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Synthesis of Sialyl Lewis^x Mimics. Modifications of the 6-Position of Galactose

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Abstract—Seven sLe^x mimics where the -CH_2OH group of the galactose moiety is replaced by -$CH_2NH_3^+$, -CH_2NHAc, -CH_2NHBz, -CH_2OSO_3Na, -COONa and -$CONH_2$ have been prepared and tested for their binding affinity to E-selectin.

The rolling of leukocytes on endothelial cells of blood vessels is the initial stage in the recruitment of leukocytes to inflamed tissue.[1] This process is mediated by the interaction of complex carbohydrate ligands on leukocytes with the carbohydrate binding receptors E- and P-selectin on the endothelial cells. The minimal structure recognized by both selectins is the tetrasaccharide sialyl Lewis^x (**1**, sLe^x, Fig. 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. In the course of the search for simplified and more potent E-selectin antagonists we[4,5] and others[6] have found that *N*-acetylglucosamine in sLe^x (**1**) can be replaced by (*R,R*)-1,2-cyclohexanediol. Concomitant replacement of *N*-acetylneuraminic acid by L-phenyl lactic acid or L-cyclohexyl lactic acid led to compounds **2**[5] ($IC_{50} = 0.35$ mM) and **3** ($IC_{50} = 0.08$ mM) which show a 3- and 12-fold increase in their in vitro biological activity[7] compared to the parent compound **1** ($IC_{50} = 1$ mM)[5] (Fig. 1). Literature reports[8a] suggest that the 6-hydroxy group of galactose as well as the fucose hydroxy groups and the sialic acid carboxy group are a prerequisite for E-selectin binding.

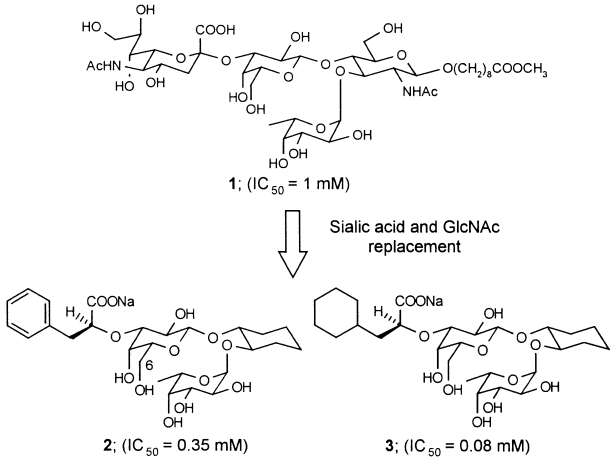

Figure 1. Mimics of sialyl Lewis^x.

In order to investigate whether additional binding can be gained by selectively modifying the 6-position of galactose, a series of modifications of **2** and **3**, respectively, with positively charged (**4**), negatively charged (**7**, **8** and **9**), neutral (**5** and **10**) and bulky (**6**) replacements for the 6-OH group were prepared (Fig. 2).

In order to prepare the 6-amino-6-deoxygalactose derivatives **4**, **5** and **6**, the tetrol **11**[5a] was regioselectively alkylated with *p*-methoxybenzylbromide at the 3-position via the intermediate stannylene acetal[9] to yield **12** (Scheme 1). Tosylation of the 6-hydroxy group and azide replacement gave **14**. Removal of the PMB group under oxidative conditions ($Ce(NH_4)_2(NO_3)_6$) yielded **15** which was alkylated at the same position with the triflate **16**[10] again via the intermediate stannylene acetal. Hydrogenation of **17** gave the ammonium salt **4**. Finally, Schotten-Baumann acylation using acetic anhydride or benzoyl chloride in aqueous base led to the acetamide **5** and the benzamide **6**, respectively.

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For the synthesis of the 6-*O*-sulfate **7**, the pseudotrisaccharide **11** was selectively silylated to **18** and alkylated to **19** (Scheme 1). Desilylation led to **20**, sulfation of which was selective for the primary 6-position. After hydrogenation of **21** and ion exchange chromatography the disodium salt **7** was obtained. The uronic acid compounds **8** and **9** were prepared by oxidation of the primary 6-hydroxy group of the galactose moiety of a precursor with a fully assembled carbon framework. In a first attempt, treatment of the triol **20** with TEMPO[11] (2,2,6,6-tetramethyl-piperidin-1-oxyl radical, which is known to selectively oxidize primary hydroxy groups in the presence of secondary ones) failed. In a second attempt, the secondary hydroxy groups in the 2- and 4-position of **19** were protected by benzoylation. By desilylation, the primary alcohol **23** (Scheme 2) was obtained. Subsequent oxidation with Dess–Martin reagent[12] gave the aldehyde **24** which decomposed upon chromatography on silica (→β-elimination of the 4-benzoyl group). Without purification it was therefore further oxidized using sodium chlorite[13] to yield the uronic acid derivative **25**. Subsequent debenzoylation could only be accomplished under harsh conditions (70 °C, 24 h) suggesting that the 2- and the 4-positions are sterically hindered. Finally, the desired compared **8** was obtained by hydrogenation over Pd/C. Further hydrogenation over Rh/Al_2O_3 eventually led to the cyclohexyllactic acid derivative **9**.

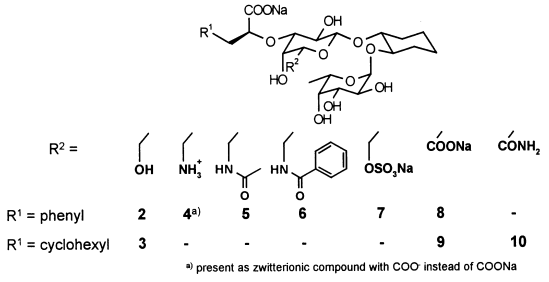
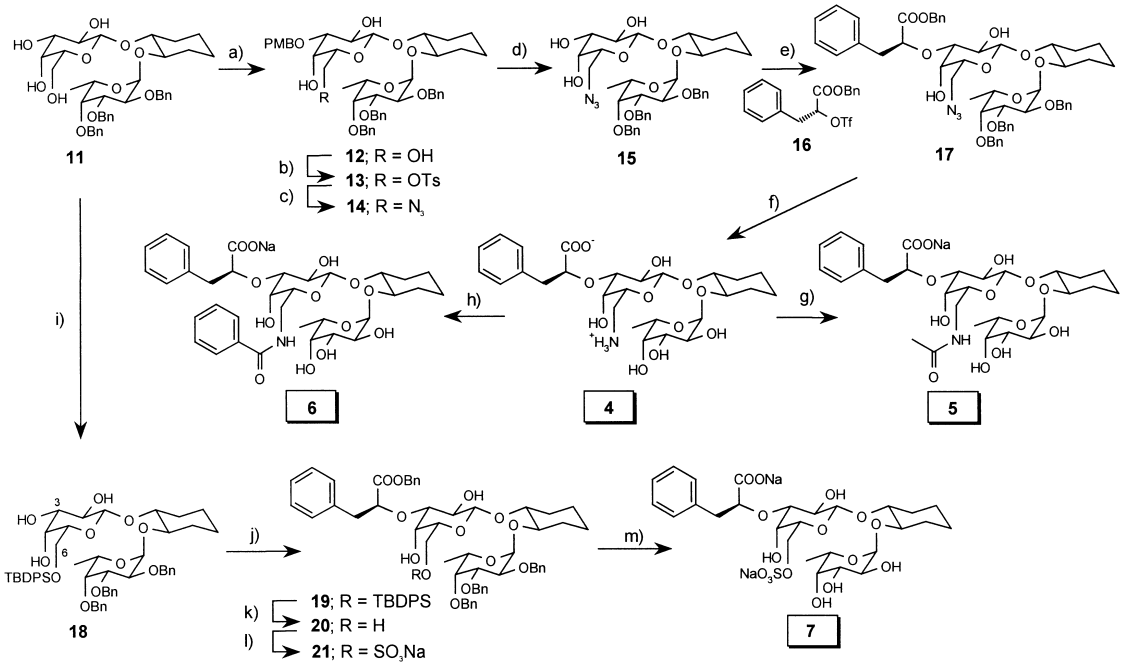


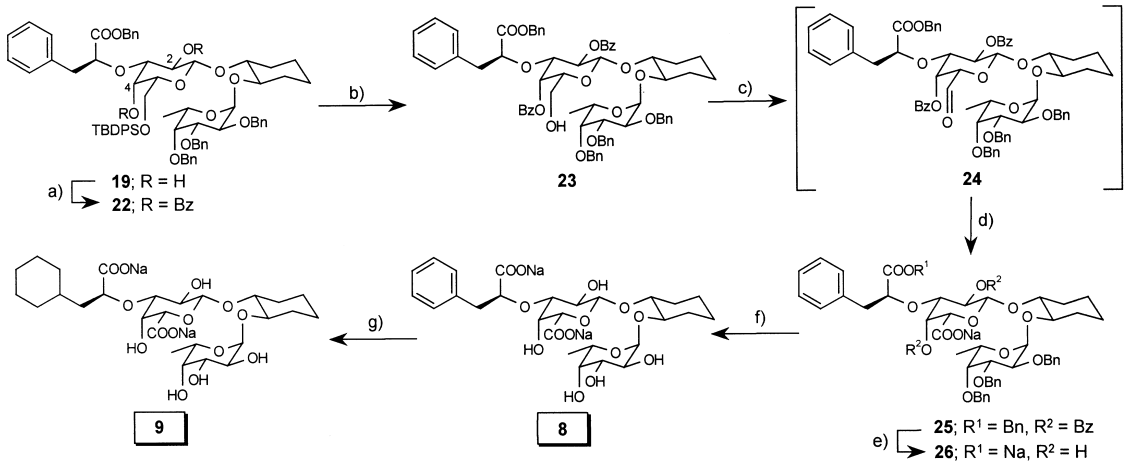
Figure 2. Modification of the galactose moiety.

In order to prepare the target **10**, the uronic acid **25** was transformed into the corresponding uronic amide (→ -COOH → -COCl → $-CONH_2$). However, all attempts to subsequently remove the benzoyl protecting groups were acompanied by β-elimination of the 4-benzoyl group. In an alternative approach benzylation of the 2- and 4-position of **19** failed due to unreactivity of the 4-position. Since this position was so unreactive towards protection it was argued that it might also be unreactive towards oxidation and that protection thereof might not be necessary. Compound **27**[14] was therefore monoprotected with $ClCH_2COCl$ to give **28** (Scheme 3). Desilylation and two-step oxidation was performed as above to give **31**. Conversion of the sodium salt to the acid and reaction with chloroenamine[15] followed by treatment with NH_3/MeOH led to the amide **32**. Deacylation with thiourea[16] to give **33** followed by hydrogenation finally yielded the uronamide **10**.

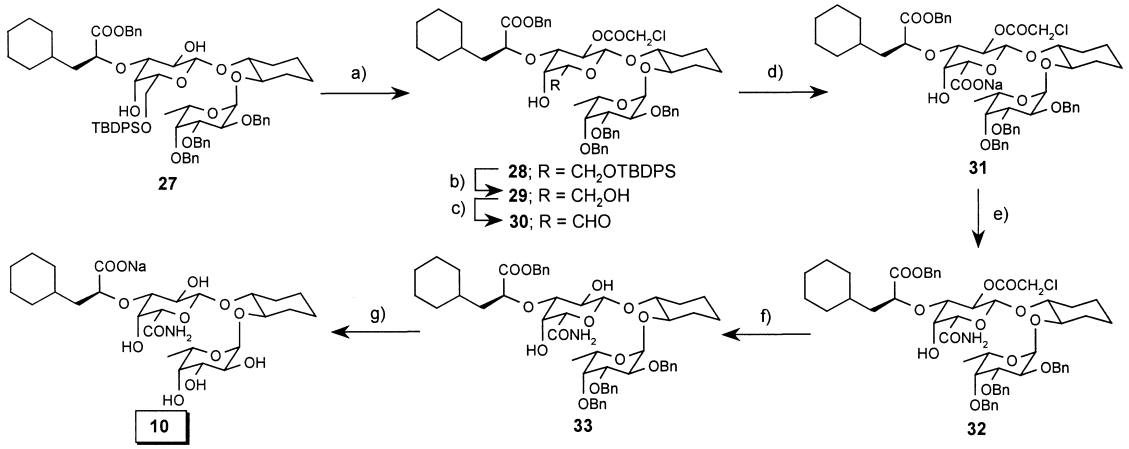
All mimics were inactive (IC_{50} >10 mM).[8] The fact that the acetamide **5**, the benzamide **6** and the sulfate **7** are inactive can be rationalized by the following argument:



Scheme 1. (a) 1. Bu_2SnO, benzene, reflux, 5 h; 2. *p*-CH_3O-BnCl (18 equiv), Bu_4NBr (1.5 equiv), 60 °C, 6 h, 81%; (b) TsCl (1.2 equiv), CH_2Cl_2, pyridine, reflux, 17 h, 75%; (c) NaN_3 (4 equiv), DMF, 60 °C, 46 h, 64%; (d) $Ce(NH_4)_2(NO_3)_6$ (3 equiv), CH_3CN/H_2O 9:1, 0 °C, 3 h, 81%; (e) 1. Bu_2SnO (1.5 equiv), MeOH, reflux, 2 h, evaporation; 2. CsF (5 equiv), **16** (5 equiv), DME, rt, 22 h, 65%; (f) H_2, $Pd(OH)_2$, 48 h, 27% and 11% (slightly impure); (g) 1. Ac_2O (10.5 equiv), H_2O, 2. adjust pH to 9–10 with NaOH, quant. (h) BzCl (1.1 equiv), NaOH, H_2O, rt, 3 h, 85%; (i) TBDPSCl, imidazole, DMF, 16 h, 87%; (j) 1. Bu_2SnO (1.5 equiv), MeOH, reflux, 2 h, 2. CsF (5 equiv), **16** (5 equiv), DME, 24 h, 60% (16% recovered starting material); (k) TBAF 1 M in THF (1.1 equiv), THF, AcOH (>1.1 equiv), 7 h, 77%; (l) 1. SO_3·py, (1.5 equiv), pyridine, 2 h, 0 °C, 1.5 h rt, 2. Dowex 50^+, >50%; (m) 1. H_2, $Pd(OH_2)$/C, MeOH, 6 h, 2. Dowex 50 Na^+.



Scheme 2. (a) BzCl (4.8 equiv), pyridine, rt, 16 h, 87%; (b) TBAF (1.1 equiv), AcOH (ca. 1.3 equiv), THF, rt, 16 h, 95%; (c) Dess–Martin periodinane (1.2 equiv), CH_2Cl_2, rt, 2 h, (used crude); (d) $NaClO_2$ (30 equiv), 2-methyl-2-butene, *i*-PrOH, NaH_2PO_4, H_2O, rt, 16 h, 88% (over two steps); (e) 1 M NaOH (9 equiv), 40 °C, 18 h, 70 °C, 24 h, 78%; (f) H_2, $Pd(OH)_2/C$, dioxane/H_2O/AcOH 24:12:1, 18 h, 70%; (g) H_2, Rh/Al_2O_3 5%, dioxane/H_2O 1:1, 4 h, 93%.



Scheme 3. (a) $ClCH_2COCl$, pyridine, CH_2Cl_2, 10 min, 87%; (b) TBAF, AcOH, THF, 16 h, 65%; (c) Dess–Martin periodinane (1.2 equiv), CH_2Cl_2, rt, 2 h, (used crude); (d) $NaClO_2$ (30 equiv), 2-methyl-2-butene, *i*-PrOH, NaH_2PO_4, H_2O, rt, 16 h, 82% (two steps); (e) 1. Dowex W X 8 H^+-form; 2. $(CH_3)_2C{=}CClN(CH_3)_2$ (1.5 equiv), CH_2Cl_2, 0 °C, 1 h, evaporate to dryness then add CH_2Cl_2 again; 3. NH_3/MeOH, 5 min, 75%; (f) H_2NCSNH_2 (3 equiv), EtOH, 48 h, 50 °C, 83%; (g) H_2, $Pd(OH)_2/C$, dioxane/water 2:1, 3 h, 90%.

as the 6-hydroxy is in close contact with the protein these substituents might be too bulky thereby preventing the close interaction between sLe^x and E-selectin. Also, the ammonium salt **4**, as well as the uronic acid compounds **8** and **9** which contain replacements of the 6-OH with comparable steric demand as 6-OH itself but with positive or negative charges, proved to be inactive. Surprisingly, even the uronic amide **10** with an uncharged 6-OH replacement which remains a hydrogen bond donor showed no activity. These results suggest that the 6-hydroxy group of the galactose moiety is optimally suited for the direct binding to E-selectin.

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7. 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. IC_{50} values greater than 10 mM are no longer detectable and the compounds are considered inactive. For more information see footnote 7 in ref 4.

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14. **27** was prepared in analogy to **19** using cyclohexyllactic acid triflate in the alkylation step instead of phenyllactic acid triflate **16**.
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