



BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY
GEORGE OLAH DOCTORAL SCHOOL

**STERESELECTIVE SYNTHESIS OF *TRANS*-
DIHYDRONARCICLASINE AND ITS ANALOGUES**

PhD Thesis

SZERZŐ:

GÁBOR VARRÓ, MSc

SUPERVISOR:

LÁSZLÓ HEGEDŰS, PhD

CONSULTANTS:

ISTVÁN KÁDAS, PhD

ANDRÁS SIMON, PhD

DEPARTMENT OF ORGANIC CHEMISTRY AND TECHNOLOGY

BUDAPEST
2019

I. INTRODUCTION

Phenanthridone alkaloids form a small group of alkaloids and, according to the modern definition of alkaloids, they are belonging to a subgroup of *Amaryllidaceae* alkaloids. Despite only 13 phenanthridone alkaloids have been isolated up to now, these compounds possess very strong potency against tumorous cell lines. Phenanthridone alkaloids have been synthesising at the Department of Organic Chemistry and Technology of BME for more than two decades. During my PhD work I joined in this research work and my aim was to synthesise the strongest cytostatic representative of this alkaloid family, *trans*-dihydro-narciclasine. Moreover, through the modification of the ring A of the phenanthridone scaffold, I also realised the total synthesis of some analogues to evaluate the variation of their biological activity depending on the substitution of ring A.

II. LITERATURE SURVEY

According to the last data of WHO, cancer is the second leading cause of decease worldwide, and was responsible for an estimated 9.6 million deaths in 2018.¹ Although there are many synthetic and naturally occurring molecules, which proved to be effective against different tumour cells, the development of new anticancer agents with decreased side effect and improved efficiency is still needed.

It has long been known that extracts from the *Amaryllidaceae* plant family have strong antitumour activity.² Members of this plant family are well favoured due to its colourful flowers, so they are grown as ornamental flowers. The ancient Greek physician, Hippocrates of Kos was the first in the history who recommended the narcissus oil for the treatment of uterine cancer. From that time up the *Narcissus* species had worldwide an important part in the treatment of cancerous diseases.²

Later it was proven that the extracts of *Amaryllidaceae* plants had powerful cytostatic activity *in vitro*, but the alkaloids, which are responsible for this activity, were unknown. The first isolated *Amaryllidaceae* alkaloid was lycorine (**1**, *Figure 1*) which was isolated from the bulbs of *Narcissus pseudonarcissus* by Gerrard,³ in 1877, but its structure was elaborated almost only 100 years later, in 1956. Thus, the isolation of phenanthridone alkaloids just begun in the middle of 20th century. However, only 13 phenanthridone alkaloids have been isolated from *Amaryllidaceae* plants and their structures have been determined by spectroscopy up to now.² Their most important representatives are shown in *Figure 2*. Intensive researches for finding new alkaloids are still in progress: for example the last alkaloid, 2-*epi*-narciclasine, was isolated in 2018.⁴

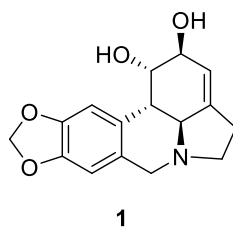


Figure 1: Structure of lycorine (1)

¹ Cancer – Key facts (WHO) (<https://www.who.int/en/news-room/fact-sheets/detail/cancer>, Sept, 2018)

² Kornienko, A.; Evidente, A. *Chem. Rev.* **2008**, *108*, 1982–2014.

³ Gerrard, A. W. *Pharm. J.* **1877**, *8*, 214–215.

⁴ Borra, S.; Lapinskaite, R.; Kempthorne, C.; Liscombe, D.; McNulty, J.; Hudlicky, T. *J. Nat. Prod.* **2018**, *81*, 1451–1459.

Phenanthridone alkaloids were tested in several disease models. The most important therapeutic goals were to examine their antiviral and cytostatic effects. It was found by Gabrielsen et al. that phenanthridone alkaloids were active against *Flaviviridae* and *Bunyaviridae* species, but their effective concentration were very close to the lethal one *in vitro*, so these alkaloids cannot be used in an antiviral therapy.^{5,6} McNulty et al. reported similar results, who tested *trans*-dihydronarciclasine (**2**), narciclasine (**3**) and pancratistatin (**7**) against Zika-virus.⁷ In addition, pancratistatin (**7**), *trans*-dihydrolycoricidine (**6**) and narciclasine (**3**) were found to be active against herpes simplex and varicella zoster viruses.⁸

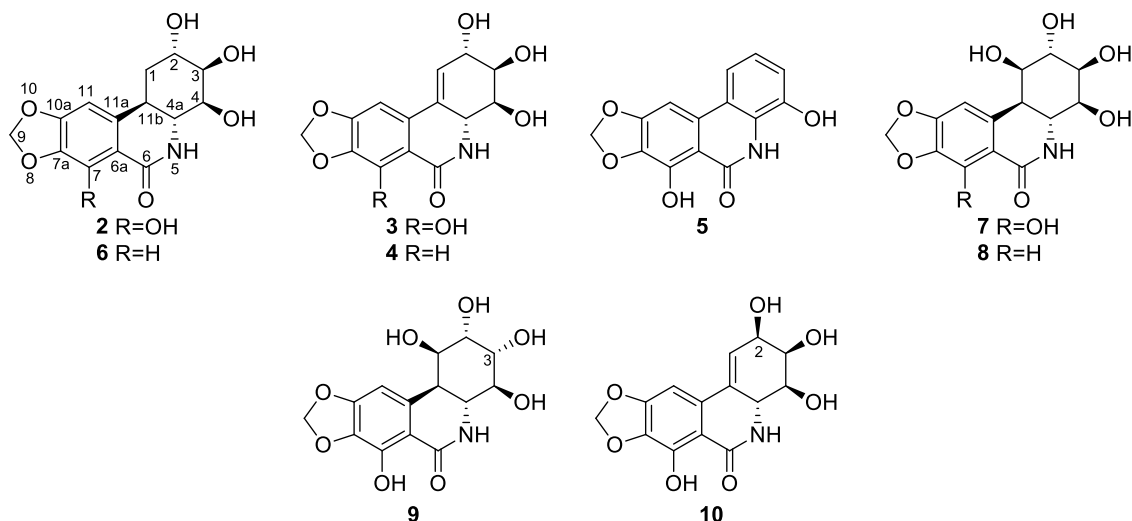


Figure 2: The most frequent phenanthridone alkaloids: *trans*-dihydronarciclasine (**2**), narciclasine (**3**), lycoricidine (**4**), narciprimine (**5**), *trans*-dihydrolycoricidine (**6**), pancratistatin (**7**), 7-deoxypancratistatin (**8**), 3-epi-pancratistatin (**9**) and 2-epi-narciclasine (**10**)

It was proved that the most important biological property of phenanthridone alkaloids is their cytostatic activity. The outstanding anticancer activity of narciclasine (**3**) and lycoricidine (**4**) was firstly discovered by Ceriotti's⁹ and Okamoto's¹⁰ research groups, as well as by Mondon and Krohn.¹¹ Later, Vazquez and co-workers observed the growth inhibition activity of narciclasine (**3**) in HeLa cells, which was attributed to its strong protein inhibition activity.¹² Other researchers examined the apoptosis induced by pancratistatin (**7**) and narciclasine (**3**).² They supposed that the cytostatic effect of **7** and **3** can be attributed to the caspase mediated apoptosis of tumour cells which occurred through the damage of mitochondria. Moreover, they found selectivity between the caspase mediated apoptosis of tumourous and non-tumourous cells induced by **3** and **7** contrary to the reference compounds, such as etoposide and paclitaxel. This was a very important observation, because most of the commercially available chemotherapeutics have a very low selectivity, because they do not

⁵ Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kirsi, J. J.; Hollingshead, M.; Shannon, W.; Pettit, G. R. *Nat. Prod. Antivir. Agents* **1992**, 121–135.

⁶ Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kefauver, D. F.; Pettit, G. R.; Groszek, G.; Hollingshead, M.; Kirsi, J. J.; Shannon, W. M.; Schubert, E. M.; DaRe, J.; Ugarkar, B.; Ussery, M. A.; Phelan, M. J. *J. Nat. Prod.* **1992**, 55, 1569–1581.

⁷ Revu, O.; Zepeda-Velázquez, C.; Nielsen, A. J.; McNulty, J.; Yolken, R. H.; Jones-Brando, L. *Med. Chem. Drug Discovery* **2016**, 1, 5895–5899.

⁸ McNulty, J.; D'Aiuto, L.; Zhi, Y.; McClain, L.; Zepeda-Velázquez, C.; Ler, S.; Jenkins, H. A.; Yee, M. B.; Piazza, P.; Yolken, R. H.; Kinchington, P. R.; Nimgaonkar, V. L. *ACS Med. Chem. Lett.* **2016**, 7, 46–50.

⁹ Ceriotti, G. *Nature* **1967**, 213, 595–596.

¹⁰ Okamoto, T.; Torii, Y.; Isogai, Y. *Chem. Pharm. Bull.* **1968**, 16, 1860–1864.

¹¹ Mondon, A.; Krohn, K. *Chem. Ber.* **1975**, 108, 445–463.

¹² Jimenez, A.; Santos, A.; Alonso, G.; Vazquez, D. *Biochim. Biophys. Acta* **1976**, 425, 342–348.

kill exclusively the cancerous cells, but the healthy ones, as well. In addition, it was proved that narciclasine (**3**) had interaction with cytochrome enzymes, which could be led to hepatotoxic effects, due to its double bond in its ring C.^{13,14}

Due to the good biological activities mentioned above, several research groups tried to understand which pharmacophores were responsible for the biological activity. The newly synthesised phenanthridone alkaloid analogues were tested on different cell lines *in vitro*, and some conclusions were drawn related to the structure–activity relationships.^{2,15}

The most examined part of the phenanthridone scaffold was ring C, because it has the most complex structure. This means that narciclasine (**3**) has four stereocentres and a double bond, *trans*-dihydronarciclasine (**2**) has five, while pancratistatin (**7**) has six stereocentres in its ring C. It was proved that the presence of C-2, C-3 and C-4 hydroxy groups with the corresponding configuration was essential for the good antitumour effect. The C=C double bond had no influence on the antiproliferative activity, but its displacement into the anellation B–C was detrimental for this activity. It seemed that C-1 hydroxy group could be negligible without changing the antiproliferative effect, but Pettit showed that its benzylation could enhance the activity of a natural representative (phenpanstatin).

It was found that the presence of a lactam structural part is necessary in the ring B. Lactone analogues and substituted lactams were practically ineffective. Furthermore, the opened *seco*-structures which have only A–C ring systems were also ineffective.

There is, however, a significantly little knowledge about the role of substituents in the ring A. It was proved that the presence of all oxygen atoms were essential for the biological activity, and the absence of C-7 phenolic hydroxy group caused decrease in the efficacy. Changing the benzene ring to *N*-heterocycles (e.g. pyridine or indole ring) was also detrimental. Lastly, it was also proved that the *ent*-form of alkaloids were ineffective.

Availability of the phenanthridone alkaloids from natural sources is limited. The most effective member of phenanthridone alkaloids, *trans*-dihydronarciclasine (**2**), can be extracted from *Zephyranthes candida*. Pettit et al. isolated compound **2** from 18 kg of grounded bulbs of *Zephyranthes candida* in an amount of 16 mg in triacetoxo form.¹⁶ It is obvious from this data that developing a synthetic method may be the best option to obtain compound **2** in a sufficient amount. Mainly semisynthetic methods were examined to prepare compound **2** from narciclasine (**3**) which was available in a higher amount than its dihydro analogue. However, the catalytic hydrogenation of C=C double bond of **3** resulted in a mixture of products in which *cis*-dihydronarciclasine and isonarciclasine were the main side products.^{11,17,18} Thus, the elaboration of a scalable total synthesis from an inexpensive starting material, using non-extreme reaction conditions has become an important synthetic task.

¹³ McNulty, J.; Nair, J. J.; Singh, M.; Crankshaw, D. J.; Holloway, A. C.; Bastida, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3233–3237.

¹⁴ McNulty, J.; Thorat, A.; Vurgun, N.; Nair, J. J.; Makaji, E.; Crankshaw, D.; Holloway, A. C.; Pandey, S. J. *Nat. Prod.* **2011**, *74*, 106–108.

¹⁵ Ghavre, M.; Froese, J.; Pour, M.; Hudlicky, T. *Angew. Chem. Int. Ed.* **2016**, *55*, 5642–5691.

¹⁶ Pettit, G. R.; Cragg, G. M.; Singh, S. B.; Duke, J. A.; Doubek, D. L. *J. Nat. Prod.* **1990**, *53*, 176–178.

¹⁷ Mondon, A.; Krohn, K. *Tetrahedron Lett.* **1972**, *21*, 2085–2088.

¹⁸ Pettit, G. R.; Ducki, S.; Eastham, S. A.; Melody, N. *J. Nat. Prod.* **2009**, *72*, 1279–1282.

III. EXPERIMENTAL METHODS

During my synthetic work I followed the classical chemical preparative and isolation methods. Thin layer chromatography was used for the monitoring of reactions. Purification of products was carried out by crystallisation, distillation, column chromatography or preparative TLC.

Structure determination of synthesised products and intermediates were carried out by one- or two-dimensional NMR spectroscopy. Single-crystal X-ray diffraction measurements was used to determine the absolute configuration of two intermediates. Besides, measurement of melting point and optical rotation, chiral HPLC methods and IR analysis were also used to characterise them.

IV. RESULTS

At first, I elaborated a scalable total synthesis of racemic *trans*-dihydonarciclasine {(±)-**2**}.¹⁹ The main intermediate of this synthesis was myristicin aldehyde (**14**), a commercially available substance, but it was expensive. Therefore vanillin (**11**) was chosen as a starting material. In the first step, compound **11** was selectively iodinated to give 5-iodovanillin (**12**). 5-Hydroxyvanillin (**13**) was obtained by hydrolysis of **12**, then the alkylation of both hydroxy groups with methylene bromide resulted in myristicin aldehyde (**14**, Figure 3).

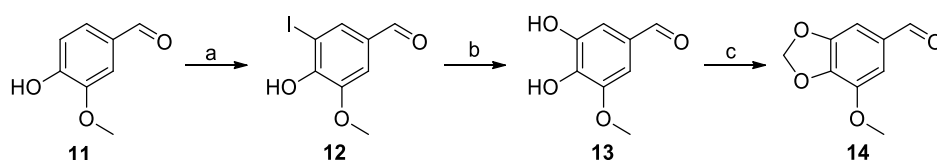


Figure 3: Reagents and conditions: (a) I_2 , KI, $NaHCO_3$, H_2O , rt, 3 h, 99%; (b) 20% $NaOH/H_2O$, $CuSO_4$, reflux, 16 h, 68%; (c) CH_2Br_2 , CuO , K_2CO_3 , DMF, 100 °C, 2 h, 94%.

In the next step, compound **14** was converted into the corresponding benzylideneacetone (**15a**). This reaction had to be carried out carefully, because a bis derivative of **15a** could be formed as a by-product in the Claisen–Schmidt reaction. To avoid the formation of dibenzylideneacetone, diluted aqueous suspension with a great excess of acetone was used. The pure arylbutenone (**15a**) was obtained after distillation. From this arylbutenone the corresponding nitropentanone {(±)-**16a**} was obtained by Michael addition of nitromethane using $NaOCH_3$ according to Walker's method²⁰ (Figure 4).

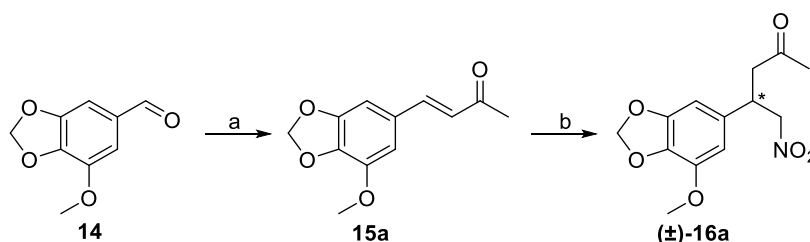


Figure 4: Reagents and conditions: (a) acetone, $NaOH$, H_2O , rt, 20 h, 67% (after distillation); (b) CH_3NO_2 , $NaOCH_3/CH_3OH$, reflux, 5.5 h, 54%.

In the next reaction step, the ring C was closed by Claisen–Henry reaction using ethyl formate to obtain the corresponding nitrocyclohexanolone {(±)-**17a**}. This reaction was fully stereoselective, which resulted in the proper anellation between the rings A–C, as well as an

¹⁹ Varró, G.; Hegedűs, L.; Simon, A.; Kádas, I. *Tetrahedron Lett.* **2016**, 57, 1544–1546.

²⁰ Walker, G. N. *J. Org. Chem.* **1965**, 30, 1416–1421.

equatorial position of the nitro group. The aromatic substituent of the formed cyclohexane ring occupies the energetically favourably equatorial position, thus the bulky, adjacent nitro group also tries to locate itself for a maximum distance from it, which can also be realised in an equatorial position. Meanwhile, a hydrogen bond is formed between the nitro and hydroxy groups, and this interaction determines the configuration of the carbon atom connected to the hydroxy group during the cyclohexane ring closure. This was evidenced by the significantly shifted peak of the $-OH$ group from 4.09 ppm to 6.02 ppm in the 1H NMR spectrum of compound **17a**, which refers to the presence of a hydrogen in a chelate bond. These facts were confirmed by single-crystal X-ray analysis of the nitrocyclohexanolone ($-$)-**17a** (Figure 5).

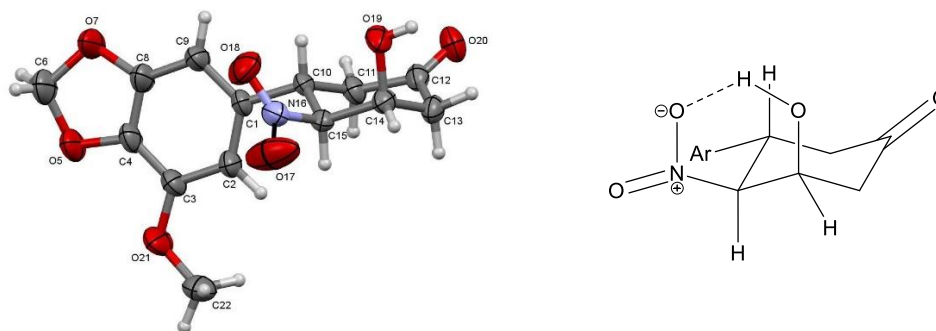


Figure 5: The structure of compound ($-$)-**17a**

Prior to the preparation of the corresponding amine $\{(\pm)\text{-19a}\}$ by catalytic hydrogenation of the nitro group, the oxo moiety had to be protected to avoid the Schiff base formation during the reduction. Its protection as a ketal was not feasible with the traditional method (ethylene glycol, azeotropic distillation of water–toluene) due to the sensitivity of compound $(\pm)\text{-17a}$, therefore applying milder conditions was necessary. Using anhydrous oxalic acid and ethylene glycol in acetonitrile, the ketal formation took place already at room temperature to give compound $(\pm)\text{-18a}$ in good yield. Then amino ketal $\{(\pm)\text{-19a}\}$ was obtained by catalytic hydrogenation over a Pd/C catalyst, at a relatively high temperature (80 °C) (Figure 6).

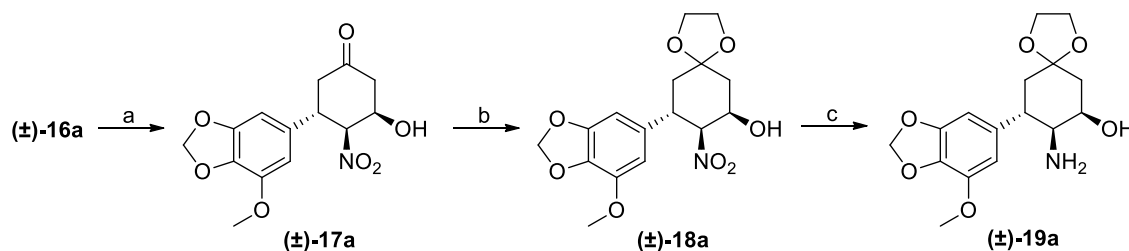


Figure 6: Synthesis of amino ketals. Reagents and conditions: (a) $HCOOEt$, $NaOCH_3$, Et_2O , rt, 20 h, 54 %; (b) $(CH_2OH)_2$, $(COOH)_2$, CH_3CN , rt, 3 d, 90%; (c) H_2 , 10% Pd/C catalyst (Selcat Q), CH_3OH , 80 °C, 7 h, quant.

In the next step, the amino group was converted regioselectively into carbamate $\{(\pm)\text{-20a}\}$, then the ketal protective group was removed by using acid catalysis in acetone. Due to the adjacent acidic proton of the oxo group, water elimination took place simultaneously. The oxo group was then reduced regio- and stereoselectively by Luche's method modified by Utimoto, using $NaBH_4$ and $CaCl_2$ in methanol (Figure 7). However, the *trans*-selective reaction gave the unwanted configuration of the hydroxy group (α -enone), therefore changing its configuration was necessary.

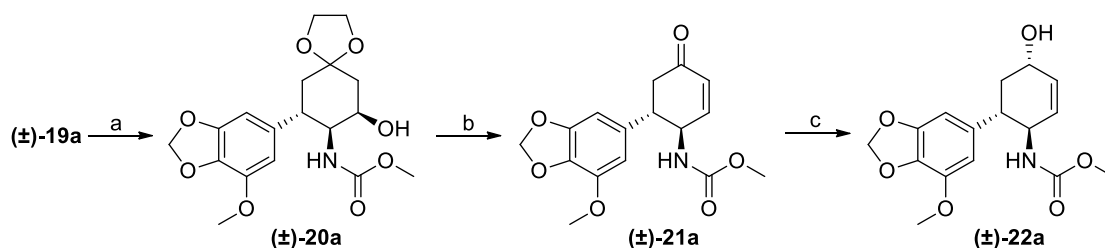


Figure 7: Synthesis of enols. Reagents and conditions: (a) ClCOOCH_3 , $\text{NaOH}/\text{H}_2\text{O}$, THF , 2 h, 99%; (b) $p\text{-TsOH}$, acetone, reflux, 1 h, 99%; (c) NaBH_4 , CaCl_2 , CH_3OH , 0 °C, 2 h, 96%.

To convert the *trans*-enol (±)-22a into *cis*-benzoate {(±)-24a} the Mitsunobu reaction was applied. The benzoyl group did not serve as a protective group only, but it also provided stereoselectivity in the next reaction step. *cis*-Dihydroxylation took place regioselectively by using Sharpless–Upjohn’s method, which attributed to the steric hindrance of benzoyl group, to afford compound (±)-25a. Then, the hydroxy groups were acetylated to protect them prior to the closure of ring B (Figure 8).

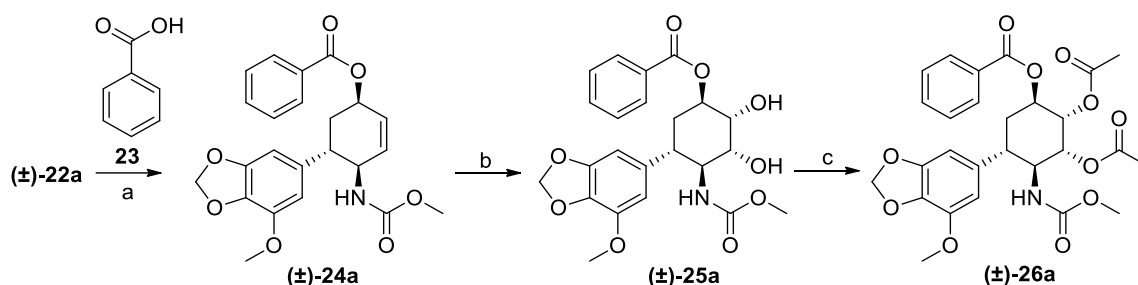


Figure 8: Synthesis of diacetoxyurethanes. Reagents and conditions: (a) **23**, DEAD , PPh_3 , THF , rt, 4 h, 63%; (b) OsO_4 , NMO , THF , H_2O , Ar atmosphere, rt, 24 h, 100%; (c) AcCl , rt, 24 h, 100%.

This cyclisation step was carried out by the Bischler–Napieralski reaction modified by Banwell, applying trifluoromethanesulphonic acid anhydride and 4-(dimethylamino)pyridine.

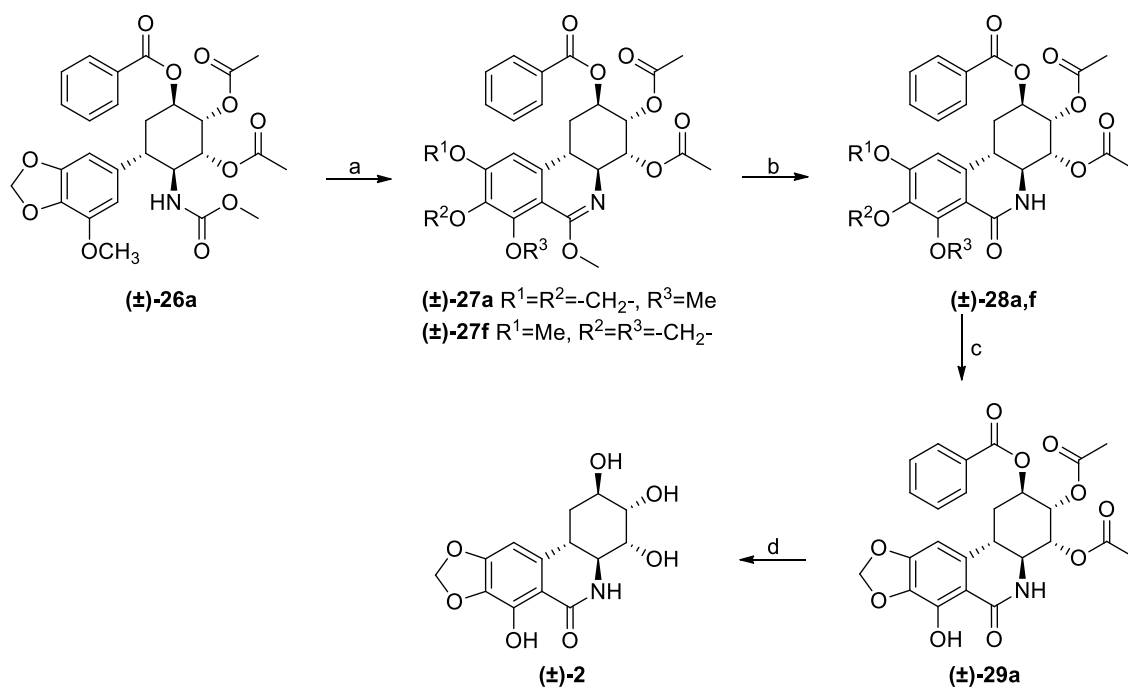


Figure 9: Last steps of racemic *trans*-dihydronarciclasine {(±)-2}. Reagents and conditions: (a) Tf_2O , DMAP , CH_2Cl_2 , 0 °C → rt, 22 h, 99%; (b) (1) $2\text{M HCl}/\text{H}_2\text{O}$, THF , rt, 21 h, (2) AcCl , rt, 21 h, 92%; (c) TMS-Cl , KI , CH_3CN , 60 °C, 1.5 h, 50%; (d) NaOCH_3 , CH_3OH , THF , rt, 1 h, 99%.

During this step, regioisomers were formed in a ratio of 3:1 in favour of the 7-methoxy analogue, presumably due to the distorted structure of the five-membered dioxole ring. However, the separation of these regioisomers was not successful in this step. Thus, the metoxyphenanthridine regioisomers were converted into lactams, and the 7-methoxy group was cleaved selectively. Lastly, racemic **2** was obtained by Zemplén's deacylation (*Figure 9*).

The next synthetic aim was the enantioselective total synthesis of **2**.²¹ For this purpose I applied the aforementioned synthesis using an organocatalyst in the asymmetric step. Firstly, I used a Jørgensen-type imidazolidine (**30**) catalyst, however, it provided only a moderate enantiomeric excess. Then, 9-amino(9-deoxy)epiquinine (**31**) and 9-amino(9-deoxy)epicinchonine (**32**) organocatalysts were tested (*Figure 10*) in the Michael addition of nitromethane onto the corresponding benzylideneacetone, even though their thiocarbamide and squaramide derivatives were traditionally used in the asymmetric Michael additions, according to the literature data. Using the results of Duan and co-workers',²² the amino derivatives of these cinchona alkaloids were applied in this organocatalytic reaction. Surprisingly, the best enantiomeric excess was obtained by using 9-amino(9-deoxy)epiquinine (**31**) in the Michael addition of nitromethane using the excess of reagent as a solvent, and without any co-solvents^{21,23,24}. Since this method is very rare in the literature further 22 benzylideneacetones, which could be substrates for newer phenanthridone derivatives, were prepared using this reaction with good or excellent enantiomeric purity.

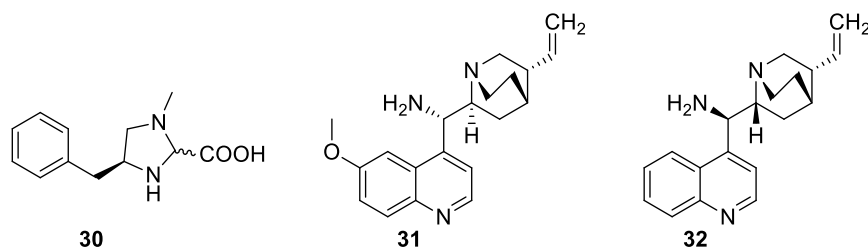


Figure 10: Structure of organocatalysts applied: Jørgensen's catalyst (30), 9-amino(9-deoxy)epiquinine (31), 9-amino(9-deoxy)epicinchonine (32)

After this step, the total synthesis was continued as described earlier (*Figure 11*). Single-crystal X-ray measurements of nitropentanone {(–)-**16a**} and nitrocyclohexanolone {(–)-**17a**}, as well as the negative optical rotation of compound **2** proved that the *ent*-form of **2** was obtained at the end of reaction route.

After the enantioselective synthesis of **2**, some analogues were synthesised to examine structure–activity relationships. Some trialkyloxy, 7-hydroxydialkyloxy and six-membered analogues containing dihydrobenzodioxine ring were prepared.^{23,24}

²¹ Varró, G.; Hegedűs, L.; Simon, A.; Balogh, A.; Grün, A.; Leveles, I.; Vértessy, G. B.; Kádas, I. *J. Nat. Prod.* **2017**, *80*, 1909–1917.

²² Liu, W.; Mei, D.; Wang, W.; Duan, W. *Tetrahedron Lett.* **2013**, *54*, 3791–3793.

²³ Varró, G.; Pogrányi, B.; Grün, A.; Simon, A.; Hegedűs, L.; Kádas, I. *Monatsh. Chem.* **2018**, *149*, 2265–2285.

²⁴ Varró, G.; Mattyasovszky, L.; Grün, A.; Simon, A.; Hegedűs, L.; Kádas, I. *Synthesis* **2018**, *50*, 625–643.

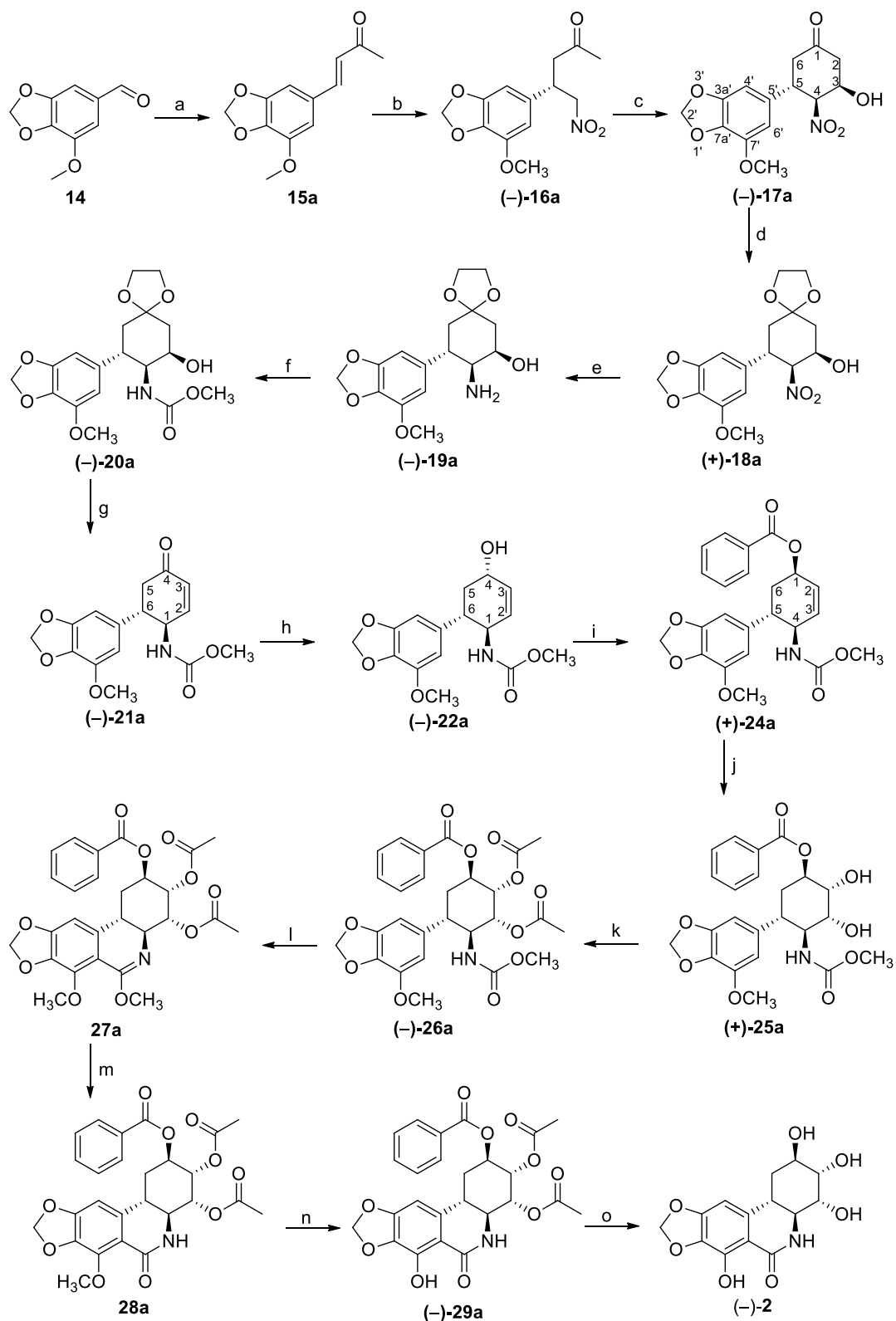


Figure 11: Reagents and conditions: (a) acetone, NaOH, H₂O, rt, 20 h, 67% (after distillation); (b) CH₃NO₂, **31**, 7 d, 57%, ee > 99%; (c) HCOOEt, NaOCH₃, Et₂O, rt, 20 h, 54 %; (d) (CH₂OH)₂, (COOH)₂, CH₃CN, rt, 3 d, 90%; (e) H₂, 10% Pd/C (Selcat Q), CH₃OH, 80 °C, 7 h, quant; (f) ClCOOCH₃, NaOH/H₂O, THF, 2 h, 99%; (g) *p*-TsOH, acetone, reflux, 1 h, 99%; (h) NaBH₄, CaCl₂, CH₃OH, 0 °C, 2 h, 96% ee 99%; (i) PhCOOH (**23**), DEAD, PPh₃, THF, rt, 4 h, 63%; (j) OsO₄, NMO, THF, H₂O, Ar atmosphere, rt, 24 h, quant.; (k) AcCl, rt, 24 h, quant.; (l) Tf₂O, DMAP, CH₂Cl₂, 0 °C → rt, 22 h, quant.; (m) (I) 2M HCl/H₂O, THF, rt, 21 h, (II) AcCl, rt, 21 h, 92%; (n) TMS-Cl, KI, CH₃CN, 60 °C, 1.5 h, 48%; (o) NaOCH₃, CH₃OH, THF, rt, 1 h, 99%.

These syntheses were also started from vanillin (**11**), ethylvanillin (**33**) or trimethoxybenzaldehyde, thus the mutual intermediates in each synthesis were the corresponding trialkoxybenzaldehydes (*Figure 12*). Their syntheses were similar to that of myristicin aldehyde (**14**). Ethylvanilline (**33**) was iodinated at position 5 and the 5-iodo derivative (**34**) was hydrolysed to 5-hydroxyethylvanillin (**35**). The next step was alkylation, where the diethylation was performed with ethyl bromide in the presence of potassium carbonate, and the dihydrobenzodioxine ring was closed with 1,2-dibromoethane. The Michael additions to the benzylideneacetones obtained from the corresponding benzaldehydes by Claisen–Schmidt condensation were carried out under both racemic and asymmetric conditions. The racemic synthesis was improved by adaption of the milder method of Peseke et al.,²⁵ who used potassium carbonate as a base. The further reaction steps, up to the Bischler–Napieralski cyclisation, were performed similarly to the synthesis of **2** (*Figure 13*). In all cases, in the modified Luche reduction step, the enantiopurity was improved by recrystallisation from hexane/EtOAc.

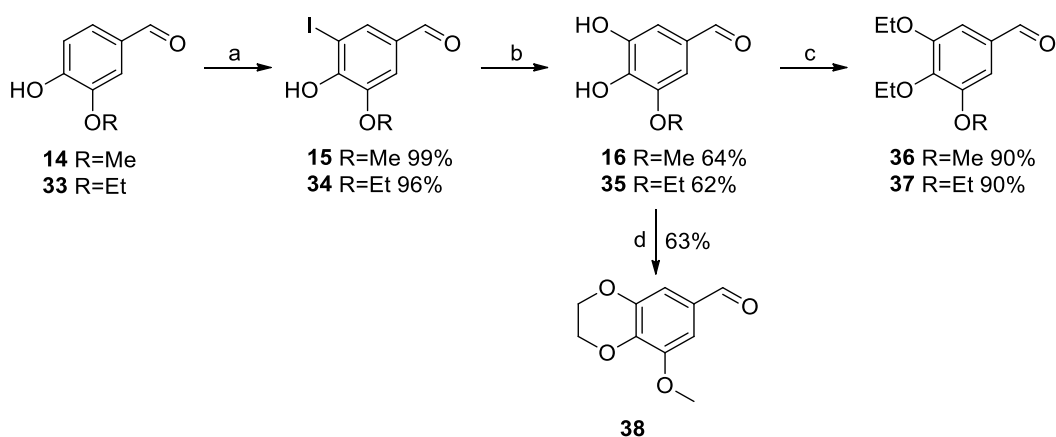


Figure 12: Preparation of benzaldehydes substituted variously. Reagents and conditions: (a) I₂, KI, NaHCO₃, H₂O, rt, 3 h; (b) 20% NaOH/H₂O, CuSO₄, reflux, 4 h; (c) EtBr, KI, K₂CO₃, DMF, rt, 20 h; (d) (CH₂)₂Br₂, K₂CO₃, DMF, 100 °C, 4 h.

During the Bischler–Napieralski reaction one methoxyphenanthridine product was obtained, when trimethoxy or triethoxy substituents were present in ring A. In other cases, regioisomers were formed in a ratio of 1:1 which could also not be separated in this step. The next step would have been the hydrolysis of methoxyphenanthridines (**27b–e**, **g–h**) into phenanthridones. Whereas, the trimethoxy derivative proved to be insoluble in the solvent used for extraction in the Zemplén’s deacylation step. Due to this solubility problem, these two steps were swapped, *i.e.* the protected methoxyphenanthridines (**27b–e**, **g–h**) were firstly deacylated. Surprisingly, the dihydrobenzodioxine regioisomers (**39e**, **g**) proved to be separable by column chromatography in this synthetic step. Then, methoxyphenanthridines were converted into lactams. Since the preparation of 7-hydroxy analogues was also our aim, the free secondary hydroxy groups had to be protected. Surprisingly, triacetoxy lactam regioisomers of methoxy-diethoxy derivatives (**47c**, **h**) proved also to be separable by column chromatography. Then, the 7-alkoxy groups were cleaved selectively by TMS-I prepared *in situ*. Lastly, target molecules (**48–51**) were obtained by Zemplén’s deacylation (*Figures 14 and 15*).

²⁵ Peseke, K.; Götze, L.; Reinke, H.; Cedeño, Q. A.; Suarez, J. Q.; Andreu, M. G., Castro, H. V. *J. Prakt. Chem.* **1997**, 339, 656–659.

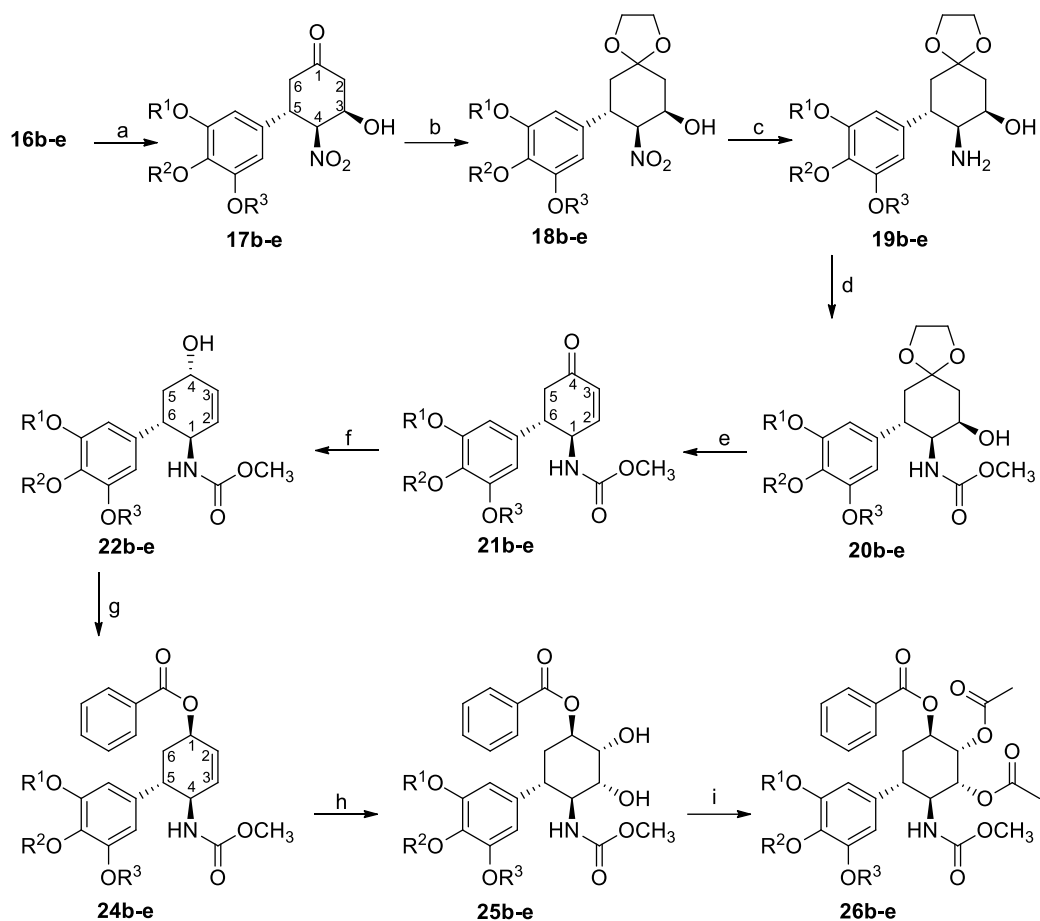


Figure 13: Reagents and conditions: (a) HCOOEt , NaOCH_3 , Et_2O , rt, 20 h; (b) $(\text{CH}_2\text{OH})_2$, $(\text{COOH})_2$, CH_3CN , rt, 3 d; (d) ClCOOCH_3 , $\text{NaOH}/\text{H}_2\text{O}$, THF , 2 h; (e) $p\text{-TsOH}$, acetone, reflux, 1 h; (f) NaBH_4 , CaCl_2 , CH_3OH , 0 °C, 2 h; (g) PhCOOH (**359**), DEAD , PPh_3 , THF , rt, 4 h; (h) OsO_4 , NMO , THF , H_2O , Ar atmosphere, rt, 24 h; (i) AcCl , rt, 24 h.

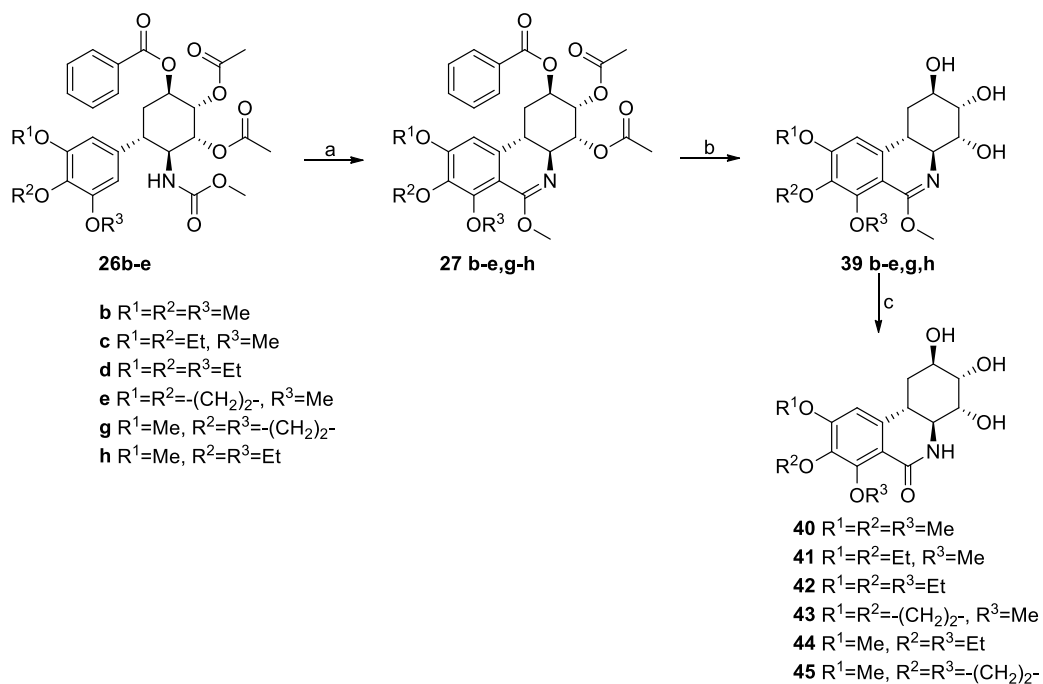


Figure 14: Synthesis of trialkoxy analogues. Reagents and conditions: (a) Tf_2O , 4-DMAP, anhydrous CH_2Cl_2 , 0 °C \rightarrow rt, 22 h; (b) NaOCH_3 , CH_3OH , anhydrous THF , rt, 2 h; (c) 2M $\text{HCl}/\text{H}_2\text{O}$, 24 h.

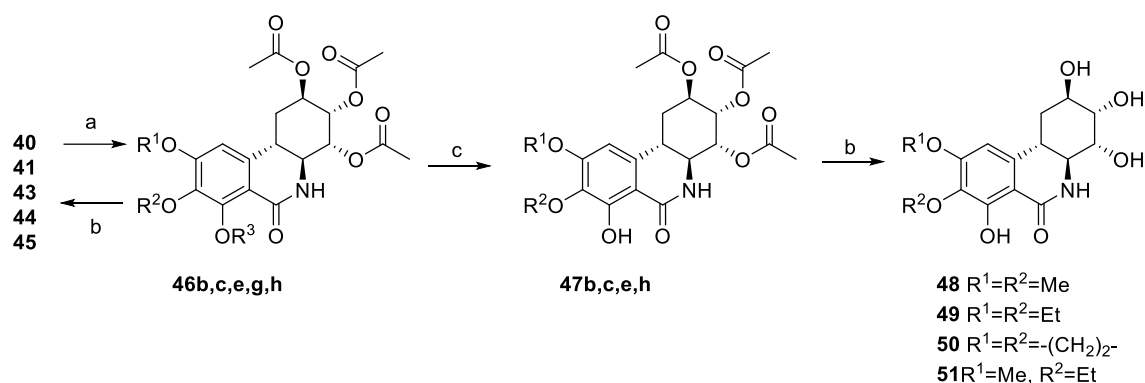


Figure 15: Synthesis of 7-hydroxy analogues. Reagents and conditions: (a) AcCl, rt, 20–24 h; (b) NaOCH₃, CH₃OH, THF, rt, 2 h; (c) TMS-Cl, KI, anhydrous CH₃CN, Δ, 3–5 h.

Antiproliferative activity of (±)-**2** and further 12 racemic analogues were evaluated by the *National Cancer Institute* in the USA. From the 13 racemic alkaloid analogues, only two compounds had significant antitumour activity which was commensurable with the naturally occurring pancratistatin. The effective analogues possess five- or six-membered ring and 7-hydroxy group as substituents in ring A. The results suggest that not only the presence of free phenolic hydroxy group at position 7 is important in respect of the anticancer activity, but the substituents of the other two phenolic hydroxy ones must be in a quasi planar arrangement with the aromatic ring.²⁶

V. THESES

- 1 I have developed an efficient and stereoselective synthetic route for the preparation of racemic *trans*-dihydronarciclasine starting from the inexpensive vanillin and containing 18 reaction steps, in 2.71% total yield. [1]
- 2 I have elaborated a method for the enantioselective Michael addition of nitromethane to benzylideneacetones using (9-amino(9-deoxy)epiquinine) as an organocatalyst. The Michael adducts were afforded with very good or excellent enantiopurity (*ee* 85–99%) in case of 22 different, di- or trisubstituted benzylideneacetones. [2–4]
- 3 I have successfully accomplished the enantioselective total synthesis of *trans*-dihydronarciclasine using asymmetric Michael addition catalysed by 9-amino(9-deoxy)epiquinine with excellent enantioselectivity. This total synthesis provided (–)-*trans*-dihydronarciclasine with 92% enantiomeric excess, in 2.37% total yield. [2]
- 4 I have proved the absolute configuration of the *ent*-form enantiomer of *trans*-dihydronarciclasine based on the single-crystal X-ray diffraction measurements of two earlier intermediates of the synthetic route, such as (*S*)-(–)-4-(7-methoxybenzo[1,3]dioxol-5-yl)-5-nitropentan-2-one and (3*R*,4*S*,5*S*)-(–)-3-hydroxy-5-(7-methoxybenzo[1,3]dioxol-5-yl)-4-nitrocyclohexanone. [2]
- 5 I have accomplished the total synthesis of further 10 analogues racemic and enantioselective way, respectively, applying the slightly modified synthetic route of *trans*-dihydronarciclasine. During the recrystallisation of enols prepared by the modified Luche reduction, in all cases, the *ent*-enantiomers with negative direction of optical rotation were afforded with excellent enantiopurity (*ee*>99%). [3,4]

²⁶ Varró, G.; Pálhuber, P.; Pogrányi, B.; Simon, A.; Hegedűs, L.; Kádas, I. *Eur. J. Med. Chem.* **2019**, *173*, 76–89.

- 6 Based on the antiproliferative tests carried out on (\pm)-*trans*-dihydronarciclasine and 12 racemic analogues, I have established some structure–activity relationships (SAR) depending on the structural modification of ring A. Among the 13 representatives, (\pm)-*trans*-dihydronarciclasine showed stronger antitumour activity than pancratistatin. [5]
- 7 I have found that the effective analogues possess five- or six-membered ring and 7-OH as substituents in ring A of the phenanthridone scaffold. Furthermore, I have concluded that the substituents of the other two phenolic hydroxy groups must be in a quasi planar arrangement with the aromatic ring. [5]

VI. APPLICATIONS

Both the racemic and the asymmetric synthetic methods may be feasible for a scalable synthesis of racemic or nearly enantiomerically pure *trans*-dihydronarciclasine after a minor optimisation which use an inexpensive starting material and a recyclable organocatalyst.

Biological results showed that the ring A of the phenanthridone scaffold has several interesting opportunities to modify the antiproliferative activity of the natural products. Syntheses of analogues with quasi planar substituted ring A and 7-hydroxy group, as well as tetrasubstituted ring A are in progress in our research group.

VII. PUBLICATIONS

7.1. Publications related to the PhD Thesis

- [1] Varró G., Hegedűs L., Simon A., Kádas I.: An efficient stereoselective total synthesis of (\pm)-*trans*-dihydronarciclasine, *Tetrahedron Letters* **2016**, *57*, 1544–1546. DOI: [10.1016/j.tetlet.2016.02.089](https://doi.org/10.1016/j.tetlet.2016.02.089), (IF: **2.193**; author's rate: 100%; citations (independent/all): 3/8.)
- [2] Varró G., Hegedűs L., Simon A., Balogh A., Grün A., Leveles I., G. Vértessy B., Kádas I.: The First Enantioselective Total Synthesis of (–)-*trans*-Dihydronarciclasine, *Journal of Natural Products* **2017**, *80*, 1909–1917. DOI: [10.1021/acs.jnatprod.7b00208](https://doi.org/10.1021/acs.jnatprod.7b00208), (IF: **3.885**; author's rate: 90%; citations (independent/all): 2/5.)
- [3] Varró G., Mattyasovszky L., Grün A., Simon A., Hegedűs L., Kádas I.: Highly Stereoselective Synthesis of *trans*-Dihydronarciclasine Analogues, *Synthesis*, **2018**, *50*, 625–643. DOI: [10.1055/s-0036-1591514](https://doi.org/10.1055/s-0036-1591514), IF: **2.722**; author's rate: 90%; citations (independent/all): 0/2.)
- [4] Varró G., Pogrányi B., Grün A., Simon A., Hegedűs L., Kádas I.: Stereoselective synthesis of *trans*-dihydronarciclasine derivatives containing a 1,4-benzodioxane moiety, *Monatshefte für Chemie – Chemical Monthly* **2018**, *149*, 2265–2285. DOI: [10.1007/s00706-018-2287-7](https://doi.org/10.1007/s00706-018-2287-7), (IF: **1.285**; author's rate: 90%; citations (independent/all): 0/1.)
- [5] Varró G., Pálchuber P., Pogrányi B., Simon A., Hegedűs L., Kádas I.: (\pm)-*trans*-Dihydronarciclasine and (\pm)-*trans*-dihydrolycoricidine analogues modified in their ring A: Evaluation of their anticancer activity and a SAR study, *European Journal of Medicinal Chemistry* **2019**, *173*, 76–89. DOI: [10.1016/j.ejmech.2019.04.010](https://doi.org/10.1016/j.ejmech.2019.04.010), (IF: **4.816**; author's rate: 80%.)

7.2. Oral presentations related to the PhD Thesis

- 1 Varró G., Hegedűs L., Simon A., Tőke L., Kádas I.: „*A (±)-transz-dihidronarciklazin sztereoszelektív totálszintézise*”, MTA Alkaloid- és Flavonoidkémiai Munkabizottság Ülése, 2014. május 12–13., Balatonalmádi
- 2 Varró G., Hegedűs L., Simon A., Kádas I.: „*A (±)-transz-dihidronarciklazin sztereoszelektív totálszintézise*”, MKE XXXVII. Kémiai Előadói Napok, 2014. november 3–5., Szeged
- 3 Varró G., Hegedűs L., Simon A., Mattyasovszky L., Kádas I.: „*A (+)-transz-dihidronarciklazin és trimetoxifenil analogonjának enantioszelektív totálszintézise*”, MKE XXXVIII. Kémiai Előadói Napok, 2015. október 26–28., Szeged
- 4 Varró G., Hegedűs L., Simon A., Grün A., Mattyasovszky L., Kádas I.: „*A transz-dihidronarciklazin és analogonjai szintézise vanillinszármazékokból*”, MTA Alkaloid- és Flavonoidkémiai Munkabizottság Ülése, 2016. április 14–15., Mátrafüred

7.3. International conference presentations (posters) related to the PhD Thesis

- 1 Varró G., Hegedűs L., Simon A., Kádas I.: „*An expedient stereoselective total synthesis of (±)-trans-dihydronarciclasine*”, 20th International Conference on Organic Synthesis (ICOS-20), June 29-July 4, 2014, Budapest, Hungary