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THE SMOOTH MUSCLE CELL ANTIPROLIFERATIVE ACTIVITY OF HEPARAN SULFATE MODEL OLIGOSACCHARIDES

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Abstract
Heparan sulfate model oligosaccharides were devised with the simplyfying assumptions that i) carboxyl-reduction and subsequent sulfation of heparan sulfate does not decrease the SMC antiproliferative activity, and ii) *N*-Sulfates in glucosamines can be replaced by *O*-sulfates. These heparan sulfate model oligosaccharides are more active than analogous heparin fractions.

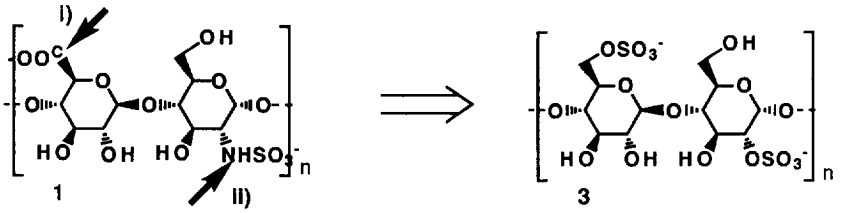
Introduction

Heparan sulfate (**1**) and heparin (**2**) interact with a high number of biomolecules. In particular, heparin inhibits the proliferation of smooth muscle cells (SMC) in culture[1] as well as after vascular injury in the rat;[2] the proliferation of SMC plays an important role in the process of restenosis.[3] Heparan sulfates with even more pronounced antiproliferative activity have been isolated from SMC[4] and endothelial cells[5] giving further evidence that heparinoids could play a physiological role in the regulation of vascular cell growth. Precise structural determinants of this activity have not been reported yet. An investigation by Castellot *et al.*[6] on size-fractionated heparin led to the conclusion that dodecasaccharide fractions are required to obtain heparin-like[7] antiproliferative activity. Oversulfation increased the activity thus lowering the size required to reach a heparin-like effect approximately by a disaccharide unit.[8] Following our discovery of highly sulfated tetrasaccharides with heparin-like activity.[9, 10] it was of interest to investigate small substructures of heparan sulfates with regard to their antiproliferative effect on SMC. Here we describe the activities of simplified heparan sulfate oligosaccharides.

Results and Discussion

To arrive at model saccharides of heparan sulfate that can be synthesized in a relatively straightforward manner we made the simplifying assumptions[11] that i) carboxyl-reduction and subsequent sulfation of heparan sulfate does not decrease the SMC antiproliferative activity, and ii) *N*-Sulfates in glucosamines can be replaced by *O*-sulfates. These reasonable[8, 12, 13] two assumptions reduce the β-D-glucuronic acid-α-(1→4)-D-glucosamine backbone of heparan sulfate to a simple (1→4)-glucan **3** with alternating α- and β-linkages (Scheme 1).
For synthetic convenience, α,β-(1→4)-glucan substructures (Scheme 2) were prepared as methyl

Scheme 1: Assumptions i) and ii) simplify the heparan sulfate backbone to an α,β-(1→4)-glucan.



glycosides. Thus, methyl β-D-glucopyranoside **4** and methyl β-maltoside **5** were chosen as simple mono- and disaccharide structures. Sulfation afforded the fully sulfated derivatives **6** and **7**.[14] Higher substructures **8** and **9**, up to the hexasaccharide **10**, were synthesized using maltosyl and glucosyl building blocks.[11] In addition, the 'frame-shifted' tri- to pentasaccharides **14 - 16** have been prepared.[15] Sulfation with sulfur trioxide complex in dimethylformamide as a solvent furnished highly, but random sulfated oligosaccharides **11 - 13** and **17 - 19**.[16]

The biological activities of these heparan sulfate model oligosaccharides are summarized in Table 1. Antiproliferative activity is expressed as relative inhibitory activity (r_i)[10] against heparin; this value compares the *in vitro* activity of substances at 100 μg/ml with that of heparin at the same concentration and in the same SMC growth inhibition experiment. In order to obtain a value for r_i within a satisfactory confidence interval compounds were tested repeatedly in independent experiments.

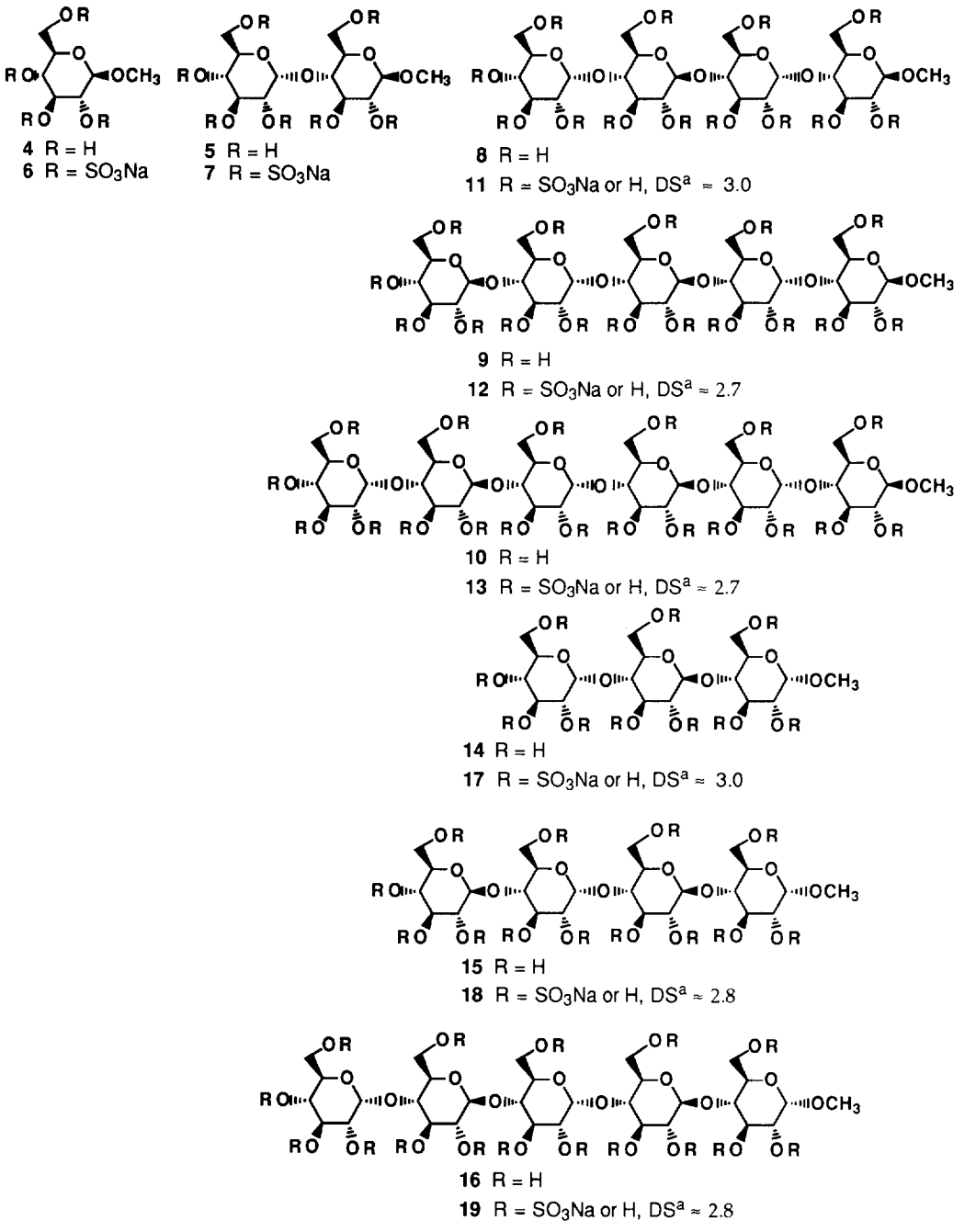
While the sulfated tetrasaccharide **11** already displays considerable antiproliferative activity, a heparin-like effect is seen with pentasaccharide **12**; this activitity is not further increased with the

Table 1: SMC Antiproliferative activities of sulfated carbohydrates.

Compound	*Degree of sulfation*	r_i [a]
2		1.0
6	100 %	0.2 ± 0.11
7	100 %	0.1 ± 0.07
11	≈ 92 %	0.8 ± 0.07
12	≈ 84 %	1.0 ± 0.12
13	≈ 85 %	1.0 ± 0.08
17	≈ 90 %	0.8 ± 0.08
18	≈ 86 %	0.7 ± 0.05
19	≈ 88 %	0.7 ± 0.07

a. Determined with rat SMC in at least 3 independent experiments; values ± standard error of mean.

Scheme 2: α,β-(1→4)-Glucan substructures.



a) DS denotes the degree of sulfation, defined as number of sulfate groups per monosaccharide unit.[12]

hexasaccharide **13**. The frame-shifted sulfated oligosaccharides **17** - **19** do not reach heparin-like activity, suggesting that a prerequisite for heparin-like activity in these heparan sulfate oligosaccharides is the presence of three β-D-glucosyl residues. These heparan sulfate model saccharides are thus more active than oversulfated heparin fragments[8] of corresponding size. Apart from the different degree of sulfation, which is most likely is not relevant when highly sulfated model saccharides are regarded, the major difference in the composition of heparan sulfate and heparin is the prevalent occurrence of L-iduronic acid in heparin *vs.* D-glucuronic acid in heparan sulfate. Although a direct comparison of the results on our model compounds with those obtained on heparin fractions is not straightforward, since the latter do not have a well-defined carbohydrate backbone, it seems that sulfated β-D-glucuronic acid components contribute more to SMC antiproliferative activity than sulfated α-L-iduronic acid components.

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16. A solution of oligosaccharide (1 mmol) in DMF (12 ml) was reacted with $SO_3 \cdot NMe_3$ (2 equ./hydroxyl group) for 20 h at 70 - 75 °C. The cooled reaction mixture was decanted, and to the resinous residue was added an aqueous NaOAc solution. The solution was concentrated, taken up with water, and concentrated again to remove trimethylamine. The residue was gelfiltrated over Sephadex LH 20, and product fractions were lyophilized. Although the oligosaccharides were not sulfated completely the sulfation protocol gave reproducible results as judged by superimposible NMR spectra.

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