Ligand Recognition by E-Selectin: Analysis of Conformation and Activity of Synthetic Monomeric and Bivalent Sialyl Lewis X Analogs[1]

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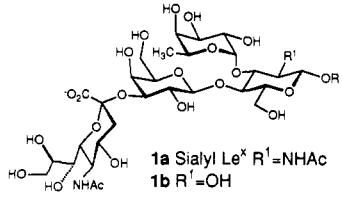
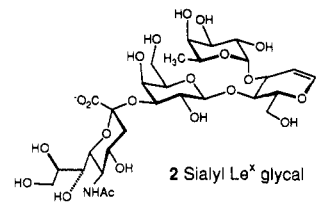
Carbohydrate-mediated cell adhesion is an important event initiated by tissue injury and infection and is involved in metastasis.[2] One of such adhesion processes discovered recently is the interaction between the glycoprotein E-selectin (formerly called endothelial leukocyte adhesion molecule-1 or ELAM-1,[2e] which is expressed on the surface of endothelial cells during inflammation) and a glycotope structure displayed on the surface of neutrophils. The ligand recognized by E-selectin has been identified to be the tetrasaccharide sialyl Lewis x (SLe^x, **1a**).[3]



Sialyl Lewis x[3a] and analogs, including the GlcNAc→Glc analog **1b**,[4a] the regioisomer sialyl Lewis a (SLe^a),[4b] and Le^x 3′-*O*-sulfate,[4c] have been shown to have similar inhibition activities for E-selectin and are thus considered to be potentially useful as new antiinflammatory antitumor agents. The large-scale synthesis of SLe^x based on glycosyltransferases[5] and its three-dimensional structure[5,6] are now available.

Table I. ^{1}H and ^{13}C Chemical Shift Assignments (ppm) of **2** and **3**

	Neu5Ac		Gal		Fuc		GlcNAc or Glucal	
carbon	H	C	H	C	H	C	H	C
				Compound **2**				
C1		173.9	4.63	101.8	5.05	96.0	6.49	144.3
C2		99.7	3.52	69.4	3.78	67.7	4.99	98.8
C3	1.72, 2.76	39.6	4.10	75.6	3.86	69.4	4.32	69.8
C4	3.68	68.3	3.95	67.3	3.80	71.8	4.16	72.3
C5	3.84	51.6	3.62	74.9	4.48	66.7	4.16	77.4
C6	3.62	72.8	3.70	61.1	1.20	15.2	3.86, 3.97	59.0
C7	3.58	68.0						
C8	3.89	71.7						
C9	3.60, 3.89	62.2						
CH_3	2.04	22.0						
C=O		175.0						
				Compound **3**				
C1		173.5	4.54	101.2	5.11	98.2	4.56	100.6
		173.5	4.55	101.2	5.12	98.2	4.72	100.8
C2		99.3	3.54	68.9	3.69	67.3	3.96	55.6
		99.3	3.54	68.9	3.69	67.3	3.96	55.6
C3	1.81, 2.77	39.4	4.10	75.3	3.90	68.8	3.83	74.6
	1.81, 2.77	39.4	4.10	75.3	3.90	68.8	3.83	74.6
C4	3.69	68.0	3.93	66.9	3.78	71.5	3.93	73.0
	3.69	68.0	3.93	66.9	3.78	71.5	3.93	73.0
C5	3.86	51.3	3.58	74.5	4.83	66.3	3.58	74.9
	3.86	51.3	3.58	74.5	4.83	66.3	3.58	74.9
C6	3.65	72.6	3.69	61.1	1.18	14.9	3.88, 4.03	59.3
	3.65	72.6	3.69	61.1	1.18	14.9	3.88, 4.03	59.3
C7	3.59	67.7						
	3.59	67.7						
C8	3.90	71.4						
	3.90	71.4						
C9	3.64, 3.87	62.2						
	3.64, 3.87	62.2						
CH_3	2.03	21.7					2.01	21.9
	2.03	21.7					2.01	21.9
C=O		174.7						173.8
		174.7						173.8



To further understand the nature of ligand recognition, we first examined the inhibition activity[7] of a Sle^x analog with glucal in the reducing end (**2**)[5a,5g] and a bivalent SLe^x (**3**) anchored on

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(7) The ELISA assays were carried out according to the procedure described reviously.[3a] Human soluble recombinant E-selection was coated on plates, followed by addition of HL-60 cells and carbohydrates. After incubation, the plates were rinsed and the adhesion determined by the cell lysis and myeloperoxidase method. IC_{50} was the concentration that inhibited cell adhesion by 50%. This method gave consistent results with 10% deviation.

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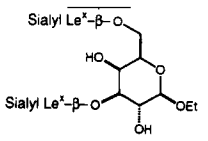
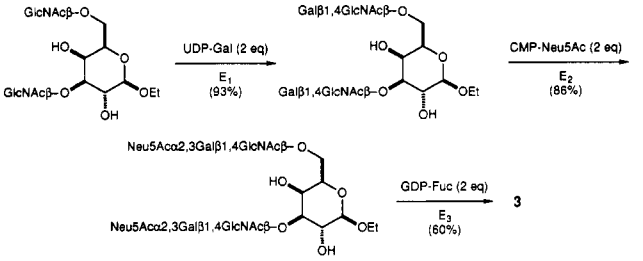
a galactose residue via β-1,3- and β-1,6-linkages.[8] Interestingly,



3 Bivalent Sialyl Le^x

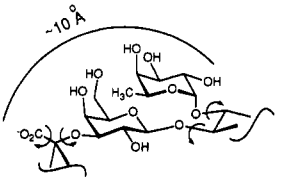
compound **2** was as active as **1a** (IC_{50} = 2.1 mM), and **3** (IC_{50} = 0.4 mM) was about 5-fold better than **1a** and 4-fold better than the pentasaccharide (IC_{50} = 1.5 mM) with Galβ*OEt* added to the reducing end of SLe^x via a β-1,3-linkage suggesting the possibility of a multivalent ligand-receptor interaction.[9] Conformational analysis of **2** with NMR[10] indicates that it is identical to **1a** and SLe^a in the space composed of Neu5Ac, Gal, and Fuc.[5a] The observations that **1b**,[4a] **2**, and SLe^a (see ref 4b for activity, and ref 5a for conformational analysis) are essential as active as **1a** suggest that the E-selectin binding domain of sialyl Le^x comes mainly from the unique space structure composed of the Neu5Ac, Gal, and Fuc residues. The *exo*-anomeric effects[11] of Gal and Fuc fix the glycosidic torsion angles and thereby the topographic structure of these two residues when attached to an ethyleneglycol unit via β- and α-glycosidic linkages, respectively. The similar inhibitory activity of Le^x 3′-*O*-sulfate to that of **1a** further suggests that the carboxylate group may be the only essential group contributed from the Neu5Ac residue.[4c] The enhanced inhibtion activity of the bivalent ligand **3** is consistent with the observation that the conformations of the two SLe^x units are essentially the same as those of **1a** and **2** (based on 1D- and 2D-NMR analyses, see Table I for assignment). It is also worth noting that the synthesis of **3** is very efficient and straightforward. Starting with a chemically synthesized trisaccharide, the sequential enzymatic addition of the other sugars (two each time) gave the nonsaccharide in 54% overall yield (Scheme I).[8]

Scheme I. Enzymatic Synthesis of Bivalent Sialyl Le^x **3**[a]



[a] E_1: β-1,4-galactosyltransferase. E_2: α-2,3-sialyltransferase. E_3: α-1,3-fucosyltransferase.

In summary, this study supports the concept that the interaction between E-selectin and SLe^x is multivalent and the active binding domain of SLe^x comes from the topostructure composed of the carboxylate, Gal, and Fuc residues as indicated in **4**.[12] While we



4 Active binding site

do not know the structure of E-selectin and the role of Ca^{2+} in ligand binding, further experiments with defined multivalent ligand analogs or mimetics with appropriate spacers should clarify the nature of E-selectin-mediated cell adhesion and may suggest new routes applicable to the discovery of antiadhesion molecules.

Supplementary Material Available: 1D spectra (^1H and ^{13}C NMR) of **2** and **3**; ROESY of **2** and **3**; procedures for the synthesis of **3** and physical data (21 pages). Ordering information is given on any current masthead page.

(8) The chemically synthesized trisaccharide was enzymatically glycosylated[5a] with 2 equiv each of the correspondinmg sugar nucleotides. The detailed procedures are described in the supplementary material.

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(10) Both 1D and 2D techniques were employed. The detailed experimental procedures were described previously.[5a]

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(12) In a separate study, we found that the $-CH_3$ group of Fuc is not essential for the activity, as Fuc can be replaced with arabinose. The three hydroxyl groups of Fuc, however, are required. Ramphal, J R.; Zheng, Z.; DeFrees, S.; Walker, L.; Gaeta, F. C. A., unpublished.