Selectin–Carbohydrate Interactions: From Natural Ligands to Designed Mimics

Eric E. Simanek, Glenn J. McGarvey, Jill A. Jablonowski, and Chi-Huey Wong*

Department of Chemistry, The Skaggs Institute for Chemical Biology and The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received September 25, 1997 (Revised Manuscript Received January 2, 1998)

Contents

I. Introduction

Carbohydrates display enormous structural and functional diversity, fulfilling roles as energy sources and as elements for recognition and for the control of the structure, function, and dynamics of proteins.[1] It is, however, the role of carbohydrates in cell adhesion processes, specifically, between the selectins (selective carbohydrate-binding proteins) and the Lewis sugars and their derivatives,[2−12] that has emerged as the cornerstone of the rapidly growing field of molecular glycobiology.

Acute diseases, such as stroke and reperfusion injury during surgery and organ transplantation, and chronic inflammatory diseases, such as psoriasis and rheumatoid arthritis, are a result of the body's overzealous recruitment of lymphocytes from the vasculature to a location suspected by the body to be a site of injury. In addition, it has been suggested that this adhesion process may be exploited by cancer cells to metastasize after entering the blood stream. To address these important health issues, considerable effort has been invested in devising methods to disrupt leukocyte recruitment. Recent efforts focus on preventing initial adhesion events between circulating leukocytes and the endothelium involving two families of cell-surface receptors: the selectins and

S0009-2665(94)00226-8 CCC: $30.00
Published on Web 03/12/1998

Eric E. Simanek was born in 1969 in Tuscola, IL. He studied chemistry at the University of Illinois in Urbana—Champaign while working in the laboratory of Professor Kenneth L. Rinehart, Jr. After finishing his B.S. degree in 1991, he went to Harvard to obtain his Ph.D. with Professor George M. Whitesides. In 1996, he joined the laboratories of Professor Chi-Huey Wong at the Scripps Research Institute as a postdoctoral fellow. His research interests center on molecular recognition in problems ranging from medicine to materials science.

Professor Glenn J. McGarvey received his B.S. in Chemistry from the University of California at Santa Barbara and then completed his Ph.D. at the University of California at Davis under the direction of R. Bryan Miller. This was followed by two years of postdoctoral research at the California Institute of Technology in the laboratories of Professor Robert E. Ireland. He then took his present position on the faculty of the Department of Chemistry at the University of Virginia where his research interests to date have been broadly based in synthetic organic chemistry.

Jill A. Jablonowski received her B.S. degree in chemistry from the Rochester Institute of Technology in Rochester, NY, working with Professor Terence C. Morrill, and her M.S. degree in organic chemistry from Indiana University with Professor William R. Roush. She went on to obtain her Ph.D. in organic chemistry under the direction of Professor James A. Marshall at the University of South Carolina. Since 1996, she has been a postdoctoral research associate at The Scripps Research Institute with Professor Wong. Her research interests include bioorganic chemistry and natural product synthesis.

Professor Chi-Huey Wong received his B.S. and M.S. degrees from National Taiwan University and his Ph.D. in Chemistry with George M. Whitesides from Massachusetts Institute of Technology. He then moved along with Professor Whitesides to Harvard University as a postdoctoral fellow for another year. He taught at Texas A&M University for six years and since 1989 has held the Ernest W. Hahn Chair in Chemistry at The Scripps Research Institute. His current interests lie in the areas of bioorganic and synthetic chemistry, especially the development of new synthetic chemistry based on enzymatic and chemoenzymatic reactions and the rational; development of mechanism-based inhibitors of enzymes and receptors.

the integrins. Our efforts focus on the generation of selectin antagonists; specifically, on the design and synthesis of mimics of sialyl Lewis X (sLe^x) and related Lewis oligosaccharides. These mimics are designed to resemble the structure and function of the natural carbohydrate ligand, but are not carbohydrates per se. Carbohydrates are not ideal drug candidates. They are difficult to synthesize, bind weakly, and have low oral availability due to labile glycosidic linkages and poor cell-entry properties.

This review begins with a discussion of the role of the selectins in inflammation and a description of their natural ligands. Strategies for the total synthesis of the sLe^x tetrasaccharide are reviewed. Next, a summary of the studies that led to the identification of the functional groups of sLe^x critical for binding to the selectins is presented. The main body of this review contains a description of the mimetics published to date (early 1997). Current efforts to increase the affinity of these molecules for their targets by the introduction of secondary groups and through multivalency are discussed. The review continues with an overview of other classes of molecules that inhibit the selectin—sLe^x interaction whose structures are not based on sLe^x. Then, assays used to evaluate binding are surveyed. Finally, this review concludes with a discussion of new targets for the inhibition of adhesion that are receiving increased attention.

A. The Role of Selectins in Inflammation

White blood cells, or leukocytes, are important species for the repair of tissue damage and defense against microbial infection. Leukocytes are brought to the site of injury by a complex series of steps, referred to as the inflammatory cascade (Figure 1).

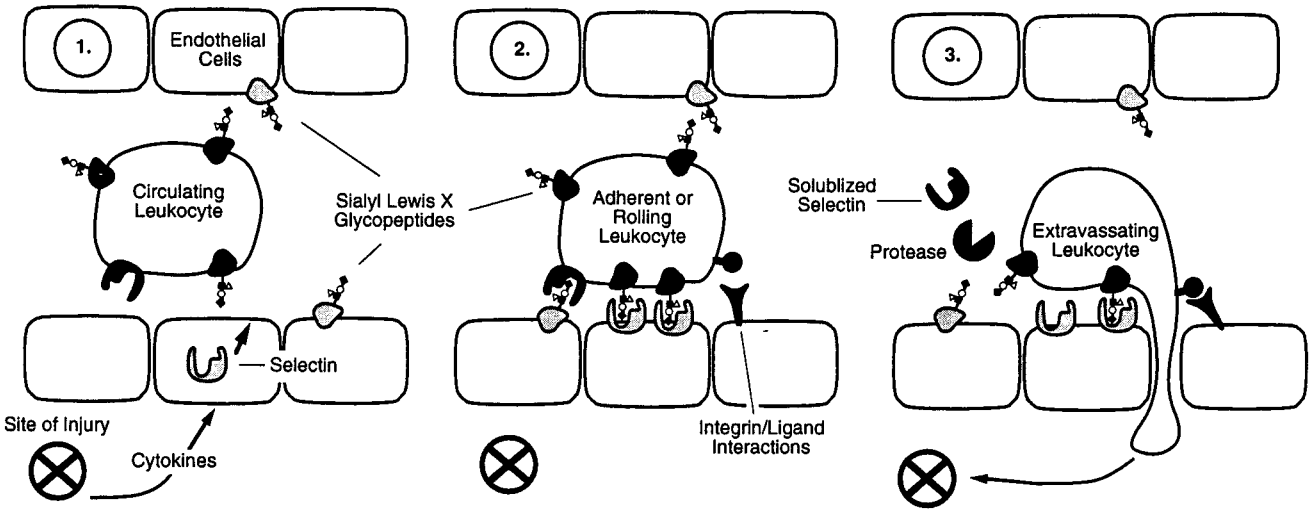


Figure 1. The inflammatory cascade. When an injury occurs (1), cytokines are released from the damaged tissue signaling the endothelium to display E- and P-selectins to the cells of the vasculature. P-selectin appears first within 30 min followed by E-selectin. Circulating leukocytes display a P-selectin glycoprotein ligand, PSGL-1 which displays sLe^x and O-sulfated tyrosine, an E-selectin ligand, ESL-1, a glycoprotein containing sLe^x, and L-selectin, an adhesion molecule that interacts with sLe^x-bearing glycoproteins of the endothelium. (2) Selectin−sLe^x interactions between the circulating leukocytes and the endothelium results in rolling and adhesion. These interactions are supplemented by stronger integrin-ligand interactions. (3) Loss of L-selectin appears to be important for extravasation, and occurs through proteolytic cleavage by a metalloprotease (or sheddase). Leukocytes then accumulate at the site of injury. See the text for more details and leading references.

The cascade begins when damaged tissues release cytokines that stimulate the endothelium to express two proteins, E-[13] and P-selectin[14] transiently on the endothelial lining.[15−21] Selectins E and P recognize sLe^x and related oligosaccharides on the surface of the leukocytes[22] and promote leukocyte adhesion to the affected endothelial cells.[23−25] L-selectin[26,27] is constitutively expressed on leukocytes,[28] and it recognizes similar carbohydrate ligands displayed on the endothelium.[29−32]

"Rolling"[33] by the leukocytes across the affected endothelium leads to further adhesion events[34,35] between integrins on the leukocytes and an endothelial protein, ICAM-1 (*i*ntercellular *a*dhesion *m*olecule-1).[11] This stronger interaction (itself the target for chemotherapies) leads to the migration of the leukocytes through the endothelial layer (extravasation) to the site of the injury.[36,37] Proteolytic cleavage of L-selectin from the surface of leukocytes occurs,[38,39] and appears to be important for extravasation.[40−43] While the mechanism of cleavage and the role of the soluble selectins are not understood,[44−46] small molecules designed to inhibit proteolysis have been reported.[47] A similar loss of P-selectin from degranulated platelets has been observed.[48]

The mechanism of the signal transduction cascade that leads to extravasation is not fully understood, but growing evidence suggests the role of an integrin-mediated pathway.[49−52] L-selectin may also play some role in signal transduction, but how the selectin−sugar interaction transduces the signal to inside the cell is not clear, although the level of cellular protein phosphorylation increases after selectin binding.[53]

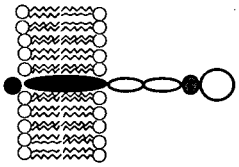
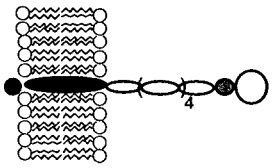
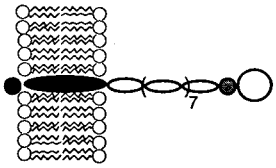
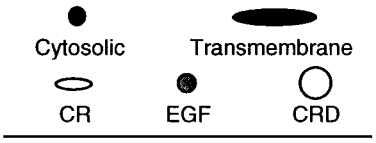
B. Structure of the Selectins

Each of the selectins comprises five domains: a cytosolic tail that may play a role in signal transduction, a transmembrane domain, a series of complement-like modules (CR), an epidermal growth factor domain (EGF), and an N-terminal, calcium-dependent carbohydrate recognition domain (CRD). Both the EGF and CRD domains are required for binding the carbohydrate ligand, although the site of binding has been localized to the CRD domain.[54] The EGF region is believed to exert its effect by holding portions of the lectin domain in the proper conformation.[55] A crystal structure of the CRD domain of E-selectin is available,[56] and its primary sequence is about 80% homologous to another structurally characterized sugar binding protein, the mannose binding protein (MBP).[57] The CRD is a globular structure that recognizes its ligands in a shallow depression that contains a Ca^{2+} ion. Models[58] of selectin−sLe^x binding in which the hydroxyl groups of fucose coordinate the Ca^{2+} (Figure 2) have been proposed on the basis of this conserved Ca^{2+} binding site, the crystal structures of the CRD and MBP, and mutagenesis studies of the CRD.[59]

C. Ligands for the Selectins

Since the initial characterization of the selectins in 1989, a number of ligands have been identified (see Table 1). While the debate over the natural ligands for each of the selectins continues, current opinion suggests that carbohydrates including a Lewis sugar (at some level of modification such as sialylation or sulfation) are the biologically relevant ligands (*vida infra*). Figure 3 shows the binding domains of PSGL-1 and GlyCAM-1 (discussed below).[60] The carbohydrate portion of PSGL-1, the natural ligand of E- and P-selectin, is a trimer of fucosylated *N*-acetyllactosamine groups with a terminal sialyl group. The pattern of tyrosine sulfation has not been

Table 1. Selectin Ligands[a]

	Selectin (Old)	[Refs]	Expressed	Small Molecule Ligands	[Refs]	GP Ligands	(Old)	[Refs]
	**E** (ELAM-1) (LECAM-2)	[13]	in response to an injury signal [15-17]	sLe^x sLe^x-SO_3^- sLe^a $sLe^aSO_3^-$	[61-66] [67] [63] [67]	ESL-1 PSGL-1		[68,69] [70]
	**L** (LECAM-1) (gp90MEL) (LAM-1)	[26] [27]	always [28]	sLe^x $Le^xSO_3^-$ sLe^a Le^a-SO_3^- sulfatide heparin sulfate fucoidin phosphomannon	[75] [77] [76] [77] [84] [85,86] [87] [88]	GlyCAM-1 CD34 MadCAM-1	(spg50) (spg90)	[71,72] [73] [74]
	**P** (CD62) (PAGDEM) (GP140) (LECAM-3)	[14]	in response to a signal (*ie.* thrombin) [18-21]	sLe^x sLe^a sulfatide heparin fucoidin dextran sulfate	[92,95] [96] [97] [98] [99] [99]	PSGL-1 [89]		
								

[a] The carbohydrate and glycoprotein ligands for the selectins are indicated with references. "(Old)" refers to old nomenclature used to identify these ligands. "Expressed" indicates when selectins appear on the cell membrane. The glycoprotein "GP" ligands are also indicated with older nomenclature and leading references.

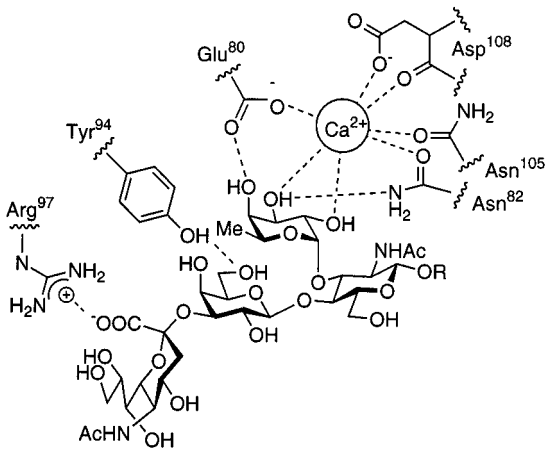


Figure 2. Hypothesized binding site for sLe^x on E-selectin. The functional groups important for binding to the selectins (vida infra) are involved in many interactions: in coordination to Ca^{2+} (the 2- and 3-hydroxyl groups of fucose); in hydrogen bonding to acid, tyrosine, or amino acid side chains (4-hydroxyl of fucose; hydroxyl groups of galactose; 3-hydroxyl group of fucose); in ion pairing with an arginine side chain (carboxylate of NeuAc). Mimetics that incorporate residues that fulfill these roles are expected to be more active than those which do not incorporate these critical functional groups.

determined. Sulfation is required for binding to P-selectin, but not E-selectin. The structure of the natural ligand on GlyCAM-1 for L-selectin is less understood. It seems clear that the carbohydrate is sulfated on the 6, 6′, or both positions. Specific details follow.

1. Ligands for E-Selectin

Preceding the isolation of the naturally occurring glycoprotein that binds to E-selectin, the carbohydrate portions of these ligands were being determined. Lowe and colleagues proposed, upon the basis of transfection of a fucosyltransferase, that the carbohydrate contained fucose.[61] Tienmeyer et al. determined the composition of the carbohydrate using mass spectrometry.[62] In 1991, four groups reported that sLe^x recognized E-selectin.[63−66] sLe^a [63] and sulfated derivatives[67] of Le^x and Le^a also bind to E-selectin. The glycoprotein receptors bearing these carbohydrates were identified in 1994 as ESL-1[68,69] and PSGL-1.[70]

2. Ligands for L-Selectin

Three glycoproteins have been identified as the natural ligands for L-selectin: GlyCAM-1,[71,72] CD34,[73] and MadCAM-1.[74] L-selectin recognizes sLe^x,[75] sLe^a,[76] and sulfated derivatives[77] which are all likely to be present in part of the carbohydrate portion of these glycoproteins. The biologically relevant pattern of sulfation is currently under investigation,[78] although the simplest carbohydrates of GlyCAM-1 appear to be sulfated on GlcNAc and Gal at equal levels.[79] However, a recent study suggests that sLe^x-6-sulfate (sulfate on the 6 position of GlcNAc, not Gal) is the ligand for L-selectin.[80] Hasegawa and colleagues have prepared the 6-sulfate, 6′-sulfate, and 6,6′-disulfate derivatives and found that the disulfate binds most tightly.[81] Efforts to characterize the

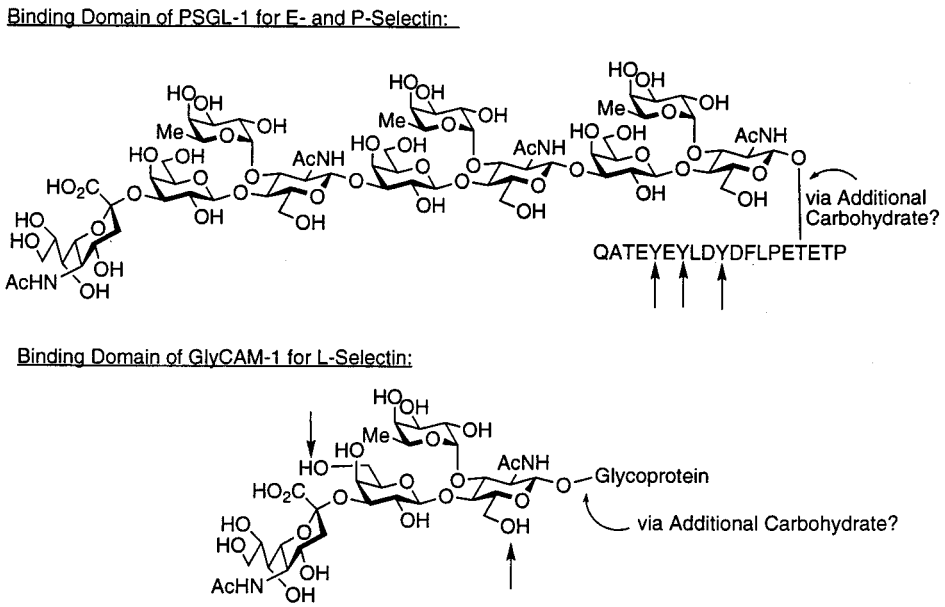


Figure 3. Natural carbohydrate ligands. The proposed structures of some of the natural ligands on PSGL-1 and GlyCAM-1 are shown. PSGL-1 recognizes E- and P-selectin, although sulfation is required for P-selectin binding, and not desired for E-selectin binding. The pattern of sulfation on tyrosine residues (indicated with arrows) has not been determined. GlyCAM-1 is sulfated on the carbohydrate portion of the molecule, although the pattern of sulfation remains a topic of debate (indicated with arrows).

extent of sulfation of the carbohydrate and to identify the entire carbohydrate portion are underway in many laboratories.[82,83] Sulfatide,[84] heparin,[85,86] fucoidin,[87] and phosphomannon[88] also bind to L-selectin. It seems likely that L-selectin presents a cationic binding site distinct from the carbohydrate binding site which could be a target for secondary groups.

3. Ligands for P-Selectin

Sako et al. identified the natural ligand for P-selectin, PSGL-1.[89] Further work by Sako[90] and Seed[91] showed that the 19 amino acid N-terminus of PSGL-1 containing acid side chains, and the O-linked sLe^x carbohydrate (established earlier by Norgard et al.)[92] was critical for binding. Sulfation of tyrosine residues is important for binding: there are three tyrosines in the 19 amino acid sequence.[93] Moore et al. reported that sLe^x is attached to PSGL-1 through a polylactose amine linker.[94] sLe^x and sLe^a have been shown to bind to P-selectin. The sulfated Lewis sugars do not bind to P-selectin.[95,96] A number of sulfated biomolecules do bind (Table 1): sulfatide,[97] heparin,[98] fucoidin,[99] and dextransulfate.[99] Whether these molecules exploit the same binding pocket on P-selectin as sLe^x or at a distinct cationic binding site remains to be determined.

II. Inhibition of Cell·Cell Interactions: Intervention of the Selectin·Sialyl Lewis^x Binding

As noted earlier, an attractive strategy for treating inflammation-related diseases, such as rheumatoid arthritis, reperfusion injury, and, perhaps, suppressing metastasis of cancer cells, is to inhibit the selectin−ligand interaction that initiates these processes. This goal provides an exceptional opportunity to apply interdisciplinary methodologies of the biological and chemical sciences to a problem in structural biology. Scheme 1 illustrates the interplay of chemical and biological disciplines in guiding the development of a potential therapeutic agent. The following discussion will address current efforts to develop antiinflammatory agents based upon inhibition of the event that launches the inflammatory cascade, the selectin−sLe^x binding event. With the use of the information gained from the examination of the structural characteristics of sLe^x binding, alternative structures will be sought that exhibit even stronger binding to selectins than the natural ligand.

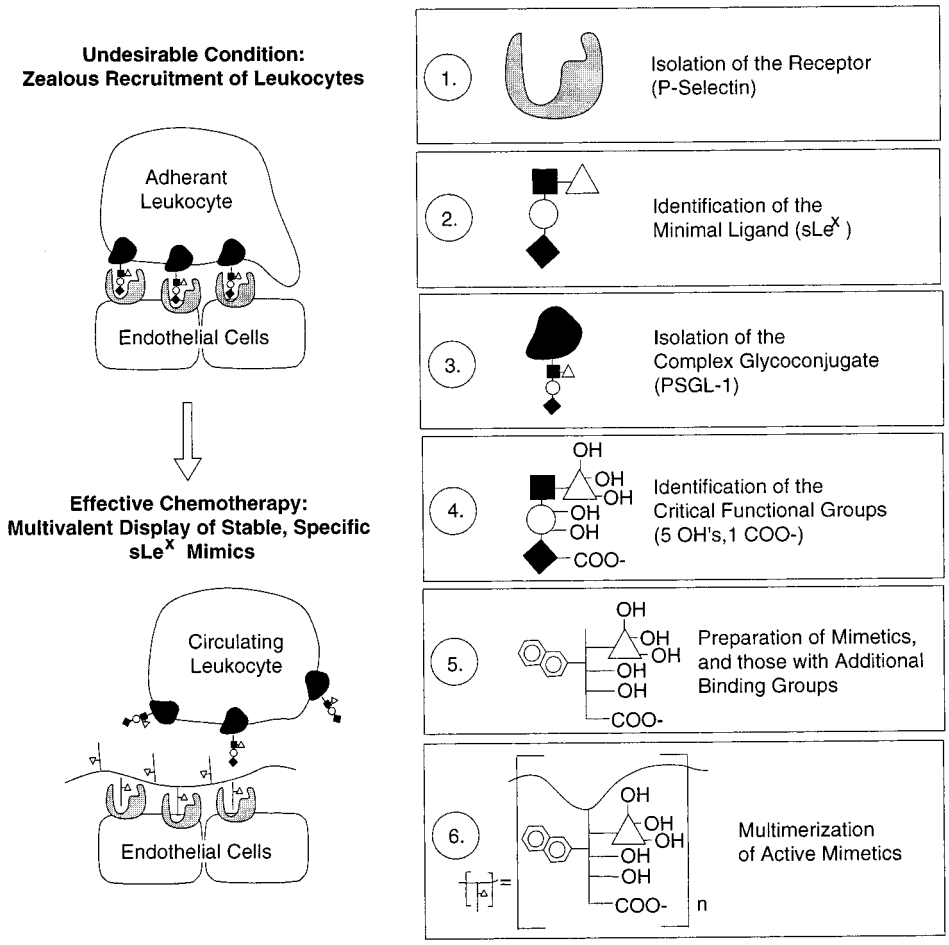
A. Synthetic Studies of Sialyl Lewis^x

The required synthetic technology for studying the structural features necessary for selectin binding, and for the subsequent preparation of analogue structures is made available through initial synthetic studies targeting the sLe^x structure itself. With the above goals in mind, the value of such a total synthesis will be judged on both efficiency and flexibility. Not only must the synthesis afford suitable quantities of sLe^x in a minimun of synthetic operations, it also must accommodate considerable structural variation to allow the preparation of analogue structures.

1. Chemical Synthesis of Sialyl Lewis^x

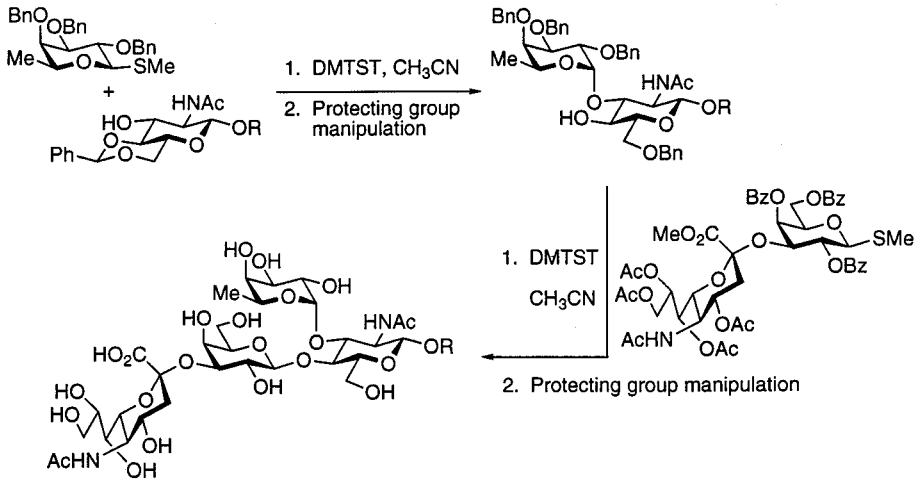
The tetrasaccharide structure of sLe^x is a demanding challenge for synthetic chemistry stemming from the need to selectively form glycosidic bonds with highly functionalized substrates. The several successful syntheses of sLe^x reported to date offer a clear demonstration of the power of modern methods of oligosaccharide synthesis.[100−104] Several noteworthy syntheses will be highlighted in the following discussion.

Scheme 1. A Biological/Chemical Protocol for the Chronology of Discovery of an Effective Chemotherapy with Specific Reference to P-Selectin[a]



[a] Isolation of the receptors began around 1989, followed by characterization of the carbohydrate portion of the natural ligand in 1991. Isolation of the natural glycoprotein ligands was described around 1992. The total syntheses of the minimal carbohydrate ligands and its derivatives useful for determining critical functional groups appeared between 1991 and 1993. The availability of these ligands allowed for the determination of the free and bound conformations of sLe^x (1994, 1997), as well as chemistries useful for the synthesis of the first generation of inhibitors in 1994. Current efforts focus on the development of sLe^x mimetics with additional binding groups and multivalent display.

Scheme 2. Hasegawa's Synthesis of Sialyl Lewis^x [a]

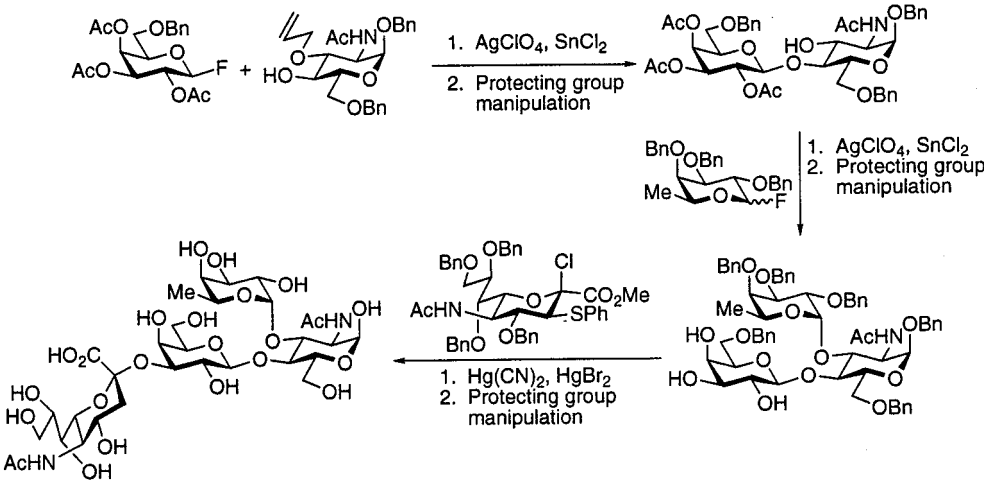


[a] Hasegawa and colleagues prepared sLe^x using thioglycosides activated with DMTST.[101]

The first synthesis[101] of sLe^x was reported in 1991 by Hasegawa and co-workers. This synthesis highlights the utility of thioglycosides for glycosidic bond formation. The use of thioglycosides with dimethyl(methylthio)sulfonium triflate (DMTST) activation was responsible for formation of all three key glycosidic bonds (Scheme 2).

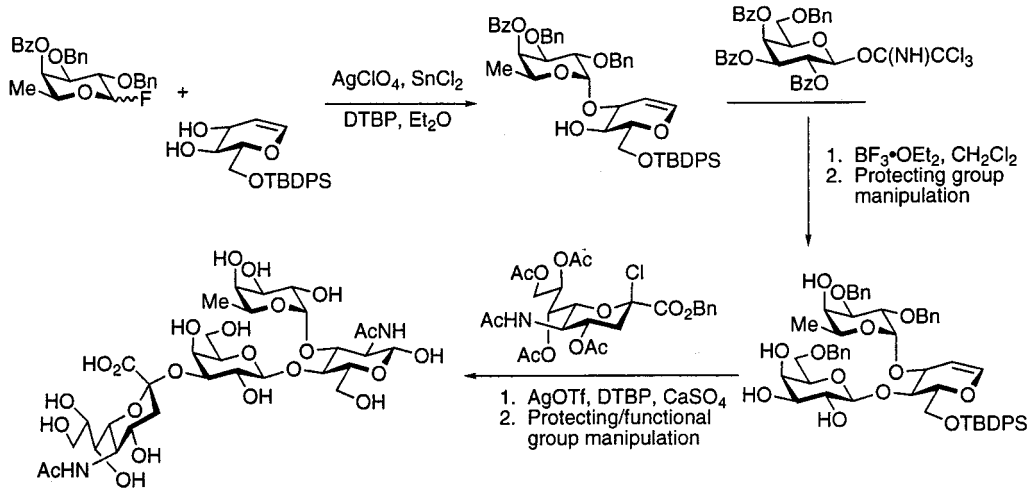
Shortly thereafter, the laboratories of Nicolaou and Wong[102a] reported a concise synthesis of sLe^x that featured the use of glycosyl fluorides to form glyco-

Scheme 3. Nicolaou and Wong's Synthesis of Sialyl Lewis^x [a]



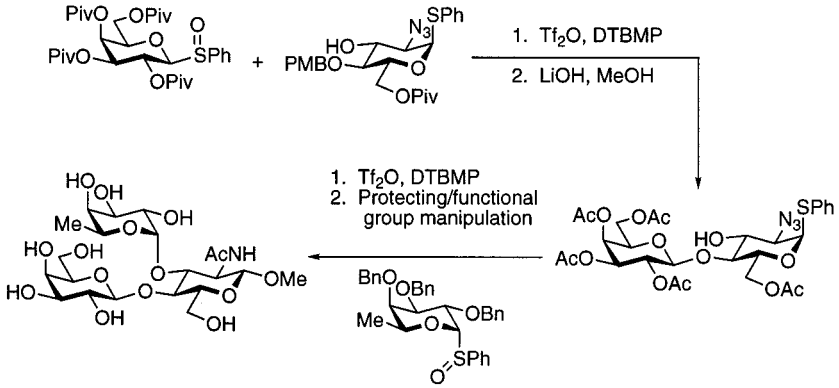
[a] Nicolaou and Wong used a variety of methodologies to complete the synthesis of sLe^x.[102]

Scheme 4. Danishefsky's Synthesis of Sialyl Lewis^x [a]



[a] Danishefsky and co-workers reported a synthesis that utilizes glycosyl fluorides, trichloroimidates, and Koenigs–Knorr methods starting with a glycal.[103]

Scheme 5. Kahne's Synthesis of Lewis^x [a]



[a] Continuing the search for universal glycosidation conditions, Yan and Kahne have prepared Le^x and Le^a using anomeric sulfoxides.[104]

sidic bonds to the galactose and fucose subunits. The special challenge presented by sialic acid was solved through the use of an anomeric chloride bearing an adjacent thiophenyl group[102b] to direct the stereochemistry of the new glycosidic bond. Desulfurization and deprotection affords the tetrasaccharide (Scheme 3).

Danishefsky's group[103] has contributed a synthesis that exploits a variety of glycosyl activation. A glycosyl fluoride and trichloroimidate were used to install the fucose and galactose subunits, respectively, while the sialic acid unit was appended using a Koenigs–Knorr procedure. A noteworthy feature of this synthesis is the use of a glucal as a precursor

to glucosamine via an azaglycosylation protocol (Scheme 4).

Kahne[104] efficiently prepared the Le^x trisaccharide through sequential glycosidic bond formation using anomeric sulfoxides with triflic anhydride activation (Scheme 5). Appending a sialic acid unit to the 3 position of the galactose with a different glycosylation method would complete the structure of sLe^x, the sulfoxide method, however, gave the undesirable β-isomer. In response to the biological importance of sialylated oligosaccharides, new methods for sialylation have been developed, including the use of thiophenyl donors from Hasegawa's laboratories and glycosyl phosphites, independently reported by the groups of Wong[105,106] and Schmidt.[107]

2. Chemoenzymatic Synthesis of Sialyl Lewis^x

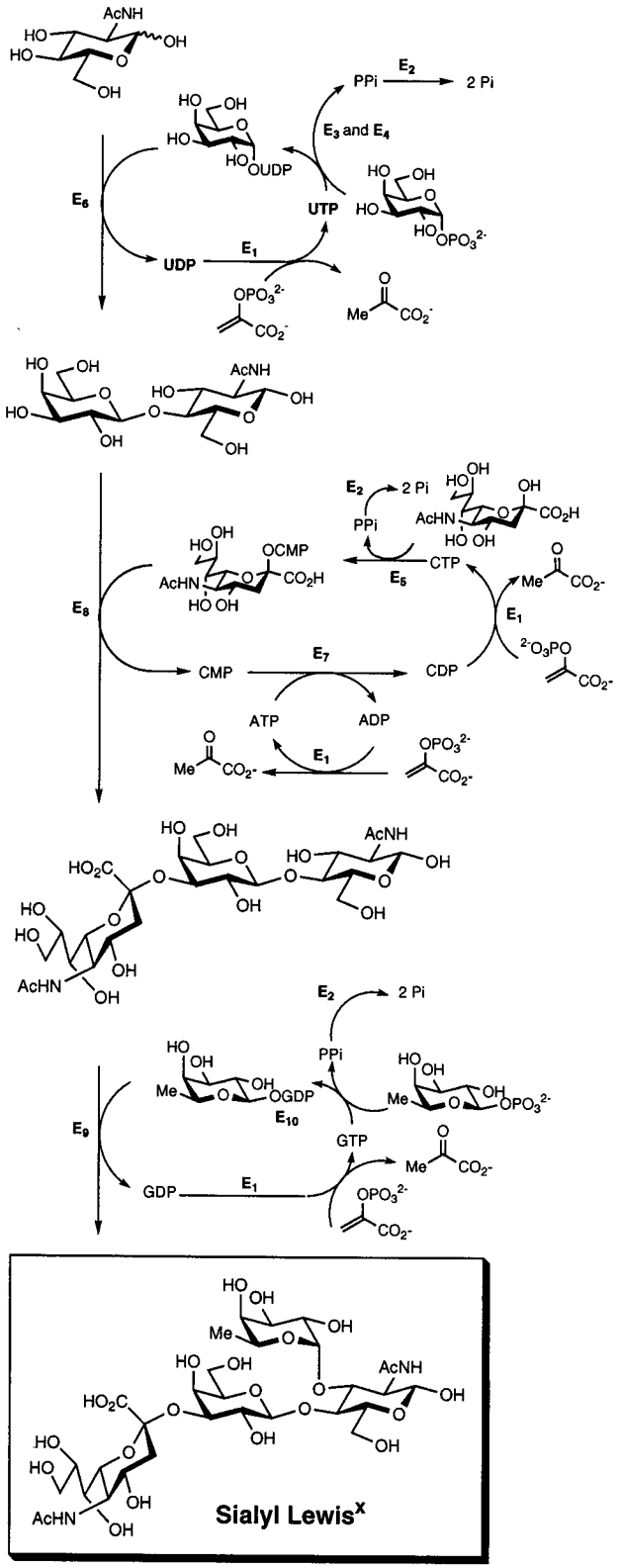
Despite the availability of effective glycosylation methodology, chemical synthesis generally suffers from the need to employ selectively protected sugars to control the position of bond formation and therefore large-scale synthesis becomes increasingly more expensive. This problem has been elegantly addressed through the application of enzymatic methods to oligosaccharide synthesis.[108] The ability to prepare oligosaccharides enzymatically has been greatly facilitated by both the growing availability of glycosyltransferases through recombinant biology[109] and the strategies developed for cofactor recycling[110] which have obviated the need for using stoichiometric amounts of the required sugar nucleotides and the problem of product inhibition. A multienzyme system used for the large scale production of sLe^x and the recycling of cofactors is shown in Scheme 6.[111] For enzymatic sulfation using phosphoadenosyl phosphosulfate (PAPS), a regeneration system has also been developed, which should be applicable to the large scale synthesis of sLe^x-6-sulfate.[112]

In cases where a suitable glycosyltransferase is not available, is unstable, or is prohibitively expensive, the reversibility of glycosidases can be exploited.[113,114] The chemoenzymatic synthesis of sLe^a using this strategy is shown in Scheme 7. An activated galactose donor is fused with 6-*O*-acetylglucal using β-galactosidase to afford a disaccharide glycal which may be chemically elaborated in a straightforward manner to the desired lactosamine for subsequent conversion into sLe^a using sialyltransferase and fucosyltransferase.[115a] Further improvements in the synthesis of *N*-acetyllactosamine using galactose oxidase/galactosidase[115b] and coupling of galactosidase with sialyltransferase[115c] have been reported.

B. Probing the Structural Details of Selectin–Sialyl Lewis^x Binding

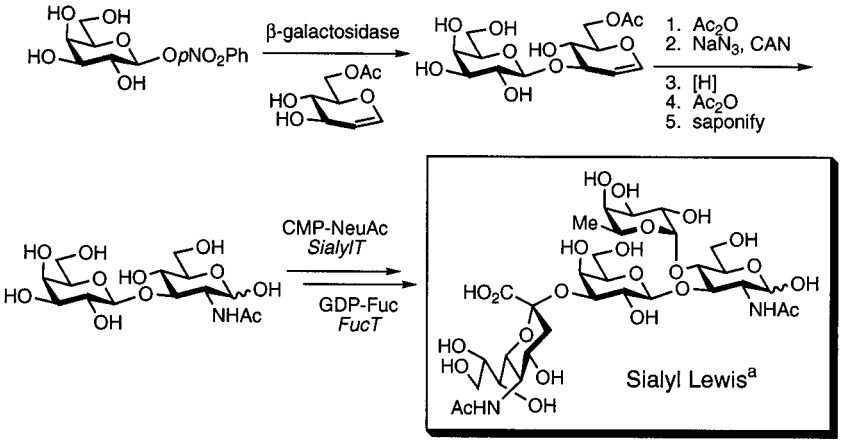
The identification of the functional groups critical for sLe^x binding to the selectins illustrates an important aspect of contemporary organic synthesis. While modern spectroscopic techniques have obviated the need for synthesis to establish proof-of-structure in most cases, these techniques lend limited insight into the relative importance of different structural features of the molecule, except in cases where the

Scheme 6. Enzymatic Synthesis of Sialyl Lewis^x [a]



[a] Using cofactor recycling, sLe^x can be prepared enzymatically in a single vessel using a 10-enzyme system: three transferases to make the glycosidic bonds (E_6 = β-1,4-galactosyltransferase, E_8 = α-2,3-sialyltransferase, and E_9 = α-1,3-fucosyltransferase); two kinases to generate the sugar nucleotide triphosphates (E_1= Pyruvate Kinase and E_7 = Myokinase); a synthetase (E_5 = CMP–NeuAc Synthetase), two phosphorylases (E_3 = UDP-Glc Pyrophosphorylase and E_{10} = GDP–Fuc Pyrophosphorylase), and an epimerase to prepare the substrates for the transferases (E_4 = UDP-Glc Epimerase); and a pyrophosphatase to drive the reactions (E_2 = Pyrophosphatase).[111]

Scheme 7. Chemoenzymatic Synthesis of Sialyl Lewis[a]



[a] In the chemoenzymatic synthesis of sLe[a], glycosidases and transferases are used to form the glycosidic linkages. Synthetic chemistry is used to elaborate the glycosidase substrate, a glucal, into GlcNAc.[115]

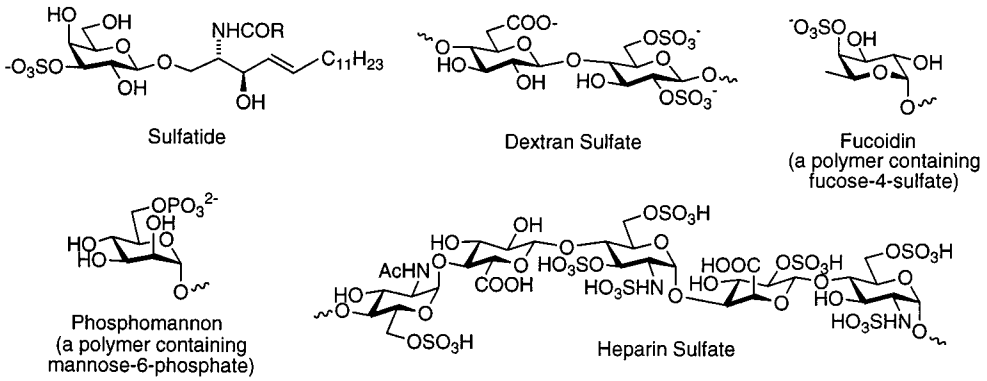


Figure 4. Fucoidin, phosphomannon, sulfatide, dextran sulfate, and heparin sulfate.

receptor·ligand complex can be determined. The synthesis of analogues incorporating functional group deletions and modifications has proven to be an invaluable method for gaining detailed information regarding the relationship between structure and function.

Efforts by the groups of Hasegawa[116] and Gaeta[117] are responsible for mapping a majority of the residues of sLe^x that are critical for binding. By systematically replacing the functional groups (OH, COO$^-$, or Me) with H, the relative contributions that these groups make to binding were determined and a "functional group map" was constructed. For sLe^x binding to E- and L-selectins, all three hydroxyl groups of the fucose,[116,117] the 2- and 6-hydroxyl groups of the galactose,[118] and the carboxylate of the neuraminic acid[116] are necessary (Figure 5). The GlcNAc residue does not play a critical role in these interactions, but is believed to be important for preorganizing the residues of the tetrasaccharide.[119–121] The binding to P-selectin is similar except that the 2- and 4- hydroxyl groups of fucose are not involved critically, and sulfation at the 6 position improves binding.[116] Table 2 reviews the results obtained for derivatives of the fucose group.

Labeled and unlabeled structures have proven useful for NMR studies to gain important conformational information.[111,122–124] Isotopically labeled sLe^x has been prepared by utilizing UDP-1-^{13}C-galactose with previously described procedures.[111] Stable un-

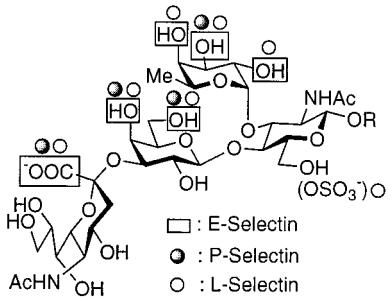
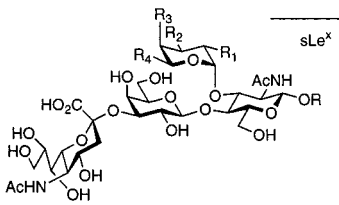


Figure 5. Structure/function map of sialyl Lewis^x. The functional groups that have been shown to be critical for binding to the selectins are identified. (See the text for additional details and literature citations.)

Table 2. Systematic Probing of the Fucose Contribution to Selectin Binding[a]



	R_1	R_2	R_3	R_4	Activity
sLe^x	OH	OH	OH	CH_3	——
	H	OH	OH	CH_3	inactive
	OH	H	OH	CH_3	inactive
	OH	OH	H	CH_3	inactive
	OH	OH	OH	H	5x less active

[a] To determine which groups were critical for binding, groups on sLe^x were systematically replaced with hydrogen and were evaluated for activity.

bound and E-selectin-bound conformations of sLe^x differ mainly in the orientation of the neuraminic acid residue (Figure 6).[111,123–124] The estimated en-

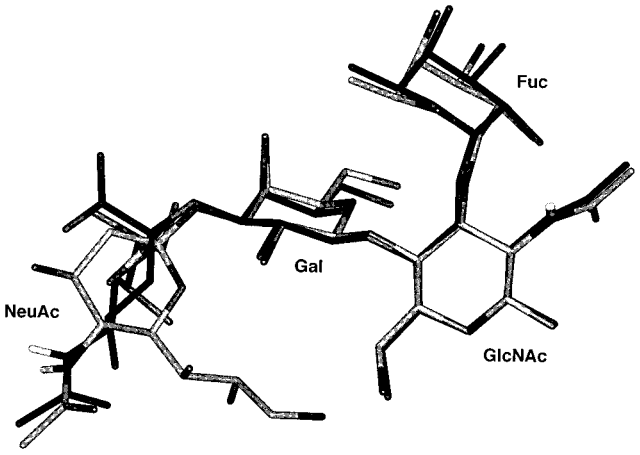


Figure 6. Bound/unbound conformations of sLe^x. Shown are a stable unbound conformation (black) and bound conformation (gray) of sLe^x with E-selectin. The dihedral angle of the glycosidic bond of NeuAc residue differs by 100°. This difference in conformation has been estimated to cost 1.5 kcal/mol.

ergy difference between these conformations is approximately 1.5 kcal/mol.[111] Recent studies by Poppe et al.[124] have shown that sLe^x adopts a similar conformation upon binding to either E- or P-selectin, but a different conformation (similar to a stable free conformation) upon binding to L-selectin. In addition to these efforts, crystal structures of Le^x and related compounds have been obtained.[125,126] Molecular dynamics calculations have been reported.[127−130]

III. Approaches to the Development of Sialyl Lewis^x Inhibitors

Cytel Corporation submitted a sLe^x pentasaccharide for clinical trials for the treatment of reperfusion injury. These compounds offered initially great promise,[131−133] showing some clinical benefit in the treatment of reperfusion injury following coronary bypass surgery. In phase II clinical trials testing the ability of this compound to treat reperfusion injury following myocardial infarction was shown to be safe, but with an activity equivalent to that of placebo. However, a positive phase II result was reported for the treatment of reperfusion injury during cardiopulmonary bypass surgery. Currently, another phase II trial has showed positive results in children for treating reperfusion injury following surgery for the repair of congenital heart defects. Cytel's ability to produce the sLe^x tetrasaccharide is a reflection of the state of the art in chemoenzymatic synthesis and cofactor regeneration strategies. The challenges met by their candidate in clinical trials has focused increased attention to the growing field of glycobiology and more specifically, to small molecule antagonists. Dimeric Le^x has also been shown to be effective against rheumatoid arthritis.[134]

The search for sLe^x antagonists can be discussed in terms of two related goals: simplification of the sLe^x structure to small molecules that are stable, inexpensive, and easy to prepare, and preservation (and ultimately enhancement) of the binding affinities and specificities of small molecules mimetics.[135] These goals have been met, in part: a tremendous number of potential selectin antagonists containing only a single carbohydrate have been described; inhibitors that have affinities equal to or exceeding that of sLe^x have been obtained.

The search for small-molecule sLe^x antagonists has proceeded in a reductionist fashion. Our discussion apes this approach. That is, with the identification of the residues critical for binding, efforts have focused on removing one or more sugars from the tetrasaccharide and replacing it with a variety of more stable linkers. We begin with inhibitors in which NeuAc has been replaced by a simple anion, then move to those inhibitors in which two carbohydrates (either NeuAc and GlcNAc, or GlcNAc and Gal) have been removed. We conclude with a discussion of inhibitors comprising a single carbohydrate. The addition of secondary groups and the use of polyvalent display are discussed separately. Along the way, we mention general trends that we have noticed in our study of the field, and indicate issues that have not been addressed completely. These dialogues are intended to represent our knowledge-based intuition of this field and are included so that the interested reader outside this discipline can rapidly access our current state of understanding.

A. Deletion of NeuAc[136−138]

One of most straightforward substitutions of sLe^x is to substitute NeuAc with a negatively charged group on the 3 position of galactose (Figure 7).

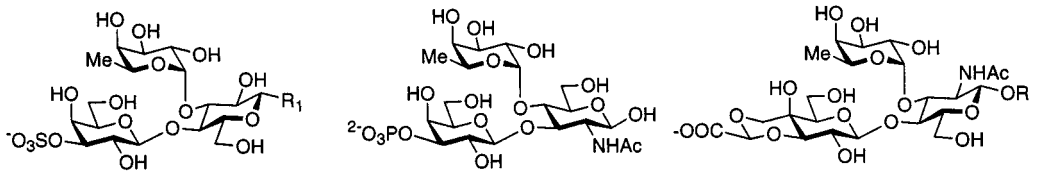
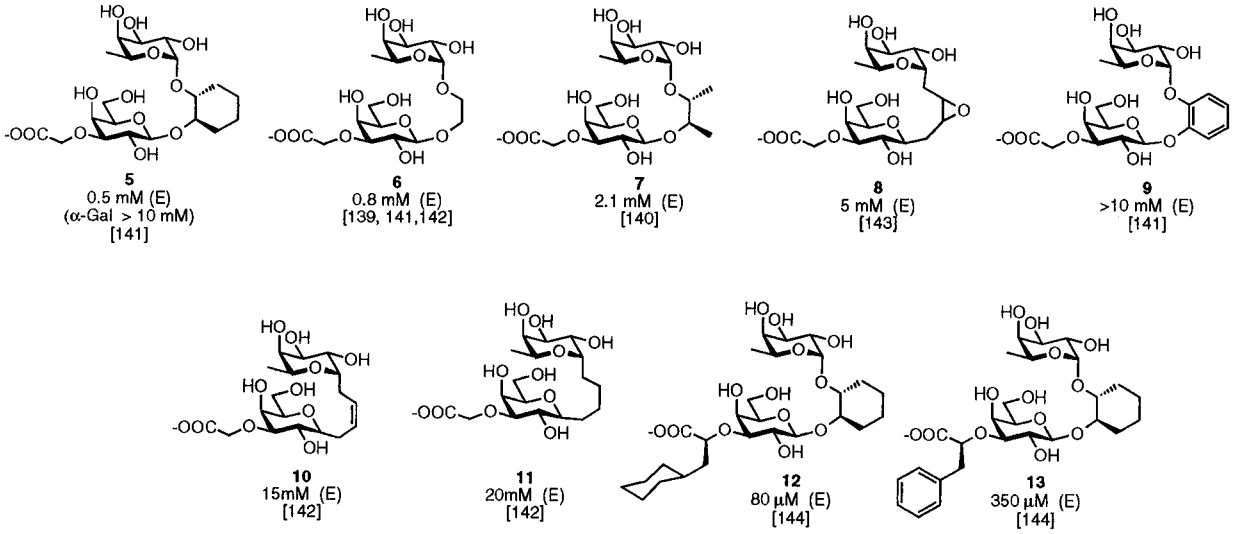


Figure 7. Trisaccharides by Hasegawa, Kiessling, and Thoma. Replacements for NeuAc include sulfate groups (yielding the known sulfo-Le^x/sulfo-Le^a natural ligands), phosphate groups, and rigid carboxylates. The most common substitution, however, is "CH_2COO^-" (see the text for more details). IC_{50} for sLe^x = 0.8 mM (E), 8.0 mM (P), 4.0 mM (L).

Table 3. Deletion of NeuAc and GlcNAc[a]



[a] NeuAc has been replaced with a variety of anions: "$CHCOO^-$" is the most common. 1,2-*trans*-Cyclohexanediol is a common and effective replacement for GlcNAc, a residue that incorporates no groups critical for binding.

Sulfation leads to sulfoLe^x, a known ligand for both E- and L-selectin. The most common substitution utilized is alkylation of the 3′ position with a "CH_2COO^-" equivalent (vida infra). The relationship of acidity of these groups and binding affinity has not been addressed rigorously.

Hasegawa and Kondo have generated mimics of Le^x, **1** and **2**, that bear phosphate and sulfate groups on the 3 position of Gal. These molecules show binding affinities similar to sLe^x.[136] Kiessling et al. prepared a phosphorylated derivative of Le^a, **3**, using a tin catalyst and dibenzylphosphoroiodate. This molecule binds to L-selectin slightly better than 3′-sulfoLe^a and 3′-sulfoLe^x. Molecule **3** binds to E-selectin with an affinity equal to that of 3′-sulfoLe^a, and both bind 20-fold better than 3′-sulfoLe^x.[137] Thoma and colleagues chose to substitute NeuAc with a rigidly fixed carboxylate; however, compound **4** showed no activity for E-selectin up to 10mM.[138]

B. Deletion of NeuAc and GlcNAc (5–13)[136,139–144]

Having established that a "CH_2COO^-" on the 3 position of galactose adequately mimics NeuAc, many groups turned their attention to replacing the GlcNAc. While this group contains none of the functional groups critical for binding, it is likely to be important for preorganizing the tetrasaccharide. Of the replacements for GlcNAc, a cyclohexyl group (as in **5**, Table 3) appears to best mimic both the shape and rigidity of the pyranose ring. It is surprising that ethanediol (**6**) is almost equally effective. The difference between these groups, however, becomes more pronounced in derivatives in which Gal has also been removed (*vida infra*).

The linking group chosen to replace GlcNAc rarely improves the binding constant: we believe that *trans*-1,2-cyclohexanediol is an energetically neutral substitution. The limited data available also suggests that there may be a 20× difference between O- and C-glycosides, but the generality of this trend has not been established.

The most effective mimic for E-selectin of this class, **12**, was reported recently by Banteli and Ernst from Novartis.[144] This molecule incorporates a novel, alklylated derivative of the "CH_2COO^-" group, and binds with 8-fold higher affinity than either **5** or sLe^x. While the generality of this substitution remains to be established, this work may reopen an old avenue of investigation.

C. Deletion of GlcNAc and Gal[145–149]

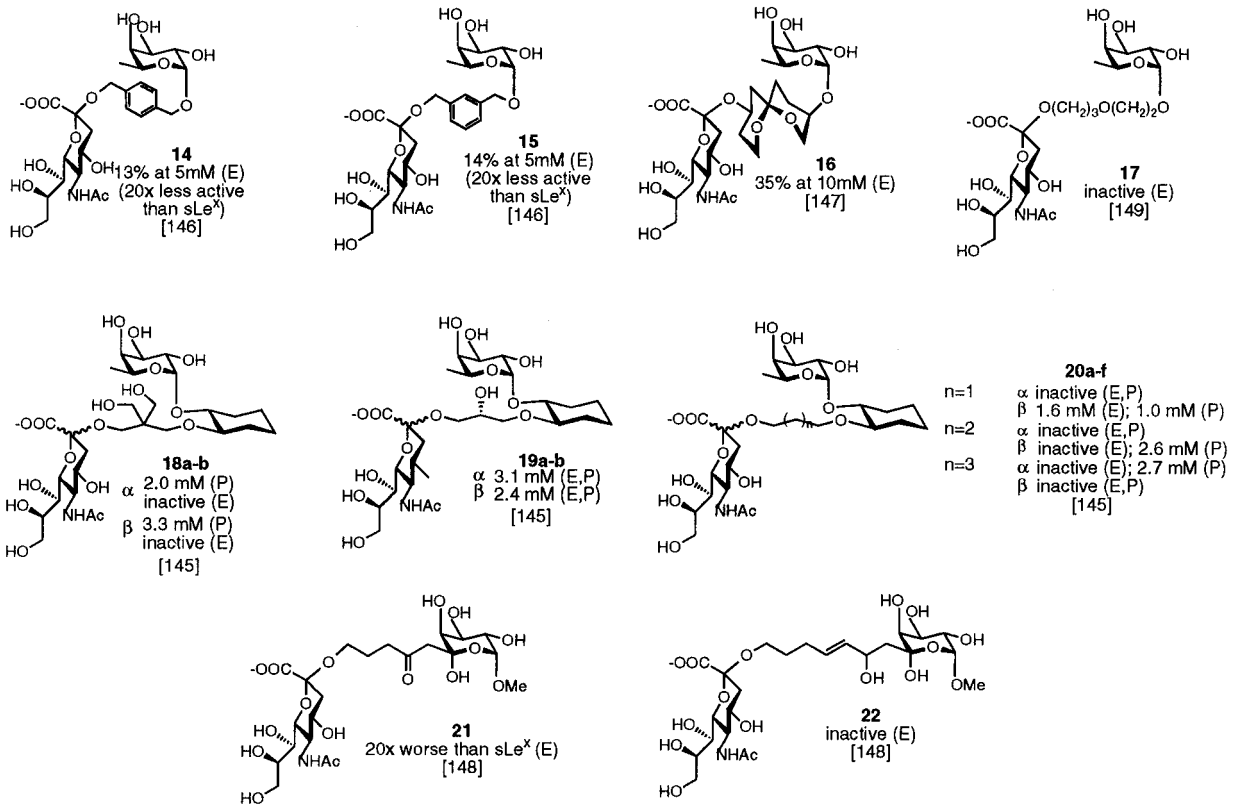
The molecules shown in Table 4, **14**–**22**, reinforce our understanding of the importance of the hydroxyl groups of galactose, and the cost of conformational freedom of linker groups. The affinity of these molecules is reflected loosely in the number of hydroxyl groups presented. That is, the tightest binding molecules, **18** and **19**, each present at least one hydroxyl group. In addition, these molecules incorporate a *trans*-cyclohexanediol group to mimic GlcNAc.

Molecules **14**–**17**, **20**, and **21** do not include any hydroxyl groups. Of those that show binding to the selectins, the tightest comes from **14** and **15** which incorporate rigid phenyl linkers, or from **20b**, which has an IC_{50} of 1.6 mM. The activity of **20b** is surprising given the inactivity of the other molecules in the series. In general, molecules **14**–**20** show greater affinity for P-selectin than E-selectin. Two molecules (**21** and **22**) which substitute galactose for fucose are also shown.

D. Deletion of NeuAc, GlcNAc, and Gal: L-Fucose-Based Inhibitors

Inhibitors containing a single carbohydrate comprise the largest number of published inhibitors investigated to date. In all of these molecules the fucose group has been retained, or replaced with either mannose or galactose: all of these simple sugars have three of the six functional groups necessary for selectin binding contained in the fucose group of sLe^x. While additional functional groups (two

Table 4. Inhibitors Comprising L-Fucose and Sialic Acid[a]



[a] Inhibitors comprising NeuAc and Fuc contain four of the six groups critical for binding. Of the inhibitors described, those which incorporate additional hydroxyl groups show greater affinities for the selectins over those with no hydroxyl groups. Molecules with more rigid groups linking NeuAc and Fuc, in general, show greater affinities than those with flexible linkers.

hydroxyls of Gal, the anion of NeuAc) have been incorporated using a variety of linkers, polyaryl and polyamide linkers being the most common.

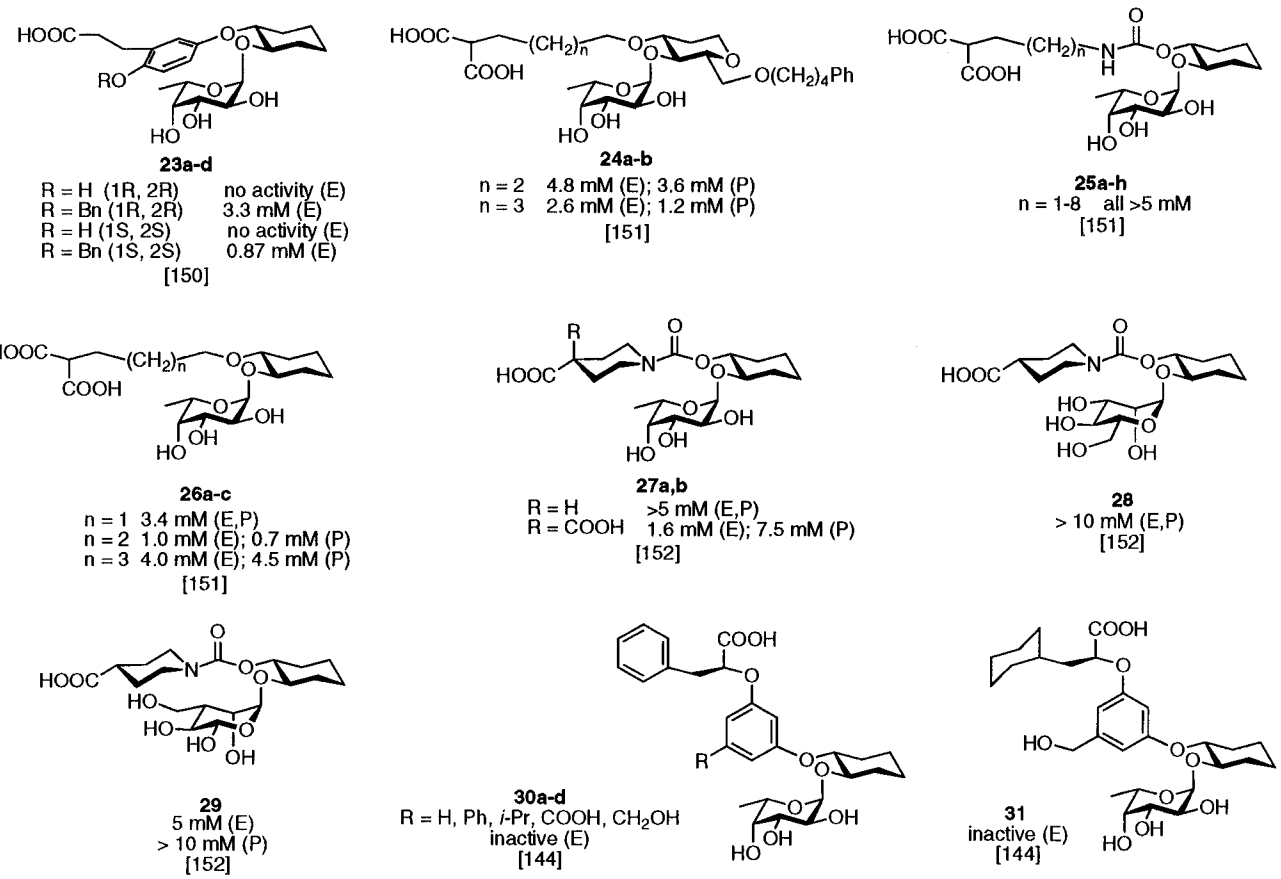
Liu and colleagues used *trans*-cyclohexanediol to replace GlcNAc and a rigid aromatic linker to install the anion of NeuAc (Table 5). Molecules **23b**,**d** showed binding affinities similar to that of sLe^x.[150] Surprisingly, debenzylation to reveal a hydroxyl group (that might mimic those of Gal) leads to a complete loss of activity (**23a**,**c**). Investigators at Hoechst recently reported derivatives containing malonic acid[151] (**24**−**26**) and piperidinecarboxylic acid[152] (**27**−**29**). While the former show binding affinities for E-selectin that are similar to that of sLe^x, it is unclear whether the extra carboxylate mimics the hydroxyl groups of Gal. Investigators at Novartis used their alkylated "CH_2COO^-" equivalent to generate a related series of molecules (**30**−**31**) which replace Gal with a substituted aromatic ring: none showed any activity.[144] In the following sections, we discuss three related classes of fucose-based mimetics: the glycopeptides of Wong and others, the glycoaromatics of Kogan, and the combinatorial libraries of Armstrong and Wong.

1. Glycopeptides of Wong[153−157]

Figure 8 shows the two design elements (*turns* **32**−**38** and *hydroxyls* **39**−**43**) that were varied in inhibitors **44**−**65** (Table 6). First, groups immediately attached to fucose were evaluated for their ability to mimic GlcNAc by orienting the remaining residues in the proper direction. In general, activity increased with rigidity: **32** < **33** < **34**...**38**. Second, groups **39**−**43** containing hydroxyl groups designed to mimic galactose were investigated. In general, those having two hydroxyl groups were tighter binding than those with one hydroxyl group: **39** < **40** < **41**...**43**. Glutaric acid (or a related diacid) was used to install the anionic group designed to mimic the carboxylate of NeuAc. To probe the effect of aromatic substitution on the activity of **53**, a series of aryl-substituted fucopeptides related to **53a** were prepared using Ru-catalyzed crossed olefin metathesis.[157b] It was found that the biphenyl-substituted mimetic showed greater inhibition than the corresponding phenylpropyl group. In addition, a library approach has been developed to optimize the activity of **56b** and it has been found that **56c** is very active against P-selectin (IC_{50} = 17 μM). When the free $-CO_2H$ group of **56b** is replaced with a phosphonate $-PO_3$, the activities against E- and P-selectins are improved (1.3, 14, and 17 μM, respectively).[157c] Two other fucose-like anchors were investigated: L-galactose (**66**−**71**; Table 7) and D-mannose (**72**−**82**, Table 8).

This series of molecules includes some of the most active inhibitors prepared to date. Specifically, molecules **48d** and **59** show IC_{50}s of 45 and 50 μM, respectively. Rationalizing the source of these activities is difficult: molecules within this series differ substantially. Clearly the hydrophobic side chains and pair of hydroxyl groups of **48d** are important, as is the rigid backbone and pair of hydroxyl groups of **59**.

Table 5. Inhibitors That Contain Only Fucose or Mannose[a]



[a] The largest number of inhibitors explored to data comprise fucose with an aromatic or peptide side chain that contains a terminal anionic group.

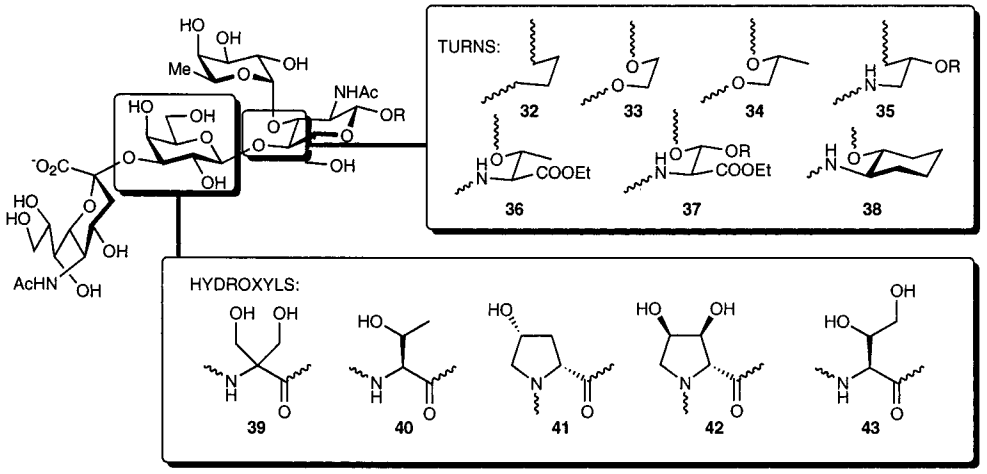
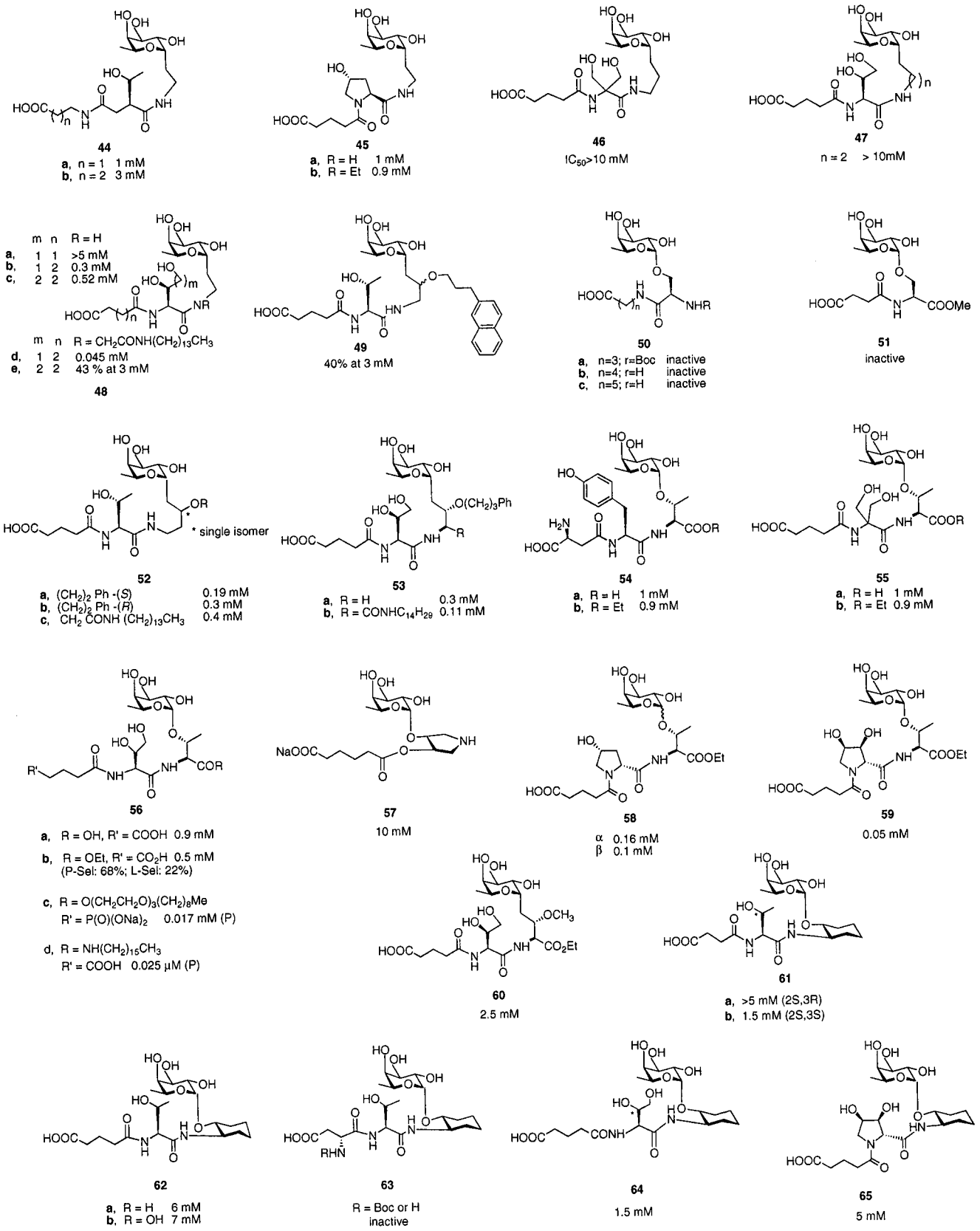


Figure 8. Groups designed to mimic *N*-acetylglucosamine and galactose.[153−157] The groups that were surveyed to replace GlcNAc (in forming turns) and Gal (in presenting hydroxyl groups are arranged crudely in order of least effective to most effective as judged by the affinities of the resulting inhibitors for the selectins.

The challenge of these efforts is illustrated by comparing **59** and **65**. From earlier studies, we might have expected the net replacement of cyclohexanediol for threonine to lead to increased binding. The lower affinity for E-selectin of **65** compared with **59** suggests that the dihydroxyproline group places new constraints on these inhibitors, contradicting our accumulated intuition. While in this case the geometric change is dramatic (proline rings are large), more subtle variations could be completely overlooked. No rigorous energetic analyses have been done, and no derivatives of the sLe^x tetrasaccharide which replace GlcNAc with a threonine-derived linker have been prepared.

In general, incorporating two hydroxyl groups instead of just one leads to tighter binding molecules. Rigidifying one portion of the molecule may require relaxing a second part: it would be interesting to see if the affinities of **30** and **31** improve by replacing the cyclohexyl ring with a threonine-based linker.

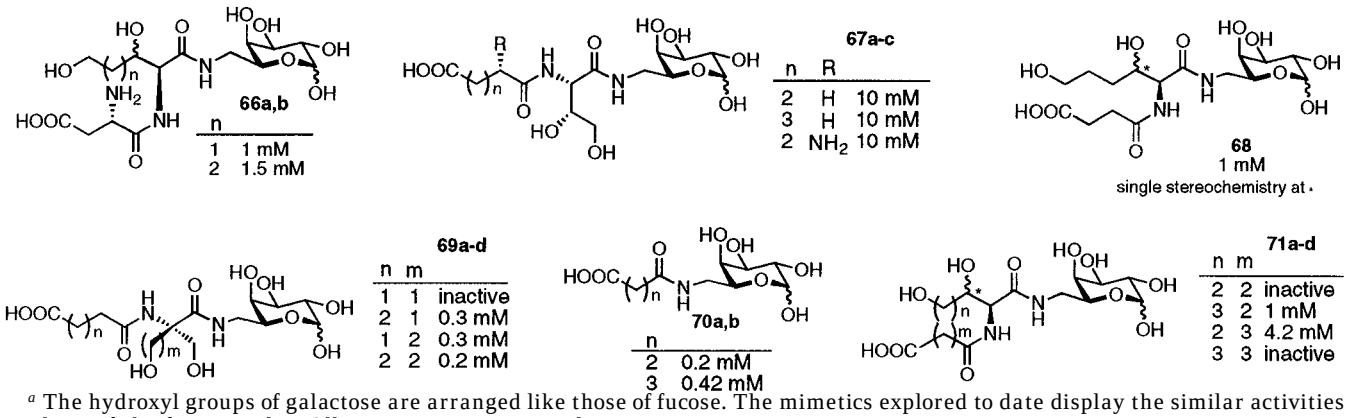
Table 6. Mimetics Based on Fucose with an Anionic Peptide[153−157,a]



[a] The activities of these *fucopeptides* are affected by the groups which mimic GlcNAc (groups **36**−**38** are best) and Gal (**42** and **43** are best). All testing values correspond to IC_{50}s using E-selectin unless otherwise mentioned.

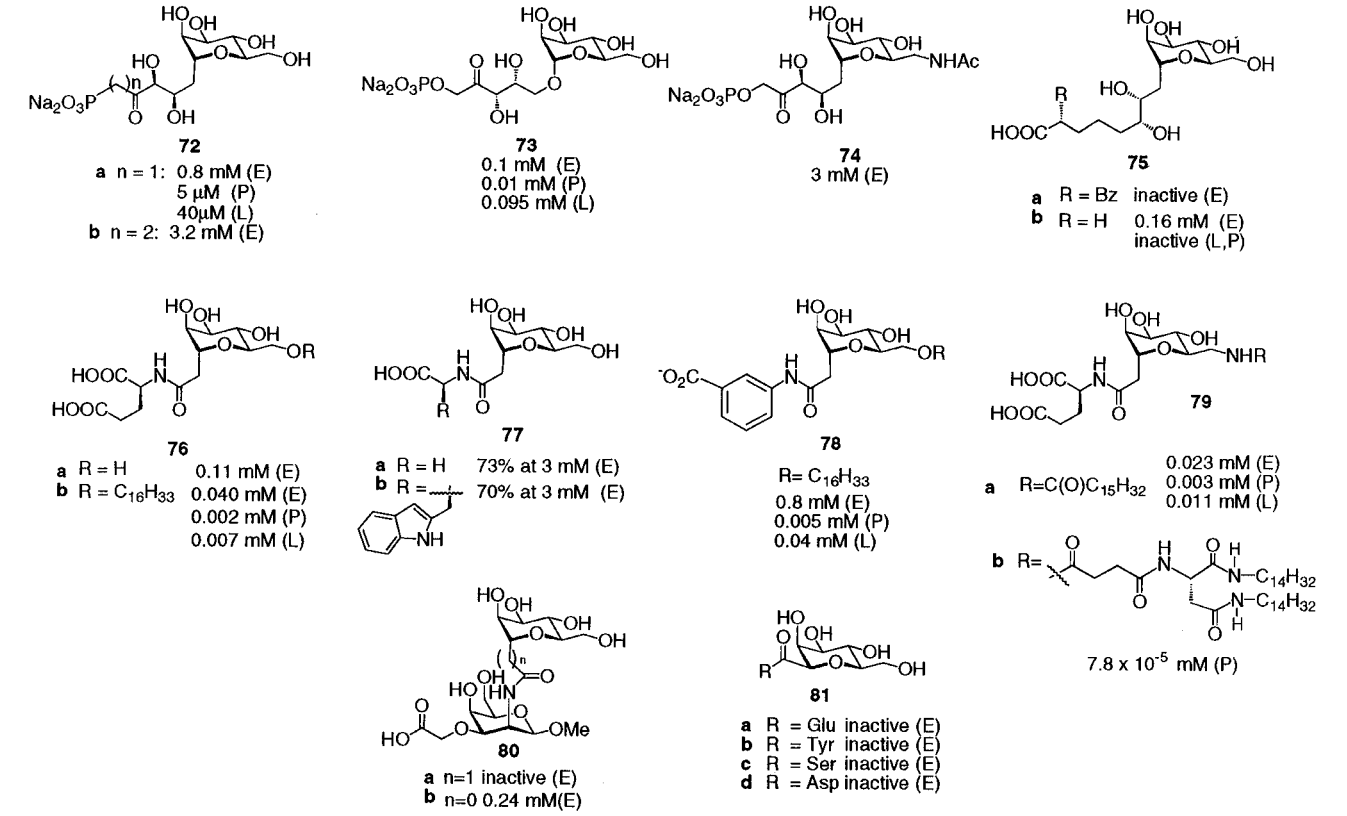
Using D-mannose or L-galactose in place of fucose leads to a series of related compounds with the similar affinities. In the case of the galactose-based mimetics (**66**−**71**), no "turn group" is required. "Turns" for the mannose-based mimetics (**72**−**81**) have not been explored. None of the galactose-based

Table 7. Mimetics Based on 6-Deoxy-6-amino-L-galactose with an Anionic Peptidic Tail[156,a]



[a] The hydroxyl groups of galactose are arranged like those of fucose. The mimetics explored to date display the similar activities as those of the fucopeptides. All tests are against E-selectin.

Table 8. Mimetics Based on a Peptidic Tail Linked to Mannose[155,157a,157d,c]



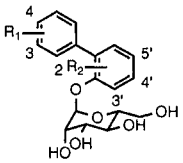
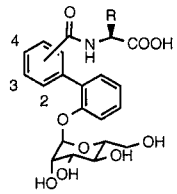
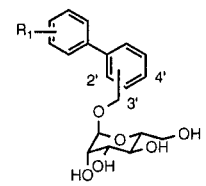
[a] Like fucose and galactose, mannose offers a similar arrangement of hydroxyl groups but is less expensive. Mimetics based on mannose have activities similar to or better than those based on fucose or galactose.

mimetics have achieved affinities less than 100 μM. Mannose-based mimetic **76b** has binding affinity that matches that of **48d** and **59**. New mannose-based mimetics in which the 6-OH group is replaced with different alkoxy chain lengths or acylated amine groups were found to be very potent with IC_{50} values in the low μM range, perhaps due to a hydrophobic effect.[155] The hydroxyacetone phosphates are also very good inhibitors, especially compound **73** ($IC_{50} \approx 0.1\ \mu$M for P-selectin). This enhancement of activity is perhaps due to the stronger interaction of the phosphate group with the nearby Lys residue or due to the formation of a Schiff base with a Lys residue.[155]

2. Glycoaromatics of Kogan[158,159]

Mannose is used as an anchoring group for three closely related classes of molecules (**82**−**84**) pursued by Kogan and colleagues (Table 9). While Kogan's strategy recognizes the importance of the hydroxyl groups of fucose and the carboxylate of NeuAc, none of his inhibitors incorporate groups that mimic the hydroxyl groups of galactose. Consistent with this fact, most inhibitors are 20 times weaker binding than sLe^x. Most compounds show similar specificity to both E- and P-selectins. Molecule **84h**, the best lead reported to date, shows 3 times better binding than sLe^x to E- and P-selectins. These molecules are

Table 9. Glycoaromatics of Kogan[a]

	R_1, R_2 =	E-Selectin	P-Selectin	L-Selectin
 **82**	a 3-CH_2CO_2H	3	2	2
	b 3-CO_2H	2	2.6	1
	c 4-CO_2H	16	0% at 27mM	15% at 6mM
	d 4-CH_2CO_2H	14.6	0% at 27mM	0% at7mM
	e 3-OCH_2CO_2H	4.9	4.9	17
	f 4-OCH_2CO_2H	6.1	20	20% at 6mM
	g 3-$CH_2OCH_2CO_2H$	9.5	0% at 24mM	30% at 6mM
	h 3-CH_2CN	7	40% at 23mM	1.5
	i 3,5-$(CH_3)_2$-4-OCH_2CO_2H	8	6.9	0% at 7mM
	j 3-CO_2CH_3	lysis	lysis	lysis
	k 3-H	lysis	lysis	lysis
	l 3-OCH_2CO_2H-5'-Me	14	-	-
	m 2-OCH_2CO_2H	20	20% at 25mM	0% at 8mM
	n 5-OCH_2CO_2H-2-Me	20.2	12	-
	o 3-CH_2CN_4H	17% at 40mM	-	-
	p 3-$NHCOCO_2H$	0% at 6mM	-	-
	q 3-OCH_2CO_2H-2-Me	0% at 20mM	0% at 20mM	0% at 8mM
	r 3-$CH(CH_3)CO_2H$	0% at 40mM	20	40% at 6mM
	s 3-$CH(Ph)CO_2H$	70% at 17mM	17	0% at 6mM
	t 3-$NHCH_2CO_2H$	lysis	lysis	lysis
	u 3-CH_2COMe	lysis	lysis	lysis
	v 4-$CO(CH_2)_2CO_2H$	40% at 20mM	0% at 20mM	0% at 20mM
 **83**	a 4-$CONHCH_2CO_2H$	4.6	0% at 20mM	0% at 6mM
	b (*S*)-4-$CONHCH(CH_2Ph)CO_2H$	3.8	0% at 20mM	0% at 5mM
	c (*S*)-3-$CONHCH((CH_2)_2CO_2H)CO_2H$	15.8	-	15% at 5mM
	d 4-$CONHCH((CH_2)_2CO_2H)CO_2H$	32	35% at 4mM	0% at 1mM
	e 4-$CONH(CH_2)_2CO_2H$	36	0% at 3mM	0% at 1mM
	f (*R*)-4-$CONHCH(CH_2Ph)CO_2H$	11.5	-	-
	g (*S*)-4-$CONHCH((CH_2)_4NH_2)CO_2H$	33% at 27mM	-	-
	h 4-$CONHCH(CH_2CO_2H)CO_2H$	0% at 16.3mM	2	5
	i 3-$CONHCH_2CO_2H$	0% at 18mM	25% at 23mM	0% at 6mM
	j (*S*)-3-$CONHCH(CH_2CO_2H)CO_2H$	0% at 32mM	40% at 20mM	0% at 5mM
	k (*S*)-3-$CONHCH(CH_2Ph)CO_2H$	14% at 1mM	5% at 20mM	0% at 6mM
	l 3-$(CH_2)_2SO_3H$	20	-	-
	m 3-$(CH_2)_3PO(OEt)OH$	34.4	12.4	-
 **84**	a 3-CH_2CO_2H 2'	>>14	-	
	b 3-CH_2CO_2H 3'	0.4	1.2	
	c 3-CH_2CO_2H 4'	>>1.5	>>2.3	
	d 3-CO_2H 3'	1.7	2.5	
	e 3-OCH_2CO_2H 3'	4.8	4.8	
	f 4-CH_2CO_2H 3'	>14	>20	
	g 4-OCH_2CO_2H 3'	4.8	4.8	
	h 4-OCH_2CO_2H-3,5-Me 3'	0.3	0.5	
	i 3-$CH_2CO_2CH_3$ 3'	lysis	lysis	
	j 4-$CO(CH_2)_2CO_2H$ 3'	>6.7	>>10	
	k 3-OH 3'	lysis	lysis	

[a] Kogan and co-workers identified the importance of the fucose group and prepared substituted biphenyl glycosides. Activities are reported as IC_{50}s (in mM) or % inhibition at a stated concentration (mM).

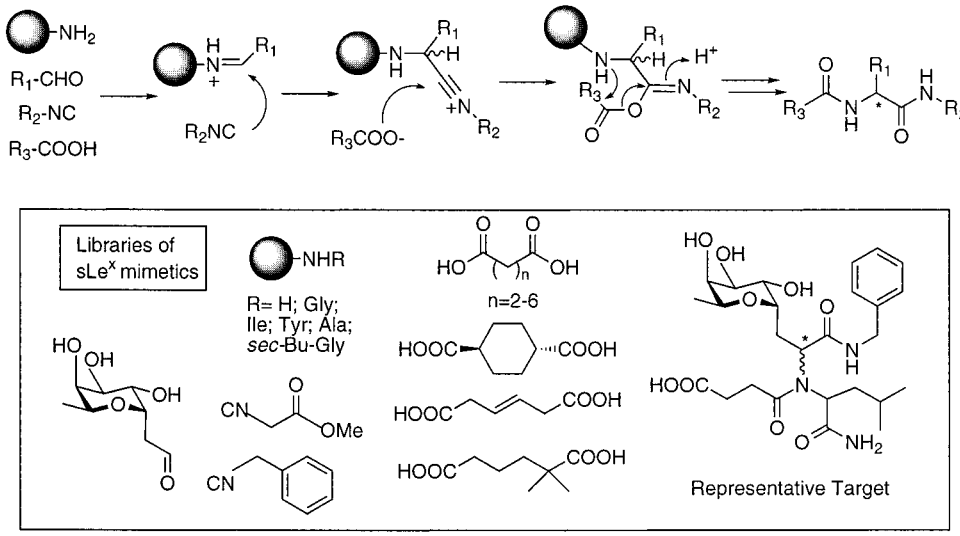


Figure 9. Armstrong's pool of mimetics. Armstrong et al. recognized the utility of the Ugi reaction in preparing libraries of mimetics. The reactive pool used is shown. No binding constants have been reported to date.

easily prepared and are potential leads for pharmaceutical agents. Derivatives that incorporate groups that mimic the hydroxyl groups of Gal would presumably increase binding by up to 10 times, but the incorporation of such groups may not be straightforward.

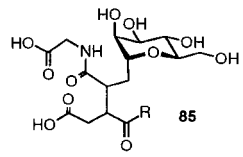
3. Combinatorial Libraries of Armstrong and Wong[160−162]

While the search for mimetics of sLe^x has identified potential leads thorough SAR studies, other efforts have focused on the generation of templates for combinatorial chemistry and the application of new methodologies for combinatorial synthesis. Armstrong and colleagues[160] showed that the Ugi reaction[161,162] could be used to make a variety of glycopeptide-like structures rapidly (Figure 9) by using the condensation of an aldehyde, primary amine, isonitrile, and carboxylic acid. This reaction offers greater diversity than previously used two-component condensations and the advantage/disadvantage of the generation of a new chiral center. Armstrong et al. reported no binding constants for these molecules. Wong et al. have exploited the two-component reaction and the four-component Ugi reaction by using a poly(ethylene glycol) amine as one component to generate a series of potential ligands (**85**−**86**; Table 10).[163] Some of these molecules show good activities against E- and P-selectins.

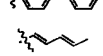
E. Secondary Binding Groups

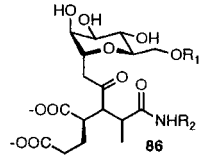
The best mimetics produced to date have eliminated labile glycosidic linkages, and bind to the selectin with affinities similar to that of sLe^x. To enhance the binding affinities of these molecules to the selectins, secondary groups have been incorporated to exploit additional binding regions on the selectins. The most common positions for installing these groups are in the GlcNAc moiety by acylation of nitrogen or additions to the reducing end. *N*-Acylation of glucosamine residues increases binding 3−10 times, with naphthoyl groups giving the best result. Hydrophobic tails added to the reducing end typically increase binding from 3- to 10-fold; tails added to the 6-OH of mannose increase binding by 10^3-fold.[155] The computational work of Hasegawa and colleagues have identified a potential role of these hydrophobic groups: instead of forming micelles, these chains appear to fit into a hydrophobic cleft running down the side of the selectins.[130] In

Table 10. Mimetics from a Multicomponent Reaction[a]

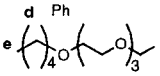
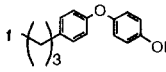


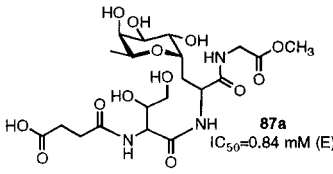
85

	R	% inhibition (E) at 3 mM
a	CH_3	28%
b	CF_3	42%
c	C_5H_{11}	52%
d	$C_{13}H_{27}$	32%
e	Ph	38%
f		42%
g		51%
h		48%
i		inactive
j		inactive
k		inactive

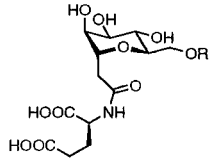


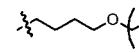
86

	R_1	R_2	IC_{50} (mM) E	P
a	H	CH_2COOMe	1.0	0.007
b	Ph	"	2.8	0.04
c	Ph	CH_2CH_2Ph	>3	0.08
d	Ph	CMe_3	>3	0.09
e		"	0.3	0.001
f		"	0.6	0.03

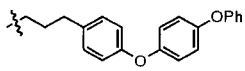


87a IC_{50}=0.84 mM (E)



87b R= 

IC_{50} = >3 mM (E), 0.02 mM (P)

87c R= 

IC_{50} = 0.3 mM (E), 0.03 mM (P)

[a] Application of the two-component and the four-component Ugi reaction by Wong et al. for the synthesis of mimetics led to the discovery of new active novel structures. The glycine moiety of **85a**−**k** was introduced as a poly(ethylene glycol) derivative in order to facilitate the product isolation by precipitation with ether.

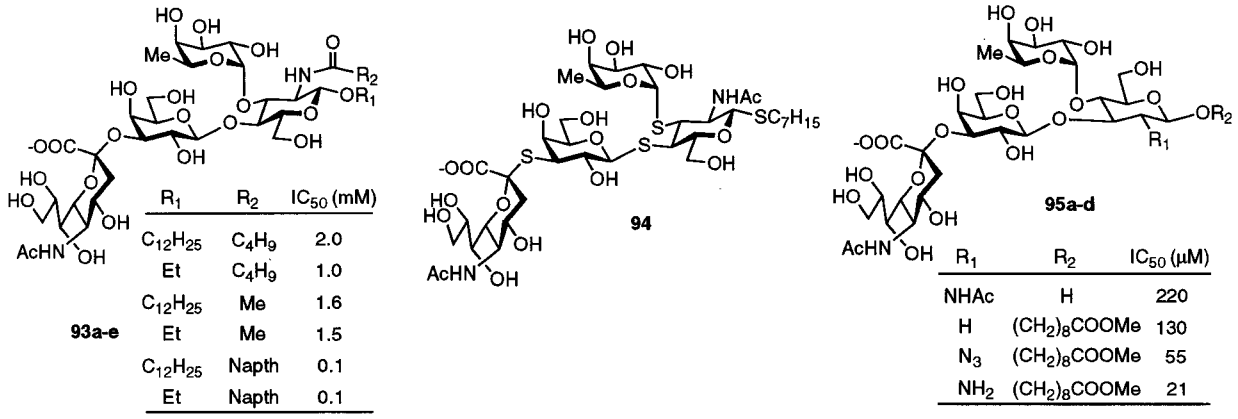
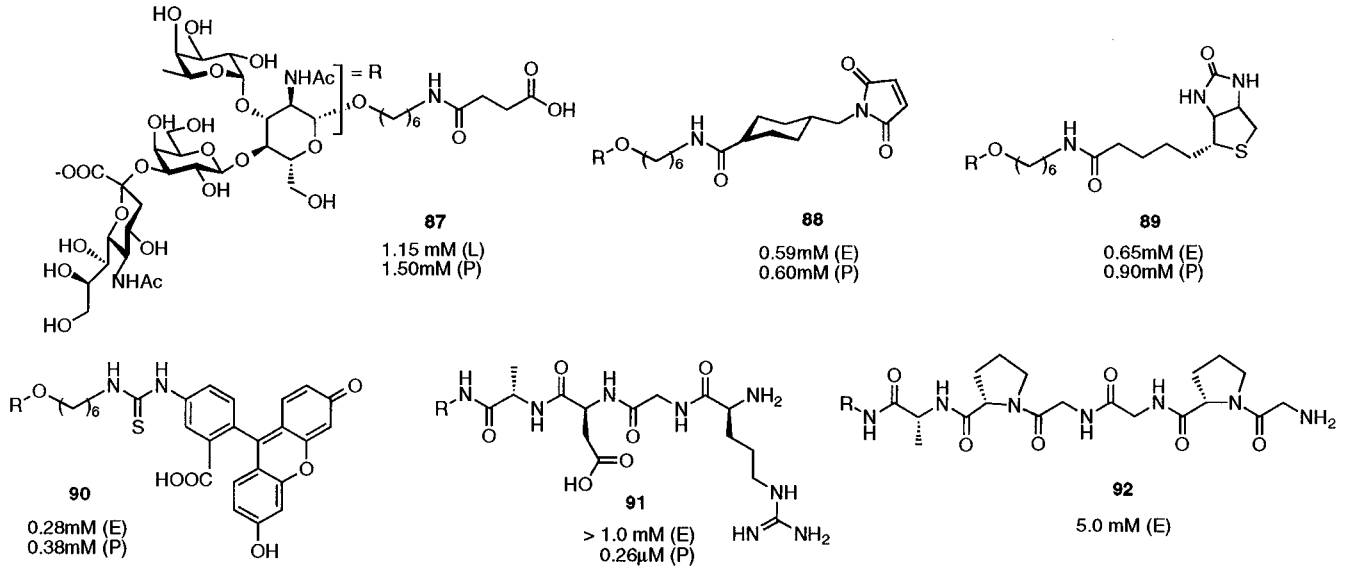


Figure 10. A variety of novel derivatizations of the sLe^x tetrasaccharide have been reported.[166]

Table 11. Attachment of Different Groups to the Reducing End of sLe^x [164]



addition, the use of an α-keto phosphophate group also enhances binding as described previously.

Sprengard et al.[164] have prepared derivatives of sLe^x in which the reducing end is modified with tethers that incorporate biotin or a fluorescent groups that may find use in analytical applications (**87**−**92**; Table 11). Kunz has prepared glycopeptides, including one that contains the sLe^x group attached to RGD (**92**) that inhibits P-selectin−HL60 cell aggregation at 260 nM.[165] It is not clear why the RGD group would enhance binding as ICAM-1 does not recognize the RGD motif. It is possible that the peptide binds to a secondary group on P-selectin. These structures were obtained by condensing the protected peptide with a partially protected sLe^x amine.

Hayashi and co-workers have investigated derivatives of sLe^x in which the reducing carbon contains hydrophobic tails, and derivatives in which the NAc group of the GlcNAc residue has been replaced with other *N*-acyl groups (Figure 10).[166] They find that *N*-naphthoyl groups increase binding affinities by almost 10-fold over that of *N*-Ac or *N*-buturyl (**93**). Similar studies were conducted by DeFrees et al. at Cytel.[167]

Eisle et al. replaced all the glycosidic linkages of sLe^x with thioglycosidic linkages (**94**).[168] No binding or stability data has been reported to date. Nelson et al. have explored the role of the *N*-acetyl group of GlcNAc in both sLe^a and sLe^x by replacing it with amine, azide, or N-propionyl groups, while leaving the reducing end as a free OH, as a methyl glycoside, or an 8-(methoxycarbonyl)octyl glycoside.[169] They found that the 8-(methoxycarbonyl)octyl glycosides bound twice as well as sLe^x or sLe^a. In addition, molecules in which the NAc was replaced with an amine or an azide were more active. The most potent molecule, **95d**, was 36-fold more active than sLe^x.

Efforts in our laboratory have focused on the combined enzymatic and solid-phase syntheses of glycopeptides bearing sLe^x.[170] The mucin domain of CD34, a 11 amino acid domain bearing a serine linked sLe^x, has recently been completed.[171] The solid-phase synthesis of a fucose-containing library has been achieved and some members have been found very active against P-selectin.[157]

F. Polyvalency

Much recent effort has gone into preparing multimers of sLe^x in hopes of mimicking Nature's use of polyvalency.[172] The route for the exploration of polyvalency mirrors that applied to monomeric in-

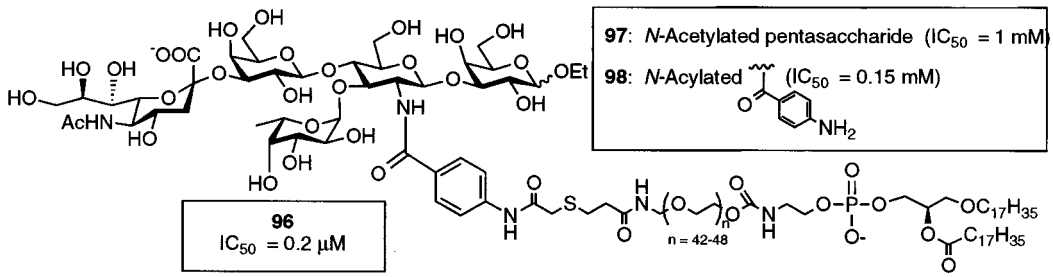


Figure 11. Cytel's liposome components. Investigators at Cytel successfully prepared liposomes presenting numerous copies of sLe^x. Controls comprising an acylated sLe^x amine have much lower affinity than Cytel's liposomes for E-selectin.

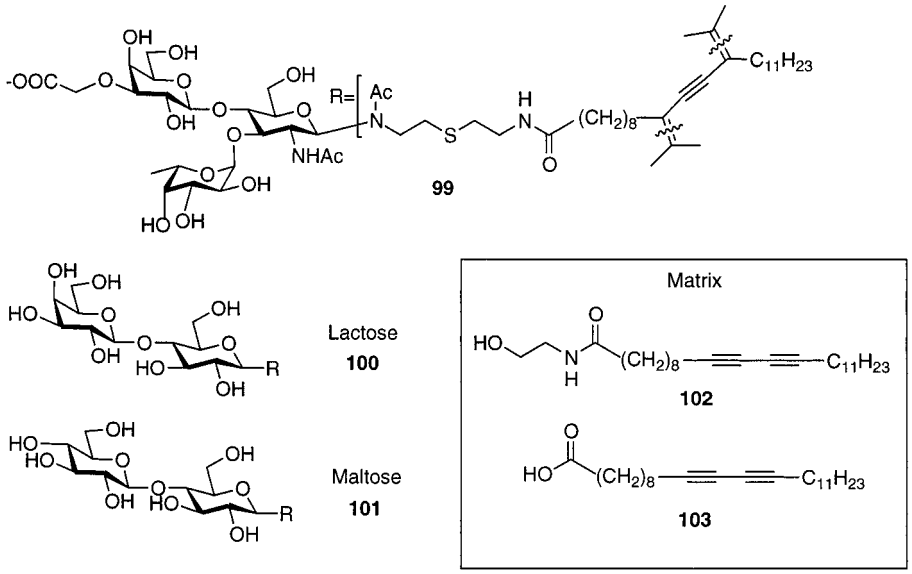


Figure 12. The liposomes of Nagy. Nagy and co-workers demonstrated the utility of polymerizable liposomes for the multivalent display of sLe^x. These assemblies had activities only slightly greater than those containing either lactose or maltose: all recognize the selectins at nM concentrations with little selectivity.

hibitors. That is, most of the work reported to date focuses on the incorporation of sLe^x or related, naturally occurring carbohydrates into polymeric displays. The challenge of these studies, especially when smaller oligomers (i.e., dimers, trimers, and tetramers) are targeted, is the choice of groups linking the binding regions of the molecule together: the structure/density of the carbohydrate groups in the natural system is unknown. In general, multivalent displays comprising small numbers of monomers—i.e., dimers and trimers—rarely show marked improvement in binding when compared to monomer perhaps due to the lack of an appropriate spacer group. Multivalent displays comprising large numbers of monomers show binding improvements, but little to no specificity.

A Note on Organization: Because these structures differ greatly in architecture, preparation, and evaluation, the discussion of multivalent systems is organized in terms of laboratory, and not the number of sLe^x groups.

1. Liposomes of Cytel

DeFrees and colleagues at Cytel Corporation developed **96**, the first polyvalent display of sLe^x. The molecule, formulated as a liposome, contains numerous copies of sLe^x each attached to the lipophilic anchoring groups through acylation of the nitrogen of glucosamine (Figure 11).[173] Molecule **96** shows marked activity over free (**97**) or acylated (**98**) monomer.

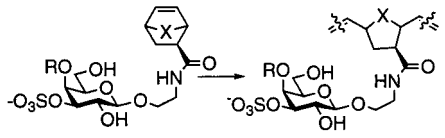
2. Liposomes of Nagy[174]

Nagy and collaborators at Glycomed prepared derivatives of sLe^x (**99**), lactose (**100**), or maltose (**101**) for their incorporation into two different matrixes (**102** and **103**; Figure 12). Unlike the liposomes of Cytel, **99**−**103** contain two adjacent acetylenic units that can be cross-linked to afford more stable, covalent displays. Liposomes of **99** inhibit selectin−sLe^x interactions at nanomolar concentrations. Maximal inhibition was achieved using covalent liposomes containing 5% of the functionalized glycolipid. Nagy's efforts, however, underscore an important aspect of multivalent display: while as monomers, the controls were inactive, as polymers they were only 10-fold less active than **99**. That is, polymers of lactose or maltose are effective at nanomolar concentrations, indicating the lack of specificity using this approach.

3. ROMP Polymers of Kiessling[175]

Kiessling and co-workers prepared polymers of sulfated galactose with ring-opening metathesis polymerization (ROMP) using the monomer shown in Table 12. Mono- and bisulfated polymers **104**−**107** that are specific for P-selectin were obtained. While differences between cyclopentyl and tetrahyrofuranyl

Table 12. Polymers of Kiessling[a]



	X =	R =	IC_{50} (mM) (against P-selectin)
104	O	H	1.3
105	CH_2	H	> 20
106	O	SO_3^-	7.8
107	CH_2	SO_3^-	0.167

[a] Kiessling and co-workers applied ROMP to the preparation of mono- and bisulfated polymers specific for P-selectin.

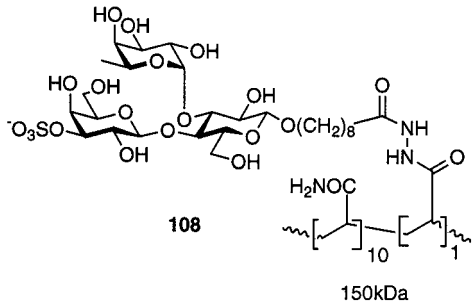


Figure 13. Polymers of Roy. Roy and colleagues prepared polymers of acrylamide displaying sLe^x that show micromolar activities.

scaffolds were observed, the limited number of polymers formulated do not support any general conclusions.

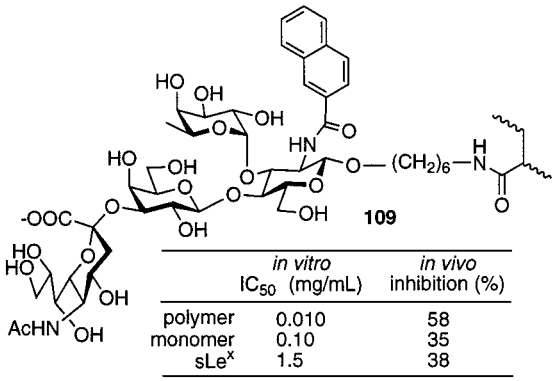
4. Polyacrylamide Polymers of Roy and Miyachi[176]

Roy and colleagues have reported the synthesis of polyacrylamide polymers bearing 3′-sulfo Lewis X mimetics (**108**, Figure 13). These mimetics, which replace GlcNAc with glucose, are attached through the reducing end of glucose to the polymer using an aliphatic linker to an acyl hydrazine group. Roy reports that in initial binding experiments with L- and E-selectins, IC_{50} values in the micromolar range have been obtained.

Miyauchi and co-workers have reported a similar system, **109**: a homopolymer (MW > 300 kDa) of sLe^x-acrylamide in which the *N*-acetyl group of GlcNAc has been replaced with a naphthoyl group (Figure 14).[176] Furthering the generality of this substitution, this polymer binds 100 times more tightly than sLe^x, and 10 times more tightly than monomer in static well tests. In a murine test system, the polymer inhibited neutrophil accumulation by 58%, while the monomer and sLe^x inhibited accumulation by 35% at equivalent doses (30 mg/kg).

5. Trimers of Kretzschmar[177]

Kretzschmar and colleagues examined the ability of trimers of sLe^x (**110**−**112**) to prevent HL-60 immobilization upon a surface coated with P− or E-selectins (Table 13). From their data, an optimal spacing, shown by the higher affinity of **110**, is revealed: the remaining two trimers show very similar affinities for both P- and E-selectin, suggesting, perhaps, similar binding. The enhancement in



	in vitro IC_{50} (mg/mL)	*in vivo* inhibition (%)
polymer	0.010	58
monomer	0.10	35
sLe^x	1.5	38

Figure 14. Polymers of Miyauchi. Miyauchi and colleagues have prepared similar homopolymers of sLe^x−acrylamide that also show strong activities measured in vivo.[176]

binding of **110**, however, corresponds to only a 3- and 5-fold increase in binding/sLe^x.

6. Glycopeptides of Ohrlein, Kunz, and Others

Starting with a glycosylated amino acid, Ohrlein and Baisch[178] prepared dimeric and trimeric scaffolds that, upon elaboration with glycosyltransferases, yielded **113a**−**f** and **114a**,**b**; complex sLe^x glycopeptides with varying distances between the sLe^x groups (Figure 15). The affinities of these molecules is similar to those reported by Kretzschmar.

Kunz[179] and co-workers have developed methods (Scheme 8) for the convergent synthesis of large glycopeptides by coupling an anomeric amine, **115**, with aspartic acid residues of a cyclic peptide, **116**. The resulting trivalent inhibitor **117** is, however, only 3-fold more active than sLe^x at preventing adhesion of HL-60 cells to a surface coated with E-selectin.

Kiessling[180] has reported a similar strategy for the synthesis of sulfated bivalent glycopeptides by an HBTU:HOBT-catalyzed coupling of aspartic acid side chains with sulfated lactosamine groups functionalized with a tether bearing a primary amine. No binding constants were reported.

7. Complex Carbohydrates of Renkonen[181,182]

Renkonen and colleagues have prepared a series of very complex sLe^x carbohydrates (**118**−**120**; Table 14) utilizing enzymatic techniques. Their most complex oligosaccharides contain four sLe^x groups and have IC_{50} values of ∼1 nM against L-selectin. Intermediates in route to the synthesis of **118**−**120** that lacked fucose or sialic acid had no activity.

8. Complex Carbohydrates of Paulson and Wong

In collaboration with Paulson, Wong has prepared dimeric sLe^x pentasaccharides **121**−**126** (Table 15).[184,195] Dimers linked by butanediol (**125**) or pentadiol (**126**) showed activities identical to that of the monomeric pentasaccharide. Dimers linked at different positions on galactose showed differing activities with 3,6-linked (5 times the activity of sLe^x pentasaccharide) > 2,3 > 4,6 > 2,6 > monomer. A similar 3,6-dimer of sLe^x (containing lactose amine

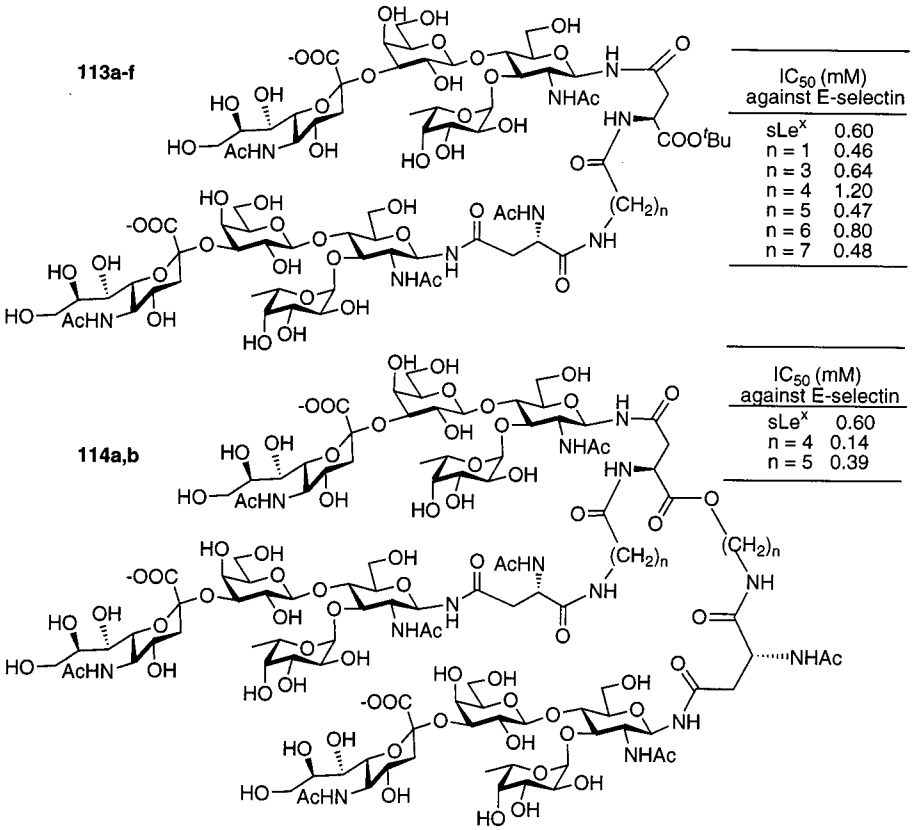
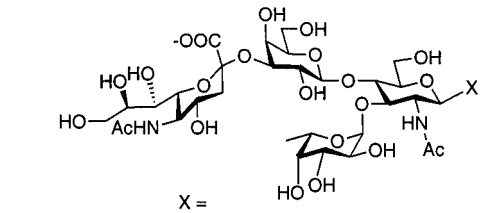
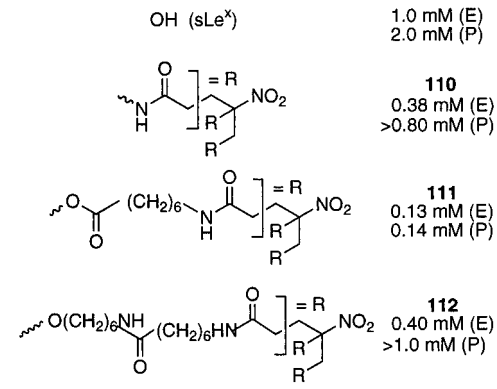


Figure 15. Dimers and trimers of Ohrlein. Similar to Kretzschmar, Baisch and Ohrlein have prepared dimers and trimers of sLe^x that differ in the spacing between residues.

Table 13. Trimers of Kretzschmar[a]



X =



[a] Kretzschmar et al. prepared trimers of sLe^x in an effort to determine the correct spacing of these molecules. These trimers, however, bind only 3−5 times more tightly per sLe^x than monomeric sLe^x.[177]

linked to asparagine) derived from egg yolk binds to E-selectin with an IC_{50} = 0.75 mM.[185]

9. Dimers of Miyauchi[186]

Miyauchi and co-workers have recently reported a similar series of sLe^x dimers, **127**−**131** (Table 14). By using *N*-naphthoyl instead of *N*-acetyl groups on glucosamine, Miyauchi and colleagues see an 8-fold increase in activity. Dimerization led to a 4−6-fold increase in activity, a value consistent with the 5-fold increase observed in the galactose linked series described above.

10. BSA Glycoconjugates of Jacob[187]

Jacob and colleagues at Monsanto attached amine-linked carbohydrates including sLe^x, to bovine serum albumin (BSA) containing 57 lysine groups, functionalized with isothiocyanate. The products were separated using high-performance anion exchange chromatography. Glycoconjugates containing 7, 11, or 16 mol of sLe^x per mole of BSA prevented the adhesion of HL-60 cells to a surface coated with E-selectin at ~20×, ~900×, and ~1000× the activity of monomeric sLe^x, respectively. The two most active species (11sLe^x/BSA, 16sLe^x/BSA) also prevented lymphocyte rolling at 300, and 1 μM, respectively. The high affinity of these glycoconjugates for the selectins allows Jacob and co-workers to use them as affinity labels to indicate the expression levels of the selectins.

11. Polymeric Fucopeptides of Wong[188]

Lin et al. recently reported that incorporation of a tight-binding mimetic (IC_{50} = 50 μM) into covalent liposomes led to only a small increase in activity (IC_{50} = 30 μM; Figure 16) on the basis of a static assay. The monomer **132** is shown. The reasons for this result are unclear and under further investigation. Perhaps the size of the liposome, the space distance

Scheme 8. The Synthesis by Kunz of a Cyclic Glycopeptide Bearing Three sLe^x [179]

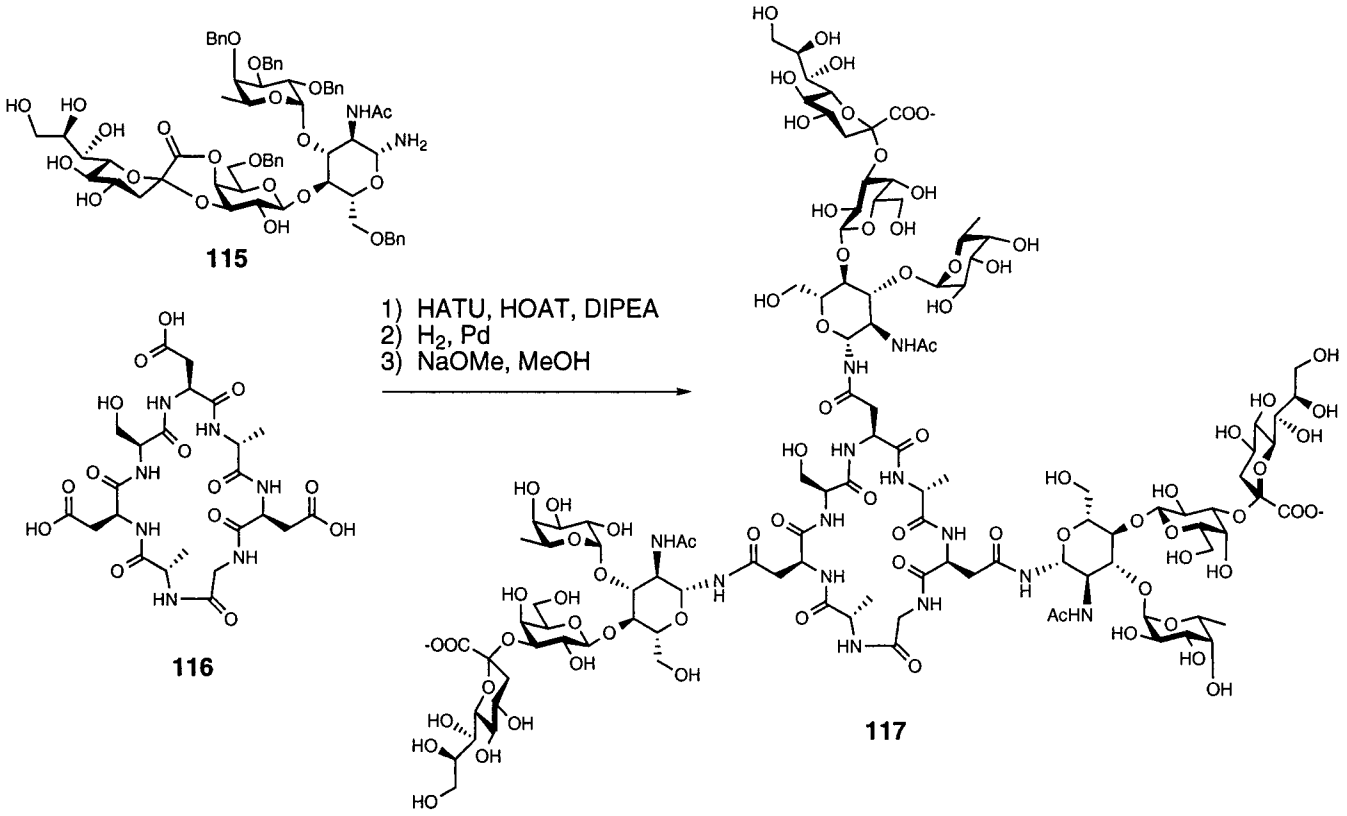
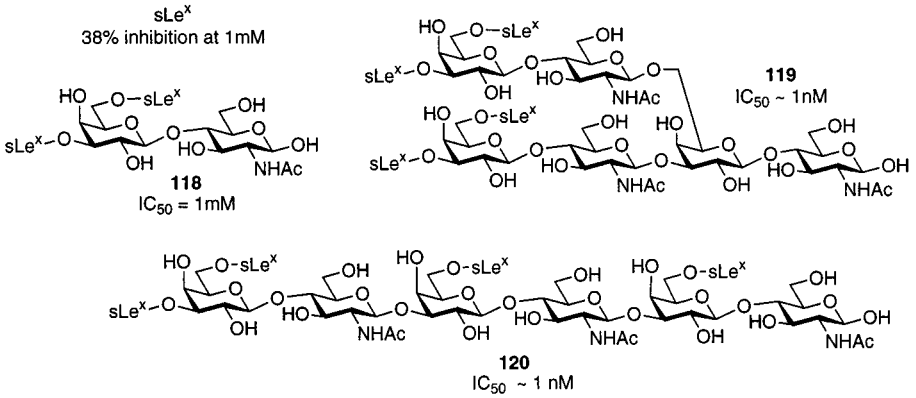


Table 14. Complex Carbohydrates of Renkonen[a]



[a] Renkonen and colleagues prepared monomeric, dimeric, and tetrameric arrays of sLe^x.

between the mimetics, and the method of the assay (ideally a rolling assay should be used) are critical and should be optimized.

IV. Inhibitors Not Based upon Sialyl Lewis^x

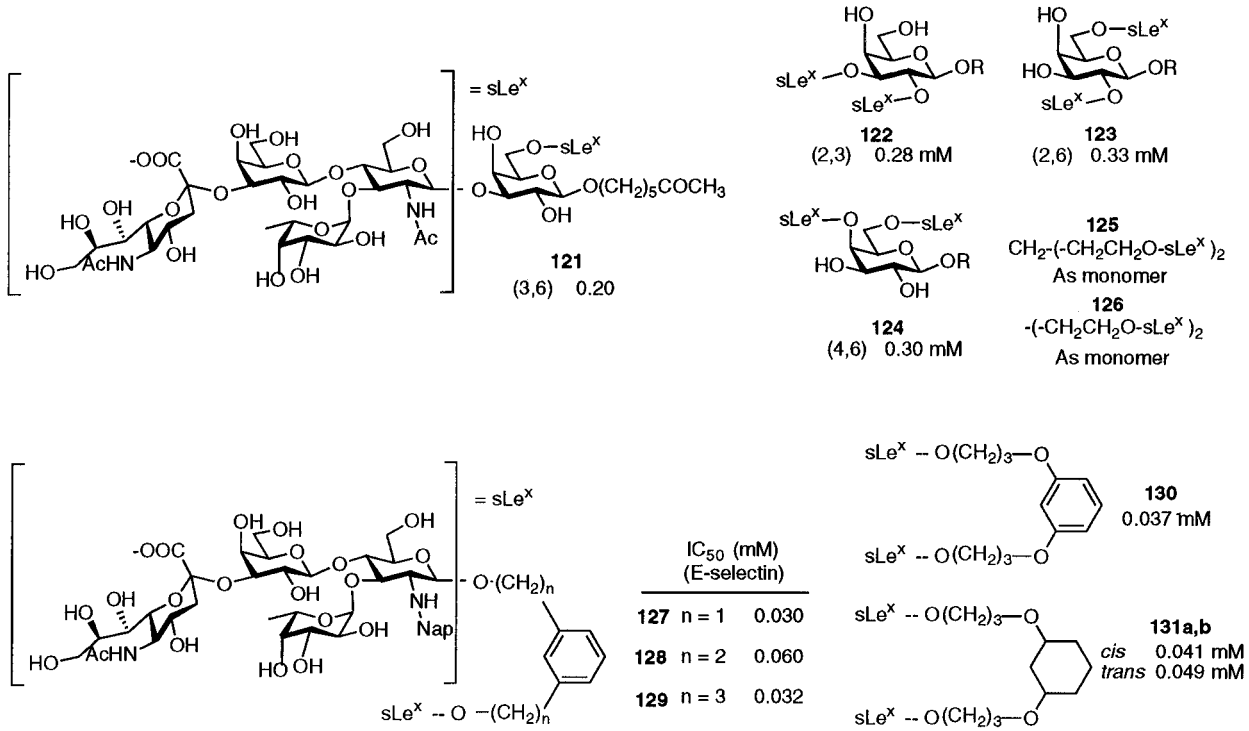
Three additional classes of molecules that bind to the selectins have been discovered that bear no resemblance to the structure of sLe^x. These will be discussed in three categories: polyanions, peptide inhibitors, and natural product derivatives.

A. Polyanions

The observations that the polyanionic fucoidin (a polymer of sulfated fucose) and heparin (a polymer of sulfated glycosaminoglycans) bind to L-selectin with high affinities, as well as the requirement of tyrosine sulfation on PSGL-1 (the natural ligand for P-selectin) has led to the investigation of other polyanionic species (Figure 17). Polysulfated cyclodextrins and myoinositol hexasulfate (or hexaphosphate) all bound to L-selectin while the CD or inositol did not.[189]

Recognizing the importance of polyanions and taking advantage of selection strategies, Varki developed a screening protocol for ribonucleotides. A pool of E-selectin-specific nucleotides were obtained.[190] The highest affinity nucleotide show 10^5 tighter binding than sLe^x and little binding to either P- or L-selectins. Common to all of these nucleotides was the consensus sequence AUGUGUGA. Whether these polyanions are selective and effective in vivo remains to be investigated.

Table 15. Dimers of Paulson and Wong, and Miyauchi[a]



[a] Using galactose as a core unit, sLe^x groups were arranged off the hydroxyl groups to obtain four different dimers of relatively similar activities. Linking sLe^x with flexible aliphatic linkers gave dimers which had activities identical to those of the monomer. Miyauchi explored the use of more rigid linkers (and a *N*-naphthoyl derivative of sLe^x) and measured ratios of dimer:monomer activities which were 8-fold more active than those of Wong and Paulson. All activities were tested against E-Selectin.

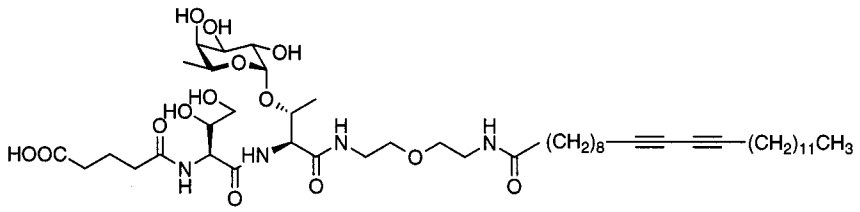


Figure 16. Wong's polymeric fucopeptide. Multivalent display of an active fucose-based monomer leads to only a slight enhancement in affinity. The reasons for this low enhancement are unclear and under investigation.

Recently, Kretzschmar et al. reported that polyanionic contaminants derived from ion-exchange resins were potent inhibitors of selectin binding in trace, and potentially undetectable, amounts.[191] Overall, these polyanions showed the highest affinity for P-selectin, perhaps interacting with the highly positively charged residues on the surface of P-selectin.

B. Peptides

Martens and colleagues at Affymax applied phage display to find peptide ligands that bind to E-selectin and prevent neutrophil adhesion (Table 16).[192] When these compounds were screened with the use of static and flow (rolling) conditions, several peptides that prevented adhesion in the nanomolar range were identified.

Glycomed has taken an alternative approach. Using sequences derived from the selectins themselves, they have obtained peptides that prevent adhesion presumably by binding to the sLe^x-containing glycopeptide.[193,194]

C. Natural Products

1. Glycorrhyzin-Type Molecules

Brandley and colleagues at Glycomed identified glycorrhyzin, a triterpene glycoside, as a potential sLe^x antagonist through a computational search of a 3-D database (Table 17).[195] Twenty-two other compounds were identified in the search and are cited. Table 16 shows the glycorrhyzin and the carbohydrate derivatives (**133**−**138**) that Brandley and colleagues prepared. Of these derivatives, the fucosylated glycorrhyzin shows the greatest activity in in vivo and in vitro tests. Two mannosyl isosteviol derivatives were designed and prepared by investigators at RIKEN (Figure 18; **139** and **140**) to target P-selectin. On the basis of a static assay **140** was selective for P-selectin ($IC_{50} = 60\ \mu M$) while **139** was

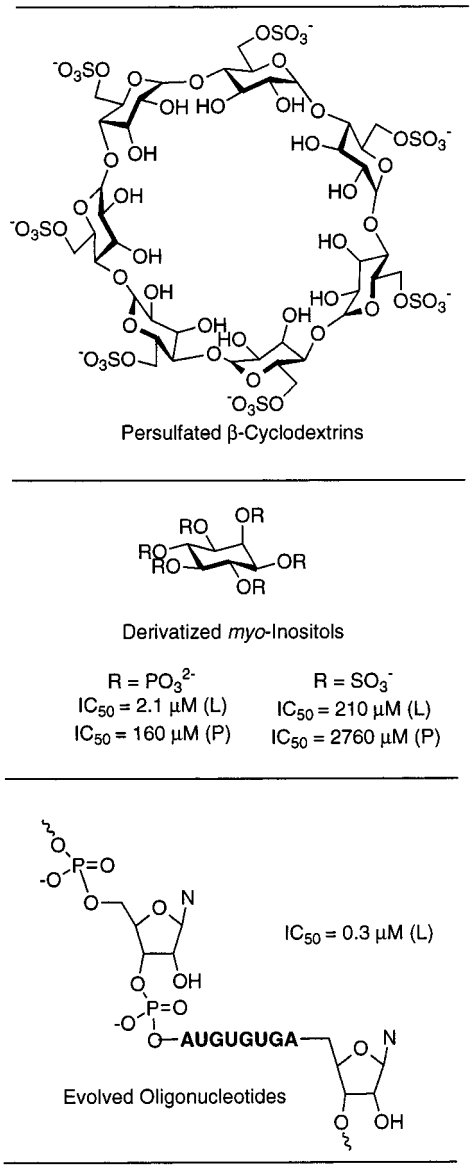


Figure 17. Polyanions.[189] The activities of a variety of polyanions have been examined. (See the text for more details.)

inactive. Molecular modeling was used to explain the experimental result.[196]

2. Deoxynojirimycin-Containing SLe^x

Hasegawa and colleagues have recently reported the synthesis of mimics sLe^x and sLe^a based on deoxynojirimycin, **141** (Figure 19).[197] Their extensive efforts in modifying the skeleton of nojirimycin skeleton suggests that a series of inhibitors can be expected.

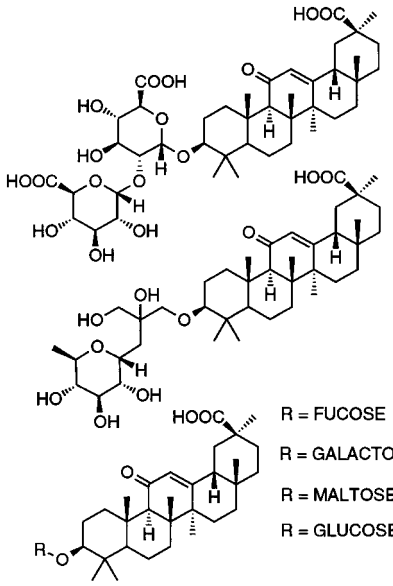
V. Evaluation of Target Structures

The lack of a universal assay for selectin−mimic binding makes direct comparisons of binding affinities difficult. In our own experience, the assays used often suffer from a lack of reproducibility most likely due to heterogeneities in the components of the assays, including HL-60 cells or polymer and the selectin structure used. ELISA assays are the most common method for determining affinity of mimics for the selectins, but protocols and components vary from group to group. Table 18 summarizes these assays in terms of the receptor used; source of the carbohydrate competitor, and method of visualization. Brief descriptions of some of the more popular assays are given below.

Table 16. Peptides Obtained by Phage Display Selection Strategies Bind to E-Selectin Effectively[192]

peptide	IC_{50} (nM)
H_2N-DITWDQLWDLMK-COOH	4.0
H_2N-DITWDELWKIMN-COOH	4.4
H_2N-DYTWFELWDMMQ-COOH	11
H_2N-QITWAQLWNMMK-COOH	16
H_2N-DMTHDLWLTLMS-COOH	23
H_2N-DYSWHDLWEMMS-COOH	57
H_2N-EITWDQLWEVMN-COOH	67
H_2N-HVSWEQLWDIMN-COOH	76
H_2N-HITWDQLWRIMT-COOH	83
H_2N-HITWDQLWNVMN-COOH	420
H_2N-DISWDDLWIMMN-COOH	620
H_2N-QITWDQLWDLMY-COOH	910
H_2N-RNMSWLELWEHMK-COOH	5.4
H_2N-AEWTWDQLWHVMNPAESQ-COOH	23
H_2N-HRAEWLALWEQMSP-COOH	47
H_2N-KKEDWLALWRIMSV-COOH	71
H_2N-KRKQWIELWNIMS-COOH	1200
AcNH-WKLDTLDMIWQD-$CONH_2$	>30000
H_2N-HITWDQLWNVMLRRAASLG-COOH	>11000

Table 17. Derivatives of Glycorrhyzin[a]

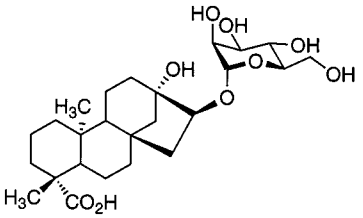


		IC_{50} (mM) E	L	P
133		>2	0.5	<0.5
134		<0.5	<0.5	<0.5
135	R = FUCOSE	>2	0.5	2.0
136	R = GALACTOSE	>2	0.6	>2
137	R = MALTOSE	>2	>2	>2
138	R = GLUCOSE	>2	1.7	>2

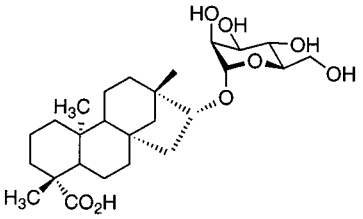
[a] Searching a structural database, Brandley and colleagues identified a series of potential selectin antagonists. The activities of the compounds pursued are reported as IC_{50}s (in mM).

A. Cytel Assay

Cytel uses a competitive ELISA assay in which solutions containing HL-60 cells and test compound are added together to wells coated with E-selectin (Table 18). After the plates are washed to remove unbound cells, the HL-60 cells are lysed and assayed for myeloperoxidase activity using phenylene diamine and H_2O_2. Absorbances are recorded at 492 nm and these values are compared to wells containing no test molecule.

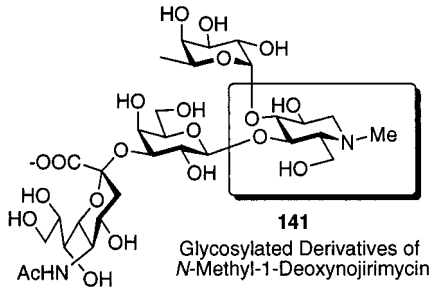


139
inactive (for E, P, L)



140
IC_{50}=60 μM (for P)
inactive (for E, L)

Figure 18. Potential inhibitors comprising mannose and a stevioside or isosteviol skeleton.[196]



141
Glycosylated Derivatives of *N*-Methyl-1-Deoxynojirimycin

Figure 19. Deoxynojirimycin containing sLe^x.[197]

B. Novartis Assay[198a]

Sandoz also uses a competitive assay in which wells coated with E-selectin are filled with the test molecule and a polyacrylamide bearing sLe^a and biotin (Table 18). After unbound ligand and polymer are washed from the plate, streptavidin conjugated to horseradish peroxidase is added. A similar colorametric determination of binding is possible. Novartis and collaborators at Glycotech have also recently published the preparation of complex glycopolymer, a polylysine bearing sLe^a, which is used in a similar assay.[198b]

C. Glycolipid Assay of Lasky, Hasegawa, and Brandley

Glycolipid (sLe^x-β-1,3-Gal-β-1,4-Glc-ceramide) is added as a solution to microtiter plates and allowed to dry. A solution containing a known amount of inhibitor is added to the wells and incubated for 45 min. A solution containing (a) selectin-Ab chimera, (b) a biotinylated anti-FC Ab, and (c) a streptavidin-alkaline phosphatase conjugate are preequilibrated for 15−30 min and then added to the wells and this mixture is incubated for another 45 min. After washing, a colorametric assay using *p*-nitrophenyl phosphate is performed to quantify binding. Other similar assays have been reported.[199]

D. Jacob's Fluorescence Polarization Assay[200]

The reproducibility of assays utilizing multivalent components such as cells or polymers can be dependent on the batch of cells (or polymer) used. These assays yield IC_{50} values rather than binding constants. Jacob et al. use a monovalent assay based on fluorescence polarization[201] in which sLe^x bearing a fluorescent label competes with test ligand for E-selectin. A similar strategy was developed by Knowles et al.[202] to examine binding between hemagglutinin and sialosides. This system avoids heterogeneities arising from poorly characterized, multivalent species including cells and polymers (whose composition can be batch-dependent).

E. Rolling Assays and in Vivo Assays

Springer and others have utilized rolling assays to study the actions of neutrophils on the vascular lining (vida infra). Most of these assays are used to study the biophysics of rolling, and are not routinely used to evaluate antagonists. Recently a rolling assay that utilizes polystyrene beads coated with sLe^x instead of whole cells has been reported.[203]

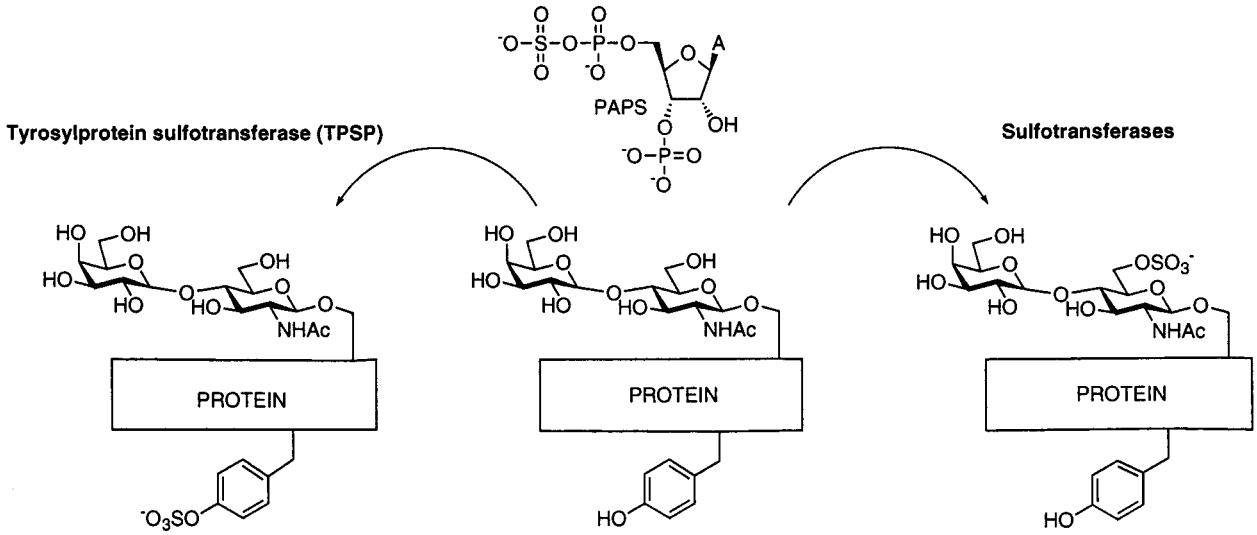
VI. Future Directions

The search for small molecules that disrupt selectin−sLe^x-mediated recognition events has met with some success. While current efforts now focus on developing small molecules with nM activity or incorporating active monomers into polyvalent displays, other areas of focus have become apparent. The following briefly summarizes these areas in the

Table 18. Assays Used for Screening Potential Inhibitors

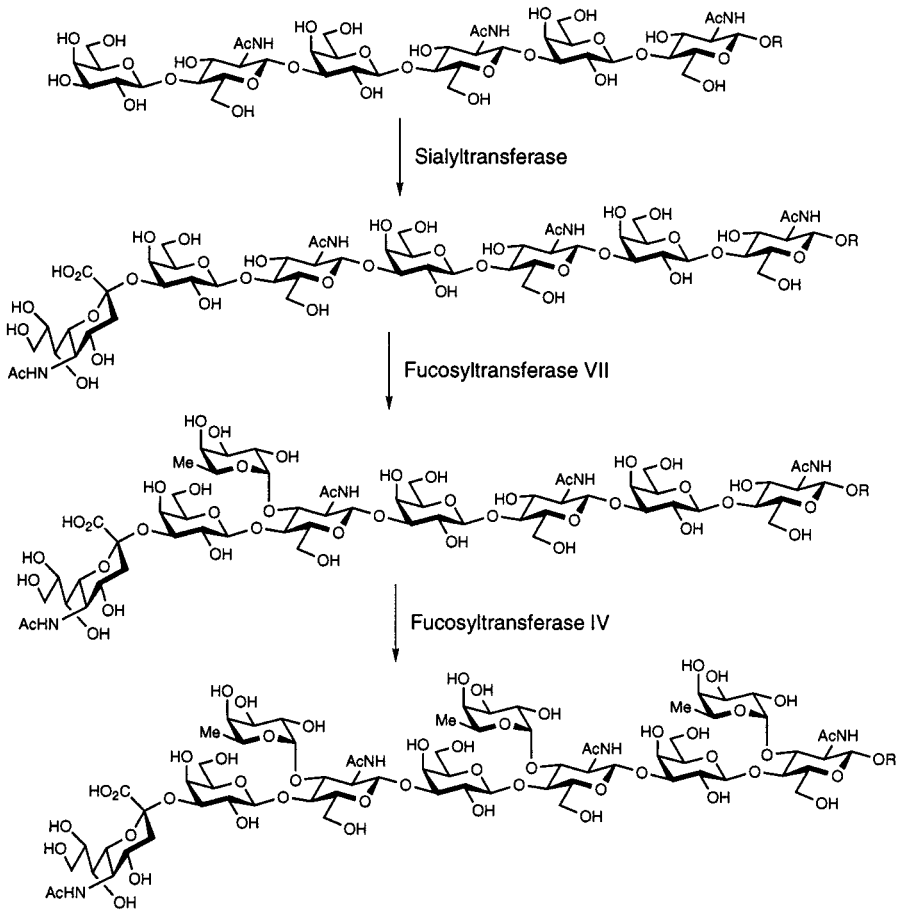
assay	selectin source	sLe^x source(s)	visualization	comment
Cytel's competitive assay	immobilized in well	HL-60 cells and ligand	peroxidase activity of lysed cells	reliability dependent on quality of stocks
Novartis's competitive assay	immobilized in well	biotinylated polymer of sLe^x and ligand	avidin−peroxidase conjugate	reproducibility may vary with polymer batch
glycolipid assay	selectin·Ab chimera	sLe^x glycolipid in well and ligand	biotinylated Anti-FC Ab and an avidin·peroxidase conjugate	reproducibility depends on an accurate quantification of glycolipid
Jacob's fluoresence assay	soluble E-selectin	ligand	fluorescense polarization	K_d can be obtained; not a competition assay
rolling assay	vascular lining	HL-60 cells; neutrophils	optical	not applied routinely for evaluating small molecules

Scheme 9. Sulfation of Proteins and Carbohydrates Often Utilizes PAPS[2,20a,b,a]



[a] Inhibiting the enzymes that are responsible for sulfation is currently being investigated for their potential use as antiinflammatory agents.

Scheme 10. Biosynthesis of the Natural Ligand of E-Selectin[214,a]



[a] The biosynthesis of sLe^x requires two fucosyltransferases and a sialyltransferase. inhibition of these enzymes may prevent cell−cell adhesion, and offer a new avenue for the discovery of antiinflammatory agents.

context of drug discovery and point the interested reader to the relevant literature.

A. Inhibition of Sulfation of Carbohydrates and Tyrosine: Sulfotransferase Inhibitors

Sulfation of the Lewis sugars, the proteins displaying these sugars, and biomolecules that appear to bind to the selectins has led to increased interest in this field. Several groups have begun to examine sulfation as a potentially important event on the outside of a cell as phosphorylation is to events on the inside of the cell.[204] Efforts have focused on small molecules that disrupt sulfation pathways (Scheme 9) in both proteins (i.e., arylsulfotransferase inhibitor)[205] and sugar (sulfotransferase inhibitors).[206]

B. Inhibition of Signal Transduction: Display and Extravasation[207]

Two different signaling pathways are receiving increased attention from cellular biologists: signals leading to the display of selectins and signals leading to the extravasation of cells. The role that leukotrienes and interleukins (IL) play in signaling presentation of selectins suggest that many leukotriene and IL antagonists may be reassayed to determine their specific role. The roles, if any, that the selectins themselves play in signal transduction are uncertain. Binding to L-selectin appears to communicate a signal to the cell interior. Whether this signaling occurs through the association of receptors on the cell membrane or is a result of occupancy numbers remains unclear.

C. Inhibition of Rolling: Metalloprotease Inhibitors

Recently, Springer and colleagues have made important advances in the understanding of the rolling phenomenon.[208−211] Alon et al. used video microscopy to visualize neutrophils rolling across a surface coated with P-selectin reconstituted in a lipid bilayer.[208] Dissociation rate constants were recorded (1 s^{-1}). The authors conclude that fast on and off rates observed and strength of the P-selectin−PSGL-1 interaction are necessary for rolling to occur. Continuing these investigations Finger et al. reported that unlike P- and E-selectin, a minimal value of shear is required for neutrophil accumulation on monolayers incorporating CD34.[209] The authors point out that requiring a minimal shear may be biologically important: neutrophils, which express L-selectin ligands as well as L-selectin, might be prone to clump together during the times when the bulk flow through the vasculature is zero, or in places where flow rates are greatly reduced. Finger et al. also note that shear in blood vessels is highest near the capillary wall and approaches zero in the center of capillary. The mechanism by which one lymphocyte nucleates rolling of circulating lymphocytes is also being addressed.[210]

Small molecules designed to inhibit rolling either through the prevention of selectin display or their subsequent shedding via the action of metalloprotease(s) are beginning to be reported. These molecules are typically leukotrienes (for display)[211] and metalloprotease inhibitors (for shedding).[203] The necessity that selectins be shed from the cell surface has led many to investigate the physiological roles of these soluble derivatives: these molecules have been shown to be cytokines and to induce angiogenesis.[212]

D. Inhibition of Biosynthesis. Fucosyltransferase and Sialyltransferase Inhibitors

Many have begun to investigate the biosynthetic pathways of sLe^x (Scheme 10). Most efforts focus on fucosyl- and sialyltransferases. A number of different fucosyltransferases have been identified, and one, FucT VII (perhaps also FucT V) appears to be important in the biosynthesis of selectin ligand.[214] Small molecules designed to inhibit specifically this enzyme are the targets of many groups.[80] The strategies developed for the inhibition of FucT III[215] and FucT V[216] perhaps can be applied to these two fucosyltransferases.

VII. References

(1) For a comprehensive review of the roles of carbohydrates in biological systems, as well as references to leading reviews in the areas mentioned see: Varki, A. *Glycobiology* **1993**, *3*, 97.
(2) Paulson, J. C. In *Adhesion: Its Role in Inflammatory Disease*; Harlan, J., Lui, D. Eds.; W. H. Freeman: New York, 1992; p19.
(3) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321.
(4) McEver, R. P.; Moore, K. L.; Cummings, R. D. *J. Biol. Chem.* **1995**, *270*, 11025.
(5) Bertozzi, C. *Chem. Biol.* **1995**, *2*, 703.
(6) Bevilacqua, M. P.; Nelson, R. M. *J. Clin. Invest.* **1993**, *91*, 379.
(7) Feizi, T. *Curr. Opin. Struct. Biol.* **1993**, *3*, 701.
(8) Fukuda, M. *Bioorg. Med. Chem.* **1995**, *3*, 207.
(9) Kansas, G. S. *Blood* **1996**, *88*, 3259.
(10) Lasky, L. A. *Science* **1992**, *258*, 964.
(11) Springer, T. A. *Nature* **1990**, *346*, 425.
(12) Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7390.
(13) Bevilacqua, M. P.; Stenglin, S.; Gimbrone, M. A., Jr.; Seed, B. *Science* **1989**, *243*, 1160.
(14) Johnston, G. I.; Cook, R. G.; McEver, R. P. *Cell* **1989**, *56*, 1033.
(15) Pober, J. S.; Bevilacqua, M. P.; Mendrick, D. L.; Lapierre, L. A.; Fiers, W.; Gimbrone, M. A., Jr. *J. Immuno.l* **1986**, *136*, 1680.
(16) Cotran, R. S.; Gimbrone, M. A., Jr.; Bevilacqua, M. P.; Mendrick, D. L.; Pober, J. S. *J. Exp. Med.* **1986**, *164*, 661.
(17) Bevilacqua, M. P.; Pober, J. S.; Wheeler, M. E.; Cotran, R. S.; Gimbrone, M. A., Jr. *J. Clin. Invest.* **1985**, *76*, 2003.
(18) Hsu-Lin, S.-C.; Berman, C. L.; Furie, B. C.; August, D.; Furie, B. *J. Biol. Chem.* **1984**, 259, 9121.
(19) Stenberg, P. E.; McEver, R. P.; Shuman, M. A.; Jacques, Y. V.; Bainton, D. F. *J. Cell Biol.* **1985**, *101*, 880.
(20) Bonfanti, R.; Furie, B. C.; Furie, B. Wagner, D. D. *Blood* **1989**, *73*, 1109.
(21) McEver, R. P.; Beckstead, J. H.; Moore, K. L.; Marshall-Carlson, L.; Bainton, D. F. *J. Clin. Invest.* **1989**, *84*, 92.
(22) Lewinsohn, D. M.; Bargatze, R. F.; Butcher, E. C. *J. Immunol.* **1987**, *138*, 4313.
(23) Larsen, E.; Celi, A.; Gilbert, G. E.; Furie, B. C.; Erban, J. K.; Bonfanti, R.; Wagner, D. D.; Furie, B. *Cell* **1989**, *59*, 305.
(24) 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. *Nature* **1990**, *343*, 757.
(25) Bevilacqua, M. P.; Pober, J. S.; Mendrick, D. L.; Cotran, R. S.; Gimbrone, M. A., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9238.
(26) Siegelman, M. H.; Van de Rijn, M.; Weissman, I. L. *Science* **1989**, *243*, 1165.
(27) Lasky, L. A.; Singer, M. S.; Yednock, T. A.; Dowbenko, D.; Fennie, C.; Rodriguez, H.; Nguyen, T.; Stachel, S.; Rosen, S. D. *Cell* **1989**, *56*, 1045.
(28) See: Tedder, T. F.; Penta, A. C.; Levine, H. B.; Freedman, A. S. *J. Immunol.* **1990**, *144*, 532, and references therein.
(29) Kishimoto, T. K.; Warnock, R. A.; Jutila, M. A.; Butcher, E. C.; Lane, C.; Anderson, D. C.; Smith, C. W. *Blood* **1991**, *78*, 805.
(30) Hallmann, R.; Jutila, M. A.; Smith, C. W.; Anderson, D. C.; Kishimoto, T. K.; Butcher, E. C. *Biochem Biophys. Res. Commun.* **1991**, *174*, 236.
(31) Spertini, O.; Luscinskas, F. W.; Kansas, G. S.; Munro, J. M.; Griffin, J. D.; Gimbrone, M. A., Jr.; Tedder, T. F. *J. Immunol.* **1991**, *147*, 2565.
(32) Spertini, O.; Luscinskas, F. W.; Gimbrone, M. A., Jr.; Tedder, T. F. *J. Exp. Med.* **1992**, *175*, 1789.
(33) Ley, K.; Gaehtgens, P.; Fennie, C.; Singer, M. S.; Lasky, L. A.; Rosen, S. D. *Blood* **1991**, *77*, 2553.
(34) von Andrian, U. H.; Chambers, J. D.; McEvoy, L. M.; Bargatze, R. F.; Arfors, K.-E.; Butcher, E. C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7538.
(35) Lawrence, M. B.; Springer T. A. *Cell* **1991**, *65*, 859.
(36) For a recent review of integrin antagonists, see: Mousa, S. A.; Cherash, D. A. *DDT* **1997**, *2*, 187.
(37) Heavner, G. A. *DDT* **1996**, *1*, 295.
(38) Kishimoto, T. K.; Jutila, M. A.; Berg, E. L.; Butcher, E. C. *Science* **1989**, *245*, 1238.
(39) Kahn, J. R.; Ingraham, R. H.; Shirley, F.; Migaki, G. I.; Kishimoto, T. K. *J. Cell. Biol.* **1994**, *125*, 461.
(40) Springer, T. A. *Cell* **1994**, *76*, 301.
(41) Butcher, E. C. *Cell* **1991**, *67*, 1033.
(42) Schleiffenbaum, B.; Spertini, O.; Tedder, T. F. *J. Cell Biol.* **1992**, *119*, 229.
(43) For a review of the role of soluble selectins, see: Gearing, A. J.; Newman, W. *Immunol. Today* **1993**, *14*, 506.

(44) Migaki, G. I.; Kahn, J.; Kishimoto, T. K. *J. Exp. Med.* **1995**, *182*, 549.
(45) Walcheck, B.; Kahn, J.; Fisher, J. M.; Wang, B. B.; Fisk, R. S.; Payan, D. G.; Feehan, C.; Betageri, R.; Darlak, K.; Spatola, A. F.; Kishimoto, T. K. *Nature* **1996**, *280*, 720.
(46) Cehn, A.; Engel, P.; Tedder, T. F. *J. Exp. Med.* **1995**, *182*, 519.
(47) Bennett, T. A.; Lynam, E. B.; Sklar, L. A.; Rogelj, S. *J. Immunol.* **1996**, *156*, 3093.
(48) Michelson, A. D.; Barnard, M. R.; Hechtman, H. B.; MacGregor, H.; Connolly, R. J.; Loscalzo, J.; Valeri, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11877, and references therein.
(49) Schlaepfer, D. D.; Hanks, S. K.; Hunter, T.; van der Geer, P. *Nature* **1994**, *372*, 786.
(50) Clark, E. A.; Brugge J. S. *Science* **1995**, *268*, 233.
(51) Hynes, R. O. *Cell* **1992**, *69*, 11.
(52) Loftus, J. C.; Smith, J. W.; Ginsberg, M. H. *J. Biol. Chem.* **1994**, *269*, 25235.
(53) Waddell, T. K.; Fialkow, L.; Chan, C. K.; Kishimoto, T. K.; Downey, G. P. *J. Biol. Chem.* **1995**, *270*, 15403.
(54) Erbe, D. V.; Wolitzky, B. A.; Presta, L. G.; Norton, C. R.; Ramos, R. J.; Burns, D. K.; Rumberger, J. M.; Rao, B. N. N.; Foxall, C. R.; Brandley, B. H.; Lasky, L. A. *J. Cell. Biol.* **1992**, *119*, 215.
(55) (a) Pigott, R.; Needham, L. A.; Edwards, R. M.; Walker, C.; Power, C. *J. Immunol.* **1991**, *147*, 130. (b) Kansas, G. S.; Saunders: K. B.; Ley, K.; Zakrzewicz, A.; Gibson, R. M.; Furie, B.; Tedder, T. F. *J. Cell. Biol.* **1994**, *124*, 609.
(56) Graves, B. J.; Crowther, R. L.; Chandran, C.; Rumberger, J. M.; Li, S.; Huang, K.-S.; Presky, D. H.; Familletti, P. C.; Wolitzky, B. A.; Burns, D. K. *Nature* **1994**, *367*, 532.
(57) Weis, W. I.; Drickamer, K.; Hendrickson, W. A. *Nature* **1992**, *360*, 127.
(58) Hiramatsu, Y.; Tsujishita, H.; Kondo, H. *J. Med. Chem.* **1996**, *39*, 4547.
(59) Kogan, T. P.; Revelle, B. M.; Tapp, S.; Beck, P. J. *J. Biol. Chem.* **1995**, *270*, 14047.
(60) For recent descriptions of the binding domain of PSGL-1, see: (a) Wilkins, P. P.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **1996**, *271*, 18732. (b) Li, F.; Wilkins, P. P.; Crawley, S.; Weinstin, J.; Cummings, R. D.; McEver, R. P. *J. Biol. Chem.* **1996**, *271*, 3255.
(61) Lowe, J. B.; Stoolman, L. M.; Nair, R. P.; Larsen, R. D.; Berhend, T. L.; Marks, R. M. *Cell* **1990**, *63*, 475.
(62) Tiemeyer, M.; Swiedler, S. J.; Ishihara, M.; Moreland, M.; Schweingruber, H.; Hirtzer, P.; Brandley, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1138.
(63) Berg, E. L.; Robinson, M. K.; Mansson, O.; Butcher, E. C.; Magnani, J. L. *J. Biol. Chem.* **1991**, *266*, 14869.
(64) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130.
(65) Tyrell, D.; James, P.; Rao, N.; Foxall, C.; Abbas, S.; Dasgupta, F.; Nashed, M.; Hasegawa, A.; Kiso, M.; Asa, D.; Kidd, J.; Brandley, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10372.
(66) Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.
(67) (a) Yuen, C.-T.; Lawson, A. M.; Chai, W.; Larkin, M.; Stoll, M. S.; Stuart, A. C. Sullivan, F. X.; Ahern, T. J.; Feizi, T. *Biochemistry* **1992**, *31*, 9126. (b) Yuen, C.-T.; Bezouska, K.; O'Brien, J.; Stoll, M.; Lemoine, R.; Lubineau, A.; Kiso, M.; Hasegawa, A.; Bockovich, N. J.; Nicolaou, K. C.; Feizi, T. *J. Biol. Chem.* **1994**, *269*, 1595.
(68) Levinovitz, A.; Muhloff, J.; Isenmann, S.; Vestweber, D. *J. Cell Biol.* **1993**, *121*, 449.
(69) Lenter M.; Levinovitz, A.; Isenmann, S.; Vestweber, D. *J. Cell Biol.* **1994**, *125*, 471.
(70) Moore, K. L.; Stults, N. L.; Diaz, S.; Smith, D. F.; Cummings, R. D.; Varki, A.; McEver, R. P. *J. Cell Biol.* **1992**, *118*, 445.
(71) Imai, Y.; Singer, M. S.; Fennie, C.; Lasky, L. A.; Rosen, S. D. *J. Biol. Chem.* **1991**, *113*, 1213.
(72) Lasky, L. A.; Singer, M. S.; Dowbenko, D.; Imai, Y.; Henzel, W. J.; Grimley, C.; Fennie, C.; Gillett, N.; Watson, S. R.; Rosen, S. D. *Cell* **1992**, *69*, 927.
(73) Baumhueter, S.; Singer, M. S.; Henzel, W.; Hemmerich, S.; Renz, M.; Rosen, S. D.; Lasky, L. A. *Science* **1993**, *262*, 436.
(74) Berg, E. L.; McEvoy, L. M.; Berlin, C.; Bargatze, R. F.; Butcher, E. C. *Nature* **1993**, *366*, 695.
(75) Foxall, C.; Watson, S. R.; Dowbenko, D.; Fennie, C.; Lasky, L. A.; Kiso, M.; Hasegawa, A.; Asa, D.; Brandley, B. K. *J. Cell Biol.* **1992**, *117*, 895.
(76) Berg, E. L.; Magnani, J.; Warnock, R. A.; Robinson, M. K.; Butcher, E. L. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 1048.
(77) Green, P. J.; Tamatani, T.; Wantabe, T.; Miyasaka, M.; Hasagawa, A.; Kiso, M.; Yuen, C.-T.; Stoll, M. S.; Feizi, T. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 244.
(78) For a review of sulfation in this system, see: Rosen, S. D.; Bertozzi, C. R. *Curr. Biol.* **1996**, *6*, 261.
(79) (a) Hemmerich, S.; Leffler, H.; Rosen, S. D. *J. Biol. Chem.* **1995**, *279*, 12035. (b) Hemmerich, S.; Bertozzi, C. R.; Leffler, H.; Rosen, S. D. *Biochemistry* **1994**, *33*, 4820. (c) Hemmerich, S.; Rosen, S. D. *Biochemistry* **1994**, *33* 4830. (d) Bertozzi, C. R.; Rosen, S. D. *Biochemistry* **1995**, *34*, 1427.
(80) Chandrasekaran, E. V.; Jain, R. K.; Larsen, R. D.; Wlasichuk, K.; Matta, K. L. *Biochemistry* **1995**, *34*, 2925−2936.
(81) (a) Komba, M. S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem.* **1996**, *4*, 1833. (b) Yoshino, K.; Ohmoto, H.; Kondo, N.; Tsujishita, H.; Hiramatsu, Y.; Inoue, Y.; Kondo, H.; Ishida, H.; Kiso, M.; Hsaegawa, A. *J. Med. Chem.* **1997**, *40*, 455−462.
(82) Scudder, P. R.; Shailubhai, K.; Duffin, K. L.; Streeter, P. E.; Jacob, G. S. *Glycobiology* **1994**, *4*, 929.
(83) (a) Vig, R.; Jain, R. V.; Piskorz, C. F.; Matta, K. V. *J. Chem. Soc., Chem. Commun.* **1995**, 2073. (b) Jain, R. K.; Vig, R.; Rampal, R.; Chandrasekaran, R. V.; Matta, K. V. *J. Am. Chem. Soc.* **1994**, *116*, 12123.
(84) Suzuki, Y.; Tamatani, T.; Watanabe, T.; Suzuki, T.; Nakao, T.; Murase, K.; Kiso, M.; Hasegawa, A.; Tadano-Aritomi, K.; Ishizuka, I.; Miyasaka, M. *Biochem. Biophys. Res. Commun.* **1993**, *190*, 426. For synthetic sulfatide and analogues as P-selectin inhibitors, see: Marinier, A.; Martel, A.; Banville, J.; Bachand, C.; Remillard, R.; Lapointe, P. Turmel, B.; Menard, M.; Harte, W. H.; Wright, J. J. K.; Todderud, G.; Tramposch, K. M.; Bajorath, J.; Hollenbaugh, D.; Aruffo, A. *J. Med. Chem.* **1997**, *40*, 3234.
(85) Norgard-Sumnicht, K. E.; Varki, N. M.; Varki, A. *Science* **1993**, *261*, 480.
(86) Norgard-Sumnicht, K. E.; Varki, A. *J. Biol. Chem.* **1995**, 12012.
(87) Stoolman, L. M.; Rosen, S. D. *J. Cell. Biol.* **1983**, *96*, 722.
(88) Stoolman, L. M.; Tenforde, T. S.; Rosen, S. D. *J. Cell. Biol.* **1984**, *99*, 1535.
(89) Sako, D.; Chang, X.-J.; Barone, K. M.; Vachino, G.; White, H. M.; Shaw, G.; Veldman, G. M.; Bean, K. M.; Ahern, T. J.; Fuire, B.; Cumming, D. A.; Larsen, G. R. *Cell* **1993**, *75*, 1179.
(90) Sako, D.; Commess, K. M.; Barone, K. M.; Camphausen, R. T.; Cumming, D. A.; Shaw, G. D. *Cell* **1995**, *83*, 323.
(91) Pouyani, T.; Seed, B. *Cell* **1995**, *83*, 333.
(92) Norgard, K. E.; Moore, K. L.; Diaz, S.; Stults, N. C.; Ushiyama, S.; McEver, R. P.; Cumming, R. D.; Varki, A. *J. Biol. Chem.* **1993**, *268*, 12764.
(93) Wilkins, P. P.; Moore, K. L.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **1995**, *270*, 22677.
(94) Moore, K. L.; Eaton, S. F.; Lyons, D. E.; Lichenstein, H. S.; Cummings, R. D.; McEver, R. P. *J. Biol. Chem.* **1994**, *269*, 23318.
(95) Polley, M. J.; Phillips, M. L.; Wayner, E.; Nudelman, E.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6224.
(96) Larsen, E.; Palabrica, T.; Sajer, S.; Gilbert, G. E.; Wagner, D. D.; Furie, B. C.; Furie, B. *Cell* **1990**, *63*, 467.
(97) Aruffo, A.; Kolanus, W.; Walz, G.; Fredman, P.; Seed, B. *Cell* **1991**, *67*, 35.
(98) Skinner, M. P.; Fournier, D. J.; Andrews, R. K.; Gorman, J. J.; Chesterman, C. N.; Berndt, M. C. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 1373.
(99) Skinner, M. P.; Lucas, C. M.; Burns, G. F.; Chesterman, C. N.; Bermdt, M. C. *J. Biol. Chem.* **1991**, *266*, 5371.
(100) For the syntheses of Le^x and sLe^x sugars, see: (a) Chowdury, U. S. *Tetrahedron* **1996**, *52*, 12775. (b) Singh, K.; Fernandez-Mayoralas, A.; Marin-Lomas, M. *J. Chem. Soc., Chem. Commun.* **1994**, 775. (c) Lubineau, A.; Le Gallic, J.; Lemoine, R. *Bioorg. Med. Chem. Lett.* **1994**, *2*, 1143.
(101) Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *209*, c1.
(102) (a) Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 870−872. (b) Nakahara, Y.; Jiyima, H.; Sibayama, S.; Ogawa, T. *Tetrahedron Lett.* **1990**, *31*, 6897.
(103) Danishefshy, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Griffith, D. A.; Oriyama, T.; Marsden, S. P. *J. Am. Chem. Soc.* **1995**, *117*, 1940.
(104) Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239.
(105) For phosphite activation of sialic acid, see: Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 8749.
(106) For phosphite activation of fucose, see: Lin, C.-C.; Shimazaki, M.; Heck, M.-P.; Aoki, S.; Wang, R.; Kimura, R.; Ritzen, H.; Takayama, S.; Wu, S.-H.; Wietz-Schmidt, G.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 6828.
(107) Martin, S. A.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123.
(108) For a recent review on the chemoenzymatic synthesis of carbohydrates, see: Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443.
(109) Ichikawa, Y.; Wong, R.; Wong, C.-H. *Methods Enzymol.* **1994**, *247*, 193.
(110) UDP-GAL: (a) Wong, C.-H.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416−5418. (b) Auge, C.; David, S.; Mathieu, C.; Gautheron, C. *Tetrahedron Lett.* **1984**, *25*, 1467. (c) Wong, C.-H.; Wang, R.; Ichikawa, Y. *J. Org. Chem.* **1992**, *57*, 4343. (d) Elling, L.; Grothus, M.; Kula, M.-R.; *Glycobiology* **1993**, *3*, 349. CMP-NeuAc: (e) Ichikawa, Y.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 4698. (f) Ichikawa, Y.; Liu, J. J.-C.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6300.

UDP-GlcNAc: Look, G. C.; Ichikawa, Y.; Shen, G.-J.; Cheng, G.-J.; Wong, C.-H. *J. Org. Chem.* **1993**, *58*, 4326. GDP-Fuc: See ref 111. UDP-GlcA: Gygax, D.; Spies, P.; Winkler, T.; Pfarr, U. *Tetrahedron* **1991**, *28*, 5119.

(111) For the large-scale production of sLe^x: Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Juneda, E.; Williams, M. A.; Bayer, R.; Ketchum, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283. For an early partial enzymatic route to sLe^x using stoichiometric sugar nucleotides: Palcic, M. M.; Venot, A. P.; Ratcliffe, R. M.; Hindsgaul, O. *Carbohydr. Res.* **1989**, *190*, 1.

(112) Lin, C.-H.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 8031−8032.

(113) For the enzymatic preparation of lactosamines for sLe^x using galactosidase, see: (a) Kimura, T.; Takayama, S.; Huang, H.; Wong, C.-H. *Angew. Chem., Int. Engl. Ed.* **1996**, *35*, 2348. (b) Takayama, S.; Shimazaki, M.; Qiao, L.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1123.

(114) For the enzymatic preparation of lactosamines for sLe^a or sLe^x using galactosidase, see: Nilsson, K. G. I.; Eliasson, A.; Larsson-Lorek, U. *Biotech. Lett.* **1995**, *17*, 717.

(115) (a) Look, G. C.; Wong, C.-H. *Tetrahedron Lett.* **1992**, *33*, 4253. (b) Kimura, T.; Takayama, S.; Huang, H.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2348. (c) Herrmann, G. F.; Ichikaya, Y.; Wandrey, C.; Gaeta, F. C. A.; Paulson, J. C.; Wong, C.-H. *Tetrahedron Lett.* **1993**, *19*, 3091.

(116) Brandley, B. K.; Kiso, M.; Abbas, S.; Nikrad, P.; Srivasatava, O.; Foxall, C.; Oda, Y.; Hasegawa, A. *Glycobiology* **1993**, *3*, 633.

(117) Ramphal, J. Y.; Zheng, Z.-L.; Perez, C.; Walker, L. E.; DeFrees, S. A.; Gaeta, F. C. A. *J. Med. Chem.* **1994**, *37*, 3459.

(118) Stahl, W.; Sprengard, U.; Kretzschmar, G.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2096.

(119) DeFrees, S. A.; Gaeta, F. C. A.; Lin, Y. C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549.

(120) Hiramatsu, Y.; Tsujishita, H.; Kondo, H. *J. Med. Chem.* **1996**, *39*, 4547.

(121) Wada, Y.; Saito, T.; Matsuda, N.; Ihmoto, H.; Yoshino, K.; Ohashi, M.; Kondo, H.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Med. Chem.* **1996**, *39*, 2055.

(122) Scheffler, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W. T.; Weisemann, R.; Peters, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1841.

(123) Cooke, R. M.; Hale, R. S.; Lister, S. G.; Shah, G.; Weir, M. P. *Biochemistry* **1994**, *33*, 10591.

(124) Poppe, L.; Brown, G. S.; Philo, J. S.; Nikrad, P. V.; Shah, B. H. *J. Am. Chem. Soc.* **1997**, *119*, 1727.

(125) For the X-ray structure of Le^x, see: Yvelin, F.; Zhang, Y.-M.; Mallet, J.-M.; Robert, F.; Jeannin, Y.; Sinay, P. *Carbohydr. Lett.* **1996**, *1*, 475. Also, see: Perez, S.; Mouhous-Rious, N.; Nifant'ev, N. E.; Nikolay, E.; tsvetov, T. E.; Bachet, B.; Imberty, A. *Glycobiology* **1996**, *6*, 537.

(126) For a discussion of the X-ray structure of a mannose binding protein mutant for E-selectin with sLe^x, see: Ng, K. K.-S.; Weis, W. I. *Biochemistry* **1997**, *36*, 979.

(127) For a discussion of molecular dynamics and NMR structures of dimeric glycolipids: Geyer, A.; Hummel, G.; Eisele, T.; Reinhardt, S.; Schmidt, R. R. *Chem. Eur. J.* **1996**, *2*, 981.

(128) Rutherford, T. J.; Soackman, D. G.; Simpson, P. J.; Homans, S. W. *Glycobiology* **1994**, *4*, 59.

(129) Kogelberg, H.; Rutherford, T. J. *Glycobiology* **1994**, *4*, 49.

(130) For a discussion of molecular dyamics of E-selectin with 3′-sulfo Lewis X with hydrophobic groups: Tsujishita, H.; Hiramatsu, Y.; Kondo, N.; Ohmoto, H.; Kondo, H.; Kiso, M.; Hasegawa, A. *J. Med. Chem.* **1997**, *40*, 362.

(131) Mulligan, M. S.; Paulson, J. C.; DeFrees, S. A.; Zheng, Z.-L.; Lowe, J. B.; Ward, P. A. *Nature*, **1993**, *364*,149.

(132) Murohara, R.; Margiotta, J.; Phillips, L. M.; Paulson, J. C.; DeFrees, S.; Zalipsky, S.; Guo, L. S.; Lefer, A. M. *Cardiovasc. Res.* **1995**, *30*, 965.

(133) Lefer, D. J.; Flynn, M. S.; Philips, M. L.; Ractcliffe, M.; Buda, A. J. *Circulation* **1994**, *90*, 2390.

(134) Ochi, T.; Hakomori, S. I.; Fujimoto, M.; Okamura, M.; Owaki, H.; Wakitani, S.; Shimaoka, Y.; Hayashida, K.; Tomita, T.; Kawamura, S.; Ono, R. *J. Rheumatol.* **1993**, 2038.

(135) DeFrees, S. A.; Phillips, L.; Guo, L.; Zapilsky, S. *J. Am. Chem. Soc.* **1996**, *118*, 6101.

(136) Ohmoto, H.; Nakamura, K.; Inoue, T.; Kondo, N.; Inoue, Y.; Yoshino, K.; Kondo, H.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Med. Chem.* **1996**, *39*, 1339.

(137) Manning, D. D.; Bertozzi, C. R.; Rosen, S. D.; Kiessling, L.L. *Tetrahedron Lett.* **1996**, *37*, 1953.

(138) Thoma, G.; Schwarzenbach, F.; Duthale, R. O. *J. Org. Chem.* **1996**, *61*, 514.

(139) Ragan, J. A.; Cooper, K. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2563.

(140) Prodger, J. C.; Bamford, M. J.; Gore, P. M.; Holmes, D. S.; Saez, V.; Ward, P. *Tetrahedron Lett.* **1995**, *36*, 2339.

(141) Bamford, M. J.; Bird, M.; Gore, P. M.; Holmes, D. S.; Priest, R.; Prodger, J. C.; Saez, V. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 239.

(142) Uchiyama, T.; Vassilev, V. P.; Kajimoto, T.; Wong, W.; Huang, H.; Lin, C.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5395.

(143) Uchiyama, T.; Woltering, T. J.; Wong, W.; Lin, C.-C.; Kajimoto, T.; Takebayashi, M.; Wietz-Schmidt, G.; Asakura, T.; Nod, M.; Wong, C.-H. *Bioorg. Chem. Med. Lett.* **1996**, *4*, 1149.

(144) (a) Huang, H.; Wong, C.-H. *J. Org. Chem.* **1995**, *60*, 3100. (b) Banteli, R.; Ernst, B. *Tetrahedron Lett.* **1997**, *38*, 4059.

(145) Toepfer, A., G.; Kretschmar, G.; Bartnik, E. *Tetrahedron Lett.* **1995**, *36*, 9161.

(146) Kaila, N.; Yu, H.-A.; Xiang, Y. *Tetrahedron Lett.* **1995**, *36*, 5503.

(147) Birkbeck, A. A.; Ley, S. V.; Prodger, J. C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2637.

(148) Allanson, N. M.; Davidson, A. H.; Floyd, C. D.; Martin, F. M. *Tetrahedron: Asymmetry* **1994**, *5*, 2061.

(149) Dekany, G.; Wright, K.; Ward, P.; Toth, I. *J. Carbohydr. Chem.* **1996**, *15*, 383.

(150) Liu, A.; Dillon, K.; Campbell, R. M.; Cox, D. C.; Huryn, D. M. *Tetrahedron Lett.* **1996**, *37*, 3785.

(151) Kretzschmar, G.; Toepfer, A.; Huls. C.; Krause, M. *Tetrahedron* **1997**, *53*, 2485.

(152) Toepfeer, A.; Kretzschmar, G. *Bioorgan. Med. Chem. Lett.* **1997**, *7*, 1997.

(153) Wu, S.-H.; Shimazaki, M.; Lin, C.-C.; Qiao, L.; Moree, W. J.; Weitz-Schmidt, G.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 88.

(154) (a) Woltering, T. J.; Weitz-Schmidt, G.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 9033. (b) Wang, R.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 5427.

(155) Wong, C.-H.; Moris-Varas, F.; Hung, S.-C.; Marron, T. G.; Lin, C.-C.; Gong, K. W.; Weitz-Schmidt, G. *J. Am. Chem. Soc.* **1997**, *119*, 8125.

(156) Cappi, M. W.; Moree, W. J.; Marron, T. G.; Weitz-Schmidt, G.; Wong, C.-H. *Angew. Chem., Int. Engl. Ed.* **1996**, *35*, 2346.

(157) (a) Marron, T. G.; Woltering, T. J.; Weitz-Schmidt, G.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 9037. (b) Huwe, C. M.; Jiricek, J.; Woltering, T. J.; Wong, C.-H. Unpublished data. (c) Lampe, T. F. J.; Weitz-Schmidt, G.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.,* submitted for publication.

(158) Kogan, T. P.; Dupre, B.; Keller, K. M.; Scott, I. L.; Bui, H.; Market, R. V.; Beck, P. J.; Voytuis, J. A.; Revelle, R. M.; Scott, D. *J. Med. Chem.* **1995**, *38*, 4976.

(159) Dupre, B.; Bui, H.; Scott, I. L.; Market, R. V.; Keller, K. M.; Beck, P. J.; Kogan, T. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 569.

(160) Sutherlin, D. P.; Stark, T. M.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 8350.

(161) Ugi, I.; Domling, A.; Horl, W. *Endeavor* **1994**, **18**, 115.

(162) Gokel, G.; Ludke, G.; Ugi, I. In *Isonitrile Chemistry;* Ugi, I., Ed.; Adacemic Press: New York, 1971; p 145.

(163) Tsai, C.-Y.; Park, W. K. C.; Wong, C.-H. *Bioorg. Med. Chem. Lett.*, in press.

(164) Spengard, U.; Kunz, H.; Huls, C.; Schmidt, W.; Sieffge, D.; Kretsschamar, G *Bioorg. Med. Chem. Lett.* **1996**, *6*, 509.

(165) Sprengard, U.; Kretschmar, G.; Bartnik, E.; Huls, C.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 990.

(166) Hayashi, M.; Tanaka, M.; Itoh, M.; Miyauchi, H. *J. Org. Chem.* **1996**, *61*, 2938.

(167) Ramphal, J. Y.; Hiroshige, M.; Lou, B.; Gaudino, J. J.; Hayashi, M.; Chen, S. M.; Chiang, L. C.; Gaeta, F. C. A.; DeFrees, S. A. *J. Med. Chem.* **1996**, *39*, 1357.

(168) Eisle, T.; Toepfer, A.; Kretschamr, G.; Schmidt, R. R. *Tetrahedron Lett.* **1996**, *37*, 1389.

(169) Nelson, R. M.; Dolich, S.; Aruffo, A.; Cecconi, O.; Bevilacqua, M. P. *J. Clin. Invest.* **1993**, *91*, 1157.

(170) Schuster, M.; Wang, P.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 1135.

(171) Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 8766.

(172) For a review of many polymeric systems, see: Kiessling, L. L.; Pohl. N. *Chem. Biol.* **1996**, *3*, 71.

(173) Eddington, S. M. *Biotechnology* **1992**, *10*, 383.

(174) Spevak, W.; Foxall, C.; Charych, D. H.; Dasgupta, F.; Nagy, J. O. *J. Med. Chem.* **1996**, *39*, 9, 1018.

(175) Manning, D. D.; Hui, C.; Beck, P.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 3161.

(176) (a) Roy, R.; Park, W. K. C.; Srivastava, O. P.; Foxall, C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1399. (b) Miyachi, H.; Tanaka, M.; Koike, H.; Kawamura, N.; Hayashi, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 985.

(177) Kretschmar, G.; Sprengard, U.; Kunz, H.; Bartnik, E.; Schmidt, W.; Toepfer, A.; Horsch, B.; Krause, M.; Seiffge, D. *Tetrahedron* **1995**, *51*, 13015.

(178) Baisch, G.; Ohrlein, R. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1812.

(179) Sprengard, U.; Schudok, M.; Schmidt, W.; Kretschmar, G.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 321.

(180) Brunig, J.; Kessling, L. L. *Tetrahedron Lett.* **1996**, *37*, 2907.

(181) Seppo, A.; Turunen, J. P.; Penttila, L.; Keane, A.; Renkonen, O.; Renkonen, R. *Glycobiology* **1996**, *6*, 65. 182. Renkonen, O.; Topipila, S.; Penttila, L.; Salminen, H.; Maaheimo, H.; Costello, C. E.; Turunen, J. P.; Renkonen, R. *Glycobiology*, **1997**, *7*, 453.

(182) Stamper, H. B.; Woodruff, J. J. *J. Exp. Med.* **1976**, *144*, 828.
(183) Defrees, S. A.; Kosch, W.; Way, W.; Paulson, J. C.; Sabesan, S.; Halcomb, R. L.; Huang, D.-H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 66.
(184) Lin, C.-H.; Shimazaki, M.; Wong, C.-H.; Koketsu, M.; Juneja, L. R.; Kim, M. *Bioorg. Med. Chem. Lett.* **1995**, *3*, 1625.
(185) Miyauchi, H.; Yuri, M.; Tanaka, M.; Kawamura, N.; Hayashi, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 989.
(186) Welpy, J. K.; Abbas, S. Z.; Scudder, P.; Keene, P. L.; Broschat, K.; Casnocha, S.; Gorka, C.; Steininger, C.; Howard, S. C.; Schmuke, J. J.; Graneto, M.; Rotsaert, J. M.; Manger, I. D.; Jacob, G. S. *Glycobiology* **1994**, *4*, 259.
(187) Lin, C.-C.; Kimura, T.; Wu, S.-H.; Weitz, Schmidt, G.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2755.
(188) Cecconi, O.; Nelson, R. M.; Roberts, W. G.; Hanasaki, K.; Mannori, G.; Schultz, C.; Ulich, T. R.; Aruffo, A.; Bevilacqua, M. P. *J. Biol. Chem.* **1994**, *269*, 15060.
(189) O'Connell, D.; Koenig, A.; Jennings, S.; Hicke, B.; Han, H.-L.; Fitzwater, T.; Chang, Y.-F.; Varki, N.; Parma, D.; Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5883.
(190) Kretzschmar, G.; Toepfer, A.; Huls, C.; Krause, M. *Tetrahedron* **1997**, *53*, 2485.
(191) Martens, C. L.; Cwirla, S. E.; Lee, R. Y.-W.; Whitehorn, E.; Chen, E. Y.-F.; Bakker, A.; Martin, E. L.; Wagstrom, C.; Gopalan, P.; Smith, C. W.; Tate, E.; Koller, K. J.; Schatz, P. J.; Dower, W. J.; Barrett, R. W. *J. Biol. Chem.* **1995**, *270*, 21129.
(192) Briggs, J. B.; Larsen, R. A.; Harris, R. B.; Sekar, K. V. S.; Macher, B. A. *Glycobiology* **1996**, *6*, 831.
(193) Briggs, J. B.; Oda, Y.; Gilbert, J. H.; Shafer, M. E.; Macher, B. A. *Glycobiology* **1995**, *5*, 583.
(194) Rao, B. N. N.; Anderson, M. B.; Musser, J. H.; Gilbert, J. H.; Schaefer, M. E.; Foxall, C.; Brandley, B. K. *J. Biol. Chem.* **1994**, *269*, 19663.
(195) Ikeda, T.; Kajimoto, T.; Kondo, H.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1997**, *4*, 22485.
(196) Kiso, M.; Furui, H.; Ishida, H.; Hasegawa, A. *J. Carbohydr. Chem.* **1996**, *15*, 1 and references therein.
(197) (a) Weitz-Schmidt, G.; Stokmaier, D.; Scheel, G.; Nifant'ev, N. E.; Tuzikov, A. B.; Bovin, N. V. *Anal. Biochem.* **1996**, *238*, 184. (b) Thoma, G.; Magnani, J. L.; Ohrlein, R.; Ernst, B.; Schwarzenbach, F.; Duthaler, R. O. *J. Am. Chem. Soc.* **1997**, *119*, 7414−7415.
(198) Charych, D.; Cheng, Q.; Reichert, A.; Kuziemko, G.; Stroh, M.; Nagy, J. O.; Spevak, W.; Stevens, R. C. *Chem. Biol.* **1996**, *3*, 113.
(199) Jacob, G. S.; Kirmaier, C.; Abbas, S. Z.; Howard, S. C.; Steininger, C. N.; Welply, J. K.; Scudder, P. *Biochemistry* **1995**. *34*, 1210.
(200) McClure, W. O.; Edelman, G. M. *Biochemistry* **1966**, *5*, 1908.
(201) Weingold, E. G.; Knowles, J. R. *J. Am. Chem. Soc.* **1992**, *114*, 9270.
(202) Brunk, D. K.; Goetz, D. J.; Hammer, D. A. *Biophys. J.* **1996**, *71*, 2902.
(203) For a review of protein tyrosine sulfation, see: Niehrs, C.; Beibwanger, R.; Huttner, W. B. *Chem.-Biol. Interact* **1994**, *92*, 257.
(204) Niehrs, C.; Huttner, W. B.; Carvallo, D.; Degryse, E. *J. Biol. Chem.* **1990**, *265*, 9314.
(205) Habuchi, O.; Suzuki, Y.; Fukut, M. *Glycobiology* **1997**, *7*, 405.
(206) Alon, R.; Hammer, D. A.; Springer, T. A. *Nature* **1995**, *374*, 539.
(207) Finger, E. B.; Puri, K. D.; Alon, R.; Lawrence, M. B.; von Andrian, U. H.; Springer, T. A. *Nature* **1996**, *379*, 266.
(208) Alon, R.; Fuhlbrigge, R. C.; Finger, E. B.; Springer, T. A. *J. Cell. Biol.* **1996**, *135*, 849.
(209) Zimmerman, B. J.; Paulson, J. C.; Arrhenius, T. S.; Gaeta, F. C. A.; Granger, D. N. *Am. J. Physiol.* **1994**, *267*, H1049.
(210) Kanwar, S.; Johnston, M. B.; Kubes, P. *Circ. Res.***1995**, *77*, 879.
(211) Koch, A. E.; Halloran, M. M.; Haskell, C. J.; Shah, M. R.; Polverini, P. J. *Nature* **1995**, *376*, 517.
(212) Maly, P.; Thall, A. D.; Petryniak, B.; Rogers, C. E.; Smith, P. L.; Marks, R. M.; Kelly, R. J.; Gersten, K. M.; Cheng, G.; Saunders: T. L.; Sullivan, F. X.; Isogai, Y.; Hindsgaul, O.; von Andrian, U. H.; Lowe, J. B. *Cell* **1996**, *86*, 643. (b) Smith, P. L.; Gersten, K. M.; Petryniak, B.; Kelly, R. J.; Rogers, C.; Natsuka, Y.; Alford, J. A., III; Scheidegger, E. P.; Nausuka, S.; Lowe, J. B. *J. Biol. Chem.* **1996**, *271*, 8250. (c) de Vries, T.; van den Eijnden, D. H. *Biochemistry* **1994**, *33*, 9937.
(213) Palcic, M. M.; Ratcliffe, R. M.; Lamontagne, L. R.; Good, A. H.; Alton, G.; Hindsgaul, O. *Carbohydr. Res.* **1990**, *196*, 133.
(214) Qiao, L.; Murray, B. W.; Shimazaki, M.; Shultz, J.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 7653.

CR940226I