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SYNTHESIS OF A POTENT ANTITHROMBIN ACTIVATING PENTASACCHARIDE: A NEW HEPARIN-LIKE FRAGMENT CONTAINING TWO 3-O-SULPHATED GLUCOSAMINES

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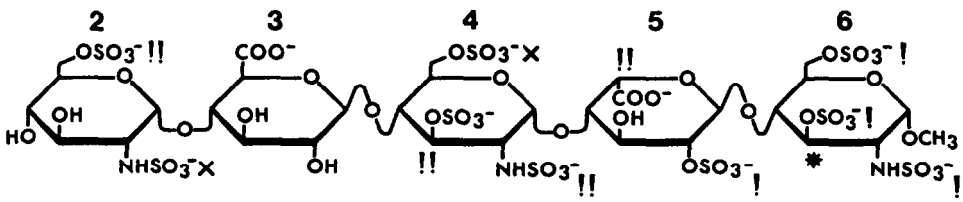
Summary
The synthesis of a pentasaccharide corresponding to the antithrombin III binding region of heparin, but containing an extra 3-O-sulphate group at the reducing end, is described. This compound elicits higher anti-Xa activity than the antithrombin III binding region of heparin.

It is well established now that the minimal antithrombin III (AT-III) binding region of heparin consists of an unique pentasaccharide fragment[1]. This pentasaccharide, which has become synthetically available[2], catalyzes the AT-III mediated inactivation of factor Xa (anti-Xa activity), but not of thrombin.

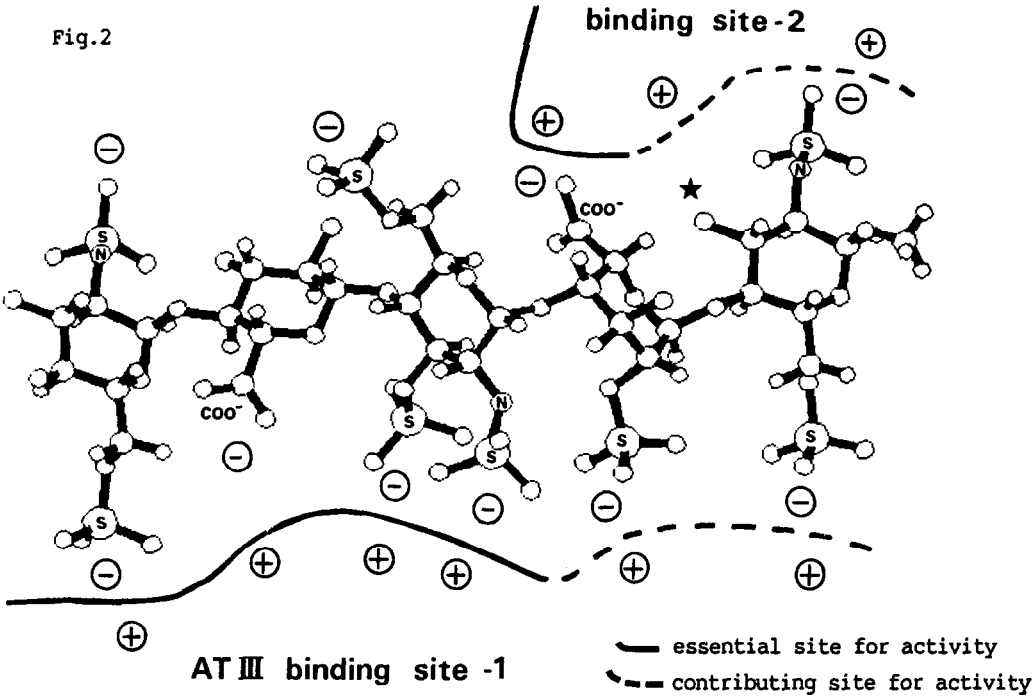
In this communication we wish to introduce a very potent synthetic analogue (i.e. compound 1 in Fig. 1) of the naturally occurring fragment, containing an additional 3-O-sulphate group at the reducing glucosamine-unit 6 [3] (see asterisk in Fig.1). This analogue displays an anti-Xa activity of about 1270 U/mg in an amidolytic assay[4], whereas the synthetic pentasaccharide corresponding to the AT-III binding site of heparin, displays 590 anti-Xa U/mg[5]. The higher activity of analogue 1 has to be attributed to the presence of the additional 3-O-sulphate group. In this respect, it is important to note that the 6-O-sulphate group at glucosamine unit 2 [6], the 3-O-sulphate[7] and N-sulphate[8] groups at glucosamine unit 4, as well as the carboxylate moiety of iduronic acid unit 5 [9] are essential (!! in Fig. 1) for activation of AT-III. In addition, the 2-O-sulphate at iduronic acid unit 5 [10] and the 6-O-sulphate[11] and N-sulphate[8] groups of glucosamine unit 6 increase (! in Fig. 1) the AT-III mediated activity. On the other hand the N-sulphate (N-acetyl) group at unit 2[1b,7] and the 6-O-sulphate at unit 4 [12] are considered to be non-essential (x in Fig. 1).

Fig.1



1

Fig.2

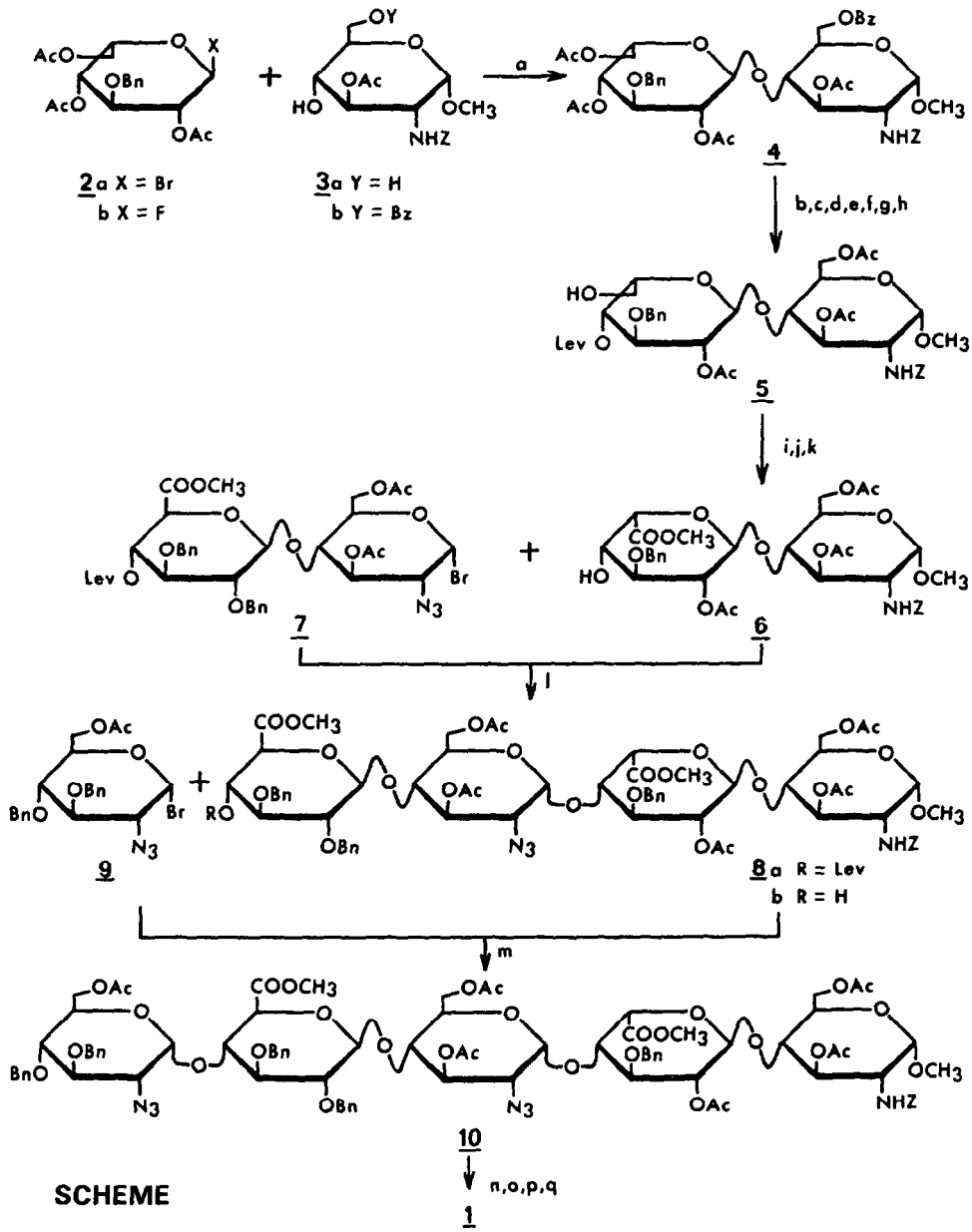


Taking into consideration these findings, the heparin-binding site of AT-III can be mapped around a molecular model[9a,13] of the naturally occurring heparin pentasaccharide (Fig. 2). In Fig.2 one can see that the pentasaccharide exhibits a linear conformation with binding areas at the south- and north site of the molecule (i.e. AT-III binding sites 1 and 2, respectively). The higher activity of compound 1, relative to the naturally occurring heparin fragment, can be tentatively ascribed to its enhanced interaction with AT-III at binding site-2, brought about by the extra 3-O-sulphate group (In Fig.2 this sulphate group would be located at the position of the asterisk ★).

The synthesis of compound 1 is outlined in the Scheme. Following a well-established strategy[2,9,11] the fully protected pentasaccharide 10 should be prepared, containing acetyl functions at hydroxyl functions to be sulphated and benzyl protective groups for unsulphated hydroxyl groups.

We started the synthesis[14] from easily available methyl 4,6-O-benzylidene-2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyranoside[15], which was acetylated in acetic anhydride/pyridine and then treated with aqueous acetic acid to afford 3a (90% yield). Compound 3a was selectively benzoylated at the primary hydroxyl group to afford compound 3b in 79% yield.

Since coupling of the unreactive compound 3b with known L-idopyranosyl bromide derivative 2a[2b] was disappointing we turned attention to the corresponding fluoride derivative 2b. Treatment of 1,2,4,6-tetra-O-acetyl-3-O-benzyl-L-idopyranose[2b] with 70% hydrogen fluoride/pyridine in dichloromethane for 4 h at 0°C, gave after work-up and chromatography pure 2b in 60% yield (^{1}H-NMR ($CDCl_3$), δ = 5.70 (dd, J=48 Hz, J=3Hz, H-1). Condensation of compounds 2b and 3b in the presence of borontrifluoride etherate gave disaccharide 4. Conversion of 4 into the desired iduronic acid-glucosamine building block 6 has been performed as described recently in similar syntheses[2b,11]. First, compound 4 was re-protected in seven steps to give 5. Oxidation of compound 5 with chromium (vi) oxide was followed by diazomethane treatment and deprotection of the levulinoyl ester to give compound 6 ($[\alpha]_D^{20}$ = 20.5, c = 1, dichloromethane).



Scheme: a) BF_3-etherate, MS 4A, CH_2Cl_2, -20°C, (73%); b) KOtBu, MeOH/dioxane, RT; c) 2,2-dimethoxy propane, pTS, DMF, RT, (b→c, 90%); d) Ac_2O, pyridine, 35°C; e) $AcOH/H_2O$, 40°C, (d→e, 93%); f) Dimethoxytrityl chloride, THF/pyridine, -6°C; g) Levulinoic acid anhydride, THF/pyridine, RT; h) $AcOH/H_2O$, RT, (f→h, 85%); i) CrO_3, acetone, 0°C; j) CH_2N_2, CH_2Cl_2, RT, (i→j, 85%); k) H_2NNH_2, AcOH, pyridine, RT, (83%); l) $AgSO_3CF_3$, MS 10A, CH_2Cl_2, -30°C, (50%); m) $AgSO_3CF_3$, MS 4A, 2,6-di-t-butylpyridine, CH_2Cl_2, -55°C, (80%); n) NaOH, $H_2O/MeOH/CHCl_3$, RT, (55%); o) $SO_3N(CH_3)_3$, DMF, 50°C, (55%); p) H_2, Pd/C, $MeOH/H_2O$, RT; q) $SO_3N(CH_3)_3$, Na_2CO_3, H_2O, RT, (p→q, 70%).

Abbreviations: Bn = benzyl, Bz = benzoyl, Ac = acetyl, Lev = levulinoyl, Z = benzyloxycarbonyl

Reaction of 6 with known glycon 7[2b], in the presence of silver triflate and molecular sieves, gave tetrasaccharide 8a. Compound 8b was obtained after hydrazinolysis of the levulinoyl group. Coupling of excess of 9 with 8b in the presence of silver triflate and 2,6-di-t-butylpyridine gave, after purification, the fully protected pentasaccharide 10 ($[\alpha]_D^{20}$ = 44.7; c = 0.9, dichloromethane)[16]. Finally, in the following four-step procedure the fully protected derivative 10 was converted into analogue 1: i) simultaneous saponification of acetyl esters and carboxyl-methyl esters; ii) O-sulphation, Sephadex LH-20 chromatography; iii) hydrogenolysis; iv) N-sulphation. The crude product was purified by Sephadex DEAE chromatography and then desalted (Sephadex G10). The structure of compound 1 was confirmed by 2-dimensional proton-proton correlated spectroscopy (2D-COSY)[17]. Most remarkably, the α-L-iduronic acid part of compound 1 adopts (in D_2O) mainly the $^2S_0$ skew boat conformation[13b,18], whereas α-L-iduronic acid in the natural occurring pentasaccharide occurs in an equilibrium between $^2S_0$ and $^1C_4$ forms (ratio about 2:1)[19,20].

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3. The unique pentasaccharide is part of an isolated octasaccharide (ref. 1) 1-[2-3-4-5-6]-7-8:-IdA-[GlcNAc(6-OSO_3)-GlA-GlcNSO_3(3,6-OSO_3)-IdA(2-O-SO_3)-GlcNSO_3(6-O-SO_3)]-IdA(2-OSO_3)-A Man.
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5. The synthetic AT-III binding site of heparin, containing α-methoxy at the anomeric center, elicits 740 anti-Xa U/mg (Th.G. van Dinther, unpublished result).
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b. Although 2-O-sulphated α-L-iduronic acid in heparin-like fragments occur in an equilibrium between $^2S_0$ and $^1C_4$ forms, we postulated that the pentasaccharide bound at AT-III displays α-L-iduronic acid in the $^1C_4$ conformation (see ref. 20).
14. Currently we introduce a methyl group at the anomeric center to avoid side-reactions at the reducing end during the last two steps of the synthesis (refs. 9 and 11).
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16. ^{1}H-NMR (360 MHz, $CDCl_3$), compound 10, δ = 4.68 (d, J = 3.5 Hz, H-1), 5.06 (d, J = 2.5 Hz, H-1'); 4.92 (d, J = 3.5 Hz, H-1''), 4.35 (d, J = 7.9 Hz, H-1'''), 5.50 (d, J = 3.6 Hz, H-1'''').
17. ^{1}H-NMR (360 MHz, D_2O) compound 1, δ = 4.97 (d, J = 3.5 Hz, H-1); 5.10 (d, J = 5.3 Hz, H-1'); 5.58 (d, J = 3.5 Hz, H-1''); 4.59 (d, J = 7.9 Hz, H-1'''); 5.59 (d, J = 3.5 Hz, H-1''''); 4.38 (dd, J = 10.8 Hz, J = 9.2 Hz, H-3); 4.32 (dd, J = 10.8 Hz, J = 9.2 HZ, H-3''). $[\alpha]_D^{20}$ =38.4 (c = 0.61, H_2O).
18. The α-L-iduronic acid part of the spectrum was computer-simulated (PANIC.84): J 1,2 = 5.3 Hz, J 2,3 = 9.4 Hz, J 3,4 = 4.4 Hz, J 4,5 = 4.4 Hz ($^2S_0$: $^1C_4$ is about 9:1).
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