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Note

Synthesis of a sulfonic acid mimetic of the sulfated Lewis A pentasaccharide

Zsolt Jakab [a,†], Anikó Fekete [a], Magdolna Csávás [a], Anikó Borbás [a,*], András Lipták [a], Sándor Antus [a,b]

[a] Research Group for Carbohydrates of the Hungarian Academy of Sciences, University of Debrecen, PO Box 94, H-4010 Debrecen, Hungary
[b] Department of Organic Chemistry, University of Debrecen, PO Box 20, H-4010 Debrecen, Hungary

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ABSTRACT

The first sulfonic acid mimetic of the sulfated Lewis A pentasaccharide in which the natural L-fucose unit is replaced by a D-arabinose ring was synthesized. Formation of the sulfonic acid moiety at a pentasaccharide level could be successfully achieved by means of introduction of an acetylthio moiety into the terminal D-galactose residue and subsequent oxidation. The equatorial arrangement of the acetylthio group linked to C-3 of the galactose ring could be obtained by double nucleophilic substitutions; efficient formation of the *gulo*-triflate derivatives required low-power microwave (MW) activation. Oxidation of the acetylthio group was carried out using Oxone in the presence of acetic acid.

Selectins are calcium-dependent adhesion molecules that are expressed on vascular endothelium and on leukocytes. Through recognition of specific carbohydrate epitopes of their ligands, the selectins mediate leukocytes rolling along the blood vessel wall which is the first step of leukocyte adhesion in the inflammation cascade.[1] E-selectin is also postulated to mediate the initial interactions with tumor cells and might be involved in tumor metastasis.[2,3] To prevent pathological recruitment of endogenous leukocytes and to decrease the incidence of metastases, soluble selectin ligands could be applied as antagonists binding competitively to the selectins and thus inhibiting the adhesion to their natural ligands.

The sialyl Lewis A tetrasaccharide (sLe^a) **1** and its positional isomer sialyl Lewis X (sLe^x) have been identified as lead compounds for binding to P- and E-selectins.[4,5] The sulfated Le^a pentasaccharide **2** isolated from an ovarian cystoadenoma glycoprotein[6] turned out to be an even more potent E-selectin ligand than the sialylated Lewis antigens,[7–10] demonstrating that the sialic acid can be advantageously substituted by a sulfate group.

We have recently described the synthesis of two new sulfonic acids containing trisaccharide mimetics of the tetrasaccharide **1**.[11] The present paper describes the preparation of the sulfonic acid pentasaccharide **3**, which being a stronger acid than the carboxylic acid-containing **1** and the sulfate ester derivative **2**,[8,10] might show high affinity to E-selectin, moreover, it is resistant against esterases and sulfatases. The L-fucose unit was also replaced by a D-arabinosyl moiety. It has been demonstrated by Kunz et al. that the essential fucose unit of the sLe^x and sLe^a could be substituted by a β-D-arabinopyranoside possessing much higher stability toward enzymatic degradation than the α-L-fucosyl residue (Fig. 1).[12,13]

Pentasaccharide backbone **6** of the planned mimetic was prepared by coupling of the D-arabinosyl-containing trisaccharide **4**[11] to the known lactoside acceptor **5**[14] upon NIS-TMSOTf activation and using a 1:2 ratio of the donor and the acceptor (Scheme 1). After complete conversion of the donor, formation of a main product and decomposition were also observed (polar components appeared on TLC). The main product could be isolated only with moderate yield, fortunately; it proved to be the desired pentasaccharide **6**. Selective removal of the chloroacetyl (CA) group of **6** with thiourea[15] resulted in **7** in a 84% yield. It is known from the literature that 3-acetylthiogalactoside can be prepared via two subsequent nucleophilic substitution reactions, and a strict substitution pattern of the galactose is required for the successful synthesis: OH-2 has to be protected by an ester group, and OH-4 and OH-6 have to be protected in the form of a benzylidene acetal.[16–18] Compound **7** being suitable for the introduction of the acetylthio moiety at position 3 of the terminal galactose residue was treated with triflic anhydride and the readily formed triflate was reacted with tetrabutylammonium nitrite ($TBANO_2$) affording the *gulo*-compound **8** in good yield. However, the next triflation step was very slow and did not go to completion at room temperature in several days. The low reactivity of the axial hydroxyl group

* Corresponding author.
E-mail address: borbas.aniko@science.unideb.hu (A. Borbás).
† Present address: Department of Carbohydrate Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Pusztaszeri út 59-67, H-1025 Budapest, Hungary.

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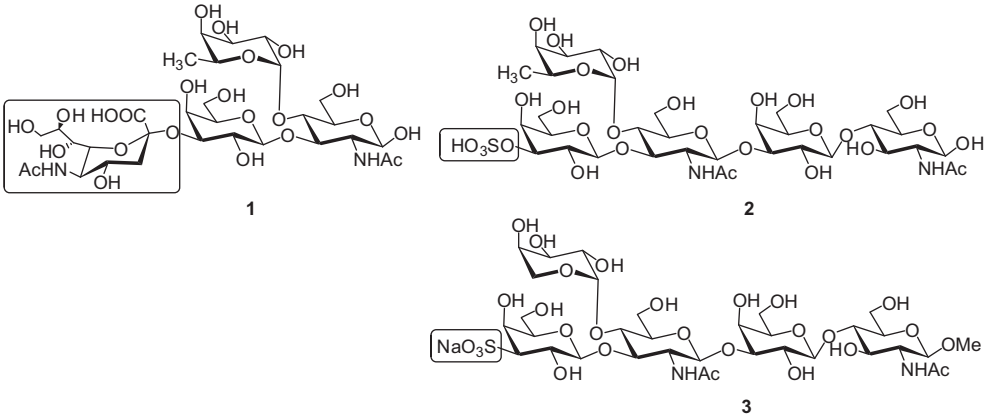
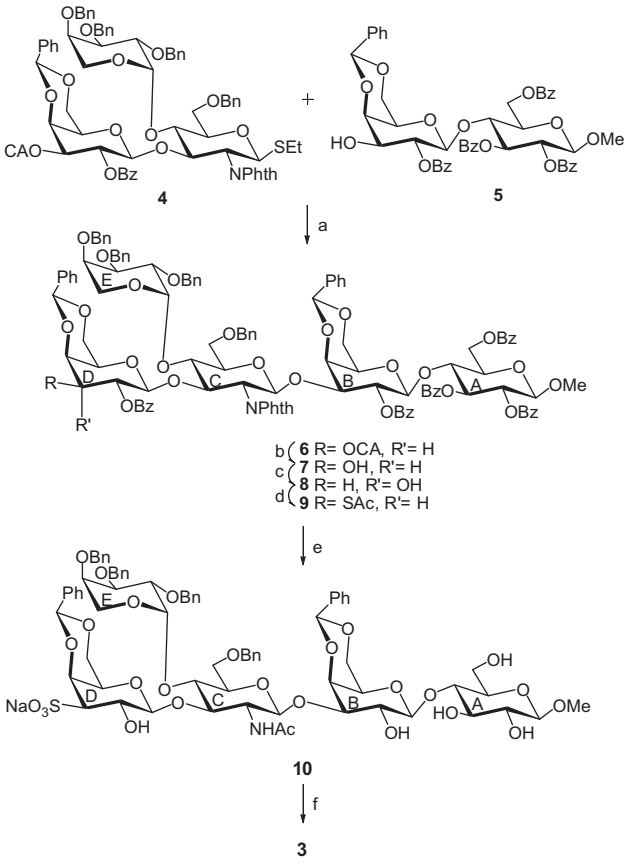


Figure 1. Sialyl Le[a] tetrasaccharide **1**, sulfated Le[a] pentasaccharide **2** and sulfonic acid mimetic **3**.



Scheme 1. Reagents and conditions: (a) **4**:**5** = 1:2, NIS, TMSOTf, dry CH_2Cl_2, THF, −45 °C, 2 h, then −20 °C overnight, 45%, (unreacted **5** was recovered with 48% yield) (b) thiourea, pyridine, CH_2Cl_2, MeOH, 1 d, 84%; (c) Tf_2O, pyridine, dry CH_2Cl_2, −20 °C to rt, 1 h, then $TBANO_2$, dry CH_3CN, rt, 1 d, 75%; (d) Tf_2O, pyridine, dry CH_2Cl_2, 0 °C, 1/2 h, then MW activation, 35 °C, 1 h, then KSAc, dry DMF, overnight, 68%; (e) EDA, dry EtOH, reflux, 1 d, then Ac_2O, MeOH, 2 h, then NaOMe, MeOH, overnight, then Oxone, cc. AcOH, KOAc, rt, 4 h, 14% over four steps; (f) Pd(C), H_2 (10 bar), EtOH, 4 days, 73%.

of **8** might arise from steric hindrance caused by the bulky phthalimido substituent.[11] Fortunately, complete formation of the *gulo*-triflate could be achieved employing low-power microwave activation in a CEM Discover Microwave reactor. Treatment of the obtained triflate with potassium thioacetate (KSAc) afforded the desired protected pentasaccharide **9** in 68% overall yield for the two steps.

Conversion of **9** into the sulfonic acid derivative **10** was carried out in a four-step synthesis involving N- and S-deacylation using ethylenediamine (EDA) followed by selective N-acetylation, fully debenzoylation, and subsequent oxidation of the 3-SH group of the terminal galactose unit into the sulfonic acid salt with Oxone.[19] Although TLC monitoring of the individual steps showed complete and efficient reactions, the overall yield was rather low, probably due to the insufficient isolation of the oxidation product from the crude reaction mixture containing high amounts of inorganic salts. The benzyl and benzylidene groups of **9** were removed by catalytic hydrogenation over Pd/C to afford **3** as the first sulfonic acid analogue of the sulfated Lewis[a] pentasaccharide **2**.[8,10]

In conclusion, sulfonic acid-containing arabino Lewis[a] pentasaccharide (**3**) was synthesized applying a 3+2 block synthesis. The sulfonic acid group at position C-3[D] was formed at a pentasaccharide level by introduction of an acetylthio moiety into the terminal galactose residue and subsequent oxidation.

1. Experimental

1.1. General methods

Optical rotations were measured at room temperature with a Perkin–Elmer 241 automatic polarimeter. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. All reactions were performed under anhydrous conditions and monitored by TLC on Kieselgel 60 F254 (Merck) visualised under UV light and charred with 5% sulfuric acid in ethanol. Column chromatography was performed on Silica Gel 60 (Merck 0.062–0.200 nm). Chemicals were purchased from Aldrich and Fluka and used without further purification. Molecular sieves were activated by heating to 360 °C overnight and were cooled over P_2O_5 in vacuo. The organic solutions were dried over $MgSO_4$, and concentrated in vacuum. The ^1H (360.13 MHz) and ^{13}C NMR (90.54 MHz) spectra were recorded with Bruker AM-360 spectrometer for solutions in $CDCl_3$. The use of a different solvent is indicated therein. Chemical shifts are referenced to Me_4Si (0.00 ppm for ^1H) or to the residual solvent signals (77.00 ppm for ^{13}C). Microwave assisted procedures took place in a CEM-Discover Focused Microwave Synthesis System (2450 MHz) with a built-in infrared temperature sensor and a CEM-Explorer computer controlled robotic sampler attaching system. Sample was measured in a 10 mL crimp-sealed, thick-walled reaction tube equipped with a magnetic stirrer. MALDI-TOF MS spectra were recorded on a Bruker Biflex III spectrometer in positive, linear mode using saturated 2,4,6-trihydroxy-acetophenone in water as matrix. Elemental analyses (C, H, N, S) were performed using an Elementar Vario MicroCube instrument.