# Structure−Activity Relationships of a New Family of Steroidal Aromatase Inhibitors. 1. Synthesis and Evaluation of a Series of Analogs Related to 19-[(Methylthio)methyl]androstenedione (RU54115)

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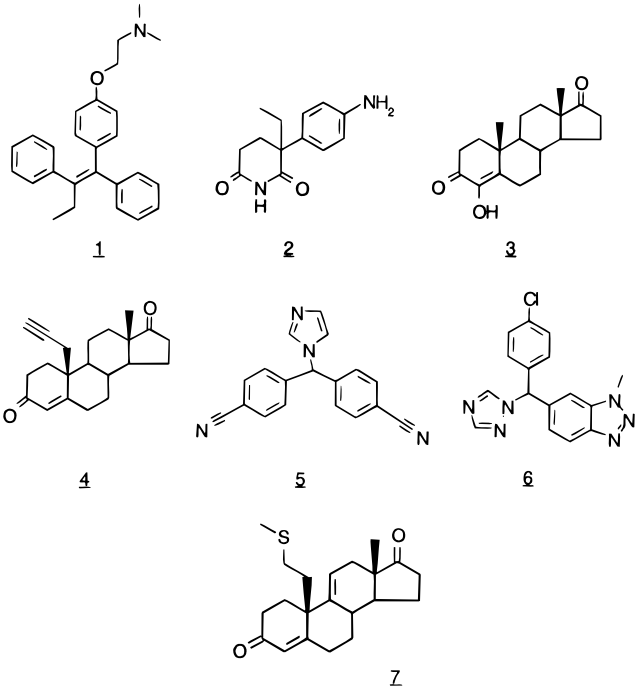
During the course of a study aimed at the search for new potent aromatase inhibitors, several new androstenedione analogs were synthesized and evaluated. This study led to the discovery of 19-[(methylthio)methyl]androsta-4,9(11)-diene-3,17-dione (**7**; RU54115) already described by our laboratory. The object of the present series of papers is to disclose the result of the structure−activity relationship studies that gave rise to this compound. This first part deals mainly with the substitution in the 19-position of the steroid nucleus. Several parameters were varied, the length of the chain and its rigidity and branching, as well as the nature of the heteroatom itself and its substitution. The interaction of these new compounds with human placental aromatase in competition with the substrate androstenedione was studied by difference visible spectroscopy. The *in vivo* aromatase-inhibiting activities were evaluated by measuring the estradiol lowering after oral administration of the compounds to PMSG-primed female rats.

## Introduction

Aromatase is a cytochrome P450 enzyme involved in the biosynthesis of estrogens from androgen precursors. The interest of inhibiting this enzyme in the treatment of advanced breast cancer is well demonstrated today. Two aromatase inhibitors are presently on the market and have proven to be as efficient as tamoxifen (**1**) or adrenalectomy in the treatment of metastatic postmenopausal breast cancer.[1] The first one, aminoglutethimide **2**,[2] however, lacks selectivity due to its inhibition of various other cytochrome P450 enzymes involved in the biosynthesis of steroid hormones and must be administered along with replacement corticosteroid. The second one, 4-hydroxyandrostenedione **3**,[3] is selective, but it must be given by intramuscular injection because of poor oral bioavailability. Many research groups are actively involved in the search for more active and selective aromatase inhibitors. This has resulted in several compounds, either steroidal such as **4**[4] or nonsteroidal such as **5**[5] and **6**,[6] some of them displaying very good kinetics and selectivities of inhibition. We now wish to report our own results in this area.

During an investigation aimed at the search for potent aromatase inhibitors, we came across a new series of thio-substituted androstenedione analogs, one of them, RU54115 (**7**), has already been disclosed.[7] This compound is an excellent tight-binding aromatase inhibitor.[7] The object of the present paper is to present the chemistry, biochemistry, and structure−activity relationships of a series of new inhibitors related to **7**.

In this first part we will describe the synthesis as well as the *in vitro* and *in vivo* evaluation of closely related analogs differing by the substitution on the sulfur atom, the length, the branching and rigidity of the chain in the 10-position, and the importance of the nature of the heteroatom itself.



## Chemistry

19-Carbethoxyandrostenedione **8**[8] was protected as 3,17-dioxolane **9** and then reduced to the alcohol **10** (Scheme 1). From there, several routes were developed to various 19-thiomethyl-substituted analogs of androstenedione. In a first route (Scheme 1, method A), the mesylate **11** of the alcohol **10** was substituted by several thiolates to afford after deprotection compounds **7** and **12**−**15** (Table 1). The alcohol function of **15** was further elaborated into a chloride (**16**) or a thiol (**17**) using standard procedures (see the experimental part).

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**Scheme 1**

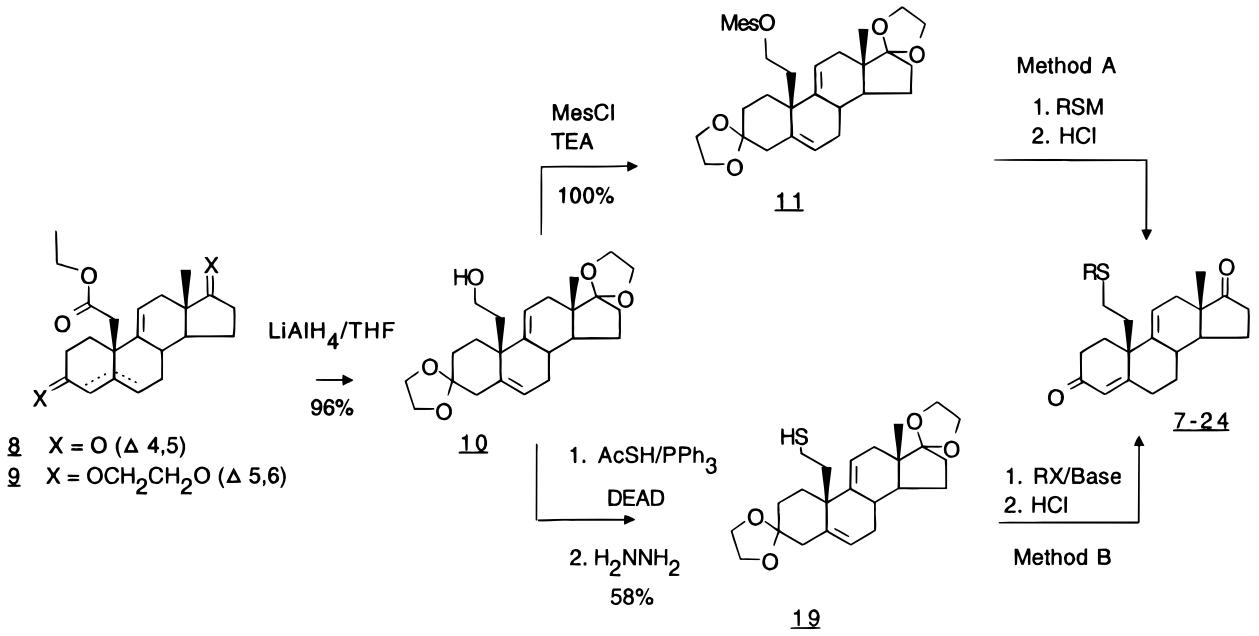
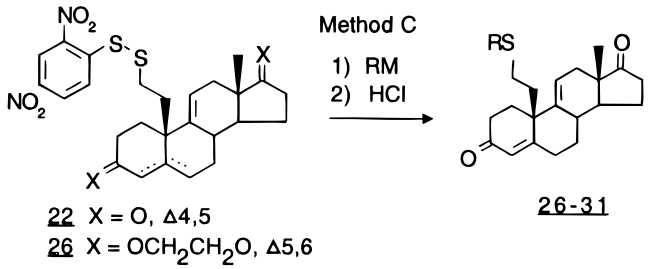



**Table 1.** Synthesis of 19-Thiomethyl-Substituted 3,17-Dioxoandrosta-9,(11)-diene Analogs

| compd | R | yield (%) | method | compd | R | yield (%) | method |
|---|---|---|---|---|---|---|---|
| **7** | Me | 75 | A | **22** | $CH_2C{\equiv}CH$ | 38 | B |
| **12** | Et | 54 | A | **23** | $CH_2SMe$ | 69 | B |
| **13** | Ac | 34 | A | **24** | $CH_2CH{=}CH_2$ | 70 | B |
| **14** | Ph | 52 | A | **26** | $CH{=}CH_2$ | 22 | C |
| **15** | $CH_2CH_2OH$ | 63 | A | **27** | SMe | 31 | C |
| **16** | $CH_2CH_2Cl$ | 27[a] | | **28** | SPh | 23 | C |
| **17** | $CH_2CH_2SH$ | 7[a] | | **29** | cPr | 20 | C |
| **20** | $CHF_2$ | 36 | B | **30** | C≡CTMS | 42 | C |
| **21** | $SPh(NO_2)_2$ | 81 | B | **31** | C≡CH | 65[b] | C |

[a] From **15** (see the Experimental Section). [b] From **30** (see the Experimental Section).

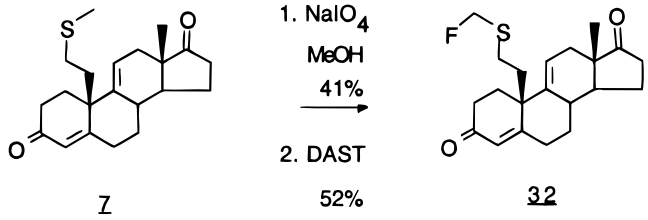
**Scheme 2**




In the cases where the thiolates were difficult or unfeasible to prepare, another way of access to the 19-thiomethyl analogs was devised (Scheme 1, method B). The free thiol **19** was obtained directly from alcohol **10** by Mitsunobu[9] reaction with thioacetic acid followed by hydrazinolysis. This thiol could then be alkylated with various alkyl halides or alkyl and aryl disulfides, giving rise to compounds **20**−**24** (Table 1).

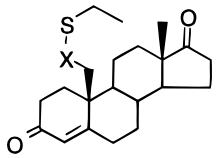
For the cases where neither the thiolates nor the desired alkyl halides were available or easy to prepare, a third way of access was studied (Scheme 2, method C).[10] Disulfide **21** or **25** was condensed with several organometallic reagents to afford after hydrolysis compounds **26**−**30** (Table 1); removal of the trimethylsilyl group from **30** using tetrabutylammonium fluoride gave the free ethynyl compound **31**. Finally, the monofluoromethyl analog **32** could best be obtained from reaction

**Scheme 3**




of the sulfoxide of **7** with DAST by the method of McCarthy et al.[11] (Scheme 3).

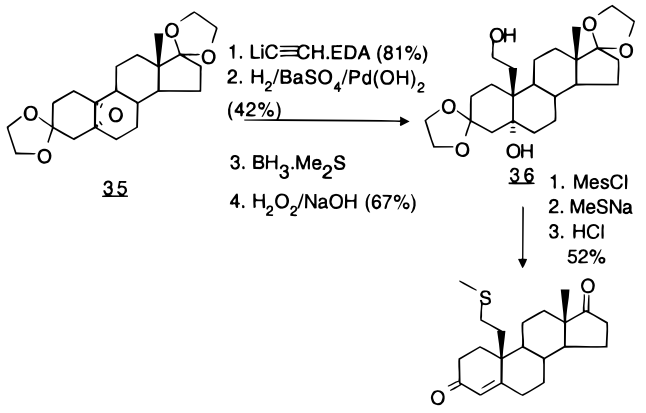
At the time we were studying these series, Organon was developing the 19-[(ethylthio)thio]androstenedione **33**,[12] and the 9,11-saturated analog of **7**, **34**, was included in the general formula of the patent[13] covering this series without any mention of its activity. From



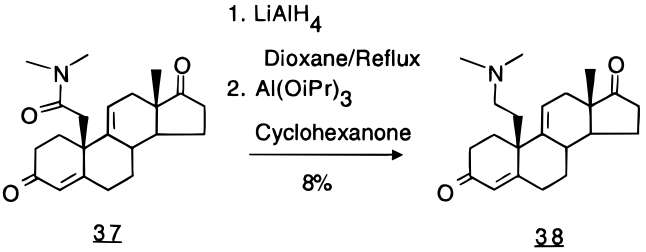

our previous experience with steroids and aromatase inhibition, we have seen little difference between analogs incorporating or not incorporating the $\Delta^{9,11}$-bond.[14] We nevertheless decided to include this compound in our tests and undertook its synthesis using a modified procedure. The epoxide **35**[15] was opened in the 10-position with lithium acetylide.[16] The triple bond was then hydrogenated, and the resulting olefin was hydroborated to afford after hydrogen peroxide oxidation the alcohol **36**. Subsequent mesylation of the alcohol and displacement with sodium thiomethoxide gave the expected compound **34** after acidic hydrolysis (Scheme 4).

Analogs of **7** where the sulfur was replaced by other heteroatoms were synthesized as follows: The *N*-dimethylamino analog **38** was obtained from amide **37**[8]

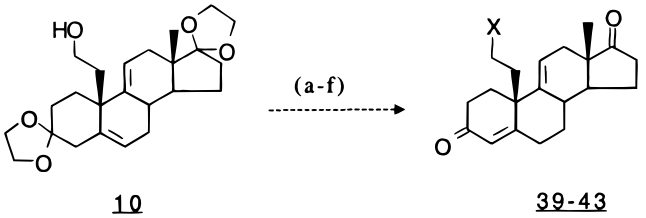
**Scheme 4**




**Scheme 5**




**Scheme 6**[a]



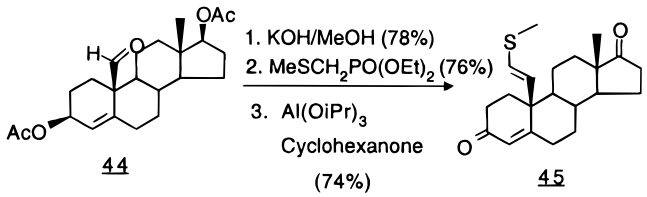

| Cpd | X | Reagents | Yield (%) |
|---|---|---|---|
| 39 | OMe | (a,b) | 79 |
| 40 | OMs | (c,b) | 61 |
| 41 | SePh | (c,d,b) | 14 |
| 42 | I | (c,b,c) | 82 |
| 43 | SCN | (c,b,e,f) | 50 |

[a] (a) tBuOK/THF, MeI; (b) 6 N HCl/EtOH; (c) MesCl/TEA/$CH_2Cl_2$; (d) PhSeH/Na/HMPA; (e) NaI/MEK; (f) KSCN/HMPA.

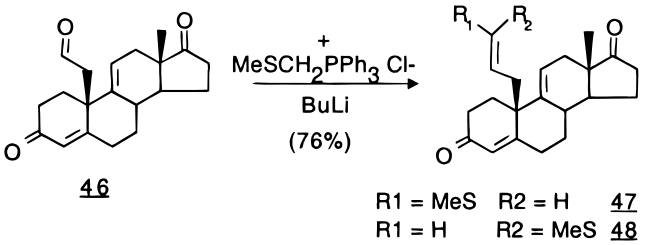
after lithium aluminum hydride reduction and reoxidation of the 3,17-ketones (Scheme 5). The oxygenated analogs **39** and **40**, the seleno compound **41**, the iodo compound **42**, and the thiocyanato compound **43** were synthesized from alcohol **10** using standard methods (Scheme 6).

In order to better ascertain the optimal position of the heteroatom in the enzymatic cavity, the sulfur was also introduced within various rigidified analogs. Thus the unsaturated analogs **45**, **47**, and **48** were obtained as shown in Schemes 7 and 8. Aldehyde **44**[17] was reacted with [(methylthio)methyl]phosphonate and subsequently oxidized to afford **45** (Scheme 7). Aldehyde **46**[8] was reacted similarly with [(methylthio)methyl]-triphenylphosphonium chloride to afford a 1:1 mixture of the *E* and *Z* isomers that were separated by chromatography (Scheme 8). The protected form (**49**) of the aldehyde **46** was obtained from alcohol **10** after Swern oxidation and used for the synthesis of the thioacetals

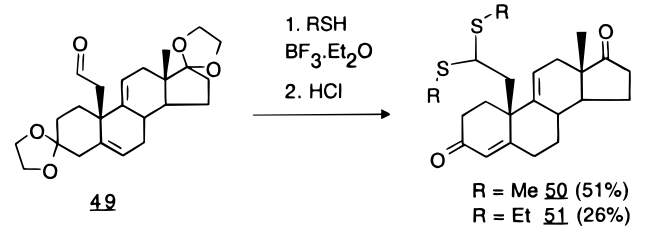
**Scheme 7**



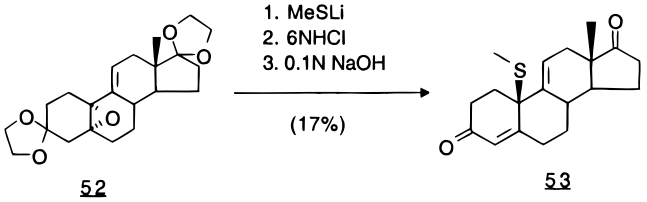

**Scheme 8**



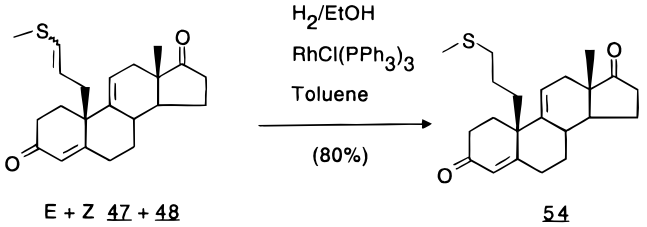

**Scheme 9**




**Scheme 10**




**Scheme 11**



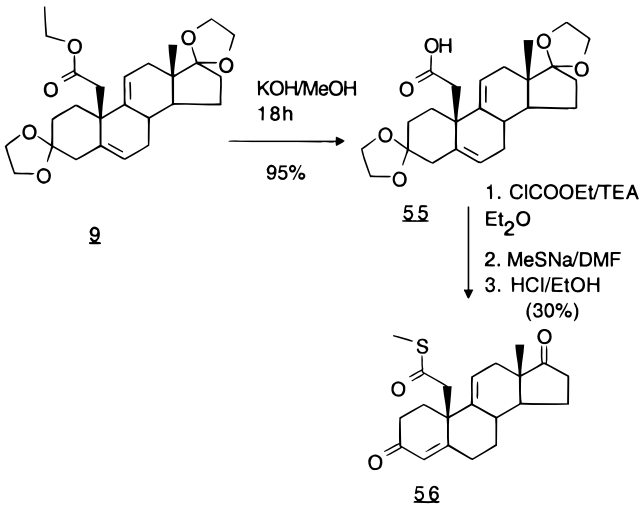

**50** and **51** by treatment with methane- or ethanethiol in the presence of boron trifluoride and deprotection of the dioxolanes (Scheme 9).

Analogs of **7** varying by the number of carbon atoms in the 10-position chain were synthesized as follows: Opening of epoxide **52**[18] with sodium thiomethoxide gave **53** after deprotection of the dimethoxyacetal and elimination of the 5α-hydroxy group (Scheme 10). The compound **54** incorporating three carbon atoms in the 10-position was obtained by reduction of a mixture of the unsaturated analogs **47** and **48** (Scheme 11). Finally, thioester **56** was obtained from **9** after ester hydrolysis to afford acid **55**, followed by chloroformate activation of the acid and reaction with sodium thiomethoxide (Scheme 12).

## Biochemistry and Pharmacology

The *in vitro* potency of the new compounds as aromatase inhibitors was assessed using human placental

**Scheme 12**

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microsomes as described previously.[19] Aromatization was followed by measuring the formation of tritiated water from [1$\beta$,2$\beta$-$^3$H]androstenedione in the presence of increasing concentrations of compounds.[20] $IC_{50}$ values of inhibition were calculated.

Spectral studies were performed with preparations of partially purified aromatase[21] from human placental microsomes. $K_s$ values of interaction with aromatase, a cytochrome P450 enzyme, were determined for some of the compounds.[22]

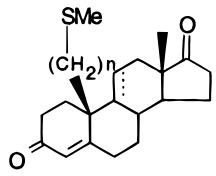
The best compounds were studied *in vivo* on the lowering of circulating estradiol in the PMSG-primed female rat.[8] For the compounds that displayed the best values of *in vitro* and *in vivo* inhibition, kinetics of inhibition were analyzed. They will be the subject of a separate paper.

## Structure−Activity Relationships

The first thing we investigated was the optimal side chain length in the 10-position for the inhibition by the thio-substituted androstenedione analogs. Table 2 summarizes the $IC_{50}$'s of a series of analogs varying by the length of the chain in the 10-position. For comparative purposes, we have included compound **57**. This compound is an analog of **7** incorporating only one carbon atom in the chain.[23] The best $IC_{50}$ values were obtained for compounds with one or two carbons in the chain regardless of the presence of the 9,11-bond (Table 2, entries 2−5), while compounds with the sulfur directly branched on the steroid skeleton (entry 1) or with three carbon atoms in the chain (entry 6) had lost all affinity for the enzyme.

At the time we started working in this project, one of the tightest aromatase inhibitors reported was the 19-(methylthio)androstenedione **57** (entry 2) described by Akthar.[23] This compound acted as a very strong inhibitor of placental aromatase with a $K_i$ of 1 nM. Moreover it was found to interact with partially purified aromatase with appearance of a difference visible spectrum characterized by a Soret peak at 430 nm.[23] This so-called "type II" difference spectrum of cytochrome P450 is generally observed upon binding of readily accessible nitrogen or sulfur of organic compounds to cytochrome P450 Fe(III) (Figure 1). Starting from this observation, Akhtar et al. were able to determine the dissociation constant, $K_s$, of **57** from aromatase. This $K_s$ was deduced from a spectral titration of aromatase in the presence of its substrate, androstenedione, upon addition of increasing concentrations of the inhibitor and found to be 42 nM.[23]

**Table 2.** *In Vitro* and *in Vivo* Aromatase-Inhibiting Activities of Analogs of RU54115 Differing by the Length of the Chain in Position 10[a,b]




| entry | compd | *n* | $\Delta^{9,11}$ | $IC_{50}$ ($\times 10^{-7}$ M) | E2 lowering[c] 2 h | E2 lowering[c] 6 h | $K_s$ (nM)[d] | ref |
|---|---|---|---|---|---|---|---|---|
| 1 | **53** | 0 | yes | 74 | nd | nd | nd | *e* |
| 2 | **57** | 1 | no | 0.42 | −37** | −49** | 62 ± 16 | 13[f] |
| 3 | **7** | 2 | yes | 0.12 | −51** | −51** | 15 ± 3 | *e* |
| 4 | **34** | 2 | no | 0.22 | −11* | −16(NS) | 44 ± 8 | 22[f] |
| 5 | **12** | 2 | yes | 8.7 | nd | nd | nd | *e* |
| 6 | **54** | 3 | yes | >100 | nd | nd | nd | *e* |

[a] Abbreviations and symbols: nd = not determined; ** = $p <$ 0.01 (Student's *t*-test); * = $p < 0.05$; NS = nonstatistically significant. [b] In the same conditions, the $IC_{50}$'s of inhibition of the reference compounds **3**[3] and **4**[4] were found to be $3.7 \times 10^{-7}$ and $4.5 \times 10^{-7}$ M, respectively. [c] Lowering of circulating estradiol after an oral dose of 5 mg/kg at times 2 and 6 h after treatment expressed as percent of control (see the Experimental Section). [d] $K_s = K_{sapp}K_m/[AD]K_m$.[21] [e] This work. [f] The compounds have been described, but the data reported in this table are from this work.

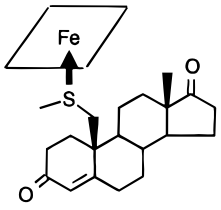



**Figure 1.** Interaction of **57** with the heme iron of aromatase.[22]

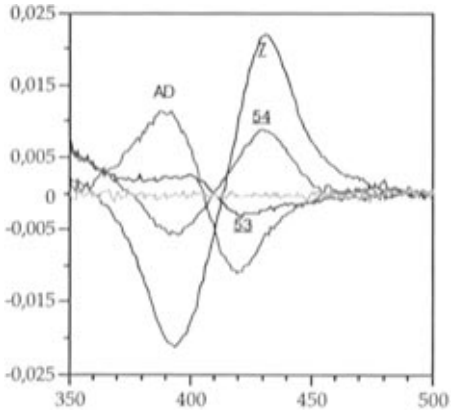



**Figure 2.** Difference visible spectra obtained upon interaction of androstenedione (AD) (100 $\mu$M) and the inhibitors **7** (5 $\mu$M) and **53** and **54** (100 $\mu$M) with partially purified aromatase from human placenta.

Addition of androstenedione to partially purified aromatase led to a difference spectrum, called type I spectrum, characterized by a peak at 390 nm and a trough at 420 nm (Figure 2). This difference spectrum corresponds to a transition from hexacoordinate low-spin aromatase Fe(III) to pentacoordinate high-spin aromatase Fe(III) after binding of androstenedione to a protein site close to the heme which should lead to

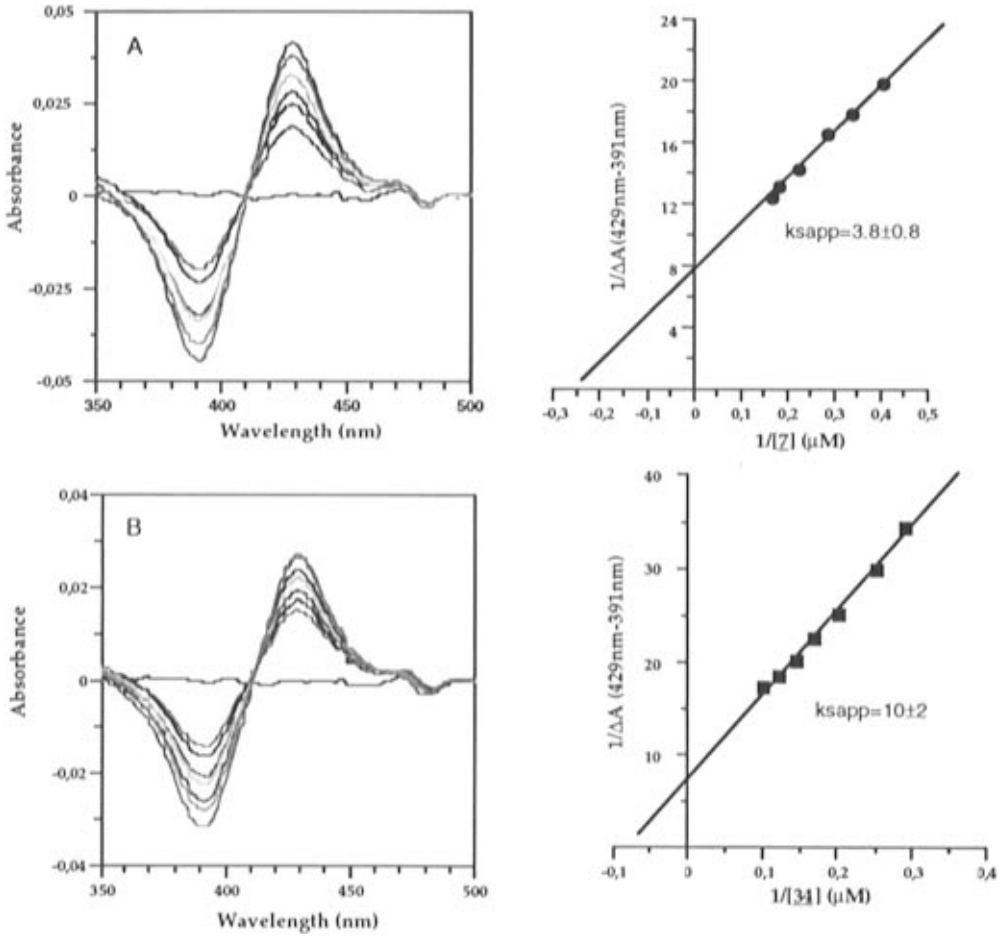


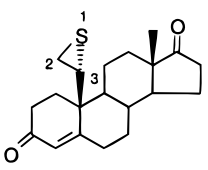

**Figure 3.** Spectral titrations curves of aromatase by **7** (A) and **34** (B) (range concentrations 2.6−6 $\mu$M). Concentrations of androstenedione (AD) and aromatase are 20 and 0.68 $\mu$M, respectively.

the removal of the $H_2O$ iron ligand. Then, addition of thioether **57**, **7**, **34**, or **54** to the aromatase−substrate complex led to the disappearance of the 390 nm spectrum and its replacement by a type II difference spectrum characterized by a peak around 430 nm and a trough around 392 nm (Figure 2), corresponding to the binding of their sulfur atom to aromatase Fe(III). Compound **53** led to a different spectral change with the appearance of a weak spectrum characterized by a flat peak around 400 nm and a trough at 420 nm, which should correspond to an interaction with the substrate-binding site without binding of the sulfur atom of **53** to iron.

Titration curves obtained upon addition of increasing concentrations of inhibitors to the aromatase−androstenedione complex (Figure 3) allowed us to calculate the $K_s$ of these inhibitors (for the method used, see ref 23). $K_s$ values obtained in our conditions were 62 nM for **57**, 15 nM for **7**, and 44 nM for its saturated analog **34** (Figure 3).

In their studies of the interaction of thiol-containing androgens with aromatase, Bednarski et al.[24] had already described that introduction of one methylene spacer between the steroid and a 10$\beta$-mercapto group markedly affected the way that the steroids interacted with the enzyme active site: The 10$\beta$-mercaptoandrostenedione gave rise to a type I difference spectrum, while the 19-mercapto analog displayed a spectrum suggestive of a direct interaction of the mercapto group with the heme iron. This different behavior was observed also in our case, namely, between **53** displaying binding only at the lipophilic steroid binding site of aromatase and **57** showing binding of the sulfur atom to the ferric ion. On the other hand, we were surprised at first by the very good affinity displayed by **7,** resulting from the introduction of one more methylene spacers, for aromatase Fe(III) with a $K_s$ 4-fold lower than that of the one-carbon analog **57**. Then we speculated that the distance between the iron and the 19-methyl of the substrate androstenedione could probably be such that the enzyme might be able to even better accommodate a compound like **7** incorporating one more carbon atom in the 19-position (Figure 4c). This reasoning was based on the fact that either during the two first steps of oxidation (Figure 4a) or during the third one (Figure 4b), the iron atom should be separated from the 19-carbon by two atoms, according to the generally accepted mechanism of aromatase.[25]

During the course of this work, two excellent aromatase inhibitors closely related to ours appeared in the literature: the 10$\beta$- and 19-thiiranylandrostenedione analogs **58**[27] and **59**[28] with respective inhibition constants ($K_i$'s) of 2 and 22 nM. Like **7**, these compounds



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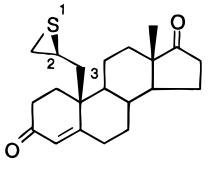

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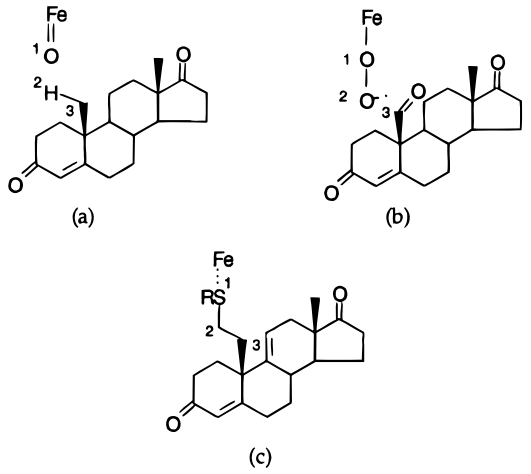
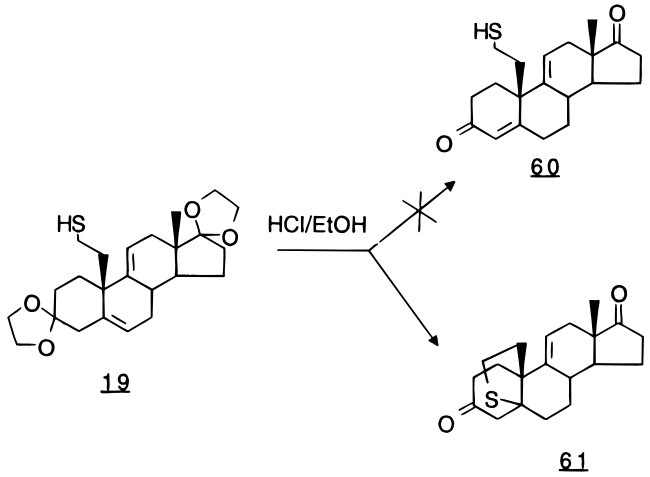



**Figure 4.**

can be regarded as having two carbon atoms between the steroidal skeleton and the sulfur. As we have not reproduced the synthesis of these compounds, we cannot compare their dissociation constants with those of our inhibitors. Yet, the fact that **59** is about 10-fold less active than **58** might mean that its one more carbon atom is somewhat detrimental to the affinity, maybe because of steric hindrance. This observation taken together with the known propensity of free thiols to irreversibly (or tightly) associate with heme iron[24] suggests that the demethylated (thiol) analog of **7, 60,** would be a very potent aromatase inhibitor. In fact, 19-mercaptoandrostenedione has been shown to be a potent suicide inhibitor of aromatase displaying split Soret peaks at 380 and 474 nm, suggesting binding of the 19-thiolate directly to the ferric ion of the enzyme.[24] Unfortunately, various attempts to obtain compound **60** failed. For instance, deprotection of the dioxolanes from **19** led instead to **61** resulting from intramolecular Michael addition[26] (Scheme 13).

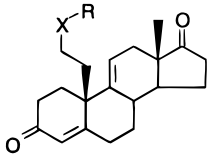
**Scheme 13**




More puzzling was the difference between **7** and its saturated analog **34**. As shown in Table 2, the analog **34** without the 9,11-double bond was a slightly less potent *in vitro* aromatase inhibitor than the unsaturated analog **7**. Molecular modeling studies did not provide explanation for this observation. This difference was enhanced *in vivo*.

We have explored analogs of **7** incorporating other heteroatoms than sulfur. Table 3 shows the $IC_{50}$'s of

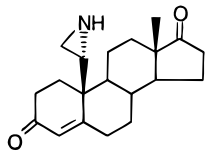
**Table 3.** *In Vitro* Aromatase-Inhibiting Activities of 19-Heteromethyl-Substituted Androstenedione Analogs




| entry | compd | R | X | $IC_{50}$ ($\times 10^{-7}$ M) |
|---|---|---|---|---|
| 1 | **7** | Me | S | 0.12 |
| 2 | **38** | Me,Me | N | >100 |
| 3 | **39** | Me | O | 100 |
| 4 | **42** | | I | >100 |
| 5 | **40** | Ms | O | >100 |
| 6 | **41** | Ph | Se | >10 |
| 7 | **14** | Ph | S | 4 |

the nitrogen, oxygen, selenium, and iodo analogs of **7**. For comparative purposes, we have included the phenyl-substituted analog of **7** (entry 7). The oxygen analog of **7** was much less potent (entry 3). This could be related to the different affinities of sulfur and oxygen atoms for the iron of the ferric porphyrin. Such differences of interactions have already been seen in the literature between oxygen and sulfur analogs of androstenedione-related compounds.[24,25]

Nitrogen atoms of amines and heterocyclic bases are known to bind to cytochrome P450 iron.[29] Robinson has shown that the inhibitory potency of (*R*)-10$\beta$-aziridinylandrostenedione **62** is comparable to that of the thiirane **59**.[30] The lack of inhibitory effect of 19-[(dimethylamino)methyl]androstenedione **38** might be due to the inability of its nitrogen atom to bind to aromatase Fe(III) for steric reasons. This has been very often observed during interaction of tertiary amines with cytochrome P450.[29] Finally, the comparison between the sulfur and selenium analogs (entries 6 and 7) was in favor of the former.

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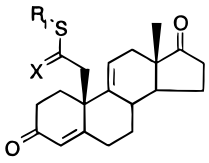
Interaction of some of these new compounds with aromatase was also studied by visible difference spectroscopy (material not shown). The oxygen analog **39** led to a very weak difference spectrum characterized by a peak around 410 nm and a trough at 390 nm (so-called reverse type I spectrum) which should correspond to the binding of the oxygen atom of **39** to pentacoordinate high-spin aromatase Fe(III). Interestingly, the selenium analog **41** gave a difference spectrum with a markedly red-shifted Soret peak at 435 nm and a trough around 395 nm. This is, to our knowledge, one of the only examples of a complex between P450 Fe(III) and a selenium-containing ligand.[31]

The influence of the substitution of the sulfur atom on the inhibition of aromatase was studied (Table 4). The best affinities were observed with compounds substituted by small groups like methyl, fluoromethyl, ethynyl, and vinyl (entries 1, 18, 17, and 13). Going from a methyl to an ethyl group resulted in 72-fold decreased activity (entry 2), probably pointing out to a very precise and narrow active site topography. Activity

**Table 4.** *In Vitro* and *in Vivo* Inhibition of Aromatase by Analogs of RU54115 Bearing Different Sulfur Substituents[a]



| entry | compd | R | X | $IC_{50}$ ($\times 10^{-7}$ M) | E2 lowering[b] 2 h | E2 lowering[b] 6 h |
|---|---|---|---|---|---|---|
| 1 | **7** | Me | | 0.12 | −51** | −50* |
| 2 | **12** | Et | | 8.7 | nd | nd |
| 3 | **13** | MeCO | | >100 | −21(NS) | −10(NS) |
| 4 | **14** | Ph | | 4 | nd | nd |
| 5 | **15** | $HO(CH_2)_2$ | | >10 | nd | nd |
| 6 | **16** | $Cl(CH_2)_2$ | | >10 | nd | nd |
| 7 | **17** | $HS(CH_2)_2$ | | >10 | nd | nd |
| 8 | **20** | $F_2CH$ | | >10 | nd | nd |
| 9 | **21** | $(O_2N)_2PhS$ | | >10 | −27** | −32** |
| 10 | **22** | $CH_2C{\equiv}CH$ | | 10 | −5(NS) | −25** |
| 11 | **23** | $MeSCH_2$ | | 5.7 | nd | nd |
| 12 | **24** | $CH_2{=}CHCH_2$ | | 2.7 | nd | nd |
| 13 | **26** | $CH_2{=}CH$ | | 0.5 | −25** | −24NS |
| 14 | **27** | MeS | | >10 | −7(NS) | +23NS |
| 15 | **28** | PhS | | >10 | −43** | −62** |
| 16 | **29** | cPr | | >10 | nd | nd |
| 17 | **31** | $C{\equiv}CH$ | | 3.3 | nd | nd |
| 18 | **32** | $FCH_2$ | | 0.5 | −31** | −41NS |
| 19 | **43** | NC | | >10 | −44**[c] | −38**[c] |
| 20 | **50** | Me | H,SMe | >10 | −18(NS) | −13(NS) |
| 21 | **51** | Et | H,SEt | >10 | nd | nd |
| 22 | **57** | Me[d] | O | >10 | nd | nd |

[a] Abbreviations and symbols: nd = not determined; ** = $p < 0.01$ (Student's *t*-test); * = $p < 0.05$; NS = nonstatistically significant. [b] Lowering of circulating estradiol after an oral dose of 5 mg/kg at times 2 and 6 h after treatment expressed as percent of control (see the Experimental Section). [c] Lowering of circulating estradiol after a subcutaneous dose of 5 mg/kg at times 2 and 6 h after treatment expressed as percent of control (see the Experimental Section). [d] No $\Delta^{9(11)}$.

was totally lost with the substituted ethyl and cyclopropyl derivatives (entries 5−7 and 16). With the allyl, phenyl, propargyl, and (methylthio)methyl groups, however (entries 12, 4, 10, and 11), some activity was regained. No doubt these subtle differences point out to a definite shape for the pocket above the steroid plane in the enzyme cavity. When electronegative functions were introduced on the methyl group, the affinity for the enzyme was lost, which can be explained in terms of sulfur lone pair decreased availability for the interaction with the iron atom. Thus, replacement of one more hydrogen of the methyl group by fluorine (difluoromethyl analog, entry 8) gave rise to more than 20-fold decreased activity over the monofluoromethyl analog (entry 18), and changing the thiomethyl function into a thiolester (entries 2 and 22) also resulted in inactive compounds. The same reason probably explained the *in vitro* inactivity of the thiocyanate analog (entry 19), although this compound displayed some *in vivo* activity when given subcutaneously. As expected, disulfides (entries 9, 14, and 15) were inactive *in vitro*, but the diphenyl disulfide analog was active *in vivo* per os. Thioacetals (entries 20 and 21) also proved to be inactive, probably mainly because of steric hindrance.

Finally, introduction of unsaturation in the 10-position side chain brought about inactive compounds: inactivities of analogs **47** and **48** are to be related to the lack of activity of their saturated analog **55** (cf. Table 2, entry 7). More interesting was the inactivity of the *E*-unsaturated analog of **7, 45**. In fact, this result could possibly rule out the extended more stable conformation of **7** as the active one, although other factors like, for instance, the electronic differences between the saturated and the unsaturated analogs might play a part in the differences of affinities for the enzyme. Unfortunately, different chemical routes failed to afford the *Z* isomer of this compound.

## Conclusion

Several new analogs of androstenedione were evaluated as aromatase inhibitors. Optimal *in vitro* and *in vivo* activities were obtained with the compounds having in the 10-position a two-carbon chain bearing a thioether function. The most active compound which bears a $CH_2$-$CH_2SMe$ chain, **7**, is one of the best steroidal aromatase inhibitors today. The key feature of these inhibitors is the sulfur atom capable of interacting in a tight fashion with the heme iron of the enzyme's porphyrin. Owing to the conformational flexibility of the chain at position 10 of these compounds, little information about the position of the iron in the active site of aromatase could be deduced. With these first data in hand, the objective was to turn our attention to cyclic analogs of **7** in which the sulfur lone pairs would point out more precisely to specific directions of the enzyme cavity. This will be the subject of part 2 of this series of papers.

## Experimental Section

Tetrahydrofuran was distilled from sodium and benzophenone. Most reagents were purchased from Aldrich and used without further purification. Sodium thiophenoxide, diethyl (thiomethyl)phosphonate, sodium thioethoxide, and vinylmagnesium chloride were purchased from Fluka. [(Methylthio)-methyl]triphenylphosphonium chloride was purchased from Lancaster. Palladium hydroxide (1%) on barium sulfate was purchased from Engelhard. Difluorochloromethane was bought from Praxair. $^1H$ NMR spectra were recorded with Bruker AC300, AM250, and WH90 spectrometers using $CDCl_3$ solution. Chemical shifts are reported in part per million (ppm) with tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) are given in hertz. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Mass spectra were recorded with a MATT311 spectrometer (ionization potential, 3 kV; electron accelerating potential, 70 eV; ion source temperature, 200 °C). Infrared spectra were recorded on a Nicolet 20SX or 5SX spectrometer using chloroform solutions. UV spectra were recorded with a CARY 2200 spectrometer. Thin layer chromatography was performed with DC Plastiekfolien Kieselgel 60 F254 (E. Merck). UV, iodine vapor, or sulfuric acid was used to visualize the developed plates. Flash chromatography[32] was performed with silica gel (Merck; 230−400 mesh grade) or reverse phase silica gel ($KC_{18}F$; Whatman). nBuLi solutions were titrated with 2,5-dimethoxybenzyl alcohol using a literature procedure.[33]

Protein concentrations were determined according to the method of Lowry.[34] For *in vitro* tests, stocks solutions of compounds were prepared in DMSO and further diluted with the appropriate buffer solutions. The inhibitors were dissolved in benzylic alcohol (5%) and diluted in sesame oil when given sc or suspended in 0.5% methocel (A-4C premium; Dow Chemical) when given orally. The PMSG was obtained from Distrivet (Roussel Uclaf Group). [1$\beta$,2$\beta$-$^3$H]Androstenedione (41.8 Ci/mmol) was purchased from NEN. Radioactivity was measured on a LKB1214 liquid scintillation counter. NADPH tetrasodium salt, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Sigma. Estradiol kit (ER155) was purchased from Baxter-Travenol.

**Ethyl 3,17-Bis(ethylenedioxy)androsta-5(6),9(11)-diene-19-carboxylate (9).** A 250 mL flask equipped with a Dean−Stark and a reflux condenser and under argon was charged with 503 mg (1.42 mmol) of ethyl 3,17-dioxoandrosta-

5(6),9(11)-diene-19-carboxylate (**8**) dissolved in 30 mL of dichloroethane; 2 mL of ethylene glycol were added to the solution, and the mixture was refluxed for 8 h; 1 mL of triethylamine was added, and the mixture was concentrated on a rotary evaporator. The crude mixture was flash chromatographed with AcOEt:cyclohexane, 3:7. The first fraction was the expected compound, obtained as a white foam (450 mg, 71.6%): $R_f = 0.47$ (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.89 (s, 18-Me), 1.21 (t, $COOCH_2CH_3$), 3.7−4.2 ($OCH_2CH_2O$, $COOCH_2$), 5.52 and 5.56 (2m, H6, H11); IR ($CHCl_3$) 1724, 1671, 1635 $cm^{-1}$.

The second fraction consisted of some 17-monoprotected derivative obtained as an oil (75 mg, 11.9%): $R_f = 0.37$ (AcOEt: cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 3.7−4.2 ($CH_2O$), 5.57 (H11), 5.81 (H4); IR ($CHCl_3$) 1725, 1666, 1616 $cm^{-1}$.

**3,17-Bis(ethylenedioxy)-19-(hydroxymethyl)androsta-5(6),9(11)-diene (10).** A 4 L 3-necked flask equipped with a mechanical stirrer, an internal thermometer, a dropping funnel, and a nitrogen inlet was charged with 21.2 g (0.56 mol, 1 equiv) of lithium aluminum hydride suspended in 2.5 L of THF. The suspension was cooled to −50 to 55 °C, and a solution of 250 g of **9** in 500 mL of THF was added over a 15 min period. After 30 min at −50 °C, the mixture was stirred for 2 h at room temperature. The excess of hydride was destroyed by slow addition of 500 mL of AcOEt, the internal temperature being kept around 25 °C. The reaction mixture was poured into 2.5 L of 2 N aqueous sodium hydroxide, and then the aqueous phase was extracted with $CH_2Cl_2$ (3 and 2 L). The organic extracts were washed with water (2 L), dried over $MgSO_4$, and concentrated to yield 225 g of crude product. The crude was purified by flash chromatography with hexane: AcOEt, 1:1, containing 1% TEA to afford 192.5 g (85.5%) of the expected **10** as a white foam: NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.83 (s, 18-Me), 2.33 (s, OH), 3.55−4.0 (m, $OCH_2CH_2O$), 5.53 (m, H6, H11); IR ($CHCl_3$) 1338, 1175 $cm^{-1}$.

**3,17-Bis(ethylenedioxy)-19-[(mesyloxy)methyl]androsta-5(6),9(11)-diene (11).** A 1 L 3-necked flask equipped with a magnetic stirrer, an internal thermometer, a dropping funnel, and a nitrogen inlet was charged with 60 g of **10** (0.144 mol) and 22.8 mL (0.16 mol) of triethylamine in solution in 600 mL of dichloromethane. The pale yellow solution was cooled to 0 °C, and 12.7 mL of methanesulfonyl chloride was added. The mixture was stirred for 45 min at 0 °C. After that time, the suspension was poured into 1 L of a saturated solution of sodium bicarbonate and the organic phase was separated. The aqueous phase was extracted with 200 mL of $CH_2Cl_2$, and the organic extracts were washed with water (200 mL), dried ($MgSO_4$), and concentrated on a rotary evaporator. The crude product was obtained as a white foam (73.5 g, 100%) ($R_f$ = 0.3, AcOEt:hexane, 1:1, 1% TEA). Owing to its relative instability, the mesylate was used without purification in the next step: NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.83 (s, 18-Me), 2.97 (s, $SO_2Me$), 3.85−4.3 (m, $OCH_2CH_2O$, $CH_2OSO_2$), 5.57 and 5.60 (2m, H6, H11); IR ($CHCl_3$) 1338, 1175 $cm^{-1}$.

**3,17-Dioxo-19-[(methylthio)methyl]androsta-4,9(11)-diene (7).** A 1 L 3-necked flask equipped with a magnetic stirrer, an internal thermometer, and a nitrogen inlet was charged with 63 g (0.131 mol) of mesylate **11** in solution in 500 mL of DMF dried over molecular sieves ($NK_{20}$); 19.6 g (0.28 mol, 2.13 equiv) of sodium thiomethoxide was added to the orange solution all at once. The mixture turned brown and heterogeneous, and the temperature rose to 35 °C. It was stirred for 1 h and then poured into 1 L of saturated aqueous solution of ammonium chloride and extracted with dichloromethane (2 × 500 mL). The organic extracts were washed twice with water (1 L total), dried over $MgSO_4$, and concentrated to dryness under vacuum (20 mmHg, bath temperature, 40 °C). The crude orange oil (69 g) was dissolved in 500 mL of 99.9% ethanol; 200 mL of 6 N hydrochloric acid was added, and the resulting yellow solution was stirred for 1 h at room temperature. A precipitate formed; it was filtered and washed twice with 50 mL of EtOH. The pale yellow crystals were dried under vacuum (26.1 g, 59%). The mother liquors were diluted under efficient stirring with 1 L of water. The precipitate was filtered, washed twice with EtOH (25 mL), and dried under vacuum to afford 5.5 g of yellow crystals (12.4%). The mother liquors were concentrated to a volume of 30 mL, and the precipitate formed was filtered and dried as before (1.5 g). The crystals were chromatographed with dichloromethane:AcOEt, 8:2, to afford 29 g of pale yellow crystals. Recrystallization from 120 mL of EtOH gave 28.1 g of microanalytically pure crystals (60.6% over the two steps): $R_f = 0.5$ ($CH_2Cl_2$:AcOEt, 8:2); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 2.10 (s, SMe), 5.56 (m, H11), 5.81 (s, H4); IR ($CHCl_3$) 1735, 1665, 1633, 1613 $cm^{-1}$; UV (EtOH) max 239−240 nM ($\epsilon$ = 16 000). Anal. ($C_{21}H_{28}O_2S$) C,H,S.

**3,17-Dioxo-19-[(ethylthio)methyl]androsta-4,9(11)-diene (12).** The above procedure was applied to 1 g (2.2 mmol) of mesylate **11**, 374 mg (4.4 mmol; Fluka) of sodium thioethoxide, and 0.044 mL (0.22 mmol, 0.1 equiv) of 15-crown-5 in 20 mL of DMF for 12 h at room temperature. After workup, the crude mixture was hydrolyzed in the same way using 2 mL of 6 N HCl in 20 mL of 99% ethanol. After 1 h of stirring at room temperature, the reaction mixture was poured into water and extracted with dichloromethane. The organic extracts were washed with 1 N HCl, saturated $NaHCO_3$, and then brine, dried, and concentrated. Flash chromatography (AcOEt:cyclohexane, 3:7) afforded 430 mg (54%) of expected thioether **12** as a colorless oil that solidified upon standing. Recrystallization from ethyl ether gave 176 mg of a white solid: mp 105 °C; $R_f = 0.57$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 5.55 (m, H11), 5.81 (d, H4); IR ($CHCl_3$) 1735, 1665, 1633, 1613 $cm^{-1}$. Anal. ($C_{22}H_{30}O_2S$) C,H,S.

**3,17-Dioxo-19-[(acetylthio)methyl]androsta-4,9(11)-diene (13).** Mesylate **11** (3.38 g, 7 mmol), 190 mg (0.1 equiv) of 18-crown-6, and 1.6 g (2 equiv) of potassium thioacetate dissolved in 40 mL of THF were refluxed for 24 h; 0.7 g of potassium thioacetate was added, and the mixture was refluxed for 12 h and then partitioned between dichloromethane and brine (100 mL each). The organic extracts were dried ($MgSO_4$), concentrated, and flash chromatographed (AcOEt: cyclohexane, 3:7) to afford 940 mg (28.5%) of thioacetate **18** as a colorless oil: $R_f = 0.74$ (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 400 MHz) 0.86 (s, 18-Me), 2.30 (s, SCOMe), 3.80−4.08 ($OCH_2CH_2O$), 5.54 (H6, H11); IR ($CHCl_3$) 1685 $cm^{-1}$.

The next fraction was the 17-monoprotected compound (500 mg, 17%): $R_f = 0.45$ (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 400 MHz) 0.88 (s, 18-Me), 2.31 (s, SCOMe), 3.80−4.08 ($OCH_2$-$CH_2O$), 5.56 (H11), 5.77 (H4); IR ($CHCl_3$) 1683, 1665, 1614 $cm^{-1}$.

This fraction was hydrolyzed in ethanol (12 mL) with 2 mL of 6 N HCl for 1 h at room temperature. Similar workup as above gave 400 mg of crude product which after flash chromatography (AcOEt:cyclohexane, 3:7) afforded 340 mg (76%) of expected thioacetate **13** as a pale brown foam. One recrystallization from AcOEt gave 78 mg of yellow crystals: mp 144.5 °C; $R_f = 0.28$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.96 (s, 18-Me), 2.33 (s, SCOMe), 5.59 (m, H11), 5.81 (s, H4); IR ($CHCl_3$) 1735, 1680, 1667, 1615 $cm^{-1}$. Anal. ($C_{22}H_{28}O_3S$) C, H, S.

**3,17-Dioxo-19-[(phenylthio)methyl]androsta-4,9(11)-diene (14).** Na (510 mg, 9 mmol) was introduced in a 100 mL round-bottomed flask equipped with a magnetic stirrer and covered with 40 mL of anhydrous THF under an atmosphere of argon; 5.6 mL (55 mmol, 6 equiv) of thiophenol was added, and the mixture was stirred for 12 h at room temperature. After that time, the sodium thiophenoxide had precipitated. The THF was removed with a pipette, and the white solid was washed with pentane (6 × 20 mL) under argon. The sodium thiophenoxide was then dried under vacuum (2.5 g, 86.2%). Mesylate **11** (300 mg, 0.66 mmol) was introduced in a 100 mL flask equipped with a magnetic stirrer and an argon inlet and dissolved in 20 mL of DMF; 165 mg of the sodium thiophenoxide (13 mmol, 2 equiv) was added. The mixture was stirred for 24 h at room temperature, and 5 drops of 15-crown-5 ether was added. After 12 h of stirring at room temperature, the mixture was evaporated to dryness and the crude was flash chromatographed with AcOEt:hexane, 8:2, to afford 281 mg of the expected compound as a white foam (91.2%): NMR ($CDCl_3$, 300 MHz) 0.80 (s, 18-Me), 1.25−2.90 (m, steroid

backbone), 3.85−3.98 (ketals), 5.52 (m, H6, H11), 7.14−7.33 (m, Ph); IR ($CHCl_3$) 1481, 1584 $cm^{-1}$.

In a 100 mL round-bottomed flask equipped with a magnetic stirrer, 260 mg of the previous compound (0.5 mmol) was introduced in 20 mL of ethanol; 2 mL of 6 N HCl was added, and the mixture was stirred for 2 h at room temperature. After neutralization by addition of an aqueous saturated solution of sodium bicarbonate, the aqueous phase was extracted with dichloromethane (2 × 20 mL). The organic extracts were dried over $MgSO_4$, concentrated, and flash chromatographed with cyclohexane:AcOEt, 8:2, to afford the expected compound as a white solid (121 mg, 56.6%) ($R_f$ = 0.55, AcOEt:cyclohexane, 1:1). It was recrystallized from ether: NMR ($CDCl_3$, 250 MHz) 0.84 (s, 18-Me), 5.57 (m, H11), 5.78 (bs, H4), 7.2−7.4 (m, Ph); IR ($CHCl_3$) 1736, 1665, 1630, 1612, 1577, 1477 $cm^{-1}$. Anal. ($C_{26}H_{30}O_2S$) C,H,S.

**3,17-Dioxo-19-[[(hydroxyethyl)thio]methyl]androsta-4,9(11)-diene (15).** A 500 mL round-bottomed flask equipped with a magnetic stirrer was charged with 4.2 mL (60 mmol) of $\beta$-mercaptoethanol in solution in 150 mL of DMF followed by 6.7 g of potassium *tert*-butoxide (60 mmol). The mixture was stirred for 1 h at room temperature under argon. Mesylate **11** (4 g, 8.3 mmol) dissolved in 50 mL of DMF was added dropwise, and the clear orange mixture was stirred for 2 h at room temperature. The DMF was evaporated on a rotavapor equipped with a membrane vacuum pump, and the resulting mixture was partitioned between 100 mL of water and 300 mL of ethyl acetate. The organic phase was dried on magnesium sulfate and concentrated to dryness to afford 2.4 g (63%) of the expected alcohol as a white foam. It was used as such in the next step: $R_f$ = 0.5 (AcOEt:cyclohexane, 7:3); NMR ($CDCl_3$, 250 MHz) 0.82 (s, 18-Me), 2.70 (t, $CH_2S$), 3.68 (t, $CH_2O$), 3.8−4 (ketals), 5.52 (m, H6, H11); IR ($CHCl_3$) 3614 $cm^{-1}$.

The previous compound (1 g, 2.16 mmol) dissolved in 50 mL of methanol was introduced in a 100 mL round-bottomed flask equipped with a magnetic stirrer. 5 mL of 6 N HCl was added, and the mixture was stirred for 1 h at room temperature. The crude reaction mixture was concentrated on a rotavapor and partitioned between aqueous bicarbonate and ethyl acetate. The organic extracts were dried, concentrated, and chromatographed to afford 0.8 g of the expected compound as a white foam (100%): $R_f$ = 0.2 (AcOEt:cyclohexane, 7:3); NMR ($CDCl_3$, 250 MHz) 0.81 (s, 18-Me), 2.65 (t, $CH_2S$), 3.66 (t, $CH_2O$), 5.49 (H11), 5.75 (H4); IR ($CHCl_3$) 3618, 1736, 1665, 1634, 1613 $cm^{-1}$. Anal. ($C_{22}H_{30}O_3S$) C,H,S.

**3,17-Dioxo-19-[[(chloroethyl)thio]methyl]androsta-4,9(11)-diene (16).** Alcohol **15** (553 mg, 1.5 mmol), 0.23 mL (1.65 mmol) of triethylamine, and 50 mL of dichloromethane were introduced in a 100 mL flask equipped with a magnetic stirrer and an argon inlet; 0.13 mL (1.65 mmol) of mesyl chloride was added dropwise, and the suspension was stirred for 2 h at room temperature and then poured into 50 mL of water. The organic phase was extracted, dried over magnesium sulfate, and concentrated to dryness. It was then dissolved in 50 mL of acetone and refluxed for 2 h in the presence of 1 g of sodium chloride. The acetone was evaporated on a rotavap, and the mixture was partitioned between ethyl acetate and water. The organic phase was dried, concentrated, and chromatographed twice (cyclohexane:ethyl acetate, 7:3, and then methylene chloride:ethyl acetate, 95:5) to afford 160 mg of the expected material as a colorless oil (27%): $R_f$ = 0.72 (AcOEt:cyclohexane, 7:3); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 2.85 (dd, $CH_2S$), 3.61 (t, $CH_2Cl$), 5.56 (m, H11), 5.82 (d, H4); IR ($CHCl_3$) 1736, 1666, 1634, 1614 $cm^{-1}$. Anal. ($C_{22}H_{29}$ Cl $O_2S$) C,H,S,Cl.

**3,17-Dioxo-19-[[(mercaptoethyl)thio]methyl]androsta-4,9(11)-diene (17).** Alcohol **15** (1.7 g, 3.7 mmol) protected as the 3,17-dioxolane (see preparation of **15**, part 1), 5.3 g (14.8 mmol) of triphenylphosphine, 1.25 mL (8 mmol) of diethyl azodicarboxylate, 0.6 mL (8 mmol) of thioacetic acid, and 40 mL of THF were introduced into a 100 mL flask equipped with a magnetic stirrer and an argon inlet. The clear yellow solution was stirred for 1 h at room temperature and then concentrated on a rotavap and partitioned between water and ethyl acetate. The organic phase was dried, concentrated, and chromatographed (cyclohexane:dichloromethane:ethyl ether, 50:50:5) to afford 900 mg of the expected thioacetate as a colorless oil (45%): $R_f$ = 0.38 (AcOEt:cyclohexane, 7:3); NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.83 (s, 18-Me), 2.33 (s, $CH_3S$), 3.04 (m, $CH_2CH_2S$), 3.75−4.00 (m, $OCH_2CH_2O$), 5.53 (m, H6, H11). The NMR spectrum showed the presence of an impurity derived from the addition of thioacetic acid to DEAD (2.5 mol equiv); this material was used as such in the next step.

The previous thioacetate (600 mg, 1.15 mmol) and 0.65 mL (8 mmol) of hydrazine hydrate were dissolved in 40 mL of THF and introduced into a 100 mL round-bottomed flask equipped with a magnetic stirrer and an argon inlet. After 1 h of stirring at room temperature, the THF was evaporated on a rotavap, and the crude mixture was partitioned between water and ethyl acetate (50 mL each). The aqueous phase was extracted with ethyl acetate, and the organic extracts were dried and concentrated on the rotavap. The crude was dissolved in 10 mL of methylene chloride, and 100 mL of methanol and 5 mL of 6 N chlorhydric acid were added. The clear yellow solution was stirred for 1 h at room temperature; then 50 mL of a saturated solution of sodium bicarbonate was added, and the methanol was evaporated on the rotavap. The crude was extracted with ethyl acetate, and the organic extracts were dried, concentrated, and chromatographed first on 60 g of $RP_{18}$ reverse phase silica gel with methanol:water, 8:2, and then on silica gel with cyclohexane:ethyl acetate, 8:2, to afford 120 mg (27%) of the expected compound as a colorless oil: $R_f$ = 0.36 (AcOEt:cyclohexane, 5:5); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 5.57 (H11), 5.82 (H4); IR ($CHCl_3$) 1736, 1666, 1634, 1614 $cm^{-1}$. Anal. ($C_{22}H_{30}O_2S_2$) C,H,S.

**3,17-Bis(ethylenedioxy)-19-[(acetylthio)methyl]androsta-5(6),9(11)-diene (18).** Alcohol **10** (8.6 g, 21 mmol) dissolved in 40 mL of THF was introduced in a 250 mL flask equipped with a magnetic stirrer and under argon; 10.8 g (38 mmol, 1.8 equiv) of triphenylphosphine and 3.6 mL (23 mmol, 1.08 equiv) of diethyl azodicarboxylate were introduced at once; then 1.65 mL (23 mmol, 1.08 equiv) of thioacetic acid was added dropwise; the dark orange mixture progressively discolored. After 2.5 h of stirring at room temperature, more diethyl azodicarboxylate (0.9 mL, 0.25 equiv) and thioacetic acid (0.42 mL, 0.25 equiv) were added and the mixture was stirred for 20 min at room temperature. The THF was evaporated on the rotavapor, and the resulting crude mixture was flash chromatographed with cyclohexane:ethyl acetate, 8:2, to afford the expected thioacetate as a colorless oil (5.88 g, 60%). $R_f$ = 0.74 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 400 MHz) 0.86 (s, 18-Me), 2.30 (s, SCOMe), 3.80−4.08 ($OCH_2$-$CH_2O$), 5.54 (H6, H11); IR ($CHCl_3$) 1685 $cm^{-1}$.

**3,17-Bis(ethylenedioxy)-19-(mercaptomethyl)androsta-5(6),9(11)-diene (19).** Thioacetate **18** (3 g, 6.5 mmol) dissolved in 150 mL of THF was introduced into a 250 mL flask equipped with a magnetic stirrer, and the solution was cooled to −20 °C; 2.3 mL of 64% hydrazine was added dropwise, and the solution was stirred for 72 h at −30 °C. The mixture was poured on water (200 mL), and the aqueous phase was extracted with methylene chloride. The organic extracts were dried and concentrated to afford 2.65 g (97%) of the expected material as a white foam. It was used as such in the next reactions: $R_f$ = 0.36 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.82 (s, 18-Me), 3.8−4.0 ($OCH_2CH_2O$), 5.49 and 5.53 (2m, H6, H11); IR ($CHCl_3$) 1672, 1635 $cm^{-1}$.

**3,17-Dioxo-19-[[(difluoromethyl)thio]methyl]androsta-4,9(11)-diene (20).** Steroid **19** (1 g, 2.4 mmol) was dissolved in 20 mL of THF and introduced into a 100 mL flask equipped with a magnetic stirrer and under argon. The solution was cooled to 0 °C, and 322 mg (2.9 mmol) of potassium *tert*-butoxide was introduced all at once. The suspension was stirred for 30 min at this temperature; then a stream of difluorochloromethane was rapidly bubbled into the flask for about 30 s. The reaction mixture was then poured in about 100 mL of a saturated solution of ammonium chloride and extracted with methylene dichloride. The organic extracts were dried, concentrated, and flash chromatographed to afford 500 mg of the expected fluorinated compound as a yellow oil (44.5%): $R_f$ = 0.79 (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300

MHz) 0.82 (s, 18-Me), 3.80−4 ($OCH_2CH_2O$), 5.54 (H6, H11), 6.76 (t, $CHF_2$, $J = 56.5$); IR ($CHCl_3$) 1638, 1615 $cm^{-1}$.

This compound (90 mg, 0.19 mmol) was hydrolyzed as described previously with 1 mL of 6 N HCl in 5 mL of 99% ethanol. After 30 min at room temperature, the mixture was worked up (methylene dichloride/aqueous saturated sodium bicarbonate) to afford after drying 61.7 mg of a yellow oil. This was chromatographed with ethyl acetate:cyclohexane, 1:1, to give 46.5 mg of a colorless oil crystallizing in pure phase: $R_f = 0.39$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 5.59 (m, H11), 5.82 (s, H4), 6.8 (t, $J = 55.5$, $CHF_2$); IR ($CHCl_3$) 1736, 1666, 1614, 1060, 1030, 867 $cm^{-1}$; HRMS calcd for $C_{21}H_{26}O_2F_2S$ 380.162159, found 380.161827. Anal. ($C_{21}H_{26}F_2O_2S$) C,H,S; F: calcd, 9.99; found, 9.2.

**3,17-Dioxo-19-[[[(2,4-dinitrophenyl)thio]thio]methyl]-androsta-4,9(11)-diene (21).** Thiol **19** (416 mg, 1 mmol) and 0.081 mL (1 mmol) of pyridine were dissolved in 15 mL of methylene dichloride and introduced in a 100 mL flask equipped with magnetic stirring and an argon inlet; 235 mg (1 mmol) of 2,4-dinitrobenzenesulfenyl chloride in 5 mL of methylene chloride was then added dropwise to the room temperature stirred solution. After 30 min of stirring, the reaction mixture was poured into 100 mL of water and 100 mL of ethyl acetate. The organic phase was dried, concentrated, and chromatographed (cyclohexane:methylene dichloride:AcOEt, 45:45:15) to afford 500 mg (81%) of the expected material **25** as a yellow foam: $R_f = 0.5$ (cyclohexane:dichloromethane:AcOEt, 45:45:15); NMR ($CDCl_3$, 300 MHz) 0.6 (s, 18-Me), 3.8−4.0 ($OCH_2CH_2O$), 5.46 and 5.53 (2d, H6, H11), 8.47 (2H, Ar), 9.11 (1H, Ar); IR ($CHCl_3$) 1594, 1526 $cm^{-1}$; MS $M^+$ 616 (FD).

The previous compound (1.25 g, 2 mmol) was dissolved in 150 mL of methanol, the solution was introduced in a 100 mL flask, and 10 mL of 6 N chlorhydric acid was added. The resulting suspension was stirred for 2 h at room temperature in an ultrasonic bath. The crude mixture was concentrated on the rotavap and then partitioned between aqueous sodium bicarbonate and ethyl acetate. The organic phase was dried, concentrated, and chromatographed (gradient of dichloromethane:ethyl ether, 100:0−90:10) to afford 1 g (100%) of the expected material as a yellow solid: mp 111.5 °C; $R_f =$ 0.38 (cyclohexane:ethyl acetate, 1:1); NMR ($CDCl_3$, 300 MHz) 0.63 (s, 18-Me), 5.51 (m, H11), 5.82 (s, H4), 8.48 (2H, Ar), 9.1 (1H, Ar); IR ($CHCl_3$) 1737, 1668, 1638, 1613, 1595, 1528 $cm^{-1}$; MS $MH^+$ 529 (FD). Anal. ($C_{26}H_{28}O_6S_2N_2$) C,H,N,S.

**3,17-Dioxo-19-[(propargylthio)methyl]androsta-4,9(11)-diene (22).** Thiol **19** (800 mg, 2 mmol) and 0.39 mL (3 mmol) of tetramethylethylenediamine were dissolved in 4 mL of THF and introduced in a 100 mL flask equipped with magnetic stirring and an argon inlet. The reaction mixture was cooled to −78 °C and 2.25 mL (2 mmol) of nBuLi (0.85 M solution in hexane) then added dropwise. After 30 min of stirring at this temperature, 0.205 mL (1.2 equiv) of propargyl bromide was added dropwise and the reaction mixture was stirred for 1.5 h at −78 °C. The reaction mixture was hydrolyzed with a saturated solution of ammonium chloride, and the aqueous phase was extracted with methylene chloride (3 × 10 mL). The organic phase was dried, concentrated, and chromatographed (cyclohexane:ethyl acetate, 8:2) to afford 462 mg (53%) of the expected material as a white foam: $R_f = 0.59$ (cyclohexane:ethyl acetate, 1:1); NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.85 (s, 18-Me), 2.21 (t, HC), 3.23 (m, $CH_2$), 5.53 (m, H6, H11); IR ($CHCl_3$) 3207, 2105 $cm^{-1}$.

The previous compound (460 mg, 1 mmol) was dissolved in 10 mL of ethanol, the solution was introduced in a 30 mL flask, and 2 mL of 6 N hydrochloric acid was added. The precipitate formed was filtered, washed with ethyl ether, redissolved in methylene dichloride, and chromatographed with cyclohexane:ethyl acetate, 8:2, to afford the expected material as a white solid (265 mg, 72%). This material was contaminated with 5% of thioacetate **13** and 8% of methylene dichloride: mp 190 °C; $R_f = 0.56$ (cyclohexane:ethyl acetate, 1:1); NMR ($CDCl_3$, 300 MHz) 0.9 (s, 18-Me), 2.23 (t, $J = 2.5$, CH), 3.26 (m, $CH_2$), 5.58 (m, H11), 5.82 (H4); IR ($CHCl_3$) 3307, 1735, 1665, 1630, 1608 $cm^{-1}$; HRMS calcd for $C_{23}H_{28}O_2S$ 368.18102, found 368.179820. Anal. ($C_{23}H_{28}O_2S$) H,S; C: calcd, 74.96; found, 73.5.

**3,17-Dioxo-19-[[[(methylthio)methyl]thio]methyl]androsta-4,9(11)-diene (23).** Thiol **19** (1 g, 2.4 mmol) was dissolved in 20 mL of degassed anhydrous THF and introduced in a 100 mL flask equipped with magnetic stirring and an argon inlet; 322 mg (2.9 mmol) of potassium *tert*-butoxide were then added portionwise. After 15 min of stirring at room temperature, 0.4 mL (4.8 mmol, 2 equiv) of methylchloromethyl sulfide was added rapidly and the reaction mixture was stirred for 5 min at room temperature The reaction mixture was hydrolyzed with a saturated aqueous solution of ammonium chloride, and the aqueous phase was extracted with methylene chloride (3 × 15 mL). The organic phase was dried, concentrated, and chromatographed (cyclohexane: AcOEt, 8:2) to afford 850 mg (74%) of the expected material as a white foam: $R_f = 0.8$ (cyclohexane:ethyl acetate, 8:2); NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.85 (s, 18-Me), 2.13 (s, SMe), 3.62 (s, $SCH_2S$), 3.8−4 ($OCH_2CH_2$), 5.53 (m, H6, H11); IR ($CHCl_3$) 1732, 1670, 1634 $cm^{-1}$.

The next fraction was characterized as the disulfide of the starting thiol **19** (200 mg, 10%): $R_f = 0.1$ (cyclohexane:ethyl acetate, 8:2); NMR ($CDCl_3$, 300 MHz) 0.83 (s, 18-Me), 3.8−4 ($OCH_2CH_2$), 5.49 and 5.53 (2d, H6, H11); IR ($CHCl_3$) 1670, 1635 $cm^{-1}$; MS $M^+$ 834.

The previous compound 350 mg, 0.7 mmol) was dissolved in 12 mL of ethanol, the solution was introduced in a 30 mL flask, and 6 mL of 6 N hydrochloric acid was added. After 1.5 h of stirring at room temperature, the reaction mixture was hydrolyzed by a saturated solution of sodium bicarbonate and the aqueous phase was extracted with methylene dichloride (3 × 10 mL). The organic extracts were dried, concentrated, and chromatographed with cyclohexane:ethyl acetate, 8:2, to afford the expected material as a white foam (270 mg, 93%): $R_f = 0.5$ (cyclohexane:AcOEt, 8:2); NMR ($CDCl_3$, 250 MHz) 0.91 (s, 18-Me), 2.13 (s), 3.63 (s, $SCH_2S$), 5.58 (m, H11), 5.82 (d, H4); IR ($CHCl_3$) 1736, 1664, 1613 $cm^{-1}$. Anal. ($C_{22}H_{30}O_2S_2$) C,H; S: calcd, 16.42; found, 16.0.

**3,17-Dioxo-19-[(allylthio)methyl]androsta-4,9(11)-diene (24).** The previous procedure was applied to 400 mg (1 mmol) of thiol **19** in 10 mL of THF, 129 mg (1.2 mmol, 1.2 equiv) of potassium *tert*-butoxide, and 0.16 mL (2 mmol, 2 equiv) of allyl bromide. After 30 min of stirring at room temperature, the same workup was applied to afford after chromatography (cyclohexane:ethyl acetate, 8:2) the expected material as a white foam (325 mg, 74%): $R_f = 0.6$ (cyclohexane: ethyl acetate, 1:1); NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.82 (s, 18-Me), 3.11 (d, $SCH_2$), 3.8−4 ($OCH_2CH_2$), 5.05−5.15 ($CH_2$═), 5.50 (m, H6, H11), 5.76 (m, CH═); IR ($CHCl_3$) 1675, 1635, 989, 919 $cm^{-1}$.

The same hydrolyzing procedure as the one described for **22** was applied to 300 mg (0.7 mmol) of the previous compound dissolved in 10 mL of ethanol and 5 mL of 6 N HCl. After 1 h at room temperature, the reaction mixture was worked up and chromatographed (cyclohexane:ethyl acetate, 8:2) to afford the expected material as an oil (230 mg, 95%). One precipitation with pentane gave the expected analytically pure material as a white solid (170 mg): $R_f = 0.5$ (cyclohexane:ethyl acetate, 8:2); mp 99 °C; NMR ($CDCl_3$, 250 MHz) 0.88 (s, 18-Me), 3.13 (dd, $J = 1, 7$), 5.05−5.11 ($CH_2C$═), 5.54 (m, H11), 5.73 (CH═), 5.81 (H4); IR ($CHCl_3$) 1736, 1665, 1634, 1613, 921 $cm^{-1}$. Anal. ($C_{23}H_{30}O_2S$) C,H,S.

**3,17-Dioxo-19-[(vinylthio)methyl]androsta-4,9(11)-diene (26).** The starting steroid **21** (265 mg, 0.5 mmol) dissolved in 50 mL of THF was introduced into a 250 mL flask equipped with a magnetic stirrer and an argon inlet. The flask was cooled to −78 °C, and vinylmagnesium bromide (1 mL of a 1 M solution in THF (Fluka), 1 mmol) was slowly added. The resulting black mixture was stirred for 1 h at this temperature and then brought to room temperature. After 30 min at room temperature, the reaction mixture was hydrolyzed with a saturated solution of ammonium chloride and concentrated on the rotavap, and the aqueous phase was extracted with ethyl acetate. The organic extracts were dried and concentrated. The crude material was crystallized from diisopropyl ether to

afford 40 mg of the expected material as a beige solid (22.4%): $R_f$ = 0.5 (ethyl acetate:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 5.12 and 5.23 (2d, $J$ = 16.5, 10, $CH_2$=), 5.59 (m, H11), 5.82 (d, H4), 6.29 (dd, $J$ = 10, 16.5, CH=) (NMR shows that the compound is contaminated with 12−15% of the product resulting from Michael cyclization of the thiol function on the 4,5-double bond); IR ($CHCl_3$) 1736, 1585, 956 $cm^{-1}$; HRMS calcd for $C_{22}H_{28}O_2S$ 356.181002, found 356.182254. Anal. ($C_{22}H_{28}O_2S$) H,S; C: calcd, 74.1; found, 73.3.

**3,17-Dioxo-19-[[(methylthio)thio]methyl]androsta-4,9(11)-diene (27).** The starting steroid **25** (625 mg, 1.01 mmol) dissolved in 50 mL of THF was introduced into a 250 mL flask equipped with a magnetic stirrer and an argon inlet. The flask was cooled to 0 °C, and sodium thiomethoxide (300 mg, 4.3 mmol; Aldrich) was added. The resulting black mixture was stirred for 1 h at this temperature and then brought to room temperature. After 30 min at room temperature, the reaction mixture was hydrolyzed with a saturated solution of ammonium chloride and concentrated on the rotavap, and the aqueous phase was extracted with ethyl acetate. The organic extracts were dried, concentrated, and chromatographed (cyclohexane:ethyl acetate, 7:3) to afford 227 mg of the expected material as a yellow oil (49%): $R_f$ = 0.8 (ethyl acetate:cyclohexane, 2:8); NMR ($CDCl_3$, 300 MHz) 0.85 (s, 18-Me), 2.36 (s, MeS), 2.52 (t, $CH_2S$), 3.82−4.0 ($OCH_2CH_2O$), 5.50 and 5.53 (H6, H11); IR ($CHCl_3$) 1672, 1632, 1086, 950 $cm^{-1}$; MS $M^+$ 417.

The previous compound (227 mg, 0.5 mmol) was dissolved in 50 mL of methanol and 5 mL of methylene dichloride and stirred with 4 mL of 6 N HCl. After 1 h at room temperature, the reaction mixture was worked up and chromatographed (dichloromethane:AcOEt, 1:1) to afford the expected material as a white solid (116 mg, 63%): $R_f$ = 0.33 (cyclohexane:ethyl acetate:methylene dichloride, 42.5:15:42.5); mp 77 °C; NMR ($CDCl_3$, 250 MHz) 0.9 (s, 18-Me), 2.38 (s, MeS), 5.56 (m, H11), 5.83 (d, H4); IR ($CHCl_3$) 1735, 1665, 1633, 1614 $cm^{-1}$. Anal. ($C_{21}H_{28}O_2S_2$) C,H,S.

**3,17-Dioxo-19-[[(phenylthio)thio]methyl]androsta-4,9(11)-diene (28).** The previous procedure was applied to 617 mg (1 mmol) of steroid **25** and 400 mg (3 mmol) of sodium thiophenoxide in 80 mL of degassed anhydrous THF. After similar workup and flash chromatography (cyclohexane: AcOEt, 75:25), the expected material was obtained as a white foam (270 mg, 46%): $R_f$ = 0.5 (cyclohexane:AcOEt, 7:3); NMR ($CDCl_3$, 300 MHz) 0.70 (s, 18-Me), 3.8−4.0 ($OCH_2CH_2O$), 5.45 and 5.50 (2m, H6, H11), 7.15−7.35 and 7.50 (m, Ar); IR ($CHCl_3$) 1581, 1477 $cm^{-1}$.

A next fraction consisted in the disulfide of the starting thiosteroid (240 mg, 51%): $R_f$ = 0.25 (cyclohexane:AcOEt, 7:3); NMR ($CDCl_3$, 300 MHz) 0.83 (s, 18-Me), 3.8−4.0 ($OCH_2CH_2O$), 5.49 and 5.53 (2m, H6, H11); IR ($CHCl_3$) 1670, 1635 $cm^{-1}$; MS $M^+$ 834.

The same hydrolyzing procedure as the one described for **21** was applied to 270 mg (0.47 mmol) of the previous compound dissolved in 50 mL of methanol and 5 mL of 6 N HCl. After 3 h at room temperature, the reaction mixture was worked up and chromatographed (cyclohexane:ethyl acetate, 7:3) to afford the expected material as a white foam (105 mg, 51%): $R_f$ = 0.5 (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.69 (s, 18-Me), 5.58 (m, H11), 5.78 (d, H4), 7.23, 7.32, and 7.5 (tm, tm, dm, Ar); IR ($CHCl_3$) 1736, 1665 $cm^{-1}$. Anal. ($C_{26}H_{30}O_2S_2$) C,H,S.

**3,17-Dioxo-19-[(cyclopropylthio)methyl]androsta-4,9(11)-diene (29).** Bromocyclopropane (0.36 mL, 4.5 mmol) dissolved in 15 mL of THF was introduced into a 100 mL flask equipped with a magnetic stirrer and an argon inlet. The flask was cooled to −78 °C, and tBuLi (0.65 mL of a 1.7 M solution in hexane, 4.5 mmol) was slowly added. The mixture was stirred for 10 min at this temperature; then 617 mg (1 mmol) of the starting steroid **25** dissolved in 15 mL of THF was added dropwise. The resulting black solution was stirred for 1 h at room temperature and then hydrolyzed with a saturated solution of ammonium chloride and concentrated on the rotavap, and the aqueous phase was extracted with ethyl acetate. The organic extracts were dried and concentrated. The crude material was chromatographed with cyclohexane:methylene dichloride:ethyl ether, 50:50:5, to afford 170 mg of the expected material as an orange oil (37%) ($R_f$ = 0.65, dichloromethane:ethyl ether, 95:5). This material was dissolved in 30 mL of methanol and hydrolyzed with 3 mL of 6 N HCl. After 2 h at room temperature, the reaction mixture was worked up and chromatographed (dichloromethane:ethyl ether, 97:3, and then cyclohexane:ethyl acetate, 8:2) to afford the expected material as a white solid (75 mg, 55%): $R_f$ = 0.5 (methylene dichloride:ethyl ether, 95:5); mp 100 °C; NMR ($CDCl_3$, 300 MHz) 0.53 and 0.84 (2m, $CH_2$-cyclopropyl), 0.90 (s, 18-Me), 5.56 (m, H11), 5.81 (s, H4); IR ($CHCl_3$) 1736, 1665, 1633, 1613 $cm^{-1}$. Anal. ($C_{23}H_{30}O_2S$) C,H,S.

**3,17-Dioxo-19-[[[(trimethylsilyl)ethynyl]thio]methyl]-androsta-4,9(11)-diene (30).** (Trimethylsilyl)acetylene (0.14 mL, 1 mmol) dissolved in 60 mL of THF was introduced into a 100 mL flask equipped with a magnetic stirrer and an argon inlet. The flask was cooled to −78 °C, and nBuLi (0.91 mL of a 1.1 M solution in hexane, 1 mmol) was slowly added. The clear resulting solution was stirred for 15 min at −78 °C; then it was cannulated into a −78 °C cooled solution of 528 mg of the starting steroid **21** in 20 mL of THF. The black solution was stirred for 1 h at this temperature and then brought to room temperature. The reaction mixture was then hydrolyzed with a saturated solution of ammonium chloride and concentrated on the rotavap, and the aqueous phase was extracted with ethyl acetate. The organic extracts were dried, concentrated, and chromatographed (cyclohexane:ethyl acetate, 1:1) to afford 180 mg of the expected material as a yellow foam (42%): $R_f$ = 0.5 (ethyl acetate:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.18 (s, TMS), 0.89 (s, 18-Me), 5.57 (m, H11), 5.84 (H4); IR ($CHCl_3$) 2092, 1736, 1666, 1636, 1616 $cm^{-1}$.

**3,17-Dioxo-19-[(ethynylthio)methyl]androsta-4,9(11)-diene (31).** Steroid **30** (110 mg, 0.26 mmol) dissolved in 20 mL of THF was introduced in a 100 mL flask equipped with a magnetic stirrer and an argon inlet; 0.3 mL (0.3 mmol) of a 1 M solution of tetrabutylammonium fluoride was added, and the resulting black solution was stirred for 15 min at room temperature. Water (50 mL) was added to the reaction mixture, and it was concentrated on the rotavap; then the aqueous phase was extracted with AcOEt. The organic extracts were dried, concentrated, and chromatographed with cyclohexane:AcOEt, 7:3, to afford 60 mg of the expected material as a white solid (65%): mp 174 °C; $R_f$ = 0.5 (cyclohexane:ethyl acetate, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 2.8 (s, CH), 5.57 (m, H11), 5.83 (d, H4); IR ($CHCl_3$) 3301, 1736, 1667, 1614 $cm^{-1}$; HRMS calcd for $C_{22}H_{27}O_2S$ 355.173177, found 355.173490. Anal. ($C_{22}H_{26}O_2S$) H,S; C: calcd, 74.6; found, 73.6.

**3,17-Dioxo-19-[[(fluoromethyl)thio]methyl]androsta-4,9(11)-diene (32).** Steroid **7** (500 mg, 1.4 mmol) was dissolved in 4 mL of methanol and introduced into a 10 mL round-bottomed flask equipped with a magnetic stirrer and an argon inlet; 373 mg (1.2 equiv, 1.7 mmol) of sodium periodate dissolved in 4 mL of water was added, and the reaction mixture was stirred for 30 min at room temperature. The resulting milky suspension was extracted with methylene dichloride, and the organic extracts were dried, concentrated, and chromatographed twice (AcOEt:methanol, 7:3) to afford 276 mg (52%) of the expected sulfoxide as a white foam. It was dried for 72 h under vacuum: $R_f$ = 0.24 (ethyl acetate:methanol, 7:3); NMR ($CDCl_3$, 300 MHz) (compound consisted of a 1:1 mixture of diasteroisomers) 0.86 and 0.92 (2s, 18-Me), 2.56 and 2.58 (2s, SMe), 5.59 (m, H11), 5.86 (s, H4); IR ($CHCl_3$) 1736, 1668, 1630, 1615, 1046 $cm^{-1}$. Anal. ($C_{21}H_{28}O_3S$) C,H,S. Microfischer: 4.3% $H_2O$.

The previous sulfoxide (482 mg, 1.34 mmol) was dissolved in 1.5 mL of chloroform and introduced into a 10 mL round-bottomed flask equipped with magnetic stirring, a reflux condenser, and an argon inlet. The flask was wrapped in aluminum foil, 12.8 mg (0.04 mmol) of zinc iodide was added under good stirring, and then 431 mg of DAST was added dropwise. The mixture was stirred for 3 h at room temperature and then at 50 °C for 1 h. The red mixture was concentrated on the rotavap and chromatographed with AcOEt:cyclohexane, 3:7. The first fraction (73.1 mg) consisted mainly of 3,17-dioxo-10-[(methylthio)vinyl]androsta-4,9(11)-

diene (16%): $R_f = 0.4$ (ethyl acetate:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 2.23 (s, $\Delta E$ MeS), 5.02 (d, CH-S), 5.7 (H11), 5.97 (H4, CH=C); IR ($CHCl_3$) 1735, 1672, 1615 $cm^{-1}$.

The next fraction was the expected material (198.4 mg, 41%). One recrystallization from ethyl ether gave 144 mg of the material as yellow crystals containing 15% of the steroid **7** (NMR): $R_f = 0.32$ (ethyl acetate:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 5.49 (d, $J = 53$, $SCH_2F$), 5.58 (H11), 5.82 (d, H4); IR ($CHCl_3$) 1735, 1667, 1614, 975 $cm^{-1}$; HRMS calcd for $C_{21}H_{27}FO_2S$ 362.171580, found 362.171982. Anal. ($C_{21}H_{27}FO_2S$) C,H,S; F: calcd, 5.24; found, 4.6.

**3,17-Dioxo-19-[(methylthio)methyl]androst-4-ene (34).** Alcohol **36** (300 mg, 0.71 mmol) and 0.3 mL (2.13 mmol, 3 equiv) of triethylamine were dissolved in 10 mL of methylene dichloride and introduced into a 50 mL flask. The flask was cooled to 0 °C, 0.3 mL (2.13 mmol, 3 equiv) of mesyl chloride added dropwise, and the mixture stirred for 45 min at 0 °C. It was poured into a saturated solution of sodium bicarbonate and extracted with dichloromethane. The organic extracts were dried and concentrated to afford 400 mg of the crude mesylate which was used as such in the next step: $R_f = 0.38$ (AcOEt:cyclohexane, 1:1).

The previous mesylate (400 mg, 0.71 mmol) 70 mg (1 mmol, 1.4 equiv) of sodium thiomethoxide, and 5 mL of DDMF were stirred at room temperature for 1 h. The reaction mixture was poured into a saturated solution of ammonium chloride and extracted with dichloromethane. The organic extracts were dried and concentrated to afford 360 mg of the crude steroid which was used as such in the next step: $R_f = 0.58$ (AcOEt: cyclohexane, 1:1).

The previous steroid (360 mg, 0.8 mmol) 1.5 mL of 6 N HCl, and 5 mL of ethanol were stirred for 3 h at room temperature. The mixture was neutralized with 2 N sodium hydroxide (pH paper) and then extracted with dichloromethane (2 × 20 mL). The organic extracts were washed with water, dried, and concentrated to afford 310 mg of crude oil. Flash chromatography with AcOEt:cyclohexane, 2:8, gave 140 mg of the expected compound (52% overall yield). This was rechromatographed on a Lobar column (Merck; Si60, 40−63 $\mu$m) with AcOEt:cyclohexane, 2:8, to afford 70 mg of the expected compound as a white foam (26%): $R_f = 0.5$ ($CH_2Cl_2$:AcOEt, 8:2); NMR ($CDCl_3$, 300 MHz) 0.93 (s, 18-Me), 2.13 (s, SMe), 5.92 (s, H4); IR ($CHCl_3$) 1735, 1667, 1617 $cm^{-1}$. Anal. ($C_{21}H_{30}O_2S$) C,H,S.

**3,17-Bis(ethylenedioxy)-5α-hydroxy-19-(hydroxymethyl)androstane (36).** A 1 L 3-necked flask equipped with a magnetic stirrer, an internal thermometer, a reflux condenser, and an argon inlet was charged with 10 g (26 mmol) of 3,17-bis(ethylenedioxy)-5α,10α-epoxyandrostane (**14**) and then with 250 mL of ethylenediamine; 50 g (0.54 mol, 20 equiv) of lithium acetylide−ethylenediamiine complex was cautiously added to this solution, and the mixture was brought to 50 °C and stirred for 96 h. The reaction mixture was cautiously poured on ice and water, and after completion of the abundant foam formation, extracted twice with ethyl acetate (total of 1 L). The organic extracts were washed with a saturated solution of ammonium chloride, dried, and then decolorized with activated carbon. The resulting yellow solution was concentrated to afford 13 g of crude brown oil. Flash chromatography with cyclohexane:ethyl acetate, 8:2, yielded 8.2 g of product (81%). This fraction was dissolved in a minimum of methylene dichloride and recrystallized in 5 vol of diisopropyl ether to afford 4.4 g of the expected compound as white crystals: $R_f = 0.7$ (AcOEt:hexane, 3:7).

The previous steroid (4.4 g, 11.3 mmol) dissolved in 150 mL of ethyl acetate and 3.5 mL of pyridine were hydrogenated over palladium hydroxide (100 mg, 10% on $BaSO_4$) under 1.4 bar of hydrogen. After 1.15 h, the absorption of hydrogen was complete. The mixture was filtered over Celite and concentrated to afford 4.5 g of the expected olefin as a white powder (42%): mp 175 °C; $R_f = 0.38$ ($CH_3CN$:$H_2O$, 8:2, reverse phase silica gel); NMR ($CDCl_3$, 300 MHz) 0.74 (s, 18-Me), 3.8−4.0 ($OCH_2CH_2O$), 4.37 (d, OH), 5.00 (dd, $J = 17.5$, 1.5) and 5.32 (dd, $J = 11$, 1.5) ($CH_2$=), 5.91 (dd, CH=); MS $M^+$ 404.

The previous compound (2 g, 5 mmol) was introduced into a 100 mL flask under argon and covered with 25 mL of THF. The mixture was cooled to 0 °C; then 1 mL (2 equiv) of the complex borane−dimethyl sulfide was added dropwise. The mixture was brought to 35 °C for 4 h and then cooled to 0 °C again, and 1.75 mL of concentrated sodium hydroxide followed by 1.75 mL of hydrogen peroxide (30% aqueous) was cautiously added. After 12 h of stirring at room temperature, the mixture was poured into 200 mL of water and extracted with ethyl acetate (2 × 200 mL). The organic extracts were dried and concentrated to afford 2.2 g of a colorless oil. Flash chromatography with ethyl acetate:cyclohexane, 2:8, yielded 1.4 g of pure alcohol **36** as a white foam (67%): $R_f = 0.26$ (AcOEt: cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.85 (s, 18-Me), 3.6−4 ($OCH_2CH_2O$, $CH_2OH$), 4.21 (s, OH); IR ($CHCl_3$) 3621, 3504 $cm^{-1}$.

**3,17-Dioxo-19-[(*N*,*N*-dimethylamino)methyl]androsta-4,9(11)-diene (38).** Amide **37**[8] (2 g, 5.57 mmol) 657 mg (15.5 equiv) of lithium aluminum hydride, and 100 mL of dioxane were refluxed under argon for 1 h. The reaction mixture was cooled to 0 °C, and the excess reagent was destroyed by slow addition of 30 mL of AcOEt followed by 5 mL of 2 M sodium hydroxide. The resulting suspension was filtered on Celite and concentrated to afford 2.26 g of the crude product as a white foam; the product consisted of a mixture of 3α,17β- and 3β,17β-diols: $R_f = 0.06$ and 0.16 (AcOEt:cyclohexane, 2:1, 10% triethylamine); NMR ($CDCl_3$, 300 MHz) 0.74 (s, 18-Me), 2.33 (s, $NMe_2$), 3.74 (t, H17), 4.13 (m, H3), 5.41 and 5.48 (m, H4, H11); IR ($CHCl_3$) 3612, 1656, 1630 $cm^{-1}$.

This material was dissolved in 200 mL of toluene and refluxed for 30 min in the presence of 10 mL of cyclohexanone; then 4 g of aluminum triisopropoxide was added and the mixture refluxed for 1 h. The mixture was cooled to room temperature, treated with 100 mL of 10% aqueous sodium potassium tartrate, and extracted with AcOEt (3 × 300 mL). The organic extracts were washed with 100 mL of brine, dried, concentrated, and chromatographed (hexane and then AcOEt: hexane, 1:1 and then 2:1, and then 10% triethylamine). The last fraction was the expected amine (156 mg, 8%). One recrystallization from ethyl ether gave 50 mg of white crystals: mp 134−136 °C; $R_f = 0.2$ (cyclohexane:AcOEt, 1:2, 10% triethylamine); NMR ($CDCl_3$, 300 MHz) 0.90 (s, 18-Me), 2.21 (s, $NMe_2$), 5.54 (H11), 5.80 (s, H4). Anal. ($C_{22}H_{31}NO_2$) C,H,N.

**3,17-Dioxo-19-(methoxymethyl)androsta-4,9(11)-diene (39).** Alcohol **10** (800 mg, 2 mmol) was dissolved in 15 mL of THF and introduced into a 50 mL flask equipped with a magnetic stirrer and an argon inlet. The mixture was cooled to 0 °C, 245 mg (2.2 mmol) of potassium *tert*-butoxide was added all at once, and the resulting suspension was stirred for 30 min; 0.62 mL (10 mmol, 5 equiv) of methyl iodide was added dropwise, and the mixture was brought to room temperature and then poured into 50 mL of a saturated solution of ammonium chloride and extracted with methylene dichloride. The organic extracts were dried and concentrated to afford 700 mg of the crude product as a white foam (84.5%). It was used in the next step without further purification: $R_f =$ 0.62 (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.84 (s, 18-Me), 3.28 (s, OMe), 3.2−3.4 ($CH_2O$), 3.8−4 ($OCH_2CH_2O$), 5.51 (H6, H11); IR ($CHCl_3$) 1111 $cm^{-1}$.

The previous steroid (700 mg, 1.7 mmol), 4 mL of 6 N HCl, and 40 mL of 99% ethanol were stirred for 1 h at room temperature and then poured into 50 mL of a saturated solution of ammonium chloride and extracted with methylene dichloride. The organic extracts were dried, concentrated, and chromatographed with ethyl acetate:cyclohexane, 3:7, to afford 500 mg (94%) of the expected compound as a colorless oil. Crystallization from ethyl ether gave a white solid: mp 94 °C; $R_f = 0.36$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 3.2−3.4 ($CH_2O$), 3.28 (s, OMe), 5.57 (m, H11), 5.81 (s, H4); IR ($CHCl_3$) 1735, 1667, 1615 $cm^{-1}$. Anal. ($C_{22}H_{28}O_3$) C,H.

**3,17-Dioxo-19-[[(methylsulfonyl)oxy]methyl]androsta-4,9(11)-diene (40).** Mesylate **11** (2.5 g) dissolved in 30 mL of 99% ethanol was introduced in a 500 mL flask equipped with magnetic stirring and cooled to 0 °C; 5 mL of 6 N HCl was added, and the mixture stirred at 0 °C for 1 h and then at

−30 °C for 72 h. The reaction mixture was hydrolyzed by a saturated solution of sodium bicarbonate, the organic phase was extracted with dichloromethane (2 × 100 mL), and the organic extracts were dried over magnesium sulfate and concentrated to dryness. The crude mixture was flash chromatographed with cyclohexane:ethyl acetate, 1:1, to afford a first fraction of starting mesylate (47 mg, 19%) followed by a fraction of the expected deprotected mesylate (1.24 g, 61%); 1 g of this material was recrystallized from ether to afford 854 mg of pure material obtained as a yellow solid: $R_f$ = 0.22 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.89 (s, 18-Me), 3.0 (s, $OSO_2Me$), 4.18 (t, $CH_2OSO_2$), 5.64 (m, H11), 5.85 (s, H4), 1.1−2.7 (m, others); IR ($CHCl_3$) 1737, 1670, 1632, 1615 $cm^{-1}$. Anal. ($C_{21}H_{28}O_5S$) C,H,S.

**3,17-Dioxo-19-[(phenylseleno)methyl]androsta-4,9(11)-diene (41).** Mesylate **11** (450 mg, 1 mmol) was dissolved in 8 mL of HMPA and treated with 0.11 mL (1.1 mmol) of selenophenol followed by 25 mg (1.1 mmol) of Na. The mixture was stirred at room temperature for 1.5 h and then hydrolyzed with water and extracted with AcOEt. After drying and concentration, the crude mixture was flash chromatographed (AcOEt:cyclohexane, 2:8) to afford 128 mg (26%) of the expected compound as a white foam: $R_f$ = 0.68 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.75 (s, 18-Me), 3.8−4.0 ($OCH_2CH_2O$), 5.50 (m, H6, and H11), 7.24 and 7.47 (2m, Ar); IR ($CHCl_3$) 1656, 1528, 1478 $cm^{-1}$; MS 542 $M^+$.

This material was dissolved in 5 mL of ethanol and hydrolyzed with 1 mL of 6 N HCl for 12 h at room temperature. After treatment with aqueous sodium bicarbonate, the mixture was extracted with dichloromethane, and the organic extracts were dried, concentrated, and flash chromatographed (AcOEt:cyclohexane, 3:7) to afford 62 mg of the expected seleno compound as a white solid: mp 87 °C; $R_f$ = 0.55 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.79 (s, 18-Me), 5.53 (m, H11), 5.77 (d, H4), 7.28 and 7.5 (2m, Ar); IR ($CHCl_3$) 1737, 1665, 1630, 1613, 1576 $cm^{-1}$. Anal. ($C_{26}H_{30}O_2Se$) C,H.

**3,17-Dioxo-19-(iodomethyl)androsta-4,9(11)-diene (42).** Mesylate **40** (200 mg, 0.5 mmol) was introduced into a 30 mL flask equipped with a reflux condenser and a magnetic stirrer and dissolved in 10 mL of methyl ethyl ketone under argon; 152 mg (1 mmol, 2 equiv) of sodium iodide was added and the mixture refluxed for 2 h. Ethyl acetate (25 mL) was added to the mixture after cooling to room temperature, and the organic phase was washed with a saturated solution of sodium thiosulfate, dried over magnesium sulfate, and concentrated to dryness to afford 181 mg (82%) of the expected iodo compound **40** as a white solid: mp 170 °C; $R_f$ = 0.84 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.89 (s, 18-Me), 3.00 (m, $CH_2I$), 5.57 (m, H11), 5.82 (d, H4); IR ($CHCl_3$) 1737, 1667, 1632, 1614 $cm^{-1}$. Anal. ($C_{20}H_{25}IO_2$) C,H,N.

**3,17-Dioxo-19-[(thiocyanato)methyl]androsta-5(6),9(11)-diene (43).** Iodo **42** (200 mg, 0.5 mmol) was introduced into a 30 mL flask equipped with a magnetic stirrer and dissolved in 10 mL of HMPA; 46 mg (0.5 mmol, 1 equiv) of potassium thiocyanate was added all at once, and the reaction mixture was stirred for 1.5 h at room temperature and then stored for 72 h at −30 °C. The reaction mixture was poured into 15 mL of water and extracted with dichloromethane (2 × 20 mL). The organic extracts were dried on magnesium sulfate, concentrated to dryness, and chromatographed (AcOEt:cyclohexane, 1:1) to afford 82.5 mg of the expected compound as a white solid (50%): $R_f$ = 0.29 (AcOEt:cyclohexane, 1:1); mp 159 °C; NMR ($CDCl_3$, 300 MHz) 0.90 (s, 18-Me), 5.60 (m, H11), 5.86 (d, H4); IR ($CHCl_3$) 2158, 1736, 1671, 1633, 1616 $cm^{-1}$. Anal. ($C_{21}H_{25}NO_2S$) C,H,N,S.

**3,17-Dioxo-(10*E*)-[(methylthio)vinyl]androsta-4,9(11)-diene (45).** Starting steroid **44**[16] (3.5 g, 9 mmol) ($R_f$ = 0.76, ethyl acetate:cyclohexane, 1:1), 2.08 g (4 equiv) of powdered potassium hydroxide, and 20 mL of methanol were stirred for 4 h at room temperature. The reaction mixture was neutralized with a saturated solution of ammonium chloride and extracted with dichloromethane, and the organic extracts were dried and concentrated to afford 2.1 g (78%) of crude diol which was used as such in the next step: $R_f$ = 0.33 (ethyl acetate:cyclohexane, 1:1).

Diethyl [(methylthio)methyl]phosphonate (6.72 g, 34 mmol) was introduced into a 250 mL flask equipped with a magnetic stirrer and an argon inlet and dissolved in 80 mL of THF. The solution was cooled to 0 °C, and 26.8 mL (37 mmol) of a 1.4 M solution of nBuLi in hexane was added dropwise. After 30 min of stirring at that temperature, 2 g (6.6 mmol) of the previous diol dissolved in 10 mL of THF was cannulated into the reaction mixture. The cooling bath was removed, and the mixture was stirred for 24 h at room temperature. A saturated solution of ammonium chloride was added and the mixture extracted with AcOEt. The organic extracts were washed with diluted ammonia, dried, concentrated, and chromatographed (AcOEt:cyclohexane, 3:7) to afford a first crop of the expected compound contaminated with the starting phosphonate (1.3 g). One crystallization from ethyl ether/pentane gave 400 mg of white crystals The next fraction (1.3 g) was pure (76%): $R_f$ = 0.44 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.66 (s, 18-Me), 2.27 (s, SMe), 3.55 (m, H3), 3.62 (t, H17), 5.25 and 5.90 (2d, $J$ = 15.5, CH=CH), 5.59 (m, H6); IR ($CHCl_3$) 3609, 1594 $cm^{-1}$.

The previous steroid (400 mg, 1.17 mmol) was introduced into a 50 mL flask equipped with a Dean−Stark trap and an argon inlet and refluxed for 4 h in the presence of 6 mL of cyclohexanone, 1.2 g of aluminum triisopropoxide, and 20 mL of toluene. The solution was then partitioned between sodium bicarbonate and methylene dichloride. The organic extracts were dried, concentrated, and chromatographed with ethyl acetate:cyclohexane, 3:7, to afford 300 mg of the expected material (74%). An analytical sample was obtained upon Lobar chromatography using the same eluting mixture (120 mg): mp 119−20 °C; $R_f$ = 0.45 (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 300 MHz) 0.89 (s, 18-Me), 2.28 (s, SMe), 5.51 and 5.96 (2d, $J$ = 15.5, CH=CH), 5.94 (m, H4); IR ($CHCl_3$) 1735, 1667, 1618, 1592 $cm^{-1}$. Anal. ($C_{21}H_{28}O_2S$) C,H,S.

**3,17-Dioxo-(19*E*)-[(methylthio)vinyl]androsta-4,9(11)-diene (47) and 3,17-Dioxo-(19*Z*)-[(methylthio)vinyl]androsta-4,9(11)-diene (48).** [(Methylthio)methyl]triphenylphosphonium chloride (5.17 g, 14.4 mmol) was introduced into a 250 mL flask equipped with a magnetic stirrer and an argon inlet and covered with 50 mL of THF; 9 mL (14.4 mmol) of a 1.6 M solution of nBuLi in hexane was added dropwise to this room temperature stirred mixture. The suspension turned yellow and then orange and finally became clear; 3 g (9.6 mmol) of aldehyde **46** in 15 mL of THF was then cannulated into the reaction flask. The mixture was stirred for 15 min at room temperature and then treated with 50 mL of a saturated solution of ammonium chloride. The aqueous phase was extracted with dichloromethane (2 × 150 mL); the organic extracts were dried, concentrated, and chromatographed (AcOEt:cyclohexane, 2:8) to afford a first fraction of the pure *Z* isomer **48** (600 mg) as an oil, a second fraction of a 1:1 mixture of the *E*:*Z* isomers (1.58 g), and a third fraction of the pure *E* isomer **47** (410 mg) as a white powder (total yield, 76%).

**47**: mp 129 °C; $R_f$ = 0.46 (cyclohexane:ethyl acetate, 1:1); NMR ($CDCl_3$, 300 MHz) 0.89 (s, 18-Me), 2.19 (s, MeS), 5.52 (m, H11), 5.81 (d, H4), 6.07 and 5.18 (d, ddd, $J$ = 15, 15, 7, 8, CH=CH); IR ($CHCl_3$) 1735, 1665, 1614 $cm^{-1}$; HRMS calcd for $C_{22}H_{28}O_2S$ 356.181002, found 356.179799. Anal. ($C_{22}H_{28}O_2S$) H,S; C: calcd, 74.11; found, 74.8.

**48**: $R_f$ = 0.53 (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.88 (s, 18-Me), 2.27 (s, MeS), 5.52 (m, H11), 5.83 (d, H4), 6.02 and 5.33 (dt, m, $J$ = 10.5, 1, CH=CH); IR ($CHCl_3$) 1735, 1665, 1614 $cm^{-1}$. Anal. ($C_{22}H_{28}O_2S$) C,H,S.

**3,17-Bis(ethylenedioxy)-19-formylandrosta-5(6),9(11)-diene (49).** Oxalyl chloride (4.84 mL, 55 mmol) was dissolved in 40 mL of methylene chloride, and the solution was cooled at −50 °C under argon; 6.68 mL (94 mmol) of dimethyl sulfoxide was added dropwise at this temperature, and the resulting mixture was stirred for 5 min; 20 g (50 mmol) of the steroid **10** was dissolved in 30 mL of methylene dichloride and added dropwise to the reaction mixture. The resulting mixture was then stirred for 15 min at −20 °C; then 38 mL (274 mmol) of triethylamine was added dropwise. After 5 min of stirring at this temperature, the reaction mixture was partitioned between sodium bicarbonate and methylene dichloride. The organic extracts were dried, concentrated, and chromato-

graphed with ethyl acetate:cyclohexane, 2:8, to afford 18 g (90%) of the expected aldehyde as a white foam: $R_f = 0.33$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.79 (s, 18-Me), 3.8−4.0 ($OCH_2CH_2O$), 5.57 and 5.63 (2m, H6, H11), 9.69 (dd, CHO); IR ($CHCl_3$) 1715, 1675, 1636 $cm^{-1}$.

**3,17-Dioxo-19-[(dimethylthio)methyl]androsta-4,9(11)-diene (50).** Aldehyde **49** (600 mg, 1.5 mmol) was dissolved in 10 mL of THF, and the solution was cooled at −78 °C in a flask equipped with magnetic stirring and an argon inlet. Methanethiol was bubbled through the solution for 1 min; then 0.185 mL (1 equiv) of boron trifluoride etherate was added dropwise and the solution brought to room temperature. A stream of methanethiol was passed again through the solution, and the mixture stirred for 3 h. The mixture was partitioned between aqueous sodium bicarbonate and methylene dichloride, and the organic extracts were dried and concentrated to afford 600 mg of crude oil. This material was dissolved in 5 mL of THF and stirred with 2 mL of 6 N HCl for 2.5 h. The mixture was partitioned between aqueous sodium bicarbonate and methylene dichloride, and the organic extracts were dried, concentrated, and chromatographed with ethyl acetate:cyclohexane, 1:2, to afford 300 mg of the expected thioacetal (51%). Recrystallization from diethyl ether/pentane gave 130 mg of white crystals: mp 144−145 °C; $R_f = 0.59$ (cyclohexane:AcOEt, 7:3); NMR ($CDCl_3$, 300 MHz) 0.98 (s, 18-Me), 2.05 and 2.08 (2s, SMe), 3.51 (dd, $J = 6$, 2.5, $CHSMe_2$), 5.65 (m, H11), 5.84 (H4). Anal. ($C_{22}H_{30}O_2S_2$) C,H,S.

**3,17-Dioxo-19-[(diethylthio)methyl]androsta-4,9(11)-diene (51).** Aldehyde **49** (500 mg, 1.25 mmol) was dissolved in 5 mL of dichloromethane and the mixture cooled to −78 °C under argon; 0.185 mL (2 equiv) of ethanethiol followed by 0.154 mL (1 equiv) of boron trifluoride etherate was added dropwise to this solution, and the mixture was stirred for 1 h; 10 mL of aqueous sodium bicarbonate was added and the mixture extracted with methylene dichloride. The organic extracts were dried and concentrated to afford 515 mg of crude oil. Crystallization from diethyl ether/pentane gave 350 mg of white crystals (55%): $R_f = 0.76$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.92 (s, 18-Me), 1.20 and 1.24 (2t, $CH_3CH_2S$), 3.8−4.0 ($OCH_2CH_2O$), 5.46 and 5.62 (2m, H6, H11); IR ($CHCl_3$) 1676, 1633 $cm^{-1}$.

The previous steroid (400 mg, 0.79 mmol) was dissolved in 5 mL of 99% ethanol and stirred for 24 h at room temperature in the presence of 3 mL of 3 N HCl. The mixture was partitioned between aqueous sodium bicarbonate and methylene dichloride, and the organic extracts were dried, concentrated, and chromatographed with ethyl acetate:cyclohexane, 2:8, to afford 200 mg of the expected thioacetal (48%) which was obtained as white crystals after one crystallization from diethyl ether/pentane: mp 99−101 °C; $R_f = 0.61$ (cyclohexane: AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.99 (s, 18-Me), 1.2 and 1.25 (2t, $CH_3CH_2S$), 2.4−2.8 ($SCH_2CH_3$), 3.70 (dd, CHS), 5.65 (m, H11), 5.83 (s, H4). Anal. ($C_{24}H_{34}O_2S_2$) C,H,S.

**3,17-Dioxo-10-(methylthio)estra-4,9(11)-diene (53).** A solution of methanethiol was prepared by passing the gas in 100 mL of THF for 30 min. To this solution was added 94 mL of a 1.6 M solution of nBuLi in hexane (0.15 mol). A white precipitate formed which was redissolved by addition of 80 mL of DMF; 5 g (15 mmol) of the starting epoxide[17] was added to this solution, and the resulting mixture was stirred for 1 h at room temperature. The mixture was poured into an aqueous solution of ammonium chloride and extracted with AcOEt; the organic extracts were dried and concentrated. The crude oil was chromatographed (AcOEt:hexane, 1:1) to give 1.82 g of the expected 3-dimethoxy-5α-hydroxy-10β-(methylthio)androst-9(11)-ene (30%): $R_f = 0.57$; NMR ($CDCl_3$, 300 MHz) 0.84 (s, 18-Me), 1.74 (s, SMe), 3.22 and 3.23 (OMe), 4.90 (OH), 5.35 (m, H11), 2.79 (m), and 1.4−2.5 (m, steroid backbone); IR ($CHCl_3$) 3436, 1733 $cm^{-1}$.

This material was dissolved in 1.5 mL of ethanol, and 0.15 mL of 6 N HCl was added. After 10 min of stirring at room temperature, a white precipitate formed. It was filtered (70 mg). The filtrate was partitioned between dichloromethane and saturated aqueous sodium bicarbonate. After drying, concentration, and flash chromatography (AcOEt:hexane, 3:7), 100 mg of product was obtained. Both precipitate and chromatographed material corresponded to 5α-hydroxy-10β-methyl-3,17-dioxoandrost-9(11)-ene (96%): $R_f = 0.25$; NMR ($CDCl_3$, 300 MHz) 0.87 (s, 18-Me), 1.86 (s, SMe), 1.80 (OH), 3.01 (s, H4), 5.53 (m, H11) ; IR ($CHCl_3$) 3588, 1734, 1718 $cm^{-1}$.

These materials were combined, dissolved in 5 mL of MeOH, and added to 28 mL of a 0.1 M methanolic solution of sodium hydroxide. After 2 h of stirring at room temperature, 50 mL of a saturated solution of ammonium chloride was added and the mixture extracted with dichloromethane. The organic extracts were dried, concentrated, and flash chromatographed to afford 96 mg (60%) of the expected product as a white powder: $R_f = 0.43$ (AcOEt:hexane, 1:1); NMR ($CDCl_3$) 0.90 (s, 18-Me), 1.91 (s, SMe), 5.57 (H11), 5.88 (H4); IR ($CHCl_3$) 1735, 1672, 1627, 1612, 890 $cm^{-1}$. Anal. ($C_{19}H_{24}O_2S$) C,H,S.

**3,17-Dioxo-19-[(methylthio)ethyl]androsta-4,9(11)-diene (54).** Steroid **48** (1 g, 2.8 mmol), 180 mg of $RhCl(PPh_3)_3$ (0.19 mmol), and 30 mL of a 1:1 mixture of ethanol:toluene were introduced into a 250 mL hydrogenation flask. The apparatus was put under 1.5 atm of hydrogen for 15 h; after that time the volume of absorbed hydrogen was 75 mL (theoretical, 65 mL). The mixture was concentrated an the rotavap and then chromatographed with ethyl acetate:cyclohexane, 1:1, to afford 800 mg (80%) of the expected material as a colorless oil that crystallized at −30 °C: mp 80 °C; $R_f = 0.47$ (cyclohexane:ethyl acetate, 1:1); NMR ($CDCl_3$, 300 MHz) 0.89 (s, 18-Me), 2.07 (s, SMe), 5.53 (m, H11), 5.79 (d, H4); IR ($CHCl_3$) 1735, 1664, 1622, 1611 $cm^{-1}$; MS $M^+$ 358. Anal. ($C_{22}H_{30}O_2S$) C,H,S.

**3,17-Bis(ethylenedioxy)androsta-5(6),9(11)-diene-19-carboxylic Acid (55).** Ester **9** (4.17 g, 9.4 mmol) dissolved in 150 mL of methanol was refluxed for 60 h in the presence of 17 mL of a 3 M methanolic solution of potassium hydroxide; 15 mL of the potassium hydroxide was re-added and the mixture refluxed for another 3 h; 200 mL of water was added, and the mixture was extracted with methylene dichloride (200 mL). The aqueous phase was acidified to pH 3 with 0.1 N HCl and extracted with diethyl ether (2 × 100 mL) and ethyl acetate (100 mL). The organic extracts were dried and concentrated to afford 3.8 g of crude acid as a colorless oil (95%): $R_f = 0.37$ (AcOEt:cyclohexane, 1:1); NMR ($CDCl_3$, 1 drop of $C_5D_5N$, 300 MHz) 0.92 (s, 18-Me), 2.58 and 2.73 (2d, $J = 13$, $COCH_2$), 3.8−4.0 ($OCH_2CH_2O$), 5.55 and 5.66 (H6, H11); IR ($CHCl_3$) 1733, 1705, 1636 $cm^{-1}$.

**3,17-Dioxo-19-[(methylthio)carbonyl]androsta-4,9(11)-diene (56).** The previous acid **55** (5 g, 12 mmol) was dissolved in 12 mL of ether, and 1.85 mL (1.1 equiv) of triethylamine was added followed by 1.28 mL (1.1 equiv) of ethyl chloroformate. The mixture was stirred for 1 h at room temperature; then 1.26 g (1.5 equiv) of sodium thiomethoxide dissolved in 2 mL of DMF was added all at once. After 2 h of stirring, the mixture was partitioned between aqueous sodium bicarbonate and methylene dichloride. The organic phase was dried and concentrated to afford 4.6 g of crude product: $R_f = 0.66$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.96 (s, 18-Me), 2.23 (s, SMe), 2.85 and 2.9 (AB, $J = 14$, $CH_2CO$), 3.95 (m, $OCH_2CH_2O$), 5.59 (m, H6, H11).

This material was dissolved in 50 mL of 99% ethanol and stirred for 20 min at room temperature in the presence of 12 mL of 6 N HCl. The reaction mixture was partitioned between methylene dichloride and aqueous sodium bicarbonate. The organic phase was washed with 3 N sodium hydroxide, dried, concentrated, and flash chromatographed to afford a first fraction ($R_f = 0.60$, AcOEt:cyclohexane, 1:1) (1.2 g, 25%) of the expected compound still protected in the 3-position and a second fraction (1.3 g, 30%) of the expected product. Crystallization from pentane gave analytically pure material as a white powder: mp = 108 °C; $R_f = 0.46$ (cyclohexane:AcOEt, 1:1); NMR ($CDCl_3$, 300 MHz) 0.96 (s, 18-Me), 2.27 (s, SMe), 2.83 and 2.97 (AB, $J = 14$, $CH_2CO$), 5.61 (m, H11), 5.84 (d, H4). Anal. ($C_{21}H_{28}O_3S$) C,H,S.

**Determination of the $IC_{50}$'s of Aromatase Inhibition.** Human placentae were processed within 1 h after delivery. Microsomes were prepared by differential centrifugation as described previously.[20] Aromatase activity was measured by the amount of tritiated water released from [1β,2β-$^3$H]andros-

tenedione as described previously.[21] All data shown were calculated using linear regression analysis.

**Partial Purification of Human Placental Aromatase.** This was performed using the method previously described by Kellis and Vickery,[22] except that the DE-52 anion-exchange step was omitted. Androstenedione-free buffers were used for the last hydroxylapatite chromatographic step. The concentration of P450arom (0.78 $\mu$M) was determined by the difference spectrum induced by 19-(thiomethyl)androstenedione.[35] The enzyme in sodium phosphate buffer (10 mM, pH = 7.4) containing 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1% (v/v) Nonidet P40, and glycerol (20%, w/v) was stored at −70 °C. This preparation was used for the spectral analysis of the aromatase−inhibitor complexes.

**Spectral Analysis of the Aromatase−Inhibitor Complexes.** P450arom was diluted into 10 mM phosphate buffer, pH = 7.4. The enzyme was equilibrated at room temperature and placed in two 1 cm path length cells. Appropriate amounts of steroids dissolved in DMSO were added in the sample cell; the reference cell received DMSO alone. Spectra were recorded at ambient temperature using a Kontron Instruments Uvikon-941 spectrophotometer.

**Titration Experiments.** We used solubilized P450arom which was subjected to ammonium sulfate fractionation as previously described.[22] This degree of purification was sufficient to allow determination of binding constants ($K_s$). The material which precipitated in 35-55% ammonium sulfate saturation was dissolved in sodium phosphate buffer (10 mM, pH = 7.4) containing 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1% (v/v) Nonidet P40, and 20% (w/v) glycerol and dialyzed against four changes of 2 L of this buffer for 12 h. The concentration of P450arom of the dialyzed ammonium sulfate cut was 0.68 $\mu$M. The enzyme was stored in the same buffer at −70 °C.

The reconstitution of aromatase activity was based on a previously described method,[36] and the conversion of androstenedione to estrone was quantified by the release of tritium from [1$\beta$-$^3$H]androstenedione. Aromatase (1.9 nM) and purified rabbit liver NADPH-P450 reductase (2.7 nM)[37] were mixed in a small volume of 10 mM phosphate buffer (pH = 7.4 mM) containing 0.002% (v/v) Nonidet P40 (based on final assay volume) and 0.4% (w/v) glycerol (also based on final assay volume), dilauroylphosphatidylcholine (10 $\mu$g/mL) in phosphate buffer was added, and the mixture was incubated at 21 °C for 5 min. The volume was adjusted to 1 mL with 50 mM potassium phosphate buffer (pH = 7.4) containing various concentrations (36−216 nM) of [1$\beta$-$^3$H]androstenedione (sa = 27.5 Ci/mmol) in methanol and NADPH (600 $\mu$M). NADPH was omitted in control assays. Incubation was conducted at 37 °C for 7 min. $^3H_2O$ isolation was performed as previously described,[38] and an aliquot (1 mL) of the $^3H_2O$ phase was quantified by counting in 5 mL of Aqualyte (Baker analyzer reagent) in a Beckman LS 5000 TD liquid scintillation analyzer. The $K_m$ and $V_{max}$ values were determined by substracting the dpm from NADPH minus incubations from their respective NADPH positive incubations followed by the least-squares analysis of the double reciprocals of background-corrected activities versus androstenedione concentrations. The enzyme had an activity of 2.5 nmol of estrone/min/nmol of P450arom and a $K_m$ of 88 nM.

***$K_s$* Determination.** The stock solution of enzyme (0.68 $\mu$M) previously equilibrated at room temperature was saturated with androstenedione (20 $\mu$M) and divided into two cells, and the base line was recorded. Steroids were added sequentially to give the desired final concentrations in the sample cell; the reference cell received DMSO. Spectra were recorded for 10 min after each addition in a Cary 2200 spectrometer at 25 °C.

***In Vivo* Potency.** Female Wistar rats weighing 180−200 g were injected sc with 100 IU of PMSG; 90 h later, blood (2 mL) was collected retroorbitally under slight $CO_2$ anesthesia. Animals were given 5 mg/kg doses of the compounds orally (or in one case sc). Blood was collected 2 h after administration. In order to minimize the variability in the PMSG induction, each animal was its own control. After 6 h, the animals were sacrificed and total blood was collected. Estradiol was measured using a direct [$^{125}$I]estradiol RIA kit (ER155 Baxter Travenol).

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