Heterobifunctional Ligands: Practical Chemoenzymatic Synthesis of a Cell Adhesive Glycopeptide That Interacts with Both Selectins and Integrins

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Received July 12, 2000

An efficient and practical synthesis of cell adhesive glycopeptides exhibiting unique properties as a novel type of modulator of cellular recognition is described. A nonnatural glycopeptide **1** composed of sialyl Lewis x and Lys-Gly-Arg-Gly-Asp-Ser that interacts with both selectins and integrins has been systematically synthesized by combined chemical and enzymatic strategy. It is suggested that glycopeptide **1** showed much higher affinity with P-selectin ($K_a = 6.6 \times 10^7\ M^{-1}$) and E-selectin ($K_a = 4.5 \times 10^5\ M^{-1}$) than sialyl Lewis x. This compound also inhibited a specific interaction between human integrin β_1 and its monoclonal antibody more effectively than the tetrapeptide Arg-Gly-Asp-Ser. Interestingly, it was demonstrated by surface plasmon resonance analysis that this heterobifunctional glycopeptide exhibited a capacity to form stable complexes with P-selectin and integrin β_1 concurrently. It is also suggested that this activity can be used for the inhibition of integrin-mediated adhesion of activated helper T cells onto collagen-coated plates as a cell migration model. These results indicate that the chemoenzymatic hybridization strategy of different biological functions of carbohydrates and peptides is a new concept for designing potent glycoconjugates as antiinflammatory and anticancer metastasis reagents.

Introduction

Cell adhesive molecules (CAMs) such as fibronectin, laminin, vitronectin, collagen, integrin, immunoglobulin, and some animal lectins participate in a variety of cell−cell interactions and cell−extracellular matrix (ECM) interactions.[1] Molecular recognition by CAMs on the cell surfaces is one of the most important biological processes not only in cell adhesion but also in fertilization, organ formation, cell migration, lymphocyte trafficking, immune response, and cancer metastasis.[2] It should be noted that multiple and complex mechanisms of the bindings between a variety of cell surface receptors with their ligands are supposed to contribute to the construction of stable and specific cellular networks. Therefore, artificial hybridization of some different functional structures of CAMs is expected to become a novel and efficient method to create useful reagents for the regulation of specific cellular recognition and adhesion.

In the present study, we selected integrins and selectins as a set of target receptors of the synthetic heterobifunctional ligand, because they play crucial roles in cellular recognition related to lymphocyte trafficking. Although individual synthetic blockers of selectin- or integrin-mediated cellular recognition have been extensively investigated,[3,4] the heteromultifunctional ligands designed by combining some different biofunctional motifs are also potential and attractive concepts for creating new types of multiple reagents.[5] Ligands that interact with both selectins and integrins are novel and potent candidates for therapeutic reagents having antiinflammatory and anticancer metastasis activities. As shown in Figure 1, we considered that the hybridization of Arg-Gly-Asp-Ser (RGDS) and sialyl Lewis x (SLex) with an appropriate flexible linker would provide us with a nonnatural glycopeptide having interesting properties as a heterobifunctional ligand. The tetrapeptide RGDS motif corresponds to natural and core sequences derived from fibronectin which has been reported to contribute to the molecular recognition in cell adhesion, spreading, and migration of cells through specific interaction with integrins, one of the most important protein families of the cell surface receptors.[2a,b] It was also suggested that the pentapeptide Gly-Arg-Gly-Asp-Ser (GRGDS) showed strong inhibitory effect on experimental metastasis of murine melanoma cells.[6] On the other hand, the tetrasaccharide SLex, Neu5Acα(2→3)Galβ(1→4)[Fucα(1→3)]GlcNAc, has been known as a common oligosaccharide ligand for the selectins that mediate adhesion of leukocytes to the endothelium cells in the early phase of an inflammatory response.[1c] Unfortunately, because the connectivity of SLex with selectins under equilibrium conditions is weak, recent efforts have been centered on the increase

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10.1021/jm000295r CCC: $20.00
Published on Web 01/09/2001

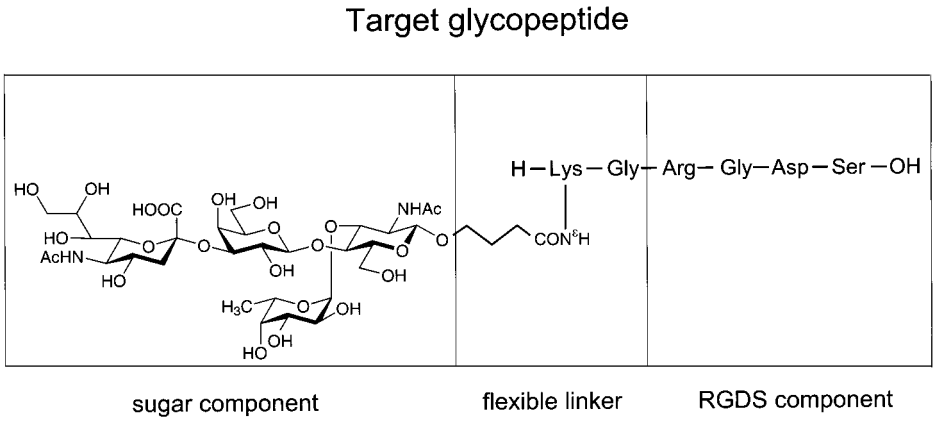


Figure 1. Chemical structure of glycopeptide **1**.

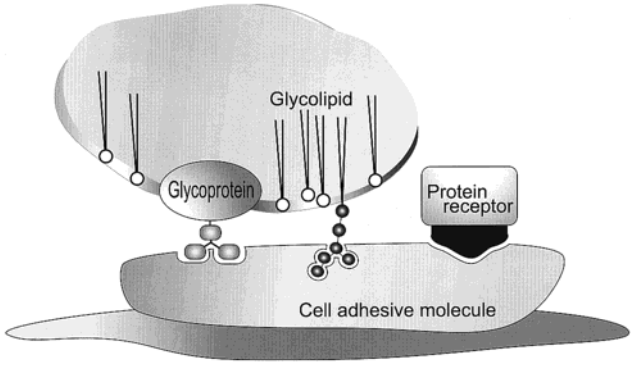


Figure 2. Cell adhesion model represented by the multiple receptor−ligand interactions.

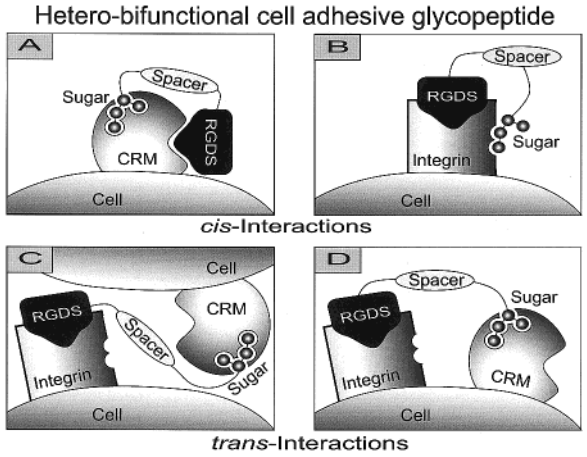


Figure 3. Some plausible mechanisms of binding of **1** with receptor molecules: (A, B) cooperative-type *cis*-interaction of **1** with selectin or integrin; (C, D) cross-linking of two different protein receptors by glycopeptide (*trans*-interaction).

of the affinity by designing mimics of SLex and through multivalency.[7] In the present work, these two functional ligands are combined by the flexible and hydrophobic dipeptide linker in order to obtain a novel class of biological response modifiers with highly improved and unique binding properties. Glycopeptide **1** that interacts with two different types of families of cell adhesive proteins such as selectins and integrins can be used for the regulation of cell adhesion and related diseases.

The basic idea has arisen from the realization that each cell adhesive protein might involve some different binding sites (domains) for their ligands as represented in Figure 2 and this must also contribute to the stable and successful cellular recognition as well as the multivalent interactions.[8] For example, although the RGDS motif has been known as a crucial ligand of integrins, it has also been reported that integrins interact with ganglioside GM3 which has a sialyl lactose moiety, Neu5Acα(2→3)Galβ(1→4)Glc. In fact, this interaction seems to enhance the affinity of integrin with fibronectin dramatically.[9] On the other hand, it was suggested that the affinity of P-selectin with SLex is strongly affected by the structure and properties of peptide segments of SLex-containing glycoproteins. For example, the peptide sequence involving sulfated tyrosine residues found in PSGL-1 (P-selectin glycoprotein ligand-1) seems to be a critical structure for the specific binding with P-selectin.[10] This clearly indicates the need of the association by peptidic binding sites in addition to the binding by the carbohydrate-based ligands. All these reports strongly suggest that the conjugation of some different CAMs or functional motifs might become a promising method to control the biological activity, specificity (targeting), and stability of each simple ligand molecule. Figure 3 summarizes plausible models for the interaction of **1** with cell adhesive proteins displayed on the cell surfaces. As illustrated in Figure 3A,B, cooperative effects (*cis*-type interactions) by artificial glycopeptide may lead to more successful and stable recognition with each receptor molecule than that by peptide (RGDS) or carbohydrate (SLex) itself. Moreover, specific cross-linking of different receptors shown in Figure 3C,D is also expected to be a novel and efficient pathway to modulate cell adhesion (*trans*-type interactions).

We report herein the full details of an efficient chemoenzymatic synthesis of the nonnatural glycopeptide **1** exhibiting specific cell adhesion activities. The concept of *heteromultifunctional ligands* described here is of growing importance for the synthesis of a novel class of bioactive glycoconjugates.

Results and Discussion

Synthesis. Although some excellent examples of the synthetic studies on the partial structures of the naturally occurring glycoproteins based on chemoenzymatic manner have been reported previously by Wong and coworkers,[11] versatility of this synthetic strategy toward nonnatural or related glycopeptide mimetics with potent biological activities has not been discussed well. We considered that an efficient and facile glycosylation might become a key step for the creation of the carbo-

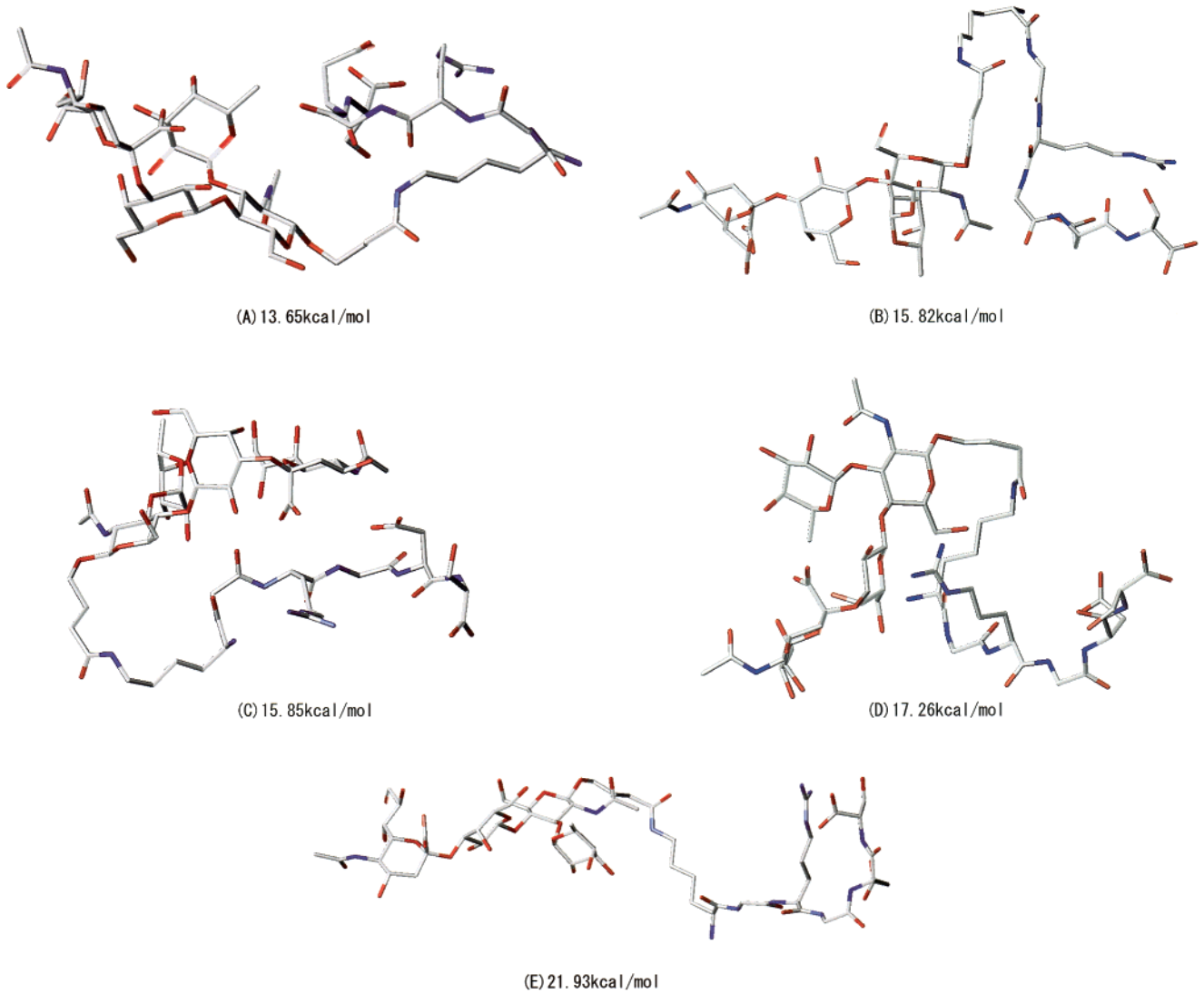
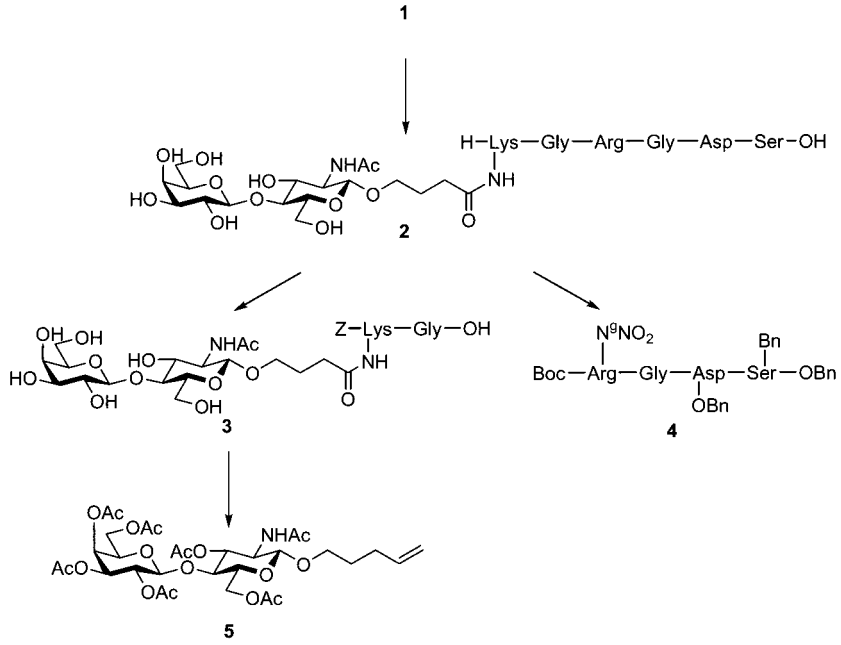


Figure 4. Stereorepresentation of the possible low-energy conformations predicted by a random conformational searching method: (A−D) four major types of the most stable conformers; (E) extended model of compound **1**.

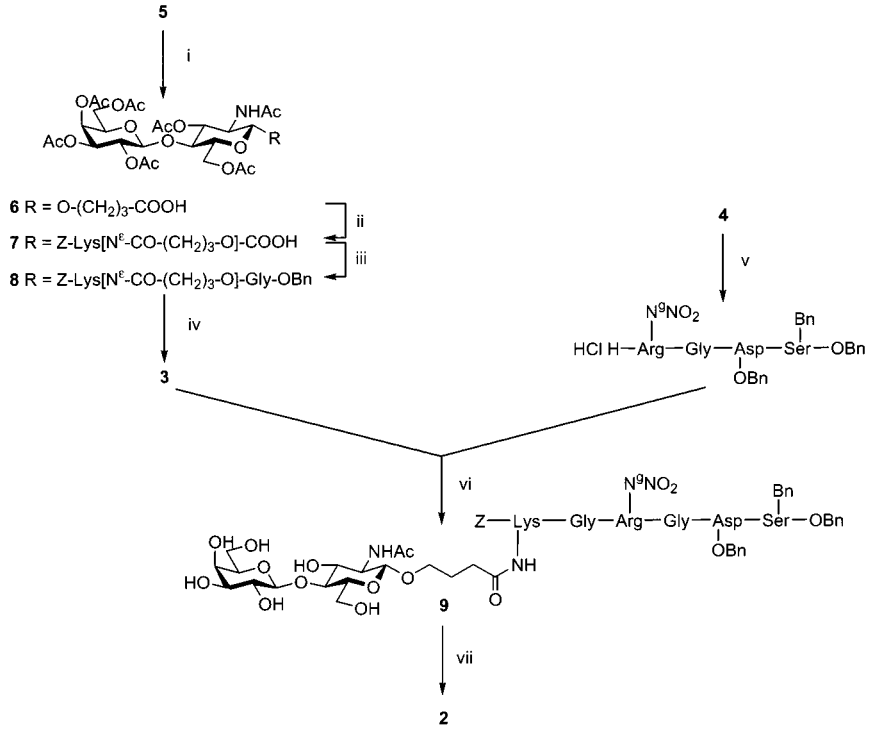
hydrate-mediated specific sorting of bioactive peptides and proteins as well as for the enhancement of the stability against the thermal denaturation or the degradation by peptidases.[12] Here, we designed an artificial heterobifunctional glycopeptide **1** as a tentative target molecule for evaluating the feasibility of the synthesis using enzyme-assisted strategy. To discuss the propriety of this compound, possible low-energy conformations were predicted by a random conformational searching method,[13] and Figure 4 shows four major types of the most stable conformers (A−D) in addition to a plausible model of the extended conformation (E). These results suggest that the flexible linker would permit the formation of hydrogen bonds between the tetrasaccharide and hexapeptide moieties and these stable conformations of the glycopeptide seem to be more suited for the successful *cis*-interactions with the receptor proteins rather than *trans*-interactions as illustrated in Figure 3.

Scheme 1 indicates a retrosynthetic pathway for the synthesis of glycopeptide **1**. The synthetic strategy is composed of chemical synthesis of a key intermediate **2**[14] and its enzymatic modifications. Since the acid lability of the L-fucose residue and the difficulty in the stereoselective glycosylation of sialic acid were crucial problems for the chemical manipulations of SLex-containing glycopeptides, the chemoenzymatic strategy greatly facilitated the synthetic scheme of the complex glycopeptide **1**. First, a key intermediate **2** was prepared by chemical synthesis from a simple *n*-pentenyl glycoside of *N*-acetyllactosamine **5**[15] and a tetrapeptide derivative **4** as starting materials (Scheme 2). The terminal double bond of compound **5** was converted into a carboxyl group by treatment with potassium permanganate in 87% yield, and the active ester of **6** was employed for the coupling reaction with the ϵ-amino group of Z-lysine to afford **7** in 71% yield. To achieve the satisfactory flexibility of the spacer arm moiety between SLex and RGDS, and the versatility in further chemical manipulation, H-Gly-OBn was combined with **7** to give *N*-acetyllactosamine dipeptide derivative **8** in 83% yield. Consequently, deprotection of the benzyl ester and *O*-acetyl groups of **8** under alkaline condition was allowed to proceed smoothly and gave compound **3** in 87% yield without racemization. Coupling reaction of the glycopeptide **3** having an unprotected disaccharide moiety with an N-terminus generated from RGDS derivative **4** was carried out by employing diphenyl phosphorazidate[16] as a selective promoter of the car-

Scheme 1. Retrosynthetic Route of CAGP **1**



Scheme 2 Synthesis of a Key Intermediate **2**[a]

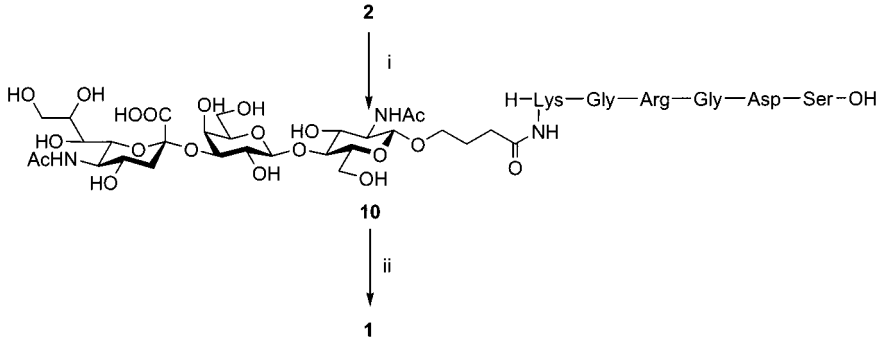


[a] Reagents and conditions: (i) $KMnO_4$, AcOH(aq), rt, 87%; (ii) HONSu, DCC, DMF, 0 °C → rt, then Z-Lys, TEA, DMF−H_2O (9:1), rt, 71%; (iii) DPPA, Gly-OBn, TEA, DMF, 0 °C → rt, 83%; (iv) NaOMe, dry MeOH, rt, then 1 N NaOH(aq), rt, ion-exchange resin (Dowex 50W-X8), 87%; (v) 2 N HCl/dioxane, 0 °C → rt; (vi) DPPA, TEA, DMF, 0 °C → rt, 77% (over all yield from **4**); (vii) Pd−C, H_2(g), MeOH−H_2O, (1:1) rt, 99%.

boxyl group of glycopeptide and afforded a hexapeptide derivative **9** in 77% yield with no side reaction. Removal of all protecting groups of the peptide moiety of **9** such as benzyl, nitro, and benzyloxycarbonyl groups was performed by usual hydrogenation in the presence of palladium on charcoal to afford the intermediate **2** in 92% yield.

Subsequently, the glycopeptide **2** was used as substrate for the corresponding glycosyltransferases without any protection/deprotection procedure. It is of interest to examine the substrate specificity of glycosyltransferases against the nonnatural glycopeptide **2**, since the acceptability of synthetic glycoconjugates to the glycosyltransferases seemed to be seriously affected by the nature of aglycons or supporting backbones. Actually, our previous studies have also shown that the substrate specificity of the glycosyltransferases is strongly dependent on the structure of backbones or scaffoldings in addition to the length and hydrophobicity of the linker moiety between the sugar and main chain.[17] For

Scheme 3. Enzymatic Sugar Elongation Reactions[a]



[a] Reagents and conditions: (i) CMP-Neu5Ac, α-2,3-sialyltransferase, calf intestine alkaline phosphatase, BSA, $MnCl_2 \cdot 4H_2O$, Triton CF-54, 50 mM sodium cacodylate buffer (pH 7.4), 37 °C, 3 days, 80%; (ii) GDP-Fuc, α-1,3-fucosyltranseferase, calf intestine alkaline phosphatase, NaN_3, $MnCl_2 \cdot 4H_2O$, 100 mM HEPES buffer (pH 7.5), 37 °C, 3 days, 56%.

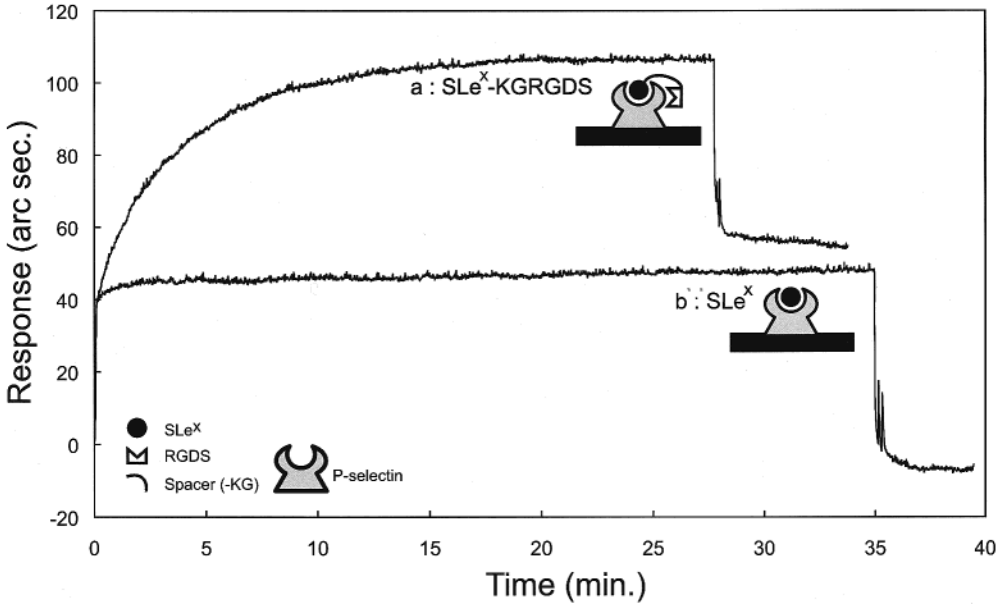


Figure 5. Binding curves for compound **1** or SLex to immobilized P-selectin at the same concentration ([ligand] = 0.66 mM). It was observed that most of compound **1** bound on the surface of the cuvette could not be replaced with 10 mM phosphate buffer solution containing 1 mM $CaCl_2$ (PBS/C), although SLex was completely removed by washing with PBS/C.

instance, the hydrophilic linkers prepared from ethyleneglycol derivatives as well as the insufficient spacing inevitably rendered unsatisfactory results of the sugar elongation reactions toward synthetic glycopolymers by some glycosyltransferases.[17c] Thus, the hexapeptide derivative bearing the *N*-acetyllactosamine side chain through an appropriate hydrophobic linker (**2**) was proven to become an excellent substrate for both recombinant rat α(2→3) sialyltransferase and recombinant human α(1→3) fucosyltransferase V. The sialylation and fucosylation of compound **2** proceeded smoothly, and the targeted glycopeptide **1** was isolated by purification with preparative HPLC in 45% overall yield from **2** (Scheme 3).

Biological Aspects. Association constants of **1** or related compounds with selectins were determined by the surface plasmon resonance (SPR) method. Since selectins are the receptors displayed on the surface of cell membranes, the interaction of the selectins immobilized on the surface of biosensor cuvettes with these synthetic ligands can be regarded as an experimental model of cell adhesion mediated by selectins and their ligands. Figure 5 is a typical binding profile between P-selectin with compound **1** or with SLex in the same ligand concentration (6.63×10^{-4} M). It was clearly suggested that compound **1** showed much higher affinity with the P-selectin immobilized cuvette than SLex. Kinetic and equilibrium constants of the interaction between selectins and all ligands used in this study are listed in Table 1. The results indicate that compound **1** binds P- and E-selectins more strongly than native SLex, and the association constants of **1** with P- and E-selectins were assumed to be 6.6×10^7 and 4.5×10^5 M, respectively. Since the SLex and simple derivatives such as glycosides and mimetics have been known to

Table 1. Kinetic and Equilibrium Constants of Synthesized Glycopeptides Against Selectins

selectin	constant	SLex–KGRGDS (**1**)	SLex	LacNAc–KGRGDS (**2**)	RGDS
P	k_{ass} (M^{-1} s^{-1})	978.1	229.6	114.5	89.1
	k_{diss} (s^{-1})	1.5×10^{-5}	2.6×10^{-4}	4.5×10^{-4}	9.2×10^{-4}
	K_a (M^{-1})	6.6×10^7	8.8×10^5	2.5×10^5	9.7×10^4
E	k_{ass} (M^{-1} s^{-1})	451.2	21.1	9.9	4.0
	k_{diss} (s^{-1})	1.1×10^{-3}	1.7×10^{-3}	1.3×10^{-3}	6.6×10^{-4}
	K_a (M^{-1})	4.1×10^5	1.2×10^4	7.6×10^3	6.1×10^3
L	k_{ass} (M^{-1} s^{-1})	180.1	312.7	29.8	4.1
	k_{diss} (s^{-1})	1.9×10^{-1}	3.6×10^{-1}	2.9×10^{-2}	7.7×10^{-4}
	K_a (M^{-1})	9.5×10^2	8.7×10^2	1.0×10^3	5.4

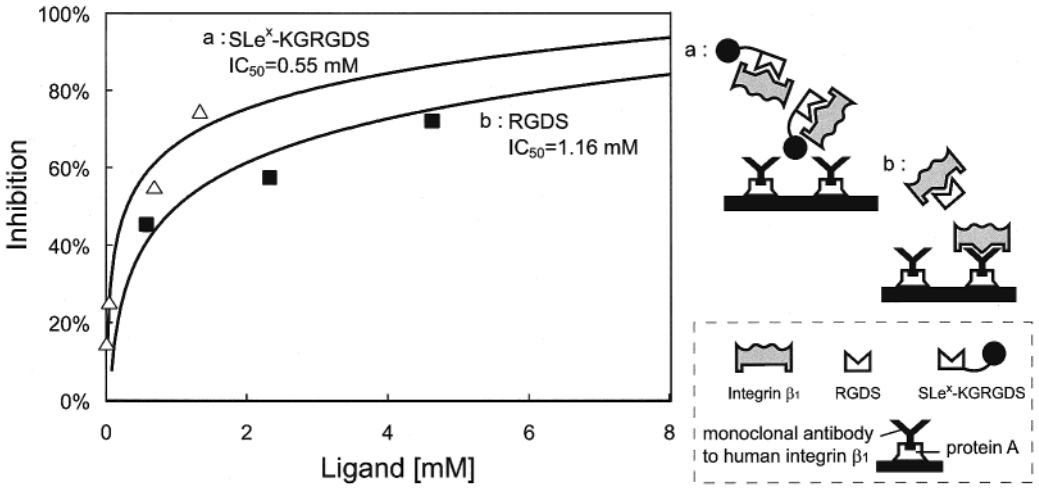


Figure 6. Inhibitory effect of glycopeptide **1** or RGDS on the interaction between integrin β_1 and immobilized monoclonal antibody to human integrin β_1.

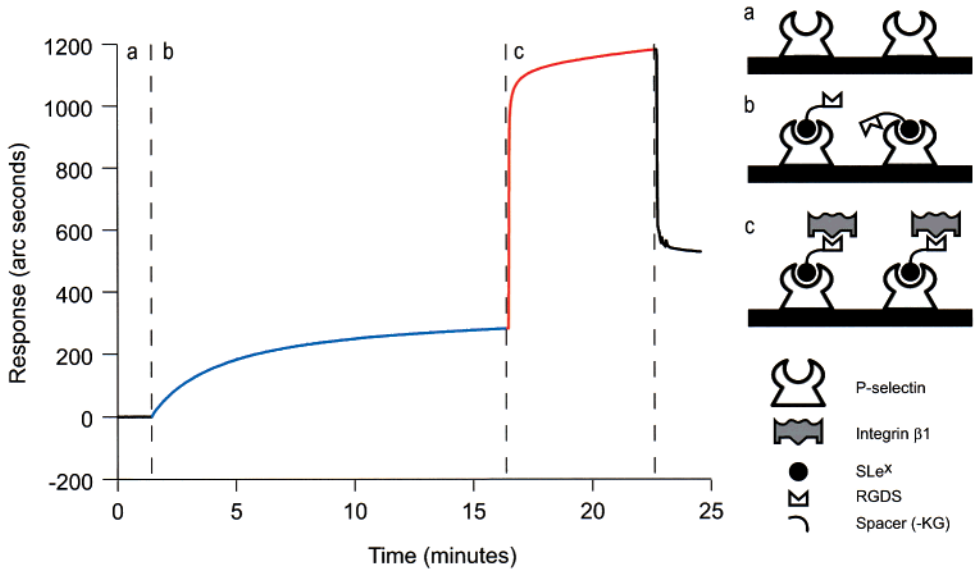


Figure 7. *Trans*-type interaction (cross-linking of selectin and integrin) by cell adhesive glycopeptide. The first line between "a" and "b" indicates the time point of the beginning of the injection of compound **1** (0.2 mL, 1.3 mg/mL) to the P-selectin- immobilized cuvette. The second line between "b" and "c" indicates the time point of the injection of integrin β_1 (0.2 mL, 9.1 μg/mL) to this cuvette. Integrin β_1 was found to bind to the glycopeptide−P-selectin complex as indicated by a significant increase in the response unit.

show the highest affinity with E-selectin among three selectins,[7] this result demonstrates that the specificity and the strength in the carbohydrate−protein interactions can be controlled by conjugating simple sugar ligands with some functional oligopeptides. Very low binding affinity with L-selectin was detected for compounds **1** and **2** as well as SLex. Next, the binding of compound **1** to integrin β_1 was discussed by the competition binding assays in comparison with the effect of RGDS. The inhibitory effect by the synthetic ligands on the binding of integrin β_1 with immobilized monoclonal antibody against integrin β_1 was measured and presented as a decrease in the binding activity, and the effect was dependent on the concentration of the ligands (Figure 6). It was found that **1** inhibited the interaction of human integrin β_1 with its monoclonal antibody more effectively than RGDS (IC_{50} = 0.55 mM). These experiments clearly demonstrate that the synthetic glycopeptide **1** exhibited an amplified affinity with selectins or integrins on the basis of the *cis*-type interactions as proposed in Figure 3A,B. Furthermore, our interest was focused on the cross-linking of these two different protein receptors by the glycopeptide **1**. Figure 7 shows a double-phase measurement of the P-selectin−compound **1**−integrin β_1 interaction carried out by using the P-selectin-immobilized optical biosensor cuvette. As shown in this binding profile, the surface of the immobilized P-selectin covered with compound **1** interacted tightly with integrin β_1, indicating that this heterobifunctional glycopeptide can combine P-selectin and integrin β_1 at the same time. Although the compound **1** has been supposed to form some stable compact structures (Figure 4A−D) rather than an extended conformation (Figure 4E) as predicted by the random conformational searching analysis, this result suggests that the spacer moiety of compound **1** is flexible enough to function as a ligand for *trans*-interaction.

As a preliminary biological application of the synthetic glycopeptide, we also examined the inhibitory effect of the glycopeptide **1** on the integrin-mediated

adhesion of activated T cells to collagen-coated plates as a cell migration model. It was found that **1** could block this interaction at the same level (52.8% inhibition) as the inhibition by a monoclonal antibody to integrin (60.7% inhibition). The SLex or RGDS did not show any significant inhibition under the same condition. Since this adhesion pathway is also very important in the regulation of lymphocyte migration into inflammatory tissues, it is strongly suggested that synthetic glycopeptide **1** will become a nice reagent, "cell adhesive glycopeptide", to modulate immune responses through its inhibitory effect on lymphocyte−endothelial cell interactions. Further biological, immunological, and clinical trials for compound **1** and analogues will be reported in the near future.

In conclusion, we succeeded in a practical chemoenzymatic synthesis of a novel class of heterobifunctional glycopeptide ligand that interacts with both selectins and integrins. The synthetic nonnatural glycopeptide showed extremely interesting biological properties as a potential candidate for a reagent to control cell adhesion. It should be noted that the concept described here is widely applicable for the synthesis of a variety of bioactive glycopeptides from the viewpoint of modulation and/or targeting of functional carbohydrates or peptides complementarily.

Experimental Section

General Procedure. Recombinant rat α-2,3-sialyltransferase, recombinant human α-1,3-fucosyltranseferase, CMP-Neu5Ac and GDP-fucose were purchased from Calbiochem Co. Ltd. Calf intestine alkaline phosphatase and protein A (soluble) were obtained from Sigma Co. Ltd. Recombinant human E-, P-, and L-selectins (soluble) were from R&D Systems Europe Ltd., U.K. Monoclonal antibodies to human integrin β_1/fibronectin receptor and integrin β_1 were obtained from TaKaRa Co. Ltd. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100.4 MHz, respectively, on a JEOL lambda 400 spectrometer; *J* values are given in Hz. Preparative HPLC was performed on a Hitachi HPLC system equipped with an L-6250 intelligent pump, L-7400 UV detector and reversed-phase C_{18} column, Inertsil ODS-3 (30 × 250 mm), at a flow rate of 30 mL min^{-1}. Analytical HPLC was performed on a Shimadzu HPLC system equipped with a LC-6A pump, SPV-6AV UV detector and reversed-phase C_{18} column, Inertsil ODS-3 (4.6 × 250 mm), at a flow rate of 0.5 mL min^{-1}. Matrix-associated laser-desorption ionization/time-of-flight/mass spectrometry (MALDI-TOF-MS) was performed on a Laser mat 2000 mass spectrometer. FAB-MS spectra were recorded on a JEOL JMS-SX102A spectrometer. The optical biosensor IAsys cuvette system with IAsys software and IAsys cuvettes coated with aminosilane were from Affinity Sensors (Cambridge, U.K.). Reactions were monitored by thin-layer chromatography (TLC) on precoated plates of silica gel 60F_{254} (layer thickness, 0.25 mm; E. Merck, Darmmstadt). Column chromatography was performed on silica gel (silica gel 60N, 100−210 μm; Kanto Chemical Co., Inc.) and flash column chromatography was performed on silica gel (silica gel 60N, 40−100 μm; Kanto Chemical Co., Inc.).

3-Carboxypropyl *O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside (6). A solution of potassium permanganate (384 mg, 2.43 mmol) in aqueous acetic acid (7 mL, H_2O: glacial acetic acid, 5:1) was cooled to 0 °C. Pentenyl glycoside **5** (533 mg, 0.76 mmol) in acetic acid (7 mL) was added to this solution in one portion and the mixture was stirred at 0 °C for 3 h. Then, the reaction mixture was diluted with ethyl acetate and to this solution were added solid sodium sulfite (623 mg, 4.94 mmol) and 1 M aqueous HCl (7 mL). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give **6** (479 mg, 87.3%): δ_H (400 MHz; $CDCl_3$) 1.90 (m, 2 H, OCH_2CH_2), 1.95−2.18 (all s, 21 H, 7 × $COCH_3$), 2.41 (m, 2 H, $OCH_2CH_2CH_2$), 3.51 (m, 2 H, OCH_2), 3.62 (m, 1 H, H-5), 3.79 (t, 1 H, *J* 7.21 and 8.79, H-4), 3.88−3.91 (m, 2 H, H-6′a and H-6′b), 4.02−4.16 (m, 3 H, H-2, H-5′ and H-6a), 4.41(d, 1 H, *J* 7.70, H-1), 4.50 (d, 1 H, *J* 8.06, H-1′), 4.48−4.51 (m, 1 H, H-6b), 4.98 (dd, 1 H, *J* 3.67 and 10.63, H-3′), 5.06 (dd, 1 H, *J* 8.25 and 9.69, H-3), 5.11 (dd, 1H, *J* 8.06 and 10.62, H-2′), 5.36 (d, 1 H, *J* 3.66, H-4′), 6.03 (d, 1 H, *J* 9.53, NH). Anal. Calcd for $C_{30}H_{43}NO_{19}$: C, 49.93; H, 6.00; N, 1.94. Found: C, 49.90; H, 5.98; N, 1.83.

Z-Lys[3-Carbamidopropyl *O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside]-OH (7). To a solution of **6** (530 mg, 0.734 mmol) in dry DMF (7 mL) at 0 °C were added *N*-hydroxysuccinimide (HONSu) (85 mg, 0.734 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) (167 mg, 0.807 mmol). The mixture was stirred at room temperature for 12 h. After filtration, the solution was evaporated under reduced pressure to give the crude active ester. To the solution of Z-Lys-OH (268 mg, 0.954 mmol) in dry DMF (4 mL) and water (1 mL) were added triethylamine (TEA) (260 μL, 1.84 mmol) and a solution of the active ester in DMF (5 mL), and the mixture was stirred at room temperature for 12 h. Next, pH of the mixture was adjusted by addition of 5% aqueous citric acid to pH 3 and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The organic layer was washed with 5% aqueous citric acid (2 × 20 mL) and brine (2 × 20 mL) and dried over $MgSO_4$. After filtration with Celite, the solution was evaporated under reduced pressure and the residue was purified by flash silica gel chromatography ($CHCl_3$−MeOH, 50:1 to 5:1, v/v) to give **7** (514 mg, 71.2%): δ_H (400 MHz; CD_3OD) 1.37 (m, 2 H, Lys-γ), 1.48 (m, 2 H, Lys-δ), 1.65 (m, 2 H, Lys-β), 1.81 (m, 2 H, OCH_2CH_2), 1.89−2.12 (all s, 21 H, 7 × $COCH_3$), 2.20 (t, 2 H, *J* 7.17 and 15.11, $OCH_2CH_2CH_2$), 3.13 (m, 2 H, Lys-ϵ), 3.51 (m, 2 H, OCH_2), 3.65 (m, 1 H, H-5), 3.75−3.85 (m, 3 H, H-4, H-6′a and H-6′b), 4.02 (dd, 1 H, *J* 4.89 and 7.94, Lys-α), 4.09−4.15 (m, 4 H, H-2, H-5′ and H-6a), 4.47−4.52 (m, 1 H, H-6b), 4.50 (d, 1 H, *J* 8.55, H-1), 4.69 (d, 1 H, *J* 7.94, H-1′), 4.99 (dd, 1 H, J 7.78 and 10.38, H-3′), 5.06−5.12 (m, 4 H, H-2′, H-3 and $CH_2C_6H_5$), 5.34 (d, 1 H, *J* 3.36, H-4′), 7.27−7.36 (m, 5 H, C_6H_5). Anal. Calcd for $C_{44}H_{61}N_3O_{22}$: C, 53.71; H, 6.25; N, 4.27. Found: C, 53.87; H, 6.13; N, 4.46.

Z-Lys[3-Carbamidopropyl *O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside]-Gly-OBzl (8). To the solution of **7** (921 mg, 0.936 mmol) and Gly-OBzl *p*-tosylate (379 mg, 1.12 mmol) in dry DMF (10 mL) were added diphenyl phosphorazidate (DPPA) (240 μL, 1.12 mmol) and TEA (290 μL, 2.06 mmol) and the mixture was stirred at 0 °C for 2 h and at room temperature for 22 h. The solution was filtrated and evaporated under reduced pressure. The residue was dissolved in ethyl acetate (10 mL) and the solution was washed with 5% aqueous citric acid (2 × 10 mL), brine (2 × 10 mL), 5% aqueous $NaHCO_3$ (2 × 10 mL) and brine (3 × 10 mL) and dried over $MgSO_4$. After filtration with Celite, the solution was evaporated under reduced pressure and the residue was purified by column chromatography ($CHCl_3$−MeOH, 30:1, v/v) to give **8** (881 mg, 83.2%): δ_H (400 MHz, $CDCl_3$) 1.10−1.36 (m, 2 H, Lys-γ), 1.38−1.51 (m, 2 H, Lys-δ), 1.58−1.72 (m, 2 H, OCH_2CH_2), 1.93−2.14 (all s, 21 H, 7 × $COCH_3$), 2.13−2.30 (m, 2 H, $OCH_2CH_2CH_2$), 3.21 (m, 2 H, Lys-ϵ), 3.47 (m, 2 H, OCH_2), 3.59 (m, 1 H, H-5), 3.78 (t, 1 H, *J* 6.84 and 13.67, H-4), 3.88 (t, 1 H, *J* 8.41 and 17.09, H-5′), 4.00−4.15 (m, 6 H, H-2, H-6a, H-6′a, H-6′b and Gly-α), 4.24 (m, 1 H, Lys-α), 4.43−4.52 (m, 2 H, H-1 and H-6b), 4.51 (d, 1 H, *J* 7.81, H-1), 4.97 (dd, 1 H, *J* 3.41 and 10.25, H-3′), 5.06−516 (m, 6 H, H-2′, H-3 and 2 × $CH_2C_6H_5$), 5.35 (d, 1 H, *J* 2.93, H-4′), 5.87 (d, 1 H, *J* 7.81, N*H*$COCH_3$), 6.47 (br, 2 H, Gly-NH and Lys-NH), 7.28−7.54 (m, 10 H, 2 × C_6H_5). Anal. Calcd for $C_{53}H_{70}N_4O_{23}$: C, 56.28; H, 6.24; N, 4.95. Found: C, 56.55; H, 6.37; N, 5.25.

Z-Lys[3-Carbamidopropyl *O*-(*β*-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-*β*-D-glucopyranoside]-Gly-OH (3). To the solution of **8** (500 mg, 0.442 mmol) in methanol

(5 mL) was added sodium methoxide (20 mg). The mixture was stirred at room temperature for 5 h and 1 M aqueous NaOH (5 mL) was added to the solution. The mixture was stirred at room temperature for 5 h, neutralized with ion-exchange resin (Dowex 50W-X8, H^+-form), filtered and evaporated under reduced pressure to give the product **3** (304 mg, 87.3%): δ_H (400 MHz, CD_3OD) 1.19−1.23 (m, 2 H, Lys-γ), 1.30−1.42 (m, 2 H, Lys-δ), 1.58 (m, 1 H, Lys-β), 1.70−1.73 (m, 3 H, OCH_2CH_2 and Lys-β), 1.87 (s, 3 H, $COCH_3$), 2.13 (m, 2 H, $OCH_2CH_2CH_2$), 3.06 (m, 2 H, Lys-ϵ), 3.27−3.89 (m, 16 H, H-2, H-2′, H-3, H-3′, H-4, H-4′, H-5, H-5′, H-6a, H-6b, H-6′a, H-6′b, OCH_2 and Gly-α), 4.03 (dd, 1 H, *J* 5.38 and 8.79, Lys-α), 4.28 (d, 1 H, *J* 7.32, H-1), 4.28 (d, 1 H, *J* 8.30, H-1′), 4.99 (q, 2 H, *J* 12.70 and 17.58, $CH_2C_6H_5$), 7.18−7.28 (m, 5 H, C_6H_5). Anal. Calcd for $C_{34}H_{52}N_4O_{17}$: C, 51.77; H, 6.65; N, 7.10. Found: C, 51.81; H, 6.67; N, 7.10.

Boc-Arg(N^gNO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (4). To a solution of Boc-Asp(OBzl) (1.26 g, 3.89 mmol) and Ser(Bzl)-OBzl hydrochloride (1.50 g, 4.67 mmol) in dry DMF (30 mL) were added DPPA (1 mL, 4.67 mmol) and TEA (1.3 mL, 9.34 mmol). The mixture was stirred at 0 °C for 2 h and at room temperature for 22 h. The solution was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and the solution was washed with 5% aqueous citric acid (2 × 30 mL), brine (2 × 30 mL), 5% aqueous $NaHCO_3$ (2 × 30 mL) and brine (3 × 30 mL) and dried over with $MgSO_4$. After filtration with Celite, the solution was evaporated under reduced pressure and the residue was purified by column chromatography with 3:1 hexane−ethyl acetate (v/v) as an eluant to give Boc-Asp-Ser(Bzl)-OBzl (2.06 g, 89.5%).

N-Protected dipeptide [Boc-Asp-Ser(Bzl)-OBzl, 1.91 g, 3.23 mmol] was dissolved in 2 M HCl/dioxane (50 mL) and the solution was stirred at room temperature for 3 h. The mixture was evaporated under reduced pressure and the residual syrup was employed for the next reaction without further purification.

To a solution of this amino component [H-Asp-Ser(Bzl)-OBzl] and Boc-Gly (622 mg, 3.55 mmol) in dry DMF (30 mL) were added DPPA (0.84 mL, 3.88 mmol) and TEA (0.99 mL, 7.11 mmol), and the mixture was stirred at 0 °C for 2 h and at room temperature for 22 h. The solution was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and the solution was washed with 5% aqueous citric acid (2 × 30 mL), brine (2 × 30 mL), 5% aqueous $NaHCO_3$ (2 × 30 mL) and brine (3 × 30 mL) and dried over $MgSO_4$. After filtration with Celite, the solution was evaporated under reduced pressure and the residue was purified by column chromatography with 3:1 hexane−ethyl acetate (v/v) as an eluant [Boc-Gly-Asp-Ser(Bzl)-OBzl, 1.89 g, 90.2%].

Boc-Gly-Asp-Ser(Bzl)-OBzl (2.18 g, 3.37 mmol) was dissolved in 2 M HCl/dioxane (50 mL) and the solution was stirred at room temperature for 3 h. The mixture was evaporated under reduced pressure and the residual syrup was used without further purification. To a solution of Boc-Arg(N^gNO_2) (1.29 g, 4.04 mmol) in dry tetrahydrofuran (THF) (30 mL) were added TEA (0.61 mL, 4.38 mmol) at room temperature and isobutyl chloroformate (IBCF) (0.631 mL, 4.85 mmol) at −15 °C, and the mixture was stirred at −15 °C for 10 min to give a mixed acid anhydride. The solution of the tripeptide [H-Gly-Asp-Ser-(Bzl)-OBzl] prepared above and TEA (0.52 mL, 3.71 mmol) in dioxane was mixed with the solution of a mixed acid anhydride and the reaction mixture was stirred at 0 °C for 2 h and room temperature for 22 h. The solution was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and the solution was washed with 5% aqueous citric acid (2 × 30 mL), brine (2 × 30 mL), 5% aqueous $NaHCO_3$ (2 × 30 mL), brine (3 × 30 mL) and dried over $MgSO_4$. After filtration with Celite, the solution was evaporated under reduced pressure and the residue was purified by column chromatography with 40:1 $CHCl_3$−methanol (v/v) as an eluant to give **4** (2.04 g, 71.3%): δ_H (400 MHz; $CDCl_3$) 1.90 (s, 9 H, *t*-Bu), 1.58−1.68 (m, 4 H, Arg-β, Arg-γ), 2.81 (dd, 1 H, *J* 4.58 and 16.63, Asp-β), 2.93 (dd, 1 H, *J* 5.90 and 16.63, Asp-β), 3.18 (m, 2 H, Arg-δ), 3.67 (dd, 1 H, *J* 3.21 and 9.61, Ser-β), 3.79 (d, 1 H, *J* 14.34, Gly-α), 3.89 (dd, 1 H, *J* 3.96 and 9.61, Ser-β), 4.04 (d, 1 H, H-2, *J* 14.34, Gly-α), 4.34−4.50 (m, 3 H, Arg-α and $OCH_2C_6H_5$), 4.70 (t, 1 H, *J* 3.90 and 7.78, Ser-α), 4.97 (dd, 1 H, *J* 6.72 and 12.82, Asp-α), 5.04−5.18 (m, 4 H, $COCH_2C_6H_5$ × 2), 7.20−7.30 (m, 15 H, C_6H_5 × 3). δ_C (100 MHz; $CDCl_3$) 24.49 (Arg-γ), 28.29 (CH_3), 30.22 (Arg-β), 36.49 (Asp-β), 40.43 (Arg-δ), 42.98 (Gly-α), 49.17 (Asp-α), 53.14 (Ser-α), 53.41 (Arg-α), 66.96, 67.31 (2 × $COCH_2C_6H_5$), 69.13 (Ser-β), 73.18 ($OCH_2C_6H_5$), 80.27 (*t*-Bu), 127.63, 127.80, 128.04, 128.37, 128.52, 135.12, 135.26, 137.31 (C_6H_5), 156.01 (Arg-ζ), 159.02, 169.18, 169.71, 170.62, 171.43, and 172.89 (CO). Anal. Calcd for $C_{41}H_{52}N_8O_{12}\cdot 0.5H_2O$: C, 57.40; H, 6.23; N, 13.06. Found: C, 57.30; H, 6.14; N, 12.99.

Z-Lys[3-Carbamidopropyl *O*-(β-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside]-Gly-Arg(N^gNO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (9). Protected RGDS derivative **4** (115 mg, 0.135 mmol) was dissolved in 2 M HCl/dioxane (2 mL) and the solution was stirred at room temperature for 3 h. The mixture was evaporated under reduced pressure and the residue was used for the next step without further purification. To a solution of H-Gly-Arg(N^g-NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl prepared from **4** and **3** (117 mg, 0.149 mmol) in DMF (5 mL) were added DPPA (32 μL, 0.149 mmol) and TEA (41.4 μL, 0.297 mmol), and the mixture was stirred at 0 °C for 2 h and at room temperature for 22 h. The solution was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (5 mL) and the solution was washed with 5% aqueous citric acid 2 × 5 mL), brine (2 × 5 mL), 5% aqueous $NaHCO_3$ (2 × 5 mL) and brine (3 × 5 mL) and dried over $MgSO_4$. After filtration with Celite, the solution was evaporated under reduced pressure and the residue was purified by column chromatography (30:1) $CHCl_3$−MeOH (v/v) as an eluant to give **9** (155 mg, 76.5%): δ_H (400 MHz; DMSO-d_6) 1.30−1.44 (m, 4 H, Lys-γ and Lys-δ), 1.53−1.63 (m, 3 H, Arg-γ and Lys-β), 1.68−1.78 (m, 5 H, Arg-β, Lys-β, OCH_2CH_2), 1.87 (s, 3 H, $COCH_3$), 2.12 (t, 2 H, *J* 7.57 and 15.14, OCH_2CH_2), 2.66 (dd, 1 H, *J* 4.88 and 16.11, Asp-β), 2.83 (dd, 1 H, *J* 8.30 and 16.11, Asp-β), 3.05 (m, 2 H, Lys-ϵ), 3.19 (m, 2 H, Arg-δ), 3.30−3.86 (m, 20 H, H-2, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6b, 6a′ and 6b′, Gly-α × 2, Ser-β, OCH_2), 4.00−4.11(m, 2 H, Arg-α and Lys-α), 4.27 (d, 1 H, *J* 7.32, H-1), 4.37 (d, 1 H, *J* 7.81, H-1′), 4.48−4.67 (m, 3 H, Ser-α, $OCH_2C_6H_5$), 4.86 (m, 1 H, Asp-α), 5.04−5.22 (m, 6 H, $COCH_2C_6H_5$ × 3), 7.30−7.44 (m, 20 H, C_6H_5 × 4). Anal. Calcd for $C_{69}H_{92}N_{12}O_{26}$: C, 55.05; H, 6.16; N, 11.16. Found: C, 55.13; H, 6.35; N, 11.36.

H-Lys[3-Carbamidopropyl *O*-(β-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside]-Gly-Arg-Gly-Asp-Ser-OH (2). To a solution of **9** (170 mg, 0.113 mmol) in (2:1) methanol−water (3 mL) was added palladium-activated carbon (Pd 10%) (10 mg). The mixture was stirred at room temperature under an atmosphere of H_2 gas for 2 days. After filtration with Celite, the solution was concentrated under reduced pressure and the residue was subjected to Sephadex G25 column chromatography using water as an eluant. Fractions containing the desired product were collected and combined, concentrated and freeze-dried to give powdery **2** (119 mg, 99.2%): δ_H (400 MHz; D_2O) 1.41−1.50 (m, 2 H, Lys-γ × 2), 1.53−1.61 (m, 2 H, Lys-δ × 2), 1.62−1.76 (m, 2 H, Arg-γ), 1.80−1.98 (m, 6 H, Arg-β, Lys-β, OCH_2CH_2), 2.03 (s, 3 H, $COCH_3$), 2.25−2.37 (m, 2 H, $OCH_2CH_2CH_2$), 2.84 (dd, 1 H, *J* 8.09 and 16.68, Asp-β), 2.97 (dd, 1 H, *J* 5.34 and 16.70, Asp-β), 3.13−3.26 (m, 2 H, Lys-ϵ), 3.55−4.11 (m, 22 H, H-2, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6b, 6a′, 6b′, Arg-δ, Gly × 2, Ser-β, OCH_2), 4.34−4.45 (m, 2 H, Arg-α and Lys-α), 4.44−4.78 (m, 3 H, H-1, H-1′ and Ser-α), 4.86 (t, 1 H, *J* 5.39 and 7.99, Asp-α). Anal. Calcd for $C_{41}H_{71}N_{11}O_{22}\cdot H_2O$: C, 45.26; H, 6.76; N, 14.16. Found: C, 45.55; H, 6.55; N, 14.52.

H-Lys[3-Carbamidopropyl *O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside]-Gly-Arg-Gly-Asp-Ser-OH (10). Bovine serum albumin (BAS) (10.0 mg) and manganese(II) chloride tetrahydrate (1.56 mg, 7.9 μmol) were dissolved in sodium cacodylate buffer (50 mM; 5.00 mL) and the pH of this solution

was adjusted by 0.25 M aqueous HCl to pH 7.40. To a solution of **2** (20 mg, 18.7 μmol) in the buffer solution (2 mL) were added Triton CF-54 (10 μL), cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP-NANA) (25 mg, 40.7 μmol), carf intestinal alkaline phosphate (CIAP) (20 units) and α-2,3-sialyltransferase (0.3 unit), and the mixture was incubated at 37 °C for 3 days. The solution was directly applied to a Sephadex G25 column (3.0 cm × 40 cm) and the fractions containing the desired product were collected, combined, concentrated and lyophilized to give crude amorphous powder. Next, the crude product was dissolved in water (1 mL) and the solution was subjected to preparative HPLC column and eluted with 65:35 acetonitrile−water (v/v) to afford **10** (20.2 mg, 79.5%): δ_H (400 MHz; D_2O) 1.12−1.24 (m, 2 H, Lys-γ × 2), 1.24−1.38 (m, 2 H, Lys-δ × 2), 1.38−1.48 (m, 3 H, Arg-γ × 2, Lys-β), 1.54−1.70 (m, 6 H, 3″ax, Arg-β × 2, Lys-β, OCH_2CH_2), 1.77 (s, 3 H, $COCH_3$), 1.82 (s, 3 H, $COCH_3$), 1.99−2.05 (m, 2 H, OCH_2-CH_2CH_2), 2.55 (dd, 1 H, *J* 4.73 and 12.52, 3″eq), 2.63 (dd, 1 H, *J* 8.09 and 16.63, Asp-β), 2.76 (dd, 1 H, *J* 5.34 and 16.63, Asp-β), 3.33−3.92 (m, 29 H, H-2, 2′, 3, 3′, 4, 4′, 4″, 5, 5′, 5″, 6a, 6b, 6a′, 6b′, 6″, 7″, 8″, 9″a and 9″b, Arg-δ × 2, Gly × 4, Ser-β × 2, OCH_2), 4.06−4.20 (m, 2 H, Arg-α and Lys-α), 4.29−4.37 (m, 3 H, H-1, H-1′ and Ser-α), 4.58−4.78 (HDO, Asp-α); FAB-MS (pos) 1361.582 ($M + H^+$), calcd 1361.581.

H-Lys{3-Carbamidopropyl *O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-(*β*-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-*O*-2-acetamido-2-deoxy-*β*-D-glucopyranoside}-Gly-Arg-Gly-Asp-Ser-OH (1). To a solution of **10** (15 mg, 10.9 μmol) in HEPES buffer (100 mM, pH 7.5; 1 mL) containing 10 mM manganese(II) chloride tetrahydrate and 1 mM sodium azide were added guanosine diphospho-fucose (GDP-Fuc) (10.3 mg, 16.4 μmol) and fucosyltransferase V (10 munit) and the solution was incubated at 37 °C. After 2 days, phosphatase alkaline (1 unit) was added to the solution and the mixture was incubated again at 37 °C for 1 day. The solution was applied to a Sephadex G25 column (3.0 cm × 40 cm) directly, and fractions containing the desired product were combined, concentrated and freeze-dried. The crude product was dissolved in water (1 mL) and purified by preparative HPLC column with 65:35 acetonitrile−water (v/v) to afford cell adhesive glycopeptide **1** (9.1 mg, 55.6%). The purity of the compound **1** was assumed to be more than 99% by using a Shimadzu HPLC system equipped with a LC-6A pump, SPV-6AV UV detector and reversed-phase C_{18} column, Inertsil ODS-3 (4.6 × 250 mm), at a flow rate of 0.5 mL min^{-1} with 65:35 acetonitrile−water (v/v): δ_H (400 MHz; D_2O) 1.00 (d, 3 H, *J* 6.52, Fuc-6), 1.12−1.25 (m, 2 H, Lys-γ × 2), 1.28−1.38 (m, 2 H, Lys-δ × 2), 1.38−1.48 (m, 2 H, Arg-γ), 1.60−1.73 (m, 7 H, NeuNAc-3ax, Arg-β, Lys-β, OCH_2CH_2), 1.85 (s, 3 H, $COCH_3$), 1.86 (s, 3 H, $COCH_3$), 2.25−2.12 (m, 2 H, OCH_2-CH_2CH_2), 2.60 (dd, 1 H, *J* 4.73 and 12.52, NeuNAc-3eq), 2.67 (dd, 1 H, *J* 7.92 and 16.76, Asp-β), 2.79 (dd, 1 H, *J* 5.36 and 16.76, Asp-β), 2.94−3.05 (m, 2 H, Lys-ϵ), 3.34−3.92 (m, 33 H, GlcNAc-2, 3, 4, 5, 5, 6a, 6b, Gal-2, 3, 4, 5, 6a, 6b, NueNAc-4, 5, 6, 7, 8, 9a, 9b, Fuc-2, 3, 4, 5, Arg-δ, Gly × 2, Ser-β, OCH_2), 4.13 (dd, 1 H, *J* 6.26 and 7.68, Lys-α), 4.24 (t, 1 H, *J* 4.58 and 5.34, Arg-α), 4.33−4.41 (m, 3 H, GlcNAc-1, Gal-1 and Ser-α), 4.63−4.71 (m, 1 H, Asp-α), 4.93 (d, 1 H, *J* 3.97, Fuc-α); δ_C (100 MHz; D_2O) 15.98 (Fuc-6), 22.34 (Lys-γ), 22.74 and 22.93 (NHAc), 24.93 (Arg-γ), 26.10 (Lys-δ), 28.69 (Arg-β), 32.02 (Lys-β), 32.03 (OCH_2CH_2), 32.89 ($OCH_2CH_2CH_2$), 36.35 (Asp-β), 40.50 (Lys-ϵ), 41.20 (Arg-δ), 42.88 and 43.15 (Gly-α × 2), 50.72 (Asp-α), 53.27 (Ser-α), 99.32 (Fuc-1), 100.37 (NeuNAc-1), 101.70 (GlcNAc-1), 102.34 (Gal-1); FAB-MS (pos) 1507.637 ($M + H^+$), calcd 1507.638.

Conformational Analysis. Random conformational searching method of the SYBYL/advanced computation module developed by Chang et al.[13] was used for conformational prediction of the glycopeptide **1**. One cycle of conformatinal searching was composed of random adjustment of selected torsions in this molecule and minimization of the structure according to the procedure reported previously.[18] After 1000 cycles of minimization calculation 79 conformations were generated as conformers with different energy minima.

Preparation of Selectin-Immobilized Optical Biosensor Cuvettes for SPR Measurements. Immobilization of recombinant P-, E-, and L-selectins onto activated aminosilane sufaces was performed according to the manufacturer's specifications.[18] Briefly, after equilibration and obtaining a stable baseline with phosphate buffer solution (PBS; 10 mM, pH 7.7), the optical biosensor cuvette coated with aminosilane was activated three times with 0.2 mL of bis(sulfosuccinimidyl)-suberate (BS^3) in PBS (0.56 mg/mL) to activate the cuvette surface for 10 min. The activation solution was removed by washing with PBS, and 0.2 mL of E-, P- or L-selectin solution (100 $\mu g/cm^{-3}$ in PBS) was added for 15 min. The remaining active sites were blocked with 0.2 mL of BSA solution (2 mg/cm^{-3} in PBS) for 15 min. Finally, selectin-immobilized cevettes were washed three times with PBS (10 mM, pH 7.2) containing 1 mM $CaCl_2$ (PBS/C) to establish a stable baseline.

Binding Assay of Interactions between Selectins and Glycoligands. All binding experiments in the IAsys instrument were carried out using 10 mM PBS/C at 25 °C. Binding of ligand onto the cuvette was monitored for 20 min using 0.200 mL of sample solution. First, 0.19 mL of PBS was added to the cuvette to obtain a stable baseline. Next, 0.01 mL of ligand solution was added to this cuvette. The association was ended by aspiration of the sample and replacing it with the same volume of PBS/C. Monitoring was continued for a couple of minutes. For displacement studies, the same protocol was used by using a PBS containing 0.05% TRITON X-100 (PBS/T) instead of PBS/C.

Immobilization of Monoclonal Antibody to Human Integrin β_1 on the Biosensor Surface (mAb-protein A cuvette). Prior to the immobilization of monoclonal antibody, optical biosensor cuvette was coated with protein A in order to achieve successful orientation of Fab domain of antibody. Protein A was immobilized according to the same manner as that of immobilization of selectins described above. Aminosilane-containing cuvette was washed three times with 0.2 mL of BS^3 in PBS (10 mM, pH 7.7) (0.56 mg/mL) to activate the surface. Next, 0.2 mL of protein A solution (100 μg/mL) was added to the activated cuvette and incuvated for 15 min. The cuvette was washed three times with PBS and followed by blocking with BSA solution (2 mg/mL). Protein A-immobilized cuvette was reactivated with BS^3 in the same way and followed by treating with 0.190 mL of PBS (pH 7.4) and 0.010 mL of mouse monoclonal antibody to human integrin β_1/fibronectin receptor (2.0 mg/mL in 10 mM PBS containing 1.0% BSA and 0.1% NaN_3, pH 7.4). After blocking with BSA solution (2 mg/mL), cuvette was washed 10 times with PBS/T. The binding activity of this cuvette with integrin β_1 was evaluated by using a solution of integrin β_1 (3.2 μg/mL) as a standard.

Inhibitory Effect of Glycopeptide 1 or RGDS on the Interaction of Human Integrin β_1 with Its Monoclonal Antibody. All measurements were carried out in 10 mM PBS containing 1 mM $CaCl_2$ (PBS/C, pH 7.2). First, 0.18 mL of PBS/C was added to mAb-protein A cuvette and baseline was stabilized. Then, to this cuvette were added 0.01 mL of ligand solution and 0.01 mL of integrin β_1 solution (3.2 μg/mL). The decrease of the binding activity of integrin β_1 to the cuvette were determined and used for evaluating the inhibition effect by ligands. Measurements were performed in the concentration range of ligands from 0 to 10 mM, respectively.

Inhibitory Effect on the Adhesion of Activated T Cells with Collagen-Coated Plate. (A) Preparation of Th2 Cells. CD4+CD45RB+ native T cells obtained from DO11.10 $OVA_{323-339}$-specific T cell receptor transgenic mice were stimulated with 10 μg/mL $OVA_{323-339}$ peptide in the presence of 1 ng/mL IL-4, 50 μg/mL anti-IFN-γ mAb, 50 μg/mL anti-IL-12 mAbs and 20 U/mL IL-2. Cells were cultured with RPMI1640 supplemented with 10% fetal calf serum (FCS).

(B) Cell Adhesion Assay. Th2 cells were suspended at 5 × 10^6 cells/mL and 1 mL of this solution was cultured using biocoat culture ware, mouse collagen IV 35 mm dish (Collaborative Biomedical Products, MA). To facilitate cell adhe-

sion, 20 ng/mL phorbol myristate acetate (PMA) was added to the culture system. After 30 min, nonadherent cells were harvested and counted for the estimation of the ratio of adherent cells/total cells cultured. This assay was carried out in the presence of synthetic glycopeptide or anti-integrin mAb.

Acknowledgment. This work was supported by a grant from NEDO for "Research and Development on Glycocluster Controlling Biomolecules" and by a grant-in-aid for scientific research from the Ministry of Education, Science, and Culture (Grants 11480157 and 12555256). We also thank Ms. Akiko Maeda, Ms. Hiroko Matsumoto, and Ms. Seiko Oka of the Center of Instrumental Analysis, Hokkaido University, for measuring elemental analyses and mass spectroscopy.

Supporting Information Available: Spectral data for all new compounds and details for biological evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM000295R