Studies on Selectin Blockers. 6. Discovery of Homologous Fucose Sugar Unit Necessary for E-Selectin Binding

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We describe a mimic of the sugar unit of the E-selectin ligand, sialyl Lewis X (sLe^X). Carbohydrates are entering the realm of rational drug design, aided by the growing understanding of the structure−function relationships. We investigated a new methodology of preparing sLe^X mimetics and developed a potent E-selectin blocker characterized by β-turn dipeptides. Another characteristic point of this E-selectin blocker is that the six-membered fucose ring was replaced with a five-membered fucose ring. Interestingly, it was found that the five-membered fucose ring could also bind to a calcium ion on the E-selectin, which could be an important role of the six-membered fucose ring. Especially, the L-Ser-D-Glu and D-Ser-L-Glu derivatives **3a**,**b** showed 65−90-fold more potent inhibitory activities than the sulfated Le^X analogue **1**. In addition, molecular dynamics (MD) studies indicated that the 2- and 3-OH groups of the six-membered fucose ring, which were necessary for the calcium binding, overlapped well with the 2- and 3-OH groups of the five-membered fucose ring. These new findings could be useful for the design of new types of selectin blockers.

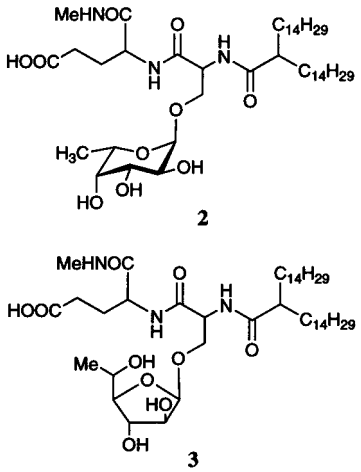
Introduction

Recently, some important vital functions of various oligosaccharides have been clarified, such as in cancer metastasis, anticancer activities, cell invasions by leukocytes, and recognition probes for the bacteria and/or viruses.[1] In addition, synthetic[2] and biological[3] studies of oligosaccharides have made remarkable progress. At the same time, oligosaccharides have become one of the best targets for new drug discovery. However, there are still some problems in applying oligosaccharides as drugs, because of the difficulties of large-scale synthesis and absorption by oral administration. Current studies of oligosaccharides and their biological activities focus on their mimics, to overcome the problems described above. Nevertheless, general methodologies and applications concerning sugar mimetics have not been established yet.

We have studied the design and synthesis of sugar mimetics, to develop a methodology for the preparaion of a sugar mimetic with a simple structure. Thus, we investigated a mimetic of a 3′-sulfo Le^X derivative (**1**) and have already found that compounds **2a,b** could mimic compound **1**; namely, the lactose unit of compound **1** could be replaced with a simple dipeptide, D-Ser-L-Glu or L-Ser-D-Glu, characterized by type II and/or type II′ β-turn formation (Figure 1).[4]

It has been reported that sLe^X itself is relatively unstable in the blood stream, due to sensitivity to glycosidases, i.e., fucosidase and sialidase.[5] Although any modifications of fucose on sLe^X have been reported,[6] there are few successful fucose mimetics. Therefore, the search for novel fucose mimetics is very important for the design of more useful selectin blockers. As part of our sugar mimetic study, we next investigated mimics of the six-membered fucose ring, as well as the application of a type II and/or type II′ β-turn dipeptide. To mimic the natural six-membered fucose ring necessary for the binding to E-selectin, we focused on an unnatural five-membered fucose ring, because it can be easily derived from the six-membered fucose ring (L-fucose).

Chart 1



In this paper, we describe the synthesis of type II and/or II′ β-turn dipeptides attached to the five-membered fucose ring and their in vitro activities against E-selectin binding. We also discuss an E-selectin/ligand complex model.

Results and Discussion

Chemistry. The five-membered fucose ring donor **7** was synthesized according to a previous paper.[7] For the synthesis of compounds **3a**,**b**, (Chart 1), we chose the easily available Glu unit **4** and Ser unit **5**. The coupling of **4** and **5** in presence of the WSC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride, and HOBt, 1-hydroxy-1*H*-benzotriazole monohydrate, pro-

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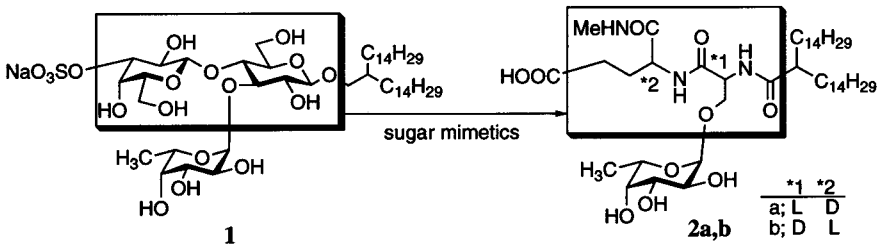
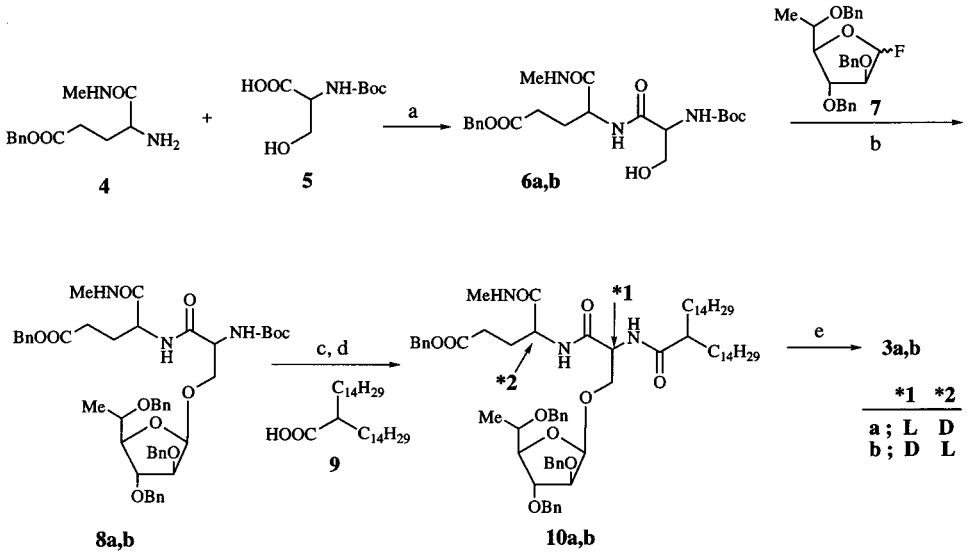


Figure 1. Successful sLe^X mimetics **1** and **2a**,**b**.

Scheme 1[a]

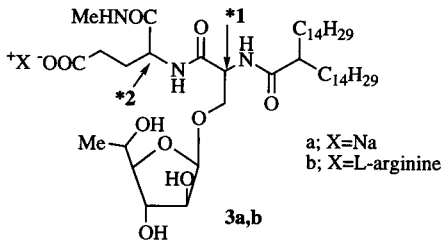


[a] Conditions: (a) WSC, HOBt, 80−88%; (b) $SnCl_2$, AgOTf, TMU, 4 Å MS/$CHCl_3$, 20−30%; (c) TFA/$CHCl_3$; (d) WSC, HOBt, 43−47% from **8a**,**b**; (e) $Pd(OH)_2$/MeOH, 90−92%.

vided the Ser-Glu dipeptide **6a**,**b** in 80−88% yields. The glycosylation of **6a**,**b** with the perbenzylfucose donor **7**, in the presence of $SnCl_2$/AgOTf as a promoter, gave mainly the α-glycosides **8a**,**b**. The deprotection of **8a**,**b** under acidic conditions, followed by the coupling with a branched alkyl chain, 2-tetradecylhexadecanoic acid (**9**), according to the general method for an amide linkage, afforded compounds **10a**,**b** in moderate yields. Finally, compounds **10a**,**b** were transformed in good yields, by the removal of the protecting groups, into the desired compounds **3a**,**b**, as a sodium salt for **3a** and an arginine salt for **3b**. (Scheme 1).

In Vitro Evaluation of Compounds 3a,b. Both compounds **3a**,**b** were potent blockers of E-selectin−sLe^X binding, with IC_{50} values of 3.1 and 4.3 μM, respectively (Table 1). Compounds **3a**,**b** were 65−90-fold more potent blockers than compound **1** (IC_{50}, 280 μM). Interestingly, both compounds **3a**,**b** showed similar activities to compounds **2a**,**b**, with IC_{50}'s of 13 μM for **2a** and 5.5 μM for **2b**. We have already reported that **2a**,**b** could bind to E-selectin with type II and/or II′ β-turn forms, respectively.[4] Since the structures of compounds **3a**,**b** are exactly the same as those of the corresponding **2** forms, except for the fucose, **3a**,**b** could also have the type II and II′ β-turn conformations such as **2a**,**b**, respectively. These conformations 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 of **3a**,**b**, for binding to E-selectin. This description indicates that the derivation of a pharmacophore conserving the essential binding groups could be a useful methodology

Table 1. E-Selectin-Blocking Activities of Compounds **1**, **2a**,**b**, and **3a**,**b**



compd	*1	*2	E-selectin (IC_{50}, μM)
3a	L	D	3.1
3b	D	L	4.3
1			280
2a	L	D	13
2b	D	L	5.5

to discover a new selectin blocker, and especially, the type II and/or II′ β-turn dipeptides could be an applicable template for sugar mimetics. In addition, it is interesting to note that the five-membered fucose ring would work as well as the six-membered fucose ring. To clarify the structural homology between the five-membered fucose ring and the six-membered fucose ring, we next performed a conformational analysis of the five-membered fucose ring using a molecular dynamics (MD) calculation method and compared it with that of the six-membered fucose ring.

Conformational Analysis of the Five-Membered Fucose Ring Using Molecular Dynamics. It has been reported that a five-membered ring occurs in two

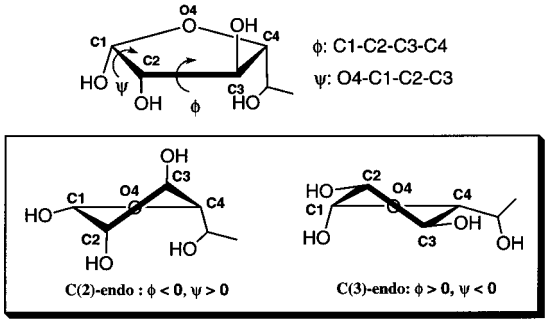


Figure 2. Diagrammatic projections of the five-membered ring fucose in two idealized twist conformations.

ranges of conformations, based on the ϕ (torsion angle ϕ: C1−C2−C3−C4) and ψ (torsion angle ψ: O4−C1−C2−C3) values, usually referred to as C(2)-endo for $\phi < 0$ and $\psi > 0$ and C(3)-endo for $\phi > 0$ and $\psi < 0$, as shown in Figure 2.[8] Especially, in the case of the C(3)-endo form ($\phi > 0$ and $\psi < 0$), the 2-OH and 3-OH groups of the five-membered fucose ring are directed toward the equatorial positions and could coordinate with the calcium ion of E-selectin. To obtain the $\phi-\psi$ plots of conformers of the five-membered fucose ring, we investigated conformation using CAMDAS.[9] First, we performed an MD calculation to explore the conformational space of the five-membered fucose ring. The calculation was performed for 200 ps with an integral time step of 1 fs, the temperature in the MD calculation system was maintained at 900 K, and the lengths of the covalent bonds were fixed with the SHAKE algorithm.[10] During the calculation, a conformer was sampled for every 0.1 ps, and the clustering of similar conformers was carried out. The criteria of the clustering involve 5° deviations of the two torsion angles, ϕ and ψ. Namely, if the deviations of the torsion angles of the two conformers sampled are smaller than 5°, then each conformer is defined as the same. Otherwise, the conformer sampled here is added to the conformer's list as a new one. Thus, we investigated the $\phi-\psi$ plots of 40 conformers of the five-membered fucose ring, in order of low energy. As a result, 9 of the first 10 stable conformers showed C(3)-endo forms, as shown in Figure 3. In addition, it was found that the 2- and 3-OH groups of the five-membered fucose ring in the C(3)-endo form overlapped well with the corresponding 2- and 3-OH groups of the six-membered fucose ring, which would be the essential binding site for calcium on E-selectin (Figure 4). Accordingly, the five-membered fucose ring would coordinate to the calcium ion and could mimic the six-membered fucose ring.

Initial Model Construction, MD Simulation, and Analysis of the Interaction between 3b and E-Selectin. The structure of the lectin domain of E-selectin was obtained from the Protein Data Bank (indentity code: 1ESL). We constructed the initial bound model of **3b** to conserve the interactions of the three functional groups with E-selectin, based on the initial bound model of **2b**/E-selectin,[4] because the structure of **3b** is the same as that of the corresponding **2b**, except for the fucose. The conformation of the five-membered fucose ring in **3b** corresponded to the lowest-energy conformer obtained from the calculation. The 2- and 3-OH groups of the fucose were coordinated to the calcium, and the carboxylate of the L-Glu was

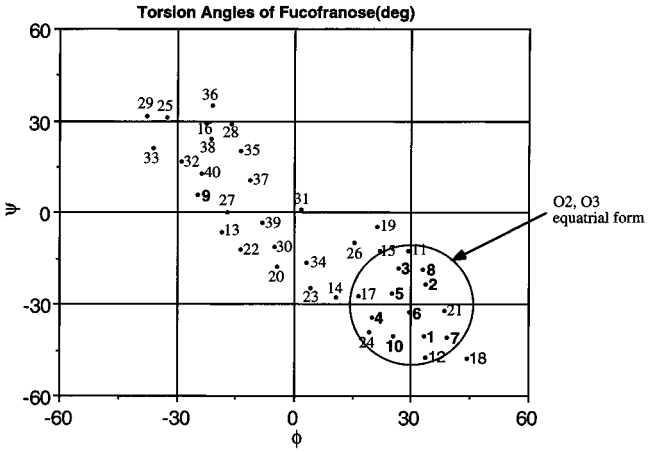


Figure 3. $\phi-\psi$ plots of the 40 conformations of five-membered ring fucose in order of low energy during the 200-ps MD calculation. These numbers show the order of low-energy conformation, and the bold numbers show the first 10 conformations. The horizontal and vertical axes represent ϕ and ψ values in degrees. ϕ: C1−C2−C3−C4. ψ: O4−C1−C2−C3. If $\phi > 0$ and $\psi < 0$, O2 and O3 are equatorial form; if $\phi < 0$ and $\psi > 0$, O2 and O3 are axial form.

Figure 4. Stereoview of superimposition of six-membered ring fucose and the most stable conformer of five-membered ring fucose obtained from CAMDAS. The thick line indicates five-membered ring fucose, and the thin line indicates six-membered ring fucose.

directed to Lys111 of E-selectin. On the other hand, one of the branched alkyl chains was directed to a hydrophobic surface consisting of Tyr44, Pro46, and Tyr48, and another alkyl chain was directed to a shallow cavity consisting of Ala9, Leu114, Lys111, Lys112, and Lys113. In addition, the initial bound model of **3b** formed a type II′ β-turn conformation.

Next, we subjected the initial bound model of **3b** to a 200-ps MD simulation with the explicit water solvent and evaluated the dynamic behavior of the model in solution. We analyzed the interaction mode between the Glu in **3b** and the basic residue on E-selectin, as well as the conformation change of the peptide portion in **3b**. As a result, during the MD calculation **3b** well-conserved the type II′ β-turn conformation, and the interaction distance between Lys111 of E-selectin and the carboxylate of **3b** was found as well, as shown in Figure 5. Additionally, we analyzed the behavior of the branched alkyl chains of **3b** during the MD simulation in solution. Since a direct estimation of the hydrophobic force is difficult, we used the ratio of the solvent-accessible surface area of the alkyl chain that was hidden by the protein as an indication of the strength of the hydrophobic interaction (see the Experimental Section). As a result, the hidden surface area of each alkyl chain was retained as the initial value and fluctuated minimally during the MD simulation. Thus, compound **3b** interacted similarly with E-selectin, as observed in the complex model[4] of **2b**/E-selectin. These

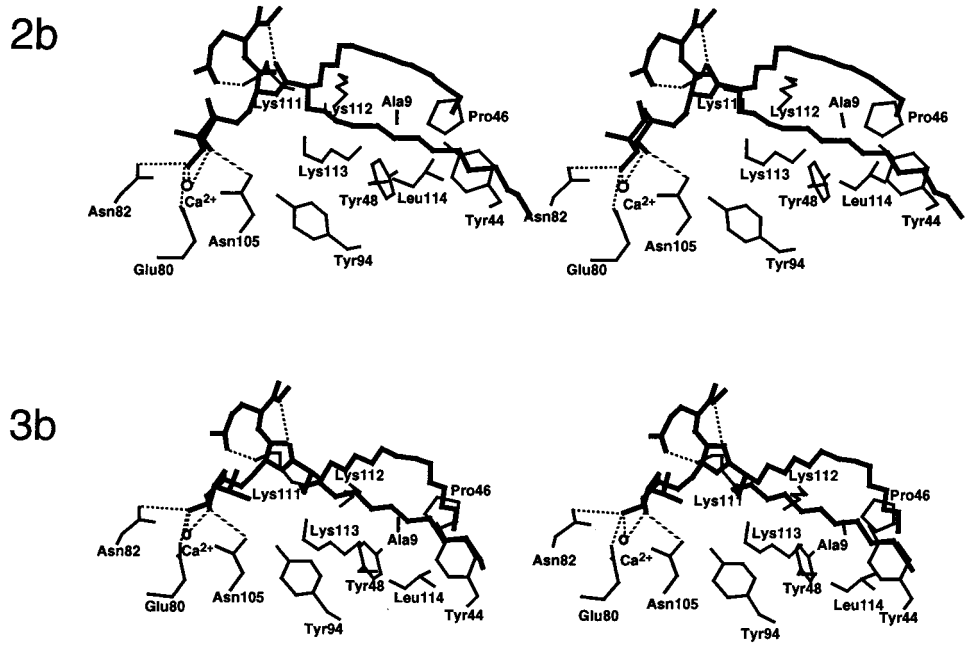


Figure 5. Stereorepresentation of the models of E-selectin and **2b** or **3b** complex models in solution. These models are the equilibrium structures during MD calculation (200 ps). The structures of **2b** and **3b** are indicated by thick lines. Thin lines indicate the structure of E-selectin. Only the residues in the ligand-binding site on the protein are shown. Dashed lines indicate interactions (hydrogen bond, electrostatic interaction, calcium coordination).

data correlate with the in vitro activity of **3b**. Namely, it was found that the six-membered fucose ring of compound **2b** could be replaced with the five-membered fucose ring. Moreover, we also constructed an initial bound model of **3b**/E-selectin and subjected this model to a 200-ps MD simulation. It was found that the final bound model of **3b**/E-selectin was also similar to that of the **2b**/E-selectin complex model[4] reported previously.

In conclusion, we succeeded in the design of sLe^X mimetics based on modified type II and/or II′ β-turn dipeptides. Especially, the L-Ser-D-Glu **3a** and D-Ser-L-Glu **3b** showed 65−90-fold more potent inhibitory activities than the sulfated Le^X analogue **1**. In addition, we discovered a homologous fucose sugar unit necessary for E-selectin binding, which is characterized by a five-membered fucose ring. Although the six-membered fucose ring might be a substrate for a fucosidase in the body, the five-membered fucose ring would not have this problem. Therefore, these new findings well be useful for the design of new types of selectin blockers, which are resistant to hydrolytic enzymes, such as a fucosidase. We are now in the progress of synthesizing a sLe^X analogue attached to a five-membered fucose ring.

Experimental Section

Simulation of the Complex in Solution Using Molecular Dynamics. The models of complexes were solvated by the addition of TIP3P water molecules[11] which were added within 25 Å from the center of each of the compounds **3a,b**. 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 of $\Delta t = 2$ fs was used. After that, all of the water molecules, compounds **3a**,**b**, 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 of $\Delta t = 2$ 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.[12] The force-field parameters for the carbohydrate portion of **3a**,**b** 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 calculation 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. 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[13] and Wesson and Eisenberg.[14] Hydrogen atoms were not included in the calculation.

Inhibition Assay of E-Selectin−sLe^x Binding. The

construction of the selectin-immunoglobulin was carried out according to a previous paper.[15]

A solution of sLe^x pentasaccharide ceramide, 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)−1 mM $CaCl_2$/50 mM imidazole buffer (pH 7.2) for 1 h at room temperature, and washed three times with 50 mM imidazole buffer (pH 7.2).

Separately, a 1:1 volumetric mixture of a 1:500 dilution in 1% BSA−1 mM $CaCl_2$/50 mM imidazole buffer (pH 7.2) of biotinylated goat F(ab′)2 anti-human IgG(γ)/streptavidin-alkaline phosphatase (Zymed Lab Inc.) 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 (**2a,b**; DMSO) at 10 mM and finally diluted by 1 mM $CaCl_2$/50 mM imidazole buffer (pH 7.2) to final concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.13, 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 50 mM imidazole buffer (pH 7.2) and distilled water, respectively, followed by addition of *p*-nitrophenyl phosphate (1 mg/mL) and 0.01% $MgCl_2$ in 1 M diethanolamine (pH 9.8) at 50 μL/well. The reactant mixture was developed for 120 min at room temperature, 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. The results of inhibitory activities are presented in Table 1 as IC_{50} values. The number of replicates is 2.

General Procedure for the Preparation of Dipeptides 6a,b: *N*-(*tert*-Butoxycarbonyl)-D-seryl-L-glutamic Acid 1-Methylamide 5-Benzyl Ester (6b). To the L-**4** (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. D-**5** (5.9 g), WSC (6.6 g, 34.2 mM), and HOBt (5.2 g, 34.2 mM) were added to the residue, 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 **6b** (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. Calcd for $C_{21}H_{31}N_3O_7$: C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-seryl-D-glutamic acid 1-methylamide 5-benzyl ester (6a):** 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. Calcd for $C_{21}H_{31}N_3O_7$; C, H, N.

General Procedure for the Preparation of 8a,b by Glycosylation of 6a,b with 2,3,5-Tri-*O*-benzylfucose Donor 7: [*N*-(*tert*-Butoxycarbonyl)-*O*-(2,3,5-tri-*O*-benzyl-α-L-fucofuranosyl)-L-seryl]-D-glutamic Acid 1-Methylamide 5-Benzyl Ester (8a). A mixture of 4 Å molecular sieve (1.0 g), AgOTf (0.89 g, 3.46 mM), and $SnCl_2$ (0.66 g, 3.48 mM) in CH_2Cl_2 (10 mL) was stirred for 5 h under nitrogen atmosphere at room temperature and cooled to −40−50 °C. TMU (1.0 g, 8.64 mM), 2,3,5-tri-*O*-benzyl-L-fucofuranosyl fluoride (**7**) (1.1 g, 2.5 mM) in CH_2Cl_2 (2 mL) and compound **6a** (0.75 g, 1.71 mM) in CH_2Cl_2 (5 mL) were added to the mixture successively and stirred for 1 h at the same temperature and for 19 h under 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 3:2 AcOEt/*n*-hexane to give **8b** (0.45 g) as a white crystal: $[\alpha]_D$ −19° (c = 0.1, MeOH); mp 122−126 °C; ^{1}H NMR (DMSO-d_6) δ 1.07 (d, 3H, J = 6.2 Hz), 1.33 (s, 9H), 1.68−1.85 (m, 1H), 1.90−2.08 (m, 1H), 2.21−2.42 (m, 2H), 2.57 (d, 3H, J = 4.5 Hz), 3.45−3.60 (m, 1H), 3.58−3.70 (m, 2H), 3.70−3.82 (m, 1H), 4.00 (d, 2H, J = 4.5 Hz), 4.10−4.30 (m, 2H), 4.35−4.65 (m, 6H), 5.03 (3, 2H), 5.05 (bs, 1H), 6.93 (d, 1H, J = 6.7 Hz), 7.15−7.40 (m, 20H), 7.79 (d, 1H, J = 4.4 Hz), 8.19 (d, 1H, J = 7.6 Hz). Anal. Calcd for $C_{48}H_{59}N_3O_{11}$: C, H, N.

[*N*-(*tert*-Butoxycarbonyl)-*O*-(2,3,5-tri-*O*-benzyl-α-L-fucofuranosyl)-D-seryl]-L-glutamic acid 1-methylamide 5-benzyl ester (8b): yield 20.5% as a syrup; $[\alpha]_D$ −21° (c=0.1, MeOH); ^{1}H NMR (DMSO-d_6) δ: 1.07 (d, 3H, J = 6.2 Hz), 1.36 (s, 9H), 1.65−1.85 (m, 1H), 1.90−2.12 (m, 1H), 2.32 (t, 2H, J = 7.2 Hz), 2.57 (d, 3H, J = 4.5 Hz), 3.45−3.70 (m, 3H), 3.78−3.91 (m, 1H), 3.95−4.05 (m, 1H), 4.10−4.30 (m, 2H), 4.35−4.72 (m, 6H), 5.02 (s, 2H), 5.09 (s, 1H), 7.16 (d, 1H, J = 7.0 Hz), 7.20−7.45 (m, 20H), 7.79 (d, 1H, J = 4.0 Hz), 8.17 (d, 1H, J = 7.9 Hz). Anal. Calcd for $C_{48}H_{59}N_3O_{11}$: C, H, N.

General Procedure for the Preparation of 10a,b: [*N*-(2-Tetradecylhexadecanoyl)-*O*-(2,3,5-tri-*O*-benzyl-α-L-fucofuranosyl)-L-seryl]-D-glutamic Acid 1-Methylamide 5-Benzyl Ester (10a). To a solution of **8a** (0.4 g, 0.47 mM) in CH_2Cl_2 (3 mL) was added TFA (3 mL) at 0 °C, and the mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated, and the residue was dissolved in $CHCl_3$ (50 mL), washed with saturated sodium hydrogen carbonate, and dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was dissolved in DMF (30 mL), 2-tetradecylhexadecanoic acid (210 mg, 0.46 mM) was added to the solution, and the mixture was dissolved by heating and then cooled to room temperature. WSC (170 mg, 0.73 mM) and HOBt (110 mg, 0.73 mM) were added to the solution. After the mixture stirred for 22 h, $CHCl_3$ (80 mL) was added to the solution, 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 20:1 $CHCl_3$/methanol to give **10a** (270 mg, 47%) as a white crystal: $[\alpha]_D$ −14° (c = 0.1, $CHCl_3$); mp 127−129 °C; ^{1}H NMR (DMSO-d_6) δ 0.75−0.95 (m, 6H), 1.06 (d, 3H, J = 6.1 Hz), 1.10−1.60 (m, 55H), 1.65−1.85 (m, 1H), 1.85−2.10 (m, 1H), 2.10−2.25 (m, 1H), 2.25−2.40 (m, 2H), 2.55 (d, 3H, J = 4.5 Hz), 3.49−3.60 (m, 1H), 3.60−3.70 (m, 2H), 3.70−3.85 (m, 1H), 3.95−4.05 (m, 2H), 4.15−4.25 (m, 1H), 4.39−4.72 (m, 7H), 5.01 (s, 2H), 5.04 (d, 1H, J = 3.3 Hz), 7.15−7.40 (m, 20H), 7.84 (d, 1H, J = 4.6 Hz), 8.11 (d, 1H, J = 6.7 Hz, 8.28 (d, 1H, J = 8.2 Hz). Anal. Calcd for $C_{73}H_{109}N_3O_{10}$: C, H, N.

[*N*-(2-Tetradecylhexadecanoyl)-*O*-(2,3,5-tri-*O*-benzyl-α-L-fucofuranosyl)-D-seryl]-L-glutamic acid 1-methylamide 5-benzyl ester (10b): yield 43.3%; $[\alpha]_D$ −19° (c = 0.1, $CHCl_3$); mp 114−117 °C; ^{1}H NMR (DMSO-d_6) δ 0.75−0.95 (m, 6H), 1.06 (d, 3H, J = 6.2 Hz), 1.10−1.50 (m, 52H), 1.65−1.85 (m, 1H), 1.88−2.12 (m, 1H), 2.15−2.30 (m, 1H), 2.30−2.42 (m, 2H), 2.55 (d, 3H, J = 4.5 Hz), 3.45−3.72 (m, 3H), 3.80−3.95 (m, 1H), 3.95−4.05 (m, 2H), 4.16−4.30 (m, 1H), 4.38−4.72 (m, 7H), 5.00 (s, 2H), 5.10 (d, 1H, J = 3.1 Hz), 7.15−7.45 (m, 20H), 7.84 (d, 1H, J = 4.7 Hz), 8.16 (d, 1H, J = 6.7 Hz), 8.26 (d, 1H, J = 8.1 Hz). Anal. Calcd for $C_{73}H_{109}N_3O_{10}$: C, H, N.

General Procedure for the Preparation of 3a,b: [*N*-(2-Tetradecylhexadecanoyl)-*O*-(α-L-fucofuranosyl)-L-seryl]-D-glutamic Acid 1-Methylamide Sodium Salt (3a). To a solution of **10a** (200 mg, 0.168 mM) in ethanol (30 mL) was added 20% $Pd(OH)_2$/C (200 mg), and the mixture was stirred

for 5 h under hydrogen (3−4 atmospheric pressure). The precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in methanol and water. To the solution was added 1 H NaOH (1 equiv), and the mixture stirred for 5 min. The reaction mixture was concentrated in vacuo. The residue was lyophilized with water to give **3a** (79 mg, 56.7%) as a white powder: mp 147−154 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.83−0.87 (m, 6H), 1.01 (d, 3H, J = 6.3 Hz), 1.10−1.55 (m, 52H), 1.60−2.05 (m, 4H), 2.10−2.32 (m, 1H), 2.55 (d, 3H, J = 3.5 Hz), 3.20−3.35 (m, 2H), 3.40−3.65 (m, 3H), 3.66−3.76 (m, 1H), 3.77−3.86 (m, 1H), 3.86−4.04 (m, 2H), 4.35 (s, 1H), 4.66 (d, 1H, J = 4.2 Hz), 8.23 (d, 1H, J = 7.4 Hz), 9.19 (d, 1H, J = 5.8 Hz). Anal. Calcd for $C_{45}H_{84}N_3O_{10}Na$: C, H, N.

[*N*-(2-Tetradecylhexadecanoyl)-*O*-(α-L-fucofuranosyl)-D-seryl]-L-glutamic acid 1-methylamide L-arginine salt (3b): yield 82%; mp 175 °C decomp; ^{1}H NMR (DMSO-d_6) δ 0.75−0.95 (m, 6H), 1.08 (d, 3H, J = 6.5 Hz), 1.1−1.5 (m, 54H), 1.5−2.2 (m, 9H), 2.55 (d, 3H, J = 4.2 Hz), 3.6−3.8 (m, 5H), 3.9−4.05 (m, 1H), 4.1−4.2 (m, 1H), 4.2−4.4 (m, 1H), 4.69 (d, 1H, J = 4.2 Hz), 7.47 (d, 1H, J = 3.9 Hz), 8.51 (d, 1H, J = 7.3 Hz), 8.85 (bs, 1H). Anal. Calcd for $C_{45}H_{85}N_3O_{10}\cdot C_5H_{12}N_4O_2$: C, H, N.

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