Novel glucocorticoid antedrugs possessing a C16,17-fused γ-lactone ring

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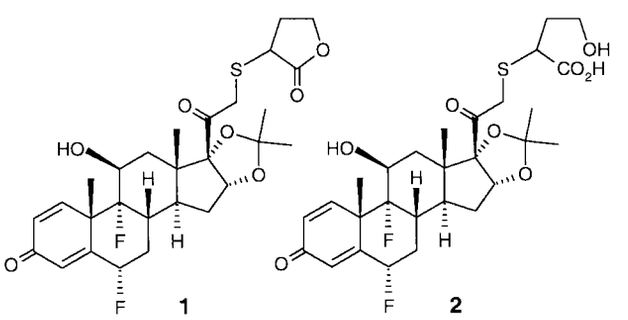
Received (in Cambridge, UK) 19th October 1999, Accepted 21st December 1999

A series of novel γ-butyrolactones fused at the 16β,17β position of the 9α-fluoro-11β-hydroxy-3-oxoandrosta-1,4-diene nucleus and possessing oxygen substituents at 16α,17α positions were prepared and tested as glucocorticoid agonists. The compounds were also tested for their lability in human plasma. Lactone **5** was found to be rapidly hydrolysed in plasma, whereas derivative **18**† was found to be a potent glucocorticoid agonist, but was stable in plasma. The structure of the C21*S* diastereoisomer of compound **18** was confirmed by an X-ray diffraction study.

Asthma is recognised as a chronic inflammatory disorder of the airways and currently, it is treated most effectively with anti-inflammatory glucocorticoids. However, long term treatment with steroids can be associated with adverse systemic effects such as growth retardation, osteoporosis, suppression of the hypothalamic–pituitary–adrenal function and of the immune system. The development of inhaled glucocorticoids which are efficiently inactivated in the liver and which show low oral bioavailability has led to a substantial reduction of the observed systemic effects.[1] Recently the terms 'antedrug'[2] or 'soft drug'[3] were introduced to describe drugs designed to act topically at the site of application but that are transformed into inactive metabolites upon entry into the systemic circulation. A number of publications have appeared that describe the synthesis of antiinflammatory glucocorticoid derivatives based on the antedrug concept.[4–11] These have generally involved the introduction of carboxylic ester functionality in the expectation that this will be hydrolysed by blood esterases to give inactive steroid carboxylic acid metabolites.

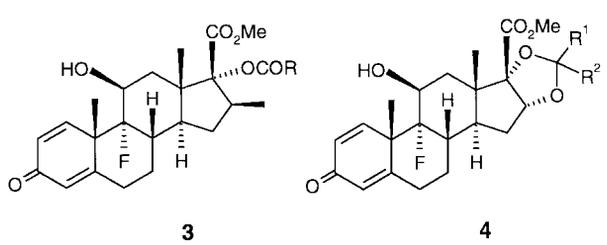
In our search for even safer inhaled glucocorticoids we have discovered that incorporation of a γ-lactone moiety provides derivatives which are extremely rapidly inactivated in plasma, but which show remarkable stability in lung S9 fraction. Thus, the potent antiinflammatory glucocorticoid **1** is hydrolysed in



1 **2**

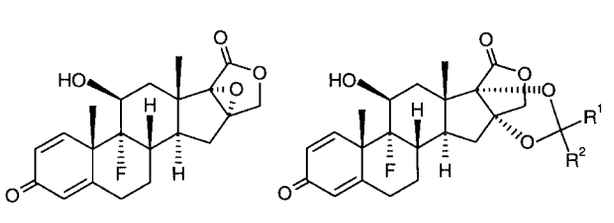
human plasma ($t_{\frac{1}{2}} < 2$ min) to the inactive hydroxy carboxylic acid **2**, but is essentially stable in human lung S9 ($t_{\frac{1}{2}} > 2$ h).[12] These ideal properties make such lactone derivatives excellent lung selective antedrug candidates for use in asthma. The enzyme responsible for the plasma hydrolysis of these γ-lactones was identified by our group as human serum paraoxonase [EC.3.1.8.1], a known enzyme that is thought to have a role in the metabolism of lipids and lipoproteins.[13] As far as we are aware, paraoxonase mediated hydrolysis of γ-lactones has not been reported prior to our disclosure. However, hydrolysis of simple γ-lactones by a mammalian enzyme described as lactonase has been reported by Fishbein and Bessman.[14] We believe that Fishbein and Bessman's lactonase and paraoxonase are probably the same enzyme.

As part of our medicinal chemistry project we have investigated the structural requirements for paraoxonase mediated hydrolysis of a variety of glucocorticoid lactone derivatives. In this paper we describe the synthesis and structure–activity relationships of a series of glucocorticoids possessing a fused γ-lactone at C16 and C17 of the steroidal nucleus. Glucocorticoids possessing a small 17β-carboxylic ester and a 17α-ester[15] **3** or 16,17-acetal/ketal[16] **4** are very potent anti-



3 **4**

inflammatory agents. We envisaged preparing analogues where the 17β-ester was locked in a rigid conformation in the form of a C16,17-fused γ-lactone and having oxygen substituents at C16 and 17 such as the epoxide **5** or acetal **6**. Metabolism of these



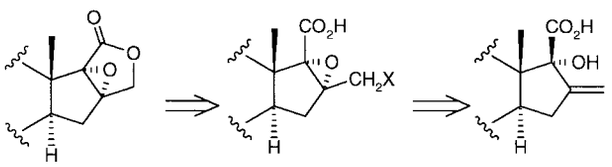
5 **6**

fused lactones would give 17β-carboxylic acid derivatives which would be expected to show only weak glucocorticoid agonist activity.[17]

† The numbering used in the discussion and NMR data is exemplified in Scheme 4 for compound **18**.

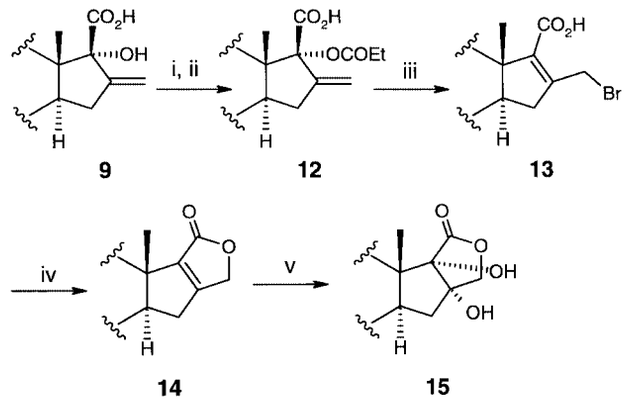
DOI 10.1039/a908358h

Our retro-synthesis of **5** involved a disconnection of the lactone ring to the 17β-carboxylic acid and a hydroxymethyl or bromomethyl group at 16β, which we expected to prepare from the known 16-*exo* methylene derivative (Scheme 1). The synthesis of **5** in the forward sense started with the allylic alcohol **7** (Scheme 2), which was obtained by the method of Taub *et al.*[18] Saponification of the acetate **7** with potassium carbonate under nitrogen gave 9α-fluoro-16-methyleneprednisolone[19] **8**, which was then oxidised with periodic acid to the key intermediate **9**.[15] An attempt to epoxidise **9** with *m*-chloroperoxybenzoic acid in chloroform for 4 days failed; only starting material was recovered. However, treatment of **9** with 1,3-dibromohydantoin in the presence of aqueous perchloric acid gave the bromomethyl epoxide **10** *via* the initial formation of the 16-*exo* bromonium ion, followed by internal trapping by the 17α-hydroxy group. The synthesis of **5** was completed by intramolecular displacement of the bromine in the 16β-bromomethyl group by the 17β-carboxylate ion (potassium carbonate in DMF) to give the required epoxy-γ-lactone in 57% overall yield for the 4 steps. Hydrolysis of **5** with sodium hydroxide gave the hydroxy acid **11**, which was used as an authentic sample for the plasma hydrolysis product of lactone **5**. We were pleased to find that the lactone **5** was rapidly hydrolysed ($t_{\frac{1}{2}}$ 15 min) by human plasma to **11**. However, lactone **5** was found to have no useful glucocorticoid agonist activity. Nevertheless this result encouraged us to investigate the synthesis of the lactones **6**.



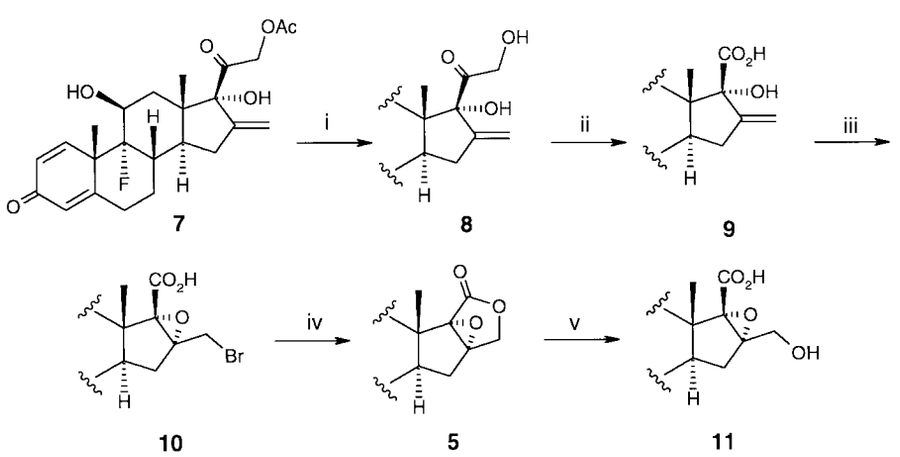
Scheme 1

The synthesis of acetals **6** required the preparation of the 16α,17α-diol **15**, and this was again derived from the key intermediate **9** as shown in Scheme 3. Selective acylation of the 17α-hydroxy group without concomitant 11β-acylation was achieved by reaction of **9** with excess propionyl chloride and triethylamine, followed by aminolysis of the resulting mixed anhydride with diethylamine.[20] Treatment of **12** with hydrogen bromide in dichloromethane furnished the allylic bromide **13** which was cyclised with potassium carbonate in DMF to the αβ-unsaturated lactone **14** in 50% overall yield. *cis*-Hydroxylation of αβ-unsaturated lactone **14** using aqueous potassium permanganate[21] in acetone in the presence of formic acid occurred exclusively from the less hindered α-face (81%). With the *cis*-diol in hand we were in a position to form ethylidene **16**, acetonide **17** and butylidene **18** derivatives with the appropriate carbonyl compound and acid catalysis (Scheme 4). The acetals **16** and **18** were obtained as diastereoisomeric mixtures at the acetal asymmetric centre. The isomers of the latter compound were separated by reversed phase HPLC and crystallisation, and their configuration at C21 was established by NOE experiments. Thus, for the C21*R* isomer **18R** NOEs were observed from 16′-H to 21-H and *vice versa*. For the C21*S*-isomer **18S** NOEs were observed from 21-H to 14-H, 22-H and 23-H, and from 16′-H to 15-H and 18-H. The structure of the C21*S* isomer (mp 317–319 °C) was confirmed by an X-ray diffraction experiment (Fig. 1).



Scheme 3 *Reagents and conditions*: i) EtCOCl, Et_3N, CH_2Cl_2; ii) Et_2NH, acetone; iii) HBr, CH_2Cl_2; iv) K_2CO_3, DMF; v) $KMnO_4$, H_2O, acetone, HCO_2H.

The glucocorticoid agonist activity of lactones **14**, **15**, **16**, **17** and the individual isomers of **18** were tested by the method of Stein and co-workers[22] in the HeLa sPAP screen, a functional *in vitro* assay. This test involves transfection of HeLa cells with a detectable reporter gene (secreted placental alkaline phosphatase, sPAP) under the control of a glucocorticoid response promoter (the LTR of the mouse mammary tumour virus, MMTV). Various concentrations of dexamethasone (used as standard) and the test compounds in Table 1 were incubated with transfected HeLa cells for 72 h. At the end of the incubation *p*-nitrophenyl acetate, used as substrate for sPAP, was added and the product concentration measured spectrophotometrically. Increased UV-absorbance reflected increased sPAP transcription and concentration–response curves were plotted so that ED_{50} values could be estimated. Dexamethasone exhibited ED_{50} values in the range of 10–15 nmol dm^{-3}. As it can be seen from the data in Table 1 the most potent activity was seen with the butylidene lactone **18** with the *R*-isomer being more active as expected.[23] The plasma stability of the compounds in



Scheme 2 *Reagents and conditions*: i) K_2CO_3, MeOH, dioxane; ii) H_5IO_6, THF, H_2O; iii) 1,3-dibromohydantoin, $HClO_4$, THF; iv) K_2CO_3, DMF; v) NaOH, H_2O, MeOH.

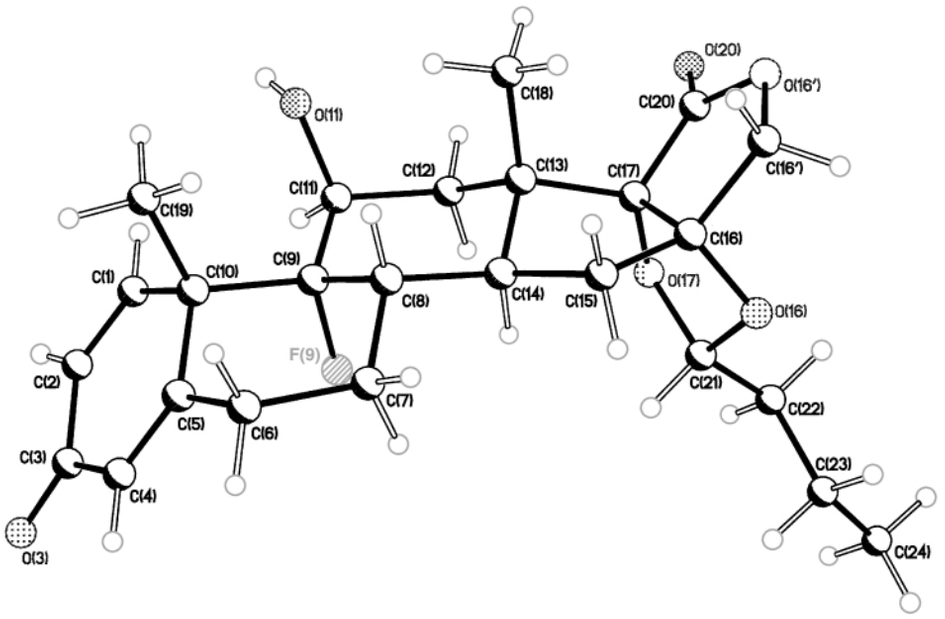
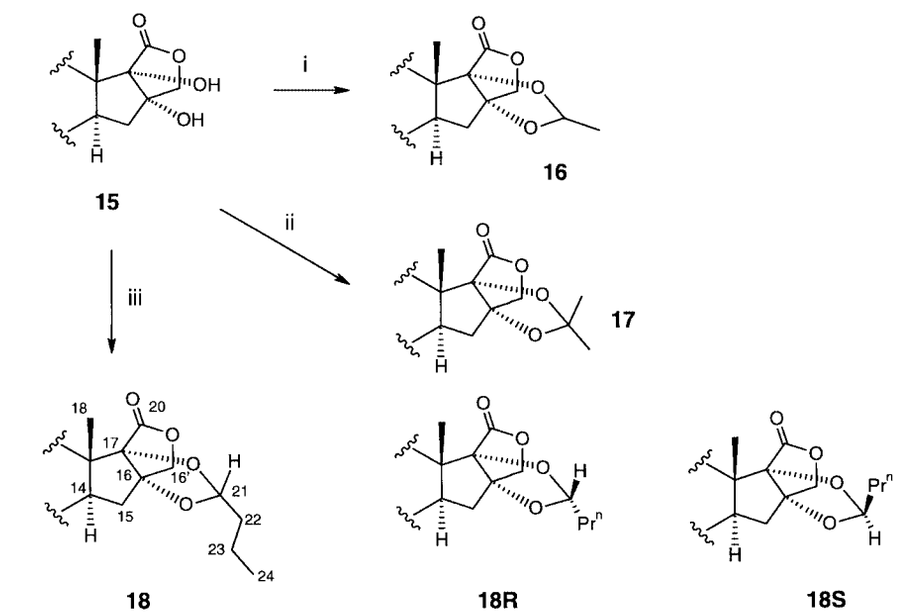


Fig. 1 X-Ray crystal structure of **18S**.



Scheme 4 *Reagents and conditions*: i) MeCHO, HCl, DMF; ii) acetone, 2,2-dimethoxypropane, HCl; iii) butyraldehyde, HCl, DMF.

Table 1 *In vitro* glucocorticoid agonist activity and human plasma stability

Compound number	HeLa/ nmol dm^{-3}	Plasma stability (%)
5	>10000	0
11	>10000	100
14	>10000	95
15	>10000	99
16	125	100
17	66	97
18	5	94
18 (*S*)	14	—
18 (*R*)	4	—
Dexamethasone	10	—

Table 1 was estimated by HPLC after their incubation in human plasma for 1 h at 37 °C. The results are expressed as % of compound remaining after 1 h. Unfortunately all the compounds in this series except the epoxy lactone **5** were stable in plasma. It is possible that the severe congestion around the steroid ring *D*, where three five-membered rings are fused together, is the most likely explanation for their plasma stability.

In conclusion, novel glucocorticoids were prepared and tested for glucocorticoid agonist activity. Butylidene lactone **18** was found to be a very potent glucocorticoid agonist. The compounds were also tested for hydrolysis in plasma. Lactone **5** was found to be rapidly hydrolysed in plasma but the 16,17-acetal derivatives were found to be stable in plasma.

Experimental

Organic solutions were dried over $MgSO_4$ and column chromatography was performed on silica gel 60 (Merck, Art no. 9385). Analytical HPLC was performed on a S5 ODS-2 column (15 × 0.46 cm) using 35% MeCN–H_2O for 25 min, increasing to 70% in 10 min and then holding at 70% for 20 min, flow rate 2 cm^3 min^{-1} and detecting at 254 nm. Preparative HPLC was conducted on a Prochrom ODS-2 column (38.5 × 5 cm) using MeCN–H_2O as eluent, flow rate 40 cm^3 min^{-1} and detecting

at 254 nm. IR spectra were recorded on a Nicolet 5SXC or a Bio-Rad FTS-7 FT-IR spectrometer. NMR spectra of compounds in DMSO-d_6 solutions were recorded on a Bruker AM500, AM250 or VarianVXR 400 spectrometer using standard pulse sequences. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. All J values are in Hz. Mass spectral abbreviations are as follows: ES+ve electrospray positive, TSP+ve thermospray positive or LSIMS+ve liquid secondary isotope mass spectrometry.

9α-Fluoro-11β,17α,21-trihydroxy-16-methylenepregna-1,4-diene-3,20-dione 8

A solution of 21-acetoxy-9α-fluoro-11β,17α-dihydroxy-16-methylenepregna-1,4-diene-3,20-dione (**7**) (500 mg, 1.16 mmol) in dioxane (10 cm^3) and methanol (10 cm^3) was deoxygenated by bubbling nitrogen through the solution for 15 min. Potassium carbonate (141 mg, 1.02 mmol) in water (1 cm^3) was added and the mixture was stirred under nitrogen for 1.5 h. Glacial acetic acid was added and the solvents were removed under reduced pressure. The residue was partitioned between ethyl acetate and water, and the organic phase was separated. The solution was washed with water, dried and evaporated to dryness. The solid was triturated in diethyl ether and the product collected by filtration to give **8** (423 mg, 93%) as a white solid: δ_H (250 MHz) 7.29 (1H, d, J 10, 1-H), 6.23 (1H, dd, J 10 and 2, 2-H), 6.02 (1H, s, 4-H), 5.51 (1H, s, 17-OH), 5.31 (1H, m, 11-OH), 5.12 (1H, s, 16′-H), 4.88 (1H, s, 16′-H), 4.69 (1H, t, J 6, 21-OH), 4.52 (1H, dd, J 19 and 6, 21-H), 4.17 (1H, m, 11-H), 4.13 (1H, dd, J 19 and 6, 21-H), 1.50 (3H, s, 19-H) and 0.80 (3H, s, 18-H) (Found: C, 66.7; H, 7.2; F, 4.7. $C_{22}H_{27}FO_5 \cdot 0.33H_2O$ requires C, 66.65; H, 7.0; F, 4.8%).

9α-Fluoro-11β,17α-dihydroxy-16-methylene-3-oxoandrosta-1,4-diene-17β-carboxylic acid 9

A suspension of **8** (500 mg, 1.28 mmol) in THF (5 cm^3) was treated with periodic acid (438 mg, 1.92 mmol) in water (1.5 cm^3) and the mixture was stirred for 6 h. A further portion of periodic acid (65 mg, 0.28 mmol) was added and the mixture was stirred for 19 h. The solvent was removed under reduced pressure and the residue was stirred in water for 2.5 h. The solid was collected by filtration, washed with water, diethyl ether and dried *in vacuo* at 40 °C for 18 h to give **9** (431 mg, 89%) as a cream solid: δ_H (250 MHz) 12.3 (1H, br s, CO_2H), 7.29 (1H, d, J 10, 1-H), 6.23 (1H, dd, J 10 and 2, 2-H), 6.02 (1H, s, 4-H), 5.27 (1H, m), 5.21 (1H, s), 5.18 (1H, s), 5.12 (1H, s), 4.16 (1H, m, 11-H), 1.51 (3H, s, 19-H) and 0.93 (3H, s, 18-H) (Found: C, 64.0; H, 7.0; F, 4.8. $C_{21}H_{25}FO_5 \cdot H_2O$ requires C, 63.9; H, 6.9; F, 4.8%).

16β-Bromomethyl-16α,17α-epoxy-9α-fluoro-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylic acid 10

A solution of **9** (600 mg, 1.59 mmol) in THF (30 cm^3) was cooled to 2 °C and treated with 3% aqueous perchloric acid (15 cm^3), followed by 1,3-dibromo-5,5-dimethylhydantoin (331 mg, 1.16 mmol). The solution was allowed to warm to 20 °C over 1 h, and then $NaHSO_3$ was added portionwise until a test with KI–starch paper was negative. The mixture was poured into water and extracted with ethyl acetate. The organic extracts were washed with water, brine, dried and evaporated to dryness to give **10** (724 mg, 100%). An analytical sample was obtained after recrystallisation from methanol to give **10** as white crystals: mp 197–199 °C (decomp.); δ_H (250 MHz) 7.28 (1H, d, J 10, 1-H), 6.23 (1H, dd, J 10 and 2, 2-H), 6.02 (1H, s, 4-H), 5.48 (1H, s), 4.12 (1H, m, 11-H), 3.99 (1H, d, J 10, 16′-H), 3.90 (1H, d, J 10, 16′-H), 1.50 (3H, s, 19-H) and 1.29 (3H, s, 18-H) (Found: C, 55.5; H, 5.4; Br, 17.3. $C_{21}H_{24}BrFO_5$ requires C, 55.4; H, 5.3; Br, 17.5%).

16α,17α-Epoxy-9α-fluoro-11β-hydroxy-2′,3′,4′,5′-tetrahydrofuro[3′,4′ : 16,17]androsta-1,4-diene-3,5′-dione 5

A solution of **10** (772 mg, 1.59 mmol) in DMF (23 cm^3) was treated with anhydrous potassium carbonate (330 mg, 2.39 mmol) and the mixture was stirred for 1.5 h, before it was poured into dilute hydrochloric acid solution. The solid was collected by filtration and washed with water. The solid was crystallised from hot acetonitrile and the crystals were triturated in ethyl acetate to give **5** (411 mg, 69%): mp 315–320 °C; ν_{max} (Nujol)/cm^{-1} 1775, 1660 and 1600; δ_H (250 MHz) 7.29 (1H, d, J 10, 1-H), 6.23 (1H, dd, J 10 and 2, 2-H), 6.02 (1H, s, 4-H), 5.62 (1H, m), 4.55 (1H, d, J 11, 16′-H), 4.46 (1H, d, J 11, 16′-H), 4.12 (1H, m, 11-H), 1.51 (3H, s, 19-H) and 1.27 (3H, s, 18-H) (Found: C, 67.7; H, 6.2. $C_{21}H_{23}FO_5$ requires C, 67.4; H, 6.2%).

16α,17α-Epoxy-9α-fluoro-11β-hydroxy-16β-hydroxymethyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid 11

A suspension of the lactone **5** (15 mg, 0.04 mmol) in methanol (0.5 cm^3) was treated with 0.1 mol dm^{-3} sodium hydroxide (0.42 cm^3) and the mixture was stirred for 24 h. More sodium hydroxide (0.1 cm^3) and methanol (0.2 cm^3) was added and the mixture was stirred for a further 18 h. The reaction mixture was diluted with ethyl acetate, acidified with dilute hydrochloric acid and washed with brine. The organic solution was dried, concentrated and triturated in diethyl ether. The solid was dried *in vacuo* for 18 h at 40 °C to give acid **11** (12 mg, 76%) as a white solid: mp 163–164 °C; δ_H (400 MHz) 7.26 (1H, d, J 10, 1-H), 6.19 (1H, dd, J 10 and 2, 2-H), 5.99 (1H, s, 4-H), 5.38 (1H, br s, 11-H), 4.08 (1H, m, 11-OH), 3.74 (1H, d, J 12, 16′-H), 3.65 (1H, d, J 12, 16′-H), 1.90 (1H, d, J 13), 1.49 (3H, s, 19-H) and 1.22 (3H, s, 18-H); MS(FAB+ve) m/z 393 $(M + H)^+$. Found: (LSIMS+ve) 393.1714 $(M + H)^+$. $C_{21}H_{26}FO_6$ requires (M + H) 393.1713.

9α-Fluoro-11β-hydroxy-16-methylene-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carboxylic acid 12

A stirred suspension of **9** (1.38 g, 3.66 mmol) in dichloromethane (36 cm^3) was cooled to 0 °C and treated with triethylamine (1.7 cm^3, 12.2 mmol). The clear solution was treated with propionyl chloride (1.12 cm^3, 12.9 mmol) and the mixture was stirred for 1 h. The reaction mixture was diluted with dichloromethane and washed with sodium bicarbonate solution, dilute hydrochloric acid and water, dried and evaporated to a white foam. This was dissolved in acetone (55 cm^3) and diethylamine (1.33 cm^3, 12.9 mmol) was added. The mixture was stirred for 1 h at 20 °C and the solid was collected by filtration. The solid was dissolved in water, acidified with hydrochloric acid and extracted with ethyl acetate. The organic extracts were dried, and evaporated to dryness to give **12** (1.576 g, 99%). An analytical sample was obtained by recrystallising a portion in acetone–light petroleum: ν_{max} (KBr)/cm^{-1} 3500–2500, 1732, 1714, 1667 and 1651; δ_H (250 MHz) 7.29 (1H, d, J 10, 1-H), 6.24 (1H, dd, J 10 and 2, 2-H), 6.03 (1H, s, 4-H), 5.63 (1H, s, 11-OH), 5.45–5.33 (2H, s, 16′-H), 4.20 (1H, m, 11-H), 1.51 (3H, s, 19-H), 0.98 (3H, s, 18-H) and 0.97 (3H, t, J 7, OCH_2CH_3); MS(ES+ve) m/z 433 $(M + H)^+$. Found: (ES+ve) $(M + H)^+$, 433.2037. $C_{24}H_{30}FO_6$ requires (M + H), 433.2026.

16-Bromomethyl-9α-fluoro-11β-hydroxy-3-oxoandrosta-1,4,16-triene-17β-carboxylic acid 13

A solution of **12** (663 mg, 1.53 mmol) in dichloromethane (90 cm^3) was treated with hydrogen bromide gas for 15 min and then stirred at 20 °C for 21 h. The mixture was diluted with dichloromethane and washed with water, dried and concentrated to a volume of about 10 cm^3, whereupon crystallisation occurred. The crystals were collected by filtration, washed with dichloromethane and dried *in vacuo* for 20 h to give **13** (488 mg,

73%) as a pale green solid: ν_{max} (Nujol)/cm^{-1} 3600–2100, 3344, 1679, 1659, 1615 and 1602; δ_H (400 MHz) 12.65 (1H, br s, CO_2H), 7.31 (1H, d, *J* 10, 1-H), 6.22 (1H, dd, *J* 10 and 2, 2-H), 6.02 (1H, br s, 4-H), 5.44 (1H, br s, 11-OH), 4.63 (1H, d, *J* 10, 16′-H), 4.47 (1H, d, *J* 10, 16′-H), 4.09 (1H, m, 11-H), 1.53 (3H, s, 19-H) and 1.21 (3H, s, 18-H); δ_C (100 MHz) 185.0 (C3), 166.4 (C20), 165.4 (C5), 152.3 (C1), 149.8 (C16), 142.7 (C17), 128.9 (C2), 124.2 (C4), 101.4 (d, *J* 175, C9), 70.7 (d, *J* 37, C11), 47.9 (d, *J* 23, C10), 47.3 (C13), 46.5 (C14), 40.1 (C12), 34.9 (C16′), 32.4 (d, *J* 19, C8), 30.0 (C15), 29.0 (C6), 26.5 (C7), 22.5 (d, *J* 6, C19) and 17.0 (C18); MS(FAB+ve) *m*/*z* 439 and 441 $(M + H)^+$; LSIMS(−ve) *m*/*z* 437 and 439 $(M - H)^-$. Found: (LSIMS+ve) 439.0917 $(M + H)^+$. $C_{21}H_{25}BrFO_4$ requires (*M* + H) 439.0920.

9α-Fluoro-11β-hydroxy-2′,5′-dihydrofuro[3′,4′ : 16,17]-androsta-1,4,16-triene-3,5′-dione 14

A solution of the bromide **13** (351 mg, 0.80 mmol) in DMF (10 cm^3) was treated with solid anhydrous potassium carbonate (122 mg, 0.88 mmol) and the mixture was stirred for 2 h at 20 °C. The mixture was diluted with ethyl acetate and washed with water, brine, dried and concentrated to about 5 cm^3 whereupon crystallisation occurred. The crystals were collected by filtration, washed with ethyl acetate, and dried *in vacuo* at 40 °C for 18 h to give **14** (197 mg, 69%) as white crystals: ν_{max} (Nujol)/cm^{-1} 3444, 1735, 1668 and 1629; δ_H (400 MHz) 7.32 (1H, d, *J* 10, 1-H), 6.24 (1H, dd, *J* 10 and 2, 2-H), 6.05 (1H, br s, 4-H), 5.56 (1H, br d, *J* 4, 11-OH), 4.91 (1H, d, *J* 18, 16′-H), 4.82 (1H, d, *J* 18, 16′-H), 4.12 (1H, m, 11-H), 1.54 (3H, s, 19-H), 1.22 (3H, s, 18-H); δ_C (100 MHz) 185.0 (C3), 173.6 (C20), 168.6 (C16), 166.2 (C5), 152.3 (C1), 143.8 (C17), 128.9 (C2), 124.3 (C4), 101.5 (d, *J* 175, C9), 70.4 (d, *J* 39, C11), 69.7 (C16′), 53.7 (C14), 47.9 (d, *J* 23, C10), 41.2 (C13), 38.9 (C12), 32.7 (d, *J* 20, C8), 29.9 (C15), 28.8 (C6), 26.5 (C7), 22.5 (d, *J* 6, C19) and 18.2 (C18); MS(TSP+ve) *m*/*z* 359 $(M + H)^+$ (Found: C, 68.95; H, 6.6; F, 5.1. $C_{21}H_{23}FO_4 \cdot 0.4C_4H_8O_2$ requires C, 68.95; H, 6.7; F, 4.8%).

9α-Fluoro-11β,16α,17α-trihydroxy-2′,3′,4′,5′-tetrahydrofuro-[3′,4′ : 16,17]androsta-1,4-diene-3,5′-dione 15

A solution of the unsaturated lactone **14** (1.444 g, 4.03 mmol) in acetone (235 cm^3) and formic acid (0.71 cm^3, 18.8 mmol) was cooled to −7 °C and then treated dropwise with a chilled solution of potassium permanganate (670 mg, 4.24 mmol) in water (7 cm^3) and acetone (21 cm^3). The solution was stirred at −6 °C for 45 min, aqueous sodium metabisulfite solution (10%, 70 cm^3) was added over 15 min, and then allowed to warm to 20 °C. The mixture was concentrated under reduced pressure and the residue was triturated in water (100 cm^3). The solid was collected by filtration, washed with water, ethyl acetate and dried at 60 °C *in vacuo* for 20 h over P_2O_5 to give **15** (1.285 g, 81%) as a cream coloured crystalline solid: mp >330 °C; ν_{max} (KBr)/cm^{-1} 3430, 1754, 1664 and 1620; δ_H (400 MHz) 7.28 (1H, d, *J* 10, 1-H), 6.22 (1H, dd, *J* 10 and 2, 2-H), 6.02 (1H, s, 4-H), 5.58 (1H, s, OH), 5.40–5.34 (2H, m), 4.34 (1H, d, *J* 11, 16′-H), 4.19 (1H, d, *J* 11, 16′-H), 4.16 (1H, m, 11-H), 1.50 (3H, s, 19-H) and 1.03 (3H, s, 18-H); δ_C (100 MHz) 185.0 (C3), 166.5 (C20), 166.4 (C5), 152.4 (C1), 128.9 (C2), 124.1 (C4), 100.8 (d, *J* 175, C9), 84.8 (C17-tentative), 81.4 (C16-tentative), 80.7 (C16′), 70.5 (d, *J* 37, C11), 47.8 (d, *J* 23, C10), 45.5 (C13), 44.7 (C14), 40.6 (C12), 35.4 (C15), 33.0 (d, *J* 20, C8), 30.0 (C6), 27.1 (C7), 22.8 (d, *J* 6, C19) and 15.6 (C18); MS(TSP+ve) *m*/*z* 393 $(M + H)^+$ (Found: C, 63.9; H, 6.3. $C_{21}H_{25}FO_6$ requires C, 64.25; H, 6.4%).

16α,17α-Ethylidenedioxy-9α-fluoro-11β-hydroxy-2′,3′,4′,5′-tetrahydrofuro[3′,4′ : 16,17]androsta-1,4-diene-3,5′-dione 16

A solution of the dihydroxylactone **15** (50 mg, 0.13 mmol) in DMF (1 cm^3) was treated with acetaldehyde (3 cm^3) and hydrogen chloride gas was bubbled through the solution for 6 min. The mixture was stirred at 20 °C for 1.5 h, diluted with ethyl acetate and poured into aqueous sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate and the combined organic extracts were washed with aqueous $NaHSO_3$ solution, water and brine, dried and concentrated. The residue was chromatographed on silica gel eluting with diethyl ether. Fractions containing the product were combined and evaporated. The residue was triturated in ether, dried *in vacuo* to give **16** (21 mg, 39%) as a fawn solid. Analytical HPLC indicated two diastereoisomers were present t_r 42.6 min 66% and 44.3 min 34%; ν_{max} (KBr)/cm^{-1} 3450, 1775, 1664 and 1620; δ_H (500 MHz) major isomer (66%) 7.27 (1H, d, *J* 10, 1-H), 6.23 (1H, dd, *J* 10 and 2, 2-H), 6.02 (1H, br s, 4-H), 5.52 (1H, m, 11-OH), 5.01 (1H, q, *J* 5, 21-H), 4.60 (1H, d, *J* 11, 16′-H), 4.51 (1H, d, *J* 11, 16′-H), 4.18 (1H, m, 11-H), 1.50 (3H, s, 19-H), 1.29 (3H, d, *J* 5, 22-H) and 1.08 (3H, s, 18-H); minor isomer (34%) 7.27 (1H, d, *J* 10, 1-H), 6.23 (1H, dd, *J* 10 and 2, 2-H), 6.02 (1H, br s, 4-H), 5.55 (1H, m, 11-OH), 5.54 (1H, q, *J* 5, 21-H), 4.49 (1H, d, *J* 11, 16′-H), 4.47 (1H, d, *J* 11, 16′-H), 4.18 (1H, m, 11-H), 1.50 (3H, s, 19-H), 1.23 (3H, d, *J* 5, 22-H) and 1.11 (3H, s, 18-H); LSIMS(+ve) *m*/*z* 419 $(M + H)^+$ (Found: C, 65.7; H, 6.4. $C_{23}H_{27}FO_6$ requires C, 66.0; H, 6.5%).

9α-Fluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-2′,3′,4′,5′-tetrahydrofuro[3′,4′ : 16,17]androsta-1,4-diene-3,5′-dione 17

A solution of the dihydroxylactone **15** (310 mg, 0.79 mmol) in DMF (6 cm^3) was treated with acetone (20 cm^3), 2,2-dimethoxypropane (20 cm^3), and then hydrogen chloride gas was bubbled through the solution for 4 min. The mixture was stirred at 20 °C for 40 min and then was diluted with ethyl acetate and aqueous sodium bicarbonate. The organic solution was washed with water and brine, dried, and concentrated. The residue was chromatographed on silica gel, eluting with ethyl acetate–cyclohexane (1 : 1) to give a yellow solid. This was triturated in diethyl ether (10 cm^3), the solid was collected by filtration, washed with more ether (5 × 2 cm^3), and dried *in vacuo* at 60 °C for 18 h to give **17** (195 mg, 57%) as a cream crystalline solid: mp 319–321 °C; ν_{max} ($CHBr_3$)/cm^{-1} 3593, 1777, 1664, 1626 and 1608; δ_H (250 MHz) 7.27 (1H, d, *J* 10, 1-H), 6.23 (1H, dd, *J* 10 and 2, 2-H), 6.02 (1H, s, 4-H), 5.52 (1H, br s, 11-OH), 4.59 (1H, d, *J* 11, 16′-H), 4.49 (1H, d, *J* 11, 16′-H), 4.19 (1H, m, 11-H), 1.50, 1.36, 1.27, 1.06 (4s, 3H each); MS(TSP+ve) *m*/*z* 433 $(M + H)^+$ (Found: C, 66.5; H, 6.7; F, 4.5. $C_{24}H_{29}FO_6$ requires C, 66.65; H, 6.75; F, 4.4%).

16α,17α-Butylidenedioxy-9α-fluoro-11β-hydroxy-2′,3′,4′,5′-tetrahydrofuro[3′,4′ : 16,17]androsta-1,4-diene-3,5′-dione 18

A solution of the dihydroxylactone **15** (316 mg, 0.805 mmol) in DMF (6.5 cm^3) and butyraldehyde (20 cm^3) was bubbled through with hydrogen chloride gas for 5 min. The mixture was stirred for 1 h and then was diluted with ethyl acetate and poured into aqueous sodium bicarbonate solution. The organic phase was washed with 10% aqueous sodium metabisulfite, water and brine, dried and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with ethyl acetate–cyclohexane (1 : 1) to give a solid (293 mg). This was triturated in diethyl ether (10 cm^3) and the solid was collected by filtration, washed with ether (3 × 5 cm^3) and dried *in vacuo* at 60 °C for 24 h to give **18** as a white solid (232 mg, 65%). Analytical HPLC indicated a mixture of the two diastereoisomers: 19.70 min 49.0% and 21.76 min 49.2%; ν_{max} (KBr)/cm^{-1} 3320, 1776, 1665 and 1620; δ_H (250 MHz) 7.28 (1H, d, *J* 10, 1-H), 6.24 (1H, d, *J* 10, 2-H), 6.03 (1H, s, 4-H), 5.56 (1H, m), 5.43 (0.5H, t, *J* 4, 21-H), 4.92 (0.5H, t, *J* 4, 21-H), 4.62 and 4.50 (2d, 1H each, *J* 11, 16′-H), 4.48 (1H, s), 4.19 (1H, m, 11-H), 1.50 (3H, s, 19-H), 1.12 and 1.09 (3H, 2s, 18-H), 0.86 and 0.85 (3H, 2t, *J* 7, 24-H); MS(TSP+ve) *m*/*z* 447 $(M + H)^+$ (Found: C, 67.05; H, 6.9; F, 4.3. $C_{25}H_{31}FO_6$ requires C, 67.25;

H, 7.0; F, 4.25%). The two diastereoisomers were separated by preparative HPLC: the more polar isomer (62 mg), which was recrystallised from ethyl acetate, gave the C21 *R*-isomer as a white solid: mp 273–277 °C; analytical HPLC t_r 18.48 min 89.3% (contains 8.5% of *S*-isomer); δ_H (400 MHz) 7.27 (1H, d, *J* 10, 1-H), 6.23 (1H, dd, *J* 10 and 2, 2-H), 6.02 (1H, br s, 4-H), 5.50 (1H, br d, *J* 4, 11-OH), 4.99 (1H, t, *J* 4, 21-H), 4.59 (1H, d, *J* 11, 16′-H), 4.50 (1H, d, *J* 11, 16′-H), 4.19 (1H, m, 11-H), 1.50 (3H, s, 19-H), 1.08 (3H, s, 18-H) and 0.86 (3H, d, *J* 7, 24-H). NOE's were observed from 16′-H to 21-H and from 21-H to 16′-H. The second isomer (87 mg) was recrystallised from ethyl acetate to give the C21 *S*-isomer as a crystalline solid: mp 317–319 °C; analytical HPLC t_r 20.42 min 97.2% (contains 2.8% of the *R*-isomer); δ_H (400 MHz) 7.27 (1H, d, *J* 10, 1-H), 6.23 (1H, dd, *J* 10 and 2, 2-H), 6.02 (1H, br s, 4-H), 5.53 (1H, dd, *J* 4 and 1, 11-OH), 5.41 (1H, t, *J* 4, 21-H), 4.47 (2H, s, 16′-H), 4.18 (1H, m, 11-H), 1.50 (3H, s, 19-H), 1.13 (3H, s, 18-H) and 0.85 (3H, d, *J* 7, 24-H). NOE's were observed from 21-H to 14-H, 22-H and 23-H, and from 16′-H to 15-H and 18-H.

Crystal structure analysis of 18.‡ Crystal data for $C_{25}H_{31}FO_6$: $M = 446.5$, monoclinic, $a = 6.103(2)$, $b = 13.105(2)$, $c = 14.153(2)$ Å, $\beta = 94.42(2)°$, $V = 1128.5(4)$ Å^3, $T = 160$ K. Space group $P2_1$ (No. 4), $Z = 2$, μ(Cu-Kα) = 0.813 mm^{-1}, 3304 reflections were measured of which 3171 were unique, $R_{int} = 0.0711$. The final $wR(F^2)$ was 0.1707 and R_{obs} was 0.0730.

Acknowledgements

We thank Mrs Y. E. Solanke and Mrs S. Walton for conducting the biological assays.

‡ CCDC reference number 207/390. See http://www.rsc.org/suppdata/p1/a9/a908358h for crystallographic files in .cif format.

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Paper a908358h