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Sialyl Lewis^x analogs based on a quinic acid scaffold as the fucose mimic

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Abstract—(−)-Quinic acid was used as a starting material for the preparation of sialyl Lewis^x mimetics in order to target E-selectin. Spatial orientation of the hydroxyl groups of quinic acid could mimic the L-fucose ones. Introduction of a side chain ending with a carboxylic acid was effected to replace the sialic acid interaction at the carbohydrate recognition domain. A first series of derivatives, incorporating amino acids linked to quinic acid, were tested for their affinity and found to interact with E-selectin with IC_{50} within the millimolar range.

1. Introduction

Carbohydrate binding proteins (lectins) are molecules involved in many important biological processes, ranging from leukocytes' recruitment at inflammatory sites to mechanisms in cancer development, as well as glycoprotein levels, control in the cell and the circulation, and other cellular interactions related to immune response.[1]

Within this class of cellular receptors, selectins (E, P, and L) are calcium-dependent lectins (C-type) involved in the events related to the action of bloodstream cells. E- and P-selectins are usually only transiently expressed on the vascular endothelium after the prime inflammatory response. They are responsible for the interaction between endothelial cells and leukocytes leading to their rolling, as the first step needed for extravasation. These molecules are also overexpressed, especially E- and P-selectins, during some pathological processes such as chronic inflammatory diseases, neoangiogenesis, and may be a way cancer cells can metastasize by adhesion to the endothelium.[2]

For E-selectin, the natural ligand by which such interactions are possible is the sialyl Lewis^x epitope (sLe^x), a terminal tetrasaccharidic appendage of a leukocytes' glycopeptide (Fig. 1). The exact conformation of sLe^x and its interaction with E-selectin have been revealed by X-ray analysis of crystals of the soluble tetrasaccharide bound to the protein.[3]

Due to the importance of sLe^x and E/P-selectins' interaction, and the possible applications to inhibit or use this adhesion for targeting, several articles related to the synthesis and use of sLe^x and its analogs have been published in the past few years.[4]

Most of the analogs synthesized so far were of saccharidic nature, with some modifications. More recent

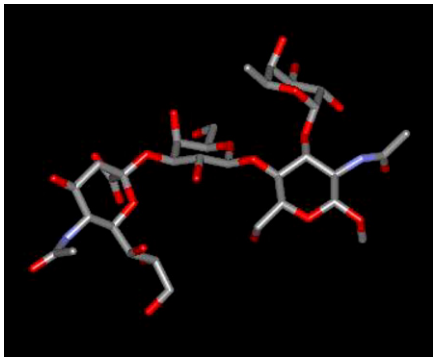

Figure 1. Sialyl Lewis^x tetrasaccharide structure at E-selectin CRD.

Keywords: E-selectin; P-selectin; Sialyl Lewis^x; Mimics; Quinic acid.
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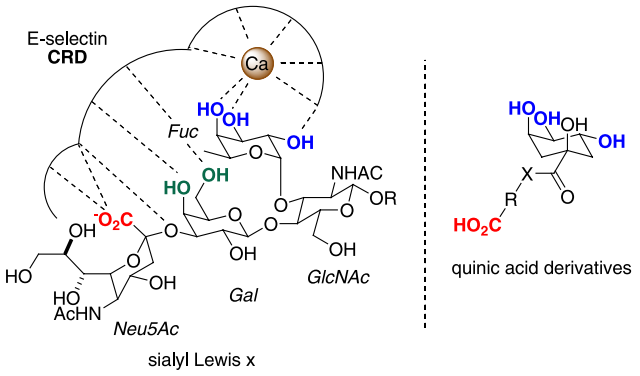

Figure 2. Minimum pharmacophores (colored groups) and structural analogies between sialyl Lewis^x and quinic acid derivatives.

examples have shown that simpler structures can be as efficient as the previously reported mimics, with a gained ease for their preparation.[5] A number of these analogs have shown an equal or much higher activity than sialyl Lewis^x, proving their value as inhibitors or targeting ligands. However, there is still some room for new sLe^x mimic, especially structures that can be easily generated, with the possibility to access larger molecular diversity.

Our approach is based on structural analogies with sLe^x active conformation and the minimum pharmacophores needed to select the basic mimic structure (Fig. 2). Within the interactions implicated, it seems that the most important features for the recognition are the hydroxyls borne by L-fucose to chelate the calcium ion, as well as the carboxyl group of sialic acid. Hydroxyls on galactose also seem to play a role, maybe at a lesser extent, while *N*-acetylglucosamine merely acts as a scaffold.[6]

Among the chiral natural products available, a carbocyclic molecule, D-(−)-quinic acid or (*1R*,*3R*,*4S*,*5R*)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid, has the required axial–equatorial–equatorial 1,2,3-triol pattern that could mimic the fucose part.[7] Furthermore, the carboxylic acid onto the cyclohexane ring can serve as an anchoring point to introduce various CO_2H-ending side chains, which have been chosen to probe carboxyl interactions with protein residues within or near the CRD.[8]

2. Synthesis of quinic acid derivatives

A first series of derivatives was prepared by coupling the quinic acid counterpart with amino acids. We selected the peracetylated quinic acid **1** as the starting material in order to graft amino acids (Fig. 3). The amino acid derivatives were selected to provide a range of substitutions between quinic acid and the terminal carboxyl. Esters of glycine (**2a**), aminobutyric (**3a**), and hexanoic (**4a**) acids were used to increase the length between both parts of the mimics. Benzyl glutamate (**5a**) was selected to place a supplementary carboxyl group in the spacer region. Alanine (**6a**) and isoleucine (**7a**) provided alkyl groups, while threonine (**8a**) and serine (**9a**) esters furnished the hydroxylated ones.

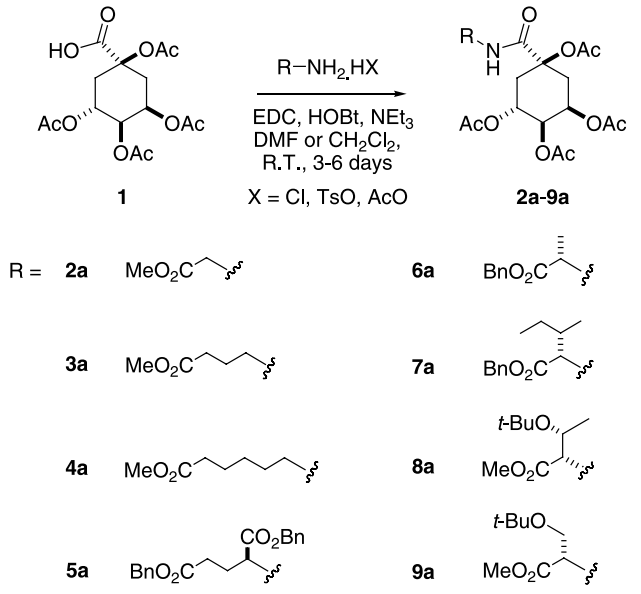

Figure 3. Coupling between quinic acid acetate **1** and some amino acids by HOBt ester activation (Method A) to obtain structures **2a**–**7a**.

The use of standard peptide coupling procedures (NHS or HOBt, EDC, and Et_3N) gave the desired compounds. However, the reactions were found to be quite slow (3–6 days) and needed supplementary purification steps to remove the by-products. The yields using this method (Method A, Table 1) ranged from low to good (39–76%).

To improve both yields and the ease of isolation, we decided to use a mild method that we had previously published for the preparation of esters and amides, via the formation of acyl chloride in the presence of a scavenging resin (Fig. 4).[9] Follow-up of the reaction (NMR) of the peracetate **1** has shown that formation of the acyl chloride was complete in 4 h.[10] Two coupling conditions were then used and compared to prepare the quinyl-aminoester derivatives (**2a**–**9a**).

In Method B (Table 1), the acyl chloride was reacted onto amino ester salts in the presence of Et_3N as a base. The reactions were conducted overnight (14 h) in order to insure complete coupling. An acido-basic aqueous

Table 1. Formation of quinyl-amino ester derivatives (**2a**–**9a**)

Compound	Method A[a] yield (%)	Method B[b] yield (%)	Method C[c] yield (%)
2a	76	65	80
3a	39	80	84
4a	76	72	86
5a	64	87	91
6a	77	70	91
7a	46	55	88
8a	—	60	96
9a	75	55	86

[a] EDC/HOBt coupling/aqueous work-up/purification.
[b] Acyl chloride/EtN_3 as a base/aqueous work-up.
[c] Acyl chloride/Amberlyst A-21 as a base/Amberlyst A-15 as scavenger/evaporation.

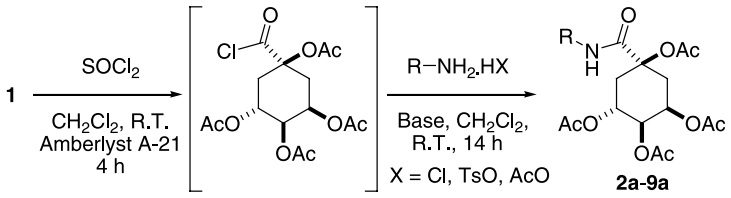

Figure 4. The reaction between quinic acid peracetate **1** and amino acids, via the acyl chloride formation (Methods B and C), to prepare compounds **2a**–**9a**.

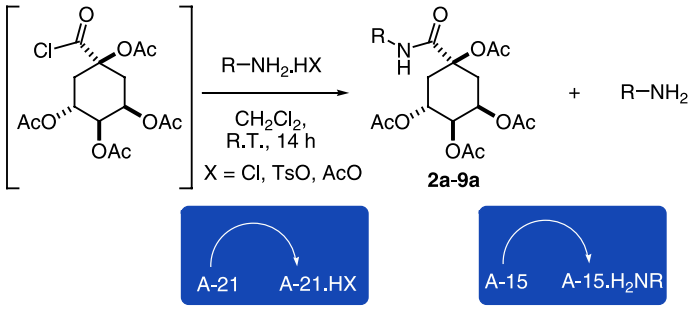

Figure 5. Synthesis of **2b**–**9b** using scavenging resins.

work-up gave access to compounds **2a**–**9a** in fair to good yield (55–80%).

The synthesis of these derivatives was made even simpler by using the last tested conditions (Method C, Table 1) based on the scavenging resins' use. The overnight reaction between the acyl chloride of peracetate **1** and the amino ester salts was cleanly effected using Amberlyst A-21 as the base, which serves to deprotonate the amino ester salt and trap the produced hydrogen chloride. The reaction mixture was filtered and contained only the desired products and the excess amino ester. Amberlyst A-15 was then added to remove the unreacted amino ester without the need for any other workup procedures (Fig. 5).

The solution was finally refiltered and evaporated to give access to products **2a**–**9a** in good yield (80–96%), but, most of all, in their pure form and without the need for any further purification.

Deprotection of the synthesized products was done by saponification, followed by aqueous TFA treatment for the *tert*-butyl ether derivatives (Fig. 6). Fully deprotected mimics **2b**–**9b** were obtained in yields ranging from 90% to quantitative.

The resulting compounds were lyophilized and all obtained as white water soluble powders.

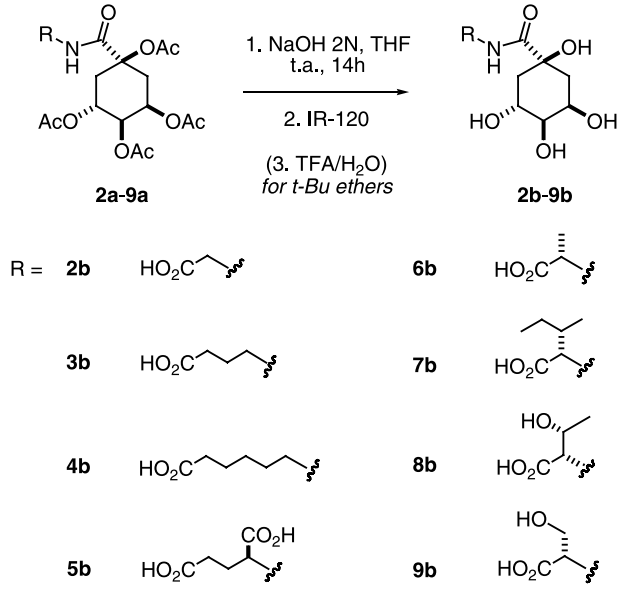

Figure 6. Deprotection of compounds **2a**–**9a** to obtain the mimics **2b**–**9b**.

3. Bioassay of quinic acid based mimics

The compounds were then tested in a competition assay for human recombinant E- and P-selectins coated to wells and using a cell-based assay, as previously described.[11] HL-60 cells expressing the selectin ligand (sialyl Lewis^x) were added together with the synthetic analog. The binding, which is relevant to the natural interaction between the selectin and the cells, leads to adhesion of HL-60 to the coated protein. The ability of the mimic to displace the interaction of the selectin (E or P) and HL-60 was monitored, and % of inhibition and IC_{50} were evaluated (Table 2).

For the control experiments, lacto-sialyl Lewis^x showed IC_{50} values within the awaited range: of 0.7 (E) and 12 mM (P). Quinic acid itself does not have any affinity towards E- and P-selectins up to 100 mM.

Regarding E-selectin, all the analogs have shown moderate affinity. Inhibitions ranged from 30 to 46% at

Table 2. Inhibition (at 50 mM) and IC_{50} for E- and P-selectins against HL-60 cells for lacto-sialyl Lewis^x, quinic acid and compounds **2b**–**9b**

Compound	E-selectin inhibition at 50 mM, %[a]	E-selectin inhibition IC_{50}, mM[a]	P-selectin inhibition at 50 mM, %[a]	P-selectin inhibition IC_{50}, mM[a]
Lacto-sLe^x	n.m.	0.7 (±0.1)	n.m.	12 (±2)
Quinic acid	n.a.	n.a.	n.a.	n.a.
2b	38 (±2)	>50	38 (±2)	>50
3b	40 (±8)	>50	83 (±2)	40 (±2)
4b	31 (±2)	>50	14 (±1)	>50
5b	43 (±2)	>50	86 (±2)	21 (±1)
6b	30 (±2)	>50	8 (±1)	>50
7b	44 (±2)	>50	92 (±2)	19 (±1)
8b	n.m.	n.m.	n.m.	n.m.
9b	46 (±1)	>50	88 (±2)	19.5 (±0.5)

[a] All essays were performed in triplicate; standard deviation is given in parentheses; n.m. = not measured; n.a. = not active up to 100 mM. Lacto-sLe^x = [α-Neu5Ac-(2,3)-β-D-Gal-(1,4)(α-Fuc-(1,3))-D-Glc].

50 mM ($IC_{50} \geqslant 50$ mM). However, the quinic acid derivatives seemed to be able to prevent the fixation of sLe^x to the selectin, whereas quinic acid alone did not, showing both the validity of the selected structures and the importance of the side chain on these compounds.

The effect is even more important while testing the competition with P-selectin. It seems that the analogs had a better affinity for this selectin with inhibition at 50 mM starting at 14% and going up to 88%. Chain length influence can be evaluated while looking at **2b**–**4b**. Only the compound with a 4-carbon chain (**3b**), derived from GABA, possesses an IC_{50} at 40 mM. The derivatives with 2- (**2b**) and 6-carbon (**4b**) chains, however, have IC_{50} much higher than 50 mM. Introduction of a second carboxylic acid function, while examining **3b** and **5b**, shows that the affinity is even better, IC_{50} falling from 40 (**3b**) to 21 mM (**5b**). Interestingly, analogs incorporating isoleucine (**7b**) and serine (**9b**) had affinities in the same range (19 mM). These results show that the basis for the preparation of analogs is once again valid. The higher affinities for P-selectin can be due to the slight modification of the geometry of the P-selectin CRD, which is anyhow highly analogous to the E-selectin one.[6]

In view of the interesting results gathered here, the search for more active analogs based on a quinic acid scaffold has to continue in order to find better candidates with an activity at least equivalent to the one of the natural ligand: sialyl Lewis^x.

4. Conclusion

We presented in this letter the preparation of analogs based on quinic acid to mimic the sialyl Lewis^x epitope. The synthesis of these derivatives was made simple and efficient by the use of scavenging resin approaches, giving access to products without the need for cumbersome purification techniques. The analogs were tested for their affinity toward markers expressed during inflammation and other diseases, such as vascular endothelium E- and P-selectins. It was found that the compounds possessed moderate affinity for both selectins. However, they were more efficient in inhibiting the fixation between P-selectin and HL-60 cells expressing the natural ligand sialyl Lewis^x. Further results and studies in this area will be reported in due course.

5. Typical experimental procedures

5.1. *N*-[(1*R*,3*R*,4*S*,5*R*)-1,3,4,5-tetraacetoxycyclohexane-1-carboxyl]glycine methyl ester (2a)

To a solution of 360 mg (1 mmol) of (1*R*,3*R*,4*S*,5*R*)-1,3,4,5-tetraacetoxycyclohexane-1-carboxylic acid (**1**)[12] in dry CH_2Cl_2 (10 mL) containing dry Amberlyst A-21 (500 mg, 2.4 mmol) was added under a nitrogen atmosphere $SOCl_2$ (145 μL, 2 mmol). The mixture was gently stirred for 4 h at room temperature. The supernatant was separated from A-21 using syringe transfer to another flask and the volatiles were removed in vacuo. The resulting acyl chloride (off-white solid) was redissolved in dry CH_2Cl_2 (10 mL) and added dropwise to a suspension of glycine methyl ester hydrochloride (151 mg, 1.2 mmol) and A-21 (625 mg, 3 mmol) in dry CH_2Cl_2 (30 mL). The mixture was stirred overnight (14 h) and filtered. Amberlyst A-15 (300 mg, 1.2 mmol) was added to the solution, which was stirred for 30 min before being filtrated and evaporated under vacuum. The product **2a** was isolated as a white solid (344 mg, 80%).

Mp = 171 °C. $[\alpha]_D = -30.5$ (*c* 1, $CHCl_3$). FT-IR (KBr): $\nu = 3370$ (NH), 2986, 2955, 2837 (CH), 1742 (C=O ester) and 1675 (NHC=O) cm^{-1}. RMN-^{1}H (300 MHz, $CDCl_3$): $\delta = 1.88$ (m, 1H), 1.97 (s, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 2.16 (s, 3H), 2.43 (dd, 1H, $J = 3.3$, 16.2 Hz), 2.53 (m, 1H), 2.84 (m, 1H), 3.73 (s, 3H), 3.98 (ddd, 1H, $J = 5.3$, 9.9, 18.3 Hz), 4.97 (dd, 1H, $J = 3.6$, 10.2 Hz), 5.41 (m, 1H), 5.57 (dd, 1H, $J = 3.3$, 6.9 Hz), and 6.47 (t, 1H, NH, $J = 5.1$ Hz) ppm. RMN-^{13}C (75 MHz, $CDCl_3$): $\delta = 20.6$, 20.9, 21.6, 30.9, 38.1, 41.3, 52.4, 66.6, 68.0, 71.9, 81.2, 169.5, 169.6, 169.9, 170.0, and 170.4 ppm. Elemental analysis: Calcd for $C_{18}H_{25}NO_{11}$: C, 50.12; H, 5.84; N, 3.25%. Found: C, 50.29; H, 5.37; N, 3.16%.

5.2. *N*-[(1*R*,3*R*,4*S*,5*R*)-1,3,4,5-tetrahydroxycyclohexane-1-carboxyl]glycine (2b)

The protected mimic **2a** (84 mg) was dissolved in THF (2 mL), cooled in an ice bath, and NaOH 2 N (2 mL)

was added. The solution was stirred for 14 h at room temperature. The mixture was treated with prewashed (MeOH and then THF) Amberlite IR-120(H+) until pH 3, filtered, and evaporated under reduced pressure. The residue is dissolved in UHQ water (5 mL), filtered on a syringe filter (0.45 μ), and lyophilized. The product **2b** was isolated as a white fluffy hygroscopic solid (44 mg, 90%).

$[\alpha]_D = -37.9$ (*c* 1, H_2O). FT-IR (KBr): $\nu = 3375$ (NH), 3164 (OH/COOH), 2950, 2843 (CH), 1731 (C=O acid) and 1651 (NHC=O) cm^{-1}. RMN-^{1}H (300 MHz, CD_3OD): $\delta = 1.97$ (m, 4H), 3.40 (dd, 1H, $J = 3.0$, 9.0 Hz), 3.92 (s, 2H), 4.01 (ddd, 1H, $J = 4.8$, 9.2, 10.8 Hz), and 4.14 (*q*, 1H, $J = 3.0$ Hz) ppm. RMN-^{13}C (75 MHz, $CDCl_3$): $\delta = 37.5$, 41.0, 41.8, 66.9, 70.9, 75.8, 76.6, 173.2, and 176.0 ppm.

5.3. E-Selectin binding inhibition measurements with HL-60 cells

Inhibition tests were conducted according to a published procedure[11] and at room temperature unless otherwise stated. Human recombinant E- or P-selectin (R&D Systems Europe, Lille) was coated onto 96-well plates (Immulon 2, Dynatech) using 50 μL of a 3 μg mL^{-1} solution (150 ng per well) of the selectin in DPBS buffer (Dulbecco's phosphate-buffered saline) for 3 h. The wells were washed three times with 200 μL DPBS buffer containing 1% of bovine serum albumin (DPBS/1% BSA), and then treated with 200 μL of the same solution for 1 h to block the uncoated surfaces.

The blocking solution was removed and 40 μL of inhibitor solutions, prepared from a 10 mM DBPS stock solution diluted in Hanks' solution containing 20 mM Hepes (pH 7.2–7.4), 0.2% glucose, and 1% BSA, was added, followed immediately by 20 μL of a 10^5 HL-60 cell suspension. After 15 min incubation time, the solution was removed and the wells were washed three times by 200 μL Hanks' solution 20 mM Hepes (pH 7.2–7.4), 0.2% glucose, 1% BSA, and 1 mM $CaCl_2$.

Lysis buffer was then added (50 μL citric acid 24 mM, dibasic sodium phosphate 51 mM, and 0.1% Nonidet P-40) and the plates were shaken for 5 min. Myeloperoxidase liberated during the lysis process was then detected by addition of *o*-phenylenediamine as a substrate (50 μL citric acid 24 mM, dibasic sodium phosphate 51 mM, 0.1% *o*-phenylenediamine, and 0.03% hydrogen peroxide). After 1 h, the reaction was stopped by addition of 40 μL of 4 N sulfuric acid, and the absorbance of the solution was measured at 492 nm. The inhibition percentage was calculated by comparison to the absorbance of a positive control in which only HL-60 was added (no inhibitor). Results are summarized in Table 1.

Sialyl Lewis^x [α-Neu5Ac-(2,3)-β-D-Gal-(1,4) (α-Fuc-(1,3))-D-GlcNAc] and "lacto-sLe^x" [α-Neu5Ac-(2,3)-β-D-Gal-(1,4)(α-Fuc-(1,3))-D-Glc] tetrasaccharides used as controls were purchased from Sigma.

Acknowledgments

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6. Sialyl Lewis^x binds to both E- and P-selectins. There is a strong homology between both selectin CRDs. There is only a slight modification in the conformation due to minor modifications of amino acids next to the CRDs (see Ref. 3b). This results, however, in a change in the spatial disposition of some amino acids (Tyr^{48} and Tyr^{94}, as an example) implicated in interactions with the Neu5Ac residue of sLe^x.
7. (a) Quinic acid has been used to mimic mannose, see: Angyalosi, G.; Grandjean, C.; Lamirand, M.; Auriault, C.; Gras-Masse, H.; Melnyk, O. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2723, and references cited therein. Quinic-style derivatives have also been tested for their affinity toward C-type lectins; (b) Schuster, M. C.; Mann, D. A.; Buchholz, T. J.; Johnson, K. M.; Thomas, W. D.; Kiessling, L. L. *Org. Lett.* **2003**, *5*, 1407; (c) Yuri, M.; Miyauchi, H. Jpn Kokai Tokkyo Koho JP 11246503 A2, 1999.
8. Some analogs can have an equivalent activity to the one of sLe^x without the COOH binding to the exact residue implicated in binding Neu5Ac. As an example, Gravel, Guindon et al. analogs of carboxylate bind to Tyr^{94} (near CH_2OH of Gal), instead of Arg^{97} (binding Neu5Ac-COOH).[5a] Furthermore, both enantiomers of their analog were found to equally bind to E-selectin, suggesting that other interactions may be involved.
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