Study on Selectin Blocker. 8. Lead Discovery of a Non-Sugar Antagonist Using a 3D-Pharmacophore Model

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Received December 6, 1999

We have developed a pharmacophore model of a ligand/E-selectin complex to screen drug candidates for selectin blockers. In a series of sugar mimetic studies of the E-selectin ligand, sialyl Lewis X (sLe^x), we have already found a potent compound, a sulfated Le^x analogue (**1**), and also have proposed how compound **1** binds to E-selectin (Tsujishita, H.; Hiramatsu, Y.; Kondo, N.; Ohmoto, H.; Kondo, H.; Kiso, M.; Hasegawa, A. *J. Med. Chem.* **1997**, *40*, 362−369). To find drug candidates that fit into the binding pocket of E-selectin, we constructed an original 3D-pharmacophore model from structural information of a compound **1**/E-selectin complex model and screened lead compounds for selectin blockers using a commercially available database ACD-3D. As a result, we discovered a lead compound (**2**) containing good selectin inhibitory activity, and in addition, we succeeded to preliminarily optimize it to a more active lead compound (**3**) with micromolar IC_{50} values, based on the 3D-pharmacophore model investigation. This methodology using the 3D-pharmacophore model could be applicable as a pre-screen system for selectin blockers.

Introduction

Recently, it was clarified that oligosaccharides play an important role in signal transduction processes involved in cell−cell adhesion.[1] In addition, syntheses[2] and biological[3] studies of oligosaccharides have made remarkable progress. Proteins interacting with oligosaccharides have been recognized as targets for new drug discovery. However, drug development of a carbohydrate itself is hindered by the complication that the synthesis of complex oligosaccharides is a rather expensive and difficult process. Thus, current studies of biologically active oligosaccharides focus on the mimic into variable low molecular compounds. However, there are very few general methodologies for sugar mimetics.

We took notice of sialyl Lewis X (sLe^x), a ligand of selectin that is one of the cell adhesion molecules, as a functional carbohydrate[4] (Figure 1). It was reported that selectin−sLe^x interaction led to acute and/or chronic inflammatory diseases.[5] Therefore, selectins are believed to be involved in the progression of the clinical manifestations of inflammatory disease. We have undertaken the discovery of a selectin antagonist based on sLe^x carbohydrate mimicking.[6]

By the way, drug discovery is a very complex process. Many different technologies can play a role in the rapid discovery of lead compounds, such as molecular modeling, a combination of combinatorial chemistry/high-throughput screening, and so on. For example, molecular modeling could be one of the useful tools for lead optimization and/or lead generation of biologically active compounds.[7] Actually, computer-docking studies are often used to simulate good inhibitors and/or promising antagonists.

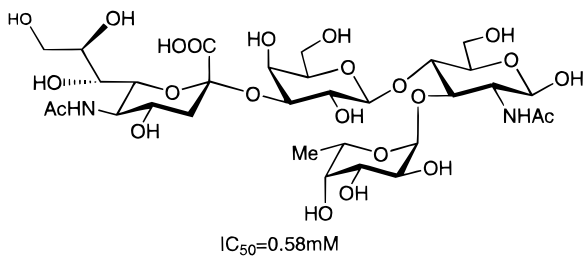

Figure 1. Chemical structure and IC_{50} of sLe^x toward E-selectin.

Understanding the selectin−sLe^x interaction is significant for discovery of good selectin antagonists. Although only a crystal structure of E-selectin itself has been determined,[8] a ligand/E-selectin complex structure has not been experimentally clarified yet. On the basis of the point mutation of E-selectin[9] and/or SAR study[10] of sLe^x, an E-selectin−sLe^x binding mode has been predicted by several groups.[11]

We have also constructed a complex model of E-selectin and a potent selectin blocker, sulfo Le^x derivative **1**.[12] Using this model, essential groups and desirable positions of compound **1** necessary for binding to E-selectin were predicted. On the basis of this bound conformation, we found a potential compound which replaced the lactose moiety of compound **1** with Ser-Glu dipeptide.[6e] This result might indicate that our pharmacophore model could be a potential tool to find other selectin antagonists.

To clarify the utility of pharmacophore models, a 3D-database search[13] is often performed. In general, 3D-database searches make it possible to generate a new scaffold, so it would be useful to discover a new lead and/or mimic an interesting skeleton.

At present, there are very few examples which showed the way to predict low molecular compounds that fit into

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10.1021/jm990342j CCC: $19.00
Published on Web 03/25/2000

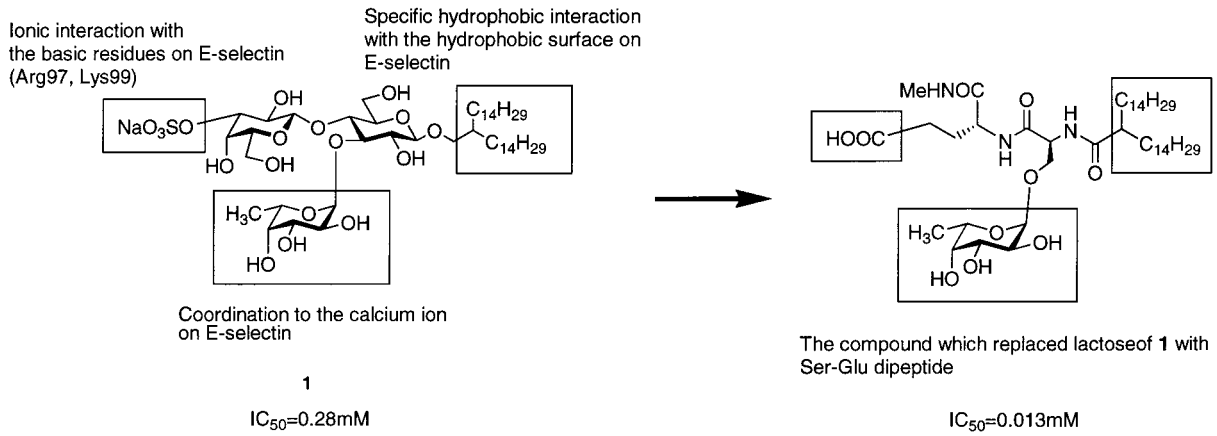


Figure 2. Chemical structure and IC_{50} of compound **1** (left). From the molecular modeling investigation, three critical interactions of **1** toward E-selectin are clarified. On the basis of the pharmacophore, Ser-Glu dipeptide derivative (right) is found.

the sugar binding pocket using the modeling studies. Therefore, we have investigated the pre-screening of structurally novel compounds which can fit into the sLe^x binding pocket using the 3D-database search method. In this paper, we describe the construction of a 3D-pharmacophore model of selectin antagonists and the lead discovery using its 3D-pharmacophore model.

Results and Discussion

3D-Pharmacophore Model and 3D-Database Search. Our previous studies[12] of molecular dynamics simulations of a compound **1**/E-selectin complex suggested the existence of three essential binding sites of compound **1** for E-selectin, as follows (Figure 2): (1) the C-2 and C-3 hydroxy groups of fucose coordinate to calcium; (2) the branched alkyl chain of compound **1** 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. Namely, it is considered that shape, functional groups, hydrogen bonding, and hydrophobic interaction affect how well compounds bind to the selectin protein. Next, we constructed a pharmacophore model which can conserve the three essential groups to the desirable positions for E-selectin binding. On the basis of this pharmacophore, we have already found an active compound which replaced the lactose moiety of compound **1** with Ser-Glu dipeptide[6e] (Figure 2). This result indicates that our pharmacophore model could have potential to find other selectin antagonists.

Thus, we tried to find other novel selectin antagonists using the 3D-database searching method. First, we selected the ACD-3D database which was a commercially available reagent database. Unfortunately, there were no compounds containing all three essential groups necessary for binding (fucose, negatively charged group, and hydrophobic part) among the database, so we constructed a 3D-pharmacophore model containing other groups which could play identical roles with the three essential groups: (1) the fucose unit, as a calcium ligand, would be replaced with a carboxylic acid;[14] (2) the hydrophobic part, branched alkyl chain, would be replaced with a single alkyl chain; (3) the negatively charged group, a sulfonic acid, would be replaced with a carboxylic acid (Figure 3).

A 3D-database search was performed using the ISIS-3D[13f] software package with the conformationally flexible search option. The fitness of compounds toward the pharmacophore model was investigated using five conformers in each compound. These conformers were obtained by rotating single bonds. Figure 3 shows the positions of the three functional groups and the distances between the groups as the query.

Screening of a Hit Compound 2 and Its in Vitro Activity against E-Selectin Binding. As a result of the 3D-database search, we found compound **2** which apparently showed inhibitory activity against E-selectin. Although its potency (IC_{50} = 1.2 mM) was weaker than that of compound **1** (IC_{50} = 0.28 mM; Figure 4),[6b] compound **2** seemed to be a very potential lead compound, because it was a completely non-sugar compound and it would have some advantages, such as ease of synthesis, oral availability, and pharmacokinetic profile. Next, we tried to investigate the optimization of compound **2**.

Although compound **2** was picked up by the 3D-database search of the pharmacophore model derived from the compound **1**/E-selectin complex model (Figure 5), it is not clear that the bound conformation of compound **2** screened by the pharmacophore model could be identical with the stable conformation of compound **2** in the presence of E-selectin. Certainly, the precision of search conditions was a little rough. Therefore, we constructed the complex model of compound **2**/E-selectin and investigated the binding possibility of compound **2** for E-selectin.

Preliminary Optimization of the Possible Bound Conformation of Compound 2. The compound **2**/E-selectin complex model was constructed by superimposing the three functional groups of compound **2** based on the compound **1**/E-selectin complex model. These graphical manipulations and representations were performed by Midas-plus 2.0[15] and Sybyl 6.5.[16] The constructed model showed the following features (Figure 6): (1) one of the carboxylic acids of isophthalic acid would coordinate to calcium; (2) the carboxylic acid in the benzoic acid moiety would form the electrostatic interaction with Arg97 and/or Lys99; (3) a C17 long alkyl chain would interact with the shallow hydrophobic region consisted of Lys114, Ala9, Tyr49, etc., on E-selectin. In this model, it was also found that one of the two carboxylic acids in the isophthalic acid moiety did not interact with the surface of the protein and was not

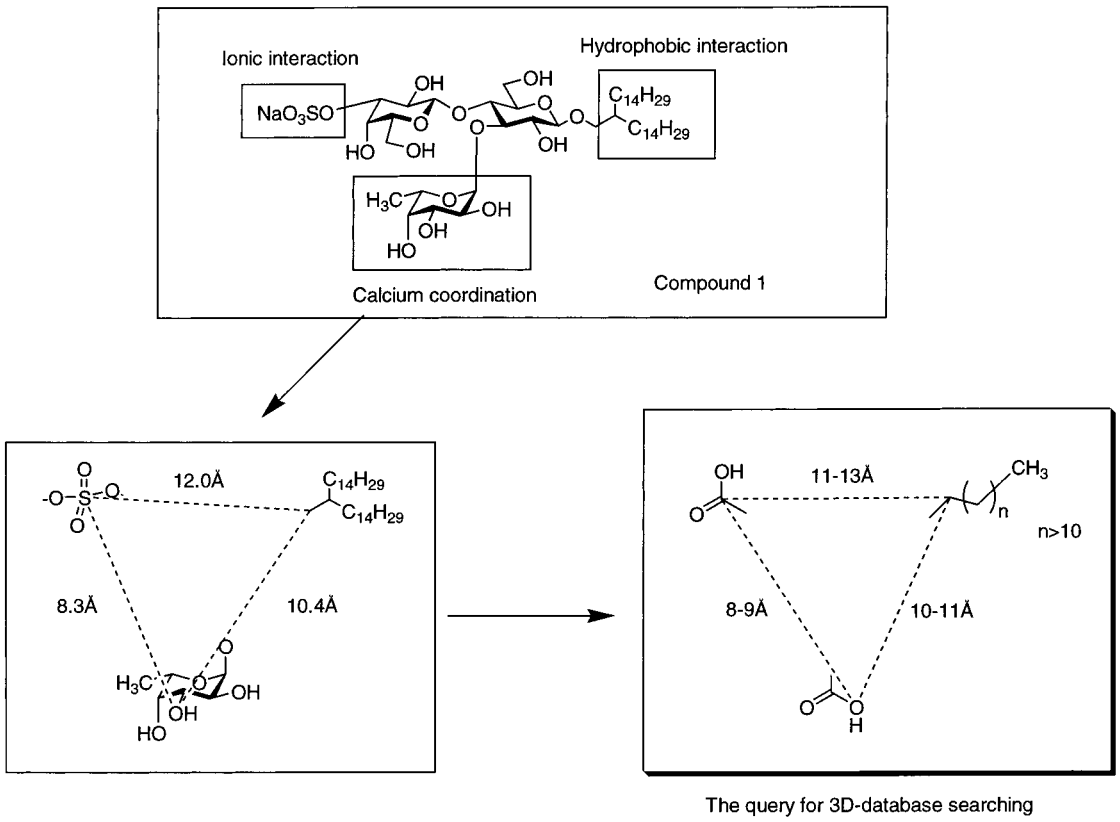


Figure 3. Retrieved relative positions of the functional groups from the complex model between compound **1** and E-selectin. Next arrow shows the pharmacophore model which is involved in the general groups (fucose to carboxylic acid, branched alkyl chain to single alkyl chain, sulfonic acid to carboxylic acid) that replaced the functional groups in compound **1**. Using this model, the 3D-database search was performed.

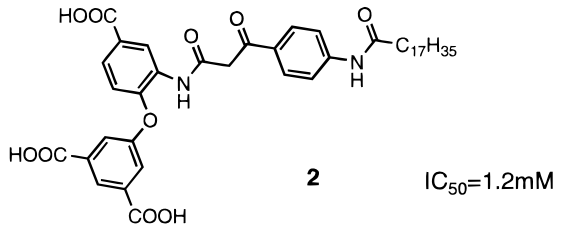


Figure 4. Chemical structure and IC_{50} of one of the hit compounds, compound **2,** toward E-selectin.

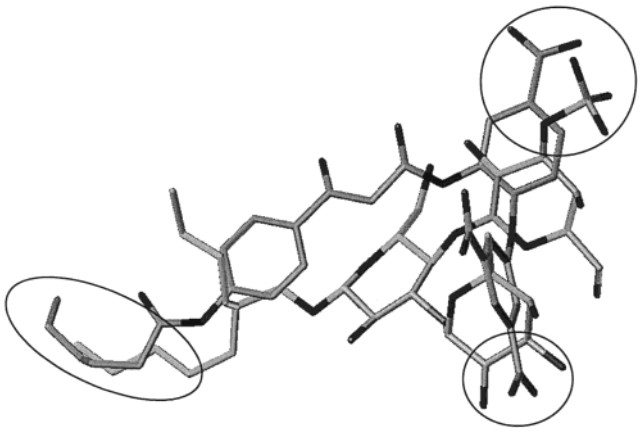


Figure 5. Superimposition of the bound conformation of compounds **1** and **2**. The circles indicate the corresponded functional groups.

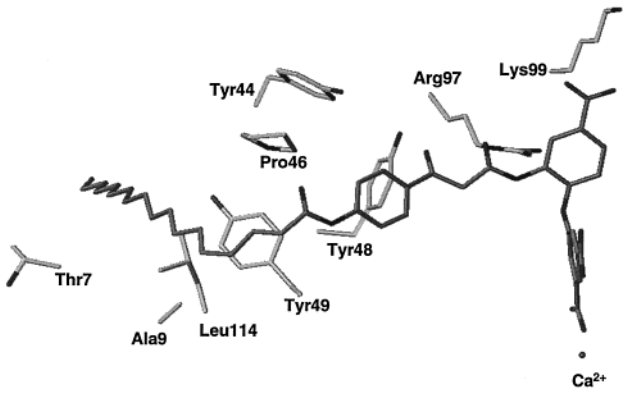


Figure 6. Complex model of compound **2** and E-selectin. Thick colored atoms show compound **2**. Thin colored atoms show E-selectin amino acid residues. Only **2** and the important residues of E-selectin are shown. This model was constructed based on the compound **1**/E-selectin complex model. The three functional groups of compound **2** superimposed with the corresponding groups in the bound conformation of compound **1**.

directed to the solvent area, but the benzene linked to the long alkyl chain seemed to interact with the hydrophobic surface consisted of Tyr44, Pro46, and Tyr48 on E-selectin. Accordingly, the original complex model constructed here showed that compound **2** could well bind to E-selectin.

However, it was experimentally shown that the potency of compound **2** was weaker than that of compound **1**. Thus, to clarify the reason for the potency of compound **2** being weaker than that of compound **1**, we should estimate the binding energy between the compound **2**/protein complex. But, in fact, it is difficult to estimate these interactions precisely and quickly (such as the coordination to metal ion and hydrophobic

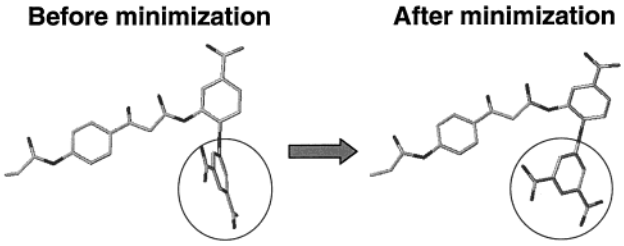


Figure 7. Retrieved compound **2** (left) from the complex model with E-selectin in Figure 6. After minimization, the right conformation was generated. The conformation of the biphenyl ether moiety has changed (in the circle). The long alkyl moiety was omitted during the minimization. The minimization was performed by MMFF94[17] force field attached with Sybyl6.5 until the root mean square of gradients was below 0.5 kcal/(mol Å). We used a nonbonded cutoff of 8 Å and dielectric constant $\epsilon = 1$.

interaction) using the current calculation technology. Therefore, we focused on the bound conformation of compound **2** in the presence of E-selectin and speculated that the desirable bound conformation of compound **2** against the target protein would not be so stable; namely the stable conformation of compound **2** would be much different from the bound conformation toward E-selectin. Thus, to clarify our hypothesis, we investigated an energy minimization of the assumed bound conformation in compound **2**. As a result, the conformation of the biphenyl ether moiety has well changed as shown in Figure 7. This finding indicates that the conformation of this moiety would not be so stable. Namely, a structural modification of this moiety, which makes its initial bound conformation so stable, would be important for lead optimization. Therefore, we have undertaken the conformational analysis of this biphenyl ether moiety in detail.

Conformational Analysis of Compound 2 and Design for a Strong Selectin Blocker. From the above description of the structural characteristics of compound **2**, we focused on the 5-(4-carboxyphenoxy)-isophthalic acid moiety (unit 1) and investigated the conformational analysis of unit 1. First, we defined two dihedral angles of unit 1, ϕ and ψ, and calculated the conformational energies every 10° of the dihedral angles (Figure 8). Figure 8 shows that the active conformation of unit 1 ($\phi = 80°$ and $\psi = 200°$) did not agree with the stable ones. This result seemed to depend on an unfavorable repulsion between the carboxylic acid of benzoic acid and a carboxylic acid of isophthalic acid. Moreover, the compound **2**/E-selectin complex model showed that the unfavorable carboxylic acid in isophthalic acid would not participate in binding with E-selectin. Thus, if this unnecessary carboxylic acid in isophthalic acid was removed from unit 1, the active bound conformation could agree with the stable one (Figure 9). To clarify our hypothesis, we designed unit 2 and performed the conformational analysis in the same way. As a result, it was of interest to note that the most stable conformation of unit 2 was almost identical to the active bound conformation of compound **2** as shown in Figure 9. To confirm the adequacy of our design in experiment, we synthesized a decarboxy compound **3** and a tricarboxyl compound **4** with very close structures to compound **2** and estimated the inhibitory activities of compounds **2**−**4**. Although we also tried the synthesis of the decarboxy derivative of compound **2**, we could not synthesize it.

Chemistry. For the syntheses of compounds **3** and **4**, the condensation of **5a**,**b** with *p*-nitrobenzoyl chloride in the presence of triethylamine in chloroform afforded compounds **6a**,**b**, in 54−73% yields. Next, compounds **6a**,**b** were subjected to hydrogenolysis in the presence of a catalyst, 10% palladium−carbon, under hydrogen atmosphere followed by the acyl condensation with stearoyl chloride in the presence of triethylamine in chloroform to afford compounds **7a**,**b**, in 31−58% yields. Finally, compounds **7a**,**b** were transformed in good yields, by removal of the protecting groups, into the desired compounds **3** and **4** (Scheme 1).

In Vitro Evaluation of Compounds 3 and 4. As a result of the in vitro assay,[6a] compound **3** showed

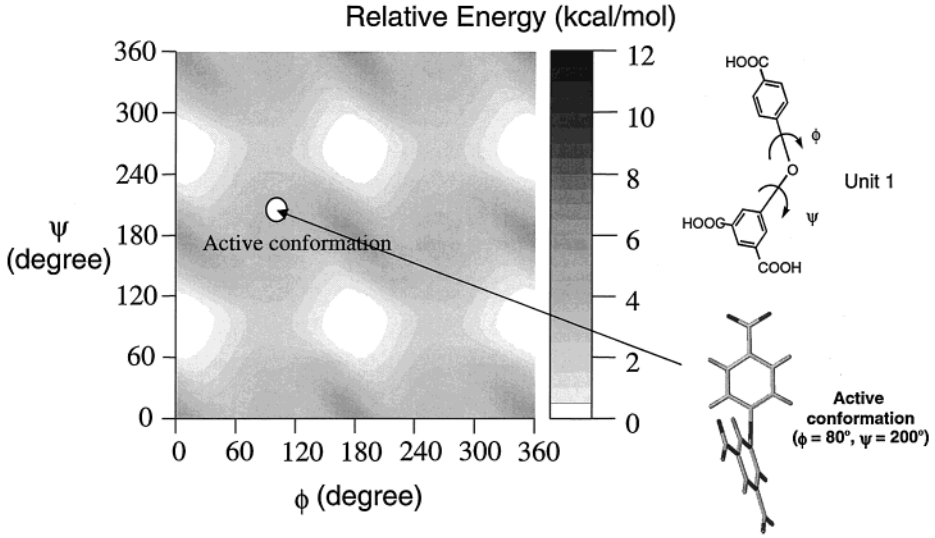


Figure 8. Potential energy surface of unit 1, as the axis of abscissa ϕ and the axis of spindle ψ. Each contour represents a relative energy from the most stable region. The white region indicates the most stable region with increasing thickness of color the growth in relative energy. We calculated the conformational energies every 10° of the dihedral angles using the molecular orbital program package MOPAC93.[18] This calculation was performed using the AM1[19] method and keywords STEP and POINT. White open circle shows the putative active conformation of unit 1 in compound **2**.

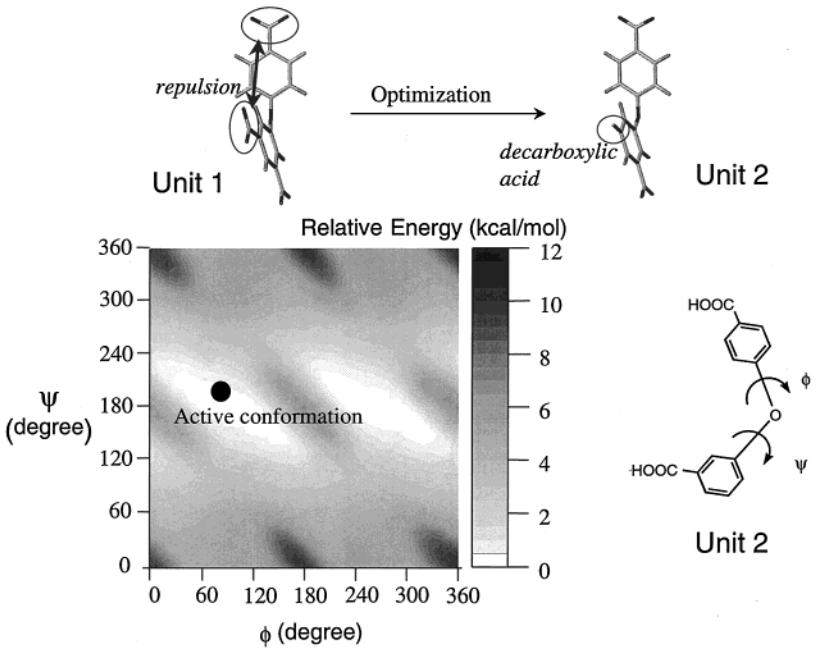
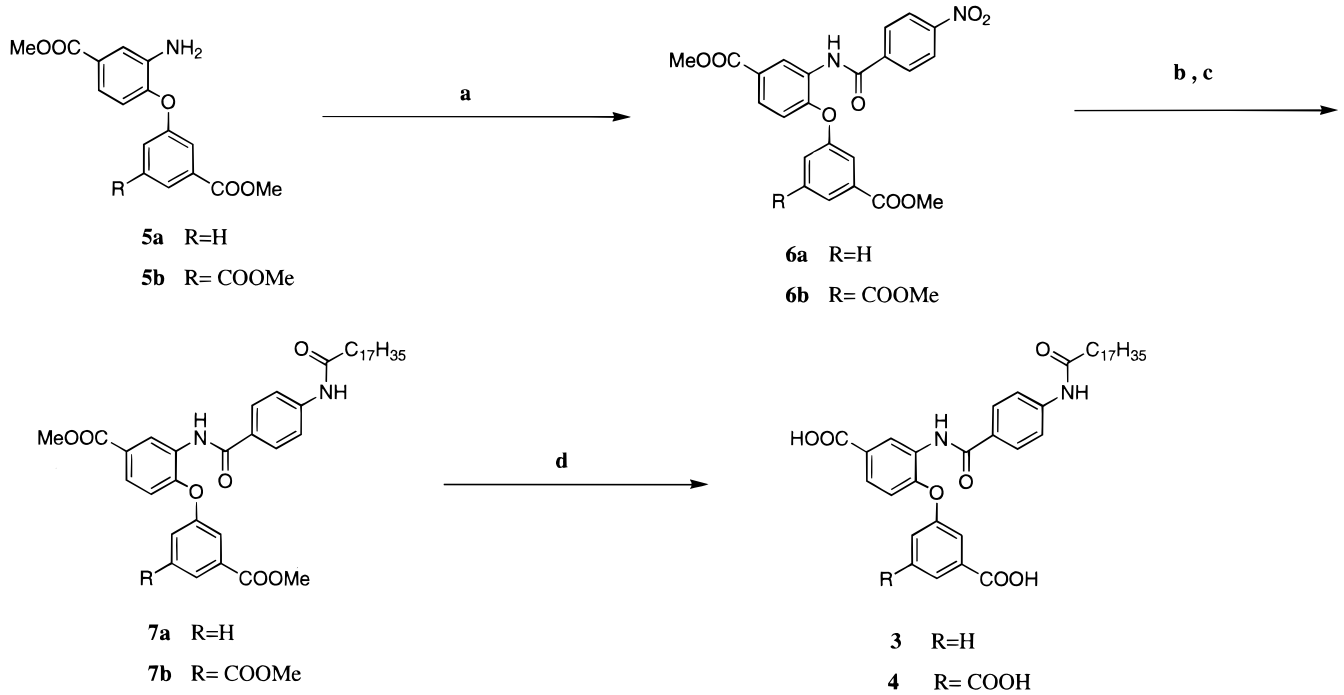


Figure 9. On the basis of the result of conformational analysis of unit 1, we designed unit 2 (decarboxylic acid derivative). Potential energy surface of unit 2 is shown. The white region indicates the most stable region with increasing thickness of color the growth in relative energy. Each contour represents a relative energy from the most stable region. This calculation was performed the same way as in Figure 8. Closed circle shows the putative active conformation of unit 2.

Scheme 1[a]



[a] Conditions: (a) *p*-nitrobenzoyl chloride, $Et_3N/CHCl_3$, 54−73%; (b) 10% Pd−C/1,4-dioxane−MeOH; (c) stearoyl chloride, $Et_3N/CHCl_3$, 31−58% from **6a**,**b**; (d) 1 N NaOH/1,4-dioxane−MeOH, 22−97%.

stronger inhibitory activity (IC_{50} = 86 μM) toward E-selectin than compound **1** (Figure 10), while the inhibitory activity of compound **4** was very weak (IC_{50} > 500 μM), the same as that for compound **2** (IC_{50} = 1200 μM). Namely, as speculated for the inhibitory activities of compounds **3** and **4** from the conformational analysis, it was found that the strong activity of compound **3** would be due to the stability of the bound conformation toward E-selectin. This finding would also suggest that the three functional groups of compound **3** could bind to E-selectin, as predicted by the complex model of compound **2**/E-selectin (Figure 6). In addition, a fucose necessary[20] for calcium binding would be replaced with a carboxylic acid that was a simple functional group. However, the precise contribution of the three functional groups of compound **3** for the binding to the protein was not clarified. To clarify this point, we need to perform a detailed SAR investigation of compound **3** derivatives and/or an estimation of binding energy between compound **3**/E-selectin based

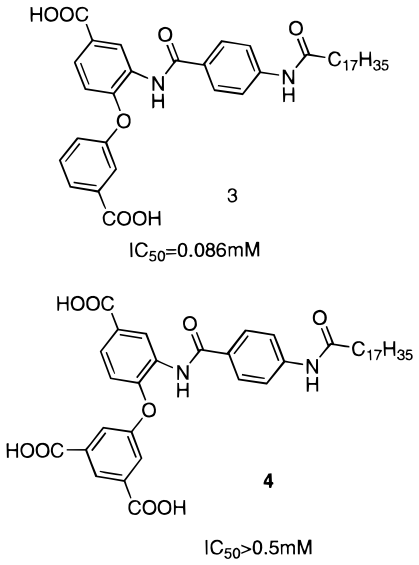


Figure 10. Chemical structure and IC_{50} of compounds **3** and **4** toward E-selectin.

on the docking study. These works are now under investigation.

In conclusion, we discovered the structurally novel selectin blocker **2** based on the 3D-pharmacophore model investigation. Namely, this pharmacophore would have potential to find a new selectin blocker and the 3D-database search using this pharmacophore model could be responsible for carbohydrate mimicking. In addition, we succeeded in optimizing the lead compound **2** preliminarily and obtained the potent selectin antagonist, compound **3**. Further optimization of compound **3** is in progress. Thus, we could present a methodology for the discovery of a new class of selectin blocker.

Experimental Section

Inhibition Assay of Selectin−sLe^x Bindings. The construction of the selectin-immunoglobulin was carried out according to a previous paper.[6a]

A solution of sLe^x pentasaccharide ceramide analogue 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(g)/streptavidin−alkaline phosphatase (Zymed Lab Inc.) and a selectin-immunoglobuline fusion protein (selectin-Ig) was incubated at room temperature for 30 min to form a complex. The test compounds were dissolved in DMSO at 10 mM and finally diluted by 1 mM $CaCl_2$/50 mM imidazole buffer (pH 7.2) to final concentrations at 1000, 500, 250, 125, 62.5, 31.3, 15.6, and 7.8 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 diluted 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 figures as IC_{50} values. The number of replicates is 2.

Dimethyl 3-Nitro-3′,4-oxydibenzoate. To a solution of methyl 4-fluoro-3-nitrobenzoate (1.99 g, 10 mM) and methyl 3-hydroxybenzoate (1.52 g, 10 mM) in DMF (20 mL) was added K_2CO_3 (1.38 g, 10 mM), and the mixture was stirred for 6 h at room temperature. AcOEt was added to the solution, and the mixture was washed with water and brine successively, dried over Na_2SO_4, and concentrated. To the resulting residue was added *n*-hexane and Et_2O, and the precipitate was filtered to afford the title compound (3.04 g, 91.8%) as a crystal: ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H), 3.96 (s, 3H), 6.99 (d, 1H, J = 8.7 Hz), 7.31 (dd, 1H, J = 8.1, 2.5 Hz), 7.51 (t, 1H, J = 7.9 Hz), 7.74 (s, 1H), 7.93 (d, 1H, J = 8.2 Hz), 8.14 (ddd, 1H, J = 0.5, 2.2, 8.8 Hz), 8.62 (d, 1H, J = 2.2 Hz).

General Procedure for the Preparation of 6a,b: Dimethyl 3-(4-Nitrobenzoylamino)-3′,4-oxydibenzoate (6a). To a solution of dimethyl 3-nitro-3′,4-oxydibenzoate (4.37 g, 13.19 mM) in MeOH (100 mL) and 1,4-Dioxane (50 mL) was added 10% Pd−C (100 mg), and the mixture was stirred for 1 h under 3 atmospheric pressure of hydrogen at room temperature. The precipitate was filtered off, and the filtrate was concentrated in vacuo to afford dimethyl 3-amino-3′,4-oxydibenzoate (**5a**) as a syrup.

On the other hand, to *p*-nitrobenzoic acid (0.90 g, 5.39 mM) was added thionyl chloride (20 g), and the mixture was stirred for 2 h at 50 °C and then for 1 h at 75 °C. The mixture was coevaporated with toluene to give *p*-nitrobenzoyl chloride as a syrup. The resulting residue and **5a** (864 mg, 2.87 mM) were dissolved in $CHCl_3$ (20 mL), triethylamine (653 mg, 6.45 mM) was added to the solution, and the mixture was stirred for 20 h at room temperature. $CHCl_3$ (50 mL) was added to the solution, and the mixture was washed with 1 N HCl, saturated sodium carbonate and brine successively, dried over $MgSO_4$, and concentrated. The resulting precipitate was filtered to afford **6a** (700 mg, 54.3%) as a colorless crystal: ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 3.87 (s, 3H), 7.07 (d, 1H, J = 8.6 Hz), 7.35−7.45 (m, 1H), 7.50−7.60 (m, 2H), 7.77 (d, 1H, J = 7.6 Hz), 7.85 (dd, 1H, J = 2.2, 8.7 Hz), 8.07 (d, 2H, J = 8.6 Hz), 8.32 (d, 2H, J = 8.6 Hz), 8.35−8.43 (m, 1H), 10.37 (s, 1H).

Dimethyl 5-[4-Methoxycarbonyl-2-(4-nitrobenzoylamino)phenoxy]isophthalate (6b). Compound **6b** was prepared from dimethyl 5-[3-amino-4-methoxycarbonylphenoxy]isophthalate (3.0 g, 8.35 mM) and *p*-nitrobenzoic acid (2.0 g, 11.97 mM) as described above: yield 72.9%; mp 226−229 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 6H), 3.88 (s, 3H), 7.18 (d, 1H, J = 8.6 Hz), 7.82 (d, 2H, J = 1.5 Hz), 7.87 (dd, 1H, J = 2.2, 8.6 Hz), 8.06 (d, 2H, J = 8.9 Hz), 8.25 (t, 1H, J = 1.5 Hz), 8.31 (d, 2H, J = 8.8 Hz), 8.39 (d, 1H, J = 2.1 Hz), 10.4 (s, 1H).

General Procedure for the Preparation of 7a,b: Dimethyl 3-(4-Octadecanoylaminobenzoylamino)-3′,4-oxydibenzoate (7a). To a solution of **6a** (650 mg, 1.44 mM) in MeOH−1,4-dioxane (10−10 mL) was added 10% Pd−C (100 mg), and the mixture was stirred for 2 h under hydrogen atmosphere at room temperature. The precipitate was filtered off, and the filtrate was concentrated in vacuo to afford dimethyl 3-aminobenzoylamino-3′,4-oxydibenzoate as a syrup.

On the other hand, to stearic acid sodium salt (607 mg, 1.98 mM) was added thionyl chloride (20 mL), and the mixture was stirred for 2 h at room temperature. The mixture was coevaporated with toluene to give stearoyl chloride as a syrup. To a solution of stearoyl chloride in $CHCl_3$ (10 mL) was added a solution of dimethyl 3-aminobenzoylamino-3′,4-oxydibenzoate in $CHCl_3$ (10 mL) and triethylamine (240 mg, 2.37 mM), and then the mixture was stirred for 21 h at room temperature. $CHCl_3$ (50 mL) was added to the solution, and the mixture

was washed with 1 N HCl, saturated sodium carbonate and brine successively, dried over $MgSO_4$, and concentrated. The residue was purified by thin-layer chromatography developing with 1:1 AcOEt/*n*-hexane to afford **7a** (310 mg, 31.3%) as a colorless crystal: ^{1}H NMR ($CDCl_3$) δ 0.70−0.90 (m, 3H), 1.25 (s, 28H), 1.40−1.80 (m, 2H), 2.20−2.40 (m, 2H), 3.80−3.95 (m, 6H), 6.70−6.90 (m, 1H), 7.15−7.30 (m, 3H), 7.30−7.95 (m, 7H), 8.44 (brs, 1H), 9.24 (brs, 1H).

Dimethyl 5-[4-Methoxycarbonyl-2-(4-octadecanoylaminobenzoylamino)phenoxy]isophthalate (7b): yield 58.2%; ^{1}H NMR ($CDCl_3$) δ 0.8−0.95 (m, 3H), 1.1−1.8 (m, 30H), 2.3−2.4 (m, 2H), 3.92 (s, 3H), 3.94 (s, 6H), 6.84 (d, 1H, J = 8.6 Hz), 7.37 (brs, 1H), 7.64 (d, 2H, J = 8.7 Hz), 7.77 (dd, 1H, J = 2.1, 8.7 Hz), 7.83 (d, 1H, J = 8.7 Hz), 7.94 (d, 2H, J = 1.4 Hz), 8.41 (brs 1H), 8.54 (t, 1H, J = 1.4 Hz), 9.24 (d, 1H, J = 2.0 Hz).

General Procedure for the Preparation of 3 and 4: 3-(4-Octadecanoylaminobenzoylamino)-3′,4-oxydibenzoic Acid (3). 7a (300 mg, 0.44 mM) was dissolved in 1,4-dioxane−MeOH (9−3 mL), and 1 N NaOH (4.1 mL) was added to the solution; then the mixture was stirred for 5.5 h at room temperature. Cold water (30 mL) was added to the mixture; the resulting mixture was adjusted to pH 1 using concentrated HCl and then extracted with AcOEt. The organic layer was washed with water and brine successively, dried over $MgSO_4$, and concentrated. The resulting precipitate was washed with *n*-hexane (30 mL) and then purified by HPLC eluting with 9:1 CH_3CN/0.1% aqueous TFA to afford **3** (63 mg, 21.9%): mp 240 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.80−0.85 (m, 3H), 1.10−1.34 (m, 28H), 1.46−1.65 (m, 2H), 2.30 (t, 2H), 7.00 (d, 1H, J = 8.6 Hz), 7.30−7.40 (m, 1H), 7.45−7.60 (m, 2H), 7.60−7.85 (m, 6H), 8.37 (d, 1H, J = 2.2 Hz), 9.79 (s, 1H), 10.07 (s, 1H).

5-[4-Carboxy-2-(4-octadecanoylaminobenzoylamino)-phenoxy]isophthalic Acid (4): yield 97.0%; mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 0.75−0.95 (m, 3H), 1.23 (s, 28H), 1.4−1.7 (m, 2H), 2.25−2.4 (m, 2H), 7.12 (d, 1H, J = 8.6 Hz), 7.66 (d, 1H, J = 8.8 Hz), 7.7−7.85 (m, 5H), 8.2−8.3 (m, 1H), 8.35−8.4(m,1H), 9.84 (s, 1H), 10.07 (s, 1H).

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JM990342J