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Synthesis of Sialyl Lewis^x Mimics: Replacement of Galactose by Aromatic Spacers

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Abstract: Six sLe^x mimics where the galactose moiety is replaced by aromatic spacers have been prepared and tested for their binding affinity to E-selectin. © 1997 Elsevier Science Ltd.

The rolling of leukocytes on endothelial cells is the initial stage in the recruitment of leukocytes to inflamed tissue[1]. This process is mediated by the interaction of complex carbohydrate structures on leukocytes with the carbohydrate binding proteins E- and P-selectin on the endothelial cells. The minimal structure that is recognized by the selectins is the tetrasaccharide sialyl Lewis^x (**1**, sLe^x, *Figure 1*)[2]. This recognition process is involved in inflammatory diseases, ischemia/reperfusion injury, metastasis and angiogenesis[3]. It is therefore of great pharmacological interest to block this process by antagonizing the binding of sLe^x to E- and P-selectin.

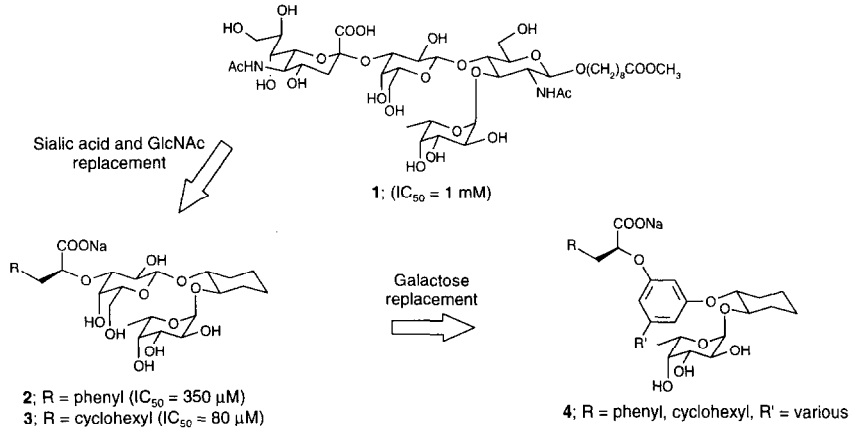


Figure 1

In the course of the search for simplified and more active E-selectin antagonists we[4] and others[5,6] have found that the N-acetylglucosamine portion of sLe^x (**1**) can be replaced by (*R,R*)-1,2-cyclohexanediol. Concomitant replacement of the N-acetyl-neuraminic acid by L-phenyl lactic acid or L-cyclohexyl lactic acid leads to compounds **2**[4] (IC_{50} = 0.35 mM)[7] and **3**[4] (IC_{50} = 0.08 mM)[7] which are three resp. twelve times as active as the parent compound **1** (IC_{50} = 1 mM)[4] (*Figure 1*). In order to further simplify the mimics **2** and **3**, the replacement of galactose by aromatic spacers was investigated (**4**, *Figure 1&2*).

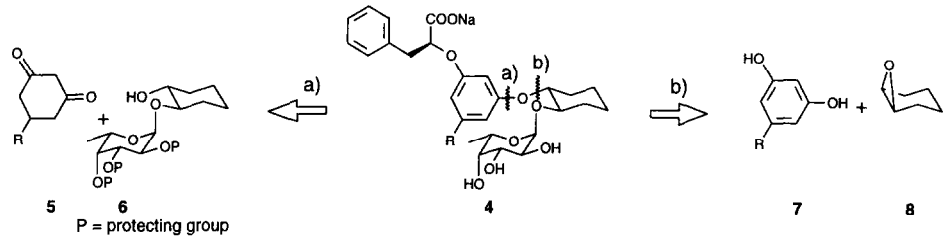
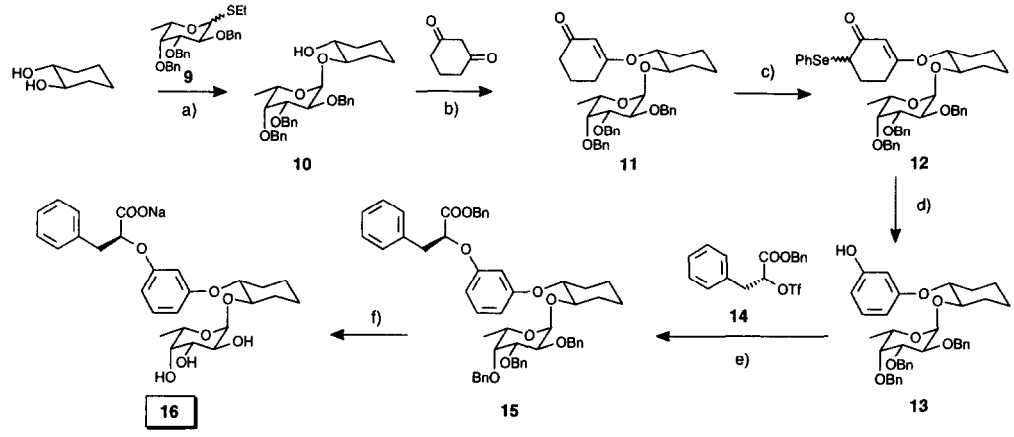


Figure 2

For compounds of type **4** there are two retrosynthetic strategies possible. Disconnecting along bond a) leads to cyclohexanediones **5** and the alcohol **6** as starting materials whereas disconnecting along bond b) leads to resorcinol derivatives **7** and cyclohexane epoxide **8**.

Compound **16** (*Scheme 1*) was prepared according to strategy a). The monofucosylation of (*R,R*)-1,2-cyclohexanediol (→ **10**) was achieved with the thiofucoside **9**[8] as glycosyl donor under bromine activation. **10** was then condensed with cyclohexanedione to give the enone **11**. Aromatization was performed in two steps: the enone was treated with *in situ* generated trimethylsilyl iodide and the intermediate silyl enol ether was trapped with phenylselenyl chloride to give the α-seleno ketone **12**. After oxidation to the seleniumoxide spontaneous elimination yielded the phenol **13**. Alkylation of **13** with the triflate **14**[9] was performed *via* the intermediate stannylene ether. Hydrogenation of **15** and ion exchange chromatography finally led to the desired mimic **16**.



Scheme 1. **a)** 1. **9**, Br_2 (1.1 eq.), CH_2Cl_2; 2. (*R,R*)-1,2-cyclohexanediol (1.5 eq.), Et_4NBr (1 eq.), 4 Å molecular sieves, CH_2Cl_2/DMF 5:3, 18 h, rt, 86%; **b)** *p*-TsOH (0.2 eq.), benzene, reflux, 20 h, 39%, (30% recovered **10**); **c)** 1. TMSCl (1.1 eq.), NaI (1.2 eq.) in MeCN, NEt_3 (5 eq.), 3 h, rt; 2. PhSeCl (1.4 eq.), 1h, rt, quant.; **d)** 30% H_2O_2 (2 eq.), pyridine (3 eq.), CH_2Cl_2, 1 h, 0°C, 91%; **e)** 1. $(Bu_3Sn)_2O$ (2 eq.), toluene, reflux, 3 h; 2. **14** (5 eq.), CsF (5 eq.), DME, 3 h, rt, 89%; **f)** 1. H_2, Pd/C, MeOH, 3 h, 2. Dowex 50 Na^+, 82%.

In a similar way the substituted derivatives **17** and **18** were prepared from the commercially available 5-phenylcyclohexane-1,3-dione and 5-isopropylcyclohexane-1,3-dione. As the initially obtained sodium salts were not soluble in water, which is a prerequisite for the bioassay[7], they were converted into their choline salts by ion exchange chromatography using choline loaded Dowex ion exchange resin.

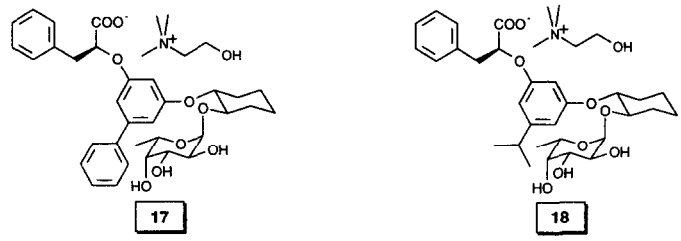
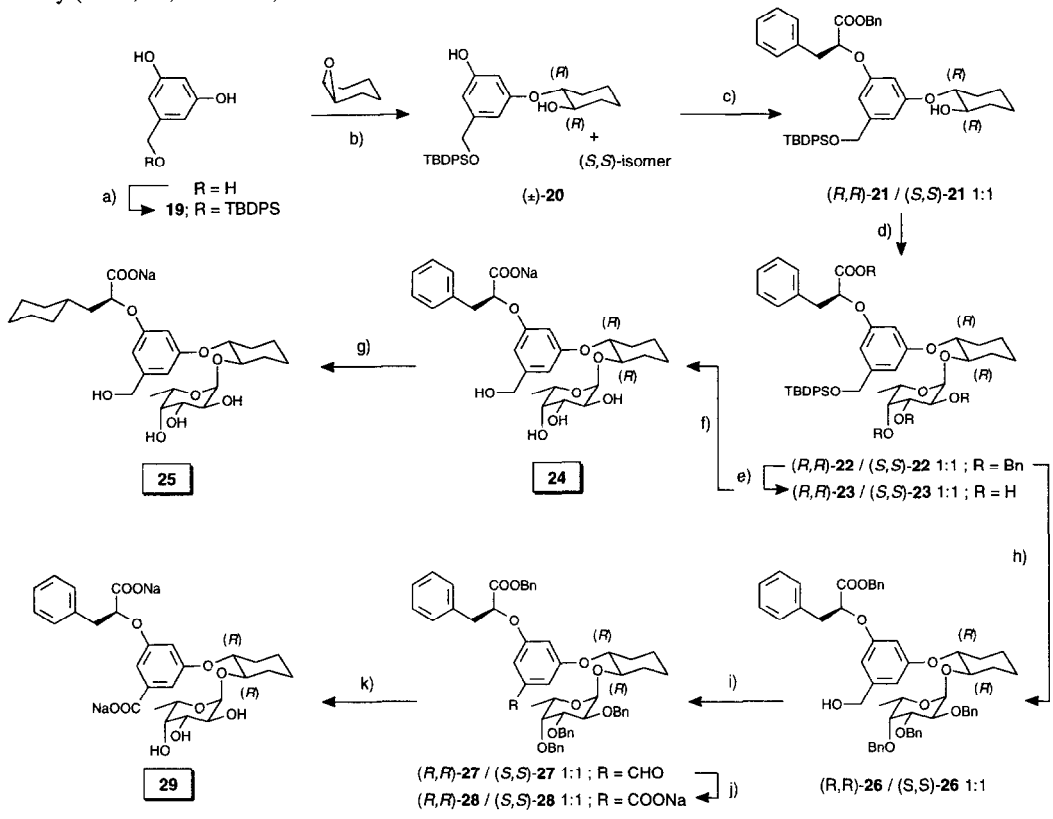


Figure 3

Since reports in the literature[10] suggest that the 6-hydroxy group of galactose is important for E-selectin binding, we planned to incorporate a hydroxymethyl substituent in the 5-position of the resorcinol moiety (→ **24**, **25**, *Scheme 2*).



Scheme 2. a) TBDPSCl (1 eq.), imidazole (6 eq.), 10 min., rt, 97%; **b)** cyclohexene epoxide (1.05 eq.), NEt_3, 100°C, 22 h, 38%; **c)** 1. $(Bu_3Sn)_2O$ (1.0 eq.), toluene, reflux, 3 h, 2. CsF (5 eq.), **13** (5 eq.), DME, 1 h, 69%; **d)** 1. **9** (1.2 eq.), Br_2 (1.3 eq.), CH_2Cl_2, 1 h, 0°C; 2. product after alkylation of **18** (1 eq.), Et_4NBr (1.2 eq.), 4 Å molecular sieves, CH_2Cl_2/DMF 5:3, 16 h, r.t., 82%; **e)** H_2, $Pd(OH)_2$/C, MeOH, 16 h, 55%; **f)** TBAF (1.1 eq.), THF, rt, 16 h, 38% **20** ((R,R)-isomer), 30% (*S,S*)-isomer; **g)** H_2, Rh/Al_2O_3, MeOH, 4 d, 80%; **h)** TBAF (1.1 eq.), THF, AcOH (1.3 eq.), rt, 4 d, 94%; **i)** Dess-Martin Periodinane (1.2 eq.), CH_2Cl_2, 1 h, rt, 96%; **j)** $NaClO_2$ (30 eq.), 2-methyl-2-butene, i-PrOH, NaH_2PO_4, H_2O, rt, 16 h, 96%; **k)** H_2, $Pd(OH)_2$/C, dioxane/H_2O 2:1, 5 h, 13% **24** ((*R,R*)-isomer), 23% (*S,S*)-isomer, 42% mixed fractions.

Following strategy b) cyclohexane epoxide was opened with the phenol **19** to give a racemic mixture of *trans* products (±)-**20** which was subsequently alkylated and fucosylated under the conditions mentioned above to give an inseparable 1:1 mixture of diastereomers (*R,R*)-**22**/(*S,S*)-**22** (latter not shown). After debenzylation and desilylation the mimic **24** could be separated from the unwanted (*S,S*)-isomer[11] by reverse

phase chromatography. The phenyllactic acid compound **24** was further hydrogenated (Rh/Al_2O_3) to the cyclohexyllactic acid compound **25**. Alternatively the mixture of primary alcohols (*R,R*)-**26**/(*S,S*)-**26** was oxidized to the aldehyde with Dess-Martin reagent[12] followed by oxidation with sodium chlorite to the corresponding diasteromeric mixture of the acid salts (*R,R*)-**28**/(*S,S*)-**28** (latter not shown). Debenzylation and separation of the two diastereomers by reverse phase chromatography gave the sodium salt **29**.

All of the prepared mimics **16**, **17**, **18** , **24**, **25** and **29** were inactive (IC_{50} > 10 mM)[7], surprisingly even the most promising one, **25**, which incorporates our best replacements for N-acetyl-neuraminic acid and N-acetyl-glucosamine (compare **3**, *Figure 1*) and a hydroxy group mimicking the 6-OH of galactose. From molecular modeling[4] and transfer NOE NMR studies[13] we know that the compounds **1**, **2** and **3** are fairly rigid as their 3-dimensional structure is ruled by the anomeric stabilization of the glycosidic bonds. The reason for the inactivity of the prepared mimics could be twofold: a) It could be that they prefer a distinct 3-dimensional structure which is unfavourable for binding to E-selectin. b) Replacing galactose by an aromatic spacer means replacing a conformationally restricted anomeric bond by a flexible ether bond. This could lead to an increased overall flexibility of these mimics. As a consequence they could lack the necessary preorganization[4] to fit into the binding site of E-selectin.

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REFERENCES

1. a) Boschelli, H. *Drugs of the Future* **1995**, *20*, 805-816. b) Spertini, O.; Luscinskas, F. W.; Gimbrone, M. A.; Tedder, T. F., *J. Exp. Med.* **1992**, *175*, 1789.
2. a) Patel, T. P.; Goelz, S. E.; Lobb, R. R.; Parekh, Raj. B., *Biochemistry* **1994**, *33*, 14815. b) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.; Paulson, J. C., *Science* **1990**, *250*, 1130.
3. a) Gallin, J. I. et. al. *Inflammation: Basic Principles and Clinical Correlates, 2nd Edition, Raven Press Ltd.* **1992**, 407-419. b) Giannis, A. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 178-181.
4. a) Kolb, H. C.; Ernst, B. *Chemistry - A European Journal*, submitted, b) Kolb, H. C.; Ernst, B. *Pure & Appl. Chem.*, submitted.
5. Toyokuni, T.; Kannagi, R. *Patent WO93/23031*, priority date 8 May 1992.
6. a) Bamford, M. J.; Bird, M.; Gore, P. M.; Holmes, D. S.; Priest, R.; Prodger, J. C.; Saez, V. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 239-244. b) Prodger, J. C.; Bamford, M. J.; Bird, M.; Gore, P. M.; Holmes, D. S.; Priest, R.; Saez, V. *Bioorg. Med. Chem.* **1996**, *4*, 793-801.
7. a) IC_{50} values are determined by GlycoTech Corp., Rockville, Maryland 20850, USA with a standard ELISA assay using an E-selectin-IgG and biotinylated polymer containing sLe^a: Wells in a microtiter plate (plate 1, Falcon probind™) are coated with E-selectin/hIg chimera by incubation of 100ml of the purified chimeric protein at a concentration of 200 ng/well in 50mM Tris, O.15M NaCl, 2mM $CaCl_2$, pH 7.4 (Tris-Ca^{2+}). After 2 hours, 100ml of a 1:1 mixture of 1% BSA in Tris-Ca^{2+} and Stabilcoat™ are added to each well and incubated at 22°C to block nonspecific binding. During this incubation, inhibitory test compounds, diluted in Tris-Ca^{2+}, 1% BSA, are titrated by a twofold serial dilution in a second U-shaped bottom low-bind microtiter plate (plate 2, Costar, Inc.). An equal volume of a preformed complex of a biotinylated sialyl $Lewis^a$ polymer and horseradish peroxidase-labeled streptavidin (KPL, Gathersburg, MD) at 1mg/ml in Tris-Ca^{2+}, 1% BSA is added to each well. After 2 hours at 22°C, plate 1 is washed with Tris-Ca^{2+} and 100ml/well are transferred from plate 2 to plate 1. The binding reaction is allowed to proceed for 2 hours at 22°C while rocking. Plate 1 is then washed with Tris-Ca^{2+} and 100ml of TMB substrate reagent (KPL, Gathersburg, MD) is added to each well. After three minutes, the colorimetric reaction is stopped by adding 100 ml/well of 1M H_3PO_4 and the optical density is determined at 450nm. IC_{50} values greater than 10 mM are no longer detectable and the compounds are considered inactive.
8. Lönn, H. *Carbohyd. Res.* **1985**, *139*, 105-113.
9. Degerbeck, F.; Fransson, B.; Grehn, L.; Ragnarsson, U. *J. Chem. Soc. Perkin Trans. 1* **1993**, 11-14.
10. a) Stahl, W.; Sprengard, U.; Kretzschmar, G; Kunz, H. *Angew. Chem.* **1994**, *106*, 2186-2188. b) Bänteli, R.; Ernst, B.; Kolb, H.; Oehrlein, R. unpublished results.
11. From the fact that for one isomer most NMR signals were almost identical with the signals of **16** (with known (*R,R*) stereochemistry) whereas for the other isomer they were clearly different it was inferred which one was **24** with (*R,R*) configuration and which one the (*S,S*)-isomer.
12. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4156-4158.
13. Scheffler, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W. T.; Weisemann, R.; Peters, T. *Angew. Chem.* **1995**, *107*, 2034-2037.

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