DOI: 10.1002/ejoc.200600825

C-Disaccharides as Probes for Carbohydrate Recognition – Investigation of the Conformational Requirements for Binding of Disaccharide Mimetics of Sialyl Lewis X

Richard W. Denton,[a,b] Xuhong Cheng,[a,b] Kurissery A. Tony,[a,b] Anna Dilhas,[a,b] Juan José Hernández,[c] Angeles Canales,[c] Jesús Jiménez-Barbero,[c] and David R. Mootoo*[a,b]

Keywords: Carbohydrates / Glycomimetics / Selectin / C-Glycoside / Oxocarbenium ion / Conformation

A set of C-disaccharide analogs was designed to probe the recognition of a known O-disaccharide mimetic of sialyl Lewis X, to P-selectin. The synthesis of the C-glycosides centered on the de novo construction of the galactose residue via an oxocarbenium ion/enol ether cyclization. Conformational analysis was performed by a combination of NMR spectroscopy and molecular mechanics (MM) and molecular dynamics (MD) calculations. The inhibition of P-selectin binding was evaluated in a P-selectin Biacore assay. At 12 mM, the O-glycoside showed 48 % inhibition of binding, while the C-glycoside analogs exhibited between 25–31 % inhibition. This data is discussed within the context of the active conformation of sLe^x and the conformational behavior of these ligands.

Introduction

The interaction of the sialyl Lewis X (sLe^x) tetrasaccharide **1** on the surface of leukocytes and E- and P-selectin on the vascular endothelieum occurs at the onset of the recruitment of leukocytes during the inflammation response (Figure 1).[1,2] This low affinity interaction slows the leukocytes to a roll, after which higher affinity integrin-based binding ensues leading to infiltration of the leukocytes into the underlying tissue. The interaction of sLe^x and the selectins has attracted considerable interest as a therapeutic target in view of the increase in selectin expression during inflammation, the association of unregulated inflammation with several pathological states (such as stroke, reperfusion injury and cardiac and allergic diseases), and the fact that sLe^x-selectin binding is low affinity relative to other cellular adhesion processes.[3,4]

One approach in the regulation of sLe^x-selectin binding is the development of small molecule mimetics of sLe^x which can act as selectin antagonists.[5–14] An attractive lead compound is the 1,1-linked disaccharide **2**, which is reported to be five and forty times more active than sLe^x against E- and P-selectin, respectively, in a cell-based assay.[15,16] The design of **2** was based on a sLe^x-selectin binding model, and it was suggested that the galactose and mannose segments mimic the galactose and fucose residues in sLe^x, respectively (Figure 2).[17–21] We were interested in the C-glycoside analogs of **2** for two reasons. First, C-glycosides are more hydrolytically stable than O-glycosides and could be more practical for drug development.[22–27] Second, the C-glycoside framework allows for design of analogs with different conformational properties with respect to the intersaccharide linkages, thereby leading to ligands with different spatial orientation between receptor contacts in the galactose and mannose segments.[28] Evaluation of the selectin affinity of such structures could provide insight on the optimal conformational requirements for binding of this disaccharide framework.[29,30] Against this backdrop we initiated an investigation on the synthesis, conformational analysis and P-selectin binding of the O- and C-disaccharides **2**–**5** (Figure 1). P-selectin has attracted special attention as a therapeutic target because it is expressed on the endothelium within minutes after stimulation and therefore inhibition of sLe^x-P-selectin binding could allow for early disruption of the inflammation cascade.[14,31,32] We have previously described the synthesis of the C-glycoside **3** and precursors to the conformationally restrained analogs **4** and **5**, and the conformational behavior of **2** and **3**.[33–36] Herein, we report the synthesis and conformational analysis of **4** and **5**, and the P-selectin binding of the four disaccharide probes **2**–**5**.

[a] Department of Chemistry, Hunter College,
695 Park Avenue New York, NY 10021, USA
Fax: +1-212-772-5332
E-mail: dmootoo@hunter.cuny.edu
[b] Department of Chemistry, The Graduate Center, CUNY, 365, 5th Avenue, New York, NY 10016, USA
[c] Centro de Investigaciones Biológicas, CSIC,
Ramiro de Maeztu 9, 28040 Madrid, Spain
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

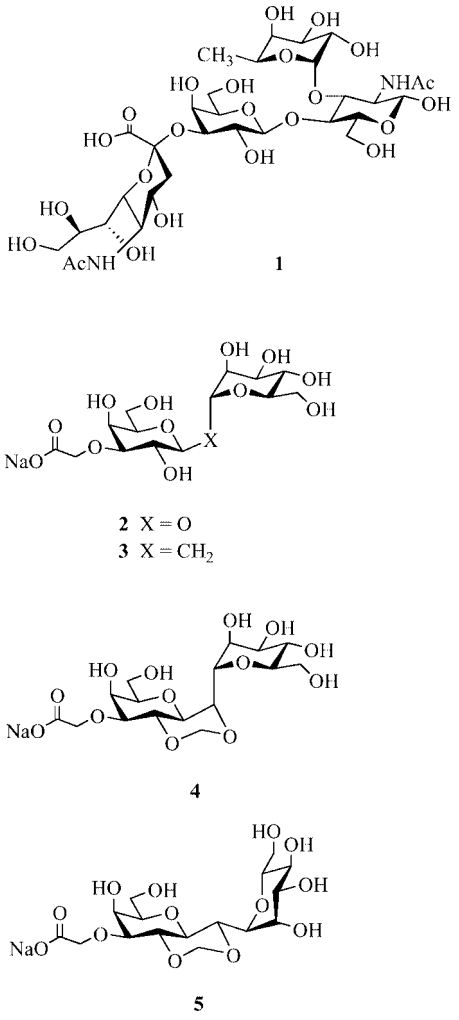


Figure 1. Disaccharide mimetics of sLe^x.

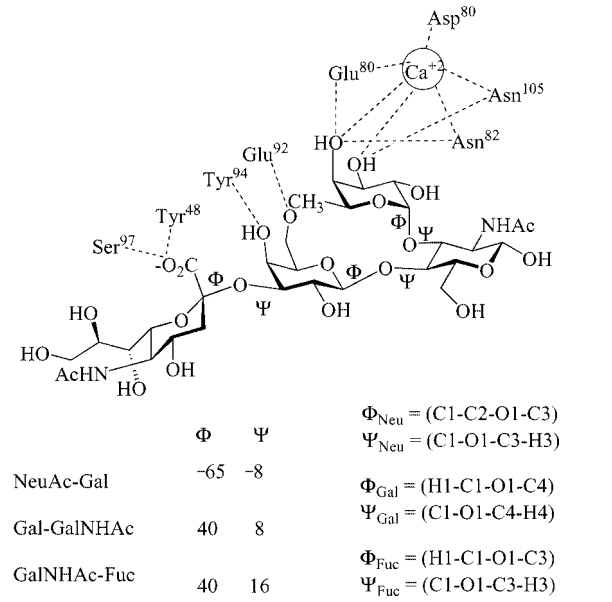
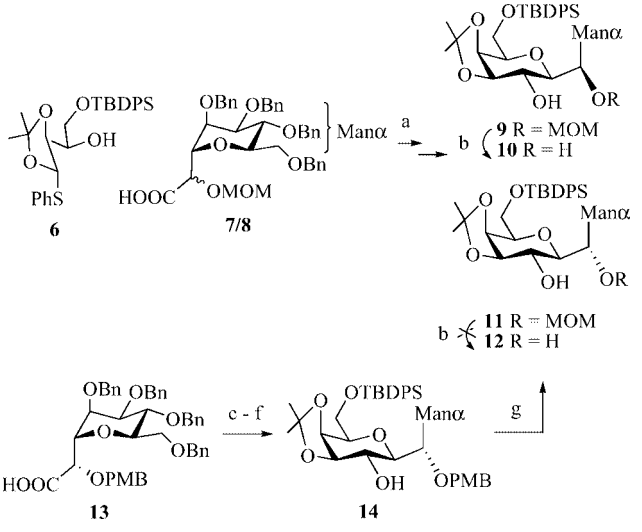


Figure 2. Model for sLe^x-P- Selectin binding.[21]

Results

Synthesis

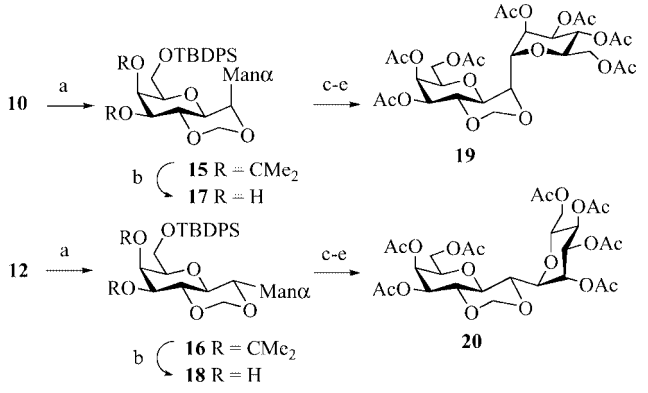
The synthesis of **3**–**5** entailed the de novo construction of the galactose residue and centered on a novel oxocarbenium ion/enol ether cyclization approach to C-glycosides. In this vein, we have previously reported the synthesis of **3**, and **9** and **11**, potential precursors to conformationally restrained analogs **4** and **5** (Scheme 1).[33,34] The initial plan was to convert the hydroxy-MOM acetals **9** and **11** in a single step to their methylene acetal derivatives.[37] However, the acidic conditions required for this transformation led to competing cleavage of the isopropylidene and silyl ether protecting groups. Therefore, in an alternative stepwise strategy, selective removal of the MOM acetals in **9** and **11** to afford the diols **10** and **12**, was next attempted. Dimethylboron bromide was found to be the optimal promoter for this transformation, but this procedure was only useful for the conversion of **9** to **10**. Application of these conditions to **11** led to a mixture of several products, with a low yield of **12**. The complications with the MOM protecting group were avoided by using the *p*-methoxybenzyl (PMB) protected hydroxy acid precursor **13** (instead of the MOM derivative **8**) in the C-glycoside synthesis, thereby leading to the PMB C-disaccharide **14**. Conversion of **14** to the diol **12** was best accomplished by first converting **14** to the acetate derivative, followed by DDQ-mediated removal of the PMB ether, and deacetylation of the product. The analogous synthesis of the diol **10** from the corresponding PMB-protected hydroxy acid precursor also proceeded smoothly.



Scheme 1. Reagents and conditions: (a) ref.[34] and supporting information; (b) Me_2BBr, DTBMP, CH_2Cl_2, –78 to 0 °C, 72%; (c) **6**, DCC, DMAP, PhH, 87%; (d) Tebbe reagent, 79%; (e) MeOTf, DTBMP, CH_2Cl_2, 87%; (f) BH_3·DMS, then Na_2O_2, 75%; (g) (i) Ac_2O, DMAP, EtOAc; (ii) DDQ, CH_2Cl_2/H_2O; (iii) NaOMe, MeOH, 74%.

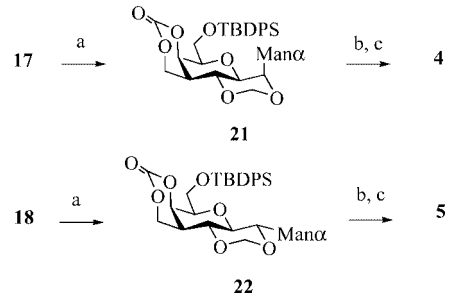
The methylene acetal derivatives **15** and **16** were obtained by individual treatment of **10** and **12** with a mixture of dibromomethane and aqueous sodium hydroxide under phase-transfer conditions (Scheme 2).[38] These reactions

were sometimes accompanied by small amounts of desilylated products, which could be easily reprotected. It was also found that small amounts of methyl ether side products were obtained, and these were suppressed by performing the reaction in the presence of 2,3-dimethylbutene. In preparation for the final alcohol protecting group modifications, the isopropylidene residues in **15** and **16** were selectively removed to give the respective diols **17** and **18**. For characterization purposes **17** and **18** were transformed via straightforward alcohol protecting group changes, to their peracetates **19** and **20**. The stereochemistry of the aglycon segment and the configuration at the intersaccharide carbon were assigned on the basis of vicinal *J* values. Thus, $J_{1,2}$ = 10.0, $J_{2,3}$ = 10.0, $J_{3,4}$ = 3.0, $J_{4,5}$ = 0 Hz for **19**, and $J_{1,2}$ = 9.8, $J_{2,3}$ = 9.8, $J_{3,4}$ = 3.0, $J_{4,5}$ = 0 Hz for **20** are consistent with the 3,4-*O*-isopropylidene-β-C-*galacto* motif.[39] A $J_{1,1'}$ value of 9.8 Hz and a NOE between H2 of the galactose residue and the intersaccharide proton for **20** pointed strongly to an equatorial-like attachment of the mannose residue onto a chair-like dioxane ring. The corresponding *J* value for **19** (6.5 Hz) is somewhat larger than expected for equatorial–axial arrangement of vicinal protons on a chair-like dioxane. It appears that the bulky pseudo-axial substituent results in a distorted, half-chair-like geometry, leading to the unexpectedly large *J* value. These stereochemical conclusions were subsequently corroborated by NMR analysis of **25** and **26**, the deacetylated derivatives of **19** and **20**, respectively (vide infra).



Scheme 2. Reagents and conditions: (a) nBu$_4$NBr, CH_2Br_2, 50% aq. NaOH, **15** (86%), **16** (83%); (b) MeOH, HCl, **17** (68%), **18** (64%); (c) nBu$_4$NF, THF, (d) H_2, Pd/C, HCOOH, MeOH; (e) Ac_2O, EtOAc, DMAP.

Finally, the diols **17** and **18** were transformed to **4** and **5** by a reaction sequence that was similar to that used in our earlier synthesis of **3** (Scheme 3)[33] Thus, selective dibutyltin oxide mediated alkylation of **17** and **18** with methyl bromoacetate led to selective 3-*O*-alkylation followed by in situ lactonization to give **21** and **22**, respectively. Exposure of these products to aqueous sodium hydroxide led to concomitant saponification and desilylation to give the corresponding dihydroxy acids, which were subjected to hydrogenolysis. The target compounds **4** and **5** were obtained after purification using both reverse and normal phase chromatography and lyophilization from aqueous solutions.



Scheme 3. Reagents and conditions: (a) Bu_2SnO, $PhCH_3$, $BrCH_2CO_2Me$, nBu$_4$NI, **21** (98%), **22** (77%); (b) aq. NaOH, EtOH, (c) H_2, Pd/C, HCOOH, MeOH, then aq. NaOH.

Conformational Analysis

Using a combination of NMR spectroscopy and molecular mechanics (MM) and molecular dynamics (MD) calculations, we had previously investigated the conformational properties of **2** and **3**, and their respective analogs without the glycolate residue at position 3 of the galactose segment, **23** and **24** (Figure 3).[36] These studies indicated that the presence or absence of glycolate substituent did not have a significant effect on the conformational behavior with respect to the intersaccharide torsions. Therefore, in the present study, for simplicity in ^{1}H NMR signal resolution, we examined **25** and **26** and assumed that the conformational properties of **23**–**26** would mimic those of **2**–**5**, respectively.

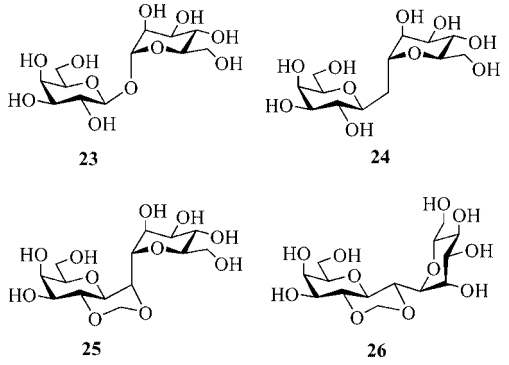


Figure 3. Analogs for conformational analysis.

To summarize our earlier result, the C-glycoside **24** in water populates five different conformational families **A**–**E**, defined by the glyconic torsions (Φ_{gal}, Φ_{man}).[40] [Figure 4]. In comparison, the O-glycoside **23** exists primarily in conformational family **A** with very minor populations of conformational families **B**, **D** and **E**.

As was the case for **23** and **24**, molecular mechanics and dynamics calculations on the conformationally restrained analogs **25** and **26** were performed using the MM3* force field (MACROMODEL 7.1).[41,42] However, unlike the case of the more flexible C-glycoside **24**, for which a time-averaged restrained MD protocol was implemented, no restraints were used for modeling the conformational behavior of **25** and **26**. Obviously, the presence of the cyclic acetal

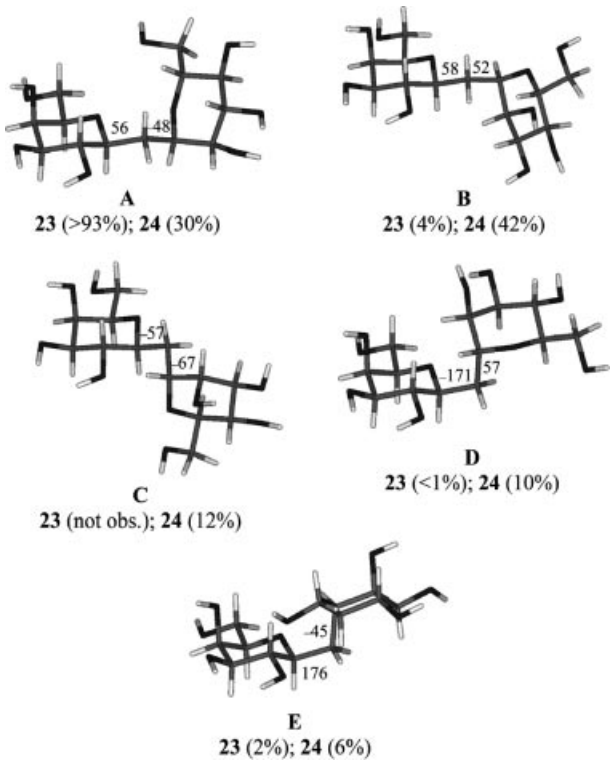
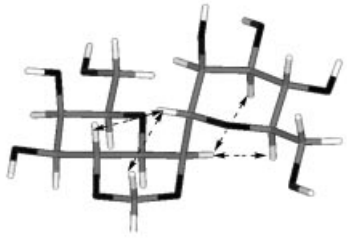


Figure 4. Conformations for **23** and **24**. Structures and exact dihedrals are shown for C-glycoside **24**. The % populations of the conformational families for **23** and **24** are given in parentheses.

in these latter structures severely restricts the conformational mobility around the Gal pseudoglycosidic linkage and only the torsional degree of freedom around the Man linkage remains. Thus, the problem is highly simplified, and the conformers around Φ_{man} were generated and optimized with MM3* with Φ_{gal} left free during the minimization process. The GB/SA solvation model for water was used.[43] The probability distribution was calculated from the energy values according to a Boltzmann function at 300 K. Three low energy minima were obtained for **25** (Figure 5). The lowest and one of the high-energy conformations (Φ_{gal},Φ_{man}: 175, 51 and 151, –59, respectively), corresponded to conformational families **D** and **E** that were previously observed for **23** and **24**. The highest energy conformation, **F** (Φ_{gal}, Φ_{man}: 168, 140) was not observed for **23** and **24**. Intersaccharide coupling constants for these three conformations were obtained from the Karplus–Altona relationship[44] and compared with the values measured from the ^{1}H NMR (Figure 5). Accordingly, **25** exists predominantly in conformation **D** (i.e. ca. 85%), with minor populations of **E** and **F** (i.e. 10 and 5%, respectively). Thus the relative population distribution was in qualitative agreement with the result predicted by MM3* calculations. The observation of strong key NOE contacts (H-intersac/H3-man, H-intersac/H5-man, OCH-axO- H1-man and H2-gal/H1-man) are also in agreement with a high population of conformation **D**. The MM3* calculations for **26** indicated low and high-energy minima corresponding to conformational families **A** and **B**, respectively (Figure 6). Analysis of the experimental J values as before indicated that **26** exists as a 20:80 ratio of **A/B**. Thus, in the case of **26**, the presence of conformation **A** is overestimated by the MM3* calculations. Observed strong key NOE contacts (H-intersac/H1-man and H1-gal/H2-man) are also in agreement with a major presence of conformation **B**.

MM3* and ^{1}H NMR data for **25**

Conformation	D	E	F	Exp.
ΔE (kJ/mol)	0	28	32	
Φ_{Gal} (°)	175	151	168	
Φ_{Man} (°)	51	–59	140	
J_{H1Gal}–$H_{intersac}$ (Hz)	5.0	7.0	5.8	5.9
J_{H1Man}–$H_{intersac}$ (Hz)	10.2	1.1	0.5	8.8



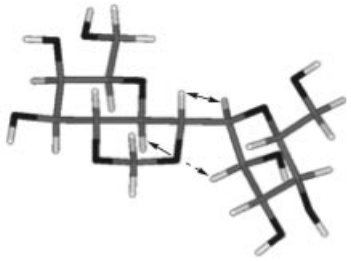
Conformation **D** for **25**

key NOE's indicated by double headed arrows

Figure 5. Conformational analysis for **25**.

MM3* and ^{1}H NMR data for **26**

Conformation	A	B	Exp.
ΔE (kJ/mol)	0.0	6.0	
Φ_{Gal} (°)	54.7	58.2	
Φ_{Man} (°)	–50.8	42.0	
J_{H1gal}–$H_{intersac}$ (Hz)	9.9	9.9	10.0
J_{H1man}–$H_{intersac}$ (Hz)	9.8	0.9	3.0



Conformation **B** for **26**

Figure 6. Conformational analysis for **26**.

P-Selectin Binding

The competitive binding of the O-disaccharide **2** and the C-disaccharides **3**–**5** to a soluble truncated form of human P-selectin was next evaluated in a Biacore assay with an immobilized monomeric truncated form of human PSGL-1 as the reference ligand.[45] At 12 mM, **2**, **3**, **4** and **5** showed 48, 26, 25 and 31% inhibition, respectively.[46] The IC_{50} of

sLe^x under these conditions was previously found to be 15 mM.[14] It should be noted that our result for the O-disaccharide **2** is in disagreement with an earlier study in which the binding of **2** to P-selectin was found to be 40 times greater than sLe^x.[16] This inconsistency could be due to the fact that the latter investigation used a dynamic cell-based assay,[47] but this explanation is not completely satisfying because the IC_{50} values for sLe^x in both the Biacore and cell-based measurement are similar (ca. 8 vs. 15 mM, respectively).

Discussion

It was anticipated that the different relative spatial positioning of the two sugar residues in **2**–**5** would lead to significantly different activities. In so far as % inhibition could be used as a measure of binding, this information could help define an optimal conformation for binding of the disaccharide framework. However, the similarity of activity of **2**–**5** makes any such conclusions somewhat conjectural. Nevertheless, analysis of possible bound conformations in terms of the sLe^x-P-selectin recognition model may be insightful with respect to the design of more active P-selectin ligands (Figure 2). In this regard, the more conformationally rigid analogs **4** and **5**, which have more limited modes of binding, are an appropriate starting point. Accordingly, the galactose and mannose residues of **4** and **5** can individually, but not simultaneously mimic the interaction of the galactose and fucose residues in sLe^x, respectively (Figure 7). The selection of which of the two subunits should be docked into the appropriate carbohydrate domain of P-selectin was guided by the crystal structure of the sLe^x-P-selectin complex and existing structure activity data, which suggests that the fucose residue accounts for the major part of the overall binding energy of sLe^x.[6,21,48,49] Therefore, we speculate that the binding of the mannose segment of these disaccharide frameworks is conserved, and closely mimics that of the fucose in sLe^x (i.e. the 2-, 3- and 4-OH of the mannose residue map to the 4-, 3- and 2-OH of fucose) [Figure 7]. In the case of the conformationally restrained analogs **4** and **5**, this means that individual galactose substituents must occupy different spatial positions relative to the mannose segment, and would therefore interact differently with the receptor sites in the galactose-binding domain of sLe^x. Inspection of the sLe^x-P selectin complex suggests these to be Tyr48, Tyr92 and Glu94, which interact with Neu-COO-, Gal-4-OH and Gal-6-OH of sLe^x.[21]

The assumption that the mannose mimics the fucose of sLe^x, the conformational restraints on **4** and **5**, and the notion that the relative position of the carboxylate of neuraminic acid in sLe^x and the fucose is critical for binding, point to conformations like **D** (Φ_{gal}, Φ_{man}; 180°, 60°) and **B** (Φ_{gal}, Φ_{man}; 60°, 60°) as possible bound orientations for **4** and **5**, respectively (Figure 7). Considering the P-selectin sites in the vicinity of the galactose-binding domain of sLe^x, conformations like **B** and **D** appear to give the best fit to the receptor, relative to their rotamers with respect to the unrestrained Φ_{man} torsion. Thus, for **4**, in which Φ_{gal} is restrained in approximately 170°, a Φ_{man} of 70° gives a **D**-like conformation (Φ_{gal}, Φ_{man}; 170°, 70°), which positions the carboxylate oxygen in **4** close to the Tyr48 binding site for Neu-COO– in sLe^x. The respective COO–/Tyr48–O distances for this conformation and that of bound sLe^x are 4.8 and 3.6 Å. In this **D**-like conformation Gal-6-O in **4**, is farther away from the carboxylate oxygen of Glu94, compared with Gal-6-O in sLe^x (3.5 vs. 2.5 Å), while the Gal-4-O/Tyr92-O distance is closer for **4** compared with sLe^x (2.5 vs. 3.5 Å). For **5**, in which Φ_{gal} is restrained to 60°, a Φ_{man} of 60° leads to conformation **B**, which places the carboxylate of the glycolate in very similar proximity to Tyr48-O compared with the carboxylate in sLe^x (3.4 vs. 3.6 Å, respectively). However, in conformation **B** the oxygen atoms at position 4 and 6 of the galactose segment is at a much further distance from Tyr92-O and Glu94-COO–, respectively, compared with the corresponding galactose positions in sLe^x, and apparently out of binding range (i.e. approximately 7 Å for gal-4-O/Tyr92-O and 9 Å for gal-6-O/Glu94-COO–). Instead, Gal-6-O in conformation **B** is relatively close to Tyr92-O (5.2 Å), and appears more likely to interact with this residue. Therefore, to summarize, the salient points on the possible binding of **B** and **D** type frameworks are an essentially identical mode of binding of their mannose segments, but differences in the receptor interactions in their galactose segments, i.e. **4** appears capable of a relatively strong Gal-4-OH/Tyr92 and weak COO–/Tyr48 and Gal-6-OH/Glu94 interactions, whereas for **5** a strong COO–/Tyr48, and weak Gal-6-OH/Tyr92 appear to be the case. Because unbound **4** and **5** favor conformations like **D** and **B**, respectively, and therefore expected to incur relatively small reorganizational energetic costs on binding, their similar binding might be an indication that these galactose interactions are relatively weak compared to the binding of the mannose segment. As stated earlier, this situation is analogous to the apparent dominant binding of the fucose residue in sLe^x. However, an alternative explanation

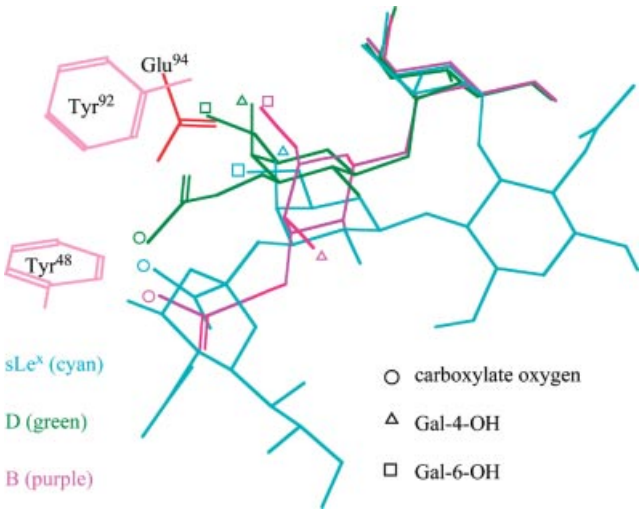


Figure 7. Comparison of binding of sLe^x, and conformations **B** and **D**.

is that the combined set of carboxylate and galactose interactions in **D** and is similar in magnitude to that for **B**. Analogs of **4** and **5** with appropriate deletions of alcohol groups may be useful in probing this issue.

It is appropriate to consider the binding of conformationally unrestrained analogs **2** and **3** in terms of conformations **B** and **D**. Because the O-glycoside **2** is preorganized in conformation **A** (Φ_{gal}, Φ_{man}; ca. 60°, –60°), which is closer in enthalpy to conformation **B** than **D**, a **B**-type bound conformation for **2** might be more favorable than a **D**-type conformation. Interestingly, minor variation in the dihedral angles for the idealized **B** conformation (Φ_{gal}, Φ_{man}; 60°, 60°), which would be energetically less demanding for **2** (compared with the more rigid framework **5**), allows for **B**-like conformations that have closer COO–/Tyr48-O and Gal-6-O/Tyr92-O contacts. Thus the larger enthalpy cost (compared to **5**), required for the O-glycoside **2** to adopt a **B**-like conformation may be offset by a more intimate fit of **2** to the receptor. The larger entropy cost (relative to **5**) that the flexible C-glycoside **3** would incur on binding in a **B**-like conformation, may be similarly compensated. Thus, a **B**-like active conformation for **2** and **3** may also account for the similarity of their binding compared with **5** (and **4**).

Summary

C-disaccharides with different conformational properties about the intersaccharide linker were synthesized and evaluated together with their parent O-disaccharide for binding to P-selectin. These analogs were found to have comparable activity to sLe^x. However, the small differences in their activity did not permit any clear conclusions on a favored bound conformation of the disaccharide framework. Possible bound conformations were considered based on the torsional constraints of conformationally restrained analogs and the X-ray structure of the sLe^x-P-selectin complex. That such conformationally different analogs show similar activity as sLe^x, raises questions about the extent to which the substituents on the galactose residue of sLe^x contribute to binding. Investigations aimed at a clearer understanding of the selectin binding of this disaccharide framework, using more finely tuned analogs of **4** and **5** are underway and will be reported in due course. On a general note the C-glycosides used in this study represent conformational mimetics of O-glycosides that could find wider use as recognition probes.

Experimental Section

General: Solvents were purified by standard procedures or used from commercial sources as appropriate. Petroleum ether refers to the fraction of petroleum ether boiling between 40 and 60 °C. Ether refers to diethyl ether. Unless otherwise stated thin layer chromatography (TLC) was done on 0.25-mm thick precoated silica gel 60 (HF-254) aluminum sheets and flash column chromatography (FCC) was performed using Silica Gel 60 (32–63 mesh). Elution for FCC usually employed a stepwise solvent polarity gradient that was correlated with TLC mobility. Chromatograms were observed under UV (short and long wavelength) light, and/or were visualized by heating plates that were dipped in a solution of ammonium(VI) molybdate tetrahydrate (12.5 g) and cerium(IV) sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL). Optical rotations were recorded at 25 °C at 589 nm (sodium D-line). ^{1}H NMR spectra were recorded on 300, 400 and 500 MHz instruments. Spectra were obtained for $CDCl_3$, C_6D_6 and D_2O solutions with $CHCl_3$, C_6H_6 and HOD, respectively, as internal standards.

2,6:8,12-Dianhydro-1,3,4,5-tetra-*O*-benzyl-13-*O*-*tert*-butyldiphenylsilyl-10,11-*O*-isopropylidene-D-*lyxo*-D-*galacto*-D-*manno*-tridecitol (10): Dimethylboron bromide (0.18 mL, 1.85 mmol) was added at –78 °C, to a mixture of MOM-protected C-glycoside **9**[50] (320 mg, 0.31 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (252 mg, 1.23 mmol), freshly activated, powdered 4-Å molecular sieves (624 mg) and anhydrous CH_2Cl_2 (20 mL). The reaction mixture was stirred at this temperature for 30 min, at room temp. for an additional 30 min, quenched by addition of a 1:1 mixture of THF and saturated aqueous $NaHCO_3$ (10 mL), and extracted with diethyl ether. After washing of the organic layer with 10% aqueous $NaHSO_4$ and brine, the organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. FCC of the crude product afforded **10** (221 mg, 72% yield based on **17**) as a colorless oil; R_f = 0.17 (30% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, C_6D_6): δ = 0.95 (s, 9 H), 1.25 (s, 3 H), 1.40 (s, 3 H), 3.13 (br. s, 1 H, D_2O ex), 3.25 (dd, J = 5.5, 9.0 Hz, 1 H), 3.45 (dd, J = 3.0, 10.0 Hz, 1 H), 3.49 (t, J = 5.0 Hz, 1 H), 3.56 (d, J = 6.0 Hz, 1 H), 3.75–3.93 (m, 5 H), 4.00 (m, 2 H), 4.11 (m, 1 H), 4.27 (dd, J = 2.0, 4.8 Hz, 1 H), 4.30 (dd, J = 4.37 Hz, 1 H), 4.45–4.63 (m, 9 H), 7.25–7.42 (m, 26 H), 7.70 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.6, 27.1, 28.6, 63.1, 69.4, 71.0, 71.2, 71.5, 72.4, 72.8, 73.1, 73.3, 73.8, 74.2, 75.0, 75.8, 76.3, 77.9, 80.3, 109.4, 127.7–129.8 (several lines), 133.7, 135.7, 137.9, 138.2, 138.5, 138.6 ppm. FAB-HRMS calcd. for $C_{60}H_{70}O_{11}NaSi$ [M + Na] 1017.4585, found 1017.4557.

2,6:8,12-Dianhydro-1,3,4,5-tetra-*O*-benzyl-13-*O*-*tert*-butyldiphenylsilyl-10,11-*O*-isopropylidene-7-*O*-*p*-methoxybenzyl-D-*lyxo*-D-*gulo*-D-*manno*-tridecitol (14): DCC (1.39 g, 6.74 mmol) was added at 0 °C to a solution of thioacetal alcohol **6**[33] (1.26 g, 2.48 mol), acid **13** (2.10 g, 2.92 mmol, see supporting information), and DMAP (59 mg, 0.49 mmol) in anhydrous benzene (30 mL). The reaction was warmed to room temp. and stirred for 3 h. The mixture was diluted with diethyl ether and filtered. The filtrate was washed with 0.1 M HCl and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC to give the derived ester (2.61 g, 87%) as a colorless oil; R_f = 0.55 (15% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, C_6D_6): δ = 1.05 (s, 9 H), 1.41 (s, 3 H), 1.47, (s, 3 H), 3.42 (dd, J = 3.0, 10.0 Hz, 1 H), 3.51 (dd, J = 3.0, 7.7 Hz, 1 H), 3.61 (dd, J = 4.7, 10.0 Hz, 1 H), 3.74 (m, 4 H), 3.87 (m, 3 H), 3.99 (t, J = 3.4 Hz, 1 H), 4.14–4.45 (m, 10 H), 4.57 (m, 3 H), 5.34 (m, 2 H), 6.75 (d, J = 8.6 Hz, 2 H), 7.12–7.55 (m, 28 H), 7.65 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.7, 27.0, 27.5, 55.3, 62.2, 69.3, 71.8, 72.0, 72.2, 72.9, 73.4, 74.3, 74.7, 75.7, 78.0, 79.1, 84.7, 111.6, 114.0, 127.3–129.8 (several lines), 132.7, 132.8, 133.0, 133.4, 135.5, 135.6, 138.3, 138.5, 138.7, 159.5, 169.6 ppm. FAB-HRMS calcd. for $C_{73}H_{80}O_{12}SiSNa$ [M + Na] 1231.5038, found 1231.5037.

Tebbe reagent in THF (12.4 mL, 0.5 M, 6.2 mmol) was added dropwise at –78 °C under argon, to a solution of ester from the previous step (1.61 g, 1.33 mmol) and pyridine (0.10 mL) in anhydrous 3:1 toluene:THF (27.0 mL). The reaction mixture was warmed to room

temp., stirred at this temperature for 2 h, then slowly poured into aqueous 1 M NaOH at 0 °C. The resulting suspension was extracted with diethyl ether, and the organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by FCC on basic alumina (Brockmann I, 150 mesh) to give the enol ether derivative (1.11 g, 79%) as a colorless oil; R_f = 0.60 (10% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, C_6D_6): δ = 1.17 (s, 9 H), 1.56 (s, 3 H), 1.60 (s, 3 H), 3.25 (s, 3 H), 3.57 (dd, J = 5.0, 11.0 Hz, 1 H), 3.68 (d, J = 11.0 Hz, 1 H), 3.75 (dd, J = 5.0, 9.5 Hz, 1 H), 3.80 (m, 1 H), 3.96 (d, J = 9.5 Hz, 1 H), 4.03 (m, 2 H), 4.19–4.29 (m, 5 H), 4.36–4.52 (m, 6 H), 4.56 (A, of ABq, J = 11.5 Hz, $\Delta\delta$ = 0.16 ppm, 1 H), 4.61 (A of ABq, J = 12.5 Hz, $\Delta\delta$ = 0.41 ppm, 1 H), 4.78, (B of ABq, J = 11.5 Hz, $\Delta\delta$ = 0.16 ppm, 1 H), 4.81, (d, J = 9.5 Hz, 1 H), 4.85 (d, J = 6.0 Hz, 1 H), 4.97 (B of ABq, J = 11.0 Hz, $\Delta\delta$ = 0.41 ppm, 1 H), 6.27 (d, J = 6.0 Hz, 1 H), 6.71 (d, J = 8.0 Hz, 2 H), 6.95–7.28 (m, 29 H), 7.32 (s, 1 H), 7.39 (t, J = 7.0 Hz, 2 H), 7.46 (d, J = 7.0 Hz, 1 H), 7.76 (m, 4 H) ppm. ^{13}C NMR (75 MHz, C_6D_6): δ = 27.6, 28.1, 55.3, 62.4, 70.6, 71.3, 71.9, 74.0, 75.0, 75.4, 75.8, 76.1, 76.4, 78.4, 81.0, 85.0, 88.2, 112.8, 114.5, 126.8–129.5 (several lines), 130.5, 132.2, 133.3, 133.8, 134.0, 135.4, 136.4, 139.7, 140.0, 157.8, 160.2 ppm. ESIHRMS calcd. for $C_{72}H_{82}O_{13}SiSNa$ [M + Na] 1229.5414, found 1229.5422.

A mixture of enol ether from the previous step (1.10 g, 0.91 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (2.24 g, 10.9 mmol), and freshly activated, powdered 4-Å molecular sieves (2.0 g) in anhydrous CH_2Cl_2 (30 mL) was stirred for 15 min at room temp. under argon and then cooled to 0 °C. Methyl triflate (1.02 mL, 9.12 mmol) was introduced, and the mixture was warmed to room temp. and stirred for an additional 16 h, at which time Et_3N (1.30 mL) was added. The mixture was diluted with diethyl ether, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by FCC on basic alumina (Brockmann I, 150 mesh) to give the glycal product (0.92 g, 87% based on recovered starting material) as a clear oil, R_f = 0.27 (10% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, C_6D_6): δ = 1.30 (s, 9 H), 1.50 (s, 3 H), 1.69 (s, 3 H), 3.38 (s, 3 H), 3.91 (dd, J = 2.3, 13.0 Hz, 1 H), 3.98 (dd, J = 4.7, 13.0 Hz, 1 H), 4.08 (dd, J = 3.0, 9.0 Hz, 1 H), 4.19 (m, 3 H), 4.32 (m, 3 H), 4.40 (t, J = 5.3 Hz, 1 H), 4.46 (d, J = 9.0 Hz, 1 H), 4.50 (d, J = 7.2 Hz, 1 H), 4.58–4.66 (m, 5 H), 4.69–4.77 (m, 5 H), 5.06 (d, J = 11.4 Hz, 1 H), 5.18 (d, J = 2.0 Hz, 1 H), 6.86 (d, J = 8.6 Hz, 2 H), 7.11–7.50 (m, 28 H), 7.90 (m, 4 H) ppm. ^{13}C NMR (125 MHz, C_6D_6): δ = .26.8, 26.9, 28.6, 54.5, 63.4, 69.5, 70.3, 71.0, 71.8, 71.9, 72.2, 73.5, 73.8, 74.5, 75.5, 76.1, 76.5, 77.4, 80.2, 101.2, 110.2, 114.0, 127.2–129.9 (several lines), 135.8 (two signals), 139.3, 153.2, 159.7 ppm. ESIHRMS calcd. for $C_{68}H_{76}O_{11}SiNa$ [M + Na] 1119.5050, found 1119.5047.

BH_3·Me_2S (1.05 mL, 10.9 mmol) was added at 0 °C to a solution of the glycal from the previous step (0.80 g, 0.73 mmol) in anhydrous THF (20 mL) under an atmosphere of argon. The mixture was warmed to room temp. and stirred for an additional 3 h at this temperature. At that time the solution was cooled to 0 °C and treated with a mixture of 3 M NaOH (4.4 mL) and 30% aqueous H_2O_2 (0.88 mL) for 0.5 h. The mixture was diluted with diethyl ether, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by FCC to give **14** (0.52 g, 75% based on recovered starting material) as a clear oil; R_f = 0.40 (30% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, C_6D_6): δ = 1.08 (s, 9 H), 1.40 (s, 3 H), 1.55 (s, 3 H), 3.56 (m, 3 H), 3.71–3.83 (m, 9 H), 3.90–4.00 (m, 4 H), 4.07 (apparent t, J = 5.8 Hz, 1 H), 4.22–4.36 (m, 4 H), 4.40 (dd, J = 2.5, 8.0 Hz, 1 H), 4.47–4.68 (m, 7 H), 4.81 (d, J = 11 Hz, 1 H), 6.77 (d, J = 8.5 Hz, 2 H), 7.11 (d, J = 8.5 Hz, 2 H), 7.17–7.44 (m, 26 H), 7.75 (m, J = 13.5 Hz, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.7, 27.1, 28.6, 55.3, 63.1, 70.1, 70.5, 71.8, 72.1, 72.5, 73.5, 74.6, 74.8, 75.2, 79.6, 80.5, 82.2, 109.3, 114.0, 127.7–129.8 (several lines), 130.1, 133.6, 133.8, 135.6, 135.7, 138.0, 138.4, 138.6, 159.4 ppm. ESIHRMS calcd. for $C_{68}H_{78}O_{12}SiNa$ [M + Na] 1137.5161, found 1137.5160.

1,3,4,5-Tetra-*O*-benzyl-13-*O*-*tert*-butyldiphenylsilyl-2,6:8,12-dianhydro-10,11-*O*-isopropylidene-D-*lyxo*-D-*gulo*-D-*manno*-tridecitol (12): The PMB-protected C-glycoside **14** (155 mg, 0.13 mmol) was dissolved in ethyl acetate (10.0 mL) and treated with acetic anhydride (0.12 mL, 1.32 mmol) and DMAP (19.0 mg, mmol) for 30 min. CH_3OH (0.5 mL) was then added to the reaction mixture, and the solvent evaporated in vacuo. FCC of the residue afforded the acetate derivative (156 mg, 98%) as a colorless oil; R_f = 0.34 (20% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.10 (s, 9 H), 1.36 (s, 3 H), 1.63 (s, 3 H), 2.06 (s, 3 H), 3.38 (dd, J = 3.0, 9.0 Hz, 1 H), 3.60 (t, J = 8.8 Hz, 1 H), 3.68 (dd, J = 6.8, 11.0 Hz, 1 H), 3.75 (dt, J = 2.5, 7.5 Hz, 1 H), 3.83–4.00 (m, 7 H), 4.02–4.09 (m, 4 H), 4.18 (dd, J = 2.0, 3.3 Hz, 1 H), 4.32–4.85 (m, 8 H), 5.34 (dd, J = 7.5, 9.7 Hz, 1 H), 6.75 (d, J = 8.6 Hz, 2 H), 7.11 (d, J = 8.6 Hz, 2 H), 7.21–7.43 (m, 26 H), 7.75 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.5, 26.6, 27.1, 27.9, 55.2, 70.3, 70.4, 71.8, 72.8, 73.6, 73.8, 74.7, 75.0, 75.2, 75.4, 75.9, 76.5, 77.4, 78.3, 80.1, 109.9, 113.9, 127.5–130.5 (several lines), 133.7, 133.8, 135.7, 138.5, 138.6, 138.7, 159.4, 169.2 ppm.

DDQ (62 mg, 0.27 mmol) was added to a solution of the product from the previous step (155 mg, 0.13 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temp. for 2 h, then diluted with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2, dried (Na_2SO_4), and evaporated under reduced pressure. FCC of the residue afforded the derived alcohol (107 mg, 77%) as a colorless oil; R_f = 0.33 (20% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.05 (s, 9 H), 1.34 (s, 3 H), 1.46 (s, 3 H), 1.99 (s, 3 H), 3.06 (d, J = 3.5 Hz, D_2O exchange, 1 H) 3.66–3.89 (m, 9 H), 3.94 (dd, J = 3.5, 9.0 Hz, 1 H), 4.00 (m, 1 H), 4.24 (br. s, 1 H), 4.30 (d, J = 6.0 Hz, 1 H), 4.48–4.63 (m, 7 H), 4.77 (d, J = 11.5 Hz, 1 H), 5.30 (t, J = 5.5 Hz, 1 H), 7.21–7.41 (m, 26 H), 7.68 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.3, 26.1, 27.1, 62.8, 68.1, 69.8, 71.2, 71.9, 72.1, 73.3, 73.5, 74.1, 74.2, 75.1, 75.3, 76.2, 78.1, 78.3, 81.4, 82.2, 110.1, 127.6–129.8 (several lines), 133.6, 133.7, 135.7, 138.5, 138.7, 170.0 ppm. ESIHRMS calcd. for $C_{62}H_{72}O_{12}SiNa$ [M + Na] 1059.4695, found 1059.4691.

NaOMe (50 mg, 1.14 mmol) was added to a solution of the product from the previous step (106 mg, 0.11 mmol) in anhydrous CH_3OH (5 mL). The reaction was stirred at room temp. for 2 h and the pH was then adjusted to 7 by addition of 2 M methanolic HCl. The mixture was concentrated under reduced pressure and the residue purified by FCC to give **12** (100 mg, 98%) as a colorless oil; R_f = 0.51 (25% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.07 (s, 9 H), 1.34 (s, 3 H), 1.51 (s, 3 H), 2.42 (d, J = 9.5 Hz, D_2O exchange, 1 H), 3.34 (dd, J = 2.0, 9.0 Hz, 1 H), 3.44 (t, J = 9.0 Hz, 1 H), 3.63 (m, 2 H), 3.77 (dd, J = 2.5, 8.5 Hz, 1 H), 3.78–4.00 (m, 6 H), 4.09 (m, 1 H), 4.15 (t, J = 2.5 Hz, 1 H), 4.21 (dd, J = 2.5, 9.5 Hz, 1 H), 4.26 (d, J = 3.0 Hz, 1 H), 4.48–4.71 (m, 8 H), 7.13–7.45 (m, 26 H), 7.71 (m, 4 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.6, 27.0, 28.5, 63.1, 69.2, 69.9, 72.2, 72.6, 73.6, 73.7, 74.0, 74.1, 75.1, 80.0 (two signals), 109.5, 127.8–129.9 (several lines), 133.5, 133.7, 135.7, 135.9, 137.3, 138.2, 138.4 ppm. FAB-HRMS calcd. for $C_{60}H_{70}O_{11}SiNa$ [M + Na] 1017.4588, found 1017.4585.

2,6:8,12-Dianhydro-1,3,4,5-tetra-*O*-benzyl-13-*O*-*tert*-butyldiphenylsilyl-10,11-*O*-isopropylidene-7,9-*O*-methylene-D-*lyxo*-D-*galacto*-D-

***manno*-tridecitol (15):** A mixture of diol **10** (260 mg, 0.26 mmol), nBu$_4$Br (42 mg, 0.13 mmol), CH_2Br_2 (0.91 mL, 13.3 mmol) and 2,3-dimethylbutene (0.70 mL) was stirred at 65 °C for 15 min. 20% aqueous NaOH (8 mL) was then added dropwise to the reaction mixture and stirring continued at 65 °C for 3 h. The mixture was then cooled to room temp. and extracted with diethyl ether. The organic layer was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure. FCC afforded **15** (204 mg, 86% based on recovered **10**) as a colorless oil; R_f = 0.44 (15% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.05 (s, 9 H), 1.39 (s, 3 H), 1.51 (s, 3 H), 3.49 (dd, J = 6.9, 10.6 Hz, 1 H), 3.63 (dd, J = 5.7, 10.1 Hz, 1 H), 3.74 (dd, J = 5.7, 10.1 Hz, 1 H), 3.81 (m, 3 H), 3.90 (m, 2 H), 3.96 (m, 2 H), 4.06 (dd, J = 5.4, 7.6 Hz, 1 H), 4.24 (dd, J = 7.8, 10.5 Hz, 1 H), 4.30–4.58 (m, 11 H), 4.82 (A of ABq, J = 5.9 Hz, $\Delta\delta$ = 0.59 ppm, 1 H), 5.42 (B of ABq, J = 5.9 Hz, $\Delta\delta$ = 0.59 ppm, 1 H), 7.15–7.40 (m, 26 H), 7.68 (m, 4 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.7, 27.0, 28.6, 62.0, 62.8, 68.9, 70.5, 71.5, 72.1, 72.6, 72.7, 73.5, 73.8, 73.9, 74.7, 74.8, 75.2, 76.0, 76.8, 77.6, 91.2, 109.9, 127.6–130.0 (several lines), 133.6, 133.7, 135.7, 135.8 (two signals), 138.5, 138.6, 138.7, 138.9 ppm. ESIHRMS calcd. for $C_{61}H_{71}O_{11}Si$ [M + H] 1007.4766, found 1007.4810.

2,6:8,12-Dianhydro-1,3,4,5-tetra-*O*-benzyl-13-*O*-*tert*-butyldiphenylsilyl-10,11-*O*-isopropylidene-7,9-*O*-methylene-D-*lyxo*-D-*gulo*-D-*manno*-tridecitol (16): Application of the procedure that was used for **10** to diol **12** (260 mg, 0.26 mmol) provided **16** (175 mg, 83% based on recovered **12**); colorless oil; R_f = 0.50 (20% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.05 (s, 9 H), 1.34 (s, 3 H), 1.55 (s, 3 H), 3.03 (t, J = 9.0 Hz, 1 H), 3.36 (t, J = 9.0 Hz, 1 H), 3.66 (m, 2 H), 3.74 (dd, J = 2.0, 9.5 Hz, 1 H), 3.80 (m, 3 H), 3.86–4.00 (m, 4 H), 4.04 (q, J = 5.0 Hz, 1 H), 4.18 (d, J = 3.5 Hz, 1 H), 4.35 (d, J = 4.0 Hz, 1 H), 4.44–4.58 (m, 7 H), 4.63 (d, J = 10.5 Hz, 1 H), 4.65 (A of ABq, J = 6.5 Hz, $\Delta\delta$ = 0.39 ppm, 1 H), 5.04 (B of ABq, J = 6.5 Hz, $\Delta\delta$ = 0.39 ppm, 1 H), 7.17–7.43 (m, 26 H), 7.69 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.6, 27.2, 28.8, 62.7, 69.7, 69.3, 70.6, 71.9, 72.2, 72.8, 73.4, 73.8, 75.4, 76.0, 77.0, 80.1, 80.4, 93.7, 109.8, 127.5–129.9 (several lines), 133.7, 133.8, 135.7, 135.8, 138.7, 138.8 ppm. FAB-HRMS calcd. for $C_{61}H_{70}O_{11}SiNa$ [M + Na] 1029.4584, found 1029.4585.

2,6:8,12-Dianhydro-1,3,4,5-tetra-*O*-benzyl-13-*O*-*tert*-butyldiphenylsilyl-7,9-*O*-methylene-D-*lyxo*-D-*galacto*-D-*manno*-tridecitol (17): A saturated solution of HCl in diethyl ether (0.1 mL) was added to a solution of **15** (200 mg, 1.29 mmol) in dry CH_3OH (30 mL). The reaction mixture was stirred at room temp. for 2.5 h then neutralized with a solution of NaOMe in methanol. Removal of the volatiles under reduced pressure and FCC of the residue provided **17** (130 mg, 68%) as a colorless oil; R_f = 0.53 (40% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.95 (s, 9 H), 2.49 (d, J = 7.5 Hz, 1 H, D_2O ex), 2.92 (d, J = 11 Hz, 1 H), 3.02 (d, J = 10.0 Hz, 1 H, D_2O ex), 3.21 (s, 1 H), 3.52 (m, 2 H), 3.59 (m, 1 H), 3.78 (m, 3 H), 3.83 (t, J = 10.0 Hz, 1 H), 3.88–4.00 (m, 3 H), 4.05 (dd, J = 3.0, 9.8 Hz, 1 H), 4.20–4.52 (m, 10 H), 4.83 (A of ABq, J = 5.5 Hz, $\Delta\delta$ = 0.45 ppm, 1 H), 5.38 (B of ABq, J = 5.5 Hz, $\Delta\delta$ = 0.45 ppm, 1 H), 7.00–7.38 (m, 26 H), 7.58 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.1, 63.0, 66.4, 69.4, 69.7, 70.4, 71.9, 72.4, 72.8, 73.2, 73.4, 73.5, 74.7, 74.8, 75.0, 75.4, 80.0, 92.1, 127.6–129.9 (several lines), 133.4, 133.5, 135.7, 137.9, 138.1, 138.3, 138.7 ppm. FAB-HRMS calcd. for $C_{58}H_{66}O_{11}NaSi$ [M + Na] 989.4272, found 989.4251.

2,6:8,12-Dianhydro-1,3,4,5-tetra-*O*-benzyl-13-*O*-*tert*-butyldiphenylsilyl-7,9-*O*-methylene-D-*lyxo*-D-*gulo*-D-*manno*-tridecitol (18): Application of the procedure that was used for **17** to **16** (175 mg, 0.17 mmol) provided **18** (92 mg, 64% based on recovered **16**) as a colorless oil; R_f = 0.44 (40% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.95 (s, 9 H), 2.44 (d, J = 6.0 Hz, 1 H, D_2O ex), 2.83 (br. s 1 H, D_2O ex), 2.94 (t, J = 9.0 Hz, 1 H), 3.25 (t, J = 5.0 Hz, 1 H), 3.50 (m, 2 H), 3.59 (m, 2 H), 3.73 (m, 4 H), 3.86 (m, 2 H), 4.07 (m, 1 H), 4.11 (d, J = 3.0 Hz, 1 H), 4.35–4.61 (m, 10 H), 4.97 (d, J = 6.0 Hz, 1 H), 7.08–7.35 (m, 26 H), 7.56 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.2, 63.6, 69.8, 70.0, 72.0, 72.2, 72.3, 72.6, 72.9, 73.6, 75.5, 76.1, 76.6, 77.4, 77.5, 78.0, 78.8, 80.4, 93.8, 127.5–130.1 (several lines), 132.9, 133.1, 135.7, 135.8, 138.6, 138.8 ppm. FAB-HRMS calcd. for $C_{58}H_{66}O_{11}NaSi$ [M + Na] 989.4272, found 989.4254.

1,3,4,5,10,11,13-Hepta-*O*-acetyl-2,6:8,12-dianhydro-7,9-*O*-methylene-D-*lyxo*-D-*galacto*-D-*manno*-tridecitol (19): Colorless oil; R_f = 0.30 (40% EtOAc/petroleum ether); (500 MHz, C_6D_6): δ = 1.57, 1.65, 1.67,1.68, 1.73, 1.96 (all s, 21 H), 3.33 (t, J = 6.0 Hz, 1 H), 3.47 (dd, J = 6.5, 10.0 Hz, 1 H), 4.00 (dd, J = 6.0, 11.6 Hz, 1 H), 4.07 (q, J = 5.7 Hz, 1 H), 4.19 (m, 2 H), 4.51 (t, J = 10.0 Hz, 1 H), 4.56 (m, 3 H), 4.71 (d, J = 6.0 Hz, 1 H), 5.06 (d, J = 6.0 Hz, 1 H), 5.19 (dd, J = 3.0, 10.0 Hz, 1 H), 5.47 (t, J = 6.0 Hz, 1 H), 5.55 (d, J = 3.0 Hz, 1 H), 5.70 (dd, J = 3.5, 6.0 Hz, 1 H), 5.78 (dd, J = 3.0, 6.0 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.7, 20.9, 21.0 (two signals), 21.1 (two signals), 61.8, 62.3, 67.4, 68.0, 68.1, 68.2, 68.7, 70.7, 71.1, 71.9, 72.6, 73.1, 75.5, 90.5, 169.3, 169.7, 170.0 (two signals), 170.2, 170.5 (two signals) ppm. FAB-HRMS calcd. for $C_{28}H_{38}O_{18}Na$ [M + Na] 685.1956, found 685.1952.

1,3,4,5,10,11,13-Hepta-*O*-acetyl-2,6:8,12-dianhydro-7,9-*O*-methylene-D-*lyxo*-D-*gulo*-D-*manno*-tridecitol (20): Colorless oil; R_f = 0.29 (50% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, C_6D_6): δ = 1.63, 1.68, 1.73,1.74, 1.75, 1.78 (all s, 21 H), 3.23 (t, J = 9.8 Hz, 1 H), 3.30 (t, J = 6.5 Hz, 1 H), 3.69 (t, J = 9.8 Hz, 1 H), 3.77 (dd, J = 2.2, 9.8 Hz, 1 H), 3.99 (dd, J = 6.5, 10.8 Hz, 1 H), 4.10 (m, 2 H), 4.28 (m, 2 H), 4.46 (s, 1 H), 4.56 (dd, J = 5.0, 11.0 Hz, 1 H), 4.62 (d, J = 6.5 Hz, 1 H), 5.15 (dd, J = 3.0, 9.8 Hz, 1 H), 5.47 (d, J = 3.0 Hz, 1 H), 5.72 (t, J = 9.0 Hz, 1 H), 5.84 (br. s, 1 H), 5.96 (dd, J = 4.0, 9.0 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.8 (two signals), 20.9 (two signals), 21.0, (two signals), 21.2, 61.7, 62.9, 67.3, 68.4 (two signals), 69.9, 71.1, 72.8, 73.7, 74.6, 75.5, 75.7, 80.4, 93.5, 169.7, 168.8 (two signals), 169.9, 170.0, 170.3, 170.7 ppm.

Lactone 21: A mixture of diol **17** (54 mg, 0.06 mmol), Bu_2SnO (27 mg, 0.11 mmol), and anhydrous toluene (5 mL) was heated at reflux using a Dean–Stark set-up for 1 h. The solution was then evaporated in vacuo and the residue was dissolved in dry toluene (3 mL). nBu$_4$NI (22 mg, 0.06 mmol) and methyl 2-bromoacetate (0.10 mL, 1.08 mmol) were added and the solution heated at reflux for 1 h, at which time the volatiles were removed under reduced pressure. FCC of the residue afforded lactone **21** (55 mg, 98%) as a colorless oil; R_f = 0.35 (20% EtOAc/petroleum ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.41 (s, 9 H), 3.42 (t, J = 7.0 Hz, 1 H), 3.38 (dd, J = 6.5, 9.0 Hz, 1 H), 3.58 (dd, J = 3.5, 10.0 Hz, 1 H), 3.84–4.48 (m, 16 H), 4.51 (d, J = 12.0 Hz, 1 H), 4.56 (d, J = 3.0 Hz, 1 H), 4.61 (dd, J = 1.5, 6.5 Hz, 1 H), 4.66 (dd, J = 1.5, 9.5 Hz, 1 H), 4.76 (d, J = 12 Hz, 1 H), 4.93 (d, J = 6.0 Hz, 1 H), 4.98 (t, J = 10.0 Hz, 1 H), 5.74 (d, J = 6.0 Hz, 1 H), 7.04–7.36 (m, 26 H), 7.82 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.1, 60.9, 61.2, 68.4, 69.0, 70.5, 71.9, 72.1, 72.7, 73.2, 73.6, 74.2, 74.3, 74.7, 78.2, 91.7, 127.2–130.0 (several lines), 133.0, 133.2, 135.6, 138.3, 138.4, 138.5, 138.7, 166.5 ppm. FAB-HRMS calcd. for $C_{60}H_{66}O_{12}NaSi$ [M + Na] 1029.4221, found 1029.4197.

Lactone 22: Application of the procedure that was used for **21** to diol **18** (90 mg, 0.09 mmol) provided **22** (75 mg, 93% based on recovered **18**) as a colorless oil; R_f = 0.33 (20% EtOAc/petroleum

ether), ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.06 (s, 9 H), 3.55 (t, J = 9.5 Hz, 1 H), 3.61–3.82 (m, 6 H), 3.90–3.97 (m, 4 H), 4.14 (d, J = 7.0 Hz, 1 H), 4.22 (s, 1 H), 4.35–4.51 (m, 11 H), 4.68 (d, J = 6.5 Hz, 1 H), 4.88 (d, J = 3.0 Hz, 1 H), 5.10 (d, J = 6.5 Hz, 1 H), 7.15–7.44 (m, 26 H), 7.67 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.2, 60.6, 61.1, 68.4, 69.5, 71.0, 71.9, 72.3, 72.8, 73.5, 73.6, 74.1, 74.2, 75.2, 75.8, 76.1, 77.5, 79.4, 93.8, 127.6–130.1 (several lines), 132.9, 133.2, 135.6, 135.7, 138.3, 138.5, 138.6, 138.7, 166.8 ppm. $C_{60}H_{66}O_{12}SiNa$ [M + Na] 1029.4222, found 1029.4221.

Sodium (2,6:8,12-Dianhydro-7,9-*O*-methylene-D-*lyxo*-D-*galacto*-D-*manno*-tridecit-10-yloxy)ethanoate (4): Lactone **21** (99 mg, 0.10 mmol) was dissolved in ethanol (5 mL) and treated with 3 M NaOH (2 mL). After 2 h the solvent was removed under reduced pressure, and the residue purified by FCC to give the dihydroxy sodium salt resulting from saponification of the lactone and cleavage of the silyl ether (56 mg, 73%), as a colorless oil; R_f = 0.46 (30% CH_3OH/acetone). ^{1}H NMR (500 MHz, CD_3OD): δ = 3.54 (dd, J = 3.0, 9.5 Hz, 1 H), 3.59 (t, J = 5.3 Hz, 1 H), 3.66 (dd, J = 7.0, 9.5 Hz, 1 H), 3.73 (m, 3 H), 3.80 (m, 2 H), 3.86 (m, 1 H), 3.91 (q, J = 5.3 Hz, 1 H), 4.11 (m, 2 H), 4.27 (A of ABq, $\Delta\delta$ = 0.11 ppm, J = 16.5 Hz, 1 H), 4.36 (apparent t, J = 5.5 Hz, 1 H), 4.38 (B of ABq, $\Delta\delta$ = 0.11 ppm, J = 16.5 Hz, 1 H), 4.51–4.60 (m, 13 H), 4.77 (d, J = 6.0 Hz, 1 H), 5.29 (d, J = 6.0 Hz, 1 H), 7.20–7.37 (m, 20 H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 61.7, 68.1, 68.4, 68.7, 70.1, 71.7, 72.7, 72.9, 73.1, 73.6, 74.0, 74.2, 74.5, 76.4, 79.3, 80.3, 90.4, 127.4, 128.1, 138.6, 174.1 ppm. ESIHRMS calcd. for $C_{44}H_{51}O_{13}$ 787.3330, found 787.3358.

A mixture of the product from the previous step (52 mg, 0.06 mmol), 10% Pd on carbon (100 mg), formic acid (0.1 mL) and CH_3OH (3.0 mL) was stirred under hydrogen (balloon), for 12 h. The reaction mixture was purged with argon, filtered through a bed of celite and the filtrate concentrated under reduced pressure. The residue was dissolved in ethanol (5 mL) and treated with 3 M NaOH (1 mL). After stirring at room temp. for 2 h the mixture was evaporated in vacuo and residue purified by sequential FCC on C18 silica gel (CH_3OH/H_2O) and Sephadex LH-20 (H_2O). Lyophilization of the eluate provided **4** as an amorphous solid (27 mg, 90%). $[\alpha]_D$ = +13.5 (c = 0.40, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 3.61 (dt, J = 2.6, 7.4 Hz, 1 H), 3.65–3.74 (m, 5 H), 3.75 (d, J = 2.6 Hz, 1 H), 3.81 (m, 2 H), 3.87 (dd, J = 3.5, 8.3 Hz, 1 H), 4.13 (ABq, J = 16.3 Hz, $\Delta\delta$ = 0.09 ppm, 2 H), 4.16 (d, J = 3.1 Hz, 1 H), 4.20 (t, J = 3.1 Hz, 1 H). 4.26 (t, J = 9.9 Hz, 1 H), 4.31 (dd, J = 3.4, 9.3 Hz, 1 H), 4.50 (dd, J = 5.9, 9.3 Hz, 1 H), 4.91 (A of ABq, J = 6.8 Hz, $\Delta\delta$ = 0.18 ppm, 1 H), 5.09 (B of ABq, J = 6.8 Hz, $\Delta\delta$ = 0.18 ppm, 1 H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 60.7, 61.7, 66.5, 67.6, 68.0, 68.5, 69.0, 70.6, 72.3, 72.4, 72.5, 76.4, 79.5, 80.4, 89.6, 178.3 ppm. FAB-HRMS calcd. for $C_{16}H_{26}O_{13}Na$ 449.1288, found 449.1271.

Sodium (2,6:8,12-Dianhydro-7,9-*O*-methylene-D-*lyxo*-D-*gulo*-D-*manno*-tridecit-10-yloxy)ethanoate (5): Lactone **22** (75 mg, 0.07 mmol) was subjected to the similar saponification procedure that was described for lactone **21**. The corresponding dihydroxy sodium salt (38 mg, 67%) was obtained as a colorless oil; R_f = 0.28 (30% CH_3OH/acetone). ^{1}H NMR (500 MHz, CD_3OD): δ = 3.16 (t, J = 9.0 Hz, 1 H), 3.45 (dd, J = 3.0, 9.5 Hz, 1 H), 3.53 (t, J = 6.3 Hz, 1 H), 3.66 (d, J = 5.0 Hz, 1 H), 3.71 (dd, J = 5.0, 11.5 Hz, 1 H), 3.77 (m, 2 H), 3.91 (d, J = 9.5 Hz, 1 H), 3.96 (q, J = 5.0 Hz, 1 H), 4.00 (m, 1 H), 4.06 (m, 3 H), 4.28 (m, 2 H), 4.48–4.69 (m, 10 H), 4.73 (d, J = 6.5 Hz, 1 H), 5.04 (d, J = 6.5 Hz, 1 H), 7.25–7.43 (m, 20 H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 63.0, 68.7, 70.8, 71.0, 73.1, 73.6, 73.8, 74.0, 74.5, 74.9, 76.6, 77.2, 78.7, 79.1, 80.7, 81.2, 81.9, 94.5, 128.5, 128.7, 128.8, 129.0, 129.1, 129.4, 139.7, 139.8, 178.4 ppm. FAB-HRMS calcd. for $C_{44}H_{51}O_{13}$ [M + H] 787.3330, found 787.3327.

The product from the previous step (37 mg, 0.05 mmol) was subjected to the hydrogenation procedure that was described for **4**. Compound **5** (17 mg, 84%) was obtained as an amorphous solid. $[\alpha]_D$ = +41.6 (c = 0.30, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 3.32 (t, J = 9.4 Hz, 1 H), 3.60 (t, J = 8.6 Hz, 1 H), 3.65 (dd, J = 2.5, 12.4 Hz, 1 H), 3.68–3.71 (m, 8 H), 3.97 (dd, J = 3.6, 8.6 Hz, 1 H), 4.02 (dd, J = 2.7, 9.6 Hz, 1 H), 4.10–4.15 (m, 5 H), 4.17 (m, 3 H), 4.79 (A of ABq, J = 6.5 Hz, $\Delta\delta$ = 0.29 ppm, 1 H), 5.08 (B of ABq, J = 6.5 Hz, $\Delta\delta$ = 0.29 ppm, 1 H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 61.2, 61.5, 66.4, 67.5 (two signals), 68.6, 71.3, 72.7, 76.1, 77.1, 77.4, 79.2, 79.3, 79.7, 93.2, 178.3 ppm. FAB-HRMS calcd. for $C_{16}H_{26}O_{13}Na$ 449.1286, found 449.1295.

Supporting Information (see also the footnote on the first page of this article): Experimental procedures and physical data for **7**, **8**, **9**, **11**, **13** and their synthetic precursors. ^{1}H and ^{13}C NMR charts for selected new compounds, procedures and data for conformational analysis on **25** and **26**. P-selectin binding results for **2**–**5**.

Acknowledgments

This investigation was supported by National Institutes of Health (NIH) (grant R01-GM57865). Support from the National Center for Research Resources of the NIH ("Research Centers in Minority Institutions" award RR-03037), which supports the infrastructure and instrumentation of the Chemistry Department at Hunter College, and from Minority Biomedical Research Support, Research Initiative for Scientific Enhancement (MBRS-RISE) (award GM60665), are also acknowledged.

[1] J. C. Paulson, in: *Adhesion: Its role in inflammatory diseases* (Eds.: J. M. Harlan, D. Y. Liu, W. H. Freeman), New York, **1992**, p. 19–42 and ref. cited.
[2] T. A. Springer, *Cell* **1994**, *76*, 301–314.
[3] S. A. Mousa, *Drugs Future* **1996**, *21*, 283–289.
[4] S. R. Chhabra, A. S. A. Rahim, B. Kellam, *Mini-Rev. Med. Chem.* **2003**, *3*, 679–687.
[5] Reviews: R. Roy, in: *Carbohydrates in Drug Design* (Eds.: Z. J. Witczak, K. A. Nieforth), Marcel Dekker, New York, **1997**, p. 83–135.
[6] E. E. Simanek, G. J. McGarvey, J. A. Jablonowski, C.-H. Wong, *Chem. Rev.* **1998**, *98*, 833–862.
[7] N. Kaila, B. E. Thomas IV, *Med. Res. Rev.* **2002**, *22*, 566–601.
[8] Selected recent studies: H. C. Kolb, B. Ernst, *Chem. Eur. J.* **1997**, *3*, 1571–1578.
[9] G. Magnani, J. L. Thoma, J. T. Patton, B. Ernst, W. Jahnke, *Angew. Chem. Int. Ed.* **2001**, *40*, 1941–1945.
[10] G. Thoma, R. Banteli, W. Jahnke, J. L. Magnani, J. T. Patton, *Angew. Chem. Int. Ed.* **2001**, *40*, 3644–3647.
[11] S. Hanessian, V. Mascitti, O. Rogel, *J. Org. Chem.* **2002**, *67*, 3346–3354.
[12] S. M. Chervin, J. B. Lowe, M. Koreeda, *J. Org. Chem.* **2002**, *67*, 5654–5662.
[13] G. Thoma, F. Schwarzenbach, *Helv. Chim. Acta* **2003**, *86*, 855–864.
[14] N. Kaila, W. S. Somers, B. E. Thomas, P. Thakker, K. Janz, S. DeBernardo, S. Tam, W. J. Moore, R. Yang, W. Wrona, P. W. Bedard, D. Keith Jr., J. C. Crommie, D. H. H. Tsao, J. C. Alvarez, H. Ni, E. Marchese, J. T. Patton, J. L. Magnani, R. T. Camphausen, *J. Med. Chem.* **2005**, *48*, 4346–4357.
[15] K. Hiruma, T. Kajimoto, G. Weitz-Schmidt, I. Ollmann, C.-H. Wong, *J. Am. Chem. Soc.* **1996**, *118*, 9265–9270.
[16] K. Shibata, K. Hiruma, O. Kanie, C.-H. Wong, *J. Org. Chem.* **2000**, *65*, 2393–2398.

[17] For early models for sLe^x-selectin recognition: R. M. Cooke, R. S. Hale, S. G. Lister, G. Shah, M. P. Weir, *Biochemistry* **1994**, *33*, 10591–10596.
[18] P. Hensley, P. J. McDevitt, I. Brooks, J. J. Trill, J. A. Field, D. E. McNulty, J. R. Connor, D. E. Griswold, N. V. Kumar, K. D. Kopple, S. A. Carr, B. J. Dalton, K. Johanson, *J. Biol. Chem.* **1994**, *269*, 23949–23958.
[19] K. Scheffler, B. Ernst, A. Katopodis, J. L. Magnani, W. T. Wang, R. Weisemann, T. Peters, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1841–1844.
[20] L. Poppe, G. S. Brown, J. S. Philo, P. V. Nikrad, B. H. Shah, *J. Am. Chem. Soc.* **1997**, *119*, 1727–1736.
[21] For recent X-ray structures which are in general agreement with ref.[17–19]: W. S. Somers, J. Tang, G. D. Shaw, T. Camphausen, *Cell* **2000**, *103*, 467–479.
[22] For reviews on synthesis and biological applications of C-glycosides: M. H. D. Postema, *C-Glycoside Synthesis*, CRC Press, Boca Raton, Fl, **1995**.
[23] D. Levy, C. Tang, *The Synthesis of C-Glycosides*, Pergamon, Oxford, **1995**.
[24] J.-M. Beau, T. Gallagher, *Top. Curr. Chem.* **1997**, *187*, 1–54.
[25] H. Togo, W. He, Y. Waki, M. Yokoyama, *Synlett* **1998**, 700–717.
[26] R. J. Linhardt, X. Yuan, *Curr. Top. Med. Chem.* **2005**, *5*, 1393–1430.
[27] M. H. D. Postema, J. L. Piper, R. L. Betts, *Synlett* **2005**, 1345–1358.
[28] J. Jimenez-Barbero, J. F. Espinosa, J. L. Asensio, F. J. Canada, A. Poveda, *Adv. Carbohydr. Chem. Biochem.* **2001**, *56*, 235–284.
[29] For examples of conformationally restrained di- and tri-saccharides as recognition probes: D. R. Bundle, R. Alibés, S. Nilar, A. Otter, M. Warwas, P. Zhang, *J. Am. Chem. Soc.* **1998**, *120*, 5317–5318.
[30] N. Navarre, N. Amiot, A. Van Oijen, A. Imberty, A. Poveda, J. Jiménez-Barbero, A. Cooper, M. A. Nutley, G.-J. Boons, *Chem. Eur. J.* **1999**, *5*, 2281–2294.
[31] J. G. Geng, M. P. Bevilacqua, K. L. Moore, T. M. McIntyre, S. M. Prescott, J. M. Kim, G. A. Bliss, G. A. Zimmerman, R. P. McEver, *Nature* **1990**, *343*, 757–760.
[32] P. Mehta, R. D. Cummings, R. P. McEver, *J. Biol. Chem.* **1998**, *273*, 32506–32513.
[33] X. Cheng, N. Khan, D. R. Mootoo, *J. Org. Chem.* **2000**, *65*, 2544–2547.
[34] R. W. Denton, D. R. Mootoo, *ACS Symp. Ser.* **2005**, *896*, 121–135.
[35] J. L. Asensio, F. J. Cañada, X. Cheng, N. Khan, D. R. Mootoo, J. Jiménez-Barbero, *Chem. Eur. J.* **2000**, *6*, 1035–1041.
[36] V. García-Aparicio, M. Fernández-Alonso, J. Angulo, J. L. Asensio, F. J. Cañada, J. Jiménez-Barbero, D. R. Mootoo, X. Cheng, *Tetrahedron Asymmetrey* **2005**, *16*, 519–527.
[37] N. A. Powell, W. R. Roush, *Org. Lett.* **2001**, *3*, 453–456.
[38] K. S. Kim, W. A. Szarek, *Synthesis* **1978**, 48–50.
[39] P. L. Barili, G. Catelani, F. D'Andrea, E. Mastrorilli, *J. Carbohydr. Chem.* **1997**, *16*, 1001–1010.
[40] The torsion angles Φ_{gal} and Φ_{man} are defined as $H1_{Gal}$–$C1_{Gal}$–X–$C1_{Man}$ and $H1_{Man}$–$C1_{Man}$–X–$C1_{Gal}$, where X represents the intersaccharide "O" or "C".
[41] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
[42] N. L. Allinger, Y. H. Yuh, J. H. Lii, *J. Am. Chem. Soc.* **1989**, *111*, 8551–8559.
[43] W. C. Still, A. Tempzyk, R. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.
[44] C. A. G. Haasnoot, F. A. A. M. de Leeuw, C. Altona, *Tetrahedron* **1980**, *36*, 2783–2794.
[45] See ref.[14] and supporting information for a detailed protocol of the binding experiments.
[46] At 6 mM **2**, **3**, **4** and **5** exhibited 33, 12, 10 and 13% inhibition, respectively.
[47] For a discussion on the pitfalls in the evaluation of selectin binding, see ref.[14] and G. Kretzschmar, A. Toepfer, C. Huls, M. Krause, *Tetrahedron* **1997**, *53*, 2485–2494.
[48] A. G. Toepfer, G. Kretzschmar, E. Bartnik, *Tetrahedron Lett.* **1995**, *36*, 9161–9164.
[49] A. G. Toepfer, G. Kretzschmar, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1311–1316.
[50] See ref.[34] and supporting information.

Received: September 20, 2006
Published Online: November 23, 2006

