Design and Synthesis of Sialyl Lewis X Mimetics

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Sialyl Lewis X (SLe^x, Figure 1), a terminal tetrasaccharide of cell-surface glycoproteins and glycolipids, is a ligand for the endothelial leukocyte adhesion molecule-1 (E-selectin), which mediates the early stage of adhesion of leukocytes to activated endothelial cells.[1] Though SLe^x has been considered to be potentially useful as an anti-inflammatory agent and its synthesis on large scales has been developed for clinical evaluation,[2] this natural saccharide can only be used in its injectable form for acute symptoms as it is orally inactive and unstable in the blood stream.[3] The search for novel SLe^x mimetics with simpler structure, higher affinity for the receptor, and better stability against glycosidases, especially fucosidase and sialidase, has been of current interest.[4] As the free[2] and bound[5] conformations of SLe^x are essentially the same, and the six functional groups required for E-selectin binding have been determined (i.e., the 2-, 3-, and 4-OH groups of Fuc, the 4- and 6-OH groups of Gal, and the $-CO_2^-$ group of NeuAc),[6] we have designed a series of small molecules (**1**−**5**, Chart 1) which contain these functional groups in space to mimic the active SLe^x conformation. Compounds **1** and **2** each contain a fucose residue and a galactose residue tethered by an ethylene glycol[7] or a butane linkage. It was felt that the *exo*-anomeric (or the gauche) effects of **1** would confine the glycosidic torsion angles to those of SLe^x, and the C-linked analog **2** is expected to have a similar conformation as the parent structure **1**.[8] Compound **3**, with a *cis*-olefin spacer, was designed to have more constrained glycosidic torsion angles than **2**, and compound **4** would have essentially the same fucosidic torsion angles as in SLe^x, though the equivalent OH groups for the Gal residue are missing. Compound **5** is a further modification of **2**, with the Gal residue replaced by an amino acid, (2*S*,3*S*)-2-amino-3,4-dihydroxybutanoic acid. Model studies indicate that the two OH groups of this amino acid in **5** overlap with the 4- and 6-OH groups of Gal in SLe^x.

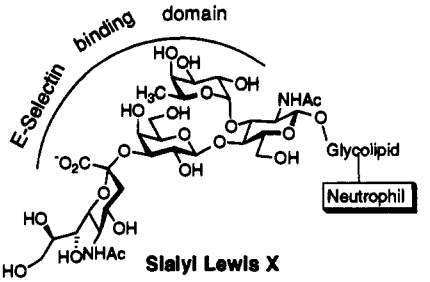
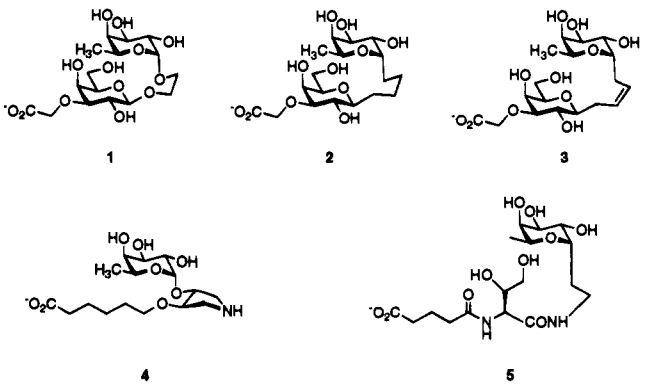
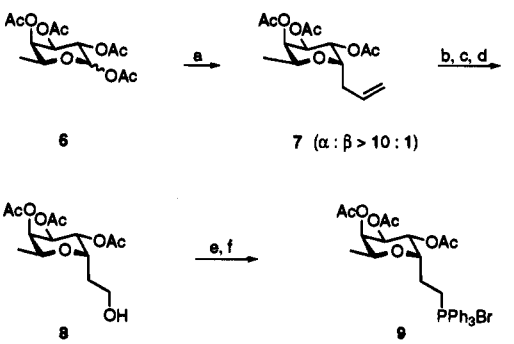


Figure 1.

Chart 1



Scheme 1[a]



[a] Conditions: (a) allyl-TMS, $BF_3{\cdot}OEt_2$, CH_3CN, 20 °C, 91%; (b) O_3, CH_2Cl_2, −78 °C; (c) DMS, room temperature; (d) $NaBH_4$, MeOH, 0 °C, 70% from **7**; (e) CBr_4, PPh_3, CH_2Cl_2; (f) PPh_3, DMF, 110 °C, 68% from **8**.

While compounds **1** and **4** can be easily prepared by conventional glycosylation reactions, the syntheses of **2**, **3** and **5** involve some interesting chemistry and deserve some comments. For the syntheses of **2** and **3** (Schemes 1 and 2), fucose was converted to the tetraacetate and treated with allyltrimethylsilane and boron trifluoride etherate in dry acetonitrile at room temperature to give the desired α-*C*-glycoside[9] in 91% yield (α:β > 10:1). Ozonolysis of **7** followed by reductive workup and further manipulation provided the Wittig reagent **9** (49% from **7**). To construct the β-*C*-galactoside, compound **10** was

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(1) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1132. Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1130. Lowe, J. B.; Stoolman, L. M.; Nair, R. P.; Larsen, R. D.; Berhend, T. L.; Marks, R. M. *Cell* **1990**, *63*, 475.

(2) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.

(3) A SLe^x derivative is in phase II clinical trials (J. C. Paulson, personal communication). The SLe^x−E-selectin interaction is weak but quite specific, resulting in a subsequent tight protein−protein interaction between integrins and ICAM-1.

(4) For previous syntheses of SLe^x mimetics: (a) Allanson, N. M.; Davidson, A. D.; Martin, F. M. *Tetrahedron Lett.* **1993**, *34*, 3945 (30-fold less active than SLe^x). (b) Ragan, J. A.; Cooper, K. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2563 (a mixture of four diastereomers with 40- to 50-fold less activity). (c) Hanessian, S.; Prabhanjan, H. *Synlett* **1994**, 868 (inactive). For active natural products inhibiting E-selectin, see: Narasinga Rao, B. N.; Anderson, M. B.; Musser, J. H.; Gilbert, J. H.; Schaefer, M. E.; Foxall, C.; Brandley, B. K. *J. Biol. Chem.* **1994**, *269*, 19663 (a semisynthetic *C*-fucoside of glycyrrhetinic acid was shown to be a potent inhibitor of E-, P-, and L-selectins).

(5) For transferred NOE study, see: Cooke, R. M.; Hale, R. S.; Lister, S. G.; Shah, G.; Weir, M. P. *Biochemistry* **1994**, *33*, 10591. The X-ray structure of E-selectin in the absence of SLe^x was reported: Graves, B. J.; Crowther, R. L.; Chandran, C.; Rumberger, J. M.; Li, S.; Huang, K.-S.; Presky, D. H.; Familletti, P. C.; Wolitzky, B. A.; Burns, D. K. *Nature* **1994**, *367*, 532.

(6) Brandley, B. K.; Kiso, M.; Abbas, S.; Nikrad, P.; Srivastava, O.; Foxall, C.; Oda, Y.; Hasegawa, A. *Glycobiology* **1993**, *3*, 633. Ramphal, J. Y.; Zheng, Z.-L.; Perez, C.; Walker, L. E.; DeFrees, S. A.; Gaeta, F. C. A. *J. Med. Chem.* **1994**, *37*, 3459; DeFrees, S. A.; Gaeta, F. C. A.; Lin, Y.-C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549. Yuen, C.-T.; Lawson, A. M.; Chai, W.; Larking, M.; Stoll, M. S.; Stuart, A.C.; Sullivan, F. X.; Ahern, T. J.; Feizi, T. *Biochemistry* **1992**, *31*, 9126.

(7) For previous work utilizing ethylene glycol spacer, see: Ats, C.-S.; Lehmann, J.; Petry, S. *Carbohydr. Res.* **1992**, *233*, 125.

(8) Kishi, Y. *Pure Appl. Chem.* **1989**, *61*, 313.

(9) Kozikowsky, A. P.; Sorgi, K. L. *Tetrahedron Lett.* **1983**, *24*, 1563.

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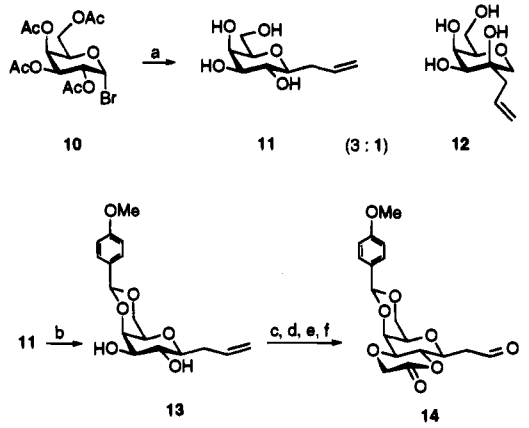
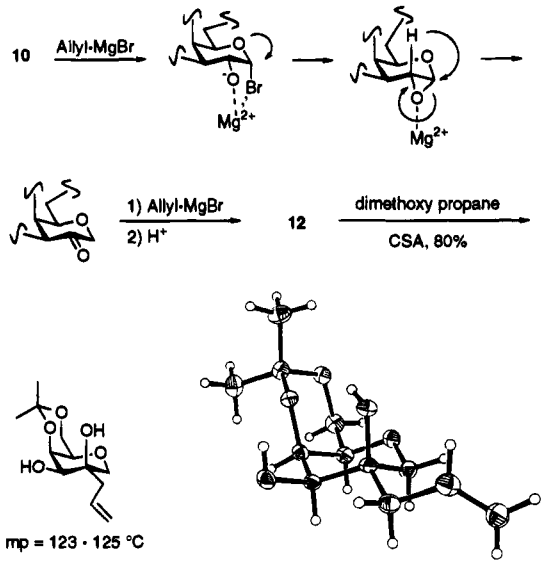
Scheme 2[a]

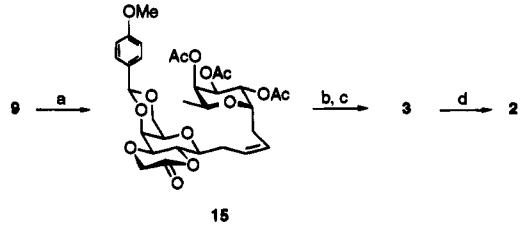


[a] Conditions: (a) 10 equiv of allyl-MgBr, THF, −78 °C, 60%; (b) *p*-methoxybenzaldehyde dimethyl acetal, 90%; (c) Bu_2SnO, Bu_4NI, toluene, reflux; (d) $MeOCOCH_2Br$, toluene, reflux; (e) O_3, CH_2Cl_2, −78 °C; (f) PPh_3, 60% from **13**.

Scheme 3



Scheme 4[a]

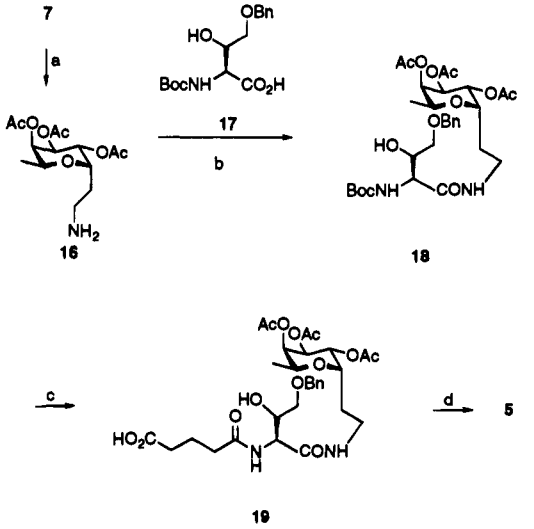


[a] Conditions: (a) NaHMDS, THF, −78 °C, then **14**, 60%; (b) 80% AcOH, room temperature; (c) NaOH, H_2O, THF, 80% from **15**; (d) H_2, Pd·C, MeOH, 100%.

treated with allylmagnesium bromide to give a 3:1 mixture of the desired *β-C*-glycoside **11** (60%), along with the side product **12** (22%), which was structurally confirmed by X-ray crystal structure analysis (Scheme 3). The formation of **12** may proceed through an epoxide intermediate (Scheme 3), as an analogous arrangement was reported[10] previously. Selective protection of the 4,6-dihydroxy groups of **11**, followed by treatment with methyl bromoacetate and ozonolysis, gave the lactonized aldehyde **14** (54% from **11**), which was coupled with **9** via a Wittig reaction to give **15** in 60% yield (Scheme 4). Deprotection of **15** gave **3** (80%), which was then reduced to **2** using H_2/Pd-C (100%).

(10) Yamamoto, H. *Tetrahedron Lett.* **1994**, *50*, 3663.

Scheme 5[a]



[a] Conditions: (a) (i) O_3, Ph_3P; (ii) Pd·C/H_2, NH_4OAc, 50%; (b) **17**, EDC·HCl, 82%; (c) (i) 4 N HCl/EtOAc; (ii) glutaric anhydride/TEA 80%; (d) (i) Pd·C/H_2; (ii) NaOMe, 62%.

For the synthesis of **5** (Scheme 5), compound **7** was ozonolyzed to provide the aldehyde for reductive amination to give amine **16** (50%), which was then coupled with the *N*-Boc-amino acid **17**[11] to form **18** (82%). After deprotection and treatment with glutaric anhydride, compound **19** was obtained (80%) and deprotected to give **5** in 62% yield.

Compounds **1**−**5** are resistant to α-fucosidase and β-galactosidase and are active as inhibitors of SLe^x glycoconjugate binding to immobilized E-selectin[12] with the following IC_{50} values: **1**, 1.5 mM;[13] **2**, 20 mM; **3**, 15 mM; **4**, 10 mM; **5**, 1.3 mM. Compounds **1**−**3** are less active than SLe^x (1 mM), probably because they are conformationally more flexible than SLe^x. Compound **4** lacks the two essential OH groups from the Gal residue. Compound **5** is, however, comparable with SLe^x. It is conformationally more stable than **2** or **3** and contains all the essential functional groups required for E-selectin binding. Replacement of any one of the OH groups in **5** with H resulted in a sharp decrease in activity (no inhibition was observed at 1 mM). This study thus confirms the important structural elements involved in E-selectin−ligand interactions and provides a new direction to the development of novel SLe^x mimetics. The synthesis of **5** is very straightforward and can be easily scaled up. Work is in progress to further modify the structure to improve the activity and to develop new novel SLe^x mimetics.

Acknowledgment. This work was partially supported by the NSF.

Supplementary Material Available: Synthetic schemes for **1** and **4**, characterization of the compounds discussed, and crystallographic data and a diagram of the 4,6-acetonide of **12** (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(11) The amino acid was prepared by L-threonine aldolase-catalyzed reaction of glycine (5 equiv) with *O*-benzylglycoaldehyde, pH 6.3 (78% yield). Vassilev, V. P.; Uchiyama, T.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.*, in press.

(12) The assay is similar to that previously reported: DeFrees, S. A.; Kosch, W.; Way, W.; Paulson, J. C.; Sabesan, S.; Halcomb, R.; Huang, D.-H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 66. Detailed inhibition analysis will be published separately.

(13) A mixture of four diastereomers was prepared previously (see ref 4b) and reported to be 40- to 45-fold less active than SLe^x.