Steroids 77 (2012) 738–744

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of ferrocene-labeled steroids via copper-catalyzed azide–alkyne cycloaddition. Reactivity difference between 2β-, 6β- and 16β-azido-androstanes

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ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form 7 March 2012
Accepted 4 April 2012
Available online 13 April 2012

Keywords:
Copper
Cycloaddition
Triazole
Ferrocene
Azidosteroid

ABSTRACT

Copper-catalyzed cycloaddition of steroidal azides and ferrocenyl-alkynes were found to be an efficient methodology for the synthesis of ferrocene-labeled steroids. At the same time, a great difference between the reactivity of 2β- or 16β-azido-androstanes and a sterically hindered 6β-azido steroid toward both ferrocenyl-alkynes and simple alkynes, such as phenylacetylene, 1-octyne, propargyl acetate and methyl propiolate, was observed.

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1. Introduction

High stability, accessibility of a great variety of derivatives and favorable electrochemical properties make ferrocene and its derivatives very suitable for biological applications and for conjugation with biomolecules [1]. Combination of a ferrocenyl moiety with structures of pharmacological importance may increase their biological activity. Low toxicity towards mammals make ferrocene derivatives ideal candidates for drug design [2]. The incorporation of the redox-active ferrocenyl moiety into a biomolecule (*e.g.* into a steroid), makes its electrochemical detection possible [3]. The copper-catalyzed 1,3-dipolar cycloaddition of azides and terminal alkynes (CuAAC, copper-catalyzed azide–alkyne cycloaddition), one of the so-called 'click reactions' (Scheme 1) [4] is an efficient methodology to attach a ferrocene label to compounds of diverse structure [5].

The CuAAC reaction has also been shown to serve as invaluable tool for the selective labeling of biological molecules with different moieties [6]. At the same time, fewer applications of this methodology for the synthesis of steroidal derivatives, compared to other biomolecules, have been reported. The conversion of 17α-ethynylestradiol to the corresponding 4-substituted 1,2,3-triazole was already mentioned by Sharpless [7]. A similar reaction was used for the synthesis of estradiol–cyclodextrin,[8] estradiol–peptide, [9] bile acid–peptide [10] or bile acid–β-lactam [11] conjugates. Cholesterol [12] and pregnane [13] derivatives with an azido group in the 17-alkyl side chain were converted to the corresponding triazoles in the presence of various terminal alkynes. Recently, some new steroid–ferrocene conjugates were synthesised in our group via the 'click reaction' of ferrocenyl azides and steroids with an ethynyl group in the side chain [14].

These steroidal triazoles were obtained either in the reaction of ethynyl/alkynyl steroids with azides of various structure, [7–11,14,15] or via the cycloaddition of simple alkynes with steroids bearing an azido group in the side chain [12,13,16]. However, there are only a few examples for the 'click reaction' of steroids with an azido group attached directly to the steroid core, at the easily accessible 3β-[17,18] or 17α-positions [19].

Although CuAAC is reported to be unaffected by steric factors, [7] we decided to explore if this methodology can be used for the attachment of a ferrocene-containing label to different positions of the steroidal skeleton. As a result, a great difference between the reactivity of 2β- or 16β-azido–androstanes and a sterically hindered 6β-azido steroid toward ferrocenyl-alkynes was observed. In order to clarify if this difference is restricted to the reaction of ferrocenyl alkynes, the 'click reaction' was also carried out with alkynes of different structures.

2. Experimental

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Inova 400 spectrometer at 400.13 MHz and 100.62 MHz, respectively. Chemical shifts are reported in ppm relative to $CHCl_3$ (7.26 and

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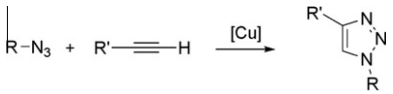
http://dx.doi.org/10.1016/j.steroids.2012.04.005



Scheme 1. Copper-catalyzed azide–alkyne cycloaddition (CuAAC).

77.00 ppm for ^{1}H and ^{13}C, respectively). Mass spectra were obtained on an Autoflex II TOF/TOF (Bruker Daltonics, Bremen, Germany) spectrometer in 2,5-dihydroxybenzoic acid matrix. Mass spectrum of **7f** was recorded on a HP-5971A MSD connected to a HP-5890/II gas chromatograph. IR spectra were made using a Thermo Nicolet Avatar 330 FT–IR instrument. Samples were prepared as KBr pellets. Elemental analyses were measured on a 1108 Carlo Erba apparatus.

2.1. Synthesis of steroidal azides *4*–*6*

To a mixture of the epoxide (**1–3**) (6 mmol), sodium azide (42 mmol, 2.73 g) and DMSO (45 ml), glacial acetic acid (9 ml) was added. The mixture was heated with stirring at 110 °C for 4 h. The mixture was poured on ice, filtered, washed with 0.1 M NaOH, water and diethyl ether and dried.

2.1.1. 16β-Azido-17α-hydroxy-5α-androstane (*4*)

^{1}H NMR(δ, $CDCl_3$): 3.75 (ddd, J = 8.3 Hz, 7.5 Hz, 0.8 Hz, 1H, 16-H); 3.65 (d, J = 0.8 Hz, 1H, 17-H); 2.15–2.25 (m, 1H, 15-H_a); 0.65–1.70 (m, 22H, ring protons, OH), 0.82 (s, 3H, 18-H_3), 0.80 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 84.7; 69.1; 54.2; 48.9; 46.9; 44.3; 38.7; 36.3; 35.3; 32.5; 32.3; 31.6; 29.0; 28.9, 26.7; 22.1; 19.9; 17.1; 12.2. IR (KBr, ν (cm^{-1})): 3371, 2093, 1256. Analysis calculated for $C_{19}H_{31}N_3O$ (317.47): C, 71.88; H, 9.84; N, 13.24. Found: C, 71.67; H, 9.95; N, 13.01. R_f (toluene/MeOH = 6/1): 0.71. Yield: 98%.

2.1.2. 2β-Azido-3α-hydroxy-5α-androstan-17-one (*5*)

^{1}H NMR(δ, $CDCl_3$): 3.83–3.86 (m, 1H, 3-H); 3.75–3.78 (m, 1H, 2-H); 2.40 (ddd, J = 0.9 Hz, 9.0 Hz, 19.0 Hz, 1H, 15-H_a); 0.71–2.09 (m, 20 H, ring protons, OH); 0.99 (s, 3H, 18-H_3); 0.84 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 221.1; 68.3; 61.6; 55.1; 51.4; 47.8; 38.7; 36.1; 35.8; 35.7; 34.5; 31.7; 31.5; 30.7; 27.7; 21.7; 20.2; 13.8; 13.0. IR (KBr, ν (cm^{-1})): 3427, 2092, 1255. Analysis calculated for $C_{19}H_{29}N_3O_2$ (331.46): C, 68.85; H, 8.82; N, 12.68. Found: C, 68.59; H, 8.95; N, 12.42 R_f (toluene/MeOH = 6/1): 0.48. Yield: 62%.

2.1.3. 6β-Azido-5α,3β-dihydroxyandrostan-17-one (*6*)

^{1}H NMR(δ, $CDCl_3$): 3.99–4.11 (m, 1H, 3-H); 3.41 (brs, 1H, 6-H); 2.45 (dd, J = 9.1 Hz, 19.4 Hz, 1H, 15-H_a); 1.20–2.12 (m, 20 H, ring protons, OH); 1.14 (s, 3H, 18-H_3); 0.87 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 220.5; 76.1; 67.3; 66.5; 50.7; 47.8; 45.6; 41.4; 38.8; 35.7; 32.3; 31.4; 30.7; 30.5; 30.0; 21.6; 20.3; 16.7; 13.9. IR (KBr, ν (cm^{-1})): 3443, 3345, 2096, 1258. Analysis calculated for $C_{19}H_{29}N_3O_3$ (347.46): C, 65.68; H, 8.41; N, 12.09. Found: C, 65.81; H, 8.18; N, 11.89. R_f (toluene/MeOH = 5/1): 0.37. Yield: 86%.

2.2. Synthesis of (E)-N-(prop-2-ynyl)-2-ethoxycarbonyl-3-ferrocenyl-2-propenamide (*7b*)

Ferrocenecarboxaldehyde (5 mmol, 1.07 g), propargyl amine (5 mmol, 343 μl) and methanol (25 ml) was stirred under argon in the presence of 4 Å molecular sieves (8–10 pieces) at room temperature for 4 h. The reaction mixture was filtered, the solvent was removed in vacuo. The product was crystallized from diethyl ether to yield N-(prop-2-ynyl)-ferrocenylideneamine (**12**). ^{1}H NMR(δ, $CDCl_3$): 8.41 (s, 1H,=C–H); 4.67 (brs, 2H, 2,5-Cp) 4.39 (brs, 2H, 3, 4-Cp); 4.30 (brs, 2H, C–CH_2); 4.18 (s, 5H, unsubstituted Cp); 2.48 (t, J = 2.3 Hz, 1H, C≡CH). MS (m/z/rel. int.): 251 (M^+)/100; 211/66; 185/2; 121/38; 56/21. IR (KBr, ν (cm^{-1})): 2110, 1643. Analysis calculated for $C_{14}H_{13}FeN$ (251.11): C, 66.96; H, 5.22; N, 5.58. Found: C, 66.89; H, 5.31; N, 5.49. Yield: 78%.

Ferrocenylimine **12** (0.6 mmol, 150.6 mg), ethyl diazoacetate (**11**) (0.6 mmol, 64 μl), $Co_2(CO)_8$ (0.03 mmol, 10 mg) and CH_2Cl_2 (4 ml) were transferred under an inert atmosphere into a stainless steel autoclave. It was charged with carbon monoxide (60 bar at room temperature) and stirred at room temperature for 24 h. (Caution! Cylinders containing CO should be used according to the safety instructions. The high pressure reactor should have the appropriate capacity and pressure rating. The reactor should be located in a laboratory hood during the reaction and should be vented carefully and slowly in the hood.) After evaporation of the solvent, the product was purified by column chromatography (silica, *n*-hexane/EtOAc = 3/1) to yield **7b**. ^{1}H NMR(δ, $CDCl_3$): 7.60 (s, 1H,=CH); 6.16 (t, J = 3.8 Hz, 1H, NH); 4.58–4.60 (m, 2H, NCH_2); 4.46 (t, J = 1.8 Hz, 2H, 2,5Cp); 4.15–4.21 (m, 9H, OCH_2, 3,4Cp, unsubstituted Cp); 2.24 (t, J = 2.5 Hz, 1H, CCH); 1.31 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR(δ, $CDCl_3$): 164.3; 163.4; 143.3; 120.7; 77.2; 73.6; 70.2; 70.1; 69.3; 68.1; 59.4; 27.5; 12.4. MS (m/z/rel. int.): 365 (M^+)/100; 320/4; 300/23; 254/12; 216/14; 173/20; 121/17; 56/9. IR (KBr, ν (cm^{-1})): 3311; 1693; 1646; 1261; 1097. Analysis calculated for $C_{19}H_{19}FeNO_3$ (365.21): C, 62.49; H, 5.24; N, 3.84. Found: C, 62.35; H, 5.11; N, 3.66 R_f (hexane/EtOAc = 3/1): 0.15. Yield: 38%.

2.3. Synthesis of 16β-(4-ferrocenyl-1,2,3-triazol-1-yl)-17-hydroxy-5-androstane (*8a*)

Method A: A mixture of the steroidal azide **4** (0.2 mmol, 63.4 mg), the alkyne **7a** (0.2 mmol, 42.0 mg), CuI (0.03 mmol, 5.7 mg) and DIPEA (4 ml) was stirred under argon at room temperature for 8 h. The solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 (8 ml) and was washed neutral with 5% HCl. The organic phase was washed with water (3 × 4 ml), dried over Na_2SO_4 and concentrated. The product was purified by column chromatography (silica, eluent: toluene/methanol = 6/1). Yield: 55%.

Method B: A mixture of the steroidal azide **4** (0.2 mmol, 63.4 mg), the alkyne **7a** (0.2 mmol, 42.0 mg), CuI (0.03 mmol, 5.7 mg), CH_3CN (4 ml) and DIPEA (0.5 mmol, 87 μl) was stirred under argon at 60 °C for 8 h. The solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 (8 ml) and was washed with 5% HCl (4 ml) and water (3 × 4 ml), dried over Na_2SO_4 and concentrated. The product was purified by column chromatography (silica, eluent: toluene/methanol = 6/1). Yield: 60%.

Method C: A mixture of the steroidal azide **4** (0.2 mmol, 63.4 mg), the alkyne **7a** (0.2 mmol, 42.0 mg), $CuSO_4.5H_2O$ (0.03 mmol, 7.5 mg), sodium ascorbate (0.076 mmol, 15.0 mg), ^{i}PrOH (2 ml) and water (2 ml) was stirred under argon at 60 °C for 8 h. The product was extracted with CH_2Cl_2 (3 × 4 ml). The combined organic phase was washed with water (3 × 4 ml) dried over Na_2SO_4 and concentrated. The product was purified by column chromatography (silica, eluent: toluene/methanol = 6/1). Yield: 70%.

Method D: A mixture of the steroidal azide **4** (0.2 mmol, 63.4 mg), the alkyne **7a** (0.2 mmol, 42.0 mg), $CuSO_4.5H_2O$ (0.03 mmol, 7.5 mg), sodium ascorbate (0.076 mmol, 15.0 mg), CH_2Cl_2 (2 ml) and water (2 ml) was stirred under argon at room temperature for 8 h. Then CH_2Cl_2 (6 ml) was added to the reaction mixture. The combined organic phase was washed with water (3 × 4 ml), dried over Na_2SO_4 and concentrated. The product was purified by column chromatography (silica, eluent: toluene/methanol = 6/1). Yield: 76%.

^{1}H NMR(δ, $CDCl_3$): 7.35 (s, 1H,=CH); 5.10 (brs, 2H, 2,3-Cp); 4.67 (brs, 2H, 3,4-Cp); 4.50 (ddd, J = 8.2 Hz, 7.5 Hz, 0.8 Hz, 1H, 16-H);

4.36 (s, 5H, unsubstituted Cp); 3.95 (d, J = 0.8 Hz, 1H 17-H); 0.69–2.33 (m, 23H, ring protons, OH); 0.89 (s, 3H, 18-H_3); 0.79 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 146.7; 120.8; 85.3; 75.5; 73.2; 71.7; 71.2; 69.7; 54.2; 49.3; 46.9; 44.4; 38.6; 36.3; 35.0; 32.8; 32.4; 31.9; 28.9; 28.8; 26.7; 22.1; 19.9; 18.0; 12.2. MS (m/z/rel. int.): 550 [(M+Na)$^+$]/6; 528/[(M+H)$^+$]/100; 527 (M$^+$)/98. Analysis calculated for $C_{31}H_{41}FeN_3O$ (527.53) C, 70.58; H, 7.83; N, 7.97. Found: C, 70.42; H, 8.07; N, 8.10. R_f (toluene/MeOH = 6/1): 0.33.

2.4. General method for the synthesis of triazolyl steroids **8 b–f**, **9 a–f** and **10 b, f** (method D)

A mixture of the steroidal azide (**4–6**) (0.2 mmol), the alkyne (**7a–f**) (0.2 mmol), $CuSO_4$ $5H_2O$ (0.03 mmol, 7.5 mg), sodium ascorbate (0.076 mmol, 15.0 mg), CH_2Cl_2 (2 ml) and water (2 ml) was stirred under argon at room temperature for 8 h. Then CH_2Cl_2 (6 ml) was added to the reaction mixture. The combined organic phase was washed with water (3 × 4 ml), dried over Na_2SO_4 and concentrated. The product was purified by column chromatography (silica, eluent: toluene/methanol = 6/1).

2.4.1. 16β-(4-((E)-Ethyl 3-ferrocenylacrylate-2-methylcarbamoyl)-1,2,3-triazol-1-yl)-17-hydroxy-5-androstane (**8b**)

^{1}H NMR(δ, $CDCl_3$): 7.78 (s, 1H,=CH); 7.54 (s, 1H,=CH); 6.89 (brs, 1H, NH); 4.55–4.66 (m, 3H, 16-H, NCH_2); 4.43 (brs, 2H, 2,5-Cp); 4.39 (brs, 2H, 3,4-Cp); 4.09–4.26 (m, 7H, OCH_2, unsubstituted Cp); 3.95 (s, 1H, 17-H); 0.69–2.41 (m, 23H, ring protons, OH); 1.24 (t, J = 7.1 Hz, 3H, CH_3); 0.87 (s, 3H, 18-H_3); 0.79 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 166.9; 165.1; 144.7; 144.1; 123.2; 122.2; 85.2; 75.5; 71.8; 70.7; 70.6; 70.2; 69.9; 61.1; 54.1; 49.3; 46.8; 44.4; 44.2; 38.6; 36.3; 35.0; 32.6; 32.3; 31.8; 28.9; 28.8; 26.7; 22.1; 19.9; 18.0; 14.2; 12.2. MS (m/z/rel. int.): 705 [(M+Na)$^+$]/49; 683/[(M+H)$^+$]/4; 682 (M$^+$)/3; 311/100; 137/41. IR (KBr, ν (cm^{-1})): 3436, 3231, 1704, 1636, 1619. Analysis calculated for $C_{38}H_{50}FeN_4O_4$ (682.68) C, 66.86; H, 7.38; N, 8.21. Found: C, 67.05; H, 7.56; N, 8.02. R_f (toluene/MeOH = 6/1): 0.23. Yield: 67%.

2.4.2. 17-Hydroxy-16β-(4-phenyl-1,2,3-triazol-1-yl)-5-androstane (**8c**)

^{1}H NMR(δ, $CDCl_3$): 7.85 (s, 1H,=CH); 7.84 (d, J = 7.3 Hz, 2H, Ph); 7.43 (t, J = 7.3 Hz, 2H, Ph); 7.34 (t, J = 7.3 Hz, 1H, Ph); 4.65 (ddd, J = 8.3 Hz, 7.6 Hz, 0.8 Hz, 1H, 16-H); 4.10 (d, J = 0.8 Hz, 1H, 17-H); 0.78–2.48 (m, 23H, ring proton, OH); 0.97 (s, 3H, 18-H_3); 0.83 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 147.4; 130.6; 128.7; 127.9; 125.5; 119.3; 85.3; 69.8; 54.1; 49.3; 46.8; 44.4; 38.5; 36.2; 34.9; 32.6; 32.2; 31.7; 28.8; 28.7; 26.6; 22.0; 19.8; 17.8; 12.1. MS (m/z/rel. int.): 442 [(M+Na)$^+$]/22; 420/[(M+H)$^+$]/100. Analysis calculated for $C_{27}H_{37}N_3O$ (419.61) C, 77.29; H, 8.89; N, 10.01. Found: C, 77.45; H, 8.99; N, 9.79.R_f (toluene/MeOH = 6/1): 0.44. Yield: 72%.

2.4.3. 16β-(4-Hexyl-1,2,3-triazol-1-yl)-17-hydroxy-5-androstane (**8d**)

^{1}H NMR(δ, $CDCl_3$): 7.40 (s, 1H,=CH); 4.60 (ddd, J = 8.5 Hz, 7.5 Hz, 0.8 Hz, 1H, 16-H); 4.00 (d, J = 0.8 Hz, 1H, 17-H); 0.75–2.75 (m, 33H, ring protons, $(CH_2)_5$, OH); 0.91 (s, 3H, 18-H_3); 0.87 (t, J = 7.8 Hz, 3H, CH_3); 0.79 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 148.1; 120.6; 85.2; 69.9; 54.2; 49.3; 46.9; 44.4; 38.6; 36.3; 35.0; 32.6; 32.3; 31.9; 31.5; 29.3; 28.9 (2C); 28.8; 26.7; 25.6; 22.5; 22.1; 19.9; 17.9; 14.0; 12.2. MS (m/z/rel. int.): 450 [(M+Na)$^+$]/5; 428/[(M+H)$^+$]/100. Analysis calculated for $C_{27}H_{45}N_3O$ (427.67) C, 75.83; H, 10.61; N, 9.83. Found: C, 75.59; H, 10.87; N, 9.72. R_f (toluene/MeOH = 6/1): 0.50. Yield: 59%.

2.4.4. 16β-(4-(Acetoxy-methyl)-1,2,3-triazol-1-yl)-17-hydroxy-5-androstane (**8e**)

^{1}H NMR(δ, $CDCl_3$): 7.79 (s, 1H,=CH); 5.20 (s, 2H, OCH_2); 4.62 (ddd, J = 8.4 Hz, 7.2 Hz, 0.8 Hz, 1H, 16-H); 4.04 (d, J = 0.8 Hz, 1H, 17-H); 0.75–2.58 (m, 23H, ring protons, OH); 2.05 (s, 3H, C(O)CH_3); 0.92 (s, 3H, 18-H_3); 0.79 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 170.9; 142.2; 124.5; 85.2; 70.2; 57.6; 54.2; 49.3; 46.9; 44.5; 38.6; 36.3; 35.0; 32.8; 32.3; 31.8; 28.9; 28.8; 26.7; 22.1; 20.9; 19.9; 17.9; 12.2. MS (m/z/rel. int.): 438 [(M+Na)$^+$]/95; 416/[(M+H)$^+$]/100. IR (KBr, ν (cm^{-1})): 3302, 1738. Analysis calculated for $C_{24}H_{37}N_3O_3$ (415.57): C, 69.37; H, 8.97; N, 10.11. Found: C, 69.51; H, 8.69; N, 10.23. R_f (toluene/MeOH = 6/1): 0.26. Yield: 75%.

2.4.5. 17-Hydroxy-16β-(4-(methoxycarbonyl)-1,2,3-triazol-1-yl)-5-androstane (**8f**)

^{1}H NMR(δ, $CDCl_3$): 8.14 (s, 1H,=CH); 4.64 (ddd, J = 8.5 Hz, 7.6 Hz, 0.8 Hz, 1H, 16-H); 4.03 (d, J = 0.8 Hz, 1H, 17-H); 3.93 (s, 3H, OCH_3); 0.69–2.47 (m, 23H, ring protons, OH); 0.89 (s, 3H, 18-H_3); 0.79 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 161.3; 142.2; 127.2; 85.4; 70.3; 54.1; 52.2; 49.3; 46.9; 44.6; 38.6; 36.3; 35.0; 32.8; 32.3; 31.7; 28.9; 28.8; 26.7; 22.1; 19.9; 17.9; 12.2. MS (m/z/rel. int.): 424 [(M+Na)$^+$]/82; 402/[(M+H)$^+$]/100. IR (KBr, ν (cm^{-1})): 3387, 1727. Analysis calculated for $C_{23}H_{35}N_3O_3$ (401.55): C, 68.80; H, 8.79; N, 10.46. Found: C, 68.95; H, 8.65; N, 10.51. R_f (toluene/MeOH = 6/1): 0.23. Yield: 72%.

2.4.6. 2β-(4-Ferrocenyl-1,2,3-triazol-1-yl)-3-hydroxy-5-androstane-17-one (**9a**)

^{1}H NMR(δ, $CDCl_3$): 7.35 (s, 1H,=CH); 5.01 (brs, 2H, 2,5-Cp); 4.81 (m, 1H, 2-H); 4.58 (m, 2H, 3,4-Cp); 4.46 (m, 1H, 3-H); 4.28 (s, 5H, unsubstituted Cp); 2.38–2.45 (m, 1H, 16-H_a); 0.75–2.23 (m, 20H, ring protons, OH); 0.81 (s, 3H, 18-H_3); 0.51 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 220.9; 144.6; 116.9; 68.9; 67.9; 66.3; 65.5; 64.4; 59.2; 53.1; 49.2; 45.9; 38.7; 37.2; 34.1; 33.9; 32.7; 31.7; 29.6; 28.7; 25.8; 19.8; 18.5; 12.0; 11.2. MS (m/z/rel. int.): 564 [(M+Na)$^+$]/19; 542 [(M+H)$^+$]/96; 541(M$^+$)/100. IR (KBr, ν (cm^{-1})): 3440, 1718. Analysis calculated for $C_{31}H_{39}FeN_3O_2$ (541.52): C, 68.76; H, 7.26; N, 7.76. Found: C, 68.61; H, 7.11; N, 7.89. R_f (toluene/MeOH = 6/1): 0.26. Yield: 64%.

2.4.7. 2β-(4-((E)-Ethyl 3-ferrocenylacrylate-2-methylcarbamoyl))-1,2,3-triazol-1-yl)-3-hydroxy-5-androstan-17-one (**9b**)

^{1}H NMR(δ, $CDCl_3$): 7.70 (s, 1H,=CH); 7.54 (s, 1H,=CH); 6.55 (s, 1H, NH); 4.72 (m, 1H, 2-H); 4.62 (m, 2H, NCH_2); 4.45–4.51 (m, 3H, 3-H, 2,5-Cp); 4.40 (brs, 2H, 3,4-Cp); 4.17–4.20 (m, 7H, OCH_2, unsubstituted Cp) 0.82–2.56 (m, 21H, ring protons, OH); 1.26 (t, J = 7.0 Hz, 3H, CH_3); 0.75 (s, 3H, 18-H_3); 0.52 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 220.8; 166.9; 165.2; 144.3; 144.2; 123.2; 122.2; 75.6; 71.9; 70.8; 69.9; 66.6; 61.5; 61.1; 55.1; 51.3; 47.7; 40.9; 39.1; 36.0; 35.7; 35.4; 34.6; 33.8; 31.5; 30.4; 27.7; 21.7; 20.4; 14.2; 13.8; 13.7. MS (m/z/rel. int.):719 [(M+Na)$^+$]/18; 697 [(M+H)$^+$]/6; 696 (M$^+$)/5; 311/100; 137/73. IR (KBr, ν (cm^{-1})): 3440, 1705, 1645. Analysis calculated for $C_{38}H_{48}FeN_4O_5$ (696.67): C, 65.51; H, 6.94; N, 8.04. Found: C, 65.71; H, 7.12; N, 8.21. R_f (toluene/MeOH = 5/1): 0.33. Yield: 63%.

2.4.8. 3-Hydroxy-2β-(4-phenyl-1,2,3-triazol-1-yl)-5-androstan-17-one (**9c**)

^{1}H NMR(δ, $CDCl_3$): 7.83 (s, 1H,=CH); 7.81 (d, J = 7.2 Hz, 2H, Ph); 7.40 (t, J = 7.2 Hz, 2H, Ph); 7.32 (t, J = 7,2 Hz, 1H, Ph); 4.85 (m, 1H, 2-H); 4.59 (m, 1H, 3-H); 0.78–2.52 (m, 21H, ring protons, OH); 0.95 (s, 3H, 18-H_3); 0.85 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 220.8; 146.9; 132.1; 128.9; 128.3; 125.8; 119.9; 66.5; 62.0; 55.1; 51.3; 47.8; 39.1; 35.8; 34.6; 34.3; 33.8; 31.6; 30.9; 30.5; 27.7; 21.7; 20.4; 13.8; 13.0. MS (m/z/rel. int.): 456 [(M+Na)$^+$]/23; 434 [(M+H)$^+$]/100. IR (KBr, ν (cm^{-1})): 3420, 1722. Analysis calculated for $C_{27}H_{35}N_3O_2$ (433.59): C, 74.79; H, 8.14; N, 9.69. Found: C, 74.71; H, 8.30; N, 9.52. R_f (toluene/MeOH = 6/1): 0.27. Yield: 63%.

*2.4.9. 2β-(4-Hexyl-1,2,3-triazol-1-yl)-3-hydroxy-5-androstan-17-one (**9d**)*

^{1}H NMR(δ, $CDCl_3$): 7.31 (s, 1H,=CH); 4.77–4.82 (m, 1H, 2-H); 4.46–4.50 (m, 1H, 3-H); 2.68 (t, J = 7.6 Hz, 2H, CH_2); 2.38–2.45 (m, 1H, 16-H_a); 0.81–2.21 (m, 28H, ring protons, $(CH_2)_4$, OH); 0.86 (t, J = 7.6 Hz, 3H, CH_3); 0.81 (s, 3H, 18-H_3); 0.50 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 220.9; 146.5; 121.2; 66.3; 62.1; 55.1; 51.3; 47.8; 40.8; 39.1; 36.0; 35.8; 34.6; 33.8; 31.5 (2C); 30.4; 29.2; 28.8; 27.7; 25.1; 22.5; 21.7; 20.4; 14.0; 13.8; 13.7. MS (m/z/rel. int.): 464 [(M+Na)$^+$]/65; 442/[(M+H)$^+$]/100. IR (KBr, ν (cm^{-1})): 3450, 1728. Analysis calculated for $C_{27}H_{43}N_3O_2$ (441.66) C, 73.43; H, 9.81; N, 9.51. Found: C, 73.22; H, 10.01; N, 9.43. R_f (toluene/MeOH = 6/1): 0.30. Yield: 47%.

*2.4.10. 2β-(4-(Acetoxy-methyl)-1,2,3-triazol-1-yl)-3-hydroxy-5-androstan-17-one (**9e**)*

^{1}H NMR(δ, $CDCl_3$): 7.76 (s, 1H,=CH); 5.21 (s, 2H, OCH_2); 4.72–4.81 (m, 1H, 2-H); 4.51–4.60 (m, 1H, 3-H); 2.38–2.44 (m, 1H, 16-H_a); 0.80–2.21 (m, 20H, ring protons, OH); 2.08 (s, 3H, $C(O)CH_3$); 0.82 (s, 3H, 18-H_3); 0.54 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 221.0; 170.8; 142.3; 124.7; 66.4; 62.2; 57.5; 55.0; 51.2; 47.7; 40.6; 39.0; 35.9; 35.7; 34.5; 33.7; 31.4; 30.4; 27.6; 21.6; 20.8; 20.3; 13.8; 13.6. MS (m/z/rel. int.): 452 [(M+Na)$^+$]/63; 430/[(M+H)$^+$]/100. IR (KBr, ν (cm^{-1})): 3430, 1735, 1720. Analysis calculated for $C_{24}H_{35}N_3O_4$ (429.56): C, 67.11; H, 8.21; N, 9.78. Found: C, 67.35; H, 8.35; N, 9.91. R_f (toluene/MeOH = 5/1): 0.37. Yield: 67%.

*2.4.11. 3-Hydroxy-2β-(4-methoxycarbonyl-1,2,3-triazol-1-yl)-5-androstan-17-one (**9f**)*

^{1}H–NMR(δ, $CDCl_3$): 8.22 (s, 1H,=CH); 4.82 (m, 1H, 2-H); 4.60 (m, 1H, 3-H); 3.95 (s, 3H, OCH_3); 0.75–2.50 (m, 21H, ring protons, OH); 0.80 (s, 3H, 18-H_3); 0.74 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 220.9; 162.1; 143.9; 127.9; 66.5; 62.6; 55.0; 52.3; 51.2; 47.8; 40.5; 39.0; 36.0; 35.7; 34.5; 33.7; 31.4; 30.4; 27.6; 21.6; 20.4; 13.8; 13.6. MS (m/z/rel. int.): 438 [(M+Na)$^+$]/30; 416 [(M+H)$^+$]/100. IR (KBr, ν (cm^{-1})): 3430, 1724, 1713. Analysis calculated for $C_{23}H_{33}N_3O_4$ (415.53): C, 66.48; H, 8.00; N, 10.11. Found: C, 66.31; H, 8.25; N, 10.27. R_f (toluene/MeOH = 5/1): 0.35. Yield: 68%.

*2.4.12. 6β-(4-((E)-Ethyl 3-ferrocenylacrylate-2-methylcarbamoyl)-1,2,3-triazol-1-yl)-3,5-dihydroxy-androstan-17-one (**10b**)*

^{1}H NMR(δ, $CDCl_3$): 7.59 (s, 1H,=CH); 7.36 (s, 1H,=CH); 6.68 (brs, 1H, NH); 4.64–4.71 (m, 2H, NCH_2); 4.02–4.58 (m, 12H, Fc, OCH_2, 3-H); 3.40 (brs, 1H, 6H); 0.75–2.60 (m, 21H, ring protons, OH); 1.28 (t, J = 7.2 Hz, 3H, CH_3); 0.95 (s, 3H, 18-H_3); 0.66 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 221.0; 167.3; 165.2; 144.3; 143.3; 124.9; 122.8; 75.7; 75.4; 72.1; 70.8; 70.5; 70.0; 67.1; 65.0; 61.3; 53.4; 51.3; 48.0; 45.1; 41.7; 38.6; 35.8; 32.7; 31.8; 31.5; 30.4; 29.3; 21.5; 20.4; 15.5; 14.1, 13.8. MS (m/z/rel. int.):735 [(M+Na)$^+$]/24; 713 [(M+H)$^+$]/5; 712 (M$^+$)/2; 311/100; 137/39. IR (KBr, ν (cm^{-1})): 3430, 1715, 1704, 1681, 1632. Analysis calculated for $C_{38}H_{48}FeN_4O_6$ (712.67): C, 64.04; H, 6.79; N, 7.86. Found: C, 64.19; H, 6.87; N, 8.00. R_f (toluene/MeOH = 5/1): 0.25. Yield: 57%.

*2.4.13. 3β,5-Dihydroxy-6β-(4-methoxycarbonyl-1,2,3-triazol-1-yl)-androstan-17-one (**10f**)*

^{1}H NMR(δ, $CDCl_3$): 8.25 (s, 1H,=CH); 4.35 (m, 1H, 3-H); 3.85 (s, 3H, OCH_3); 3.45 (brs, 1H, 6-H); 1.25–2.60 (m, 21H, ring protons, OH); 0.92 (s, 3H, 18-H_3); 0.66 (s, 3H, 19-H_3). ^{13}C NMR(δ, $CDCl_3$): 220.7; 207.0; 141.5; 127.8; 76.1; 67.3; 66.5; 51.3; 50.7; 47.8; 45.6; 41.4; 38.8; 35.7; 32.3; 31.4; 30.9; 30.4; 30.0; 21.7; 20.3; 16.8; 13.9. MS (m/z/rel. int.): 454 [(M+Na)$^+$]/100; 432 [(M+H)$^+$]/70. IR (KBr, ν (cm^{-1})): 3414, 1734, 1718. Analysis calculated for $C_{23}H_{33}N_3O_5$ (431.53): C, 64.02; H, 7.71; N, 9.74. Found: C, 64.23; H, 7.99; N, 9.95. R_f (toluene/MeOH = 5/1): 0.33. Yield: 47%.

2.5. Computational details

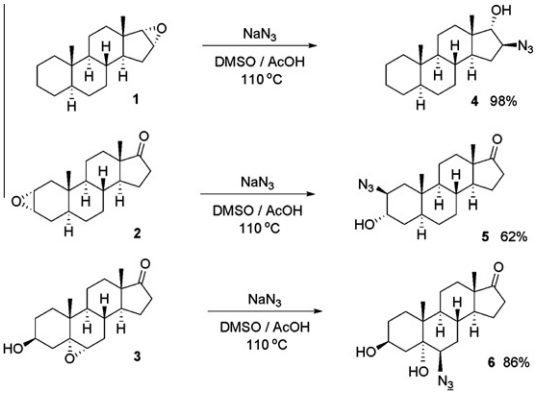
All the geometries were calculated without any symmetry constraints by using the gradient-corrected exchange functional developed by Perdew, Burke and Ernzerhof in combination with a correlation functional also developed by the same authors and denoted as PBEPBE [20]. The 6–31G(d,p) basis set [21] was employed throughout this study. For all stationary points the Hessian was evaluated to characterize the genuine minima (no imaginary frequency). Thermal correction for the Gibbs free energy (at 298 K) has been estimated on the basis of the frequency calculations at the optimization level. For the calculations the Firefly (formerly known as PC GAMESS) [22] software was used.

3. Results and discussion

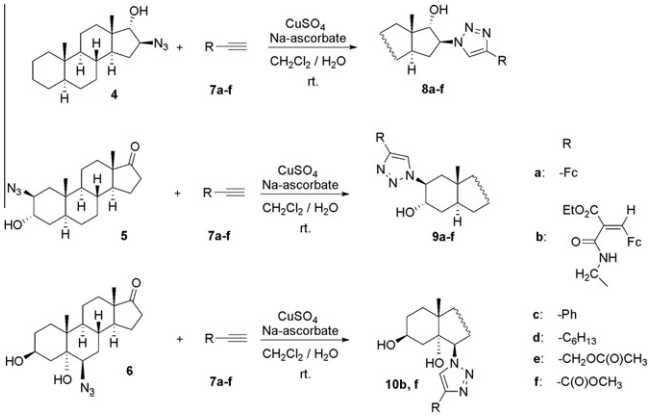
Three steroids (**4–6**), bearing the azido group in different positions of the steroidal skeleton, were synthesised as model compounds by a known procedure [23], starting from epoxides **1–3** (Scheme 2). The ring-opening was completely selective in each case, leading to the 16β- (**4**), 2β- (**5**) and 6β-azido-androstanes (**6**) in moderate to excellent yields. No formation of other epimers could be detected.

First, two alkynyl-ferrocene derivatives, ethynylferrocene (**7a**) and a ferrocenylmethylidene–malonic acid derivative (**7b**) were used as reaction partners (Scheme 3).

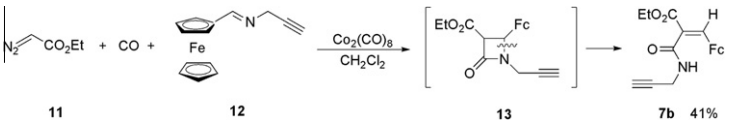
Compound **7b** was obtained by the cobalt-catalyzed domino reaction developed in our group (Scheme 4) [24]. The cobalt-catalyzed carbonylation of ethyl diazoacetate (**11**) leads to ethoxycarbonyl ketene that reacts rapidly with ferrocenylimine **12** to yield



Scheme 2. Synthesis of azido-androstanes **4–6**.



Scheme 3. CuAAC reaction of steroids **4–6** and alkynes **7a–f**.



Scheme 4. Synthesis of **7b** by the domino reaction of ethyl diazoacetate (**11**), ferrocenylimine **12** and carbon-monoxide.

Table 1
CuAAC reaction of alkyne **7a** and steroid **4** under different conditions[a].

Entry	Catalyst	Additive	Solvent	r. temperature (°C)	Yield of **8a** (%)
1	CuI	DIPEA[b]	DIPEA	rt	55
2	CuI	DIPEA[b]	CH_3CN	60	60
3	$CuSO_4$	Na-ascorbate	iPrOH/H_2O	60	70
4	$CuSO_4$	Na-ascorbate	CH_2Cl_2/H_2O	rt	76

[a] Reaction conditions: **4**/**7a**/Cu = 0.2 mmol/0.2 mmol/0.03 mmol in 4 ml solvent, 8 h.
[b] DIPEA: *N,N*-diisopropylethylamine.

β-lactam **13**. An immediate N(1)-C(4) cleavage of the latter compound results in the formation of **7b**. Compound **7b** might have preferential applications in click reactions, since its use leads to steroidal triazoles connected to the bulky ferrocene moiety through a linker, which may be desirable in order not to hamper the attachment of the steroid to a receptor (*vide infra*). 17α-ferrocenyl-17β-estradiol and 17α-ferrocenylethynyl-17β-estradiol were found to have a relative binding affinity of 8% and 28% to the estrogen α-receptor, respectively [1]. This shows a favorable effect of the introduction of a linker between the steroidal skeleton and the ferrocenyl moiety.

In the CuAAC reaction the key intermediate is a Cu(I) acetylide that is obtained in the reaction of the precursor Cu(I) salt and the terminal acetylene [6]. The Cu(I) catalyst can also be obtained in situ, starting from a Cu(II) salt and sodium ascorbate.

Cycloaddition of **7a** and **4** (Scheme 4) was used as a model reaction to determine the optimal conditions (Table 1). The activities of CuI + base (entries 1,2) and $CuSO_4$ + Na–ascorbate catalyst systems (entries 3,4), commonly used for similar reactions, were compared. The application of the Cu(II) precursor was found to be more efficient, so the subsequent reactions were carried out under the conditions used in entry 4 (method D).

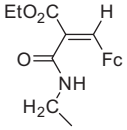
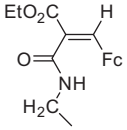
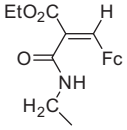
Similar yields were obtained in the CuAAC reaction of **4** and **7b** (Table 2, entry 2), as well as in the cycloaddition of **5** and alkynes **7a**,**b** (Table 2, entries 7,8).

At the same time, a different behavior of the 6β-azido derivative **6** was observed. The target compound was detected only in traces by TLC in the CuAAC reaction of **6** with ethynylferrocene **7a**. The application of other conditions, corresponding to those used for the cycloaddition of **4** and **7a** (Table 1, entries 1–3), did not improve the course of the cycloaddition, either. On the other hand, cycloaddition of **6** with **7b**, where the alkyne and the bulky ferrocene moieties are separated by a spacer, led to product **10b** with acceptable yield (Table 2, entry 14).

In order to explore if this difference in the reactivity of the steroids **4**–**6** is restricted to the click reactions of the bulky ferrocenyl alkynes, cycloaddition of the azides **4**–**6** with simple alkynes (**7c–f**) were also investigated.

The reactions of azides **4** and **5** gave the corresponding triazoles **8c–f** and **9c–f** in good yields (Table 2, entries 3–6 and 9–12, respectively). The results obtained with **4** were slightly better. Azide **6** was proved to be considerably less reactive again. No reaction was observed with **7c** and **7e** and the substrate was recovered unchanged in both cases. Triazole **10f** was obtained in moderate yield using the activated alkyne **7f** (Table 2, entry 17).

Table 2
CuAAC reaction of steroids **4**–**6** with alkynes **7a–f** [a].

Entry	Steroid	Alkyne	R	Product	Yield (%)
1	**4**	**7a**	Fc	**8a**	76
2	**4**	**7b**		**8b**	67
3	**4**	**7c**	Ph	**8c**	72
4	**4**	**7d**	C_6H_{13}	**8d**	59
5	**4**	**7e**	$CH_2OC(O)CH_3$	**8e**	75
6	**4**	**7f**	$C(O)OCH_3$	**8f**	72
7	**5**	**7a**	Fc	**9a**	64
8	**5**	**7b**		**9b**	63
9	**5**	**7c**	Ph	**9c**	63
10	**5**	**7d**	C_6H_{13}	**9d**	47
11	**5**	**7e**	$CH_2OC(O)CH_3$	**9e**	67
12	**5**	**7f**	$C(O)OCH_3$	**9f**	68
13	**6**	**7a**	Fc		traces
14	**6**	**7b**		**10b**	57
15	**6**	**7c**	Ph		no reaction
16	**6**	**7e**	$CH_2OC(O)CH_3$		no reaction
17	**6**	**7f**	$C(O)OCH_3$	**10f**	47

[a] Reaction conditions catalyst: 15% $CuSO_4$, 38% Na-ascorbate in CH_2Cl_2/H_2O, 24 h, rt.

According to these experiments, the reactivity of steroidal azides decreased in the order **4** > **5** >> **6**. Although steric crowding caused by the proximity of the angular methyl group seems to be similar in the 2β and 6β positions, the steric hindrance hampering the addition of alkyne to the azide moiety might be reduced by the flipping of the corresponding ring of the steroid scaffold from conformers **5-ax** and **6-ax** to conformers **5-eq**, and **6-eq**, respectively (Fig. 1.). As a consequence, the carbon atom in position 2 (compound **5**) and in position 6 (compound **6**) is puckered out of plane arranging the A and B rings in twisted boat conformation, respectively. Consequently, the azido groups are allocated in equatorial positions with significantly less steric hindrance. The free energy difference between **5-ax** and **5-eq** is 3.4 kcal/mol in favor of the former structure, with all the cyclohexane rings in chair conformation. For species **6-ax**, however, the ring flipping is a far less favored process. The resulting structure **6-eq**, with the B ring in twisted boat conformation, is higher in free energy by 11.6 kcal/mol. Thus, it can be stated that in spite of the presumably higher reactivity of **6-eq**, its population is almost negligible resulting in slower reaction of **6** towards the addition of alkynes.

For species **4**, however, only one conformer was found to be a genuine minimum (depicted also in Fig. 1) with the D ring in

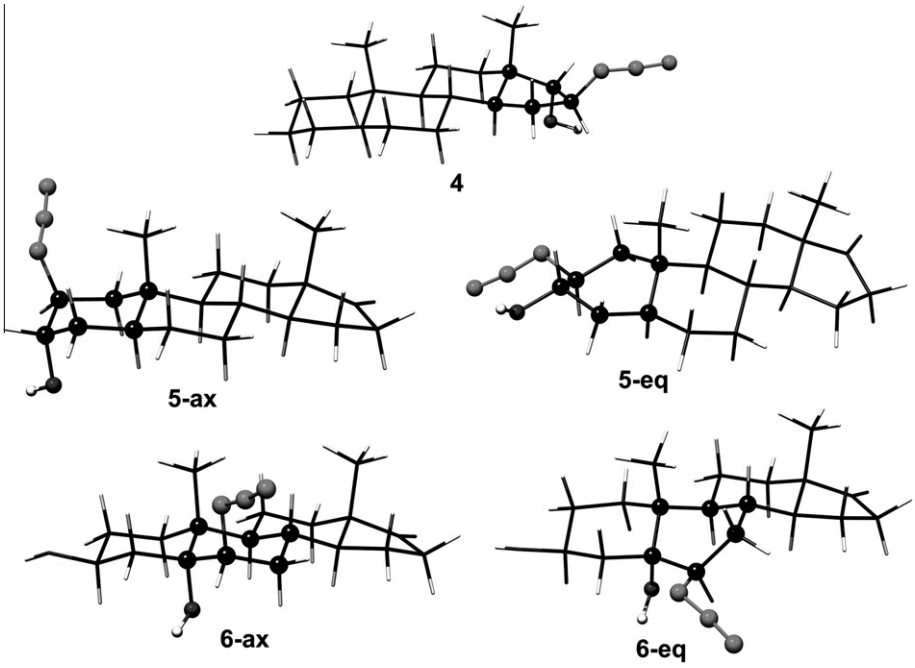


Fig. 1. Conformations of steroidal azides **4**, **5** and **6**.

half-chair conformation. No local minimum was found for the envelope conformation possessing the azido moiety in a clearly axial position. At the same time, the azido group is sterically the least hindered compared to all conformers of **5** and **6** which may explain its higher reactivity.

4. Conclusions

Copper-catalyzed azide–alkyne cycloaddition can efficiently be used for the synthesis of ferrocene-labeled steroids with triazole groups attached directly to the 2β and 16β positions. At the same time, considerably lower reactivity of azide **6** towards both ferrocenyl and simple alkynes was observed. According to the computational studies, the azido group is in the least hindered position in the 16β azido-steroid **4**. Flipping of ring A of steroid **5** into a twisted boat conformer with the 2β azido group in equatorial position is still feasible. At the same time, there is a great difference in the free energies of the twisted boat and the chair conformations of the B ring in steroid **6**, in favor of the latter structure. So steric hindrance cannot be released by a conformational change in compound **6** that may result in a considerably lower reactivity.

Acknowledgments

The authors thank the Hungarian National Science Foundation (OTKA NK71906) and project 'Developing Competitiveness of Universities in the South Transdanubian Region' (SROP-4.2.1.B-10/2/KONV-2010-0002) for financial support. Z.C. thanks the Bolyai Grant of the Hungarian Academy of Sciences. The support of the project TÁMOP-4.2.2/B-10/1-2010-0025, realized with the support of the Hungarian Government and the European Union, with the co-funding of the European Social Fund, is also acknowledged.

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