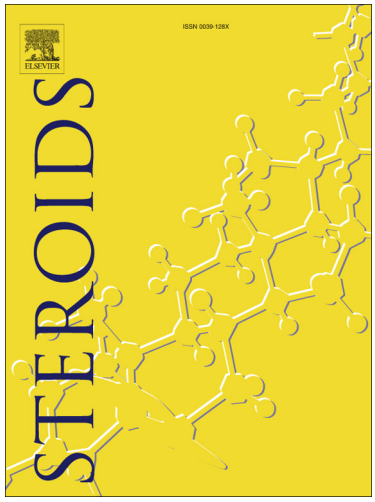

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Synthesis of 17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-trien-3-one: a long-term metandienone metabolite

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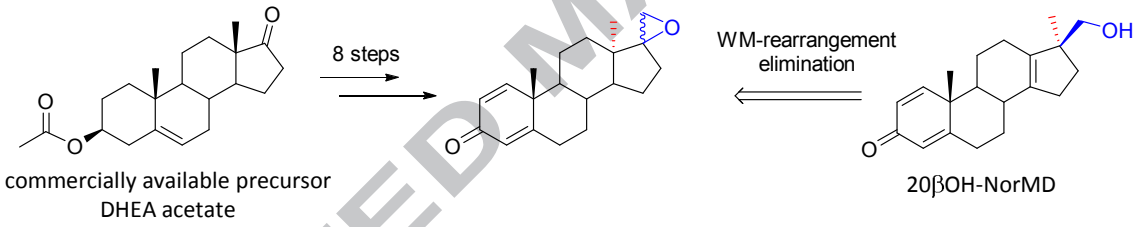
Abstract:

The goal of this work was a good-yielding chemical synthesis of a metandienone metabolite which is of interest in doping analysis. 20ßOH-NorMD (IUPAC: 17ß-hydroxymethyl-17--methyl-18-norandrosta-1,4,13-triene-3-one) has been identified as a long-term urinary metabolite which can be detected and attributed to metandienone up to almost 3 weeks after exposure. The chemical synthesis of its epimer 20αOH-NorMD has been described before, as was an enzymatic synthesis of 20ßOH-NorMD, but no chemical synthesis was published.

Graphical abstract:

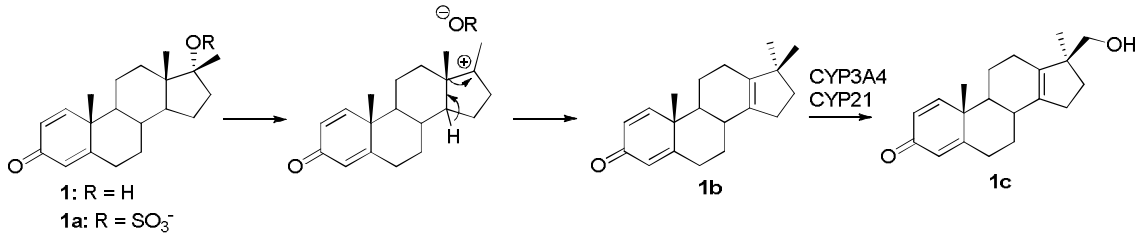


Keywords: metandienone, metabolism, wagner-meerwein rearrangement,

1. Introduction

Metandienone (**1**) is a synthetic anabolic androgenic steroid (AAS) that was first synthesized in 1955[1] and is still being used by professional athletes today. It is one of the highly abused synthetic AAS together with Nandrolone and Stanozolol judging from adverse analytical findings (AAF) from 2004-2007[2]. It is also reportedly used by amateur athletes.[3] Since the discovery of the first metabolite in 1963[4] the metabolism has been extensively investigated[5,6,7,8,9].

As reported by Schänzer *et al*. in 1992 the 17-sulphate conjugate (**1a**) of metandienone can epimerize and/or rearrange (Wagner-Meerwein-Rearrangement) to give primarily NorMD (17,17-dimethyl-18-norandrosta-1,4,13-trien-3-one) (**1b**). This intermediate is further hydroxylated by Cytochrome P450 enzymes CYP21 and CYP3A4 to 20βOH-NorMD (**1c**).[10] Hydroxylation can also take place at C-16 (CYP21) and on the 17α-methyl group (CYP3A4) giving the epimer.[11] Both 17-epimers have identical detection limits. Additionally it was reported that the metabolite is also formed by C-18 hydroxylation *via* CYP11B2 and subsequent rearrangement.[12]



Scheme 1: Suspected *in vivo* formation of the metabolite

Compound **1c** (IUPAC: 17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-triene-3-one, also called "nightwatch" and "20βOH-NorMD") has been identified as a long-term urinary metabolite which can be detected and attributed to metandienone up to 19 days after administration of a single dose.[13] This constitutes a significantly longer window of detection than with metandienone metabolites used before. When screening routines using this new metabolite were implemented in 2006 the number of AAF rose from 15 to 68 for metandienone (450% increase).[14]

In 2011 a small quantity (10 mg) of this metabolite was synthesized from NorMD using recombinant strains of the fission yeast *Schizosaccharomyces pombe* expressing CYP21.[11] The epimer of this metabolite: 17α-hydroxymethyl-17β-methyl-18-norandrosta-1,4,13-triene-3-one has been reportedly synthesized in 2012 in 5 steps with an overall yield of about 0.1%.[12] Thus far there is no report on the chemical synthesis of this compound. We hereby report the first synthesis of this metabolite which is needed as a reference material for metandienone abuse in antidoping laboratories around the world.

2. Experimental

Dehydroepiandrosterone acetate and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone were purchased from FluoroChem. MSTFA was supplied by Machery & Nagel (Düren, Germany). Silica gel (0–63 µm, 60Å) for chromatography was from Merck. Titanium tetrachloride, anhydrous dimethylformamide and anhydrous dimethylsulfoxide were from Acros Organics. Sodium sulfate was from VWR. All other non-specified chemicals were from Sigma Aldrich. Anhydrous methylene chloride, diethyl ether, toluene and 1,4-dioxane were retrieved from an Innovative Technologies PureSolv system. Anhydrous tetrahydrofuran was pre-dried using an Innovative Technologies PureSolv system, refluxed over sodium/benzophenone and freshly distilled.

NMR spectra were recorded on a Bruker AC400. IR spectra were recorded on a Perkin Elmer Spectrum 65. TLC-analysis was performed with precoated aluminium-backed plates (Silica gel 60 F_{254}, Merck). Compounds were visualized by submerging in an acidic phosphomolybdic acid / cerium sulfate solution and heating. Melting points were determined with a Kofler hot-stage apparatus.

Preparative HPLC was carried out on a Reveleris® Prep system by Grace using a Luna® 10 µm, C18 (TMS endcapping) 100 Å, LC Column (250 x 21.2 mm).

GC-EI-MS analyses were performed using a Thermo Trace GC coupled to a Thermo Trace MS instrument (Thermo Quest, Austin, USA) equipped with a Restek RTX-5ms GC column (length 15 m, inner diameter 0.25 mm, film thickness 0.25 µm). The GC oven temperature program started at 150°C was increased at 20°/min to 320°C using Helium as carrier gas 100 kPa,

constant pressure). The injector temperature was set to 270°C, the interface temperature to 280°C and the ion source temperature to 250°C. Ionization was accomplished using EI (70 eV), and full scan analysis (m/z 60-600) was employed at 2 scans/s. A volume of 1 µL of a derivatization mixture consisting of 5 µg of the target product in 200 µl of a mixture of MSTFA/ammonium iodide/ethanethiol- TMS 1000:2:6 (v/w/v) was injected in split mode (1 / 40) into the GC-MS system.

High resolution/high accuracy mass spectra were recorded on a Thermo Q Exactive Focus mass spectrometer (Thermo Scientific, Austin, Texas) by direct injection of a 10 µg/ml solution of the target product in acetonitrile.

The mass spectrometer was operated in electrospray ionization mode at a spray voltage of 4 kV at 400°C. The instrument was calibrated using the manufacturers calibration mixture allowing or mass accuracies < 3 ppm. Full scan mass spectra were recorded in profile mode at a resolution of 70.000 at a scan range of m/z 100 – 350.

2.1 3β-Hydroxy-13α-methylandrost-5-en-17-one acetate (**2**)

A solution of dehydroepiandrosterone acetate (10 g, 30.26 mmol) in acetic acid (125 mL) and 1,2-phenylenediamine (5.43 g 50.2 mmol) was refluxed for 24 hours. The color changed from light brown to dark green in about 2 h and then proceeded to turn very dark with blue complexion which disappeared after cooling the reaction. The solution was then flooded with 150 mL deionised water upon which a beige precipitate formed. This was extracted with 150 mL ethyl acetate and the organic phase washed ten times with small portions of water and four times with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and the solvent evaporated *in vacuo*. The crude product was recrystallized from diisopropylether, giving 6 g of product, and the mother liquors concentrated and separated on a 180 g silica gel column with 5/1 v/v petroleum ether/diethyl ether as eluent to give 1.16 g starting material and in total 8.2 g (82%) of the title compound **2** as white solid, m.p. 141 – 143 °C.

TLC: R_f: (petroleum ether/diethyl ether 3/1 v/v) 0.55. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.33 (1H, tt, *J* = 2.56), 4.53 (1H, h, *J* = 5.4), 2.18 – 2.38 (4H, m), 2.0 – 2.19 (3H, m), 1.96 (3H, s), 1.68 – 1.87 (3H, m), 1.45 – 1.63 (3H, m) 1.15 (1H, td, *J* = 13.44, 3.61), 0.98 – 1.1 (2H, m), 0.93 (3H, s), 0.92 (1H, m), 0.80 (3H, s), 0.8 (1H, m). ^{13}C-NMR (100MHz, $CDCl_3$): δ= 222.09, 170.43, 139.19, 121.88, 73.72, 50.98, 50.02, 47.86, 37.86, 36.81, 36.61, 34.12, 34.05, 33.03, 31.56, 27.56, 25.12, 22.91, 22.04, 21.39, 19.05. IR [cm^{-1}]: 2935, 1731, 1235, 1024. $[\alpha]^{20}_D$ = -154.3 (c 0.78, dichloromethane)

2.2 3β-Hydroxy-13α-methylandrost-5-en-17-one (**3**)

A solution of **2** (1.15 g, 3.48 mmol) and potassium carbonate (1 g, 7.24 mmol) in methanol (50 mL) was refluxed for an hour and TLC showed full conversion. The reaction mixture was diluted with deionised water and extracted with dichloromethane. The pooled extracts were washed with brine, dried over Na_2SO_4 and evaporated to give 982 mg (98%) of the title compound as white crystals, m.p. 180 – 181 °C.

TLC: R_f: (petroleum ether/ethyl acetate 5/1 v/v) 0.24. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.35 (1H, tt, *J* = 5.10, 2.55), 3.50 (1H, h, *J* = 5.28), 2.25 – 2.41 (3H, m), 2.03 – 2.24 (4H, m), 1.71 –

1.91 (4H, m), 1.41 – 1.67 (4H, m), 1.19 (1H, td, *J* = 13.56, 4.04), 1.0 – 1.14 (2H, m), 0.97 (3H, s), 0.94 (1H, td, *J* = 11.94, 2.1), 0.85 (1H, td, *J* = 12.9, 3.55), 0.83 (3H, s). ^{13}C-NMR (100MHz, $CDCl_3$): δ= 222.50, 140.44, 121.03, 71.74, 51.18, 50.18, 48.07, 42.12, 36.98, 36.85, 34.29, 34.21, 33.17, 31.73, 31.57, 25.26, 23.09, 22.17, 19.25. IR [cm^{-1}]: 3456, 2932, 1722. $[\alpha]^{20}_D$ = -167.4 (c 0.81, dichloromethane)

2.3 3β-Hydroxy-13α-methylandrost-5-en-17-one pivalate (**4**)

To a solution of **3** (1.63 g, 5.64 mmol) in pyridine (10 mL) there was added trimethylacetyl chloride (815 mg, 6.77 mmol) dropwise at 0°C. The reaction was stirred for 3 hours. The solvent was evaporated *in vacuo* and the residue dissolved in dichloromethane and saturated $NaHCO_3$ solution. After phase separation the aqueous layer was extracted three times with small portions of dichloromethane, the pooled organic phases washed with deionised water and brine and dried over Na_2SO_4 to give 1.94 g (92%) as off-white solid, m.p. 167 – 170 °C.

TLC: R_f: (petroleum ether /ethyl acetate 7/1 v/v) 0.55. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.38 (1H, tt, *J* = 2.58), 4.56 (1H, h, *J* = 5.42), 2.04 – 2.42 (7H, m), 1.74 – 1.93 (3H, m), 1.49 – 1.7 (5H, m), 1.21 (1H, td, *J* = 13.37, 3.59), 1.17 (9H, s), 1.04 – 1.13 (1H, m), 0.98 (3H, s), 0.98 (1H, td, *J* = 11.54, 2.73), 0.87 (1H, td, *J* = 13.29, 3.53), 0.85 (3H, s). ^{13}C-NMR (100MHz, $CDCl_3$): δ = 222.45, 178.17, 139.49, 121.86, 73.48, 51.15, 50.20, 47.99, 38.75, 37.89, 36.97, 36.74, 34.30, 34.20, 33.18, 31.70, 27.58, 27.28, 25.26, 23.07, 22.16, 19.23. IR [cm-1]: 2957, 1732, 1717, 1480, 1284, 1161. $[\alpha]^{20}_D$ = -131 (c 0.96, dichloromethane).

2.4 17-Methylene-13α-methylandrost-5-en-3β-ol pivalate (**5**)

Nysted reagent suspension (30.6 g, 20 wt%, 13.42 mmol) in THF (20 mL) was stirred in a Schlenk flask at 0 °C and $TiCl_4$ (8.05 mmol, 8 mL, 1M solution) in dichloromethane was added dropwise. The white milky suspension turned yellow and then light brown over the course of 10 minutes. Ketone **5** (1 g, 2.7 mmol) was added after formation of the reagent and the cooling bath was removed. The reaction was complete after stirring at room temperature for 18 hours and was quenched with 100 mL 2M hydrochloric acid and extracted with diethyl ether. The pooled organic phases were washed with brine, dried over Na_2SO_4 and evaporated to dryness. A yellow oil (1.28 g) was obtained as crude product. This was purified over a 60 g silica gel column using 15/1 v/v petroleum ether/ethyl acetate as eluent to recover 230 mg (23%) starting material and give 586 mg (59%) of product as colorless crystals, m.p. 108 - 111°C.

TLC: R_f: (petroleum ether/ethyl acetate 15/1 v/v) 0.34. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.37 (1H, t, *J* = 4.65), 4.84 (1H, t, *J* = 2.02), 4.69 (1H, t, *J* = 2.02), 4.57 (1H, h, *J* = 4.65), 2.18 – 2.54 (5H, m), 1.78 – 1.95 (4H, m), 1.39 – 1.63 (5H, m), 1.36 (1H, dd, 13.4, 3.24), 1.27 (1H, dd, 9.64, 6.2), 0.98 – 1.20 (3H, m), 1.17 (9H, s), 0.95 (3H, s), 0.89 (3H, s). ^{13}C-NMR (100MHz, $CDCl_3$): δ = 178.15, 157.51, 139.36, 122.47, 103.05, 73.66, 54.52, 48.93, 46.08, 38.75, 37.95, 37.02, 36.86, 34.12, 33.42, 33.19, 31.54, 29.85, 27.70, 27.30, 25.30, 21.55, 19.36. IR [cm^{-1}]: 2942, 1724. $[\alpha]^{20}_D$ = -131 (c 0.96, dichloromethane)

2.5 17-Methylene-13α-methylandrost-5-en-3β-ol (**6**)

A solution of **5** (506 mg, 1.37 mmol) in THF (15 mL) was stirred at room temperature and lithium aluminium hydride (129 mg, 3.41 mmol) was added at once. After 15 minutes TLC analysis indicated complete consumption of starting material. The grey suspension was quenched with 1 mL deionised water, dried over Na_2SO_4 and the solids were filtered off and thoroughly washed with diethyl ether. Evaporation of the pooled organic phases gave 392 mg (100%) product as colorless solid, m.p. 150 – 152 °C.

TLC: R_f: (petroleum ether/ethyl acetate 5/1 v/v) 0.17. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.35 (1H, tt, *J* = 2.51), 4.84 (1H, t, *J* = 2.23), 4.69 (1H, t, *J* = 2.23), 3.51 (1H, h, *J* = 5.28), 2.13 – 2.53 (5H, m), 1.77 – 1.95 (4H, m), 1.38 – 1.6 (6H, m), 1.35 (1H, dd, *J* = 13.40, 3.28), 0.99 – 1.29 (5H, m), 0.94 (3H, s), 0.93 (1H, m), 0.86 (3H, s). ^{13}C-NMR (100MHz, $CDCl_3$): δ = 157.47, 140.30, 121.69, 121.86, 103.06, 71.92, 54.57, 49.03, 46.05, 42.23, 37.11, 36.90, 34.17, 33.44, 33.18, 31.70, 31.55, 29.84, 25.30, 21.57, 19.37. IR [cm^{-1}]: 3229, 2936, 2893. $[\alpha]^{20}_D$ = -168.9 (c 0.7, dichloromethane).

2.6 17-Methylene-13α-methylandrost-4-en-3-one (**7**)

To a solution of **6** (707 mg, 2.47 mmol) in toluene (50 mL) there were added cyclohexanone (4.9 g, 50 mmol) and the solution was heated on a Dean-Stark trap for 1 h. At this point aluminium *iso*-propoxide (1.26 g, 6.17 mmol) was added and the reaction mixture quickly turned yellow. After 2 hours the reaction was complete. It was then washed with water (15 mL) and 0.1 M sulfuric acid (100 mL), dried over Na_2SO_4 and the now clear solution evaporated *in vacuo*. Upon standing over night at room temperature the product crystallized as white needles. The crystals were washed with cold petroleum ether and the filtrate concentrated and purified *via* column chromatography on 25 g silica gel with 90/10 v/v petroleum ether/ethyl acetate as eluent. There were obtained in total 670 mg (95%) of the title compound as white solid, m.p. 166 – 167 °C.

TLC: R_f: (petroleum ether/ethyl acetate 5/1 v/v) 0.33. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.7 (1H, t, *J* = 0.68), 4.86 (1H, t, *J* = 2.34), 4.71 (1H, t, *J* = 2.34), 2.21 – 2.57 (6H, m), 2.0 – 2.1 (2H, m), 1.96 (1H, dt, *J* = 13.54, 3.19), 1.8 – 1.92 (1H, m), 1.52 – 1.75 (3H, m), 1.44 (1H, dq, *J* = 12.3, 3.15), 1.35 (1H, td, *J* = 19.73, 3.55), 1.13 – 1.27 (2H, m), 1.05 (3H, s), 0.97 – 1.03 (1H, m), 0.94 (3H, s), 0.91 – 0.97 (1H, m). ^{13}C-NMR (100MHz, $CDCl_3$): δ = 199.78, 171.59, 156.87, 123.78, 103.35, 53.35, 52.71, 45.90, 38.90, 37.40, 35.67, 34.30, 34.06, 33.28, 32.95, 31.28, 29.46, 24.76, 21.35, 17.85. IR [cm^{-1}]: 2931, 1662. $[\alpha]^{20}_D$ = 72.9 (c 1.01, dichloromethane). HRMS(ESI+) 285.221 $[M+H]^+$ (Calcd. 285.2213).

2.7 17-Methylene-13α-methylandrost-1,4-dien-3-one (**8**)

To compound **7** (232 mg, 0.82 mmol) dissolved in 1,4-dioxane (5 mL) was added *tert*-Butyldimethylsilyl chloride (6 mg, 0.04 mmol) and then the mixture was cooled to 0 °C. To the solidified solution there was added DDQ (204 mg, 0.9 mmol) in two portions. The mixture was slowly allowed to reach room temperature and was stirred for 96 hours. The solvent was evaporated, the residue dissolved in dichloromethane and washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and evaporated in vacuo to give 227 mg (99%) of a yellow solid which was used in the next step without further purification, m.p.: 108 – 110 °C.

TLC: R_f: (petroleum ether/ethyl acetate 3/1 v/v) 0.75. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.07 (1H, d, *J* = 10.12), 6.23 (1H, dd, *J* = 10.14, 1.86), 6.06 (1H, t, *J* = 1.52), 4.89 (1H, t, J = 2.06), 4.75 (1H, t, J = 2.38), 2.3 – 2.59 (4H, m), 2.14 – 2.22 (1H, m), 1.95-2.01 (1H, m), 1.81 – 1.94 (1H, m), 1.6 – 1.67 (1H, m), 1.35 – 1.42 (2H, m), 1.13 – 1.28 (2H, m), 1.1 (3H, s), 0.97 – 1.13 (3H, m), 0.95 (3H, s). ^{13}C-NMR (100MHz, $CDCl_3$): δ = 186.54, 169.46, 156.63, 155.81, 127.66, 123.80, 103.55, 53.30, 50.82, 46.09, 43.58, 37.24, 34.59, 34.12, 33.14, 31.10, 29.33, 24.84, 22.83, 18.93. $[\alpha]^{20}_D$ = -9.1 (c 1.02, dichloromethane)

2.8 Spiro(13α-methylandrost-1,4-dien-17,2'-oxirane)-3-one (**9**)

To a solution of **8** (200 mg, 0.71 mmol) in $CHCl_3$ (3 mL) and buffer solution (di-sodium hydrogen phosphate/potassium dihydrogen phosphate) pH 6.88 (2 mL) at 0 °C there was added *meta*-Chloroperoxybenzoic acid (218 mg, 0.88 mmol) in 1 mL chloroform dropwise. The temperature was held for 3 hours. The reaction was then quenched with saturated aqueous $NaHCO_3$ and $Na_2S_2O_3$ solutions and washed twice with saturated aqueous $NaHCO_3$. After back extraction of the aqueous phases the pooled organic phases were dried over Na_2SO_4 and evaporated in vacuo to give 209 mg (99%) of **9** as a 54:46 mixture of diastereomers. These can be used directly without further purification. An analytical sample was obtained via column chromatography (2 g silica, 5/1 v/v petroleum ether/ethyl acetate).

Major diastereomer:

m.p. 128 – 130 °C. TLC: R_f: (petroleum ether/ethyl acetate 3/1 v/v) 0.33. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.06 (1H, d, *J* = 10.12), 6.22 (1H, dd, *J* = 10.12, 1.88), 6.07 (1H, t, *J* = 1.56), 2.73 (1H, d, *J* = 4.56), 2.64 (1H, d, *J* = 4.64), 2.32 – 2.52 (2H, m), 2.19 – 2.3 (2H, m), 1.87 – 1.97 (1H, m), 1.59 – 1.86 (4H, m), 1.43 (1H, td, *J* = 16.88, 3.67), 1.19 – 1.41 (3H, m), 1.17 (3H, s), 0.95 – 1.13 (2H, m), 0.92 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 186.55, 169.60, 155.96, 127.59, 123.69, 68.35, 53.16, 49.75, 47.54, 43.58, 41.43, 37.21, 34.77, 32.96, 31.50, 30.00, 27.96, 24.68, 24.34, 18.98. IR [cm^{-1}]: 3402, 2924, 1652, 1610, 1597. $[\alpha]^{20}_D$ = -65.8 (c 0.6, dichloromethane). HRMS(ESI+) 299.201 $[M+H]^+$ (Calcd. 299.2006).

Minor diastereomer:

m.p. 132 – 133 °C. TLC: R_f: (petroleum ether/ethyl acetate 3/1 v/v) 0.18. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.04 (1H, d, *J* = 10.12), 6.24 (1H, dd, *J* = 10.14, 1.9), 6.08 (1H, t, *J* = 1.50), 2.83 (2H, dd, *J* = 18.54, 4.94), 2.34 – 2.5 (2H, m), 2.18 – 2.27 (1H, m), 2.02 – 2.14 (2H, m), 1.88 – 2 (1H, m), 1.68 – 1.77 (1H, m), 1.56 – 1.67 (2H, m), 1.18 – 1.41 (5H, m), 1.15 (3H, s), 1 – 1.13 (1H, m), 0.99 (3H, s). ^{13}C-NMR (100MHz, $CDCl_3$): δ = 186.38, 168.91, 155.28, 127.88, 123.94, 66.58, 55.19, 53.14, 49.70, 43.33, 41.11, 37.62, 34.65, 32.88, 31.79, 29.36, 26.23, 24.34, 23.58, 18.92. IR [cm^{-1}]: 2930, 2900, 1667, 1602. $[\alpha]^{20}_D$ = -11 (c 0.6, dichloromethane)

2.9 17β-Hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-triene-3-one (**1c**)

To a mixture of epoxides **9** (210 mg, 0.7 mmol) in THF/H_2O 1/1 (5 mL) were added 5 drops of concentrated phosphoric acid and the solution was heated to reflux for 2 hours. After completion of the reaction THF was evaporated and the aqueous phase extracted four times with 20 ml dichloromethane, washed with saturated $NaHCO_3$ solution and brine and dried over Na_2SO_4.The organic solvent was evaporated to yield 209 mg crude product as oily

crystals. This was separated on the preparative HPLC (water + 0.1 % TFA / methanol = 2/3) to give 47 mg (22%) of the title compound as light yellow oily solid.

TLC: R_f: (petroleum ether/ethyl acetate 1/1 v/v) 0.44. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.14 (1H, d, *J* = 10.16), 6.26 (1H, dd, *J* = 10.16, 1.88), 6.09 (1H, t, *J* = 1.6), 3.48 (1H, d, *J* = 10.54), 3.34 (1H, d, *J* = 10.54), 2.52 (1H, tdd, *J* = 20.09, 5.03, 1.51), 2.37 – 2.47 (2H, m), 2.14 – 2.34 (3H, m). 1.86 – 2.1 (4H, m), 1.5 – 1.72 (4H, m). 1.30 (1H, td, J = 11.3, 2.06), 1.21 (3H, s), 0.92 (3H, s). ^{13}C-NMR (100MHz, $CDCl_3$): δ = 186.44, 168.76, 155.62, 138.95, 137.60, 127.83, 124.51, 69.02, 51.67, 49.51, 43.45, 36.74, 33.96, 33.36, 32.62, 30.82, 24.03, 22.48, 21.82, 18.65. IR [cm^{-1}]: 3395, 2923, 2855, 1655, 1615, 1600. $[\alpha]^{20}_D$ = 17.4 (c 0.88, dichloromethane).

EI fragmentation MS: The mass spectra of the bis-TMS derivative of the title compound equals to already published spectra by Schänzer et al[13], having abundantly discussed the relevant fragments after deuteration as well as multi-dimensional mass spectrometry experiments.

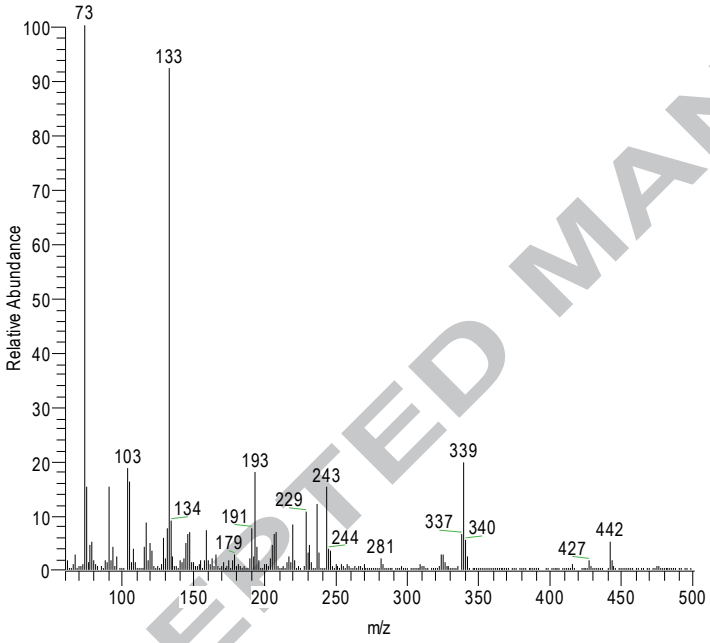


Figure 1: EI mass spectrum of bis-TMS-20βOH-NorMD

ESI(+)-MS: Elemental composition of the target compound was obtained by high resolution/high accuracy MS analysis, yielding the mass of the pseudomolecular ion as 299.2002 u with a mass accuracy < 2 ppm (theoretical mass: 299.2006, error = 1.2 ppm) as well as the elemental composition (C20H27O2).

In addition the mass spectrum shows a sodium adduct as m/z 321.1820. Only a few fragments are visible at 281.1897 (loss of water) and 269.1898 (loss of formaldehyde); m/z 147.0803 is a typical A ring fragment indicating a 3-keto-1,4-diene structural element.[13]

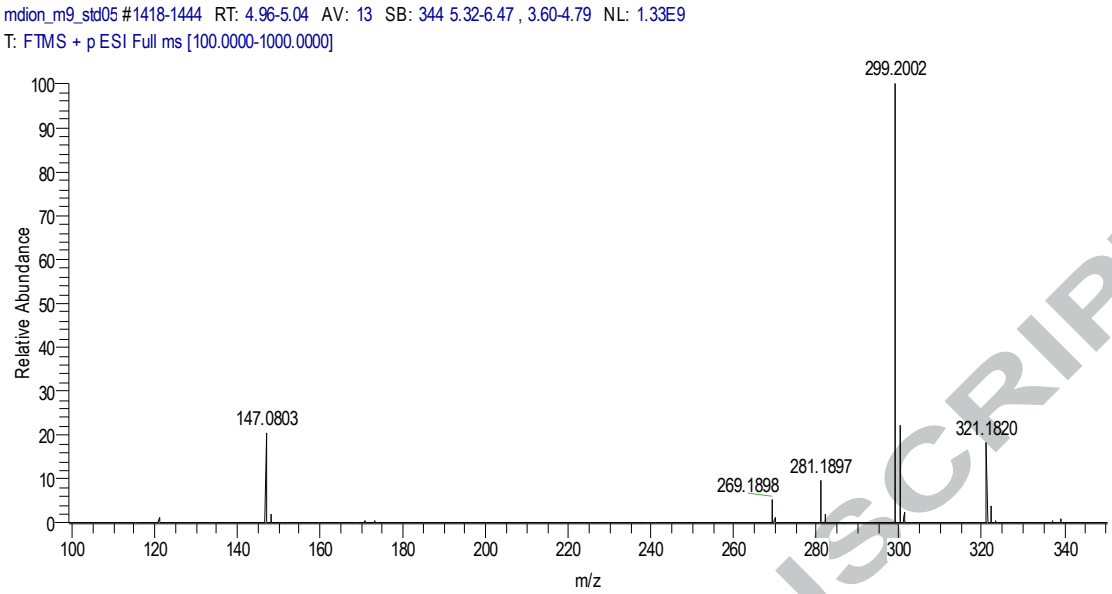
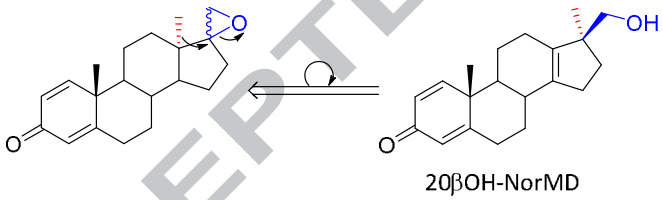


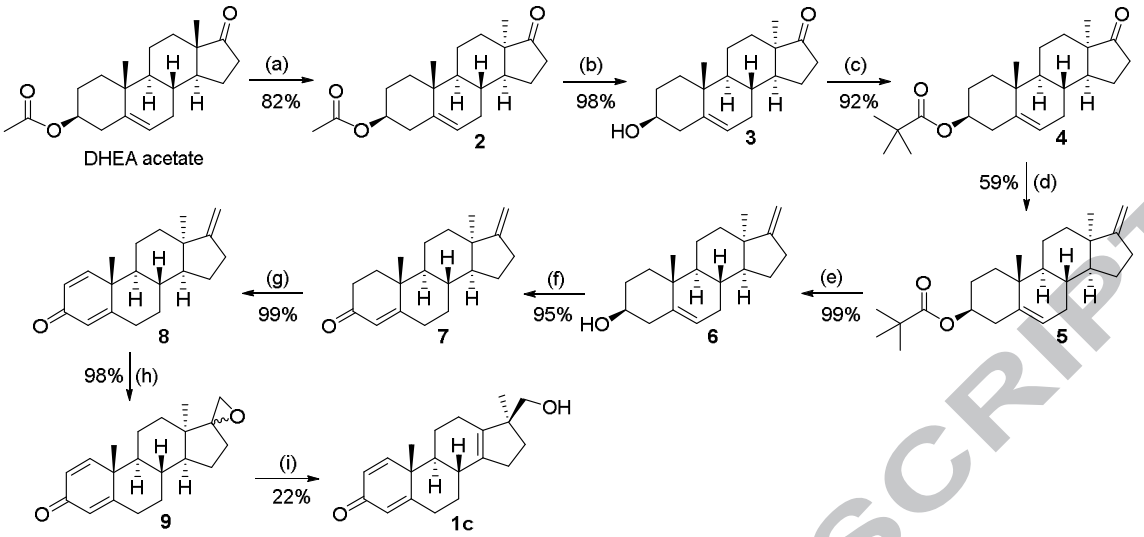
Figure 2: ESI(+) mass spectrum of 20βOH-NorMD

3. Results and discussion

Keeping in mind the biological transformation that leads to the formation of the target molecule our retrosynthetic strategy for introduction of the C-17 substituents is closely related. The C-13 methyl group can migrate when a carbocation is formed on the adjacent carbon C-17. A spiroepoxide on C-17 can form a carbocation and upon opening directly furnishes the hydroxymethyl group that is needed.



Scheme 2: Retrosynthetic analysis of the key-step



Scheme 3: Synthetic route and conditions: (a) 1,2-phenylendiamine, AcOH, reflux 24h. (b) K_2CO_3, MeOH, reflux 2h. (c) $(CH_3)_3CCOCl$, DMAP, pyridine, 0°C, 4h. (d) Nysted reagent, THF, 0°C then $TiCl_4$, rt o/n. (e) $LiAlH_4$, THF, 0°C, 15 min. (f) $Al(O\text{-}iPr)_3$, cyclohexanone, toluene, reflux, 4h. (g) DDQ, TBSCl, dioxane, rt, 96h. (h) *m*-CPBA, $CHCl_3$/buffered H_2O, rt, 2h. (i) THF/H_2O (1:1), phosphoric acid, reflux, 3h.

The synthesis started with epimerization of the angular methyl group on C-13. Only if the methyl group is in the α-position it should give the correct stereochemistry after the rearrangement. The C-13-epimerization which is suspected to proceed via an ion-radical mechanism gives about 85% of 13α-methyl (**2**) and 15% 13β-methyl when it reaches equilibrium.[15a,b,] First we used Corey-Chaykovsky[16] conditions for the direct transformation of the ketone to the spiroepoxides. Since this gave a complex mixture and low yields instead an *exo*-cyclic double bond was installed on the C-17.

Since cleavage of the acetate was observed in some of the screened reactions, a change in protecting groups was necessary and was easily realized by cleaving the acetate under mild basic conditions and subsequent protection of the alcohol as a pivalate (**4**). Peterson olefination[17] using (trimethylsilyl)methyllithium as well as the Tebbe reagent[18] proved unsuccessful. Most likely the low reactivity towards a nucleophilic attack at the ketone stems from the steric hindrance due to the *cis*-configuration of the C and D ring as well as enolization taking place due to basic reaction conditions. After intensive investigation, the Nysted reagent[19] was able to methylenate the substrate efficiently and selectively to provide **5**.

Deprotection of the pivalate protecting group was accomplished under reductive conditions with lithium aluminium hydride and gave alcohol **6** in quantitative yield. In order to oxidize the C-3 and isomerize the double-bond to form an enone we chose Oppenauer oxidation conditions which smoothly furnished enone **7**. Dehydrogenation to the dienone **8** was accomplished via a *tert*-Butyldimethylsilyl chloride catalyzed DDQ oxidation.[20]

Finally selective epoxidation of the *exo*cyclic electron-rich double was achieved using *meta*-Chloroperoxybenzoic acid in chloroform/water (buffered). When using only a non-polar solvent system considerable (>10%) amounts of Baeyer-Villiger oxidation products were observed. Both diastereomers of the epoxides were used in the next reaction.

The epoxide opening / rearrangement reaction was first tested under anhydrous conditions using boron trifluoride etherate in tetrahydrofurane. This gave only miniscule amounts of product, but switching to an aqueous acidic system increased yields greatly. In the end phosphoric acid in tetrahydrofurane/water was used. The complex mixture was separated on RP-HPLC. The crude mixture consisted of approximately 40% product (estimated from ^{1}H-NMR data); after purification 22% of pure product **1c** could be isolated. Analytical data of the synthesized material is in accordance to previously published data on this metabolite.[11,13]

4. Conclusions.

The first chemical synthesis of 20βOH-NorMD was achieved in nine steps and 8.9% overall yield of The main features of our synthesis include epimerization of the C-13 methyl group, introducing of *exo*-cyclic double bond at C-17, regioselective epoxidation of exomethylen double bond followed by acid mediated epoxide opening and subsequent rearrangement to create the desired 20βOH-NorMD. The described synthesis can provide doping laboratories with an essential reference material for their antidoping performance.

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Synthesis of 17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-trien-3-one: a long-term metandienone metabolite

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Highlights:

-) First chemical synthesis of compound 20βOH-NorMD

-) Efficient introduction of 17β-hydroxymethyl-17α-methyl substitution pattern

-) The crucial Wagner-Meerwein rearrangement is initiated by acidic epoxide opening