Research Final Report

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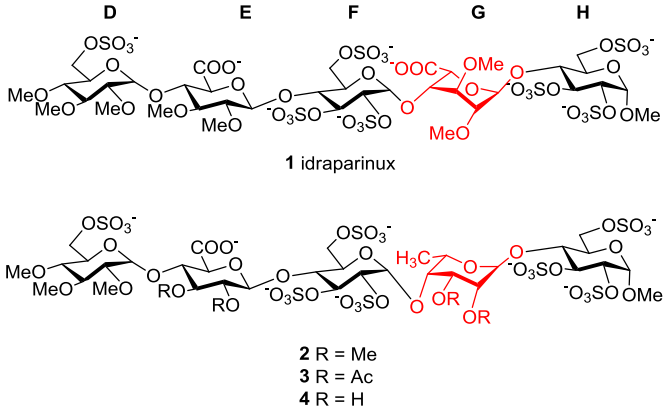
Project title: Synthesis of 6-deoxy-L-talopyranoside-containing idraparinux derivatives with potential anticoagulant activity

Project ID: **PD 115645**

Our research group, currently uniquely in the country is involved in the synthesis of oligosaccharides. We have been examining the development of new synthetic anticoagulant pentasaccharides for several years. Many heparin-analogue oligosaccharides (di-, tri- and pentasaccharides) have been synthesized within this research. These derivatives represent the basis of the collaboration with the research groups of Dr. Zsuzsanna Bereczky (UD, Department of Laboratory Medicine, anticoagulant activity measurements, docking studies, SPR measurements, ATBp3 mutant antithrombin assay) and Prof. Dr. E. Kövér Katalin (UD, Department of Inorganic and Analytical Chemistry, NMR and ITC measurements).

During my post-doctoral application period, L-idose, the most expensive and most problematic building block of the synthesis of heparin-analogues, was at the centre of my research. My job had a dual purpose. On the one hand, my research aimed at the synthesis of heparin analogue oligosaccharides of anticoagulant activity in which the L-iduronic acid moiety is replaced by a more easily and more economically synthesizable building block (6-deoxy-L-talopyranose). On the other hand, my goal was to develop a simpler, faster and more economical synthetic route to prepare L-idose.

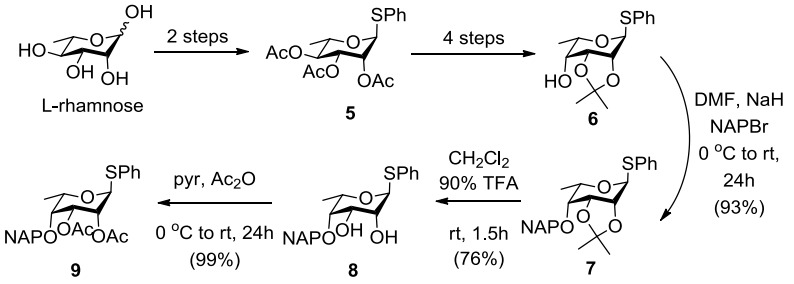
Three new idraparinux analogue derivatives (**2**, **3** and **4**) were successfully synthesized during the research project, in which the L-idose part was replaced by a 6-deoxy-L-talopyranose unit since this monosaccharide can be prepared much faster and more economically than the L-idose.



Scheme 1. The structures of idraparinux (**1**) and the synthesized pentasaccharides (**2**, **3**, **4**)

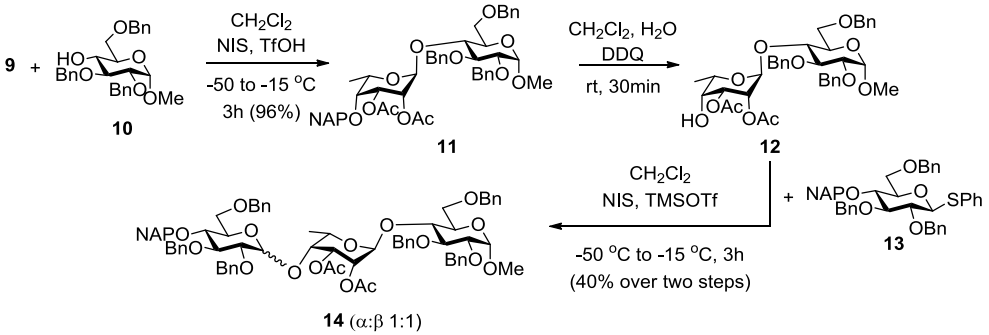
In our research some synthetic pathway were developed for the preparation of the protected pentasaccharide. The L-talose residue was formed on the monosaccharide level starting from

L-rhamnose in 9 steps. (In the literature the synthesis of the L-idose donor takes minimum 14-15 steps). The phenylthio-L-talopyranoside derivative which is usable in glycosylation reactions was successfully prepared (**9, Scheme 2**.), then was applied in coupling reaction for the synthesis of disaccharide acceptor **GH** (14 steps).



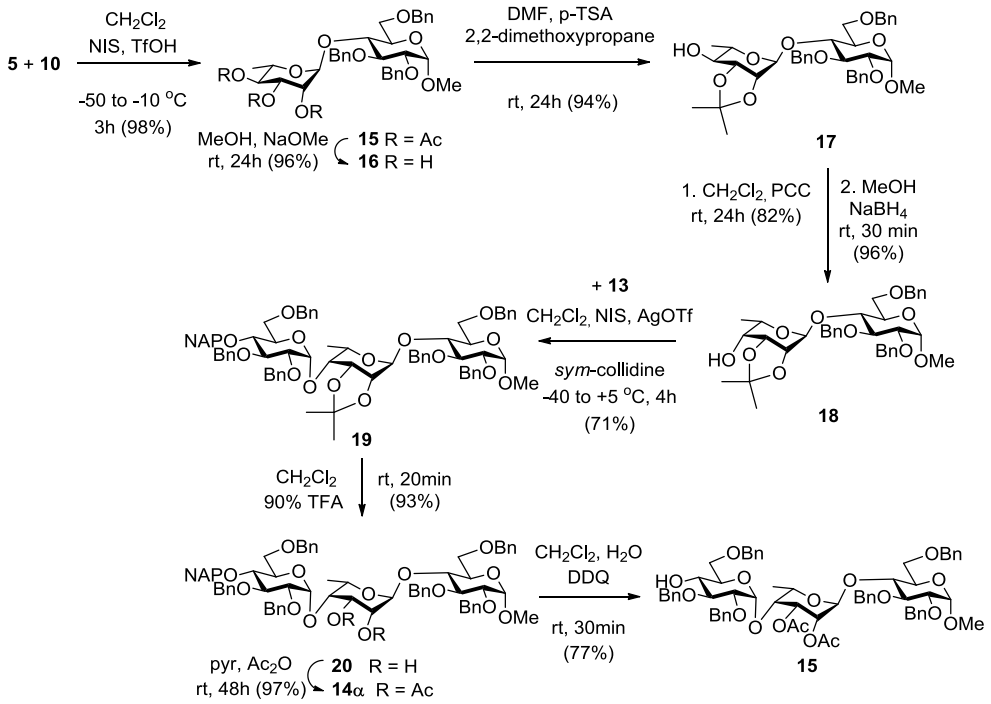
Scheme 2. The synthesis of the 6-deoxy-L-talopyranozide (**9**) derivative

Glycosylation of disaccharide **GH** with compound **13** resulted in the needed protected trisaccharide **FGH** (22 steps). However the expected product was formed as an anomeric mixture, therefore the required trisaccharide was isolated with low yield.

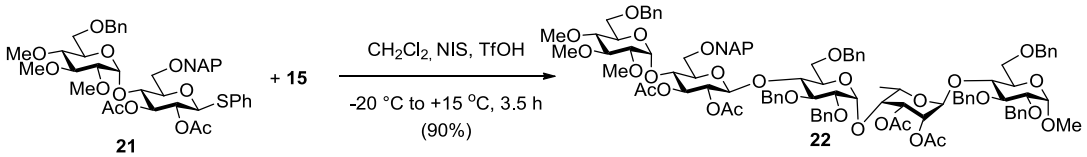


Scheme 3. The preparation of the **FGH** trisaccharide I.

Because we needed a larger amount of trisaccharide **FGH** for the synthesis of the planned pentasaccharides, a new, shorter and more economical pathway was worked out (**Scheme 4.**). To prepare the trisaccharide, firstly, disaccharide **15** was synthesized starting from peracetylated L-rhamnose, then the corresponding protecting groups were introduced. Subsequently, the rhamnose residue was converted to the *talo*-configured product by an oxidation/reduction processes. Next the disaccharide acceptor **18** was glycosylated with compound **13,** which was synthesized earlier, to result in the trisaccharide **FGH**. Owing to the applied protecting groups the expected compound was formed with good yield and stereoselectivity. Thereafter, the expected trisaccharide (**14α**) was successfully produced in overall 13 steps. This compound was then converted to acceptor (**15**) by the oxidative cleavage of the NAP-group followed the coupling with the disaccharide donor **DE** (**21**) which has been produced in our previous work. As a result, the fully protected pentasaccharide **22** was obtained with an excellent yield and complete stereoselectivity. Finally, the desired three end-products were successfully synthesized by the removal of temporary protecting groups of the protected pentasaccharide and the introduction of permanent groups.



Scheme 4. The preparation of the **FGH** trisaccharide II.



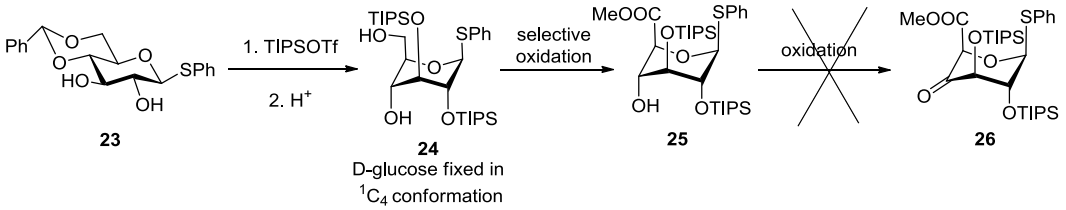
Scheme 5. The synthesis of the protected pentasaccharide

We also investigated the anticoagulant activity of the synthesized compounds (**2**, **3** and **4**) in normal human blood plasma, but unfortunately owing to the performed transformations the pentasaccharides have lost their inhibitory effect. The detailed carbohydrate-protein interaction studies (NMR and ITC assays with normal antithrombin) showed that the conformation of the newly built 6-deoxy-L-talose unit (which was almost completely $^{1}C_{4}$ state based on NMR measurements) did not resemble to the conformational state provided by the L-idose ($^{2}S_{O}$ conformer which is necessary for the biological activity). These results are in line with the loss of anticoagulant activity. The results of our research were published in the *Scientific Reports* journal in this year. The SPR-based carbohydrate-protein interaction studies requiring a small amount of antithrombin are already in progress with normal antithrombin and idraparinux, however the SPR measurements of the novel heparin analogue oligosaccharides with the normal and ATBp3 mutant antithrombins will be performed later. In the light of the activity and interaction studies we started to design the structure of the synthetically feasible oligosaccharides by docking tests. The produced oligosaccharides have attracted the attention of American company Schrödinger, which is involved in molecular modelling and get involved in the work, helping us to select potentially promising structures (4-5 oligosaccharides). These docking measurements are still in progress. Because of the loss

of anticoagulant activity, the synthesis of sulfonic-acid containing pentasaccharides will not be continued, but after evaluating the results of molecular modelling and docking studies, the synthesis of other derivatives can be started.

Also we have an opportunity to investigate the cell growth inhibitory effects of our heparin analogue oligosaccharides. The investigations have shown that some trisaccharide derivatives have excellent cell growth inhibitory effects on cancer cells and no cytotoxicity is observed on healthy cell lines. These results were presented in the form of lecture at the *„Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium '18*" in Szeged and in the form of poster at the international conference on "*Chemistry towards Biology - Biomolecules as potential drugs"* in Budapest.

The oxidation/reduction pathway in the research plan to prepared L-idose was failed since the protected glucuronic acid derivative containing the keto-group in the planned position 4 could not be produced (**Scheme 6.**). In addition to the designed protective groups, due to steric congestion at position 4, several methods were tested but we could not oxidized the OH group.

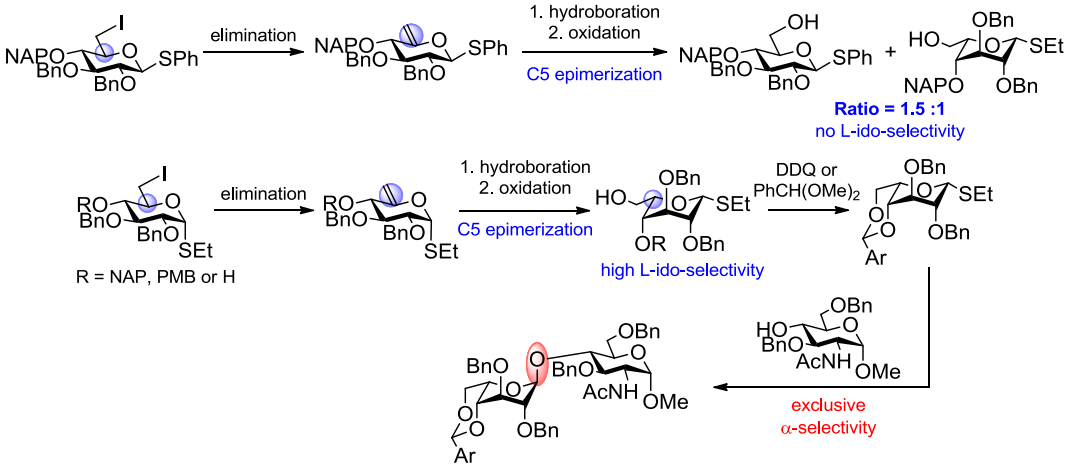


Scheme 6. The planned oxidation/reduction reaction pathway

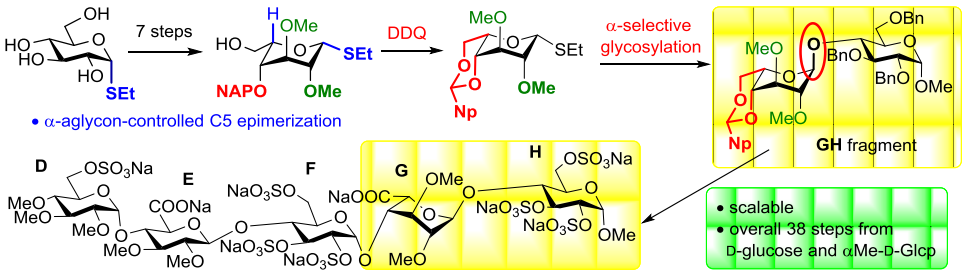
However, by changing the protecting-group strategy and the reactions, we have developed a new method for preparing L-idose donors (**Scheme 7.**). Using our new method, we have synthesized properly protected L-idose moieties with activated anomeric group from 5,6-unsaturated D-glucose derivatives with good yields and more economically than before. The developed method is based on the configuration inversion of C5 carbon. The cheap D-glucose was used as a starting material. In the anomeric position, alkylthio groups (-SPh, -SEt) were used, which are easily activated in the designed glycosylation reactions. Other positions of the molecule were protected with various protecting groups (-Bn, -Me), thus investigating their effect. The hydroboration/oxidation reactions were also carried out on β-phenylthio and α-ethylthio derivatives. For β-phenylthio-glucosides, hydroboration reactions were not stereoselective at all and yields were not satisfactory.

In contrast, in the case of α-glycosides, the expected L-ido configuration was generated with good yield and stereoselectivity. From the successfully prepared α-ethylthio derivatives subsequently we formed glycosyl donors containing arylmethylidene acetal ((2-naphthyl)methylene, benzylidene, *p*-methoxybenzylidene) at position 4,6 and examined their utility in coupling reactions. In the test reactions, the desired α-1,4-linked heparin analogue disaccharides were obtained with excellent stereoselectivity and yield. Based on these experiments we can say that we have successfully synthesized orthogonally protected L-idose derivatives which are also suitable for the synthesis of higher oligosaccharides containing α-idosidic bonds. In this research, hydroboration/oxidation reactions have been studied on

thioglycosides for the first time. Finally, to demonstrate the applicability of this new method, we synthesized a new L-idose donor molecule and successfully developed a new and shorter reaction pathway to prepare idraparinux (**Scheme 8.**). The first half of this research was published in the journal *European Journal of Organic Chemistry* and the second part was also sent here for publication.



Scheme 7. The preparation of the L-idose unit with hydroboration/oxidation reaction and applying in glycosylation



Scheme 8. The new synthesis of idraparinux

As a continuation of my research work, we are planning to extend our newly developed method to prepare further L-sugars and to build up these monosaccharides into heparin analogue oligosaccharides and to plan biological studies of these derivatives. During my post-doctoral period I won the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and I have successfully entered the Preliminary Postdoctoral Research Program of HAS, so I have the opportunity to continue and extend the researches that I started.