Synthesis of an arabinogalactan-type oligosaccharide series

theses of doctoral (PhD) dissertation

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I. Introduction and objectives of the dissertation

Carbohydrates play important roles in the living organisms not only as energy resources, but also as biological information carriers. This fact was recognized only recently, since the techniques for isolation, purification and elucidation, as well as the sensitivity of the devices (HPLC, MS, GC-MS, NMR) have developed revolutionary in the last decades. Owing to this development numerous biologically active carbohydrates could be isolated from glycoconjugates, and their structures were determined by modern methods.

The studies of glycoconjugates help us to understand the role of the carbohydrate portion of glycoconjugates: the communication of the cells with their environment, the cell-cell interaction, and the organisation of cells into tissues.

The recognition of the biological roles of the oligosaccharides has brought new challenges for chemists. The synthesis of oligosaccharides with longer or branched chains necessitated new block syntheses, protecting group strategies, stereospecific glycosylation methods and further developments in the techniques for the isolation of compounds and the elucidation of their structures. The syntheses and studies of the biologically active natural compounds or their units and analogues, open up options to examine the connection between the structure and the biological activity.

The present work describes the synthesis of an arabinogalactan-type oligosaccharide series which play important roles in the field of fitobiology and immunology. Arabinogalactan polysaccharides were isolated from the cell-culture exudates of *Echinacea purpurea*, but the exact structures of the active compounds are unknown.

My main goal was to prepare the anticipated repeating unit of the isolated natural arabinogalactans. During the synthesis of these oligosaccharides the compatibility of the used protecting groups was examined. I could successfully tune the protecting group strategy which reduced the large number of the synthetic steps. I hope the method will find application in the synthesis of other complex oligosaccharides, too.

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II. Methods applied

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

Thin layer-, high pressure liquid-, and gas chromatography were applied to follow the reactions, to control the purity of the substances and to determine the ratios of the products of the reactions. In addition to classical crystallization, column chromatography was used for the purification of the crude products and for the separation of the isomers.

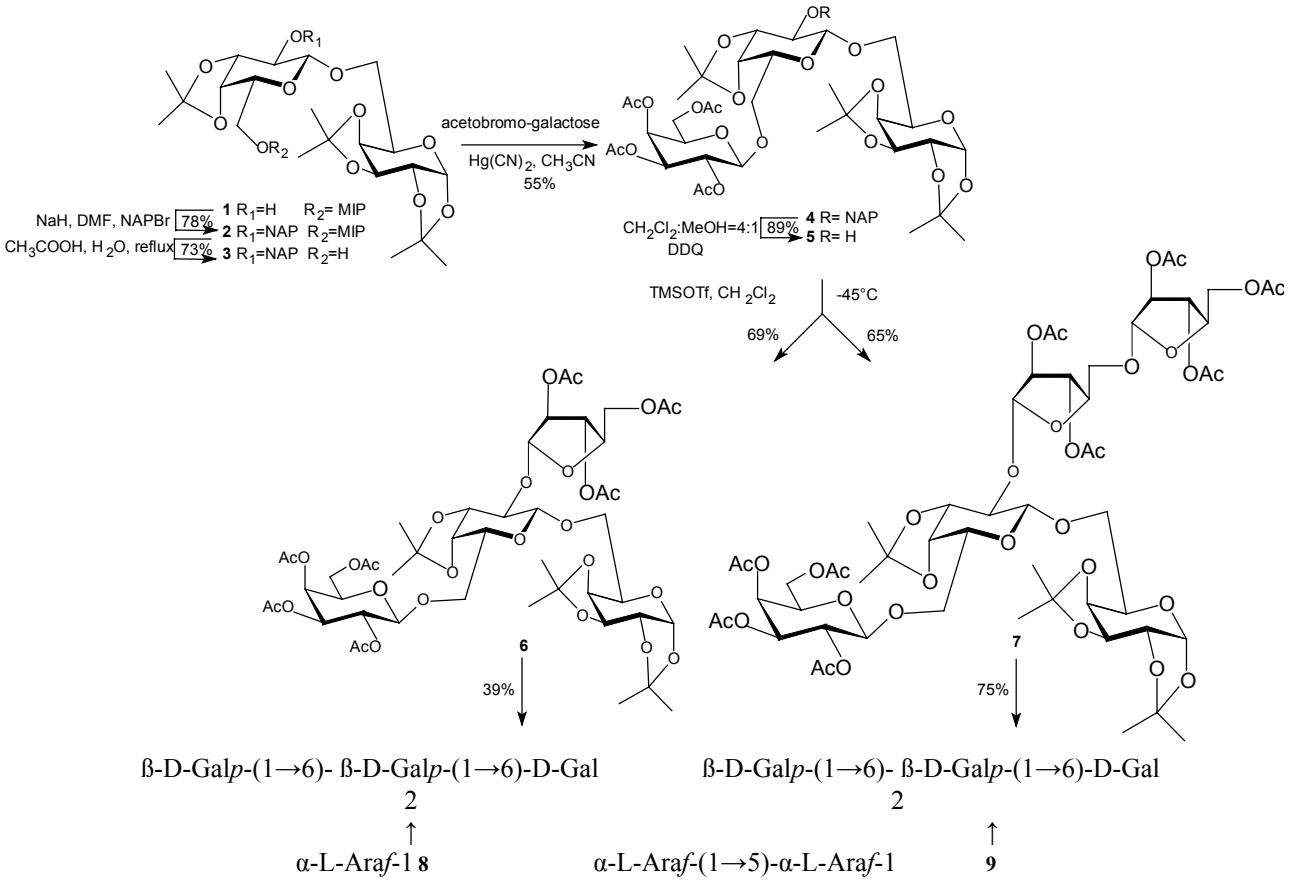
Besides classical methods (elemental analysis, melting point and optical rotation determination) modern spectroscopic methods (one- and two dimensional NMR and mass spectrometry) were applied for the verification of the structures of the compounds synthesized.

III. New scientific results of the dissertation

The syntheses of the presumed repeating units of the cell-cultured exudates of *Echinacea purpurea* are discussed in the dissertation. A ß-(1→6)-linked galactan skeleton is anticipated with α-L-arabinofuranosyl or α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl branch in every second or third galactopyranosyl units at position 2.

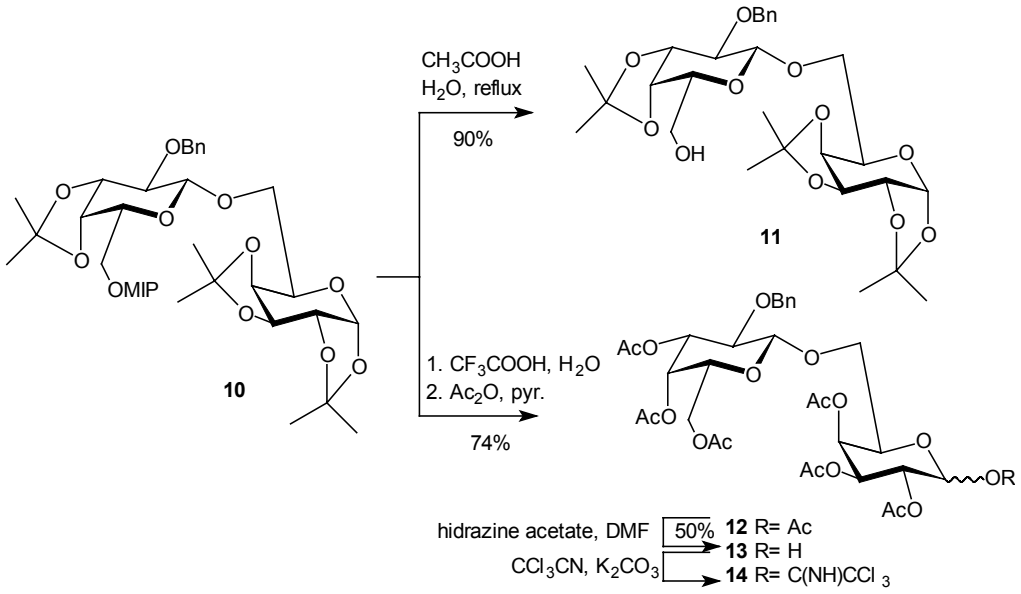
III.1. Synthesis of the arabinogalactan-type tetra- and pentasaccharide

The synthesized free tetra- and pentasaccharide have common ß-(1→6)-linked trigalactan skeleton, which was prepared from the known disaccharide **1** as follows: the OH-2 group of compound **1** was protected with (2-naphthyl)methyl (NAP) group (**2**), and after the removal of the 6'-*O*-MIP group (**3**), it was glycosylated with acetobromo-galactose donor compound. The NAP group of the fully protected trisaccharide **4** was removed by oxidative way, so we could use the obtained compound **5** as a glycosyl acceptor. Trisaccharide **5** reacted with mono- and diarabinosyl donor compound, thus we could isolate the fully protected tetra- (**6**) and pentasaccharide (**7**) in an acceptable yield. Common procedure was followed for the deprotection: first, the acetyl groups were removed by Zemplén-method and second, acid hydrolysis was done and the free arabinogalactan-type tetra- (**8**) and pentasaccharide (**9**) were isolated.

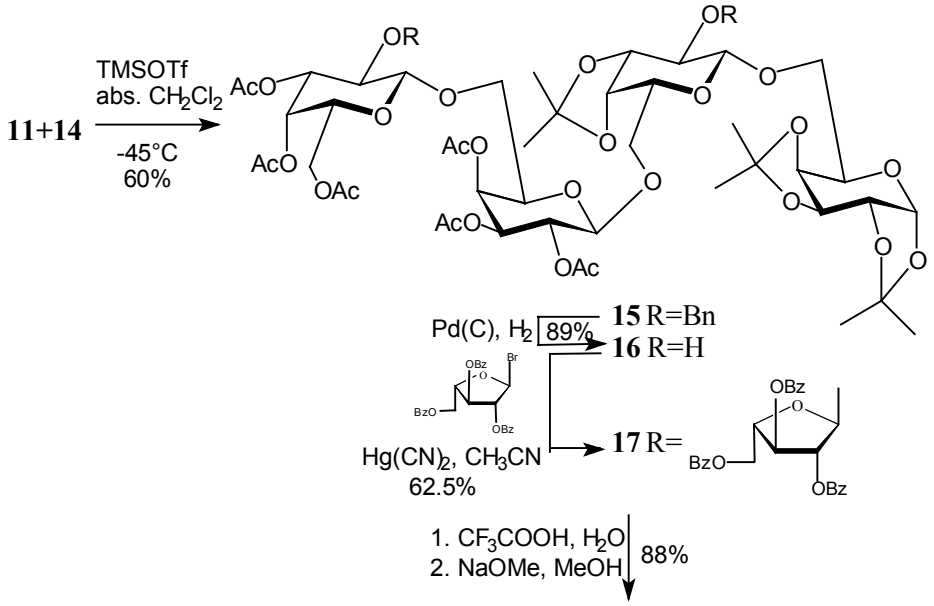


III.2. Synthesis of the arabinogalactan hexasaccharide

Our main goal was to prepare a hexasaccharide compound which has a ß-(1→6)-linked tetragalactan skeleton with an α-L-arabinofuranosyl branch in the second and the fourth galactopyranosyl units at position 2.



Compound **10,** already known, seemed to be suitable for the synthesis of the glycosyl acceptor and the donor compound as a common starting material. Removal of the MIP-group with acetic acid from the disaccharide **10** the glycosyl acceptor compound (**11**) could be obtained. For the synthesis of the donor, on the other hand, we removed the isopropylidene acetals and a consecutive acetylation gave compound **12**, which was transformed into trichloroacetimidate donor (**14**) after removing of the anomeric acetyl group.



α-L-Ara*f*-(1→2)-ß-D-Gal*p*-(1→6)- ß-D-Gal*p*-(1→6)- ß-D-Gal*p*-(1→6)-D-Gal

2

↑

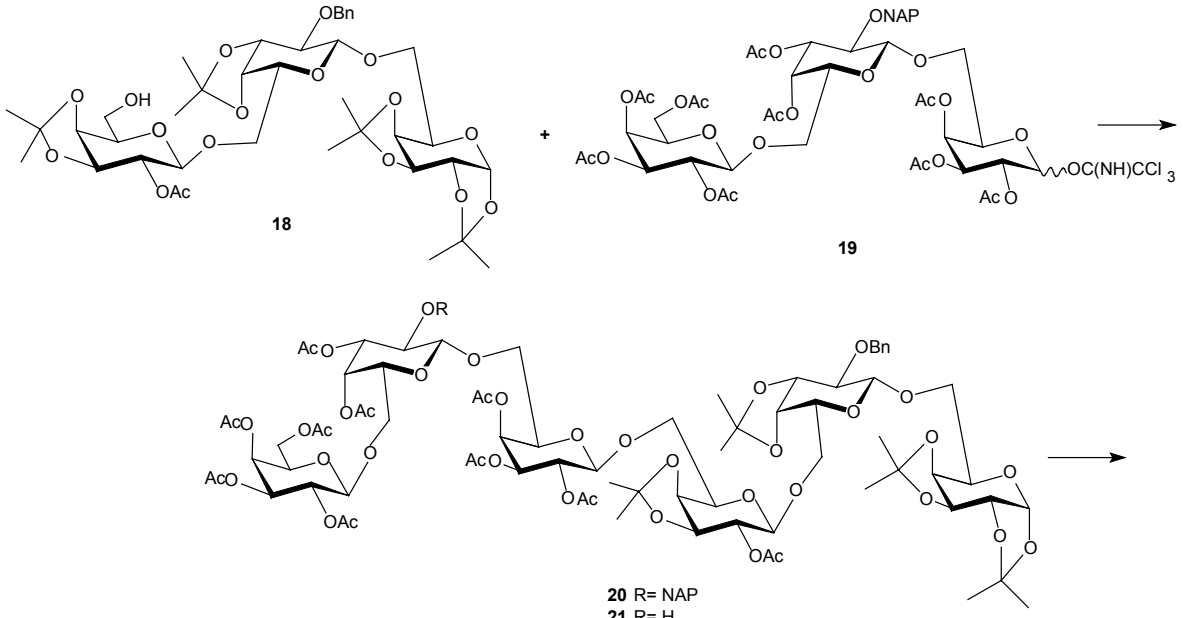
α-L-Ara*f*-1 **18**

TMSOTf-activated coupling reaction resulted in the fully protected tetragalactan **15**. The two –OBn groups were removed by hydrogenolysis (compound **16**), which allowed to introduce the two α-L-arabinofuranosyl branches in one step. The isolated hexasaccharide **17** was deacetylated by Zemplén-method and a consecutive acid hydrolysis gave the free arabinogalactan hexasaccharide **18**.

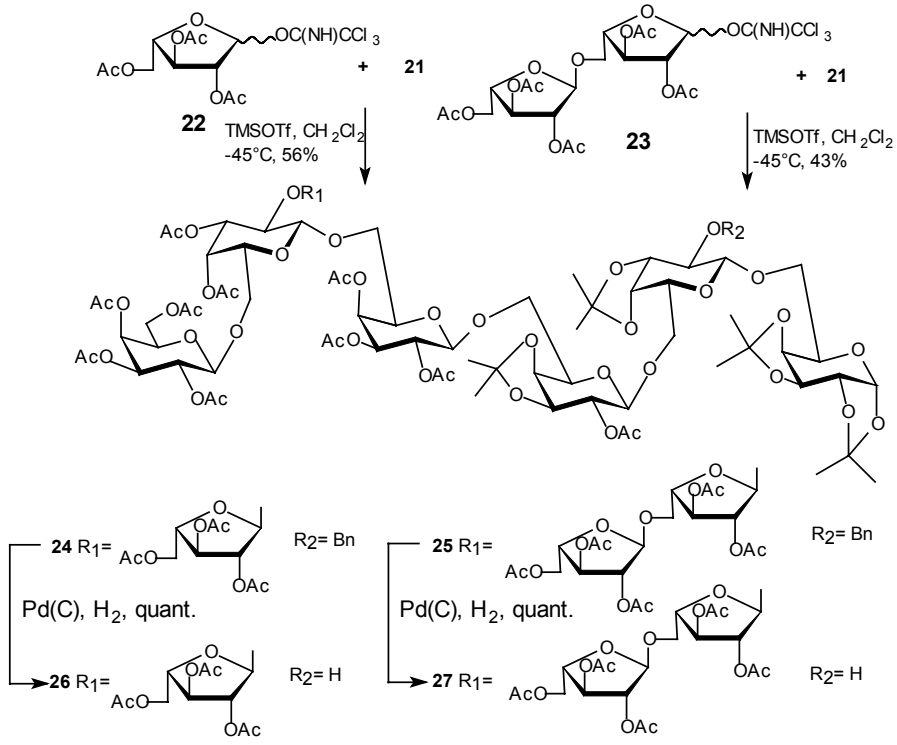
III.3. Synthesis of the arabinogalactan-type octa- and two isomeric nonasaccharides. Suitable tuning of protecting groups.

The planned oligosaccharides have common ß-(1→6)-linked hexagalactan skeleton, which have mono- or diarabinosyl branch in every third galactopyranosyl unit at position 2. The first step was the synthesis of the hexagalactan skeleton by a 3+3 block synthetic

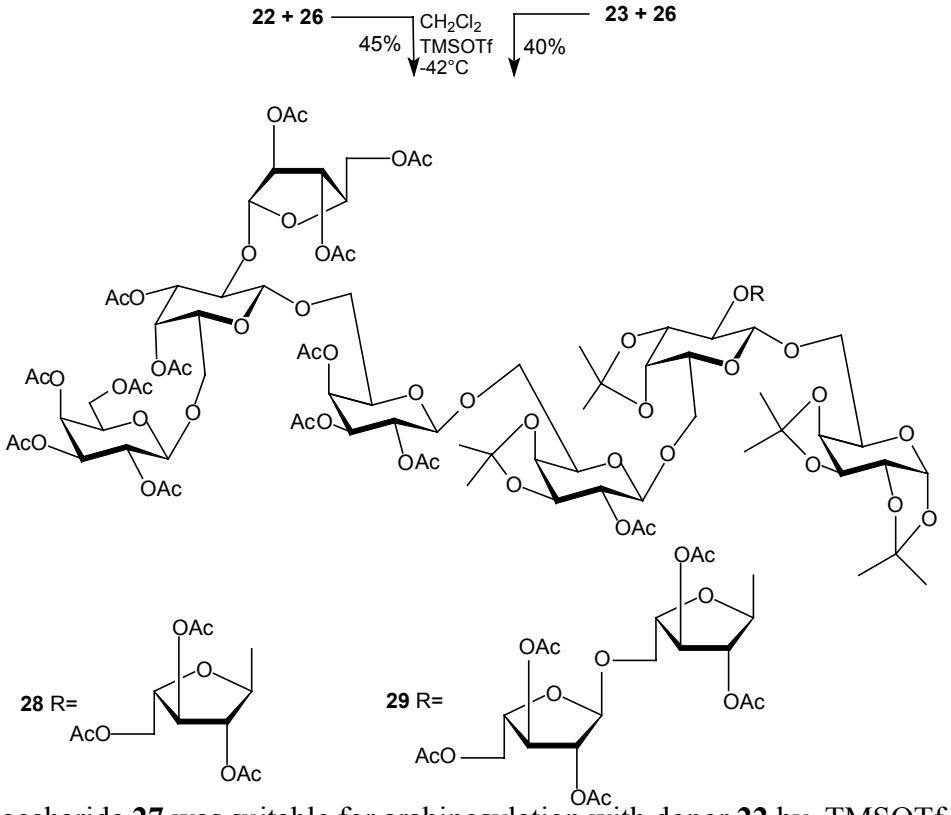
strategy. First we prepared the trisaccharide acceptor (**18**) carrying a 2'-*O*-Bn protecting group, and then the trigalactan donor (**19**) was synthesized having a 2'-*O*-NAP group. TMSOTf-activated glycosylation resulted in the protected hexagalactan skeleton (**20**) in a yield of 59%.



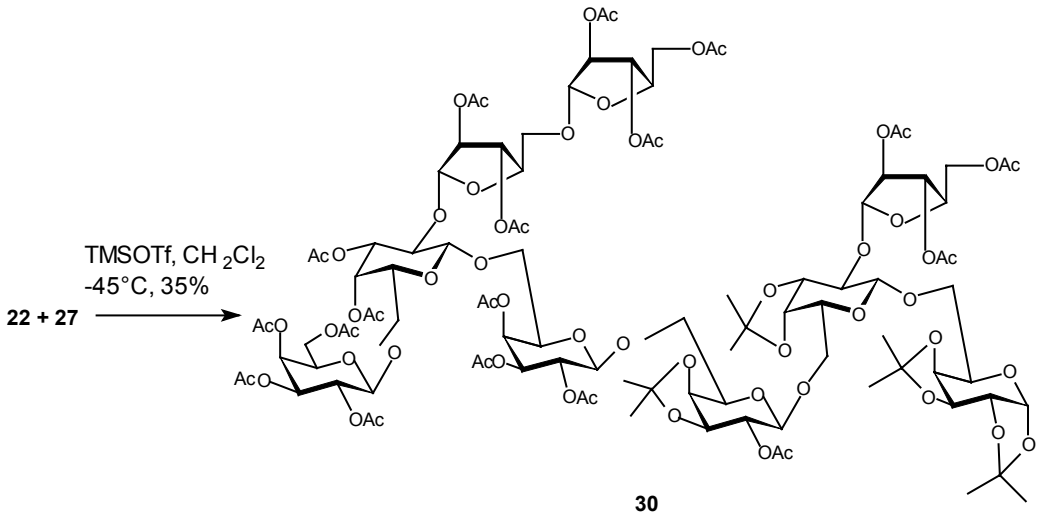
The (2-naphtyl)methyl group can selectively be removed in the presence of benzyl ether by oxidative way using DDQ (70%), so we could prepare a hexasaccharide acceptor which is suitable for introducing branch with either donor **22** or **23**.



The obtained **24** hepta- and **25** octasaccharide can easily be transformed into glycosyl acceptor compounds **26** and **27** by the hydrogenolysis of the benzyl ethers. The heptasaccharide **26** was ready for further arabinosylation, so we could introduce not only mono- but diarabinofuranosyl branches at position 2' to give the fully protected octa- (**28**) and one of the planned nonasaccharides (**29**) .



Finally, octasaccharide **27** was suitable for arabinosylation with donor **22** by TMSOTf activation agent and the isomer nonasaccharide **30** could be isolated in an acceptable yield.



Common procedure was followed for the deprotection of all the three oligosaccharides: Zemplén-deacetylation and acid hydrolysis of the isopropylidene acetals gave the free arabinogalactan-type compounds (**31**, **32** and **33**).

ß-D-Gal*p*-(1→6)- ß-D-Gal*p*-(1→6)-ß-D-Gal*p*-(1→6)- ß-D-Gal*p*-(1→6)-ß-D-Gal*p*-(1→6)-D-Gal

2 2

↑ ↑

1 1

R_1 R_2

31 R_1=R_2=α-L-Ara*f*

32 R_1= α-L-Ara*f* R_2= α-L-Ara*f*-(1→5)- α-L-Ara*f*

33 R_1= α-L-Ara*f*-(1→5)-α-L-Ara*f* R_2= α-L-Ara*f*

IV. Summary

Among the plant tissue proteins the arabinogalactan proteins are the most wide-spread representatives in nature. Their presumed biological functions are participation in cell-cell adhesion, communication or morphogenesis.

Most recently an arabinogalactan fraction possessing biological activity was isolated from the cell-cultured exudates of *Echinacea purpurea*. The exact stucture of these polysaccharides is unknown, although monoclonal antibodies directed against epitopes of the polysaccharides can provide useful and fast structural information.

The present work summarizes the results concerning the synthesis of an arabinogalactan-type oligosaccharide series which have the anticipated repeating unit. The presumed structure is: ß-(1→6)-linked galactan skeleton and each second or third unit was thought to be α-L-arabinofuranosylated or α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosylated at position 2.

We provide an efficient synthesis of a branched arabinogalactan-type oligosaccharide series. Oligosaccharides remain challenging synthetic targets and the suitable tuning of protecting groups will hopefully find application in the synthesis of other complex oligosaccharides.

V. List of publications

V.1. Publications in the field of the dissertation

1. Csávás, M., Borbás, A., Jánossy, L., Batta, Gy., Lipták, A.
Synthesis of the α-L-Ara*f*-(1→2)-ß-D-Gal*p*-(1→6)- ß-D-Gal*p*-(1→6)-[α-L-Ara*f*-(1→2)]- ß-D-Gal*p*-(1→6)-D-Gal hexasaccharide as a possible repeating unit of the cell-cultured exudates of *Echinacea purpurea* arabinogalactan
Carbohydr. Res., **336** (2001) 107-115.
2. Csávás, M., Borbás, A., Szilágyi, L., Lipták, A.
Succesful combination of (methoxydimethyl)methyl (MIP) and (2-naphthyl)methyl (NAP) ethers for the synthesis of arabinogalactan-type oligosaccharides
Synlett, **6** (2002) 887-890.
3. Csávás, M., Borbás, A., Jánossy, L., Lipták, A.
Synthesis of an arabinogalactan-type octa- and two isomeric nonasaccharides. Suitable tuning of protecting groups
Tetrahedron Lett., **44** (2003) 631-635.

V.2. Publications in other fields

1. Borbás, A., Szabovik, G., Antal, Zs., Fehér, K., Csávás, M., Szilágyi, L., Herczegh, P., Lipták, A.
Sulfonic acid analogues of the sialyl Lewis X tetrasaccharide
Tetrahedron:Asymmetry, **11** (2000) 549-566.

V.3. Lectures and posters in the field of the dissertation

1. Csávás, M., Borbás, A., Jánossy, L., Batta, Gy., Lipták, A.
Anticipated repeating unit of the cell-cultured exudates of *Echinacea purpurea* arabinogalactan. Synthesis of the α-L-Ara*f*-(1→2)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-[α-L-Ara*f*-(1→2)]-β-D-Gal*p*-(1→6)-D-Gal hexasaccharide
Annual Meeting of the Committee of Carbohydrates of Chemistry of the Hungarian Academy of Sciences. 2001, Mátrafüred, Hungary.

2. Csávás, M., Borbás, A., Jánossy, L., Batta, Gy., Lipták, A.

Az *Echinacea purpurea* sejttenyészetéből izolált arabinogalaktánban ismétlődő egységként előforduló hexaszacharid szintézise

MKE-Vegyészkonferencia , 2001, Hajdúszoboszló, Hungary.

3. Csávás, M., Borbás, A., Jánossy, L., Batta, Gy., Lipták, A.

Synthesis of the anticipated repeating hexasaccharide unit of the cell-cultured exudates of *Echinacea purpurea* arabinogalactan

11th European Carbohydrate Symposium, 2001, Lisszabon, Portugal.

4. Csávás, M., Borbás, A., Jánossy, L., Lipták, A.

Synthesis of an arabinogalactan-type oligosaccharide series

Annual Meeting of the Committee of Carbohydrates of Chemistry of the Hungarian Academy of Sciences. 2002, Mátrafüred, Hungary.

5. Csávás, M., Borbás, A., Jánossy, L., Lipták, A.

Suitable tuning of protecting groups. Synthesis of an arabinogalactan-type oligosaccharide series

Summer Course Glycosciences. 7th European Training Course on Carbohydrates, 2002, Wageningen, The Netherlands.

V.4. Lectures and posters in other fields

1. Csávás, M., Borbás, A., Lipták, A.

Synthesis of the sulfonic-acid analogues of the sialyl Lewis X tetrasaccharide

2nd East-European Carbohydrate Workshop, 1999, Güstrow, Germany.

2. Csávás, M., Borbás, A., Lipták, A.

Szénhidrát ligandumok szulfonsav analógjainak szintézise

OTDK Konferencia, 2001, Gödöllő, Hungary.