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Synthesis and Biological Evaluation of a New Sialyl Lewis X Mimetic Derived from Lactose

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A sialyl Lewis X (sLe^x) mimetic compound, 2-(trimethylsilyl)ethyl 3-*O*-carboxymethyl-*β*-D-galactopyranosyl-(1→4)-[α-L-fucosyl-(1→6)]-*β*-D-glucopyranoside (**2a**), has been synthesized in 14 steps from D-lactose. This synthesis features the use of the activated glycosylating donor, lactosyl iodide, in a Koenigs−Knorr sequence, the regioselective derivatization at the C-3 position of the galactose moiety, and the stereoselective construction of a fucose-α(1→6)-lactose linkage. The mimetic was tested for its ability to inhibit human polymorphonuclear leukocyte (hPMNL) adhesion to immobilized recombinant human E-selectin under shear stress conditions.

Introduction

The regulated recruitment of leukocytes, the disease-fighting cells of the immune system, to damaged or infected tissue has been the subject of extensive investigation.[1] The passage of the leukocytes from the blood stream to the tissue matrix depends on an initial cell recognition event between the leukocyte and the vascular wall. The damaged tissue initiates this important event by releasing signaling cytokines that stimulate the endothelial cells of the nearest postcapillary venules to express the leukocyte adhesion molecules, E- and P-selectin. The C-type lectin domains of E- and P-selectin recognizes and weakly binds the blood antigen sialyl Lewis X (sLe^x), **1**, the tetrasaccharide found on the terminus of certain leukocyte surface glycoproteins expressed on the tips of microvilli. The leukocyte surface is decorated with multiple copies of sLe^x and the multivalent nature of this weak selectin−carbohydrate interaction causes the leukocyte to reduce its flow velocity and roll along the endothelial wall. This initial rolling interaction promotes the subsequent firm adhesion mediated by a protein−integrin interaction of the leukocyte to the endothelial cell surface. The adhesion of the leukocyte to the vascular wall achieved, the leukocyte migrates through the endothelial cell surface of the vessel, travels through the tissue matrix to the damaged cells, and releases its arsenal of oxygen radicals, proteases, and antimicrobial peptides.

While the migration of leukocytes is highly regulated by the healthy body, the unregulated migration of leukocytes from the vascular structures to healthy tissues does occur and can have destructive consequences. In such circumstances, the aggregation of disease-fighting leukocytes destroys healthy tissues and promotes a variety of inflammatory conditions such as rheumatoid arthritis, asthma, lupus, inflammatory bowel disease, and reperfusion injury. The pharmaceutical industry has shown great interest in the development of low molecular weight agents that could inhibit the sLe^x-mediated cellular adhesion event between the vascular cell wall and the leukocyte. Such agents may provide effective therapy for the above conditions that are caused at least in part by the unregulated migration of leukocytes. The study of the E-selectin sLe^x interaction has uncovered the minimal structural requirements for recognition by E-selectin and has led to a model of the binding coordination of sLe^x to E-selectin.[2] On the basis of these data, the synthetic community has designed a large number of sLe^x mimetics and some have demonstrated impressive inhibitory effects.[3]

We proposed the synthesis of sLe^x mimetic **2**. It was envisioned that our mimetic **2** could be effective alone as a selectin inhibitor or, if linked to a common antiinflammatory drug compound, could serve as a targeting device for the delivery of the drug (e.g., **2b**). It was

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(1) (a) Tyrell, D.; James, P.; Rao, N.; Foxall, C.; Abbas, S.; Dasgupta, F.; Nashed, M.; Hasegawa, A.; Kiso, M.; Asa, D.; Kidd, J.; Brandley, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10372−10376. (b) Picker, L. J.; Warnock, R. A.; Burns, A. R.; Doerschuk, C. M.; Berg, E. L.; Butcher, E. C. *Cell* **1991**, *66*, 921−933. (c) Rice, G. E.; Bevilacqua, M. P. *Science* **1989**, *246*, 1303−1306. (d) Bevilacqua, M. P.; Nelson, R. M. *J. Clin. Invest.* **1993**, *91*, 379−387. (e) Lowe, J. B. In *Molecular Glycobiology*; Fukada, M., Hindsgaul, O., Eds.; Oxford University Press: Oxford, UK, 1994; pp 163−205. (f) Vestweber, D.; Blanks, J. E. *Physiol. Rev.* **1999**, *79*, 181−213. (g) Lefer, D. J. *Annu. Rev. Pharmacol. Toxicol.* **2000**, *40*, 283−294.

(2) (a) Wormald, M. R.; Edge, C. J.; Dwek, R. A. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1214−1221. (b) Ball, G. E.; O'Neill, R. A.; Schultz, J. E.; Lowe, J. B.; Weston, B. W.; Nagy, J. O.; Brown, E. G.; Hobbs, C. J.; Bednarski, M. D. *J. Am. Chem. Soc.* **1992**, *114*, 5449−5451. (c) Miller, K. E.; Mukhopadhyay, C.; Cagas, P.; Bush, C. A. *Biochemistry* **1992**, *31*, 6703−6709. (d) Lin, Y.-C.; Hummel, C. W.; Huang, D.-H.; Ichikawa, Y.; Nicolaou, K. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 5452−5454. (e) Rutherford, T. J.; Spackman, D. G.; Simpson, P. J.; Homans, S. W. *Glycobiology* **1994**, *4*, 59−68.

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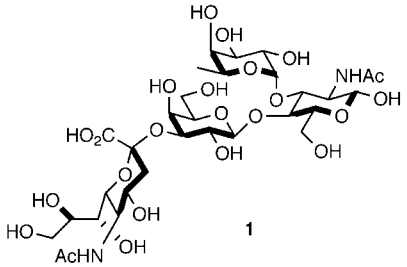

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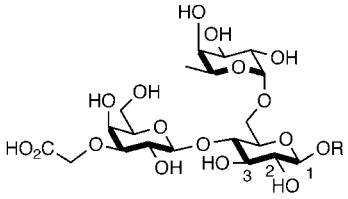
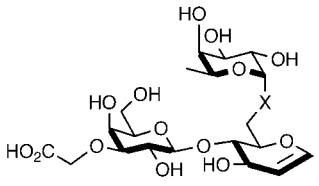


2a: R = CH_2CH_2TMS
2b: R = salicylic acid or diflusinal
2c: 2,3-dideoxy-2,3-didehydro-1α -OR; R = CH_2CH_3



3a: X = O
3b: X = C

envisioned that the carbohydrate portion of a sLe^x mimetic-drug conjugate could interact with the selectins expressed by endothelial cells stimulated by nearby damaged tissue, thereby delivering the antiinflammatory drug directly to the site of injury. This mimetic design replaces the glucosamine residue of sLe^x with a glucose residue scaffold and replaces the sialic acid residue with an ethanoic acid substituent at the C-3 position of the galactose ring.

Our mimetic design is predicated upon literature data which implicated that the glucosamine residue of **1** only served as a rigid spacer between the fucose and galactose residues and exhibited no coordination to the binding cavity of E-selectin.[2,3w,3x,4] Accordingly, the β-D-lactosamine core in **1** was supplanted with the chemically more robust β-D-lactose core. The L-fucose residue was retained, as reports indicated that the hydroxyl groups at positions C-2 and C-3 of the fucose residue are involved in coordination to the Ca^{2+} ion in the binding pocket,[5,6] and linked to the lactose scaffold by an α (1→6) glycoside bond. Literature reports also revealed that only the carboxylic acid group of the *N*-acetylneuraminic acid residue of **1** was involved in coordination to the binding site.[2,3w,3x,4] In 1995, the structure of the E-selectin-bound sLe^x was postulated on the basis of transferred nuclear Overhauser experiments.[7] These NMR results suggest that the tortional angles of the glycosidic bond between Gal and NeuAc of sLe^x change dramatically upon binding with E-selectin to assume the conformer where the COO^- group of NeuAc nearly juxtaposes the 2-OH of Gal. This provides the direct distances of roughly 8.9 and 9.1 Å between the NeuAc carboxylate C atom and C-1 and C-2 atoms of Fuc, respectively (results obtained by Macromodel Calculations using the parameters given in ref 7a, vide infra).

The fact that the core structure of the Galβ1→4GlcNAc moiety in sLe^x is the inexpensive disaccharide D-lactose prompted us to consider its possible use as a building block for rapid assembly of sLe^x mimetics. In keeping with substantial literature evidence, it was decided to append the entire L-fucose unit to this lactose core. For the eventual in vivo studies, the attachment of the fucose ring needs to be made through a *C*-glycoside bond to achieve better in vivo stability against fucosidase digestion. Moreover, the entire NeuAc group was replaced with the flexible β-methylene carboxylic acid group at C-3 of the galactose residue.

It was further postulated that a drug molecule with an accessible hydroxyl group (e.g., salicylic acid or

(3) (a) Roche, D.; Bänteli, R.; Winkler, T.; Casset, F.; Ernst, B. *Tetrahedron Lett.* **1998**, *39*, 2545−2548. (b) Tsai, C.-Y.; Park, W. K. C.; Weitz-Schmidt, G.; Ernst, B.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2333−2338. (c) Ida, Y.; Satoh, Y.; Katsumata, M.; Nagasao, M.; Hirai, Y.; Kajimoto, T.; Katada, N.; Yasuda, M.; Yamamoto, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2555−2558. (d) Hanessian, S.; Huynh, H. K.; Reddy, G. V.; McNaughton-Smith, G.; Ernst, B.; Kolb, H. C.; Magnani, J.; Sweeley, C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2803−2808. (e) Murphy, P. V.; Hubbard, R. E.; Manallack, D. T.; Wills, R. E.; Montana, J. G.; Taylor, R. J. K. *Bioorg. Med. Chem.* **1998**, *6*, 2421−2439. (f) Tsukida, T.; Moriyama, H.; Kurokawa, K.; Achida, T.; Inoue, Y.; Kondo, H. *J. Med. Chem.* **1998**, *41*, 4279−4287. (g) Hiruma, K.; Kanie, O.; Wong, C.-H. *Tetrahedron* **1998**, *54*, 15781−15792. (h) Lin, C.-C.; Morís-Varas, F.; Weitz-Schmidt, G.; Wong, C.-H. *Bioorg. Med. Chem.* **1999**, *7*, 425−433. (i) Hume, C. M.; Woltering, T. J.; Jiricek, J.; Weitz-Schmidt, G.; Wong, C.-H. *Bioorg. Med. Chem.* **1999**, *7*, 773−788. (j) Thorma, G.; Kinzy, W.; Bruns, C.; Patton, J. T.; Magnani, J. L.; Bänteli, R. *J. Med. Chem.* **1999**, *42*, 4909−4913. (k) Düffels, A.; Green, L. G.; Lenz, R.; Ley, S. V.; Vincent, S. P.; Wong, C.-H. *Bioorg. Med. Chem.* **2000**, *8*, 2519−2525. (l) Mootoo, D. R.; Cheng, X.; Khan, N. *J. Org. Chem.* **2000**, *65*, 2544−2547. (m) Hiramatsu, Y.; Tsukida, T.; Nakai, Y.; Inoue, Y.; Kondo, H. *J. Med. Chem.* **2000**, *43*, 1476−1483. (n) Gordon, E. J.; Gestwicki, J. E.; Strong, L. E.; Kiessling, L. L. *Chem. Biol.* **2000**, *7*, 9−16. (o) Bänteli, R.; Herold, P.; Bruns, C.; Patton, J. T.; Magnani, J. L.; Thoma, G. *Helv. Chim. Acta* **2000**, *83*, 2893−2907. (p) Tsai, C.-Y.; Huang, X.; Wong, C.-H. *Tetrahedron Lett.* **2000**, *41*, 9499−9503. (q) Kaila, N.; Thomas, B. E.; Thakker, P.; Alvarez, J. C.; Camphausen, R. T.; Crommie, D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 151−155. (r) Bänteli, R.; Ernst, B. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 459−462. (s) De Vleeschauwer, M.; Vaillancourt, M.; Goudreau, N.; Guidon, Y.; Gravel, D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1109−1112. (t) Slee, D. H.; Romano, S. J.; Yu, J.; Nguyen, T. N.; John, J. K.; Raheja, N. K.; Axe, F. U.; Jones, T. K.; Ripka, W. C. *J. Med. Chem.* **2001**, *44*, 2094−2107. (u) Thoma, G.; Magnani, J. L.; Patton, J. T.; Ernst, B.; Jahnke, W. *Angew. Chem., Int. Ed.* **2001**, *40*, 1941−1945. (v) Thoma, G.; Bänteli, R.; Jahnke, W.; Magnani.; Patton, J. T. *Angew. Chem., Int. Ed.* **2002**, *40*, 3644−3647. (w) Ernst, B.; Dragic, Z.; Marti, S.; Müller, C.; Wagner, B.; Jahnke, W.; Magnani, J. L.; Norman, K. E.; Oehrlein, R.; Peters, T.; Kolb, H. C. *Chimia* **2001**, *55*, 268−274. For a review of literature prior to 1998, see: (x) Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833−862.

(4) Rosen, S. V.; Bertozzi, C. R. *Curr. Opin. Cell Biol.* **1994**, *6*, 663−673.

(5) (a) Yuen, C.-T.; Lawson, A. M.; Chai, W.; Larkin, M.; Stoll, M. S.; Stuart, A. C.; Sullivan, F. X.; Ahern, T. J.; Feizi, T. *Biochemistry* **1992**, *31*, 9126−9131. (b) Brandley, B. K.; Kiso, M.; Abbas, S.; Nikrad, P.; Srivasatava, P.; Foxall, C.; Oda, Y.; Hasegawa, A. *Glycobiology* **1993**, *3*, 633−639. (c) DeFrees, S. A.; Gaeta, F. C. A.; Lin, Y.-C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549−7550. (d) Ramphal, J. Y.; Zheng, Z.-L.; Perez, C.; Walker, L. E.; DeFrees, S. A.; Gaeta, F. C. A. *J. Med. Chem.* **1994**, *37*, 3459−3463.

(6) For the proposed coordination of Ca^{2+} with the 2-OH of Fuc and the 6-OH of Gal in the E-selectin-bound sLe^x, see: Siuzdak, G.; Zheng, Z.-L.; Ramphal, J. Y.; Ichikawa, Y.; Nicolaou, K. C.; Gaeta, F. C. A.; Chatman, K. S.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2863−2866.

(7) (a) Scheffler, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W. T.; Weisemann, R.; Peters, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1841−1844. (b) Scheffler, K.; Brisson, J.-R.; Weisemann, R.; Magnani, J. L.; Wong, W. T.; Ernst, B.; Peters, T. *J. Biomol. NMR* **1997**, *9*, 423−436.

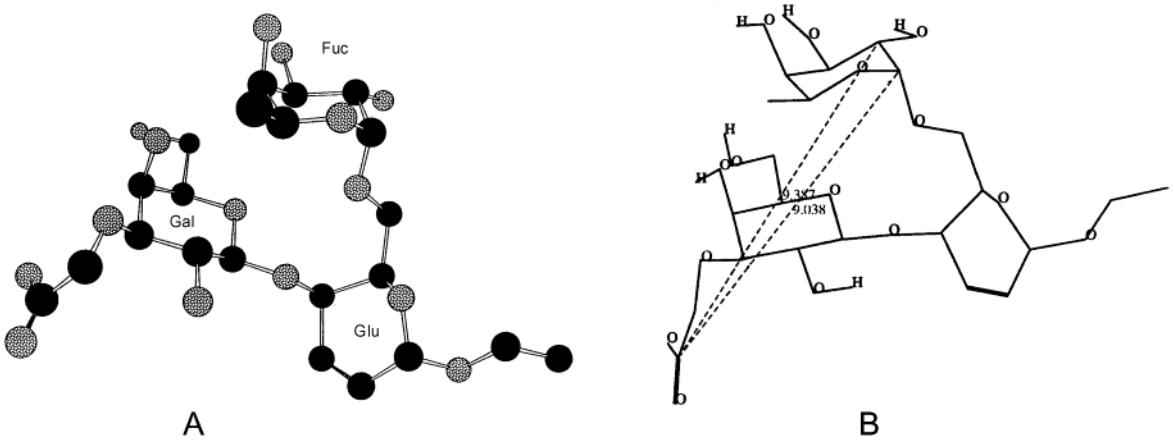


FIGURE 1. Calculated local minimum energy conformation of carboxylate **2c** (R = CH_2CH_3).

diflusinal) could be attached to our mimetic by either acid-promoted glycosylation of **2a**[8] or the Ferrier glycosylation of the glycal analogue **3a**.[9]

The global and local lowest energy conformers of this new *O*-fucoside mimetic and a few analogues were sought by a Macromodel Conformational Search using the Monte Carlo method (AMBER* Force Field) in a vacuum. The energy allowed for bond rotation was set as 50 kJ/mol. Interestingly, the conformational structure virtually identical with that drawn for **2c** was found to be the most stable conformer of carboxylate **2c**. Figure 1 shows the calculated local minimum energy conformation of carboxylate **2c** (R = OCH_2CH_3) where the carboxylate end is pointed down as in the case E-selectin-bound sLe^x. The results of the calculations revealed that the conformer matching structure **2c** (i.e., with the carboxylate end pointing up) was estimated to be more stable by only 4.10 kJ/mol. Direct distances between the carboxylate carbon and C-1 and C-2 in Fuc in the conformer shown in Figure 1B were calculated to be 9.0 and 9.4 Å, respectively, which are quite close to those values estimated for the biologically active conformation of sLe^x.

Results and Discussion

Synthesis of 2a. Our planned synthesis of the sLe^x mimetic **2a** required access to the D-lactose-derived requisite intermediate **10** in which only the 6-OH group is unprotected for the conjugation with the L-fucose unit. Access to this lactose derivative involved the selective protection of hydroxyl groups as well as the incorporation of the ethanoic acid unit onto the 3′-OH group (see Scheme 1). In an effort first to block the anomeric hydroxyl group of D-lactose, the inexpensive starting material D-lactose (**4a**) (α and β mixture) was converted to the per-*O*-acetyl derivative **4b** (≈100%). The installation of the 2-(trimethylsilyl)ethyl (TMSET) anomeric blocking group by the two-step Koenig−Knorr method[10] required the transformation of the per-*O*-acetyl lactose to the 1α-lactosyl halide (e.g., **5**) which was accomplished with I_2/CH_3COSH[11] in good yield (77%). The TMSET group was introduced by the treatment of the lactosyl iodide **5** with 2-(trimethylsilyl)ethanol in the presence of $HgCl_2$ and HgO to give exclusively the β-glycoside, **6a** (58%).[10b] The removal of the acetate protecting groups with the use of the Zemplén conditions[12] proceeded cleanly to give **6b** (≈100%). The application of the well-established, mild, and highly regioselective dialkylstannylene acetal methodology[13] was next planned for the installation of the methylene carboxylic acid group at the 3′-hydroxyl position. Although the selective alkylation and acylation of the axial 3-OH over the equatorial 4-OH of the galactose and similar systems[14] are well documented, treatment of the 3′,4′-stanylene intermediate derivative of the lactose system with less bulky esters of bromoacetic acid such as methyl and ethyl esters is reported to result in the formation of the lactone derivatives involving 2′- and 4′-OH groups in addition to the desired 3-OH derivative.[15] Therefore, in an effort to circumvent this lactone formation problem, the 3,4-stanylene derivative of **6b** was treated with *tert*-butyl bromoacetate[16] in the presence of catalytic TBAI, result-

(8) See, e.g.: Jansson, K.; Noori, G.; Magnusson, G. *J. Org. Chem.* **1990**, *55*, 3181−3185.

(9) (a) Koreeda, M.; Houston, T. A.; Shull, B. K.; Klemke, E.; Tuinman, R. J. *Synlett* **1995**, 90−92. (b) Ferrier, R. J.; Prasad, N.; Sankey, G. H. *J. Chem. Soc. C* **1969**, 587−591.

(10) (a) Lipshutz, B. H.; Pegram, J. J.; Morey, M. C. *Tetrahedron Lett.* **1981**, *22*, 4603−4606. (b) Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629−5647. (c) Ekberg, T.; Magnusson, G. *Carbohydr. Res.* **1993**, *246*, 119−136. For reviews on the Koenigs−Knorr glycosidation reaction see: (d) Igarashi, K. *Adv. Carbohydr. Biochem.* **1977**, *34*, 243−283. (e) Schmidt, R. R. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon Press: Oxford, UK, 1991; Vol. 6, pp 37−49.

(11) Chervin, S. M.; Abada, P.; Koreeda, M. *Org. Lett.* **2000**, *2*, 369−372.

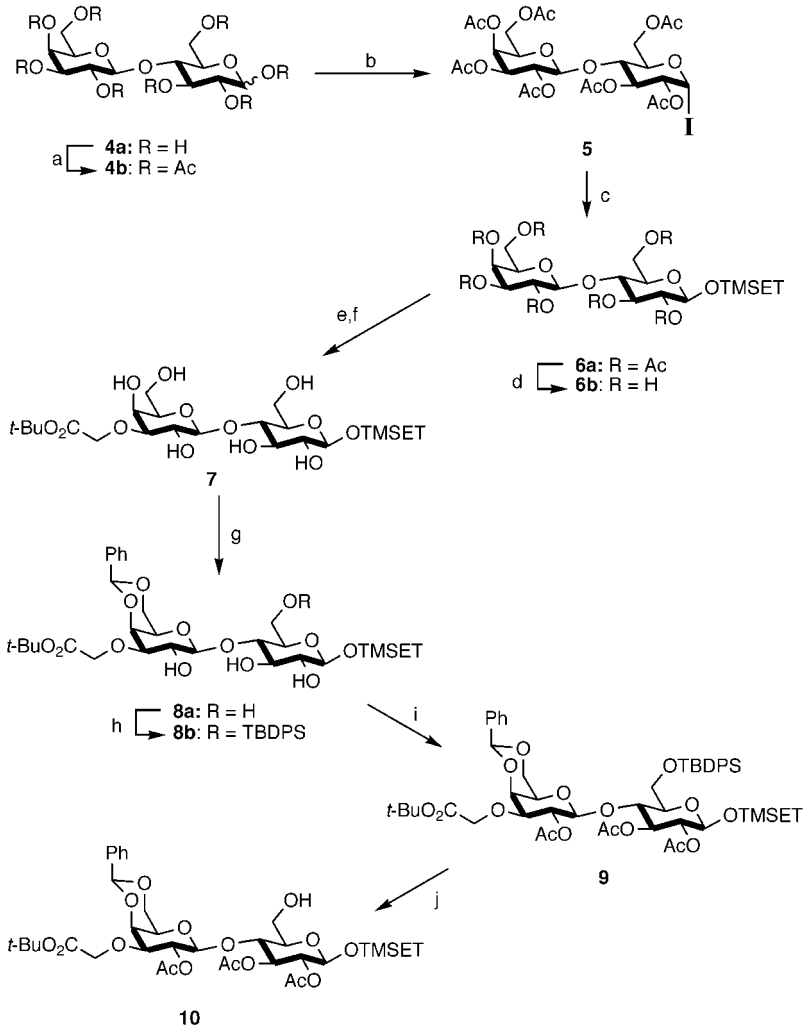
(12) (a) Zemplén, G.; Pascu, E. *Ber. Dtsch. Chem. Ges.* **1929**, *62*, 1613−1614. See also: (b) Collins, P.; Ferrier, R. *Monosaccharides: Their Chemistry and Their Roles in Natural* Products; Wiley: Chichester, UK, 1995; p 361.

(13) Reviews: (a) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643−663. (b) Stanek, J. *Top. Curr. Chem.* **1990**, *154*, 209−253. (c) Davies, A. G. In *Organotin Chemistry*; VCH: Weinheim, Germany, 1997; pp 173−180. (d) Grindley, T. B. *Adv. Carbohydr. Chem. Biochem.* **1998**, *53*, 17−142.

(14) (a) Nashed, M. A.; Anderson, L. *Carbohydr. Res.* **1977**, *56*, 419−422. (b) David, S.; Thieffry, A. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1568−1573. (c) Tsuda, Y.; Haque, Md. E.; Yoshimoto, K. *Chem. Pharm. Bull.* **1983**, *31*, 1612−1624.

(15) (a) Huang, H.; Wong, C.-H. *J. Org. Chem.* **1995**, *60*, 3100−3106. (b) Cheng, X.; Khan, N.; Mootoo, D. R. *J. Org. Chem.* **2000**, *65*, 2544−2547. For the clean formation of the lactone formation involving the 4-OH in the galactose system with methyl bromoacetate, see: Hiruma, K.; Kajimoto, T.; Weitz-Schmidt, G.; Ollman, I.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 9265−9270.

SCHEME 1. Elaboration of Lactose Core to Key Intermediate 10[a]



[a] Reagents and conditions: (a) Ac_2O, Et_3N, DMAP, EtOAc. 100%. (b) I_2, CH_3COSH, CH_2Cl_2 77%. (c) 2-(Trimethylsilyl)ethanol, HgO, $HgBr_2$, $CaSO_4$, $CHCl_3$, 58%. (d) $NaOCH_3$, CH_3OH, 100%. (e) Bu_2SnO, PhH. (f) $BrCH_2CO_2t$-Bu, PhH, 66%. (g) $PhCH(OCH_3)_2$, *p*-TsOH, CH_2Cl_2, 90%. (h) *t*-Bu(Ph)$_2$SiCl, DMAP, CH_2Cl_2, 79%. (i) Ac_2O, DMAP, CH_2Cl_2, 92%. (j) TBAF, AcOH, THF, 95%.

ing in the highly regioselective alkylation of the 3′-hydroxy group (**7**) in 66% yield (or 88% based on the recovered **6b**) together with the recovery of the starting disaccharide **6b** (25%). The 4′- and 6′-hydroxy groups next were protected smoothly as the diastereochemically pure benzylidine acetal **8a** (83%). The primary 6-hydroxy group was protected as the *tert*-butyldiphenylsilyl (TBDPS) ether **8b** (80%). For ease and uniformity of final deprotection steps, protection of the remaining hydroxyl groups as benzyl ethers was deemed most desirable. However, conversion of **8b** to the tri-2,3,2′-*O*-benzyl ether could not be realized under the standard basic (NaH/BnBr/TBAI/DMF) or acidic protocols [$Cl_3CC(=NH)OCH_2$-Ph/TfOH],[17] presumably due to unfavorable steric hindrance imposed by the juxtaposing (*tert*-butoxycarbonyl)-methylene ether at C′-3 and glucose residues at C′-1. In contrast, acetylation of **8b** with Ac_2O/DMAP in CH_2Cl_2 under reflux for 20 min afforded cleanly the 2,3,2′-triacetate **9** in 92% yield. Finally, the selective removal of the 6-TBDPS ether group was achieved with refluxing TBAF/AcOH/THF for 2 h to provide cleanly the desired alcohol **10** in 95% yield.

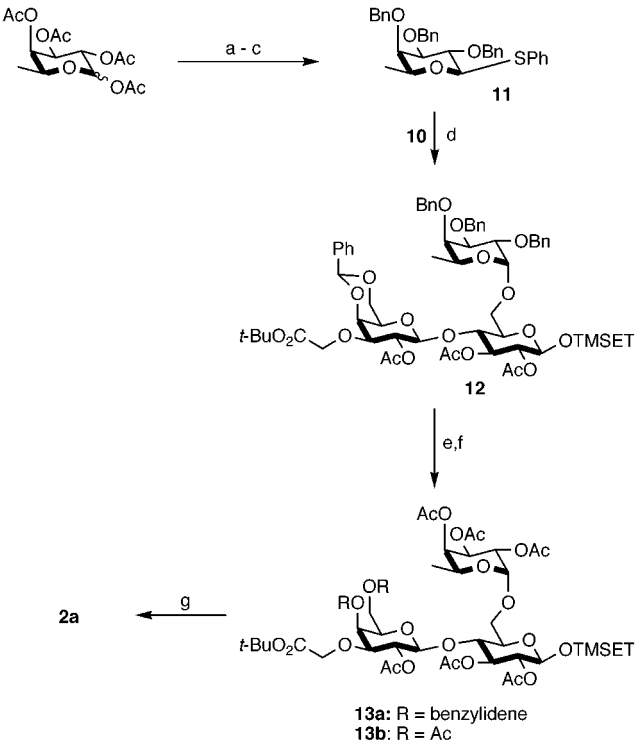
The *N*-iodosuccinimide-promoted coupling of thiophenyl per-*O*-benzyl-β-L-fucoside (**11**)[18] with **10** proceeded smoothly with high α-selectivity to give trisaccharide **12** (80%, α:β = 10:1) (Scheme 2). Interestingly, the use of the α-anomer of **11**, thiophenyl per-*O*-benzyl-α-L-fucoside, resulted in predominant formation of a β-fucoside linkage (α:β = 1:3) in a similar yield. The removal of the three benzyl and benzylidine protecting groups in trisaccharide **12** required a long reaction period (2 d, H_2, Pd/C, ethanol). Hydrogenolysis for shorter reaction periods (<10 h) resulted in the selective cleavage of the three benzyl ether groups leading, upon subsequent acetylation, to benzylidene **13a**. To facilitate in isolation and identification of the deprotected trisaccharide, the crude trisaccharide

(16) (a) Prodger, J. C.; Bamford, M. J.; Gore, P. M.; Holmes, D. S.; Saez, V.; Ward, P. *Tetrahedron Lett.* **1995**, *36*, 2339−2342. (b) Prodger, J. C.; Bamford, M. J.; Bird, M. I.; Gore, P. M.; Holmes, D. S.; Priest, R.; Saez, V. *Bioorg. Med. Chem.* **1996**, *4*, 793−801.

(17) Wessel, H.-P.; Iverson, T.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2247−2249.

(18) (a) Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem.* **1996**, *4*, 1833−1847. (b) Paquette, L. A.; Barriault, L.; Pissarnitski, D.; Johnson, J. N. *J. Am. Chem. Soc.* **2000**, *122*, 619−631.

SCHEME 2. Synthesis of *O*-Glycoside Mimetic Compound 2a[a]



[a] Reagents and conditions: (a) $BF_3 \cdot Et_2$, PhSH, THF, 95%.[18] (b) $NaOCH_3$, CH_3OH, 100%.[18] (c) NaH, BnBr, THF, DMF, 90%. (d) NIS, *p*-TsOH, CH_2Cl_2, 80%, $\alpha:\beta$ = 10:1. (e) H_2, Pd/C. CH_3OH. (f) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 71%. (g) KO*t*-Bu, Et_2O, H_2O, 100%.

was converted immediately to the per-*O*-acetyl derivative **13b** (71%, purified yield for 2 steps). The complete structure of this trisaccharide product **13b** was ascertained by ^1H and ^{13}C NMR and all NMR signal assignments were verified by $^1H-^1H$ gCOSY and HSQC ($^1H-^{13}C$ correlation) experiments on a 500 MHz NMR instrument.

The hydrolysis of the eight acetate esters and the *tert*-butyl ester in trisaccharide **16b** remained. In view of the inherent acid sensitivity of the trisaccharide, the use of the typical Lewis acid-mediated methods for deprotection of the *tert*-butyl ester (e.g., CF_3CO_2H; AcOH; TMSOTf/TEA; $TiCl_4$) was suspected to result in extensive decomposition of the trisaccharide. Indeed, exposure of the trisaccharide to these acidic conditions resulted in massive decomposition of the sugar. In search for mild, nonacidic conditions for the hydrolysis of a *tert*-butyl ester, we adopted the method of Gassman[19] that used the highly nucleophilic anhydrous hydroxide ion generated from potassium *tert*-butoxide and stoichiometric water in anhydrous diethyl ether at room temperature for the deprotection of simple hindered esters. Accordingly, octaacetate *tert*-butyl ester **13b** was subjected to 36 mol equiv each of KO*t*-Bu and H_2O in Et_2O at room temperature, which resulted in the formation of the fully deprotected trisaccharide acid **2a** in quantitative yield. TLC analysis of the crude reaction mixture indicated that the *tert*-butyl group was sluggish to hydrolyze but did hydrolyze after long reaction periods (14 h, 20 °C). Interestingly, the use of nonanhydrous ethyl ether made the transformation extremely sluggish and no hydrolysis product formation was visible by TLC after 3 days of stirring. Upon completion of exhaustive hydrolysis, the crude potassium salt was taken up into water and carefully protonated to pH 5 with cationic acid exchange resin. Purification of the compound by Sephadex G-10 chromatography gave pure trisaccharide **2a** in quantitative yield. The structure of the trisaccharide was confirmed by ^1H and ^{13}C NMR spectroscopy, and NMR peak assignments were validated by $^1H-^1H$ COSY and $^1H-^{13}C$ HSQC experiments.

Biological Evaluation. Compound **2a** was tested for its ability to inhibit human polymorphonuclear leukocyte (hPMNL) adhesion to immobilized recombinant human E-selectin[20] under shear stress conditions.[21] The assay involved pumping a suspension of hPMNL in buffer containing a concentration of the small molecule inhibitor over a parallel plate flow chamber coated with hE-selectin-hIgG chimera. The flowing and rolling of the hPMNL cells were recorded by video over 2 min, and the total number of rolling cells at three time intervals were quantified with the aid of an analysis program. The inhibitory activity of compound **2a** was tested against the negative control compound 3′-*N*-acetylneuraminyl-*N*-acetyllatosamine (3′-SLN)[22] and the native ligand sLe^x (**1**) (both purchased from Calbiochem, San Diego). The native ligand, sLe^x, showed a 68% reduction of activity at 1 mM vs the negative control compound **14**, respectively. However, compound **2a** showed no inhibition at a concentration of 1 mM, and likewise no significant inhibition at concentrations of up to 4 mM.

Conclusions

A new sLe^x mimetic compound, 2-(trimethylsilyl)ethyl 3-*O*-carboxymethyl-*β*-D-galactopyranosyl-(1→4)-[α-L-fucosyl-(1→6)]-*β*-D-glucopyranoside (**2a**), was designed and has been synthesized from D-lactose in 14 steps (longest linear sequence). Our route features the use of an activated glycosylating agent, per-*O*-acetylated lactosyl iodide, prepared with $I_2/CH_3C(=O)SH$ in the initial Koenigs–Knorr sequence, the regioselective derivatization at C-3′, and the stereoselective construction of the fucose-α(1→6)-lactose linkage. However, biological testing revealed that this sLe^x mimetic was inactive up to 4 mM in an E-selectin binding assay. In light of the unremarkable E-selectin inhibitory property of compound **2a**, it appears unlikely that the trisaccharide moiety would perform well as the targeting device for the delivery of an antiinflammatory drug. The results of a Macromodel Conformational Search (Monte Carlo/AMBER* Force Fields/vacuum) of sLe^x indicated the distance between the sialic acid carboxylate carbon and the fucose anomeric carbon of sLe^x in its bound form to be 9.1 Å. This value is similar to literature estimates of the distance between the two carbon centers.[7] A similar Macromodel Confor-

(19) Gassman, P. G.; Schenk, W. N. *J. Org. Chem.* **1977**, *42*, 918–920.

(20) Watsin, S. R.; Imai, Y.; Fennie, C.; Geoffroy, J. S.; Rosen, S. D. *J. Cell Biol.* **1990**, *110*, 2221–2229.

(21) (a) Lawrence, M. B.; Mcintire, L. V.; Eskin, S. G. *Blood* **1987**, *70*, 1284–1290. (b) Lawrence, M. B.; Springer, T. A. *J. Immunol.* **1993**, *151*, 6338–6346.

(22) Ichikawa, Y.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 4698–4700.

mational Search carried out on the projected Ferrier reaction product **2c** resulted in the crucial distance of 9.0 Å between the carboxylate carbon and the fucose anomeric carbon. On these grounds, analogue **2a** had been anticipated to be comparable to sLe^x in its E-selectin inhibitory activity. It is interesting to note that more recent NMR studies[23] through the use of ^{13}C-enriched sLe^x have shown that the orientation of the NeuAc−Gal linkage in the E-selectin-bound sLe^x differs from that postulated earlier by Sheffler et al.[7] Although our flexible mimetic could adopt an equivalent conformation even more easily, the considerably flexible nature of our mimetic in terms of its entire conformation may in turn be the drawback of this mimetic. This line of analysis seems quite relevant in light of recent successes by Thoma and co-workers in the design of E-selectin antagonists guided by the principle of preorganization of the bioactive conformation of sLe^x.[3u,v] Additionally, the X-ray-based structure of E-selectin bound to sLe^x recently achieved by Camphausen[24] should provide a further guide for our design of sLe^x mimetic compounds.

Experimental Section

General. All reactions were performed under an atmosphere of N_2 unless otherwise indicated. The reaction solvents diethyl ether (over benzophenone/Na), tetrahydrofuran (over benzophenone/Na), and dichloromethane (over $CaCl_2$) were distilled freshly from laboratory stills. Other reaction solvents (benzene, toluene, methanol, ethanol, *N*,*N*-dimethylformamide, dimethyl sulfoxide) were distilled as needed and stored in sealed bottles over molecular sieves. All glassware was dried in a laboratory oven (100 °C) prior to use. Solutions were concentrated at room temperature by rotary evaporation. Flash column chromatography was performed with E. Merck 200−300 silica gel and N_2 gas pressure. Analytical thin-layer chromatography was performed with EM Science Silica Gel 60 F_{254} 0.2-mm precoated silica gel aluminum sheets with UV indicator. Compounds were visualized by using carbazole/sulfuric acid stain.

Nuclear magnetic spectra were recorded on either a Varian Inova 400 MHz or a Varian Inova 500 MHz spectrometer. The chemical shifts are reported from low field to high field with respect to tetramethylsilane as the internal standard (δ = 0.00). The data for ^1H spectra are recorded as follows: chemical shift (peak shape, integration, H-assignment if unambiguous, and *J* values if resolvable). Peak shapes are described as follows: s for singlet, d for doublet, dd for doublet of doublets, ddd for doublet of doublet of doublets, q for quartet, m for multiplet, dq for doublet of quartets, ABq for AB quartet, and br for broadened. Coupling constants are given as *J* values in hertz. The data for ^{13}C spectra are reported from low field to high field with respect to tetramethylsilane as the internal standard ($\delta = 0.00$) as follows: chemical shift (C-assignment). $^1H-^1H$ (gCOSY) and $^1H-^{13}C$ (HSQC) 2D experiments were performed on many compounds to aid in peak assignments.

2-(Trimethylsilyl)ethyl [3-*O*-[(*tert*-Butoxycarbonyl)-methyl]-*β*-D-galactopyranosyl]-(1→4)-*β*-D-glucopyranoside (7). To a 500-mL, round-bottomed flask equipped with a Dean−Stark trap, a condenser, a stir bar, a rubber septum, and a N_2 inlet were added lactoside **6b** (4.41 g, 9.96 mmol), dibutyltin oxide (2.9 g, 1.2 equiv), and benzene (325 mL). The reaction mixture was heated at reflux with removal of water for 5.5 h. The reaction mixture was cooled, and the Dean−Stark trap was removed. Tetrabutylammonium iodide (1.84 g, 0.50 equiv) and *tert*-butyl bromoacetate (5.8 mL, 4.0 equiv) were added. The reaction mixture was heated at reflux for 16 h and then cooled to room temperature. The solid residue obtained upon removal of the organic solvent was purified by silica gel column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 95:5) to give the *tert*-butyl ester **7** as a white powder (3.68 g, 66%), together with the recovered starting disaccharide **6b** (1.10 g). Data for **7**: ^1H NMR [400 MHz, acetone-d_6 (1.5 mL)/D_2O (2 drops)] 4.42 (d, 1H, H-1′, $J_{1',2'}$ = 7.7 Hz), 4.31 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.26 and 4.22 (ABq, 2H, $-CH_2CO_2t$-Bu, J_{AB} =17.2 Hz), 4.06 (dd, 1H, H-4′, $J_{4',3'}$ = 3.3 Hz, $J_{4',5'}$ = 1.1 Hz), 3.96 (ddd, 1H, $-OCHHCH_2TMS$, *J* = 10.6, 9.5, 5.9 Hz), 3.84 and 3.82 (ABq, 2H, H-6, H-7, J_{AB} = 12.5 Hz, the 3.84 and 3.82 peaks further split by 3.5 Hz), 3.79 and 3.75 (ABq, 2H, H-6′, H-7′, J_{AB} = 11.0 Hz, the 3.79 and 3.74 peaks further split by 7.0 and 5.1 Hz, respectively), 3.72 (dd, 1H, H-2′, $J_{2',3'}$ = 9.5 Hz, $J_{2',1'}$ = 7.7 Hz), 3.64 (ddd, 1H, H-5′, $J_{5',6'}$ = 7.0 Hz, $J_{5',7''}$ = 5.1 Hz, $J_{5',4'}$ = 1.1 Hz), 3.59 (ddd, 1H, $-OCHHCH_2TMS$, *J* = 10.3, 9.5, 5.9 Hz), 3.51−3.47 (m, 2H, H-3, H-4), 3.45 (dd, 1H, H-3′, $J_{3',2'}$ = 9.5 Hz, $J_{3',4'}$ = 3.3 Hz), 3.41−3.37 (m, 1H, H-5), 3.21 (dd, 1H, H-2, $J_{2,3}$ = 9.2 Hz, $J_{2,1}$ = 7.7 Hz), 1.48 (s, 9H, $-C(CH_3)_3$), 0.89−1.02 (m, 2H, $-OCH_2CH_2TMS$), 0.03 (s, 9H, $-Si(CH_3)_3$); ^{13}C NMR [100.6 MHz, acetone-d_6 (1.5 mL)/D_2O (2 drops)] 172.64, 105.09 (C-1′), 103.49 (C-1), 84.40 (C-3′), 82.85 ($-C(CH_3)_3$), 82.71 (C-4), 76.31 (C-5′), 76.25 (C-3), 75.75 (C-5), 74.54 (C-2), 70.86 (C-2′), 68.18 ($-CH_2CO_2t$-Bu), 67.21 ($-OCH_2CH_2TMS$), 66.84 (C-4′), 62.62 (C-6), 62.08 (C-6′), 28.21 ($-C(CH_3)_3$), 18.76 ($-CH_2CH_2TMS$), −1.23 ($-Si(CH_3)_3$). $[\alpha]^{25}_D$ −6.2 ($CHCl_3$, *c* 0.75). Anal. Calcd for $C_{23}H_{44}O_{13}Si$: C, 49.63; H, 7.97. Found: C, 49.35; H, 7.81. $^1H-^1H$ gCOSY, $^1H-^{13}C$ HSQC.

2-(Trimethylsilyl)ethyl 4,6-*O*-Benzylidine-[3-*O*-[(*tert*-butoxycarbonyl)methyl]-*β*-D-galactopyranosyl]-(1→4)-*β*-D-glucopyranoside (8a). To a 250-mL, round-bottomed flask equipped with a condenser, a stir bar, rubber septum, and a N_2 inlet were added **7** (2.37 g, 4.25 mmol), CH_2Cl_2 (150 mL), benzaldehyde dimethylacetal (1.3 mL, 2.0 equiv), and *p*-TsOH·H_2O (0.10 g). The reaction mixture was heated at reflux for 20 min. The reaction mixture was cooled and was washed with aq satd $NaHCO_3$, water, and brine, dried (Na_2SO_4), and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 95:5) to give the product as a colorless oil (2.27 g, 83%): ^1H NMR (400 MHz, $CDCl_3$) 7.50−7.47 (m, 2H, Ar), 7.38−7.32 (m, 3H, Ar), 5.54 (s, 1H, $-CHPh$), 4.90 (s, 1H, −OH), 4.50 (d, 1H, H-1′, $J_{1',2'}$ = 7.7 Hz), 4.34 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 4.32 and 4.03 (ABq, 2H, $-CH_2CO_2t$-Bu, J_{AB} = 17.2 Hz), 4.30−4.27 (m, 3H, H-4′, H-6′, −OH), 3.89 (ddd, 1H, H-6, $J_{6,7}$ = 12.5 Hz, $J_{6,-OH}$ = 5.8 Hz, $J_{6,5}$ = 4.0 Hz), 4.09−3.97 (m, 4H, H-2′, H-7, H-7′, $-OCHHCH_2TMS$), 3.72 (dd, 1H, H-3, $J_{3,2}$ = 8.8 Hz, $J_{3,4}$ = 8.8 Hz), 3.66 (dd, 1H, H-4, $J_{4,5}$ = 9.1 Hz, $J_{4,3}$ = 8.8 Hz), 3.60 (ddd, 1H, $-OCHHCH_2TMS$, *J* = 15.4, 9.5, 5.8 Hz), 3.53 (br, 1H, H-5′), 3.47 (ddd, 1H, H-5, $J_{5,4}$ = 9.1 Hz, $J_{5,6}$ = 4.0 Hz, $J_{5,7}$ = 3.7 Hz), 3.42 (dd, 1H, H-3′, $J_{3',2'}$ = 9.9 Hz, $J_{3',4'}$ = 3.6 Hz), 3.41 (dd, 1H, H-2, $J_{2,3}$ = 8.8 Hz, $J_{2,1}$ = 7.7 Hz), 3.20 (dd, 1H, $-CH_2OH$, $J_{OH,7}$ = 8.8 Hz, $J_{OH,6}$ = 5.8 Hz), 2.49 (s, 1H, −OH), 1.47 (s, 9H, $-C(CH_3)_3$), 1.10−0.95 (m, 2H, $-OCH_2CH_2TMS$), 0.26 (s, 9H, $-Si(CH_3)_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 170.96 (R_2CO), 137.22 (Ar), 128.98 (Ar), 128.11 (Ar), 126.28 (Ar), 104.19 (C-1′), 102.04 (C-1), 101.24 ($-CHPh$), 82.90 ($-C(CH_3)_3$), 81.39, 81.38, 74.68 (C-3), 74.52 (C-5), 73.56 (C-2), 73.29 (C-4′), 69.01 (C-6′), 68.90 (C-2′), 66.85 (C-5′), 67.54 ($-CH_2CO_2t$-Bu), 67.49 ($-OCH_2CH_2TMS$), 62.30 (C-6), 28.13 ($-C(CH_3)_3$), 18.32 ($-OCH_2CH_2TMS$), −1.33 ($-Si(CH_3)_3$). $[\alpha]^{22}_D$ +8.3 ($CHCl_3$, *c* 0.58,). Anal. Calcd for $C_{30}H_{48}O_{13}Si$: C, 55.88; H, 7.50. Found: C, 55.80; H, 7.75. $^1H-^1H$ gCOSY, $^1H-^{13}C$ HSQC.

2-(Trimethylsilyl)ethyl 4,6-*O*-Benzylidine-[3-*O*-[(*tert*-butoxycarbonyl)methyl]-*β*-D-galactopyranosyl]-(1→4)-6-*O*-*tert*-butyldiphenylsilyl-*β*-D-glucopyranoside (8b). To a 250-mL round-bottomed flask equipped with a condenser, a stir bar, rubber septum, and a N_2 inlet were added **8a** (2.27 g,

(23) Scheffller, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W.-T.; Weisemann, R.; Peters, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1841−1844.

(24) Somers, W. S.; Tang, J.; Shaw, G. D.; Camphausen, R. T. *Cell* **2000**, *103*, 467−479.

3.52 mmol), THF (180 mL), *tert*-butylchlorodiphenylsilane (3.7 mL, 4.0 equiv), and DMAP (0.86 g, 2.0 equiv). The reaction mixture was heated at reflux for 21 h and then cooled to room temperature and quenched with water. The organic layer was washed with satd aq $NaHCO_3$, water, and brine and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone 5:1) to obtain the TBDPS ether as a colorless oil (2.46 g, 79%): ^{1}H NMR (400 MHz, $CDCl_3$) 7.80−7.76 (m, 4H, Ar), 7.51−7.48 (m, 2H, Ar), 7.43−7.31 (m, 9H, Ar), 5.53 (s, 1H, −C*H*Ph), 4.51 and 3.93 (ABq, 2H, −CH_2CO$_2$*t*-Bu, J_{AB} = 17.2 Hz), 4.49 (d, 1H, H-1′, $J_{1',2'}$ = 7.7 Hz), 4.32 (d, 1H, H-1, $J_{1,2}$ = 7.3 Hz), 4.28−4.26 (m, 2H, H-4′, H-6′), 4.16 (dd, 1H, H-6, $J_{6,7}$ = 11.5 Hz, $J_{6,5}$ = 4.0 Hz), 4.06 and 3.98 (m, 3H, H-7, H-7′, −OC*H*HCH$_2$TMS), 3.96 (ddd, 1H, H-2′, $J_{2',3'}$ = 9.7 Hz, $J_{2',1'}$ = 7.7 Hz, $J_{2',OH}$ = 1.7 Hz), 3.74 (dd, 1H, H-2, $J_{3,2}$ = 9.0 Hz, $J_{3,4}$ = 9.0 Hz), 3.69 (dd, 1H, H-2, $J_{4,3}$ = 9.0 Hz, $J_{4,5}$ = 9.0 Hz), 3.60−3.58 (m, 1H, −OCH*H*CH$_2$TMS), 3.49−3.44 (m, 3H, H-2, H-5, H-5′), 3.36 (dd, 1H, H-3′, $J_{3',2'}$ = 9.7 Hz, $J_{3',4'}$ = 3.5 Hz), 1.48 (s, 9H, −C(CH_3)$_3$), 1.09−1.02 (m, 2H, −OCH$_2$CH_2TMS), 1.04 (s, 9H, −Si(Ph)$_2$C-(CH_3)$_3$), 0.03 (s, 9H, −Si(CH_3)$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 170.99 (R$_2$*C*O), 137.50 (Ar), 135.89 (Ar), 135.70 (Ar), 133.73 (Ar), 133.16 (Ar), 129.60 (Ar), 129.53 (Ar), 129.12 (Ar), 128.23 (Ar), 127.67 (Ar), 127.55 (Ar), 126.49 (Ar), 104.18 (C-1′), 101.78 (C-1), 101.34 (−*C*HPh), 82.69 (−*C*(CH$_3$)$_3$), 81.57 (C-3′), 80.67 (C-3), 75.08, 74.51 (C-4), 73.86, 73.43 (C-4′), 69.59 (C-2′), 68.89 (C-6′), 67.54 (−*C*H$_2$CO$_2$*t*-Bu), 66.97 (−O*C*H$_2$CH$_2$TMS), 66.81 (C-2), 62.66 (C-6), 28.10 (−C(*C*H$_3$)$_3$), 26.78 (−SiC(*C*H$_3$)$_3$), 19.34 (−Si*C*(CH$_3$)$_3$), 18.35 (−OCH$_2$*C*H$_2$TMS), −1.37 (−Si(CH_3)$_3$). [α]$^{22}_D$ −4.6 ($CHCl_3$, *c* 0.63). Anal. Calcd for $C_{46}H_{66}O_{13}Si_2$: C, 62.56; H, 7.53. Found: C, 62.49; H, 7.64. ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C HSQC.

2-(Trimethylsilyl)ethyl [2-*O*-Acetyl-4,6-*O*-benzylidine-[3-*O*-(*tert*-butoxycarbonyl)methyl]-β-D-galactopyranosyl]-(1→4)-2,3-di-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (9). To a 250-mL, round-bottomed flask equipped with a stir bar, a condenser, rubber septum, and a N_2 inlet were added triol **8b** (4.46 g, 5.0 mmol), CH_2Cl_2 (150 mL), DMAP (3.7 g, 6.0 equiv), and acetic anhydride (2.85 mL, 6.0 equiv). The reaction mixture was stirred and heated at reflux for 20 min. The reaction mixture then was quenched with water and the organic layer washed first with satd aq $NaHCO_3$ and then with water, dried ($NaSO_4$), and concentrated. Purification of the residue by silica gel column chromatography (hexanes/ethyl acetate 10:1 to 1:1) afforded the triacetate **9** as a colorless oil (4.6 g, 92%): ^{1}H NMR (400 MHz, $CDCl_3$) 7.79−7.74 (m, 3H, Ar), 7.47−7.32 (m, 12H, Ar), 5.51 (s, 1H, −C*H*Ph), 5.25 (dd, 1H, H-2′, $J_{2',3'}$ = 10.3 Hz, $J_{2'1'}$ = 8.0 Hz), 5.19 (dd, 1H, H-3, $J_{3,4}$ = 9.8 Hz, $J_{3,2}$ = 9.5 Hz), 4.98 (dd, 1H, H-2, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 8.0 Hz), 4.76 (d, 1H, H-1′, $J_{1',2'}$ = 8.0 Hz), 4.47 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.43 (d br., 1H, H-4′, $J_{4',3'}$ = 3.3 Hz, $J_{4',5'}$ < 1.0 Hz), 4.32 (dd, 1H, H-6′, $J_{6',7'}$ = 12.4 Hz, $J_{6',5'}$ = 1.5 Hz), 4.29 and 3.90 (ABq, 2H, −CH_2CO$_2$*t*-Bu, J_{AB} = 16.8 Hz), 4.12 (dd, 1H, H-4, $J_{4,3}$ = 9.8 Hz, $J_{4,5}$ = 9.8 Hz), 4.06−3.94 (m, 4H, H-6, H-7, H-7′, −OC*H*HCH$_2$TMS), 3.58−3.52 (m, 1H, −OCH*H*CH$_2$TMS), 3.55 (dd, 1H, H-3′, $J_{3',2'}$ = 10.3 Hz, $J_{3',4'}$ = 3.3 Hz), 3.35 (d br, 1H, H-5, $J_{5,4}$ = 9.8 Hz), 3.25 (br, 1H, H-5′), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.86 (s, 3H, Ac), 1.41 (s, 9H, −C(CH_3)$_3$), 1.07 (s, 9H, −Si(Ph)$_2$C(CH_3)$_3$), 1.02−0.94 (m, 2H, −OCH$_2$CH_2TMS), 0.02 (s, 9H, −Si(CH_3)$_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$) 170.69 (R$_2$*C*O), 169.80 (R$_2$*C*O), 169.76 (R$_2$*C*O), 168.71 (R$_2$*C*O), 137.62 (Ar), 136.03 (Ar), 135.50 (Ar), 133.76 (Ar), 132.25 (Ar), 129.86 (Ar), 129.82 (Ar), 129.11 (Ar), 128.21 (Ar), 127.83 (Ar), 127.66 (Ar), 126.66 (Ar), 101.52 (−*C*HPh), 100.54 (C-1′), 100.21 (C-1), 81.61 (−*C*(CH$_3$)$_3$), 78.47 (C-3′), 75.24 (C-5), 74.93 (C-4′), 74.32 (C-4), 72.53 (C-3), 71.76 (C-2), 70.38 (C-2′), 68.75 (C-6′), 67.32 (−*C*H$_2$CO$_2$*t*-Bu), 66.68 (−O*C*H$_2$CH$_2$TMS), 66.52 (C-5′), 61.23 (C-6), 28.07 (−C(*C*H$_3$)$_3$), 26.82 (−Si(Ph)$_2$C(*C*H$_3$)$_3$), 19.40 (−Si(Ph)$_2$*C*(CH$_3$)$_3$), 20.85, 20.80, 20.78, 17.97 (−OCH$_2$*C*H$_2$TMS), −1.32 (−Si(*C*H$_3$)$_3$). [α]$^{23}_D$ +17.6 ($CHCl_3$, *c* 0.86). Anal. Calcd for $C_{52}H_{72}O_{16}Si_2$: C, 61.88; H, 7.19. Found: C, 61.43; H, 7.40. HRMS Calcd for $C_{52}H_{72}O_{16}NaSi_2$: *m*/*z* 1031.4257. Found: *m*/*z* 1031.4222. ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C HSQC.

2-(Trimethylsilyl)ethyl [2-*O*-Acetyl-4,6-*O*-benzylidine-3-*O*-[(*tert*-butoxycarbonyl)methyl]-β-D-galactopyranosyl]-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranoside (10). To a 250-mL, round-bottomed flask equipped with a stir bar, a condenser, a rubber septum, and a N_2 inlet were placed TBDPS ether **9** (1.75 g, 1.73 mmol) and anhydrous THF (100 mL). Acetic acid (0.20 mL, 2.0 equiv) and tetrabutylammonium fluoride (3.46 mL of 1 M solution in THF, 2.0 equiv) were added, and the reaction mixture was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and quenched with water. The organic layer was first washed with satd aq $NaHCO_3$ and then with water. The organic phase was concentrated, and the residue thus obtained was purified by silica gel column chromatography (hexanes/ethyl acetate 2:1) to afford alcohol **10** as a white foam (1.21 g, 91%): ^{1}H NMR (400 MHz, $CDCl_3$) 7.47−7.45 (m, 2H, Ar), 7.37−7.32 (m, 3H, Ar), 5.53 (s, 1H, −C*H*Ph), 5.25 (dd, 1H, H-2′, $J_{2',3'}$ = 10.2 Hz, $J_{2',1'}$ = 8.0 Hz), 5.20 (dd, 1H, H-3, $J_{3,2}$ = 9.9 Hz, $J_{3,4}$ = 9.5 Hz), 4.88 (dd, 1H, H-2, $J_{2,3}$ = 9.9 Hz, $J_{2,1}$ = 8.1 Hz), 4.55 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 4.54 (d, 1H, H-1′, $J_{1',2'}$ = 8.0 Hz), 4.48 (d br., 1H, H-4′, $J_{4',3'}$ = 3.3 Hz, $J_{4',5'}$ < 1.0 Hz), 4.29 and 4.05 (ABq, 2H, H-6′, H-7′, J_{AB} = 12.4 Hz), 4.17 and 4.01 (ABq, 2H, −CH_2-CO$_2$*t*-Bu, J_{AB} = 16.8 Hz), 3.91 (dd, 1H, H-4, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 9.5 Hz), 3.91 and 3.82 (ABq, 2H, H-6, H-7, J_{AB} = 12.1 Hz), 3.93 (ddd, 1H, −OCH*H*CH$_2$TMS, *J* = 9.9, 9.9, 6.6 Hz), 3.69 (dd, 1H, H-3′, $J_{3',2'}$ = 10.2 Hz, $J_{3',4'}$ = 3.3 Hz), 3.54 (ddd, 1H, −*OCH*HCH$_2$TMS, *J* = 9.9, 9.9, 6.6 Hz), 3.41 (m br., 2H, H-5, H-5′), 2.02 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.42 (s, 9H, −C(CH_3)$_3$), 0.96−0.88 (m, 2H, −OCH$_2$CH_2TMS), 0.00 (s, −Si(CH_3)$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 170.59 (R$_2$*C*O), 169.95 (R$_2$*C*O), 169.86 (R$_2$*C*O), 169.16 (R$_2$*C*O), 137.81 (Ar), 129.30 (Ar), 128.40 (Ar), 126.86 (Ar), 101.70 (−*C*HPh), 101.16 (C-1′), 100.63 (C-1), 81.89 (−*C*(CH$_3$)$_3$), 78.35 (C-3′), 75.22 (C-5), 74.94 (C-4′), 74.68 (C-4), 72.79 (C-3), 70.95(C-2′), 70.90 (C-2), 68.90 (C-6′), 67.94 (−O*C*H$_2$CH$_2$TMS), 67.54 (−*C*H$_2$CO$_2$*t*-Bu), 66.75 (C-5′), 60.61 (C-6), 28.29 (−C(*C*H$_3$)$_3$), 21.18 (−O*C*H$_3$), 20.99 (−O*C*H$_3$), 20.93 (−O*C*H$_3$), 18.22 (−OCH$_2$*C*H$_2$TMS), 1.23 (−Si(*C*H$_3$)$_3$). [α]$^{21}_D$ −5.5 ($CHCl_3$, *c* 0.87). Anal. Calcd for $C_{36}H_{54}O_{16}Si$: C, 56.09; H, 7.06. Found: C, 55.85; H, 7.24. ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C HSQC.

Phenyl 2,3,4-Tri-*O*-benzyl-1-thio-β-L-fucopyranose (11). To a 10-mL, round-bottomed flask equipped with a stir bar, rubber septum, and N_2 inlet was added phenyl 1-thio-β-L-fucopyranoside (42 mg, 0.16 mmol) and DMF (3 mL). Sodium hydride (38 mg of 60% suspension in mineral oil, 6.0 mol equiv) was added to the flask and the suspension stirred for 10 min at room temperature. Benzyl bromide (0.11 mL, 6.0 mol equiv) and tetra(*n*-butyl)ammonium iodide (cat.) were added, and the mixture was stirred for 2 h at room temperature. The reaction mixture was then quenched with water (4 mL) and diluted with ether (10 mL). The layers were separated and the aqueous layer was back extracted with ether (10 mL). The combined organic layers were washed with satd aq NH_4Cl and concentrated. The resulting oil was purified by silica gel column chromatography (hexanes → hexanes/ethyl acetate 3:1) to give benzyl ether **11**[18a] as a white solid (74 mg, 90%).

2-(Trimethylsilyl)ethyl [2-*O*-Acetyl-4,6-*O*-benzylidine-3-*O*-[(*tert*-butoxycarbonyl)methyl]-β-D-galactopyranosyl]-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (12). Fucoside **11** (38.0 mg, 0.0700 mmol) and lactoside **10** (64.6 mg, 1.2 equiv) were dissolved in CH_2Cl_2 (5 mL) in a 10-mL, round-bottomed flask containing molecular sieves and equipped with a rubber septum and N_2 inlet. To the flask were added *N*-iodosuccinimide (23 mg, 2.0 equiv) and *p*-TsOH·H_2O (cat.). The mixture became purple instantly, indicating the generation of molecular iodine. After the solution was stirred for 15 min, the reaction mixture was decanted by pipet into 3 mL of aq satd $Na_2S_2O_3$. The organic layer was concentrated by rotary evaporation. The residue was

purified by silica gel column chromatography to give the trisaccharide as a foam (46.9 mg, 56%): ^{1}H NMR (500 MHz, $CDCl_3$) 7.48−7.30 (m, 20H, Ar), 5.31 (s, 1H, −C*H*Ph), 5.15 (dd, 1H, H-3, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 9.5 Hz), 5.09 (dd, 1H, H-2′, $J_{2',3'}$ = 10.0 Hz, $J_{2',1'}$ = 8.1 Hz), 4.99 and 4.69 (ABq, 2H, −CH_2Ph, J_{AB} = 11.5 Hz), 4.94 (d, 1H, H-1″, $J_{1'',2''}$ = 3.4 Hz), 4.92 (dd, 1H, H-2, $J_{2,3}$ = 10.0 Hz, $J_{2,1}$ = 7.8 Hz), 4.88 (s br, 2H, −CH_2-Ph), 4.87 and 4.67 (ABq, 2H, −CH_2Ph, J_{AB} = 11.7 Hz), 4.62 (d, 1H, H-1′, $J_{1',2'}$ = 8.1 Hz), 4.44 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 4.09−3.93 (m, 9H, H-4, H-7, H-5′, H-6′, H-7′, H-2″, H-5″, −CH_2-CO_2*t*-Bu), 3.91−3.87 (m, 1H, −OC*H*HCH_2TMS), 3.85 (dd, 1H, H-4′, $J_{4',3'}$ = 3.2 Hz, $J_{4',5'}$ < 1.0 Hz), 3.75 (dd, 1H, H-4″, $J_{4'',3''}$ = 1.7 Hz, $J_{4'',5''}$ < 1.0 Hz), 3.71 (dd, 1H, H-6, $J_{6,7}$ = 10.5 Hz, $J_{6,5}$ = 2.2 Hz), 3.58−3.50 (m, 1H, −OCH*H*CH$_2$TMS), 3.43 (dd, 1H, H-5, $J_{5,4}$ = 9.7 Hz, $J_{5,6}$ < 1.0 Hz), 3.35 (dd, 1H, H-3′, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 3.2 Hz), 2.12 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.39 (s, 9H, −C(CH_3)$_3$), 1.10 (d, 3H, H-6″, $J_{6'',5''}$ = 6.3 Hz), 0.92−0.86 (m, 2H, −OCH$_2$CH_2TMS), −0.02 (s, 9H, −Si(CH_3)$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 171.91 (R_2CO), 170.99 (R_2CO), 170.32 (R_2CO), 168.20 (R_2CO), 140.44 (Ar), 139.90 (Ar), 139.19 (Ar), 130.36 (Ar), 129.91 (Ar), 129.85 (Ar), 129.84 (Ar), 129.73 (Ar), 129.62 (Ar), 129.52 (Ar), 129.22 (Ar), 129.16 (Ar), 129.13 (Ar), 128.99 (Ar), 128.94 (Ar), 127.97 (Ar), 101.24 (−*C*HPh), 100.26 (C-1), 99.93 (C-1′), 96.68 (C-1″), 81.29 (−*C*(CH$_3$)$_3$), 79.31, 77.89 (C-3′), 77.30, 74.93 (−*C*H$_2$Ph), 74.27 (C-4′), 73.94, 73.86, 73.81 (C-5), 72.68, 72.47 (C-3), 71.68 (C-2), 70.65 (C-2′), 68.63 (C-3″), 68.63 (overlap), 66.86, 66.52, 66.16, 65.65, 63.11 (C-6), 27.99 (−C(*C*H$_3$)$_3$), 21.11 (Ac), 20.74 (Ac), 20.65 (Ac), 17.90 (−OCH$_2$*C*H$_2$TMS), 16.48 (C-6″), −1.44 (−Si(*C*H$_3$)$_3$). [α]$^{20}_D$ −20.4 ($CHCl_3$, *c* 0.99). HRMS Calcd for $C_{63}H_{82}O_{20}NaSi$: *m*/*z* 1209.5066. Found: *m*/*z* 1209.5073. ^{1}H−^{13}C HSQC.

2-(Trimethylsilyl)ethyl [2-*O*-Acetyl-4,6-*O*-benzylidine-3-*O*-[(*tert*-butoxycarbonyl)methyl]-β-D-galactopyranosyl]-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-fucosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (13a). To a 5-mL, round-bottomed flask equipped with a stir bar and balloon was added **12** (0.047 g, 4.0×10^{-2}mmol), ethanol (4 mL, absolute, distilled), and 10% Pd/C (8.5 mg, 20 mol %); the balloon was filled with H_2 four times over a 23-h period. The reaction mixture was filtered through Celite, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (4 mL). To the solution was added triethylamine (excess), acetic anhydride (excess), and DMAP (cat.). The reaction mixture was allowed to stir for 1.25 h at room temperature. Column chromatography of the reaction mixture gave hexaacetate **13a** as a colorless film (24 mg, 57%, 2 steps): ^{1}H NMR (500 MHz, $CDCl_3$) 7.48−7.46 (m, 2H, Ar), 7.36−7.34 (m, 3H, Ar), 5.53 (s, 1H, −C*H*Ph), 5.37 (dd, 1H, H-3″, $J_{3'',2''}$ = 10.7 Hz, $J_{3'',4''}$ = 3.4 Hz), 5.31 (dd, 1H, H-4″, $J_{4'',3''}$ = 3.4 Hz, $J_{4'',5''}$ = 1.0 Hz), 5.23 (dd, 1H, H-2′, $J_{2',3'}$ = 10.3 Hz, $J_{2',1'}$ = 8.0 Hz), 5.19 (dd, 1H, H-3, $J_{3,2}$ = 9.7 Hz, $J_{3,4}$ = 9.5 Hz), 5.16 (d, 1H, H-1″, $J_{1'',2''}$ = 3.6 Hz), 5.12 (dd, 1H, H-2″, $J_{2'',3''}$ = 10.7 Hz, $J_{2'',1''}$ = 3.6 Hz), 4.88 (dd, 1H, H-2, $J_{2,3}$ = 9.7 Hz, $J_{2,1}$ = 8.0 Hz), 4.57 (d, 1H, H-1′, $J_{1',2'}$ = 8.0 Hz), 4.47 (dd, 1H, H-4′, $J_{4',3'}$ = 3.4 Hz, $J_{4',5'}$ < 1.0 Hz), 4.46 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.35−4.32 (m, 2H, H-5″, H-7′), 4.18 and 3.98 (ABq, 2H, −CH_2-CO_2*t*-Bu, J_{AB} = 16.8 Hz), 4.08 (d br, 1H, H-6′, $J_{6',7'}$ = 10.9 Hz), 3.97−3.91 (m, 1H, −OCH*H*CH$_2$TMS), 3.93−3.84 (m, 2H, H-6, H-7), 3.81 (dd, 1H, H-4, $J_{4,5}$ = 9.7 Hz, $J_{4,3}$ = 9.5 Hz), 3.69 (dd, 1H, H-3′, $J_{3',2'}$ = 10.3 Hz, $J_{3',4'}$ = 3.4 Hz), 3.54−3.48 (m, 3H, H-5, H-5′, −OC*H*HCH$_2$TMS), 2.18, 2.10, 2.07, 2.03, 2.02, 2.01, 1.39 (s, 9H, −OC(CH_3)$_3$), 1.12 (d, 3H, −CH_3, $J_{6'',5''}$ = 6.3 Hz), 0.98−0.83 (m, 2H, −CH$_2$CH_2TMS), 0.00 (s, −Si(CH_3)$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 170.50 (R_2CO), 170.49 (R_2CO), 170.27 (R_2CO), 169.82 (R_2CO), 169.81 (R_2CO), 169.61 (R_2CO), 168.97 (R_2CO), 137.67 (Ar), 129.08 (Ar), 128.20 (Ar), 126.61 (Ar), 101.41 (−*C*HPh), 100.59 (C-1′), 100.29 (C-1), 96.46 (C-1″), 81.61 (−*C*(CH$_3$)$_3$), 77.95 (C-3′), 75.07 (C-4), 74.70 (C-4′), 74.31, 72.56 (C-3), 71.93 (C-2), 71.36 (C-4″), 70.74 (C-2′), 68.87 (C-6′), 68.87 (C-2″), 67.70 (C-3″), 67.21 (−OCH$_2$CH$_2$TMS), 66.80 (−*C*H$_2$CO$_2$*t*-Bu), 66.55, 66.27 (C-6), 64.90 (C-5″), 28.07 (−C(*C*H$_3$)$_3$), 21.01, 21.01, 20.73, 20.73, 20.73, 20.64, 17.93 (−OCH$_2$*C*H$_2$TMS), 15.72 (C-6″), −1.38 (−Si(*C*H$_3$)$_3$). HRMS. Calcd for $C_{48}H_{70}O_{23}NaSi$: *m*/*z* 1065.3975. Found: *m*/*z* 1065.4015. ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C HSQC.

2-(Trimethylsilyl)ethyl [2,4,6-Tri-*O*-acetyl-3-*O*-(*tert*-butoxycarbonyl)methyl-β-D-galactopyranosyl]-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-fucosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (13b). Triacetate **12** (46.9 mg, 0.0487 mmol) was placed in a 10-mL, round-bottomed flask equipped with a stir bar. To the flask were added methanol (distilled, 6 mL) and 10% Pd/C (53 mg, 1.0 mol equiv). The flask was equipped with a gas inlet adapter and H_2-filled balloon. The suspension was stirred for 2 d with replenishment of the H_2 balloon when necessary. The reaction mixture was then passed through a short column of Celite to remove the particulates, and the solvent was evaporated. The resulting residue was dissolved in CH_2Cl_2 (3 mL) and stirred with triethylamine (69 μL, 10 mol equiv), acetic anhydride (46 μL, 10 mol equiv), and DMAP (cat.). The mixture was stirred at room temperature under an atmosphere of nitrogen for 2 h until TLC analysis indicated that no starting material remained. The reaction mixture was quenched with the addition of water, and the organic layer was concentrated. Purification of the crude material by silica gel column chromatography (CH_2Cl_2) gave the octaacetate **13b** as a colorless film (37 mg, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) 5.39 (dd, 1H, H-4′, $J_{4',3'}$ = 3.4 Hz, $J_{4',5'}$ < 1.0 Hz), 5.35 (dd, 1H, H-3″, $J_{3'',2''}$ = 10.5 Hz, $J_{3'',4''}$ = 3.1 Hz), 5.32 (dd, 1H, H-4″, $J_{4'',3''}$ = 3.4 Hz, $J_{4'',5''}$ < 1.0 Hz), 5.18 (dd, 1H, H-3, $J_{3,2}$ = 9.7 Hz, $J_{3,4}$ = 9.2 Hz), 5.16 (d, 1H, H-1″, $J_{1'',2''}$ = 3.6 Hz), 5.12 (dd, 1H, H-2″, $J_{2'',3''}$ = 10.5 Hz, $J_{2'',1''}$ = 3.6 Hz), 4.99 (dd, 1H, H-2′, $J_{2',3'}$ = 10.0 Hz, $J_{2',1'}$ = 8.1 Hz), 4.86 (dd, 1H, H-2, $J_{2,3}$ = 9.7 Hz, $J_{2,1}$ = 8.1 Hz), 4.54 (d, 1H, H-1′, $J_{1',2'}$ = 8.1 Hz), 4.45 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 4.26 (dq, 1H, H-5″, $J_{5'',6''}$ = 6.6 Hz, $J_{5'',4''}$ < 1.0 Hz), 4.14−4.11 (m, 2H, H-6′, H-7′), 3.98−3.90 (m, 2H, H-6, −OCH*H*CH$_2$TMS), 3.89 and 3.29 (ABq, 2H, −CH_2CO$_2$*t*-Bu, J_{AB} = 17.1 Hz), 3.78−3.74 (m, 2H, H-4, H-7), 3.81 (ddd br, 1H, H-5′, $J_{5',6'}$ = 7.3 Hz, $J_{5',7'}$ = 6.8 Hz, $J_{5',4'}$ < 1.0 Hz), 3.61 (dd, 1H, H-3′, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 3.4 Hz), 3.54−3.49 (m, 2H, H-5, −OC*H*HCH$_2$TMS), 2.17 (s, 3H, Ac), 2.14 (s, 6H, Ac), 2.08 (s, 6H, Ac), 2.04 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.44 (s, 9H, −OC(CH_3)$_3$), 1.15 (d, 3H, −CH_3, $J_{6'',5''}$ = 6.6 Hz), 1.00−0.83 (m, 2H, −CH$_2$CH_2TMS), 0.00 (s, −Si(CH_3)$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 175.75 (R_2CO), 175.24 (R_2CO), 173.16 (R_2CO), 170.70 (R_2CO), 170.32 (R_2CO), 170.20 (R_2CO), 170.68 (R_2CO), 169.82 (R_2CO), 169.80 (R_2CO), 100.77 (C-1′), 100.08 (C-1), 96.50 (C-1″), 81.53 (−*C*(CH$_3$)$_3$), 78.26 (C-3′), 75.92 (C-4), 74.21 (C-5), 73.17 (C-3), 72.06 (C-2), 71.27 (C-4″), 70.92 (C-2′), 70.67 (C-5′), 68.63 (C-2″), 67.85 (C-3″), 67.25 (−O*C*H$_2$-CH$_2$TMS), 66.43 (−*C*H$_2$CO2*t*-Bu), 66.22 (C-6), 65.42 (C-4′), 64.81 (C-5″), 61.49 (C-6′), 28.12 (−C(*C*H$_3$)$_3$), 21.01, 20.90, 20.84, 20.79, 20.78, 20.68, 20.67, 20.64, 17.91 (−OCH$_2$*C*H$_2$TMS), 15.79 (C-6″), −1.39 (−Si(*C*H$_3$)$_3$). [α]$^{20}_D$ −6.1 ($CHCl_3$, *c* 0.87). HRMS. Calcd for $C_{45}H_{70}O_{25}NaSi$: *m*/*z* 1061.3873. Found: *m*/*z* 1061.3844. ESIHRMS. Calcd for $C_{45}H_{70}O_{25}NaSi$: *m*/*z* 1061.3873. Found: *m*/*z* 1061.3868. ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C HSQC.

2-(Trimethylsilyl)ethyl 3-*O*-Carboxymethyl-β-D-galactopyranosyl-(1→4)-[α-L-fucosyl-(1→6)]-β-D-glucopyranoside (2a). Octaacetate *tert*-butyl ester **13b** (16 mg, 1.6×10^{-2} mmol) was dissolved in dry diethyl ether (4 mL). Water (10.1 μL, 36 mol equiv) and potassium *tert*-butoxide (63 mg, 36 mol equiv) were added. The resulting slurry was stirred for 14 h at room temperature until TLC analysis indicated that the starting material had been consumed. The reaction mixture was quenched by the addition of water (2 mL). The aqueous phase was acidified to pH 5 with cationic acid-exchange resin. The resin was removed by filtration, and the filtrate was concentrated. The syrup thus obtained was purified by gravity chromatography with water as the eluent (Sephadex G-10, 1-cm-diameter column, 3-in. high). The water was removed under high vacuum to give the trisaccharide **2a** as a white powder (19 mg): ^{1}H NMR (500 MHz, D_2O) 4.77 (d,1H, H-1″, $J_{1'',2''}$ = 3.6 Hz), 4.41 (d, 1H, H-1′, $J_{1',2'}$ = 7.8 Hz), 4.35 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 3.97 (dq, 1H, H-5″, $J_{5'',6''}$ = 6.6 Hz, $J_{5'',4''}$

<1.0 Hz), 3.94−3.92 (m, 3H, H-4′, −CH_2), 3.89−3.82 (m, 2H, −C*H*H, −C*H*HCH$_2$TMS), 3.77 (dd, 1H, H-3″, $J_{3'',2''}$ = 10.2 Hz, $J_{3'',4''}$ = 2.9 Hz), 3.72−3.50 (m, 9H, H-4, H-5, H-5′, H-2“, H-4”, −CH*H*, −CH_2, −OCH*H*CH$_2$TMS), 3.49−3.32 (m, 2H, H-3, H-2′), 3.34 (dd, 1H, H-3′, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 2.7 Hz), 3.15 (dd, 1H, H-2, $J_{2,3}$ = 8.3 Hz, $J_{2,1}$ = 8.1 Hz), 1.08−0.95 (m, 1H, −OCH$_2$C*H*HTMS), 0.85−0.78 (m, 1H, −OCH$_2$CH*H*TMS), −0.16 (s, 9H, −Si(CH_3)$_3$); ^{13}C NMR (100.6 MHz, D2O) 86.16 (R$_2$*C*O), 103.02 (C-1′), 102.03 (C-1), 99.58 (C-1″), 82.31 (C-3′), 78.12, 75.46, 74.82, 74.01, 73.35 (C-2), 72.30, 70.22, 69.90, 69.88 (C-3″), 68.74 (−*C*H$_2$), 68.56 (−O*C*H$_2$CH$_2$TMS), 67.04 (C-5″), 66.72 (−*C*H$_2$), 65.76 (C-4′), 61.60 (−*C*H$_2$), 18.08 (−OCH$_2$*C*H$_2$TMS), 15.86 (C-6″), −2.43 (−Si(*C*H$_3$)$_3$). $[\alpha]^{20}_D$ −10.7 (H$_2$O, *c* 0.46). ESIHRMS. Calcd for $C_{25}H_{46}O_{17}SiNa$: *m*/*z* 669.2402. Found: *m*/*z* 669.2445. ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C HSQC**.**

Determination of E-Selectin/Leukocyte Inhibition. One week prior to performing the rolling assay, the tissue culture plates to be used with the parallel plate flow assembly were coated with recombinant human E-selectin. Each plate (35 mm, polystyrene) was spotted with 40 μL of a solution of Protein A (Sigma) in Tris-HCl buffer (20 μg/mL, pH 9.0) and incubated for 18 h at 4 °C. The Protein A solution was removed (aspiration) and each plate was washed with TBS buffer (3 × 40 μL). Each plate was spotted with 80 μL of BSA/TBS/NaN_3 blocking buffer (pH 7.5) and incubated for 3 h at room temperature. The blocking buffer was removed (aspiration) and each plate was washed with TBS buffer (5 × 80 μL). The plates were spotted with varying concentrations of chimera IgG-human E-selectin (IgG-hE-sel) in BSA/TBS/NaN_3 buffer (3 plates each at dilutions of 1:10, 1:20, and 1:30). The plates were incubated for 2 h at 4 °C, washed with TBS buffer (3 × 40 μL), and stored over BSA/TBS/NaN_3 buffer at 4 °C until use. Human polymorphonuclear leukocytes were collected from fresh blood (20 mL) and suspended in rolling media (0.2% BSA/HEPES/2 mM $CaCl_2$) at a concentration of 1 × 10^6 cells/mL and used within 4 h of isolation.

The experimental apparatus was assembled as described by Lawrence.[21] A culture plate coated with the 1:10 dilution of IgG-hE-sel solution (which was determined to give the optimum number of binding events by prior analysis) was mounted on the flow chamber and clamped to the stage of the inverted-stage microscope. Solutions of the test compounds in water (10 mM) and a test solution containing no inhibitor were prepared. Each test solution (100 μL) was combined with rolling media (400 μL) and neutrophil suspension (500 μL) and agitated immediately before analysis. One-third of the resulting mixture was transferred to the assay inlet. The mixture was perfused through the chamber at a shear rate of 0.38 dynes/cm^2 for 2 min and the rolling action recorded on video. Each experiment was performed in triplicate and the chamber was washed with rolling media between each run.

The video was analyzed at snapshot intervals of 60, 90, and 120 s for each run. Interacting cells appeared as spherical, well-defined objects whereas noninteracting flowing cells appeared as long horizontal segmented streaks. The number of interacting cells was tabulated for each shot snapshot image and the average of three runs for each test suspension was calculated.

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Supporting Information Available: NMR spectral data (^{1}H, ^{13}C, ^{1}H−^{1}H gCOSY, and HSQC) for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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