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A Solid-Phase Synthesis for β-Turn Mimetics of Sialyl Lewis X

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Abstract—A solid-phase synthesis of heterocyclic β-turn mimetics of sialyl Lewis X, which is a natural carbohydrate ligand of selectins, was established. This synthetic method could be very useful for drug discovery of selectin antagonists using combinatorial chemistry techniques. © 2000 Elsevier Science Ltd. All rights reserved.

Sialyl Lewis X (sLe^X) is a natural carbohydrate ligand of cell adhesion molecules such as E-, P-, and L-selectins. Many studies indicated that the sLe^X/selectin interaction played an important role in the migration of inflammatory cells from the blood stream to inflammatory sites, and the interaction participates in various inflammatory diseases[1–5] such as asthma, rheumatoid arthritis, ischemia/reperfusion injury, and cutaneous inflammatory disorders containing atopic dermatitis and psoriasis. Therefore, a number of selectin blockers have been focused on as a new type of anti-inflammatory agents and sLe^X-based drug design has attracted the interest of medicinal chemists.

In the series of mimetic studies of sLe^X, we have already reported that a fucopyranosyl Ser-Glu dipeptide (**1a**), a fucofuranosyl Ser-Glu dipeptide (**1b**), and a mannosyl Ser-Glu dipeptide (**1c**) showed potent inhibitory activities against selectins. In particular, the inhibitory activities of compounds **1a**–**c** against P- and L-selectins were 1000 times more potent than that of sLe^X.[6–8] In addition, computational investigations regarding the bound form of **1a**–**c**/selectin complex indicated that the active forms of compounds **1a**–**c** were characterized by type II and/or type II′ β-turn formation.[6] There is, however, little experimental evidence to support that the type II and/or type II′ β-turn conformation would be an active scaffold for the sLe^X mimetics.

On the other hand, the research of peptidomimetics based on the β-turn structure is very in fashion and has attracted interest in the field of medicinal chemistry.[9] However, very few β-turn mimetics of biologically active carbohydrate motif have been developed.[10]

Ellman et al. recently reported that a small molecule heterocyclic mimetic of the biologically important peptide somatostatin was identified in β-turn mimetic libraries constructed by solid-phase synthesis.[9] Solid-phase synthesis is an applicable tool for drug discovery and/or drug optimization using the combinatorial chemistry technique.[11] Therefore, we focused on the design and synthesis of heterocyclic scaffold (**2**) for sLe^X mimetics based on solid-phase synthesis as shown in Figure 1.

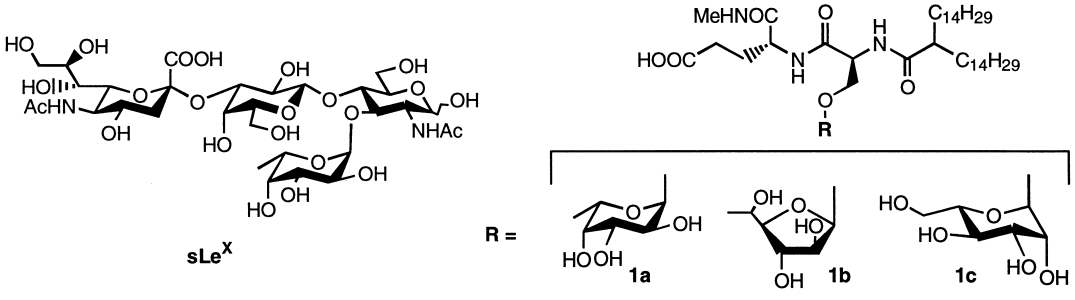

Chart 1.

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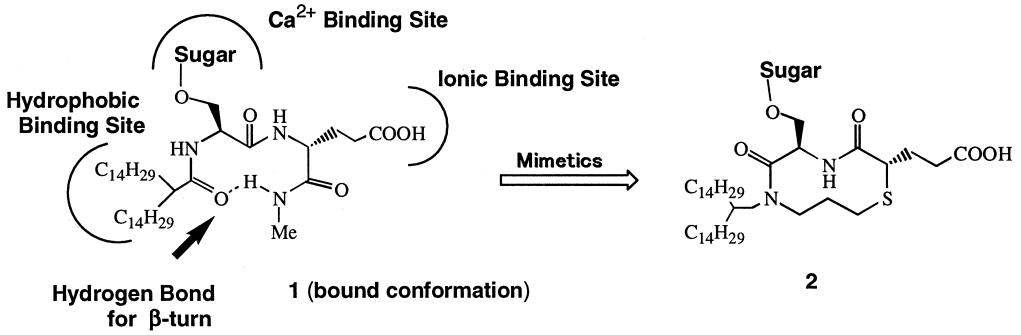


Figure 1. Heterocyclic β-turn mimetics.

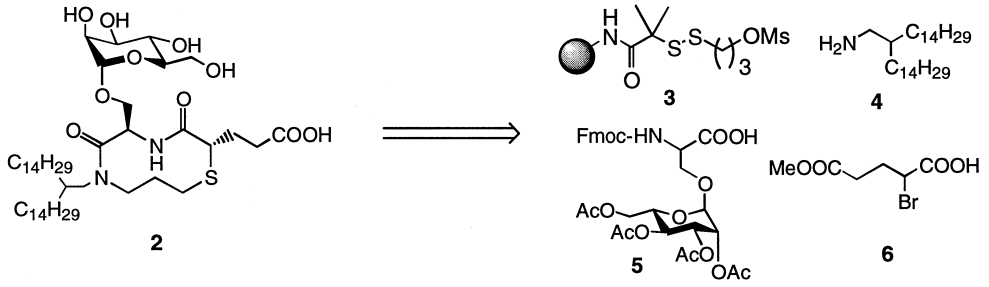
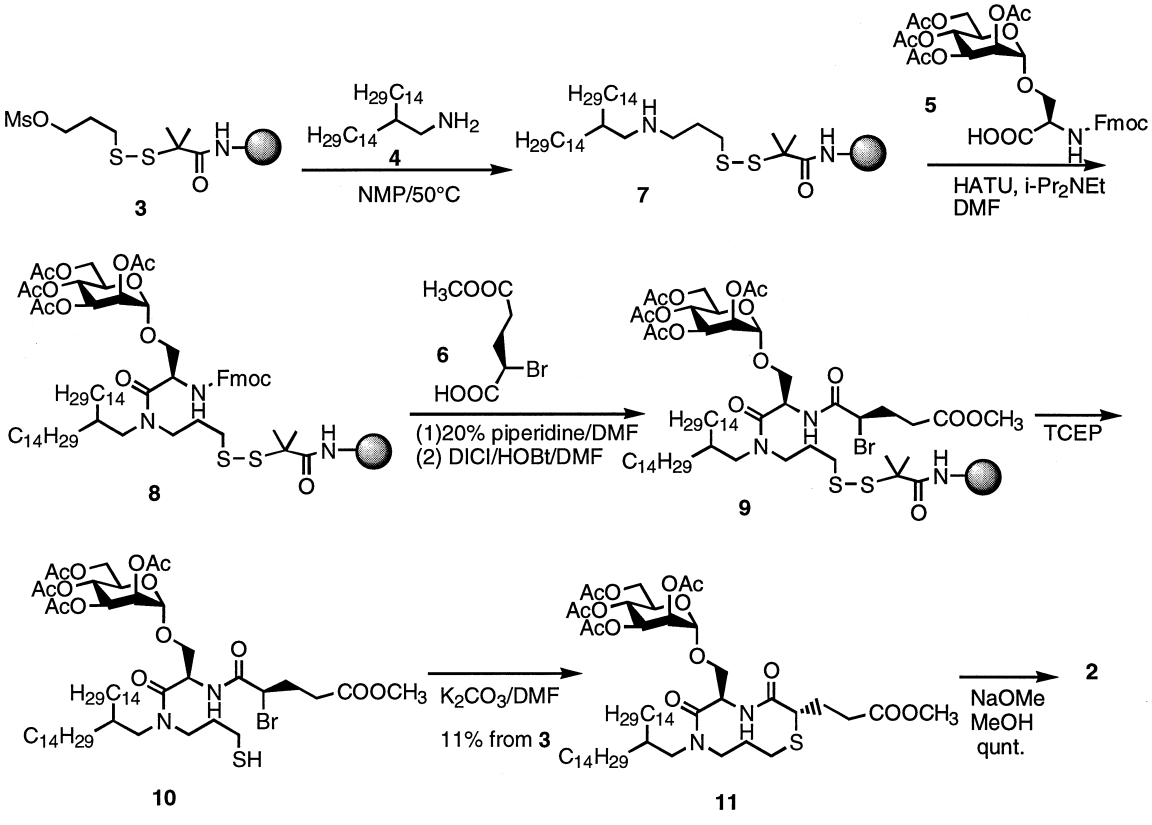


Figure 2. Retrosynthesis for heterocyclic β-turn mimetics.



Scheme 1.

Although the solid-phase synthesis of a heterocyclic β-turn mimetic containing a carbohydrate has never been reported, basically the synthesis of compound **2** was achieved according to the retrosynthesis as shown in Figure 2. An activated key-linker **3** with a *gem*-dimethyl disulfide unit was prepared according to a previous paper.[9] It is well known that the absence of the *gem*-dimethyl group in **3** results in reduced yields presumably

Table 1. In vitro activity of compounds **1c**, **2**, and sLe^X

Compounds	IC_{50} μM		
	E-selectin	P-selectin	L-selectin
1c	3.5	0.45	4.0
2	>100	2.57	2.34
sLe^X	600	>1000	>1000

due to the lability of the disulfide bond to amine bases under reaction conditions.[12] As shown in Scheme 1, the solid-phase synthesis of the heterocyclic β-turn mimetic (**2**) was established and its in vitro activity was evaluated (Table 1).

The activated support-linker **3** was first treated with a branched alkyl amine **4** in *N*-methyl pyrrolidone for one night at 50 °C to afford **7**. Condensation of an Fmoc-protected serine derivative **5**[,8] and the secondary amine **7** in the presence of *o*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[13] gave a coupling product **8** in a good manner. Deprotection of the Fmoc group of compound **8**, followed by the coupling with an α-bromo acid **6** afforded compound **9** in a moderate yield. The α-bromo acid **6** used here was prepared in highly enantioselective form in a single step from glutamic acid.[14] Next, the support-linker release from compound **9** and the following cyclization reaction into compound **11** was investigated. It has already been reported that tris-(2-carboxyethyl)-phosphine (TCEP) provided successfully the linker release and cyclization in the presence of *N*-methylmorpholine which worked as a scavenger of acid such as HBr.[9] According to this procedure, a similar reaction was achieved. As a result, it was found that the heterocyclization reaction of compound **10** containing a carbohydrate did not work in the presence of organic bases such as *N*-methylmorpholine or diisopropylethylamine, but it was found that K_2CO_3 was a successful base to obtain the desirable compound **11**. Although the yield should be more improved (11% from compound **3**), the cyclic compound **11** was obtained in a highly stereoselective manner. Deprotection of compound **11** was carried out under mild conditions to afford a target compound **2**.[15]

In order to clarify the inhibitory activity of compound **2** against selectin/sLe^X binding, the biological activity of compound **2** was evaluated using in vitro ELISA assays[16] and its activity was compared to that of the β-turn mimetic compound **1c** (Table 1). Toward the E-selectin, compound **2** showed weak activity compared to compound **1c** (IC_{50} values 3.5 μM for **1c** and >100 μM for **2**). On the other hand, toward the P- and L-selectins, compound **2** showed potent inhibitory activity as well as compound **1c** and more potent than sLe^X. These findings indicated that the heterocyclic skeleton such as compound **2** could be a useful scaffold for sLe^X mimetics. Although compound **2** has a 10-membered ring, other membered-ring compounds might bind more tightly to target selectins. We are now in the process of synthesizing and evaluating several derivatives with other membered-rings using the divergent solid-phase synthesis.

References and Notes

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15. Typical procedure: A linker **3** was treated with branched alkyl amine **4** (1.5 mmol) in *N*-methyl pyrrolidone (3 mL) for one night at 50 °C. The resin was isolated by filtration and rinsed with DMF, chloroform, and MeOH to afford **7**. Resin **7** was dissolved in a solution of Fmoc-Ser(OMan(OAc))-OH **5** (1.5 mmol), HATU (1.5 mmol), and *i*-Pr_2NEt (1.5 mmol) in DMF (3 mL) and the reaction vessel was shaken for three days. The resin was collected by filtration and rinsed with DMF, chloroform, and MeOH. A solution of piperidine (20% v/v) in DMF was used to deprotect the Fmoc group. The deprotected resin was added in a solution of 2-bromopentanedioic acid 5-methyl ester **6** (1.5 mmol) in DMF (3 mL) and 1-hydroxy-7-azabenzotriazole (1.5 mmol) and *N,N*-diisopropylcarbodiimide (1.5 mmol) was added to resin mixture. After shaking for 20 h, the resin was isolated by filtration and rinsed as above to provide **9**. The solid support disulfide linkage of **9** was reduced by treatment with a solution of TCEP (1.5 mmol) and *i*-Pr_2NEt (3.0 mmol) in a 9:1 dioxane H_2O solution (6 mL). After shaking for 20 h, the mixture was filtered to remove the resin and the filtrate was added to chloroform, and washed with aqueous $NaHCO_3$ and satd NaCl. The organic layer was dried over $MgSO_4$, and was concentrated. To the obtained residue was added DMF (1.5 mL) and K_2CO_3 (1.5 mmol). After stirring for 5 h, to the solution was added chloroform, and the mixture was washed with water and satd NaCl. The organic solution was dried over $MgSO_4$ and concentrated to afford **11** (65 mg); ^{1}H NMR ($CDCl_3$): 0.79–0.93 (m, 6H), 1.06–1.43 (m, 52H), 1.54–1.75 (m, 2H), 1.75–1.91 (m, 2H), 1.92–2.01 (m, 3H), 2.04 (s, 3H), 2.11 (s, 3H), 2.13–2.62 (m, 3H), 2.35–2.61 (m, 4H), 2.75–3.00 (m, 1H), 3.10–3.16 (m, 1H), 3.30–3.45 (m, 3H), 3.67 (s, 3H), 3.90–4.10 (m, 3H), 4.30–4.38 (m, 2H), 5.20–5.34 (m, 3H). Hydrolysis of **11**: To a solution of **11** (0.06 mmol) in MeOH (2.0 mL) was added NaOMe (0.55 mmol). After stirring for 3 h, H_2O (0.55 mmol) and dioxane (1 mL) were added to the reaction mixture, and the mixture was stirred for 3 h at room temperature. The reaction

mixture was neutralized with 1N HCl, and to the mixture was added chloroform, and washed with H_2O. The organic solution was dried over $MgSO_4$, and concentrated under reduced pressure. The crude mixture was purified by column chromatography to obtain **2**; ^1H NMR ($CDCl_3$): 0.75–0.95 (m, 6H), 1.00–1.75 (m, 49H), 3.00–4.35 (m, 13H), 4.75–5.50 (m, 3H). MS (TOF-MS); $m/z = 895.959$ (M + Na).

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