Summary of Ph.D. thesis

Synthesis of steroidal azides and their Cu(I)-catalyzed dipolar cycloadditions

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1. Introduction and aims

One of the main driving force towards the preparation of steroidal compounds nowadays is the development of novel analogs with a biological target other than a hormone receptor, and therefore the reduction or elimination of unwanted hormonal effects. The synthetic tools for achievement of the above purpose are i) the synthesis of molecules lacking the functionalities necessary for effective binding to the hormone receptors; ii) modification of the binding ability by chemical transformation of the extant functional groups; iii) steric hindrance of the substrate-receptor interaction by chemical substitution; iv) altering the primary stereostructure or the number of ring members; and v) the design of heterocyclic derivatives that are not recognized by the receptor protein in consequence of their specific structure or the fact that their geometry differs from that of the natural hormones. Furthermore, experimental results during the past few years have revealed that a number of natural or synthetic steroidal heterocycles play important roles in complex signal transduction mechanisms, and therefore affect the proliferation of human cancer cells without influencing the division of intact cells.

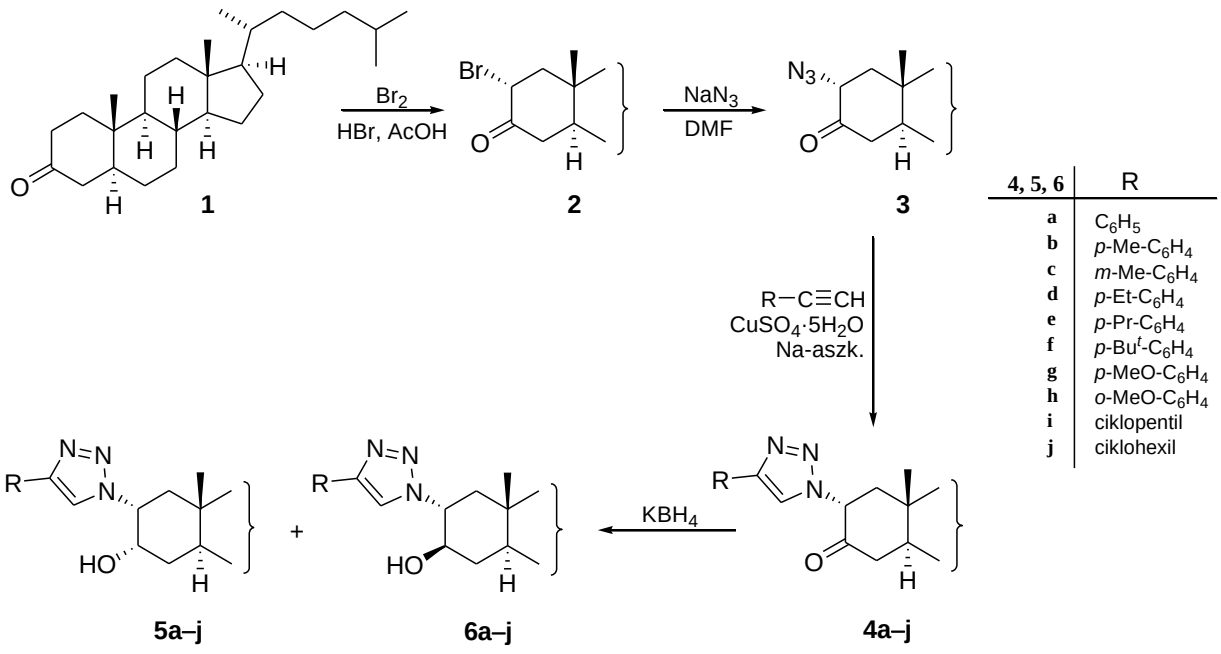
Based on the previous results as mentioned above, we set out to prepare novel *exo*-heterocyclic steroidal derivatives containing a triazole ring. Our synthetic modifications were performed on ring A and ring D of the sterane skeleton, at the positions C-1, C-2, C-15, C-16 and C-17 respectively. Furthermore, determination of the optimal reaction conditions of 1,3-dipolar cycloadditions and investigation the influence of the alkyne substituent on the intermolecular ring-closures were also planned. Our aim was to confirm the structures of all synthetized compounds by various spectroscopic methods and to screen these triazolyl derivatives *in vitro* for their activities against a panel of human cancer cell lines.

2. Experimental methods

Most of the reactions were carried out in millimolar scale, and monitored by thin-layer chromatography. The structures of all synthetized compounds were confirmed by ^{1}H and ^{13}C NMR measurements and other spectroscopic methods (MS and in some cases IR). The crude products were purified by flash chromatography.

3. Scientific results*

3.1. In our initial research, 2α-azido-5α-cholestan-3-one (**3**), readily available from cholestanone (**1**), was subjected to intermolecular ring-closure reactions with various terminal acetylenes. The Cu(I)-catalyzed regioselective cycloadditions afforded exclusively 1,4-disubstituted triazolyl derivatives (**4a**–**j**) in good yields. The catalyst was generated *in situ* by the reduction of $CuSO_4 \cdot 5H_2O$ with sodium ascorbate. Furthermore, an unusual solvent system (CH_2Cl_2/H_2O) was applied to simplify the reaction protocol, in contrast with the generally used solvents (H_2O/*t*-BuOH, THF, MeCN). Subsequently, reduction of the synthetized 2α-triazolyl compounds (**4a**−**j**) with KBH_4 resulted in two diastereomeric alcohols (**5a**−**j** and **6a**−**j**), which could be separated by flash chromatography (*Scheme 1*).



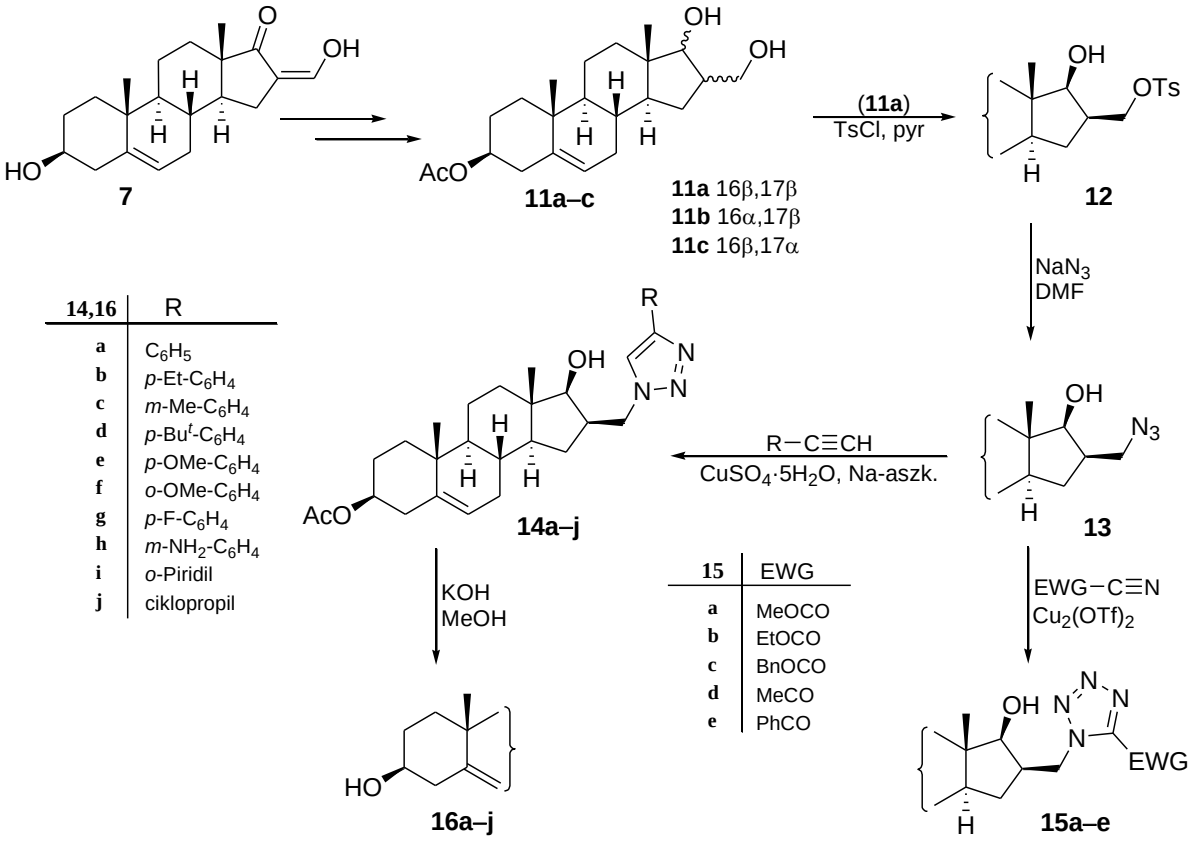
Scheme 1

3.2. In the next stage of our work, a diastereomeric mixture of three diols (**11a**−**c**) was prepared from an earlier synthetized compound (3β-hydroxy-16-hydroxymethylideneandrost-5-en-17-one, **7**) in a two-step pathway. After separation of the 16β,17β isomer (**11a**), the azido group was introduced by tosylation and subsequent S_N2 substitution with NaN_3 to afford the desired steroidal azide (**13**) in good yield. Several D-ring-substituted androst-5-ene derivatives containing a triazole ring (**14a**−**j**) were synthetized by the reaction of **13** with

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

various terminal alkynes through use of the *"click"* chemistry approach. According to our observation, the steroid heterocycles bearing an OH group usually proved to be more potent antiproliferative agents than their analogs containing an OAc group. Thus, the novel triazolyl derivatives (**14a**–**j**) were deacetylated in alkaline methanol to the corresponding 3β-hydroxy compounds (**16a**−**j**) (*Scheme 2*).

3.3. Intermolecular [3+2] cycloadditions of the steroid azide (**13**) with different nitriles containing an electron-withdrawing group (EWG) were carried out to furnish the desired 1,5-disubstituted steroidal tetrazoles (**15a**−**e**). It has been found that highly electrophilic nitrile carbon atoms (e.g. acyl cyanides, cyanoformates) and 10 mol% copper(I) catalyst ($Cu_2(OTf)_2 \cdot C_6H_6$) were required for successful addition (*Scheme 2*).



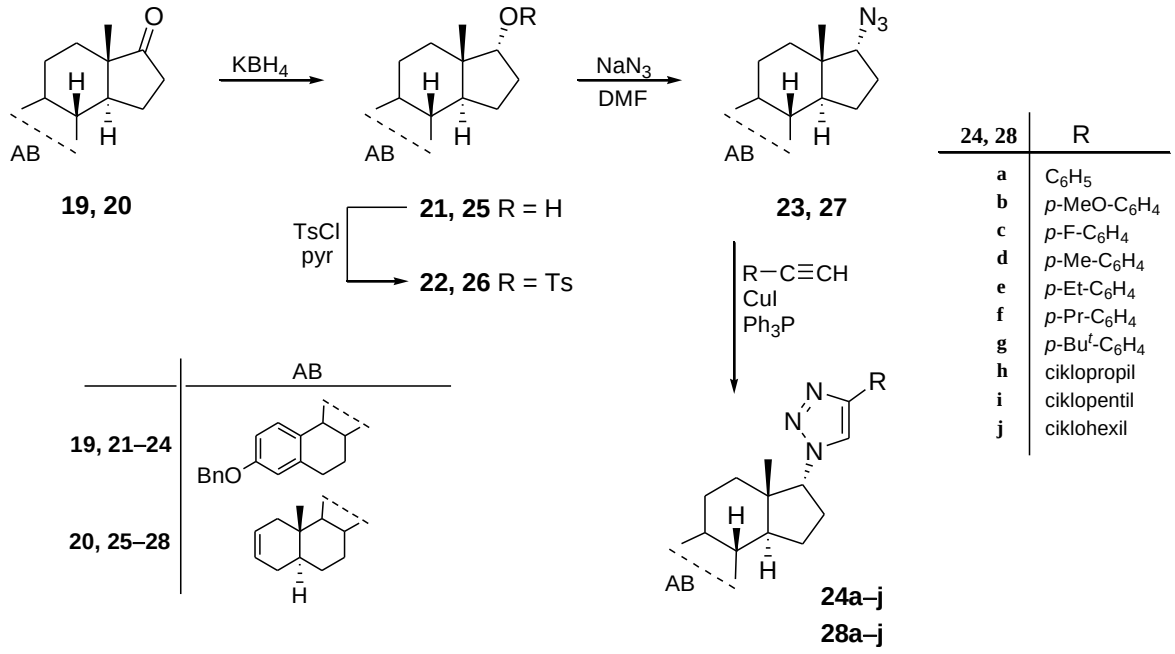
Scheme 2

3.4. For the preparation of steroidal 17α-azides, estrone-3-benzyl ether (**19**) and 5α-androst-2-en-17-one (**20**) were used as starting materials. Stereoselective reduction of the 17-keto group with KBH_4 leading to **21** and **25** was followed by tosylation to give **22** and **26**. The

crude products (**22**, **26**) were then used for further nucleophilic substitution with NaN_3 to provide the corresponding 17α-azido compounds (**23**, **27**) (*Scheme 3*).

3.5. The Cu(I)-catalyzed azide-alkyne cycloadditions of the isolated 17α-azido derivatives (**23**, **27**) with ten different terminal acetylenes were subsequently carried out with CuI as catalyst (*Scheme 3*).

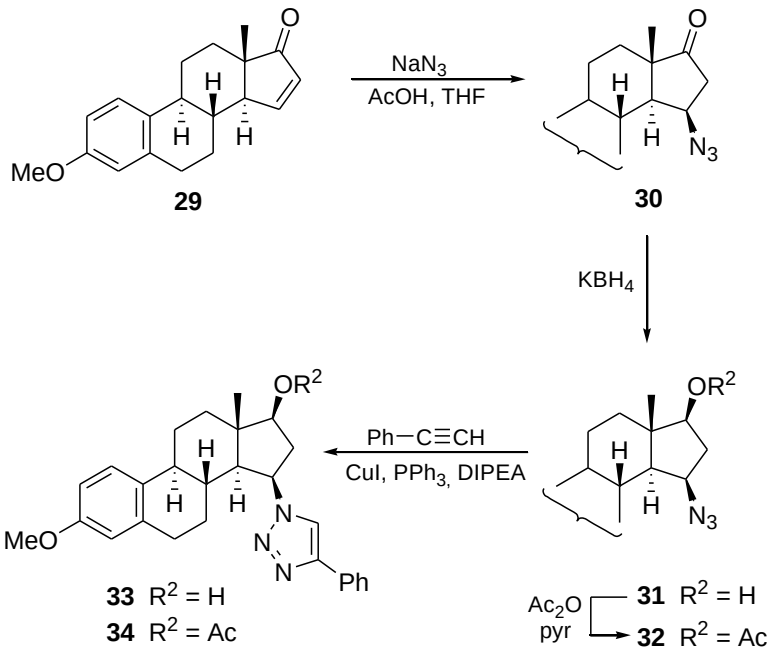
3.6. It has been found that amine base additive was not required for adequate formation of the Cu-acetylide complex, however dipolar cycloadditions were carried out in refluxing solvent (40 °C) to furnish 17α-triazolyl compounds (**24a**−**j** and **28a**−**j**). Moreover, a complexing ligand (PPh_3) was employed in order to enhance the activity and to improve the solubility of the catalyst (*Scheme 3*).



Scheme 3

3.7. An azido group was introduced onto the unconventional position 15β of 3-methoxy-1,3,5(10),15-estratetraen-17-one (**29**) by the 1,4-*Michael* addition of *in situ* generated azoimide. Since β-substituted ketones are often susceptible to elimination and undergo facile transformation to the corresponding enone, azidoketone (**30**) was reduced with KBH_4 so as to avoid this adverse side-reaction. The resultant *cis*-azidoalcohol (**31**) was then reacted with phenylacetylene under various reaction conditions in order to determine the parameters

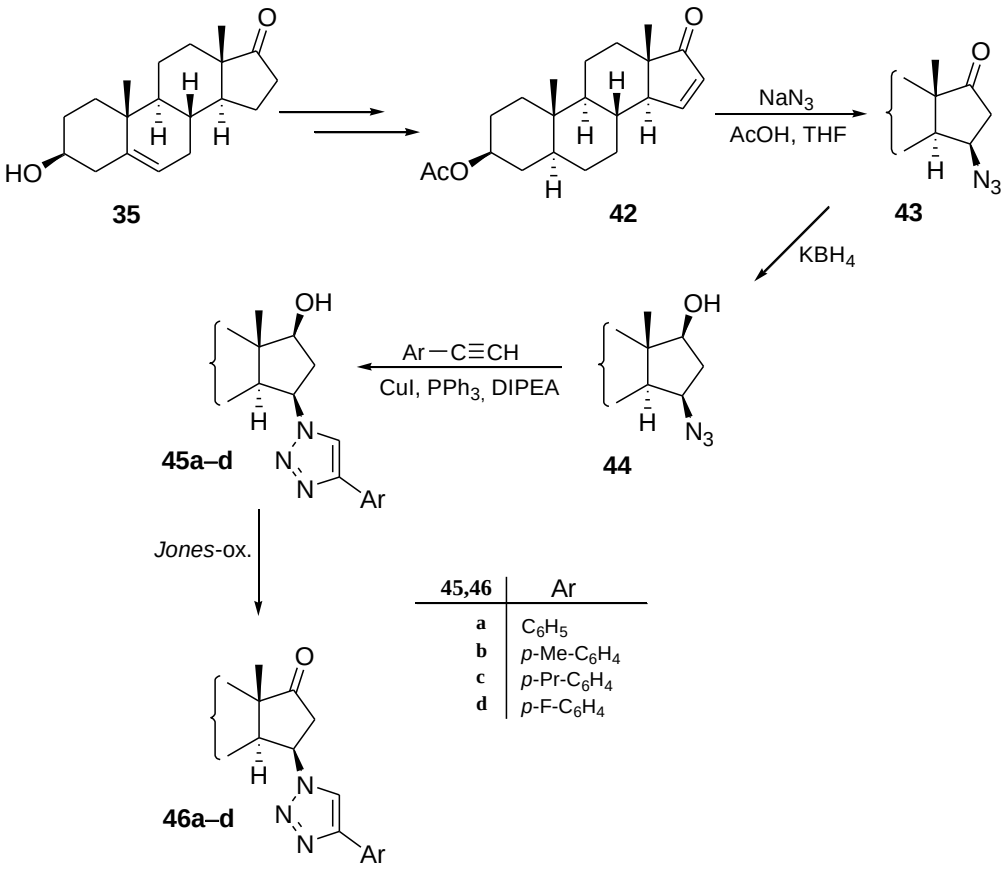
(catalyst, additives, solvent, etc.) needed for optimal yields. The best conversion was found on the use of a catalytic amount of CuI with the simultaneous addition of PPh_3 as stabilizing ligand and excess DIPEA as amine base (*Scheme 4*).



Scheme 4

3.8. After determination of the optimal reaction conditions, an azidoalcohol (**44**) in the 5α-androstane series, readily available from dehydroepiandrosterone (DEA) in a multistep pathway, was subjected to similar cycloadditions with different aryl-substituted acetylenes. This resulted in steroidal 15β-*exo*-triazolyl derivatives (**45a**−**d**) in yields of 70−75%, independently of the substituent on the alkyne dipolarophile (*Scheme 5*).

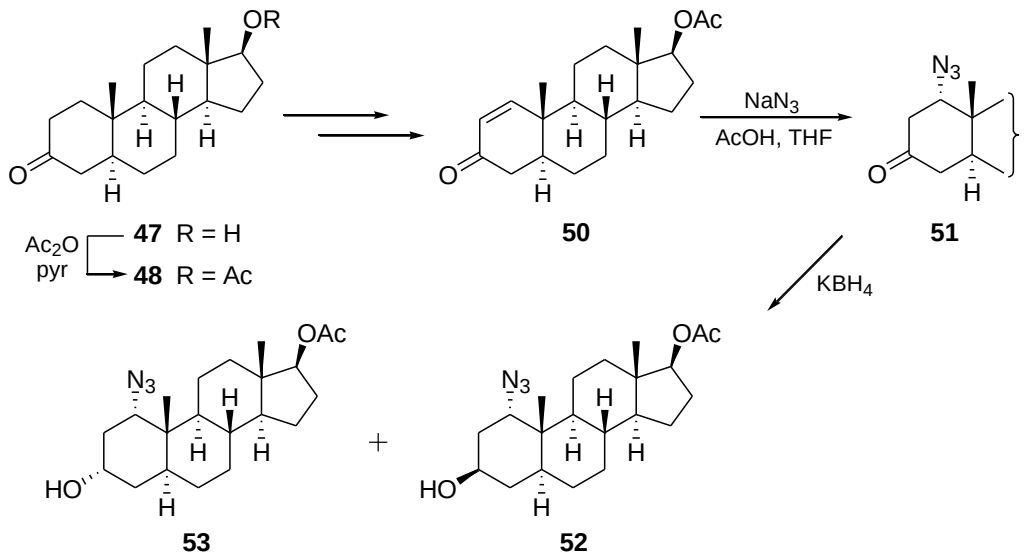
3.9. In this particular case the isolated yields proved to be lower than usual in CuAAC. The lack of full conversion of the starting materials may be attributed to the OH group on C-17, which is *cis* and therefore spatially close to the azide dipole, presumably causing a crowded transition state in the ring closure process. Subsequent *Jones* oxidation of triazolyl alcohols (**45a**−**d**) furnished the corresponding 17-keto analogs (**46a**−**d**) in good yields (*Scheme 5*).



Scheme 5

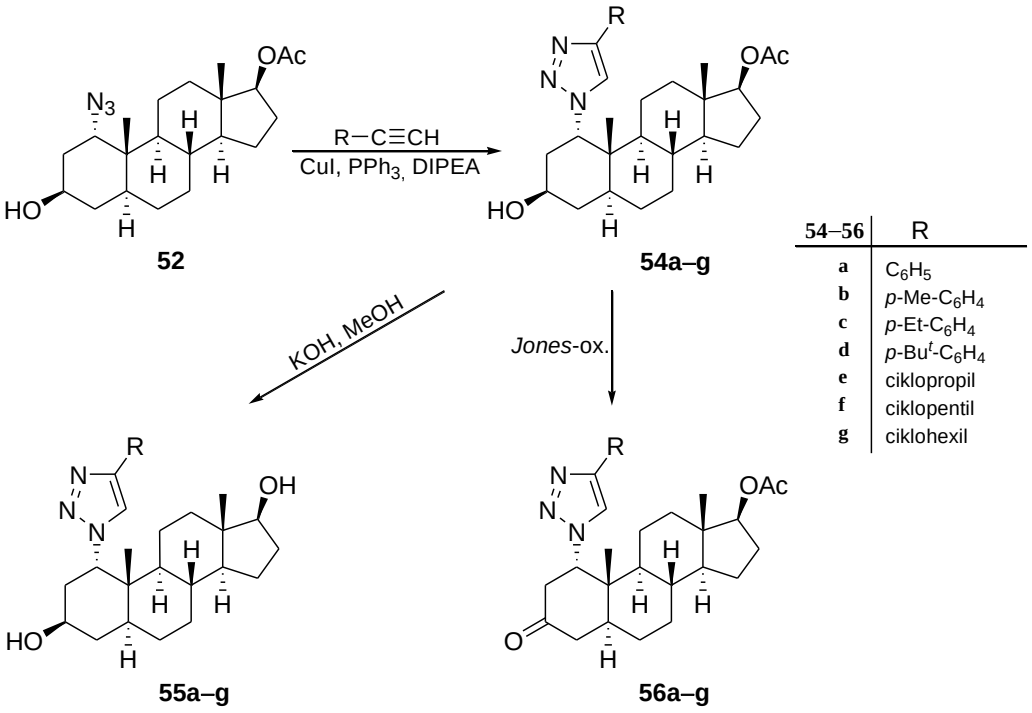
3.10. In a continuation of our work, the starting material applied was 17β-acetoxy-5α-androst-1-en-3-one (**50**), which is readily available from stanolone acetate (**48**) in a two-step pathway. Subsequent 1,4-*Michael* addition of the azoimide afforded stereoselectively the corresponding 1α-azido derivative (**51**), which is not surprising considering the steric bulk of the adjacent angular β-methyl group on C-10. The β-substituted ketone (**51**) was then reduced under pH-controlled conditions to give epimeric azidoalcohols in a ratio of 5:2, and the diastereomeric mixture was separated by column chromatography to yield **52** and **53** (*Scheme 6*).

3.11. Although CuAAC is generally not affected by the steric features of the alkyne and azide components, it has been found that the *trans* (**52**) and *cis* (**53**) azidoalcohols displayed considerably different behavior under similar reaction conditions. The cycloadditions of **52** with different aryl- and cycloalkyl-substituted acetylenes furnished steroidal 1α-*exo*-triazolyl derivatives (**54a**–**g**) in yields exceeding 90%, independently of the substituents on the alkyne dipolarophile (*Scheme 7*).



Scheme 6

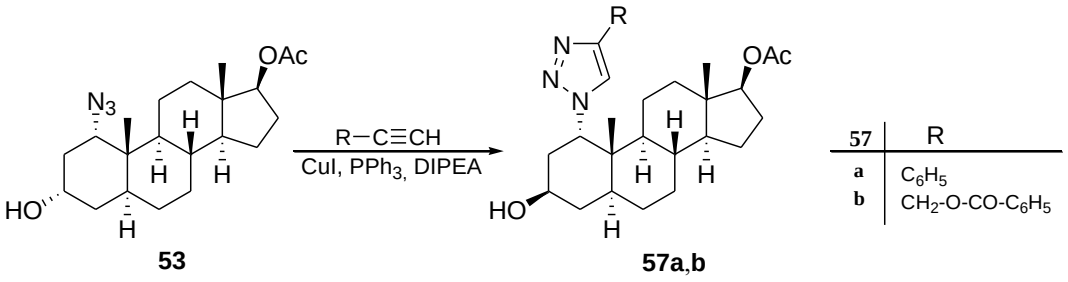
3.12. The resultant *"click"* products (**54a**–**g**) were deacetylated in alkaline methanol to the corresponding 3β,17β-diols (**55a**–**g**), while the 3-keto analogs (**56a**–**g**) were also obtained by *Jones* oxidation, during which a slight formation of enone (**50**) was observed (*Scheme 7*).



54–56	R
a	C_6H_5
b	*p*-Me-C_6H_4
c	*p*-Et-C_6H_4
d	*p*-Bu^t-C_6H_4
e	ciklopropil
f	ciklopentil
g	ciklohexil

Scheme 7

3.13. In contrast, the reaction of the *cis*-azidoalcohol (**53**) with phenylacetylene was not complete even after a longer time, and the purified product (**57a**) was obtained in a yield of only 61%. Nevertheless, treatment of substrate **53** with benzoic acid propargyl ester, in which the aromatic ring is situated farther from the reaction center than in phenylacetylene, resulted in the triazolyl derivative (**57b**) in a higher isolated yield (83%). These results suggest that the intermolecular ring closure is significantly influenced by the 3α-OH group, spatially close to the azide dipole on C-1, and especially by the steric bulk of the alkyne substituent, which presumably causes a crowded transition state in the Cu(I)-catalyzed process (*Scheme 8*).



53 **57a**,**b**

Scheme 8

3.14. In our research, a number of novel *exo*-heterocyclic steroid derivatives were obtained by applying CuAAC, and their structures were confirmed by various spectroscopic methods (IR, MS, ^{1}H and ^{13}C NMR).

3.15. The vast majority of the novel compounds were subjected to *in vitro* pharmacological studies at the Department of Pharmacodynamics and Biopharmacy. The calculated IC_{50} values revealed that several newly-prepared triazolyl derivatives (**45a**−**c**, **46a**−**d**, **56a**−**g**) exhibit substantial antiproliferative activity against malignant human cell lines.

3. Publications directly related to the dissertation

1. **Z. Kádár**, É. Frank, Gy. Schneider, J. Molnár, I. Zupkó, J. Kóti, B. Schönecker, J. Wölfling
   Efficient synthesis of novel A-ring-substituted 1,2,3-triazolylcholestane derivatives via catalytic azide-alkyne cycloaddition
   Arkivoc **2012**, *(iii)*, 279–296.
   Impact factor (2011): **1.252**

2. **Z. Kádár**, D. Kovács, É. Frank, G. Schneider, J. Huber, I. Zupkó, T. Bartók, J. Wölfling
   Synthesis and *in vitro* antiproliferative activity of novel androst-5-ene triazolyl and tetrazolyl derivatives
   Molecules **2011**, *16*, 4786–4806.
   Impact factor: **2.386**

3. É. Frank, J. Molnár, I. Zupkó, **Z. Kádár**, J. Wölfling
   Synthesis of novel steroidal 17α-triazolyl derivatives via Cu(I)-catalyzed azide-alkyne cycloaddition, and an evaluation of their cytotoxic activity *in vitro*
   Steroids **2011**, *76*, 1141–1148.
   Impact factor: **2.829**

4. **Z. Kádár**, J. Molnár, Gy. Schneider, I. Zupkó, É. Frank
   A facile ″click″ approach to novel 15β-triazolyl-5α-androstane derivatives, and an evaluation of their antiproliferative activities *in vitro*
   Bioorg. Med. Chem. **2012**, *20*, 1396–1402.
   Impact factor (2011): **2.921**

5. **Z. Kádár**, Á. Baji, I. Zupkó, T. Bartók, J. Wölfling, É. Frank
   Efficient approach to novel 1α-triazolyl-5α-androstane derivatives as potent antiproliferative agents
   Org. Biomol. Chem. **2011**, *9*, 8051–8057.
   Impact factor: **3.696**

Total impact factor: **13.084**

4. Lectures and posters related to the dissertation

1. **Z. Kádár**
   Triazolgyűrűt tartalmazó koleszténvázas vegyületek előállítása 1,3-dipoláris cikloaddícióval
   A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 10. tudományos előadóülése, 5. May 2010, Szeged, Hungary

2. D. Kovács, J. Wölfling, **Z. Kádár**
   16β-Triazolilmetil-androsztének szintézise 1,3-dipoláris cikloaddícióval
   XXXIII. Kémiai Előadói Napok, 25−27. October 2010, Szeged, Hungary

3. É. Frank, **Z. Kádár**, J. Wölfling, Gy. Schneider, I. Zupkó
   Synthesis of novel 1α-triazolyl derivatives in the 5α-androstane series and an evaluation of their antiproliferative activity *in vitro*
   14th Asian Chemical Congress, 5−8. September 2011, Bangkok, Thailand (Pg. 486)

4. É. Frank, D. Kovács, J. Wölfling, **Z. Kádár**
   Regioselective approach to novel steroidal triazoles and tetrazoles by 1,3-dipolar cycloaddition
   17th European Symposium on Organic Chemistry, 10−15. July 2011, Crete (P−1.036)

5. **Z. Kádár**, D. Kovács, Gy. Schneider, É. Frank, J. Wölfling
   Efficient synthesis of novel steroid triazole and tetrazole derivatives using click chemistry approach
   German-Austrian-French-Hungarian-Italian Conference in Organic and Biomolecular Chemistry, 26−29. May 2011, Goslar, Germany (Pg. 52)

6. **Z. Kádár**, K. Fodor, B. Juracsek, Gy. Schneider, J. Molnár, I. Zupkó, J. Wölfling
   Koleszténvázas α-azidoketon előállítása és 1,3-dipoláris cikloaddíciói terminális acetilénekkel
   Vegyészkonferencia és 53. Magyar Spektrokémiai Vándorgyűlés
   2010, Hajdúszoboszló, Hungary, Book of Abstracts p. 117.

7. **Z. Kádár**, D. Kovács, Gy. Schneider, J. Wölfling
Diszubsztituált triazol és tetrazol gyűrűk kiépítése androszténvázas szteroid azidon
MKE 1. Nemzeti Konferencia, 22–25. May 2011, Sopron, Hungary (P−12)

8. **Z. Kádár**, D. Kovács, Gy. Schneider, I. Zupkó, É. Frank
Synthesis of novel 15β-triazolyl-5α-androstane derivatives as potent antiproliferative agents
13th Tetrahedron Symposium, 25−29. June 2012, Amsterdam (P−1.41)

6. Other publications

1. É. Frank, Gy. Schneider, **Z. Kádár**, J. Wölfling
   Intramolecular hydro-*N*-alkylation of hydrazones and oxime ethers: synthesis of novel D-secoestrone isoquinuclidines *via* domino 1,5-hydride shift/cyclization
   Eur. J. Org. Chem. **2009**, 3544−3553.
   Impact factor: **3.096**

2. G. Montsko, A. Váczy, G. Maasz, E. Mernyák, É. Frank, C. Bay, **Z. Kádár**, R. Ohmacht, J. Wölfling, L. Márk
   Analysis of nonderivatized steroids by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using C70 fullerene as matrix
   Anal. Bioanal. Chem. **2009**, *395*, 869−874.
   Impact factor: **3.480**

3. D. Kovács, **Z. Kádár**, G. Mótyán, Gy. Schneider, J. Wölfling, I. Zupkó, É. Frank
   Synthesis, characterization and biological evaluation of some novel 17-isoxazoles in the estrone series
   Steroids **2012**, *77*, 1075−1085.
   Impact factor (2011): **2.829**

Total impact factor: **9.405**

7. Other posters

1. **Z. Kádár**, A. Sárközy, Gy. Schneider, J. Molnár, I. Zupkó, J. Wölfling
   A 16α-azidoösztron-3-metiléter klikk reakciói terminális acetilénekkel
   Vegyészkonferencia és 53. Magyar Spektrokémiai Vándorgyűlés
   2010, Hajdúszoboszló, Hungary, Book of Abstracts p. 116.

2. G. Mótyán, **Z. Kádár**, Gy. Schneider, É. Frank, J. Wölfling
   Új típusú, triazol gyűrűt tartalmazó dehidroepiandroszteron származékok előállítása 1,3-dipoláris cikloaddícióval
   Vegyészkonferencia és 53. Magyar Spektrokémiai Vándorgyűlés
   2010, Hajdúszoboszló, Hungary, Book of Abstracts p. 132.

3. I. Zupkó, J. Molnár, Á. Berényi, **Z. Kádár**, J. Wölfling
   Antiproliferative action of novel triazole-containing estranes
   Frontiers in Medicinal Chemistry, Joint German-Swiss Meeting on Medicinal Chemistry
   20−23. March 2011, Saarbrücken, Germany (Pg. 78)

4. D. Kovács, **Z. Kádár**, Gy. Schneider, J. Wölfling, I. Zupkó, É. Frank
   Efficient approach to novel isoxazolyl steroids by Cu(I)-catalyzed 1,3-dipolar cycloaddition
   13th Tetrahedron Symposium, 25−29. June 2012, Amsterdam (P−1.42)

5. I. Zupkó, J. Molnár, R. Minorics, I. Ocsovszki, **Z. Kádár**, É. Frank, J. Wölfling
   Antiproliferative effect and mechanism of action of novel triazole-containing estranes
   6th European Congress of Pharmacology, 17−20. July 2012, Granada, Spain (P−425)