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# MALONIC ACID DERIVATIVES AS SIALYL LEWIS X MIMETICS

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***Abstract:*** Malonic acid derivatives designed as mimetics of the sialyl Lewis X determinant were synthesized. Their inhibitory potency for selectin mediated cell adhesion was evaluated in cell culture assays and *in vivo*. The receptor binding affinity for E- and P-selectins of simple mimetics was found to be comparable to the potency of the tetrasaccharide sialyl Lewis X. © 1997 Elsevier Science Ltd.

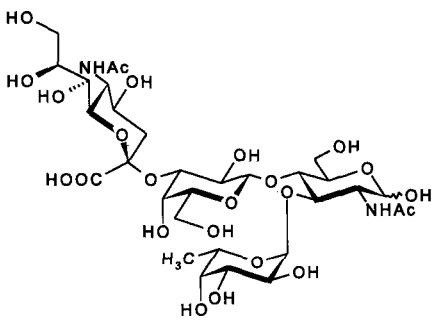


**Figure 1:** *sialyl Lewis X tetrasaccharide*

## Introduction

The role of selectin mediated adhesion of leukocytes to the activated endothelium of blood vessels is generally recognized.[1] The selectin family of adhesion molecules bind to specific carbohydrate ligands and are important in a variety of physiological and pathological processes including inflammation and cancer metastasis. Particularly E- and P-selectin are believed to interact with sialyl Lewis X (Figure 1) and its positional isomer sialyl Lewis A. Therefore, modulation of this interaction by small mimetics of the natural oligosaccharide ligands may be of use for the treatment of acute and/or chronic diseases in which excessive adherence of neutrophils occurs at inflamed tissue sites.[2] Excellent reviews about mimetics of sialyl Lewis X synthesized until now were published recently[2], but none of the simplified non-oligosaccharide or non-peptide mimetics described up to now[3] showed significantly higher affinity[4] to one of the selectins compared to sialyl Lewis X or A. E-selectin binds to carbohydrates including sialyl Lewis X , sialyl Lewis A as well as 3'-sialyl-3-fucosyl lactose.[5] It seems to be obvious that a common epitope[5,6] of these tetrasaccharides is responsible for this interaction. The determination of the minimum recognition sites of sialyl Lewis X resulted in the finding that the 4- and 6-hydroxyls of galactose, the 2-, 3-, and 4-hydroxyls of fucose and the carboxylate group of the sialic acid are important. Improvements were achieved by attaching hydrophobic aglycons and/or by replacing the

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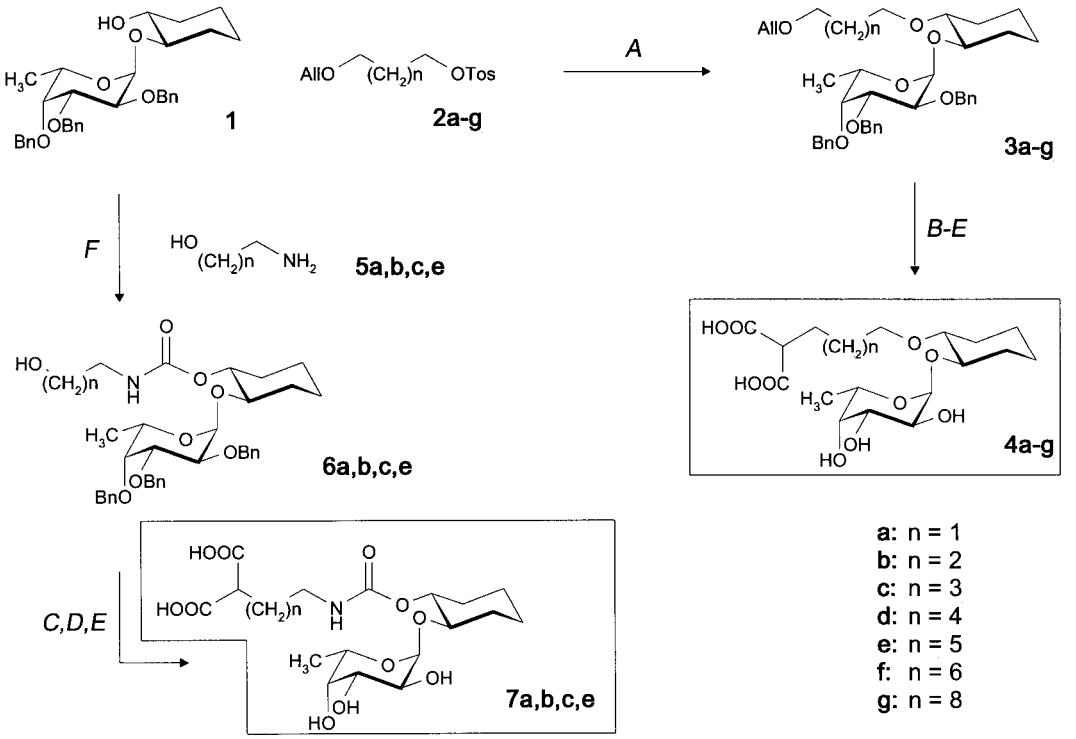
NHAc groups in N-acetyl-glucosamine and in the sialic acid by amino or azido groups.[7] Furthermore, substitution of the N-acetyl-glucosamine by a (1R,2R)-*trans*-1,2-cyclohexanediol moiety resulted in compounds with slightly improved affinities to E- and P-selectin.[8] We now achieved in the synthesis of even more simplified mimetics in which three sugars of the tetrasaccharide were replaced by non-sugar moieties. Particulary, the sialic acid was now replaced by malonic acid which was anticipated to bind to the two lysine side chains (Lys-111 and Lys-113) in E-selectin. From a three-dimensional model of P-selectin, site-specific mutagenesis studies and chemical modifications, a possible interaction of the ligand with the two lysine side chains was previously identified as critical for binding.[9] For the same reasons, and as it was suggested by our

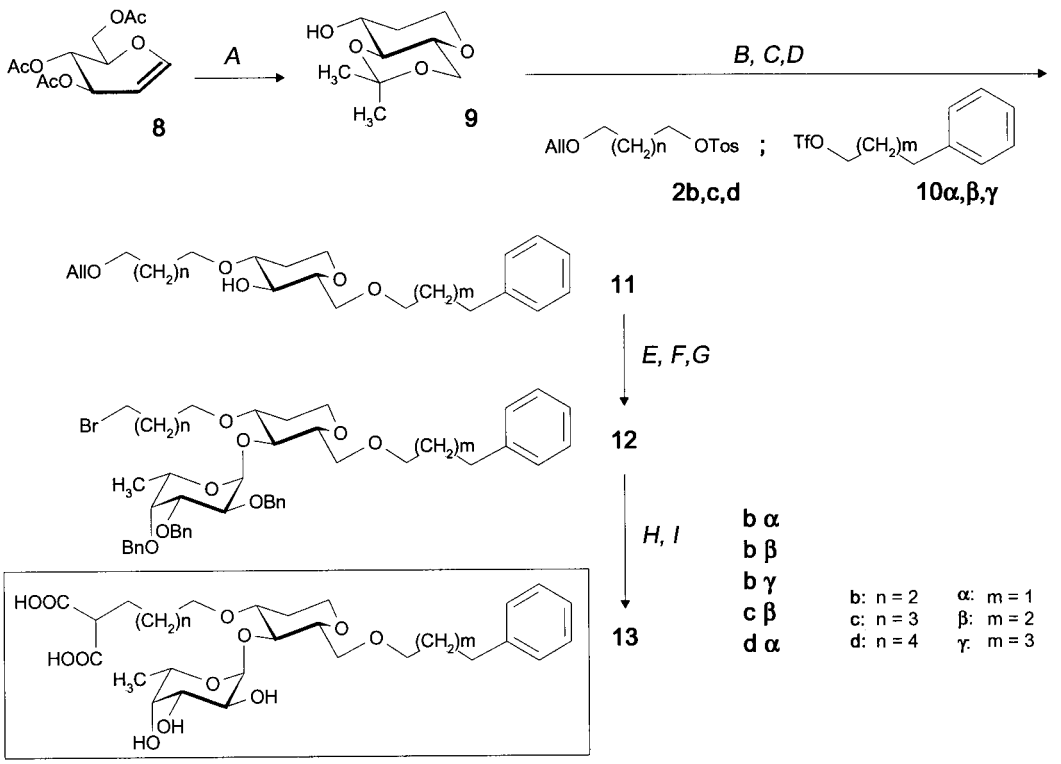



**Scheme 1**: *A.* NaH, DMF, 20 h, 85-92%; *B.* $[(C_6H_5)_3P]RhCl$, $EtOH/H_2O$ (9:1) reflux, 1 h, 64-90%; *C.* $PPh_3$, $NEt_3$, $BrCCl_2CCl_2Br$, $CH_2Cl_2$, 0 °C → 20 °C, 2 h, 86%; *D.* $H_2C(COOMe)$, $K_2CO_3$, dibenzo-18-crown-6, 80 C, 5 h, 88%; *E.* Pd on carbon/$H_2$, $CH_3OH$/dioxane (10:1), 18 h; NaOH (1M), 3 h, 85-92%; *F.* $NEt_3$, DMAP, 4-nitrophenyl-chloroformate, $CH_2Cl_2$, 18 h; N-ethyl-diisopropylamine, **5a,b,c,e**, 18 h, 76-88%.

molecular modeling studies, we then introduced a second carboxylic group at the piperidine moiety, namely diethyl piperidine 4,4-dicarboxylate.[10] In the first step [(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→1)]-(1R,2R)-*trans*-1,2-cyclohexanediol **1**[8] was alkylated with **2a-g**, easily available by monoallylation and

subsequent tosylation of the corresponding diols, to yield **3a-g**. After cleavage of the allyl group, the resulting primary hydroxyl group was transformed into the bromide. Treatment with dimethyl malonate under phase transfer conditions and then cleavage of all protecting groups led to the desired mimetics **4a**[11]**-g**. For the synthesis of mimetics **7a,b**[12]**,c,e**, compound **1** was treated with nitrophenyl chloroformate and subsequently with **5a,b,c,e**. The final reaction steps were analogous to the synthesis of compounds **4a-g** (Scheme 1).

Furthermore, compounds **13bα-dα** with an additional hydrophobic moiety were prepared as outlined in Scheme 2. The synthesis started with the hydrogenation of tri-O-acetyl-D-glucal **8**. Subsequent deacetylation followed




**Scheme 2**: *A.* i.) $PdC/H_2$, dioxane, 24 h, ii.) NaOMe/MeOH, iii.) dimethoxypropane, p-TosOH, 80%; *B.* NaH, **2b,c,d**, DMF, 20 h, 89%; *C.* $CF_3COOH$ (20%), $CH_2CCl_2$, 4 h, 90%; *D.* **10a,b,g**, $K_2CO_3$, dibenzo-18-crown-6, toluene, 18 h, 73%; *E.* O-(2,3,4-tri-O-benzyl-L-fucopyranosyl)-trichloroacetimidate, TMSOTf, 87%; *F.* $[C_6H_5)_3P]RhCl$, $EtOH/H_2O$ (9:1), reflux, 1 h, 62%; *G.* $PPh_3$, $NEt_3$, 1,2-dibromotetrachloroethane, $CH_2Cl_2$, 0 °C → 20 °C, 2 h, 85%; *H.* $H_2C(COOMe)$, $K_2CO_3$, dibenzo-18-crown-6, 80 °C, 5 h, 93%; *I.* $PdC/H_2$, $CH_3OH$/dioxane (10:1), 18 h; NaOH (1M), 3 h, 86%.

by isopropylidenation gave the alcohol **9**. Alkylation with **2b-d** followed by acidic cleavage of the isopropylidene group and selective alkylation with the tosylates **10α-γ**, which were easily available by tosylation of the

commercially available phenylalkanols, led to **11bα-dα**. These intermediates then were glycosylated with O-(2,3,4-tri-O-benzyl-L-fucopyranosyl)-trichloroacetimidate[14] by the inverted procedure.[15] The further steps are analogous to the synthesis of compounds **4a-g**. The inhibitory potency of potential selectin antagonists on HL60 cell binding to immobilized E- and P-selectin-IgG was measured as already described.[16] The results are summarized in Table 1.

**Table 1**: *Inhibition of HL60 cell adhesion to recombinant E- and P-selectin-IgG fusion proteins on plates.*

| inhibitor | $IC_{50}$ (mM) | |
|---|---|---|
| | E-selectin | P-selectin |
| sLeX-tetra | 1.5 | 3.0 |
| **4a-g** | >5.0 | >5.0 |
| **7a** | 3.4 | 3.5 |
| **7b** | 1.0 | 0.7 |
| **7c** | 4.0 | 4.5 |
| **13bβ** | 4.8 | 3.6 |
| **13cβ**[13] | 2.6 | 1.2 |

$IC_{50}$-values are concentrations of inhibitors required to block adhesion of 50% of the cells compared with the nonspecific binding to the CD4-IgG fusion protein as negative control. sLeX-tetra is the reference tetra-saccharide described in ref.16, and each $IC_{50}$ determination was repeated on another day at least once.[16]

The effect of lipopolysaccharide (LPS) induced leukocyte adhesion in rat mesenteric venules *in vivo* was measured by intravital microscopy and an analogous video image processing system as already described.[16] The results are shown in Figure 2.

## Discussion

Especially the highly simplified mimetic **7b**, compared to the very complex sLeX tetrasaccharide, exhibited remarkable activity in both cell assay systems. Three of the four saccharides in sLeX could be successfully replaced by much simplified moieties whereby the overall weak affinity of sLeX was still retained. Compounds **4a-g** were inactive in our assays, probably due to the substitution of the galactose by a very flexible, hydrophobic linker moiety. An additional hydrophobic moiety at an appropriate position, as present in mimetics **13bβ** and **13cβ**, surprisingly seems to compensate for this disadvantage. However, mimetics in which the carboxylic groups are connected by a more rigid spacer, especially a cyclic spacer, are of special interest, because some of them were reasonable active in the cell-based selectin assays and they are available by a very short synthesis sequence.[9] Most interestingly, the simple mimetic **7b** which was the most active in the cell-based assay proved to be very active in the *in vivo* model of LPS-induced inflammation model, which confirms the structure-activity relationships established in our study. Therefore, it cannot be ruled out that the sialic acid

replacement carried out here may be the result of binding to the two lysine side chains of the receptor protein as was suggested. The design and synthesis of next generation non-peptide and non-glycoside mimetics are currently underway and will be reported in due course.

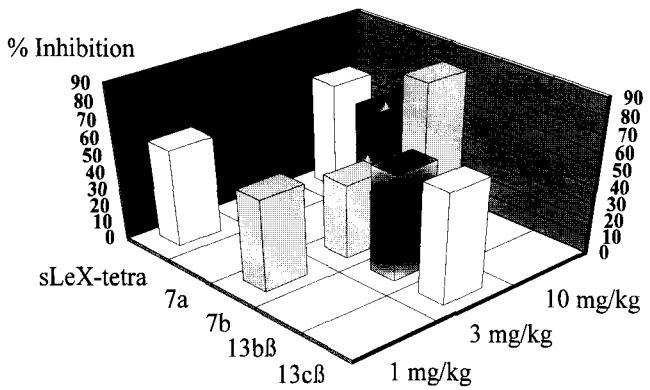



**Figure 2**: *Inhibition of leukocyte adhesion as measured by intravital microscopy.* The inhibition of leukocyte adhesion in rat mesenteric venules by application (i.v.) of compounds **7a** (58% at 10 mg/kg), **7b** (54, 44 and 85% at 1, 3 and 10 mg/kg), **13bβ (62**% at 3 mg/kg) and **13cβ** (65% at 3 mg/kg), respectively, in response to LPS-induced adhesion; sLeX-tetra is the reference tetrasaccharide described.[16]

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11. $^1H$ NMR ($D_2O$): **4a**: d = 1.06 (d, m, 7 H, 6-$H_{fuc}$, 4-$H_{cyclohex}$, 5-$H_{cyclohex}$), 1.48 (m, 2 H, $CH_2$), 1.56 (m, 2 H, $CH_2$), 1.76 (m, 2 H, $CH_2$), 1.98 (m, 2 H, $CH_2$), 3.20, 3.33, 3.43, 3.58-3.74 (4 m, 1-$H_{cyclohex}$, 2-$H_{cyclohex}$, O-$CH_2$, 2-$H_{fuc}$, 3-$H_{fuc}$, 4-$H_{fuc}$), 4,13 (q, 1 H, 5-$H_{fuc}$), 4.90 (d, 1 H, 1-$H_{fuc}$).
12. $^1H$ NMR ($D_2O$): **7b**: d = 1.08 (d, 3 H, 6-$H_{fuc}$), 1.16-1.36 (m, 6 H, 4-$H_{cyclohex}$, 5-$H_{cyclohex}$,, $CH_2$), 1.59 (m, 4 H, 2 $CH_2$), 1.82, 2.01 (2 m, 4 H, 3-$H_{cyclohex}$, 6-$H_{cyclohex}$), 3.01 (m, 2 H), 3.41 (m, 1 H), 3.61 (m, 3 H), 3.90 (q, 1 H, 5-$H_{fuc}$), 4.51 (m, 1 H, 2-$H_{cyclohex}$), 4.88 (bs, 1 H, 1-$H_{fuc}$).
13. $^1H$ NMR ($D_2O$): **13cβ**: d = 1.05 (d, 3 H, 6-$H_{fuc}$), 1.19 (m, 4 H, 2 $CH_2$), 1.44 (m, 7 H, 3 $CH_2$, $CH_{cyclohex}$), 1.70 (m, 2 H, $CH_2$), 2.06 (m, 1 H, $CH_{cyclohex}$), 2.50 (bs, 2 H, $CH_2Ph$), 3.20-3.84 (m, 15 H), 4,20 (q, 1 H, 5-$H_{fuc}$), 4.75 (d, 1 H, 1-$H_{fuc}$), 7.16 (m, 5 H, Ph).
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