Synthesis of pentasaccharide haptens from glycopeptidolipid antigens of *Mycobacteria*

theses of doctoral (PhD) dissertation

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Debrecen, 2001

I. Introduction and objectives of the dissertation

In the living organisms carbohydrates play important roles not only as energy resource, but also as carriers of biological information. This fact could be recognized only recently, as the techniques for isolation, purification and structure determination, 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 clarified connections which 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 determination of their structures. The syntheses and studies of the biologically active natural compounds, their units, and analogues, open up options to examine the connection between the structure and the biological activity.

Researchers of the Department of Biochemistry at Faculty of Sciences, University of Debrecen and the Research Group for Carbohydrates of the Hungarian Academy of Sciences have had considerable results in the field of the synthesis of oligosaccharide antigens from *Mycobacteria*. They synthesized the oligoglycosyl part of the trehalose-containing lipooligosaccharides from *Mycobacterium smegmatis* and *Mycobacterium kansasii*; the phenolic glycolipid from *Mycobacterium leprae;* and the glycopeptidolipids from the serovariants 8, 14, and 20 of the *Mycobacterium avium* complex.

The present describes the syntheses of the pentasaccharide haptens of the glycopeptidolipids from the serovariant 12 and 17 of the *Mycobacterium avium* complex.

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 substances and to determine the ratios of the products. Besides 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 synthesized compounds.

III. New scientific results of the dissertation

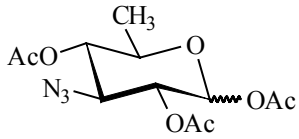
The synthesis of the pentasaccharide from the serovar 17 of the *Mycobacterium avium* complex acylated with (2*S*,3*S*) nilic acid, and the fully protected pentasaccharide from serovar 12 are discussed in the dissertation.

III.1. Synthesis of the pentasaccharide hapten of the glycopeptidolipid antigen from serovar 17 of the Mycobacterium avium *complex*

The synthesis of the terminal monosaccharide unit was carried out starting from D-glucose. The main tasks of the synthesis were a) to introduce an amino group into position 3, and b) to deoxygenate at position 6. The amino function was introduced as an azido precursor, and the 6-deoxy derivative was obtained by the reduction of the 6-O-*p*-toluenesulfonyl group (→**1**).

The key step of the syntheses of the B and C L-*rhamno* carbohydrate units was the regioselective benzylation at position 2 by the two-phase method to yield the synthons **2** and **3**.

The trisaccharide **4** was obtained from these monosaccharide units, and the glycosylation of the disaccharide **5** with the trisaccharide yielded the pentasaccharide **6**.



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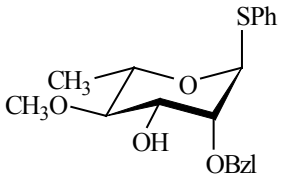

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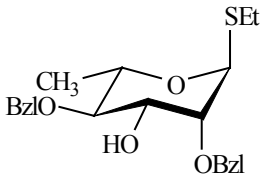

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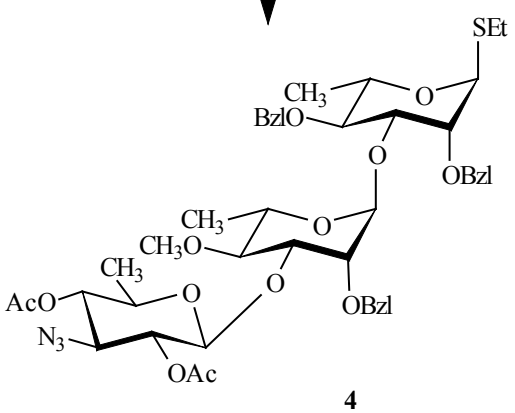

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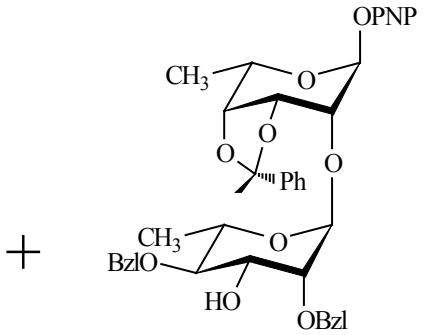

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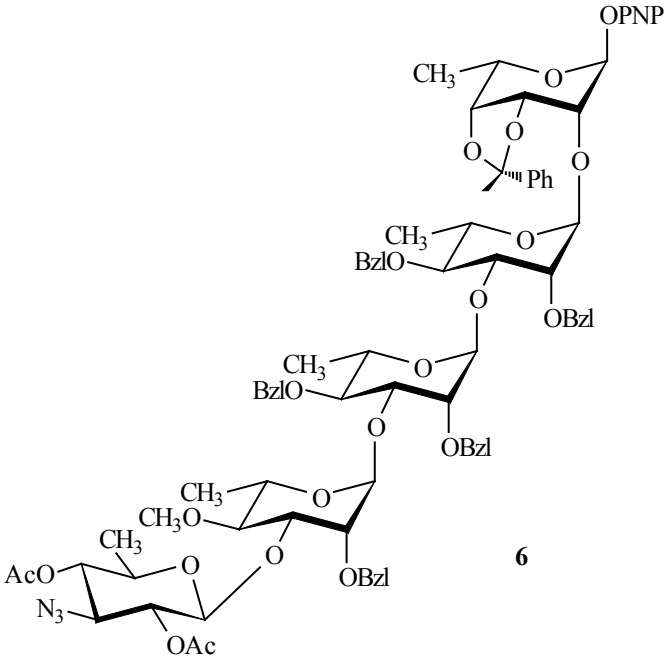

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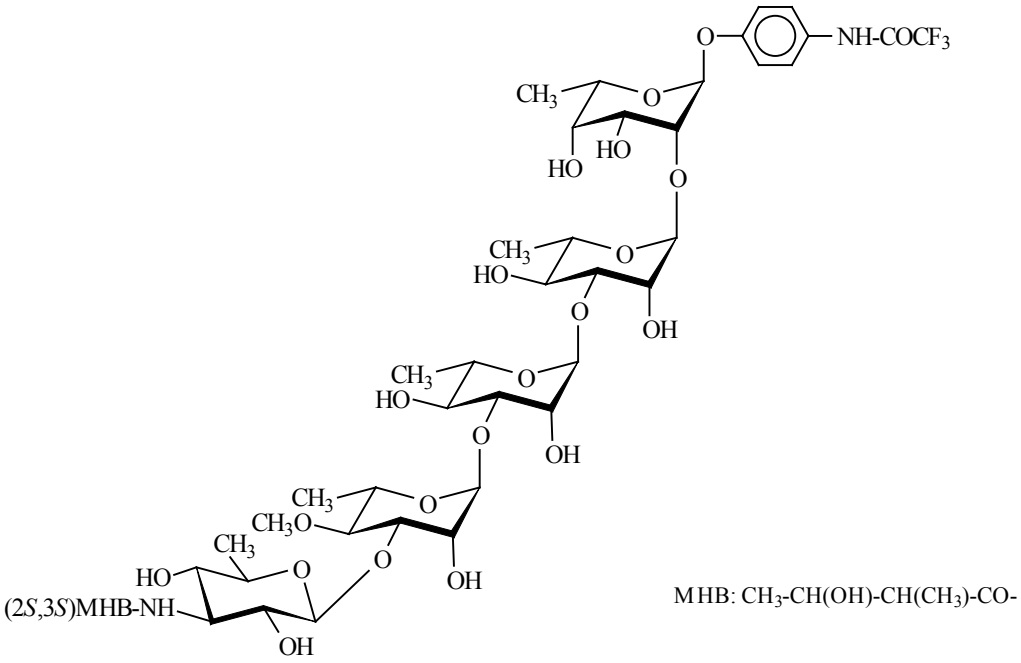
The azido group was reduced to amino, which was acylated with (2*S*,3*S*) nilic acid, then transformation of the aglycone and removal of the protecting groups yielded the target compound **7**.



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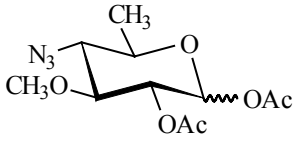
III.1. Synthesis of the pentasaccharide hapten of the glycopeptidolipid antigen from serovar 12 of the **Mycobacterium avium** *complex*

The synthesis of the terminal monosaccharide unit was carried out starting from D-galactose. The main tasks of the synthesis were a) methylation at position 3, b) introduction of an amino group into position 3, and c) deoxygenation at position 6. The OH-3 was methylated with diazomethane, the amino group was introduced as an azido precursor, and the 6-deoxy derivative was obtained by the reduction of the 6-O-*p*-toluenesulfonyl group (→**8**). A new type of the anomalous Zemplén-deacylation was observed: the 4-O-benzoate of the D-*galacto* derivative **9**, carrying isolated benzoyl groups at positions both 2 and 4, proved to be more stable than the 2-O-benzoate under the Zemplén conditions.



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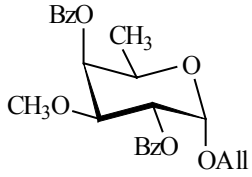

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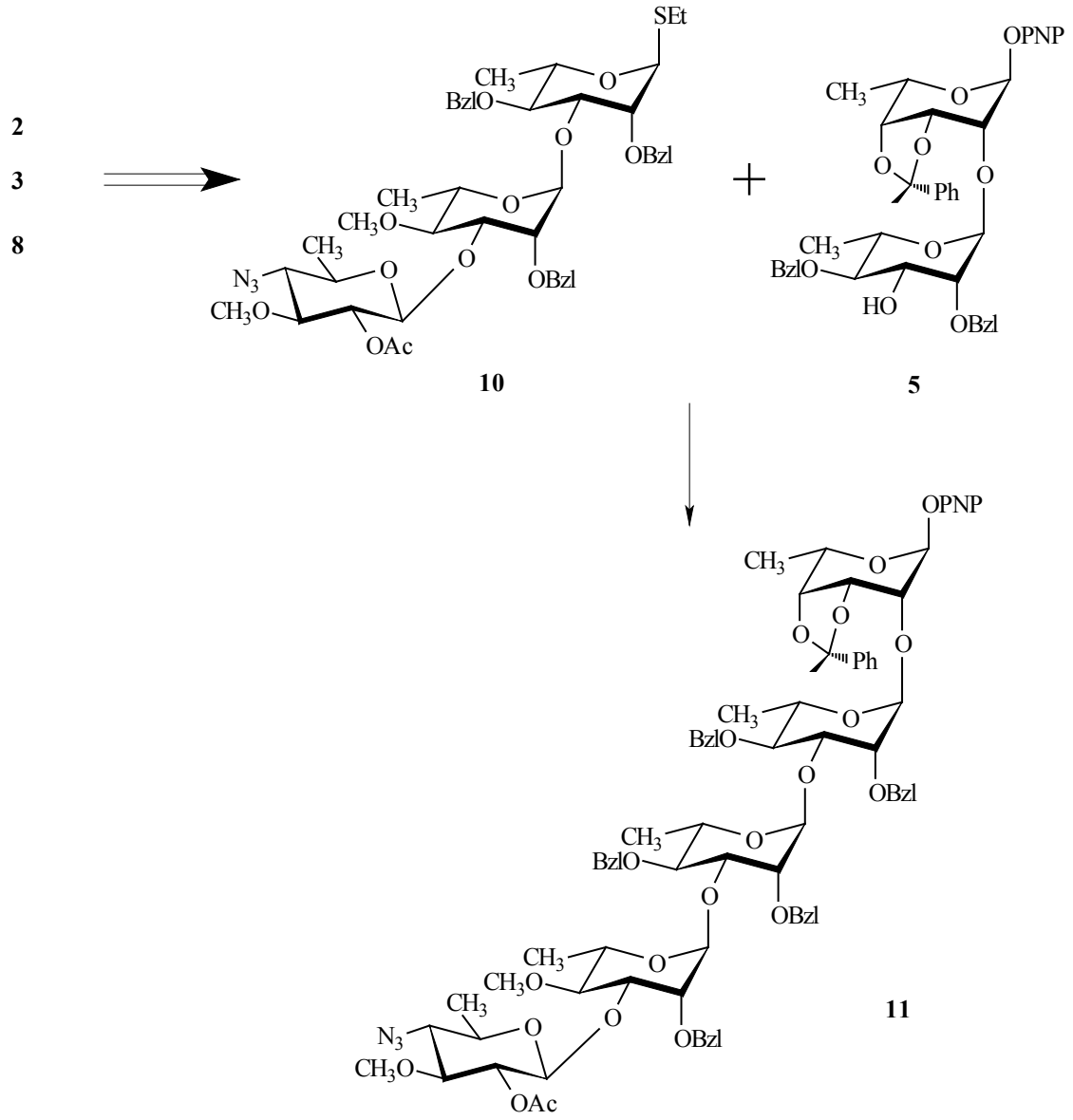
The trisaccharide **10** was obtained from the monosaccharide units **2**, **3** and **8**, and glycosylation of the disaccharide **5** with the trisaccharide yielded the pentasaccharide **11**.



IV. Possibilities for utilization of the results

The synthesized pentasaccharide haptens – connected to suitable spacers – could be used for immunological studies in the serodiagnosis of *Mycobacterial* infections.

The new type of the anomalous Zemplén *O*-deacylation reaction may help in the elaboration of suitable protecting group strategies for another syntheses.

V. List of publications

V.1. Publications in the field of the dissertation

1. Bajza István, Borbás Anikó, Hajkó János, Ron Legas, Szabovik Gabriella, **Varga Zsolt**, Lipták András: Glikolipid és glikopeptidolipid típusú mycobacteriális antigének kémiája. *Magyar Kémikusok Lapja,* **1996**, *51,* 464-475.

2. **Zsolt Varga**, István Bajza, Gyula Batta, András Lipták: Synthesis of the pentasaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serovar 17. *Tetrahedron Letters*, **2001**, *42*, 5283-5286. **IF 2000: 2,558**

3. **Zsolt Varga**, István Bajza, Gyula Batta, András Lipták: Synthesis of the pentasaccharide hapten from the glycopeptidolipid antigen of *Mycobacterium avium* serovar 12. *Tetrahedron Letters*, **2002**, *43*, 3145-3148. **IF 2000: 2,558**

V.2. Publications in other fields

1. István F. Pelyvás, Mária Mádi-Puskás, Zoltán G. Tóth, **Zsolt Varga**, Gyula Batta, Ferenc Sztaricskai: Novel aminocyclitol antibiotics derived from natural carbohydrates. *Carbohydrate Research,* **1995**, *272*, C5-C9. **IF 1995: 1,506**

2. István F. Pelyvás, Mária Mádi-Puskás, Zoltán G. Tóth, **Zsolt Varga**, Gyula Batta, Miklós Hornyák, Ferenc Sztaricskai: Synthesis of New Pseudodisaccharide Aminoglycoside Antibiotics from Carbohydrates. *The Journal of Antibiotics,* **1995**, *48*, 683-695. **IF 1995: 1,436**

3. László Pál, Mádiné Puskás Mária, Tóth Zoltán Gábor, **Varga Zsolt**, Hornyák Miklós, Batta Gyula, Pelyvás F. István, Sztaricskai Ferenc: Antibiotikumok szintézise szénhidrátokból. Pszeudodiszacharid aminoglikozidok. *Magyar Kémiai Folyóirat*, **1997**, *103*, 147-162. **IF 1997: 0,215**

4. Gabriella Szabovik, Adél Medgyes, Zsuzsa Antal, **Zsolt Varga**, W. Knott, András Lipták: The Use of a New Magnesium-Derived Hydride Reagent for Carbohydrate Derivatives.
Polish Journal of Chemistry, **1999**, *73*, 1003-1009. **IF 1999: 0,595**

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

1. **Zsolt Varga**: Synthesis of the terminal aminosugars of the surface antigens of *Mycobacterium avium* serovars 12 and 17; *Annual Meeting of Carbohydrate Working Group of Hungarian Academy of Sciences, Debrecen, 1994* – lecture

2. **Zsolt Varga**, András Lipták: Synthesis of the terminal aminosugars and their acyl components of the surface antigens of *Mycobacterium avium* serovars 12 and 17; *3rd European Training Course on Carbohydrates, Kerkrade, 1994* – poster (P-42)

3. András Lipták, **Zsolt Varga**, Gabriella Szabovik, István Bajza: Synthesis of the terminal aminosugars and their acyl components of the surface antigens of *Mycobacterium avium* serovars 12 and 17; *XVII. International Carbohydrate Symposium, Ottawa, 1994* – poster (Book of Abstracts B-2.6.)

4. András Lipták, **Zsolt Varga**, István Bajza: Surface antigens of the MAIS complex. Synthesis of two pentasaccharides characteristic of serovars 14 and 17; *207th ACS National Meeting, San Diego, California, 1994* – poster (CARB 0016)

5. **Varga Zsolt**, Lipták András: A *Mycobacterium avium* 12-es és 17-es szerovariánsa terminális aminocukrainak és azok acil komponenseinek szintézise; *MKE XVII. Kémiai Előadói Napok, Szerves, Gyógyszer- és Biokémiai Szimpózium, Szeged, 1994* – lecture (Book of Abstracts 36.)

6. **Varga Zsolt**: A *Mycobacterium avium* 12-es és 17-es szerovariánsai terminális aminocukrainak és azok acil komponenseinek szintézise; *Pro Scientia Érmesek Második Szakmai Konferenciája, Budapest, 1994* – lecture

7. **Zsolt Varga**, Zsuzsa Antal, András Lipták: Novel synthesis of the terminal aminosugars of the surface antigens of *Mycobacterium avium* serovars 12 and 17; *Annual Meeting of Carbohydrate Working Group of Hungarian Academy of Sciences, Debrecen, 1995* – lecture

8. **Zsolt Varga**, Zsuzsa Antal, András Lipták: Novel synthesis of the terminal aminosugars of the surface antigens of *Mycobacterium avium* serovars 12 and 17; *VIII. European Carbohydrate Symposium, Seville, 1995* – poster (Book of Abstracts A-140)

9. **Varga Zsolt**, Antal Zsuzsa, Lipták András: A *Mycobacterium avium* 12-es és 17-es szerovariánsa terminális aminocukrainak szintézise; *MKE Vegyészkonferencia, Debrecen, 1995* – poster (Book of Abstracts 174.)

10. **Zsolt Varga**, András Lipták: Synthesis of pentasaccharide antigen from *Mycobacterium avium* serovariant 17; *Annual Meeting of Carbohydrate Working Group of Hungarian Academy of Sciences, Mátrafüred, 1997* - lecture

11. **Varga Zsolt**, Bajza István, Lipták András: Mycobacteriális sejtfelszíni haptének előállítása; *MKE Vegyészkonferencia, Hajdúszoboszló, 2001* – poster (P-92)

V.4. Lectures and posters in other fields

1. Pelyvás F. István, László Pál, **Varga Zsolt**, Sztaricskai Ferenc: Pszeudodiszacharid típusú antibiotikumok szintézise; *VIII. Fermentációs Kollokvium, Hajdúszoboszló, 1992* – poster (Book of Abstracts 99.)

2. **Varga Zsolt**, Pelyvás F. István, Sztaricskai Ferenc: Pszeudodiszacharid antibiotikum modellek szintézise; *MTA Antibiotikum-kémiai Munkabizottságának előadó ülése, Debrecen, 1993* – lecture

3. **Varga Zsolt**: Pszeudodiszacharid antibiotikum modellek szintézise; *XXI. Országos Tudományos Diákköri Konferencia, Nyíregyháza, 1993* – lecture

4. István F. Pelyvás, **Zsolt Varga**, Pál László, Ferenc Sztaricskai: Synthesis of pseudodisaccharide-type antibiotic models; VII. European Carbohydrate Symposium, Cracow, 1993 – poster (Book of Abstracts A-115)

5. **Zsolt Varga**: Synthesis of pseudodisaccharide-type antibiotic models; *Scientific Meeting of Dutch and Hungarian PhD Students, Debrecen*, 1993 – lecture

6. **Varga Zsolt**: Pszeudodiszacharid antibiotikum modellek szintézise; *MKE Kémiai Előadói Napok XVI. Tudományos Szimpózium, Szeged, 1993* – lecture (Book of Abstracts 17.)

7. **Zsolt Varga**, István F. Pelyvás, Zoltán G. Tóth, Ferenc Sztaricskai: Synthesis of Pseudodisaccharide Antibiotic Models; *Annual Meeting of Carbohydrate Working Group of Hungarian Academy of Sciences, Debrecen, 1994* – lecture

8. Zoltán G. Tóth, István F. Pelyvás, Mária Mádi-Puskás, **Zsolt Varga**, Miklós Hornyák, Gyula Batta, Ferenc Sztaricskai: Synthesis of new aminoglycoside antibiotics from carbohydrates; *Annual Meeting of Carbohydrate Working Group of Hungarian Academy of Sciences, Debrecen, 1995* - lecture

9. István F. Pelyvás, Zoltán G. Tóth, Mária Mádi-Puskás, **Zsolt Varga**, Miklós Hornyák, Gyula Batta, Ferenc Sztaricskai: Synthesis of pseudodisaccharide-type antibiotic models; *VIII. European Carbohydrate Symposium, Seville, 1995* – poster (Book of Abstracts A-152)

10. Heinz-Ulrich May, Berndt Werner, **Zsolt Varga**, Frieder W. Lichtenthaler: Hexopyranos-2-ulosyl bromides as versatile glycosyl donors for the construction of oligosaccharides containing β-D-mannose and β-D-mannosamine units; *XVIII. International Carbohydrate Symposium, Milano, 1996* – poster (Book of Abstracts BP267)

11. Heinz-Ulrich May, Berndt Werner, **Zsolt Varga**, Frieder W. Lichtenthaler: Hexopyranos-2-ulosyl bromides as versatile glycosyl donors for the construction of oligosaccharides containing β-D-mannose and β-D-mannosamine units; *5th International Conference on Chemical Synthesis of Antibiotics, Debrecen, 1996* – poster (Book of Abstracts P-32)

12. **Varga Zsolt**, Lipták András, Frieder W. Lichtenthaler: Az ulozil-bromid stratégia: β-D-mannopiranozid tartalmú oligoszacharidok szintézise; *MKE XIX. Kémiai Előadói Napok, Szerves, Gyógyszer- és Biokémiai Szimpózium, Szeged, 1996* – lecture (Book of Abstracts 58-60.)