ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

A New Approach to Explore the Binding Space of Polysaccharide-Based Ligands: Selectin Antagonists

Mickael Calosso,[†] Daniel Charpentier,[†] Marc Vaillancourt,[†] Mohammed Bencheqroun,[†] Gabrielle St-Pierre,[†] Brian C. Wilkes,[‡] and Yvan Guindon*[,†,§,∥]

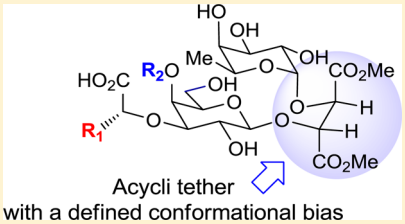
[†]Bio-organic Chemistry and [‡]Chemical Biology and Peptide Research, Institut de recherches cliniques de Montréal (IRCM), Montréal, Québec H2W 1R7, Canada

[§]Département de Chimie, Université de Montréal, C.P. 6128, succursale Centre-ville, Montréal, Québec H3C 3J7, Canada

[∥]Department of Chemistry, McGill University, Montréal, Québec H3A 2K6, Canada

Supporting Information

ABSTRACT: The discovery of molecules that interfere with the binding of a ligand to a receptor remains a topic of great interest in medicinal chemistry. Herein, we report that a monosaccharide unit of a polysaccharide ligand can be replaced advantageously by a conformationally locked acyclic molecular entity. A cyclic component of the selectin ligand Sialyl Lewis^x, GlcNAc, is replaced by an acyclic tether, tartaric esters, which link two saccharide units. The conformational bias of this acyclic tether originates from the minimization of intramolecular dipole−dipole interaction and the gauche effect. The evaluation of the binding of these derivatives to P-selectin was measured by surface plasmon resonance spectroscopy. The results obtained in our pilot study suggest that the discovery of tunable tethers could facilitate the exploration of the carbohydrate recognition domain of various receptors.



KEYWORDS: *polysaccharide-based ligands, selectin antagonists, Sialyl Lewis^x, surface plasmon resonance spectroscopy, carbohydrate recognition domain*

The identification of deregulated molecular events associated with disease and the discovery of medicinal prototypes aimed at restoring the molecular homeostasis are topics of intense multidisciplinary research. Interfering with ligand−receptor has been one of the strategies successfully used in this regard. Of particular interest to us are cases where O- and N-linked carbohydrate motifs on the ligand are involved in the binding of a receptor to a carbohydrate recognition domain (CRD), such as identified in the binding of E- and P-selectins and their ligands.[1] P-selectins interact with P-selectin glycoprotein ligand-1 (PSGL-1), while E-selectins interact with both E-selectin ligand-1 (ESL-1) and PSGL-1. These interactions are involved in the capture and rolling of circulating leukocytes on the vascular wall at the site of inflammation. E- and P-selectins both, albeit with different kinetics, transmigrate to the surface of the vascular endothelium in response to inflammatory stimuli. ESL-1 and PSGL-1 are expressed on the surface of most circulating leukocytes and have been shown to play a key role in leukocytes−endothelial cell interactions. Displayed at the ESL-1 and PSGL-1 terminus is a sialylated and fucosylated tetrasaccharide termed Sialyl Lewis X (sLe^x) that is recognized by E-, P-, and L-selectins (Figure 1a). Interfering with these molecular binding events was suggested to represent an interesting drug target for inflammatory diseases and tumors metastasis.[2] Recently, selectin antagonists have been shown to reverse acute vascular occlusions in sickle cell[3] and atherothrombosis disease models. Binding to E-selectin is also considered as a target for drug delivery and molecular imaging.[4]

At the tactical level, the design of a competitive ligand is often based on the finding of the bioactive conformation of the natural ligand interacting with a receptor. Determining the chemical functionalities of the ligand involved in the binding to the receptor and other binding sites on the receptor surrounding the ligand are also both critical. Ultimately, we aim at identifying a molecular scaffold locked in the bioactive conformation bearing a sufficient number of pharmacophores to have a better affinity to the receptor than the natural ligand. These molecules should also have druglike physicochemical properties to achieve acceptable pharmacokinetic and pharmacodynamic profiles.

X-ray diffraction analysis of complexes involving E- and P-selectin fragments was realized with sLe^x or the N-terminal domain of human PSGL-1 (hPSGL-1) modified by both tyrosine sulfatation and glycosylation (Figure 1a).[5] These studies indicate that the acid group on the NeuAc is the only functionality showing interaction with P-selectins and that no interactions are found with E-selectin. From a drug design standpoint, the removal of the NeuAc subunit could therefore be envisaged. Furthermore, no interaction with the selectins

Received: August 31, 2012
Accepted: October 11, 2012
Published: October 11, 2012

   dx.doi.org/10.1021/ml300263x | ACS Med. Chem. Lett. 2012, 3, 1045−1049

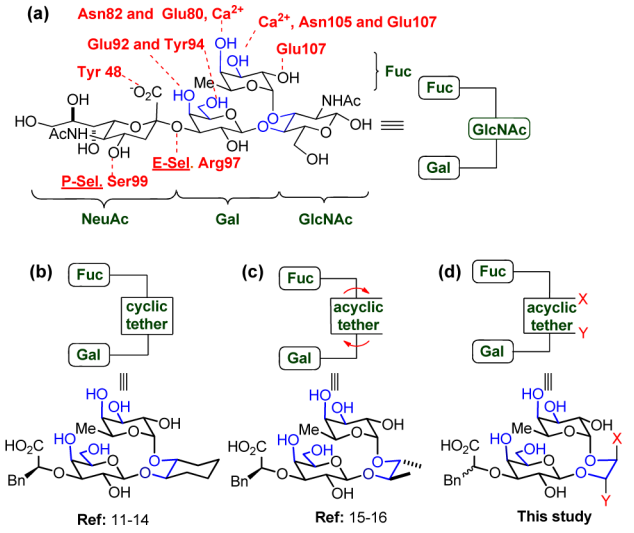


Figure 1. Strategy. (a) Structure of sLe^x. The functionalities involved in binding to E- and P-selectin proteins and the amino acid residues implicated are illustrated. (b) Example of a cyclic tether. (c) Example of an acyclic tether. (d) Acyclic tether with a defined conformational bias.

was noted for the GlcNAc moiety, confirming its role as a tether holding in space the fucose and galactose. Similar conclusions were reached independently through the synthesis of sLe^x analogues.[6−10] The pharmacophores of sLe^x being identified, the modification of the GlcNAc, and the addition of supplementary binding sites on the galactose and the fucose were considered by us and others.

Two scenarios are classically explored to replace a cyclic structural subunit, not directly involved in binding, of a given biologically active molecule, such as sLe^x. The first consists of replacing the cyclic GlcNAc by another cyclic tether.[11−14] This strategy has the advantage of allowing the rapid generation of molecules with activity similar to the original ligand (e.g., sLe^x). Although used successfully to achieve potent antagonists (Figure 1b),[12] this approach renders difficult the full exploration of the chemical space of the CRD, because the cyclic tether that holds the two sugars imposes a conformational bias that cannot be easily modified. Notwithstanding the positive impact of these modifications on the overall biological profile of a given analogue (stability), we are facing the same lack of molecular plasticity that was present with the original ligand. A second scenario consists of linking the fucose and galactose derivatives by an acyclic tether, which could enable a better probing of the CRD (Figure 1c).[15,16] Obviously, with this strategy, the molecule could reach potentially new binding sites because of its plasticity. However, the lack of a preorganized "bioactive"[17] conformation in the ground state could lead to entropic penalties and a decrease in potency.

Herein, we propose a novel and complementary strategy that involves the replacement of the cyclic subunit of the motif by an acyclic tether with a defined conformational bias (the ATC-B strategy). This strategy, as depicted in (Figure 1d), aims at taking advantage of the two approaches previously described. On one hand, we will benefit from an alignment of the pharmacophores similar to the one induced by GlcNAc (panels a and b). On the other hand, we could benefit from the increased plasticity of the acyclic tether. This could be advantageous in the context of the induced fit occurring during binding, whereas conformational changes in both the receptor and the ligand occur. The first step was to find acyclic tether molecules that have a population of conformers similar to the ones induced by the cyclic tether (Figure 1d), which in the present study would require that the two oxygens linked to the carbohydrate be gauche to each other. Ideally, these acyclic tethers should be tunable. Conservative modification of the factors at the origin of the conformational bias (e.g., X and Y in Figure 1d) should lead to different populations of conformers, thus allowing for a more extensive exploration of the surrounding chemical space. The conformation of those molecules should also be verifiable using spectroscopic techniques.

The use of a polysaccharide as a starting point for the development of a ligand is challenging. These complex molecules have often low affinity and poor druglike properties. The presence of cyclic monosaccharides, which are quite rigid from a conformation standpoint, also complicates the exploration of the surrounding binding space of the ligand. Replacing some of these monosaccharides, not directly involved in the binding to the receptor, by simple semirigid tethers with a given conformational bias should facilitate the exploration of the receptor, while decreasing the carbohydrate character of the competing putative ligand.

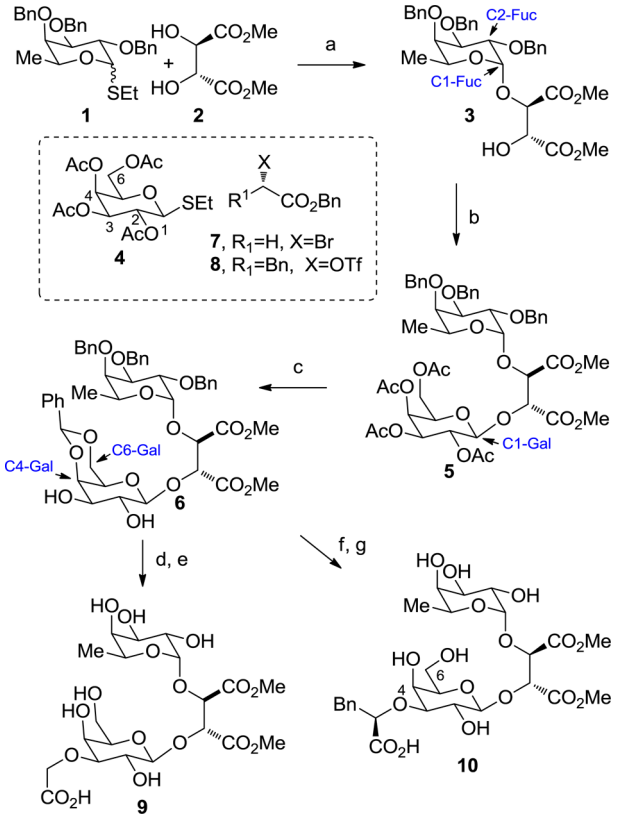
The interest of developing an acyclic tether to replace the GlcNAc unit stemmed from our research program in synthetic methodologies. X-ray analysis of tartrate esters derivatives shows that the two carbon−oxygen bonds at C2 and C3 are gauche to each other, while the esters are anti.[18,19] Two factors could be at the origin of this conformational bias: the minimization of the dipole−dipole effect induced by the esters and the gauche effect resulting from the vicinal diol. To further maximize the biological activity of our pseudodisaccharide, we also considered optimizing the galactose subunit. Sialyl Lewis^x analogues having a cyclic tether replacing GlcNAc, wherein one or more hydroxyl groups of the galactose have been replaced by benzoate esters, was shown to have, in certain cases, improved potencies.[17] To examine this phenomenon with our analogues, we decided to evaluate in this pilot study a compound bearing a benzoate at C4, a docking experiment suggesting a favorable interaction with the Tyr 94 of P-selectine. An analogue bearing a C4-naphtoate group was also examined. While having similar physicochemical properties, its binding to Tyr 94 of the P-selectin might be precluded for steric encumbrance reasons.

The synthesis of sLe^x analogues bearing an acyclic tartrate unit offers significant challenges, including the stereochemical control of the O-glycosidation reactions at the anomeric position of the galactose and the fucose subunits. The first series of analogues were prepared by introducing the tartrate tether on benzylated thioethyl fucoside **1** by a kinetic α-selective glycosylation in presence of NIS to give **3** (Scheme 1).[20] The latter was coupled to thioethyl galactoside **4** with an anchimeric C2 group assistance to generate **5** with high 1,2-trans selectivity on the galactose unit.[21] After Zemplen deacetylation of **5**, the C4-Gal and C6-Gal alcohols were protected with a benzylidene acetal yielding **6**. C3-regioselective alkylations through in situ formation of organotin oxides derivatives of **6** in the presence of **7** or **8** and CsF led to sLe^x analogues **9** and **10**, after Pd/C-catalyzed hydrogenations.

The synthesis of sLe^x analogues **18** and **19** with benzoate or naphtoate at the C4-Gal position was more challenging because the use of a C2-Gal ester protecting group, which would allow anchimeric participation favoring the β-attack of the incoming nucleophile, was precluded (Scheme 2). Another protecting

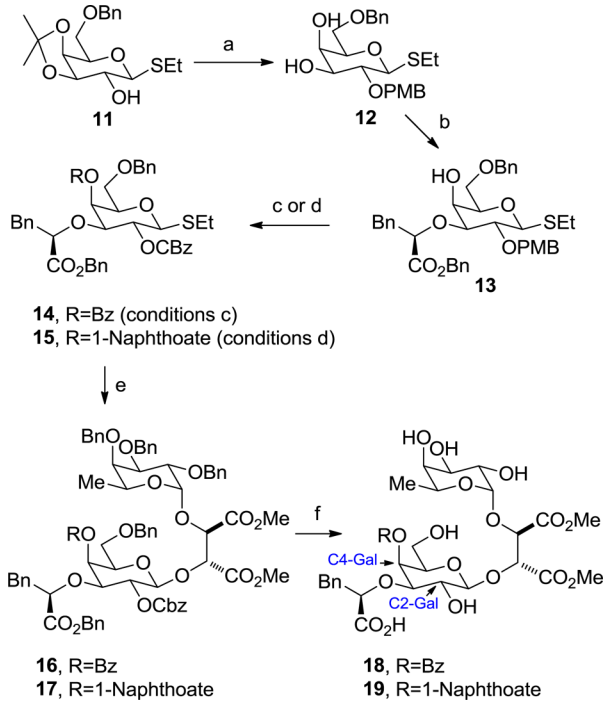
dx.doi.org/10.1021/ml300263x | ACS Med. Chem. Lett. 2012, 3, 1045−1049

Scheme 1. Synthesis of sLe^x Analogues 9 and 10[a]



[a](a) NIS/CF_3SO_3H, CH_2Cl_2, −30 °C, 4 Å mol sieves, 88%. (b) NIS/CF_3SO_3H, CH_2Cl_2, −30 °C, 4 Å mol sieves, 60%. (c) (i) MeONa, MeOH; (ii) $PhCH(OMe)_2$, MeCN, CSA (60% over two steps). (d) Bu_2SnO, MeOH, then CsF and **7** in THF, 100%. (e) Pd(C)/H_2, dioxane, 75%. (f) Bu_2SnO, MeOH, then CsF and **8** in THF, 75%. (g) Pd(C)/H_2, dioxane, 77%.

Scheme 2. Synthesis of sLe^x Analogues 18 and 19[a]



[a](a) (i) NaH, PMBCl, DMF, 74%; (ii) AcOH/H_2O (80/20), 73%. (b) Bu_2SnO, MeOH, then CsF and **8** in THF, 85%. (c) (i) BzCl, DMAP, DCM; (ii) DDQ, DCM, H_2O; (iii) CBZCl, DMAP, DCM (47% over three steps for **14**). (d) (i) 1-Naphthoyl chloride, DMAP, DCM; (ii) DDQ, DCM, H_2O; (iii) CBZCl, DMAP, DCM (40% over three steps for **15**). (e) Br_2, AgOTf, **3**, DCM (64% for **16** and 58% for **17**). (f) Pd(C)/H_2, THF:MeOH (30% for **18** and 13% for **19**).

group allowing anchimeric assistance that could be cleaved in the presence of an ester group had to be identified. We thus turn our attention to the use of a carbonate protecting group.[22,23] The reaction sequence was elaborated from previously reported *β*-thioethyl galactoside **11** (Scheme 2). The C2-Gal alcohol group was protected with a PMB group, and the acetonide was cleaved under mild acidic condition to furnish **12**. The selective introduction of an oxyacetic chain was achieved through the displacement of **8** by a tin acetal intermediate derived from **12**. An excellent 85% yield of the O3-alkylated product was obtained. The hydroxyl at C4-Gal was then either benzoylated or naphtolated, the PMB was cleaved by an oxidative process (DDQ), and the CBZ group was installed to provide compound **14** or **15**. These thioglycosides were then respectively coupled with **3** in presence of Br_2 and AgOTf at −78 °C to give sLe^x analogues **18** and **19**, after Pd/C catalytic debenzylation. The high selectivity obtained for the *β*-anomers formation represents a remarkable improvement that we will be exploring in a separate study.

A plethora of downstream events resulting from the selectin-dependent rolling of leukocytes on the vascular endothelium are observed. For instance, a dynamic clustering of PSGL-1 at the surface of leukocytes[24] and the reorganization of their cytoskeleton and intracellular signaling[25,26] are noted. These molecular events play a critical role in regulating the transient adhesions and represent potential targets to disrupt the cell−cell adhesion phenotypes. They could be at the origin of false-positive results when cell-based assays are used for the evaluation of putative selectin antagonists, a concern that was alleviated in this study by using direct biophysical measurements with surface plasmon resonance (SPR) spectroscopy.[27]

In our first assay, reported herein, we immobilized a monomeric hPSGL-1 fused with the Fc portion of a human IgG (chimeric protein termed rhPSGL-Ig) covalently attached to the sensor chip (Figure 2). Its binding to soluble P-selectin was evaluated. The kinetic parameters measured by SPR were similar to kinetic data previously reported for various forms of PSGL-1 interacting with sP-selectin (Table 1).[28−30]

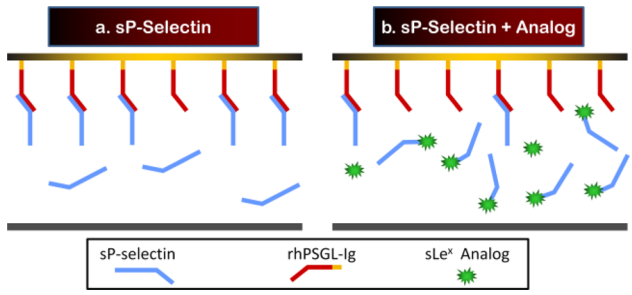


Figure 2. SPR approach, where rhPSGL-Ig is fixed to the sensor chip in the flow cell. (a) Affinity and kinetic constants determination for the binding to sP-selectin. (b) IC_{50} determination of sLe^x analogues.

dx.doi.org/10.1021/ml300263x | ACS Med. Chem. Lett. 2012, 3, 1045−1049

Table 1. Affinity and Kinetic Constants of the Binding of sP-Selectin to rPSGL-Ig

$K_A \pm$ SD	$(1.69 \pm 0.19) \times 10^6$ M^{-1}
$K_D \pm$ SD	$(591 \pm 73) \times 10^{-9}$ M
$k_{on} \pm$ SD	$(1.1 \pm 0.1) \times 10^6$ M^{-1} s^{-1}
$k_{off} \pm$ SD	0.65 ± 0.07 s^{-1}

These calcium-dependent interactions were abrogated by blocking antibodies to either hPSGL-1 or hP-selectin, and their dependency on post-translational modifications was supported by the loss of any significant interaction when rhPSGL-lg was treated with sialylidase or sulphatase (Table 2).

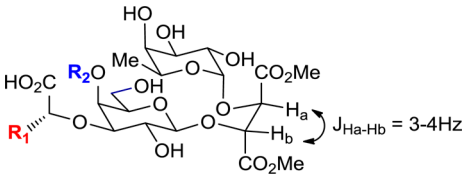
Table 2. Relative Response (After Treatment/Before Treatment) Obtained Using SPR Following Treatment with Sialidase, Sulfatase, Blocking Anti-hPSGL-1, or Blocking Anti-hP-selectin

treatment	relative response (%)
sialidase treatment	3.8 ± 0.3
sulfatase treatment	7.4 ± 0.4
anti-hPSGL-1	0.9 ± 0.3
anti-hP-selectin	0.4 ± 0.2

We next evaluated our compounds by SPR in a competition assay. Various concentrations of our analogues in a solution containing a constant amount of sP-selectin were injected on the flow cell containing rhPSGL-lg linked to the sensor chip. In this assay, the sialyl Lewis^x analogues competed with the carbohydrate motifs attached on the immobilized proteins for binding to sP-selectin (Figure 2b). The objective of this study was therefore to evaluate the biological and pharmacological effect of the replacement of the GlcNAc moiety by the tartaric ester as a strategy to develop P-selectin antagonist.

The first tartrate analogue **9** was inactive, but analogue **10** bearing a benzyl on the oxyacetic chain at O3-Gal position showed an IC_{50} comparable to the one obtained with sLe^x (Table 3, entries 1 and 2). Interestingly, both compounds

Table 3. P-Selectin Dynamic Assay (Competition Based Assay Using SPR) for siLe^x Analogues



entry	compd	R_1	R_2	$IC_{50} \pm$ SD (μM)	ratio[a]
1	**9**	H	H	>1000	
2	**10**	Bn	H	880	0.75
3	**18**	Bn	Bz	18	37.0
4	**19**	Bn	naphtyl	>1000	

[a]Ratio (relative IC_{50}) = IC_{50}(compound)/IC_{50}(sLe^x).

displaced spectroscopic NMR data consistent with the desired gauche orientation between the fucose and the galactose sugar units (J_{Ha-Hb} = 3−4HZ Hz, suggesting a 44−52° dihedral angle) that is important for binding.

The pseudo disaccharide **18** bearing the benzoate at C4-Gal has an IC_{50} of 18 μM, which represents a 37-fold improvement over Sialyl Lewis^x in the same assay (Table 3, entry 3). The naphtoate derivative **19** was not active in our assay (Table 3, entry 4), suggesting that bulky proximal functionality at O4-Gal interferes with binding at the CRD of the selectine receptor.

In conclusion, we have demonstrated in our pilot study that an acyclic tether with a conformational bias could replace GlucNAc to furnish effective Sialyl Lewis^x mimetics. We also have shown that a derivative of the fucose subunit can improve significantly the potency of these pseudo disaccharides. We are now studying the effects of altering the substituents of the tartrate moiety to modulate the conformation, plasticity, and resulting biological activity of sLe^x analogues.

■ ASSOCIATED CONTENT

Supporting Information

Details for surface plasmon resonace assays, methods of organic synthesis, and spectroscopic data of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 514-987-5785. Fax: 514-987-5789. E-mail: yvan.guindon@ircm.qc.ca.

Funding

Funding for this research has been granted from Natural Sciences and Engineering Research Council (NSERC) and Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to our colleague Dr. Michel Prévost for advice and suggestions in writing this manuscript.

■ REFERENCES

(1) Bevilacqua, M. P. Endothelial-leukocyte adhesion molecules. *Annu. Rev. Immunol.* **1993**, *11*, 767−804.

(2) Barthel, S. R.; Gavino, J. D.; Descheny, L.; Dimitroff, C. J. Targeting selectins and selectin ligands in inflammation and cancer. *Expert Opin. Ther. Targets* **2007**, *11*, 1473−1491.

(3) Chang, J.; Patton, J. T.; Sarkar, A.; Ernst, B.; Magnani, J. L.; Frenette, P. S. GMI-1070, a novel pan-selectin antagonist, reverses acute vascular occlusions in sickle cell mice. *Blood* **2010**, *116*, 1779−1786.

(4) Jubeli, E.; Moine, L.; Vergnaud-Gauduchon, J.; Barratt, G. E-selectin as a target for drug delivery and molecular imaging. *J. Controlled Release* **2012**, *158*, 194−206.

(5) Somers, W. S.; Tang, J.; Shaw, G. D.; Camphausen, R. T. Insights into the Molecular Basis of Leukocyte Tethering and Rolling Revealed by Structures of P- and E-Selectin Bound to SLeX and PSGL-1. *Cell* **2000**, *103*, 467−479.

(6) Kaila, N.; Thomas, Iv, B. E. Design and synthesis of sialyl Lewisx mimics as E- and P-selectin inhibitors. *Med. Res. Rev.* **2002**, *22*, 566−601.

(7) Thoma, G.; Schwarzenbach, F. Simplified Sialyl Lewisx Analogues with Improved E-Selectin Inhibition. *Helv. Chim. Acta* **2003**, *86*, 855−864.

(8) Kaila, N.; Somers, W. S.; Thomas, B. E.; Thakker, P.; Janz, K.; DeBernardo, S.; Tam, S.; Moore, W. J.; Yang, R.; Wrona, W.; Bedard, P. W.; Crommie, D.; Keith, J. C.; Tsao, D. H. H.; Alvarez, J. C.; Ni, H.; Marchese, E.; Patton, J. T.; Magnani, J. L.; Camphausen, R. T. Quinic Acid Derivatives as Sialyl Lewisx-Mimicking Selectin Inhibitors: Design, Synthesis, and Crystal Structure in Complex with E-Selectin. *J. Med. Chem.* **2005**, *48*, 4346−4357.

(9) De Vleeschauwer, M.; Vaillancourt, M.; Goudreau, N.; Guindon, Y.; Gravel, D. Design and synthesis of a new Sialyl Lewis X Mimetic: How selective are the selectin receptors? *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1109−1112.
(10) Hanessian, S.; Mascitti, V.; Rogel, O. Synthesis of a Potent Antagonist of E-Selectin. *J. Org. Chem.* **2002**, *67*, 3346−3354.
(11) Kolb, H. C.; Ernst, B. Development of Tools for the Design of Selectin Antagonists. *Chem.—Eur. J.* **1997**, *3*, 1571−1578.
(12) Kolb, H. C.; Ernst, B. Recent progresses in the glycodrug area. *Pure Appl. Chem.* **1997**, *69*, 1879−1884.
(13) Ragan, J. A.; Cooper, K. Synthesis of a galactose-fucose disaccharide mimic of sialyl Lewis X. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2563−2566.
(14) Binder, F. P. C.; Lemme, K.; Preston, R. C.; Ernst, B. Sialyl Lewisx: A "Pre-Organized Water Oligomer"? *Angew. Chem., Int. Ed.* **2012**, *51*, 7327−7331.
(15) Prodger, J. C.; Bamford, M. J.; Gore, P. M.; Holmes, D. S.; Saez, V.; Ward, P. Synthesis of a novel analogue of sialyl Lewis X. *Tetrahedron Lett.* **1995**, *36*, 2339−2342.
(16) Prodger, J. C.; Bamford, M. J.; Bird, M. I.; Gore, P. M.; Holmes, D. S.; Priest, R.; Saez, V. Mimics of the sialyl Lewis X tetrasaccharide. Replacement of the N-Acetylglucosamine sugar with simple C2-symmetric 1,2-diols. *Bioorg. Med. Chem.* **1996**, *4*, 793−801.
(17) Thoma, G.; Bänteli, R.; Jahnke, W.; Magnani, J. L.; Patton, J. T. A Readily Available, Highly Potent E-Selectin Antagonist. *Angew. Chem., Int. Ed.* **2001**, *40*, 3644−3647.
(18) Guindon, Y.; Ogilvie, W. W.; Bordeleau, J.; Cui, W. L.; Durkin, K.; Gorys, V.; Juteau, H.; Lemieux, R.; Liotta, D.; Simoneau, B.; Yoakim, C. Opening of Tartrate Acetals Using Dialkylboron Bromide: Evidence for Stereoselectivity Downstream from Ring Fission. *J. Am. Chem. Soc.* **2002**, *125*, 428−436.
(19) Drouin, M.; Michel, A. G.; Guindon, Y.; Ogilvie, W. Structure of dimethyl (2R,3R)-2-{[(S)-bromo(cyclohexyl)methyl]oxy}-3-[(diphenylboryl)oxy]-1,4-butanedioate. *Acta Crystallogr., Sect. C* **1993**, *49*, 75−77.
(20) Zegelaar-Jaarsveld, K.; van der Marel, G. A.; van Boom, J. H. Iodonium ion assisted synthesis of a common inner core trisaccharide fragment corresponding to the cell-wall phenolic glycolipid of Mycobacterium kansasii. *Tetrahedron* **1992**, *48*, 10133−10148.
(21) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. Iodonium ion promoted reactions at the anomeric centre. II An efficient thioglycoside mediated approach toward the formation of 1,2-trans linked glycosides and glycosidic esters. *Tetrahedron Lett.* **1990**, *31*, 1331−1334.
(22) Morère, A.; Mouffouk, F.; Leiris, S.; Leydet, A.; Montero, J. L. Influence of the Benzyloxycarbonyl Protective Group on Glycosylation with Mannopyranosyl Donors. *J. Carbohydr. Chem.* **2006**, *25*, 451−459.
(23) Grathwohl, M.; Schmidt, R. R. Solid Phase Syntheses of Oligomannosides and of a Lactosamine Containing Milk Trisaccharide Using a Benzoate Linker. *Synthesis* **2001**, *2001*, 2263−2272.
(24) Setiadi, H.; Sedgewick, G.; Erlandsen, S. L.; McEver, R. P. Interactions of the Cytoplasmic Domain of P-Selectin with Clathrin-coated Pits Enhance Leukocyte Adhesion under Flow. *J. Cell Biol.* **1998**, *142*, 859−871.
(25) Crockett-Torabi, E. Selectins and mechanisms of signal transduction. *J. Leukocyte Biol.* **1998**, *63*, 1−14.
(26) Zarbock, A.; Lowell, C. A.; Ley, K. Spleen Tyrosine Kinase Syk Is Necessary for E-Selectin-Induced $\alpha L\beta 2$ Integrin-Mediated Rolling on Intercellular Adhesion Molecule-1. *Immunity* **2007**, *26*, 773−783.
(27) Homola, J. Present and future of surface plasmon resonance biosensors. *Anal. Bioanal. Chem.* **2003**, *377*, 528−539.
(28) Alon, R.; Hammer, D. A.; Springer, T. A. Lifetime of the P-selectin-carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature* **1995**, *374*, 539−542.
(29) Leppänen, A.; Mehta, P.; Ouyang, Y.-B.; Ju, T.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D. A Novel Glycosulfopeptide Binds to P-selectin and Inhibits Leukocyte Adhesion to P-selectin. *J. Biol. Chem.* **1999**, *274*, 24838−24848.
(30) Fritz, J.; Katopodis, A. G.; Kolbinger, F.; Anselmetti, D. Force-mediated kinetics of single P-selectin/ligand complexes observed by atomic force microscopy. *Proc. Natl. Acad. Sci.* **1998**, *95*, 12283−12288.