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SELECTIVELY DEOXYGENATED SULFATED TETRASACCHARIDES AS PROBES FOR THE INVESTIGATION OF SMOOTH MUSCLE CELL ANTIPROLIFERATIVE ACTIVITY

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Abstract

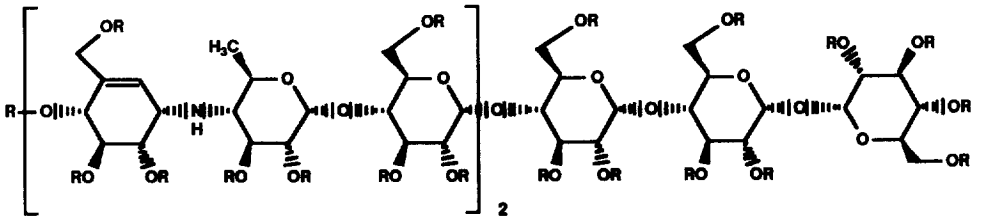
Selectively deoxygenated analogues of highly (ca. 80 %) sulfated β-maltosyl-(1→4)-α,α-trehalose were investigated to map the importance of the individual sulfates for biological activity. Single deoxygenations led to activity losses up to 30 %; the essential sulfates are located on the outer glucopyranosyl rings of the molecule.

Introduction

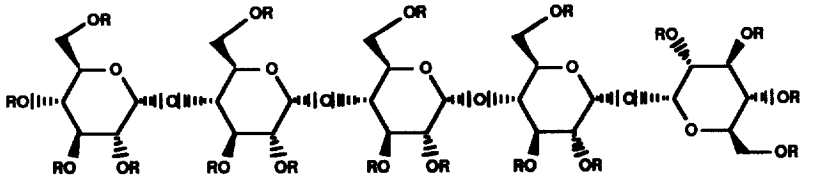
Migration and proliferation of smooth muscle cells (SMC) play an important role in the process of restenosis,[1] a renarrowing of the arterial lumen which occurs with high incidence after angioplasty, an often used procedure to restore blood flow in stenotic coronary arteries. Heparin inhibits the proliferation of SMC in culture[2] as well as after vascular injury in the rat,[3] and heparan sulfates with strong antiproliferative activity have been isolated from cells of the vascular wall.[4] This suggests that heparinoids could play a physiological role in the regulation of vascular cell growth. Since the antiproliferative activity of heparin is distinct from its antithrombin III (AT_{III}) mediated anticoagulant activity,[5] a non-anticoagulant heparinoid could be a potential drug for the prevention of restenosis.

In our search for heparinoid mimetics we have, departing from heparin derivatives, identified sulfated Trestatin A (**1**), a pseudo-nonasaccharide, as a potent SMC antiproliferative agent *in vitro* devoid of antithrombin III mediated anticoagulant activity.[6] Attempts to further reduce the molecular weight led to sulfated α-maltotriosyl-(1→4)-α,α-trehalose (**2**), a pentasaccharide substructure of sulfated Trestatin A, and, by modification of the α-D-linked substructures, to the sulfated β-maltosyl-(1→4)-α,α-trehalose (**3**) tetrasaccharide.[7] Both sulfated oligosaccharides are readily synthesized[8, 9] and retain heparin-like[10] antiproliferative activity.[7] While an isomaltosyl analogue of **3** with a similar conformation[11, 12] has the same antiproliferative activity, other relatively small modifications of the tetrasaccharide backbone of **3** may result in a drastic loss of anti-

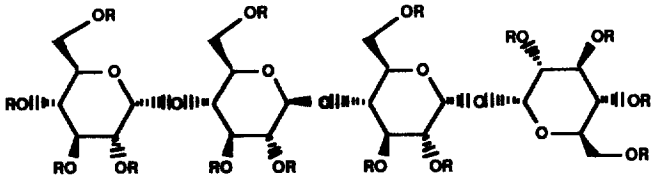
Scheme 1: Known lead structures.



1 R = SO_3Na or H, DS ≈ 2.4 [a], Trestatin A sulfate



2 R = SO_3Na or H, DS ≈ 2.9 [a]



3 R = SO_3Na or H, DS ≈ 2.8 [a]

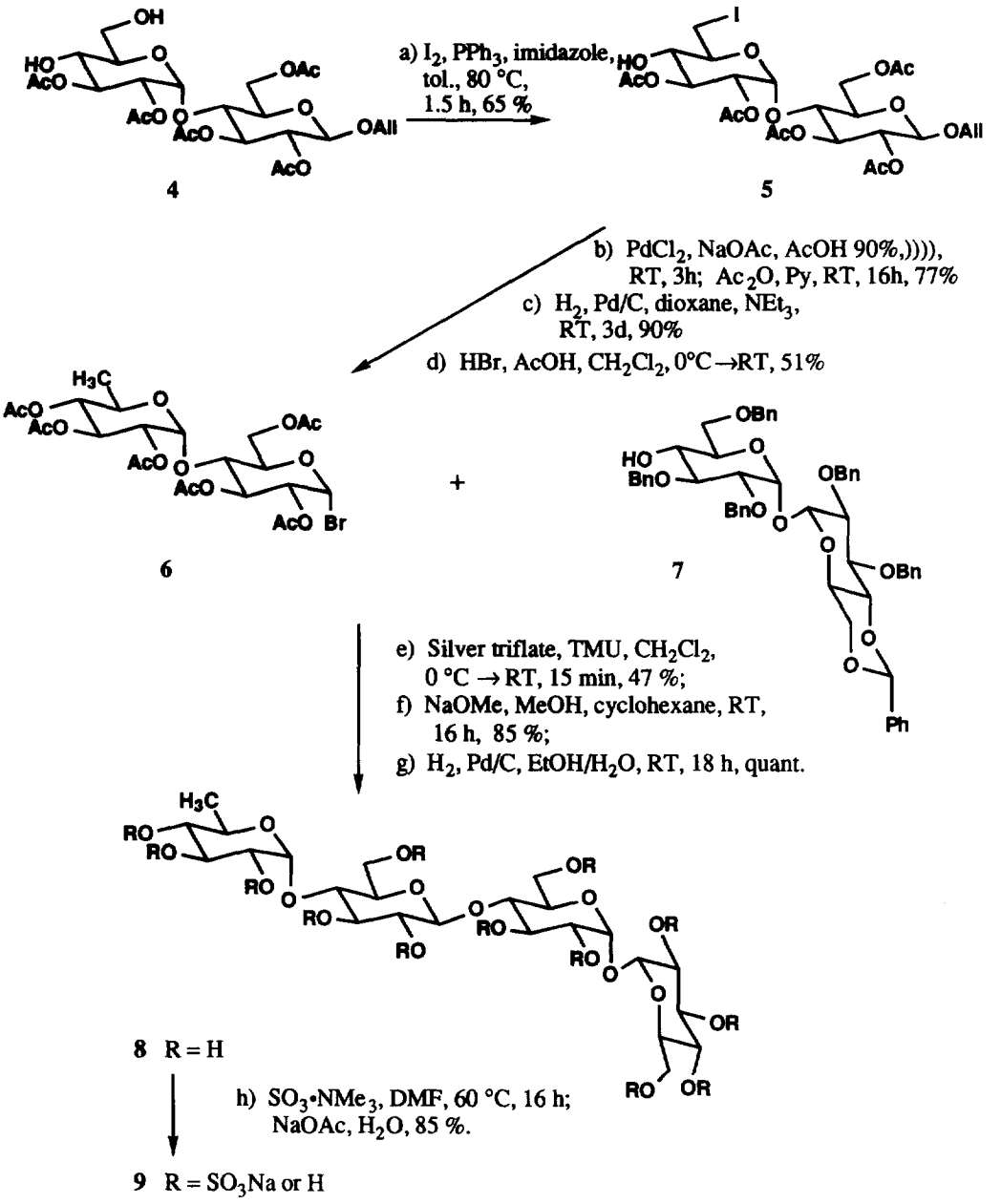
a) DS denotes the degree of sulfation, defined as number of sulfate groups per monosaccharide unit.[6]

proliferative activity. It was therefore our working hypothesis that the spatial arrangement of sulfate groups is crucial. Here we describe the results of our work on the impact of the various sulfate groups of **3** on SMC antiproliferative activity.

Results and Discussion

The oligosaccharides **1** - **3** are highly, but not completely sulfated and thus mixtures of compounds with different sulfate group substitution patterns.[13] To finally arrive at chemically defined compounds it was of interest to determine the relative importance of sulfates at the different positions in the molecule. For this study the tetrasaccharide **3** was selected because it is the compound with the lowest molecular weight and best synthetic availability, together with a high heparin-like antiproliferative activity. We chose a deoxygenation approach to eliminate sulfates at

Scheme 2: Synthesis of 6'''H-β-Mal-(1→4)-α,α-Tre



specific positions of the tetrasaccharide. Deoxygenations of (unsubstituted) saccharides have been carried out, pioneered by the work of Lemieux and collaborators,[14, 15] to study interactions with antibodies, lectins,[16] and enzymes.[17]

For the synthesis of deoxygenated analogues of **3** the [2+2] block synthesis successfully employed for the parent compound was maintained. In most cases it was appropriate to introduce or preform deoxy functions on the disaccharide level, maltose and trehalose being readily available starting materials. A synthetic example using an iodinated intermediate is depicted in Scheme 2. Starting from the known[18] maltosyl derivative **4**, iodine was introduced at the primary 6'-position using the procedure of Garegg and Samuelsson.[19] After removal of the anomeric protective group and hydrogenation of the iodide the reaction scheme channelled into the procedure described[9] for the synthesis of tetrasaccharide **3** employing the established[8, 20] trehalose glycosyl acceptor **7** in a silver triflate mediated[21] Koenigs-Knorr glycosylation reaction.[22, 23] Other procedures employed for the formation of an deoxygenated sugar included the Barton-McCombie deoxygenation[24] and, for 2-de-

Table 1: SMC Antiproliferative activities of selectively deoxygenated derivatives of β-Mal-(1→4)-α,α-Tre

Compound (Degree of sulfation)	*Site of De-oxygenation*	r_i [a]	*Compound (DS)*	*Site(s) of De-oxygenation*	r_i [a]
3 (≈ 80 %)	-	0.9 ± 0.05	**18** (≈ 71 %)	6"	0.7 ± 0.05
10 (≈ 83 %)	2	0.8 ± 0.06	**19** (≈ 71 %)	2"'	0.9 ± 0.09
11 (≈ 80 %)	4	**0.5** ± 0.05	**20** (≈ 86 %)	3"'	0.8 ± 0.05
12 (≈ 83 %)	6	0.9 ± 0.05	**21** (≈ 85 %)	4"'	0.7 ± 0.04
13 (≈ 80 %)	2'	0.7 ± 0.03	**9** (≈ 74 %)	6"'	**0.6** ± 0.07
14 (≈ 83 %)	3'	0.7 ± 0.16	**22** (≈ 84 %)	2,3,2',3'	**0.6** ± 0.09
15 (≈ 86 %)	6'	0.9 ± 0.05	**23** (≈ 88 %)	3,3'	**0.5** ± 0.04
16 (≈ 71 %)	2"	1.0 ± 0.07	**24** (≈ 88 %)	3",3"'	0.8 ± 0.07
17 (≈ 83 %)	3"	0.7 ± 0.04	**25** (≈ 77 %)	4"', 6"'	0.7 ± 0.05

a. Determined in a proliferation assay with rat SMC in at least 3 independent experiments; values ± SEM.

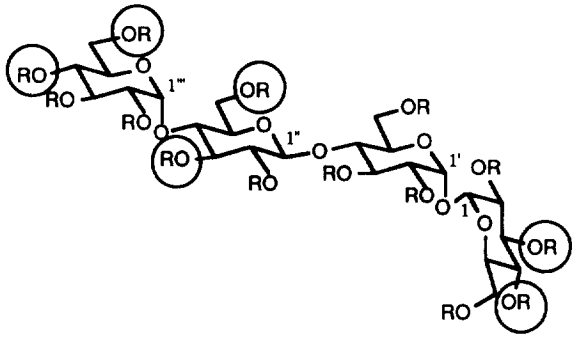
oxy sugars, the NIS[25] and the glycosyloxyselenation methods.[26] The deprotected tetrasaccharides were reacted with sulfur trioxide trimethylamine complex in DMF[6] to provide the sulfated trehalose tetrasaccharides **9** - **25**.

The results from the biological assay are summarized in Table 1. Antiproliferative activities are expressed as relative inhibitory activity (r_i); this value compares the *in vitro* activity of substances at 100 μg/ml in a proliferation assay with rat SMC with that of heparin at the same concentration and in the same experiment. In order to obtain a value for r_i within a satisfactory confidence interval most compounds were tested repeatedly in independent experiments. It is seen that a single deoxy function either has little or no influence on activity or may also lead to a substantial drop as for tetrasaccharide **11**. Values for the oligodeoxygenated tetrasaccharides **22** - **25** are in keeping with the data from the monodeoxygenated analogues. The activity of the missing tetrasaccharide deoxygenated at C-3 can therefore be estimated to be $r_i < 0.7$.

The interpretation of these data is not straightforward because all selectively deoxygenated tetrasaccharides are, as expected, random sulfated and exhibit some variation in their degree of sulfation. Furthermore, the mechanism by which these sulfated compounds inhibit the proliferation of SMC is not known, it is even conceivable that different proteins play a role in the mediation of antiproliferative activity. With these considerations, it is interesting that a single deoxygenation could lead to a strong loss in activity ($r_i = 0.5$). Values of $r_i \leq 0.6$ are below the activity of the parent compound **3** with high significance; positions at which a single deoxygenation led to such significant decrease in activity are highlighted in Scheme 3. It can be assumed that the sulfates at these positions of the tetrasaccharide **3** contribute to the interaction with a target protein.

As depicted in Scheme 3, the inner glucopyranosyl rings of the molecule do not carry any of the essential sulfates; these results, among other findings, led us to the investigation of 'spaced' sulfated saccharides in which a non-carbohydrate moiety is placed between the saccharides.

Scheme 3: Sulfated β-Mal-(1→4)-α,α-Tre: positions at which a single deoxygenation leads to a decrease in antiproliferative activity by > 30 % are circled.



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