SYNTHESIS OF FERROCENE-LABELLED STEROID DERIVATIVES VIA HOMOGENEOUS CATALYTIC REACTIONS

THESES OF THE PhD DISSERTATION

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I. PRELIMINARIES AND AIMS OF THE WORK

The coupling of ferrocene with biomolecules could be interesting for many reasons. Due to the reversible one electron oxidation of ferrocene, its derivatives are applicable as biosensors, molecular- and ion receptors. There are many literature examples for ferrocene derivatives with biological activity. At the same time, many steroids with heterocyclic ring show antiproliferative and enzyme inhibitory effect. Some steroid-ferrocene derivatives have favorable biological activity and are applicable in analytical chemistry and materials science.

The basic goal of my work was the synthesis of steroidal-ferrocene derivatives bearing a heterocyclic group, starting from natural and unnatural steroids. The new compounds were obtained via homogeneous catalytic reactions that usually ensure high selectivity and mild reaction conditions.

In our research group carbonylative Sonogashira coupling of iodoferrocene with terminal alkynes was investigated. It was proved that the alkynyl ketone products could be converted efficiently to heterocyclic compounds. There are no representatives for the conversion of ethynyl-steroids via this method, therefore it was studied first.

Because of its many advantages, azide-alkyne cycloaddition was planned as another route for the synthesis of steroidal-ferrocene derivatives. The aim was the transformation of ethynyl- and alkynyl-steroids to ferrocene containing compounds with a highly stable triazole ring.

In our laboratory a new synthetic pathway was developed starting from 16α,17α-androstanes and -estranes via Wagner-Meerwein rearrangement in ionic liquids. The reaction led to unnatural, 16-oxo-18-nor-13α-steroids. Steroids with this skeleton may have interesting biological properties, therefore the derivatization of these epi-steroids was planned in different pathways: starting from iodoalkenes via palladium-catalyzed aminocarbonylation, moreover using an aminocarbonylation-azide-alkyne cycloaddition reaction sequence.
II. EXPERIMENTAL METHODS APPLIED

A stainless steel autoclave was used in carbonylative Sonogashira reactions. Other homogeneous catalytic reactions were carried out applying Schlenk technique.

The reactions were followed by gas chromatography, thin layer chromatography and in some cases by $^1$H-NMR.

The products were purified by column chromatography.

The products were characterized by $^1$H-, $^{13}$C-NMR, 2D-NMR (COSY, NOESY, HMBC and HSQC), IR, MS measurements and elemental analysis. The structure of four compounds was proved by X-ray crystallography.

III. NEW SCIENTIFIC RESULTS

1. Ferrocene-labelled steroids were obtained via palladium-catalyzed carbonylative Sonogashira coupling and copper-catalyzed azide-alkyne cycloaddition starting from 29a-c ethynyl-steroids.

![Chemical structures](image)

The conversion of 33a alkynyl-ketone to a pyrazole derivative was attempted, but only traces of the desired product could be isolated. The azide-alkyne cycloaddition was found to be a more effective methodology for the synthesis of C-17 heterocyclic steroid-ferrocene derivatives.

2. Alkynyl-steroids were synthesized via palladium-catalyzed aminocarbonylation reaction from 41a-e iodoalkenes.
The cycloaddition step from alkynyl-steroids 42a-e led to 45a-e ferrocene derivatives selectively. The two reaction steps provided 12 previously unknown steroid compounds.

3. An unnatural 16-oxo-18-nor-13α-steroid (18) was converted to iodoalkenes by Barton’s methodology.

It was found that the oxidation with iodine resulted in the formation of 16-iodo-16-ene (52a) and 16-iodo-15-ene (53a) isomers (52a/53a = 45/55). The two regioisomeric products could not be separated from each other by column chromatography, therefore their mixture was used as a starting material in aminocarbonylation reactions.

4. Selective aminocarbonylation of 52a/53a steroids was carried out with various N-nucleophiles, moreover 52b-g and 53b-g carboxamides were isolated in good yields. A marked difference in the reactivities of iodoalkenes was observed, steroid 52a was less reactive especially in the reaction with secondary amines.
(a) It was found that during aminocarbonylation of 52a, the reaction rate depends on
the steric bulk of the amine rather than its basicity. Lower reactivity had been explained by
a steric hindrance caused by the planar disposition and close proximity of the 17-methyl
and 16-iodo groups. In the presence of a heterogeneous silica-palladium catalyst the
greatest bulk of the heterogeneous catalyst may enhance the difference between the
reactivity of 52a and 53a.

(b) The reduction of 52b and 53b carboxamides led to a 16α-carboxamido-17α-
methyl derivative.

(c) 13 New 13α-18-nor-16-karboxamido steroids were isolated via this method.

5. 16-Azido steroids (61a/61b) were prepared starting from a 16-oxo-18-nor-13α-
steroid (18) in a three-step reaction sequence.

The reduction of 18 led to an epimeric mixture of 16-hydroxy steroids (58a/58b =
93/7). Contrary to the literature data, in case of Appel reaction the main product was the
16β-iodo derivative (59a). The conversion of 59a/59b iodo-steroids to azide derivatives
led to an isomer mixture too. The two isomers could not be separated from each other by
column chromatography.

6. The cycloaddition of unnatural 61a/61b steroids and 53h alkynyl-steroid was
studied in the presence of the appropriate ferrocene derivatives.
It was found that azide group in a sterically hindered position may withhold the formation of the triazole group. In case of N-propargyl-carboxamide 53h excellent yield was achieved, which can be explained by the fact that in this case the click reaction takes place farther away from the steroid backbone.

7. Biological activity of some new compounds was evaluated in two fields.

(a) Androstane (42e, 45e) and estrane based (42c, 45c) alkynyl steroids and their ferrocene derivatives were studied for the inhibition of 17β-HSD1 enzyme in vitro. The steroid-ferrocene conjugates showed higher inhibitory effect than the starting steroids.

(b) It was found that unnatural 53h alkynyl-steroid and its ferrocene derivative (64) were able to decrease the activation of TRPV1 receptor on trigeminal ganglion neurons.
IV. SIGNIFICANCE OF THE SCIENTIFIC RESULTS

The main goal of my PhD work was the synthesis of new steroid-ferrocene conjugates containing a heterocyclic ring.

Steroids with natural and unnatural backbone were coupled with ferrocene via homogeneous catalytic reactions. These synthetic methods are selective and work under mild reaction conditions. The applied coupling reactions were not used earlier for the connection of steroids with ferrocene. The reaction conditions were optimized in order to achieve high yields.

The new steroid-ferrocene derivatives may have favorable biological activity due to the presence of the heterocyclic ring or the ferrocenyl moiety. In addition the unnatural steroid backbone could be responsible for the biological activity too. Some new compounds shown moderate 17β-HSD1 inhibitory effect, others were able to decrease the activation of TRPV1 receptor.
V. SCIENTIFIC PUBLICATIONS AND PRESENTATIONS RELATED TO THE DISSERTATION

PUBLICATIONS:

1. E. Szánti-Pintér, J. Balogh, Z. Csók, L. Kollár, Á. Gömöry, R. Skoda-Földes
   Synthesis of steroid-ferrocene conjugates of steroidal 17-carboxamides via a palladium-catalyzed aminocarbonylation - Copper catalyzed azide-alkyne cycloaddition reaction sequence
   Steroids 2011, 76, 1377-1382.
   IF: 2.829 (2011)

2. E. Szánti-Pintér, Z. Csók, L. Kollár, K. Vékey, R. Skoda-Földes
   Synthesis of ferrocene-labelled steroid derivatives via homogeneous catalytic methods
   IF: 2.000 (2012)

3. E. Szánti-Pintér, Z. Csók, Z. Berente, L. Kollár, R. Skoda-Földes
   Synthesis of novel 13α-18-nor-16-carboxamido steroids via a palladium-catalyzed aminocarbonylation reaction
   Steroids 2013, 78, 1177-1182.
   IF: 2.716 (2013)

4. E. Szánti-Pintér, J. Wouters, Á. Gömöry, É. Sághy, É. Szőke, Zs. Helyes, L. Kollár, R. Skoda-Földes
   Synthesis of novel 13α-18-norandrostane-ferrocene conjugates via homogeneous catalytic methods and their investigation on TRPV1 receptor activation
   Steroids 2015, 104, 284-293.
   IF: 2.639 (2014/15)

PRESENTATIONS:

1. E. Szánti-Pintér, K. Fehér, J. Balogh, R. Skoda-Földes
   Synthesis of steroid-ferrocene conjugates via copper-catalyzed azide-alkyne cycloaddition (P21) (poster)
   Innovation-V, Workshop on Innovative Catalysis (COST D40), Valletta, Malta, 14-16 June, 2011.

2. E. Szánti-Pintér, R. Skoda-Földes
   Palladium-catalyzed aminocarbonylation of steroidal alkyl halogenides (P154) (poster)

3. Szánti-Pintér, E.; Skoda-Földes, R.
   Új szerkezetű 13-epi-szteroid származékok szintézise aminokarbonilezési reakcióval (poster)
4. **E. Szánti-Pintér**, J. Herczig, R. Skoda-Földes

*Synthesis of novel 13α-steroid-ferrocene conjugates (P41B) (poster)*


5. **E. Szánti-Pintér**, J. Wouters, R. Skoda-Földes

*Synthesis of unnatural 13α-steroid-ferrocene derivatives (2P134) (poster)*


*Synthesis and pharmacological evaluation of steroid-ferrocene derivatives (P43) (poster)*


7. **Szánti-Pintér, E.**; Skoda-Földes, R.

*Szteroid-ferrocén konjugátumok előállítása (presentation)*


8. **Szánti-Pintér, E.**; Skoda-Földes, R.

*Szterénvázás alkinek 1,3-dipoláris cikloaddiciójának vizsgálata (presentation)*


9. **Szánti-Pintér, E.**; Skoda-Földes, R.

*Újfajta 13α-szteroid származékok szintézise homogénkatalitikus módszerekkel (presentation)*


10. **Szánti-Pintér, E.**; Skoda-Földes, R.

*Új szerkezetű 13α-szteroid származékok szintézise aminokarbonilézési reakcióval (presentation)*


11. **Szánti-Pintér, E.**; Skoda-Földes, R.

*Új szerkezetű 13α-szteroid-ferrocén származékok szintézise homogénkatalitikus reakciókkal (presentation)*


12. **Szánti-Pintér, E.**; Skoda-Földes, R.

*Ferrocénnel jelzett szteroidok előállítása (presentation)*


13. **Szánti-Pintér, E.**; Fehér, K.; Balogh, J.; Skoda-Földes R.

*Ferrocénnel jelzett szteroidok előállítása (presentation)*