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IDENTIFICATION OF A SULFATED TETRASACCHARIDE WITH HEPARIN-LIKE ANTIPROLIFERATIVE ACTIVITY

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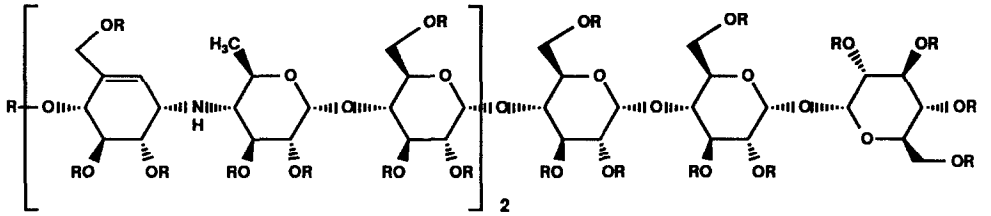
Abstract. - Investigation of the smooth muscle cell antiproliferative activities of Trestatin A sulfate substructures showed that a sulfated pentasaccharide is required to maintain a heparin-like effect. The modification of one glycosidic bond led to a synthetically readily available tetrasaccharide, namely sulfated β-maltosyl trehalose **12**, with similar antiproliferative but devoid of anticoagulant activity.

Introduction

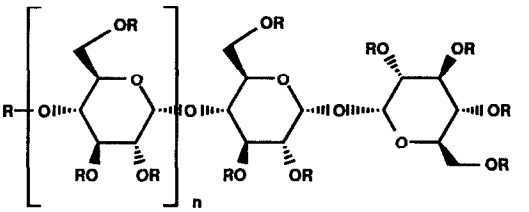
Heparin inhibits the proliferation of smooth muscle cells (SMC) in culture[1] as well as intimal thickening after vascular injury in the rat.[2] This model is thought to reflect the mechanism of restenosis,[3] a renarrowing of the arterial lumen which occurs with high incidence after angioplasty (i.e. opening of an arteriosclerotic artery) and in which SMC migration and proliferation play an important role. Since heparan sulfates with strong antiproliferative activity could be isolated from endothelial cells,[4] heparinoids may also play a physiological role in the regulation of cell growth. The antiproliferative activity of heparin can be separated from its antithrombin III (AT_{III}) mediated anticoagulant activity.[5] With that, a non-anticoagulant heparin analogue could be a drug candidate for the prevention of restenosis.

A molecular mechanism for the growth-inhibitory action of heparin is not yet known and, moreover, the discussion is still ongoing whether heparin acts in a highly specific manner or rather unspecifically through cooperative electrostatic associations.[6,7] Since a unique pentasaccharide responsible for the AT_{III} mediated anticoagulant activity had been identified,[8,9] one aspect of research was to determine the size of oligosaccharides required for antiproliferative activity. A study on heparin oligosaccharides obtained from nitrite degradation followed by gel filtration showed that a dodecamer is necessary for heparin-like antiproliferative activity *in vitro*.[10] Furthermore it has been demonstrated[11] that O-sulfation of heparin saccharides increases their antiproliferative activity; however, it seems that O-oversulfated octasaccharide fractions do not reach the activity of heparin. Sulfated cyclodextrins, originally prepared as anti-hypercholesterolemic agents,[12] also proved to elicit SMC growth inhibitory activity,[13] and notably β-cyclodextrin tetradecasulfate was investigated in this respect.[14] We have published data on Trestatin A sulfate, a pseudo-nonasaccharide with high antiproliferative activity.[15] In this study we present work on Trestatin A sulfate substructures and analogues, resulting in the first description of a compound as small as a tetrasaccharide with heparin-like antiproliferative activity *in vitro*.

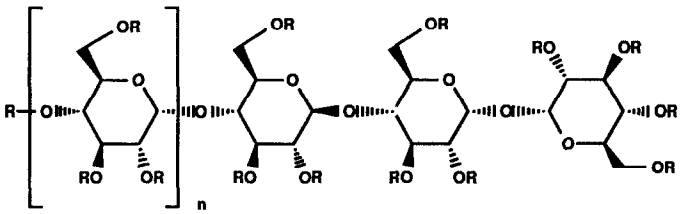
Scheme 1[16,17]



1 R = H
2 R = SO_3Na or H, DS ≈ 2.4



3 n = 1, R = H
4 n = 1, R = SO_3Na or H, DS ≈ 3.0
5 n = 2, R = H
6 n = 2, R = SO_3Na or H, DS ≈ 2.9
7 n = 3, R = H
8 n = 3, R = SO_3Na or H, DS ≈ 2.4



9 n = 0, R = H
10 n = 0, R = SO_3Na or H, DS ≈ 3.0
11 n = 1, R = H
12 n = 1, R = SO_3Na or H, DS ≈ 2.8
13 n = 2, R = H
14 n = 2, R = SO_3Na or H, DS ≈ 2.6

Results and Discussion

Trestatin A (**1**) had been developed as an amylase inhibitor[18,19] and, as such, very likely has an amylose-like helical conformation.[20,21] The obvious thing to do was the investigation of amylose saccharides. However, these maltooligosaccharides, after sulfation, had no or low antiproliferative activity (data not shown here). On the other hand, molecular modelling experiments[21] suggested that the trehalose end of Trestatin A bends out from the helical conformation which led to the hypothesis that this moiety of Trestatin A sulfate might be responsible for the biological activity of the molecule.

The preparation of Trestatin A tri- and tetrasaccharide substructures **3** and **5** has been described.[22,23] Compound **7** is a 6''''-hydroxylated pentasaccharide substructure analogue,[23] which is more readily synthesized

Scheme 2

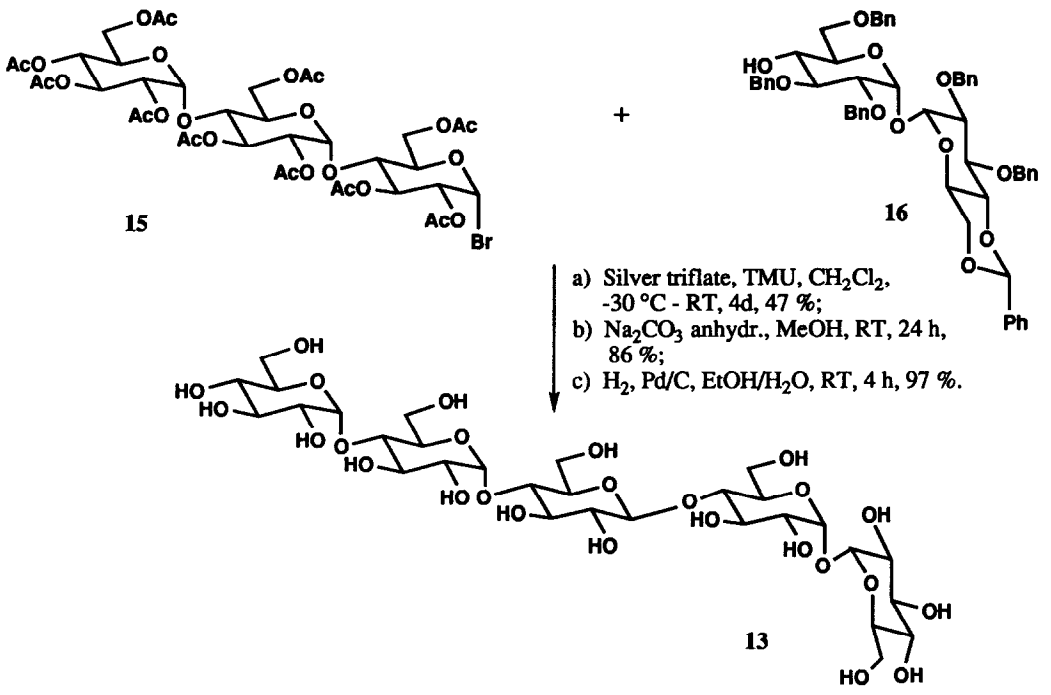


Table 1

Compound	*Antiproliferative Activity*[a] Growth Inhibition at 100 µg/mL [%]	*Anticoagulant Activity*[b] IC_{50} in Chromogenic Tests Anti-IIa [µg/mL]	Anti-Xa [µg/mL]
2	76	>1000	>1000
4	inactive	>1000	>1000
6	<12	>1000	>1000
8	57 ±2.1	>1000	960
10	38 ±1.6	>1000	>1000
12	59 ±0.7	>1000	>1000
14	64 ±2.2	>1000	>1000
Heparin[c]	47 ±5.5	1.9±0.2	2.6±0.1

a. Determined in triplicate with rat SMC, protocol according to ref.15; values ± standard error of mean.
b. Determined as reported in ref.15. c. 4th International Standard, values ± standard error of mean.

than the exact 6""-deoxy substructure. Standard sulfation[15] of these oligosaccharides led to **4**, **6**, and **8**. As shown in Table 1, a pentasaccharide substructure is required to reach a heparin-like antiproliferative effect.

With the glycosyl acceptor **16** in hand, we prepared the analogous β-D-linked oligosaccharides in a block synthesis approach using the respective per-O-acetyl-glycosyl bromides as glycosyl donors. Condensations were carried out with silver triflate as promoter.[24] Standard deblocking gave the free saccharides **9**, **11**, and **13**. A synthetic example is depicted in Scheme 2. Sulfation of **9**, **11**, and **13** furnished **10**, **12**, and **14**. In this series, already the sulfated tetrasaccharide led to heparin-like antiproliferative activity *in vitro* (cf. Table 1), the pentasaccharide being only slightly more active. None of these synthetic sulfated oligosaccharides exhibits AT_{III} mediated anticoagulant activity. The different antiproliferative activities in the α-D- *vs.* the β-D-linked oligosaccharide series show that the positions of sulfates are of importance and thus indicate specificity of binding.

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