Synthetic Glycoconjugates. 6.[1] Preparation and Biochemical Evaluation of Novel Cluster-Type Glycopolymers Containing Gal β(1→4)GlcNAc (*N*-Acetyllactosamine) Residue

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ABSTRACT: A simple and facile method for the syntheses of cluster-type homopolymers from ω-(acrylamido)alkyl glycosides of *N*-acetyllactosamine [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc] is described. An efficient procedure for the preparation of poly[ω-(acrylamido)alkyl *O*-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside] was established on the basis of radical polymerization of the novel glycosides in the presence of ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine. These synthetic cluster glycopolymers exhibited good solubility in water and had sugar densities much higher than the glycopolymers derived from known *n*-pentenyl glycosides. The association constants of *Erythrina corallodendron* lectin (ECorL) with these cluster glycopolymers were evaluated by measuring the changes in fluorescence intensity of fluorescent-labeled ECorL induced by the addition of the polymeric ligands. Addition of the cluster-type glycopolymers having different degrees of *N*-acetyllactosamine density to ECorL induced a significant decrease of the fluorescence intensity at 520 nm, and this phenomenon was used for the determination of binding constants between the glycopolymers and ECorL.

Introduction

Major oligosaccharides of asparagine-linked-type glycoproteins existing on the cell surfaces are known as multi-antennary chains such as high mannose-, complex-, and hybrid-type structures. It is suggested that the branching structure greatly contributes to the appearance in a variety of specific cell−cell interactions. A significance of sugar density regulated by the multiplicity of sugar chains on the glycoproteins was first proposed and reported by Lee et al. on the basis of chemically designed cluster glycosides[2] or neoglycoproteins.[3] The importance of sugar cluster effects has been widely demonstrated by using synthetic multivalent ligands and neoglycoconjugates.[4] Moreover, this concept may be applicable for creating a novel class of specific drugs based on sugar−protein or sugar−sugar interactions.[5,6]

In the preceding papers, we have reported an efficient method for the syntheses of biochemically useful water-soluble polymers containing a variety of oligosaccharides by radical copolymerization of *n*-pentenyl glycosides with acrylamide.[1,7−9] This method has been widely applicable for the preparation of glycopolymers having sugar residues such as *N*-acetyl-D-glucosamine (Glc*p*NAc),[7] *N,N′*-diacetylchitobiose [Glc*p*NAcβ(1→4)Glc*p*NAc],[8] *N*-acetyllactosamine [Gal*p*β(1→4)Glc*p*NAc],[8] and Le^x-type trisaccharide {Gal*p*β(1→4)[Fuc*p*α(1→3)]Glc*p*NAc} known as a sequence of tumor-associated carbohydrate antigens.[9] It was also demonstrated that chemically synthesized glycopolymers are enzymatically modified to afford sialic acid-containing glycoconjugates.[10] Using a variety of *N*-acetyllactosamine-related polymers from *n*-pentenyl glycosides, it was clearly suggested that *Erythrina corallodendron* lectin specifically recognizes β(1→4) linkage between galactose and *N*-acetyl-D-glucosamine and structural isomers such as β(1→3)- and β(1→6)-type models showed much lower affinity to the lectin than the native lactosamine structure.[1]

Next, our attention was directed toward sugar cluster effects on the interaction of lectins with glycopolymer ligands. Although *n*-pentenyl glycosides were useful for the preparation of simple and low-sugar-density ligands, higher sugar-density ligands could not be prepared owing to the inert reactivity of the α-olefin structure. Recently, we reported the feasibility of ω-(acrylamido)-alkyl glycosides for creating cluster-type high-density ligands having *N*-acetyl-D-glucosamine.[11] These polymeric cluster ligands were used for the characterization of the specific interaction of wheat germ agglutinin (WGA) with synthetic glycopolymers.

In this paper, we describe the versatility of the ω-(acrylamido)alkyl groups for the synthesis of novel cluster glycopolymers having *N*-acetyllactosamine with different degrees of sugar density. Biochemical availability of these novel glycoligands will be evaluated on the basis of fluorometric analyses of the binding between FITC-labeled *E. corallodendron* lectin (ECorL)[12] and glycopolymers in relation to the significance of the inter-lactosamine distance on the polymer main chains for the successful binding.

Results and Discussion

Synthesis of ω-(acrylamido)alkyl Glycosides having *N*-Acetyllactosamine. Scheme 1 indicates the synthetic route of *N*-acetyllactosamine derivatives **1** and **2** having polymerizable aglycon at the reducing end. Initially, compounds **6** and **7** were efficiently prepared by glycosylation of oxazoline derivative **5**[8] derivated from *N*-acetyllactosamine octaacetate with *N*-protected amino alcohols, 3-[*N*-[(benzyloxy)carbonyl]amino]propan-1-ol (**3**)[11] and 6-[*N*-[(benzyloxy)carbonyl]amino]-hexan-1-ol (**4**),[11,13] in the presence of 10-camphorsulfonic acid (CSA) as the promoter. General *N*-deprotection by hydrogenation and the following *N*-acryloylation afforded derivatives **8** and **9** as peracetates. Finally,

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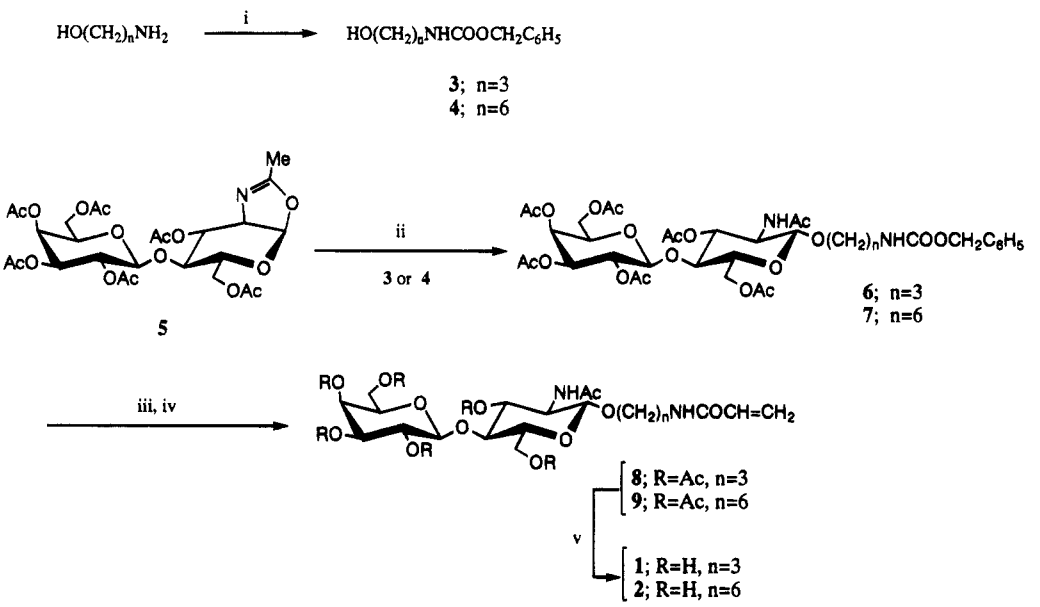
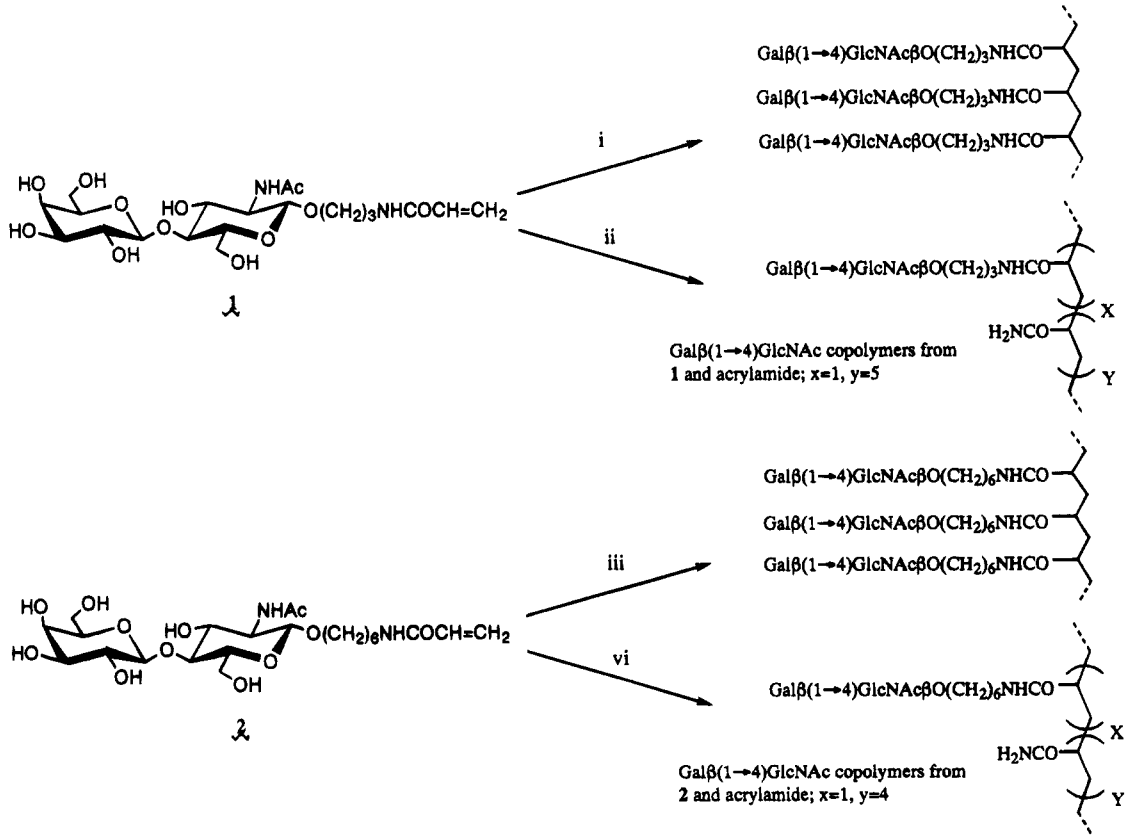
Scheme 1[a]



[a] Reagents and conditions: (i) $C_6H_5CH_2OCOCl$, $NaHCO_3$(aq), room temperature, 3 h; (ii) CSA, $ClCH_2CH_2Cl$, 70 °C, 3 h; (iii) H_2/Pd–C, MeOH, room temperature, 15 h; (iv) CH_2=CHCOCl, Et_3N, THF, 0 → 25 °C, 3 h; (v) NaOMe/MeOH, room temperature, 3 h.

Scheme 2[a]



[a] Reagents and conditions: (i) TEMED, APS, H_2O, 25 °C, 20 h; (ii) CH_2=$CHCONH_2$, TEMED, APS, H_2O, 25 °C, 20 h; (iii) TEMED, APS, H_2O–DMSO, 50 °C, 20 h; (iv) CH_2=$CHCONH_2$, TEMED, APS, H_2O–DMSO, 50 °C, 20 h.

O-deacetylation by the usual Zemplen procedure gave polymerizable monomers **1** and **2**, respectively. All new compounds prepared here gave satisfactory analytical and spectroscopic data.

Preparation of Water-Soluble Polymers Containing *N*-Acetyllactosamine Residue. Radical polymerization of the ω-(acrylamido)-type monomer **1** was carried out in deionized water in the presence of ammonium peroxodisulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) as the initiators at room temperature (Scheme 2). On the other hand, the polymerization of monomer **2** was found to proceed in deionized water–dimethyl sulfoxide (DMSO) solution at 50 °C because of the lower solubility of compound **2** to water. Subsequently, these polymers were purified and characterized according to the method reported previ-

Table 1. Polymerizations of ω-(Acrylamido)alkyl Glycosides of *N*-Acetyllactosamine

sugar monomer	monomer ratio[a]	total yield (%)	polymer compos[a]	sugar (wt %)	$[\alpha]_D$ (deg)	$10^{-3}M_w$[b]
1	1:0[c]	79.3	1:0	100	−15.5	280
1	1:4	86.4	1:5	59.2	−10.0	200
2	1:0[c]	78.3[d]	1:0	100	−15.1	60
2	1:4	89.3[d]	1:4	64.5	−8.5	230

[a] Ratio of carbohydrate monomer to acrylamide. [b] M_w's were determined by the GPC method with an Asahipack GS-510 column [pullulans (5.8K, 12.2K, 23.7K, 48.0K, 100K, 186K, and 380K, Shodex Standard P-82) were used as standards]. [c] Homopolymerization of glycoside (polymerization without acrylamide). [d] 1:1 (v/v) DMSO−H_2O was used for the polymerization solvent owing to a poor solubility in water.

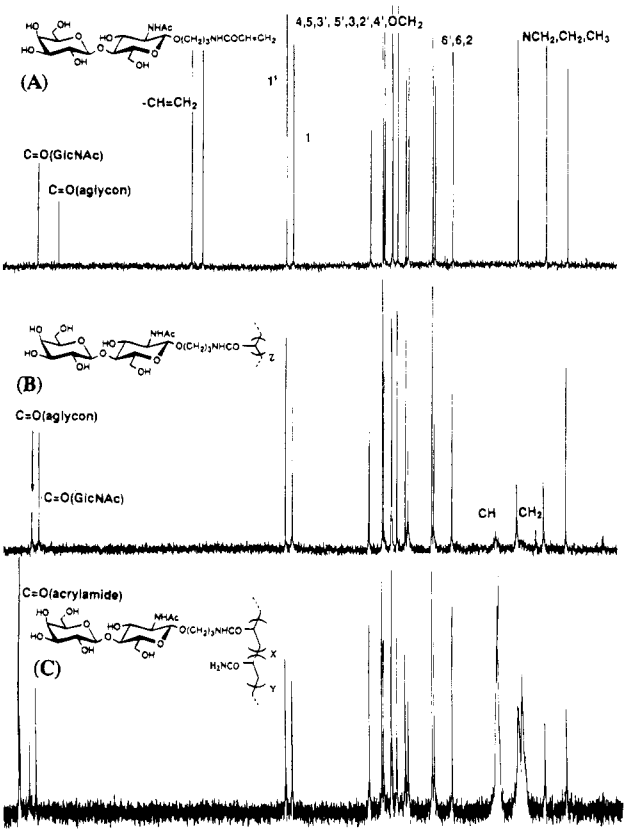


Figure 1. ^{13}C-NMR spectra of (A) 3-(acrylamido)propyl glycoside **1**, (B) copolymer from glycoside **1** with acrylamide, and (C) homopolymer from glycoside **1**, in H_2O at 50 °C.

Table 2. ^{13}C Chemical Shifts of Glycosides and Other Polymers (in ppm from TSP [a])

	carbohydr monomers		homopolymer		copolymer	
compds	**1**	**2**	**1**	**2**	**1**	**2**
C-1	103.8	103.8	103.8	103.7	103.9	103.9
C-2	57.9	57.9	58.0	57.9	57.9	58.1
C-3	75.2	75.2	75.2	75.2	75.2	75.4
C-4	81.6	81.3	81.8	81.5	81.3	81.8
C-5	78.1	78.0	78.1	78.1	78.2	78.3
C-6	63.0	62.9	63.1	62.9	62.9	63.2
C-1′	105.7	105.7	105.8	105.7	105.7	105.8
C-2′	73.8	73.7	73.8	73.7	73.8	73.9
C-3′	77.6	77.4	77.6	77.5	77.6	77.7
C-4′	71.4	71.3	71.4	71.4	71.4	71.5
C-5′	75.4	75.2	75.4	75.3	75.3	75.6
C-6′	63.7	63.7	63.7	63.7	63.8	63.9
CH_2=CH	132.9	132.9				
CH_2=*C*H	129.7	129.6				
CH_2	31.1	31.2	31.6	31.5	31.2	31.3
		31.0		31.4		31.5
		28.4		28.9		28.8
		27.4		27.7		27.6
OCH_2	70.7	73.1	70.5	73.0	70.6	73.3
NCH_2	39.2	42.0	39.3	42.3	39.1	42.3
CH[b]			45.9	45.4	44.6	44.9
CH_2[b]			38.0	38.2	37.7	37.8
C=O[c]	177.2	177.0	176.8	176.6	177.3	177.1
C=O[d]	171.3	171.1	178.9	178.6	179.3	179.1
$CONH_2$					182.3	182.2
CH_3	24.9	25.0	25.3	25.2	25.0	25.3

[a] 3-(Trimethylsilyl)propanesulfonic acid sodium salt. [b] Methylene carbons due to main chain. [c] Signals due to the carbonyl group of the Glc*p*NAc residue. [d] Signals due to the carbonyl groups of aglycons.

ously.[1,7-9] As expected, all new compounds showed an excellent polymerizability compared with those of the glycosides having simple ω-alkenyl-type aglycons. Polymerization of glycosides with acrylamide gave the corresponding copolymers containing desirable amounts of *N*-acetyllactosamine residues in high yields (Table 1). Moreover, the polymerization reaction proceeded efficiently even in the absence of acrylamide and afforded homopolymers from derivatives **1** and **2** in 79 and 78% yields, respectively. Both 3-(acrylamido)propyl and 6-(acrylamido)hexyl[14] groups seem to be practically available polymerizable aglycons having high reactivity and good solubility in water. Fully assigned ^{13}C-NMR spectra of the carbohydrate monomer **1** and polymers derived from **1** are shown in Figure 1. The spectra show a disappearance of signals due to the C−C double bond of glycosides after polymerization. In addition, all data of chemical shifts of these polymers and each monomer glycoside prepared here are assigned and summarized in Table 2.

Interaction of Cluster Glycopolymers with ECorL. We have already reported that the homopolymer-bearing clustered GlcNAc residues can interact tightly with subsites in the binding sites of WGA.[11] Since WGA showed a much higher affinity for this clustered GlcNAc polymer due to polymeric sugar cluster effects than the known chitooligosaccharides such as $(GlcNAc)_2$, $(GlcNAc)_3$, and $(GlcNAc)_4$,[12,15] cluster-type glycopolymers seemed to be high-performance ligands for investigating sugar−lectin interactions by amplified binding specificity which could not be detected in cases of simple sugar ligands with lower molecular weights.

E. Corallodendron lectin (ECorL) is one of the well-characterized plant lectins which mainly recognize lactose and *N*-acetyllactosamine (LacNAc).[13,16-18] Although the importance of the interglycosidic bond between galactose and *N*-acetyl-D-glucosamine residues in the effective binding was clearly demonstrated by measuring the inhibitory effects of glycopolymers containing LacNAc or related regioisomers on the hemagglutination of blood cells induced by ECorL,[1] there is no attempt to possess information on the LacNAc density or its distribution on the globular glycoproteins in the binding process. Therefore, our interest was focused on the versatility of novel LacNAc glycopolymers prepared here for the characterization of the binding site of lectins, since the binding constants and the combining curves of synthetic glycopolymers and lectins were significantly affected by the multiplicity of interac-

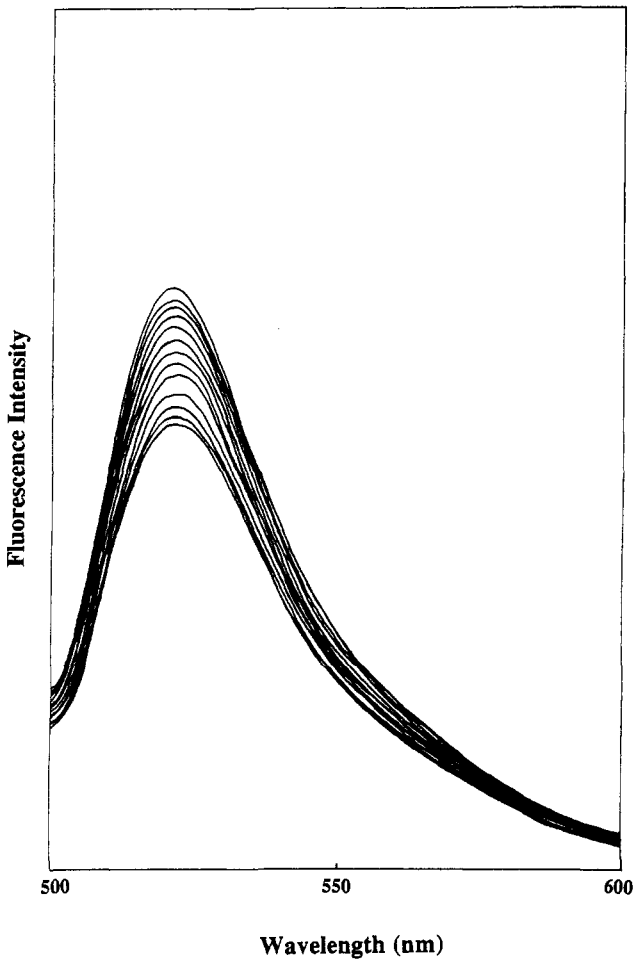


Figure 2. Changes in fluorescence spectrum of FITC-labeled ECorL (0.86 μM, 1.0 mL of 0.15 M phosphate-buffered saline containing 50 mM Na_2HPO_4 and 50 mM KH_2PO_4, pH 7.3, 5.0 °C) upon addition of 10 μL aliquots of the cluster *N*-acetyllactosamine polymer (0.10 mM).

tion, the subsite structure, and the cooperativity of intersubunit structure.[19] In the present study, we selected a fluorescein isothiocyanate-labeled ECorL (FITC-ECorL) as a modified lectin for the binding study, because ECorL has no tryptophan residue which is usually available for fluorescence analyses.[20]

Figure 2 shows the emission spectra of FITC-ECorL and its complexes with cluster LacNAc polymer (homopoly-LacNAc) at pH 7.3. The emission spectrum of FITC-ECorL was gradually reduced by addition of 10 μL aliquots of cluster LacNAc polymer. When lectin was saturated with the polymers, the maximum fluorescence intensities were decreased by approximately 9–22% but no significant shift of the wavelength at the maximum fluorescence spectrum was observed. Therefore, it was suggested that the environment of the fluorescent probes of the lectin altered from hydrophobic to relatively more hydrophilic upon interaction with the glycopolymer. In the course of the combining of LacNAc polymer with the binding sites of lectin, there may be some conformational changes in the protein structure. Moreover, these fluorescent probes seemed to locate apart from the binding sites, since it is commonly reported that the emission maximum is shifted to shorter wavelengths and the intensity is enhanced if a fluorescent probe locates at or near the binding sites of ECorL.

The maximum intensities of the fluorescence spectra measured here were replotted against the glycopolymer concentration [S] (Figure 3), and the association constant was calculated using a Steck–Wallack equation (eq 1).

$$\frac{[S]}{\Delta F} = \frac{1}{\Delta F_{max}}[S] + \frac{1}{\Delta F_{max} K_a} \quad (1)$$

We have performed a preliminary survey of the affinity of FITC-ECorL for several LacNAc polymers having different sugar densities in order to determine the binding specificity of this lectin. The compounds used all exhibited a similar decrease in fluorescence as observed in the case of the LacNAc homopolymer upon interaction with FITC-ECorL (Figure 3). From the Steck–Wallack analyses, we obtained the association constants (K_a), and the K_a values of low-density LacNAc polymers were proved to be slightly higher than that of the LacNAc homopolymer (Table 3). Figure 4 shows a relationship between the K_a value and average inter-LacNAc distance (r). The affinity of the low-LacNAc-density polymer derived from *n*-pentenyl glycoside (r = 67.7) was approximately 2-fold higher than that of the LacNAc homopolymer. Moreover, the K_a value seems to be linearly enhanced by increasing the intersugar spatial distance. Since no significant difference was observed in the inhibitory effects of these glycopolymers on the hemagglutination by ECorL (data not shown), it should be noted that the fluorometric titration examined here is regarded as one of the most sophisticated methods for investigating the binding specificity of lectins with glycoconjugates. The results in these combining experiments can be summarized as follows: (i) All polymeric LacNAc ligands prepared here showed strong cluster effects of up to 1000-fold compared with the corresponding LacNAc monomer or oligosaccharide ligands.[21] (ii) Since the K_a values were enhanced by increasing the ratio of the acrylamide as a partner monomer, the maximal and spatial distance between the two LacNAc residues on the macromolecular ligand may exist for the successful binding with each binding site of the two subunits of ECorL. (iii) At the least, the much shorter spacing of inter-LacNAc residues as in the case of LacNAc homopolymer reduced the binding affinity of glycopolymer, suggesting that the binding pocket of each subunit locates independently with an appropriate spacing.[17] In contrast, it was demonstrated recently using GlcNAc polymers that wheat germ agglutinin (WGA) exhibits a specific and cooperative binding affinity with a clustered GlcNAc homopolymer.[11] Such strong affinity enhancements probably occurred because of multipoint-type binding between the subsite structure of WGA and high-density GlcNAc residues. It appears, therefore, that the binding characteristics of polymeric sugar-cluster ligands with lectins are mainly dependent upon the number, multivalency (subunit), and subsite structures of sugar binding proteins.

In conclusion, the versatility of ω-(acrylamido)alkyl LacNAc derivatives for the facile preparation of novel cluster LacNAc polymers was demonstrated. It was clearly suggested that fluorometric titration of FITC-ECorL with a variety of LacNAc polymers revealed the importance of the suitable spacing of the intersugar residues on the glycoprotein models for the efficient interaction with the binding sites of lectins. The stoichiometry of the interaction between ECorL and glycoligands including cross-linking[19] by the multivalent glycopolymers is under investigation, and the results will be discussed elsewhere.

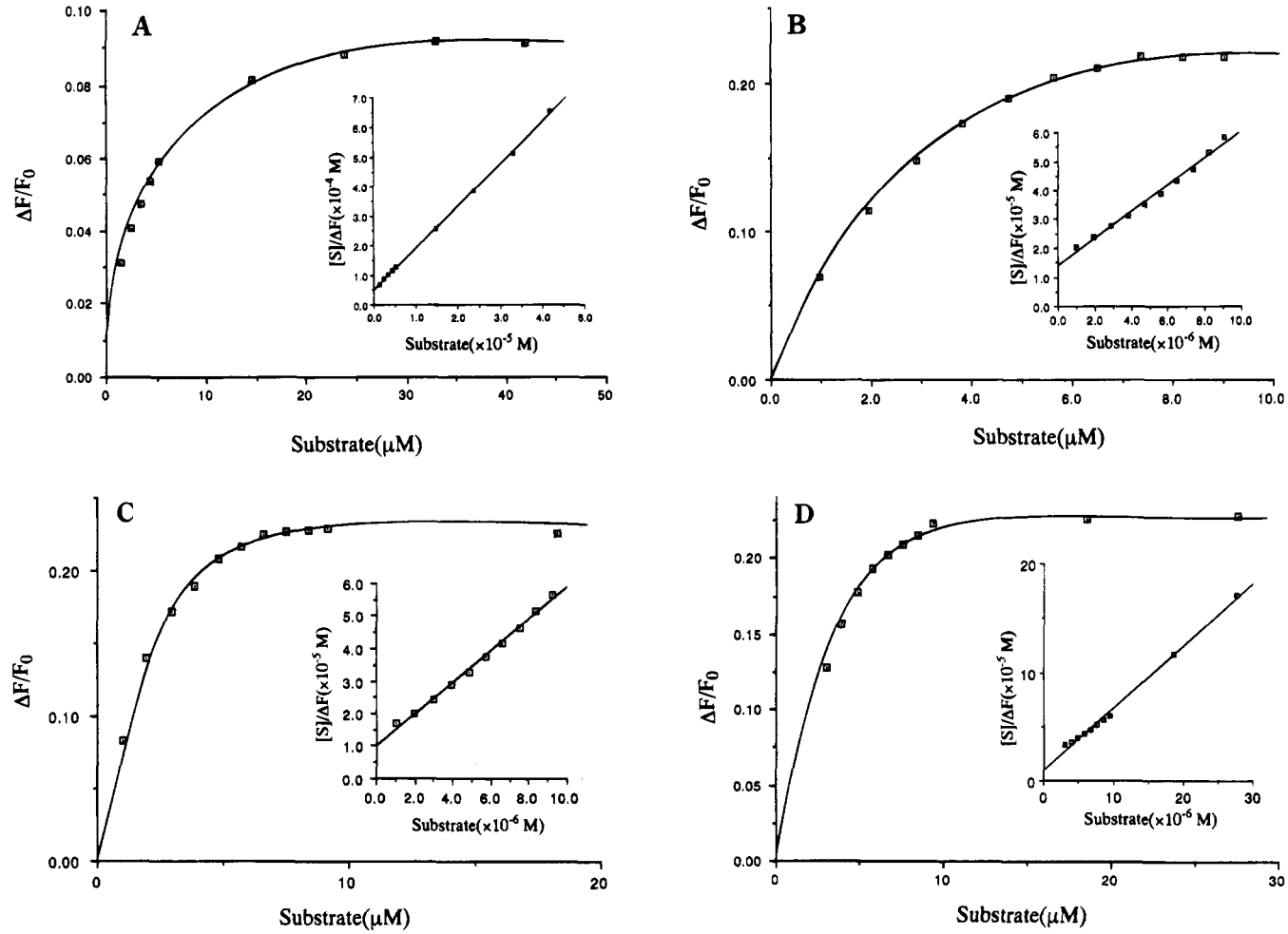


Figure 3. Replots in fluorescence emission spectra of FITC-labeled ECorL upon addition of 10 μL aliquots of (A) *N*-acetyllactosamine-homopolymer (0.10 mM), (B) *N*-acetyllactosamine copolymer (0.10 mM) (LacNAc: 59.2 wt %), (C) *N*-acetyllactosamine copolymer (0.10 mM) (LacNAc: 30.6 wt %), and (D) *N*-acetyllactosamine copolymer (0.10 mM) (LacNAc: 23.0 wt %). ΔF is a change of the fluorescence intensity at the fluorescence maximum at 520 nm of a solution containing the lectin obtained by exciting at 494 nm with a total ligand concentration [S], and F_0 is the fluorescence intensity of lectin alone.

Table 3. K_a of the Glycopolymers Containing *N*-Acetyllactosamine Residue

sugar monomer	monomer ratio[a]	polymer compos[a]	sugar (wt %)	$10^{-3}M_w$[b]	$10^{-5}K_a$[c] (M^{-1})
1	1:0[d]	1:0	100	280	2.82
1	1:4	1:5	59.2	200	3.33
Pent–LacNAc[e]	1:4	1:14	30.6	180	4.73
Pent–LacNAc[e]	1:10	1:21	23.0	>300	5.67

[a] Ratio of carbohydrate monomer to acrylamide. [b] M_w's were determined by the GPC method with an Asahipack GS-510 column [pullulans (5.8K, 12.2K, 23.7K, 48.0K, 100K, 186K, and 380K, Shodex Standard P-82) were used as standards]. [c] K_a is calculated from a Steck–Wallack plot using average residual molecular weights calculated from polymer composition. [d] Homopolymerization of glycoside (polymerization without acrylamide). [e] Polymer containing the *n*-pentenyl glycosides of *N*-acetyllactosamine.

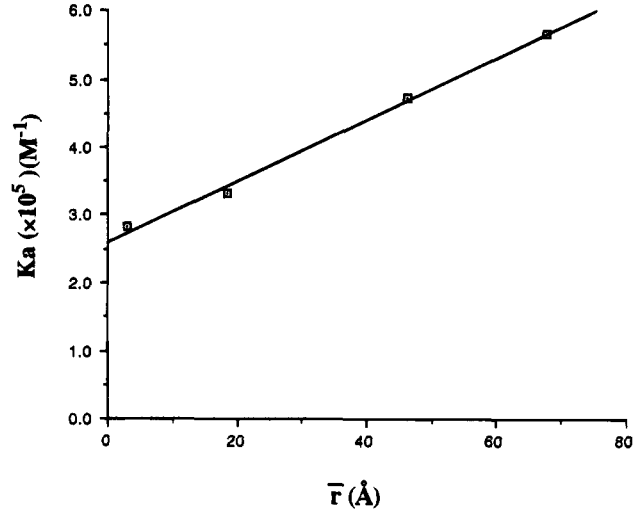


Figure 4. Relationship between K_a and sugar density. r is the average inter-LacNAc distance in the polymers.

Experimental Section

General Procedure. Melting points were determined with a Laboratory Devices melting point apparatus and are uncorrected. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. 1,2-Dichloroethane, ethyl acetate, and pyridine were stored over molecular sieves (4 Å) for several days before use. Acrylamide was recrystallized from benzene before use. *E. corallodendron*–fluorescein isothiocyanate (FITC-labeled ECorL) was purchased from Sigma. Optical rotations were determined with a Horiba SEPA-200 digital polarimerter at 25 °C. ^{1}H and proton decoupled carbon NMR spectra were recorded at 300 and 75.47 MHz, respectively, on a Bruker AMX-300 spectrometer in chloroform-*d*, dimethyl sulfoxide-d_6, or deuterium oxide, using tetramethylsilane (TMS), methanol, or 3-(trimethylsilyl)-propanesulfonic acid sodium salt (TSP) as internal standards. The average molecular weight was estimated by gel permeation chromatography (GPC) with an Asahipak GS-510 column, and pullulans (5.8K, 12.2K, 23.7K, 48.0K, 100K, 186K, and 380K, Shodex Standard P-82) were used as standards. Elemental analyses were performed with a Sartorius 4503

Micro 7079 printer on the samples extensively (ca. 24 h) dried in vacuo (50 °C, 0.1 Torr) over phosphorus pentoxide. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmmstadt, Germany). For detection of the components, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) methanol–concentrated sulfuric acid–*p*-anisaldehyde and heated for a few minutes (for carbohydrates), (b) a solution of 5 wt % ninhydrin in ethanol and heated for a few minutes (for amino groups), or (c) an aqueous solution of 5 wt % potassium permanganate and heated similarly (for C–C double bonds). Column chromatography was performed on silica gel (Wakogel C-200; 100–200 mesh, Wako Pure Chemical Industries Co. Ltd., Japan). All extractions were concentrated below 40 °C under diminished pressure.

3-[[(Benzyloxy)carbonyl]amino]propyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*- acetyl-2-deoxy-β-D-glucopyranoside (6). A solution of oxazoline derivative **5**[8] (1.0 g, 1.62 mmol) and **3**[11] (1.02 g, 4.86 mmol) in 1,2-dichloroethane (15 mL) was stirred under a nitrogen atmosphere for 3 h at 70 °C in the presence of CSA (200 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice water. The organic layer was washed successively with aqueous sodium hydrogen carbonate and brine, dried over magnesium sulfate, filtered, and evaporated. The residue was purified by chromatography on silica gel first with 1:0 and then with 50:1 (v/v) chloroform–methanol to afford compound **6** (950 mg, 71%): mp 86 °C; $[\alpha]_D$ −7.5 (*c* 0.327 g/dL, chloroform); ^{1}H-NMR δ ($CDCl_3$) 1.60 and 1.76 (m, 2 H, CH_2), 1.94–2.15 (all s, 21 H, 7 $COCH_3$), 3.07 (m, 2 H, NCH_2), 3.33 (ddd, 1 H, H-5), 3.49 (m, 1 H, OCH_2), 3.77 (t, 1 H, *J* = 8.9 Hz, H-4), 3.84–3.93 (m, 2 H, H-6′a and H-6′b), 4.05 (m, 2 H, H-6a and H-6b), 4.11–4.17 (m, 3 H, H-2, H-5′ and OCH_2), 4.46 (d, 1 H, *J* = 9.5 Hz, H-1), 4.48 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.96 (dd, 1 H, *J* = 3.3 and 10.5 Hz, H-3′), 5.01 (br s, 1 H, NHCOO), 5.03–5.16 (m, 3 H, H-3, and $PhCH_2$), 5.15 (d, 1 H, *J* = 7.5 Hz, H-2′), 5.35 (d, 1 H, *J* = 2.6 Hz, H-4′), 6.42 (d, 1 H, *J* = 8.9 Hz, NH), and 7.29–7.39 (m, 5 H, aromatic). Anal. Calcd for $C_{37}H_{50}O_{19}N_2{\cdot}0.5\ H_2$:; C, 53.17; H, 6.15; N, 3.35. Found: C, 53.02; H, 6.10; N, 3.46.

6-[[(Benzyloxy)carbonyl]amino]hexyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (7). A solution of oxazoline derivative **5** (1.5 g, 2.43 mmol) and **4**[11] (1.83 g, 7.29 mmol) in 1, 2-dichloroethane (40 mL) was stirred under a nitrogen atmosphere for 3 h at 70 °C in the presence of CSA (200 mg). The solution was cooled to room temperature, diluted with chloroform, and poured into ice water. The extract with chloroform was washed successively with aqueous sodium hydrogen carbonate and water, dried over magnesium sulfate, filtered, and evaporated. The residue was purified by chromatography on silica gel first with 1:0 and then with 80:1 (v/v) chloroform–methanol to afford compound **7** (1.5 g, 71%): mp 70–72 °C; $[\alpha]_D$ −2.7 (*c* 0.326 g/dL, chloroform); ^{1}H-NMR δ ($CDCl_3$) 1.32 and 1.51 (m, 8 H, CH_2), 1.94–2.15 (all s, 21 H, 7 $COCH_3$), 3.18 (m, 2 H, NCH_2), 3.43 (m, 1 H, H-5), 3.56 and 3.81–3.90 (m, 4 H, H-6′a, H-6′b, and OCH_2), 3.77 (t, 1 H, *J* = 8.4 Hz, H-4), 4.02 (dd, 1 H, *J* = 7.4 Hz, H-2), 4.07–4.13 (m, 3 H, H-5′, H-6a, and H-6b), 4.40 (d, 1 H, *J* = 7.5 Hz, H-1), 4.49 (d, 1 H, *J* = 7.5 Hz, H-1′), 4.93 (br s, 1 H, NHCOO), 4.97 (dd, 1 H, *J* = 3.3 and 10.5 Hz, H-3′), 5.07 (t, 1 H, *J* = 9.5 Hz, H-3), 5.11 (m, 2 H, $PhCH_2$), 5.14 (d, 1 H, *J* = 8.9 Hz, H-2′), 5.35 (d, 1 H, *J* = 2.6 Hz, H-4′), 5.98 (d, 1 H, *J* = 9.5 Hz, NH), and 7.30–7.36 (m, 5 H, aromatic). Anal. Calcd for $C_{40}H_{56}O_{19}N_2{\cdot}$ CH_3OH: C, 54.65; H, 6.71; N, 3.11. Found: C, 54.66; H, 6.88; N, 3.28.

3-(*N*-Acryloylamino)propyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*- acetyl-2-deoxy-β-D-glucopyranoside (8). Compound **6** (190 mg, 0.230 mmol) was hydrogenated in the presence of 5% palladium on carbon (100 mg) in methanol (10 mL) for 15 h at room temperature. The reaction was monitored by TLC in 5:4:1 (v/v/v) chloroform–ethyl acetate–methanol. The mixture was filtered and evaporated to give the crude 3-aminopropyl glycoside.

To a solution of the crude 3-aminopropyl glycoside in DMF (5.0 mL) were added triethylamine (38.5 μL) and acryloyl chloride (22.4 μL) at 0 °C, and the mixture was stirred for 3 h at room temperature. The mixture was evaporated under reduced pressure, and the residue was poured into ice water and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtrated, and evaporated. The residue was subjected to silica gel chromatography first with 1:0 and then with 20:1 (v/v) chloroform–methanol as eluant and was crystallized from chloroform–ether–*n*-hexane to give **8** (120 mg, 70%) as an amorphous powder: mp 94–96 °C; $[\alpha]_D$ −27.4 (*c* 0.316 g/dL, chloroform); ^{1}H-NMR δ ($CDCl_3$) 1.70 and 1.84 (m, 2 H, CH_2), 1.97–2.17 (all s, 21 H, 7 $COCH_3$), 3.19 and 3.43 (m, 2 H, NCH_2), 3.59 (m, 1 H, H-5), 3.67 and 3.96 (m, 2 H, OCH_2), 3.81 (t, 1 H, H-4), 3.88 (dd, 2 H, H-6′a and H-6′b), 4.03–4.17 (m, 4 H, H-2, H-5, H-6a, and H-6b), 4.33 (d, 1 H, *J* = 8.2 Hz, H-1), 4.51 (d, 1 H, *J* = 7.5 Hz, H-1′), 4.97 (dd, 1 H, *J* = 3.3 and 10.2 Hz, H-3′), 5.07 (d, 1 H, *J* = 7.9 Hz, H-3), 5.14 (d, 1 H, *J* = 7.9 Hz, H-2′), 5.35 (d, 1 H, *J* = 2.6 Hz, H-4′), 5.63 [dd, 1 H, *J* = 3.4 and 7.4 Hz, CH=CH_2 (cis)], and 6.27–6.31 [m, 4 H, CH=CH_2 (trans), CH_2NHCO, and NH]. Anal. Calcd for $C_{32}H_{46}O_{18}N_2{\cdot}H_2O$: C, 50.25; H, 6.33; N, 3.66. Found: C, 50.28; H, 6.13; N, 3.57.

6-(*N*-Acryloylamino)hexyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (9). Compound **7** (1.7 g, 1.96 mmol) was hydrogenated in the presence of 5% palladium on carbon (300 mg) in methanol (30 mL) for 12 h at room temperature. The reaction was monitored by TLC in 5:4:1 (v/v/v) chloroform–ethyl acetate–methanol. The mixture was filtered and evaporated to give the crude 6-aminohexyl glycoside.

To a solution of the crude 6-aminohexyl glycoside in DMF (20 mL) were added triethylamine (328 μL) and acryloyl chloride (191 μL) at 0 °C, and the mixture was stirred for 3 h at room temperature. The mixture was evaporated under reduced pressure, and the residue was poured into ice water and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtrated, and evaporated. The residue was subjected to silica gel chromatography first with 1:0 and then with 50:1(v/v) chloroform–methanol as eluant and was crystallized from chloroform–ether–*n*-hexane to give **9** (1.28 g, 82.8%) as an amorphous powder: mp 79–81 °C; $[\alpha]_D$ −14.1 (*c* 0.326 g/dL, chloroform); ^{1}H-NMR δ ($CDCl_3$) 1.35 and 1.57 (m, 8 H, CH_2), 1.96–2.13 (all s, 21 H, 7 $COCH_3$), 3.27 and 3.40 (m, 2 H, NCH_2), 3.44 (m, 2 H, OCH_2), 3.61 (m, 1 H, H-5), 3.78 (t, 1 H, H-4), 3.88 (m, 2 H, H-6′a and H-6′b), 4.00 (m, 1 H, H-2), 4.12 (m, 2 H, H-6a and H-6b), 4.44 (d, 1 H, *J* = 7.5 Hz, H-1), 4.50 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.97 (dd, 1 H, *J* = 3.3 and 10.5 Hz, H-3′), 5.09 (m, 1 H, H-3), 5.13 (d, 1 H, *J* = 7.9 Hz, H-2′), 5.35 (d, 1 H, *J* = 2.6 Hz, H-4′), 5.64 [dd, 1 H, *J* = 1.6 and 9.9 Hz, CH=CH_2 (cis)], 6.07–6.23 [m, 2 H, CH=CH_2 (trans)], 6.17 (d, 1 H, *J* = 9.8 Hz, NH), and 6.32 (m, 1 H, CH_2NHCO). Anal. Calcd for $C_{35}H_{52}O_{18}N_2$: C, 53.29; H, 6.64; N, 3.55. Found: C, 53.13; H, 6.62; N, 3.73.

3-(*N*-Acryloylamino)propyl *O*-(β-D-Galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (1). To a solution of compound **8** (300 mg, 0.402 mmol) in dry methanol (10 mL) was added sodium methoxide (20 mg), and the mixture was stirred for 3 h at room temperature. It was neutralized by Dowex 50W-X8 (H^+) resin, filtered, and evaporated in vacuo to give **1** (170 mg; 86%): mp 188 °C; $[\alpha]_D$ −18.2 (*c* 0.250 g/dL, water); ^{1}H-NMR δ (D_2O) 1.82 (m, 2 H, CH_2), 2.04 (s, 3 H, $COCH_3$), 3.28 and 3.35 (m, 2 H, NCH_2), 4.48 (d, 1 H, *J* = 7.8 Hz, H-1), 4.53 (d, 1 H, *J* = 7.9 Hz, H-1′), 5.76 [dd, 1 H, *J* = 1.8 and 9.7 Hz, CH=CH_2 (cis)], 6.14–6.32 [m, 2 H, CH=CH_2 (trans),], 6.25 (d, 1 H, *J* = 9.7 Hz, NH), and 6.30 (m, 1 H, CH_2NHCO). Anal. Calcd for $C_{20}H_{34}O_{12}N_2{\cdot}H_2O$: C, 48.87; H, 7.08; N, 5.47. Found: C, 46.70; H, 6.80; N, 5.31.

6-(*N*-Acryloylamino)hexyl *O*-(β-D-Galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (2).[11] To a solution of compound **9** (290 mg, 0.368 mmol) in dry methanol (10 mL) was added sodium methoxide (10 mg), and the mixture was stirred for 2 h at room temperature. It was neutralized by Dowex 50W-X8 (H^+) resin, filtered, and evapo-

rated in vacuo to give **2** (187 mg, 95%): mp 176 °C; ^{1}H-NMR δ ($CDCl_3$) 1.26 and 1.47 (m, 8 H, CH_2), 1.96 (s, 3 H, $COCH_3$), 3.20 and 3.40 (m, 2 H, NCH_2), 3.37 (m, 2 H, OCH_2), 4.41 (d, 1 H, J = 7.7 Hz, H-1), 4.45 (d, 1 H, J = 7.3 Hz, H-1′), 5.68 [dd, 1 H, J = 1.8 and 9.9 Hz, CH=CH_2 (cis)], 6.06–6.24 [m, 2 H, CH=CH_2 (trans)], 6.17 (d, 1 H, J = 9.8 Hz, N*H*), and 6.22 (m, 1 H, CH_2NHCO). Anal. Calcd for $C_{23}H_{40}O_{12}N_2 \cdot 0.5H_2O$: C, 50.63; H, 7.57; N, 5.13. Found: C, 50.80; H, 7.37; N, 5.06.

Homopolymerization of 1. A solution of monomer **1** (140 mg, 0.283 mmol) in deionized water (2.0 mL) was deaerated by using a water aspirator for 20 min, followed by addition of TEMED (4.24 μL) and APS (2.58 mg). The mixture was stirred for 20 h at room temperature, diluted with 1.5 mL of 0.1 M pyridine–acetic acid buffer (pH 5.1), dialyzed against deionized water for 24 h, and freeze-dried to give a white powdery polymer (111 mg; 79%).

Copolymerization of 1. A solution of monomer **1** (150 mg, 0.303 mmol) and acrylamide (86.2 mg, 1.21 mmol) in deionized water (5.0 mL) was deaerated by using a water aspirator for 20 min, followed by addition of TEMED (4.55 μL) and APS (2.77 mg). The mixture was stirred for 20 h at room temperature, diluted with 2.0 mL of 0.1 M pyridine–acetic acid buffer (pH 5.1), dialyzed against deionized water for 24 h, and freeze-dried to give a white powdery polymer (204 mg; 86%).

Homo- and Copolymerization of 2. A solution of monomer **2** (140 mg, 0.224 mmol) in deionized water (2.0 mL) and dimethyl sulfoxide (2.0 mL) was deaerated by using a water aspirator for 20 min, followed by addition of TEMED (5.04 μL) and APS (4.09 mg). The mixture was stirred for 20 h at room temperature, diluted with 2.0 mL of 0.1 M pyridine–acetic acid buffer (pH 5.1), dialyzed against deionized water for 24 h, and freeze-dried to give a white powdery homopolymer (94 mg; 78%).

Monomer **2** (150 mg, 0.280 mmol) and acrylamide (79.6 mg, 1.12 mmol) were dissolved in deionized water (3.0 mL) and dimethyl sulfoxide (3.0 mL), and the mixture was treated with the procedure described above to give a white powdery copolymer (205 mg; 89%).

Fluorescence Measurements. Emission spectra of a FITC in the ECorL induced by excitation at 494 nm were recorded with a Hitachi 650-60 fluorescence spectrophotometer. The solutions were contained in 1 cm quartz cuvettes, and the measurements were carried out at 5 °C in order to remove the effect of nonspecific binding on the spectra. The concentration of FITC-labeled ECorL was estimated to be 0.87 μM by using an absorption coefficient at 280 nm (1.53 mg^{-1} cm^2 in 50 mM Na_2HPO_4/KH_2PO_4, 0.15 M NaCl, pH 7.3).[22]

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