SYNTHESIS OF SYMMETRICAL BIS-STEROID PYRAZINES CONNECTED *via* D-RINGS+

Ivan ČERNÝ[1,*], Vladimír POUZAR[2], Miloš BUDĚŠÍNSKÝ[3] and Pavel DRAŠAR[4]

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic; e-mail: [1] cerny@uochb.cas.cz, [2] pouzar@uochb.cas.cz, [3] budesinsky@uochb.cas.cz, [4] drasar@uochb.cas.cz

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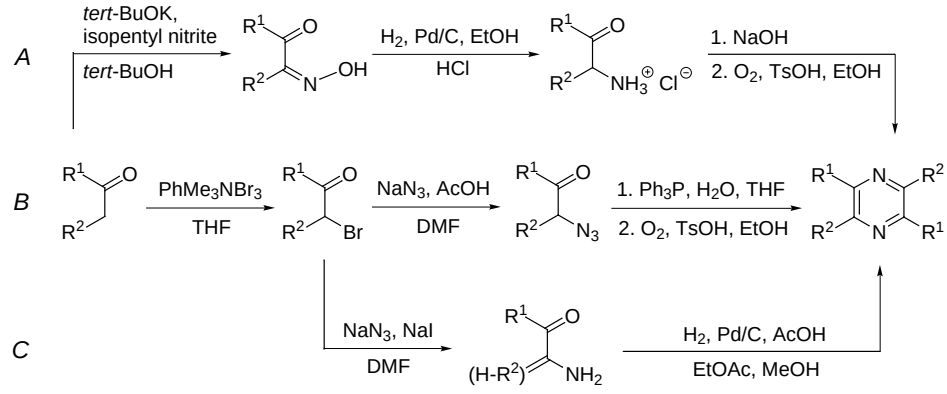
Bis-steroid pyrazines fused through rings D of the steroid skeleton were synthesized. Methods for the preparation of the corresponding rings A fused derivatives (cephalostatin type) were checked on simple androstanes and then a symmetrical D fused analogue, 5α-androstano[16″,17″-5′,6′]pyrazino[2′,3′-16,17]-5α-androstane-3β,3″β-diol, was prepared. Partially substituted bis-steroid pyrazines in both series were prepared and their use for the preparation of higher fused compounds was discussed. No significant cytostatic activity was found on parent bis-steroid pyrazines both A and D fused.

Key words: Steroids; Cephalostatin analogues; Symmetrical bis-steroid pyrazines; NMR spectroscopy; Cytostatic activity.

The size and rigidity of a steroid skeleton render this unit a potential building block for constructing larger molecular structures with interesting biological properties (membrane modifications, ion hosting and transport, self-assembly, *etc.*) Many examples of dimeric and oligomeric steroids, both synthetic and natural, are known[2]. From this class of compounds, those having two steroid skeletons fused together through pyrazine ring were very intensively studied in connection with cephalostatin[3] and its analogues. Several synthetic approaches made available both symmetrical and unsymmetrical bis-steroid pyrazines[4–9]. Most of the syntheses of symmetrical pyrazines used vicinal amino ketones, available from other nitrogen derivatives, such as oximes[4], enamines[5] or azides[6] (Scheme 1). The aim of our work was first to check selected methods for condensation through both ring A and ring D on simple androstane derivatives. Secondly, we intended to prepare some partially substituted derivatives suitable for an additional condensation cycle, which may deliver highly fused systems.

+ Part CDIX in the series On Steroids; Part CDVIII see ref.[1]

The pyrazine derivative **3** in ring A fused series is known: a thorough description of experiments is given in ref.[4], whereas ref.[10] mentioned the synthesis only briefly. An unsuccessful attempt at the synthesis of ring D fused pyrazine **12** was mentioned[4].



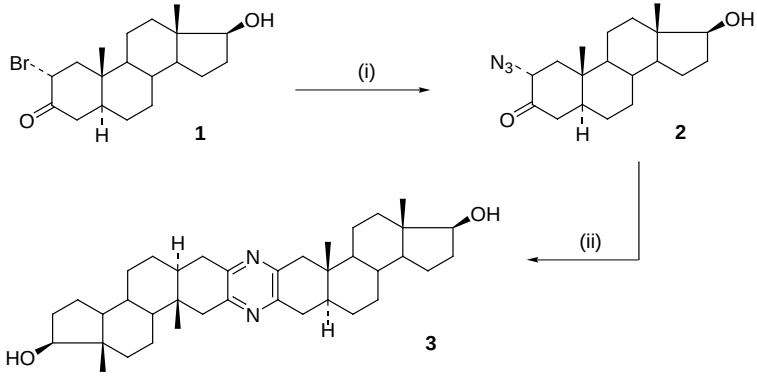
SCHEME 1

RESULTS AND DISCUSSION

In the ring A fused series, we tested methods starting from bromoketone **1**. Preliminary experiments were based on its reaction with sodium azide in dimethylformamide in the presence of sodium iodide at elevated temperature (Scheme 1: method *C*, ref.[5]). The foamy enamine product on subsequent hydrogenation on palladium on carbon in a mixture of ethyl acetate and acetic acid gave pyrazine **3**, but only in a low yield. Better results were achieved by using nucleophilic substitution of bromine in bromoketone **1** with sodium azide in dimethylformamide in the presence of acetic acid[11] suppressing the resulting azide destruction (Scheme 1: method *B*). In agreement with the finding on cholestane derivatives[11], azide **2** with 2α-configuration was formed. The reduction of azide **2** was performed by hydrogenation on palladium on carbon in ethanol with the addition of hydrochloric acid. The crude intermediate (amine hydrochloride) was transformed into pyrazine **3** by alkalinization with a concomitant condensation to a dihydropyrazine mixture and subsequent aromatization by air oxidation in ethanol with 4-toluenesulfonic acid (Scheme 2).

When compared with the published procedures leading to **3**, the use of azide instead of oxime circumvented problems with oximation selectivity (*cf.* ref.[4]) and the overall yield was in our case better; for another ap-

proach[10], very close to ours, only synthetic scheme without experimental data was given.



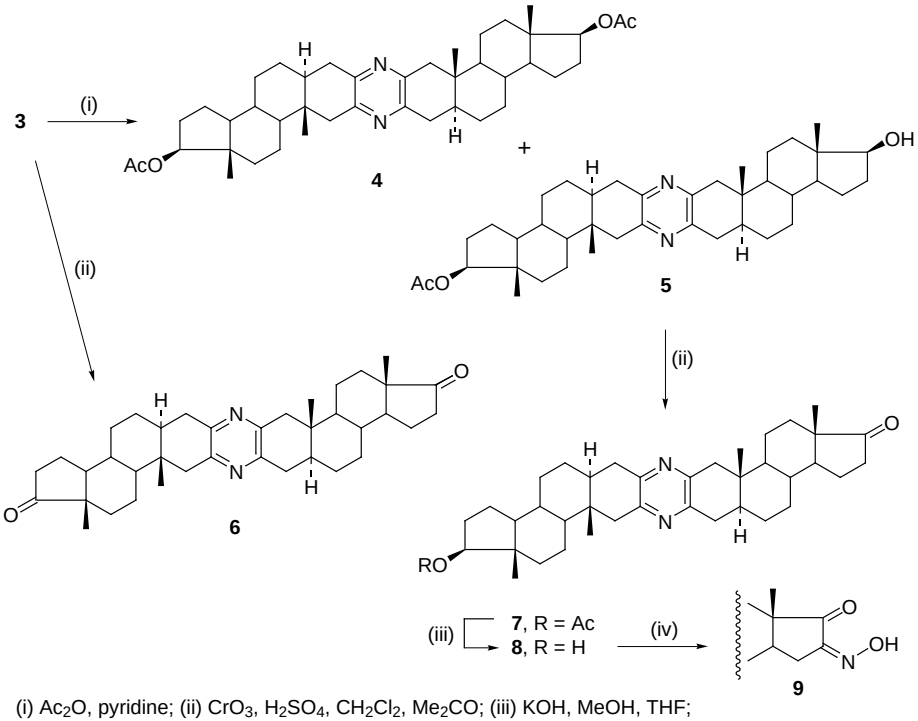
(i) NaN_3, AcOH, DMF; (ii) 1. H_2, Pd/C, EtOH, HCl; 2. K_2CO_3, H_2O; 3. O_2, TsOH, EtOH

SCHEME 2

The pyrazine **3** was partially acetylated with acetic anhydride in pyridine: in a mixture of diacetate **4**, monoacetate **5** and unreacted starting pyrazine diol **3**, the monoacetate **5** prevailed (Scheme 3) and could be separated by column chromatography.

Oxidation of the free hydroxy groups on bis-steroid pyrazines **3** and **4** with chromium(IV) oxide in sulfuric acid (Jones reagent) did not affect the pyrazine ring; we prepared both diketone **6** and ketone **7**. Deacetylation of compound **7** gave bis-steroid pyrazine **8** with hydroxy and keto groups on remote sides of the molecule.

At this stage, we planned to repeat the above synthetic route in order to obtain a molecule containing four steroid units. We failed, however, in the preparation of the corresponding bromoketone from **7** or **8** both by the reaction with trimethylphenylammonium bromide perbromide in tetrahydrofuran and with copper(II) bromide in a methanol–benzene mixture. It is necessary to note that all bis-steroid pyrazines under study have limited solubility in common organic solvents and this complicates the treatment and narrows the reaction selection. The oxime approach was a little more promising: the reaction of **8** with potassium *tert*-butoxide and isopentyl nitrite in *tert*-butyl alcohol or in a *tert*-butyl alcohol–pyridine mixture gave oxime **9** (see NMR in Experimental) but only in very low yield (about 10%, most of the unreacted starting compound was recovered). Even an attempt



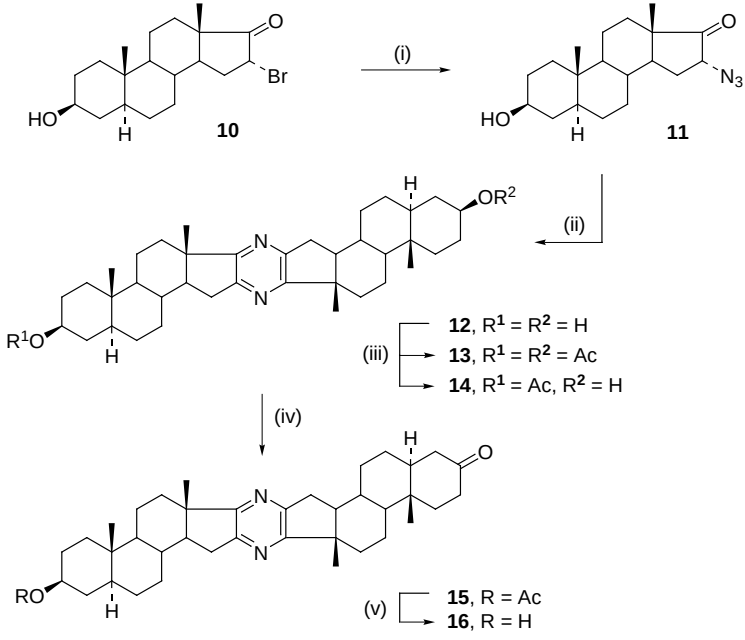
(i) Ac_2O, pyridine; (ii) CrO_3, H_2SO_4, CH_2Cl_2, Me_2CO; (iii) KOH, MeOH, THF; (iv) 1. *tert*-BuOK, *tert*-BuOH, DMF; 2. isopentyl nitrite

SCHEME 3

at increasing solubility by a *tert*-butyldimethylsilyl protection of the hydroxy group failed; the resulting protected derivative was insoluble as well (derivative of **8**, R = TBDMS). Despite the preparation problems, we tried to hydrogenate oxime **9** over palladium on carbon in acetic acid. The experiment was done on the scale of several milligrams, so the results are only tentative, but no fused product was identified after alkali addition. The only separated product seems to be the 17-hydroxy-16-oxo derivative of the starting bis-steroid pyrazine (MS FAB: 587 [M + 1], similar derivatives of simple steroids were found in trace amounts in the mixture after the reduction of azide **2** by the triphenylphosphine method).

The above results posed a question about the stability of bis-steroid pyrazines fused through rings D. To address this issue, we prepared azide **11** from ketone **10** *via* the same route as in the case of compounds **1** and **2** in the ring A fused series (Scheme 4). Hydrogenation and subsequent treatment as in the case of **3** did not give any crystalline product directly from

the reaction mixture. After purification by column chromatography, we obtained a crystalline material, but it was a nonseparable mixture, in which, in addition to the bis-steroid pyrazine **12**, other compounds were present, most probably dihydropyrazines with a similar chromatographic mobility. After some unsuccessful experiments, we changed the method of azide reduction and using a procedure[6] based on the triphenylphosphine method, we were able to prepare the bis-steroid pyrazine **12**, however, in a yield lower than in ring A fused series. To promote the oxidation/dehydrogenation step, we varied the conditions, but neither prolonged stirring, elevated temperature, change of the solvent, nor addition of copper(II) acetate had any marked effect on the yield.



(i) NaN_3, AcOH, DMF; (ii) 1. Ph_3P, THF; 2. H_2O; 3. O_2, TsOH, EtOH; (iii) Ac_2O, pyridine; (iv) CrO_3, H_2SO_4, CH_2Cl_2, Me_2CO; (v) KOH, MeOH, THF

SCHEME 4

Chemical properties of ring D fused bis-steroid pyrazines are similar to their ring A fused counterparts; after acetylation, it was possible to separate diacetate **13** and monoacetate **14**. Oxidation of monoacetate **14** gave ketone **15** and its deacetylation hydroxyketone **16**. When compared with

ring A fused analogues, the ring D fused bis-steroid pyrazines are little more soluble. However, preliminary experiments with bromination gave the same results as in the ring A fused series and nitrosation of **15** afforded only a 2,4-bis oxime derivative without any practical use for further condensation.

In summary, we accomplished simple syntheses of model bis-steroid pyrazines and prepared ring D fused derivatives. The extension of the pyrazine coupling of steroids to more than two steroid units will be a difficult task due to an extremely low solubility of bis-steroid pyrazines and to a relatively high chemical reactivity of the pyrazine nucleus.

Cytostatic activity of the parent bis-steroid pyrazines **3** and **12** was tested on their *in vitro* inhibition of the cell growth using the methodology described in ref.[12]. No significant inhibition on L1210, L929, HeLa S3 and CCRF-CEM cell cultures were found at the concentration of 10 μmol l^{-1} (precipitation in culture medium was observed).

EXPERIMENTAL

Melting points were determined on a Boetius micro melting point apparatus (Germany). Optical rotations were measured on a Perkin–Elmer 141 MC polarimeter; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer. ^{1}H NMR spectra were taken on a Varian UNITY-200 (200 MHz) or on Varian UNITY-500 (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) instruments at 23 °C, in deuteriochloroform, with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and width of multiplets (*W*) in Hz. Homonuclear 2D-COSY spectra were used for structural assignment of proton signals in compounds **3**, **6**, **9** and **13**. Partial assignment in 1D-spectra of other compounds was done on the basis of chemical shift values and the comparison with data on structurally similar compounds. Carbon signals in compounds **3**, **6**, **9**, **12**, **13** and **15** were assigned using: (i) distinguishing of CH_3-, CH_2-, CH- and C-type carbon atoms in APT spectra, (ii) literature data on structuraly similar compounds and (iii) ^{1}H–^{13}C correlation in an HMQC spectrum of compound **13**. The signals in NMR spectra of symmetrical bis-steroid pyrazines are numbered with only one set of numbers. Mass spectra (FAB) were recorded on a VG Analytical ZAB-EQ spectrometer. Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biomedicals, detection by spraying with concentrated sulfuric acid followed by heating); preparative TLC was done on 200 × 200 mm plates (layer thickness 0.4 mm). For column chromatography, neutral silica gel 60–120 μm was used. Prior to evaporation on a rotary evaporator *in vacuo* (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous $MgSO_4$.

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

To a solution of bromoketone **1** (ref.[13]; 1.5 g, 4.06 mmol) in DMF (25 ml) acetic acid (0.25 ml, 4.37 mmol) and sodium azide (1.0 g, 15.38 mmol) were added. The mixture was stirred at room temperature for 20 min and then poured on ice. Crystalline material was collected by

filtration, washed with water and dried. Yield of **2** was 1.03 g (77%), analytical sample was purified by column chromatography in a mixture of benzene–acetone (20 : 1) and crystallized from hot acetone, m.p. 170–173 °C (dec.), $[\alpha]_D^{25}$ –83 (*c* 0.95, chloroform). IR: 3 614 (O–H); 2 107 ($-N_3$); 1 723 (C=O); 1 054 (C–O). ^{1}H NMR (200 MHz): 3.99 dd, 1 H, *J* = 6.3, 13.0 (H-2β); 3.65 t, 1 H, *J* = 8.2 (H-17α); 1.10 s, 3 H (3 × H-19); 0.76 s, 3 H (3 × H-18). For $C_{19}H_{29}N_3O_2$ (331.5) calculated: 68.85% C, 8.82% H, 12.68% N; found: 68.98% C, 8.90% H, 12.42% N.

5″α-Androstano[2″,3″-5′,6′]pyrazino[2′,3′-2,3]-5α-androstane-17β,17″β-diol (**3**)

A solution of azide **2** (1.0 g, 3.02 mmol) in ethanol (100 ml) and 6 M hydrochloric acid (1 ml) was hydrogenated over 10% palladium on carbon (100 mg) at atmospheric pressure and at room temperature for 2 h. The catalyst was filtered off on celite, which was washed with ethanol, and the combined solutions were evaporated. Water (5 ml) and aqueous saturated $KHCO_3$ were added, the yellow solid was filtered off, dissolved in ethanol and evaporated. The residue was twice coevaporated with a mixture of benzene and ethanol (1 : 1, 20 ml) and then ethanol (20 ml) and 4-toluenesulfonic acid monohydrate (50 mg) were added. The mixture was shortly boiled to dissolve all solids and then intensively stirred in an open flask at room temperature for 24 h. During this time a solid was precipitating. The product was filtered off and washed with aqueous ethanol to give 480 mg (55%) of **3**, m.p. 340 °C (dec.). IR (KBr): 3 394 (O–H); 1 398 (pyrazine ring); 1 059, 1 046 (C–O). ^{1}H NMR (500 MHz): 3.66 t, 2 H, *J* = 8.7 (H-17); 2.92 d, 2 H, *J* = 16.5 (H-1β); 2.78 dd, 2 H, *J* = 17.5, 5.1 (H-4α); 2.59 bdd, 2 H, *J* = 17.5, 12.5 (H-4β); 2.52 bd, 2 H, *J* = 16.5 (H-1α); 2.07 ddt, 2 H, *J* = 13.5, 5.8, 9.2 (H-16β); 1.86 ddd, 2 H, *J* = 12.5, 3.9, 2.8 (H-12β); 1.75 dq, 2 H, *J* = 12.8, 3.5 (H-6α); 1.70 m, 2 H (H-11α); 1.66 m, 2 H (H-5); 1.64 m, 2 H (H-7β); 1.62 m, 2 H (H-15α); 1.46 m, 2 H (H-16α); 1.45 m, 2 H (H-11β); 1.40 m, 2 H (H-8); 1.35 m, 2 H (H-7α); 1.28 dq, 2 H, *J* = 5.9, 12.2 (H-15β); 1.12 dt, 2 H *J* = 4.1, 12.8 (H-12α); 0.99 ddd, 2 H, *J* = 12.2, 11.0, 7.2 (H-14); 0.94 m, 2 H (H-6β); 0.88 ddd, 2 H, *J* = 12.2, 10.6, 4.0 (H-9); 0.813 s, 6 H (6 × H-19); 0.766 s, 6 H (6 × H-18). ^{13}C NMR: 148.86 (C-2), 148.47 (C-3), 81.92 (C-17), 53.81 (C-9), 50.90 (C-14), 45.99 (C-1), 42.89 (C-13), 41.84 (C-5), 36.69 (C-12), 35.70 (C-10), 35.50 (C-4), 35.40 (C-8), 31.18 (C-7), 30.50 (C-16), 28.30 (C-6), 23.40 (C-15), 20.82 (C-11), 12.01 (C-19), 11.06 (C-18). MS, *m/z*: 573 (M + 1). For $C_{38}H_{56}N_2O_2$ (572.9) calculated: 79.67% C, 9.85% H, 4.89% N; found: 79.73% C, 9.90% H, 4.71% N.

5″α-Androstano[2,3″-5′,6′]pyrazino[2′,3′-2,3]-5α-androstane-17β,17″β-diol Diacetate (**4**) and 5″α-Androstano[2″,3″-5′,6′]pyrazino[2′,3′-2,3]-5α-androstane-17β,17″β-diol 17-Acetate (**5**)

To a stirred solution of diol **3** (1 g, 1.75 mmol) in pyridine (25 ml), acetic anhydride (50% solution in pyridine, 0.6 ml, 3.18 mmol) was added dropwise and stirring was continued at room temperature for 48 h. The mixture was poured on ice, the solid was filtered off, washed with water and dried. Chromatography on silica gel (100 ml) in a mixture of chloroform–ethyl acetate (100 : 1 to 50 : 1) gave successively diacetate **4** (50 mg, 4%), monoacetate **5** (460 mg, 43%) and recovered pyrazine **3** (372 mg, 37%).

Diacetate **4**: m.p. 330 °C (dec., ethanol), $[\alpha]_D^{25}$ +61 (*c* 0.5, chloroform). IR: 1 724 (C=O); 1 399 (pyrazine ring); 1 258, 1 032. ^{1}H NMR (200 MHz): 4.62 t, 2 H, *J* = 8.3 (H-17); 2.91 d, 2 H, *J* = 16.4 (H-1β); 2.79 dd, 2 H, *J* = 18.0, 5.8 (H-4α); 2.57 dd, 2 H, *J* = 18.0, 11.9 (H-4β);

2.52 bd, 2 H, *J* = 16.4 (H-1α); 2.04 s, 6 H (CH_3CO); 0.81 s, 12 H (6 × H-18, 6 x H-19). MS, *m/z*: 657 (M + 1). For $C_{42}H_{60}N_2O_4$ (657.0) calculated: 76.97% C, 9.21% H, 4.26% N; found: 77.12% C, 9.29% H, 4.10% N.

Monoacetate **5**: m.p. 345 °C (dec., ethanol). IR: 3 450 (O–H); 1 738 ($C=O_{acetate}$); 1 397 (pyrazine ring); 1 247, 1 239, 1 030 (C–O); 1 045 (C–OH). ^{1}H NMR (200 MHz): 4.62 t, 1 H, *J* = 8.3 (H-17α); 3.66 bt, 1 H, *J* = 8.3 (H-17″α); 2.95 d, 2 H, *J* = 17.4 (H-1β); 2.82 dd, 2 H, *J* = 18.2, 4.3 (H-4α); 2.60 dd, 2 H, *J* = 18.2, 12.2 (H-4β); 2.53 d, 2 H, *J* = 17.4 (H-1α); 2.04 s, 3 H (CH_3CO); 0.81 s, 9 H (6 × H-19, 3 × H-18); 0.77 s, 3 H (3 × H-18″). MS, *m/z*: 615 (M + 1). For $C_{40}H_{58}N_2O_3$ (614.9) calculated: 78.13% C, 9.51% H, 4.56% N; found: 78.27% C, 9.61% H, 4.29% N.

5″α-Androstano[2″,3″-5′,6′]pyrazino[2′,3′-2,3]-5α-androstane-17β,17″β-dione (**6**)

To diol **3** (120 mg, 0.21 mmol) stirred in a mixture of dichloromethane (5 ml) and acetone (5 ml) the Jones reagent (0.3 ml, 0.54 mmol) was added dropwise. After 10 min, the excess reagent was destroyed by propan-2-ol (0.3 ml), aqueous saturated $KHCO_3$ (about 10 ml) was added and organic solvents were removed on a rotary evaporator. The aqueous suspension was extracted with chloroform (3 × 5 ml), the combined extracts were washed with aqueous saturated $KHCO_3$ and water, dried and the solvent was evaporated. Boiling with acetone left 93 mg (78%) of ketone **6**. Analytical sample was recrystallized from a chloroform–ether mixture, m.p. 320 °C (dec.). IR (KBr): 1 743 (C=O); 1 399 (pyrazine ring). ^{1}H NMR (500 MHz): 2.93 d, 2 H, *J* = 16.7 (H-1β); 2.81 dd, 2 H, *J* = 18.0, 5.0 (H-4α); 2.61 bdd, 2 H, *J* = 18.0, 12.0 (H-4β); 2.54 bd, 2 H, *J* = 16.7 (H-1α); 2.46 ddd, 2 H, *J* = 19.2, 9.0, 0.8 (H-16β); 2.09 dt, 2 H, *J* = 19.2, 9.0 (H-16α); 1.98 dddd, 2 H, *J* = 12.2, 9.0, 5.8, 0.8 (H-15α); 1.89 m, 2 H (H-7β); 1.78 ddd, 2 H, *J* = 13.0, 4.0, 3.0 (H-11α); 1.70 m, 2 H (H-5); 1.68 m, 2 H (H-6α); 1.57 dq, 2 H, *J* = 3.7, 11.0 (H-8); 1.54 tt, 2 H, *J* = 12.4, 9.0 (H-15β); 1.49 dq, 2 H, *J* = 4.0, 13.0 (H-11β); 1.39 dq, 2 H, *J* = 3.8, 13.3 (H-6β); 1.31 ddd, 2 H, *J* = 12.6, 11.0, 5.8 (H-14); 1.31 dt, 2 H, *J* = 4.0, 13.0 (H-12α); 1.06 ddt, 2 H, *J* = 12.0, 3.6, 13.0 (H-7α); 0.95 ddd, 2 H, *J* = 12.5, 10.5, 4.0 (H-9); 0.895 s, 6 H (6 × H-18); 0.832 s, 6 H (6 × H-19). ^{13}C NMR: 220.94 (C-17), 148.72 (C-2), 148.41 (C-3), 53.78 (C-5), 51.33 (C-14), 47.64 (C-13), 45.89 (C-1), 41.76 (C-9), 35.81 (C-16), 35.76 (C-4), 35.41 (C-10), 34.90 (C-8), 31.54 (C-12), 30.45 (C-6), 28.11 (C-7), 21.78 (C-15), 20.50 (C-11), 13.71 (C-18), 11.98 (C-19). MS, *m/z*: 569 (M + 1). For $C_{38}H_{52}N_2O_2$ (568.9) calculated: 80.24% C, 9.21% H, 4.82% N; found: 79.97% C, 9.30% H, 4.75% N.

17″-Oxo-5″α-androstano[2″,3″-5′,6′]pyrazino[2′,3′-2,3]-5α-androstane-17β-yl Acetate (**7**)

To monoacetate **5** (400 mg, 0.65 mmol) stirred in a mixture of dichloromethane (10 ml) and acetone (10 ml) the Jones reagent (0.4 ml, 0.72 mmol) was added dropwise. After 20 min, the excess reagent was destroyed by propan-2-ol (0.4 ml), aqueous saturated $KHCO_3$ (about 50 ml) was added and organic solvents were removed on a rotary evaporator. The aqueous suspension was extracted by chloroform (3 × 15 ml), the combined extracts were washed by aqueous saturated $KHCO_3$ and water, dried and the solvent was evaporated. The product was purified on preparative TLC plates in a mixture of chloroform–methanol (50 : 1) yielding 310 mg (78%) of ketone **7**, m.p. 330 °C (dec., methanol), $[\alpha]_D^{25}$ +117 (*c* 0.5, chloroform). IR (KBr): 1 740 (C=O); 1 398 (pyrazine ring); 1 246, 1 032 (C–O). ^{1}H NMR (200 MHz): 4.62 t, 1 H, *J* = 8.3 (H-17α); 2.04 s, 3 H (CH_3CO); 0.89 s, 3 H (3 × H-18″); 0.83 s, 3 H (3 × H-19″); 0.81 s, 6 H (3 × H-19, 3 × H-18). MS, *m/z*: 613 (M + 1). For $C_{40}H_{56}N_2O_3$ (612.9) calculated: 78.39% C, 9.21% H, 4.57% N; found: 78.46% C, 9.28% H, 4.41% N.

17″β-Hydroxy-5″α-androstano[2″,3″-5′,6′]pyrazino[2′,3′-2,3]-5α-androstan-17-one (**8**)

Acetate **7** (200 mg, 0.32 mmol) in a mixture of benzene (10 ml) and methanol (10 ml) was deacetylated with sodium methoxide (100 mg Na in 5 ml of methanol; 0.6 ml, 0.52 mmol) at room temperature for 24 h. Solid CO_2 (about 0.5 cm^3) was added and the solvents were evaporated. The residue was extracted with chloroform (3 × 10 ml), the combined extracts were concentrated and the product was purified on the preparative TLC plates in a chloroform–methanol (50 : 1) mixture. Boiling with a mixture of methanol–water (100 : 1) gave 136 mg (73%) of ketone **8**, m.p. 335 °C (dec.), $[\alpha]_D^{25}$ +134 (*c* 0.5, chloroform). IR (KBr): 3 461 (O–H); 1 742, 1 729 (C=O); 1 397 (pyrazine ring); 1 048 (C–O). ^{1}H NMR (200 MHz): 3.65 t, 1 H, *J* ≈ 8 (H-17″); 0.90 s, 3 H (3 × H-18); 0.83 s, 3 H (3 × H-19); 0.82 s, 3 H (3 × H-19″); 0.77 s, 3 H (3 × H-18″). MS, *m/z*: 571 (M + 1). For $C_{38}H_{54}N_2O_2$ (570.9) calculated: 79.95% C, 9.53% H, 4.91% N; found: 79.92% C, 9.37% H, 4.73% N.

17″β-Hydroxy-5″α-androstano[2″,3″-5′,6′]pyrazino[2′,3′-2,3]-5α-androstan-16,17-dione 16-Oxime (**9**)

^{1}H NMR (500 MHz): 10.05 bs (oxime H); 3.66 t, 1 H, *J* ≈ 9.3 (H-17α); 2.97 dd, 1 H, *J* = 17.5, 6.6 (H-15″α); 2.94 d, 1 H, *J* = 17.0 (H-1″β); 2.92 d, 1 H, *J* = 17.0 (H-1β); 2.83 bdd, 1 H, *J* = 17.5, 5.2 (H-4″α); 2.79 bdd, 1 H, *J* = 17.5, 5.2 (H-4α); 2.62 bdd, 1 H, *J* = 17.5, 11.5 (H-4″β); 2.60 bdd, 2 H, *J* = 17.5, 11.5 (H-4β); 2.55 bd, 1 H, *J* = 17.0 (H-1″α); 2.53 bd, 1 H, *J* = 17.0 (H-1α); 2.16 dd, 1 H, *J* = 17.5, 13.2 (H-15″β); 2.07 dddd, 1 H, *J* = 13.6, 9.3, 9.0, 6.0 (H-16β); ≈1.68 m, 2 H (H-5α, H-5″α); ≈1.62 m, 1 H (H-15α); ≈1.46 m, 1 H (H-16α); ≈1.45 m, 1 H (H-14″α); ≈1.27 m, 1 H (H-15β); 0.988 s, 3 H (3 × H-18″); 0.852 s, 3 H (3 × H-19″); 0.814 s, 3 H (3 × H-19); 0.768 s, 3 H (3 × H-18″). ^{13}C NMR: 205.09 (C-17″), 156.55 (C-16″), 149.16 (C-2″), 148.73 (C-3), 148.49 (C-2), 148.26 (C-3″), 81.90 (C-17), 53.78 (C-9), 53.58 (C-9″), 50.89 (C-14), 48.76 (C-13″), 46.39 (C-14″), 45.86 (C-1), 45.56 (C-1″), 42.87 (C-13), 41.77 (C-5), 41.68 (C-5″), 36.67 (C-12), 35.79 (C-10″), 35.66 (C-10), 35.38 (C-8), 35.34 (C-4), 35.22 (C-4″), 34.30 (C-8″), 31.14 2 C (C-7, C-15″), 30.46 2 C (C-7″, C-16), 28.25 (C-6), 27.98 (C-6″), 25.50 (C-12″), 23.38 (C-15), 20.80 (C-11), 20.34 (C-11″), 14.08 (C-18″), 12.00 (C-19), 11.97 (C-19″), 11.03 (C-18).

16β-Azido-3β-hydroxy-5α-androstan-17-one (**11**)

To a solution of bromoketone **10** (ref.[14]; 3.0 g, 8.12 mmol) in DMF (60 ml) acetic acid (0.5 ml, 8.74 mmol) and sodium azide (1.8 g, 27.69 mmol) were added. The mixture was stirred at room temperature for 20 min and then poured on ice. Crystalline material was collected by filtration, washed with water and dried. Yield of **11** was 2.65 g (98%), analytical sample was crystallized from hot acetone, m.p. 184–185 °C (dec., ref.[15] gives m.p. 113–116 °C), $[\alpha]_D^{25}$ –28 (*c* 1.0, chloroform). IR: 3 610 (O–H); 2 102 (–N_3); 1 749 (C=O); 1 034 (C–O). ^{1}H NMR: 3.71 t, 1 H, *J* = 8 (H-16α); 3.58 m, 1 H, *W* = 32 (H-3α); 2.32 ddd, 1 H, *J* = 11.6, 8.4, 4.6 (H-15α); 0.92 s, 3 H (3 × H-19); 0.83 s, 1 H (3 × H-18). For $C_{19}H_{29}N_3O_2$ (331.5) calculated: 68.85% C, 8.82% H, 12.68% N; found: 69.03% C, 8.85% H, 12.57% N.

5″α-Androstano[16″,17″-5′,6′]pyrazino[2′,3′-16,17]-5α-androstane-3β,3″β-diol (**12**)

A dry mixture of azide **11** (1.0 g, 3.02 mmol) and triphenylphosphine (2.0 g, 7.62 mmol) was stirred under argon for 10 min and then tetrahydrofuran (20 ml) was added through

septum. The mixture was stirred for additional 1 h; during this period a gas was evolving from the solution. Water (1.0 ml, 55.56 mmol) was added and the mixture was stirred for 24 h. The solution was transferred to a 250 ml round flask, the reaction vessel was washed with tetrahydrofuran and the combined solutions were concentrated. The product was coevaporated twice with toluene (20 ml), then ethanol (10 ml) and 4-toluenesulfonic acid monohydrate (50 mg) were added. The mixture was shortly boiled to dissolve all solids and intensively stirred in an open flask at room temperature for 24 h. The solvents were evaporated, the residue was coevaporated twice with toluene (20 ml) and chromatographed on silica gel (50 ml) in a mixture of chloroform–methanol (100 : 1). The glassy main fraction was boiled with ethanol to give 160 mg (18%) of crystalline solid **12**, m.p. >340 °C (from 320 °C subl.), $[\alpha]_D^{25}$ +53 (*c* 0.5, chloroform). IR (KBr): 3 415 (O–H); 1 041 (C–O). ^{1}H NMR (200 MHz): 3.61 m, 2 H, *W* = 32 (H-3); 2.78 dd, 2 H, *J* = 15.8, 5.6 (H-15α); 2.59 dd, 2 H, *J* = 15.8, 10.2 (H-15β); 2.21 m, 2 H, *W* = 12 (H-12β); 1.01 s, 6 H (6 × H-19); 0.89 s, 6 H (6 × H-18). ^{13}C NMR: 163.84 (C-17), 155.51 (C-16), 71.21 (C-3), 54.83 (C-9), 54.72 (C-14), 44.99 (C-5), 44.11 (C-13), 38.13 (C-4), 36.72 (C-1), 35.80 (C-10), 34.12 (C-8), 33.56 (C-12), 31.86 (C-15), 31.58 (C-2), 31.45 (C-7), 28.45 (C-6), 20.77 (C-11), 17.56 (C-18), 12.32 (C-19). MS, *m/z*: 573 (M + 1). For $C_{38}H_{56}N_2O_2$ (572.9) calculated: 79.67% C, 9.85% H, 4.89% N; found: 79.76% C, 9.91% H, 4.68% N.

5″α-Androstano[16″,17″-5′,6′]pyrazino[2′,3′-16,17]-5α-androstane-3β,3′β-diol Diacetate (**13**) and 5″α-Androstano[16″,17″-5′,6′]pyrazino[2′,3′-16,17]-5α-androstane-3β,3″β-diol 3-Acetate (**14**)

To a stirred solution of diol **12** (1 g, 1.75 mmol) in pyridine (25 ml), acetic anhydride (50% solution in pyridine; 0.6 ml, 3.18 mmol) was added dropwise. Stirring was continued at room temperature for 48 h. The mixture was poured on ice, the solid was filtered off, washed with water and dried. Chromatography on silica gel (100 ml) in a mixture of chloroform–ethyl acetate (50 : 1 to 30 : 1) gave successively diacetate **13** (70 mg, 6%), monoacetate **14** (527 mg, 49%) and recovered diol **12** (278 mg, 27%).

Diacetate **13**: m.p. 342–343 °C (ethanol), $[\alpha]_D^{25}$ +42 (*c* 0.5, chloroform). IR: 1 724 (C=O); 1 261, 1 254, 1 025. ^{1}H NMR (500 MHz): 4.77 tt, 2 H, *J* = 11.4, 5.0 (H-3); 2.77 dd, 2 H, *J* = 15.0, 6.4 (H-15α); 2.59 m, 2 H, *W* = 30 (H-15β); 2.21 m, 2 H (H-12β); 2.03 s, 6 H (CH_3CO); 1.85 m, 2 H (H-2α); 1.78 m, 2 H (H-7α); 1.75 m, 2 H (H-1β); 1.73 m, 2 H (H-11α); 1.70 m, 2 H (H-8); 1.67 m, 2 H (H-14); 1.62 m, 2 H (H-4α); 1.52 m, 4 H (H-11β, H-12α); 1.50 m, 2 H (H-2β); 1.37 m, 2 H (H-4β); 1.35 m, 4 H (H-6α, H-6β); 1.23 m, 2 H (H-5); 1.05 m, 2 H (H-1α); 1.03 m, 2 H (H-7β); 1.005 s, 6 H (6 × H-18); 0.903 s, 6 H (6 × H-19); 0.85 m, 2 H (H-9). ^{13}C NMR: 170.66 (C=O), 163.77 (C-17), 155.45 (C-16), 73.54 (C-3), 54.66 (C-9), 54.59 (C-14), 44.79 (C-5), 44.09 (C-13), 36.47 (C-1), 35.81 (C-10), 34.11 (C-8), 33.97 (C-4), 33.51 (C-12), 31.85 (C-15), 31.49 (C-7), 28.34 (C-6), 27.41 (C-2), 21.44 (**C**H_3CO), 20.72 (C-11), 17.56 (C-18), 12.22 (C-19). MS, *m/z*: 657 (M + 1). For $C_{42}H_{60}N_2O_4$ (657.0) calculated: 76.97% C, 9.21% H, 4.26% N; found: 77.03% C, 9.35% H, 4.17% N.

Monoacetate **14**: m.p. 312–313 °C (ethanol), $[\alpha]_D^{25}$ +47 (*c* 0.1, chloroform). IR: 3 437 (O–H); 1 738 ($C{=}O_{acetate}$); 1 247, 1 030 (C–O). ^{1}H NMR (500 MHz): 4.70 m, 1 H, *W* = 32 (H-3); 3.61 m, 1 H, *W* = 32 (H-3″); 2.77 dd, 2 H, *J* = 6.5, 15.0 (H-15α, H-15″α); 2.60 m, 2 H (H-15β, H-15″β); 2.21 m, 2 H (H-12β, H-12″β); 2.03 s, 3 H (CH_3CO); 1.007 s, 6 H (3 × H-18, 3 × H-18″); 0.904 s, 3 H (3 × H-19); 0.888 s, 3 H (3 × H-19″). MS, *m/z*: 615 (M + 1). For

$C_{40}H_{58}N_2O_3$ (614.9) calculated: 78.13% C, 9.51% H, 4.56% N; found: 78.31% C, 9.65% H, 4.38% N.

3″-Oxo-5″α-androstano[16″,17″-5′,6′]pyrazino[2′,3′-16,17]-5α-androstan-3β-yl Acetate (**15**)

To monoacetate **14** (80 mg, 0.13 mmol) stirred in a mixture of dichloromethane (3 ml) and acetone (3 ml) the Jones reagent (0.2 ml, 0.36 mmol) was added dropwise. After 10 min the excess reagent was destroyed with propan-2-ol (0.2 ml), aqueous saturated $KHCO_3$ was added and organic solvents were removed on a rotary evaporator. The aqueous suspension was extracted with chloroform (3 × 2 ml), the combined extracts were washed by aqueous saturated $KHCO_3$ and water, dried and the solvent was evaporated. Boiling with ethanol left 55 mg (69%) of ketone **15**, m.p. 330–331 °C, $[\alpha]_D^{25}$ +55 (*c* 0.5, chloroform). IR (KBr): 1 738 ($C{=}O_{acetate}$); 1 713 ($C{=}O_{ketone}$); 1 250, 1 027 (C–O). ^{1}H NMR (500 MHz): 4.70 m, 1 H, *W* = 32 (H-3); 2.78 m, 2 H (H-15α, H-15″α); 2.60 m, 2 H (H-15β, H-15″β); 2.02 s, 3 H (CH_3CO); 1.092 s, 3 H (3 × H-19″); 1.035 s, 3 H (3 × H-18″); 1.008 s, 3 H (3 × H-18); 0.905 s, 3 H (3 × H-19). ^{13}C NMR: 211.70 (C-3″); 170.70 (C=O); 163.95, 163.61 (C-17, C-17″); 155.61, 155.33 (C-16, C-16″); 73.55 (C-3); 54.68, 54.61, 54.48, 54.26 (C-9, C-9″, C-14, C-14″); 46.75 (C-5′); 44.80 (C-5); 44.63 (C-4″); 44.12, 44.08 (C-13, C-13″); 38.21 (C-1″); 38.08 (C-2″); 36.49 (C-1); 35.98, 35.81 (C-10, C-10″); 34.12, 34.04 (C-8, C-8″); 33.98 (C-4); 33.52, 33.50 (C-12, C-12″); 31.85, 2 C (C-15, C-15″); 31.50 (C-7); 31.24 (C-7″); 28.69 (C-6″); 28.34 (C-6); 27.41 (C-2); 21.42 (CH_3CO); 21.00, 20.72 (C-11,C-11″); 17.56, 2 C (C-18, C-18″); 12.22 (C-19); 11.47 (C-19). MS, *m/z*: 613 (M + 1). For $C_{40}H_{56}N_2O_3$ (612.9) calculated: 78.39% C, 9.21% H, 4.57% N; found: 78.46% C, 9.28% H, 4.41% N.

3″β-Hydroxy-5″α-androstano[16″,17″-5′,6′]pyrazino[2′,3′-16,17]-5α-androstan-3-one (**16**)

Acetate **15** (100 mg, 0.16 mmol) in a mixture of benzene (10 ml) and methanol (10 ml) was deacetylated with sodium methoxide (100 mg Na in 5 ml of methanol; 0.3 ml, 0.26 mmol) at room temperature for 24 h. Solid CO_2 (about 0.5 cm^3) was added and the solvents were evaporated. The residue was extracted with chloroform (3 × 2 ml), the combined extracts were concentrated and the product was purified on preparative TLC plates in a chloroform–methanol (10 : 1) mixture. Boiling with a mixture of methanol–water (100 : 1) gave 50 mg (54%) of ketone **16**, m.p. 320 °C (dec.), $[\alpha]_D^{25}$ +65 (*c* 0.5, chloroform). IR (KBr): 3 446 (O–H); 1 713 (C=O); 1 045 (C–O). ^{1}H NMR (200 MHz): 3.60 m, 1 H, *W* = 32 (H-3″); 2.78 m, 2 H (H-15″α, H-15α); 2.60 m, 2 H (H-15′β, H-15β); 1.09 s, 3 H (3 × H-19); 1.03 s, 3 H (3 × H-18); 1.01 s, 3 H (3 × H-18″); 0.88 s, 3 H (3 × H-19″). MS, *m/z*: 571 (M + 1). For $C_{38}H_{54}N_2O_2$ (570.9) calculated: 79.95% C, 9.53% H, 4.91% N; found: 79.85% C, 9.38% H, 4.62% N.

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