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THE HEPARIN-LIKE ANTIPROLIFERATIVE ACTIVITY OF SULFATED TETRASACCHARIDES IS SENSITIVE TO STRUCTURAL VARIATION

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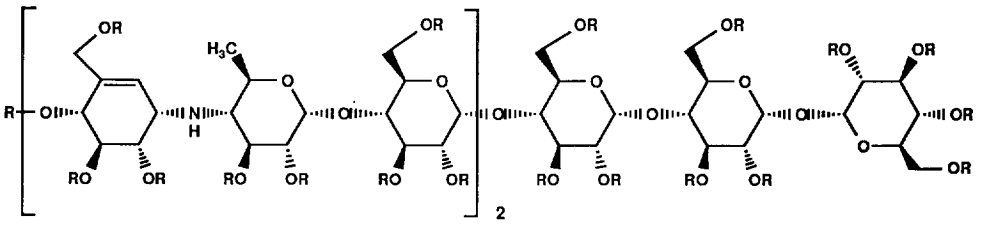
Abstract
The sulfated tetrasaccharide β-maltosyl-(1→4)-α,α-trehalose **2** with high antiproliferative activity on smooth muscle cells was modified in the maltosyl moiety by linking eight different disaccharides equatorially to trehalose; the biological activity varied strongly between different structures.

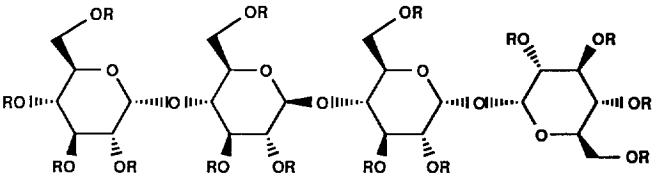
Introduction

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 (i.e. opening of an arteriosclerotic artery). Heparin inhibits the proliferation of SMC in culture[2] as well as after vascular injury in the rat.[3] Heparan sulfates with strong antiproliferative activity have been isolated from endothelial cells[4] which 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.

Our investigation on carboxyl-reduced sulfated heparin (CRS-heparin) showed that carboxyl groups are not required for heparin-like antiproliferative activity.[6] Since heparins with their dispersion of molecular weight and structural and stereochemical heterogeneity[7] were believed not to be an ideal starting point for drug development, our further research was directed to the identification of active compounds of lower molecular weight. A screening programme on non-uronic sulfated oligosaccharides led to the identification of sulfated Trestatin A (**1**), a sulfated pseudo-nonasaccharide, which had high antiproliferative activity on SMC and was concomitantly non-anticoagulant. Recently, we could show that also sulfated α-maltotriosyl-(1→4)-α,α-trehalose, a pentasaccharide substructure of sulfated Trestatin A, conveys heparin-like antiproliferative activity.[8] Moreover, modification of the α-D-linked substructures to β-D-linked analogues led to the sulfated β-maltosyl-



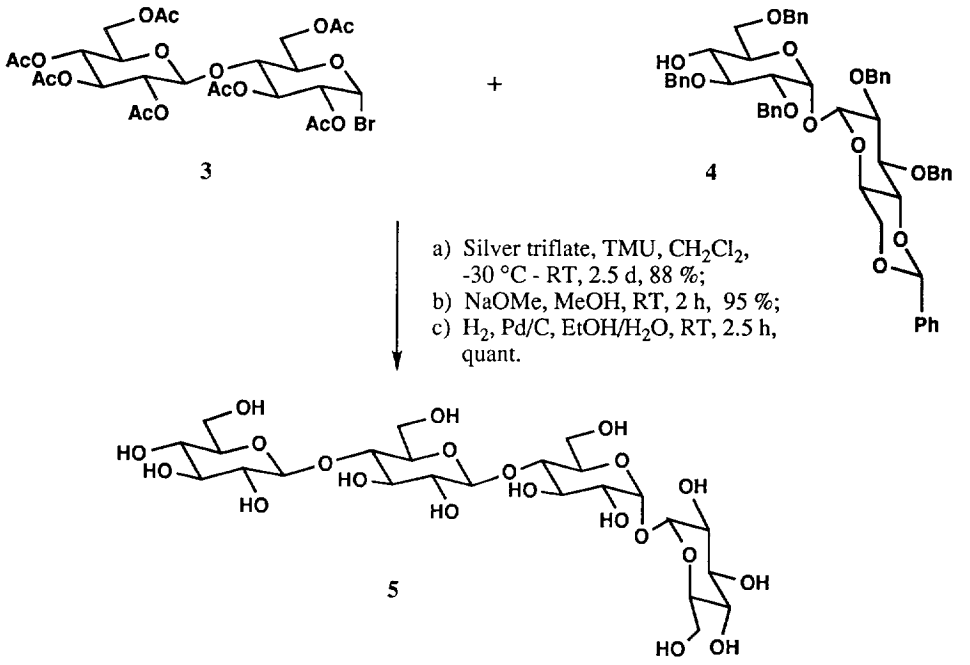
1 R = SO_3Na or H, DS ≈ 2.4 (ref. 9), **Trestatin A sulfate**



2 R = SO_3Na or H, DS ≈ 2.8 (ref. 9)

(1→4)-α,α-trehalose (**2**) tetrasaccharide, so far the smallest sulfated oligosaccharide with heparin-like antiproliferative, but no anticoagulant activity *in vitro*.[8] Here we describe modifications of this highly active tetrasaccharide which illustrate the sensitivity of biological activity towards structural variation and thus hint to specific interactions.

Scheme 1: Synthesis of β-Cel-(1→4)-α,α-Tre



3 **4** **5**

Results and Discussion

In order to study the structure - activity relationship a series of sulfated tetrasaccharides closely related to **2** was prepared. β-Maltosyl-(1→4)-α,α-trehalose, the synthetic precursor of sulfated compound **2**, is readily available in a block glycoside synthesis coupling maltose and trehalose building blocks.[8,10] In a similar fashion, analogues were synthesized replacing the maltosyl donor by another suitable disaccharide glycosyl donor. As a typical example, Scheme 1 shows the reaction of known[11] cellobiosyl bromide **3** with the established[12,13] trehalose glycosyl acceptor **4** to yield the tetrasaccharide **5** in a silver triflate mediated[14] Koenigs-Knorr reaction.[15,16] The stereochemistry of the glycosidation reactions was controlled by the presence of a participating[17] ester neighbouring group at the C-2 position of the glycosyl donor to guarantee the β-D-linkage between the two disaccharide building blocks. Thus, besides cellobiose, the disaccharides isomaltose [α-D-Glc-(1→6)-D-Glc], melibiose [α-D-Gal-(1→6)-D-Glc], rutinose [α-L-Rha-(1→6)-D-Glc], gentiobiose [β-D-Glc-(1→6)-D-Glc], and lactose [β-D-Gal-(1→4)-D-Glc] were β-(1→4)-linked to trehalose. The synthesis of the corresponding β-sophorosyl [β-D-Glc-(1→2)-β-D-Glc] trehalose, which was less straightforward due to the non-participating neighbouring group at the C-2 position, was described elsewhere.[18] As shown in Scheme 2, the new 2,4-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-arabinopyranosyl bromide (**6**)[19] was employed to furnish the α-D-linked tetrasaccharide **7**.[15] Despite the different stereochemistry of the newly formed glycosidic linkage, **7** can be compared to the previously described tetrasaccharides because the α-D-arabinopyranosyl moiety is attached

Scheme 2: Synthesis of β-D-Gal-(1→3)-β-D-Ara-(1→4)-α,α-Tre

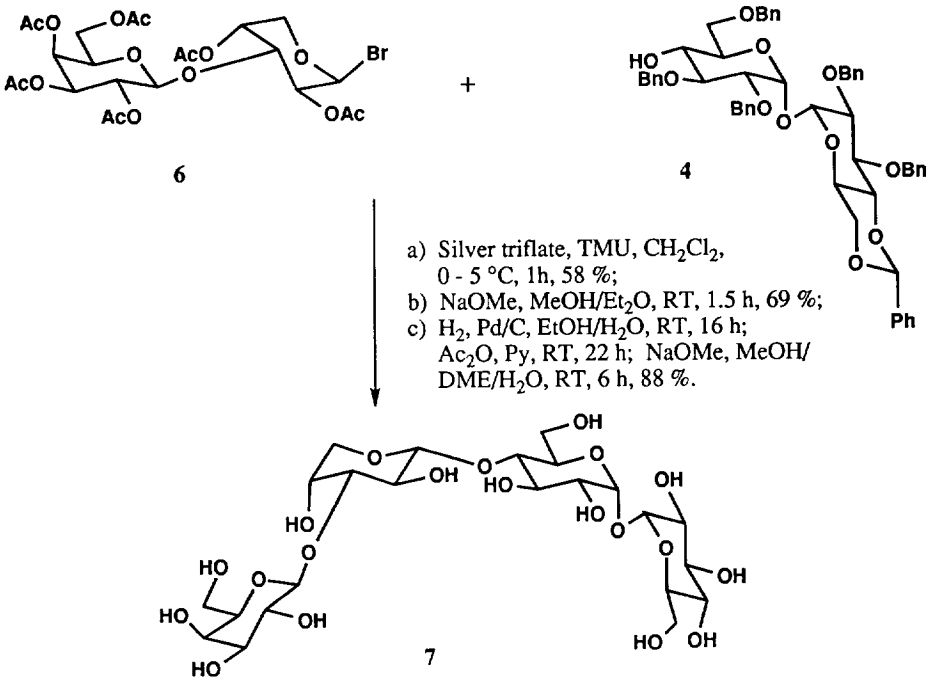
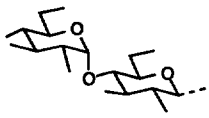
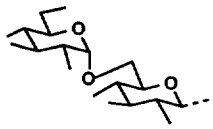
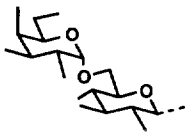
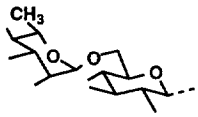
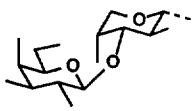
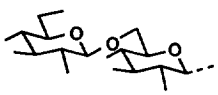
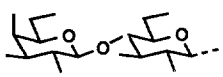
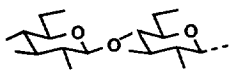


Table 1: Biological activities of sulfated tetrasaccharides of the type (disaccharide)-(1→4)-α,α-trehalose

Compound *(DS)*[9]	*Disaccharide structure*	*Disaccharide Configuration*	*Anti-proliferative activity*[a] r_i	*Anticoagulant activity*[b] IC_{50} [μg/ml] anti-IIa / anti-Xa
2 (DS ≈ 2.8)		αDGlc(1→4)βDGlc (Mal)	0.9 ± 0.05	> 1000 / > 1000
8 (DS ≈ 3.0)		αDGlc(1→6)βDGlc (Iso-Mal)	0.9 (n=1)	> 1000 / > 1000
9 (DS ≈ 3.0)		αDGal(1→6)βDGlc (Mel)	0.8 ± 0.01	> 1000 / > 1000
10 (DS ≈ 3.0)		αLRha(1→6)βDGlc (Rut)	0.7 (n=1)	> 1000 / > 1000
11 (DS ≈ 2.7)		βDGal(1→3)αDAra	0.7 (n=1)	> 1000 / > 1000
12 (DS ≈ 3.3)		βDGlc(1→6)βDGlc (Gen)	0.6 ± 0.12	> 1000 / > 1000
13 (DS ≈ 2.9)		βDGal(1→4)βDGlc (Lac)	0.5 ± 0.21	> 1000 / > 1000
14 (DS ≈ 2.8)		βDGlc(1→2)βDGlc (Soph)	0.5 ± 0.05	> 1000 / > 1000
15 (DS ≈ 2.7)		βDGlc(1→4)βDGlc (Cel)	0.3 ± 0.13	> 1000 / > 1000
Heparin[c]			1.0 ± 0	1.9±0.2 / 2.6±0.1

a. Determined with rat SMC in at least 3 independent experiments; values ± standard error of mean.
b. Determined as reported in ref.6. c. 4th International Standard, values ± standard error of mean.

equatorially due to the favoured $^1C_4$ conformation as evidenced by ^1H NMR data.[20] The deprotected tetrasaccharides were reacted with sulfur trioxide trimethylamine complex in DMF[6] to result in the sulfated trehalose tetrasaccharides **8 - 15**. The modified disaccharide structures are summarized in Table 1 along with their biological activities

The antiproliferative activity of the compounds was measured with SMC in culture. Since the responsiveness of the cells to heparin and small sulfated carbohydrates varied in different experiments, i.e. inhibition of cell growth by a given substance in separate experiments was inconsistent, we calculated a value r_i – the *relative inhibitory activity*. 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 assay. In order to obtain a value for r_i within a satisfactory confidence interval most compounds were tested in this SMC proliferation assay repeatedly in independent experiments.

Inspection of Table 1 shows that none of the disaccharide variations led to an increased antiproliferative activity. On the contrary, a single modification, such as the attachment of the terminal glucose in β-D- instead of α-D-fashion through the replacement of maltose by cellobiose, led to a significant drop in activity (r_i = 0.9 to r_i = 0.3). Since all tetrasaccharides are sulfated to a similar degree, it can be concluded that the positioning of sulfates and thus the overall conformation of the molecule is of importance. This is supported by the finding that the sulfated isomaltosyl trehalose **8** has the same activity as the maltosyl derivative **2**, since the similarity in the overall conformation of maltose and isomaltose are known. It is thus clear that the antiproliferative effect of the sulfated carbohydrates discussed here is not mediated through an *unspecific* charge-charge interaction. So far, however, the molecular mechanism of this effect is not known yet, and it remains to be seen whether a highly specific interaction with one protein is responsible as it was shown for the well-investigated interaction of heparin with antithrombin III.[21]

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9. DS denotes degree of sulfation, defined as mean number of sulfates per monosaccharide unit; cf. ref. 6.
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15. All new structures are supported by ^{1}H-NMR and mass spectra as well as elemental analyses.
16. Selected physical data for **5**: Colourless foam, $[\alpha]_D^{20}$ + 34.7° (*c* 0.3, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$, δ in ppm) 5.13 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1); 5.12 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1'); 4.47 (d, 1H, $J_{1'',2''}$ 8.1 Hz, H-1''); 4.37 (d, 1H, $J_{1''',2'''}$ 7.7 Hz, H-1''').
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19. Selected physical data for **6**: Colourless foam, $[\alpha]_D^{20}$ - 148.8° (*c* 0.5, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$, δ in ppm) 6.67 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.06 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2); 4.57 (d, 1H, $J_{1',2'}$ 7.7 Hz, H-1'), 5.12 (dd, 1H, $J_{2',3'}$ 10.9 Hz, H-2').
20. Selected physical data for **7**: Colourless foam, $[\alpha]_D^{20}$ + 42.0° (*c* 0.5, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$, δ in ppm) 5.16 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1); 5.19 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1'); 4.94 (d, 1H, $J_{1'',2''}$ 7.0 Hz, H-1''), 5.10 (dd, 1H, $J_{2'',3''}$ 10.0 Hz, H-2''); 4.50 (d, 1H, $J_{1''',2'''}$ 7.8 Hz, H-1''').
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