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Note

Synthesis and characterization of a sulfated pentasaccharide containing the Lewis^x motif

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Abstract

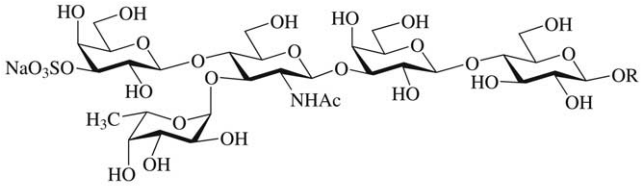
We report the synthesis of a sulfated pentasaccharide containing the Lewis^x motif used for an NMR study described in Carbohydr. Res. 2003, 338, this issue, see following communication: doi:10.1016/S0008-6215(03)00243-X, using the dibutylstannylene acetal methodology.

Keywords: Lewis^x; Oligosaccharide; Sulfated Lewis^x pentasaccharide

Sulfated Lewis^x oligosaccharides have been abundantly synthesized owing to the biological importance of these compounds as potential ligands for selectins.[1] Indeed the sialyl group of this motif has been shown to be important for the trisaccharide to maintain a high-affinity for E-selectin,[2] but the replacement of the sialic acid residue by a sulfate group retains this affinity.[1,3] The first total synthesis of 3′-sulfated (position 3 of the outer galactose) Lewis^x pentaosyl ceramide (R = Ceramide, **2**) was reported by Ogawa's group.[4] Lubineau and coworkers synthesized a 3′-sulfated Lewis^x pentasaccharide with R = H, **3**.[5] We describe here the synthesis of the β-methyl glycoside of the 3′-sulfated pentasaccharide Lewis^x **1**. This oligosaccharide was in depth studied by NMR for determining its structure in solution.[6]

1. Synthesis

Our previous work on Lewis^x derivatives led to the synthesis of a Lewis^x pentasaccharide.[7,8] We thus started the synthesis of the target compound **1** from a known intermediate,[8] namely, methyl *O*-(β-D-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-*O*-(2-acetamido-6-*O*-benzyl-2-O-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4**) (Fig. 1 and Scheme 1). In order to introduce regioselectively a sulfate group at position 3 of the unprotected galactose (a) in pentasaccharide **4**, the classical stannylene methodology was used. This method was demonstrated efficient in the preparation of the 3′-sulfated galactose-containing oligosaccharides.[9–12] Indeed, in the standard stannylene conditions, compound **4** was converted to the 3′-*O*-sulfate **5** in 79% yield by reaction of the intermediate 3′,4′ di-*O*-butylstannylene (prepared in situ by stirring **4** with dibutyltin oxide in refluxing methanol followed by removal of the solvent under diminished pressure) with the sulfur trioxide–



1 R = CH_3
2 R = Ceramide
3 R = H

Fig. 1. Structures of the sulfated Lewis^x oligosaccharides **1**–**3**.

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trimethylamine complex. A more polar compound was also obtained (16%) and identified as a 3′,6′-di-*O*-sulfated derivative which will be described elsewhere. Catalytic hydrogenolysis of **5**, followed by purification on Sephadex G10, and chromatography with a cation exchange resin (Na^+ form) gave, after freeze-drying, the sodium salt **1** in 97% yield.

2. Experimental

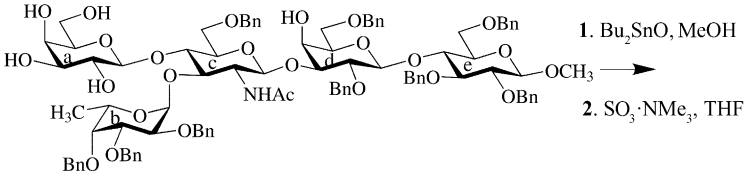
The detailed synthetic procedure leading to the pentasaccharide **1** was the following:

2.1. Methyl *O*-(3-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-benzyl-2-*O*-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside sodium salt (5)

A mixture of methyl *O*-(β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-benzyl-2-*O*-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4**) (100 mg, 59.5 μmol) and dibutyltin oxide (14.8 mg, 59.5 μmol) in dry MeOH (3 mL) was refluxed for 2 h (the solution became clear). Then the solvent was distilled at 80 °C to give a yellowish syrup, which was evaporated to dryness under diminished pressure (2 h). $SO_3 \cdot NMe_3$ (16.5 mg, 119 μmol) and dry tetrahydrofuran (3 mL) were introduced, the mixture was stirred under argon for 40 h at room temperature. Methanol (1 mL) was added. After stirring for 10 min, the reaction mixture was concentrated. Flash chromatography of the residue on a column of silica gel (8:1, 6:1, 4:1 CH_2Cl_2–MeOH) followed by cation exchange chromatography (Dowex 50X8-200, Na^+ form) using MeOH afforded **5** as a white amorphous solid (84 mg, 79%): $R_f = 0.61$ (6:1 CH_2Cl_2–MeOH); $[\alpha]_D$ −40° (*c* 1, MeOH); ^1H NMR (400 MHz, CD_3OD): δ 7.62–7.38 (m, 45 H, aromatic H), 5.53 (d, $J = 3.7$ Hz, 1 H, H-1b), 3.67 (s, 3 H, OMe), 1.95 (s, 3 H, NHAc), 1.39 (d, $J = 6.4$ Hz, 3 H, H-6b); ^{13}C NMR (100.6 MHz, CD_3OD): δ 173.35 (C=O, NAc), 140.61, 140.55, 140.48, 140.39, 140.19, 140.11, 139.68, 139.59 (aromatic C), 129.98, 129.83, 129.75, 129.59, 129.52, 129.51, 129.47, 129.42, 129.25, 129.23, 129.05, 129.02, 128.94, 128.80, 128.75, 128.72, 128.68, 128.59 (aromatic *C*H), 105.90, 104.16, 103.83, 103.45 (4 C-1), 97.85 (C-1b), 83.96, 83.03, 82.96, 82.28, 80.37, 80.21, 79.96, 77.41, 77.36, 77.09, 76.24, 75.95, 75.83, 75.07, 74.53, 71.38, 70.54, 68.58, 68.00 (19 ring-*C*H), 76.67, 76.49, 76.04, 76.04, 74.66, 74.43, 74.36, 74.08, 73.72 (9 Ph*C*H_2), 70.59, 69.68, 69.09 (C-6c, C-6d, C-6e), 63.46 (C-6a), 57.59 (C-2c, OMe), 23.80 (NAc), 17.18 (C-6b). MS for $C_{96}H_{110}NNaO_{28}S$ (FAB^-): *m/z* 1758.38 [M−Na]$^-$.

2.2. Methyl *O*-(3-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-2-*O*-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside sodium salt (1)

A mixture of compound **5** (60 mg, 33.7 μmol), 10% Pd/C (80 mg) and MeOH (12 mL) was stirred at 15 °C for 1 h under hydrogen (140 kPa). The reaction mixture was filtered through Celite, and the Celite pad was rinsed with MeOH and water. The filtrate was concentrated under diminshed pressure, and the residue purified on a Sephadex column (G10-120) using water as eluent. After ion exchange chromatography with Dowex 50X8-200 (Na^+ form) and lyophilization, the target compound **1** was obtained as a white amorphous solid (32 mg, 97%): $R_f = 0.27$ (3:3:2 EtOAc–isopropanol–water); $[\alpha]_D$ −29°



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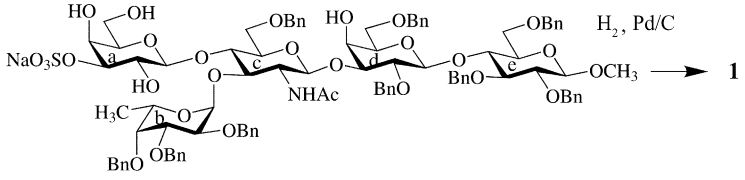

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Scheme 1. Preparation of the sulfated Lewis^x oligosaccharides **1**.

(c 0.8, water); MS for $C_{33}H_{56}NNaO_{28}S$: MALDI-TOF$^+$ m/z 992 $[M+Na]^+$, MALDI-TOF$^-$ m/z 946 $[M-Na]^-$.

Acknowledgements

The NMR study of pentasaccharide **1** was performed in collaboration with the groups in CEA/Saclay. Their results are reported in an article of this issue.[6]

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