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DESIGN AND SYNTHESIS OF SIALYL Le^x MIMETICS BASED ON CARBOCYCLIC SCAFFOLDS DERIVED FROM (–) QUINIC ACID

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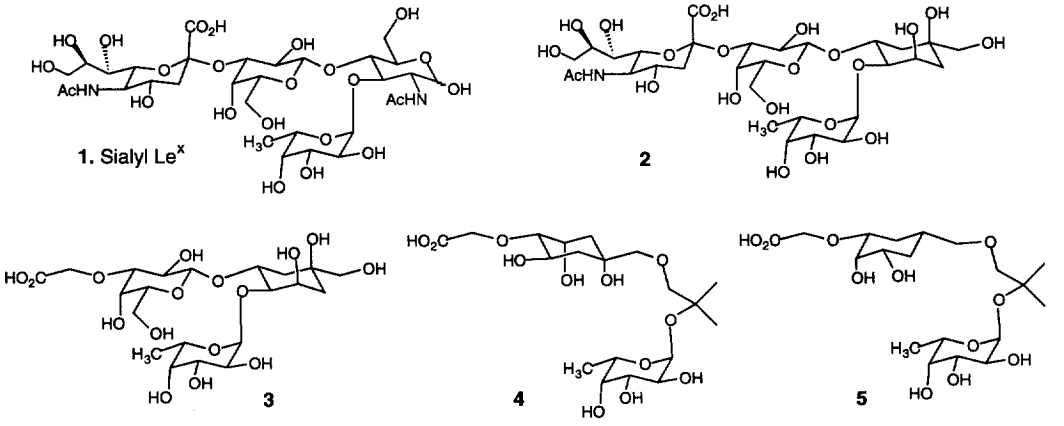
Abstract: The synthesis of sialyl Lewis^x mimetic anologs in which the D-glucosamine, D-galactose, and sialic acid residues are replaced individually with appropriate glycomimetics is described. A computational model for predicting bioactive conformations was tested. © 1997 Elsevier Science Ltd.

Sialyl Lewis^x (sLe^x) **1** (Figure 1) is the terminal tetrasaccharide structural unit of the physiological selectin ligands that are present on the surface of leukocytes.[1] This unique structure is responsible for the initial interaction of leukocytes with the surface of blood vessels at sites of inflammation or injury.[2] Much effort has been focused in recent years to antagonize this interaction which is a prerequisite for the inflammatory response to take place. In an effort to obtain simpler and more effective analogs, numerous potential sLe^x mimetics have been synthesized.[3]

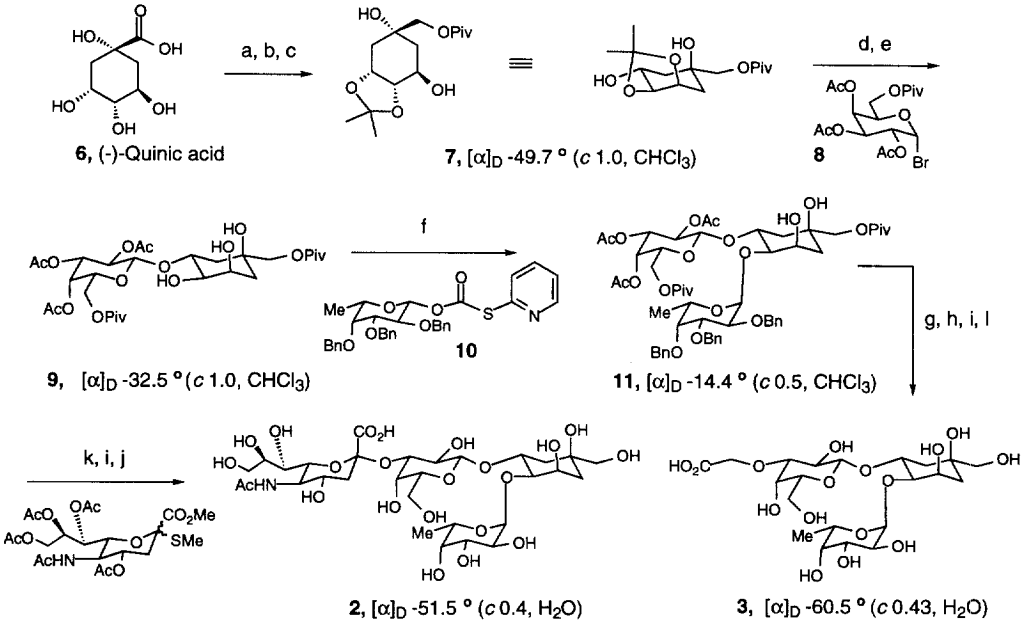
Figure 1



In previous studies directed at probing the functional and structural requirements for activity of sLe^x, we utilized pentaerythritol as an anchoring subunit for the attachment of α-L-fucosyl and α-sialyl residues.[4] These sugar residues are essential for effective recognition by the E-selectins, although carboxylic acid derivatives appear to replace the sialic acid part.[5,6] The N-acetyl-D-glucosamine residue in **1** has shown no preponderant contribution towards the binding process, apparently acting as a scaffold to provide a spacially suitable arrangement for the molecule as a whole.[7] It is also known that the D-galactosyl residue is subject to considerable functional manipulations.[8]

Consideration of available data on the bioactive conformation of sLe^x in its free or bound forms to proteins,[9] and model studies in our laboratory, led us to consider the incorporation of carbocyclic units as glycomimetic scaffolds to replace the N-acetyl-D-glucosamine and D-galactose residues individually in sLe^x. To this end, we have used quinic acid as the carbocyclic chiron, in which the spacial disposition of some hydroxyl groups, would provide a binding environment similar to the natural residues in sLe^x. Structures **2** and **3** (Figure 1) are representative of N-acetyl glucosamine replacements in a full sLe^x structure and a glycolic ether surrogate, respectively. Compounds **4** and **5** focus on carbocycles as D-galactose mimics, with conformationally biased ethylenedioxy tethers, and a pendant glycolic acid ether unit as a sialyl carboxylate mimic.

Scheme 1



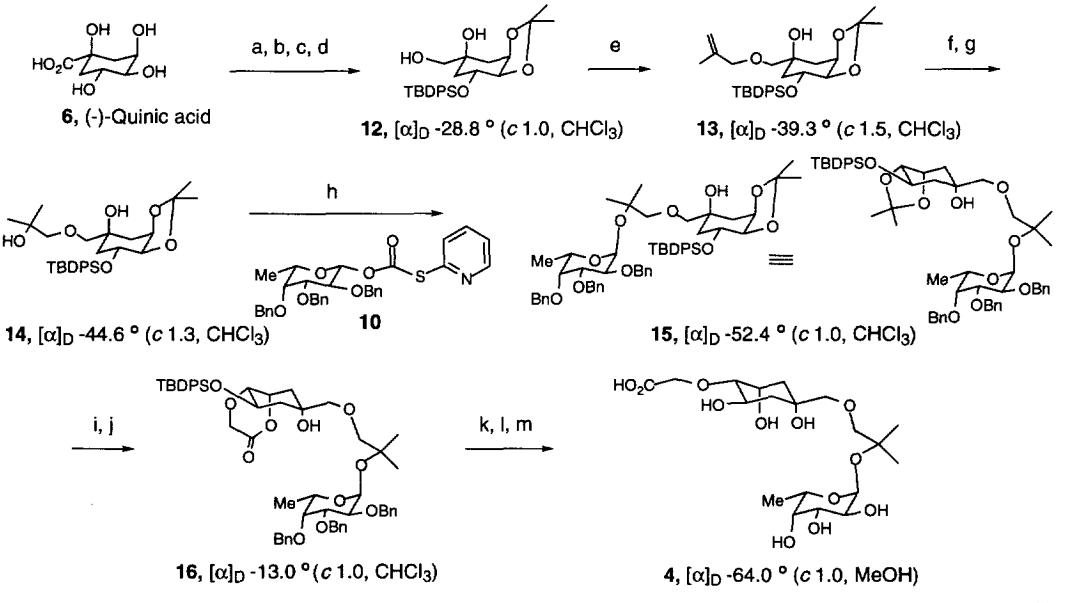
a. Acetone, H^+, rt, 16 h, 90%; b. $LiBH_4$, rt, 16 h, 98%; c. Bu_2SnO, 100 °C, 16 h, then, trimethylacetylchloride, rt, 30 min, 85%; d. AgOTf, tetramethylurea (TMU), –78 °C, 3 h, 79%; e. aq AcOH, 80 °C, 3 h, 80%; f. **10**, AgOTf, tetramethylurea (TMU), –78 °C, 3 h, 50%; g. 0.1 N NaOMe MeOH, rt, 2 h, 85%; h. Bu_2SnO, 100 °C, 16 h, then methyl bromoacetate, 70 °C, 16 h, 42%; i. $Pd(OH)_2/C$, H_2, ethanol, 16 h, 85%; j. 0.1 N NaOMe, MeOH, rt, 24 h, then, H_2O, 90%; k. NIS, TfOH, –40 °C, CH_3CN, 5 h, 48%; l. see j, 91%.

The known acetonide of quinic acid[10] was reduced and pivaloylated via the stannylidene acetal[11] to afford **7** in excellent yield (Scheme 1). Glycosylation with **8** in the presence of silver triflate and tetramethylurea[12] led to the corresponding pseudodisaccharide which was selectively deprotected under acidic conditions to give the triol derivative **9**. It was now possible to effect a selective α-fucosylation on the equatorially disposed hydroxy group utilizing the recently discovered α-selective glycosylation procedure that involves 2-pyridylthiocarbonyl activation.[13] Thus, treatment of **9** with 2,3,4-tri-*O*-benzyl-β-L-fucopyranosyl 2-pyridylthiocarbonate **10** in the presence of silver triflate gave the expected pseudotrisaccharide **11**. Selective deacetylation, followed by conversion of the polyol to the 3,4-*O*-stannylidene acetal on the D-galactose residue,

and subsequent alkylation with methyl bromoacetate led to the desired glycolate ether. Catalytic hydrogenation followed by base-catalyzed hydrolysis of the ester function led to the sLe^x mimetic **3**. Treatment of **11** with base followed by sialylation according to the general procedure of Hasegawa,[14] gave the corresponding pseudotetrasaccharide. Classical deprotection, followed by chromatography on a Sephadex column ($CHCl_3$:MeOH:H_2O, 5:4:1), led to the carbocyclic sLe^x mimetic **2**. In spite of the relatively modest yields of fucosylation and sialylation, it is noteworthy to mention that the reactions were done on triol and tetrol acceptors without the need for protection.

Replacement of the D-galactose residue with hydroxylated cyclohexanes was achieved by chemically modifying quinic acid in two perspective representations. The acetonide derivative **12**, easily available from quinic acid,[15] was methallylated selectively on the primary hydroxyl group to afford **13** (Scheme 2). Ozonolysis, and treatment of the resulting ketone with methyl lithium gave the tertiary alcohol side-chain as in **14**. Selective α-fucosylation was achieved in excellent yield to afford **15**. Removal of the isopropylidene group

Scheme 2



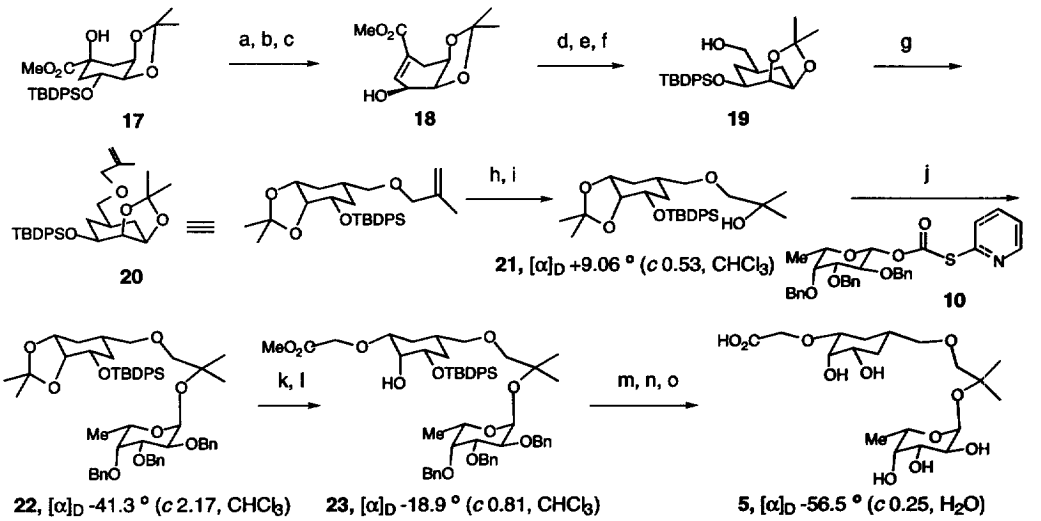
a. Acetone, H$^+$, rt, 16 h, 90%: b. NaOMe, MeOH, 0 °C, 3 h, 80%; c. TBDPSCl, DMAP, rt, 16 h, 95%; d. $NaBH_4$, EtOH, rt, 3 h, 90%; e. methallyl bromide, NaH, rt, 1 h, 73%; f. O_3, –78 °C, DMS, 1 h, 90%; g. MeLi, –78 °C, 4 h, 78%; h. **10**, AgOTf, 0 °C, 76%; i. aq AcOH, 80 °C, 2 h, 86%; j. Bu_2SnO, 100 °C, 16 h, then methyl bromoacetate, 100 °C, 16 h, 72%; k. TBAF, AcOH buffer, 50 °C, 16 h, 90%; l. Pd(OH)2/C, H_2, ethanol, 16 h, 90%; m. 0.1 N NaOH, rt, 2 h, quant.

and conversion of the resulting diol to the cyclic lactone ether **16** was achieved by a stannylidenation-etherification process. Finally, debenzylation and basic hydrolysis led to the pseudodisaccharide **4**.

The second carbocyclic D-galactose mimic was synthesised according to the protocol shown in Scheme 3. Thus, quinic acid was converted to the methyl ester derivative **17**, which was subjected to oxidation, followed by β-elimination to afford the α,β- unsaturated ester derivative **18**. Catalytic hydrogenation followed by

reduction of the ester function gave the alcohol **19** which was methallylated in the usual way to afford **20**. An oxidation-branching sequence led to the tertiary alcohol **21**, which was α-fucosylated in excellent yield to give **22**. Acidic cleavage of the acetonide group followed by the introduction of methyl glycolic ether group via the stannylidene acetal gave **23**. Finally, catalytic hydrogenation and hydrolysis afforded the pseudodisaccharide **5**.

Scheme 3



a. PDC, rt, 16 h, 85%; b. $POCl_3$, rt, 3 h, 84%; c. $NaBH_4$, EtOH, rt, 3 h, 92%; d. TBDPSCl, DMF, rt, 16 h, 80%; e. H_2, Pd/C, rt, 1 h, 98% ; f. LAH, rt, 88%; g. methallyl bromide, NaH, rt, 1 h, 86%; h. O_3, DMS, -78 °C, 1 h, 75%; i. Methyl lithium, -78 °C, 4 h, 90%; j. AgOTf, 0 °C, 82%; k. aq AcOH, 80 °C, 2 h, 86%; l. Bu_2SnO, 100 °C, 16 h, then methyl bromoacetate, 100 °C, 16 h, 91%; m. TBAF/AcOH buffer, 50 °C, 16 h, 90%; n. $Pd(OH)_2/C$, H_2, ethanol, 16 h, 90%; o. 0.1 N NaOH, rt, 2 h, quant.

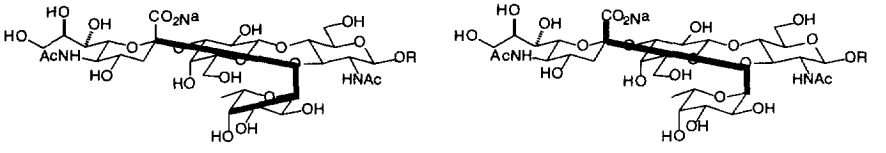
Biological data and conformational analysis: Compounds **2**, **3**, **4** and **5** were tested for their binding to E-selectin.[16] An activity of 1.0 mM was found for the compound **2**, which was equal to natural sLe^x. Unfortunately, the glycolic acid derivative **3**, as well as the tethered gal-mimetic structures **4** and **5** were found to be inactive (>10 mM).

Kolb and Ernst have recently reported a molecular modelling procedure for the rationalization and prediction of activity trends towards E-selectin.[6] The large contact area between the receptor[17] and its ligand causes the free binding energy to be greatly influenced by entropic factors. Consequently, the ligands ability to adopt the bioactive conformation (i.e. its preorganization for binding) is one of the fundamental requirements for bioactivity. The bioactive conformation of sLe^x has previously been determined by transfer-NOE studies.[9c] Experience shows that only those mimics that populate the bioactive conformation in torsional space are likely to be active. Conversely, mimics that do not populate the bioactive window are likely to be inactive. [6] The modelling tool assesses a mimic's preorganization by probing the energy surface in a 'Monte–Carlo (Jumping between Wells)/stochastic dynamics[18] [MC(JBW)/SD] simulation. The data from this simulations are used to

calculate the probability for being at any point of conformational space, hence the probability for being in the area responsible for bioactivity which is a measure for the ligand's preorganization for binding.

A two-dimensional internal coordinate system (cf. Figure 2) is employed to define the spatial arrangement of the relevant pharmacophores, i.e. the COOH group relative to the fucose moiety.

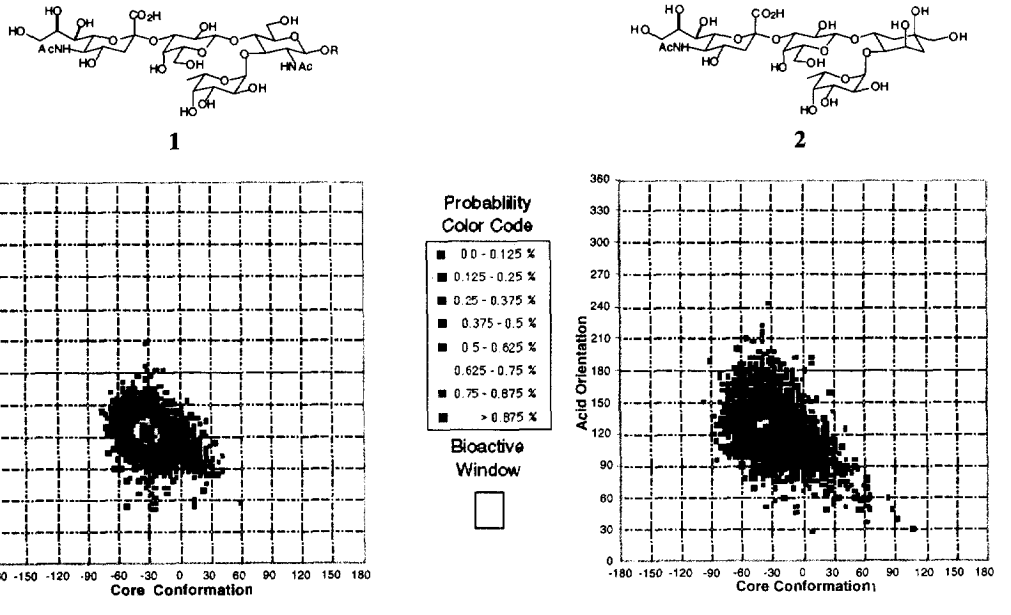
Figure 2. Internal Coordinate System



(a) Core Conformation (b) Acid Orientation

Figure 3 shows the results for sLe^x and for compound **2**. The box in each diagram marks the bioactive window. Both sLe^x and mimic **2** populate this area and both compounds are, therefore, preorganized for binding to E-selectin. Compounds **3**, **4** and **5** are much more flexible than sLe^x and mimic **2** and the low preorganization for binding may be the reason for the lack of activity of these compounds.

Figure 3. 10 ns MC(JBW)/SD Results for Sialyl Lewis^x **1** and Mimic **2**



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