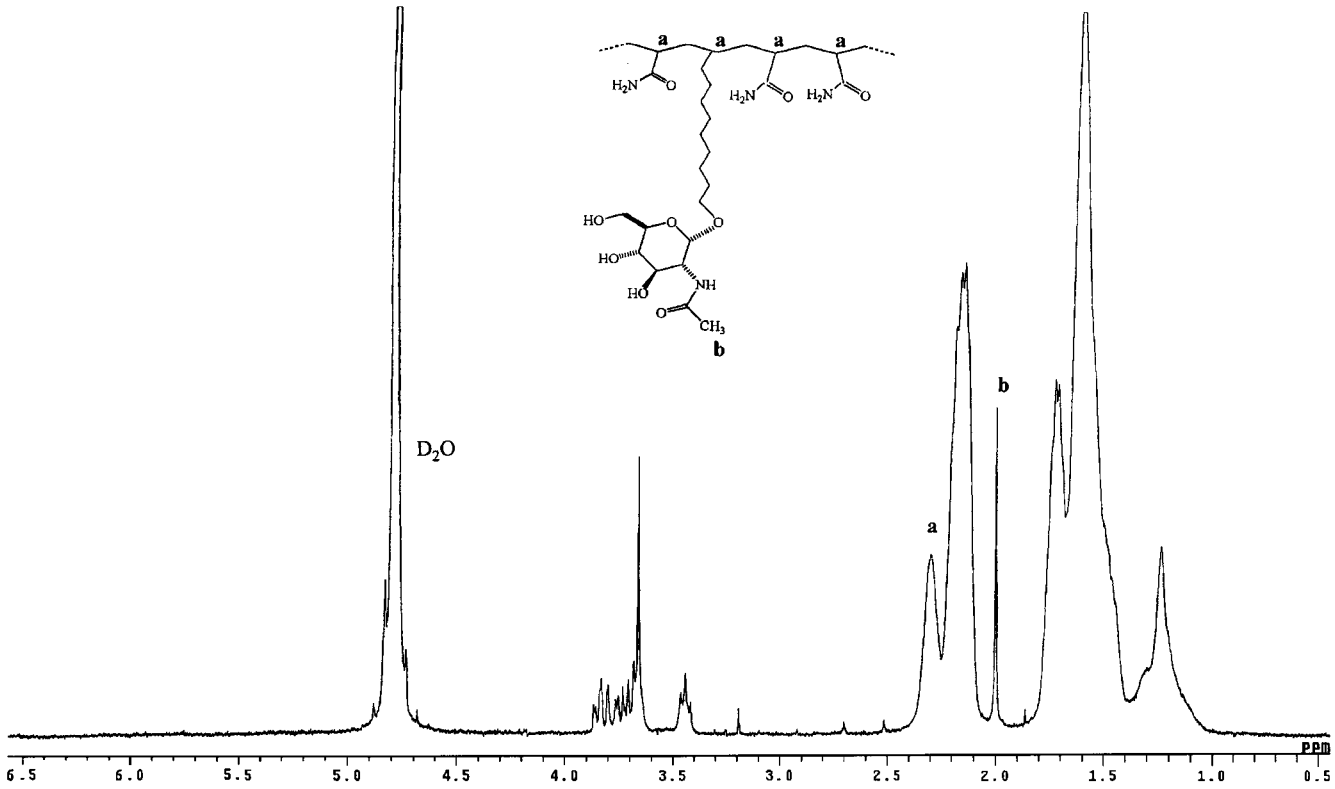

Figure 1. ^{1}H NMR spectrum of a **1**(C-9)/AM glycopolymer sample. The spectrum was recorded at room temperature with a Varian INOVA 400 spectrometer with a magnetic field strength of 400 MHz. The sample concentration was 10 mg/mL, and D_2O was used as the solvent and internal standard, 4.8 ppm.

indexes (1.7−2.0). Moreover, increasing the polymerization time from 1.5 to 16 h increased the polydispersity index but otherwise had little effect on either monosaccharide content or molar mass. These investigations illustrate the anticipated absence of control over the copolymerization process using the classical APS/TMEDA initiating system, while confirming the significant role of cyanoxyl radicals in promoting a controlled form of polymerization.

Conclusions. We have synthesized a series of *N*-acetyl-D-glucosamine-containing glycomonomers as an initial step in the synthesis of glycosaminoglycan-mimetic glycopolymers. This report demonstrates that cyanoxyl-mediated free-radical polymerization is an original and straightforward approach for obtaining water-soluble glycopolymers with high monosaccharide contents and low polydispersity indexes.

We believe that well-characterized glycopolymers will serve as useful model systems for investigating protein−carbohydrate interactions relevant to endothelial regeneration and angiogenesis. The design of biomaterials capable of promoting these physiological processes may have significant impact in the areas of wound repair and tissue regeneration, as well as other phenomena influenced by glycosaminoglycans.

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