



**BUDAPESTI MŰSZAKI ÉS GAZDASÁGTUDOMÁNYI EGYETEM
VEGYÉSZMÉRNÖKI ÉS BIOMÉRNÖKI KAR
OLÁH GYÖRGY DOKTORI ISKOLA**

**SYNTHESIS AND REACTIONS OF SOME BIOLOGICALLY ACTIVE
NATURAL COMPOUNDS**

Thesis of the PhD dissertation

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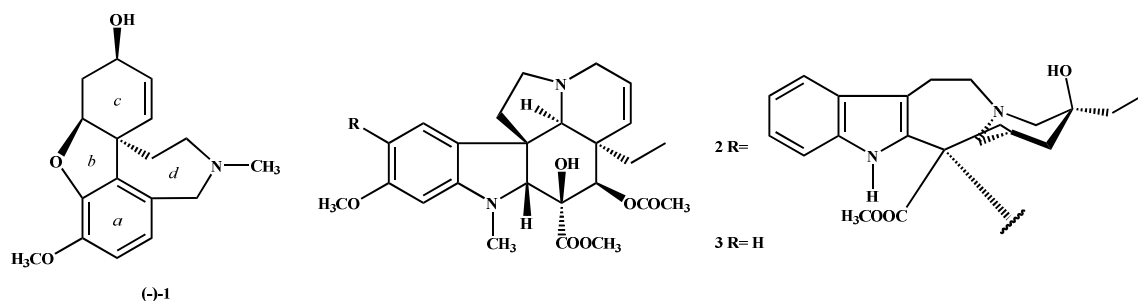
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Department of Organic Chemistry and Technology

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1. Introduction

Investigations of biologically active natural compounds comprise two main fields: (i) the total synthesis of alkaloids and their analogues, (ii) the modification of the structures for producing more selective, more effective, or less toxic derivatives.

In my PhD theses, the results obtained during the studies on two known natural compounds in accordance to the above-mentioned classification are presented. (i) For the preparation of (-)-galanthamine (**1**) and its analogues substituted in the aromatic A ring and on azepine nitrogen, respectively, we developed a flexible synthesis using simple reaction steps, (ii) and synthesized new derivatives of vinblastine (**2**), which showed significant cytostatic effect, as well as one of its components vindoline (**3**), which has been found less active so far.



2. Literature

2.1. Galanthamine

The biological effect of (-)-galanthamine (**1**), one of the important *Amaryllidaceae* alkaloids, is based on the inhibition of acetylcholine esterase as well as on the modulation of the nicotine receptors in the brain¹. The decreased level of acetylcholine plays a significant role in the development of Alzheimer disease. The release of acetylcholine may therefore slow down the progress of the disease as a result of its role played in the modulation of neuronal nicotine receptors. In addition, it is the only medicine for the treatment of Alzheimer disease which has a positive effect on the cognitive functions (process of learning and remembering), routine activities and behavioural disorders. Due to the increasing number of patients, the high prices and the limited supply of raw material, several research groups are trying to develop a number of synthetic methods to produce galanthamine.

¹ Maelicke, A.; Samochocki, M.; Jostock, R.; Fehrenbacher, A.; Ludwig, J.; Albuquerque, E. X.; Zerlin, M.: *Biol. Psychiatry*, **2001**, 49, 279-288

In 2006 a review was published² presenting in detail the synthetic ways to produce galanthamine and its derivatives, as well as their pharmacological results. Although several research groups have attempted new synthetic ways to prepare galanthamine, only a few methods have proved to be useful for the preparation of its derivatives. The publications can be classed into three groups: (i) the tetracycle characteristic of the *Amaryllidaceae* alkaloids was built up by phenolic oxidative coupling³; (ii) the benzofuran skeleton was synthesized by intramolecular Heck reaction followed by the formation of the azepine ring⁴; (iii) the spiro derivatives were prepared by other methods⁵ (e.g. photochemical reaction, Grewe cyclization, etc.).

The interest in the development of successful acetylcholine esterase inhibitors aims at the synthesis of new derivatives on the basis of galanthamine as "lead" molecule. Most of the synthetic approaches developed so far, however, provide little possibility for wider range of variations in the structure.

In our research group, we have developed a new synthetic route using simple reactions and suitable for the preparation not only of galanthamine, but also of its derivatives.

2.2. Literature on Vindoline

The chemistry of vinblastine and vincristine represents one of the most exciting fields of alkaloid research, however, very few results have been reported on vindoline and related analogues substituted in the A ring (nitration, halogenation⁶). Vindoline is applied mainly for the preparation of dimeric alkaloids.

Since the monomer alkaloid vindoline has been considered and found to be quite inactive, a new project has been launched to investigate the possibility of synthesizing biologically active vindoline derivatives through the modification of the reactive parts of its structure. Since

² Marco-Contelles, J.; Carreiras, M.; Rodríguez, C.; Villaroya, M.; Garcia, A. G.: *Chem. Rev.*, **2006**, *106* (1), 116-133

³ (a) Czollner, L.; Frantsits, W.; Küenburg, B.; Hedenig, U.; Fröhlich, J.; Jordis, U.: *Tetrahedron Letters*, **1998**, *39*, (15), 2087-2088. (b) Kodama, S.; Hamashima, Y.; Nishide, K.; Node, M.: *Angew. Chem. Int. Ed.*, **2004**, *43* (20), 2659-2661. (c) Node, M.; Kodama, S.; Hamashima, Y.; Katoh, T.; Nishide, K.; Kajimoto, T.: *Chem. Pharm. Bull.*, **2006**, *54* (12), 1662-1679

⁴ (a) Trost, B. M.; Tang, W.; Toste, F. D.: *J. Am. Chem. Soc.*, **2005**, *127* (42), 14785-14803. (b) Guillou, C.; Beunard, J.—L.; Gras, E.; Thal, C.: *Angew. Chem.*, **2001**, *113* (24), 4881-4882

⁵ (a) Holton, R. A.; Sibi, M. P.; Murphy, W. S.: *J. Am. Chem. Soc.*, **1988**, *110* (1), 314-316. (b) Sanchez, I. H.; Soria, J. J.; Lopez, F. J.; Larraza, M. I.; Flores, H. J.: *J. Org. Chem.*, **1984**, *49* (1), 157-163. (c) Lukács, A.; Szabó, L.; Hazai, L.; Ifj. Szántay, Cs.; Mák, M.; Gorka Á.: *Tetrahedron*, **2001**, *57*, 5843-5850.

⁶ (a) Bölskei, H.; Ifj. Szántay, Cs.; Mák, M.; Balázs, M.; Szántay, Cs.: *Acta Pharm. Bull.*, 1998, 6887-93. (b) Kutney, J. P.; Bunzli-Trepp, U.; Honda, T.; Katsube, J.; Worth, B. R.: *Helv. Chim. Acta*, 1987, 61. (c) Lewin, G.; Rolland, Y.; Schaffer, C.; Poisson, J.: *Heterocycles*, 1994, *38* (11), 2401-2405. (d) Kutney, J. P.; Beck, J.; Bylsma, F.; Cook, J.; Cretney, W. J.; Fuji, K.; Imhof, R.; Treasurywala, A. M.: *Helv. Chim. Acta*, 1975, *58* (6), 1690-1719. (e) Fekete, M.; Kolonits, P.; Novák, L.: *Heterocycles*, 2005, *65* (1), 165-171.

conjugating vinblastine with aminoacid-esters was more beneficial regarding its cytotoxic effect, we tried to produce new derivatives by the modification of the position 16 ester group, in which the vindoline molecule was conjugated with aminoacid derivatives⁷. Moreover, we also have studied the reactivity of positions 10 and 12 of the aromatic ring in electrophilic substitution reactions, e.g. nitration and halogenation.

3. Methods

In the course of my synthetic work I applied the regular preparative and separation methods of “classic” organic chemistry. I used thin layer chromatography for monitoring the reactions. For the isolation of the products, crystallization, distillation and preparative thin layer chromatography as well as column chromatography were used.

The structure of the compounds prepared was identified by spectroscopic methods (IR, ¹H- and ¹³C-NMR, MS).

4. Results

My research work presented in these theses can be divided into the following main parts:

- 4.1. The development of a model synthesis resulting in galanthamine, as well as the preparation of the key intermediates of the synthesis, and
- 4.2. The preparation of new vindoline and vinblastine derivatives.

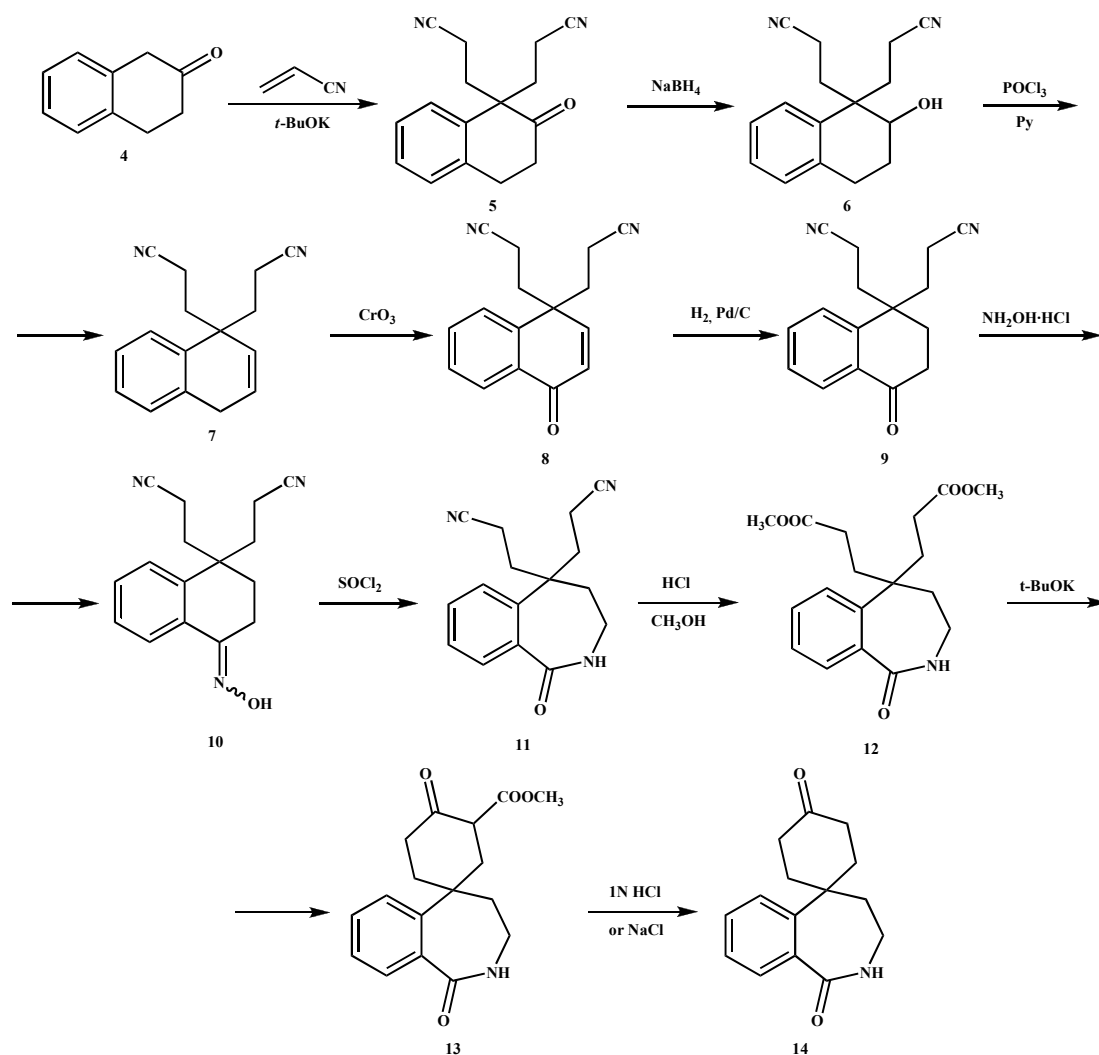
4.1. Development of a model synthesis resulting in galanthamine, as well as the preparation of the key intermediates of the synthesis

4.1.1. Development of the model synthesis

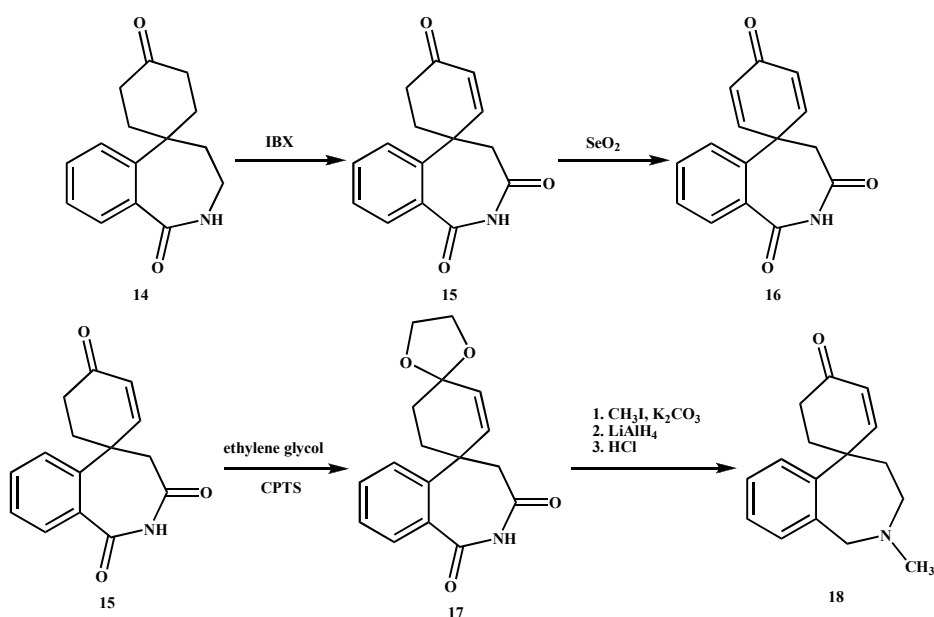
In the course of the synthesis, the reduction of the ketone (**5**) obtained from β-tetralone (**4**) by cyanoethylation resulted in the formation of alcohol (**6**). After the dehydration of the latter compound, the unsaturated derivative (**7**) was oxidized and after catalytic hydrogenation saturated ketone (**9**) was obtained. The Beckmann rearrangement of the oxim (**10**) prepared from ketone (**9**) resulted in the required bis(cyano-ethyl)benzo[*c*]azepinone (**11**). Then the diester (**12**) obtained in the Pinner reaction of dinitrile (**11**) was converted to ketoester (**13**) by

⁷ Rao, B. K. S. P.; Collard, M. P. M.; Dejonghe, J. P. C.; Atassi, G.; Hannart, J. A.; Trouet, A.: *J. Med. Chem.*, **1985**, 28 (8), 1079–1088

the Dieckmann condensation. Hydrolysis and decarboxylation or demethoxycarbonylation of ketoester (**13**) resulted in the expected spiro-substituted benzo[*c*]azepinone (**14**).

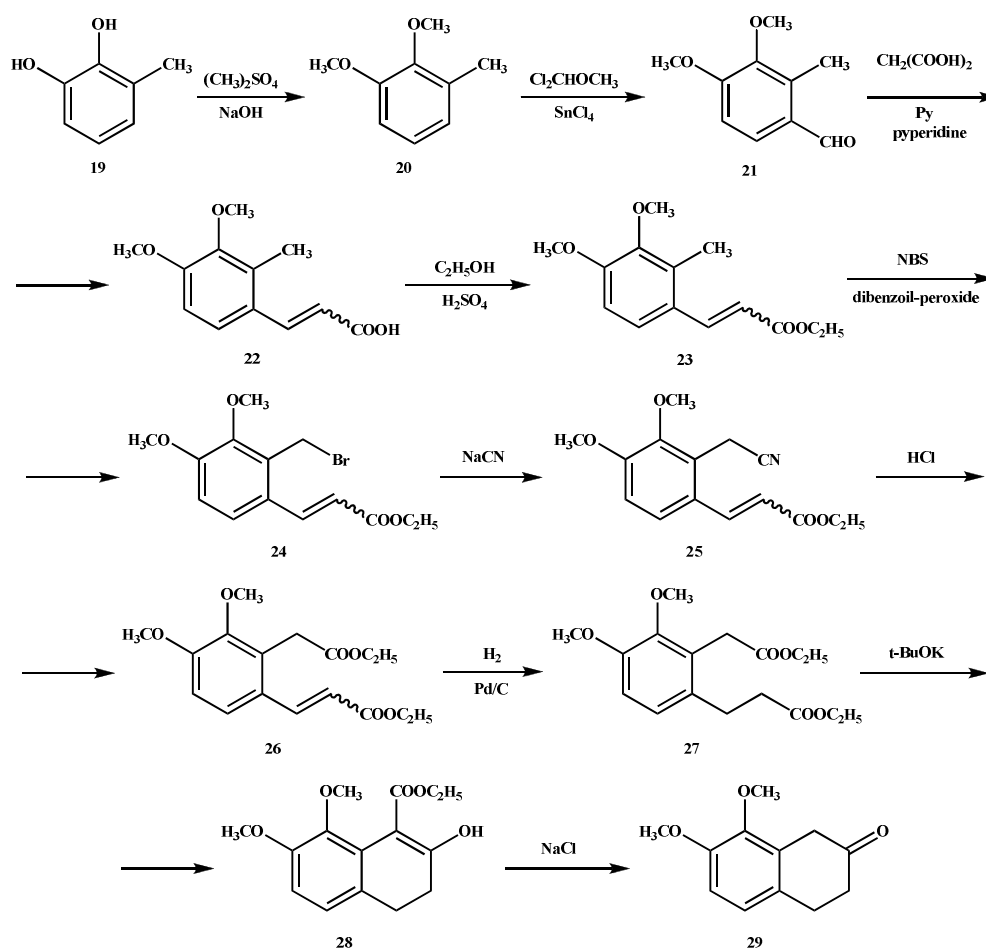


The spiroketon-lactam (**14**) was oxidized by *o*-iodobenzoic acid and accordingly, enone (**15**) was obtained. The second C=C double bond was introduced by means of oxidation with selenium dioxide resulting in the $\alpha,\beta : \alpha',\beta'$ -dienone (**16**). Ketal (**17**) from ketone (**15**) was formed with ethylene glycol in the presence of collidinium-*p*-toluol sulphonate. Then a methylation and a reduction step were carried out and the target molecule (**18**) of the model synthesis without methoxy substituent(s) was obtained in 15 steps with 3.3% overall yield.



4.1.2. Synthesis of 7,8-dimethoxy-2-tetralone

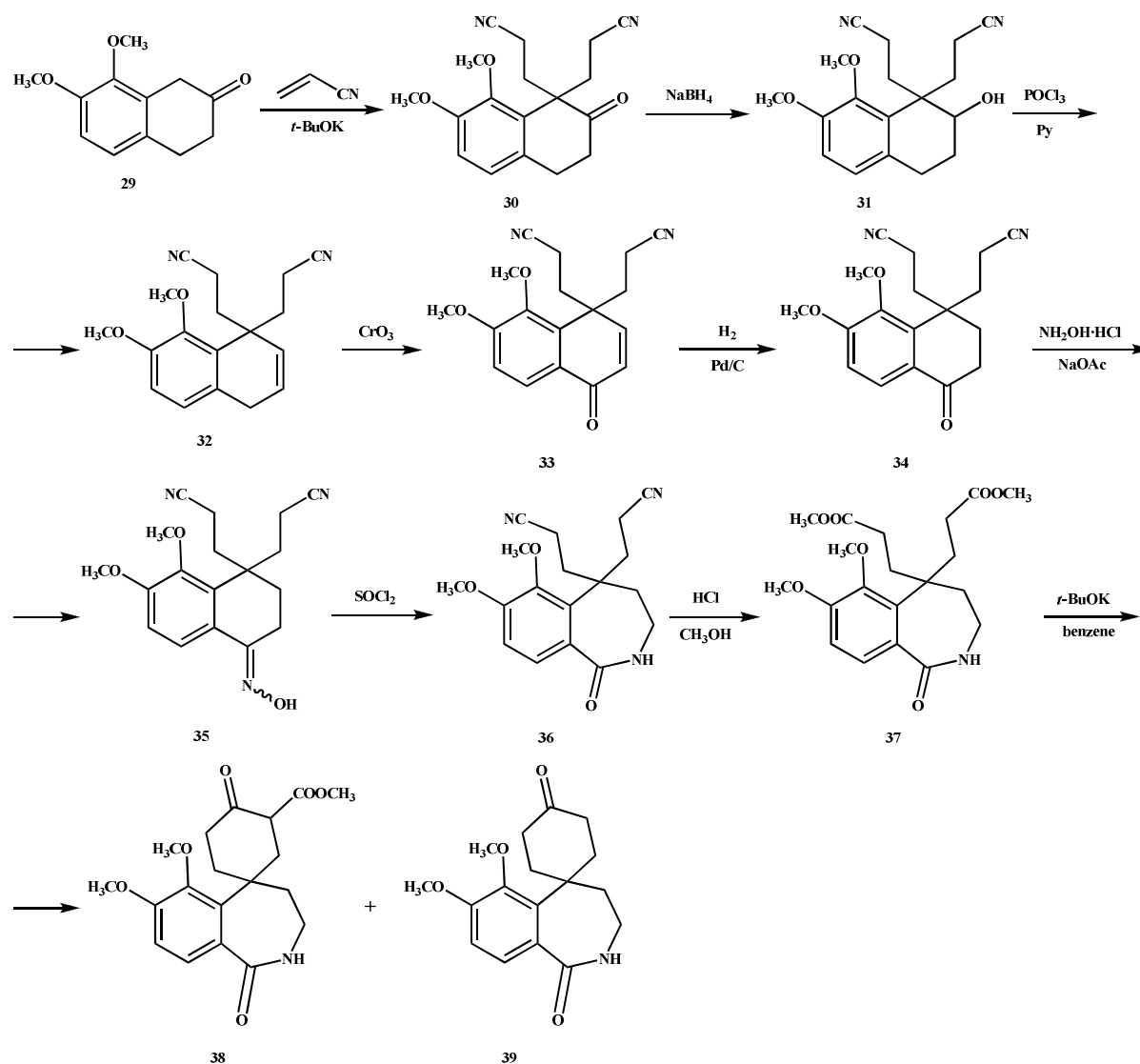
The *O*-methylation of 3-methylpyrocatechin (**19**) furnished compound **20**, which was then converted to aldehyde (**21**). The important key intermediate (**24**) was prepared *via* the Knoevenagel condensation and concomitant decarboxylation reaction of aldehyde **21** with



malonic acid, followed by the esterification and consecutive bromination with NBS. After replacing the halogen atom with cyano group, the obtained nitrile (**25**) was converted to the unsaturated diester (**26**) *via* the Pinner reaction. Then catalytic hydrogenation was carried out and the saturated diester (**27**) was subjected to the Dieckmann condensation reaction. After de-ethoxycarbonylation reaction of compound (**28**) in position 1 the required tetralon (**29**) was obtained. In this way a new synthesis was carried out by simple reaction steps and using commercially available reagents for the preparation of the 7,8-dimethoxy-2-tetralone (**29**) with 24% overall yield.

4.1.3. Preparation of key intermediates resulting in galanthamine

The synthesis elaborated for the model compound (**18**) as well as the preparation of 7,8-dimethoxy-2-tetralone (**29**) gave the possibility to prepare the key intermediates for the synthesis of galanthamine. 7,8-Dimethoxy-2-tetralone (**29**) was cyanoethylated by

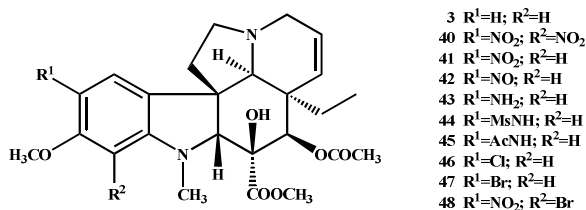


acrylonitrile, then the ketone (**30**) was reduced and the obtained alcohol (**31**) was converted to (**32**) by dehydration with phosphorus oxychloride. After the oxidation of the olefin (**32**) in allylic position followed by the saturation of the double bond by catalytic hydrogenation, the ketone (**34**) obtained was turned into the corresponding oxim (**35**). This was then subjected to the Beckmann rearrangement reaction resulting in the required benzo[*c*]azepinone (**36**). The Pinner reaction of the dinitrile (**36**) gave the diester (**37**). In the the Dieckmann cyclization reaction of the diester (**37**) surprisingly provided the de-methoxycarbonylated spiroketone (**39**) beside the expected spiro- β -oxo-ester (**38**). The compounds obtained can be considered as key intermediates in the preparation of narwedine as the precursor of galanthamine.

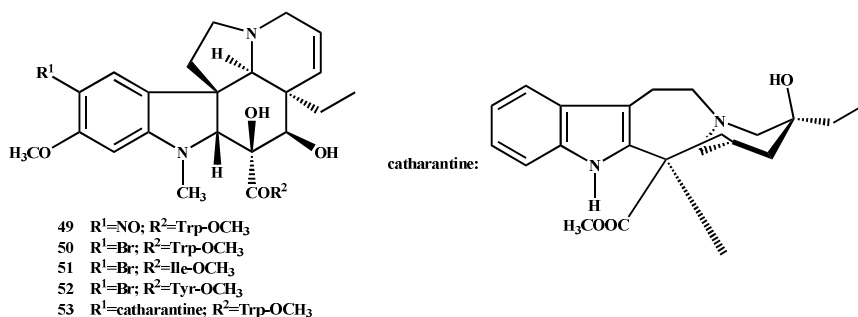
4.2. Preparation of new vindoline and vinblastine derivatives

4.2.1. Examination of the aromatic electrophilic substitution reactions on the aromatic ring of vindoline

10,12-Dinitro- (**40**); 10-nitro- (**41**); 10-nitroso- (**42**); 10-bromo- (**47**) and 10-chlorovindoline(**46**) were prepared by electrophilic substitution of the aromatic ring. During the nitration of 10-bromovindoline an unexpected rearrangement reaction was observed, which is known in the literature as the Reverdin reaction, and in this way 10-nitro-12-bromovindoline (**48**) was obtained. The reduction of 10-nitro- (**41**), and 10-nitrozovindoline (**42**) resulted in the corresponding 10-aminovindoline (**43**). Acylation of (**43**) provided mezilamino- (**44**), and acetylaminovindoline (**45**). The amino-group in position 10 unlocks the possibility to couple vindoline with various amino acids.



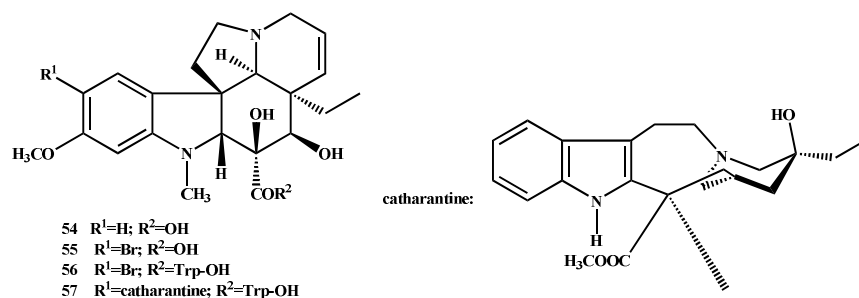
4.2.2. The coupling reactions of the ester group of vindoline and its derivatives



After activating the position 16 of vindoline and vinblastine by converting the ester group into carboxylic acid azide, amino acid esters were coupled with vindoline. Using this procedure I managed to prepare the derivative of 17-dezacyl-10-nitrovindoline coupled to tryptophan-methyl-ester (**49**), the derivative of 17-dezacyl-10-bromovindoline coupled to tryptophan-methyl-ester (**50**), isoleucine-methyl-ester (**51**), tyrosine-methyl-ester (**52**) and the derivative of vinblastine coupled to tryptophan-methyl-ester (**53**).

4.2.3. The examination of the hydrolysis of the ester group

In order to perform biological assays, the acetyl group was removed from the position 17 of vinblastine, and vindoline (**3**), 10-bromovindoline (**47**), 10-bromovindoline-tryptophan-methyl-ester derivative (**50**), as well as the vinblastine-tryptophan-methyl-ester derivative (**53**) ester group were subjected to hydrolysis to the corresponding carboxylic acid derivatives.



4.2.4. Biological results

The new compounds were tested by the NIH, the Research Group of Peptide Chemistry of HAS, as well as by the Research Group of Pathobiochemistry of HAS.

Among the new vindoline and vinblastine derivatives prepared by us, several compounds were found to have significant cytotoxic effect. Their further investigations are in progress.

5. Theses

1. Starting from 2-tetralone, I worked out a synthetic route resulting in the preparation of the spirobenzo[*c*]azepine as the basic skeleton of galanthamine.⁸
2. I achieved the preparation of 7,8-dimethoxy-2-tetralone using simple reaction steps.⁹
3. In accordance with the synthesis route I worked out, starting from 7,8-dimethoxy-2-tetralone I prepared spiro[*N*-methylbenzo[*c*]azepin-cyclohexenone], the key intermediate of the galanthamine synthesis.
4. During the study of electrophilic substitution reactions of vindoline I obtained new nitrated and halogenated derivatives in the aromatic ring, as well as derivatives containing acyl-amino group.¹⁰
5. Vindoline and vinblastine were coupled with various amino acid esters, and thereafter the hydrolysis of the ester group was also performed. The biological tests of the compounds obtained showed promising results.

⁸ Gorka, Á.; Hazai, L.; Ifj. Szántay, Cs.; Háda, V.; Szabó, L.; Szántay, Cs.: Synthesis of spiro-substituted benzo[*c*]azepinones, *Heterocycles*, **2005**, 65 (6), 1359-1371

⁹ Gorka, Á.; Czuczai, B.; Szoleczky, P.; Hazai, L.; Ifj. Szántay, Cs.; Háda, V.; Szántay, Cs.: Convenient synthesis of 7,8-dimethoxytetralin-2-one, *Synthetic Communications*, **2005**, 35 (18), 2371-2378

¹⁰ Gorka-Kereskényi, Á.; Szabó, L.; Hazai, L.; Lengyel, M.; Ifj. Szántay, Cs.; Sánta, Zs.; Kalas, Gy.; Szántay, Cs.: Aromatic electrophilic substitutions on vindoline, *Heterocycles*, **2007**, 71 (7), 1553-1563

6. Publications

Related publications to PhD thesis

Articles:

1. **Á. Gorka**; L. Hazai; Cs. Szántay, Jr.; V. Háda; L. Szabó; Cs. Szántay: Synthesis of spiro-substituted benzo[c]azepinones, *Heterocycles*, **2005**, 65 (6), 1359-1371. [IF: 1,07; Citations: 2]
2. **Á. Gorka**; B. Czuczai; P. Szoleczky; L. Hazai; Cs. Szántay, Jr.; V. Háda; Cs. Szántay: Convenient synthesis of 7,8-dimethoxytetralin-2-one, *Synthetic Communications*, **2005**, 35 (18), 2371-2378. [IF: 0,86; Citations: 2]
3. **Á. Gorka-Kereskényi**; L. Szabó; L. Hazai; M. Lengyel; Cs. Szántay, Jr.; Zs. Sánta; Gy. Kalaus; Cs. Szántay: Aromatic electrophilic substitutions on vindoline, *Heterocycles*, **2007**, 71 (7), 1553-1563. [IF: 1,066; Citations: 0]

Presentations and posters:

4. **Á. Gorka**; J. Hajgató; L. Szabó; L. Hazai; Cs. Szántay, Jr.; Cs. Szántay: Vindolin nitrálása. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2000. ORAL PRESENTATION
5. L. Szabó; **Gorka, Á.**; Hazai, L.; Ifj. Szántay, Cs.; Kalaus, Gy.; Szántay, Cs.: Biológiaiilag aktív vindolinok szintézise. MKE Vegyészkonferencia, Hajdúszoboszló, 2001. ORAL PRESENTATION
6. **Gorka, Á.**; Hazai, L.; Szabó, L.; Ifj. Szántay, Cs.; Milen, M.; Mándi, R.; Szántay, Cs.: Kísérletek a galanthamin szintézisére. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2002. ORAL PRESENTATION
7. Novák, L.; Hazai, L.; Szabó, L.; Fekete, M.; **Gorka, Á.**; Szántay, Cs.: Új vindolin-származékok előállítása és vizsgálata. II. Medichem Konferencia, Kémiai Kutatóközpont, Budapest, 2002. ORAL PRESENTATION
8. Szabó, L.; **Gorka, Á.**; Hazai, L.; Ifj. Szántay, Cs.; Kalaus, Gy.; Szántay, Cs.: Biológiaiilag aktív vindolinok szintézise. Ipari Nyílt Nap, BME, 2002. POSTER PRESENTATION
9. **Gorka, Á.**; Milen, M.; Mándi, R.; Czuczai, B.; Hazai, L.; Szabó, L.; Ifj. Szántay, Cs.; Szántay, Cs.: Galanthamin és származékainak szintézise. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2003. ORAL PRESENTATION
10. Novák, L.; Hazai, L.; Szabó, L.; Fekete, M.; **Gorka, Á.**; Szántay, Cs.: Központi idegrendszerre ható és sejtnövekedést gátló új anyagok előállítása. III. Medichem Konferencia, MTA Kémiai Kutatóközpont, Budapest, 2004. ORAL PRESENTATION
11. **Gorka, Á.**; Milen, M.; Czuczai, B.; Hazai, L.; Szabó, L.; Ifj. Szántay, Cs.; Szántay, Cs.: Kísérletek a galanthamin és származékainak előállítására. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2004. ORAL PRESENTATION
12. Hazai, L.; **Gorka, Á.**; Milen, M.; Mándi, R.; Czuczai, B.; Szabó, L.; Ifj. Szántay, Cs.; Szántay, Cs.: Kísérletek a galanthamin-típusú vegyületek előállítására. Bruckner termi előadások, Budapest, 2004. ORAL PRESENTATION

13. Hazai, L.; Szabó, L.; **Gorka, Á.**; Tóth, A.; Ifj. Szántay, Cs.; Bökönyi, Gy.; Kéri, Gy.; Reményi, J.; Mező, G.; Hudecz, F.; Szántay, Cs.: Biológiai aktív vindolin-származékok szintézise. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2005. ORAL PRESENTATION
14. **Gorka Á.**: Új szintézis a 7,8-dimetoxi-2-tetralon előállítására. Budapesti Műszaki és Gazdaságtudományi Egyetem Vegyészmérnöki és Biomérnöki Kar, Doktoráns Konferencia, Budapest, 2006. ORAL PRESENTATION
15. **Gorka-Kereskényi, Á.**; Czuczai, B.; Szoleczky, P.; Prepok, A.; Hudák, M. Sz.; Gorka-Kereskényi, L.; Hazai, L.; Szabó, L.; Ifj. Szántay, Cs.; Szántay, Cs.: Galanthamin intermedierek szintézise. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2006. ORAL PRESENTATION
16. Novák, L.; Hazai, L.; Szabó, L.; **Gorka-Kereskényi, Á.**; Fekete, M.; Szántay, Cs.: Új vindolin-származékok szintézise. Medichem2 Konferencia, Kémiai Kutatóközpont, Budapest, 2006. ORAL PRESENTATION
17. Hudák, M. Sz.; **Gorka, Á.**; Prepok, A.; Hazai, L.; Ifj. Szántay, Cs.; Szántay, Cs.: Ciklohexán gyűrűvel spiro-helyettesített benzo[c]azepinekhez vezető reakcióutak vizsgálata. Kémiai Előadói Napok, Szeged, 2006. ORAL PRESENTATION
18. **Gorka-Kereskényi, Á.**; Hudák, M. Sz.; Prepok, A.; Hazai, L.; Szabó, L.; Ifj. Szántay, Cs.; Szántay, Cs.: Galanthamin-származékok szintézise. Újabb eredmények. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2007. ORAL PRESENTATION
19. Bánóczy, Z.; **Gorka-Kereskényi, Á.**; Reményi, J.; Szabó, L.; Hazai, L.; Hudecz, F.; Szántay, Cs.: Vinblasztin-származékot tartalmazó oligoarginin konjugátumok szintézise és in vitro tumorellenes hatásának vizsgálata szenitív és rezisztens HL-60 sejteken. MKE Vegyészkonferencia, Sopron, 2007. ORAL PRESENTATION
20. Novák, L.; Hazai, L.; Szabó, L.; Fekete, M.; Törincsi, M.; **Gorka-Kereskényi, Á.**; Hornyánszky, G.; Szántay, Cs.: Új sejtsztódást gátló és vírus ellenes anyagok szintézise. MediChem2, MTA Kémiai Kutatóközpont, Budapest, 2007. ORAL PRESENTATION
21. **Gorka-Kereskényi, Á.**: Kísérletek a galanthamin előállítására. sanofi-aventis PhD beszámoló, Budapest, 2008. ORAL PRESENTATION
22. Herke, K.; Hazai, L.; Milen, M.; Prepok, A.; **Gorka-Kereskényi, Á.**, Szabó, L.; ifj. Szántay, Cs.; Szántay, Cs.: Galanthamin-származékok szintézise, II. MTA Alkaloidkémiai Munkabizottság, Balatonfüred, 2008. ORAL PRESENTATION
23. **Gorka-Kereskényi, Á.**: Synthesis and reactions of biologically active natural compounds. sanofi-aventis PhD ösztöndíjas beszámoló, Budapest, 2008. POSTER PRESENTATION

Other publications

24. A., Lukács; L., Szabó; L., Hazai; Cs., Szántay, Jr.; M., Mák; **Á., Gorka:** *Tetrahedron*, **2001**, 57, 5843-5850. [IF: 2,276; Citations: 4]