Novel glucocorticoid antedrugs possessing a 21-(γ-lactone) ring

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A series of novel pregnane derivatives bearing γ-butyrolactones at C21 were prepared and tested as glucocorticoid agonists. The compounds were also tested for their lability in human plasma, and found to be rapidly hydrolysed by the enzyme paraoxonase to the respective hydroxyacids.

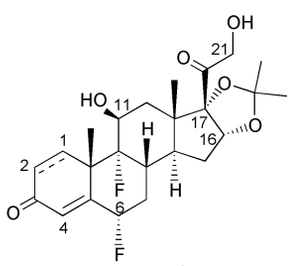
Introduction

The incidence of asthma in developed countries is increasing and is responsible for an increasing proportion of healthcare costs. Inhaled glucocorticoids are currently used for the treatment of all types of asthma, including mild forms of the disease, and for this reason the search for new and even safer glucocorticoids is continuing.

The terms 'antedrug'[1] and 'soft drug'[2] were introduced to describe drugs designed to act topically at the side of application but that are transformed into inactive metabolites upon entry into the systemic circulation. Recently our group has discovered that the incorporation of a γ-lactone moiety provides derivatives which are extremely rapidly inactivated in plasma but which show remarkable stability in human lung S9 fraction.[3,4] These ideal properties make such derivatives excellent lung selective antedrug candidates for use in asthma. The enzyme responsible for the plasma hydrolysis of these lactones was identified by our group[3] as human serum paraoxonase (EC 3.1.8.1), an organophosphate-detoxifying enzyme whose natural substrates have recently been shown to include homocysteine thiolactone.[5]

As part of our medicinal chemistry programme we have investigated the scope for paraoxonase-mediated hydrolysis of a variety of glucocorticoid lactone derivatives and have recently reported on some 16,17-fused γ-lactones,[6] and on 17β-sulfur linked butyrolactones.[4]

Substitution of the 21-hydroxy group of fluocinolone acetonide (**1a**) by a small alkylthio group such as methylthio was reported by Mitsukuchi *et al.* to enhance topical antiinflammatory activity measured by vasoconstrictive activity in humans.[7] In addition butixocort propionate, a C21 thiopropionate ester,[8] was progressed by Jouveinal to phase II clinical studies as an inhaled antiinflammatory agent for the treatment of asthma. Furthermore, butixocort 21-propionate was found to undergo ester hydrolysis in rat lung to the corresponding 21-thiol, and subsequently methylation of the resulting thiol to the *S*-methyl derivative. Both metabolites were found to be active with topical activity similar to that of the parent compound.[9] We envisaged that incorporation of a γ-lactone linked *via* sulfur at C21 would retain glucocorticoid activity, however, the metabolism of such analogues was not easily predicted. In our preliminary communication[3] we have reported that the lactone **2a** was stable in human lung S9 fraction, but rapidly hydrolysed in human plasma ($t_{½}$ < 1 min) to the corresponding hydroxyacid **3a**. In this paper we report the synthesis and structure–activity relationships of a series of pregnanes possessing a γ-lactone at C21.



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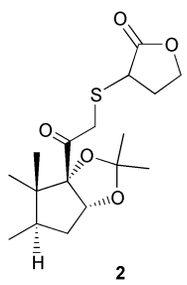
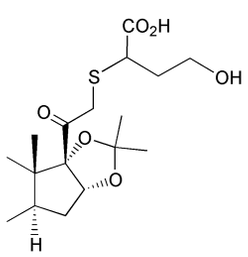


2

a = 1,4-diene
b = 1,2-dihydro



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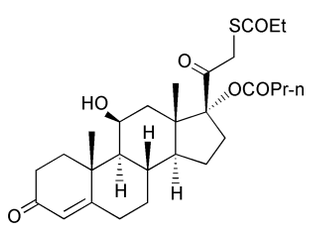

Butixocort propionate

Results and discussion

Lactone **2a** (1 : 1 mixture of diastereoisomers) was prepared by displacement of the 21-methanesulfonate **4a**[7] with α-mercapto-γ-butyrolactone **5**[10] or alternatively from the 21-mercaptopregnane **6a**[7] and commercially available α-bromo-γ-butyrolactone **7**. The resulting diastereoisomers **2aS** and **2aR** were readily separated by reverse phase preparative HPLC (Scheme 1). The absolute configuration of the individual diastereoisomers was assigned following diastereoselective synthesis of **2aS** starting from the commercially available (*R*)-(+)-α-hydroxy-γ-butyrolactone **8R** as shown in Scheme 2. Hydroxylactone **8R**, which can also be conveniently prepared from (*R*)-malic acid,[11] was converted to the corresponding crystalline methanesulfonate **9R** with methanesulfonyl chloride in the presence of triethylamine. The absolute configuration of **2aS** was confirmed by an X-ray diffraction study (Fig. 1). Similarly the (*R*)-diastereoisomer **2aR** was obtained from the (*S*)-(−)-α-hydroxy-γ-butyrolactone **8S** *via* the corresponding methanesulfonate **9S**.

The β-linked lactone **11a** was prepared from the thiol **6a** and furan-2(5*H*)-one (**10**) in the presence of potassium carbonate

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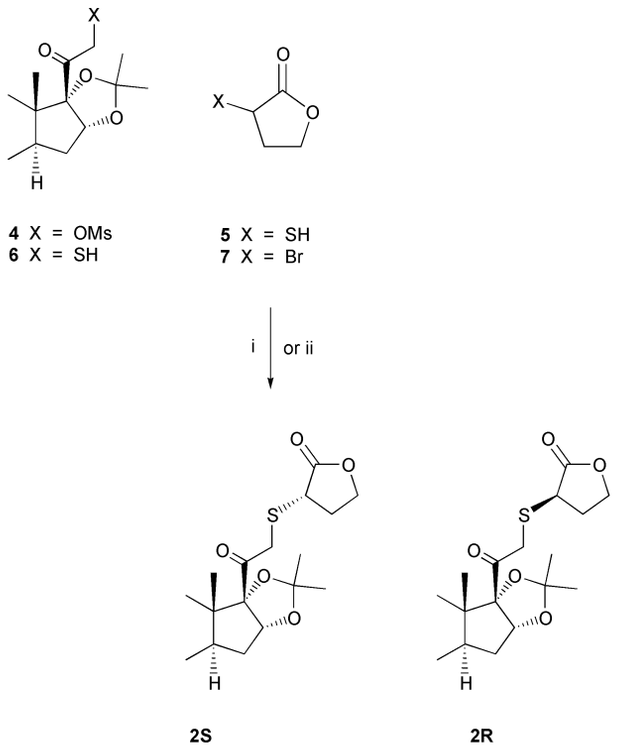
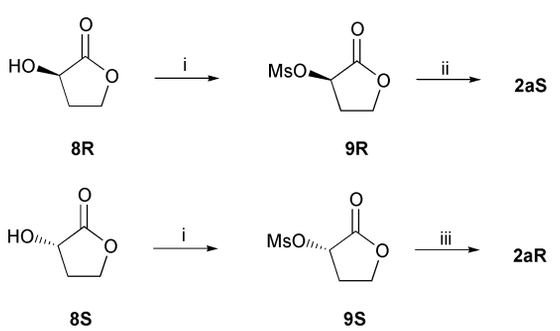


Scheme 1 *Reagents and conditions*: i) NaH, THF, 0 °C; ii) Et_3N, CH_2Cl_2, 0 °C.



Scheme 2 *Reagents and conditions*: CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C; ii) **6a**, NaH, DMF, 0 °C; iii) **6a**, K_2CO_3, DMF, 20 °C.

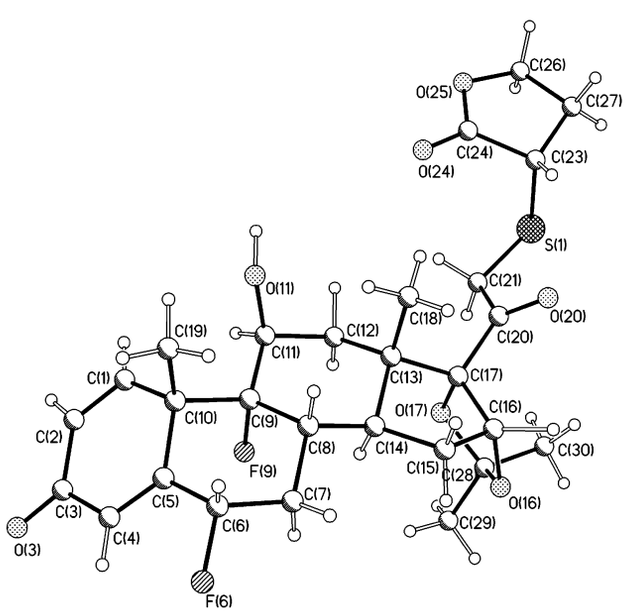
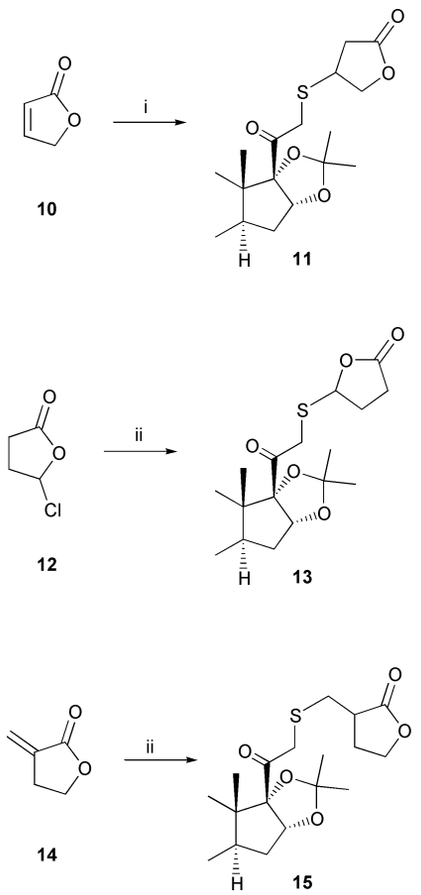


Fig. 1 X-Ray crystal structure of **2aS**.

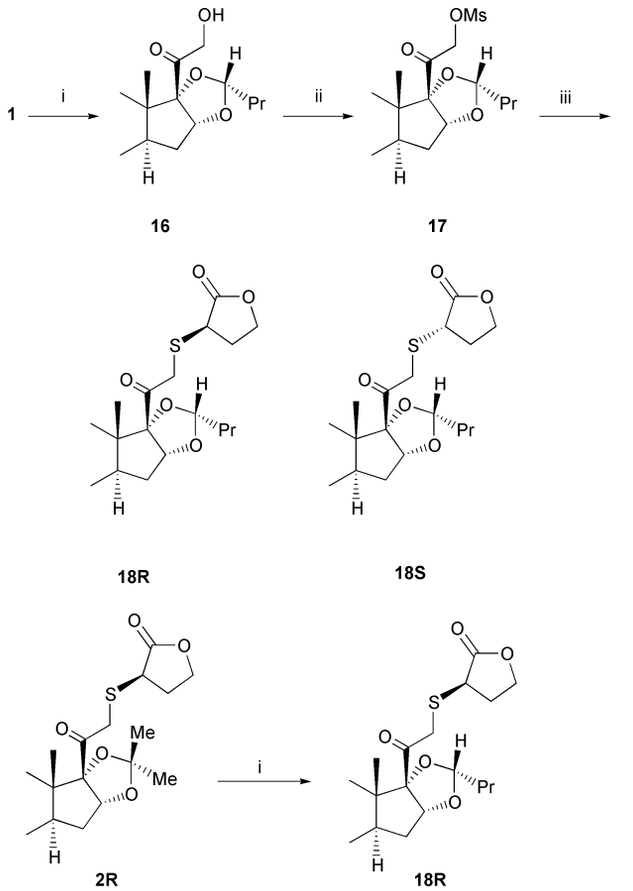


Scheme 3 *Reagents and conditions*: i) K_2CO_3, **6**, DMF, 20 °C; ii) Et_3N, **6**, THF, 20 °C.

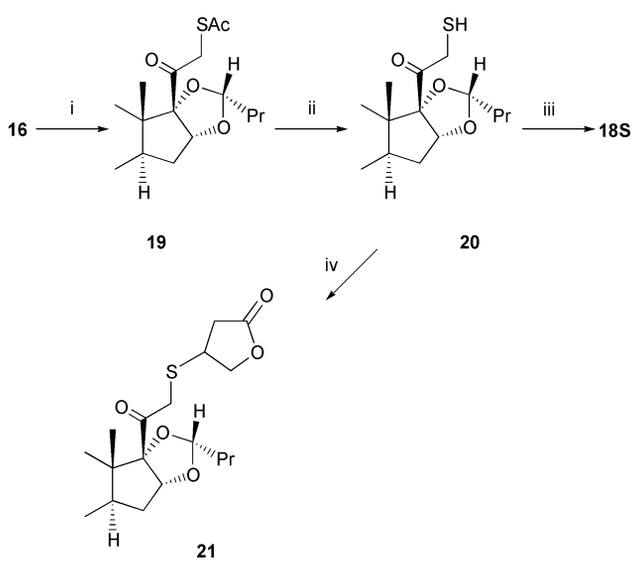
in DMF, and was obtained as an inseparable mixture of diastereoisomers in 79% yield (Scheme 3). The analogous γ-linked lactones **13a** were prepared from thiol **6a** and γ-chloro-γ-butyrolactone **12**.[12] The individual diastereoisomers were separable by silica gel chromatography (26 and 13% respectively), but their configuration at the lactone asymmetric centre was not established. Addition of **6a** to α-methylene-γ-butyrolactone (**14**) in the presence of triethylamine provided the homologous lactone **15a** as a 3 : 1 mixture of diastereoisomers (55%).

The dihydro α-linked lactones **2bS** and **2bR** were prepared from dihydro methanesulfonate **4b**[7] and thiol **5**. The diastereoisomers were separated by preparative HPLC (34 and 28% respectively). Glucocorticoids with the 16,17-butylidene moiety are generally more potent than the corresponding 16,17-isopropylidenes and it was thus important to synthesise such analogues. The lactones in the butylidene series were prepared in a similar way to the acetonide series starting from **1a**. The acetonide group was replaced by the (*R*)-butylidene group by the Astra method[13] of transacetalisation to form **16a**, then converted to the methanesulfonate **17a** (97%), followed by displacement of the methanesulfonate with thiol **5** to give a mixture of diastereoisomers **18aR** and **18aS** (Scheme 4). The diastereoisomers were separated by HPLC and their configuration was established following diastereoselective synthesis of **18aR** from **2aR** using the Astra procedure.[13]

The dihydro analogues in the butylidene series were prepared from dihydro alcohol **16b**.[14] Thus reaction with methanesulfonyl chloride in pyridine gave the methanesulfonate **17b** (63%) which was reacted with thiol **5** to give the dihydro α-linked lactones **18bR** and **18bS** after preparative HPLC (18 and 45% respectively). The absolute configuration of **18bR** and **18bS** was established by diastereoselective synthesis (Scheme 5). Thus reaction of **16b** with thioacetic acid under Mitsunobu



Scheme 4 *Reagents and conditions*: i) n-PrCHO, sand, 70% $HClO_4$, heptane; ii) CH_3SO_2Cl, pyridine; iii) **5**, NaH, THF, 0 °C.



Scheme 5 *Reagents and conditions*: i) Ph_3P, diisopropyl azodicarboxylate, AcSH, THF, 0 °C; ii) H_2NNH_2, THF, −15 to 20 °C; iii) **9R**, K_2CO_3, DMF, or NaH, THF; iv) **10**, Et_3N, THF.

conditions gave the thioacetate **19b** (100%), which was converted to the thiol **20b** by treatment with hydrazine (91%). Reaction of **20b** with the (*R*)-methanesulfonate lactone **9R** gave the lactone **18bS** (37%). Reaction of thiol **20b** with furan-2(5*H*)-one (**10**) gave the β-linked lactone **21b** (71%) as a mixture of diastereoisomers (Scheme 5).

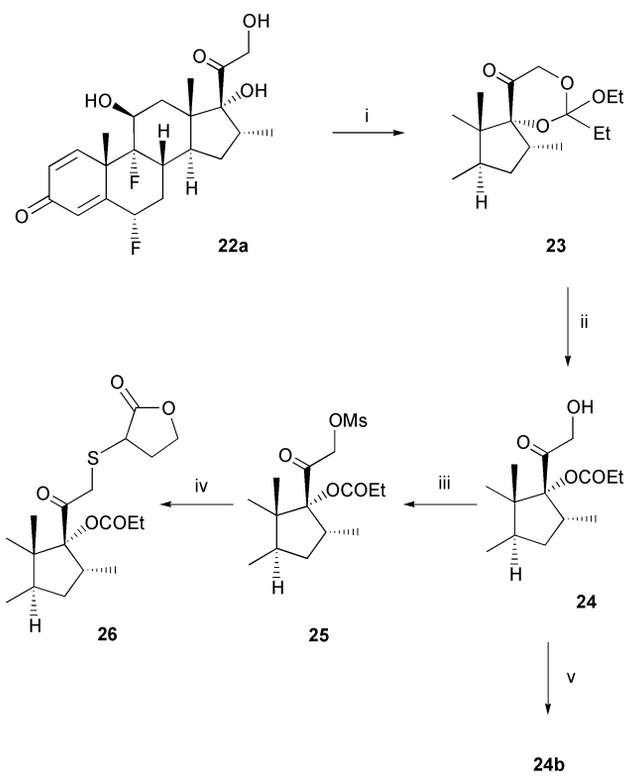
In addition to 16,17-acetonides and butylidenes, 16α-methyl-17α-propionate derivatives were synthesised, as these analogues are generally more potent glucocorticoids. Reaction of flumethasone **22a** with triethyl orthopropionate in the presence

Table 1 Human plasma stability and *in vitro* glucocorticoid agonist activity

Compound number	Plasma stability[a]	Relative potency
2aS	0	4.5
2aR	0	6.9
2bS	29	11.7
2bR	NT[b]	29
11a	0	7.9
13a isomer A	36	1.6
13a isomer B	49	1.2
15a	22	2.5
18aS	0	1.0
18aR	44	0.9
18bS	57	3.2
18bR	NT[b]	1.3
21b	0	2.4
26a	23	1.4
26b	76	2.7
Dexamethasone	—	1.0

[a] % remaining at 60 min. [b] NT = not tested.

of a catalytic amount of toluene-*p*-sulfonic acid gave the cyclic orthoester **23a** which was hydrolysed by aqueous acetic acid to give selectively the 17α-propionate **24a** (Scheme 6).[15,16]



Scheme 6 *Reagents and conditions*: i) $(EtO)_3CEt$, TsOH, DMF, PhMe; ii) AcOH, H_2O, MeOH; iii) CH_3SO_2Cl, pyridine; iv) **5**, NaH, THF; v) H_2, $(Ph_3P)_3RhCl$, EtOH.

Reaction of 17β-hydroxymethyl ketone **24a** with methanesulfonyl chloride gave methanesulfonate **25a**, which was reacted with thiol **5** to give an inseparable mixture of diastereoisomers **26a**. Finally, dihydro analogue **26b** was obtained from **24a** by selective hydrogenation using Wilkinson's catalyst (85%), followed by mesylation to **25b** (100%) and displacement of methanesulfonate with thiol **5** to give **26b** (43%).

The compounds listed in Table 1 were tested *in vitro* for their stability in human plasma and for their glucocorticoid agonist activity. The chemical stability of the compounds in Krebs buffer was examined by HPLC after incubating for 1 h and then their stability in human plasma after incubating for 1 h at 37 °C. The identity of the metabolites was obtained by LCMS and by

chemical synthesis in some cases (**2a**, **11a**). The results are expressed as a percentage (%) of compound remaining. All the compounds were found to be essentially stable in Krebs buffer after 1 h incubation, and to hydrolyse to the corresponding hydroxy carboxylic acids in plasma. The metabolite of the γ-linked lactones **13a** is a hemiacetal, which then collapses to the 21-mercapto derivative **6a**. As this metabolite is a potent glucocorticoid these analogues were not of any further interest. In addition the β-linked lactones **11a** and **21b** were found to undergo to a small extent retro-Michael reaction on prolonged storage to again produce the corresponding 21-mercapto derivatives. The *C*-linked lactone **15a** was found unexpectedly to hydrolyse in plasma, albeit more slowly than the *S*-linked lactones (22% lactone remaining after 1 h incubation) in contrast to our earlier findings in the 17β-sulfur linked butyrolactone series.[4]

The pharmacological activity was assessed in a functional *in vitro* assay of glucocorticoid agonist activity, which is generally predictive of antiinflammatory or anti-allergic activity *in vivo*. The functional assay was based on that described by Farrow *et al.*[17] A549 cells stably transfected with a reporter gene containing the NF-κB responsive elements from the ELAM gene promoter coupled to sPAP (secreted alkaline phosphatase) were treated with test compounds at appropriate doses for 1 h at 37 °C. The cells were then stimulated with tumour necrosis factor (TNF, 10 ng ml^{-1}) for 16 h, after which time the amount of alkaline phosphatase produced was measured by a standard colorimetric assay. Dose response curves were constructed from which EC_{50} values were estimated. The relative potencies of the compounds in Table 1 are expressed as the ratio of the EC_{50} value of the test compound to that of dexamethasone which was used as a standard.

The potencies of the individual diastereoisomers at the lactone asymmetric centre were found to be similar. Butyrolactones linked to the steroidal nucleus at the γ-position were generally more potent than those linked at the α-and β-positions based on the acetonide series where a complete set of α-, β- and γ-linked analogues were examined. However, because of the active metabolite associated with the β- and γ-linked analogues, the latter were of less interest. The 1,4-dien-3-one analogues (**a** series) were found to be more potent than the 4-en-3-one analogues (**b** series) *e.g.* **2a** > **2b**, **18a** > **18b**, **26a** > **26b**, confirming our findings in the 17β-lactone series.[4] Similarly, the potency across the series was found to be in the order of 17α-propionates ≈ 16α,17α-butylidenes > 16α,17α-isopropylidenes.

Conclusions

In conclusion, a series of novel lactone-containing pregnanes were synthesised and tested as glucocorticoid agonists. The 1,4-dien-3-one analogues possessing a 17α-propionate ester or the 16α,17α-butylidene were found to be the most potent glucocorticoids in this series. In addition, the butyrolactones were found to hydrolyse in human plasma to the respective hydroxy carboxylic acids.

Experimental

Organic solutions were dried over anhydrous magnesium sulfate. TLC was performed on Merck Kieselgel 60 F_{254} plates, and column chromatography was performed on Merck Kieselgel 60 (art. 7734 or 9385). Analytical HPLC was conducted on a Phenomenex Prodigy ODS-2 column (15 cm × 0.46 cm) eluting with 15–95% MeCN–H_2O over 16 min. Preparative HPLC was performed on a Gilson Medical Electronics system using a reversed-phase Dynamax 60Å C18 column (25 cm × 5 cm) flow rate 45 ml min^{-1}, detecting at 230 nm. Appropriate fractions were combined and evaporated under reduced pressure. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured with an Optical Activity AA100 digital polarimeter at 20 °C and are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded by reflectance from KBr on a Bio-Rad FTS-7 FT-IR spectrometer. ^{1}H NMR spectra were recorded at 250 MHz, and the chemical shifts are expressed in ppm relative to tetramethylsilane. All *J* values are in Hz. MS (TSP +ve) and MS (ES +ve) refer to mass spectra run in positive mode using thermospray or electrospray techniques, respectively. Residual solvents reported in microanalytical data were observed in the NMR spectra of such samples.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-(2-oxotetrahydrofuran-3-ylsulfanyl)pregna-1,4-diene-3,20-dione (2a)

Method A using α-mercapto-γ-butyrolactone 5. A solution of **5** (80 mg, 0.68 mmol) in anhydrous tetrahydrofuran (2 ml) was added dropwise to a stirring suspension of sodium hydride (60% oil dispersion; 27 mg, 0.68 mmol) in anhydrous tetrahydrofuran (1 ml) at 0 °C under a nitrogen atmosphere. The resulting solution was stirred at 0 °C for 30 min by which time effervescence had ceased. A solution of 6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-methylsulfonyloxy-pregna-1,4-diene-3,20-dione (**4a**) (300 mg, 0.57 mmol) in anhydrous tetrahydrofuran (10 ml) was added and the reaction mixture was stirred for a further 1 h at 0–21 °C. The reaction mixture was poured into water (30 ml) and extracted with ethyl acetate (10 ml × 3). The combined organic extracts were washed with saturated brine (20 ml) and dried. Removal of the solvent under reduced pressure yielded a white solid, which was separated by HPLC (50% MeCN–H_2O) to give 6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-[(3*S*)-2-oxotetrahydrofuran-3-ylsulfanyl]pregna-1,4-diene-3,20-dione (**2aS**) (88 mg, 28%) as a white solid: analytical HPLC t_r 8.11 min; mp 226–229 °C; ν_{max} (KBr)/cm^{-1} 3391, 1766, 1716 and 1668; δ_H (DMSO-d_6) includes 7.30 (1H, d, *J* 10, 1-H), 6.30 (1H, d, *J* 10, 2-H), 6.11 (1H, s, 4-H), 5.74 and 5.54 (1H, 2 m, 6-H), 5.60 (1H, d, *J* 4.5, 16-H), 4.91 (1H, br s), 4.39–4.15 (3H, m), 4.22 (1H, d, *J* 17.5, 21-H), 3.93 (1H, dd, *J* 8 and 6, SC*H*CO), 3.83 (1H, d, *J* 17.5, 21-H), 1.48 (3H, s), 1.35 (3H, s), 1.08 (3H, s) and 0.82 (3H, s); MS (TSP +ve) *m*/*z* 553 $(M + H)^+$ (Found: C, 60.0; H, 6.1; S, 5.6. $C_{28}H_{34}F_2O_7S{\cdot}0.5H_2O$ requires C, 59.9; H, 6.3; S 5.7%) and 6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-[(3*R*)-2-oxotetrahydrofuran-3-ylsulfanyl]-pregna-1,4-diene-3,20-dione (**2aR**) (72 mg, 23%): analytical HPLC t_r 8.34 min; mp 204–205 °C; ν_{max} (KBr)/cm^{-1} 3474, 1762, 1719, 1668 and 1633; δ_H (DMSO-d_6) includes 7.27 (1H, d, *J* 10, 1-H), 6.30 (1H, d, *J* 10, 2-H), 6.11 (1H, s, 4-H), 5.69 and 5.54 (1H, 2 m, 6-H), 5.58 (1H, m), 4.90 (1H, br s), 4.36–4.15 (4H, m), 3.90 (1H, dd, *J* 8 and 6.5, SC*H*CO), 3.82 (1H, d, *J* 18, 21-H), 1.48 (3H, s), 1.35 (3H, s), 1.10 (3H, s) and 0.79 (3H, s); MS (TSP +ve) *m*/*z* 553 $(M + H)^+$ (Found: C, 57.3; H, 6.4; S, 5.4. $C_{28}H_{34}F_2O_7S{\cdot}2H_2O$ requires C, 57.1; H, 6.5; S, 5.45%).

Method B using α-bromo-γ-butyrolactone 7. Triethylamine (45 μl, 0.32 mmol) was added to a stirring suspension of 6α,9α-difluoro-11β-hydroxy-21-mercapto-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione[7] (**6a**) (50 mg, 0.11 mmol) in dichloromethane (1 ml) under a nitrogen atmosphere. The suspension was then cooled to 0 °C and α-bromo-γ-butyrolactone (**7**) (27 μl, 0.32 mmol) was added. The resulting reaction mixture was then stirred at 0–21 °C for 4 h. The solvent was evaporated under reduced pressure and the residue was partitioned between ethyl acetate (25 ml) and 0.5 M hydrochloric acid (25 ml). The organic layer was washed with water (25 ml × 2) and saturated brine (25 ml) and dried. Removal of the solvent under reduced pressure yielded a colourless residue which was purified by HPLC (50–95% MeCN–H_2O) to give **2aS** (27 mg, 46%): mp 224–228 °C; δ_H ($CDCl_3$) includes 7.12 (1H, d,

J 10, 1-H), 6.44 (1H, s, 4-H), 6.37 (1H, d, *J* 10, 2-H), 5.48 and 5.29 (1H, 2 m, 6-H), 5.06 (1H, d, *J* 4.5, 16-H), 4.55–4.33 (3H, m), 4.04 (1H, d, *J* 19, 21-H), 3.88 (1H, d, *J* 19, 21-H), 3.83 (1H, dd, *J* 8.5 and 3.5, SC*H*CO), 1.53 (3H, s), 1.43 (3H, s), 1.16 (3H, s) and 0.94 (3H, s) and **2aR** (29 mg, 49%): mp 203–206 °C; δ_H ($CDCl_3$) includes 7.12 (1H, d, *J* 10, 1-H), 6.44 (1H, s, 4-H), 6.38 (1H, d, *J* 10, 2-H), 5.48 and 5.29 (1H, 2 m, 6-H), 5.05 (1H, d, *J* 4.5, 16-H), 4.55–4.30 (3H, m), 4.27 (1H, d, *J* 17.5, 21-H), 3.93 (1H, d, *J* 17.5, 21-H), 3.68 (1H, dd, *J* 8 and 4.5, SC*H*CO), 1.53 (3H, s), 1.43 (3H, s) 1.19 (3H, s) and 0.90 (3H, s).

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-[(3*S*)-2-oxotetrahydrofuran-3-ylsulfanyl]pregna-1,4-diene-3,20-dione (2aS)

A solution of 6α,9α-difluoro-11β-hydroxy-21-mercapto-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (**6a**) (100 mg, 0.21 mmol) in anhydrous DMF (2 ml) was added to a stirring suspension of sodium hydride (60% oil dispersion; 9 mg, 0.21 mmol) in anhydrous DMF (1 ml) at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred at 0 °C for 0.5 h at which point a solution of **9R** (38 mg, 0.21 mmol) in anhydrous DMF (3 ml) was added. The reaction mixture was stirred at 0 °C for 0.5 h and at 21 °C for a further 1 h. The mixture was partitioned between water (20 ml) and ethyl acetate (20 ml). The aqueous layer was extracted with ethyl acetate (20 ml) and the combined organic extracts were washed with saturated brine (20 ml), dried and evaporated under reduced pressure to yield a yellow oily residue. This material was purified by flash column chromatography eluting with ethyl acetate to give **2aS** as a white solid (38 mg, 32%): mp 220–222 °C; chromatographic and spectroscopic data were identical to those obtained above.

Crystal structure analysis of 2aS. Suitable crystals were obtained from methyl isobutyl ketone. Crystal data† for $C_{28}H_{34}F_2O_7S{\cdot}nC_6H_{12}O$ (where $n \approx 1$, disordered solvent present in crystal): M = 552.64, orthorhombic, a = 7.939(2), b = 14.627(3), c = 29.719(6) Å, U = 3451.0(12) Å^3, T = 293 K, space group $P2_12_12_1$ (no. 19), Z = 4, μ(Cu-Kα) = 1.316 mm^{-1}; 2624 unique reflections measured, of which 1781 were observed [$>2\sigma(I)$], (R_σ = 0.0694). The final $wR(F^2)$ was 0.1620 (observed reflections) and R_{obs} was 0.0684.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-[(3*R*)-2-oxotetrahydrofuran-3-ylsulfanyl]pregna-1,4-diene-3,20-dione (2aR)

Potassium carbonate (15 mg, 0.11 mmol) was added in a single portion to a stirring solution of 6α,9α-difluoro-11β-hydroxy-21-mercapto-16α,17α-isopropylidenedioxypregna-1,4-diene-3, 20-dione (**6a**) (100 mg, 0.21 mmol) and **9S** (38 mg, 0.21 mmol) in anhydrous DMF (2 ml). The resulting reaction mixture was stirred under a nitrogen atmosphere for 1.5 h at which point another quantity of potassium carbonate (15 mg, 0.11 mmol) was added. The mixture was stirred for a further 2.5 h. Ethyl acetate (10 ml) and 2 M hydrochloric acid (10 ml) were added and the organic phase was separated and washed with water (10 ml), saturated brine (10 ml) and then dried. Removal of the solvent under reduced pressure yielded a yellow residue, which was purified by flash column chromatography eluting with diethyl ether to give **2aR** as a white solid (91 mg, 77%); chromatographic and spectroscopic data were identical to those reported above.

Methanesulfonic acid (3*S*)-2-oxotetrahydrofuran-3-yl ester (9S)

Methanesulfonyl chloride (0.49 ml, 6.37 mmol) was added to a stirring solution of (*S*)-3-hydroxy-2-oxotetrahydrofuran (500 mg, 4.90 mmol) and triethylamine (0.88 ml, 6.37 mmol) in anhydrous dichloromethane (20 ml) at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred at 0 °C for 0.5 h and at 21 °C for a further 5 h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography eluting with ethyl acetate–cyclohexane (3 : 2) to give **9S** as a white crystalline solid (341 mg, 39%): mp 74–76 °C; $[\alpha]_D^{20}$ −57 (*c* 1.1 in $CHCl_3$); ν_{max} (KBr)/cm^{-1} 1787 and 1363; δ_H ($CDCl_3$) 5.34 (1H, t, *J* 9, 3-H), 4.53 (1H, dt, *J* 3 and 9, 5-H), 4.35 (1H, dt, *J* 6 and 9, 5-H), 3.29 (3H, s, *Me*SO_3), 2.86–2.71 (1H, m, 4-H) and 2.66–2.47 (1H, m, 4-H); MS (TSP +ve) *m*/*z* 198 (M + NH_4)$^+$ (Found: C, 33.5; H, 4.9; S, 17.8. $C_5H_8O_5S$ requires C, 33.3; H, 4.5; S, 17.8%).

Methanesulfonic acid (3*R*)-2-oxotetrahydrofuran-3-yl ester (9R)

White crystalline solid (214 mg, 30%): mp 73–74 °C; $[\alpha]_D^{20}$ +58 (*c* 0.96 in $CHCl_3$); ν_{max} (KBr)/cm^{-1} 1774 and 1363; δ_H ($CDCl_3$) 5.33 (1H, t, *J* 9, 3-H), 4.53 (1H, dt, *J* 3 and 9, 5-H), 4.35 (1H, dt, *J* 6 and 9, 5-H), 3.30 (3H, s, *Me*SO_3), 2.88–2.72 (1H, m, 4-H) and 2.66–2.47 (1H, m, 4-H); MS (TSP +ve) *m*/*z* 198 (M + NH_4)$^+$ (Found: C, 33.5; H, 4.2; S, 17.55. $C_5H_8O_5S$ requires C, 33.3; H, 4.5; S, 17.8%).

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-(2-oxotetrahydrofuran-4-ylsulfanyl)pregna-1,4-diene-3,20-dione (11a)

A suspension of 6α,9α-difluoro-11β-hydroxy-21-mercapto-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (**6a**) (100 mg, 0.23 mmol) and anhydrous potassium carbonate (32 mg, 0.23 mmol) in dry DMF (2 ml) was treated with furan-2(5*H*)-one (**10**) (0.035 ml, 0.5 mmol) and the mixture was stirred at 20 °C under a nitrogen atmosphere for 4 h. The reaction mixture was poured into 0.5 M hydrochloric acid (20 ml) and extracted with ethyl acetate (40 ml). The organic phase was washed with water (10 ml), aqueous sodium bicarbonate solution, water, saturated brine (20 ml each) and dried. Removal of the solvent under reduced pressure and trituration in diethyl ether (3 × 2 ml) yielded **11a** (100 mg, 79%): analytical HPLC t_r 7.77 min; ν_{max} (KBr)/cm^{-1} 3480, 1780, 1715 1668 and 1631; δ_H (DMSO-d_6) includes 7.30 (1H, d, *J* 9, 1-H), 6.31 (1H, d, *J* 9, 2-H), 6.13 (1H, s, 4-H), 5.70 and 5.58 (1H, 2 m, 6-H), 5.51 (1H, d, *J* 4, 16-H), 4.93 (1H, d, *J* 4, 16-H), 4.58 (1H, dd, *J* 9 and 8), 4.23 (2H, m), 4.03 and 4.01 (1H, 2 d, *J* 17, 21-H), 3.68 and 3.66 (1H, 2 d, *J* 17, 21-H), 3.90 (1H, m), 3.83 (1H, d, *J* 17), 3.02 (1H, dd, *J* 8 and 2), 1.51 (3H, s), 1.37 (3H, s), 1.11 (3H, s) and 0.83 (3H, s); δ_H ($CDCl_3$) includes 7.13 (1H, d, *J* 10, 1-H), 6.44 (1H, s, 4-H), 6.38 (1H, d, *J* 10, 2-H), 5.50 and 5.30 (1H, 2 m, 6-H), 5.03 (1H, d, *J* 5, 16-H), 4.64 (1H, m), 4.42 (1H, m), 4.24 (1H, m), 3.87 (1H, m), 3.71 and 3.68 (1H, 2 d, *J* 16, 21-H), 3.57 and 3.53 (1H, 2 d, *J* 16, 21-H), 2.96 (1H, m), 1.53 (3H, s), 1.43 (3H, s), 1.15 (3H, s) and 0.93 (3H, s); δ_C (DMSO-d_6) includes 206.2, 184.2, 175.4 (3 × C=O); MS (ES +ve) *m*/*z* 553 (M + H)$^+$ (Found: C, 59.25; H, 6.1; S, 5.7. $C_{28}H_{34}F_2O_7S{\cdot}0.75H_2O$ requires C, 59.4; H, 6.3; S 5.7%).

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-(2-oxotetrahydrofuran-5-ylsulfanyl)pregna-1,4-diene-3,20-dione (13a)

A solution of 6α,9α-difluoro-11β-hydroxy-21-mercapto-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (**6a**) (400 mg, 0.85 mmol) and γ-chloro-γ-butyrolactone (**12**)[10] (103 mg, 0.85 mmol) in anhydrous tetrahydrofuran (10 ml) was treated with triethylamine (0.12 ml, 0.85 mmol) and the mixture was stirred at 20 °C under a nitrogen atmosphere for 24 h. Removal of the solvent under reduced pressure yielded a brown foam. The crude product was purified by column chromatography eluting with dichloromethane–ethyl acetate (3 : 1) to

† CCDC reference number 177055. See http://www.rsc.org/suppdata/p1/b2/b200190j/ for crystallographic files in .cif or other electronic format.

give **13a** isomer A (124 mg, 26%): mp 238–239 °C; ν_{max} (KBr)/cm^{-1} 1781, 1719 and 1669; δ_H ($CDCl_3$) includes 7.19 (1H, d, *J* 10, 1-H), 6.43 (1H, s, 4-H), 6.37 (1H, d, *J* 10, 2-H), 5.87 (1H, m, SC*H*O), 5.49 and 5.29 (1H, 2 m, 6-H), 5.02 (1H, d, *J* 4, 16-H), 4.42 (1H, m, 11-H), 3.92 (1H, d, *J* 17, 21-H), 3.78 (1H, d, *J* 17, 21-H), 1.53 (3H, s), 1.42 (3H, s), 1.17 (3H, s) and 0.90 (3H, s); MS (TSP +ve) *m*/*z* 553 (M + H)$^+$ (Found: C, 59.55; H, 6.2; S, 5.6. $C_{28}H_{34}F_2O_7S{\cdot}0.2CH_2Cl_2$ requires C, 59.5; H, 6.1; S, 5.6%) and **13a** isomer B (62 mg, 13%): mp 244–246 °C; ν_{max} (KBr)/cm^{-1} 1784, 1715 and 1688; δ_H ($CDCl_3$) includes 7.14 (1H, d, *J* 10, 1-H), 6.43 (1H, s, 4-H), 6.38 (1H, d, *J* 10, 2-H), 5.92 (1H, m, SC*H*O), 5.49 and 5.29 (1H, 2 m, 6-H), 5.06 (1H, d, *J* 4, 16-H), 4.42 (1H, m, 11-H), 3.91 (1H, d, *J* 18, 21-H), 3.72 (1H, d, *J* 18, 21-H), 1.52 (3H, s), 1.42 (3H, s), 1.17 (3H, s) and 0.90 (3H, s); MS (TSP +ve) *m*/*z* 553 (M + H)$^+$ (Found: C, 61.0; H, 6.2; S, 5.6. $C_{28}H_{34}F_2O_7S$ requires C, 60.9; H, 6.2; S, 5.8%).

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-(2-oxotetrahydrofuran-3-ylmethylsulfanyl)pregna-1,4-diene-3,20-dione (15a)

Triethylamine (0.12 ml, 0.85 mmol) was added to a solution of **6** (400 mg, 0.85 mmol) and α-methylene-γ-butyrolactone (**14**) (0.075 ml, 0.85 mmol) in tetrahydrofuran (10 ml) and the mixture was stirred at 20 °C under nitrogen for 22 h. The solvent was removed under reduced pressure and the residue was purified by chromatography eluting with ethyl acetate–cyclohexane (3 : 2) to give **15a** (268 mg, 55%) as a white solid: analytical HPLC indicated a 3 : 1 mixture of diastereoisomers; δ_H ($CDCl_3$) includes 7.16 (1H, d, *J* 10, 1-H), 6.44 (1H, s, 4-H), 6.38 (1H, d, *J* 10, 2-H), 5.49 and 5.30 (1H, 2 m, 6-H), 5.03 (1H, d, *J* 4, 16-H), 4.52–4.37 (2H, m), 4.28 (1H, dt, *J* 7 and 9, C*H*$_2$OCO), 3.76 (0.5H, s, 21-H, minor isomer) and 3.68 (1.5H, AB q, *J* 16, 21-H, major isomer), 1.54 (3H, s), 1.43 (3H, s), 1.15 (3H, s) and 0.98 (3H, s); MS (ES +ve) *m*/*z* 567 (M + H)$^+$ (Found: C, 61.1; H, 6.5; S, 5.5. $C_{29}H_{36}F_2O_7S$ requires C, 61.5; H, 6.4; S, 5.7%).

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-methylsulfonyloxypregn-4-ene-3,20-dione (4b)

Methanesulfonyl chloride (0.85 ml, 11 mmol) was added to a stirring solution of 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (**1b**) (1 g, 2.2 mmol) in anhydrous pyridine (10 ml) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 2 h and was then poured into ice-cold 1 M hydrochloric acid (40 ml). The resulting suspension was extracted with ethyl acetate (20 ml × 4) and the combined organic extracts were washed with saturated brine (20 ml) and dried. Removal of the solvent under reduced pressure yielded a pale yellow residue which was purified by HPLC (55% MeCN–H_2O) to give **4b** as a colourless foam (843 mg, 72%): ν_{max} (KBr)/cm^{-1} 3453, 1732 and 1669; δ_H (DMSO-d_6) includes 5.82 (1H, s, 4-H), 5.61 and 5.42 (1H, 2 m, 6-H), 5.35 (1H, d, *J* 18, 21-H), 5.30 (1H, br s), 4.93 (1H, br s), 4.90 (1H, d, *J* 18, 21-H), 4.19 (1H, m, 11-H), 3.32 (3H, s, *Me*SO_3), 1.48 (3H, s), 1.38 (3H, s), 1.15 (3H, s) and 0.80 (3H, s); MS (ES +ve) *m*/*z* 533 (M + H)$^+$ (Found: C, 55.7; H, 6.3; S, 5.9. $C_{25}H_{34}F_2O_8S{\cdot}0.4H_2O$ requires C, 55.6; H, 6.5; S, 5.9%).

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-21-(2-oxotetrahydrofuran-3-ylsulfanyl)pregn-4-ene-3,20-dione (2b)

A solution of α-mercapto-γ-butyrolactone (**5**) (213 mg, 1.80 mmol) in anhydrous tetrahydrofuran (2 ml) was added, dropwise, to a stirring suspension of sodium hydride (60% oil dispersion; 72 mg, 1.80 mmol) in anhydrous tetrahydrofuran (1 ml) at 0 °C under a nitrogen atmosphere. The resulting solution was stirred at 0 °C for 30 min by which time effervescence had ceased. A solution of **4b** (800 mg, 1.50 mmol) in anhydrous tetrahydrofuran (10 ml) was added and the reaction mixture was stirred for a further 1 h at 0 °C. The reaction mixture was poured into water (20 ml) and extracted with ethyl acetate (20 ml × 4). The combined organic extracts were then washed with saturated brine (20 ml) and dried. Removal of the solvent under reduced pressure yielded a residue which was purified by HPLC (55% MeCN–H_2O) to give **2b** isomer A (282 mg, 34%): analytical HPLC t_r 7.82 min; mp 124–128 °C; ν_{max} (KBr)/cm^{-1} 3468, 1760, 1716 and 1669; δ_H (DMSO-d_6) includes 5.80 (1H, s, 4-H), 5.60 and 5.41 (1H, 2 m, 6-H), 5.30 (1H, br s), 4.90 (1H, br s), 4.40–4.10 (3H, m), 4.08–3.73 (3H, m), 1.47 (3H, s), 1.37 (3H, s), 1.08 (3H, s), and 0.79 (3H, s); MS (TSP +ve) *m*/*z* 555 (M + H)$^+$ (Found: C, 59.7; H, 6.4; S, 5.6. $C_{28}H_{36}F_2O_7S{\cdot}0.5H_2O$ requires C, 59.7; H, 6.6; S, 5.7%) and **2b** isomer B (235 mg, 28%): analytical HPLC t_r 7.54 min; mp 224–228 °C; ν_{max} (KBr)/cm^{-1} 3482, 1762, 1714 and 1669; δ_H (DMSO-d_6) includes 5.81 (1H, s, 4-H), 5.60 and 5.41 (1H, 2 m, 6-H), 5.28 (1H, br s), 4.91 (1H, br s), 4.40–4.10 (4H, m), 3.95–3.73 (2H, m), 1.47 (3H, s), 1.38 (3H, s), 1.11 (3H, s) and 0.77 (3H, s); MS (TSP +ve) *m*/*z* 555 (M + H)$^+$ (Found: C, 59.8; H, 6.6; S, 5.6. $C_{28}H_{36}F_2O_7S{\cdot}0.5H_2O$ requires C, 59.7; H, 6.6; S, 5.7%).

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-methylsulfonyloxypregna-1,4-diene-3,20-dione (17a)

Methanesulfonyl chloride (0.41 ml, 5.35 mmol) was added to a stirring solution of 16α,17α-[(*R*)-butylidenedioxy]-6α,9α-difluoro-11β,21-dihydroxypregna-1,4-diene-3,20-dione (**16a**) (500 mg, 1.07 mmol) in anhydrous pyridine (6 ml) under a nitrogen atmosphere. The reaction mixture was stirred for 2 h and was then poured into ice-cold 1 M hydrochloric acid (30 ml). The resulting precipitate was collected by filtration and dried at reduced pressure over phosphorus pentaoxide to yield **17a** as a pale yellow solid (566 mg, 97%): δ_H (DMSO-d_6) includes 7.27 (1H, d, *J* 10, 1-H), 6.30 (1H, d, *J* 10, 2-H), 6.10 (1H, s, 4-H), 5.73 and 5.53 (1H, 2 m, 6-H), 5.61 (1H, br s), 5.27 (1H, d, *J* 18, 21-H), 4.99 (1H, d, *J* 18, 21-H), 4.75 (2H, m), 4.20 (1H, m, 11-H), 3.29 (3H, s, *Me*SO_3), 1.48 (3H, s), 0.87 (3H, t, *J* 7.5, CH_2CH_3) and 0.84 (3H, s); MS (TSP +ve) *m*/*z* 545 (M + H)$^+$ (Found: C, 56.4; H, 6.2; S, 6.0. $C_{26}H_{34}F_2O_8S{\cdot}0.4H_2O$ requires C, 56.6; H, 6.4; S, 5.8%).

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-[(3*R*)-2-oxotetrahydrofuran-3-ylsulfanyl]pregna-1,4-diene-3,20-dione (18aR)

A mixture of sand (2 g), **2aR** (100 mg, 0.018 mmol), *n*-butyraldehyde (0.032 ml, 0.036 mmol) and perchloric acid (11.7 M; 0.062 ml) was stirred vigorously in heptane (10 ml) overnight. The sand was collected by filtration and washed with heptane. The sand was then stirred in a mixture of aqueous sodium bicarbonate solution (50 ml) and ethyl acetate (50 ml). The organic phase was separated, washed with brine, dried and filtered. The filtrate was evaporated and the residue was triturated in diethyl ether to give **18aR** as a solid (92 mg, 90%): analytical HPLC t_r 11.40 min; mp 219–221 °C; ν_{max} (KBr)/cm^{-1} 3356, 1768, 1721, 1665, 1625 and 1609; δ_H ($CDCl_3$) includes 7.12 (1H, d, *J* 10, 1-H), 6.44 (1H, s, 4-H), 6.40 (1H, d, *J* 10, 2-H), 5.48 and 5.29 (1H, 2 m, 6-H), 4.92 (1H, d, *J* 5, 16-H), 4.67 (1H, t, *J* 4.5, OC*H*O), 4.55–4.30 (3H, m), 4.19 (1H, d, *J* 18, 21-H), 3.81 (1H, d, *J* 18, 21-H), 3.68 (1H, m), 1.53 (3H, s), 0.92 (6H, s and t, *J* 7.5, CH_2CH_3); MS (TSP +ve) *m*/*z* 567 (M + H)$^+$ (Found: C, 61.35; H, 6.4; S, 5.6. $C_{29}H_{36}F_2O_7S$ requires C, 61.5; H, 6.4; S, 5.7%).

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-(2-oxotetrahydrofuran-3-ylsulfanyl)pregna-1,4-diene-3,20-dione (18a)

A solution of α-mercapto-γ-butyrolactone (**5**) (144 mg, 1.22 mmol) in anhydrous tetrahydrofuran (4 ml) was added dropwise to a stirring suspension of sodium hydride (60% oil

dispersion; 49 mg, 1.22 mmol) in anhydrous tetrahydrofuran (2 ml) at 0 °C under a nitrogen atmosphere. The resulting solution was stirred at 0 °C for 30 min by which time effervescence had ceased. A solution of **17a** (557 mg, 1.02 mmol) in anhydrous tetrahydrofuran (15 ml) was added and the reaction mixture was stirred for 18 h at 0–21 °C. Further quantities of sodium hydride (24 mg, 0.61 mmol) and α-mercapto-γ-butyrolactone (72 mg, 0.61 mmol) were added and the mixture stirred for another 1 h. The reaction mixture was poured into water (30 ml) and extracted with ethyl acetate (30 ml × 2). The combined organic extracts were then washed with saturated brine (30 ml) and dried. Removal of the solvent under reduced pressure yielded a yellow residue which was purified by HPLC (60% MeCN–H_2O) to give **18aS** (152 mg, 26%): analytical HPLC t_r 11.82 min; mp 221–223 °C; ν_{max} (KBr)/cm^{-1} 3356, 1766, 1715, 1665 and 1607; δ_H ($CDCl_3$) includes 7.12 (1H, d, *J* 10, 1-H), 6.44 (1H, s, 4-H), 6.38 (1H, d, *J* 10, 2-H), 5.48 and 5.28 (1H, 2 m, 6-H), 4.93 (1H, d, *J* 5.5, 16-H), 4.62 (1H, t, *J* 4.5, OC*H*O), 4.55–4.32 (3H, m), 4.05 (1H, d, *J* 19, 21-H), 3.71 (1H, d, *J* 19, 21-H), 3.78 (1H, m), 1.52 (3H, s), 0.95 (3H, s) and 0.92 (3H, t, *J* 7.5, CH_2CH_3); MS (TSP +ve) *m*/*z* 567 (M + H)$^+$ (Found: C, 61.0; H, 6.6; S, 5.3. $C_{29}H_{36}F_2O_7S{\cdot}0.2H_2O$ requires C, 61.1; H, 6.4; S, 5.6%) and **18aR** (92 mg, 16%): analytical HPLC t_r 11.40 min, co-elutes with authentic **18aR**; NMR spectrum identical to **18aR** prepared above.

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-methylsulfonyloxypregn-4-ene-3,20-dione (17b)

Methanesulfonyl chloride (0.82 ml, 10.65 mmol) was added to a stirring solution of 16α,17α-[(*R*)-butylidenedioxy]-6α,9α-difluoro-11β,21-dihydroxypregn-4-ene-3,20-dione (**16b**) (1 g, 2.13 mmol) in anhydrous pyridine (12 ml) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 0.5 h at 0 °C and 2.5 h at 21 °C and was then poured into ice-cold 1 M hydrochloric acid (60 ml). The resulting precipitate was collected by filtration and dried at reduced pressure over phosphorus pentaoxide. The resulting pale yellow solid was purified by HPLC (55% MeCN–H_2O) to give **17b** (730 mg, 63%): δ_H ($CDCl_3$) includes 6.15 (1H, s, 4-H), 5.37 and 5.18 (1H, 2 m, 6-H), 5.04 (2H, s, 21-H), 4.89 (1H, d, *J* 5, 16-H), 4.65 (1H, t, *J* 4.5, OC*H*O), 4.41 (1H, m, 11-H), 3.26 (3H, s, *Me*SO_3), 1.52 (3H, s), 0.95 (3H, t, *J* 7.5, CH_2CH_3) and 0.94 (3H, s); MS (TSP +ve) *m*/*z* 547 (M + H)$^+$ (Found: C, 56.6; H, 6.4; S, 5.8. $C_{26}H_{36}F_2O_8S{\cdot}0.3H_2O$ requires C, 56.6; H, 6.7; S, 5.8%).

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-(2-oxotetrahydrofuran-3-ylsulfanyl)pregn-4-ene-3,20-dione (18b)

A solution of α-mercapto-γ-butyrolactone (**5**) (187 mg, 1.58 mmol) in tetrahydrofuran (2 ml) was added dropwise to a stirring suspension of sodium hydride (60% oil dispersion; 63 mg, 1.58 mmol) in anhydrous tetrahydrofuran (1 ml) at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred at 0 °C for 45 min by which time effervescence had ceased. A solution of **17b** (722 mg, 1.32 mmol) in anhydrous tetrahydrofuran (10 ml) was added and the reaction mixture was stirred for a further 1.5 h at 0–21 °C. The reaction mixture was poured into water (30 ml) and extracted with ethyl acetate (30 ml × 2). The combined organic extracts were then washed with saturated brine (30 ml) and dried. Removal of the solvent under reduced pressure yielded a white solid which was purified by HPLC (60% MeCN–H_2O) to yield **18bS** (335 mg, 45%): analytical HPLC t_r 11.92 min; mp 159–161 °C; ν_{max} (KBr)/cm^{-1} 1762, 1715 and 1669; δ_H ($CDCl_3$) includes 6.14 (1H, s, 4-H), 5.37 and 5.17 (1H, 2 m, 6-H), 4.95 (1H, d, *J* 5, 16-H), 4.63 (1H, t, *J* 4.5, OC*H*O), 4.55–4.29 (3H, m), 4.08 (1H, d, *J* 18, 21-H), 3.77 (1H, t, *J* 4, SC*H*CO), 3.72 (1H, d, *J* 18, 21-H), 1.52 (3H, s), 0.95 (3H, t, *J* 7, CH_2CH_3) and 0.92 (3H, s); MS (TSP +ve) *m*/*z* 569 (M + H)$^+$ (Found: C, 60.3; H, 6.9; S, 5.5. $C_{29}H_{38}F_2O_7S{\cdot}0.4H_2O$ requires C, 60.5; H, 6.8; S 5.6%) and **18bR** (137 mg, 18%): analytical HPLC t_r 11.66 min; mp 99–102 °C; ν_{max} (KBr)/cm^{-1} 1760, 1714 and 1668; δ_H ($CDCl_3$) includes 6.14 (1H, s, 4-H), 5.37 and 5.18 (1H, 2 m, 6-H), 4.92 (1H, d, *J* 5, 16-H), 4.69 (1H, t, *J* 4.5, OC*H*O), 4.54–4.30 (3H, m), 4.22 (1H, d, *J* 18, 21-H), 3.80 (1H, d, *J* 18, 21-H), 3.70 (1H, t, *J* 4, SC*H*CO), 1.52 (3H, s), 0.95 (3H, t, *J* 7.5, CH_2CH_3) and 0.90 (3H, s); MS (TSP +ve) *m*/*z* 569 (M + H)$^+$ (Found: C, 59.5; H, 6.6; S, 5.4. $C_{29}H_{38}F_2O_7S{\cdot}0.8H_2O$ requires C, 59.7; H, 6.85; S, 5.5%).

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-[(3*S*)-2-oxotetrahydrofuran-3-ylsulfanyl]pregn-4-ene-3,20-dione (18bS)

Potassium carbonate (238 mg, 1.72 mmol) was added to a solution of **20b** (835 mg, 1.72 mmol) and **9R** (310 mg, 1.72 mmol) in anhydrous dimethylformamide (15 ml). The mixture was stirred at 20 °C under nitrogen for 3 h. Ethyl acetate (100 ml) and 2 M hydrochloric acid (100 ml) were added and the organic phase was separated, washed with water (100 ml), brine (100 ml), dried and evaporated to dryness. The residue was purified by chromatography, eluting with ethyl acetate–dichloromethane (1 : 3) and purified further by HPLC (60% MeCN–H_2O) to give the (*S*) isomer **18bS** (358 mg, 37%) as a white crystalline solid: analytical HPLC t_r 11.88 min; spectroscopic data identical to those reported earlier.

21-Acetylsulfanyl-16α,17α-[(*R*)-butylidenedioxy]-6α,9α-difluoro-11β-hydroxypregn-4-ene-3,20-dione (19b)

Diisopropyl azodicarboxylate (0.91 ml, 4.62 mmol) was added to a stirring solution of triphenylphosphine (1.21 g, 4.62 mmol) in anhydrous tetrahydrofuran (20 ml) at 0 °C under a nitrogen atmosphere. The resulting yellow suspension was stirred at 0–5 °C for 0.5 h after which time a solution of 16α,17α-[(*R*)-butylidenedioxy]-6α,9α-difluoro-11β,21-dihydroxypregn-4-ene-3,20-dione (**16b**) (1.40 g, 3.08 mmol) and thioacetic acid (0.26 ml, 3.70 mmol) in anhydrous tetrahydrofuran (10 ml) was added dropwise over 15 min. The reaction mixture was stirred for a further 0.5 h at 0–5 °C and for 18 h at 20 °C. The reaction mixture was poured into 2 M hydrochloric acid (100 ml) and extracted with ethyl acetate (100 ml). The organic phase was washed with aqueous sodium bicarbonate solution (100 ml), water (100 ml), and saturated brine (100 ml) and dried. Removal of the solvent under reduced pressure yielded a yellow solid, which was purified by column chromatography eluting with ethyl acetate–cyclohexane (2 : 3) to give **19b** (1.63 g, 100%): δ_H ($CDCl_3$) includes 6.16 (1H, s, 4-H), 5.37 and 5.18 (1H, 2 m, 6-H), 4.90 (1H, d, *J* 5,16-H), 4.68 (1H, t, *J* 4, OC*H*O), 4.42 (1H, m, 11-H), 3.94 (2H, s, 21-H), 2.40 (3H, s, AcS), 1.52 (3H, s), 0.95 (3H, t, *J* 7, CH_2CH_3) and 0.90 (3H, s); MS (TSP +ve) *m*/*z* 527 (M + H)$^+$.

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-mercaptopregn-4-ene-3,20-dione (20b)

Hydrazine hydrate (0.18 ml, 3.08 mmol) was added to a stirring solution of 21-acetylsulfanyl-16α,17α-[(*R*)-butylidenedioxy]-6α,9α-difluoro-11β-hydroxypregn-4-ene-3,20-dione (**19b**) (1.62 g, 3.08 mmol) in anhydrous tetrahydrofuran (25 ml) at −15 °C under a nitrogen atmosphere. The reaction mixture was stirred at −15 to −10 °C for 0.5 h and then at 20 °C for 5 h. The reaction mixture was poured into 2 M hydrochloric acid (75 ml) and extracted with ethyl acetate (75 ml). The organic phase was washed with water (75 ml), saturated brine (75 ml) and dried. Removal of the solvent under reduced pressure yielded **20b** (1.35 g, 91%): δ_H ($CDCl_3$) includes 6.15 (1H, s, 4-H), 5.37 and 5.18 (1H, 2 m, 6-H), 4.94 (1H, d, *J* 5, 16-H), 4.65 (1H, t, *J* 4, OC*H*O), 4.40 (1H, m, 11-H), 3.68 (1H, dd, *J* 16 and 7, 21-H), 3.42 (1H, dd, *J* 16 and 7, 21-H), 1.52 (3H, s), 0.94 (3H, t, *J* 7, CH_2CH_3) and 0.90 (3H, s); MS (TSP +ve) *m*/*z* 485 (M + H)$^+$.

16α,17α-[(*R*)-Butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-(2-oxotetrahydrofuran-4-ylsulfanyl)pregn-4-ene-3,20-dione (21b)

A solution of 16α,17α-[(*R*)-butylidenedioxy]-6α,9α-difluoro-11β-hydroxy-21-mercaptopregn-4-ene-3,20-dione (**20b**) (500 mg, 1.03 mmol) and furan-2(5*H*)-one (**10**) (0.073 ml, 1.03 mmol) in anhydrous tetrahydrofuran (10 ml) was treated with triethylamine (0.14 ml, 1.03 mmol) and the mixture was stirred at 20 °C under a nitrogen atmosphere for 48 h. Removal of the solvent under reduced pressure yielded a cream foam, which was purified by column chromatography eluting with ethyl acetate–cyclohexane (3 : 2) to give **21b** (413 mg, 71%): ν_{max} (KBr)/cm^{-1} 1779, 1712 and 1668; δ_H ($CDCl_3$) includes 6.16 (1H, s, 4-H), 5.37 and 5.18 (1H, 2 m, 6-H), 4.91 (1H, d, *J* 5, 16-H), 4.70 (1H, t, *J* 4, OC*H*O), 4.62 (1H, dd, *J* 9 and 7), 4.40 (1H, m, 11-H), 4.22 (1H, m), 3.82 (1H, m), 3.63 and 3.60 (1H, 2 d, *J* 15), 3.43 and 3.39 (1H, 2 d, *J* 16, 21-H), 2.95 (1H, dd, *J* 18 and 8), 1.52 (3H, s), 0.94 (3H, t, *J* 7, CH_2CH_3) and 0.91 (3H, s); MS (TSP +ve) *m*/*z* 569 (M + H)$^+$ (Found: C, 62.4; H, 7.4; S, 5.0. $C_{29}H_{38}F_2O_7S\cdot0.3C_6H_{12}$ requires C, 62.3; H, 7.1; S 5.4%).

6α,9α-Difluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione, cyclic 17,21-(ethyl orthopropionate) (23a)

Toluene (140 ml) was added to a solution of flumethasone (**22a**) (10.26 g, 25 mmol) in dry dimethylformamide (14 ml) whereupon some precipitation occurred to give a cream suspension. Toluene-*p*-sulfonic acid (50 mg) was then added followed by triethyl orthopropionate (10 ml, 50 mmol) and the mixture was stirred at 20 °C. After 2 h the suspension was partitioned between water (250 ml) and ethyl acetate (350 ml) and the solid was collected by filtration and washed with water (20 ml) and ethyl acetate (20 ml). The solid was dried over phosphorus pentaoxide under reduced pressure to give the orthoester **23a**[15,16] (9.41 g, 76%) as a white solid: δ_H (DMSO-d_6) 7.28 (1H, d, *J* 10, 1-H), 6.29 (1H, dd, *J* 10, 2-H), 6.10 (1H, s, 4-H), 5.55 and 5.75 (1H, 2 d, 6-H), 5.40 (1H, d, *J* 4, OH), 4.20 (1H, m, 11-H), 4.09 (1H, d, *J* 16, 21-H), 3.95 (1H, d, *J* 16, 21-H), 3.45 (2H, q, *J* 7, OCH_2CH_3), 1.48 (3H, s), 1.03 (3H, *t*, *J* 7), 0.90 (3H, *t*, *J* 7), 0.87 (3H, s), 0.86 (3H, d, *J* 7, 16-H); MS (TSP +ve) *m*/*z* 495 (M + H)$^+$. Additional **23a** (1.73 g, 14%) was obtained by evaporation of the filtrate and trituration with ethyl acetate.

6α,9α-Difluoro-11β,21-dihydroxy-16α-methyl-17α-propionyloxypregna-1,4-diene-4,20-dione (24a)

A suspension of **23a** (11.1 g, 22.4 mmol) in methanol (400 ml) was diluted with 5% aqueous acetic acid (80 ml) and the mixture was heated to reflux for 30 min. The solution was concentrated under reduced pressure to a volume of about 100 ml by which time the product started to crystallise. Water was added (100 ml) and the solid was collected by filtration, washed well with water (50 × 3 ml) and dried *in vacuo* over phosphorus pentaoxide to give **24a**[15,16] (10.09 g, 96%) as a white solid: ν_{max} (KBr)/cm^{-1} 3424, 1729, 1715, 1668 and 1631; δ_H (DMSO-d_6) 7.25 (1H, d, *J* 10, 1-H), 6.28 (1H, d, *J* 10, 2-H), 6.10 (1H, s, 4-H), 5.70 and 5.50 (1H, 2 m, 6-H), 5.47 (1H, m), 5.07 (1H, t, *J* 5.5, 21-OH), 4.20 (1H, m), 4.10 (2H, m), 3.20 (1H, m, 16-H), 2.36 (2H, q, *J* 7, $COCH_2CH_3$), 1.50 (3H, s), 1.00 (3H, t, *J* 7, $COCH_2CH_3$), 0.91 (3H, s) and 0.83 (3H, d, *J* 7,16-*Me*); MS (TSP +ve) *m*/*z* 467 (M + H)$^+$.

6α,9α-Difluoro-11β-hydroxy-21-methylsulfonyloxy-16α-methyl-17α-propionyloxypregna-1,4-diene-3,20-dione (25a)

Methanesulfonyl chloride (1.66 ml, 21 mmol) was added dropwise to a stirring solution of 6α,9α-difluoro-11β,21-dihydroxy-16α-methyl-17α-propionyloxypregna-1,4-diene-3,20-dione (**24a**) (2 g, 4.29 mmol) in anhydrous pyridine (20 ml) under a nitrogen atmosphere. The reaction mixture was stirred for 2 h and was then poured into ice-cold 2 M hydrochloric acid (40 ml). The resulting precipitate was collected by filtration, washed with water (30 ml × 3) and dried at reduced pressure over phosphorus pentaoxide to yield **25a** (2.658 g, 100%): mp 185–187 °C; ν_{max} (KBr)/cm^{-1} 3544, 1731, 1667 and 1633; δ_H (DMSO-d_6) includes 7.25 (1H, d, *J* 10, 1-H), 6.30 (1H, d, *J* 10, 2-H), 6.11 (1H, s, 4-H), 5.73 and 5.53 (1H, 2 m, 6-H), 5.54 (1H, br s), 5.07 (1H, d, *J* 16, 21-H), 4.83 (1H, d, *J* 16, 21-H), 4.18 (1H, m, 11-H), 3.26 (3H, s, *Me*SO_3), 3.23 (1H, m), 2.40 (2H, q, *J* 7.5, $COCH_2CH_3$), 1.48 (3H, s), 1.00 (3H, t, *J* 7.5, $COCH_2CH_3$), 0.96 (3H, s) and 0.85 (3H, d, *J* 7, 16-*Me*); MS (TSP +ve) *m*/*z* 545 (M + H)$^+$ (Found: C, 57.0; H, 6.3; S, 5.4. $C_{26}H_{34}F_2O_8S\cdot0.3H_2O$ requires C, 56.8; H, 6.3; S, 5.8%).

6α,9α-Difluoro-11β-hydroxy-16α-methyl-21-(2-oxotetrahydrofuran-3-ylsulfanyl)-17α-propionyloxypregna-1,4-diene-3,20-dione (26a)

A solution of α-mercapto-γ-butyrolactone (**5**) (220 mg, 1.84 mmol) in anhydrous tetrahydrofuran (10 ml) was added to a stirring suspension of sodium hydride (60% oil dispersion; 74 mg, 1.84 mmol) in anhydrous tetrahydrofuran (2 ml) at 0 °C under a nitrogen atmosphere. The resulting solution was stirred at 0 °C for 30 min by which time effervescence had ceased. A solution of **25a** (1 g, 1.84 mmol) in anhydrous tetrahydrofuran (20 ml) was added and the reaction mixture was stirred for 16 h at 0–21 °C. Further quantities of sodium hydride (37 mg, 0.92 mmol) and α-mercapto-γ-butyrolactone (110 mg, 0.92 mmol) were added and the mixture stirred for another 6 h. The reaction mixture was poured into water (30 ml) and extracted with ethyl acetate (30 ml × 4). The combined organic extracts were then washed with saturated brine (20 ml) and dried. Removal of the solvent under reduced pressure yielded a pale yellow residue which was purified by HPLC (50% MeCN–H_2O) to give **26a** (177 mg, 17%): ν_{max} (KBr)/cm^{-1} 3484, 1761, 1731, 1669 and 1629; δ_H ($CDCl_3$) includes 7.14 (1H, d, *J* 10, 1-H), 6.44 (1H, s, 4-H), 6.38 (1H, d, *J* 10, 2-H), 5.49 and 5.30 (1H, 2 m, 6-H), 4.53–4.28 (3H, m), 4.00–3.75 (3H, m), 3.60–3.30 (2H, m), 2.43 (1H, q, *J* 7.5, $COCH_2CH_3$), 2.40 (1H, q, *J* 7.5, $COCH_2CH_3$), 1.53 (3H, s), 1.14 (3H, t, *J* 7.5, $COCH_2CH_3$), 1.06 and 1.05 (3H, 2 s), 0.94 (3H, d, *J* 7.5, 16-*Me*); MS (ES +ve) *m*/*z* 567 (M + H)$^+$ (Found: C, 61.6; H, 6.3; S, 5.75. $C_{29}H_{36}F_2O_7S$ requires C, 61.5; H, 6.4; S, 5.7%).

6α,9α-Difluoro-11β,21-dihydroxy-16α-methyl-17α-propionyloxypregn-4-ene-3,20-dione (24b)

A stirring solution of 6α,9α-difluoro-11β,21-dihydroxy-16α-methyl-17α-propionyloxypregna-1,4-diene-3,20-dione (**24a**) (3 g, 6.43 mmol) was hydrogenated over tris(triphenylphosphine)rhodium(I) chloride (0.6 g, 0.64 mmol) in ethanol (100 ml) at 1 atmosphere for 60 h. The solvent was removed under reduced pressure and the residue was purified by chromatography eluting with ethyl acetate–cyclohexane (3 : 1) to give **24b** as a pale yellow solid (2.57 g, 85%): analytical HPLC t_r 7.06 min; ν_{max} (KBr)/cm^{-1} 3428, 1730, 1717 and 1669; δ_H (DMSO-d_6) includes 5.82 (1H, s, 4-H), 5.60 and 5.42 (1H, 2 m, 6-H), 5.20 (1H, br s), 5.07 (1H, t, *J* 6), 4.25–3.95 (3H, m), 3.24 (1H, m), 2.39 (2H, q, *J* 7.5, $COCH_2CH_3$), 1.48 (3H, s), 1.03 (3H, t, *J* 7.5 $COCH_2CH_3$), 0.90 (3H, s) and 0.84 (3H, d, *J* 7, 16-*Me*); MS (TSP +ve) *m*/*z* 469 (M + H)$^+$; HRMS (ESI +ve) found 469.2401 (M + H)$^+$. $C_{25}H_{35}F_2O_6$ requires 469.2417.

6α,9α-Difluoro-11β-hydroxy-21-methylsulfonyloxy-16α-methyl-17α-propionyloxypregn-4-ene-3,20-dione (25b)

Methanesulfonyl chloride (1.66 ml, 21 mmol) was added dropwise over 2 min to a stirring solution of **24b** (2g, 4.27 mmol) in anhydrous pyridine (20 ml) under a nitrogen atmosphere. The reaction mixture was stirred for 2 h and was then poured into ice-cold 2 M hydrochloric acid (40 ml). The resulting precipitate was collected by filtration, washed with water (30 ml × 3) and dried at reduced pressure over phosphorus pentaoxide to yield

25b (2.390 g, 100%): mp 157–159 °C (decomp.); ν_{max} (KBr)/cm^{-1} 3532, 1731 and 1668; δ_H (DMSO-d_6) includes 5.81 (1H, s, 4-H), 5.60 and 5.41 (1H, 2 m, 6-H), 5.24 (1H, br s), 5.05 (1H, d, *J* 16, 21-H), 4.82 (1H, d, *J* 16, 21-H), 4.16 (1H, m, 11-H), 3.26 (3H, s, *Me*SO_3), 2.43 (2H, q, *J* 7.5, COC*H*$_2$CH$_3$), 1.48 (3H, s), 1.02 (3H, t, *J* 7.5, COCH$_2$C*H*$_3$), 0.94 (3H, s) and 0.85 (3H, d, *J* 7, 16-*Me*); MS (TSP +ve) *m*/*z* 547 (M + H)$^+$ (Found: C, 56.1; H, 6.5; S, 5.7. $C_{26}H_{36}F_2O_8S{\cdot}0.5H_2O$ requires C, 56.4; H, 6.7; S, 5.8%).

6α,9α-Difluoro-11β-hydroxy-16α-methyl-21-(2-oxotetrahydrofuran-3-ylsulfanyl)-17α-propionyloxypregn-4-ene-3,20-dione (26b)

A solution of α-mercapto-γ-butyrolactone (**5**) (220 mg, 1.84 mmol) in anhydrous tetrahydrofuran (10 ml) was added dropwise to a stirring suspension of sodium hydride (60% oil dispersion; 74 mg, 1.84 mmol) in anhydrous tetrahydrofuran (2 ml) at 0 °C under a nitrogen atmosphere. The resulting solution was stirred at 0 °C for 30 min by which time effervescence had ceased. A solution of **25b** (1 g, 1.84 mmol) in anhydrous tetrahydrofuran (20 ml) was added and the reaction mixture was stirred for 16 h at 0–21 °C. Further quantities of sodium hydride (37 mg, 0.92 mmol) and **5** (110 mg, 0.92 mmol) were added and the mixture stirred for another 6 h. The reaction mixture was poured into water (30 ml) and extracted with ethyl acetate (30 ml × 4). The combined organic extracts were then washed with saturated brine (20 ml) and dried. Removal of the solvent under reduced pressure yielded a yellow residue which was purified by HPLC (55% MeCN–H_2O) to give **26b** (455 mg, 43%): mp 171–175 °C; ν_{max} (KBr)/cm^{-1} 3505, 1757, 1732 and 1669; δ_H ($CDCl_3$) includes 6.14 (1H, s, 4-H), 5.37 and 5.18 (1H, 2 m, 6-H), 4.52–4.26 (3H, m), 4.03–3.74 (2H, m), 3.59–3.32 (2H, m), 1.52 (3H, s), 1.17 (3H, t, *J* 7.5, COCH$_2$C*H*$_3$), 1.03 and 1.02 (3H, 2 s) and 0.94 (3H, d, *J* 7, 16-*Me*); MS (ES +ve) *m*/*z* 569 (M + H)$^+$ (Found: C, 61.2; H, 7.0; S, 5.5. $C_{29}H_{38}F_2O_7S$ requires C, 61.25; H, 6.7; S, 5.6%).

Metabolite of 11a

Sodium hydroxide solution (0.1 M, 4.52 ml) was added to a solution of **11a** (250 mg, 0.452 mmol) in methanol (15 ml) and the mixture was stirred overnight. The solvent was removed under reduced pressure and the residue was dissolved in tetrahydrofuran (10 ml) and water (2 ml) and purified by HPLC (80% MeCN–H_2O) to give the corresponding hydroxy carboxylic acid sodium salt (115 mg, 43%) as a beige solid: analytical HPLC t_r 8.22 min; ν_{max} (KBr)/cm^{-1} 1715, 1669, 1634, 1576 and 1383; δ_H (DMSO-d_6) includes 7.27 (1H, d, *J* 10, 1-H), 6.28 (1H, dd, *J* 10 and 1, 2-H), 6.10 (1H, s), 5.74 and 5.55 (1H, 2 m, 6-H), 4.89 (1H, br s), 4.19 (1H, d, *J* 10), 1.50 and 1.49 (3H, 2 s), 1.34 (3H, s), 1.09 and 1.07 (3H, 2 s), 0.88 and 0.82 (3H, 2 s); MS (ES +ve) *m*/*z* 571 (M + H)$^+$ (Found: C, 51.4; H, 6.2; N, 1.1. $C_{28}H_{35}F_2NaO_8S{\cdot}3.4H_2O{\cdot}0.6C_2H_3N$ requires C, 51.7; H, 6.5; N, 1.2%).

Metabolite of 2a

A diastereoisomeric mixture of **3a**[3] was separated by HPLC (35% MeCN–H_2O containing 0.1% formic acid) to give 21-[(1*R*)-1-carboxy-3-hydroxypropyl)sulfanyl]-6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione sodium salt (**3aR**) as a white solid after trituration with ethyl acetate and then with diethyl ether: δ_H (DMSO-d_6) includes 7.34 (1H, d, *J* 10, 1-H), 6.27 (1H, dd, *J* 10 and 2, 2-H), 6.18 (1H, s), 6.10 (1H, s), 5.73 and 5.53 (1H, 2 m, 6-H), 4.89 (1H, br s), 4.21 (1H, br), 4.03 (1H, d, *J* 18, 21-H), 3.67 (1H, dd, *J* 18, 21-H), 1.50 (3H, s), 1.30 (3H, s), 1.07 (3H, s), and 0.80 (3H, s); MS (ES +ve) *m*/*z* 571 (M + H)$^+$ (Found: C, 53.25; H, 6.3; S, 4.9. $C_{28}H_{35}F_2NaO_8S{\cdot}2H_2O$ requires C, 53.5; H, 6.25; S, 5.1%) and 21-[(1*S*)-1-carboxy-3-hydroxypropyl)sulfanyl]-6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione sodium salt (**3aS**): δ_H (DMSO-d_6) includes 7.36 (1H, d, *J* 10, 1-H), 6.37 (1H, br), 6.26 (1H, d, *J* 10, 2-H), 6.10 (1H, s, 4-H), 5.73 and 5.53 (1H, 2 m, 6-H), 4.89 (1H, br s), 4.21 (1H, br), 3.94 (1H, d, *J* 18, 21-H), 3.75 (1H, d, *J* 18, 21-H), 1.50 (3H, s), 1.30 (3H, s), 1.03 (3H, s), and 0.82 (3H, s); MS (ES +ve) *m*/*z* 571 (M + H)$^+$ (Found: C, 53.9; H, 6.2; S, 4.8. $C_{28}H_{35}F_2NaO_8S{\cdot}1.6H_2O$ requires C, 54.1; H, 6.2; S, 5.15%). The configurations of **3aR** and **3aS** were established by re-lactonisation of the sodium salts to **2aR** and **2aS** respectively under acid conditions.

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