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PIPERIDINE CARBOXYLIC ACID DERIVATIVES AS SIALYL LEWIS X MIMETICS

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Abstract: Four piperidine carboxylic 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, *in vivo* and in a reperfusion model. Compound **7** showed strong inhibition of leukocyte adhesion in rats and a poweful decrease of the incidence of arrhythmias in a reperfusion model in isolated rabbit hearts. © 1997 Elsevier Science Ltd.

Introduction

Carbohydrate recognition by selectins is an early event in the adhesion of leukocytes to the activated endothelium of blood vessels.[1] Sialyl Lewis X (Figure 1) and its positional isomer sialyl Lewis A are

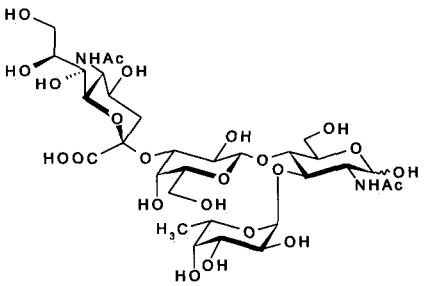

Figure 1:
Sialyl Lewis X tetrasaccharide

believed to interact with adhesion molecules, particularly the E- and P-selectins. Modulation of this interaction by small mimetics of the natural oligosaccharide ligands may therefore be useful for the treatment of acute and/or chronic diseases in which excessive adherence of neutrophils occurs at inflamed tissue sites.[2] However, the simplified mimetics reported thus far,[3] showed only slightly improved[4] or partially amazing affinities[5] compared to sialyl Lewis X or A in cell-based or in cell-free selectin adhesion assays. Substitution of the GlcNAc domain by a (1R,2R)-*trans*-1,2-cyclohexandiol moiety resulted in compounds with slightly better affinities to E- and P-selectin.[4j] We now report on a series of sLeX mimetics in which three sugars of the tetrasaccharide were replaced by non-sugar moieties. The spatial orientation of the acid function and the hydroxyl groups in these rigid compounds is very similar to that of sialyl Lewis X or A.

Preparative Results

The synthesis started with the transformation of (1R,2R)-*trans*-1,2-cyclohexandiol **1** to the C_2-symmetrical carbonate **2**. Nucleophilic attack of the piperidine **3A** led to intermediate **4A** which was alternatively

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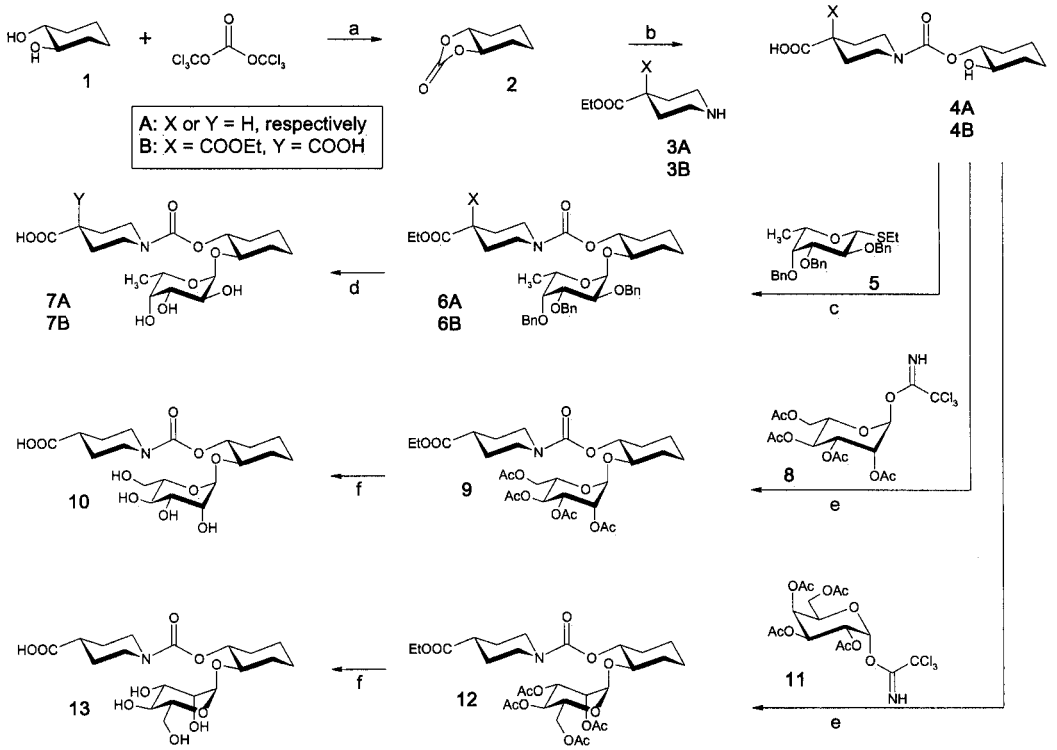
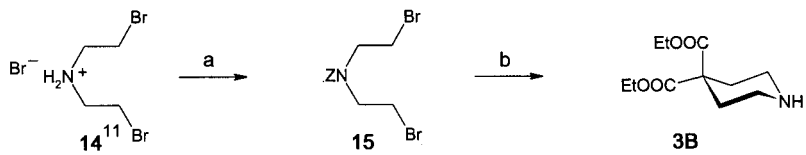
glycosylated by the fucosyl donor **5**[6], the L-mannosyl donor **8**[7] or the D-mannosyl donor **11**[7] to give the compounds **6A**, **9** and **12**, respectively. Cleavage of the protecting groups led to the desired mimetics **7A**[8], **10**[9] and **13**[10] which could be prepared easily in multigram quantities.



Scheme 1: a) CH_2Cl_2, NEt_3, 0 °C (96%); b) DMF, 80 °C, 8 h (75%); c) CH_2Cl_2/DMF 5:1, $BrN(n\text{-}Bu)_4$, $CuBr_2$ (76%); d) i.: Pd/C , H_2, CH_3OH/dioxane/AcOH (10:1:0.5), 18 h, ii.: 1 M NaOH/MeOH (1:1), 45 min, then AcOH, RP-18 silica gel (81%); e) TMSOTf (0.05 eq), diethylether (82-88%); f) i.: NaOMe/MeOH, ii.: 1 M NaOH/MeOH (1:1), 45 min, then AcOH, RP-18 silica gel (78-81%).



Scheme 2: a) benzyl chloroformate, H_2O, NaOH, 0 °C (81%); b) i.: $CH_2(COOEt)_2$, NaH, DMF, 50 °C, 12 h, ii.: PdC, H_2, MeOH (71%).

From a three-dimensional model of P-selectin, site-specific mutagenesis studies and chemical modifications, a possible interaction of the ligand with two lysine side chains (Lys-111 and Lys-113) was previously

identified as critical for binding.[14] For this reason we introduced a second carboxylic group at the piperidine moiety, namely diethyl piperidine 4,4-dicarboxylate **3B**.[11] The synthesis of this compound is shown in Scheme 2. The further steps to mimetic **7B**[13] are analogous to the mimetics described above.

Biological Results

Inhibition of sLeX dependent binding of HL60 cells to E- or P-selectin fusion protein: The inhibitory potency of the potential selectin antagonists on HL60 cell binding to immobilized E- and P- selectin-IgG was measured as already described.[4i] The results are summarized in Table 1. Despite the good accordance with sLeX in our modeling studies, compound **7A** was inactive in this assay system. Only the activity of the dicarboxylated compound **7B** was comparable to the lead compound sLeX in these assays.

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
7A	> 5.0	>5.0
10	5.0	>10.0
13	> 10.0	> 10.0
7B	1.6	7.5

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.

Inhibitory effects of compounds 7A, 10, 13 and 7B on lipopolysaccharide (LPS) induced leukocyte adhesion in the microcirculation of rats: The effect of LPS induced leukocyte adhesion in rat mesenteric venules was measured by intravital microscopy and an analogous video image processing system.[4i] The results are shown in Figure 2. Surprisingly, compound **7A** was found to be a very effective inhibitior in this *in vivo* model despite its inefficiency in the selectin assays. Indeed, the potency of compound **7A** in this model is even higher then the potency of the parent lead compound sLeX.

Effects of compounds 7A, 10, 13 and 7B on the incidence of arrhythmia and ventricular fibrillation.[15] Isolated spontaneously beating rabbit hearts, perfused with saline solution at constant pressure according to the Langendorff technique, were treated with 15 min infusion of autologous neutrophils. 10 min after the start of this infusion the hearts wer submitted to coronary occlusion (LAD) for 30 min followed by 30 min reperfusion. Four groups were investigated: 1) saline-perfused control hearts, 2) leukocyte-perfused hearts, 3) leukocyte-perfused hearts treated with sLeX-tetrasaccharide, 4) leukocyte-perfused hearts treated with

compound **7A**. In all experiments epicardial potential mapping was carried out (256 unipolar leads). The results are shown in Figure 3.

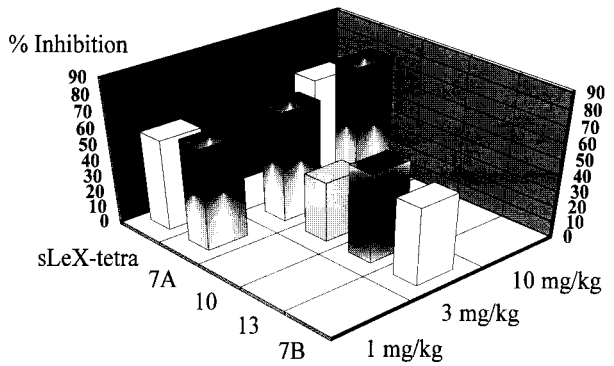


Figure 2: *Inhibition of leukocyte adhesion.* The inhibition of leukocyte adhesion in rat mesenteric venules by application (i.v.) of the sLeX-tetrasaccharide (57% and 63% at 1 and 10 mg/kg) compound **7A** (64, 69 and 81% at 1, 3 and 10 mg/kg), **10** (38% at 3 mg/kg), **13** (56% at 3 mg/kg) and **7B** (49% at 3 mg/kg), respectively, in response to lipopolysaccharide (LPS) induced adhesion.

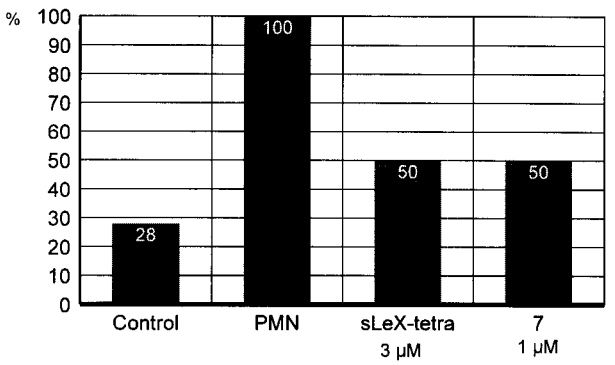


Figure 3: *Incidence of heart arrhythmia and ventricular fibrillation.* SLeX-tetrasaccharide (3 µmol/l) and compound 7 (1 µmol/l).

It was found that arrhythmogenesis can be reduced even at very low concentrations of the sLeX-tetrasaccharide (3 μmol/l) and especially compound **7A** (1 μmol/l). In summary, compound **7A** was found to be very effective in inhibition of leukocyte adhesion in rat mesenteric venules and decrease of arrhytmogenesis in isolated rabbit hearts. However, compound **7A** failed in inhibition of HL60 cell adhesion to recombinant E- and P-selectin-IgG fusion proteins. Thus the mechanism of action of this compound which was designed as a SLeX mimetic remains to be investigated.

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8. ^{1}H NMR (D_2O): **7A**: δ = 1.05 (d, 3 H, 6-H_{fuc}), 1.56 (m, 2 H, CH_2), 1.78 (m, 3 H, 1½ CH_2), 2.00 (m, 1 H, ½ CH_2), 2.45 (m, 1 H, CHCOOH), 2.80 (m, 2 H, CH_2), 4.50 (m, 1 H, 2-$H_{cyclohex}$), 4.87 (bs, 1 H, 1-H_{fuc}).
9. ^{1}H NMR (D_2O): **10**: δ = 1.18-1.48 (m, 6 H, 3 CH_2), 1.62 (m, 2 H, CH_2), 1.72-2.03 (m, 4 H, 2 CH_2), 2.28 (m, 1 H, CHCOOH), 2.83 (m, 2 H, CH_2), 3.50-3.82 (m, 7 H), 3.98 (m, 2 H), 4.47 (m, 1 H, 2-$H_{cyclo-hex}$), 4.94 (d, 1 H, 1-H_{man}).
10. ^{1}H NMR (D_2O): **13**: δ = 1.10-1.50 (m, 6 H, 3 CH_2), 1.63 (m, 2 H, CH_2), 1.71-1.92 (m, 3 H, 1½ CH_2), 2.10 (m, 1 H, ½ CH_2), 2.24 (m, 1 H, CHCOOH), 2.82 (m, 2 H, CH_2), 3.50-3.80 (m, 7 H), 3.96 (m, 2 H), 4.53 (m, 1 H, 2-$H_{cyclohex}$), 4.91 (d, 1 H, 1-H_{man}).
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13. ^{1}H NMR (D_2O): **7B**: δ = 0.95 (d, 3 H, 6-H_{fuc}), 1.46 (m, 2 H, CH_2), 1.74 (m, 4 H, 2 CH_2), 1.90 (m, 1 H, ½ CH_2), 3.69 (q, 1 H, 5-H_{fuc}), 4.41 (m, 1 H, 2-$H_{cyclohex}$), 4.78 (bs, 1 H, 1-H_{fuc}); ESI-MS: m/e = 460.2 $[M-H]^-$.
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