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Toward Synthesis of the Isosteric Sulfonate Analogues of the AT-III Binding Domain of Heparin

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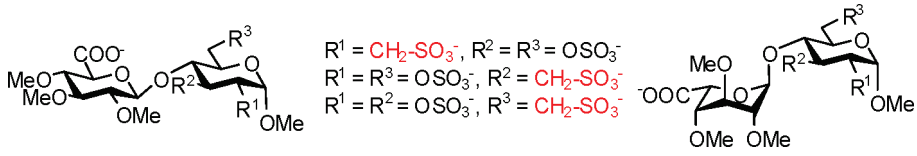
ABSTRACT



D-Glucuronate and L-iduronate containing disaccharides related to the antithrombin-binding pentasaccharide of heparin, in which one of the sulfate esters is systematically replaced by a sodium sulfonatomethyl moiety, were synthesized. The sulfonic acid group was introduced by stereoselective radical addition onto the exomethylene moiety of the appropriate glycoside derivatives, and the resulting sulfonatomethyl glucosides were used as acceptors.

Heparin, a linear, highly sulfated glycosamino-glycan polysaccharide is a well-known blood anticoagulant and has been employed in medical practice since the late 1930s.[1] A characteristic pentasaccaharide domain of heparin termed DEFGH (**1**, Figure 1) binds and activates the coagulation inhibitor antithrombin III (AT-III) through an allosteric mechanism, the activated AT-III then inhibits both the procoagulant serine proteinase factor Xa and another coagulation enzyme, thrombin, by forming an inactive ternary complex.[2,3] Fondaparinux (**2**), a closely related synthetic counterpart of the AT-III binding pentasaccharide, does not inhibit thrombin and exhibits only factor Xa inhibitory activity via binding to AT-III. However, because of superior antithrombotic activity it became the first of a new class of antithrombotic agents (Arixtra).[4] Replacement of the N-sulfate groups by O-sulfates has subsequently led to non-glycosaminoglycan analogues, such as idraparinux (**3**), having simplified structure much easier to synthesize and possessing increased anticoagulant activity and longer dura-

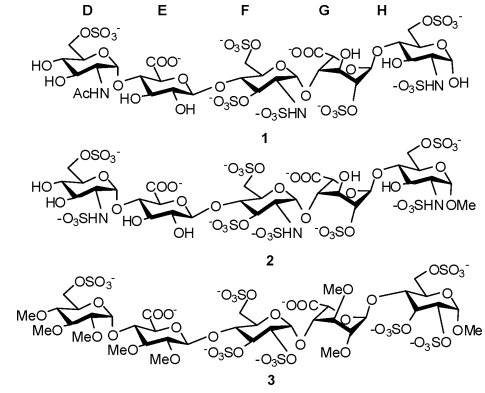


Figure 1. Structure of the antithrombin binding domain of heparin (**1**), synthetic antithrombotic agent fondaparinux (**2**), and the non-glycosaminoglycan analogue idraparinux (**3**).

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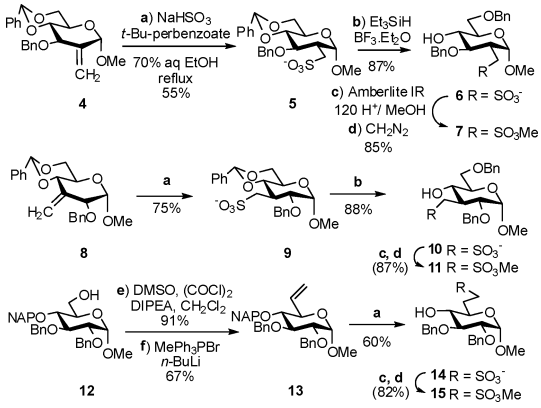
tion of action as compared to the natural pentasaccharide.[5] Idraparinux can be regarded as a lead compound for the development of second-generation synthetic antithrombotics.

Structure−activity relationship studies of synthetic analogues of the heparin pentasaccharide revealed that four sulfate groups and two carboxylate groups are essential for the activation of AT-III, and an extra sulfate group at the position O-3 of unit H improves the activity.[4,6] The type of charge is also crucial; an essential sulfate group cannot be replaced by a phosphate, and the carboxylate groups may not be exchanged for $CH_2OSO_3^-$ residues.[4] Replacement of the sulfate groups with isosteric sulfonatomethyl moieties has not been investigated until now, although this replacement may result in bioactive mimetics. It has been shown, for example, that the isosteric phosphonate analogues[7−9] of mannose-6-phosphate binds with high affinity to the cation-independent mannose 6-phosphate receptor. Isosteric sulfonate analogues of the AT-III binding pentasaccharide of heparin may provide further information on structure−activity relationship. Therefore, we decided to prepare analogues of **3**, in which the sulfate esters are partially replaced with sulfonatomethyl moieties. As a beginning of this program we now describe the synthesis of three sulfonatomethyl analogues of the EF fragment and three sulfonatomethyl analogues of the GH fragment of compound **3**.

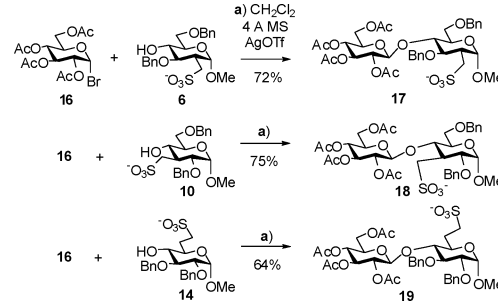
The sulfonatomethyl glucoside acceptors in the form of salts (**6**, **10**, **14**), as well as in the form of esters (**7**, **11**, **15**), were synthesized as shown in Scheme 1. The 2-exomethylene

Scheme 1. Synthesis of Glucoside Acceptors Carrying a Sulfonatomethyl Moiety



derivative **4**[10] was reacted with $NaHSO_3$ to furnish the desired 2-sulfonatomethyl-glucoside **5**, in a stereoselective radical addition,[11] the moderate yield was a consequence of partial hydrolysis of the benzylidene acetal under the slightly acidic conditions. Regioselective opening of the 4,6-O-acetal ring of **5** afforded acceptor **6** in salt form, which was converted into the sulfonic acid ester **7** via a two-step procedure involving liberation of the sulfonic acid with ion-exchange resin followed by methylation with diazomethane. The 3-sulfonatomethyl-glucoside acceptors (salt **10** and methyl ester **11**) were prepared from **8**[12] by applying the reactions discussed for the synthesis of **7**. For the preparation of the 6-sulfonatomethylglucoside acceptors (**14** and **15**) compound **12**[13] was oxidized, and then Wittig reaction of the resulting 6-aldehyde afforded the 6,7-unsaturated heptoside **13**. In the course of the addition of the radical anion formed from $NaHSO_3$ onto the double bond of **13**, the 2-naphthylmethyl (NAP) group was also split off from position 4, resulting in the sulfonic acid salt **14**, which was converted into the sulfonic acid ester **15** in two steps. Synthesis of analogues of the glucuronic acid containing EF fragment was carried out by means of coupling of the sulfonic acid salt acceptors **6**, **10**, and **14** with acetobromo glucose donor **16** to afford disaccharides **17**, **18**, and **19** (Scheme 2) and a subsequent oxidation into uronic acid at a later stage of the synthesis.

Scheme 2. Glycosylation of Sulfonatomethyl Acceptors **6**, **10**, and **14** with Acetobromo Glucose **16** To Obtain Fully Protected Disaccharides **17**, **18**, and **19**



In the case of disaccharides **17** and **18**, deacetylation and 6′-O-triphenylmethylation followed by introduction of the methyl groups into the secondary positions and subsequent hydrolysis of the trityl moiety afforded compounds **20** and **23**, respectively. Oxidation of the primary position of the glucose unit of **20** and **23** followed by catalytic hydrogenation of the benzyl protecting groups resulted in diols **21** and **24**, which upon sulfation with

(4) van Boeckel, C. A. A.; Petitou, M. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1671.
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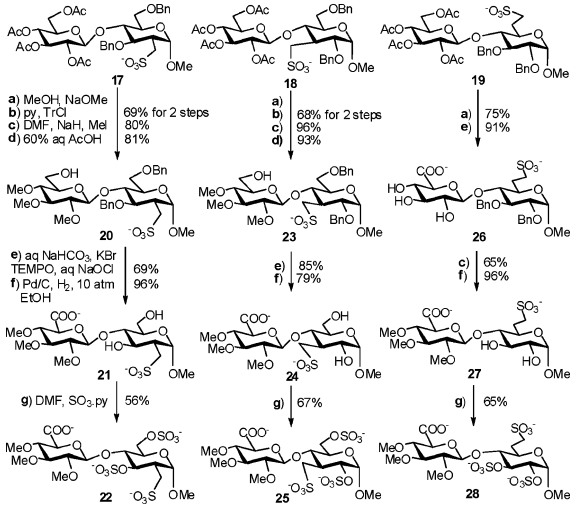
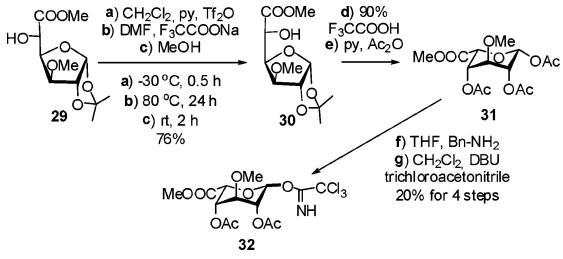
the SO_3·pyridine complex furnished the target compounds **22** and **25** with high overall yields (Scheme 3). Disaccharide **19** was also deacetylated; however, subsequent tritylation was unsuccessful. Therefore, the tetrahydroxy compound was converted into **26** by selective oxidation. Methylation of compound **26** carried a risk of β-elimination of the base-sensitive glucuronic residue. However, it proceeded smoothly, and no elimination could be observed. Removal of the benzyl groups by catalytic hydrogenation afforded compound **27** quantitatively. Subsequent sulfation of the diol **27** gave the third analogue **28** of the EF fragment (Scheme 3). For the synthesis of the GH analogues, L-iduronic acid trichloroacetimidate **32** was chosen as the donor prepared from **29**[14] by a two-step epimerization of C-5[15] (**30**), followed by deisopropylidenation, acetylation (**31**), selective deacetylation of the anomeric position, and imidate formation (Scheme 4).

Scheme 3. Synthesis of 2-, 3-, and 6-Sulfonatomethyl Analogues of the EF Disaccharide

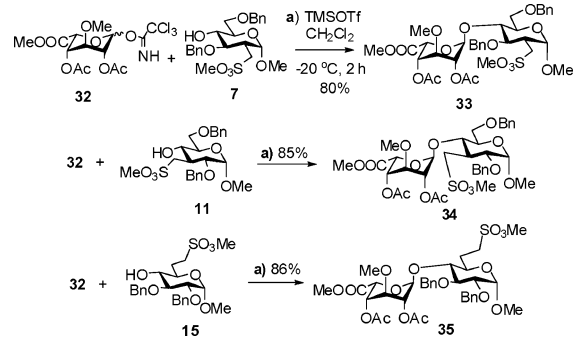


Scheme 4. Synthesis of the L-Iduronic Acid Donor



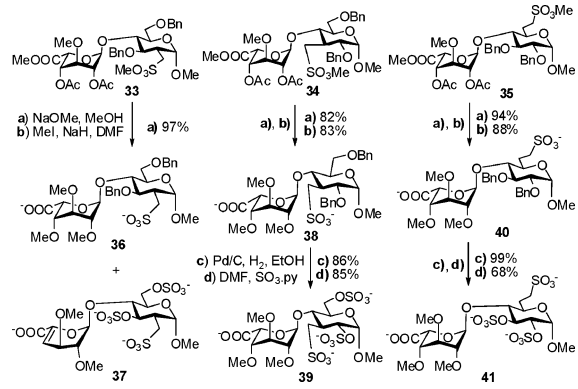
The sulfonatomethyl glucoside acceptors were then glycosylated in form of the methyl esters **7**, **11**, and **15** with the donor **32** to afford the desired α-linked iduronic acid containing disaccharides **33**, **34**, and **35** (Scheme 5). Synthesis of the target GH analogues involving introduction of two methyl groups into the iduronic acid unit and two sulfate ester functions into the glucose unit was attempted as depicted in Scheme 6, to furnish the 3- and 6-sulfonatomethyl disaccharides **39** and **41** with good yields.

Scheme 5. Glycosylation of Methylsulfonatomethyl Acceptors **7**, **11**, and **15** with Donor **32** To Obtain Fully Protected Disaccharides **33**, **34**, and **35**



In the case of the 2-sulfonatomethyl derivative, however, upon methylation an inseparable mixture of the desired product **36** and the byproduct **37** (as a result of β-elimination of the base-sensitive iduronic acid residue) were formed in a ratio of 3:1.

Scheme 6. Synthesis of Sulfonatomethyl Analogues (**39** and **41**) of the GH Disaccharide



To overcome this difficulty, compound **33** was deacetylated, and the resulting diol was methylated with diazomethane in the presence of silica gel as catalyst[16] to give **42** with high yield. The sulfonic ester was converted into salt with sodium iodide, and subsequent treatment of the crude product with sodium hydroxide solution gave the disodium salt, which was then debenzylated, and the resulting diol was sulfated to afford the desired analogue **43** (Scheme 7).

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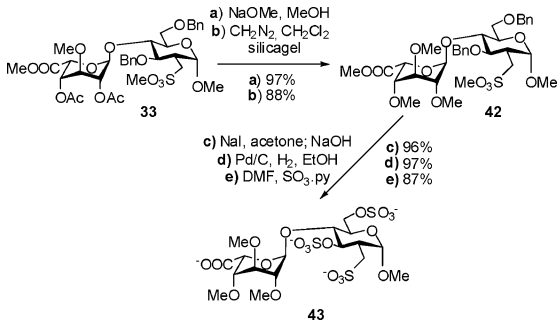
Scheme 7. Synthesis of the 2-Sulfonatomethyl Analogue of the GH Disaccharide



In conclusion, we synthesized three sulfonatomethyl analogues of the EF fragment and three sulfonatomethyl analogues of the GH fragment of the nonglycosaminoglycan blood-anticoagulant **3**. The sulfonic acid group was introduced by $NaHSO_3$ addition onto the exomethylene moiety of the appropriate glycoside derivatives. The glycosylation reactions were carried out either with sulfonic acid salt or with sulfonic acid ester acceptors. The carboxylic function of the uronic acid unit was formed on a disaccharide level in the case of the EF analogues but on a monosaccharide level in the case of the GH analogues. Methylation reactions were carried out both under basic and acidic conditions. Synthesis of tri- and pentasaccharide analogues applying the presented methods is underway.

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Supporting Information Available: Experimental procedures, characterization data and selected NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org

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