

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

**Synthesis of Antibiotic Analogues and Derivatives  
Using Diels–Alder Cycloaddition Reactions**

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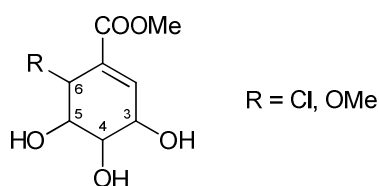
The Ph.D. Defense takes place at the Study Center of the Central Pharmacy,  
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## I. INTRODUCTION AND AIMS

After the discovery of the Diels–Alder cycloaddition, this reaction has acquired enormous value in organic chemistry. This is mainly due to the fact that more and more naturally occurring biologically active compounds have been being published which had a polycyclic structure. For the construction of these polycyclic moieties the Diels–Alder reaction, which is capable of forming a carbon-carbon bond and heterocycles as well, is highly suitable. It is very important that as a result of the cycloaddition as many as four chiral centers can be generated in one step. Using this reaction steroids, alkaloids and many other naturally occurring compounds have been synthesized. The Diels–Alder cycloaddition has become the subject of theoretical studies that help to understand of the nature of this reaction, also support the optimal use in synthetic chemistry. The structures of many secondary metabolites indicate that the Diels–Alder reaction might take place (by Diels–Alderase) in living organisms, too.

My PhD thesis consists of two main parts, with the utilization of Diels–Alder reactions as the common point. In the first part my goal was to synthesize a bridged analogue of the pericosine antibiotics using Diels–Alder cycloaddition, targeting a biologically active compound.

Pericosines are naturally occurring compounds with a cyclohexene skeleton (**Scheme 1.**) They show *in vitro* cytotoxicity against murine P388 lymphocytic leukemia cells. Pericosine A (**Scheme 1.**, R=Cl, 3*S*,4*S*,5*S*,6*S* configuration) is active *in vivo* too and, in addition, selectively inhibits the growth of HBC-5 and SNB-75 human tumor cells.



**Scheme 1.** General structure of the pericosines

In the second part of the dissertation I studied the participation of polyene macrolide antibiotics in a hetero Diels–Alder reaction and the regioselectivity of the cycloaddition.

The [4+2] type cycloaddition reactions of polyenes are definitely less studied, and the reactions of cyclic polyenes, such as polyene macrolides, have not been investigated at all.

Among the antibiotics used to treat fungal infections polyene macrolides have the most important role. They have a characteristic macrolactone ring containing 3–8 conjugated carbon-carbon double bonds capable of Diels–Alder reaction.

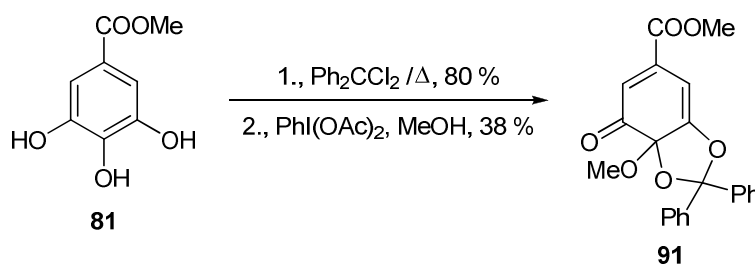
## II. SYNTHETIC METHODS

During my synthetic work I applied preparative methods of organic chemistry. To track the reactions and check the purity of compounds thin-layer chromatography was applied. For purifying crude products flash column chromatography was used. For the structure determination of the synthesized compounds one- and multidimensional NMR spectroscopy ( $^1\text{H}$ - $^1\text{H}$  COSY,  $^{13}\text{C}$ - $^1\text{H}$  HSQC, NOESY), UV-VIS spectroscopy, CD spectroscopy, X-ray diffraction and MALDI-TOF / ESI-TOF mass spectrometry were applied. Melting point and specific rotation determination were also used for characterising the compounds.

## III. NEW SCIENTIFIC RESULTS

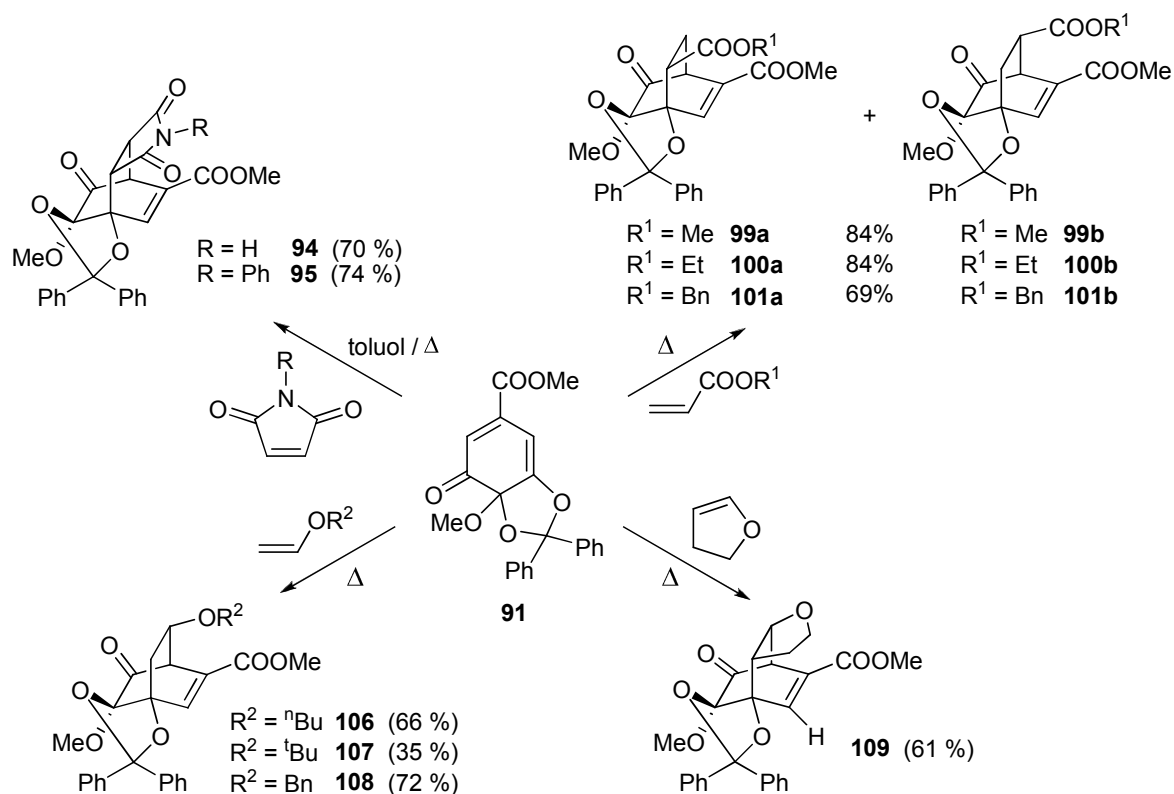
### III.1. Synthesis of a new, bridged pericosine analogue

The synthesis of the pericosine analogue has been accomplished starting from a simple, achiral compound, methyl gallate (**81**). The starting material was chosen by taking its simple structure, ready availability and the similarity with the pericosines considering the substituents into account. One of the key steps, the oxidative dearomatization, was carried out on the protected derivative of **81**, leading to the racemate cyclohexadienone derivative **91** (Scheme 2.)



**Scheme 2.** Oxidative dearomatization of **81**

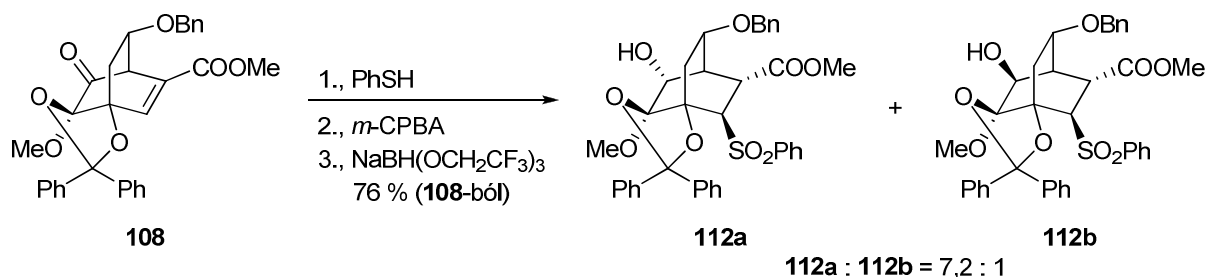
Regarding the electronic nature of the substituents of **91**, Diels–Alder reactions with both normal and inverse electron demand are possible. To confirm the dual character of **91**, we prepared cycloadducts (**94–109**, **Scheme 3.**) using both electron-rich and electron-deficient dienophiles. The electron demand of these reactions were also determined by quantum chemical calculations.



**Scheme 3.** Diels–Alder reactions of **91**

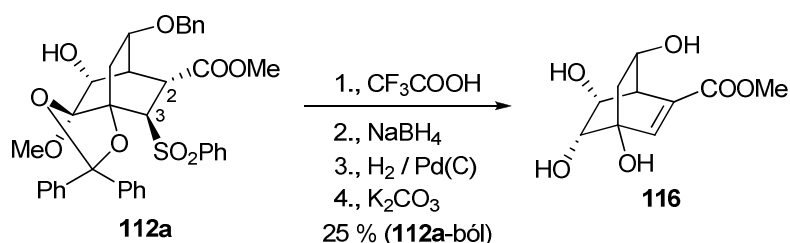
None of the inverse reactions followed the Houk's rule, since only the unexpected regioisomers were formed. Theoretical calculations on the cycloadduct **108** revealed a secondary bonding interaction which could explain the deviancy from the Houk's rule.

From the intermediate **108** a bridged pericosine analogue has been synthesized in seven reaction steps. The carbon-carbon double bond of **108** was protected by means of a stereoselective conjugate addition of thiophenol and oxidation of the so-formed thioether. Reducing the oxo group led to diastereomers **112a** and **112b** (**Scheme 4.**) which were separated. To achieve good stereoselectivity and reproducibility of the reduction, various reducing agents ( $\text{NaBH}_4$ ,  $\text{LiBH}_4$ ,  $\text{BH}_3 \cdot \text{DMS}$ ,  $\text{NaBH}_3\text{CN}$ ,  $\text{NaBH}(\text{OAc})_3$ ,  $\text{NaBH}_4/\text{CeCl}_3$ ,  $\text{NaBH}(\text{OCH}_2\text{CF}_3)_3$ ) were tried out. The stereostructure of **112b** was determined by X-ray diffraction.



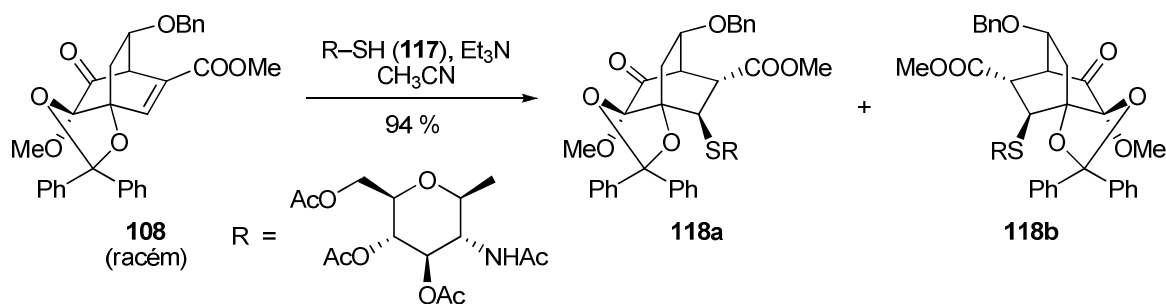
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Acidic hydrolysis of mixed acetal **112a**, stereoselective reduction of the resulted  $\alpha$ -hydroxyketone with NaBH<sub>4</sub>, catalytic removal of the benzyl group then eliminating the phenylsulfonyl group under basic condition gave rise to the bridged pericosine analogue **116** (**Scheme 5.**). **116** showed no cytotoxicity on various cell lines (HeLa, HEL, CRFK, MDCK, Vero).



Scheme 5.

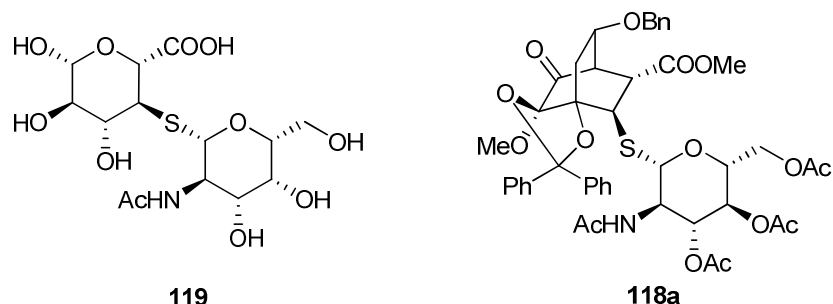
For preparing **116** in enantiomerically pure form, racemate Diels–Alder adduct **108** was reacted with a chiral, enantiomer pure thiosugar (**117**) to form the diastereomers **118a** and **118b** (**Scheme 6.**) which were separated from each other.



Scheme 6.

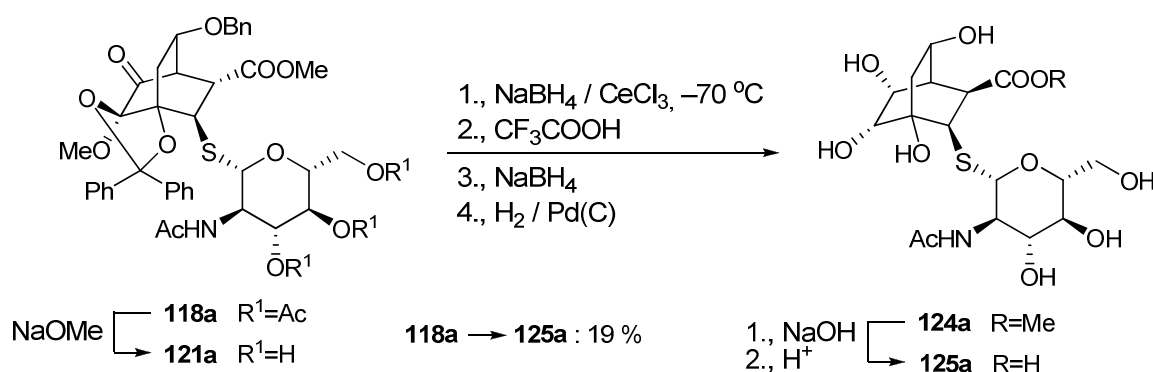
However, the fact observed meanwhile, that **116** is biologically inactive, made my efforts for the further derivatizations of **118a/118b** aimless. **118a/118b** can be regarded as pseudothiodisaccharides showing similarity with **119** found in the literature. Since **119** is a

weak inhibitor of chondroitin lyase, the potential use of the derivatized **118a/118b** also as a lyase inhibitor arose.



Scheme 7.

De-*O*-acetylated **118a** and **118b** were converted into **125a** and **125b** (Scheme 8.), respectively, using transformations outlined earlier at the preparation of **116**. (The preparation of **125b** is the same as of **125a** and is not shown for simplicity reason.)



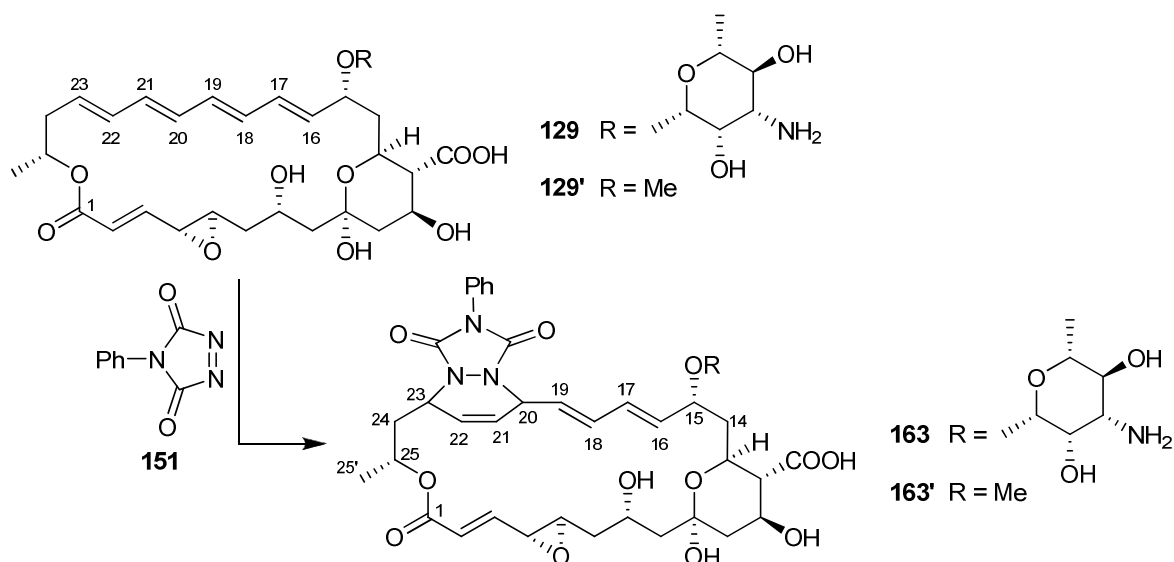
Scheme 8.

The inhibitory constants ( $K_i$ ) of **125a** and **125b** turned out to be 0.9 and 8.2 mM, respectively, which indicates a weak inhibitory property of these compounds.

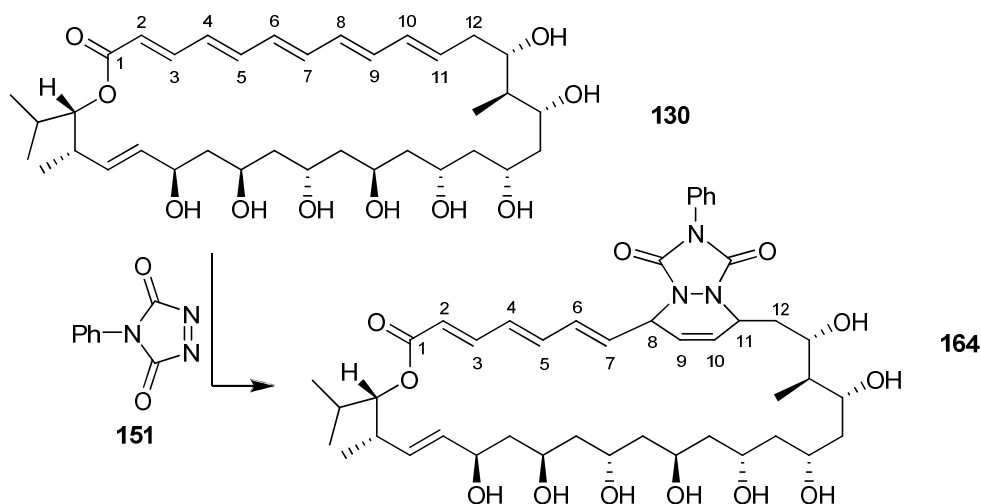
### III.2. Diels–Alder reactions of polyene macrolides

Two polyene macrolide antibiotics, natamycin (or pimaricin, **129**) and flavofungin (**130**) were reacted with a reactive azadienophile, 4-phenyl-1,2,4-triazoline-3,5-dione (**151**).

In the case of **129** the cycloadduct **163** (Scheme 9.) was isolated, the product of the reaction of **130** and **151** was the compound **164** (Scheme 10.). Structures of these adducts were determined by mass spectrometry and UV and NMR spectroscopy. I concluded that both Diels–Alder reactions were regio- and diastereoselective.



**Scheme 9.** Cycloaddition reaction of natamycin (**129**) with **151** and the model reaction



**Scheme 10.** Cycloaddition reaction of flavofungin (**130**) with **151**

The cycloaddition of the natamycin model **129'** with **151** (Scheme 9.) was studied by means of theoretical calculations and it was concluded that independently of the applied method the transition state involving the C<sub>8</sub>–C<sub>11</sub> diene part proved to be the most stable one. This is in accordance with the experimental finding. Besides, it was concluded that the conformation of **163** greatly differs from that of **129**.

The possible transition states in the case of flavofungin also were examined. The results were dependent on the applied method, only the PM3 and the HF/6-31G containing ONIOM calculations gave results consistent with the experimental finding.

The directing effect of the lactone carbonyl group was theoretically studied using several polyenoic acids as models of flavofungin.



The antifungal activities of **163** and **164** were also tested. **163** proved to be inactive, the activity of **164** was diminished compared to that of **130** or it had no activity at all, dependently on the fungal strain.

#### IV. SUMMARY

Pericosines are naturally occurring compounds with antitumour activity. In my doctoral work I report on the synthesis of a bridged analogue having bicyclo[2.2.2]octane skeleton. Dealing with Diels–Alder cycloaddition reactions, too, regioselectivity in a hetero Diels–Alder reaction of two polyene macrolide antibiotics was also studied.

The pericosine analogue has been synthesized from a simple aromatic precursor, methyl gallate. The key steps of the synthesis are an oxidative dearomatization and a regio- and diastereoselective Diels–Alder cycloaddition.

The participation of the cyclohexadienone intermediar, obtained in the dearomatization step, in [4+2] type cycloadditions has been studied in detail by means of both experimental methods and quantum chemical calculations. It has been pointed out that the diene has dual character as it reacts in Diels–Alder reactions with both normal and inverse electron demand. The regioselectivity of the products in inverse electron demand reactions did not follow the Houk's rule. The reason of this discrepancy and the importance of the secondary orbital interactions were pointed out with the use of theoretical calculation applied for the reaction of the diene with benzyl vinyl ether.

From the cycloadduct containing four chiral centers, obtained with benzyl vinyl ether, a bridged pericosine analogue having five chiral centers has been synthesized in seven reaction steps with full diastereoselectivity has been achieved. Unfortunately, the target compound has not proved to be active on different types of cell lines.

From the racemate cyclohexadienone intermediate two enantiomerically pure pseudothiodisaccharides with potential chondroitin lyase inhibitory effect have been synthesized. These pseudothiodisaccharides, in which the bicyclooctane scaffold contains seven chiral centers, showed poor inhibitory activity.

In the second part of this work, a Diels–Alder reaction of two polyene macrolide antibiotics, natamycin and flavofungin with 4-phenyl-1,2,4-triazoline-3,5-dione has been examined. According to the results of detailed spectroscopic studies the cycloaddition proceeded with high regio- and diastereoselectivity in both cases. Using theoretical

calculations on a natamycin model we confirmed that the C<sub>20</sub>–C<sub>23</sub> section (situated the farthest from the sugar moiety) of the polyene part is the most reactive. In the case of flavofungin the directing effect of the carbonyl group has been pointed out using polyenoic acids as model compounds.

## V. SCIENTIFIC PUBLICATIONS

### V.1. Publications related to the subject of the dissertation

- 1., Zsolt Fejes, Attila Mándi, István Komáromi, Attila Bényei, Lieve Naesens, Ferenc Fenyvesi, László Szilágyi, Pál Herczegh  
Synthesis of a pericosine analogue with a bicyclo[2.2.2]octene skeleton  
*Tetrahedron*, **2009**, 65(39), 8171-8175.  
**IF: 3.219**
- 2., Zsolt Fejes, Attila Mándi, István Komáromi, László Majoros, Gyula Batta, Pál Herczegh  
A synthetic and *in silico* study on the highly regioselective Diels–Alder reaction of the polyenic antifungal antibiotics natamycin and flavofungin  
*Tetrahedron Letters*, **2010**, 51(38), 4968-4971.  
**IF: 2.660** (2009)

### V.2. Lectures related to the subject of the dissertation

- 1., Zs. Fejes, Gy. Gyémánt, L. Kandra, S. Antus, T. Kurtán, L. Szilágyi, F. Fenyvesi, L. Majoros, P. Herczegh  
Synthesis of antibiotic analogs using cycloaddition reaction: the case of pericosin and flavofungin  
*3<sup>rd</sup> German-Hungarian Workshop – Synthesis, Isolation and Biological Activity of Natural Products*, Paderborn, 2008.05.15-17.
- 2., Zs. Fejes, Gy. Gyémánt, L. Kandra, S. Antus, T. Kurtán, L. Szilágyi, F. Fenyvesi, L. Majoros, P. Herczegh  
Synthesis of antibiotic analogs using cycloaddition reaction: the case of pericosin and flavofungin  
*Kisfaludy Lajos Alapítvány előadói ülése*, Budapest, 2009.03.09.
- 3., Fejes Zsolt, Mándi Attila, Komáromi István, Antus Sándor, Kurtán Tibor, Szilágyi László, Bényei Attila, Lieve Naesens, Fenyvesi Ferenc, Herczegh Pál  
A perikozin antibiotikum analógjainak szintézise  
*MTA Antibiotikum és MTA Nukleotid Munkabizottsági Ülés*, Debrecen, 2009.11.19-20.
- 4., Fejes Zsolt  
Antibiotikumok szintézise és szerkezetmódosítása Diels–Alder reakcióval  
*MTA Szerves és Biomolekuláris Kémiai Bizottság Bruckner termi előadása*, Budapest, 2010.05.28.

### V.3. Posters related to the subject of the dissertation

- 1, P. Herczegh, Zs. Fejes  
Synthesis of the analogues of the antibiotic pericosine  
*23<sup>rd</sup> IUPAC - International Symposium on the Chemistry of Natural Products*,  
Firenze, 2002.07.28-08.02
- 2, P. Herczegh, Zs. Fejes  
Synthesis of the analogues of the antibiotic pericosin  
*1<sup>st</sup> Austrian-Hungarian Carbohydrate Conference*, Burg Schlaining, 2003.09.24-26.
- 3, Fejes Zsolt, Gyémánt Gyöngyi, Kandra Lili, Antus Sándor, Kurtán Tibor,  
Szilágyi László, Fenyvesi Ferenc, Herczegh Pál  
Perikozin antibiotikum analógok szintézise  
*Vegyészkonferencia 2008*, Hajdúszoboszló, 2008.06.19-21.

### V.4. Other publications

Sándor Kéki, Gyula Batta, Ilona Bereczki, Zsolt Fejes, Lajos Nagy, Ágnes Zajác, Lili Kandra, Imre Kiricsi, György Deák, Miklós Zsuga, Pál Herczegh  
New types of  $\alpha$ -amylase enzyme-inhibitory polysaccharides from D-glucal  
*Carbohydrate Polymers*, **2006**, 63(1), 136-140.

**IF: 1.784**

**Total IF: 7.663**