Selectin–Saccharide Interactions: Revealing Structure–Function Relationships with Chemical Synthesis

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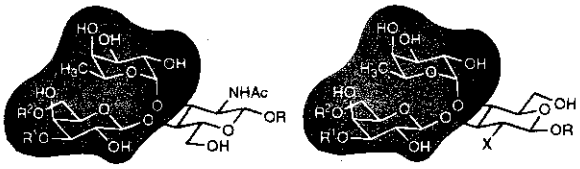
The benefits of modulating the inflammatory response have fueled efforts to understand the role of specific carbohydrate structures in this process. In the early stages of inflammation, proteins termed selectins facilitate leukocyte rolling along the vascular endothelium. The selectins possess a C-type lectin domain, which can bind surface oligosaccharide residues on apposing cells.[1] Minimum oligosaccharide determinants that interact with all three of the known selectins, E-, P-, and L-, are sialylated and/or sulfated derivatives of Lewis a (Le^a) and Lewis x (Le^x) (compounds **1**–**4**, Figure 1). Of these naturally occurring ligands, 3′-sulfo Le^a (**4**) has the highest affinity for the selectins, yet it still binds relatively weakly ($K_a \approx 10^4\ M^{-1}$).[2] Higher affinity glycoprotein ligands for the selectins have been identified. One of these, the mucin GlyCAM-1, presents the novel dianionic oligosaccharide 6′-sulfo sialyl Lewis x (6′-sulfo sLe^x (**5**)).[3,4] The high affinity of L-selectin for GlyCAM-1,[3] the importance of sulfation for L-selectin binding,[5] and the identification of this determinant led to the hypothesis that the 6′ sulfo group enhances L-selectin affinity.[3] To test the role of 6′ sulfation on the affinity and specificity of selectin–ligand interactions, we developed a chemical synthesis of Le^a trisaccharides **6**[6] –**8** (Figure 1), and we determined the selectin inhibitory abilities of these molecules.

To test the effect of 6′ sulfation on selectin recognition, we designed target molecules based on the structurally well-characterized Le^a trisaccharide core.[7] Previous experimental results suggest that Le^a and Le^x derivatives bind in the same mode to the selectins. We chose to generate the Le^a scaffold because its derivatives bind more tightly to the selectins than do the corresponding Le^x variants.[8,9] In addition, substitution of sulfate for sialic acid affords ligands with enhanced selectin affinity.[9,10] By incorporating the features that were known to lead to higher selectin affinity into our targets, we anticipated that they would ultimately faciliate structural characterization of the complexes. The positions of sulfation on the Le^a scaffold were modeled after the sites of charge displayed on the GlyCAM-1 determinant **5**. We examined the effect of anions at two sites of the Le^a scaffold by generating trisaccharides substituted with sulfate groups at the 3- or 6-position of galactose, compounds **6** and **7**, or at both sites, analog **8**.



sLe^x 1: R^1=sialic acid, R^2=H
sulfo-Le^x 2: R^1=SO_3H, R^2=H
5: R^1=sialic acid, R^2=SO_3H

sLe^a 3: R^1=sialic acid, R^2=H, X=NHAc
sulfo-Le^a 4: R^1=SO_3H, R^2=H, X= NHAc
6: R= Pr, R^1=SO_3Na, R^2=H, X=OH
7: R= Pr, R^1= H, R^2=SO_3Na, X=OH
8: R= Pr, R^1= R^2=SO_3Na, X=OH

Figure 1. Structures of naturally-occurring selectin ligands (**1**–**5**) and the synthetic target molecules **6**–**8**. The outlined area highlights the common structural features of these molecules.

An important feature of our synthetic strategy is the generation of all three sulfate derivatives through precursors **15** and **16** (Scheme 1). An appropriately functionalized Le^a scaffold was assembled from compounds **10**, **11**, and **13**.[11] Instrumental to the success of our strategy was the synthesis of glycosyl acceptor **10**, which was obtained from cyclic sulfite **9**.[12] Glycosylation of **10** with trichloroacetimidate **11a** or **11b**[13] afforded high yields of the β-linked disaccharides. Selective reduction of a benzylidene acetal to produce **12a** or **12b** could be effected with standard protocols or with triethylsilane.[14] The trisaccharides **14a** and **14b** were formed by fluoride activation with "dibutyltin ditriflate".[15] Removal of the protecting groups afforded sulfate precursors **15** and **16**.

Selective sulfation was achieved through the use of stannylating agents dibutyltin oxide and bis(tributyltin) oxide (Scheme 1).[16] Treatment of **15** with dibutyltin oxide followed by sulfur trioxide•pyridine complex afforded the 3′-monosulfate adduct, which upon removal of the pro-

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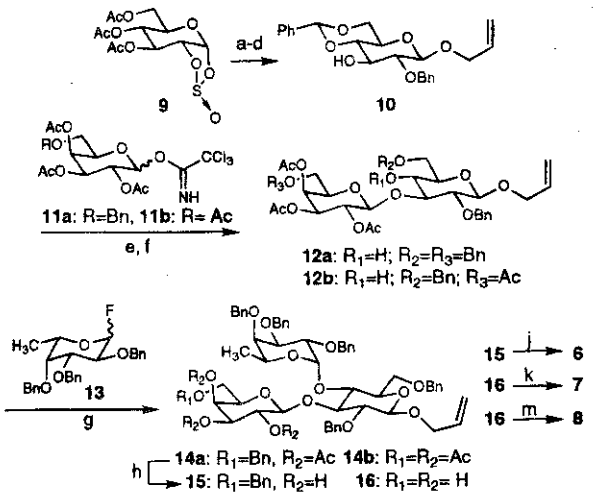
Scheme 1[a]



[a]Key: $Yb(OTf)_3$ (cat.), 3 Å ms, allyl alcohol, toluene, Δ (82%); (b) benzyl trichloroacetimidate, CH_2Cl_2–cyclohexane, TfOH, (97%); (c) K_2CO_3, MeOH; (d) *p*-TsOH, DMF, $PhCH(OMe)_2$, 50 °C, reduced pressure, (two steps, 77%); (e) **11a** or **11b**, 0.1 M TMSOTf, CH_2Cl_2, (from **11a**: 85%; from **11b**: 87%); (f) **12a**: (i) $NaCNBH_3$, THF, 3 Å ms, (ii) ethereal HCl, (72%); **12b**: Et_3SiH, TFA, 0 °C, (77%); (g) **13**, Bu_2SnCl_2, AgOTf, 2,6-di-*tert*-butyl-4-methylpyridine, 4 Å ms, toluene, 0 °C, (**14a**, 90%; **14b**, 90%); (h) K_2CO_3, 1:1 MeOH:THF (**15**, 86%; **16**, 91%); (j) Bu_2SnO, PhH, Δ; Pyr•SO_3, DMF; H_2, $Pd(OH)_2$/C, (three steps, 57%); (k) $(Bu_3Sn)_2O$, PhH, Δ; Pyr•SO_3, PhH; H_2, $Pd(OH)_2$/C, (three steps, 59%); (m) $(Bu_3Sn)_2O$, PhH, Δ; Pyr•SO_3, Pyr; H_2, $Pd(OH)_2$/C, (three steps, 54%).

tecting groups yielded **6**. Similarly, reaction of **16** with bis(tributyltin) oxide and subsequent sulfation in benzene produced the 6′-monosulfate. The 3′,6′-disulfate was obtained when the sulfation reaction was conducted in pyridine. Cleavage of the benzyl ether groups with concomitant reduction of the alkene afforded target molecules **7** and **8**. Compounds **6**–**8** were fully characterized by a combination of NMR experiments.[17]

The relative abilities of compounds **6**–**8** to block binding of L-, E-, and P-selectin–IgG fusion proteins to immobilized GlyCAM-1 were evaluated by ELISAs.[18] First, we found that the 3′ sulfo derivative **6** was more effective in blocking E- and L-selectin and at least as effective for P-selectin than the related 3′ sulfo Le^x, a trisaccharide known to bind to all three selectins.[9] This result is consistent with previous data.[9] In the case of L-selectin, all three Le^a sulfates prevented binding with similar IC_{50} values of 1.5–2.0 mM. Thus, anionic substitution at the 6′ position of the Le^a scaffold is comparable to 3′-substitution in conferring L-selectin binding activity. We anticipated that the 3′,6′-disulfate **8**, a predicted mimic of the GlyCAM-1 determinant, would be a more effective inhibitor of the L-selectin–GlyCAM-1 interaction than the monosulfates. Surprisingly, the 3′,6′-disulfate **8** bound no more tightly to L-selectin than did the monosulfates **6** and **7**. Similar results were obtained with P-selectin; all three compounds blocked GlyCAM-1 binding with IC_{50} values of 2–4 mM. These results, along with other findings,[19] support a model wherein P- and L-selectin interact with a broad spectrum of negatively charged oligosaccharides. Our results do not substantiate the hypothesis that the 6′ sulfo group in concert with the 3′ sulfo group would augment L-selectin affinity; however, it is possible that a 6′ sulfation on the Le^a core does not lead to the same enhanced L-selectin affinity as sulfation on the Le^x core. Alternatively, other features of GlyCAM-1, such as sulfation at other positions, may be responsible for high affinity binding.[20]

In contrast to the results with P- and L-selectin, the three compounds differed markedly in their binding activity with E-selectin. The 3′-sulfate **6** (IC_{50}: 140 μM) was considerably more potent than was the 6′-sulfate **7** (IC_{50}: 3 mM) or the 3′,6′-disulfate **8** (IC_{50}: 5 mM). Thus, anionic substitution at the 6′ position of the Le^a scaffold disrupts binding to E-selectin, while enabling the interaction with both L- and P-selectin. The dramatic destabilizing effect of the 6′ sulfo group observed for the E-selectin complex is consistent with a model of the protein–carbohydrate complex in which the galactose 6′ position is directed toward the negatively charged residues, Glu 80 and Asp 100.[21] In an alternative model, the galactose 6′ position is directed toward Lys 111.[22] If the carbohydrate binds in this mode, it is difficult to rationalize the large destabilization of the E-selectin complex that arises from sulfation of the 6′ position.

In conclusion, we efficiently synthesized three new sulfated derivatives of Le^a (**6**–**8**) to test the hypothesis that charged groups at the galactose 6′ as well as the 3′ position in a selectin ligand would confer higher selectin affinity. We obtained the unexpected result that 3′,6′-disulfo Le^a, the compound designed to mimic the GlyCAM-1 determinant 6′-sulfo sLe^x, did not exhibit high L-selectin affinity. Additionally, our results with E-selectin indicate that placement of anionic charges on a carbohydrate template can have a marked affect on selectin recognition.

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Supporting Information Available: Spectral, analytical, and ELISA data including selected HMQC, HMBC, and TOCSY NMR data, preparations for all new compounds, and a description of ELISAs (33 pages).

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(20) NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAc6OSO_3β1→3 Gal is also a GlyCAM-1 determinant.[3] This compound exhibits enhanced binding to L-selectin relative to sLe^x. See: Scudder, P. R.; Shailubhai, D.; Duffin, K. L.; Streeter, P. R.; Jacob, G. S. *Glycobiology* **1994**, *4*, 929–933.

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