Theses of doctoral (PhD) dissertation

MODULAR SYNTHESIS OF ANTICOAGULANT PENTASACCHARIDES

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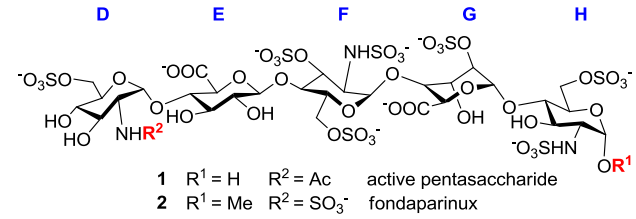
Debrecen, 2015

1. Introduction

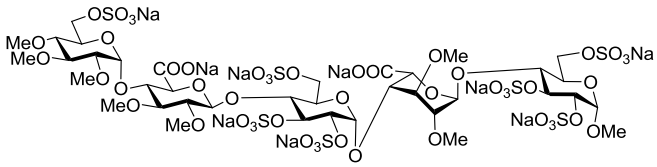
Venous and arterial thromboembolic disorders (e.g. pulmonary embolism, deep vein thrombosis) are quite serious problem all over the world. Vessels occluding blood clot can be formed by coagulation of blood, which causes deep vein thrombosis and/or pulmonar embolism in most cases. Untreated thromboembolism lead to cardiac or cerebral infarction or in more severe case to death. For about 10% of the hospital mortality these disorders are responsible.

Anticoagulants are used for the prevention and treatment of these diseases, which inhibit the undisered coagulation processes. In the last decades many anthitrombotic drugs were developed for inhibition of enzymes in the coagulation pathways.

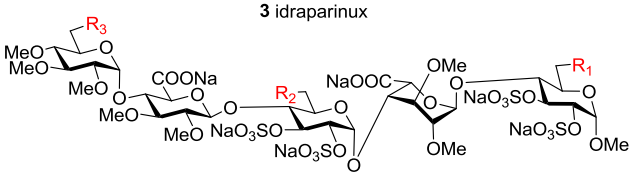
Heparin has been present on the market for decades until today and is one of the most often applied anticoagulants, even though it has a number of limitation including the risk of life-threatening heparin-induced thrombocytopaenia. Heparin is a sulfated glycosaminoglycan (GAGs) consisting of 1→4 linked hexuronic acids (D-glucuronic and L-iduronic acids in a ratio of 1:9) and glucosamine with varying degrees of sulfation. This negatively charged linear polysaccharide is found inside cells and in the extracellular matrix. It is an indirect inhibitor of trombin through binding to antithrombin (AT, a serine protease inhibitor) which is a regulator protein in the coagulation cascade. Presence of unique pentasaccharide unit (**DEFGH**, **1**) within a polysaccharide is nessecery for the anticoagulant effect. Its synthetic analogue (**2**, fondaparinux sodium) has been used under the name Arixtra® in the medicine since 2001. Some analogues of fondaparinux were prepared in the last decades. One of the most promising molecule is idraparinux (**3**) which is a non-glycoseaminoglycan analogue of fondaparinux and it is a more easier obtainable derivative. Its main advantage is the excellent anticoagulation effect (**Scheme 1.**).



1 R^1 = H R^2 = Ac active pentasaccharide
2 R^1 = Me R^2 = SO_3^- fondaparinux



3 idraparinux



44 R^1 = R^2 = CH_2SO_3Na, R^3= OBn
45 R^1 = R^2 = R^3 = CH_2SO_3Na

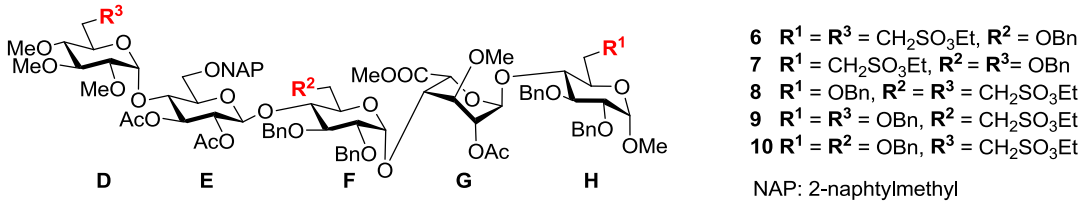
	Anti-Xa activity (U/mg)
Arixtra (2)	1195±189
idraparinux (3)	1911± 193
pentasaccharide disulfonic acid (44)	2153±153
pentasaccharide trisulfonic acid (45)	384±139

Scheme 1. Structure and anticoagulant activity of the active pentasaccharide domain (1) of heparin and its synthetic derivatives (2, 3, 44 and 45)

Our group has been dealing with the synthesis of bioisosteric sulfonic acid analogues of idraparinux in order to obtain novel selective factor Xa inhibitors. Advantage of these molecules is the higher resistance against sulfatase and hydrolase enzyms, therefore they are able to exert their

anticogulant effect at a constant level. Two pentasaccharide sulfonic acids (**44** and **45**) and the reference compound idraparinux (**3**) have been prepared by now. Evaluation of the inhibitory activities of pentasaccharides (**3**, **44** and **45**) towards the blood-coagulation proteinase factor-Xa revealed that the disulfonate analogue **44** displayed higher activity than idraparinux, however, introduction of the third sulfonic-acid moiety (**45**) resulted in a notable decrease in anti-Xa activity. It revealed that the position and number of the replaced sulfate groups are very important. To gain deeper insight into the structure–activity relationship of the anticoagulant action of the sulfonic acid derivatives we decided to prepare a series of heparinoid pentasaccharides by systematic replacement of the primary sulfate esters with a sodium sulfonatomethyl moiety.

The aim of my PhD project was the preparation of this series of pentasaccharide sulfonic acids (**6**-**10**) in protected form (**Scheme 2.**). Moreover, we also aimed at preparing compounds **45** in a sufficient amount for detailed STD NMR studies of its interactions with antithrombin.



Scheme 2. Structure of the designed pentasaccharide di- and monsulfonic acids in protected form

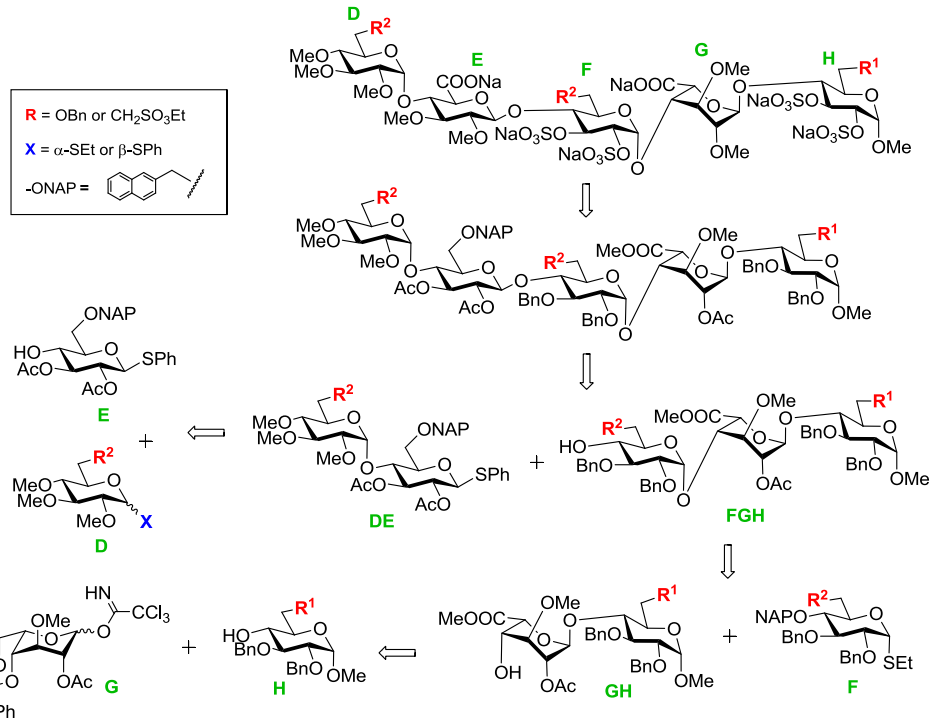
2. Methods

In the course of synthetic work, macro, semimicro and micro methods of modern preparative organic chemsitry were applied. The purity of the substances, the ratio of products were controlled and the reactions were monitored by thin-layer chromatography. Purification of the crude products and separation of the isomers were carried out either by crystallization, column chromatography, gel chromatography or ion exchange chromatography. The characterisation and the elucidation of the compounds were carried out by elemental analysis, melting point- and optical rotation determination, and by one and two-dimensional (1H-1H-COSY, 13C-1H-HSQC, TOCSY, ROESY) NMR spectroscopy and MALDI/ESI-TOF mass spectrometric methods, respectively.

3. The new scientific results of the dissertation

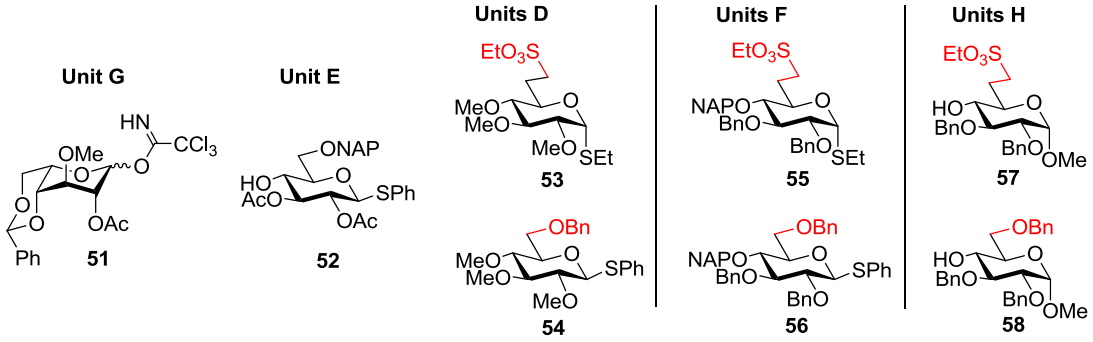
3.1. Elaboration of modular synthetic route

We elaborated a modular synthetic pathway to obtain the new pentasaccharides **6**-**10** containing 6-sulfonic acid moieties (**Scheme 3.**). Based on the retrosynthetic analysis the target molecules can be disconnected to two **DE** dissacharide donor and four **FGH** trisaccharide acceptor blocks, which can be built up from only eight monosaccharide units (**51**-**58**).



Scheme 3. Retrosynthetic analysis of the series of pentasaccharide 6-sulfonic acids

Thus we synthesized all of the monosaccharide units in sufficient amounts for the preparation of the targeted pentasaccharide series (**Scheme 4.**).

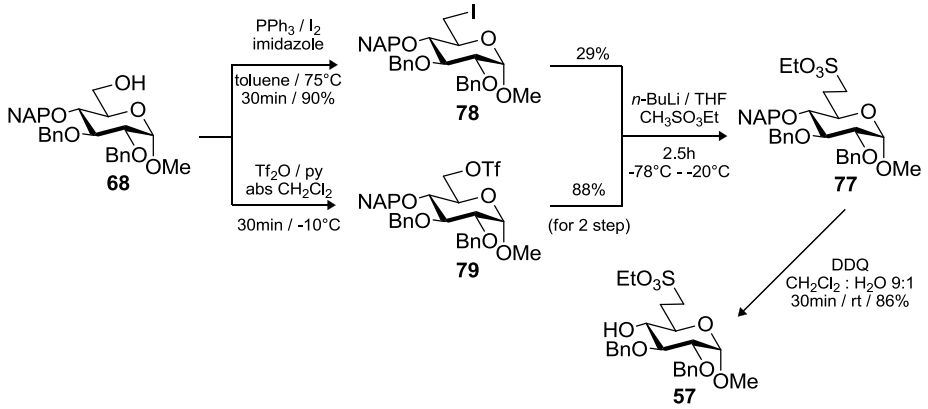


Scheme 4. Monosaccharide building blocks

3.2. Synthesis of monosaccharides containing a sulfonic acid

3.2.1. Two synthetic routes for unit H

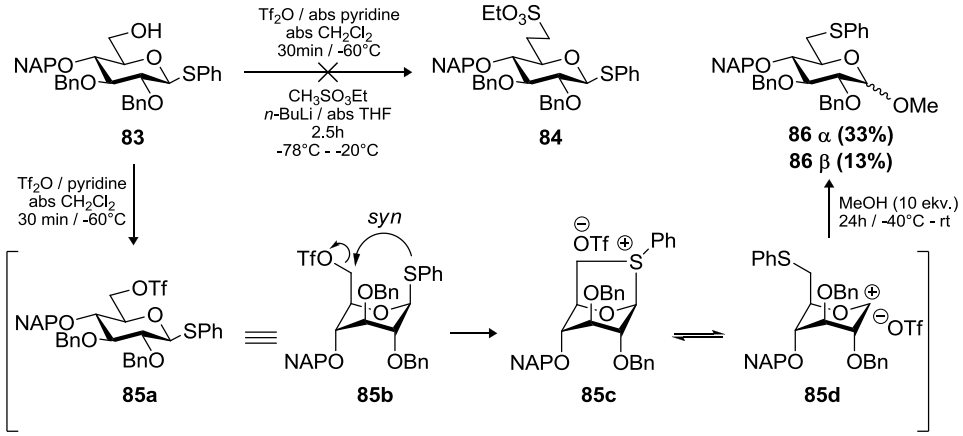
For large scale synthesis of monosaccharide sulfonic acids (**53**, **55** and **57**), an effective method was developed including nucleophilic substitution of the corresponding glucoside-6-*O*-triflate with lithiated ethyl methanesulfonate. This reaction was excellent for the synthesis of *O*-glucoside derivative (**Scheme 5.**) and we produced unit **H** (**57**) in almost 10 grams. The synthesis of the target molecule **57** by using ioidine as a leaving group (**78**) was also attempted. Although the synthesis was sucsessful, the efficacy of this route was low.



Scheme 5. Synthesis of unit H containing a sulfonic acid moiety

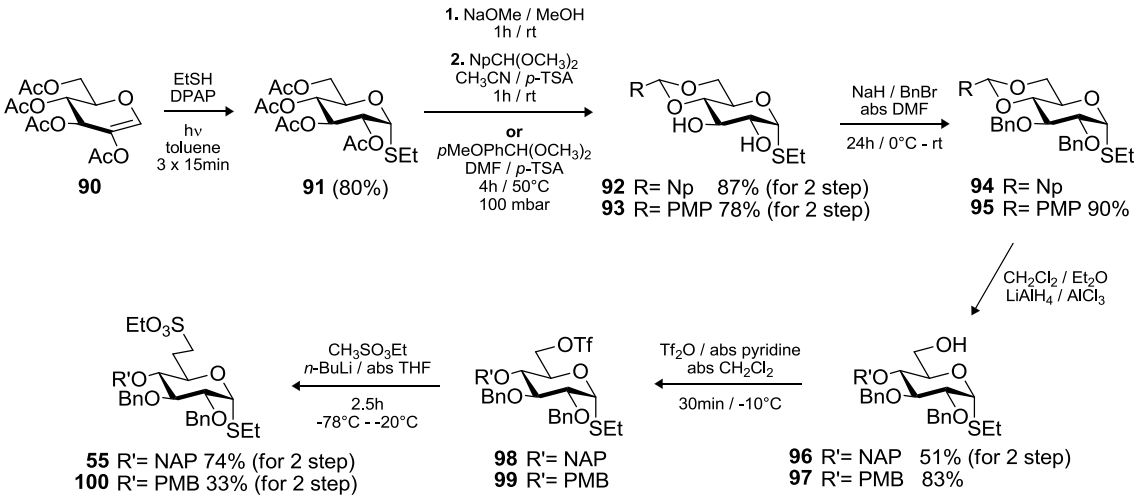
3.2.2. Synthesis of unit F by using two temporary protecting groups

For the synthesis of glycosyl donor **F** containing a sulfonatomethyl group (**84**), we used β-thioglucoside derivative **83** as the starting molecule (**Scheme 6.**). However the product (**84**) was not formed. Because of an undesired 1→6 thio-migration was able took place, due to an intramolecular substitution reaction. We elaborated two methodes to avoid the intramolecular side-reaction of the anomeric thio group. In one case, we locked the $^4C_1$ conformation of the sugar ring to prevent the conformational change. Alternatively the corresponding α-thioglycoside (**55**), was used for synthesis of the end-product.



Scheme 6. 1→6 Thio-migration through a sulfonium ion intermedier

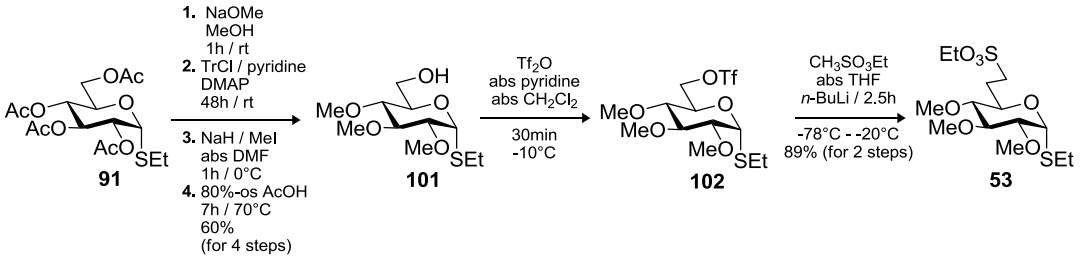
Starting from α-thioglucoside derivative **91** we could synthesize the unit **F** with high yield using 2-NAP as the temporary protecting group. Two temporary protecting groups were tried in the reaction route (**Scheme 7.**).



Scheme 7. Formation of unit F containing a sulfonic acid moiety from α-thioglucoside by using either Np- or PMP-acetal protection

3.2.2. Synthesis of unit D

The synthesis of monosaccharide sulfonic acid unit **D** was carried out also from the starting α-thioglucoside derivative (**91**). We prepared the target molecule (**53**) with an excellent yield and nearly in 10 grams (**Scheme 8.**).

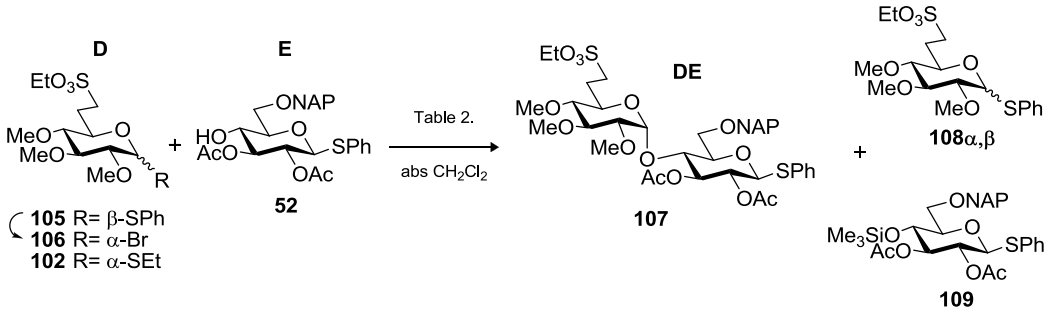


Scheme 8. Formation of unit D containing 6-sulfonatomethyl group

Building blocks (**51**, **52**, **54**, **56**, **58**), which do not contain the sulfonatomethyl group, were prepared according to the literature methods.

3.3. Large scale synthesis of DE building blocks, optimization of the glycosylation reaction

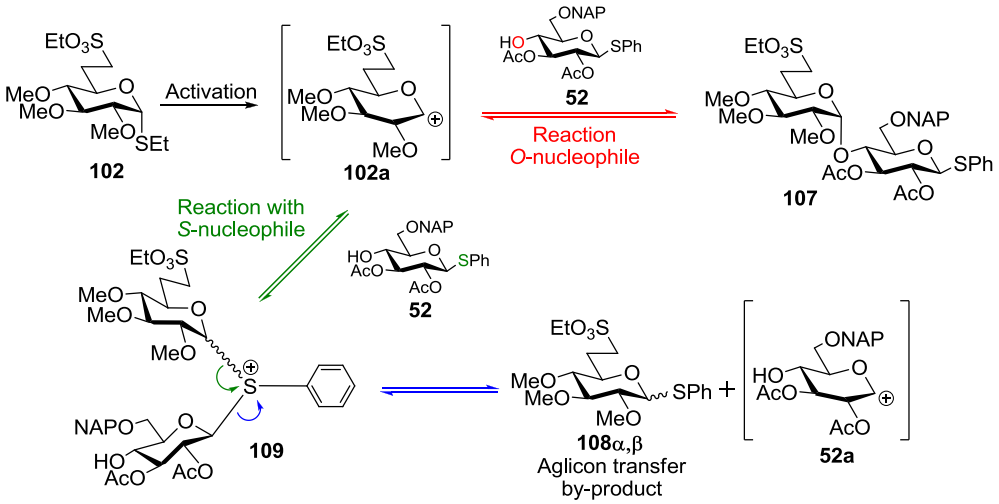
The synthesis of **DE** disaccharide containing sulfonic acid (**107**) was completed in a large scale in a chemo- and stereoselective way (**Scheme 9.**). A wide variety of glycosyl donors (**102**, **105**, **106**) and promoters were tested in the glycosylation of units **D** and **E**.



	Donor	Promoter (ekv.)	T (°C)	Time	Yield (39)	by-products
1	β-SPh (**105**)	NIS (1.1) – AgOTf (0.2)	−75 - −15	1.5h	11	**108α,β** (9%)
2	α-Br (**106**)	AgOTf (1.5)	−30	1.5h	21	**108α,β** (6%)
3	α-SEt (**102**)	NIS (1.5) – TfOH (0.1)	−75 - −50	3h	31	degradation products
4	α-SEt (**102**)	NIS (1.5) – TMSOTf (0.2)	−75 - −55	2h	44	**109** (8%)
5	α-SEt (**102**)	NIS (1.1) – AgOTf (0.2)	−75 - −55	45min	66	
6	α-SEt (**102**)	NIS (1.1) – AgOTf (0.2)	−75 - −65	45min	89	

Scheme 9. Glycosylation reactions for synthesis of disaccharide donor DE containing a sulfonatomethyl group

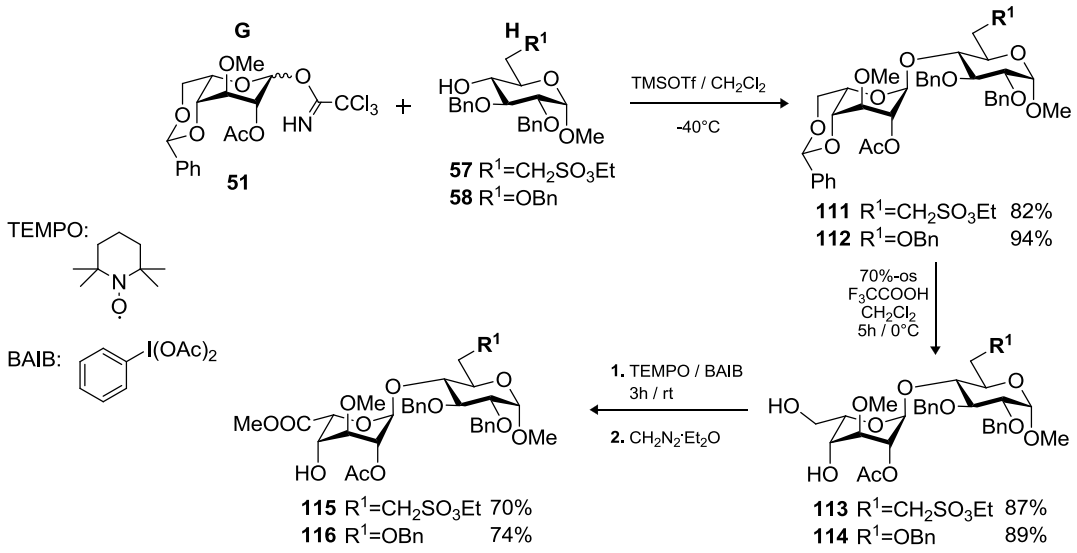
Formation of an aglycon transfer side product (**108α,β**) was observed when glycosyl bromide (**106**) and β-thioglycoside (**105**) were used as the donors **(Scheme 10.**). This competitive side reaction could be avoided with the use of α-thioglycoside at a low temperature and a short reaction time.



Scheme 10. Mechanism of glycosyl transfer side reaction during synthesis of disaccharide donor DE

Based on our previous experiments, the suitable **DE** disaccharide was prepared in appropriate amount for the pentasaccharides which do not contain sulfonatomethyl moiety on the unit **D**.

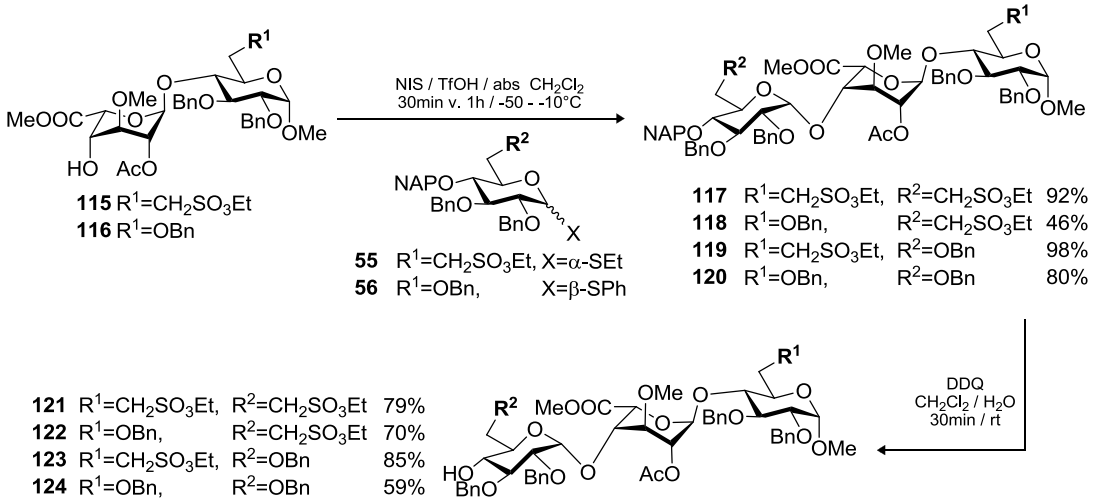
3.4. Synthesis of GH building blocks



Scheme 11. Preparation of the GH buliding blocks

We carried out the formation of two diaccharide acceptors **GH** (**111**, **112**) by the glycosylation of units **H** (**57**, **58**) with the L-idose derivative (**51**), and subsequent formation of the L-iduronic acid moiety at a disaccharide level (**Scheme 11.**).

3.5. Synthesis of FGH trisaccharide acceptors

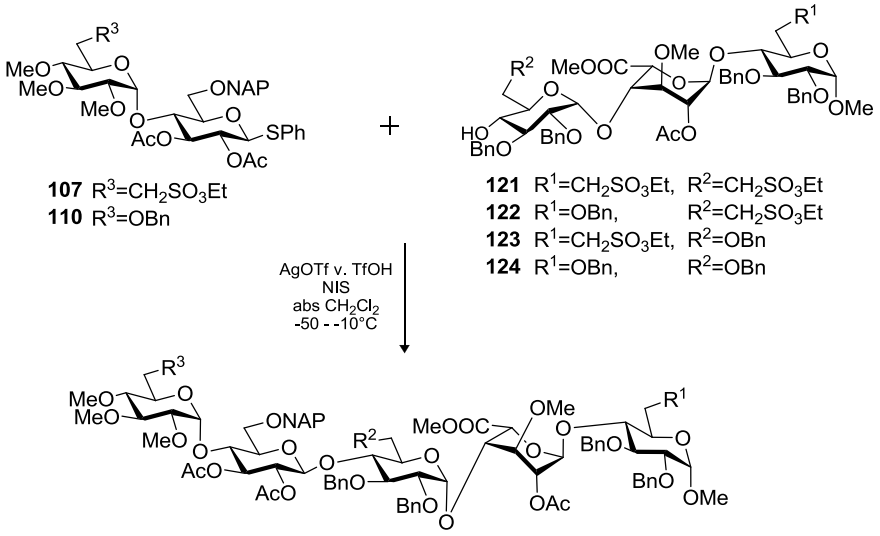


Scheme 12. Preparation of the FGH building blocks

FGH trisaccharide acceptor precursors (**117-120**) were prepared by condensation of **GH** disaccharide acceptors (**115**, **116**) and **F** monosaccharide donors (**55**, **56**) respectively. In all cases the promoter was TfOH-NIS system, and except for synthesis of **118**, the coupling reactions took place with good yields. We could increase the yield of derivative **118** to 73% by using AgOTf-NIS as the promoter. **FGH** trisaccharide acceptors were achieved by removal of the 2-naphtylmethyl group from the position 4 of unit **F** (**Scheme 12.**).

3.6. Synthesis of new pentasaccharides containing sulfonic acid moieties

We synthesized the target pentasaccharides (**46**-**50**) and the pentasaccharide trisulfonic acids (**125**) in protected form by the glycosylation of the **FGH** trisaccharide acceptors (**121-124**) with **DE** disaccharide donors (**107**, **110**) respectively. All glycosylation reactions took place in a stereoselective way and we obtained the products in good yields (**Scheme 13**).



107 R^3=CH_2SO_3Et
110 R^3=OBn

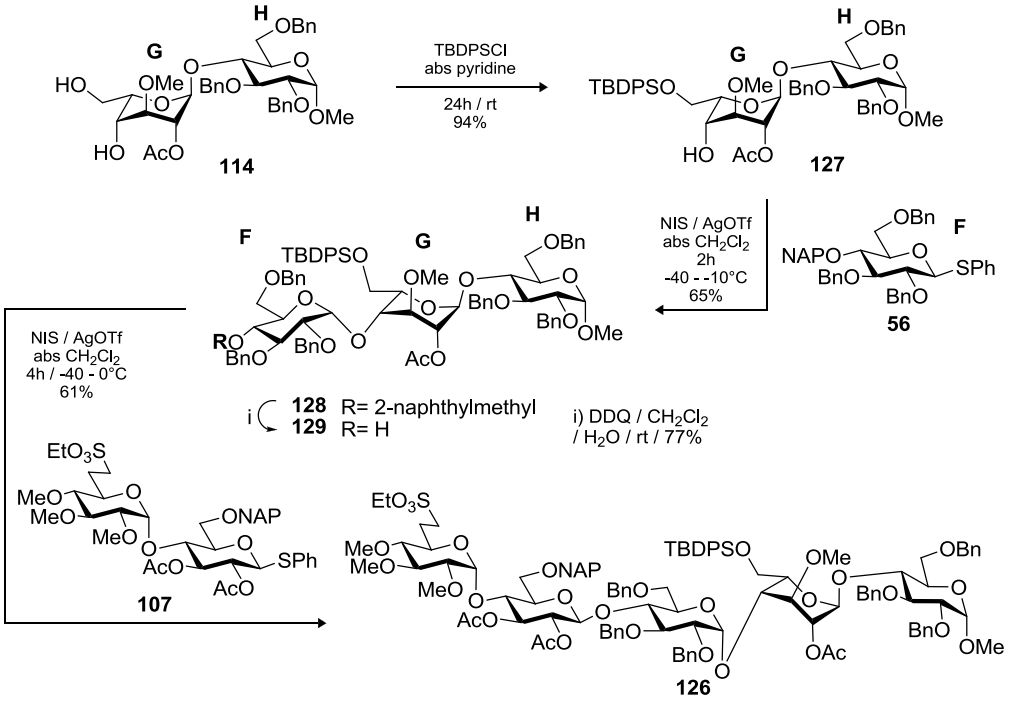
121 R^1=CH_2SO_3Et, R^2=CH_2SO_3Et
122 R^1=OBn, R^2=CH_2SO_3Et
123 R^1=CH_2SO_3Et, R^2=OBn
124 R^1=OBn, R^2=OBn

125	R^1=CH_2SO_3Et,	R^2=CH_2SO_3Et,	R^3=CH_2SO_3Et	65%
46	R^1=CH_2SO_3Et,	R^2=OBn,	R^3=CH_2SO_3Et	63%
47	R^1=CH_2SO_3Et,	R^2=OBn,	R^3=OBn	72%
48	R^1=OBn,	R^2=CH_2SO_3Et,	R^3=CH_2SO_3Et	59%
49	R^1=OBn,	R^2=CH_2SO_3Et,	R^3=OBn	63%
50	R^1=OBn,	R^2=OBn,	R^3=CH_2SO_3Et	68%

Scheme 13. Synthesis of the new pentasaccharide derivatives

3.7. Elaboration of a new synthetic route: synthesis of a L-idose-containing pentasaccharide

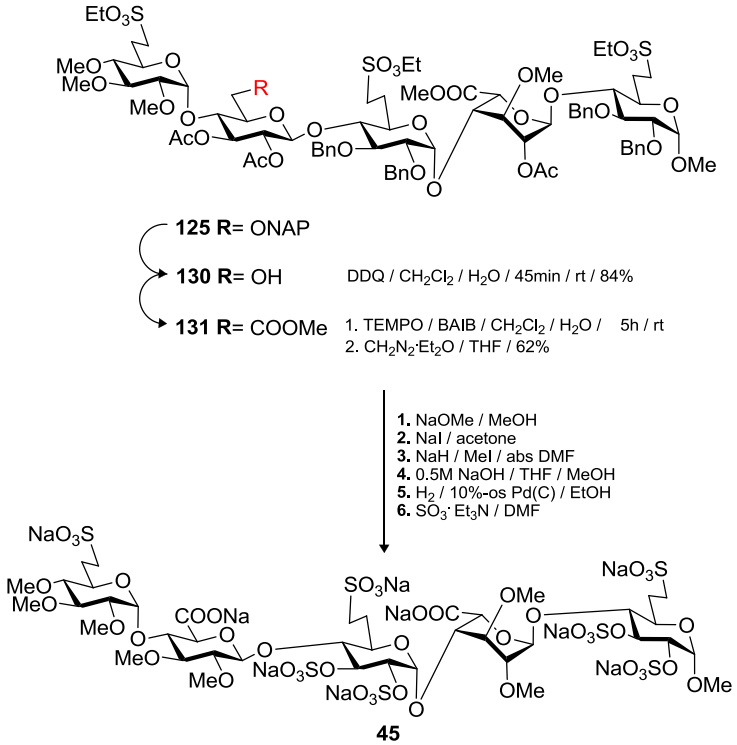
Furthermore, we carried out the synthesis of a sixth pentasaccharide derivative (**126**) as well, which is the non-oxidised analouge of compound **50** containing the D-glucuronic and L-iduronic acids in their precursor forms (**Scheme 14**). Therefore it can be suitable for the investigation of simultaneous oxidation of both units at a pentasaccharide level. For the preparation of pentasaccharide (**126**), the 6-hydroxyl group of the **GH** disaccharide was protected with terc-butyl-diphenylsilyl ether group instead of oxidation. Then the **GH** disaccharide acceptor (**127**) was glycosylated with **F** monosaccharide donor (**56**). After the of the 2-naphtylmethy protecting group, the **FGH** trisaccharide acceptor was glycosylated with the **DE** disaccharide donor containing a sulfonatomethyl moiety (**107**).



Scheme 14. Preparation of pentasaccharide 126 containing non-oxidised precursors of the uronic acid units

3.8. New synthesis of pentasaccharide trisulfonic acid 45 and antithrombin-carbohydrate interaction studies

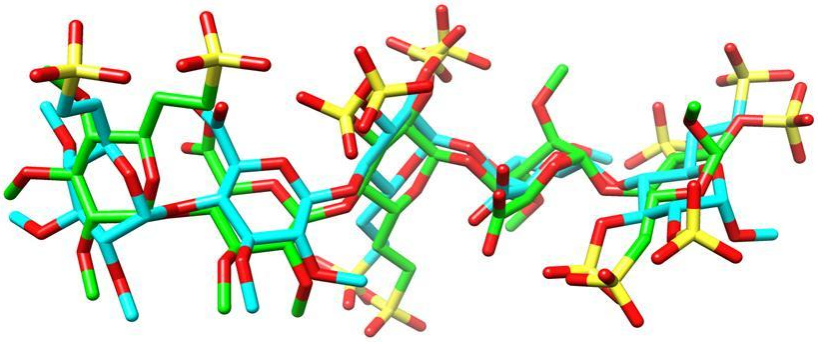
Second part of my work was the transformation of the pentasaccharide trisulfonic acid (**125**) into the trisulfonic acid end-product (**45**) in eight steps, in 100 mg scale (**Scheme 15**).



Scheme 15. Modification of pentasaccharide 125 to the corresponding target molecule (45)

This compound was utilized by our co-workers for antithrombin-carbohydrate interaction studies. Three-dimensional structures of the free and AT-binded forms of the trisulfonate analogue were

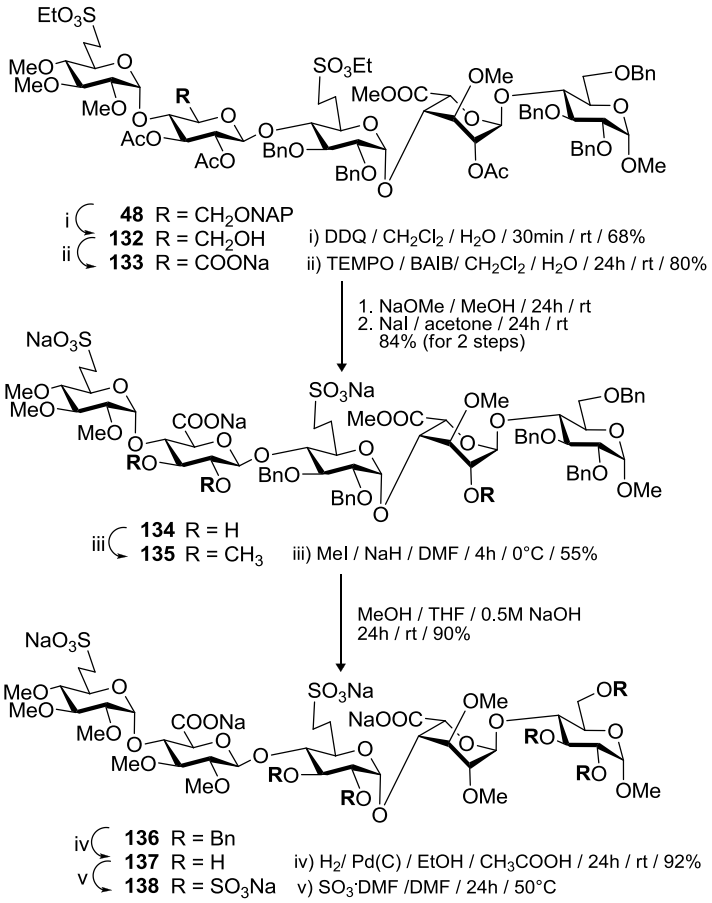
determined by using different NMR spectroscopic data and dynamics simulations (**Scheme 16.**). A significant difference in the structure and the conformational flexibility of the idraparinux and its analogue was observed. There is also a notable difference in the 3D structures of the free and bound form of the trisulfonic analogue, revealing that a change in the conformation is required for the activation of antithrombin.



Scheme 16. Molecular model of free (green) and AT-bound (blue) forms of pentasaccharide trisulfonic acid (45)

3.9. Synthesis of the new idraparinux analogue

As a contiunation of my PhD work, I plan to convert the protected pentasaccharides into the corresponding idraparinux-analogue end-products. We have already started the modification of pentasaccharide **48** which contains sulfonic acid moieties on units **D** and **F** (**Scheme 17**).



Scheme 17. Modification of the protected pentasaccharide disulfonic acid (48) to the corresponding end-product

Registry number: DEENK/178/2015.PL
Subject: Ph.D. List of Publications

Candidate: Erika Mező
Neptun ID: ZSN98G
Doctoral School: Doctoral School of Chemistry

List of publications related to the dissertation

Foreign language scientific article(s) in international journal(s) (4)

1. **Mező, E.**, Herczeg, M., Eszenyi, D., Borbás, A.: Large-scale synthesis of 6-deoxy-6-sulfonatomethyl glycosides and their application for novel synthesis of a heparinoid pentasaccharide trisulfonic acid of anticoagulant activity.
Carbohydr. Res. *388*, 19-29, 2014. ISSN: 0008-6215.
DOI: http://dx.doi.org/10.1016/j.carres.2014.02.012
IF:1.929

2. Herczeg, M., **Mező, E.**, Eszenyi, D., Antus, S., Borbás, A.: New synthesis of idraparinux, the non-glycosaminoglycan analogue of the antithrombin-binding domain of heparin.
Tetrahedron. *70* (18), 2919-2927, 2014. ISSN: 0040-4020.
DOI: http://dx.doi.org/10.1016/j.tet.2014.03.033
IF:2.641

3. Herczeg, M., **Mező, E.**, Eszenyi, D., Lázár, L., Csávás, M., Bereczki, I., Antus, S., Borbás, A.: Synthesis of 6-Sulfonatomethyl Thioglycosides by Nucleophilic Substitution: Methods to Prevent 1 6 Anomeric Group Migration of Thioglycoside 6-O-Triflates.
Eur. J. Org. Chem. *2013* (25), 5570-5573, 2013. ISSN: 1434-193X.
DOI: http://dx.doi.org/10.1002/ejoc.201300681
IF:3.154

4. Herczeg, M., **Mező, E.**, Lázár, L., Fekete, A., Kövér, K.E., Antus, S., Borbás, A.: Novel syntheses of Idraparinux, the anticoagulant pentasaccharide with indirect selective factor Xa inhibitory activity.
Tetrahedron. *69* (15), 3149-3158, 2013. ISSN: 0040-4020.
DOI: http://dx.doi.org/10.1016/j.tet.2013.02.076
IF:2.817

List of other publications

Hungarian scientific article(s) in Hungarian journal(s) (1)

5. **Mező E.**, Herczeg M., Eszenyi D., Antus S., Borbás A.: Antikoaguláns hatású pentaszacharidszulfonsav sorozat moduláris szintézise: Problémák és megoldások. *Magyar Kém. L. 69* (6), 184-187, 2014. ISSN: 0025-0163.

Foreign language scientific article(s) in international journal(s) (1)

6. Lázár, L., **Mező, E.**, Herczeg, M., Lipták, A., Antus, S., Borbás, A.: Synthesis of the non-reducing end trisaccharide of the antithrombin-binding domain of heparin and its bioisosteric sulfonic acid analogues.
Tetrahedron. 68 (36), 7386-7399, 2012. ISSN: 0040-4020.
DOI: http://dx.doi.org/10.1016/j.tet.2012.06.081
IF:2.803

Total IF of journals (all publications): 13,344
Total IF of journals (publications related to the dissertation): 10,541

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

07 September, 2015

Conference Participations

Oral presentations

1.) Borbás. A., Herczeg. M., Lázár. L., Mező. E., Bereczky. Zs., Lipták. A., Antus S.: **Sulfonic acid analogues of the antithrombin-binding domain of Heparin**. *4th European Conference on Chemistry for Life Sciences. Budapest. Hungary. August 31.- September 3. 2011*

2.) Mező E., Lázár L., Herczeg M., Borbás A., Lipták A., Antus S.: **Szulfonsav-tartalmú heparinoid triszacharid szintézise.** *XXXIV. Kémiai Előadói Napok. Szeged. 2011.11.02.-04. (konferencia kiadvány 166. oldal)*

3.) Herczeg M., Mező E., Lázár L., Borbás A., Antus S.: **Szulfonátometil-csoportot tartalmazó heparin analóg oligoszacharidok szintézise és biológiai vizsgálata.** *Molekulatudomány. Egészség- és környezettudomány alprojektek előadói ülése. Debrecen. 2012. 04. 19.*

4.) Herczeg M., Mező E., Lázár L., Borbás A., Antus S.: **Antitrombotikus hatású szulfonátometil-csoportot tartalmazó heparin analóg pentaszacharidok szintézise.** *Bruckner-termi előadások. Budapest. 2012. 04. 27.*

5.) Mező E., Herczeg M., Lázár L., Antus S., Borbás A.: **Szulfonátometil-csoportot tartalmazó építőelemek szintézise heparinoid oligoszacharidokhoz.** *MTA Szénhidrát-. Antibiotikum-. és Nukleotidkémiai Munkabizottsági ülés. Debrecen. 2012. 05. 31.-06. 01.*

6.) Herczeg M., Mező E., Lázár L., Antus S., Borbás A.: **Az antitrombotikus hatású idraparinux szulfonsav-tartalmú analogonjainak újabb szintézise.** *MTA Szénhidrát-. Antibiotikum-. és Nukleotidkémiai Munkabizottsági ülés. Debrecen. 2012. 05. 31.-06. 01.*

7.) E. Mező., M. Herczeg., D. Eszenyi., L. Lázár., I. Bereczki., A. Borbás: **Synthesis of 6-sulfonatomethyl thioglycosides by nucleophilic substitution and their application in the synthesis of heparinoid trisaccharides.** *Working Committee for Carbohydrates. Nucleic Acids and Antibiotics. Mátrafüred. May 22–24. 2013*

8.) M. Herczeg., E. Mező., D. Eszenyi., L. Lázár., S. Antus., A. Borbás: **Synthesis of new 6-sulfonic-acid-containing analogues of Idraparinux.** *Working Committee for Carbohydrates. Nucleic Acids and Antibiotics. Mátrafüred. May 22–24. 2013*

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10.) Herczeg M., Mező E., Eszenyi D., Lázár L., Borbás A., Antus S.: **Antitrombotikus hatású heparin-analóg pentaszacharid-szulfonsavak szintézise.** *Vegyészkonferencia. Hajdúszoboszló. 2013. 06. 26-28.*

11.) Mező E., Herczeg M., Eszenyi D., Borbás A.: **Újabb eredmények a szulfonsav-tartalmú heparinoid pentaszacharidok szintézisének terén.** *XXXVI. Kémiai Előadói Napok. Szeged. 2013.10.28.-30. (konferencia kiadvány 362. oldal*)

12.) Mező E., Herczeg M., Eszenyi D., Antus S., Borbás A.: **Antikoaguláns hatású pentaszacharid-szulfonsav-sorozat moduláris szintézise. Problémák és megoldások.** *Bruckner-termi előadások. Budapest. 2013. 11. 29.*

13.) D. Eszenyi., M. Herczeg., E. Mező., A. Borbás: **Toward synthesis of a C-2 sulfonatomethyl group containing anticoagulant pentasaccharide.** *Working Committee for Carbohydrates. Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences. Mátrafüred. May 21–23. 2014*

Posters

1.) E. Mező., L. Lázár., M. Herczeg., A. Borbás., A. Lipták., S. Antus: **Synthesis of Bioisoteric Sulfonic Acid Analogues of the Nonreducing-end Trisaccharide of the Antithrombin-binding Domain of Heparin.** *4th European Conference on Chemistry for Life Sciences. Budapest. Hungary. August 31.- September 3. 2011*

2.) Mező. E., Herczeg. M., Eszenyi. D., Borbás A.: **Building blocks for heparinoid pentasaccharide sulfonic acids of anticoagulant activity.** *5th European Conference on Chemistry for Life Sciences. Barcelona. Spain. Juny 9.-12. 2013*

3.) Mező. E., Herczeg. M., Eszenyi. D., Lázár. L., Bereczki. I., Borbás A.: **Synthesis of 6-sulfonatomethyl thioglycosides by nucleophilic substitution and their application in the synthesis of heparinoid trisaccharides.** *5th European Conference on Chemistry for Life Sciences. Barcelona. Spain. Juny 9.-12. 2013*

4.) Eszenyi. D., Herczeg. M., Mező. E., Borbás A.: **Toward synthesis of a C-2 sulfonatomethyl group containing anticoagulant pentasaccaride.** *13th Bratislava Symposium on Saccharides „Recent Advances in Glycomics". Smolenice. Slovakia. June 22-26. 2014*

5.) D. Eszenyi, M. Herczeg, E. Mező, Borbás A.: **Toward synthesis of an idraparinux analogue bearing a secondary sulfonatomethyl moiety.** *18th European Carbohydrate Symposium. Moskow, Russia, August 2-6. 2015*

Other oral presentations

1.) Csávás M., Lázár L., Hadházi Á., Demeter T., Nábrádi P., Herczeg M., Eszenyi D., Mező E., Herczegh P., Borbás A.: **Tio-diszacharidok és glikokonjugátumok szintézise tio-click módszerrel**. *Bruckner-termi előadások. Budapest. 2013. 05. 31.*

2.) M. Herczeg., E. Mező., F. Demeter., R. Pataki., A. Borbás**: Simultaneous application of 1.3- and 1.4-dioxane acetal groups for protection of hexopyranosides. Synthesis and chemoselective ring opening reactions**. *Working Committee for Carbohydrates. Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences. Mátrafüred. May 21–23. 2014*

Other poster

3.) E. Mező., M. Herczeg., F. Demeter., R. Pataki., A. Borbás: **Simultaneous application of two different dioxane-acetal groups for protection of hexopyranosides**. *13th Bratislava Symposium on Saccharides „Recent Advances in Glycomics". Smolenice. Slovakia. June 22-26. 2014*