C_2-Symmetric Lewis Antigen Mimetics Exhibiting the Common Structural Motif

Armin Geyer,*,† Stefan Reinhardt,† G. Bendas,‡ U. Rothe,‡ and Richard R. Schmidt*,†

Fakultät für Chemie, Universität Konstanz Postfach 5560, D-78434 Konstanz, Germany Fachbereich Pharmazie, Universität Halle Langenbeckstrasse 4, D-06120 Halle, Germany

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Lewis carbohydrate determinants are involved in transient cell adhesion associated with inflammatory processes;[1] therefore, quite a few glycomimetics have been synthesized with potential anti-inflammatory activity.[2] Because ligand flexibility strongly influences receptor binding, internal motions of the Lewis antigens were thoroughly investigated by experimental and computational methods.[3] Average structures of the Lewis antigens are determined with high fidelity, but the extent of rotational flexibility of individual glycosidic bonds cannot be quantified in NMR spectra which may be influenced by fast conformational averaging or by molecular dynamics simulations which are restricted to the nanosecond time scale.[4] The C_2-symmetric tetrasaccharide scaffold described here is stabilized by the same intramolecular interactions as the natural Lewis b (Le^b) and y (Le^y) moieties and thus also exhibits a similar conformational behavior. This tetrasaccharide scaffold forms the basis for the design of a new class of rigid Lewis antigen mimetics.[5]

Until now, the pseudo-C_2-symmetry, inherent to Le^b and Le^y tetrasaccharides, was not considered. The four pyranose rings are positioned at the apices of a slightly distorted tetrahedron (Figure 1), and this pseudo-C_2-symmetry promotes cooperative stabilization of interglycosidic interactions: the globular arrangement minimizes the total hydrophobic surface, and van der Waals contacts are found between the hydrophobic ring plane of each α-configurated fucose and either β-D-galactose or β-D-*N*-acetylglucosamine of parallel orientation (stacking interaction). Furthermore, glycosidic torsion angles assume values for which sterical interactions between directly connected monosaccharides are minimized and bond-dipoles cancel (exo-anomeric effect[6]). Double fucosylation of the central disaccharide unit (D-Galβ(1→3)D-GlcNAc for Le^b and D-Galβ-(1→4)D-GlcNAc for Le^y) results in tightly packed tetrasaccharide scaffolds. In both cases, four hexopyranoses interact to display an exceedingly rigid overall structure.

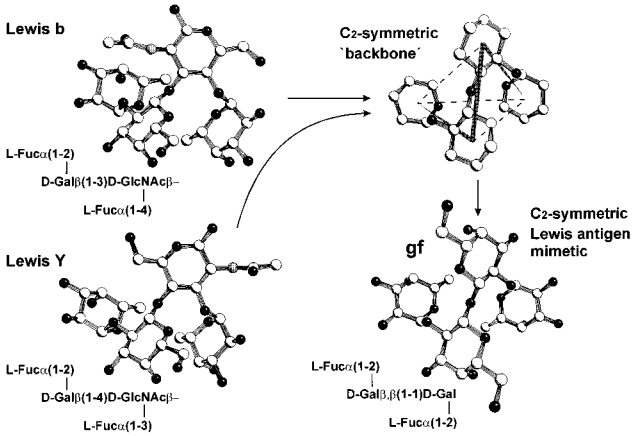
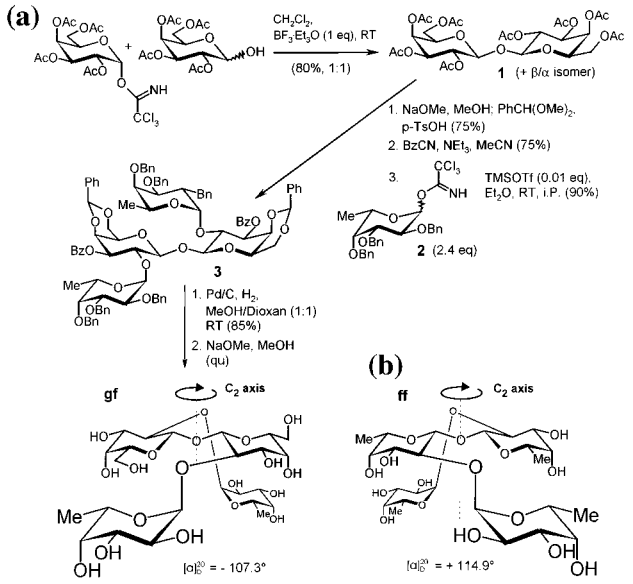


Figure 1. Design of a Lewis antigen mimetic exhibiting the common structural motif of the Lewis carbohydrates. The C_2-symmetric backbone is visualized by deleting all ring substituents of the Le^b and Le^y tetrasaccharide moieties. The four pyranose rings describe a negative overall torsion (shaded) and the four rings span a slightly distorted tetrahedron. The same spatial structure is formed by the bis-α-fucosylated Galβ,β-trehalose **gf**. This tetrasaccharide can serve as a scaffold for Lewis carbohydrate antigens and other oligosaccharides which are stabilized by stacking interactions. The (pseudo-)C_2-axis runs in the direction of view through the oxygen of the central glycosidic linkage of each tetrasaccharide.

Scheme 1



The common carbohydrate scaffold allows the design of fully C_2-symmetric tetrasaccharides such as **gf** (Figure 1). The core disaccharides of the Le^b and Le^y antigens are replaced by β,β-trehalose and fucosylation is performed in one step on both homotopic halves of the molecule. The synthesis is outlined in Scheme 1a: 2,3,4,6-tetra-*O*-acetyl-D-galactose[7] and the corresponding trichloroacetimidate[8] are ligated to trehalose **1**. The β,β-isomer of **1** is isolated, and after the protecting group pattern is changed, the Galβ,β-trehalose is selectively bis-α-fucosylated with fucosyl donor **2** at 2-O to afford the tetrasaccharide intermediate **3**. Deprotection yields **gf**[9] which is depicted twice to indicate the C_2-axis.

A 2D-ROESY spectrum of **gf** yields an average distance of 240 pm between fucose 1-H and galactose 2-H and an average

† Universität Konstanz.
‡ Universität Halle.

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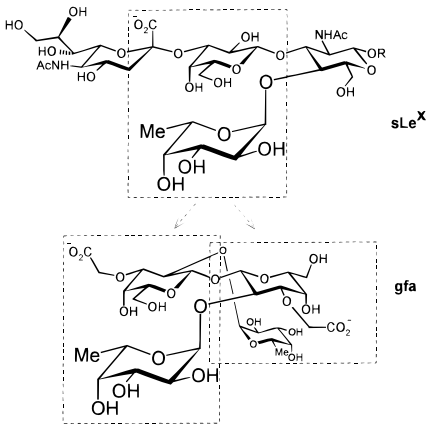
Scheme 2



distance of 280 pm between fucose 6-H_3 and 2-H of the opposite galactose moiety. Similar distances have been found for Le^b and Le^y.[10] Average glycosidic angles of fucose are $\phi = 45°$ ($\phi = H^{1'}-C^{1'}-O^2-C^2$) and $\psi = 25°$ ($\psi = C^{1'}-O^2-C^2-H^2$). The ϕ angle of galactose was determined to 40°.[11]

This tetrasaccharide scaffold allows the design of a new class of sialyl Lewis X (sLe^x) mimetics which exhibit a bivalent binding domain (**gfa**, Scheme 2).[12] The D-GlcNAc residue of the natural Le^x trisaccharide, which is not essential for the binding of sLe^x to E-Selectin,[2b,d] is substituted by a second Gal/Fuc recognition domain. The sialic acid moiety of the natural sLe^x may be substituted by hydroxyacetic acid, because only the carboxylic group is needed for activity.[2] Synthesis of **gfa** started from 3-*O*-allyl-2,4,6-tri-*O*-acetyl-D-galactose,[13] trehalose formation and fucosylation proceeded along the same route as described that for **gf**. Oxidative cleavage of the allylic double bond was performed with catalytic amounts of OsO_4, followed by excess $NaIO_4$. The aldehyde was isolated (75%) and subsequently oxidized with pyridinium dichromate in DMF (49%). Final hydrogenolysis yielded **gfa** (83%).[14]

The sLe^x mimetic **gfa** was tested for its activity to inhibit the adhesion of E-Selectin presenting cells on a model membrane.[15] In this binding assay, **gfa** exhibited 90% of the activity of soluble sLe^x. From comparable testing systems it is known that the E-Selectin binding of HO_2C-CH_2-3-*O*-Galβ(1→4)-[Fucα(1→3)]Glc−OH is less than 50% of the affinity of sLe^x.[16] Thus, the bivalent sLe^x mimetic **gfa** demonstrates the significance of these readily available C_2-symmetric trehalose derivatives in biological studies.

In order to show the structural variability of the tetrasaccharide scaffold, we also synthesized tetrasaccharide **ff** (Scheme 1b).[17] This compound exhibits a positive overall torsion and the pyranose rings are oriented like a mirror image of the Lewis antigens. Interresidue ROE intensities within **ff** (D-Fuc-1H → L-Fuc-2H = 230 pm, D-Fuc-6H_3 → l-Fuc-2H = 280 pm) prove the stacking of the D- and L-configured pyranose rings as found for the natural Lewis antigens and for **gf**. The average glycosidic torsions are negative (D-Fuc $\phi = -45°$, $\psi = -25°$; L-Fuc $\phi = -40°$).

In conclusion, the C_2- or pseudo-C_2-symmetric tetrahedron can be assembled from various six-membered rings, as long as the vicinal bis-equatorial substitution pattern of α-L- and β-D-pyranoses, or their mirror images, is retained. Thus, the Le^b and Le^y fragments and compounds **gf** and **ff** are representatives of a secondary structure in which two α-configured fucoses rigidify a central disaccharide unit. Stacking interactions between pyranose rings are frequent in branched carbohydrates and the tetrasaccharide scaffold of the figure which is stabilized by "twofold stacking" is not restricted to Lewis determinants but can be employed for the generation of glycomimetics of broad applicability.

Acknowledgment. This research was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. The authors thank Dr. Beat Ernst for helpful discussions.

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(9) For **gf**: TLC (Li-Chroprep NH_2, Merck; EtOH/H_2O, 5:1) $R_f = 0.45$; $[\alpha]^{20}_D = -107.3°$ ($c = 1$, H_2O); MALDI-MS (positive mode, 2,5-dihydroxybenzoic acid) 657 [M + Na^+]; ^{1}H NMR (600 MHz, D_2O; D-Galβ = a, L-Fucα = b) δ 1.14 (d, $J_{5,6} = 6.6$ Hz, 3H, 6b-H3), 3.53 (dd, 1H, $J_{5,6'} = 4.9$ Hz, $J_{5,6''} = 7.0$ Hz, 5a-H), 3.59 (dd, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 9.4$ Hz, 1H, 2a-H), 3.61−3.66 (m, 2H, 6a-H_2), 3.67−3.70 (m, 2H, 2b-H, 4b-H), 3.75 (dd, $J_{2,3} = 9.7$ Hz, $J_{3,4} = 3.4$ Hz, 1H, 3a-H), 3.78−3.81 (m, 2H, 4a-H, 3b-H), 4.55 (q, $J_{5,6} = 6.7$ Hz, 1H, 5b-H), 4.72 (d, $J_{1,2} = 7.8$ Hz, 1H, 1a-H), 5.14 (d, $J_{1,2} = 3.8$ Hz, 1H, 1b-H).

(10) NOE-derived distances for Le^b from ref 6: Fuc^1-1H → GlcNAc-4H = 260 pm; Fuc^1-6H_3 → Gal-2H = 280 pm, Fuc^2-1H → Gal-3H = 250 pm, Fuc^2-5H → GlcNAc-2H = 260 pm; with Le^b = $Fuc^2\alpha$(1→2)Galβ-(1→3)[$Fuc^1\alpha$(1→4)]GlcNAcβ(1→O-).

(11) A 100 ps molecular dynamics simulation with the experimental proton−proton distances included as weak (7 kcal mol^{-1} $Å^{-2}$) additional restraints was performed with the MM+ force field which is included in the HyperChem program package (Hypercube, Inc.). Structure sampling and energy minimization were carried out according to ref 5.

(12) Multivalent binding is expected to enhance the efficacy of carbohydrate binding to selectin receptors. See references 2a,c and Van der Merwe, P. A.; Barclay, A. N. *Trends Biol. Sci.* **1994**, *19*, 354.

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(14) For **gfa**: TLC (Li-Chroprep NH_2, Merck; MeOH/$CHCl_3$/H_2O, 5:4:2) $R_f = 0.25$; $[\alpha]^{20}_D = -111°$ ($c = 0.5$, H_2O); MALDI-MS (positive mode, 2,5-dihydroxybenzoic acid) 751 [M + H^+], 773 [M + Na^+]; ^{1}H NMR (600 MHz, D_2O; D-Galβ = a, L-Fucα = b) δ 1.14 (d, $J_{5,6} = 6.4$ Hz, 3H, 6b-H_3), 3.53 (m, 1H, 5a-H), 3.60 (dd, $J_{2,3} = 9.5$ Hz, $J_{3,4} = 2.8$ Hz, 1H, 3a-H), 3.63−3.69 (m, 6H, 3b-H, 4b-H, 2a-H, 2b-H, 6a-H_2), 3.79 (dd, $J_{2,3} = 10.4$ Hz, $J_{3,4} = 3.2$ Hz, 3b-H), 4.01 (d, $J_{3,4} = 2.9$ Hz, 1H, 4a-H), 4.05 (d, $J_{gem} = 16.4$ Hz, 1H, CH_2CO_2H), 4.11 (d, $J_{gem} = 16.4$ Hz, 1H, CH_2CO_2H), 4.58 (q, $J_{5,6} = 6.4$ Hz, 1H, 5b-H), 4.74 (d, $J_{1,2} = 7.7$ Hz, 1H, 1a-H), 5.14 (d, $J_{1,2} = 3.8$ Hz, 1H, 1b-H).

(15) A parallel plate chamber with defined wall shear rates contained planar model bilayers with high concentrations of sLe^x ceramide. E-Selectin-presenting Chinese hamster ovary cells (CHO cells) were incubated with sLe^x or **gfa**, and the adhesion of the CHO cells to the model membrane under constant shear forces was quantified by counting adherent cells. The flow chamber is described in the following: Bendas, G.; Vogel, J.; Bakowski, U.; Krause, A.; Müller, J.; Rothe, U. *Biochem. Biophys. Acta* **1997**, *1325*, 297.

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(17) For **ff**: TLC (Li-Chroprep NH_2, Merck; EtOH/H_2O, 5:1) $R_f = 0.55$; $[\alpha]^{20}_D = +114.9°$ ($c = 1$, H_2O); MALDI-MS (positive mode, 2,5-dihydroxybenzoic acid) 626 [M + Na^+]; ^{1}H NMR (600 MHz, D_2O; L-Fucβ = a, D-Fucα = b) $\delta = 1.12$ (d, $J_{5,6} = 6.7$ Hz, 3H, 6a-H_3), 1.14 (d, $J_{5,6} = 6.7$ Hz, 3H, 6b-H_3), 3.53 (dd, 1H, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.7$ Hz, 2a-H), 3.59−3.63 (m, 2H, 4a-H, 5a-H), 3.67 (dd, $J_{1,2} = 3.9$ Hz, $J_{2,3} = 10.3$ Hz, 1H, 2b-H), 3.70 (d, $J_{3,4} = 3.4$ Hz, 1H, 4b-H), 3.74 (dd, $J_{2,3} = 9.7$ Hz, $J_{3,4} = 3.4$ Hz, 1H, 3a-H), 3.82 (dd, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 3.4$ Hz, 1H, 3b-H), 4.53 (q, $J_{5,6} = 6.7$ Hz, 1H, 5b-H), 4.61 (d, $J_{1,2} = 7.9$ Hz, 1H, 1a-H), 5.14 (d, $J_{1,2} = 3.8$ Hz, 1H, 1b-H).