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Synthesis of E-Selectin Inhibitors: Use of an Aryl-Cyclohexyl Ether as a Disaccharide Scaffold

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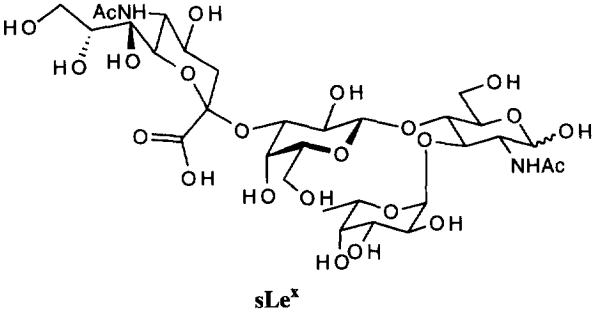
Abstract: A series of potential E-selectin antagonists were designed and synthesized based on the bound conformation of sLe^x, as well as the functional group requirements for anti-adhesion activity. A non-carbohydrate scaffolding using an aryl-cyclohexyl ether which can orient the critical acid and fucose residues in appropriate positions has been investigated. The inhibitory activity of members of this series suggests the feasibility of non-carbohydrate E-selectin antagonists.

E-Selectin-mediated cell adhesion via the protein's interaction with carbohydrates on the surface of cells is an early event in the recruitment of leukocytes to sites of inflammation. Evidence also exists that E-selectin may play a role in the interaction of tumor cells with the endothelium of blood vessels, thereby mediating mechanisms of tumor metastasis.[1] Sialyl Lewis X (**sLe^x**) appears to be the minimal carbohydrate determinant expressed on the surface of neutrophils which interacts with E-selectin, and the isolated tetrasaccharide can act as an antagonist to prevent E-selectin-mediated adhesion (IC_{50} ~ 1mM).[2] Inhibition of E-Selectin by use of sLe^x analogs may therefore present opportunities for new therapeutic agents for inflammatory diseases and for the prevention of metastasis of certain types of tumors.

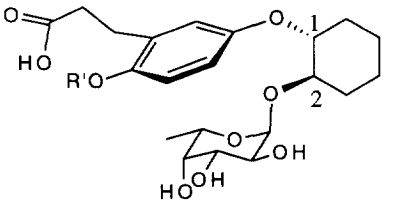


sLe^x

Previous studies on sLe^x and its analogs suggest that the acid moiety of the sialic acid and the fucose group play an essential role in binding to E-selectin, as their removal or modification completely abolishes inhibitory activity.[3] Modifications at the glucosamine and galactose groups are quite well tolerated, and this observation forms the basis of the hypothesis that these two carbohydrates act mainly as a scaffold which

maintains the acid and fucose groups in an appropriate orientation for binding.[4] We have set out to design and synthesize a series of sLe^x mimetics[5] in which a non-carbohydrate moiety replaces the internal galactose-glucosamine unit, while the critical acid and fucose groups are maintained.

The galactose-glucosamine unit has been replaced with an aryl-cyclohexyl ether as shown in compounds **1** - **4**. This new scaffold allows the fucose and acid groups to adopt an orientation similar to that observed in



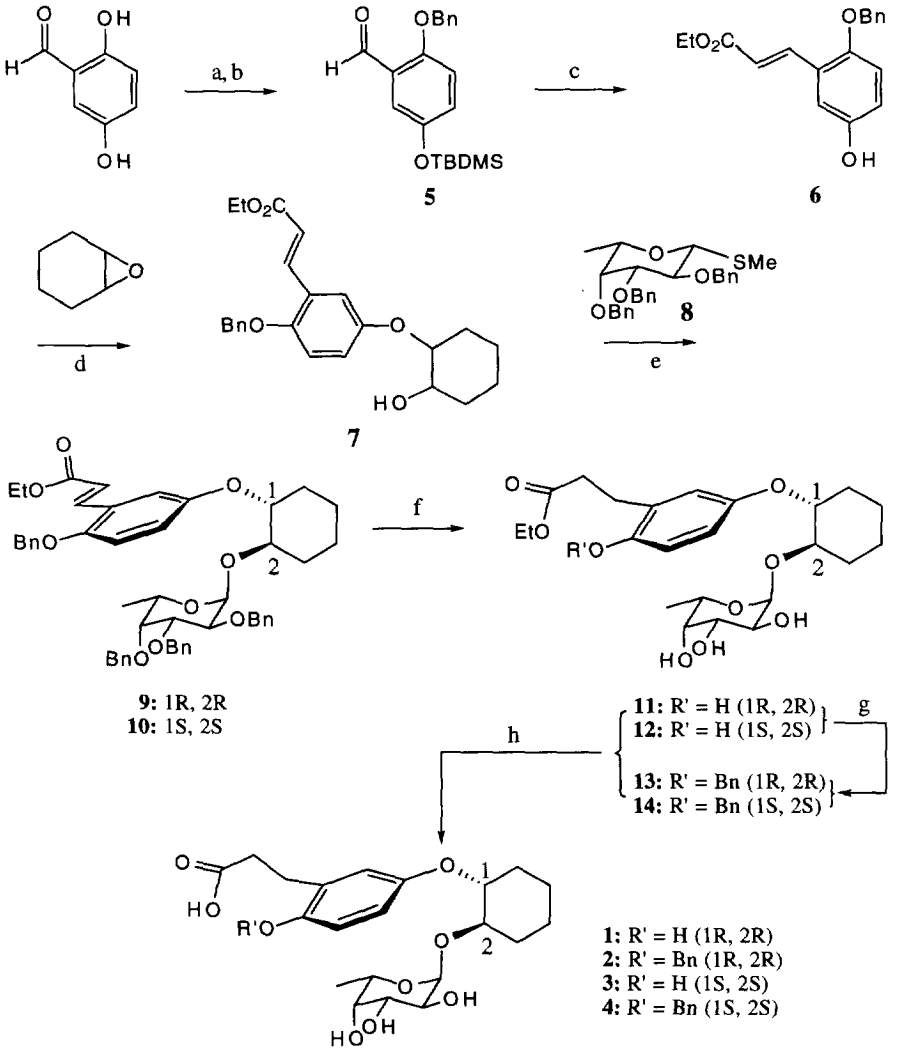
1: R' = H (1R, 2R)
2: R' = Bn (1R, 2R)
3: R' = H (1S, 2S)
4: R' = Bn (1S, 2S)

the conformation of sLe^x bound to E-selectin.[6] While the conformation of the fucose unit would be expected to be quite flexible, we envisioned that in highly polar media such as water, the more hydrophobic α-surface of the fucose ring would prefer to stay near the aromatic ring due to hydrophobic collapse[7] in water. This pre-organization could orient the critical acid and the fucose groups in a conformation appropriate for binding to E-selectin. Interactions of an aromatic ring with a face of a pyranose ring has been previously observed in x-ray structures of protein-carbohydrate complexes.[8] An NMR study of the compound **4** in D_2O revealed an NOE effect between the two aromatic rings and the methyl function of the fucose. It has been reported that introduction of a hydrophobic substituent such as a methyl or a phenyl to the methylene position of a carboxyl methyl group, which serves as a viable replacement for the sialic acid in sLe^x, would increase the binding affinity.[9] This fact, coupled with the possibility of increasing hydrophobic environment of the aromatic center, led to the attachment of a benzyl group to the phenolic oxygen.

Synthesis of the aryl-cyclohexyl ether mimetic is illustrated in **Scheme 1**. 2,4-Dihydroxybenzaldehyde was selectively protected at the meta-position with a *t*-butydimethyl silyl group under mild conditions to generate **5**. Under more strongly basic conditions, with a mixture of DMSO and toluene as a solvent, the *ortho*-hydroxyl group was protected as its benzyl ether. Elaboration of the aldehyde function via a Horner-Emmons reaction allowed introduction of an acid precursor, and at the same time, removed the TBDMS protecting group to provide **6**, which was suitable for further coupling.

The phenolic intermediate **6** was heated together with an excess of cyclohexene oxide under basic conditions to afford **7** as a racemic mixture. Coupling of the phenol **7** with the fucosyl bromide derivative[10] **8** yielded a mixture of diastereomers **9** and **10**, which were easily separable by flash chromatography. Hydrogenation using Pd(C) as a catalyst removed all benzyl protecting groups, and reduced the double bond at the same time. The acids **1** and **3** were prepared by direct basic hydrolysis of the hydrogenated intermediates **11** and **12**. To synthesize the benzyl substituted analogs **2** and **4**, the phenolic hydoxyl groups of the hydrogenated intermediates **11** and **12** were re-attached with a benzyl group selectively with a mild condition, and the resultant compounds **13** and **14** were then hydrolyzed under the basic condition. X-ray structures of the two intermediates **11** and **12** were obtained to determine the absolute stereo chemical orientation of the substituents in the final products.[11]

Scheme 1[a]



[a] Conditions: (a) TBDMSCl, imidazole, CH_2Cl_2, RT, 88%; (b) NaH, BnBr, toluene and DMSO, 0 °C - RT, 90%; (c) **5** was added to a solution of $(EtO)_2P(O)CH_2CO_2Et$ and KH in DMF, RT, 80%; (d) excess epoxide, K_2CO_3, DMF, 95 °C, 90%; (e) (i) **8**, Br_2, CH_2Cl_2, 0 °C; (ii) **7**, Et_4NBr, ground sieves (4 Å), DMF, the solution made in (i), RT, **9** (44%) and **10** (38%) were separated by flash chromatography; (f) H_2 (1 atm), Pd (C), EtOAc, RT, 92% for both; (g) Cs_2CO_3, BnBr, 18-Crown-6, DMF, RT, 82% for both; (h) 0.5 N aq. NaOH, 1,4-dioxane, RT, purified by reverse phase flash chromatography, 42% - 59% for **1** - **4**.

All four compounds[12] were tested in a competitive cell-free ELISA assay that measures inhibition of soluble E-selectin binding to sLe^x-polyacryamides.[4c] Compound **4** showed activity equivalent to sLe^x with an IC_{50} = 0.87 mM, while its diastereomer **2** exhibited less potent activity (IC_{50} ~ 3.3 mM). The compounds **1** and **3** did not exhibit any inhibitory activity under these assay conditions.

The activity displayed by **2** and **4** further strengthens the hypothesis of the role of the galactose-glucosamine in interactions of sLe^x with E-selectin. To date, carbohydrate-based antagonists of E-selectin binding appear to exhibit potencies similar to those of sLe^x itself ($IC_{50} \sim 1mM$).[4c, 13] The potencies observed for the compounds described, containing only one carbohydrate unit, suggest that non-carbohydrate antagonists of E-selectin (carbohydrate-mimetics) are a feasible alternative to sugar-based analogs.

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10. Compound **8** was prepared via adaptation of the procedures in this paper: Yamazaki, F.; Sato, S.; Nukada, T.; Ito, Y.; Ogawa, T. *Carbohydrate Research*, **1990**, *201*, 31-50.
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12. All spectral data for new compounds are in accordance with the assigned structure. The data for the four final products are:

    1: ^1H NMR (200 MHz, D_2O), δ 1.10 (d, 3H, J=6.4Hz), 1.28-1.50 (m, 4H,), 1.76 (d, 2H, J=10.6Hz), 2.14 (d, 2H, J=13.4Hz), 2.50 (t, 2H, J=7.3Hz), 2.82 (t, 2H, J=7.4Hz), 3.43-3.48 (m, 2H), 3.63-3.71 (m, 2H), 3.82-3.83 (m, 1H), 4.32 (m, 1H), 5.01 (d, 1H, J=3.8Hz), 6.84 (s, 2H), 6.90 (s, 1H); HRMS (Fab) obs'd for $C_{21}H_{29}O_9Na+H$, 449.1780; calc'd, 449.1788.

    2: ^1H NMR (200 MHz, D_2O), δ 1.04 (d, 3H, J=6.5 Hz), 1.20-1.50 (m, 4H), 1.70-1.80 (m, 2H), 2.10-2.20 (m, 2H), 2.42 (t, 2H, J=8.0Hz), 2.86 (t, 2H, J=8.0Hz), 3.38 (m, 2H), 3.55-3.77 (m, 3H), 4.30-4.40 (m, 1H), 4.98 (d, 1H, J=3.4Hz), 5.14 (s, 2H), 6.82-7.00 (m, 3H), 7.38-7.50 (m, 5H); HRMS (Fab) obs'd for $C_{28}H_{35}O_9Na+H$, 539.2254; calc'd, 539.2257.

    3: ^1H NMR (200 MHz, D_2O), δ 1.16 (d, 3H, J=6.4Hz), 1.32 (m, 3H), 1.50 (m, 1H), 1.71 (m, 2H), 2.10 (m, 2H), 2.49 (t, 2H, J=7.2Hz), 2.82 (t, 2H, J=7.3Hz), 3.63-3.84 (m, 4H), 4.10-4.25 (m, 2H), 5.18 (d, 1H, J=2.3Hz), 6.83 (s, 2H), 6.93 (s, 1H); HRMS (Fab) obs'd for $C_{21}H_{29}O_9Na+H$, 449.1790; calc'd, 449.1788

    4: ^1H NMR (200 MHz, D_2O), δ 1.14 (d, 3H, J=6.4Hz), 1.32 (m, 3H), 1.52 (m, 1H), 1.71 (m, 2H), 2.20 (m, 2H), 2.42 (t, 2H, J=7.6Hz), 2.86 (t, 2H, J=8.0Hz), 3.64-3.82 (m, 4H), 4.10-4.18 (m, 1H), 4.19-4.28 (m, 1H), 5.17 (s, 2H), 5.18 (d, 1H, J=3.5Hz), 6.87-7.06 (m, 3H), 7.38-7.53 (m, 5H); HRMS (Fab) obs'd for $C_{28}H_{35}O_9Na+H$, 539.2233; calc'd, 539.2257.
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