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SYNTHESIS OF A GALACTOSE-FUCOSE DISACCHARIDE MIMIC OF SIALYL LEWIS X

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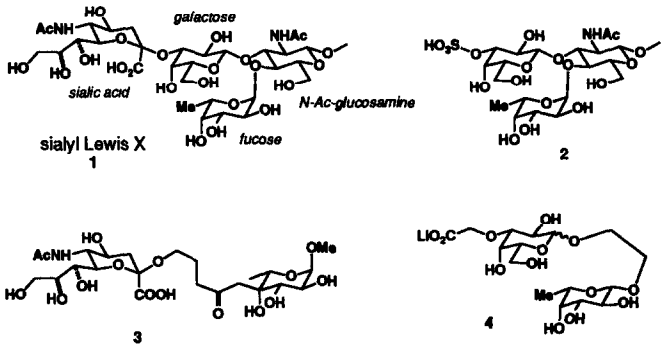
Abstract: Disaccharide **4** was designed as a structurally simplified, potential mimic of sialyl Lewis X (**1**). **4** was prepared utilizing Schmidt's trichloroacetimidate methodology in 10 linear steps from methyl galactoside **5**. **4** exhibited activity in an E-selectin binding assay at concentrations 40 to 45-fold higher than that observed for monomeric sialyl Lewis X.

Introduction

Sialyl Lewis X (sLe^x, **1**) has been suggested to play a role in cell adhesion through interactions with its putative receptor, E-selectin.[1] Cell adhesion plays a pivotal role in several disease states, including arthritis, asthma, and cancer.[2] For example, the recruitment of neutrophils to the site of injured tissue, an important component of a variety of inflammatory processes, is thought to possibly be mediated by the interaction of E-selectin with sLe^x.[1] Thus, blocking the sLe^x / E-selectin interaction is an attractive strategy for treatment of neutrophil mediated inflammatory diseases such as arthritis. We are interested in designing simplified sLe^x analogs which retain the ability to block neutrophil rolling along the endothelium, but which have smaller molecular weights and are more synthetically accessible; this paper describes the design and synthesis of one such mimic, disaccharide **4**.

Feizi et al. have shown that sLe^x retains its activity as an antagonist when the sialic acid residue is removed if an acidic group is retained at C-3 of the galactose (e.g. sulfate **2**).[3] Other workers

Figure 1

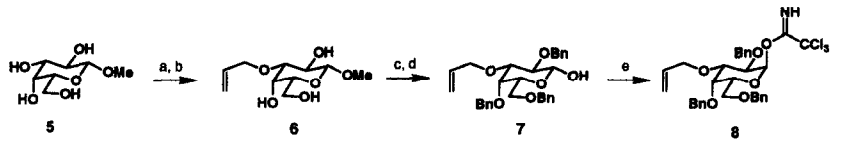


have shown that the galactose and fucose moieties are critical, but that the N-acetylglucosamine tolerates some variation (e.g. reductive opening of the pyranoside or replacement with a simple glucose residue).[4] These observations led us to design disaccharide mimic **4**, in which a 3-substituted galactose and a fucose are linked through a simple two carbon tether (our initial synthetic target was the analog of **4** in which C-1 of the fucose moiety is the axial, or α anomer; an unexpected β-selective glycosylation forced us to alter the stereochemistry at this position). Following the initiation of this project, Allanson and co-workers reported the preparation of disaccharide mimic **3**, in which they linked the sialic acid and fucose residues through a five carbon tether.[5]

Results and Discussion

Preparation of the differentially 3-substituted galactose moiety is shown in Scheme 1.[6] Selective allylation of the 3-hydroxyl of methyl galactoside was achieved *via* the stannylene acetal, providing **6** in 87% yield.[7] The remaining hydroxyls were masked as the corresponding benzyl ethers (84% yield), and the methyl glycoside was removed *via* acid hydrolysis (62% yield) to provide 3-allyl-2,4,6-tribenzyl galactose (**7**). Schmidt's trichloroacetimidate glycosidation methodology[8] had worked well in our hands in ealier applications, leading us to utilize it in the present study. Thus, treatment of **7** with trichloroacetonitrile, K_2CO_3, and NaH in CH_2Cl_2 provided trichloroacetimidate **8**, as an 8:1 mixture of axial / equatorial acetimidate anomers (100% yield).[9]

Scheme 1

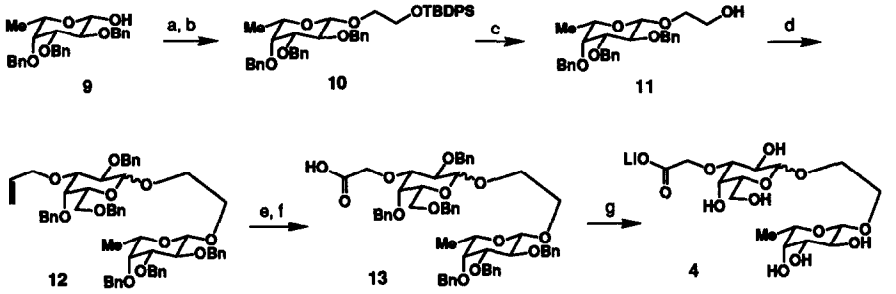


5 6 7 8

Key: (a) Bu_2SnO, PhH; (b) allyl bromide, Bu_4NI, PhH (87% overall); (c) NaH, $PhCH_2Br$, DMF (84%); (d) 1N HCl, aq AcOH, 100 °C (62%) (e) Cl_3CCN, K_2CO_3, NaH, CH_2Cl_2 (100%).

Preparation of the fucose portion of the target began with 2,3,4-tribenzyl fucose, which was prepared by the published synthesis.[10] Treatment with trichloroacetonitrile, K_2CO_3, and NaH in CH_2Cl_2 provided the corresponding trichloroacetimidate as a 4:1 mixture of axial / equatorial anomers (Scheme 2). This material was then coupled with the t-butyldiphenylsilyl ether of ethylene glycol (prepared from TBDPSCl, imidazole and ethylene glycol in DMF in 86% yield), using 10 mol% triflic acid in toluene at -20 °C (syringe pump addition of the TfOH in toluene over 70 min).[11] Based on a literature precedent with the same trichloroacetimidate,[9] we had anticipated that the glycosylation would proceed with net retention of configuration to provide the axial glycosylation product. Thus, we were surprised to find the major coupling product (4:1 ratio) to be the equatorial anomer **10**. (This assignment was based on the H_1-H_2 coupling constants: the major product exhibited a 7.7 Hz coupling, whereas the minor, axial anomer displayed a 3.9 Hz coupling). This result demonstrates the sensitivity of these glycosylations to solvent and catalyst; whereas the earlier glycosylation[9] proceeded primarily

Scheme 2



Key: (a) Cl_3CCN, K_2CO_3, NaH, CH_2Cl_2 (100%); (b) $HOCH_2CH_2OSi^tBuPh_2$ (1.5 eq), TfOH (10 mol%), $PhCH_3$, -20 °C (65%); (c) Bu_4NF, THF (62%); (d) **8** (1.6 eq), TfOH (10 mol%), $PhCH_3$, -20 °C (76%); (e) OsO_4 (5 mol%), N-methylmorpholine-N-oxide (2.0 eq), aq THF (61%); (f) $NaIO_4$ (1.1 eq), then $AgNO_3$ (2.5 eq), KOH (5.0 eq), aq THF (84% overall); (g) 40 psi H_2, $Pd(OH)_2$ / C, EtOH, then LiOH, MeOH, aq THF.

(9:1) with retention of stereochemistry (consistent with an S_N1 type mechanism), our coupling appears to be proceeding with inversion of configuration (consistent with an S_N2 type mechanism). One explanation for this dichotomy is the difference in solvent polarity (toluene in the present study, *vs.* Et_2O in the earlier work). It should be noted that in Myers' studies on the catalytic TfOH-mediated Schmidt glycosidation, he also observed net inversion of stereochemistry (albeit with a 2-azidoglucosyl rather than a fucosyl trichloroacetimidate).[11]

Removal of the silyl ether was effected by treatment with Bu_4NF in THF, providing alcohol **11** in 62% yield (deprotection with NaOH in EtOH[12] was also investigated, but after 3 days had proceeded in only 20% yield, with 55-60% recovered starting material). The final glycosidation was also mediated using Schmidt's trichloroacetimidate methodology; coupling of alcohol **11** with 1.6 equivalents of acetimidate **8** in toluene with catalytic triflic acid proceeded in 76% yield. The product was found to be a 3:2 mixture of equatorial / axial anomers at the C-1 of galactose, which could not be separated by chromatography.[13] Disaccharide **12** was thus carried on as a mixture of galactose anomers.[14] Oxidative cleavage of the terminal olefin could be realized by exposure to catalytic $RuCl_3$ and $NaIO_4$ in CCl_4-CH_3CN-H_2O[15] in low yield (<20%), or by a more efficient three-step procedure: dihydroxylation with OsO_4 / NMO (61% yield) followed by $NaIO_4$ cleavage to the aldehyde and immediate oxidation to the acid with $AgNO_3$ / KOH (84% yield).[16] Hydrogenolysis of the benzyl ethers was effected by treatment with Pearlman's catalyst in EtOH under 40 psi H_2 to provide the desired disaccharide **4** as a mixture of free acid and ethyl ester; this material was then saponified (LiOH, MeOH, aq THF) to provide the lithium carboxylate (**4**) as a viscous, glassy foam.

This material was tested at concentrations of 0.050 to 100 mM in a static assay which measured the Ca^{++} mediated binding of HL-60 cells to E-selectin adsorbed onto a multi-well plate. **4** was found to inhibit calcium-dependent binding at 40 to 45-fold higher concentrations than monomeric sialyl Lewis X; this is approximately two-fold less active than compound **3** (which displayed inhibition at 25 to 30-fold higher concentrations than sialyl Lewis X).[5] The reduced potency of **4** (as well as **3**)

suggests that the increased conformational flexibility arising from the tether connecting the two pyranose rings leads to an increase in entropic penalties for binding. The relatively weak inhibition displayed by disaccharides **3** and **4** is also consistent with the hypothesis that multiple sLe^x epitopes must be presented for efficient binding to E-selectin.[17]

Acknowledgements: The authors gratefully acknowledge Mr. Phil Reiche for timely assistance in obtaining mass spectral data on numerous intermediates, Dr. Earl Whipple for ^1H NMR assignments of intermediates **10** and **12**, and Dr. Chris Gabel for testing compound **4** in the E-selectin binding assay as well as for numerous insights and stimulating discussions. Professor Andrew Myers (Cal Tech) provided valuable advice concerning the TfOH-mediated glycosylations.

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14. While there is no direct literature precedent for the coupling of acetimidate **8**, there are at least two examples of Schmidt glycosidations with the corresponding perbenzylated galactose derivative. These couplings displayed widely varying levels of stereoselectivity, from 7:1 equatorial:axial ($BF_3{\cdot}OEt_2$ in CH_2Cl_2-hexane, ref. 14a), to 1:4 equatorial:axial (TMSOTf in ether, ref. 14b): (a) Kinzy, W.; Schmidt, R. R. *Carbohydr. Res.* **1987**, *164*, 265. (b) Schaubach, R.; Hemberger, J.; Kinzy, W. *Liebigs Ann. Chem.* **1991**, 607.
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