BUDAPESTI UNIVERSITY OF TECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY
GYÖRGY OLÁH PHD-SCHOOL

Synthesis of pyrrolo-isoquinoline-, indolizino[8,7-*b*]indole and phenanthridone alkaloids and their analogues

Ph. D. thesis

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1. Introduction

The syntheses of novel alkaloid analogues having potential cytostatic activity have been studied for decades at the Department of Organic Chemistry and Technology, Budapest University of Technology and Economics.

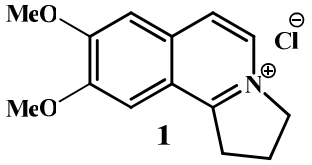
My ongoing studies were first focused on the synthesis of pyrroloisoquinolines and indolizino[8,7-*b*]indoles via 1,3-dipolar cycloaddition and the investigation of the stereochemistry thereof. We aimed at preparing novel derivatives of these heterocycles which may have cytostatic activity according to previous analogies. After that, my work was extended to finding new synthetic approaches for the most important phenantridone alkaloid family, the *Amaryllidaceae* alkaloids and their analogues.

2. Literature

2.1. Pyrrolo-isoquinolines and indolizino[8,7-*b*]indoles

According to the literature, the alkaloids having pyrrolo-isoquinoline skeleton, whose majority has cytostatic activity, can be found in terranean plants and in sea animals. The structure of molecules planned is similar to that of compounds being in plants. In this chapter a short summary is given for this class of compounds.

Chinese scientists isolated pyrrolo-isoquinoline derivatives from *Carduus crispus* in 2002, which were named as Crispine A and B. [1] Among these compounds Crispine B (**1**) showed cytostatic activity against various human cancer cells.



1

Crispine B

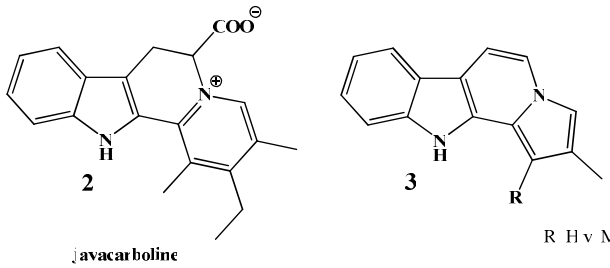
Numerous total synthesis can be found for the synthesis of **1**, but it is not possible to go in details here. Nevertheless, compounds having ciano group on the pyrrole ring have not been prepared and investigated in connection with the aspect of biological activity up to now.

Among the other class of compounds which was studied in my PhD-thesis, the indolizino[8,7-*b*]indoles obtainable from 3,4-dihydro-ß-carboline, numerous analogues having cytostatic activity can be found.

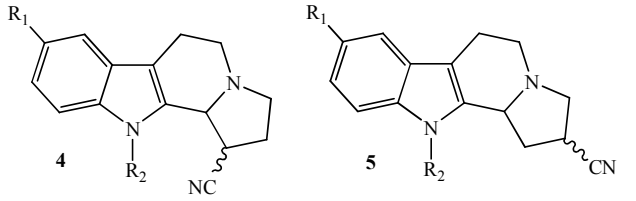
Koike and co-workers isolated an alkaloid, javacarboline having ß-carboline skeleton from *Picrasma javanica*. The cytostatic activity against PC-6 human carcinoma cells and P-388 leukemia was reported. [2]

Beside this compound *Koike*, *Nikaido* és co-workers synthesized indolizino[8,7-*b*]indole molecules (**3**) having different skeleton in comparison to that of javacarboline, and these derivatives showed cytostatic activity against P-388 limphocytic leukemia and PC-6 human carcinoma cell lines. [3]

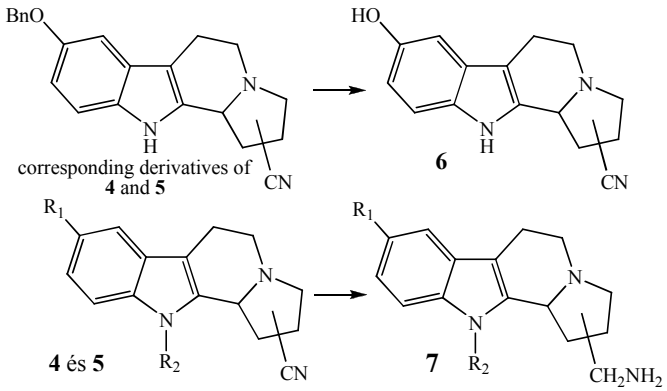
[1] Q. Zhang, Q.; Tu, G.; Zhao, Y.; Cheng, T. *Tetrahedron* **2002**, *58*, 6795.
[2] Koike, K.; Ohmoto, T.; Uchida, A.; Oonishi, I. *Heterocycles* **1994**, *38*, 1413.
[3] Yoshino, H.; Koike, K.; Nikaido, T. *Heterocycles* **1999**, *51*, 281.



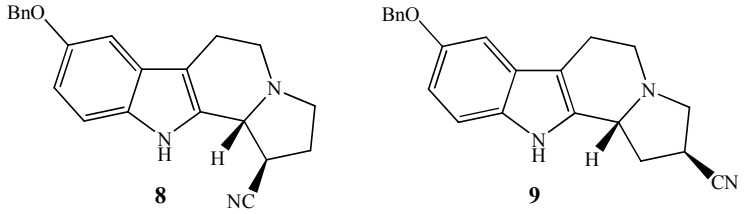
The previously mentioned and further ß-carboline-based compounds (manzamines, eudistomine, azatoxine) encouraged french sciencists to prepare further indolizino[8,7-*b*]indole derivatives and investigate their cytostatic activity. [4]



R_1, R_2: H, Bn, v. OBn



The inhibitor activity of the synthesized compounds were tested toward the fission of L1210 cancer cells, and the two most active compounds were chosen (**8** and **9**), and their cytostatic activity were investigated in multidrug-resistent cell lines. The results were significant, as the K562R human eritro-leukemia cells which were resistent to doxorubucin, were sensible to **8** and **9**.

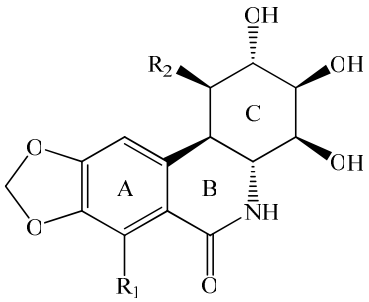


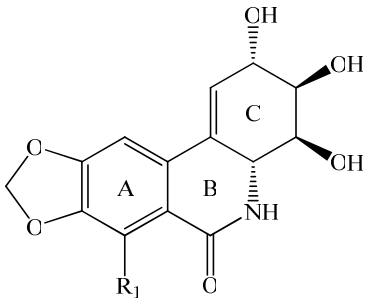
2.2. Phenantridone-based alkaloids belonging to the *Amaryllidaceae* family

Among the *Amaryllidaceae* alkaloids, the phenantridones form a sub-group consisting of 15 isolated members.[5] Most of them have significant cytostatic activity, and among these, **10**-**15** can be emphasized.

[4] Poissonnet, G.; Thret-Bettiol, M.-H.; Dodd, R. H. *J. Org. Chem.* **1996**, *61*, 2273.
Bertrand, M., Poissonnet, G., Théret-Bettiol, M.-M., Gaspard Ch., Werner, G.M., Pfeiffer, B., Rendard, P., Léonce, S., Dodd, R.M. *Bioorg. Med. Chem.* **2001**, *9*, 2155.

[5] Hoshino, O. In „The Alkaloids" Cordell, G. A.,, Ed.,; Academic Press: New York. p323, 1998.





10 R_1=OH, R_2=OH pancratistatin
12 R_1=H, R_2=OH 7-deoxy-pancratistatin
13 R_1=H, R_2=H 7-deoxy-*trans*-dihydronarciclasine
14 R_1=OH, R_2=H *trans*-dihydronarciclasine

11 R_1=OH narciclasine
15 R_1=H lycoricidine

These compounds containing an amide (in our case: lactam) unit, in contrast with the majority, are of neutral character instead of basic, and their isolation can be carried out from the neutral fraction.

The above mentioned six compounds have significant antitumor activity, and among them pancratistatin passed to clinical trials.[6] According to the literature, the di- and trideoxy analogues of these compounds also have cytostatic activity, so we aimed at synthesizing further derivatives not described before, which may help finding the minimal pharmacophore.

Despite that the cytotoxic activity of 7-deoxy-*trans*-dihydronarciclasine is commensurate with that of pancratistatin, there are only three total syntheses of this alkaloid, which apply sophisticated steps, catalyst difficult to obtain, with low overall yields. For these reasons we attempted to establish a total synthesis for alkaloid **13** consisting of easy steps from a cheap starting material and furthermore, applying an enantioselective organocatalyst in an early step to make the synthesis enantioselective.

3. Scientific methods

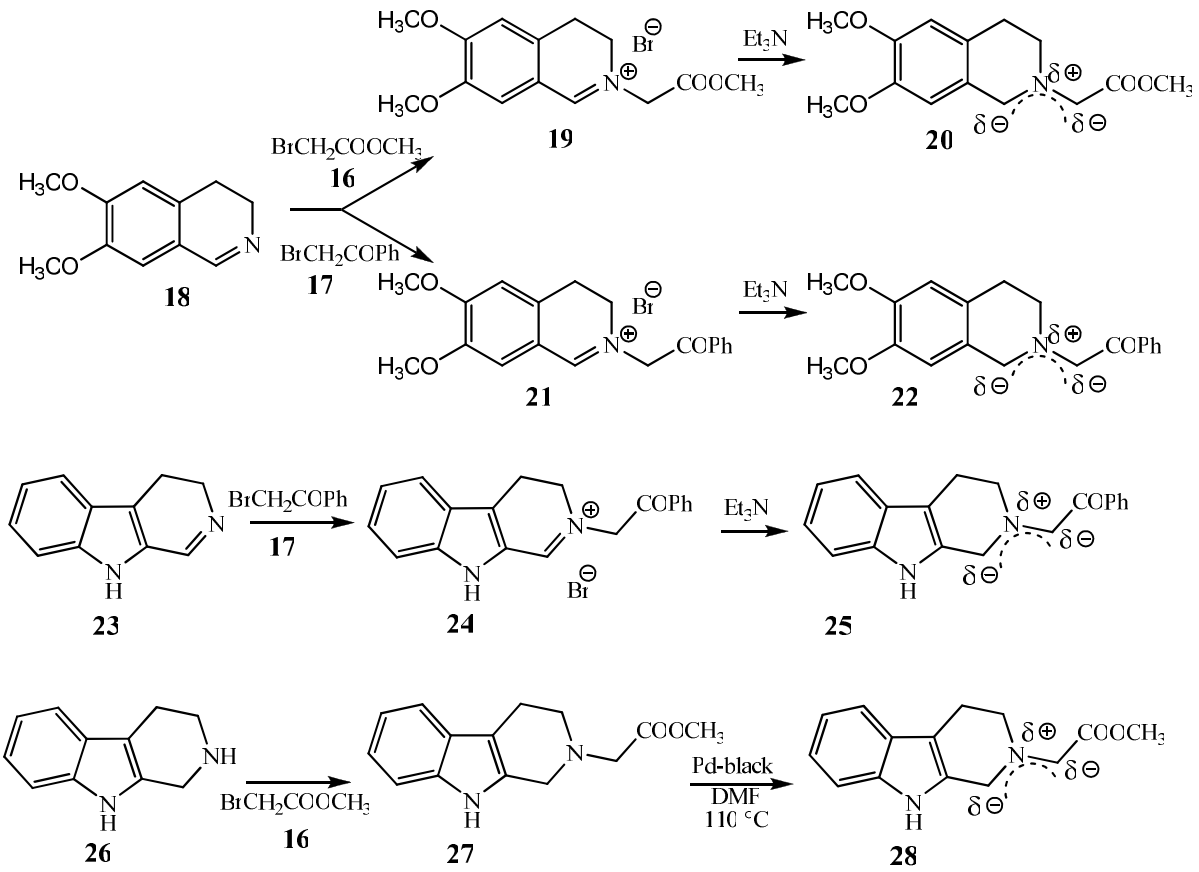
During my synthetic works classical organic preparative methods and separating techniques were used. The reactions were monitored by thin layer chromatography, the products were purified by destillation, recrystallization or coloumn chromatography.

The structure elucidation of the prepared new compounds was accomplished by one- and two-dimensional NMR techniques. The characterization of the intermediates and endproducts were carried out by melting points, chiral HPLC techniques, IR spectroscopy and measuring the optical rotation power.

4. Results*

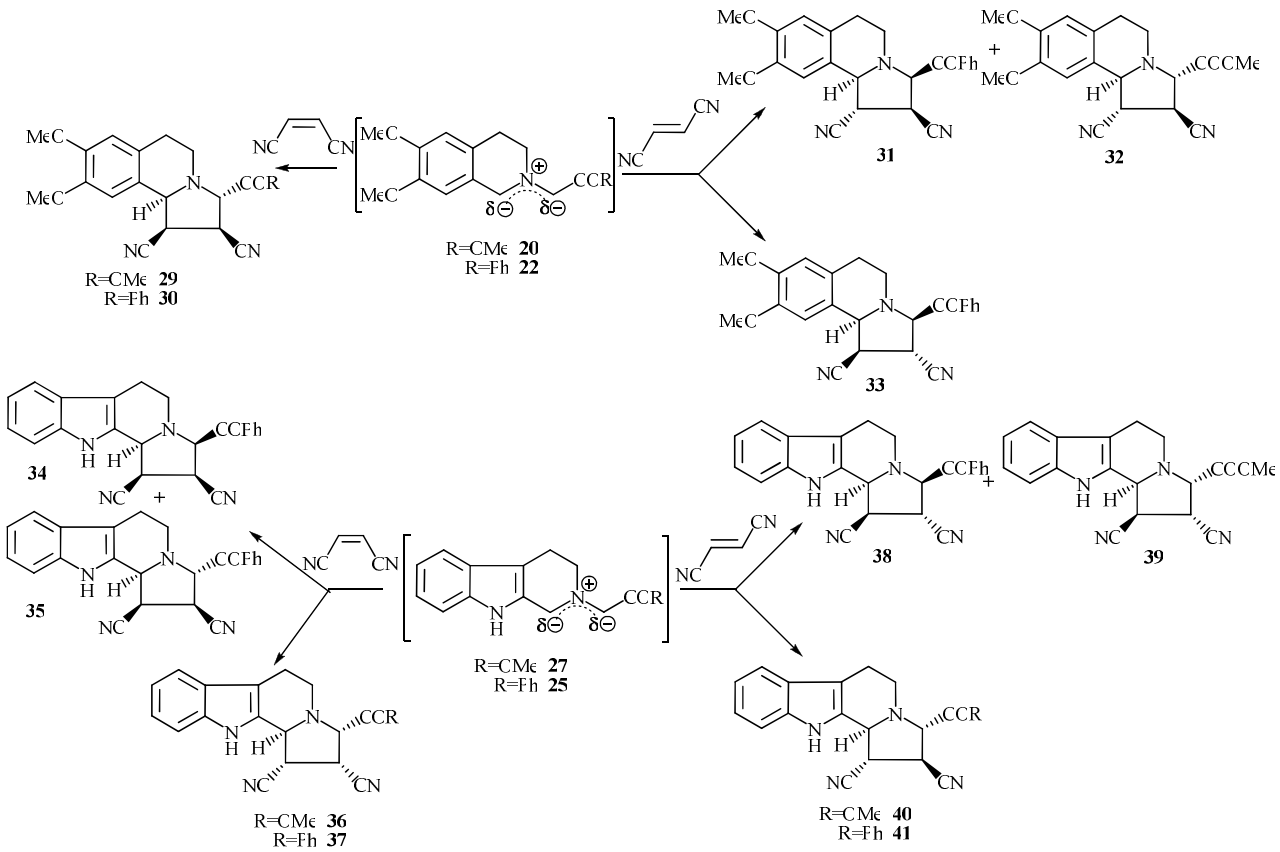
In the first part of my work the reaction of four cyclic azomethin ylides: two obtainable from 6,7-dimethoxy-3,4-dihydroisoquinoline (**18**) and two from 3,4-dihydro-ß-carboline (**23**) were investigated with malono-and fumaronitrile, and some potentially cytostatic pyrrolo-isoquinoline and indolizino[8,7-*b*]indole derivatives (**29-41**) were prepared.

[6] NCI Investigational Drugs. Chemical Information, National Institutes of Health, 273-275, 1988
* In case of racemic compounds only one enantiomer can be seen.

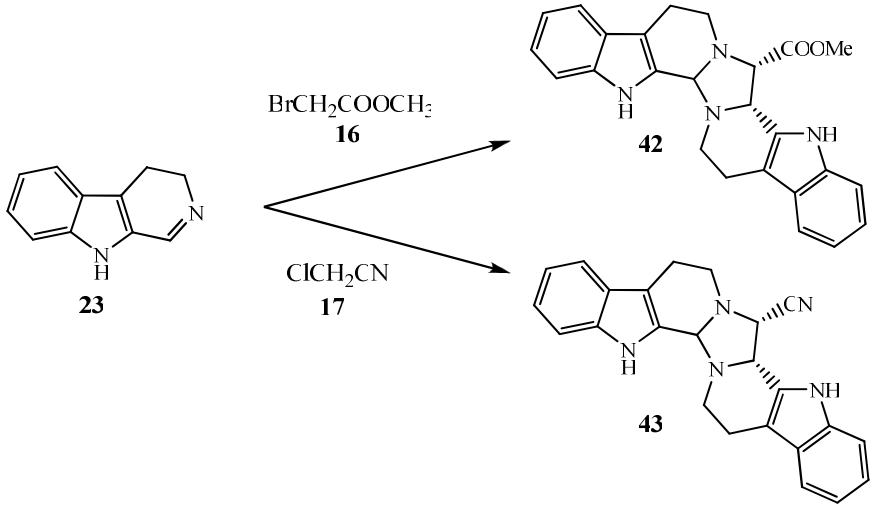


The generation of azomethin-ylides were carried out by the deprotonation[7] except one case, when the dehydrogenation method discovered by Grigg and co-workers was applied.[8]

[7] Bende, Z.; Simon, K.; Tóth, G.; Tőke, L.; Weber, L. *Liebigs Ann. Chem.* **1982**, 924.
[8] Grigg, R.; Kemp, J. *Tetrahedron Lett*. **1980**, *21*, 2461

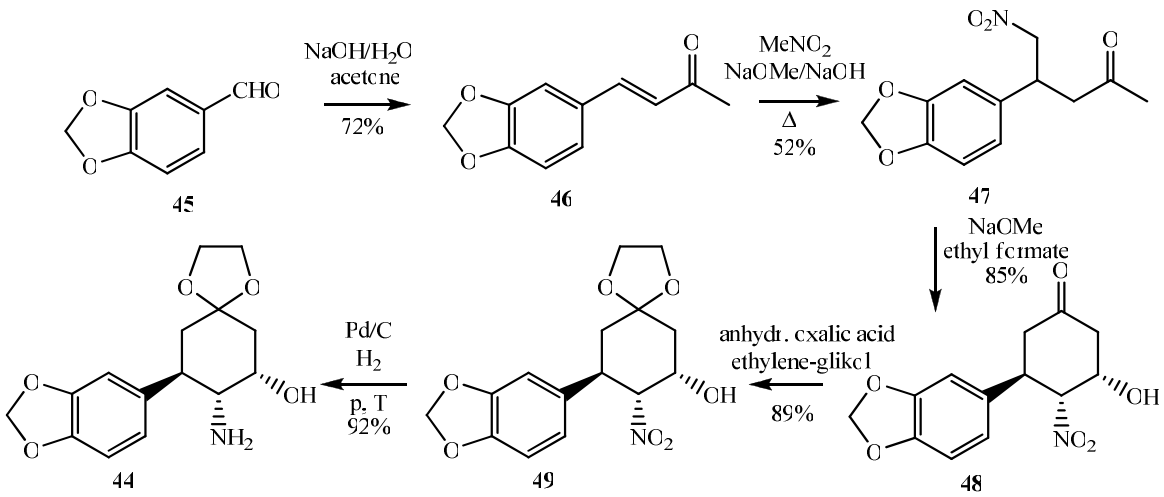


In the case of **23** the deprotonation method resulted in the formation of dimer-like cycloadducts (**42, 43**), the stereochemistry of which were also studied

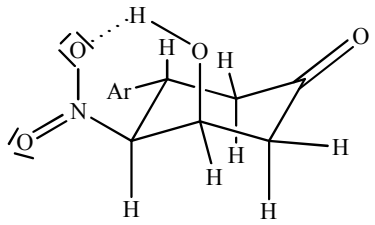


In the second part of my work the stereoselective total synthesis of a natural phenanthridone alkaloid (±)-**13** 7-deoxy-*trans*-dihydronarciclasine having significant cytostatic activity was elaborated. This novel approach allowed to find an enantioselective synthesis for (+)-**13** not described before.

The key intermediate of the synthesis is ketal **44** containing the A and C ring of the phenanthridone skeleton. The literature preparation of **44** was too difficult and circumstantial, so a more practical synthesis starting from piperonal (**45**) was worked out:



The key step of the reaction route is the diastereoselective cyclization to cyclohexanolone **48** due to the stabilisation effect of the hydrogen bridge outlined below:



Ar: methylenedioxyphenyl

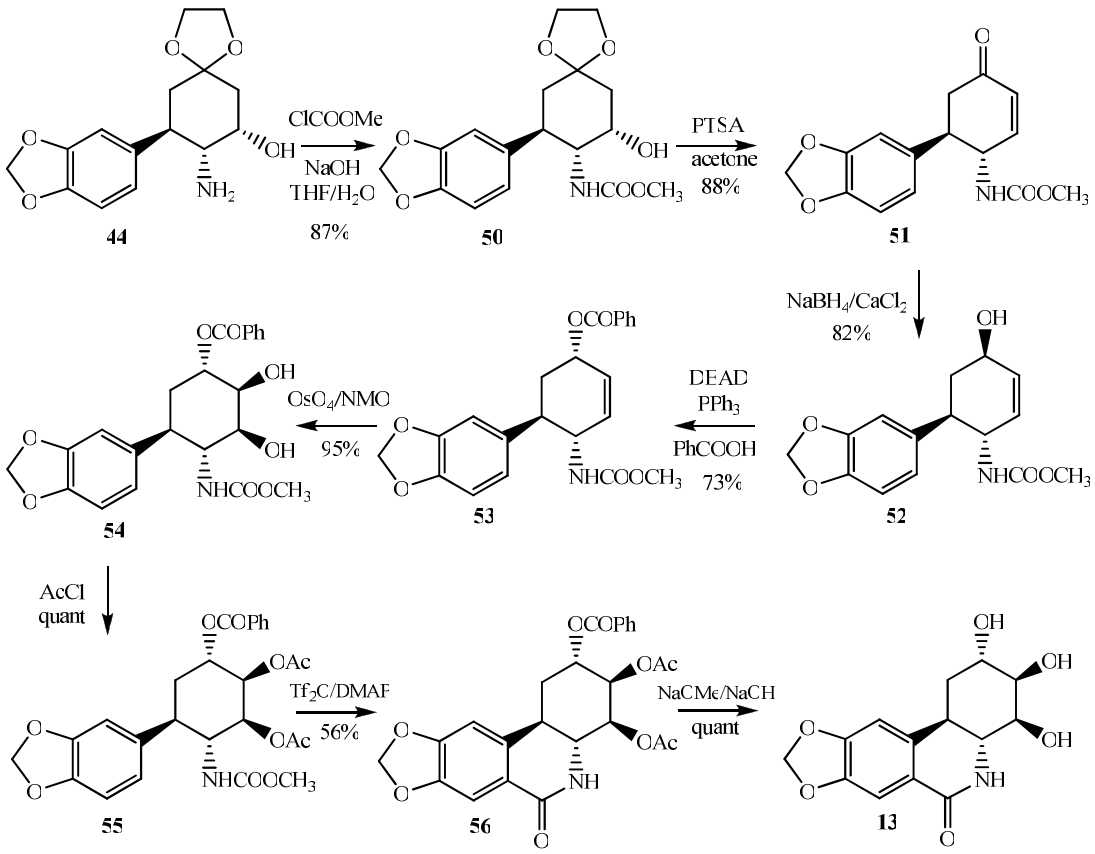
During the total synthesis **44** ketal was converted to urethane **50** followed by deketalization to **51** enone. Although it is known that the reduction of enones to enoles by complex metal hydrides are difficult but using $NaBH_4$ in methanol with chelate forming $CaCl_2$ a chemo- and stereoselective reduction took place affording exclusively one product, where the orientation of the hydroxy group of **52** was *cis*-equatorial (β).

Since the orientation of the hydroxy group in the target molecule is quasi *trans*-axial, inversion of the hydroxy group was necessary. For this purpose **52** was converted to the quasi *trans*-axial benzoate **53** by Mitsunobu reaction, then **54** *cis*-diol was obtained using *N*-methyl-morpholine-*N*-oxide and catalytic amount of OsO_4. The orientation of the OH-groups in the main product was β,β due to the neighbouring bulky groups.

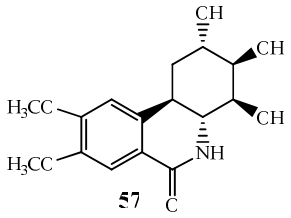
The B-ring closure was carried out by the *Banwell* modification of the Bischler-Napieralski reaction.[9]

The second step of the total synthesis (**46→47**) was accomplished by enantioselective catalysis, and from this point we were able to access to (-)-7-deoxy-*trans*-dihydronarciclasine. The total synthesis of **13** and (-)-**13** can be seen on the following scheme:

[9] Banwell, M. G.; Bissett, B. D.; Busato, S; Cowden, C. J.; Hockless, D. C. R.; Holman, J. W.; Read, R. W.; Wu, A. W. *J. Chem. Soc., Chem. Commun.* **1995**, 2551.



After synthesizing **13**, the following analogues were prepared with slight modifications of the above route:



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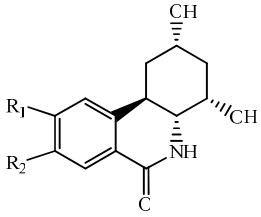
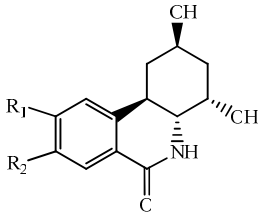
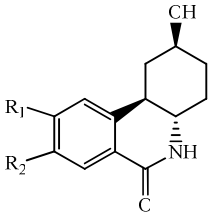


R_1,R_2=-OCH_2O-: **58a**
R_1,R_2=OMe: **59a**

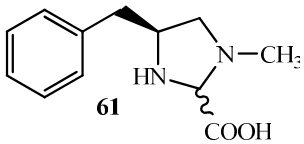


R_1,R_2=-OCH_2O-: **58b**
R_1,R_2=OMe: **59b**



R_1,R_2=-OCH_2O-: **60**
R_1,R_2=OMe: **61**

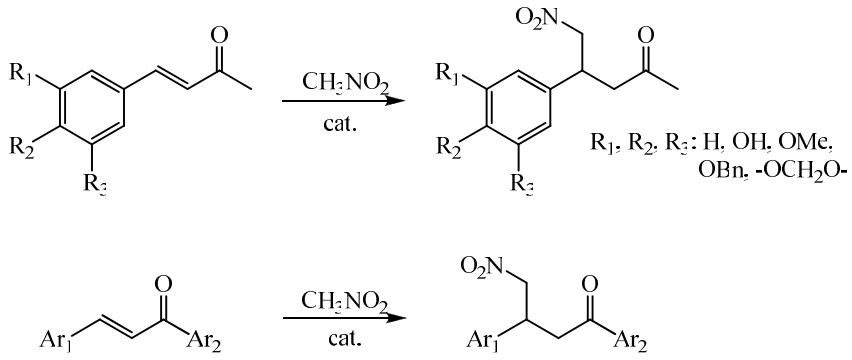
The above mentioned early enantioselective step allowing the synthesis of (+)-**13** was performed by asymmetric organocatalysis using catalyst **61** developed by *Jørgensen*.[10]



61

[10] Halland, N.; Hazell, R. G.; Jørgensen, K. A. *J. Org. Chem.* **2002**, *67*, 8331.

The addition of nitromethane to **46** benzilidene acetone was achieved with excellent, practically 100% ee value. The Michael addition was carried out with several other substrates (benzilidene- and chalcone-derivatives), and good enantioselectivites were experienced.



5. Theses

1. Potentially cytostatic pyrrolo-isoquinoline and indolizino[8,7-*b*]indole derivatives were synthesized via stereoselective 1,3-dipolar cycloaddition of azomethin-ylides.[11]

2. The structure elucidation for dimer-type cycloadduct from the reaction of 3,4-dihydro-ß-carboline and methyl bromoacetate was accomplished. The investigation was extended to the reaction of chloroacetonitrile.

3. A practical, simple and stereoselective total synthesis for the natural 7-deoxy-*trans*-dihydronarciclasine was elaborated.[12]

4. Known organocatalyst was applied for the enantioselective Michael addition of nitromethane to benzilidene acetones and good to excellent enantioselectivites were achieved.[13]

5. The total synthesis of *ent*-(-)-7-deoxy-*trans*-dihydronarciclasine, the enantiomer of the natural product was worked out from piperonyl-nitropentanone with almost 100% enantioselectivity.[14]

6. Further analogues containing less OH-groups were synthesized with the modifications of the original synthetic route.

7. The enantioselective organocatalyst was applied for the Michael addition of nitromethane to various chalcones, and established that electron donor groups on either phenyl ring is required to achieve good enantioselectivity.

6. Applications

The synthesized compounds may have cytostatic acivity, the biological evaluation will be performed in the near future. The biological evaluation of either compounds synthesized by 1,3-

[11] Kádas, I.; Szántó, G.; Tőke, L.; Simon, A.; Tóth, G. *J. Het. Chem.* **2007**, *44*, 1373.
[12] Szántó, G.; Hegedűs, L.; Mattyasovszky, L.; Simon, A.; Simon, Á.; Kádas, I. *Tetrahedron Lett.* **2009**, *50*, 2857.
[13] Szántó, G.; Bombicz, P.; Grün, A.; Kádas, I. *Chirality* **2008**, *20*, 1120.
[14] Szántó, G.; Hegedűs, L.; Mattyasovszky, L.; Simon, A.; Simon, Á.; Bitter, I.; Tóth, G.; Tőke, L.; Kádas, I. *Tetrahedron*, **2009**, *65*, 8412.

dipolar cycloaddition, or the phenanthridone alkaloid analogues on human cell lines could help in establishing structure-activity relationship, finding and preparing more effective molecules.

Besides, the recognized selectivities during the total synthesis of phenanthridone compounds widen the knowledge and toolbar of organic chemistry following the tendency of finding and using simpler reagents and catalysts even in complex chemo- and stereoselective reactions.

7. Publications

7.1. Publications related to the PhD dissertation

1. István Kádas, Gábor Szántó, László Tőke, András Simon, Gábor Tóth: Stereochemistry of 1,3-dipolar-cycloaddition of 3,4-dihydro-isoquinoline- and 3,4-dihydro-carboline-*n*-methoxycarbonyl- and *N*-phenacyl- methylides with maleic and fumaric nitrile, *J. Het. Chem*., **2007**, *44*, 1373. (IF: 0,813)

2. Gábor Szántó, Alajos Grün, Petra Bombicz, István Kádas: Highly enantioselective organocatalytic conjugate addition of nitromethane to benzylidene acetones, *Chirality,* **2008**, *20*, 1120. (IF: 2,436)

3. Gábor Szántó, László Hegedűs, Lenke Mattyasovszky, András Simon, Ákos Simon and István Kádas: Stereoselective total synthesis of (±)-7-deoxy-*trans*-dihydronarciclasine, a potent antineoplastic phenanthridone alkaloid, *Tetrahedron Lett.*, **2009**, *50*, 2857. (IF: 2,615)

4. Gábor Szántó, László Hegedűs, Lenke Mattyasovszky, András Simon, Ákos Simon, István Bitter, Gábor Tóth, László Tőke and István Kádas: An expedient total synthesis of *ent*-(–)-7-deoxy-*trans*-dihydronarciclasine, *Tetrahedron*, **2009**, *65*, 8412. (IF: 2,869)

5. Gábor Szántó, István Kádas, Tamás Kárpáti, László Hegedűs: Hydrogenation of (±)-*Trans*-2-arynitrocyclohexane derivatives over palladium, *Reaction Kinetics and Catalysis Letters*, **2009**, accepted for publication. (IF: 0,584)

7.2. Presentations

1. István Kádas, Gábor Szántó és Áron Szöllősy: Investigation of preparation and 1,3-dipolar cycloaddition reaction of cyclic azomethin ylides. Chemical Presentations' Day, Szeged, 25-27th of October, 2004. (oral presentation)

2. István Kádas, Gábor Szántó, Áron Szöllősy, Gábor Tóth és László Tőke: Synthesis of new, cycloc azomethin ylides and investigation of their cycloaddition reactions. Working Committee of Alkaloid Chemistry, HAS, Balatonfüred, 9-10th of May, 2005. (oral presentation)

3. István Kádas, Gábor Szántó, Áron Szöllősy, Gábor Tóth és László Tőke: Synthesis of new, potential bioactive heterocyclic molecules with 1,3-dipolar cycloaddition reactions of cylic azomethin ylides. Working Committee of Heterocyclic Chemistry, HAS, Balatonszemes, 25-27th of May, 2005. (oral presentation)

4. Gábor Szántó, István Kádas, András Simon és Gábor Tóth: Synthesis of potent bioactive indolizino[8,7-*b*]indole alkaloid analogues with 1,3-dipolar cycloaddition. Conference of the Hungarian Scientific Pharmaceutical Society, Dobogókő, 13-15th of October, 2005. (poster)

5. István Kádas, Gábor Szántó és Áron Szöllősy: Investigation of the 1,3-dipolar cycloaddition of cyclic azomethin ylides. Chemical Presentations' Day, Szeged, 24-26th of October, 2005. (oral presentation)

6. István Kádas, Gábor Szántó, András Simon és Gábor Tóth: Investigation of the stereochemistry of 1,3-dipolar cycloaddition of cyclic azomethin ylides. Working Committee of Alkaloid Chemistry, HAS, Balatonfüred, 15-16th of May, 2006. (oral presentation)

7. István Kádas, Gábor Szántó, András Simon és Gábor Tóth: Stereochemistry of 1,3-dipolar cycloaddition of cyclic azomethin ylides and their application for the synthesis of new bioactive molecules. Working Committee of Heterocyclic Chemistry, HAS, Balatonszemes, 2006. június 7-9. (oral presentation)

8. Gábor Szántó, István Kádas, László Tőke, András Simon és Gábor Tóth: Investigation of the stereochemistry of 1,3-dipolar cycloaddition of cyclic azomethin ylides to electron-deficient olefines. PhD-conference of the György Oláh PhD-school, Faculty of Chemical Technology and Biotechnology, BME, Budapest, 7th of February, 2007. (oral presentation)

9. István Kádas, Gábor Szántó, Alajos Grün and Bombicz Petra: Synthesis of 4-aryl-5-nitro-pentan-2-ones as chiral intermediates for the synthesis of phenanthridone alkaloids. Working Committee of Alkaloid Chemistry, HAS, Balatonfüred, 2007. május 15-16. (oral presentation)

10. István Kádas, Gábor Szántó, László Tőke, András Simon és Gábor Tóth: Investigation of the stereochemistry of 1,3-dipolar cycloaddition of cyclic azomethin ylides to electron-deficient olefines. Centenary Conference of Chemists, HAS, Sopron, 29th of May – 1th of June, 2007. (poster)

11. István Kádas, Gábor Szántó, Alajos Grün and Petra Bombicz: Synthesis of 4-aryl-5-nitro-pentan-2-ones from 4-phenyl-buten-2-ones with enantioselective organocatalyst. Centenary Conference of Chemists, HAS, Sopron, 29th of May – 1th of June, 2007. (poster)

12. István Kádas, Gábor Szántó, László Hegedűs, András Simon, Gábor Tóth, László Tőke: Stereoselective synthesis of potential cytostatic phenanthridone alkaloid analogues. Working Committee of Alkaloid Chemistry, HAS, Balatonfüred, 13-14th of May, 2008. (oral presentation)

13. István Kádas, Gábor Szántó, László Hegedűs, András Simon, Gábor Tóth, László Tőke: Stereoselective synthesis of potential cytostatic phenanthridone alkaloid analogues. Working Committee of Heterocyclic Chemistry, HAS, Balatonszemes, 21-23th of May, 2008. (oral presentation)