

Summary of Ph.D. Thesis

**Synthesis and biological investigation of nitrogen- and  
oxygen-containing heterocyclic steroids**

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## 1. Introduction and aims

Steroid derivatives are applied for clinical purposes worldwide. The medical applications of cardenolides and bufadienolides are associated with high risk, due to their exceptional toxicity and the small difference between the therapeutic and toxic doses. In the past few years, increasing attention has been paid to the synthesis of cardenolide analogues containing a heterocyclic ring at C-17, which are expected to have better therapeutic indices. Besides cardiotonic activity, a large number of *exo*-heterocyclic steroids at position C-17 display high inhibitory activity towards 17 $\alpha$ -hydroxylase-C<sub>17,20</sub>-lyase (P450<sub>17 $\alpha$</sub> ) and 5 $\alpha$ -reductase. Androgens are known to play a critical role in the development and progression of prostatic diseases such as benign prostatic hyperplasia and prostate carcinoma. P450<sub>17 $\alpha$</sub>  and 5 $\alpha$ -reductase are key enzymes in the androgen biosynthesis pathway. Inhibition of these pivotal enzymes in the androgen synthesis cascade would diminish the circulating androgens and is a useful approach for the development of therapeutic agents for the treatment of prostatic diseases and other androgen-dependent diseases.

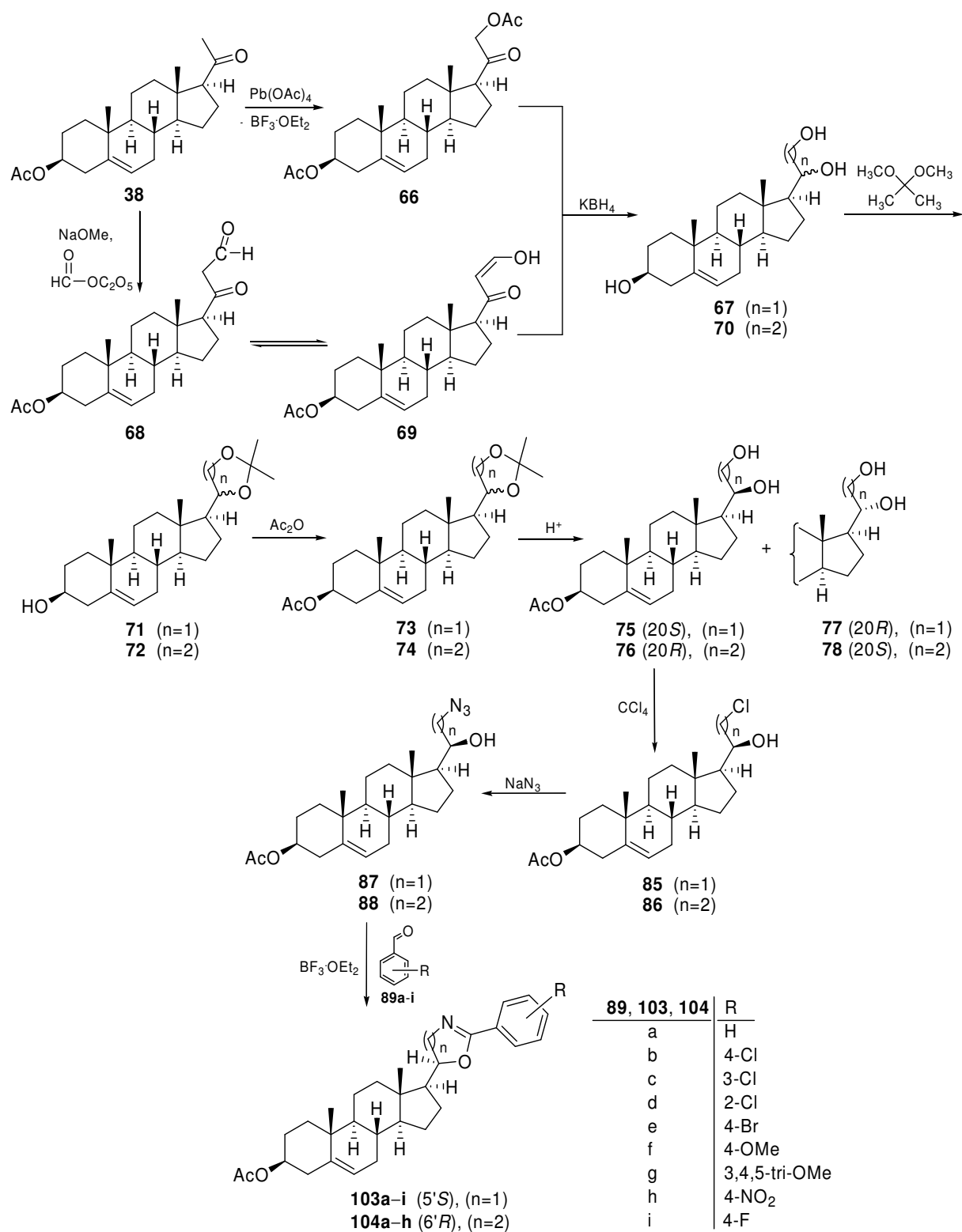
In view of the available literature knowledge, we set out to synthesize *exo*-heterocyclic steroids containing N and O, as novel presumed inhibitors of P450<sub>17 $\alpha$</sub>  and/or 5 $\alpha$ -reductase. The inhibitory effects of these compounds were investigated with an *in vitro* radioincubation technique for both P450<sub>17 $\alpha$</sub>  and 5 $\alpha$ -reductase.

## 2. Experimental methods

Reactions were monitored on the mmol scale, by thin-layer chromatography. The crude products were purified by flash chromatography. The structures of the compounds obtained were determined via the <sup>1</sup>H and <sup>13</sup>C NMR (including J-MOD, NOE, COSY, HMBC and HSQC) spectra, EI- and DCI-MS techniques and elemental analysis.

### 3. Scientific results \*

- 3.1. Preparation of the  $\alpha,\beta$ - and  $\alpha,\gamma$ -diol systems on the side-chain of the sterane skeleton from  $3\beta$ -acetoxypregn-5-en-20-one (**38**) as starting material was achieved by elongation of the side-chain of **38**, by means of oxidation (**66**) and CLAISEN condensation (**69**), followed by reduction with  $\text{KBH}_4$  to the triols (**67**, **70**) (Scheme 1).
- 3.2. For selective acetylation of the  $3\beta$ -OH group, the cyclic acetonides of the mixture (**71**, **72**) should first be prepared (**73**, **74**). Under acidic conditions, the cyclic ketals produced were transformed to chromatographically separable mixtures of diols (**75**, **76** and **77**, **78**) (Scheme 1).
- 3.3. APPEL reactions of the pure isomers  $20S$  and  $20R$  (**75**, **76**) with  $\text{CCl}_4$  and  $\text{PPh}_3$  produced **85** and **86**. We found that the reaction was regioselective; only 21- (**85**) and 22-chloro derivatives (**86**) were formed (Scheme 1).
- 3.4. To prepare the  $\alpha,\beta$ - and  $\alpha,\gamma$ -systems in the side-chain, nucleophilic exchange with  $\text{NaN}_3$  in dimethylformamide led to the required ( $20S$ )- $3\beta$ -acetoxy-21- (**87**) and ( $20R$ )- $3\beta$ -acetoxy-22-azidopregn-5-en-20-ol (**88**), as key compounds (Scheme 1).
- 3.5. We found that the SCHMIDT reactions of the  $\alpha,\beta$ - (**87**) and the  $\alpha,\gamma$ -azidoalcohol (**88**) with appropriately substituted aromatic aldehydes (**89a-i**) activated by  $\text{BF}_3\cdot\text{OEt}_2$  as LEWIS acid catalyst proceeded cleanly to the corresponding dihydrooxazolines (**103a-i**) and dihydrooxazines (**104a-i**) in good yields (Scheme 1).

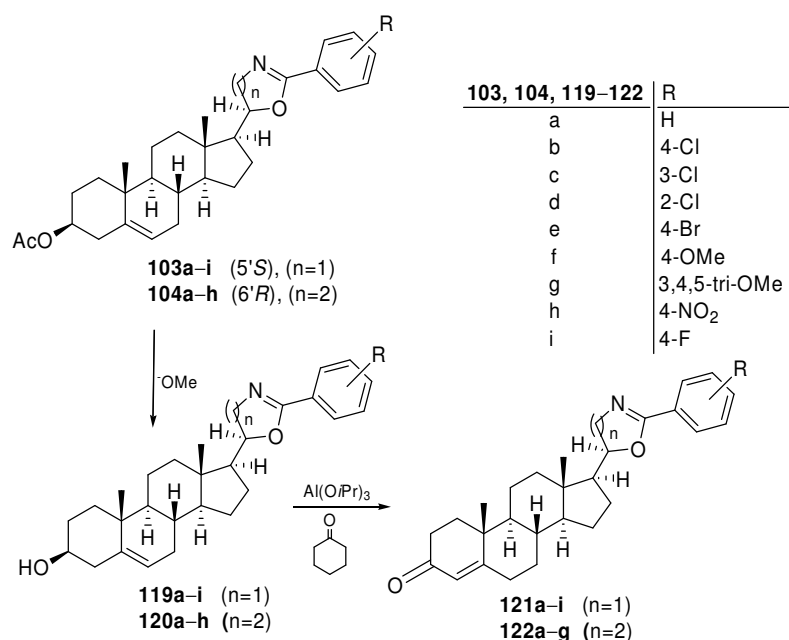


Scheme 1

3.6. Deacetylation of the newly synthesized  $3\beta$ -acetoxy compounds **103a-i** and **104a-h** in methanol in the presence of NaOMe by the ZEMPLÉN method

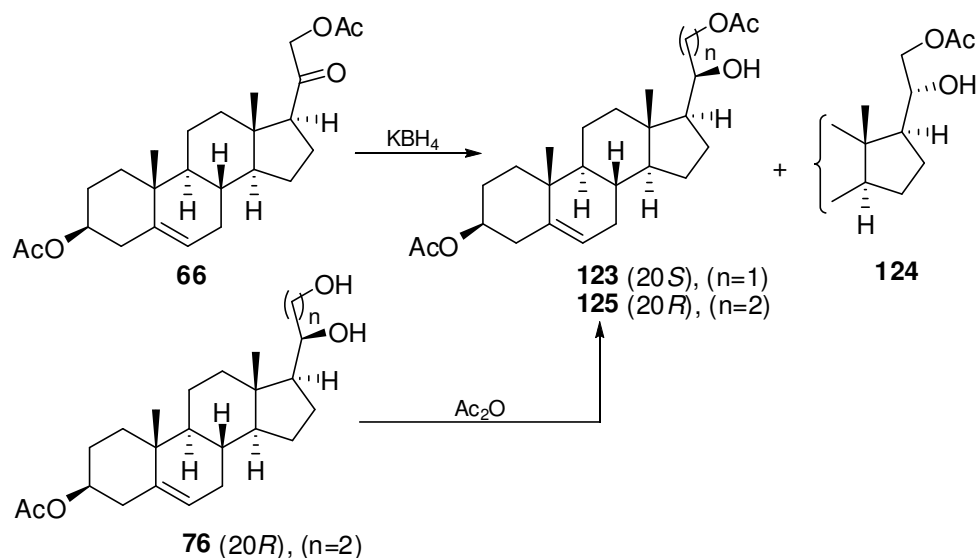
resulted in the 3 $\beta$ -hydroxy derivatives (**119a-i**, **120a-h**), without opening of the heterocyclic ring. OPPENAUER oxidation of the 3 $\beta$ -hydroxy-*exo*-heterocyclic steroids yielded the corresponding  $\Delta^4$ -3-ketosteroids (**121a-i**, **122a-g**) in moderate yields (Scheme 2).

3.7. A substituent effect was observed during the reaction. The speed of the ring closure depends on the electronic properties of the substituents on the aromatic aldehyde. In the case of aldehydes containing electron-withdrawing groups (**89b-e**, **h**, **i**) the ring closure was facilitated. In contrast, hydrogen (**89a**) or electron-donating groups (**89f**, **g**) on the aromatic ring retarded the SCHMIDT reaction (Scheme 2).



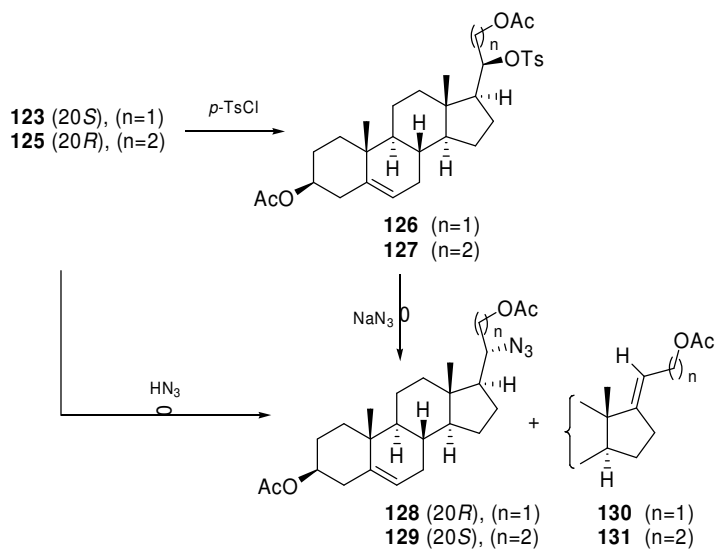
Scheme 2

3.8. We established that the hydroxy group at position 20 was formed by controlled reduction of 3 $\beta$ ,21-diacetoxypregn-5-en-20-one (**66**) at about pH 6.6, which gave a mixture of 20-hydroxy isomers (**123** and **124**) in a ratio of 9:1. These epimers could be separated by flash chromatography. On the other hand, the 22-acetoxy,20-hydroxy compound (**125**) was formed from 3 $\beta$ -acetoxy-20,22-dihydroxypregn-5-ene (**76**) via selective esterification (Scheme 3).



Scheme 3

3.9. We found that the azido group at position C-20 could be formed in two different ways. First, in a two-step reaction, *p*-tosyl ester formation from **123** and **125** resulted in **126** and **127**, which in subsequent reactions with  $\text{NaN}_3$  were converted to the corresponding 20-azido compounds (**128**, **129**). In the other method, **123** and **124** were transformed with  $\text{HN}_3$ ,  $\text{PPh}_3$  and diethyl diazenedicarboxylate into the corresponding azides (**128**, **129**) in moderate yields. The MITSUNOBU reaction was accompanied by elimination to afford 17(20)*E*-unsaturated compounds (**130**, **131**) (Scheme 4).

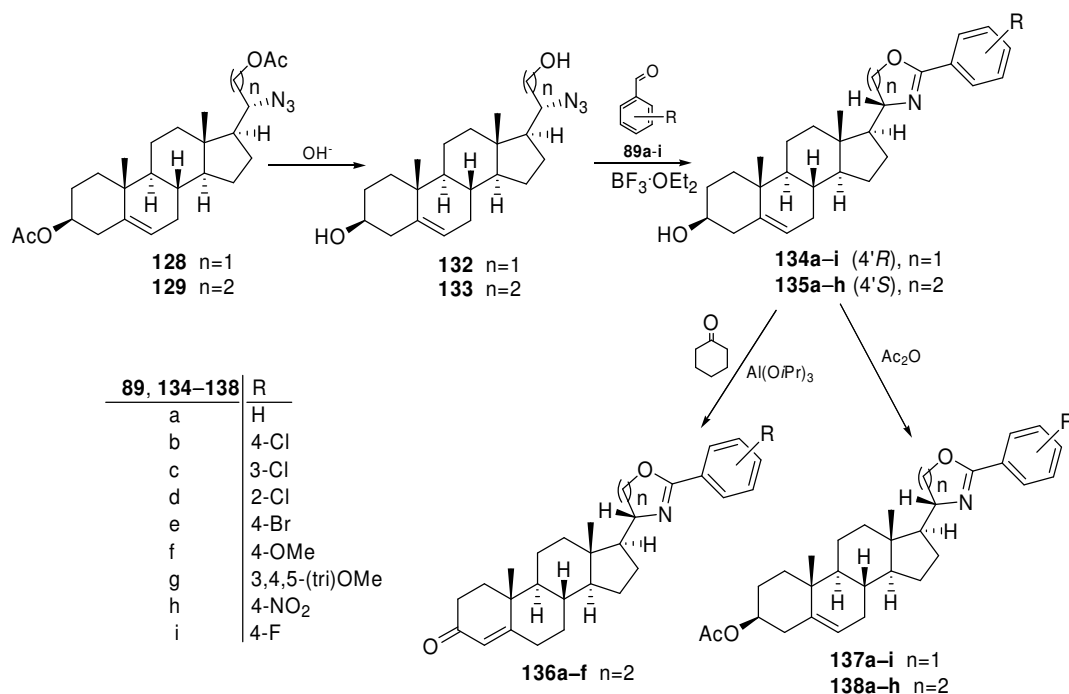


Scheme 4

3.10. Compounds **132** and **133** for cyclization were obtained after deacetylation of **128** and **129** (Scheme 8). We observed that in the 20-azido series, the  $\alpha,\beta$ - and  $\alpha,\gamma$ -azidoalcohols with differently substituted aromatic aldehydes (**89a-i**) furnished the corresponding dihydrooxazolinyll (**134a-i**) and dihydrooxazinyll (**135a-h**) steroids, similarly to the regioisomeric azidoalcohols (**87**, **88**) (Scheme 5).

3.11. In the SCHMIDT reaction, it was proved that the ring closure depended on the electrophilic character of the substituent. Aromatic aldehydes containing electron-withdrawing groups (**89b-e**, **h**, **i**) took part in the ring-closure reaction more responsively, while aldehydes bearing electron-donating substituents (**89f**, **g**) or hydrogen (**89a**) were changed to cyclic products slowly.

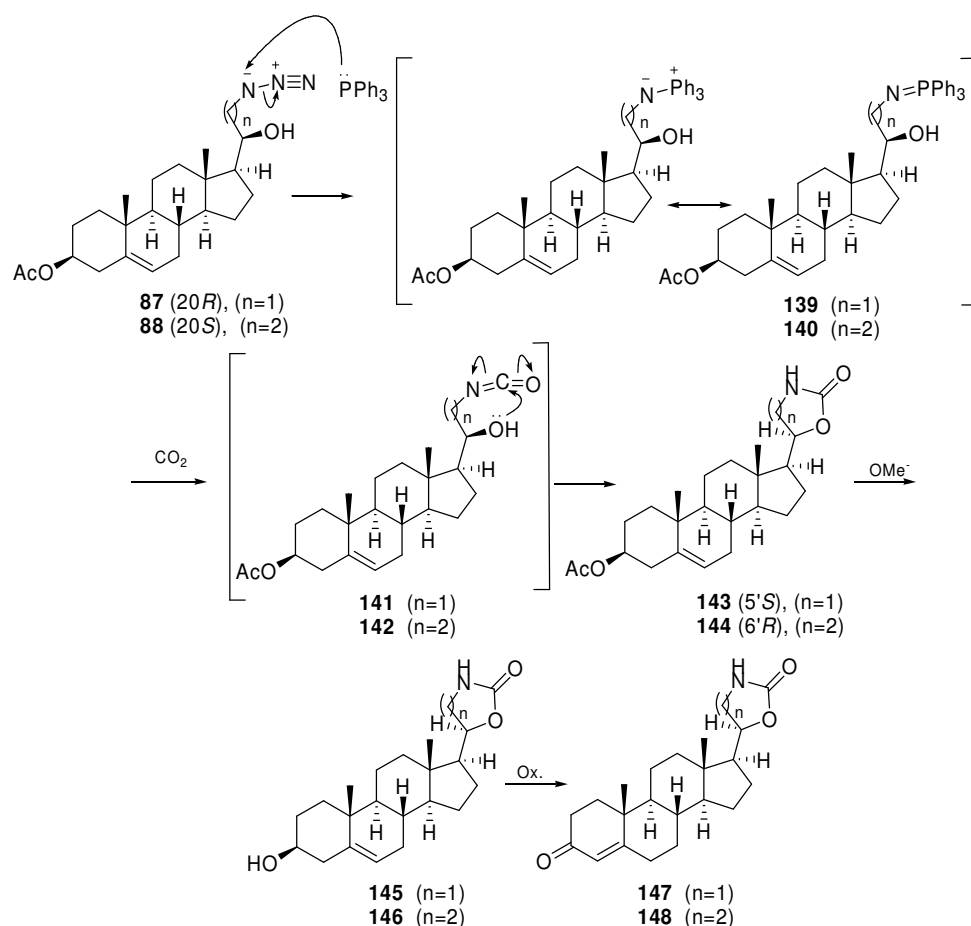
3.12. OPPENAUER oxidation of the  $3\beta$ -hydroxy-*exo*-heterocyclic steroids (**135a-h**) yielded the corresponding  $\Delta^4$ -3-ketosteroids (**136a-f**). Acetylation of **134a-i** and **135a-h** gave the  $3\beta$ -acetoxy compounds (**137a-i**, **138a-h**) (Scheme 5).



Scheme 5

3.13. For the synthesis of the unsubstituted steroidal cyclic carbamates (**143**, **144**), we used a different method. Under the conditions of the STAUDINGER reaction,

**87** and **88** with  $\text{Ph}_3\text{P}$  gave the 21- and 22-phosphimino compounds (**139**, **140**), which were converted into the corresponding isocyanates (**141**, **142**) with  $\text{CO}_2$ . Their deacetylation in methanol in the presence of  $\text{NaOMe}$  by the ZEMPLÉN method furnished **145** and **146**. OPPENAUER oxidation of the  $3\beta$ -hydroxy-*exo*-heterocyclic steroids (**145**, **146**) yielded the corresponding  $\Delta^4$ -3-ketosteroids (**147**, **148**) (Scheme 6).



Scheme 6

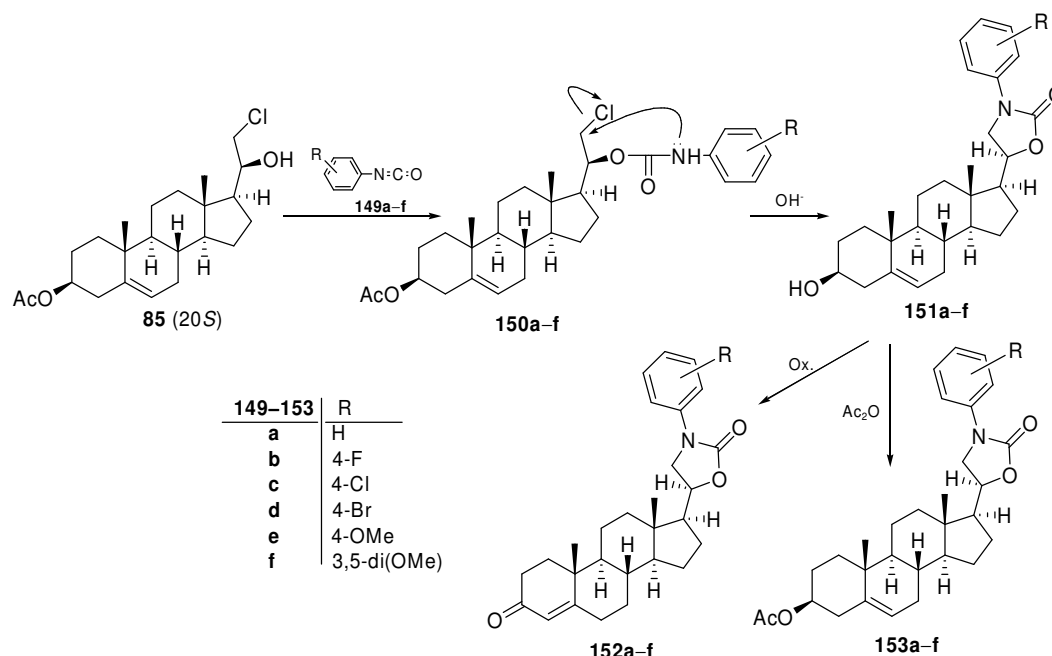
3.14. We found that the 21-chloro derivative (**85**) was a suitable starting material for cyclization into steroid carbamates. **85** was reacted with phenyl isocyanate (**149a**) or substituted phenyl isocyanates (**149b–f**) in the presence of triethylamine to afford the desired 21-chloromethylpregn-5-ene-20-arylurethanes (**150a–f**). During the alkaline methanolysis of  $3\beta$ -acetoxy-21-chloropregn-5-ene-20-*N*-phenylurethane (**150a**) or its 4-monosubstituted (**150b–e**) or 3,5-disubstituted (**150f**) phenyl derivatives, cyclization occurred, in



the course of which phenyl- (**151a**) and (*N*-substituted phenyl)-oxazolidinone derivatives (**151b-f**) were formed (Scheme 7). The cyclization took place with (*N*-5) neighbouring group participation.

3.15. The substituent effect was observed under the ring-closure reactions. The *N*-phenylcarbamates bearing electron-withdrawing groups reacted rapidly, while the ring closure with electron-donating substituents took place slowly.

3.16. OPPENAUER oxidation of the 3 $\beta$ -hydroxy-*exo*-heterocyclic steroids (**152a-f**) yielded the corresponding  $\Delta^4$ -3-ketosteroids (**152a-f**). Esterification of **152a-f** by acetylation furnished the 3 $\beta$ -acetoxy compounds (**153a-f**) (Scheme 7).



Scheme 7

3.17. The inhibitory effects ( $IC_{50}$ ) of these *exo*-heterocyclic compounds on rat testicular P450<sub>17 $\alpha$</sub>  and 5 $\alpha$ -reductase were investigated with an *in vitro* radioligand incubation technique. Both 3 $\beta$ -hydroxy- and  $\Delta^4$ -3-ketosteroids were tested.

3.18. Most of our newly synthesized *exo*-heterocyclic steroids displayed moderate or weak inhibitory action in the tests. However, there were some (5'S)-dihydrooxazinyll derivatives whose IC<sub>50</sub> values approximated that of the reference inhibitor (ketoconazole, **31**). The most effective inhibitor in the investigation of the inhibitory effects of P450<sub>17α</sub> and 5αR2 was an unsubstituted oxazolidinone derivative (**147**).

#### 4. Papers forming the basis of the dissertation

1. Stereoselective synthesis of some  $17\beta$ -dihydrooxazinyll steroids, as novel presumed inhibitors of  $17\alpha$ -hydroxylase- $C_{17,20}$ -lyase  
János Wölfling, Éva Andrea Oravec, **Dóra Ondré**, Erzsébet Mernyák, Gyula Schneider, István Tóth, Mihály Szécsi, János Julesz  
*Steroids* **2006**, 71, 809-816.  
Impact factor: **2.849**
2. Neighboring group participation, Part 17. Stereoselective synthesis of some steroidal 2-oxazolidones, as novel potential inhibitors of  $17\alpha$ -hydroxylase- $C_{17,20}$ -lyase  
**Dóra Ondré**, János Wölfling, Zoltán Iványi, Gyula Schneider, István Tóth, Mihály Szécsi, János Julesz  
*Steroids* **2008**, 73, 1375-1384.  
Impact factor: **2.588**
3. Stereoselective synthesis of some steroidal oxazolines, as novel potential inhibitors of  $17\alpha$ -hydroxylase- $C_{17,20}$ -lyase  
**Dóra Ondré**, János Wölfling, Gyula Schneider, István Tóth, Mihály Szécsi, János Julesz  
*Steroids* **2009**, doi:10.1016/j.steroids.2009.08.001.  
Impact factor (2008): **2.588**
4. Synthesis of steroidal dihydrooazines and 2-oxazolidones, as novel potential inhibitors of  $17\alpha$ -hydroxylase- $C_{17,20}$ -lyase  
**Dóra Ondré**, Gyula Schneider, Zoltán Iványi, István Tóth, Mihály Szécsi, János Julesz, János Wölfling  
1<sup>st</sup> Hungarian-Singaporean Workshop on Drug Discovery and Biomaterials  
Budapest, 10–11 March 2008.  
Proceedings, pp. 98-100.

5. Determination of rat 5 $\alpha$ -reductase type 1 isozyme activity and its inhibition by novel *N*-aryl substituted 17 $\beta$ -oxazolidonyl-androst-4-en-3-one derivatives  
Mihály Szécsi, **Dóra Ondré**, István Tóth, Sándor Magony, János Wölfling, Gyula Schneider, János Julesz  
*Acta Biologica Hungarica*, prepared for publication, 2009.  
Impact factor (2008): 0.619

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**Total impact factor for the publications that have already appeared: 8,025**

## 5. Scientific lectures and posters forming the basis of the dissertation

### Lectures:

1. Gyűrűs szteroid-karbamátok szintézise  
(Synthesis of cyclic steroidal carbamates)  
**Dóra Ondré**, János Wölfling, Gyula Schneider  
A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 7. Tudományos Előadóülése (2<sup>nd</sup>. prize)  
Szeged, 18 January 2007.
2. Szteroid-oxazolidinonok szintézise  
(Synthesis of steroidal oxazolidinones)  
**Dóra Ondré**, János Wölfling, Gyula Schneider  
XXX. Kémiai Előadói Napok  
Szeged, 29-31 October 2007.
3. Szteroid-oxazolinok előállítása  
(Synthesis of steroidal oxazolines)  
**Dóra Ondré**, János Wölfling, Gyula Schneider, Mihály Szécsi  
MTA Szteroidkémiai Munkabizottsági Ülés  
Szeged, 20 November 2008.
4. Nitrogén- és oxigéntartalmú heterociklusos szteroidok szintézise és biológiai hatásvizsgálata  
(Synthesis and biological investigation of nitrogen- and oxygen-containing heterocyclic steroids)  
**Dóra Ondré**  
Bruckner-termi előadás  
Budapest, 30 October 2009.

Posters:

1. 17 $\beta$ -Dihidrooxazinil-szteroidok sztereoselektív szintézise  
(Stereoselective synthesis of 17 $\beta$ -dihydrooxazolanyl steroids)  
**Dóra Ondré**, Gyula Schneider, János Wölfling, István Tóth, Mihály Szécsi,  
János Julesz  
Centenárium Vegyészkonferencia  
Sopron, 29 May – 1 June 2007.  
Program és előadásösszefoglalók, p. 360.
2. Stereoselective synthesis of some 17 $\beta$ -dihydrooxazolanyl steroids, as novel  
presumed inhibitors of 17 $\alpha$ -hydroxylase-C<sub>17,20</sub>-lyase  
Gyula Schneider, János Wölfling, **Dóra Ondré**, István Tóth, Mihály Szécsi,  
János Julesz  
5<sup>th</sup> Joint Meeting on Medicinal Chemistry  
Portorož, Slovenia, 17–21 June 2007.  
*Farmaceutski Vestnik* **2007**, 58, 158.
3. C<sub>17,20</sub>-liáz enzim gátlása új 17 $\beta$ -oxazolidinil-androszteron származékokkal  
(Inhibition of C<sub>17,20</sub>-lyase enzyme with some novel 17 $\beta$ -oxazolidinyl  
androsterone derivatives)  
Mihály Szécsi, János Wölfling, **Dóra Ondré**, Gyula Schneider, István Tóth,  
János Julesz  
A Magyar Endokrinológiai és Anyagcsere Társaság 22. Kongresszusa  
Eger, 5-7 June 2008.  
*Magyar Belorvosi Archívum* **2008**, 61, 264.

4. Új szteroid-oxazolidinonok szintézise  
(Synthesis of novel steroidal oxazolidinones)  
**Dóra Ondré**, János Wölfling, Gyula Schneider, Mihály Szécsi, István Tóth, János Julesz  
MKE Vegyészkonferencia (Poster session, 1<sup>st</sup> prize)  
Hajdúszoboszló, 19–21 June 2008.  
Program és előadásösszefoglalók, p. 97.
  
5. Synthesis of new steroidal 2-oxazolidones, as novel potential inhibitors of 17 $\alpha$ -hydroxylase-C<sub>17,20</sub>-lyase  
**Dóra Ondré**  
European School of Medicinal Chemistry (XXVIII. Advanced Course of Medicinal Chemistry and „E. Duranti” National Seminar for PhD Students)  
Urbino, Italy, 6–11 July 2008.  
Proceedings of Ph.D. Student Poster Session, p. 84.
  
6. Stereoselective synthesis of some new steroidal oxazolines  
**Dóra Ondré**, Mihály Szécsi, István Tóth, János Wölfling, Éva Frank, Gyula Schneider, János Julesz  
German-French-Hungarian Congress in Organic and Biomolecular Chemistry  
Budapest, 20–23 June 2009.  
Book of Abstracts, p. 46.
  
7. Inhibition of C<sub>17,20</sub>-lyase activity by new 17 $\beta$ -oxazoline derivatives in the androsterone series  
**Dóra Ondré**, Mihály Szécsi, István Tóth, János Wölfling, Gyula Schneider, János Julesz  
6<sup>th</sup> Hungarian-Austrian-Czech-German-Greek-Italian-Polish-Slovak-Slovenian Joint Meeting on Medicinal Chemistry  
Budapest, 24–27 June 2009.  
Book of Abstracts, p. 106.

