Steroids 158 (2020) 108601

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

The synthesis of 4-chloro-17β-hydroxymethyl-17α-methyl-18-norandrosta-4,13-diene-3α-ol – Proposed long term metabolite (M4) of oralturinabol

D. Yu. Shostko, A.I. Liubina, Yu.Yu. Kozyrkov*, S.A. Beliaev

National Anti-Doping Laboratory, Lesnoy 31, 223040, Belarus

ARTICLE INFO

Keywords:
Dehydrochloromethyltestosterone
Oral turinabol
Long-term metabolite
Total synthesis

ABSTRACT

4-Chloro-17β-hydroxymethyl-17α-methyl-18-norandrosta-4,13-diene-3α-ol is one of proposed long term metabolites of oralturinabol (anabolic androgenic steroid restricted in sport). The synthesis of 4-chloro-17β-hydroxymethyl-17α-methyl-18-norandrosta-4,13-diene-3α-ol was achieved. Isomerisation of configuration of 13-carbon was used for construction of 17β-hydroxymethyl-17α-methyl fragment. The proposed route of synthesis allows to obtain 3β-hydroxy isomer as well.

1. Introduction

The search for new long-term metabolites of the substances prohibited in sports is one of the fields of anti-doping research [1,2]. Together with a growing sensitivity of analytical instrumentation, it allows to expand a detection window and increase chances of revealing cases of illicit usage of performance-enhancing drugs [3]. For example, finding of metabolites containing 17β-hydroxymethyl-17α-methyl fragment [4] of 17-methylated androgenic-anabolic steroids (AAS) allowed to expand significantly the detection window of such drugs as metandienone [5], oxandrolone [6], and especially oralturinabol [7] (dehydrochloromethyltestosterone, DHCMT) **1**. In the latter case, it led to an increase in the number of adverse analytical finding (AAF) more than 10 times in 2013 [4]. At the same time, discovering of new metabolites makes it necessary to characterise them [8]. Structure assignment usually performed on the basis of previous knowledge of metabolism and MS-spectrometry data [7]. However, an assigned structure can be inexact and should be proved by compound isolation and characterization or by comparison with synthesized reference material [9]. For instance, recent synthesis of the DHCMT metabolite M3 [10] **2** (Fig. 1), which was discovered by Sobolevsky in 2012 [7], and its comparison [9] with reference urine showed the difference in the C-4 and C-5 stereocenters.

The M3 metabolite is used [4] for anti-doping analysis and has the detection window more than 40 days [7]. The other new DHCMT metabolites M2 **4** and M4 **3** containing 17β-hydroxymethyl-17α-methyl fragment are left without attention and their proposed structures are not proven, although they are at least as valuable as previously known long-term metabolites [7]. The content of metabolites in a biological sample is very low [11], thus the way to prove an assigned structure by synthesis is more preferred [8]. Synthesis of these substances have not been reported yet. The aim of the present study was to synthesise and characterise the structure of proposed for DHCMT long-term metabolite M4.

2. Experimental

2.1. General information

Commercially available reagents were obtained from Aldrich, Acros Organics, Fluka or Merck. Anhydrous tetrahydrofuran was dried by refluxing over $LiAlH_4$ and freshly distilled. NMR spectra were obtained on a Bruker AVANCE 500 MHz spectrometer using TMS as an internal standard. IR spectra (4000–400 cm^{-1}) were recorded as KBr pellets on a Nicolet iS10 IR spectrometer. Melting point was measured in open capillary with melting point apparatus MPM-HV2 in manual mode. High-resolution mass measurements were performed on an LTQ Orbitrap XL, with the external calibration of the instrument, having accuracy up to 5 ppm. Ionisation was carried out in the positive mode using the electrospray source at voltage of 4.5 kV. Samples were used as a solution in methanol or acetonitrile. TLC-analysis was performed with pre-coated aluminium plates (Silica gel 60 UV254, Macherey–Nagel). The spots were visualized under 254 nm UV light and/or submerging in an phosphomolybdic acid solution and heating. Column chromatography was performed through silica gel (200–300 mesh).

* Corresponding author.
E-mail address: kozyrkovyu@gmail.com (Y.Y. Kozyrkov).

https://doi.org/10.1016/j.steroids.2020.108601
Received 5 December 2019; Received in revised form 29 January 2020; Accepted 5 February 2020
Available online 19 February 2020
0039-128X/

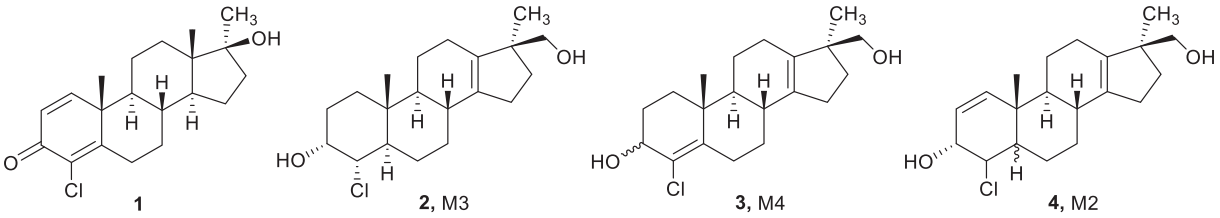


Fig. 1. Structures of oral turinabol (DHCMT) (**1**) and long-term metabolites proposed for it: M3 (**2**), M4 (**3**) and M2 (**4**) [7].

2.2. Chemical synthesis

2.2.1. 3β-Hydroxy-13α-androst-5-en-17-one acetate (14)

A solution of dehydroepiandrosterone **13** (4.16 g, 14.32 mmol) in acetic acid (40 mL) and 1,2-phenylenediamine (*o*-PDA) (2.32 g 21.48 mmol) was refluxed for 20 h. The resulting mixture was then poured into ice water (80 mL) and the beige precipitate formed was separated by filtration. The precipitate was washed with water and dried to give crude product (1.5 g). The filtrate was extracted with CH_2Cl_2 (2 × 40 mL) and the organic phase washed with water (2 × 20 mL) and with saturated $NaHCO_3$ solution (3 × 15), dried over Na_2SO_4 and the solvent evaporated under reduced pressure to give 3 g of crude product. The combined solids was chromatographed on silica gel (petroleum ether/ethyl acetate 90:10) to give **14** (2.66 g, 56%), 3β-Hydroxy-13α-androst-5-en-17-one (0.53 g, 11%) and starting material (0.30 g, 7%). m.p. 143–144 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.43–5.37 (m, 1H), 4.65–4.55 (m, 1H), 2.42–2.22 (m, 4H), 2.25–2.07 (m, 3H), 2.03 (s, 3H), 1.93–1.81 (m, 2H), 1.83–1.75 (m, 1H), 1.69–1.51 (m, 4H), 1.22 (td, J = 13.5, 3.6 Hz, 1H), 1.17–1.04 (m, 2H), 1.02–0.93 (m, 1H), 0.99 (s, 3H), 0.92–0.80 (m, 1H), 0.86 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 222.42, 170.65, 139.32, 122.00, 73.89, 51.12, 50.19, 47.99, 37.98, 36.94, 36.73, 34.28, 34.18, 33.16, 31.68, 27.68, 25.25, 23.05, 22.16, 21.54, 19.18. HRMS (ESI): *m/z* calcd for $C_{21}H_{31}O_3$ $[M+H]^+$: 331.22677; found: 331.22729 (Δ = 1.6 ppm). IR [cm^{-1}]: 2939, 1735, 1239, 1026.

2.2.2. 13α-Androst-4-en-3,17-dione (15)

A solution of **14** (1.29 g, 3.92 mmol) and potassium carbonate (1.14 g, 8.23 mmol) in methanol (50 mL) was refluxed for an hour. The reaction mixture was diluted with deionised water and extracted with dichloromethane. The pooled extracts were washed with brine, dried over Na_2SO_4. The solvents were removed under reduced pressure and the resulting crude 3β-Hydroxy-13α-methylandrost-5-en-17-one (1.13 g, 100%) was dissolved in dry toluene (70 mL) and cyclohexanone (7.7 g, 78.4 mmol) The solution was heated to reflux and after 15 min aluminium isopropoxide (2.0 g, 9.79 mmol) were added, upon which the solution turned yellow. After 2 h the reaction was complete. It was then washed with water (25 mL), 5% sulfuric acid (120 mL), saturated $NaHCO_3$ solution and brine. After drying over Na_2SO_4 and evaporating under reduced pressure the mixture was left to crystallize at 3 °C overnight. The crystals were washed with cold petroleum ether and dried to give 830 mg of crude product. The filtrate was left to crystallize at −18 °C overnight and after filtration, washing and drying additional 230 mg were collected. Combined solids purified via column chromatography on silica gel (petroleum ether/ethyl acetate 70:30) to afford product **15** (795 mg, 71%) as white solid, m.p. 147–149 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.76–5.72 (m, 1H), 2.46–2.28 (m, 5H), 2.27–2.04 (m, 5H), 1.90–1.83 (m, 1H), 1.69 (td, J = 13.8, 5.0 Hz, 1H), 1.60–1.54 (m, 2H), 1.21 (td, J = 13.5, 3.9 Hz, 1H), 1.16–1.05 (m, 2H), 1.04 (s, 3H), 1.01–0.93 (m, 1H), 1.00 (s, 3H), 0.91–0.81 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 221.70, 199.51, 170.44, 124.07, 51.62, 50.02, 49.99, 38.77, 38.13, 35.56, 33.94, 33.83, 32.93, 32.86, 31.91, 25.18, 22.81, 21.62, 17.70. HRMS (ESI): *m/z* calcd for $C_{19}H_{27}O_2$ $[M+H]^+$: 287.20056; found: 287.19977 (Δ = −2.7). IR [cm^{-1}]: 2922, 1735, 1667, 1619, 1447, 1229, 1190, 1095, 871.

2.2.3. 4-Chloro-13α-androst-4-en-3,17-dione (16)

To a solution of 13α-androst-4-en-17-one (**15**) (700 mg, 2.45 mmol) in dry pyridine (7 mL) SO_2Cl_2 (0.32 mL, 3.92 mmol) was added at 0 °C. The resulting mixture was stirred at the same temperature for 1 h and quenched with water (20 mL). The mixture was extracted with EtOAc (3 × 10 mL), combined organic layers were washed with 5% HCl, saturated $NaHCO_3$ solution and brine and then dried over Na_2SO_4. Solvents were evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether/ethyl acetate, 88:12–84:16) to afford compound **16** (650 mg) as yellowish crystal. Additional recrystallization from mixture hexane/ethyl acetate allows to obtain white crystals of **16** (580 mg, 74%). m.p. 191–193 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.31–3.23 (m, 1H), 2.63–2.49 (m, 2H), 2.47–2.38 (m, 1H), 2.29–2.03 (m, 6H), 1.93–1.85 (m, 1H), 1.74 (td, J = 13.6, 5.5 Hz, 1H), 1.63–1.54 (m, 2H), 1.26–1.15 (m, 1H), 1.16–1.06 (m, 1H), 1.09 (s, 3H), 1.06–0.99 (m, 2H), 1.00 (s, 3H), 0.92–0.80 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 221.52, 190.73, 164.00, 127.45, 51.83, 50.00, 49.94, 41.51, 37.73, 34.28, 33.99, 33.76, 31.95, 31.85, 29.14, 25.10, 22.86, 21.55, 18.11. HRMS (ESI): *m/z* calcd for $C_{19}H_{26}ClO_2$ $[M+H]^+$: 321.16158; found: 321.16136 (Δ = −0.7 ppm). IR [cm^{-1}]: 2953, 1720, 1692, 1581, 1292, 1238, 1188, 1094, 1070, 955, 808, 575.

2.2.4. 3β-Hydroxy-4-chloro-13α-androst-4-en-17-one (17)

To a solution of 4-chloro-13α-androst-4-en-3,17-dione **(16)** (470 mg, 1.46 mmol) in dry THF (20 mL) under Ar atmosphere was added a 1 M solution of L-Selectride in THF (1.48 mmol, 1.5 mL) dropwise at −50 °C. The mixture was stirred at −50 °C for 2 h and quenched with saturated aqueous NH_4Cl (1.5 mL) and then was warmed to room temperature. After concentrating under reduced pressure the mixture was diluted with water and extracted with EtOAc. Combined organic layers were washed with water and brine and then dried over Na_2SO_4. Solvents were evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether: ethyl acetate, 86:14) to give allyl alcohol **17** (435 mg, 92%) with ratio of diastereomers as 9:1. Analytical data were recorded for single isomer obtained after recrystallization from MeOH: m.p. 157–160 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.16 (ddd, J = 9.4, 6.2, 3.0 Hz, 1H), 2.94 (dt, J = 14.1, 3.2 Hz, 1H), 2.48 (d, J = 3.0 Hz, 1H), 2.38 (dd, J = 19.1, 9.0 Hz, 1H), 2.26–2.03 (m, 5H), 1.99–1.90 (m, 1H), 1.90–1.82 (m, 1H), 1.81–1.74 (m, 1H), 1.73–1.61 (m, 1H), 1.57–1.47 (m, 2H), 1.35 (td, J = 13.4, 2.9 Hz, 1H), 1.16 (td, J = 13.4, 3.9 Hz, 1H), 1.04–0.91 (m, 2H), 0.97 (s, 3H), 0.95 (s, 3H), 0.90–0.71 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 222.05, 142.12, 128.51, 69.68, 51.76, 50.26, 50.07, 40.60, 38.02, 33.88, 33.39, 32.55, 32.09, 28.04, 27.28, 25.24, 22.98, 21.54, 19.54. HRMS (ESI): *m/z* calcd for $C_{19}H_{28}ClO_2$ $[M+H]^+$: 323.17723; found: 323.17745 (Δ = 0.7 ppm). IR [cm^{-1}]: 3540, 2949, 2839, 1720, 1451, 841, 454.

2.2.5. 3α-Hydroxy-4-chloro-13α-androst-4-en-17-one (18)

To a solution of 3β-alcohol **17** (400 mg, 1.24 mmol) and PPh_3 (650 mg, 2.48 mmol) in dry THF (12 mL) under Ar atmosphere neat formic acid (95 μL, 2.48 mmol) was added at once, followed by solution of diethyl azodicarboxylate in toluene (1.0 mL, 2.48 mmol, 40%). The resulted light-yellowish solution was left stirred at room temperature for 2 h. After concentrating under reduced pressure the reaction

mixture was divided between ethyl ether (10 mL) and aqueous $NaHCO_3$ (5%, 10 mL). The organic phase was washed with water, then methanolic NaOH (1 M, 4 mL) was added and resulting mixture was stirred for 30 min. The reaction mixture was diluted with water and extracted with EtOAc. Combined organic layers were washed with water and brine and then dried over Na_2SO_4. Solvents were evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether: ethyl acetate, 80:20) to afford 3α-alcohol **18** (345 mg, 86%) as white crystals with ratio of diastereomers as 9:1. M.p. 169–171 °C (MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.15 (q, J = 2.6, Hz, 1H), 2.96 (dt, J = 14.5, 3.4 Hz, 1H), 2.44–2.33 (m, 1H), 2.27–2.05 (m, 5H), 1.96–1.77 (m, 4H), 1.65 (dt, J = 13.3, 3.8 Hz, 1H), 1.61–1.49 (m, 3H), 1.18 (td, J = 13.5, 4.0 Hz, 1H), 1.04–0.94 (m, 2H), 0.97 (s, 3H), 0.92–0.74 (m, 2H), 0.89 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 222.08, 143.05, 127.30, 69.77, 52.10, 50.12, 49.99, 40.58, 37.97, 33.83, 32.26, 32.00, 31.35, 27.45, 26.80, 25.17, 23.26, 21.49, 18.29. HRMS (ESI): *m/z* calcd for $C_{19}H_{28}ClO_2$ $[M+H]^+$: 323.17723; found: 323.17783 (Δ = 1.8 ppm). IR [cm^{-1}]: 3542, 2947, 2881, 1726, 1446, 1274, 1077, 1054, 1005, 966, 774, 636.

2.2.6. 3α-[(tert-Butyldimethylsilyl)oxy]-4-chloro-13α-androst-4-en-17-one (19)

To a solution of 3α-hydroxy-4-chloro-13α-androst-4-en-17-one (**18**) (345 mg, 1.07 mmol) in DMF (3.5 mL) imidazole (182 mg, 2.67 mmol) and TBSCl (201 mg, 1.34 mmol) were sequentially added at room temperature. The reaction mixture was stirred overnight and quenched with water (15 mL). The mixture was extracted with mixture of EtOAc: PE (1:1) (3 × 5 mL). The combined extracts were washed with water (5 mL), brine (5 mL) and dried over Na_2SO_4. Concentration and column chromatography on silica gel (petroleum ether:ethyl acetate, 97:3 to 95:4) afforded **19** (440 mg, 94%) as white crystals, m.p. 163–165 °C ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.11–4.06 (m, 1H), 2.95 (dt, J = 14.4, 3.3 Hz, 1H), 2.42–2.32 (m, 1H), 2.25–1.99 (m, 4H), 1.94–1.81 (m, 2H), 1.80–1.46 (m, 6H), 1.27–1.12 (m, 1H), 1.06–0.93 (m, 2H), 0.97 (s, 3H), 0.92–0.71 (m, 2H), 0.90 (s, 9H), 0.85 (s, 3H), 0.13 (s, 3H), 0.09 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 222.20, 141.65, 127.73, 70.47, 51.91, 50.07, 50.00, 40.37, 38.01, 33.86, 32.20, 32.01, 31.21, 29.24, 26.74, 25.92(×3), 25.15, 23.22, 21.51, 18.27, 18.22, −4.32, −4.73. HRMS (ESI): *m/z* calcd for $C_{25}H_{42}ClO_2Si$ $[M+H]^+$: 437.26371; found: 437.26424 (Δ = 1.3 ppm). IR [cm^{-1}]: 2948, 2856, 1732, 1470, 1358, 1070, 934, 837, 776.

2.2.7. 3α-[(tert-butyldimethylsilyl)oxy]-4-chloro-17-methylene-13α-androst-4-en (20)

Diiodomethane (0.42 mL, 5.26 mmol) was added dropwise to a stirred suspension of activated Zn (1.17 g, 17.9 mmol) and PbI_2 (78 mg, 0.17 mmol) in THF (5 mL) and the resulting mixture was maintained at self-reflux during the addition. The reaction mixture was stirred for a further 30 min at rt before cooling to 0 °C. $TiCl_4$ (0.22 mL in 1 mL CH_2Cl_2, 1.97 mmol) was added dropwise, and the mixture was allowed to stir at rt for a further 1 h after the addition. A solution of ketone **19** (230 mg, 0.53 mmol) in THF (0.7 mL + 0.7 mL washing) was added and the resultant mixture was stirred at rt for 20 h. The reaction was quenched by slow addition of saturated aqueous NH_4Cl (15 mL) at 0 °C and stirred for 20 min. Resulting suspension was filtered through a pad of Celite, filter cake was washed with EtOAc (50 mL), then the layers were separated. The organic phase was washed with water, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel (petroleum ether: ethyl acetate, 99:1) to afford **20** (152 mg, 66%) as white crystals, m.p. 130–132 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.87–4.81 (m, 1H), 4.73–4.67 (m, 1H), 4.09 (dt, J = 4.1, 2.2 Hz, 1H), 2.91 (dt, J = 13.9, 3.1 Hz, 1H), 2.53–2.34 (m, 2H), 1.93 (ddt, J = 13.5, 10.4, 3.0 Hz, 2H), 1.89–1.78 (m, 2H), 1.75 (dt, J = 13.8, 3.5 Hz, 1H), 1.69–1.53 (m, 4H), 1.45 (dq, J = 12.4, 3.3 Hz, 1H), 1.37 (td, J = 13.4, 3.7 Hz, 1H), 1.21 (dd, J = 9.6, 6.2 Hz, 1H), 1.13–1.04 (m, 1H), 0.96–0.83 (m, 3H), 0.94 (s, 3H), 0.91 (s, 9H), 0.88 (s, 3H), 0.14 (s, 3H), 0.10 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.40, 142.50, 127.35, 103.04, 70.69, 53.46, 53.03, 45.94, 40.58, 37.32, 34.50, 32.30, 31.43, 31.39, 29.57, 29.47, 27.09, 26.06(×3), 24.75, 21.89, 18.55, 18.36, −4.18, −4.59. HRMS (ESI): *m/z* calcd for $C_{26}H_{44}ClOSi$ $[M+H]^+$: 435.28445; found: 435.28479 (Δ = 0.8 ppm). IR [cm^{-1}]: 2950, 2855, 1080, 838, 774.

2.2.8. 3α-[(tert-Butyldimethylsilyl)oxy]-4-chloro-17-spiro[13α-androst-4-en-17,2′-oxirane] (21, 22)

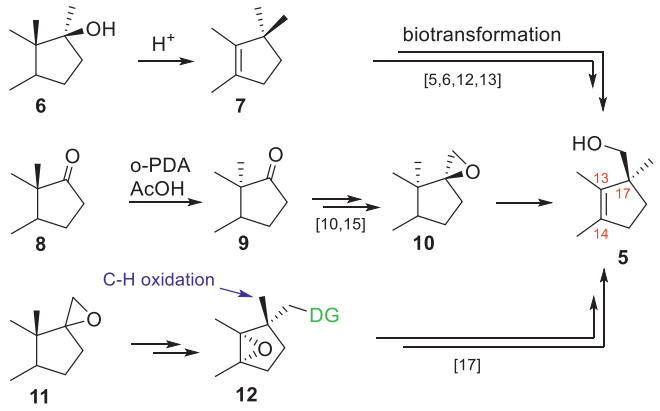
$NaHCO_3$ (335 mg, 4.0 mmol) and MCPBA (70%, 99 mg, 0.40 mmol) were sequentially added to an ice cooled stirring solution of **20** (174 mg, 0.40 mmol) in CH_2Cl_2 (12 mL). The reaction mixture was stirred for 1 h at the same temperature and then 15 h at room temperature, diluted with CH_2Cl_2 (25 mL) and quenched with saturated aqueous $Na_2S_2O_3$ (1 mL). The organic layer was separated, washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel preparative plate (20 × 20 × 0.1 cm) (petroleum ether: ethyl acetate, 96:4; two eluations) to afford compound **21** (21 mg, 12%) as a white solid and **22** (81 mg, 45%) as a white solid. TLC on SiO_2: (petroleum ether/ethyl acetate 15/1 v/v) R_f (**21**): 0.51, R_f (**22**): 0.42.

2.2.9. 3α-[(tert-Butyldimethylsilyl)oxy]-4-chloro-17β-hydroxymethyl-17α-methyl-18-nor-androst-4,13-dien (23)

A mixture of dry CH_2Cl_2 (1.3 mL) and 2,6-lutidine (12 μL, 0.11 mmol) under Ar atmosphere was cooled to −80 °C. Then TMSOTf (16 μL, 0.09 mmol) was added at that temperature. After five minutes, the solution of minor epoxide **21** (20 mg, 0.04 mmol) in CH_2Cl_2 (0.2 μL + 0.2 μL washings) was added dropwise to the chilled reaction mixture. The reaction mixture is stirred at −80–−70 °C for 1 h and then quenched by the addition of methanol (1 mL) followed by 2 M HCl (0.2 mL). The reaction mixture was warmed to room temperature and stirred for 30 min. The mixture was diluted with water, extracted with CH_2Cl_2 and the combined organic phases were washed with saturated $NaHCO_3$ solution, brine and dried over Na_2SO_4. Solvent were evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether: ethyl acetate, 90:10) to afford **23** (13 mg, 65%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.10 (dt, J = 3.8, 1.9 Hz, 1H), 3.48 (d, J = 10.5 Hz, 1H), 3.31 (d, J = 10.5 Hz, 1H), 3.01 (ddd, J = 13.8, 4.4, 2.0 Hz, 1H), 2.34–2.25 (m, 1H), 2.25–2.12 (m, 2H), 2.03–1.85 (m, 6H), 1.82 (tt, J = 13.8, 3.9 Hz, 1H), 1.74–1.58 (m, 3H), 1.55 (ddd, J = 12.8, 9.4, 5.5 Hz, 1H), 1.32–1.22 (m, 1H), 1.17 (s br, 1H) 1.15–1.02 (m, 2H), 0.98 (s, 3H), 0.94 (s, 3H), 0.92 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.94, 140.59, 136.80, 128.17, 70.61, 69.13, 51.93, 51.71, 40.41, 36.75, 34.12, 31.25, 30.68, 30.55, 29.40, 27.22, 26.04, 23.22, 22.66, 21.89, 18.34, 17.83, −4.21, −4.61. HRMS (ESI): *m/z* calcd for $C_{26}H_{44}ClO_2Si$ $[M+H]^+$: calc: 451.27936; found: 451.28015 (Δ = 1.7 ppm).

2.2.10. 4-Chloro-17β-hydroxymethyl-17α-methyl-18-nor-androst-4,13-dien-3α-ol (24)

To a solution of TBS-ether **(23)** (13 mg, 0.044 mmol) in THF (0.5 mL) was added TBAF·$3H_2O$ (18 mg, 0.06 mmol) and stirred for 44 h at room temperature. The mixture was diluted with water (2 mL) and extracted with EtOAc. Combined organic layers were washed with brine and dried over Na_2SO_4. Solvents were evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether: ethyl acetate, 80:20 to 70:30) to give diol **24** (**7** mg, 78%) as colorless oil. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.16 (q, J = 2.7 Hz, 1H), 3.48 (d, J = 10.5 Hz, 1H), 3.32 (d, J = 10.5 Hz, 1H), 3.01 (ddd, J = 13.8, 4.4, 2.0 Hz, 1H), 2.37–2.09 (m, 4H), 2.06–1.82 (m, 8H), 1.71 (dt, J = 13.4, 3.6 Hz, 1H), 1.65–1.52 (m, 2H), 1.35–1.23 (m, 1H), 1.18 (s br, 1H), 1.12 (ddd, J = 12.8, 10.7, 2.0 Hz, 1H), 1.08–1.02 (m, 1H), 1.01 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.36, 140.24, 137.07, 127.74, 69.95, 69.05, 52.30, 51.68, 40.64, 36.68, 34.03, 31.43, 30.67, 30.62, 27.51, 27.28, 23.20, 22.69, 21.89, 17.81. HRMS (ESI): *m/*



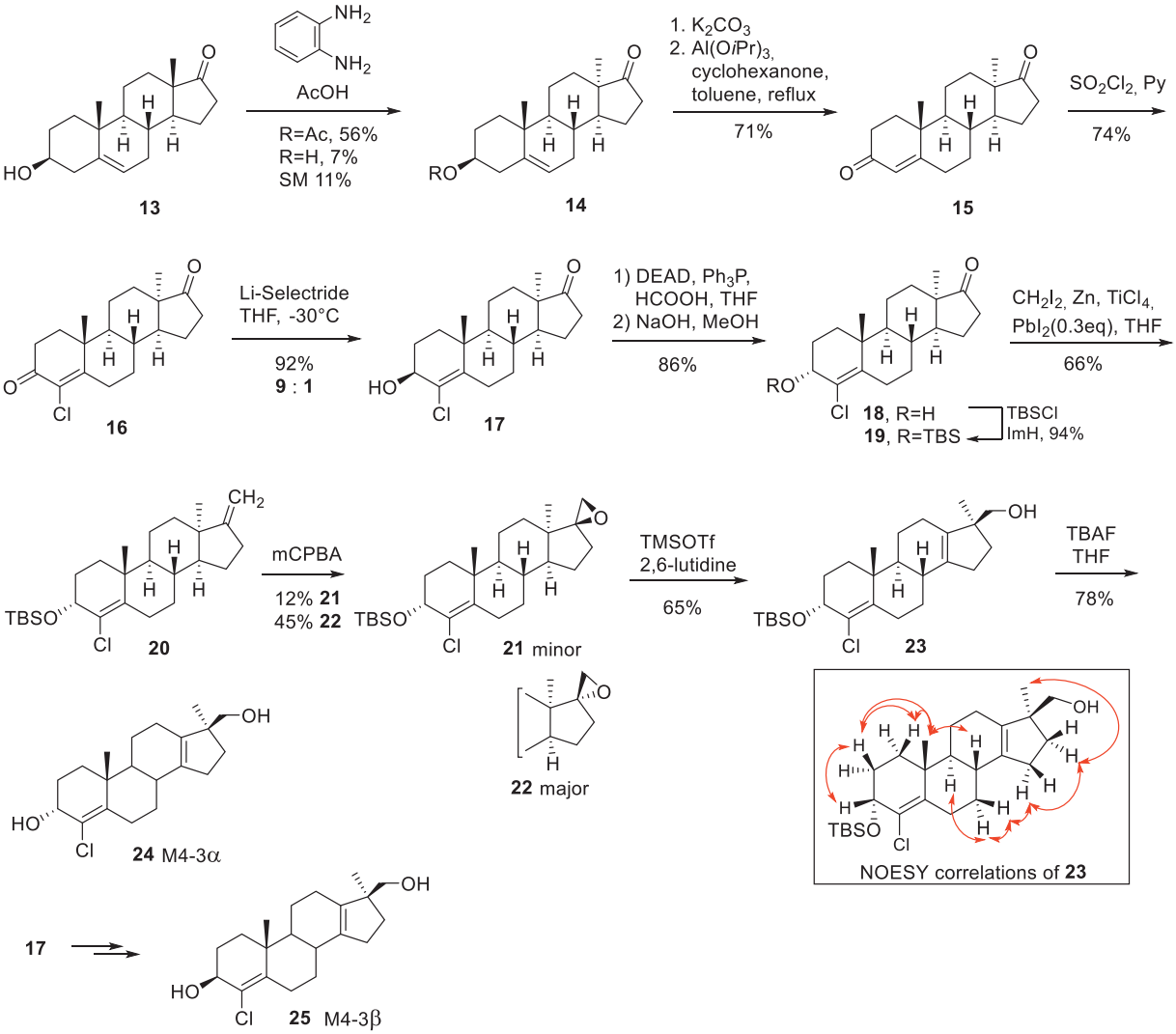
Scheme 1. Approaches to the construction of the steroidal fragment **5**.

z calcd for $C_{20}H_{30}ClO_2$ $[M+H]^+$: 337.19288 found: 337.19290 (Δ = 0.04 ppm); calcd for $C_{20}H_{30}{}^{37}ClO_2$: 339.18993; found: 339.18998 (Δ = 0.15 ppm).

3. Results and discussion

There are only a handful of approaches to form challenging C-17 stereocenter of 17-methylated steroids long-term metabolites **5** in literature (Scheme 1). One of them is the biotechnological approach that is based on cytochrome P450 [5,12,13] or fungus [6] oxidation of 17,17-dimethyl fragment **7**. In addition, there are two chemical approaches. First includes isomerization of the C-13 *β*-methyl group of 17-ketosteroids **8** to **9** by o-PDA in acetic acid media [14]. After installation of epoxide function, **10** undergoes ring-opening and cationic migration of C-13 α-methyl group [10,15,16]. Second is based on Pd-catalysed C–H oxidation of the 17β-methyl substituent in **12** using directing oxazoline group established at 17α-methyl [17].

For the synthesis of M4, pathway including epimerization of C-13 and cationic rearrangement was chosen. The synthesis was started from the commercially available dehydroepiandrosterone **13** that at the first stage was exposed to radical epimerization by o-PDA under refluxing in acetic acid with formation of 13α-DHEA acetate **14** as the main product (Scheme 2). The basic deprotection of acetate with subsequent Oppenauer oxidation leads to enone **15**. To incorporate chlorine function in α-position of **15**, it was subjected to SO_2Cl_2 [18] in pyridine at 0 °C give **16** in 72% yield. With chloroenone in hand, we tried to selectively reduce conjugated carbonyl group with $NaBH_4$, $NaBH_4/CeCl_3{\cdot}7H_2O$, DIBAL-H, L-Selectride. Only L-Selectride [19] at −30 °C allowed to reduce selectively 3-carbonyl group in presence of 17-ketone with the formation of isomers **17** as 9:1. It is known that reduction of 4-en-3-on fragment by Selectride reagents in steroid substrates without substituents in A and B ring leads mostly to *β*-configuration of hydroxyl group [20–22], whereas reduction of substrates with 2α-, 6α- or 7α-



Scheme 2. Rout of the synthesis of 4-chloro-17β-(hydroxymethyl)-17α-methyl-18-norandrosta-4,13-diene-3α-ol.

substituents gives α-alcohols [23,24]. At this stage configuration of C-3 was assigned by implication from our previous results [25] as *β*. Since the recent research [10] reports that DHCMT metabolite M3 has 3α-OH, the next step was the inversion of the hydroxyl group configuration. Inversion of C-3 was performed by Mitsunobu reaction [26] of **17** with formic acid followed by treatment with methanolic NaOH solution to afford compound **18** with the 3α-hydroxyl group in 86% yield that was subsequently protected as TBS-ether. The next stage was to transform the 17-carbonyl group into some functionality that could be rearranged in mild conditions to give proper C–17 stereocenter.

Because of steric hindrance and enolization of C-17 ketone in steroids with *cis*-C/D rings, such substrate is inactive in Corey-Chaykovsky reaction [15,16]. Attempts to attach other nucleophiles as bromomethyllithium [27] and cyanide failed. When TMSCl was added to ketone **19** and cyanide in DMSO [28], slight formation of products was observed, although with low conversion. Therefore, further study was focused on methylenation of carbonyl group.

We observed that similar and much cheaper combination of reagents $Zn/CH_2I_2/TiCl_4$ [29] was as effective in methylenation of **19** as the Nysted reagent [10,30]. In this way, compound **20** was obtained in 66% yield (76% BRSM). Then exocyclic alkene was effected epoxidation by mCPBA to afford two epoxides with close chromatographic mobility on SiO_2 ($\Delta R_f < 0.1$). Separation of these epoxides by preparative TLC was accompanied with partial decomposition. Single isomers **21** and **22** were obtained only in 12% and 45% yields respectively. The next stage to be performed was the epoxide ring-opening followed by Wagner-Meerwein rearrangement. Rearrangement of major epoxide **22** under TMSOTF/2.6-lutidine condition resulted in an inseparable mixture of isomers on the position of the double bond in C or D rings with low yield. Whereas rearrangement of minor epoxide under the same conditions led to compound **23** with target fragment 13-en-18-nor-17β-hydroxymethyl-17α-methyl in 65% yield, as described in the previous work [10]. At this stage, structure confirmation was done by 2D ^1H–^1H NOESY. After TBS-deprotection of **23**, target diol **24** was obtained.

Furthermore, 3*β*-isomer **25** was synthetized from **17** in the same way as 3α-M4 (**24**) without inversion stage of C-3.

To summarise, we have performed the first synthesis of two isomers (relative to 3-hydroxy group) **24** and **25** of potential long-term metabolite (M4) of DHCMT. Comparison of this compounds with endogenous metabolite will allow us to determine exact structure of it.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2020.108601.

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