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SYNTHESIS OF AN ANALOGUE OF THE ANTITHROMBIN BINDING REGION OF HEPARIN CONTAINING α-L-IDOPYRANOSE.

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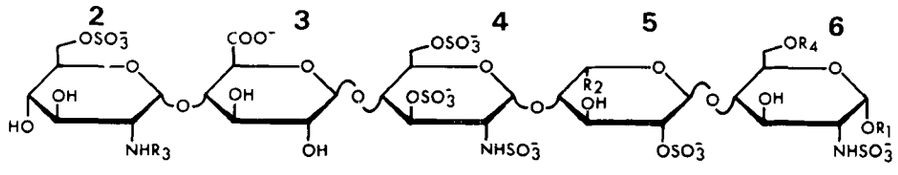
ABSTRACT. The synthesis of a heparin-like pentasaccharide, containing 2,6--di-O-sulphated α-L-idopyranose instead of the naturally occurring 2-O-sulphated α-L-idopyranuronate, is described. Although conformational properties of these compounds are similar, the analogue is not biologically active.

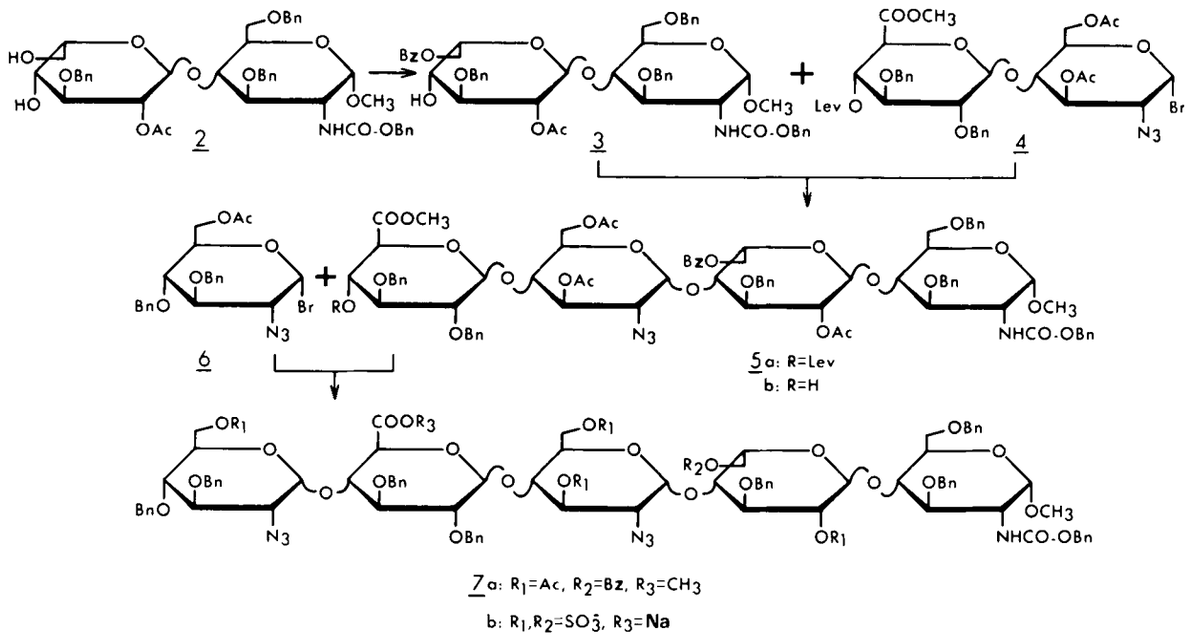
Heparin, an important drug in anticoagulant therapy consists of a mixture of sulphated glycosaminoglycans. About one-third of the heparin polysaccharides includes an unique pentasaccharide subunit 1a[1], which constitutes the minimal binding site for the coagulation inhibitor AT-III. It is well established which sulphate groups of compound 1a are essential for catalyzing the AT-III mediated inactivation of blood-coagulation factor Xa (anti-Xa activity)[1,2,6]. Recently, it has been suggested that also the conformational properties of the flexible carbohydrate moiety α-L-iduronic acid play an important role in the activation process of AT-III[1,3,4].

Since it is known that L-idopyranose can adopt similar conformations as L-idopyranuronate[5], we decided to prepare an analogue of fragment 1a containing 2,6-di-O-sulphated α-L-idopyranose instead of 2-O-sulphated α-L-idopyranuronate (i.e. compound 1c, see Figure).

Actually, compound 1c is an analogue of the synthetic heparin fragment 1b[6], which reducing glucosamine unit lacks the 6-O-sulphate group and which anomeric hydroxyl group is methylated.



1 a: R_1=H, R_2=COO^-, R_3=Ac, R_4=SO_3^-
b: R_1=CH_3, R_2=COO^-, R_3=SO_3^-, R_4=H
c: R_1=CH_3, R_2=$CH_2OSO_3^-$, R_3=SO_3^-, R_4=H



The synthesis of pentasaccharide 1c could be realized by using the building blocks 2[6], 4[7] and 6 (see Scheme), following a similar approach as described in previous heparin-syntheses[6,7,8]. Disaccharide 2 was regioselectively benzoylated with benzoyl chloride in pyridine at -35°C to give compound 3 in 72% yield. Aglycon 3 was coupled with known glycon 4[7] in dichloromethane at -35°C, using silver triflate as promoter and 2,6-di-t-butylpyridine as acid scavenger to give crude tetrasaccharide 5a. After silica gel chromatography (toluene/acetone; 9/1, v/v) pure α-tetrasaccharide 5a was obtained in 22% yield. The levulinoyl group was cleaved from 5a with hydrazine hydrate to afford 5b in 80% yield. Compound 5b was then coupled with monosaccharide 6 at -60°C in the presence of silver triflate as promoter and 2,4,6-collidine as acid scavenger to give, after purification by silica gel chromatography (toluene/acetone; 9/1, v/v), fully protected pentasaccharide 7a in 70% yield[9]. The ester groups of compound 7a were simultaneously saponified with sodium hydroxide in a mixture of chloroform/methanol/water. O-sulphation was performed with sulphurtrioxide trimethylamine complex in N,N-dimethylformamide at 50°C to afford, after silica gel chromatography (methylene chloride/methanol, 9/1, v/v) compound 7b in 60% yield. Hydrogenolysis of benzyl functions and azido groups in methanol/ water, followed by selective N-sulphation (sulphurtrioxide trimethylamine complex, water, r.t., pH 9.5) gave crude compound 1c. Final purification was executed by ion-exchange chromatography over Sephadex DEAE; after desalting over Sephadex G10, pure pentasaccharide 1c was obtained in 40% yield. $[\alpha]_D^{20}$ = +51.3 (water, c=0.75). The structure of compound 1c was fully ascertained by two dimensional ^1H-^1H correlated NMR-spectroscopy[10] (see Table).

Table ^{1}H-NMR (360 MHz, D_2O, δ in ppm, J in Hz)

	Unit 2 δ (J)	Unit 3 δ (J)	Unit 4 δ (J)	Unit 5[10] δ (J)	Unit 6 δ (J)
H-1	5.61 (3.6)	4.62 (7.8)	5.48 (3.6)	5.095 (5.25)	5.02 (3.6)
H-2	3.25 (10.1)	3.42 (9.0)	3.49 (10.5)	4.291 (7.82)	3.27 (10.2)
H-3	3.62 (9.0)	3.84 (c)	4.45 (9.0)	4.198 (5.93)	3.72 (c)
H-4	3.57 (9.5)	3.77 (c)	4.01 (9.5)	4.042 (4.37)	3.66 (c)
H-5	3.87 (m)	3.84 (c)	4.09 (m)	4.583 (5.39)	3.78 (c)
H-6A	4.14 (2.0)	-	4.47 (3.0)	4.267 (6.96)	3.91 (c)
H-6B	4.36 (-11,0)	-	4.32 (-12.0)	4.332 (-10,84)	3.91 (c)

Unfortunately, analogue 1c displayed only very low AT-III mediated anti-Xa activity (i.e. 1.0 anti-Xa U/mg) with respect to the L-iduronic acid containing analogue 1b (150 anti-Xa U/mg) or compound 1a (600 anti-Xa U/mg). The loss of activity can hardly be ascribed to conformational differences[11] between 1c and 1a,b, since the vicinal coupling constants[10] of the L-idopyranose moiety in 1c are indicative for the presence of $^1C_4$, ^2S_o and $^4C_1$ conformations (ratio about 1:2:1), while the L-idopyranuronate of heparin-like fragments 1a,b occur in a $^1C_4 \rightleftharpoons {}^2S_o$ equilibrium (ratio about 1:2)[3,4]. Therefore, we presume that the carboxylate function of α-L-idopyranuronate (unit 5) in the natural compound 1a interacts with AT-III to bring about activation of the inhibitor. This hypothesis is further supported by the fact that another analogue of 1a, which lacks the carboxylate of iduronic acid (i.e. containing 2-O-sulphated-β-D-xylopyranose), does not exhibit significant anti-Xa activity[12] either.

In conclusion, as established so far the functional groups of heparin fragment 1a, that are essential for activation of AT-III, are the 6-O-sulphate group[1,2] at unit 2, the 3-O-sulphate and N-sulphate groups at unit 4[1,2], and the carboxylate function of unit 5.

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9. ^{1}H-NMR (360 MHz, $CDCl_3$) compound 7a: δ = 4.66 (d, H-1, J = 3.6 Hz); 5.02 (d, H-1', J = 3.0 Hz); 4.77 (d, H-1'', J = 3.6 Hz); 4.32 (d, H-1''', J = 7.9 Hz); 5.49 (d, H-1'''', J = 3.6 Hz).
10. Accurate vicinal coupling constants of the Idose moiety (7-spin system) were obtained by computer-simulation of the proton spectrum, using a LAOCOON-type computer program (PANIC.84).
11. Carbohydrate Units 2,3,4 and 6 of compound 1c display regular $^4C_1$ conformations as was also observed for compounds 1a,b.
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