# Selectin Ligands: Synthesis of 3′-*O*-Sialyl-6′-*O*-Sulfo Lewis$^a$, NeuAcα2→3(6-*O*-$SO_3$Na)Galβ1→3 (Fucα1→4) GlcNAcβ–OMe

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The stereoselective synthesis of 3′-*O*-sialyl-6′-*O*-sulfo-Le$^a$, a stereoisomer of the major capping group of GLYCAM-I is described.

L-Selectin is a membrane bound lectin which mediates the initial attachment of lymphocytes to the high endothelial venules (HEV) of lymph nodes.[1] It functions in leukocyte trafficking, thrombosis and inflammation. GLYCAM-I and $CD_{34}$, two HEV associated selectin ligands, are mucin-like *O*-linked glycoproteins with carbohydrate chains[2,3] containing fucose, sialic acid and sulfate. Selectins recognize carbohydrate ligands comprised of these elements, such as, the sialyl Le$^x$, sialyl Le$^a$, sulfated Le$^a$ and sulfated Le$^x$ structures.[4] In a previous paper we described the chemical synthesis of 3′-*O*-sialyl-6′-*O*-sulfo Le$^x$-β-OMe,[5] the carbohydrate moiety which has been reported to be a major capping group of GLYCAM-I.[6] Thus, in a continuing effort to shed more light on the ligand specificities of L-selectin, we describe herein the synthesis of the title compound. Our purpose is to determine what effect an alteration in the position of interglycosidic linkages will have on selectin binding.

Compounds **1** and **2** (Fig. 1) were prepared from key intermediates (**3**–**6**)[7] by stereoselective transformation, as described in Schemes 1 and 2, respectively. Reaction of the easily accessable 6-*O*-pivaloyl derivative **6** with bromide **3** (Scheme 1) in benzene–nitromethane (1 : 1, *v*/*v*) at 55 °C afforded **7** in 80% yield. α-L-Fucopyranosylation of **7** by **4** under $CuBr_2$–$Bu_4NBr$[8] followed by selective removal of the *O*-acetyl group furnished the appropriately protected trisaccharide **9** in 67% yield. It is noteworthy that the 6-*O*-pivaloyl group remains intact. Isopropylidenation of **9** according to Catelani's procedure[9] afforded the 3,4-*O*-isopropylidene derivative **10** in 70% yield. Chloroacetylation[10] of **10** and removal of isopropylidene afforded the triol derivative **11** in 60% yield. Condensation of the sialic acid donor **5**[7c] with **11** under *N*-iodosuccinimide–triflic acid[11] conditions at −70 °C[5] provided **12** in 67% yield. Removal of the chloroacetyl group gave compound **13** in 76% yield. The selective sulfation of **13** with $SO_3$–pyridine complex at 5 °C provided the 6-*O*-sulfo compound which, after removal of *O*-benzyl (10% Pd–C), and the *O*-acetyl group (MeOH–MeONa), addition of water to hydrolyse the ester, afforded the target compound **1**.

The synthesis of the trisaccharide **2** involved the selective removal of the *O*-acetyl group of **15** followed by isopropylidenation to give compound **16**. The synthesis of **21** from **17**

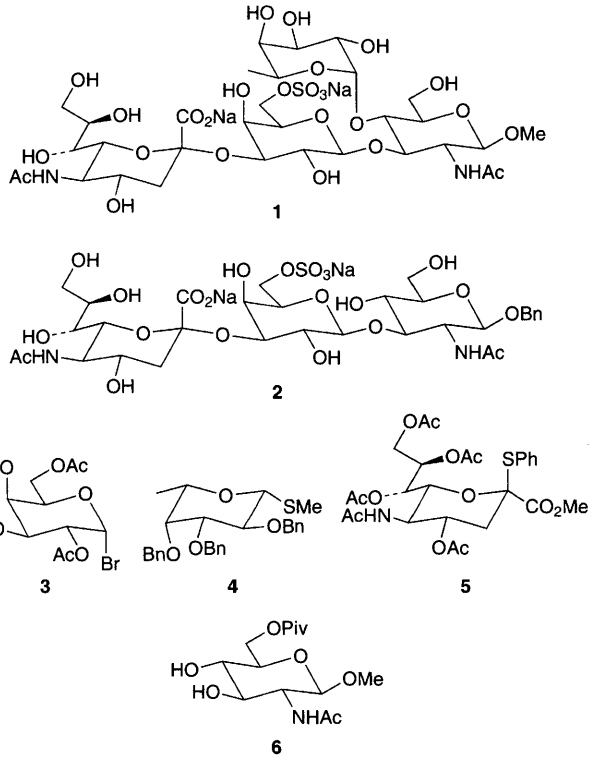
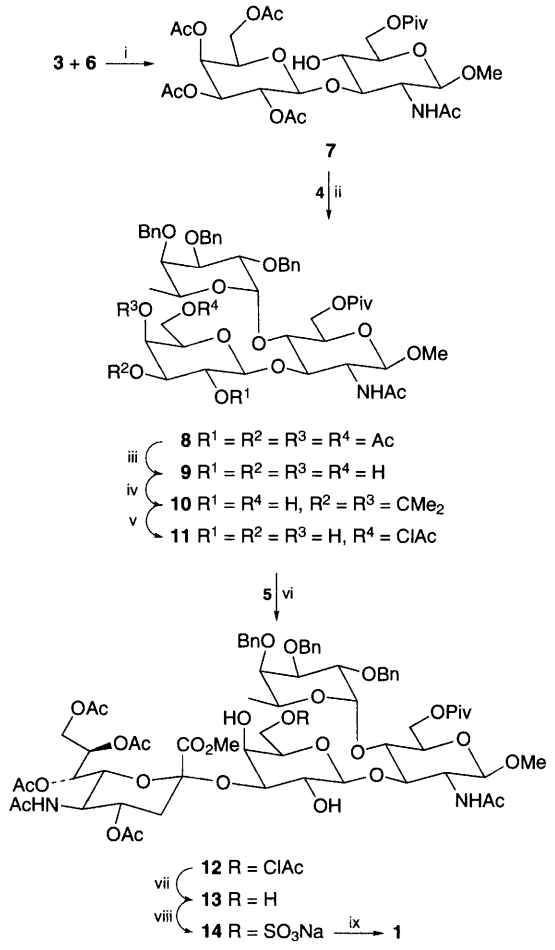


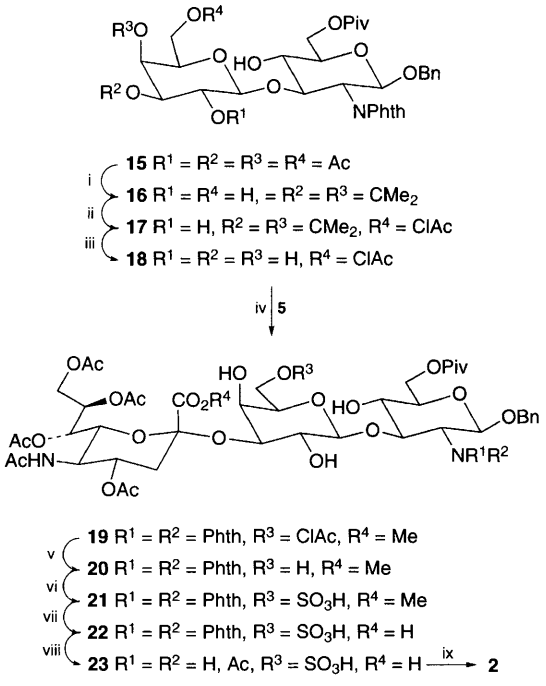

**Fig. 1** Sulfated Sialyl Le$^a$ **1** and Sulfated Sialyl Lacto-*N*-biose **2** target molecules and key intermediates (**3**–**6**) involved in their synthesis




**Scheme 1** *Reagents and conditions*: i, **3** (1.5 equiv.), **6** (1.0 equiv.), $Hg(CN)_2$ (1.5 equiv.), benzene–nitromethane 1 : 1 (*v*/*v*), 55 °C, 16 h, 80%; ii, **7** (1.0 equiv.), **4** (2.0 equiv.), $CuBr_2$, (3.0 equiv.), $Bu_4NBr$ (3.0 equiv.), $ClCH_2CH_2Cl$–DMF 5 : 1 (*v*/*v*), 4 Å molecular sieves, 16 h; iii, MeOH–MeONa, 16 h, 67%; iv, 0.15% camphorsulfonic acid, DMP, 20 °C, 24 h, MeOH–$H_2O$ 10 : 1 (*v*/*v*), 100 °C, 6 h, 70%; v, chloroacetic anhydride (1.2 equiv.), $NaHCO_3$ (5 equiv.), DMF, −30 °C, 3 h, 60% aq. AcOH, 70 °C, 3 h, 60%; vi, **5** (3 equiv.), *N*-iodosuccinimide–triflic acid (3 equiv.), propionitrile, −75 °C, 3 h 67%; vii, thiourea (5 equiv.), 2,6-lutidine (2.5 equiv.), EtOH–$CH_2Cl_2$ 1 : 1 (*v*/*v*), 80 °C, 3 h, 76%; viii, $SO_3$–pyridine complex–DMF (1.5 equiv.), 5 °C, 4 h; ix, MeOH, 10% Pd–C, 24 h, MeOH–MeONa, 24 h, $H_2O$, 4 h, $Na^+$ resin, 50% from **13**

was achieved by a reaction sequence similar to that described for the preparation of **14** from **10**. The formation of **22** from its methyl ester **21** was achieved by a lithium iodide–pyridine[12] procedure. Further treatment of **22** with hydrazine hydrate in ethanol at 80 °C followed by *N*-acetylation with excess acetic anhydride in methanol–dichloromethane and removal of the *O*-acetyl group with MeOH–MeONa provided compound **2** in 14% yield from **21**. The structures of **1** and **2** were confirmed by NMR and FAB MS.† The synthesis of Le$^a$ type derivatives are convenient and economical as compared to the corresponding Le$^x$ structures.

In a collaborative study with Dr Varki *et al.* a series of compounds were examined for binding properties with E-, L- and P-Selectins in an ELISA competition assay where relative inhibitory concentrations (RIC) were determined against that of NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAc and 3-*O*-$SO_3$Na-Galβ1→4(Fucα1→3)GlcNAc [unpublished data].




**Scheme 2** *Reagents and conditions*: i, $CH_2Cl_2$–MeOH 1 : 1 (*v/v*)–MeONa, 0.15% comphorsulfonic acid, DMP, 24 h, MeOH–$H_2O$ 10 : 1 (*v/v*), 100 °C, 6 h, 60%; ii, chloroacetic anhydride (1.2 equiv.), $NaHCO_3$ (5 equiv.), DMF, −30 °C, 3 h; iii, 60% aq. AcOH, 70 °C, 3 h, 54%; iv, **5** (3 equiv.), *N*-iodosuccinimide–triflic acid (3 equiv.), propionitrile, −45 °C, 2 h, 59%; v, thiourea (5 equiv.), 2,6-lutidine (2.5 equiv.), EtOH–$CH_2Cl_2$ 1 : 1 (*v/v*), 80 °C, 3 h, 77%; vi, $SO_3$–pyridine complex–DMF (1.5 equiv.), 5 °C, 4 h; vii, LiI, (8 equiv.), pyridine, 120 °C, 3 h; viii, MeOH-hydrazine hydrate (5 : 1, *v/v*), 80 °C, 6 h, $Ac_2O$ (excess), MeOH–$CH_2Cl_2$ 1 : 1, 0 °C, 1 h; ix, MeOH–MeONa, 48 h, $Na^+$ resin, 14% from **22**. Phth = phthalimido group.

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## Footnote

† *Selected NMR* data for **1**: $^1$H NMR ($D_2O$, 400 MHz) δ 5.05 (d, *J* 4 Hz, H-1″), 4.56 (d, *J* 7.8 Hz, H-1′), 3.52 (s, OMe), 2.79 (dd, *J* 4.5 Hz, H-3‴ e), 2.06 and 2.05 (each s, 2 × NAc), 1.79 (t, *J* 12.2 Hz, H-3‴ a), 1.19 (d, *J* 6.5 Hz, H-6″); $^{13}$C NMR ($D_2O$, 100.6 MHz) δ 101.59 (C-1′), 100.82 (C-1), 98.35 (C-2‴), 97.11 (C-1″), 75.29 (C-3), 74.49 (C-3′), 74.45 (C-4), 66.98 (C-6′), 61.35 (C-9‴), 58.69 (C-6), 56.07 (OMe), 14.35 (C-6″), FABMS *m/z* 959.6 [M + H]$^+$.

For **2**: $^1$H NMR ($D_2O$, 400 MHz) δ 4.86 (d, *J* 8.1 Hz, H-1), 4.60 (d, *J* 8.6 Hz, H-1′), 2.76 (dd, *J* 4.5 Hz, H-1″e), 2.05 and 1.94 (each s, 2 × NAc), 1.79 (t, *J* 12.2 Hz, H-3″a); $^{13}$C NMR ($D_2O$, 100.6 MHz) δ 102.47 (C-1′), 98.77 (C-2″), 98.71 (C-1), 82.12 (C-3′), 74.38 (C-3), 66.58 (C-6′), 61.46 (C-9″), 59.74 (C-6).

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