

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

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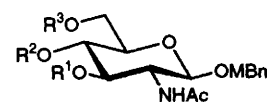
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The trisaccharide 3'-OSO₃Na-Gal β(1 → 3)[Fuc α(1 → 4)]GlcNAc (3'-sulfated Le^a) is prepared from 4-methoxybenzyl β-D-N-acetylglucosaminide.

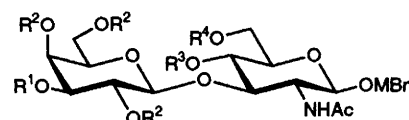
The L-selectin (homing receptor), an adhesion molecule on leucocytes, plays a key role in lymphocyte extravasation into peripheral lymph nodes¹ and neutrophil recruitment at inflammatory sites.² It has been shown that this adhesion protein recognizes 3'-sialyl Lewis^a (SLe^a) and 3'-sialyl Lewis^x (SLe^x),³ but Feizi *et al.* recently⁴ 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

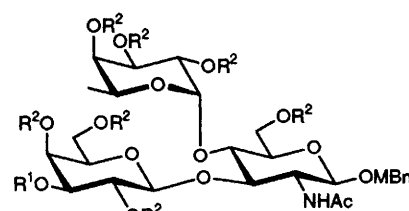
reducing position relies on the possibility to remove it selectively, if necessary, to further extend the oligosaccharide structure. After de-*O*-acetylation (NEt₃-MeOH-H₂O, 1:8:1), reaction with benzaldehyde dimethyl acetal in tetrahydrofuran (THF) in the presence of TsOH afforded the acetal 3[†] (93%) which was glycosylated⁵ with acetobromo-



- 1; R¹ = R² = R³ = Ac
2; R¹ = R² = R³ = H
3; R¹ = H, R², R³ = CHPh



- 4; R¹ = R² = Ac, R³, R⁴ = CHPh
5; R¹ = R² = H, R³, R⁴ = CHPh
6; R¹ = All, R² = H, R³, R⁴ = CHPh
7; R¹ = All, R² = Ac, R³, R⁴ = CHPh
8; R¹ = All, R² = Bn (benzyl), R³, R⁴ = CHPh
9; R¹ = All, R² = Bn, R³ = H, R⁴ = Bn



- 10; R¹ = All, R² = Bn, R³ = MBn
11; R¹ = H, R² = Bn, R³ = MBn
12; R¹ = SO₃Na, R² = Bn, R³ = MBn
13; R¹ = SO₃Na, R² = R³ = H

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

1: m.p. 164–165 °C, [α]_D²⁰ –49 (c 0.9 CH₂Cl₂), 2: m.p. 210–211 °C, [α]_D²⁰ –41 (c 1, H₂O), 3: m.p. 285 °C, [α]_D²⁰ –54 (c 1, pyridine), 4: m.p. 136–137 °C, [α]_D²⁰ –39 (c 1.07, CH₂Cl₂), 5 m.p. 251 °C, [α]_D²⁰ –67 (c 1, DMF), 6: m.p. 247 °C, [α]_D²⁰ –74 (c 0.5, DMF), 7 m.p. 175 °C, [α]_D²⁰ –14 (c 1.5, CHCl₃), 8: m.p. 137 °C, [α]_D²⁰ –34 (c 1.5, CHCl₃), 9: m.p. 167 °C, [α]_D²⁰ –11 (c 1, CHCl₃), 10: [α]_D²⁰ –40 (c 1.5, CHCl₃), 11: [α]_D²⁰ –54 (c 1, CH₂Cl₂), 12: [α]_D²⁰ –42 (c 1, CH₂Cl₂), 13: as a mixture of α and β anomers (α/β, 1.5:1), [α]_D³⁰ –38 (c 0.5, MeOH); ¹H NMR (250 MHz, D₂O) 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-6αβ, H-6bβ), 3.61 (H-5β), 3.61 (H-2'), 3.55 (H-5'), 2.05 (CH₃CONH), 1.18 (CH₃ Fuc).

galactose [$\text{Hg}(\text{CN})_2$, molecular sieves (4 Å), nitromethane-toluene, 1:1]. The blocked disaccharide obtained in quantitative yield was then treated with NEt_3 in aqueous methanol ($\text{NEt}_3\text{-MeOH-H}_2\text{O}$, 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 procedure⁶ ($1\text{-Bu}_2\text{SnO}$, toluene; 2- AlIBr , BrNBu_4 , toluene) to give the crystalline compound **6**[†] in 77% yield purified as the peracetylated derivative **7**[†]. After de-*O*-acetylation ($\text{NEt}_3\text{-MeOH-H}_2\text{O}$, 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- $\text{HCl}(\text{g})$ in THF,⁷ gave the compound **9**[†] in 81% yield. Then, the fucose residue was introduced from freshly prepared perbenzyl fucosyl bromide⁸ under *in situ* anomerization⁹ 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 [$1\text{-(Ph}_3\text{P)}_3\text{RhCl}$, 2- $\text{HgCl}_2\text{-HgO}$, 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 ($\text{Pr}^i\text{OH-AcOEt-H}_2\text{O}$, 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 trisaccharide¹⁰ 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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References

- 1 W. M. Gallatin, I. L. Weissman and E. C. Butcher, *Nature*, 1983, **304**, 30.
- 2 S. R. Watson, C. Fennie and L. A. Lasky, *Nature*, 1991, **349**, 164.
- 3 E. L. Berg, J. Magnani, R. A. Warnock, M. K. Robinson and E. C. Butcher, *Biochem. Biophys. Res. Comm.*, 1992, **184**, 1048.
- 4 P. J. Green, T. Tamatani, T. Watanake, M. Miyasaka, A. Hasegawa, M. Kiso, C.-T. Yuen, M. S. Stoll and T. Feizi, *Biochem. Biophys. Res. Comm.*, 1992, **188**, 244.
- 5 C. Augé and A. Veyrières, *Carbohydr. Res.*, 1976, **46**, 293.
- 6 S. David, A. Thieffry and A. Veyrières, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1796.
- 7 P. J. Garegg, H. Hultberg and S. Wallin, *Carbohydr. Res.*, 1982, **108**, 97.
- 8 H. Lönn, *Carbohydr. Res.*, 1985, **139**, 105.
- 9 R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, 1975, **97**, 4063.
- 10 B. Bechtel, A. J. Wand, K. Wroblewski, H. Koprowski and J. Thuring, *J. Biol. Chem.*, 1990, **265**, 2028.