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Drug Design, Synthesis, and Evaluation of a Non-sugar-based Selectin Antagonist

Kyoko Fukunaga, Takahiro Tsukida, Hideki Moriyama and Hirosato Kondo*

Department of Chemistry, R&D Labs, Nippon Organon K.K., Tomobuchi-cho, Miyakojima-ku, Osaka 534-0016, Japan

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Abstract—We have designed a series of simple rigid compounds (**2**) having a phenyl ring attached to three essential groups necessary for selectin binding, i.e., a fucose unit, a carboxylic acid, and the hydrophobic part. In this series of compound **2**, **2a** exhibited strong inhibitory activity in in vitro P-selectin mediated cell adhesion assay. The novel type of compound **2a** would be a potential lead compound for selectin antagonist. © 2001 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. It has been reported that the sLe^X/selectin interaction plays an important role in the migration of inflammatory cells from the blood stream to inflammatory sites, and the interaction participates in various inflammatory diseases.[1–8] Therefore, it is expected that selectin inhibitors based on sLe^X or sLe^X mimetics could be a new type of anti-inflammatory agent. However, drug development of a carbohydrate itself is hindered by the complication that the synthesis of complex oligosaccharides is rather expensive and difficult. Thus, one of the current studies of biologically active oligosaccharides is directed to mimic sugar with low molecular-weight compounds having simple and drug-like structures (Fig. 1).

There has been a substantial amount of work on this subject. Recently, we have succeeded in discovering a Ser-Glu dipeptide inhibitor (**1**) for selectin/sLe^X binding.[9,10] From the SAR study, it has been found that compound **1** has three essential groups for the selectin binding: (1) the fucose unit, as a calcium ligand; (2) the hydrophobic part with a branched alkyl chain; and (3) the negatively charged group, a carboxylic acid for interaction with the target molecule.[10] In addition, compound **1** is characterized by the type II β-turn structure, which is supported by NMR studies.[11]

We focused on the type II β-turn motif of compound **1**. If the type II β-turn structure played an important role in orienting the desired positions of three essential groups for binding to selectins, the β-turn motif may be replaced by other templates to keep the desired positions for the three functional groups. To this end, we designed a simple rigid compound (**2**) having a phenyl ring attached to the three essential groups necessary for selectin binding, that is a fucose unit, a carboxylic acid, and the hydrophobic part.

Synthesis of compounds **2** was achieved as shown in Scheme 1. Diethyl 5-(hydroxymethyl)-isophthalate (**3**) was treated with triphenylphosphine and CBr_4 in methylene chloride to afford bromide **4** in 83% yield.

Glycosylation of **4** with 1-thio-L-fucose derivative **5**[12] in the presence of 20% NaOEt gave compound **6** in 92% yield. Hydrolysis of compound **6** with KOH (1.5 equiv) in ethanol afforded compound **7** in 90% yield. Next, condensation of **7** with a branched alkylamine in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide (WSC) and 1-hydroxybenzotriazole (HOBt) gave compound **8** in 47% yield. Deprotection of compound **8** in the presence of 1 N NaOH afforded target compound **2a**. At the same time, compound **2a** is a key intermediate for compounds **2b** and **2c**. So, condensation

*Corresponding author. Tel.: +81-6-6921-8920; fax: +81-6-6921-9078; e-mail: kondo.hirosata@organon.co.jp

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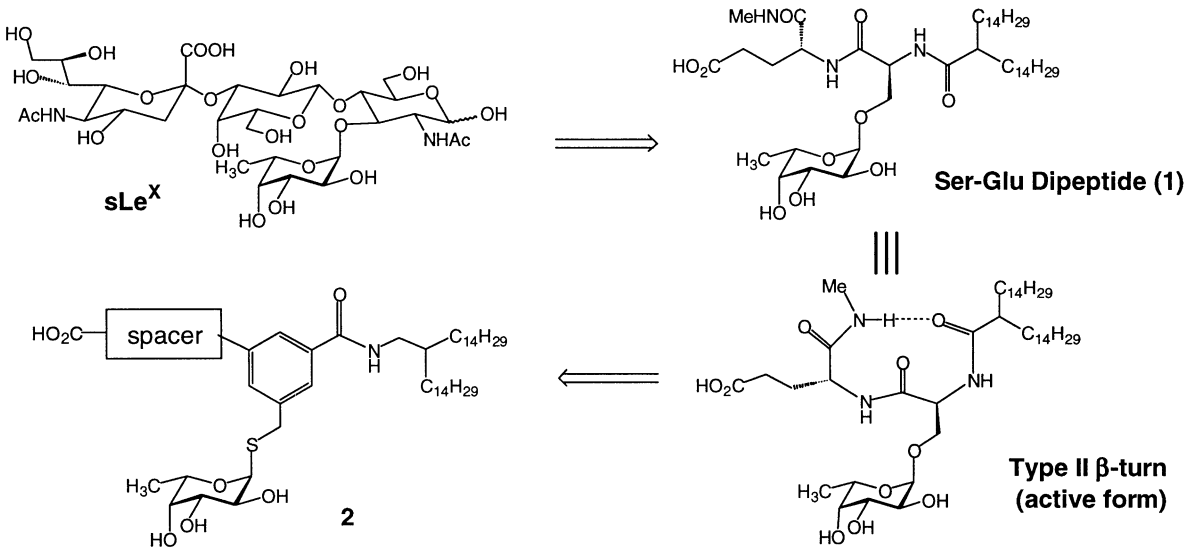


Figure 1. Design of novel selectin antagonist.

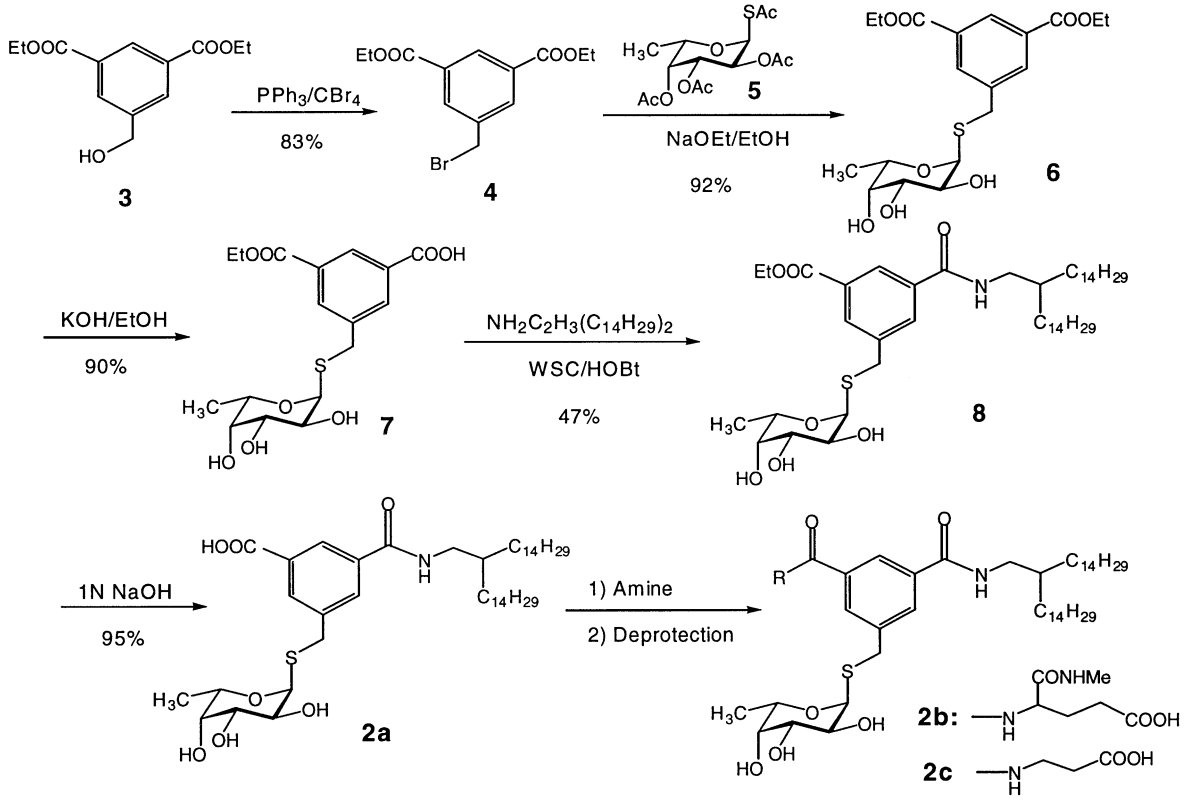
of **2a** with C-terminal protected amino acids, followed by deprotection according to the general condition yielded target compounds **2b** and **2c** in 82 and 88% yields from **2a**, respectively.

In order to examine the inhibitory activity of compounds **2a–c** against selectin/sLe^X binding, the biological activities of compounds **2a–c** were evaluated in an in vitro P-selectin mediated cell adhesion assay[13] and the cell toxicities of compounds **1** and **2a–c** was also investigated. As shown in Table 1, all compounds did not show significant cell toxicity at 30 μM concentration.

Table 1. In vitro P-selectin mediated cell adhesion assay and cell toxicology

	P-selectin cell adhesion assay and cell toxicology		
Compd	% inhibition @ 30 μM	% death @ 30 μM	IC_{50} (μM)
2a	75.7	1.9	10.6
2b	−0.9	5.3	ND[a]
2c	31.3	2.8	ND
1	42.8	1.7	ND

[a]ND, not determined.



Scheme 1.

Compound **1** exhibited 42.8% inhibition at 30 μM against P-selectin mediated cell adhesion. On the other hand, compound **2a** showed the strongest inhibition against the cell-based assay, 75.7% inhibition at 30 μM and IC_{50} value 10.6 μM. However, other compounds, **2b**, **2c**, were weaker than compound **2a**; in particular compound **2b** did not show inhibition at all. From the data, it was found that the length between the carboxylic acid and the phenyl ring of compound **2** would be very important to interact with the target molecule and compound **2a** was found to become a good lead compound for selectin antagonists. Optimization of compound **2** is in progress.

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13. Cell adhesion assay: Anti-human IgG(Fc) mAb was diluted to 10 μg/mL in PBS and 50 μL aliquots were incubated overnight in each well of 96-well microtiter plate (Microtest96TM3075; Becton Dickinson Labware, Flanklin Lakes, NJ, USA) at 4 °C. The well were washed with PBS containing 0.05% Tween 20 and blocked with 2% BSA in PBS for 1 h at room temperature. The wells were then washed with T-PBS and incubated with 5 μg/mL P-selectin-IgG chimera. The wells were incubated with 1 mg/mL goat F(ab′)2 anti-human IgG(Fc) to block Fc receptors. Compounds dissolved in DMSO or distilled water at 10 mM were diluted by RPMI 1640 containing 10% FCS to final concentration at 0.1 mM. BCECF-AM (5 μM; Dojindo, Osaka, Japan)-labeled HL-60 cells (1×10^5 cells/well) were then added to P-selectin-IgG chimera coated wells and allowed to bind for 30 min at 37 °C. The wells were then filled with RPMI1640 containing 10% FCS, and the plate was inverted and placed for 30 min at 37 °C. After removal of the unbound cells by gentle aspiration, 1% NP-40 in PBS was added to each well and the fluorescence intensity was measured at 490 nm of excitation and 530 nm of emission by MTP-32F fluorescence microplate reader (Corona Electric, Hitachinaka, Japan).