PROJECT DESCRIPTION

Development of a synthetic method for the preparation of L-hexoses for synthesis of oligosaccharides and their applications for the economical synthesis of heparin analogues

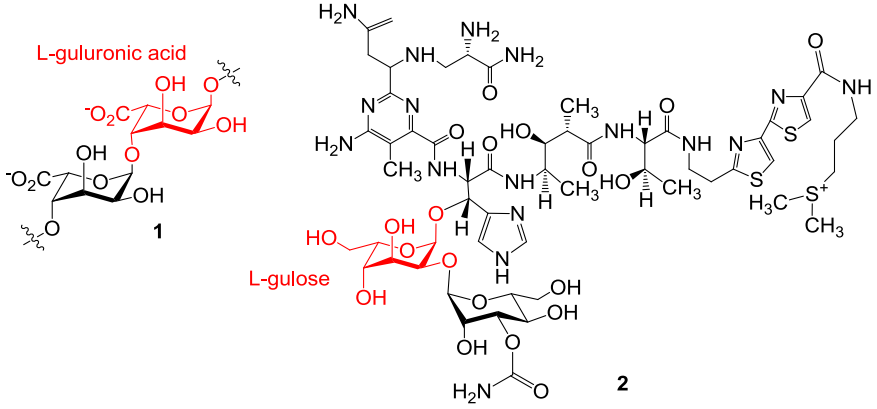
1. Introduction

The rare, but biologically widespread L-hexoses and their corresponding 6-deoxy counterparts play important roles in nature[1]. Although L-hexoses are not as prevalent as their enantiomers, the D-hexoses, numerous significant biomolecules contain L-sugars. The L-sugars are commercially available but most of them are very expensive (**Table 1.**). Therefore, it is important to produce L-sugars from common carbohydrates, which has always been a major challenge for chemists in the history of carbohydrate chemistry.

L-sugar	**Price/1g (EUR)**	**L- and D-sugars**	**Price/1g (EUR)**
L-idose	**1390**	L-glucose	49
L-altrose	**1210**	L-gulose	40
L-talose	**1060**	L-fucose	11
L-allose	**840**	L-rhamnose	0.21
L-galactose	260	**D-mannose**	**0.23**
L-mannose	53	**D-glucose**	**0.008**

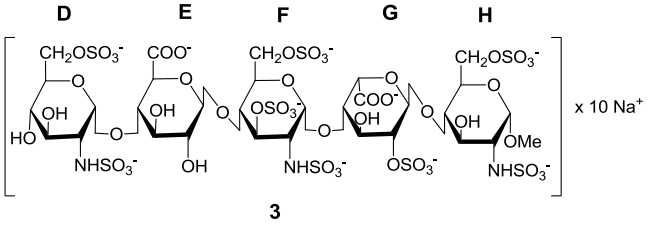
Table 1.: The commercial prices of L-sugars[2] and the prices our planned starting materials D-mannose and D-glucose

L-sugars are found in numerous important natural products, e.g.: alginates[3] (**1**, a highly anionic polysaccharide from the cell wall of brown algae, containing L-guluronic acid monomers) or in the well-known potent antitumor antibiotic Bleomycin A_2 (**2**) produced by *Streptomyces verticillus* contains an L-gulose unit [4].



Scheme 1.: Structures of the monomers of alginate (**1**) and the Bleomicin A_2 (**2**)

Another important L-sugar is L-iduronic acid, which plays a crucial role in the construction of repeating disaccharide units of some glycosaminoglycans. In these repeating units, one molecule is always an amino-carbohydrate (*N*-acetylglucosamine or *N*-acetylgalactosamine), while the other molecule is generally an uronic acid (D-glucuronic acid or L-iduronic acid). Such glycosaminoglycans are e.g.: heparin, hyaluronic acid, heparan-, chondroitin- and dermatan sulphate[5,6].Because of their anticoagulant activity, heparin and its derivatives (e.g.: the synthetic Fondaparinux, **3**) are among the most widely used drugs for the prevention and treatment of thromboembolic disorders in these days. Fondaparinux (**3**), under the name Arixtra®, is the only synthetic heparin analogue molecule used in the medical practice. For its preparation, Sanofi and Organon pharmaceutical companies have developed a 55-step synthesis first[7]. One lasting challenge in the synthesis of heparin derivatives, including Fondaparinux, is the efficient preparation of the L-idose/L-iduronic acid building block.



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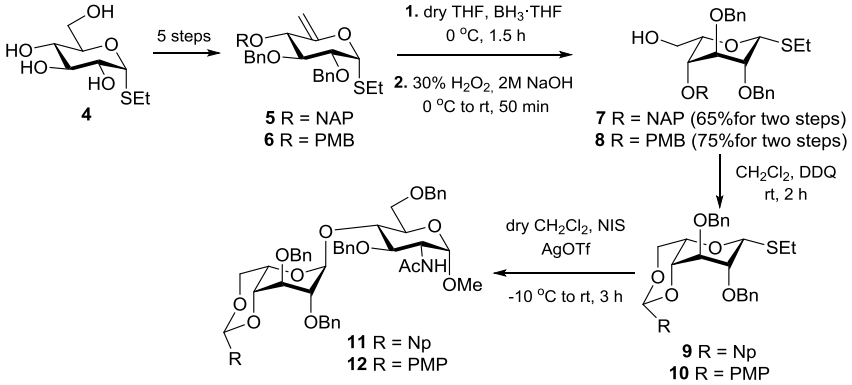
Scheme 2.: The structure of Fondaparinux (Arixtra®)

Our research group, currently uniquely in the country, is dealing with synthetic oligosaccharide chemistry, with the main focus on heparinoid synthesis. 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[8-10]. These derivatives represent the basis of the collaboration with the research groups of Dr. Zsuzsanna Bereczky (UD, Department of Laboratory Medicine, anticoagulant activity measurements) and Prof. Dr. Katalin E. Kövér (UD, Department of Inorganic and Analytical Chemistry, NMR and ITC measurements), with whom we are working on a more accurate mapping of the functioning of the AT Budapest 3 (ATBp3), a mutant antithrombin occurring in Hungary. In the framework of our run-out OTKA PD project we have successfully developed a new L-idose synthesis starting from α-thioglucosides[11]. Applying this method, we have prepared L-idose thioglycosides (**7**, **8**) in good yields and a more economical way than before, from suitably protected, 5,6-unsaturated ethylthio-D-glucose derivatives (**5**, **6**). Furthermore, using these compounds, stereoselective α-glycosylation reactions were performed[11]. On the basis of these new results, the obtained L-idosyl thioglycosides are efficient α-selective glycosyl donors that are suitable for the synthesis of heparin analogue oligosaccharides.



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5 R = NAP
6 R = PMB

7 R = NAP (65%for two steps)
8 R = PMB (75%for two steps)

9 R = Np
10 R = PMP

11 R = Np
12 R = PMP

Scheme 3.: The syntheses of the L-idose derivatives and their glycosylation reactions

Based on these preliminary results, we assume that this method can be extended for the synthesis of all eight L-hexoses as their thioglycoside donors, ready for oligosaccharide synthesis. Due the importance of L-sugars, various methods have been explored for their synthesis.[2] A major disadvantage of these syntheses is the necessity for complicated post functionalization in order to turn the L-sugar into a proper donor ready for glycosylation. In 2014, Bols *et* al.[12] published a new method for the synthesis of all eight L-hexose thioglycoside donors. However, due to the lengthy, multistep reaction paths and the high price of the used starting materials (L-fucose and L-rhamnose) and reagents (e.g.: iridium catalyst), these syntheses are very expensive and complicated, thus, they are not economically feasible in large scale.

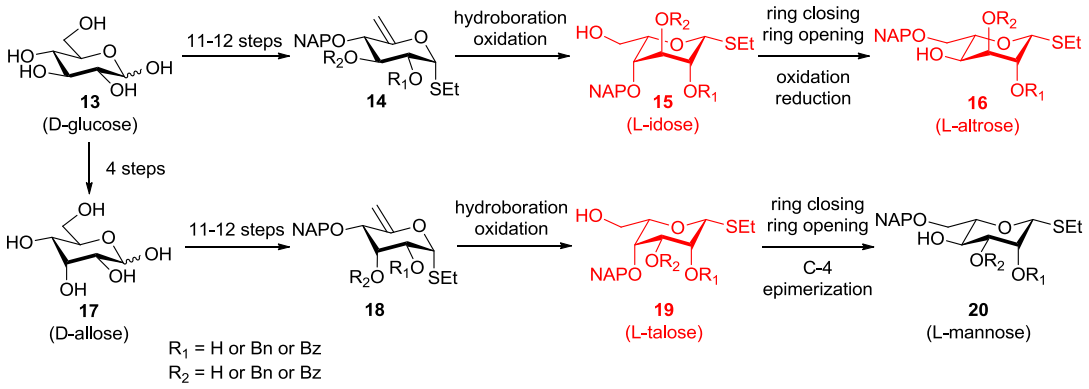
Our goal is to develop a new, easy and economically feasible synthetic method which, after optimization, is suitable for the preparation of all eight L-hexoses in form of thioglycosides. The thioglycosides can be used directly as glycosyl donors in oligosaccharide synthesis, which greatly extends the synthetic utility of these carbohydrate derivatives. Our further aim is to establish a new and effective reaction pathway for the synthesis of the commercially available Fondaparinux (**3**) using the L-idose donor synthesized by our method.

2. The synthesis of all eight L-hexoses from 5,6-unsaturated α-thioglycosides

The preparation of the suitably protected monosaccharides is planned by using the cheapest and most readily available D-hexoses (D-glucose and D-mannose) as the starting materials. Our aim is to economically synthesize orthogonally protected L-hexose glycosyl donors useful for the stereoselective synthesis of oligosaccharides containing α- or β-glycosidic bonds. The key step of the synthesis is the C-5-epimerisation of thioglycosides which is planned by hydroboration-oxidation reaction[13,14] of the corresponding 5,6-unsaturated hexopyranosides[15]. It is already known that the α-position of the anomeric group is crucial for the efficient C-5-epimerization of D-sugars by the hydroboration method[14]. Therefore, we plan to perform all syntheses by starting from α-thioglycosides. It is very important to note, that although the hydroboration-oxidation of *O*-glycosides is well-known from the literature[13,14], we are the first who tested the applicability of this method on thioglucosides[11].

2.1 Synthesis of L-hexoses from D-glucose

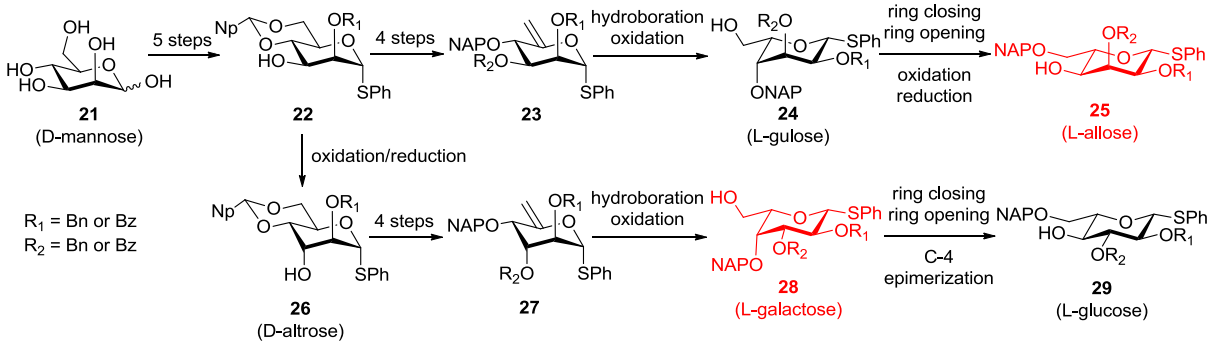
The preparation of the first two L-hexoses, L-idose and L-altrose, is planned by starting from D-glucose. Furthermore, through diisopropyl-glucofuranose, D-glucose can easily be converted to D-allose by C-3-epimerization, from which two further L-hexoses, L-talose and L-mannose, can also be prepared (**Scheme 4.**). First, α-ethylthio glycosides will be prepared from both derivatives, then the obtaining thioglycosides will be equipped with orthogonal protecting groups, appropriate for oligosaccharide syntheses. The 5,6-exomethylene derivatives will be prepared from the corresponding 6-deoxy-6-iodo-glycosides[15], then they will be converted to the corresponding L-configured products by hydroboration-oxidation reaction. We plan to test the impact of ether (benzyl, Bn) and ester groups (benzoyl, Bz) on the efficacy of epimerization reaction as well as on the stereoselectivity of the glycosylation reactions. On the planned pathway, two derivatives (L-*ido* and L-*talo*) could be prepared directly. Then, in a few steps, L-*altro* and L-*manno* derivatives can also be synthesized from these two compounds.



Scheme 4.: Syntheses from D-glucose

2.2 Synthesis of L-hexoses from D-mannose

The syntheses of the further four L-hexoses can be accomplished from D-mannose (**Scheme 5.**). First, we also plan to produce an α-thioglycoside, preparation of which is much easier from mannose than glucose. Then, after the introduction of the protecting groups, the synthesis will continue from **22** via 5,6-exomethylene **23** which can be converted to the L-gulose derivative **24**, and further transformation of this, including C-4-epimerisation, will provide the L-allose thioglycoside **25**. By inversion of the C-3 configuration of compound **22**, it is possible to prepare the D-altrose derivative **26** from which the L-galactose and L-glucose can be synthesized via the route shown in **Scheme 5**.

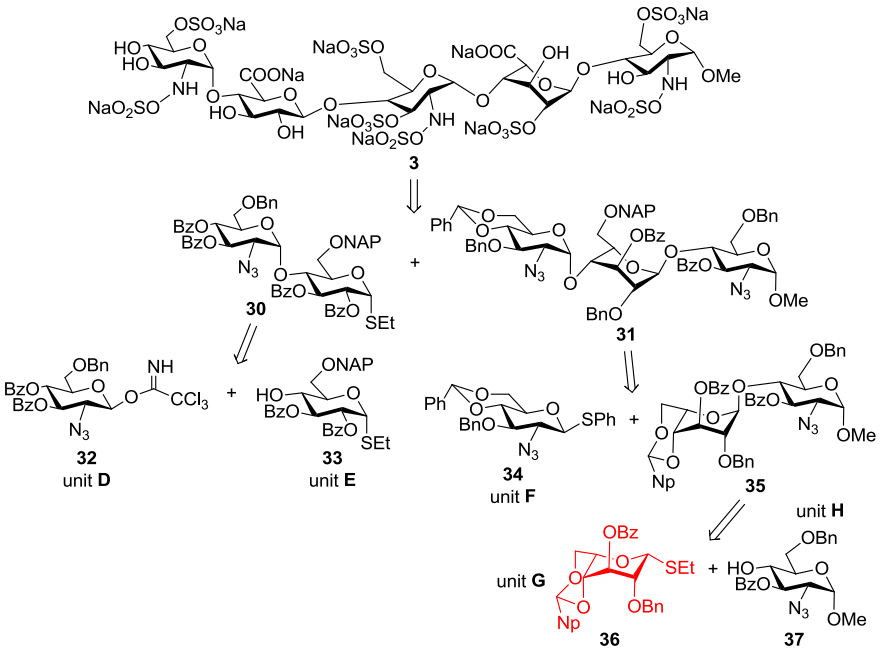


Scheme 5.: Syntheses from D-mannose

3. Development of a new reaction pathway for the synthesis of Fondaparinux

3.1 Retrosynthetic plan for the preparation of Fondaparinux

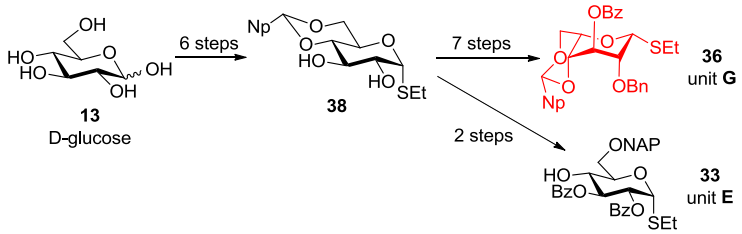
The assembly of the protected pentasaccharide is planned by [2+3] coupling reaction between the corresponding **DE** disaccharide donor and **FGH** trisaccharide acceptor (**Scheme 6.**). For the synthesis of disaccharide unit **DE**, the thioglycoside acceptor unit **E** (**33**) is designed to glycosylate with a trichloracetimidate donor (**32**). The **FGH** trisaccharide can be prepared by coupling of the **GH** disaccharide acceptor (**35**) with the monosaccharide donor **F** (**34**). The synthesis of the **GH** disaccharide is planned to be accomplished by using the orthogonally protected L-idose (**36**) and also orthogonally protected D-glucosamine (**37**) derivatives. Those hydroxyl groups which will be sulphated in the final product will be masked with benzyl groups. The OH-groups to be free in the final product are protected with benzoyl groups and the amino groups are protected as azides. The proposed synthetic route consists of 39 steps, which is currently one of the shortest new synthesis pathways in the literature.



Scheme 6.: Retrosynthetic analysis of [2+3] block syntheses of Fondaparinux (**3**)

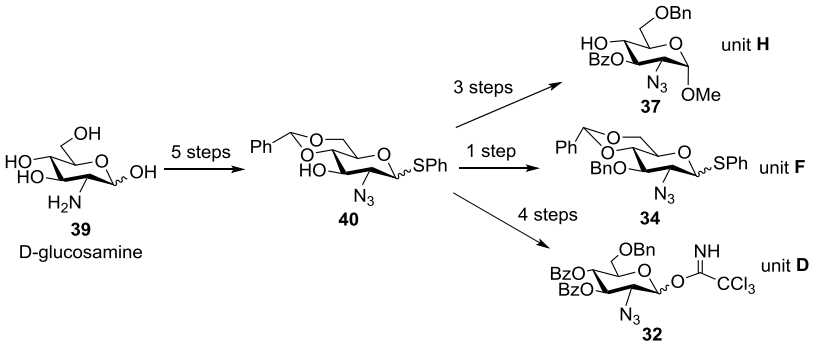
3.2 Synthesis of the monosaccharide building blocks

The preparation of Fondaparinux (**3**) is planned to accomplish from two commercially available monosaccharides: D-glucose and D-glucosamine (**Figure 7.**). The synthesis of units **G** and **E** will be carried out from D-glucose. For the preparation of unit **G** (L-iduronic acid precursor) our new method discussed above will be applied. Then, the orthogonally protected L-idose donor **36** will be used in the stereoselective glycosylation reaction. From **38**, one of the intermediates of this pathway unit **G** (D-glucuronic acid precursor) can be prepared in two steps. The uronic acid functions are plans to be prepared at a pentasaccharide level.



Scheme 7.: The synthesis of the uronic acid precursor

The synthesis of the amino-sugar building blocks is planned in a similar manner to those of the literature[16], except that changing the position of the benzyl and benzoyl protecting groups in the molecules.



Scheme 8.: The synthesis of the amino-sugar building blocks

4. Expected results

In case of successful completion of our research, we can prepare all L-hexoses and their derivatives in highly cost-friendly and efficient routes. Furthermore, the efficient synthesis of L-idose and Fondaparinux can give new impetus to the heparin-based drug discovery as well. In an optimal case, the commercialization of the synthesized L-hexoses can come to the front. However, it must be taken into account the uncertainty of synthetic organic chemistry, if it turns out during the synthesis that certain reactions are not feasible on the tested compounds. In this case, usage of other protective groups or other alternative pathways/reactions may come to the fore. In addition, the synthesis of the planned compounds can contribute to the development of carbohydrate chemistry methods (glycosylation reactions, protecting group manipulations). Using the L-hexoses biologically active molecules can be prepared (e.g.: aminodeoxy L-sugars, aminoglycoside antibiotics), which may be utilized in further pharmaceutical development. The synthetic work, together with biological and structural determination results, will be published in at least 8-10 research papers in peer-reviewed journals, one PhD thesis and some diploma works. We also plan to present our results at national and international conferences in form of oral presentations or posters.

5. Work plan

First year

- Preparation of the α-ethylthio-glucopyranoside in large scale, introduction of the proposed protecting groups (ester, ether), synthesis of 5,6-unsaturated d-glucose derivatives, optimization of the elimination reaction
- Synthesis of the D-allose from D-glucose in large scale, preparation of the α-ethylthio-alloside and formation of the intended protecting groups, synthesis of 5,6-unsaturated D-allose derivatives
- Perform of the hydroboration/oxidation test reactions, optimization; characterization of the obtained L-sugars
- Testing of the 4,6-acetal ring closure reactions and transformation of the L-sugar into donors
- Further transformation of the L-*ido* and L-*talo* configured derivatives
- Publication of the results in referred international journal (2 publication)

Second year

- Preparation of the L-*altro* and L-*manno* derivatives, characterization of the obtained L-sugars
- Synthesis of the α-ethylthio-mannopyranoside in large scale, introduction of the proposed protecting groups (ester, ether), synthesis of 5,6-unsaturated D-glucose derivatives, optimization of the elimination reaction
- Synthesis of the L-idose donor in large scale for the preparation of Fondaparinux
- Preparation of D-aldose derivative in large scale from the planned D-mannose derivative
- Synthesis and transformation of the L-gulose and L-galactose derivatives
- Publication of the results in referred international journal (3 publications)
- Publication of the results in a PhD dissertation

Third year

- Synthesis of the 5,6-unsaturated D-altrose derivatives, optimization of the elimination reaction on the given derivatives

- Preparation of the glucosamine monomers in large scale for the synthesis of Fondaparinux
- Preparation of the L-allose and L-glucose derivatives, configuration analysis
- Synthesis of glucuronic acid precursor for the production of Fondaparinux
- Optimization of the glycosylation reactions, synthesis of the **DE** disaccharide donor and the **FGH** trisaccharide acceptor
- Preparation of the protected pentasaccharide by [2+3] block synthesis, formation of the final groups, structural analysis
- Publication of the results in referred international journal (3 publications)

References

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