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Synthetic heparin-like antithrombotics

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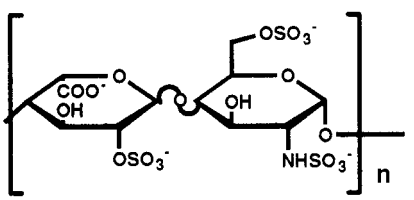
Abstract: After the discovery of a unique pentasaccharide domain in heparin that is responsible for activation of antithrombin III, many analogues have been synthesised which stimulate the inhibition of factor Xa. Some aspects of the synthesis of heparin like pentasaccharides are presented. Furthermore, pentasaccharide conjugates could be designed and synthesised which stimulate the antithrombin III mediated inhibition of both factor Xa and thrombin.

INTRODUCTION

Heparin (**1**), is an anticoagulant drug composed of a mixture of glycosaminoglycans (GAGs) purified from biological sources such as intestinal mucosa. The antithrombotic action of heparin mainly results from interactions with the protease inhibitor antithrombin III (AT-III), the heparin/AT-III complex inactivates the blood coagulation factors Xa and thrombin.

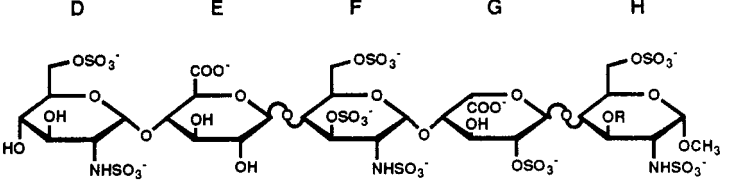
1 = Heparin



AT-III binding pentasaccharides

The shortest fragment in the heparin polymer with high affinity for AT-III was discovered (1) to be an unique pentasaccharide domain, also known as the DEFGH fragment of heparin. This pentasaccharide was shown to accelerate the AT-III mediated inactivation of factor Xa (anti-Xa activity) but not that of thrombin. The antithrombotic activity of the synthetic counterpart of this pentasaccharide, i.e. **2a** (2), was demonstrated in animal models, while no bleeding was induced at therapeutic doses. In order to get insight into the structure-activity relationships of this new class of selective factor Xa inhibitors a program (3) towards the preparation of pentasaccharide analogues was started, giving detailed information on the functional groups essential for activation of AT-III. These studies (2) led to the design and synthesis of a more potent analogue containing extra 3-O-sulphated glucosamine residues at its reducing end, i.e. **2b** (4).

2a : R=H
2b : R=SO_3^-



It is attractive to use the highly active, well-defined compounds **2a** and **2b** as leads for drug development although their chemical complexity renders large-scale synthesis difficult. Hence, the synthesis of simplified analogues of the natural pentasaccharide was pursued (5,6).

Such compounds (e.g. compound **3**) feature O-sulphate instead of N-sulphate esters and O-alkyl ethers instead of hydroxyl groups and are even somewhat more active than the parent compounds **2a** and **2b**.

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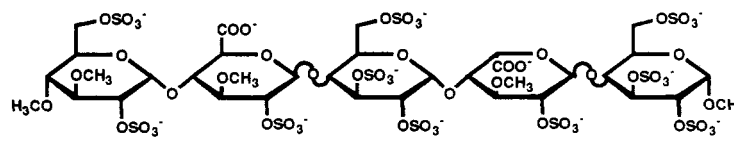

Mixed-profile analogues

The next challenge was to extend the concept of AT-III-mediated inhibition of factor Xa by pentasaccharides towards synthetically feasible derivatives displaying both anti-factor Xa and anti-thrombin activity.

It is known that for AT-III-mediated inhibition of thrombin a heparin fragment comprising at least 18 saccharide units is required to facilitate the binding of AT-III and thrombin to the same polysaccharide chain (the so-called "bridge" or "template" mechanism). In the formation of the heparin/AT-III/thrombin ternary complex the unique pentasaccharide sequence interacts specifically with AT-III, and any sulphated oligosaccharide fragment along the heparin chain interacts in a less specific mode with thrombin. Our recent model (7) of the ternary complex reveals that heparin analogues may be obtained when a thrombin-binding oligosaccharide is tethered to the non-reducing terminus of the AT-III-binding pentasaccharide with a spacer of about 50 atoms in length. For instance glycoconjugate **4** has been synthesised (8), which indeed displays a substantial anti-thrombin activity, thus demonstrating that the synthesis of large, complex heparin-like oligosaccharides can be circumvented efficiently by the introduction of molecular spacers.

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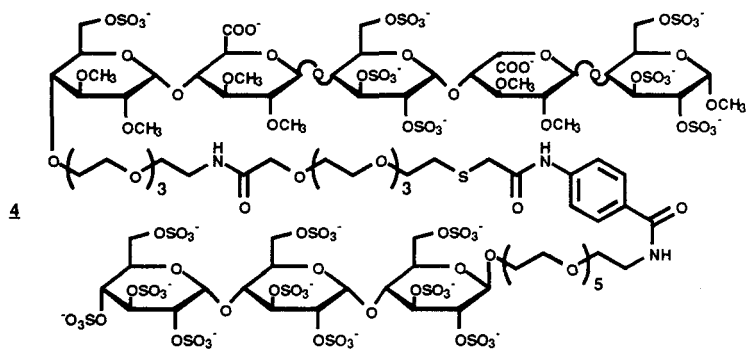

Other synthetic analogues

Organic synthesis also allowed us to prepare heparin analogues with special modifications in order to get deeper insight in the AT-III-pentasaccharide interaction at the molecular level.

For instance analogue **5** (see Fig. 4) containing a 6-O-phosphate instead of a 6-O-sulphate group at the non-reducing terminus taught us (9) that essential sulphate groups cannot be replaced by phosphate groups. The synthetic derivatives **6** and **7** (see Fig. 5) containing opened uronic acid moieties provided evidence (10) that for AT-III recognition the D-glucuronic acid part of the pentasaccharide should be rigid, while the L-iduronic acid part may be flexible. Recently, it was shown (11) that analogue **8** (see Fig. 6) containing a rigid L-iduronic acid moiety is hardly active thus indicating the importance of the conformational flexibility of L-iduronic acid in heparin.

SYNTHETIC ASPECTS

Synthetic pentasaccharides

In the 1980s the synthesis of pentasaccharides **2a** and **2b** was carried out (2,4) according to the strategy depicted in Fig. 1, in which fully protected pentasaccharides were prepared first and then converted into **2a** and **2b**. The fully protected pentasaccharides were obtained by coupling of a trisaccharide imidate (DEF)

donor with a (GH) acceptor. In the selection of protective groups the following issues were taken into account: a) The precursors of the glucosamine units D and F should contain 2-azido protective groups to facilitate formation of 1,2-cis linked (α) glycosidic bonds; b) The free hydroxyl groups of the heparin fragment should be discriminated from the sulphated ones. To this end benzyl groups were used to protect the former class, whereas acetyl esters are used to protect the latter. As a consequence the fully protected precursor of **2a** contains a 3-O-benzyl function at the reducing end while for the synthesis of **2b** a 3-O-acetyl group is required at this position; c) The carboxylate groups should be protected as methyl esters.

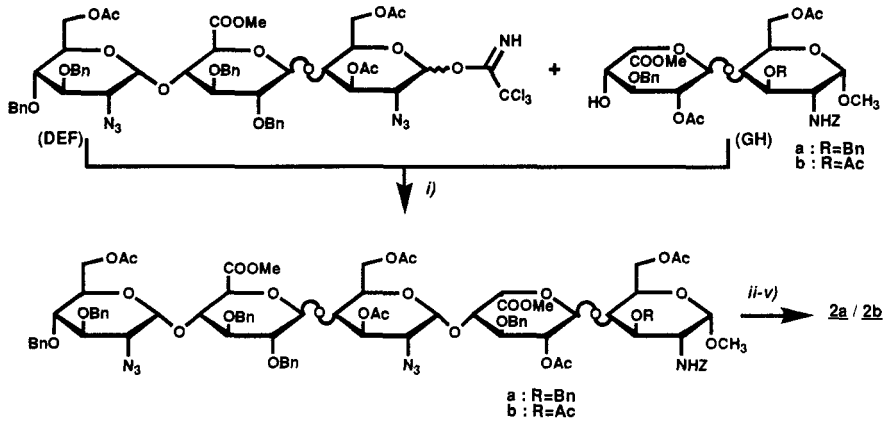

Fig. 1: i) TMSOTf; ii) $LiOOH/OH^-$; iii) $Et_3N.SO_3$; iv) H_2/Pd; v) pyridine.SO_3

After obtaining the protected pentasaccharides the following sequence of reactions had to be performed: a) saponification of the ester groups; b) sulphation of the resulting free hydroxyl groups; c) hydrogenolysis to generate the amino groups of the glucosamine units and to liberate hydroxyl groups, and d) selective N-sulphation of amino groups.

The synthesis (6) of "non-glycosamino" glycan analogue **3** is much easier than that of heparin fragments **2a** and **2b** because: a) the synthetic strategy is more flexible in that both acyl esters and benzyl ethers can be used for the protection of hydroxyl groups to be sulphated; b) no amino sugars have to be introduced which require elaborate synthetic routes for the preparation of azide containing building blocks; c) at the end of the synthesis no selective N-sulphation has to be performed; d) the substitution pattern of disaccharide moiety EF corresponds to that of GH.

Hence a route could be developed in which a properly protected GH fragment is transformed into its counterpart EF by base-catalysed epimerization. The synthetic strategy leading to the fully protected precursor of compound **3** is outlined schematically in Fig. 2. The EF disaccharide is obtained by epimerization of the suitably protected GH disaccharide with sodium methoxide. Subsequently, the EF glycosyl donor requires protection of its 4′-hydroxyl group (by levulinoylation) and acetolysis of the O-methylated anomeric center.

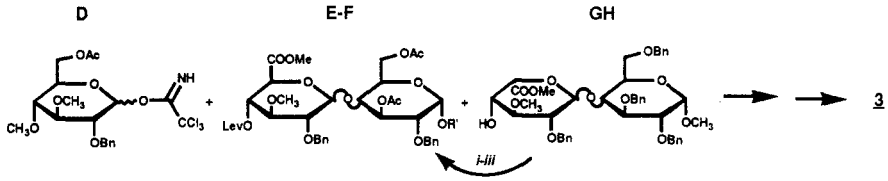

Fig. 2: i) NaOMe (epimerization); ii) Lev_2O; iii) Ac_2O/H^+

Synthesis of conjugates

As an example the last steps in the synthesis (8) of conjugate **4**, showing both anti-factor Xa and anti-thrombin activity, is outlined in Fig. 3.

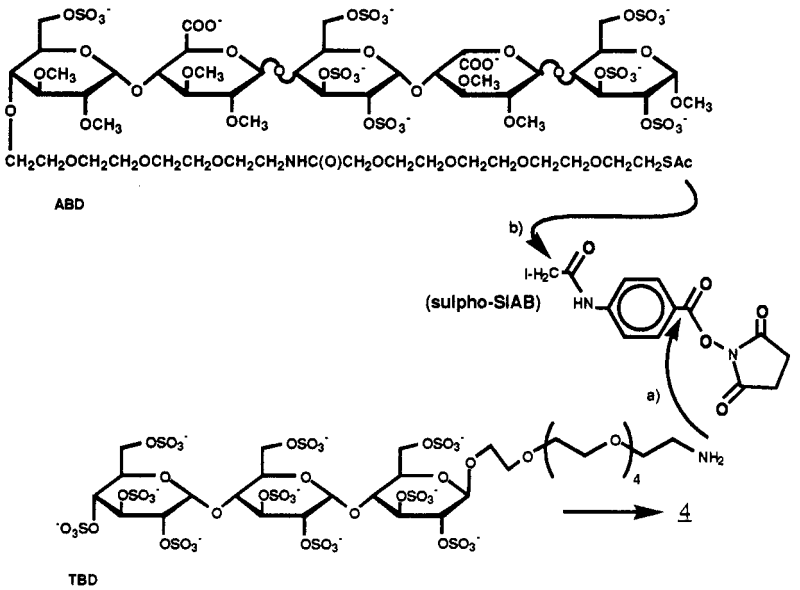

Fig. 3: a) pH = 8.0; b) NH_2OH

The synthesis of the protected pentasaccharide (protected ABD) was according to procedures used in the synthesis of **3** but slightly modified in order to introduce a 13-azido-tetraethyleneglycol moiety at the non-reducing end. After hydrogenolysis of benzyl and azido groups followed by saponification of the remaining acetyl esters the resulting 13-amino-tetraethyleneglycol moiety was reacted with a bifunctional tetraethyleneglycol derivative containing a succinimide activated methylenecarboxylate at one end and an acetyl protected thiol at the other end. After the sulphation of the hydroxyl groups pentasaccharide ABD is obtained. The thrombin-binding domain (TBD) was prepared by reacting deca-acetylmaltotriose imidate with 1-azido-17-hydroxy-hexaethyleneglycol, followed by saponification, sulphation of hydroxyls, and finally hydrogenolysis. Subsequently, the amino function of the thrombin-binding domain was reacted with sulpho-SIAB. Finally, the TBD-SIAB adduct and pentasaccharide were allowed to react (under exclusion of O_2) in the presence of NH_2OH to afford the synthetic conjugate **4**.

Synthesis of various other analogues

For the synthesis (9) of analogue **5**, in which the 6-hydroxyl group of unit D is phosphorylated a levulinoyl ester was employed for temporary protection (see Fig. 4). After controlled hydrazinolysis a fully protected pentasaccharide is obtained in which only the 6-hydroxyl group at unit D is liberated. In the next step the hydroxyl group is phosphorylated using phosphoramidite chemistry. Suitable phosphate protecting groups were required that are compatible with the subsequent deprotection and sulphation steps. A 2-cyanoethyl protective group in combination with a benzyl protective group gave the best results. Thus, the 2-cyanoethyl group is cleaved during the saponification step, while the benzyl group is removed during hydrogenolysis.

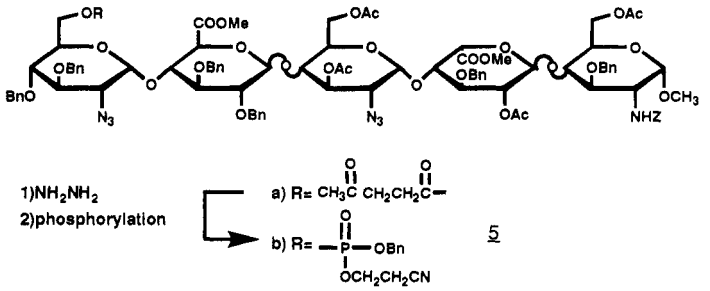

Fig. 4

In order to answer the question (10) as to whether the pyran rings of the two uronic acid moieties are essential the "ring-opened" analogues **6** and **7** were prepared (see Fig. 5). To this end, two suitably protected and activated building blocks **A** and **B** were prepared from (S)- and (R)-glyceric acid, respectively. The methyl-fluoride moiety acts as "pseudo-anomeric" center and could be coupled to the acceptor by the action of boron trifluoride. Subsequently, the levulinoyl group can be removed selectively by hydrazinolysis, after which the acceptor thus obtained can be coupled with the required donor.

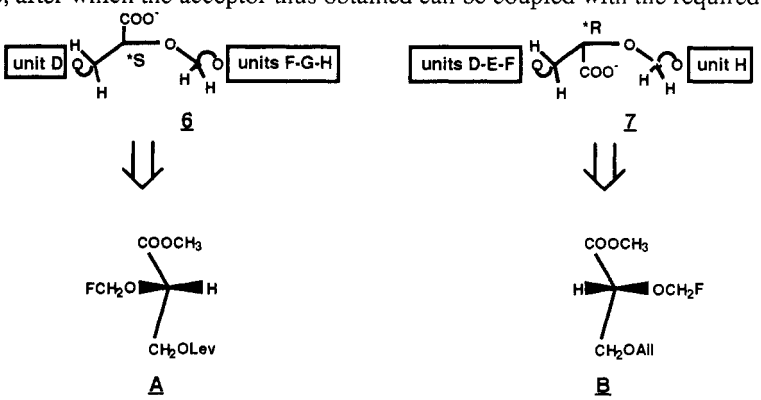

Fig. 5

In order to study the importance of the flexibility of the L-iduronic acid moiety in the heparin pentasaccharide, we were anxious to synthesise (11) an analogue containing the L-iduronic acid moiety in a fixed $^1C_4$ conformation (see Fig. 6). The target pentasaccharide **8** was designed having a rigid 3-O, 5-C-methylidene L-idopyranuronate residue. Its retrosynthetic analysis is illustrated in Fig 6. Antithesis of pentasaccharide **8** led to a disaccharide acceptor **A**. The propenyl group at C-5′ and the tosyl group at C-6′ are envisioned as precursors of the carboxylate group and methylidene bridge, respectively. The C-5′ propenyl of building block **A** could be introduced through radical C,C-bond formation between the glycosyl bromide **B** and allyl tri-n-butyltin followed by olefin isomerization.

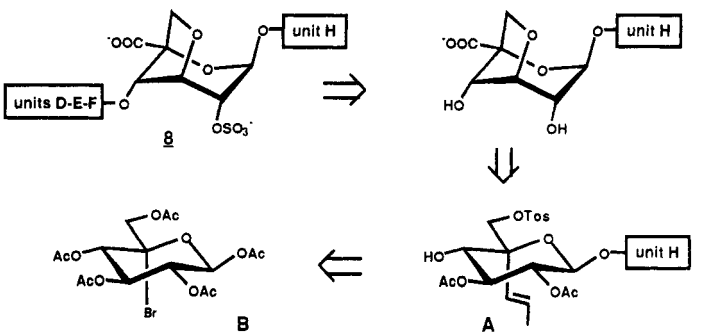

Fig. 6

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REFERENCES

1 a) L. Thunberg, G. Bäckström, U. Lindahl. *Carbohydr. Res.* **100**, 393 (1982).
b) J. Choay, J.C. Lormeau, M. Petitou, P. Sinay, J. Fareed. *Ann. N.Y. Acad. Sci.* **370**, 644 (1981).
2. C.A.A. van Boeckel, M. Petitou. *Angew. Chem. Int. Ed. Engl.* **32**, 1671 (1993).
3. M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, P. Sinay, J.C. Jacquinet and G. Torri. *Carbohydrate Research* **167**, 67 (1987).
4. C.A.A. van Boeckel, T. Beetz and S.F. van Aelst. *Tetrahedron Lett.* **29**, 803 (1988).
5. J.E.M. Basten, G. Jaurand, B. Olde-Hanter, M. Petitou, C.A.A. van Boeckel. *Bioorg. Med. Chem. Lett.* **2**, 901 (1992).
6. H. Lucas, J.E.M. Basten, P. Konradsson, C.A.A. van Boeckel. *Angew. Chem. Int. Ed. Engl.* **32**, 434 (1993).
7. P.D.J. Grootenhuis, P. Westerduin, D. Meuleman, M. Petitou, C.A.A. van Boeckel. *Nature Struct. Biol.* **2**, 736 (1995).
8. P. Westerduin, J.E.M. Basten, M.A. Broekhoven, V. de Kimpe, W.H.A. Kuijpers, C.A.A. van Boeckel. *Angew. Chem. Int. Ed. Engl.* **35**, 331 (1996).
9. J.N. Vos, P. Westerduin, C.A.A. van Boeckel. *Bioorg. Med. Chem. Lett.* **1**, 143 (1991).
10. H. Lucas, J.E.M. Basten, T.G. van Dinther, D.G. Meuleman, S.F. van Aelst, C.A.A. van Boeckel. *Tetrahedron.* **46**, 8207 (1990).
11. N. Sakairi, J.E.M. Basten, G.A. van der Marel, C.A.A. van Boeckel, J.H. van Boom. *Chemistry.* submitted.

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