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Note

Chondroitin-4-*O*-sulfatase from *Bacteroides thetaiotaomicron*: exploration of the substrate specificity

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ABSTRACT

Bacterial sulfatases can be good tools to increase the molecular diversity of glycosaminoglycan synthetic fragments. A chondroitin 4-*O*-sulfatase from the human commensal bacterium *Bacteroides thetaiotaomicron* has recently been identified and expressed. In order to use this enzyme for synthetic purposes, the minimal structure required for its activity has been determined. For that, four 4-O-sulfated monosaccharides and one 4-O-sulfated disaccharide have been synthesized and used as substrates with the sulfatase. The minimum structure was shown to be a disaccharide but in contrast to the natural substrate, which must have a 4,5-insaturation, the enzyme accepts as substrate, a disaccharide with a saturated glucuronic acid at the non-reducing end and even a glucopyranosyl moiety without the carboxylic acid functionality.

Chondroitin sulfate (CS) is a glycosaminoglycan composed of β-D-GlcA-(1–3)-β-D-GalNAc-(1–4) repeating units and found both in invertebrates and vertebrates. The position 4 or 6 of the GalNAc units is commonly sulfated, while position 2 or 3 of the GlcA units is sulfated to a minor extent. Several bacterial strains, such as *Proteus vulgaris* or *Bacteroides thetaiotaomicron* can use CS as their sole source of carbohydrate.[1,2] In this process, the polymer is first cleaved into 4′-5′-unsaturated and sulfated disaccharides by a lyase, then a series of sulfatases act sequentially to remove sulfate esters on these disaccharides. Nevertheless, if genomic analysis has revealed that these bacteria possess a large number of sulfatase coding genes,[3] their substrate specificity and selectivity are not currently understood. Interestingly, it has been demonstrated that such bacterial sulfatases may be used as tools for the structural analysis of glycosaminoglycan oligosaccharides.[1,4] Furthermore, in view of increasing the molecular diversity of synthetic fragments, chemoenzymatic diversification could benefit from the regioselectivity that some bacterial sulfatases may exhibit. Thus, the identification of new glycosaminoglycan sulfatases appears as a new opportunity to reinforce the potential of these two approaches.

In this regard, using two chondroitin disaccharide libraries as substrates[5] and a panel of cloned sulfatases, notably identified from the human commensal bacterium *Bacteroides thetaiotaomicron*,[3] we have recently described a sulfatase able to selectively remove sulfate at the 4 position of a CS *N*-acetylgalactosaminyl residue.[6] In order to better characterize the substrate specificity of this enzyme, as a prerequisite to its use in chemoenzymatic syntheses, we decided to determine the minimal structure required for its activity. We describe here the synthesis of four 4-O-sulfated monosaccharides (**1**, **7**, **13,** and **19**) and one 4-O-sulfated disaccharide (**25**) and their use to determine the minimal structure that can be accepted as substrate by the chondroitin-4-*O*-sulfatase from *Bacteroides thetaiotaomicron*.

Our first monosaccharide substrate, benzyl 4-*O*-sulfonato-β-D-galactose sodium salt **1**, was obtained following a known procedure starting from benzyl-β-D-galactopyranoside **2** (Scheme 1).[7–9]

Compound **7**, benzyl 4-*O*-sulfonato-α-D-glucose sodium salt, was prepared starting from benzyl 2,3-di-*O*-benzoyl-α-D-glucopyranoside **5** (Scheme 2).[7] Regioselective acylation of the primary hydroxyl was achieved, in 83% yield, using *Candida antartica* lipase and isopropenyl acetate.[10] Structural confirmation was achieved by ^{1}H NMR, which showed characteristic downfield shifts of the H-6, H-6′, and H-5′ protons. Sulfation using sulfurtrioxide–pyridine complex followed by transesterification with methanolic sodium methoxide gave benzyl 4-*O*-sulfonato-α-D-glucopyranoside, sodium salt **7** in 77% yield.

Benzyl 2-acetamido-2-deoxy-4-*O*-sulfonato-β-D-galactopyranoside sodium salt **13** and 2-acetamido-2-deoxy-3-*O*-methyl-4-*O*-sulfonato-β-D-galactopyranose sodium salt **19** were obtained from their *gluco* counterparts using, as key steps, the known and elegant stereospecific inversion of configuration at C-4 of D-glucosamine (Schemes 3 and 4).[11] To this aim, benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside **8** was first obtained by O-anomeric alkylation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucose using NaH and allylbromide in CH_2Cl_2.[12] Zemplèn deacet-

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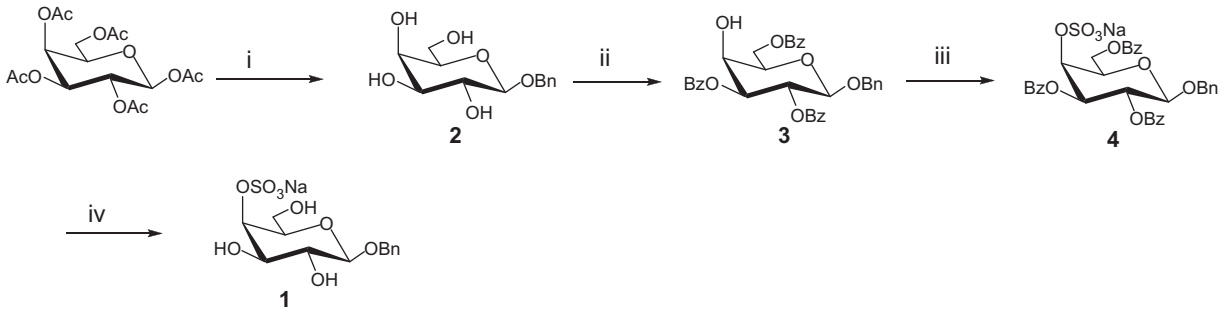
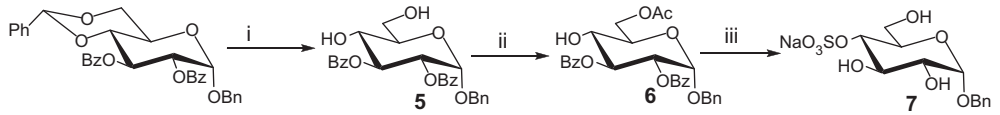
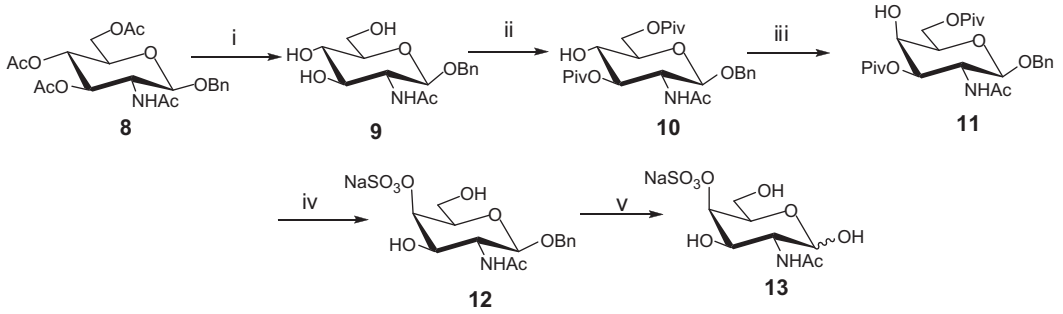


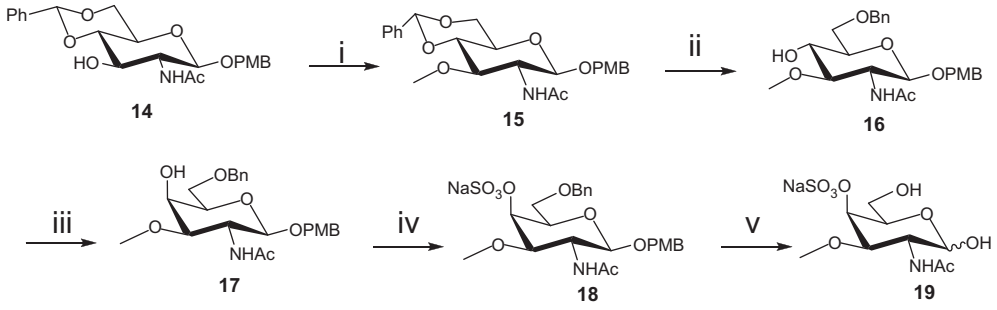
Scheme 1. Reagents and conditions: (i) BnOH (1.8 equiv) $BF_3{\cdot}Et_2O$ (1.2 equiv), CH_2Cl_2, t.a., 15 h; NaOMe (0.4 M), MeOH, t.a., 2 h, 56.5%; (ii) BzCl (4.2 equiv), pyridine, −40 °C, 2 h, then 15 h at 4 °C , 27%; (iii) $SO_3{\cdot}$pyr (7.6 equiv), pyridine, 65 °C, 30 min, 57%; (iv) MeONa (0.2 M), MeOH, 15 h, rt, 97%.



Scheme 2. Reagents and conditions: (i) AcOH 60%, 70 °C, 4 h, 68%; (ii) isopropenyl acetate (7 equiv), *Candida antartica* lipase, THF, 24 h, 40 °C, 83%; (iii) $SO_3{\cdot}$pyr (7 equiv), pyridine, 65 °C, 15 min; then MeONa, (0.2 M), MeOH, 15 h, rt, 77%.



Scheme 3. Reagents and conditions: (i) MeONa (0.3 M), MeOH, 90 min, rt, 95%; (ii) PivCl (2.8 equiv), pyridine, 0 °C, 2 h, 69%; (iii) Tf_2O (1.3 equiv), pyridine/1,2 dichloroethane (1:9), −15 0°C to rt, 15 h; then NBu_4NO_2 (4 equiv), DMF, 3 h, 57%; (iv) $SO_3{\cdot}$pyridine (2.6 equiv), pyridine, 65 °C; then MeONa (0.4 M), MeOH, 16 h, rt, 76%; (v) H_2, Pd/C, EtOH–H_2O (9:1), 15 h, 95%.



Scheme 4. Reagents and conditions: (i) CH_3I (3 equiv), BaO (12 equiv), $Ba(OH)_2{\cdot}8H_20$ (1.8 equiv), DMF, 16 h, rt (64%); (ii) CH_3COOH 60%, 5 h, 50 °C; Bu_2SnO (1.1 equiv), toluene, 5 h, Dean-start; then $C_6H_5CH_2Br$ (2 equiv), Bu_4NBr (1 equiv), 36 h, 70 °C, 82%; (iii) Tf_20 (1.3 equiv), pyridine (6 equiv), 1,2 dichloroethane, −15 °C, 2 h; then NBu_4NO_2 (4 equiv), DMF, 3 h, rt (51%); (iv) $SO_3{\cdot}$pyridine (2.6 equiv), pyridine, 65 °C, 1 h, 85%; (v) H_2, Pd/C, EtOH–H_2O (4:1), 15 h, 61%.

ylation of **8** further gave triol **9**, which was then treated with pivaloyl chloride and pyridine in dichloromethane at 0 °C to give **10** in 69% yield.[11a] Inversion of the configuration at C-4 of **10** was achieved by first treatment with triflic anhydride and pyridine in dichloromethane at −15 °C, followed by nucleophilic displacement of the intermediate 4-*O*-triflate with tetrabutylammonium nitrite in DMF affording, after aqueous workup, the D-*galacto* derivative **11** in 57%[14] yield. Treatment of alcohol **11** with the sulfurtrioxide–pyridine complex in pyridine gave the 4-*O*-sulfonato derivative, which after transesterification with methanolic sodium methoxide, gave monosaccharide **12** in 76% yield.

Compounds **19** and **25** (Schemes 4 and 5) were obtained starting from the known benzylidene **14**.[13] On one hand, chemoselective 3-O-methylation of **14**, with methyl iodide and barium hydroxide in