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Synthesis and Biological Evaluation of a Sialyl Lewis X Mimic with Significantly Improved E-selectin Inhibition

Gebhard Thoma,[a,*] John L. Magnani[b] and John T. Patton[b]

[a]*Novartis Pharma AG, PO Box, CH-4002 Basel, Switzerland*
[b]*GlycoTech Corporation, 14915 Broschart Road, Rockville, MD 10850, USA*

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Abstract—The synthesis of the highly potent E-selectin inhibitor **5** is described. Sialyl Lewis X mimic **5** was rationally designed by combining two previously disclosed beneficial sLe^x modifications in a single molecule. The compound was found to be 30-fold more potent than sLe^x in a static, cell-free equilibrium assay. Furthermore, compound **5** was highly active ($IC_{50} = 10\,\mu M$) in a dynamic non-equilibrium assay in which sLe^x did not inhibit neutrophil rolling at up to 1000 μM.

Introduction

The recruitment of leukocytes to sites of inflammation is a multistep process.[1] The selectins are cell-surface glycoproteins which promote both the initial attachment of leukocytes (tethering) and their movement (rolling) over the blood vessel wall.[2] Integrins mediate the subsequent firm adhesion preceding extravasation into the underlying tissue. L-Selectin is expressed constitutively on leukocytes whereas E- and P-selectin are induced on the surface of vascular endothelium in response to inflammatory stimuli. Excessive infiltration of leukocytes can cause acute or chronic reactions as observed in reperfusion injuries, stroke, psoriasis, rheumatoid arthritis, or respiratory diseases. Thus, the adverse effects could be prevented by selectin blockade.[3] The tetrasaccharide sialyl Lewis X (sLe^x **1**, Fig. 1)[4] is a physiologically relevant recognition component for all three members of the selectin family.[5] It is a weak inhibitor of E-selectin ($K_D \approx 1000\,\mu M$) and became a prominent lead stucture for the design of more potent mimics which could be of therapeutic interest.[6] Thus, the introduction of *N*-acyl aromatic substituents into sLe^x led to up to 10-fold more potent analogues.[7] We have found the 3,4-dimethoxybenzoate **2** (Fig. 1) to be 6-fold more potent than sLe^x.[8] Previously, we have reported on potent sLe^x mimetics, such as **3** and **4** (Fig. 1), containing S-cyclohexyllactic acid as a replacement for sialic acid.[9] Here we describe the synthesis of the novel, potent E-selectin inhibitor **5** (Fig. 1) which combines both the glucosamine modification and the sialic acid replacement. Compound **5** was found to be 30-fold more potent than sialyl Lewis X. Furthermore, under in vitro flow conditions compound **5** inhibited rolling of polymorphonuclear leukocytes (PMNs) on stimulated human umbilical vein endothelial cells (HUVECs) with an IC_{50} value of 10 μM.

Synthesis

Compound **5** was prepared starting from suitably protected glucosamine derivative **6**[10] (Scheme 1). Treatment of a mixture of **6** and alcohol **7** (1.3 equiv) with TMS triflate (2.5 equiv) at −35 °C gave selectively the β-glycoside **8** (85%) which was subsequently deacetylated applying catalytic amounts of NaOMe in methanol. The crude triol intermediate was reacted with benzaldehyde dimethylacetal in acetonitrile in the presence of catalytic amounts of PTSA to selectively block the 4- and 6-hydroxyl functions as a benzylidene acetal leaving the 3-hydroxyl group unprotected. Compound **9** was isolated in 88% yield over two steps. Fucose was introduced using fucosyl donor **10**[11] which was transformed into the corresponding glycosylbromide by slow addition of a small excess of bromine at 0 °C to a solution of **10** in methylene chloride. Unreacted bromine was quenched with cyclohexene. This solution was added to compound **9** dissolved in methylene chloride/DMF (3:2). The mixture was kept at ambient temperature for 16 h. Disaccharide **11**[12] was isolated by flash chromatography in 89% yield. The benzylidene acetal was opened by

*Corresponding author. Tel.: +41-61-3243342; fax: +41-61-3246735; e-mail: gebhard.thoma@pharma.novartis.com

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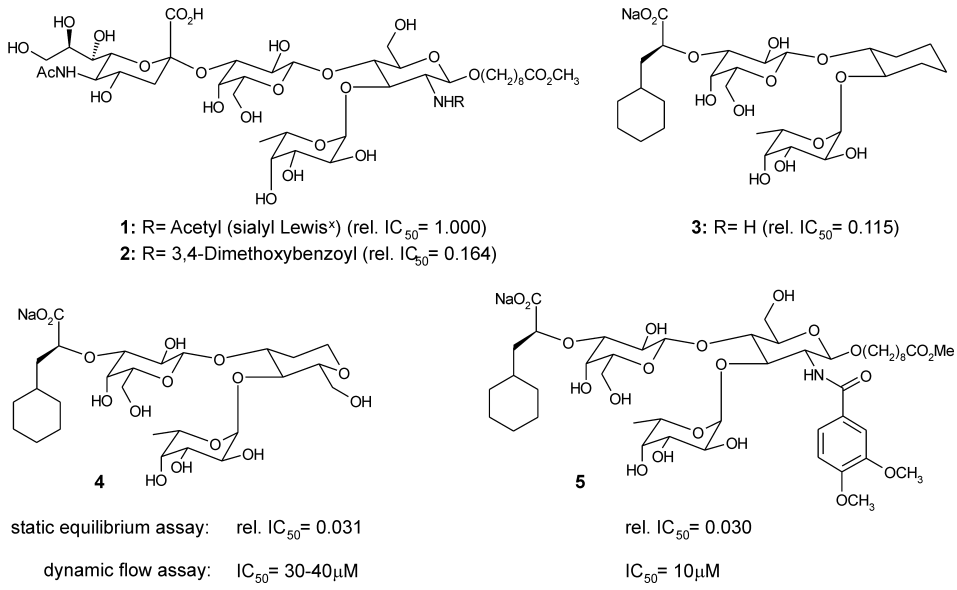
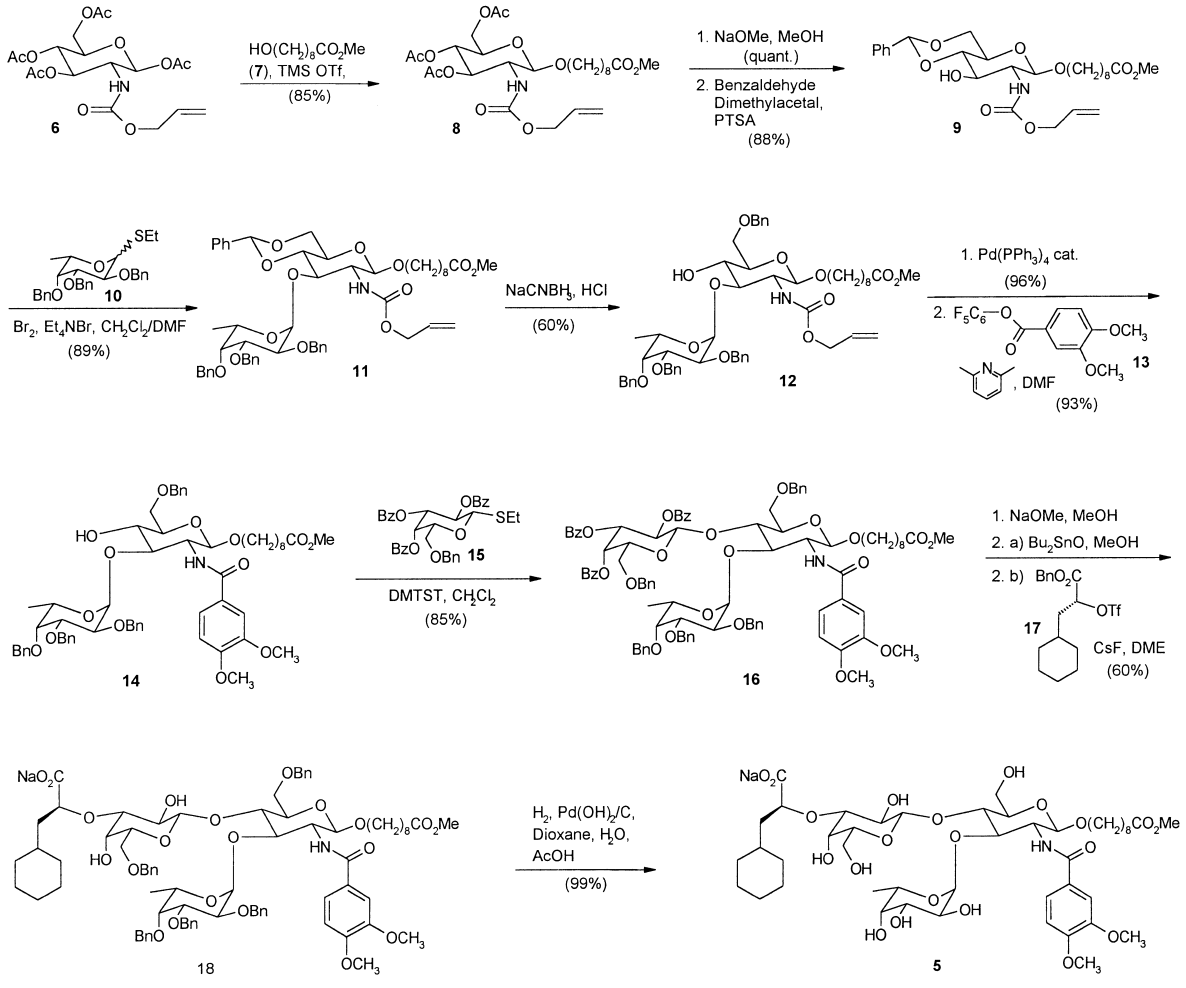


Figure 1. Structures of selected E-selectin inhibitors.



Scheme 1. Synthesis of E-selectin antagonist **5**.

slowly adding a freshly prepared saturated solution of HCl in ether to a mixture of **11**, molecular sieves (3 Å) and $NaCNBH_3$ (10 equiv) in THF to afford compound **12**. Complete conversion of **11** gave only low yields of **12** due to cleavage of the acid labile α-glycosidic linkage. Thus, the reaction had to be monitored carefully (TLC) and stopped at 70–80% conversion. Compound **12** was isolated in 60% yield along with 20% of unreacted **11**.

It is worthwhile to mention that this rather simple protecting group manipulation turned out to be the most critical step in the whole synthesis. Next, the amine protecting group was removed using catalytic quantities of $Pd(PPh_3)_4$ (0.1 equiv) in THF in the presence of morpholine (30 equiv). The corresponding amine was isolated in 96% yield. Treatment with active ester **13** (2.5 equiv) in a mixture of DMF/2,6-lutidine (5:1) at 80 °C for 16 h gave benzamide **14** in 93% yield. Galactose was introduced activating a mixture of **14**, selectively protected thiogalactoside **15**[11] (1.5 equiv) and molecular sieves (3 Å) in CH_2Cl_2 with dimethyl-(methylthio)sulfonium triflate (DMTST). Trisaccharide **16**[12] was obtained in 85% yield. The benzoates were removed applying catalytic amounts of NaOMe in methanol (quant.), the triol heated in methanol for 16 h in the presence of dibutyltin oxide[13] (1.75 equiv), the solvent removed and the residue dried. This material and triflate **17**[9c] (3.0 equiv) were dissolved in DME, CsF (2.5 equiv) was added and the mixture kept at ambient temperature for 4 h. Compound **18** was isolated in 60% yield. Hydrogenation of **18** in a mixture of dioxane/water/acetic acid (0.7:0.25:0.05) using $Pd(OH)_2$ (20%) on charcoal as a catalyst followed by transformation of the carboxylic acid into the sodium salt afforded the potent E-selectin inhibitor **5**[12] in 99% yield.

Biological Evaluation

Compound **5** was tested in a static, cell-free ligand binding assay which measures E-selectin inhibition under equilibrium conditions.[14] To compare the data for different compounds obtained on different test plates sLe^x **1** (IC_{50} = 1000–1500 μM) was assayed on each plate as a reference. This allows the determination of IC_{50} values relative to sLe^x which are defined as relative $IC_{50} = IC_{50}(\text{test compound})/IC_{50}(sLe^x)$. The relative IC_{50} for **5** was determined in three independent measurements to be 0.030 ± 0.015. In addition, **5** was profiled in a dynamic in vitro assay which allows monitoring of E-selectin-dependent rolling of neutrophils on activated endothelial cells and, hence, mimics the non-equilibrium in vivo conditions.[15] sLe^x (**1**) showed no inhibition in this more relevant assay at up to 1000 μM. Compound **5** was tested at 200, 50 and 10 μM and showed 97, 93 and 54% inhibition of the number of rolling cells, respectively. Thus, the IC_{50} was estimated to be ~10 μM.

Conclusion

E-selectin antagonist **5** was rationally designed combining two previously discovered beneficial structural elements (cyclohexyllactic acid and amide modification) in a single molecule. In a static equilibrium assay **5** (relative $IC_{50} = 0.030$) was found to be equally potent as compound **4** (rel. $IC_{50} = 0.031$). In the dynamic flow assay **5** (IC_{50} = ~10 mM) is even more potent than **4** (IC_{50} = 30–40 μM). Thus, compound **5** is one of the most potent E-selectin inhibitors to date. Simplified analogues of **5** are currently being evaluated.

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12. **11**: MS/EI 936 $(M-H)^-$; ^{1}H NMR (400 MHz, $CDCl_3$) selected signals δ 0.75 (3H, d, J = 6.5 Hz, *H*-6 Fuc), 3.67 (s, 3H, CO_2CH_3), 4.82 (1H, d, J = 8.0 Hz, *H*-1 Glc), 5.15 (1H, d, J = 3.5 Hz, *H*-1 Fuc), 5.18 (1H, dq, J = 10.5, 1.5 Hz, $OCH_2CH{=}CH_EH_Z$), 5.27 (1H, dq, J = 17.5, 1.5 Hz, $OCH_2CH{=}CH_EH_Z$), 5.48 (1H, s, Ar-C*H*-), 5.87 (1H, ddt, J = 17.5, 1.5 Hz, $OCH_2CH{=}CH_EH_Z$); **16**: MS/EI 1582 $(M-H)^-$; ^{1}H NMR (400 MHz, $CDCl_3$) selected signals δ 1.17 (3H, d, J = 6.5 Hz, *H*-6 Fuc), 3.64 (s, 3H, CO_2CH_3), 3.74 (3H, s, $ArOCH_3$), 3.87 (3H, s, $ArOCH_3$), 4.35 (1H, m, *H*-1 Glc), 4.91 (1H, d, J = 8.0 Hz, *H*-1 Gal), 5.47 (1H, s (br), *H*-1 Fuc), 5.89 (1H, d, J = 3.5 Hz, *H*-4 Gal), 6.62 (1H, d, J = 8.5 Hz, Ar-*H*), 6.63 (1H, d, J = 8.0 Hz, N*H*); **5**: MS/EI 974 $(M-Na)^-$; ^{1}H NMR (400 MHz, D_2O) selected signals δ 1.06 (3H, d, J = 6.5 Hz, *H*-6 Fuc), 3.54 (s, 3H, CO_2CH_3), 3.77 (3H, s, $ArOCH_3$), 3.76 (3H, s, $ArOCH_3$), 4.38 (1H, d, J = 8.0 Hz, *H*-1 Gal), 4.55 (1H, m, *H*-1 Glc), 4.70 (1H, q, J = 6.5 Hz, *H*-5 Fuc), 5.01 (1H, s (br), *H*-1 Fuc), 7.00 (1H, d, J = 8.5 Hz, Ar-*H*), 7.32 (1H, d, J = 2.0 Hz, Ar-*H*), 7.32 (1H, dd, J = 8.5, 2.0 Hz, Ar-*H*).
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