Expedited Articles

Rational Design and Synthesis of Small Molecule, Non-oligosaccharide Selectin Inhibitors: (α-D-Mannopyranosyloxy)biphenyl-Substituted Carboxylic Acids

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The calcium dependent E-selectin/sialyl Lewis^x (sLe^x) interaction plays a key role in inflammation where it mediates the rolling of leukocytes prior to firm adhesion and extravasation from the vasculature. A model of E-selectin/sLe^x binding, along with previously reported structure-activity relationships of sLe^x-related oligosaccharide, was used in the rational design of non-oligosaccharide inhibitors of this pivotal interaction. A palladium-mediated biaryl-coupling (Suzuki) reaction was used as the key step to prepare a number of substituted biphenyls which were assayed for their ability to inhibit the binding of E-, P-, and L-selectin–IgG fusion proteins to sLe^x expressed on the surface of HL60 cells. Some of the compounds developed had greater *in vitro* potency than the parent sLe^x tetrasaccharide and are currently being evaluated in *in vivo* models of inflammation to select a candidate for clinical development.

Introduction

Cell adhesion molecules play a crucial role in inflammatory conditions and several immune system disorders by recruiting leukocytes to the injured area within the body.[1–5] The selectins are one family of cell adhesion molecules comprised of three structurally related carbohydrate-binding proteins.[1] These proteins are expressed on the surface of vascular endothelial cells (E- and P-selectin), platelets (P-selectin), and leukocytes (L-selectin) and function in binding oligosaccharide ligands present on the surface of heterologous cells to promote intercellular adhesion. E-Selectin is thought to be expressed only on activated endothelial cells where transcription is induced by cytokines,[2] and the protein is present in significant quantities from around 4 h after induction until 24 h or more. Conversely, P-selectin is contained in storage granules and appears on the cell surface in the period of a few minutes until several hours after injury, while L-selectin is constitutively expressed on leukocytes. The binding of E-selectin to sialyl Lewis^x (sLe^x) and related epitopes on neutrophils, monocytes, a specific subset of T lymphocytes, eosinophils, and basophils[6] is believed to aid in the recruitment of these cells in response to inflammatory stimuli. Modulation of this recognition and binding process by inhibiting the selectins would potentially have clinical applications in the treatment of a number of immune system-mediated disease states including reperfusion injury, septic shock, rheumatoid arthritis, psoriasis, asthma, lupus, diabetes, cancer metastasis, ARDS, and inflammatory bowel disease.[7,8]

E-Selectin possesses an amino-terminal calcium dependent lectin domain that is essential for ligand binding and recognizes known carbohydrate antigens such as sLe^x [Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc] and sialyl Lewis^a (sLe^a) [Neu5Acα2–3Galβ1–3(Fucα1–4)GlcNAc] present on the surface of targeted cells. We have developed a model of E-selectin binding to the sLe^x tetrasaccharide (Figures 1 and 2)[9] using the NMR-determined bound conformation of sLe^x [10] together with the X-ray crystallographic structures of E-selectin[11,12] (ligand unbound) and a homologous C-type lectin, the rat mannose-binding protein, coordinated to its oligomannose ligand.[13] This hypothesis was supported by detailed site-directed mutagenesis data,[9] including the alteration of one E-selectin residue, Ala-77, to a lysine which altered binding specificity from sLe^x to oligomannose. Furthermore, the observation that mutation of Lys-113 to either Glu or Gln did not abrogate ligand binding argued against a previous hypothesis[11] that Lys-113 is involved in a charge-paired interaction with the sialyl carboxylate of sLe^x and further supported our model.[9]

In this paper, we describe the synthesis and *in vitro* activity of a novel class of non-oligosaccharide selectin inhibitors based upon our model of the interactions of E-selectin with its cognate ligand sLe^x. Studies have previously been reported that determined the relevant structural features of sLe^x necessary for binding to E- and P-selectin.[14–18] This binding has been shown to be dependent mostly on the presence of the sialic acid carboxylate and the fucosyl hydroxyl groups, while other functionality on the sLe^x motif did not appear as relevant. The critical spatial relationship between the sialic acid and fucose moieties of sLe^x was determined from an examination of our model, which hypothesized that the fucose 2- and 3-hydroxyl groups were coordinated to the calcium ion, while the sialyl carboxylate formed a salt bridge with the side chain of Arg-97 and incorporated the bound conformation of sLe^x determined

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by NMR studies.[10] Furthermore, our previous site-directed mutagenesis results indicating that an Ala-77–Lys mutant of E-selectin bound oligomannose structures rather than sLe^x indicated that the local environment of the calcium site of E-selectin maintained the fucose *or* mannose recognition of the related C-type lectin, rat mannose-binding protein. This information supported the design of a series of potential selectin inhibitors in which a mannosyl unit was designed as a calcium ligand, while a carboxylic acid was presented for coordination with Arg-97 *via* use of a substituted biphenyl spacer. It should be mentioned, however, that the design of most compounds reported here was achieved on the basis of a homology-built model of E-selectin lectin domain (based on rat mannose-binding protein) which maintained most of the key secondary structural elements subsequently confirmed by the X-ray crystal structure.[11] The homology model is not discussed in detail, since the publication of the X-ray coordinates permits a more rational discussion of the design aspects of this work using real biophysical data (the X-ray structure of E-selectin lectin and EGF domains). Positive confirmation that the selectin site could indeed recognize mannose in addition to fucose arose directly from the results of compounds reported here and was subsequently confirmed by molecular genetic studies.[9]

While there have been recent reports of synthetic sLe^x analogs where either the glucosamine moiety[19] or the lactosamine unit[20] had been replaced, these modifications had resulted in a reduction in relative binding affinity to the parent sLe^x tetrasaccharide. We believe this paper represents the first report of the rational design of non-oligosaccharide glycomimetic compounds based on a model of carbohydrate–protein interactions and provides a route to a novel class of anti-inflammatory agents of great therapeutic potential.

Modeling and Structural Design

Modeling work was conducted on either a high-performance stereoscopic CAChe WorkSystem using commercially available software for constructing and editing molecules or a Silicon Graphics Indigo-2 workstation using commercial Biosym software.

A model of E-selectin/sLe^x interactions was generated using the X-ray crystallographic coordinates of E-selectin[11] (Figures 1 and 2) and the recently reported E-selectin-bound solution conformation (NMR) of the sLe^x tetrasaccharide.[10] The sLe^x tetrasaccharide was constructed using the CAChe default MM2 force field incorporating the torsion angles of Cooke *et al.*[10] that were determined from a transfer nuclear Overhauser enhancement (tNOE) experiment for the three glycosidic linkages and approximated the previously modeled GESA-C conformer,[21] which differed from the reported solution conformation.[21–23] Introduction of the tetrasaccharide was achieved by coordinating the fucose residue to the calcium ion by analogy to the mannose/rat mannose-binding protein (rMBP) coordination.[13] Docking of the oligosaccharide was achieved by a series of superimpositions using the mannose/rat mannose-binding protein interactions as a template. Specifically, the calcium ion, the calcium-coordinated mannose residue (*via* C-3 and C-4 hydroxyl oxygens), and five calcium-coordinating oxygens from rMBP [Glu-185, Asn-187, Asn-205, and Asp-206 (two oxygens, one from the carboxylate along with the main chain carbonyl)] were superimposed on the equivalent calcium ion and ligands in E-selectin [Glu-80, Asn-82, Asn-105, and Asp-106 (two oxygens, one from the carboxylate along with the main chain carbonyl)]. The root-mean-square (rms) deviation for the five oxygen atoms and the calcium ion was 0.134 Å. The mannose residue was then used as a template for superimposing the fucose residue of sLe^x using the ring oxygen, C-2, and C-3 hydroxyls of fucose to superimpose the ring oxygen, C-4, and C-3 hydroxyls of mannose, respectively. The rms deviation for this superimposition was 0.060 Å. Finally the 2MSB (rMBP)-derived template was removed, and an energy minimization was carried out to relieve close steric contacts. As shown, the resulting model had the critical sialyl carboxylate coordinated in close proximity to the side chain of Arg-97, a residue that has been previously shown to be important for sLe^x recognition, since site-directed mutation to alanine abrogated carbohydrate recognition.[8,11] Other close contacts derived from the model included Gal-6-OH coordinated to Tyr-94; however, we elected not to consider these interactions during the initial stages of compound design in order to simplify the modeling process.

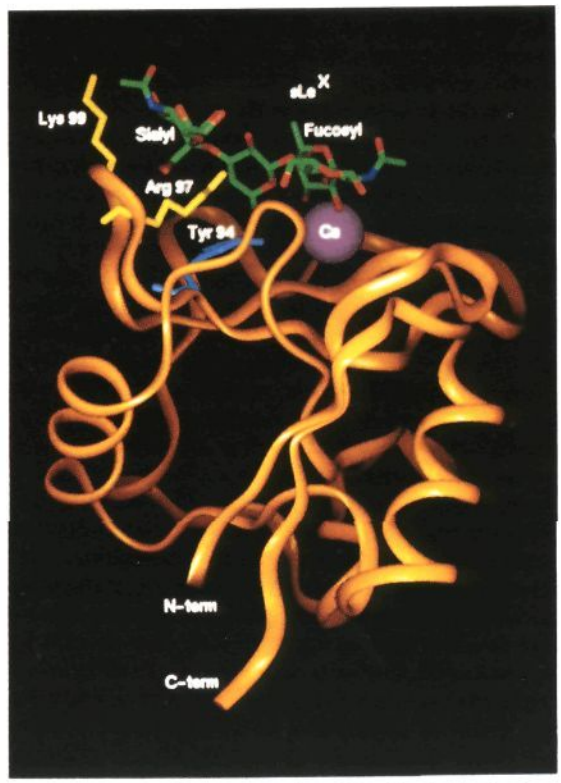


Figure 1. Model of the interaction of E-selectin lectin domain and sLe^x. The protein is illustrated in a backbone ribbon format for clarity, with several of the key residues (Tyr-94, Arg-97, and Lys-99) involved in interaction with sLe^x (multicolored, green = carbon, red = oxygen, blue = nitrogen) shown. Binding is calcium dependent (magenta space-filling sphere).

The design of the 2-substituted biphenyl template was achieved by first considering the two monosaccharide units that were predicted to recognize the E-selectin calcium site, fucose and mannose, along with a consideration of the known preferred orientation of the glycosidic linkage of an α-pyranose. The choice of a mannose-based compound over a fucose-based target was indicated by several important considerations.

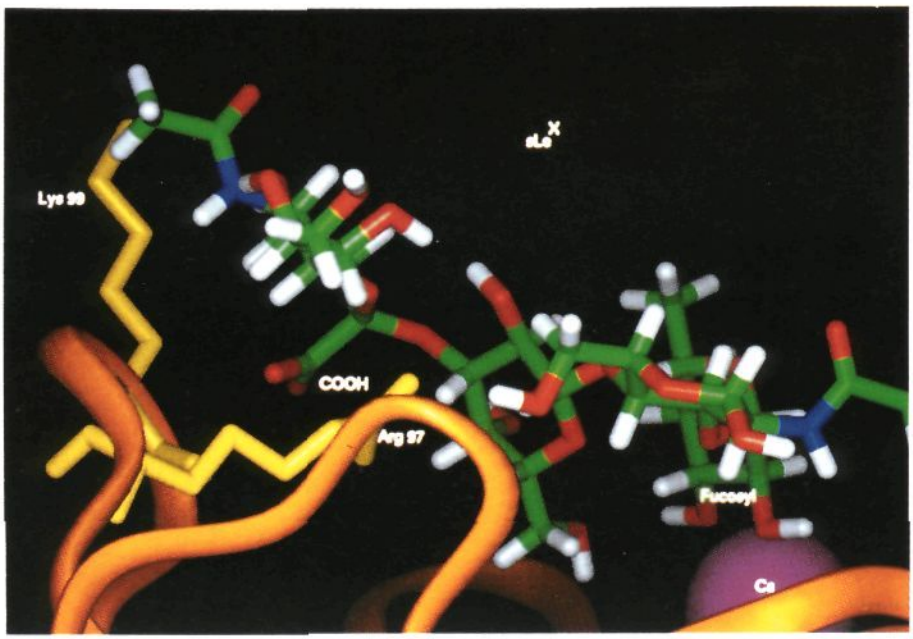


Figure 2. Detailed view of sLe^x/E-selectin lectin domain interactions (closer view of the interactions shown in Figure 1). The fucosyl residue is coordinated to the functional calcium ion *via* the 2- and 3-hydroxyl groups. The sialic acid carboxylate is in close proximity to the Arg-97 and Lys-99 side chains, while the 6-hydroxyl of galactose is close to Tyr-94.

Firstly, the distance from the C-1 position of mannose to the desired position of the carboxylate was shorter than the corresponding distance for fucose and provides an advantage for the design of smaller templates. This difference arises because the C-1 position of mannose corresponds to the C-5 position of fucose when coordinated to the E-selectin calcium ion. Secondly, mannose is a less expensive starting material than fucose, which may be important in the generation of cost effective therapeutics, and furthermore control of α-mannosylation is much more straightforward than control of α-fucosylation.[24]

Having considered these factors, we envisioned that a 2-(α-D-mannopyranosyloxy)biphenyl or a 3-[(α-D-mannopyranosyloxy)methyl]biphenyl, appended on the remote ring by a side chain which terminates in a carboxylic acid, would maintain the through-space geometry between these key residues in a manner similar to that observed in the model of protein-bound sLe^x (Figure 3). Thus, our synthetic efforts were directed toward the construction of a series of suitably substituted biphenyl systems.

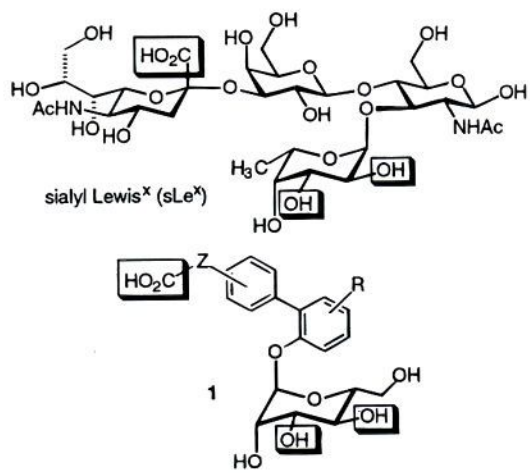


Figure 3. (Mannopyranosyloxy)biphenyl-based sLe^x mimetics. The carboxylic acid serves as a mimetic of the sialic acid group, while the 4-and 3-hydroxyls of mannose mimic the fucose 2- and 3-hydroxyls of sLe^x , respectively.

Synthetic Chemistry

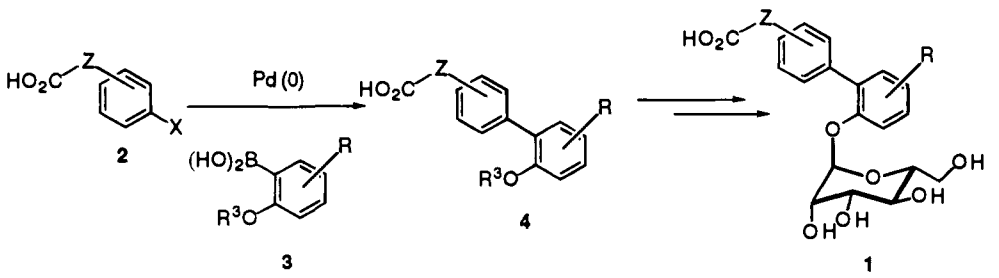
The general synthetic strategy for the preparation of the target compounds involved a Suzuki palladium-mediated coupling of suitably functionalized aromatic components[25] followed by further elaboration of the biphenyl core (Scheme 1).

The phenolic biphenyl **7** was constructed by coupling either 2-methoxybenzeneboronic acid (**3a**) or 2-hydroxybenzeneboronic acid (**3b**) with the appropriately substituted aryl bromide (or triflate, in the case of compound **9i**) as outlined (Scheme 2). Where the methyl ethers were used, demethylation was achieved by use of boron tribromide. Subsequent glycosylation of the phenols **7** produced intermediates **8**, which were deprotected to provide compounds **9a**–**w** (Table 1). The amino acid derivatives **12a**–**l** were synthesized from the bis-acetonides **10** (Scheme 3), available from either reprotection of tetraol **9** or conversion of the protected intermediates **8**. Condensation of **10** with the appropriate amino esters gave the amides **11**. Final deprotection of the hydroxyl groups and removal of the ester produced compounds **12a**–**k** (Table 2).

Earlier reports[26] that a sulfated Lewis^x analog of sLe^x possessed high-affinity binding to E-selectin prompted us to apply this finding to the present systems under study. Thus, the acetate groups of **8a** were removed, and the product was reprotected as the bis-acetonide **14**. Reduction of the ester, followed by conversion of the primary alcohol to the mesylate, gave intermediate **16**, which was treated with potassium thioacetate to give the *S*-acetate **17**. Oxidation of the sulfur using Oxone followed by deprotection gave the sulfonic acid **18** (Scheme 4).

To further investigate the role of acidic functionality

Scheme 1



Scheme 2

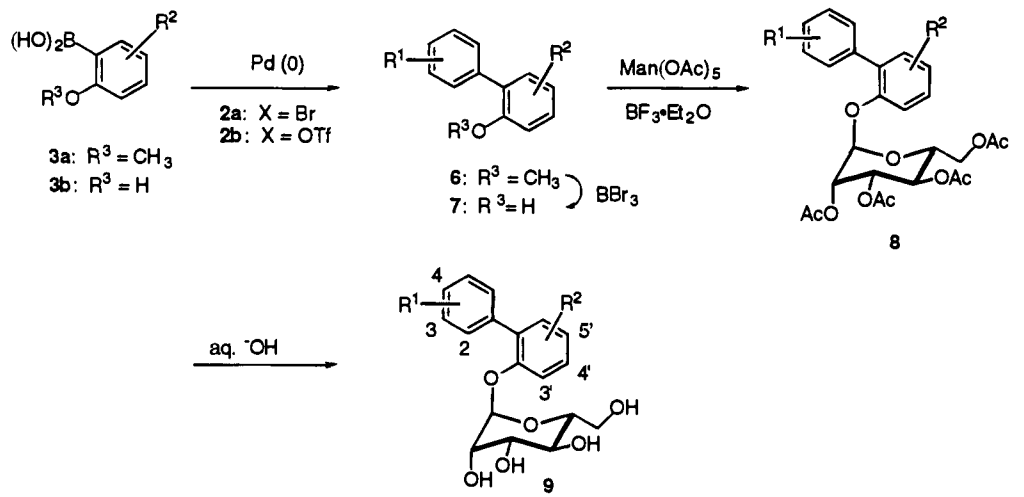


Table 1. *In Vitro* Activity Data for Substituted Biphenyls **9a–w**[a]

compd	R^1, R^2 =	IC_{50} (mM) or % inhib at (mM) E-selectin	P-selectin	L-selectin
sLe^x		3	2	2
9a	3-CH_2CO_2H	2	2.6	1
9b	3-CO_2Li	16	0 @ 27	15 @ 6
9c	4-CO_2H	14.6	0 @ 20	0 @ 7
9d	4-CH_2CO_2H	2.6	0 @ 20	0 @ 6
9e	3-OCH_2CO_2H	4.9	4.9	17
9f	4-OCH_2CO_2H	6.1	20	20 @ 6
9g	3-$CH_2OCH_2CO_2H$	9.5	0 @ 24	30 @ 6
9h	3-CH_2CN	7	40 @ 23	1.5
9i	3,5-$(CH_3)_2$, 4-OCH_2CO_2H	8	6.9	0 @ 7
9j	3-CO_2CH_3	nd	nd	nd
9k	H	nd	nd	nd
9l	3-OCH_2CO_2H, 5′-Me	14	na	na
9m	2-OCH_2CO_2H	20	20 @ 25	0 @ 8
9n	5-OCH_2CO_2H, 2-Me	20.2	12	na
9o	3-CH_2CN_4H	17 @ 40	na	na
9p	3-$NHC(O)CO_2H$	0 @ 6	na	na
9q	3-OCH_2CO_2H, 2-Me	0 @ 20	0 @ 20	0 @ 8
9r	3-$CH(CH_3)CO_2H$	0 @ 40	20	40 @ 6
9s	3-$CH(CH_3)CO_2H$	20	30 @ 10	0 @ 6
9t	3-$OCH(Ph)CO_2H$	70 @ 17	17	0 @ 6
9u	3-$NHCH_2CO_2H$	nd	nd	nd
9v	3-$CH_2CO_2CH_3$	nd	nd	nd
9w	4-$C(O)(CH_2)_2CO_2H$	40 @ 20	0 @ 20	0 @ 3

[a] nd = not determined; the compound causes cell lysis at high concentration and prevents a determination of its inhibitory activity in this assay. na = not assayed; this assay was not available when this compound was synthesized.

present, the phosphonate ester **21** was prepared following Scheme 5. The bis-acetonide **15** was oxidized to the aldehyde **19** using the Dess–Martin periodinane. Wadsworth–Emmons reaction of the aldehyde with the anion of tetraethylmethylene diphosphonate gave the α,β-unsaturated phosphonate **20** which was reduced and deprotected to give the half-ester **21**.

In Vitro Biological Evaluation

The assay format used consisted of evaluating HL60 cell (sLe^x expressing) binding to a recombinant fusion protein containing the human E-selectin lectin, EGF, and two complement repeats fused to a mouse IgG near the constant region and bound to Dynal magnetic beads as reported elsewhere.[9] To purify the recombinant proteins, goat anti-mouse antibody-coated Dynabeads (Dynal Inc.) were added to culture media harvested from transfected cells and incubated overnight with rocking at 4 °C. The beads were concentrated by centrifugation at 600*g* for 5 min, and the culture media were removed after the incubation tubes were placed on a magnetic separator. The beads were resuspended in 1 mL of PBS (4×10^6 beads/mL final concentration) and stored at either 0 or 4 °C. The amount of recombinant protein recovered on the beads was monitored by ELISA using BBA8, a biotinylated version of BBA1 (R & D Research) which recognizes E-selectin.

E-Selectin–HL60 cell-binding assays were performed in Falcon 96-well flexible assay plates. The wells were first incubated briefly with PBS supplemented with 3% BSA. After aspirating the buffer, 7 μL of the test compound of interest suspended in PBS, and 7 μL of HL60 cells (10^7 cells/mL suspended in RPMI 1640 with 10% FCS) that had been fluorescently labeled with Calcein AMC-3099 (Molecular Probes) were added to each well followed by 7 μL of beads (4×10^6 beads/mL). The cells, compound, and beads were incubated together at room temperature for 10 min. The plate was then placed on a magnetic separator and incubated for an additional 2 min. While the assay plate remained on

Scheme 3

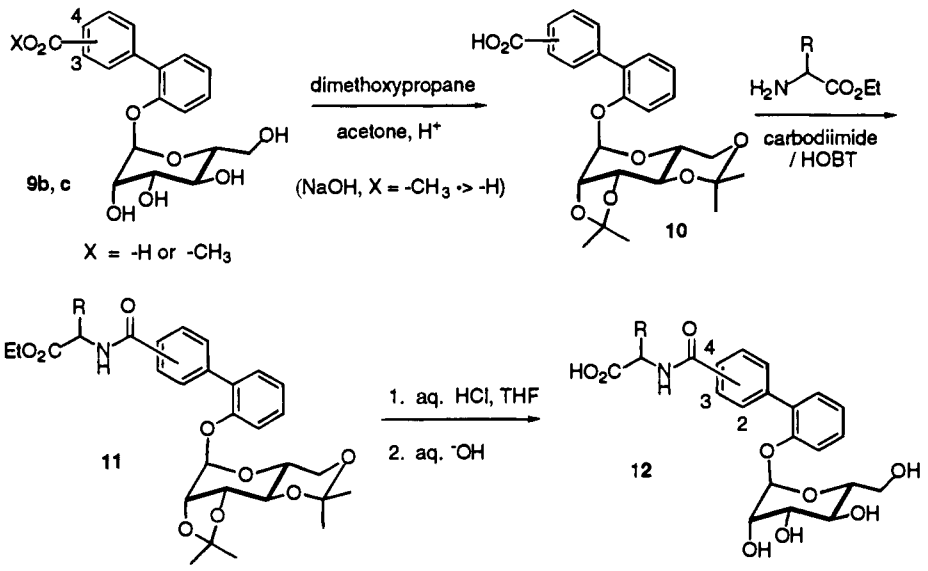
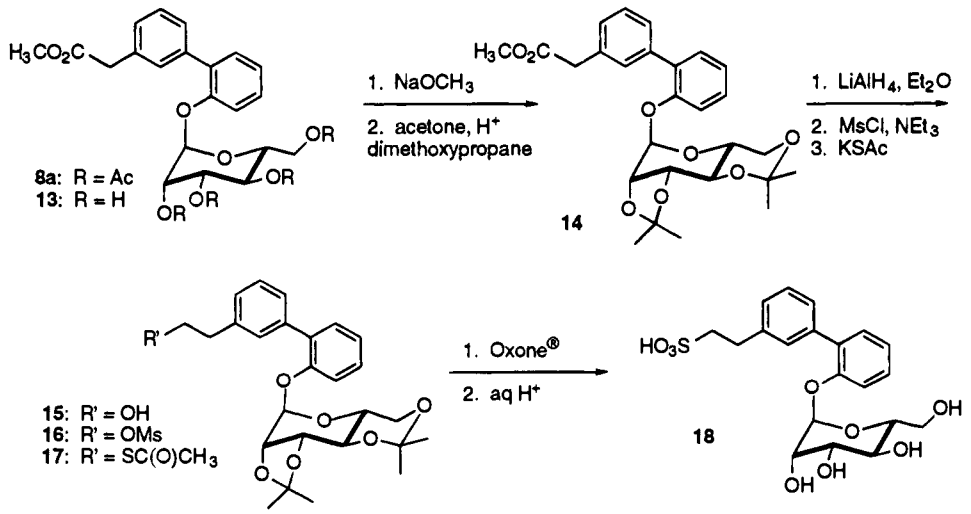


Table 2. *In Vitro* Data for Biphenyls **12a–k**, **18**, and **21**

		IC$_{50}$ (mM) or % inhib @ (mM)		
compd	R =	E-selectin	P-selectin	L-selectin
12a	4-C(O)NHCH$_2$CO$_2$H	4.6	0 @ 20	0 @ 6
12b	(*S*)-4-C(O)NHCH(CH$_2$Ph)CO$_2$H	3.8	0 @ 20	0 @ 5
12c	(*S*)-3-C(O)NHCH((CH$_2$)$_2$CO$_2$H)CO$_2$H	15.8	na	15 @ 5
12d	4-C(O)NHCH((CH$_2$)$_2$CO$_2$H)CO$_2$H	32	35 @ 4	0 @ 1
12e	4-C(O)NH(CH$_2$)$_2$CO$_2$H	36	0 @ 3	0 @ 1
12f	(*R*)-4-C(O)NHCH(CH$_2$Ph)CO$_2$H	11.5	na	na
12g	(*S*)-4-C(O)NHCH((CH$_2$)$_4$NH$_2$)CO$_2$H	33 @ 27	na	na
12h	4-C(O)NHCH(CH$_2$CO$_2$H)CO$_2$H	0 @ 16.3	2	5
12i	3-C(O)NHCH$_2$CO$_2$H	0 @ 18	25 @ 23	0 @ 6
12j	(*S*)-3-C(O)NHCH(CH$_2$CO$_2$H)CO$_2$H	0 @ 32	40 @ 20	0 @ 5
12k	(*S*)-3-C(O)NHCH(CH$_2$Ph)CO$_2$H	14 @ 1	5 @ 20	0 @ 6
18	3-(CH$_2$)$_2$SO$_3$H	20	na	na
21	3-(CH$_2$)$_3$P(O)(OEt)OH	34.4	12.4	na

[a] na = not assayed; this assay was not available when this compound was synthesized.

Scheme 4



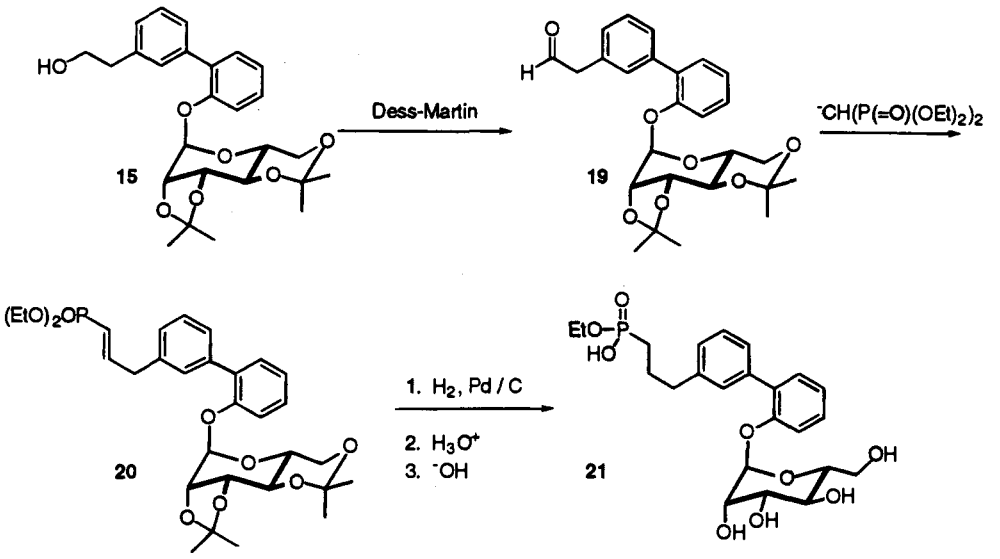
the separator, excess unbound HL60 cells were removed, and the wells were washed twice with PBS to remove any remaining unbound cells. The HL60 cells remaining bound to the beads were inspected by microscopy and then lysed by adding 50 μL of a 1% solution of NP40 in PBS. Binding was quantitated fluorimetrically using a Millipore Cytofluor 2350 fluorimeter.

Analogous assays were developed for P- and L-selectin using the equivalent sequences for P- and L-selectin lectin, EGF, and two complement repeat domains fused to a mouse IgG.

Structure–Activity Relationships

Several of the compounds synthesized were able to inhibit HL60 cell (bearing sLe^x oligosaccharides) binding to magnetic beads bearing an E-selectin fusion protein

Scheme 5



with greater potency than the sLe^x tetrasaccharide itself (Table 1). This is particularly remarkable in that these compounds were designed strictly on the basis of a model of the protein–sugar interactions and do not contain any of the monosaccharide units of the parent sLe^x. Compound **9a** has a molecular weight of 390 (vs 821 for sLe^x), has five chiral centers (vs 21 for sLe^x), and lacks the galactosyl hydroxyl groups reported by others to be important for selectin binding.[17,27]

Further examination of the data presented in Tables 1 and 2 reveals several interesting factors. First, the position of substitution on the A-ring of the biphenyl (**9e**,**f**,**m**) is important for binding, with slight favor being given to the 3-substituted isomers over the 4-substituted compounds, while the 2-substituted is poor. Next, in the series comprised of **9a**,**b**,**e**,**g** and **9c**,**d**,**f**,**w**, it was determined that maximum activity is obtained with one carbon linking the carboxylic acid to the biphenyl. These observations are in direct correlation with our computer models, in which **9a** was predicted to mimic sLe^x bound in the manner previously described. Replacement of the acid functionality with nitrile (**9h**), ester (**9v**), or tetrazole (**9o**) reduced activity significantly, which reinforced the necessity of the acid group and supported our proposed mimicry of bound sLe^x with these compounds. The modest loss in activity for the nitrile **9h** for E- and L-selectin compared to **9a** requires further comment. One possible hypothesis would involve interaction of the nitrile with Glu-98 in E- and L-selectin, where the equivalent residue in P-selectin is a proline. Since either the sulfonate **18** or phosphonate half-ester **21** decreased activity from equivalent carboxylic acid-containing derivatives, these acid replacements may have adverse effects due to either the different pK_a of these groups or alternatively the different geometry presented.

Two compounds (**9n**,**q**) were prepared which were designed to test the effect of restricted rotation about the central biaryl bond. Examination of models of these compounds indicates that the two phenyl rings must be rotated out of planarity in the lowest energy conformation, which has the effect of reorienting the acid group away from the desired position in the bound state. The lack of activity for these two compounds confirms this suboptimal geometry.

Of the amino acid derivatives **12a**–**k** (Table 2), only the 4-substituted glycine and phenylalanine derivatives **12a**,**b** displayed activity comparable to sLe^x, and no further improvement in activity was observed for the rest of the series, which also may be a consequence of distance to the acid functionality, effects of added steric bulk, or conformational influences. Thus, it is evident from the series of compounds studied that the lectin binding domain for E-selectin has distinct geometric requirements for inhibitors, and **9a** appears to satisfy these requirements. Other variations in the biphenyl system include modifications of the mannose unit and alteration of the attachment of the mannose with respect to the ring bearing the carboxylic acid, and these results will be reported shortly.

Summary

A series of (α-D-mannopyranosyloxy)biphenyl-substituted carboxylic acids were designed as small molecule mimetics of the sLe^x tetrasaccharide from a model of the bound tetrasaccharide and the E-selectin lectin domain X-ray crystal structure developed in our laboratories. The compounds were synthesized by a Suzuki coupling followed by Lewis acid-catalyzed mannosylation. *In vitro* assay data demonstrated the ability of this novel class of compounds to inhibit the binding of HL60 cells (which express sLe^x-bearing glycoproteins on their surface) to a recombinant E-selectin–IgG fusion protein bound to magnetic beads.

The results presented demonstrate that potent selectin inhibitors can be designed on the basis of the structural information available which utilize only the calcium recognition site (fucose) and a carboxylic acid (sialic acid replacement) to achieve interactions competitive with the native sLe^x tetrasaccharide. These results further support the recent knowledge[9] that the calcium site in E-selectin is capable of recognizing both mannose- as well as fucose-based compounds, and this ability to recognize mannose extends to the other members of the selectin family.

While there have been major advances in the design of peptidomimetics, we believe the work presented here represents the first successful report of glycomimetic rational design. Furthermore the first designed target

using the approach described (**9a**) was active and showed better *in vitro* potency than the parent sLe^x oligosaccharide. Since glycomimetics of this class may have advantages of increased stability, *in vivo* half-life, bioavailability, and more facile synthesis with fewer chiral centers, they offer considerable advantages over oligosaccharides for the development of therapeutic agents. We believe the results presented in this paper are highly significant with regard to the rational design of small molecule glycomimetic analogs.

Experimental Section

Solvents and reagents were used as received unless specified otherwise. All reactions involving organometallic reagents or other air or moisture sensitive compounds were conducted under a nitrogen atmosphere using oven-dried glassware. Preparative reverse-phase HPLC was conducted on a Dynamax 300 Å 5 μm (21 mm i.d. × 25 cm) C_{18} column. A gradient of 5–50% solvent B was run over 20 min at a flow rate of 10 mL/min, where solvent A was composed of 5% acetonitrile/water with 0.1% TFA and solvent B was composed of 95% acetonitrile/water with 0.1% TFA. The effluent was monitored at 254 nm. Pure fractions were pooled and lyophilized. ^{1}H NMR spectra were recorded on either a JEOL 400 MHz spectrometer or a GE-Bruker 300 MHz spectrometer using tetramethylsilane as an internal standard. NMR data are reported in δ units. Infrared spectra were recorded on a Bruker IFS-25 instrument as thin films on sodium chloride plates or as KBr pellets. Melting points were determined using a Fisher-Johns hot stage apparatus and are uncorrected.

3-[2-(α-D-Mannopyranosyloxy)phenyl]phenylacetic Acid (9a). Step 1: 3-Bromophenylacetic acid (2.0 g, 9.3 mmol) was dissolved in methanol (20 mL), sulfuric acid (2 drops) was added, and the mixture was heated at reflux for 10 h and then concentrated under reduced pressure. The residue was mixed with dichloromethane (20 mL) and saturated sodium bicarbonate solution (10 mL); the organic phase was separated, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was flushed through silica gel with hexane/ethyl acetate (3:1) and concentrated which provided 2.12 g (99%) of methyl 3-bromophenylacetate which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): 7.15–7.48 (m, 4H), 3.70 (s, 3H), 3.59 (s, 2H). IR (NaCl): 1731 cm^{-1}.

Step 2: Anisole (2.16 g, 20.0 mmol) was dissolved in dry THF (50 mL) and cooled to −78 °C , *n*-butyllithium (10.9 mL of a 2.3 M solution in hexanes, 25 mmol) was added, and then the reaction mixture was warmed to 0 °C. The reaction mixture was stirred for 1 h at 0 °C, then trimethyl borate (2.3 mL, 20 mmol) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was treated with 2 N aqueous HCl to pH 3, mixed well for 30 min, and then extracted with ether (3 × 15 mL). The organic materials were combined, dried ($MgSO_4$), and then concentrated under reduced pressure which gave 2.88 g (95%) of 2-methoxybenzeneboronic acid as a clear oil which was used without further purification. Methyl 3-bromophenylacetate (2.06 g, 9.0 mmol), tetrakis(triphenylphosphine)palladium(0) (115 mg), sodium carbonate (2.61 g, 25 mmol in 2 mL of water), and toluene (10 mL) were degassed under nitrogen in a 25 mL flask fitted with a reflux condenser. 2-Methoxybenzeneboronic acid (1.5 g, 9.87 mmol) in toluene (1 mL) was added, and the mixture was heated at reflux overnight and then mixed with 1:1 saturated sodium chloride/ethyl acetate (15 mL). The organic materials were separated, dried ($MgSO_4$), and then concentrated under reduced pressure which gave 2.81 g of methyl 3-(2-methoxyphenyl)phenylacetate. ^{1}H NMR (300 MHz, $CDCl_3$): 7.15–7.50 (m, 6H), 6.93–7.05 (m, 2H), 3.79 (s, 3H), 3.68 (s, 3H), 3.67 (s, 2H). IR (NaCl): 1741 cm^{-1}.

Step 3: Methyl 3-(2-methoxyphenyl)phenylacetate (2.0 g, 7.8 mmol) was dissolved in dichloromethane (100 mL) under nitrogen and chilled in a dry ice/2-propanol bath. Boron tribromide (2.2 mL, 24 mmol) was added slowly, and the mixture was kept at −10°C for 14 h and then mixed with ice–water (100 mL). The organic material was separated, washed with saturated sodium bicarbonate solution (50 mL), water (50 mL), and saturated sodium chloride (60 mL), and then dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 3:1 hexane/ethyl acetate) which gave 1.25 g (66% from methyl 3-bromophenylacetate) of methyl 3-(2-hydroxyphenyl)phenylacetate. ^{1}H NMR (300 MHz, $CDCl_3$): 7.15–7.50 (m, 6H), 6.95–7.05 (m, 2H), 5.29 (br s, 1H), 3.72 (s, 3H), 3.69 (s, 2H). IR (NaCl): 3417, 1715 cm^{-1}.

Step 4: Methyl 3-(2-hydroxyphenyl)phenylacetate (1.28 g, 5.28 mmol) was dissolved in 1,2-dichloroethane (20 mL) in a dry 50 mL flask; α-D-mannose pentaacetate (2.08 g, 5.34 mmol) was added followed by slow addition of boron trifluoride etherate (2.32 mL, 18.5 mmol). The mixture was stirred under nitrogen overnight at room temperature and then mixed with water (50 mL). The organic material was separated, and the aqueous portion was extracted with dichloromethane (3 × 5 mL). The extracts were combined with the original organic fraction, dried ($MgSO_4$), and then concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, gradient elution hexane to 3:1 hexane/ethyl acetate) which provided 2.74 g (91%) of methyl 3-[2-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)oxy]phenyl]phenylacetate contaminated with a small amount of unreacted α-D-mannose pentaacetate which coeluted with the product. ^{1}H NMR (300 MHz, $CDCl_3$): 7.02–7.50 (m, 8H), 5.48 (d, J = 0.8 Hz, 1H), 5.25–5.40 (m, 3H), 3.90–4.30 (m, 3H), 3.72 (s, 2H), 3.70 (s, 3H), 2.16 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H). IR (NaCl): 1749 cm^{-1}.

Step 5: Methyl 3-[2-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)oxy]phenyl]phenylacetate (2.74 g, 4.78 mmol) was dissolved in acetonitrile (25 mL) in a 50 mL flask and treated with a solution of lithium hydroxide monohydrate (1.1 g, 26.3 mmol) in water (10 mL), and the mixture was stirred at room temperature overnight and then acidified to pH 2 with concentrated hydrochloric acid. The mixture was concentrated under reduced pressure, and the residue was purified by reverse-phase HPLC which gave 0.87 g (47%) of **9a** as a white solid, mp 85–86°C. ^{1}H NMR (300 MHz, DMSO-d_6): 7.02–7.40 (m, 8H), 5.31 (s, 1H), 3.25–4.00 (m, 12 H). IR (KBr): 1713 cm^{-1}. Anal. ($C_{20}H_{22}O_8$) C, H.

***N*-[4-[2-(α-D-Mannopyranosyloxy)phenyl]benzoyl]glycine (12a). Step 1:** Acetone (5.6 mL) and dimethoxypropane (5.6 mL) were added to 4-[2-(α-D-mannopyranosyloxy)phenyl]benzoic acid (0.54 g, 1.43 mmol) to form a heterogeneous mixture. A catalytic amount of *p*-toluenesulfonic acid monohydrate was introduced, and the reaction mixture was stirred at room temperature for 45 min, at which point a clear homogeneous solution was obtained. The solvent was removed under reduced pressure, and the oily residue was dissolved in ethyl acetate, washed with saturated sodium bicarbonate and then saturated sodium chloride, dried ($MgSO_4$), and concentrated under reduced pressure to afford 4-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]benzoic acid (0.7 g). ^{1}H NMR (300 MHz, $CDCl_3$): 8.24 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 7.8 Hz, 2H), 7.41 (m, 2H), 7.25 (m, 2H), 5.73 (s, 1H), 4.26 (d, J = 5.4 Hz, 1H), 4.15 (m, 1H), 3.75 (m, 4H), 3.55 (m, 2H), 1.60 (s, 3H), 1.55 (s, 3H), 1.44 (s, 3H), 1.40 (s, 3H). IR (NaCl): 1724 cm^{-1}.

Step 2: A solution of crude 4-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]benzoic acid (0.7 g) in dry methylene chloride (4 mL) was added to a slurry of glycine ethyl ester hydrochloride (0.2 g, 1.44 mmol) and triethylamine (0.4 mL, 2.88 mmol) in dry methylene chloride (93 mL). *N*-Hydroxysuccinimide (0.16 g, 1.44 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (0.32 g, 1.55 mmol) were added, and the mixture was stirred under nitrogen at room temperature for 4 h. Precipitated dicyclohexylurea was filtered away, and the filtrate was diluted with methylene chloride. The resulting solution was washed successively with water, 1 N HCl, saturated sodium bicarbonate, and brine, and then dried ($MgSO_4$) and concentrated under reduced pressure to afford 0.69 g (89% overall) of *N*-[4-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]benzoyl]glycine ethyl ester. ^{1}H NMR (300 MHz, $CDCl_3$): 7.92 (d, J = 8.1 Hz, 2H), 7.63 (d, J = 8.1 Hz, 2H), 7.39 (m, 2H), 7.23 (m, 2H), 5.73 (s, 1H), 4.31

(m, 5H), 4.13 (m, 1H), 3.73 (m, 3H), 3.51 (m, 1H), 1.59 (s, 3H), 1.54 (s, 3H), 1.44 (s, 3H), 1.38 (m, 6H). IR (NaCl): 1744 cm^{-1}.

Step 3: *N*-[4-[2-[(2,3:4,6-di-*O*-Isopropylidene-α-D-mannopyranosyl)oxy]phenyl]benzoyl]glycine ethyl ester (0.69 g, 1.28 mmol) was dissolved in THF (2.5 mL). An equal volume of 1 N HCl was added, and the reaction mixture was stirred at room temperature overnight. Sodium hydroxide (2 mL of 2 N) was added, and the reaction mixture was stirred for another 8 h. The solution was then reacidified to pH 4.5 with 1 N HCl, and the product was isolated by preparative reverse-phase HPLC to yield 0.33 g (56%) of **12a** as a white solid, mp 127–129 °C. ^1H NMR (300 MHz, D_2O): 7.85 (d, J = 7.8 Hz, 2H), 7.61 (d, J = 7.8 Hz, 2H), 7.41 (m, 2H), 7.32 (d, J = 9.0 Hz, 1H), 7.20 (dd, J = 6.9, 7.8 Hz, 1H), 5.48 (s, 1H), 4.11 (s, 2H), 3.94 (s, 1H), 3.60 (br m, 4H), 3.28 (br m, 1H). IR (KBr): 1734 cm^{-1}. Anal. ($C_{21}H_{23}NO_9$) C, H, N.

2-[3-[2-(α-D-Mannopyranosyloxy)phenyl]phenyl]ethanesulfonic Acid (18). Step 1: Methyl 3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenylacetate (1.28 g, 2.6 mmol) was dissolved in ether (50 mL) and cooled to 0 °C. Lithium aluminum hydride (1 M solution in THF, 50 mL, 5 mmol) was added dropwise and the solution stirred for 10 min. The reaction was quenched by careful addition of water followed by ice-cold dilute sulfuric acid. The organic layer was washed with water and then saturated sodium bicarbonate solution, dried ($MgSO_4$), and concentrated under reduced pressure to give 1.13 g (94%) 2-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]-ethanol. ^1H NMR (300 MHz, $CDCl_3$): 7.1–7.5 (m, 8H), 5.68 (s, 1H), 4.34 (d, 1H), 4.10 (dd, J = 6 Hz, 1H), 3.9–4.0 (m, 2H), 3.5–3.8 (m, 3H), 3.28 (ddd, J = 9, 9, 6 Hz, 1H), 2.95 (t, J = 6 Hz, 2H), 1.58 (s, 3H), 1.49 (s, 3H), 1.40 (s, 3H), 1.38 (s, 3H).

Step 2: 2-[3-[2-[(2,3:4,6-di-*O*-Isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]ethanol (0.73 g, 1.6 mmol) was dissolved in methylene chloride (50 mL) and cooled to 0 °C. Triethylamine (0.33 mL, 2.4 mmol) was added followed by methanesulfonyl chloride (0.15 mL, 1.9 mmol). After 5 min at 0 °C, the reaction mixture was diluted with methylene chloride, washed with dilute hydrochloric acid, water, and then saturated sodium bicarbonate solution, dried ($MgSO_4$), and concentrated under reduced pressure to give 0.77 g (90%) of 2-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]-phenyl]phenyl]ethanol 1-*O*-methanesulfonate. ^1H NMR (300 MHz, $CDCl_3$): 7.1–7.5 (m, 8H), 5.68 (s, 1H), 4.52 (t, J = 7.5 Hz, 2H), 4.27 (d, J = 6 Hz, 1H), 4.09 (dd, J = 6.8 Hz, 1H), 3.6–3.8 (m, 3H), 3.43 (m, 1H), 3.16 (t, J = 7.5 Hz, 2H), 2.95 (s, 3H), 1.58 (s, 3H), 1.53 (s, 3H), 1.43 (s, 3H), 1.39 (s, 3H).

Step 3: 2-[3-[2-[(2,3:4,6-di-*O*-Isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]ethanol 1-*O*-methanesulfonate (0.22 g, 0.4 mmol) was dissolved in ethanol (5 mL), potassium thioacetate (0.1 g, 0.88 mmol) was added, and the mixture was heated at 80°C for 30 min and then cooled to room temperature. The reaction mixture was partitioned between ethyl acetate and water, and the organic layer was washed twice with water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by chromatography (SiO_2, 3:1 hexane/ethyl acetate) to give 0.17 g (80%) of 2-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]ethanethiol *S*-acetate. ^1H NMR (300 MHz, $CDCl_3$): 7.1–7.5 (m, 8H), 5.68 (s, 1H), 4.38 (d, J = 6 Hz, 1H), 4.11 (t, J = 7.5 Hz, 2H), 3.5–3.8 (m, 3H), 3.49 (m, 1H), 3.20 (t, J = 8 Hz, 2H), 2.96 (t, J = 8 Hz, 2H), 2.48 (s, 3H), 1.58 (s, 3H), 1.52 (s, 3H), 1.43 (s, 3H), 1.37 (s, 3H).

Step 4: 2-[3-[2-[(2,3:4,6-di-*O*-Isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]ethanethiol *S*-acetate (0.15 g, 0.29 mmol) was dissolved in methanol (1 mL), and a solution of Oxone (*ca.* 1 mequiv/mL in aqueous methanol, 1.5 mL) was added dropwise at room temperature over a course of 90 min. After stirring for 7 days, another portion of Oxone solution (1 mL) was added over the course of 1 h, and stirring was continued for another 3 days. The product was isolated by preparative reverse-phase HPLC to yield 50.8 mg (38%) of **18**. ^1H NMR (300 MHz, D_2O): 7.36 (m, 8H), 7.17 (t, J = 7.5 Hz, 1H), 5.44 (s, 1H), 3.93 (s, 1H), 3.61 (m, 1H), 3.57 (m, 4H), 3.25 (br m, 1H), 3.10 (m, 4H). MS spectrum (CI, CH_4): m/e 279, 163. Anal. ($C_{20}H_{24}SO_9$) C, H.

Ethyl [3-[3-[2-(α-D-Mannopyranosyloxy)phenyl]phenyl]-propyl]phosphonate (21). Step 1: A solution of 2-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]-ethanol (1.12 g, 2.46 mmol) in methylene chloride (15 mL) was added slowly to a suspension of Dess–Martin periodinane (4.95 g, 11.7 mmol) in dry methylene chloride (5 mL), and the reaction mixture was stirred at room temperature overnight. The mixture was then diluted with diethyl ether and filtered. The filtrate was washed twice with saturated sodium bicarbonate solution and brine and then dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by chromatography (SiO_2, 4:1 hexane/ethyl acetate) to afford 0.63 g (57%) of [3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]ethanal. ^1H NMR (300 MHz, $CDCl_3$): 9.82 (s, 1H), 7.37 (m, 8H), 5.66 (s, 1H), 4.24 (d, 1H, J = 5.4 Hz), 4.07 (dd, 1H, J = 7.8, 5.7 Hz), 3.70 (m, 6H), 3.40 (m, 1H), 1.55 (s, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.36 (s, 3H).

Step 2: Tetraethyl methylenediphosphonate (0.37 g, 1.28 mmol) was dissolved in dry THF (3.8 mL) under nitrogen, and the solution was cooled to −78 °C. A 0.5 M solution of potassium hexamethyldisilazide in toluene (2.56 mL, 1.28 mmol) was added dropwise, and the mixture was stirred for 10 min. A solution of [3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]ethanal (0.58 g, 1.28 mmol) in THF (3.8 mL) was then added, and the reaction mixture was allowed to warm to room temperature with stirring overnight. Water was then added, and the mixture was extracted with ethyl acetate. The extracts were combined, washed with water, 1 N HCl, saturated sodium bicarbonate solution, and brine, and then dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by chromatography (SiO_2, 1:2 hexane/ethyl acetate) to afford 0.46 g (63%) of diethyl [3-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]prop-1-enyl]phosphonate. ^1H NMR (300 MHz, $CDCl_3$): 7.30 (m, 8H), 6.58 (dd, 1H, J = 15.6, 5.1 Hz), 6.22 (m, 1H), 5.63 (s, 1H), 4.140 (m, 6H), 3.71 (m, 3H), 3.52 (m, 1H), 2.80 (dd, 2H, J = 22.2, 7.5 Hz), 1.55 (s, 3H), 1.50 (s, 3H), 1.40 (s, 3H), 1.33 (m, 9H).

Step 3: Diethyl [3-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]prop-1-enyl]phosphonate (0.46 g, 0.8 mmol) was dissolved in ethanol (25 mL) and hydrogenated (40 psi H_2, 10% Pd/C) for 3 h. The suspension was filtered through Celite, and the filtrate was concentrated under reduced pressure to afford 0.46 g (100%) of diethyl [3-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]propyl]phosphonate. ^1H NMR (300 MHz, $CDCl_3$): 7.28 (m, 8H), 5.61 (s, 1H), 4.21 (d, 1H, J = 5.4 Hz), 4.08 (m, 4H), 3.69 (m, 4H), 3.46 (q, 1H, J = 8.7 Hz), 2.76 (t, 2H, J = 7.2 Hz), 1.97 (m, 2H), 1.78 (m, 2H), 1.53 (s, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.31 (s, 9H).

Step 4: Diethyl [3-[3-[2-[(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)oxy]phenyl]phenyl]propyl]phosphonate (0.23 g, 0.4 mmol) was dissolved in methanol (2 mL); 2 N hydrochloric acid (0.5 mL) was added, and the mixture was stirred at room temperature overnight. The solution was made pH 10 with 2 N NaOH and allowed to stir at room temperature for 3 h. The reaction mixture was heated at 60 °C for 18 h and then at 80 °C for 116 h. The reaction mixture was cooled and acidified with 1 N HCl, and the product was isolated by preparative reverse-phase HPLC to yield 76.4 mg (40%) of **21** as a white solid, mp 67–70 °C. ^1H NMR (300 MHz, D_2O): 7.25 (m, 8H), 5.41 (s, 1H), 3.88 (m, 3H), 3.61 (m, 4H), 3.26 (m, 1H), 2.64 (m, 2H), 1.78 (m, 2H), 1.66 (m, 2H), 1.16 (t, J = 6.9 Hz, 3H). IR (KBr): 1222 cm^{-1}.

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