Ph. D. THESIS

Zsuzsanna Csíki

Synthesis of heparanase inhibitors

Application of *N*-nosyl protected azasugar acceptors for the synthesis of heparin disaccharide analogs

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BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS

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1. INTRODUCTION

The structurally related heparin and heparan sulfate (HS) are heterogeneous polysaccharide chains, members of the glycosaminoglycan family of polysaccharides.[1;2] Heparan sulfate binds to a large number of proteins, thereby influencing a variety of normal and pathological processes including tumor growth and metastasis, tissue repair, angiogenesis, and inflammation. Cleavage of HS chains alters its interaction with proteins and thus influences the above processes.

The most important cleavage enzyme in the catabolism degradation of heparan sulfate chains is heparanase. It has been recognized that tumor metastasis occurs *via* complex multistage processes, which involves tumor cell adhesion to various basement membrane components, and degradation of the extracellular matrix and basement membranes. As HS is an important constituent in these structures, cleavage of HS by heparanase plays an important role in cell invasion of some malignant tumors through basement membranes.

The natural substrates of the heparanase enzyme are heparin and heparan sulfate polysaccharides (Figure 1.). Heparanase is an *endo*-β-glucuronidase, which specifically cleaves the HS chains at a limited number (8-10 oligosaccharides) of sites.

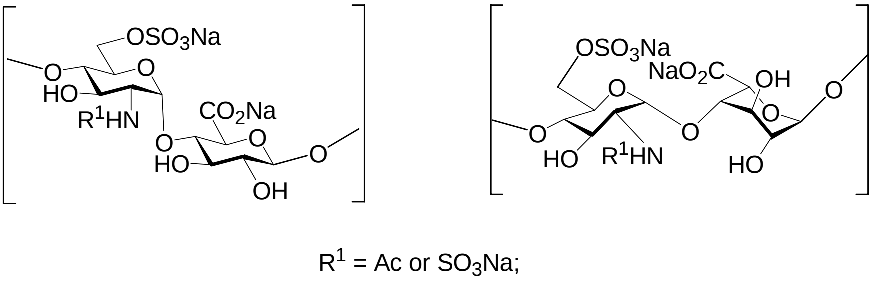
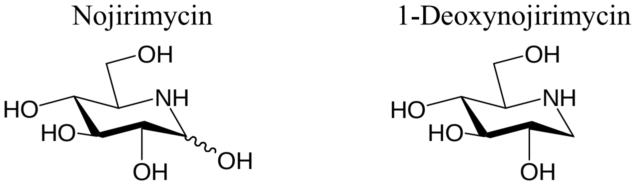


Figure 1. The heparin and heparan sulfate polysaccharides chains.

Heparanase is over expressed in a number of human tumors. The expression level of the enzyme is of diagnostic and prognostic value, and has been negatively correlated to the survival time of cancer patients. The inhibition of heparanase forms the basis of potential antimetastatic cancer therapy and therefore, it has been intensively investigated.

1.1. OBJECTIVES OF THE Ph. D. THESIS

Azasugars are monosaccharide analogs having a nitrogen atom instead of oxygen in the ring. Azasugars have received significant attention as carbohydrate mimetics. Compounds of this type, such as nojirimycin and 1-deoxynojirimycin (Figure 2.),[3] are potent inhibitors of various glycosidases.

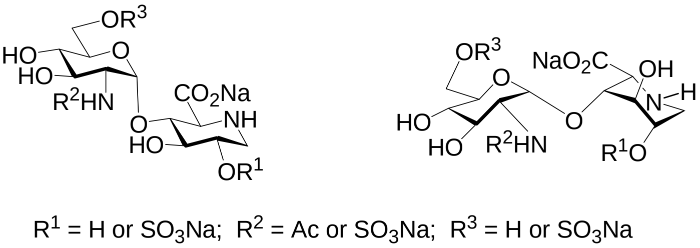

Nojirimycin

1-Deoxynojirimycin

Figure 2. Structures of nojirimycin and 1-deoxynojirimycin.

Though monosaccharidic iminosugars, in general, nojirimycin and 1-deoxynojirimycin show some specificity to inhibit certain types of glycosidases. This specificity is still fairly broad, which limits their potential therapeutic applications. One way to increase specificity is to use larger molecules which closer mimic of the natural substrates of the enzymes.

In order to incorporate specificity in iminosugars towards heparanase, we have designed pseudooligosaccharides mimicking the structure of heparin and heparan sulfate (Figure 3.).



R^1 = H or SO_3Na; R^2 = Ac or SO_3Na; R^3 = H or SO_3Na

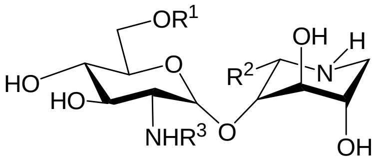
Figure 3. Designed heparanase inhibitors.

Compounds of designed azapseudodisaccharides contain a D-glucosamine unit α-(1→4)-linked to an azasugar analog of D-glucuronic acid and L-iduronic acid, respectively. Though heparanase is a β-glucuronidase, the compounds designed by us having an L-*ido* configured azasugar might also be of interest as potential inhibitors. The rationale for this is that there are several examples reported that azasugars of the "wrong" configuration are proper inhibitors of glycosidases having specificity for a different configuration.[4] Additionally, because of the known conformational mobility of L-idose and L-iduronic acid,[5] compounds might fit into the active site of the enzyme.

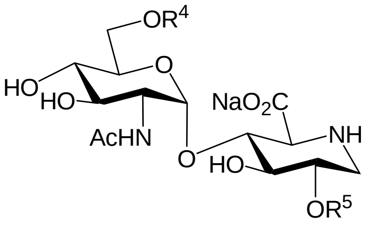
It was also of interest, that an L-iduronic acid-type 1-*N*-iminosugar has been reported to have inhibitory activity on cancer metastasis.[6]

The designed compounds are structurally new type heparanase inhibitors. Previously, only an aza-D-glucuronic acid-containing disaccharide heparanase inhibitor of has been reported.[7] To our knowledge, L-*ido*-configured azasugar-containing oligosaccharides have not been synthesized before.

In my thesis, the synthesis of six differently substituted disaccharides (**1**, **2**, **3**, **4**, **5** and **6**) and one already known aza-L-iduronic acid monosaccharide (**7**)[8] (Figure 4.) are discussed.



1 R^1= H ; R^2= CH_2OH; R^3= Ac
2 R^1= H ; R^2= COONa; R^3= Ac
3 R^1= SO_3Na ; R^2= COONa; R^3= Ac
4 R^1= SO_3Na ; R^2= COONa; R^3= SO_3Na



5 R^4= H ; R^5= SO_3Na
6 R^4= SO_3Na ; R^5= H

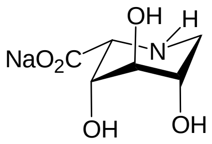


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Figure 4. Planed potential heparanase inhibitors.

In order to prepare the designed pseudodisaccharides, new methods were required for the synthesis of azasugar acceptors with L-*ido*- and D-*gluco* configuration.

For the protection of the ring nitrogen in the glycosyl acceptors, the benzyloxycarbonyl group was replaced with the 4-nitrobenzenesulfonyl (Ns) group.

The O-4 and O-6 positions were protected by (1-naphthyl)methylene acetal, which have not been used before in this field. The use of this protecting group allows further selective manipulations of the intermediers.

To synthesize the D-*gluco* configured azasugar disaccharides, an orthogonally protected disaccharide was designed, which lead an easy access to the 2-*O*-sulfated and the 6-*O*-sulfated heparanase inhibitors.

A short and unambiguous syntheses of 3-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol and -L-iditol were designed and realized.

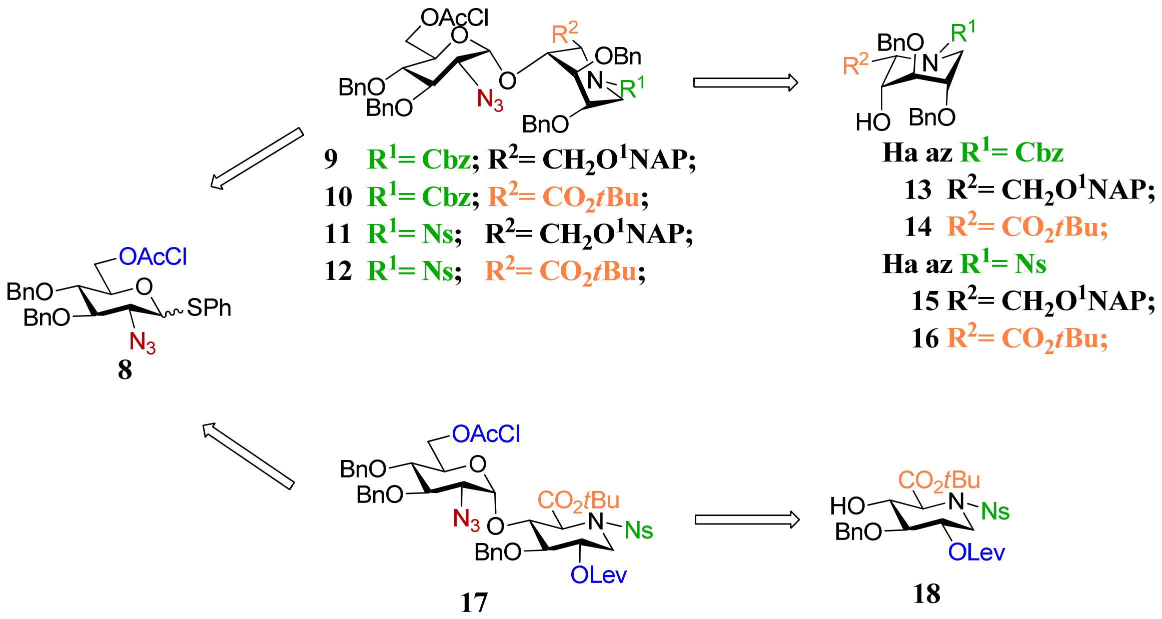
2. EXPERIMENTAL METHODS

The macro- and micro methods of modern preparative organic chemistry were applied in the synthetic work.

Thin layer chromatography was applied to monitor reactions. Crystallization, column chromatography and gel chromatography were used for the purification of the crude products. Modern spectroscopic methods (one- and two-dimensional nuclear magnetic resonance spectroscopy, IR spectroscopy, mass spectroscopy) and classical methods (melting point determination, optical rotation determination and elemental analysis) were applied for the verification of the structures of the synthesized compounds.

3. RESULTS AND DISCUSSION

The target structures (**1**, **2**, **3**, **4**, **5** and **6**) are available due to the synthesis of **9**, **10**, **11**, **12** and **17** fully protected disaccharides. These disaccharides are derived from the 2-azido-2-deoxy-D-glucopyranosyl donor (**8**) and the L-idose (**13** and **15**) and L-iduronic acid (**14** and **16**) azasugar glycosyl acceptors (Scheme 1.).



Scheme 1. Synthesis of heparanase inhibitory disaccharides.

In the glycosyl donor **8**, O-6 is masked temporary by the chloroacetyl group in the synthesis of L-*ido* configured disaccharides.

The carboxyl functions in **14**, **16** and **18** were protected as the *tert*-butyl ester. For the protection of the ring nitrogen in the glycosyl acceptors (**13**, **14**), the benzyloxycarbonyl group was selected originally, as this is used most commonly for this purpose in the azasugar synthesis. However, at a later stage of this work for the synthesis of **15**, **16** and **18** glycosyl acceptors, the 4-nitrobenzenesulfonyl (Ns) group was used for the protection of the ring nitrogen.

The synthesis of glycosyl acceptors requires selective manipulations at O-4 and O-6, which should be available from the (1-naphthyl)methylene acetal by different reductive acetal openings. Both O-4 and O-6 (1-naphthyl)methyl derivatives were glycosylable at O-4 position. The O-6 position was converted to *tert*-butyl ester derivative then gave **14**, **16** and **18** glycosyl acceptors.

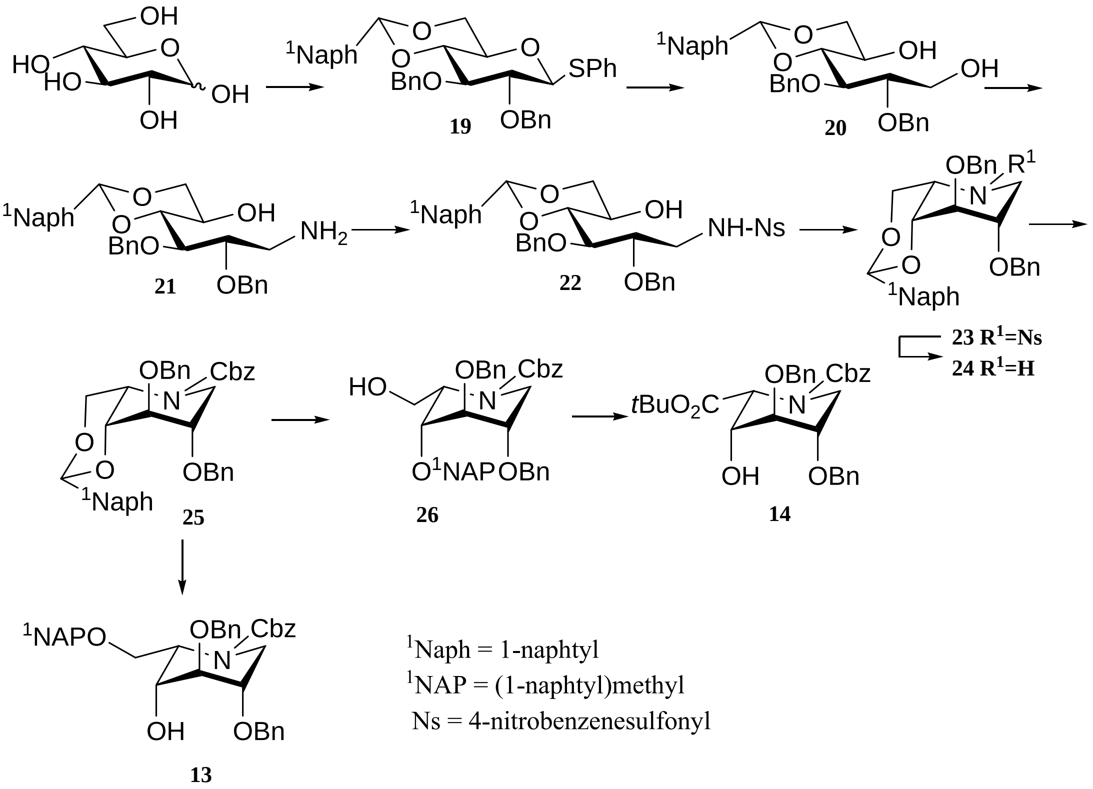
The D-*gluco* configured disaccharides (**5** and **6**) were prepared from an orthogonally protected precursor (**17**). For the orthogonally protected precursor, an aza-D-glucoronic acid glycosyl acceptor (**18**), with levulinoyl orthogonal protecting group in O-2 position was synthesized.

3.1. Synthesis of 1,5-dideoxy-1,5-imino-L-iditol glycosyl acceptors

3.1.1. Synthesis of N-carbobenzyloxy protected 1,5-dideoxy-1,5-imino-L-iditol glycosyl acceptors

Starting from commercially available D-glucose, the thioglycoside derivative (**19**) was prepared. Two different synthetic methods were developed for the synthesis of **21** precursor from the **20** alditol. For the incorporation of nitrogen into the ring, the *Mitsunobu* reaction was selected. As for this reaction an acidic nucleophile is required, we chose to protect the amino group as a sulphonamide (4-nitrobenzenesulfonyl, nozil, Ns),[9] which makes the amid proton sufficiently acidic for the S_N2 nucleophilic reaction to the place. Cyclization of **22** 4-nitrobenzenesulfonamide using the *Mitsunobu* reaction took place with configurationally inversion at C-5 and provided the L-*ido* derivative (**23**). The 4-nitrobenzenesulfonyl group was exchanged to benzyloxycarbonyl (**25**). The conversion of **25** to the glycosyl acceptors (**13**, **14**) was performed in the following ways (Scheme 2): reductive ring opening of the (1-naphthyl)methylene acetal with $NaBH_3CN$-HCl[10] afforded **13** directly.

For the synthesis of **14**, the 4-*O*-(1-naphthyl)methyl ether was prepared by reduction by BH_3·THF-TMSOTf,[11] which gave **26**. One-step oxidation-esterification using pyridinium dichromate in the presence of acetic anhydride and *tert*-butanol (*Corey-Samuelsson* method)[12] afforded the *tert*-butyl uronate, then the (1-naphthyl)methyl group was removed.



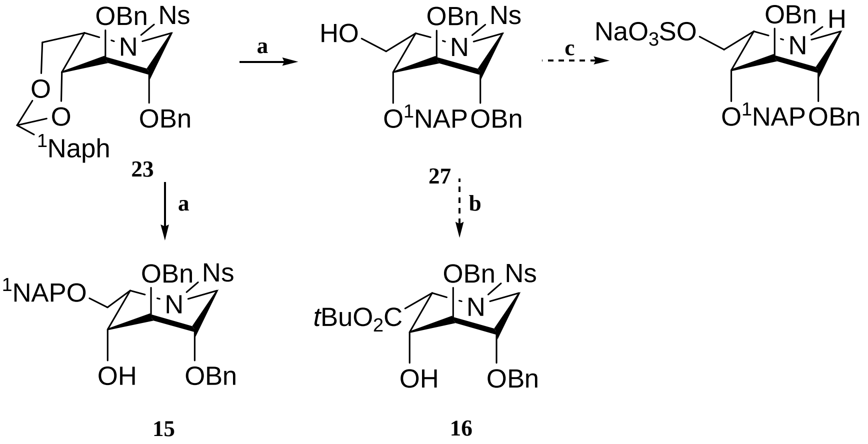
¹Naph = 1-naphtyl
¹NAP = (1-naphtyl)methyl
Ns = 4-nitrobenzenesulfonyl

Scheme 2. Synthesize of *N*-carbobenzyloxy protected 1,5-dideoxy-1,5-imino-L-iditol glycosyl acceptors.

3.1.2. Synthesis of N-nosyl protected 1,5-dideoxy-1,5-imino-L-iditol glycosyl acceptors

Our original choice for the protection of the ring nitrogen of azasugars was the benzyloxycarbonyl group which is the most commonly used group for this purpose. During the previous syntheses, however, we have experienced a series of drawbacks associated with the use of this protecting group. A major inconvenience was the doubling of signals in the NMR spectra of the *N*-benzyloxycarbonyl derivatives. This is due to the existence of rotamers around the amide bond. Another problem with the *N*-benzyloxycarbonyl group was the undesired side product. In case of the *N*-nosyl intermediate, the NMR spectrum was simpler, better-resolved. For these purposes the benzyloxycarbonyl group was replaced with the 4-nitrobenzenesulfonyl group.

To check whether the nosyl group is stable in reactions used for heparin oligosaccharide synthesis, we performed some experiments: the (1-naphthyl)methylene acetal was opened with $NaBH_3CN$-HCl[10] that gave the 6-O-naphthylmethyl derivative (**15**), whereas reduction with BH_3·THF-TMSOTf,[11] led to the 4-O-naphthylmethyl ether (**27**) (Scheme 3. a steps). Compound **23** was converted into the glycosyl acceptor via a one-step oxidation with PDC-Ac_2O-*t*-BuOH[12] to give the *tert*-butyl uronate, followed by removal of the (1-naphthyl)methyl group to yield **16** (Scheme 3. b steps). The O-4 (1-naphthyl)methyl derivative was used as a model compound, to investigate if the nosyl group can be removed in the presence of a sulfate. We found that the nosyl can be effectively removed without affecting the *O*-sulfate (Scheme 3. c step).

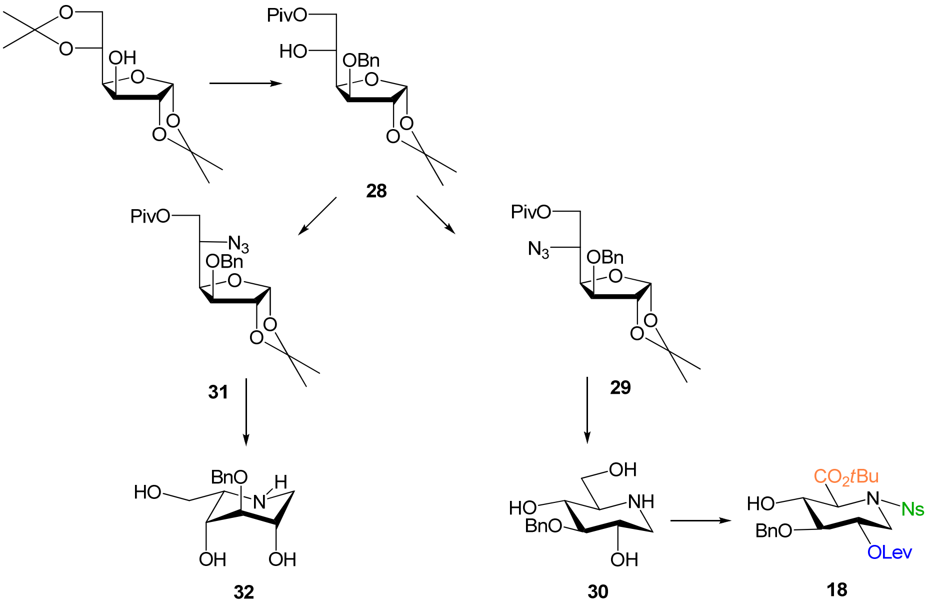


Scheme 3. Synthesize of *N*-nosyl protected 1,5-dideoxy-1,5-imino-L-iditol glycosyl acceptors.

3.2. Synthesis of D-glucuronitol glycosyl acceptor

We realized that the compound reported previously by *Roy* and coworkers[13] as 3-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol is actually 3-*O*-benzyl-1,5-dideoxy-1,5-imino-L-iditol. We envisaged the synthesis of both **30** (3-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol) and **32** (3-*O*-benzyl-1,5-dideoxy-1,5-imino-L-iditol) from the partially protected D-glucofuranose derivative **28** (Scheme 4.). We have developed a short and unambiguous synthetic route to 3-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol and -L-iditol, which are useful synthetic intermediates for azasugar containing oligosaccharides. We have shown that the compound previously reported to have the D-*gluco* configuration (**30**) is in fact the L-*ido* derivative **32**.

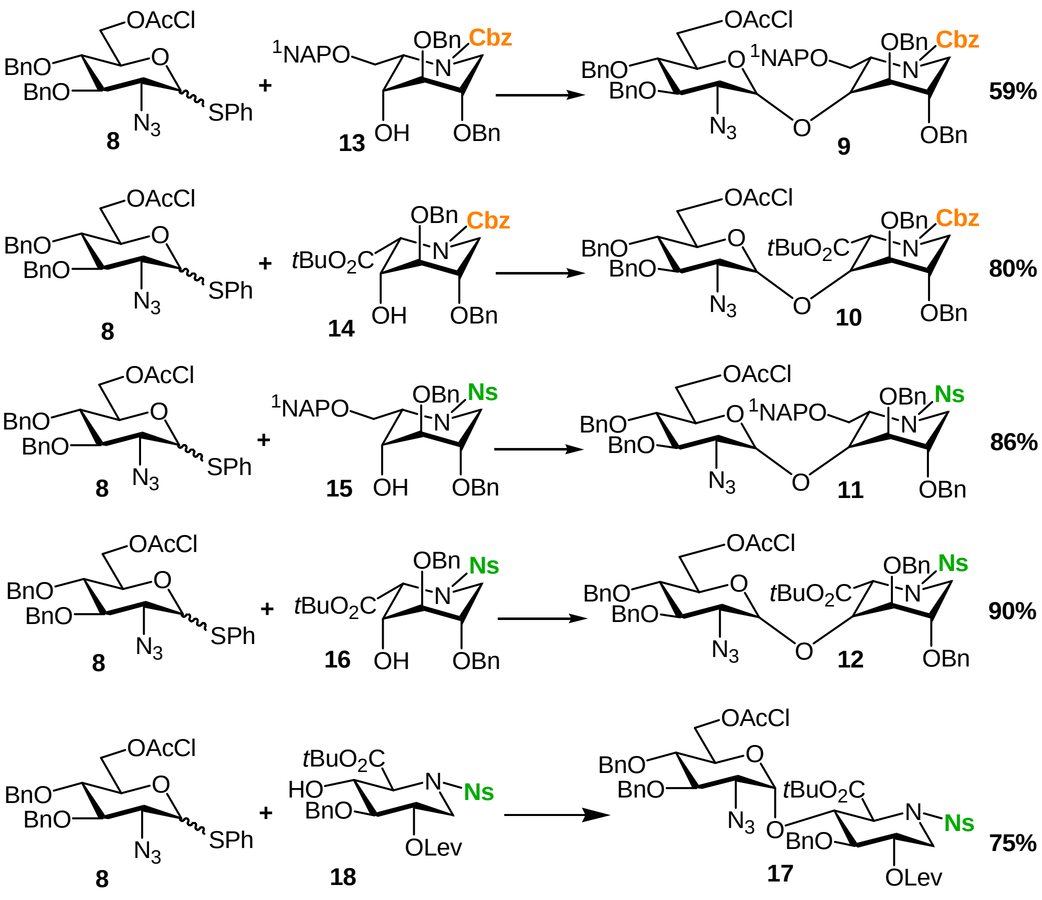
The D-glucitol glycosyl acceptor **(18)** was synthesized from **30** intermediate (Scheme 4.).



Scheme 4. Synthesize of 3-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (**30**), -L-iditol (**32**) intermediates and D-glucuronitol glycosyl acceptor (**18**).

3.3. Glycosylations: synthesis of fully protected disaccharides

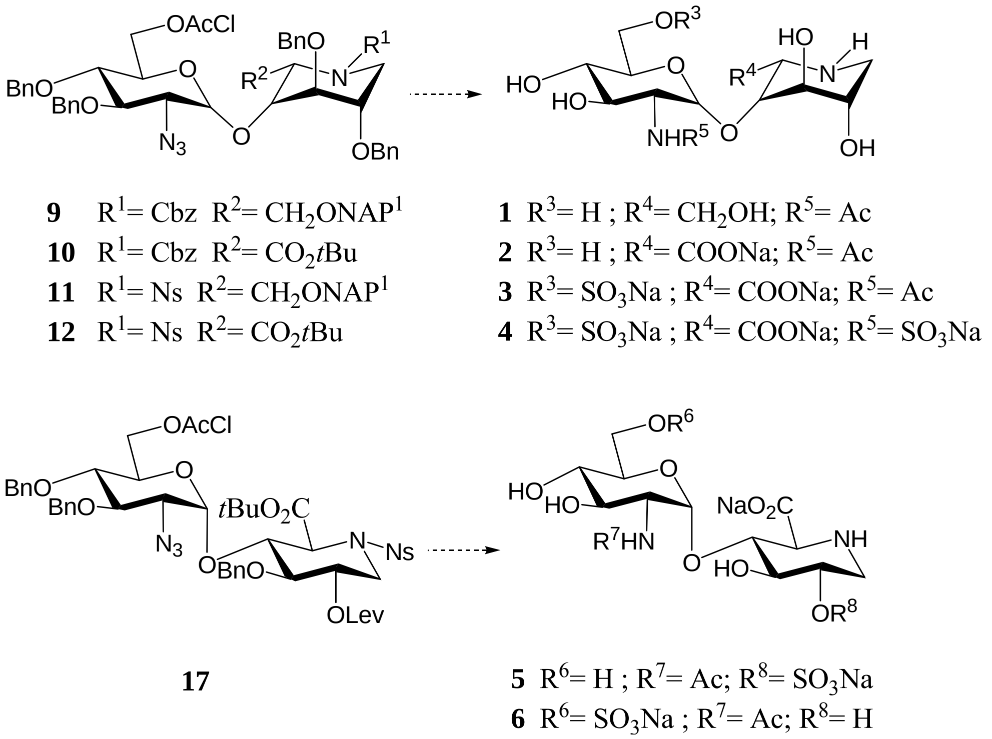
The **8** glycosyl donor was glycosylated with **13** *N*-benzyloxycarbonyl protected azasugar acceptor using DMTST[14] as promoter. For further glycosylations Me_2S_2-Tf_2O[15] promoter was used, because this promoter combination was more reactive and thus the reaction time was shorter, moreover the yields of glycosylations were higher. In each glycosylation reaction, the solvents were diethyl ether-dichloromethane and the α-linked disaccharides were obtained in high yield (Scheme 5.).



Scheme 5. Synthesis of the fully protected disaccharides.

3.4. Synthesis of azapseudodisaccharides

The azasugar containing disaccharides with L-*ido* configuration were obtained from **9**, **10**, **11** and **12** fully protected disaccharides. The selective removal of the protecting groups, *N*,*O*-disulfation followed by catalytic hydrogenolysis of permanent protecting groups afforded the target compounds, the **1**, **2**, **3** and **4** potential heparanase inhibitors (Scheme 6.).



9 R^1= Cbz R^2= CH_2ONAP^1

10 R^1= Cbz R^2= CO_2tBu

11 R^1= Ns R^2= CH_2ONAP^1

12 R^1= Ns R^2= CO_2tBu

1 R^3= H ; R^4= CH_2OH; R^5= Ac

2 R^3= H ; R^4= COONa; R^5= Ac

3 R^3= SO_3Na ; R^4= COONa; R^5= Ac

4 R^3= SO_3Na ; R^4= COONa; R^5= SO_3Na

17

5 R^6= H ; R^7= Ac; R^8= SO_3Na

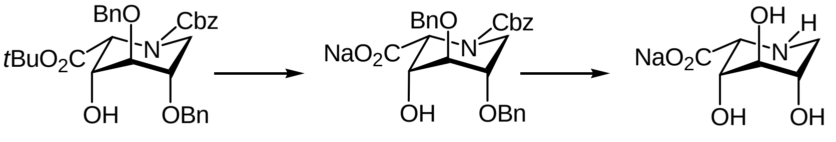
6 R^6= SO_3Na ; R^7= Ac; R^8= H

Scheme 6. Synthesis of azapseudodisaccharides.

The D-*gluco* configured azapseudodisaccharides (**5** and **6**) were synthesized from **17** orthogonally protected precursor. We have demonstrated that the chloroacetyl and levulinoyl protecting groups are orthogonal and can be used together. We have developed an orthogonal protecting-based synthesis strategy for the preparation of differently sulfated azapseudodisaccharides (Scheme 6.).

3.5. Synthesis of 1,5-dideoxy-1,5-imino-L-iduronitol

In order to preparation of the free monosaccharide 1-deoxynojirimycin derivative (**7**)[8] the *tert*-butyl ester was hydrolyzed and then followed by catalytic hydrogenation of **31** afforded **7** (Scheme 7.).



14 **31** **7**

Scheme 7. Synthesis of 1,5-dideoxy-1,5-imino-L-iduronitol.

4. THESIS STATEMENTS

1. In order to incorporate specificity in azasugars towards heparanase, we have designed and synthesized azasugar-containing oligosaccharides mimicking the structure of heparin and heparan sulfate [**1**, **2** and **3**].

2. During the thesis work, six differently substituted, L-*ido* [**1** and **3**] and D-*gluco* configured azapseudodisaccharides and one 1-deoxynojirimycin monosaccharide [**3**] derivative of the designed potential heparanase inhibitors were synthesized.

We have reported the first L-*ido*-configured azasugar-containing oligosaccharides [**1** and **3**].

3. We have developed an efficient synthesis strategy for the preparation of L-*ido* [**1** and **3**] and D-*gluco* configured azasugar glycosyl acceptors for the access of designed heparanase inhibitors.

We have successfully glycosylated the 2-azido-2-deoxy-D-glucopyranosyl thioglycoside donor with L-*ido* and D-*gluco* configured aza acceptors and then afforded disaccharides in good yields [**1** and **3**].

4. We have introduced the 4-nitrobenzenesulfonyl group for the protection of the ring nitrogen in iminosugars. The usefulness of the *N*-nosyl protection was demonstrated by the synthesis of four differently substituted (**3**, **4**, **5** and **6**) azapseudodisaccharides. This group can be conveniently introduced and it proved to be stable under a series of common carbohydrate transformations including reductive acetal openings, glycosylations, oxidations, sulfations and removal of some common protecting groups (Ac, Bz, ClAc, Lev, *t*Bu). The nosyl group can be removed under mild conditions without affecting *O*- and *N*-sulfate groups. The orthogonality of the *N*-(4-nitrobenzenesulfonyl) group with the azido function was demonstrated. An additional advantage is that the NMR spectra of the nosylated compounds are simpler than those of the *N*-benzyloxycarbonyl protected ones [**1**].

5. We have used first the (1-naphthyl)methylene acetal for the protection of O-4 and O-6 in the synthesis of azasugar derivatives. We have demonstrated that different reductive ring-openings allow further selective manipulations at O-4 and O-6 positions [**1** and **3**].

6. We have developed a short and unambiguous synthetic route to 3-O-benzyl-1,5-dideoxy-1,5-imino-D-glucitol and -L-iditol, which are useful synthetic intermediates of azasugar containing oligosaccharides. We have shown that a compound previously reported to have D-gluco configuration in fact, is the L-ido derivative [**2**].

PUBLICATIONS ON THE SUBJECT OF THE THESIS

[1] Zsuzsanna Csíki, Péter Fügedi: The 4-nitrobenzenesulfonyl group as a convenient N-protecting group for iminosugars - synthesis of oligosaccharide inhibitors of heparanase
Tetrahedron Lett. **2010**, *51*, 391-395.
IF: 2.538 (2008)

[2] Zsuzsanna Csíki, Péter Fügedi: An unambiguous synthesis of 3-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol and -L-iditol
Synthesis **2010**, (Received April 6, 2010).
IF: 2.447 (2008)

[3] Zsuzsanna Csíki, Péter Fügedi: Synthesis of aza-L-iduronic acid containing analogs of heparan sulfate oligosaccharides as heparanase inhibitors
Tetrahedron **2010**, (Received minor corrections May 24, 2010).
IF: 2.897 (2008)

[4] Zsuzsanna Csíki, Péter Fügedi: Synthesis of heparanase inhibitors, heparin disaccharides possessing an azasugar unit
Per. Pol. Chem. Eng., **2007,** *51/2,* 80.

[5] Zsuzsanna Csíki and Péter Fügedi: Synthesis of a heparanase inhibitor molecule possessing an azasugar component
Per. Pol. Chem. Eng., **2005,** *49/1,* 25-89.

ORAL AND POSTER PRESENTATIONS ON THE SUBJECT OF THE THESIS ON THE INTERNATIONAL CONFERENCES

Oral presentation:

1. Zsuzsanna Csíki, Péter Fügedi: Heparanase inhibitors: the use of *N*-nosyl as an azasugar protecting group in oligosaccharide synthesis (Abstract: OP-035)
   14th European Carbohydrate Symposium, Lübeck, Germany, September 2-7, **2007**.

Poster presentations:

1. János Tatai, Zsuzsanna Csíki, Péter Fügedi: Synthesis of L-iduronic acid containing heparin disaccharides by orthogonal protecting group strategy
   1st Austrian-Hungarian Carbohydrate Conference, Burg Schlaining, Austria, September 24-26, **2003**.

2. Zsuzsanna Csíki, Péter Fügedi: Synthesis of protected azasugar derivatives for the synthesis of heparanase inhibitors
   8st European Training Course on Carbohydrates , Wageningen, The Netherlands, June 28-July 1, **2004**.

3. Zsuzsanna Csíki, Péter Fügedi: Synthesis of a heparanase inhibitor molecule possessing an azasugar component
   13th European Carbohydrate Symposium, Bratislava, Slovakia, August 21-26, **2005**.

4. Zsuzsanna Csíki and Péter Fügedi: Synthesis of azadisaccharides as heparanase inhibitors (A-P077)
   XXIV International Carbohydrate Symposium, Oslo, Norway, July 27 - August 1, **2008**.

5. Zsuzsanna Csíki, Péter Fügedi: Synthesis of azadisaccharides as heparanase inhibitors (P-77)
   4th Central European Conference, Chemistry towards Biology, Dobogókő, Hungary, September 8-11, **2008**.

ORAL PRESENTATIONS ON THE SUBJECT OF THE THESIS

1. Zsuzsanna Csíki: Biological function of heparin and heparane sulfate
   VI. Chemical School, Tahitótfalu, April 29-30, **2003.**

2. Zsuzsanna Csíki, Péter Fügedi: Synthesis of protected azasugar derivatives for the synthesis of heparanase inhibitors
VII. Chemical School, Tahitótfalu, April 27-28, **2004**.

3. Zsuzsanna Csíki, Péter Fügedi: Synthesis of a heparanase inhibitor molecule possessing an azasugar component
2nd *Conference of PhD students at faculty of chemical engineering (BME), Budapest, November 24,* **2004**.

4. Zsuzsanna Csíki, Péter Fügedi: Synthesis of a heparanase inhibitor molecule possessing an azasugar component
VIII. Chemical School, Tahitótfalu, May 5-6, **2005**.

5. Zsuzsanna Csíki, Péter Fügedi: Synthesis of a heparanase inhibitor molecule possessing an azasugar component
2nd *Austrian-Hungarian Carbohydrate Conference, Somogyaszaló, Hungary May 24-26,* **2005**.

6. Zsuzsanna Csíki, Péter Fügedi: Synthesis of heparanase inhibitors, heparin disaccharides possessing an azasugar unit
3nd *Conference of PhD students at faculty of chemical engineering (BME), Budapest, February 7,* **2006**.

7. Zsuzsanna Csíki, Péter Fügedi: Synthesis of heparanase inhibitors, heparin disaccharides possessing an azasugar unit
HAS-Seminar of Organic Chemistry, Budapest, February 20, **2006**.

8. Zsuzsanna Csíki, Péter Fügedi: Using *N*-nosyl group for the synthesis of azasugar containing heparin disaccharide
IX. Chemical School, Tahitótfalu, April 24-25, **2006**.

9. Zsuzsanna Csíki and Péter Fügedi: The 4-nitrobenzenesulfonyl as a convenient *N*-protecting group in azasugar synthesis
Annual Meeting of the Committee of Carbohydrate Chemistry, Mátrafüred, May 31 – June 2, **2006**.

10. Zsuzsanna Csíki and Péter Fügedi: Heparanase inhibitors: application of *N*-nosylated azasugar unit for the synthesis of heparin disaccharide analogs
Annual Meeting of the Committee of Carbohydrate Chemistry, Mátrafüred, May 23-25, **2007**.

11. Zsuzsanna Csíki, Péter Fügedi: Heparanase inhibitors: application of *N*-nosylated azasugar unit for the synthesis of heparin disaccharide analogs
Chemical Research Center Scientific Days, Budapest, May 23-24, **2007**.

12. Zsuzsanna Csíki, Péter Fügedi: Heparanase inhibitors: the use of *N*-nosyl as an azasugar protecting group in oligosaccharide synthesis
Gedeon Richter Pharmaceutical Factory, Lajos Kisfaludy Annual Meeting, Budapest, February 18. **2008**.

13. Zsuzsanna Csíki, Péter Fügedi: New synthesis method for the preparation of heparanase inhibitors. The use of nosyl protected azasugar acceptors in the synthesis of heparin disaccharid analogs
HAS-Seminar of Organic Chemistry, Budapest, May 25, **2009**.

Other oral presentations:

1. Zsuzsanna Csíki, Erzsébet Czinege, Péter Fügedi: Development of a new manufacturing procedure for PEIm
1st Annual Meeting Vaccine Therapy Cluster , Mátraháza, October 25-28, **2006**.

2. Zsuzsanna Csíki, Péter Fügedi: Redesigned PEIm analogs
3rd Annual Meeting NanoMedicine Cluster, Mátraháza, October 15-18, **2008**.

3. Zsuzsanna Csíki, Béla Iván, Lajos Kemény, Péter Fügedi: Synthesis carbohydrate modified polymers for targeted gene therapy
Chemical Research Center Scientific Days, Budapest, December 3-5, **2008**.

4. Zsuzsanna Csíki, Péter Fügedi: Synthesis carbohydrate modified polymers for targeted gene therapy
Annual Meeting of the Committee of Carbohydrate Chemistry, Mátrafüred, May 28-29, **2009**.

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