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DESIGN AND SYNTHESIS OF AN α-MANNOSYL TERPENOID AS SELECTIVE INHIBITOR OF P-SELECTIN

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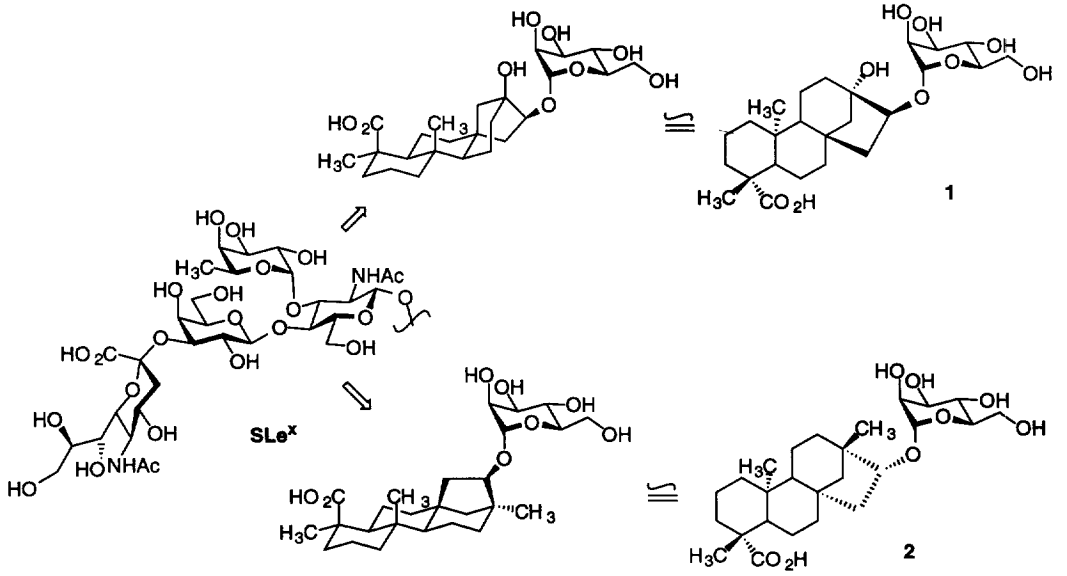
Abstract: In an effort to develop the structural and functional mimics of the tetrasaccharide sialyl Lewis x as selective inhibitors of P-selectin, we have designed a mannosyl terpenoid derivative that selectively inhibits P-selectin with an IC_{50} value of 60 μM, but exhibits no inhibition activity against E- and L-selectins.

Recent study in the selectin-sialyl Lewis x (SLe^x) tetrasaccharide ligand interaction in response to tissue injury and reperfusion has stimulated an intensive search for small molecule inhibitors as structural and functional mimics of the parent carbohydrate ligand.[1] Of the three selectins (i.e., E-, P-, and L-selectins) known to recognize SLe^x as a common ligand, P-selectin represents the most interesting target for inhibition as it is expressed at a very early stage (prior to E-selectin expression) of the inflammatory reaction cascade.[2] Selective inhibition of this selectin is, however, a significant challenge as all three selectins recognize SLe^x as a common ligand, though with small differences in conformation.[3] With the P-selectin-SLe^x binding model available recently,[1c,4] we have begun to search for natural product skeletons that can serve as a template for the synthesis of P-selectin antagonists. We have focused our attention on the space distance and orientation between the negative charge of sialic acid and the three essential hydroxyl groups of the fucose residue. We found that the terpenoid moiety of stevioside and isosteviol (both are available from Wako Pure Chem. Inc., Osaka) can be used as a template if an α-mannosyl residue is placed at the glycosylation site (Scheme 1) of stevioside or at the OH-group of the reduced isosteviols (based on modeling using Macromodel). The conversion of stevioside and isosteviol to the designed α-mannosyl derivatives are shown in Scheme 2. Ozonolysis and reduction followed by acid hydrolysis to remove the sugars and esterification, stevioside was converted to a diol derivative (**5**) that was selectively mannosylated using the Schmidt imidate chemistry[5] to give the protected desired product (**6**), which was then deprotected to give the final

product (**1**). Acid hydrolysis of stevioside gave isosteviol,[6] which after reduction and esterification was mannosylated similarly to give the desired protected (**9**) and unprotected products (**2**).[7] Inhibition analysis of compounds **1** and **2** indicated that **1** is inactive against all three selectins and compound **2** is active against P-selectin only with IC_{50} = 60 μM, corresponding to a 100-fold increase in activity compared to SLe^x. To further understand the origin of selective inhibition, a more detailed computer modeling was performed, and it was found that **2** fits into the SLe^x binding site very well, with the 3-OH group of mannose interacting with the Ca^{2+} ion and the carboxyl groups interacting with the Lys113 side-chain amino group. The binding of **1**, however, possesses some steric problem as the 5-methyl group of **1** is too close to the side-chain of Lys113 (Figure 1), thereby disrupting the interaction between Lys113 and the negative charge.

Scheme 1. Design of mannosylated terpenoids (**1** and **2**) from Stevioside and Isosteviol as structural and functional mimics of Sialyl Lewis X tetrasaccharide for P-Selectin Inhibition



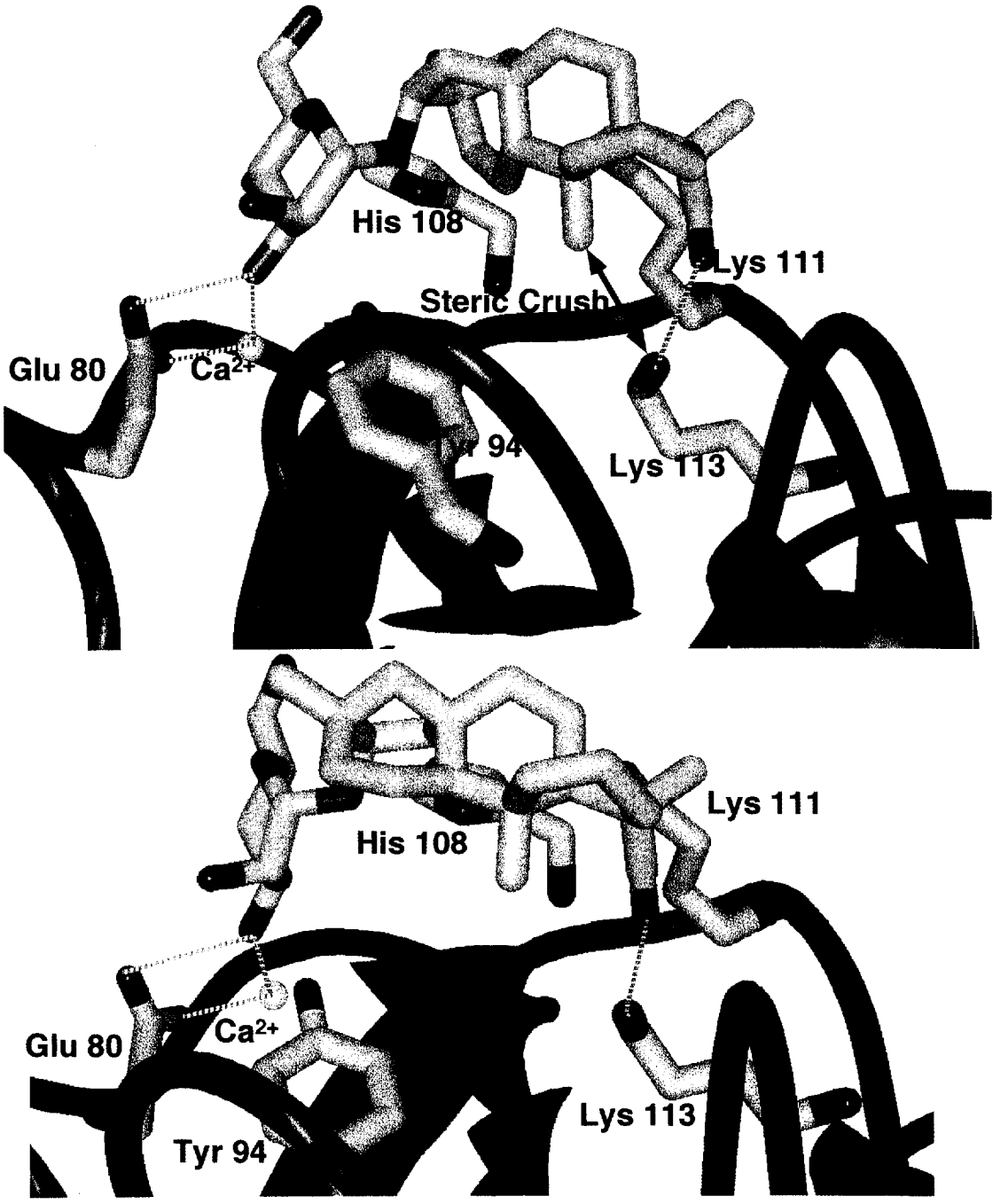
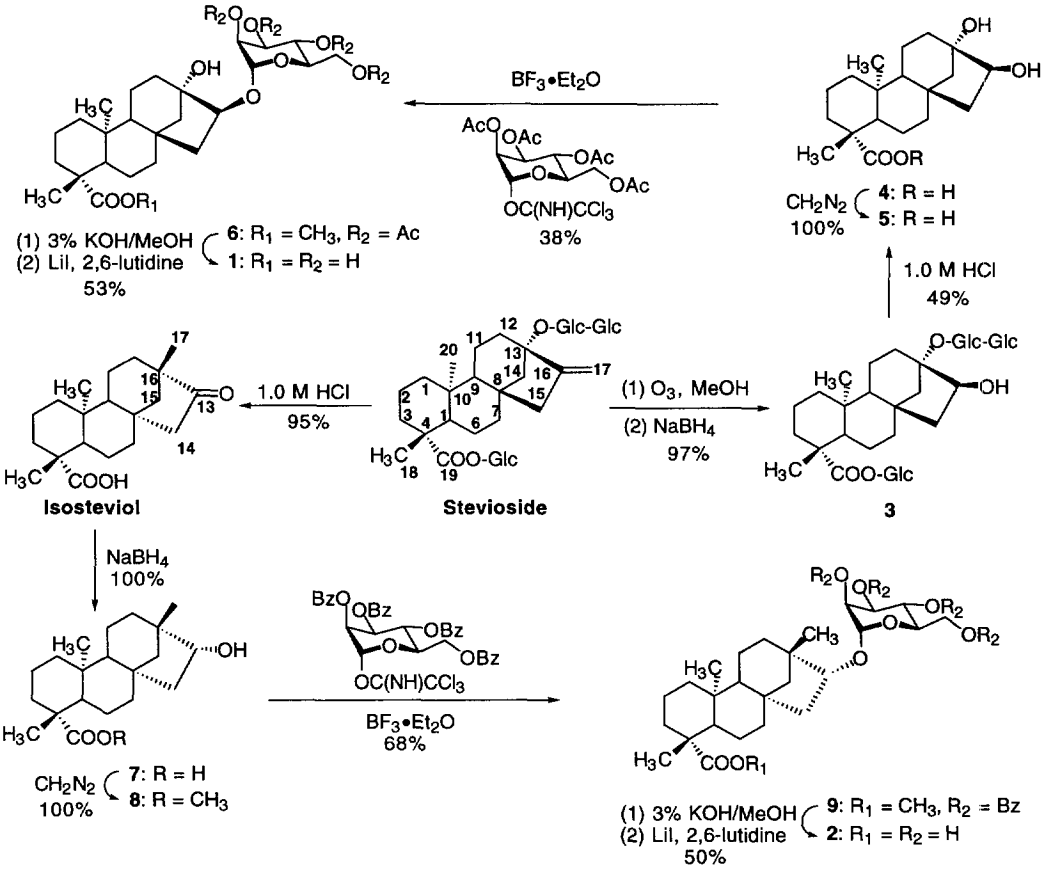


Figure 1. Modeling of P-selectin binding to **1** (top, inactive) and **2** (bottom, IC_{50} = 60 μM). Compound **1** does not fit the SLe^x binding site as the 5-CH_3 group is too close to the Lys-113 side chain. Compound **2** fits well in the active site with the carboxyl group interacting with Lys-113 and mannose-3OH interacting with Glu-80 carboxyl and Ca^{2+}.

Scheme 2. Synthesis of Mimics **1** and **2**



In summary, this study illustrates that selective P-selectin inhibitors can be developed using isosteviol as a template. Though previous studies on the development of selective selectin inhibitors have shown some success, this study represents the first design and synthesis of a highly selective SLe^x mimic inhibitor of P-selectin. Other C-linked fucosyl terpenoids have also been reported[8] to be active against the three selectins but the activities and selectivity are relatively low. This study has also demonstrated that the P-selectin-SLe^x binding model is useful for the design of new SLe^x mimics. Work is in progress to develop more potent P-selectin inhibitors.

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7. Physical data for **6**, **9** and **1**, **2**.

   Compound **6** $[a]_D$ –9.4° (*c* 0.29, $CHCl_3$); ^1H NMR (C_5D_5N) δ 0.83, 1.17 (each s, 3H, H-18, 20), 2.02, 2.06x2, 2.09 (each s, 3H, CH_3COO-), 3.61 (s, 3H, -$COOCH_3$), 4.57 (m, 1H, H-16), 5.32 (br s, 1H, Mannose H-1); ^{13}C NMR (see Table 1); $^3J_{CH}$ (Mannose H-1, C-1) = 174.6 Hz; Positive FABMS *m/z* 667 $[M+H]^+$; HR positive FABMS 667.3330 $[M+H]^+$ ($C_{34}H_{51}O_{13}$, Calcd for M, 667.3329); MALDI TOF-MS *m/z* 689 $[M+Na]^+$.

   Compound **1**: $[a]_D$ +5.8° (*c* 0.26, MeOH); ^{1}HNMR (C_5D_5N) δ 1.18, 1.35 (each s, 3H, H-18, 20), 4.54 (m, 1H, H-16), 5.48 (br s, 1H, Mannose H-1); ^{13}C NMR (Table 1). $^3J_{CH}$ (Mannose, H-1, C-1) = 167.2 Hz; Positive FABMS *m/z* 485 $(M+H)^+$; HR positive FABMS 485.2748 $[M+H]^+$ ($C_{25}H_{41}O_9$, Calcd for M, 485.2750); MALDI TOF-MS *m/z* 507 $[M+Na]^+$.

   Compound **9**: $[a]_D$ –53.7° (*c* 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.82 (s, 3H, H-17), 1.02 (s, 3H, H-20), 1.18 (s, 3H, H-18), 3.57 (s, 3H, -$COOCH_3$), 3.89 (dd, 1H, *J* = 4.0, 10.9 Hz, H-13), 4.48–4.55 (m, 2H, Mannose H-5, 6), 4.62–4.66 (m, 1H, Mannose H-6), 5.14 (d, 1H, *J* = 1.7 Hz, Mannose H-1), 5.70 (m, 1H, Mannose H-2), 5.89 (dd, 1H, *J* = 3.3, 10.0 Hz, Mannose H-3), 6.11 (t, 1H, *J* = 10.0 Hz, Mannose H-4), 7.24–8.14 (20H, Aromatic); ^{13}C NMR (Table 1). $^3J_{CH}$ (Mannose H-1, C-1) = 172.1 Hz; Positive FABMS *m/z* 913 $[M+H]^+$; HR positive FABMS 913.4160 $[M+H]^+$ ($C_{55}H_{61}O_{12}$, Calcd for M, 913.4163), MALDI TOF-MS *m/z* 935 $[M+Na]^+$.

   Compound **2**: $[a]_D$ –9.9° (*c* 0.30, MeOH); ^1H NMR (C_5D_5N) δ 0.93 (s, 3H, H-17), 1.11 (s, 3H, H-20), 1.36 (s, 3H, H-18), 3.87 (dd, 1H, *J* = 4.2, 10.8 Hz, H-13), 4.72 (t, 1H, *J* = 9.2 Hz, Mannose H-4), 5.36 (br s, 1H, Mannose H-1); ^{13}C NMR (Table 1). $^3J_{CH}$ (Mannose H-1, C-1) = 167.3 Hz; Positive FABMS *m/z* 483 $[M+H]^+$; HR positive FABMS 483.2957 $[M+H]^+$ ($C_{26}H_{43}O_8$, Calcd for M, 483.2958).

Table 1: ^{13}C-NMR of Compounds **1-9**, stevioside (**A**) and isosteviol (**B**)

	A[a]	**3**[a]	**4**[a]	**5**[b]	**6**[b]	**1**[a]	**B**[a]	**7**[b]	**8**[b]	**9**[a]	**2**[b]
C-1	40.8	40.8	41.1	40.6	40.7	41.1	41.4	42.3	41.7	41.6	41.7
2	19.4	19.4	19.9	19.0	19.5	19.9	18.8	19.6	18.9	18.9	19.6
3	38.4	38.5	38.8	38.0	38.2	38.7	37.6	38.6	38.0	38.1	38.7
4	44.0	44.1	43.9	44.0	43.9	43.9	43.6	43.8	43.8	43.8	43.9
5	57.4	57.4	57.1	56.8	56.8	57.1	57.0	57.2	57.1	57.1	57.3
6	22.2	22.0	22.5	21.6	22.0	22.4	21.6	22.5	21.1	21.6	22.5
7	41.7	42.4	42.7	41.8	42.1	42.5	39.7	40.3	39.9	39.9	40.3
8	42.6	43.4	43.2	42.9	43.3	43.1	39.4	42.4	42.0	42.3	42.5
9	54.0	55.5	55.9	55.0	55.0	55.1	56.8	56.3	55.8	55.8	56.2
10	39.9	39.9	39.9	39.2	39.5	39.9	38.1	38.5	38.0	38.1	38.5
11	20.6	20.0	20.1	19.4	20.0	20.1	20.3	20.8	20.4	20.5	20.8
12	36.9	30.4	34.4	33.0	34.4	34.5	37.3	34.6	33.7	34.4	34.9
13	86.5	87.2	80.1	80.5	79.1	79.4	222.7	79.6	80.6	88.1	87.2
14	44.5	42.7	45.7	43.8	45.1	46.4	48.7	43.8	42.8	41.1	42.1
15	44.7	49.7	50.1	47.8	45.6	45.4	54.2	55.8	55.2	54.9	55.2
16	154.3	75.5	77.1	76.6	81.1	81.2	48.4	42.5	42.1	42.4	42.5
17	104.6						19.8	25.6	24.9	25.5	25.8
18	28.3	28.4	29.4	28.7	28.6	29.3	28.9	29.4	28.9	28.9	29.5
19	177.1	177.1	180.3	178.1	177.7	180.2	183.9	180.2	178.1	178.1	180.1
20	15.5	15.6	16.0	15.3	15.4	15.9	13.3	13.8	13.1	13.2	13.8
-$CO_2\underline{C}H_3$				51.2	51.1				51.2	51.2	
Man-1					96.4	100.5			96.4	98.9	103.4
2					70.7	72.8			70.7	70.7	72.5
3					70.2	73.3			70.2	70.2	73.2
4					66.6	69.4			66.6	67.0	69.1
5					69.1	75.1			69.1	69.3	75.7
6					62.7	63.2				63.0	63.1

[a] in C_5D_5N; [b] in $CDCl_3$

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