# Glycosaminoglycan Mimetic Biomaterials. 2. Alkene- and Acrylate-Derivatized Glycopolymers via Cyanoxyl-Mediated Free-Radical Polymerization

**Daniel Grande, Subramanian Baskaran, and Elliot L. Chaikof***

*Laboratory for Biomolecular Materials Research, Departments of Surgery and Bioengineering, Emory University School of Medicine, Atlanta, Georgia 30322, and School of Chemical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30320*

*Received September 27, 2000; Revised Manuscript Received January 5, 2001*

ABSTRACT: Cyanoxyl (•OC≡N)-mediated free-radical polymerization provides an effective and versatile method for engineering a diverse array of water-soluble glycopolymers with high saccharide contents and low polydispersity indexes ($1.1 < M_w/M_n < 1.6$). Cyanoxyl persistent radicals can be used as chain-growth moderators of the statistical copolymerization of acrylamide with either nonsulfated or sulfated *N*-acetyl-D-glucosamine-carrying alkene- and acrylate-derivatized unprotected glycomonomers. Moreover, monosaccharide-containing homopolymers can be prepared with some degree of control by polymerization of the acrylic glycomonomers.

## Introduction

Glycosaminoglycans (GAGs) are naturally occurring linear polysaccharides encountered both in the extracellular matrix and on cell surfaces, where they form a carbohydrate coating referred to as the glycocalyx. GAGs are involved in a wide array of physiological processes, including cell proliferation and migration, as well as modulation of angiogenesis and inflammatory responses.[1−3] The diverse bioactivities of GAGs are a consequence of unique binding sequences that facilitate local sequestration of biologically active proteins, such as growth factors and antithrombin III. In this manner, GAGs function as delivery vehicles for the controlled local release of a variety of proteins and, in select circumstances, potentiate the activity of the bound protein.[4−6] The inability to generate GAGs through recombinant genetic engineering strategies, combined with the inherent complexity that has been associated with their direct chemical synthesis, has stimulated the development of a variety of biomimetic synthetic approaches for the generation of carbohydrate-based macromolecules.[7,8]

The recent identification that smaller oligosaccharide sequences may be responsible for the unique biological activities of the parent polysaccharides holds the promise of generating relatively small molecule GAG equivalents through a total synthesis strategy.[9,10] While advantages of such an approach exist, an inherent limitation is the loss of spatially controlled organization of multiple target saccharide sequences. Indeed, the observation of enhanced protein binding affinity derived from multivalent oligosaccharide ligands has been termed the "cluster glycoside effect".[11−13] Consequently, an alternative glycomimetic strategy has consisted of the design of synthetic polymers that contain a hydrocarbon backbone with biologically active pendant saccharides. Fundamental studies on the synthesis and properties of model "glycopolymers" have already proven to be useful in the characterization of specific biomolecular recognition processes that hold relevance for both pharmaceutical and biomaterial applications.[14]

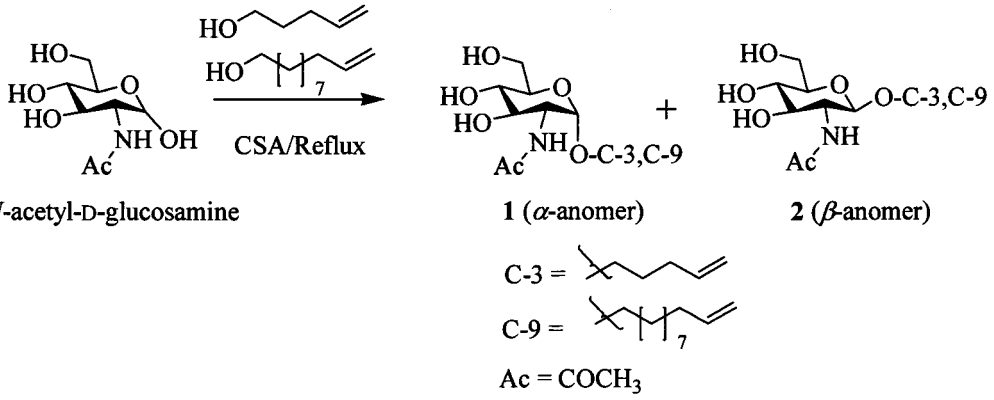
Optimization of glycopolymer properties has required the utilization of biomolecular architectures that exhibit low fluctuations both in polymer size and in composition. A large variety of "living"/controlled polymerization techniques have recently emerged for this purpose, including ring-opening polymerization of sugar-substituted *N*-carboxyanhydrides,[15] ring-opening metathesis polymerization (ROMP) of sugar-derivatized norbornenes,[16,17] cationic polymerization of saccharide-carrying vinyl ethers,[18] anionic polymerization of styrene derivatives containing monosaccharide residues,[19] nitroxide-mediated free-radical polymerization of sugar-carrying styryl[20] and acryloyl[21] monomers, and atom transfer radical polymerization (ATRP) of carbohydrate-based methacrylates.[22,23] Nevertheless, due to the incompatibility of hydroxyl groups from the saccharide moieties with either initiators or controlling agents, all of these approaches require the use of protected monomers and the subsequent deprotection of polymer chains to generate the desired glycopolymers.

To circumvent these limitations, we have examined the applicability of cyanoxyl (•OC≡N)-mediated free-radical polymerization in the synthesis of model glycopolymers from unprotected glycomonomers. Indeed, it should be stressed that this straightforward polymerization technique can be conducted in aqueous solution, is tolerant of a broad range of functional groups (−OH, $-NH_2$, −COOH, etc.), yields low-polydispersity polymers, and can be applied to the synthesis of block and graft copolymers.[24,25] In this report, we first describe the preparation of a series of model *N*-acetyl-D-glucosamine-containing glycomonomers, both nonsulfated and sulfated, that are either alkene- or acrylate-derivatized. Cyanoxyl-mediated free-radical polymerization is then exploited to create a wide variety of water-soluble glycopolymers with high monosaccharide contents and low polydispersity indexes. Specifically, we demonstrate that cyanoxyl persistent radicals can be effectively

* Corresponding author. Phone (404) 727-8413; Fax (404) 727-3660; E-mail echaiko@emory.edu.

10.1021/ma001680t CCC: $20.00 
Published on Web 02/13/2001

**Scheme 1. Synthesis of Nonsulfated Alkene-Derivatized Glycomonomers**




employed as chain-growth moderators of the statistical copolymerization of acrylamide with either alkene- or acrylate-based glycomonomers. Further, in association with a cyanoxyl-mediated polymerization strategy, acrylic monosaccharides can be utilized to generate homoglycopolymers with some degree of control.

## Experimental Section

**Materials.** All solvents and reagents were purchased from commercial sources and were used as received, unless otherwise noted. Deionized water with a resistivity of 18 MΩ·cm was used as solvent in all polymerization reactions.

**Methods.** All reactions were performed in flame-dried glassware under an atmosphere of dry argon. The reaction medium solutions were evaporated under reduced pressure with a rotary evaporator, and the residue was chromatographed on a silica gel (230−400 mesh) column.

Analytical thin-layer chromatography (TLC) was performed on Whatman silica gel aluminum backed plates of 250 $\mu$m thickness on which spots were visualized with UV light or charring the plate before and/or after dipping in a $H_2SO_4$−EtOH mixture.

Melting point (mp) measurements were performed with a Thomas-Hoover melting point apparatus in open capillary tubes and were uncorrected.

Mass spectra (MS/FAB) were obtained at an ionizing voltage of 70 eV.

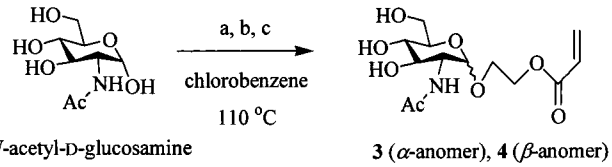
Optical rotations were determined with a Perkin-Elmer-2 GIMC polarimeter.

$^1$H and $^{13}$C NMR spectra were recorded at room temperature with a Varian INOVA 400 spectrometer (magnetic field strengths of 400 and 100 MHz for $^1$H and $^{13}$C NMR analyses, respectively). In all cases, the sample concentration was 10 mg/mL, and the appropriate deuterated solvent was used as internal standard.

The size-exclusion chromatography (SEC) equipment comprised a Waters model 510 HPLC pump, a Waters Ultrahydrogel 250 column, and a Wyatt Technology Optilab 903 refractometer. The eluent consisted of a 0.1 mol/L $NaNO_3$ deionized water solution containing 0.05 wt % sodium azide at a flow rate of 0.7 mL/min. The actual molar masses of the glycopolymer samples were determined from the response of the DAWN F (Wyatt Technology) multiangle laser light-scattering (LLS) detector that was connected to the outlet of the SEC apparatus.

**Synthesis of Nonsulfated Alkene-Derivatized Glycomonomers.** To a mixture of *N*-acetyl-D-glucosamine (10 g) and either 4-penten-1-ol or 10-undecen-1-ol (large excess, ∼50−75 mL) was added a catalytic amount of 10-camphorsulfonic acid (CSA) (∼400 mg), and the mixture was refluxed at 110 °C for 9 h. The reaction mixture was then cooled and neutralized with triethylamine, and the excess of alcohol was removed under vacuum. Moreover, the rest of the solid mass was rinsed with hot petroleum ether to remove the rest of $\omega$-alkenyl alcohol. The residue was purified by column chromatography using a chloroform/methanol (97/3) mixture to afford the expected α- and $\beta$-anomers (Scheme 1, **1**(C-3) or **1**(C-9) and **2**(C-3) or **2**(C-9), respectively).

**Scheme 2. Synthesis of Nonsulfated Acrylic Glycomonomers**[a]




[a] a = 2-hydroxyethyl acrylate; b = phosphomolybdic acid; c = 1-chloro-2,4-dinitrobenzene.

***n*-Pentenyl 2-Acetamido-2-deoxy-α-D-glucopyranoside, 1**(C-3)**.** Yield 46%; mp 146−148 °C; $[\alpha]_D^{20}$ = +142.2° (c 1.3, MeOH). $^1$H NMR ($CDCl_3$ + 5% $CD_3OD$), $\delta_{ppm}$: 1.68 (m, 2H), 2.02 (s, 3H, NHCOC*H*$_3$), 2.12 (m, 2H), 3.41 (dt, 1H, $J$ = 6.4 and 10 Hz), 3.68 (m, 4H), 3.78 (d, 1H, $J$ = 3.2 and 12.4 Hz), 4.00 (dd, 1H, $J$ = 3.2 and 12.4 Hz), 4.02 (m, 1H), 4.77 (d, 1H, $J_{1,2}$ = 3.6 Hz, *H-1*), 5.01 (m, 2H, C*H*$_2$=), 5.80 (m, 1H, C*H*=), 6.61 (d, 1H, $J$ = 8.8 Hz, N*H*COCH$_3$). $^{13}$C NMR ($CDCl_3$ + 5% $CD_3OD$), $\delta_{ppm}$: 22.9, 28.4, 30.2, 53.6, 61.2, 67.2, 70.1, 71.6, 72.4, 97.3, 114.9, 137.9, 171.7. MS/FAB, *m/z*: 289 ($M^+$).

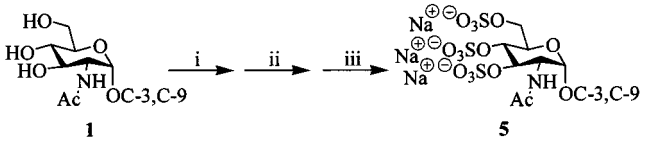
***n*-Undecenyl 2-Acetamido-2-deoxy-α-D-glucopyranoside, 1**(C-9)**.** Yield 50%; mp 152−154 °C; $[\alpha]_D^{20}$ = −9.0° (c 0.2, MeOH). $^1$H NMR ($CDCl_3$ + 5% $CD_3OD$), $\delta_{ppm}$: 1.34 (m, 9H), 1.54 (m, 2H), 2.02 (m and s, 5H), 3.33 (m, 1H), 3.68 (m, 4H), 3.77 (br d, 1H, $J$ = 9.6 Hz), 3.92 (br d, 1H, $J$ = 9.6 Hz), 4.06 (m, 1H), 4.78 (d, 1H, $J_{1,2}$ = 3.6 Hz, *H-1*), 4.95 (m, 2H, C*H*$_2$=), 5.80 (m, 1H, C*H*=), 6.43 (d, 1H, $J$ = 8.8 Hz, N*H*COCH$_3$). $^{13}$C NMR ($CDCl_3$ + 5% $CD_3OD$), $\delta_{ppm}$: 23.3, 26.1, 28.9, 29.1, 29.3, 29.4, 29.5, 29.6, 33.8, 53.7, 61.2, 68.0, 69.9, 71.6, 72.8, 97.4, 114.1, 139.1, 171.7. MS/FAB, *m/z*: 373 ($M^+$).

***n*-Pentenyl 2-Acetamido-2-deoxy-β-D-glucopyranoside, 2**(C-3)**.** Yield: 15%. Detailed characterization data have been previously reported by Nishimura.[26b]

***n*-Undecenyl 2-Acetamido-2-deoxy-β-D-glucopyranoside, 2**(C-9)**.** Yield 16%; mp 175−177 °C; $[\alpha]_D^{20}$ = −7.0° (c 0.23, MeOH). $^1$H NMR ($CDCl_3$ + 5% $CD_3OD$), $\delta_{ppm}$: 1.32 (m, 9H), 1.54 (m, 2H), 2.01 (m and s, 5H), 3.32 (m, 1H), 3.47 (m, 2H), 3.54 (m, 2H), 3.84 (m, 3H), 4.47 (d, 1H, $J_{1,2}$ = 8.0 Hz, *H-1*), 4.96 (m, 2H, C*H*$_2$=), 5.81 (m, 1H, C*H*=). MS/FAB, *m/z*: 373 ($M^+$).

**Synthesis of Nonsulfated Acrylic Glycomonomers.** A mixture of *N*-acetyl-D-glucosamine (10 g, 45 mmol), 2-hydroxyethyl acrylate (52 g, 450 mmol), chlorobenzene (10 g, 90 mmol), phosphomolybdic acid (0.82 g, 0.45 mmol), and 1-chloro-2,4-dinitrochlorobenzene (1 g, 4.5 mmol) was heated to 110 °C. The reaction was monitored by TLC; after 5 h, the reaction mixture was cooled and then neutralized with a saturated $NaHCO_3$ aqueous solution. The crude product obtained was purified by column chromatography with a chloroform/methanol (96/4) mixture whereby the α-anomer (Scheme 2, compound **3**) was recovered as a viscous oil and the $\beta$-anomer (Scheme 2, compound **4**) was isolated as a white powder.

**Scheme 3. Synthesis of Sulfated Alkene-Derivatized Glycomonomers**[a]




1 5

[a] i = $SO_3$−$NMe_3$, DMF, 60 °C; ii = DEAE-sephacel anion-exchange resin column; iii = trisacryl size-exclusion resin column.

**2*S*-(4,5-Dihydroxy-6-hydroxymethyl-3-methylcarboxamidotetrahydro-2*H*-2-pyranyloxy)ethyl Acrylate, 3.** Yield 37%; $[\alpha]_D^{20} = -32.5°$ (c 2, $CHCl_3$). $^1$H NMR ($CDCl_3$), $\delta_{ppm}$: 1.88 (s, 3H, NHCOC*H*$_3$), 3.38−3.66 (m, 7H), 3.81 (m, 1H), 4.17 (m, 3H), 4.70 (d, 1H, $J_{1,2} = 3.5$ Hz, *H-1*), 5.79 (d, 1H, $J$ = 10.5 Hz, C$H_2$=), 6.05 (dd, 1H, $J$ = 10.5 and 17.2 Hz, C*H*=), 6.29 (d, 1H, $J$ = 17.2 Hz, C$H_2$=). $^{13}$C NMR ($CDCl_3$), $\delta_{ppm}$: 22.5, 53.3, 61.2, 63.1, 65.7, 68.6, 70.3, 71.9, 97.5, 127.7, 131.6, 167.0, 172.0. MS/FAB, *m*/*z*: 320 ($M^+ + H^+$).

**2*R*-(4,5-Dihydroxy-6-hydroxymethyl-3-methylcarboxamidotetrahydro-2*H*-2-pyranyloxy)ethyl Acrylate, 4.** Yield 11%; mp 138−140 °C; $[\alpha]_D^{20} = +24.5°$ (c 2, $CHCl_3$). $^1$H NMR ($CDCl_3$), $\delta_{ppm}$: 1.89 (s, 3H, NHCOC*H*$_3$), 3.28−3.48 (m, 5H), 3.35 (m, 1H), 3.44 (m, 1H), 3.64−3.78 (m, 3H), 3.91 (m, 1H), 4.17 (m, 1H), 4.28 (m, 1H), 4.43 (d, 1H, $J_{1,2} = 7.9$ Hz, *H-1*), 5.80 (d, 1H, $J$ = 10.4 Hz, C$H_2$=), 6.05 (dd, 1H, $J$ = 10.4 and 17.5 Hz, C*H*=), 6.36 (d, 1H, $J$ = 17.5 Hz, C$H_2$=). $^{13}$C NMR ($CDCl_3$), $\delta_{ppm}$: 22.7, 56.2, 61.5, 63.1, 66.8, 70.6, 74.7, 75.8, 100.3, 127.8, 131.5, 166.2, 172.6. MS/FAB, *m*/*z*: 320 ($M^+ + H^+$).

**Preparation of Sulfated Glycomonomers.** Under argon atmosphere, the appropriate amount of sulfur trioxide−trimethylamine ($SO_3$−$NMe_3$) complex (4 equiv for each hydroxyl group) was added to a nonsulfated glycomonomer in DMF, and the mixture was stirred at 60 °C for 12 h. The reaction medium was then cooled to 0 °C, and a saturated $NaHCO_3$ aqueous solution was added. The crude mixture was stirred for 1 h and concentrated to a smaller volume that was passed through a diethylaminoethyl (DEAE)-sephacel anion-exchange resin column. It was first eluted with a 10 mmol/L sodium phosphate buffer (pH ∼ 7.0), thereby removing the unreacted nonsulfated compound. The sulfated homologue was then eluted with a 1 mol/L NaCl buffer (pH ∼ 7.0) and recovered as a mixture of its trisodium with a NaCl excess. The latter eluate was concentrated, redissolved in a minimum amount of water, and passed through a Trisacryl (Gf05 M grade, Sigma-Aldrich) size-exclusion resin column for isolation of the sulfated compound free of NaCl (Scheme 3). Appropriate fractions were pooled and freeze-dried to provide the pure sulfated glycomonomer in 30−35% yield.

***n*-Pentenyl 2-Acetamido-2-deoxy-3,4,6-trisulfoxy-α-D-glucopyranoside, Trisodium Salt, 5**(C-3)**.** $[\alpha]_D^{23} = +27.8°$ (c 1.2, $H_2O$). $^1$H NMR ($D_2O$), $\delta_{ppm}$: 1.73 (m, 2H), 2.00 (s, 3H, NHCOC*H*$_3$), 2.17 (m, 2H), 3.51 (m, 1H), 3.76 (m, 1H), 3.90 (m, 1H), 4.23 (m, 2H), 4.25(m, 1H), 4.59 (m, 2H), 4.90 (d, 1H, $J$ = 3.6 Hz, *H-1*), 5.05 (m, 2H, C$H_2$=), 5.90 (m, 1H, C*H*=). $^{13}$C NMR ($D_2O$), $\delta_{ppm}$: 24.8, 30.4, 32.4, 55.3,70.1, 70.4, 71.5, 77.2, 79.4, 99.1, 117.5, 147.7, 177.1. MS/FAB, *m*/*z*: 595 ($M^+ + 3Na^+ - 3H^+$), 572 ($M^+ + 2Na^+ - 2H^+$).

***n*-Undecenyl 2-Acetamido-2-deoxy-3,4,6-trisulfoxy-α-D-glucopyranoside, Trisodium Salt, 5**(C-9)**.** $[\alpha]_D^{23} = +32.0°$ (c 0.2, $H_2O$). $^1$H NMR ($D_2O$), $\delta_{ppm}$: 1.35 (m, 12H), 1.61 (m, 2H), 2.05 (m, 5H), 3.50 (m, 1H), 3.73 (m, 1H), 3.91 (m, 1H), 4.19 (m, 2H), 4.28 (m, 1H), 4.58 (m, 2H), 4.91 (d, 1H, $J$ = 3.7 Hz, *H-1*), 5.04 (m, 2H, C$H_2$=), 5.91 (m, 1H, C*H*=). $^{13}$C NMR ($D_2O$), $\delta_{ppm}$: 24.8−35.8, 55.3, 70.0, 71.1, 71.2, 79.4, 99.0, 116.7, 143.2, 177.1. MS/FAB, *m*/*z*: 679 ($M^+ + 3Na^+ - 3H^+$).

**2*S*-(4,5-Disulfoxy-6-sulfoxymethyl-3-methylcarboxamidotetrahydro-2*H*-2-pyranyloxy)ethyl Acrylate, Trisodium Salt, 6.** $[\alpha]_D^{23} = -39.0°$ (c 0.8, $H_2O$). $^1$H NMR ($D_2O$), $\delta_{ppm}$: 1.92 (s, 3H, C*H*$_3$), 3.34−3.69 (m, 7H), 3.89 (m, 1H), 4.23 (m, 3H), 4.80 (d, 1H, $J$ = 3.5 Hz, *H-1*), 5.82 (d, 1H, $J$ = 10.3 Hz, C$H_2$=), 6.07 (dd, 1H, $J$ = 10.3 and 17.4 Hz, C*H*=), 6.38 (d, 1H, $J$ = 17.4 Hz, C$H_2$=).

**Statistical Copolymerization of Alkene-Derivatized Glycomonomers and Acrylamide Initiated by $ClC_6H_4N{\equiv}N^+BF_4^-$/NaOCN.** In a three-neck flask, $6.03 \times 10^{-5}$ mol. (0.008 g) of *p*-chloroaniline was reacted with $9.04 \times 10^{-5}$ mol of $HBF_4$ (actually 0.017 g of 48 wt % aqueous solution), at 0 °C, in 2 mL of water and under argon atmosphere. The diazonium salt $ClC_6H_4N{\equiv}N^+BF_4^-$ was then generated by adding $7.2 \times 10^{-5}$ mol (0.005 g) of sodium nitrite ($NaNO_2$) to the reaction medium. After 30 min, a degassed mixture of $6.03 \times 10^{-4}$ mol (0.225 g) of glycomonomer **1**(C-9), $2.41 \times 10^{-3}$ mol (0.171 g) of acrylamide, and $6.03 \times 10^{-5}$ mol (0.004 g) of sodium cyanate (NaOCN), dissolved in 1 mL of water/tetrahydrofuran (1/1), was introduced into the flask containing the diazonium salt. The polymerization medium was then heated to 50 °C. The statistical copolymers formed after 1.5 and 16 h of reaction were isolated by precipitation in a 10-fold excess of cold methanol, dried, and weighed so as to determine the conversion.

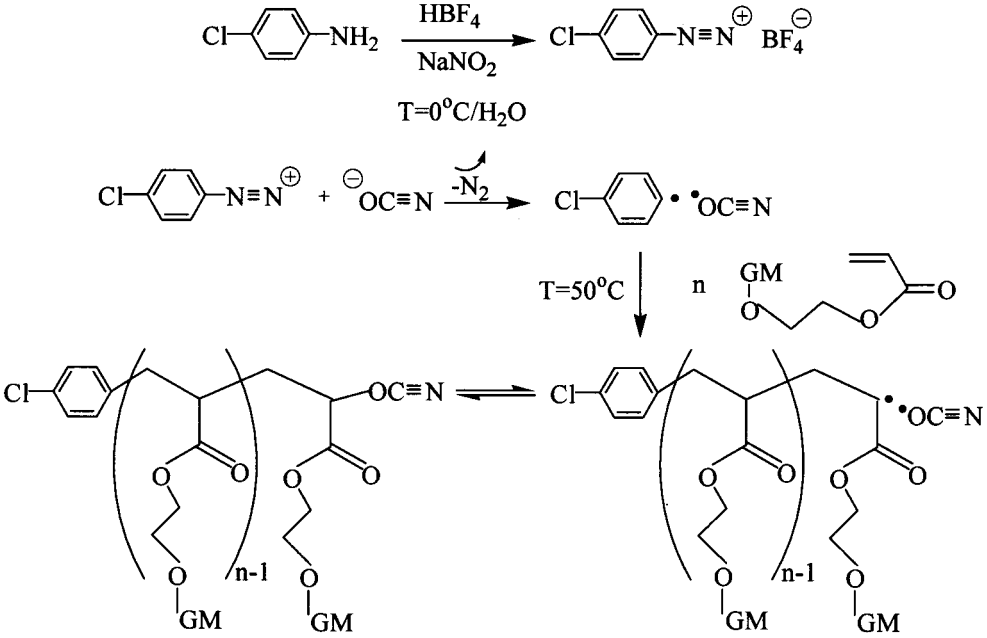
**Homopolymerization of Acrylic Glycomonomers Initiated by $ClC_6H_4N{\equiv}N^+BF_4^-$/NaOCN.** The general mechanism of this reaction is depicted in Scheme 4, and a typical polymerization is described hereafter. In a three-neck flask, $2.45 \times 10^{-5}$ mol (0.003 g) of *p*-chloroaniline was reacted with $3.67 \times 10^{-5}$ mol of $HBF_4$ (actually 0.007 g of 48 wt % aqueous solution), at 0 °C, in 2 mL of water and under argon atmosphere. The diazonium salt $ClC_6H_4N{\equiv}N^+BF^{4-}$ was generated by adding $2.93 \times 10^{-5}$ mol (0.002 g) of $NaNO_2$ to the reaction medium. After 30 min, a degassed mixture of $1.22 \times 10^{-3}$ mol (0.39 g) of glycomonomer **3** and $2.45 \times 10^{-5}$ mol (0.002 g) of NaOCN, dissolved in 0.5 mL of water, was introduced into the flask containing the arenediazonium salt. The polymerization medium was then heated to 50 °C for 4 h. The resulting glycopolymer was isolated by precipitation in a 10-fold excess of cold methanol and dried to yield a white cotton wool-like material.

## Results and Discussion

**1. Synthesis of Alkene- and Acrylate-Derivatized Glycomonomers.** As a starting point for our synthetic studies, nonsulfated alkene-derivatized unprotected glycomonomers were directly synthesized from *N*-acetyl-D-glucosamine. Three different glycosidation methods have been previously reported. Monosaccharides with side carbon chains from C-1 to C-3 have been synthesized using $FeCl_3$ as a catalyst.[27] Similarly, trifluoromethanesulfonic acid[28] as well as 10-camphorsulfonic acid (CSA)[29] has been utilized for the synthesis of spacer-armed glycosides from glucose or an oxazoline derivative.[26] For our purpose, we resorted to the CSA/reflux method that provided a facile means of yielding the desired spacer arm-containing glycomonomer. Hence, *N*-acetyl-D-glucosamine was refluxed with a catalytic amount of CSA and a large excess of either *ω*-pentenyl or *ω*-undecenyl alcohol to provide a mixture of α- and *β*-anomers (Scheme 1, **1**(C-3) or **1**(C-9) and **2**(C-3) or **2**(C-9), respectively) in an average 60−70% crude yield. The latter compounds were separated by column chromatography with a ratio (α/*β*) of 3/1 for both cases. The yield was improved by varying the temperature and reaction time with respect to our previously reported studies.[30a,b] The α- and *β*-anomeric configurations of the separated products were determined from $^1$H NMR data, namely $J_{1,2}$ coupling constants of 3.6 and 8.0 Hz, respectively.

To introduce the acrylate functionality into the glycomonomer structure, we adopted a published method[31] for methyl glycosides with slight modifications. Thus, acrylic monomer **3** was directly synthesized from *N*-

**Scheme 4. Cyanoxyl-Mediated Free-Radical Polymerization of Acrylic Glycomonomers**[a]




[a] GM = nonsulfated or sulfated *N*-acetyl-D-glucosamine residue.

acetyl-D-glucosamine as depicted in Scheme 2. Treatment of this carbohydrate with 2-hydroxyethyl acrylate in the presence of phosphomolybdic acid as catalyst and 1-chloro-2,4-dinitrobenzene as polymerization inhibitor yielded a crude $\alpha,\beta$-anomeric mixture (compounds **3** and **4**, respectively) in an overall yield close to 50%. Column chromatography allowed both anomers to be separated with a ratio ($\alpha/\beta$) of 3.5/1. They were characterized by $^1$H and $^{13}$C NMR, as well as by mass spectrometry.

Chemoselective sulfation of all hydroxyl groups on $\alpha$-anomers, namely **1**(C-3), **1**(C-9), and **3**, was achieved by treating these monosaccharides with $SO_3-NMe_3$ complex at 60 °C. As expected, a relative downfield shift in the $^1$H NMR spectra of the sulfated glycomonomers (compounds **5**(C-3), **5**(C-9), and **6**) was observed when compared with their nonsulfated homologues.

**2. Synthesis of Alkene- and Acrylate-Derivatized Glycopolymers.** Druliner[24] in the early 1990s, and more recently Grande and Gnanou,[25] have noticed that a certain degree of control could be achieved when polymerizing a broad range of (meth)acrylic monomers in the presence of cyanoxyl (•OC≡N) persistent radicals. In contrast with nitroxide-controlled free-radical polymerization or ATRP processes, these oxygen-centered radicals function under mild conditions (25−70 °C).

In the present study, cyanoxyl radicals were generated by an electron-transfer reaction between cyanate anions ($^-$OC≡N), from a NaOCN aqueous solution, and *p*-chlorobenzene-diazonium salts ($ClC_6H_4N{\equiv}N^+BF_4^-$) that were previously prepared in situ through a diazotization reaction of *p*-chloroaniline in water (Scheme 4). In addition to cyanoxyl persistent radicals, aryl-type active radicals were simultaneously produced, and only the latter species is capable of initiating chain growth. A large variety of water-soluble glycopolymers were generated by varying the nature of the $\omega$-alkenyl glycomonomer (nonsulfated/sulfated, C-3/C-9 spacer arm), as well as the initial molar ratio of glycomonomer (GM) to acrylamide (AM) in the statistical cyanoxyl-mediated copolymerization of both comonomers (Table 1). Statistical copolymers were characterized by $^1$H NMR spectroscopy (see Figure 1, as an example) as well as by size-exclusion chromatography (SEC) coupled with both refractive index (RI) and LLS detection systems (see Figure 2, as an example). $^1$H NMR made it possible to verify the absence of residual comonomers, particularly glycomonomer, in the purified glycopolymers. The ratio of resonance signal intensities due to methyl protons from *N*-acetyl groups (2.0 ppm) and methine protons (2.1−2.4 ppm) from the hydrocarbon skeleton allowed determination of monosaccharide content. It is noteworthy that the spacer-arm length of $\omega$-alkenyl glycomonomer influenced the copolymerization behavior. Indeed, the proportion of incorporated carbohydrate in the final copolymer increased with decreasing spacer-arm length from *n*-nonyl (C-9) to *n*-propyl (C-3). This suggests that C-3 spacer-armed glycomonomers have a higher reactivity than their C-9 homologues.

It is most noteworthy that the degree of control over the macromolecular structure is significantly better with cyanoxyl-mediated free-radical polymerization than that observed by resorting to classical free-radical processes.[30] Polydispersity indexes for all copolymers remained below 1.5, which defines a theoretical lower limit for a conventional free-radical mechanism. Moreover, regardless of the glycomonomer used and the initial GM/AM molar ratio, monosaccharide contents in the resulting glycopolymers as well as their molar masses increased with comonomer conversion. As previously shown by Grande and Gnanou,[25a] cyanoxyl persistent radicals impart some "livingness" to the polymerization process by scavenging growing radicals and forming dormant species reversibly. In this manner, polymer chains can grow with reaction time, and an increasing number of carbohydrate monomeric units could potentially be incorporated within the copolymer structure. Yet, it should be stressed that, contrary to a truly "living"/controlled radical polymerization, cyanoxyl-terminated samples of predetermined molar masses cannot be designed by ending the polymerization reaction at a certain conversion. Indeed, actual molar masses ($M_{n,SEC}$ obtained from SEC/RI/LLS) were sys-

**Table 1. Statistical Free-Radical Copolymerization of AM with Alkene-Derivatized Unprotected 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 | 38 | 24 100 | 1.46 |
| | | 16 | 30 | 1/5 | 50 | 43 000 | 1.47 |
| | 1/20 | 1.5 | 23 | 1/90 | 04 | 94 000 | 1.17 |
| | | 16 | 30 | 1/70 | 05 | 112 100 | 1.20 |
| **1**(C-9) | 1/4 | 1.5 | 15 | 1/16 | 25 | 43 400 | 1.25 |
| | | 16 | 20 | 1/6 | 46 | 99 300 | 1.45 |
| | 1/20 | 1.5 | 21 | 1/166 | 03 | 25 800 | 1.14 |
| | | 16 | 29 | 1/100 | 05 | 28 200 | 1.24 |
| **5**(C-3) | 1/4 | 1.5 | 21 | 1/10 | 44 | 16 100 | 1.13 |
| | | 16 | 35 | 1/6 | 55 | 57 300 | 1.37 |
| | 1/20 | 1.5 | 35 | 1/93 | 07 | 25 400 | 1.10 |
| | | 16 | 51 | 1/58 | 11 | 47 200 | 1.29 |
| **5**(C-9) | 1/4 | 16 | 26 | 1/10 | 45 | 57 200 | 1.20 |
| | 1/20 | 16 | 11 | 1/42 | 17 | 16 300 | 1.17 |

[a] $T$ = 50 °C, $[M]_0 = [GM]_0 + [AM]_0$ = 1 mol/L, $[I]_0 = [ClC_6H_4N{\equiv}N^+BF_4^-]_0 = [NaOCN]_0$ = 0.02 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/RI/LLS.

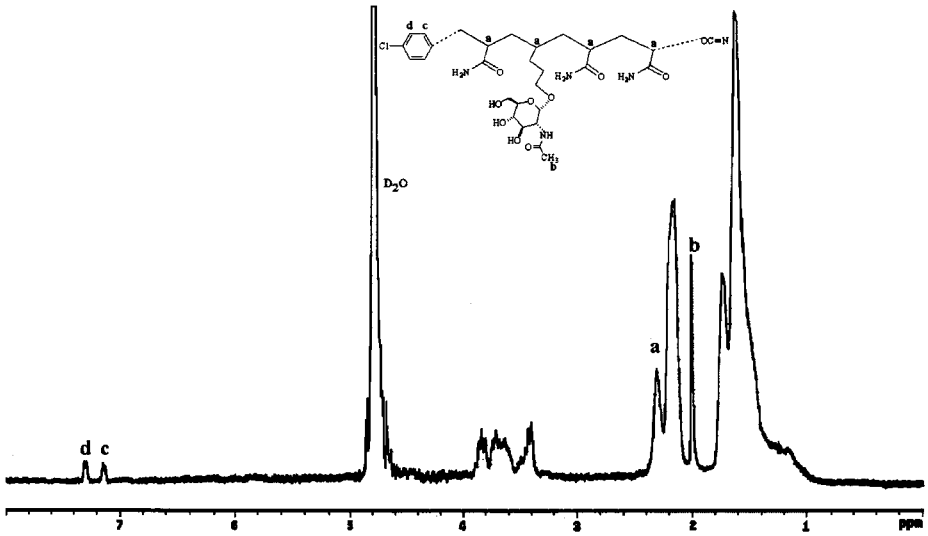



**Figure 1.** $^1$H NMR ($D_2O$) spectrum of a **1**(C-3)/AM glycopolymer sample (**1**(C-3)/AM = 1/16 (mol) **1**(C-3): 20 wt %, $M_{n,NMR}$ = 3700 g/mol, $M_{n,SEC}$ = 4800 g/mol).

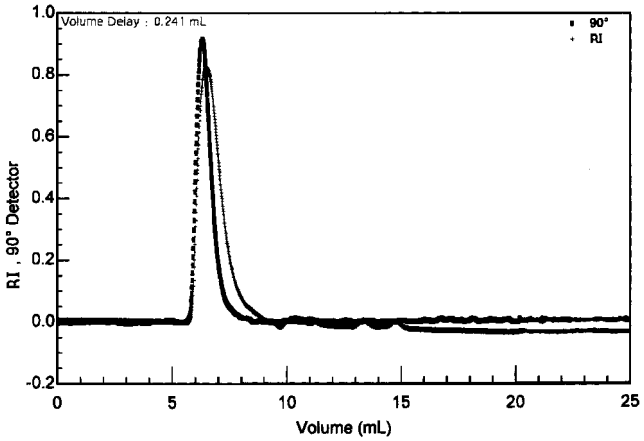



**Figure 2.** SEC/RI/LLS chromatogram of a **1**(C-3)/AM glycopolymer sample ($M_n$ = 112 100 g/mol, $M_w/M_n$ = 1.20).

tematically much higher than theoretical values ($M_{n,th} = M_0([M]_0/[I]_0)\pi$, where $M_0$ stands for the molar mass of a monomeric unit and $\pi$ for monomer conversion, assuming a complete initiator efficiency of $f$ = 1). Likewise, a large discrepancy between experimental and targeted molar masses has been previously observed for the cyanoxyl-mediated polymerization of (meth)acrylic monomers.[25a] We presume that a large proportion of the moderately reactive phenyl-type initiating radicals are lost in irreversible primary termination reactions at the initial stages of the polymerization process, thus decreasing initiator efficiency ($f$). Values as low as 0.1 were indeed estimated for this parameter by taking the ratio of theoretical to actual molar masses ($f = M_{n,th}/M_{n,SEC}$). On the other hand, it is also interesting to point out that the use of an initial GM/AM molar ratio of $^1/_4$ enabled the design of a copolymer that exhibited a monosaccharide content in close agreement with that expected, after 16 h of reaction. Mass compositions of sugar residues as high as 50 wt % were thus reached. Nonetheless, a higher carbohydrate content in the resulting copolymers was associated with a broadening of the molar mass distributions ($M_w/M_n$). This is probably attributable to some loss of control over the copolymerization process in the presence of increasing amounts of $\omega$-alkenyl glycomonomers, considering the innate low chemical reactivity of the unactivated vinyl group in these saccharide monomers.

The low level of polymerizability associated with alkene-derivatized monosaccharides in free-radical processes, and particularly in cyanoxyl-mediated polymerization, implies two major limitations. First, that the

**Table 2. Free-Radical Homopolymerization of Acrylic Unprotected Glycomonomers and Statistical Copolymerization with AM Using $ClC_6H_4N{\equiv}N^+BF_4^-$/NaOCN as Initiating System**

| glycomonomer (GM) | monomer ratio GM/AM (mol) | time (h) | yield[e] (%) | polymer composition[f] (mol) | monosaccharide content (wt %) | $M_n$[g] (g/mol) | $M_w/M_n$ SEC |
|---|---|---|---|---|---|---|---|
| **3**[a] | 1/0 | 4 | 25 | 1/0 | 100 | 15 400 | 1.26 |
| **3**[b] | 1/0 | 1.5 | 40 | 1/0 | 100 | 77 100 | 1.45 |
| | | 4 | 70 | 1/0 | 100 | 127 000 | 1.56 |
| **3**[b] | 1/1 | 1.5 | 88 | 1/1.5 | 75 | 47 400 | 1.57 |
| | 1/4 | 1.5 | 33 | 1/7 | 40 | 30 600 | 1.35 |
| | | 4 | 93 | 1/6 | 43 | 51 700 | 1.55 |
| **6**[c] | 1/0 | 4 | 35 | 1/0 | 100 | 9900 | 1.13 |
| **6**[d] | 1/0 | 4 | 25 | 1/0 | 100 | 20 800 | 1.49 |
| **6**[c] | 1/1 | 1.5 | 20 | 1/4 | 66 | 48 000 | 1.45 |
| | | 4 | 25 | 1/2 | 80 | 59 500 | 1.47 |
| | 1/4 | 1.5 | 95 | 1/9 | 42 | 115 600 | 1.57 |

[a] $T = 50$ °C, $[M]_0 = [GM]_0 + [AM]_0 = 1$ mol/L, $[I]_0 = [ClC_6H_4N{\equiv}N^+BF_4^-]_0 = [NaOCN]_0 = 0.1$ mol/L. [b] $T = 50$ °C, $[M]_0 = 1$ mol/L, $[I]_0 = 0.02$ mol/L. [c] $T = 50$ °C, $[M]_0 = 0.5$ mol/L, $[I]_0 = 0.05$ mol/L. [d] $T = 50$ °C, $[M]_0 = 0.5$ mol/L, $[I]_0 = 0.01$ mol/L. [e] Total monomer conversion as determined by gravimetry. [f] Molar ratio of monosaccharide to acrylamide monomeric units in the resulting copolymer as determined by $^1$H NMR. [g] $M_n$ obtained from SEC/RI/LLS.

yield of copolymers with AM is low even after a reaction time of 16 h and second, that homopolymers cannot be derived from the polymerization of these glycomonomers, as confirmed by further investigation. Consequently, nonsulfated and sulfated acrylate-based glycomonomers were synthesized. As summarized in Table 2, cyanoxyl-mediated homopolymerization of these monosaccharides could be achieved with some degree of control. This is illustrated by the low polydispersity indexes ($1.13 < M_w/M_n < 1.56$) observed for nonsulfated and sulfated homo-glycopolymers. Samples of different molar masses were also prepared by varying either monomer conversion or the initial ratio of monomer to initiator concentrations ($[M]_0/[I]_0$). Acrylic glycomonomers were also copolymerized with AM to yield low-polydispersity (∼1.5) statistical copolymers with saccharide contents as high as 75 wt %. Because of the much higher reactivity of acrylic monomers compared with that of their $\omega$-alkenyl counterparts, higher conversions were reached within 4 h, or even 1.5 h in some instances (Table 2). However, polydispersity indexes of both nonsulfated and sulfated acrylic glycopolymers were generally higher than those obtained for alkene-derivatized homologues. Presumably, this arises from the nonnegligible contribution of irreversible bimolecular termination reactions that characterizes free-radical polymerization of acrylates. The presence of cyanoxyl radicals as chain-growth moderators, nonetheless, makes it possible to minimize the extent of these side reactions.

## Conclusions

We have synthesized a series of model *N*-acetyl-D-glucosamine-carrying unprotected glycomonomers as an initial step in the design of glycosaminoglycan mimetic architectures. The present study broadens the family of vinyl monomers that are polymerizable with some degree of control by cyanoxyl-mediated free-radical polymerization. Both nonsulfated and sulfated alkene-derivatized as well as acrylate-derivatized monosaccharides were polymerized using this straightforward methodology, which demonstrates the effectiveness and versatility of this approach for preparing water-soluble glycopolymers with high carbohydrate contents and low polydispersity indexes ($1.1 < M_w/M_n < 1.6$). We believe that tailored glycopolymers will contribute to the progress of glycotechnology, including the design of nonthrombogenic biomaterials that enhance tissue regeneration and wound healing responses.

**Acknowledgment.** This work was supported by grants from the NIH. The authors acknowledge the Emory University NMR and Mass Spectrometry Centers for their facilities. They are also indebted to Mrs. Chitra Baskaran and Dr. Suri Iyer for their kind technical assistance.

## References and Notes

(1) Clowes, A. W.; Karnovsky, M. J. *Nature* **1977**, *265*, 625.
(2) Jackson, R. L.; Busch, S. J.; Cardin, A. L. *Physiol. Rev.* **1991**, *71*, 481.
(3) Linhardt, R. J.; Kerns, R. J.; Vlahov, I. R. In *Biomedical Functions and Biotechnology of Natural and Artificial Polymers*; ATL Press: 1996; p 45.
(4) Kjellen, L.; Lindahl, U. *Annu. Rev. Biochem.* **1991**, *60*, 443.
(5) Faham, S.; Hileman, R. E.; Fromm, J. R.; Linhardt, R. J.; Rees, D. C. *Science* **1996**, *271*, 1116.
(6) Bitomsky, W.; Wade, R. C. *J. Am. Chem. Soc.* **1999**, *121*, 3004.
(7) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.
(8) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692. (b) Roy, R. *Top. Curr. Chem.* **1997**, *187*, 241.
(9) Van Boeckel, C. A. A.; Petitou, M. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1671. (b) Westerduin, P.; Basten, J. E. M.; Broekhoven, M. A.; De Kimpe, V.; Kuijpers, W. H. A.; Van Boeckel, C. A. A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 331. (c) Petitou, M.; Duchaussoy, P.; Driguez, P.-A.; Jaurand, G.; Hérault, J.-P.; Lormeau, J.-C.; Van Boeckel, C. A. A.; Herbert, J.-M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 3009. (d) Petitou, M.; Hérault, J.-P.; Bernat, A.; Driguez, P.-A.; Duchaussoy, P.; Lormeau, J.-C.; Herbert, J.-M. *Nature* **1999**, *398*, 417.
(10) Westman, J.; Nilsson, M.; Ornitz, D. M.; Svahn, C.-M. *J. Carbohydr. Chem.* **1995**, *14*, 95. (b) Ornitz, D. M.; Herr, A. B.; Nilsson, M.; Westman, J.; Svahn, C.-M.; Waksman, G. *Science* **1995**, *268*, 432.
(11) Lee, Y. C. In *Synthetic Oligosaccharides: Indispensable Probes for the Life Sciences*; ACS Symposium Series 560; American Chemical Society: Washington, DC, 1994; Chapter 1, p 6.
(12) Dimick, S. M.; Powell, S. C.; McMahon, S. A.; Moothoo, D. N.; Naismith, J. H.; Toone, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 10286.
(13) Suda, Y.; Koshida, S.; Arano, A.; Morichika, T.; Fukui, Y.; Kusumuto, S.; Sobel, M. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **2000**, *41* (2), 1624.
(14) Miyata, T.; Nakamae, K. *Trends Polym. Sci.* **1997**, *5*, 198.
(15) Aoi, K.; Suzuki, H.; Okada, M. *Macromolecules* **1992**, *25*, 7073. (b) Aoi, K.; Tsutsumiuchi, K.; Okada, M. *Macromolecules* **1994**, *27*, 875.
(16) Fraser, C.; Grubbs, R. H. *Macromolecules* **1995**, *28*, 7248.
(17) Nomura, K.; Schrock, R. R. *Macromolecules* **1996**, *29*, 540.
(18) Minoda, M.; Yamaoka, K.; Yamada, K.; Takaragi, A.; Miyamoto, T. *Macromol. Symp.* **1995**, *99*, 169. (b) Yamada, K.; Yamaoka, K.; Minoda, M.; Miyamoto, T. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 255. (c) Yamada, K.; Minoda, M.; Miyamoto, T. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*,

751. (d) Yamada, K.; Minoda, M.; Miyamoto, T. *Macromolecules* **1999**, *32*, 3553.
(19) Loykulnant, S.; Hayashi, M.; Hirao, A. *Macromolecules* **1998**, *31*, 9121. (b) Loykulnant, S.; Hirao, A. *Macromolecules* **2000**, *33*, 4757.
(20) Ohno, K.; Tsujii, Y.; Miyamoto, T.; Fukuda, T.; Goto, M.; Kobayashi, K.; Akaike, T. *Macromolecules* **1998**, *31*, 1064. (b) Ohno, K.; Tsujii, Y.; Fukuda, T. *Macromol. Chem. Phys.* **1998**, *199*, 2193.
(21) Ohno, K.; Izu, Y.; Yamamoto, S.; Miyamoto, T.; Fukuda, T. *Macromol. Chem. Phys.* **1999**, *200*, 1619.
(22) Ohno, K.; Tsujii, Y.; Fukuda, T. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 2473. (b) Ejaz, M.; Ohno, K.; Tsujii, Y.; Fukuda, T. *Macromolecules* **2000**, *33*, 2870.
(23) Bon, S. A. F.; Haddleton, D. M. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1999**, *40* (2), 248. (b) Marsh, A.; Khan, A.; Haddleton, D. M.; Hannon, M. J. *Macromolecules* **1999**, *32*, 8725.
(24) Druliner, J. D. *Macromolecules* **1991**, *24*, 6079. (b) Druliner, J. D.; Fryd, M. U.S. Patent 5,059,657, 1991.
(25) Grande, D. Ph.D. Thesis, Université Bordeaux-I (France)/ Universidad Autónoma de Coahuila (Mexico), 1998. (b) Gnanou, Y.; Grande, D.; Guerrero, R. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1999**, *40* (2), 99.
(26) Nishimura, S.-I.; Matsuoka, K.; Kurita, K. *Macromolecules* **1990**, *23*, 4182. (b) Nishimura, S.-I.; Matsuoka, K.; Furuike, T.; Ishii, S.; Kurita, K. *Macromolecules* **1991**, *24*, 4236.
(27) Peter, M. G.; Boldt, P. C.; Peterson, S. *Liebigs Ann. Chem.* **1992**, 1275.
(28) Udodong, U. E.; Rao, C. S.; Fraser-Reid, B. *Tetrahedron* **1992**, *48*, 4713.
(29) Konradsson, P.; Roberts, C.; Fraser-Reid, B. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 23.
(30) Grande, D.; Baskaran, S.; Chaikof, E. L. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **2000**, *41* (1), 1000. (b) Grande, D.; Baskaran, S.; Baskaran, C.; Gnanou, Y.; Chaikof, E. L. *Macromolecules* **2000**, *33*, 1123. (c) Grande, D.; Baskaran, S.; Chaikof, E. L. *Polym. Mater. Sci. Eng.* **2001**, *84*, in press.
(31) Kitazawa, S.; Okumura, M.; Kinomura, K.; Sakakibara, T. *Chem. Lett.* **1990**, 1733.

MA001680T