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

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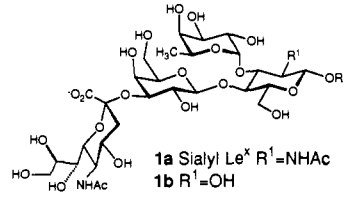
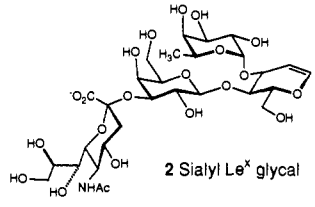
Carbohydrate-mediated cell adhesion is an important event initiated by tissue injury and infection and is involved in metastasis.[2] One of such adhesion processes discovered recently is the interaction between the glycoprotein E-selectin (formerly called endothelial leukocyte adhesion molecule-1 or 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

(1) Part of this work was supported by the NIH (GM44154). We thank Dr. J. C. Paulson for his kind help and advice on the subject and Dr. Les Walker and Diane LaPonte for the inhibition analysis.

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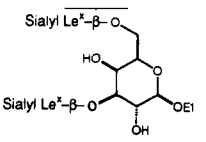
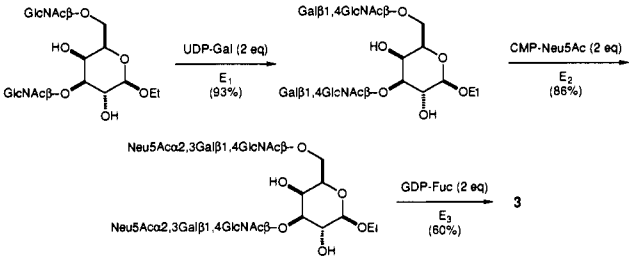
a galactose residue via β-1,3- and β-1,6-linkages.[8] Interestingly,



3 Bivalent Sialyl Le^x

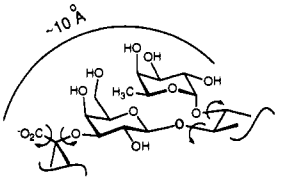
compound **2** was as active as **1a** (IC_{50} = 2.1 mM), and **3** (IC_{50} = 0.4 mM) was about 5-fold better than **1a** and 4-fold better than the pentasaccharide (IC_{50} = 1.5 mM) with Galβ*OEt added to the reducing end of SLe^x via a β-1,3-linkage suggesting the possibility of a multivalent ligand-receptor 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.