Synthesis of and Chemical Model Reaction Studies with 3-Deoxyandrogens: Evidence Supporting a 2,3-Enolization Hypothesis in Human Placental Aromatase Catalysis

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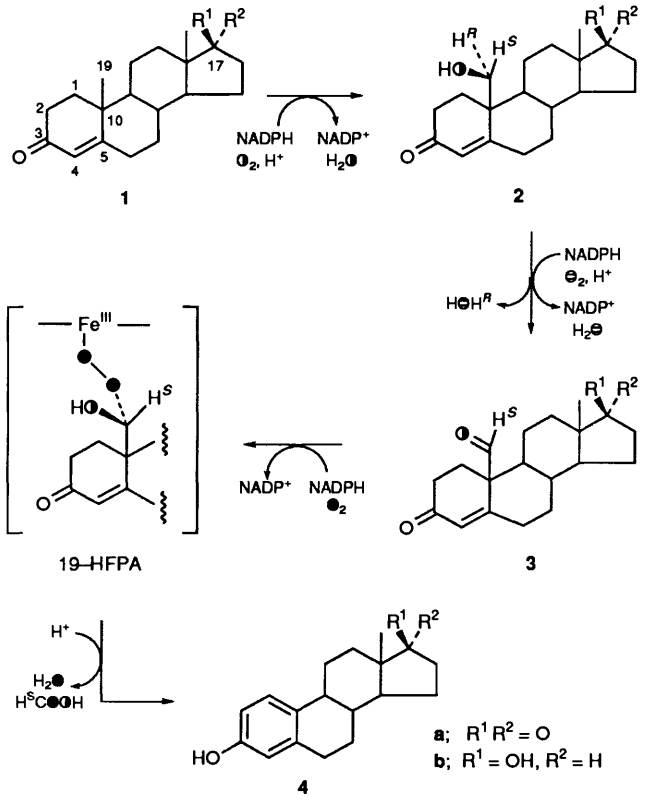
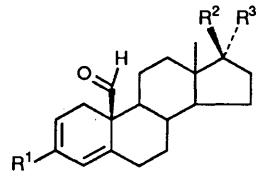
A number of hitherto undescribed Δ^2- and Δ^3-3-deoxy-5α-androgen derivatives (17β,19-diols **12** and **13**, 19-hydroxy-17-ketones **16** and **17**, 19-oxo-17-ketones **18** and **19**) were synthesized in good yield. The lithium–ammonia reduction of 19-(tetrahydropyran-2-yloxy)androst-4-ene-3,17-dione **7** followed by Shapiro reaction allowed easy construction of both Δ^2- and Δ^3-3-deoxy-5α-steroid systems. An improved synthesis of the known Δ^4-3-deoxyandrogen derivatives (**28**, **30**, **31**) was accomplished in high yield. Masking of the 19-hydroxy group was necessary in order to generate the Δ^4-3-deoxyandrogen system in good yield, in contrast to the account of an earlier synthesis of compounds **28** and **30** by Numazawa. Chemical model reactions of the third oxidative process in aromatase action were carried out with Δ^2-, Δ^3-, Δ^4- and $\Delta^{2,4}$-3-deoxy-19-oxo-17-keto steroids (**18**, **19**, **31** and **5**, respectively). The findings illustrate the need for both Δ^2- and Δ^4-unsaturation in order to generate $\Delta^{1(10)}$-unsaturation under the model reaction conditions. This study supports our 2,3-enolization hypothesis in the proposed catalytic mechanism for human placental aromatase. The importance of the stereoelectronic nature of substrate 1β-H in the enzyme-catalysed third oxidative process is discussed.

Human placental aromatase (HPA) is a cytochrome *P*-450 enzyme complex which catalyses three sequential oxidations[1] of androgens (**1**) to give estrogens (**4**), and several aspects of the catalysis[2] are summarized in Scheme 1. The first two oxidation steps (**1** ⟶ **2** ⟶ **3**) can be viewed as typical *P*-450 hydroxylations. The third oxidative process (**3** ⟶ **4**) may be rationalized with a concept originally formulated by Akhtar,[3] which features formation of a 19-hydroxy-19-ferricperoxy androgen (19-HFPA) following attack by the postulated haem ferric [iron(III)] peroxide species (Fe^{III}–OOH)[4] on the biosynthetic intermediate **3**.



Scheme 1 Conversion of androgens into estrogens by human placental aromatase

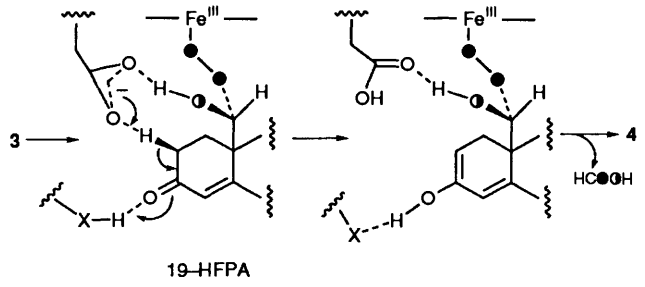
This laboratory has described[5] a chemical model reaction that supported Akhtar's concept. The model reaction, carried out with hydrogen peroxide, was faithful to the enzyme's third oxidative process with respect to aromatization of the steroid A-ring, production of formic acid, ^{18}O-incorporation pattern, and stereospecific loss of 1β-H. The model reaction proceeded well when $\Delta^{2,4}$-19-oxo steroids **5** and **6** were the substrates,[6] but was ineffective with 3,17-dioxoandrost-4-en-19-al (19-OxoA) **3a**.[7] It was shown[7b] that, in the case of compound **3a**, the predominant process under the model conditions was intramolecular Michael addition of the 19-peroxide to the steroid's 4-en-3-one grouping, after formation of the 19-hydroxy-19-peroxy species. Our previous studies with $\Delta^{2,4}$-19-oxo steroids had suggested that 2,3-enolization might be required before or during the cleavage of the C(10)–C(19) bond in the HPA-catalysed conversion of enone **3** into the phenol **4**.



5 R^1 = H, R^2R^3 = O
6 R^1 = OTBDMS, R^2 = OTHP, R^3 = H

Recently, we proposed[8] a catalytic mechanism and active-site model of HPA consistent with the experimental and particularly the stereochemical findings to date. The proposal featured hydrogen-bonding hypotheses involving 3-ketone, 19-

hydroxy, and 19-oxo groups of substrates and active-site residues. In the third oxidative step (Scheme 2), enzyme-catalysed 2,3-enolization of Akhtar's postulated intermediate, 19-HFPA, was proposed.



Scheme 2 Illustration of proposed mechanism for the third oxidative process

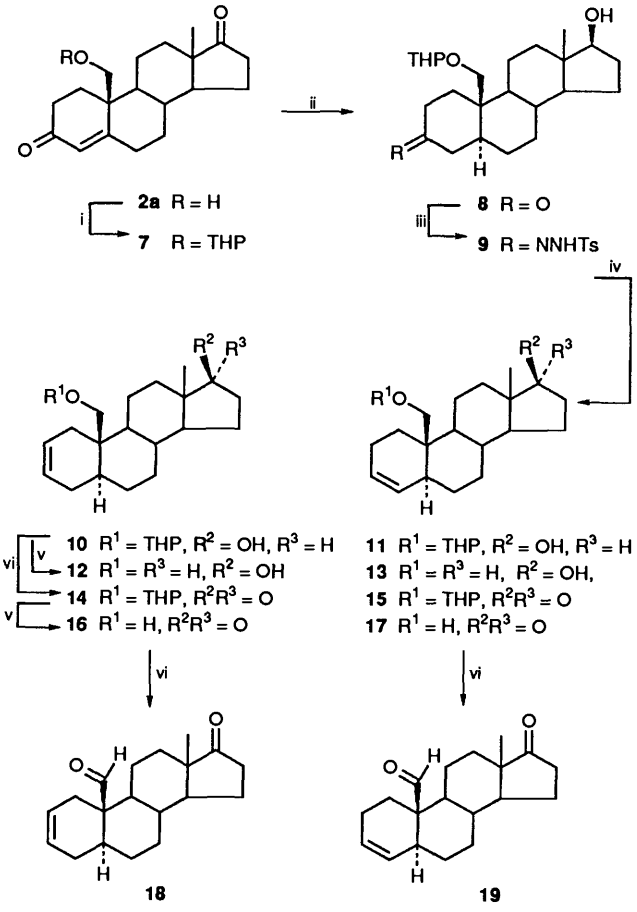
For further testing of our catalytic mechanism and active-site model hypotheses, we required a number of 3-deoxysteroid olefins. In this paper, we describe the first synthesis of 19-oxygenated Δ^2- and Δ^3-3-deoxy-5α-androgens **12**, **16**, **18**, **13**, **17** and **19** and an improved synthesis of Δ^4-3-deoxyandrogens **28**, **30** and **31**. Furthermore, we provide evidence supporting the 2,3-enolization hypothesis, based on chemical-model-reaction studies carried out with substrates **5**, **18**, **19** and **31**.

Results and Discussion

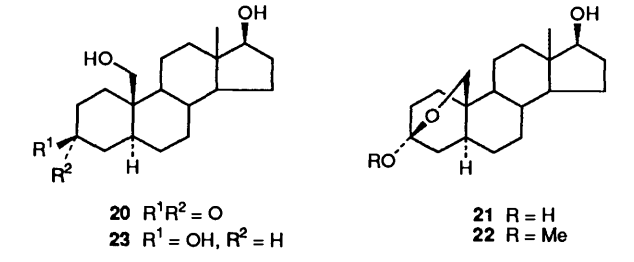
Synthesis of Δ^2- *and* Δ^3-3-*Deoxy*-5α-*androgens*.—Easy construction of the 5α-androstane **8** from 19-hydroxy androst-4-ene-3,17-dione (19-OHA) **2a** was realized by modification of a Syntex procedure[9] (Scheme 3). Protection of the alcohol **2a** as its tetrahydropyran-2-yl (THP) ether **7** was effected in 97% yield. Metal–ammonia reduction of enone **7** afforded ketone **8** and its corresponding 17-ketone in 84 and 5% yield, respectively, without significant reduction of the 3-ketone group.

The 5α-stereochemistry of compound **8** was determined by spectroscopic and chemical methods. The CD spectrum of ketone **8** exhibited a positive Cotton effect (λ_{max} 295 nm), characteristic of a 3-keto-5α-steroid.[10] Moreover, when the THP ether **8** was unmasked with toluene-*p*-sulfonic acid monohydrate (PTSA) in MeOH–tetrahydrofuran (THF), a mixture (50% yield) of ketone **20** and internal hemiketal **21** as well as pure ketal **22** (10% yield) were obtained. ^{1}H and ^{13}C NMR spectroscopic studies of the mixture unambiguously demonstrated that isomers **20** and **21** were in equilibrium (~1:1 ratio in $CDCl_3$ at 25 °C) as suggested earlier by a Syntex group[9] from m.p. and IR spectroscopic properties of a very similar mixture of 5α-steroids. Reduction of the mixture of isomers **20** and **21** with lithium aluminium hydride (LAH) afforded triol **23** in 63% yield. The equilibrium between isomers **20** and **21**, and the conversion into triol **23**, confirmed that ketone **8** was a 5α-steroid.

Both Δ^2- and Δ^3-3-deoxysteroids were efficiently formed from ketone **8** by the Shapiro reaction.[11] The partially purified tosylhydrazone **9**, formed from ketone **8** in more than 95% yield, was decomposed with excess of BuLi in 1:1 *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (TMEDA)–THF at 0 °C for 3 h to afford a mixture of regioisomers **10** and **11** in 46% yield (2 steps from ketone **8**). The product mixture also contained starting material **9** and its hydrolysed counterpart **8**. The ratio of regioisomers **10** to **11** was estimated as 1.3:1.0 by ^{1}H NMR spectroscopy. The decomposition of hydrazone **9** was slower at lower temperature and in different solvent systems. Protection of the 17β-hydroxy group as the *tert*-butyldimethylsilyl (TBDMS) ether did not improve the yield. We suspected that the THP ether group might be causing steric hindrance during the reaction. However, an attempt to remove selectively the THP protecting group from compound **9** with PTSA in MeOH–THF was unsuccessful, resulting in formation of ketal **22** in 65% yield due to solvolysis of the desired product.



Scheme 3 *Reagents and conditions*: i, DHP, PPTS, THF, room temp.; ii, Li, NH_3, THF, Ar, −78 °C; then NH_4Cl, room temp.; iii, $TsNHNH_2$, H^+, THF–MeOH, 0 °C; iv, BuLi, TMEDA, THF, 0 °C; v, PTSA, MeOH–THF, room temp.; vi, TPAP, NMO, MeCN–CH_2Cl_2, room temp.



The mixture of regioisomeric ethers **10** and **11** was readily converted into diols **12** and **13** by treatment with PTSA in 94% yield. Following successive chromatographic separations, pure diols **12** and **13** were obtained. Oxidation of the mixture of alcohols **10** and **11** with tetrapropylammonium perruthenate (TPAP)[12] resulted in a mixture of ketones **14** and **15**. Subsequent deprotection of the mixture afforded a mixture of the 17-ketones **16** and **17** (90% yield, 2 steps from alcohols **10** and **11**), which was separated by chromatography. The pure 19-aldehydes **18** (86%) and **19** (89%) were obtained upon oxidation of 19-hydroxy-17-ones **16** and **17**, respectively, with TPAP. When stored at −20 °C under N_2, pure aldehydes **18** and **19** were stable for over a week.

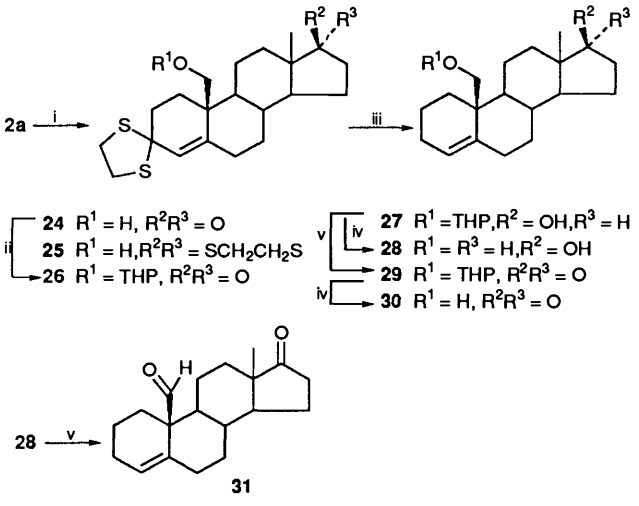
Table 1 Chemical model reactions[a]

Reactant	Reaction conditions[b] H_2O_2 (50 mol equiv.) 4 °C, 3.5 days	H_2O_2 (500 mol equiv.) 25 °C, 3.5 days	H_2O_2 (100 mol equiv.) 37 °C, 1 day
18	NR[c]	NR	NR
19	NR	NR	NR
31	NR	NR	NR
5	33[d]		33[e]

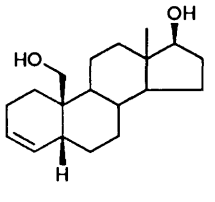
[a] Determined by ^{1}H NMR spectroscopy. [b] 30% H_2O_2, $NaHCO_3$, MeOH–CH_2Cl_2 (9:1). [c] NR: no reaction. [d] Quantitative conversion. [e] Quantitative conversion in 6 h.

Synthesis of Δ^4*-3-Deoxyandrogens.*—The two-step synthesis of compounds **28** and **30** from 19-OHA (**2a**) reported by Numazawa *et al.*[13] was not readily reproducible in our hands. Protection of the 3-ketone **2a** as the dithioketal **24** under their reaction conditions resulted in a very poor yield of compound **24** along with a number of other major products including the bis-dithioketal **25**. We found that the second step, in which dissolving-metal reduction of dithioketal **24** was stated[13] to afford compounds **28** and **30** in 75 and 15% yield, respectively, was much worse. In fact, reaction of compound **24** under their conditions resulted in formation of diol **28** (13%) and the 5β-steroid **32** (>50%) as major products. Formation of the undesired product **32** can be ascribed to the ability of the 19-hydroxy group to transfer internally a hydrogen to C-5 from the β-face during the dissolving-metal reduction.[9]

We therefore modified the reaction conditions. Protection of the 3-ketone **2a** proceeded smoothly by treatment of substrate **2a** with ethane-1,2-dithiol in THF in the presence of PTSA to afford dithioketal **24** in high yield (81%) with minimal formation of bis-dithioketal **25** (2%) (Scheme 4). We then argued that masking of the 19-hydroxy group as the THP ether



Scheme 4 *Reagents and conditions:* i, $HSCH_2CH_2SH$, PTSA, THF, room temp.; ii, DHP, PPTS, THF, room temp.; iii, Na, NH_3, THF, Ar, −78 °C; then EtOH, room temp.; iv, PTSA, MeOH–THF, room temp.; v, TPAP, NMO, MeCN–CH_2Cl_2, room temp.



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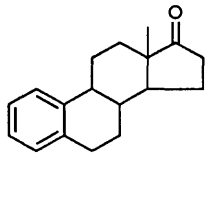

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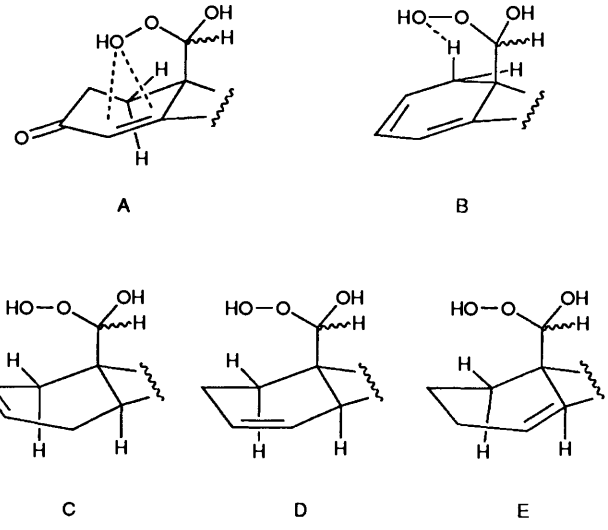

Fig. 1 19-Hydroxy-19-peroxy species of compounds **3a** (A), **5** (B), **18** (C), **19** (D) and **31** (E)

might favour the necessary desulfurization and improve the overall yield. Conversion of the alcohol **24** into the THP ether **26** was effected in 99% yield, and indeed desulfurization of compound **26** with sodium–ammonia in THF at −78 °C for 2 min afforded the alcoholic ether **27** in high yield (70%). Oxidation of the alcohol **27** to ketone **29** (93%), followed by removal of the THP ether group, gave hydroxy ketone **30** in 99% yield. The diol **28** was formed in 76% yield upon deprotection of the THP ether **27**. Oxidation of diol **28** to keto aldehyde **31** with TPAP was quantitative, as observed by TLC and ^{1}H NMR spectroscopy, but chromatographic purification resulted in only a 67% isolated yield due to the unstable nature of the compound. Compound **31** was best stored in ethanol under N_2 at −20 °C.

Chemical-model Reaction Studies.—Earlier chemical-model studies[5–7] with substrates **3a**, **5** and **6** suggested that 2,3-enolization might be necessary prior to C(10)–C(19) bond cleavage in the HPA reaction. Owing to a major competing process (intramolecular Michael addition, Fig. 1A) in the chemical model reaction of 19-OxoA **3a**, however, those studies did not permit direct comparison between the $\Delta^{2,4}$-system (**5** or **6**) and the Δ^4-system (**3a**). Our chemical-model studies have now been extended to include the 3-deoxy-19-oxosteroids **18**, **19** and **31** which lack the electrophilic conjugated ketone grouping, and thus might provide a valid comparison with diene **5**.

The results of chemical-model reactions with substrates **18**, **19**, **31** and **5** are summarized in Table 1. While the Δ^2-, Δ^3- and Δ^4-3-deoxysteroids (**18**, **19** and **31**) were unchanged, the $\Delta^{2,4}$-3-deoxysteroid **5** was converted quantitatively into 3-deoxyestrone **33** under the model reaction conditions as demonstrated[6b] previously. The findings clearly illustrate that both

Δ^2- and Δ^4-unsaturation are required to afford $\Delta^{1(10)}$-unsaturation under our model reaction conditions. This may be rationalized by considering the stereoelectronic features of the 1β-hydrogen atoms of these 3-deoxysteroids. The 1β-H of the 19-hydroxy-19-peroxy species (Fig. 1B) postulated to be formed in the model reaction of diene **5** would be labile not only because it is allylically activated, but it also adopts a quasi-axial conformation due to the $\Delta^{2,4}$-unsaturations of the steroid A-ring. We believe that this stereoelectronic property of the 1β-H should favour spontaneous decomposition of the 19-hydroxy-19-peroxy functionality through a six-membered cyclic transition state to afford the aromatic steroid **33** and formic acid.

In the case of the Δ^2-steroid **18**, the allylically activated 1β-H should be in a quasi-equatorial conformation. The 1β-H and the dioxygen bond of the intermediate shown in Fig. 1C would then be too far apart for an internal 1β-H transfer, and thus spontaneous decomposition of the 19-hydroxy-19-peroxy functionality would be an unlikely process. Lacking both the electronic (allylic 1β-H) and steric (quasi-axial 1β-H) features, the Δ^3-steroid **19** and the Δ^4-steroid **31** were expected and indeed observed to be unreactive. For the isomeric keto aldehydes **18** (Fig. 1C), **19** (Fig. 1D) and **31** (Fig. 1E), the postulated 19-hydroxy-19-peroxy species would presumably simply equilibrate back to the starting materials.

We turn now to the enzymic process. For the third oxidative process in the HPA-catalysed reaction, we argue that a chemical process similar to that described for the model reaction of aldehyde **5** may be taking place. Upon formation of 19-HFPA (see Scheme 2), subsequent enzyme-catalysed 2,3-enolization would produce a species that would be equivalent to that illustrated in Fig. 1B. Decomposition of this species to the phenol **4** and formic acid would then be an energetically favoured process.

In the present work, we have synthesized a number of previously undescribed 3-deoxy-5α-androgens **12**, **13**, **16**, **17**, **18** and **19** in high yield. An alternate synthesis of Δ^4-3-deoxysteroids **28**, **30** and **31** has been accomplished under improved reaction conditions. The chemical-model reaction of the HPA-catalysed third oxidative process was carried out with substrates **5**, **18**, **19** and **31**. The study demonstrated the necessity of both Δ^2- and Δ^4-unsaturation in forming $\Delta^{1(10)}$-unsaturation in the steroid A-ring under the model reaction conditions. The findings strongly support our 2,3-enolization hypothesis.[8] This chemical model reaction may indeed provide a useful picture of the third oxidative process in HPA catalysis.

Experimental

General.—^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 spectrometer for samples in $CDCl_3$ and in one case in CD_3OD and referenced to $SiMe_4$ unless noted otherwise. Coupling constants (J) are reported in Hz. ^{13}C Peak assignments were based on broadband and DEPT[14] experiments. IR spectra were recorded on a Perkin-Elmer 710B spectrometer. Mass spectra were recorded at 70 eV on a VG70S instrument. The CD spectrum was recorded on a JASCO J-600 spectropolarimeter for a sample in 1,4-dioxane. Flash column chromatography (FCC) was performed by the method of Still[15] using Baker silica gel. Analytical TLC was performed on Whatman glass plates (Silica gel 60A) with fluorescent indicator (254 nm). HPLC was performed on a Beckman instrument equipped with a Partisil-10 (Whatman) analytical column. M.p.s were determined on a Kofler micro hot-stage and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

19-OHA **2a** was obtained as a generous gift from Dr. D. F. Covey of Washington University School of Medicine. All other reagents were commercially available. THF was freshly distilled from sodium benzophenone ketyl. TMEDA was distilled and stored over KOH. CH_2Cl_2 and MeCN were dried over activated molecular sieves 4 Å. NH_3 was dried over NaOH. N_2 and Ar were dried over $CaSO_4$. BuLi was titrated by the method of Watson and Eastham.[16]

Reactions were run under positive pressure of N_2, and anhydrous Na_2SO_4 was used as a drying agent in the work-up of a reaction mixture, unless specified otherwise. The following work-up procedure was followed in oxidation reactions involving TPAP: the reaction mixture was concentrated to dryness under reduced pressure, the residue was taken up in the minimum amount of CH_2Cl_2, and the solution was filtered through a pad of silica with excess of EtOAc. The filtrate was concentrated under reduced pressure to afford crude product. During work-up, solution pH was assessed using pH papers.

19-(*Tetrahydropyran-2-yloxy*)*androst-4-ene-3,17-dione* **7**.—A solution of the alcohol **2a** (1.02 g, 3.37 mmol) in THF was treated with 3,4-dihydro-2*H*-pyran (DHP) (2.50 cm^3, 27.4 mmol) and pyridinium toluene-*p*-sulfonate (PPTS) (426 mg, 1.70 mmol) and was stirred at room temp. for 15 h. Upon concentration under reduced pressure, the reaction mixture was taken up in EtOAc, washed successively with water and brine, dried, and concentrated under reduced pressure to obtain a crude oil. FCC [EtOAc–hexane (1:1)] of the crude product afforded a diastereoisomeric mixture **7** (1.26 g, 97%) as a solid, δ_H 5.90 (m, vinyl H of diastereoisomers A and B), 0.93 (s, 18-H_3 of diastereoisomer A) and 0.92 (s, 18-H_3 of diastereoisomer B); ν_{max}(KBr)/cm^{-1} 2920, 1740 (C-17 CO) and 1660 (C-3 CO); EIMS *m/z* 386 (M^+) and 302 (Found: M^+, 386.2461; C, 74.65; H, 9.0. Calc. for $C_{24}H_{34}O_4$: M, 386.2457; C, 74.58; H, 8.87%).

17-*Hydroxy*-19-*tetrahydropyran*-2-*yloxy*-5α-*androstan*-3-*one* **8**.—To a stirred solution of Li (365 mg, 52.6 mmol) in liquid NH_3 (300 cm^3), generated at −78 °C over a period of 30 min under Ar, was quickly added a solution of dione **7** (1.16 g, 3.00 mmol) in THF (120 cm^3). After being stirred for 5 min, the reaction mixture was treated with NH_4Cl (16 g, 300 mmol) and warmed to room temp. during 1 h, allowing NH_3 to escape. The resulting mixture was diluted with EtOAc (300 cm^3) and water (300 cm^3) and was acidified to pH 5 with 30% HCl. After extractions with EtOAc, the combined extracts were washed with brine, dried, and concentrated under reduced pressure to obtain crude product. FCC of the crude product in acetone–$CHCl_3$ (1:9) afforded a diastereoisomeric mixture **8** (984 mg, 84%) as an oil, δ_H 4.61 (m, 2′-H), 4.16 (2 H, m, 6′-H^a), 3.83 (m, 19-H^a), 3.64 (m, 17α-H), 3.59 (m, 19-H^b and 6′-H^b), 0.78 (s, 18-H_3) and 0.76 (s, 18-H_3); ν_{max}(film)/cm^{-1} 3420, 2920 and 1710; EIMS *m/z* 390 (M^+) and 306 (Found: M^+, 390.2768; C, 73.8; H, 9.8. $C_{24}H_{38}O_4$ requires M, 390.2770; C, 73.81; H, 9.81%).

Deprotection of the THP Ether **8**.—A solution of the THP ether **8** (48.0 mg, 123 μmol) in THF (2 cm^3) was treated with MeOH (16 cm^3) and PTSA (140 mg, 736 μmol) and was then stirred for 4.5 h. The reaction mixture was concentrated under reduced pressure and the residue was taken up in EtOAc and washed successively with saturated aq. $NaHCO_3$, water, and brine. Drying, and concentrating under reduced pressure, afforded a crude product, which was purified by FCC [acetone–$CHCl_3$ (1:5 ⟶ 1:2 gradient)] to afford a mixture of hydroxy ketone **20** and hemiketal **21** (18.9 mg, 50%) as a solid, and pure ketal **22** (3.9 mg, 10%) as an oil. The mixture of isomers **20** and **21** was crystallized twice from acetone (m.p. 134–136 °C); δ_H 4.21–4.09 (br m, 19-H^a), 4.00 (br m, 19-H^b), 3.63 (m, 17α-H) and 0.74 (br s, 18-H); ν_{max}(film)/cm^{-1} 3370 and 1700; EIMS *m/z* 306 (M^+) and 288 (Found: M, 306.2203. $C_{19}H_{30}O_3$ requires M, 306.2195).

LAH Reduction of the Mixture of Compounds **20** *and* **21**.—An equilibrating mixture of isomers **20** and **21** (24.4 mg, 79.6 μmol) in THF (5 cm^3) was added dropwise to a stirred suspension of LAH in THF (5 cm^3), and the resulting reaction mixture was refluxed for 1 h. Upon cooling to 0 °C, the mixture was treated first with aq. acetone, to destroy excess of LAH, and then with saturated aq. Na_2SO_4 to coagulate aluminium salts. The resulting mixture was dried, filtered, concentrated under reduced pressure, and purified by FCC [acetone–$CHCl_3$ (1 : 1 ⟶ 3 : 2 gradient)] to yield triol **23** (15.4 mg, 63%) as a solid. Recrystallization from acetone (twice) afforded needles, m.p. 221–222 °C (lit.,[9] 233–234 °C); $\delta_H(CD_3OD)$ 3.87 (1 H, d, *J* 11.7, 19-H^a), 3.74 (1 H, d, *J* 11.7, 19-H^b), 3.59–3.50 (2 H, m, 3α- and 17α-H) and 0.77 (3 H, s, 18-H_3); $\delta_C(CD_3OD)$ 82.7 (C-19), 71.9 (C-3), 60.8 (C-17), 56.7, 52.9, 46.6, 44.3, 40.4, 39.4, 38.8, 37.4, 33.0, 32.8, 32.6, 30.8, 29.5, 24.4, 23.6 and 11.9 (C-18) (Found: M^+, 308.2344. Calc. for $C_{19}H_{32}O_3$: M, 308.2351).

Mixture of 19-(*Tetrahydropyran*-2-*yloxy*)-5α-*androst*-2-*en*-17β-*ol* **10** *and* 19-(*Tetrahydropyran*-2-*yloxy*)-5α-*androst*-3-*en*-17β-*ol* **11**.—To a solution of ketone **8** (432 mg, 1.11 mmol) in THF (8 cm^3) were added MeOH (15 cm^3), toluene-*p*-sulfonohydrazide (227 mg, 1.22 mmol) and 10% HCl–MeOH (100 mm^3). The reaction mixture was stirred for 30 min at 0 °C, diluted with water, and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated under reduced pressure to afford crude product. Further purification by FCC [EtOAc–hexane (1 : 1)] afforded hydrazone **9** (> 95% yield) as a mixture with toluene-*p*-sulfonohydrazide (> 80% of the mixture was compound **9** as determined by ^1H NMR spectroscopy).

The partially purified hydrazone **9** was dissolved in 1 : 1 THF–TMEDA (20 cm^3) and treated with BuLi [(1.40 mol dm^{-3} hexane solution) 7.40 cm^3, 10.3 mmol] dropwise at 0 °C. The orange coloured reaction mixture was stirred for 3 h at 0 °C. Upon dilution with water (150 cm^3), the reaction mixture was acidified to pH 6 with 30% HCl, then extracted with EtOAc, and the extract was dried, and concentrated under reduced pressure to afford crude product. Purification of the crude product by FCC [EtOAc–hexane (1 : 3)] yielded a solid mixture of unsaturated alcohols **10** and **11** (193 mg, 46%) in 1.3 : 1.0 ratio (by ^1H NMR spectroscopy), δ_H 5.61 (m, 2- and 3-H of **10**), 5.61 (m, vinyl H of **11**), 5.30 (m, vinyl H of **11**), 4.60 (m, 2′-H of **10**), 4.52 (m, 2′-H of **11**), 0.79 (s, 18-H_3), 0.77 (s, 18-H_3), 0.77 (s, 18-H_3) and 0.75 (s, 18-H_3); EIMS *m*/*z* 374 (M^+) and 356 (Found: M^+, 374.2825. $C_{24}H_{38}O_3$ requires M, 374.2821).

Deprotection of the THP Ether **9**.—The partially purified ether **9** (20.0 mg, 35.8 μmol) was dissolved in 3 : 1 MeOH–THF (2 cm^3) and treated with PTSA (20.4 mg, 107 μmol). The reaction mixture was stirred for 2.5 h at room temp., diluted with water, and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated under reduced pressure to obtain crude product, which was purified by FCC [acetone–$CHCl_3$ (1 : 5)] to yield ketal **22** (7.5 mg, 65%) as an oil, and unchanged ether **9** (6.6 mg, 33% recovery). For ketal **22**, δ_H 4.18 (1 H, dd, *J* 8.8 and 3.0, 19-H^a), 3.89 (1 H, dd, *J* 8.8 and 1.6, 19-H^b), 3.61 (1 H, m, 17α-H), 3.30 (3 H, s, 3-OMe) and 0.70 (3 H, s, 18-H_3); ν_{max}(film)/cm^{-1} 3400 and 2930; EIMS *m*/*z* 320 (M^+) and 302 (Found: M^+, 320.2357. $C_{20}H_{32}O_3$ requires M, 320.2351).

5α-*Androst*-2-*ene*-17β,19-*diol* **12** *and* 5α-*Androst*-3-*ene*-17β,19-*diol* **13**.—A mixture of ethers **10** and **11** (26.0 mg, 69.4 μmol) dissolved in 5 : 1 MeOH–THF (8 cm^3) was treated with PTSA (52.8 mg, 278 μmol) and stirred for 2 h at room temp. The reaction mixture was diluted with water (10 cm^3) and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated under reduced pressure to obtain crude product, which was purified by FCC [acetone–$CHCl_3$ (1 : 5)] to afford a mixture of diols **12** and **13** (19.0 mg, 94%) as a solid. Further chromatographic separations afforded pure 2-ene **12** (m.p. 134–135 °C) and 3-ene **13** (m.p. 139–141 °C). For compound **12**: δ_H 5.70 (2 H, m, 2- and 3-H), 3.82 (1 H, d, *J* 11.7, 19-H^a), 3.75 (1 H, d, *J* 11.7, 19-H^b), 3.63 (1 H, m, 17α-H) and 0.77 (3 H, s, 18-H_3); δ_C 127.2 (C-3), 126.5 (C-2), 82.0 (C-17), 62.1 (C-19), 54.4 (C-9), 51.4 (C-14), 42.9 (C-13), 41.6 (C-5), 38.9 (C-10), 37.1, 36.2, 34.2, 31.3, 30.8, 30.5, 28.5, 23.4, 21.6 and 11.2 (C-18); EIMS *m*/*z* 290 (M^+), 272 and 259 (Found: M^+, 290.2242. $C_{19}H_{30}O_2$ requires M, 290.2246).

For compound **13**: δ_H 5.68 (1 H, m, vinyl H), 5.35 (1 H, m, vinyl H), 3.91 (1 H, d, *J* 11.2, 19-H^a), 3.76 (1 H, d, *J* 11.2, 19-H^b), 3.64 (1 H, m, 17α-H) and 0.79 (3 H, s, 18-H_3); δ_C 131.1 (C-4), 127.0 (C-3), 81.9 (C-17), 62.4 (C-19), 53.8 (C-9), 51.4 (C-14), 45.7 (C-5), 43.2 (C-13), 39.1 (C-10), 37.3, 36.1, 31.6, 30.5, 29.6, 27.2, 24.0, 23.4, 22.2 and 11.4 (C-18); EIMS *m*/*z* 290 (M^+), 272 and 259 (Found: M^+, 290.2239).

19-*Hydroxy*-5α-*androst*-2-*en*-17-*one* **16** *and* 19-*Hydroxy*-5α-*androst*-3-*en*-17-*one* **17**.—A mixture of THP ethers **10** and **11** (152 mg, 407 μmol) dissolved in 1 : 9 MeCN–CH_2Cl_2 (3.0 cm^3) was treated with 4-methylmorpholine *N*-oxide (NMO) (71.5 mg, 610 μmol), activated molecular sieves 4 Å (205 mg, powdered), and TPAP (7.1 mg, 20.2 μmol) under Ar. The reaction mixture was stirred for 1 h at room temp., followed by work-up to afford a crude mixture of THP ether 17-ones **14** and **15** as an oil. This material was then dissolved in 4 : 1 MeOH–THF (30 cm^3) and treated with PTSA (232 mg, 1.22 mmol). After being stirred for 3 h at room temp., the reaction mixture was diluted with water (100 cm^3) and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated under reduced pressure to afford crude product, which was purified by FCC [EtOAc–hexane (1 : 3)] to yield a mixture of hydroxy ketones **16** and **17** (106 mg, 90%) as a solid. Further chromatographic separations [acetone–$CHCl_3$ (1 : 20)] afforded pure compounds **16** (m.p. 126–128 °C) and **17** (m.p. 125–127 °C). For compound **16**: δ_H 5.70 (2 H, m, 2- and 3-H), 3.84 (1 H, d, *J* 11.7, 19-H^a), 3.75 (1 H, d, *J* 11.7, 19-H^b) and 0.91 (3 H, s, 18-H_3); δ_C 221.2 (C-17), 127.1 (C-3), 126.2 (C-2), 62.1 (C-19), 54.4 (C-9), 51.9 (C-14), 47.8 (C-13), 41.6 (C-5), 39.0 (C-10), 35.8, 35.7 (C-8), 34.2, 31.9, 30.7, 30.6, 28.4, 21.8, 21.2 and 13.9 (C-18); ν_{max}(KBr)/cm^{-1} 3480, 2900 and 1725; EIMS *m*/*z* 288 (M^+), 270 and 257 (Found: M^+, 288.2090. $C_{19}H_{28}O_2$ requires M, 288.2089). For compound **17**: δ_H 5.69 (1 H, m, vinyl H), 5.36 (1 H, m, vinyl H), 3.91 (1 H, d, *J* 11.4, 19-H^a), 3.78 (1 H, d, *J* 11.4, 19-H^b) and 0.92 (3 H, s, 18-H_3); δ_C 221.1 (C-17), 130.9 (C-4), 127.0 (C-3), 62.2 (C-19), 53.8 (C-9), 51.8 (C-14), 48.0 (C-13), 45.6 (C-5), 39.2 (C-10), 35.8 (C-8), 35.6, 32.1, 30.9, 29.5, 27.1, 23.9, 21.8 (2 C) and 14.1 (C-18); ν_{max}(KBr)/cm^{-1} 3490, 2925 and 1725; EIMS *m*/*z* 288 (M^+), 270 and 257 (Found: M^+, 288.2096).

17-*Oxo*-5α-*androst*-2-*en*-19-*al* **18** *and* 17-*Oxo*-5α-*androst*-3-*en*-19-*al* **19**.—To a solution of the alcohol **16** (8.7 mg, 30.2 μmol) in 1 : 9 MeCN–CH_2Cl_2 (200 mm^3) were added NMO (5.3 mg, 45.2 μmol), activated molecular sieves 4 Å (15 mg, powdered), and TPAP (531 μg, 1.51 μmol) under Ar. The reaction mixture was stirred for 1 h at room temp., and the crude product was obtained following work-up. Further purification by FCC [EtOAc–hexane (1 : 5)] afforded the aldehyde **18** (7.4 mg, 85%) as an oil; δ_H 9.88 (1 H, s, 19-H), 5.71 (2 H, m, 2- and 3-H) and 0.86 (3 H, s, 18-H_3); δ_C 220.7 (C-17), 207.9 (C-19), 127.3 (C-3), 125.0 (C-2), 52.4 (C-9), 51.4 (C-14), 50.7 (C-10), 47.6 (C-13), 40.6 (C-5), 35.9 (C-8), 35.7, 33.8, 31.6, 30.6, 30.2, 28.5, 21.6, 21.1 and 13.8 (C-18); ν_{max}(film)/cm^{-1} 2940, 1740 (C-17 CO) and 1715 (C-19 CHO); EIMS *m*/*z* 286 (M^+), 268, 258 and 257 (Found: M^+, 286.1935. $C_{19}H_{26}O_2$ requires M, 286.1933).

A solution of the alcohol **17** (7.2 mg, 25.0 μmol) in 1:9 MeCN–CH_2Cl_2 (170 mm^3) was treated with NMO (4.4 mg, 37.6 μmol), activated molecular sieves 4 Å (12.5 mg, powdered), and TPAP (440 μg, 1.25 μmol) under Ar. The reaction mixture was stirred for 100 min at room temp., and a crude product was obtained following work-up. Further purification by FCC [EtOAc-hexane (1:5)] afforded aldehyde **19** (6.4 mg, 89%) as an oil; δ_H 9.94 (1 H, s, 19-H), 5.66 (1 H, m, vinyl H), 5.56 (1 H, m, vinyl H) and 0.83 (3 H, s, 18-H_3); δ_C 220.7 (C-17), 207.4 (C-19), 130.4 (C-4), 128.0 (C-3), 51.7 (C-9), 51.4 (C-14), 51.1 (C-10), 47.7 (C-13), 43.2 (C-5), 36.7 (C-8), 35.7, 31.4, 30.7, 29.3, 27.6, 23.4, 21.6, 20.7 and 13.7 (C-18); ν_{max}(film)/cm^{-1} 2935, 1740 (C-17 CO) and 1715 (C-19 CHO); EIMS *m/z* 286 (M^+), 268, 258 and 257 (Found: M^+, 286.1932).

3,3-*Ethylenebis(sulfanediyl)*-19-*hydroxyandrost*-4-*en*-17-*one* **24**.—A solution of enone **2a** (514 mg, 1.70 mmol) in THF (7 cm^3) was treated with PTSA (970 mg, 5.10 mmol) and ethane-1,2-dithiol (171 mm^3, 2.04 mmol) and was then stirred for 80 min at room temp. The reaction mixture was diluted with both water (50 cm^3) and brine (20 cm^3) and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated under reduced pressure to give an oil, which was purified by FCC [EtOAc–hexane (1:2)] to obtain compounds **24** (521 mg, 81%) and **25** (16.8 mg, 2%) as solids. Compound **24** had m.p. 169–171 °C (lit.,[13a] 170–171 °C); ^1H NMR and IR data were identical with those reported;[13a] δ_C 140.8 (C-5), 129.7 (C-4), 65.7 (C-19), 65.1, 53.9, 51.5, 47.7, 42.1, 40.0, 39.6, 38.5, 36.0, 35.6, 33.7, 32.3, 31.9, 31.6, 21.7, 21.1 and 13.8 (C-18); EIMS *m/z* 378 (M^+) and 360 (Found: M^+, 378.1692. Calc. for $C_{21}H_{30}O_2S_2$: M, 378.1687).

Compound **25** had m.p. 133–135 °C; δ_H 5.85 (1 H, m, 4-H), 3.94 (1 H, d, *J* 10.8, 19-H^a), 3.63 (1 H, d, *J* 10.8, 19.H^b), 3.42–3.34 (3 H, m, SCH_2CH_2S), 3.31–3.06 (5 H, m, SCH_2CH_2S) and 0.93 (3 H, s, 18-H_3); δ_C 140.9 (C-5), 129.8 (C-4), 65.8 (C-19), 65.1, 53.2, 52.3, 49.1, 42.8, 42.0, 40.1, 39.7, 39.6, 39.3, 38.6, 37.7, 34.0, 32.4, 23.7, 21.4 and 17.3 (C-18); ν_{max}(KBr)/cm^{-1} 3450 and 2950; EIMS *m/z* 454 (M^+) (Found M^+, 454.1495. $C_{23}H_{34}OS_4$ requires M, 454.1493).

3,3-*Ethylenebis(sulfanediyl)*-19-(*tetrahydropyran*-2-*yloxy*)-*androst*-4-*en*-17-*one* **26**.—A solution of the alcohol **24** (586 mg, 1.55 mmol) in THF (15 cm^3) was treated with PPTS (195 mg, 776 μmol) and DHP (1.13 cm^3, 12.4 mmol) and was stirred for 15 h at room temp. The reaction mixture was concentrated under reduced pressure and the residue was taken up in EtOAc (100 cm^3), poured into water (100 cm^3) and neutralized with saturated aq. $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were dried, and concentrated under reduced pressure to afford an oil, which was purified by FCC [EtOAc–hexane (1:4)] to yield a diastereoisomeric mixture **26** (710 mg, 99%) as a solid; δ_H 5.68 (m, 4-H), 4.56 (m, 2′-H), 4.00 (d, *J* 9.6, 19-H^a of diastereoisomer A), 3.88 (d, *J* 9.8, 19-H^a of diastereoisomer B), 3.78 (m, 6′-H^a), 3.55–3.42 (m, 19-H^b and 6′-H^b of both diastereoisomers), 3.40–3.18 (4 H, m, SCH_2CH_2S) and 0.88 (s, 18-H); ν_{max}(KBr)/cm^{-1} 2930 and 1740; EIMS *m/z* 462 (M^+), 377 and 362 (Found: M^+, 462.2258; C, 67.2; H, 8.5. $C_{26}H_{38}O_3S_2$ requires M, 462.2262; C, 67.49; H, 8.28%).

19-(*Tetrahydropyran*-2-*yloxy*)*androst*-4-*en*-17β-*ol* **27**.—To a stirred solution of Na (1.11 g, 48.3 mmol) in liquid NH_3 (200 cm^3) generated at −78 °C over a period of 30 min under Ar, was added a solution of ketone **26** (675 mg, 1.46 mmol) in THF (30 cm^3) all at once. After being stirred for 2 min, the reaction mixture was treated with EtOH (100 cm^3) and was warmed to room temp. during 1.5 h, allowing NH_3 to escape. The mixture was acidified to pH 5 with 10% HCl and was then poured into cold water (100 cm^3). After extraction with EtOAc, the combined extracts were washed with brine, dried, and concentrated under reduced pressure to afford a crude oil, which was purified by FCC [EtOAc–hexane (1:3)] to yield a diastereoisomeric mixture **27** (383 mg, 70%) as a solid, δ_H 5.44 (m, 4-H), 4.57 (m, 2′-H), 3.97 (d, *J* 9.6, 19-H^a of diastereoisomer A), 3.88 (d, *J* 9.8, 19-H^a of diastereoisomer B), 3.84 (m, 6′-H^a), 3.62 (m, 17α-H), 3.52–3.40 (m, 19-H^b and 6′-H^b of both diastereoisomers) and 0.77 (s, 18-H); EIMS *m/z* 374 (M^+), 356 and 290 (Found: M^+, 374.2828. $C_{24}H_{38}O_3$ requires M, 374.2821).

19-(*Tetrahydropyran*-2-*yloxy*)*androst*-4-*en*-17-*one* **29**.—To a solution of the alcohol **27** (145 mg, 387 μmol) in 1:9 MeCN–CH_2Cl_2 (1.0 cm^3) were added NMO (68.0 mg, 580 μmol), activated molecular sieves 4 Å (194 mg, powdered), and TPAP (6.8 mg, 19.3 μmol) under Ar. The reaction mixture was stirred for 1 h at room temp., and the crude product was obtained following work-up. Further purification by FCC [EtOAc–hexane (1:6)] afforded a diastereoisomeric mixture **29** (134 mg, 93%) as an oil; δ_H 5.47 (m, 4-H), 4.56 (m, 2′-H), 3.97 (d, *J* 9.6, 19-H^a of diastereoisomer A), 3.91 (d, *J* 9.9, 19-H^a of diastereoisomer B), 3.83 (m, 6′-H^a), 3.52 (m, 6′-H^b), 3.48 (d, *J* 9.9, 19-H^b of diastereoisomer B), 3.43 (d, *J* 9.6, 19-H^b of diastereoisomer A) and 0.90 (s, 18-H_3); ν_{max}(film)/cm^{-1} 2920 and 1740; EIMS *m/z* 372 (M^+), 354, 342 and 288 (Found: M^+, 372.2669. $C_{24}H_{36}O_3$ requires M, 372.2664).

19-*Hydroxyandrost*-4-*en*-17-*one* **30**.—A solution of THP ether **29** (15 mg, 40.3 μmol) in 9:1 MeOH–THF (5 cm^3) was treated with PTSA (30.0 mg, 158 mmol) and was stirred for 90 min. The reaction mixture was concentrated under reduced pressure, the residue was taken up in EtOAc (10 cm^3), and the solution was diluted with water (10 cm^3) and neutralized with saturated aq. $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic phases were washed with brine, dried, and concentrated under reduced pressure to afford crude product, which was purified by FCC [EtOAc–hexane (2:5)] to yield compound **30** (11.5 mg, 99%) as a solid, m.p. 153–155 °C (lit.,[13a] 154–156 °C); ^1H NMR and IR data were identical with those reported;[13a] δ_C 222.3 (C-17), 139.0 (C-5), 125.9 (C-4), 66.0 (C-19), 53.8 (C-14), 51.5 (C-9), 47.8 (C-10), 42.6 (C-13), 36.1 (C-8), 35.8, 34.5, 32.7, 31.9 (2 C), 25.3, 21.7, 20.9, 20.0 and 13.9 (C-18); EIMS *m/z* 228 (M^+), 270, 258, 257 and 239 (Found: M^+, 288.2090. Calc. for $C_{19}H_{28}O_2$: M, 288.2089).

Androst-4-*ene*-17β,19-*diol* **28**.—A solution of the THP ether **27** (187 mg, 500 μmol) in 9:1 MeOH–THF (30 cm^3) was treated with PTSA (285 mg, 1.50 mmol) and was stirred for 90 min. The reaction mixture was concentrated under reduced pressure, the residue was taken up in EtOAc (50 cm^3), and the solution was diluted with water (50 cm^3) and neutralized with saturated aq. $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried, and concentrated under reduced pressure to afford crude product, which was purified by FCC [acetone–$CHCl_3$ (1:5)] to yield diol **28** (110 mg, 76%) as a solid, m.p. 99–100 °C (lit.,[13b] 99–101 °C); ^1H NMR and IR data were identical with those reported;[13b] δ_C 139.3 (C-5), 125.6 (C-4), 81.8 (C-17), 66.0 (C-19), 53.8 (C-14), 51.1 (C-9), 47.6 (C-10), 43.1 (C-13), 37.1, 36.6 (C-8), 34.7, 32.9, 32.6, 30.6, 25.4, 23.3, 21.2, 20.0 and 11.2 (C-18); EIMS *m/z* 290 (M^+), 272, 259, 258 and 241 (Found: M^+, 290.2248. Calc. for $C_{19}H_{30}O_2$: M, 290.2246).

17-*Oxoandrost*-4-*en*-19-*al* **31**.—To a solution of diol **28** (25 mg, 86.1 μmol) in 1:9 MeCN–CH_2Cl_2 (1.0 cm^3) were added

NMO (30.3 mg, 259 μmol), activated molecular sieves 4 Å (40 mg, powdered), and TPAP (6.1 mg, 17.4 μmol) under Ar. The reaction mixture was stirred for 2.5 h at room temp., and the crude product was obtained following work-up. The oxidation of diol **28** to aldehyde **31** was quantitative, as observed by TLC and ^{1}H NMR spectroscopy. Further purification by FCC [EtOAc–hexane (1:5)] afforded pure aldehyde **31** (16.5 mg, 67%) as an oil: ^{1}H NMR, IR and EIMS data were identical with those reported;[8] δ_C 221.8 (C-17), 204.3 (C-19), 135.6 (C-5), 125.7 (C-4), 53.4 (C-14), 51.4 (C-9), 47.7 (C-10), 43.8 (C-13), 36.6 (C-8), 35.7, 33.1, 31.7, 31.5, 30.8, 25.1, 21.7, 21.3, 19.4 and 13.8 (C-18) (Found: M^+, 286.1936. Calc. for $C_{19}H_{26}O_2$: M, 286.1933).

Desulfurization of Dithioketal **24** *by Dissolving-metal Reduction.*—The dithioketal **24** was subjected to reaction conditions described by Numazawa.[13] The major product obtained was diol **32** (> 50% yield by ^{1}H NMR spectroscopy) as a solid, m.p. 159–161 °C; δ_H 5.66 (1 H, m, 3-H), 5.38 (1 H, m, 4-H), 3.93 (1 H, d, *J* 11.0, 19-H^a), 3.61 (1 H, m, 17α-H), 3.59 (1 H, d, *J* 11.0, 19-H^b) and 0.73 (3 H, s, 18-H$_3$); δ_C 132.4 (C-3), 126.6 (C-4), 81.9 (C-17), 66.0 (C-19), 51.2, 40.6, 37.2, 35.8, 35.6, 27.6, 27.2, 26.6, 24.0, 23.4, 22.0, 20.9 and 11.1 (C-18); ν_{max}(film)/cm^{-1} 3300 and 2930; EIMS *m/z* 290 (M^+), 272, 260, 259 and 241 (Found: M^+, 290.2250. $C_{19}H_{30}O_2$ requires M, 290.2246).

Chemical Model Reactions of 3*-Deoxy-*19*-oxosteroids* **18**, **19**, **31** *and* **5**.—The reactants **18**, **19**, **31** and **5** were purified by HPLC prior to use. A solution of a 3-deoxy-19-oxosteroid (3.5 μmol) in 9:1 MeOH–CH_2Cl_2 in a 1-fluidram* vial was treated with 30% H_2O_2 and $NaHCO_3$ (1.0 mg, powdered). The capped vial containing the reaction mixture was vortexed and stored under air. The following conditions were employed: 175 μmol (50 mol equiv.) of H_2O_2, at 4 °C for 3.5 days; 1.75 mmol (500 mol equiv.) of H_2O_2, at 25 °C for 3.5 days (not applicable to substrate **5**); 350 μmol (100 mol equiv.) of H_2O_2, at 37 °C for 1 day (in the case of substrate **5**, the reaction was run for 6 h). The reaction was periodically monitored by TLC, and at the end of the reaction the mixture was concentrated under a stream of N_2 and then under reduced pressure. The crude mixture so obtained was analysed by TLC and ^{1}H NMR spectroscopy. The reactants were essentially unchanged in the case of substrates **18**, **19** and **31**. When compound **5** reacted with H_2O_2, trienone **33**[6b] was formed quantitatively.

* 1 fluidram = 3.697 cm^3.

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