First Synthesis of Glycopeptide Macromonomers and Graft-Type Sugar-Containing Polymers with Glycopeptide Side Chains

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This paper deals with the first synthesis of well-defined glycopeptide macromonomers by living ring-opening polymerization of sugar-substituted α-amino acid *N*-carboxyanhydrides (NCAs). Glycopeptide macromonomers are of great importance, since they provide a variety of carbohydrate polymers with sugar residues having molecular information and biological function. Actually, development of a new class of artificial glycoconjugates with glycopeptide side chains, which show high recognition ability to a protein receptor, is achieved by copolymerization using glycopeptide macromonomers.

Glycoproteins comprise several essential classes of macromolecules such as enzymes, hormones, immunoglobulins, cell adhesion molecules, and transport proteins, exhibiting various biological activities. The interest in the synthetic glycoconjugates, especially glycopeptide-containing polymers, has risen tremendously in the past decade, together with the progress in the field of glycotechnology.[1] We have already proposed a new general methodology to synthesize stereoregular glycopeptides by the NCA method.[2] A glycopeptide-containing AB-type block copolymer has also been reported.

Structural control of glycopolymers has a significant meaning in their recognition functions. Fully sugar-substituted globular dendrimers, "Sugar Balls", have been created based on three-dimensional architectures of synthetic glycoconjugates.[3] The structure−recognition ability relationship has been investigated in a system between sugar balls and lectins. Versatile synthetic utilities of living polymerization have been demonstrated for design of well-characterized carbohydrate-bearing biofunctional materials.[2,4] Recently, glycopolymers of controlled molecular weight have been prepared by ring-opening metathesis polymerization of sugar-substituted norbornenes.[5] In the present study, glycopeptide macromonomers and related graft-type glycopolymers were designed with motivation from arranged terminal sugar residues of natural multiantennary oligosaccharides. Glycopeptide macromonomers should be important as a building block of artificial arrayed sugar-bearing units. Graft copolymers having monodisperse glycopeptide side chains have not been hitherto reported to our knowledge.

Glycopeptide macromonomers with an α-styryl group were synthesized by using D-glucose and *N*-acetyl-D-glucosamine derivative-carrying monomers, i.e., *O*-(tetra-*O*-acetyl-*β*-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1a**) and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-serine *N*-carboxyanhydride (**1b**).[2,6] Significant functions of glycosides of glycopeptides are currently known. Intracellular *O*-linked *N*-acetyl-D-glucosamine is, for example, implicated in cellular stimuli[7] and in the pathogenesis of Alzheimer's disease.[8] Ring-opening polymerization of **1** with *p*-vinylbenzylamine as an initiator was carried out in dry dichloromethane under a nitrogen atmosphere (Scheme 1).[9,10] The structure of macromonomer **2** was determined by ^{1}H, ^{13}C NMR, and IR measurements.[9] No side reaction such as production of hydantoic acid was observed.[11] A sole unimodal peak was detected by direct size exclusion chromatography (SEC) of a reaction mixture at the end of polymerization. Results of polymerization are summarized in Table 1. Degrees of polymerization (DPs) were almost regulated by the monomer/initiator feed molar ratios. SEC analysis indicated the relatively narrow molecular weight distribution of **2**, taking a Poisson distribution into account. The oligomer **2a** obtained in entry nos. 1 and 4 showed M_w/M_n values smaller than the theoretical values, which is probably caused by the removal of the low molecular weight fraction by a reprecipitation procedure. We have already reported that polymerization of **1a** with *n*-hexylamine proceeds without chain termination and transfer.[2] In the present system, the nucleophilicity of *p*-vinylbenzylamine is somewhat lower than that of *n*-hexylamine. However, initiation is sufficiently fast to control their DPs, since initiation in entry no. 3 was nearly complete within 5 min and then propagation proceeded gradually for over 100 h.[12]

Table 1. Preparation of Glycopeptide-Based Macromonomer 2[a]

	monomer			product			
entry no.		[monomer]$_0$/[initiator]$_0$	time, h	yield, %	$\bar{M}_n$[b]	$\overline{DP}$[b]	$\bar{M}_w/\bar{M}_n$[c]
1	**1a**	5.0	24	95.4	2090	4.7	1.06
2	**1a**	10.7	48	99.7	4820	11.2	1.09
3	**1a**	31.7	120	99.2	13900	33.0	1.07
4	**1b**	4.0	24[d]	77.8	1840[e]	4.1[e]	1.18

[a] Initiator, *p*-vinylbenzylamine; solvent, CH_2Cl_2; [monomer]$_0$, 0.10 mol/L; temperature, 27 °C; under nitrogen. [b] Determined by the ^{1}H NMR spectra in $CDCl_3$ at 50 °C. [c] Estimated from the SEC curve, using standard polystyrenes for calibration (in $CHCl_3$ at 38 °C). [d] At 25 °C. [e] In CD_3OD at 25 °C.

A glycopeptide macromonomer **3** was derived from **2** as shown in Scheme 2. Deacetylation of **2a** was performed by using hydrazine monohydrate in methanol at 0 °C to afford glycopeptide macromonomer **3a** in 81.5% yield.[13] The quantitative deprotection was confirmed by ^{1}H, ^{13}C NMR, and IR spectra. The terminal vinyl group did not change during the deacetylation procedure.[13]

Water-soluble glycopeptide-containing polymeric materials are expected to be useful in medical and biomedical applications.[1−4] A new type of synthetic glycoconjugate **5** with glycopeptide side chains was prepared by copolymerization of **3** and acrylamide (path A in Scheme 2). Radical copolymerization between **3a** and acrylamide was carried out with 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPD) as an initiator in water at 55 °C.[14] Results are listed in Table 2. Graft copolymer **5** was also derived from macromonomer **2** via protected glycopeptide-carrying graft copolymer **4** as illustrated in path B of Scheme 2. Judging from the data of Table 2, the macromonomer **2a** was efficiently introduced into the copolymer due to the higher reactivity of the styryl group than that of acrylamide. Deacetylation of **4a** using hydrazine monohydrate was readily

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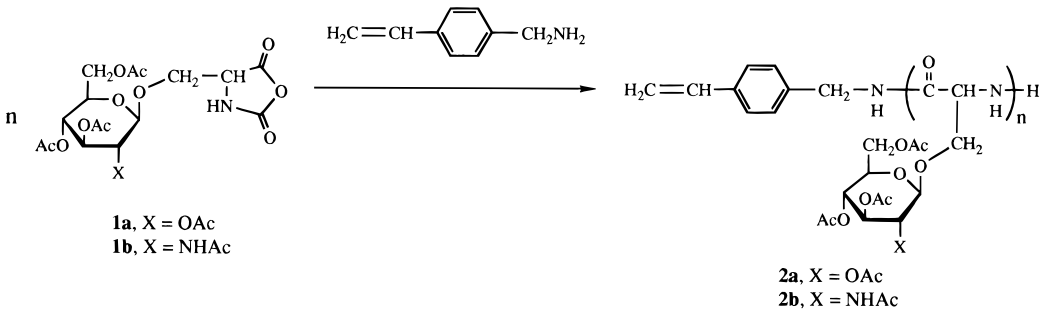
Table 2. Copolymerization of Glycopeptide-Based Macromonomers and Acrylamide

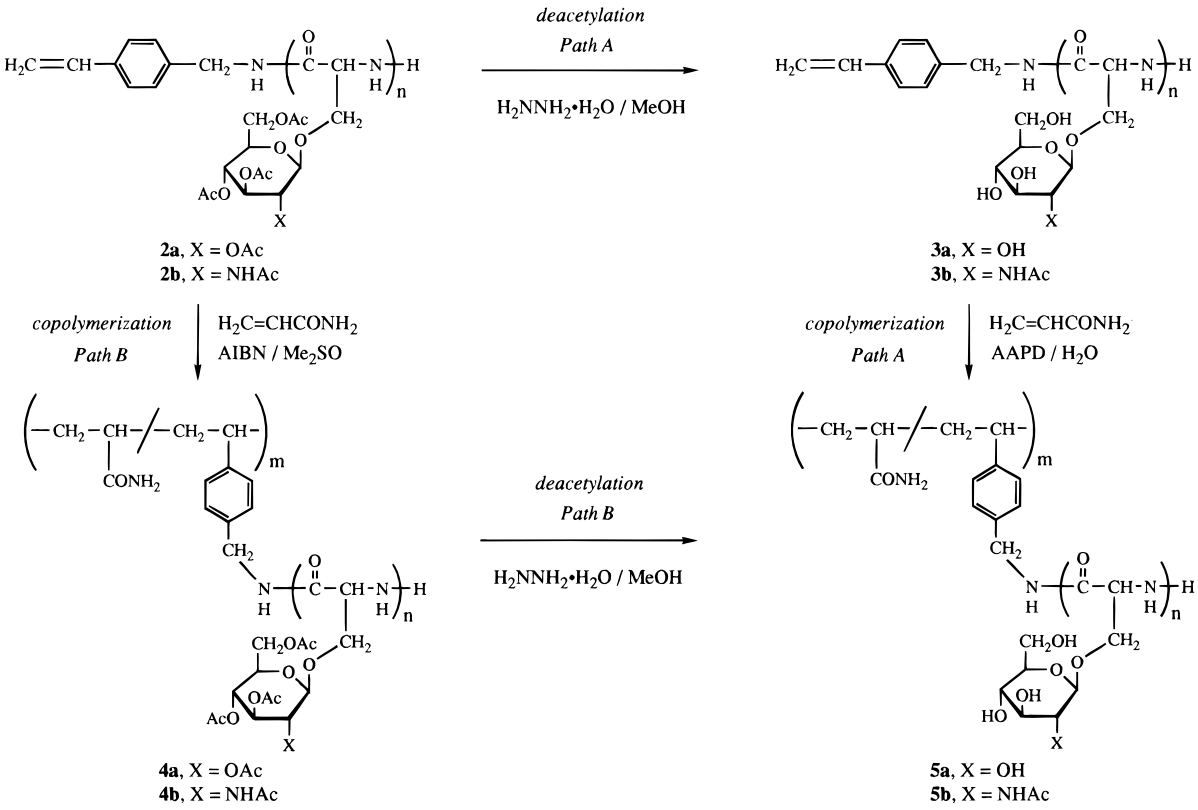
	macromonomer[a]			feed ratio [macromonomer]$_0$/[acrylamide]$_0$	initiator[d]	solvent	temp, °C	time, h	product graft copolymer		
		$\overline{DP}$[b]	$\bar{M}_w/\bar{M}_n$[c]							yield, %	unit ratio[e] macromonomer/acrylamide
path A[a]	**3a**	4.1[f]	1.04[g]	0.010	AAPD[h]	H_2O[i]	55	0.5	**5a**	68[m]	0.015[f]
path B[a]	**2a**	4.7	1.06	0.010	AIBN	Me_2SO[j]	60	15	**4a**	91[n]	0.010
path B[a]	**2a**	11.2	1.09	0.010	AIBN	Me_2SO[k]	60	3.3	**4a**	13	0.045
path B[a]	**2a**	11.2	1.09	0.010	AIBN	Me_2SO[k]	60	15	**4a**	63	0.013
path B[a]	**2b**	4.1	1.18	0.010	AIBN	Me_2SO[l]	60	15	**4b**	59	0.0077[o]

[a] See Scheme 2. [b] Determined by the ^{1}H NMR spectra in $CDCl_3$ at 50 °C. [c] Measured by SEC in $CHCl_3$ at 38 °C (polystyrene standard). [d] [Initiator]$_0$/[acrylamide]$_0$ = 0.0050. [e] Determined by the ^{1}H NMR spectra in CF_3CO_2H at 27 °C. [f] In D_2O at 27 °C. [g] In Me_2SO at 27 °C. [h] 2,2′-Azobis(2-amidinopropane) dihydrochloride. [i] Acrylamide]$_0$ = 1.5 mol/L. [j] [Acrylamide]$_0$ = 1.3 mol/L. [k] [Acrylamide]$_0$ = 0.50 mol/L. [l] [Acrylamide]$_0$ = 2.0 mol/L. [m] [η] = 13 dL/g (in H_2O at 25 °C). [n] [η] = 0.85 dL/g (in H_2O at 25 °C). [o] In D_2O at 25 °C.

Scheme 1



Scheme 2



performed in methanol at 20 °C for 12 h to give artificial glycoconjugate **5a**. Similarly, **5b** was also derived from **4b**.

In order to clarify the molecular recognition ability, inhibition activities of erythrocyte agglutination by wheat germ agglutinin (WGA) lectin was examined with multivalent glycopeptide-carrying polymer **5b** and control monovalent *N*-acetyl-D-glucosamine. WGA is known to have several sugar-binding sites,[15] which specifically recognize *N*-acetyl-D-glucosamine, *N*,*N*′-diacetylchitobiose, and *N*,*N*′,*N*″-triacetylchitotriose. Extracellular carbohydrates of erythrocyte interact with the lectin to make agglutination. In the hemagglutination inhibition assay,[16] *N*-acetyl-D-glucosamine did not inhibit aggregate formation between erythrocyte and WGA up to the *N*-acetyl-D-glucosamine concentration of 2.3×10^{-2} mol/L. On the other hand, the minimum concentration of **5b** to inhibit agglutination was as low as 4.9×10^{-5} mol/L based on a monomeric sugar unit. Highly efficient interaction between **5b** and WGA is apparently

ascribed to the multivalency of the sugar moieties of a **5b** molecule.

The living character of the polymerization of sugar-carrying NCA monomers **1** offered key glycopeptide macromonomers and then new diverse directions of glycopeptide-containing macromolecular design. These glycopeptide-based materials should contribute to rapid progress in broad areas including biology, medical, and the life sciences.

References and Notes

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(6) **1b** was prepared according to previous papers: (a) Rüde, E.; Meyer-Delius, M. *Carbohydr. Res.* **1968**, *8*, 219. (b) Garg, H. G.; Jeanloz, R. W. *Carbohydr. Res.* **1976**, *49*, 482.

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(9) Ring-opening polymerization of **1a** with *p*-vinylbenzylamine was carried out as follows. In a test tube with a three-way stopcock was suspended 440 mg (0.953 mmol) of **1a** in 8.86 mL of dichloromethane, followed by the addition of 4.0 mg (0.0030 mmol) of *p*-vinylbenzylamine. The mixture was stirred at 27 °C under nitrogen. The product polymer **2a** was obtained by precipitation from diethyl ether and then purified further by repeated reprecipitation from chloroform to diethyl ether. After drying in vacuo, 374 mg of **2a** was isolated (99.2% yield). **2a**: IR ($CHCl_3$ solution) 3345 (ν_{N-H}), 2950 (ν_{C-H}), 1760 ($\nu_{C=O}$(ester)), 1660 ($\nu_{C=O}$(amide)), 1500 (δ_{N-H}), 1230 ($\nu_{C-C(=O)-O}$), 1045 (ν_{C-O-C}) cm^{-1}; ^1H NMR ($CDCl_3$, 50 °C, 270 MHz) δ 1.96−2.09 (methyl protons of acetyl groups), 3.66−4.43 (H-5, H-6 of the pyranose ring, and H-α, H-β of poly(L-serine)), 4.61−5.23 (H-1, H-2, H-3, H-4 of the pyranose ring, $C_6H_4CH_2$, and HC*H*=CHC$_6$H$_4$-(*trans*)), 5.73 (*H*CH=CHC$_6$H$_4$(*cis*)), 6.72 (CH_2=C*H*C$_6$H$_4$), 7.36 (C_6H_4), 7.48 (NH); ^{13}C NMR ($CDCl_3$, 25 °C, 100 MHz) δ 20.6 (methyl carbons of acetyl groups), 43.2 ($C_6H_4CH_2$), 52.9 (α-carbon of poly(L-serine)), 62.1 (CH_2OAc), 68.5 (C-4 of the pyranose ring), 71.2 (C-5 of the pyranose ring and the methylene carbon of poly(L-serine)), 72.0 (C-2 of the pyranose ring), 72.9 (C-3 of the pyranose ring), 102.1 (C-1 of the pyranose ring), 113.5 (CH_2=CH), 126.5 and 127.7 (ortho, meta positions of aromatic carbons), 130.2 (CH_2=CH*C*), 136.8 (CH_2=*C*H), 138.0 (*C*CH$_2$NHCO), 168.7−170.9 (carbonyl carbons).

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(12) Monomer conversion was easily estimated by the characteristic absorptions of NCA (1800 and 1860 cm^{-1}) in the IR spectra. ^1H NMR analysis also supported the observation.

(13) Deacetylation of **2a** (a fractionated sample of entry 1, DP = 4.0, M_w/M_n = 1.02, 153 mg, 0.0842 mmol) was carried out in 13.3 mL of methanol at 0 °C by adding 340 mg (6.79 mmol) of hydrazine monohydrate dropwise. After 55 h at 0 °C, 680 mg (13.6 mmol) of acetone was added to quench hydrazine. The mixture was evaporated, and then the product was purified by repeated reprecipitations from water to ethanol. The yield was 78.2 mg (81.5%, DP = 4.1, M_w/M_n = 1.04). **3a**: IR (KBr disk) 3355 (ν_{O-H}, ν_{N-H}), 2910 (ν_{C-H}), 1655 ($\nu_{C=O}$ (amide)), 1535 (δ_{N-H}), 1080, 1040 (ν_{C-O-C}) cm^{-1}; ^1H NMR (D_2O, 27 °C, 270 MHz) δ 3.09−4.66 ($C_6H_4CH_2$ and protons of the pyranose ring and poly(L-serine), 5.29 (HC*H*=CHC$_6$H$_4$(*trans*)), 5.83 (*H*CH=CHC$_6$H$_4$(*cis*)), 6.79 (CH_2=C*H*C$_6$H$_4$), 7.25−7.49 (C_6H_4); ^{13}C NMR (D_2O, 27 °C, 67.8 MHz) δ 45.2 ($C_6H_4CH_2$), 56.1 (α-carbon of poly(L-serine)), 63.2 (CH_2OH), 72.2 (C-4 of the pyranose ring and the methylene carbon of poly(L-serine)), 75.5 (C-5 of the pyranose ring)), 78.0 (C-2 of the pyranose ring), 78.4 (C-3 of the pyranose ring), 105.0 (C-1 of the pyranose ring), 116.9 (CH_2=CH), 129.0 and 129.9 (ortho, meta positions of aromatic carbons), 138.8 (CH_2=CH*C*), 139.2 (CH_2=*C*H), 139.7 (*C*CH$_2$NHCO), 173.7 (carbonyl carbons), 177.1 (C$_6$H$_4$-CH$_2$NH*C*O).

(14) In an ampule, 103 mg (1.45 mmol) of acrylamide and 16.7 mg (0.0147 mmol) of **3a** (DP = 4.1, M_w/M_n = 1.04) were dissolved in 1.00 mL of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPD) aqueous solution (7.34 mmol/L). Freezing and degassing of the solution were repeated, and the ampule was sealed. Polymerization was carried out at 55 °C for 30 min. The product was purified by the dialysis. After lyophilization, 81.9 mg of **5a** was obtained (68.2% yield). **5a**: IR (KBr disk) 3400 (ν_{O-H} , ν_{N-H}), 2950 (ν_{C-H}), 1665 ($\nu_{C=O}$(amide)), 1610 (δ_{N-H}), 1080, 1040 (ν_{C-O-C}) cm^{-1}; ^1H NMR (D_2O, 50 °C, 270 MHz) δ 1.61 (CH_2 of main chain), 2.17 (CH of main chain), 3.09−4.66 ($C_6H_4CH_2$ and protons of the pyranose ring and poly(L-serine)), 7.18 (C_6H_4); ^{13}C NMR (D_2O, 50 °C, 100 MHz) δ 36.8−38.7 (CH_2 of main chain), 44.2−45.0 (CH of main chain), 56.3 (α-carbon of poly-(L-serine)), 63.6 (CH_2OH), 71.3 (methylene carbon of poly-(L-serine)), 72.4 (C-4 of the pyranose ring), 75.8 (C-5 of the pyranose ring)), 78.4 (C-2 of the pyranose ring), 78.7 (C-3 of the pyranose ring), 105.2 (C-1 of the pyranose ring), 173.5 (carbonyl carbon of poly(L-serine)), 182.1 (carbonyl carbon of acrylamide units).

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(16) The agglutination inhibition assays were carried out according to the literature: Osawa, T.; Matsumoto, I. *Methods Enzymol.* **1972**, *28*, 323. The minimum inhibition concentration of **5b** or *N*-acetyl-D-glucosamine was determined within 2-fold serial dilutions. Wheat germ agglutinin (WGA) (Sigma Chemical Co.) was used at a final concentration of 2.0×10^{-4} g/L in a phosphate buffer solution. Substrates were incubated for 1 h at 25 °C prior to the addition of an actinase E treated human erythrocyte suspension (blood type A). The mixture was kept for 12 h at 25 °C and then examined for hemagglutination.

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