Theses of doctoral (PhD) dissertation

Synthesis of sulfur-containing carbohydrate mimetics

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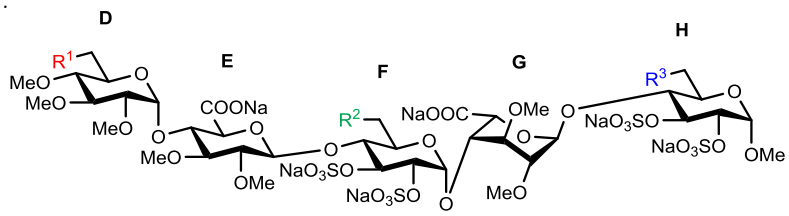
1 Introduction

Heparin and its derivatives are used for decades to treat and prevent thrombosis due to the excellent antithrombotic activity. The heparinoid anticoagulants exert their activity by binding to and activation of antithrombin. In this process there is a key role of the ionic bonds formed between the sulfate esters of heparin and the lysine and arginine units of antithrombin. Our research group synthesizes heparinoid antithrombotic sulfonic acids for years.



idraparinux (**1**): $R^1 = R^2 = R^3 = OSO_3Na$
pentasaccharide-disulfonic acid (**2**): $R^1 = OSO_3Na$; $R^2 = R^3 = CH_2SO_3Na$
pentasaccharide-trisulfonic acid (**3**): $R^1 = R^2 = R^3 = CH_2SO_3Na$

	Anti-Xa activity (U/mg)
idraparinux (1)	1911 ± 193
pentasaccharide disulfonic acid (2)	2153 ± 153
pentasaccharide trisulfonic acid (3)	384 ± 139

Table 1. Antithrombotic effect of the previously synthesized (**2**,**3**) sulfonic acids

We have prepared idraparinux (**1**) derivatives on which the pharmacophore sulfate esters are systematically changed to sulfonic acids. Our previous work showed that this change can increase or decrease the anticoagulant activity, depending on the position (Table 1). Based on the above results we aimed to prepare isomers of **2**, in which secondary sulfonatomethyl groups are present on unit **H** and **F** at position *C*-2 or *C*-3. Because there is not any general method in the literature for the synthesis of sulfonic acid-containing building blocks, first we wished to develop a synthetic method for the preparation of secondary sulfonatomethyl derivatives of *O*- and *S*-glycosides.

During the synthesis of sulfonic acid containing unit **F**, we applied a free radical hydrothiolation on 2-acetoxyglycal. When reviewing the literature, we found that the hydrothiolation of unsaturated carbohydrates is an unexploited area of chemistry, so we aimed to synthesize stable disaccharide mimetics by free radical thiol-ene reaction. In the frame of this research we try to discover the scope and limitations of the reaction both on *endo*- and *exo*-glycals.

2 New scientific results of the dissertation

2.1 Formation of a secondary sulfonatomethyl group on O- and S-glycosides

Previously, sulfonic acid formation was carried out by *tert*-butyl-perbenzoate catalyzed free radical addition of $NaHSO_3$ to a carbohydrate *exo*-methylene derivative. The process is lengthy and cannot be applied on thioglycosides due to the oxidability of the sulfur atom. My goal was to develop a method for sulfonic acid formation, that can be used on thioglycosides. After reviewing the literature, we found that Horner-Wadsworth-Emmons (HWE) reaction followed by reduction is suitable for this purpose. First, we wished to optimize the reaction conditions on an *O*-glycoside, and apply the optimized conditions on *S*-glycosides.

2.1.1 Optimization of the HWE-reaction on an *O*-glycoside

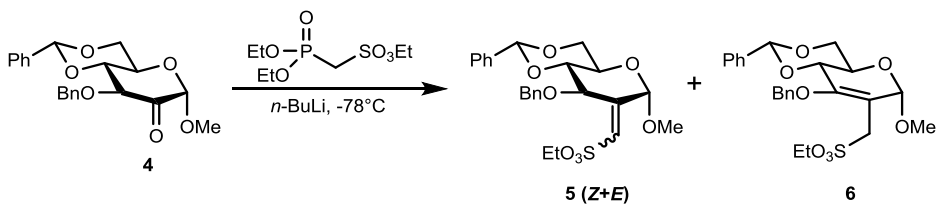
- For the experiments we synthesized the protected 2-ulose derivative **4** and the phosphonate derivative required for HWE-reaction. After that we formed an ethyl-sulfonatomethylene group at *C*-2 position on **4** by HWE-reaction (Scheme 1).

- We optimized the reaction conditions (reaction time, temperature, solvent), results are summarized in Table 2.

- Applying higher temperature and longer reaction time led to isomerization of **5** into undesired endocyclic derivative **6**. According to DFT calculations, isomerization is favoured because of greater stability of **6**.

- Configuration of compounds **5(*E*)** and **5(*Z*)** was determined by ROESY NMR experiments. *Z* configuration of **5(*Z*)** can be concluded from the cross-peak of the sulfonatomethylene proton and H-3 on the ROESY spectrum.

- The undesired isomerization of **5** can be avoided by controlling the reaction time and temperature. Best yield was obtained in tetrahydrofurane with reaction time of 6 hours (Table 2, Entry c).



Scheme 1. Synthesis of unit **H** I

Entry	Solvent	T /°C	Reaction time	Yield /%		
				5 (*Z*)	**5 (*E*)**	**6**
(**a**)	THF	−78 → −15	4 h	52	15	-
(**b**)	THF	−78 → rt	16 h	46	7	25
(**c**)	THF	−78 → +10	6 h	61	17	-
(**d**)	Et_2O	−78 → rt	6 h	10	6	2
(**e**)	Bu_2O	−78 → rt	6 h	11	3	4
(**f**)	*t*-BuOMe	−78 → rt	6 h	23	16	6

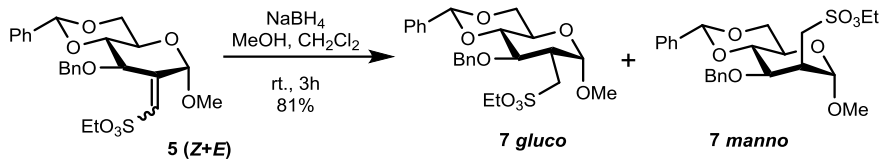
Table 2. WHE-reaction of **4** under different conditions

- We studied the reduction of **5(*Z*)** and **5(*E*)** (Scheme 2). We found that the ratio of products **7 *gluco*** and **7 *manno*** significantly depends on neither the method of reduction nor the configuration of the alkene (Table 3).

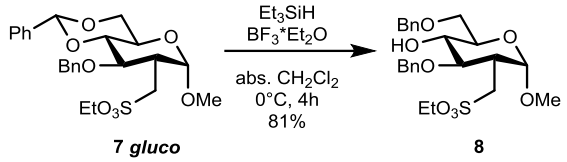
Compound	Reagent	Conditions	Ratio of products %	
			7 *gluco*	**7 *manno***
5(*Z*)	Pd(0)/C, H_2	CH_2Cl_2, rt. 2h	95	5
5(*E*)	Pd(0)/C, H_2	CH_2Cl_2, rt. 2h	89	11
5(*Z*)	$NaBH_4$	MeOH, rt. 3h	87	13

Table 3. Results of the reduction of compound **5**

- When hydrogenating **5** in large scale $NaBH_4$ proved to be more efficient than catalytic hydrogenation.



Scheme 2. Reduction of **5**



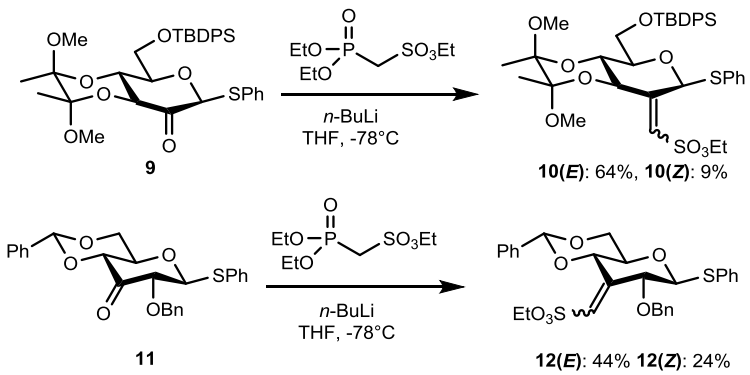
Scheme 3. Synthesis of unit **H**

- After hydrogenation, by the ring-opening reaction of **7** ***gluco*** we synthesized the secondary sulfonatomethyl-bearing glycosyl acceptor building block **8** (Scheme 3).

2.1.2 Formation of secondary sulfonatomethyl group on thioglycosides

- We were the first, who performed HWE-reaction at secondary positions of a thioglycoside.

- We have found that on compounds **9** and **11** sulfonatomethylene group can be formed in *C*-2 and *C*-3 positions with good yields, using the optimized conditions (Scheme 4).



Scheme 4. HWE reaction at secondary positions of thioglycosides

- We extensively studied the reduction of **10(*E*)** and **12(*E*)**. Catalytic hydrogenation of **10(*E*)** and **12(*E*)** did not result in the desired *gluco* product. Typically *exo-endo* isomerisation and/or desulfuration occured (Table 4).

- Reduction of **10** with sodium-borohydride resulted in the *manno* configured **13** with medium yield. It is the first secondary sulfonic acid-containing thioglycoside in the literature. This can be used as a building block to synthesize a sulfonic acid analogue of PI-88 antimetastatic agent.

- Reduction of **12(*E*)** with sodium-borohydride was successfull, however the 4:1 mixture of *allo* and *gluco* products were nearly inseparable with column chromatography, only a small amount of **19** could be isolated (Table 4 / Entry e).

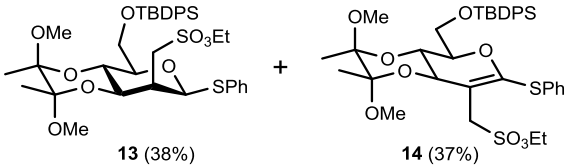
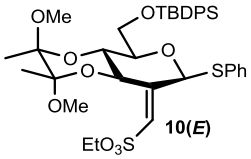
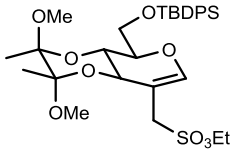
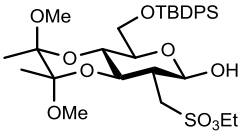
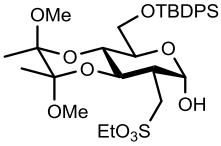
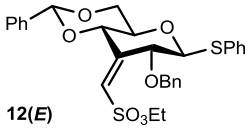
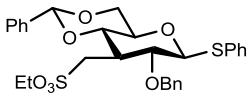
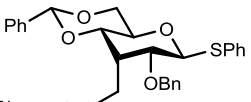
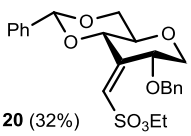
Starting material	Conditions	Product(s)
	(**a**) $NaBH_4$, MeOH, rt., 3h	 **13** (38%) + **14** (37%)
 **10(*E*)**	(**b**) Pd(0)C, 10 bar H_2, rt. 60h (**c**) Raney-Ni, H_2, 16h	 **15** (b: 35%; c: 78%)
	(**d**) Pd(0)C, 10 eq. Et_3SiH, rt., 1h	**15** (24%) +  **16** (10%) +  **17** (12%)
 **12(*E*)**	(**e**) $NaBH_4$, MeOH, rt., 3h	 **18** +  **19** összesen: 69% **18**:**19** = ~1:4
	(**f**) Raney-Ni, H_2, 16h	 **20** (32%)

Table 4. Reduction of **10(*E*)** and **12(*E*)** under different conditions

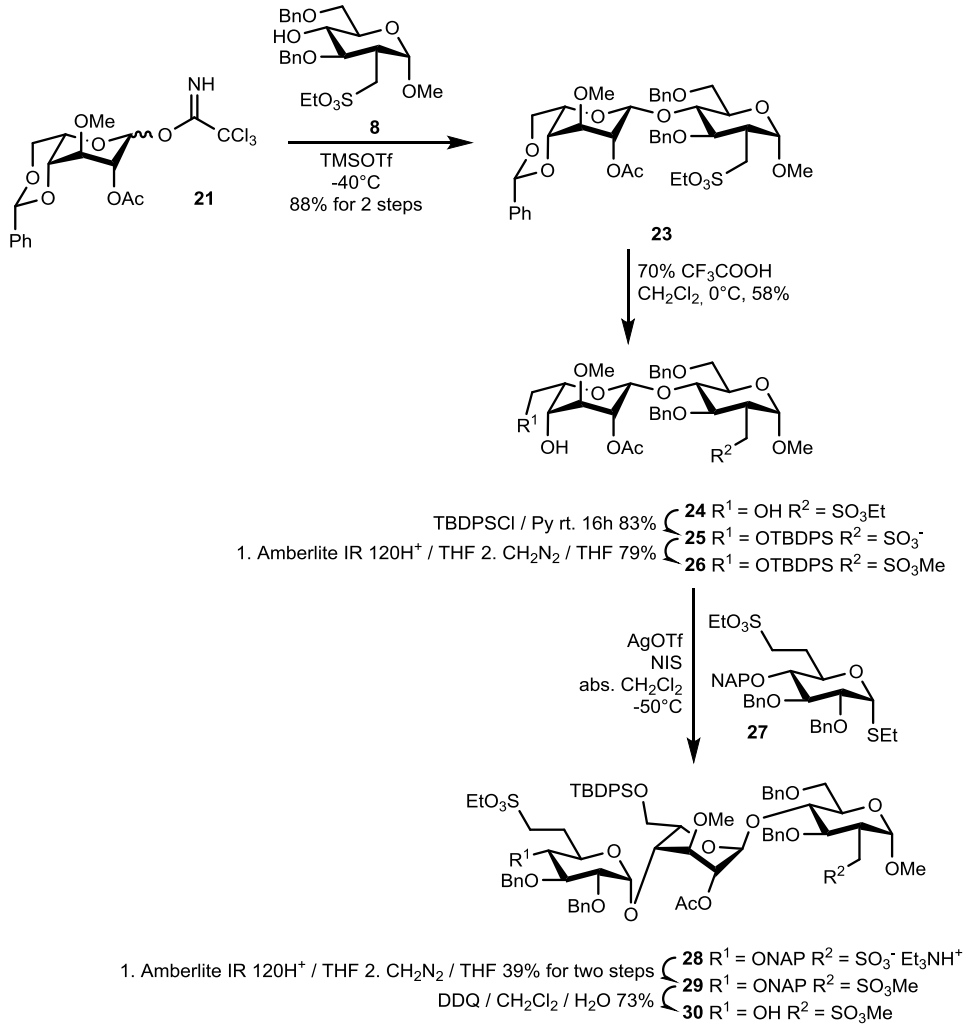
- We found that HWE reaction followed by reduction with $NaBH_4$ is a convenient method for the synthesis of thioglycoside bearing a secondary sulfonatomethyl group.

- With β-anomeric configuration and/or with the protecting groups applied, upon reduction, the formation of an axial sulfonatomethyl group is favoured.

2.2 Synthesis of a secondary sulfonic acid-containing idraparinux-analogue pentasaccharide

- We planned the synthesis of a novel idraparinux-analogue pentasaccharide disulfonic acid, using building block **8**. As a new characteristic of the synthesis , we wished to form the D-glucuronic- an L-iduronic acid moieties on pentasaccharide level with the simultaneous oxidation of D-glucose and L-idose precursors.

- According to [2+3] (**DE**+**FGH**) block synthetic plan we synthesized the **GH** disaccharide acceptor (**26**), which was glycosylated with donor **27**. The fully protected derivative **29** was converted to **30** by removing the 4''-*O*-NAP group (Scheme 5).

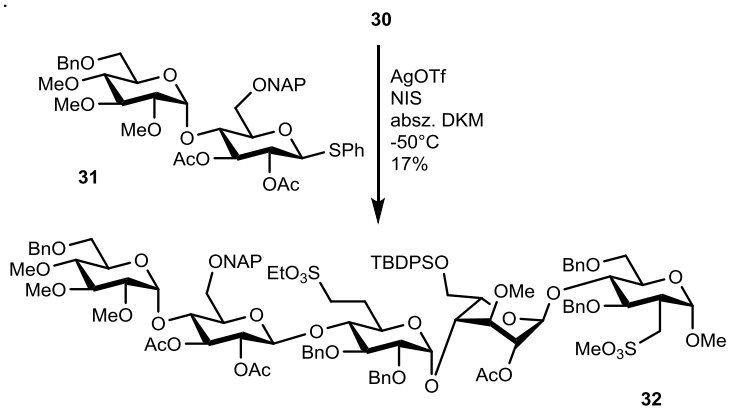


Scheme 5. Synthesis of the protected **FGH** trisaccharide

- During the synthesis, *C*-2 sulfonic acid ester was cleaved two times, lengthening the synthetic path with two steps and decreasing the yield.

- With the reaction of glycosyl donor **31** and glycosyl acceptor **30** we synthesized the protected secunder sulfonic acid-bearing pentasaccharide (Scheme 6).

- The overall yield of the synthesis was 1.6%. To continue our work, we need to synthesize **32** in a more efficient way.



Scheme 6. Synthesis of the protected pentasaccharide

2.3 Photoinitiated free radical hydrothiolation

2.3.1 Thioladdition onto *exo*-methylene derivatives

- We studied the free radical hydrothiolation on pyranose *C*-2 and *C*-4 *exo*-methylene derivatives.
- Addition of **34** to **36** resulted in a complex mixture from which product cannot be isolated (Table 5 / Entry 2).

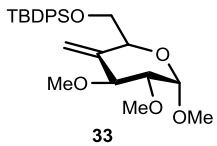
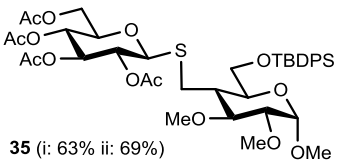
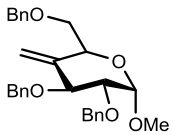
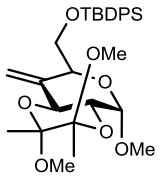
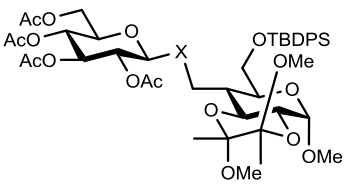
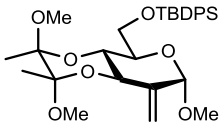
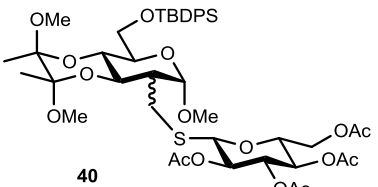
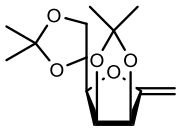
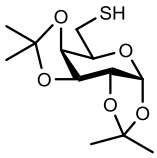
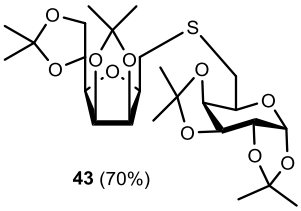
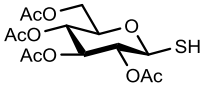
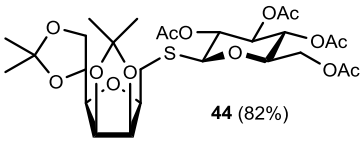
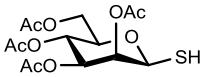
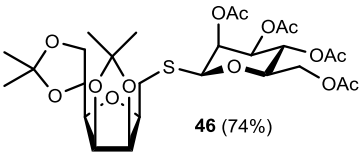
Entry	Glycal	Thiol	Product / conditions (**i**) 1.2 eq. Et_3B, 1.4 eq catechol, CH_2Cl_2 rt. 45 min	Product / conditions (**ii**) 3*0.1 eq. DPAP, hν, toluene, rt. 3*15 min
(**a**)	 **33**	 **34**	 **35** (i: 63% ii: 69%)	
(**b**)	 **36**	 **34**	i:complex reaction mixture	ii: complex reaction mixture
(**c**)	 **37**	 **34**		**38a** X: S **38b** X: S=O i: n/a ii: **38a+38b**= 74 %
(**d**)	 **39**	 **34**	 **40** i: n/a ii: 80 % (*gluco*:*manno* = ~1:1)	

Table 5. Hydrothiolation of sugar exomethylene derivatives

- Addition of thiol **34** onto 4-exo-methylene derivatives **33** and **37** took place with good yields and complete diastereoselectivity (Table 5 / Entry a and c).

- We found that compound **38** tends to be oxidized, which makes difficult the isolation and structure determination of **38a**. The tendency of oxidation may be due to the butanedione dimethyl acetal protecting group.

- On *C*-2 *exo*-methylene **39** we observed the formation of *gluco* and *manno* products in a nearly 1:1 ratio (Table 5 / Entry d).

Glycal	Thiol	Product
 **41**	 **42**	 **43** (70%)
	 **34**	 **44** (82%)
	 **45**	 **46** (74%)

Conditions: thiol:**41** = 1.2:1; DPAP, hν, toluene, 3*15 min

Table 6. Thioladdition to a furanoid exo-mannal

- Reactions of furanoside 1-*exo*-glycal **41** with thiols **34**, **42** and **45** resulted in the β-mannofuranoside-containing *C-S* disaccharide mimetics with complete stereoselectivity and good yields (Table 6).

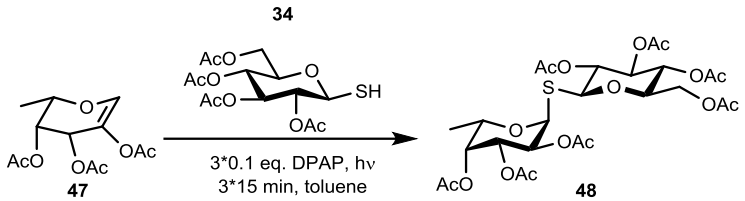
2.3.2 Thioladdition onto *endo*-glycals

- Previously, thiol-ene coupling reactions have been only carried out on *endo*-glycals of D-series. We extended the addition to L-sugars.

- When examining the thiol-ene reaction of 2-acetoxy-L-fucal (**47**) we found that the low conversion observed at room temperature could be improved by cooling the reaction mixture (Table 7.). According to our knowledge, this is the first example in the literature for a succesful radical mediated thioladdition at such a low temperature.

- We developed a new, simple excellent-yielding synthetic method to obtain α-L-fucoside thiomimetics.

- To further improve the conversion, we examined the solvent-dependence of the reaction of **47** and **34** at 0°C, however changing the solvent did not improve the yield.



Temperature	Conversion
(**a**) Non controlled	~5% (according to TLC)
(**b**) 0-5°C	33% (according to ^{1}H NMR)
(**c**) −40°C	66% (according to ^{1}H NMR)
(**d**) −80°C	92% (according to ^{1}H NMR) 90% (isolated yield)

Table 7. Effect of temperature to thiol-ene reaction of **47** and **34**

- We examined the addition of further thiols onto **47** at −85 ºC (Table 8.). Depending on the reactivity of the thiol we observed medium to excellent yields.

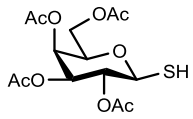
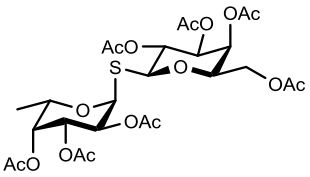
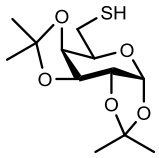
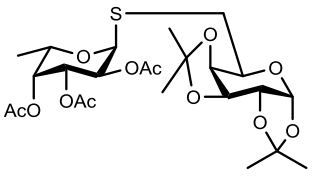
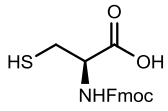
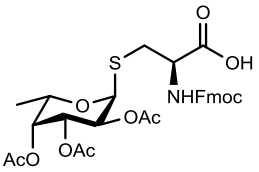
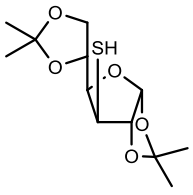
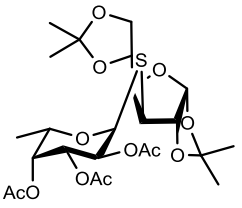
Thiol	Product	
49 		**50** -85°C: 95% (rt.: 33%)
42 		**51** (91%)
52 		**53** (75%)
54 		**55** (58%)

Table 8. Further thioladditions to **47** at −85 ºC

- The above conditions were applied for hydrothiolation of 2-acetoxygalactal and high yields were achieved (Table 9).

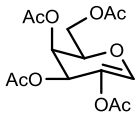
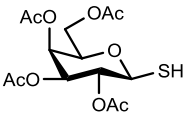
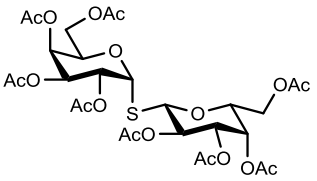
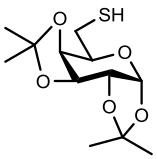
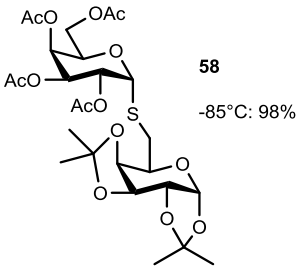
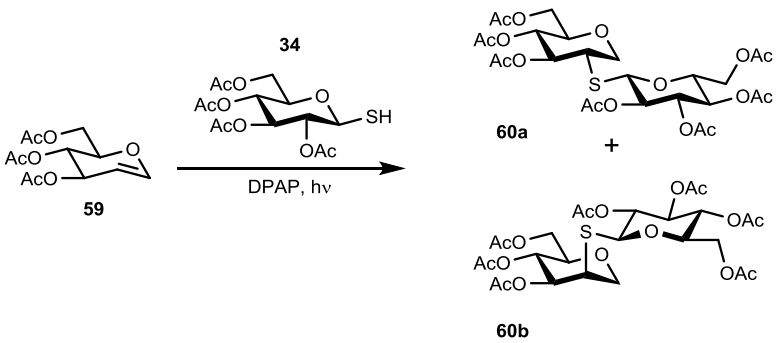
2-acetoxygalactal	Thiol	Product
 **56**	 **49**	 **57** -85°C: 95% rt.: 50%
	 **42**	 **58** -85°C: 98%

Table 9. Thioladdition to 2-acetoxygalactal

- We examined the effect of cooling on a reaction, which has a poor stereoselectivity at room temperature (Table 10). We found that cooling the reaction mixture improves the stereoselectivity and allows to decrease the excess of **34**.



Temperature	excess of 34	Yield	60a / 60b
room temperature	6 eq.	80%	57:43
−80°C	1.2 eq.	84%	89:11
−120°C	1.2 eq.	72%	80:20

Table 10. Temperature dependence of stereoselectivity and yield of the addition of **34** to **59**

Registry number: DEENK/19/2017.PL
Subject: PhD Publikációs Lista

Candidate: Dániel Eszenyi
Neptun ID: LOCPIS
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10038073

List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. Mező, E., **Eszenyi, D.**, Varga, E., Herczeg, M., Borbás, A.: A Modular Synthetic Approach to Isosteric Sulfonic Acid Analogues of the Anticoagulant Pentasaccharide Idraparinux. *Molecules. 21* (11), 1497-1515, 2016. EISSN: 1420-3049.
DOI: http://dx.doi.org/10.3390/molecules21111497
IF: 2.465 (2015)

2. **Eszenyi, D.**, Mándi, A., Herczeg, M., Bényei, A., Komáromi, I., Borbás, A.: Synthesis of C-2- and C-3-Sulfonatomethyl O- and S-Glycosides by Horner-Wadsworth-Emmons Olefination. *Eur. J. Org. Chem. 2016* (22), 3884-3893, 2016. ISSN: 1434-193X.
DOI: http://dx.doi.org/10.1002/ejoc.201600526
IF: 3.068 (2015)

List of other publications

Hungarian scientific articles in Hungarian journals (2)

3. Herczeg, M., Csávás, M., Bereczki, I., Mező, E., **Eszenyi, D.**, Kicsák, M., Hadházi, Á., Tollas, S., Varga, E., Szilágyi, E., Molnár, J. D., Bege, M., Pénzes, A., Herczegh, P., Borbás, A.: Gyógyhatású szénhidrátok: a véralvadástól a géncsendesítésig. *Magy. Kém. F. 121* (1), 13-21, 2015. ISSN: 0025-0155.

4. 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 articles in international journals (4)

5. 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

6. 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

7. Fekete, A., **Eszenyi, D.**, Herczeg, M., Pozsgay, V., Borbás, A.: Preparation of synthetic oligosaccharide-conjugates of poly-[beta]-(1-6)-N-acetyl glucosamine.
Carbohydr. Res. 386, 33-40, 2014. ISSN: 0008-6215.
DOI: http://dx.doi.org/10.1016/j.carres.2013.12.022
IF: 1.929

8. 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

Total IF of journals (all publications): 15,186
Total IF of journals (publications related to the dissertation): 5,533

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.

03 February, 2017