Regio- and stereoselective access to novel ring-condensed steroidal isoxazolines

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Novel 5α-androstanes containing an isoxazoline moiety condensed to ring A or D were efficiently synthesized by 1,3-dipolar cycloadditions of aryl nitrile oxides to steroidal αβ-unsaturated ketones. During the ring closures, regioisomers in which the O terminus of the nitrile oxide dipoles is attached to the β-carbon of the dipolarophile were formed in a stereoselective manner to furnish exclusively 1α,2α- or 15β,16β-condensed heterocycles. The cyclic enone moiety of the six-membered ring A proved to be less reactive than that of the five-membered ring D, but all the reactions were affected significantly by the substitution pattern of the nitrile oxide. 17-Decaetylation of the primary products resulted in aromatization or simultaneous hydroxylation, depending on the base applied for the ring A-fused heterocycles, while retro-Dieckmann-like fragmentation was observed partially or completely for the ring D-fused analogues during 3-decaetylation.

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1. Introduction

In view of the diversity of their biological activities, heterocyclic steroids continue to be attractive target molecules for stereoselective syntheses [1–6]. The main driving force towards the preparation of such compounds is the modification of the receptor-binding ability by chemical transformation of the extant functional groups and therefore the reduction or elimination of the undesirable hormonal effects [7]. Significant changes in the pharmacodynamic and pharmacokinetic properties can result from the introduction of a hetero ring into the sterane core.

Among the five-membered heterocyclic compounds, 2-isoxazolines have attracted interest as structural building blocks of biologically active molecules and they also serve as versatile intermediates in organic chemistry [8–10]. Thanks to their stability, the hetero ring can be functionalized or can be reductively cleaved to furnish a number of important bifunctional units, such as α,β-unsaturated ketones [11], β-hydroxycarbonyl compounds [12] or 1,3-aminoalcohols [13]. The outstanding role of 2-isoxazolines, however, is due to their straightforward synthetic availability from chemically divergent alkenes and nitrile oxides by 1,3-dipolar cycloadditions [14].

In consequence of their instability and high reactivity, most nitrile oxides have to be protected in situ. In the absence of a dipolarophile, sterically unhindered nitrile oxides tend to dimerize to furoxanes at ambient temperatures, or (especially the sterically stabilized ones) undergo rearrangement to isocyanates at elevated temperature [15]. Although most aryl nitrile oxides possess relatively long half-lives at room temperature, the presence of an electron-withdrawing substituent on the aromatic ring facilitates dimerization, while the resistance to furoxane formation is enhanced by electron-donating groups and sterically by ortho substituents [5]. These side-reactions may also occur to a certain extent even in the presence of an alkene component, which can reduce the yield of the desired isoxazoline. Hydroximidoyl chlorides, the most frequently applied precursors of nitrile oxides, are usually prepared from aldoximes by oxidative halogenation with N-chlorosuccinimide and subsequent base-induced dehydrohalogenation [16].

A number of steroid isoxazolines with diverse pharmacological activities (i.e. anti-inflammatory, hypcholesterolemic, antiviral, antibacterial, antifungal and antiproliferative effects) have been reported to date [17–19], but there are no examples of isoxazolines condensed to positions 1,2 or 15,16 of sterane ring A or D. The addition of steric bulk adjacent to the extant functional groups on C-3 or C-17, essential for hormone-receptor binding, may contribute to a change in biological activity and these derivatives may therefore deserve attention from a pharmacological aspect. Moreover, the vicinity of the angular methyl groups on C-3 or C-17, essential for hormone-receptor binding, may contribute to a change in biological activity and these derivatives may therefore deserve attention from a pharmacological aspect. Moreover, the vicinity of the angular methyl groups on C-10 and C-13 to the reaction centre and also the rigidity of the sterane skeleton overall may have a significant influence on the stereo- and regiocontrol of the processes.
As an extension of our work on the synthesis of steroid-fused heterocycles [20–23], we set out to prepare novel ring-fused isoazolines from \( \alpha, \beta \)-unsaturated steroidal 17-ketones with aryl nitrile oxides via intermolecular 1,3-dipolar cycloadditions. A further goal was to investigate the regio- and stereoselectivity of the processes and to compare the reactivities of rings A and D against nitrile oxides. The influence of steric and electronic factors on the ring closures and the behaviour of the cycloadducts under conventional deacetylation conditions were also studied. Determination of the stereostructures of the synthesized compounds was also planned.

2. Experimental

2.1. General

Melting points (Mps) were determined on an SMS Optimelt digital apparatus. Elemental analysis data were obtained with a Perkin Elmer CHN analyser model 2400. NMR spectra were recorded at room temperature with a Bruker DRX 500 instrument. Chemical shifts are reported in ppm (\( \delta \) scale), and coupling constants (\( J \)) in Hz. For the determination of multiplicities, the \( J \)-MOD pulse sequence was used. Automated flow injection analyses were performed by using an HPLC/MSD system. The system comprised an Agilent 1100 micro vacuum degasser, a quaternary pump, a micro-well plate autoinjector and a 1946A MSD equipped with an electrospray ion source (ESI) operated in positive ion mode. Samples (0.2 \( \mu l \)) were injected with an automated needle wash directly into the solvent flow (0.3 ml/min) of MeCN/H2O 70:30 (v/v) supplemented with 0.1% formic acid. The system was controlled by Agilent LC/MSD Chemstation software. All solvents were distilled immediately prior to use. Reagents and materials were obtained from reliable commercial suppliers and were used without purification. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F254) layers (0.25 mm thick); solvent systems (ss): (A) CH2Cl2 (B) EtOAc/CH2Cl2 (2:98 v/v), (C) EtOAc/CH3Cl (5:95 v/v) or (D) EtOAc/CH3Cl (10:90 v/v). The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The \( R_F \) values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: Merck silica gel 60, 40–63 \( \mu m \).

2.2. General procedure for the synthesis of ring-condensed isoazolines

2.2.1. 17\(^{\alpha} \)Acetoxy-5\(^{\alpha} \)-androsten-1-3-one (5a, 330 mg, 1.00 mmol), 3\(^{\alpha} \)-acetoxy-5\(^{\alpha} \)-androsten-15-\( \alpha \)-17-one (2, 330 mg, 1.00 mmol), 17\(^{\alpha} \)-hydroxy-5\(^{\alpha} \)-androsten-1-3-one (13, 288 mg, 1.00 mmol) and the appropriate aromatic imidoyl chloride (3a–g, 1.50 mmol) were dissolved in toluene (15 ml), and DIPEA (0.52 ml, 3.00 mmol) was added dropwise to the reaction mixture at room temperature, with subsequent refluxing for 5 h (for 1 and 13) or for 2 h (for 2 and 14). The solvent was then evaporated off in vacuo and the resulting crude product was purified by column chromatography with CH2Cl2 for 5 and 6 and with EtOAc/CH3Cl = 5:95 for 9 and 11.

2.2.2. 17\(^{\alpha} \)-Acetoxy-3\'-phenyl-2\'-isoazolino[4,5-\( \delta \):d:2x,1x]-5\(^{\alpha} \)-androstan-3-one (5a)

Compound 1 and N-hydroxybenzenecarboximidoyl chloride (3a, 233 mg) were used for the synthesis as described in the General Procedure. The crude product 5a (247 mg, 55%) was obtained as a white precipitate. Mp 222–224°C; \( R_F \) = 0.54 (ss B).

The spots observed by illumination at 254 and 365 nm. Flash chromatography: Merck silica gel 60, 40–63 \( \mu m \). Samples (0.2 \( \mu l \)) were injected with an automated needle wash directly into the solvent flow (0.3 ml/min) of MeCN/H2O 70:30 (v/v) supplemented with 0.1% formic acid. The system was controlled by Agilent LC/MSD Chemstation software. All solvents were distilled immediately prior to use. Reagents and materials were obtained from reliable commercial suppliers and were used without purification. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F254) layers (0.25 mm thick); solvent systems (ss): (A) CH2Cl2 (B) EtOAc/CH2Cl2 (2:98 v/v), (C) EtOAc/CH3Cl (5:95 v/v) or (D) EtOAc/CH3Cl (10:90 v/v). The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The \( R_F \) values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: Merck silica gel 60, 40–63 \( \mu m \).
9.49 (s, 3H, 19-H), 0.98 (m, 1H), 1.13 (m, 1H), 1.24–1.33 (m, 3H), 1.39–1.80 (m, 9H), 2.04 (s, 3H, Ac-CH3), 2.12 (m, 1H), 2.17–2.32 (m, 3H), 2.36 (s, 3H, 3-C6H5), 4.30 (d, 1H, J = 10.0 Hz, 2-H), 4.60 (t, 1H, J = 8.4 Hz, 17-H), 4.85 (d, 1H, J = 10.0 Hz, 11-H), 7.20 (d, 1H, J = 7.3 Hz), 7.26 (t, 1H, J = 7.3 Hz, 5″-H), 7.53 (d, 1H, J = 7.3 Hz), 7.58 (s, 1H, 2″-H) ppm; 13C NMR (125 MHz, CDCl3): δ 12.0 (C-18), 12.2 (C-19), 20.4 (CH3), 21.1 (Ac-CH3), 23.6 (CH2), 27.5 (CH2), 28.5 (CH2), 30.5 (CH3), 34.8 (CH), 35.9 (CH2), 36.3 (CH3), 38.4 (C-10), 42.4 (C-13), 42.7 (CH2), 46.4 (CH), 50.2 (CH), 57.5 (C-2), 82.6 (C-17), 89.3 (C-1), 123.7 (2C), 128.3 (2C), 135.0 (C-1″), 148.4 (C-4″), 154.6 (C-2″), 171.1 (Ac-CO), 205.4 (C-3″) ppm; ESI-MS 495 [M+H]+.

2.2.8. 3j-Acetoxy-3′-phenyl-2′-isoxazolino[4,5-d′:2′,1′j]-5x-androstan-17-one (6a)

Compound 1 and N-hydroxy-4-methylbenzenecarboximidoxy chloride (3a, 255 mg) were used for the synthesis as described in the General Procedure. The crude product 6a (423 mg, 94%) was obtained as a white precipitate. Mp 281–283 °C; Rf = 0.43 (ss A). Anal. Calc. for C28H23O2N5: C, 74.80; H, 7.85. Found: C, 74.95; H, 7.74.

3j-Acetoxy-3′-phenyl-2′-isoxazolino[4,5-d′:2′,1′j]-5x-androstan-17-one (6b)

Compound 2 and N-hydroxy-4-methylbenzenecarboximidoxy chloride (3b, 279 mg) were used for the synthesis as described in the General Procedure. The crude product 6b (470 mg, 98%) was obtained as a white precipitate. Mp 232–234 °C; Rf = 0.33 (ss A). Anal. Calc. for C29H25O3N4: C, 72.62; H, 7.78. Found: C, 72.45; H, 7.70. 1H NMR (CDCl3, 500 MHz): δ 0.81 (m, 1H, 19-H), 1.05 (m, 1H and s, 3H, 18-H), 1.11–1.32 (overlapping m, 3H), 1.34–1.43 (overlapping m, 4H), 1.52 (m, 1H, 1.59–1.69 (overlapping m, 3H), 1.75 (m, 2H), 1.83 (m, 2H, 2-OH), 2.02 (s, 3H Ac-CH3), 2.07 (m, 1H), 2.14 (m, 1H), 3.81 (s, 3H, 4′-OMe), 4.11 (t, 1H, J = 9.1 Hz, 16-H), 4.70 (m, 1H, 3-OH), 5.38 (dd, 1H, J = 9.1 Hz, J = 4.2 Hz, 15-H), 6.92 (d, 2H, J = 8.8 Hz, 3′-H and 5′-H), 7.86 (d, 2H, J = 8.8 Hz, 2′-H and 6′-H) ppm; 13C NMR (CDCl3, 125 MHz): δ 121.2 (C-19), 179.1 (C-18), 202.0 (CH2), 214.0 (Ac-CH3), 27.4 (CH2), 28.0 (CH3), 30.4 (CH3), 32.1 (CH), 33.2 (CH2), 33.9 (CH2), 35.8 (C-10), 36.6 (CH2), 44.8 (CH), 48.9 (C-13), 54.3 (CH), 55.0 (CH), 55.3 (4′-OMe), 62.4 (CH), 73.4 (C-3′), 84.6 (C-15), 127.6 (2C, C-3″ and C-5″), 128.3 (C-1′), 128.7 (2C, C-2″ and C-6″), 130.3 (C-3′), 155.3 (C-3″), 170.6 (Ac-CO), 212.3 (C-17) ppm; ESI-MS 450 [M+H]+.

2.2.9. 3j-Acetoxy-3′-methoxyphenyl-2′-isoxazolino[4,5-d′:2′,1′j]-5x-androstan-17-one (6d)

Compound 1 and N-hydroxy-4-methylbenzenecarboximidoxy chloride (3b, 255 mg) were used for the synthesis as described in the General Procedure. The crude product 6d (450 mg, 97%) was obtained as a white precipitate. Mp 273–276 °C; Rf = 0.27 (ss A). Anal. Calc. for C30H25O3N5: C, 75.13; H, 8.04. Found: C, 75.25; H, 8.10. 1H NMR (CDCl3, 500 MHz): δ 0.81 (m, 1H, 19-H), 1.05 (m, 1H and s, 3H, 18-H), 1.12–1.32 (overlapping m, 3H), 1.35–1.42 (overlapping m, 4H), 1.52 (m, 1H), 1.60–1.67 (overlapping m, 3H), 1.78 (m, 2H), 1.83 (m, 2H, 2-OH), 2.03 (s, 3H Ac-CH3), 2.07 (m, 1H), 2.15 (m, 1H), 2.37 (3H, 4′-CH3), 4.2 (s, 3H, J = 9.1 Hz, 16-H), 4.71 (m, 1H, 3-H), 5.39 (dd, 1H, J = 9.1 Hz, J = 4.1 Hz, 15-H), 7.21 (d, 2H, J = 7.8 Hz, 3′-H and 5′-H), 7.81 (d, 2H, J = 7.8 Hz, 2′-H and 6′-H) ppm; 13C NMR (CDCl3, 125 MHz):
δ12.1 (C-19), 17.8 (C-18), 20.1 (CH2), 21.4 (2C, Ac-CH3 and 4-Ch3), 27.4 (CH3), 28.0 (CH3), 30.4 (CH2), 32.1 (CH), 33.2 (CH3), 33.9 (CH3), 35.8 (C-10), 36.6 (CH2), 44.8 (CH), 48.9 (C-13), 54.3 (CH), 55.0 (CH), 62.2 (CH), 73.4 (C-3), 84.3 (C-15), 125.5 (C-1'), 127.6 (2C, C-2' and C-6'), 129.4 (2C, C-3' and C-5'), 140.6 (C-4'), 155.3 (C-3'), 170.6 (Ac-Co), 212.4 (C-17) ppm; ESI-MS 464 [M+H]+.

2.2.11. 3β-Acetoxy-3'4'-chlorophenyl-2'-isosoilino[4',5'-d:6',5'j]-5α-androstan-17-one (6f)

Compound 2 and N-hydroxy-4-chlorobenzencarboximidoxy chloride (3f, 292 mg) were used for the synthesis as described in the General Procedure. The crude product 6f (378 mg, 78%) was obtained as a white precipitate. Mp 242–245°C; Rf = 0.50 (ss A). Anal. Calcld. for C28H25ClN2O5 (484.09): C, 69.48; H, 7.08. Found: C, 69.37; H, 7.18. 1H NMR (CDCl3, 500 MHz): 8.02 (m, 1H), 0.90 (3, 3H, 19-H3), 1.03 (s, 3H, 18-H3), 1.04 (m, 1H), 1.13–1.43 (overlapping m, 7H), 1.52 (m, 1H), 1.61–1.84 (overlapping m, 6H), 2.02 (3, 3H Ac-CH3), 2.07 (m, 1H), 2.14 (m, 1H), 2.09 (d, 1H, J = 8.9 Hz, 18-H3), 8.11 (d, 2H, J = 7.3 Hz, 3'-H and 5'-H) ppm; 13C NMR (CDCl3, 125 MHz): δ = 245.2, 225.7, 129.4, 128.0, 126.2, 127.5, 124.2, 120.4, 118.1, 107.8, 103.5, 29.7, 25.7, 22.6, 19.3, 19.1, 17.8, 17.6, 14.1, 12.1 ppm.

2.2.12. 3β-Acetoxy-3'4'-nitrophenyl-2'-isosoilino[4',5'-d:6',5'j]-5α-androstan-17-one (6g)

Compound 2 and N-hydroxy-4-nitrobenzencarboximidoxy chloride (3g, 300 mg) were used for the synthesis as described in the General Procedure. The crude product 6g (222 mg, 45%) was obtained as a yellow precipitate. Mp > 250°C (decomp.); Rf = 0.38 (ss A). Anal. Calcld. for C29H24N2O5 (549.58): C, 68.00; H, 6.93. Found: C, 67.87; H, 7.05. 1H NMR (CDCl3, 500 MHz): δ = 8.22 (m, 1H), 0.90 (3, 3H, 19-H3), 1.03 (s, 3H, 18-H3), 1.06 (m, 1H), 1.19–1.43 (overlapping m, 7H), 1.51 (m, 1H), 1.65–1.81 (overlapping m, 6H), 2.03 (3, 3H Ac-CH3), 2.08 (m, 1H), 2.14 (m, 1H), 4.14 (d, 1H, J = 9.1 Hz, 16-H), 4.70 (m, 1H, 3-H), 5.50 (dd, 1H, J = 9.1 Hz, J = 4.0 Hz, 15-H), 8.11 (d, 2H, J = 8.6 Hz, 2'-H and 6'-H) ppm; 13C NMR (CDCl3, 125 MHz): δ = 12.2 (C-19), 17.9 (C-18), 20.1 (CH2), 21.4 (Ac-CH3), 27.3 (CH3), 28.0 (CH3), 30.4 (CH2), 32.1 (CH), 33.2 (CH3), 33.8 (C-10), 36.6 (CH2), 44.8 (CH), 48.9 (C-13), 54.3 (CH), 61.9 (CH), 73.3 (C-3), 84.3 (C-15), 126.8 (C-1'), 128.2 (2C, C-2' and C-5'), 128.9 (2C, C-2'' and C-6''), 136.4 (C-4''), 154.4 (C-3'), 170.6 (Ac-Co), 212.3 (C-17) ppm; ESI-MS 485 [M+H]+.

2.2.13. 17β-Hydroxy-3'4'-methylenophenyl-2'-isosoilino[4',5'-d:2',2'a,1'j]-5α-androstan-3-one (9a)

Compound 13 and N-hydroxy-4-methylbenzenecarboximidoxy chloride (3e, 255 mg) were used for the synthesis as described in the General Procedure. The crude product 9a (223 mg, 53%) was obtained as a white precipitate. Mp 232–234°C; Rf = 0.45 (ss B).
General Procedure. The crude product (3f, 292 mg) was used for the synthesis as described in the General Procedure. The crude product 9f (164 mg, 37%) was obtained as a white precipitate. M.p 210–212 °C; Rf = 0.41 (ss B). Anal. Calcld. for C27H37NO3: C, 70.65; H, 7.82. Found: C, 70.65; H, 7.52. 1H NMR (500 MHz, CDCl3): δ 0.76 (s, 3H, 18-H3), 0.96 (s, 3H, 19-H3), 1.04 (m, 1H), 1.17–1.32 (m, 4H), 1.40–1.54 (m, 9H), 2.04–2.34 (m, 4H), 3.67 (t, 1H, J = 8.3 Hz, 2-H), 4.85 (d, 1H, J = 10.1 Hz, 1-H), 3.49 (d, 2H, J = 9.3 Hz, 3”-H and 5”-H), 3.65 (d, 2H, J = 8.3 Hz, 2”-H and 6”-H) ppm; 13C NMR (125 MHz, CDCl3): δ 11.0 (C-18), 12.2 (C-19), 20.5 (CH3), 21.4 (C4”), 23.4 (CH2), 28.7 (CH3), 30.3 (CH2), 30.6 (CH2), 35.1 (CH), 35.9 (CH), 36.2 (CH2), 38.7 (C-10), 42.7 (CH2), 42.8 (C-13), 46.4 (CH), 50.6 (CH), 58.8 (C-2), 81.7 (C-17), 88.5 (C-1), 125.9 (C-1”), 127.4 (2C), 129.2 (2C), 140.5 (C-4”), 155.3 (C-5”), 206.1 (C-3) ppm; ESI-MS 422 [M+H]+.

Compound 13 and N-hydroxy-4-chlorobenzencarboximidoyl chloride (3f, 292 mg) were used for the synthesis as described in the General Procedure. The crude product 9f (164 mg, 37%) was obtained as a white precipitate. M.p 210–212 °C; Rf = 0.41 (ss B). Anal. Calcld. for C27H37NO3: C, 70.65; H, 7.82. Found: C, 70.65; H, 7.52. 1H NMR (500 MHz, CDCl3): δ 0.76 (s, 3H, 18-H3), 0.96 (s, 3H, 19-H3), 1.04 (m, 1H), 1.17–1.32 (m, 4H), 1.40–1.54 (m, 9H), 2.04–2.34 (m, 4H), 3.67 (t, 1H, J = 8.3 Hz, 2-H), 4.85 (d, 1H, J = 10.1 Hz, 1-H), 3.49 (d, 2H, J = 9.3 Hz, 3”-H and 5”-H), 3.65 (d, 2H, J = 8.3 Hz, 2”-H and 6”-H) ppm; 13C NMR (125 MHz, CDCl3): δ 11.0 (C-18), 12.2 (C-19), 20.5 (CH3), 21.4 (C4”), 23.4 (CH2), 28.7 (CH3), 30.3 (CH2), 30.6 (CH2), 35.1 (CH), 35.9 (CH), 36.2 (CH2), 38.7 (C-10), 42.7 (CH2), 42.8 (C-13), 46.4 (CH), 50.6 (CH), 58.8 (C-2), 81.7 (C-17), 88.5 (C-1), 125.9 (C-1”), 127.4 (2C), 129.2 (2C), 140.5 (C-4”), 155.3 (C-5”), 206.1 (C-3) ppm; ESI-MS 443 [M+H]+.

2.19. 3β-Hydroxy-3’-phenyl-2’-isoxazolino[4,5’;d:2,1,9]-5x-androstan-3-one (11a)

Compound 14 and N-hydroxybenzenecarboximido chloride (3a, 233 mg) were used for the synthesis as described in the General Procedure. The crude product 11a (357 mg, 92%) was obtained as a white precipitate. M.p > 280 °C (decomp.); Rf = 0.43 (ss D). Anal. Calcld. for C27H37NO3: C, 76.85; H, 8.22. Found: C, 76.85; H, 8.16. 1H NMR (CDCl3, 500 MHz): δ 0.81 (m, 1H), 0.90 (s, 3H, 19-H3), 1.02 (m, 1H), 1.07 (s, 3H, 18-H3), 1.19 (m, 2H), 1.26–1.47 (overlapping m, 6H), 1.62 (m, 2H), 1.68–1.85 (overlapping m, 4H), 2.08 (m, 1H), 2.16 (m, 1H), 3.61 (m, 1H, 3-H), 4.14 (d, 1H, J = 9.2 Hz, 16-H), 5.43 (dd, 1H, J = 9.2 Hz, J = 4.3 Hz, 15-H), 7.42 (m, 3H, 3”-H, 4”-H and 5”-H), 7.94 (m, 2H, 2”-H and 6”-H) ppm; ESI-MS 408 [M+H]+.

2.20. 3β-Hydroxy-3’-4’-methoxyphenyl-2’-isoxazolino[4,5’;d:2,1,9]-5x-androstan-3-one (11b)

Compound 14 and N-hydroxy-4-methoxycarboximido chloride (3b, 279 mg) were used for the synthesis as described in the General Procedure. The crude product 11b (424 mg, 97%) was obtained as a white precipitate. M.p 205–208 °C; Rf = 0.24 (ss D). Anal. Calcld. for C27H35NO4: C, 74.11; H, 8.06. Found: C, 73.95; H, 8.22. 1H NMR (CDCl3, 500 MHz): δ 0.79 (m, 1H), 0.89 (s, 3H, 19-H3), 1.06 (m, 1H and s, 3H, 18-H3), 1.12–1.33 (overlapping m, 3H), 1.35–1.43 (overlapping m, 4H), 1.53 (m, 1H), 1.62 (m, 2H), 1.67–1.84 (overlapping m, 4H), 2.08 (m, 1H), 2.14 (m, 1H), 3.61 (m, 1H, 3-H), 3.84 (s, 3H, 4’-OMe), 4.11 (d, 1H, J = 9.1 Hz, 16-H), 5.39 (dd, 1H, J = 9.1 Hz, J = 4.2 Hz, 15-H), 6.93 (d, 2H, J = 8.8 Hz, 3”-H and 5”-H), 7.88 (d, 2H, J = 8.8 Hz, 2”-H and 6”-H) ppm; ESI-MS 438 [M+H]+.

Method A: Compound 5b (150 mg, 0.31 mmol) was dissolved in MeOH (10 mL), and KOH (50 mg, 0.89 mmol) was added. The solution was stirred at room temperature for 3 h and then diluted with water, and the precipitate that formed was filtered off, washed with water and purified by flash chromatography with EtOAc/CH2Cl2. C18 = 20:80 as eluant to obtain 7b (100 mg, 74%) and 8b (25 mg, 18%) as white crystals {sequence of elution: 7b > 8b).

Method B: Compound 5b (150 mg, 0.31 mmol) was dissolved in DMSO (10 mL), and ‘BuOK (80 mg, 0.71 mmol) was added. The solution was stirred at 80 °C for 1 h and then diluted with water and extracted with EtOAc (2 × 10 mL). The crude product was purified by flash chromatography with EtOAc/CH2Cl2 = 20:80 as eluant to obtain 7b (120 mg, 89%).
1H NMR (125 MHz, CDCl3) \(\delta 11.4\) (C-18), 13.6 (C-19), 234 (CH3), 238 (CH2), 265 (CH2), 287 (C-10), 302 (CH2), 362 (CH), 366 (CH2), 414 (C-13), 429 (CH2), 451 (CH), 495 (CH), 508 (CH), 553 (4’-OCH3), 816 (C-17), 1132 (C-2’, C-3’ and C-5’), 1197 (C-1’), 1308 (2C, C-2’ and C-6’), 1595 (C-3’), 1613 (C-1’), 1898 (C-1), 1919 ppm; ESI-MS: 436 [M + H]+.

Method C: Compound 6a (135 mg, 0.30 mmol) was dissolved in DMSO (10 mL), and BuOK (80 mg, 0.71 mmol) was added. The solution was stirred at 80°C for 1 h and then diluted with water and extracted with EtOAc (2 × 10 mL). The crude product was purified by flash chromatography with EtOAc/CH2Cl2 = 5:95 as eluent to obtain 11a (106 mg, 87%). See 2.2.19.

3. Results and discussion

Since conjugation of the alkylene moiety with a C=O bond has been demonstrated to have a strong promoting effect on the reactivity of the dipolarophile [14], the steroidal unsaturated ketones 17β-acetoxy-5α-androst-1-en-3-one (1) [24] and 3β-acetoxy-5α-androst-15-en-17-one (2) [25], readily available from 5α-dihydrotestosterone and dehydroepiandrosterone, respectively, in a multistep pathway, were applied for the transformations (Table 1). Aromatic hydroximidoyl chlorides (3a–g), as relatively stable precursors of nitrole oxides (4a–g), were synthesized in two steps by the general protocol from benzaldehyde or its substituted derivatives [26]. The 1,3-dipoles (4a–g) can be generated in situ from 3a–g by dehydrochlorination with a base.

Preliminary ring-closure experiments on 1 and 2 with benzoni-azole oxides 4a were first carried out in order to determine the optimum conditions. The steroidal enone (1 or 2) and an excess of N-hydroxybenzenecarboximidoyl chloride (3α) were dissolved in toluene and 3 equivalents of N,N-diisopropylamylamine (DipeA) was slowly added in order to ensure a low stationary concentration of the dipole and to minimize undesired furoxane formation. After the completion of DipeA addition at room temperature, the solution was refluxed to achieve complete conversion for compound 2 within 2 h, leading to a single product 6a in a yield of 94% after chromatographic purification (Table 1, entry 8). However, the similar reaction of 1 with 4a was not complete after a 5–h heating period, as indicated by TLC, and the yield of the purified isoxazoline (5a) was only 55% (entry 1), together with a considerable amount (42%) of the residual starting material 1. The rate of cycloaddition was much slower at room temperature or below, and refluxing of the mixtures was therefore necessary for sufficient conversions. Similar cycloadditions of 1 and 2 with various substituted benzoni-azole oxides (4b–g) were subsequently performed under the same conditions to furnish novel ring-condensed isoxazolines (5b–g, 6b and 6e–g) in moderate to excellent yields (entries 2–7 and 9–12).

Depending on the dipole orientation relative to the double bond, two regioisomers, each involving four diastereoisomers (two cis and two trans) as concerns the newly-formed stereogenic centres on C-1 and C-2 in 5a–g or on C-15 and C-16 in 6a–e can be conceived as possible products of the cycloadditions. However, the trans–like connection of the 1,3-dipole in both orientations is precluded because of the ring strain. The attack of the dipole from above the general plane of the sterane framework (the β side) in consequence of the steric interaction between the dipole and 14-H3, is unlikely in 1 in consequence of the same spatial orientation of the 19-CH3 [27]. Similarly, the introduction of the nitrole oxide in 2 is unlikely to occur from beneath (the α side) in consequence of the steric interaction between the dipole and 14-H3 [28,29]. Accordingly, for both reactions only two regioisomers are probable (depicted in Table 1), containing a 1α,2α-cis (5 and 5′) or a 1β,16β-cis ring junction (6 and 6′). The formation of regioisomers (5′ and 6′) in which the 0 terminal of the dipole is attached to the α-carbon of the dipolarophile, and the aromatic ring is therefore bent towards the sterane portion, is considered to be hampered.
by steric repulsions. The most facilitated isomers are undoubtedly 5 and 6, in which the anionic pole of the nitrile oxide is connected to the β carbon of 1 or 2. Both the regio- and the stereoselectivity of the ring closures are therefore influenced by steric factors, in good agreement with earlier observations that the electronic character of the dipolarophile has only a minor effect on such reactions [30].

The overall yields of the products (5a–g, 6a, 6b and 6e–g) were found to depend on the electronic features of the substituents on the aromatic ring of the dipoles 4a–g (Table 1), and this effect was more pronounced in the case of the ring A-fused derivatives 5a–g. The electron-donating substituents in 4b and 4e favoured cycloaddition to 1 and 2 (Table 1, entries 2, 5, 9 and 10), in consequence of the lower tendency of these dipoles to undergo dimerization to furoxanes, while the yields of the desired products (5f and 6f, or 5g and 6g) were decreased by the presence of the electron-withdrawing groups on the aromatic moiety in 4f and 4g (entries 6, 7, 11 and 12). The lowest conversions were achieved for the reactions of 1 and 2 with p-nitrobenzonitrile oxide 4g, which resulted in the desired products (5g and 6g) in yields of only 19% and 45%, respectively. In order to investigate both the electronic and the steric effects of the substituents on the reactivity of the dipoles, the cycloadditions of o- and m-methyl-substituted benzonitrile oxides (4c and 4d) to 1 were also carried out (entries 3 and 4). The highest yield of the tolyl-substituted cycloadducts (5c–e) was that for 5c, which was comparable to that of the methoxy-substituted analogue 5b (entry 3). This is indicative of the steric hindrance of the o-methyl group and hence the increased resistance of this dipole (4c) against dimerization to furoxane [14]. The five-membered ring D of 2 proved to be more reactive against nitrile oxides than the six-membered ring A of 1, and the related products were obtained in higher yields within shorter reaction times. A similar difference in reactivity was earlier observed for the reactions of cyclopentene and cyclohexene, the former being more reactive due to ring strain and conformational effects [16].

The structures of all the synthetized compounds were confirmed by 1H and 13C NMR measurements. The presence of the phenyl or substituted phenyl ring derived from the nitrile oxides (4a–g) was demonstrated by the signals in the aromatic range of the ppm scale in 5a–g, 6a, 6b and 6e–g. In the 1H NMR spectra of 5a–g, the signals of 2-H and 1-H appeared as two doublets at around 4.27 and 4.85 ppm. The coupling constant 2JHCH of about 10.0 Hz was consistent with the 1α,2α-(cis)-anellation of the hetero ring. At the same time, the doublet of 16-H and the doublet of 15-H were detected at around 4.12 and 5.42 ppm in the spectra of 6a, 6b and 6e–g. The exact configurations of the newly formed stereocentres were established with the aid of homonuclear 2D NMR (COSY and NOESY) and heteronuclear 2D NMR (HSQC and HMBC) measurements.

### Table 1
Regio- and stereoselective synthesis of ring A- and ring D-fused steroidal isoxazolines.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Hydroximidoyl chloride/Nitrile oxide</th>
<th>R</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3a/4a</td>
<td>H</td>
<td>5a</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3b/4b</td>
<td>p-OMe</td>
<td>5b</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3c/4c</td>
<td>o-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5c</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>3d/4d</td>
<td>m-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5d</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>3e/4e</td>
<td>p-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5e</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>3f/4f</td>
<td>p-Cl</td>
<td>5f</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>3g/4g</td>
<td>p-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5g</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>3a/4a</td>
<td>H</td>
<td>6a</td>
<td>94</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>3b/4b</td>
<td>p-OMe</td>
<td>6b</td>
<td>98</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>3c/4c</td>
<td>p-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6c</td>
<td>97</td>
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<tr>
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<td>2</td>
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<td>p-Cl</td>
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<tr>
<td>12</td>
<td>2</td>
<td>3e/4e</td>
<td>p-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6g</td>
<td>45</td>
</tr>
</tbody>
</table>

* Determined after purification by column chromatography.
The deacetylations of one cycloadduct from both sets of compounds (5b and 6a) were carried out by using general conditions so as to obtain the corresponding 17-OH analogs of 5 and 3-OH derivatives of 6. However, the transformations led to unexpected products in particular cases. 17-Deacetylation of 5b in alkaline MeOH at room temperature resulted in the simultaneous formation of a heteroaromatic isoxazole (7b) and a 4’-hydroxylated isoxazoline (8b) in an approximate ratio of 4:1, the structures of which were confirmed by NMR spectroscopy after separation (Scheme 1). The formation of the 4’-OH derivative 8b can be explained by the oxidation of the corresponding enolate produced in alkaline medium. Such hydroxylation has already been observed, especially for ketones containing a tertiary carbon at the α position [31]. The spontaneous aromatization of the isoxazoline ring of 5b to isoxazole 7b is quite unusual, however, since the oxidation of such rings is more difficult than that of pyrazolines to pyrazoles [32], and the application of different oxidizing reagents is usually needed [33] even for the dehydrogenation of six-membered ring-condensed analogues [34]. Interestingly, repeated deacetylation of 5b with tBuOK in DMSO at 80 °C led to the formation of 7b alone, which can be attributed to the fact that the enolate needed for α-hydroxylation is less favoured in more polar solvents. Although 8b proved to be quite stable in alkaline medium, it could be converted to 7b by elimination in the presence of p-toluenesulfonic acid or sulfuric acid at elevated temperature. However, similar reaction by applying an inert atmosphere led to the desired product 9b exclusively.

When the 3-deacetylation of the ring D-fused analogue 6a was carried out at room temperature, the major formation of a D-seco ester (10a) was observed, together with the desired product (11a) in a ratio of about 6:4 (Scheme 2), while 10a was the sole product when either 6a or 11a was refluxed in alkaline MeOH. However, exclusively the seco-carboxylic acid 12a was obtained when MeOH was replaced by tBuOH presuming due to the poor nucleophilicity of ‘BuO−’ which precludes its attack on C-17. The desired 3β-hydroxy compound 11a was obtained as sole product by applying tBuOK in DMSO for repeated deacetylation.

The observed (α1α) fragmentation is similar to the retro-Dieckmann reactions of 1,3-diketones and ketoesters [35], where the attack of the MeO− or HO− nucleophile, derived from either the solvent or the reagent, on the carbonyl-C induces the cleavage of ring D between C-16 and C-17, as depicted in Fig. 1. Simultaneous deacetylation on C-3 also occurs to furnish 10a or 12a. The formation of the D-seco derivatives (10a and 12a) serves as indirect evidence of the regioselectivity of the 1,3-cycloaddition of 2 with 4, because the fragmentation would not be possible in the cases of 6 (see Table 1).

The different behaviour observed for 5b and 6a under deacetylation conditions may be attributed to the higher rigidity and sterically more hindered character of ring D in 6a compared to the flexible six-membered ring A in 5b. Aromatization of the hetero ring should further enhance the ring strain of ring D in 6a, therefore, fragmentation induced by a nucleophile attack on C-17 instead of oxidation is more favourable in this case.

The 1,3-cycloadditions of nitrile oxides generated in situ from 3a–g were also carried out with the corresponding 17- and 3-OH analogues (13 and 14) of 1 and 2 under the same conditions as applied earlier (Table 2). Similar tendencies concerning the reaction rates and the electronic and steric effects of the substituents on the aromatic ring of the nitrile oxides 4a–g and therefore the
substituted compound 5g was isolated in only a moderate yield (19%) from the reaction of 1 with 4g, the similar cycloaddition of 13 and 4g was not performed in this case.

4. Conclusions

In summary, novel types of steroidal isoxazolines condensed to either ring A or ring D of the sterane framework were synthetized through the 1,3-dipolar cycloaddition of 5α-androstiones and aromatic nitrile oxides. The ring closures proved to occur in a regio- and stereoselective manner to furnish a single isomer in all cases, in moderate to excellent yields. The higher reactivity of the five-membered ring D led to higher yields of the corresponding products, though all the reactions were affected by the substitution pattern of the nitrile oxides. The 17- and 3-deacetylation of the isoxazolines in alkaline medium opened the way for interesting oxidation and fragmentation pathways, resulting in aromatization of the hetero ring for the ring A-fused derivatives, and skeletal cleavage of the ring D-condensed analogues without affecting the isoxazoline moiety.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2014.05.019.

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