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APPLICATION OF CYCLIC *N*-ACYLAMINOCARBINOLS IN THE SYNTHESIS OF NATURAL PRODUCTS

PhD Thesis Summary

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1. Introduction and objectives

Today's drug discovery is largely based on medicinal chemistry, which – through close cooperation with related disciplines – makes it possible to systematically synthesize and test a vast number of new compounds as potential drug candidates for a specific pharmacological activity. The foundation of medicinal chemistry is synthetic organic chemistry, whose toolbox comprises a wide array of synthetic methodologies. The continuous advance of these methodologies facilitates the synthesis of more and more complex chemical structures, complying with the increasing demands of medicinal chemistry. The need for the exploration of wider chemical spaces is posed by the criteria of patentability, which are getting more difficult and laborious to achieve as a large number of new compounds are patented every year, restricting the exploitable chemical space around appealing targets. Therefore, due to the increasing demands of medicinal chemistry, the research and advance of efficient synthetic methodologies is of utmost importance for the success of drug discovery efforts.

A key area of organic chemistry is the total synthesis and structural modification of natural products, which still retains an emphasized importance within the field of drug discovery. The synthesis of natural products or any compounds with a similar complexity is often a challenging task for organic chemists due to the typically complicated ring systems and the frequent abundance of stereogenic centers within the molecules. The synthesis of such compounds requires advanced methodologies, which facilitate highly effective, rational synthetic routes towards the target molecules.

A particularly important methodology in this respect is the application of iminium chemistry. The versatility and efficiency of the use of iminium and *N*-acyliminium reagents facilitates key reactions in the construction of heterocyclic frameworks highly relevant to natural products and medicinal chemistry. Thus, even though the application of iminium and *N*-acyliminium species is far from being a novelty, the advance of this methodology and the widening of its scope through application in the synthesis of structurally complex natural products and medicinally relevant heterocyclic systems is still a relevant field of research today.

During my PhD work I set the aim of widening the scope of iminium chemistry by extending its application to the synthesis of various types of natural products that have not been synthesized so far. The targeted natural products were selected from diverse, medicinally relevant natural product families such as *Aspidosperma* alkaloids, flavonoid alkaloids and a set of potentially bioactive compounds isolated from the medicinal plant *Lilium candidum*. The chosen target compounds all contain a pyrrolidone or pyrrolinone moiety, creating a possibility for their synthesis *via* the application of the appropriate five-membered cyclic *N*-acyliminium species, which can be generated from a suitable *N*-acylaminocarbinol precursor.

2. Experimental methods

During our synthetic work we have applied the classical methods of preparative organic chemistry. The progress of the reactions was monitored by TLC and the purification of the products was performed by distillation, crystallization, column chromatography, preparative TLC and preparative HPLC. The chemical structures of the synthesized substances were elucidated by HR-MS and NMR, which were recorded and evaluated at the Spectroscopic Research Department of Gedeon Richter Plc. The synthesized products were also characterized by their melting points, IR spectra and specific rotations in the case of chiral compounds.

3. Results and discussion

To give a more thorough introduction to the targeted natural products, the first subsection of this chapter will be devoted to our first synthetic endeavor in the field, the synthesis of the simple *N*-acylaminocarbinol ether sessiline, which is composed of a hydroxymethylfurfural unit connected to a pyrrolidone ring through a hemiaminal moiety. In the second subsection, the surprisingly simple synthesis of the *Aspidosperma* alkaloid bannucine will be presented from its natural pentacyclic precursor (–)-vindoline. The third subsection will address the family of flavonoid alkaloids, including the synthesis of five medicinally relevant flavoalkaloids isolated from various plant sources originating from the Far East. And lastly, the fourth subsection will feature the synthesis of five aminal-type pyrrolinone derivatives from a special family of lily alkaloids, as well as the synthesis of the flavoalkaloid lilaline.

3.1. (±)-Sessiline: isolation, structure, synthesis[1]

Sessiline (**1**, **Figure 1**) was isolated in 2002 by Lee and co-workers from the dried fruits of *Acanthopanax sessiliflorus* (also known as *Eleutherococcus sessiliflorus*) (**Figure 2**), an herbaceous plant native to East Asia.[2] Its structure was elucidated by spectroscopic methods such as UV, MS and NMR. The molecule consists of two five-membered heterocyclic units joined together by an *N*-acylaminocarbinol ether type bond. The alkaloid[3] was isolated from the plant as a racemate.

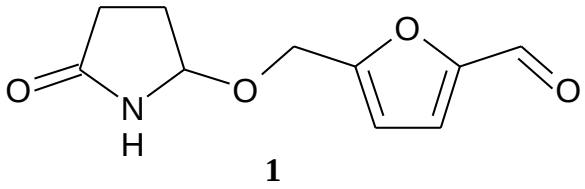

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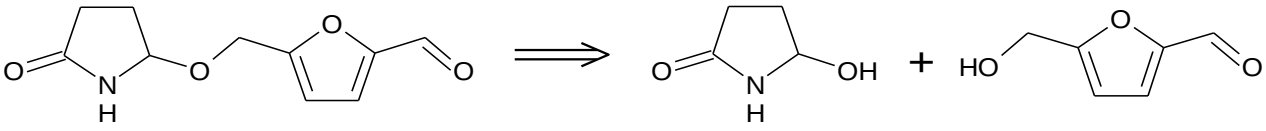
Figure 1. The structure of (±)-sessiline (**1**)

Figure 2. Fruits of *Acanthopanax sessiliflorus*

Due to its unique structure and potential bioactivity, we decided to synthesize (±)-sessiline (**1**). The retrosynthetic analysis of sessiline is pretty simple: by disconnecting the ether bond, we obtain two known compounds: 5-hydroxypyrrolidin-2-one (**2**) and 5-hydroxymethylfurfural (**3**) (**Scheme 1**), delineating a simple, straightforward synthesis.



1 **2** **3**

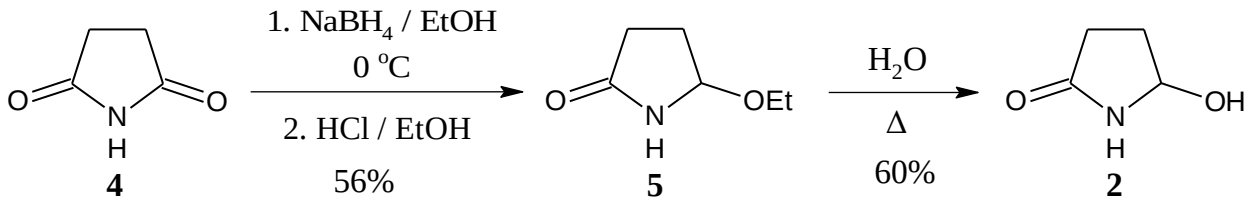
Scheme 1. Retrosynthetic analysis of sessiline (**1**)

[1] The synthesis of sessiline was carried out in collaboration with Kornél Faragó.

[2] S. Lee, J. Ji, K. H. Shin, B.-K. Kim; *Planta Med.* **68**, 939–941 (2002)

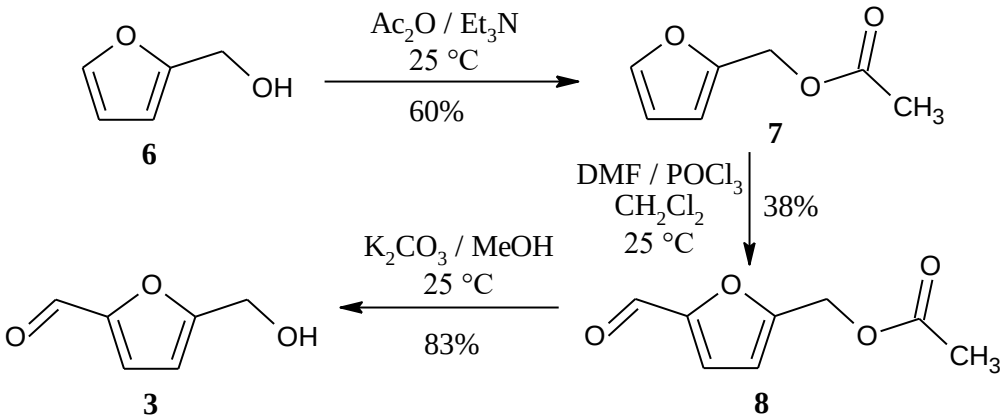
[3] The definition of the term 'alkaloid' was originally meant to refer to plant-derived natural compounds with a basic character and marked physiological activity. The definition had a stepwise evolution over the decades and was extended with several amendments by several authors over the years. Today, the common ground generally acceptable for all compounds classified as alkaloids would be nitrogen-containing organic substances of natural origin with a potentially – but not necessarily – basic character.

In order to accomplish our goal, first we needed to synthesize 5-hydroxypyrrolidin-2-one (**2**) and 5-hydroxymethylfurfural (**3**). Compound **2** was prepared from succinimide (**4**) in two steps. The partial reduction of **4** with sodium borohydride yielded 5-ethoxypyrrolidin-2-one (**5**),[4] which was hydrolyzed in boiling water furnishing cyclic *N*-acylaminocarbinol **2** (**Scheme 2**).[5]



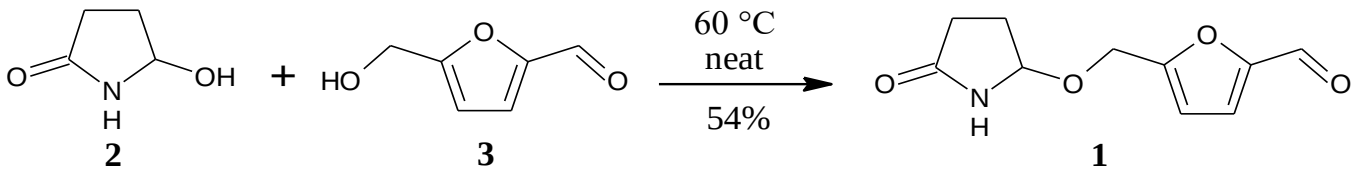
Scheme 2. Synthesis of 5-hydroxypyrrolidin-2-one (**2**) in 2 steps from succinimide (**4**)

Next, we prepared 5-hydroxymethylfurfural (**3**) from furfuryl alcohol (**6**) in three steps (**Scheme 3**). First, **6** was protected by acetylation to give furfuryl acetate (**7**),[6] which was subjected to *Vilsmeier formylation* to yield 5-(formyl)furfuryl acetate (**8**).[7] Then, **3** was obtained by deacetylation.[8]



Scheme 3. Synthesis of 5-hydroxymethylfurfural (**3**) in three steps from furfuryl alcohol (**6**)

Next, we synthesized our target molecule **1** by coupling **2** and **3**. Compound **2** was allowed to react with an excess of **3** at 60 ˚C in a neat reaction. The reaction gave (±)-sessiline (**1**) in a medium yield (**Scheme 4**).



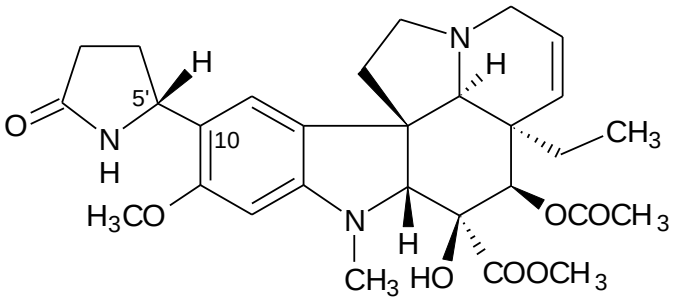
Scheme 4. Synthesis of (±)-sessiline (**1**)

[4] J. C. Hubert, J. B. P. A. Wijnberg, W. N. Speckamp; *Tetrahedron* **31**, 1437–1441 (1975)
[5] B. W. Cue Jr., N. Chamberlain; *Org. Prep. Proced. Int.* **11**, 285–286 (1979)
[6] I. Renvall, T. Mattila; US Patent No. 4008256 (1977)
[7] A. Mehner, A. L. Montero, R. Martinez, S. Spange; *Molecules* **12**, 634–640 (2007)
[8] D. Schinzer, E. Bourguet, S. Ducki; *Chem. Eur. J.* **10**, 3217–3224 (2004)

3.2. Bannucine: isolation, structure, synthesis

Catharanthus roseus is an herbaceous evergreen plant endemic to Madagascar. It is grown around the globe as an ornamental plant and also as a medical plant used for the treatment of a wide range of diseases including hypertension, malaria, diabetes and Hodgkin's lymphoma.[9] Numerous alkaloids have been isolated from *C. roseus*, among which the most important ones are vinblastine and vincristine, which are used in the clinical treatment of certain types of cancer including leukaemias, non-small cell lung cancer and Hodgkin's lymphoma. The isolation and structural analysis of potentially antineoplastic alkaloids derived from *C. roseus* is still a relevant field of research today. The structure elucidation, rational synthesis and chemical modification of the isolated compounds provide a constant challenge for organic chemists.

The *Aspidosperma* alkaloid bannucine (**(–)-9**, **Figure 3**) was isolated by Atta-ur-Rahman and co-workers from the dried leaves of *Catharanthus roseus* (**Figure 4**) in 1986.[10] The molecule is a derivative of vindoline (**(–)-10**) bearing a C10 substituent, a pattern common to the antineoplastic dimeric indole alkaloids. In bannucine, a 2-pyrrolidone moiety is attached at C5' to the aromatic ring of the vindoline core at C10.



(–)-9

Figure 3. The structure of bannucine (**(–)-9**)

Figure 4. *Catharanthus roseus*

Apart from spectroscopic structure elucidation the authors also described the new compound by giving its melting point (152-154 °C) and specific rotation ($[\alpha]_D$ = –33 (c = 0.26; chloroform)). Based on their NMR measurements the authors assigned a β-orientation to the proton attached to C5'.

During our work we set the aim of synthesizing bannucine from natural (–)-vindoline (**(–)-10**). The electron-rich aromatic ring of vindoline (**(–)-10**) is highly activated toward electrophilic substitution. Its most reactive position is C10, owing to the directing effects of the methoxy and the dialkylamino group. With this reactivity in mind, we assumed that the use of the strongly electrophilic *N*-acyliminium reagents provides an opportunity for the synthesis of bannucine (**(–)-9**).

<u>Synthesis of bannucine[11]</u>

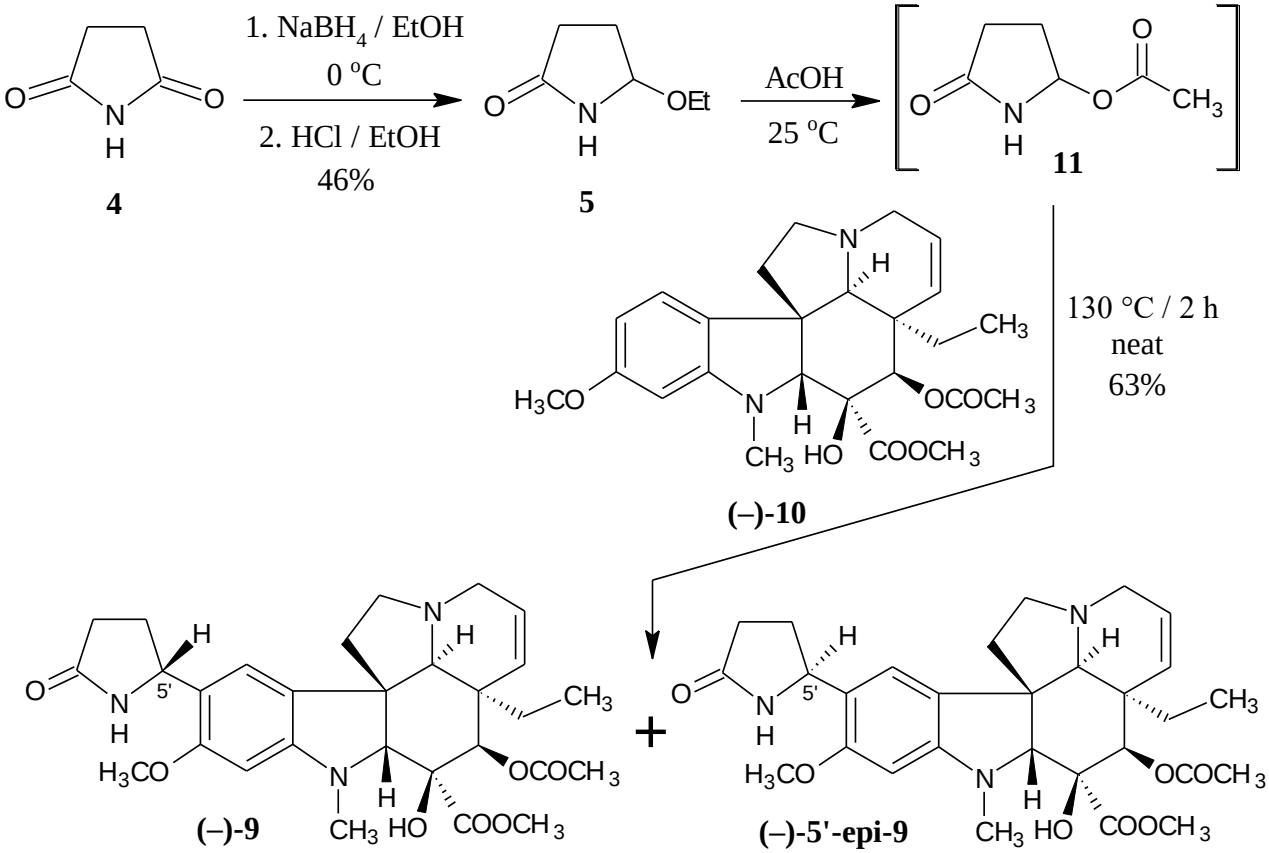
We first synthesized 5-acetoxypyrrolidine-2-one (**11**) from succinimide (**4**) in two steps (**Scheme 5**). The partial reduction of **4** yielded 5-ethoxypyrrolidine-2-one (**5**),[4] which was converted to acetate **11** by stirring

[9] in *Flora of China* Vol. 16, W. Zhengyi, P. H. Raven, H. Deyuan (Eds.); Science Press: Beijing & Missouri Botanical Garden Press: St. Louis, 1994; pp 157

[10] Atta-ur-Rahman, I. Ali, M. I. Chaudhary; *J. Chem. Soc. Perkin Trans. 1* **1986**, 923–926

[11] Microwave experiments in this topic were carried out in collaboration with Péter Bana.

in acetic acid at ambient temperature.[12] The acetate **11** was used immediately as an oil in the next reaction. Vindoline (**(–)-10**) was allowed to react with an excess of acetate **11** at 130 °C under neat conditions for 2 hours. The reaction gave a mixture of bannucine (**(–)-9**) and 5'-epibannucine (**(–)-5'-epi-9**) in an acceptable yield (**Scheme 5**). Purification of the epimeric mixture was carried out by column chromatography. The pure epimeric mixture was crystallized by trituration in ether and characterized by its melting point (151-153 °C) and specific rotation ($[\alpha]_D = -52.3$ (c = 0.26; $CHCl_3$)).



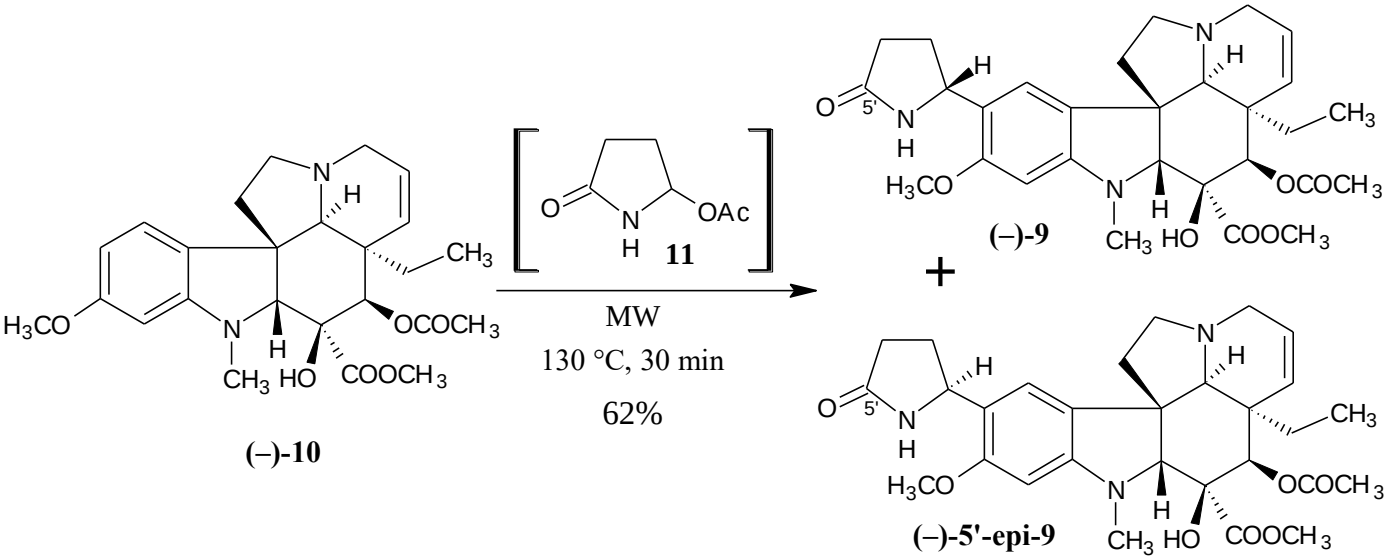
Scheme 5. Synthesis of bannucine epimers under neat conditions

Optimization of the excess of reagent **11** was carried out under neat conditions. Since raising the amount of the *N*-acylaminocarbinol reagent to more than 5 equivalents relative to vindoline did not result in a higher yield, experiments hereafter were conducted using 5 equivalents of acetate **11** relative to **(–)-10**. Next, we investigated the possibility of preparing the epimeric mixture using different methods. Applying microwave heating to neat mixtures of five-fold excess of the *N*-acylaminocarbinol acetate **11** and vindoline (**(–)-10**) at 130 °C for different durations gave oily reaction mixtures (**Scheme 6**). A shorter reaction time resulted in a clear reaction mixture, while longer reaction times gave lower yields presumably due to the thermal degradation of the product, which resulted in the formation of tarry impurities in the reaction mixture. The highest yield (62%) was reached after a 30 min run at 130 °C, which was virtually equal to that of the conventional heating method. Compared to conventional heating, the advantage of using microwave heating

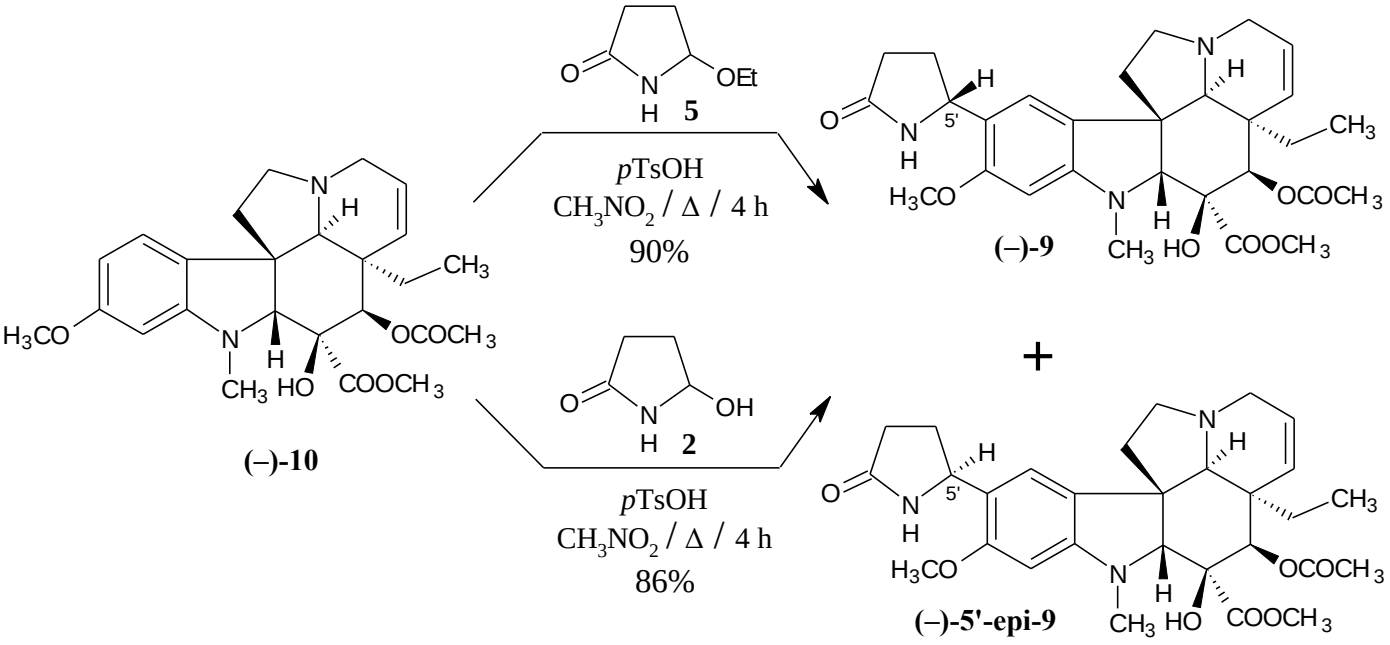
[12] T. Nagasaka, M. Abe, N. Ozawa, Y. Kosugi, F. Hamaguchi; *Heterocycles* **20**, 985–990 (1983)

was a significantly shorter reaction time and a reduced amount of tarry side-products, owing to the direct and uniform heating of the reaction mixture.

Next, we investigated the acid catalyzed solution-phase synthesis of the bannucine epimers. Small-scale model experiments were conducted to establish the most suitable solvent for the reaction. Reactions were run in boiling solvents for 4 hours, with catalytic amounts of *para*-toluenesulfonic acid. The best solvent was found to be nitromethane, with the isolated yield 77%. A larger scale (100 mg) experiment in nitromethane gave the title compounds in an improved 90% overall yield, likely due to decreased loss of material during isolation (**Scheme 7**). Under the same conditions, *N*-acylaminocarbinol **2**, which was prepared by hot water hydrolysis of **5** (**Subsection 3.1**, **Scheme 2**),[5] gave virtually the same yield, which comes as no surprise, considering that the same *N*-acyliminium intermediate is involved in the reaction.



Scheme 6. Synthesis of bannucine epimers using microwave irradiation



Scheme 7. Acid catalyzed solution-phase synthesis of bannucine epimers

According to the NMR measurements of the product the mixture contained the two epimers in an approximate ratio of 2:3. Separation of the epimers was accomplished by multiple consecutive chromatographic purifications on preparative TLC plates. The structures of the pure epimers were elucidated by spectroscopic methods including MS and NMR measurements, and the configuration of the stereogenic carbon at C5' was elucidated by X-ray crystallography. The absolute configuration of C5' in the epimer with the lower R_f value turned out to be *S*, which translates to the α-orientation of the hydrogen at C5'. Therefore the examined isomer was 5'-epibannucine (**(–)-5'-epi-9**), and the ratio of epimers in the original mixture was **(–)-9** : **(–)-5'-epi-9** = 2:3. Further reactions yielded the epimers in approximately the same ratio.

The pure epimers of bannucine were characterized by their melting points and specific rotations. Bannucine (**(–)-9**) melted at 284-286 °C with a specific rotation $[\alpha]_D = -12.3$ (c = 0.26; $CHCl_3$), while 5'-epibannucine (**(–)-5'-epi-9**) melted at 191-193 °C and had specific rotation $[\alpha]_D = -72.3$ (c = 0.26; $CHCl_3$). Comparison of the melting points and specific rotations we measured for the pure epimers to those from the literature[10] gave no leads in the identification of the epimers (**Figure 5**). Based on the fact that the specific rotation reported by Atta-ur-Rahman and co-workers for the substance they regarded as pure bannucine ($[\alpha]_D = -33$ (c = 0.26; $CHCl_3$))[10] significantly differs from the value measured by us, and that it falls between the specific rotations we measured for bannucine (**(–)-9**) ($[\alpha]_D = -12.3$ (c = 0.26; $CHCl_3$)) and 5'-epibannucine (**(–)-5'-epi-9**) ($[\alpha]_D = -72.3$ (c = 0.26; $CHCl_3$)), it is likely that the substance which Atta-ur-Rahman *et al.* isolated from *Catharanthus roseus* was not pure bannucine, but in fact a mixture of the two epimers. This assumption is corroborated by the fact that the melting point given by Atta-ur-Rahman *et al.* for their substance (152-154 °C)[10], is very close to the one we measured for the solid epimeric mixture that we synthesized (151-153 °C), and it is much lower than the melting point we have measured for each pure epimer (bannucine (**(–)-9**) m.p.: 284-286 °C; 5'-epibannucine (**(–)-5'-epi-9**) m.p.: 191-193 °C).

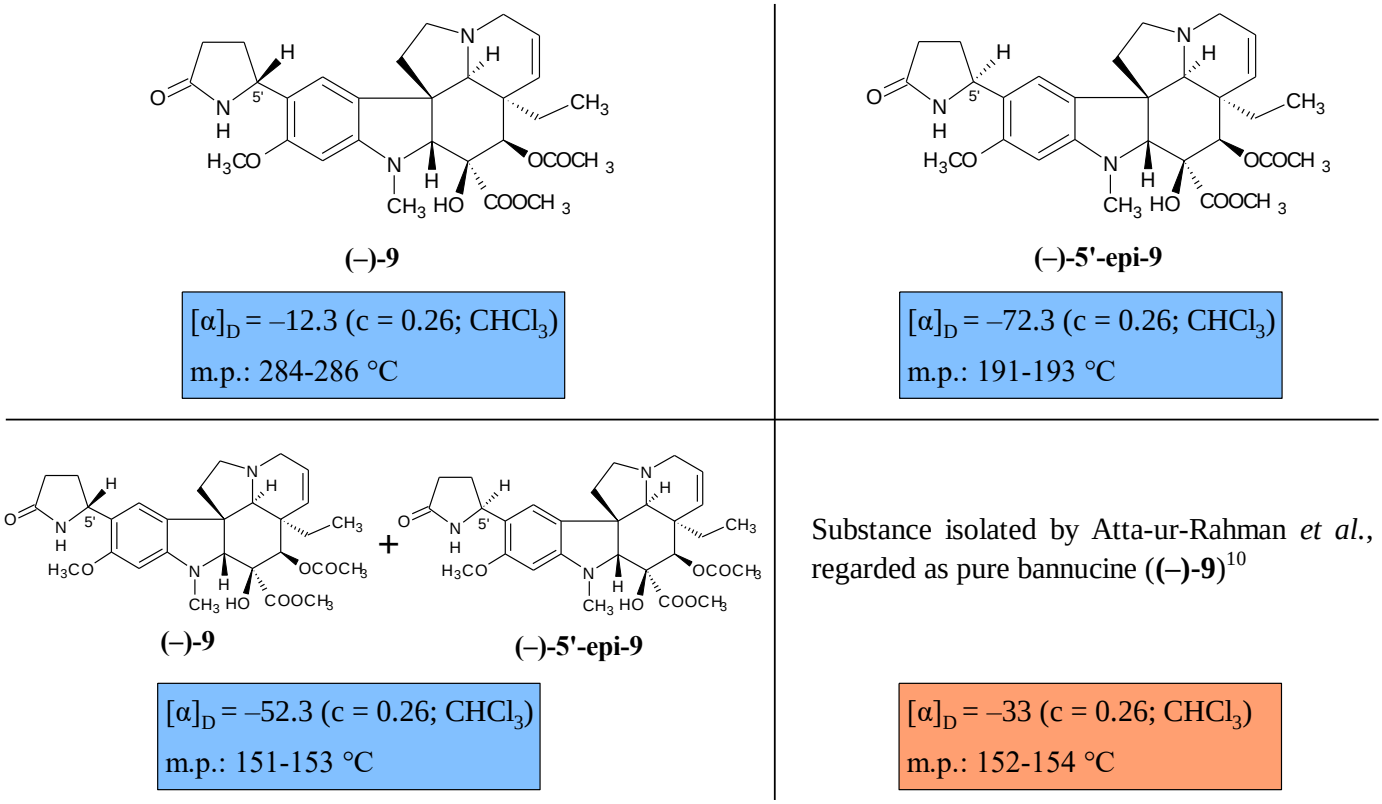


Figure 5. Comparison of own and literature data: melting points and specific rotations of the epimeric mixture and of the pure epimers

3.3. Flavonoid alkaloids: structure, synthesis

Flavonoid alkaloids (also known as flavoalkaloids) constitute a unique group of natural products as a crossing of two big natural product families: flavonoids and alkaloids. The structure of these hybrid molecules consists of a flavonoid core and a nitrogen-containing moiety (in most cases a nitrogen heterocycle), which is usually attached to the aromatic A-ring of the flavonoid skeleton at the C6 or C8 position. The A-ring of the molecules also contains two hydroxyl groups at the C5 and C7 positions, which are sometimes present as methyl ethers. The variability of the flavonoid core is demostrated in the diverse set of compounds comprising this special family of natural products: their flavonoid backbone encompasses almost all the distinct types of flavonoids ranging from flavans and flavanols through flavanones to flavones and flavonols. This variability is represented by dashed lines in the general structures in **Figure 6**. The dashed *N*-heterocycle indicates the variability of the nitrogen-containing moiety, which is a 5- or 6-membered ring in most of the cases. We know of more than 50 molecules today that can be classified as flavonoid alkaloids, and their numbers are growing day by day as new compounds are isolated from different kinds of plant sources and identified as novel flavoalkaloids.[13]

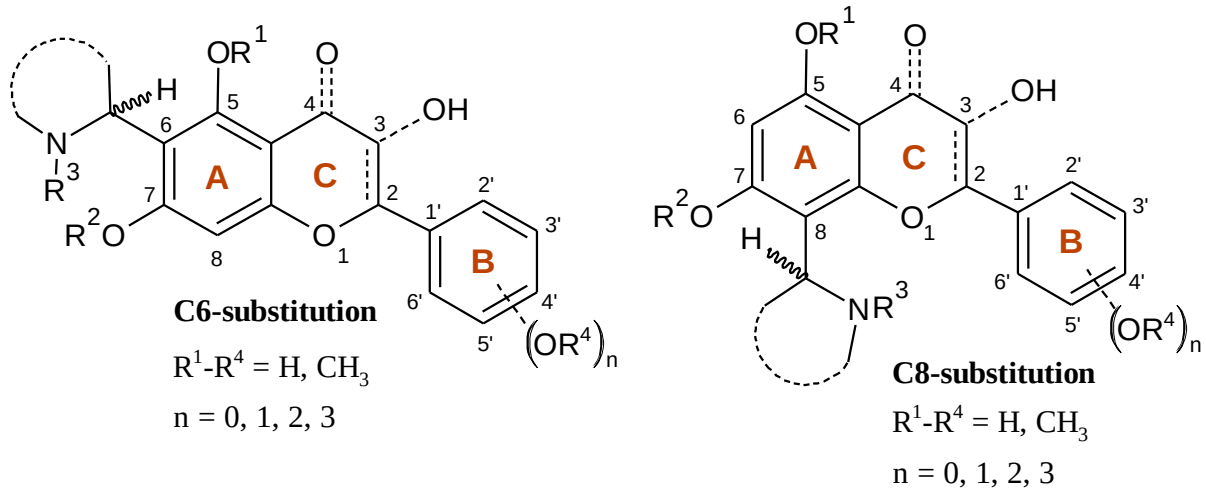


Figure 6. General structure of flavonoid alkaloids

<u>Synthesis of flavoalkaloids with naringenin, quercetin and (–)-epicatechin skeletons[14]</u>

The importance of the research of flavoalkaloids for supporting drug discovery efforts is unquestionable. The recently conducted successful phase II clinical trials of synthetic flavoalkaloid analogues as antineoplastic agents has proven that the isolation, structural modification and biological evaluation of flavoalkaloids and their derivatives is an important field of research today. In an effort to expand the synthetic availability of the flavanoid alkaloids known today, we endeavored to synthesize some of the compounds whose synthesis has not been described in the literature. Since some of these compounds have been reported to show important

[13] The classical definition of *alkaloids* requires compounds classified as such to have a basic character owing to the basic nitrogen atoms present in the molecule. However, many flavoalkaloids contain non-basic nitrogen atoms as parts of amide functionalities, while the phenolic hydroxyl groups endow the compounds with a slightly acidic character altogether. Accordingly, the meaning of the name *flavoalkaloid* has shifted towards a structural definition referring to nitrogen-containing flavonoids, similarly to how nowadays the meaning of the term *alkaloid* tends to shift towards nitrogen-containing natural product.

[14] Synthetic work in this field was carried out in collaboration with András Spaits.

biological activity, and others could also potentially possess valuable pharmacological traits as well, we set the aim of synthesizing the stereoisomers of dracocephins A (**12**) and B (**13**),[15] 6-(2”-pyrrolidinone-5”-yl)-(–)-epicatechin (**14**) and 8-(2”-pyrrolidinone-5”-yl)-(–)-epicatechin (**15**)[16] and 8-(2”-pyrrolidinone-5”-yl)quercetin (**16**).[17] The above listed target molecules are shown in **Figure 7**.

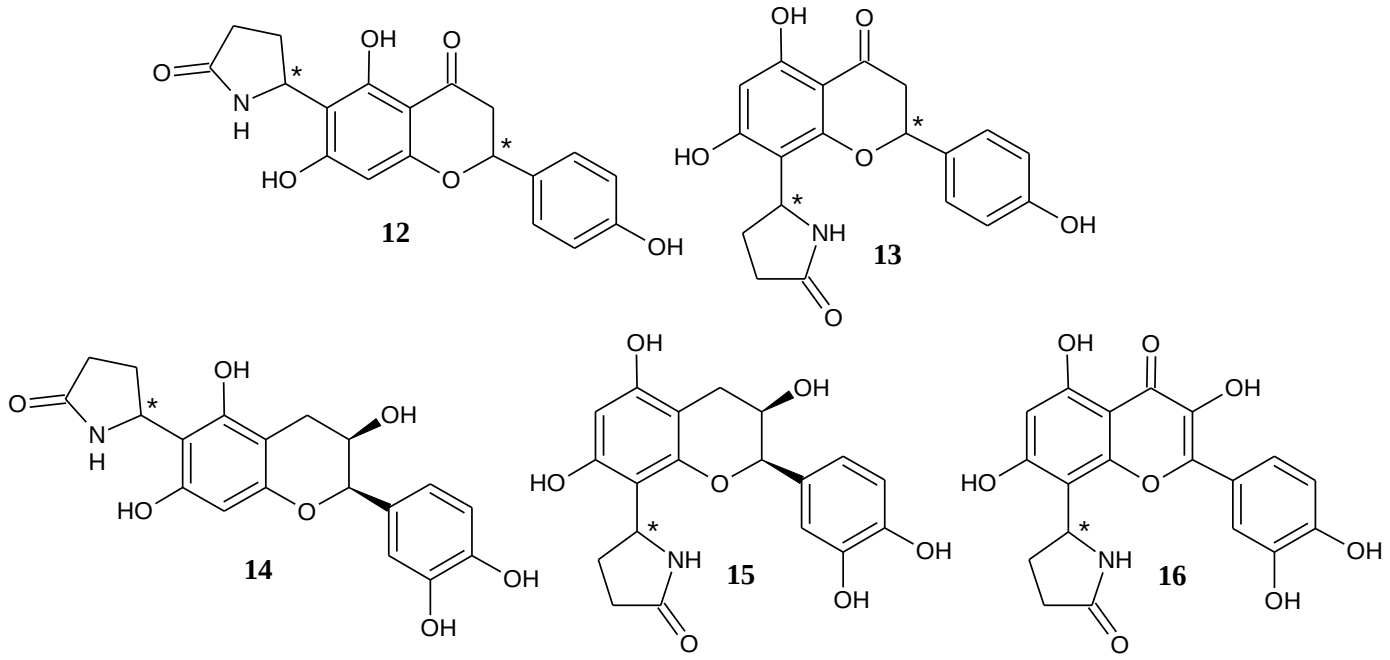
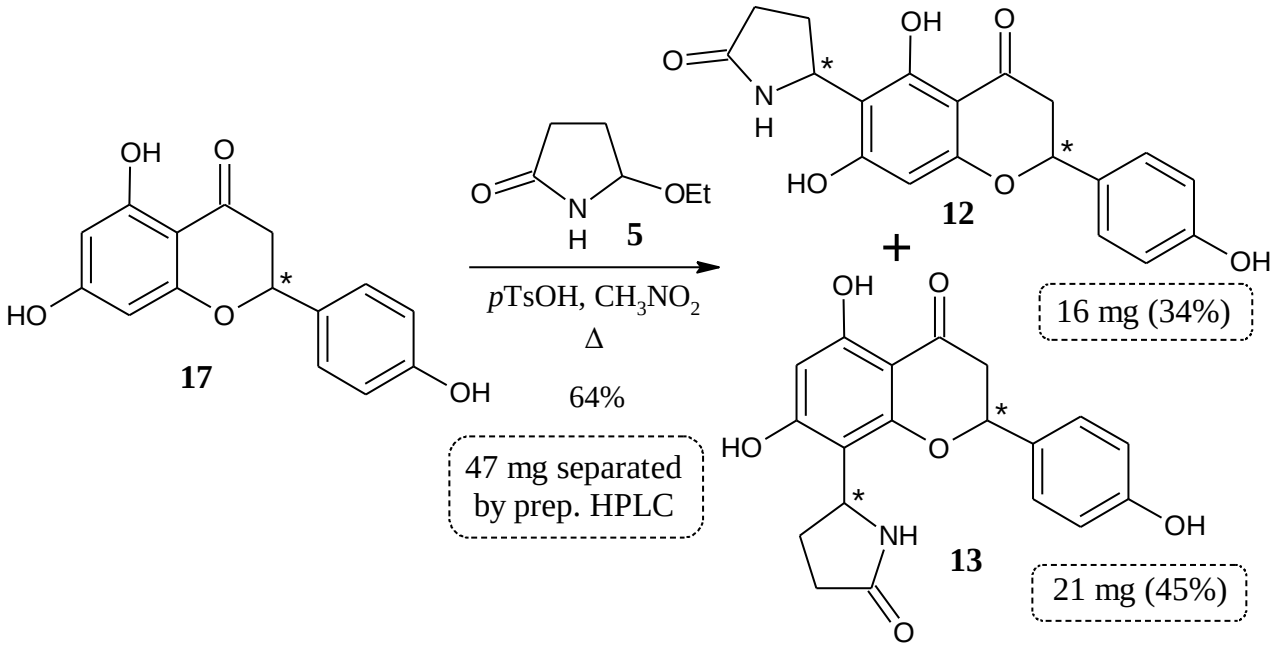


Figure 7. Structures of our flavoalkaloid target compounds

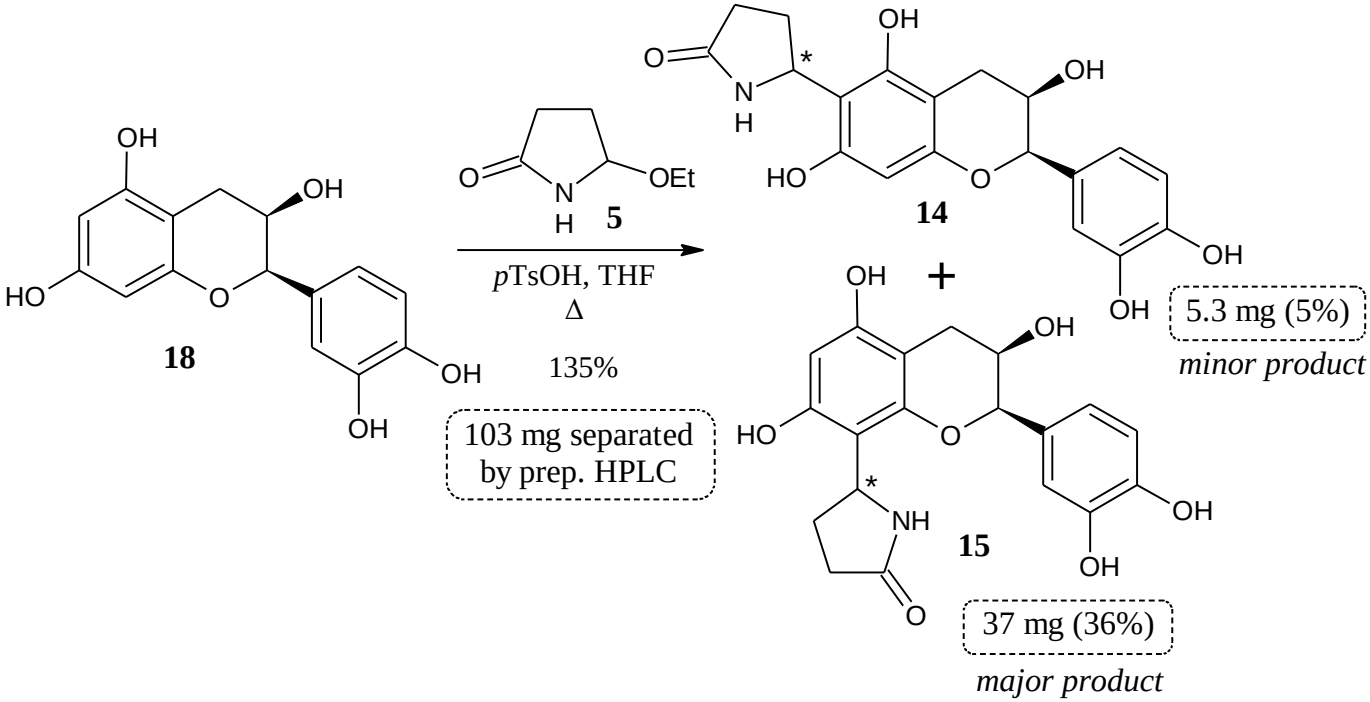
The synthesis of dracocephins A (**12**) and B (**13**) was accomplished using commercially available racemic naringenin (**17**), which was suspended in nitromethane and made to react with a slight excess of 5-ethoxy-2-pyrrolidinone (**5**) under reflux in the presence of catalytic amounts of *para*-toluenesulfonic acid. The reaction afforded an isomeric mixture of the C6- and C8-substituted products (**12** and **13**), which were purified by column chromatography on silica, furnishing a white crystalline solid in 64% yield (**Scheme 8**). The C6- and C8-regioisomers were separated by preparative HPLC from a small sample of the purified isomeric mixture, giving dracocephin A (**12**) and B (**13**) as white crystalline powders in a ratio of 43:57, respectively. The separated isomers were subsequently identified by NMR. In order to separate and identify the stereoisomers of **12** and **13**, a chiral HPLC-UV and coupled -ECD technique was applied, according to which in both cases the ratio of the 4 stereoisomers was approximately 1:1:1:1. The ECD spectra of the acquired peaks was compared to the CD spectra of the isomers described in the literature by Ren *et al*.[15] The comparison of the ECD spectra of our synthetic stereoisomers with the CD spectra described in the literature allowed us to assign the configuration of each stereoisomer of the synthesized flavoalkaloids dracocephins A (**12**) and B (**13**). The configurational analysis of the stereoisomers was further supported with TDDFT-ECD calculations.

[15] D. M. Ren, H. F. Guo, W. T. Yu, S. Q. Wang, M. Ji, H. X. Lou; *Phytochemistry* **69**, 1425–1433 (2008)
[16] D. S. Jang, G. Y. Lee, Y. M. Lee, Y. S. Kim, H. Sun, D. Kim, J. S. Kim; *Chem. Pharm. Bull.* **57**, 397–400 (2009)
[17] N. Li, L. Shao, C. Zhang, M. Zhang; *J. Asian Nat. Prod. Res.* **10**, 1143–1146 (2008)



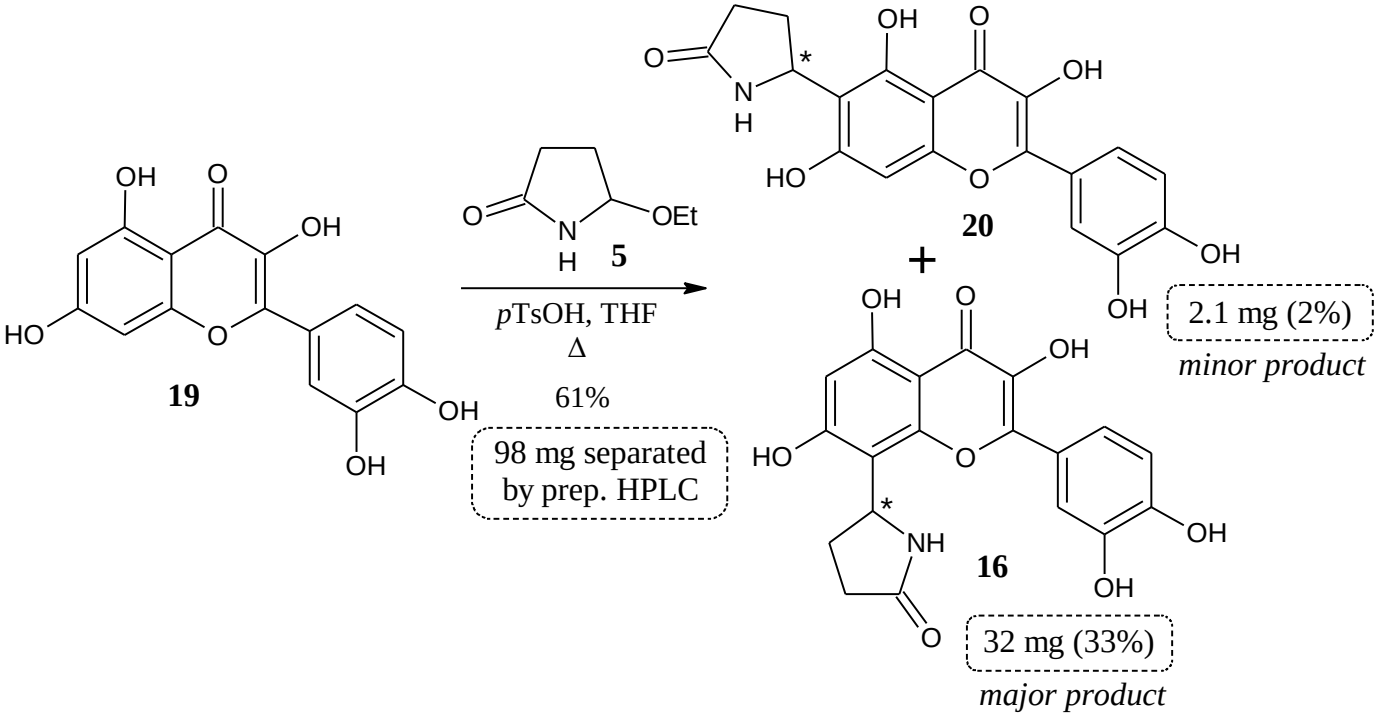
Scheme 8. Synthesis and separation of dracocephins A (**12**) and B (**13**)

The synthesis of 6-(2”-pyrrolidinone-5”-yl)-(–)-epicatechin (**14**) and 8-(2”-pyrrolidinone-5”-yl)-(–)-epicatechin (**15**) was achieved from commercially available (–)-epicatechin (**18**), which was reacted with 1.2 eq. of 5-ethoxy-2-pyrrolidinone (**5**) in refluxing THF in the presence of *para*-toluenesulfonic acid as catalyst (**Scheme 9**). A sample of the crude isomeric mixture was purified by preparative HPLC, giving the epimeric mixtures of the pure C6-isomer (**14**) and the C8-isomer (**15**) as white solids in a ratio of 13:87, respectively. The epimers of the regioisomers could not be separated by the applied preparative HPLC method, but their ratio could be determined by NMR (ca. 50-50% in the case of **14** and ca. 58-42% in the case of **15**).



Scheme 9. Synthesis and separation of 6-(2”-pyrrolidinone-5”-yl)-(–)-epicatechin (**14**) and 8-(2”-pyrrolidinone-5”-yl)-(–)-epicatechin (**15**)

The synthesis of 8-(2”-pyrrolidinone-5”-yl)quercetin (**16**) was accomplished from commercially available quercetin (**19**), which was made to react with a slight excess of 5-ethoxy-2-pyrrolidinone (**5**) in refluxing THF in the presence of catalytic amounts of *para*-toluenesulfonic acid (**Scheme 10**). A sample of the crude product was purified by preparative HPLC, furnishing the racemates of the C6-isomer (**20**) and the C8-isomer (**16**) as yellow solids in an approximate ratio of 6:94, respectively, indicating a high degree of regioselectivity. It should be noted that only the C8-isomer (**16**) was isolated from its natural source, *Senecio argunensis*.



Scheme 10. Synthesis and separation of 6-(2”-pyrrolidinone-5”-yl)quercetin (**20**) and 8-(2”-pyrrolidinone-5”-yl)quercetin (**16**)

The above presented three syntheses all yielded isomeric mixtures of the products consisting of the C6- and C8-substituted isomers of the desired flavoalkaloids in varying ratios, indicating a notable difference in the regioselectivity of the *Betti reactions* leading to the products. **Table 1** shows the natural and the synthetic isomeric ratios of the synthesized products, which follow a similar pattern among the different flavoalkaloids, implying that a potentially biomimetic mechanism might be involved in the syntheses.

Table 1. Comparison of the natural and synthetic isomeric ratios of the synthesized flavoalkaloids

Flavoalkaloid isomers	Natural isomeric ratio (C6 : C8)	Synthetic isomeric ratio (C6 : C8)
Dracocephins A (**12**) and B (**13**)	**58 : 42**	**43 : 57**
Pyrrolidinonyl epicatechins (**14** and **15**)	**22 : 78**	**13 : 87**
Pyrrolidinonyl quercetins (**20** and **16**)	**– : 100**	**6 : 94**

3.4. Lily alkaloids: isolation, structure, retrosynthetic analysis and synthesis

Lilium candidum – commonly known as the *Madonna lily* – is a perennial plant in the true lily family. It has a high-growing leafy stem, and it bears white, strongly fragrant flowers in the summer (**Figure 8**). The *Madonna lily* is native to the Balkans and the Middle East, but it is also naturalized in other parts of the world. In folk medicine the extract of the plant is used for the treatment of burns, ulcers, inflammation and for healing wounds.

Figure 8. Flowers of *Lilium candidum*

The phytochemical constituents of *Lilium* species comprise several interesting heterocyclic compounds, many of them containing a hemiaminal or aminal function (**Figure 9**). Most members of this special family of lily alkaloids have not yet been synthesized. Several natural products isolated from *Lilium candidum* and other closely related lily species contain a 3-pyrrolin-2-one moiety (see **Figure 9**, highlighted in blue). Among these, the simplest molecule is jatropham [5-hydroxy-3-methyl-3-pyrrolin-2-one] (**21**), which was first isolated from *Jatropha macrorhiza* by Wiedhopf and co-workers,[18] who assigned a wrong structure to the molecule, which was later revised by Yakushijin and co-workers.[19]

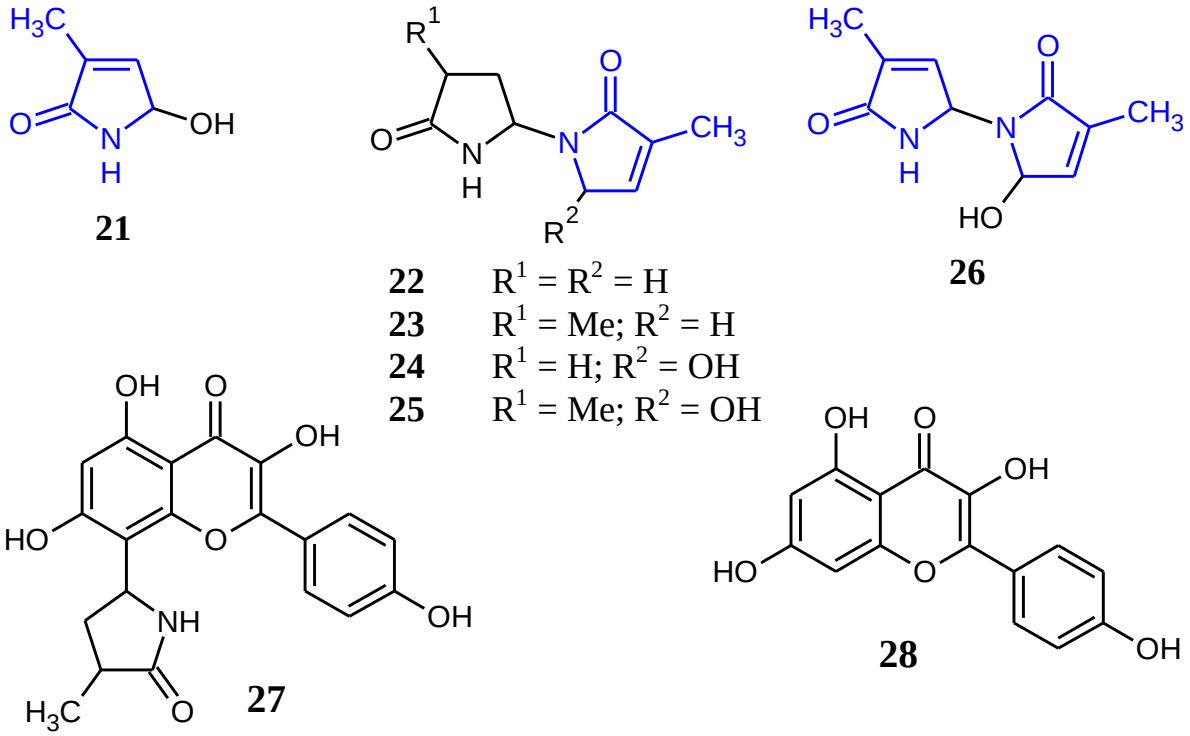


Figure 9. Pyrroline alkaloids and the flavonoid constituents isolated from *Lilium candidum* (3-pyrrolin-2-one moieties are highlighted in blue)

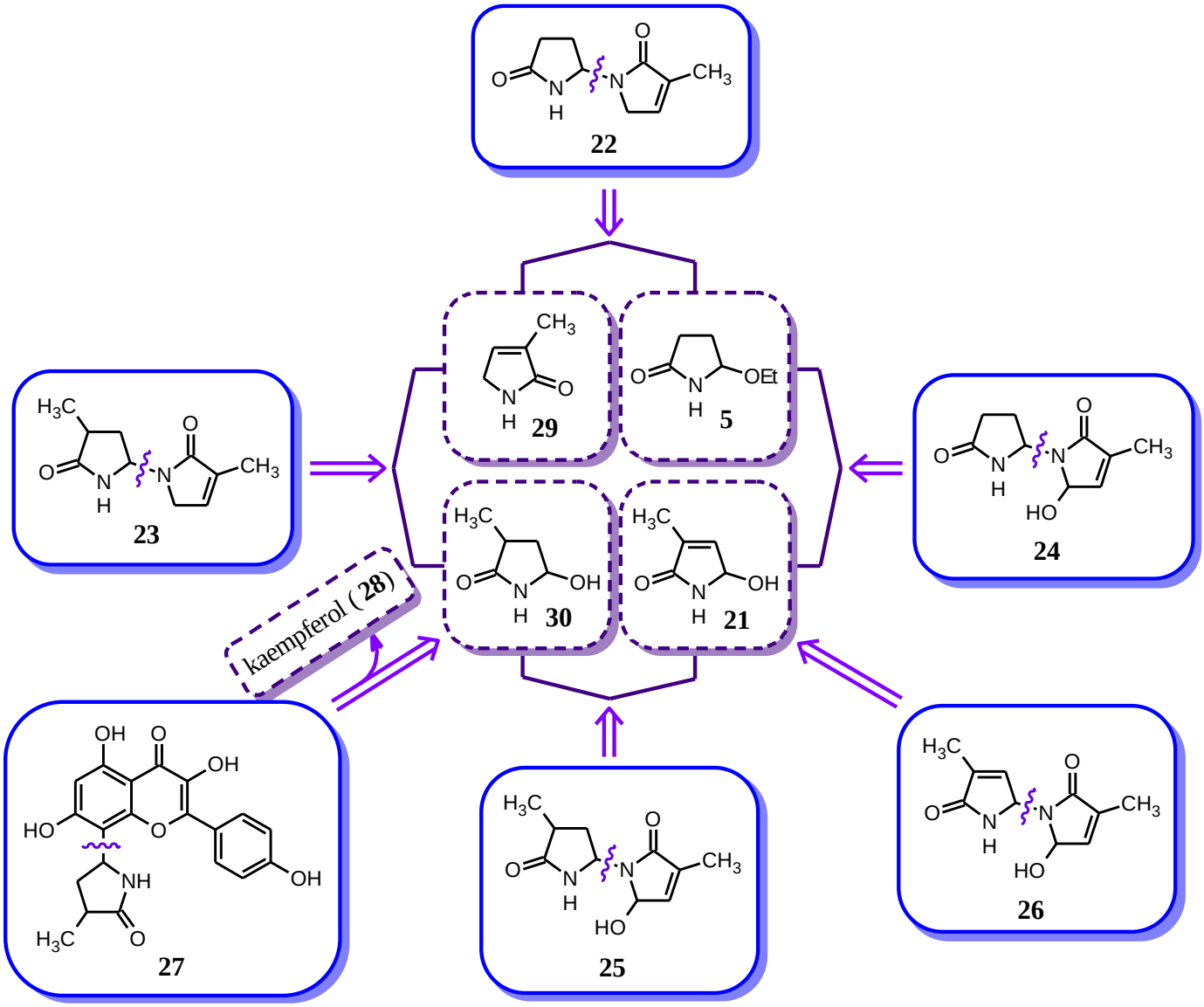
[18] R. M. Wiedhopf, E. R. Trumbull, J. R. Cole; *J. Pharm. Sci.* **62**, 1206–1207 (1973)
[19] K. Yakushijin, M. Kozuka, Y. Ito, R. Suzuki, H. Furukawa; *Heterocycles* **14**, 1073–1076 (1980)

The pyrroline-pyrrolidine alkaloids **22**, **23** and **25** were isolated by Haladová and co-workers from *Lilium candidum*.[20] The optical rotations of compounds **23** and **25** were provided by the authors, but no information on the isomeric composition or stereochemistry of the newly isolated alkaloids was disclosed in their report. In a different communication, Pavelčík unambiguously determined the absolute configuration of compound **23** as (*R, R*) using X-ray analysis.[21] The *N*-pyrrolidonyl jatropham derivative **24** was also isolated from *Lilium candidum* by Eisenreichová *et al.*, but the optical rotation or the stereochemistry of the compound was not described.[22] The jatropham dimer **26** was isolated by Haladová and co-workers from *Lilium candidum*.[23] No information on the stereochemistry of **26** was disclosed by the authors. No synthesis of the above discussed dimeric pyrroline-alkaloids has been published so far, with the exception of jatropham dimer **26**, whose formation was observed by Yakushijin *et al.* upon the acid treatment of an *N*-protected derivative of **21**.[24] Lilaline (**27**) is a flavonoid alkaloid, whose isolation from *Lilium candidum* was reported by Mašterová and co-workers.[25] The flavonoid core of lilaline (**27**) is kaempferol (**28**) – also a constituent of *L. candidum*[26] –, which is connected at its C8 position to a saturated five-membered lactam ring. Although the optical rotation of lilaline was provided by the authors, the isomeric composition and the stereochemistry of the natural product was not clarified.

Driven by our synthetic curiosity, we endeavored to synthesize some of these interesting heterocyclic compounds, focusing primarily on the pyrroline–pyrrolidine type dimers (**22**–**26**) and the flavonoid alkaloid lilaline (**27**), which were all isolated from *Lilium candidum*.

The retrosynthetic concept of the target compounds is outlined in **Scheme 11**. Disconnection of the C–N bonds (marked by wavy lines) of the aminal-type lily alkaloids (**22**–**26**) leads to an *N*-acylaminocarbinol reagent and a five-membered lactam, which is in some cases also an *N*-acylaminocarbinol itself. The disconnection of the lactam and the flavonol moieties in lilaline (**27**) leads to kaempferol (**28**) and again an *N*-acylaminocarbinol reagent, predictive of a reaction analogous to the synthesis of the flavoalkaloids described in **Subsection 3.3**, **Schemes 8–10**. We can see that only five reactants are required for the synthesis of the six lily alkaloids (**Scheme 11**), which clearly demonstrates the versatility of these building blocks in the context of lily alkaloids. These building blocks are 3-methyl-3-pyrrolin-2-one (**29**), 5-ethoxypyrrolidin-2-one (**5**), 5-hydroxy-3-methylpyrrolidin-2-one (**30**), (±)-jatropham [5-hydroxy-3-methyl-3-pyrrolin-2-one] (**21**) and the flavonol kaempferol (**28**) (**Scheme 11**), which is the only building block available at a reasonable price.

[20] M. Haladová, E. Eisenreichová, A. Bučková, J. Tomko, D. Uhrín; *Collect. Czech. Chem. Commun.* **53**, 157–160 (1988)
[21] F. Pavelčík; *Acta Cryst.* C**45**, 956–957 (1989)
[22] E. Eisenreichová, M. Haladová, A. Bučková, J. Tomko, D. Uhrín, K. Ubik; *Phytochemistry* **31**, 1084–1085 (1992)
[23] M. Haladová, E. Eisenreichová, A. Bučková, J. Tomko, D. Uhrín, K. Ubik; *Collect. Czech. Chem. Commun.* **56**, 436–438 (1991)
[24] K. Yakushijin, R. Suzuki, R. Hattori, H. Furukawa; *Heterocycles* **16**, 1157–1160 (1981)
[25] I. Mašterová, D. Uhrin, J. Tomko; *Phytochemistry* **26**, 1844–1845 (1987)
[26] (a) E. Eisenreichová, I. Mašterová, A. Bučková, M. Haladová, J. Tomko; *Československ. Farm.* **34**, 408–409 (1985); (b) E. Eisenreichová, M. Haladová, P. Mučaji, D. Grančai; *Acta Fac. Pharm. Univ. Comen.* **51**, 27–37 (2004)



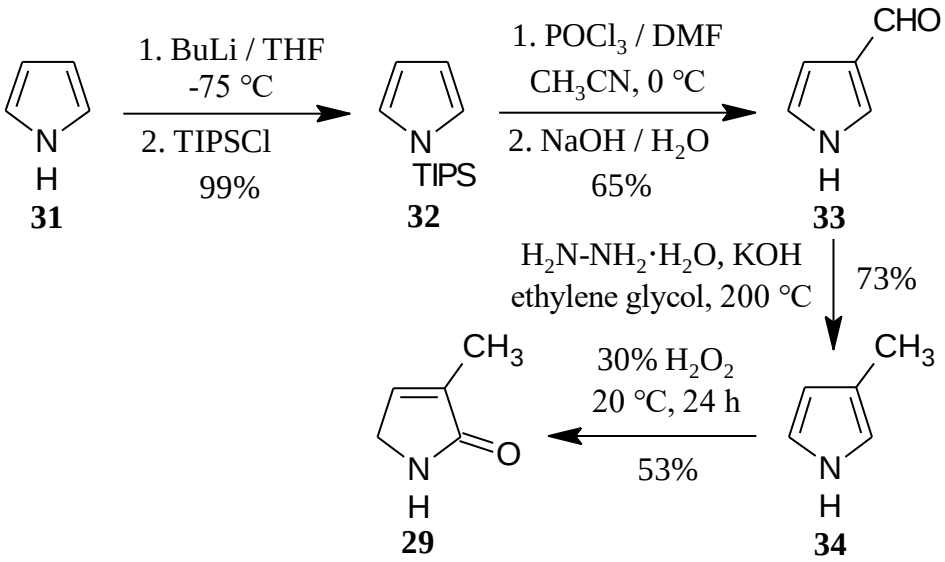
Scheme 11. Retrosynthetic concept of target compounds **22**–**27**

Synthesis of aminal-type lily alkaloids and lilaline[27]

The synthesis of the four pyrroline and pyrrolidine building blocks was achieved in a few steps from cheap, easily accessible starting materials. 5-Ethoxypyrrolidin-2-one (**5**) was synthesized in a single step from succinimide according to the procedure described in the literature (*see* **Subsection 3.1**, **Scheme 2**).[4] 3-Methyl-3-pyrrolin-2-one (**29**) was synthesized in 4 steps from pyrrole (**Scheme 12**). The nitrogen atom of pyrrole (**31**) was silylated with the bulky triisopropylsilyl (TIPS) group to yield TIPS-pyrrole (**32**),[28] in order to shield its α positions, thereby directing the formylation in the subsequent *Vilsmeier reaction* in the C3 position.[28, 29] Work-up effected desilylation, yielding pyrrole-3-carboxaldehyde (**33**), which was converted

[27] The synthesis of lily alkaloids was carried out in collaboration with Sándor Nagy.
[28] B. L. Bray, P. H. Mathies, R. Naef, D. R. Solas, T. T. Tidwell, D. R. Artis, J. M. Muchowski; *J. Org. Chem.* **55**, 6317–6328 (1990)
[29] I. M. Downie, M. J. Earle, H. Heaney, K. F. Shuhaibar; *Tetrahedron* **49**, 4015–4034 (1993)

to 3-methylpyrrole (**34**) in a *Wolff-Kishner reduction*.[30] Oxidation of **34** by aqueous hydrogen peroxide[31] gave lactam **29** in medium yield.



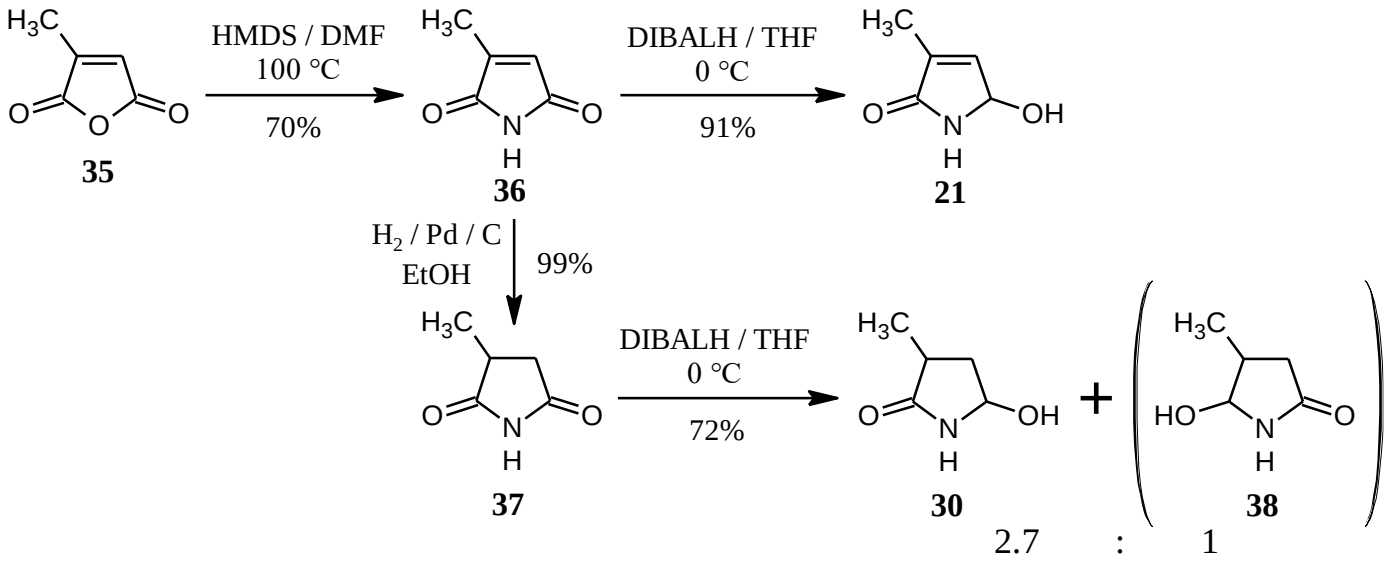
Scheme 12. Synthesis of 3-methyl-3-pyrrolin-2-one (**29**)

The cyclic *N*-acylaminocarbinol reagents **21** and **30** were synthesized from citraconic anhydride (**35**) in 2 and 3 steps, respectively (**Scheme 13**). Citraconic anhydride (**35**) was converted to citraconimide (**36**) with hexamethyldisilazane (HMDS) in DMF at 100 °C,[32] which was reduced with diisobutyl aluminium hydride (DIBALH) in THF at 0 °C to yield (±)-jatropham (**21**).[33] For the synthesis of the saturated *N*-acylaminocarbinol **30**, citraconimide (**36**) was first reduced by catalytic hydrogenation to (±)-2-methylsuccinimide (**37**),[34] which was then reduced with DIBALH at 0 °C to give an inseparable mixture of 5-hydroxy-3-methylpyrrolidin-2-one (**30**) and its isomer **38**, which had been reduced at the more hindered carbonyl. In contrast to the DIBALH reduction of **36**, this reduction proceeded with lower regioselectivity, yielding the major isomer **30** and the minor isomer **38** in an approximate ratio of 2.7:1 (according to NMR data). Despite the presence of significant amounts (ca. 27%) of the isomeric *N*-acylaminocarbinol **38** in the obtained isomeric reagent mixture, it could be utilized in the syntheses of the targeted lily alkaloids.

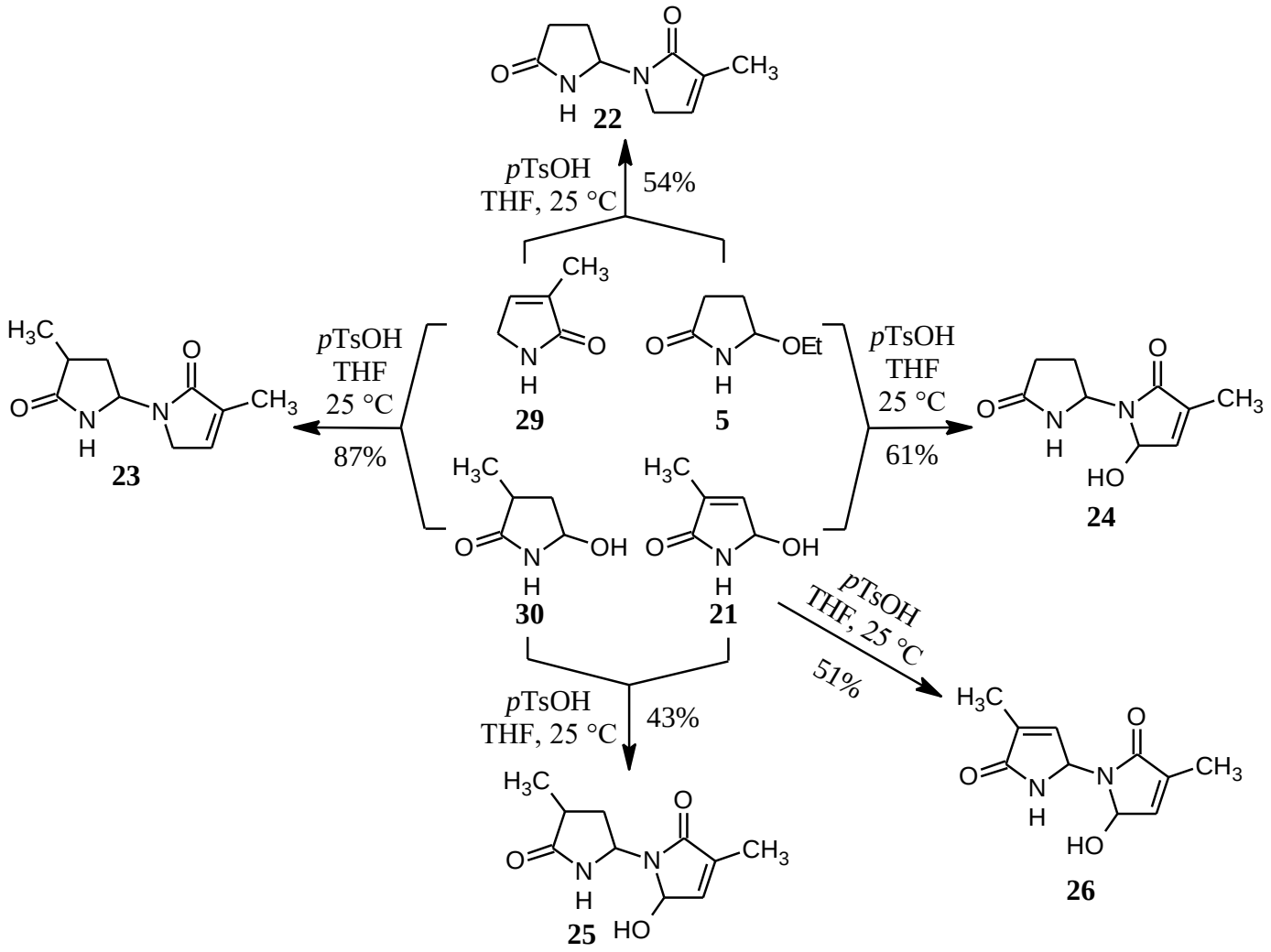
With the four pyrroline-pyrrolidine building blocks in our hands, we synthesized the desired lily alkaloids by the reaction of the appropriate two compounds in THF in the presence of catalytic amounts of *para*-toluenesulfonic acid. The reactants were employed in equimolar amounts and the reactions proceeded to full conversion at room temperature in 1 h. The reactions gave the desired lily alkaloids **22**–**26** as the major products in medium to high yields (**Scheme 14**). In the case of **22** and **26**, the precipitated crystalline products were isolated simply by filtration of the reaction mixture, while in the case of **23** and **24**, the products were crystallized after evaporation of the solvent. The pyrroline alkaloid **25** was purified by column chromatography. Compound **22** was isolated as a single racemic compound, while in the case of **23**–**26** due

[30] D. O. A. Garrido, G. Buldain, M. I. Ojea, B. Frydman; *J. Org. Chem.* **53**, 403–407 (1988)
[31] G. P. Gardini, V. Bocchi; *Gazz. Chim. Ital.* **102**, 91–101 (1972)
[32] J. Bessho, Y. Shimotsu, S. Mizumoto, N. Mase, H. Yoda, K. Takabe; *Heterocycles* **63**, 1013–1016 (2004)
[33] (a) N. Mase, T. Nishi, M. Hiyoshi, K. Ichihara; J. Bessho; H. Yoda, K. Takabe; *J. Chem. Soc. Perkin Trans. 1* **2002**, 707–709; (b) N. Mase, T. Nishi, Y. Takamori, H. Yoda, K. Takabe; *Tetrahedron: Asymmetry* **10**, 4469–4471 (1999)
[34] P. Kuehne, M. Hesse; *Tetrahedron* **49**, 4575–4580 (1993)

to the presence of multiple stereogenic centres the synthesized lily alkaloids were all isolated as racemic mixtures of diastereomers in different compositions.

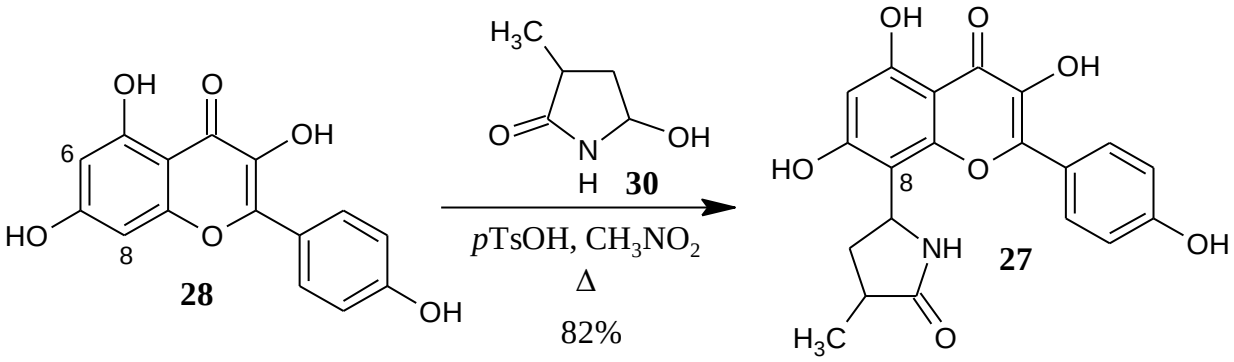


Scheme 13. Synthesis of the cyclic *N*-acylaminocarbinols **21** and **30**



Scheme 14. Synthesis of lily alkaloids **22**–**26**

The synthesis of lilaline (**27**) was accomplished from commercially available kaempferol (**28**), which was made to react with a slight excess (1.2 eq.) of 5-hydroxy-3-methylpyrrolidin-2-one (**30**) in refluxing nitromethane in the presence of catalytic amounts of *para*-toluenesulfonic acid, furnishing the target compound (**27**) in high yield (**Scheme 15**). Lilaline (**27**) was purified by preparative HPLC and was obtained as a diastereomeric mixture in the form of a yellow solid. The ratio of the major *trans* diastereomer (CH_3 and aryl *trans*) and the minor *cis* diastereomer (CH_3 and aryl *cis*) in the final product was found to be approx. 3:1 (according to the ^{1}H NMR data).



Scheme 15. Synthesis of the flavonoid alkaloid lilaline (**27**)

4. Thesis points

1. We have executed the first synthesis of the simple *N*-acylaminocarbinol ether sessiline (**1**) *via* the *O*-amidoalkylation of 5-hydroxymethylfurfural (**3**) by 5-hydroxypyrrolidin-2-one (**2**) under neat conditions. **[VI-1]**

2. We have accomplished the first synthesis of the *Aspidosperma* alkaloid bannucine (**(–)-9**) and its 5'-epimer (**(–)-5'-epi-9**) through the electrophilic aromatic substitution of natural vindoline (**(–)-10**) at C10 by the appropriate *N*-acyliminium species. We have optimized the synthetic procedure to provide the epimeric mixture in an excellent 90% yield. We performed the chromatographic separation of the epimers and characterized them with their melting points and specific rotations, based on which we postulated a corrigendum of the results of Atta-ur-Rahman *et al.*[10] proposing that the substance they isolated from *Catharanthus roseus* was not pure bannucine (5'*R* configuration), but most probably a mixture of the two epimers. **[VI-2]**

3. We have achieved the first synthesis of the flavoalkaloids dracocephins A (**12**), dracocephins B (**13**), 6-(2"-pyrrolidinone-5"-yl)-(–)-epicatechin (**14**), 8-(2"-pyrrolidinone-5"-yl)-(–)-epicatechin (**15**), 8-(2"-pyrrolidinone-5"-yl)quercetin (**16**) and lilaline (**27**) *via* the *Betti reaction* of their 5,7-dihydroxylated flavonoid precursors with the appropriate *N*-acyliminium species. **[VI-3, VI-4, VI-6]**

4. We have elaborated the first synthesis of five aminal-type pyrroline- and pyrrolidine-alkaloids isolated from *Lilium candidum*, namely 3-methyl-1-(2'-oxopyrrolidin-5'-yl)-3-pyrrolin-2-one (**22**), 3-methyl-1-(3'-methyl-2'-oxopyrrolidin-5'-yl)-3-pyrrolin-2-one (**23**), 5-hydroxy-3-methyl-1-(2'-oxopyrrolidin-5'-yl)-3-pyrrolin-2-one (**24**), 5-hydroxy-3-methyl-1-(3'-methyl-2'-oxopyrrolidin-5'-yl)-3-pyrrolin-2-one (**25**) and 5-hydroxy-3-methyl-1-(3'-methyl-2'-oxo-3'-pyrrolin-5'-yl)-3-pyrrolin-2-one (**26**). **[VI-6]**

5. Potential applications

The synthetic work presented in this thesis contributes to the broadening of the scope of iminium chemistry by extending the application of cyclic *N*-acylaminocarbinols to the synthesis of various types of natural products that had not been synthesized before. The methodology can potentially be used in the synthesis of further members of the discussed natural product families.

By providing the first feasible synthetic procedures to the involved natural products, this work facilitates the synthetic availability of these substances, enabling researchers in the field to synthesize them in ample amounts for the purpose of exploring their potential bioactivity. Since some of these compounds have been reported to show noteworthy biological activity, and their analogues could potentially possess valuable pharmacological traits as well, the synthesis and pharmacological evaluation of these natural products could be a promising possibility for supporting drug discovery efforts.

6. Publications

6.1. Publications related to the PhD thesis

VI-1 Ilkei, V., Faragó, K., Sánta, Zs., Dékány, M., Hazai, L., Szántay Jr., Cs., Szántay, Cs., Kalaus, Gy.; **The First Synthesis of Sessiline.** *International Journal of Organic Chemistry* **4**, 309–313 (2014). DOI: 10.4236/ijoc.2014.45033 (IF: -; authorship contribution: 90%), independent citations: 2

VI-2 Ilkei, V., Bana, P., Tóth, F., Palló, A., Holczbauer, T., Czugler, M., Sánta, Zs., Dékány, M., Szigetvári, Á., Hazai, L., Szántay Jr., Cs., Szántay, Cs., Kalaus, Gy.; **A simple synthesis of bannucine and 5'-epibannucine from (−)-vindoline.** *Tetrahedron* **71**, 9579–9586 (2015). DOI: 10.1016/j.tet.2015.10.020 (IF in 2015: 2,645; authorship contribution: 90%), independent citations: 0

VI-3 Ilkei, V., Spaits, A., Prechl, A., Szigetvári, Á., Béni, Z., Dékány, M., Szántay Jr., Cs., Müller, J., Könczöl, Á., Szappanos, Á., Mándi, A., Antus, S., Martins, A., Hunyadi, A., Balogh, Gy. T., Kalaus, Gy., Bölcskei, H., Hazai, L., Kurtán, T.; **Biomimetic synthesis and HPLC–ECD analysis of the isomers of dracocephins A and B.** *Beilstein Journal of Organic Chemistry* **12**, 2523–2534 (2016). DOI: 10.3762/bjoc.12.247 (IF 2016-ban: 2,337; authorship contribution: 70%), independent citations: 1

VI-4 Ilkei, V., Spaits, A., Prechl, A., Müller, J., Könczöl, Á., Lévai, S., Riethmüller, E., Szigetvári, Á., Béni, Z., Dékány, M., Martins, A., Hunyadi, A., Antus, S., Szántay Jr., Cs., Balogh, Gy. T., Kalaus, Gy., Bölcskei, H., Hazai, L.; **C8-selective biomimetic transformation of 5,7-dihydroxylated flavonoids by an acid-catalysed phenolic *Mannich reaction*: Synthesis of flavonoid alkaloids with quercetin and (–)-epicatechin skeletons.** *Tetrahedron* **73**, 1503–1510 (2017). DOI: 10.1016/j.tet.2017.01.068 (IF 2017-ben: 2,377; authorship contribution: 80%), independent citations: 7

VI-5 Ilkei, V., Hazai, L., Antus, S., Bölcskei, H.; **Flavonoid Alkaloids: Isolation, Bioactivity and Synthesis.** In *Studies in Natural Products Chemistry* **Vol. 56**, Atta-ur-Rahman (Ed.); Elsevier,

2018; pp 247–285. DOI: 10.1016/B978-0-444-64058-1.00008-X (authorship contribution: 100%), independent citations: 5

VI-6 Nagy, S., Szigetvári, Á., Ilkei, V., Krámos, B., Béni, Z., Szántay Jr., Cs., Hazai, L.; **Synthesis of aminal-type Lilium candidum alkaloids and lilaline; determination of their relative configuration by the concerted use of NMR spectroscopy and DFT conformational analysis.** *Tetrahedron* **81**, 131827 (2021). DOI: 10.1016/j.tet.2020.131827 (IF: 2,233; authorship contribution: 49%), independent citations: 0

6.2. Further publications

VI-7 Sepsey Für, Cs., Keglevich, P., Bölcskei, H., Ilkei, V., Hazai, L.; **Eredmények a természetes szerves anyagok kutatásában – Új, daganatellenes hatású *Vinca* alkaloid származékok előállítása és flavon alkaloidok szintézise.** *Magyar Kémiai Folyóirat* **124**, 71–77 (2018)

VI-8 Mayer, Sz., Keglevich, A., Sepsey Für, Cs., Bölcskei, H., Ilkei, V., Keglevich, P., Hazai, L.; **Results in Chemistry of Natural Organic Compounds. Synthesis of New Anticancer Vinca Alkaloids and Flavone Alkaloids.** *Chemistry* **2**, 714–726 (2020)

6.3. Presentations

Oral presentations related to the PhD thesis (held in Hungarian)

1. Ilkei, V., Spaits, A., Hazai, L., Bölcskei, H., Kalaus, Gy. (†), Szappanos, Á., Mándi, A., Kurtán, T., Antus, S., Balogh, Gy. T., Prechl, A., Szigetvári, Á., Béni, Z., Dékány, M., Szántay, Cs. Jr., Müller, J., Könczöl, Á., Hunyadi, A., Martins, A.; *Flavonoid alkaloidok biomimetikus szintézise, gyógyszerkémiai és in vitro biológiai jellemzése.* **Presenters' Session of the HAS Working Committee for Alkaloid and Flavonoid Chemistry** (Mátrafüred, 14-15. April 2016)

2. Ilkei, V., Spaits, A., Vámosi, P., Sánta, Zs., Béni, Z., Szigetvári, Á., Dékány, M., Balogh, Gy. T., Prechl, A., Hazai, L., Antus, S., Szántay, Cs., Kalaus, Gy. (†); *Flavonoid alkaloidok: irodalmi összefoglaló és kezdeti eredmények.* **Presenters' Session of the HAS Working Committee for Alkaloid and Flavonoid Chemistry** (Balatonalmádi, 18-19. May 2015)

3. Ilkei, V., Faragó, K., Hazai, L., Sánta, Zs., Dékány, M., Czugler, M., Szántay, Cs., Kalaus, Gy.; *Egy amidokarbinol elektrofil partnerként történő alkalmazása alkaloidok előállításában. A (–)-bannucin, a (–)-5'-epibannucin és a (±)-szesszilin első szintézise.* **XXXVI. Chemistry Lectures** (Szeged, 28-30. October 2013)

4. Ilkei, V., Faragó, K., Hazai, L., Sánta, Zs., Dékány, M., Szántay, Cs., Kalaus, Gy.; *Acilaminokarbinol – mint elektrofil prekurzor – alkalmazása alkaloidok előállításában. A (–)-bannucin és a (–)-5'-epibannucin, valamint a (±)-szesszilin első szintézise.* **Presenters' Session of the HAS Working Committee for Alkaloid and Flavonoid Chemistry** (Balatonalmádi, 13-14. May 2013)