equiv of maleic acid, and the reaction mixture was kept at the boiling point for 2 min and cooled to afford the trimaleate precipitates, which were collected and air-dried. When the pure bases were taken up in ethanol solutions of hydrochloric acid, usual workup led to the crystalline trihydrochlorides.

Chemical shifts of compounds **20**, **23**, **26**, and **27** are given as typical examples of ^1H NMR spectra in D_2O.

20: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-$CH_2\delta$-N(CH_2CH_3)$_2$) δ 1.50 (t, 2 × 3 H, CH_2CH_3), 1.97–2.25 (m, 2 × 2 H, CH_2-β + CH_2-γ), 2.53 (s, 3 H, CH_3-4), 3.31–3.62 (m, 3 × 2 H, CH_2CH_3 + CH_2-δ), 3.68–3.97 (m, 2 H, CH_2-α), 7.94 (s, 1 H, H-3), 8.20 (d, 1 H, H-6, J_{6-7} = 8 Hz), 8.88 (d, 1 H, H-7), 9.95 (s, 1 H, H-9).

23: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-N(CH_{2a}-CH_{2b})$_2$) δ 2.20–2.35 (m, 3 × 2 H, $(CH_2b)_2$ + CH_2-β), 2.45 (s, 3 H, CH_3-4), 3.2–3.7 (m, 3 × 2 H, $(CH_{2a})_2$ + CH_2-γ), 3.82 (t, 2 H, CH_2-α), 7.85 (s, 1 H, H-3), 7.89 (d, 1 H, H-6, J_{6-7} = 6 Hz), 8.73 (d, 1 H, H-7), 9.70 (s, 1 H, H-9).

26: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-N(CH_3)$_2$) δ 2.35–2.59 (m, 2 H, CH_2-β), 2.87 (d, 3 H, CH_3-4, $J_{CH_3\text{-}4\text{-}H\text{-}3}$ = 1 Hz), 3.07 (s, 2 × 3 H, N(CH_3)$_2$), 3.46–3.66 (m, 2 H, CH_2-γ), 3.94 (t, 2 H, CH_2-α), 4.44 (s, 3 H, CH_3-5), 8.0 (d, 1 H, H-3), 8.38 (d, 1 H, H-6, J_{6-7} = 7 Hz), 8.88 (d, 1 H, H-7), 10.0 (s, 1 H, H-9).

27: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-$CH_2\delta$-N(CH_2CH_3)$_2$) δ 1.40 (t, 2 × 3 H, CH_2CH_3), 1.81–2.12 (m, 2 × 2 H, CH_2-β + CH_2-γ), 2.84 (d, 3 H, CH_3-4, $J_{CH_3\text{-}4\text{-}H\text{-}3}$ = 1 Hz), 3.23–3.53 (m, 3 × 2 H, CH_2CH_3 + CH_2-δ), 3.72–3.94 (m, 2 H, CH_2-α), 4.39 (s, 3 H, CH_3-5), 7.89 (d, 1 H, H-3), 8.33 (d, 1 H, H-6, J_{6-7} = 9 Hz), 8.84 (d, 1 H, H-7), 9.86 (s, 1 H, H-9).

DNA Binding Measurements. The DNA binding constants were determined by competition with ethidium bromide, as described,[27] in 20 mM Na_2NPO_4/NaH_2PO_4, 0.1 M NaCl, 1 mM EDTA, pH 7.4. DNA (calf thymus, Sigma) concentration was 10 μg/mL.

L1210 Cytotoxicity Determination. L1210 cells in exponential phase of growth were grown in RPMI 1640 medium supplemented with 2 mM glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% fetal calf serum, in a 5% CO_2 atmosphere at 37 °C. The drugs were dissolved in distilled water and added to the cells (0.8×10^5 cells/mL) for 48 h. The cells were then counted, and results were expressed as the drug concentration that inhibited by 50% the cell proliferation (ID_{50}). The ID_{50}'s were estimated by regression analysis of the dose–response data.

In Vivo Murine Tumor Models. The murine tumors were provided by Prof. G. Atassi (Institut Jules Bordet, Bruxelles, Belgium), and NCI protocols[25] were used throughout the drug evaluation tests.

P388 and L1210 leukemia viable cells, respectively 10^6 and 10^5, were inoculated ip on day 0 in CDF1 hybrid male or female mice (10 mice for test group). Compounds, dissolved in distilled water, were administered ip (0.1 mL/10 g of body weight), at various doses, for 5 days (D1–5). Vehicle, 5-fluorouracil (positive control of test), and BD40 (reference compound) were administered under the same conditions. The antitumor activity (T/C) was evaluated, according to the formula T/C = (median day of survival of treated animals at a given dose of product/median day of survival of "negative control" animals) × 100. Mice surviving for 30 days were considered as cured; they were included in the calculation of the median survival time.

The NCI criteria for activity in the in vivo murine models were used:

tumor model	act. criterion	good act. criterion
P388 leukemia	T/C > 127%	T/C > 175%
L1210 leukemia	T/C > 125%	T/C > 150%

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Aldosterone Antagonists. 2. Synthesis and Biological Activities of 11,12-Dehydropregnane Derivatives

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Several steroid derivatives having the Δ^{11}-pregnane skeleton with a 17-γ-spirolactone function were synthesized to evaluate their antialdosterone activity and to elucidate the relation between their binding affinity to mineralocorticoid receptor (MR) and their mineralo- and/or antimineralocorticoid activity. Although many of the synthesized compounds showed strong binding affinity for the MR and aldosterone agonist activity, 3-(17β-hydroxy-3-oxoandrosta-1,4,6,11-tetraen-17α-yl)propionic acid γ-lactone (**12**) exhibited good aldosterone antagonist activity in an in vivo assay. Its in vivo antiandrogenic activity was also found to be relatively weak.

Antimineralocorticoid therapy has been considered to be effective for treating edematous diseases and essential hypertension with primary and secondary aldosterone excess.[1] Aldosterone (Ald), the most potent mineralocorticoid hormone,[2] is synthesized in the zona glomerulosa cells of the adrenal cortex from deoxycorticosterone (DOC) via corticosterone and 18-hydroxycorticosterone by stepwise hydroxylations mediated by cytochrome P-450.[3] This hormone regulates the electrolyte balance of body fluids by promoting excretion of potassium and retention of sodium ions.[4] Treatment of aldosterone excess has been attempted with spironolactone (Sp)[5] and potassium canrenoate (soldactone),[6] well-established aldosterone antagonists, but their clinical usefulness is often limited by

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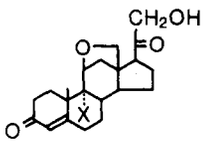
Table I. Comparison of the Relative Binding Affinity (RBA) for the Mineralocorticoid Receptor (MR) and Mineralocorticoid Activity in Vivo of Δ^{11}-Deoxycorticosterone (4) with Other Natural Mineralocorticoids

compound	RBA for MR (0 °C)	rel mineralocorticoid effect in vivo
corticosterone	14	0.14[a]
11-deoxycorticosterone	58	1
18-deoxyaldosterone	50	–
19-nordeoxycorticosterone	170	1.5–5.1[b]
Δ^{11}-deoxycorticosterone	150	1
aldosterone	100	30–50[b]

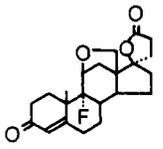
[a] Value is quoted from ref 24. [b] Values are quoted from ref 25.

adverse side effects attributed to their antiandrogenic and progestational properties.[7] Therefore, increasing interest is being focused on the search for new aldosterone antagonists that will not produce such side effects.[8] Spirorenone[9] and mespirenone[10] are representative results of these efforts.

18-Deoxyaldosterone (21-hydroxy-11β,18-epoxypregn-4-ene-3,20-dione, 1),[11,12] an analogue of Ald in which the aldehyde hemiacetal structure is replaced by a stable 11β,18-epoxy ring, was shown to possess high binding affinity for the cytoplasmic mineralocorticoid receptor (MR) of rat kidney (about one-third of the binding affinity of Ald) and to exhibit about a 2:1 antagonist to agonist ratio in both toad bladder and adrenalectomized rat bioassay systems by Ulick et al.[13] Prompted by the interesting



1, X=H
2, X=F



3

findings that a similar analogue, 18-deoxy-9α-fluoroaldosterone (2), showed extremely high binding affinity for the MR and sodium-retaining activity about as potent as that of Ald, we recently conducted structural modification of 18-deoxyaldosterone and 18-deoxy-9α-fluoroaldosterone by replacing their 17β-hydroxyacetyl side chain with 17β-hydroxy-17-propionic acid γ-lactone (17-γ-spirolactone) to improve their antagonistic nature.[14] The biological properties of these compounds both in vitro and in vivo were evaluated, and 3-(9α-fluoro-17β-hydroxy-11β,18-epoxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone (3) was shown to possess fairly strong binding affinity for the MR. It also exhibited good aldosterone antagonist activity in an in vivo assay. However, its agonistic nature cannot be ignored.

Table II. Comparison of the Relative Binding Affinity (RBA) for the Progestin Receptor (PR) and Progestational Activity in Vivo of Δ^{11}-Progesterone (5) with Other Progesterone Derivatives

compound	RBA for PR (0 °C)	rel progestational effect in vivo
progesterone	100	1
19-norprogesterone	650 (120[a])	10.7[a]
Δ^{11}-progesterone	220	3[b]

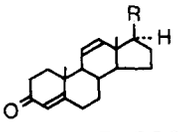
[a] Values are quoted from ref 20. [b] Value is quoted from ref 26.

Table III. Comparison of the Relative Binding Affinity (RBA) for the Androgen Receptor (AR) and Androgenic Activity in Vivo of Δ^{11}-Testosterone (6) with Other Testosterone Derivatives

compound	RBA for AR (0 °C)	rel androgenic effect in vivo (ventral prostate)
testosterone	37	0.4
dihydrotestosterone	100	1
19-nortestosterone	80	0.2[a]
19-nordihydrotestosterone	59	0.1[a]
Δ^{11}-testosterone	120	0.7
Δ^{11}-dihydrotestosterone	81	1.0

[a] Values are quoted from ref 27.

The important role of the degree of planarity of the A ring is generally accepted for the androgen receptor (AR), progestin receptor (PR), and MR bindings.[15] Supporting this is the apparent correlation between the strength of the binding affinity for the MR or the potency of in vivo mineralocorticoid activity and the degree of flatness of conformations of the several naturally occurring mineralocorticoid hormones and synthetic 18-deoxyaldosterone. Strong receptor binding and high in vivo mineralocorticoid activity seem to appear after removal of substituents that would cause bending toward the α-face (e.g., 19-methyl or 11β-hydroxy group) and also after formation of an ether bridge or an acetal bridge that would induce the molecule into a flat conformation (Table I). We presumed that a similar conformational effect would occur upon introduction of a double bond between the C(11) and C(12) positions. Thus, 21-hydroxypregna-4,11-diene-3,20-dione (Δ^{11}-DOC, 4) was expected to have a high affinity for the



4, R=COCH$_2$OH
5, R=COMe
6, R=OH

MR and thus display a more potent mineralocorticoid effect than DOC. First, we used X-ray crystallographic analysis to determine the molecular conformation of the newly synthesized Δ^{11}-DOC, prepared by a method we developed,[16] and the already-known 19-nordeoxycorticosterone (19-nor-DOC).[17] We compared the conformations of corticosterone, DOC, 19-nor-DOC, Δ^{11}-DOC, 18-deoxyaldosterone, and Ald obtained from X-ray diffraction

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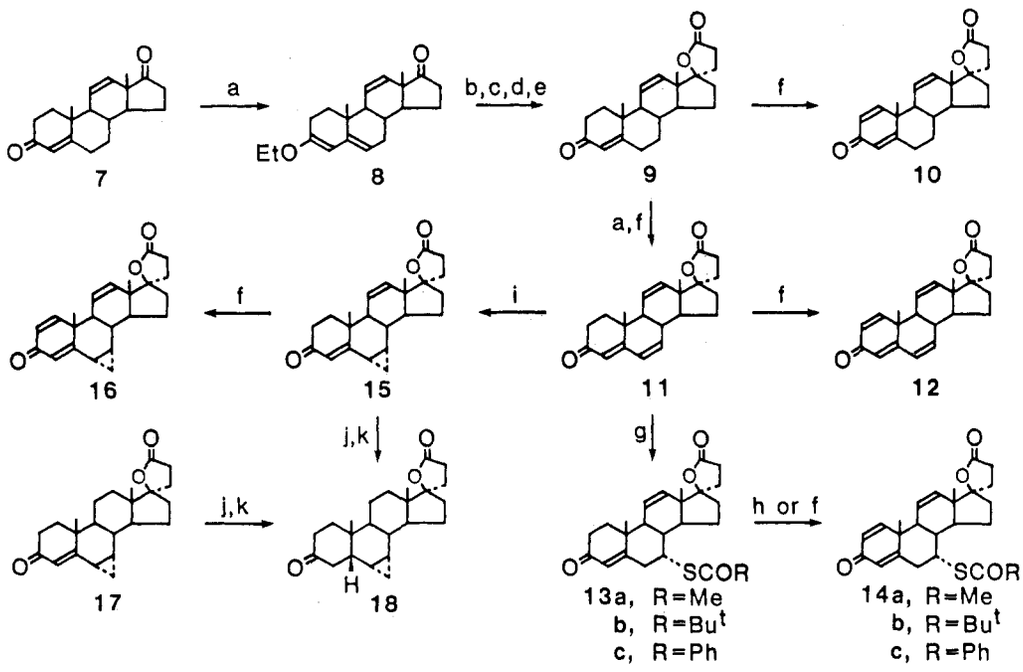
Scheme I[a]



[a] Reagents are given in footnote 28.

analysis and from empirical force field calculation using the MMP1 program based on the crystallographically observed structures, and then we examined the factors affecting the binding affinity for the MR. The results, detailed in another paper,[18] clearly showed that Δ^{11}-DOC is really a very flat molecule and its binding affinity, as expected, is about 3 times greater than that of DOC. The in vivo mineralocorticoid activities of both compounds were almost the same[19] (Table I). In addition, introduction of the Δ^{11}-double bond to progesterone (Prog) and testosterone was also found to generally produce a slight to strong increase in both in vitro receptor affinity and in vivo hormonal activity. Δ^{11}-Progesterone (**5**), reported by Meystre et al. to be 2–3 times more potent than Prog in progestational activity,[20] showed a 120% increase in receptor affinity for the PR compared with Prog (Table II). The relative binding affinity (RBA) for AR and the relative in vivo androgenic activity of Δ^{11}-testosterone (**6**),[16] 5α-dihydro-Δ^{11}-testosterone, and other testosterone derivatives to dihydrotestosterone (DHT) are summarized in Table III. Δ^{11}-Testosterone exhibited 2–2.5 times stronger affinity than testosterone and about a 50% increase of in vivo androgenic activity.[19] These observations provided further evidence that steroid receptors such as the MR, PR, and AR, with the exception of the glucocorticoid receptor (GR), might have a rather narrow space to accommodate the hormone. The flatness of the A ring with the 4-en-3-one function is important, at least for the initiation of strong binding for the receptors.

Thus, Δ^{11}-DOC was found to be a new potent synthetic mineralocorticoid that offers great potential for the discovery of a new mineralocorticoid antagonist having improved hormonal balance upon replacement of its 17β-hydroxyacetyl side chain with 17-γ-spirolactone and subsequent structural modifications. We therefore synthesized several Δ^{11}-steroid derivatives with a 17-γ-spirolactone function and examined their in vitro receptor affinity for the MR, AR, PR, GR, and estrogen receptor (ER), in vivo mineralo- and/or antimineralocorticoid activity, and also some of their in vivo antiandrogenic activity.

Chemistry

The 3,5-dien-3-ol ethyl ether **8** of androsta-4,11-diene-3,17-dione (**7**) was converted to 17-γ-spirolactone derivative **9** by a sequence of reactions involving methyl transfer reaction to the 17-keto function with dimethylsulfonium methylide, alkylation of the produced spirooxirane with the 17β-oriented oxygen with diethyl malonate and base, hydrolysis of the ester, decarboxylation, and acid-catalyzed lactonization of the resulting γ-hydroxypropionate. The compound was further converted to 1,4,11-trien-3-one derivative **10**, 4,6,11-trien-3-one derivative **11**, and 1,4,6,11-tetraen-3-one derivative **12**, by the usual dehydrogenation method using DDQ or selenium dioxide as the reagent. 7α-Acetylthio derivatives **13a** and **13b** and 7α-benzoylthio derivative **13c** were obtained by reaction with the corresponding thiol acids and then further converted to the corresponding 1,2-dehydro derivatives **14a–c**. For the 1,2-dehydrogenation reaction, selenium dioxide and pyridine in a *tert*-butyl alcohol system generally gave a better yield than DDQ oxidation (Scheme I). In contrast to the 11,12-saturated analogue, **11** gave only 6α,7α-methylene compound **15** by reaction with dimethylsulfoxonium methylide. The configuration of the 6α,7α-methylene function was confirmed when compound **18**, obtained by reduction of **15** with platinum catalyst in methanol followed by Jones oxidation, proved to be identical with the compound obtained from α-prorenone (SC-4441, **17**)[21] by the same procedures. The structure of

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(19) The discrepancy of the results of in vitro and in vivo bioassays might have arisen because the manifestation of the in vivo biological response of the administered drugs is a reflection of the sum of their inherent hormonal properties and the distribution factors, such as absorption, metabolism, and plasma binding, which influence the drug concentration level in the vicinity of the active site.

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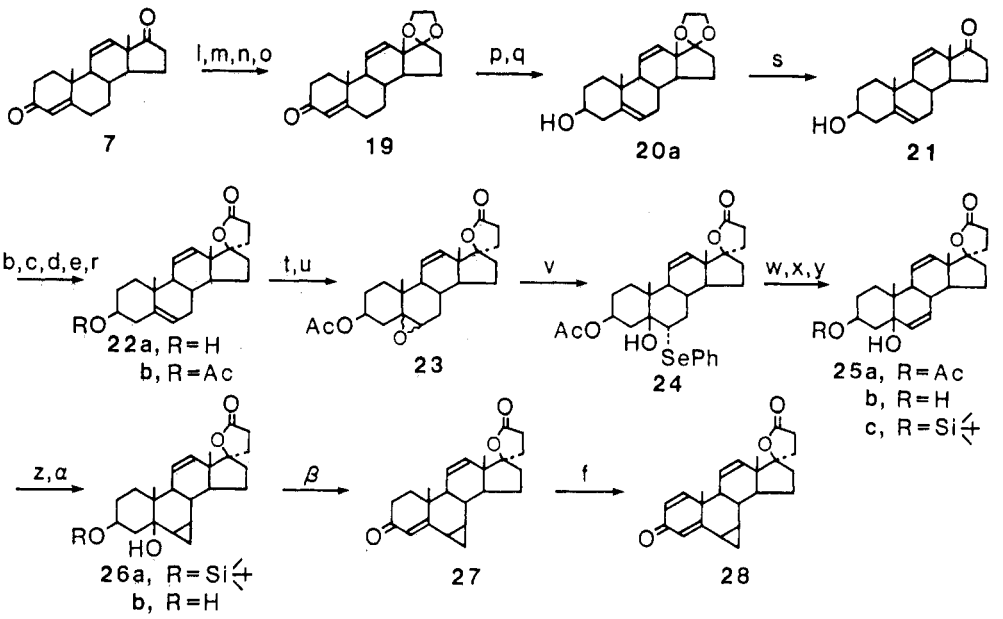
(21) Chinn, L. J.; Salamon, K. W.; Desai, B. N. *J. Med. Chem.* **1981**, *24*, 1103.

Scheme II[a]



[a] Reagents are given in footnote 28.

18 was decisively confirmed as 3-(5β-dihydro-17β-hydroxy-3-oxo-6α,7α-methyleneandrostan-17α-yl)propionic acid γ-lactone by X-ray crystallographic analysis. Compound **15** was further converted to the 1,2-dehydro derivative **16** (Scheme I).

To obtain the 6β,7β-methylene derivatives, the following syntheses were conducted (Scheme II). The 17-(monoethylene ketal) derivative **19** was obtained from androsta-4,11-diene-3,17-dione (**7**) by the sequence of reactions described in Scheme II and then transformed to 3β-hydroxyandrosta-5,11-dien-17-one (**21**) by deconjugation of the 4-en-3-one function by kinetic protonation of the corresponding enolate, followed by reduction of the 3-ketone and deketalization. Compound **21** was further converted to the corresponding 17-γ-spirolactone derivative **22a** as described above for the transformation of **8** to **9**. As the allylic oxidation of **22b** at C(7) with Collins' reagent failed, the 5,6-epoxy derivative **23**, obtained from **22b** by base treatment of the intermediate 5,6-bromohydrin derivative, was allowed to react with benzeneselenol, giving a 7:3 mixture of 5β-hydroxy-6α-(phenylseleno) compound **24** and its 5α-hydroxy-6β-(phenylseleno) isomer. Compound **24** was separated and converted to 5β-hydroxy 6,11-diene derivatives **25**. Stereocontrolled cyclopropanation of **25c** by Simmons–Smith reagent prepared with a Zn(Ag) couple and diiodomethane gave **26a**. Desilylated compound **26b** was further oxidized with pyridinium dichromate to obtain **27**, which was further converted to **28**.

The 15β,16β-methylene function was reported[22] to protect 17-γ-spirolactone compounds from inactivation arising from ring opening, to improve the durability of action, and also to enhance the binding affinity for MR while preserving the favorable antagonistic properties. We therefore tried to synthesize derivatives with this function (Scheme III). The 15-en-17-one derivative **29b**, obtained from **20b** by the sequence of reactions involving bromination, dehydrobromination, and deketalization of the 17-(ethylene ketal) function, was converted to 15β,16β-methylene derivative **30** by reaction with dimethylsulfoxonium methylide. As methylene transfer reaction of **30** with dimethylsulfonium methylide exclusively gave D-ring homologated derivative **32** and with dimethylsulfoxonium methylide exclusively gave a mixture of the desired epoxy derivative **31** and **32** in poor yield, we adopted another method to introduce the 17-γ-spirolactone function into **30** to obtain **33a**. This method consists of sequential ethynylation of the 17-keto function, carboxylation of the lithium acetylide, stepwise hydrogenation of the acetylenic function catalyzed with palladium on barium sulfate and palladium on charcoal, and acid-catalyzed lactonization. Oxidation of **33b** by Swern oxidation gave **34**, which was further converted to the 17-γ-spirolactone derivatives with functions such as 1,4,11-trien-3-one (**35**), 4,6,11-trien-3-one (**36**), 1,4,6,11-tetraen-3-one (**37**), 7α-acetylthio 4,11-dien-3-one (**38**), and 7α-acetylthio 1,4,11-trien-3-one (**39**). To obtain derivatives with the 6β,7β:15β,16β-bis(methylene) function, the reaction sequence used to obtain **26a** was used on **33c**, and **43a** was obtained smoothly as expected. Pyridinium dichromate oxidation of **43b** gave **44**, which was subsequently converted to **45** by SeO_2 oxidation.

Methods

Details of the methods used for the receptor binding studies and for in vivo mineralo- and antimineralocorticosteroid studies were described in the previous paper.[14] The MR assay used kidneys from male Sprague–Dawley rats (4–8 weeks old) that had been adrenalectomized bilaterally 1 or 2 days earlier, and the AR assay used ventral prostates from rats (4–8 weeks old) that had been castrated 1 day earlier. As PR, ER, and GR sources, uteri from rats primed with estradiol, uteri from immature rats, and livers from adrenalectomized male rats were used. Binding assays were performed by using ^{3}H-labeled Ald, dihydrotestosterone (DHT) or methyltrienolone, promegestone, estradiol, and dexamethasone as ligands. The antimineralo- and/or mineralocorticoid effect was determined in vivo by a bioassay system modified from the method of Kagawa[5b] in which male Wistar rats (7 days) were bilaterally adrenalectomized.

To determine antiandrogenic activity, male Sprague–Dawley rats (3 weeks old) that had been castrated 3 days earlier received 20 μg of testosterone propionate (TP) and an appropriate amount of the test compound in 0.1 mL

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Table IV.[a] Relative Binding Affinity for Mineralocorticoid, Androgen, and Progestin Receptors (MR, AR, and PR) and Antimineralo- and/or Mineralocorticoid Activity in Vivo of Δ^{11}-Spirolactones

compound	antagonist activity sc	antagonist activity po	agonist activity (sc)	RBA, % MR (Ald = 100%) 0 °C	RBA, % MR (Ald = 100%) 25 °C	RBA, % AR; (DHT = 100%; 0 °C)	RBA, % PR; (Prog = 100%; 0 °C)
Sp	+[b]	+	-[b]	3.2 (7)	15 (6)	4.7 (6)	2.6 (6)
canrenone	+	-		1.7 (2)	2.9	0.1 (2)	1.6 (3)
prorenone	+	+		58 (3)	30 (3)	0.5 (5)	4.3 (4)
α-prorenone	+	+			4.0	2.5	0.6
9	-		+	570	440	13 (3)	53 (2)
10	-		+	120 (3)	71 (2)	10 (2)	7.5 (3)
11	+	-	-	70 (5)	64 (6)	4.8 (4)	4.7 (4)
12	+	+	-	22 (4)	20 (6)	0.4 (3)	1.6 (4)
13a	-		+	43 (4)	169 (3)	200	14 (5)
13b	+			46 (3)	42 (2)	25 (2)	38 (2)
13c	-			11 (3)	18	140	25
14a	-	+	+	23 (2)	71 (2)	2.7 (2)	3.9
14b	+	+	-	1000	530 (3)	0.5 (2)	18
14c	-		+	3.7	37	1.3	
15	+		-	88 (2)	110 (3)	44 (3)	6.7 (5)
16	+	-	-	32	48 (2)	7.1	1.9 (2)
27	-		+	580	30	15	56
28	-		+	320	300	4	11
34				310	650		
35				220	440		
36	-			290 (2)	320 (2)	8.2	210
37	-		+	160 (2)	200 (2)	4.8	19
38	-			370 (2)	>1000	>11	89
39	-	-	+	91	350	7.7	23
44	-			300	760	44	540
45	-	-	+	250	480	11	150

[a] Values in parentheses are the numbers of measurements. Rat renal (or prostatic or uterine) cytosol was incubated with various concentrations of the compounds and about 1 nM [^{3}H]Ald (or [^{3}H]DHT or [^{3}H]methyltrienolone or [^{3}H]promegestone) for 42–48 h at 0 °C or for 100–120 min at 25 °C for MR (or for 16–20 h at 0 °C for AR and PR). Bound and free steroids were separated by the DCC method. The relative binding affinity (RBA) was determined from the equation RBA = 100 × ${}^{50}_{0}C/{}^{50}C$ (%), where ${}^{50}_{0}C$ = concentration of the standard at 50% inhibition of [^{3}H]ligand bindings of control and ${}^{50}C$ = concentration of the competitor at 50% inhibition of [^{3}H]ligand bindings of the control. [b] (+) positive, (-) negative.

Table V. Comparison of Aldosterone-like Activity of Δ^{11}-Spirolactones

compd	rel potency	95% confidence limits (%)
Ald	1.00	
9	1/177–1/1340	(333–44)
13a	1/53–1/97	(135–74)
27	1/14–1/224	(407–25)
28	1/4–1/187	(680–15)
37	1/72–1/216	(173–58)
39	1/4.3–1/23	(230–43)
45	1/1.2–1/1.6	(130–77)

of sesame oil/rat subcutaneously once daily for 7 days. The rats were sacrificed on the day following the last injection. The ventral prostate and seminal vesicle were removed and weighed. The potency was evaluated from the inhibitory effect of the test compound on TP-induced increase in organ weight.

Results

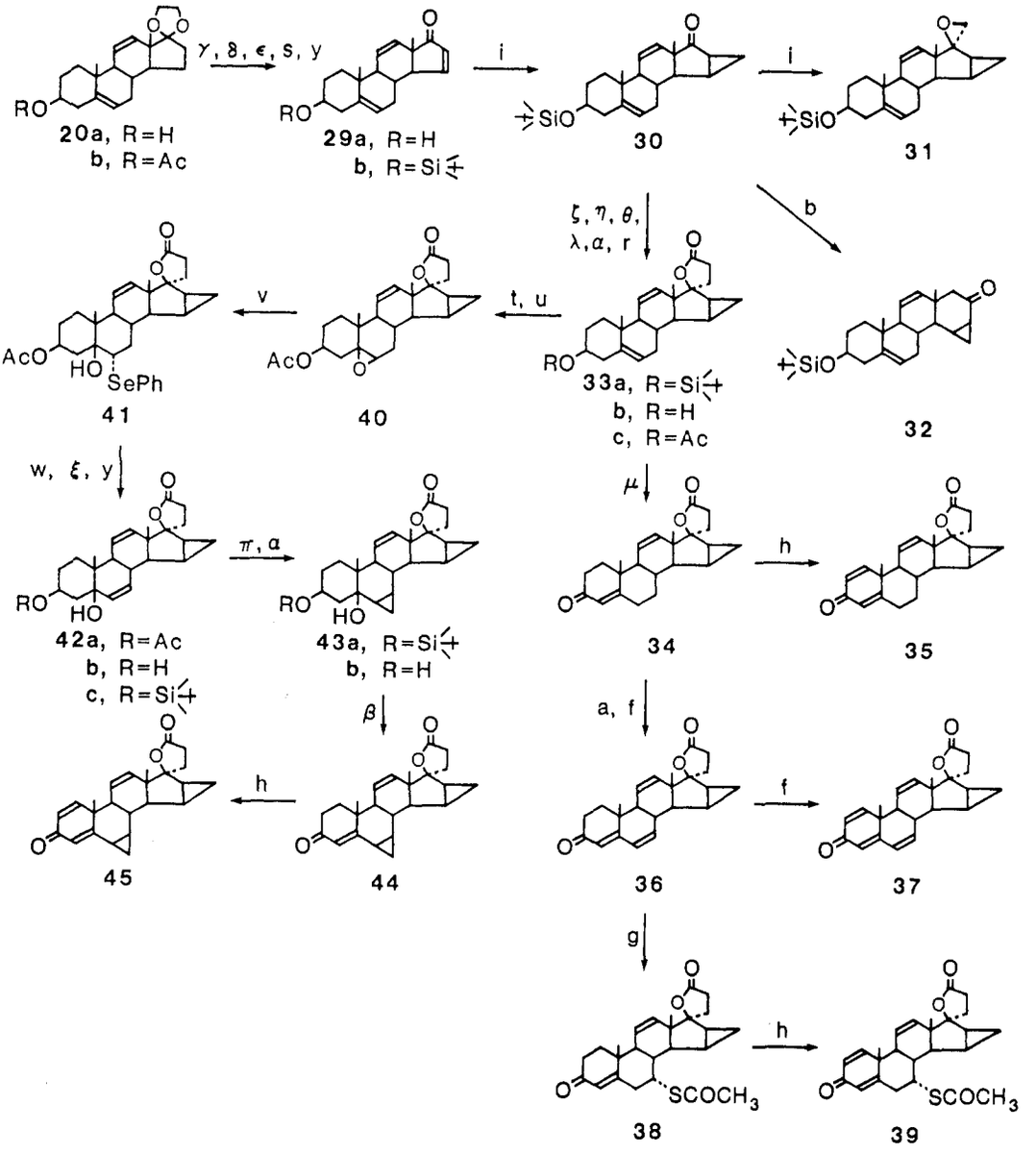
The binding affinity for the MR, AR, and PR and in vivo mineralo- and/or antimineralocorticoid activity examined with these Δ^{11}-steroids with the 17-γ-spirolactone ring are summarized in Table IV. Those with the saturated 6,7-position generally showed high binding affinity for the MR and thus had agonistic rather than antagonistic properties. In most cases, substitution of a 6β,7β-methylene or a 7α-acetylthio function even further enhanced the binding affinity for the MR and the agonistic nature. Combination of the Δ^{11} function and the 15β,16β-methylene function very strongly enhanced the binding affinity for the MR, but contrary to our expectation, all of the synthesized compounds exhibited strong aldosterone-like activity in

Table VI.[a] Comparison of Antialdosterone Activity of Δ^{11}-Spirolactones (Sc or Po)

compound	relative potency vs. Sp: sc rel potency	sc 95% conf limits (%)	po rel potency	po 95% conf limits (%)
Sp	1.00		1.00	
canrenone	0.29	0.46–0.18 (160–63)		
prorenone	2.08[b]	2.80–1.54 (135–74)		
α-prorenone	0.64[b]	1.01–0.42 (158–66)		
11	2.00[b]	2.80–1.42 (140–71)		
12	2.08[b]	2.58–1.68 (124–81)	0.93	0.70–1.25 (134–75)
14a			0.77	
15	1.48	2.58–0.85 (174–57)		
16	1.31	2.15–0.80 (164–61)		

[a] Ald (1 μg/kg of body weight) was injected subcutaneously to the adrenalectomized rats, and immediately thereafter the test compound in sesame oil was given subcutaneously (sc) or orally (po). Urine was collected for the 4-h period between 2 and 6 h after steroid administration. The potency was evaluated from the urinary Na/K ratio of the sample to the control. [b] Combined potency of two assays.

Scheme III[a]



[a] Reagents are given in footnote 28.

the in vivo assay. The results of the comparison of aldosterone-like activity of some of these compounds relative to Ald are summarized in Table V. Introduction of a double bond at the C(6) and then at the C(1) position decreases the binding affinity for the MR, but in the case of **11** and **12**, enough binding affinity is preserved to have stronger antialdosterone activity than that of Sp in the in vivo assay. Some of the compounds showing antialdosterone activity were compared with Sp, and the results are summarized in Table VI. Compound **12** displayed about 2–3 times stronger antialdosterone activity when administered subcutaneously but almost the same activity when administered orally. Interestingly, compound **14a**, which was expected to be metabolized to **12**, showed aldosteron-like activity when administered subcutaneously but antialdosterone activity when administered orally. As compound **12** shows weaker antiandrogenic activity than Sp in the in vivo assay (Table VII) and also weak to negligible affinity for the PR, GR,[23] and ER,[23] it is being evaluated further as a possibly useful antialdosterone agent.

Table VII.[a] Relative Antiandrogenic Activity of Δ^{11}-Spirolactones

compound	ventral prostate	seminal vesicles
Sp	1.00	1.00
canrenone	0.46 (0.78–0.27)[b]	0.84 (1.29–0.55)
prorenone	0.96 (2.04–0.45)	0.93 (1.18–0.73)
11	1.00	2.86 (4.46–1.86)
12	0.42 (0.98–0.18)	0.14 (0.31–0.06)

[a] Potency was evaluated by the suppressive effect on the increase of organ weight of castrated immature rats given testosterone propionate. [b] 95% confidence limits.

(23) Data are not given in detail in this paper, as the affinities for ER and GR of almost all the compounds examined were very weak or negative.

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Discussion

The present study was done to obtain some insights into the functions or conformational requirements responsible for initiating and/or maintaining binding for a receptor and also to find the factors that correlate the in vitro binding affinity for the receptors of new compounds with in vivo biological agonist and/or antagonist response. Introduction of the Δ^{11}-double bond into certain pregnane derivatives causes the molecules to adopt a flat conformation, which significantly increases the binding affinity for the MR, but even substitution of the 17β-hydroxyacetyl substituent with 17-γ-spirolactone does not always induce an antagonistic nature. Except for a few compounds, such as **11** and **12**, we have not yet found compounds showing in vivo antimineralocorticoid activity with high binding affinity for the MR. Strong MR binders found in many of our synthesized compounds generally displayed an agonistic rather than antagonistic nature with the exception of **14b**. Thus, the question remains of how to predict the factors that differentiate the antagonistic from the agonistic response of synthesized compounds on the basis of the strength or nature of binding affinity for the receptor. We are encouraged by the fact that many of our Δ^{11}-17-γ-spirolactone compounds, which show extremely strong binding affinity for the MR and in vivo mineralocorticoid activity, offer great potential for the discovery of new aldosterone antagonists by their further modifications.

Experimental Section

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere with dry solvents being used under anhydrous conditions and with anhydrous $MgSO_4$ being used as a drying agent for extracts. The organic solvents were removed by evaporation under reduced pressure with a rotary evaporator. Medium-pressure column chromatographies on Merck "Lobar" prepacked columns packed with LiChroprep Si 60 [size A (240–10 mm, 40–63 μm), size B (310–25 mm, 40–63 μm), and size C (440–37 mm, 40–63 μm)] were carried out for separation and purification of the products. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were determined with a Hitachi Model 260-10 spectrophotometer, and NMR spectra were determined on a Varian EM-390 spectrometer. Analytical results indicated by elemental symbols were within 0.4% of the theoretical values.

3-Ethoxyandrosta-3,5,11-trien-17-one (8). A solution of 37.25 g (131 mmol) of androsta-4,11-diene-3,17-dione (**7**),[16] 53 mL (319 mmol) of ethyl orthoformate, and 3 g of *p*-toluenesulfonic acid monohydrate in 450 mL of dioxane was stirred at room temperature for 1.5 h. Pyridine (10 mL) was added to the mixture, and the product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and 27.68 g (67.6%) of **8** was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 163–164 °C; IR ($CHCl_3$) 1730 cm^{-1}; NMR ($CDCl_3$) δ 0.97 (3 H, s), 1.31 (3 H, t, J = 7 Hz), 3.75 (2 H, q, J = 7 Hz), 5.12 (1 H, s), 5.12–5.35 (1 H, m), 5.57 (1 H, d, J = 10 Hz), 6.07 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{21}H_{28}O_2$) C, H.

3-(17β-Hydroxy-3-oxoandrosta-4,11-dien-17α-yl)propionic Acid γ-Lactone (9). A suspension of 14.15 g (35.4 mmol) of 60% sodium hydride in mineral oil in 190 mL of dimethyl sulfoxide (Me_2SO) was stirred and heated at 70 °C for 1 h. The cooled mixture was diluted with 190 mL of THF and further cooled to −5 °C. Next a solution of 75.8 g (35.4 mmol) of trimethylsulfonium iodide in 350 mL of Me_2SO was added followed by a solution of 27.68 g (88.6 mmol) of **8** in 200 mL of THF, which was introduced dropwise with stirring while the reaction temperature was maintained below 0 °C. The mixture was then stirred at 0 °C for 2 h and at room temperature for 15 h. The product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and 21.4 g (74.0%) of 3-ethoxy-17,20-epoxy-17α-methylandrosta-3,5,11-triene was obtained on recrystallization from CH_2Cl–ether–*n*-pentane. To an EtOH solution of sodium ethoxide prepared from 2.26 g (98.4 mol) of sodium and 150 mL of EtOH was added 32.37 g (200 mmol) of diethyl malonate dropwise at room temperature. After the reaction had proceeded for another 10 min at room temperature, 21.4 g (65.6 mmol) of 3-ethoxy-17,20-epoxy-17α-methylandrosta-3,5,11-triene was added and the mixture was stirred under reflux for 5 h. After cooling, the solvent was evaporated and then a solution of 36 g (800 mmol) of NaOH in 500 mL of a H_2O–EtOH (9:1) mixture was added to the residue, and the mixture was stirred vigorously at room temperature for 15 h. The mixture was acidified by the addition of concentrated HCl, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The crude product was dissolved in 300 mL of xylene, and the mixture was heated under reflux in a flask equipped with a Dean–Stark water separator containing molecular sieves 4A for 3 h. After evaporation of xylene, the product was purified by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent, and 15.96 g (52.9% from **8**) of **9** was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 187–189 °C; IR ($CHCl_3$) 1615, 1668, 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.03 (3 H, s), 1.14 (3 H, s), 5.60 (1 H, d, J = 10 Hz), 5.77 (1 H, s), 5.93 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{22}H_{28}O_3$) C, H.

3-(17β-Hydroxy-3-oxoandrosta-1,4,11-trien-17α-yl)propionic Acid γ-Lactone (10). A solution of 100 mg (0.29 mmol) of **9** and 70 mg (0.28 mmol) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (90% purity) in 1 mL of benzene was stirred under reflux for 6 h and cooled. The insoluble material was removed by filtration, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with 5% aqueous NaOH and saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent, and 56 mg (57%) of **10** was obtained on recrystallization from CH_2Cl_2–ether: mp 97–100 °C; IR ($CHCl_3$) 1600, 1618, 1659, 1762 cm^{-1}; NMR ($CDCl_3$) δ 1.09 (3 H, s), 1.22 (3 H, s), 5.76 (1 H, d, J = 10.5 Hz), 5.96 (1 H, dd, J = 10.5 and 2 Hz), 6.13 (1 H, s), 6.28 (1 H, dd, J = 10.5 and 2 Hz), 7.15 (1 H, d, J = 10.5 Hz). Anal. ($C_{22}H_{26}O_3$) C, H.

3-(17β-Hydroxy-3-oxoandrosta-4,6,11-trien-17α-yl)propionic Acid γ-Lactone (11). A solution of 1.70 g (5 mmol) of **9**, 2.22 g (15 mmol) of ethyl orthoformate, and 15 mg of *p*-toluenesulfonic acid monohydrate in 10 mL of dioxane was stirred at room temperature for 1.5 h. After addition of 0.5 mL of pyridine, the mixture was poured into ice water and the product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and 1.50 g (81.2%) of 3-(3-ethoxy-17β-hydroxyandrosta-3,5,11-trien-17α-yl)propionic acid γ-lactone was obtained. DDQ (90% purity; 1.02 g, 4.06 mmol) was added to a solution of 1.50 g (4.07 mmol) of the product obtained in the previous reaction in 15 mL of an acetone–water (9:1) mixture containing several drops of pyridine, and the mixture

(28) Reagents (Schemes I–III): a, $(EtO)_3CH$, *p*-TsOH (cat.), dioxane. b, $CH_2{=}SMe_2$, Me_2SO–THF. c, NaOEt, $CH_2(CO_2Et)_2$, EtOH. d, NaOH, EtOH–H_2O. e, aqueous HCl. f, DDQ, dioxane. g, RCOSH (R = Me, *t*-Bu, Ph). h, SeO_2, pyridine–*t*-BuOH. i, $CH_2{=}S(=O)Me_2$, Me_2SO–THF. j, Pt/H_2, MeOH. k, Jones oxidation. l, pyrrolidine, MeOH. m, HCl, $CHCl_3$. n, $HO(CH_2)_2OH$, *p*-TsOH (cat.). o, aqueous NH_3. p, KO-*t*-Bu, Me_2SO; AcOH. q, $NaBH_4$, Me_2SO–MeOH–H_2O. r, Ac_2O, pyridine. s, aqueous $HClO_4$, dioxane. t, NBS, aqueous $HClO_4$, dioxane. u, KOAc, EtOH. v, PhSeH, EtOH. w, H_2O_2, pyridine–CH_2Cl_2–MeOH; pyridine–benzene, Δ. x, $KHCO_3$, MeOH. y, *t*-$BuMe_2SiCl$, imidazole, DMF. z, Zn(Ag)–CH_2I_2, THF. α, $(n\text{-Bu})_4NF$, THF. β, pyridinium dichromate, DMF; NaOH, MeOH. γ, $C_5H_5NHBr_3$, THF. δ, K_2CO_3, MeOH–H_2O. ϵ, KO-*t*-Bu, Me_2SO. ζ, HC≡CLi, THF–ether. η, *n*-BuLi, THF; CO_2. θ, Pd–$BaSO_4$/H_2, dioxane. λ, Pd–C/H_2, dioxane–EtOH. μ, Me_2SO, $(COCl)_2$, CH_2Cl_2–THF; Et_3N. ξ, NaOMe, MeOH; AcOH. π, Et_2Zn, CH_2I_2, THF.

was stirred at room temperature for 30 min. The solvents were evaporated, and the product was isolated by CH_2Cl_2 extraction. The material insoluble in CH_2Cl_2 was removed by filtration through a layer of Hyflo Super Cel (John Manville Sales Co.), and the solvent of the filtrate was evaporated. Purification of the residue by column silica gel chromatography using AcOEt–benzene (2:1) as an eluent and recrystallization of the product from CH_2Cl_2–ether gave 919 mg (66.7% from **9**) of **11**: mp 184–186 °C; IR ($CHCl_3$) 1580, 1615, 1660, 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.08 (6 H, s), 5.68 (1 H, d, J = 10 Hz), 5.70 (1 H, s), 5.87 (1 H, dd, J = 10 and 3 Hz), 6.20 (2 H, m). Anal. ($C_{22}H_{26}O_3$) C, H.

3-(17β-Hydroxy-3-oxoandrosta-1,4,6,11-tetraen-17α-yl)propionic Acid γ-Lactone (12). A 300-mg (0.88 mmol) sample of **11** was oxidized with 277 mg (1.1 mmol) of DDQ, and the product was purified by the same procedures as described for the preparation of **10**. Recrystallization of the product from CH_2Cl_2–ether gave 233 mg (79%) of **12**: mp 85–88 °C; IR ($CHCl_3$) 1600, 1654, 1764 cm^{-1}; NMR ($CDCl_3$) δ 1.12 (3 H, s), 1.20 (3 H, s), 5.93 (2 H, s), 6.05 (1 H, s), 6.11 (1 H, dd, J = 8.5 and 2 Hz), 6.29 (1 H, dd, J = 9 and 2 Hz), 6.32 (1 H, dd, J = 8.5 and 2 Hz), 7.17 (1 H, d, J = 9 Hz). Anal. ($C_{22}H_{24}O_3$) C, H.

3-[7α-(Acetylthio)-17β-hydroxy-3-oxoandrosta-4,11-dien-17α-yl]propionic Acid γ-Lactone (13a). A mixture of 338 mg (1 mmol) of **11** and 2 mL of thiolacetic acid was heated under reflux for 1.5 h. The excess thiolacetic acid was removed by distillation in vacuo, and the residue was purified by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent. Recrystallization from CH_2Cl_2–MeOH gave 403 mg (97.5%) of **13a**: mp 137–139 °C; IR ($CHCl_3$) 1620, 1675, 1690, 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.06 (3 H, s), 1.17 (3 H, s), 2.40 (3 H, s), 4.03 (1 H, m), 5.56 (1 H, d, J = 10 Hz), 5.70 (1 H, s), 5.88 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{24}H_{30}O_4S$) C, H.

3-[17β-Hydroxy-3-oxo-7α-[(trimethylacetyl)thio]androsta-4,11-dien-17α-yl]propionic Acid γ-Lactone (13b). A 169-mg (0.5 mmol) sample of **11** and 0.75 mL of thiopivalic acid were allowed to react, and purification was by the same procedures as described for the preparation of **13a**. Recrystallization from CH_2Cl_2–ether gave 222 mg (97.7%) of **13b**: mp 185–186 °C; IR ($CHCl_3$) 1620, 1685, 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.07 (3 H, s), 1.18 (3 H, s), 1.24 (9 H, s), 4.00 (1 H, m), 5.58 (1 H, d, J = 10 Hz), 5.75 (1 H, s), 5.93 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{27}H_{36}O_4S$) C, H.

3-[7α-(Benzoylthio)-17β-hydroxy-3-oxoandrosta-4,11-dien-17α-yl]propionic Acid γ-Lactone (13c). A 100-mg (0.3 mmol) sample of **11** and 0.44 mL of thiobenzoic acid were allowed to react, and purification was by the same procedures as described for the preparation of **13a**. Compound **13c** was obtained as a noncrystalline powder (119 mg, 84.0%): IR ($CHCl_3$) 1660, 1761 cm^{-1}; NMR ($CDCl_3$) δ 1.05 (3 H, s), 1.15 (3 H, s), 4.2–4.4 (1 H, m), 5.60 (1 H, dd, J = 10 and 2 Hz), 5.94 (1 H, dd, J = 10 and 3 Hz), 7.2–7.7 (3 H, m), 7.99 (1 H, dd, J = 8 and 2 Hz).

3-[3α-(Acetylthio)-17β-hydroxy-3-oxoandrosta-1,4,11-trien-17α-yl]propionic Acid γ-Lactone (14a). A solution of 500 mg (1.21 mmol) of **13a**, 0.05 mL of pyridine, and 274 mg (2.47 mmol) of SeO_2 (purified by sublimation) in 50 mL of *tert*-butyl alcohol was stirred and heated under reflux for 26 h. After cooling, the insoluble material was removed by filtration through Hyflo Super Cel and the product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. The residue was purified by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent, and 332 mg (67%) of **14a** was obtained on recrystallization from MeOH: mp 230–233 °C; IR ($CHCl_3$) 1620, 1670, 1690, 1770 cm^{-1}; NMR ($CDCl_3$) δ 1.10 (3 H, s), 1.23 (3 H, s), 2.34 (3 H, s), 4.12 (1 H, m), 5.69 (1 H, d, J = 10 Hz), 5.95 (1 H, dd, J = 10 and 3 Hz), 6.06 (1 H, s), 6.27 (1 H, dd, J = 12 and 3 Hz), 7.07 (1 H, d, J = 12 Hz). Anal. ($C_{24}H_{28}O_4S$) C, H.

3-[17β-Hydroxy-3-oxo-7α-[(trimethylacetyl)thio]androsta-1,4,11-trien-17α-yl]propionic Acid γ-Lactone (14b). A 436-mg (0.96 mmol) sample of **13b** was oxidized with 450 mg (1.59 mmol) of DDQ in 5 mL of dioxane, and the product was purified by the procedure described for the preparation of **10**. Recrystallization from CH_2Cl_2–isopropyl ether gave 200 mg (46%) of **14b**: mp 255–258 °C; IR ($CHCl_3$) 1620, 1660, 1760 cm^{-1}; NMR ($CDCl_3$) δ 1.10 (3 H, s), 1.20 (3 H, s), 1.23 (9 H, s), 4.06 (1 H, m), 5.71 (1 H, d, J = 10 Hz), 5.95 (1 H, dd, J = 10 and 2 Hz), 6.05 (1 H, s), 6.8 (1 H, dd, J = 12 and 2 Hz), 7.09 (1 H, d, J = 12 Hz). Anal. ($C_{27}H_{34}O_4S$) C, H.

3-[7α-(Benzoylthio)-17β-hydroxy-3-oxoandrosta-1,4,11-trien-17α-yl]propionic Acid γ-Lactone (14c). A 200-mg (0.42 mmol) sample of **13c** was oxidized with 140 mg (1.22 mmol) of SeO_2 and 0.02 mL of pyridine in 20 mL of *tert*-butyl alcohol, and the product was purified by the procedure described for the preparation of **14a**, giving 152 mg (76%) of **14c** as a noncrystalline powder: IR ($CHCl_3$) 1658, 1661, 1762 cm^{-1}; NMR ($CDCl_3$) δ 1.10 (3 H, s), 1.26 (3 H, s), 4.25–4.6 (1 H, m), 5.75 (1 H, d, J = 10 Hz), 5.99 (1 H, dd, J = 10 and 2 Hz), 6.10 (1 H, s), 6.31 (1 H, dd, J = 10 and 2 Hz), 7.15 (1 H, d, J = 10 Hz), 7.3–7.7 (3 H, m), 7.97 (2 H, dd, J = 8 and 2 Hz).

3-(17β-Hydroxy-6α,7α-methylene-3-oxoandrosta-4,11-dien-17α-yl)propionic Acid γ-Lactone (15). To an ice-cooled and stirred solution of 1.0 g (25 mmol) of 60% sodium hydride in mineral oil and 5.5 g (25 mmol) of trimethylsulfoxonium iodide was added 25 mL of Me_2SO dropwise, and the mixture was stirred at 0 °C for another 10 min and at room temperature for 1 h. To the mixture was added dropwise a solution of 1.69 g (5 mmol) of **11** in 10 mL of Me_2SO while the temperature was maintained at 0 °C. The mixture was then allowed to react at 0 °C for 30 min and at room temperature for 17 h, and it was poured into ice water containing 2 N HCl. The product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. The residue was separated by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent, giving 620 mg (67.5%) of **15** as a noncrystalline powder: IR ($CHCl_3$) 1602, 1652, 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.06 (3 H, s), 1.09 (3 H, s), 5.63 (1 H, d, J = 11 Hz), 5.85 (1 H, dd, J = 11 and 2 Hz), 5.93 (1 H, s).

3-(17β-Hydroxy-6α,7α-methylene-3-oxoandrosta-1,4,11-trien-17α-yl)propionic Acid γ-Lactone (16). A 40-mg (0.11 mmol) sample of **15** was oxidized with 31 mg (0.12 mmol) of DDQ in 1 mL of dioxane, and the product was purified by the procedure described for **10**, giving 19 mg (47.8%) of **16** as a noncrystalline powder: IR ($CHCl_3$) 1600, 1620, 1660, 1667 cm^{-1}; NMR ($CDCl_3$) δ 1.10 (3 H, s), 1.24 (3 H, s), 5.79 (1 H, d, J = 12 Hz), 5.90 (1 H, dd, J = 12 and 3 Hz), 6.21 (1 H, dd, J = 12 and 1 Hz), 6.30 (1 H, S), 7.07 (1 H, d, J = 12 Hz).

3-(17β-Hydroxy-6α,7α-methylene-3-oxo-5β-androstan-17α-yl)propionic Acid γ-Lactone (18). A solution of 126 mg (0.36 mmol) of **15** containing 30 mg of PtO_2 in 2 mL of MeOH was hydrogenated under a hydrogen atmosphere for 40 h. The catalyst was separated by filtration, and the filtrate was evaporated. The residue was dissolved in 2 mL of acetone and cooled with an ice–water bath, and 0.13 mL (0.36 mmol) of 2.76 M solution of Johns reagent was added. After 5 min of reaction at 0 °C, the mixture was poured into ice water and the product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–cyclohexane (1:2) as an eluent, and 62 mg (48.3%) of **18** was obtained on recrystallization from ether: mp 146–147 °C; IR ($CHCl_3$) 1707, 1760 cm^{-1}; NMR ($CDCl_3$) δ 1.01 (6 H, s). Anal. ($C_{23}H_{32}O_3$) C, H. The product proved to be identical with the reaction product obtained from **17** by the same reaction procedures.

17-(Ethylenedioxy)androsta-4,11-dien-3-one (19). To a hot solution of 17.2 g (60.5 mmol) of **7** in 360 mL of MeOH was added 5.62 mL of pyrrolidine (67.3 mmol). After cooling, the resulting crystals were filtered and washed with cold MeOH to obtain 19.38 g (95%) of 3-pyrrolidinoandrosta-3,5,11-trien-17-one: mp 206–209 °C; IR ($CHCl_3$) 1592, 1618, 1728 cm^{-1}; NMR ($CDCl_3$) δ 0.97 (6 H, S), 3.0–3.3 (4 H, m), 4.82 (1 H, s), 5.05–5.25 (1 H, m), 5.64 (1 H, dd, J = 10 and 1.5 Hz), 6.13 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{23}H_{31}ON$) C, H, N. A solution of 22.26 g (65.9 mmol) of 3-pyrrolidinoandrosta-3,5,11-trien-17-one obtained in the previous reaction in 600 mL of a $CHCl_3$–MeOH (1:1) mixture was saturated with dry HCl gas. Excess HCl gas was removed by passing N_2 gas, and the solvents were evaporated. The residue and 1.55 g (8.15 mmol) of *p*-toluenesulfonic acid monohydrate were dissolved in 460 mL of ethylene glycol, and the mixture was heated at 90 °C. The excess ethylene glycol was removed under reduced pressure at 90 °C for 4 h. The residue was dissolved in 600 mL of water, and 50 mL of 25% NH_4OH was added. After the mixture was allowed to react for a while, the product was isolated by

CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:4) as an eluent, and 14.9 g (69%) of **19** was obtained. A portion of the product was recrystallized from CH_2Cl_2–MeOH: mp 139–140 °C; IR ($CHCl_3$) 1656 cm^{-1}; NMR ($CDCl_3$) δ 0.96 (3 H, s), 1.13 (3 H, s), 3.94 (4 H, s), 5.56 (1 H, d, J = 10 Hz), 5.78 (1 H, s), 5.92 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{21}H_{28}O_3$) C, H.

17-(Ethylenedioxy)-3β-hydroxyandrosta-5,11-diene (20a). A mixture of 1.46 g (4.45 mmol) of **19** and 4.37 g (39.2 mmol) of potassium *tert*-butoxide in 60 mL of Me_2SO was stirred at room temperature for 2.5 h. The mixture was poured into a solution of 9 mL (15.7 mmol) of acetic acid in 70 mL of CH_2Cl_2 which was cooled with an ice–water bath at 0 °C, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated to obtain the crude product of 17-(ethylenedioxy)androsta-5,11-dien-3-one. To a cooled solution of 693 mg (18.2 mmol) of $NaBH_4$ in 110 mL of a mixture of MeOH–H_2O (10:1) at 0 °C was added a solution of the crude product obtained in the previous reaction in 110 mL of Me_2SO, and the mixture was stirred at 5–7 °C for 10 min. The mixture was poured into ice water, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with dilute aqueous HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, dried, and evaporated. The residue was purified by column silica gel chromatography using AcOEt–benzene (1:4–1:2) as an eluent, and 1.02 g (70%) of **20a** was obtained on recrystallization from CH_2Cl_2–ether: mp 217 °C; NMR ($CDCl_3$) δ 0.91 (3 H, s), 0.96 (3 H, s), 3.3–3.8 (1 H, m), 3.90 (3 H, s), 5.3–5.6 (1 H, m), 5.58 (1 H, d, J = 10 Hz), 5.90 (1 H, d, J = 10 Hz). Anal. ($C_{23}H_{30}O_3$) C, H.

3β-Hydroxyandrosta-5,11-dien-17-one (21). A mixture of 941 mg (2.85 mmol) of **20a** and 4 mL of 60% perchloric acid in 100 mL of a mixture of dioxane–H_2O (10:1) was stirred at room temperature for 3 h, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried, and evaporated. The residue was purified by column silica gel chromatography using AcOEt–benzene (1:2) as an eluent, and 800 mg (98%) of **21** was obtained on recrystallization from CH_2Cl_2–ether: mp 153–154 °C; IR ($CHCl_3$) 1732 cm^{-1}; NMR ($CDCl_3$) δ 0.95 (3 H, s), 0.99 (3 H, s), 3.3–3.8 (1 H, m), 5.3–5.5 (1 H, m), 5.56 (1 H, d, J = 10 Hz), 6.10 (1 H, d, J = 10 Hz). Anal. ($C_{19}H_{26}O_2$) C, H.

3-(3β-Acetoxy-17β-hydroxyandrosta-5,11-dien-17α-yl)-propionic Acid γ-Lactone (22b). An 800-mg (2.79 mmol) sample of **21** was converted to 3-(3β,17β-dihydroxyandrosta-5,11-dien-17α-yl)propionic acid γ-lactone (**22a**) and purified by the procedures described for the conversion of **8** to **9**. The crude product thus obtained was acetylated with acetic anhydride in pyridine in the presence of a catalytic amount of 4-(dimethylamino)-pyridine. The product was purified in the usual manner, and 441 mg (41% from **21**) of **22b** was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 134–138 °C; IR ($CHCl_3$) 1726, 1765 cm^{-1}; NMR ($CDCl_3$) δ 1.00 (6 H, s), 2.02 (3 H, s), 4.6 (1 H, m), 5.48 (1 H, m), 5.62 (1 H, d, J = 10 Hz), 5.89 (1 H, d, J = 10 Hz). Anal. ($C_{24}H_{32}O_4$) C, H.

3-(3β-Acetoxy-5β,6β-epoxy-17β-hydroxyandrost-11-en-17α-yl)propionic Acid γ-Lactone (23). To a solution of 3.30 g (8.58 mmol) of **22b** and 6.7 mL of 0.5 N perchloric acid in 67 mL of dioxane at 15 °C was added 1.39 g (9.90 mmol) of *N*-bromoacetamide. After the reaction had continued for 45 min at 15 °C, aqueous $Na_2S_2O_3$ was added and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The residue was dissolved in a solution of 1.26 g (12.9 mmol) of sodium acetate in 165 mL of EtOH, and the mixture was stirred under reflux for 30 min. The product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ and aqueous saturated NaCl, dried, and evaporated. The residue was separated by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and a mixture of 5α,6α- and 5β,6β-epoxy derivatives was obtained. This mixture was subjected to the next reaction without further separation.

3-[3β-Acetoxy-5β,17β-dihydroxy-6α-(phenylseleno)-androst-11-en-17α-yl]propionic Acid γ-Lactone (24). To a solution of 8.08 g (25.9 mmol) of diphenyl diselenide in 135 mL of EtOH was added 2.07 g (54.7 mmol) of sodium borohydride, and the mixture was stirred at room temperature for 30 min. To this reagent solution were added 3.4 mL (59 mmol) of acetic acid and a solution of 2.66 g (6.64 mmol) of **23** in 25 mL of THF, and the mixture was stirred under reflux for 15 h. After cooling, the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The residue was separated by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent. From the less polar fraction was obtained 2.42 g (65%) of **24** as a noncrystalline powder: IR ($CHCl_3$) 1729, 1758, 3550 cm^{-1}; NMR ($CDCl_3$) δ 0.95 (6 H, s), 2.07 (3 H, s), 3.4–3.8 (1 H, m), 5.2 (1 H, m), 5.51 (1 H, d, J = 10 Hz), 5.75 (1 H, dd, J = 10 and 2 Hz). From the polar fraction was obtained 1.07 g (29%) of the 5α-hydroxy-6β-(phenylseleno) isomer of **24**. A portion of the product was recrystallized from CH_2Cl_2: mp 147–150 °C; IR ($CHCl_3$) 1720, 1761 cm^{-1}; NMR ($CDCl_3$) δ 1.07 (3 H, s), 1.12 (3 H, s), 2.00 (3 H, s), 3.05–3.3 (1 H, m), 4.9–5.4 (1 H, m), 5.51 (1 H, d, J = 10 Hz), 5.77 (1 H, d, J = 10 Hz). Anal. ($C_{30}H_{38}O_5Se$) C, H.

3-[3β-[(*tert*-Butyldimethylsilyl)oxy]-5β,17β-dihydroxyandrosta-6,11-dien-17α-yl]propionic Acid γ-Lactone (25c). A mixture of 2.42 g (4.34 mmol) of **24**, 0.77 mL (9.5 mmol) of pyridine, and 5 mL of 30% aqueous hydrogen peroxide in 76 mL of a mixture of CH_2Cl_2–MeOH (1:1) was allowed to react at room temperature for 1 h, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried, and evaporated. The residue was dissolved in 25 mL of benzene containing 0.77 mL (9.5 mmol) of pyridine, and the mixture was stirred under reflux for 4 h. After cooling, the product was isolated again by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:2) as an eluent, and 1.52 g (87%) of **25a** was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 185–188 °C; IR ($CHCl_3$) 1738, 1762, 3560 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (3 H, s), 1.01 (3 H, s), 2.09 (3 H, s), 5.10 (1 H, m), 5.49 (1 H, s), 5.64 (1 H, d, J = 10 Hz), 5.79 (1 H, d, J = 10 Hz). Anal. ($C_{24}H_{32}O_5$) C, H. A 1.52-g sample of **25a** was hydrolyzed in a refluxing solution of 1.14 g (11.4 mmol) of $KHCO_3$ in 100 mL of a MeOH–H_2O (4:1) mixture, and the hydrolyzed product was then silylated by the reaction with 1.43 g (9.48 mmol) of *tert*-butyldimethylsilyl chloride and 0.65 g (9.48 mmol) of imidazole in 20 mL of dimethylformamide (DMF). Purification by column silica gel chromatography and recrystallization from CH_2Cl_2–ether of the product gave 1.38 g (77%) of **25c**: mp 185–187 °C; IR ($CHCl_3$) 1761, 3490 cm^{-1}; NMR ($CDCl_3$) δ 0.91 (12 H, s), 1.0 (3 H, s), 4.16 (1 H, m), 5.50 (2 H, s), 5.73 (2 H, s). Anal. ($C_{28}H_{44}O_4Si$) C, H.

3-[3β-[(*tert*-Butyldimethylsilyl)oxy]-5β,17β-dihydroxy-6β,7β-methyleneandrost-11-en-17α-yl]propionic Acid γ-Lactone (26a). To a hot stirred solution of 28 mg (0.17 mmol) of silver acetate in 30 mL of acetic acid was added 4.8 g (73 mmol) of zinc powder in one portion. The mixture was stirred for 30 s, and the zinc–silver couple formed was isolated by decantation and was washed with ether (5 × 30 mL). A solution of 4.2 mL (53 mmol) of diiodomethane in 10 mL of THF was added dropwise over 1 h to a mixture of the zinc–silver couple product and 1.38 g (2.92 mmol) of **25c** in 20 mL of THF, while the temperature was kept at 20–30 °C. The mixture was stirred at room temperature for another 3 h and diluted with 30 mL of CH_2Cl_2, and the insoluble material was filtered off through a pad of Hyflo Super Cel. The filtrate was poured into cooled saturated aqueous NH_4Cl, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:15–1:9) as an eluent, and 0.89 g (63%) of **26a** was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 179–181 °C; IR ($CHCl_3$) 1760, 3450 cm^{-1}; NMR ($CDCl_3$) δ 0.80 (3 H, s), 0.91 (9 H, s), 0.99 (3 H, s), 4.09 (1 H, m), 5.55 (1 H, d, J = 10 Hz), 5.73 (1 H, d, J = 10 Hz). Anal. ($C_{29}H_{46}O_4Si$) C, H.

3-(17β-Hydroxy-6β,7β-methylene-3-oxoandrosta-4,11-dien-17α-yl)propionic Acid γ-Lactone (27). A 1.10-g sample (2.26 mmol) of **26a** was desilylated by the reaction with 767 mg (2.94 mmol) of tetrabutylammonium fluoride in 20 mL of THF, and

the product was isolated by CH_2Cl_2 extraction in the usual manner. The product obtained in the previous reaction dissolved in 40 mL of DMF was oxidized with 2.55 g (6.78 mmol) of pyridinium dichromate at 80 °C for 10 h. The product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:4–1:2) as an eluent, and 438 mg (55%) of **27** was obtained on recrystallization from CH_2Cl_2–ether: mp 224–225 °C; IR ($CHCl_3$) 1652, 1770 cm^{-1}; NMR ($CDCl_3$) δ 1.02 (6 H, s), 5.61 (1 H, d, J = 10 Hz), 5.89 (1 H, dd, J = 10 and 3 Hz), 6.06 (1 H, s). Anal. ($C_{23}H_{28}O_3$) C, H.

3-(17β-Hydroxy-6β,7β-methylene-3-oxoandrosta-1,4,11-trien-17α-yl)propionic Acid γ-Lactone (28). A 245-mg (0.7 mmol) sample of **27** was oxidized with 244 mg (0.97 mmol) of DDQ in 3 mL of dioxane, and the product was purified by the procedure described for the preparation of **10**, giving 193 mg (79%) of **28** as a noncrystalline powder: IR ($CHCl_3$) 1661, 1766, cm^{-1}; NMR ($CDCl_3$) δ 1.05 (3 H, s), 1.06 (3 H, s), 5.77 (1 H, d, J = 10 Hz), 5.92 (1 H, dd, J = 10 and 2 Hz), 6.23 (1 H, dd, J = 10 and 1.5 Hz), 6.36 (1 H, d, J = 1.5 Hz), 7.04 (1 H, d, J = 10 Hz).

3β-(*tert*-Butyldimethylsiloxy)androsta-5,11,15-trien-17-one (29b). To a solution of 80.1 g (215 mmol) of 3β-acetoxy-17-(ethylenedioxy)androsta-5,11-diene (**20b**) in 270 mL of THF at 0–15 °C was added dropwise a solution of 161 g (495 mmol) of pyridinium hydrobromide perbromide in 270 mL of THF. After the mixture was stirred at room temperature for another 1.5 h, 52 mL (645 mmol) of pyridine and a solution of 68 g (430 mmol) of $Na_2S_2O_3$ in 270 mL of water were added, the mixture was stirred, and the product was isolated by AcOEt extraction in the usual manner. The residue and 97 g (645 mmol) of NaI were allowed to react in 540 mL of THF at room temperature for 3 h, and then 68 g (430 mmol) of $Na_2S_2O_3$ and 17 mL (215 mmol) of pyridine were added. After the mixture was allowed to react for a while, the product was isolated by AcOEt extraction in the usual manner and crude 16α-bromo derivative was obtained. A mixture of the crude product obtained from the previous reaction and 54 g (391 mmol) of K_2CO_3 in 1.4 L of a MeOH–THF–H_2O (5:1:1) mixture was stirred under reflux for 1 h. After cooling, the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:1) as an eluent, and 76.0 g (86.4%) of 16α-bromo-17-(ethylenedioxy)-3β-hydroxyandrosta-5,11-diene was obtained on recrystallization from CH_2Cl_2–ether: mp 98–99.5 °C. A mixture of 76 g (186 mmol) of the 16α-bromo derivative obtained in the previous reaction and 45 g (400 mmol) of potassium *tert*-butoxide in 1.3 L of Me_2SO was allowed to react at 40–50 °C for 5 h. After cooling, the product was isolated by CH_2Cl_2 extraction in the usual manner, and the isolated product was hydrolyzed in 1.1 L of an acetone–H_2O (10:1) mixture containing 1.12 g (5.9 mmol) of *p*-toluenesulfonic acid monohydrate at 10 °C for 72 h. The product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated, and 38.7 g (73.3%) of 3β-hydroxyandrosta-5,11,15-trien-17-one (**29a**) was obtained on recrystallization from CH_2Cl_2–ether: mp 210–213 °C; IR ($CHCl_3$) 1710 cm^{-1}; NMR ($CDCl_3$) δ 1.03 (3 H, s), 1.20 (3 H, s), 3.2–3.8 (1 H, m), 5.3–5.6 (1 H, m), 5.58 (1 H, dd, J = 10 and 3 Hz), 6.05 (1 H, dd, J = 6 and 3 Hz), 6.26 (1 H, dd, J = 10 and 3 Hz), 7.51 (1 H, J = 6 Hz). Anal. ($C_{19}H_{24}O_2$) C, H. A 5.42-g (19 mmol) sample of **29a** was converted to the *tert*-butyldimethylsiloxy derivative **29b** by reaction with 3.40 g (22.8 mmol) of *tert*-butyldimethylsilyl chloride and 1.93 g (28.5 mmol) of imidazole in 80 mL of DMF. The product was purified in the usual manner, and 6.34 g (83.7%) of **29b** was obtained.

3β-(*tert*-Butyldimethylsiloxy)-15β,16β-methyleneandrosta-5,11-dien-17-one (30). A suspension of 62 mg (1.5 mmol) of 60% sodium hydride in mineral oil in 3 mL of a mixture of Me_2SO–THF (1:1) was stirred and heated at 70 °C for 1.5 h. To the solution of sodium (methylsulfinyl)methide in Me_2SO at room temperature was added 345 mg (1.5 mmol) of trimethylsulfoxonium iodide, and the mixture was stirred at room temperature for 1.5 h. A solution of 400 mg (1 mmol) of **29b** in 5 mL of Me_2SO was added to the reagent solution with the temperature being maintained at 15–20 °C, and the mixture was allowed to react at 15–20 °C for another hour. The product was isolated by AcOEt extraction, and the AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and 262 g (63.6%) of **30** was obtained. A portion of the product was recrystallized from CH_2Cl_2–petroleum ether: mp 165–167 °C; IR ($CHCl_3$) 1718 cm^{-1}; NMR ($CDCl_3$) δ 0.90 (9 H, s), 1.00 (3 H, s), 1.10 (3 H, s), 3.3–3.7 (1 H, m), 5.35–5.55 (1 H, m), 5.39 (1 H, dd, J = 9 and 3 Hz), 6.21 (1 H, dd, J = 9 and 3 Hz). Anal. ($C_{26}H_{40}O_2Si$) C, H.

3-[3β-(*tert*-Butyldimethylsiloxy)-17β-hydroxy-15β,16β-methyleneandrosta-5,11-dien-17α-yl]propionic Acid γ-Lactone (33a). A mixture of 580 mL of THF and 290 mL of ether was saturated with acetylene at −40 °C. To the solution was added 48 mL (72.5 mmol) of a solution of 1.52 M *n*-butyllithium in *n*-hexane in 10 min, and then a solution of 6 g (14.5 mmol) of **30** in 30 mL of THF was added dropwise. The mixture was stirred at −40 °C for 15 min and at 0 °C for 1 h, and then saturated aqueous NH_4Cl was added. The product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and 5.49 g (86.3%) of 3β-(*tert*-butyldimethylsiloxy)-17α-ethynyl-17β-hydroxy-15β,16β-methyleneandrosta-5,11-diene was obtained on recrystallization from petroleum ether: mp 182.5–184 °C; IR ($CHCl_3$) cm^{-1}; NMR ($CDCl_3$) δ 0.9 (9 H, s), 0.98 (3 H, s), 1.01 (3 H, s), 2.53 (1 H, s), 3.3–3.75 (1 H, m), 5.3–5.55 (1 H, m), 5.35 (1 H, d, J = 10 Hz), 6.10 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{28}H_{42}O_2Si$) C, H.

A solution of 10.05 g (22.9 mmol) of 3β-(*tert*-butyldimethylsiloxy)-17α-ethynyl-17β-hydroxy-15β,16β-methyleneandrosta-5,11-diene obtained in the previous reaction in 350 mL of THF was cooled to −75 °C, and 38 mL of a solution of 1.52 M *n*-butyllithium in *n*-hexane was added dropwise. After the reaction had proceeded for 30 min at −75 °C and for 15 min at −60 °C, dry ice was added at once, the temperature of the reaction mixture was allowed to rise to room temperature, and saturated aqueous NH_4Cl was added. After the mixture was acidified with 2 N HCl, the product was isolated by AcOEt extraction. The AcOEt layer was washed with saturated aqueous NaCl, dried, and evaporated. Recrystallization of the residue from CH_2Cl_2–petroleum ether gave 10.67 g (96.5%) of 3-[3β-(*tert*-butyldimethylsiloxy)-17β-hydroxy-15β,16β-methyleneandrosta-5,11-dien-17α-yl]propynoic acid: mp 196–197.5 °C; IR ($CHCl_3$) 1710, 2225 cm^{-1}; NMR ($CDCl_3$) δ 0.9 (9 H, s), 1.00 (3 H, s), 1.06 (3 H, s), 3.3–3.8 (1 H, m), 5.35–5.55 (1 H, m), 5.35 (1 H, dd, J = 10 and 2 Hz), 6.10 (1 H, dd, J = 10 and 2 Hz).

A solution of 10.94 g (22.7 mmol) of 3-[3β-(*tert*-butyldimethylsiloxy)-17β-hydroxy-15β,16β-methyleneandrosta-5,11-dien-17α-yl]propynoic acid obtained in the previous reaction in 300 mL of dioxane containing 2.5 g of 5% Pd–$BaSO_4$ was hydrogenated under a hydrogen atmosphere for 3 h. The catalyst was separated by filtration and washed with dioxane. The solvent was evaporated, the residue was dissolved again in 300 mL of a dioxane–EtOH (4:1) mixture containing 500 mg of 5% Pd–C, and the mixture was hydrogenated under a hydrogen atmosphere for 1.5 h. The catalyst was separated by filtration and washed with dioxane. The filtrate was evaporated, and the residue was dissolved in 300 mL of toluene and heated under reflux for 2 h. The solvent was evaporated, the residue was purified by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and 9.3 g (87.8%) of **33a** was obtained. A portion of the product was recrystallized from CH_2Cl_2–petroleum ether: mp 194–195 °C; IR ($CHCl_3$) 1750 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (9 H, s), 0.94 (3 H, s), 1.03 (3 H, s), 3.2–3.75 (1 H, m), 5.2–5.53 (1 H, m), 5.39 (1 H, d, J = 10 Hz), 5.83 (1 H, dd, J = 10 and 2 Hz). Anal. ($C_{29}H_{44}O_3Si$) C, H.

3-(3β,17β-Dihydroxy-15β,16β-methyleneandrosta-5,11-dien-17α-yl)propionic acid γ-lactone (33b): mp 248–253 °C (CH_2Cl_2–ether); IR ($CHCl_3$) 1760 cm^{-1}; NMR ($CDCl_3$) δ 0.98 (3 H, s), 1.06 (3 H, s), 5.43 (1 H, dd, J = 10 and 2 Hz), 5.49 (1 H, s), 5.87 (1 H, dd, J = 10 and 3 Hz). Anal. ($C_{23}H_{30}O_3$) C, H.

3-(3β-Acetyl-17β-hydroxy-15β,16β-methyleneandrosta-5,11-dien-17α-yl)propionic acid γ-lactone (33c): mp 252–253 °C (CH_2Cl_2–ether); IR ($CHCl_3$) 1720, 1758 cm^{-1}; NMR ($CDCl_3$) δ 0.98 (3 H, s), 1.05 (3 H, s), 2.03 (1 H, s), 4.35–4.9 (1 H, m), 5.41 (1 H, dd, J = 10 and 2 Hz), 5.4–5.65 (1 H, m), 5.89 (1 H, dd, J

= 10 and 2 Hz). Anal. ($C_{25}H_{32}O_4$) C, H.

3-(17β-Hydroxy-15β,16β-methylene-3-oxoandrosta-4,11-dien-17α-yl)propionic Acid γ-Lactone (34). To a cooled solution of 0.021 mL (0.24 mmol) of oxalyl chloride in 0.6 mL of CH_2Cl_2 with a dry ice–acetone bath at −30 °C was added 0.033 mL (0.47 mmol) of Me_2SO, and the mixture was stirred at −30 °C for 5 min. To the mixture was added a solution of 23 mg (0.065 mmol) of **33b** in 2 mL of a CH_2Cl_2–THF (1:1) mixture dropwise. After the reaction mixture was allowed to react at −30 °C for 1 h, 0.1 mL (0.72 mmol) of Et_3N was added and the temperature of the reaction mixture was allowed to rise to room temperature. The oxidation product was isolated by CH_2Cl_2 extraction, and the CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product obtained in the previous reaction was dissolved in 1 mL of a CH_2Cl_2–THF (1:1) mixture containing three drops of 5 M sodium methoxide solution in MeOH, and the mixture was stirred at room temperature for 10 min. The product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:2) as an eluent, and 14 mg (61%) of **34** was obtained. A portion of the product was recrystallized from CH_2Cl_2–ether: mp 189–190 °C; IR ($CHCl_3$) 1610, 1660, 1758 cm^{-1}; NMR ($CDCl_3$) δ 1.09 (3 H, s), 1.12 (3 H, s), 5.40 (1 H, dd, J = 10 and 2 Hz), 5.80 (1 H, s), 5.92 (1 H, dd, J = 10 and 3 Hz). Anal. ($C_{23}H_{28}O_3$) C, H.

3-(17β-Hydroxy-15β,16β-methylene-3-oxoandrosta-1,4,11-trien-17α-yl)propionic Acid γ-Lactone (35). A 40-mg (0.11 mmol) sample of **34** was oxidized with 0.01 mL of pyridine and 39 mg (0.34 mmol) of SeO_2 (purified by sublimation) in 6 mL of *tert*-butyl alcohol, and the product was purified by the procedure described for the preparation of **14a**. Recrystallization from THF–ether gave 30 mg (76%) of **35**: mp 224–226 °C; IR ($CHCl_3$) 1602, 1620, 1661, 1761 cm^{-1}; NMR ($CDCl_3$) δ 1.00 (3 H, s), 1.18 (3 H, s), 5.49 (1 H, dd, J = 10 and 2 Hz), 5.90 (1 H, dd, J = 10 and 3 Hz), 6.10 (1 H, s), 6.24 (1 H, dd, J = 10 and 2 Hz), 7.03 (1 H, d, J = 10 Hz). Anal. ($C_{23}H_{26}O_3$) C, H.

3-(17β-Hydroxy-15β,16β-methylene-3-oxoandrosta-4,6,11-trien-17α-yl)propionic Acid γ-Lactone (36). An 84-mg (0.24 mmol) sample of **34** was converted to the 3-ethoxy 3,5-diene derivative by the reaction with 178 mg (1.2 mmol) of ethyl orthoformate and 5 mg of *p*-toluenesulfonic acid monohydrate in 1 mL of dioxane, and the product was isolated in the usual manner. The product obtained in the previous reaction was then oxidized with 66 mg (0.26 mmol) of DDQ (90% purity) in 6 mL of an acetone–H_2O (9:1) mixture containing several drops of pyridine, and the product was purified by the same procedure as described for the preparation of **11**. Recrystallization from CH_2Cl_2–ether gave 41 mg (49%) of **36**: mp 177–181 °C; IR ($CHCl_3$) 1608, 1650, 1760 cm^{-1}; NMR ($CDCl_3$) δ 1.07 (3 H, s), 1.11 (3 H, s), 5.46 (1 H, dd, J = 10 and 2 Hz), 5.72 (1 H, s), 5.87 (1 H, dd, J = 10 and 3 Hz), 6.20 (1 H, dd, J = 9 and 3 Hz), 6.49 (1 H, dd, J = 9 and 2 Hz). Anal. ($C_{23}H_{26}O_3$) C, H.

3-(17β-Hydroxy-15β,16β-methylene-3-oxoandrosta-1,4,6,11-tetraen-17α-yl)propionic Acid γ-Lactone (37). A 420-mg (1.2 mmol) sample of **36** was oxidized with 420 mg (1.85 mmol) of DDQ in 4 mL of dioxane. The product was purified by the procedure described for the preparation of **10**, giving 219 mg (50.1%) of **37** as a noncrystalline powder: IR ($CHCl_3$) 1600, 1654, 1766 cm^{-1}; NMR ($CDCl_3$) δ 1.16 (3 H, s), 1.20 (3 H, s), 5.66 (1 H, d, J = 11 Hz), 5.95 (1 H, dd, J = 11 and 3 Hz), 6.05 (1 H, s), 6.29 (1 H, dd, J = 10 and 3 Hz), 6.35 (2 H, s), 7.12 (1 H, d, J = 10 Hz).

3-[7α-(Acetylthio)-17β-hydroxy-15β,16β-methylene-3-oxoandrosta-4,11-dien-17α-yl]propionic Acid γ-Lactone (38). A 320-mg (0.91 mmol) sample of **36** and 2 mL of thiolacetic acid were heated under reflux for 1 h, and the product was purified by the procedure described for the preparation of **13a**. Recrystallization from CH_2Cl_2–ether gave 256 mg (65.7%) of **38**: mp 273–277 °C; IR ($CHCl_3$) 1620, 1674, 1690, 1763 cm^{-1}; NMR ($CDCl_3$) δ 1.10 (3 H, s), 1.16 (3 H, s), 2.39 (3 H, s), (1 H, m), 4.30 (1 H, m), 5.38 (1 H, dd, J = 10 and 2 Hz), 5.79 (1 H, s), 5.98 (1 H, dd, J = 10 and 3 Hz). Anal. ($C_{25}H_{30}O_4S$) C, H.

3-[7α-(Acetylthio)-17β-hydroxy-15β,16β-methylene-3-oxoandrosta-1,4,11-trien-17α-yl]propionic Acid γ-Lactone (39). A 340-mg (0.8 mmol) sample of **38** was oxidized with 0.034 mL of pyridine and 182 mg (1.59 mmol) of SeO_2 (purified by sublimation) in 34 mL of *tert*-butyl alcohol, and the product was purified by the procedure described for the preparation of **14a**. Recrystallization from CH_2Cl_2–ether gave 261 mg of **39**: mp 288–291 °C dec; IR ($CHCl_3$) 1621, 1661, 1689, 1760 cm^{-1}; NMR ($CDCl_3$) δ 1.14 (3 H, s), 1.23 (3 H, s), 2.36 (3 H, s), 4.3–4.5 (1 H, m), 5.49 (1 H, dd, J = 10 and 2 Hz), 5.99 (1 H, dd, J = 10 and 3 Hz), 6.12 (1 H, s), 6.31 (1 H, dd, J = 10 and 2 Hz), 7.06 (1 H, d, J = 10 Hz). Anal. ($C_{25}H_{28}O_4S$) C, H.

3-(3β-Acetoxy-5β,6β-epoxy-17β-hydroxy-15β,16β-methyleneandrost-11-en-17α-yl)propionic Acid γ-Lactone (40). A 270-mg (0.68 mmol) sample of **33c** was treated with 213 mg (1.5 mmol) of *N*-bromoacetamide in 1.5 mL of a dioxane–THF (2:1) mixture containing 1.4 mL of 0.5 N perchloric acid and then with 196 mg (2 mmol) of sodium acetate in 10 mL of EtOH, and the product was purified by the procedure described for the preparation of **23**. The product consisting of a mixture of 5α,6α- and 5β,6β-epoxy derivatives was subjected to the next reaction without further separation.

3-[3β-Acetoxy-5β,17β-dihydroxy-15β,16β-methylene-6α-(phenylseleno)androst-11-en-17α-yl]propionic Acid γ-Lactone (41). A 160-mg (0.39 mmol) sample of **40** was treated with a reagent prepared from 468 mg (1.5 mmol) of diphenyl diselenide and 120 mg (3.2 mmol) of sodium borohydride in 10 mL of EtOH, and the products were separated by the procedure described for the preparation of **24**. The main product was 119 mg (54%) of **41**, obtained as noncrystalline powder: IR ($CHCl_3$) 1726, 1758 cm^{-1}; NMR ($CDCl_3$) δ 0.94 (3 H, s), 0.99 (3 H, s), 2.10 (3 H, s), 3.63 (1 H, dd, J = 9 and 4 Hz), 5.20 (1 H, s), 5.32 (1 H, d, J = 10 Hz), 5.80 (1 H, d, J = 10 Hz), 7.1–7.5 (3 H, m), 7.5–7.8 (2 H, m).

3-[3β-[(*tert*-Butyldimethylsilyl)oxy]-5β,17β-dihydroxy-15β,16β-methyleneandrosta-6,11-dien-17α-yl]propionic Acid γ-Lactone (42c). A 2.11-g (3.7 mmol) sample of **41** was oxidized with 0.82 mL (10 mmol) of pyridine and 5.3 mL (46 mmol) of 30% aqueous hydrogen peroxide in 80 mL of a mixture of CH_2Cl_2–MeOH (1:1) and then treated in 50 mL of benzene containing 0.82 mL (10 mmol) of pyridine. The product, purified by the same procedure as described for the preparation of **25a**, was further hydrolyzed by the reaction with 760 mg (14 mmol) of sodium methoxide in 70 mL of MeOH and then silylated with 1.23 g (8.18 mmol) of *tert*-butyldimethylsilyl chloride and 0.56 g (8.18 mmol) of imidazole in 20 mL of DMF. Purification by column silica gel chromatography and recrystallization from CH_2Cl_2–petroleum ether gave 1.33 g (74%) of **42c**: mp 196–197 °C; IR ($CHCl_3$) 1759 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (12 H, s), 1.08 (3 H, s), 4.05–4.30 (1 H, m), 4.20 (1 H, s), 5.48 (1 H, d, J = 10 Hz), 5.49 (1 H, dd, J = 11 and 3 Hz), 5.75 (1 H, d, J = 10 Hz), 5.76 (1 H, dd, J = 11 and 3 Hz). Anal. ($C_{29}H_{44}O_4Si$) C, H.

3-[3β-[(*tert*-Butyldimethylsilyl)oxy]-5β,17β-dihydroxy-6β,7β:15β,16β-bis(methylene)androst-11-en-17α-yl]propionic Acid γ-Lactone (43a). To a stirred mixture of 1.06 g (2.19 mmol) of **42c** and 5.4 mL (10.9 mmol) of diethylzinc in 25 mL of THF was added dropwise 1.32 mL (16.4 mmol) of methylene iodide. The stirred mixture was maintained at 60–70 °C for 15 h and then cooled. The mixture was poured into a large quantity of saturated aqueous NH_4Cl with stirring, and the product was isolated by CH_2Cl_2 extraction. The CH_2Cl_2 layer was washed with saturated aqueous NaCl, dried, and evaporated. The product was purified by column silica gel chromatography using AcOEt–benzene (1:9) as an eluent, and 746 mg (68.3%) of **43a** was obtained on recrystallization from CH_2Cl_2–petroleum ether: mp 188.5–191 °C; IR ($CHCl_3$) 1760 cm^{-1}; NMR ($CDCl_3$) δ 0.81 (3 H, s), 0.90 (9 H, s), 1.01 (3 H, s), 3.97 (1 H, s), 4.0–4.3 (1 H, m), 5.33 (1 H, d, J = 10 Hz), 5.75 (d, J = 10 Hz). Anal. ($C_{30}H_{46}O_4Si$) C, H.

3-(17β-Hydroxy-6β,7β:15β,16β-bis(methylene)-3-oxoandrosta-4,11-dien-17α-yl)propionic Acid γ-Lactone (44). A 708-mg sample (1.42 mmol) of **43a** was desilylated by the reaction with 565 mg (2.16 mmol) of tetrabutylammonium fluoride in 10 mL of THF, and the product was isolated in the usual manner. The product obtained in the previous reaction was oxidized by the reaction with 1.62 g (4.31 mmol) of pyridinium dichromate in 33 mL of DMF. The resulting product was purified by the procedure described for the preparation of **27**. Recrystallization from CH_2Cl_2–ether gave 258 mg (50%) of **44**: mp 182–184 °C; IR ($CHCl_3$) 1598, 1656, 1763 cm^{-1}; NMR ($CDCl_3$) δ 1.01 (3 H, s),

1.06 (3 H, s), 5.39 (1 H, d, J = 10 Hz), 5.89 (1 H, dd, J = 10 and 2 Hz), 6.06 (1 H, s). Anal. ($C_{24}H_{28}O_3$) C, H.

3-(17β-Hydroxy-6β,7β:15β,16β-bis(methylene)-3-oxoandrosta-1,4,11-trien-17α-yl)propionic Acid γ-Lactone (45). A 201-mg (0.55 mmol) sample of **44** was oxidized with 185 mg (1.6 mmol) of SeO_2 and 0.03 mL of pyridine in 20 mL of *tert*-butyl alcohol. The product was purified by the procedure described for the preparation of **14a**, giving 116 mg (58.2%) of **45** as a noncrystalline powder: IR ($CHCl_3$) 1594, 1619, 1660, 1767 cm^{-1}; NMR ($CDCl_3$) δ 1.08 (6 H, s), 5.52 (1 H, d, J = 10 Hz), 5.92 (1 H, dd, J = 10 and 2 Hz), 6.18 (1 H, dd, J = 10 and 2 Hz), 6.34 (1 H, d, J = 2 Hz), 6.97 (1 H, d, J = 10 Hz).

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Analgesic Dipeptide Derivatives. 3. Synthesis and Structure–Activity Relationships of *o*-Nitrophenyl-Modified Analogues of the Analgesic Compound H-Lys-Trp(NPS)-OMe[1]

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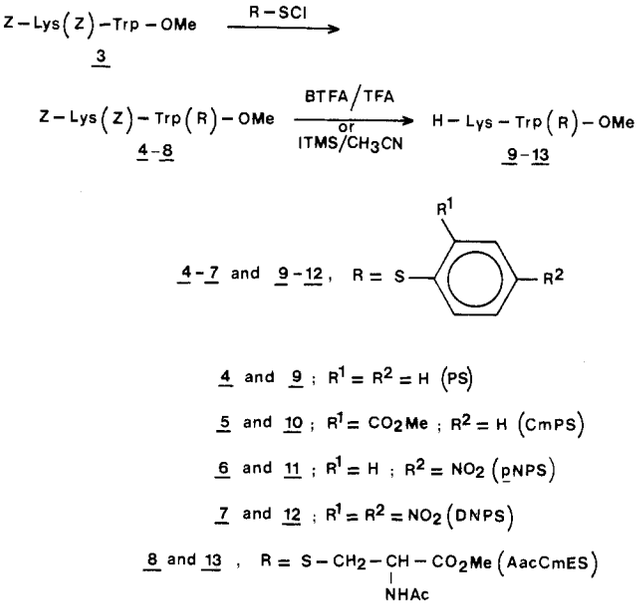
A series of analogues of the analgesic dipeptide derivative H-Lys-Trp(NPS)-OMe has been designed to determine the influence of the (2-nitrophenyl)sulfenyl (NPS) moiety on the activity. The syntheses and antinociceptive effects of these analogues of general formula H-Lys-Trp(R)-OMe [R = phenylsulfenyl (PS) (**9**); R = (2-carbomethoxyphenyl)sulfenyl (CmPS) (**10**); R = (4-nitrophenyl)sulfenyl (*p*NPS) (**11**); R = (2,4-dinitrophenyl)sulfenyl (DNPS) (**12**); R = [2-(acetylamino)-2-carbomethoxyethyl]sulfenyl (AacCmES) (**13**); R = [2-(acetylamino)phenyl]sulfenyl (AacPS) (**17**); R = *tert*-butylsulfenyl (*t*-BuS) (**23**); R = (2-carbomethoxyethyl)sulfenyl (CmES) (**24**)] are described. Reaction of Z-Lys(Z)-Trp-OMe (**3**) with PS-, CmPS-, *p*NPS-, DNPS-, and AacCmES-Cl afforded the corresponding 2-(sulfenyl)tryptophan derivatives, which on treatment with boron–tris(trifluoroacetate)/trifluoroacetic acid or trimethylsilyl iodide in acetonitrile (Me_3SiI/CH_3CN) provided **9–13**, respectively. Sulfenylation of **3** with NPS-Cl gave Z-Lys(Z)-Trp(NPS)-OMe, which, on catalytic hydrogenation of the nitro group using 10% Pd/C followed by acetylation of the resulting amino function and removal of the protecting Z groups, gave **17**. Condensation of 2-(*tert*-butylsulfenyl)- and 2-[(2-carbomethoxyethyl)sulfenyl]tryptophan methyl ester, obtained by reaction of methyl 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylate with the corresponding thiol, with Z-Lys(Z)-OSu afforded Z-Lys(Z)-Trp(*t*-BuS)-OMe and Z-Lys(Z)-Trp(CmES)-OMe, which on treatment with Me_3SiI/CH_3CN provided **23** and **24**, respectively. Intracerebroventricular administration of **10** elicited a naloxone-reversible antinociceptive effect in mice similar to that of H-Lys-Trp(NPS)-OMe. No analgesia was however found with the phenylsulfenyl or acyclic sulfenyl substituted dipeptides **9**, **11**, and **17** or **13**, **23**, and **24**. The Trp(DNPS)-containing analogue was neurotoxic. Structure–activity studies indicate that the role of the NPS and CmPS moieties could be related to the adoption of a preferential active conformation.

In the first paper of this series,[2] we have shown that the synthetic dipeptide derivative H-Lys-Trp(NPS)-OH [NPS = (*o*-nitrophenyl)sulfenyl] (**1**) exhibited a naloxone-reversible analgesia in mice, when administered intracerebroventricularly, comparable with that of the enkephalin analogue D-Ala[2]-Met-enkephalinamide (DAME) regarding both the maximum effect and the time-course of analgesia. A similar antinociceptive effect has also been found with the corresponding dipeptide methyl ester, H-Lys-Trp(NPS)-OMe (**2**). However, no analgesia was observed with Trp(NPS) or with the unsubstituted dipeptide H-Lys-Trp-OH. Preliminary studies to establish the structural requirements for the antinociceptive effect of **1** and **2** revealed, besides the importance of the NPS moiety, the need for a basic amino acid, although the side-chain length was not a critical factor.[2,3] In view of the peculiar structure of **1** and **2** as compared to other directly or indirectly acting opioid peptides, we have considered it of interest to gain further insight into the structure-activity relationships. In this sense, the role of the NPS moiety seemed to us rather intriguing, and, therefore, we have now investigated the effect of replacing this portion by various related groups.

The present paper describes the synthesis and analgesic activity in mice of a series of novel H-Lys-Trp(R)-OMe in which the substituent R at the 2-position of Trp is a phenylsulfenyl or an acyclic sulfenyl moiety. In the first case, the *o*-nitrophenyl portion of **2** has been modified by

Scheme I



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(1) For previous papers in this series see ref 2 and 3.

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