Preparation of analogues of biologically active carbohydrate sulfate esters: Synthesis of sugar-sulfonates and methylene-sulfonates

Ph.D. Theses

László Lázár

Supervisor: Dr. András Lipták

Debreceni Egyetem, Természettudományi Kar

Debrecen, 2006.

1. Introduction

Sulfated carbohydrates are widespread in nature, predominantly represented on cell surfaces and in the extracellular space. Many of these sulfated molecules have been implicated as important mediators of extracellular traffic and cell-cell communication. These biological processes are facilitated by sulfate ester groups, found in the biologically active molecule part, forming ionic bonds due to their anionic character. Since sugar sulfate esters are susceptible to the hydrolytic effect of sulfatases and esterases our aim was to substitute sulfate esters by sulfonate and methylene sulfonate groups. These negatively charged sulfonic acid derivatives are more resistant to the aforementioned enzymes, thus the desired biological effect in the organism can be sustained for a longer period of time.

There are only a few examples in the literature to the synthesis of secondary sugar sulfonic and methylene sulfonic acids. In the past few years our group carried out intensive research in the field of the preparation of carbohydrate sulfonates. My task was to develop generally applicable methods for the preparation of sulfonate and methylene sulfonate functions on carbohydrates, firstly on the level of monosaccharides.

A 6-deoxy-L-talose component, sulfated at position 4, is found in the core region of the glycopeptidolipid of *Mycobacterium avium*. Firstly, this component and its sulfonate and methylene sulfonate analogue was planned to be prepared as methyl glycosides to compare their behaviour in a biological medium. Since the synthesis of the desired talopyranose derivatives was planned from L-rhamnose, it was also interesting to examine the preparation of the appropriate *rhamno*-4-O-sulfate ester, the 4-sulfonate and 4-methylenesulfonate derivatives.

Gluco- and *manno*-2-sulfonic acid derivatives were also successfully prepared in our research group by using the advantages of the 1,2-thiomigration reaction. This method involves a suitable thio group (STr, SPMBn) at the anomeric position and a group of good leaving property (OMs) at position 2. Whenever, it is reacted with the appropriate nucleophile ($^-$OMe), the thio group migrates into position 2. An SH group from the thus obtained 2-thio group can be regenerated and can be readily oxidized to a sulfonate, which may occur simultaneously (in situ) or in two steps. Only by the use of the tritylthio group, out of the examined thio groups, it is possible to obtain a 2-sulfonate in good yield.

Therefore, our goal was to find and apply such new-type thiol protecting groups, that can be easily oxidized to a 2-sulfonate, after migration. For this purpose, acetylthio, 2-(trimethylsilyl)ethylthio-, and allylthio groups were chosen.

2. Applied methods

The macro-, semimicro- and micro methods of modern preparative organic chemistry were applied in the synthetic work. Reactions were monitored by thin layer-chomatography, the isolation and purification of the crude products were carried out by crystallization or by column chomatography.

Elemental analyses, melting point and optical rotation measurements, NMR spectroscopy and mass spectrometry (MALDI–TOF MS) were applied for the identification and characterization of the compounds prepared. Complete assignments of ^{1}H- and ^{13}C-spectra were achieved by the combined analysis of various 1D and 2D measurements such as ^{1}H-^{1}H COSY, TOCSY and ^{13}C-^{1}H HSQC.

3. New scientific results

3.1. Synthesis of *talo*- and *ramno*-4-*O*-sulfate, -4-sulfonate and -4-methylene sulfonate derivatives

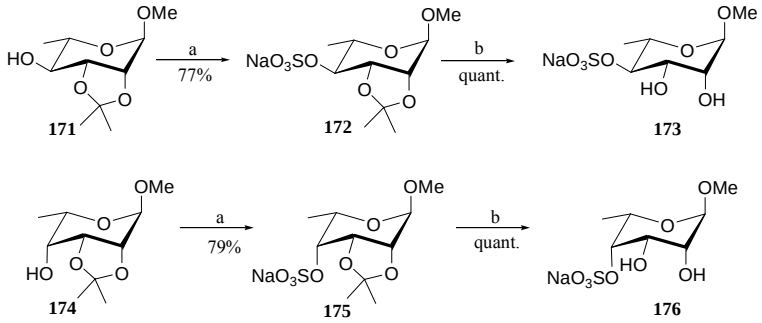
In accordance with our aims firstly, the monosaccharide component, found in the core region of the glycopeptidolipid of *Mycobacterium avium*, the 6-deoxy-L-talopyranose 4-*O*-sulfate was prepared as a methyl glycoside (**176**[*]) and its methylene sulfonate (**181**) and sulfonate (**197**) analogues were also synthesized. The appropriate *rhamno* analogues, namely the *rhamno*-4-*O* sulfate ester (**173**), the 4-methylene sulfonate (**183**) and the 4-sulfonate (**189**) were prepared, too.

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

3.1.1. Synthesis of *talo*- and *ramno*-4-*O*-sulfate esters

For the preparation of the desired *rhamno*-4-*O*-sulfate ester derivative (**173**), methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**171**) was treated with the SO_3·Pyridine complex in DMF for 1h at room temperature to give methyl 2,3-*O*-isopropylidene-4-*O*-(sodium sulfonato)-α-L-rhamnopyranoside (**172**) in 77% yield. Hydrolysis of the isopropylidene group was achieved with acetic acid at rt for 30 min, to give methyl 4-*O*-(sodium sulfonato)-α-L-rhamnopyranoside (**173**) with quantitative yield.

Similar treatment of methyl 6-deoxy-2,3-O-isopropylidene-α-L-talopyranoside (**174**) with the SO_3·Pyridine complex resulted in methyl 6-deoxy-2,3-O-isopropylidene-4-O-(sodium sulfonato)-α-L-talopyranoside (**175**). Hydrolysis of the isopropylidene acetal resulted in the target compound **176** with quantitative yield (Scheme 1).



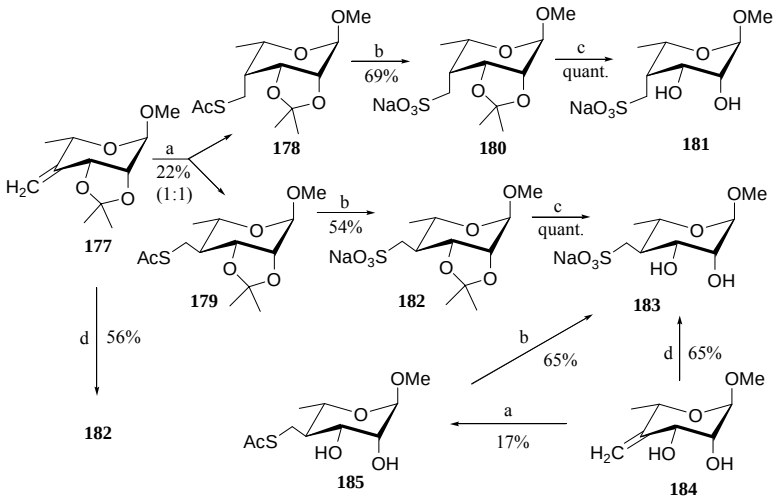
a) 10 equiv. SO_3·pyridine, DMF, 1 h; $NaHCO_3$; b) 96% AcOH, 30 min.

Scheme 1

3.1.2. Synthesis of *talo*- and *ramno*-4-methylene sulfonates

In the course of the preparation of the desired *talo*- and *rhamno*-4-methylene sulfonates two different methods were used. The first method involved the addition of a thioacetic acid onto the appropriate 4-exomethylene derivatives (**177** and **184**) in the presence of AIBN radical initiator, then the obtained acetylthiomethyl compounds (**178**, **179** and **185**) were oxidized with Oxone ($2KHSO_5$, $KHSO_4$, K_2SO_4) to afford **180**, **182** and **183** methylene sulfonates. The deprotection of the isopropylidene protecting groups from **180** and **182** gave **181** and **183** target compounds (Scheme 2).

The second method involved the addition of $NaHSO_3$ onto **177** and **184** exomethylene derivatives in the presence of *t*-butyl perbenzoate, thus giving rise to the formation of **182** and **183** methylene sulfonic acid derivatives in one synthetic step. In the case of the $NaHSO_3$ addition the equatorial product was always obtained out of the two possible products. However, in the case of the addition of thioacetic acid such a simple stereoselectivity could not be observed. While **184** exomethylene derivative reacted to give selectively the equatorial acetylthiomethyl (**185**) compound, **177**, however, reacted to give both the axial (**178**) and the equatorial (**179**) products in a 1:1 ratio.



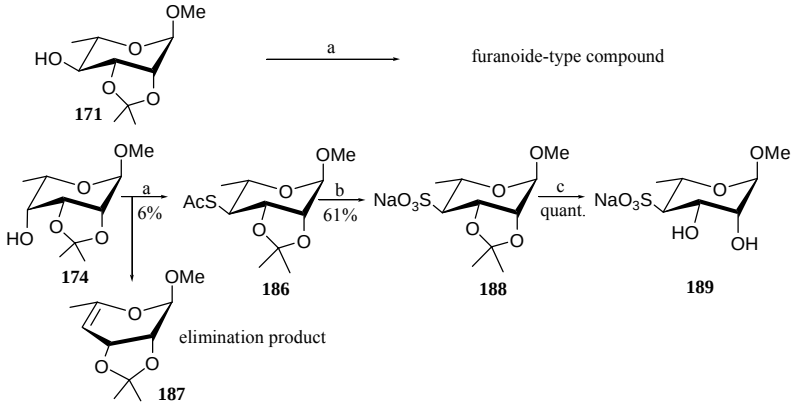
a) 5 equiv. AcSH, AIBN, toluene, 80°C, 8 h; b) 2.5 equiv. Oxone, 20 equiv. KOAc, AcOH; c) 96% AcOH, 60°C, 1 h; d) 10 equiv. $NaHSO_3$, *t*-butyl-peroxybenzoate, EtOH-H_2O, reflux, 4 h.

Scheme 2

3.1.3. Synthesis of *talo*- and *ramno*-4-sulfonates

For the preparation of the *talo*- and *rhamno*-4-sulfonates intermolecular nucleophilic substitution reactions were used. Firstly, *rhamno* and *talo* derivatives (**171** and **174**), bearing a free OH group in position 4, were treated with trifluoromethanesulfonic anhydride and the obtained 4-*O*-triflate compounds were treated with potassium thioacetate. Carrying out of reactions compound **171** yielded a furanoide-type compound instead of the desired *talo*-4-*S*-acetyl derivative. Starting from **174** the desired *rhamno*-4-*S*-

acetyl compound (**186**) was isolated, however, with a moderate yield only, because **187** elimination product was the main product of this reaction. Thioacetyl derivative **186** was reacted with oxone in acetic acid medium and deprotection of the isopropylidene group from the thus obtained **188** yielded **189** as the target compound (Scheme 3).

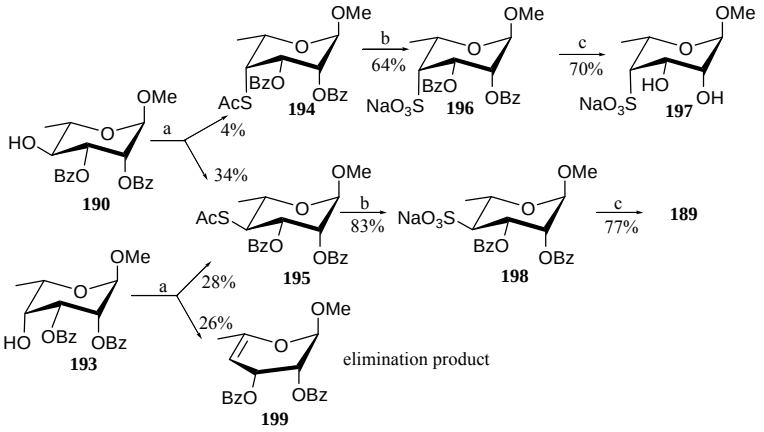


a) Tf_2O, CH_2Cl_2, pyridine; 2.5 equiv. KSAc, DMF, 60°C, 2 h; b) 2.5 h. Oxone, 20 h. KOAc, AcOH; c) AcOH 96%, 60°C, 1 h.

Scheme 3

Because of the unsuccessful preparation of the *talo*-4-*S*-acetyl compound and the low yielding preparation of the *rhamno*-4-*S*-acetyl derivative the isopropylidene group, used in positions 2 and 3, was exchanged into benzoyl protective groups.

Starting from the rhamno-2,3-di-*O*-benzoyl derivative (**190**) and using the aforementioned procedure it was possible to prepare the desired *talo*-4-*S*-acetyl derivative (**194**), but, surprisingly, the main product of the reaction proved to be the *rhamno* (**195**) isomer. This can only happen, when this transformation does not strictly follows the S_N2-type mechanism. The *talo*- and *rhamno*-4-acetylthio derivatives (**194** and **195**), thus obtained, were oxidized with hydrogen peroxide to afford sulfonates (**196** and **197**) and after the deprotection of the benzoyl groups **189** and **197** target compounds were obtained. Next, similarly to the case of **190** the appropriate *talo*-2,3-di-*O*-benzoyl (**193**) derivative was treated with potassium thioacetate, following a triflate formation, and the reaction yielded the desired *rhamno*-4-*S*-acetyl derivative (**195**) and a large amount of the elimination product (**199**, Scheme 4).



a) Tf_2O, CH_2Cl_2, pyridine; 2.5 equiv. KSAc, DMF, 60°C, 2 h; b) 10 equiv. 30% H_2O_2, 1 equiv. NaOAc, AcOH, 50°C, 24 h; c) NaOMe, MeOH.

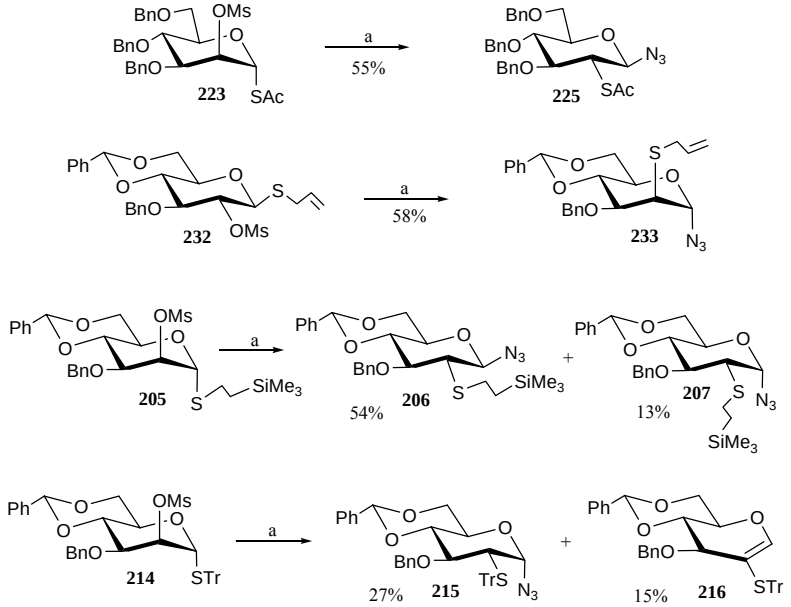
Scheme 4

3.2. Synthesis of 2-sulfonic acids

In accordance with our aims, intramolecular nucleophilic substitution reactions (thio-migration) were also used for the preparation of secondary sulfonates. Derivatives, bearing a suitable thio group at the anomeric position and a good leaving group at position 2, undergo a transition, in the presence of a nucleophile, when the alkyl/acyl thio group migrates into position 2. The formed 2-thio group can be converted into an SH-group and it can be readily oxidized to give a sulfonate. This result can be accomplished in one or two steps. Following this procedure *gluco*- and *manno*-2-sulfonic acid derivatives (**208**, **209**, **226** and **239**) were successfully prepared. Four new-type thio protective groups (trityl, 2-(trimethylsilyl)ethyl, acetyl, and allyl) were used in the course of these reactions, the last three of the mentioned ones were first tested in our laboratory as protecting groups in thio migration reactions. The 2-thio groups, successfully obtained after migration, were converted into the appropriate 2-sulfonate.

The migration reactions of 2-*O*-mesyl derivatives (**205**, **214**, **223** and **232**), in the case of all four thio protecting groups, were carried out in the presence of sodium-azide as the nucleophile and DMF as the solvent. 1,2-*Trans* products (**225** and **233**) were formed in

the case of acetyl thio and allylthio groups, while the use of 2-(trimethylsilyl)ethyl group gave a 4:1 ratio of the 1,2-*trans* (**206**) and 1,2-*cis* (**207**) products. However, the reaction in the case of the tritylthio group resulted in a 2:1 mixture of the 1,2-*cis* (**215**) and the elimination product (**216**, Scheme 5).

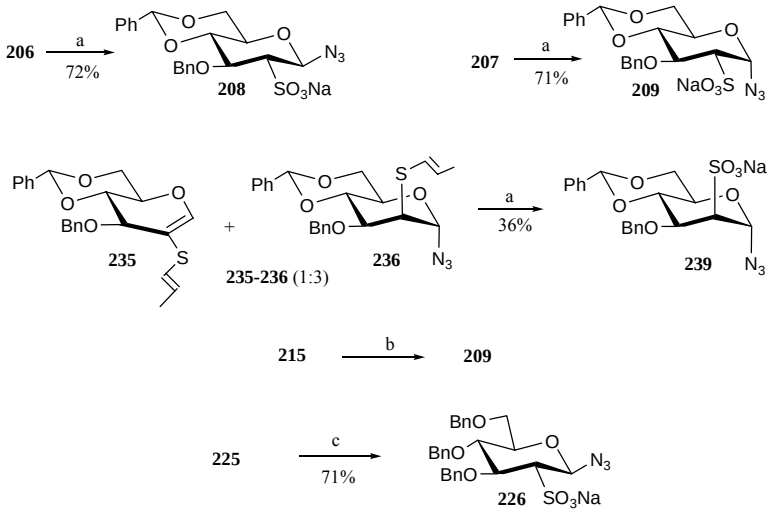


a) 10 equiv. NaN_3, DMF, 0 °C, 1 h (**223**), 70 °C, 8 h (**205** and **232**), 80 °C, 72 h (**214**).

Scheme 5

It is worth to mention, that a 70-80 °C temperature was necessary for the thioglycosides to undergo the migration, while in the case of the 1-*S*-acetyl group the same process took place rapidly at 0 °C.

From 2-*S*-[2'-(trimethylsilyl)ethyl] compounds (**206** and **207**) and a 1:3 mixture of **235** and **236**, obtained after the isomerisation of **233**, the appropriate sulfonic acid derivatives (**208**, **209** and **239**) could be prepared in two steps, by using mercuric-trifluoroacetate and Oxone, afterwards. The desired 2-sulfonates (**209** and **226**) were obtained in one synthetic step from the 2-*S*-trityl (**215**) and from the 2-*S*-acetyl (**225**) using Oxone and hydrogen peroxide as oxidizing agents, respectively (Scheme 6). To our best knowledge the preparation of bifunctional molecules of such type has not been reported yet.



a) 1.5 equiv. $Hg(CF_3COO)_2$, CH_2Cl_2, H_2O, 6 h; 2.5 equiv. Oxone, 20 equiv. KOAc, AcOH, 4 h; b) 2.5 equiv. Oxone, 20 equiv. KOAc, AcOH, 16 h; c) 10 equiv. H_2O_2, 1 equiv. NaOAc, AcOH, 50 °C, 24 h.

Scheme 6

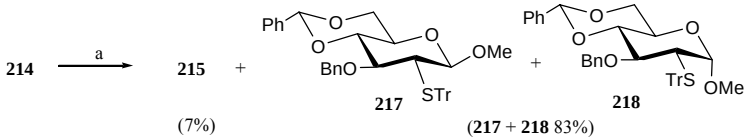
The thio-migration reactions were also tested with different nucleophiles, as well. The 1-*S*-acetyl compound (**223**) was reacted with potassium thioacetate, the allylthioglycoside (**232**) with sodium acetate and both reactions yielded only the 1,2-*trans* products (**227** and **234**) similarly to the reaction, that involved sodium-azide as the nucleophile (Scheme 7).

a) 10 equiv. NaOAc, DMF, 70°C, 5 h; b) 10 equiv. KSAc, DMF, 0 °C, 1 h.

Scheme 7

In the case of triphenylmethyltio group the influence of different solvents on the result of the migration reaction was also examined. The 72 hour-long reaction time, experienced by the use of DMF was reduced to 24 hours by exchanging the solvent either to DMSO, to acetonitril, or to methyl-ethyl ketone. In the case of methanol as the solvent the reaction took place in 12 hours at reflux temperature. The ratio and quality of the products, depending on the solvent, were different from that of the products obtained in DMF. In the case of DMSO the yield of the 1,2-*cis* (**215**) product was severely increased

and a smaller amount of elimination product (**216**) was formed, while the use of acetonitril and methyl-ethyl ketone resulted in the formation of the **216** glycal as the only product. The most surprising result was obtained in the case of methanol as the solvent, since the desired product (**215**) was only formed with a yield of 7 % and **217** and **218** β- and α-methyl glucosides with an 83 % yield, in a 5:1 ratio were obtained as main products. The formation of methyl glycosides could only be explained by the fact, that methanol, being present in a large excess, also acted as a nucleophile (Scheme 8, Table 1).



a) 10 equiv. NaN_3, MeOH, reflux, 12 h.

Scheme 8

1,2-Thio-migration reactions of tritylthio-glycoside with 10 equiv. sodium-azide at 80 °C, using different kinds of solvents

solvent	**215** (yield %)	**216** (yield %)	**217** and **218** (overall yield)	reaction time
DMSO	47	8	0	24 h
DMF	27	15	0	72 h
acetonitril	0	78	0	24 h
methyl-ethyl ketone	0	72	0	24 h
methanol	7	0	83%	12 h

Table 1

Finally, to summarize the behaviour of the 1,2-thio-migration reactions in the presence of different nucleophiles the followings can be stated: usually, in the course of the migration reactions the formation of the 1,2-*trans* product could be observed, but in the case of the bulky tritylthio group the stereoselectivity disappeared. According to the reactions carried out it is clearly seen, that the quality of the thio group, the nucleophile and the solvent used highly influences the outcome of these reactions. It was also found, that out of the prepared 1-thio compounds the trityl thio, the 2-(trimethylsilyl)ethylthio and the acetylthio derivatives are excellent starting materials for the preparation of sugar-2-sulfonates, since they are readily oxidized into sulfonates after migration.

By the use of sodium-acetate as the nucleophile the preparation of such a 2-thio group (oxidizable into a sulfonate) containing compound became possible, that has an O-

acetyl group at the anomeric position and, therefore, directly, or after minimal transformations (e.g. trichloroacetimidate preparation) can be used as a glycosyl donor. It is planned, that such type of donors will be used in the synthesis of oligosaccharides with an analogous structure to the glycosaminoglycan oligosaccharides.

4. Summary

In conclusion, the synthesis of the 6-deoxy-L-talopyranose part, sulfated at position 4, of the core region of the glycopeptidolipid of *M. avium* and its *rhamno* counterpart, namely the methyl-4-*O*-sodiumsulfonato-α-L-rhamnopyranoside were successfully synthesized as methyl glycosides. The 4-sulfonate and 4-methylenesulfonate analogues of both compounds were also prepared.

The synthesis of the *talo* and *rhamno*-4-methylene sulfonate target compounds were accomplished from the appropriate 4-exomethylene derivatives in two different methods. In the first method the acetylthiomethyl derivative, obtained by addition of thioacetic acid, was oxidized to a methylene sulfonate either with Oxone, or with hydrogen peroxide. In the second method the addition of $NaHSO_3$ gave the desired compound in one step.

The *talo* and *rhamno* sulfonates were achieved by an intermolecular nucleophilic substitution, by the use of a good leaving group (triflate), and potassium thioacetate as the nucleophile and by the oxidation of the thus formed thioacetate to a sulfonate.

Intramolecular nucleophilic substitution reactions (thio-migration) were also used in the preparation of secondary sulfonates. Four new-type thiol protecting groups (trityl, 2-(trimethylsilyl)ethyl-, acetyl- and allyl-) were used. The 2-thio groups, obtained via the migration, were successfully transformed into the appropriate 2-sulfonate in all cases. Following this procedure 2-sulfonates of *gluco* and *manno* configuration were prepared.

5. List of publications

Papers related to the subject of the dissertation

1. **L. Lázár**, M. Csávás, A. Borbás, Gy. Gyémánt and A. Lipták; Synthesis of Methyl 6-Deoxy-4-*O*-(sodium sulfonato)-α-L-talopyranoside, Its C-4 Epimer and Both Isosteric [4-C-(Potassium sulfonatomethyl)] Derivatives; *ARKIVOC*, vii (**2004**)196-207.

2. F. Sajtos, **L. Lázár**, A. Borbás, I. Bajza and A. Lipták; Glycosyl Azides of Sugar 2-Sulfonic Acids; *Tetrahedron Letters*; 46 (**2005**) 5191-5194.

3. **L. Lázár**, I. Bajza, Zs. Jakab and A. Lipták; 1,2-*trans*-Glycosyl Azides of Sugar 2-Sulfonic Acids; *Synlett*; 14 (**2005**) 2242-2244.

Lectures (L) and posters (P) related to the subject of the dissertation

1. A. Lipták, **L. Lázár**, F. Sajtos, E. Balla and A. Borbás; Sugar C-sulfonic acids and sugar methylene-sulfonic acids; XII. European Carbohydrate Symposium, Grenoble, France, July 6-11, 2003. (P)
2. **L. Lázár**; A. Borbás and A. Lipták; Synthesis of sulfonic acid and sulfate ester derivatives of methyl 6-deoxy-α-L-manno- and α-L-talopyranosides; 1st Austrian-Hungarian Carbohydrate Conference, Burg Schlaining, Austria, 2003. (P)

3. A. Lipták, A. Borbás, **L. Lázár** and M. Csávás; Different tipe of sugar C-sulfonic acids; 6th Hungarian-Korean Symposium on Organic Chemistry; Incheon, Korea, 2004, Abstract Book, p: 22-32. (E)

4. A. Lipták, A. Borbás, **L. Lázár**, M. Csávás and F. Sajtos: New types of sugars: sugar sulfonic acids and sugar methylene sulfonic acids; 2nd International Symposium of Rare Sugars, Takamatsu, Kagava, Japan, May 27-29, 2004. (E)

5. **Lázár L**.; Bajza I. és Lipták A.; 2-Szulfonsav-glükozil-azidok előállítása 1→2 tiovándorlási reakciók felhasználásával; MTA Szénhidrátkémiai Munkabizottság Előadóülése, Debrecen, 2004. november 5. (E)

6. **Lázár L**.; Bajza I. és Lipták A.; Cukor 2-szulfonsavak előállítása új tiolvédőcsoportok vándoroltatása és a nyert termékek oxidációja révén; Vegyészkonferencia, Hajdúszoboszló, 2005. június 28-30. (P)

7. **L**. **Lázár**; I. Bajza and A. Lipták; Synthesis of sugar 2-sulfonic acids by 1,2-thio-migration and subsequent oxidation; 8th Summer School on Green Chemistry, Venice, Italy, September 4-10, 2005. (P)