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Design and Synthesis of Sialyl Lewis x Mimics as E-Selectin Inhibitors

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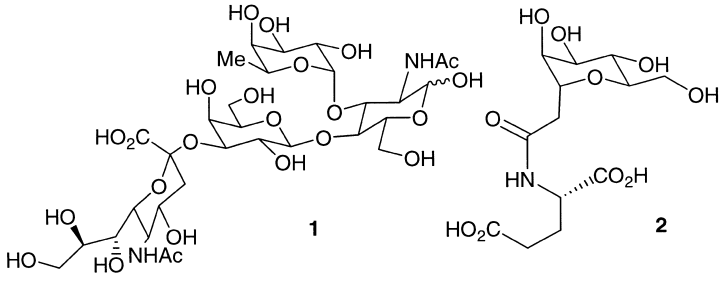
Abstract—The design and synthesis of novel β-C-mannosides that inhibit the binding of sialyl Lewis x to E-selectin are described. Compounds that contained a phenyl substituent at the C-6 position were found to have increased potency.

The selectins are a family of calcium dependent, cell adhesion molecules that mediate the initial rolling of leukocytes during an inflammatory response. Two members of the selectin family, E- and P-selectin, are expressed on the activated endothelium at sites of inflammation. They recognize and bind to a minimal lactosaminoglycan carbohydrate epitope, sLe^x (sialyl Lewis x, **1**) on the leukocytes. The excess recruitment of leukocytes can contribute to several acute diseases, such as stroke and reperfusion injury, and chronic diseases, such as psoriasis and rheumatoid arthritis. The development of a small molecule inhibitor that blocks the interaction of selectins with sLe^x has become an important therapeutic target.[1–7]

The role of the individual sugars of sLe^x in selectin–ligand interactions has been identified.[8–11] Sialic acid, L-fucose and galactose have been shown to be critical for the binding of sLe^x to selectins. The *N*-acetylglucosamine moiety permits some variations. Several reports of small molecules that mimic sLe^x and show in vitro activity can be found in the literature.[12–21]

As part of our ongoing effort in development of an E-selectin antagonist, we are interested in synthetically accessible sLe^x mimics.[22] Some of the sLe^x mimetics in the literature contain a mannose moiety[13–18] that functionally replaces the fucose of sLe^x and a carboxylate connected by a linker that plays the role of sialic acid. In one such report by Wong et al.,[18] α-mannosyl glutamate **2** was shown to be five times more potent than sLe^x. We were interested in using the C-mannose core due to its chemical stability and ease of availability. We synthesized **2**[22] as well as the β-mannosyl glutamate **3** and tested them in our ELISA-based assay, which measures the binding of E-selectin to human recombinant AGP (α-1 acid glycoprotein) containing sLe^x. We were surprised to find that β-mannosyl glutamate **3** showed activity. Further probing was done using molecular modeling.



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The conformation of sLe^x in solution has been studied by a number of groups using 2-D NMR and molecular dynamics simulation.[23] The crystal structure of E-selectin has also been determined.[24] Consequently, a number of proposed complex structures of sLe^x bound to E-selectin have appeared in the literature.[23,25–28] We used the proposed structure of Poppe et al.[23] as a starting point for modeling the bound structures of mannosides **2** and **3** to E-selectin, respectively. In the proposed sLe^x–E-selectin complex structure, the 2- and 3-hydroxyls of the L-fucose moiety, which are both in equatorial orientations, are bound to the essential selectin-bound calcium. The mannose rings of **2** and **3** were overlaid onto the L-fucose in the proposed complex structure. The glutamate side chains of **2** and **3** were then orientated such that they were pointed in the general direction of the sialic acid binding site. Using the program QXP,[29] these initial structures of **2** and **3** bound to E-selectin were energy minimized, followed by a Monte Carlo simulation in which the ligand was allowed to rotate, translate and flex torsions for up to 5000 steps while the protein was held rigid. In both the minimizations and the Monte Carlo simulations the 3- and 4-hydroxyls of the mannose moiety were constrained to be proximal to the selectin-bound calcium through the use of zero-order bonds. The resulting structures, shown in Figure 1, were significantly different from the starting point. Instead of the mannose moiety remaining overlaid on the fucose moiety in the sLe^x structure, it relaxes to lie on the surface of E-selectin, optimizing the van der Waals and electrostatic interactions.

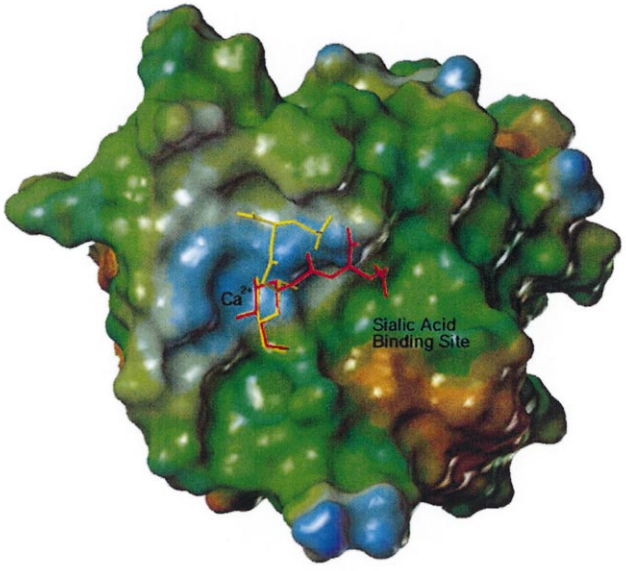
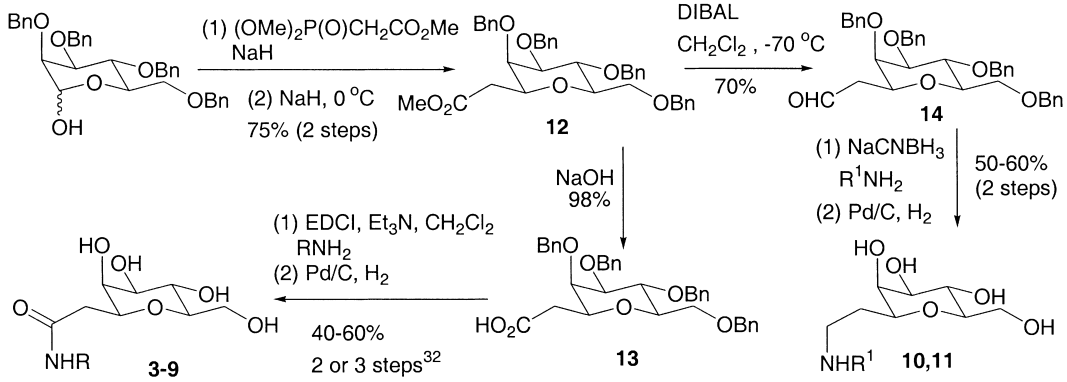


Figure 1. The Monte Carlo structures of **2** (yellow) and **3** (red) bound to E-selectin are shown. The surface of E-selectin is represented by a Connelly surface that is colored by lipophilicity. The locations of the bound calcium and the sialic acid binding site are indicated.

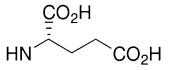
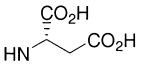
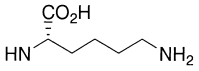
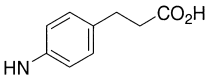
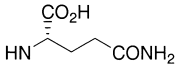
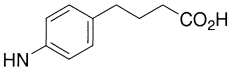
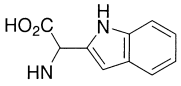
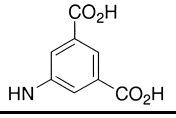
The bias for α-substituents is grounded in the structure of sLex, which has an α linkage between fucose and glucose, and the proposed complex structures of sLe^x bound to E-selectin, which indicate that substituents in the α position of the fucose (or similarly a mannose) are directed towards the sialic acid binding site while substituents in the β position are directed towards solvent. The structures that we obtained from our Monte Carlo simulations show a different trend. β substituents are pointed in the general direction of the sialic acid binding site, while substituents in the α position are directed away from the sialic acid binding site. This occurs because the tetrasaccharide has been clipped to a substituted monosaccharide. In the absence of the rigid confirmation of the tetrasaccharide the mannose relaxes onto the surface of E-selectin in order to optimize interactions between the mannose hydroxyls and the protein. The relaxation process results in the anomeric carbon being closer to the surface of E-selectin. The interpretation of these structures does not preclude α substitutents from interacting with the binding site, as they must be given the number of α substituted mannose derivatives that have been reported in the literature. It does suggest that β substituted mannose derivatives may be potential inhibitors. Based on the modeling results and the fact that they had not been previously explored in the literature, we decided to use the β-mannose scaffold.

Accordingly, several C-1 substituted β-mannosides were prepared (Table 1). The synthetic approach used to prepare C-mannosides **3**–**11** is outlined in Scheme 1. Methyl-2-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl) acetate **12** was prepared from 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose in two steps via a known procedure.[30] Hydrolysis of ester **12** yielded 2-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl) acetic acid **13**, which on coupling



Scheme 1. Synthesis of C-1 substituted mannosides **3**–**11**.

Table 1. C-1 Mannosides in the E-selectin assay

Compound	NHR or NHR[1]	rAGP/ESel Assay IC_{50} (mM)
	sLe^x	0.3
4	NH_2	NA at 10 mM
3		7
5		6
6		7
7		10
8		>10
9		>10
10		NA at 10 mM
11		NA at 10 mM

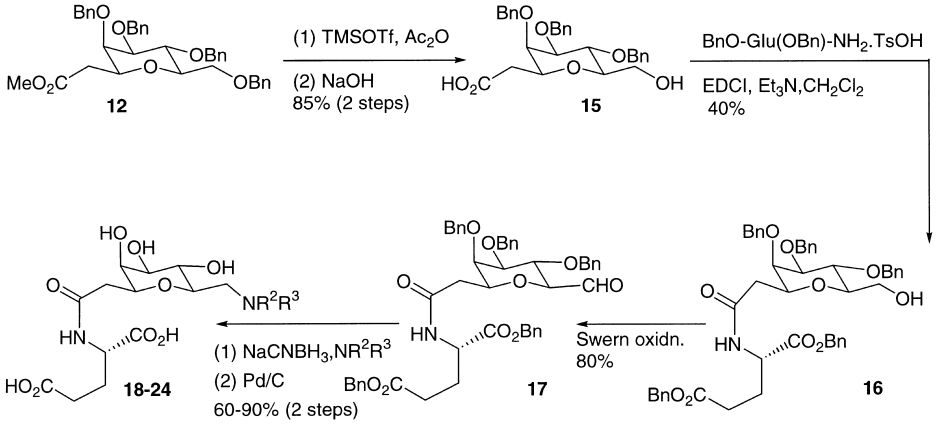
with a variety of protected amino acids gave amides in 40–60% yields. After purification the amides were subjected to hydrogenolysis to give the desired products (**3**–**9**)[31,32] in quantitative yield. Reduction of ester **12** with DIBAL at −70 °C furnished aldehyde **14** in 70% yield. Reductive aminations using sodium cyanoborohydride gave amines **10** and **11**.[31,32]

Biological evaluation of these compounds in our ELISA produced disappointing results. A majority of the compounds showed IC_{50}s greater than 10 mM. Glycoside **3** was found to be the most potent compound (Table 1).[33] There are several possible explanations for their lack of activity.[34] The sLe^x binding site on E-selectin is relatively shallow, which results in the sLe^x–E-selectin interaction resembling a protein–protein interaction more than a protein–small molecule interaction. If this is truly the case, our ligands may need to be larger to occupy more of the sLe^x binding site in order to out compete sLe^x. In order to address these concerns, we decided to explore a different site on the E-selectin surface.

Using the lowest energy Monte Carlo structure of **3** bound to E-selectin as a starting point, a virtual library that corresponds to derivatization of C-6 of mannose was generated in the presence of E-selectin using QXP. Each initial structure was minimized, followed by a Monte Carlo simulation of up to 1000 steps in which the ligand was allowed to rotate, translate and flex while the protein was held rigid. The 3- and 4-hydroxyls of the mannose moiety of each construct were constrained to be proximal to the selectin-bound calcium through the use of zero-order bonds. The low energy structure of each construct was retained and ranked using the QXP scoring function.[29] The top 50 structures were visually screened and the best 10 structures were retained for synthesis.

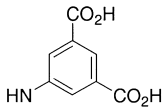
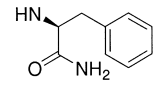
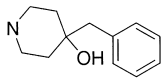
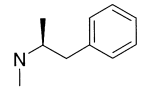
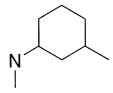
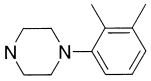
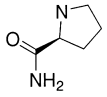
The C-6 substituted derivatives of β-mannosyl glutamate **3**, were prepared by the procedure shown in Scheme 2 (compounds **18**–**24**). Glycoside **12** on treatment with trimethylsilyl triflate and excess acetic anhydride gave exclusively the C-6 acetyl glycoside,[35] which on hydrolysis with sodium hydroxide gave acid **15**. The latter on coupling with dibenzyl glutamate resulted in **16**. The C-6 hydroxyl was then converted to the aldehyde by Swern oxidation. Reductive aminations of core **17** produced amines in 60–90% yields. The latter on further hydrogenolysis yielded mannosides **18**–**24**.[31]

These compounds were tested in our in vitro E-selectin assay (Table 2). Compounds **18**, **19**, **20**, and **22** that contain phenyl substituents at the C-6 position showed activity. Glycoside **20** was the most active; being four times more active than the earlier lead compound **3**. These results indicate that we have possibly accessed a hydrophobic patch on the E-selectin surface with the phenyl substituents at C-6 position. Recently several



Scheme 2. Synthesis of C-6 substituted mannosides **18**–**24**.

Table 2. C-6 Mannosides in the E-selectin assay

Compounds	NR^2R^3	rAGP/Esel Assay IC_{50} (mM)
	sLe^x	0.3
18		6.0
19		6.7
20		1.6
21		NA at 10 mM
22		8
23		NA at 10 mM
24		NA at 10 mM

groups have reported a new hydrophobic binding site on the selectins.[16,36] Further efforts are in progress to explore the new hydrophobic area in the sLe^x-binding site and optimize the potency of this series of molecules.

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31. Final compounds were purified on silica gel using ethyl acetate:methanol:acetonitrile:water: 6/1.5/1.5/1.5. All compounds gave spectroscopic data consistent with the proposed structures. Satisfactory HRMS data (EI) was obtained for all the final products.
32. The acid groups in amines **3**, **5**, **6**, and **10** were protected as benzyl esters, which were removed in the final hydrogenolysis step. Amine **8** was used as a *t*-butyl ester; in this case

an additional deprotection step using TFA/CH_2Cl_2/water was performed before hydrogenolysis. Amines **7**, **9**, and **11** were used as methyl esters; in these cases an additional deprotection step using NaOH/THF/MeOH was performed before hydrogenolysis.

33. Mannoside **5** showed the same activity as **3** but was not stable for long periods of time.

34. The activity of α-mannoside **2** ($IC_{50} = 3$ mM) was not significantly different from β-mannoside **3** ($IC_{50} = 7$ mM) in our assays.

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