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 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) and 5α-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. $P450_{17\alpha}$ and 5α-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 synthetize *exo*-heterocyclic steroids containing N and O, as novel presumed inhibitors of $P450_{17\alpha}$ and/or 5α-reductase. The inhibitory effects of these compounds were investigated with an *in vitro* radioincubation technique for both $P450_{17\alpha}$ and 5α-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 ^{1}H and ^{13}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 α,β- and α,γ-diol systems on the side-chain of the sterane skeleton from 3β-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 KBH_4 to the triols (**67**, **70**) (Scheme 1).

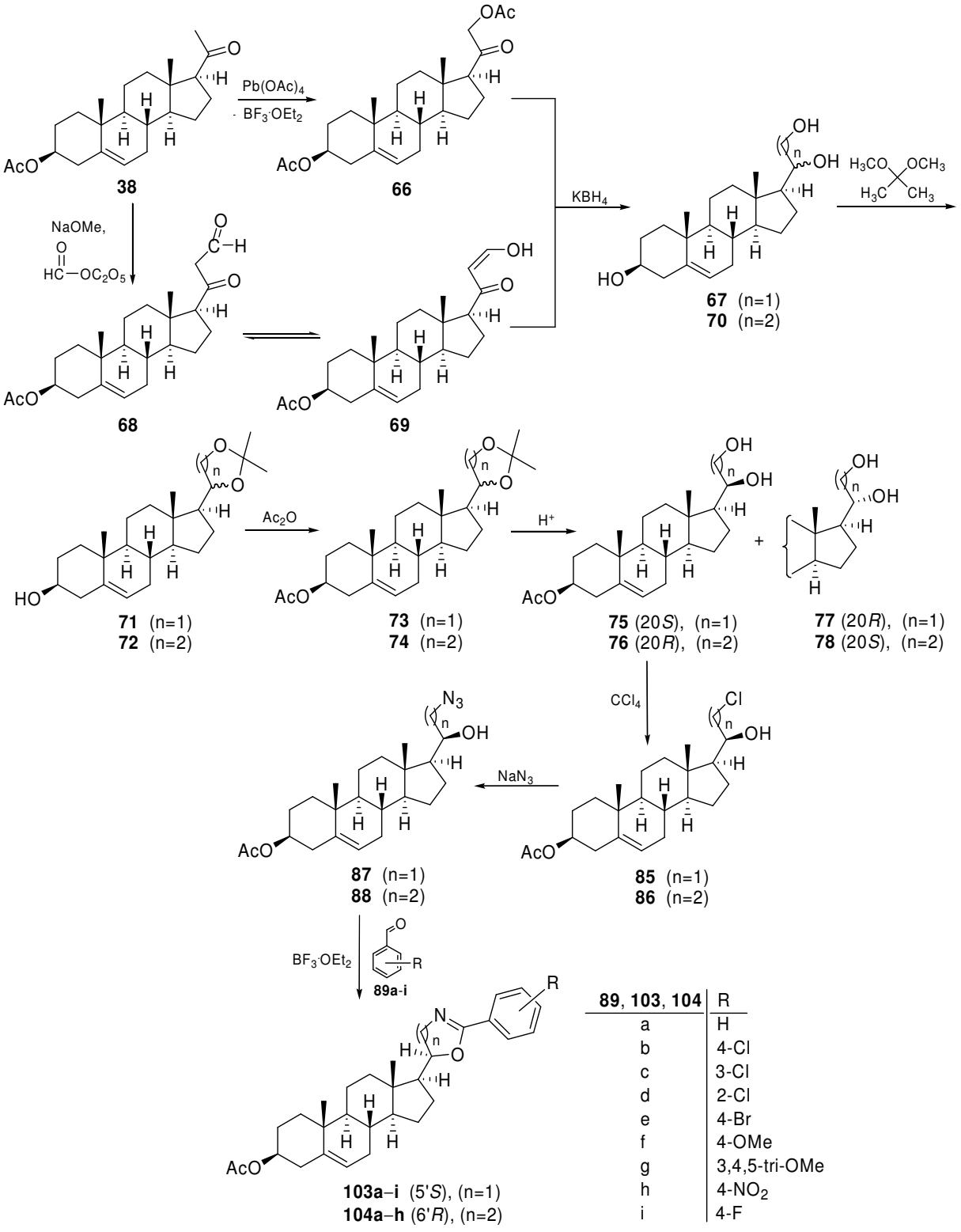
3.2. For selective acetylation of the 3β-OH group, the cyclic acetonides of the mixture (**71**, **72**) should first be prepared (**73**, **74**). Under acidic conditions, the cyclic ketals produced were transformed to chromatographically separable mixtures of diols (**75**, **76** and **77**, **78**) (Scheme 1).

3.3. APPEL reactions of the pure isomers 20*S* and 20*R* (**75**, **76**) with CCl_4 and 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 α,β- and α,γ-systems in the side-chain, nucleophilic exchange with NaN_3 in dimethylformamide led to the required (20*S*)-3β-acetoxy-21- (**87**) and (20*R*)-3β-acetoxy-22-azidopregn-5-en-20-ol (**88**), as key compounds (Scheme 1).

3.5. We found that the SCHMIDT reactions of the α,β- (**87**) and the α,γ-azidoalcohol (**88**) with appropriately substituted aromatic aldehydes (**89a–i**) activated by $BF_3{\cdot}OEt_2$ as LEWIS acid catalyst proceeded cleanly to the corresponding dihydrooxazolines (**103a–i**) and dihydrooxazines (**104a–i**) in good yields (Scheme 1).

* The numbering of the compounds accords with that in the Ph.D. Thesis



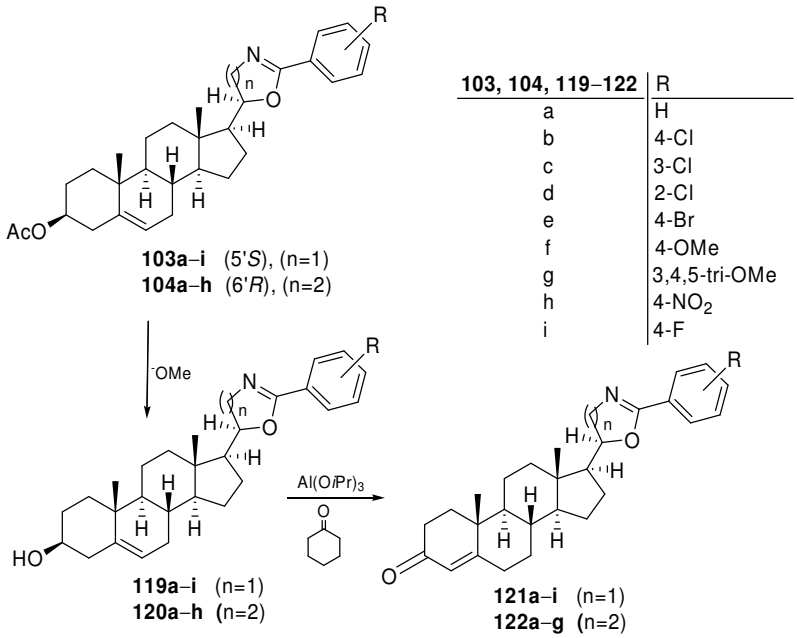
89, 103, 104	R
a	H
b	4-Cl
c	3-Cl
d	2-Cl
e	4-Br
f	4-OMe
g	3,4,5-tri-OMe
h	4-NO_2
i	4-F

Scheme 1

3.6. Deacetylation of the newly synthetized 3β-acetoxy compounds **103a–i** and **104a–h** in methanol in the presence of NaOMe by the ZEMPLÉN method

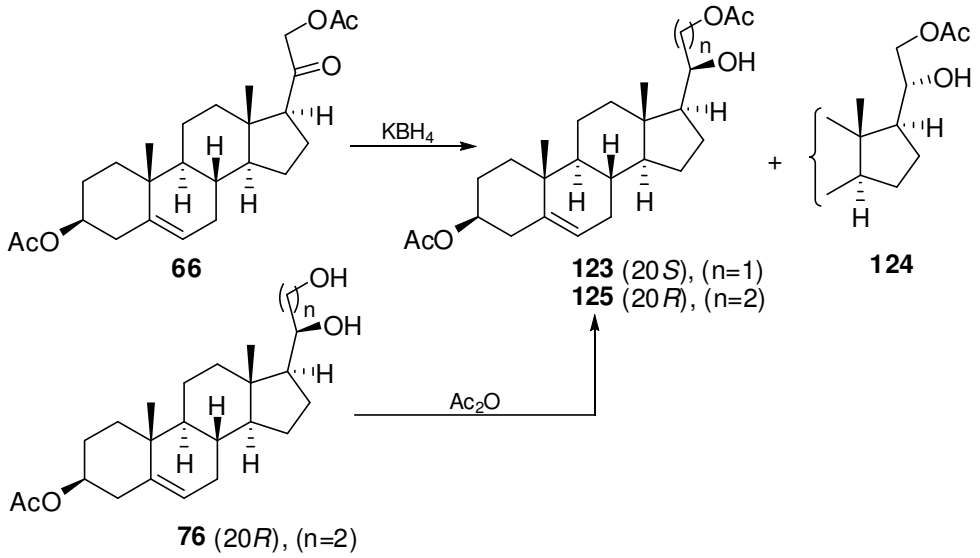
resulted in the 3β-hydroxy derivatives (**119a–i**, **120a–h**), without opening of the heterocyclic ring. OPPENAUER oxidation of the 3β-hydroxy-*exo*-heterocyclic steroids yielded the corresponding Δ^4-3-ketosteroids (**121a–i**, **122a–g**) in moderate yields (Scheme 2).

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).



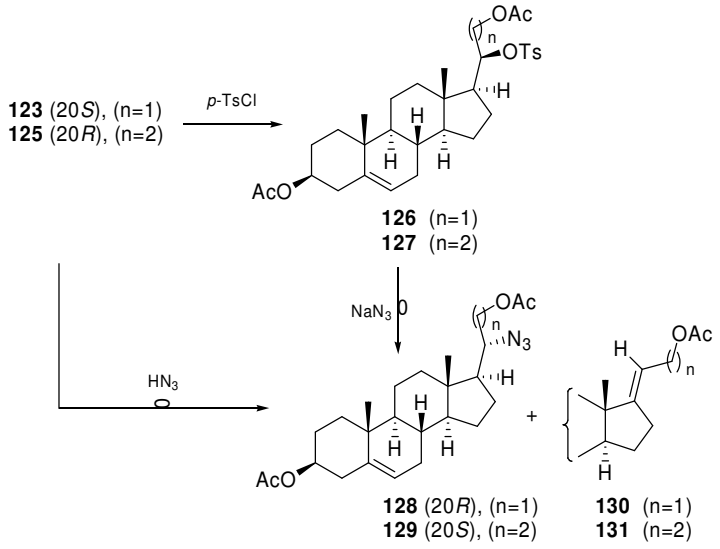
Scheme 2

3.8. We established that the hydroxy group at position 20 was formed by controlled reduction of 3β,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 3β-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 NaN_3 were converted to the corresponding 20-azido compounds (**128**, **129**). In the other method, **123** and **124** were transformed with HN_3, 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).

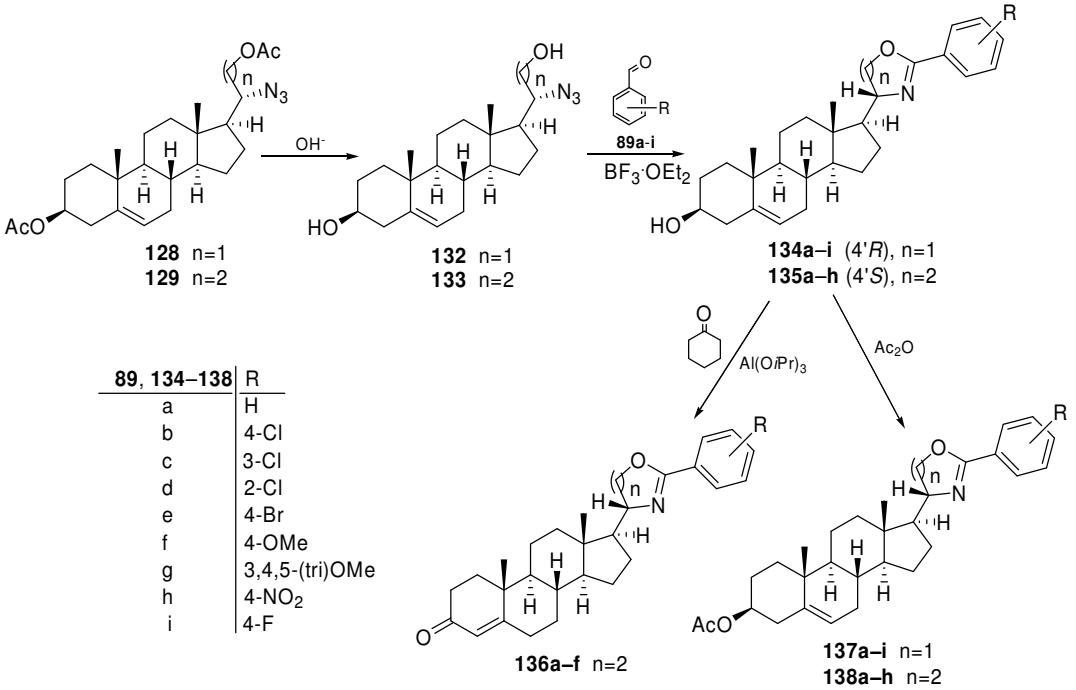


Scheme 4

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 α,β- and α,γ-azidoalcohols with differently substituted aromatic aldehydes (**89a–i**) furnished the corresponding dihydrooxazolinyl (**134a–i**) and dihydrooxazinyl (**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 3β-hydroxy-*exo*-heterocyclic steroids (**135a–h**) yielded the corresponding Δ^4-3-ketosteroids (**136a–f**). Acetylation of **134a–i** and **135a–h** gave the 3β-acetoxy compounds (**137a–i**, **138a–h**) (Scheme 5).

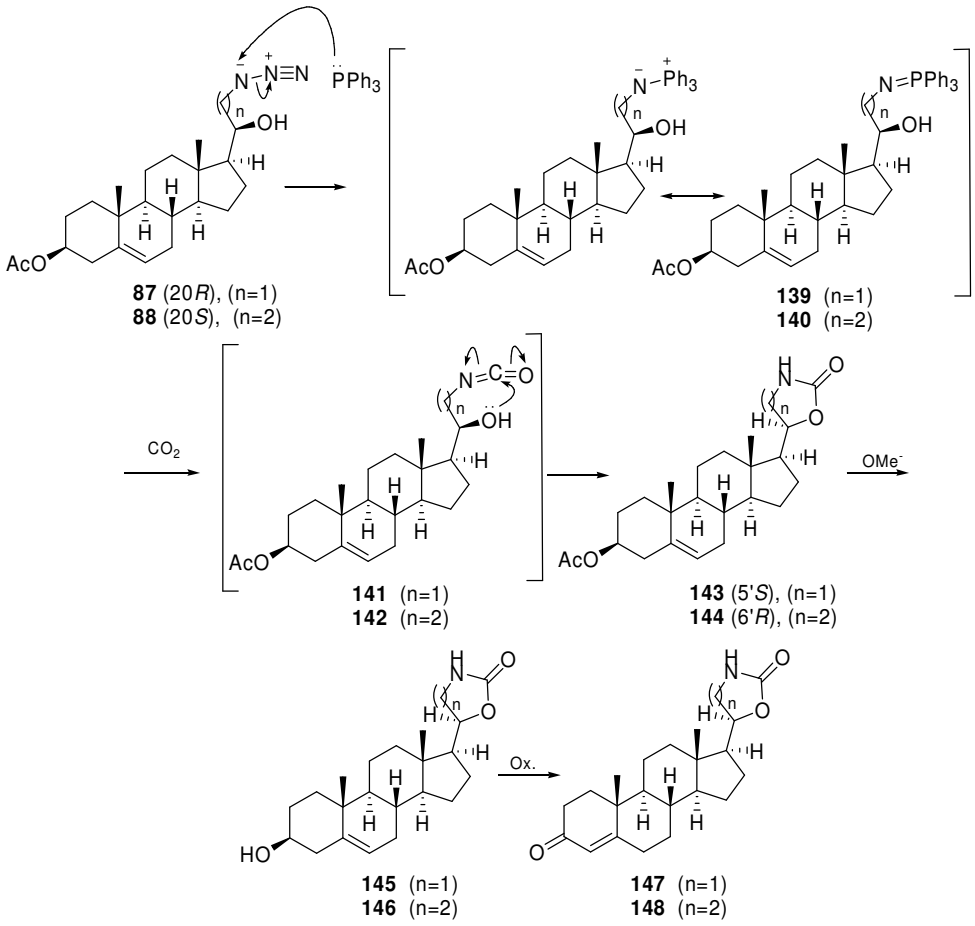


89, **134–138**	R
a	H
b	4-Cl
c	3-Cl
d	2-Cl
e	4-Br
f	4-OMe
g	3,4,5-(tri)OMe
h	4-NO_2
i	4-F

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 Ph_3P gave the 21- and 22-phosphimino compounds (**139**, **140**), which were converted into the corresponding isocyanates (**141**, **142**) with CO_2. Their deacetylation in methanol in the presence of NaOMe by the ZEMPLÉN method furnished **145** and **146**. OPPENAUER oxidation of the 3β-hydroxy-*exo*-heterocyclic steroids (**145**, **146**) yielded the corresponding Δ^4-3-ketosteroids (**147, 148**) (Scheme 6).



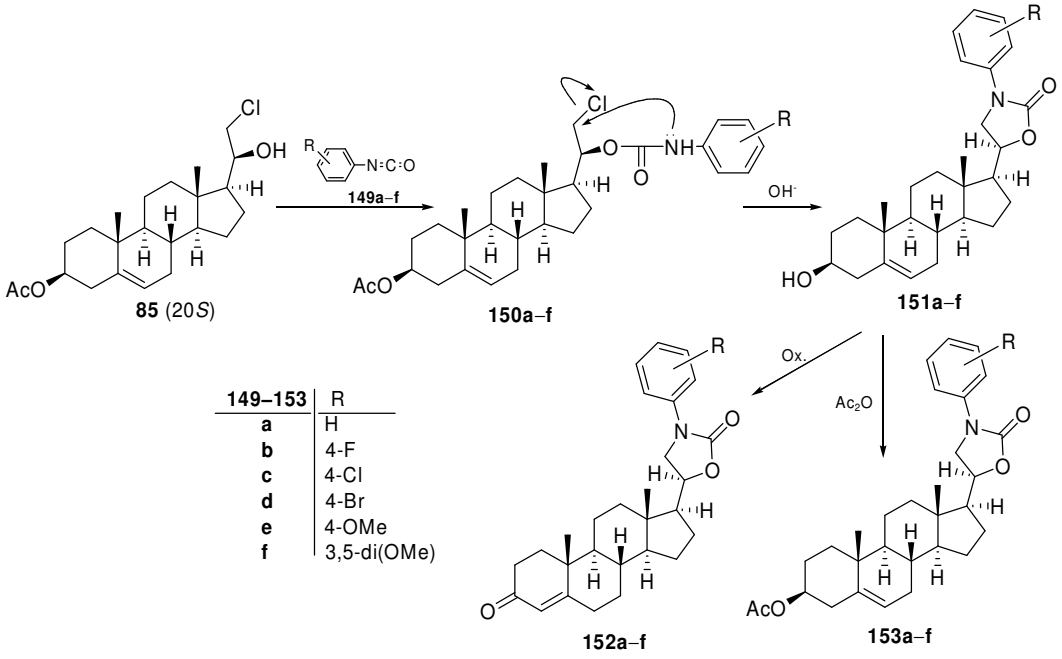
Scheme 6

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 3β-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 (1**51b–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 3β-hydroxy-*exo*-heterocyclic steroids (**152a–f**) yielded the corresponding Δ^4-3-ketosteroids (**152a–f**). Esterification of **152a–f** by acetylation furnished the 3β-acetoxy compounds (**153a–f**) (Scheme 7).



Scheme 7

3.17. The inhibitory effects (IC_{50}) of these *exo*-heterocyclic compounds on rat testicular $P450_{17\alpha}$ and 5α-reductase were investigated with an *in vitro* radioligand incubation technique. Both 3β-hydroxy- and Δ^4-3-ketosteroids were tested.

3.18. Most of our newly synthetized *exo*-heterocyclic steroids displayed moderate or weak inhibitory action in the tests. However, there were some (5′*S*)-dihydrooxazinyl derivatives whose IC_{50} values approximated that of the reference inhibitor (ketoconazole, **31**). The most effective inhibitor in the investigation of the inhibitory effects of $P450_{17\alpha}$ and 5αR2 was an unsubstituted oxazolidinone derivative (**147**).

4. Papers forming the basis of the dissertation

1. Stereoselective synthesis of some 17β-dihydrooxazinyl steroids, as novel presumed inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase
   János Wölfling, Éva Andrea Oravecz, **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 17α-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 17α-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 17α-hydroxylase-$C_{17,20}$-lyase
   Dóra Ondré, Gyula Schneider, Zoltán Iványi, István Tóth, Mihály Szécsi, János Julesz, János Wölfling
   1st Hungarian-Singaporean Workshop on Drug Discovery and Biomaterials
   Budapest, 10–11 March 2008.
   Proceedings, pp. 98-100.

5. Determination of rat 5α-reductase type 1 isozyme activity and its inhibition by novel *N*-aryl substituted 17β-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**

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 (2nd. 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. 17β-Dihidrooxazinil-szteroidok sztereoszelektív szintézise
   (Stereoselective synthesis of 17β-dihydrooxazolinyl steroids)
   Dóra Ondré, Gyula Schneider, János Wölfling, István Tóth, Mihály Szécsi, János Julesz
   Centenáriumi Vegyészkonferencia
   Sopron, 29 May – 1 June 2007.
   Program és előadásösszefoglalók, p. 360.

2. Stereoselective synthesis of some 17β-dihydrooxazinyl steroids, as novel presumed inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase
   Gyula Schneider, János Wölfling, **Dóra Ondré**, István Tóth, Mihály Szécsi, János Julesz
   5th Joint Meeting on Medicinal Chemistry
   Portorož, Slovenia, 17–21 June 2007.
   Farmacevtski Vestnik **2007**, *58*, 158.

3. $C_{17,20}$-liáz enzim gátlása új 17β-oxazolidinil-androszteron származékokkal
   (Inhibition of $C_{17,20}$-lyase enzyme with some novel 17β-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, 1st prize)
Hajdúszoboszló, 19–21 June 2008.
Program és előadásösszefoglalók, p. 97.

5. Synthesis of new steroidal 2-oxazolidones, as novel potential inhibitors of 17α-hydroxylase-$C_{17,20}$-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 $C_{17,20}$-lyase activity by new 17β-oxazoline derivatives in the androsterone series
Dóra Ondré, Mihály Szécsi, István Tóth, János Wölfling, Gyula Schneider, János Julesz
6th Hungarian-Austrian-Czech-German-Greek-Italian-Polish-Slovak-Slovenian Joint Meeting on Medicinal Chemistry
Budapest, 24–27 June 2009.
Book of Abstracts, p. 106.