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Design and Synthesis of a New Sialyl Lewis X Mimetic: How Selective Are the Selectin Receptors?

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Abstract—The present paper reports the molecular modeling-based design and synthesis of an optically pure noncarbohydrate mimetic of sialyl Lewis X to inhibit E-selectin. Biological evaluation of the designed substance as well as that of its enantiomer gave, contrary to expectations, comparable IC_{50} values. Results are discussed in terms of receptor binding specificity and the molecular modeling protocol used.

The selectins are key players in the fight against pathogens. They play an essential role in the immune system as they are involved in the migration of leukocytes from the blood vessels to the sites of inflammation.[1] They are responsible for the rolling of leukocytes on the endothelial cells of post-capillary veinules leading to leukocyte extravasation from the blood stream. E- and P-selectins are expressed on activated endothelial cells while L-selectins are constitutively expressed on leukocytes. It is known that overrecruitment of leukocytes can cause health problems such as rhumatoid arthritis, septic shock, asthma, diabetes, psoriasis, and reperfusion injury.[2] Consequently, much effort has been expended towards the study of these receptors and their ligands.

One of the natural ligands of selectins is sialyl Lewis X (sLe^x), a tetrasaccharide motif found on specialized glycoproteins expressed on the surface of endothelial cells (Fig. 1).[3] Following a number of molecular biology and medicinal chemistry studies on E-selectin such as mutagenesis and ligand modification, it is now widely recognized that the 2- and 3-hydroxyl groups on the fucose unit of sLe^X are essential for binding to the calcium atom located within the receptor binding site, and that the carboxyl group of the sialic acid moiety is involved in the formation of a salt bridge with a neighboring arginine (Arg-97) side chain.[4] In addition, it was proposed that the 4-hydroxyl of the galactose unit binds to the side chain of tyrosine (Tyr-94) through a hydrogen bond, but the energy involved seems less important than that for the other two previously described interactions.[5] An examination of the literature revealed that efforts in designing inhibitors were concentrated mainly on the binding to the calcium atom and Arg-97, and have been mostly directed towards modified carbohydrates.[6] This endeavor has given good results, but carbohydrate derivatives, and particularly glycosides, are not considered good drug candidates as they are usually too sensitive to enzymatic hydrolysis to be orally active and too hydrophilic to show good bioavailability. The obvious solution then becomes the creation of small

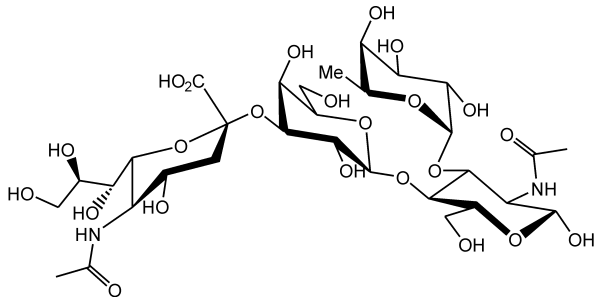

Figure 1. Structural drawing of the sLe^X active conformation.[9]

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noncarbohydrate molecules that can effectively mimic sLe^X and bind efficiently and selectively to one of the selectins.[7] In the present study, we used a molecular modeling approach to design a low molecular weight noncarbohydrate inhibitor, which was then synthesized and tested against sLe^X.

The general approach used in the design of this new inhibitor is similar to that of other research groups and focused on exploiting some of the key interactions with the selectin receptors such as the binding to the active site calcium atom and binding to the side chain of Arg-97 through the use of a vicinal diol and a carboxylic acid moiety, respectively. In addition, our candidate structure was also designed to mimic as much as possible the E-selectin bound conformation of sLe^X as determined by transfer NOE experiments.[8] Therefore, in order to access proper spatial orientation and distance between the pharmacophoric groups, we used a conformationally semi-rigid *cis*-decalinic scaffold that nicely mimics the stacked fucose and galactose rings in the sLe^X active conformation (Fig. 2).

Finally, in order to evaluate this mimic, we docked it on to a model of the E-selectin receptor. Since there was no published X-ray structure of sLe^x or any other molecule complexed to E-selectin, we first followed the Kogan approach to generate an E-selectin-sLe^X model to serve as template for the docking of our candidate inhibitor (Fig. 3).[5] This initial model was created using the published X-ray structure of the lectin domain of apo E-selectin and the published bound conformation of sLe^X, and is based on the homology between E-selectin and the rat-mannose binding protein for which a co-crystal structure with its natural ligand exists.[10] Our modeled ligand was then built and minimized while constraining the essential binding groups in a conformation that mimics the active conformation of sLe^X.[11] This allowed

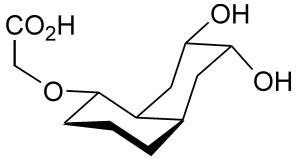


Figure 2. *cis*-Decalinic mimic of sLe^X.

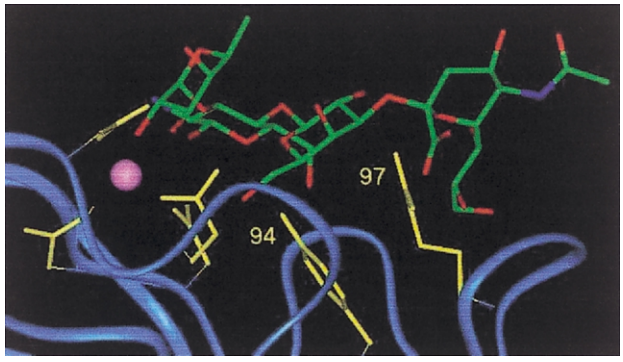


Figure 3. Docking of sLe^X on E-selectin (blue). The pink sphere is the calcium atom. Acetyl group of GluNAc is deleted for clarity. Distance of COOH to Arg-97: 2.22 Å.

the docking of the candidate molecule on the active site of the E-selectin complex model by superimposing the vicinal diol of the candidate structure with the calcium binding diol of the fucose moiety of sLe^X (Fig. 4). The sialyl Lewis X ligand was then removed and the new E-selectin–candidate structure complex minimized. It is important to note here that the forcefield does not properly take into account the interaction between the ligand and the calcium atom, so a constraint is introduced in the minimization process to keep the distance and the angle of coordination of the diol of the candidate structure with the calcium identical to that in the docked sLe^X. The resulting calculated electrostatic and van der Waals energies showed favorable interaction of the mimic to the receptor (Fig. 5). It is interesting to note that in Figure 5 the carboxyl of the mimic hydrogen bonds to both Arg-97 and Tyr-94 whereas in sLe^X (cf. Fig. 3), the neuraminic acid carboxyl binds only to Arg-97 and from a different orientation. In summary, the synthetic target validated by this process is the *cis*-decalinic diol bearing a carboxylic acid side chain represented in Figure 2.

A first attempt at elaborating an enantioselective synthesis of the target compound (Fig. 2 or structure **8**; Scheme 1) having proven inefficient, the target dihydroxyacid **8** was synthesized in nine steps starting with the known 4-benzyloxycyclohexanone **1** via a racemic

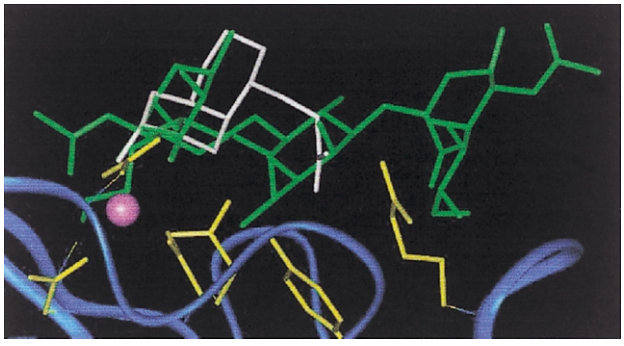


Figure 4. Superimposition of the *cis*-decalinic mimic of Figure 2 (white) and sLe^X (green) on E-selectin.

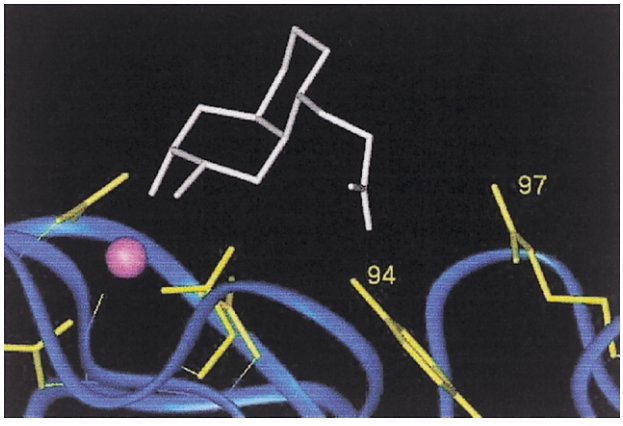
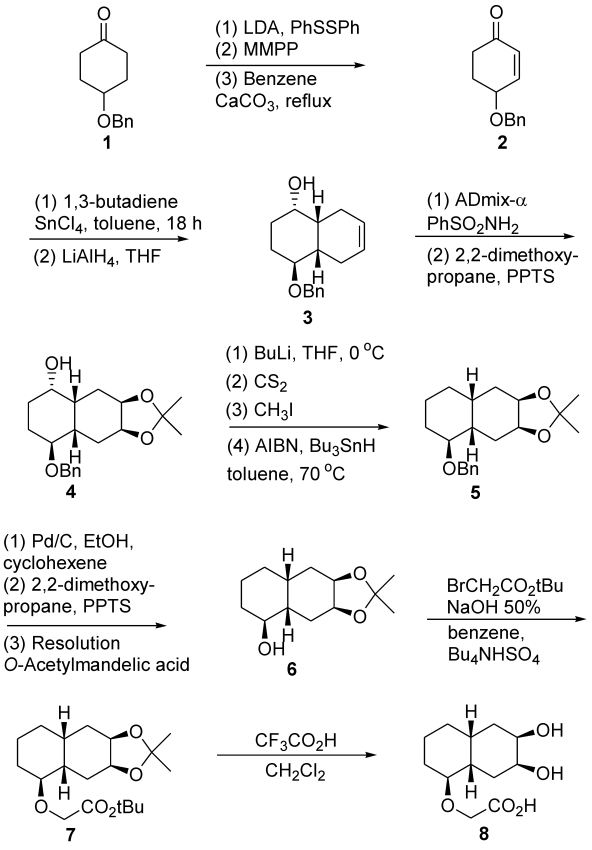


Figure 5. Docking of the *cis*-decalinic mimic of Figure 2 (white) on E-selectin (blue). Distances of COOH to Arg-97 and Tyr-94: respectively 2.36 Å and 2.23 Å.

approach (Scheme 1).[12] Conversion to the corresponding enone **2** was effected by the Trost procedure using diphenyldisulfide and the magnesium salt of monoperoxyphthalic acid (MMPP).[13,14] The next step was the most critical one in this sequence and involved carrying out a Lewis acid catalyzed Diels–Alder reaction with 1,3-butadiene that established the *cis* ring juncture of the decalin and the *exo* position of the benzyloxy group. This reaction was carefully monitored to maximize the selectivity towards the *endo* product, and to minimize epimerization of one of the hydrogens at the ring junction in the Diels–Alder adduct. In order to prevent this epimerization, we avoided using an aqueous work-up; instead, we quenched the Diels–Alder keto-adduct by cannulating the reaction mixture into a lithium aluminum hydride suspension in THF to reduce the ketone, thus securing the ring junction by generating the oxy-adduct **3**. The optimal conditions for this two-step one-pot reaction were obtained by using $SnCl_4$ as catalyst, which resulted in a 51% yield of a 5:2 ratio of the *cis* and *trans* junction of the desired *endo* hydroxy-adduct **3**.[15]



Scheme 1. Synthesis of sLe^X mimic **8**.

Table 1. Biological activity of the enantiomeric pair

Compound	IC_{50} (mM)	
	E-selectin	P-selectin
sLe^X	5.0	5.5
Enantiomer **A**	7.0	5.0
Enantiomer **B**	5.0	4.5

The next step in the synthesis involved the introduction of the *exo-cis* vicinal diol functionality. This reaction proceeded using the Sharpless AD-mix α reagent where the bulky ligand ensured *exo* dihydroxylation.[16] The resulting *exo*-diol was converted to acetonide **4** and then deoxygenated by the Barton procedure.[17,18] Debenzylation of **5** required two sequential treatments with palladium on charcoal to obtain the free alcohol **6**, probably because the starting material was contaminated with sulfur impurities from the deoxygenation reaction. It should be mentioned here that deacetonization also occurred during this reaction, so the diol had to be reprotected before pursuing the sequence.[19]

At this point in the sequence, resolution of racemic **6** was carried out by chromatographic separation of the *O*-acetylmandelate diastereoisomeric mixture prepared by coupling alcohol **6** with *O*-acetylmandelic acid using DCC and DMAP. The optically pure alcohols **6** were then obtained by hydrolyzing the diastereoisomeric pair of esters with aqueous potassium carbonate.[20] Finally, *O*-alkylation of each enantiomer **6** under phase transfer catalysis[21] yielded the two enantiomers of the product **7** which were totally deprotected, in one step, using trifluoroacetic acid in dichloromethane.[22] Final purification of the resulting enantiomeric dihydroxyacids **8** by chromatography on Sephadex® G-10 yielded the pure enantiomeric pair of **8** for testing.[23]

The compounds were tested in competition assays. Expression vectors coding for E-selectin-Ig and P-selectin-Ig[24] were used to produce the chimeric proteins in COS cells. The proteins were then coated to wells and used in a cell assay based on previously described methodology.[25] Briefly, HL-60 cells containing radioactive tritium and expressing the P- and E-selectin ligands with the natural sLe^X on their surface are added. The binding, which is relevant to the natural interaction between the selectins and the cells leads to the adhesion of HL-60 cells to the coated wells. The ability of the mimic to displace the interaction of selectins and HL-60 cells is monitored and IC_{50}'s are evaluated. If the mimic has a better or similar affinity to the selectins compared to sLe^X, cells are not able to adhere to the wells in a dose-dependent concentration manner. The activity of the mimics is measured by counting the change in radioactivity as a function of concentration. A parallel control experiment is also performed by measuring the activity of sLe^X in this assay.

Much to our surprise and as indicated in Table 1, both enantiomers gave, more or less, the same activity as sLe^X for both E- and P- selectins. This result was at the same time rewarding and puzzling! It was rewarding because it justified the modeling approach to generate simple, noncarbohydrate and low-molecular weight mimics of sLe^X. On the other hand, it was puzzling because, as indicated above, our modeling protocol followed that of Kogan which involved a superimposition of the vicinal diol of the candidate structure with the calcium binding diol of the fucose moiety of sLe^X. In our case, it happens that *such a superimposition of the vicinal diol moiety is possible for one enantiomer only*

raising the possibility that chelation of the calcium by the vicinal diol may adopt different orientations of similar energies depending on the enantiomer tested. On the other hand, there remains the more remote possibility where the 'wrong' enantiomer could bind in a totally different manner but fortuitously with the same energy.

Whatever the exact reason, the present experimental results do not justify a choice at the moment but they clearly demonstrate that low molecular weight noncarbohydrate mimics of sLe^X can be generated using the simple *cis*-decalinic scaffold. Furthermore, because of its simplicity and the diversity of access routes to a large number of its analogues, it is expected that additional pharmacophoric groups can be introduced to access additional binding and better selectivity.

Acknowledgements

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