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Design and Synthesis of E-Selectin Antagonists

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Abstract: Selectin-mediated recruitment of leukocytes plays a crucial role in a number of diseases and pathological situations, for example in inflammation, reperfusion injury, rheumatoid arthritis and respiratory diseases. Substantial research efforts are directed toward development of carbohydrate-derived drugs that interfere with the inflammatory response by blocking the selectin binding site. This article describes two approaches for the improvement of the inhibitory potency of the lead structure sialyl Lewis^x (sLe^x). One approach is based on the preorganization of mimics in their bioactive conformation to reduce entropic costs. For the conformational analysis of mimics, molecular modeling based tools were developed. They allow the rational design of selectin antagonists with simplified structures, but increased inhibitory potency. Alternatively, additional carbohydrate/lectin contacts can be identified for the improvement of enthalpic contributions. Following this approach, an additional hydrophobic interaction of the antagonists with E-selectin leads to a 60-fold improvement of E-selectin affinity. Antagonists have been synthesized using chemical or chemo-enzymatic methods. Finally, a flow chart for the biological evaluation of the antagonists is presented.

Keywords: Bioactive conformation · Chemical and chemo-enzymatic synthesis of glycomimetics · Molecular modeling · Preorganization · Selectin antagonists · Sialyl Lewis^x

Introduction

Excessive infiltration of leukocytes from blood vessels into inflamed tissues can cause acute or chronic reactions as observed in reperfusion injuries, stroke, psoriasis, rheumatoid arthritis, and respiratory diseases [1]. Using intravital microscopy, the migration of leukocytes from the circulation to the inflamed tissue can be directly observed. This process takes place following a regulated chain of events (rolling, firm adhesion, diapedesis, and migration through the interstitium). Leukocyte rolling – the first event in the inflammatory cascade – is mediated largely by the selectin family of adhesion molecules and their carbohydrate bearing ligands [2]. The contribution of carbohydrate-selectin interactions to inflammation is exemplified by the impaired inflammatory responses seen in selectin knockout mice [3a–c], and LAD-type-2 patients [3d]. Although the nature of the carbohydrate ligands and their interaction with the selectins is not fully understood, it is accepted that expression of fucosylated and sialylated glycans such as sialyl Lewis^x (sLe^x) is required for function [4]. In addition to findings that sLe^x inhibits leukocyte binding to selectins *in vitro*, a beneficial effect of this tetrasaccharide in inflammatory disease models has also been demonstrated [5].

Despite promising biological findings, Cylexin, a sialyl Lewis^x analogue, proved to be an unsuccessful drug candidate [6]. The reason for its failure is, most likely, a combination of low biological affinity [7] and limited bioavailability [8]. A third hurdle for clinical application of sialyl Lewis^x and derivatives thereof is synthetic availability, since only gram quantities are generally obtained by chemical syntheses [9]. The latter problem, however, was solved by Wong and coworkers [10], who developed a large-scale enzymatic approach.

Structure-Activity Relationship – Bioactive Conformation – Docking to E-Selectin

As a consequence of the aforementioned problems, sLe^x was not a drug candidate itself but served as a lead structure for numerous industrial and academ-

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ic research groups. Their major aim was to design sLe^x mimetics with simplified structures but increased inhibitory potency and improved bio-availability. According to various structure-activity studies [11], only a selected subset of the functional groups of sLe^x are required for E-selectin recognition. In Fig. 1 these functional groups are highlighted in red. Groups colored in blue are not essential and can therefore be altered or even removed without any loss in bioactivity.

The bioactive conformation (= spatial orientation of the pharmacophores when bound to the receptor) was determined from transfer-NOE NMR studies on the sLe^x/E-selectin complex [12]. The structures shown in Fig. 2 were generated from the distance constraints obtained from the NMR experiments. These conformations reflect the experimental precision with which the bound conformation of sLe^x can be obtained. The most striking feature of this arrangement is a stacking of the galactose and the fucose units, with the Glc*N*Ac portion acting as a spacer. The carboxy function of the neuraminic acid residue points out of the plane and, as a consequence, the C(7)-C(9) side chain of neuraminic acid is placed directly above the galactose ring system. This result contrasts with NMR [9][13] and molecular modeling studies [13a][14] on the free sugar that suggest that several conformations exist in aqueous solution.

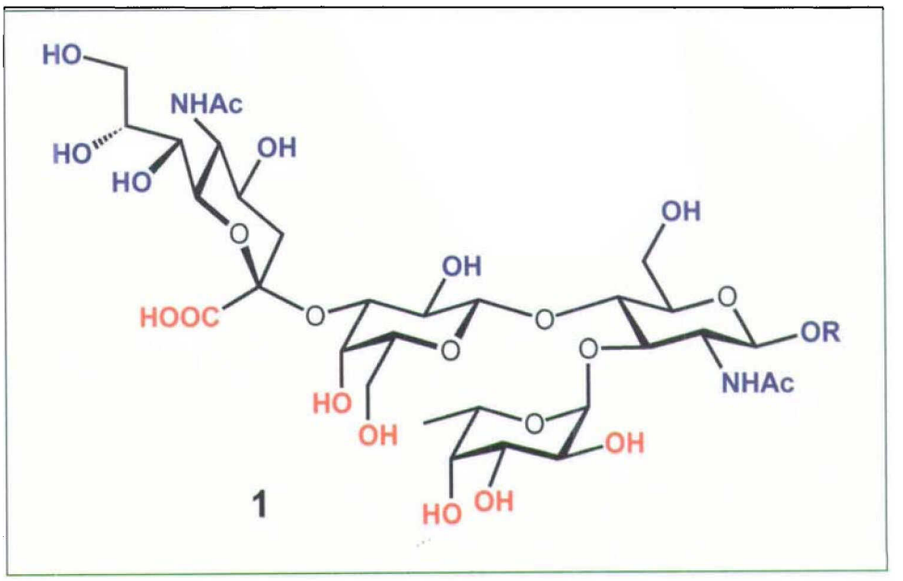


Fig. 1. Sialyl Lewis^x tetrasaccharide (pharmacophores required for binding to E-selectin are highlighted in red, functional groups not required for binding are colored in blue).

A two-dimensional internal coordinate system was used for the analysis of the structural data [15]. It allows the spatial arrangement of the relevant pharmacophores, namely that of the COOH group relative to the fucose moiety to be defined. One coordinate, the Fuc(C4)-Fuc(C1)-Fuc(O1)-Acid(Cα) angle (core conformation), describes the Lewis^x core. The other coordinate, the angle Fuc(C1)-Fuc(O1)-Acid(Cα)-Acid (C=O) (acid orientation), defines the orientation of the COOH group relative to the core (see plot in Fig. 2).

The three-dimensional structure of the ligand-binding region of human E-selectin has been determined at 2.0 Å resolution by Graves *et al.* [16]. By mapping the amino acid substitution that eliminates neutrophile adhesion to E-selectin, a finite region of the lec domain of E-selectin in the vicinity of the bound calcium ion was identified as the most likely carbohydrate contact surface.

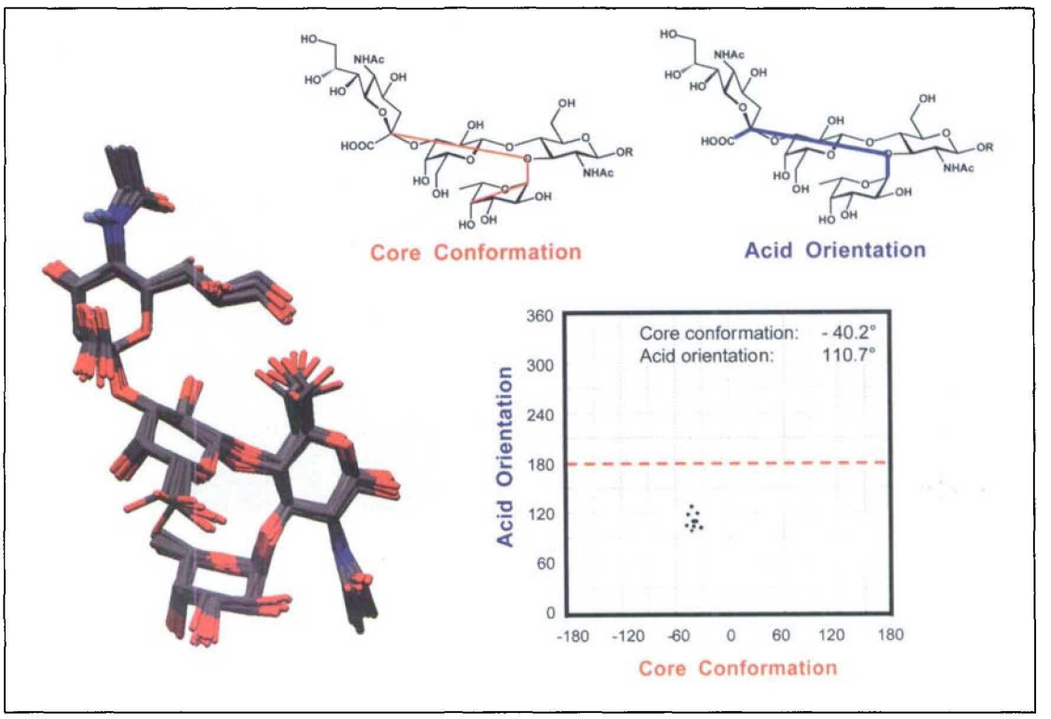


Fig. 2. Bioactive conformation of sialyl Lewis^x determined by transfer-NOE NMR [12a].

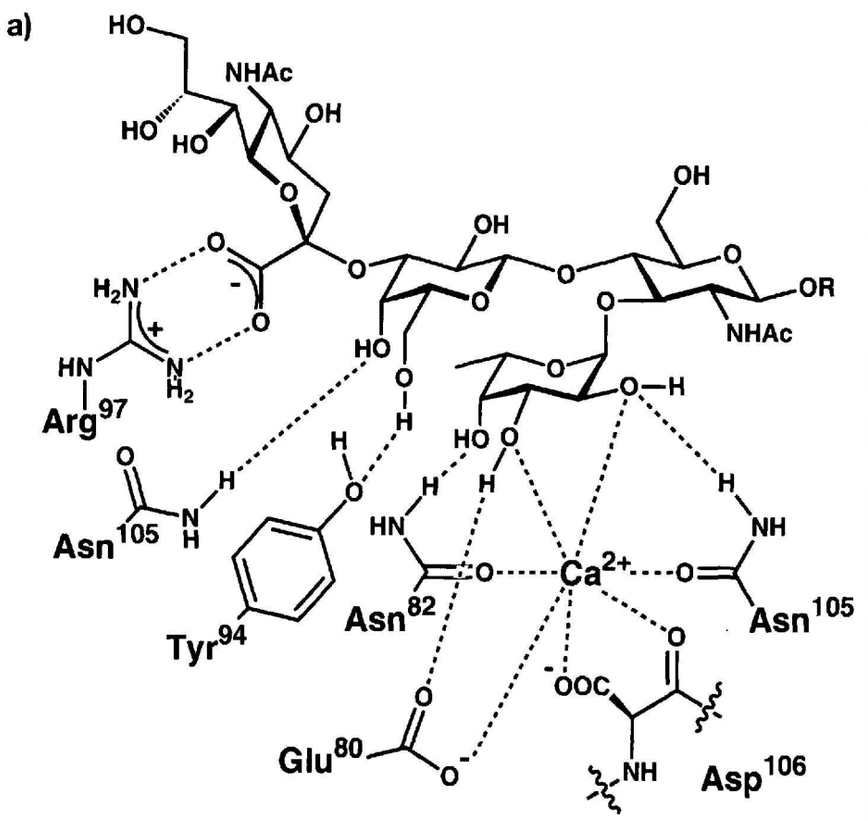


Fig. 3. a) Model showing the functional groups of sLe^x interacting with E-selectin [17]; b) docking of sialyl Lewis^x with E-selectin (shown in a colored grid, where red represents surface areas that act as hydrogen bridge acceptors and blue those that act as hydrogen bridge donors; hydrophobic areas are indicated in yellow).

Docking of the bioactive conformation of the sialyl Lewis^x tetrasaccharide was performed as shown in Fig. 3 [17] in analogy to the mannose-binding protein [18]. In this docking mode all pharmacophores are in contact with the rather shallow binding site and all functional groups not involved in binding (see Fig. 1) point towards the surrounding water. Although the crystal structure of E-selectin co-complexed with sialyl Lewis^x [19] was recently published, the X-ray data are not yet available, and in the discussion of [19] no data are presented for the bioactive conformation of sLe^x that could be compared to prior NMR studies.

Rational Design of Antagonists

The free binding energy between the carbohydrate ligand and E-selectin is a function of the binding enthalpy and the binding entropy. The binding enthalpy is directly dependent on the number and strength of carbohydrate/lectin contacts, while the binding entropy is influenced by the flexibility of the carbohydrate ligand and its propensity to adopt the bioactive conformation. We first focused on conformational aspects by developing a molecular modeling tool [Monte Carlo (jumping between wells)/stochastic dynamics simulation] for assessing the flexibility and the preorganization of sialyl Lewis^x mimics for binding to E-selectin [15].

Our approach is illustrated by the following three examples. Based on the structure-activity relationships (see Fig. 1, 2, and 3), Glc*N*Ac was replaced by (*R,R*)-cyclohexan-1,2-diol and sialic acid by glycolic acid. Computational analysis (Fig. 4a) suggested that the core conformation remains unchanged compared to the lead structure sLe^x. For the acid orientation, however, a dramatic change was observed. Since the carboxylic acid function can freely rotate around the ether bond to the galactose, the bioactive conformation is still occupied, but with a much lower probability than in the case of the lead structure. When, however, cyclohexyl lactic acid (Fig. 4b) was introduced instead of glycolic acid, the computational model showed a surprising result. With the (*S*)-cyclohexyl lactic acid substituent, the core conformation and acid orientation remain very close to that of the lead structure. With the (*R*)-cyclohexyl lactic acid substituent, however, it was postulated that the bioactive window is no longer populated at all, suggesting that **3** would show a higher affinity for E-selectin than **2** or even **4**.

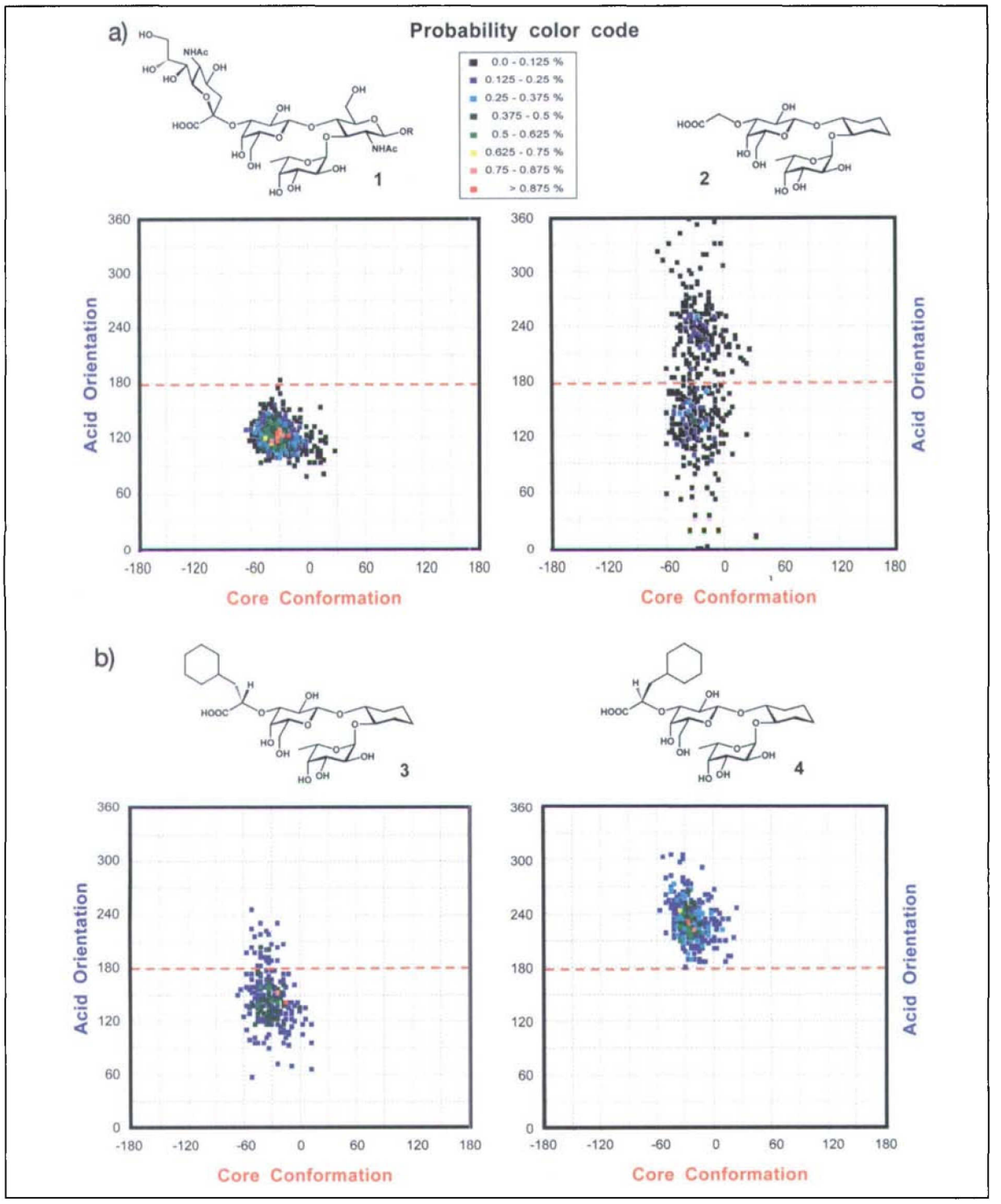


Fig. 4. a) Conformational analysis of sialyl Lewis^x (**1**) and mimic **2** by MC(JBW)/SD simulation [15]; the probability of being at any point in the two-dimensional torsional space at a resolution of 3° x 3° is indicated by color code: bright colors represent high probability and dark colors low probability; b) conformational analysis of mimics **3** and **4** by MC(JBW)/SD simulation [15]; the color code used to indicate the probability of being at any point in the two-dimensional torsional space is the same as in Fig. 4a.

Flow Chart

Biological evaluation of compounds **1–4** was performed according to the flow chart in Fig. 5. A primary target-based screen for E-selectin activity in a cell-free, competitive assay was developed using a polylysine/sialyl Lewis^a conjugate as a multivalent ligand [20]. Immobilized E-selectin was incubated with the polymer and then treated with the test compound. The measurement of the dose-dependent displacement of the polymer by potential inhibitors in comparison with the sialy Lewis^x tetrasaccharide allows the determination of their rIC_{50} values (relative IC_{50} values; concentration to achieve an inhibitory effect of 50% relative to the concentration of sLe^x producing the same effect) under static conditions. The second screen was function-based and was conducted under flow conditions, imitating the *in vivo* situation in a blood vessel. For this purpose a flow chamber-assay allowing the observation of neutrophile rolling on activated endothelial cells under the light microscope was developed [21]. The third assay was based on our findings that soluble sLe^x selectively disrupts E-selectin dependent

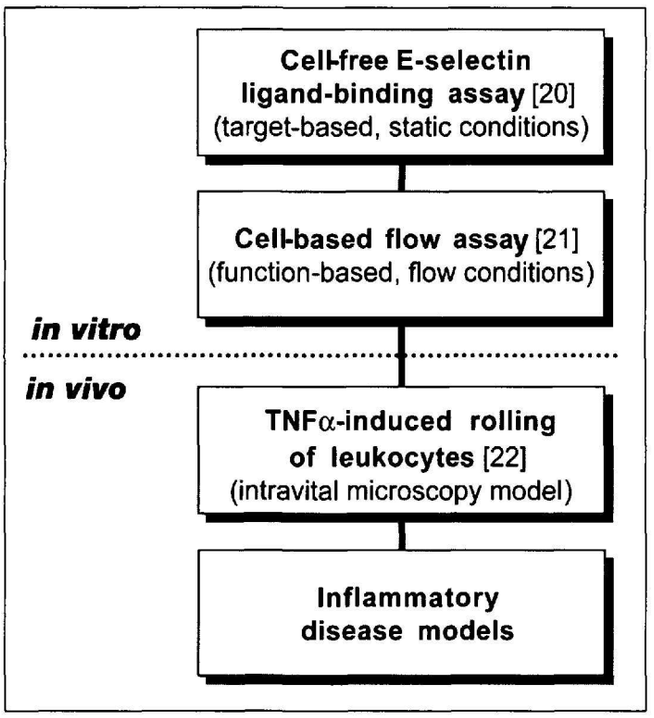


Fig. 5: Flow chart for the biological evaluation of selectin antagonists.

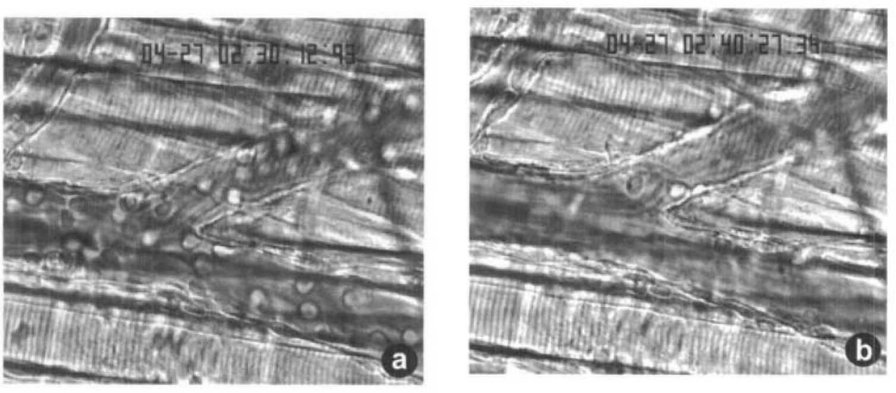


Fig. 6. a) Leukocyte rolling and firm adhesion in a TNFα stimulated P-selectin knockout mouse; b) clearance of rolling after addition of 30 mg/kg E-selectin antagonist **3**.

Table. For the static cell-free assay and the dynamic cell assay relative IC_{50}s (rIC_{50}) are reported which are standardized relative to sLe^x. The percentage of leukocytes which are involved in E-selectin dependent rolling two hours after TNFα-stimulation was determined in the intravital microscopy model.

Compounds	Static cell-free assay rIC_{50}	Dynamic cell assay rIC_{50}	Leucocytes engaged in TNFα-induced rolling measured by intravital microscopy
sLe^x (**1**)	1	1	~ 40%
2	10	n.d.	n.d.
3	0.1	0.1–0.15	< 6%
4	> 15	> 15	n.d.
16i	0.016	0.02	< 6%
Control (Siaα(2-3)LacNAc)	> 15	> 15	~ 40%

rolling in the mouse cremaster muscle, causing increased rolling velocity of leukocytes on the endothelial surface [22]. An intravital microscopy model, where the percentage of leukocytes engaged in TNFα-induced rolling interaction with the blood vessel wall can be measured before and after application of inhibitors (Fig. 6), was used for *in vivo* screening of sLe^x mimetics.

The biological evaluation (Table) of compounds **1–4** confirmed the predictions of the molecular modeling-based approach described above. Compound **3**, the best mimic identified, is approximately 10 times more active than the parent sLe^x. According to its bioactive conformation, which was determined by transfer-NOE NMR [23] (Scheme 1), the pharmacophores bind to E-selectin in the same three-dimensional conformation as in the lead structure sLe^x.

The agreement between predicted and observed activity trends suggests that the free binding energy is dominated by the preorganization of the mimic and consequently by the entropy. The calculated preorganization can therefore be used as a descriptor for quantitative structure-activity models.

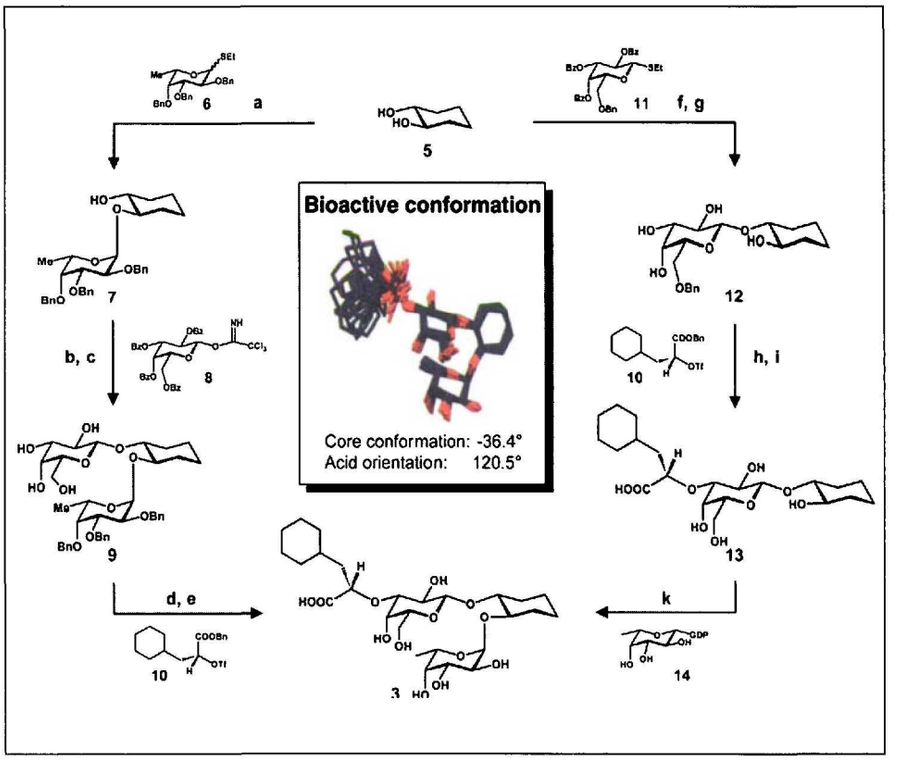
Compared to sLe^x (**1**), the synthesis of mimic **3** is dramatically simplified. Starting from (*R,R*)- cyclohexan-1,2-diol (**5**), fucosylation is achieved stereoselectively in an excellent yield with the *in situ* anomerization procedure. For the introduction of galactose, the trichloracetimidate procedure was successfully applied. After hydrolysis of the benzoates (→**9**), exclusive alkylation of the 3-position with benzyl (*R*)-3-cyclohexyl-2-trifluoromethansulfonyloxy-propionate was achieved by regioselective opening of the corresponding stannylene acetal. After deprotection the (*S*)-lactic acid derivative **3** was obtained in 62% yield.

Compound **3** can also be synthesized chemo-enzymatically. The glycosyl acceptor was obtained by galactosylation of (*R,R*)-cyclohexan-1,2-diol (**5**) with ethyl 6-O-benzyl-2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside **11** using DMTST as promoter (→**12**) followed by the removal of benzoate protecting groups by transesterification. Finally, alkylation with benzyl (*R*)-3-cyclohexyl-2-trifluoromethansulfonyloxy-propionate (**10**) and deprotection by hydrogenolysis gave substrate **13** for the enzymatic fucosylation. Fucose was transferred from GDP-fucose (**14**) to the hydroxy group of the cyclohexane moiety [25] with fucosyl transferase III [24a] yielding **3** in good overall yield.

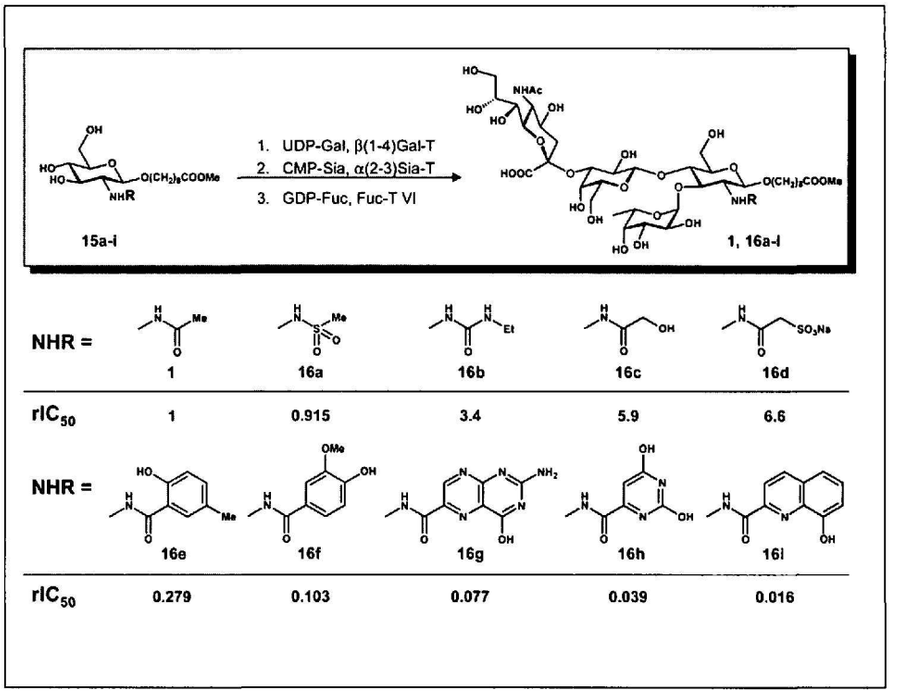
A possibility for the improvement of the enthalpic component of the sLe^x/E-selectin interaction was suggested by a publication by DeFrees and coworkers [26], who reported that *N*-benzoate and *N*-naphthoate analogues of sLe^x showed slightly increased inhibitory potency when tested as inhibitors of E-selectin-mediated cell adhesion. Conformational analysis by NMR indicates that the topographic orientation of the pharmacophores required for binding is not influenced by the modification of the acyl group at the glucosamine nitrogen [27]. The increase in potency may therefore result from a direct interaction of the aromatic acyl groups with E-selectin, presumably through a hydrophobic mechanism.

To further investigate the effect of non-natural acyl substituents at the glucosamine nitrogen, derivatives **16a–i** were synthesized chemo-enzymatically. Since the modification of the acyl group in the final tetrasaccharides *via* an exchangeable allyl carbamate protection of the glucosamine nitrogen was not generally applicable, a non-convergent approach was chosen. Based on their broad substrate specificity, we have explored with β(1-4) galactosyltransferase [28], α(2-3)sialyltransferase [29], and α(1-3,4)fucosyltransferase (FucT VI) [30], the modified *N*-acylglucosamine derivatives **15a–i** were submitted for enzymatic glycosylation. In the first enzymatic step, galactose was transferred to Glc*N*Ac, the natural substrate, but also to glucosamines acylated with various aliphatic, aromatic and heteroaromatic acyl groups. The cloned α(2-3)SiaT [31] also accepted a whole range of *N*-acyl-lactosamines. Although DMSO had to be added in some cases to obtain sufficient solubility, neuraminic acid was enzymatically transferred by α(2-3)SiaT without any adverse effects. Finally, all trisaccharides were well tolerated by Fuc-T VI [24b] (Scheme 2).

Whereas the aliphatic substitutions (see **16a–d**) do not improve E-selectin affinity, a substantial increase was observed with aromatic or heteroaromatic acyl groups (see **16e–i**). The most active representative, **16i**, shows an approximately 60-fold increase (compared with sLe^x) in E-selectin affinity in the static and dynamic *in vitro* assays and also improved activity *in vivo* [27].



Scheme 1. Chemical and chemo-enzymatic synthesis of sLe^x-mimic **3**: a) **6**, Br_2, Bu_4NBr (87%); b) **8**, BF_3•Et_2O, CH_2Cl_2 (84%); c) NaOMe, MeOH (75%); d) $^nBu_2SnO$, MeOH, rfl., 3h; **10**, CsF, DMF, r.t., 3h (78%); e) H_2, Pd/C, MeOH (88%); f) **11**, DMTST, CH_2Cl_2, 4 Å molecular sieves, 3h (59%); g) cat. NaOMe, MeOH, r.t., 3h (96%); h) $^nBu_2SnO$, MeOH, rfl., 2h; **10**, CsF, DMF, r.t., 3h (76%); i) 20% $Pd(OH)_2$, H_2, dioxane-H_2O-AcOH; Dowex 50 (Na^+ form) (80%); k) GDP-fucose (**14**), FucTIII (84%).



Scheme 2. Chemo-enzymatic synthesis of sialyl Lewis^x derivatives; relative IC_{50}s (rIC_{50}) which are standardized relative to sLe^x (**1**) are reported.

Conclusions

Carbohydrates traditionally have had only limited use as therapeutic agents, because of their structural complexity and synthetic difficulties. Only recently have their biological functions and their role in disease been decoded. One of these roles – that of carbohydrate-selectin interactions in inflammation – is currently the focus of numerous industrial and academic research groups. Because of the inherently low affinity between lectins and carbohydrates, the design of high affinity inhibitors is difficult. Our combined approach tackles this problem simultaneously from different directions. Structural information about the bioactive conformation of the lead structure sialyl Lewis^x by transfer-NOE NMR, conformational analysis of proposed mimics by molecular modeling, and chemo-enzymatic synthesis of the mimetics was key for the successful development of carbohydrate mimetics with simplified structures and improved target affinity.

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[1] a) S.A. Mousa, *Drugs Fut.* **1996**, *21*, 283; b) S.A. Mousa, D.A. Cheresh, *Drug Discovery Today* **1997**, *2*, 187; c) D.B. Cines, E.S. Pollak, C.A. Buck, J. Loscalzo, G.A. Zimmermann, R.P. McEver, J.S. Pober, T.M. Wick, B.A. Konkle, B.S. Schwartz, E.S. Barnathan, K.R. McCrae, B.A. Hug, A.-M. Schmidt, D.M. Stern, *Blood* **1998**, *91*, 3527.

[2] a) R.R. Lobb in 'Adhesion. Its Role in Inflammatory Disease', Eds. J.M. Harlan, D.Y. Liu, W.H. Freeman: New York, **1992**, Chapter 1, p. 1; b) J.C. Paulson in 'Adhesion. It's Role in Inflammatory Disease', Eds. J.M. Harlan, D.Y. Liu, W.H. Freeman: New York **1992**; Chapter 2, p. 19; c) G.S. Kansas, *Blood* **1996**, *88*, 3259. d) A. Varki, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390.

[3] a) T.F. Tedder, *FASEB J.* **1995**, *9*, 866; b) R. McEver, K. Moore, R.D. Cummings, *J. Biol. Chem.* **1995**, *270*, 11025; c) L. Lasky, *Annu. Rev. Biochem.* **1995**, *64*, 113; d) U.H. van Adrian, E.M. Berger, L. Ramezani, J.D. Chambers, H.D. Ochs, J.M. Harlan, J.C. Paulson, A. Etzioni, K.-E. Arfors, *J. Clin. Invest.* **1993**, *91*, 2893.

[4] a) L. Phillips, E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S.-L. Hakomori, J.C. Paulson, *Science* **1990**, *250*, 1130; b) G. Walz, A. Aruffo, W. Kolanus, M. Bevilacqua, B. Seed, *Science* **1990**, *250*, 1132; c) E.L. Berg, M.K. Robinson, O. Mansson, E.C. Butcher, J.L. Magnani, *J. Biol. Chem.* **1991**, *266*, 14869.

[5] a) D. Mihelcic, B. Schleiffenbaum, T.F. Tedder, S.R. Sharar, J.M. Harlan, R.K. Winn, *Blood*, **1994**, *84*, 2322; b) D.M. Flynn, A.J. Buda, P.R. Jeffords, D.J. Lefer, *Am. J. Physiol.* **1996**, *271*, H2086; c) H. Sagara, C. Ra, T. Okada, S. Shinohara, T. Fukuda, K. Okumura, S. Makino, *Int. Arch. Allergy Immunol.* **1996**, *111*, 32; d) I.Y. Park, D.S. Lee, M.H. Song, W. Kim, J.M. Won, *Transplant. Proc.* **1998**, *30*, 2927.

[6] In the first quarter of 1999, Cytel Corporation, La Jolla, CA announced the results from the Cylexin (CY1503, sialyl Lewis^x analogue) Phase II/III clinical trials (treatment of reperfusion injury in infants undergoing corrective surgery for congenital heart disease) which indicated that Cylexin showed no benefit over the placebo.

[7] A. Koenig, R. Jain, R. Vig, K.E. Norgard-Sumnicht, K.L. Matta, A. Varki, *Glycobiology* **1997**, *7*, 79.

[8] D.J. Lefer, D.M. Flynn, M.L. Phillips, M. Ratcliffe, A.J. Buda, *Circulation* **1994**, *90*, 2390.

[9] a) A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.* **1991**, *209*, c1; b) K.C. Nicolaou, C.W. Hummel, Y. Iwabuchi, *J. Am. Chem. Soc.* **1992**, *114*, 3126; c) S. J. Danishefsky, J. Gervay, J.M. Peterson, F.E. McDonald, K. Koseki, T. Oriyama, D.A. Griffith, *J. Am. Chem. Soc.* **1992**, *114*, 8329.

[10] Y. Ichikawa, Y.-C. Lin, D.P. Dumas, G.-J. Shen, E. Garcia-Junceda, M.A. Williams, R. Bayer, C. Ketcham, L.E. Walker, J.C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 9283.

[11] a) J.T. Ramphal, Z.-L. Zheng, C. Perez, L.E. Walker, S.A. DeFrees, F.C.A. Gaeta, *J. Med. Chem.* **1994**, *37*, 3459; b) W. Stahl, U. Sprengard, G. Kretschmar, H. Kunz, *Angew. Chem. Int. Ed.* **1994**, *33*, 2096; c) B.K. Brandley, M. Kiso, S. Abbas, P. Nikrad, O. Srivasatava, C. Foxall, Y. Oda, A. Hasegawa, *Glycobiology* **1993**, *3*, 633; R. Bänteli, B. Ernst, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 459.

[12] a) K. Scheffler, B. Ernst, A. Katopodis, J.L. Magnani, W.T. Wong, R. Weisemann, T. Peters, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1841; b) K. Scheffler, J.-R. Brisson, R. Weisemann, J.L. Magnani, W.-T. Wong, B. Ernst, T. Peters, *J. Biomol. NMR* **1997**, *9*, 423; c) R. Harris, G.R. Kiddle, R.A. Field, B. Ernst, J.L. Magnani, S.W. Homans, *J. Am. Chem. Soc.* **1999**, *121*, 2546.

[13] a) T.J. Rutherford, D.G. Spackman, P.J. Simpson, S.W. Homans, *Glycobiology* **1994**, *4* 59; b) C. Mukhopadhyay, K.E. Miller, C.A. Bush, *Biopolymers* **1994**, *34*, 21; c) H.C. Siebert, G. Reuter, R. Schauer, C.W. v.d. Lieth, J. Dabrowski, *Biochemistry* **1992**, *31*, 6962; d) G.E. Ball, R.A. O'Niell, J.E. Schultz, J.B. Lowe, B.W. Weston, J.O. Nagy, E.G. Brown, C.J. Hobbs, M.D. Bednarsky, *J. Am. Chem. Soc.* **1992**, *114*, 5449.

[14] F. Bizik, I. Tvaroska, *Chem Papers* **1996**, *50*, 84.

[15] a) H.C. Kolb, B. Ernst, *Chem. Eur. J.* **1997**, 1571; b) H.C. Kolb, B. Ernst, *Pure & Appl. Chem.* **1997**, *69*, 1879.

[16] B.J. Graves, R.L. Crowther, C. Chandran, J.M. Rumberger, S. Li, K.-S. Huang, D.H. Presky, P.C. Familletti, B.A. Wolitzky, D.K. Burns, *Nature* **1994**, *367*, 532.

[17] a) D.V. Erbe, B.A. Wolitzky, L.G. Presta, C.R. Norton, R.J. Ramos, D.K. Burns, J.M. Rumberger, B.N.N. Rao, C. Foxall, B.K. Brandley, L.A. Lasky, *J. Cell Biol.* **1992**, *119*, 215; b) T.P. Kogan, B.M. Revelle, S. Tapp, D. Scott, P.J. Beck, *J. Biol. Chem.* **1995**, *270*, 14047.

[18] a) W.I. Weis, K. Drickamer, W.A. Hendrickson, *Nature* **1992**, *360*, 127; b) O. Blanck, S.T. Iobst, C. Gabel, K. Drickamer, *J. Biol. Chem.* **1996**, *271*, 7289.

[19] W.S. Somers, J. Tang, G.D. Shaw, R.T. Camphausen, *Cell* **2000**, *103*, 467.

[20] G. Thoma, J.L. Magnani, R. Oehrlein, B. Ernst, F. Schwarzenbach, R.O. Duthaler, *J. Am. Chem. Soc.* **1997**, *119*, 7414.

[21] G. Thoma, J.T. Patton, J.L. Magnani, B. Ernst, R. Oehrlein, R.O. Duthaler, *J. Am. Chem. Soc.* **1999**, *121*, 5919.

[22] K.E. Norman, G.P. Anderson, H.C. Kolb, K. Ley, B. Ernst, *Blood* **1998**, *91*, 475.

[23] W. Jahnke, H.C. Kolb, M.J.J. Blommers, J.L. Magnani, B. Ernst, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2603.

[24] a) B.W. Weston, P.L. Smith, R.J. Kelly, J.B. Lowe, *J. Biol. Chem.* **1993**, *267*, 24575; b) K.L. Koszdin, B.R. Bowen, *Biochem. Biophys. Res. Commun.* **1992**, *187*, 152.

[25] B. Ernst, B. Wagner, G. Baisch, A. Katopodis, T. Winkler, R. Oehrlein, *Can. J. Chem.* **2000**, *78*, 891.

[26] J.Y. Ramphal, M. Hiroshige, B. Lou, J.J. Gaudino, M. Hayashi, S.M. Chen, L.C. Chiang, F.C.A. Gaeta, S.A. DeFrees, *J. Am. Chem. Soc.* **1996**, *39*, 1357.

[27] B. Ernst, M. Blommers, W. Jahnke, R. Oehrlein, J.L. Magnani, unpublished results.

[28] a) R. Oehrlein, B. Ernst, E.G. Berger, *Carbohydr. Res.* **1992**, *236*, 335; b) G. Baisch, R. Oehrlein, B. Ernst, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 749.

[29] G. Baisch, R. Oehrlein, M. Streiff, B. Ernst, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 755.

[30] G. Baisch, R. Oehrlein, A. Katopodis, B. Ernst, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 759.

[31] D.X. Wen, B.D. Livingston, K.F. Medziharadszky, S. Kelm, A.L. Burlingame, J.C. Paulson, *J. Biol. Chem.* **1992**, *267*, 21011.