First Synthesis of the 3′-Sulfated Lewis^a Trisaccharide, Putative Ligand for the Leucocyte Homing Receptor

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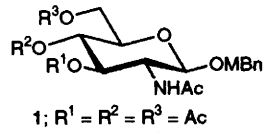
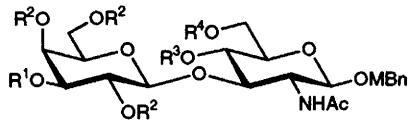
The trisaccharide 3′-OSO_3Na-Gal β(1 → 3)[Fuc α(1→4)]GlcNAc (3′-sulfated Le^a) is prepared from 4-methoxybenzyl β-D-*N*-acetylglucosaminide.

The L-selectin (homing receptor), an adhesion molecule on leucocytes, plays a key role in lymphocyte extravasation into peripheral lymph nodes[1] and neutrophile recruitement at inflammatory sites.[2] It has been shown that this adhesion protein recognizes 3′-sialyl Lewis^a (SLe^a) and 3′-sialyl Lewis^x (SLe^x),[3] but Feizi *et al.* recently[4] found that the 3′-sulfated analogues of SLe^a and SLe^x are even better ligands. However, in these crucial studies, an equimolecular inseparable mixture of 3′-sulfated Le^a and 3′-sulfated Le^x tetrasaccharides derived from an ovarian cystadenoma glycoprotein was used, which precluded determination of the most potent of the two sulfated oligosaccharides as an L-selectin ligand. In order to answer this question, we decided to chemically synthesize the terminal 3′-sulfated Le^a trisaccharide on a preparative scale. It must be emphasized that structures of all of the ligands of the three selectins (E, L and P) have not yet been determined and that chemical synthesis will surely help to solve these questions and provide carbohydrates with adverse inflammatory inhibition properties.

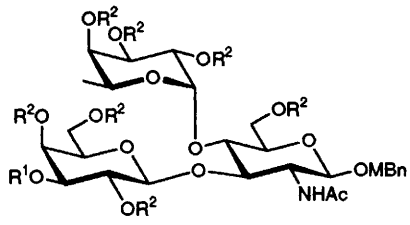
The new 4′-methoxy benzyl glycoside **1**† was readily prepared from the common *O,N*-peracetylated glucosaminyl chloride in 96% yield using classical conditions [$Hg(CN)_2$, toluene]. The use of the 4-methoxybenzyl group at the reducing position relies on the possibility to remove it selectively, if necessary, to further extend the oligosaccharide structure. After de-*O*-acetylation (NEt_3–MeOH–H_2O, 1:8:1), reaction with benzaldehyde dimethyl acetal in tetrahydrofuran (THF) in the presence of TsOH afforded the acetal **3**† (93%) which was glycosylated[5] with acetobromo-



1; $R^1 = R^2 = R^3 = Ac$
2; $R^1 = R^2 = R^3 = H$
3; $R^1 = H$, R^2, $R^3 = CHPh$



4; $R^1 = R^2 = Ac$, R^3, $R^4 = CHPh$
5; $R^1 = R^2 = H$, R^3, $R^4 = CHPh$
6; $R^1 = All$, $R^2 = H$, R^3, $R^4 = CHPh$
7; $R^1 = All$, $R^2 = Ac$, R^3, $R^4 = CHPh$
8; $R^1 = All$, $R^2 = Bn$ (benzyl), R^3, $R^4 = CHPh$
9; $R^1 = All$, $R^2 = Bn$, $R^3 = H$, $R^4 = Bn$



10; $R^1 = All$, $R^2 = Bn$, $R^3 = MBn$
11; $R^1 = H$, $R^2 = Bn$, $R^3 = MBn$
12; $R^1 = SO_3Na$, $R^2 = Bn$, $R^3 = MBn$
13; $R^1 = SO_3Na$, $R^2 = R^3 = H$

† All new compounds gave satisfactory elemental analysis. Physical data are given below:

1: m.p. 164–165 °C, $[\alpha]_D^{20}$ −49 (*c* 0.9 CH_2Cl_2), **2**: m.p. 210–211 °C, $[\alpha]_D^{20}$ −41 (*c* 1, H_2O). **3**: m.p. 285 °C, $[\alpha]_D^{20}$ −54 (*c* 1, pyridine). **4**: m.p. 136–137 °C, $[\alpha]_D^{20}$ −39 (*c* 1.07, CH_2Cl_2). **5** m.p. 251 °C, $[\alpha]_D^{20}$ −67 (*c* 1, DMF). **6**: m.p. 247 °C, $[\alpha]_D^{20}$ −74 (*c* 0.5, DMF). **7** m.p. 175 °C, $[\alpha]_D^{20}$ −14 (*c* 1.5, $CHCl_3$). **8**: m.p. 137 °C, $[\alpha]_D^{20}$ −34 (*c* 1.5, $CHCl_3$). **9**: m.p. 167 °C, $[\alpha]_D^{20}$ −11 (*c* 1, $CHCl_3$). **10**: $[\alpha]_D^{20}$ −40 (*c* 1.5, $CHCl_3$). **11**: $[\alpha]_D^{20}$ −54 (*c* 1, CH_2Cl_2). **12**: $[\alpha]_D^{20}$ −42 (*c* 1, CH_2Cl_2). **13**: as a mixture of α and β anomers (α/β, 1.5:1), $[\alpha]_D^{30}$ −38 (*c* 0.5, MeOH); ^1H NMR (250 MHz, D_2O) δ 5.11 (H-1α), 5.02 (H-1″), 4.86 (H-5″), 4.71 (H-1β), 4.62 (H-1′α), 4.58 (H-1′β), 4.29 (H-3′, H-4′), 4.16 (H-3α), 4.13 (H-2α), 4.07 (H-3β), 3.98 (H-5α), 3.89 (H-3″), 3.86 (H-2β), 3.79 (H-2″, H-4″), 3.76 (H-4α), 3.74 (H-4β, H-6aβ, H6bβ), 3.61 (H-5β), 3.61 (H-2′), 3.55 (H-5′), 2.05 (CH_3CONH), 1.18 (CH_3 Fuc).

galactose [$Hg(CN)_2$, molecular sieves (4 Å), nitromethane–toluene, 1:1]. The blocked disacccharide obtained in quantitative yield was then treated with NEt_3 in aqueous methanol (NEt_3–MeOH–H_2O, 1:8:1) for 48 h at room temp. to give **5**† (93%). The 3′-position was then protected with an allyl group through the stannylene procedure[6] (1-Bu_2SnO, toluene; 2-AllBr, $BrNBu_4$, toluene) to give the crystalline compound **6**† in 77% yield purified as the peracetylated derivative **7**.† After de-*O*-acetylation (NEt_3–MeOH–H_2O, 1:8:1, for 60 h at 80 °C), perbenzylation is performed in dimethylformamide (DMF) with benzyl bromide (4 equiv. *per* OH) in the presence of HNa (1.1 equiv. *per* OH) added portionwise during 5 h to avoid *N*-benzylation. In these conditions, compound **8**† was obtained in 65% yield (along with a mixture of dibenzylated derivatives which is recycled to furnish finally compound **8** in 78% yields). Regioselective opening of the benzylidene acetal using sodium cyanoborohydride–HCl(g) in THF,[7] gave the compound **9**† in 81% yield. Then, the fucose residue was introduced from freshly prepared perbenzyl fucosyl bromide[8] under *in situ* anomerization[9] conditions using tetraethylammonium bromide in DMF–CH_2Cl_2 in the presence of diisopropylethylamine. The fully protected tetrasaccharide **10**† was thus obtained in 80% yield. Two-step deallylation [1-$(Ph_3P)_3RhCl$, 2-$HgCl_2$–HgO, acetone–water] delivered compound **11**† in 67% yield (90% based on starting material recovery). Then sulfation with the sulfur trioxide–trimethylamine complex in DMF (12 h at 55 °C) gave the sulfated trisaccharide **12**† (82%). Complete deprotection [10% Pd/C, H_2 (1 atm)] afforded in 80% yield the sodium salt of 3′-sulfated Lewis^a trisaccharide **13** as an amorphous white powder after purification by silica gel chromatography (Pr^iOH–AcOEt–H_2O, 3:5:2) followed by cation exchange chromatography (AG50W-X8, Na^+) and lyophilisation of the aqueous solution. Compound **13**, obtained as a mixture of α and β anomers (α/β, 1.5:1) gave satisfactory elemental analysis and has been fully characterized by NMR using a COSY (correlation spectroscopy) experiment.† In particular, the H-3′ is found at δ 0.7 downfield compared with H-3′ of the Le^a trisaccharide[10] showing unambiguously the presence of the sulfate on galactose at O-3. We have thus prepared 60 mg of the title compound, the biological properties of which are currently being studied.

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