Synthesis of anionic oligosaccharides using 1-deoxy-1-ethoxysulfonyl-hept-2-ulopyranoside building blocks. Investigation of anomeric configuration of ketopyranosyl glycosides

theses of doctoral (PhD) dissertation

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1. Introduction

Selectins are carbohydrate-binding transmembrane glycoproteins and their role is to mediate the first steps of the recruitment of leukocytes from the blood stream in a series of normal and pathologic situations. Control of these processes by inhibiting the adhesion steps have been considered as a new anti-inflammatory and anti-metastatic strategy. Sialyl Lewis X tetrasaccharide is one of the major natural ligands of selectins. The acid function in the sialic acid moiety is crucial in binding. Our department described the synthesis of sulfonic acid analogues of the sLe X tetrasaccharide in which the sialic acid is replaced by an anomeric sulfonomethyl-type sugar moiety. For the substitution of *N*-acetylneuraminic acid with sulfonomethyl derivatives in the above mentioned analogues the ethyl 3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-ethoxysulfonyl-2-thio-α-D-*gluco*-hept-2-ulopyranoside (**37***) was used as glycosyl donor. During my PhD work, my task was to investigate glycosylation conditions, and improve glycosylation potential of the donor **37** and related compounds, and utilization of these derivatives in the syntheses of oligosaccharides having potential biological activities.

Gram-negative bacterium *Helicobacter pylori,* causative agent in chronic active gastritis, gastric and duodenal ulcers and presumably gastric malignancies uses different, highly specific carbohydrate-protein recognition and adhesion interactions to attack gastrointestinal epithelial cells. The 3'-sialylated and 3'-sulfated lactose and lactoseamin are ligands of adhesins of *H. pylori.* Our aim was to replace the sulfate ester group of the 3'-sulfated lactose by sugar C-sulfonic acid moiety

In the course glycosylation reactions with donor **37**, a considerable amount of *exo*-glycal **(40)** was always formed *via* elimination side-reaction. The glycosylium cation, which is formed during glycosylation reaction, can react with the aglycon into glycoside or can transform into the *exo*-glycal (**40**) *via* elimination of the labile proton next to the electron withdrawing sulfonic acid moiety. The lower was the reactivity of the acceptor, the higher was the rate of the elimination, which resulted in decreased yield of glycosylation reactions.

Our aim was to synthesize different glycosyl donors from 3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-ethylsulfonato-α-D-*gluco*-hept-2-ulose (**36**), to investigate the glycosylation reactions, to increase the yield of glycoside formation and to decrease the rate of the elimination reaction. Another aim was the synthesis of maltose type, polianionic oligosaccharides having α(2→5) interglycosidic linkage. It is known that polysulfated maltooligomers have

* Numbering of compounds refers to that used in the dissertation.

heparin-like, anti-metastatic and anti-viral effect. The planned polysulfonated maltose-type oligosaccharides may also display heparin like, antiviral and antimetastatic activity.

2. Methods

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

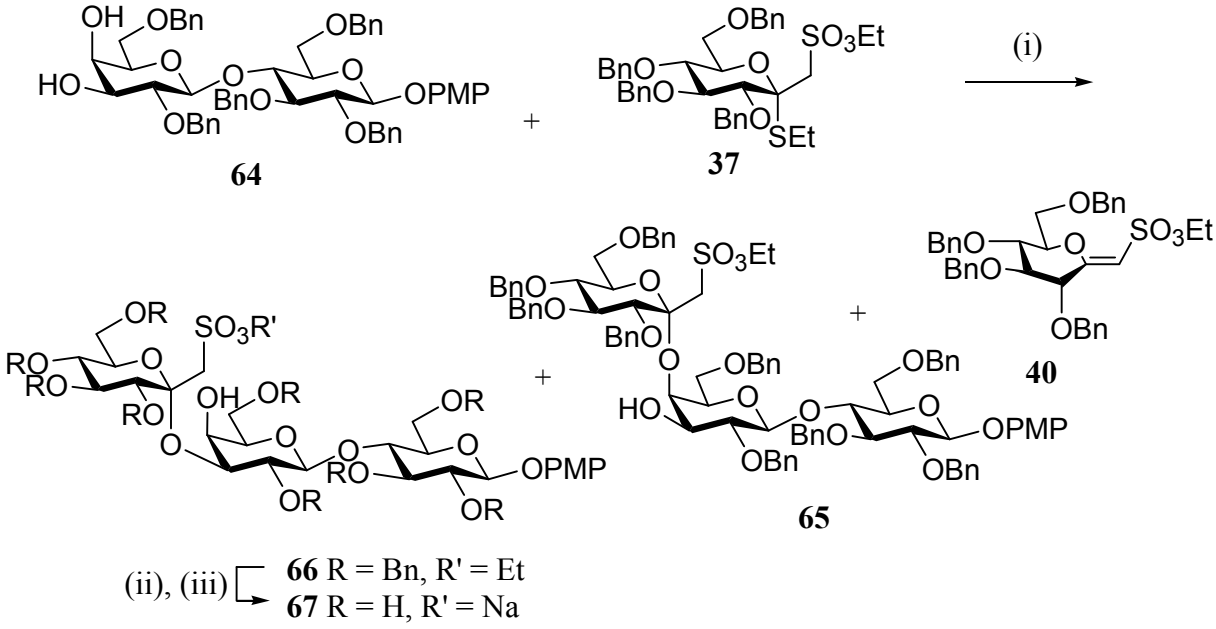
The reactions were monitored by thin layer-chromatography, the purity of the substances and the ratios of the products were controlled by high pressure liquid-, and gas chromatography. The purification of the crude products and the separation of the isomers were carried out by crystallization or by column chromatography.

Elemental analysis, melting point- and optical rotation determination, ^{1}H and ^{13}C NMR spectroscopic methods, X-ray spectroscopy and mass spectrometry were applied for the identification of the prepared compounds.

3. New scientific results

3.1. Synthesis of sulfonic acid analogue of the natural ligand of *Helicobacter pylori*

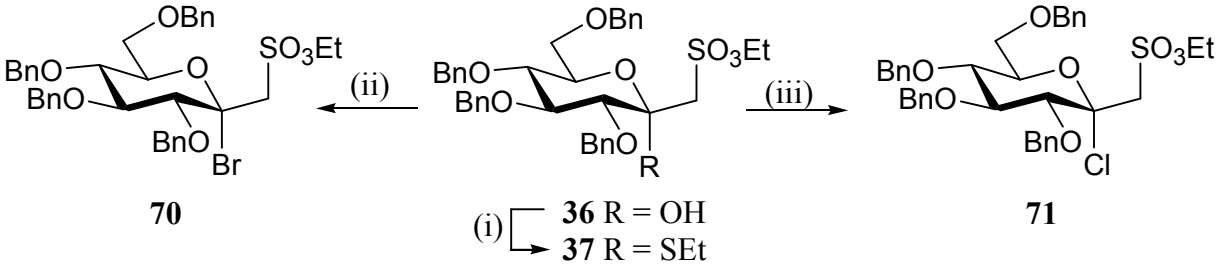
Glycosylation of protected lactoside acceptor (**64**) with donor **37** afforded the regioisomeric mixture of the corresponding trisaccharides. Next, by deprotection of the trisaccharide **66**, the sulfonic acid analogue of the natural ligand was synthesized, which can inhibit adhesion between *H. pylori* and the epithelial cells of the gastric antrum.



Scheme 1. (i) NIS – TfOH, CH_2Cl_2, -40 °C, (ii) NaI, acetone, (iii) H_2-Pd/C, EtOH – H_2O

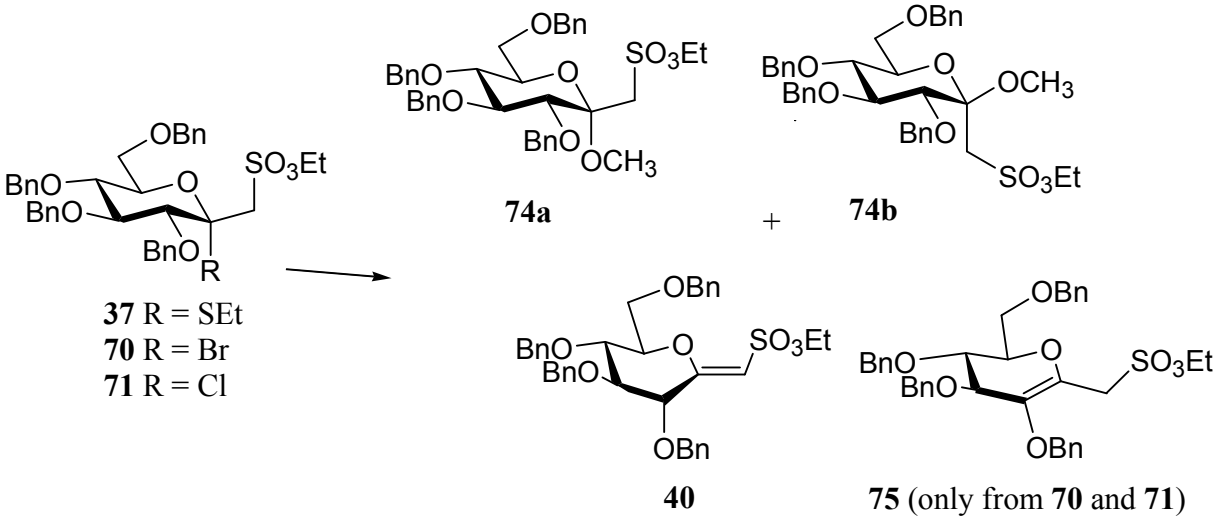
3.2. Investigation of glycosylation properties of 1-deoxy-1-ethoxysulfonyl-hept-2-ulopyranosyl derivatives

Beside the thioglycoside **37**, the appropriate bromo (**70**) and chloro (**71**) derivatives were also synthesized (**Scheme 2.**). These derivatives were used as donors in glycosylation reactions with methanol (**Scheme 3.**), with acceptor **51** containing primary free OH group (**51**), and with acceptors **72** and **73** containing free secondary OH group (**Scheme 4.** and **5.**), respectively, aiming at investigate of the effect of acceptor reactivity on the rate of the formed glycoside and glycal products.



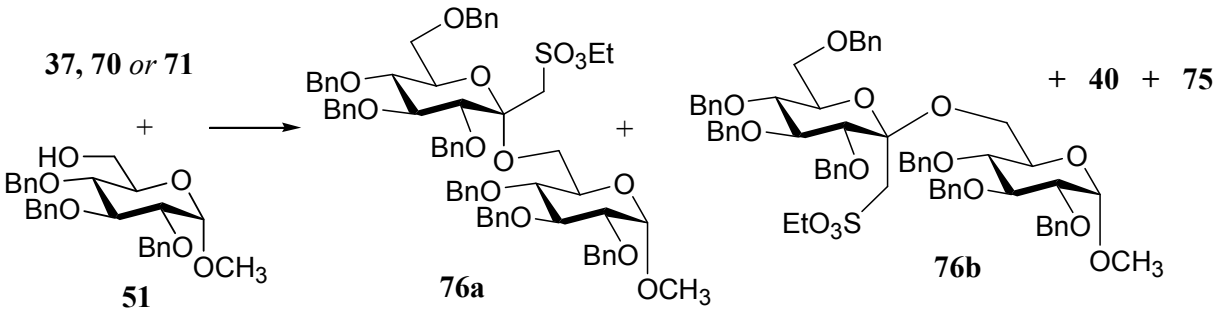
Scheme 2. (i) EtSH, $BF_3 \cdot Et_2O$, 0 °C, (ii) from **37,** Br_2, 0 °C, (iii) from **36,** pyridine, $SOCl_2$, RT

The reactions of the thioglycoside donor, using NIS-TfOH activation was carried out by using different amount of acid at different temperatures, furthermore, DMTSB and MeOTf promoters were also used as promotors. Halide donors (**72**, **73**) were activated with heavy metal salts (AgOTf, $Hg(CN)_2$, and $Hg(CN)_2 - HgBr_2$), or were reacted with neat methanol without any promoter (methanolysis). The ratios of the products were determined by HPLC chromatography.



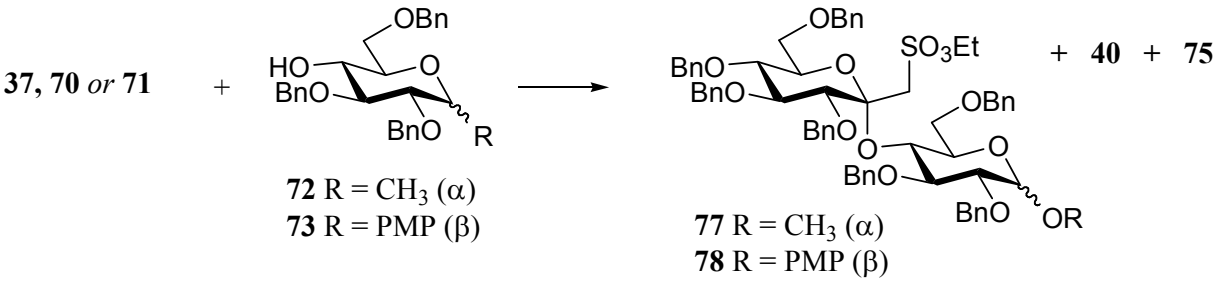
Scheme 3.

Glycosylation of the reactive acceptors (methanol, 6-OH of **51**) afforded anomeric mixtures of the glycosides (**74a**, **74b** and **76a**, **76b**) with poor stereoselectivity. Decrease of the reactivity of the acceptor results in increasing of the selectivity. By using of thioglycoside donor and increase of the temperature the stereoselectivity also increases. The α:β anomeric ratio of the methyl glycosides varied between 11:1 to 2:3, while the α-glycoside was the main product in a ratio of 12:1 - 3:1 in case of the 2→6 linked disaccharides (**76a**, **76b**). In the course of glycosylation of the unreactive acceptors (**72**, **73**) the α-glycosides were formed exclusively.



Scheme 4.

The ratio of the glycosylation and the elimination was determined by the rate of formation of the glycosylium cation, its reaction with the acceptor and the elimination reaction from the oxocarbenium ion. Using methanol as acceptor, elimination was negligible, independently of the applied donor or promoter, due to the immediate reaction of the glycosylium cation with the highly reactive acceptor. In case of reactions with 6-OH acceptor (**51**) at lower temperature and using low acid-concentration thioglycoside proved to be a little bit better donor than the halide donors. The rate of elimination was moderate at lower temperature, thus the formed glycosylium cation reacted with the fairly reactive acceptor in a ratio of 60-90%. Slow activation of halide donors resulted in lower average concentration of the glycosylium cation, however, elimination exceeded the glycoside formation at higher temperature.



Scheme 5.

In case of relatively unreactive acceptors, halide donors gave better results by activation at 0 °C. At this temperature, reactivity of the acceptor was satisfactory, thus, competition of the formation of the glycoside and the elimination reaction resulted in the mixture of the glycoside and the glycal products in 3:7 to 1:1 ratio.

The thioglycoside was not suitable for glycosylation of the unreactive acceptors. Reactivity of the secondary hydroxyl groups was not satisfactory at lower temperature (-40 °C), while increasing the temperature resulted in the increased rate of both glycosylation and elimination, however, elimination outruned the glycosylation. Therefore, elimination occurred exclusively.

The *endo*-type glycal (**75**) was identified beside the *exo*-glycal from the halide donors (**70, 71**), and three different kind of mechanism were proposed for the formation: *via* E1 mechanism from the donor, due to elimination of the H-3 proton from the glycosylium cation, or *via* acid catalyzed rearrangement of the double bond of the formed *exo*-glycal.

3.3. Synthesis of ketopyranosyl glycosides and determination of their anomeric configuration on the basis of the three-bond carbon–proton couplings

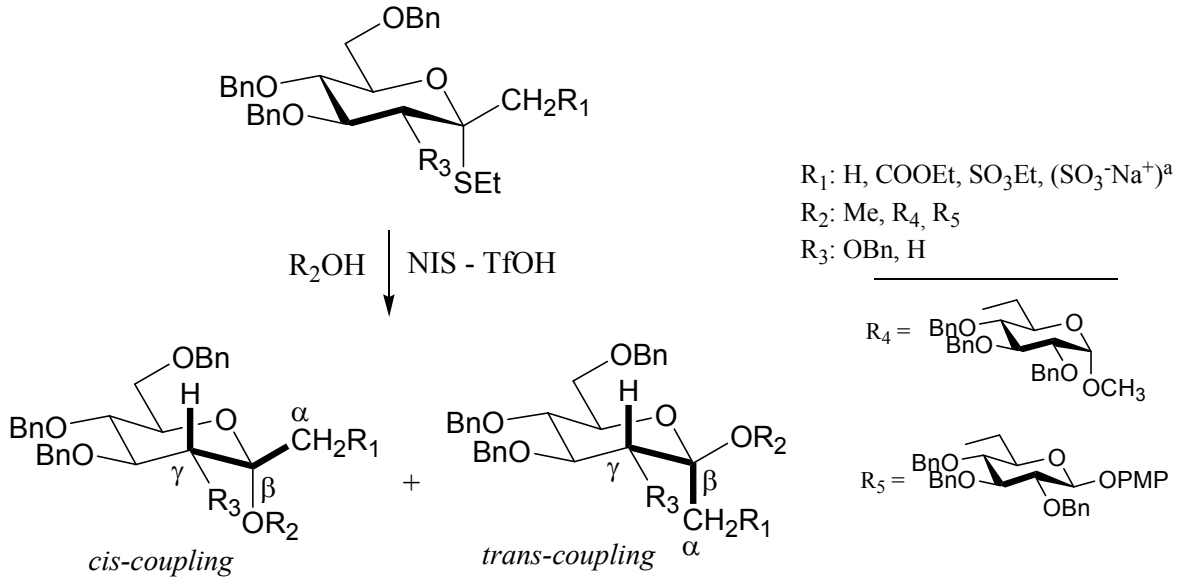
Because of the lack of the anomeric proton of the formed ketopyranosyl glycosides (**74a,b**, **76a,b**, **77** and **78**) the anomeric configuration was determined on the basis of the NMR C1-H3 coupling constant, which is dependent on the dihedral angle in a manner similar to $^{3}J_{H,H}$. Consequently, small couplings were expected for the α-anomers (**74a**, **76a**, **77** and 7**8**) whereas larger ones for the β-anomers.

However, in course of structural determination of our ketosyl glycosides very similar coupling constant values could be obtained for both the α and β anomers, the couplings varied between 1 Hz and 2.7 Hz for the α-anomers, while ~2.5 Hz was for the β-anomers. To test the applicability of the $^{3}J_{C,H}$ values for the determination of anomeric configuration, we decided to synthesize and investigate the coupling constants of anomeric pairs of ketopyranosyl glycosides bearing various substituents along the coupling way (**Table I**.).

Comparing the values for the anomeric pairs we find that, these couplings tend to be for the α-glycosides always smaller (~1 Hz), than that of the β-glycosides (~2.5 Hz). However, $^{3}J_{C1,H3}$ values can be used for reliable determination of the anomeric configuration only when both anomers of a particular derivative are available. Structural assignments based on a single coupling constant or from data measured on derivatives with different substituents and/or different substitution patterns along the coupling path are, however, unreliable.

Therefore, in such cases it is recommended to seek independent structural evidence such as NOEs, chemical shifts or X-ray determination.

Table I. *Ketopyranosyl glycoside anomeric pairs and their anomeric three-bond carbon–proton couplings ($^3J_{C\alpha,H\gamma}$)*



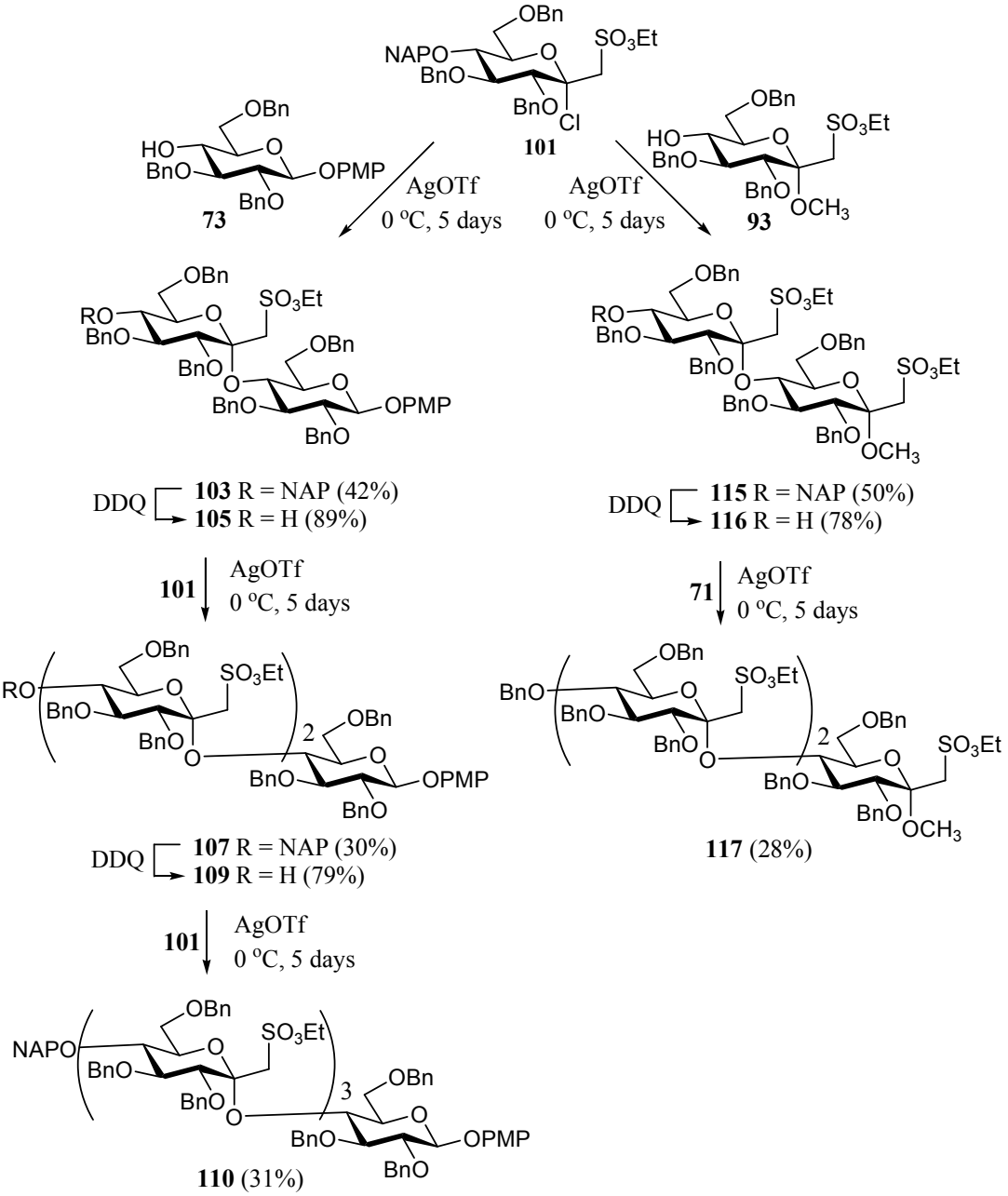
[a]**82a** and **82b** sodium-sulfonato sugars were derived from **74a** and **74b** ester-derivatives by using NaI reagent in acetone

Entry	R_1	R_2	R_3	Compound	$^3J_{C\alpha,H\gamma}$ (Hz) *cis*	Compound	$^3J_{C\alpha,H\gamma}$ (Hz) *trans*
1.	SO_3Et	*CH_3*	OBn	**74a**[a]	~1	**74a**	2.4
2.	SO_3Et	*R_4*	OBn	**76a**	≤ 1	**76a**	2.5
3.	SO_3Et	*Bn*	OBn	**79a**[a]	≤ 1	**79a**	2.3
4.	SO_3Et	*R_5*	OBn	**81a**	~ 2	**81a**	n.a.
5.	*SO_3Na*[a]	CH_3	OBn	**82a**	≤ 1	**82a**	2.4
6.	SO_3Et	CH_3	*H*	**86a**	≤ 1	**86a**	4.8
7.	*COOEt*	CH_3	OBn	**89a**	~1	**89a**	2.6
8.	*H*	CH_3	OBn	**91a**	1.8	**91a**	2.6

3.4. Synthesis of polysulfonated maltose-type oligosaccharides

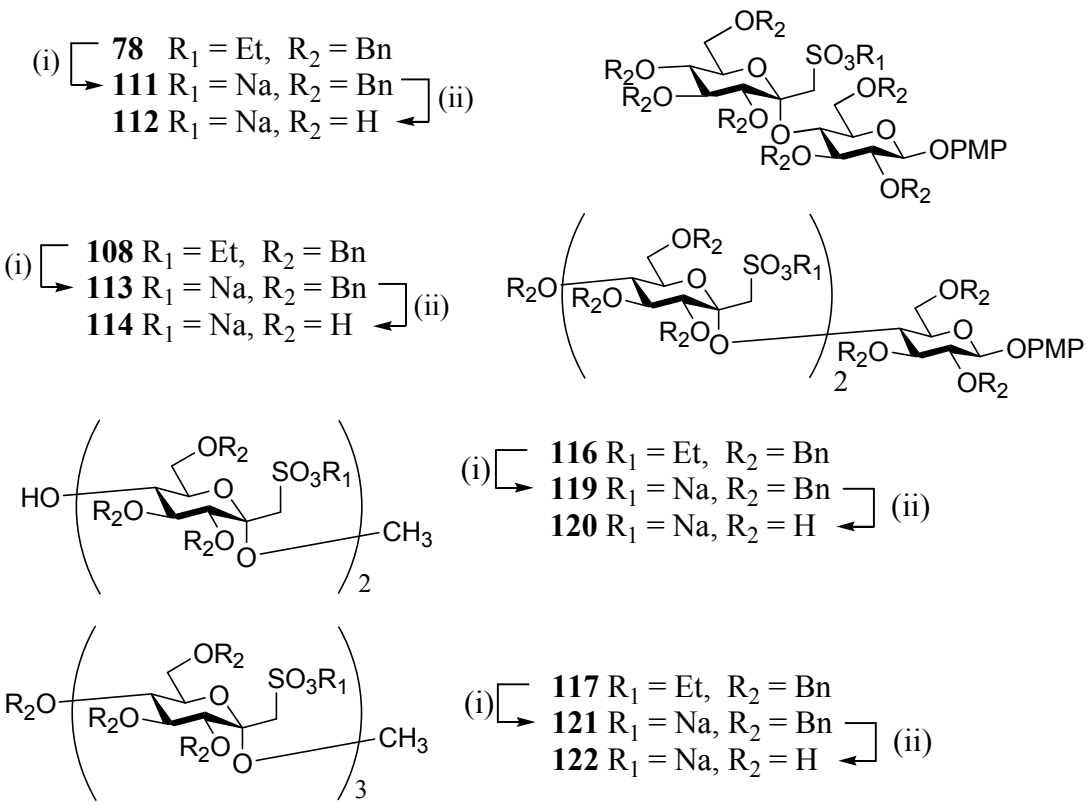
The last part of this paper describes the synthesis of polysulfonated, maltose-type keto-oligosaccharides by using of 1-deoxy-1-ethoxysulfonyl building blocks. For the

synthesis, a glycosyl chloride donor molecule (**101**) was prepared from 3,4,7-tri-*O*-benzyl-5-*O*-(2-naphthyl)methyl-1-deoxy-1-ethoxysulfonyl-α-D-gluco-hept-2-ulopyranose (**98**). Compound **101** containing selectively removable (2-naphthyl)methyl (NAP) protecting group at the C-5 position, could be used as donor in glycosylation reaction to get glycoside, which, after removal of the NAP group, could be used as acceptor (**Scheme 6.**). Glycosylation of acceptors **73** and **93**, and subsequent removal of the 5'-NAP group from the non-reducting terminal of the formed glycosides (**103**, **115**) resulted in two disaccharide acceptors (**105**, **116**), which were also glycosylated with **101**. That iterative glycosylation sequence was used for the synthesis of two different series of homologous oligosaccharides up to tetra- (**110**) and trisaccharide (**117**).



Scheme 6

Sodium-sulfonato derivatives of the protected sugars were formed *via* nucleophilic attack by iodide ion, then subsequent removal of the benzyl protecting groups was performed by catalytic hydrogenation.



Scheme 7. (i) NaI (1.1 equiv.), acetone, 3 h, reflux, 97% **111**, 84% **113,** 82% **119**, 72% **121**; (ii) Pd/C (20 m/m %), H_2, AcOH, EtOH – H_2O, 62% **112,** 81% **114,** 78% **120,** 72% **122**.

4. Summary

1) A sulfonic acid trisaccharide analogue of the natural ligand of *Helicobacter pylori* was synthesized, which can inhibit adhesions of the bacteria responsible for gastric antrum diseases.

2) A suitable glycosyl donor and reaction conditions were developed by investigation of glycosylation reactions of the thio, bromo, and chloro derivatives of 1-deoxy-1-ethoxysulfonyl-hept-2-ulopyranosyl donors with more or less reactive acceptors. The best yields of disaccharides from unreactive acceptors were achieved by using chloro sugar donor and silver-triflate, as promoter at 0 °C.

3) To test the applicability of the $^3J_{C,H}$ values for the determination of anomeric configuration, we synthesized of anomeric pairs of ketopyranosyl glycosides with various substituents along the coupling path. Comparison of the coupling constants of the ketosyl glycosides suggested that, structural assignments based on a single coupling constant or from

data measured on derivatives with different substituents and/or different substitution patterns along the coupling path are, however, unreliable.

4) By suitable protecting group strategy, a 1-deoxy-1-ethoxysulfonyl-hept-2-ulopyranoside donor building block was prepared, and used for the synthesis of polysulfonated maltose-type oligosaccharides. Two different series of homologous oligosaccharides up to tetra- (**110**) and trisaccharide (**117**) were synthesized, which were transformed to deprotected sodium salt derivatives, and which could be elongated, too. The polysulfonated oligosaccharides may have potential antiviral activity.

5. List of publications

5.1 Publications

1. A. Borbás, M. Csávás, L. Szilágyi, **G. Májer**, A. Lipták; Replacement of carbohydrate sulfates by sugar *C*-sulfonic acid derivatives; *J. Carbohydr. Chem.,* 23 (**2004**) 133-146.

2. **G. Májer**, A. Borbás, T. Z. Illyés, L. Szilágyi, A. Cs. Bényei, A. Lipták; Synthesis of ketopyranosyl glycosides and determination of their anomeric configuration on the basis of the three-bond carbon–proton couplings; *Carbohydr. Res.*, 342 (**2007**) 1393–1404.

5.2. Lectures and posters

1. A. Borbás, M. Csávás, **G. Májer**, A. Lipták; Synthesis of lactose sulfonic acids; *First Austrian-Hungarian Carbohydrate Conference*, Burg-Schlaining, Austria, 24-27 September 2003. (E)
2. Borbás A., **Májer G**., Szilágyi L., Lipták A.; Ketopiranozil glikozidok szintézise és anomer konfigurációjuk meghatározása; *Szénhidrátkémiai Munkabizottsági Ülés*, Debrecen, 2004. XI. 5. (E)
3. A. Borbás, **G. Májer**, Z. B. Szabó, A Lipták; Investigation of glycosylation properties of 1-ethoxysulfonyl-hept-ulopyranosyl derivatives; *2nd Austrian-Hungarian Carbohydrate Conference*, Somogyaszaló, Hungary, 24-26 May 2005. (E)
4. Borbás A., **Májer G.**, Illyés T. Z., Szilágyi L., Lipták A.; Ketopiranozil-glikozidok szintézise és anomer konfigurációjuk meghatározása; *Vegyészkonferencia*, Hajdúszoboszló, 2005. június 28.-30. (E)

5. A. Borbás, Z. B. Szabó, **G. Májer**, A. Lipták; Synthesis of saccharide sulfonic acids; *2nd German-Hungarian Workshop*, Debrecen, Hungary, 4 April, 2006. (E)
6. **G. Májer**, A. Borbás, A. Lipták; Synthesis of maltose-type oligosaccharides containing sulfonic acid moiety at the anomeric configuration; *Szénhidrátkémiai Munkabizottsági Ülés*, Mátrafüred, 2006. május 31.-június 2. (E)
7. A. Borbás, F. Sajtos, **G. Májer**, A. Lipták; Preparation of C-sulfated sugar donors for the synthesis of C-sulfate containing Sialyl Lewis X analogues; *Third Pan-Pacific Conference on Sialoglycoscience and Other Novel Forms of Glycosylation,* Gold Coast, Australia, 14-17 July 2002. (P)
8. Borbás A., **Májer G.**, Szeghy G., Lipták A.; Polianionos maltooligomerek építőelemeinek szintézise; *Vegyészkonferencia*, Hajdúszoboszló, 2001. június 27-29, P-96. (P)
9. Borbás A., Csávás M., **Májer G.**, Lipták A.; Szulfonilezett laktóz származékok szintézise; *Vegyészkonferencia*, Hajdúszoboszló, 2003. június 26-28, P-13. (P)
10. **G. Májer,** A. Borbás, A. Lipták; Synthesis of ketopyranosyl glycosides and determination of their anomeric configuration; *First German-Hungarian workshop*, Hannover, Germany, July 5-6, 2004, P-07. (P)
11. **G. Májer,** A. Borbás, Z. B. Szabó, A. Lipták; Investigation of glycosylation properties of 1-deoxy-1-ethoxysulfonyl-hept-ulopyranosyl derivatives; *13th European Carbohydrate Symphosium*, Bratislava, Slovakia, 21-26 August, 2005, P-37. (P)
12. **G. Májer,** A. Borbás, Z. B. Szabó, A. Lipták; Investigation of glycosylation reactions using 1-deoxy-1-ethoxysulfonyl-hept-2-ulopyranosyl donors;, *2nd German Hungarian Workshop*, Debrecen, Hungary, 4 April, 2006, P-2. (P)