Summary of PhD Thesis

Syntheses of Nitrogen-Containing Ring D-Modified Steroids

Angéla Magyar

University of Szeged
Department of Organic Chemistry

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1 Introduction and Aims

Ever since the discovery of the pharmacological activity and diversity of nitrogen-containing steroids, there has been great scientific interest in this class of compounds. Our work has focused on the chemical synthesis of artifical seco- and homosteroids and their subsequent transformation into nitrogen-containing derivatives.

In the group directed by *Gyula Schneider* at the Department of Organic Chemistry, University of Szeged, Hungary, we have investigated the possibility of extension of the applicability of the fragmentation steps found previously by this group. Out of the numerous possible reactions, we were primarily interested in the condensation and ring-closing behavior of the fragmentation product derived from pregnane. We have succeeded in synthesizing novel, pharmacologically promising compounds.

Together with the group directed by *Bruno Schönecker* at the Institute of Organic and Macromolecular Chemistry, Friedrich-Schiller-University Jena, Germany, we aimed at the synthesis of new, variously substituted homo- and secosteroidal amines and imines. Depending on the number and position of the introduced nitrogen atoms, these compounds can act as bi- or tridentate ligands for metal coordination. In order to mimic the function of the enzyme dopamine-β-hydroxylase, we have synthesized copper complexes bearing such steroidal ligands. These complexes are capable of activating molecular oxygen and we have investigated the possible mechanisms of hydroxylation.

2 Methods Applied

The courses of reactions were controlled by thin-layer chromatography (TLC). Synthesized substances were separated by column chromatography. Structures were determined by ^{1}H- and ^{13}C-NMR (J-MOD, DEPT, COSY, TOCSY or NOESY) spectroscopy, by mass spectroscopy (EI, DEI, DCI or ESI), by elemental analysis and by X-ray diffraction measurements.

3 Summary of Scientific Achievements

1. We have demonstrated, that 3β,16α-dihydroxy-5-pregnen-20-one and its derivatives **6a**–**d** are readily obtainable by chemo- and stereoselective epoxidation of pregnadienolone acetate **4b** and subsequent reductive opening of the epoxide ring (Figure 1).

2. By the alkaline methanolysis of 3β,16α-diacetoxy-5-pregnen-20-one (**6c**), pregnenolone (**4a**) and 3β-hydroxy-16α-methoxy-5-pregnen-20-one (**6d**) were obtained in a ratio of 2:5. We explain this in terms of *Michael* addition of the solvent methanol to the primarily formed α,β-unsaturated 20-ketone **4a**, which affords the methylether **6d**.

3. We found that the sodium borohydride reduction of the 3β,16α-diacetoxy derivative **6c** furnished the (20*R*)- and (20*S*)-3β,16α-diacetoxy-20-hydroxy-5-pregnene (**7a** and **8a**) in a ratio of 19:1 in high yield. The considerable amount of the 20*R*-product **7a** formed is in conformity with the behavior of 20-oxopregnanes usually shown under these conditions.

4. Alkaline *Grob* fragmentation of (20*R*)-3β,16α-diacetoxy-20-*p*-tolylsulfonyloxy-5-pregnene (**7b**) occurred immediately and afforded *trans*-3β-hydroxy-16,17-secopregna-5,17(20)-dien-16-al (**9a**). The reaction was complete without the formation of any by-products. Since **9a** was the only product and because of its structural features, the fragmentation must follow a synchronous *trans*-elimination mechanism.

5. We synthesized the novel compounds **14a**–**21a** by condensation of secosteroid **9b** with variously substituted anilines **10**–**13** and subsequent *in situ Lewis* acid-catalyzed ring closure (Figure 2).

6. We obtained different product mixtures, depending on the nature of the substituents on the aniline applied. Generally, the reaction proceeds in two different directions. Besides two different *Diels-Alder* products **14a**–**17a** and **18a**–**20a** an aza-*Prins* product **21a** can be formed.

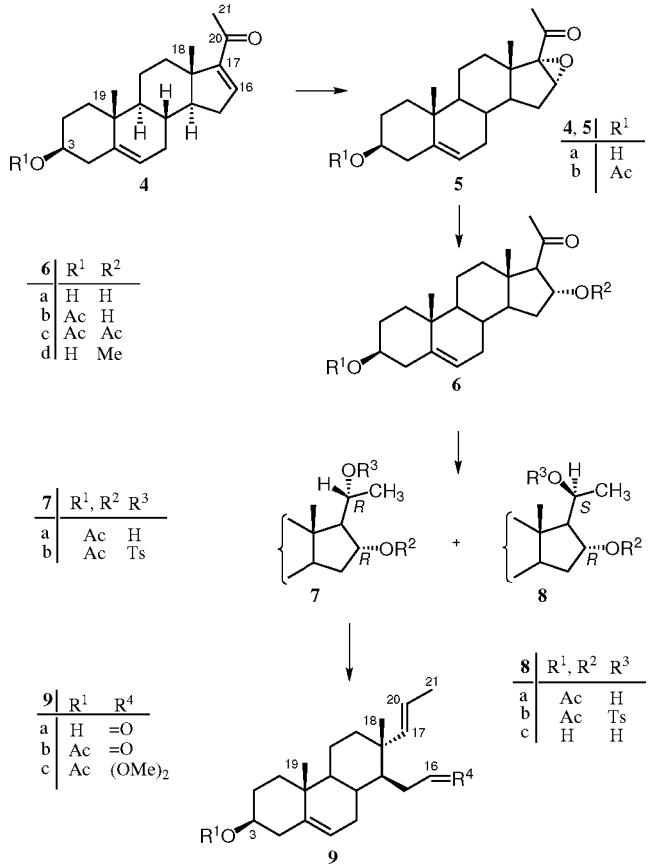
4
5
6
7
8
9
4, 5 | R1
a | H
b | Ac
6 | R1 R2
a | H H
b | Ac H
c | Ac Ac
d | H Me
7 | R1, R2 R3
a | Ac H
b | Ac Ts
8 | R1, R2 R3
a | Ac H
b | Ac Ts
c | H H
9 | R1 R4
a | H =O
b | Ac =O
c | Ac (OMe)2

Figure 1

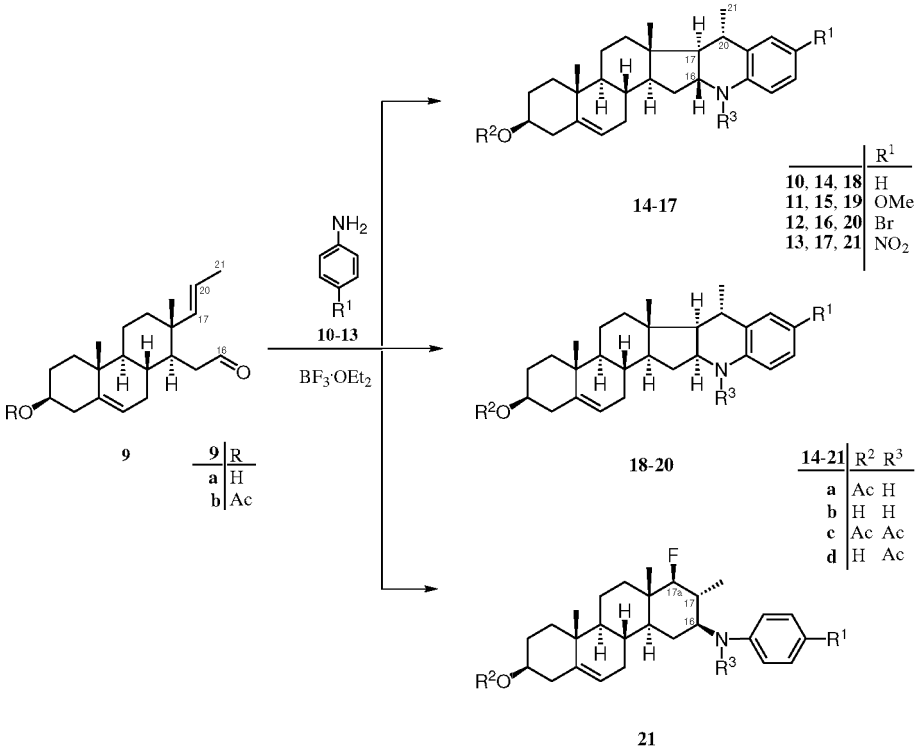
NH2
R1
10-13
BF3·OEt2
RO
9
9 | R
a | H
b | Ac
R2O
R1
R3
14-17
10, 14, 18 | H
11, 15, 19 | OMe
12, 16, 20 | Br
13, 17, 21 | NO2
18-20
14-21 | R2 R3
a | Ac H
b | H H
c | Ac Ac
d | H Ac
F
21

Figure 2

7. The imines **22** obtained by condensation reaction of secoaldehyde **9b** and anilines **10**–**13** are capable of reacting with the 17,20-alkene moiety by *Lewis* acid catalysis. We established that this reaction can be explained by an intramolecular ionic mechanism.

8. The intermediates **24** and **25** stemming from intramolecular attack of the alkene moiety on iminium ion **23** are in equilibrium (Figure 3).

9. In reactions with aniline derivatives bearing activating substituents, e.g. **11** and **12** or no substituents at all, e.g. **10**, the affinity of the aromatic ring toward electrophilic substitution directs the reaction toward *Friedel-Crafts* alkylation, affording the tetrahydroquinolino-steroidal hybrid molecules **14a**–**20a**.

10. For *p*-nitrophenylimino derivative **22** ($R^1 = NO_2$), the electrophilic substitution affinity of the aromatic ring is reduced. Ion association of a fluoride ion with carbocation **25** yielded aza-*Prins* product **21a**.

11. Acetylation at the nitrogen atom in the tetrahydroquinolino- or *N*-arylamino compounds was strongly influenced by the substituent on the aromatic ring. For steroids with the *p*-nitro substituent, e.g. **17a** or **21a**, more vigorous conditions were required.

12. Structure determination of the novel condensed ring system in **14**–**20** and 16-*N*-arylamino-D-homosteroids **21** was accomplished by means of NMR spectroscopy and X-ray crystallography.

13. During our work on biomimetic hydroxylation, new compounds (i.e. **40**, **49**, **63**, **64**, **67**, **69** and **70**) were prepared for Cu-complexing experiments (Figures 4–7).

14. We observed different results of the *Beckmann* rearrangement of estrone 3-methyl ether 17-oximes **35** and **43** in the *normal* and *epi* series. We synthesized the valuable 3-methoxy-17a-aza-D-homoestra-1,3,5(10)-trien-17-one (**36**) in a yield of 90 %,

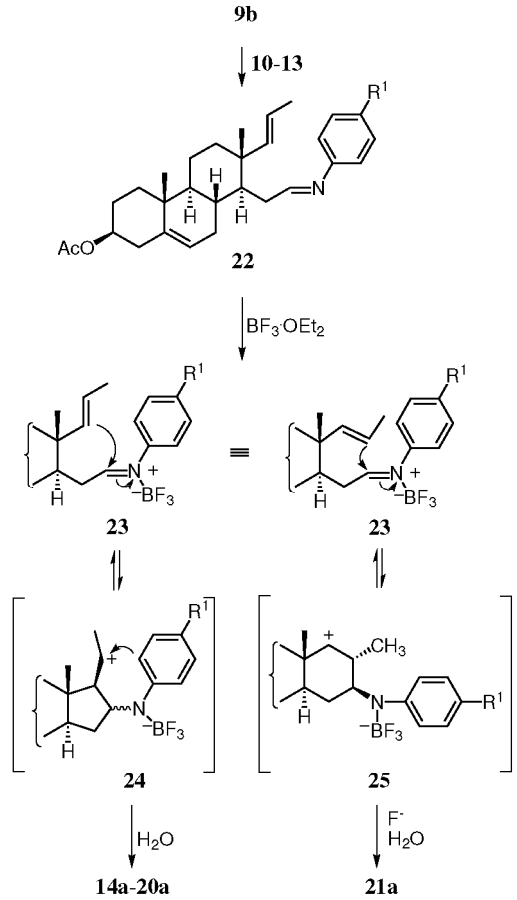
9b
10-13
R1
H
H
H
N
AcO
22
BF3·OEt2
R1
N +
−BF3
H
23
≡
R1
N +
−BF3
H
23
R1
+
N
−BF3
H
24
+
CH3
N
−BF3
H
R1
25
H2O
14a-20a
F-
H2O
21a

Figure 3

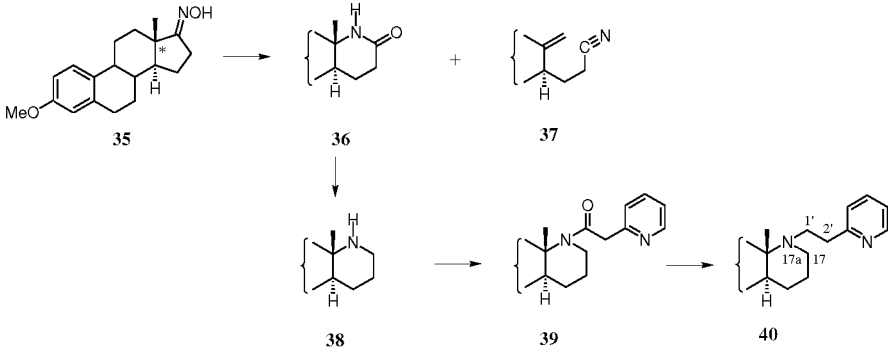

Figure 4

together with the 3-methoxy-13,17-secoestra-1,3,5(10),13(18)-tetraen-16-nitrile (**37**) in the *normal* series (Figure 4). In the *epi* series, we obtained the *epi*-lactam **44** in a much lower yield (55 %), together with all three 13,17-secosteroidal fragmentation by-products: **37**, **45** and **46** (Figure 5).

15. Synthesis of *N*-substituted steroids **40** and **49** was accomplished in good yield by a two step method starting from *normal*- or *epi*-3-methoxy-17a-aza-D-homoestra-1,3,5(10)-triene (**38** and **47**). These secondary amines were *N*-acylated and reduced with BH_3·THF.

16. *Jones* oxidation of the secosteroidal aldehyde nitrile **52** afforded not only carbonitrile **53**, but also lactone **54** with a novel bridged structure. We assume that an inorganic carboxylic anhydride, formed from chromic acid and carbonitrile **53**, is able to oxidize the benzylic carbon atom at position 9α (Figure 6).

17. 3-Methoxy-16,17-secoestra-1,3,5(10)-triene 16-nitrile 17-carboxylic acid (**53**) was also synthesized by fragmentation of 16,17-dioxo 16-oxime **50** by using *p*-toluenesulfonyl chloride/pyridine and also titanium tetrachloride.

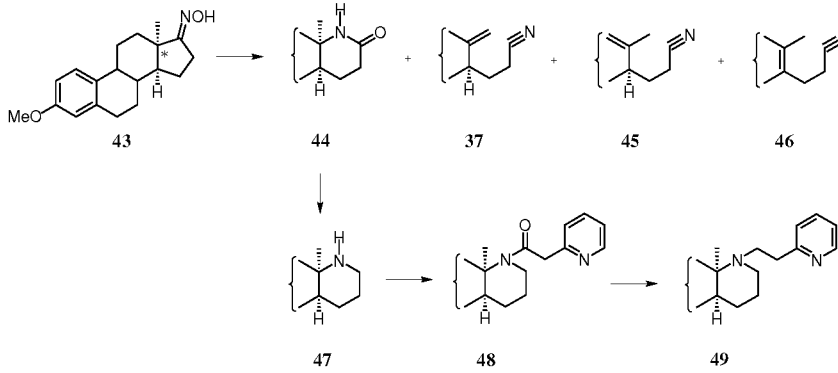

Figure 5

18. We observed cleavage of the 16-oxime and reduction of the 17-keto function upon treatment of 16,17-dioxo 16-oxime **50** with titanium trichloride/hydrochloric acid. This provides a simple method for the stereo- and regioselective preparation of 3-methoxy-17β-hydroxyestra-1,3,5(10)-trien-16-one (**56**).

19. *In situ Curtius* rearrangement of carboxylic acid **53** afforded 13α-amino-3-methoxy-16,17-seco-17-nor-estra-1,3,5(10)-triene 16-nitrile (**61**) in good yield. The most noteworthy feature of this compound is the 13α-amine function, which is directly attached to a chiral tertiary carbon atom.

20. The condensations of the appropriate aldehyde and amino compounds and subsequent sodium borohydride reduction gave secosteroids with a pyridyl side-chain: **64**, **67** and **70** (Figure 7). The intermediates of the *N*-(pyridylmethyl) compounds **64** and **70**, steroidimines **63** and **69**, could be isolated, whereas this was impossible for *N*-pyridylimine **66**. In contrast with **63** and **69**, in the latter case condensation was carried out by *Lewis* acid catalysis. The amidine character of 2-aminopyridine renders this reaction even more difficult.

21. Experiments with 17a-aza-*N*-alkylpyridyl-D-homosteroids **40**

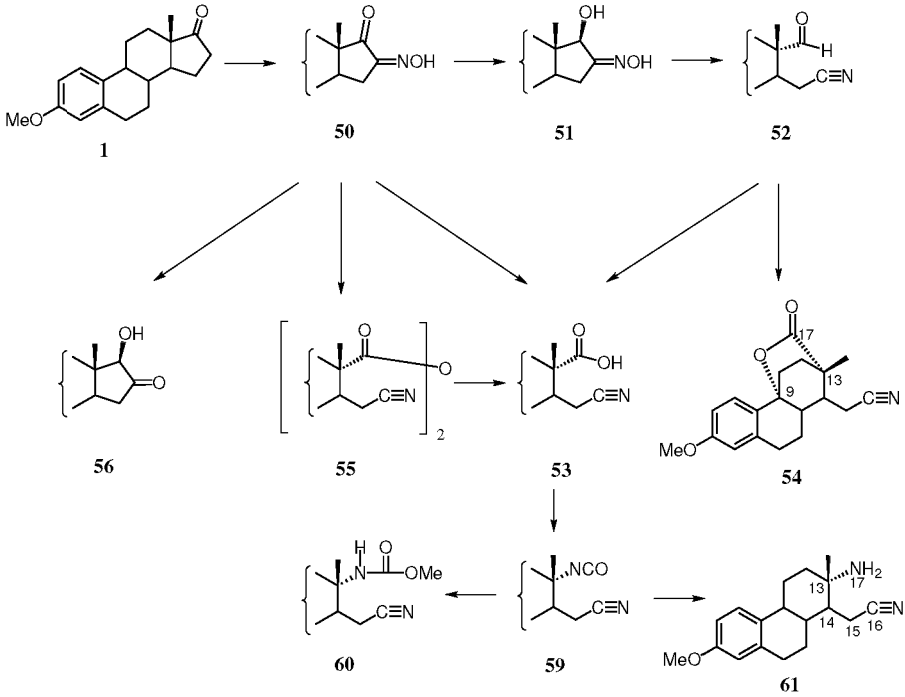

Figure 6

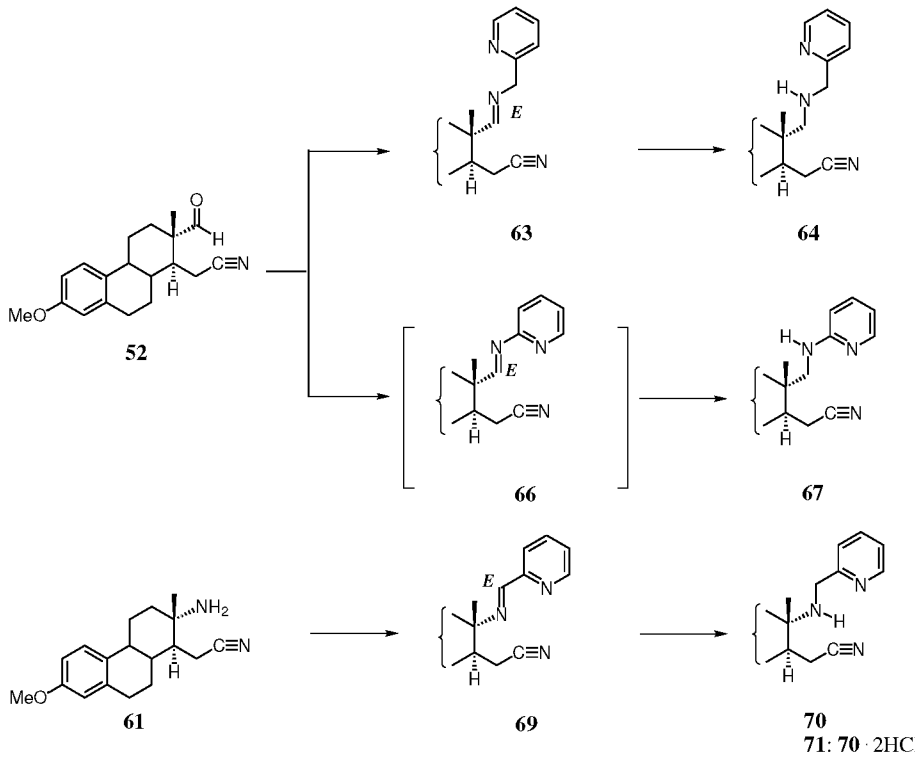

Figure 7

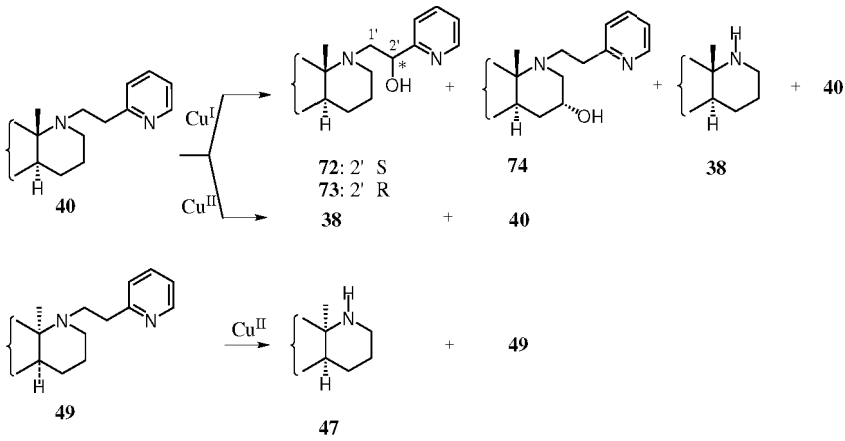

Figure 8

and **49** demonstrated that the structure and configuration of the ligand have a great influence on the reaction behavior (Figure 8).

22. Hydroxylation is also affected by the method of preparation of the activated Cu complexes. We obtained different reaction mixtures depending on whether we started from Cu(I) or Cu(II) salts.

23. Besides α- and β-hydroxylation of the activated methylene group, we achieved a novel β-hydroxylation of a non-activated methylene group of 3-methoxy-*N*-[2-(2-pyridyl)ethyl]-17a-aza-D-homoestra-1,3,5(10)-triene (**40**).

24. Hydroxylation at the α-position of 3-methoxy-*N*-[2-(2-pyridyl)ethyl]-17a-aza-13α-D-homoestra-1,3,5(10)-triene (**49**) was performed using the method starting from a Cu(I) complex.

25. We observed that the conversion is higher for the methods involving the use of Cu(II) than for those involving Cu(I). However, in the former case only secondary amines **38** and

47, resulting from α-hydroxylation, were separable from the starting ligands **40** and **49**.

26. Examination of the hydroxylation behavior of the imino-secosteroids **63** and **69** did not reveal the expected reaction products. This is due to an unfavorable influence of the nitrile group of the 16,17-secosteroid on the active complex, hindering activation and the transfer of molecular oxygen.

4 References Related to the Thesis

1. Angéla Magyar, Bruno Schönecker, János Wölfling, Gyula Schneider, Wolfgang Günther, Helmar Görls

   Synthesis of N-[2-(2-pyridyl)ethyl]-17a-aza-D-homosteroids and biomimetic copper-mediated ligand hydroxylation with molecular oxygen

   Tetrahedron: Asymmetry **2003**, *14*, 1925–34

   Impact factor: 2.163

2. Angéla Magyar, Bruno Schönecker, János Wölfling, Gyula Schneider, Wolfgang Günther, Helmar Görls.

   Synthesis of 16,17-secosteroids with iminomethyl-2-pyridine and aminomethylene-2-pyridine structures as chiral ligands for copper ions and molecular oxygen activation

   Tetrahedron: Asymmetry **2003**, *14*, 2705–15

   Impact Factor: 2.163

3. János Wölfling, Angéla Magyar, Gyula Schneider

   Synthesis of novel D-secopregnenes

   Monatshefte für Chemie **2003**, *134*, 1387–93

   Impact Factor: 0.813

4. Angéla Magyar, János Wölfling, Melanie Kubas, Jose Antonio Cuesta Seijo, Madhumati Sevvana, Regine Herbst-Irmer, Péter Forgó, Gyula Schneider

   Synthesis of novel steroid-tetrahydroquinolines hybrid molecules and D-homosteroids by intramolecular cyclization reactions

   Steroids **2003**, accepted for publication

   Impact factor: 2.524

Total impact factor (2002): 7.663

5 Scientific Presentations and Posters Related to the Thesis

1. Magyar Angéla, Wölfling János, Schneider Gyula, Bruno Schönecker

   Ösztránvázas komplexképző ligandumok szintézise

   MKE Vegyészkonferencia (June 27–29, 2001, Hajdúszoboszló, Hungary)

   Book of Abstracts, p. 85

2. Magyar Angéla, Wölfling János, Schneider Gyula

   Egy új D-szekoszteroid szintézis

   MKE Vegyészkonferencia (June 27–29, 2001, Hajdúszoboszló, Hungary)

   Book of Abstracts, p. 86

3. Angéla Magyar, János Wölfling, Gyula Schneider

   Synthesis of a new D-secosteroid Derivative

   Hungarian-German-Italian-Polish Joint Meeting on Medicinal Chemistry (September 2–6, 2001, Budapest, Hungary)

   Book of Abstracts, p. 117

4. Magyar Angéla, Wölfling János, Schneider Gyula

   Gyűrűfelnyitás és gyűrűzárás a pregnánvázas vegyületek sorában

   A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alaítvány és a SZAB Szerves és Gyógyszerkémiai Munkabizottság tudományos előadóülése (January 17, 2002, Szeged, Hungary)

5. Magyar Angéla, Wölfling János, Schneider Gyula

   Szekosteroidok sztereoszelektív szintézise és további átalakításuk

   A SZAB Kémiai Szakbizottságának előadóülése a Magyar Tudomány Napja alkalmából (November 5–11, 2001, Szeged, Hungary).

6. Magyar Angéla, Wölfling János, Schneider Gyula

   Fragmentálódás a pregnánvázas vegyületek sorában

   MKE XXIV. Kémiai Előadói Napok (October 29–31, 2001, Szeged, Hungary)

   Book of Abstracts, p. 84–85

7. Magyar Angéla, Matthias Rost, Yong Liu, Bruno Schönecker

   Übergangsmetallinduzierte diastereoselektive und enantioselektive Hydroxylierungen mit chiralen stickstoffhaltigen Steroidliganden

   Klausurtagung des Sonderforschungsbereiches 436 (April 25–26, Thalbürgel, Germany)

8. Angéla Magyar, Tatjana Zheldakova, Yong Liu, Corinna Lange, Manuela Kötteritzsch, Matthias Rost, Wolfgang Günther, Bruno Schönecker

   Synthesis of N-containing steroids as chiral ligands for copper complexation and subsequent O_2 activation

   2nd International SFB Congress "Metal Mediated Reactions Modelled after Nature" (September 15–19, 2002, Jena, Germany)

   Homepage of 2nd International SFB Congress (http://www2.uni-jena.de/chemie/sfb/congress2002Poster.htm) PB2

9. Angéla Magyar, Tatjana Zheldakova, Bruno Schönecker

   Stickstoffhaltige Steroidliganden für Kupfer-Komplexierung und O_2-Aktivierung

   Symposium des Sonderforschungsbereiches 436 (September 26–28, 2002, Großkochberg, Germany)

10. Magyar Angéla, Wölfling János, Forgó Peter, Schneider Gyula

    Új nitrogéntartalmú szteroid heterociklusok szintézise

    MKE Vegyészkonferencia (June 24–26, 2003, Hajdúszoboszló, Hungary)

    Book of Abstracts, p. 116

6 Further Scientific Publications and Posters

1. Angéla Magyar, Zsuzsa Szendi, Péter Forgó, Marianna Mák, Helmar Görls, Frederick Sweet

   Stereoselective epoxidation and bromination of 3,4-dihydro-2H-pyranyl/pregnenolone conjugates

   Steroids **2003**, in press

   Impact factor: 2.524

2. Angéla Magyar, Zsuzsa Szendi, János T. Kiss, István Pálinkó

   Intra- and intermolecular hydrogen bondings in steroids — a combined experimental study

   Journal of Molecular Structure (THEOCHEM) **2003**, in press

   Impact factor: 1.014

3. Magyar Angéla

   C/D-gyűrűs prekurzor szintézise a D_3-vitamin és analógjainak előállításához

   OTDK (April 7–9, 1999, Veszprém, Hungary)

   Book of Abstracts, p. 101

4. Magyar Angéla, Szendi Zsuzsanna, Frederick Sweet

   Koleszterinanalógok előállítása sztigmaszterinből

   MKE Vegyészkonferencia (June 22–24, 1999, Eger, Hungary)

   Book of Abstracts, p. 138

5. Magyar Angéla, Szendi Zsuzsanna, Kövér Katalin, Vincze Irén

   Oxidációk a sztigmaszterin A,B-gyűrűjén

   MKE Vegyészkonferencia (June 22–24, 1999, Eger, Hungary)

   Book of Abstracts, p. 136

6. Zsuzsanna Szendi, Péter Forgó, Angéla Magyar

   Structure elucidation of side-chain modified oxysterol derivatives

   Hungarian-German-Italian-Polish Joint Meeting on Medicinal Chemistry (September 2–6, 2001, Budapest, Hungary)

   Book of Abstracts, p. 172

7. Angéla Magyar, Zsuzsa Szendi, János T. Kiss, István Pálinkó

   Intra- and intermolecular hydrogen bond forming properties of steroids (pregnene derivatives) in solution and in the solid state

   XXVI European Congress on Molecular Spectroscopy (September 1–6, 2002, Villeneuve d'Ascq, France)

   Book of Abstracts, p. 124

8. Magyar Angéla, Szendi Zsuzsanna, Forgó Péter

   Az A,B-gyűrűben telítetlen ketoszteroidok olefinkötésének oxidációja

   MKE Vegyészkonferencia (June 24–26, 2003, Hajdúszoboszló, Hungary)

   Book of Abstracts, p. 115