Synthesis and structural analysis of sugar sulfonic acids

Ph.D. Theses

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1. Introduction

The goal of our work was to develop general methods for the synthesis of sugar sulfonic acids. Sulfonated carbohydrates can be mimetics of different negatively charged sugars, due to their strong ionic character, allowing them to interact with proteins. At the same time, they can stay in the circulation for longer time because they resist hydrolytic effect of esterases. Another motivation of our research was the fact that there have been no report on the synthesis of secondary sugar sulfonic acids in the literature, except of an uncharacterised one.

Sugar 2-sulfonic acids can be prepared by 1,2-thiomigration reactions using thioglycosides containing good leaving group at position 2. Migration reaction generated by a nucleophile can result in the corresponding 2-*S*-alkyl/aryl/aralkyl compound. From this, the 2-SH group can be liberated, and it can be oxidized to the desired compound. First, the 1,2-thiomigration reactions were investigated in the case of different model compounds and nucleophiles. Our intention with these examinations was to clarify all the details of the 1,2-thiomigration reaction.

In order to prepare carbohydrates sulfonated at other positions than C-2 nucleophilic displacement reactions were also examined. According to the literature sugar containing good leaving group at primary position can be reacted with KSAc as a nucleophile, and then oxidized into sulfonic acid. Applying this method, theoretically, AcS group can be formed at secondary position, and thus the corresponding sulfonic acid can be obtained.

Having succeeded to develop certain method in case of monosaccharides, with the help of which sulfonic acid can be introduced into each position our aim was to extend these methods for disaccharides, too, and meanwhile to collect experiences about different procedures. As the first step of this project a sulfonated disaccharide was produced which was analogue of repeating unit of hialuronic acid.

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.

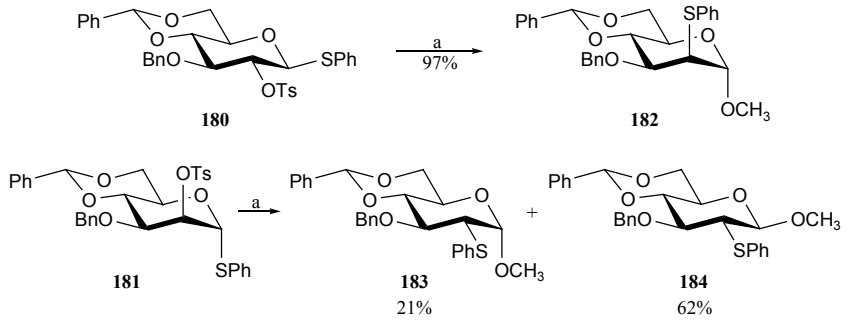
3. New scientific results

3.1. Migration reactions of thiophenyl glycosides

In accordance with our aims the 1,2-thiomigration reactions were investigated in the case of hexopyranosides. Phenyl 1-thio-β-D-glucoside (**180**[*]) and α-D-mannoside (**181**) were prepared containing tosyloxy group at C-2 as starting materials for the 1,2-thiomigration reactions. In the first case, the aglycon and the leaving group at C-2 are in *diequatorial* position, and in the second one in *diaxial*.

These compounds were reacted with 5 equiv. of sodium methylate in methanol at reflux temperature. From the *gluco* compound (**180**) one product was formed after four hours: methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*S*-phenyl-2-thio-α-D-mannopyranoside (**182**). On the other hand, from the *manno* starting material (**181**) two anomers were formed in an overnight reaction: methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*S*-phenyl-2-thio-α-D- (**183**) and β-D-glucopyranoside (**184**) (Scheme 1).

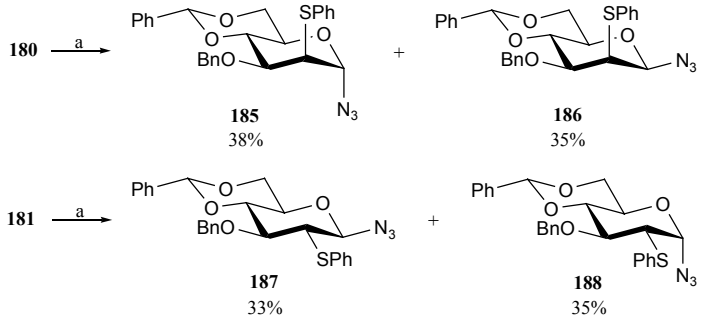
[*] Numbering of compounds refers to that used in the dissertation.



(a) $NaOCH_3$, CH_3OH, reflux.

Scheme 1

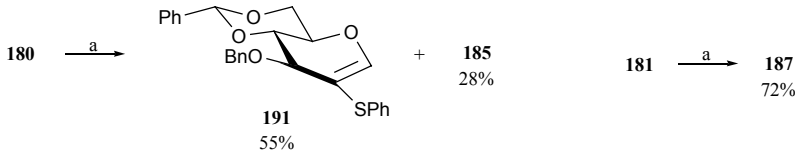
The 1,2-thiomigration reactions were also studied in the presence of azide nucleophile. The same thioglycosides (**180** and **181**) were treated with NaN_3 at 80 °C in *N*,*N*-dimethylformamide for overnight. From the reaction of **180** two compounds (**185** and **186**) were isolated, and **181** gave also two products (**187** and **188**) in both instances, in a 1:1 ratio (Scheme 2). The tosylates **180** and **181** were also reacted with trimethylsilyl-azide to study the effect of the counter-ion on the reaction. Starting from **180**, the 1,2-*trans* product (**185**) was isolated only in a yield of 28%, and the major product was the glycal **191** (55%). In contrast, compound **181** gave only the 1,2-*trans* product **187** in good yield (72%) and no glycal formation was observed (Scheme 3).



(a) NaN_3, DMF, 80 ºC, overnight.

Scheme 2

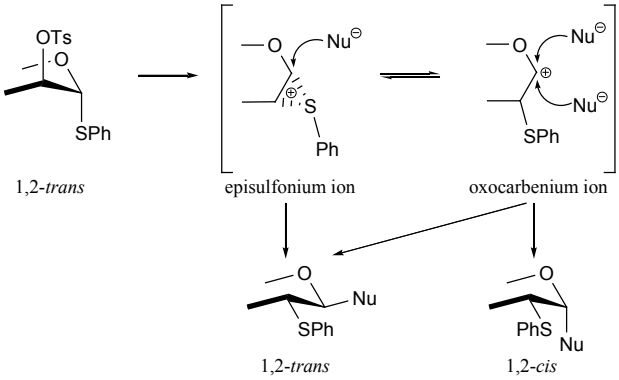
In those cases when only 1,2-*trans* products were formed, in our opinion, the episulfonium ion was the intermediate of the migration reactions. This covered the corresponding side of the sugar ring, so the nucleophile could attack solely from the



(a) $(CH_3)_3SiN_3$, DMF, 80 °C, overnight.

Scheme 3

other side. If the episulfonium ring opened, the oxocarbenium ion was formed, which could be attacked from both sides. It may have been the reason for the formation of the 1,2-*cis* products. The glycal **191** could be formed from the episulfonium ion by loss of the proton at C-2 and cleavage of the C-1–S bond (Scheme 4).

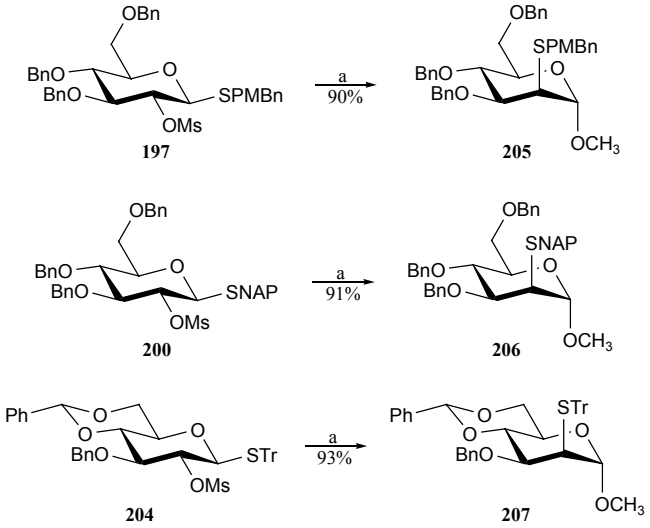


Scheme 4

3.2. Synthesis of sugar 2-sulfonic acids

After the model studies compounds were synthesized, which were suitable for the synthesis of sugar 2-sulfonic acids. For this reason, *p*-methoxybenzyl (**197**), (2-naphthyl)methyl (**200**) and trityl 2-*O*-methanesulfonyl-1-thio-β-D-glucopyranosides (**204**) were prepared. Having utilized the previous observations, the sodium methylate was chosen as nucleophile in the migration reactions. The same reaction conditions were used like in the case of thiophenyl glucoside (**180**). All of the three reactions gave

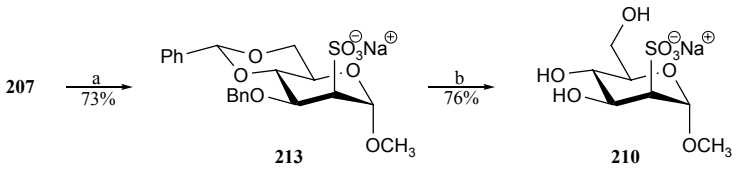
the corresponding methyl 2-*S*-aralkyl-2-thio-α-D-mannopyranosides (**205**-**207**) in high yields and with complete stereoselectivity, resembling the model reaction (Scheme 5).



(a) $NaOCH_3$, CH_3OH, reflux, 4 h.

Scheme 5

Deprotection of the S-protected compounds was carried out by different methods, and the oxidation reactions gave the corresponding sugar sulfonic acids. The most suitable starting material was the 2-*S*-derivative (**207**), which could be oxidized directly by Oxone, in the presence of KOAc in acetic acid (→**213**). Protecting groups were hydrogenolyzed in ethanol in the presence of 10% Pd(C) catalyst to give methyl 2-deoxy-2-sodiumsulfonato-α-D-mannopyranoside (**210**) (Scheme 6).

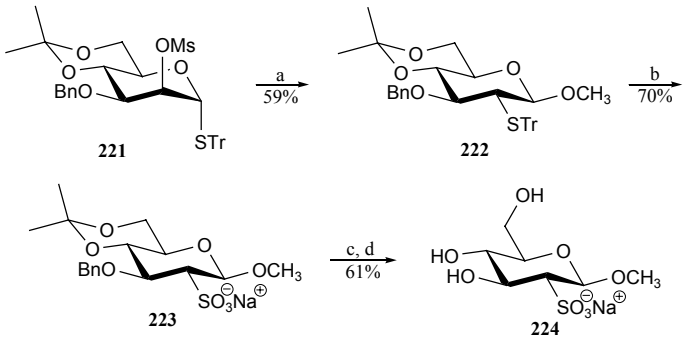


(a) Oxone, KOAc, AcOH, RT, 5 h; (b) H_2-Pd(C), AcOH, EtOH, RT, 24 h.

Scheme 6

The easy transformation of the sugar thiotrityl ether into sugar sulfonic acid prompted us to prepare suitably protected trityl 1-thio-α-D-mannopyranoside to be used

for the synthesis of 2-sulfonic acid of D-glucose. Trityl 3-*O*-benzyl-4,6-*O*-isopropylidene-2-*O*-mesyl-1-thio-α-D-mannopyranoside (**221**) was prepared, and treated with 10 equiv. of $NaOCH_3$ in dichloromethane–methanol (1:1) at reflux for 24 h. The intramolecular thiotrityl migration proceeded with excellent stereoselectivity and methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-2-*S*-trityl-2-thio-β-D-glucopyranoside (**222**) was isolated. Direct oxidation of the thiotrityl ether gave the protected 2-sulfonic acid of methyl β-D-glucoside (**223**). The isopropylidene group was hydrolyzed with diluted TFA in dichloromethane, and the benzyl group could be removed by catalytic hydrogenolysis (→**224**) (Scheme 7).



221 **222** **223** **224**

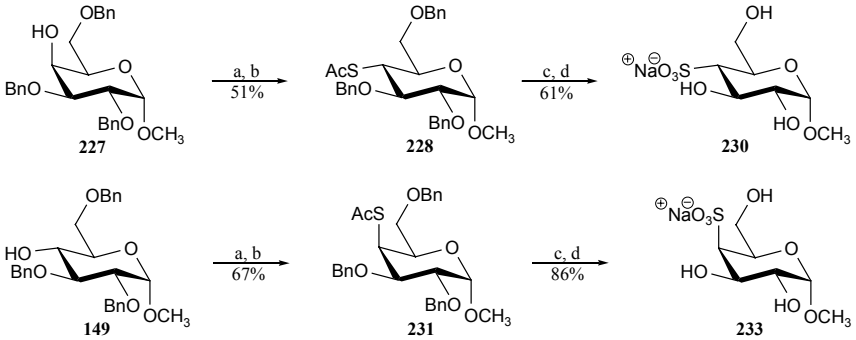
(a) $NaOCH_3$, CH_2Cl_2-CH_3OH (1:1), reflux, 24 h; (b) Oxone, KOAc, AcOH, RT, 5 h; (c) TFA, H_2O, CH_2Cl_2, RT, 3 h; (d) H_2-Pd(C), AcOH, EtOH, RT, 24 h.

Scheme 7

3.3. Preparation of sugar 4- and 6-sulfonic acids by nucleophilic displacement reactions

Nucleophilic displacement reactions were also used for the synthesis of sugar sulfonic acids. *Gluco* (**149**, **234**) and *galacto* (**227**, **237**) derivatives containing free 4-OH or 6-OH group were trifluoromethanesulfonylated, and the crude products were treated with KSAc, respectively. According to the mechanism of S_N2 type reactions, from compound **227** 4-*S*-acetyl-glucoside (**228**), and from the **149** 4-*S*-acetyl-galactoside (**231**) were isolated. Oxidation of these compounds followed by removal of

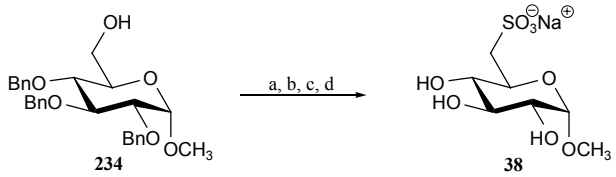
the benzyl groups yielded the methyl 4-deoxy-4-sodiumsulfonato-α-D-gluco- (**230**) and galactopyranosides (**233**) (Scheme 8).



(a) Tf_2O, Py, CH_2Cl_2, -10 °C, 1 h; (b) KSAc, DMF, 90 °C, 3 h;
(c) Oxone, KOAc, AcOH, RT, 5 h; (d) H_2-Pd(C), AcOH, EtOH, RT, 24 h.

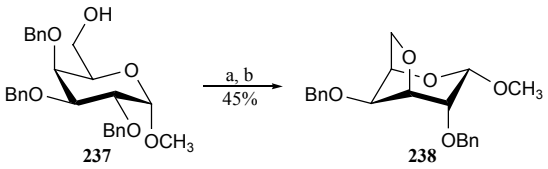
Scheme 8

During synthesis of the primary derivatives there is no inversion. From the *gluco* compound (**234**) in the same sequence of above mentioned reactions methyl 6-deoxy-6-sodiumsulfonato-α-D-glucopyranoside was obtained (**38**) (Scheme 9).



(a) Tf_2O, Py, CH_2Cl_2, -10 °C, 1 h; (b) KSAc, DMF, 90 °C, 3 h;
(c) Oxone, KOAc, AcOH, RT, 5 h; (d) H_2-Pd(C), AcOH, EtOH, RT, 24 h.

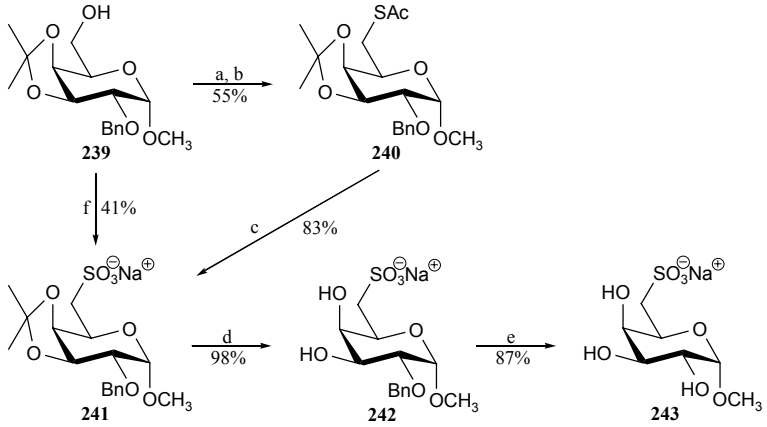
Scheme 9



(a) Tf_2O, Py, CH_2Cl_2, -10 °C, 1 h; (b) KSAc, DMF, 90 °C, 3 h.

Scheme 10

Triflation of the galactoside compound (**237**) gave the 6-*O*-triflate derivative, but an intramolecular displacement reaction occured during treatment with potassium thioacetate, and methyl 3,6-anhydro-2,4-di-*O*-benzyl-α-D-galactopyranoside (**238**) was formed (Scheme 10). The presence of a 3,4-*O*-isopropylidene ring can prevent the $^4C_1 \rightarrow {}_1C^4$ conformational flip. Based on this observation a new route was worked out for the synthesis of methyl 6-deoxy-6-sodiumsulfonato-α-D-galactopyranoside (**243**). Compound **239** was converted into the thioacetyl derivative **240**, and its oxidation gave the fully protected sulfonic acid **241**. This was transformed into the methyl 6-deoxy-6-sulfo-α-D-galactopyranoside sodium salt (**243**) after removal of the isopropylidene (→**242**) and benzyl groups. In another approach, the triflate from **239** was treated with Na_2SO_3 in ethanol–water (1:1). The yield of this sequence was similar to that of the first procedure, but it was five times faster, and simpler (Scheme 11).

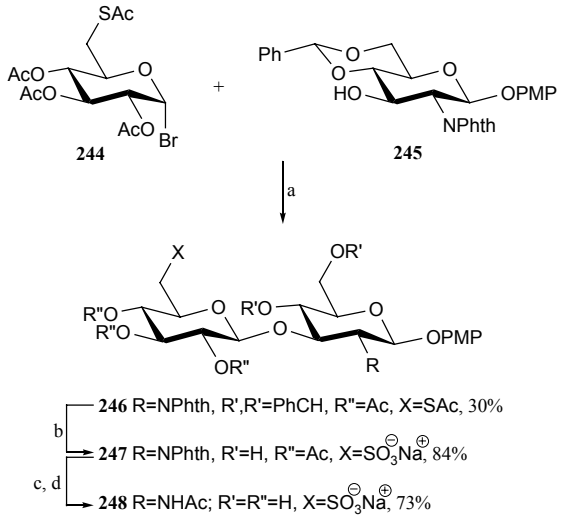


(a) Tf_2O, Py, CH_2Cl_2, -10 ºC, 1 h; (b) KSAc, DMF, 90 ºC, 3 h;
(c) Oxone, KOAc, AcOH, RT, 5 h; (d) AcOH, CH_2Cl_2, H_2O, RT, 3 h;
(e) H_2-Pd(C), AcOH, EtOH, RT, 24 h; (f) Na_2SO_3, EtOH-H_2O (1:1), reflux, 45 min.

Scheme 11

3.4. Synthesis of a disaccharide containing sulfonic acid

In accordance with the primary goals a sulfonated disaccharide (**248**) was also synthesized, which is a mimetic of hialuronic acid. Suitably protected glucosamine derivative (**245**) was glucosylated with the bromosugar **244**, containing acetylthio group at position 6. In the coupling reaction the AcS group did not change, so after the isolation of the disaccharide **246** was oxidized with hydrogen peroxide in acetic acid and simultaneously the benzylidene acetal was removed (→**247**). Phthaloyl and acetyl groups were removed with ethylenediamine, and the desired compound **248** was prepared with subsequent *N*-acetylation.



(a) AgOTf, CH_2Cl_2, toluene, -40 ºC, 3 h; (b) 30% H_2O_2, NaOAc, AcOH, 40 ºC, overnight; (c) $NH_2CH_2CH_2NH_2$, EtOH, reflux, 5 h; (d) Ac_2O, CH_3OH, 0 ºC, 1 h.

Scheme 12

4. Summary

In summary, the preparation of 1,2-*trans*-2-sulfonic acids was solved based on the studies of thiomigration reactions in the case of model derivatives. The most

successful compounds were the 1,2-*trans*-thiotrityl glycosides from which the desired sugar 2-sulfonic acids colud be obtained after migration reactions, oxidations, and removal of the protecting groups.

Nucleophilic displacement reactions were also used for the synthesis of sugar sulfonic acids. With the help of this method glucose and galactose derivatives containing 4- and 6-sulfonic acids were prepared.

A sulfonated disaccharide was also synthesized by glucosylation reaction of a glucosyl donor containing acetylthio group, and subsequent oxidation. This way seems to be very promising later on, and the combination of it with migration reaction additional sulfonic acid containing mimetics can be prepared.

5. List of publications

Papers related to the subject of the dissertation

1. András Lipták, **Ferenc Sajtos**, Lóránt Jánossy, Dietmar Gehle and László Szilágyi: A General Method for the Synthesis of Sugar 2-*C*-Sulfonic Acids by 1→2 Arylthio Group Migration in Acid-Sensitive Thioglycosides. Direct Transformation of Thiotrityl Ethers into *C*-Sulfonic Acids;
   Org. Lett., **5** (2003) 3671-3674.

2. András Lipták, Edit Balla, Lóránt Jánossy, **Ferenc Sajtos** and László Szilágyi: The First Synthesis of Secondary Sugar Sulfonic Acids by Nucleophilic Displacement Reactions;
   Tetrahedron Lett., **45** (2004) 839-842.

Other paper

3. **Ferenc Sajtos**, János Hajkó, Katalin E. Kövér, András Lipták:
   Synthesis of the α-D-GlcpA-(1→3)-α-L-Rhap-(1→2)-L-Rha trisaccharide isolated from the cell wall hydrolyzate of green alga *Chlorella vulgaris*;
   Carbohydr. Res., **334** (2001) 253-259.

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

1. **Sajtos Ferenc**, Lipták András:
   2-*O*-Tozil csoportot tartalmazó tioglikozidok átrendeződése;
   XXIV. Kémiai Előadói Napok, 2001. október 29-31., Szeged. (L)

2. András Lipták, Dietmar Gehle, **Ferenc Sajtos** and Edit Balla:
   Thioglycoside rearrangement by 1→2-thiomigration into 2-thio sugars and their oxidation to sugar 2-sulfonic acids;
   XXIst International Carbohydrate Symposium, 7-12 July 2002, Cairns, Australia. (P)

3. Anikó Borbás, **Ferenc Sajtos**, Gábor Májer and András Lipták:
   Preparation of *C*-sulfated sugar donors for the synthesis of *C*-sulfate containing Sialyl Lewis X analogues;
   Third Pan-Pacific conference on „Sialoglycosience and Other Novel forms of glycosylation", 14-17 July 2002, Gold Coast, Australia. (P)

4. András Lipták, László Lázár, **Ferenc Sajtos**, Edit Balla and Anikó Borbás:
   Sugar *C*-sulfonic acids and sugar methylene-sulfonic acids;
   XII. European Carbohydrate Symposium, 6-11 July 2003, Grenoble, France. (P)

5. András Lipták, Anikó Borbás, László Lázár, Magdolna Csávás and **Ferenc Sajtos**:
   New types of sugars: sugar sulfonic acids and sugar methylene sulfonic acids;
   2nd International Symposium of Rare Sugars, 27-29 May 2004, Takamatsu, Kagava, Japan. (L)

6. **Ferenc Sajtos**:
   Preparation of unknown sugar sulfonic acids;
   Summer Course Glycosciences, 28 June - 1 July 2004, Wageningen, The Netherlands. (P)

7. **Sajtos Ferenc**, Bajza István, Lipták András:
   Fenil-1-tio-2-*O*-tozil-β-D-glüko- és α-D-mannopiranozidok nukleofil szubsztitúciós reakciói;
   MTA Kém. Tud. Oszt., Szénhidrátkémiai Munkabizottság Előadóülése, 2004. november 5., Debrecen. (L)