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BIOLOGICALLY ACTIVE HEPARIN-LIKE FRAGMENTS WITH A "NON-GLYCOSAMINO"GLYCAN STRUCTURE. Part 1: A PENTASACCHARIDE CONTAINING A 3-O-METHYL IDURONIC ACID UNIT.

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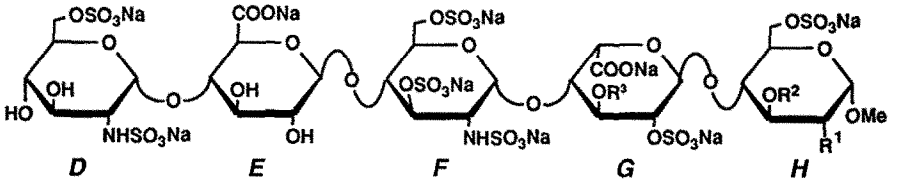
Abstract. *We describe a new approach towards biologically active analogues of glycosaminoglycan fragments where N-sulphates are replaced by O-sulphates and free hydroxyls are substituted by alkyl ethers. Here we demonstrate that introduction of a methyl group at the 3 position of L-iduronic acid residue neither affects the AT III mediated anti-factor Xa activity nor alters the conformational properties of a unique heparin pentasaccharide sequence.*

The unique pentasaccharide sequence **1** is responsible for the binding of the polysaccharide heparin to the plasma protein antithrombin III (AT III), a member of the serine protease inhibitors super family (serpins). Binding of heparin to AT III induces a conformational change which results in inhibition of several blood coagulation factors, particularly factor Xa and thrombin. This phenomenon constitutes the rationale for the clinical use of heparin as a drug in the prevention of venous thrombosis[1]. The chemical synthesis of **1** has been achieved[2-5] and, most remarkably, it was found that this pentasaccharide sequence induces a similar conformational change in AT III as heparin, although it promotes selectively factor Xa inhibition[6]. Compound **1** displays antithrombotic properties in animal models[7,8] and therefore represents a potential new class of antithrombotic drugs, acting by sole inhibition of blood coagulation factor Xa.



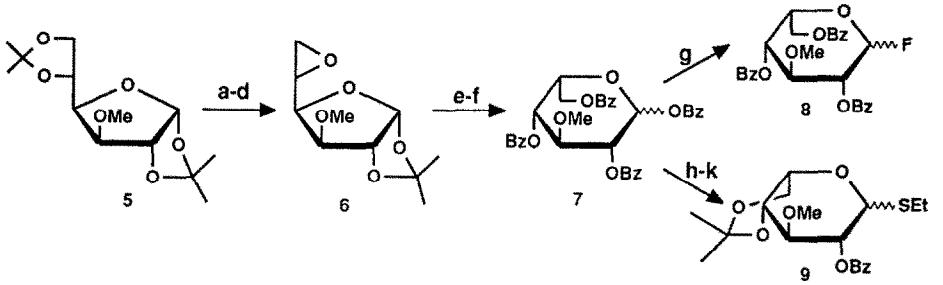
1: R^1=$NHSO_3Na$ R^2=H R^3=H **3**: R^1=OSO_3Na R^2=SO_3Na R^3=H
2: R^1=$NHSO_3Na$ R^2=SO_3Na R^3=H **4**: R^1=OSO_3Na R^2=SO_3Na R^3=Me

We have studied the structure activity relationship in **1** and have found that some structural modifications are allowed while others result in a dramatic loss of activity[9]. In view of the pharmaceutical development, we have been searching for highly active and simplified analogues. Thus the synthesis of a pentasaccharide containing two 3-O-sulphated glucosamine units (**2**) was achieved[10] and led to a compound with a very high affinity for AT III, resulting in a very potent

antithrombotic compound with a prolonged period of action[11]. We also reported the synthesis of an analogue (**3**) of this pentasaccharide, displaying similar biological properties, but containing a glucose residue instead of a glucosamine[12]. This constituted a first approach towards biologically active "non-glycosamino"glycan fragments.

We now reasoned that if hydroxyl functions in the active compounds are substituted by alkyl groups, e.g. methyl, then shorter and more efficient routes towards such compounds would be possible. The stable alkyl groups can be introduced at the very beginning of the synthesis, a larger choice of temporary protective groups (including benzyl) would be at one's disposal, carboxylic acid can be blocked as benzyl esters (thus allowing their removal by smooth hydrogenolysis rather than by basic treatment which sometimes results in an elimination reaction); finally, sulphation can be achieved in a single operation.

In the new approach mentioned above, we investigated first 3-O-methylation of the L-iduronic acid residue in **3**. The reason for this choice lies in the unique conformational behaviour of L-iduronic acid in heparin and heparin oligosaccharides: there exists an equilibrium between the three conformers $^1C_4$, $^4C_1$ and $^2S_0$, which equilibrium is governed by the nature of the ring substituents, including the adjacent monosaccharide residues[13-15]. Although the bearing of the conformation on the biological properties is not well understood, it was important to check that introducing a new substituent at the 3 position of G was not detrimental to the activity. Thus we describe here the preparation and biological properties of **4**. The accompanying papers[16] further demonstrate the advantages of this approach.

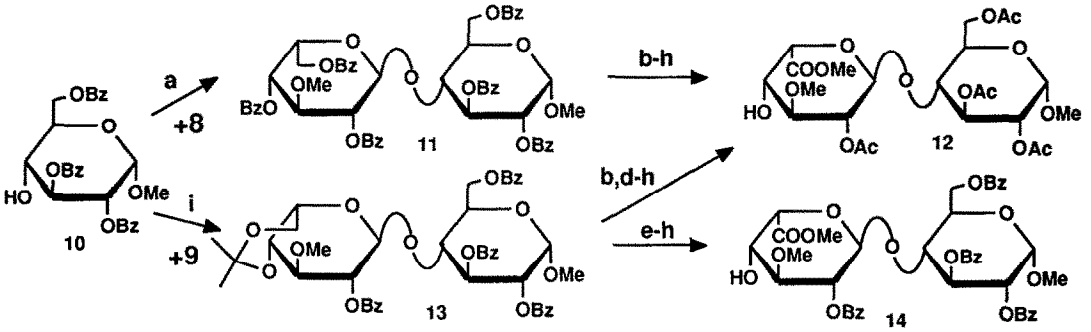


Scheme 1. a) 60% AcOH, rt, 30h (95%). **b)** MsCl, pyridine, 16h (91%). **c)** AcOK, DMF, 80°C, 16h (82%). **d)** tBuOK, tBuOH, CH_2Cl_2, 0°C, 2h (89%). **e)** 0.1M H_2SO_4, 60°C, 5h (70%). **f)** BzCl, pyridine (90%). **g)** 70% HF/pyridine, CH_2Cl_2, DMAP, 0-8°C, 16h (70%). **h)** EtSH, BF_3/Et_2O, toluene, 4h (81%). **i)** MeONa. j) Dimethoxypropane, camphorsulphonic acid, 1h (80%). **k)** BzCl, pyridine 1h (93%)

According to the usual strategy used in this kind of synthesis, the fully protected pentasaccharide **16** constitutes the key compound towards **4**. Using a recently published method, **16** can be obtained from the trisaccharide imidate[12] **15** and the disaccharide **12.** Two routes towards **12** were devised. In the first **11** is obtained after coupling of the fluoro idose donor **8** (prepared in 6 steps from the known[17] **5**; scheme 1) and acceptor[12] **10**. Using boron trifluoride etherate and dichloromethane the α L-linked disaccharide **11** is formed in 77% yield. The configuration of the newly established interglycosidic bond is confirmed by ^{1}H-NMR analysis of **11,** particularly by the long range coupling

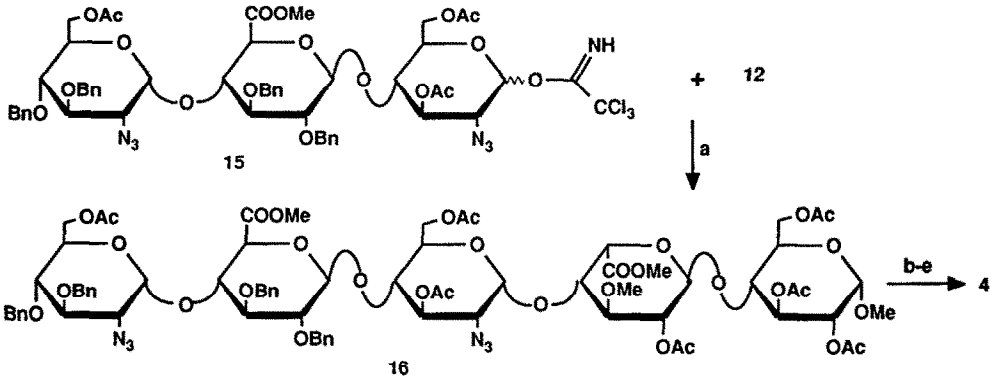
observed between H-1' and H-3' of the L-idose residue which stands in the $^1C_4$ conformation: $^4J_{1,3}=^4J_{2,4}\sim 1Hz$. In the second route[18] the thioethyl glycoside **9**, prepared from **7** was coupled with **10** to give the disaccharide **13** in excellent (93%) yield. Again ^{1}H-NMR analysis of **13** confirmed the anomery of the product. Compound **13** can be converted into **12** or, in a more efficient way, into the tetra-O-benzoylated analogue **14**.

Compound **11** was then converted into **12** as described in scheme 2. Coupling of **15** and **12** gives the α linked[19] pentasaccharide **16** in excellent yield. The fully protected pentasaccharide **16** was then saponified using lithium hydroperoxide, to avoid elimination reaction, then O-sulphated, hydrogenolysed/reduced and N-sulphated (scheme 3).



Scheme 2. a) BF_3/Et_2O, CH_2Cl_2, -10°C (77%). **b)** MeONa, MeOH, CH_2Cl_2, 3h (91%). **c)**: PhCHO, CF_3COOH, 1h (81%). **d)** Ac_2O, DMAP, Et_3N, CH_2Cl_2, 16h (89%). **e)** 70%CF_3COOH, CH_2Cl_2, 3h (83%). **f)** TBDMSCl, DMAP, Et_3N, CH_2Cl_2, 1h; then Lev_2O (81%). **g)** CrO_3, H_2SO_4, acetone; then CH_3I, $KHCO_3$, DMF (75%). **h)** NH_2NH_2, AcOH, pyridine, 30min (89%). **i)** NIS, triflic acid, toluene, -20°C, 2h (93%).

^{1}H-NMR analysis of **4**, the final compound, confirms its structure[20] and reveals a strong contribution of the $^2S_0$ partner in the conformational equilibrium of the iduronic acid residue. This strong shift towards the $^2S_0$ conformer is usually observed in biologically active pentasaccharides[9]. Indeed **4** displays as high an anti-factor Xa (1110 u/mg) activity and an affinity for antithrombin III (Kd = 4.10^{-9}M) as the potent compounds **2** and **3**. Thus replacement of the hydroxyl group of the iduronic acid residue by a methoxy does not affect the biological properties of this kind of pentasaccharides.



Scheme 3. a) TMSOTf, CH_2Cl_2, -20°C (80%). **b)** 30%H_2O_2, LiOH, THF (85%). **c)** Et_3N-SO_3, DMF. **d)** H_2, Pd/C. **e)** pyridine-SO_3, H_2O (32% 3 steps).

Acknowledgement.

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19. ^{1}H-nmr data for **16** (500 MHz, $CDCl_3$, δ, ppm): Unit **D**: 5.49 (d, $J_{1,2}$ 3.76 Hz, H-1). Unit **E**: 4.39 (d, $J_{1,2}$ 8 Hz, H-1). Unit **F**: 5.11 (d, $J_{1,2}$ 3.48 Hz, H-1). Unit **G**: 5.08 (d, $J_{1,2}$ 2.95 Hz, $J_{1,3}$ 0.8 Hz, H-1). Unit **H**: 4.90 (d, $J_{1,2}$ 3.68 Hz, H-1).
20. ^{1}H-nmr data for **4** (500 MHz, D_2O, δ, ppm). Unit **D**: 5.68 (d, $J_{1,2}$ 3.72 Hz, H-1); 3.31 (dd, $J_{2,3}$ 9.9 Hz, H-2); 3.67, H-3; 3.62 ($J_{4,5}$ 9.55 Hz, H-4); 3.95, H-5; 4.42, H-6; 4.21, H-6'. Unit **E**: 4.68 (d, $J_{1,2}$ 7.82 Hz, H-1); 3.47, H-2; 3.90, H-3; 3.87, H-4; 3.87, H-5. Unit **F**: 5.48 (d, $J_{1,2}$ 3.39 Hz, H-1); 3.50, H-2; 4.40 (t, $J_{3,4}$ 9.6 Hz, H-3); 4.03 (t, $J_{4,5}$ 9.6 Hz); 4.07, H-5; 4.48, H-6, H-6'. Unit **G**: 5.22 (d, $J_{1,2}$ 5.03 Hz, H-1); 4.47 (dd, $J_{2,3}$ 9 Hz, H-2); 3.86 (dd, $J_{3,4}$ 3.9 Hz, H-3); 4.27 (dd, $J_{4,5}$ 3.55 Hz, H-4); 4.91, H-5. Unit **H**: 5.19 (d, $J_{1,2}$ 3.57 Hz, H-1); 4.41 (dd, $J_{2,3}$ 9Hz, H-2); 4.66 (dd, $J_{3,4}$ 9.2 Hz, H-3); 4.09 (dd, $J_{4,5}$ 10 Hz, H-4); 4.05, H-5; 4.54, H-6; 4.41, H-6'.