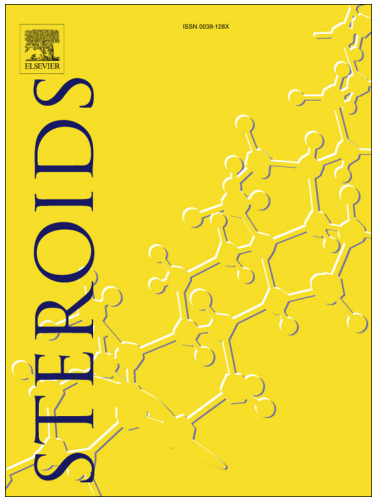

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Synthesis of a human long-term oxymetholone metabolite

Nicolas Kratena[1], Nina Biedermann[1], Biljana Stojanovic[3], Lorenz Göschl[3], Matthias Weil[2], Valentin S. Enev*[1], Günter Gmeiner[3], Peter Gärtner*[1]

[1] Institute of Applied Synthetic Chemistry, Vienna University of Technology

[2] Institute for Chemical Technologies and Analytics, Vienna University of Technology

[3] Doping Control Laboratory, Seibersdorf Labor GmbH

* valentin.enev@tuwien.ac.at, peter.gaertner@tuwien.ac.at,

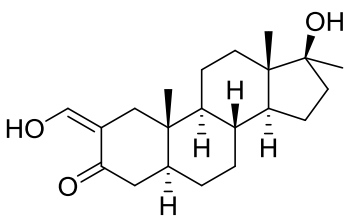
Abstract:

A long-term metabolite of the doping agent oxymetholone (OXM-M2, 17β-hydroxymethyl-2,17α-methyl-18-norandrost-13-en-3-one) which has been identified by GC-MS/MS was synthesized from commercially available materials. Two efficient synthetic routes to access both C-17 epimers of tentative metabolites were developed. The identity and molecular configuration of the *in vivo* metabolite: 17β-hydroxymethyl-2α,17α-methyl-18-norandrost-13-en-3-one was confirmed by single crystal X-ray diffraction.

1. Introduction

Oxymetholone (Anadrol, Anapolon, **1**) is an anabolic androgenic steroid (AAS), more specifically a 17α-methyltestosterone derivative. It was first described in 1959 by Ringold and coworkers at Syntex [1] and has since been used to treat anemia, osteoporosis and to promote muscle growth in different disorders like HIV-related wasting. It was considered to be one of the strongest AAS available in medicine. [2-4] This lead to abuse of the substance for performance enhancing purposes by bodybuilders and professional athletes. Consequently, it is listed as "prohibited at all times" by the World Anti-Doping Agency (WADA). [5]

The metabolism of oxymetholone has been thoroughly investigated. [6-16] The A-ring can be transformed *in vivo* in a number of ways, some leading to oxidation and decarboxylation at C-2, others reducing the C-2 substituent as well as the C-3 ketone. Not all of these metabolites could be traced unilaterally to oxymetholone, and the detection timeframe for the polyhydroxylated metabolites was generally short (max. 5 days). In doping analysis, special interest is bestowed on specific long-term metabolites (retained for more than two weeks) to maximize the window of detection. Two such metabolites (OXM-M1 and OXM-M2) were discovered by Sobolevsky and Rodchenkov and reported in 2012. [16] The assigned structures are depicted in Figure 1. The metabolite OXM-M2 (**2**) was selected as a target compound for synthesis to elucidate C-2 and C-17 configuration and secure its identity.



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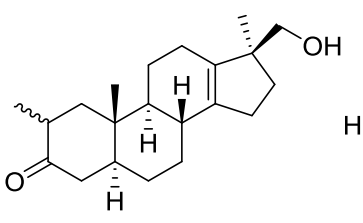
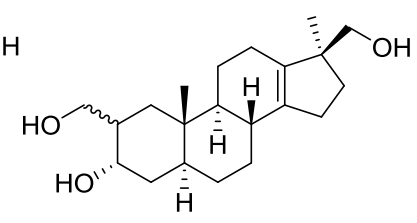


OXM-M2 **(2)**



OXM-M1 **(3)**

Figure 1: Molecular structures of relevant compounds

The C-17 substituents and the 13,14-double bond were planned to be introduced in the same fashion as in earlier projects [17, 20] concerning long-term metabolites with rearranged D-ring. Further, it was envisaged to first prepare the 2β-methyl (axial) derivatives of **2** *via* cuprate addition to an epoxide. This method would grant absolute stereocontrol over the newly formed center and does not allow immediate epimerization at that carbon to occur. After oxidation of the C-3 hydroxy group to a carbonyl, basic or acidic [21] epimerization would provide the more stable 2α-methyl derivatives. Dehydroepiandrosterone (DHEA) acetate was chosen as a commercially available starting material.

2. Experimental

Dehydroepiandrosterone acetate was purchased from FluoroChem. Dry toluene, dichloromethane, dimethylformamide and *m*-CPBA are from Acros Organics. HPLC grade solvents (acetonitrile, methanol, isopropanol, benzene) were from VWR. All other non-specified chemicals were from Sigma-Aldrich. Anhydrous tetrahydrofuran was pre-dried using an Innovative Technologies PureSolv system, degassed and stored over 3Å molecular sieves. Potassium dihydrogen phosphate, disodium hydrogen phosphate dihydrate, potassium hydrogen carbonate, potassium carbonate, diphosphorus pentoxide were purchased from Merck. N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was purchased from Macherey-Nagel, and beta-glucuronidase obtained from Roche. The internal standard D_3-Testosterone was provided by the National Measurement Institute (Sydney, Australia).

NMR spectra were recorded on a Bruker AC400 and AC600 using TMS as internal standard. IR spectra were recorded on a Perkin Elmer Spectrum 65 as thin films (ATR FT-IR). TLC-analysis was performed with pre-coated aluminium-backed plates (Silica gel 60 F254, Merck). Compounds were visualized by submerging in an acidic phosphomolybdic acid/cerium sulphate solution and heating. Melting points were determined with a Kofler hot-stage apparatus.

Sample preparation for GC-MS/MS analysis: To 5 mL of urine 100µl of D3-Testosterone (IS), 1 mL of 0.8M phosphate buffer pH 7 and 25 µL of β-glucuronidase were added. Hydrolysis was performed at 50°C for 2h. After hydrolysis, 1 mL of 20% potassium carbonate buffer pH 9.0 and 7 mL of MTBE were added, and a liquid-liquid extraction was performed by shaking for 10 min. After that, sample was centrifugated for 7 min with 2100 rpm, the organic layer was transferred and evaporated. Subsequently the samples were dried for 15 min over diphosphorus pentoxide. Derivatization was performed by adding 100 µL working solution for derivatization and heating at 60°C for 20 min. For derivatization, a trimethylsilyl iodide stock solution was prepared by mixing 5 mL of MSTFA with 300 µL of ethanethiol. Subsequently, 100 mg of NH_4I were dissolved in this mixture. A working solution for derivatization was prepared by adding 1 mL of the trimethylsilyl iodide stock solution to 9 mL of MSTFA. Solutions of internal standard (1µg/ml) and analytes (1µg/ml) were prepared in methanol and stored at -20°C.

The GC-MS/MS measurements were performed with a Trace-1300 gas chromatograph coupled to a TSQ-8000 Evo triple quadrupole mass spectrometer, and a TriPlus-100

autosampler (Thermo Fisher, Austin, USA). For chromatographic separation a Rtx-1MS fused silica capillary column, 15 m x 0.25 mm ID, 0.11 µL film thickness was used (Restek, CP-Analytica, Mistelbach, Austria). Injections of 2.5 µL were performed in split mode with an injector temperature of 270°C, using a split flow of 40 mL/min. The temperature program for the GC run was following: 170°C initial column temperature, 3°C/min to 210°C, held for 1 min, 25°C/min to 305°C, held for 3 min. High purity helium gas was used as carrier gas with a constant pressure of 80 kPa. The temperature of the transfer line and ion source was set to 270°C. Electron impact ionization was used with an electron energy of 70 eV and data were acquired in selected reaction monitoring (SRM) mode. Transition m/z 435 → 209 was used for internal standard D3-Testosterone.

For single crystal X-ray diffraction analysis, crystals were embedded in perfluorinated polyether and mounted on MITGEN™ loops. X-ray diffraction data were measured in a cold stream of nitrogen at *T* = 100 K on a Bruker APEX-II diffractometer with Mo-*K*α radiation. The collection strategy for the measurement was optimized with APEX-3 using ω- and φ-scans. After integration of the data with SAINT, a semi-empirical absorption correction was performed with SADABS. The crystal structures were solved by Direct Methods and refined using the SHELXTL program package. All H atoms were placed geometrically and refined in a riding model approximation. H atoms of hydroxyl groups were taken from difference maps and refined freely. Crystallographic data for the structure(s) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-1900498-1900499. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax.: (internat.) +44 1223/336-033; e-mail: deposit@ccdc.cam.ac.uk]

2.1 3β-(*p*-Toluenesulfonyloxy)-13α-androstan-17-one (**5**)

Starting material **4** (2.05 g, 6.74 mmol) was charged in a round bottom flask and dry pyridine (15 mL) was added until complete dissolution. After addition of a catalytic amount of 4-dimethylaminopyridine (4-DMAP, ca. 0.005 g), tosyl chloride (2.57 g, 13.48 mmol) was added in three portions over 15 minutes. The reaction was stirred at room temperature for 18 hours, then quenched by addition of water (20 mL) followed by CH_2Cl_2 (50 mL). The mixture was extracted with CH_2Cl_2 (3x) and the pooled organic extracts were dried over $MgSO_4$ and evaporated. Crude product was purified via column chromatography (75 g SiO_2, LP:EtOAc = 5:1) to give 2.59 (86%) of product **5** as colorless crystals. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 7.79 (2H, d, *J* = 8.07 Hz), 7.32 (2H, d, *J* = 8.07 Hz), 4.42 (1H, m), 2.44 (3H, s), 2.33 (1H, m), 1.96 – 2.20 (4H, m), 1.69 – 1.85 (3H, m), 1.42 – 1.66 (5H, m), 1.02 – 1.31 (4H, m), 0.95 (3H, s), 0.82 – 0.93 (3H, m), 0.62 (3H, s), 0.52 – 0.72 (2H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 222.33, 144.49, 134.91, 129.86, 127.71, 82.39, 51.52, 50.78, 50.22, 44.37, 37.78, 36.69, 35.49, 34.81, 33.91, 32.96, 32.17, 28.38, 28.29, 25.36, 22.73, 21.77, 21.41, 11.94. IR [cm^{-1}]: 2916, 1730, 1341, 1171. α_D^{20}: -84.77 (c 1.35, CH_2Cl_2). m.p.: 130 – 131 °C. HRMS: (M-OTs) Calcd.: 273.2213, found: 273.2229.

2.2 13α-Androst-2-en-17-one (**6**, **7**)

Tosylate **5** (2.39 g, 5.38 mmol) was weighed into a round bottom flask and dissolved in dry DMF (60 mL). The reaction was heated to 50 °C and LiBr (2.8 g, 32.3 mmol) and Li_2CO_3 (2.38

g, 32.3 mmol) were added at once. The reaction was further heated on an oil bath at reflux for 1 hour, then allowed to cool. The reaction mixture was poured onto 1 M HCl (100 mL) and the mixture diluted with CH_2Cl_2 (60 mL). After extraction with CH_2Cl_2 (2x), washing the pooled extracts with water, drying over $MgSO_4$ and evaporating under reduced pressure the crude product was obtained. This could be purified by recrystallizing from MeOH/H_2O = 2:1, yielding 1.33 g (91%) of a mixture of regioisomeric products **6** and **7** in a mixture of 6:1 (determined by ^{1}H-NMR). HRMS: (M+H) Calcd.: 273.2213, found: 273.2208.

2.3 2α,3α-Epoxy-13α-androstan-17-one (**8**, **9**)

Product **6** (1.18 g, 4.33 mmol) was charged in a round bottom flask and dissolved in CH_2Cl_2 (40 mL). There was added $KHCO_3$ (0.87 g, 8.7 mmol), and *meta*-chloroperoxybenzoic acid (1.17 g, 4.76 mmol, ca. 70%) in one portion. The suspension was stirred at room temperature for 3 hours and quenched with saturated $Na_2S_2O_3$ solution. Extracted with CH_2Cl_2 (3x) and washed with saturated $NaHCO_3$ solution before drying over $MgSO_4$ and evaporating under reduced pressure. Purification via column chromatography (55 g SiO_2, LP:Et_2O = 7:1) gives 1.04 g of epoxides **8** and **9** (83%). Compound **8** was not isolated in a pure form. Analytical data of **9** obtained after the cuprate addition (See 2.4.) in accordance with literature [17]. HRMS: (M+H) Calcd.: 289.2162, found: 289.2165.

2.4 3α-Hydroxy-2β-methyl-13α-androstan-17-one (**10**)

In a Schlenk flask there was charged dry CuI (2.36 g, 12.4 mmol) and dry Et_2O (15 mL). The mixture was chilled to -20 °C and 1.6 M methyllithium (15.5 mL, 24.75 mmol) was added dropwise over the course of 15 minutes. After complete addition a clear solution formed and starting material **8** (0.51 g, 1.77 mmol) was added as a solution in dry Et_2O (5 mL). The reaction was allowed to warm to room temperature, sealed and stirred for 18 hours at room temperature. The resulting suspension was poured onto saturated NH_4Cl solution (100 mL) and treated with conc. NH_3 (2 mL). The mixture was stirred vigorously for 30 minutes and then extracted with Et_2O (4x). The pooled organic phases were washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification via column chromatography (30 g SiO_2, LP:CH_2Cl_2:CH_3CN = 25:25:1) provided recovered 69 mg starting material (13%, unreacted **9**) as well as 272 mg of a mixture of product **10** and 3-oxo byproduct **11** (51%). This was used directly in the next step. HRMS: (M+H) Calcd.: 305.2475, found: 305.2479.

2.5 3α-Hydroxy-2β-methyl-13α-androstan-17-one pivalate (**12**)

To mixture of **10** and **11** (0.54 g, 1.77 mmol) in a round bottom flask was added dry pyridine (15 mL) and 4-DMAP (ca. 0.005 g). The clear solution was chilled to 0 °C and trimethylacetyl chloride (0.175 mL, 1.4 mmol) was added dropwise. The mixture was stirred for 16 hours and taken up in H_2O (50 mL) and CH_2Cl_2 (50 mL). The mixture was shaken and extracted with CH_2Cl_2 (2x), washed with 1 M NaOH, dried over $MgSO_4$ and evaporated to dryness. Column chromatography of the crude product (25 g SiO_2, LP:CH_2Cl_2:CH_3CN = 40:40:1) provided 337 mg pivalate **12** (65% single step, 33% over two steps) as white crystalline solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 4.68 (1H, m), 2.31 (1H, m), 2.08 – 2.20 (2H, m), 1.87 – 2.05 (3H, m), 1.80 (1H, m), 1.65 (1H, m), 1.30 – 1.58 (6H, m), 1.23 (3H, s), 1.08 – 1.28 (3H, m), 1.18 (9H, s), 1.00 (3H, d, *J* = 7.70 Hz), 0.94 (3H, s), 0.84 – 0.92 (2H, m), 0.67 (3H, s), 0.56 – 0.72 (2H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 222.51, 177.96, 74.27, 53.16, 50.94, 50.31, 41.21, 39.96, 39.01,

37.52, 36.07, 33.97, 33.05, 32.79, 32.39, 29.78, 28.18, 27.41, 26.70, 25.45, 22.58, 21.37, 20.10, 15.50. IR [cm^{-1}]: 2916, 1733, 1720, 1284. α_D^{20}: -24.04 (c 0.8, CH_2Cl_2). m.p.: 108 °C. HRMS: (M+H) Calcd.: 389.3050, found: 389.3050.

2.6 2β-Methyl-17-methylene-13α-androstan-3α-ol pivalate (**13**)

In a Schlenk flask there was weighed in zinc (2.18 g, 33.4 mmol) and dry THF (15 mL) was added. Dibromomethane (1.93 g, 11.12 mmol) and $PbCl_2$ (< 0.005 g) were added and the mixture was cooled to -25 °C. Neat $TiCl_4$ (1.19 g, 6.25 mmol) was added dropwise under vigorous stirring and the resulting suspension was stirred at -10 °C for 2 hours. Starting material **12** (0.515 g, 1.33 mmol) dissolved in dry THF (5 mL) was added dropwise at 0 °C, the vessel was then sealed and stirred for 16 hours at room temperature. The mixture was poured onto ice/1 M HCl (150 mL) and extracted with Et_2O (4x) and EtOAc (1x). The organic phases were washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated under reduced pressure. Crude product was purified via flash chromatography (10 g SiO_2, LP:Et_2O = 10:1) to give 300 mg of pivalate **13** (59%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 4.80 (1H, s), 4.70 (1H, m), 4.67 (1H, s), 2.28 – 2.52 (2H, m), 1.85 – 2.00 (2H, m), 1.79 (1H, m), 1.67 (1H, m), 1.56 (1H, m), 1.40 – 1.51 (3H, m), 1.28 – 1.39 (2H, m), 1.15 – 1.27 (2H, m), 1.20 (9H, s), 1.11 (1H, m), 1.02 (3H, d, *J* = 7.61 Hz), 0.88 – 0.98 (1H, m), 0.77 – 0.87 (2H, m), 0.72 (3H, s), 0.62 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 177.98, 157.56, 102.84,74.36, 54.19, 54.15, 46.09, 41.31, 40.00, 38.96, 36.64, 36.03, 34.68, 32.91, 32.80, 31.50, 29.84, 29.48, 28.39, 27.35, 24.39, 21.10, 20.09, 15.65. IR [cm^{-1}]: 2926, 1722, 1148, 873. α_D^{20}: 1.03 (c 0.98, CH_2Cl_2). HRMS: (M-OPiv) Calcd.: 285.2577, found: 285.2590.

2.7 2β-Methyl-17-methylene-13α-androstan-3α-ol (**14**)

Pivalate **13** (0.47 g, 1.22 mmol), was charged in a round bottom flask and dissolved in dry THF (10 mL). The solution was chilled to 0 °C and $LiAlH_4$ (0.09 g, 2.4 mmol) was added under argon flow. The suspension was stirred for 30 minutes and then quenched by addition of water (2 mL), followed by Et_2O (50 mL). The suspension was stirred and excess $MgSO_4$ was added. The solids were filtered and washed thoroughly with Et_2O (3x 30 mL). The filtrate was evaporated to provide 370 mg pure alcohol **14** (>99%) as white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 4.80 (1H, m), 4.68 (1H, m), 3.71 (1H, m), 2.28 – 2.51 (2H, m), 1.85 – 1.95 (3H, m), 1.77 (1H, m), 1.67 (1H, m), 1.51 – 1.60 (2H, m), 1.48 (1H, m), 1.36 – 1.43 (2H, m), 1.27 – 1.35 (2H, m), 1.17 – 1.24 (2H, m), 1.12 (1H, m), 1.01 (3H, d, *J* = 7.47 Hz), 0.93 (3H, s), 0.80 – 0.96 (4H, m), 0.70 (3H, s), 0.68 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 157.70, 102.77, 72.12, 54.24, 54.05, 46.11, 40.61, 38.84, 36.67, 36.27, 35.93, 34.73, 32.85, 32.75, 31.52, 29.51, 28.49, 24.38, 21.12, 20.59, 15.66. IR [cm^{-1}]: 3393, 2922, 997, 887. α_D^{20}: 24.65 (c 0.6, CH_2Cl_2). m.p.: 117 – 118 °C. HRMS: (M-OH) Calcd.: 285.2577, found: 285.2594.

2.8 2β-Methylspiro[13α-androstan-17ξ,2'-oxiran]-3α-ol (**15**, **16**)

To alcohol **14** (0.37 g, 1.22 mmol) in CH_2Cl_2 (12 mL) was added $KHCO_3$ (0.25 g, 2.5 mmol) followed by *meta*-chloroperoxybenzoic acid (0.36 g, 1.47 mmol, 70%) at 0 °C. The mixture was stirred at room temperature for 2 hours and quenched with saturated $Na_2S_2O_3$ and saturated $NaHCO_3$ solution. After extraction with CH_2Cl_2 (2x) and drying over $MgSO_4$ the organic phases were evaporated to dryness and the residue purified via column

chromatography (20 g SiO_2, LP:EtOAc = 12:1) giving 73 mg 17β-epoxide **15** (19%) as colorless oil and 290 mg 17α-epoxide **16** (74%) as white foam.

17β-Epoxide (**15**): ^{1}H-NMR (400 MHz, CD_2Cl_2): δ_H = 3.66 (1H, m), 2.63 (1H, d, *J* = 4.71 Hz), 2.55 (1H, d, *J* = 4.71 Hz), 2.13 (1H, dd, *J* = 11.12 Hz, 9.56 Hz), 2.00 (1H, s), 1.98 (1H, m), 1.84 (2H, m), 1.66 – 1.76 (3H, m), 1.53 – 1.64 (2H, m), 1.36 – 1.52 (3H, m), 1.29 (2H, m), 1.24 (2H, m), 1.17 (1H, m), 1.10 (1H, m), 1.00 (3H, d, *J* = 7.67 Hz), 0.90 (3H, s), 0.86 (1H, m), 0.77 (3H, s), 0.66 (1H, m), ^{13}C-NMR (400 MHz, CD_2Cl_2): δ_C = 72.30, 68.66, 54.68, 53.32, 47.83, 41.67, 41.00, 39.25, 37.08, 36.67, 36.42, 33.48, 33.23, 32.43, 30.61, 28.84, 28.58, 24.62, 23.00, 20.74, 15.99. IR [cm^{-1}]: 3414, 2919, 737. α_D^{20}: 3.86 (c 1.32, CH_2Cl_2). HRMS: (M-OH) Calcd.: 301.2526, found: 301.2545.

17α-Epoxide (**16**): ^{1}H-NMR (400 MHz, CD_2Cl_2): δ_H = 3.67 (1H, m), 2.77 (1H, d, *J* = 5.29 Hz), 2.68 (1H, d, *J* = 5.29 Hz), 1.93 – 2.04 (3H, m), 1.62 – 1.90 (4H, m), 1.54 – 1.61 (5H, m), 1.31 – 1.42 (2H, m), 1.25 (3H, m), 1.11 – 1.21 (2H, m), 1.07 (1H, m), 1.00 (3H, d, *J* = 7.80 Hz), 0.94 (3H, s), 0.86 (1H, m), 0.75 (3H, s), 0.70 (1H, m). ^{13}C-NMR (100 MHz, CD_2Cl_2): δ_C = 72.21, 67.03, 55.55, 54.60, 53.49, 41.39, 40.91, 39.19, 37.36, 36.62, 36.37, 33.41, 33.16, 32.73, 29.89, 28.77, 26.74, 24.12, 22.25, 20.75, 15.90. IR [cm^{-1}]: 3336, 2920, 1442, 1036. α_D^{20}: -7.477 (c 0.65, CH_2Cl_2). m.p.: 102 – 103 °C. HRMS: (M-OH) Calcd.: 301.2526, found: 301.2537.

2.9 2β-Methylspiro[13α-androstan-17ξ,2'-oxiran]-3-one (**17**)

To a solution of epoxide **15** (0.085 g, 0.27 mmol) in CH_2Cl_2 (5 mL) at room temperature Dess-Martin periodinane (0.136 g, 0.32 mmol) was added in one portion. The mixture was stirred for 30 minutes and quenched by addition of saturated $Na_2S_2O_3$ and saturated $NaHCO_3$ solution. After extraction with CH_2Cl_2 (3x) and drying over $MgSO_4$ the crude product was obtained after evaporation of the solvent. This was purified via column chromatography (5 g SiO_2, LP:EtOAc = 10:1) to give 60 mg ketone **17** (71%) as white solid. ^{1}H-NMR (400 MHz, CD_2Cl_2): δ_H = 2.64 (1H, d, *J* = 4.69 Hz), 2.58 (1H, m), 2.56 (1H, d, *J* = 4.69 Hz), 2.09 – 2.21 (2H, m), 1.91 – 2.07 (4H, m), 1.85 (1H, m), 1.65 – 1.79 (2H, m), 1.49 (1H, m), 1.35 – 1.44 (2H, m), 1.17 – 1.34 (4H, m), 1.06 – 1.16 (2H, m), 0.98 (3H, d, *J* = 6.89 Hz), 0.91 (3H, s), 0.69 (3H, s), 0.76 – 0.95 (2H, m). ^{13}C-NMR (100 MHz, CD_2Cl_2): δ_C = 215.74, 68.51, 54.28, 52.96, 47.87, 47.12, 43.56, 42.16, 41.69, 41.53, 37.66, 36.24, 33.20, 32.41, 30.62, 28.81, 28.60, 24.83, 23.69, 16.14, 15.12. IR [cm^{-1}]: 2929, 1710, 931. α_D^{20}: 95.54 (c 1.2, CH_2Cl_2). m.p.: 146 – 148 °C. HRMS: (M+H) Calcd.: 317.2475, found: 317.2492.

2.10 17β-Hydroxymethyl-2α,17α-methyl-18-norandrost-13-en-3-one (**2a**)

In a Schlenk flask under argon atmosphere there was added dry CH_2Cl_2 (10 mL). The solvent was chilled to -78 °C and 2,6-lutidine (0.044 ml, 0.38 mmol) and trimethylsilyl triflate (0.058 ml, 0.32 mmol) were added. Starting material **17** (0.06 g, 0.19 mmol) dissolved in dry CH_2Cl_2 (1 mL) was added dropwise. After 1 hour the reaction was warmed to room temperature and methanol (2 mL) was added followed by saturated $NaHCO_3$ solution. The reaction mixture was extracted with CH_2Cl_2 (3x) and the pooled extracts were washed with water and brine, dried over $MgSO_4$ and evaporated to dryness. The residue was taken up in acetonitrile (3 mL) and H_2SiF_6 (0.027 mL, 0.09 mmol) was added. After 45 minutes the reaction was quenched by addition of saturated $NaHCO_3$ solution. Extraction with CH_2Cl_2 (2x) followed by drying over $MgSO_4$ and evaporating under reduced pressure provided crude product.

Purification via column chromatography (6 g SiO_2, LP:EtOAc 10:1) gave 45 mg of **2a** (76%) as white crystals. A single crystal for x-ray analysis was crystallized from methanol/H_2O. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 3.46 (1H, d, *J* = 10.31 Hz), 3.29 (1H, d, *J* = 10.37 Hz), 2.48 (1H, m), 2.21 – 2.37 (2H, m), 2.04 – 2.20 (4H, m), 1.83 – 2.01 (5H, m), 1.47 – 1.60 (2H, m), 1.17 – 1.42 (5H, m), 1.03 (3H, s), 1.00 (3H, d, *J* = 6.61 Hz), 0.89 – 1.04 (2H, m), 0.93 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 213.28, 140.71, 136.68, 69.06, 51.68, 51.37, 48.30, 47.84, 44.70, 41.18, 36.61, 36.39, 34.06, 31.00, 30.64, 29.09, 23.01, 22.57, 21.85, 14.72, 12.06. IR [cm^{-1}]: 3410, 2926, 1713. α_D^{20}: -3.91 (c 0.9, CH_2Cl_2). m.p.: 135 – 136 °C. HRMS: (M+H) Calcd.: 317.2475, found: 317.2469.

EI fragmentation MS:

The mass spectrum of the bis-TMS derivative of the title compound shown below is in accordance with already published spectra in the literature [16]. The most abundant fragment 357.3 m/z is suggested to result from the loss of CH_2OTMS (Δ = 105). The smaller, less abundant fragment ions were assigned and discussed by Sobolevsky and Rodchenkov [16].

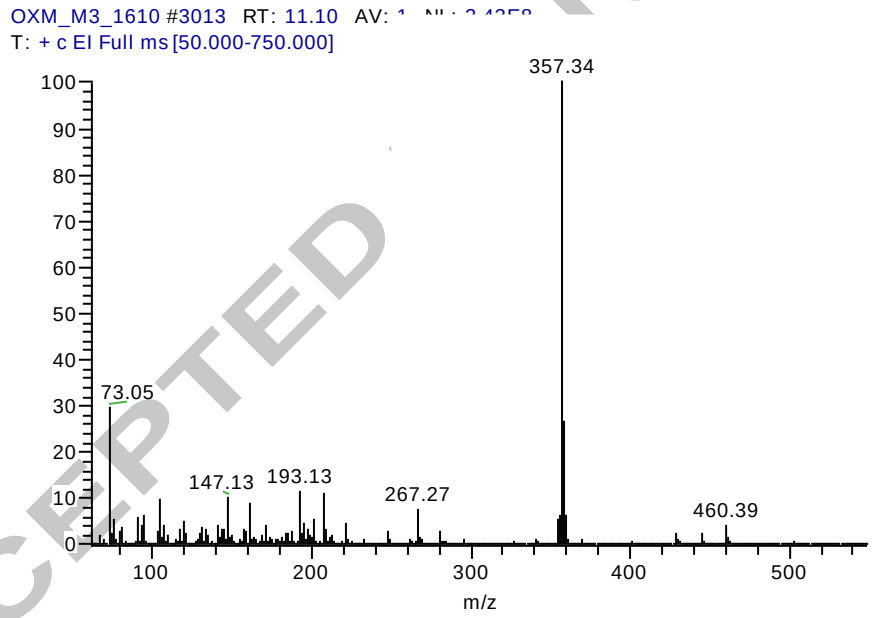


Figure 2: EI spectrum of TMS-**2a**

Crystal data (CCDC-1900498). $C_{21}H_{32}O_2$, *M* = 316.47, orthorhombic, *a* = 7.7653(6), *b* = 9.7987(7), *c* = 22.7256(17) Å, *Z* = 4, *T* = 100 K, space group $P2_12_12_1$ (No. 19), 70505 reflections measured, 6263 unique (R_{int} = 0.042), which were used in all calculations. Flack parameter using 2440 quotients is -0.2(2), the final wR(F^2) is 0.090.

2.11 3β-Hydroxyandrostan-17-one acetate (**18**)

Dehydroepiandrosterone acetate (3 g, 9.08 mmol) was charged in a three-necked flask and dissolved in dry EtOH (470 mL). Pd/C (10%, 0.483 g, 0.45 mmol) was added under argon flow, the atmosphere was exchanged for hydrogen and the vessel sealed and stirred for 18 hours. The mixture is filtrated over Celite, solids washed with EtOAc (2x 100 mL) and the filtrate evaporated under reduced pressure. Flash chromatography (70 g SiO_2, LP:Et_2O = 3:1) of the crude product gave 2.25 g of acetate **18** (75%) as colorless solid.

Analytical data in accordance with literature [18].

2.12 17-(1,3-Dioxolan-2-yl)-androstan-3β-ol acetate (**19**)

Epiandrosterone acetate (2.25 g, 6.87 mmol) was dissolved in benzene (150 mL). Ethylene glycol (8.55 g, 137 mmol) and *para*-toluenesulfonic acid monohydrate (0.013 g, 0.07 mmol) were added to the solution and the mixture was heated to reflux on a Dean-Stark trap. After 16 hours the reaction was allowed to cool to room temperature and treated with saturated $NaHCO_3$ solution (50 mL) and shaken in a separatory funnel. The phases were separated and the aqueous phase extracted with EtOAc (3x). The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude product was crystallized from MeOH:EtOH = 1:1 to give 2.1 g acetate **19** (81%) as polycrystalline material.

Analytical data in accordance with literature [19].

2.13 17-(1,3-Dioxolan-2-yl)-androstan-3β-ol (**20**)

In a round bottom flask was charged starting material **19** (3.04 g, 8.08 mmol) and MeOH (150 mL). The mixture was heated to reflux and K_2CO_3 (2.23 g, 16.17 mmol) was added to the clear solution. After 45 minutes the reaction was concentrated and the residue taken up in CH_2Cl_2 (150 mL) and water (50 mL). After separation of the phases the aqueous phase was extracted with CH_2Cl_2 (2x 50 mL) and the pooled organics were washed with water, brine, dried over $MgSO_4$ and evaporated under reduced pressure to give 2.8 g alcohol **20** (>99%) as white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 3.87 (4H, m), 3.57 (1H, m), 1.95 (1H, m), 1.49 – 1.82 (9H, m), 1.15 – 1.42 (9H, m), 1.09 (1H, m), 0.85 – 1.00 (2H, m), 0.81 (3H, s), 0.79 (3H, s), 0.65 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 119.58, 71.32, 65.26, 64.63, 54.26, 50.44, 46.06, 44.95, 38.28, 37.15, 35.86, 35.62, 34.28, 31.58, 31.44, 30.81, 28.68, 22.75, 20.76, 14.52, 12.42. IR [cm^{-1}]: 3508, 3254, 2931, 1310. α_D^{20}: -15.74 (c 1.0, CH_2Cl_2). m.p.: 152 – 155 °C. HRMS: (M+H) Calcd.: 335.2581, found: 335.2595.

2.14 17-(1,3-Dioxolan-2-yl)-androstan-3β-ol tosylate (**21**)

Starting material **20** (2.83 g, 8.46 mmol) was dissolved in dry pyridine (30 mL) and chilled to 0 °C. To this solution DMAP (0.005 g), followed by tosyl chloride (2.02 g, 10.6 mmol) was added and the reaction stirred for 5 hours at room temperature. The resulting suspension was concentrated under reduced pressure and the residue dissolved in CH_2Cl_2 (100 mL) and water (50 mL). Phases were separated and the aqueous phase extracted twice with CH_2Cl_2. Pooled organic phases were washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography (200 g SiO_2, LP:Et_2O = 2:1) to give 3.97 g of tosylate **21** (96%) as colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 7.77 (2H, d, *J* = 8.25 Hz), 7.31 (2H, d, *J* = 8.25 Hz), 4.40 (1H, m), 3.78 – 3.95 (4H, m), 2.43 (3H, s), 1.94 (1H, m), 1.43 – 1.80 (8H, m), 1.28 – 1.39 (3H, m), 1.14 – 1.26 (4H, m), 1.05 (1H, m), 0.78 – 0.96 (2H, m), 0.80 (3H, s), 0.76 (3H, s), 0.61 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 144.40, 134.86, 129.82, 127.67, 119.45, 82.52, 65.26, 64.60, 53.97, 50.31, 45.98, 44.86, 36.89, 35.70, 35.33, 34.91, 34.24, 31.23, 30.68, 28.47, 28.36, 22.69, 21.72, 20.67, 14.47, 12.19. IR [cm^{-1}]: 2939, 1356, 933. α_D^{20}: -18.68 (c 1.0, CH_2Cl_2). m.p.: 163 – 165 °C. HRMS: (M+H) Calcd.: 489.2669, found: 489.2694.

2.15 17-(1,3-Dioxolan-2-yl)-androst-2-en (**22**, **23**)

Tosylate **21** (3.97 g, 8.08 mmol) was weighed into a round bottom flask and dissolved in dry DMF (100 mL). LiBr (4.23 g, 48.8 mmol) and Li_2CO_3 (3.86 g, 48.8 mmol) were added at once. The reaction was heated on an oil bath at reflux for 1.5 hours, then allowed to cool. The reaction mixture was poured onto 1 M HCl (150 mL) and the mixture diluted with CH_2Cl_2 (80 mL). After extraction with CH_2Cl_2 (2x), washing the pooled extracts with water, drying over $MgSO_4$ and evaporating under reduced pressure the crude product was obtained. This could be purified by recrystallizing from MeOH/H_2O = 3:2, yielding 2.18 g of a mixture of regioisomeric products **22** and **23** (85%) in a mixture of 6:1 (determined by ^{1}H-NMR). HRMS: (M+H) Calcd.: 317.2475, found: 317.2490.

2.16 17-(1,3-Dioxolan-2-yl)-2α,3α-epoxyandrostan (**24**, **25**)

Mixture of products **22** and **23** (1.58 g, 4.74 mmol) was charged in a round bottom flask and dissolved in CH_2Cl_2 (40 mL). There was added $KHCO_3$ (0.96 g, 9.5 mmol), and *meta*-Chloroperoxybenzoic acid (1.29 g, 5.21 mmol, ca. 70%) in one portion. The resulting suspension was stirred at room temperature for 2.5 hours and quenched with saturated $Na_2S_2O_3$ solution. Extracted with CH_2Cl_2 (3x) and washed with saturated $NaHCO_3$ solution and brine, before drying over $MgSO_4$ and evaporating to dryness. Purification *via* flash chromatography (37 g SiO_2, LP:Et_2O = 7:1) gives 1.47 g of epoxides **24** and **25** (94%). HRMS: (M+H) Calcd.: 333.2424, found: 333.2450.

2.17 17-(1,3-Dioxolan-2-yl)-2β-methylandrostan-3α-ol (**26**)

Dry CuI (1.97 g, 10.36 mmol) was charged in a Schlenk flask (100 mL) and dry Et_2O (20 mL) was added to form a suspension. The reaction was chilled to -20 °C and 1.5 M methyllithium-LiBr complex in Et_2O (13.81 mL, 20.72 mmol) was added dropwise until a clear, colorless solution formed. Starting material **24** and **25** (0.49 g, 1.48 mmol) was dissolved in Et_2O (5 mL) and added dropwise to the solution at -20 °C. The reaction was then allowed to warm to room temperature, sealed and stirred for 20 hours. Reaction was quenched by pouring onto a mixture of 30 ml saturated NH_4Cl, 30 ml saturated $NaHCO_3$, 30 ml water and 2 ml conc. NH_3 and stirring for 1 hour. The aqueous phase was extracted with Et_2O (3x), the pooled organic phases washed twice with dilute NH_3 and dried over $MgSO_4$. After evaporating the solvent the crude product was purified via column chromatography (25 g SiO_2, LP:Et_2O = 2:1) to give 207 mg of alcohol **26** (40%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 3.81 – 3.93 (4H, m), 3.71 (1H, m), 1.84 – 2.00 (2H, m), 1.77 (1H, m), 1.62 – 1.69 (2H, m), 1.54 – 1.61 (2H, m), 1.46 – 1.53 (2H, m), 1.42 (1H, m), 1.34 – 1.41 (3H, m), 1.31 (1H, m), 1.16 – 1.28 (5H, m), 1.03 (3H, d, *J* = 7.53 Hz), 0.94 (1H, m), 0.84 (3H, s), 0.83 (1H, m), 0.82 (3H, s), 0.73 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 119.61, 72.05, 65.28, 64.67, 55.65, 50.34, 46.19, 40.62, 39.26, 36.26, 35.93, 35.56, 34.32, 32.75, 31.32, 30.90, 28.31, 22.74, 20.66, 20.44, 15.73, 14.61. IR [cm^{-1}]: 2917, 1003, 737. α_D^{20}: 2.18 (c 1.0, CH_2Cl_2). m.p.: 119 – 121 °C. HRMS: (M+H) Calcd.: 349.2737, found: 349.2757.

2.18 3α-Hydroxy-2β-methylandrostan-17-one (**27**)

To a solution of starting material **26** (0.6 g, 1.71 mmol), in 1,4-dioxane (20 mL) was added 0.5 M H_2SO_4 (10 mL) and the solution was stirred at room temperature. After 2 hours the reaction was neutralized with saturated $NaHCO_3$ solution and extracted with Et_2O (3x). Pooled organic phases were washed with brine, dried over $MgSO_4$ and evaporated under

reduced pressure. The crude product was crystallized from boiling *n*-Hexane/MTBE to give 427 mg of ketone **27** (82%) as white crystals. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 3.74(1H, m), 2.41 (1H, dd, *J* = 8.78 Hz, 18.93 Hz), 2.05 (1H, m), 1.85 – 1.96 (2H, m), 1.72 – 1.81 (2H, m), 1.65 – 1.72 (2H, m), 1.61 (1H, m), 1.46 – 1.57 (3H, m), 1.36 – 1.46 (2H, m), 1.19 – 1.34 (6H, m), 0.97 – 1.07 (1H, m), 1.03 (3H, d, *J* = 7.40 Hz), 0.86 (3H, s), 0.85 (3H, s), 0.75 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 221.58, 71.93, 55.98, 51.48, 48.03, 40.49, 39.25, 36.36, 35.97, 35.92, 34.86, 32.55, 31.75, 30.89, 28.12, 21.87, 20.61, 20.29, 15.71, 14.03. IR [cm^{-1}]: 3546, 2909, 1723, 1007. α_D^{20}: 94.03 (c 1.0, CH_2Cl_2). HRMS: (M+H) Calcd.: 305.2475, found: 305.2476.

2.19 2β-Methyl-17-methyleneandrostan-3α-ol (**28**)

A Schlenk flask (100 mL) was charged with zinc (2.69 g, 41.2 mmol), dry THF (35 mL), $PbCl_2$ (0.005 g), and CH_2Br_2 (2.38 g, 13.72 mmol). The mixture was stirred vigorously and chilled to -20 °C. At that temperature neat $TiCl_4$ (1.62 g, 8.57 mmol) was added dropwise over 10 minutes. The mixture was further stirred at -10 °C for 3 hours. The reaction was then warmed to 0 °C and starting material **27** (0.52 g, 1.71 mmol) was added in dry THF (10 mL). The reaction was stirred for 1.5 hours at room temperature and then quenched with 1 M HCl (50 mL) and ice (50 g). The mixture was extracted with Et_2O (3x) and EtOAc (1x) and the pooled organic phases washed with $NaHCO_3$ solution and brine. After drying over $MgSO_4$ and evaporating the solvent the crude product was purified by flash chromatography (13 g SiO_2, LP:EtOAc = 20:1) to give 438 mg of olefin **28** (85%) as polycrystalline material. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 4.60 (2H, m), 3.72 (1H, m), 2.46 (1H, m), 2.22 (1H, m), 1.89 (1H, m), 1.78 (1H, m), 1.60 – 1.73 (4H, m), 1.47 – 1.59 (3H, m), 1.35 – 1.44 (2H, m), 1.15 – 1.33 (6H, m), 1.03 (3H, d, *J* = 7.68 Hz), 0.89 – 1.00 (2H, m), 0.86 (3H, s), 0.76 (3H, s), 0.72 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 162.15, 100.68, 72.04, 56.10, 54.45, 44.34, 40.58, 39.31, 36.32, 35.92, 35.90, 35.25, 32.68, 31.89, 29.49, 28.38, 24.19, 20.90, 20.64, 18.73, 15.75. IR [cm^{-1}]: 3358, 2911, 874. α_D^{20}: 40.37 (c 1.0, CH_2Cl_2). m.p.: 126 – 128 °C. HRMS: (M-OH) Calcd.: 285.2577, found: 285.2591.

2.20 2β-Methylspiro[androstan-17α,2'-oxiran]-3α-ol (**29**)

A round bottom flask was charged with olefin **28** (0.44 g, 1.45 mmol). This was dissolved in CH_2Cl_2 (12 mL) and $KHCO_3$ (0.29 g, 2.9 mmol), followed by *meta*-chloroperoxybenzoic acid (0.43 g, 1.74 mmol) was added in one portion at room temperature. After 1 hour the reaction was quenched by addition of saturated $Na_2S_2O_3$ solution and $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (3x) and the extracts washed with water, brine and dried over $MgSO_4$. The solvent was evaporated and the crude product purified *via* column chromatography (32 g SiO_2, LP:EtOAc = 10:1) to give 185 mg desired 17α-epoxide **29** (40%). The other stereoisomer (17β-epoxide) was not isolated in a pure form. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 3.72 (1H, m), 2.72 (1H, d, *J* = 4.94 Hz), 2.64 (1H, d, *J* = 4.94 Hz), 2.25 (1H, m), 1.77 – 1.92 (2H, m), 1.65 – 1.76 (2H, m), 1.47 – 1.64 (4H, m), 1.34 – 1.44 (3H, m), 1.10 – 1.32 (8H, m), 1.02 (3H, d, *J* = 7.49 Hz), 0.97 – 1.07 (1H, m), 0.84 (3H, s), 0.79 (3H, s), 0.75 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 72.04, 70.16, 55.66, 52.69, 47.47, 41.71, 40.62, 39.26, 36.30, 35.94, 35.41, 32.72, 31.82, 30.45, 30.05, 28.35, 24.09, 20.66, 20.07, 16.41, 15.75. IR [cm^{-1}]: 3385, 2922, 929, 731. α_D^{20}: 16.81 (c 1.0, CH_2Cl_2). m.p.: 130 – 132 °C. HRMS: (M-H) Calcd.: 317.2486, found: 317.2468.

2.21 2β-Methylspiro[androstan-17ξ,2'-oxiran]-3-one (**30**)

A solution of starting material **29** (0.16 g, 0.49 mmol) in CH_2Cl_2 (10 mL) was chilled to 0 °C and Dess-Martins periodinane (0.25 g, 0.59 mmol) was added at that temperature as a solid in one portion. After 1 hour the reaction was quenched with saturated $NaHCO_3$ and $Na_2S_2O_3$ solution and extracted with CH_2Cl_2 (3x). Pooled organic phases were washed with $NaHCO_3$ solution, water and brine and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure the crude product was purified *via* flash chromatography (9 g SiO_2, LP:EtOAc = 15:1) to provide 87 mg of ketone **30** (55%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 2.73 (1H, d, *J* = 4.52 Hz), 2.66 (1H, d, *J* = 4.52 Hz), 2.59 (1H, m), 2.20 – 2.32 (2H, m), 1.92 – 2.12 (3H, m), 1.84 (1H, m), 1.74 (1H, m), 1.45 – 1.60 (3H, m), 1.11 – 1.44 (8H, m), 0.93 – 1.06 (1H, m), 1.02 (3H, d, *J* = 6.75 Hz), 0.85 (1H, m), 0.80 (3H, s), 0.75 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 215.96, 70.01, 54.97, 52.57, 47.41, 46.72, 43.33, 41.97, 41.66, 41.18, 35.71 (2C), 31.34, 30.39, 29.99, 28.32, 24.06, 20.64, 16.24, 15.99, 14.61. IR [cm^{-1}]: 2913, 1705, 927. α_D^{20}: 115.14 (c 1.0, CH_2Cl_2). m.p.: 136 – 140 °C. HRMS: (M+H) Calcd.: 317.2475, found: 317.2483.

2.22 17β-Hydroxymethyl-2α,17α-methyl-18-norandrost-13-en-3-one (**2b**)

In a round bottom flask (25 mL) there was charged epoxide **30** (0.04 g, 0.13 mmol) and acetic acid (3 mL) was added. The solution was stirred for 1 hour, then diluted with water (10 mL) and neutralized by addition of $KHCO_3$. The neutral aqueous solution was extracted with CH_2Cl_2 (3x) and the pooled extracts washed with water and brine. After drying over $MgSO_4$ and evaporating to dryness the crude product was purified *via* column chromatography (3 g SiO_2, LP:EtOAc = 5:1) to give 28 mg of ketone **2b** (69%) as colorless crystals. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 3.39 (1H, d, *J* = 10.63 Hz), 3.29 (1H, d, *J* = 10.63 Hz), 2.61 (1H, m), 2.22 – 2.34 (2H, m), 2.02 – 2.14 (2H, m), 1.96 – 2.02 (3H, m), 1.88 – 1.95 (2H, m), 1.73 – 1.86 (2H, m), 1.51 – 1.61 (2H, m), 1.16 – 1.40 (5H, m), 1.09 (1H, m), 1.03 (3H, d, *J* = 6.57 Hz), 1.01 (1H, m), 0.96 (3H, s), 0.70 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 215.86, 140.97, 136.42, 69.13, 52.80, 51.67, 46.47, 43.14, 41.49, 40.96, 37.03, 35.57, 34.21, 30.97, 30.72, 28.69, 23.27, 22.78, 21.85, 15.80, 14.48. IR [cm^{-1}]: 3429, 1703, 1037. α_D^{20}: 75.24 (c 1.0, CH_2Cl_2). HRMS: (M+H) Calcd.: 317.2475, found: 317.2474.

2.23 17-(1,3-Dioxolan-2-yl)-2β-methylandrostan-3α-ol 4-nitrobenzoate (**31**)

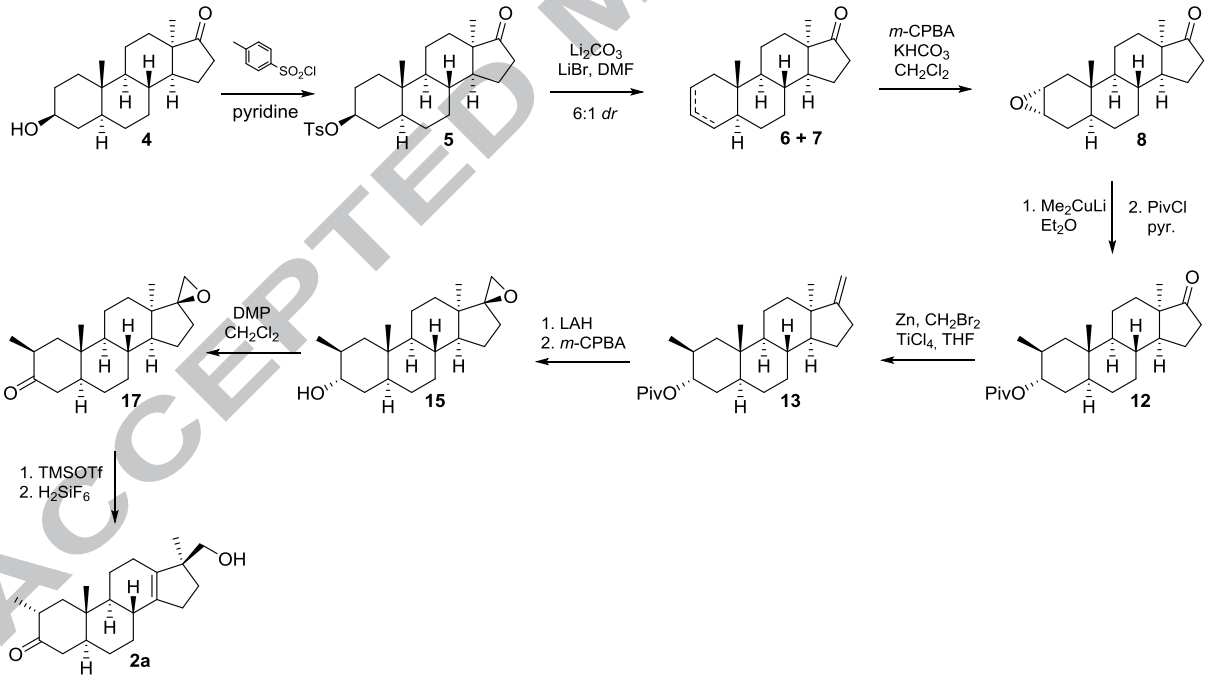
In a round-bottom flask (25 mL) starting material **26** (0.03 g, 0.086 mmol) was dissolved in dry CH_2Cl_2 (5 mL). There was added 4-DMAP (0.012 g, 0.1 mmol), 4-nitrobenzoic acid (0.017 g, 0.1 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.019 g, 0.1 mmol) in that order at room temperature. The reaction was stirred at room temperature and was quenched after 4 hours by addition of saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (2x) and organic phases dried over $MgSO_4$ and evaporated. Flash column chromatography (2.5 g SiO_2, LP:EtOAc = 10:1) provided 41 mg of ester **31** (96%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 8.30 (2H, d, *J* = 9.00 Hz), 8.19 (2H, d, *J* = 9.00 Hz), 5.06 (1H, m), 3.81 – 3.95 (4H, m), 2.15 (1H, m), 2.03 (1H, s), 1.96 (1H, m), 1.72 – 1.85 (2H, m), 1.58 – 1.70 (3H, m), 1.52 – 1.56 (3H, m), 1.50 (1H, m), 1.33 – 1.46 (3H, m), 1.26 (1H, m), 1.19 – 1.24 (2H, m), 1.13 (3H, d, *J* = 8.03 Hz), 0.92 (3H, s), 0.82 – 0.95 (2H, m), 0.84 (3H, s), 0.77 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C = 164.08, 150.57, 136.61, 130.73, 123.69, 119.52, 65.34, 64.65,

55.94, 50.44, 46.14, 41.25, 40.71, 36.06, 35.46, 34.25, 33.00, 31.29, 30.83, 29.68, 28.03, 22.67, 20.51, 20.14, 15.81, 14.64. IR [cm^{-1}]: 2912, 1686, 881.

Crystal data (CCDC-1900499). $C_{29}H_{39}NO_6$, M = 497.61, monoclinic, a = 10.9205(11). b = 11.4099(12), c = 20.867(2) Å, Z = 4 (Z' = 2), T = 100 K, space group $P2_1$ (No. 4), 37131 reflections measured, 12209 unique (R_{int} = 0.077), which were used in all calculations. Flack parameter using 2391 quotients is 0.0(7), the final wR(F^2) is 0.126.

3. Results and discussion

The first synthetic route served to prepare only the 17β-hydroxymethyl isomer **2a**. Synthesis of **2a** commenced from **4** (3 steps from DHEA acetate [20], 75% yield) which was converted into tosylate **5** in excellent yield. Elimination to the desired 2,3-olefin was achieved with LiBr and Li_2CO_3 in refluxing DMF [22]. The product was isolated as a 6:1 mixture of regioisomeric olefins **6** and **7** (determined from ^{1}H-NMR integrals of olefinic protons). The mixture was epoxidized with *m*-CPBA and the α-epoxides **8** and **9** were isolated exclusively, again as a mixture (See Scheme 1: compounds **9**, **10**, **11**, **14**, **16** not shown). Treatment of this epoxide with lithium dimethylcopper (Me_2CuLi) in Et_2O [23-24] provided the desired 2β-methyl alcohol **10** as a chromatographically inseparable mixture together with the rearranged 3-oxo product **11**. Protection of the hydroxy group (pivaloyl chloride, pyridine) allowed for isolation as a pure product (**12**, 33% over two steps).



Scheme 1: Synthesis of target compound **2a**

With A-ring substituents in place, the D-ring was now elaborated. Methylenation of the ketone at C-17 with Takai-Lombardo reagent (Zn, $TiCl_4$, CH_2Br_2) in THF [25] gave ester **13** in 59% yield. Cleavage of the protecting group with $LiAlH_4$ provided alcohol **14** in quantitative yield. The 17-*exo*-methylene double bond was epoxidized with *m*-CPBA to yield a mixture of diastereomers, of which the minor 17β-epoxide **15** is the desired product (19% yield). The alcohol was now oxidized to the ketone with Dess-Martins periodinane (DMP) in CH_2Cl_2 to give ketone **17** in 71% yield. Finally, Wagner-Meerwein rearrangement was initiated by

treating **17** with TMSOTf and 2,6-lutidine in CH_2Cl_2 at -78 °C. In order to minimize epimerization of the C-2 stereocenter deprotection of the resulting primary TMS ether was effected using H_2SiF_6 in acetonitrile, arriving at product **2a** (76% over two steps). [26] Single crystal X-ray analysis revealed 2α-configuration of the isolated product (*vide infra*: Figure 3). This indicates that during the last two steps equilibration of the C-2 carbon had already occurred.

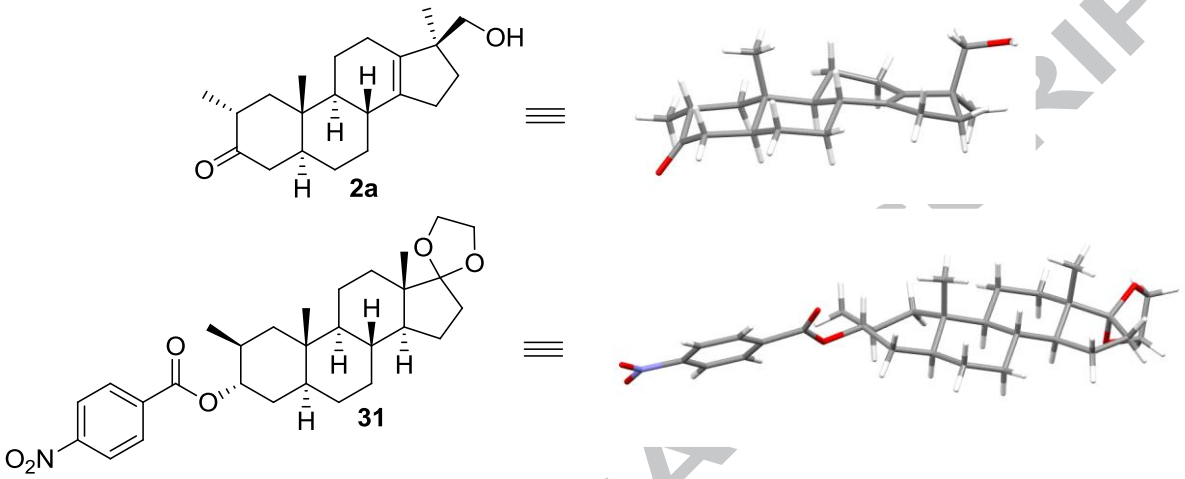
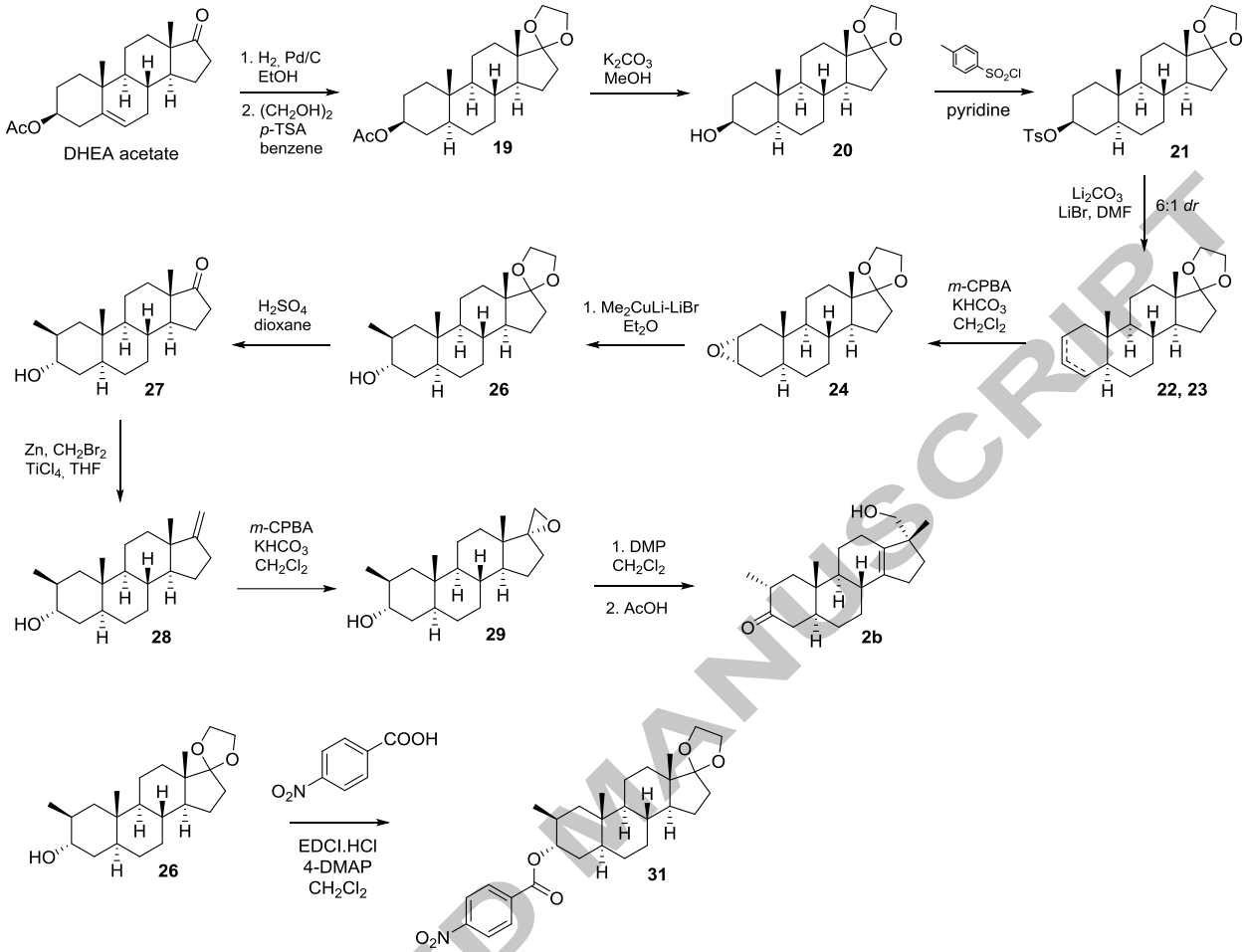


Figure 3: Molecular structures determined by X-ray single crystal analysis

The second synthetic route allows access to the 17α-hydroxymethyl epimer **2b** and was carried out using the same methodology. Starting from DHEA acetate which was hydrogenated over Pd/C in ethanol in 75% yield followed by protection of the ketone moiety by ketal formation giving acetate **19** (See Scheme 2: compounds **18**, **25**, **30** not depicted). Next, the ester was cleaved using K_2CO_3/methanol (99% yield) and alcohol **20** was tosylated, eliminated and the formed olefin epoxidized as described before (77% over three steps). Opening of the epoxide using excess Me_2CuLi in Et_2O gave a 1:1 mixture of rearranged 3-oxo product and desired 2β-methyl-3α-alcohol **26**, the latter could be isolated in 40% yield after column chromatography. The configuration of A-ring substituents was secured by X-ray analysis of the 4-nitrobenzoate **31** (Figure 2) of this alcohol. After deprotection of the cyclic ketal (H_2SO_4, dioxane) the synthesis proceeded as shown before. Methylenation of the 17-ketone using Takai-Lombardo reagent was successful and delivered alcohol **28** in 85% yield without the need for a protecting group. Epoxidation with *m*-CPBA gives the desired 17β-epoxide **29** (40% yield), which was again oxidized with DMP and rearranged in acetic acid (38% over two steps) [27] to furnish **2b**.



Scheme 2: Synthesis of target compound **2b** and nitrobenzoate **31**

Only the 2α-isomers of the series of putative metabolites could be accessed by this route since the 2β-methyl-3-oxo isomers equilibrated rapidly under acidic conditions and reasonably fast under basic conditions (ca. 8 hours in $KOH/CD_3OD/D_2O$). With these observations in mind it is conceivable that over a long time in an *in vivo* system the metabolite would also equilibrate to its most stable isomers: **2a** or **2b**.

Due to the method chosen for analytical comparison with excretion studies (GC-MS/MS), TMS-derivatization is required, possibly destroying the stereochemical information at C-2 by enolization at that carbon ([16] suggests enolization at C-4). The comparative measurements including a positive sample, a blank urine sample and a spiked blank sample are shown below in Figure 4. For metabolite **2a** transitions: m/z 357 → 161, m/z 357 → 193 and m/z 357 → 207 were selected.

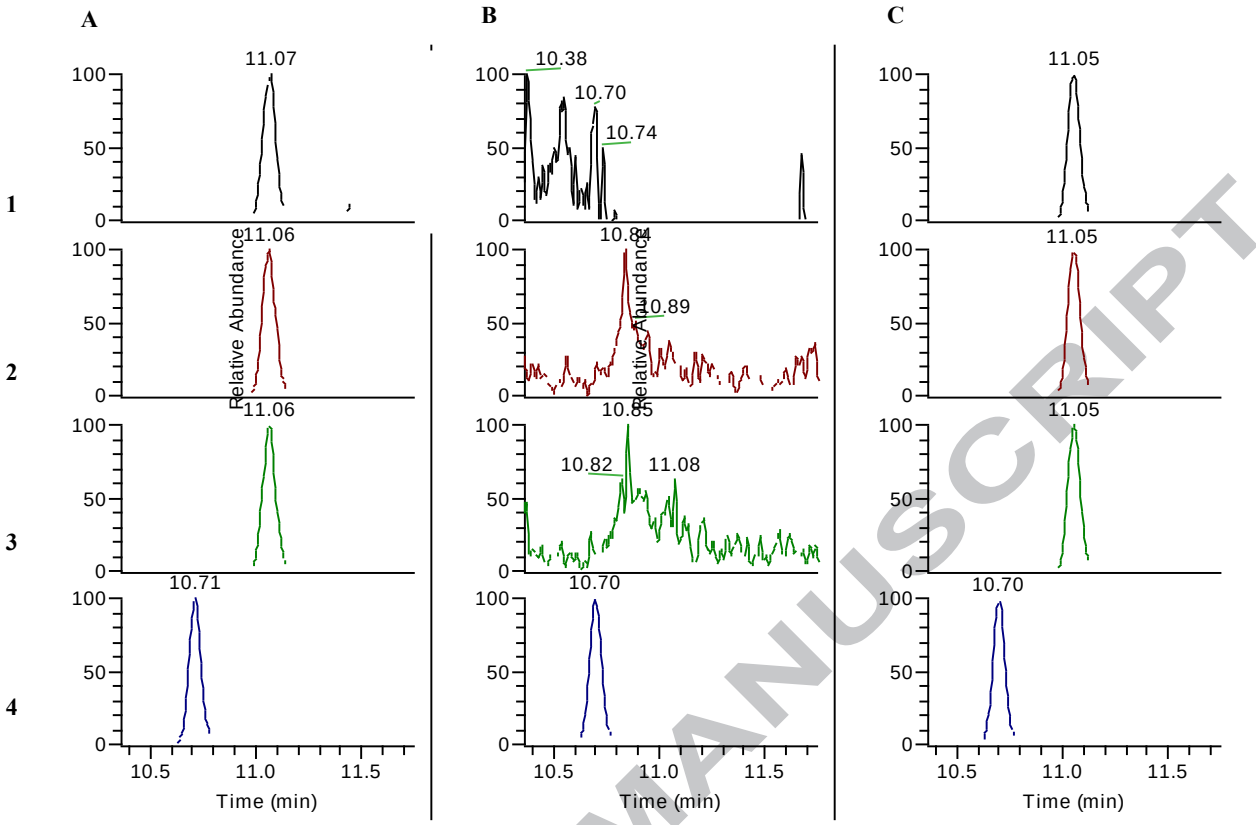


Figure **4:** Detection of metabolite **2a** in positive urine sample by GC-MS/MS; Row 1-3: mass transitions for metabolite 2a (357 → 161, 357 → 193, 357 → 207); Row 4: mass transition for internal standard D3-Testosterone (435 → 209); **A:** positive urine sample; **B:** blank urine sample; **C:** blank urine sample spiked with 5 ng/mL metabolite **2a**

4. Conclusion:

To summarize, we accomplished the first chemical synthesis of compounds **2a** and **2b** in 13 and 12 steps respectively (from DHEA acetate). These two putative metabolites were examined and showed accordance of **2a** with the *in vivo* formed metabolite **2**.

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Synthesis of a human long-term oxymetholone metabolite

Nicolas Kratena[1], Nina Biedermann[1], Matthias Weil[2], Valentin S. Enev*[1], Günter Gmeiner[3], Peter Gärtner*[1]

[1] Institute of Applied Synthetic Chemistry, Vienna University of Technology

[2] Institute for Chemical Technologies and Analytics, Vienna University of Technology

[3] Doping Control Laboratory, Seibersdorf Labor GmbH

* valentin.enev@tuwien.ac.at, peter.gaertner@tuwien.ac.at,

Highlights:
-) First chemical synthesis of reported metabolite OXM-M2
-) Two epimeric putative metabolites investigated
-) Identity and structure of *in vivo* metabolite secured

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[1] Institute of Applied Synthetic Chemistry, Vienna University of Technology

[2] Institute for Chemical Technologies and Analytics, Vienna University of Technology

[3] Doping Control Laboratory, Seibersdorf Labor GmbH

* valentin.enev@tuwien.ac.at, peter.gaertner@tuwien.ac.at,

Graphical abstract: