Steroidal Spiro-γ-lactones That Inhibit 17β-Hydroxysteroid Dehydrogenase Activity in Human Placental Microsomes

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The important enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) is known to regulate intracellular levels of biologically active steroids, namely, androgens and estrogens. In an effort to develop potent inhibitors of 17β-HSD for reducing the levels of active steroids, we found that steroidal spiro-γ-lactones inhibit 17β-HSD activity. In this report, we describe the synthesis of 11 spiro-γ-lactone analogs containing a steroidal C-18 or C-19 nucleus and compare their relative inhibitory effects on 17β-HSD activity in the human placenta microsomes that catalyze the interconversion of androgens and estrogens. To avoid the interaction of the cytosolic 17β-HSD activity that is specific for the interconversion of estrone and estradiol, we used 4-androstenedione as substrate. Analysis of the inhibitory effect exerted by these analogs on microsomal 17β-HSD activity indicates that spiro-γ-lactones containing the C-18 nucleus are more potent inhibitors than C-19 nucleus analogs. The best inhibition was obtained with the phenolic spiro-γ-lactone **7** (3-hydroxy-19-nor-17α-pregna-1,3,5(10)-triene 21,17-carbolactone), which has an IC_{50} value of 0.27 μM, and was much lower than the competitive effect of the unlabeled substrate 4-androstenedione, which has an IC_{50} value of 1.40 μM. Preincubation with lactone **7** did not inactivate 17β-HSD activity. The results thus suggest that lactone **7** is a reversible inhibitor. Lactone **7** is selective for microsomal 17β-HSD activity, as no inhibition was observed for cytosolic 17β-HSD activity.

Introduction

The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) plays an essential role in the biosynthesis and metabolism of steroid hormones (Figure 1), in particular androgens and estrogens. These hormones are more active in their 17β-hydroxy configuration by binding with higher affinity to the receptors than their 17-keto analogs. 17β-HSD activities are widespread in human tissues, not only in classic steroidogenic tissues, such as the testis,[1] ovary,[2] and placenta,[3] but also in a large series of peripheral intracrine tissues,[4] including adipose tissue,[5] endometrium,[6] ileum,[1] liver,[7] lung,[8] skin, and vaginal mucosa,[9] as well as red blood,[10] breast cancer,[11,12] and prostatic cancer.[13] It is estimated that 50% of the androgens in adult men, 75% of the estrogens in premenopausal women, and 100% of the estrogens in postmenopausal women are synthesized in the peripheral tissues from the adrenal C-19 steroids.[14] It is now well accepted that intracellular formation and degradation of androgens and estrogens play important roles in the regulation of cell function and proliferation. This new area of endocrinology has been termed intracrinology.[15] It describes the high level of sex steroids synthesized in peripheral target cells and executing their action in the same cells where synthesis takes place without release into the surrounding space or the circulation.

Three types of 17β-HSD have been identified in humans. Types 1[3,16] and 3[17] are substrate specific and catalyze almost exclusively the transformation of estrone to estradiol and 4-androstenedione to testosterone, respectively. In contrast, type 2 17β-HSD[18] possesses broad subtrate specificity and catalyzes the interconversion of estrogen and androgen substrates. Impairment of 17β-HSD activity led to the well-known male pseudohermaphroditism due to the absence of the internal male reproductive structures (epididymes, seminal vesicles, and vas deferens) that are formed from wolffian duct under the control of testosterone. The 17β-HSD deficiency is due to mutations in the type 3 17β-HSD, which is expressed selectively in the testis.[17] As there are multiple 17β-HSD genes that are expressed selectively in various tissues, other types of intracrine dysfunction of 17β-HSD activity in the peripheral tissues could also be expected.[14] 17β-HSD activity is indeed an obligatory step for the biosynthesis of andro-

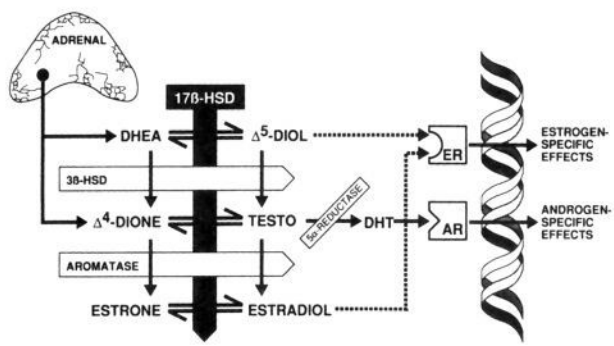

Figure 1. Biosynthetic steps involved in the formation in peripheral target tissues of the estrogens Δ5-diol (androst-5-ene-3β,17β-diol) and estradiol and of the androgens testosterone (testo or T) and DHT (5α-dihydrotestosterone). DHEA, dehydroepiandrosterone; Δ4-dione, 4-androstenedione; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; ER, estrogen receptor; AR, androgen receptor (from F. Labrie, Intracrinology, ref 14).

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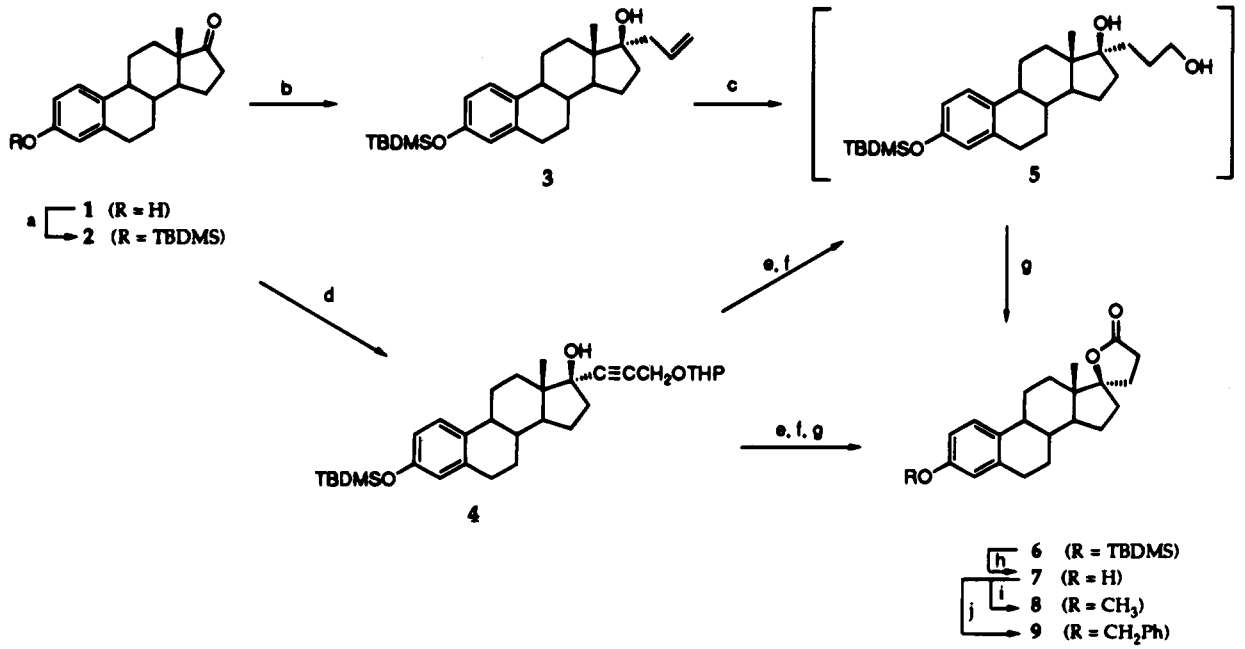
Scheme 1. Synthesis of Spiro-γ-lactones **7**–**9** (with a C-18 Steroidal Nucleus).[a]



[a] Reagents: (a) TBDMS-Cl, imidazole, DMF, room temperature; (b) $BrMgCH_2CH{=}CH_2$, THF, −78 °C; (c) i. BH_3·THF, THF, 0 °C, ii. NaOH, H_2O_2 (30%); (d) $HC{\equiv}CCH_2OTHP$, *n*-BuLi, THF, −78 °C; (e) H_2, Pd/C−Pd/$CaCO_3$ (1:1), EtOAc, room temperature; (f) amberlyst acidic resin, MeOH, room temperature; (g) Jones' reagent (2.7 M), acetone, 0 °C to room temperature; (h) Bu_4NF, THF, 0 °C to room temperature; (i) CH_3I, K_2CO_3, 18-crown-6, THF, reflux; (j) benzyl bromide, K_2CO_3, $CHCl_3$, MeOH, reflux.

gens and estrogens, namely, estradiol, 5-androstenediol, testosterone, and dihydrotestosterone.

In the placenta, which because of its availability is the most studied organ for steroid hormone metabolism, it is well established that at least two 17β-HSD isoenzymes are present. A soluble enzyme purified from cytosol catalyzes the interconversion of estrone and estradiol and is referred to as type 1 17β-HSD. It is functional as a dimer of identical subunits.[19] A microsomal 17β-HSD activity that catalyzes the interconversion of 4-androstenedione and testosterone was first evidenced by Blomquist et al.,[20] Lehmann et al.,[21] and Pollow et al.[22] It probably corresponds to the recently cloned type 2 17β-HSD,[18] which catalyzes the interconversion of 4-androstenedione and testosterone as well as that of estrone and estradiol.

To control the production of androgens and estrogens in peripheral target tissues, we synthesized several steroidal spiro-γ-lactone analogs. In this paper, we report their synthesis and characterize their ability to inhibit the human placenta microsomal 17β-HSD activity.

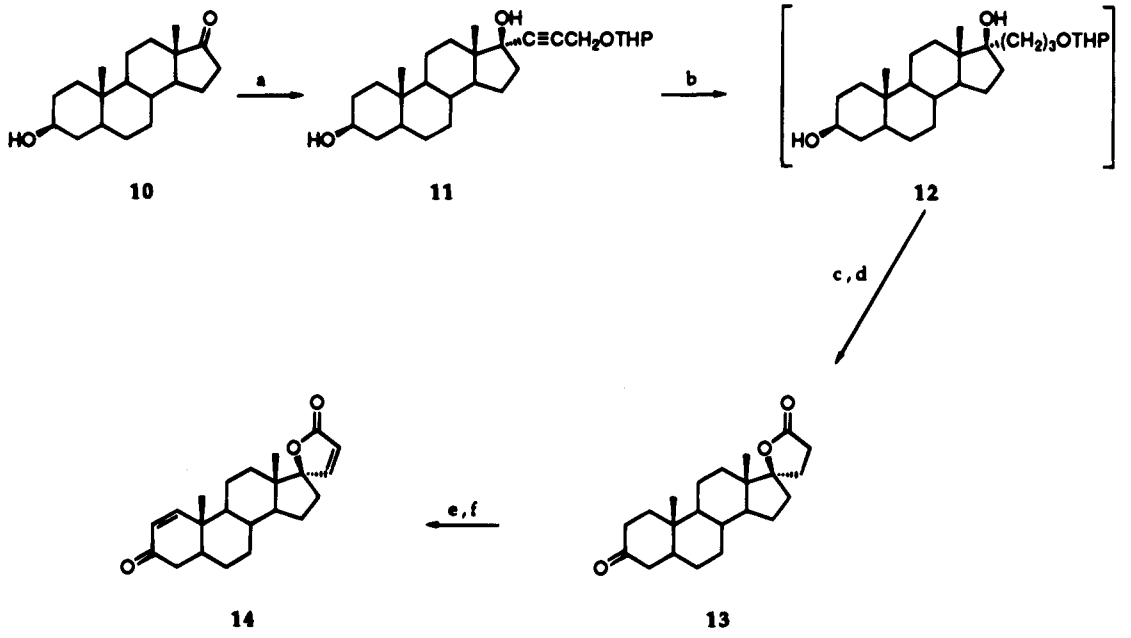
Chemistry

Synthesis of Spiro-γ-lactones 7–9, with a C-18 Steroidal Nucleus (Scheme 1). The synthesis of lactones **7**–**9** is depicted in Scheme 1. Two different synthetic approaches were employed for preparation of intermediate alcohol **5**. In the first approach, previously reported by us[23] and Garrouj et al.,[24] allylmagnesium bromide was used to alkylate TBDMS-estrone (**2**) and provide allyl derivative **3**. Reduction of the latter with borane in THF followed by oxidation with H_2O_2 (30%) and NaOH allowed the formation of alcohol **5**. The second approach was performed by alkylation of TBDMS-estrone (**2**) with tetrahydro(propynyloxy)-2*H*-pyran and *n*-BuLi to give alkyne **4** exclusively as a 17α-isomer.[25] The triple bond was then hydrogenated with a 1:1 mixture of Pd/C−Pd/$CaCO_3$ in ethyl acetate, and the THP group was hydrolyzed by treatment with an acidic resin (amberlyst) in methanol to allow the formation of alcohol **5**. Oxidation of **5** with Jones' reagent in acetone gave the TBDMS spiro-γ-lactone **6**. Herein the carboxylic acid resulting from Jones' oxidation of primary alcohol underwent an intramolecular cyclization with the tertiary 17β-hydroxy to generate the corresponding spiro-γ-lactone (a five-member ring). When the second approach was used, we did not generally isolate the intermediate alcohol **5**; rather, we immediately performed the Jones' oxidation to obtain the spiro-γ-lactones **6** and **7** in good overall yields. The TBDMS group of lactone **6** was cleaved by treatment with Bu_4NF to give the phenolic spiro-γ-lactone **7**. The two other lactones, **8** and **9**, were synthesized from **7**. The phenolic group of **7** was then alkylated by CH_3I and K_2CO_3 in refluxing THF or benzyl bromide and K_2CO_3 in refluxing $CHCl_3$/MeOH (2:1) to provide the methoxy lactone **8** or benzyloxy lactone **9**, respectively.

Synthesis of Spiro-γ-lactones 13, 14, 19, 20, 25, 29, and 33 with a C-19 Steroidal Nucleus (Schemes 2–6). Scheme 2 shows the synthetic pathway to obtain lactones **13** and **14**. Epiandrosterone (**10**) was first alkylated at position 17α with tetrahydro(propynyloxy)-2*H*-pyran and *n*-BuLi to give the diol **11**. Then the three-step sequence described above (catalytic hydrogenation of the triple bond, cleavage of the THP group, and Jones' oxidation) was used to transform diol **11** to 3-keto spiro-γ-lactone **13**. Unsaturated lactone **14** was obtained from lactone **13** by introducing double bonds in the α-position of both carbonyl groups (3-ketone and lactone). Phenylselenylation with LDA and PhSeBr and subsequent oxidation with H_2O_2 (30%) in refluxing THF, which allowed elimination of the phenyl selenoxide, gave the di-α,β-unsaturated compound **14**, with an unfortunately poor yield (5%) after two steps.

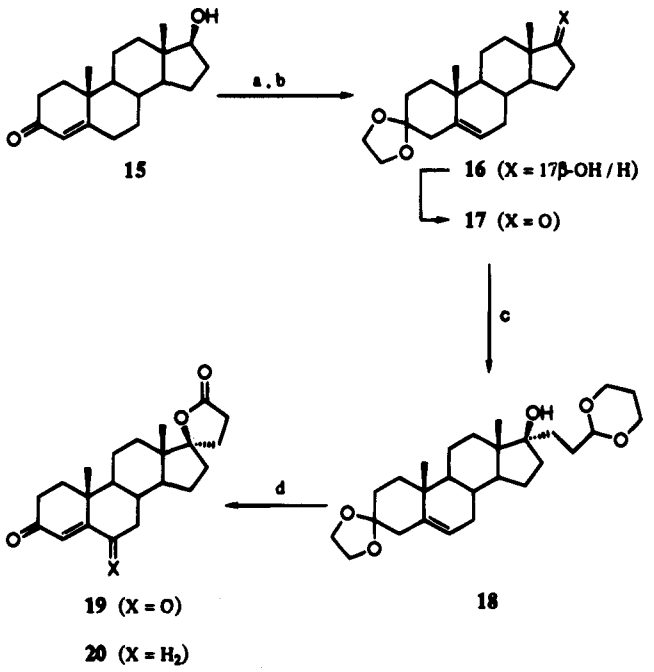
The synthesis of lactone **19** from testosterone (**15**) is shown in Scheme 3. By a classic two-step sequence,[26]

Scheme 2. Synthesis of Spiro-γ-lactones **13** and **14** (with a C-19 Steroidal Nucleus).[a]



[a] Reagents: (a) HC≡CCH$_2$OTHP, *n*-BuLi, THF, −78 °C; (b) H$_2$, Pd/C−Pd/CaCO$_3$ (1:1), EtOAc, room temperature; (c) amberlyst acidic resin, MeOH, room temperature; (d) Jones' reagent (2.7 M), acetone, 0 °C to room temperature, 30 min; (e) LDA, PhSeBr, THF, −78 °C; (f) H$_2$O$_2$ (30%), reflux.

Scheme 3. Synthesis of Spiro-γ-lactones **19** and **20**.[a]



[a] Reagents: (a) HO(CH$_2$)$_2$OH, *p*-TSA, toluene, reflux; (b) (*n*-Pr$_4$N)(RuO$_4$), 4-methylmorpholine *N*-oxide, CH$_2$Cl$_2$, molecular sieves, room temperature; (c) *t*-BuLi, 2-(2-iodoethyl)-1,3-dioxane, THF, −78 °C; (d) Jones' reagent (2.7 M), acetone, 0 °C.

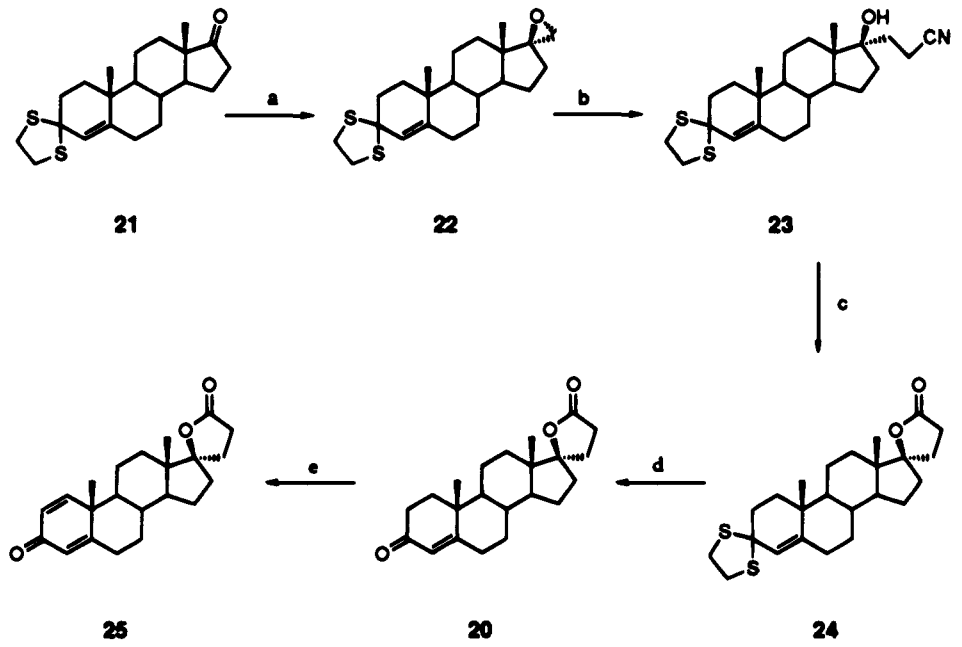
protection of ketone at position 3 using ethylene glycol and *p*-TSA in refluxing toluene and oxidation of the 17β-hydroxyl group, ketone **17** was obtained in good yield. Alkylation at position 17α by primary alkyl halide lithium interchange of 2-(2-iodoethyl)-1,3-dioxane with *t*-BuLi in a mixture of diethyl ether/pentane (2:3)[27] afforded 53% of alkylated compound **18**. Treatment of **18** with Jones' reagent gave lactone **19** (3,6-dioxo) in 39% yield and lactone **20** (3-oxo) in 21% yield. The latter was also synthesized in better yield by another synthetic sequence, represented in Scheme 4.

The synthesis of lactones **20** and **25** (Scheme 4) began with compound **21** (1, 3-dithiolane-protected testosterone).[28] This ketone was treated with trimethylsulfoniumylide (NaH in DMSO followed by Me$_3$S$^+$I$^-$ in THF)[29] as a methyl transfer agent to give **22** in 80% yield. The epoxide **22** was opened by alkylation with lithium acetonitrile[29] to obtain a three-carbon side chain derivative (**23**) in 81% yield. Hydrolysis of the nitrile group gave carboxylic acid, which underwent an intramolecular cyclization with the tertiary alcohol to afford the spiro-γ-lactone **24**. Removal of 1,3-dithiolane group was performed with silver nitrate and *N*-chlorosuccinimide in acetonitrile[29] to give lactone **20** in 74% yield. According to a known methodology (DDQ in refluxing toluene),[30] dienone lactone **25** was obtained from enone lactone **20**. These neutral conditions allowed the selective introduction of a double bond in positions 1 and 2 instead of positions 6 and 7.

For comparison, we also synthesized the saturated analog of lactone **19**, the 3,6-diketo lactone **29** (Scheme 5). Our synthetic route began with dehydroepiandrosterone-3-acetate (**26**) as starting material. Alkylation at the 17α-position with allylmagnesium bromide in THF gave the diol intermediate (without acetate group), which was submitted to TBDMS-Cl and imidazole treatment to protect selectively the hydroxyl group at C-3 and allow the formation of compound **27**. Oxidative hydroboration (BH$_3$ and H$_2$O$_2$) of double bonds generated two additional hydroxyl groups (triol **28**). This compound was transformed upon treatment with Bu$_4$NF to the highly polar intermediate tetraol, which without purification was treated with Jones' reagent to give the less polar saturated diketo lactone **29**.

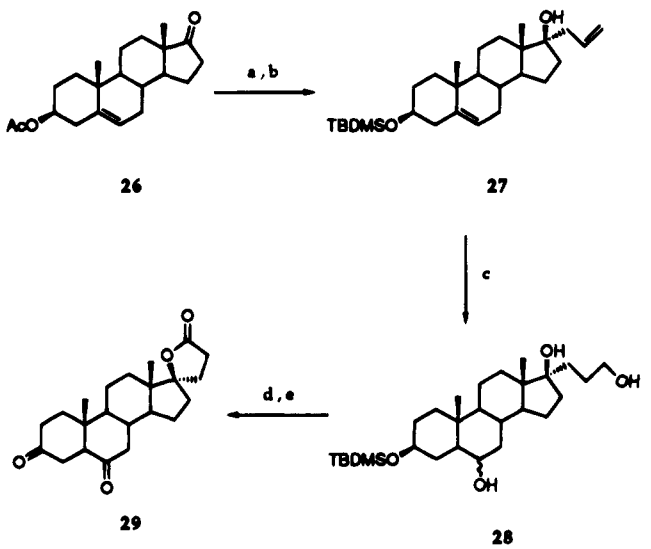
Because we wondered about the role of the carbonyl group at C-3 and the role of the lactone group, we synthesized dilactone **33** (Scheme 6). Both carbonyls of androstanedione **30** were alkylated by acetylenide, resulting from *n*-BuLi and the three-carbon side chain tetrahydro(propynyloxy)-2*H*-pyran, to give the dialky-

Scheme 4. Synthesis of Spiro-γ-lactones **20** and **25**.[a]



[a] Reagents: (a) i. NaH (60%), DMSO, −78 °C, ii. $Me_3S^+I^-$, DMSO, THF, −5 °C, 35 min, iii. room temperature; (b) LDA, CH_3CN, THF, −10 °C to room temperature; (c) i. KOH (6 M), reflux, ii. concentrated HCl, reflux; (d) H_2O, CH_3CN, *N*-chlorosuccinimide, $AgNO_3$, room temperature; (e) DDQ, toluene, reflux.

Scheme 5. Synthesis of 3,6-Dioxy Spiro-γ-lactone **29**.[a]



[a] Reagents: (a) $BrMgCH_2CH{=}CH_2$, THF, 0 °C; (b) TBDMS-Cl, imidazole, DMF, room temperature; (c) i. BH_3-THF, 0 °C, ii. NaOH (3 N), H_2O_2 (30%); (d) Bu_4NF, THF, 0 °C to room temperature; (e) Jones' reagent (2.7 M), acetone, 0 °C.

lated compound **31**. In this transformation, C-18 and C-19 methyls efficiently induced the attack of lithium acetylenide on the less hindered steroidal α-face, leading to 3α- and 17α-orientation of the side chain. Catalytic hydrogenation of **31**, as reported above, gave the saturated diol **32**. Without purification, cleavage of the THP protecting groups with *p*-TSA in methanol and oxidation of primary alcohol with Jones' reagent allowed the formation of dispiro-γ-lactone **33** in 50% yield (three steps).

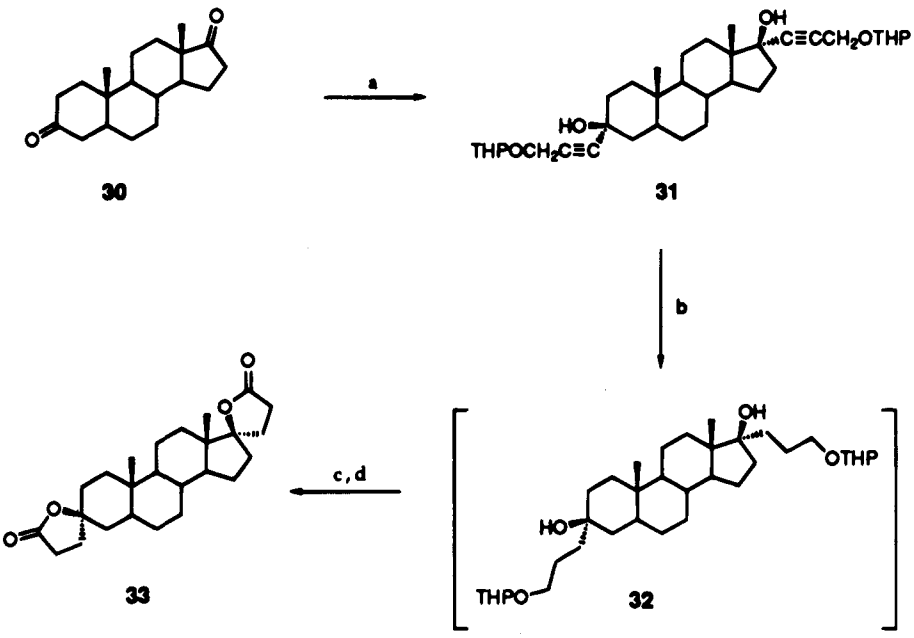
Biological Results and Discussion

The inhibitory effect of newly synthesized lactones was determined using a 17β-HSD-enriched fraction obtained from microsomes of human placenta. An aromatase inhibitor was added to block the aromatization of the substrate Δ^4-dione into estrone. Preliminary tests showed that a 10 μM concentration of the aromatase inhibitor allowed a full blokade of estrone formation (Δ^4-dione to estrone) without significant inhibition of 17β-HSD activity. For screening purposes, 1 μM of tested compounds was used to determine their ability to inhibit the transformation of 4-androstenedione to testosterone by microsomal 17β-HSD activity.

Starting with about 40 16α- or 17α-estradiol derivatives, initially synthesized for inhibition of cytosolic 17β-HSD,[31] we observed no inhibitory activity toward microsomal 17β-HSD except for one compound bearing a spiro-γ-lactone function in position 17 (unreported results). Those findings led us to synthesize 11 spiro-γ-lactone analogs with a C-18 estrogenic or C-19 androgenic nucleus (Schemes 2−6). As shown in Table 1, a higher inhibition of microsomal 17β-HSD was obtained with the phenolic derivative **7** (81%), whereas we observed an important decrease of inhibiting activity with methoxy (**8**, 55%) and benzyloxy (**9**, 11%) derivatives. It appears the bulkier the chemical group in position 3, the lower the inhibition. The presence of the spiro-γ-lactone group in position 17 is also essential because a spiro-γ-cycloether analog exhibited only a weak inhibition of microsomal 17β-HSD (unreported result).

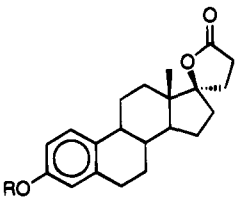
We also synthesized lactone analogs with a C-19 steroid nucleus. Since a C-19 nucleus possesses a structure similar to the substrate 4-androstenedione, it is likely that a C-19 steroid nucleus bearing the spiro-γ-lactone might be a better inhibitor than C-18 analogs. A series of spiro-γ-lactones was thus synthesized with modification mainly on the A and/or B androgenic ring and tested for their inhibitory effects. Surprisingly, only a weak inhibitory effect was observed (Table 2). At 1 μM, only three lactones showed 25−28% inhibition of microsomal 17β-HSD activity. In contrast, the C-18 analog spiro-γ-lactone **7** showed 81% inhibition (Table

Scheme 6. Synthesis of Dispiro-γ-lactone **33**.[a]



[a] Reagents: (a) HC≡CCH$_2$OTHP, *n*-BuLi, THF, −78 °C; (b) H$_2$, Pd/C, EtOAc, room temperature; (c) *p*-TSA, MeOH, room temperature; (d) Jones' reagent (2.7 M), acetone, 0 °C.

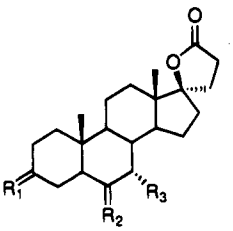
Table 1. Inhibition of Microsomal 17β-HSD by Spiro-γ-lactones **7**–**9** (with a C-18 Steroidal Nucleus)



no.	R	inhibition of 17β-HSD (%)[a]
7	H	81
8	CH_3	55
9	CH_2Ph	11

[a] Error ±5%; inhibitor concentration 1 μM.

Table 2. Inhibition of Microsomal 17β-HSD by Spiro-γ-lactones **13**, **14**, **19**, **25**, **34**, **20**, **29**, and **33** (with a C-19 Steroidal Nucleus)



no.	R_1 (functional group at C-3)	R_2	R_3	double bond position	inhibition of 17β-HSD (%)[a]
13	O	H/H	H		25
14	O	H/H	H	1–2	0
19	O	H/H	H	4–5	3
25	O	H/H	H	1–2, 4–5	6
34	O	H/H	$SCOCH_3$	4–5	25
20	O	O	H	4–5	3
29	O	O	H		28
33	lactone	H/H	H		11

[a] Error ±5%; inhibitor concentration 1 μM.

1). Among those C-19 androgenic derivatives, the well-known spironolactone (**34**) showed the highest inhibitory effect. The backbone of spironolactone resembles spiro-γ-lactone **19**, but the presence of an acetylthio moiety at position 7α seemed to improve the inhibition of microsomal 17β-HSD (compare **19** with **34**). The results suggest also that introducing one or two double bonds in the A-ring decreased the inhibitory effect of the spiro-γ-lactones (compare **13** with **14**; **19** with **25**; and **29** with **20**). We also synthesized dispiro-γ-lactone **33** and observed that the presence of the additional lactone group at position 3 of the A-ring lowered the inhibitory effect (compare **33** with **13**).

According to our screening exploratory study with 11 spiro-γ-lactones (Tables 1 and 2), spiro-γ-lactone **7** (with a C-18 estradiol nucleus) is the best inhibitor of microsomal 17β-HSD activity. Indeed, as shown in Figure 2, spiro-γ-lactone **7** is better as a competitor than unlabeled substrate (4-androstenedione). The IC_{50} value of spiro-γ-lactone **7** (0.27 μM) is 5-fold better than the IC_{50} value of unlabeled 4-androstenedione (1.40 μM).

When compound **7**, at a concentration of 1 or 5 μM, was incubated with microsomal 17β-HSD and cofactor, no inactivation of enzyme activity was observed (Figure 3). In fact, enzymatic activity is entirely restored after the removal of inhibitor, suggesting that spiro-γ-lactone **7** acts as a reversible inhibitor. Taking into account this result, the inhibition constant (K_i) can be calculated from the IC_{50} value by the Cheng–Prusoff equation.[32] For microsomal 17β-HSD, the K_i of spiro-γ-lactone **7** was equal to 0.25 μM. On the other hand, this inhibitor is selective for microsomal 17β-HSD rather than for cytosolic 17β-HSD (type 1). Indeed, a K_i value higher than 40 μM was obtained when spiro-γ-lactone **7** was tested using a cytosolic fraction of human placenta (17β-HSD type 1).[31,33]

In conclusion, the C-19 steroid nucleus bearing a spiro-γ-lactone in position 17 is not an efficient inhibitor of microsomal 17β-HSD. On the other hand, the C-18 estradiol nucleus with the spiro-γ-lactone moiety at position 17 is a more potent inhibitor, exhibiting a selective and reversible inhibitory effect toward mi-

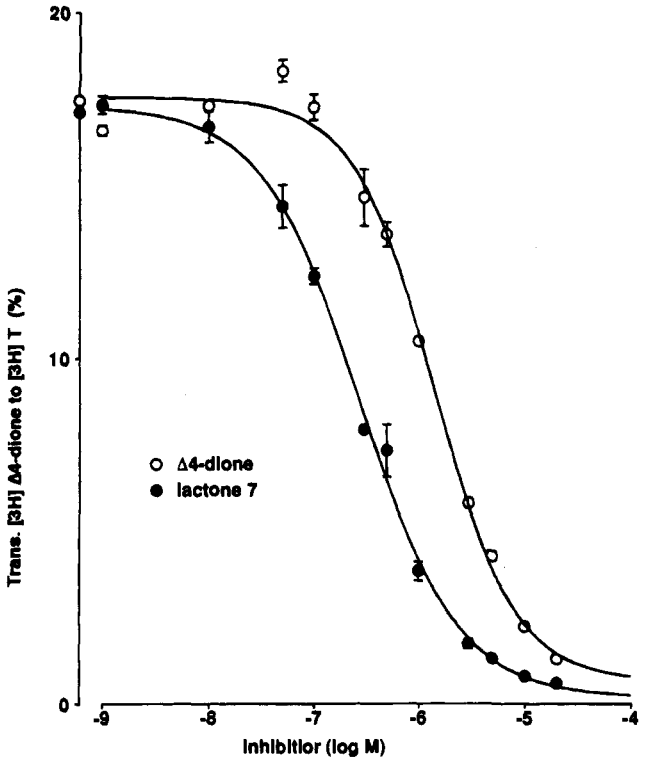


Figure 2. Inhibition of microsomal 17β-HSD by increasing concentrations of spiro-γ-lactone **7** (IC_{50} = 0.27 μM) or 4-androstenedione (Δ^4-dione) (IC_{50} = 1.40 μM). See the Experimental Section for the conditions used in this enzymatic assay.

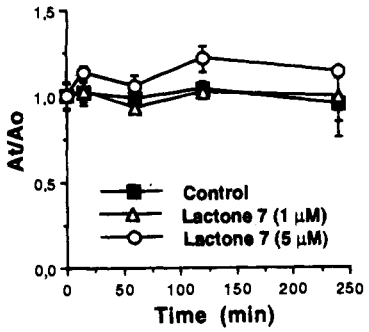


Figure 3. Inactivation of microsomal 17β-HSD by spiro-γ-lactone **7**. A_t, enzymatic activity at time t, and A_o, initial activity. See the Experimental Section for the conditions used in this enzymatic assay.

crosomal 17β-HSD. The combination of a phenolic group and a spiro-γ-lactone is important to the efficient inhibition of enzyme activity, but the precise role of the lactone group is not fully understood. The availability of spiro-γ-lactone **7** (3-hydroxy-19-nor-17α-pregna-1,3,5-(10)-triene 21,17-carbolactone), the first inhibitor of human placenta microsomal 17β-HSD, is a valuable tool for the study of 17β-HSD isoenzymes and the control of androgen and estrogen formation in peripheral tissues.

Experimental Section

Chemical Synthesis. General Procedure. Chemical reagents and starting materials (estrone, epiandrosterone, testosterone, epiandrosterone-3-acetate, and androstanedione) were purchased from Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), or Steraloids (Wilton, NH), and solvents were obtained from BDH Chemicals (Montréal, Canada) or Baker Chemicals (Montréal, Canada). Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany), and 230–400 mesh ASTM silica gel 60 (E. Merck) was used for flash-column chromatography. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Infrared spectra (IR) are expressed in cm^{-1} and were obtained on a Perkin-Elmer 1600 (series FTIR) spectrophotometer. ^1H nuclear magnetic resonance spectra (^1H NMR) were recorded with a Bruker AC/F 300 (300 MHz) spectrometer, and ^{13}C NMR spectra were recorded with a Bruker AC/F 300 spectrometer at 75.47 MHz. The chemical shifts (δ) are expressed in ppm and referenced to $CDCl_3$ (7.26 ppm for ^1H and 77.00 ppm for ^{13}C) or acetone-d_6 (2.05 ppm for ^1H and 29.83 ppm for ^{13}C). For ^1H NMR, only characteristic signals are reported. For ^{13}C NMR, all signals are indicated, generally with full assignments. Assignment of ^{13}C NMR signals was made easier by literature data[34,35] and additional NMR experiments (COSY, DEPT, HETCOR, and COLOC).[35,36] High-resolution electron impact mass spectra (EIMS) were provided by the Centre Régional de Spectrométrie de Masse (Université de Montréal, Montréal, Canada). Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN).

Synthesis of Spiro-γ-lactones 7–9 (Scheme 1). 3-[(*tert*-Butyldimethylsilyl)oxy]-17β-hydroxy-17α-[3′-[(tetrahydro-2″*H*-pyranyl)oxy]propynyl]-1,3,5(10)-estratriene (4). To a solution of tetrahydro-2-(2-propynyloxy)-2*H*-pyran (1.1 mL, 7.8 mmol) in 20 mL of dry THF, under argon atmosphere and at 0 °C, was added 4.8 mL of a standard solution of *n*-BuLi 1.6 M (7.7 mmol), and the mixture was stirred for 40 min. A solution of TBDMS-estrone (**2**)[33] (1.0 g, 2.6 mmol) in 7 mL of THF was then added dropwise at −78 °C, and the reaction mixture was stirred for 11 h, after which time, a solution of aqueous $NaHCO_3$ (5%) was poured into the mixture and the aqueous phase extracted with EtOAc. Combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (hexane/EtOAc, 8:2) yielded the alkylated compound **4** (959 mg, 70%): white foam; IR (KBr) ν 3430 (OH, alcohol), 1608, 1572 and 1497 (C=C, aromatic ring); ^1H NMR ($CDCl_3$) δ 0.19 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 3H, CH_3-18), 0.98 (s, 9H, $SiC(CH_3)_3$), 2.80 (m, 2H, CH_2-6), 3.54 and 3.86 (2m, 2H, CH_2O of THP), 4.35 (s, 2H, CH_2OTHP), 4.87 (t, J = 3.2 Hz, 1H, CH of THP), 6.56 (d, J = 2.4 Hz, 1H, CH-4), 6.62 (dd, J_1 = 2.5 Hz, J_2 = 8.4 Hz, 1H, CH-2), 7.13 (d, J = 8.5 Hz, 1H, CH-1); ^{13}C NMR ($CDCl_3$) δ −4.42 ($Si(CH_3)_2$), 12.79 (C-18), 18.15 ($SiC(CH_3)_3$), 19.09 (THP), 22.85 (C-15), 25.35 (THP), 25.70 ($SiC(CH_3)_3$), 26.36 (C-11), 27.29 (C-7), 29.63 (C-6), 30.29 (THP), 32.95 (C-12), 38.93 (C-16), 39.39 (C-8), 43.62 (C-9), 47.30 (C-13), 49.57 (C-14), 54.39 ($C{\equiv}CCH_2OTHP$), 62.07 (OCH_2 of THP), 79.87 (C-17), 81.81 and 89.55 (C≡C), 96.65 (CH of THP), 117.12 (C-2), 119.90 (C-4), 126.12 (C-1), 132.96 (C-10), 137.81 (C-5), 153.30 (C-3); EIMS calcd for $C_{32}H_{48}O_4Si$ (M^+) 524.3322, found 524.3313.

3-[(*tert*-Butyldimethylsilyl)oxy]-19-nor-17α-pregna-1,3,5-(10)-triene 21,17-Carbolactone (6). To a solution of compound **4** (930 mg, 1.77 mmol) in EtOAc (25 mL) was added 100 mg of a 1:1 mixture of palladium on activated charcoal (10%) and palladium on calcium carbonate (5%), and the reaction mixture was stirred under hydrogen atmosphere for 9 h. Then it was filtered through Celite, washed with EtOAc, and evaporated to dryness. The resulting white foam was dissolved in 25 mL of methanol, and 90 mg of amberlyst 15R acidic resin (Aldrich Chemical Co.) was added. After 12 h at room temperature, the reaction mixture was filtered and evaporated to dryness. The crude solid was dissolved in acetone (50 mL), and 2.8 mL of Jones' reagent (2.7 M) was added dropwise at 0 °C. After the addition was completed, the mixture was stirred at room temperature for 50 min. Then, 3 mL of isopropyl alcohol was added, and the resulting green solution was evaporated to dryness. The solid was dissolved in water and EtOAc, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography (hexane/EtOAc, 9:1 (2000 mL), 8:2 (750 mL), and 7:3 (700 mL), yielded two compounds, 135 mg (23%, three steps) of the deprotected lactone **7** (described later) and 169 mg (22%, three steps) of the TBDMS protected lactone **6**: white foam; IR (KBr) ν 1782 (C=O, lactone), 1606, 1570 and 1495 (C=C, aromatic

ring); ^{1}H NMR ($CDCl_3$) δ 0.19 (s, 6H, $Si(CH_3)_2$), 0.97 (s, 12H, CH_3-18, $SiC(CH_3)_3$), 2.81 (m, 2H, CH_2-6), 6.55 (d, J = 2.3 Hz, 1H, CH-4), 6.62 (dd, J_1 = 2.5 Hz, J_2 = 8.4 Hz, 1H, CH-2), 7.11 (d, J = 8.4 Hz, CH-1); ^{13}C NMR ($CDCl_3$) δ −4.43 ($Si(CH_3)_2$), 14.52 (C-18), 18.12 ($SiC(CH_3)_3$), 22.61 (C-15), 25.67 ($SiC(CH_3)_3$), 25.95 (C-11), 27.22 (C-7), 29.35 (C-21), 29.50 (C-6), 31.33 (C-20), 31.90 (C-12), 35.64 (C-16), 38.97 (C-8), 43.55 (C-9), 45.98 (C-13), 48.79 (C-14), 96.01 (C-17), 117.22 (C-2), 119.94 (C-4), 126.07 (C-1), 132.36 (C-10), 137.63 (C-5), 153.41 (C-3), 176.78 (C=O, lactone); EIMS calcd for $C_{27}H_{40}O_3Si$ (M^+) 440.2747, found 440.2742.

3-Hydroxy-19-nor-17α-pregna-1,3,5(10)-triene 21,17-Carbolactone (7). To a solution of compound **6** (155 mg, 0.35 mmol) in dry THF (15 mL) was added 0.6 mL of a 1 M tetrabutylammonium fluoride solution, and the mixture was stirred at 0 °C for 40 min, after which time water was added and the aqueous phase extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography yielded 130 mg (88%) of the deprotected lactone **7**:[23] colorless needles; mp 244−246 °C (acetone/hexane); IR (KBr) ν 3350 (OH, phenol), 1754 (C=O, lactone), 1609, 1592 and 1506 (C=C, aromatic ring); ^{1}H NMR (acetone-d_6) δ 0.95 (s, 3H, CH_3-18), 2.52 (m, 2H, CH_2C=O), 2.77 (m, 2H, CH_2-6), 6.53 (d, J = 2.6 Hz, 1H, CH-4), 6.60 (dd, J_1 = 2.6 Hz, J_2 = 8.4 Hz, 1H, CH-2), 7.10 (d, J = 8.5 Hz, 1H, CH-1), 7.97 (s, 1H, OH phenol); ^{13}C NMR (acetone-d_6) δ 15.00 (C-18), 23.24 (C-15), 27.00 (C-11), 28.04 (C-7), ~29 (C-6, C-21 under solvent peaks), 31.74 (C-20), 32.61 (C-12), 36.16 (C-16), 40.26 (C-8), 44.34 (C-9), 46.73 (C-13), 49.30 (C-14), 96.00 (C-17), 113.70 (C-2), 116.00 (C-4), 127.09 (C-1), 131.61 (C-10), 138.40 (C-5), 156.06 (C-3), 176.62 (C=O of lactone); EIMS calcd for $C_{21}H_{26}O_3$ (M^+) 326.1882, found 326.1877. Anal. ($C_{21}H_{26}O_3$) C, H.

3-Methoxy-19-nor-17α-pregna-1,3,5(10)-triene 21,17-Carbolactone (8). To a mixture of lactone **7** (55 mg, 0.17 mmol) in dry THF were added 33 mg (0.24 mmol) of K_2CO_3, 105 μL (1.68 mmol) of CH_3I, and 18 mg (0.067 mmol) of dibenzo-18-crown-6, and the reaction mixture heated to reflux. After 3 h, 210 μL (3.36 mmol) of CH_3I was added and the mixture refluxed for an additional 10 h. Next, water was added and the aqueous phase extracted with Et_2O. The combined organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (hexane/EtOAc, 8:2) afforded 49 mg (86%) of methoxy lactone **8**: white solid; IR (KBr) ν 1777 (C=O, lactone), 1609, 1580 and 1499 (C=C, aromatic ring); ^{1}H NMR ($CDCl_3$) δ 0.97 (s, 3H, CH_3-18), 2.86 (m, 2H, CH_2-6), 3.78 (s, 3H, CH_3O), 6.63 (d, J = 2.6 Hz, 1H, CH-4), 6.72 (dd, J_1 = 2.7 Hz, J_2 = 8.6 Hz, 1H, CH-2), 7.20 (d, J = 8.6 Hz, 1H, CH-1); ^{13}C NMR ($CDCl_3$) δ 14.51 (C-18), 22.61 (C-15), 26.02 (C-11), 27.21 (C-7), 29.36 (C-21), 29.70 (C-6), 31.32 (C-20), 31.91 (C-12), 35.63 (C-16), 39.04 (C-8), 43.54 (C-9), 45.99 (C-13), 48.78 (C-14), 55.17 (CH_3O), 96.02 (C-17), 111.54 (C-2), 113.83 (C-4), 126.27 (C-1), 131.95 (C-10), 137.78 (C-5), 157.54 (C-3), 176.78 (C=O, lactone); EIMS calcd for $C_{22}H_{28}O_3$ (M^+) 340.2039, found 340.2026. Anal. ($C_{22}H_{28}O_3$) C, H.

3-(Benzyloxy)-19-nor-17α-pregna-1,3,5(10)-triene 21,17-Carbolactone (9). To 33 mg (0.10 mmol) of lactone **7** in 10 mL of a mixture of chloroform−methanol (2:1) were added 77 mg (0.55 mmol) of K_2CO_3 and 20 μL (0.16 mmol) of benzyl bromide (BnBr), and the mixture was refluxed for 4 h. Then solvents were evaporated, and water was added. The aqueous phase was extracted with EtOAc, and the combined organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification of the crude residue by flash chromatography afforded 36 mg (86%) of benzyloxy lactone **9**: white solid; IR (KBr) ν 1767 (C=O, lactone), 1612, 1577 and 1500 (C=C, aromatic ring); ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 3H, CH_3-18), 2.84 (m, 2H, CH_2-6), 5.04 (s, 2H, OCH_2Ph), 6.72 (d, J = 2.9 Hz, 1H, CH-4), 6.79 (dd, J_1 = 2.9 Hz, J_2 = 8.8 Hz, 1H, CH-2), 7.20 (d, J = 8.5 Hz, 1H, CH-1), 7.32−7.44 (m, 5H, OCH_2Ph); ^{13}C NMR ($CDCl_3$) δ 14.55 (C-18), 22.66 (C-15), 26.05 (C-11), 27.25 (C-7), 29.41 (C-21), 29.73 (C-6), 31.36 (C-20), 31.95 (C-12), 35.68 (C-16), 39.06 (C-8), 43.60 (C-9), 46.04 (C-13), 48.82 (C-14), 69.97 (OCH_2Ph), 96.06 (C-17), 112.41 (C-2), 114.90 (C-4), 126.34 (C-1), 127.44 (2×) (OCH_2Ph), 127.87 (OCH_2Ph), 128.56 (2×) (OCH_2Ph), 132.30 (C-10), 137.28 (OCH_2Ph), 137.88 (C-5), 156.87 (C-3), 176.84 (C=O, lactone); EIMS calcd for $C_{28}H_{32}O_3$ (M^+) 416.2351, found 416.2367. Anal. ($C_{28}H_{32}O_3$) C, H.

Synthesis of Spiro-γ-lactones 13 and 14 (Scheme 2). 3β-Hydroxy-17α-[3′-[(tetrahydro-2″H-pyranyl)oxy]propynyl]androstane (11). To a solution of 2.9 g (20.7 mmol) of tetrahydro-2-(2-propynyloxy)-2H-pyran in 30 mL of dry THF was added 12.8 mL of n-BuLi (1.6 M), and the reaction mixture was stirred at −78 °C for 20 min. Then a solution of 1.5 g (5.16 mmol) of epiandrosterone (**10**) in 10 mL of dry THF was added dropwise over 15 min, and the mixture was allowed to stir at −78 °C for 10 h. The cooling bath was removed, and 30 mL of a saturated solution of $NaHCO_3$ and 50 mL of brine were added. The aqueous phase was extracted with EtOAc, and the organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude solid was purified by flash chromatography (hexane/EtOAc, 8:2) to give 2.13 g (96% yield) of alkylated compound **11**: white solid; IR (KBr) ν 3335 and 3415 (OH, alcohols), 2215 weak (C≡C); ^{1}H NMR ($CDCl_3$) δ 0.79 and 0.81 (2s, 3H, CH_3-18, CH_3-19), 3.54 (m, 2H, CH_2O of THP), 3.84 (t_{app}, 1H, $CHOH$), 4.31 (s, 2H, CH_2OTHP), 4.83 (s_{app}, 1H, CH of THP); ^{13}C NMR ($CDCl_3$) δ 12.29 (C-19), 12.85 (C-18), 19.14 (THP), 20.27 (C-11), 23.11 (C-15), 25.33 (THP), 28.53 (C-6), 30.28 (THP), 31.42 (C-7), 31.57 (C-2), 32.85 (C-12), 35.50 (C-8), 36.10 (C-10), 37.01 (C-1), 38.09 (C-4), 38.85 (C-16), 44.87 (C-5), 47.02 (C-13), 50.46 (C-14), 54.05 (C-9), 54.39 (CH_2OTHP), 62.14 (CH_2O of THP), 71.19 (C-3), 79.81 (C-17), 81.54 and 89.73 (C≡C), 96.62 (CH of THP); EIMS calcd for $C_{27}H_{42}O_4$ (M^+) 430.3083, found 430.3062.

3-Oxo-17α-pregnane 21,17-Carbolactone (13). To a solution of 1.92 g (4.46 mmol) of alkyne derivative 11 in 35 mL of EtOAc was added 190 mg of a 1:1 mixture of palladium on charcoal (10%) and palladium in calcium carbonate (5%), and the reaction mixture was stirred at room temperature under hydrogen atmosphere. After 10 h, the mixture was filtered through Celite and washed with EtOAc. The solvent was evaporated under reduced pressure. The white solid (one spot by TLC) was used without purification for the next step. At room temperature, the solid was dissolved with 50 mL of methanol, and 6 g of amberlyst 15R acidic resin (Aldrich Chemical Co.) was added. After 6 h, the mixture was filtered and the filtrate evaporated to dryness. The white solid was dissolved in 60 mL of acetone and the solution cooled to 0 °C before the dropwise addition of a 4.8 mL of Jones' reagent (2.7 M) over 10 min. The reaction was complete after 2 h, and workup was done as described above for typical Jones' oxidation. Purification by flash chromatography (hexane/EtOAc, 55:45) yielded 878 mg (57% after three steps) of lactone **13**: white solid; IR (KBr) ν 1773 (C=O, lactone), 1710 (C=O, ketone); ^{1}H NMR ($CDCl_3$) δ 0.94 (s, 3H, CH_3-19), 1.01 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 11.43 (CH_3-19), 14.10 (CH_3-18), 20.81 (C-11), 22.93 (C-15), 28.66 (C-6), 29.30 (C-21), 31.22 (C-7, C-20), 31.83 (C-12), 35.56 (C-16), 35.70 (C-8 and C-10), 38.05 (C-2), 38.54 (C-1), 44.56 (C-4), 45.73 (C-13), 46.65 (C-5), 49.62 (C-14), 53.49 (C-9), 95.97 (C-17), 176.75 (C=O, lactone), 211.58 (C-3); EIMS calcd for $C_{22}H_{32}O_3$ (M^+) 344.2351, found 344.2347. Anal. ($C_{22}H_{32}O_3$) C, H.

3-Oxo-17α-pregna-1,20-diene 21,17-Carbolactone (14). To a solution of 1.75 mL (12.5 mmol) of freshly distilled diisopropylamine in 25 mL of dry THF was added 7.35 mL of n-BuLi (1.6 M), and the mixture was stirred at −10 °C under argon for 50 min. Then a solution of compound **13** (810 mg, 2.35 mmol) in 7 mL of dry THF was added dropwise and stirred for 1.5 h at −10 °C. After cooling at −78 °C, a solution of 2.5 g (10.6 mmol) of phenylselenium bromide in 10 mL of dry THF was added dropwise. The mixture was stirred for 14 h at −78 °C under an atmosphere of argon, after which the reaction mixture was stirred for 10 min at room temperature before adding 50 mL of water. The aqueous phase was extracted with EtOAc and the organic layer washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting yellow paste was dried under vacuum and used without purification for the next step. The crude yellow paste was dissolved in CH_2Cl_2 (40 mL), and 0.64 mL of

H_2O_2 (30%) was added dropwise. The mixture was stirred for 30 min at room temperature. Then water was added and the aqueous phase extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure to give a crude yellow oil. Purification by flash chromatography (hexane/EtOAc, 87:13) yielded 38 mg (4.8% after two steps) of lactone **14**: amorphous white solid; IR (film) ν 1754 (C=O, conjugated lactone), 1674 (C=O, conjugated ketone), 1602 weak (C=C); ^{1}H NMR ($CDCl_3$) δ 1.01 and 1.06 (2s, 6H, CH_3-18, CH_3-19), 5.84 (d, J = 10.2 Hz, 1H, CH-2), 5.94 (d, J = 5.6 Hz, 1H, CH-21), 7.08 (d, J = 10.2 Hz, 1H, CH-1), 7.43 (d, J = 5.6 Hz, 1H, CH-20); ^{13}C NMR ($CDCl_3$) δ 13.00 (CH_3-19), 15.09 (CH_3-18), 20.62 (C-11), 23.55 (C-15), 27.35 (C-6), 30.91 (C-7), 31.30 (C-12), 33.24 (C-16), 35.88 (C-8), 38.94 (C-10), 40.85 (C-4), 44.32 (C-5), 46.91 (C-13), 49.85 (C-14), 51.40 (C-9), 98.46 (C-17), 118.54 (C-21), 127.57 (C-2), 157.58 (C-1), 158.90 (C-20), 172.50 (C=O, conjugated lactone), 199.70 (C-3); EIMS calcd for $C_{22}H_{28}O_3$ (M^+) 340.2039, found 340.2031.

Synthesis of Spiro-γ-lactones 19 and 20 (Scheme 3). The synthesis of the starting material, 3,3-(ethylenedioxy)-5-androsten-17-one (**17**), was performed from testosterone (**15**) according to an already described procedure.[26] In the first step, the carbonyl group was protected by the formation of a 1,3-dioxolane group with migration of a double bond from the 3,4- to 5,6-position. In the second step, the 17β-hydroxyl group was oxidized to the corresponding ketone, compound **17**.

3,3-(Ethylenedioxy)-17β-hydroxy-17α-[3′-(propylenedioxy)propyl]-5-androstene (18). To 60 mL of diethyl ether–pentane (2:3) in anhydrous conditions and under argon at −78 °C was added 2.41 g (9.9 mmol) of 2-(2-iodoethyl)-1,3-dioxane, and the mixture was stirred for 15 min. Then 5.23 mL of a 1.7 M solution of t-BuLi was added dropwise and the mixture allowed to stir for 1 h at −78 °C before adding a solution of 0.7 g (2.1 mmol) of ketone **17** dissolved in 10 mL of dry THF, 20 mL of pentane, and 13 mL of diethyl ether. After 11 h at - 78 °C, the reaction mixture was poured into 20 mL of a 5% $NaHCO_3$ solution followed by the addition of water and extraction of the aqueous phase with EtOAc. Organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and evaporated to give a yellow solid. Purification by flash chromatography (hexane/EtOAc, 8:2 and 7:3) allowed us to obtain 0.5 g (53% yield) of alkylated compound **18**: white solid; IR (KBr) ν 3465 (OH, alcohol); ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3H, CH_3-18), 1.02 (s, 3H, CH_3-19), 3.74, 3.83, and 4.09 (3m, 4H, 2 × CH_2O of 1,3-dioxane), 3.92 (s_{app}, 4H, 2 × CH_2O of 1,3-dioxolane), 4.55 and 5.00 (m, t, J = 4.8 Hz, 1H, CH of 1,3-dioxane), 5.33 (m, 1H, CH-6); ^{13}C NMR ($CDCl_3$) δ 14.30 (C-18), 18.83 (C-19), 20.68 (C-11), 23.80 (C-15), 25.68, 29.58, 30.51, 31.02 (2×), 31.55 (C-7), 32.79, 34.51, 36.29, 36.70 (C-10), 41.73, 46.22 (C-13), 49.59 (C-14), 50.75 (C-9), 64.36 and 64.15 (CH_2O of dioxolane), 66.87 (CH_2O of dioxane), 82.81 (C-17), 102.67 (CH of dioxane), 109.38 (C-3), 121.86 (C-6), 140.11 (C-5); EIMS calcd for $C_{27}H_{42}O_5$ (M^+) 446.3032, found 446.3033.

3,6-Dioxo-17α-pregn-4-ene 21,17-Carbolactone (19). To a solution of compound **18** (480 mg, 1.07 mmol) in acetone (20 mL) was added dropwise 2 mL of a 2.7 M solution of Jones' reagent. After 40 min at 0 °C, 10 mL of isopropyl alcohol was added and organic solvents were removed under reduced pressure. The resulting green solid was dissolved in water, and the aqueous phase was extracted with EtOAc. Combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography (hexane/EtOAc, 8:2) yielded 77 mg (21% yield) of lactone **20** (described later, see Scheme 4) and 148 mg (39% yield) of lactone **19**: light yellow oil; IR (film) ν 3440 (OH, alcohol), 1768 (C=O, lactone), 1682 (C=O, conjugated ketone); ^{1}H NMR ($CDCl_3$) δ 0.95 (s, 3H, CH_3-18), 1.14 (s, 3H, CH_3-19), 6.12 (s, 1H, CH-4); ^{13}C NMR ($CDCl_3$) δ 14.38 (C-18), 17.44 (C-19), 20.22 (C-11), 22.57 (C-15), 29.06 (C-21), 30.95 and 31.13 (C-12, C-20), 33.77 and 34.26 (C-2, C-8), 35.18 (C-1), 35.39 (C-16), 39.50 (C-10), 45.50 (C-13), 45.84 (C-7), 49.85 (C-14), 50.33 (C-9), 95.21 (C-17), 125.61 (C-4), 160.14 (C-5), 176.32 (C=O, lactone), 199.03 (C-3), 201.06 (C-6); EIMS calcd for $C_{22}H_{28}O_4$ (M^+) 356.1988, found 356.1984. Anal. ($C_{22}H_{28}O_4$) H.

Synthesis of Spiro-γ-lactones 20 and 25 (Scheme 4). 3,3-(Ethylenedithio)spiro[4-androstene-17,2′-oxirane] (22). A suspension of 60% sodium hydride (0.83 g, 34.5 mmol) and mineral oil in 39 mL of DMSO was vigorously stirred and heated at −78 °C for 1 h. The cooled mixture was diluted with 55 mL of THF and further cooled to −5 °C. After 5 min of rapid stirring, a solution of trimethylsulfonium iodide (3.17 g, 15.5 mmol) in 27 mL of DMSO was added, and the reaction mixture was stirred for 35 min at −5 °C followed by treatment with a solution of compound **21** (2.0 g, 5.5 mmol) in 22 mL of THF. The mixture was stirred at −5 °C for 2 h and then at room temperature for 20 h. The reaction mixture was poured into cold aqueous NH_4Cl solution and extracted with EtOAc. Combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (hexane/EtOAc, 95:5) yielded 1.66 g (80%) of compound **22**: white solid; mp 168−170 °C (from hexane/CH_2Cl_2, 70:30); IR (KBr) ν 1640 weak (C=C); ^{1}H NMR ($CDCl_3$) δ 0.88 (s, 3H, CH_3-18), 1.01 (s, 3H, CH_3-19), 2.58 and 2.87 (2d, J = 5.1 Hz, 2H, CH_2 of oxirane diastereotopic protons), 3.20−3.40 (m, 4H, SCH_2CH_2S), 5.48 (s, 1H, CH-4); ^{13}C NMR ($CDCl_3$) δ 14.22 (C-18), 18.51 (C-19), 20.62 (C-11), 23.47 (C-15), 28.96, 31.92, 32.08, 33.78, 35.87, 36.65 (C-10), 37.28, 37.98, 39.52 and 40.03 (SCH_2CH_2S), 39.95 (C-13), 52.35 (C-14), 53.54 (CH_2O), 54.16 (C-9), 65.71 (C-3), 70.35 (C-17), 124.27 (C-4), 146.16 (C-5); EIMS calcd for $C_{22}H_{32}OS_2$ (M^+) 376.1894, found 376.1860.

3-(3′,3′-(Ethylenedithio)-17′β-hydroxy-4′-androsten-17′α-yl) propionitrile (23). To a solution of 0.9 mL (6.4 mmol) of diisopropylamine in dry THF (20 mL), at −40 °C and under argon, was added 4.0 mL of n-BuLi (1.6 M) in hexane, and the mixture was stirred for 35 min. A solution of acetonitrile (0.34 mL, 6.4 mmol) in THF (6 mL) was added, and the reaction mixture was stirred at −15 °C for 1 h. A solution of oxirane **22** (0.81 g, 2.15 mmol) in 15 mL of THF was added dropwise over 5 min, and the reaction mixture was stirred at room temperature for 20 h. Then it was poured into 20 mL of saturated NH_4Cl solution and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by flash chromatography (hexane/EtOAc/CH_3CN/acetone, 95:2:0.5:2.5−90:4:1:5 as eluant) to give 725 mg (81% yield) of nitrile derivative **23**: white solid; mp 199−200 °C; IR (KBr) ν 3460 (OH, alcohol), 2246 (C≡N), 1638 (C=C); ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3H, CH_3-18), 1.01 (s, 3H, CH_3-19), 3.30−3.40 (m, 4H, SCH_2CH_2S), 5.47 (s, 1H, CH-4); ^{13}C NMR ($CDCl_3$) δ 12.00 (C-21), 14.12 (C-18), 18.50 (C-19), 20.83 (C-11), 23.46 (C-15), 31.41, 31.88, 32.28, 32.58, 34.61, 36.57, 37.25 (2×), 37.94, 39.50 and 39.97 (SCH_2CH_2S), 46.39 (C-13), 50.06 (C-14), 53.94 (C-9), 65.65 (C-3), 82.51 (C-17), 120.85 (C≡N), 124.38 (C-4), 145.89 (C-5); EIMS calcd for $C_{24}H_{35}ONS_2$ (M^+) 417.2160, found 417.2208.

3,3-(Ethylenedithio)-17α-pregn-4-ene 21,17-Carbolactone (24). To a solution of nitrile **23** (500 mg, 1.2 mmol) in 0.8 mL of methanol was added 0.7 mL of aqueous KOH (6 M), and the mixture was heated to reflux for 4 h. The cooled mixture was acidified with concentrated HCl (0.8 mL) and refluxed for 1.5 h. The solution was diluted with water (20 mL) and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude yellow solid was purified by flash chromatography (hexane/EtOAc, 95:5) to afford 478 mg (95% yield) of lactone **24**: white solid; mp 194−196 °C: IR (KBr) ν 1768 (C=O, lactone), 1642 weak (C=C); ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3H, CH_3-18), 1.00 (s, 3H, CH_3, 19), 3.20−3.40 (m, 4H, SCH_2CH_2S) 5.47 (s, 1H, CH-4); ^{13}C NMR ($CDCl_3$) δ 14.48 (C-12), 18.51 (C-19), 20.62 (C-11), 22.86 (C-15), 29.28 (C-21), 31.16, 31.70, and 31.79 (C-7, C-12, C-20), 32.12, 35.48, 36.02, 36.54 (C-10), 37.25, 37.90, 39.49 and 39.94 (SCH_2CH_2S), 45.57 (C-13), 49.31 (C-14), 53.81 (C-9), 65.61 (C-3), 95.93 (C-17), 124.39 (C-4), 145.83 (C-5), 176.74 (C=O, lactone); EIMS calcd for $C_{24}H_{34}O_2S_2$ (M^+) 418.2000, found 418.2035.

3-Oxo-17α-pregn-4-ene 21,17-Carbolactone (20). To a solution of 350 mg (0.84 mmol) of **24** in 37 mL of acetonitrile were added 9 mL of water, 240 mg (1.8 mmol) of N-chlorosuccinimide, and 321 mg (1.9 mmol) of silver nitrate, and the

yellowish mixture was stirred for 30 min at room temperature. Next the mixture was poured into 20 mL of a saturated solution of Na_2SO_3 and 10 mL of brine. The aquous phase was extracted with CH_2Cl_2, and the organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (hexane/EtOAc, 75:25) yielded 213 mg (76%) of lactone **20**: white solid; IR (film) ν 1770 (C=O, lactone), 1672 (C=O, congugated ketone), 1615 weak (C=C); ^{1}H NMR ($CDCl_3$) δ 0.97 (s, 3H, CH_3-18), 1.19 (s, 3H, CH_3-19), 5.72 (s, 1H, CH-4); ^{13}C NMR ($CDCl_3$) δ 14.43 (C-18), 17.30 (C-19), 20.36 (C-11), 22.79 (C-15), 29.17 (C-21), 31.08, 31.35, and 31.53 (C-7, C-12, C-20), 32.53 (C-6), 33.81 (C-2), 35.39 (C-8), 35.63 (C-1), 35.81 (C-16), 38.46 (C-10), 45.43 (C-13), 49.14 (C-14), 53.34 (C-9), 95.66 (C-17), 123.88 (C-4), 170.48 (C-5), 176.60 (C=O, lactone), 199.21 (C=O, conjugated ketone); EIMS calcd for $C_{22}H_{30}O_3$ 342.2195, found 342.2182. Anal. ($C_{22}H_{30}O_3$) C, H.

3-Oxo-17α-pregna-1,4-diene 21,17-Carbolactone (25). To a solution of lactone **20** (150 mg, 0.44 mmol) in 7 mL of dry toluene was added 170 mg (0.73 mmol) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and the reaction mixture was heated at 100 °C for 5 h under argon atmosphere. Then diethyl ether was added to the cooled solution, and the organic layer was washed with a Na_2CO_3 solution and water and then dried over $MgSO_4$. After evaporation to dryness, the crude product was purified by flash chromatography (hexane/EtOAc, 75:25) to give 104 mg (70% yield) of dienone lactone **25**: pink foam; IR (film) ν 1770 (C=O, lactone), 1662 (C=O, conjugated ketone), 1622 and 1601 weak (C=C); ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 3H, CH_3-18), 1.22 (s, 3H, CH_3-19), 6.04 (d, J = 2.1 Hz, 1H, CH-4), 6.20 (dd, J_1 = 2.1 Hz, J_2 = 10.2 Hz, 1H, CH-2), 7.02 (d, J = 10.2 Hz, 1H, CH-1); ^{13}C NMR ($CDCl_3$) δ 14.57 (C-18), 18.61 (C-19), 22.17 (C-11), 22.99 (C-15), 29.15 (C-21), 31.04, 31.48, and 32.96 (C-7, C-12, C-20), 32.54 (C-6), 35.34 (C-8), 35.74 (C-16), 43.30 (C-10), 45.69 (C-13), 48.86 (C-14), 51.92 (C-9), 95.47 (C-17), 123.92 (C-4), 127.55 (C-2), 155.32 (C-1), 168.47 (C-5), 176.50 (C=O, lactone), 186.07 (C-3); EIMS calcd for $C_{22}H_{28}O_3$ 340.2039, found 340.2035. Anal. ($C_{22}H_{28}O_3$) C, H.

Synthesis of Spiro-γ-lactone 29 (Scheme 5). 3β-[(*tert*-Butyldimethylsilyl)oxy]-17β-hydroxy-17α-allyl-5-androstene (27). To a solution of 2.0 g (6.0 mmol) of dehydroepiandrosterone acetate (**26**) in 20 mL of dry THF was added 13.3 mL of a solution of allylmagnesium bromide (1 M) at 0 °C, and the mixture was stirred for 11 h. Then another 5.0 mL of allylmagnesium bromide solution was added, and the reaction mixture was stirred for 6 h. The mixture was poured into a saturated solution of NH_4Cl, water was added, and the aqueous phase was extracted with EtOAc. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure to provide a white solid, which was used for the next step without further purification. The crude solid was dissolved in 30 mL of DMF, and 2.06 g (30.3 mmol) of imidazole and 1.82 g (1.21 mmol) of *tert*-butyldimethylsilyl chloride were added. The mixture was stirred for 14 h before pouring into ice. The white precipitate was filtered through a buchner funnel, and the solid was purified by flash chromatography (hexane/EtOAc, 9:1) to yield 2.42 g (89%, two steps) of compound **27**: white solid; IR (KBr) ν 3260 (OH, alcohol), 1638 weak (C=C); ^{1}H NMR ($CDCl_3$) δ 0.06 (s, 6H, $Si(CH_3)_2$), 0.89 (s, 9H, $SiC(CH_3)_3$), 0.90 (s, 3H, CH_3-18), 1.02 (s, 3H, CH_3-19), 3.48 (m, 1H, CH-3), 5.18 (m, 2H, CH=CH_2), 5.31 (d, J = 5.1 Hz, 1H, CH-6), 6.00 (m, 1H, CH=CH_2); ^{13}C NMR ($CDCl_3$) δ −4.57 ($Si(CH_3)_2$), 14.25 (C-18), 18.23 ($SiC(CH_3)_3$), 19.43 (C-19), 20.72 (C-11), 23.87 (C-15), 25.93 ($SiC(CH_3)_3$), 31.73, 31.79, 32.07, 32.79, 35.01, 36.69 (C-10), 37.44, 41.78, 42.80 (C-1′), 46.00 (C-13), 50.23 (C-14), 51.03 (C-9), 72.57 (C-3), 82.45 (C-17), 119.09 (C-3′), 120.83 (C-6), 134.90 (C-2′), 141.65 (C-5).

3β-[(*tert*-Butyldimethylsilyl)oxy]-6α,β,17β-dihydroxy-17α-(3′-hydroxypropyl)androstane (28). To a solution of 0.61 g (1.36 mmol) of compound **27** in 20 mL of dry THF was added 3.0 mL of BH_3 (1.0 M) dropwise was added at 0 °C, and the mixture was stirred for 3 h. Then 1.2 mL of NaOH (3 N) and 0.45 mL of H_2O_2 (30%) were added at 0 °C, and the mixture was stirred at reflux for 1.5 h. The mixture was poured into 10 mL of HCl (10%) and 10 mL of H_2O. The aqueous phase was extracted with EtOAc, and the organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography (hexane/EtOAc/methanol, 4:5.8:0.2) afforded 0.63 g (96% yield) of triol **28**: white solid; mp 209−211 °C; IR (KBr) ν 3325 (OH, alcohols); ^{1}H NMR (CD_3OD) δ 0.07 (s, 6H, $Si(CH_3)_2$), 0.85 (s, 3H, CH_3-18), 0.90 (s, 9H, $SiC(CH_3)_3$), 3.30 (m, 1H, CH-6), 3.57 (m, 3H, CH_2OH, CH-3); ^{13}C NMR (CD_3OD) δ −4.42 ($Si(CH_3)_2$), 13.84 (C-19), 15.16 (C-18), 19.02 ($SiC(CH_3)_3$), 22.01 (C-11), 24.70 (C-15), 26.41 ($SiC(CH_3)_3$), 27.97 (C-2′), 32.73, 32.82, 33.78, 34.05, 34.24, 36.57 (C-8), 37.38 (C-10), 38.73, 42.42, ~49 (C-13, under solvent peaks), 51.87, 53.02, and 55.37 (C-5, C-9, C-14), 63.83 (C-3′), 69.96 (C-6), 73.70 (C-3), 84.02 (C-17).

3,6-Dioxo-17α-pregnane 21,17-Carbolactone (29). To a solution of triol **28** (500 mg, 1.0 mmol) in dry THF was added 1.34 mL (1.3 mmol) of tetrabutylammonium fluoride (1.0 M) at 0 °C, and the mixture was stirred for 2 h. Then the THF was removed under reduced pressure and acetone (40 mL) was added followed by the dropwise addition of 1.9 mL of Jones' reagent (2.7 M). The reaction mixture was allowed to stir for 3 h at 0 °C. Then isopropyl alcohol was added dropwise until a persistent green color remained, and the solvent was removed under reduced pressure. Water and EtOAc were added, and the aqueous phase was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (hexane/EtOAc, 5:5) afforded 239 mg (65% yield, two steps) of lactone **29**: colorless crystals; mp 183−185 °C; IR (KBr) ν 1770 (C=O, lactone), 1706 (C=O, ketone); ^{1}H NMR ($CDCl_3$) δ 0.94 and 0.95 (2s, 6H, CH_3-18, 19); ^{13}C NMR ($CDCl_3$) δ 12.50 (C-19), 14.54 (C-18), 21.00 (C-11), 22.65 (C-15), 29.14 (C-21), 31.10 (C-20), 31.40 (C-12), 35.30 (C-16), 36.83 (C-4), 37.15 and 38.01 (2×) (C-1, C-2, C-8), 40.99 (C-10), 45.81 (C-7), 46.02 (C-13), 49.95 (C-14), 52.99 (C-9), 57.38 (C-5), 95.33 (C-17), 176.38 (C=O, lactone), 207.98 (C-3), 210.68 (C-6); EIMS calcd for $C_{22}H_{30}O_4$ (M^+) 358.2144, found 358.2152. Anal. ($C_{22}H_{28}O_3$) C, H.

Synthesis of Dilactone 33 (Scheme 6). 3β,17β-Dihydroxy-3α,17α-bis[3′-[(tetrahydro-2″*H*-pyranyl)oxy]propynyl]androstane (31). To a mixture of 1.5 g (10.75 mmol) of tetrahydro-2-(2-propynyloxy)-2*H*-pyran in 25 mL of dry THF was added 6.72 mL of *n*-BuLi (1.6 M) at 0 °C, and the reaction mixture was stirred for 40 min. Then the mixture was cooled to −78 °C, and a solution of androstane-3,17-dione (**30**) (620 mg, 2.15 mmol) in 10 mL of THF was added dropwise over 15 min. The mixture was then allowed to stir for 11 h at −78 °C. The cooling bath was removed, and 5 mL of a saturated solution of $NaHCO_3$, 10 mL of a saturated solution of NH_4Cl, and 20 mL of H_2O were poured into the mixture. The aqueous phase was extracted with EtOAc, and the organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (hexane/EtOAc, 8:2) of the crude oil gave 0.83 g (68% yield) of dialkylated compound **31**: white foam; IR (film) ν 3415 (OH, alcohols); ^{1}H NMR ($CDCl_3$) δ 0.81 and 0.82 (2s, 6H, CH_3-18, 19), 3.55 and 3.85 (2m, 4H, 2 × CH_2O of THP), 4.31 and 4.32 (2s, 4H, 2 × C≡CCH_2OTHP), 4.86 (s_{app}, 2H, 2 × CH of THP); ^{13}C NMR ($CDCl_3$) δ 11.77 (C-19), 12.63 (C-18), 18.79 (2×) (THP), 20.6 (C-11), 22.87 (C-15), 25.06 (2×) (THP), 27.85 (C-6), 29.96 (2×) (THP), 31.23 (C-7), 32.56, 35.33, 35.67, 35.82, 35.96, 38.58, 41.90, 43.55, 46.74, 50.14 (C-14), 53.56 (C-9), 54.06 (2×) (CH_2OTHP), 61.68 (2×) (CH_2O of THP), 69.09 (C-3), 79.27 (C-17), 80.93 (2×) and 89.79 (2×) (C≡C), 96.20 (2×) (CH of THP).

3α,3β-(1-Oxo-1,3-propanediyloxy)-17α-pregnane 21,17β-Carbolactone (33 or Dilactone 33). To a solution of 3.67 g (6.41 mmol) of compound **31** in 200 mL of EtOAc was added 200 mg of palladium on charcoal (10%), and the mixture was stirred at room temperature with hydrogen atmosphere for 16 h. Then the mixture was filtered through Celite, washed with CH_2Cl_2 and EtOAc, and evaporated under reduced pressure to give a white foam, which was used for the next step (deprotection of THP groups). Without purification, the white foam was dissolved in methanol (200 mL), *p*-TSA (300 mg) was added, and the mixture was stirred at room temperature for

3 h, after which water was added and methanol evaporated under reduced pressure. The white solid was filtered and dried under vacuum pump for 18 h. The crude solid was dissolved in 400 mL of acetone and the solution cooled to 0 °C before the dropwise addition of 9.0 mL of Jones' reagent (2.7 M). After 2 h, isopropyl alcohol was added, and the mixture was evaporated to dryness. Water (200 mL) was added, and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give a crude white foam. Purification by flash chromatography (hexane/EtOAc, 50:50) yielded 1.67 g (65%, three steps) of dilactone **33**: white solid; IR (KBr) ν 1780 (C=O, large band including both lactones); ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3H, CH_3-19), 0.93 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 11.65 (C-19), 14.55 (C-18), 20.51 (C-11), 22.81 (C-15), 28.18 (C-6), 28.45 (C-2'), 29.24 (C-21), 31.15 (C-1', C-20), 31.32 (C-7), 31.76 (C-12), 32.19 (C-2), 35.40 (C-16), 35.46 (C-1, C-10), 35.63 (C-8), 39.2 (C-4), 43.23 (C-5), 45.64 (C-13), 49.65 (C-14), 53.85 (C-9), 87.16 (C-3), 95.93 (C-17), 176.36 and 176.69 (C=O, two lactones); EIMS calcd for $C_{25}H_{36}O_4$ 400.2614, found 400.2615. Anal. ($C_{25}H_{36}O_4$) C, H.

Inhibition of Microsomal 17β-HSD. Preparation of Enzymatic Source (Microsomes of human placenta). Human placenta, with the membranes cleared out, was homogenized with two portions of buffer (KH_2PO_4 (50 mM), glycerol (40%), EDTA (1 mM) at pH 7.4). After 15 min of 1000*g* centrifugation, the supernatant was collected and submitted for 30 min to 10000*g* centrifugation to separate the mitochondria. The supernatant was cleaned by a 100000*g* centrifugation for 60 min to retrieve microsomes. The latter was dissolved in the buffer and submitted to 100000*g* centrifugation. This operation was repeated until no more hemoglobin remained in the solution. Then the concentrated microsomes were dissolved in the same phosphate-based buffer (as above) and stored at −80 °C.

Enzymatic Assay. Tritiated 4-androstenedione ([^{3}H]-Δ^4-dione) was used as the enzyme substrate, and the amount of tritiated testosterone ([^{3}H]T) formed as well as [^{3}H]-4-androstenedione remaining was evaluated by counting radioactivity. We first prepared a stock solution containing [^{3}H]-4-androstenedione (3−5 nM), NADH (1 mM), and the phosphate-based buffer (glycerol (20%), KH_2PO_4 (50 mM), EDTA (1 mM) at pH 7.4). For the assay, 890 μL of a stock solution and 10 μL of an ethanolic solution of test compound (spiro-γ-lactones or unlabeled 4-androstenedione) were added in a tube. The reaction was started by adding 100 μL of a solution containing the microsome preparation of 17β-HSD (2.7 mg of protein/mL) and the aromatase inhibitor (100 μM). The mixture was incubated for 1 h at 37 °C, and the reaction was stopped by adding a solution of unlabeled 4-androstenedione and T before extraction with diethyl ether and evaporation of organic solvent. The residue was dissolved with CH_2Cl_2 so as to be spotted on a silica gel plate (TLC, 20 × 20 cm × 0.2 mm Kieselgel 60 F_{254}) and eluted with CH_2Cl_2/EtOAc, 90:10. Less polar 4-androstenedione and more polar T were identified on TLC as two rows of visible spots under UV light. Each spot on the plate was cut and stored in a vial with 1 mL of ethanol and 10 mL of scintillating solution. Radioactivity was measured in β-counter. The percent of transformation and the percent of inhibition were calculated from eqs 1 and 2, respectively:

$$\% \text{ transformation} = \frac{[^3\text{H}]\text{T (cpm)}}{[^3\text{H}]\text{T (cpm)} + [^3\text{H}]\text{-}\Delta^4\text{-dione (cpm)}} \times 100 \quad (1)$$

$$\% \text{ inhibition} = \frac{\% \text{ transf (without inhibitor)} - \% \text{ transf (with inhibitor)}}{\% \text{ transf (without inhibitor)}} \times 100 \quad (2)$$

When several concentrations of an inhibitor were used in the enzymatic assay, an inhibition curve was plotted using the percent of transformation versus the concentration of inhibitor. From this inhibition curve, the IC_{50} value (the concentration of inhibitor that provokes 50% of enzymatic inhibition) was calculated by computer (DE_{50} program, CHUL Research Center, Québec, Canada).

Inactivation of Microsomal 17β-HSD by Spiro-γ-lactone 7. A solution containing the microsomal preparation of enzyme (24.3 mg of protein/mL), NADH (100 μM), and ethanol (control) or ethanolic solution of spiro-γ-lactone **7** (test compound, final concentration of 1 or 5 μM) was prepared and used for the inactivation test. Then the tubes containing 100 μL of this stock solution were incubated at 37 °C. At specific intervals, the sample tubes (duplicate) were diluted 20-fold with 1.9 mL of phosphate buffer (as above) solution containing an aromatase inhibitor (10 μM) and [^{3}H]-4-androstenedione (5 mM), and an enzymatic assay was performed as described in the previous section. The data were plotted according to Kitz and Wilson.[37]

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