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Synthesis of new C-25 and C-26 steroidal acids as potential ligands of the nuclear receptors DAF-12, LXR and GR

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ABSTRACT

A new methodology to obtain C-25 and C-26 steroidal acids starting from pregnenolone is described. Construction of the side chain was achieved by applying the Mukaiyama aldol reaction with a non-hydrolytic work-up to isolate the trapped silyl enol ether with higher yields. Using this methodology we synthesized three new steroidal acids as potential ligands of DAF-12, Liver X and Glucocorticoid nuclear receptors and studied their activity in reporter gene assays. Our results show that replacement of the 21-CH_3 by a 20-keto group in the side chains of the cholestane scaffold of DAF-12 or Liver X receptors ligands causes the loss of the activity.

1. Introduction

Life span of nematodes such as *Caenorhabditis elegans* is controlled by a family of C-27 steroidal acids, called dafachronic acids (DAs), **1** and **2** being the most active and abundant (Fig. 1) [1,2]. These compounds inactivate the nuclear hormone receptor DAF-12 and thus, lead to reproductive development of worms. In *daf9* mutant worms, incapable of DAs biosynthesis, DAF-12 is active constitutively and worms enter the diapause state which is specially adapted for long-term survival. Liver X receptors (LXRα and LXRβ) were identified as one of the human nuclear receptor (NHRs) with the highest protein sequence similarity to *ce*DAF-12 and recently LXRα has been proposed as a candidate target to affect human life span [3]. Interestingly, cholestenoic acids 25S (**3a**) and 25R (**3b**), closely related to DAs, bind to *ce*DAF-12 receptor and LXR respectively [4,5]. The fact that similar cholesterol metabolites are able to activate such evolutionary related receptors suggests that a conserved ligand-receptor mechanism may be considered to investigate the structure-activity relationship in both receptors. Although the configuration of C-25 of DAs is an important determinant on the activity of the ligand-DAF-12 receptor complex, 25S-DAs being more active than 25R diastereomers [6], we have recently shown that removal of the C-25 methyl does not impede the strong interaction of the 26-carboxylate moiety with the *ce*DAF-12 receptor binding site [7,8]. Thus, we found that the 27-nor-Δ^4-dafachronic acid **4** induces response of GAL4-LUC reporter by the chimera pGAL4-DAF12 on HEK-293T cells. Besides, its activity was evaluated *in vivo* where it rescued the Mig phenotype of *daf-9(rh50) C. elegans* mutants. Based on the homology between the LXR and DAF-12 receptors, we have recently evaluated the activity of the 27-nor-steroid **5** as a ligand of the LXR [9]. The results obtained revealed that compound **5** behaves as a dual LXRα/β inverse agonist. This steroid strongly reduced *per se* basal levels of luciferase activity at 10 μM in HEK-293T cells co-transfected with CMX-hLXRα or pSG5-hLXRβ, and pLRE-LUC reporter gene. Furthermore, it downregulates the basal expression of FASN and SREBP-1a/c in HepG2 cells. Willson et al. [10] investigated the structural requirements for activation of the LXR using a set of oxysterols which included all the potential regio- and stereoisomers between C22 and C26, at end of the sterol side chain. They proposed a model in agreement with the activities observed that could explain how small changes, such as the inversion of the C-22 configuration, may flip a full agonist into an antagonist.

The results mentioned above prompted us to evaluate how the replacement of the 21-methyl group for a carbonyl group on steroids **4** and **5** would affect their activity profile. Thus, we designed and prepared the 27-nor-steroids **7** and **8** (see Fig. 2) which are 20-keto analogues of **4** and **5** and examined their biological activity

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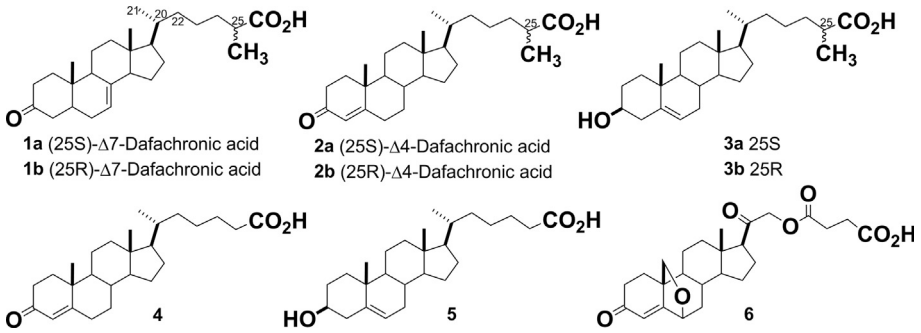


Fig. 1. Structures of compounds **1**–**6**.

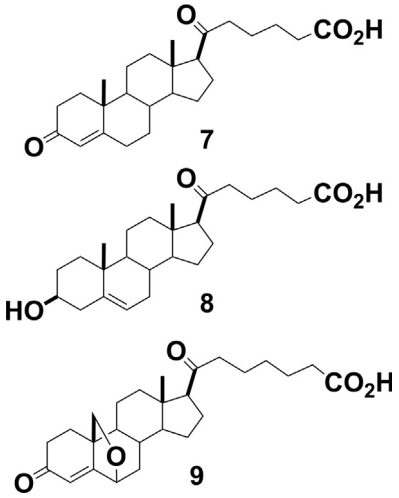


Fig. 2. Structures of compounds **7** and **8**, 20-keto analogues of bioactive steroids **4** and **5** respectively and compound **9**, a non-hydrolysable analogue of **6**.

as potential ligands of the DAF-12 and LXR receptors respectively. On this purpose we have developed a new methodology for the preparation of steroidal acids based on the catalysed Mukaiyama aldol addition, using BF_3-EtOEt as Lewis acid. Moreover, we extended the application of this methodology to the synthesis of the highly constrained steroid **9**, which is a non-hydrolyzable analogue of the hemisuccinate **6**, a selective modulator of the glucocorticoid receptor (GR) previously developed in our group [11], and evaluated its biological activity.

2. Experimental

2.1. General

Optical rotations were measured on a PerkinElmer 343 polarimeter (λ = 589 nm, T = 20 °C) using a 1 ml Micro cell, light path 100 mm. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna 550 FT-IR spectrophotometer, values are given in cm^{-1}. NMR spectra were recorded on Bruker AC-200 (^{1}H at 200.13 MHz, ^{13}C at 50.32 MHz) or Avance II 500 (^{1}H at 500.13 MHz, ^{13}C at 125.77 MHz) spectrometers. Chemical shifts are given in ppm downfield from TMS as internal standard, *J* values are given in Hz. Multiplicity determinations and 2D spectra (COSY, NOESY, HSQC and HMBC) were obtained using standard Bruker software. Exact mass spectra were obtained using a Bruker micrO-TOF-Q II mass spectrometer, equipped with an ESI source operating in positive mode. Flash column chromatography was carried out on silica gel S 0.040–0.063 mm. Thin layer chromatography (TLC) analysis was performed on silica gel 60 F254 (0.2 mm thick). The homogeneity of all compounds was confirmed by TLC. Solvents were evaporated at reduced pressure and *ca.* 40–50 °C. Compound **11** was synthesized from pregnenolone (**10**, Steraloids Inc., USA) as previously described [12]. Compound **18** was prepared as previously described by us [13]. The C-4 and C-5 aldehydes methyl 4-oxobutanoate (**13**) and methyl 5-oxopentanoate (**21**) were synthesized from butyrolactone and valerolactone following the procedure described by Duffy et al. [14].

2.2. Chemistry

2.2.1. 6-(3β-t-Butyldimethylsilyloxy-androst-5-ene-17β-il)-6-oxo-4-hydroxymethylhexanoate (**14**)

To a solution of compound **11** (800 mg, 1.9 mmol) in anhydrous THF (25 ml), LDA in THF (4.5 ml, 2 M, 9.0 mmol, Sigma) was added and the resulting mixture was stirred at −78 °C, under argon atmosphere. After 20 minutes chlorotrimethylsilane (0.94 μl, 7.5 mmol) was added and the mixture was allowed to warm to room temperature·THF was removed by a nitrogen stream, anhydrous chloroform (40 ml) was added and the salts were filtered out through a sintered-glass funnel under argon atmosphere, to give a solution of the silyl enol ether **12**. An analytical sample was taken and characterized. RMN: ^{1}H NMR (500.13 MHz): 5.32 (1H, dt, *J* = 1.5 and 5.3 Hz, H-6), 4.08 (1H, d, *J* = 0.7 Hz, H-21a), 4.04 (1H, bs, H-21b), 3.48 (1H, m, H-3), 2.27 (1H, m, H-4β), 2.17 (1H, ddd, *J* = 13.2, 4.9 and 2.1, H-4α), 2.01 (1H, m, H-17), 1.99 (1H, m, H-7β), 1.97 (1H, m, H-12β), 1.81 (1H, dt, *J* = 13.2 and 3.5 Hz, H-1β), 1.72 (1H, m, H-2β), 1.69 (3H, m, H-16 and H-15β), 1.55 (1H, m, H-7), 1.54 (1H, m, H-11α), 1.53 (1H, m, H-2β), 1.45 (1H, m, H-8), 1.44 (1H, m, H-12α), 1.19 (1H, m, H-15α), 1.14 (1H, m, H-12α), 1.04 (1H, m, H-1α), 1.03 (1H, m, H-14), 1.00 (3H, s H-19), 0.93 (1H, m, H-9), 0.89 (9H, s, 3-$(CH_3)_3$Si), 0.62 (3H, s, H-18), 0.06 (6H, s, 3-$(CH_3)_2$Si); ^{13}C NMR (125.77 MHz): 160.1 (C-20), 141.7 (C-5), 121.1 (C-6), 89.6 (C-21), 72.6 (C-3), 56.3 (C-17), 56.2 (C-14), 50.4 (C-9), 43.0 (C-13), 42.8 (C-4), 38.6 (C-12), 37.4 (C-1), 36.7 (C-10), 32.2 (C-8), 32.1 (C-2), 31.9 (C-7), 25.9 (($\mathbf{C}H_3)_3$CSi), 24.6 (C-15), 24.4 (C-16), 21.1 (C-11), 19.5 (C-19), 18.3 (**C**-Si), 12.7 (C-18), -4.6 (**C**H_3CSi).

Aldehyde **13** (4.6 mmol) and borom trifluoride etherate (0.51 ml, 4.1 mmol) were added successively to the solution of **12** in chloroform obtained above. The reaction mixture was stirred at −78 °C, under an argon atmosphere for 30 min, neutralized with a saturated solution of sodium bicarbonate and extracted with dichloromethane. The organic layer was washed with water and dried with sodium sulphate. Evaporation of the solvent followed by flash chromatography (ethyl acetate–hexane 5:95) gave steroid **14** as a mixture of epimers (7:3) at C-22 (620 mg, 61%). From the flash chromatografhy an analitical sample of the major epimer was obtained and characterized: $[\alpha]_D^{20}$ = +26,5 (c = 0,7, methanol); IR (KBr) υ_{max}: 3466, 2935, 2859, 1731, 1704, 1645, 1381,

1256 cm^{-1}; ^1H NMR (500.13 MHz): 5.31 (1H, bs, H-6), 4.05 (1H, m, H-22), 3.68 (3H, s, 25-$COOCH_3$), 3.48 (1H, m, H-3), 2.57 (1H, dd, *J* = 17.7 and 2.8 Hz, H-21a), 2.52 (1H, t, *J* = 8.3 Hz, H-17), 2.51 (1H, m, H-21b), 2.50 (2H, m, H-24), 2.27 (1H, m, H-4β), 2.18 (2H, m, H-4α and H-16α), 2.04 (1H, m, H-12β), 2.00 (1H, m, H-7β), 1.81 (1H, m, H-1β), 1.77 (2H, m, H-23), 1.74 (1H, m, H-2α), 1.70 (1H, m, H-15α), 1.66 (1H, m, H-16β), 1.62 (1H, m, H-11α), 1.57 (1H, m, H-7α), 1.54 (1H, m, H-2β), 1.48 (1H, m, H-8), 1.46 (1H, m, H-11β), 1.42 (1H, m, H-12α), 1.25 (1H, m, H-15β), 1.15 (1H, m, H-14), 1.06 (1H, m, H-1α), 1.00 (3H, s H-19), 0.97 (1H, m, H-9), 0.89 (9H, s, 3-$(CH_3)_3Si$), 0.63 (3H, s, H-18), 0.06 (6H, s, 3-$(CH_3)_2Si$); ^{13}C NMR (125.77 MHz): 212.6 (C-20), 174.1 (C-25), 141.5 (C-5), 120.7 (C-6), 72.5 (C-3), 66.7 (C-22), 63.4 (C-17), 57.0 (C-14), 51.6 (COO**C**H_3), 50.7 (C-21), 50.0 (C-9), 44.5 (C-13), 42.7 (C-4), 38.8 (C-12), 37.3 (C-1), 36.6 (C-10), 32.0 (C-2), 31.83 (C-7), 31.75 (C-8), 31.2 (C-23), 30.1 (C-24), 25.9 (($\mathbf{C}H_3)_3CSi$), 24.5 (C-15), 22.6 (C-16), 21.0 (C-11), 19.4 (C-19), 18.2 (**C**-Si), 13.2 (C-18), -4.6 (**C**H_3CSi); HR MS-ESI: calculated for $C_{32}H_{55}O_5Si$ 547.38133, found 547.38192.

*2.2.2. 6-(3β-t-Butyldimethylsilyloxy-androst-5-ene-17β-il)-6-oxo-methyl-trans-4-hexenoate (**15**)*

To a solution of **14** (330 mg, 0.60 mmol) in dry pyridine (3.0 ml) at 0 °C, mesyl chloride (147 μl, 1.9 mol) was added under an argon atmosphere and the reaction mixture was stirred for 2 h at 0 °C. After diluting with dichloromethane (6.0 ml), HCl 1 N (18.0 ml) was added and the mixture was extracted with dichloromethane. The organic layer was washed with water and dried with sodium sulphate. Evaporation of the solvent followed by flash chromatography (ethyl acetate–hexane 5:95) gave the 22-mesylate (365 mg, 98%); ^1H NMR (200.13 MHz): 5.31 (1H, d, *J* = 5.5 Hz, H-6), 5.17 (1H, m, H-22), 3.69 (3H, s, 25-$COOCH_3$), 3.49 (1H, m, H-3), 3.04 (3H, s, 22-CH_3SO_2), 0.99 (3H, s, H-19), 0.89 (9H, s, 3-$(CH_3)_3Si$), 0.62 (1H, s, H-18), 0.06 (6H, s, 3-$(CH_3)_2Si$); ^{13}C NMR (50,32 MHz): 212.0 (C-20), 141.8 (C-5), 120.7 (C-6), 74.0 (C-25), 72.52 (C-3), 68.0 (C-2), 62.9 (C-17), 57.0 (C-14), 51.9 (C-21), 50.0 (C-9), 44.6 (C-13), 42.7 (C-4), 38.9 (C-12), 38.3 (22-CH_3SO_2), 37.4 (C-1), 36.6 (C-10), 31.8 (C-8), 31.8 (C-7), 31.0 (C-2), 29.5 (C-23), 25.9 (3-$(CH_3)_3Si$), 24.5 (C-15), 22.8 (C-16), 21.1 (C-11), 20.2 (C-24), 19.4 (C-19), 18.2 (3-$(CH_3)_3Si$), 13.4 (C-18), -4.6 (3-$(CH_3)_2Si$).

To a solution of the 22-mesylate obtained above (395 mg, 0.63 mmol) in acetone (9.8 ml) at 0 °C, DBU (208 μl, 1.39 mmol) was added. After stirring for 10 min at 0 °C, the reaction mixture was diluted with water and concentrated to a third of its volume. The solution was washed with a saturated solution of ammonium chloride and then extracted with ethyl ether. The organic layer was dried with sodium sulphate and the solvent evaporated under vacuum. The resulting solid was purified by flash chromatography (ethyl acetate-hexane 5:95 → 20:80) to give compound **15** (269 mg, 81%) as an amorphous solid; $[\alpha]_D^{20}$ = +31.0 (c = 0.3, methanol); IR (KBr) υ_{max}: 2935, 2890, 2855, 1731, 1690, 1617, 1443, 1381, 1066 cm^{-1}; ^1H NMR (500.13 MHz): 6.78 (1H, dt, *J* = 15.5 and 6.0 Hz, H-22), 6.18 (1H, dt, *J* = 15.5 and 1.5 Hz, H-21), 5.30 (1H, bs, H-6), 3.69 (3H, s, 25-OCH_3), 3.49 (1H, m, H-3), 2.72 (1H, t, *J* = 9.0 Hz, H-17), 2.50 (4H, m, H-24 and H-23), 2.28 (1H, m, H-16α), 2.25 (1H, m, H-4β), 2.17 (1H, ddd, *J* = 2.1, 4.9 and 13.3 Hz, H-4α), 2.00 (1H, m, H-7β), 1.91 (1H, m, H-12β), 1.81 (1H, m, H-1β), 1.72 (1H, m, H-2α), 1.70 (1H, m, H-15α) 1.63 (1H, m, H-16β), 1.60 (1H, m, H-11α), 1.58 (1H, m, H-7α), 1.56 (1H, m, H-2β), 1.48 (1H, m, H-8), 1.44 (1H, m, H-11β), 1.40 (1H, m, H-12α), 1.24 (1H, m, H-15β), 1.19 (1H, m, H-14), 1.05 (1H, m, H-1α), 1.00 (3H, s H-19), 0.98 (1H, m, H-9), 0.89 (9H, s, 3-$(CH_3)_3Si$), 0.58 (3H, s, H-18), 0.06 (6H, s, 3-$(CH_3)_2Si$); ^{13}C NMR (125.77 MHz): 200.3 (C-20), 172.8 (C-25), 143.4 (C-22), 141.5 (C-5), 131.4 (C-21), 120.9 (C-6), 72.5 (C-3), 61.3 (C-17), 57.2 (C-14), 51.8 (25-COO**C**H_3), 50.1 (C-9), 44.7 (C-13), 42.8 (C-4), 39.1 (C-12), 37.4 (C-1), 36.6 (C-10), 32.4 (C-23), 32.04 (C-2), 31.99 (C-8), 31.9 (C-7), 27.4 (C-24), 25.9 (($\mathbf{C}H_3)_3CSi$), 24.6 (C-15), 22.7 (C-16), 21.1 (C-11), 19.4 (C-19), 18.3 (C-Si), 13.4 (C-18), -4.6 (**C**H_3CSi); HR MS-ESI: calculated for $C_{32}H_{53}O_4Si$ 529.3708, found 529.3728.

*2.2.3. 6-(3β-Hydroxyandrost-5-ene-17β-il)-6-oxo-methylhexanoate (**17**)*

To a solution of **15** (132 mg, 0.25 mmol) in ethyl acetate (20 ml) Pd/C 10% w/w (13.2 g) was added and the resulting mixture was hydrogenated at 1 bar at room temperature for 20 min. The reaction mixture was then filtered through a silicagel pad and washed with ethyl acetate. The filtrate was evaporated under reduced pressure to give compound **16** (127 mg, 96%) as an amorphous solid; $[\alpha]_D^{20}$ = +29.2 (c = 0.5, methanol); IR (KBr) υ_{max}: 2933, 2859, 1732, 1692, 1465, 1385, 1249, 1093 cm^{-1}; ^1H NMR (500.13 MHz): 5.31 (1H, bs, H-6), 3.67 (3H, s, 25-OCH_3), 3.48 (1H, m, H-3), 2.51 (1H, t, *J* = 9.0 Hz, H-17), 2.39 (2H, t, *J* = 6.7 Hz, H-21), 2.32 (2H, t, *J* = 7.1 Hz, H-24), 2.27 (1H, m, H-4β), 2.18 (1H, m, H-16α), 2.17 (1H, m, H-4α), 1.99 (2H, m, H-12β and H-7β), 1.82 (1H, m, H-1β), 1.73 (1H, m, H-2α), 1.67 (2H, m, H-15α and H-16β), 1.62 (4H, m, H-22 y H-23), 1.61 (1H, m, H-11α), 1.57 (1H, m, H-7α), 1.54 (1H, m, H-2β), 1.47 (1H, m, H-8), 1.45 (1H, m, H-11β), 1.42 (1H, m, H-12α), 1.23 (1H, m, H-15β), 1.13 (1H, m, H-14), 1.05 (1H, m, H-1α), 1.00 (3H, s H-19), 0.96 (1H, m, H-9), 0.89 (9H, s, 3-($\mathbf{C}H_3)_3CSi$), 0.61 (3H, s, H-18), 0.06 (6H, s, 3-($\mathbf{C}H_3)_2CSi$); ^{13}C NMR (125.77 MHz): 211.0 (C-20), 173.9 (C-25), 141.5 (C-5), 120.8 (C-6), 72.5 (C-3), 62.9 (C-17), 57.0 (C-14), 51.5 (25-COO**C**H3), 50.05 (C-9), 44.2 (C-13), 43.8 (C-21), 42.7 (C-4), 39.0 (C-12), 37.4 (C-1), 36.6 (C-10), 33.9 (C-24), 32.0 (C-2), 31.84 (C-8), 31.80 (C-7), 25.9 (($\mathbf{C}H_3)_3CSi$), 24.6 (C-22 o C-23), 24.5 (C-15), 23.1 (C-23 o C-22), 22.9 (C-16), 21.1 (C-11), 19.4 (C-19), 18.2 (**C**-Si), 13.4 (C-18), -4.60 (**C**H_3CSi); HR MS-ESI: calculated for $C_{32}H_{54}NaO_4Si$ 553.3684, found 553.3695.

To a solution of **16** (125 mg, 0.233 mmol) in THF (6.25 ml) and acetonitrile (6.25 ml), was added 40% hydrofluoric acid (2.25 ml) and the solution was stirred for 20 minutes at room temperature. The reaction mixture was neutralized with a saturated solution of potassium bicarbonate and extracted with ethyl acetate. The organic layer was washed with water, dried with sodium sulphate and the solvent evaporated under vacuum. The resulting solid was purified by flash chromatography (ethyl acetate - hexane 40:60) to give **17** (88.3 mg, 90%) as an amorphous solid; $[\alpha]_D^{20}$ = +26.9 (c = 0.2, methanol); IR (KBr) υ_{max}: 3235, 2947, 2905, 2853, 1746, 1705, 1439, 1365, 1241, 1063 cm^{-1}; ^1H NMR (500.13 MHz): 5.35 (1H, bs, H-6), 3.67 (3H, s, 25-OCH_3), 3.53 (1H, m, H-3), 2.51 (1H, t, *J* = 9.0 Hz, H-17), 2.39 (2H, t, *J* = 6.7 Hz, H-21), 2.33 (2H, t, *J* = 7.1 Hz, H-24), 2.32 (1H, m, H-4α), 2.25 (1H, m, H-4β), 2.19 (1H, m, H-16α), 2.01 (2H, m, H-12β and H-7β), 1.86 (1H, m, H-1β), 1.85 (1H, m, H-2α), 1.68 (1H, m, H-15α), 1.64 (1H, m, H-16β), 1.62 (4H, m, H-22 y H-23), 1.61 (1H, m, H-11α),1.58 (1H, m, H-7α), 1.51 (1H, m, H-2β), 1.48 (1H, m, H-8), 1.47 (1H, m, H-11β), 1.44 (1H, m, H-12α), 1.24 (1H, m, H-15β), 1.14 (1H, m, H-14), 1.10 (1H, m, H-1α), 1.01 (3H, s H-19), 0.99 (1H, m, H-9), 0.61 (3H, s, H-18); ^{13}C NMR (125.77 MHz): 211.0 (C-20), 173.9 (C-25), 140.8 (C-5), 121.4 (C-6), 71.7 (C-3), 62.9 (C-17), 57.0 (C-14), 51.5 (25-COO**C**H_3), 50.0 (C-9), 44.2 (C-13), 43.8 (C-21), 42.2 (C-4), 38.9 (C-12), 37.2 (C-1), 36.5 (C-10), 33.9 (C-24), 31.84 (C-8), 31.77 (C-7), 31.6 (C-2), 24.6 (C-22 o C-23), 24.5 (C-15), 23.1 (C-23 o C-22), 23.0 (C-16), 21.1 (C-11), 19.4 (C-19), 13.4 (C-18); HR MS-ESI: calculated for $C_{26}H_{40}NaO_4$ 439.2819, found 439.2829.

*2.2.4. 6-(3-Oxo-androst-4-ene-17β-il)-6-oxo-hexanoic acid (**7**)*

A suspension of pyridinium chlorochromate (107 mg, 0.22 mmol), barium carbonate (47 mg) and 3 Å molecular sieves (88 mg) in anhydrous dichloromethane (5 ml) was stirred for 5 min under a nitrogen atmosphere. A solution of **17** (54 mg, 0.13 mmol) in anhydrous dichloromethane (3 ml) was added and

stirring continued at room temperature for 2 h. The reaction mixture was diluted with ether and percolated through silicagel with ether-ethyl acetate (1:1) to give the corresponding 3-ketosteroid (39.1 mg, 80%) as an amorphous solid; ^{1}H NMR (200.13 MHz): 5.35(1H, bs, H-6), 3.67 (3H, m, 25-COOCH$_3$), 3.30 (1H, d, *J* = 17 Hz, H-4a), 2.83 (1H, d, *J* = 17 Hz, H-4b), 1.19 (3H, s, H-19), 0.65 (3H, s, H-18).

To a solution of the solid obtained above in THF (1 ml), HCl 6 N (170 μL) was added. The reaction mixture was stirred 24 h at room temperature, diluted with water, concentrated to a third of its volume, and extracted with ethyl acetate. The organic layer was dried with sodium sulphate and the solvent evaporated under vacuum. The resulting solid was purified by preparative TLC (ethyl acetate-hexane 20:80) to give compound **7** as an amporphous solid (20.7 mg, 55%) and the 3-ketosteroid used as starting material (15.6 mg, 40%). Compound **7**: $[\alpha]_D^{20}$ = +20.9 (c = 0.2, methanol); IR (KBr) υ_{max}: 3401, 2938, 1720, 1705, 1458, 1386, 1259, 1051, 1022 cm^{-1}; ^{1}H NMR (500.13 MHz): 5.74 (1H, s, H-4), 2.51 (1H, t, *J* = 9.0 Hz, H-17), 2.42 (1H, m, H-24a), 2.40 (1H, m, H-6β), 2.39 (2H, m, H-21), 2.37 (3H, m, H-24b and H-2), 2.29 (1H, ddd, *J* = 2.5, 4.0 and 14.5 Hz, H-6α),2.19 (1H, m, H-16α), 2.04 (1H, m, H-1β), 2.03 (1H, m, H-12β), 1.87 (1H, m, H-7β), 1.71 (2H, m, H-1α and H-15α), 1.67 (1H, m, H-16β), 1.63 (4H, m, H-22 y H-23), 1.62 (1H, m H-11α), 1.56 (1H, m, H-8), 1.44 (1H, m, H-11β), 1.43 (1H, m, H-12α), 1.27 (1H, m, H-15β), 1.16 (1H, m, H-14), 1.19 (3H, s H-19), 1.06 (1H, td, *J* = 12.9 and 4.2, H-7α), 0.98 (1H, m, H-9), 0.65 (3H, s, H-18); ^{13}C NMR (125.77 MHz): 210.8 (C-20), 199.5 (C-3), 177.3 (C-25), 171.0 (C-5), 123.9 (C-4), 62.7 (C-17), 56.1 (C-14), 53.65 (C-9), 44.1 (C-13), 43.8 (C-21), 38.8 (C-10), 38.6 (C-12), 35.7 (C-1), 35.6 (C-8), 33.9 (C-24), 33.5 (C-2), 32.8 (C-6), 31.9 (C-7), 24.4 (C-22), 24.3 (C-15), 23.00 (C-16), 22.99 (C-23), 21.0 (C-11), 17.4 (C-19), 13.5 (C-18); HR MS-ESI: calculated for $C_{25}H_{37}O_4$ 401.2686, found 401.2697.

*2.2.5. 6-(3β-Hydroxy-5-androstene-17β-il)-6-oxo-hexanoic acid (**8**)*

To a solution of compound **17** (24 mg, 0.058 mmol) in methanol (0.6 ml) and THF (0.6 ml), 5% aqueous LiOH (0.12 ml, 0.6 mmol) was added. The reaction mixture was stirred for 18 hs at room temperature, diluted with water and concentrated to a third of its volume. After addition of HCl 1 N (pH 3) the mixture was extracted with ethyl acetate. The organic layer was washed with water, dried with sodium sulphate and the solvent evaporated under vacuum. The resulting solid was purified by preparative TLC (ethyl acetate – hexane 7:3) to give compound **8** (22 mg, 92%) as an amorphous solid; $[\alpha]_D^{20}$ = +18.2 (c = 0.4, methanol); IR (KBr) υ_{max}: 3396, 2937, 2164, 1718, 1698, 1463, 1226, 1049 cm^{-1}; ^{1}H NMR (500.13 MHz): 5.35 (1H, bs, H-6), 3.52 (1H, m, H-3), 2.51 (1H, t, *J* = 9.0 Hz, H-17), 2.40 (2H, t, *J* = 2.4 Hz, H-21), 2.32 (2H, m, H-24), 2.30 (1H, m, H-4α), 2.23 (1H, m, H-4β), 2.18 (1H, m, H-16α), 2.00 (2H, m, H-12β and H-7β), 1.85 (2H, m, H-1β and H-2α), 1.69 (1H, m, H-15α), 1.65 (1H, m, H-16β), 1.62 (1H, m, H-11α), 1.60 (4H, m, H-22 and H-23), 1.59 (1H, m, H-7α), 1.52 (1H, m, H-2β), 1.48 (1H, m, H-8), 1.47 (1H, m, H-11β), 1.43 (1H, m, H-12α), 1.23 (1H, m, H-15β), 1.14 (1H, m, H-14), 1.09 (1H, m, H-1α), 1.01 (3H, s H-19), 0.99 (1H, m, H-9), 0.61 (3H, s, H-18), ^{13}C NMR (125.77 MHz): 211.3 (C-20), 174.0 (C-25), 140.7 (C-5), 121.3 (C-6), 71.6 (C-3), 62.9 (C-17), 56.9 (C-14), 49.9 (C-9), 44.2 (C-13), 43.8 (C-21), 42.1 (C-4), 38.9 (C-12), 37.2 (C-1), 36.5 (C-10), 33.9 (C-24), 31.80 (C-8), 31.72 (C-7), 31.4 (C-2), 24.5 (C-22), 24.46 (C-15), 23.1 (C-23), 22.9 (C-16), 21.0 (C-11), 19.3 (C-19), 13.3 (C-18); HR MS-ESI: calculated for $C_{25}H_{38}NaO_4$ 425.2662, found 425.2678.

*2.2.6. 3α-t-Butyldimethylsilyloxy-6,19-epoxypregn-4-ene-20-one (**19**)*

Imidazole (47 mg, 0.68 mmol) and *t*-butyldimethylsilyl chloride (69 mg, 0.45 mmol) were added successively to a solution of **18** (75 mg, 0.23 mmol) in anhydrous DMF (1.8 ml) and the solution was stirred for 4 h at room temperature under nitrogen atmosphere. The reaction mixture was extracted with ether. The organic layer was washed successively with brine and water and dried with sodium sulphate. Evaporation of the solvent followed by flash chromatography (ethyl acetate–hexane 30:70) gave the 3-silyl ether **19** (93 mg, 92%) as an amorphous solid; IR (KBr) υ_{max}: 2933, 2853, 1707, 1076, 1007 cm^{-1}; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 5.37 (1H, d, *J* = 1.8, H-4), 4.41 (1H, d, *J* = 4.8, H-6), 4.36 (1H, m, H-3), 4.02 (1H, d, *J* = 7.7, H-19), 3.28 (1H, d, *J* = 7.7, H-19), 2.51 (1H, t, *J* = 9.0, H-17), 2.17 (1H, m, H-16β), 2.11 (3H, s, H-21), 2.04 (1H, m, H-12β), 1.97 (1H, m, H-1α), 1.89 (1H, dt, *J* = 12.8 and 5.0 H-7β), 1.80 (1H, m, H-2β), 1.79 (1H, m, H-8), 1.64 (2H, m, H-11α and H-16α), 1.60 (1H, m, H-15β), 1.59 (1H, m, H-9), 1.58 (1H, m, H-2α), 1.45 (1H, m, H-12α), 1.32 (1H, m, H-14), 1.31 (1H, m, H-1β), 1.29 (1H, m, H-11β), 1.26 (1H, m, H-15α), 1.25 (1H, m, H-7α), 0.91 (9H, s, $(CH_3)_3CSi$), 0.67 (3H, s, H-18), 0.10 (3H, s, $(CH_3)_2Si$), 0.09 (3H, s, $(CH_3)_2Si$); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 209.4 (C-20), 147.8 (C-5), 117.0 (C-4), 77.1 (C-6), 75.5 (C-19), 68.5 (C-3), 63.5 (C-17), 55.1 (C-14), 49.5 (C-9), 44.8 (C-10), 44.3 (C-13), 39.2 (C-7), 38.8 (C-12), 34.4 (C-8), 31.4 (C-21), 29.2 (C-2), 26.0 (($\mathbf{C}H_3)_3CSi$), 25.4 (C-1), 23.9 (C-15), 22.9 (C-16), 22.9 (C-11), 18.4 (($CH_3)_3\mathbf{C}Si$), 13.7 (C-18), -4.48 (($\mathbf{C}H_3)_2Si$), -4.55 (($\mathbf{C}H_3)_2Si$); Analysis $C_{27}H_{44}O_3Si$: calcd C, 72.9, H, 10.0 Found C, 72.4, H, 10.1.

*2.2.7. 7-(3α-t-Butyldimethylsilyloxy-6,19-epoxyandrost-4-ene-17β-il)-7-oxo-5-hydroxymethylheptanoate (**22**)*

The silyl enol ether **20** was obtained from compound **19** (102 mg, 0.23 mmol) following the procedure described for compound **12**. Compound **20**: ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 5.35 (1H, bs, H-4), 4.40 (1H, d, *J* = 4.5, H-6), 4.35 (1H, m, H-3), 4.08 (1H, d, *J* = 1.0, H-21a), 4.03 (1H, d, *J* = 1.0, H-21b), 4.03 (1H, d, *J* = 7.5, H-19a), 3.27 (1H, d, *J* = 7.5, H-19b), 2.00 (1H, t, *J* = 9.5, H-17), 1.99 (1H, m, H-16β), 1.98 (1H, m, H-1α), 1.97 (1H, m, H-12β), 1.86 (1H, m, H-7β), 1.79 (1H, m, H-2β), 1.76 (1H, m, H-8), 1.68 (1H, m, H-16α), 1.61 (1H, m, H-12α), 1.56 (2H, m, H-2α and H-15β), 1.55 (2H, m, H-11α and H-9), 1.28 (1H, m, H-1β), 1.25 (1H, m, H-11β), 1.22 (1H, m, H-7α), 1.20 (2H, m, H-4 and H-15α), 0.90 (9H, s, (($CH_3)_3CSi$)), 0.66 (3H, s, H-18), 0.19 (9H, s, (($CH_3)_3Si$)), 0.09 (3H, s, (($CH_3)_2Si$)), 0.08 (3H, s, (($CH_3)_2Si$)); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 160.0 (C-20), 148.0 (C-5), 116.8 (C-4), 89.7 (C-21), 77.2 (C-6), 75.6 (C-19), 68.7 (C-3), 56.1 (C-17), 54.3 (C-14), 49.8 (C-9), 44.4 (C-10), 43.9 (C-13), 39.3 (C-7), 38.5 (C-12), 34.7 (C-8), 29.2 (C-2), 25.9 (($\mathbf{C}H_3)_3CSi$), 25.4 (C-1), 24.6 (C-16), 23.7 (C-15), 23.0 (C-11), 18.4 (($CH_3)_3\mathbf{C}Si$), 13.1 (C-18), 0.15 (($CH_3)_3Si$), -4.5 (($CH_3)_2Si$), -4.6 (($CH_3)_2Si$).

Compound **22** was obtained from the silyl eno ether **20** chloroform solution obtained above and aldehyde **21**, following the procedure described for compound **14**. Purification by flash chromatography (ethyl acetate–hexane 5:95) gave steroid **22** as a mixture of epimers (7:3) at C-22 (70 mg, 64%). From the flash chromatography an analytical sample of the major epimer was obtained and characterized: $[\alpha]_D^{20}$ = +108.0 (c = 0.9, methanol); IR (KBr) υ_{max}: 3455, 2928, 2855, 1728, 1704, 1246, 1072, 1045, 854 cm^{-1}; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 5.38 (1H, bs, H-4), 4.41 (1H, d, *J* = 4.7, H-6), 4.35 (1H, m, H-3), 4.04 (1H, m, H-22), 4.02 (1H, d, *J* = 8.0, H-19a), 3.66 (3H, s, CO_2CH_3), 3.28 (1H, d, *J* = 8.0, H-19b), 2.55 (1H, dd, *J* = 17.8 and 2.6, H-21a), 2.50 (1H, t, *J* = 9.0, H-17), 2.45 (1H, m, H-21b), 2.34 (2H, t, *J* = 7.4, H-25), 2.15 (1H, m, H-16β), 2.04 (1H, m, H-12β), 1.97 (1H, m, H-1α), 1.89 (1H, m, H-7α), 1.79 (1H, m, H-2β), 1.78 (2H, m, H-24a and m, H-8), 1.69 (1H, m, H-24b), 1.65 (2H, m, H-11α and H-16α), 1.61 (1H, m, H-15β), 1.57 (1H, m, H-9), 1.55 (1H, m, H-2α), 1.53 (1H, m, H-23a), 1.43 (1H, m, H-23b), 1.41 (1H, m, H-12α), 1.30 (2H, m, H-1β and H-14), 1.27 (1H, m, H-11β), 1.26 (1H, m, H-15α), 1.24 (1H, m, H-7β), 0.91 (9H, s, (($CH_3)_3CSi$)), 0.67 (3H, s, H-18),

0.10 (3H, s, $((CH_3)_2Si)$), 0.09 (3H, s, $((CH_3)_2Si)$); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 212.8 (C-20), 174.0 (C-26), 147.7 (C-5), 117.1 (C-4), 77.0 (C-6), 75.5 (C-19), 68.5 (C-3), 67.0 (C-22), 63.2 (C-17), 55.2 (C-14), 51.5 ($CO_2\mathbf{C}H_3$), 50.6 (C-21), 49.5 (C-9), 45.3 (C-13), 44.2 (C-10), 39.2 (C-7), 38.8 (C-12), 35.5 (C-23), 34.4 (C-8), 33.7 (C-25), 29.2 (C-2), 26.0 ($(\mathbf{C}H_3)_3CSi$), 25.3 (C-1), 23.9 (C-15), 22.80 (C-11), 22.76 (C-16), 20.9 (C-24), 18.4 ($(CH_3)_3\mathbf{C}Si$), 13.7 (C-18), -4.49 ($(CH_3)_2Si$), -4.56 ($(CH_3)_2Si$); HR MS-ESI: calculated for $C_{33}H_{54}O_6NaSi$ 597.3582, found 597.3591.

2.2.8. 7-(3α-t-Butyldimethylsilyloxy-6,19-epoxyandrost-4-ene-17β-il)-7-oxo-methyl-trans-5-heptenoate (**23**)

To a solution of **22** (170 mg, 0.30 mmol) in anhydrous dichloromethane (1.2 ml), a solution of triflic anhydride (60 μL mg, 0.35 mmol) in dry pyridine (1.4 ml) was added and the resulting solution was stirred at 0 °C, under argon atmosphere for 30 min. Then DBU (97 μL, 0.65 mmol) was added and the mixture was stirred for 2 additional hours, diluted with dichloromethane and washed with HCl (1 N). The organic layer was dried with anhydrous sodium sulphate, and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography (ethyl acetate–hexane 5:95 → 1:9) to give compound **23** as an amorphous solid (77.5 mg, 47%); $[\alpha]_D^{20}$ = +122.0 (c = 0.5, methanol); IR (KBr) υ_{max}: 2923, 2854, 1738, 1680, 1246, 1074, 835 cm^{-1}; ^1H NMR (500.13 MHz, $CDCl_3$) δ: 6.77 (1H, dt, *J* = 15.5 and 7.0, H-22), 6.15 (1H, dt, *J* = 16.0 and 1.5, H-21), 5.38 (1H, m, H-4), 4.41 (1H, d, *J* = 4.5, H-6), 4.36 (1H, m, H-3), 4.02 (1H, d, *J* = 7.7, H-19a), 3.68 (3H, s, CO_2CH_3), 3.28 (1H, d, *J* = 7.5, H-19b), 2.71 (1H, t, *J* = 8,8, H-17), 2.34 (2H, t, *J* = 7.4 H-25), 2.26 (1H, m, H-16), 2.24 (2H, m, H-23), 1.96 (1H, m, H-1α), 1.92 (1H, m, H-12β), 1.90 (1H, m, H-7β), 1.81 (2H, m, H-24), 1.80 (1H, m, H-2β), 1.78 (1H, m, H-8), 1.64 (1H, m, H-16), 1.62 (1H, m, H-11α), 1.61 (1H, m, H-15β), 1.59 (1H, m, H-9), 1.57 (1H, m, H-2α), 1.42 (1H, m, H-12α), 1.35 (1H, m, H-14), 1.30 (1H, m, H-1β), 1.27 (1H, m, H-15), 1.26 (1H, m, H-7α), 1.25 (1H, m, H-11β), 0.98 (3H, s, $((CH_3)_2Si)$), 0.92 (9H, s, $((CH_3)_3CSi)$), 0.64 (3H, s, H-18), 0.10 (3H, s, $((CH_3)_2Si)$), 0.09 (3H, s, $((CH_3)_2Si)$); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 200.2 (C-20), 173.6 (C-26), 147.8 (C-5), 144.7 (C-22), 131.3 (C-21), 117.0 (C-4), 77.1 (C-6), 75.5 (C-19), 68.5 (C-3), 61.1 (C-17), 55.4 (C-14), 51.6 ($CO_2\mathbf{C}H_3$), 49.6 (C-9), 45.4 (C-13), 44.3 (C-10), 39.3 (C-7), 39.0 (C-12), 34.5 (C-8), 33.3 (C-25), 31.6 (C-23), 29.2 (C-2), 26.0 ($(\mathbf{C}H_3)_3CSi$), 25.4 (C-1), 24.1 (C-15), 23.4 (C-24), 22.9 (C-11), 22.8 (C-16), 18.4 ($(CH_3)_3\mathbf{C}Si$), 13.9 (C-18), -4.47 ($(CH_3)_2Si$), −4.54 ($(CH_3)_2Si$); HR MS-ESI: calculated for $C_{33}H_{52}O_5NaSi$ 579.3349, found 579.3376.

2.2.9. 7-(3α-t-Butyldimethylsilyloxy-6,19-epoxyandrost-4-ene-17β-il)-7-oxo-methylheptanoate (**24**)

Compound **23** (65 mg, 0.12 mmol) was hydrogenated as previously described for the synthesis of **16**. The residue was purified by flash chromatography (ethyl acetate–hexane 5:95) to give compound **24** as an amorphous solid (50 mg, 77%); $[\alpha]_D^{20}$ = +98.4 (c = 0.3, methanol); IR (KBr) υ_{max}: 2929, 2859, 1741, 1705, 1432, 1072, 830, 780 cm^{-1}; ^1H NMR (500.13 MHz, $CDCl_3$) δ: 5.37 (1H, m, H-4), 4.41 (1H, d, *J* = 4.5, H-6), 4.36 (1H,m, H-3), 4.02 (1H, d, *J* = 8.0, H-19a), 3.66 (3H, s, (CO_2CH_3)), 3.28 (1H, d, *J* = 8.0, H-19b), 2.48 (1H, t, *J* = 9.0, H-17), 2.35 (2H, t, *J* = 7.4, H-21), 2.31 (2H, t, *J* = 7.5, H-25), 2.16 (1H, m, H-16β), 2.00 (1H, m, H-12β), 1.97 (1H, m, H-1α), 1.89 (1H, m, H-7β), 1.80 (1H, m, H-2β), 1.78 (1H, m, H-8), 1.65 (1H, m, H-11α), 1.63 (2H, m, H-24), 1.63 (1H, m, H-16α), 1.59 (1H, m, H-15α), 1.58 (1H, m, H-9), 1.57 (1H, m, H-2α), 1.57 (2H, m, H-22), 1.42 (1H, m, H-12α), 1.31 (1H, m, H-1β), 1.31 (2H, m, H-23), 1.29 (1H, m, H-11β), 1.28 (1H, m, H-14), 1.25 (1H, m, H-7α), 1.24 (1H, m, H-15β), 0.91 (9H, s, $((CH_3)_3CSi)$), 0.65 (3H, s, 18), 0.10 (3H, s, H-$((CH_3)_2Si)$), 0.09 (3H, s, $((CH_3)_2Si)$); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 211.3 (C-20), 174.1 (C-26), 147.8 (C-5), 117.0 (C-4), 77.1 (C-6), 75.5 (C-19), 68.5 (C-3), 62.7 (C-17), 55.2 (C-14), 51.5 ($CO_2\mathbf{C}H_3$), 49.5 (C-9), 45.0 (C-13), 44.3 (C-10), 43.9 (C-21), 39.3 (C-7), 38.9 (C-12), 34.4 (C-8), 33.9 (C-25), 29.2 (C-2), 28.8 (C-23), 26.0 ($(\mathbf{C}H_3)_3CSi$), 25.4 (C-1), 24.8 (C-24), 23.9 (C-15), 23.3 (C-22), 23.1 (C-16), 22.9 (C-11), 18.4 ($(CH_3)_3\mathbf{C}Si$), 13.9 (C-18), -4.47 ($(\mathbf{C}H_3)_2CSi$), -4.54 ($(CH_3)_2Si$); HR MS-ESI: calculated for $C_{33}H_{54}O_5NaSi$ 581.3633, found 581.3649.

2.2.10. 7-(3α-Hydroxy-6,19-epoxyandrost-4-ene-17β-il)-7-oxo-heptanoic acid (**25**)

To a solution of steroid **24** (50 mg, 0.09 mmol) in THF (1 ml), hydrochloric acid (6 N, 0.50 ml) was added and the solution was stirred for 2 h at room temperature. The sodium hydroxide (50% aq) was added (pH 14) and the resulting mixture was stirred for 1 additional hour. Finally hydrochloric acid was added (pH 2) and the mixture extracted with ethyl acetate. The organic layer was dried with sodium sulphate and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography (acetic acid-ethyl acetate–hexane 1:80:20) to give compound **25** as an amorphous solid to (25 mg, 65%); $[\alpha]_D^{20}$ = +62.0 (c = 0.2, methanol); IR (KBr) υ_{max}: 3403 (broad), 2928, 1732, 1699, 1449, 1036, 736 cm^{-1}; ^1H NMR (500.13 MHz, $CDCl_3$) δ: 5.48 (1H, m, H-4), 4.45 (1H, d, *J* = 4.9, H-6), 4.39 (1H, m, H-3), 4.04 (1H, d, *J* = 7.8, H-19a), 3.31 (1H, d, *J* = 7.9, H-19b), 2.49 (1H, t, *J* = 9.0, H-17), 2.37 (2H, t, *J* = 7.1, H-21), 2.36 (2H, t, *J* = 7.4, H-25), 2.16 (1H, m, H-16β), 2.00 (1H, m, H-1α), 1.99 (1H, m, H-12β), 1.95 (1H, m, H-2β), 1.93 (1H, m, H-7β), 1.80 (1H, m, H-8), 1.66 (1H, m, H-11α), 1.65 (1H, m, H-15β and H-16α), 1.64 (2H, m, H-24), 1.61 (2H, m, H-22), 1.56 (1H, m, H-2α), 1.52 (1H, m, H-9), 1.43 (1H, m, H-12α), 1.35 (1H, m, H-1β), 1.34 (2H, m, H-23), 1.32 (1H, m, H-11β), 1.28 (1H, m, H-14), 1.27 (1H, m, H-15α), 1.21 (1H, m, H-7α), 0.67 (3H, s, H-18); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 211.2 (C-20), 176.9 (C-26), 149.4 (C-5), 115.7 (C-4), 77.2 (C-6), 75.3 (C-19), 67.5 (C-3), 62.7 (C-17), 55.3 (C-14), 50.1 (C-9), 45.0 (C-13), 44.3 (C-10), 43.8 (C-21), 39.5 (C-7), 38.7 (C-12), 34.3 (C-8), 33.3 (C-25), 28.9 (C-2), 28.7 (C-23), 25.1 (C-1), 24.5 (C-24), 23.9 (C-15), 23.2 (C-22), 23.1 (C-16), 22.9 (C-11), 13.9 (C-18); HR MS-ESI: calculated for $C_{26}H_{38}O_5Na$ 453.2612, found 453.2613.

2.2.11. 7-(3-Oxo-6,19-epoxyandrost-4-ene-17β-il)-7-oxo-heptanoic acid (**9**)

Manganese dioxide (145 mg, 1.65 mmol) was added to a solution of steroid **25** (20 mg, 0.05 mmol) in dichloromethane (2.0 ml) and the solution was stirred for 24 h at room temperature. The reaction mixture was filtered through celite layer. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (acetic acid-ethyl acetate–hexane 1:50:50) to give compound **9** (12.5 mg, 62%) as an amorphous solid; $[\alpha]_D^{20}$ = −1.3 (c = 0.4, methanol); IR (KBr) υ_{max}: 3242 (broad), 2934, 2876, 1735, 1699, 1671, 1455, 1027, 741 cm^{-1}; ^1H NMR (500.13 MHz, $CDCl_3$) δ: 5.82 (1H, s, H-4), 4.70 (1H, d, *J* = 5.2, H-6), 4.20 (1H, d, *J* = 8.2, H-19a), 3.51 (1H, d, *J* = 8.2, H-19b), 2.50 (1H, t, *J* = 9.1, H-17), 2.39 (2H, m, H-21), 2.35 (2H, t, *J* = 7.4, H-25), 2.23 (1H, m, H-1α), 2.18 (1H, m, H-16β), 2.10 (1H, m, H-7β), 2.08 (1H, m, H-12β), 1.89 (1H, m, H-8), 1.84 (1H, m, H-1β), 1.69 (1H, m, H-16α), 1.67 (1H, m, H-11α), 1.65 (2H, m, H-24), 1.65 (1H, m, H-15β), 1.65 (1H, m, H-9), 1.60 (2H, m, H-22), 1.49 (1H, m, H-12α), 1.49 (1H, m, H-11β), 1.35 (2H, m, H-23), 1.31 (1H, m, H-14), 1.30 (1H, m, H-15α), 1.28 (1H, m, H-7α), 0.69 (3H, s, H-18); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 210.9 (C-20), 198.9 (C-3), 178.3 (C-26), 171.8 (C-5), 115.0 (C-4), 77.2 (C-6), 75.6 (C-19), 62.4 (C-17), 55.0 (C-14), 50.2 (C-9), 45.9 (C-10), 44.9 (C-13), 43.8 (C-21), 41.1 (C-1), 38.6 (C-12), 33.7 (C-8), 33.6 (C-25), 33.2 (C-2), 28.7 (C-23), 26.5 (C-7), 24.5 (C-24), 24.0 (C-15), 23.9 (C-11), 23.2 (C-16), 23.1 (C-22), 13.8 (C-18); HRMS (ESI) calculated for $C_{26}H_{36}O_5Na$ 451.2455, found 451.2450.

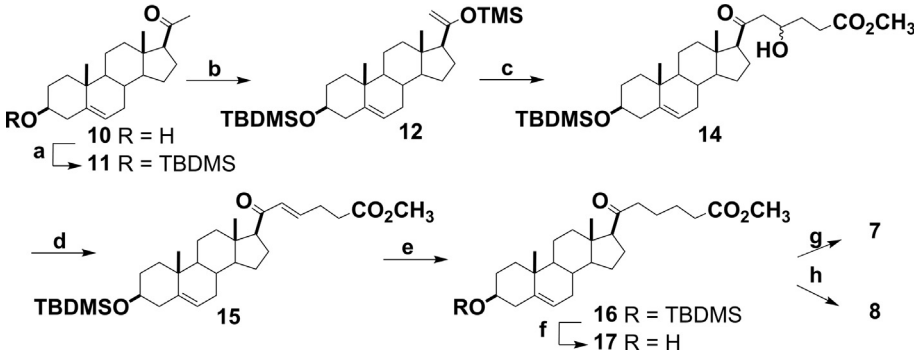
3. Results and discussion

Compounds **7** and **8** were obtained from the commercially available pregnenolone, the strategy to introduce an alkyl side chain at position 21 is shown in Scheme 1. The key step of this transformation involved a C—C bond formation step using the Lewis acid mediated Mukaiyama aldol addition [15,16] of the silyl enol ether **12** to methyl 4-oxobutanoate **13** ($HCOCH_2CH_2COOCH_3$). As a first attempt we followed the methodology used by Kirk *et al.* to prepare a silyl enolate at C-21 [17]. After protection of the 3-hydroxy group in **10** as the *tert*-butyldimethylsilyl ether, followed by treatment with LDA in THF at −78 °C, the enolate formed under kinetic control was trapped by reaction with trimethyl silyl chloride to give **12**. The signals at 4.08 (J = 0.7 Hz) and 4.04 (bs) observed in the ^{1}H NMR spectrum of **12** were assigned to the vinylic hydrogens at position 21. The presence of the enol ether was confirmed by the correlations observed between CH_2-21 and C-21 (89.6 ppm) in the HSQC and CH_2-21 and C-20 (160.1 ppm) in the HMBC. Even though the ^{1}H NMR analysis of the reaction before the work-up showed total conversion, we observed that an important amount of the silyl enol ether was hydrolyzed during the work-up used by Kirk et al., which involved several extraction steps with aqueous solutions. To avoid hydrolysis during the extraction steps we developed a new water free work-up method. After formation of the silyl enol ether we evaporated the THF with nitrogen, re-dissolved the solid residue in chloroform or dichloromethane and the insoluble salts that precipitated with the addition of the chlorinated solvent were filtered out under argon atmosphere. Hydrolysis of the silyl enol ether was not observed in this case and the resulting solution was used in the next step without further treatment. Then we studied the Mukaiyama aldol addition of **12** to the C-4 aldehyde **13** promoted by Lewis acids. As a first attempt we choose the boron trifluoride ethyl ether, a Lewis acid commonly used with very good results [15]. The BF_3-Et_2O mediated reaction was carried out in chloroform rendering the 22-hydroxy steroid **14** as a mixture of epimers at C-22 with 61% yield calculated from **11**, recovering a 10% of the 20-ketone precursor **11**. It is commonly accepted that the function of the Lewis acid is to activate the carbonyl substrate (electrophile) by coordinating to the carbonyl oxygen, rending it more susceptible to a nucleophilic attack. In this way, various experimental studies found in literature suggest that the mechanism involved, and so the success of the reaction, is likely to depend on the Lewis acid properties of the catalyst [16,18,19]. With this in mind, and with the purpose of improving the performance of the aldol addition, we tested a set of six commercially available Lewis acids: $ZnCl_2$, $TiCl_4$, Ti(*i*-PrO)$_4$, $SnCl_4$, $AlCl_3$ and $ZnBr_2$.Under the same conditions none of these catalysts gave the desired 22-hydroxi steroid **14**, the hydrolysis of the silyl enol ether being the main reaction. Given these results, from here on all Mukaiyama reactions were carried out using boron trifluoride ethylether as Lewis acid.To obtain the olefin **15** from **14**, the 22-hydroxy group was converted to the corresponding mesylate (MsCl, Py) and it was eliminated *in situ* under basic conditions (DBU, acetone). Regioselective hydrogenation under mild conditions (H_2, Pd 10%C, EtOAc, 1Bar, room temperature, 20 min), followed by cleavage of the TBDMS ether with HF 40% in CH_3CN gave **17**. Compound **7** was obtained from **17** by oxidation of the 3-hydroxy group with PCC followed by hydrolysis of the methyl ester under acid conditions, with 17% yield from pregnenolone. Treatment of **17** with LiOH in H_2O-THF-MeOH gave **8** with 36% yield from pregnenolone.

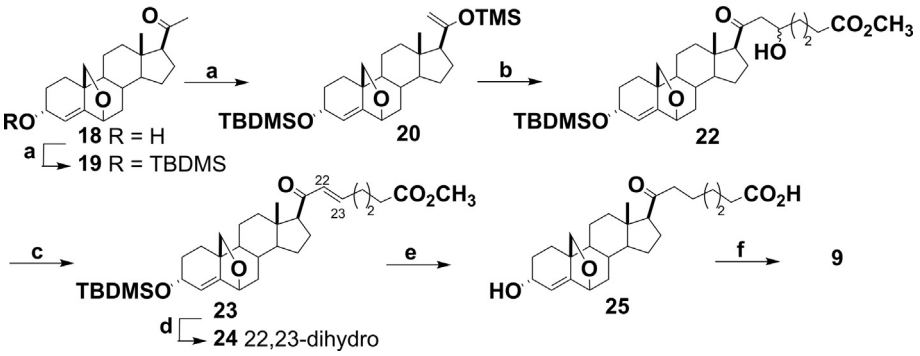
Although 21-hemisuccinate-6,19-epoxyprogesterone (**6**) is a selective modulator of the glucocorticoid receptor and the hemisuccinate moiety is stable *in vitro*, the ester is hydrolysable *in vivo*. With the purpose of overcome this problem, we synthesized compound **9** as a potential non hydrolysable analogue starting from 6,19-epoxy steroid **18** (Scheme 2). Compound **18** was obtained from commercially available pregnenolone acetate following essentially the procedure described previously by us [13]. In this case the addition of the silyl enol ether **20** to methyl 5-oxopentanoate **21** ($HCO(CH_2)_3COOCH_3$) gave the 22-hydroxy steroid **22** as a mixture of epimers at C-22 in 64% yield from **19** and 15% of the 20-ketone **19** was recovered. Elimination of the 22-hydroxy group in **22** was tested using the corresponding mesylate and triflate derivatives, the use of the last leaving group gave the highest yield. Regioselective hydrogenation, followed by cleavage of the TBDMS ether (HCl 6 N, THF) and hydrolysis of the methyl ester in basic conditions (NaOH 50% aq) gave steroid **9** with 8.6% yield from the 6,19 oxide **18**.

The biological activity of compounds **7**, **8** and **9** was evaluated on the nuclear receptors DAF-12, LXR and GR, respectively. As mentioned before, compound **4** induces DAF-12 response in HEK-293T cells [8], however the loss of the activity was observed when the 21-CH_3 in **4** was replaced by a carbonyl group at the 20 position in **7**. Similar results were observed for steroid **8** on Liver X receptors (LXRα and β), while compound **5** showed an agonist activity when co-incubated with the LXR agonist GW3965 in HEK-293T cells [9], the 20-ketoanalogue **8** was inactive.

Finally, we evaluated the activity of steroid **9** on the GR in direct transactivation assays using BHK cells [11], in this case replacement of the hemisuccinate ester moiety on **6** for a hydrocarbonated side chain lead to a complete loss of activity highlighting the importance of this functional group in the modulation of the glucocorticoid receptor.



Scheme 1. Reagents and conditions: a) TBDMSCl, imidazol, DMF, 92.0%; b) i) LDA-THF, ii) TMSCl, −78 °C to room temperature; c) $HCOCH_2CH_2COOCH_3$ (**13**), 61.0% from **11**; d) i) MsCl, Py, 98.0%, ii) DBU, acetone, 81.0%; e) H_2, Pd 10%C, EtOAc, 1 Bar, room temperature, 96.0%; f) HF 40%, CH_3CN, 90.0%; g) i) PCC, CH_2Cl_2, 80.0%: ii) HCl 1 N, THF, 55.0%; h) LiOH, H_2O-THF-MeOH, 92.0%.



Scheme 2. Reagents and conditions: a) i) TBDMSCl, imidazol, DMF, 92.0%, ii) 1)LDA-THF, 2) TMSCl, −78 °C to room temperature; b) $HCO(CH_2)_3COOCH_3$ (**21**), 64% from **19**; c) i) triflic anhydride, CH_2Cl_2-Py, ii) DBU, acetone, 47.0%; d) H_2, Pd 10%C, EtOAc, 1 Bar, room temperature, 77.0%; e) i) ClH 6 N, THF, ii) NaOH 50% (aq), 65%; f) MnO_2, CH_2Cl_2, 62%.

4. Conclusion

In conclusion, we have developed an efficient and general method for the synthesis of C-25 and C-26 steroidal acids from commercially available pregnenolone using as a key step a Mukaiyama aldol reaction. This methodology involves a water-free work-up to isolate the silyl enol ether in order to prevent its hydrolysis. Based on the results of the biological tests, we can conclude that the replacement of the 21-CH_3 by a 20-keto group in the side chains of **4** and **5** (ligands of DAF-12 receptor and LXR respectively), causes the complete loss of the activity. These results are in agreement with the fact that the evolutionary conserved DAF-12 receptor and Liver X receptors are activated by similar cholesterol metabolites as mentioned above. Moreover the replacement of the hemisuccinate ester moiety on the bioactive steroid **6** for a non-hydrolysable hydrocarbonated side chain leads to the complete loss of activity which highlights the crucial role of the ester moiety on steroid **6** in the modulation of the glucocorticoid receptor activity.

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Appendix A. Supplementary data

^1H and ^{13}C NMR spectra of compounds **7**–**9**, **14**, **15**, **17**, **20**, **22**–**24** are available on the online version. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2017.03.003.

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