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DESIGN AND SYNTHESIS OF A MACROCYCLIC E-SELECTIN ANTAGONIST

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Abstract: The macrocyclic sialyl Lewis^x mimic **2** has been synthesized starting from L-galactose. The core of **2** is extremely rigid as indicated by molecular modelling. This compound functions as a structural probe for exploring the spatial orientation of the functional groups involved in binding to E-Selectin. © 1997 Elsevier Science Ltd.

E-, P- and L-selectin belong to a family of Ca^{2+} dependent carbohydrate binding proteins.[1] They play an important role in leukocyte recruitment to sites of injury or inflammation. Consequently, it may be possible to treat inflammatory and respiratory diseases by blocking these receptors.[1]

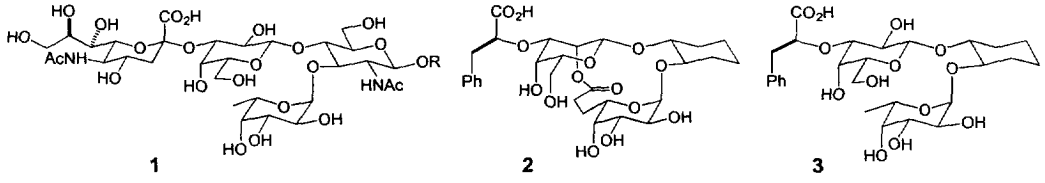

Figure 1: Sialyl Lewis^x (**1**) and its mimics

The development of potent selectin antagonists[2] is a challenging task due to the low binding affinity of the natural lead structure, the tetrasaccharide sialyl Lewis^x (**1**) (Figure 1). Recently, the conformation of sialyl Lewis^x bound to E-selectin (bioactive conformation) has been determined with transfer-NOE NMR studies[3] (Figure 2a). This information is fundamental for our molecular modelling tools,[2] which are designed to identify compounds that adopt the bioactive conformation and that are, therefore, *pre-organized* for binding.

Macrocyclic sialyl Lewis^x analogs are structural probes to explore the spatial orientation of the functional groups in the ligand bound to E-selectin (Figure 2b). In addition, the rigid macrocyclic core may provide the basis for enhanced bioactivity due to a potentially high pre-organization of the functional groups involved in binding. We now report the synthesis and biological evaluation of the macrocyclic sialyl Lewis^x analog **2** which combines the following design elements:

(1) The Lewis^x core is rigidified by linking the 6-position of fucose to the 2-position of galactose. Previous work had revealed the receptor's tolerance to modifications at these centers.[4]

(2) In analogy to our previous studies[2] we planned to simplify sialyl Lewis^x by replacing N-acetyl glucosamine and neuraminic acid by *R,R*-1,2-cyclohexanediol and *S*-phenyllactic acid, respectively (cf. Figure 1, compound **3**).

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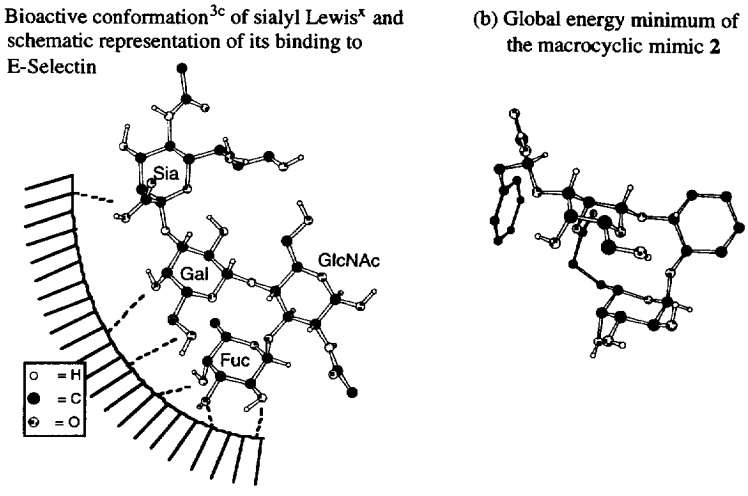


Figure 2: Three dimensional structures of sialyl Lewis[x] and macrocycle **2**.

Our recently developed computational method[2] was used for the analysis of the energy surface of sialyl Lewis[x] (**1**) and its macrocyclic mimic **2**. It is based on the 'Jumping between Wells' [MC(JBW)/SD] simulation technique[5,6] implemented in MacroModel 5.0.[7] A Boltzman weighted ensemble of states is generated by jumping between different energy wells (conformations) and performing stochastic dynamics simulations within each well. The total simulation time was 2 ns for compound **2** and 10 ns for **1**. The conformations used for the MC(JBW)/SD simulations were obtained in preceeding 5000 step internal coordinate systematic pseudo-Monte-Carlo (systematic, unbounded multiple minimum search, SUMM) simulations.[8] All calculations were performed with an augmented AMBER* force field,[7] containing our α-alkoxyacid parameters,[2] in conjunction with the GB/SA continuum water model.[9] The data analysis is based on a two-dimensional *internal coordinate* system to define the spatial orientation of the relevant pharmacophores, i.e. the COOH group relative to the fucose moiety.[2] 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 other 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.

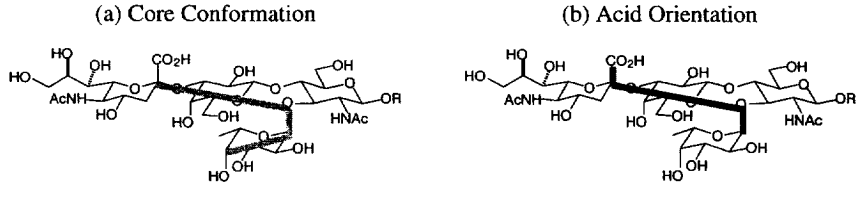


Figure 3: Internal Coordinates

The several thousand structures obtained by sampling the MC(JBW)/SD simulations every 1 ps were used to evaluate the probability for being at any point of the two-dimensional torsional space at a resolution of 3° by 3°. The probability data are displayed using a grey-scale code shown in Figure 4. These plots represent the *free energy surfaces* of the molecules and they reveal that the locations of the high probability areas are indeed very

similar for both compounds. Furthermore, the probability distribution of the macrolide **2** with respect to the core (horizontal axis) is much more narrow than in the uncyclized compound **1**. Thus, linking the galactose portion to the fucose locks the core in the stacked conformation (Figure 2b). However, the acid orientation (vertical axis) of the macrolide is shifted slightly towards larger angles, probably due to steric interactions between the acid residue and the macrolide chain. Thus, macrolide **2** is structurally closely related to the lead structure **1**, but its core is considerably more rigid.

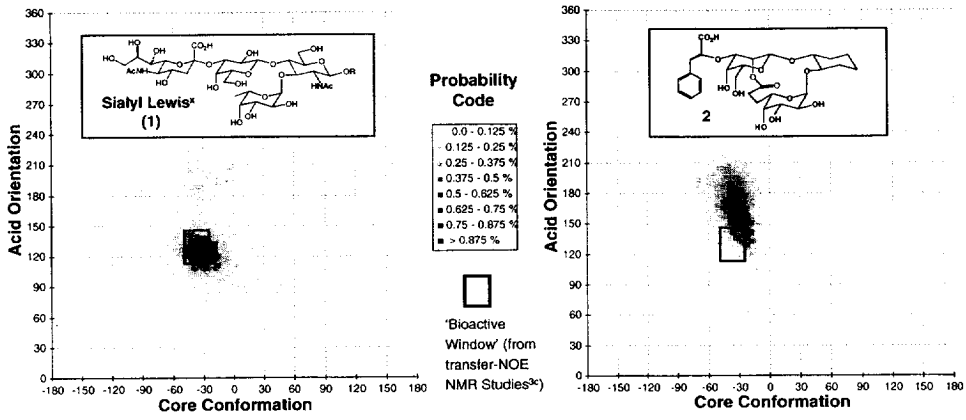
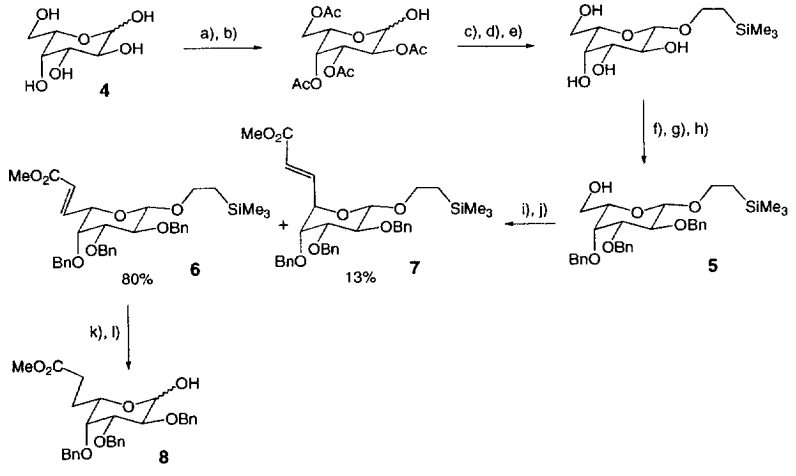


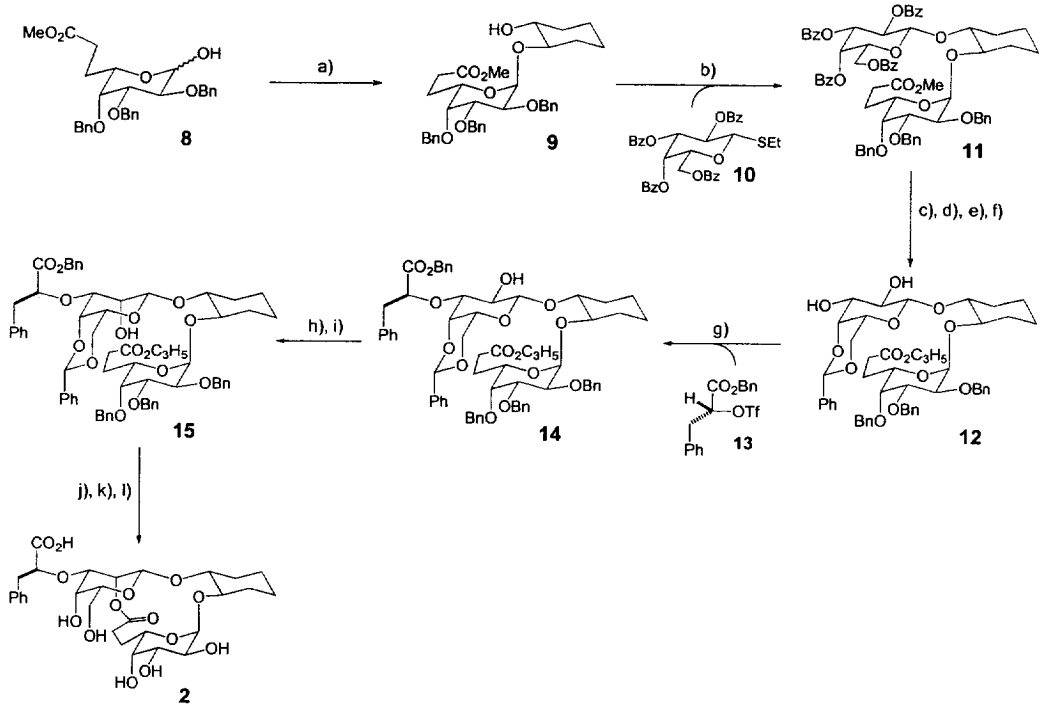
Figure 4: Internal coordinate probability plots of sialyl Lewis^x (**1**) and macrolide **2**.

The synthesis of macrolide **2** required homologation of fucose at the 6 position. Building block **8** was obtained in 12 steps and 19.6 % yield starting from L-galactose **4** (Scheme 1).



Scheme 1. Reagents and conditions: a) Ac_2O, pyridine, 98%; b) N_2H_4•HOAc, DMF, r.t., 55%; c) Cl_3CCN, Cs_2CO_3, CH_2Cl_2, r.t., 100%; d) $Me_3SiC_2H_4OH$, Me_3SiOTf, CH_2Cl_2, -20°C, 92%; e) NaOMe, MeOH, r.t., 100%; f) Ph_3CCl, pyridine, DMAP, r.t., 73%; g) BnBr, NaH, DMF, r.t., 79%; h) 80 % TFA-H_2O, CH_2Cl_2, r.t., 94%; i) $(COCl)_2$, DMSO, Et_3N, -78°C to r.t.; j) Ph_3P=$CHCO_2Me$, CH_2Cl_2, -20°C; k) H_2, PtO_2, MeOH, 100%; l) TFA, CH_2Cl_2, 0°C, 91%.

Key steps in the synthesis were the homologation of alcohol **5** at C6 by Swern oxidation followed by Wittig olefination of the resulting aldehyde. The C5 epimer **7** could be readily separated from the desired isomer **6** by flash chromatography. Selective hydrogenation of the double bond was achieved with PtO_2 as the catalyst. Removal of the 2-trimethylsilylethyl protecting group at the anomeric center by treatment with anhydrous trifluoroacetic acid in CH_2Cl_2, provided the homologated fucose building block **8** in 87 % yield. Activation of building block **8** with oxalyl bromide and *in situ* coupling of the resulting anomeric bromide with *R,R*-1,2-cyclohexanediol in the presence of tetra-*n*-butyl ammonium bromide[10] provided the α-glycoside **9** with a good stereoselectivity (α:β ≈ 10:3) (Scheme 2). This *in situ* anomerization protocol[10] gave superior results to the trichloroacetimidate method which afforded the separable α,β-anomers as a 1:1 mixture.



Scheme 2. Reagents and conditions: a) $(COBr)_2$, CH_2Cl_2, DMF, 0°C; then nBu$_4$NBr, 4 Å molecular sieves, *R,R*-1,2-cyclohexanediol, CH_2Cl_2, DMF, r.t., α:β=10:3, 59% α-isomer; b) $[MeSSMe_2]^+OTf^-$, 4 Å molecular sieves, CH_2Cl_2, r.t., 75%; c) NaOMe, MeOH, r.t., 100%; d) $PhCH(OMe)_2$, CSA, MeCN, r.t., 88%; e) LiOH, EtOH, H_2O, 60°C, 95%; f) Cs_2CO_3, allyl bromide, r.t., 87%; g) nBu$_2$SnO, C_6H_6, reflux, 10 h, followed by evaporation and addition of CsF, 1,2-dimethoxyethane, 6 eq. triflate **13** at r.t., 70 % (+ 21% starting material); h) Dess-Martin Periodinane, CH_2Cl_2, r.t.; i) $NaBH_4$, 0°C, iPrOH, 75%; j) $Pd(PPh_3)_4$, morpholine, THF; k) trichlorobenzoyl chloride, Et_3N, THF; then slowly added to a solution of DMAP in refluxing C_6H_6 over 24 h; 80% over 2 steps; l) H_2, $Pd(OH)_2$/C, dioxane, H_2O, HOAc, 84%.

Cyclohexanediol derivative **9** was galactosylated with **10** as donor and the resulting galactoside **11** was converted to the diol **12** in a series of protecting group manipulations, including the switch of the methylester to the allyl ester. This switch was required to ensure orthogonal protection at a later stage. Activation of the diol **12**

with di-*n*-butyltin oxide[11] and treatment with an excess of the triflate **13**,[12] derived from *R*-phenyllactic acid, resulted in exclusive etherification at the 3-position, allowing the ether **14** to be isolated in good yield. Conversion of the β-galactoside **14** to the β-taloside **15** was accomplished with an excellent stereoselectivity by oxidation at the 2-position with Dess-Martin periodinane reagent,[13] followed by reduction with $NaBH_4$. Removal of the allyl protecting group with $Pd(PPh_3)_4$ afforded the corresponding seco acid which was subjected to macrocyclization under the *Yamaguchi* protocol.[14] Thus, activation with trichlorobenzoic acid followed by DMAP catalyzed cyclization under conditions of high dilution gave the macrocyclic ester in excellent overall yield (80%). Finally, hydrogenation in the presence of Perlman's catalyst provided the target molecule **2**.[15]

The reference compound **16** (Table) was prepared from **14** in similar fashion by omitting the galactose/talose inversion and macrocyclization steps.

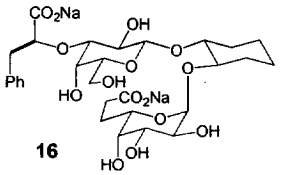
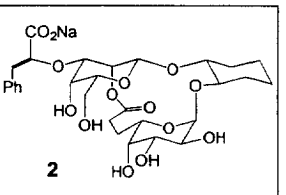
Compound	Sialyl Lewis^x-O-$(CH_2)_8CO_2Me$ (1)	 **16**	 **2**
RIC_{50}	1.0	0.39	2.8

Table. *In Vitro* Affinity towards E-Selectin (RIC_{50} values[16])

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 a competitive inhibitor.[16] The results are summarized in the Table. The macrolide **2** is indeed recognised by E-selectin even though with a 2.8 times lower affinity than sialyl Lewis^x (1) and a 7.2 times lower affinity than the open chain reference compound **16**. This result suggests the bioactive conformation with respect to the core to be very similar to the structure shown in Figure 2b. The lower bioactivity may be due to several factors, but our analysis suggests that the slight distortion of the acid orientation from the optimum (Figure 4) may be the actual culprit.

In summary, we have prepared a rigid macrocyclic sialyl Lewis^x analog which is recognised by E-selectin. The design is based on the analysis of the free energy surface in order to assess the pre-organization for binding. The positive result may be regarded as a further validation of the computational method, thereby providing the means for the rational design of structurally more simplified selectin antagonists.

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[15] Spectroscopic data of the macrolide **2**: ^{1}H NMR (500 MHz, D_2O) δ 7.33-7.27 (m, 2H, Ph), 7.26-7.21 (m, 3H, Ph), 5.12 (d, J=3.5 Hz, 1H, Tal-2), 4.87 (d, J=4.1 Hz, 1H, Fuc-1), 4.75 (m, 1H, Fuc-5), 4.60 (s, 1H, Tal-1), 4.05 (dd, J=3.0, 9.8 Hz, 1H, H-α acid), 3.91 (d, J=3.6 Hz, 1H, Tal-4), 3.86-3.81 (m, 2H, Fuc-3, Fuc-4), 3.76-3.67 (m, 3H, Tal-6, Fuc-2), 3.61 (ddd, J=5.0, 9.8, 11.2 Hz, 1H, chx-diol-1 or 2), 3.56 (t, J=3.6 Hz, 1H, Tal-3), 3.45 (ddd, J=1.0, 3.8, 7.6 Hz, 1H, Tal-5), 3.31 (m, 1H, chx-diol 2 or 1), 3.01 (dd, J=3.0, 13.8 Hz, 1H, H-β acid), 2.77 (dd, J=9.8, 13.8 Hz, 1H, H-β acid), 2.25 (ddd, J=3.0, 8.8, 15.0 Hz, 1H, Fuc-7), 2.16 (ddd, J=3.0, 9.4, 15.0 Hz, 1H, Fuc-7), 1.98 (m, 1H, chx-diol), 1.94 (m, 1H, chx-diol), 1.81 (m, 1H, Fuc-6), 1.60 (m, 2H, chx-diol), 1.55 (m, 1H, Fuc-6), 1.09 (m, 4H, chx-diol); ^{13}C NMR (90.6 MHz, D_2O) δ 180.0 (C_q), 175.8 (C_q), 138.9 (C_q), 129.8 (CH), 128.8 (CH), 127.0 (CH), 95.6 (CH), 95.5 (CH), 80.5 (CH), 78.0 (CH), 77.0 (CH), 75.4 (CH), 74.3 (CH), 70.1 (CH), 69.3 (CH), 68.5 (CH), 67.8 (CH), 66.8 (CH), 62.0 (CH_2), 39.6 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 29.8 (CH_2), 25.5 (CH_2), 23.7 (CH_2); MS (FAB, thioglycerin) 657 (M+Na), 635 (M+H).

[16] The ligand binding assays were performed by Dr. J. Magnani at GlycoTech Corp., Rockville, USA. The binding affinities are quoted as relative IC_{50} values (RIC_{50}) which are obtained by dividing the antagonist concentration at 50 % inhibition (IC_{50}) by the IC_{50} value of the reference compound sialyl Lewis^x (**1**) (R = $(CH_2)_8CO_2Me$). See reference 2 for details.

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