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SYNTHESIS OF LABELLED [$^{13}C_6$]TESTOSTERONE AND [$^{13}C_5$]19-NORTESTOSTERONE

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SUMMARY

The condensation of ethyl acetoacetate-$^{13}C_4$ and ethyl bromoacetate-$^{13}C_2$ afforded, in seven steps, (1,2,3,4,5-$^{13}C_5$) 5-(diethylphosphono)-2-pentanone ethylene ketal **9**. The reaction of this labelled compound with 7-[[(1,1-dimethylethyl)-dimethylsilyl]oxy]-1,6,6a,7,8, 9, 9a, 9b-octahydro-6a-methyl-[6aS-(6aa,7a,9aß,9ba)] cyclopenta[f][1]benzopyran-3 (2H)-one **13** gave the benzindenone **14** which was converted to (1,2,3,4,10,19-$^{13}C_6$)testosterone **17** then, into (1,2,3,4,10-$^{13}C_5$)19-nortestosterone **18** by a reductive alkylation method.

Key words : Testosterone, Nortestosterone, Steroid, Carbon-13, Diethylphosphono-pentanone ethylene ketal.

INTRODUCTION

The measurement of steroids at low concentrations in biological fluids by mass spectrometric methods is necessary for many applications. These are: medical analysis (determination of definitive method of dosage), anti-doping control and medical research (utilization as tracers for *in vivo* metabolic and kinetic studies). To realize these measurements, internal standards are required. The multilabelled carbon-13 standards seems to be the best for this purpose. The carbon-13 atom presents some advantages over deuterium: the stability of incorporation of carbon-13 is better than that of deuterium and the properties of the molecule are hardly altered (*in vitro* and *in vivo*) with carbon-13. In this paper we described the synthesis of two ^{13}C-labelled steroids: [1,2,3,4,10,19-$^{13}C_6$]testosterone and [1,2,3,4,10-$^{13}C_5$]19-nortestosterone. We have firstly synthesized a labelled synthon: 5-(diethylphosphono)-pentan-2-one ethylene ketal by an original synthetic pathway. DeGraw et al (1) reported the synthesis of this compound by an alternative route. Then, the key step was the reaction of 5-(diethylphosphono)-pentan-2-one ethylene ketal with the enol lactone **13** for the construction of the A-ring of steroids (2-4).

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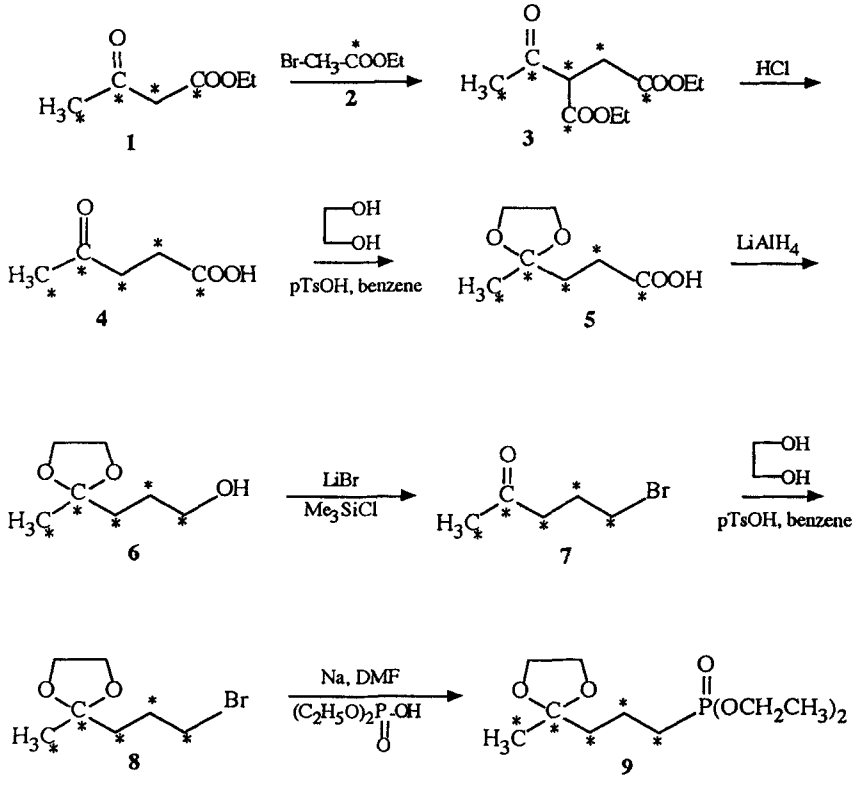
DISCUSSION

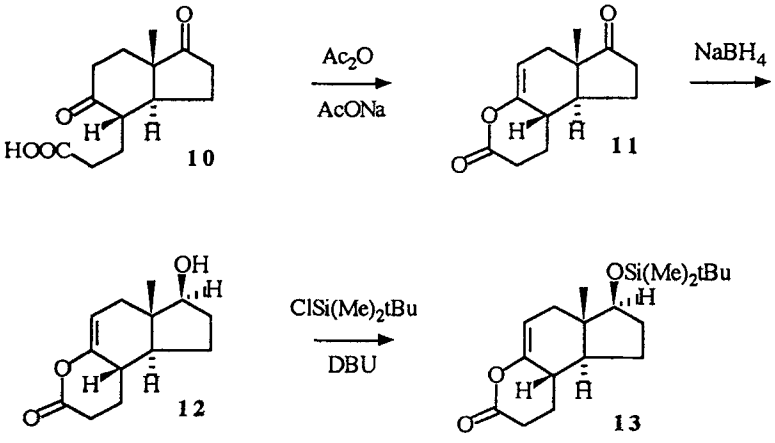
For the starting material, ($^{13}C_4$) ethyl acetoacetate and ($^{13}C_2$) ethyl bromoacetate were chosen. Condensation of (1,2,3,4-$^{13}C_4$) ethyl acetoacetate **1** with (1,2-$^{13}C_2$) ethyl bromoacetate **2** catalysed by sodium (5) afforded (1,2,3,4,1',2'-$^{13}C_6$) 1,4-diethyl-2-acetyl succinate **3**. The treatment of **3** with HCl gave (1,2,3,4,5-$^{13}C_5$) levulinic acid **4** in 77% yield. This acid was then converted to its ethylene ketal **5** by refluxing with ethylene glycol and p-toluenesulfonic acid in benzene with water separation. The acid function of **5** was reduced by $LiAlH_4$ in diethyl ether to afford (1,2,3,4,5-$^{13}C_5$) 1-hydroxypentan-4-one ethylene ketal **6**. Bromination of **6** with trimethylsilyl chloride and lithium bromide resulted in deprotection of the ketone function to yield (1,2,3,4,5-$^{13}C_5$)-5-bromopentan-2-one **7**. This ketone protection was required for the next condensation with enol lactone **13**. Therefore, the bromo ketone was converted to its ethylene ketal **8** by azeotropic distillation in benzene with ethylene glycol and p-toluenesulfonic acid. We think that the deprotection could be avoided by use of selenium bromide. Reaction of the bromo ketal **8** with sodium diethyl phosphite in DMF at 95°C afforded (1,2,3,4,5-$^{13}C_5$)-5-(diethylphosphono)-pentan-2-one ethylene ketal **9** (6). This compound is the main intermediate of our synthesis (Scheme 1).



Scheme 1: Preparation of (1,2,3,4,5-$^{13}C_5$) 5-(diethylphosphono)-pentan-2-one ethylene ketal **9**

Preparation of the enol lactone **13** was realized in three steps from 3-[(+)-3a,4,5,6,7,7a-hexahydro-7aß-methyl-1,5-dioxo-4-indanyl] propionic acid **10** (Scheme 2). Compound **10**, in the

presence of sodium acetate and acetic anhydride at 100°C, was converted to the enol lactone **11** (7). The lactone **11** was stereoselectively reduced by sodium borohydride to give **12**. The treatment of **12** by *t*-butyldimethylsylil chloride and DBU afforded **13**.



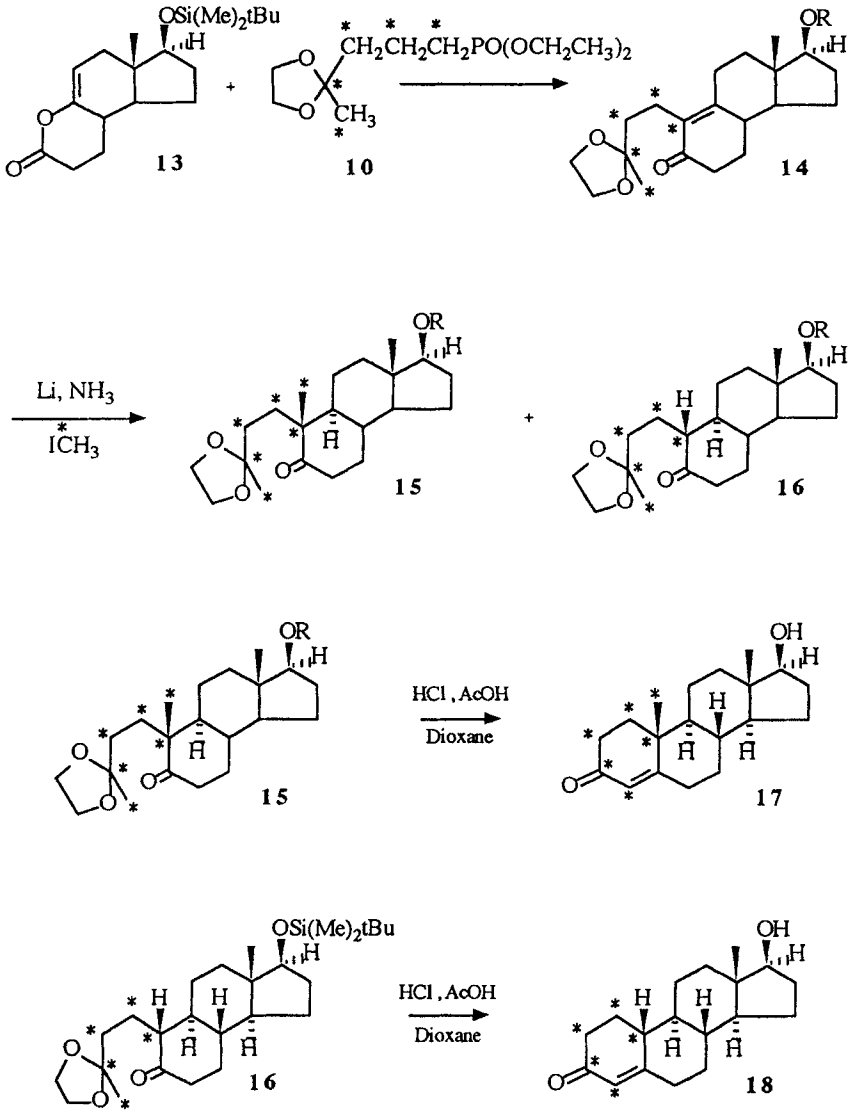
Scheme **2** : Preparation of the enol lactone **13**

The condensation of the labelled intermediate **9** with **13** (Scheme **3**) was the key step in the synthesis. Compound **13** was condensed with the anion of the ketal phosphonate (2,3) giving **14** in very low yield (8-9 %). Aristoff (4) described an improved method for the conversion of enol lactones to cyclic α,β-unsaturated ketones, but we were unable to improve the yield. However, nearly all the unreacted phosphonate reagent and nearly 40% of the keto acid corresponding to **13** were recovered.

From **14**, compound **15** having the methyl angular group at the C-10ß position was obtained by the reductive alkylation method described by Stork et al (8). The reduction of α,β-unsaturated ketone with lithium in liquid ammonia gave a nucleophilic β-carbon atom which can be alkylated by ($^{13}C_1$) methyl iodide (suitable electrophilic center). This alkylation-trapping method converts **14** into a single ß-isomer. Another compound **16** is also produced in this reaction. The last steps of this scheme are the deprotection of the alcohol and ketone functions and the ring cyclization. They were realized by treatment of **15** and **16** in acetic acid and HCl at room temperature to give (1,2,3,4,10, 19-$^{13}C_6$) testosterone **17** and (1,2,3,4,10-$^{13}C_5$)-19-nortestosterone **18**.

Experimental

IR spectra werre obtained on a Perkin-Elmer 1310 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 200 spectrometer using TMS as internal standard and $CDCl_3$ as solvent. Chemical shifts values are reported in parts per million (δ) downfield from tetramethyl silane. Coupling constants (J) are given in Hz. Analytical thin layer chromatography was carried out on Merck 0.25 mm precoated silica-gel plates (60 F-254) using various solvent systems with UV detection. The (1,2,3,4-$^{13}C_4$) ethyl acetoacetate and (1,2-$^{13}C_2$) ethyl bromoacetate were purchased from Isotec, France.



Scheme 3 : [$^{13}C_6$]Testosterone **17** and [$^{13}C_5$]-19-Nortestosterone **18**

(1,2,3,4,1',2'-$^{13}C_6$) 1,4-diethyl-2-acetyl succinate [**3**]. To a solution of (1,2,3,4-$^{13}C_4$) ethyl acetoacetate (99 atom % ^{13}C minima, 10 g, 74.63 mmol, 1 eq) in anhydrous toluene (200 ml), sodium metal (1.80 g, 78.36 mmol, 1.05 eq) was added gradually with stirring. This mixture was heated at 60°C to complete the dissolution of sodium (3-4 h). Then, (1,2-$^{13}C_2$) ethyl bromoacetate (99 atom % ^{13}C minimum, 14 g, 82.84 mmol, 1.1 eq) in 5 ml of toluene was added dropwise and the mixture heated at 90°C for 4 h. After cooling, the inorganic precipitate formed was filtered and washed with benzene. The combined filtrate was evaporated to give an oily residue of (1,2,3,4,1',2' -$^{13}C_6$) 1,4-diethyl-2-acetyl succinate (15.45 g).

(1,2,3,4,5-$^{13}C_5$) levulinic acid **4**. The residue **3** was heated with 7 % HCl acid (200 ml) at 90°C for 4 h. After cooling, the mixture was extracted with diethyl ether (5 x 200 ml). The etheral extract was evaporated to give (1,2,3,4,5-$^{13}C_5$) levulinic acid (7 g, 77 % from **1**) as a yellow oil. Its purity was determined on silica gel with chloroform-acetone-acetic acid (90/10/1 v/v) (R_F = 0.22). The product was used without any purification.

Unlabelled compound: Unlabelled runs averaged similar yield. IR (liquid film, cm^{-1}): 3700-2400, 1720 (C=O). ^1H NMR (δ ppm): 2.18 (s, 3H); 2.61 (m, 2H); 2.74 (m, 2H). ^{13}C NMR (50 MHz): 27.74 (CH_2); 29.76 (CH_3); 37.67 (CH_2); 178.19 (COO); 204.03 (CO).

(1,2,3,4,5-$^{13}C_5$) 4-oxo-pentanoic acid ethylene ketal **5**: A mixture of levulinic acid **4** (7 g, 57.79 mmol, 1 eq), ethylene glycol (3.9 ml, 69.35 mmol, 1.2 eq), 100 mg of *p*-toluene sulfonic acid and 150 ml of benzene was heated under reflux for 5 hours with a Dean Stark water separator. After cooling, the mixture was washed with 50 ml of a saturated solution of Na_2CO_3. The organic layer was washed with water (2 x 50 ml) and the solvent removed under reduced pressure. An amount of 6.53 g (68.4%) of (1,2,3,4,5-$^{13}C_5$)4-oxo-pentanoic acid ethylene ketal **5** was obtained as a yellow oil. The formation of the product was confirmed on TLC silica gel with chloroform-ethyl acetate (9/1) (R_F = 0.34) and it was used without any purification.

Unlabelled product: Unlabelled runs averaged similar yield. ^1H NMR (δ ppm): 1.28 (s, 3H); 2.00 (m, 2H); 2.33 (m, 2H); 3.89 (m, 4H). ^{13}C NMR (50 MHz): 23.84 (CH_3); 28.99 (CH_2); 37.84 (CH_2); 64.65 (($CH_2O)_2$); 109.04 (C); 173.49 (COOH).

(1,2,3,4,5-$^{13}C_5$) 4-oxo-pentan-1-ol ethylene ketal **6**: A solution of **5** (6.35 g, 39.57 mmol, 1 eq) in 75 ml of anhydrous diethyl ether was stirred at 0°C under a nitrogen atmosphere. $LiAlH_4$ (2.26 g, 59.36 mmol, 1,5 eq) was added in small portions and the mixture stirred at 0°C for 0.5 h. The residue was treated with a saturated solution of Na_2SO_4. The white emulsion was extracted with diethyl ether (5 x 50 ml). The organic layers were evaporated under reduced pressure to leave 3.04 g of (1,2,3,4,5-$^{13}C_5$)-4-oxo-pentan-1-ol ethylene ketal (50.8%) as a yellow oil. The TLC [chloroform-ethyl acetate (9/1) (R_F = 0.18)] of **6** was similar to that of unlabelled compound.

Unlabelled product: Unlabelled runs averaged similar yield. IR (liquid film, cm^{-1}): 3400 (OH); 2940 (CH_2). ^1H TLC (δ ppm): 1.28 (s, 3H); 1.67 (m, 4H); 3.57 (t, 2H); 3.90 (s, 4H). ^{13}C NMR (50 MHz): 23.56 (CH_3); 27.03 (CH_2); 35.58 (CH_2); 62.64 (CH_2OH); 64.46 (($CH_2O)_2$); 109.87 (C).

(1,2,3,4,5-$^{13}C_5$)-1-bromopentan-4-one **7**: To a solution of lithium bromide (3.49 g, 40.26 mmol, 2 eq) in 50 ml of dry acetonitrile at 0-5°C, was added dropwise trimethylsilyl chloride (6.39 ml, 50.33 mmol, 2.5 eq). To this yellow solution were added 3.04 g of **6** (20.13 mmol, 1 eq) and the mixture heated under reflux for 15 h. After cooling, the mixture was diluted with diethyl ether and washed with water to pH7. The etheral layer was evaporated to give 2.69 g (78%) of **7** as a dark solution.

Unlabelled product: Unlabelled runs averaged similar yield. IR (liquid film, cm^{-1}): 1720 (CO). ^1H NMR (δ ppm): 2.12 (t, 2H; J_1 = 6.7 Hz); 2.17 (s, 3H); 2.65 (t, 2H; J_1 = 6.9 Hz); 3.45 (t, 2H; J_1 = 6.6 Hz).

(1,2,3,4,5-$^{13}C_5$)-5-bromopentan-2-one ethylene ketal **8**. A mixture of **7** (2.69 g, 14 mmol, 1 eq), ethylene glycol (0.95 ml, 16.81 mmol, 1.2 eq) and 50 mg of p-toluenesulfonic acid in 40 ml

of benzene was heated under reflux for 5 h with a Dean-Stark water separator. After cooling to room temperature, 20 ml of saturated Na_2CO_3 solution were added to the mixture with stirring. The aqueous phase was extracted with diethyl ether (2 x 30 ml). The organic extracts were washed with water to pH7. Evaporation of benzene and ether afforded 2.59 g (96 %) of **8** as a brown oil. The product was used without any purification. The TLC [toluene-ethyl acetate (95/5) (R_F = 0.54)] of **8** was similar to that of unlabelled compound.

Unlabelled product: Unlabelled runs averaged similar yield. IR (liquid film, cm^{-1}): no CO band at 1700. ^1H NMR (δ ppm): 1.33 (s, 3H); 1.80 (m, 2H); 1.97 (m, 2H); 3.44 (t, 2H; J_1 = 6.5 Hz); 3.95 (m, 4H). ^{13}C NMR (50 MHz): 23.93 (CH_3); 27.48 (CH_2); 33.95 (CH_2Br); 37.56 ($\underline{C}H_2C$); 64.67 (($CH_2O)_2$); 109.52 (C).

(1,2,3,4,5-$^{13}C_5$)-5-(diethylphosphono)pentan-2-one ethylene ketal **9**: To a solution of diethylphosphite (1.22 g, 13.91 mmol, 1.15 eq) in 20 ml of dry diethyl ether was added 0.345 g (15.13 at/g, 1.25 eq) of small cuts of sodium. The mixture was stirred till consumption of the sodium. A solution of **8** (2.59 g, 12.10 mmol, 1 eq) in 15 ml of dry DMF was added dropwise. The mixture was heated first to distil off diethyl ether, then the temperature was raised to 95°C for 1 h. After cooling, the solvent was removed in vacuo. The brown mixture was extracted with diethyl ether followed by filtration to remove sodium bromide. Evaporation of the solvent afforded 2.73 g of a dark oil which was separated by chromatography using ethyl acetate-ethanol (96:4) to give 1.05 g (32%) of (1,2,3,4,5-$^{13}C_5$)-5-(diethylphosphono)-pentan-2-one ethylene ketal which was purified by TLC [ethyl acetate-ethanol (96/4) (R_F = 0.32)].

Unlabelled product: Unlabelled runs averaged similar yield. ^1H NMR (δ ppm): 1.30 (s, 3H; C-CH_3); 1.31 (t, 6H; O-CH_2-$\underline{C}H_3$; J_1 = 7 Hz); 1.72 (s, 6H; $(CH_3)_2$); 3.93 (m, 4H; $(CH_2O)_2$); 4.08 (dq, 4H; $(OC\underline{H}_2CH_3)_2$; J_1 = 7 Hz). ^{13}C NMR (50 MHz): 16.42 (d, $CH_2\underline{C}H_3$; J ^{13}C-^{13}C-^{16}O-^{31}P = 5.8 Hz); 17.12 (d, $CH_2\underline{C}H_2CH_2$; J ^{13}C-^{13}C-^{31}P = 4.4 Hz); 23.76 (s, CH_3); 25.72 (d, CH_2-P; J ^{13}C-^{31}P = 140 Hz); 39.73 (d, $\underline{C}H_2$-C; J ^{13}C-^{13}C-^{13}C-^{31}P = 16Hz); 61.36 (d, O-CH_2; J ^{13}C-^{16}O-^{31}P = 6.6 Hz); 64.61 (s, $(CH_2O)_2$); 109.74 (s, C-). MS (EI, 70 eV, 158°C), m/z (%): M^+ 267 (52); 252 (49.8); 236 (10.7); 221 (56.7); 206 (25.7); 193 (20.9); 165 (31); 151 (54.7); 125 (55.6); 109 (49.7); 105 (45.4); 98 (43.7); 89 (60.7); 87 (100); 77 (47.8); 67 (50.9); 55 (40.1) ; 43 (65.1).

Labelled product **9**: ^1H NMR (δ ppm): 1.26 (d, 3H; C-*CH_3; J ^1H-^{13}C = 126 Hz); 1.28 (t, 6H; CH_2-$\underline{C}H_3$; J_1 = 7 Hz); 1.68 (d, 6H; $(*CH_2)_3$; J ^1H-^{13}C = 124 Hz); 3.89 (m, 4H; $(C\underline{H}_2O)_2$); 4.04 (dq, 4H; $(OC\underline{H}_2CH_3)_2$; J_1 = 7 Hz). ^{13}C NMR (50 MHz): 17.06 (dt, $\underline{C}H_2CH_2P$; J ^{13}C-^{13}C = 34 Hz; J ^{13}C-^{13}C-^{31}P = 4 Hz); 23.69 (dd, CH_3; J ^{13}C-^{13}C = 46 Hz; J_2 ^{13}C-^{13}C = 6 Hz); 25.72 (ddd, CH_2P; J ^{13}C-^{13}C = 33 Hz; J_2 ^{13}C-^{13}C = 4 Hz; J ^{13}C-^{31}P = 140 Hz); 39.66 (m, C-$\underline{C}H_2$; J ^{13}C-^{13}C = 44 Hz; J_2 ^{13}C-^{13}C = 6 Hz; J ^{13}C-^{13}C-^{13}C-^{31}P = 16 Hz); 109.51 (dt , $\underline{C}$-CH_2; J ^{13}C-^{13}C = 45 Hz). MS (EI, 70 eV, 185°C), m/z (%): M^+ 272 (1.2); 255 (17.0); 241 (0.6); 226 (6.0); 211 (1.0); 198 (0.8); 182 (2.0); 167 (0.6); 154 (3.0); 133 (1.3); 126 (6.1); 109 (5.3); 98 (4.3); 89 (100); 81 (9.0) ; 72 (10.2); 45 (32.4).

1,6,6a,7,8,9,9a,9b-octahydro-6a-methyl-[6aS-(6aα,9aβ,9bα)] cyclopenta[f] [1] benzo pyran-3 (2H)-7-dione **11**: A mixture of 3-[(+)-3a,4,5,6,7,7a-hexahydro-7aß-methyl-1,5-dioxo-4-indanyl] propionic acid **10** (5 g, 21 mmol, 1 eq) and sodium acetate (2.58 g, 31.5 mmol, 1.5 eq) in 100 ml of acetic anhydride was heated under reflux for 4 h under an argon atmosphere. After cooling, acetic anhydride was removed under high vacuum. A solution of benzene and diethyl ether was added to the mixture which was washed with water (3 x 30 ml). After drying over sodium sulfate, the solvents were removed in vacuo to leave 4.3 g (93%) of **11**. This compound was used

without further purification [R_F (dichloromethane-ethyl acetate-ethanol: 5/1/1 = 0.7]. Mp = 132°C. IR (KBr 1%, cm^{-1}): 2900 (CH_2); 1720 (C=O) 1760; 1650 (C=C). ^1H NMR (δ ppm): 0.99 (s, 3H); 1.5-2.3 (m, 10H); 2.5-2.8 (m, 2H); 5.3 (m, 1H; C=C<u>H</u>). ^{13}C NMR (50 MHz): 14.44 (CH_3); 21.78 (CH_2); 23.06 (CH_2); 30.13 (CH_2); 30.32 (CH_2); 34.69 (CH); 36.04 (CH_2); 46.56 (C); 46.85 (CH); 105.10 (<u>C</u>H=C); 150.19 (CH=<u>C</u>); 167.41 (COO); 219.01 (CO). MS (EI, 70eV, 200°C), m/z (%): M^+ 220 (46); 205 (60); 191 (8); 177 (9); 161 (12); 149 (35); 134 (10); 121 (14); 109 (16); 95 (10); 91 (27); 79 (30); 67 (15); 55 (100); 41 (22); 27 (19).

7-hydroxy-1,6,6a,7,8,9,9a,9b-octahydro-6a-methyl-[6aS-(6aα,7α,9aβ,9bα)] cyclo penta [f] [1]benzopyran-3 (2H)-one **12**: To a solution of 4.3 g (19.5 mmol, 1 eq) of enol lactone **11** in 40 ml of anhydrous DMF at -10°C, was added under vigorous agitation, 1.48 g (39 mmol, 2 eq) of sodium borohydride in small portions. The temperature was allowed to arise to 0°C in 15 min and 30 ml of saturated sodium chloride solution was slowly added to the mixture. The reaction product was extracted with chloroform (3 x 50 ml), and the organic layer dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. A slightly yellow oil was obtained and purified by chromatography on silica gel using dichloromethane-ethyl acetate (5:1) to give 2.36 g (55%) of a pure white solid (R_F = 0,3). Mp = 92°C.

IR (KBr 2%; cm^{-1}): 3600-3200 (OH); 2960-2860 (CH_2); 1750 (COO); 1660 (C=C). ^1H NMR (δ ppm): 0.82 (s, 3H); 1.3-2.3 (m, 10H); 2.45-2.85 (m, 2H); 3.80 (t, 1H: C-17); J_1 = 8Hz); 5.29 (m, 1H; C=CH). ^{13}C NMR (50 MHz): 10.86 (CH_3); 23.23 (CH_2); 23.47 (CH_2); 30.53 (CH_2); 30.90 (CH_2); 35.03 (CH_2); 35.32 (CH); 42.34 (C); 46.77 (CH); 80.89 (CH: C-17); 105.45 (<u>C</u>H=C: C-11); 150.42 (CH=<u>C</u>: C-9); 167.89 (COO: C-5). MS (EI, 70 eV, 200°C), m/z (%): M^+ 222 (67); 207 (2); 189 (8); 178 (13); 163 (30); 147 (16); 133 (17); 121 (24); 107 (18); 93 (34); 79 (37); 67 (26); 55 (100); 41 (37); 27 (23).

7-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1,6,6a,7,8,9,9a,9b-octahydro-6a-methyl-[6aS-(6aα,7α,9aβ,9bα)]cyclopenta[f][1]benzopyran-3(2H)-one **13**: To a solution of **12** (2.36, 10.6 mmol, 1 eq) in 50 ml of anhydrous dichloromethane, was added dropwise, under an inert atmosphere, 8 g (53 mmol, 5 eq) of *t*-butyl dimethylsilyl chloride and 4.85 g (32 mmol, 3 eq) of DBU. The mixture was stirred for 2 days at room temperature, then it was washed with $AlCl_3$ (3 x 20 ml), $NaHCO_3$ (3 x 20 ml) and NaCl (2 x 20 ml) in saturated solutions. The extract was dried over Na_2SO_4 and the solvent was evaporated. The product was purified by silica gel chromatography using hexane-ethyl acetate (4:1) to give 2.56 g (72 %) of pure **13** [R_F = 0.88].

IR (liquid film, cm^{-1}): 2940-2840 (CH_2); 1740 (C=O); 1660 (C=C). ^1H NMR (δ ppm): 0.01 (s, 6H; $(CH_3)_2Si$); 0.82 (s, 3H; C-18); 0.88 (s, 9H; *t*-Bu); 3.70 (t, 1H; C-17α; J_1 = 8 Hz); 5.28 (m, 1H; C-11). ^{13}C NMR (50 MHz): - 4.89 (CH_3Si); - 4.52 (CH_3Si); 11.19 (CH_3: C-18); 18.02 (C: *t*-Bu); 23.40 (CH_2); 23.49 (CH_2); 25.77 ((CH_3)$_3$; *t*-Bu); 30.58 (CH_2); 31.22 (CH_2); 35.38 (CH_2); 35.45 (CH); 42.71 (C: C-13); 46.38 (CH: C-14); 80.83 (CH: C-17); 105.67 (<u>C</u>H=C: C-11); 150.53 (CH=<u>C</u>: C-9); 168.01 (COO: C-5). MS (EI, 70 eV, 200°C), m/z (%): M^+ 336 (9); 321 (1); 279 (62); 261 (3); 237 (15); 219 (4); 203 (12); 161 (17); 141 (27); 91 (17); 81 (34); 75 (100); 55 (58); 41 (16).

(1',2',3',4',10-$^{13}C_5$) Des-A-17β-t-butyldimethylsilyloxy-10-(3',3'-dioxyethylene butyl)-9-estren-5-one **14** (Chem. Abstr. nomination: *3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1,2,3,3a,4,5, 8,9,9a,9b-decahydro-3a-methyl-6-[2-(2-methyl-1,3-dioxolan-2-yl)ethyl]-[3S-(3a,3aa,9aa,9bβ)]-7H -benz[e]inden-7-one*): A solution of **9** (1.05 g, 3.97 mmol, 1 eq) in 50 ml of

dry THF was chilled at -78°C in a three-necked flask fitted with a dropping funnel, a distillation head and a thermometer. Then, 1.6 M butyl lithium (7.25 ml, 11.62 mmol, 3 eq) was added dropwise and the mixture was stirred for 1 h. To this mixture, a solution of **13** (1.04 g, 3.09 mmol, 0.8 eq) in 5 ml of THF was added dropwise. The mixture was stirred at -78°C for 4 h and the temperature was slowly heated to 20°C. After a contact of 12 h, the solvent was removed and 30 ml of ethyl acetate was added. HCl was added to pH7 and the mixture was extracted with 30 ml of ethyl acetate. Evaporation of the solvent afforded a yellow oil which was purified by chromatography using hexane-ethyl acetate (4:2) to give 145 mg (8.27 %) of pure **14**. Unreacted compounds **9** and **13** were recovered.

Unlabelled compound: ^{1}H NMR (δ ppm): 0.01 (s, 6H; $(CH_3)_2Si$); 0.87 (s, 3H; CH_3); 0.89 (s, 9H; $(CH_3)_3$); 1.36 (s, 3H; CH_3: C-4); 3.59 (t, 1H; CH: C-17α; J_1 = 8 Hz); 3.94 (m, 4H; $(CH_2O)_2$).^{13}C NMR (50 MHz): - 4,83 (CH_3Si); - 4,48 (CH_3Si); 10.65 (CH_3: C-18); 18.08 (C-*t*-Bu); 20.14 (CH_2: C-1); 23.49 (CH_3: C-4); 23.65 (CH_2); 25.82 (*t*-Bu); 26.63 (CH_2); 26.83 (CH_2); 30.94 (CH_2); 36.54 (CH_2); 37.08 (CH_2); 38.10 (CH_2); 39.05 (CH: C-8); 42.64 (C: C-13); 50.51 (CH: C-14 ; 64.60 ($(CH_2O)_2$); 81.18 (CH: C-17); 109.82 (C: C-3); 134.11 (C=C: C-10); 159.25 (C=C: C-9); 198.49 (C=O: C-5). MS (EI, 70eV, 200°C), m/z (%): M^+ 448 (11); 433 (1.7); 404 (3.9); 388 (2.3); 359 (1.4); 347 (3.6); 329 (1.6); 289 (4); 273 (4.2); 255 (2.5); 229 (1.2); 213 (7.4); 197 (3.2); 185 (2.7); 171 (4.2); 159 (4.5); 145 (4.2); 131 (2.6); 115 (2.7); 105 (1.8); 87 (100); 73 (19); 59 (5); 43 (19).

Labelled compound: ^{1}H NMR (δ ppm): 0.008 (s, 6H; $(CH_3)_2Si$); 0.86 (s, 3H; CH_3: C-18); 0.88 (s, 9H, *t*-Bu); 1.35 (m, 3H; *CH_3: C-4; $J\ ^{1}H\text{-}^{13}C$ = 126 Hz); 3.58 (t, 1H; CH: C-17α; J_1 = 8 Hz); 3.93 (m, 4H; $(CH_2O)_2$). ^{13}C NMR (50 MHz): 20.06 (dd; CH_2: C-1; $J\ ^{13}C\text{-}^{13}C$ = 34 Hz); 23.45 (dd, CH_3: C-4; $J\ ^{13}C\text{-}^{13}C$ = 46 Hz; $J_2\ ^{13}C\text{-}^{13}C$ = 6Hz); 38.04 (ddd or m; CH_2: C-2; $J\ ^{13}C\text{-}^{13}C$ = 41 Hz; $J_2\ ^{13}C\text{-}^{13}C$ = 6 Hz); 109.76 (dt, C: C-3; $J\ ^{13}C\text{-}^{13}C$ = 46 Hz; $J_2\ ^{13}C\text{-}^{13}C$ = 4 Hz); 134.11 (dd, C=C: C-10; $J\ ^{13}C\text{-}^{13}C$ = 47 Hz; $J_2\ ^{13}C\text{-}^{13}C$ = 3,4 Hz). MS (EI, 70 eV, 185°C), m/z (%): M^+ 453 (5); 437 (0.7); 410 (0.3); 393 (1); 362 (0.6); 352 (0.3); 334 (0.9); 308 (0.5); 291 (1.2); 278 (2.6); 260 (1.3); 232 (0.6); 215 (3); 197 (0.8); 190 (0.8); 175 (0.7); 149 (0.6); 131 (1.2); 119 (1.7); 101 (1.2); 89 (100); 73 (16); 57 (5); 45 (11).

(1',2',3',4',10,19-$^{13}C_6$)Des-A-17β-t-butyl dimethylsilyloxy-10-(3',3'-dioxyethylene butyl) androstan-5-one **15** and *(1',2',3',4',10-$^{13}C_5$)Des-A-17β-t-butyldimethylsilyloxy-10-(3',3'-dioxy ethylene butyl) estran-5-one* **16**: In a three necked flask fitted with a dropping funnel, a condenser and a delivery pipe of ammoniac, were stirred 30 ml of ammoniac (evolved from the pipe and condensed on a solid carbon dioxide condenser) and lithium (55.5 mg, 8.0 mmol, 25 eq) at -70°C. After 20 min, a solution of **14** (145 mg, 0.32 mmol, 1 eq) in 5 ml of dry diethyl ether was added dropwise and the mixture stirred at -70°C for 3 h. The temperature was allowed to rise to 20°C and the ammoniac was evolved. The residue was treated with 10% HCl to pH7 and extracted with chloroform (5 x 20 ml). After evaporation, the residue was chromatographed on silica gel using hexane-ethyl acetate (4:2) to give 39 mg (23%) of (1',2',3',4',10,19-$^{13}C_6$)Des-A-17ß-*t*-butyl di methylsilyloxy-10-(3',3'-dioxyethylene butyl)androstan-5-one **15** [R_F (hexane-ethyl acetate 8:2) = 0.35], 34 mg (23%) of (1',2',3',4',10-$^{13}C_5$) Des-A-17ß-*t*-butyl dimethylsilyloxy-10-(3',3'-dioxyethylene butyl)estran-5-one **16** [R_F (hexane:ethyl acetate 8:2) = 0.31] and 32 mg (22 %) of recovered starting material.

Unlabelled compound: ^{1}H NMR (δ ppm): 0.008 (s, 6H; $(CH_3)_2Si$); 0.77 (s, 3H); 0.88 (s, 9H; *t*-Bu); 1.10 (s, 3H); 1.36 (s, 3H); 2.25 (m, 1H); 2.52 (m, 1H); 3.57 (t, 1H: C-17α; J_1 = 7 Hz); 3.94 (d, 4H). ^{13}C NMR (50 MHz): - 4.84 (CH_3Si); - 4,50 (CH_3Si); 11.27 (CH_3: C-18); 18.11 (C: *t*-Bu); 20.93 (CH_2: C-1); 21.09 (CH_3: C-19); 23.37

(CH_3: C-4); 23.52 (CH_2); 25.80 ((CH_3)3: *t*-Bu); 29.02 (CH_2); 29.64 (CH_2); 30.73 (CH_2); 33.04 (CH_2); 34.89 (CH: C-8); 36.65 (CH_2); 38.07 (CH_2); 43.30 (C: C-13); 47.56 (CH: C-14); 50.11 (CH: C-9); 50.57 (C: C-10); 64.40 (CH_2O); 64.47 (CH_2O); 81.48 (CH: C-17); 110.28 (C: C-3); 214.72 (C=O: C-5). MS (EI, 70eV, 185°C), m/z (%): M^+ 464 (0.2); 449 (1); 407 (1.8); 393 (0.2); 377 (0.4); 350 (0.3); 289 (0.7); 269 (2.7); 253 (1.8); 241 (0.4); 231 (0.8); 213 (2.4); 197 (1); 187 (0.7); 173 (1); 159 (0.6); 115 (12); 99 (11); 87 (100); 75 (23); 55 (5.7); 43 (15).
Unlabelled compound: ^{1}H NMR (δ ppm): 0.006 (s, 3H; CH_3Si); 0.009 (s, 3H; CH_3Si); 0.78 (s, 3H; CH_3: C-18); 0.88 (s, 9H; *t*-Bu); 1.33 (s, 3H; CH_3: C-4); 3.56 (t, 1H; C-17α); 3.93 (m, 4H; $(CH_2O)_2$). ^{13}C NMR (50 MHz): 11.39 (CH_3: C-18); 18.08 (C: *t*-Bu); 19.95 (CH_2: C-1); 23.43 (CH_3: C-4); 23.56 (CH_2); 25.83 (CH_3)3: *t*-Bu); 27.16 (CH_2); 30.77 (CH_2); 31.34 (CH_2); 35.44 (CH_2); 36.78 (CH_2); 40.74 (CH: C-8); 41.69 (CH_2); 43.47 (C: C-13); 48.43 (CH: C-14); 49.33 (CH: C-9); 54.52 (CH: C-10); 64.51 ((CH_2O)2); 81.52 (CH: C-17); 110.20 (C: C-3); 212.06 (C: C-5). MS (EI, 70 eV, 185°C), m/z (%): M^+ 450 (0.6); 435 (2.8); 393 (8.7); 363 (0.4); 349 (0.3); 331 (1.2); 317 (1.1); 299 (2.2); 291 (3.7); 275 (6.2); 255 (5.7); 239 (2.8); 227 (1); 215 (2.4); 199 (5.6); 183 (1.6); 171 (1.8); 159 (1.8); 145 (3.7); 131 (4.7); 115 (22); 99 (23); 87 (100); 75 (45); 55 (11); 43 (26).

(1,2,3,4,10,19-$^{13}C_6$)testosterone **17**: A mixture of **15** (39 mg, 0.083 mmol) and 0.28 ml (1.66 mmol, 20 eq) of 6N HCl in 5 ml of dioxane was heated under reflux for 5 h. After cooling, the mixture was neutralized with a saturated $NaHCO_3$ solution and extracted with chloroform. The solvent was removed under reduced pressure and the product was purified by chromatography on preparative TLC plate using benzene-acetone (8:2) to leave 19 mg (77.86 %) of pure (1,2,3,4,10,19-$^{13}C_6$) testosterone. [R_F (benzene:acetone / 8:2) = 0.35].
Unlabelled compound: Mp = 148°C. $[\alpha]_D^{24}$ = + 104 (c = 0.4 alc.). ^{1}H NMR (δ ppm): 0.78 (s, 3H: C-18); 1.18 (s, 3H: C-19); 3.64 (t, 1H: C-17α; J_1 = 8 Hz); 5.71 (d, 1H: C-4). ^{13}C NMR (50 MHz): 11.02 (CH_3: C-18); 17.39 (CH_3: C-19); 20.63 (CH_2: C-11); 23.31 (CH_2: C-15); 30.42 (CH_2: C-16); 31.52 (CH_2: C-7); 32.76 (CH_2: C-6); 33.91 (CH_2: C-2); 35.68 (CH_2: C-8); 35.71 (CH_2: C-1); 36.41 (CH_2: C-12); 38.64 (C: C-10); 42.80 (C: C-13); 50.48 (CH: C-14); 53,90 (CH: C-9); 81.58 (CH: C-17); 123.83 (CH=C: C-4); 171.17 (CH=C: C-5); 199.46 (C=O: C-3). MS (EI, 70 eV, 185°C), m/z (%): M^+ 288 (96); 273 (9); 270 (7); 246 (57); 228 (17); 203 (24); 185 (8); 165 (5); 147 (13); 133 (19); 124 (95); 109 (36); 91 (100); 79 (80); 67 (49); 55 (46); 41 (45).
Labelled compound: ^{1}H NMR (δ ppm): 0.80 (s, 3H: C-18); 3,66 (t, 1H: C-17α); 5.73 (d, 1H: *C-4; J ^{1}H-^{13}C = 160 Hz). ^{13}C NMR (50 MHz): 17.42 (d, CH_3: C-19; J ^{13}C-^{13}C = 34 Hz); 33.81 (ddd, CH_2: C-2; J_1 ^{13}C-^{13}C = 38 Hz; J_2 ^{13}C-^{13}C = 11 Hz); 35.78 (t, CH_2: C-1; J ^{13}C-^{13}C = 32 Hz); 38.66 (dd, C: C-10; J_1 ^{13}C-^{13}C = 33 Hz; J_2 ^{13}C-^{13}C = 3 Hz); 123.88 (dd, CH: C-4; J_1 ^{13}C-^{13}C = 52 Hz; J_2 ^{13}C-^{13}C = 11 Hz); 199.41 (dd, C=O: C-3; J_1 ^{13}C-^{13}C = 52 Hz; J_2 ^{13}C-^{13}C = 36 Hz). MS (EI, 70 eV), m/z (%): M^+ 294 (73); 276 (7); 250 (47); 232 (15); 204 (23); 165 (15); 147 (31); 143 (10); 133 (10); 130 (100); 93 (23); 81 (25); 69 (25); 55 (31); 41 (40).

(1,2,3,4,10-$^{13}C_5$)-19-nortestosterone **18**: A mixture of **16** (34 mg, 0.075 mmol) and 0.25 ml (1,49 mmol, 20 eq) of 6N HCl in 5 ml of dioxane was heated under reflux for 5 h. After cooling, the mixture was neutralized with a saturated Na_2CO_3 solution and extracted with chloroform. The solvent was removed under reduced pressure and the product was purified by chromatography on preparative TLC using benzene-acetone (8:2) to leave 19 mg (77.86 %) of pure (1,2,3,4,10-$^{13}C_5$)-19-nortestosteron [R_F benzene:acetone (8:2) = 0.31].
Labelled compound: ^{1}H NMR (δ ppm): 0.80 (s, 3H; C-18); 0.93-2.65 (m, 21H); 3.66 (t, 1H; C-17α); 5.81 (d, 1H; *C-4; J ^{1}H-^{13}C = 161 Hz). ^{13}C NMR (50 MHz): 26.50 (t, CH_2: C-1; J ^{13}C-^{13}C = 33 Hz); 36.42 (ddd,

CH_2; C-2; J_1 ^{13}C-^{13}C = 40 Hz; J_2 ^{13}C-^{13}C = 32 Hz; J_3 ^{13}C-^{13}C = 12 Hz); 42.57 (d, CH; C-10; J ^{13}C-^{13}C = 35 Hz); 124.50 (dd, C=CH; C-4; J_1 ^{13}C-^{13}C = 52 Hz; J_2 ^{13}C-^{13}C = 12 Hz); 200.03 (dd, C=O; C-3; J_1 ^{13}C-^{13}C = 52 Hz; J_2 ^{13}C-^{13}C = 39 Hz). MS (EI, 70 eV), m/z (%): M^+ 279 (98.8); 261 (18.8); 251 (74.7); 235 (17.3); 221 (24.6); 153 (44.9); 149 (48.8); 136 (49.9); 123 (58.5); 115 (71.7); 107 (80.1); 93 (83.0); 87 (100); 81 (85.5); 67 (69.7); 55 (66.1); 47 (68.1); 43 (89.2).

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