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Stereoselective synthesis of spiro and condensed pyrazolines of steroidal α,β-unsaturated ketones and nitrilimines by 1,3-dipolar cycloaddition

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ABSTRACT

Effective syntheses of endo- and exocyclic α,β-unsaturated ketones as C=C dipolarophiles were carried out in the 13α-estrone series. The 1,3-dipolar cycloadditions of 15,16α,β-unsaturated ketones of 13α-estrone 3-methyl and 3-benzyl ether with nitrilimines stereoselectively furnished two regioisomers of new condensed pyrazolines in a ratio of 2:1. The main product was the isomer obtained by the attack of the N-terminus of the 1,3-dipole on the carbon atom β to the carbonyl group of the dipolarophile. The nitrilimine cycloadditions to the 16-methylene-17-ketones of 13α-estrone 3-methyl and 3-benzyl ether stereo- and regioselectively furnished spiropyrazolines. The attack of the N-terminus of the dipole occurred on the α-carbon of the α,β-unsaturated ketones. The reactions were performed under both homogeneous and heterogeneous conditions. Silver acetate as a base proved more effective than its triethylamine counterpart. Changes in regio- and stereoselectivities were not observed on variation of the conditions of the cycloaddition reactions. The structures of the new products were determined by NMR (one- and two-dimensional) and MALDI TOF MS techniques, with C_{70} fullerenes as matrix in the latter case.

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1. Introduction

Synthesis of derivatives of naturally occurring terpenes including pyrazolo analogs has recently received considerable attention. Natural terpenes can suppress the proliferation of murine B16 melanoma and other carcinogenic processes [1–3]. Moreover, the pyrazole ring comprises the core structure of a number of drugs [4]. The reactions of nitrilimine 1,3-dipoles with dipolarophiles provide an option for the construction of substituted pyrazoles [5]. Nitrilimines are well-known intermediates generated *in situ* by base-promoted dehydrohalogenation of the corresponding hydrazonoyl halides [6–9]. The nitrilimine cycloadditions of certain enones or allylic alcohols were recently reported under both homogeneous and heterogeneous conditions [7,8]. For α,β-unsaturated ketones containing a trisubstituted C=C bond, only the cycloadducts in which the C-terminus of the 1,3-dipole is bonded to the carbon atom α to the carbonyl group were obtained. This structure results from the electronic demands of the cycloaddition. Nitrilimine cycloaddition to 2-arylmethylidene-indanones or -tetralones leads to spiropyrazolines with high regioselectivity, but differences in diastereoselectivity, depending on the conformational arrangement of substituents of the dipolarophile [10–12].

13α-Estrone derivatives possess a quasi-equatorial angular methyl group and their conformation depends on the substitution pattern of ring D [13–17]. The target binding properties of these derivatives are strongly dependent on the conformation. The conformational control provides an option to influence the binding activities of the molecules. Only two types of condensed N-containing heterocyclic 13α-estrone derivatives (condensed aza-D-homoestrones and tetrahydroquinolines) are known [18–20]; accordingly, we are interested in the synthesis and stereochemical investigation of such compounds. A few 16,17-condensed steroidal pyrazolines of pharmacological significance have been described [21–24], but steroidal 16-spiro- or 15,16-condensed 17-ketopyrazolines are not known.

2. Experimental

Melting points (mps) were determined with a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were performed with a PerkinElmer CHN analyzer model 2400. Thin-layer chromatography: silica gel 60 F_{254}; layer thickness 0.2 mm (Merck);

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detection with iodine or UV (365 nm) after spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid and heating at 100–120 °C for 10 min. Flash chromatography: silica gel 60, 40–63 μm (Merck). ^{1}H NMR spectra were recorded in $CDCl_3$ solution (if not otherwise stated) with a Bruker DRX-500 instrument at 500 MHz, with Me_4Si as internal standard. ^{13}C NMR spectra were recorded with the same instrument at 125 MHz under the same conditions. The mass spectrometer used was an Autoflex II TOF/TOF (Bruker Daltonics, Bremen, Germany) operated in reflector mode. The ions were accelerated under delayed extraction conditions (80 ns) in positive and negative ion modes, with an acceleration voltage of 20.00 kV. The instrument uses a 337 nm pulsed (50 Hz) nitrogen laser. 1 μl aliquots of the standard solutions were loaded onto the target plate (MTP 384 target plate ground steel TF, Bruker Daltonics, Bremen, Germany) by mixing with the same volume of a saturated matrix solution prepared by dissolving C_{70} fullerenes in toluene.

2.1. Synthesis of *3a* or *3b*

To a suspension of **1a** (10 g, 44 mmol) or **1b** (15.9 g, 44 mmol) in triethyl orthoformate (15 ml, 88 mmol) and ethylene glycol (6 ml, 110 mmol), a catalytic amount of *p*-TsOH was added, and the mixture was gently heated until the suspension became a clear solution (0.5 h). The warm reaction mixture was then poured into a saturated solution of sodium bicarbonate (100 ml). The white precipitate formed was filtered off, washed with water and dried in the air. To a solution of the crude product **2a** (10 g, 30 mmol) or **2b** (15 g, 37 mmol) in abs. tetrahydrofuran (100 ml), 10 g (30 mmol) of pyridinium hydrobromide perbromide was added. A white precipitate was formed immediately from the yellow solution. The reaction mixture was stirred for 2 h at rt, and then diluted with a saturated aqueous solution of sodium bicarbonate (0.5 l). The precipitate formed was filtered off, washed with water and dried in the air. The crude product **3a** or **3b** was transformed without purification.

2.2. Synthesis of *4a* or *4b*

To a solution of **3a** (4.07 g, 10 mmol) or **3b** (4.83 g, 10 mmol) in dimethyl sulfoxide (40 ml) potassium *tert*-butylate (2.24 g, 20 mmol) was added. The suspension was vigorously stirred and heated (80 °C) until it became a clear yellow-brown solution (5 h). The mixture was poured into ice-water (1 l), and the resulting precipitate was filtered off, washed with water and dried in the air. The crude product **4a** or **4b** was subjected to chromatographic separation, with 30% diisopropyl ether/70% hexane as eluent.

Data for **4a**: 2.45 g, 75%; mp 92–95 °C; R_f = 0.47 (CH_2Cl_2). Anal. Calcd. for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03. Found: C, 77.42; H, 7.92. ^{1}H NMR 1.10 (s, 3H, 18-H_3), 2.80 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 3.90 (m, 3H) and 4.00 (m, 1H): ketal-OCH_2, 5.71 (d, 1H, J = 5.9 Hz, 16-H), 6.33 (m, 1H, 15-H), 6.58 (d, 1H, J = 2.3 Hz, 4-H), 6.71 (dd, 1H, J = 8.5 Hz, J = 2.3 Hz, 2-H), 7.20 (d, 1H, J = 8.5 Hz, 1-H). ^{13}C NMR 27.7 (C-18), 28.1, 29.6, 30.3, 31.3, 40.5, 44.5, 44.9 (C-13), 55.2 (3-OMe), 57.3, 64.5 and 64.9 (2× OCH_2), 111.9 (C-4), 113.4 (C-2), 120.8 (C-17), 127.6 (C-1), 130.5 and 139.1 (C-15 and C-16), 132.6 (C-10), 138.1 (C-5), 157.2 (C-3). MS positive mode: 327 (20%, $[M+H]^+$).

Data for **4b**: 3.42 g, 85%; mp 137–140 °C; R_f = 0.58 (CH_2Cl_2). Anal. Calcd. for $C_{27}H_{30}O_3$: C, 80.56; H, 7.51. Found: C, 80.68; H, 7.66. ^{1}H NMR 1.12 (s, 3H, 18-H_3), 2.81 (m, 2H, 6-H_2), 3.90 (m, 3H) and 4.02 (m, 1H): ketal-OCH_2, 5.04 (s, 2H, benzyl-OCH_2), 5.72 (d, 1H, J = 5.8 Hz, 16-H), 6.34 (m, 1H, 15-H), 6.69 (d, 1H, J = 2.3 Hz, 4-H), 6.80 (dd, 1H, J = 8.5 Hz, J = 2.3 Hz, 2-H), 7.22 (d, 1H, J = 8.5 Hz, 1-H), 7.32 (t, 1H, J = 7.1 Hz, 4′-H), 7.39 (t, 2H, J = 7.1 Hz, 3′-H and 5′-H), 7.44 (d, 2H, J = 7.1 Hz, 2′-H and 6′-H). ^{13}C NMR 27.7 (C-18), 28.1, 29.6, 30.2, 31.3, 40.5, 44.5, 45.0 (C-13), 57.3, 64.5 and 64.9 (2× OCH_2), 70.0 (benzyl-OCH_2), 112.7 (C-4), 114.5 (C-2), 120.8 (C-17), 127.4 (2C) and 128.5 (2C): C-2′, C-3′, C-5′ and C-6′, 127.7 and 127.8 (C-1 and C-4′), 130.5 and 139.1 (C-15 and C-16), 132.9 (C-10), 137.4 (C-1′), 138.1 (C-5), 156.5 (C-3). MS positive mode: 403 (20%, $[M+H]^+$).

2.3. Synthesis of *5a* or *5b*

To a solution of **4a** (1.30 g, 4 mmol) or **4b** (1.61 g, 4 mmol) in acetone (25 ml), formalin (5 ml, 60 mmol formaldehyde) and a catalytic amount of *p*-TsOH were added. The mixture was stirred for 1.5 h at rt, and then poured into water (100 ml). The precipitate formed was filtered off, washed with water and dried in the air. The crude product **5a** or **5b** was subjected to chromatographic separation, with 50% diisopropyl ether/50% hexane as eluent.

Data for **5a**: 1.10 g, 98%; mp 70–73 °C; R_f = 0.25 (CH_2Cl_2). Anal. Calcd. for $C_{19}H_{22}O_2$: C, 80.82; H, 7.85. Found: C, 80.97; H, 7.72. ^{1}H NMR 1.21 (s, 3H, 18-H_3), 2.81 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OMe), 6.17 (dd, 1H, J = 5.8 Hz, J = 1.4 Hz, 16-H), 6.59 (d, 1H, J = 2.4 Hz, 4-H), 6.73 (dd, 1H, J = 8.6 Hz, J = 2.4 Hz, 2-H), 7.14 (d, 1H, J = 8.6 Hz, 1-H), 7.78 (dd, 1H, J = 5.8 Hz, J = 2.5 Hz, 15-H). ^{13}C NMR 24.2 (C-18), 27.0, 28.2, 29.1, 29.8, 38.4, 41.2, 46.5 (C-13), 55.2 (3-OMe), 56.6, 112.3 (C-4), 113.2 (C-2), 128.0 (C-1), 131.2 (C-15), 132.7 (C-10), 137.2 (C-5), 157.4 (C-3), 163.9 (C-16), 214.1 (C-17). MS positive mode: 282 (15%, $[M+H]^+$).

Data for **5b**: 1.38 g, 96%; mp 111–113 °C; R_f = 0.24 (CH_2Cl_2). Anal. Calcd. for $C_{25}H_{26}O_2$: C, 83.76; H, 7.31. Found: C, 83.94; H, 7.26. ^{1}H NMR 1.21 (s, 3H, 18-H_3), 2.80 (m, 2H, 6-H_2), 5.03 (s, 2H, OCH_2), 6.18 (dd, 1H, J = 5.8 Hz, J = 1.7 Hz, 16-H), 6.68 (d, 1H, J = 2.6 Hz, 4-H), 6.80 (dd, 1H, J = 8.6 Hz, J = 2.6 Hz, 2-H), 7.14 (d, 1H, J = 8.6 Hz, 1-H), 7.31 (t, 1H, J = 7.0 Hz, 4′H), 7.38 (t, 2H, J = 7.0 Hz, 3′-H and 5′-H), 7.42 (d, 2H, J = 7.0 Hz, 2′-H and 6′-H), 7.77 (dd, 1H, J = 5.8 Hz, J = 2.5 Hz, 15-H). ^{13}C NMR 24.2 (C-18), 27.0, 28.2, 29.2, 29.9, 38.4, 41.2, 46.5 (C-13), 56.7, 70.0 (OCH_2), 113.1 (C-4), 114.4 (C-2), 127.4 and 128.5 (2 × 2C, C-2′, C-3′, C-5′ and C-6′), 127.8 and 128.0 (C-1 and C-4′), 131.3 (C-15), 133.1 (C-10), 137.1 (C-1′), 137.2 (C-5), 156.7 (C-3), 163.9 (C-16), 214.1 (C-17).

MS positive mode: 357 (10%, $[M-H]^+$), 91 (100%, benzylic cation).

2.4. Synthesis of *7a* or *7b*

To a suspension of **6a** (3.12 g, 10 mmol) or **6b** (3.72 g, 10 mmol) in acetone (50 ml), formalin (31 ml, 372 mmol formaldehyde) was added, and the mixture was maintained at rt for 0.5 h. Powdered sodium carbonate (7.13 g, 66 mmol) was next added and the mixture was vigorously stirred for 24 h, diluted with water, and acidified with hydrochloric acid until pH 5. The resulting precipitate was filtered off, washed with water, and dried in the air. The crude product **7a** or **7b** was subjected to chromatographic purification, with dichloromethane as eluent.

Data for **7a**: 26.6 g, 90%; mp 120–124 °C; R_f = 0.52 (CH_2Cl_2). Anal. Calcd. for $C_{20}H_{24}O_2$: C, 81.04; H, 8.16. Found: C, 80.95; H, 8.27. ^{1}H NMR 1.06 (s, 3H, 18-H_3), 2.80 (m, 2H, 6-H_2), 3.76 (s, 3H, 3-OMe), 5.38 and 6.08 (2 × s, 2s1H, 16a-H_2), 6.57 (d, 1H, J = 2.6 Hz, 4-H), 6.68 (dd, 1H, J = 8.6 Hz, J = 2.6 Hz, 2-H), 7.16 (d, 1H, J = 8.6 Hz, 1-H). ^{13}C NMR 25.1 (18-Me), 28.0, 28.1, 29.9, 30.2, 32.1, 41.2, 42.2, 46.5, 50.1 (C-13), 55.1 (3-OMe), 111.6 (C-4), 113.4 (C-2), 119.3 (C-16a), 126.8 (C-1), 131.8 (C-10), 137.9 (C-5), 143.0 (C-16), 157.4 (C-3), 208.5 (C-17). MS positive mode: 296 (50%, $[M]^+$).

Data for **7b**: 34.2 g, 92%; mp 155–158 °C; R_f = 0.64 (CH_2Cl_2). Anal. Calcd. for $C_{26}H_{28}O_2$: C, 83.83; H, 7.58. Found: C, 83.65; H, 7.38. ^{1}H NMR 1.08 (s, 3H, 18-H_3), 2.84 (m, 2H, 6-H_2), 5.02 (s, 2H, OCH_2), 5.39 (s, 1H) and 6.10 (s, 1H): 16a-H_2, 6.68 (d, 1H, J = 2.4 Hz, 4-H), 6.77 (dd, 1H, J = 8.6 Hz, J = 2.4 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.31 (t, 1H, J = 7.3 Hz, 4′-H), 7.37 (t, 2H, J = 7.3 Hz, 3′-H and 5′-H), 7.41 (d, 2H, J = 7.3 Hz, 2′-H and 6′-H). ^{13}C NMR 25.3 (C-18), 28.1, 28.2, 30.1, 30.3,

32.3, 41.3, 42.3, 46.6, 50.2 (C-13), 70.0 (OCH_2), 112.5 (C-4), 114.6 (C-2), 119.4 (C-16a), 126.9 and 127.8 (C-1 and C-4′), 127.4 and 128.5 (2 × 2C, C-2′, C-3′ C-5′ and C-6′), 132.2 (C-10), 137.3 (C-1′), 138.0 (C-5), 143.1 (C-16), 156.8 (C-3), 208.5 (C-17). MS positive mode: 371 (5%, $[M-H]^+$), 91 (100%, benzylic cation).

2.5. Synthesis of **8**

Diazonium salt of *p*-toluidine: 1.9 g (18 mmol) of *p*-toluidine was suspended in water (25 ml) and cc. sulfuric acid (2.5 ml, 47 mmol) was added. The mixture was cooled below 10 °C, and 1.25 g (18 mmol) of sodium nitrite in water (6 ml) was added in 5 amounts under vigorous stirring. After 20 min of stirring, a brown solution was obtained. A solution of 2.5 ml (18 mmol) of ethyl-2-chloroacetoacetate in 50% pyridine-water (20 ml) was added and the mixture was stirred for an additional 15 min. The orange precipitate that resulted was filtered off, washed with water, dried in the air and recrystallized from methanol.

2.6. General procedures for the synthesis of condensed pyrazolines and spiropyrazolines

Procedure A: a solution of hydrazonoyl chloride **8a** (170 mg, 1 mmol) and enone **5a** (140 mg, 0.5 mmol) or **5b** (180 mg, 0.5 mmol) or **7a** (150 mg, 0.5 mmol) or **7b** (186 mg, 0.5 mmol) in dry toluene (5 ml) was treated with triethyl amine (0.28 ml, 2 mmol) and refluxed under a nitrogen atmosphere for 48 h. The solvent was then evaporated off and the residue was subjected to column chromatography with 30% diisopropyl ether/70% hexane.

Procedure B: a solution of hydrazonoyl chloride **8a** (170 mg, 1 mmol) and enone **5a** (140 mg, 0.5 mmol) or **5b** (180 mg, 0.5 mmol) or **7a** (150 mg, 0.5 mmol) or **7b** (186 mg, 0.5 mmol) in dry toluene (5 ml) was treated with silver acetate (200 mg, 1 mmol) and stirred in the dark at rt under a nitrogen atmosphere for 24 h. The solvent was then evaporated off and the residue was subjected to column chromatography with 30% diisopropyl ether/70% hexane.

2.6.1. Synthesis of ***9a*** and ***10a***

Following the reaction of **5a** with **8** as in General Procedure B, the chromatographic elution first yielded **9a**, which was obtained as a white solid (106 mg, 44%). Mp 179–182 °C; R_f = 0.62 (CH_2Cl_2). Anal. Calcd. for $C_{30}H_{34}N_2O_4$: C, 74.05; H, 7.04. Found: C, 73.92; H, 7.18.^{1}H NMR 1.06 (s, 3H, 18-H_3), 1.43 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3), 2.30 (s, 3H, tolyl-Me), 2.88 (m, 2H, 6-H_2), 3.77 (s, 3H, 3-OMe), 4.16 (d, 1H, *J* = 13.1 Hz, 16-H), 4.42 (m, 2H, OCH_2CH_3), 4.69 (d, 1H, *J* = 13.1 Hz, 15-H), 6.62 (d, 1H, *J* = 2.3 Hz, 4-H), 6.71 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 7.11 (d, 2H, *J* = 8.3 Hz, 2′-H and 6′-H), 7.18 (d, 1H, *J* = 8.5 Hz, 1-H), 7.41 (d, 2H, *J* = 8.3 Hz, 3′-H and 5′-H). ^{13}C NMR δ ppm 14.4 (OCH_2CH_3), 20.6 (tolyl-Me), 26.3 (18-Me), 28.0 (2C), 30.4, 33.6, 41.3, 46.2, 50.6 (C-13), 50.9, 51.4, 55.2 (3-OMe), 61.3 (OCH_2CH_3), 67.9, 111.9 (C-4), 113.6 (C-2), 115.1 (2C, C-2′ and C-6′), 126.9 (C-1), 129.6 (2C, C-3′ and C-5′), 131.3 (C-4′), 131.8 (C-10), 137.9 (C-5), 140.2 and 141.4 (C-1′ and C=N), 157.7 (C-3), 162.4 ($COOCH_2CH_3$), 212.3 (C-17). MS positive mode: 486 (20%, $[M]^+$), 441 (80%, M^+-C_2H_5O).

Continued elution yielded **10a** (53 mg, 22%). Mp 210–215 °C; R_f = 0.18 (CH_2Cl_2). Anal. Calcd. for $C_{30}H_{34}N_2O_4$: C, 74.05; H, 7.04. Found: C, 74.18; H, 6.95.^{1}H NMR 1.05 (s, 3H, 18-H_3), 1.38 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3), 2.34 (s, 3H, tolyl-Me), 2.90 (m, 2H, 6-H_2), 3.78 (s, 3H, 3-OMe), 4.22 (d, 1H, *J* = 13.1 Hz, 15-H), 4.37 (m, 2H, OCH_2CH_3), 5.12 (d, 1H, *J* = 13.1 Hz, 16-H), 6.62 (d, 1H, *J* = 2.4 Hz, 4-H), 6.73 (dd, 1H, *J* = 8.6 Hz, *J* = 2.4 Hz, 2-H), 7.18 (overlapping doublets, 3H, 1-H, 2′-H and 6′-H), 7.25 (d, 2H, *J* = 8.6 Hz, 3′-H and 5′-H). ^{13}C NMR *δ* ppm 14.3, 20.6, 26.8, 28.1, 28.3, 30.3, 33.9, 41.3, 44.0, 50.6, 51.8, 55.2 (3-OMe), 56.4 (C-15), 61.3 (OCH_2CH_3), 68.4 (C-16), 112.1 (C-2), 113.5 (C-4), 115.4 (2C, C-2′ and C-6′), 127.1 (C-1), 129.9 (2C, C-3′ and C-5′), 130.9 (C-4′), 132.1 (C-10), 135.2 (C=N), 137.1 (C-5), 139.7 (C-1′), 157.7 (C-3), 161.7 ($COOCH_2CH_3$), 212.2 (C-17). MS positive mode: 486 (10%, M^+), 441 (50%, M^+-C_2H_5O).

2.6.2. Synthesis of ***9b*** and ***10b***

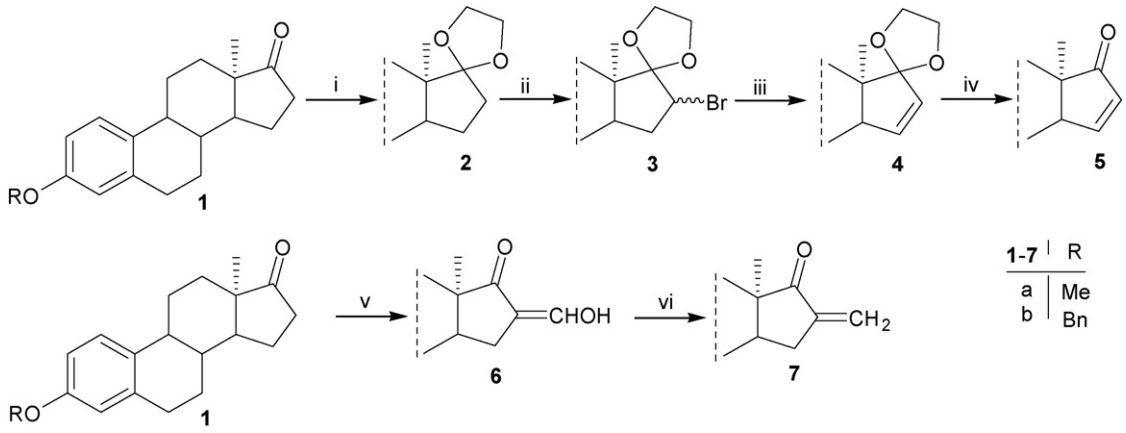
Following the reaction of **5b** with **8** as in General Procedure B, the chromatographic elution first yielded **9b**, which was obtained as a white solid (136 mg, 48%). Mp 186–190 °C; R_f = 0.55 (CH_2Cl_2). Anal. Calcd. for $C_{36}H_{38}N_2O_4$: C, 76.84; H, 6.81. Found: C, 76.73; H, 6.98. ^{1}H NMR 1.06 (s, 3H, 18-H_3), 1.43 (t, 3H, *J* = 7.1 Hz, OCH_2CH_3), 2.30 (s, 3H, tolyl-Me), 2.88 (m, 2H, 6-H_2), 4.16 (d, 1H, *J* = 13.1 Hz, 16-H), 4.41 (m, 2H, OCH_2CH_3), 4.68 (d, 1H, *J* = 13.1 Hz, 15-H), 5.03 (s, 2H, benzyl-OCH_2), 6.72 (d, 1H, *J* = 2.4 Hz, 4-H), 6.79 (dd, 1H, *J* = 8.6 Hz, *J* = 2.4 Hz, 2-H), 7.12 (d, 2H, *J* = 9.4 Hz, 2′-H and 6′-H), 7.18 (d, 1H, *J* = 8.6 Hz, 1-H), 7.32 (t, 1H, *J* = 7.3 Hz, 4″-H), 7.38 (t, 2H, *J* = 7.3 Hz, 3″-H and 5″-H), 7.41 (overlapping multiplets, 4H, 2″-H, 6″-H, 3′-H and 5′-H). ^{13}C NMR *δ* ppm 14.4 (OCH_2CH_3), 20.6 (tolyl-Me), 26.3 (18-Me), 27.8, 27.9, 30.3, 33.5, 41.2, 46.1, 50.6 (C-13), 50.7, 51.1 (C-16), 61.4 (OCH_2CH_3), 67.8 (C-15), 70.0 (benzyl-OCH_2), 112.6 (C-2), 114.4 (C-4), 114.9 (2C, C-2′ and C-6′), 126.9 (C-1), 127.4 and 128.5 (2 × 2C, C-2″, C-3″, C-5″ and C-6″), 127.9 (C-4″), 129.6 (2C, C-3′ and C-5′), 131.4 (C-4′), 131.7 (C-10), 137.0 (C-1″), 137.9 (C-5), 139.9 (C-1′), 141.2 (C=N), 156.8 (C-3), 162.3 ($COOCH_2CH_3$), 212.4 (C-17). MS positive mode: 562 (20%, $[M]^+$), 517 (40%, M^+-C_2H_5O), 304 (25%, M^+-259), 91 (100%, benzylic cation). Continued elution yielded **10b** (68 mg, 24%). Mp 216–220 °C; R_f = 0.20 (CH_2Cl_2). Anal. Calcd. for $C_{36}H_{38}N_2O_4$: C, 76.84; H, 6.81. Found: C, 76.63; H, 6.95.^{1}H NMR 1.05 (s, 3H, 18-H_3), 1.38 (t, 3H, *J* = 7.1 Hz, OCH_2CH_3), 2.33 (s, 3H, tolyl-Me), 2.91 (m, 2H, 6-H_2), 4.22 (d, 1H, *J* = 11.8 Hz, 15-H), 4.37 (m, 2H, OCH_2CH_3), 5.03 (s, 2H, benzyl-OCH_2), 5.11 (d, 1H, *J* = 11.8 Hz, 16-H), 6.71 (d, 1H, *J* = 2.4 Hz, 4-H), 6.79 (dd, 1H, *J* = 8.6 Hz, *J* = 2.4 Hz, 2-H), 7.18 (overlapping doublets, 3H, 1-H, 2′-H and 6′-H), 7.25 (d, 2H, *J* = 8.7 Hz, 3′-H and 5′-H), 7.32 (t, 1H, *J* = 7.3 Hz, 4″-H), 7.38 (t, 2H, *J* = 7.3 Hz, 3″-H and 5″-H), 7.42 (d, 2H, *J* = 7.3 Hz, 2″-H and 6″-H). ^{13}C NMR *δ* ppm 14.3 (OCH_2CH_3), 20.6 (tolyl-Me), 26.8 (18-Me), 28.1, 28.3, 30.3, 33.9, 41.3, 44.0, 50.6, 51.8 (C-13), 56.4 (C-15), 61.3 (OCH_2CH_3), 68.5 (C-16), 70.0 (benzyl-OCH_2), 112.9 (C-4), 114.5 (C-2), 115.4 (2C, C-2′ and C-6′), 127.1 and 127.8 (C-1 and C-4″), 127.4 and 128.5 (2 × 2C, C-2″, C-3″, C-5″ and C-6″), 129.9 (2C, C-3′ and C-5′), 131.2 (C-4′), 132.1 (C-10), 135.2 (C=N), 137.0 (C-1″), 137.2 (C-5), 139.7 (C-1′), 157.0 (C-3), 161.6 ($COOCH_2CH_3$), 212.1 (C-17). MS positive mode: 562 (10%, $[M]^+$), 517 (50%, M^+-C_2H_5O), 304 (20%, M^+-259), 91 (100%, benzylic cation).

2.6.3. Synthesis of ***11a***

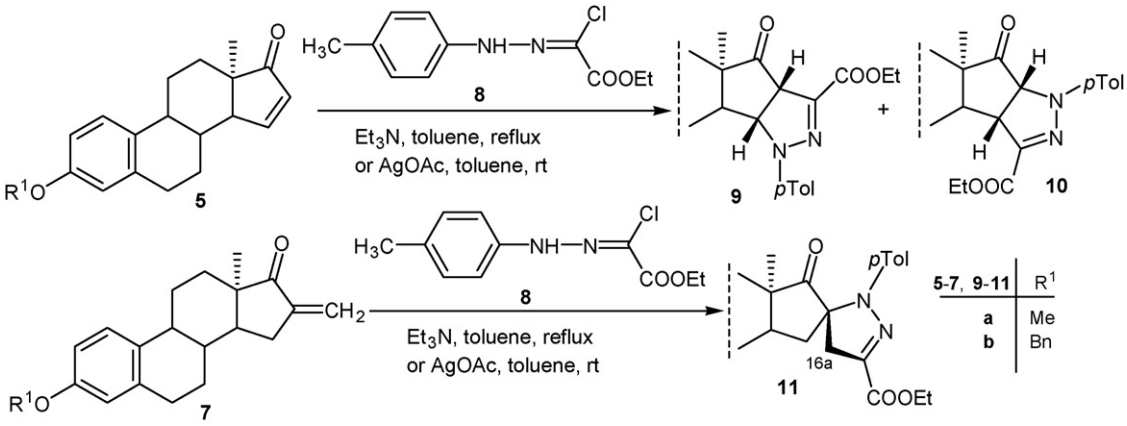
Following the reaction of **7a** with **8** as in General Procedure B, **11a** was obtained as a white solid (198 mg, 78%). Mp 178–180 °C; R_f = 0.40 (CH_2Cl_2). Anal. Calcd. for $C_{31}H_{36}N_2O_4$: C, 74.37; H, 7.25. Found: C, 74.46; H, 7.18.^{1}H NMR 0.59 (s, 3H, 18-H_3), 1.32 (t, 1H, *J* = 7.1 Hz, OCH_2CH_3), 2.32 (s, 3H, tolyl-Me), 2.85 (m, 2H, 6-H_2), 3.06 and 3.41 (2 × d, 2 × 1H, *J* = 16.9 Hz): 16a-H_2, 3.78 (s, 3H, 3-OMe), 4.28 (m, 2H, OCH_2CH_3), 6.63 (d, *J* = 2.4 Hz, 4-H), 6.72 (dd, 1H, *J* = 8.6 Hz, *J* = 2.4 Hz, 2-H), 7.11 (s, 4H, tolyl aromatic protons), 7.17 (d, 1H, *J* = 8.6 Hz, 1-H). MS positive mode: 501 (5%, $[M+H]^+$), 455 (40%, M^+-C_2H_5O).

2.6.4. Synthesis of ***11b***

Following the reaction of **7b** with **8** as in General Procedure B, **11b** was obtained as a white solid (233 mg, 81%). Mp 221–223 °C; R_f = 0.55 (CH_2Cl_2). Anal. Calcd. for $C_{31}H_{36}N_2O_4$: C, 74.37; H, 7.25. Found: C, 74.51; H, 7.38. ^{1}H NMR *δ* ppm 0.62 (s, 3H, 18-H_3), 0.96 (m, 1H, one 11-H), 1.10 (m, 1H, 8-H), 1.35 (t, 3H, $COOCH_2CH_3$), 1.38 (overlapping multiplets, 2H, one 7-H and 12-H), 1.66 (m, 1H, 14-H), 2.11 (m, 1H, other 7-H), 2.20 (m, 1H, 9-H), 2.23 (m, 1H, one 15-H), 2.25 (m, 1H, 11-H), 2.33 (t, 1H, tolyl-H_3), 2.35 (m, 1H, other 12-H), 2.46 (m, 1H, other 15-H), 2.85 (m, 2H, 6-H_2), 3.09 and 3.45 (2 × d, 2 × 1H,



Scheme 1. Reagents and conditions: (i) 2.5 equiv. of ethylene glycol; 2 equiv. of triethyl orthoformate; catal. amount of *p*-TsOH; rt; 2 h; (ii) 1 equiv. of pyridinium hydrobromide perbromide; THF; rt; (iii) 2 equiv. of KOtBu; DMSO; 80 °C; 5 h; (iv) 15 equiv. of H_2CO; catal. amount of *p*-TsOH; acetone; rt; 30 min; (v) 2 equiv. of $NaOCH_3$; 15 equiv. of HCOOEt; benzene; 50 °C; 6 h; (vi) 50 equiv. of H_2CO; 8 equiv. of Na_2CO_3; acetone; 24 h.



Scheme 2. 1,3-Dipolar cycloadditions of steroidal α,β-unsaturated ketones with nitrilimines.

J = 16.9 Hz, 16a-H_2), 4.30 (q, 2H, *J* = 7.0 Hz, O*C*H_2CH$_3$), 5.06 (s, 2H, benzyl-O*C*H_2), 6.71 (d, 1H, *J* = 2.0 Hz, 4-H), 6.79 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, 2-H), 7.10 (s, 4H, 2′-H, 3′-H, 5′-H and 6′-H), 7.16 (d, 1H, *J* = 8.4 Hz, 1-H), 7.32 (t, 1H, *J* = 7.2 Hz, 4″-H), 7.38 (t, 2H, *J* = 7.2 Hz, 3″-H and 5″-H), 7.42 (d, 2H, *J* = 7.2 Hz, 2″-H and 6″-H). ^{13}C NMR δ ppm 14.4 (O*C*H_2*C*H$_3$), 21.0 (tolyl-*C*H$_3$), 25.6 (C-18), 28.3 (C-7), 28.5 (C-11), 30.2 (C-6), 33.3 (C-12), 34.0 (C-15), 41.1 (C-9), 42.4 (C-8), 46.2 (C-14), 47.9 (C-16a), 49.4 (C-13), 61.0 (O*C*H_2CH$_3$), 70.0 (benzyl-O*C*H_2), 78.2 (C-16), 112.9 (C-2), 114.7 (C-4), 127.1 (3C, C-1 and C-2′,6′), 127.4 (2C) and 128.5 (2C): C-2″, C-3″, C-5″and C-6″, 127.9 (C-4″), 129.4 (2C, C-3′ and C-5′), 131.6 (C-10), 136.9 (C=N), 137.2 (C-4′), 137.3 (C-1″), 137.8 (C-5), 139.0 (C-1′), 157.0 (C-3), 162.5 (*C*OOEt), 218.8 (C-17). MS positive mode: 576 (10%, $[M]^+$), 531 (30%, M^+-C_2H_5O), 91 (100%, benzylic cation).

3. Results and discussion

Our aim was to build up pyrazolines from steroidal α,β-unsaturated ketone dipolarophiles and hydrazonoyl chlorides as nitrilimine precursors. Two different types of dipolarophiles (endo- and exocyclic C=C) were synthesized.

The new endocyclic α,β-unsaturated ketones were prepared from 13α-estrone-3-methyl or 3-benzyl ether **1a** or **1b**, which were obtained by the epimerization of estrone 3-methyl or 3-benzyl ether by the method of Yaremenko and Khvat [13,25,26]. 13α-Estrone is available in one step by irradiation of estrone with monochromatic ultraviolet light [27].

The first step was the protection of the 17-keto function in the form of ethylene ketal in order to prevent the formation of undesired side-products (Scheme 1). Ketals are commonly used to protect the carbonyl function during multistep syntheses, because of the ease of removal of the ketal protecting group [28]. After protection of the 17-keto function, α-bromination was carried out with pyridinium hydrobromide perbromide, which yielded a stereoisomeric mixture of 16-bromo derivatives **3** [29]. Subsequent dehydrohalogenation with potassium *tert*-butylate and deprotection of the 17-ketone led to endocyclic α,β-unsaturated ketone dipolarophiles **5**. 3-Benzyloxy derivative **5b** is of value for pharmacological experiments, because of the ease of removal of the benzyl function. 3-Methoxy- and 3-benzyloxy-estra-1,3,5(10),15-tetraen-17-one have been reported previously, but their 13α counterparts are unknown [30]. The new exocyclic unsaturated ketones **7** were synthesized in excellent yields from the known 16-

Table 1
Synthesis of unsaturated ketones **5** and **7**.

Entry	Substrate	Product	Yield (%)
1	**1a**	**3a**	75
2	**1b**	**3b**	79
3	**3a**	**4a**	75
4	**3b**	**4b**	85
5	**4a**	**5a**	98
6	**4b**	**5b**	96
7	**6a**	**7a**	90
8	**6b**	**7b**	92

Table 2
Reactions between hydrazonoyl chloride **8** and enones **5** and **7** in dry toluene.

Entry	Substrate	Base (equiv.) + reagent	Reaction time (h)	Temperature (°C)	Products	Yields (%)
1	**5a**	Et_3N (4) + **8**	48	110	**9a** + **10a**	27 + 13
2	**5a**	AgOAc (2) + **8**	24	25	**9a** + **10a**	44 + 22
3	**5b**	Et_3N (4) + **8**	48	110	**9b** + **10b**	30 + 15
4	**5b**	AgOAc (2) + **8**	24	25	**9b** + **10b**	48 + 24
5	**7a**	Et_3N (4) + **8**	48	110	**11a**	39
6	**7a**	AgOAc (2) + **8**	24	25	**11a**	78
8	**7b**	Et_3N (4) + **8**	48	110	**11b**	56
9	**7b**	AgOAc (2) + **8**	24	25	**11b**	81

hydroxymethylidene derivatives **6** in one step, using formalin and sodium carbonate in acetone [31]. No side-products were formed, in contrast to the similar reactions in the natural estrone series [31].

The hydrazonoyl chloride **8** was prepared via the *Japp–Klingemann* reaction [32–34]. The diazonium sulfate of 4-methylaniline was coupled to ethyl 2-chloroacetoacetate. The nitrilimine precursor **8** was formed in high yield in a short reaction time and was purified by crystallization from methanol.

The 1,3-dipolar cycloadditions were carried out in dry toluene, under both homogeneous and heterogeneous reaction conditions, with triethylamine or silver(I) acetate as base (Scheme 2). Reactions with triethylamine were carried out under reflux, but only poor yields were obtained (Table 1). The silver salt proved more effective: the reaction mixture was stirred in the dark at rt, under a nitrogen atmosphere. Some starting enone was always recovered and some tarry material was always formed under both conditions.

The reaction of **5** with **8** led to a regioisomeric mixture of **9** and **10**. The main product was the pyrazoline **9** in which the N-terminus of the 1,3-dipole is attached to the β-carbon of the enone **5**. The ratio of the products **9** and **10** was 2:1. The condensed pyrazolines **9** and **10** were formed stereoselectively with the same type of D/E ring junction. The two regioisomers could be easily separated by flash chromatography. The cycloaddition of **7** to **8** under heterogeneous conditions was completely regio- and diastereoselective, yielding one spiropyrazoline, **11**. The dipole and the dipolarophile were joined in the same way as in the minor product of the previous reaction (Table 2).

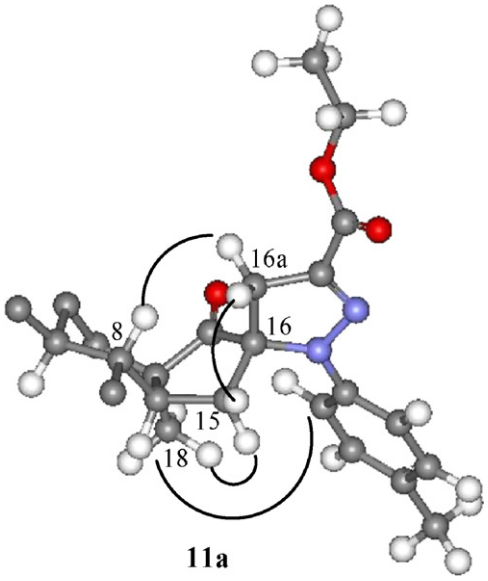


Fig. 1. NOE effects between the relevant protons in rings C, D and E.

4. Structure determination

The structures of the newly formed cycloadducts **9**, **10** and **11** were determined by NMR spectroscopy. The ^{1}H NMR spectra of **9a** and **9b** displayed two doublets, at around δ = 4.2 and 4.7 ppm, due to 16-H and 15-H with a coupling constant of 13 Hz. In the similar spectra of the other regioisomer **10a** and **10b**, an upfield shift and the reversed sequence of the signals of 15-H (4.22 ppm) and 16-H (5.12 ppm) were observed, with a coupling constant of 12 Hz. The regio- and stereochemistry of the pyrazoline products **9**, **10** and **11** were established from 2D NMR (NOESY, COSY, HSQC and HMBC) measurements. NOESY experiments on cycloadducts **9** and **10** showed cross-peaks between the signals of 15-H and 16-H, which proved *cis* ring junctions in the condensed pyrazolines **9** and **10**. The stereochemistry of spiropyrazolines **11** was established from the NOE cross-peaks: 18-H_3 and 15α-H; 18-H_3 and tolyl aromatic proton 2′-H; 8β-H and *cis* 16a-H; 15β-H and *trans* 16a-H (*cis* and *trans* referring to the orientation relative to the carbonyl group). The NOE effects between the marked protons in rings C, D and E are indicative of the *S* configuration of C-16 (Fig. 1). In the HMBC spectra of spiro derivatives **11**, the cross-peak between C-16a and C═N reflects the structure in which the carbon atom of the dipole is connected to the β-carbon of the enone.

Neutral steroids are difficult to analyze by matrix-assisted laser desorption/ionization (MALDI) TOF, and there have been only a few literature reports on the analysis of derivatized steroids through the MALDI TOF technique [35–37]. We reported earlier the successful measurement of some heterocyclic steroids with a MALDI TOF mass spectrometer without further chemical derivatization, using C_{70} fullerenes as a matrix. We now describe the results of the positive mode detections of the synthesized non-heterocyclic and heterocyclic compounds. The analytical method applied was similar to that used in our previous work. We observed that non-heterocyclic derivatives were also effectively detected: molecular or positively charged quasimolecular ions appeared in all cases. In the spectra of the 3-benzylic derivatives, the benzylic cation was always seen. The regioisomeric counterparts of the heterocyclic cycloadducts exhibited similar fragmentations. In the spectra of condensed pyrazolines **9a** and **10a**, molecular ions and fragment ions resulting from the cleavage of the *O*-ethyl function from the ester group were seen. Two additional characteristic peaks could be observed in the spectra of 3-benzyl ethers **9b** and **10b**: that of the benzylic cation and that of the fragment ion obtained by cleavage of the *O*-ethyl, benzyl and tolyl groups and two oxygen atoms.

5. Conclusion

In summary, we have presented a simple and highly stereoselective method for the preparation of novel condensed pyrazolines and spiropyrazolines of 13α-estrone ethers. The reaction rates and yields are strongly influenced by the structure of the starting enone and the nature of the base used. Through substitution of the enone, various derivatives can be synthesized. Known 13α-estrone derivatives with 17α substituents or a 17-keto group possess the usual ring

C chair conformation, whereas 17β substituents or double bonds in ring D are responsible for the unusual, twist-boat conformation in ring C [13–17]. Conformational analysis of the α,β-unsaturated ketones **5** and **7** prepared and the cycloadducts **9**, **10** and **11** would be of particular interest, because there are no literature indications of the conformations of such compounds. Recent investigations have shown that benzyl ester cleavage in pyrazoline, or benzyl ether cleavage in pyrazol derivatives under hydrogen pressure in methanol or tetrahydrofurane, in the presence of palladium on charcoal catalyst can be successfully performed, while the pyrazoline or pyrazol moiety remains unchanged [23,38]. Thus, the removal of the benzyl protecting group under similar reaction conditions in the compounds synthetized, is expected to lead to steroids of potential pharmacological significance.

Acknowledgement

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