Synthesis of 6α,7α- and 6β,7β-aziridinoandrost-4-ene-3,17-diones and related compounds: potential aromatase inhibitors

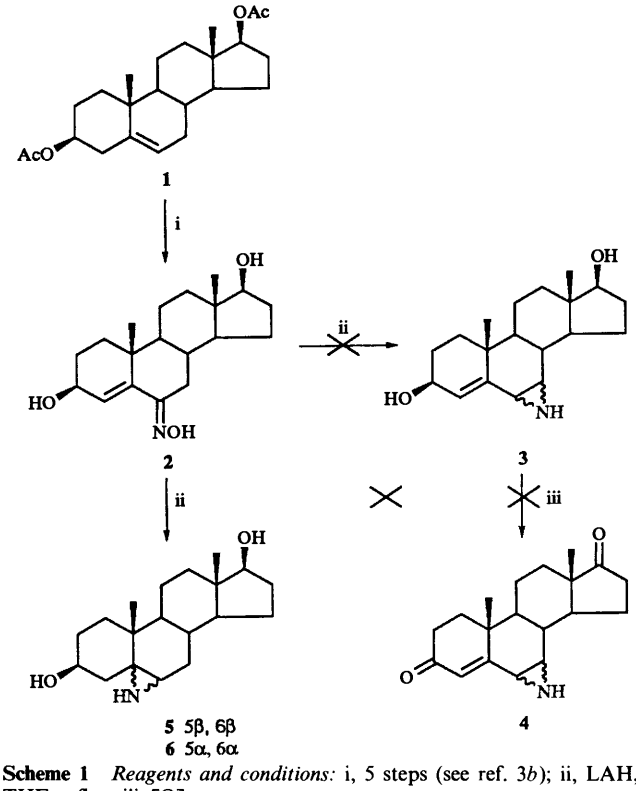
Vincent C. O. Njar,[a] Rolf W. Hartmann[b] and Cecil H. Robinson *,[c]

[a] *Department of Chemistry, University of Ibadan, Ibadan, Nigeria*
[b] *Fachrichtung 12.1 Pharmazeutische Chemie, Universität des Saarlandes, 66041 Saarbrücken, Germany*
[c] *Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205-2185, USA*

The stereospecific synthesis of the novel 6β,7β- and 6α,7α-aziridinoandrost-4-ene-3,17-dione **21** and **23** has been accomplished by treatment of vicinal azidohydrins **19** and **20** respectively with triphenylphosphane. Several related compounds (fused steroidal oxiranes, azidohydrins and an azide) were also synthesized. Reaction of (*E*)-6-hydroxyiminoandrost-4-ene-3β,17β-diol **2** with lithium aluminium hydride (LAH) gave, respectively, 5β,6β- and 5α,6α-aziridinoandrostane-3β,17β-diol **5** and **6**. Although the 6,7-aziridines and their N-derivatives are poor inhibitors of human placental microsomal aromatase, most of the other compounds are modest inhibitors, while 7α-acetoxy-6β-azidoandrost-4-ene-3,17-dione **24** is a potent inhibitor of the enzyme (IC_{50}-value = 0.40 μ mol dm^{-3}).

Human placental aromatase is a cytochrome *P*-450 enzyme complex which catalyses the conversion of androgens into estrogens. The potential therapeutic value of aromatase inhibitors in the treatment of estrogen-dependent diseases (*e.g.*, breast cancer) has led to much interest in this area.[1] Most of the steroids which have been studied as aromatase inhibitors are analogues of androstenedione (AD) with substitutions at C-4,[2] -6,[3] -7[4] and -19.[5] These studies have resulted in the discovery of potent aromatase inhibitors,[1] and, indeed, 4-hydroxyandrostenedione has recently been approved for clinical use in the treatment of breast cancer in the United Kingdom and a number of other countries.[6]

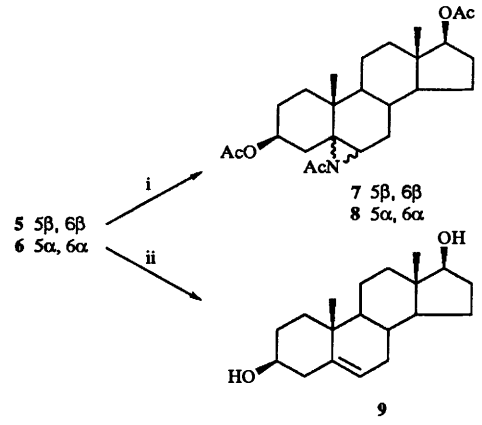
Several 6- and 7-substituted analogues of AD are powerful inhibitors of human placental aromatase. For example, 6β-bromo-,[7] 6β-ethyl-[8] and 7α-(4'-aminophenyl)sulfanyl-androstenedione[9] have been found to be among the most potent inhibitors produced to date (K_i/K_m = 0.35, 0.08 and 0.35 respectively). In addition, 3-deoxy-6α,7α-cyclopropa[6,7]AD has recently been shown to be a potent aromatase inhibitor (K_i/K_m = 0.09).[10] Consequently, we embarked on the synthesis of the hitherto undescribed 6,7-aziridino steroids **21** and **23** (see Scheme 5), thinking that they might also bind tightly to the enzyme. We also considered the possibility that, because of the reactivity of the aziridine ring,[11] these aziridino steroids might react covalently at the active site of the enzyme, resulting in irreversible inhibition. This paper describes the synthesis of novel aziridino steroids and other related compounds (fused steroidal oxiranes, azidohydrins and an azide), and their preliminary evaluation as inhibitors of human placental aromatase.[12]



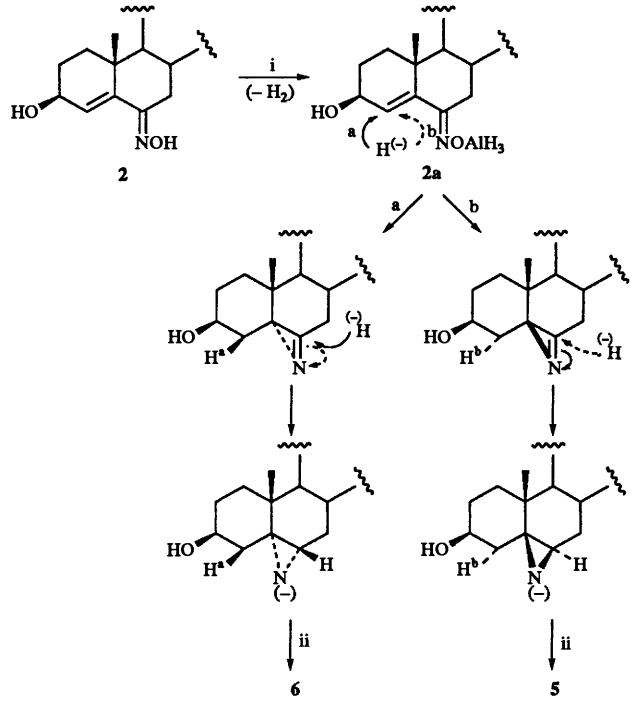
Scheme 1 *Reagents and conditions:* i, 5 steps (see ref. 3*b*); ii, LAH, THF, reflux; iii, [O]

Results and discussion

Although 6,7-aziridino steroids are known,[13] the 6,7-aziridino-3-oxo-4-enes are a class of steroids not hitherto reported. The planned synthetic route is shown in Scheme 1. Previous work of one of us[3b] made available the (*E*)-oxime **2**. Reduction[5c] of oxime **2** with LAH in dry tetrahydrofuran (THF) under nitrogen at reflux for 4 h gave no 4-ene-6,7-aziridines, but instead gave two products in quantitative yield. These were separated (silica gel, TLC or neutral alumina, column) and identified by spectral data as 5β,6β-aziridinoandrostane-3β,17β-diol **5** (35%) and the 5α,6α-isomer **6** (57%). The stereochemistry of the aziridine ring in products **5** and **6** was assigned by comparison of their ^1H NMR data with those of closely related 5,6-oxiranes and thiiranes.[8,14] The 5β,6β- and 5α,6α-aziridinoandrostane-3β,17β-diols **5** and **6** were further characterized as the corresponding *N*,*O*-triacetyl derivatives **7** and **8** respectively (acetic anhydride–pyridine; room temp.; 15 h) (Scheme 2). As a final confirmation of structure, the aziridines **5** and **6** were each smoothly deaminated by treatment at 25 °C with aq. sodium nitrite[5c] in acetic acid to give the known androst-5-ene-3β,17β-diol **9**. We are aware of other α,β-unsaturated cyclohexanone oximes which gave saturated aziridines on reduction with LAH but in most of these cases unsaturated aziridines and primary amines



Scheme 2 *Reagents and conditions:* i, Ac_2O, Py, room temp., 15 h; ii, AcOH, $NaNO_2$, room temp., 0.5 h

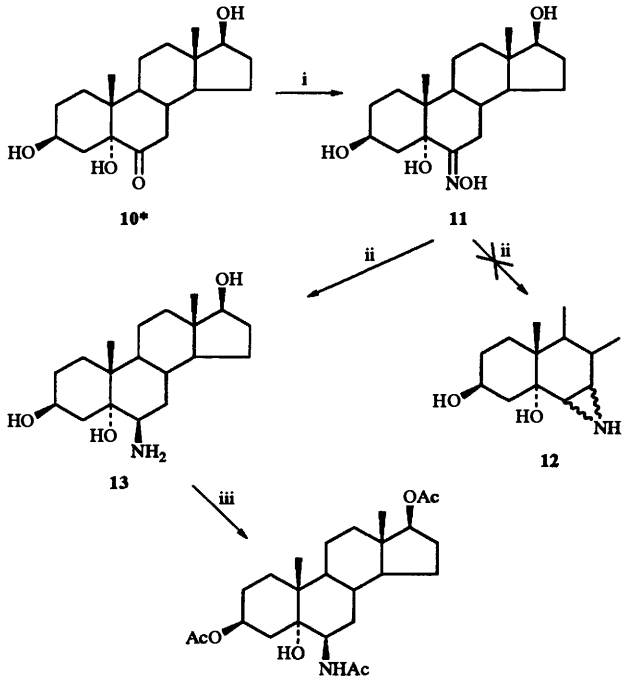


Scheme 3 a and b = attack of hydride ion (H^-) at C-4 of intermediate **2a** from above (a) and below (b) the plane of the molecule, respectively. *Reagents:* i, LAH; ii, water.

were also obtained.[15,16] A possible mechanism for the formation of the isomeric 5,6-aziridines **5** and **6** is shown in Scheme 3. It is necessary to state here that the related 5α,6α-aziridinocholestan-3β-ol has been synthesized by reduction (LAH) of 5α-azido-6β-chlorocholestan-3β-ol.[17] At this point it became clear to us that for the synthesis of compounds **4**, the Δ^4 double bond had to be introduced after formation of the 6,7-aziridine.

As shown in Scheme 4, the (*E*)-oxime triol **11** was chosen as the intermediate for introducing the 6,7-aziridine ring prior to formation of the Δ^4 double bond. However, reduction of compound **11** with LAH following the usual procedure gave no aziridine **12** but instead gave 6β-aminoandrostane-3β,5α,17β-triol **13** (12.5%), recovered **11** (38%) and many unidentified polar products. Spectral data were consistent with the assigned structure of product **13** which was further characterized as the *N,O*-triacetyl derivative **14**. Reduction of ketoximes with LAH is known[16,18-20] to give primary amines and aziridines but in this case no aziridines were obtained.

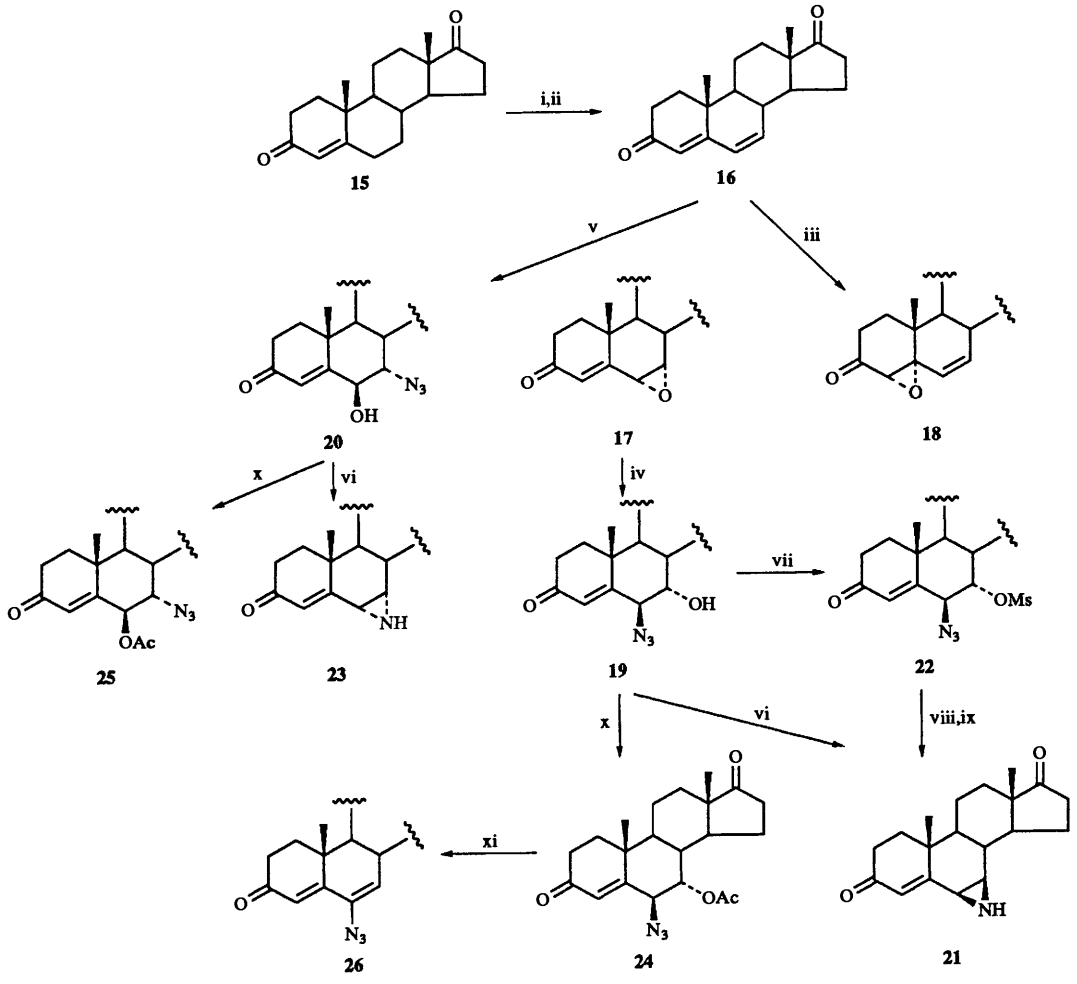
We eventually synthesized the desired 6,7-aziridino steroids



Scheme 4 *Reagents and conditions:* * obtained by hydrolysis of its 3β,17β-diacetate (see ref. 3*b*); i, NH_2OH·HCl; ii, LAH, THF, reflux, 15 h; iii, Ac_2O, Py, room temp., 15 h

(Scheme 5) by the methodology of Ittah *et al.*[21] which involved separate treatment of the key intermediates 6β-azido-7α-hydroxyandrost-4-ene-3,17-dione **19** and 7α-azido-6β-hydroxyandrost-4-ene-3,17-dione **20** with triphenylphosphane. The preparation of intermediates **19** and **20** is also outlined in Scheme 5. The commercially available androstenedione **15** was converted by a standard procedure[22] into androsta-4,6-diene-3,17-dione **16** in 80% yield. Treatment of dienone **16** with *m*-chloroperbenzoic acid (MCPBA)[23] in freshly distilled chloroform at reflux gave the desired 6α,7α-oxiranoandrost-4-ene-3,17-dione **17** (57.3%), together with 4α,5α-oxiranoandrost-6-ene-3,17-dione **18** (7.6%). Treatment of the oxirane **17** with NaN_3/conc.H_2SO_4[24] in dimethyl sulfoxide (DMSO) at room temp. afforded 6β-azido-7α-ol **19** in 84% yield. It should be noted that in order to obtain compound **19** in high yield the exact molar ratio of H_2SO_4 and the oxirane was necessary. The configurations of the azide and alcohol groups of compound **19** were established by ^1H NMR spectroscopy and are consistent with related diaxial openings of 6α,7α-epoxides.[25,26] Unfortunately the azido alcohol **19** under the reported reaction conditions (Ph_3P, reflux in diethyl ether or THF) failed to give the expected aziridine. However, we found that compound **19**, when heated in dry toluene at 105–110 °C with 2 mol equiv. Ph_3P for 3 h, afforded the desired 6β,7β-aziridine **21** in 68% yield. The high temperature, it seems, was necessary for the cyclization to occur. The assignment of the β-configuration to the 6,7-aziridine ring is based in part on the shift of the C-19 protons NMR resonance to lower field. The C-19 protons of compound **21** appear at δ 1.25, while the corresponding protons of androstenedione resonate at δ 1.22. The large body of ^1H NMR data available for the analogous 6α,7α-epoxide reveal that in this case the C-19 protons are shifted to higher field and they appear as a singlet at δ 1.09–1.13.†[,23,27,28] Further confirmation for the 6β,7β-stereochemistry of compound **21** was provided by its synthesis *via* reduction (LAH) of the 6β-

† This work. See ^1H NMR spectrum of compound **17**.



Scheme 5 *Reagents and conditions:* i, $HC(OEt)_3$, 1,4-dioxane, *p*-TSOH; ii, DDQ, acetone; iii, MCPBA, $CHCl_3$, reflux, 4 h; iv, NaN_3, DMSO, H_2SO_4, room temp; v, NaN_3, CrO_3, AcOH; vi, Ph_3P, toluene, 105–110 °C; vii, MsCl, Py, 4 °C, 20 h; viii, LAH, THF, N_2, reflux, 2 h; ix, PDC, DMF, 4 °C, 3 h; x, Ac_2O, Py, room temp. 15 h; xi, TMAF, MeCN, N_2, room temp., 20 h

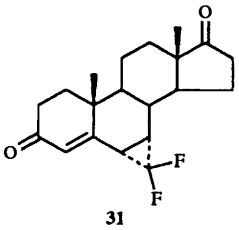
azido-7α-mesylester **22** (Scheme 5), a well established procedure in which the aziridine ring retains the original configuration of the azido group.[29] The 6β,7β-aziridine **21** was further characterized as the *N*-acetyl derivative **27** (Scheme 6). As a final confirmation of structure, the aziridine **21** was smoothly deaminated to the known[22] androsta-4,6-diene-3,17-dione **16** as described above for the 5,6-aziridines.

We then turned our attention to the synthesis of the 6α,7α-isomer **23** of compound **21**. Reaction of androsta-4,6-diene-3,17-dione **16** with NaN_3 and 1 mol equiv. of CrO_3[30] in acetic acid at room temp. gave 7α-azido-6β-hydroxyandrost-4-ene-3,17-dione **20** in 42% yield. When compound **20** was treated with Ph_3P as described above, we obtained 6α,7α-aziridinoandrost-4-ene-3,17-dione **23** in 84% yield. Spectral data were consistent with the assigned structure **23**. Faithful to the influence of a 6,7-oxirane/aziridine ring on the ^1H NMR resonance of the C-19 protons of AD, the C-19 protons of compound **23** appeared at δ 1.14, an upfield shift with respect to the ^1H NMR resonance of C-19 protons of AD. These ^1H NMR data of compound **23** are consistent with those of the analogous 6α,7α-epoxide **17**. The 6α,7α-aziridine **23** was also further characterized as the *N*-acetyl derivative **28** and it also underwent smooth deamination to give dienone **16** (Scheme 6).

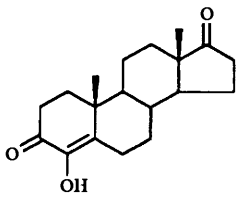
The stereochemical aspects of the formation of the aziridines **21** and **23** from the respective azido alcohols **19** and **20** are of interest. In each case, the azido alcohol yields only one product, and the aziridine ring retains the original configuration of the azido group. The two-step procedure developed by Ittah *et al.*[21] (i, NaN_3; ii, Ph_3P) for the transformation of an epoxide into the corresponding aziridine is reported to occur with retention of configuration (*i.e.*, α-epoxide ⟶ α-aziridine). In contrast, our transformation **17** ⟶ **21** results in an aziridine with inversion of configuration (*i.e.*, 6α,7α-epoxide ⟶ 6β,7β-aziridine). Similar observations have recently been reported.[31,32]

The IC_{50}-values of the isomeric aziridines **21** and **23** were determined (Table 1) to be 49.25 and ⩾250 μ mol dm^{-3} respectively towards human placental aromatase. In addition, the IC_{50}-values of their derivatives (*N*-acetates **27** and **28** and *N*-tosylesters **29** and **30**) were also determined. These were also found (Table 1) to be poor inhibitors of the enzyme. Some of the intermediates described in this work for the synthesis of the 6,7-aziridines are novel and have not been studied as aromatase inhibitors, and it appeared worthwhile to evaluate them for aromatase inhibitory properties. In addition, some of their derivatives and a transformed product were also assayed for aromatase inhibition. Specifically, we examined the aromatase inhibitory effects of the following: compounds **17**–**20**; acetyl derivatives, **24** and **25** respectively, of the isomeric azidohydrins **19** and **20**, and 6-azidoandrosta-4,6-diene-3,17-dione **30** obtained from acetate **24** by base-catalysed elimination[26] of the 7-acetate substituent. It was found (Table 1) that most of the compounds were modest inhibitors of aromatase. On the other hand, 7α-acetoxy-6β-azidoandrost-4-ene-3,17-dione **24** was a potent inhibitor of the enzyme. The IC_{50}-values of some known inhibitors of aromatase were also determined (Table 1) for comparison. Detailed biochemical results of our inhibitors will be reported elsewhere.[12]

Table 1 Aromatase inhibition by analogues of androstenedione



31



32

Compound	IC_{50} (10^{-6} mol dm^{-3})[a]
17	39.0
18	15.3
19	47.0
20	3.03
21	49.25
23	≫250
24	0.40
25	23.13
26	4.55
27	>250
28	64.50
29	53.8
30	93.0
for comparison	
Androsta-4,6-diene-3,17-dione **16**	2.20
3′,3′-Difluoro-3′H-cyclopropa[6,7]-androstenedione **31**	0.89
4-Hydroxyandrostenedione **32**	0.55

[a] Substrate: 2.5 μmol dm^{-3} [1β,2β-^{3}H]testosterone.

Experimental

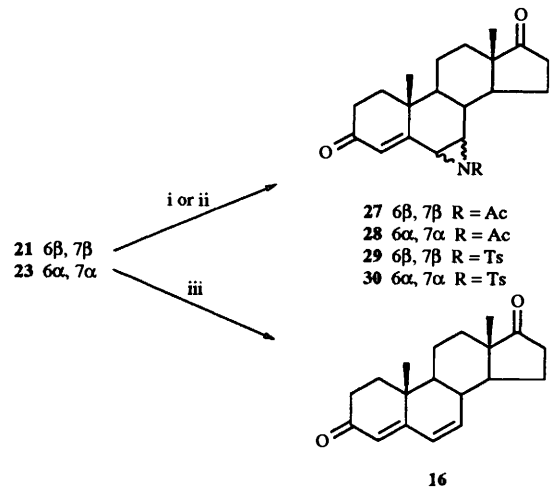
General procedures and techniques were identical with those previously described.[5c]

(*E*)-6-(Hydroxyimino)androst-4-ene-3β,17β-diol 2

This compound, prepared as previously reported,[3b] provided spectral and analytical data as described.[3b]

Reduction of (*E*)-6-(hydroxyimino)androst-4-ene-3β,17β-diol 2 with LAH. 5α,6α-Dihydro-1′H-azirino[5,6]-5α-androstane-3β,17β-diol 5 5β,6β-dihydro-1′H-azirino[5,6]-5α-androstane-3β,17β-diol 6

A solution of the oxime **2** (500 mg, 1.57 mmol) in dry THF (20 cm^3) was added dropwise to a suspension of LAH (350 mg, 9.22 mmol) in dry THF (35 cm^3) which was then refluxed under nitrogen. Stirring and heating were continued for 4 h, after which excess of LAH was decomposed with water (3 cm^3). Insoluble inorganic material was filtered off, and washed with 10% methanol in EtOAc (100 cm^3). The combined filtrate and washings were washed with brine (25 cm^3 × 2), dried, and evaporated to give a light yellow solid (460 mg). Chromatography on neutral column (30 g; 2 cm i.d.; gradient elution with $CHCl_3$–EtOH) gave two products in the following order: (i) the 5β,6β-*aziridinoandrostane*-3β,17β-*diol* **5** (163 mg, 35%), mp 214–218 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3400 and 3150; δ_H(300 MHz; CD_3OD) 0.72 (3 H, s, 18-H_3), 0.99 (3 H, s, 19-H_3), 3.53 (1 H, t, *J* 8.5, 17α-H) and 3.67 (1 H, m, 3α-H) [Found: M^+, 305.2359 (17%). $C_{19}H_{31}NO_2$ requires *M*, 305.2355]; and (ii) the 5α,6α-*aziridinoandrostane*-3β,17β-*diol* **6** (273 mg, 57%), mp (crystals fragment 119–122 °C) 169–172 °C; ν_{max}(KBr)/cm^{-1} 3400; δ_H(300 MHz; CD_3OD) 0.68 (3 H, s, 18-H_3), 1.11 (3 H, s, 19-H_3), 3.53 (1 H, t, *J* 8.7, 17α-H) and 3.69 (1 H, m, 3α-H) [Found: M^+, 305.2362 (65%)].



Scheme 6 *Reagents and conditions:* Ac_2O, Py, room temp., 15 h; ii, *p*-TSCl, Et_3N, CH_2Cl_2, room temp., 12 h; iii, AcOH, $NaNO_2$, room temp., 0.5 h

3β,5,17β-Trihydroxy-5α-androstan-6-one 10

3β,5,17β-Trihydroxy-6-oneandrostan-6-one3β,17β-diacetate[3b] (1.85 g, 4.56 mmol) was dissolved in methanol (20 cm^3) under nitrogen and the resulting solution was treated with 10% aq. potassium hydroxide (55 cm^3). The mixture was stirred at room temp. for 45 min, and then concentrated under reduced pressure, without being heated, to a volume of 15 cm^3. This solution was then poured into ice–water (200 cm^3), and the resulting precipitate was filtered off, washed with water (100 cm^3), and dried *in vacuo* to give compound **10** (1.35 g, 91%), mp 222–225 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3330, 1695, 1070 and 1060; δ_H(400 MHz; CD_3OD) 0.72 (3 H, s, 18-H_3), 0.79 (3 H, s, 19-H_3), 3.62 (1 H, t, *J* 9, 17α-H) and 3.90 (1 H, m, 3α-H) [Found: M^+, 322.2149 (50%). $C_{19}H_{30}O_4$ requires *M*, 322.2144].

(*E*)-6-(Hydroxyimino)-5α-androstane-3β,5,17β-triol 11

A solution of the ketone **10** (1.2 g, 3.7 mmol) in ethanol (25 cm^3) was treated with a solution of hydroxylamine hydrochloride (940 mg) and anhydrous sodium acetate (940 mg) in 50% aq. ethanol (45 cm^3). The reaction mixture was stirred at room temperature for 16 h, and was then concentrated under reduced pressure at room temperature to a volume of ~20 cm^3. This solution was diluted with ice-cold water (180 cm^3) and the resulting precipitate was processed in the usual manner to give *oxime* **11** (1.14 g, 91.2%), mp 278–282 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3420–3300, 1650, 1075 and 1060; δ_H(300 MHz; CD_3OD) 0.72 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 3.12 (1 H, dd, *J* 13.5 and 4.6, 7β-H), 3.59 (1 H, t, *J* 8.6, 17α-H) and 3.96 (1 H, m, 3α-H) [Found: M^+, 337.2261 (19.5%). $C_{19}H_{31}NO_4$ requires M, 337.2252].

Reduction of (*E*)-6-(hydroxyimino)-5α-androstane-3β,5,17β-triol 11 with LAH: 6β-amino-5α-androstane-3β,5α,17β-triol 13

Treatment of oxime **11** (1.0 g, 2.96 mmol) in dry THF (25 cm^3) with LAH (700 mg, 18.42 mmol) in THF (40 cm^3) at reflux for 26 h as described above gave unchanged oxime **11** (380 mg recovery) and 6β-*amino*-5α-*androstane*-3β,5,17β-*triol* **13** (120 mg, 12.5%); mp 260–263 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3400, 3150 and 1595 (N–H bending, typical for primary amine); δ_H(300 MHz; CD_3OD) 0.76 (3 H, s, 18-H_3), 1.17 (3 H, s, 19-H_3), 2.64 (1 H, m, 6α-H), 3.57 (1 H, t, *J* 8.5, 17α-H) and 4.02 (1 H, m, 3α-H); EIMS *m/z* 323 (M^+, 57%), 305 (3), 287 (3) and 152 (100) (Found: M^+, 323.2451. $C_{19}H_{33}NO_2$ requires M, 323.2460).

Androsta-4,6-diene-3,17-dione 16

This compound, prepared as reported,[22] provided spectral and analytical data as described.[22]

Epoxidation of androsta-4,6-diene-3,17-dione 16 with MCPBA: 6α,7α-dihydrooxireno[6,7]androst-4-ene-3,17-dione 17 and 4β,5β-dihydrooxireno[4,5]androst-6-ene-3,17-dione 18

A mixture of the dienone **16** (2.5 g, 8.8 mmol) and MCPBA (80–90% ex Janssen Chemica; 2.6 g) in freshly distilled $CHCl_3$ (120 cm^3) was refluxed for 2 h. More MCPBA (600 mg) was added to the reaction mixture, which was refluxed for a further 1 h. After cooling of the mixture, excess of MCPBA was destroyed by the addition of saturated aq. sodium sulfite (50 cm^3). After being vigorously stirred for 20 min, the layers were separated, and the organic phase was washed successively with saturated aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by flash chromatography [silica gel; (1:1) light petroleum–EtOAc] to give pure compound **17** (1.5 g, 57.3%), compound **18** (200 mg, 7.6%) and mixed fractions. The 6α,7α-oxirane, **17**, had mp 206–210 °C; ν_{max}(KBr)/cm^{-1} 1735, 1670, 1270 and 1230; δ_H(300 MHz; $CDCl_3$) 0.95 (3 H, s, 18-H_3), 1.13 (3 H, s, 19-H_3), 3.46 (1 H, br s, 6β-H), 3.53 (1 H, d, *J* 3.7, 7β-H) and 6.14 (1 H, s, 4-H); λ_{max}(EtOH)/nm 237 (ε 11 420); EIMS *m/z* 300 (M^+, 63.1%), 285 (35.2), 267 (34.8), 138 (66.2) and 41 (100) (Found: M^+, 300.1727. $C_{19}H_{24}O_3$ requires M, 300.1725). Compound **18** had mp 121–124 °C; ν_{max}(KBr)/cm^{-1} 1750, 1720 and 1240; δ_H(300 MHz; CD_3OD) 0.95 (3 H, s, 18-H_3), 0.98 (3 H, s, 19-H_3), 3.36 (1 H, s, 4β-H), 5.22 (1 H, dd, *J* 9.9 and 2.6, 7-H) and 6.16 (1 H, d, *J* 9.9, 6-H); EIMS *m/z* 300 (M^+, 79%), 285 (26), 267 (25) and 138 (100) (Found: M^+, 300.1724).

6β-Azido-7α-hydroxyandrost-4-ene-3,17-dione 19

To a solution of sodium azide (1.38 g, 20 mmol) in DMSO (9 cm^3)–conc. H_2SO_4 (97 mm^3) at room temperature was added the 6α,7α-epoxide **17** (400 mg, 1.33 mmol). The mixture was stirred for 5 h, then was poured into cold water (100 cm^3) and the resulting precipitate was filtered off, washed (water), and dried under reduced pressure to give pure azide **19** (382 mg, 84%), mp 245–250 °C (decomp); ν_{max}(KBr)/cm^{-1} 3410, 2100, 1735, 1665 and 1245; δ_H(300 MHz; $CDCl_3$) 0.94 (3 H, s, 18-H_3), 1.39 (3 H, s, 19-H_3), 3.87 (1 H, m, 7β-H), 4.12 (1 H, d, *J* 2.9, 6α-H) and 5.92 (1 H, s, 4-H); λ_{max}(EtOH)/nm 232 (ε 11 590); EIMS *m/z* 343 (M^+, 0%), 315 ($M^+ - N_2$, 25), 299 (58), 284 (100) and 240 (35) [Found: MH^+, 344.1978 (30%). $C_{19}H_{26}N_3O_3$ requires MH, 344.1974].

7α-Azido-6β-hydroxyandrost-4-ene-3,17-dione 20

Sodium azide (7.41 g, 114 mmol), then chromium trioxide (285.1 mg, 2.85 mmol), were added to a magnetically stirred solution of androsta-4,6-diene-3,17-dione **16** (810 mg, 2.85 mmol) in glacial acetic acid (36 cm^3) and the mixture was stirred for 30 min at room temperature before being diluted with water (140 cm^3) and extracted well with 10% methanol in EtOAc. The combined extracts were washed successively with saturated aq. $NaHCO_3$ and brine, and dried (Na_2SO_4). Evaporation of the solvent gave a light green solid, which was purified by flash chromatography [silica gel; (18:1) $CHCl_3$–EtOH] to give pure azide **20** (405 mg, 42%), mp 250–254 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3430, 2110, 1720 and 1670; δ_H(300 MHz; $CDCl_3$) 0.94 (3 H, s, 18-H_3), 1.37 (3 H, s, 19-H_3), 3.91 (1 H, m, 7β-H), 4.42 (1 H, m, 6α-H) and 5.94 (1 H, s, 4-H); λ_{max}(EtOH)/nm 231.7 (ε 11 590); CIMS *m/z* 344 (MH^+, 8.5%), 300 (32.2), 286 (100), 272 (77.3) and 227 (32.7) (Found: M^+, 343.1897. $C_{19}H_{25}N_3O_3$ requires M, 343.1896).

6β-Azido-7α-mesyloxyandrost-4-ene-3,17-dione 22

A solution of the azido alcohol **19** (250 mg, 0.729 mmol) in pyridine (35 cm^3) at 0 °C was treated with methanesulfonyl chloride (228 mm^3, 2.94 mmol) and the reaction mixture was then left in a cold-room for 16 h. Water was then added and the product was recovered with EtOAc. The extract was processed in the conventional manner to give pure ester **22** (263 mg, 95%); mp 160–162 °C (decomp.); ν_{max}(KBr)/cm^{-1} 2105, 1725, 1675, 1355 and 1108; δ_H(300 MHz; $CDCl_3$) 0.95 (3 H, s, 18-H_3), 1.42 (3 H, s, 19-H_3), 3.03 (3 H, s, 7α-OMs), 4.47 (1 H, d, *J* 3.1, 6α-H), 4.48 (1 H, m, 7β-H) and 5.95 (1 H, s, 4-H); λ_{max}(EtOH)/nm 229 (ε 997); CIMS *m/z* 422 (MH^+, 12.5%), 404 (37.5), 326 (71.7), 298 (100) and 280 (41) (Found: MH^+, 422.1756. $C_{20}H_{28}N_3O_5$ requires MH, 422.1777).

6α,7α-Dihydro-1'H-azirino[6,7]androst-4-ene-3,17-dione 21

(A) From alcohol 19. A mixture of the alcohol **19** (120 mg, 0.3499 mmol) and Ph_3P (186 mg, 0.7154 mmol) in sodium-dried toluene (6 cm^3) under nitrogen was maintained at 105–110 °C for 4 h. After evaporation, the residue was purified by flash chromatography [silica gel; $CHCl_3$–EtOH (18:1)] to give the pure *aziridine* **21** (71 mg, 68%); mp 256–259 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3260, 1700 and 1660; δ_H(400 MHz; $CDCl_3$) 0.94 (3 H, s, 18-H_3), 1.25 (3 H, s, 19-H_3), 2.60 (1 H, m, 7α-H), 2.69 (1 H, d, *J* 5.8, 6α-H) and 6.1 (1 H, s, 4-H); λ_{max}(EtOH)/nm 253 (ε 9010); EIMS *m/z* 299 (M^+, 100%), 284 (28), 271 (10) and 256 (15) (Found: M^+, 299.1789. $C_{19}H_{25}NO_2$ requires M, 299.1894).

(B) From ester 22. A solution of ester **22** (200 mg, 0.4750 mmol) in dry THF (3 cm^3) was added slowly to a stirred suspension of LAH (180 mg, 4.74 mmol) in dry THF (4 cm^3) at room temperature under nitrogen. The mixture was stirred for 2.5 h, after which excess of LAH was decomposed with water (2 cm^3). Processing as described above for compounds **5** and **6** gave a solid (124 mg, crude 6α,7α-dihydroazirino[6,7]androst-4-ene-3,17-diol **3**), which was oxidized without purification.

A solution of the crude 6β,7β-aziridino diol **3** (100 mg, ~0.33 mmol) in dry DMF (4 cm^3) at 4 °C was treated with pyridinium dichromate (PDC, 1.12 g, 2.96 mmol) and the reaction mixture was stirred in a cold-room for 12 h. The mixture was diluted with water (25 cm^3) and basified with 2 mol dm^{-3} NaOH (10 cm^3) followed by extraction with 10% MeOH in EtOAc (25 cm^3 × 3). The combined extract was washed successively with water and brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a yellow semi-solid (85 mg). Purification of this by preparative TLC (PLC) [silica gel; $CHCl_3$–EtOH (18:1) × 2] gave pure compound **21** (52 mg). All spectral and analytical data were identical with those of compound **21** from the alcohol **19** described above.

6β,7β-Dihydro-1'H-azirino[6,7]androst-4-ene-3,17-dione 23

Compound **20** (150 mg, 0.4373 mmol) was subjected to the Ph_3P reductive cyclization as described above for compound **21** to give *compound* **23** (110 mg, 84%), mp 202–205 °C; ν_{max}(KBr)/cm^{-1} 3290, 1740 and 1660; δ_H(400 MHz; $CDCl_3$) 0.95 (3 H, s, 18-H_3), 1.14 (3 H, s, 19-H_3), 2.59 (1 H, m, 7β-H), 2.79 (1 H, d, *J* 5.2, 6β-H) and 6.06 (1 H, s, 4-H); λ_{max}(EtOH)/nm 238 (ε 21 580); EIMS *m/z* 299 (M^+, 28.3%), 284 (8.1), 256 (31.2) and 43 (100) (Found: M^+, 299.1894. $C_{19}H_{25}NO_2$ requires M, 299.1894).

Acetyl derivatives of aziridines 5, 6, 21 and 23, amine 13 and azidohydrins 19 and 20

Each of these compounds was separately acetylated (Ac_2O–pyridine; room temp., 17 h) and, following work-up and purification [PLC, silica gel; $CHCl_3$–EtOH (18:1)], yielded the corresponding *N*-acetyl, *N,O*-triacetyl or *O*-acetyl derivatives. Analytical and spectroscopic data for these derivatives were as follows:

***N,O*-Triacetyl derivative of compound 5.** Compound **7**, mp (crystals fragment 80–85 °C) 104–108 °C; ν_{max}(KBr)/cm^{-1} 1725, 1680 and 1240; δ_H(300 MHz; $CDCl_3$) 0.77 (3 H, s, 18-H_3), 1.09 (3 H, s, 19-H_3), 2.02 (3 H, s, 17β-OAc), 2.03 (3 H, s, 3β-OAc), 2.07 (3 H, s, NAc), 2.40 (1 H, m, 6α-H), 2.61 (1 H, s), 4.57 (1 H, t, *J* 8.5, 17α-H) and 4.86 (1 H, m, 3α-H); CIMS *m/z* 432 (MH^+, 21.9%), 404 (16), 372 (100) and 312 (35.2) (Found: MH^+, 432.2749. $C_{25}H_{38}NO_5$ requires MH, 432.3116).

***N,O*-Triacetyl derivative of compound 6.** Compound **8**, mp 159–162 °C; ν_{max}(KBr)/cm^{-1} 1720, 1660 and 1240; δ_H(300 MHz; $CDCl_3$) 0.75 (3 H, s, 18-H_3), 1.07 (3 H, s, 19-H_3), 1.99 (3 H, s, 17β-OAc), 2.03 (3 H, s, 3β-OAc), 2.15 (3 H, s, NAc), 2.26 (1 H, m, 6β-H), 2.39 (1 H, d, *J* 4.8 6α-H), 4.55 (1 H, t, *J* 8.5, 17α-H) and 5.33 (1 H, m, 3α-H); CIMS *m/z* 432 (MH^+, 22.5%), 372 (100), 328 (21.7), 312 (39.8) and 253 (32.6) (Found: MH^+, 432.2753).

***N,O*-Triacetyl derivative of compound 13.** Compound **14**, mp 275–278 °C; ν_{max}(KBr)/cm^{-1} 3400, 1730, 1665 and 1250; δ_H(300 MHz; $CDCl_3$) 0.82 (3 H, s, 18-H_3), 1.14 (3 H, s, 19-H_3), 2.01 (3 H, s, 17β-OAc), 2.02 (3 H, s, 3β-OAc), 2.04 (3 H, s, NAc), 4.02 [1 H, m (d with NaOD), 6α-H], 4.60 (1 H, t, *J* 8.5, 17α-H), 5.15 (1 H, m, 3α-H) and 5.55 [1 H, d (exchanges with NaOD), *J* 10, N*H*Ac]; EIMS *m/z* 449 (M^+, 9%), 431 (2), 371 (60), 356 (24), 335 (32), 312 (27) and 276 (42) (Found: M^+, 449.2758. $C_{25}H_{39}NO_6$ requires M, 449.2786).

***N*-Acetyl derivative of compound 21.** Compound **27**, mp 156–159 °C; ν_{max}(KBr)/cm^{-1} 1735, 1700, 1660 and 1610; δ_H(300 MHz; $CDCl_3$) 0.95 (3 H, s, 18-H_3), 1.25 (3 H, s, 19-H_3), 2.14 (3 H, s, NAc), 2.95 (1 H, m, 7α-H), 3.01 (1 H, d, *J* 5.8, 6α-H) and 6.15 (1 H, s, 4-H); λ_{max}(EtOH)/nm 243 (ε 13 100); EIMS *m/z* 341 (M^+, 43.9%), 313 (14.3), 299 (100), 284 (55.6) and 256 (24.7) (Found: M^+, 341.1994. $C_{21}H_{29}NO_3$ requires M, 341.2000).

***N*-Acetyl derivative of compound 23.** Compound **28**, mp 160–164 °C; ν_{max}(KBr)/cm^{-1} 1740, 1690 and 1675; δ_H(400 MHz; $CDCl_3$) 0.95 (3 H, s, 18-H_3), 1.25 (3 H, s, 19-H_3), 2.14 (3 H, s, NAc), 2.95 (1 H, dd, *J* 5.9 and 1.5, 7β-H), 3.01 (1 H, d, *J* 5.9, 6β-H) and 6.16 (1 H, s, 4-H); λ_{max}(EtOH)/nm 234 (ε 17 030); EIMS *m/z* 341 (M^+, 37.4%), 314 (100), 299 (36.4) and 272 (39.3) (Found: M^+, 341.2002).

***O*-Acetyl derivative of compound 19.** Compound **24**, mp 164–166 °C; ν_{max}(KBr)/cm^{-1} 2100, 1730, 1670 and 1230; δ_H(400 MHz; $CDCl_3$) 0.94 (3 H, s, 18-H_3), 1.41 (3 H, s, 19-H_3), 2.06 (3 H, s, 7α-OAc), 4.14 (1 H, d, *J* 2.9, 6α-H), 4.94 (1 H, m, 7β-H) and 5.85 (1 H, s, 4-H); λ_{max}(EtOH)/nm 230.4 (ε 16 520); CIMS *m/z* 386 (MH^+, 1.83%), 368 (34.8), 343 (20.8), 327 (21), 326 (100), 298 (20.2), 285 (16.8) and 280 (5) (Found: M^+, 385.2024. $C_{21}H_{27}N_3O_4$ requires M, 385.2028).

***O*-Acetyl derivative of compound 20.** Compound **25**, mp 183–185 °C; ν_{max}(KBr)/cm^{-1} 2102, 1736, 1680 and 1230; δ_H(400 MHz; $CDCl_3$) 0.95 (3 H, s, 18-H_3), 1.29 (3 H, s, 19-H_3), 2.12 (3 H, s, 6β-OAc), 3.86 (1 H, t, *J* 3.0, 7β-H), 5.46 (1 H, d, *J* 3.1, 6α-H) and 6.08 (1 H, s, 4-H); λ_{max}(EtOH)/nm 233 (ε 18 380); CIMS *m/z* 386 (MH^+, 5.7%), 371 (16.7), 344 (34.3), 343 (100) and 301 (20) (Found: MH^+, 386.2048. $C_{21}H_{28}N_3O_4$ requires MH, 386.2106).

N-Tosyl derivatives of aziridines 21 and 23

Each of the aziridines (10 mg, 0.0334 mmol) was separately tosylated [toluene-p-sulfonyl chloride (freshly recrystallized from $CHCl_3$, 12.7 mg, 0.0668 mmol); triethylamine (0.2672 mmol), CH_2Cl_2 (0.5 cm^3); room temp; 12 h] and, following work-up and purification [PLC, silica gel; light petroleum (40–60 °C)–EtOAc (1 : 1)], gave the corresponding *N*-tosyl derivative in quantitative yield. Analytical and spectroscopic data for the two derivatives were as follows:

***N*-Tosyl derivative of compound 21.** Compound **29**, mp 180–184 °C; ν_{max}(KBr)/cm^{-1} 1740, 1675, 1158 and 680; δ_H(400 MHz; $CDCl_3$) 0.88 (3 H, s, 18-H_3), 1.17 (3 H, s, 19-H_3), 2.43 (3 H, s, Ar*Me*), 3.22 (1 H, m, 7α-H), 3.27 (1 H, d, *J* 6.8, 6α-H), 5.96 (1 H, s, 4-H), 7.36 (2 H, d, *J* 8.3, ArH) and 7.82 (2 H, d, *J* 8.1, ArH); λ_{max}(EtOH)/nm 228 (ε 23 870); CIMS *m/z* 454 (MH^+, 100%), 300 (9.6), 285 (17.7) and 172 (15.3) (Found: MH^+, 454.2077. $C_{26}H_{32}NO_4S$ requires MH, 454.2061).

***N*-Tosyl derivative of compound 23.** Compound **30**, mp 196–200 °C; ν_{max}(KBr)/cm^{-1} 1735, 1675, 1160 and 675; δ_H(400 MHz; $CDCl_3$) 0.91 (3 H, s, 18-H_3), 1.09 (3 H, s, 19-H_3), 2.45 (3 H, s, Ar*Me*), 3.31 (1 H, dd, *J* 6.4 and 2.7, 7β-H), 3.43 (1 H, d, *J* 6.7, 6β-H), 5.79 (1 H, s, 4-H), 7.35 (2 H, d, *J* 8.2, ArH) and 7.82 (2 H, d, *J* 8.3, ArH); λ_{max}(EtOH)/nm 229 (ε 33 300); CIMS *m/z* 454 (MH^+, 100%), 436 (25.8, 285 (30.1) and 172 (16) (Found: MH^+, 454.2088. $C_{26}H_{32}NO_4S$ requires MH, 454.2061).

Deamination of the 5β,6β- and 5α,6α-aziridinoandrostane-3β,17β-diols 5 and 6 and of the 6β,7β- and 6α,7α-aziridinoandrost-4-ene-3,17-diones 21 and 23

Each of the aziridines was separately deaminated as previously[5c] described. Compounds **5** and **6** each gave androst-4-ene-3β,17β-diol **9**, identified by its mp, IR and ^{1}H NMR spectra and TLC comparison with authentic material, while compounds **21** and **23** each gave androsta-4,6-diene-3,17-dione **16**, identified by its mp, IR, UV and ^{1}H NMR spectra and TLC comparison with authentic material.

6-Azidoandrosta-4,6-diene-3,17-dione 26.

This compound, prepared from acetate **24** following the procedure of Teutsch *et al.*,[26] provided spectral and analytical data as described.[26]

3′,3′-Difluoro-3′H-cyclopropa[6,7]androst-4-ene-3,17-dione 31

This compound, prepared as previously reported,[10] provided spectral and analytical data as described.[10]

4-Hydroxyandrost-4-ene-3,17-dione 32

This compound, prepared from androstenedione following the procedure of Mann and Pietrzak,[3c] provided spectral and analytical data as described.[3c]

Acknowledgements

We thank Dr. H. Ł. Holland, Dr. J. Kachinski, Dr. R. Graf, Mr. A Weber and Mr. J. Schröder for the mass spectra, Mr. M. Schommer for the 400 MHz NMR spectra, the School of Public Health, Johns Hopkins University for access to their 300 MHz NMR spectrometer; Miss M. Palzer and Miss G. Grün for enzyme assays and Dr. P. Dorfmüller for useful discussions. We also thank the Alexander von Humboldt Foundation for the award of a Fellowship to V. C. O. N. This work was supported by NIH Grant HD 11840, Verband der Chemischen Industrie, Fonds der Chemischen Industrie and Wellcome Nigeria Fund.

References

1 P. A. Cole and C. H. Robinson, *J. Med. Chem.*, 1990, **33**, 2933.
2 A. M. H. Brodie, W. M. Garrett, J. R. Hendrickson, C. H. Tsai-Morris, P. A. Marcotte and C. H. Robinson, *Steroids*, 1981, **38**, 393; D. F. Covey and W. F. Hood, *Mol. Pharmacol.*, 1982, **21**, 173; Y. J. Abul-Hajj, *J. Med. Chem.*, 1986, **29**, 582; *J. Steroid Biochem.*, 1990, **35**, 139; M. G. Rowlands, A. B. Foster, J. Mann, B. Pietrzak, J. Wilkinson and R. C. Coombes, *Steroids*, 1987, **49**, 371.
3 (*a*) M. Numazawa, M. Tsuji and A. Mutsumi, *J. Steroid Biochem.*, 1987, **28**, 337; (*b*) H. L. Holland, S. Kumaresan, L. Tan and V. C. O. Njar, *J. Chem. Soc., Perkin Trans 1*, 1992, 585; (*c*) J. Mann and B. Pietrzak, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2681.
4 C. E. Snider and R. W. Brueggemeier, *J. Biol. Chem.*, 1987, **262**, 8685.
5 J. T. Kellis, W. E. Childers, C. H. Robinson and L. E. Vickery, *J. Biol. Chem.*, 1987, **262**, 4421; (*b*) J. N. Wright, P. T. vanLeersum, S. G. Chamberlin and M. Akhtar, *J. Chem. Soc., Perkin Trans. 1*,

1989, 1647; (*c*) V. C. O. Njar, E. Safi, J. V. Silverton and C. H. Robinson, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1161; (*d*) D. F. Covey, W. F. Hood and V. D. Parikh, *J. Biol. Chem.*, 1981, **256**, 1076 (*e*) B. W. Metcalf, C. L. Wright, J. P. Burkhart and J. O. Johnston, *J. Am. Chem. Soc.*, 1981, **103**, 3221; (*f*) J. Mann and B. Pietrzak, *J. Chem. Soc., Perkin Trans. 1*, 1987, 385.
6 A. M. H. Brodie, *J. Steroid Biochem. Mol. Biol.*, 1994, **49**, 281.
7 Y. Osawa, Y. Osawa and M. J. Coon, *Endocrinology*, 1987, **121**, 1010.
8 M. Numazawa and M. Oshibe, *J. Med. Chem.*, 1994, **37**, 1312.
9 R. W. Brueggemeier, E. E. Floyd and R. E. Counsell, *J. Med. Chem.*, 1978, **21**, 1007; R. W. Brueggemeier, P. P. Moh, S. Ebrahimian and M. V. Darby, *J. Steroid Biochem. Mol. Biol.*, 1993, **44**, 357.
10 M. Numazawa and A. Mutsumi, *Biochem. Biophys. Res. Commun.*, 1991, **177**, 401.
11 J. A. Deyrup, *Heterocyclic Compounds*, ed. A. Hassner, Wiley, New York, 1983, vol. 42, part 1. ch 1, p. 116.
12 Part of this work has been presented by V. C. O. Njar, R. W. Hartmann and C. H. Robinson, at the 2nd European Congress of Pharmaceutical Sciences, Berlin, Germany, September, 1994; Abstract published in *Eur. J. Pharm. Sci.*, 1994, **2**, 101. Detailed biochemical results will be published in *J. Enz. Inhib.*
13 K. Ponsold, *Chem. Ber.*, 1964, **97**, 3524.
14 K. Tori, T. Komeno and T. Nakagawa, *J. Org. Chem.*, 1964, **29**, 1136.
15 Shinongi and Co. Ltd., *Neth. Appl.*, 6 515 376, March 1966 (*Chem. Abstr.*, **65**, 115324e).
16 L. Ferrero, S. Geribaldi, M. Rouillard and M. Azzaro, *Can. J. Chem.*, 1975, **53**, 3227.
17 Y. Houminer, *J. Chem. Soc., Perkin Trans. 1*, 1976, 1037.
18 N. J. Harper, A. F. Casy and J. R. Dimmock, *J. Chem. Soc.*, 1964, 4280.
19 S. R. Landor, O. O. Sonola and A. R. Tatchell, *J. Chem. Soc., Perkin Trans 1*, 1974, 1294.
20 A. Tzikas, C. Tamm, A. Boller and A. Fürst, *Helv. Chim. Acta*, 1976, **59**, 1850.
21 Y. Ittah, Y. Sasson, I. Shakah, S. Tsaroom and J. Blum, *J. Org. Chem.*, 1978, **43**, 4271.
22 S. K. Pradhan and H. J. Ringold, *J. Org. Chem.*, 1964, **29**, 601.
23 J. A. Saboz, T. Lizuka, H. Wehrli, K. Schaffner and O. Jeger, *Helv. Chim. Acta*, 1968, **51**, 1362.
24 G. Nathaansohn, G. Winters and A. Vigevani, *Gazz. Chim. Ital.*, 1965, **95**, 1338 (*Chem. Abstr.*, 1966, **64**, 9791b).
25 G. Teutsch, E. L. Shapiro and H. L. Herzog, *J. Med. Chem.*, 1970, **13**, 750.
26 G. Teutsch, L. Weber, G. Page, E. L. Shapiro, R. Neri and E. J. Collins, *J. Med. Chem.*, 1973, **16**, 1370.
27 G. W. Krakower and H. Ann van Dine, *J. Org. Chem.*, 1966, **31**, 3467.
28 A. M. M. Hossain, D. N. Kirk and G. Mitra, *Steroids*, 1976, **27**, 603.
29 G. J. Matthews and A. Hassner in *Organic Reactions in Steroid Chemistry*, ed. J. Fried and J. A. Edwards, Van Nostrand Reinhold, New York, 1972, vol. 2, pp. 27–29.
30 R. W. Draper, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2781.
31 D. Tanner and P. Somfai, *Tetrahedron Lett.*, 1987, **28**, 1211.
32 J. Ahman and P. Somfai, *J. Am. Chem. Soc.*, 1994, **116**, 9781.

Paper 4/07315K
Received 30*th November* 1994
Accepted 30*th November* 1994