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Recent progress in the glycodrug area

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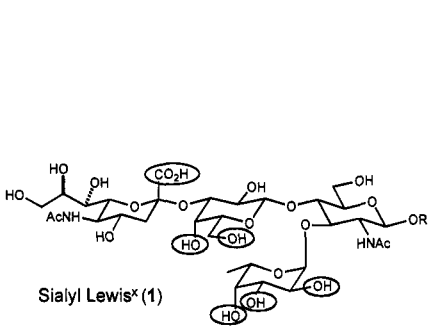
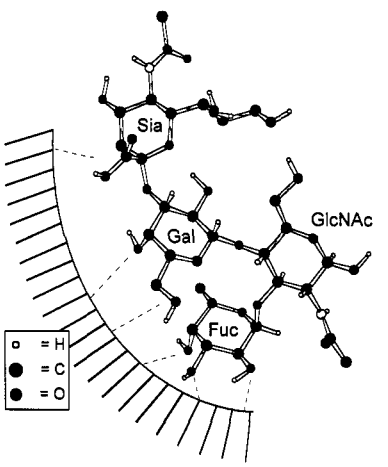
Abstract: A new molecular modelling tool for analysing the pre-organization of sialyl Lewis^x mimics for binding to E-selectin has been developed. The *pre-organization* is quantified as the probability for being in the *bioactive window* of torsional space. The probability data can be correlated with bioactivity, since carbohydrate mimics which populate the bioactive window are likely to be active towards E-selectin, whereas compounds which do not populate the bioactive window are generally inactive. Work is in progress with the aim to further refine this model and to develop quantitative structure activity relationships using the probability data as descriptors. The computational tool has guided our search for new and more active E-selectin antagonists. One of our most active carbohydrate mimics, the cyclohexyl derivative *S*-**5**, combines an over ten-fold higher affinity towards E-selectin with a considerably lower molecular weight and a lower hydrophilicity compared to sialyl Lewis^x.

Numerous diseases and pathological situations are related to excessive influx of leukocytes into the tissue.[1] The complex process which leads to the recruitment of leukocytes from the blood stream is initiated by the interaction of carbohydrates on the leukocyte's cell surface and the selectins. The latter are a family of C-type lectins, of which P- and E-selectin are expressed on endothelial cells after stimulation while L-selectin is present on leukocyte cell surfaces. The carbohydrate epitope recognized by all selectins, albeit with different affinities, is the tetrasaccharide sialyl Lewis^x. A new strategy for the treatment of inflammatory and respiratory diseases is based on the inhibition of the carbohydrate/selectin interactions.[1]

Currently, we are searching for low molecular weight carbohydrate mimics which are designed to block the selectin binding site in order to inhibit the process of leukocyte recruitment. A prerequisite for the rational design of high affinity selectin antagonists is a thorough understanding of the role of all the functional groups[2] and their spatial orientation in the lead structure, sialyl Lewis^x. *Figure* 1 summarizes the functional groups involved in binding (*figure* 1a) and their orientation (*figure* 1b) in the 'bioactive conformation', as established by transfer-NOE NMR experiments.[3]



(a) The functional groups involved in binding



(b) Bioactive conformation of sialyl Lewis^x and schematic representation of its binding to E-Selectin

Fig. 1 Three-dimensional functional group requirements for bioactivity. The most important functional groups are circled.

The X-ray structure of E-selectin[4] reveals a relatively flat binding site. This is consistent with our experimental observation[2a] that only some of the ligand's functional groups are involved in direct interactions with the protein. Preliminary computational docking studies show that interactions are distributed over a large surface area. Thus, the three-dimensional orientation of the relevant functional groups is very important, suggesting that binding is largely influenced by the pre-organization of the ligand, i.e. by entropy factors.

We have, therefore, developed a computational tool for assessing a sialyl Lewis^x mimic's pre-organization for binding to E-selectin.[5] It is based on the 'Jumping between Wells/stochastic Dynamics' [MC(JBW)/SD] algorithm[6,7] and the systematic pseudo-Monte-Carlo (SUMM) simulation[8] technique recently developed by Still *et al.* (*figure* 2). These methods are implemented in MacroModel 5.0.[9] First, the locations of the most relevant energy minima (conformations) of a mimic are determined in a 5000 step internal coordinate systematic pseudo-Monte-Carlo (systematic, unbounded multiple minimum search, SUMM) simulation[8] (*figure* 2a-c). The shape of the potential energy surface is then probed in a subsequent MC(JBW)/SD simulation[6,7] which uses the information obtained in the SUMM analysis (*figure* 2d). Thus, a Boltzman weighted ensemble of states is generated in this MC(JBW)/SD simulation by jumping between different energy wells, i.e. conformations found in the preceeding SUMM analysis, and performing stochastic dynamics simulations within each well. The simulations are performed employing an augmented AMBER* force field,[7] containing optimized α-alkoxy acid parameters,[5] and the GB/SA continuum water model.[10]

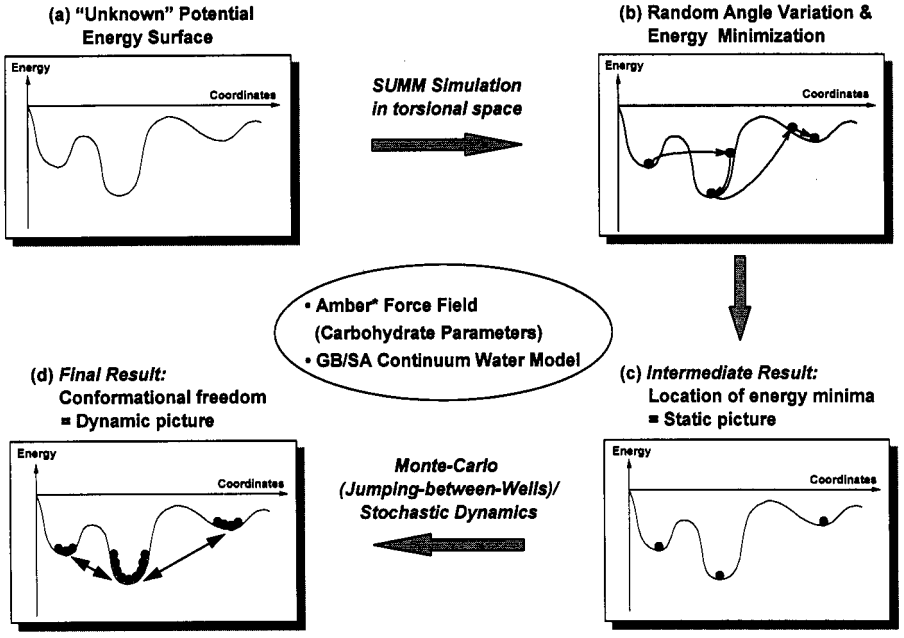
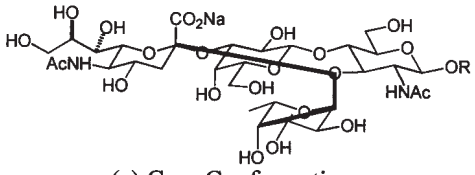


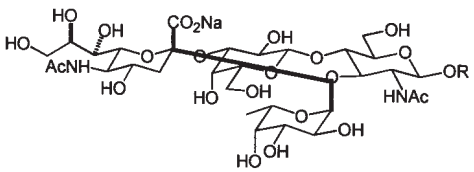
Fig. 2 Analysis of the potential energy surface and the pre-organization of a mimic.

As a result a dynamic picture of the flexibility and the pre-organization of a mimic is obtained. The structural data are analysed using a two-dimensional *internal coordinate* system to define the spatial arrangement of the relevant pharmacophores, i.e. the COOH group relative to the fucose moiety. The Fuc(C4)–Fuc(C1)–Fuc(O1)–Acid(Cα) angle (*figure* 3a) describes the conformation of the Lewis^x core. This coordinate is independent of the actual nature of the core. The second coordinate, the angle Fuc(C1)–Fuc(O1)–Acid(Cα)–Acid(**C**=O) (*figure* 3b), defines the orientation of the COOH group relative to the core.

The several thousand structures which were obtained by sampling the 2-10 ns MC(JBW)/SD simulations every 1 ps were used to evaluate the probability of the test compound for being at any point of the two-dimensional torsional space at a resolution of 3° by 3°. These probability data were generated by dividing the number of structures within each angle segment by the total number of structures and they are displayed in the above coordinate system using a color code (*figure* 4): Bright colors represent high probability and dark colors low probability.



(a) Core Conformation



(b) Acid Orientation

Fig. 3 Internal Coordinates

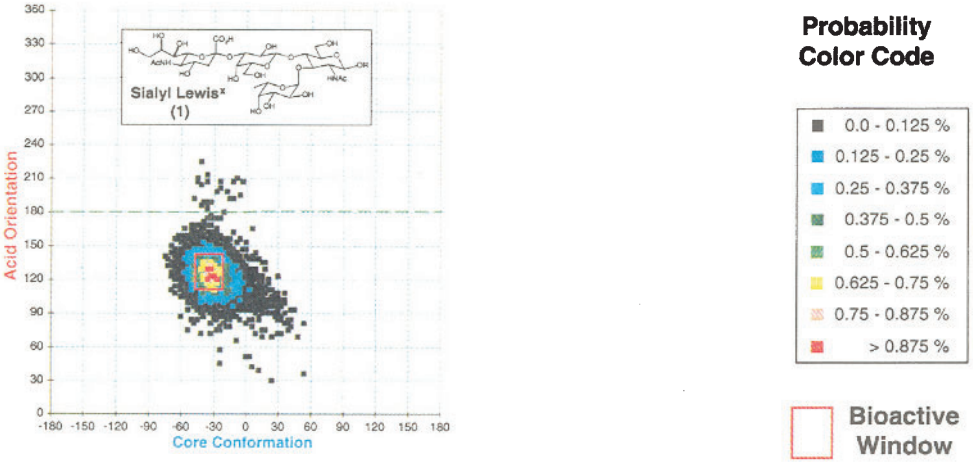
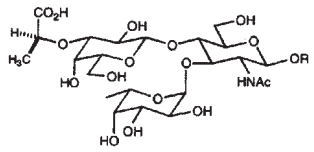
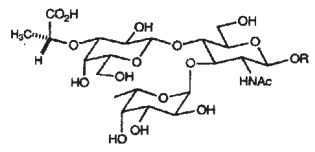


Fig. 4 Results from a 10 ns MC(JBW)/SD simulation of sialyl Lewis^x.

(a) *S*-Lactic Acid Derivative *S*-**2**



(b) *R*-Lactic Acid Derivative *R*-**2**



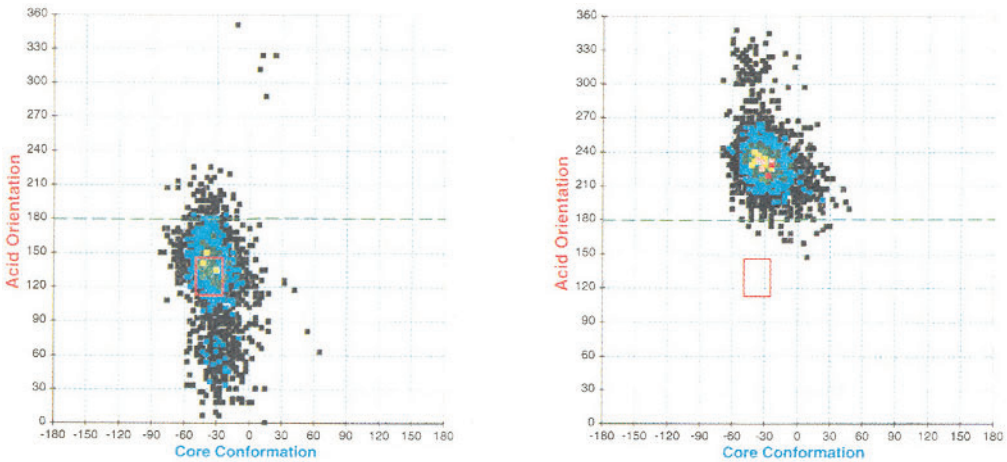


Fig. 5 Sialic Acid Replacement – Predictions

Figure 4 shows the results from a 10 ns analysis of sialyl Lewis^x. The region of highest probability lies between -20° and -50° with respect to the core conformation and between 110° and 140° with respect to the acid orientation. A representative structure is shown in *figure* 1. Interestingly, this area of highest probability in torsional space matches closely with the bioactive conformation determined by transfer-NOE measurements.[3c] Consequently, sialyl Lewis^x in aqueous solution is well *pre-organized* for binding to E-selectin, because it has a high probability for being in the *bioactive window*.

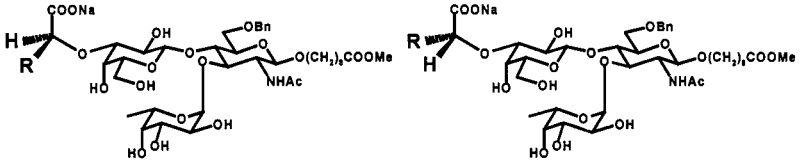
The following conclusion can be drawn from this probability analysis. Firstly, a glycomimetic with a high probability for being in the bioactive window of torsional space has a good chance of being active, since it is pre-organized for binding. Conversely, a compound which populates the bioactive window only partially or not at all has a low probability of being active.

We have applied this computational tool for the rational design of sialyl Lewis^x mimics. First, our efforts concentrated on the search for suitable replacements of the neuraminic acid portion. In a second step we applied our tool for the design of sialyl Lewis^x mimics with a modified LacNAc core.

Our structure-activity studies[2a] show that the presence of a carboxy group is a prerequisite for activity towards E-selectin. Lactic acid is one of the structurally most simple mimics of neuraminic acid and the configuration at the α-position may be used to control the spatial orientation of the COOH group. The results from the 2 ns MC(JBW)/SD simulations of the lactic acid derivatives *S*-**2** and *R*-**2** indicate (*figure* 5) that the configuration does indeed influence the preferred three-dimensional orientation: The *S*-configured mimic *S*-**2** populates the bioactive window and its probability plot looks similar to that of the lead structure (**1**). In contrast, the *R*-configured mimic *R*-**2** does not populate the bioactive window. As a consequence, the *S*-diastereomer should be active while its epimer *R*-**2** should be inactive.

The affinities of the target compounds to E-selectin were determined in a cell-free ELISA assay using a polymeric sialyl Lewis^a derivative as competitive inhibitor and they are quoted as RIC_{50} values.[11] These RIC_{50} values are IC_{50} values relative to the lead structure sialyl Lewis^x whose RIC_{50} is, consequently, equal to 1. The results in *table* 1 show the predicted trends: The *S*-isomers are active, while the *R*-stereoisomers are completely inactive. The *S*-phenyllactic acid derivative *S*-**3** is slightly more active active than S-**2**, probably due to the larger steric bulk of the acid residue and consequently a better pre-organization.

Table 1. Bioactivities (RIC_{50}) of Mimics with Sialic Acid Replacement – Experimental Validation



S-isomers *R-isomers*

Sialyl Lewis^x	**(1)**	***1***	
R = Me	**(2)**	***3.7***	***>15***
R = CH_2Ph	**(3)**	***2.2***	***>15***

As the next step, the feasibility of replacing the GlcNAc portion of sialyl Lewis^x by *R*,*R*-1,2-cyclohexanediol was investigated. The 2 ns MC(JBW)/SD simulation of the mimic *S*-**4**, having both neuraminic acid and GlcNAc replaced, indicates that the bioactive window is indeed populated and consequently this compound should be active (*figure* 6). Thus, the cyclohexanediol portion should function as a suitable GlcNAc mimic.

The observed bioactivities (*table* 2) confirm this prediction and the best mimic in this series, the cyclohexyl derivative S-**5**, is over 10 times more active than sialyl Lewis^x. As a consequence for not being pre-organized for binding, the epimeric compounds, bearing the *R*-configuration in the lactic acid portion, are completely inactive.

In summary, we have developed a molecular modelling tool for analysing the pre-organization of sialyl Lewis^x mimics for binding to E-selectin. The pre-organization is quantified as the probability for being in the *bioactive window* of torsional space. The model has predictive value in a qualitative sense: carbohydrate mimics which populate the bioactive window are likely to be active towards E-selectin, whereas compounds which do not populate the bioactive window are generally inactive. Work is in progress with the aim to further refine this model and to develop quantitative structure activity relationships using the probability data as descriptors.

The computational tool has guided our search for new and more active E-selectin antagonists. The highly active cyclohexyl derivative *S*-**5**, is a very promising E-selectin antagonist, since it combines an over ten-fold higher affinity towards E-selectin compared to sialyl Lewis^x with a considerably lower

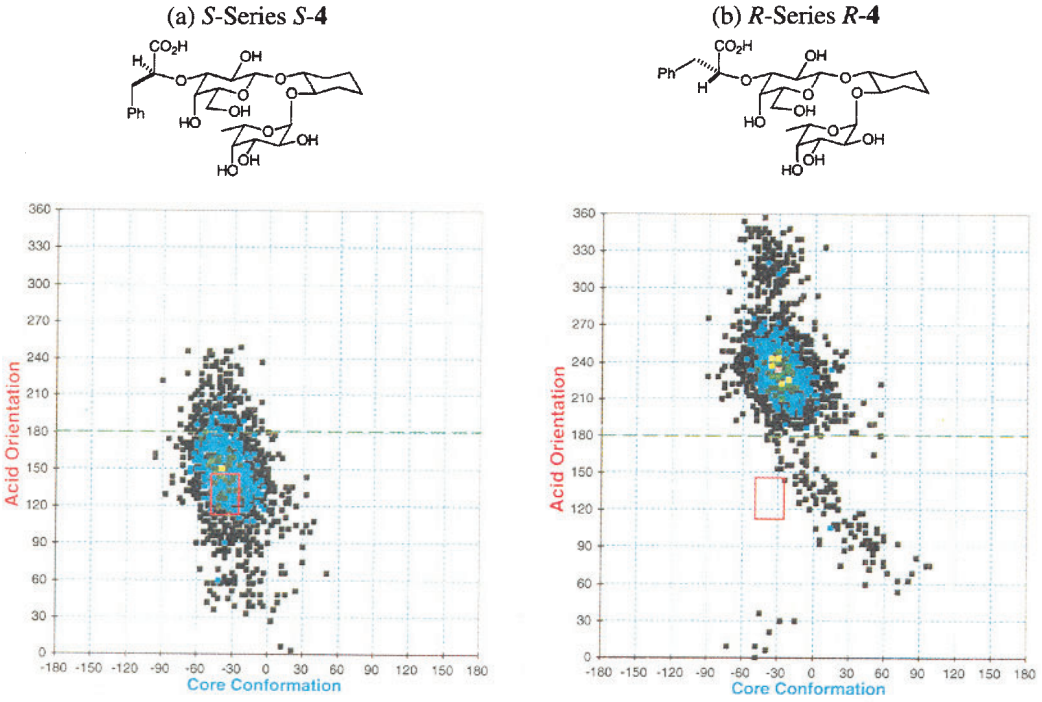
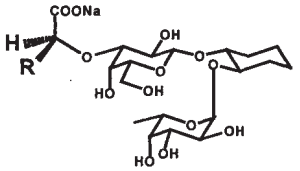


Fig. 6 GlcNAc Replacement – Prediction

Table 2 Bioactivities (RIC_{50} values) of mimics with GlcNAc Replacement – Results



S-isomers *R-isomers*

Sialyl Lewis^x	(1)	*1*	
R : CH_2Phenyl	(4)	*0.3*	*>15*
R : CH_2Cyclohexyl	(5)	*0.09*	*>15*

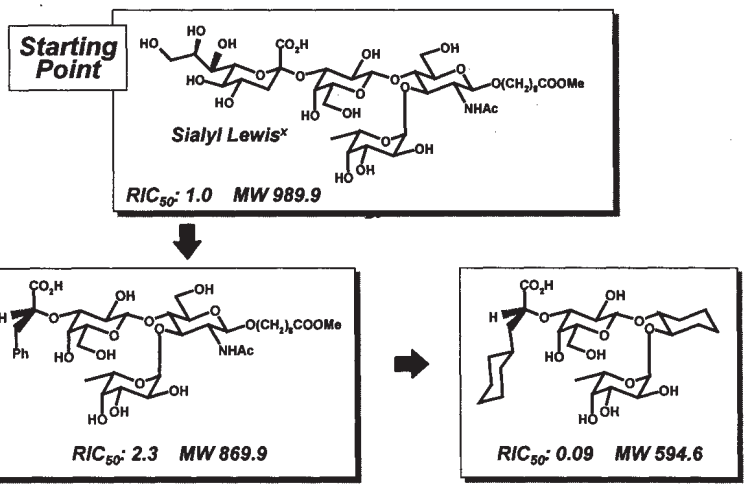


Fig. 7 Peripheral and core modification lead predictably to more potent glycomimetics

molecular weight and lower hydrophilicity (*figure* 7). The biological properties of this mimics are currently under further evaluation.

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11. Cf. reference 5 for details on the ligand binding assay.