# Selective manipulation of steroid hydroxyl groups with boronate esters: efficient access to antigenic C-3 linked steroid-protein conjugates and steroid sulfate standards for drug detection

**Natasha L. Hungerford,[a] Andrew R. McKinney,[b] Allen M. Stenhouse[b] and Malcolm D. McLeod*[a]**

[a] *School of Chemistry, F11, The University of Sydney, NSW 2006, Australia. Fax: +61-2-9351-6650; Tel: +61-2-9351-5877; E-mail: m.mcleod@chem.usyd.edu.au*

[b] *Australian Racing Forensic Laboratory, P.O. Box 528, Kensington, NSW 1465, Australia.*

## Experimental

### General experimental

Optical rotations were measured using a PolAAR 2001 polarimeter set at the 589.3 nm sodium D line, in a 0.25 dm cell, in the solvent indicated, and at the concentration (g / 100 mL) and temperature indicated. Optical rotations are quoted in $10^{-1}$.deg.cm$^2$.g$^{-1}$. Infrared spectra were recorded on a Shimadzu FTIR-8400S Fourier transform infrared spectrometer. Compounds were prepared as thin films on a 0.5 cm NaCl plate, or as KBr disks, seated on a custom-made perch in the apparatus. Absorption maxima are expressed as wavenumbers (cm$^{-1}$).

$^1$H Nuclear magnetic resonance spectra were recorded using a Bruker Avance 200 (200.13 MHz), a Bruker Avance 300 (300.13 MHz) or a Bruker DRX 400 (400.13 MHz) spectrometer. Spectra were recorded in $CDCl_3$ or $CD_3OD$ and chemical shifts were recorded as δ values in parts per million (ppm). Signals arising from residual protio-forms of the solvent were used as internal standard (δ7.26, or δ3.30, respectively). Data are reported as chemical shift (δ), relative integral, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, m = multiplet), coupling constant (*J* in Hz) and assignment. All coupling constants and multiplicities reported are apparent values.

$^{13}$C Nuclear magnetic resonance spectra were recorded using a Bruker Avance 200 (50.3 MHz), a Bruker Avance 300 (75.5 MHz) or a Bruker DRX 400 (100.62 MHz) spectrometer at ambient temperature, with complete proton decoupling. Spectra were recorded in $CDCl_3$, $CD_3OD$ or in $D_6$-DMSO and chemical shifts were recorded as δ values in parts per million (ppm). Signals arising from the solvent were used as internal standard (δ77.0, δ49.0 or δ39.5 respectively).

Low resolution mass spectra were recorded by the Mass Spectrometry Unit, School of Chemistry, The University of Sydney. Major fragments are quoted as mass to charge ratio (assignment, percentage of base peak). Low resolution mass spectra were recorded on a Finnigan Polaris Q ion trap mass spectrometer, using electron impact (+EI) ionisation mode at 70 eV, or on a Finnigan LCQ ion trap mass spectrometer, using positive electrospray ionisation (+ESI), or negative electrospray ionisation (-ESI). High resolution mass spectra were recorded on a Kratos MS25 RFA mass spectrometer, using electron impact (+EI) ionisation, operating at 70 eV, in magnetic scan, with PFK as standard (The University of Queensland), or on a Spectrospin 7T FTICR, using positive or negative electrospray ionisation (The University of New South Wales).

Analytical thin layer chromatography (TLC) was performed using 0.2 mm thick, aluminium-backed, pre-coated silica gel plates (Merck Silica gel 60 $F_{254}$). Compounds were visualised by staining with Goofy's Dip (15 g phosphomolybdic acid, 15 mL conc. sulfuric acid, 485 mL water, 2.5 g cerium sulfate) or an anisaldehyde solution (7.4 mL anisaldehyde, 383 mL ethanol 95%, 10 mL sulfuric acid, 3 mL acetic acid). Flash chromatography was performed using Merck Silica gel 60 (230 – 400 mesh ASTM), under a positive pressure of nitrogen, with the solvents indicated. Solvent compositions were mixed v/v as specified.

All solvents and reagents were purified according to standard procedures. Moisture sensitive reactions were carried out in oven-dried glassware under a dry, inert nitrogen atmosphere. Reaction temperatures were controlled using dry ice : acetone (-78 °C) or ice : water (0 – 5 °C) cooling baths. Concentration under reduced pressure refers to evaporation of solvent using a rotary evaporator connected to a water aspirator. Removal of residual solvent where desired, was achieved by evacuation (0.1 – 0.01 mmHg) with a high-stage, oil-sealed vacuum pump.

Epiandrosterone (3β-hydroxy-5α-androstan-17-one) was obtained from Steraloids (Newport, RI, U.S.A.). 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), carboxymethoxylamine hemihydrochloride, *N*-hydroxysuccinimide and lithium acetylide-ethylenediamine complex, were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

### Synthesis

**5α,17α-pregn-20-yne-3β,16β,17β-triol (19).** 3β,16β-Diacetoxy-5α-androstane-17-one **22**[2,3] (0.973 g, 2.49 mmol) in THF (100 mL) was added drop-wise to a stirred suspension of lithium acetylide-ethylenediamine complex (2.76 g, 29.9 mmol) in THF (40 mL). After stirring for 20 hours, TLC (ethyl acetate/hexane, 1/1) analysis suggested an absence of diacetate **22** ($R_f$ 0.8) and complete conversion to product. The reaction mixture was poured into saturated $NH_4Cl$ solution (600 mL) and extracted into ethyl acetate (4 x 100 mL). The combined ethyl acetate extracts were washed with pH 7 buffer (80 mL) and saturated NaCl solution (80 mL), then dried ($Na_2SO_4$) and concentrated. The resulting residue was pre-adsorbed onto silica from DCM/MeOH and subjected to flash chromatography (ethyl acetate/hexane, 1/3 then 1/2 then 1/1.2), which gave 17α-ethynyl triol **19** (0.592 g, 71%), as a

colourless, amorphous solid. $R_f$ 0.3 (ethyl acetate/hexane, 1/1); **mp** 205-208 °C; $[\alpha]_D^{22}$ -26.4 (*c* 0.50, MeOH); $\nu_{max}$/ cm$^{-1}$ (KBr disk) 3650-3000 (OH), 3275 (C≡CH), 2920, 2851, 2110 (C≡C), 1385, 1366, 1049; $\delta_H$ (300 MHz, MeOD) 4.10 (1H, dd, *J* 7.9, 4.6, H16), 3.57-3.44 (1H, m, H3), 2.87 (1H, s, H21), 2.25-2.15 (1H, m), 1.82-0.80 (21H, m), 0.84 (3H, s, $CH_3$), 0.82 (3H, s, $CH_3$) 0.74-0.62 (1H, m); $\delta_C$ (75 MHz, MeOD) 87.7, 79.7, 78.4, 71.8, 75.6, 55.8, 48.3, 47.3, 46.2, 38.9, 38.3, 37.0, 36.7, 35.7, 35.0, 33.1, 32.1, 29.8, 21.8, 13.5, 12.8; **m/z** (ESI-) 331.2281 (M-H$^-$, $C_{21}H_{31}O_3$ requires 331.2273, 100%), 306 (M-C≡CH$^-$, 70%).

**5α,17α-pregnane-3β,16β,17β-triol (20).** 17α-Ethynyl triol **19** (0.570 g, 1.72 mmol) was dissolved in MeOH (20 mL). 10% Pd/C (0.080 g) was added and the reaction mixture was stirred under an atmosphere of $H_2$ (balloon pressure) for 16 hours. The reaction mixture was diluted with ethyl acetate/MeOH (1/1) and filtered through celite, to afford 17α-ethyl triol **20** (0.552 g, 96%), as a colourless, amorphous solid. $R_f$ 0.33 (ethyl acetate/hexane, 1/1); **mp** 218-221°C; $[\alpha]_D^{22}$ -13.0 (*c* 0.43, MeOH); $\nu_{max}$/ cm$^{-1}$ (KBr disk) 3650-3100 (OH), 2932, 2854, 1450, 1381, 1038; $\delta_H$ (300 MHz, MeOD) 3.77 (1H, dd, *J* 8.1, 6.2, H16), 3.56-3.43 (1H, m, H3), 2.12 (1H, ddd, *J* 12.6, 8.1, 6.2), 1.80-0.80 (20H, m), 0.90 (3H, t, *J* 7.2, H21), 0.84 (3H, s, $CH_3$), 0.81 (3H, s, $CH_3$) 0.68-0.57 (1H, m); $\delta_C$ (75 MHz, MeOD) 81.2, 73.4, 71.8, 55.9, 48.0, 47.1, 46.2, 38.9, 38.2, 37.1, 36.7, 36.3, 33.8, 33.3, 32.1, 29.9, 28.2, 21.9, 15.0, 12.7, 7.5; **m/z** (ESI+) 336 (MH$^+$, 30%), 271(25), 243 (30), 231 (70), 216 (55), 135 (55), 107 (57), 91(100), 79 (84); **m/z** (ESI-) 335.2600 (M-H$^-$, $C_{21}H_{35}O_3$ requires 335.2592).

**[(*S*)-16β,17β-benzylidenedioxy]-17α-methyl-5α-androstane-3β-ol.** 17α-Methyl triol **18**[1] (0.50 g, 1.55 mmol) was stirred (the triol was partially insoluble) in a mixture of DMF/DCM (1/1, 12 mL). Benzaldehyde dimethyl acetal (0.35 mL, 0.354 g, 2.33 mmol) was added, followed by conc. $H_2SO_4$ (10 drops). After 2 hours, a homogenous solution resulted. After 4 hours, TLC (ethyl acetate / hexane, 1/2) showed an absence of starting material ($R_f$ 0.1) and complete conversion to product. The reaction mixture was poured into $H_2O$ (150 mL) and extracted with ethyl acetate (5 x 80 mL). The organic extracts were washed with saturated NaCl solution, dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue obtained was pre-adsorbed onto silica from DCM and subjected to flash chromatography (ethyl acetate/hexane, 1/5, 1/4 then 1/3), which gave [(*S*)-16β,17β-benzylidenedioxy]-17α-methyl-5α-androstane-3β-ol (0.607 g, 95%). $R_f$ 0.2 (ethyl acetate / hexane, 1/2); **mp** 66-68°C; $[\alpha]_D^{22}$ -23.5 (*c* 0.46, DCM); $\nu_{max}$/ cm$^{-1}$ (film) 3600-3100 (OH), 2927, 1445, 1406, 1067, 1045; $\delta_H$ (300 MHz, $CDCl_3$) 7.58-7.52 (2H, m, ArH), 7.40-7.33 (3H, m, ArH), 5.94 (1H, s, O-CH-O), 4.14 (1H, dd, *J* 7.6, 5.5, H16), 3.65-3.52 (1H, m, H3), 2.08 (1H, dd, *J* 13.0, 7.6, 6.2), 1.85-0.78 (19H, m), 1.34 (3H, s, $CH_3$), 1.02 (3H, s, $CH_3$), 0.83 (3H, s, $CH_3$), 0.68-0.55 (1H, m); $\delta_C$ (75 MHz, $CDCl_3$) 136.6, 129.3, 128.3, 126.9, 105.6, 90.7, 88.5, 71.3, 54.2, 53.3, 44.9, 44.7, 38.2, 37.0, 35.7, 35.6, 33.4, 32.0, 31.5, 31.0, 28.5, 21.0, 20.3, 15.8, 12.3; **m/z** (EI+) 410.2828 (M$^+$, $C_{27}H_{38}O_3$ requires 410.2821, 10%), 409 (15), 367 (90), 349 (55), 331 (76), 323 (85), 305 (55), 105 (100), 91 (96), 77 (68).

**[(*S*)-16β,17β-benzylidenedioxy]-17α-methyl-5α-androstan-3-one (26).** [(*S*)-16β,17β-Benzylidenedioxy]-17α-methyl-5α-androstane-3β-ol (0.50g, 1.22 mmol) in DMSO (2 mL) was stirred for 16 hours with 4Å molecular sieves. $Et_3N$ (2.55 mL, 1.85 g, 18.3 mmol) was added *via* syringe. Sulfur trioxide-pyridine complex (1.75 g, 11.0 mmol) in DMSO (6 mL) was added by cannula, and the reaction stirred for 2.5 hours. The mixture was poured into saturated $NH_4Cl$ solution (450 mL) and extracted into ethyl acetate (5 x 100 mL). The combined organic extracts were washed with saturated NaCl solution (200 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude product was pre-adsorbed onto silica from DCM and subjected to flash chromatography (ethyl acetate / hexane, 1/5), which gave ketone **26** (0.386 g, 78%). $R_f$ 0.60 (ethyl acetate / hexane, 1/2); **mp** 179-181°C; $[\alpha]_D^{22}$ -12.4 (*c* 0.55, DCM); $\nu_{max}$/ cm$^{-1}$ (film) 2943, 1709 (C=O), 1458, 1402, 1059, 1001, 989; $\delta_H$ (200 MHz, $CDCl_3$) 7.60-7.51 (2H, m, ArH), 7.41-7.33 (3H, m, ArH), 5.94 (1H, s, O-CH-O), 4.15 (1H, dd, *J* 7.7, 5.4, H16), 2.50-1.95 (6H, m), 1.76-0.65 (14H, m), 1.34 (3H, s, $CH_3$), 1.04 (3H, s, $CH_3$), 1.03 (3H, s, $CH_3$); $\delta_C$ (50 MHz, $CDCl_3$) 211.7, 136.5, 129.3, 128.3, 126.9, 105.6, 90.6, 88.3, 53.6, 53.0, 46.6, 44.6, 38.5, 38.1, 35.7, 35.5, 33.3, 31.6, 31.0, 28.7, 21.1, 20.2, 15.7, 11.5; **m/z** (EI+) 408.2663 (M$^+$, $C_{27}H_{36}O_3$ requires 408.2664, 5%), 407 (10), 365 (100), 347 (45), 321 (75), 285 (35), 217 (45), 105 (65), 91 (57), 77 (46).

**16β,17β-Dihydroxy-17α-methyl-5α-androstan-3-one (β-hydroxymestanolone) (15).** Method 2. Ketone **26** (0.170 g, 0.42 mmol) was dissolved in THF (3 mL). 20 % $Pd(OH)_2$/C (0.030 g) was added and the reaction mixture was stirred under an atmosphere of $H_2$ (balloon pressure) for 8 hours. TLC (ethyl acetate / hexane, 1/1) suggested mostly starting material **26**. The reaction mixture was filtered through celite, which was washed with ethyl acetate. After concentration *in vacuo*, the residue was re-dissolved in THF (3 mL) and stirred with $Pd(OH)_2$/C (0.030 g) under an $H_2$ atmosphere for 16 hours. Additional $Pd(OH)_2$/C (0.050 g) was added and stirring was continued under an $H_2$ atmosphere for 4 hours. TLC (ethyl acetate / hexane, 1/1) then showed complete conversion to a lower $R_f$ product. Filtration through celite (ethyl acetate elution) and concentration yielded crude product. This was dissolved in DCM and pre-adsorbed onto silica for flash chromatography (ethyl acetate / hexane, 1/2 then 1/1), which gave β-hydroxymestanolone (**15**) (0.124 g, 93%).

**5α,17α-pregn-20-yne-3β,17β-diol (32).**[4] Epiandrosterone (**21**) (1.627 g, 5.61 mmol) in THF (100 mL) was added drop-wise to a stirred suspension of lithium acetylide-ethylenediamine complex (2.066 g, 22.4 mmol) in THF (50 mL). After stirring for 20 hours, TLC (ethyl acetate/hexane, 1/2) showed a spot of the same $R_f$ as **21** ($R_f$ 0.2). The reaction mixture was poured into saturated $NH_4Cl$ solution (500 mL) and extracted into ethyl acetate (4 x 100 mL). The combined ethyl acetate extracts were washed with pH 7 buffer (80 mL) and saturated NaCl solution (80 mL), then dried ($Na_2SO_4$) and concentrated. The resulting

residue was pre-adsorbed onto silica from DCM/MeOH and subjected to flash chromatography (ethyl acetate/hexane, 1/2 then 1/1), which gave diol **32** (1.659 g, 94%), as a colourless solid. $\boldsymbol{R_f}$ 0.4 (ethyl acetate / hexane, 1/1); **mp** 265-267°C (lit.,[4] 260-261°C); $\boldsymbol{[\alpha]_D^{20}}$ -42.8 (*c* 0.43, $CHCl_3$ / MeOH, 5/1); $\boldsymbol{\nu_{max}}$/ **cm**$^{-1}$ (film) 3500-3300 (OH), 3263 (≡C-H), 2930, 2100 (C≡C), 1383, 1053, 1026; $\boldsymbol{\delta_H}$ (300 MHz, $CDCl_3$ / MeOD, 5/1) 3.43-3.29 (1H, m, H3), 2.42 (1H, s, ≡CH), 2.06 (1H, ddd, *J* 13.9, 9.6, 5.6), 1.81-0.60 (20H, m), 0.65 (3H, s, $CH_3$), 0.64 (3H, s, $CH_3$), 0.56-0.42 (1H, m); $\boldsymbol{\delta_C}$ (75 MHz, $CDCl_3$ / MeOD, 5/1) 87.5, 79.0, 73.0, 70.5, 53.7, 50.1, 46.5, 44.6, 38.4, 37.3, 36.7, 35.8, 35.2, 32.3, 31.3, 30.6, 28.3, 22.7, 20.6, 12.3, 11.8; **m/z** (EI+) 316.2406 ($M^+$, $C_{21}H_{32}O_2$ requires 316.2402, 8%), 283 (30), 265 (45), 215 (90), 147 (60), 124 (90), 91 (100), 79 (70), 77 (70).

**17β-hydroxy-5α,17α-pregn-20-yn-3-one (30)**[4] Diol **32** (0.676 g, 2.14 mmol), dissolved in a mixture of DCM (9 mL) and DMSO (9 mL) was stirred for 16 hours with 4Å molecular sieves. $Et_3N$ (5.97 mL, 4.33 g, 42.8 mmol) was added *via* syringe. Sulfur trioxide-pyridine complex (4.09 g, 25.7 mmol) in DMSO (15 mL) was added by cannula, and the reaction stirred for 16 hours. The mixture was poured into saturated $NH_4Cl$ solution (300 mL) and ethyl acetate (100 mL) was added, followed by $CHCl_3$/$^i$PrOH (3/1, 200 mL). The mixture was filtered through celite and the layers separated. The aqueous phase was further extracted with $CHCl_3$/$^i$PrOH (3/1, 3 x 100 mL). The combined organic extracts were dried ($Na_2SO_4$) and concentrated *in vacuo*. The crude product was triturated with $Et_2O$ (4 x 80 mL) and then $H_2O$ (2 x 50 mL) which afforded ketone **30** (0.503 g, 75%). $\boldsymbol{R_f}$ 0.6 (ethyl acetate / hexane, 1/1); **mp** 294-296°C (decomp.) (lit.,[4] 296-297°C); $\boldsymbol{[\alpha]_D^{24}}$ -44.7 (*c* 0.17, $CHCl_3$ / MeOH, 4/1); $\boldsymbol{\nu_{max}}$/ **cm**$^{-1}$ (KBr disk) 3700-3100 (OH), 3271 (≡C-H), 2924, 2106 (C≡C), 1693 (C=O), 1381, 1053; $\boldsymbol{\delta_H}$ (300 MHz, $C_5D_5N$) 3.44 (1H, s, ≡C-H), 2.58-0.62 (22H, m), 1.10 (3H, s, $CH_3$), 0.92 (3H, s, $CH_3$); $\boldsymbol{\delta_C}$ (50 MHz, $C_5D_5N$) 209.9, 88.9, 78.8, 73.8, 53.3, 50.3, 47.1, 46.4, 44.5, 39.6, 38.3, 37.9, 35.9, 35.5, 33.1, 31.2, 28.6, 23.2, 21.1, 13.1, 10.9; **m/z** (EI+) 314.2250 ($M^+$, $C_{21}H_{30}O_2$ requires 314.2246, 5%), 285 (15), 229 (30), 173 (25), 145 (26), 124 (100).

**17β-hydroxy-5α,17α-pregnan-3-one (31).** 17α-Ethynyl ketone **30** (0.145 g, 0.46 mmol) was dissolved in DCM/MeOH (3/1, 30 mL) and stirred with decolourising charcoal for 2 hours. The mixture was filtered through celite and concentrated. The residue was stirred in a mixture of MeOH (3.5 mL), 1,4-dioxane (2.5 mL), $H_20$ (1.5 mL) and THF (5 mL). $NaHCO_3$ ( 0.150 g) and 10% Pd/C (0.050 g) was added and the reaction mixture was stirred under an atmosphere of $H_2$ (balloon pressure) for 16 hours. The reaction mixture was filtered through celite and concentrated. $^1$H NMR revealed partial reduction. The residue was dissolved in THF (15 mL) and $H_2O$ (2 mL) and stirred with 10% Pd/C (0.050 g), under an atmosphere of $H_2$ (balloon pressure), for 16 hours. Filtration, concentration and repetition of the latter procedure afforded 17α-ethyl ketone **31** (0.095 g, 65%), as a colourless, amorphous solid. $\boldsymbol{R_f}$ 0.65 (ethyl acetate / hexane, 1/1); **mp** 145-148°C; $\boldsymbol{[\alpha]_D^{25}}$ +12.6 (*c* 0.70, $CHCl_3$); $\boldsymbol{\nu_{max}}$/ **cm**$^{-1}$ (film) 3700-3100 (OH), 2935, 1713 (C=O), 1447, 1000, 980; $\boldsymbol{\delta_H}$ (300 MHz, $CDCl_3$) 2.45-2.16 (3H, m), 2.12-1.88 (3H, m), 1.76-0.78 (18H, m), 1.02 (3H, s, $CH_3$), 0.97 (3H, t, *J* 7.2, $C_{21}$-$H_3$), 0.89 (3H, s, $CH_3$), 0.75-0.64 (1H, m); $\boldsymbol{\delta_C}$ (75 MHz, $CDCl_3$) 211.9, 83.5, 53.8, 50.3, 46.8, 46.4, 44.7, 38.6, 38.1, 36.3, 35.7, 33.6, 31.5, 28.9, 28.8, 23.6, 21.1, 14.5, 11.5, 7.8 (1C overlapping); **m/z** (EI+) 318.2554 ($M^+$, $C_{21}H_{34}O_2$ requires 318.2559, 5%), 289 ($M^+$-Et, 100), 271 (40), 229 (30), 213 (25).

**General procedure for preparation of (carboxymethyl)oximes 33-36.** Carboxymethoxylamine hemihydrochloride (2 mmol) was added to a solution of **16**, **17, 30** or **31** (1 mmol) in pyridine (10 mL). The reaction mixture was heated to 80 °C and stirred under a nitrogen atmosphere for 4-5 hours. The reaction mixture was partially concentrated *in vacuo*, and then poured into distilled water (100 mL) and extracted into ethyl acetate (3 x 60 mL). The combined organic layers were washed with pH 7 buffer (1 x 60 mL) and saturated sodium chloride solution (1 x 60 mL), and then dried ($Na_2SO_4$) and concentrated to give the product **33**, **34**, **35** or **36**.

**3-(Carboxymethoxyimino)-5α,17α-pregn-20-yne-16β,17β-diol (33).** 17α-Ethynyl ketone **16** (0.063 g, 0.19 mmol) was treated as per the general procedure. The residue obtained was pre-adsorbed onto silica from DCM/MeOH and subjected to flash chromatography (ethyl acetate / MeOH / $H_2O$, 50/2/1 then 10/2/1) which afforded a mixture of *E* and *Z* isomers of **33** (0.058 g, 75%), as colourless crystals. $\boldsymbol{R_f}$ 0.3 (ethyl acetate/MeOH/$H_2O$, 50/2/1); **mp** 138-140°C; $\boldsymbol{[\alpha]_D^{23}}$ -29.1 (*c* 0.22, MeOH); $\boldsymbol{\nu_{max}}$/ **cm**$^{-1}$ (KBr disk) 3700-2300 (COOH, OH), 3279 (≡C-H), 2922, 1728 (C=O), 1630, 1452, 1385, 1248, 1148, 1092, 1049; $\boldsymbol{\delta_H}$ (400 MHz, MeOD) 4.47 (4H, s, $OCH_2CO_2$), 4.10 (2H, dd, *J* 5.0, H16), 3.26-3.18 (1H, m), 3.04-2.97 (1H, m), 2.88 (2H, s, ≡C-H), 2.27-0.85 (36H, m), 0.95 (6H, s, $CH_3$), 0.84 (6H, s, $CH_3$), 0.76-0.67 (2H, m); $\boldsymbol{\delta_C}$ (100 MHz, MeOD) 174.7, 163.1, 87.6, 79.7, 78.4, 75.7, 70.9, 55.4, 55.3, 48.2, 47.9, 47.32, 47.30, 46.7, 39.7, 38.5, 37.3 37.28, 36.9, 35.7, 35.1, 35.0, 32.9, 32.8, 29.8, 29.6, 29.1, 28.4, 22.4, 21.8, 13.5, 11.8, 11.7 (13C overlapping); **m/z** (ESI+) 845 ($2M+K^+$, 35%), 829 ($2M+Na^+$, 45), 426 ($M+Na^+$, 80), 404.2421 ($M+H^+$, $C_{23}H_{34}NO_5$ requires 404.2432, 100%).

**3-(Carboxymethoxyimino)- 5α,17α-pregnane-16β,17β-diol (34).** 17α-Ethyl diol **17** (0.110 g, 0.33 mmol) was treated as per the general procedure. The residue obtained was pre-adsorbed onto silica from DCM/MeOH and subjected to flash chromatography (ethyl acetate / MeOH / $H_2O$, 50/2/1 then 20/2/1 then 10/2/1) which afforded a mixture of *E* and *Z* isomers of **34** (0.112 g, 84%), as colourless crystals. $\boldsymbol{R_f}$ 0.3 (ethyl acetate/MeOH/$H_2O$, 50/2/1); **mp** 158-160°C; $\boldsymbol{[\alpha]_D^{23}}$ -25.3 (*c* 0.19, MeOH); $\boldsymbol{\nu_{max}}$/ **cm**$^{-1}$ (KBr disk) 3700-2300 (COOH, OH), 2937, 2856, 1736 (C=O), 1452, 1383, 1217, 1097; $\boldsymbol{\delta_H}$ (400 MHz, MeOD) 4.48, 4.47 (4H, 2 x s, $OCH_2CO_2$), 3.81-3.75 (2H, m, H16), 3.26-3.18 (1H, m), 3.03-2.96 (1H, m), 2.27-0.85 (40H, m), 0.95 (3H, s, $CH_3$), 0.94 (3H, s, $CH_3$), 0.91 (3H, t, *J* 7.2, $C_{21}$-$H_3$), 0.90 (3H, t, *J* 7.2, $C_{21}$-$H_3$) 0.83 (3H, s, $CH_3$), 0.828 (3H, s, $CH_3$), 0.74-0.64 (2H, m); $\boldsymbol{\delta_C}$ (50

MHz, MeOD) 174.3, 163.2, 81.2, 73.3, 70.7, 55.5, 55.4, 48.0, 47.9, 47.1, 46.7, 39.7, 38.5, 37.3, 37.0, 36.2, 35.1, 33.7, 33.0, 29.9, 29.7, 29.1, 28.4, 28.2, 22.4, 21.9, 15.0, 11.8, 11.7, 7.6 (16C overlapping); **m/z** (ESI+) 837 (2M+$Na^+$, 45%), 430.2572 (M+$Na^+$, $C_{23}H_{37}NO_5Na$ requires 430.2564, 90%), 408 (M+$H^+$, 100).

**3-(Carboxymethoxyimino)-5α,17α-pregn-20-yn-17β-ol (35).** 17α-Ethynyl ketone **30** (0.092 g, 0.29 mmol) was treated as per the general procedure. The residue obtained was pre-adsorbed onto silica from DCM/MeOH and subjected to flash chromatography (ethyl acetate / MeOH / $H_2O$, 100/2/1 then 80/2/1 then 60/2/1) which afforded a mixture of *E* and *Z* isomers of **35** (0.061 g, 54%), as colourless crystals. $R_f$ 0.25 (ethyl acetate/MeOH/$H_2O$, 100/2/1); **mp** 198-200°C (decomp.); $[\alpha]_D^{24}$ -32.0 (*c* 0.20, MeOH); $\nu_{max}$/ **cm$^{-1}$** (KBr disk) 3700-2600 (COOH, OH), 3290 (≡C-H), 2932, 2851, 1713 (C=O), 1383, 1356, 1088; $\delta_H$ (300 MHz, $CDCl_3$ / MeOD, 1/1) 4.48 (4H, s, $OCH_2CO_2$), 3.27-3.15 (1H, m), 3.03-2.94 (1H, m), 2.67 (2H, s, ≡C-H), 2.26-0.64 (42H, m), 0.91 (6H, s, $CH_3$), 0.80 (6H, s, $CH_3$); $\delta_C$ (75 MHz, $CDCl_3$ / MeOD, 1/2) 173.6, 163.1, 163.07, 88.4, 79.9, 74.1, 74.0, 70.3, 54.6, 54.5, 51.1, 47.5, 47.4, 46.2, 39.3, 39.2, 38.1, 36.8, 34.7, 33.4, 32.1, 30.3, 29.4, 29.2, 28.8, 28.1, 23.7, 22.1, 21.6, 13.2, 11.8, 11.7 (14C overlapping); **m/z** (ESI-) 773 (2M-$H^-$, 55%), 386 (M-$H^-$, 100); **m/z** (ESI+) 388.2480 (M+$H^+$, $C_{23}H_{34}NO_4$ requires 388.2483).

**3-(Carboxymethoxyimino)-5α,17α-pregnan-17β-ol (36).** 17α-Ethyl ketone **31** (0.099 g, 0.31 mmol) was treated as per the general procedure. The residue obtained was pre-adsorbed onto silica from DCM/MeOH and subjected to flash chromatography (ethyl acetate / MeOH / $H_2O$, 80/2/1) which afforded a mixture of *E* and *Z* isomers of **36** (0.105 g, 86%), as colourless crystals. $R_f$ 0.3 (ethyl acetate/MeOH/$H_2O$, 80/2/1); **mp** 154-156°C; $[\alpha]_D^{23}$ -8.0 (*c* 0.2, MeOH); $\nu_{max}$/ **cm$^{-1}$** (KBr disk) 3700-2300 (COOH, OH), 2932, 2854, 1751 (C=O), 1408, 1095, 970, 874; $\delta_H$ (300 MHz, $CDCl_3$ / MeOD, 2/1) 4.23 (4H, s, $OCH_2CO_2$), 3.05-2.94 (1H, m), 2.82-2.72 (1H, m), 2.04-1.97 (2H, m), 1.83-0.80 (42H, m), 0.72 (6H, t, *J* 7.2, $C_{21}$-$H_3$), 0.69 (6H, s, $CH_3$), 0.62 (6H, s, $CH_3$), 0.48-0.37 (2H, m); $\delta_C$ (75 MHz, $CDCl_3$ / MeOD, 2/1) 172.4, 162.1, 83.0, 69.3, 53.6, 53.5, 50.1, 46.2, 46.1, 45.0, 38.0, 36.9, 35.9, 35.7, 33.6, 32.1, 31.2, 31.1, 28.3, 28.2, 27.8, 27.0, 23.1, 21.0, 20.5, 14.0, 10.9, 10.7, 7.0 (17C overlapping); **m/z** (ESI-) 781 (2M-$H^-$, 90%), 390 (M-$H^-$, 100); **m/z** (ESI+) 392.2730 (M+$H^+$, $C_{23}H_{38}NO_4$ requires 392.2717).

**General procedure for synthesis of *N*-hydroxysuccinimide derivatives 37-40.** *N*-hydroxysuccinimide (1.1 mmol) was added to a solution of **33**, **34, 35** or **36** (1 mmol) in dioxane (12 mL) and dichloromethane (12 mL). EDC (1.1 molar eq.) was added and the reaction mixture was stirred at room temperature under nitrogen, for 16-24 h. The reaction mixture was concentrated under reduced pressure. The residue obtained was partitioned between water (250 mL) and ethyl acetate (150 mL). The aqueous layer was further extracted with ethyl acetate (2 x 150 mL). The combined organic phases were washed with pH 7 buffer (80 mL) and saturated sodium chloride solution (80 mL), then dried ($Na_2SO_4$) and concentrated to give product **37**, **38**, **39** or **40**.

**3-(Carboxymethoxyimino)-5α,17α-pregn-20-yne-16β,17β-diol *N*-hydroxysuccinimide ester (37).** Compound **33** (0.056 g, 0.14 mmol) was treated as per the general procedure. Precipitation of the resulting solid from ethyl acetate/hexane gave **37** (0.058 g, 84%) as a 1:1 mixture of isomers. $R_f$ 0.8 (ethyl acetate); **mp** 119-121°C; $[\alpha]_D^{27}$ -10.0 (*c* 0.20, $CH_2Cl_2$); $\nu_{max}$/ **cm$^{-1}$** (film) 3600-3200 (OH), 3302 (≡C-H), 2937, 1824, 1786, 1740 (C=O), 1207, 1076; $\delta_H$ (300 MHz, $CDCl_3$) 4.88 (4H, s, $OCH_2CO_2$), 4.20 (2H, dd, *J* 8.0, 4.1, H16), 3.27-3.15 (1H, m), 3.02-2.94 (1H, m), 2.84 (8H, s, $COCH_2CH_2CO$), 2.56 (2H, s, ≡C-H), 2.36-0.83 (40H, m), 0.90 (6H, s, $CH_3$), 0.86 (6H, s, $CH_3$), 0.81-0.68 (2H, m); $\delta_C$ (50 MHz, $CDCl_3$) 168.7, 165.8, 163.1, 162.8, 85.8, 78.8, 77.4, 74.8, 67.8, 53.6, 53.5, 46.8, 46.4, 46.2, 45.2, 38.2, 37.3, 36.2, 35.4, 34.6, 33.9, 33.6, 31.4, 28.5, 28.4, 28.2, 27.4, 25.6, 21.5, 20.6, 12.7, 11.5, 11.3 (21C overlapping); **m/z** (ESI+) 559 (55%), 523.2410 (M+$Na^+$, $C_{27}H_{36}N_2O_7Na$ requires 523.2415, 32%), 501 (M+$H^+$, 30%), 488 (100).

**3-(Carboxymethoxyimino)- 5α,17α-pregnane-16β,17β-diol *N*-hydroxysuccinimide ester (38).** Compound **34** (0.069 g, 0.17 mmol) was treated as per the general procedure. Precipitation of the resulting solid from diethyl ether/hexane gave **38** (0.081 g, 95%) as a 1:1 mixture of isomers. $R_f$ 0.8 (ethyl acetate); **mp** 88-90°C; $[\alpha]_D^{24}$ -16.0 (*c* 0.20, $CH_2Cl_2$); $\nu_{max}$/ **cm$^{-1}$** (film) 3600-3000 (OH), 2936, 1830, 1786, 1740 (C=O), 1205, 1076; $\delta_H$ (300 MHz, $CDCl_3$) 4.88 (4H, s, $OCH_2CO_2$), 3.81 (2H, dd, *J* 7.8, 6.2, H16), 3.26-3.14 (1H, m), 3.02-2.93 (1H, m), 2.84 (8H, s, $COCH_2CH_2CO$), 2.55-0.80 (44H, m), 0.92 (6H, t, *J* 7.2, $C_{21}$-$H_3$), 0.90 (6H, s, $CH_3$), 0.86 (6H, s, $CH_3$) 0.73-0.59 (2H, m); $\delta_C$ (75 MHz, $CDCl_3$) 168.7, 165.8, 163.1, 162.9, 80.5, 73.2, 67.9, 54.0, 53.9, 46.5, 45.8, 45.3, 38.2, 37.3, 36.2, 36.1, 35.5, 35.3, 34.0, 32.4, 31.7, 28.6, 28.4, 28.2, 27.4, 27.3, 25.6, 21.5, 20.7, 14.3, 11.5, 11.4, 7.2 (21C overlapping); **m/z** (ESI+) 563 (100%), 527.2729 (M+$Na^+$, $C_{27}H_{40}N_2O_7Na$ requires 527.2828, 55%), 505 (M+$H^+$, 30%), 492 (55).

**3-(Carboxymethoxyimino)-5α,17α-pregn-20-yn-17β-ol *N*-hydroxysuccinimide ester (39).** Compound **35** (0.036 g, 0.093 mmol) was treated as per the general procedure. Precipitation of the resulting solid from ethyl acetate/hexane gave **39** (0.042 g, 95%) as a 1:1 mixture of isomers. $R_f$ 0.8 (ethyl acetate); **mp** 170-172°C (decomp.); $[\alpha]_D^{23}$ -42.0 (*c* 0.20, $CHCl_3$); $\nu_{max}$/ **cm$^{-1}$** (film) 3600-3300 (OH), 3296 (≡C-H), 2922, 1828, 1784, 1740 (C=O), 1205, 1078; $\delta_H$ (300 MHz, $CDCl_3$) 4.88 (4H, s, $OCH_2CO_2$), 3.27-3.16 (1H, m), 3.02-2.93 (1H, m), 2.84 (8H, s, $COCH_2CH_2CO$), 2.57 (2H, s, ≡C-H), 2.34-0.85 (42H, m), 0.90 (6H, s, $CH_3$), 0.84 (6H, s, $CH_3$), 0.79-0.68 (2H, m); $\delta_C$ (75 MHz, $CDCl_3$) 168.7, 165.8, 163.1, 163.0, 87.5, 79.9, 73.94, 73.90, 67.9, 53.5, 53.4, 50.3, 46.8, 46.5, 45.3, 38.9, 38.3, 37.3, 36.1, 36.0, 34.0, 32.6, 31.3, 28.6, 28.4, 28.2, 27.4, 25.6, 23.1, 21.5, 20.8, 12.8, 11.5, 11.4 (20C overlapping); **m/z** (ESI+) 543 (70%), 507 (M+$Na^+$, 55), 485.2657 (M+$H^+$, $C_{27}H_{37}N_2O_6$ requires 485.2646, 100%).

**3-(Carboxymethoxyimino)-5α,17α-pregnan-17β-ol *N*-hydroxysuccinimide ester (40).** Compound **36** (0.048 g, 0.12 mmol) was treated as per the general procedure. Precipitation of the resulting solid from ethyl acetate/hexane gave **40** (0.058 g, 97%) as a 1:1 mixture of isomers. $\boldsymbol{R_f}$ 0.7 (ethyl acetate); **mp** 69-72°C; $\boldsymbol{[\alpha]_D^{23}}$ -8.0 (*c* 0.20, $CHCl_3$); $\boldsymbol{\nu_{max}}$**/ cm$^{-1}$** (film) 3600-3200 (OH), 2937, 1823, 1784, 1740 (C=O), 1205, 1078; $\boldsymbol{\delta_H}$ (300 MHz, $CDCl_3$) 4.88 (4H, s, $OCH_2CO_2$), 3.26-3.14 (1H, m), 3.02-2.93 (1H, m), 2.84 (8H, s, $COCH_2CH_2CO$), 2.35-0.78 (46H, m), 0.97 (6H, t, *J* 7.3, $C_{21}$-$H_3$), 0.90 (6H, s, $CH_3$), 0.87 (6H, s, $CH_3$), 0.71-0.59 (2H, m); $\boldsymbol{\delta_C}$ (75 MHz, $CDCl_3$) 168.7, 165.8, 163.1, 163.0, 83.5, 67.9, 53.9, 53.8, 50.4, 46.6, 46.4, 45.4, 38.3, 37.4, 36.3, 36.2, 36.1, 34.0, 33.6, 31.5, 28.8, 28.7, 28.5, 28.2, 27.5, 25.6, 23.6, 21.5, 20.8, 14.5, 11.5, 11.4, 7.8 (21C overlapping); **m/z** (ESI+) 547 (100%), 511 ($M+Na^+$, 25), 489.2969 ($M+H^+$, $C_{27}H_{41}N_2O_6$ requires 489.2959, 65%).

**Sodium 3β-(*tert*-butyldimethylsilyloxy)-16β,17β-dihydroxy-17α-methyl-5α-androstane 16-sulfate (47).** Diol **46** (0.061 g, 0.14 mmol) was dissolved in DMF (4.5 mL). Pyridine (0.225 mL, 0.220 g, 2.78 mmol) and 4Å molecular sieves (20) were added and the mixture was stirred overnight. Sulfur trioxide-pyridine complex (0.156 g, 0.98 mmol) was added and the reaction mixture was stirred for 50 minutes at 45ºC. TLC (ethyl acetate / hexane, 1/1) analysis showed an absence of starting material. The molecular sieves were removed and the reaction mixture was poured into saturated $NaHCO_3$ solution (50 mL), which was extracted with ethyl acetate (5 x 20 mL). The organic extracts were dried ($Na_2SO_4$) and concentrated to give a residue which was triturated with $Et_2O$ (2 mL) to give silyl protected 16-sulfate **47** (0.046 g, 61%). $\boldsymbol{R_f}$ 0.5 (MeOH / DCM, 1/4); **mp** 200-202°C; $\boldsymbol{[\alpha]_D^{22}}$ -55.2 (*c* 0.21, $CHCl_3$ / MeOH, 1/2); $\boldsymbol{\nu_{max}}$**/ cm$^{-1}$** (film) 3700-3200 (OH), 2928, 1377, 1252 (O-$SO_2$), 1090, 1011; $\boldsymbol{\delta_H}$ (400 MHz, $CDCl_3$ / MeOD, 1/1) 4.30 (1H, dd, *J* 8.2, 5.8, H16), 3.58-3.48 (1H, m, H3), 2.26-2.16 (1H, m), 1.69-0.78 (18H, m), 1.16 (3H, s, $CH_3$), 0.85 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, s, $CH_3$), 0.79 (3H, s, $CH_3$), 0.64-0.55 (1H, m), 0.03 (6H, s, $(CH_3)_2Si$); $\boldsymbol{\delta_C}$ (100 MHz, $CDCl_3$ / MeOD, 1/1) 84.4, 80.2, 73.0, 55.1, 47.8, 45.6, 39.0, 37.7, 36.3, 36.1, 33.7, 32.8, 32.5, 32.3, 29.1, 26.2, 24.4, 21.2, 18.7, 14.1, 12.6 (1C overlapping); **m/z** (ESI+) 561.2666 ($MNa^+$, $C_{26}H_{47}Na_2O_6SSi$ requires 561.2656, 100%).

**Sodium 3β,16β,17β-trihydroxy-17α-methyl-5α-androstane 16-sulfate (44).** Silyl protected 16-sulfate **47** (0.020 g, 0.037 mmol) was dissolved in acetic acid / $H_2O$ (4/1, 1.1 mL) and stirred for 1 hour. TLC (MeOH / DCM, 1/4) showed complete conversion to product ($R_f$ 0.2). The mixture was poured into saturated $NaHCO_3$ solution (60 mL) and extracted into ethyl acetate (3 x 20 mL) and then into $CHCl_3$/$^i$PrOH (3/1, 5 x 15 mL). The combined organic extracts were dried ($Na_2SO_4$) and concentrated *in vacuo*. The residue was dissolved in DCM/MeOH and pre-adsorbed onto silica for flash chromatography (DCM/MeOH/$H_2O$, 60/15/1), which afforded 16-sulfate **44** (0.011 g, 69%). $\boldsymbol{R_f}$ 0.4 (DCM/MeOH/$H_2O$, 60/15/1); **mp** 279-280 °C (decomp.); $\boldsymbol{[\alpha]_D^{23}}$ -28.6 (*c* 0.21, MeOH); $\boldsymbol{\nu_{max}}$**/ cm$^{-1}$** (film) 3700-3100 (OH), 2922, 1379, 1248 (O-$SO_2$); $\boldsymbol{\delta_H}$ (300 MHz, $CDCl_3$ / MeOD, 1/1) 4.30 (1H, dd, *J* 8.3, 5.6, H16), 3.56-3.42 (1H, m, H3), 2.28-2.15 (1H, m), 1.78-0.78 (18H, m), 1.16 (3H, s, $CH_3$), 0.83 (3H, s, $CH_3$), 0.80 (3H, s, $CH_3$), 0.66-0.55 (1H, m); $\boldsymbol{\delta_C}$ (100 MHz, $CDCl_3$ / MeOD, 1/1) 84.4, 80.2, 71.3, 55.1, 47.9, 45.6, 45.5, 38.2, 37.6, 36.4, 36.2, 33.8, 32.8, 32.5, 31.5, 29.2, 24.5, 21.2, 14.1, 12.6; **m/z** (ESI-) 401.1987 ($C_{20}H_{33}O_6S$ requires 401.1998, 100%).

## Notes and references


1 N.L. Hungerford, B. Sortais, C.G. Smart, A.R. McKinney, D.D. Ridley, A.M. Stenhouse, C.J. Suann, K.J. Munn, M.N. Sillence, M.D. McLeod, *J. Steroid Biochem. Mol. Biol.*, 2005, **96**, 317-34.

2 T. Takegoshi, *Chem. Pharm. Bull.*, 1972, **20**, 1260-71.

3 N.S. Leeds, D.K. Fukushima, T.F. Gallagher, *J. Am. Chem. Soc.,* 1954, **76**, 2943-48. (c) M. Numazawa, M. Shelangouski, M. Nakakoshi, *Steroids,* 2001, **66**, 743-48.

4 M. Salman, G.C. Chamness, *J. Med. Chem.*, 1991, **34**, 1019-24.