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SYNTHESIS OF SULFO AND SIALYL LEWIS^X ANALOGS WITH A MANNOSE AT THE REDUCING END

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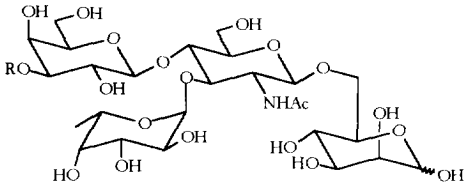
Abstract: Stereoselective syntheses of 3-O-sulfo and 3-O-sialyl Lewis^X analogs **1** and **2** were accomplished. Compound **1** showed greater inhibitory activity than the natural ligand sialyl Lewis^X against P and L-selectin.

The recruitment of leukocytes to sites of inflammation is mediated by multiple adhesion molecules, such as integrins and super-immunoglobulins which interact on the basis of protein - protein recognition.[1] Selectins, a third family of leukocyte adhesion receptors, are involved in an early state of the leukocyte recruitment cascade, the so-called rolling.[2] In contrast to integrins and super-immunoglobulins, selectins recognize complex carbohydrate structures in a Ca^{2+}-dependent manner. It is well known that complex glycoproteins bearing the sialyl Lewis^X epitope [NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc] are ligands of E-, P- and L-selectin.

A variety of sialyl Lewis^X analogs have already been synthesized.[3] Many studies indicate that sialic acid and fucose residues are essential for recognition, and, that a sulfate ester can replace sialic acid in some instances.[4] Interestingly, mannose-6-phosphate binds to L-selectin with affinity comparable to that of sialyl Lewis^X, and the E-selectin lectin domain is remarkably similar to the lectin domain determined for the mannose binding protein.[5,6] These findings suggested that a mannose residue could be beneficial for selectin binding.

Figure 1



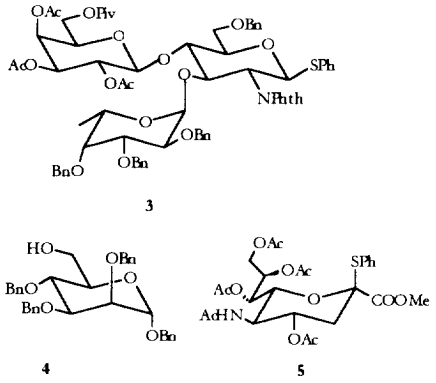
1 R = SO_3Na
2 R = NeuAc

We initially synthesized 3-sulfo Lewis^X with Man at the reducing end (**1**). Preliminary selectin inhibitory studies indicated that compound **1** was 1.5-fold and 2-fold more active than sialyl Lewis^X against L- and P-selectin, respectively. In light of these results we were encouraged to synthesize the 3-sialyl Lewis^X 1,6-linked Man structure (**2**) for comparative studies. Additionally, these molecules when linked to protein can be employed as immunogens to generate antibodies against sulfo Lewis^X or sialyl Lewis^X.[7,8] This communication reports the chemical synthesis of target molecules **1** and **2** (Fig. 1).

In designing our approach to the target molecules we decided to prepare a Lewis^x donor **3** and subsequently link this building block to the 6-hydroxy mannose acceptor **4** (Fig. 2). A pivaloyl group at the 6-position of galactose in **3** enabled us to introduce a sulfate or sialic acid residue at the 3-position after selective removal of acetate.

The donor **3** was prepared by a procedure reported from this laboratory with further modification, as depicted in Scheme 1.[9] The glycosyl donor **6** was coupled with the acceptor **7**[10] ($AgOTf\text{-}SnCl_2$) to form, selectively, the β-linked 1→4 glycoside **9** along with a small amount of 1→3 glycoside **8** in 71% yield with **8**:**9**=

Figure 2

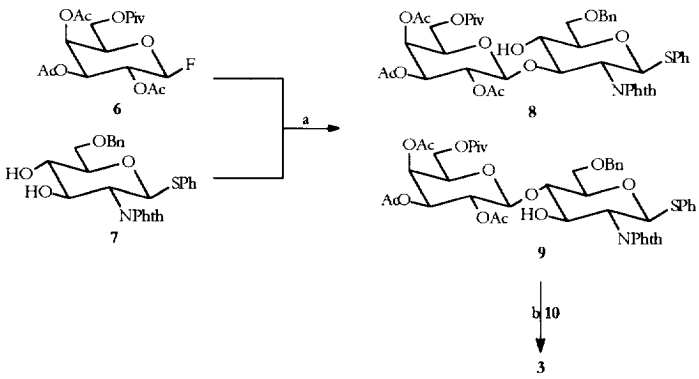


3 4 5

1:4. The disaccharide **9** was reacted with methyl 2, 3, 4-tri-O-benzyl-1-thio-L-fucopyranoside **10** ($CuBr_2$-Bu_4NBr) to give, stereoselectively, trisaccharide **3** (60%) with the desired α-fucose anomeric linkage.

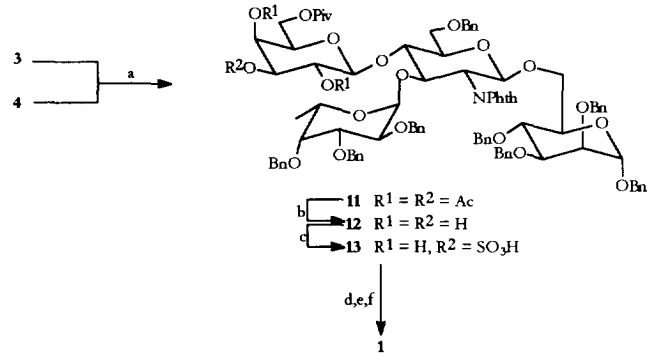
The synthesis of the sulfated and sialylated analogs were accomplished as described in Scheme 2 and Scheme 3, respectively. Thus, glycosylation of mannose acceptor **4** with Lewis^x donor **3** was carried out in CH_2Cl_2 with NIS-TfOH as mediators. Selective deacetylation of tetrasaccharide **11** with NaOMe in MeOH-CH_2Cl_2 (v/v, 1:1) at 0 °C afforded the 2,3,4-triol derivative **12** (44% from **3**), which was converted to the 3-O-sulfated compound **13** in 85% yield by exposure to SO_3-pyridine complex in anhydrous pyridine. Removal of both the phthalimido and acetate groups from **13** by treatment with $NH_2NH_2 \cdot H_2O$ followed by acetylation of the generated amino group gave the amide. Final deprotection to produce the target molecule **1** was acheived by hydrogenolysis (51% from **13**).[11]

Scheme 1



Reagents and conditions: (a) 1.7 equiv of **6**, 3.0 equiv of $SnCl_2$, 3.0 equiv of AgOTf, CH_2Cl_2-toluene (v/v, 5:1), -20 °C→rt, 4 h, 71%, **8**:**9** = 4:1; (b) 2.0 equiv of **10**, 2.0 equiv of $CuBr_2$, 2.0 equiv of Bu_4NBr, $ClCH_2CH_2Cl$:DMF (v/v, 5:1), rt, 16 h, 60%.

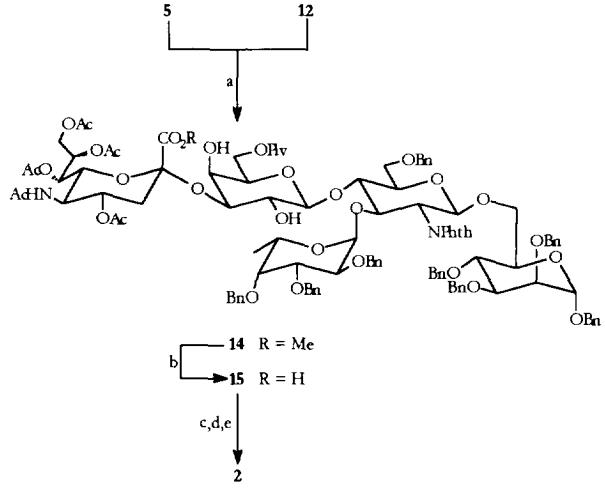
Scheme 2



Reagents and conditions: (a) NIS-TfOH, CH_2Cl_2, -75 °C, 2 h; (b) NaOMe, MeOH:CH_2Cl_2 (v/v, 1:1), 0 °C, 3 h, 44% from **3**; (c) 20 equiv of SO_3-pyridine complex, pyridine, 5 °C, 48 h, 85%; (d) hydrazine hydrate-MeOH (v/v, 5:1), 80 °C, 6 h; MeOH:Et_3N:Ac_2O (4:2:1), rt, 2 h; (e) NaOMe-MeOH, rt, 48 h; (f) MeOH, H_2, 10% Pd-C, rt, 24 h; Na^+ resin, 51% from **13**.

In the synthesis of the sialylated saccharide **2** glycosylation of **12** with sialic acid donor **5** promoted with NIS-TfOH at -75 °C led to the formation of α-sialic acid glycoside product **14** in 40% yield. The conversion of the methyl ester in **14** into free carboxylic acid derivative **15** was accomplished with lithium iodide-pyridine in 75% yield. Deprotection of **15** gave **2** (24% from **15**) following procedures similar to that described above for the synthesis of **1** from **13**. The structures of compounds **1** and **2** were confirmed by NMR and MS. Selectin inhibitory studies of **1** and **2**, along with a series of other compounds, are being conducted in the laboratory of Dr. Ajit Varki. The details of those investigations will be reported elsewhere.

Scheme 3



Reagents and conditions: (a) 3.0 equiv of **5**, NIS-TfOH, CH_3CH_2CN, -75 °C, 3 h, 40%; (b) 8.0 equiv of LiI, pyridine, 120 °C, 4 h, 75%; (c) hydrazine hydrate-MeOH (v/v, 5:1), 80 °C, 6 h; MeOH:Et_3N:Ac_2O (4:2:1), rt, 2 h; (d) NaOMe-MeOH, rt, 48 h; (e) MeOH, H_2, 10% Pd-C, rt, 48 h, 24% from **15**.

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10. Compound **7** was prepared from phenyl 2-deoxy-2-phthalimido-1-thio-D-glucoside in 70% yield under the following condition[1.1 equiv $(Bu_3Sn)_2O$, toluene, reflux, 4 h; 3.0 equiv BnBr, 1.1 equiv Bu_4NBr, 80°C, 16 h] instead of 2-steps procedure, Jain, R. K; Matta, K. L. *Carbohydr. Res.* **1992**, *226*, 91.
11. Compounds **1** and **2** were characterized by ^1H, ^{13}C NMR and MS data. Selected data: **1**. $[\alpha]_D$ +0.5° (*c* 0.82, H_2O), ^1H NMR (D_2O, 400 MHz) δ 5.14 (d, 1H, H-1''', *J* = 3.9 Hz), 4.59 (d, 1H, H-1'', *J* = 7.7 Hz), 2.07 (s, 3H, NHAc), 1.20 (d, 3H, H-6'', *J* = 6.5 Hz); ^{13}C NMR (D_2O, 100.6 MHz) δ 100.6 (C-1'''), 100.4 (C-1'), 97.6 (C-1''), 93.2 (C-1), 79.3 (C-3'''), 60.4 (C-6'''), 58.9 (C-6'), 54.9 (C-2'), 21.4 (NHAc), 14.3 (C-6''), MS (m/z): 770.3 $(M-Na)^-$; **2**. $[\alpha]_D$ +10.3° (*c* 0.41, H_2O); ^1H NMR (D_2O, 400 MHz) δ 5.15 (s, 1H, H-1'''), 4.55 (d, 1H, H-1'', *J* = 8.0 Hz), 2.80 (dd, 1H, H-3''''e, *J* = 3.6 Hz), 2.08 (2s, 6H, 2xNHAc), 1.83 (t, 1H, H-3''''a, *J* = 12.4 Hz), 1.27 (d, 3H, H-6''); ^{13}C NMR (D_2O, 100.6 MHz) δ 176.3, 175.8, 175.1 (3x CO), 103.0 (C-1'''), 102.8 (C-1'), 102.6 (C-1''), 101.0 (C-1), 99.8 (C-2''''), 76.9 (C-3'''), 62.6 (C-9''''), 61.02 (C-6'''), 57.1 (C-6'), 53.0 (C-2'), 23.6, 23.4 (2x NHAc), 16.6 (C-6''), MS (m/z): 982.4 $(M+H)^+$.

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