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Acyclic Tethers Mimicking Subunits of Polysaccharide Ligands: Selectin Antagonists

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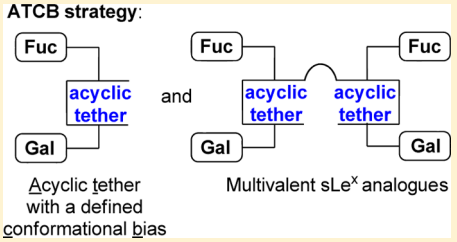
Supporting Information

ABSTRACT: We report on the design and synthesis of molecules having E- and P-selectins blocking activity both in vitro and in vivo. The GlcNAc component of the selectin ligand sialyl Lewis^X was replaced by an acyclic tether that links two saccharide units. The minimization of intramolecular dipole−dipole interactions and the gauche effect would be at the origin of the conformational bias imposed by this acyclic tether. The stereoselective synthesis of these molecules, their biochemical and biological evaluations using surface plasmon resonance spectroscopy (SPR), and *in vivo* assays are described. Because the structure of our analogues differs from the most potent E-selectin antagonists reported, our acyclic analogues offer new opportunities for chemical diversity.



KEYWORDS: *Polysaccharide-based ligands, selectin antagonists, sialyl Lewis^X, surface plasmon resonance spectroscopy, carbohydrate recognition domain*

The design of molecules mimicking natural ligands that interact with biologically relevant receptors is a widely used approach in medicinal chemistry. However, improving the potency of these natural molecules is challenging, particularly with polysaccharide compounds. These molecules are structurally complex and possess many stereocenters with different functionalities that complicate the identification of the pharmacophores involved in the binding to the receptor. Sialyl Lewis^X (**1**, sLe^X), a sialylated and fucosylated tetrasaccharide, represents a particularly interesting target for the development of novel pharmaceutical agents and has not surprisingly been the subject of numerous medicinal studies (Figure 1).[1−16] A sLe^X antagonist (GMI-1070, **2**) was recently shown to reverse vascular occlusions in sickle cell animal model and in preliminary clinical trials, when given intravenously (Figure 1).[17]

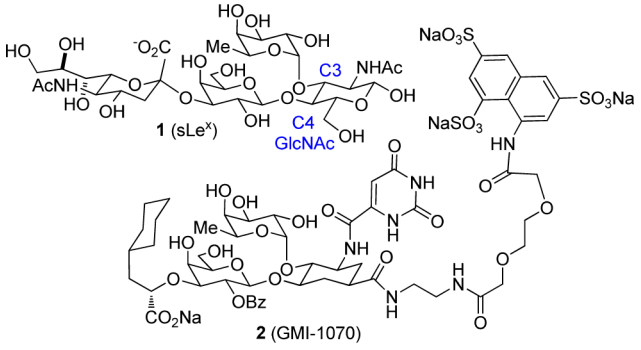


Figure 1. Sialyl Lewis^X (sLe^X 1) and GMI-1070.[17]

sLe^X is found on leukocytes at the terminus of P-selectin glycoprotein-1 ligand (PSGL-1) and E-selectin ligand-1 (ESL-1). E- and P-selectin proteins are expressed on the vascular walls responding to various inflammatory signals resulting from hypertension, atherosclerosis, and other traumas.[18] The interactions between the vascular receptors and the sLe^X ligands on the circulating cells induce the rolling of the leukocyte on the vascular walls, followed by their arrest and extravasation to inflammatory sites. Up-regulation of the β-2 integrin Mac-1 on the leukocytes surface after binding to vascular selectins would promote the aggregation of red blood cells (RBC) and eventually trigger the occlusion of small vessels.[19] This is a particularly dangerous phenotype for sickle cell disease patients. Interestingly, E-selectins are also expressed by the bone marrow endothelial cells in the vascular hematopoietic stem cells (HSCs) niche.[20] Binding to E-selectin seemingly induces HSC proliferation. Deletion of E-selectin in KO mice or blockade by GMI-1070 retarded their proliferation in this niche, allowing protection of the mice HSC from a systemic exposure to cytotoxic anticancer agents or irradiation. Enhanced survival relative to the control was observed in the

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recuperation phase of these animals. Another noteworthy biological role of selectin antagonists concerns their putative implication in reducing myocardial damage after percutaneous coronary intervention (PCI).[21]

We have reported on the replacement of the sLe^X GlucNAc saccharide unit with an acyclic tether possessing a conformational bias (ATCB strategy, Figure 2).[22] A promising P-selectin

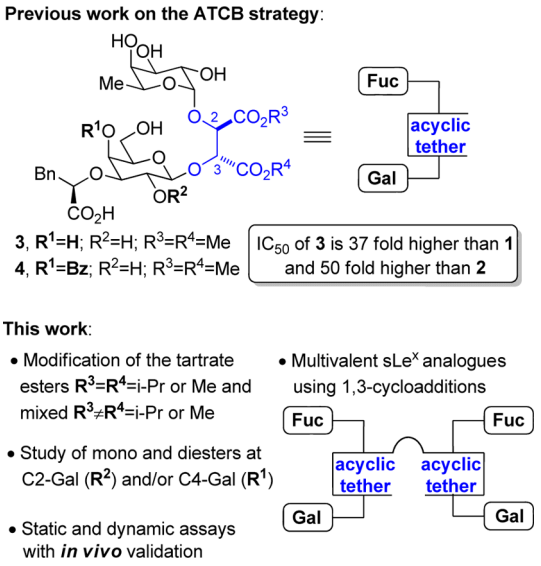


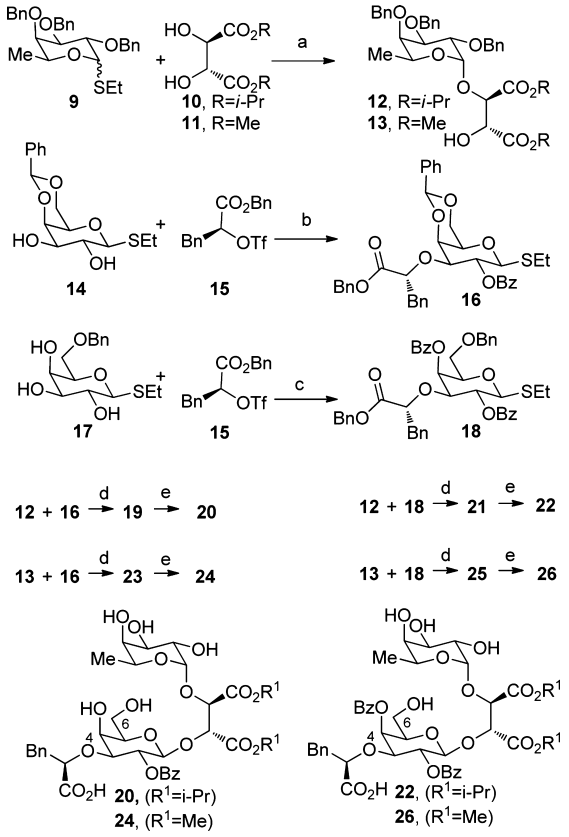
Figure 2. Acyclic tether with a defined conformational bias (ATCB strategy).

antagonist with *in vitro* activity (**3**) was identified in this pilot study. X-ray and NMR analyses suggested that the acyclic L-tartrate methyl ester subunit of **3** (Figure 2) was orienting the two sugar moieties attached at C2 and C3 in a gauche conformation, similar to the one imposed by the GlcNAc unit of sLe^X (Figure 1).[22−24] This first study confirmed that the relative plasticity of our tether contrasts with the rigidity of cyclic tethers more generally employed, allowing a productive binding to the target receptors. A significant increase of activity was also observed when a benzoate group was introduced at C4 of the galactose subunit.

The present work aimed at improving the properties of our acyclic tether and at increasing the potency of the lead compound **3** that was identified previously. We hypothesized that bulkier ester groups could induce an orientation of the fucose and galactose sugar moieties to increase the binding to selectins. The impact of installing a benzoate group at the C2-galactose position (R^2) was also examined. Other groups observed that this modification enhances significantly the potency of their sLe^X analogues.[7,9,16] Another avenue that we have begun to explore herein involves the preparation and biological evaluation of multivalent sLe^X analogues (Figure 2).

Synthesis of sLe^X Analogues with Acyclic Tethers. The first series of analogues was prepared by coupling the fucosides **12** and **13**, bearing the acyclic tether, with galactoside donors **16** and **18** (Scheme 1). The former were prepared by adding L-tartrate ester **10** or **11** to perbenzylated thioethyl fucoside **9** in the presence of NIS (Scheme 1).[25] The β-thioethyl galactoside with C4 and C6 hydroxyls protected by a benzylidene acetal was obtained by a regioselective C3 O-alkylation of **14** with triflate **15** using *in situ* formation of organotin acetals. The benzoate at C2 was then installed to give **16**. A similar approach

Scheme 1. Synthesis of sLe^X Analogues 20, 22, 24, and 26[a]



[a](a) NIS/CF_3SO_3H, CH_2Cl_2, −30 °C, 4 Å mol sieves (88% for **12** and 75% for **13**); (b) i. Bu_2SnO, MeOH, then CsF and **15** in THF; ii. BzCl, DMAP, DCM, 93% over 2 steps; (c) i. Bu_2SnO, MeOH, then CsF and **15** in THF; ii. BzCl, DMAP, DCM, 70% over 2 steps; (d) NIS/TMSOTf, CH_2Cl_2, −30 °C, 4 Å mol sieves (60% for **19**, 77% for **21**, 67% for **23**, and 72% for **25**); (e) Pd/C, H_2, dioxane (80% for **20**, 65% for **22**, 53% for **24**, and 53% for **26**).

was employed from β-thioethyl galactoside **17** to generate **18**. Both **16** and **18** were then coupled to **12** and **13** in the presence of NIS/TMSOTf at −30 °C.

The β-selectivities for these glycosylations are attributed to anchimeric assistance of the ester at C2.[26] After debenzylation with Pd/C in the presence of H_2, the targeted products **20**, **22**, **24**, and **26** were obtained.

The selective differentiation of the tartrate esters was challenging (Scheme 2). A dioxolanone intermediate was prepared by hydrolyzing **13** with an NaOH solution and treating the resulting product with an excess of 2,2-dimethoxy propane and a catalytic amount of PTSA.[27] The crude mixture was then dissolved in DMF and reacted with Cs_2CO_3 and isopropyl iodide to give **28**. Hydrolysis of the latter with AcOH in water at 50 °C and treatment with $TMSCH_2N_2$ provided **31**. Inverting the order of the esterification steps led to **30**, the structure of which was confirmed by X-ray analysis of a *para*-nitrobenzoate derivative.[28] Both **30** and **31** were then coupled with **18** using a mixture of TMSOTf/NIS in CH_2Cl_2 to generate **32** and **34**. Removal of the four benzyl groups by hydrogenolysis yielded the final products **33** and **35**.

E- and P-Selectin Static Assays and P-Selectin Dynamic Assay. sLe^X analogues were first evaluated in E- and P-selectin cell-based adhesion assays (static assay, Table 1).[29] We also performed a more direct competition assay using

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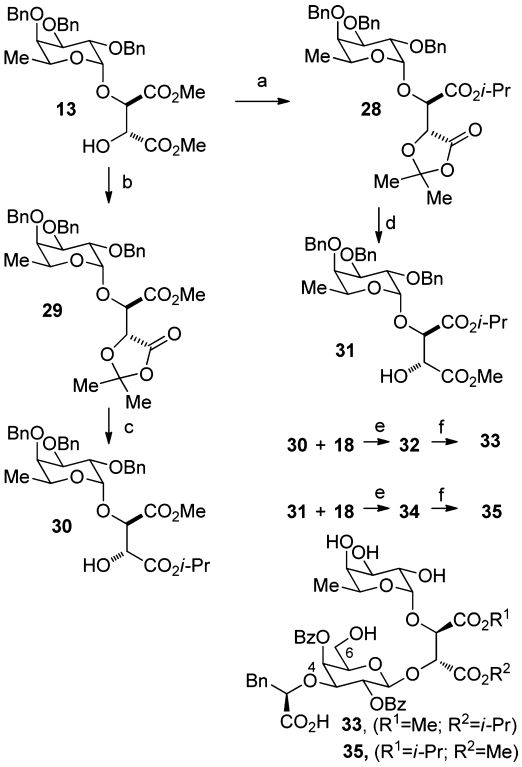
Scheme 2. Synthesis of 33 and 35[a]



[a](a) i. NaOH solution (10%), THF, 25 °C; ii. 2,2-dimethoxy-propane, PTSA, DCM, 25 °C, no purification; iii. Cs_2CO_3, i-PrI, DMF, 25 °C, 56% over 3 steps; (b) i. NaOH solution (10%), THF, 25 °C; ii. 2,2-dimethoxypropane, PTSA, DCM, 25 °C; iii. $TMSCH_2N_2$, MeOH, 25 °C, 72% over 3 steps; (c) i. $AcOH/H_2O$ (80:20), 50 °C; ii. Cs_2CO_3, i-PrI, DMF, 25 °C, 59% over 2 steps; (d) i. $AcOH/H_2O$ (80:20), 50 °C; ii. $TMSCH_2N_2$, DCM, 25 °C, 66% over 2 steps; (e) TMSOTf, NIS, DCM, −25 °C, 81% for **32** and 75% for **34**; (h) H_2, Pd/C, dioxane, 23% for **33** and 20% for **35**.

surface plasmon resonance spectroscopy (SPR, dynamic assay, Table 1).[22,30] The extracellular monomeric human PSGL-1 (hPSGL-1) fused with the Fc portion of a human IgG (rPSGL-Ig) was covalently attached to a sensor chip. A constant amount of soluble P-selectin with variable concentrations of one of our molecules was then injected in the flow cell. In this assay, the tested analogues compete with the carbohydrate motifs attached on the immobilized protein for binding to P-selectins (P-selectin dynamic assay).

Biological evaluations of the analogues by static and dynamic assays are presented at Table 1. sLe^X was used as a control in each assay. A ratio of the relative potency of the tested analogues and sLe^X was calculated [IC_{50}(Cpd)/IC_{50}(sLe^X)]. As reported in the previous pilot study, replacing the GlucNAc subunit by an acyclic tether provides molecular prototype **3** with an antagonist activity slightly lower than sLe^X with both P- and E-selectin (entry 1). Compound **4**, bearing a benzoate at the C4-Gal position, is 37 times more potent than sLe^X (entry 2). We hypothesized that this improvement originates from a favorable interaction of the benzoate with the Tyr94 in the carbohydrate binding domain (CRD) of P-selectin.[22]

The potency of sLe^X **24** bearing a benzoate at C2 of galactose was next examined (entry 3, Table 1). Only a slight improvement of the potency was noted with **24**, as compared to **3**. The installation of benzoates at C2 and C4 provided a product (**26**) with high potency in the three assays (entry 4). Both **26** and **4** have, however, the same potency in the P-selectin binding assay, which indicates the importance of the benzoate at C4.

Di-isopropyl esters displayed improved IC_{50} in the static and dynamic assays. Compound **20** (entry 5) bearing a benzoate at C2-Gal was more potent than its dimethyl ester counterpart **24** (entry 5 versus entry 3). The dibenzoate derivative **22** (bearing diisopropyl esters) provided exciting results (entry 6). Potency ratios ranging from 47 to 79 were observed. We then sought to rationalize the increase of potency noted. As indicated by preliminary NMR spectroscopy experiments, the relative alignment of the fucose and galactose moieties was modified in the ground state conformation. Contrary to the methyl ester series, intramolecular nuclear Overhauser effect (NOE) interactions between the methyl of fucose and the methylene

Table 1. IC_{50} and Relative IC_{50} versus sLe^X in E- and P-Selectin Static Assays and P-Selectin Dynamic Assay

						Static assays				Dynamic assays	
						E-Selectin		P-Selectin		P-Selectin	
Entry	Cpd	R_1	R_2	R_3	R_4	IC_{50}(mM)	Ratio[a]	IC_{50}(mM)	Ratio[a]	IC_{50}(mM)	Ratio[a]
1	**3**	H	H	Me	Me	5.46	0.66	4.840	0.71	0.880	0.75
2	**4**	Bz	H	Me	Me	N.D.	N.D.	N.D.	N.D.	0.018	37
3	**24**	H	Bz	Me	Me	2.08	1.8	1.9	1.78	0.52	1.25
4	**26**	Bz	Bz	Me	Me	0.14	23.8	0.11	29.8	0.018	36.0
5	**20**	H	Bz	*i*-Pr	*i*-Pr	0.22	16.1	0.19	17.6	0.034	9.0
6	**22**	Bz	Bz	*i*-Pr	*i*-Pr	0.076	46.8	0.067	52.5	0.008	79.3
7	**36**	Bz	H	*i*-Pr	*i*-Pr	N.D.	N.D.	N.D.	N.D.	0.019	28.0
8	**33**	Bz	Bz	Me	*i*-Pr	N.D.	N.D.	N.D.	N.D.	0.021	31.6
9	**35**	Bz	Bz	*i*-Pr	Me	N.D.	N.D.	N.D.	N.D.	0.016	40.5
10	**46**	See Scheme 3				N.D.	N.D.	N.D.	N.D.	0.008	82.0

[a]Ratio or relative IC_{50} = IC_{50}(Cpd)/IC_{50}(sLe^X).

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(C6) of galactose are now present, suggesting a proximal stacking of these units. These stacking interactions have been identified as important in the pioneering work of Thoma, Ernst, and others.[4,9,12]

We then prepared the C4−OBz **36** using the same sequence of reactions described in our previous study, changing only the esters (entry 2 versus entry 7). From this point on, only the P-selectin dynamic assay (SPR) was performed. No significant change of the potency was noted, a ratio of 28 being obtained for **36** (entry 7), as opposed to 37 for **4** (entry 2). This result shows the importance of the benzoate at C2 regarding the increase of potency induced by diisopropylester groups. The replacement of one of the isopropyls by a methyl was then evaluated. For each molecule **33** or **35** a significant reduction of potency was measured (entries 8 and 9). The variation of the nature of the esters and its replacement by other functionalities are avenues to be explored in a subsequent study.

***In Vivo* Evaluations of sLe^X Analogues.** We have begun the *in vivo* evaluation of our molecules. Leukocyte rolling flux was measured using intravital microscopy and tumor necrosis factor (TNFα) stimulated mouse cremaster. The monobenzoate di-isopropyl ester **20** dissolved in a saline solution was evaluated for its capacity to inhibit the decreased leukocyte rolling flux induced by TNFα. As seen in Figure 3, the addition

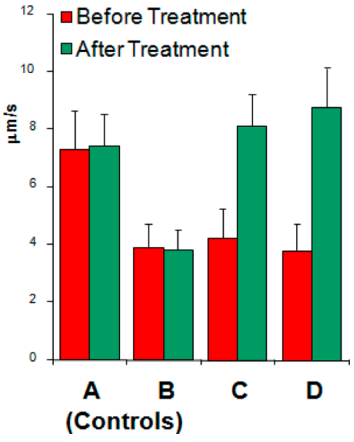
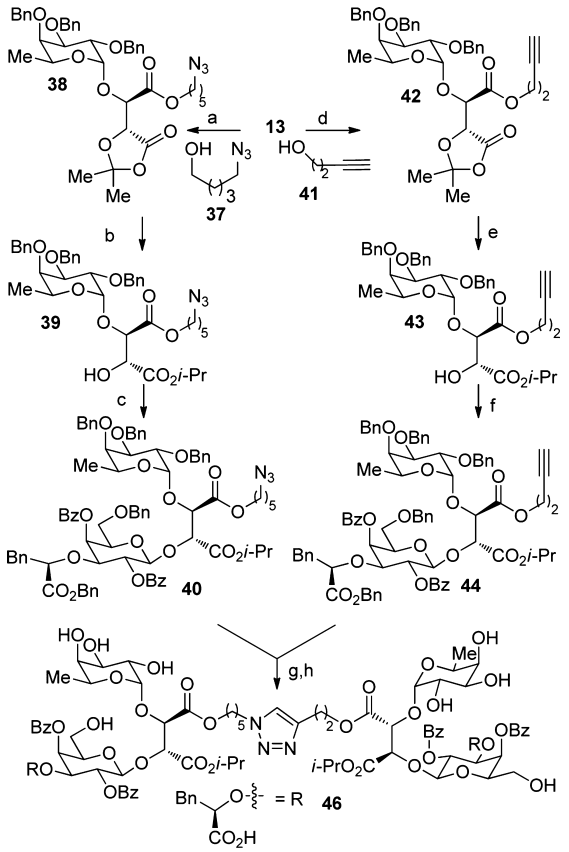


Figure 3. Control mice (red) were injected with 150 μL of saline (A) and 150 μL of saline containing 500 mg of rmTNFα (B−D). Results show rolling velocity of leukocytes before (red) and 10 min after the intrajugular injection of saline (B), sLe^X (C), and 20 (D) at 100 mg/kg (green). Results are the averages of 5 readings per venule, 10 venules per mouse, and 5−6 mice per tested conditions (±SEM).

of TNFα led to a decreased rolling velocity (B, red, versus A, red), which was not reversed by a subsequent saline control (B, green). Sialyl Lewis^X reversed the effect of TNFα (C, green). Similarly, a significant increase of rolling velocity was noted when analogue **20** was injected (D, green).

Multivalent sLe^X Analogues. Multivalent ligands have attracted considerable attention in the carbohydrate community in the past decade.[31−33] Divalent or trivalent ligands harboring sLe^X have been previously synthesized; some showing increased potency.[33−36] As illustrated in Scheme 3, we intended to prepare a bivalent ligand taking advantage of the ester groups on the acyclic tether moiety of our analogues to introduce other chemical entities. We planned to use a 1,3-dipolar cycloaddition to link two fragments by forming a triazole.[37] The first fragment was generated from the acid **13** to which an azido pentanol was coupled. The corresponding ester **38** was then treated under

Scheme 3. Synthesis of Dimer 45[a]



[a](a) i. NaOH solution (10%), THF, 25 °C; ii. 2,2-dimethoxy-propane, PTSA, DCM, 25 °C, no purification; iii. **37**, DCC, DMAP, DCM, 52% over 3 steps; (b) i. AcOH/H_2O (80:20), 50 °C; ii. Cs_2CO_3, DMF, then *i*-PrI, 25 °C, 66% over 2 steps; (c) **18**, NIS, TMSOTf, DCM, −25 °C, 69%; (d) i. NaOH solution (10%), THF, 25 °C; ii. 2,2-dimethoxy-propane, PTSA, DCM, 25 °C, no purification; iii. **41**, DCC, DMAP, DCM, 25 °C, 53% over 3 steps; (e) i. AcOH/H_2O (80:20), 50 °C; ii. Cs_2CO_3, DMF, then *i*-PrI, 25 °C, 58% over 2 steps; (f) **18**, NIS, TMSOTf, DCM, −25 °C, 64%; (g) CuI, DIEA, THF, 25 °C, 60%; (h) $Pd(OH)_2$, THF, 25 °C, 19%.

acidic conditions to hydrolyze the dioxolanone. The free acid was then esterified to the isopropyl ester **39** and coupled to the dibenzoate donor to give the corresponding β-anomer **40**. A similar reaction sequence was realized after adding the propargylic alcohol to the acid **13**, which could be efficiently converted to **44**. The azide **40** and alkyne **44** were then reacted in the presence of CuI and DIEA in THF at room temperature.[37] The 1,3-triazole dimer was obtained in a 60% yield. The eight benzyl groups were then removed to give **46**.

As seen in Table 1, our divalent ligand **46** showed a relative potency ratio of 82 in the P-selectin assay (entry 10, Table 1). Compared to analogue **35**, this represented a more than 2-fold increase in potency (entry 9 versus entry 10). We are considering varying the length and the nature of the triazole tether chain by introducing different substituents to improve further this multivalent approach.

In conclusion, we have shown herein that by using an acyclic tether we were able to generate potent E- and P-selectin antagonists. The representative member of this series demonstrates *in vivo* activity in modifying the rolling of leukocytes induced by an inflammatory stimulus. We are now evaluating other acyclic tethers in order to probe the CRD of

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the selectin and to improve the resulting biological properties of this promising family 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.

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Notes

The authors declare no competing financial interest.

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(28) Details are provided as Supporting Information.

(29) HL-60 cells were radiolabeled by adding [H3] thymidine in the incubation medium. Wells were precoated with goat anti-human1gG antibodies. E- or P-selectin 1gG were added. Radiolabeled cells were incubated in the presence of the immobilized selectins. After carefully washing the nonadhered cells, the radioactivity was measured. In the presence of putative selectin antagonist, the number of these radiolabeled cells will decrease in a dose-dependent way.

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