Novel Glucocorticoid Antedrugs Possessing a 17β-(γ-Lactone) Ring

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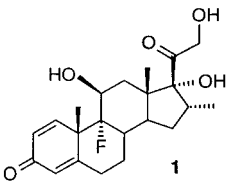
The chemical synthesis and structure−activity relationships of a novel series of 17β-glucocorticoid butyrolactones possessing either a 16α,17α-isopropylidene or -butylidene group are described. The sulfur-linked γ-lactone group was incorporated onto the 17β-position of the androstane nucleus via Barton ester decarboxylation and trapping the generated 17-radical with butyrolactone disulfides. The glucocorticoid butyrolactones were hydrolyzed in human plasma by the enzyme paraoxonase to the respective hydroxy acids, which were very weak glucocorticoid agonists. The rate of hydrolysis in plasma was very rapid ($t_{1/2} = 4-5$ min) in the case of lactones possessing a sulfur atom in the α-position of the butyrolactone group, whereas carbon-linked lactones were stable in plasma. 16α,17α-Butylidenes were more potent glucocorticoid agonists than the corresponding isopropylidene derivatives. Similarly, 1,4-dien-3-ones were more potent than the corresponding 4-en-3-ones. The butyrolactones linked to the steroidal nucleus via the β-position were more potent glucocorticoid agonists than those linked through the α-position of the lactone. The most potent compounds were also shown to be stable in human lung S9 fraction, showed much lower systemic effects than budesonide in the thymus involution test, and possessed topical antiinflammatory activity in the rat ear edema model.

Introduction

Topical glucocorticoids are extensively used for the treatment of a variety of inflammatory diseases such as rhinitis, inflammatory bowel disease, asthma, and several dermatological diseases. Asthma is recognized as a chronic inflammatory disease of the airways which is characterized by an eosinophil- and lymphocyte-rich accumulation of activated inflammatory cells in the airway wall. Over 100 million people worldwide suffer from asthma, and the most effective antiinflammatory treatment of the disease is provided by glucocorticoids.[1] Oral glucocorticoids such as dexamethasone (**1**) and prednisolone (**2**) are still used in patients with severe



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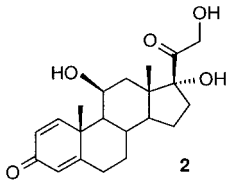

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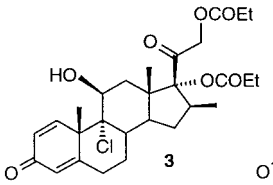
asthma; however, the high doses required mean that these agents are associated with significant adverse systemic effects such as growth retardation, osteoporosis, and suppression of the hypothalamic−pituitary−adrenal function and of the immune system. Inhaled glucocorticoid therapy was introduced in 1972 with beclomethasone dipropionate (**3**), and this provided asthma control with much lower doses and dramatically reduced systemic effects. A large proportion of the inhaled dose (ca. 80%) is, however, swallowed and therefore available for absorption into the systemic circulation from the gastrointestinal tract. Glucocorticoids such as **3** and budesonide (**4**) retain significant oral bioavailability (>10%), and together with drug absorbed via the lung still have the potential to cause systemic side effects at therapeutic doses. Fluticasone propionate (**5**) (launched in 1993) is very efficiently inactivated in the liver and exhibits low oral bioavailability leading to a further reduction in systemic exposure.[2]



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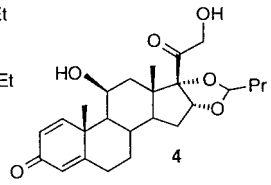

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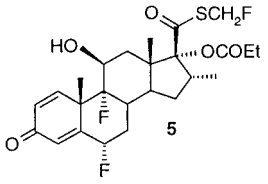

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The incidence of asthma in developed countries is increasing and is responsible for an increasing proportion of healthcare costs. Inhaled glucocorticoids are currently used for treatment of all types of asthma including mild forms of the disease, and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

The terms 'antedrug'[3] and 'soft drug'[4] 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

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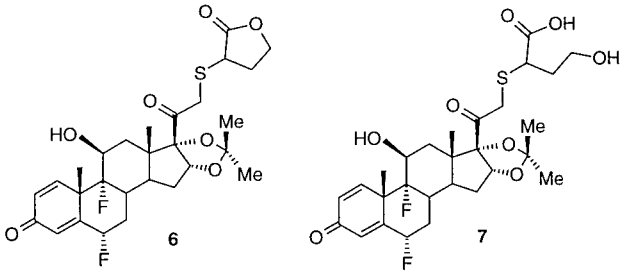
number of publications have appeared that describe the synthesis of antiinflammatory glucocorticoid derivatives based on the antedrug concept.[5−12] These have generally involved the introduction of a carboxylic ester functionality in the expectation that hydrolysis by blood esterases will give inactive steroid carboxylic acid metabolites. 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. Thus, the potent antiinflammatory glucocorticoid **6** is hydrolyzed in human plasma ($t_{1/2}$ < 2 min) to the inactive hydroxycarboxylic acid **7** but is essentially stable in human lung S9 ($t_{1/2}$ > 2 h).[13] These



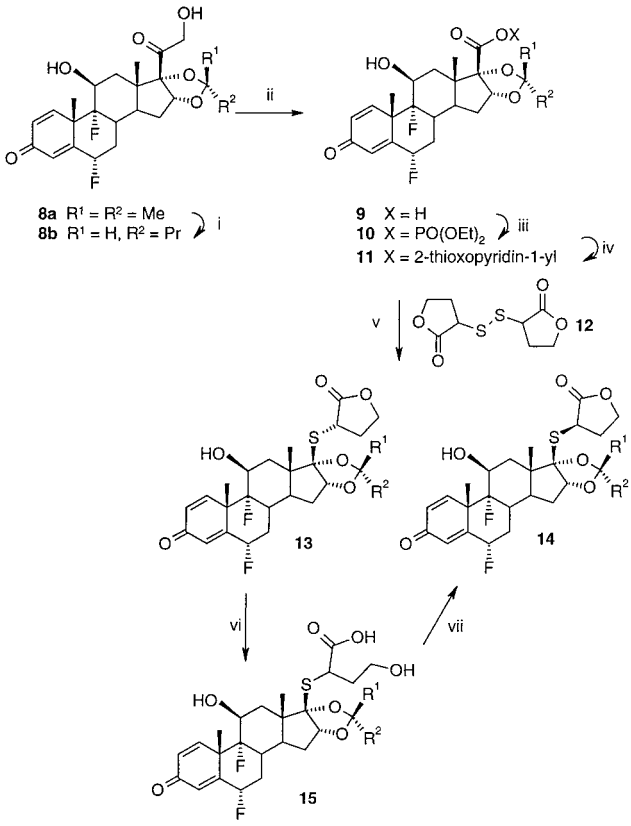
ideal properties make such lactone derivatives excellent lung-selective antedrug candidates for use in asthma. The enzyme responsible for the plasma hydrolysis of these γ-lactones was identified by our group as human serum paraoxonase (EC 3.1.8.1), an organophosphate-detoxifying enzyme whose natural substrate and function were unknown but which is thought to have a role in the metabolism of lipids and lipoproteins.[14] 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.[15] We believe that Fishbein's lactonase and paraoxonase are probably the same enzyme. Very recently the enzyme human serum homocysteine thiolactone hydrolase, a detoxifying enzyme responsible for the hydrolysis of homocysteine thiolactone, has been isolated and again identified as paraoxonase.[16]

As part of our medicinal chemistry program we have investigated the structural requirements for paraoxonase-mediated hydrolysis of a variety of glucocorticoid lactone derivatives and have recently reported on some C16,17-fused γ-lactones.[17] In this paper we report a series of C17-linked γ-lactones as analogues of the highly potent antiinflammatory 17β-thioalkylandrostane derivatives reported recently by the Rhône-Poulenc Rorer group.[18]

Chemistry

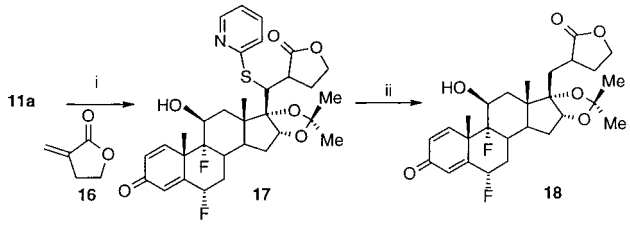
Fluocinolone acetonide (**8a**) was the most convenient commercially available starting material for the synthesis of our target acetonide, butylidene, and their respective 1,2-dihydro analogues. Fluocinolone acetonide was converted to the carboxylic acid **9a** by the K_2CO_3-catalyzed air oxidation developed by Kertesz and Marx[19] (Scheme 1). The carboxylic acid **9a** was then activated as the mixed anhydride **10a**[18] before reaction with 2-mercaptopyridine *N*-oxide sodium salt.[20] The resulting Barton ester **11a** was decarboxylated in the

Scheme 1[a]



[a] (i) PrCHO, sand, heptane, $HClO_4$; (ii) air, K_2CO_3, EtOH, H_2O; (iii) $ClPO(OEt)_2$, Et_3N, THF; (iv) 2-mercaptopyridine *N*-oxide sodium salt, DMF; (v) **12**, DMF, light; (vi) NaOH, H_2O, THF; (vii) EtOAc, TsOH, HCl.

Scheme 2[a]

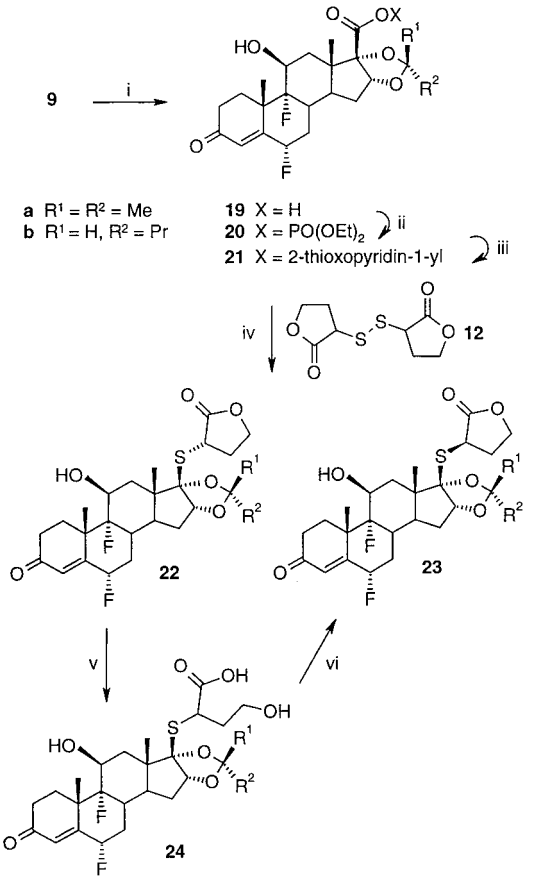


[a] (i) Light, DMF; (ii) Raney Ni, THF, H_2O.

presence of the disulfide **12**[21] to give a 1:1 mixture of diastereoisomers of the androstane-17β-(γ-lactone) sulfides **13a** and **14a**. The two diastereoisomers were separated by HPLC, and the configuration of the diastereoisomer **13a** with the 3.93 ppm triplet in the ^{1}H NMR spectrum (isomer with the shorter HPLC retention time) was shown to be the *S* isomer by an X-ray diffraction study. Base-catalyzed hydrolysis of isomer **13a** gave an 8:1 (*R*:*S*) mixture of the hydroxy acid **15a** in 70% yield indicating the facile epimerization at the lactone asymmetric center. Acid-catalyzed relactonization of this hydroxy acid mixture gave predominantly the lactone **14a** (9:1 **14a**:**13a**).

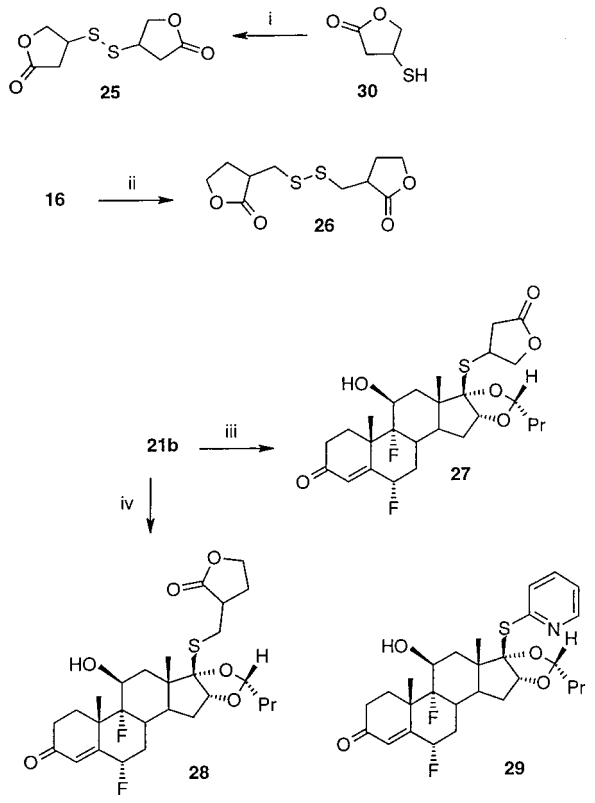
Decarboxylation of the Barton ester **11a** in the presence of α-methylene-γ-butyrolactone (**16**) gave the γ-butyrolactone pyridyl sulfide **17** (Scheme 2) which was desulfurized with Raney nickel to provide the C-linked γ-butyrolactones **18** as a 2:1 mixture of diastereoisomers. The individual diastereoisomers were separated by preparative HPLC.

Scheme 3[a]



[a] (i) H_2, $(PPh_3)_3RhCl$, EtOH; (ii) $ClPO(OEt)_2$, Et_3N, THF; (iii) 2-mercaptopyridine *N*-oxide sodium salt, DMF; (iv) **12**, DMF, light; (v) NaOH, H_2O, THF; (vi) EtOAc, HCl.

Scheme 4[a]



[a] (i) I_2, CH_2Cl_2; (ii) Na_2S_2, H_2O, EtOH; (iii) **25**, DMF, light; (iv) **26**, DMF, light.

The 16,17-butylidene derivatives were prepared in a fashion similar to the acetonides (Scheme 1). Thus fluocinolone acetonide **8a** was converted to the (*R*)-butylidene **8b** by the Astra method[22] and then oxidized to the carboxylic acid **9b**.[23] Activation of **9b** to **10b** and reaction with mercaptopyridine *N*-oxide as before gave Barton ester **11b** which was then decarboxylated in the presence of disulfide **12** to give the γ-lactone sulfides **13b** and **14b**. The two diastereoisomers were separated by preparative HPLC, and the configuration of the isomer with the shorter retention time (**13b**, more polar) was assigned the *S* configuration at the lactone asymmetric center (vide infra).

Selective hydrogenation of the carboxylic acids **9a**,**b** with Wilkinson's catalyst gave the corresponding 1,2-dihydro derivatives **19a,b** (Scheme 3). Conversion of the acids **19a,b** to the corresponding mixed anhydrides **20a**,**b**, followed by Barton ester formation gave **21a**,**b**. Decarboxylation of **21a,b** in the presence of **12** gave 1:1 diastereoisomeric mixtures of γ-lactone sulfides **22a,b** and **23a,b** which were separated by preparative HPLC. The structure of **22a** with the 3.95 ppm triplet in the ^{1}H NMR spectrum (isomer with the shorter HPLC retention time) was shown to be the *S* isomer by an X-ray diffraction study. The butylidenes were generally less crystalline than isopropylidenes, and crystals suitable for X-ray diffraction study to confirm the assignments of configuration could not be obtained. The butylidene **22b** was assigned the *S* configuration at the lactone asymmetric center (isomer with shorter HPLC retention time) by analogy with the corresponding acetonides. Base-catalyzed hydrolysis of lactone **22b** gave predominantly the (*R*)-hydroxy acid **24b**. Lactonization of the hydroxy acid mixture gave mainly the (*R*)-lactone **23b** confirming again that an epimerization occurs during hydrolysis. Additional confirmation of the configuration of diastereoisomers **22b** and **23b** was obtained from the chemical shift of the 16β-H of **24b** (minor isomer, giving **22b** on relactonization) which appeared 0.23 ppm further downfield than the major isomer (giving **23b** on relactonization). The analogous minor isomer of isopropylidene **15a** (*S* isomer) had a chemical shift 0.19 ppm further downfield than the corresponding major isomer (*R* isomer).

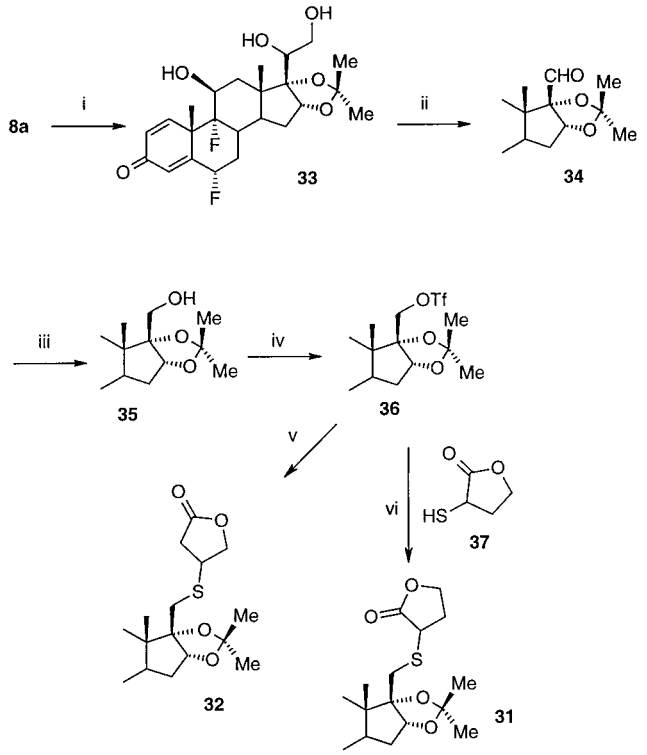
Barton ester **21b** was decarboxylated in the presence of disulfides **25** and **26** to give the steroidal sulfide lactones **27** and **28**, respectively, as mixtures of diastereoisomers (Scheme 4). The diastereoisomers of **27** were separated by preparative HPLC, the diastereoisomers of **28**, however, were separated only after extensive chromatography by preparative TLC in order to remove the 2-pyridyl sulfide impurity **29**. Disulfide **25** was prepared from 3-mercapto-γ-butyrolactone (**30**)[24] by oxidation with iodine in CH_2Cl_2, whereas disulfide **26** was prepared by Michael addition of disodium disulfide to **16**.

The homologous acetonide lactones **31** and **32** were prepared by the synthetic route shown in Scheme 5. Fluocinolone acetonide (**8a**) was selectively reduced to the diol **33** with $NaBH_4$ in THF in 99% yield. The use of THF was critical for the selective reduction of the C20-carbonyl; reduction in EtOH was nonselective, reducing the C3-carbonyl as well. The diol **33** was

Table 1. Stability of Compounds in Krebs Buffer and Human Plasma and Relative Potency in Vitro

entry	compd no.	stability in Krebs buffer (% compd remaining) after 60 min	stability in human plasma (% compd remaining) after 10 min	stability in human plasma (% compd remaining) after 60 min	rel potency (HeLa)	$t_{1/2}$ in human plasma (min)
1	**13a**	74	24	0	13	4
2	**14a**	90	45	15	2	4
3	**13b**	77	0	0	5	
4	**14b**	100	19	7	1	
5	**18** isomer A	100	100	100	3	
6	**18** isomer B	100	100	99	19	
7	**22a**	89	20	0	23	
8	**23a**	92	28	0	8	
9	**22b**	82	0	0	10	
10	**23b**	91	19	0	1.5	5
11	**27** isomer A	99	28	19	2	
12	**27** isomer B	100	56	0	0.5	
13	**28**	100	100	100	0.2	>240
14	**31**	96	0	0	>73	
15	**32**	89	24	0	52	
16	**38** isomer A	97	53	13	>250	
17	**38** isomer B	98	17	8	>250	
18	**41**	NT	NT	NT	7	
19	**15a**	NT	NT	NT	1200	
20	**24b**	NT	NT	NT	73	

Scheme 5[a]



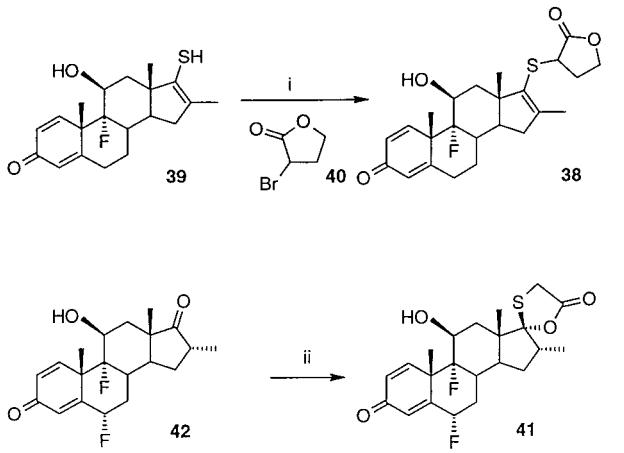
[a] (i) $NaBH_4$, THF; (ii) $NaIO_4$, EtOH, H_2O; (iii) $NaBH_4$, EtOH; (iv) Tf_2O, pyridine, CH_2Cl_2; (v) **30**, NaH, THF; (vi) **37**, NaH, THF.

cleaved to the aldehyde **34** with $NaIO_4$ and then reduced to the alcohol **35** with $NaBH_4$ in EtOH (45% for the last two steps). Reaction of alcohol **35** with trifluoromethanesulfonic acid anhydride gave the triflate **36** (93%) which was then displaced with 2-mercapto-γ-butyrolactone (**37**)[25] or 3-mercapto-γ-butyrolactone (**30**) to give **31** and **32**, respectively, as mixtures of diastereoisomers.

The lactone **38** (Scheme 6) was obtained by alkylation of the thioketone **39**[26] with 2-bromo-γ-butyrolactone (**40**) using K_2CO_3 in DMF, and the isomers were separated by chromatography followed by preparative HPLC.

Finally the spirocyclic analogue **41** was prepared from ketone **42**[27] and thioglycolic acid (**43**) using trimethylsilyl trifluoromethanesulfonate (TMSOTf) which acts as a Lewis acid and converts **43** to its bis-trimethylsilyl derivative in situ.[28] The spirocycle **41** was obtained as a single diastereoisomer whose configuration at C17 was assumed to be as drawn based on the mechanism of the reaction, where the mercapto group attacked the 17-keto group to form an ene-sulfide, followed by attack by the carboxylic acid from the least hindered α-side. The structure could not, however, be confirmed by NOE experiments.

Scheme 6[a]



[a] (i) K_2CO_3, **40**, DMF; (ii) $HSCH_2CO_2H$ (**43**), TMSOTf, CH_2Cl_2.

Biology

Compounds were tested in vitro for their stability in human plasma and for their glucocorticoid agonist activity. The stability of the compounds shown in Table 1 was examined by HPLC in Krebs buffer after incubating for 1 h and then their stability in human plasma after incubating for 10 and 60 min at 37 °C. The results are expressed as a percent (%) of compound remaining. For a selection of compounds more accurate half-lives in human plasma were determined and are shown in Table 1. The glucocorticoid agonist activity of the compounds in Table 1 was tested by the method of Stein[29] in the HeLa MMTV sPAP screen, a functional

Table 2. Inhibition of Rat Ear Edema by **13a** and **14a** Compared to Fluticasone Propionate

test compd	dose (μg)	mean wt[a] (±SEM) (mg)	% decrease	*p*
13a	1.56	56.03 ± 1.41	0	NS
13a	6.25	54.86 ± 1.35	1.46	NS
13a	25.00	50.65 ± 1.18	9.01	0.007
13a	100.00	44.05 ± 1.18	20.87	<0.001
14a	1.56	58.02 ± 1.46	0	NS
14a	6.25	47.85 ± 1.09	14.04	<0.001
14a	25.00	47.21 ± 1.28	15.20	<0.001
14a	100.00	43.03 ± 1.19	22.70	<0.001
5	0.25	45.29 ± 1.16	18.64	<0.001
5	1.00	39.79 ± 0.99	28.53	<0.001
5	4.00	40.57 ± 1.10	27.12	<0.001

[a] The mean weight of the control group was 55.67 ± 1.22 mg. Each group had $n = 44$.

Table 3. Inhibition of Rat Ear Edema by **23b** Compared to Fluticasone Propionate

test compd	dose (μg)	mean wt[a] (±SEM) (mg)	% decrease	*p*
23b	1.56	71.65 ± 2.81	0.83	NS
23b	6.25	55.94 ± 3.31	22.57	<0.001
23b	10.00	54.84 ± 2.18	24.10	<0.001
23b	25.00	49.76 ± 2.12	31.13	<0.001
23b	100.00	46.46 ± 2.86	35.70	<0.001
5	0.06	64.86 ± 2.70	10.23	0.04
5	0.25	47.21 ± 1.49	34.66	<0.001
5	1.00	44.71 ± 1.53	38.12	<0.001
5	4.00	44.39 ± 2.26	38.56	<0.001

[a] The mean weight of the control group was 72.25 ± 2.24 mg. Each group had $n = 28$.

Table 4. Effect on Thymus Weight by Compounds **14a** and **23b** Compared to Vehicle and Budesonide

compd	dose (μg)	thymus wt (mg)	% reduction
vehicle		482.22 ± 20.57	0
14a	1000	166.91 ± 13.91	65
23b	1000	304.95 ± 12.20	37
4	50	248.99 ± 23.19	48

in vitro assay. This test involves transfection of HeLa cells (human epithelial fibroblast cells established from a patient with cervical carcenoma) with a detectable reporter gene (secreted placental alkaline phosphatase, sPAP) under the control of a glucocorticoid response promoter (the LTR of the mouse mammary tumor virus, MMTV). The relative potencies of the compounds in Table 1 are expressed as the ratio of the ED_{50} of the test compound to that of dexamethasone which exhibited ED_{50} values in the range of 10−18 nM. Relative human glucocorticoid receptor affinities were determined for **23b** and its metabolite **24b** in competition studies with [^{3}H]dexamethasone. The stability of **23b** in human lung S9 fraction was examined by HPLC. Compounds **13a**, **14a**, and **23b** were examined in the croton oil-induced ear edema bioassay in rats, and the results are shown in Tables 2 and 3. Finally, systemic glucocorticoid activity was examined in the thymus involution test, following intratracheal (it) administration of 1 mg/day of **14a** and **23b** to male CD rats for 3 days (Table 4).

Results and Discussion

All compounds tested were essentially stable in Krebs solution. All lactones in Table 1 except **18** and **28** were hydrolyzed in human plasma to their respective hydroxy acids. Metabolites **15a** and **24b** were specifically synthesized as authentic materials for comparison with plasma hydrolysis products, whereas base-catalyzed hydrolysis caused epimerization at the lactone asymmetric center and plasma hydrolysis proceeded without epimerization. Lactone **28** was stable in plasma even after incubating for 4 h. Both **18** and **28** are lactones linked to the steroidal nucleus via carbon rather than sulfur, and this suggests that paraoxonase, the enzyme responsible for the lactone hydrolysis in human plasma, favors the presence of a heteroatom linkage to the lactone moiety. Typical half-lives of labile lactones in human plasma (such as **13a**, **14a**, and **23b**) were 4−5 min.

Lactone isomers **38**, lacking oxygen functionality at 17α, were very weak agonists. The androstane-17β-(methyl lactones) **31** and **32** were also weak agonists. The diene analogues were more potent than the dihydro versions (**14a-23a**, **13a-22a**, **14b-23b**, **13b-22b**), and the *R* isomers were more potent than the *S* isomers at the lactone asymmetric center (**14a-13a**, **23a-22a**, **14b-13b**, **23b-22b**). Additionally the butylidene analogues were more potent than the isopropylidene analogues (**14b-14a**, **23b-23a**, **13b-13a**, **22b-22a**). The β-linked lactone isomers **27** were more potent than their respective α-linked isomers **22b** and **23b**. The homologous lactone **28** was an extremely potent compound, more potent than its parent **22/23b** and much more active than its isomeric analogue **31**. The carbon-linked lactone **18** was equipotent with its sulfur-linked analogue **14a**; however, both **18** and **28** were stable in plasma as already mentioned. The metabolites, hydroxy acids **15a** and **24b**, showed very weak glucocorticoid agonist activity. The spirolactone **41**, which was prepared as a 17α-ester analogue, was a relatively weak agonist. From the above in vitro data lactones **14a**,**b**, **23b**, and **27** were identified as the most promising. However, the β-linked lactone **27**, although being the most potent compound, was not progressed any further because of concerns regarding its long-term stability (retro-Michael reaction). From the remaining compounds **14a** and **23b** were selected as examples possessing the diene-acetonide and the 1,2-dihydrobutylidene steroidal nuclei, and their relative human glucocorticoid receptor affinity was determined. Both parent lactones **14a** and **23b** had high affinity for the glucocorticoid receptor (IC_{50} = 1.4 and 6.4 nM, respectively), whereas their respective metabolites **15a** and **24b** had much lower affinity (IC_{50} = 289 and 400 nM, respectively). Additionally, both lactones were shown to be stable in human lung S9 fraction for at least 2.5 h making these compounds ideal antedrug candidates. The antiinflammatory activity of **13a**, **14a**, and **23b** was examined in the rat ear edema in vivo model and compared with that of fluticasone propionate **5**. The data are shown in Tables 2 and 3. Lactone **13a**, as expected from its in vitro data, possessed very weak antiinflammatory activity, whereas **14a** possessed slightly higher antiinflammatory activity. Compound **23b** was significantly more potent than the previous two compounds; however, it was less potent than **5**. Finally, lactones **14a** and **23b** were examined in the thymus involution test for the presence of any systemic effects in vivo. The weight of the thymi of rats treated with 1 mg/day for 3 days with **14a** and **23b** was reduced by

65% and 37%, respectively, whereas budesonide at a 20-fold lower dose (50 μg) caused a 48% reduction. Considering the greater topical antiinflammatory activity and its cleaner systemic profile, **23b** was the most promising candidate for progression from this series of compounds.

Conclusion

A series of novel lactone-containing glucocorticoid agonists have been identified displaying remarkably rapid and selective inactivation in human plasma. The butyrolactones were hydrolyzed in plasma to the respective hydroxy acids, which were very weak agonists. The rate of hydrolysis in plasma was very rapid in the case of lactones linked via sulfur, whereas carbon-linked lactones were stable in plasma. 16,17-Butylidene derivatives were more potent in vitro than the corresponding isopropylidenes. Similarly, 1,4-dien-3-ones were more potent than the coresponding 4-en-3-ones. The butyrolactones linked to the steroidal nucleus via the β-position were more potent glucocorticoid agonists than those linked through the α-position of the lactone. The most potent compounds were also stable in human lung S9 fraction, showed much lower systemic effects than budesonide in the rat thymus involution test, and possessed topical antiinflammatory activity in the rat ear edema inhibition test. Lactone **23b** was identified as a compound suitable for further progression from this series of glucocorticoids.

Experimental Section

Organic solutions were dried over anhydrous $MgSO_4$. 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 C18 column (25 cm × 5 cm), flow rate 45 mL/min, detecting at 240 nm, unless otherwise stated. Appropriate fractions were combined and evaporated under reduced pressure. The residue was dissolved in EtOAc, and the solution was dried, evaporated, and triturated in Et_2O. Melting points were determined on a Kofler block and are uncorrected. 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 or 400 MHz, and the chemical shifts are expressed in ppm relative to tetramethylsilane. MS(TSP+ve) and MS(ES+ve) refer to mass spectra ran 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-17β-(2-oxotetrahydrofuran-3-ylsulfanyl)androsta-1,4-dien-3-one (13a and 14a). A solution of 6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-3-oxoandrosta-1,4-diene-17β-carboxylic acid, 2-thioxo-2*H*-pyridin-1-yl ester (**11a**)[30] (2.50 g, 4.57 mmol) in dry DMF (15 mL) was added to a stirred suspension of **12**[21] (2.15 g, 9.17 mmol) in dry DMF (15 mL) under nitrogen at 0 °C and then subjected to irradiation by two 200 W tungsten filament light bulbs for approximately 4 h. The reaction mixture was diluted with EtOAc (650 mL) and washed with brine, water, 2 M HCl, water, saturated $NaHCO_3$, water and brine (200 mL each). The organic solution was dried, the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel eluting with $CHCl_3$−MeOH (50:1) and further purified by HPLC (60% MeCN−H_2O) to give **13a** (635 mg, 27%) as a white crystalline solid: Anal. HPLC t_R 7.71 min; mp 296−302 °C; IR ν_{max} 1774, 1668 cm^{-1}; NMR δ ($CDCl_3$) 7.08 (1H, dd, *J* 10 and 1 Hz), 6.45 (1H, s), 6.38 (1H, dd, *J* 10 and 2 Hz), 5.45 and 5.35 (1H, 2m), 4.7 (1H, t, *J* 3 Hz), 4.43−4.26 (3H, m), 3.93 (1H, t, *J* 8 Hz), 1.69, 1.52, 1.44, 1.20 (4s, 3H each); MS(TSP+ve) *m*/*z* 511 (M + H)$^+$. Anal. ($C_{26}H_{32}F_2O_6S$) C, H, S. And **14a** (602 mg, 26%) as a white solid: Anal. HPLC t_R 8.10 min; mp 262−275 °C; IR ν_{max} 1768, 1668 cm^{-1}; NMR δ ($CDCl_3$) 7.25 (1H, dd, *J* 10 and 1 Hz), 6.43 (1H, s), 6.37 (1H, dd, *J* 10 and 2 Hz), 5.46 and 5.33 (1H, 2 m), 4.56 (1H, t, *J* 3 Hz), 4.38−4.26 (3H, m), 3.88 (1H, t, *J* 8 Hz), 1.62, 1.54, 1.44, 1.27 (4s, 3H each); MS(TSP+ve) *m*/*z* 511 (M + H)$^+$. Anal. ($C_{26}H_{32}F_2O_6S\cdot0.6C_4H_{10}O\cdot0.5H_2O$) C, H, S.

21-[(1-Carboxy-3-hydroxypropyl)sulfanyl]-6α,9α-difluoro-11β-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione, Sodium Salt (15a). A solution of **13a** (100 mg, 0.19 mmol) in THF (3 mL) was treated with sodium hydroxide (1 M, 0.19 mL) and the mixture was stirred for 2 h at 20 °C. The reaction mixture was evaporated to dryness under reduced pressure, the residue was dissolved in water (16 mL) and washed with Et_2O (35 mL) and EtOAc (25 mL). The aqueous phase was then freeze-dried to give **15a** (76 mg, 70%): Anal. HPLC t_R 6.08 min, 10% and 6.26 min, 79.5%; NMR δ (DMSO-d_6) 7.28 (1H, d, *J* 10 Hz), 6.25 (1H, dd, *J* 10 and 2 Hz), 6.10 (1H, s), 5.69 and 5.56 (1H, 2m), 4.50 (0.1 H, d, *J* 5 Hz), 4.31 (0.9H, d, *J* 5 Hz), 4.12 (1H, m), 3.67 (1H, dd, *J* 9 and 3 Hz), 3.55 (1H, m), 3.42 (1H, m), 1.55, 1.50, 1.28, 1.17 (4s, 3H each); MS(ES+ve) *m*/*z* 529 (M + H)$^+$. Anal. ($C_{26}H_{33}F_2O_7$-SNa) C, H, S.

Acidification of the above sodium salt of **15a** with *p*-toluenesulfonic acid in a mixture of EtOAc and 2 M HCl (1:1, 20 mL) over 18 h gave lactone **14a**. Anal. HPLC t_R 7.75 min, 7% (**13a**) and t_R 8.12 min, 63% (**14a**).

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-17β-[(2-oxotetrahydrofuran-3-yl)(2-pyridylsulfanyl)-methyl]androsta-1,4-dien-3-one (17). A solution of **11a** (370 mg, 0.67 mmol) and **16** (0.09 mL, 1.0 mmol) in dry DMF (5 mL) was stirred under an atmosphere of nitrogen at −5 to 0 °C and was subjected to irradiation by two 200-W tungsten filament light bulbs for 5.5 h. During this time **16** (0.09 mL, 1.0 mmol) was added three times at 1.5-h intervals. More **16** (0.27 mL, 3.1 mmol) was added and the reaction mixture was stirred under nitrogen with irradiation at −5 to 0 °C for 1 h and then allowed to warm to room temperature. The reaction mixture was diluted with EtOAc (30 mL) and water (30 mL). The organic phase was separated, washed with brine (30 mL) and dried. The solvent was removed under reduced pressure, and the residue was purified by HPLC (55% MeCN−H_2O) to give **17** (39 mg, 10%): NMR δ ($CDCl_3$) 8.52 (1H, d, *J* 5 Hz), 7.64 (1H, m), 7.44 (1H, d, *J* 7.5 Hz), 7.22 (1H, m), 7.09 (1H, d, *J* 10 Hz), 6.41 (1H, br s), 6.33 (1H, d, *J* 10 Hz), 5.46 and 5.26 (1H, 2m), 4.53−4.24 (5H, m), 2.86 (1H, s), 1.50, 1.46, 1.38, 1.10 (4s, 3H each); MS(TSP+ve) *m*/*z* 502 (M + H)$^+$.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-17β-[(2-oxotetrahydrofuran-3-yl)methyl]androsta-1,4-dien-3-one (18). Raney nickel (80 mg) in water (1 mL) was added to a solution of **17** (38 mg, 0.06 mmol) in THF (4 mL). The reaction mixture was stirred under an atmosphere of nitrogen at room temperature for 24 h and then heated to reflux for 18 h. After cooling to room temperature, Raney nickel (136 mg) in water−THF (1:1, 1 mL) was added and the reaction mixture stirred for 20 h. More Raney nickel (30 mg) in water (1 mL) was added and the reaction mixture stirred for 4 h. The mixture was filtered and washed with EtOAc (3 × 5 mL). Water (20 mL) was added and the organic phase was separated, washed with brine (20 mL) and dried. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC eluting with EtOAc−cyclohexane (1:3) to give **18** isomer A (7 mg, 23%): Anal. HPLC t_R 7.47 min; NMR δ ($CDCl_3$) 7.09 (1H, d, *J* 10 Hz), 6.43 (1H, bs), 6.36 (1H, d, *J* 10 Hz), 5.44 and 5.32 (1H, 2m), 4.36 (3H, m), 4.16 (1H, m), 1.53, 1.39, 1.24, 1.17 (4s, 3H each); MS(TSP+ve) *m*/*z* 493 (M + H)$^+$; HRMS(ES+ve) 493.2394 $C_{27}H_{35}F_2O_6$ requires 493.2402. And **18** isomer B (15 mg, 48%): Anal. HPLC t_R 7.31 min; IR ν_{max} 1770, 1666 cm^{-1}; NMR δ ($CDCl_3$) 7.09 (1H, d, *J*

10 Hz), 6.43 (1H, br s), 6.36 (1H, d, *J* 10 Hz), 5.45 and 5.32 (1H, 2m), 4.44 (1H, d, *J* 5 Hz), 4.35 (2H, m), 4.14 (1H, m), 2.83 (1H, m), 1.53, 1.47, 1.39, 1.04 (4s, 3H each); MS(TSP+ve) *m*/*z* 493 $(M + H)^+$.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylic Acid, Diethyl Phosphoric Anhydride (10b). A solution of **9b**[23] (4.00 g, 8.84 mmol) in THF (100 mL) was treated with 4 Å powdered molecular sieves (3 g), followed by triethylamine (2.64 mL, 17.7 mmol) and the mixture was stirred for 2 h under nitrogen at 20 °C. Diethyl chlorophosphate (1.92 mL, 13.3 mmol) was then added and the reaction mixture was stirred for a further 16 h at 20 °C. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in EtOAc (150 mL) and washed with 1 M HCl (2 × 100 mL), aqueous K_2CO_3, water and brine (100 mL each). The organic phase was dried and the solvent was removed under reduced pressure, to give **10b** (4.35 g, 84%): NMR δ ($CDCl_3$) 7.16 (1H, d, *J* 10 Hz), 6.44 (1H, s), 6.37 (1H, d, *J* 10 Hz), 5.49 and 5.29 (1H, 2m), 5.00 (1H, d, *J* 5 Hz), 4.78 (1H, t, *J* 4 Hz), 4.47−4.02 (5H, m), 1.55 (3H, s), 1.39 (6H, t, *J* 7 Hz), 1.08 (3H, s), 0.90 (3H, t, *J* 7.5 Hz); MS(TSP+ve) *m*/*z* 589 $(M + H)^+$.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylic Acid, 2-Thioxo-2*H*-pyridin-1-yl Ester (11b). A solution of **10b** (1.0 g, 1.7 mmol) in DMF (8.5 mL) in a flask protected from light was treated with 2-mercaptopyridine *N*-oxide sodium salt (304 mg, 2.04 mmol) at 20 °C under nitrogen for 16 h. The reaction mixture was then poured into EtOAc (100 mL) and the mixture was washed with brine (100 mL), water (3 × 100 mL), saturated $NaHCO_3$, water and brine (100 mL each). The organic phase was dried, the solvent was removed under reduced pressure, and the residue was purified by HPLC (diol column 25 cm × 5 cm) eluting with 90% EtOAc−heptane to give **11b** (580 mg, 61%): IR ν_{max} 1788, 1736, 1705, 1670 cm^{-1}; NMR δ (DMSO-d_6) 8.24 (1H, d, *J* 7 Hz), 7.60 (1H, d, *J* 8 Hz), 7.67 (1H, t, *J* 8 Hz), 7.29 (1H, d, *J* 10 Hz), 6.94 (1H, t, *J* 7 Hz), 6.31 (1H, d, *J* 10 Hz), 6.13 (1H, s), 5.73 and 5.56 (1H, 2m), 5.58 (1H, br s), 5.22 (1H, t, *J* 4 Hz), 5.02 (1H, d, *J* 5 Hz), 4.24 (1H, m), 1.51 (3H, s), 1.15 (3H, s), 0.89 (3H, t, *J* 7.5 Hz); MS-(TSP+ve) *m*/*z* 562 $(M + H)^+$. Anal. ($C_{29}H_{33}F_2NO_6S \cdot C_4H_8O_2$) C, H, N, S.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11β-hydroxy-17β-(2-oxotetrahydrofuran-3-ylsulfanyl)androsta-1,4-dien-3-one (13b and 14b). A solution of **11b** (300 mg, 0.53 mmol) in dry DMF (5.5 mL) was reacted with **12** (199 mg, 1.07 mmol) in dry DMF (2 mL) in a way similar to that for the preparation of **13a**. The crude product was purified by HPLC (65% MeCN−H_2O) to give **13b** (83.3 mg, 30%): Anal. HPLC t_R 8.245 min; mp 204−207 °C; IR ν_{max} 1774, 1668 cm^{-1}; NMR δ ($CDCl_3$) 7.08 (1H, d, *J* 10 Hz), 6.44 (1H, s), 6.37 (1H, d, *J* 10 Hz), 5.45 (1H, t, *J* 4.5 Hz), 5.47 and 5.28 (1H, 2m), 4.80−4.21 (4H, m), 3.81 (1H, t, *J* 8.5 Hz), 1.54 (3H, s), 1.19 (3H, s), 0.93 (3H, t, *J* 7.5 Hz); MS(TSP+ve) *m*/*z* 525 $(M + H)^+$. Anal. ($C_{27}H_{34}F_2O_6S \cdot 0.5C_4H_8O_2$) C, H, S. And **14b** (75 mg, 27%): Anal. HPLC t_R 8.72 min; mp 183−185 °C; IR ν_{max} 1772, 1669 cm^{-1}; NMR δ ($CDCl_3$) 7.13 (1H, d, *J* 10 Hz), 6.43 (1H, s), 6.37 (1H, d, *J* 10 Hz), 5.48 and 5.28 (1H, 2 m), 5.26 (1H, t, *J* 4.5 Hz), 4.41−4.21 (4H, m), 3.85 (1H, t, *J* 8.5 Hz), 1.54 (3H, s), 1.29 (3H, s), 0.94 (3H, t, *J* 7.5 Hz); MS(TSP+ve) *m*/*z* 525 $(M + H)^+$. Anal. ($C_{27}H_{34}F_2O_6S$) C, H, S.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11β-hydroxy-3-oxoandrosta-4-ene-17β-carboxylic Acid (19b). A solution of **9b** (4.66 g, 10.3 mmol) in EtOH (180 mL) was hydrogenated over tris(triphenylphosphine)rhodium chloride (466 mg) at atmospheric pressure for 65 h. A further quantity of catalyst (500 mg) was added and the reaction mixture was hydrogenated for a further 48 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was dissolved in EtOAc (150 mL) and extracted with aqueous K_2CO_3 (3 × 100 mL). The combined aqueous extracts were washed with EtOAc (100 mL) and acidified to pH 2 with 7 M hydrochloric acid. The resulting suspension was extracted with EtOAc (3 × 100 mL) and the combined organic extracts were washed with water (2 × 100 mL) and brine (2 × 150 mL). The organic phase was dried and the solvent was removed under reduced pressure, to give **19b** (3.93 g, 84%): mp 145−150 °C; IR ν_{max} 1736 cm^{-1}; NMR δ (DMSO-d_6) 5.81 (1H, s), 5.60 and 5.41 (1H, 2m), 5.17 (1H, br s), 4.87 (1H, br s), 4.68 (1H, t, *J* 4 Hz), 4.15 (1H, m), 1.49 (3H, s), 0.93 (3H, s), 0.87 (3H, t, *J* 7 Hz); MS(TSP+ve) *m*/*z* 455 $(M + H)^+$. Anal. ($C_{24}H_{32}F_2O_6 \cdot H_2O$) C, H.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-3-oxoandrosta-4-ene-17β-carboxylic Acid, Diethyl Phosphoric Anhydride (20a). A solution of **19a**[31] (3.00 g, 6.81 mmol) in THF (75 mL) was reacted with diethyl chlorophosphate (1.08 mL, 7.49 mmol) in a way similar to that for the preparation of **10b**, to give **20a** (3.64 g, 92%): IR ν_{max} 1768, 1669 cm^{-1}; NMR δ ($CDCl_3$) 6.14 (1H, s), 5.37 and 5.18 (1H, 2m), 5.12 (1H, d, *J* 4.5 Hz), 4.41−4.19 (5H, m), 1.52 (3H, s), 1.48 (3H, s), 1.39 (3H, t, *J* 6.5 Hz), 1.38 (3H, t, *J* 6.5 Hz), 1.28 (3H, s), 1.05 (3H, s); MS(TSP+ve) *m*/*z* 577 $(M + H)^+$. Anal. ($C_{27}H_{39}F_2O_9P \cdot 0.18C_4H_8O_2 \cdot 0.2H_2O$) C, H.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-3-oxoandrosta-4-ene-17β-carboxylic Acid, 2-Thioxo-2*H*-pyridin-1-yl Ester (21a). A solution of **20a** (1.5 g, 2.6 mmol) in DMF (12 mL) was reacted with 2-mercaptopyridine *N*-oxide sodium salt (465 mg, 3.12 mmol) in a similar way to that for the preparation of **11b** to give **21a** (1.13 g, 79%): IR ν_{max} 1805, 1715, 1669 cm^{-1}; NMR δ (DMSO-d_6) 7.92 (1H, d, *J* 6 Hz), 7.60 (1H, d, *J* 8 Hz), 7.45 (1H, t, *J* 8 Hz), 6.88 (1H m), 5.83 (1H, s), 5.62 and 5.43 (1H, 2m), 5.33 (1H, d, *J* 5 Hz), 5.13 (1H, br s), 4.21 (1H, m), 1.51, 1.43, 1.39 and 1.19 (4s, 3H each); MS(TSP+ve) *m*/*z* 550 $(M + H)^+$. Anal. ($C_{28}H_{33}F_2NO_6S \cdot 1.1H_2O \cdot 0.15C_3H_7NO$) C, H, N, S.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-17β-(2-oxotetrahydrofuran-3-ylsulfanyl)androsta-4-en-3-one (22a and 23a). A solution of **21a** (400 mg, 0.73 mmol) in dry DMF (7.5 mL) was reacted with **12** (271 mg, 1.45 mmol) in a way similar to that for the preparation of **13a**. The crude product was purified by HPLC (60% MeCN−H_2O), and further purified by HPLC (53% MeCN−H_2O) to give **22a** (33.1 mg, 9%): Anal. HPLC t_R 7.65 min; mp 237−240 °C; IR ν_{max} 1774, 1668 cm^{-1}; NMR δ ($CDCl_3$) 6.14 (1H, s), 5.37 and 5.18 (1H, 2m), 4.68 (1H, d, *J* 4.5 Hz), 4.45−4.21 (3H, m), 3.95 (1H, t, *J* 8 Hz), 1.67, 1.53, 1.47, 1.16 (4s, 3H each); MS(TSP+ve) *m*/*z* 513 $(M + H)^+$. Anal. ($C_{26}H_{34}F_2O_6S$) C, H. And **23a** (46.6 mg, 12%): Anal. HPLC t_R 8.18 min; mp 258−263 °C; IR ν_{max} 1772, 1669 cm^{-1}; NMR δ ($CDCl_3$) 6.14 (1H, s), 5.38 and 5.19 (1H, 2 m), 4.57 (1H, br s), 4.4−4.2 (3H, m), 3.89 (1H, t, *J* 8.5 Hz), 1.64, 1.53 1.48, 1.25 (4s, 3H each); MS(TSP+ve) *m*/*z* 513 $(M + H)^+$. Anal. ($C_{26}H_{34}F_2O_6S$) C, H, S.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11β-hydroxy-3-oxoandrosta-4-ene-17β-carboxylic Acid, Diethyl Phosphoric Anhydride (20b). A solution of **19b** (3.0 g, 6.6 mmol) in THF (75 mL) was reacted with diethyl chlorophosphate (1.05 mL, 7.26 mmol) in a way similar to that for the preparation of **10b**, to give **20b** (3.34 g, 86%): IR ν_{max} 1767, 1668 cm^{-1}; NMR δ ($CDCl_3$) 6.14 (1H, s), 5.37 and 5.18 (1H, 2m), 5.10 (1H, d, *J* 5 Hz), 4.79 (1H, t, *J* 4.5 Hz), 4.4−4.2 (5H, m), 1.53 (3H, s), 1.39 (6H, t, *J* 7 Hz), 1.06 (3H, s), 0.93 (3H, t, *J* 7 Hz); MS(ES+ve) *m*/*z* 591 $(M + H)^+$. Anal. ($C_{28}H_{41}F_2O_9P$) C, H.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11β-hydroxy-3-oxoandrosta-4-ene-17β-carboxylic Acid, 2-Thioxo-2*H*-pyridin-1-yl Ester (21b). A solution of **20b** (1.50 g, 2.54 mmol) in DMF (12 mL) was reacted with 2-mercaptopyridine *N*-oxide sodium salt (455 mg, 3.05 mmol) in a way similar to that for the preparation of **11b**, to give **21b** (1.25 g, 87%): IR ν_{max} 1800, 1718, 1684, 1669 cm^{-1}; NMR δ (DMSO-d_6) 8.23 (1H, d, *J* 7 Hz), 7.6 (1H, dd, *J* 9 and 2 Hz), 7.46 (1H, dt, *J* 7 and 2 Hz), 6.93 (1H, dt, *J* 7 and 2 Hz), 5.83 (1H, s), 5.62 and 5.43 (1H, 2m), 5.28 (1H, d, *J* 4.5 Hz), 5.24 (1H, t, *J* 4.5 Hz), 5.0 (1H, d, *J* 4.5 Hz), 4.26−4.16 (1H, m), 1.51 (3H, s), 1.14 (3H, s), 0.91 (3H, t, *J* 7.5 Hz); MS(TSP+ve) *m*/*z* 564 $(M + H)^+$. Anal. ($C_{29}H_{35}F_2NO_6S \cdot 0.3C_4H_8O_2$) C, H, N, S.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11β-hydroxy-17β-(2-oxotetrahydrofuran-3-ylsulfanyl)androsta-

4-en-3-one (22b and 23b). A solution of **21b** (400 mg, 0.71 mmol) in dry DMF (7.5 mL) was reacted with **12** (264.5 mg, 1.42 mmol) in a way similar to that for the preparation of **13a.** The crude product was purified by HPLC (65% MeCN−H_2O) to give **22b** (91 mg, 24%): Anal. HPLC t_R 8.49 min; mp 209−213 °C; IR ν_{max} 3493, 1774, 1668 cm^{-1}; NMR δ ($CDCl_3$) 6.14 (1H, s), 5.47 (1H, t, *J* 4.5 Hz), 5.36 and 5.17 (1H, 2 m), 4.5−4.2 (4H, m), 3.84 (1H, t, *J* 8.5 Hz), 1.53 (3H, s), 1.17 (3H, s), 0.93 (3H, t, *J* 7.5 Hz); MS(TSP+ve) *m*/*z* 527 $(M + H)^+$. Anal. ($C_{27}H_{36}F_2O_6S \cdot 0.25C_4H_{10}O \cdot 0.15C_4H_8O_2$) C, H, S. And **23b** (79.4 mg, 21%): Anal. HPLC t_R 9.11 min; mp 120−122 °C; IR ν_{max} 1768, 1682, 1668 cm^{-1}; NMR δ ($CDCl_3$) 6.14 (1H, s), 5.36 and 5.17 (1H, 2 m), 5.27 (1H, t, *J* 4 Hz), 4.4−4.2 (4H, m), 3.88 (1H, t, *J* 8.5 Hz), 1.53 (3H, s), 1.27 (3H, s), 0.97 (3H, t, *J* 7.5 Hz); MS(TSP+ve) *m*/*z* 527 $(M + H)^+$. Anal. ($C_{27}H_{36}F_2O_6S \cdot 0.2H_2O$) C, H, S.

21-[(1-Carboxy-3-hydroxypropyl)sulfanyl]-6α,9α-difluoro-11*β*-hydroxy-16α,17α-(*R*)-butylidenedioxypregna-4-ene-3,20-dione, Sodium Salt (24b). To a stirring solution of **22b** (71.0 mg, 0.135 mmol) in MeOH (6 mL) was added sodium hydroxide (0.05 M, 2.70 mL) over 5 min. The reaction mixture was stirred for 2 h and then the solvent was removed under reduced pressure. EtOAc (4 mL) was added to the residue and triturated for 20 min. The solid was collected by filtration, washed with EtOAc (2 × 1 mL) and dried under reduced pressure to give **24b** (57 mg, 73%), as a 2:1 mixture of *R*:*S* isomers: Anal. HPLC t_R 9.00min, 33% and 9.57 min, 67%; NMR δ (DMSO-d_6) 5.80 (1H, s), 5.59 and 5.39 (1H, 2m), 5.38 (0.33H, t, *J* 4 Hz), 5.15 (0.67H, t, *J* 4 Hz), 4.32−4.00 (2H, m), 3.69−3.49 (2H, m), 1.50 (3H, s), 1.15 (3H, s), 0.88 (3H, t, *J* 7.5 Hz); MS(ES+ve) *m*/*z* 545 $(M + H)^+$. A small amount of the product was relactonized in MeOH and 2 M HCl to give a 2:1 mixture of **23b**:**22b**.

4,4′-Disulfanediylbis(dihydrofuran-2-one) (25). A solution of **30**[24] (106 mg, 0.90 mmol) in CH_2Cl_2 (2 mL) was treated with a solution of iodine (253 mg, 1.00 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred for 18 h at 20 °C. The reaction mixture was diluted with CH_2Cl_2 and washed with aqueous $NaHSO_3$ solution, dried, the solvent was removed under reduced pressure, and the residue was purified by preparative TLC on a single plate (20 cm × 20 cm) run in EtOAc−cyclohexane (2:1) to give **25** (30 mg, 28%): IR ν_{max} 1778, 1771 cm^{-1}; NMR δ ($CDCl_3$) 4.6 (2H, dd, *J* 9 and 6 Hz), 4.35 (2H, dt, *J* 5 and 4 Hz), 3.86−3.72 (2H, m), 2.96 (2H, dd, *J* 17 and 8 Hz), 2.66 (2H, dt, *J* 18 and 5 Hz); MS(TSP+ve) *m*/*z* 252 $(M + NH_4)^+$; HRMS(EI) found 234.0017, $C_8H_{10}O_4S_2$ requires 234.0020.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11*β*-hydroxy-17*β*-(2-oxotetrahydrofuran-4-ylsulfanyl)androst-4-en-3-one (27). A solution of **21b** (1.3 g, 2.3 mmol) in dry DMF (13 mL) was reacted with **25** (450 mg, 1.92 mmol) in a way similar to that for the preparation of **13a**. The crude product was purified by column chromatography on silica gel eluting with EtOAc−cyclohexane (1:1) and then by HPLC (65% MeCN−H_2O) to give **27** isomer A (54 mg, 4%): mp 117−119 °C; IR ν_{max} 1782, 1667 cm^{-1}; NMR δ ($CDCl_3$) 6.15 (1H, s), 5.37 and 5.18 (1H, 2 m), 5.22 (1H, t, *J* 5 Hz), 4.63 (1H, dd, *J* 9 and 8 Hz), 4.37 (1H, m), 4.24 (1H, d, *J* 5 Hz), 4.2−4.1 (1H, br), 4.14 (1H, t, *J* 9 Hz), 4.05−3.88 (1H, m), 2.85 (1H, dd, *J* 17 and 8 Hz), 1.53 (3H, s), 1.2 (3H, s), 0.97 (3H, t, *J* 8 Hz); MS(TSP+ve) *m*/*z* 527 $(M + H)^+$, 443 $(MH - C_4H_4O_2)^+$. Anal. ($C_{27}H_{36}F_2O_6S \cdot H_2O$) C, H, S. And **27** isomer B (29 mg, 2%): mp 112−113 °C; IR ν_{max} 1782, 1669 cm^{-1}; NMR δ ($CDCl_3$) 6.14 (1H, s), 5.25 (1H, t, *J* 4 Hz), 5.35 and 5.17 (1H, 2 m), 4.58 (1H, dd, *J* 9 and 7 Hz), 4.37 (1H, m), 4.28 (1H, d, *J* 5 Hz), 4.05 (1H, t, *J* 9 Hz), 4.0−3.86 (1H, m), 2.93 (1H, dd, *J* 18 and 8 Hz), 2.62 (1H, dd, *J* 18 and 9 Hz), 1.53 (3H, s), 1.17 (3H, s), 0.96 (3H, t, *J* 7 Hz); MS(TSP+ve) *m*/*z* 527 $(M + H)^+$, 443 $(MH - C_4H_4O_2)^+$. Anal. ($C_{27}H_{36}F_2O_6S \cdot 0.5CH_2Cl_2$) C, H, S.

3-({[(2-Oxotetrahydrofuran-3-yl)methyl]dithio}methyl)-dihydrofuran-2(3*H*)-one (26). Disodium sulfide nonahydrate (8.6 g, 36 mmol) and sulfur (1.14 g, 35.8 mmol) were heated in water (30 mL) to 126 °C (oil bath temperature) for 2 h. A solution of **16** (5.0 mL, 57 mmol) in EtOH (10 mL) was added at 120 °C (oil bath temperature) and then the mixture was stirred at 20 °C for 65 h. Concentrated H_2SO_4 (18 M; 3 mL) was added and the mixture was stood at 20 °C for 24 h. The reaction mixture was partitioned between CH_2Cl_2 and water and the organic phase was washed with water, aqueous $NaHCO_3$, dried and concentrated. The residue was chromatographed on silica gel eluting with EtOAc−cyclohexane (1:3 to 1:1) to give **26** (2.76 g, 36%) as a gum: IR ν_{max} 1768 cm^{-1}; NMR δ ($CDCl_3$) 4.43 (2H, dt, *J* 9, 2 Hz), 4.27 (2H, dd, *J* 9, 7 Hz), 3.30 (1H, t, *J* 3 Hz), 3.25 (1H, t, *J* 3 Hz), 3.13−2.90 (2H, m), 2.83−2.69 (2H, m), 2.62−2.48 (2H, m), 2.25−2.05 (2H, m); MS-(TSP+ve) *m*/*z* 280 $(M + NH_4)^+$.

16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11*β*-hydroxy-17*β*-{[(2-oxotetrahydrofuran-3-yl)methyl]sulfanyl}-androst-4-en-3-one (28) and 16α,17α-(*R*)-Butylidenedioxy-6α,9α-difluoro-11*β*-hydroxy-17*β*-(2-pyridylsulfanyl)-androst-4-en-3-one (29). A solution of **21b** (509 mg, 0.90 mmol) in DMF (9 mL) was reacted with **26** (524 mg, 2.00 mmol) in a way similar to that for the preparation of **13a**. The crude product was purified by HPLC (65% MeCN−H_2O), and further purified by preparative TLC eluting with EtOAc−cyclohexane (1:1) to give **28** isomer A (22 mg, 4%): Anal. HPLC t_R 9.88 min; NMR δ ($CDCl_3$) 6.14 (1H,s), 5.23 (1H, t, *J* 4 Hz), 5.35 and 5.17 (1H, 2m), 4.48−4.20 (4H, m), 3.39 (1H, dd, *J* 13, 4 Hz), 3.02 (1H, dq, *J* 9, 4 Hz), 2.68 (1H, dd, *J* 13, 9 Hz), 1.53 (3H, s), 1.24 (3H, s), 0.97 (3H, t, *J* 7 Hz); MS(TSP+ve) *m*/*z* 541 $(M + H)^+$. Anal. ($C_{28}H_{38}F_2O_6S \cdot H_2O$) C, H, S. And **28** isomer B (31 mg, 6%) as a white foam: Anal. HPLC t_R 9.78 min; NMR δ ($CDCl_3$) 6.15 (1H, s), 5.22 (1H, t, *J* 4 Hz), 5.35 and 5.18 (1H, 2m), 4.5−4.20 (4H, m), 3.29 (1H, d, *J* 8 Hz), 2.82−2.63 (2H, m), 1.52 (3H, s), 1.20 (3H, s), 0.95 (3H, t, *J* 7 Hz); MS(TSP+ve) *m*/*z* 541 $(M + H)^+$. Anal. ($C_{28}H_{38}F_2O_6S \cdot 0.25C_4H_8O_2 \cdot 0.5H_2O$) C, H, S. And **29** (30 mg, 6%): Anal. HPLC t_R 7.60 min; IR ν_{max} 1668 cm^{-1}; NMR δ ($CDCl_3$) 8.50 (1H, d, *J* 5 Hz), 7.68 (1H,d, *J* 8 Hz), 7.58 (1H,dt, *J* 8, 2 Hz), 7.20 (1H, dt, *J* 5, 1 Hz), 6.13 (1H, s), 5.38 (1H, t, *J* 4 Hz), 5.35 and 5.15 (1H, 2m), 4.49 (1H, d, *J* 5 Hz), 4.16 (1H, br d), 1.49 (3H, s), 1.27 (3H, s), 0.96 (3H, t, *J* 7 Hz); MS(TSP+ve) *m*/*z* 520 $(M + H)^+$. Anal. ($C_{28}H_{35}F_2NO_4S \cdot 0.5H_2O$) C, H, N, S.

6α,9α-Difluoro-11*β*,20,21-trihydroxy-16α,17α-isopropylidenedioxypregnane-1,4-dien-3-one (33). A solution of **8a** (22.7 g, 50.2 mmol) in THF (250 mL) was treated with $NaBH_4$ (1.90 g, 50.2 mmol) and the mixture was stirred under nitrogen for 18 h at 20 °C. The mixture treated with 2 M HCl (100 mL) and diluted with water (1L). The solid that formed was collected by filtration, and dried. Additional product was obtained from the filtrate by extraction with EtOAc to give **33** (22.5 g, 99%) as a white solid: NMR δ (DMSO-d_6) 7.27 (1H, d, *J* 10 Hz), 6.29 (1H, d, *J* 10 Hz), 6.12 (1H, s), 5.75 and 5.57 (1H, 2m), 5.01 (1H, d, *J* 6 Hz), 4.79 (0.67H, d, *J* 5 Hz), 4.74 (0.33H, t, *J* 5 Hz), 4.57 (0.67H, t, *J* 5 Hz), 4.48 (0.33H, t, *J* 5 Hz), 1.51, 1.36, 1.351 and 1.13 (4s, 3H each); MS(TSP+ve) *m*/*z* 455 $(M + H)^+$. Anal. ($C_{26}H_{32}F_2O_60 \cdot 5H_2O$) C, H.

6α,9α-Difluoro-17*β*-formyl-11*β*-hydroxy-16α,17α-isopropylidenedioxyandrosta-1,4-dien-3-one (34). A solution of **33** (22.0 g, 48.4 mmol) in EtOH (750 mL) was reacted with a suspension of $NaIO_4$ (18.5 g, 86.5 mmol) in H_2O (75 mL). The mixture was stirred at 20 °C for 4 h. The white solid was removed by filtration and washed with EtOAc. The combined filtrate and washings were evaporated under reduced pressure and the residue was redissolved in EtOAc. The solution was washed with water, brine, dried and evaporated to dryness to give **34** (20.4 g, 100%) as a white foam: IR ν_{max} 1726, 1668 cm^{-1}; NMR δ ($CDCl_3$) 9.8 (1H, s), 7.1 (1H, dd, *J* 10, 1 Hz), 6.45 (1H, s), 6.38 (1H, dd, *J* 10, 2 Hz), 5.48 and 5.30 (1H, 2m), 4.96 (1H, d, *J* 5 Hz), 4.42 (1H, m), 1.53, 1.45, 1.18, 1.00 (4s, 3H each); MS(TSP+ve) *m*/*z* 423 $(M + H)^+$. Anal. ($C_{23}H_{28}F_2O_5 \cdot 0.25H_2O$) C, H.

6α,9α-Difluoro-11*β*-hydroxy-17*β*-hydroxymethyl-16α,17α-isopropylidenedioxyandrosta-1,4-dien-3-one (35). A solution of **34** (3.16 g, 7.48 mmol) in EtOH (100 mL) was cooled to 0 °C and treated with $NaBH_4$ (283 mg, 7.48 mmol). After 0.5 h the reaction mixture was treated with 2 M HCl and evaporated to dryness. The residue was partitioned between EtOAc and 2 M HCl. The organic phase was washed with 2 M

HCl, aqueous $NaHCO_3$, brine, dried, concentrated and chromatographed on silica gel eluting with EtOAc to give **35** (1.43 g, 45%) as a white solid: mp 286−290 °C; IR ν_{max} 1668 cm^{-1}; NMR δ ($CDCl_3$) 7.15 (1H, d, *J* 10 Hz), 6.44 (1H, s), 6.38 (1H, d, *J* 10 Hz), 5.48 and 5.30 (1H, 2m), 4.40−4.28 (1H, m), 4.32 (1H, d, *J* 5 Hz), 3.98−3.80 (2H, m), 1.54 (3H, s), 1.43 (6H, s), 1.14 (3H, s); MS(TSP+ve) *m/z* 425 $(M + H)^+$. Anal. ($C_{23}H_{30}F_2O_5$) C, H.

6α,9α-Difluoro-11β-hydroxy-17β-hydroxymethyl-16α,17α-isopropylidenedioxy-3-oxoandrosta-1,4-dien-17-yl Trifluoromethanesulfonate (36). A solution of **35** (1.42 g, 3.34 mmol) in CH_2Cl_2 (35 mL) and pyridine (0.32 mL, 3.9 mmol) was cooled to 0 °C and then treated with Tf_2O (0.67 mL, 4.0 mmol). After 1.5 h the mixture was diluted with EtOAc and 0.5 M HCl. The organic phase was washed with $NaHCO_3$, water, dried and evaporated under reduced pressure to give **36** (1.73 g, 93%) as a yellow solid: NMR δ ($CDCl_3$) 7.12 (1H, d, *J* 10 Hz), 6.44 (1H, s), 6.39 (1H, d, *J* 10 Hz), 5.50 and 5.30 (1H, 2m), 4.74 (2H, m), 4.45 (1H, d, *J* 5 Hz), 4.38 (1H, m), 1.54,1.44, 1.43, 1.17 (4s, 3H each); MS(ES+ve) *m/z* 557 $(M + H)^+$. Anal. ($C_{24}H_{29}F_5O_7S \cdot H_2O$) C, H, S.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-17β-[(2-oxotetrahydrofuran-3-ylsulfanyl)methyl]-androsta-1,4-dien-3-one (31). 2-Mercapto-γ-butyrolactone (100 mg, 0.85 mmol) was added to a suspension of NaH (60% oil dispersion, 24 mg, 0.60 mmol) in THF (1 mL) under nitrogen. After 5 min a solution of **36** (81 mg, 0.14 mmol) in THF (3 mL) was added and the mixture was stirred for 16 h at 20 °C. The reaction mixture was diluted with EtOAc and 2 M HCl. The organic phase was washed with brine, dried and evaporated under reduced pressure to an orange solid, which was purified by HPLC (65% MeCN−H_2O) to give **31** (32 mg, 43%) as a white foam: Anal. HPLC indicated a mixture of two diastereoisomers t_R 9.46 min, 34% and 9.58 min, 66%; IR ν_{max} 1760, 1664 cm^{-1}; NMR δ (DMSO-d_6) 7.26 (1H, d, *J* 10 Hz), 6.30 (1H, d, *J* 10 Hz), 6.12 (1H, s), 5.75 and 5.56 (1H, 2m), 5.48 (1H, d, *J* 3.5 Hz), 4.46 (1H, m), 4.30 (2H, br t, *J* 6 Hz), 4.13 (1H, br), 3.9−3.8 (1H, m), 1.51 (3H, s), 1.40 (2H, s), 1.36 (1H, s), 1.30 (2H, s), 1.16 (1H, s), 1.10 (1H, s), 1.05 (2H, s); MS(TSP+ve) *m/z* 525 $(M + H)^+$; HRMS(ES+ve) found 525.2098, $C_{27}H_{35}F_2O_6S$ requires 525.2122.

6α,9α-Difluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-17β-[(2-oxotetrahydrofuran-4-ylsulfanyl)methyl]-androsta-1,4-dien-3-one (32). A solution of **30** (180 mg, 1.52 mmol) in THF (2 mL) was added to a suspension of NaH (60% oil dispersion, 42 mg, 1.05 mmol) in THF (1 mL) under nitrogen. After 5 min a solution of **36** (310 mg, 0.14 mmol) in THF (15 mL) was added and the mixture was stirred for 18 h at 20 °C. The reaction mixture was diluted with EtOAc and 2 M HCl. The organic phase was washed with brine, dried and evaporated under reduced pressure to an orange gum, which was purified by HPLC (65% MeCN−H_2O) to give **32** (62 mg, 21%) as a white solid: IR ν_{max} 1774, 1663 cm^{-1}; NMR δ ($CDCl_3$ + CD_3OD) 7.17 (1H, d, *J* 10 Hz), 6.43 (1H, s), 6.38 (1H, d, *J* 10 Hz), 5.5 and 5.3 (1H, 2m), 4.61 (1H, q, *J* 9 Hz), 4.40 (1H, d, *J* 5 Hz), 4.35−4.26 (1H, m), 4.22 (1H, dd, *J* 10, 5 Hz), 3.9−3.7 (1H, m), 2.98 and 2.91 (1H each, dd, *J* 8, 2 Hz), 1.54, 1.43, 1.41, 1.12 (4s, 3H each); MS(TSP+ve) *m/z* 441 $(MH - C_4H_4O_2)^+$. Anal. ($C_{27}H_{34}F_2O_6S$) C, H, S.

9α-Fluoro-11β-hydroxy-16-methyl-17-(2-oxotetrahydrofuran-3-ylsulfanyl)androsta-1,4,16-trien-3-one (38). A mixture of enethiol **39**[26] (322 mg, 0.92 mmol) and K_2CO_3 (276 mg, 2.00 mmol) in DMF (3 mL) was stirred for 10 min and then reacted with **40** (166 mg, 1.00 mmol). The mixture was stirred at 20 °C for 14 days and then diluted with EtOAc and 2 M HCl. The organic phase was washed with brine, dried, concentrated and chromatographed on silica gel eluting with EtOAc−cyclohexane (1:) and then further purified by HPLC (60% MeCN−H_2O) to give **38** isomer A (41 mg, 11%) as a white solid: Anal. HPLC t_R 10.91 min; NMR δ ($CDCl_3$) 7.26 (1H, d, *J* 10 Hz), 6.35 (1H, dd, *J* 10, 2 Hz), 6.13 (1H, s), 4.5−4.26 (3H, m), 3.70 (1H, dd, *J* 10, 6 Hz), 1.90 (3H, s), 1.58 (3H, s), 1.19 (3H, s); MS(ES+ve) *m/z* 433 $(M + H)^+$; HRMS(ES+ve) found 433.1853, $C_{24}H_{30}FO_4S$ requires 433.1849. And **38** isomer B (45 mg, 12%) as a white foam: Anal. HPLC t_R 10.73 min; IR ν_{max} 1770, 1666 cm^{-1}; NMR δ ($CDCl_3$) 7.25 (1H, d, *J* 10 Hz), 6.33 (1H, dd, *J* 10, 2 Hz), 6.13 (1H, s), 3.58 (1H, dd, *J* 10, 5 Hz), 1.87 (3H, s), 1.58 (3H, s), 1.15 (3H, s); MS(ES+ve) *m/z* 433 $(M + H)^+$. Anal. ($C_{24}H_{29}FO_4S$) C, H.

6α,9α-Difluoro-11β-hydroxy-16α-methyl-17-spiro[androsta-1,4-diene-17,2′-[1,3]oxathiolane]-3,5′-dione (41). A mixture of 6α,9α-difluoro-11β-hydroxy-16α-methylandrosta-1,4-diene-3,17-dione (**42**) (1.16 g, 3.30 mmol) and thioglycolic acid (0.46 mL, 6.6 mmol)in CH_2Cl_2 (10 mL) was treated with a solution of TMSOTf in CH_2Cl_2 (1 M; 2 mL) and the mixture was stirred for 15 h at 20 °C. More TMSOTf (1 M; 1 mL) was added and after 0.5 h stirring a further portion of TMSOTf (1 M; 1 mL) was added (total 4 mmol). After 1 h stirring the dark solution was diluted with CH_2Cl_2 and aqueous $NaHCO_3$. The organic phase was separated, washed with aqueous $NaHCO_3$ water, dried and evaporated to a yellow solid (470 mg) which was chromatographed on silica gel eluting with EtOH−CH_2Cl_2 (1:99) to give **41** (170 mg, 12%) as a white solid: Anal. HPLC t_R 9.78 min; IR ν_{max} 3463, 1769, 1667 cm^{-1}; NMR δ ($CDCl_3$) 7.12 (1H, dd, *J* 10, 2 Hz), 6.43 (1H, s), 6.38 (1H, dd, *J* 10, 2 Hz), 5.48 and 5.28 (1H, 2m), 4.40 (1H, m), 3.75 (1H, d, *J* 17 Hz), 3.60 (1H, d, *J* 17 Hz), 1.54 (3H, s), 1.20 (3H, s), 1.09 (3H, d, *J* 7 Hz); MS(ES+ve) *m/z* 425 $(M + H)^+$. Anal. ($C_{22}H_{26}F_2O_4S$) C, H.

Assay Methods. 1. Krebs and Human Plasma Incubations. Whole blood was collected from 4 volunteers, pooled in order to reduce variations between different plasma preparations, treated with heparin solution (1.2 units/mL) to prevent clotting, and then centrifuged at 3000*g* for 10 min at 4 °C. The upper plasma layer was stored at −20 °C. Krebs buffer and human plasma aliquots were incubated at 37 °C and spiked with test compound solution to give a final concentration of 30 μg/mL. Control tubes were spiked with DMSO only. At 10- and 60-min time points for the plasma solutions, and after 60 min for the Krebs buffer, citric acid solution (2 M, 20 μL) was added. For the zero time points citric acid solution (2 M, 20 μL) was added to the plasma before the test compound solution was added. All samples were stored at −20 °C overnight, centrifuged and aliquots (2 μL) of the supernatants were injected onto a Spherisorb S3 ODS-2 column (10 cm × 0.2 cm) eluting with 0.1% v/v H_3PO_4 (A) and 95:5 v/v MeOH−H_2O containing 0.1% v/v H_3PO_4 (B) using the following gradient: 0−100% B over 12 min, 100% B for 4 min, returned to 0% B over 2 min, flow rate 0.2 mL/min detecting at 240 nm. All samples and controls were assayed in duplicate. Internal standards were used as a quality control check of the reproducibility of the chromatography. In most cases dexamethasone (1 mg/mL in 2-propanol) was used as the internal standard except in cases where the retention time for dexamethasone coincided with that for the test compound in which case hydrocortisone (1 mg/mL in 2-propanol) was used instead. Half-lives of a selection of test compounds in human plasma were determined by obtaining more time points as appropriate in the above plasma hydrolysis assay. The identity of the metabolites of **14a** and **23b** was confirmed by spiking the plasma hydrolysis products with the appropriate authentic hydroxy acids **15a** and **24b**.

2. HeLa MMTV sPAP Cell Assay. HeLa cells were grown as monolayer cultures. The cells were cultured in modified Earles medium (10% heat-inactivated fetal calf serum, 1% L-glutamine, 0.1% benzylpenicillin sodium, 0.1% streptomycin sulfate, 1% nonessential amino acids, 250 μg/mL Geneticin G418 sulfate). The cells were plated at 10^6 cells/mL in 96-well plates and incubated for 72 h to grow to confluency. The medium was removed and compounds were added to triplicate wells at concentrations ranging from 10 μM to 25.6 pM (9 points/curve). Dexamethasone was used as standard and a DMSO control was also included as a background control. The plate was incubated for 72 h in the incubator. An aliquot of sample medium was removed and heat inactivated at 65 °C for 0.5 h. The plate was spun at 450*g* and then *p*-nitrophenyl phosphate solution (5 mM, 200 μL) was added to each well. The plate was incubated in the dark at 37 °C for 10 min, read

at 405 nm on a microplate reader, and the sPAP units were calculated. From the sPAP calculations a dose−response curve was constructed and the ED_{50} value was calculated. An average value of the ratio of the test compound's ED_{50} to that of dexamethasone's from the same experiment (minimum of two separate experiments in triplicate) gave the relative potency.

3. Human Glucocorticoid Receptor Binding Assay. Cytosolic lysate, prepared from human glucocorticoid receptor-infected baculovirus Tni cells (5 μg total protein), was incubated in a total volume of 100 μL with [^{3}H]dexamethasone (Amersham International; 86Ci/mmol; TRK645) (2 nM) in 50 mM potassium phosphate buffer pH 7.0 containing; 100 μM EDTA, 20 mM $NaMoO_4$, 500 μM Pefobloc (Sigma), 1 μM Leupeptin, 1 μM pepstatin, 1 mM dithiothreitol, 10% v/v glycerol. For competition experiments, compounds (10 μL) were added to the assay mixture in a 10% v/v DMSO solution. After a 2-h incubation at 20 °C, samples were filtered through a polyethylenimine-treated glass microfiber filter mat B (Wallac, cat. no. 1204-404) prewetted with ice-cold wash buffer (50 mM Tris, pH 7.4). Polyethylenimine-treated filters were prepared by presoaking mats in a 0.2% v/v solution for 3 h with drying at 20 °C. Samples were washed through the filter with a further 1 mL of ice-cold buffer and the filtermats dried at 20 °C 12 h. Dried filtermats were melted to a solid scintillant sheet (Wallac MeltiLex, 90 °C, 15 min) and counted in a scintillation counter (Wallac Betaplate). The relative receptor potencies were expressed as the concentration required to inhibit the binding of [^{3}H]dexamethasone by 50%. Values were derived from 10-point competition curves and are the mean (±SE) of two separate experiments.

4. Human Lung S9 Incubations. Human lung S9 preparation was mixed with 10 mM pH 7.4 phosphate buffer, in the ratio of 1:1. Incubations were performed in 500 μL of the above mixed with 50 μL of 30 mg/mL NADPH in 10 mM pH 7.4 phosphate buffer and carried out at 37 °C. After preincubating for 5 min the reactions were started by the addition of 5 μL of drug solution, nominally 5 mg/mL in DMSO. Aliquots were removed immediately after mixing and at intervals up to 6 h and mixed with an equal volume of MeCN. These samples were centrifuged and then aliquots (20 μL) of the supernatants were injected onto a Zorbax Rx C8 column (25 cm × 0.46 cm) maintained at 30 °C and eluted with MeCN:25 mM aqueous ammonium formate containing 0.1% HCO_2H (70:30), at a flow rate of 1 mL/min, detecting at 240 nm.

5. Antiinflammatory Activity. Inhibition of rat ear edema: A solution of the test compounds dissolved in acetone containing croton oil (5% v/v, 40 μL) was applied simultaneously to the inner surface of each of the ears of male rats (60−100 g). The control groups of animals were treated with croton oil only or vehicle only (40 μL acetone), three groups of animals were treated with standard (fluticasone propionate) at three different doses (0.25, 1 and 4 μg), and four or five groups of animals were treated with test compounds at 1.56, 6.25, 10, 25 and 100 μg. The animals were sacrificed 6 h later and the ears removed. Standard size disks (5 mm diameter) were punched out and the disks weighed. Mean weight of the ear disks was calculated and from this the percentage decrease in ear weight from the croton oil controls was obtained.

6. Systemic Effects. Thymus involution test: Isofluorane-anesthetized male CD rats (90−120 g) (n = 8) were dosed with test compound suspension daily for 3 days 1 mg/day (0.2 mL). Budesonide (50 μg/d) was used as standard and the control group was dosed with vehicle (0.2% Tween 80 in 100 mL saline) (0.2 mL; n = 6). The animals were sacrificed on day 4 and the thymus removed and weighed. The percentage reduction of the mean weights ±SE of thymi of animals treated with test compounds and standard was calculated from the mean weights of vehicle-treated animals.

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Supporting Information Available: Additional IR data, microanalytical data, and X-ray data for compounds **13a** and **22a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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