Ph.D. Theses

Synthesis of heteroatom-containing ring D modified steroids

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1. Introduction and aims

Steroid derivatives are applied for clinical purposes worldwide. The halogen-containing estrogens are useful for the treatment of estrogen-dependent breast cancer. The halogen-containing D-homosteroid derivatives are of great importance in the diagnosis and chemotherapy of human cancers. Moreover, the heterocyclic steroids are potential inhibitors of 17β-hydroxysteroid dehydrogenase, which transforms the estrone into estradiol. An other important area of clinical research is the study and application of androgens.

We have achieved the synthesis of bromo-containing estradiol derivatives by means of substitution reactions. Furthermore, our work on the ring-closure reactions of D-*seco*-steroids in the 13β and 13α series resulted in D-homosteroids and D-heteroannulated compounds. Additionally, we have developed procedures for the synthesis of oxygen-, nitrogen- and sulphur-containing ring D-modified steroids in the 13α series.

Our modifications of the estrone and androstane skeletons were performed with two aims: to study the chemo-, regio- and stereoselectivies of the reactions in the 13α and 13β series, and to synthetize novel, pharmacologically active compounds.

2. Methods applied

Reactions were monitored by thin-layer chromatography. The substances produced were separated by flash chromatography. The structures of the compounds obtained were determined via the ^{1}H and ^{13}C NMR (*J*-MOD, NOE, NOESY and COSY) spectra, EI- and DCI-MS techniques, elemental analysis and single-crystal X-ray diffraction measurements.

3. Summary of the scientific achievements

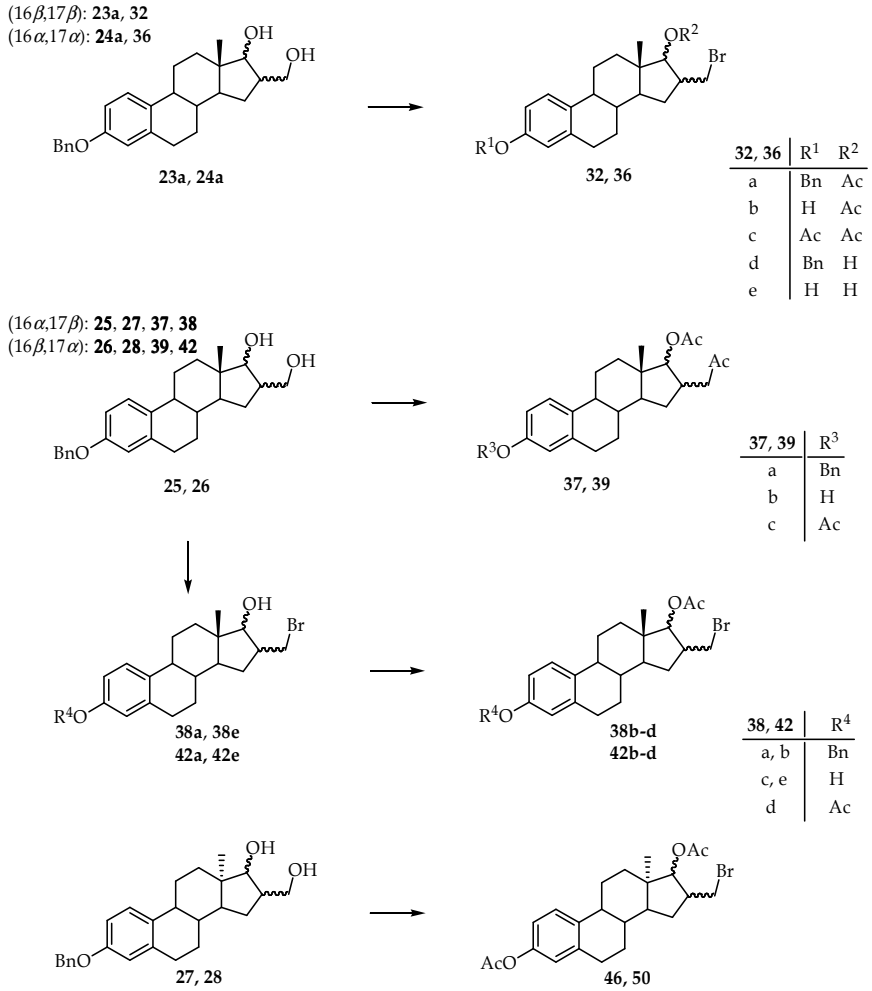
3.1. The reactions of the four isomers of 16-hydroxymethylestra-1,3,5(10)-trien-17-ol (**23a**, **24a**, **25**, **26**) with HBr and acetic acid in the 13β series proved to be stereospecific. The *cis* isomers (**23a**, **24a**) were transformed via a six-membered acetoxonium cation into the 17-acetoxy-16-bromomethyl derivatives (**32a**, **36a**). The mechanism of the process can be interpreted as involving front-side neighbouring group participation. In contrast with the *cis* isomers (**23a**, **24a**) under similar experimental conditions the *trans* isomers (**25**, **26**) were converted into 17-acetoxy-16-acetoxymethyl estradiol derivatives (**37a**, **39a**), (*Scheme 1*).

3.2. In the 13α series, treatment of the *trans* isomers of 16-hydroxymethyl-13α-estra-1,3,5(10)-trien-17-ol (**27**, **28**) with HBr and acetic acid furnished the 17-acetoxy-16-bromomethylestradiol derivatives (**46**, **50**), similarly to the *cis*-isomers (**23a**, **24a**), yielded 17-acetoxy-16-bromomethylestradiol derivatives (**32a**, **36a**), in the 13β series. The mechanism of the process can likewise be interpreted as involving front-side neighbouring group participation.

3.3. The cause of the lack of front-side neighbouring group participation in the reactions of the *trans* isomers in the 13β series is the steric hindrance of the methyl group on C-13. The steric position of this methyl group proved to have a significant effect on the reaction, as confirmed by study of the front-side neighbouring group participation in the 13α series.

3.4. The *trans* isomers of 17-acetoxy-3-benzyloxy-16-bromomethylestra-1,3,5(10)-triene (**38b**, **42b**) in the 13β series were synthetized from the corresponding 16-hydroxymethylestra-1,3,5(10)-trien-17-ol (**25**, **26**) via the APPEL reaction as a key step.

3.5. Modification of the conditions of the above reactions of the four isomers of 16-hydroxymethylestra-1,3,5(10)-trien-17-ol (**23a**, **24a**, **25**, **26**) allowed cleavage of the benzyl-protecting group. 17-Acetoxy-16-bromomethyl derivatives containing a hydroxy (**32b**, **36b**, **38c**, **42c**) or an acetoxy



32, 36	R^1	R^2
a	Bn	Ac
b	H	Ac
c	Ac	Ac
d	Bn	H
e	H	H

37, 39	R^3
a	Bn
b	H
c	Ac

38, 42	R^4
a, b	Bn
c, e	H
d	Ac

Scheme 1

group (**32c**, **36c**, **38d**, **42d**) on C-3, and of 17-acetoxy-16-acetoxymethyl derivatives containing a hydroxy group (**37b**, **39b**) or acetoxy group (**37c**, **39c**) on C-3 were also synthetized.

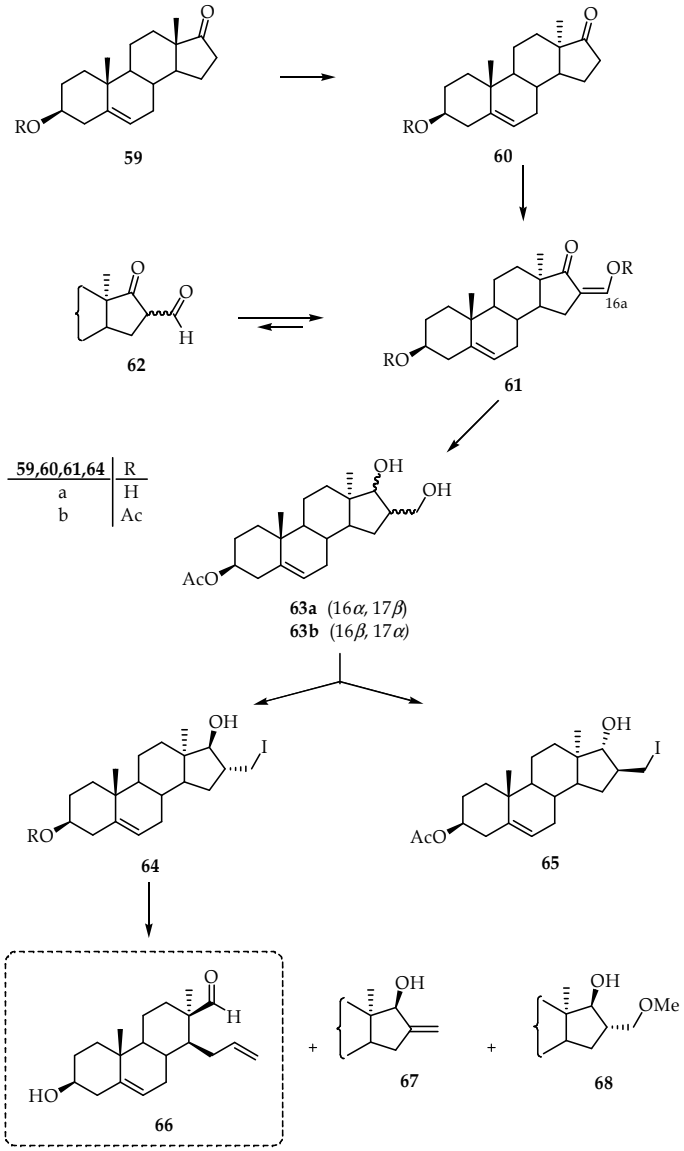
3.6. Debenzylation of the four isomers of 3-benzyloxy-16-bromomethylestra-1,3,5(10)-trien-17-ol (**32d**, **36d**, **38a**, **42a**) in the presence of Pd/C as catalyst resulted in the 16-bromomethyl estradiol derivatives (**32e**, **36e**, **38e**, **42e**).

3.7. CLAISEN condensation of 3β-acetoxy-13α-androst-5-en-17-one (**60b**) with ethyl formate furnished the 16-hydroxymethylidene derivative **61a**, which tautomerized into the 16-formyl compound **62**. The ^{1}H NMR spectrum demonstrated that **61a** was the main form present in $CDCl_3$ (*Scheme 2*).

3.8. The selective reduction of 3β-acetoxy-16-acetoxymethylidene-13α-androst-5-en-17-one (**61b**) with $NaBH_4$ afforded the two *trans* isomers of 16-hydroxymethyl-13α-androst-5-en-17-one (**63a**, **63b**). These products were separated after derivatization.

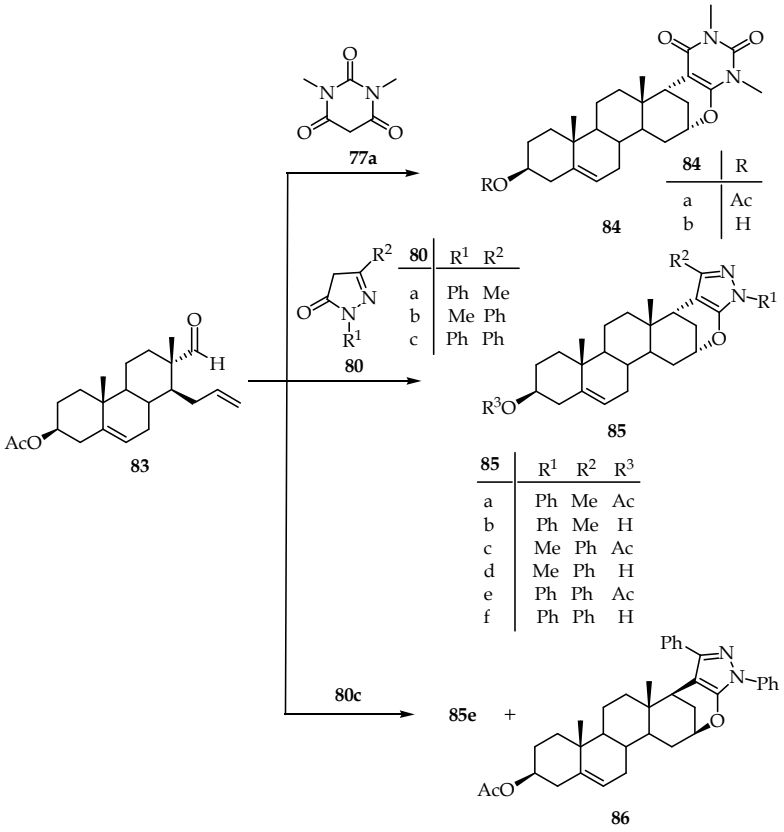
3.9. For GROB fragmentation, the 16-iodomethyl derivatives (**64b**, **65**) of the *trans* isomers of 16-hydroxymethyl-13α-androst-5-en-17-ol (**63a**, **63b**) were synthetized by means of the APPEL reaction.

3.10. Solvolysis of 3β-acetoxy-16α-iodomethyl-13α-androst-5-en-17β-ol (**64b**) under alkaline reaction conditions yielded the *cis* D-*seco*-steroid (**66**). As by-products, 16-methylidene (**67**) and 16-methoxymethyl (**68**) derivatives were formed.

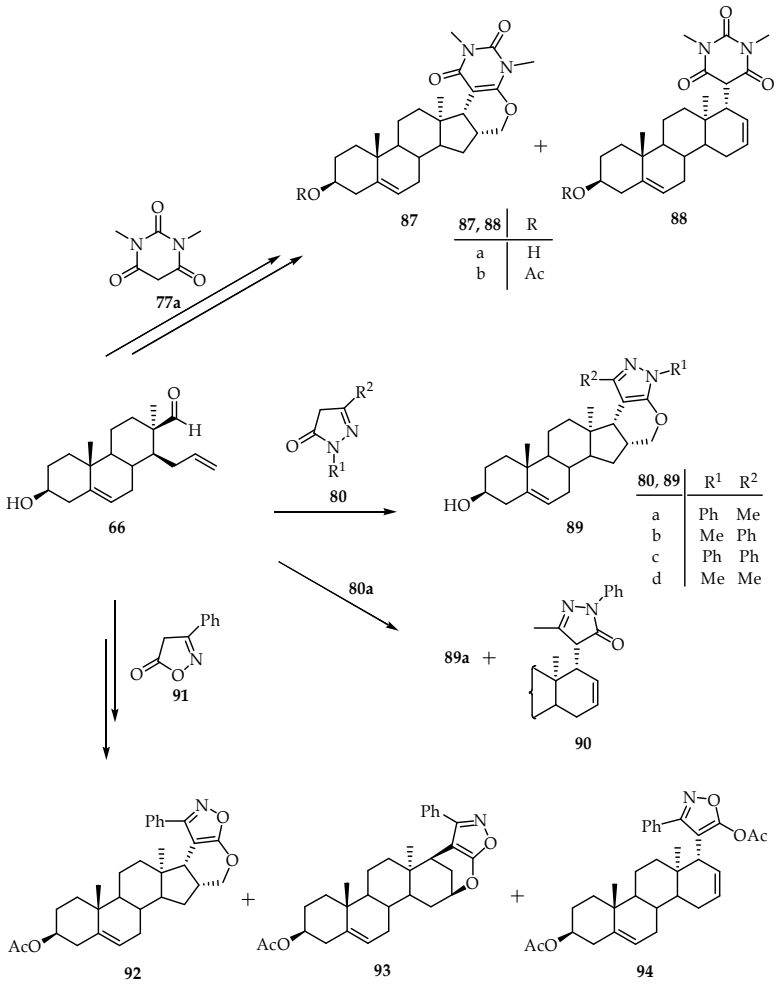


Scheme 2

3.11. The chemo-, regio- and stereoselective domino KNOEVENAGEL–intramolecular hetero DIELS-ALDER (DK-IMHDA) reaction of the *trans* 16,17-*seco*-aldehyde (**83**) in the 13β androstane series with *N*,*N*-dimethylbarbituric acid (**77a**) and disubstituted pyrazol-5-one (**80a-c**) in the presence of EDDA as catalyst furnished 16α,17aα-substituted D-homosteroid derivatives (**84**, **85**). With 1,3-diphenylpyrazol-5-one as reagent (**80c**), we obtained the 16β,17aβ isomer (**87**) as a by-product (*Scheme 3*).



Scheme 3



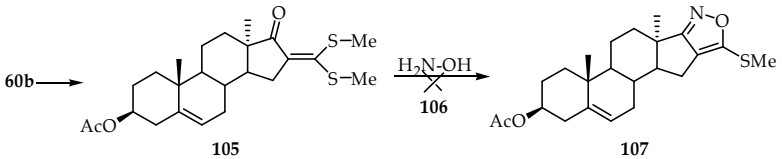
Scheme 4

3.12. The DK-IMHDA reaction in the 13α series was characterized by lower chemoselectivity than in the 13β series (*Scheme 4*). The stereoselective DK-IMHDA reaction of the *cis* 16,17-*seco*-aldehyde (**66**) with *N*,*N*-dimethylbarbituric acid (**77a**) and disubsituted pyrazol-5-one reagents

(**80a-d**) in the presence of EDDA as catalyst gave the 16α,17α-substituted D-heteroannulated derivatives (**87**, **89** and **92**). The use of 3-phenylisoxazol-5-one as reagent (**91**) yielded the 16β,17aβ-substituted bridged D-homosteroid (**93**) as a by-product. Under the reaction conditions of the DK-IMHDA reaction, unsaturated D-homosteroid derivatives (**88**, **90** and **94**) were also formed.

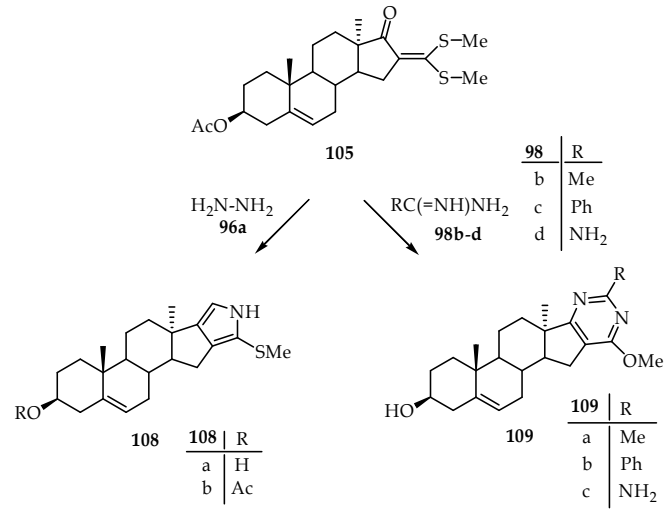
3.13. Differences in chemo- and regioselectivity of the DK-IMHDA reaction in the 13β and 13α series, were found to be caused by differences in structure flexibility of the sterane skeleton in the 13β and 13α series.

3.14. In contrast with our expectation from the experiment in the 13β estrone series, the α-oxoketene dithioacetal (**105**) did not react with hydroxylamine (**106**) in the 13α androstene series; the izoxazolo[5′,4′:16,17]-13α-androst-5-en-17-one derivative was not formed (*Scheme 5*). The non-occurrence of the condensation of the 17-keto function in the 13α series can be explained by steric hindrance of the methylthio group, caused by the modification in the sterane skeleton in the 13α series. This was confirmed by X-ray diffraction.



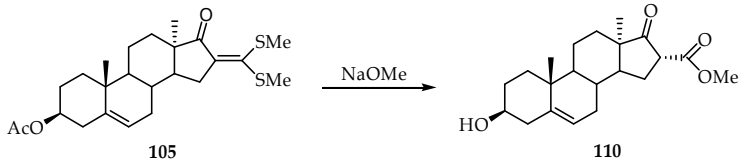
Scheme 5

3.15. The α-oxoketene dithioacetal derivative (**105**) of 3β-hydroxy-13α-androst-5-en-17-one (**60a**) is an excellent synthon for the synthesis of heterocycles. The condensations of **105** with hydrazine (**96a**), amidine (**98c**), benzidine (**98d**) and guanidine (**98e**) dinucleophile reagents gave steroid heterocycles containing a pyrazole (**108**) or pyrimidine (**109a-c**) ring E (*Scheme 6*).



Scheme 6

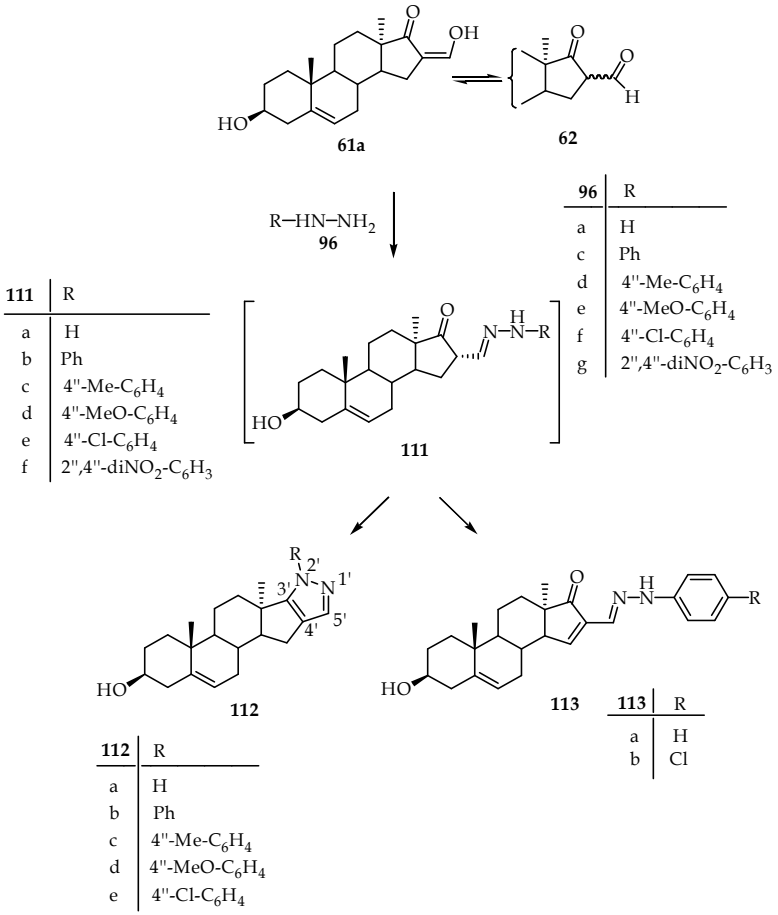
3.16. The α-oxoketene dithioacetal derivative (**105**) of 3β-hydroxy-13α-androst-5-en-17-one (**60a**) was transformed into the steroid derivative **111** in the presence of NaOMe in methanol as solvent (*Scheme 7*).



Scheme 7

3.17. The reactions of 16-hydroxymethylidene-13α-androst-5-en-17-one (**61a**) with substituted hydrazines (**96a-g**) proved to depend on the mono- or dinucleophile character of the reagent, which in turn depends on the electrophilic character of the substituent. With an aromatic ring bearing electron-donating substituents (CH_3 and OCH_3) or hydrogen, the hydrazine (**96d**, **96e, 96a**) behaved as a dinucleophile, resulting in D-heteroannulated derivatives (**112c**, **112d** and **112a**). Phenylhydrazine (**96c**) reacted as a mononucleophile and gave the 16a-phenylhydrazone

113a containing an unsaturated ring D. 2,4-Dinitrophenylhydrazine (**96g**) also reacted as a mononucleophile with **61a,** and furnished 16a-phenylhydrazone derivative **111f**. *p*-Chlorophenylhydrazine (**96f**) has an ambivalent electronic character: as a dinucleophile, it gave the D-heteroannulated derivative **112e**, and as a mononucleophile, it gave the 16a-(*p*-chlorophenyl)hydrazone derivative **113e** containing an unsaturated ring D (*Scheme 8*).



111	R
a	H
b	Ph
c	4''-Me-C_6H_4
d	4''-MeO-C_6H_4
e	4''-Cl-C_6H_4
f	2'',4''-diNO_2-C_6H_3

96	R
a	H
c	Ph
d	4''-Me-C_6H_4
e	4''-MeO-C_6H_4
f	4''-Cl-C_6H_4
g	2'',4''-diNO_2-C_6H_3

112	R
a	H
b	Ph
c	4''-Me-C_6H_4
d	4''-MeO-C_6H_4
e	4''-Cl-C_6H_4

113	R
a	H
b	Cl

Scheme 8

3.18. The condensation of 16-hydroxymethylidene-13α-androst-5-en-17-one (**61a**) with phenylhydrazine yielded the D-heteroannulated derivatives **112b** only in the presence of a LEWIS acid catalyst. In the case of 2,4-dinitrophenylhydrazine no reaction was observed in the presence of a LEWIS acid catalyst.

3.19. An X-ray diffraction study of 3β-acetoxy-13α-androst-5-en-17-one (**60b**), α-oxoketene dithioacetal (**105**) and derivative **89c** of *cis* 16,17-*seco*-aldehyde confirmed that rings A and C of the sterane skeleton have a chair, ring B has a distorted half-chair, and the ring D has a 14β-envelope conformation. This afforded an opportunity to study the structure of the androstene skeleton in the 13α series.

4. Scientific publications

1. **Ágota Szájli**, János Wölfling, Erzsébet Mernyák, Renáta Minorics, Árpád Márki, George Falkay, Gyula Schneider
Neighboring group participation. Part 16. Stereoselective synthesis and receptor-binding examination of the four stereoisomers of 16-bromomethyl-3,17-estradiols
Steroids, **2006**, *71,* 141-153.
Impact factor: **2,337**

2. János Wölfling, **Ágota Szájli**, László Vörös, Mónika Gáspár and Gyula Schneider
Synthesis of D-seco-13α-androst-5-ene derivatives
Monatshefte für Chemie, DOI: 10.1007/s00706-005-0500-y
Impact factor: **0,904**

3. **Ágota Szájli**, János Wölfling
The synthesis of D-heteroannulated 3β-hydroxy-13α-androst-5-ene derivatives via α-oxoketene dithioacetal and α-oxohydroxymethylidene synthons
Monatshefte für Chemie, accepted for publication
Impact factor: **0,904**

4. Gábor Bunkóczi, J. A. Cuesta Seijo, **Ágota Szájli**, Gyula Schneider, János Wölfling
3β-acetoxy-13a-androst-5-en-17-one
Acta Crystallographyca, Section E, ***2006****,* submitted for publication

Impakt faktor: **0,491**

Total impact factor for the publications (2004): **4.145**
+0.491

5. Scientific presentations and posters

Presentations:

1. **Szájli Ágota**, Wölfling János, Schneider Gyula
A védőcsoport egyidejű változtatásával járó cserélődési reakciók az ösztron sorban
MKE XXV. Kémiai Előadói Napok
October 2002, Szeged
Book of abstracts p 170-172.

2. **Szájli Ágota**, Wölfling János, Schneider Gyula
A védőcsoport egyidejű változtatásával járó szubsztitúciós reakciók a szteroidok sorában
XXVII. Országos Tudományos Diákköri Konferencia
April 2003, Budapest
Book of abstracts p 84.

3. **Szájli Ágota**, Wölfling János, Schneider Gyula
Szomszédcsoport részvétel vizsgálata az ösztron sorban
Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 3. tudományos előadóülése
January 2003, Szeged

4. **Szájli Ágota**, Wölfling János, Schneider Gyula
16-Brómmetilösztra-3,17-diolok kemo- és sztereoszelektív szintézise
MTA Szteroidkémiai Munkabizottsági előadóülés
June 2004, Szeged

5. **Szájli Ágota**, Wölfling János, Schneider Gyula
Heteroatomot tartalmazó 13-epi-DHEA származékok szintézise
A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 4. tudományos előadóülése
January 2005, Szeged (prize 1st)

6. **Szájli Ágota**, Wölfling János, Schneider Gyula
Androsztánvázas vegyületek előállítása dominó Knoevenagel-intramolekuláris hetero Diels-Alder reakcióval
MTA Szteroidkémiai Munkabizottsági előadóülés
June 2006, Szeged

Posters:

1. **Szájli Ágota**, Wölfling János, Schneider Gyula
   Benzil-védőcsoport csere a szteroidok sorában
   MKE Vegyészkonferencia
   June 2003, Hajdúszoboszló
   Book of abstracts p 149.

2. **Ágota Szájli**, Emília Szájli, János Wölfling, Gyula Schneider
   Synthesis of estrone derivatives with halogen content
   The Vth International symposium „Young people and multidisciplinary research",
   November 2003, Temesvár (Rumania)
   Book of abstracts 42-43.

3. **Ágota Szájli**, János Wölfling, Gyula Schneider and Lutz F. Tietze
   Syntheis of 13α-dehidroepiandrosterone derivatives by domino Knoevenagel hetero Diels-Alder reaction
   Joint Meeting on Medicinal Chemistry
   June 2005, Bécs (Austria)
   Book of abstracts p 101.

4. Renáta Minorics, Árpád Márki, Pál Tapolcsányi, Erzsébet Mernyák, **Ágota Szájli**, János Wölfling, Gyula Schneider and George Falkay
   Pharmacological evaluation of originally synthesized estrone and estradiol stereoisomers
   Joint Meeting on Medicinal Chemistry
   June 2005, Bécs (Austria)
   Book of abstracts p 99.

5. **Szájli Ágota**, Wölfling János, Schneider Gyula
   Heterociklusos 13α-dehidro-epiandroszteron származékok szintézise
   MKE Vegyészkonferencia
   June 2005, Hajdúszoboszló
   Book of abstracts p 138.