Quinic Acid Derivatives as Sialyl Lewis^x-Mimicking Selectin Inhibitors: Design, Synthesis, and Crystal Structure in Complex with E-Selectin

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A search for noncarbohydrate sLe^x mimics led to the development of quinic acid derivatives as selectin inhibitors. At Wyeth we solved the first cocrystal structure of a small molecule, quinic acid, with E-selectin. In the cocomplex two hydroxyls of quinic acid mimic the calcium-bound fucose of the tetrasaccharide sLe^x. The X-ray structure, together with structure based computational methods, was used to design quinic acid based libraries that were synthesized and evaluated for their ability to block the interaction of sLex with P-selectin. A large number of analogues were prepared using solution-phase parallel synthesis. Selected compounds showed decrease in leukocyte rolling in the IVM mouse model. Compound **2** inhibited neutrophil influx in the murine TIP model and demonstrated good plasma exposure.

Background

Excessive recruitment of leukocytes from the circulation can result in pathological conditions such as stroke, reperfusion injury, and cardiovascular and allergic diseases. Leukocyte migration into underlying tissues is an orderly process mediated initially by low-affinity, transient interactions of P- and E-selectin cell adhesion molecules expressed on the vascular endothelium with tetrasaccharide sialyl Lewis x (sLex)-containing ligands on the leukocyte cell surface. Leukocytes thus slowed to a roll by interactions with the selectins subsequently arrest as a consequence of high-affinity integrin-based interactions and then extravasate into the interstitium where inflammatory reactions manifest. Constituting the earliest adhesion event in the leukocyte recruitment cascade, central to virtually all inflammatory processes, and of low-affinity relative to other cell adhesion events, selection-sLex interactions are appealing drug discovery targets.[1−10]

Recently, we published the crystal structures of both E- and P-selectin (lectin and EGF domains) in complex with sLex, and P-selectin LE cocomplexed with the PSGL-1 N-terminus.[11] These structures have clearly defined the interactions between the selectins and sLex and are consistent with information gleaned from NMR experiments and binding studies of selectively modified sLex.[5] Sialic acid, L-fucose and galactose are vital for the calcium-mediated binding of sLex to selectins. In addition to binding directly to the calcium ion, the 3- and 4-hydroxyls of fucose make interactions with protein side chains that bind the calcium. Some of the hydroxyls of galactose and sialic acid also interact with the selectins, as does the required acid group of sialic acid, which binds to an arginine (E-selectin) or a serine (P-selectin) side chain.

There are numerous reports of selectin inhibitors in the literature. One popular strategy has been the design of sLex mimetics with better pharmacokinetic properties than the tetrasaccharide sLex. This has been accomplished by replacing some or all of the sugars with other moieties such that the key interactions are conserved.[5] Our search for a noncarbohydrate mimic of fucose led to the identification of quinic acid as a replacement for fucose. In this paper, we describe the design, synthesis and biological evaluation of quinic acid derivatives as sLex mimetics through the use of X-ray crystallography and molecular modeling. This is the first report of the crystal structure of E-selectin in complex with a small molecule.

Structural Studies

A number of small molecules that potentially mimic the fucose residue of sLex were evaluated. Quinic acid was chosen as the preferred template in anticipation that this noncarbohydrate would prove more stable than a carbohydrate-based inhibitor; at the time, there were no literature references to quinic acid derivatives having been evaluated as selectin inhibitors. Derivatization was synthetically straightforward. It was expected that the 4- and 5-hydroxyls of quinic acid (one in an equatorial position and one in an axial position) would coordinate the calcium in a fashion similar to the 3- and 4-hydroxyls of fucose in sLex. To confirm this hypothesis, we soaked quinic acid into preformed crystals of a human E-selectin construct consisting of the lectin and

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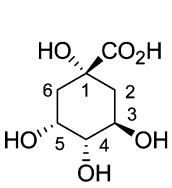
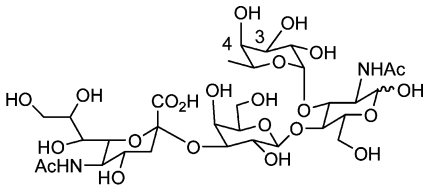


(-) Quinic Acid

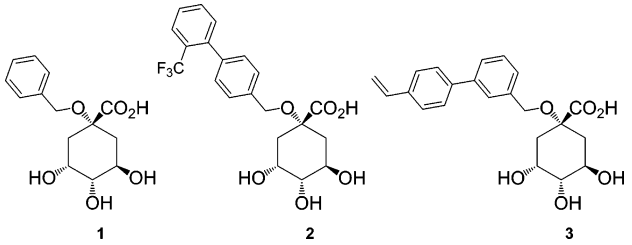


Sialyl Lewis x (sLex)

EGF domains (E-LE). The protein structure in the resulting complex is essentially identical to those previously described[11,12] (Figure 1). However, close examination of the complex structure revealed the unexpected result that the 3- and 4-hydroxyls of quinic acid (both in an equatorial position) bind the calcium ion, while the 5-hydroxyl forms a hydrogen bond with the side chain of Glu107. The consequences of the 3- and 4-hydroxyls binding to the calcium ion are significant. The geometry of the sLex molecule requires that the fucose ring be perpendicular to the surface of the protein when it is bound to the calcium ion. Quinic acid, which is not constrained as part of a tetrasaccharide, binds to the calcium ion with its ring parallel to the surface of the protein. This significantly changes the directional vector of substituents that are attached to the 1-position of quinic acid.

Our focus was to find a P-selectin inhibitor, since it is expressed on the endothelium within minutes after stimulation and plays a key role in the early adhesion cascade.[13] Attempts to obtain a cocrystal structure of quinic acid with P-selectin failed; the high concentrations of ligand used in the soaking experiments caused loss of diffraction with the P-selectin crystals.[14] Since E- and P-selectin bind to sLex in a similar fashion, we used the cocrystal of E-selectin-quinic acid to generate a model of the P-selectin−quinic acid complex. This model was also used to generate virtual libraries analoging the tertiary alcohol and carboxylic acid functionalities to improve potencies.

Initial modeling efforts were directed toward exploring simple modifications of the 1-hydroxy group of quinic acid. One particularly interesting modification was the addition of a benzyl group (**1**). Modeling suggested that



1 **2** **3**

a benzyl group would cause very little change in the orientation of the quinic acid group with respect to P-selectin but would result in a stacking interaction between the benzyl group and the side chain of Tyr-94. Compound **1** was synthesized and showed 61% inhibition at 10 mM in the P-selectin ELISA assay. Quinic acid shows marginal activity in this assay at 10 mM. At this time the Biacore assay was developed which proved to be more stringent, robust, and reproducible (see details for the Biacore assay in the results section). The ELISA relied on amplification of signal by formation

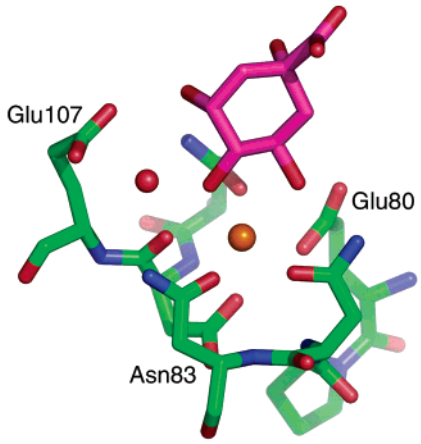


Figure 1. The structure of quinic acid shown with purple carbon atoms bound to E-selectin. The bound calcium ion is shown in orange and an ordered water in red. The figure was made using PYMOL.[25]

of multimeric complexes of an Fc construct of the P-selectin ligand (PSGL-1) and a conjugated antibody. The formation of these complexes is quite variable, making it difficult to evaluate small gains in activity. This problem is avoided in the Biacore assay, which looks at monomeric binding of P-selectin to PSGL-1. Compound **1** showed marginal activity at 10 mM in the Biacore assay. All subsequent compounds were tested in the Biacore assay.[15]

Next, we modeled simple modifications of **1** that could be made through amide formation or a Suzuki reaction, adding an additional aryl group. We hoped to obtain the same kind of increase in potency and change in physical properties that had resulted from adding the first aryl group to quinic acid. Library A and library B were the first two libraries made (Figure 2). Library A utilized an amide group as a spacer between the two aryl groups, while library B investigated the direct addition of an additional aryl group to **1**. Each library consisted of three subgroups, with the new linker ortho, meta, or para to quinic acid. The parent structures were each modeled onto the P-selectin surface using a Monte Carlo algorithm in QXP.[16] Using the lowest energy structure of each parent as a starting point, a virtual library corresponding to the appropriate adducts was generated in the presence of P-selectin using QXP. This method of reagent selection was utilized for every library except library H.

Modeling studies for Library B showed that in the ortho position, the new aryl ring does not make any significant interactions with the protein surface, but does orient possible substitution positions on the ring toward the protein surface where new interactions could be made. In the meta position, the new aryl ring lies along the surface of the protein, interacting with the side chain of Tyr-48. Possible substitution points on this new ring are directed along the protein surface.

Several potent compounds were identified out of library B. On the basis of this result, we decided to replace the phenyl ring attached to quinic acid with a heterocycle, giving library C. The attachment point for substitutents presents a similar though slightly different vector than the meta position on an aryl group. Thus, molecular modeling suggested that substituents

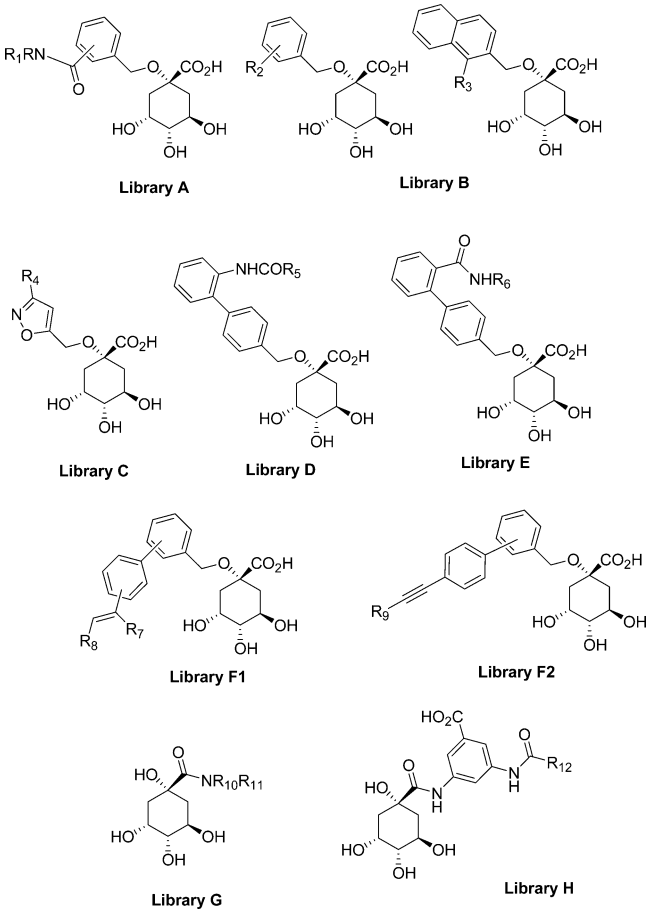


Figure 2. Quinic acid libraries.

would have slightly altered interactions with the protein relative to similar compounds from library B.

Library D and E were based on further modification of compound **2**, a hit from library B. Modeling predicted that the trifluoromethyl group from biphenyl **2** was oriented in the direction of a small hydrophobic tunnel (or pseudo pocket) on the surface of P-selectin. It was proposed that the alkyl group from the amides might interact with this hydrophobic tunnel.

Library F is based on **3**, a potent hit from library B. When **3** was modeled onto the P-selectin surface we found that the first aryl ring stacks onto Tyr94, while the second aryl group makes a T-interaction with the side chain of Tyr48 (Figure 3). All three substitution sites on the second aryl ring are directed along the surface of the protein, allowing for the opportunity to find the appropriate substituent that makes additional productive interactions.

Library G explored amide substitution at the carboxylic acid of quinic acid. While the directional vector with respect to the protein surface is not as favorable for a substituent attached to the carboxylic acid as for one attached to the hydroxyl we still decided to explore this option. We selected amines with substituents that could reach back and make interactions with the surface of the protein and then used previously described molecular modeling experiments to choose reagents out of this set. Results from library G showed that a carboxyl functionality was essential for activity. Thus, we designed library H, which kept an amide linked

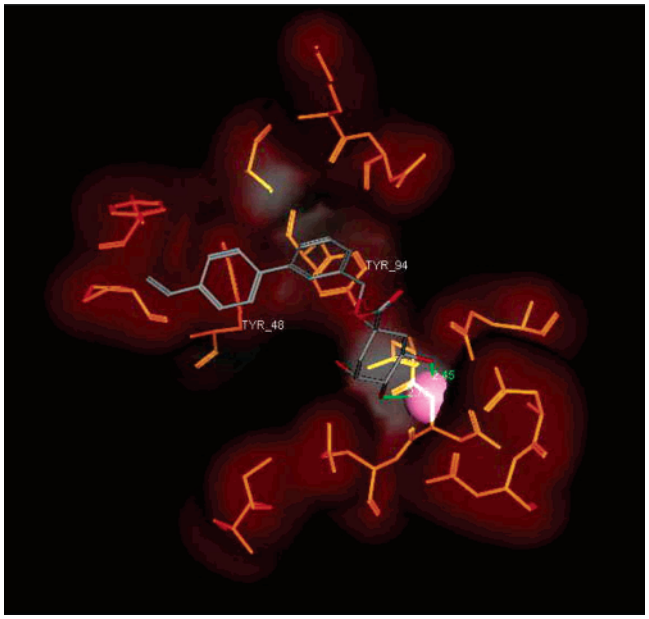
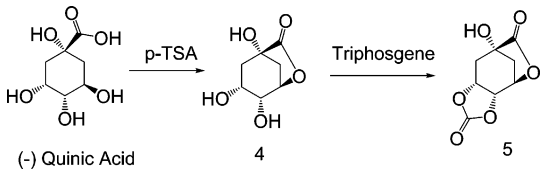


Figure 3. A proposed binding mode of compound **3** to P-selectin is shown. The proximal residues of the protein are shown in orange with a Connelly surface. The bound calcium is shown in pink. Tyr94 and Tyr48 are labeled to highlight the interaction between the side chains of these residues and the aryl groups of **3**.

Scheme 1



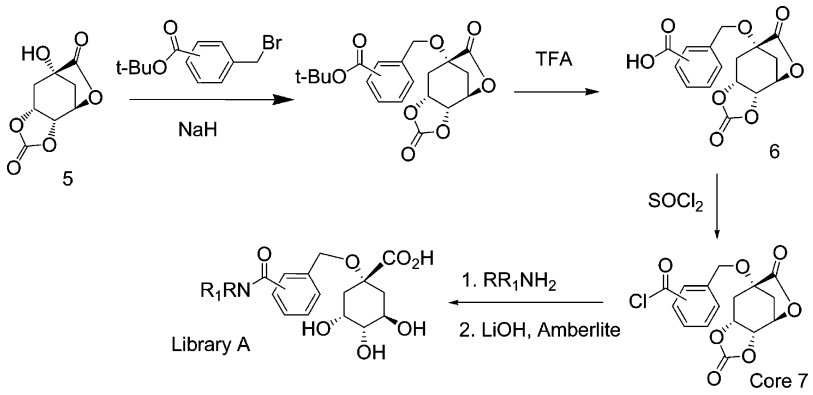
substituent (as in library G), but incorporated a carboxylic acid group. Diverse reagents were chosen for this library.

Chemistry

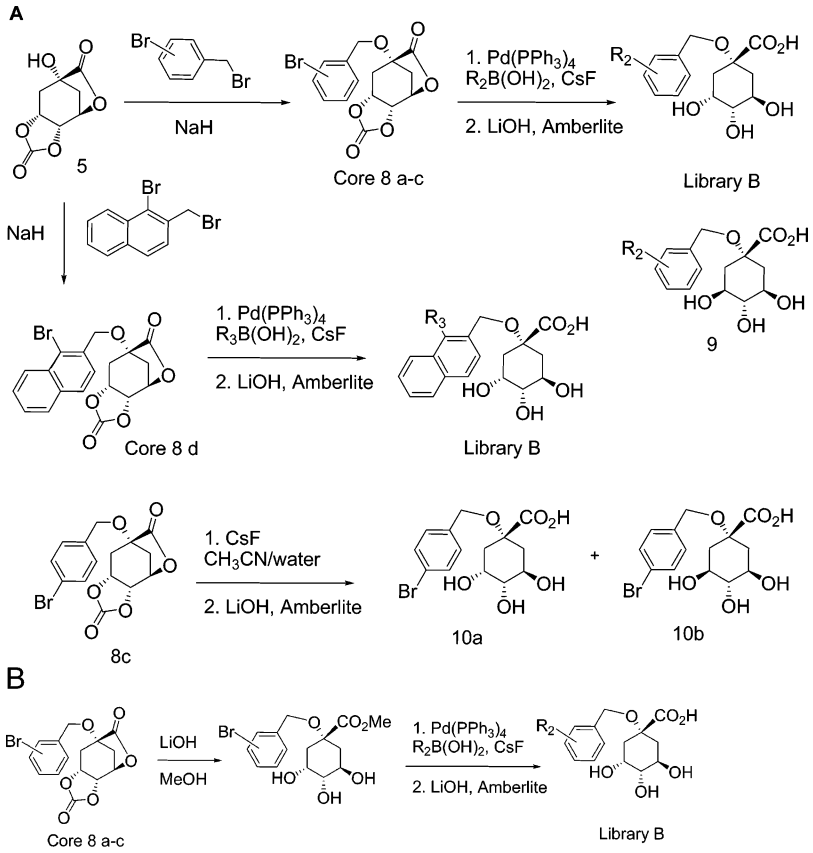
Libraries A−F, the Tertiary Alcohol-Substituted Libraries. To rapidly prepare libraries of quinic acid in a parallel format, we were looking for protecting groups, which could be removed in such a manner as to eliminate the need for one-by-one workup of each reaction. For the tertiary alcohol libraries, we used the carbonate-lactone functionality to protect the secondary alcohols and carboxylic acid group (Scheme 1). Commercially available quinic acid was converted to the corresponding lactone **4** in refluxing DMF/benzene with catalytic *p*-toluenesulfonic acid.[17] Lactone **4** was then treated with triphosgene in pyridine to form lactone carbonate **5**, which is the common intermediate for the synthesis of libraries A−F. Treatment with lithium hydroxide and quenching with acidic resin can remove these groups in one step.

Library A (Scheme 2): Intermediate **5** was alkylated with ortho, meta, or para substituted bromomethylbenzoic acid *tert*-butyl ester to form ethers. Removal of the *tert*-butyl group with TFA provided the free acids, **6**. Due to the wide range of amines selected for this library, there was no universal set of conditions effective for every amide coupling. However, a large percentage of the compounds could be prepared through the interme-

Scheme 2



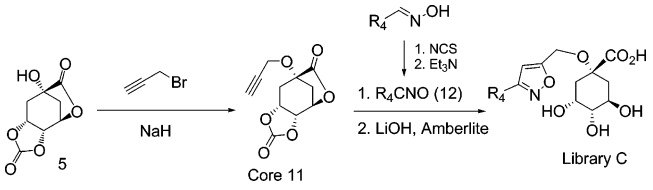
Scheme 3



diacy of acid chloride **7**. The amide products were hydrolyzed with LiOH and quenched with acidic resin to give library A, which contained 256 compounds.

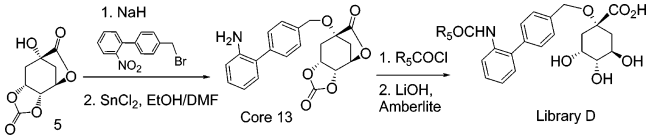
Library B (Scheme 3A): Intermediate **5** was alkylated with ortho, meta, or para substituted bromobenzyl bromide or 1-bromo-2-bromomethylnaphthalene to give intermediates **8a**−**d** respectively. Suzuki coupling of intermediates **8a**−**d** was a challenging reaction for parallel synthesis. It was difficult to find coupling conditions that worked for all four cores **8a**−**d**. The conditions shown in Scheme 3 worked reasonably well; other conditions tried were $Pd(OAc)_2/CsCO_3$ and $Pd_2(dba)_3/PtBu_3/CsCO_3$. The four cores, **8a**−**d,** were crossed with several boronic acids; hydrolyzed with LiOH, and quenched with acidic resin to give library B, which contained 140 compounds. During the purification of some of these compounds, approximately 5−20% of epimerized product **9** was isolated. Further studies showed that the CsF in the Suzuki reaction was causing this epimerization. Simply refluxing the core **8c** in CH_3CN/water in the presence of CsF, followed by LiOH hydrolysis, gave triol **10a** that contained about 20% of epimerized product **10b**. For the resynthesis of biologically active compounds from library B, modified Scheme 3B was used in order to avoid **9**-like byproducts.

Scheme 4

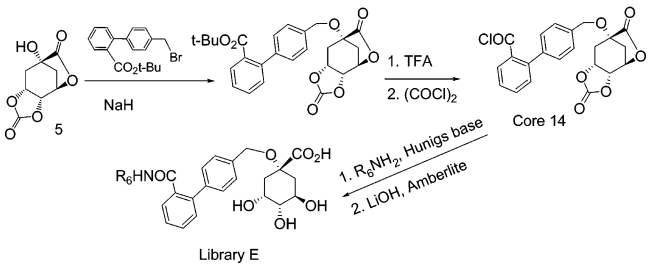


Library C (Scheme 4): The common intermediate **11** was easily synthesized by alkylation of **5** with propargyl bromide. Reaction of **11** with nitrile oxides **12** gave

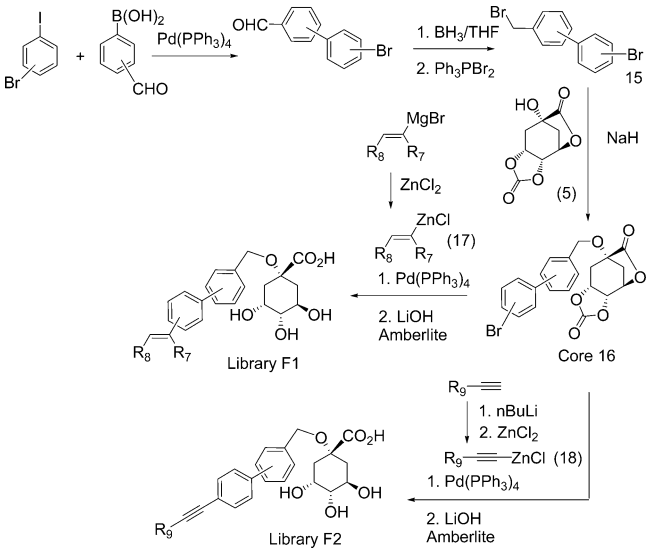
Scheme 5



Scheme 6



Scheme 7



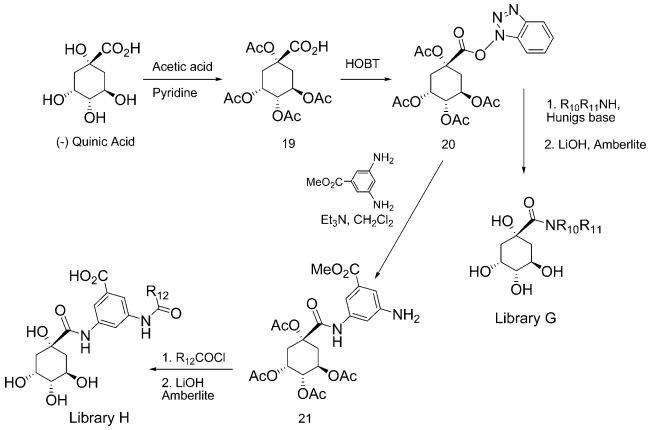
isoxazoles which, upon deprotection with LiOH, resulted in library C. The required nitrile oxides **12** were formed in situ by chlorination of oximes with *N*-chlorosuccinimide followed by dehydrohalogenation with triethylamine. Library C contained 21 compounds.

Library D (Scheme 5): Alkylation of intermediate **5** with 4-(2-nitrophenyl)benzyl bromide, followed by reduction, gave the aniline core **13**. Coupling of a variety of acid chlorides with core **13,** followed by hydrolysis with LiOH, resulted in library D (37 compounds). It was found that LiOH hydrolysis gave the methyl ester as a byproduct when carried out in MeOH/ water solution; therefore, the hydrolysis for this library was performed in THF/water.

Library E (Scheme 6): Acid chloride core **14** was prepared from intermediate **5** in three steps. Alkylation with 4-(2-*tert*-butoxycarbonylphenyl)benzylbromide was followed by TFA removal of the *tert*-butyl group, then conversion to the acid chloride using oxalyl chloride. Coupling of core **14** with several amines followed by LiOH deprotection gave 41 new compounds.

Library F (Scheme 7): The common core for this library, **16,** was prepared by alkylation of intermediate **5** with biphenyl benzylbromides **15**. Compounds **15** were prepared in three steps starting with the Suzuki coupling of a bromoiodobenzene with a formylboronic acid.

Scheme 8



The aldehyde group was then reduced to the corresponding alcohol, which was converted to the desired bromide **15** with triphenylphophine dibromide. Core **16** was coupled with vinylzinc (**17**) and alkynylzinc (**18**) reagents to give vinyl- and alkynyl-substituted biphenyl quinic acids, respectively.

Commercially available Grignard reagents were used to obtain the vinyl zinc reagents. Grignard reagents were first reacted with $ZnCl_2$ to form the vinylzinc, followed by $Pd(PPh_3)_4$ catalyzed coupling with core **16**. Hydrolysis with LiOH gave the final vinyl-substituted products (Library F1, nine compounds).

Commercially available terminal alkynes were used to obtain the alkynylzinc reagents. Terminal alkynes were first treated with *n*-butyllithium. The alkynyllithium reagents were then reacted with zinc chloride to generate alkynylzinc reagents in situ, which were coupled to core **16** using the previously established conditions for vinyl substituted quinic acids. Deprotection with LiOH gave the final alkynyl substituted products (Library F2, 4 compounds).

Libraries G and H, the C-1 Carboxylic Acid Substituted Libraries. In these libraries the alcohol groups were protected as acetates. Library G (Scheme 8): Quinic acid was treated with acetic anhydride /pyridine to give the tetraacetyl intermediate **19**. The acid group was then activated by conversion to the HOBt ester **20**. Compound **20** was stable for long periods of time and could be coupled with a variety of amines. Hydrolysis under basic conditions gave library G (70 compounds).

Library H (Scheme 8): Coupling of intermediate **20** with methyl 3,5-diaminobenzoate gave the core for library H, the reaction of core **21** with acid chlorides worked well using PVP (polyvinylpyridine) as the catalyst. Final deprotection with LiOH also hydrolyzed the methyl ester to give a free acid in library H (42 compounds).

Coupling of **21** with sulfonyl chlorides was sluggish. We could not find optimal conditions for the synthesis of sulfonamides, and therefore this approach was abandoned.

Results and Discussion

Compounds were evaluated for inhibition of P-selectin in a Biacore assay. This is an equilibrium assay, which measures interactions between monomeric, soluble P-selectin, and immobilized PSGL-1 under flow condi-

tions. Compounds from our libraries with IC_{50}s from 1 to 10 mM in the Biacore assay were considered reasonable hits, as the natural ligand sLex has an IC_{50} of 15 mM in this assay.[18]

Compounds with $IC_{50} \leq 10$ mM in the Biacore assay were further evaluated in our secondary assays, an NMR assay, and a cell-based flow assay. In the NMR assay, transfer NOE (trNOE) experiments were performed using P-selectin. The trNOE experiment is a well-established technique for studying protein−ligand interactions, including the determination of bound ligand structures.[19] In the trNOE experiment, the protein−ligand system must be in rapid exchange for the bound small molecule to be easily detectable. In this case, strong negative trNOEs are observed. Compounds that do not bind to the protein display a weak positive NOE.

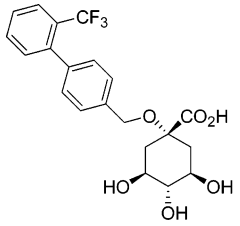
The cell-based in vitro flow assay measures selectin-mediated rolling of human neutrophils on stimulated human umbilical vein endothelial cells (HUVEC) in a parallel plate flow chamber. Digital image analysis of rolling and arrested cells is performed to quantitate inhibition.

Addition of an extra aryl group to compound **1** using an amide linker (Library A) did not result in any hits. Many of these compounds had additional atoms between the amide and the added aryl ring, in the form of small alkyl chains. While the flexibility inherent in these molecules allows for them to have a conformation that fits the surface of the protein well and to produce a good interaction energy with the protein from a molecular force field, it is likely that the additional negative entropy of binding counteracts any positive enthalpic considerations.

In contrast to library A, library B produced several hits. Adding the aryl ring directly to **1** was a success. It should be noted that palladium contamination of the products resulted in false positives. When the library was resynthesized, ensuring that the compounds were free of palladium contamination, eight compounds (**2** and **3**, **22**−**27**) were identified with $IC_{50} < 10$mM in the Biacore assay. Unfortunately, **3** ($IC_{50} = 1$ mM) could not be further evaluated due to the difficulty in synthesizing this compound on a larger scale. All efforts toward its scaled up synthesis resulted in polymerization of the vinyl boronic acid. Seven compounds, **2** and **22**−**27**, were evaluated in the NMR assay. Five compounds (**2**, **22**−**25**) showed positive binding by transfer NOE with P-selectin. Addition of small amounts of protein to **26** and **27** results in a significant broadening of the tNOE peaks, much more than is observed for compounds of similar Biacore potency. This suggests that these compounds bind to P-selectin with high stoichiometry, which is an unfavorable property for a drug. From our data it is difficult to speculate how many additional potential binding sites might exist on the P-selectin surface.

2 and **22**−**27** were also evaluated in the cell-rolling assay. Six compounds had IC_{50}s between 100 and 1000 μM. One compound caused cell lysis. It is difficult to deduce a meaningful SAR from our data. The only conclusion that can be drawn is that a hydrophobic substituent on the biphenyl results in a modest increase in activity. Nonetheless, a 500 μM hit in the rolling assay is exciting and worthy of evaluation in vivo.

As mentioned above, the cesium fluoride used during the synthesis of this library caused hydrolysis of the carbonate protecting group and partial epimerization of the C-3 hydroxyl group. The diastereomers for two representative examples were separated and tested and were found to have similar activities (**2** versus **32** and **22** versus **33**).



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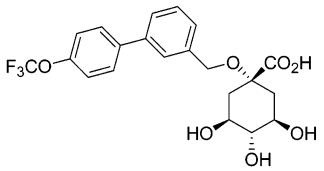

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The isoxazole compounds from library C did not show significant activity. No further effort was invested in this class of compounds.

Library D produced one hit, **28**. This compound was very potent in the cell based flow assay. Unfortunately, **28** showed nonstoichiometric binding to P-selectin in the NMR assay. Library E, the reverse amide library of D, produced no hits.

One hit, **29**, was obtained from library F1. **29** showed strong positive tNOE with P-selectin and had an IC_{50} of 1 mM in the cell rolling assay.

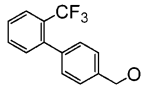
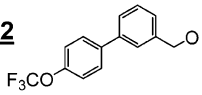
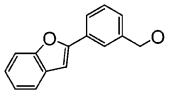
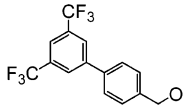
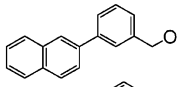
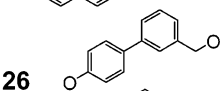
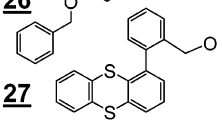
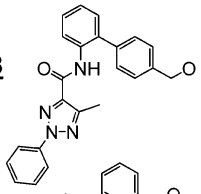
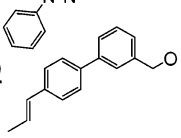
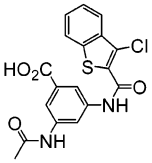
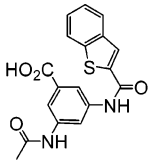
Library G produced no hits. The most potent compound in this library showed only 20% inhibition at 10 mM. Two hits were obtained from library H, **30** and **31**. They were well behaved in both the NMR and cell rolling assay. Replacement of the benzothiophene ring in **31** with a benzofuran or thiophene ring resulted in decreased activity.

The hits from all the libraries are summarized in Table 1. There were seven compounds that were well behaved in all three assays. These seven compounds were evaluated for iv PK in the rat (Table 2). Several of the quinic acid derivatives showed good pharmacokinetic properties. Our decision to choose a noncarbohydrate fucose mimic seems to have been a good one. A comparison of AUC/IC_{50} in the cell-rolling assay (Figure 4) showed that **2** and **22** scored the highest, and these two compounds were advanced to in vivo studies based on these results.

The compounds were first evaluated in intravital microscopy (IVM). In this study, the mesentery vessels of mice are surgically exteriorized. This surgical manipulation results in up-regulation of P-selectin on the vessel endothelium and causes leukocytes to roll on the endothelium via the P-selectin PSGL-1 interaction. Fifteen minutes after surgery, leukocyte rolling is video recorded. At 50 mg/kg iv compounds **2** and **22** showed a 49% and 48% decrease in leukocyte rolling respectively, compared to vehicle (Figure 5). In comparison, a P-selectin antibody completely abated rolling at a dose of 2.5 mg/kg iv. These results clearly show that quinic acid derivatives inhibit P-selectin dependent leukocyte rolling in vivo.

Compound **2**, which has somewhat better iv PK than **22**, was evaluated in the murine thioglycolate induced

Table 1. Hits from the Quinic Acid Libraries

	R	R_1	Biacore* IC_{50} (mM)	TNOE for P-Selectin by NMR	Cell-Flow IC_{50} (uM)
2		OH	10	Positive	625
22		OH	10	Positive	500
23		OH	6	Positive	1200
24		OH	4	Positive	550
25		OH	6**	Positive	1000
26		OH	2	Non-Stoichiometric	225
27		OH	2	Non-Stoichiometric	1000***
28		OH	2	Non-Stoichiometric	100
29		OH	6	Positive	1000
30	H		3	Positive	900
31	H		2	Positive	625

*IC_{50} for sLex ~ 15 mM ** not well behaved *** cell lysis

Table 2. Abbreviated Pharmacokinetic Parameters after Single Dose 20 mg/kg iv

compound	CL, mL/min/kg	AUC, min·μM	C_{max}, μM
2	4	13159	211.47
22	5	9376	245.71
23	10	5233	60.69
24	24	1899	48.7
29	6	8480	114
30	12	5028	73.95
31	35	1332	57.69

peritonitis (TIP) model. **2** at 50 mg/kg iv inhibited neutrophil influx by 58% compared to vehicle (Figure 6). In conclusion we have identified small molecule P-selectin inhibitors with in vivo results comparable to PSGL-1.

Experimental Section

Crystallography. Crystals of E-LE were grown using the hanging drop vapor diffusion method at 18 °C. The solution contained protein at 30 mg/mL, 15% (w/v) PEG 4000, 200 mM $CaCl_2$, 10 mM Tris-HCl, and 100 mM HEPES at pH 7.5. The crystals were transferred to an intermediate stabilizing solution consisting of 30% (w/v) PEG 4000, 200 mM $CaCl_2$, 100 mM HEPES, and 200 mM quinic acid which was adjusted to

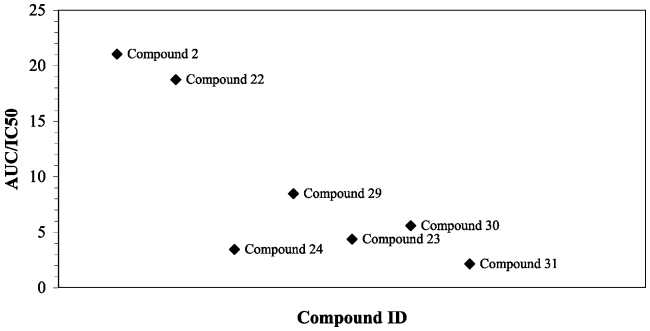


Figure 4. Quinic acid monomer lead compounds, AUC/IC_{50}.

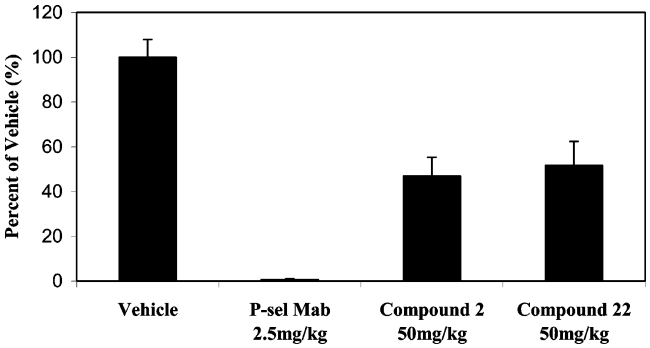


Figure 5. Effect of quinic acid derivatives **2** and **22** on leukocyte rolling in mouse mesenteric.

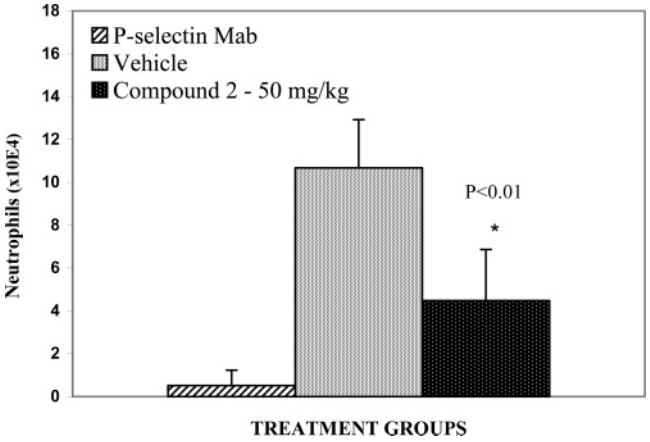


Figure 6. Compound **2** in the TIP model.

pH 7.5 with the addition of small amounts of HCl and NaOH. After 15 h the crystals were transferred into an identical solution but with the $CaCl_2$ concentration reduced to 2 mM. After 1 h in the second solution the crystals were rapidly cooled by plunging into liquid propane held at liquid nitrogen temperatures. Diffration data were collected on a Raxis II detector mounted on a Rigaku RU200 generator with Yale/Molecular Structure Corp. focusing mirrors. The data were reduced using DENZO and Scalepack (HKL research INC) giving the statistics in Table 3.

Rigid body refinement in XPLOR[20] resulted in phases that gave clear electron density for a bound Quinic acid. Refinement of the complex was performed using XPLOR giving the statistics in Table 3.

Molecular Modeling. Parent structures for each library were modeled onto the P-selectin site by overlaying the quinic acid moiety of the ligand onto quinic acid in the P-selectin/quinic acid model structure. Each structure was optimized using a Monte Carlo simulation of 1000 steps in which the ligand was allowed to rotate, translate, and flex while the protein was held rigid using QXP.[16] The 3- and 4-hydroxyls of the quinic acid moiety were constrained to be proximal to the selectin-bound calcium through he use of zero-order bonds. The lowest energy structure of each parent structure was used as a starting point for designing the library. A virtual library for each parent structure was generated in the presence of P-selectin using QXP employing the same method as described above. The low energy structure of each construct was retained and ranked using the QXP scoring function.[16] The top 200 structures were visually screened and the best structures retained for library synthesis. For library G, a diverse set of amines was chosen using molecular fingerprints and atom-pair fingerprints as descriptors, and hierarchal clustering through Selector.[21]

Table 3. Statistics for the Complex of Quinic Acid with E-LE

space group	$P2_12_12_1$
unit cell parameters	
a (Å)	34.5
b (Å)	72.0
c (Å)	77.9
maximum resolution (Å)	2.0
reflections	65483
unique reflections	12915
R_{merge} (%)	5.5
completeness (%)	94.2
shell 2.00−2.07 Å	
R_{merge} (%)	21.1
completeness (%)	73.7
model refinement	
R-value	0.201
R_{free}-value	0.237
rmsd bonds (Å)	0.010
rmsd angles (deg)	1.44

Chemistry. Reactions were run using commercially available starting materials and anhydrous solvents, without further purification. Proton NMR spectra were recorded at 300 MHz on a Varian Gemini 2000 or on a 400 MHz Bruker AV-400 spectrometer using TMS (δ 0.0) as a reference. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. CHN analyses were carried out by Robertson-Microlit. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were ±0.4 of the theoretical values. Low resolution mass spectra were obtained using a Micromass Platform Electrospray Ionization Quadrupole mass spectrometer. High resolution mass spectra were obtained using a Bruker APEXIII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an actively shielded 7 T superconducting magnet (Magnex Scientific Ltd., UK) and an external Bruker APOLLO electrospray ionization (ESI) source. Preparative HPLC was run using a Shimadzu reverse phase prep HPLC with Bischoff C18 SH (10 μm) column. The flow rate was 40 mL/min and mobil phase A was water with TFA as modifier; mobile phase B was CH_3CN. Purity in two solvent systems [H_2O-CH_3CN (method 1) and $H_2O-MeOH$ (method 2)] was determined using an Agilent 1100 HPLC instrument, and all compounds analyzed were >98% pure.

General Procedure for Library Synthesis. To a 2-dram vial were added all the reagents. The vials were then capped, shaken, and heated (if needed) overnight. The volatiles were removed under vacuum, and the residue was redissolved in EtOAc (2 mL) and water (1 mL) added. The vials were vortexed and centrifuged. The organic layer was collected and the aqueous layer extracted with EtOAc (1 mL × 2). At this stage, for some of the libraries, resins were added to remove the excess acidic or basic reagents. For example, for library B, Amberlite basic resin was added, and the vials were shaken for 1 h at room temperature to remove the excess boronic acid. The solvent was then transferred to another vial, and solvent was removed on Savant. The residues were redissolved in 2 mL of THF, 250 μL of water, and 800 μL of 0.5 M LiOH in water and the resulting mixture was shaken at room-temperature overnight (for some of the libraries, methanol was used instead of THF). Amberlite acidic resin was added to the vial, and the vial was shaken for 1 h at room temperature. The solution was transferred to a tared vial and an aliquot removed for QC analysis. Products with less than 90% purity were

purified by HPLC. For compounds with greater than 90% purity, the solvent was removed and the vial weighed, and a determined volume of DMSO was added to the product to make a 60 mM solution, which was formatted into 96-well polypropylene microtiter plates. These solutions were further diluted as described in the Experimental Section for the Biacore assay. If these compounds showed ≤10 mM IC_{50} in the Biacore assay they were resynthesized as powders and evaluated in the NMR and cell-based flow assay. Procedures for the resynthesis of hits (i.e. compounds from Table 1) are given below.

1,3,4-Trihydroxy-6-oxa-bicyclo[3.2.1]octan-7-one (4). In a 500 mL round-bottom flask fitted with a stirring bar, reflux condensor, Dean−Stark trap, and argon inlet, 20 g of quinic acid (104 mmol) was placed and 20 mL of dry DMF was added via syringe and the slurry stirred at room temperature. Next, benzene (200 mL, added via cannula) and *p*-toluenesulfonic acid (2.0 g, 10.4 mmol) were added, and the slurry was heated to reflux for 25 h. TLC after 25 h showed completion of reaction. A 1:1 mixture of EtOAc and heptane (100 mL) was added to the cooled reaction mixture. The mixture stirred for 1 h at room temperature and filtered. The collected solid was again stirred with a 1:1 mixture of EtOAc and heptane (100 mL) for 1 h at room temperature and filtered. Tituration was repeated one more time with a 1:1 mixture of EtOAc and heptane (100 mL) and the precipitate collected to give 16.9 g of intermediate **4** (93% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.89 (t, J = 11.7 Hz, 1H), 2.01−2.08 (m, 1H), 2.21−2.27 (m, 1H), 2.49 (d, J = 11.3 Hz, 1H), 3.72 (ddd, J = 11.3, 6.8, 4.5 Hz, 1H), 4.00 (t, J = 4.6 Hz, 1H), 4.72 (t, J = 5.4 Hz, 1H).

8-Hydroxy-3,5,10-trioxa-tricyclo[6.2.1.0^{2,6}]undecane-4,9-dione (5). To intermediate **4** (15.0 g, 86.2 mmol) in 42.5 mL of dry pyridine (kept over KOH) at 0 °C was added triphosgene (10.2 g, 34.5 mmol) portionwise over about 5 min. The ice bath was removed and the reaction stirred at room temperature for 1 h. TLC showed some unreacted starting material. Another 1 g of triphosgene was added and the reaction stirred for another 1 h. TLC showed completion of reaction. Pyridine was removed on the high vaccum pump at room temperature, and then the thick syrup was poured into water. The aqueous layer was repeatedly extracted with EtOAc until TLC showed no leftover product in the aqueous layer. The organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The resulting solid was extracted with hot methanol and filtered while hot. The filtrate was evaporated under reduced pressure to give the pure product (13.1 g, 76% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 2.16 (dd, J = 14.9, 3.8 Hz, 1H), 2.34 (d, J = 12.6 Hz, 1H), 2.50−2.64 (m, 1H), 4.90−5.00 (m, 2H), 5.16 (dt, J = 11.6, 3.8 Hz, 1H).

8-(3-Bromobenzyloxy)-3,5,10-trioxa-tricyclo[6.2.1.0^{2,6}]-undecane-4,9-dione (8c). To intermediate **5** (3.0 g, 15.0 mmol) in 16 mL of dry DMF at 0 °C (ice/NaCl) was added NaH (900 mg, 22.5 mmol, 60% dispersion in oil). The reaction was stirred at 0 °C for 30 min. Next the 4-bromobenzyl bromide (7.5 g, 30 mmol) was added and the reaction left in the ice bath for 30 min. The ice bath was then removed and the reaction stirred at room temperature for 1 h. TLC showed no starting material, and the reaction was worked up by quenching with saturated aqueous ammonium chloride and extracting into EtOAc. Column chromatography using silica gel and eluting with hexanes/EtOAc gave pure product in 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$), δ 2.06 (m, 1H), 2.22 (dd, J = 15 Hz, 3.6 Hz, 1H), 2.57−2.64 (m, 2H), 4.51(dd, J = 3.4, 9.9 Hz, 2H), 4.84 (m, 2H), 5.03 (dt, J = 7.8, 3.6 Hz, 1H), 7.12 (d, J = 7.54 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H). MS(electrospray), 369 $(M + H)^+$.

(3*R*,5*R*)-3,4,5-Trihydroxy-1-(2′-trifluoromethyl-biphenyl-3-ylmethoxy)-cyclohexanecarboxylic Acid (2). Intermediate **8c** (369 mg, 1 mmol) was dissolved in methanol (10 mL), followed by addition of LiOH solution (1 M, 2 mL, 2 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 2 h. The solution was then concentrated. To the residue was added *o*-trifluoromethylphenylboronic acid (209 mg, 1.1 mmol). $NaHCO_3$ (3.3 mmol), $Pd(PPh_3)_4$ (50 mg), CH_3CN (30 mL), and water (10 mL). The reaction system was evacuated and then filled with nitrogen. This was repeated at least three times. Then the resulting mixture was refluxed overnight under nitrogen. After cooling to room temperature, the reaction was quenched with aqueous NH_4Cl solution and extracted with EtOAc. After the organic layer was concentrated, the residue was dissolved in methanol (40 mL), followed by addition of LiOH (1 M, 4 mL). The resulting mixture was stirred at room-temperature overnite. Amberlite acidic resin was added to neutralize the reaction mixture to a pH around 5. After filtration, the solution was concentrated and the residue was purified by reverse phase HPLC to give compound **2** (300 mg, 70%). ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.93 (m, 3 H), 2.09 (d, J = 13.1 Hz, 1 H), 3.34−3.42 (m, 1 H), 3.75−3.84 (m. 1 H), 3.86−3.94 (m, 1 H), 4.27 (d, J = 10.9 Hz, 1 H), 4.54 (d, J = 11.1 Hz, 1 H), 7.23 (d, J = 7.6 Hz, 2 H), 7.35 (t, J = 8.7 Hz, 3 H), 7.57 (t, J = 7.6 Hz, 1 H), 7.67 (t, J = 7.58 Hz, 1 H), 7.79 (d, J = 8.1 Hz, 1 H). Anal. ($C_{21}H_{21}F_3NaO_6.CH_3OH$) C, H. HRMS calcd. for $C_{21}H_{21}F_3O_6$ $(M + H)^+$ 427.1363, found: 427.1351.

3,4,5-Trihydroxy-1-(4′-trifluoromethoxy-biphenyl-3-ylmethoxy)-cyclohexanecarboxylic Acid (22). This compound was prepared using the same procedure as for compound **2**. ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.97 (m, 2 H), 2.01 (m, 1 H), 2.03 (m, 1 H), 3.43 (dd, J = 6.4, 3.16 Hz, 1 H), 3.78−3.88 (m, 1 H), 3.91−4.02 (m, 1 H) 7.38 (d, J = 7.6 Hz, 1 H), 7.48 (t, J = 7.8 Hz, 3 H), 7.59 (d, J = 7.6 Hz, 1 H), 7.64 (s, 1 H), 7.78 (d, J = 8.6 Hz, 2 H). Anal. ($C_{21}H_{21}F_3O_7 \cdot H_2O$) C, H. HRMS calcd. for $C_{21}H_{21}F_3O_7$ $(M + H)^+$ 443.1312, found: 443.1325.

1-(3-Benzofuran-2-yl-benzyloxy)-(3*R*,5*R*)-3,4,5-trihydroxy-cyclohexanecarboxylic Acid (23). This compound was prepared using the same procedure as for compound **2**. ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.85−2.03 (m, 3 H), 2.05−2.15 (m, 1 H), 3.38 (dd, J = 6.6, 3.0 Hz, 1 H), 3.75−3.84 (m, 1 H), 3.82−3.90 (m, 1 H), 4.31 (d, J = 11.1 Hz, 1 H), 4.57 (d, J = 11.12 Hz, 1 H), 7.24 (t, J = 7.45 Hz, 1 H), 7.27−7.36 (m, 3 H) 7.45 (t, J = 7.71 Hz, 1 H), 7.59 (d, J = 8.1 Hz, 1 H), 7.64 (d, J = 7.6 Hz, 1 H), 7.79 (d, J = 7.8 Hz, 1 H), 7.83 (s, 1 H). Anal. ($C_{22}H_{22}O_7.CH_3OH$) C, H. HRMS calcd. for $C_{22}H_{22}O_7$ $(M + H)^+$ 399.1439, found: 399.1447.

1-(3′,5′-Bis-trifluoromethyl-biphenyl-4-ylmethoxy)-(3*R*,5*R*)-3,4,5-trihydroxy-cyclohexanecarboxylic Acid (24). This compound was prepared using the same procedure as for compound **2**. ^{1}H NMR (400 MHz, DMSO-d_6d_6+D_2O) δ ppm 1.95 (dd, J = 13.0, 7.45 Hz, 2 H), 1.99−2.08 (m, 1 H), 2.10−2.20 (m, 1 H), 3.44 (dd, J = 6.3, 3.0 Hz, 1 H), 3.81−3.88 (m, 1 H), 3.94−4.02 (m, 1 H), 4.35 (d, J = 11.1 Hz, 1 H), 4.61 (d, J = 11.4 Hz, 1 H), 7.52 (d, J = 8.3 Hz, 2 H), 7.81 (d, J = 8.3 Hz, 2 H), 8.08 (s, 1 H), 8.30 (s, 2 H). Anal. ($C_{22}H_{20}F_6O_6 \cdot H_2O$) C, H. HRMS calcd. for $C_{22}H_{20}F_6O_6$ $(M + H)^+$ 495.1237, found: 495.125.

(3*R*,5*R*)-3,4,5-Trihydroxy-1-(3-naphthalen-2-yl-benzyloxy)-cyclohexanecarboxylic Acid (25). This compound was prepared using the same procedure as for compound **2**. ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.91−2.08 (m, 3 H), 2.16 (dd, J = 13.1, 3.54 Hz, 1 H), 3.43 (dd, J = 6.3, 3.0 Hz, 1 H), 3.81−3.88 (m, 1 H), 3.94−4.02 (m, 1 H), 4.39 (d, J = 11.1 Hz, 1 H), 4.64 (d, J = 10.9 Hz, 1 H), 7.38 (d, J = 7.6 Hz, 1 H), 7.48−7.60 (m, 3 H), 7.74 (d, J = 7.8 Hz, 1 H), 7.76−7.88 (m, 2 H), 7.96 (d, J = 7.3 Hz, 1 H), 7.99−8.08 (m, 2 H), 8.17−8.26 (m, 1 H). HPLC (Method 1: 100%, Method 2: 100%); HRMS calcd for $C_{24}H_{24}O_6$ $(M + H^+)$ 409.1646, found: 409.1649.

1-(4′-Benzyloxy-biphenyl-3-ylmethoxy)−(3*R*,5*R*)-3,4,5-trihydroxy-cyclohexanecarboxylic Acid (26). This compound was prepared using the same procedure as for compound **2**. ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.83−2.03 (m, 3 H), 2.04−2.16 (m, 1 H), 3.37 (dd, J = 6.1, 2.8 Hz, 1 H), 3.72−3.79 (m, 1 H), 3.87−3.96 (m, 1 H), 4.28 (d, J = 11.1 Hz, 1 H), 4.53 (d, J = 11.1 Hz, 1 H), 5.11 (s, 2 H), 7.07 (d, J = 8.6 Hz, 2 H), 7.23 (d, J = 7.3 Hz, 1 H), 7.39 (m, 6 H), 7.50−7.56 (m, 4 H). Anal. ($C_{27}H_{28}O_7$) C, H. HRMS calcd. for $C_{27}H_{28}O_7$ (M + H465.1908, found: 465.1901.

(3*R*,5*R*)-3,4,5-Trihydroxy-1-(2-thianthren-1-yl-benzyloxy)-cyclohexanecaroxylic Acid (27). This compound was prepared using the same procedure as for compound **2**. ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.72−1.94 (m, 4 H), 3.31−3.40 (m, 1 H), 3.67−3.76 (m, 1 H), 3.76−3.86 (m, 1 H), 3.93 (d, J = 12.0 Hz, 1 H), 4.35 (d, J = 11.4 Hz, 1 H), 7.13 (d, J = 7.6 Hz, 1 H), 7.20 (d, J = 7.6 Hz, 1 H), 7.28−7.45 (m, 5 H), 7.49 (t, J = 7.6 Hz, 1 H), 7.57−7.70 (m, 3 H). Anal. ($C_{26}H_{23}NaO_6S_2$) C, H. HRMS calcd. for $C_{26}H_{24}O_6S_2$ $(M + H)^+$ 514.1352, found: 514.1360.

8-(2′-Amino-biphenyl-4-ylmethoxy)-3,5,10-trioxa-tricyclo[6.2.1.02,6]undecane-4,9-dione (13). Intermediate **5** (3.00 g, 14.9 mmol) was dissolved in dry DMF under argon at 0 °C. To the cold solution was added NaH (900 mg, 22.4 mmol), and the reaction was allowed to stir for 30 min. To the mixture was added 3′-bromomethyl-2-nitro-biphenyl[22] (4.37 g, 14.9 mmol) dissolved in dry DMF, via syringe under argon. The reaction was allowed to reach room-temperature overnight. The mixture was poured into a cold solution of saturated aqueous ammonium chloride (200 mL) and extracted with EtOAc (2 × 200 mL). The combined organic phases were dried over sodium sulfate and filtered. The organics were concentrated to an oil and titurated in hexanes to yield 2.63 g of a yellow powder. The yellow solid (2.63 g, 6.4 mmol) was dissolved in EtOH/DMF (1:1). To the solution was added $SnCl_2 \cdot 2H_2O$ (8.65 g, 38.3 mmol), and the reaction was allowed to stir at room-temperature overnight. Solid $NaHCO_3$ was added until basic and the mixture stirred for 60 min. The mixture was poured into water and extracted with EtOAc (2 × 200 mL). The combined extracts were dried over sodium sulfate, filtered, and concentrated under vacuum to provide an oil. The oil was purified by column chromatography (5:1 hexane/EtOAc) to give intermediate **13** as a yellow powder (3.1 g, 55% yield). ^{1}H NMR (300 MHz, DMSO) δ ppm 2.11 (dd, J = 14.8, 3.8 Hz, 1H), 2.42 (d, J = 12.5 Hz, 1H), 2.35−2.52 (m, 2 H), 4.29 (d, J = 10.9 Hz, 1 H), 4.58 (d, J = 10.8 Hz, 1 H), 4.95−5.1 (m, 2 H), 5.15−5.26 (m, 1 H), 6.90−7.02 (m, 2 H), 7.12−7.25 (m, 2 H), 7.37−7.44 (m, 4 H). MS(electrospray), 382 $(M + H)^+$.

(3*R*,5*R*)-3,4,5-Trihydroxy-1-{2′-[(5-methyl-2-phenyl-2*H*-[1,2,3]triazole-4-carbonyl)-amino]- biphenyl-4-ylmethoxy}-cyclohexanecarboxylic Acid (28). To intermediate **13** (760 mg, 2 mmol) in 25 mL of CH_3CN was added 1.4 mL of Hunig's base followed by 5-methyl-2-phenyl-2*H*-[1,2,3]triazole-4-carbonyl chloride (556 mg, 2.5 mmol). The reaction was heated at 60 °C for 48 h. The volatiles were removed, and the residue was dissolved in methylene chloride and water. The water layer was extracted twice with methylene chloride. The combined organics were dried with $MgSO_4$ and concentrated under vacuum. The residues were redissolved in 20 mL of THF, followed by addition of LiOH (8 mL of 1 M solution in water). The resulting solution was stirred at room-temperature overnight. Amberlite acidic resin was added to neutralize the solution to pH around 5. After filtration, the solution was concentrated and the residue was purified by reverse phase HPLC to give compound **28** (650 mg, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.69−1.83 (m, 2 H), 1.85−1.93 (m, 1 H), 2.11 (d, J = 11.6 Hz, 1 H), 2.46 (s, 3H), 3.39−3.49 (m, 1 H), 3.58−3.76 (m, 2 H, 4.43 (s, 2 H), 7.29 (t, J = 7.3 Hz, 1 H), 7.32−7.45 (m, 7 H), 7.59 (t, J = 8.0 Hz, 2 H), 7.80 (d, J = 7.8 Hz, 2 H), 8.03 (d, J = 7.8 Hz, 1 H). HPLC (Method 1: 99%, Method 2: 99%); HRMS calcd for $C_{30}H_{30}N_4O_7$ $(M + H^+)$ 559.2187, found: 559.2193.

8-(4′-Bromo-biphenyl-4-ylmethoxy)-3,5,10-trioxa-tricyclo[6.2.1.0.^{2,6}]undecane-4,9-dione (16). To 4′-bromo-biphenyl-3-carbaldehyde[23] (2.6 g, 10.0 mmol) in 30 mL THF was added 14 mL of a 1 molar solution of borane in THF (14.0 mmol). The reaction was stirred overnight and then quenched with water and extracted with EtOAc (3×). After removal of the solvent, the crude reaction mixture was dissolved in 15 mL of CH_2Cl_2 and to this was added a suspension of triphenylphosphine dibromide (4.6 g, 11 mmol) in 20 mL of CH_2Cl_2. The reaction was stirred for 4 h and then washed with water. The organic layer was dried with magnesium sulfate and the solvent evaporated. The crude mixture was purified by column chromatography using hexane/EtOAc to give intermediate **15**. To intermediate **5** (3.0 g, 15.0 mmol) in 16 mL of dry DMF at 0 °C (ice/NaCl) was added NaH (900 mg, 22.5 mmol, 60% dispersion in oil). The reaction was stirred at 0 °C for 30 min. Next, intermediate **15** (9.8 g, 30.0 mmol) was added and the reaction left in the ice bath for 30 min. The ice bath was then removed and the reaction stirred at room temperature for 1 h. The reaction was worked up by quenching with saturated aqueous ammonium chloride and extracting into EtOAc. Column chromatography using hexane/EtOAc gave pure product **16** in 70% yield. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 2.29 (d, J = 13.5 Hz, 1H), 2.38 (dd, J = 17.0, 3.1 Hz, 1H), 2.69−2.88 (m, 2H), 4.68 (dd, J = 2.5, 11.0 Hz, 2H), 4.86(d, J = 8.1 Hz, 1H), 4.96 (m, 1H), 5.04−5.14 (m, 1H), 7.18−7.62 (m, 8H). MS(electrospray), 445 $(M + H)^+$.

(3*R*,5*R*)-3,4,5-Trihydroxy-1-(4′-propenyl-biphenyl-4-yl-methoxy)cyclohexanecarboxylic Acid (29). Under argon, $ZnCl_2$ solution (0.5 M in THF, 0.5 mL) was added to propenylmagnesium bromide solution (0.5 M in THF, 0.5 mL) at room temperature. After 5 min, this mixture was transferred to a solution of intermediate **16** (50 mg, 0.1 mmol) and $Pd(PPh_3)_4$ (12 mg, 0.01 mmol) in THF (2 mL) under argon. The resulting mixture was refluxed for 3 h. The reaction was quenched with aqueous NH_4Cl solution and extracted with EtOAc (2 mL × 2). After concentration of the organic layer, the residue was dissolved in methanol (2 mL), followed by addition of LiOH (1 M in water, 0.6 mL). The resulting solution was stirred at r.t. overnight. Amberlite acidic resin was added to neutralize the solution to a pH around 5. After filtration, the solution was concentrated and the residue was purified by reverse phase HPLC to give **29** (25.4 mg, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.88 (d, J = 6.3 Hz, 1 H), 1.92 (dd, J = 7.2, 1.64 Hz, 3 H), 1.94−2.08 (m, 2 H), 2.10−2.18 (m, 1H), 3.43 (dd, J = 6.4, 3.2 Hz, 1 H), 3.80−3.88 (m, 1 H), 3.93−4.00 (m, 1 H), 4.35 (d, J = 10.9 Hz, 1 H), 4.60 (d, J = 11.1 Hz, 1 H), 5.79−5.92 (m, 1 H), 6.43−6.52 (m, 1 H), 7.30−7.37 (m, 1 H), 7.42−7.47 (m, 2 H), 7.48 (d, J = 7.3 Hz, 1 H), 7.55−7.71 (m, 4 H). Anal. ($C_{23}H_{26}O_6 \cdot H_2O$) C, H. HRMS calcd for $C_{23}H_{26}O_6$ $(M + H^+)$ 399.1802, found: 399.1799.

3-Amino-5-[(1,3,4,5-tetraacetoxy-cyclohexanecarbonyl)-amino]-benzoic Acid Methyl Ester (21). To a stirred solution of 5.00 g (26.0 mmol) quinic acid in 50 mL anhydrous pyridine, cooled in an ice bath under argon, was added dropwise 50 mL acetic anhydride. At the end of the addition the reaction was allowed to warm slowly to room temperature. After 24 h the solvents were removed under vacuum at <35 °C and the residue was azeotroped twice with toluene−CH_3CN, then dried under high vacuum to a yellow foam, which was used directly in the next step. MS(electrospray), 359 $(M - H)^-$.

The crude, peracetylated quinic acid **19** (26 mmol) and 3.5 g (26.0 mmol) 1-hydroxybenzotriazole were dissolved in a mixture of 120 mL of dry CH_2Cl_2 and 120 mL of dry THF, and the solution was cooled under argon in an ice bath. After addition of 5.4 g (28.8 mmol) of dicyclohexylcarbodiimide, the reaction was stirred at 0−4 °C for 1 h, then 2 h at room temperature. The solid dicyclohexylurea was removed by filtration and washed with CH_2Cl_2, and the combined filtrate and washings were evaporated under reduced pressure. The residue was taken up in 100 mL of CH_2Cl_2, and some residual dicyclohexylurea was removed by filtration. After evaporation of the filtrate, the residue was dried under high vacuum to a pale yellow foam, which was used directly in the next step.

The crude activated ester **20** (26 mmol) was dissolved under argon in 110 mL of dry CH_2Cl_2, and the solution transferred via syringe into a flame dried 1 L, three-necked flask under argon. After addition of 17.0 g (78.0 mmol) of 3,5-diaminobenzoic acid methyl ester, the stirred suspension was cooled under argon in an ice−water bath for 20 min. A solution of 5.7 mL (40.6 mmol) of triethylamine in 80 mL of anhydrous DMF was then added from a dropping funnel over 0.5 h while stirring at 0−4 °C under argon. Care should be taken that the pH does not exceed ~10 during the addition. At the end of the addition,

the solution was stirred in ice−water for 1 h, then at room-temperature overnight.

The reaction was diluted with 800 mL CH_2Cl_2 and washed in sequence with 2 × 400 mL of H_2O, 2 × 500 mL of 0.1 N HCl, 500 mL of diluted (1:10) brine, and 500 mL of semisaturated brine. The organic phase was dried (Na_2SO_4) and the solvent evaporated under vacuum. The residue was dissolved in 60 mL of CH_2Cl_2, and the solution was purified by flash chromatography on silica gel eluting with cyclohexane/EtOAc. Evaporation of the pertinent fractions afforded a residue which crystallized from hexanes/EtOAc to afford 7.22 g of pure intermediate **21** as colorless crystals. A second crop (0.30 g) of pure material was obtained from the mother liquors. The total yield was 60% over the three steps. ^{1}H NMR (300 MHz, DMSO-d_6+D_2O) δ ppm 1.88−1.99 (m, 10 H), 2.04 (s, 3 H), 2.11 (s, 3 H), 3.76 (s, 3 H), 5.04 (dd, J = 10.0, 3.7 Hz, 1 H), 5.22−5.37 (m, 1 H, 5.37−5.47 (m, 1 H), 6.89 (s, 1 H), 7.11 (s, 1 H), 7.30 (s, 1 H), 9.70 (s, 1 H). MS(electrospray), 509 $(M + H)^+$.

3-[(3-Chloro-1-benzothien-2-ylcarbonyl)amino]-5-({[3*R*,5*R*)-1,3,4,5-tetrahydroxylcyclohexyl]carbonyl}-amino)benzoic Acid (30). To a solution of 509 mg (1.0 mmol) of intermediate **21** in 6 mL dry CH_3CN was added 0.35 mL (2.0 mmol) of diisopropylethylamine, dropwise via syringe, while stirring at 0 °C under argon, followed by a solution of 246 mg (1.3 mmol) benzo[*b*]thiophene-2-carbonyl chloride in 4 mL of anhydrous CH_3CN, over 5 min. The reaction was stirred 15 min in the ice−water bath, then 7 h at room temperature, after which point no starting material was present. The solution was concentrated under vacuum to a small volume, diluted with 150 mL of EtOAc, and washed in sequence with water (50 mL), ice cold 0.5 N HCl (50 mL), ice-cold semisaturated $NaHCO_3$ (50 mL), and semisaturated brine (2 × 50 mL). All the aqueous washings were extracted with EtOAc (150 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography over silica gel, eluting with cyclohexane/EtOAc. Evaporation of the appropriate fractions gave 582 mg of a pale tan solid. To a suspension of this solid in 15 mL of CH_3CN was added a solution of $LiOH·H_2O$ (177 mg, 4.20 mmol) in 10 mL water. After stirring 8 h at room temperature, a second portion (60 mg, 1.4 mmol) of $LiOH·H_2O$ was added. One hour later, the conversion was complete. Stirring with 7 g of Amberlite acidic resin for one minute quenched the reaction. After removal by filtration, the resin was washed with 1:1 CH_3CN /H_2O. The combined filtrate and washings were evaporated under vacuum, and the residue was re-evaporated several times from CH_3CN to afford, after drying under vacuum over P_2O_5, 390 mg (75%) of compound **30**. ^{1}H NMR (400 MHz, DMSO-d_6+D_2O) δ ppm 1.80 (dd, J = 12.8, 11.0 Hz, 1 H), 1.85−1.95 (m, 3 H), 3.26 (dd, J = 8.8, 2.8 Hz, 1 H), 3.75−3.80 (m, 1 H), 3.97−4.03 (m, 1 H), 7.57−7.63 (m, 2 H), 7.91−7.98 (m, 3 H), 8.05−8.13 (m, 1 H), 8.26 (t, J = 1.8 Hz, 1 H). Anal. ($C_{23}H_{21}ClN_2O_8S·H_2O$) C, H. HRMS calcd for $C_{23}H_{21}ClN_2O_8S$ $(M + H^+)$ 521.078, found: 521.0781.

3-[(1-Benzothien-2-ylcarbonyl)amino]-5-({[3*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexyl]carbonyl}amino)-benzoic Acid (31). This compound was prepared using the same procedure as for compound **30**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.84 (dd, J = 13.0, 10.5 Hz, 1 H), 1.89−1.98 (m, 3 H), 3.28 (dd, J = 8.6, 2.8 Hz, 1 H), 3.75−3.93 (m, 1 H), 4.00−4.08 (m, 1 H), 4.78 (s, 2 H), 5.33 (s, 2 H),5.76 (s, 1 H), 7.45−7.55 (m, 2 H), 7.99−8.10 (m, 3 H), 8.13 (t, J = 1.8 Hz, 1 H), 8.43 (s, 1 H), 8.49 (t, J = 2.0 Hz, 1 H), 10.7 (s, 1 H). Anal. ($C_{23}H_{22}N_2O_8S·2H_2O$) C, H.

Transferred NOE (trNOE) Experiment. The NMR samples were prepared in D_2O buffer, with 20 mM Imidazole, pH = 7.4, 150 mM NaCl, 5 mM $CaCl_2$, 0.02% NaN_3, with 1 mM small molecule and 40 μM P-selectin-hIgG chimera protein. The 2D trNOE experiments were performed on a Varian Unity Plus 600 MHz instrument at 25 °C, with 170 ms mixing time. The data were processed with nmrPipe software.[19] Negative control trNOE experiments were also performed either without any added protein, or in the presence of IgG instead of P-selectin.FC, run under the above conditions.

Pharmacokinetics in Rats. Jugular vein cannulated Sprague Dawley male rats weighing between 195 and 215 g were used. Animals were fasted and housed at constant temperature-humidity environment at 12−12 h day-night cycles. Three rats were administered single 20 mg/kg iv bolus (10 mg/mL, 50% DMSO:50% PEG400). Blood was collected from each rat at 5 min, 0.5, 0.75, 1, 2, 4, 7, 24 h after dosing. Equal volume of saline was given to replace the lost blood. Plasma levels of test compounds were assayed via LC/MS method. In this method an aliquot of plasma was treated (with 2× of CH_3CN to precipitate plasma protein) after centrifugation, the supernatant was then further diluted with format buffer (50 mM) and inject onto an LC/MS. A linear trapezoid noncompartmental analysis was used in pharmacokinetic analysis.

Biology. Biacore P-Selectin/PSGL-1 Inhibition Assay. All surface plasmon resonance (Biacore) assays were performed at 25 °C on a Biacore 3000 instrument (Biacore Inc. Piscataway, NJ). 19ek, a purified, monomeric, truncated form of human PSGL-1 that contains all the necessary P-selectin binding determinants, was biotinylated via amine chemistry (Sulfo-NHS-LC−Biotin, Pierce) at a unique C-terminal lysine residue and immobilized on a Biacore streptavidin-coated SA sensor chip.[11] Small molecule P-selectin inhibitors were incubated for 1 h in 100 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.05% P40, 10% DMSO. Solutions were filtered to remove any possible precipitate. A soluble recombinant truncated form of human P-selectin comprised of the lectin and EGF domains (P-LE), expressed in CHO cells, was delivered to the 19ek coated sensor chip at 30 μL/minute in the presence and absence of small molecule inhibitors. The final P-LE solution contained 500 nM P.LE, 100 mM HEPES, 150 mM NaCl, 1mM $CaCl_2$, 1 mM $MgCl_2$, 0.05% P40, 10% DMSO and compound from 0 to 6 mM. Sample injections were run in duplicate, and each compound was assayed at least twice. Percent inhibition of binding was calculated by dividing the inhibited signal, measured in resonance units (RU), by the uninhibited signal, subtracting this value from one, then multiplying by one hundred. Inhibitors, with greater than 50% inhibition at 6mM, were assayed again using a series of 2-fold dilutions. The data from this titration were plotted RU vs concentration, and the IC_{50} concentration was determined by extrapolation from the plot. All RU values were blank and reference subtracted prior to percent inhibition and IC_{50}.

In Vitro Leukocyte Rolling Assay. A parallel plate flow chamber (GlycoTech, Rockville, MD) was used to measure neutrophil/endothelial cell adhesion events under flow as previously described,[24] with changes in protocol noted as follows. Confluent monolayers of HUVECs (human umbilical cord endothelial cells) were activated with IL-4 to up-regulate P-selectin expression on the cell surface just prior to the experiment. Freshly isolated human neutrophils were incubated for 20 min with or without compound in 4 mL of serum-free cell media containing histamine (to further activate the HUVEC once introduced into the flow chamber). This solution was flowed over the HUVEC monolayer to generate a shear stress of at approximately 1 dyn/cm^2. For each test article, data was collected by digital imaging and videotape on three different monolayers. The number of rolling cells was an average value from 10 fields in each of the three monolayers. Active compounds were titrated to determine an IC_{50} concentration. Glycyrrhizin trisodium salt (TCI) was used as a positive control, inhibiting 50% of rolling flux at 1 mM.

Intravital Microscopy in Mouse Mesentery Venules. C57/black mice were placed briefly under a heat lamp to dilate blood vessels and then injected with small molecule in 8% PEG400, 0.2% Tween80/water (w/w) pH 6−8 or vehicle by intravenous tail vein injection. The mice were then anesthetized using avertin, and the mesentery was exteriorized through a midline abdominal incision. Venules were visualized with a Zeiss Axiovert 135-inverted microscope (32x, 0.4NA; Zeiss, Oberkochen, Germany). Messenteric tissues were bathed periodically in PBS warmed to 37 °C, during the experiment. Fifteen minutes after the mesentery was exteriorized, vessels

measuring 100−200 μm were identified and leukocyte rolling was video-recorded in the selected vessels. The number of rolling cells/minute was determined by counting the number of cells passing a given plane perpendicular to the length of the vessel. This number was measured in five vessels per mouse and averaged. Blood samples were collected for analysis of plasma levels of the small molecules just before the animals were euthanized with 100% CO_2 gas.

Thioglycollate-Induced Peritonitis. Female Balb/c E-selectin -/- mice were injected intravenously, by tail vein injection with small molecules in 8% PEG400/ water (w/w) pH 4−9, vehicle or antibody. Ten minutes after injection of test article, 1 mL of 3% thioglycollate (Sigma) solution was injected into the peritoneal cavity. Two hours later the animals were deeply anesthetized with CO_2 gas, and peritoneal lavage was performed by injecting 5 mL of complete RPMI containing 10 units mL^{-1} heparin into the peritoneal cavity, massaging the abdominal wall, and removing the injected liquid. Total cell counts were calculated in the lavage fluid using a Baker Hematology Analyzer. Cytospins were prepared and stained with Diff-Quick, to perform differential counts. Just before the animals were euthanized with 100% CO_2 gas, blood samples were collected for the analysis of plasma levels of the test articles. A blocking anti-P-selectin antibody, RB40.34 (Pharmingen), was used as a positive control (2.5 mg/kg).

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Supporting Information Available: List of compounds prepared for Libraries A−H. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ebnet, K.; Vestweber, D. Molecular mechanisms that control leukocyte extravasation: the selectins and the chemokines. *Histochem. Cell Biol.* **1999**, *112*, 1−23.
(2) Sears, P.; Wong, C.-H. Carbohydrate Mimetics: A New Strategy for Tackling the Problem of Carbohydrate-Mediated Biological Recognition. *Angew. Chem., Int. Ed.* **1999**, *38*, 2300−2324.
(3) Ley, K. Integration of inflammatory signals by rolling neutrophils *Immunol. Rev.* **2002**, *186*, 8−18.
(4) Thoma, G.; Banteli, R.; Jahmke, W.; Magnani, J. L.; Patton, J. T. Preorganization of the Bioactive Conformation of Sialyl Lewis x Analogues Correlates with Their Affinity to E-Selectin. *Angew. Chem., Int. Ed.* **2001**, *40*, 3644−3647.
(5) For a summary on design and synthesis of selectin inhibitors see refs 5−9 and references therein. Kaila, N.; Thomas IV, B. E. Design and Synthesis of Sialyl Lewis x Mimics as E- and P−Selectin Inhibitors. *Med. Res. Rev.* **2002**, *22*, 566−601.
(6) Dey, P. M.; Witczak, Z. J. Funtionalized S− Thio-di- and S−Oligosaccharide Precursors as Templates for Novel Sle$^{x/a}$ Mimetic antimetastatic Agents. *Mini Rev. Med. Chem.* **2003**, *3*, 271−280.
(7) Kaila, N.; Thomas, B. E. Selectin Inhibitors *Expert Opin. Ther. Pat.* **2003**, *13*, 305−317.
(8) Chhabra, S. R.; Rahim, A. S. A.; Kellam, B. Recent Progress in the Design of Selectin Inhibitors *Mini Rev. Med. Chem.* **2003**, *3*, 679−687.
(9) Thoma, G.; Schwarzenbach, F. Simplified sialyl Lewis x analogue.s with improved E-selectin Inhibition. *Helv. Chim. Acta* **2003**, *86*, 855−864.
(10) Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. Selectin-Carbohydrate Interactions: From Natural Ligands to Designed Mimics. *Chem. Rev.* **1998**, *98*, 833−862 and references therein.
(11) 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 sLe^x and PSGL-1. *Cell* **2000**, *103*, 467−479.
(12) Graves, B. J.; Crowther R. L.; Chandram C.; Rumberger J. M.; Li S.; Huang K.-S.; Presky D. H.; Familletti P. C.; Wolitzky B. A. Burns D. K. Insight into e-selectin/ligand interaction from the crystal structure and mutagenesis of the lec/EGF domains. *Nature* **1994**, *367*, 532−538.
(13) Geng J. G.; Bevilacqua M. P.; Moore K. L.; McIntyre T. M.; Prescott S. M.; Kim J. M.; Bliss G. A.; Zimmerman G. A.; McEver R. P. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* **1990**, *343*, 757−760.
(14) Somers, W. Unpublished results towards P-selectin-quinic acid soaks.
(15) The IC_{50} for natural ligand sLex in the Biacore and ELISA assay is ∼15 and 8 mM, respectively.
(16) McMartin, C.; Bohacek, R. S.QXP: powerful, rapid computer algorithms for structure-based drug design *J. Comput.-Aided Mol. Des.* **1997**, *11*, 333−334.
(17) Hanessian, S., Pan, J., Carnell, A., Bouchard, H., Lesage, L., Total synthesis of (−) Reserpine using the chiron approach *J. Org. Chem.* **1997**, *62*, 465−473.
(18) It is well demonstrated in the literature that due to the nature of the selection biology this high IC_{50} translates to in vivo efficacy.[1−10]
(19) (a) Poppe, L.; Brown, G. S.; Philo, J. S.; Nikrad, P. V.; Shah, B. H. Conformation of sLe^x Tetrasaccharide, Free in Solution and Bound to E-, P- and L-Selectin. *J. Am. Chem. Soc.* **1997**, *119*, 1727−1736. (b) Ni, F. Recent developments in transferred NOE methods. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 517−606. (c) Meyer, B.; Thomas, W.; Peters, T. Screening mixtures for biological activity by NMR. *Eur J. Biochem.* **1997**, *246*, 705−709.
(20) Brunger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J.-S.; Kuszewski, J.; Nilges, N.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. Crystallography & NMR System: a new software suite for macromolecular structure determination *Acta Crystallogr.* **1998**, *D54*, 905−921.
(21) Tripos, Inc., St. Louis, MO.
(22) DeVita, R. J.; Bochis, R.; Frontier, A. J.; Kotliar, A.; Fisher, M. H.; Schoen, W. R.; Wyvratt, M. J.; Cheng, K.; Chan W.-S.; Butler, B.; Jacks, M. T.; Hickey, G. J.; Schleim, K. D.; Leung, K.; Chen, Z.; Chiu, S.-H.; Feeney, W. P.; Cunningham P. K.; Smith R. G. A Potent, Orally Bioavailable Benzazepinone Growth Hormone Secretagogue. *J. Med. Chem.* **1998**, *41*, 1716−1728.
(23) This compound was prepared by Suzuki coupling of 1-bromo-4-iodobenzene with 3-formylphenylboronic acid. Simoni, D.; Gianni, G.; Baraldi, P. G.; Romagnoli, R.; Roberti, M.; Rondanin, R.; Baruchello, R.; Grisolia, G.; Rossi, M.; Mirizzi, D.; Invidiata, F. P.; Grimaudo, S.; Tolomeo, M. A convenient synthesis of unsymmetrically substituted terphenyls of biologically active stilbenes via a double Suzuki cross-coupling protocol *Tetrahedron Lett.* **2003**, *44*, 3005−3008.
(24) Thoma, G.; Patton, J. T.; Magnain, J. L.; Ernst, B.; Ohrlein, R.; Duthaler, R. O. Versatile Functionalization of Polylysine: Synthesis, Characterization, and Use of Neoglycoconjugates. *J. Am. Chem. Soc.* **1999**, *121*, 5919−5929.
(25) DeLano, W. L. The PyMOL Molecular Graphics System 2002, DeLano Scientific, San Carlos, CA. (http://www.pymol.org.).

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