Studies on Selectin Blockers. 5. Design, Synthesis, and Biological Profile of Sialyl Lewis x Mimetics Based on Modified Serine−Glutamic Acid Dipeptides

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We have rationally designed a sLe^x mimetic based on molecular modeling, synthesized type II and type II′ β-turn dipeptides (**3a,b**), and evaluated their biological profiles both *in vitro* and *in vivo*. Against E-selectin−sLe^x binding, the type II β-turn dipeptide L-Ser-D-Glu **3a** (IC_{50}, 13 μM) and the type II′ β-turn dipeptide D-Ser-L-Glu **3b** (IC_{50}, 5.5 μM) were 20−100-fold more potent blockers than sLe^x (**1**; IC_{50}, 600 μM) and a 3′-sulfated Le^x analog (**2**; IC_{50}, 280 μM). On the other hand, other stereoisomers, such as L-Ser-L-Glu **3c** and D-Ser-D-Glu **3d**, were very weak blockers, with IC_{50} > 1000 μM for both **3c,d**. Against the P- and L-selectins, despite much different stereochemistry of compounds **3a**−**d**, the dipeptides **3a**−**d** were all more potent blockers than either sLe^x or compound **2**. Interestingly, compound **3b** provided significant *in vivo* efficacy against an immunoglobulin E-mediated skin reaction in a mouse model. These findings indicate that sLe^x mimetics with type II and type II′ β-turn dipeptides could be useful in the design of an active selectin blocker *in vitro* and/or *in vivo*.

Introduction

Selectins are believed to be involved in the progression of the clinical manifestations of complicated diseases, such as chronic inflammation,[1] and have demonstrated a role in the inflammatory response. In addition, a recent exciting report described that sialyl Lewis x (**1**, sLe^x), which is a ligand for endothelial-leukocyte adhesion molecules, inhibited angiogenesis both *in vitro* and *in vivo*.[2] Such a biologically integral function makes sLe^x mimetics an attractive target for the therapy of these diseases.

We have already reported the design, synthesis, and biological profile of a 3′-O-sulfated Le^x (**2**) containing a branched alkyl chain, such as a natural ceramide, a potent selectin blocker.[3] In addition, our previous studies[4] of molecular dynamics simulations of the compound **2**/E-selectin complex suggested the existence of three essential binding sites of compound **2** for E-selectin, as follows (Figure 1): (1) The C-2 and C-3 hydroxy groups of fucose coordinate to calcium. (2) The branched alkyl chain of **2** interacts with two hydrophobic regions on the surface of E-selectin. (3) The negatively charged group of the ligand interacts with the basic residues of E-selectin.[5] On the basis of the information concerning the essential binding sites of compound **2**, we sought to mimic compound **2** by a simple skeleton. To design simple sLe^x mimetics, we focused on compound libraries with simple peptide backbones, due to the wealth of three-dimensional (3-D) analysis data of dipeptides.[6] In docking models of compounds/E-selectin, we have screened dipeptide backbones conserving the positions of the three essential functional groups for binding to E-selectin and have designed a dipeptide backbone, Ser-Glu, as a rational mimetic of compound **2**. To provide support for the structural hypothesis that led to the design of the rational mimetics based on molecular modeling, we synthesized the type II and type II′ β-turn dipeptides (**3a,b**) (Chart 1) and evaluated their biological profiles *in vitro* and *in vivo*.

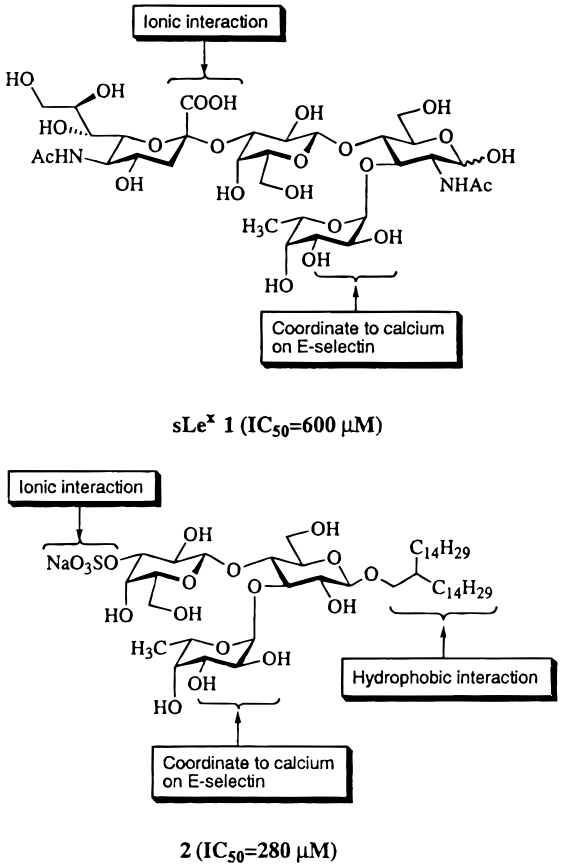

Figure 1. Binding sites and IC_{50} of sLe^x and compound **2** toward E-selectin.

In this paper, we describe the derivation strategy for an active pharmacophore and a conceptual design of sLe^x mimetics using MD simulation. We also describe the synthesis of sLe^x mimetics and the evaluations *in vitro* and *in vivo*.

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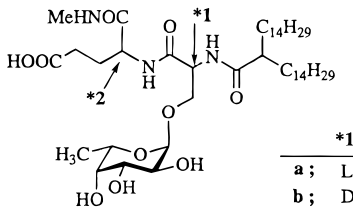
Chart 1



3a-d

	*1	*2
a;	L	D
b;	D	L
c;	L	L
d;	D	D

Results and Discussion

Initial Model Construction and Molecular Dynamics Simulation of the Interaction Between Compounds 3a−d and E-Selectin. The initial attempt at docking a peptide backbone containing the three essential functional groups, the fucose, the carboxylic acid, and the hydrophobic branched alkyl chain, on E-selectin produced almost the same interaction modes as those observed in our previously reported complex model of compound **2**/E-selectin.[4] Next, we explored the three essential binding sites to E-selectin by conformational analyses using a molecular dynamics (MD) technique.[7] The crystal structure[8] of the lectin domain of E-selectin was obtained from the Protein Data Bank (identity code: 1ESL). We undertook the model building of the complex of compounds **3a−d** with E-selectin.

First, we constructed the initial bound model of **3a** to conserve the interactions of the three functional groups with E-selectin. The 2- and 3-OH groups of the fucose were coordinated to the calcium, and the carboxylate of the D-Glu was directed to Arg97. Both alkyl chains (chain A and chain B) were also directed to each hydrophobic region on the surface of E-selectin; namely, chain A interacted with one hydrophobic region consisting of Lys111, Lys112, Lys113, Ala9, and Leu114, and chain B interacted with another hydrophobic region consisting of Tyr44, Pro46, and Tyr48.

Next, we subjected the initial bound model of **3a** to a 200-ps MD simulation with the explicit water solvent and evaluated the dynamic behavior of the model in solution. Figure 2 shows the analytical data of the interactions between the Glu in compound **3a** and the basic residue on E-selectin, as well as the conformational change of the peptide portion in compound **3a**. As illustrated in Figure 2, the initial interaction between the carboxylate of **3a** and Arg97 in E-selectin was broken immediately following the MD calculation. Instead, the interaction of **3a** with Lys111 was observed at about 130 ps. The hidden surface area of A chain retained the initial value and fluctuated minimally. On the other hand, although the hidden surface area of chain B fluctuated greatly, the hydrophobic interaction of chain B with proteins would be retained, because the hidden surface area of chain B retained more than one-half of the initial value (Figure 3). Surprisingly, although **3a** did not assume the β-turn conformation in the initial bound model, it was found that the final form of **3a** bound to E-selectin was characterized by the type II β-turn formation.

The initial bound model of **3b** was constructed in a similar manner to **3a**; however, the carboxylate of L-Glu was directed to Lys111 like the bound form of **3a**. In the case of **3b**, the initial bound conformation was

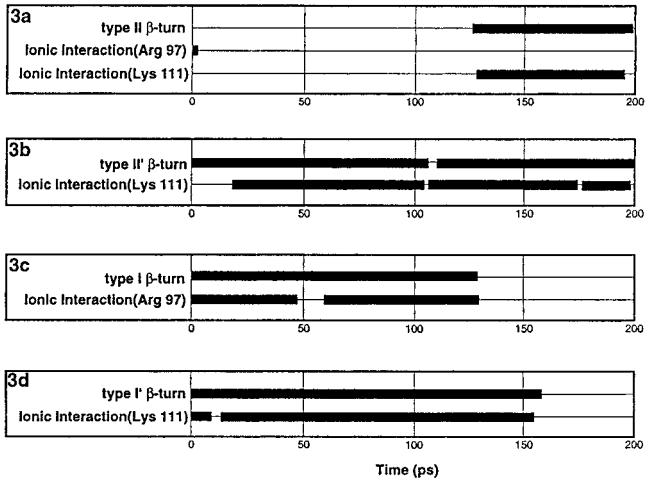


Figure 2. Evaluation of dynamic behavior of the model in solution. Bold lines indicate the existence of the β-turn formation and the ionic interaction between compounds **3a−d** and E-selectin.

characterized by the type II′ β-turn formation. As shown in Figure 2, the ionic interaction between L-Glu−Lys 111 and the type II′ β-turn conformation were conserved during the simulation. The hydrophobic interaction between this chain and the protein still existed and did not completely disappear (Figure 3). The snapshots of the trajectory show the stability of the interactions involved in the branched alkyl chain (Figure 4).

We also constructed the initial bound models of **3c,d** and subjected their initial bound models to a 200-ps MD simulation. However, stable bound forms with E-selectin were not observed.

Drug Design Based on Molecular Modeling. Using the MD simulation, we screened some conformers involving a tight binding form with E-selectin at the three essential binding sites. The MD calculation was carried out to investigate the binding mode of **3a−d** with E-selectin, including the explicit solvent water. As a result, we found that the dipeptides **3a,b** could bind to E-selectin (Figure 4); namely, the important three binding sites of **3a,b**, e.g., the fucose, the branched alkyl chain, and the negatively charged group, tightly interacted with E-selectin. Especially, the negatively charged groups of **3a,b**, the carboxylic acids of D-Glu and/or L-Glu, directly formed an ion pair with Lys111 on E-selectin. This interaction was different from that of compound **2**.[5] As illustrated in Figure 4, the most characteristic structures of compounds **3a,b** involve β-turn formations: the type II β-turn for **3a** and the type II′ β-turn for **3b**. Although the β-turn formation is often observed at a tetrapeptide unit, as an intramolecular hydrogen bond between the *i*th amide oxygen and the *i*+3th amide proton, it is also possible that dipeptides modified at the N- and C-termini with amide bonds could form a β-turn involving an intramolecular hydrogen bond.[9] Figure 5 shows the torsion angle values of **3a,b** and the type II and type II′ β-turn tetrapeptides. Both torsion angle values of **3a,b** were similar to those of the type II and type II′ β-turn tetrapeptides, respectively. These β-turn structures would play important roles in conserving the positions of the three essential functional groups, e.g., the fucose, the branched alkyl chain, and the negatively charged group, for binding to

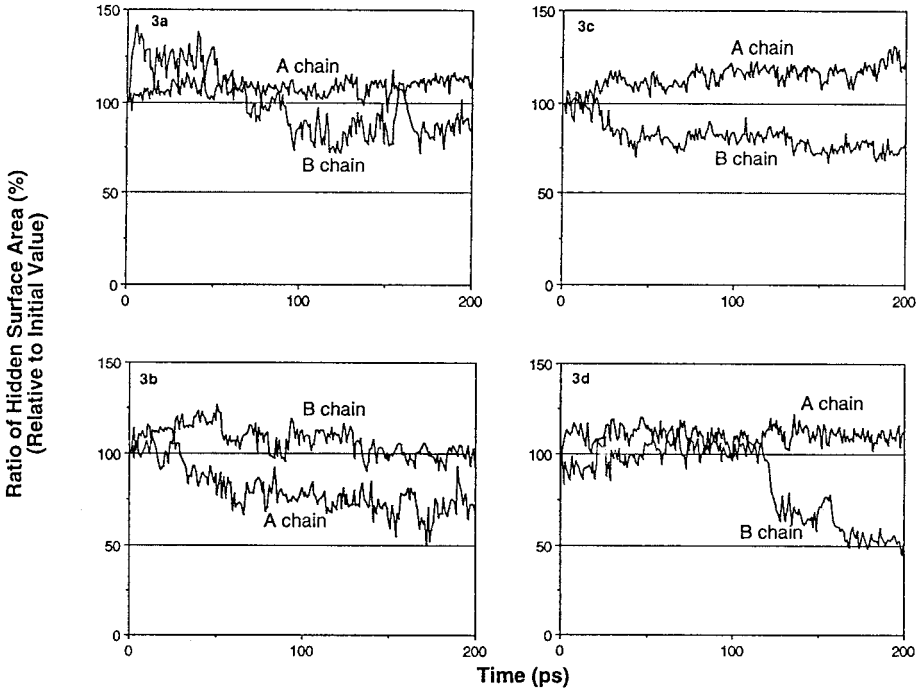


Figure 3. Change of the ratio of hidden surface area of alkyl chain portions in **3a**−**d** during the simulation in solution: ratio of the alkyl chain buried in the cavity consisting of Lys111, Lys112, Lys113, Ala9, and Leu114 (A chain) and ratio of the alkyl chain on the surface of Tyr44, Pro46, and Tyr48 (B chain). Values relative to those in the initial model are shown.

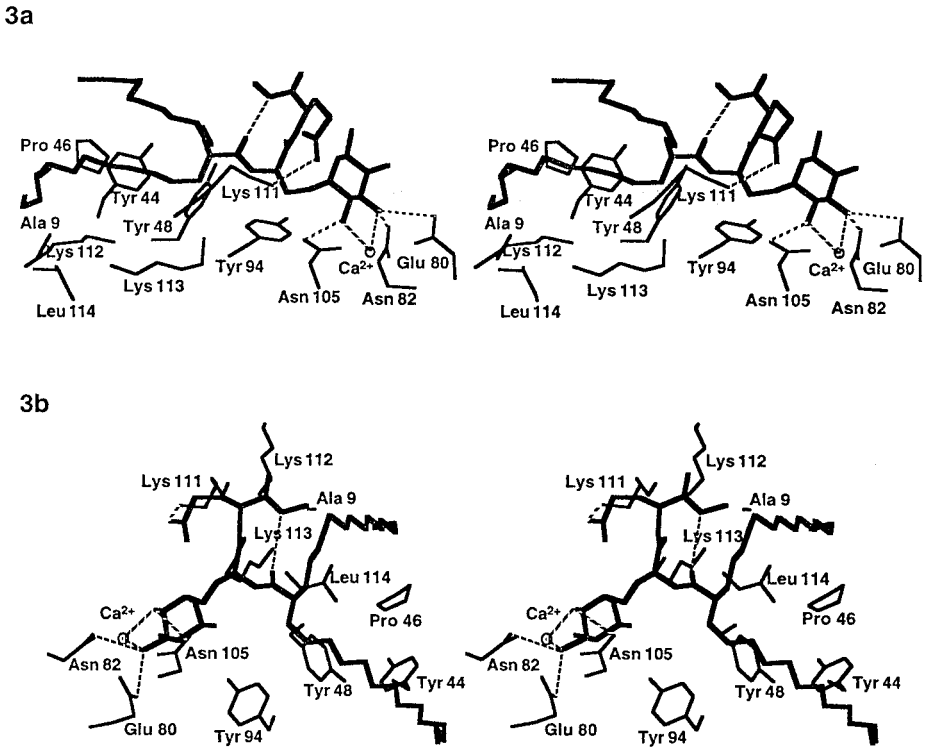
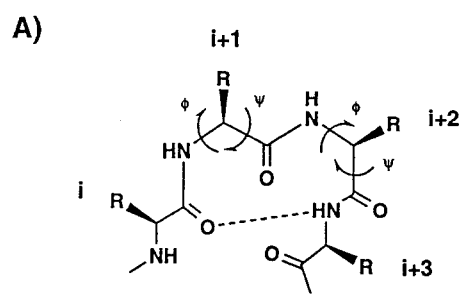


Figure 4. Stereorepresentation of the models of E-selectin and **3a** or **3b** complex models in solution. These models are the equilibrium structures during MD calculation (200 ps). The structures of **3a,b** are indicated by thick lines. Thin lines indicate the structure of E-selectin. Only the residues in the ligand-binding site on the protein are shown. Dashed lines indicate interactions (hydrogen bond, electrostatic interaction, calcium coordination).

E-selectin. On the other hand, the other stereoisomers (**3c,d**) did not bind to E-selectin.

Chemistry. For the syntheses of the intermediates **7a**−**d** for dipeptides **3a**−**d** (Scheme 1), enantiomeric Glu units, an L-Glu unit (L-**4**) and a D-Glu unit (D-**4**), which are commercially available, were treated with 40% methylamine/methanol using a mixed-anhydride method[10] to afford the corresponding enantiomers (L-**5**, D-**5**) in quantitative yields. The deprotection of the Boc groups of the enantiomers (L-**5**, D-**5**) with 4 N HCl in 1,4-dioxane at room temperature followed by the coupling with enantiomeric Ser units (L-**6**, D-**6**) in the presence of WSC, 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride, and HOBt, 1-hydroxy-1*H*-benzotriazole monohydrate, gave the Ser-Glu dipeptides **7a**−**d** in 80−88% yields.

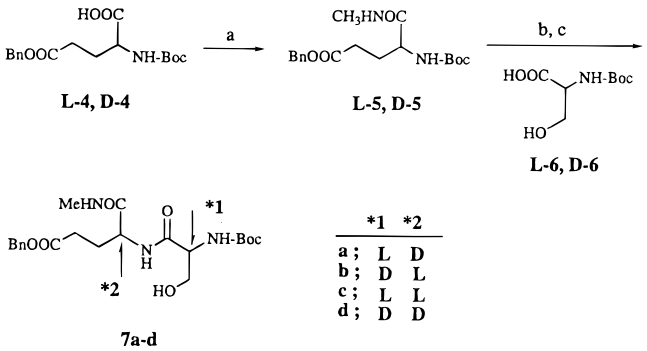


	i+1		i+2	
	φ	ψ	φ	ψ
type II	−60	120	80	0
type II′	60	−120	−80	0

			Ser		Glu	
	Ser	Glu	φ	ψ	φ	ψ
3a (type II)	L	D	−55	107	63	17
3b (type II′)	D	L	65	−129	−83	−5

Figure 5. (A) Torsion angles (deg) of two types of β-turns (type II and type II′) with the tetrapeptide. Each residue is denoted as *i*, *i*+1, *i*+2, *i*+3. The types of β-turns are defined by the main chain torsion angles of the *i*+1th and *i*+2th residues. (B) Torsion angles (deg) of **3a,b** Ser-Glu main chain of the complex models with E-selectin. **3a** structure is classified as type II β-turn; **3b** is type II′.

Scheme 1[a]



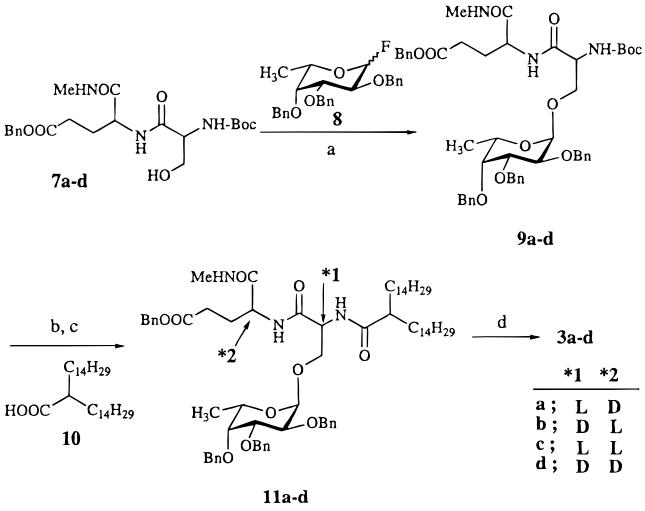
	*1	*2
a;	L	D
b;	D	L
c;	L	L
d;	D	D

[a] Conditions: (a) 40% CH_3NH_2/MeOH, Cl-COOEt, Et_3N, 86−90%; (b) 4 N HCl/1,4-dioxane; (c) **L-6**, **D-6**, WSC, HOBt, 80−88% from **L-5**, **D-5**.

For the syntheses of compounds **3a**−**d**, the glycosylation of **7a**−**d** with 2,3,4-tri-*O*-benzylfucose (**8**),[11] in the presence of $SnCl_2$/AgOTf as promoters and TMU, tetramethylurea, as a weak base, yielded mainly the α-glycosides **9a**−**d**. The deprotection of the Boc groups of **9a**−**d** with TFA in chloroform followed by the coupling with 1-tetradecylhexadecanoic acid (**10**) in the presence of WSC and HOBt for the amide linkage afforded compounds **11a**−**d** in 65−72% yields. Finally, compounds **11a**−**d** were transformed, by removal of the protecting benzyl group under general catalytic reduction conditions, into the desired compounds **3a**−**d**, as shown in Scheme 2.

Biological Activities: Inhibition Assay of E-, P-, and L-Selectin−sLe^x Binding. The method using selectin-IgG chimeras reported by Foxall et al. was followed.[12] As shown in Table 1, both dipeptides **3a,b** were more potent (IC_{50}, 13 μM for **3a**, 5.5 μM for **3b**) than compound **2** (IC_{50}, 280 μM) in the ligand−E-selectin competitive binding assay. In contrast, the other stereoisomers (**3c,d**) showed very weak blocking activities (IC_{50}, >1000 μM). It was interesting to note that the biological activities of compounds **3a**−**d** corresponded well with the predictions from the molecular modeling studies, and the remarkable change of the activities was due to the stereochemistry of the Ser-Glu dipeptide backbones; namely, the heterochiral amino acids, **3a** (L-Ser-D-Glu) and **3b** (D-Ser-L-Glu), were type II and type II′ β-turn dipeptides, respectively, and the β-turn formations of compounds **3a,b** could play important roles in favorable binding to E-selectin. In addi-

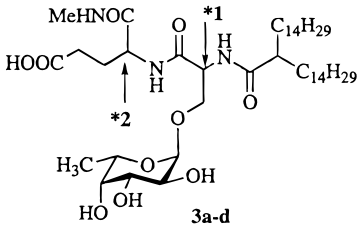
Scheme 2[a]



	*1	*2
a;	L	D
b;	D	L
c;	L	L
d;	D	D

[a] Conditions: (a) $SnCl_2$, AgOTf, TMU, 4A MS/$CHCl_3$, 60−66%; (b) TFA/$CHCl_3$; (c) WSC, HOBt, 65−72% from **9a**−**d**; (d) $Pd(OH)_2$/MeOH, 72−97%.

Table 1. Blocking Activity of Compounds **2**, **3a**−**d**, and sLe^x (**1**)



			IC_{50}, μM		
compd	*1	*2	E-selectin	P-selectin	L-selectin
3a	L	D	13	0.5	6.0
3b	D	L	5.5	0.7	9.4
3c	L	L	>1000	1.9	39
3d	D	D	>1000	2.1	18
sLe^x			600	>1000	>1000
2			280	100	30

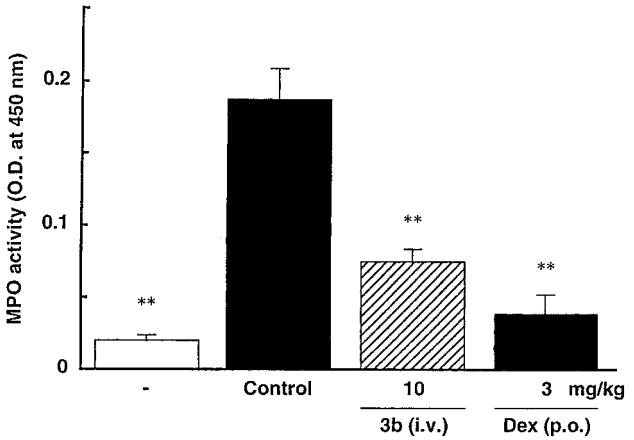


Figure 6. Effects of compound **3b** and dexamethasone (Dex) on leukocyte infiltration into skin tissue induced by antigen in actively sensitized mice. Compound **3b** was given intravenously immediately before the antigen challenge. Dexamethasone was given orally 2 h before the antigen challenge. Each column represents the mean of 5 or 6 animals. Vertical bars indicate SE; $^{**}p < 0.01$, significantly different from control group; −, nonsensitized mice.

tion, the inhibitory activities of the other stereoisomers, **3c** (L-Ser-L-Glu) and **3d** (D-Ser-D-Glu), supported the importance of the type II and type II′ β-turn formations for the binding to E-selectin, because homochiral amino acids, such as L-Ser-L-Glu and/or D-Ser-D-Glu, generally do not assume type II and type II′ β-turn formations, owing to the unfavorable steric interaction between the carbonyl oxygen of the i+1th central amide group and the side chain of the i+2th residue.[13]

On the other hand, all of the dipeptides **3a**−**d** (IC_{50}s, 0.5−2.1 μM) were more potent blockers toward P-selectin than compound **2** (IC_{50}, 100 μM); especially, dipeptides **3a,b** showed 140−200-fold more potent blocking activity (IC_{50}, 0.5 μM for **3a**, 0.7 μM for **3b**) than compound **2**. Furthermore, the blocking activities of compounds **3c,d** toward L-selectin were similar to that of compound **2** (IC_{50}, 36 μM); however, compounds **3a,b** still showed 4−6-fold more potent blocking activity (IC_{50}, 6.0 μM for **3a**, 9.4 μM for **3b**) than compound **2**. Toward the P- and L-selectins, all of the dipeptides **3a**−**d** showed potent blocking activities, in spite of their different stereochemical features. These findings indicate that the ligand-binding site to E-selectin would be different from those to the P- and L-selectins.

***In Vivo* Activities: Effects on IgE-Mediated Skin Reaction in Mouse Ears.** Next, we estimated the *in vivo* activity of compound **3b**, which was the most potent *in vitro*. The *in vivo* efficacy of compound **3b** was assessed according to a previous paper.[3] Compound **3b** was administered intravenously at 5 h after an ovalbumin (OA) challenge. Measurements of neutrophil infiltration in mouse ears were made at 24 h, as assessed by myeloperoxidase (MPO). As indicated in Figure 6, the IgE-mediated skin reactions in the mouse ears were significantly inhibited by dexamethasone at 3 mg/kg, po. In addition, compound **3b** (10 mg/kg, iv) also showed a reduced MPO content in the mouse ears. The IgE-mediated skin reactions have been studied in an experiment using mouse E-, P-, and L-selectin mAb, and the requisite role of selectins in this model has been confirmed.[14] Therefore, the *in vivo* efficacy of compound **3b** in the IgE-mediated skin reaction seems to be due to the selectin blocking activities.

In conclusion, we succeeded in creating rational mimetics of a sugar epitope by a simple peptide backbone, based on molecular modeling. The sLe^x mimetics developed involve the dipeptide backbones, the L-Ser-D-Glu, and the D-Ser-L-Glu, which are characterized by type II and type II′ β-turns. Both β-turn dipeptides **3a,b** showed 50−100-fold more potent inhibitory activity than sLe^x and a 3′-sulfated Le^x derivative (**2**) *in vitro*. In addition, compound **3b** also showed significant *in vivo* activity in an IgE-mediated mouse skin reaction model. These findings indicate that compound **3b** could be an effective antiinflammatory candidate.

Experimental Section

Simulation of the Complex in Solution Using Molecular Dynamics. The models of complexes were solvated by the addition of TIP3P water molecules[15] which were added within 25 Å from the center of each compound **3a**−**d**. The water molecules were then minimized until the root mean square of the gradients was below 0.05 kcal/(mol·Å). Following the minimization, the water molecules were equilibrated by a 5-ps MD calculation at a temperature of 298 K. An integral time step $\Delta t = 2$ fs was used. After that, all of the water molecules, compounds **3a**−**d**, and the binding sites on E-selectin (Ser6-Met10, Ser43-Tyr94, Trp76-Arg84, Val91-Val101, Trp104-Ala115) were minimized until the root mean square of gradients was below 0.05 kcal/(mol·Å). Part of the system was then subjected to a 200-ps MD simulation at a temperature of 298 K. An integral time step $\Delta t = 2$ fs was used. The coordinates of the system were recorded at every 0.2 ps during the calculation, for the following analyses of the trajectory. In the calculations with the explicit water a dielectric constant, $\epsilon = 1$, was used. During MD calculations, all of the bond lengths were fixed by SHAKE algorithm. A nonbonded cutoff of 9 Å was used in all of the above calculations. The atoms coordinated to the calcium ion were fixed by harmonic position constraints with a force constant of 50 kcal/(mol·Å). All calculations were performed using AMBER 4.0.1 with the force field published by Cornell et al.[16] The force-field parameters for the carbohydrate protion of **3a**−**d** were derived from GLYCAM-93, a general force field for carbohydrates that is suitable to AMBER.

Analyses of the Trajectory. Various analyses of the trajectory from MD calculations in solution were performed with several in-house programs. For the interactions observed in the initial model complex, we calculated the distances of each interaction from the sets of coordinates in the trajectory. For the analysis of the behavior of the branched alkyl chains, we calculated the "ratio of hidden surface area" as a measure of hydrophobic interactions with the protein. This indicator was calculated in the following way: for each set of coordinates in the trajectory, we calculated the solvent accessible surface area of the alkyl chain portions. The area calculations were performed in two ways: one was the area of free alkyl chains (A_{free}), which was calculated after the deletion of the protein coordinates, and the other was the area of bound alkyl chains ($A_{complex}$), calculated in the presence of the protein. Then, the ratio of hidden surface areas was calculated by the following equation:

$$\text{ratio of hidden surface area (\%)} = (A_{free} - A_{complex})/A_{free} \times 100$$

As indicated by the above equation, the ratio of hidden surface area is the ratio of the solvent accessible surface area of the alkyl chains that was buried in the protein. A larger percentage of hidden surface area reflects wider alkyl chain contacts with the protein surface. The calculation of solvent accessible surface area was done by the methods of Richmond[17] and Wesson and Eisenberg.[18] Hydrogen atoms were not included in the calculation.

Inhibition Assay of E-, P-, and L-Selectin−sLe^x Binding. The construction of the selectin-immunoglobulin was

carried out according to the previous paper. A solution of sLe^x-pentaceramide, in a 1:1 mixture of methanol and distilled water, was pipetted into microtiter plate wells (96 wells, Falcon PRO-BIND) at 100 pmol/50 μL/well and was adsorbed by evaporating the solvent. The wells were washed twice with distilled water, blocked with 5% BSA (bovine serum albumin)−PBS (phosphate-buffered saline) for 1 h at room temperature, and washed three times with PBS.

Separately, a 1:1 volumetric mixture of a 1:500 dilution in 1% BSA−PBS of biotin-anti-human IgG (Fc) (BioSource International Inc., lot 1201)/streptavidin-alkaline phosphatase (Zymed Lab Inc., lot 50424702) and a E-selectin-immunoglobulin fusion protein (E-selectin-Ig) was incubated at room temperature for 30 min to form a complex. The test compounds were dissolved in distilled water at 10 mM and finally diluted to final concentrations at 100, 25, 6.25, and 1.56 μM, respectively. Reactant solutions were prepared by incubating 30 μL of this solution at each concentration with 30 μL of the above complex solution for 30 min at room temperature. This reactant solution was then added to the above microtiter wells at 50 μL/well and incubated at 37 °C for 45 min. The wells were washed three times with PBS and distilled water, respectively, followed by addition of *p*-nitrophenylphosphate (1 mg/mL) and 0.01% $MgCl_2$ in 1 M diethanolamine (pH 9.8) at 50 mL/well. The reactant mixture was developed for 120 min at 23 °C, and absorbance at 405 nm was measured. Percent binding was calculated by the following equation:

$$\% \text{ binding} = (X - C)/(A - C) \times 100$$

wherein X is the absorbance of wells containing the test compounds at each concentration, C is the absorbance of wells not containing the selectin-Ig and test compounds, and A is the absorbance of control wells not containing the test compounds. Inhibition of P- or L-selectin−sLex binding was repeated except the P-selectin-Ig or L-selectin-Ig was replaced for E-selectin-Ig. The results of inhibitory activities are presented in Table 1 as IC_{50} values. The number of replicates is two.

Protective Effects on IgE-Mediated Skin Reaction in Mice Ears. Eight week-old female BALB/c mice were sensitized by intraperitoneal injection with 3 μg of ovalbumin (OA) and 4 mg of alum. Reactions to OA were elicited by intracutaneous injection of 10 μg of OA to each ear of mice 2 weeks after the sensitization. After 24 h, the mice were killed, and 6-mm diameter biopsies of the ears were taken and weighed as an index of tissue swelling. As an index of neutrophil infiltration into tissue, myeloperoxidase activity in biopsy homogenates (in 50 mM potassium phosphate, pH 6.0, with 0.5% hexadecyltrimethylammonium bromide) was measured as the degradation of H_2O_2 in the presence of *o*-dianisidine. Compound **3b** dissolved in 0.1 N NaOH−water solution was given intravenously immediately before the antigen challenge. Dexamethasone suspended in 0.5% carboxymethyl cellulose was given orally 2 h before the OA challenge. Results are expressed as mean ± SE. A one-way analysis of variance with Dunnett's test was used to determine statistical significance.[19]

General Procedure for the Preparations of Enantiomeric Glu Units (L-5, D-5): *N*-(*tert*-Butoxycarbonyl)-L-glutamic Acid 1-Methylamide 5-Benzyl Ester (L-5). To a solution of *N*-(*tert*-butoxycarbonyl)-L-glutamic acid 5-benzyl ester (**L-4**, 10.0 g, 29.6 mM) in THF (100 mL) was added Et_3N (3.9 g, 38.5 mM), and then the mixture cooled to 0 °C. Ethyl chlorocarbonate (4.2 g, 38.7 mM) was added to the mixture, this was stirred for 2 min, and then 40% methylamine/methanol (6.9 g, 74.0 mM) was added to the mixture. After the mixture stirred for 2 h gradually returning to room temperature, AcOEt (200 mL) was added to the mixture, and the mixture was washed with 1 N HCl, saturated sodium hydrogen carbonate, and brine, dried ($MgSO_4$), and concentrated. The precipitate was filtered and washed with Et_2O to give **L-5** (9.3 g, 90.3%) as a white crystal: $[\alpha]_D$ −6° (c = 0.1, $CHCl_3$); mp 124−125 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 9H), 1.66−1.82 (m, 1H), 1.83−2.00 (m, 1H), 2.35 (t, 2H, J = 7.6 Hz), 2.57 (d, 3H, J = 4.5 Hz), 3.83−3.95 (m, 1H), 5.08 (s, 2H), 6.90 (d, 1H, J = 8.2 Hz), 7.25−7.45 (m, 5H), 7.76 (d, 1H, J = 4.6 Hz). Anal. ($C_{18}H_{26}N_2O_5$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-D-glutamic acid 1-methylamide 5-benzyl ester (D-5):** yield 86.7%; $[\alpha]_D$ +6° (c = 0.1, $CHCl_3$); mp 120−123 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 9H), 1.65−1.82 (m, 1H), 1.83−2.00 (m, 1H), 2.36 (t, 2H, J = 7.7 Hz), 2.57 (d, 3H, J = 4.5 Hz), 3.82−3.98 (m, 1H), 5.08 (s, 2H), 6.90 (d, 1H, J = 8.2 Hz), 7.25-7.45 (m, 5H), 7.76 (d, 1H, J = 4.5 Hz). Anal. ($C_{18}H_{26}N_2O_5$) C, H, N.

General Procedure for the Preparation of Dipeptides 7a−d: *N*-(*tert*-Butoxycarbonyl)-D-seryl-L-glutamic Acid 1-Methylamide 5-Benzyl Ester (7b). To the **L-5** (10.0 g, 28.5 mM) was added 4 N HCl/1,4-dioxane (30 mL), and the solution was stirred for 0.5 h at room temperature. The mixture was concentrated, and the residue was dissolved in DMF (200 mL), added to Et_3N (5.4 mL), and then cooled to 0 °C. WSC (6.6 g, 34.2 mM) and HOBt (5.2 g, 34.2 mM) were added to the mixture, and this was stirred for 18 h at room temperature. The solvent was removed under reduced pressure, and the oily residue was dissolved in AcOEt (300 mL), washed with 0.1 N HCl, saturated sodium hydrogen carbonate, and brine, dried ($MgSO_4$), and concentrated. The precipitate was filtered, and recrystallization from AcOEt−*n*-hexane gave **7b** (10.3 g, 82.5%) as a white crystal: $[\alpha]_D$ −5° (c = 0.1, $CHCl_3$); mp 127−128 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H), 1.65−1.85 (m, 1H), 1.90−2.15 (m, 1H), 2.30−2.40 (m, 2H), 2.58 (d, 3H, J = 4.6 Hz), 3.54 (t, 2H, J = 5.6 Hz), 3.85−4.00 (m, 1H), 4.13−4.29 (m, 1H), 4.88 (t, 1H, J = 5.6 Hz), 5.07 (s, 2H), 6.82 (d, 1H, J = 6.9 Hz), 7.30−7.45 (m, 5H), 7.77 (d, 1H, J = 4.6 Hz), 8.03 (d, 1H, J = 8.3 Hz). Anal. ($C_{21}H_{31}N_3O_7$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-seryl-D-glutamic acid 1-methylamide 5-benzyl ester (7a):** yield 80.6%; $[\alpha]_D$ +5° (c = 0.1, $CHCl_3$); mp 124−127 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H), 1.66−1.88 (m, 1H), 1.92−2.12 (m, 1H), 2.30−2.42 (m, 2H), 2.57 (d, 3H, J = 4.6 Hz), 3.54 (t, 2H, J = 5.6 Hz), 3.86−4.00 (m, 1H), 4.13−4.30 (m, 1H), 4.88 (t, 1H, J = 5.7 Hz), 5.07 (s, 2H), 6.82 (d, 1H, J = 6.9 Hz), 7.30−7.45 (m, 5H), 7.77 (d, 1H, J = 4.5 Hz), 8.03 (d, 1H, J = 8.1 Hz). Anal. ($C_{21}H_{31}N_3O_7$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-seryl-L-glutamic acid 1-methylamide 5-benzyl ester (7c):** yield 85.2%; $[\alpha]_D$ −22° (c = 0.1, $CHCl_3$); mp 110−113 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 9H), 1.68−1.86 (m, 1H), 1.90−2.11 (m, 1H), 2.30−2.45 (m, 2H), 2.56 (d, 3H, J = 4.6 Hz), 3.55 (t, 2H, J = 5.4 Hz), 3.90−4.15 (m, 1H), 4.20−4.35 (m, 1H), 4.98 (t, 1H, J = 5.4 Hz), 5.10 (s, 2H), 6.79 (d, 1H, J = 7.5 Hz), 7.25−7.45 (m, 5H), 7.78 (d, 1H, J = 4.6 Hz), 7.96 (d, 1H, J = 8.5 Hz). Anal. ($C_{21}H_{31}N_3O_7$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-D-seryl-D-glutamic acid 1-methylamide 5-benzyl ester (7d):** yield 82.9%; $[\alpha]_D$ +18° (c = 0.1, $CHCl_3$); mp 110−112 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 9H), 1.70−1.85 (m, 1H), 1.90−2.10 (m, 1H), 2.30−2.45 (m, 2H), 2.56 (d, 3H, J = 4.5 Hz), 3.55 (t, 2H, J = 5.6 Hz), 3.90−4.05 (m, 1H), 4.15−4.30 (m, 1H), 4.99 (t, 1H, J = 5.5 Hz), 5.07 (s, 2H), 6.80 (d, 1H, J = 7.5 Hz), 7.25−7.45 (m, 5H), 7.78 (d, 1H, J = 4.6 Hz), 7.97 (d, 1H, J = 8.1 Hz). Anal. ($C_{21}H_{31}N_3O_7$) C, H, N.

General Procedure for the Preparation of 9a−d by Glycosylation of 7a−d with 2,3,4-Tri-*O*-benzylfucose Donor 8: *N*-(*tert*-Butoxycarbonyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-seryl-L-glutamic Acid 1-Methylamide 5-Benzyl Ester (9b). A mixture of 4A molecular sieves (2.0 g), AgOTf (0.59 g, 2.28 mM), and $SnCl_2$ (0.43 g, 2.28 mM) in $CHCl_3$ (15 mL) was stirred for 5 h under nitrogen atmosphere at room temperature and cooled to 40 − 50 °C. TMU (0.66 g, 5.70 mM), 2,3,4-tri-*O*-benzyl-L-fucosyl fluoride (**8**) (1.00 g, 2.28 mM) in $CHCl_3$ (1 mL), and compound **7b** (0.50 g, 1.14 mM) in $CHCl_3$ (3 mL) were added to the mixture successively, and the mixture stirred for 1 h at the same temperature and then for 20 h gradually returning to room temperature. The precipitate was filtered off, and the filtrate was concentrated. The residue was purified by thin-layer chromatography developing with 1:1 AcOEt/cyclohexane to give **9b** (0.60 g, 62.0%) as a white crystal: $[\alpha]_D$ −59° (c = 0.1, $CHCl_3$); mp 77−81 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (d, 3H, J = 6.5 Hz), 1.43 (s, 9H), 1.7−2.1 (m, 1H), 2.1−2.3 (m, 1H), 2.3−2.6 (m, 2H), 2.67 (d, 3H, J = 4.8 Hz), 3.5−3.7 (m, 2H), 3.8−4.0 (m, 3H), 4.0−4.25 (m, 1H), 4.25−4.45 (m, 1H), 4.5−5.0 (m, 7H),

5.0−5.2 (m, 2H), 5.5 (bs, 1H), 6.07 (bs, 1H), 7.1−7.45 (m, 20H). Anal. ($C_{48}H_{59}N_3O_{11}$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-L-seryl-D-glutamic acid 1-methylamide 5-benzyl ester (9a):** yield 66.0% as a syrup; $[\alpha]_D$ −43° (*c* = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.11 (d, 3H, *J* = 6 Hz), 1.45 (s, 9H), 1.8−2.0 (m, 1H), 2.0−2.25 (m, 1H), 2.3−2.6 (m, 2H), 2.71 (d, 3H, *J* = 5 Hz), 3.47 (dd, 1H, *J* = 5, 10 Hz), 3.65 (d, 1H, *J* = 2 Hz), 3.81 (q, 1H, *J* = 7 Hz), 3.92 (dd, 1H, *J* = 3, 10 Hz), 4.03 (dd, 1H, *J* = 4, 10 Hz), 7.0−7.4 (m, 20H). Anal. ($C_{48}H_{59}N_3O_{11}$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-L-seryl-L-glutamic acid 1-methylamide 5-benzyl ester (9c):** yield 64.2%; $[\alpha]_D$ −53° (*c* = 0.17, $CHCl_3$); mp 141−142 °C; ^1H NMR ($CDCl_3$) δ 1.09 (d, 3H, *J* = 6.5 Hz), 1.45 (s, 9H), 1.65−1.9 (m, 1H), 2.05−2.55 (m, 3H), 2.74 (d, 3H, *J* = 4.8 Hz), 3.4−3.5 (m, 1H), 3.63 (d, 1H, *J* = 1.6 Hz), 3.75−3.9 (m, 2H), 4.02 (dd, 1H, *J* = 3.6, 10.1 Hz), 4.12−4.28 (m, 2H), 4.35−4.5 (m, 1H), 4.5−4.95 (m, 7H), 5.06 (s, 2H), 6.04 (bs, 1H), 6.33 (bs, 1H), 7.05 (d, 1H, *J* = 8.3 Hz), 7.2−7.4 (m, 20H). Anal. ($C_{48}H_{59}N_3O_{11}\cdot 0.5H_2O$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-seryl-D-glutamic acid 1-methylamide 5-benzyl ester (9d):** yield 61.8%; $[\alpha]_D$ −42° (*c* = 0.1, $CHCl_3$); mp 109−116 °C; ^1H NMR ($CDCl_3$) δ 1.13 (d, 3H, *J* = 6.5 Hz), 1.44 (s, 9H), 1.6−1.75 (m, 1H), 2.0−2.15 (m, 1H), 2.15-2.4 (m, 2H), 2.71 (d, 3H, *J* = 4.8 Hz), 3.55−3.7 (m, 2H), 3.75−4.0 (m, 3H), 4.07 (dd, 1H, *J* = 3.7, 10.2 Hz), 4.1−4.25 (m, 1H), 4.25−4.4 (m, 1H), 4.6−5.0 (m, 7H), 5.07 (s, 2H), 5.52 (bs, 1H), 6.4 (bs, 1H), 7.1−7.5 (m, 20H). Anal. ($C_{48}H_{59}N_3O_{11}$) C, H, N.

General Procedure for the Preparation of 11a−d: *N*-(2-Tetradecylhexadecanoyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-seryl-L-glutamic Acid 1-Methylamide 5-Benzyl Ester (11b). To a solution of **9b** (0.5 g, 0.59 mM) in CH_2Cl_2 (10 mL) was added TFA (10 mL) at 0 °C, and the mixture was stirred for 2 h at room temperature and then concentrated. The residue was dissolved in AcOEt (100 mL), washed with saturated sodium carbonate, and dried ($MgSO_4$), and the solvent was removed *in vacuo*. The residue was dissolved in DMF (50 mL), 2-tetradecylhexadecanoic acid (294 mg, 0.65 mM) was added to the solution, and the mixture was dissolved by heating and then cooled to room temperature. WSC (170 mg, 0.89 mM) and HOBt (136 mg, 0.89 mM) were added to the solution. After the mixture stirred for 20 h, AcOEt (100 mL) was added to the solution, and the mixture was washed with 1 N HCl, saturated sodium hydrogen carbonate, and brine successively, dried ($MgSO_4$), and concentrated. The residue was purified by thin-layer chromatography developing with 25:1 $CHCl_3$/methanol to give **11b** (456 mg, 65.0%) as a white crystal: $[\alpha]_D$ −44° (*c* = 0.1, $CHCl_3$); mp 118−120 °C; ^1H NMR ($CDCl_3$) δ 0.8−0.95 (m, 6H), 1.12 (d, 3H, *J* = 6.5 Hz), 1.15−1.7 (m, 52H), 1.75−1.92 (m, 1H), 1.92−2.1 (m, 1H), 2.1−2.3 (m, 2H), 2.3−2.6 (m, 2H), 2.66 (d, 3H, *J* = 4.8 Hz), 3.45−3.65 (m, 2H), 3.78−3.95 (m, 3H), 4.08 (dd, 1H, *J* = 4.2, 10.2 Hz), 4.15−4.25 (m, 2H), 4.25−4.4 (m, 1H), 4.6−5.0 (m, 7H), 5.05 (d, 1H, *J* = 11.9 Hz), 5.1 (d, 1H, *J* = 12.3 Hz), 6.0−6.1 (m, 1H), 6.47 (d, 1H, *J* = 6.7 Hz), 7.15−7.4 (m, 20H). Anal. ($C_{73}H_{109}N_3O_{10}$) C, H, N.

***N*-(2-Tetradecylhexadecanoyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-L-seryl-D-glutamic acid 1-methylamide 5-benzyl ester (11a):** yield 61.1%; $[\alpha]_D$ −29° (*c* = 0.1, $CHCl_3$); mp 119−120 °C; ^1H NMR ($CDCl_3$) δ 0.75−0.95 (m, 6H), 1.13 (d, 3H, *J* = 6 Hz), 1.1−1.7 (m, 52H), 1.75−2.0 (m, 2H), 2.0−2.2 (m, 1H), 2.25−2.6 (m, 2H), 2.73 (d, 3H, *J* = 5 Hz), 3.37 (dd, 1H, *J* = 5, 9 Hz), 3.67 (s, 1H), 3.85 (q, 1H, *J* = 6 Hz), 3.97 (dd, 1H, *J* = 2, 10 Hz), 4.06 (dd, 1H, *J* = 3, 10 Hz), 7.0−7.4 (m, 20H). Anal. ($C_{73}H_{109}N_3O_{10}$) C, H, N.

***N*-(2-Tetradecylhexadecanoyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-L-seryl-L-glutamic acid 1-methylamide 5-benzyl ester (11c):** yield 71.8%; $[\alpha]_D$ −44° (*c* = 0.24, $CHCl_3$); mp 133−135 °C; ^1H NMR ($CDCl_3$) δ 0.8−0.95 (m, 6H), 1.13 (d, 3H, *J* = 6.5 Hz), 1.1−1.6 (m, 52H), 1.7−2.0 (m, 2H), 2.0−2.25 (m, 1H), 2.25−2.55 (m, 2H), 2.71 (d, 3H, *J* = 4.8 Hz), 3.37 (dd, 1H, *J* = 5.9, 9.4 Hz), 3.67 (d, 1H, *J* = 1.7 Hz), 3.85−3.95 (m, 2H), 4.06 (dd, 1H, *J* = 3.6, 10.1 Hz), 4.21 (dd, 1H, *J* = 2.8, 9.5 Hz), 4.3−4.45 (m, 1H), 4.5−4.6 (m, 1H), 4.55−4.95 (m, 7H), 5.06 (s, 2H), 6.2−6.3 (m, 1H), 6.82 (d, 1H, *J* = 6.7 Hz), 7.06 (d, 1H, *J* = 8 Hz), 7.15-7.4 (m, 20H). Anal. ($C_{73}H_{109}N_3O_{10}$) C, H, N.

***N*-(2-Tetradecylhexadecanoyl)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-seryl-D-glutamic acid 1-methylamide 5-benzyl ester (11d):** yield 54.5%; $[\alpha]_D$ −19° (*c* = 0.1, $CHCl_3$); mp 130−132 °C; ^1H NMR ($CDCl_3$) δ 0.8−0.95 (m, 6H), 1.13 (d, 3H, *J* = 6.5 Hz), 1.1−1.7 (m, 53H), 1.9−2.4 (m, 4H), 2.69 (d, 3H, *J* = 4.8 Hz), 3.56 (dd, 1H, *J* = 9.1, 10.8 Hz), 3.69 (d, 1H, *J* = 1.8 Hz), 3.75−4.0 (m, 3H), 4.1 (dd, 1H, *J* = 3.5, 6.5 Hz), 4.2−4.35 (m, 1H), 4.45−4.55 (m, 1H), 4.63 (d, 1H, *J* = 11.6 Hz), 4.68−4.9 (m, 4H), 4.95 (d, 1H, *J* = 11.6 Hz), 5.03 (d, 1H, *J* = 3.7 Hz), 5.07 (s, 2H), 6.34 (q, 1H, *J* = 4.8 Hz), 6.43 (d, 1H, *J* = 6.1 Hz), 7.1−7.5 (m, 20H). Anal. ($C_{73}H_{109}N_3O_{10}$) C, H, N.

General Procedure for the Preparation of 3a−d: *N*-(2-Tetradecylhexadecanoyl)-O-(α-L-fucopyranosyl)-D-seryl-L-glutamic Acid 1-Methylamide (3b). To a solution of **11b** (80 mg, 0.067 mM) in ethanol (50 mL) was added 20% $Pd(OH)_2$/C (80 mg), and the mixture was stirred for 4 h under 3−4 atmospheric pressure of hydrogen. The precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was crystallyzed with water to give **3b** (46 mg, 82.5%) as a white crystal: $[\alpha]_D$ −62° (*c* = 0.1, MeOH); mp 179−183 °C; ^1H NMR (DMSO-d_6) δ 0.75−0.9 (m, 6H), 1.06 (d, 3H, *J* = 6.4 Hz), 1.1−1.55 (m, 52H), 1.6−1.8 (m, 1H), 1.8−2.1 (m, 1H), 2.1−2.3 (m, 3H), 2.58 (d, 3H, *J* = 4.5 Hz), 3.4−3.6 (m, 3H), 3.6−3.85 (m, 2H), 4.1−4.3 (m, 1H), 4.34 (d, 1H, *J* = 4.5 Hz), 4.4−4.55 (m, 1H), 7.45 (d, 1H, *J* = 4.4 Hz), 7.97 (d, 1H, *J* = 7.0 Hz), 8.05 (d, 1H, *J* = 8.0 Hz). Anal. ($C_{45}H_{85}N_3O_{10}$) C, H, N.

***N*-(2-Tetradecylhexadecanoyl)-*O*-(α-L-fucopyranosyl)-L-seryl-D-glutamic acid 1-methylamide (3a):** yield 85.0%; $[\alpha]_D$ −40° (*c* = 0.1, MeOH); mp 183−187 °C; ^1H NMR (DMSO-d_6) δ 0.75−0.95 (m, 6H), 1.05 (d, 3H, *J* = 6.5 Hz), 1.1−1.55 (m, 52H), 1.55−1.75 (m, 1H), 1.75−2.0 (m, 1H), 2.05−2.3 (m, 3H), 2.57 (d, 3H, *J* = 4.5 Hz), 3.3−3.55 (m, 4H), 3.67 (q, 1H, *J* = 6.8 Hz), 3.85 (dd, 1H, *J* = 4.3, 9.2 Hz), 4.1−4.35 (m, 3H), 4.45−4.55 (m, 2H), 7.68 (d, 1H, *J* = 8 Hz), 7.86 (q, 1H, *J* = 4.5 Hz), 8.13 (d, 1H, *J* = 7.9 Hz), 11.9 (bs, 1H). Anal. ($C_{45}H_{85}N_3O_{10}\cdot 1H_2O$) C, H, N.

***N*-(2-Tetradecylhexadecanoyl)-*O*-(α-L-fucopyranosyl)-L-seryl-L-glutamic acid 1-methylamide (3c):** yield 72.8%; $[\alpha]_D$ −45° (*c* = 0.08, MeOH); mp 171−173 °C; ^1H NMR (DMSO-d_6) δ 0.75−0.95 (m, 6H), 1.06 (d, 3H, *J* = 6.5 Hz), 1.1−1.6 (m, 52H), 1.6−2.0 (m, 2H), 2.1−2.3 (m, 3H), 2.58 (d, 3H, *J* = 4.5 Hz), 3.4−3.6 (m, 3H), 3.74 (q, 1H, *J* = 6.9 Hz), 3.86 (dd, 1H, *J* = 5.7, 9.3 Hz), 4.15−4.4 (m, 3H), 4.45−4.6 (m, 2H), 7.63 (d, 1H, *J* = 8.2 Hz), 7.82 (q, 1H, *J* = 4.4 Hz), 8.09 (d, 1H, *J* = 8.7 Hz), 11.9 (bs, 1H). Anal. ($C_{45}H_{85}N_3O_{10}$) C, H, N.

***N*-(2-Tetradecylhexadecanoyl)-*O*-(α-L-fucopyranosyl)-D-seryl-D-glutamic acid 1-methylamide (3d):** Yield 96.7%; $[\alpha]_D$ −32° (*c* = 0.1, MeOH); mp 155−157 °C; ^1H NMR (DMSO-d_6) δ 0.75−0.9 (m, 6H), 1.05 (d, 3H, *J* = 6.5 Hz), 1.1−1.55 (m, 52H), 1.6−2.05 (m, 2H), 2.1−2.3 (m, 3H), 2.57 (d, 3H, *J* = 4.5 Hz), 3.55−3.8 (m, 2H), 4.1−4.25 (m, 1H), 4.4−4.6 (m, 1H), 4.6−4.7 (m, 1H), 7.66 (q, 1H, *J* = 4.4 Hz), 7.83 (d, 1H, *J* = 8.4 Hz), 7.92 (d, 1H, *J* = 8.3 Hz). Anal. ($C_{45}H_{85}N_3O_{10}\cdot 1.5H_2O$) C, H, N.

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