Total Synthesis of 3′-*O*-Sialyl, 6′-*O*-Sulfo Lewis^x, NeuAcα2→3(6-*O*-SO_3Na)Galβ1→4(Fucα1→3)-GlcNAcβ-OMe: A Major Capping Group of GLYCAM-I

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The selectins are a family of three Ca^{2+}-dependent membrane-bound lectins.[1] Two high endothelial venules (HEV) associated ligands of L-selectin, GLYCAM-I and CD_{34}, are mucin like O-linked glycoproteins.[2] These selectins recognize carbohydrate type ligands such as sialyl Lewis^x, sialyl Lewis^a, sulfated Lewis^x, and sulfated Lewis^a type structures.[3] Our laboratory[4] was the first to chemically synthesize 3-*O*-sulfo Lewis^x and related compounds. Subsequently, other laboratories have reported the synthesis of these types of compounds.[5] Fucosyl, sialyl, and sulfate groups have been shown to be constitutively involved in the interaction of these ligands with L-selectin,[6] Recently, Rosen et al.[7] have reported that GLYCAM-I contains NeuAcα2→3(6-*O*-SO_3)Galβ1→4(Fucα1→3)GlcNAc as a major capping group, and they also found Galβ1→4(6-*O*-SO_3)GlcNAc to be a predominant structure in this O-linked glycoprotein. We hereby communicate the total synthesis of the above capping group, NeuAcα2→3(6-*O*-SO_3Na)Galβ1→4(Fucα1→3)GlcNAcβ-OMe (**1**) and also the precursor structure, NeuAcα2→3(6-*O*-SO_3Na)Galβ1→4GlcNAcβ-OMe (**2**).

Compounds **1** and **2** were prepared from key intermediates **3**−**7**[8] (Figure 1) by stereoselective transformation as described in Schemes 1 and 2, respectively. Glycosylation of methyl 6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside with **3** under Mukaiyama's conditions[9] ($SnCl_2$−AgOTf) afforded β(1→4)-linked disaccharide **9** in 52% yield and the β(1→3)-linked disaccharide **8** in 17% yield. α-L-Fucopyranosylation of **9** with

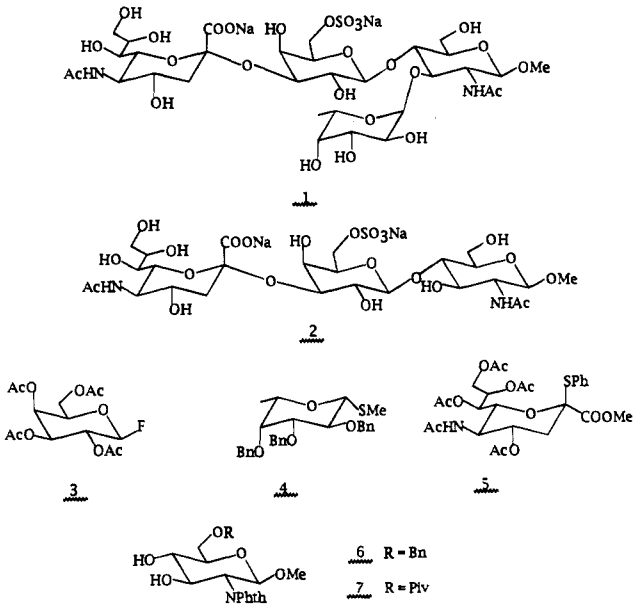

Figure 1. Sulfated sialyl Le^x (**1**) and sulfated sialyl lactosamine (**2**) target molecules and key intermediates (**3**−**7**) involved in their synthesis.

4 under $CuBr_2$−Bu_4NBr[10] furnished the fully protected trisaccharide **10** in 72% yield. Removal of both the phthalimido and acetate groups from **10** was accomplished by treatment with hydrazine hydrate in ethanol at 100 °C followed by N-acetylation to give **11** in 70% yield. Isopropylidenation of **11** according to Catelani's procedure[11] afforded the 3,4-*O*-isopropylidene derivative **12** in 80% yield along with the 4,6-*O*-isopropylidene compound in 8% yield. Chloroacetylation[12] of **12** furnished the 2,6-di-O-chloroacetylated compound **13** and 6-*O*-chloroacetylated compound **14** in 43% and 46% yields, respectively. Deacetonation of **14** afforded key intermediate **15** in 71% yield. Condensation of the sialic acid donor **5** with **15** under NIS−triflic acid[13] conditions at −40 to −50 °C resulted in the loss of the α-L-fucopyranosyl residue from both the acceptor moiety and the initially formed product. A similar reaction at −75 °C was more encouraging and gave **16** in 64% yield. Removal of the chloroacetyl group provided the trihydroxy compound **17** in 71% yield. The selective sulfation of **17** with SO_3−pyridine complex at 5 °C provided the 6-*O*-sulfo compound, which after removal of *O*-benzyl (10% Pd/C) and de-O-acetylation (MeOH−MeONa) and addition of water to hydrolyze ester to acid afforded the target compound **1**.

The synthesis of the trisaccharide **2** involved the glycosylation of **7** with fluoride **3** under conditions similar to those described for the preparation of **9** (from **6**) to give β(1→3)-linked **19** and β(1→4)-linked **20** in 22% and 47% yields, respectively. Selective de-O-acetylation in the presence of a pivaloyl group followed by isopropylidenation provided **21** in 65% yield.

The synthesis of **27** from **21** was achieved by a sequence of reactions similar to that described for the preparation of **18** from **12**. The formation of **28** from its methyl ester **27** was accomplished with lithium iodide−pyridine[14] in 81% yield. Further treatment of **28** with hydrazine hydrate in methanol at

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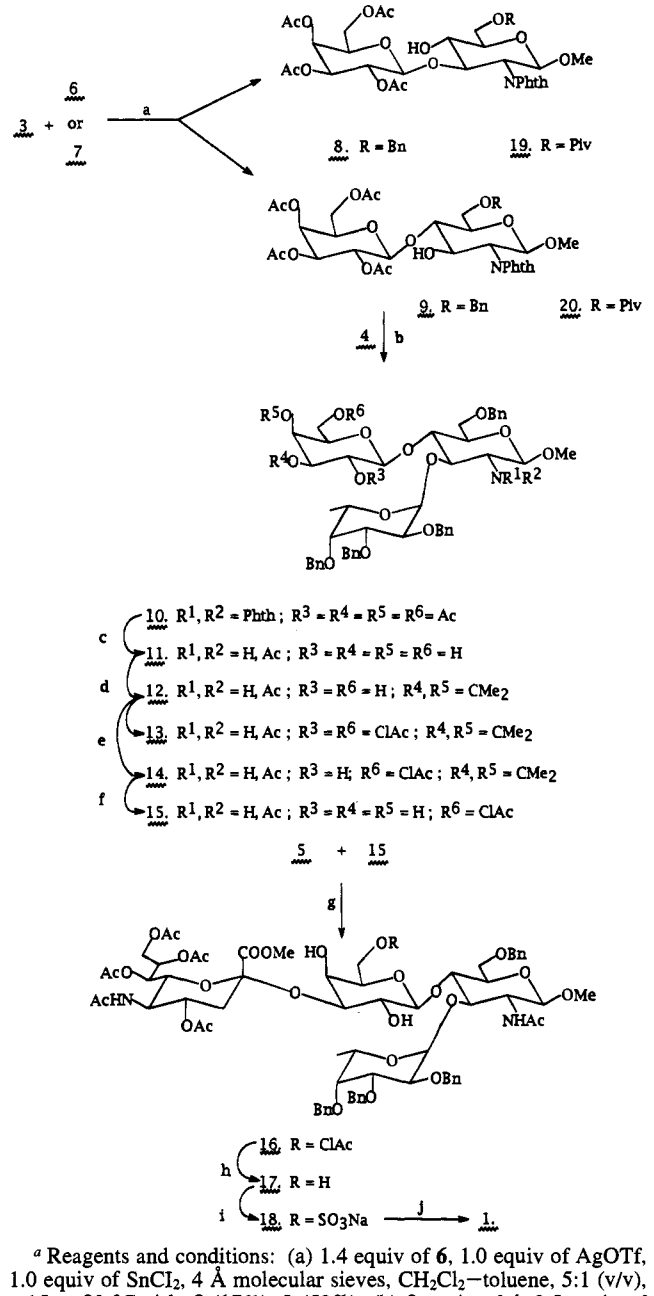
Scheme 1[a]

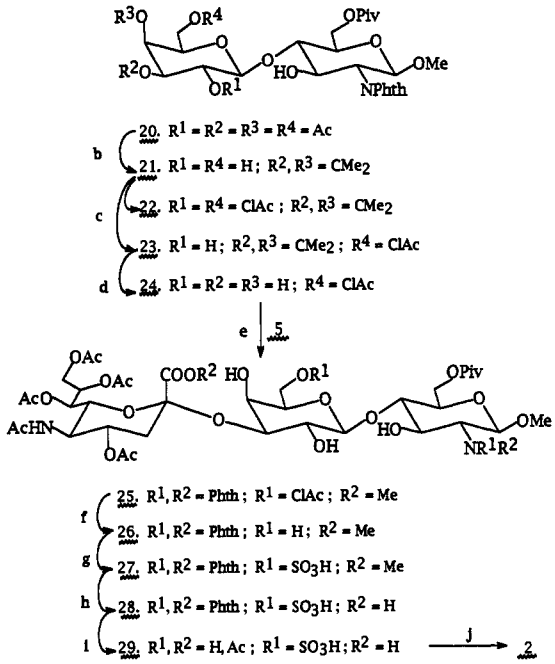


[a] Reagents and conditions: (a) 1.4 equiv of **6**, 1.0 equiv of AgOTf, 1.0 equiv of $SnCl_2$, 4 Å molecular sieves, CH_2Cl_2–toluene, 5:1 (v/v), −15 to 20 °C, 4 h, **8** (17%), **9** (52%); (b) 2 equiv of **4**, 2.5 equiv of Bu_4NBr, 2.5 equiv of $CuBr_2$, 4 Å molecular sieves, CH_2Cl–CH_2Cl/DMF, 5:1 (v/v), 20 °C 48 h, 72%; (c) EtOH/hydrazine hydrate, 4:1 (v/v), 100 °C, 6 h, MeOH/Et_3N/Ac_2O, 4:2:1 (v/v) 0 to 20 °C, 2 h, 70%; (d) 1.5% camphorsulfonic acid in DMP, 20 °C, 24 h, MeOH–H_2O, 10:1 (v/v), 100 °C, 6 h, **12** (80%) and 4,6-*O*-isopropylidene derivative (8%); (e) 1.2 equiv of chloroacetic anhydride, 5 equiv of $NaHCO_3$/DMF, −30 °C, 3 h, **13** (43%), **14** (46%); (f) 70% aqueous AcOH, 65 °C, 3 h, 71%; (g) 3 equiv of **5**, 3 equiv of *N*-iodosuccinimide/triflic acid in propionitrile, −75 °C, 1 h, 64%; (h) 5 equiv of thiourea, 2.5 equiv of 2,6-lutidine, EtOH–CH_2Cl_2 (1:1), 80 °C, 2 h, 71%; (i) 10 equiv of SO_3–pyridine complex–DMF, 5 °C, 16 h; (j) 90% aqueous EtOH, 10% Pd–C, 48 h, MeOH–MeONa, 24 h, H_2O, 5 h, Na^+ resin, 39% from **17**.

80 °C followed by N-acetylation with excess acetic anhydride in methanol–methylene chloride provided **29**, which was converted to **2** in 16% yield from **28**. The structures of **1** and **2** were confirmed by NMR and FAB mass spectroscopy.[15]

Recent advances in the utilization of glycosyltransferases in a combined chemical–enzymatic approach for oligosaccharide syntheses prompted us to attempt the synthesis of **1** by utilizing **2** as an acceptor for α-L-fucosyltransferase. Our studies indicate that our synthetic compound **2** and also Fucα1→2(6-*O*-SO_3-Na)Galβ1→3/4GlcNAc do not act as acceptors for α-(1,3/4)-L-fucosyltransferases from different sources, e.g., Colo 205 (colon), HL60 (erythroid), and human serum. Our enzyme preparations showed activities toward the acceptors (devoid of the 6-*O*-sulfate group) such as Fucα1→2Galβ1→4GlcNAc and NeuAcα2→3Galβ1→4GlcNAc. However, there may be yet unidentified novel α-L-fucosyltransferases capable of converting **2** to **1**. Our present findings suggest that the presence of the 6-*O*-sulfate group on the galactose residue of the acceptor abolishes the enzymatic transfer of Fuc to the GlcNAc moiety by α-(1,3)-L-fucosyltransferases.

Scheme 2[a]



[a] Reagents and conditions: (a) 1.4 equiv of **3**, 1.0 equiv of AgOTf, 1.0 equiv of $SnCl_2$, 4 Å molecular sieves, CH_2Cl_2–toluene, 5:1 (v/v), −15 to 20 °C, 4 h, **19** (22%), **20** (47%); (b) MeOH–CH_2Cl_2, 1:1 (v/v), MeONa (pH ~ 10), 0 °C, 2 h, 73%, DMP–camphorsulfonic acid, MeOH–H_2O, 10:1 (v/v), 100 °C, 6 h, 75%; (c) 1.3 equiv of chloroacetic anhydride, 5 equiv of $NaHCO_3$ in DMF, −30 °C, 2 h, **22** (35%), **23** (59%); (d) 5% trifluoroacetic acid in $CHCl_3$ saturated with H_2O, 88%; (e) 2 equiv of **5**, 3 equiv of *N*-iodosuccinimide triflic acid in propionitrile, −45 °C, 2 h, 43%; (f) 5 equiv of thiourea, 2.4 equiv of 2,6-lutidine, EtOH–CH_2Cl_2 (1:1), 70 °C, 7 h, 73%; (g) 5 equiv of SO_3–pyridine complex in DMF, 0 °C, 3 h, 85%; (h) 8 equiv of LiI in pyridine, 120 °C, 3 h, 81% along with little starting material; (i) MeOH–hydrazine hydrate, 5:1 (v/v), 80 °C, 7 h; (j) Ac_2O (excess), MeOH–CH_2Cl_2 (1:1), 0 °C, 1 h; MeOH–MeONa, 48 h, Na^+ resin, 16% from **28**.

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(15) **1**: ^{1}H-NMR (D_2O) δ 5.12 (d, J = 4 Hz, 1 H, H-1′), 4.58 (d, J = 7.8 Hz, 1 H, H-1″), 3.54 (s, 3 H, OMe), 2.78 (dd, $J_{3'''e,4'''}$ = 4.5 Hz, 1 H, H-3‴e), 2.08 (s, 3 H, NAc), 2.06 (s, 3 H, NAc), 1.85 (t, $J_{3'''a,4'''} = J_{3'''e,3'''a}$ = 12.1 Hz, 1 H, H-3‴a), 1.21 (d, J = 6.6 Hz, 1 H, CMe); ^{13}C-NMR δ 173.97, 173.31, 172.65 (3 CO); 100.97 (C-1″), 100.54 (C-1), 98.54 (C-2‴), 97.51 (C-1′), 74.36 (C-3), 74.19 (C-3″), 74.06 (C-4), 66.20 (C-6″), 61.59 (C-9‴), 58.78 (C-6), 56.02 (OMe), 54.59 (C-2), 50.65 (C-5‴), 38.72 (C-3‴), 21.19 and 20.99 (2 NAc); MS *m/z* 959.6 (M + H)$^+$, 981.8 (M + Na)$^+$. **2**: ^{1}H-NMR (D_2O) δ 4.61 (d, J = 7.9 Hz, 1 H, H-1″), 3.52 (s, 3 H, OMe), 2.77 (dd, $J_{3''e,4''}$ = 4.6 Hz, 1 H, H-3″e), 2.05 (s, 6 H, 2 NAc), 1.82 (t, $J_{3''a,4''} = J_{3''a,3''e}$ = 12.1 Hz, H-3″a); ^{13}C-NMR δ 173.99, 173.37, 172.78 (3 CO), 101.43 (C-1′), 99.85 (C-1), 98.81 (C-2″), 77.34 (C-3), 77.29 (C-3′), 66.14 (C-6′), 61.60 (C-9″), 59.06 (C-6), 56.05 (OMe), 54.04 (C-2), 50.68 (C-5″), 38.63 (C-3″), 21.11 and 21.02 (2 NAc); MS *m/z* 814.1 (M + H)$^+$, 836.0 (M + Na)$^+$.

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