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Conformationally Constrained Anesthetic Steroids That Modulate $GABA_A$ Receptors

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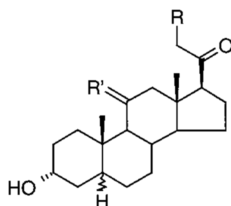
Various cyclic ether and other 3α-hydroxyandrostane derivatives bearing a conformationally constrained hydrogen-bonding moiety were prepared. Their anesthetic potency and their binding affinity for $GABA_A$ receptors, measured by intravenous administration to mice and inhibition of [^{35}S]TBPS binding to rat whole brain membranes, were compared with that of known anesthetic 3α-hydroxypregnan-20-ones. Synthetic steroids with similar in vitro and in vivo activities to the endogenous 3α-hydroxypregnan-20-ones all had an ether oxygen on the *β*-face of the steroid D-ring. These results suggest that for optimal $GABA_A$ receptor modulation, the hydrogen bond-accepting substituent should be near perpendicular to the plane of the D-ring on the *β*-face of the steroid.

Introduction

It is well-established that neurosteroids such as (3α,5α)-3-hydroxypregnan-20-one (**1**) and (3α,5α)-3,21-dihydroxypregnan-20-one (**2**) and neuroactive steroids such as (3α,5*β*)-3-hydroxypregnan-20-one (pregnanolone, **3**) modulate *γ*-aminobutyric $acid_A$ ($GABA_A$) receptor



function.[1] *γ*-Aminobutyric acid (GABA) is the major inhibitory neurotransmittter within the CNS (central nervous system), and steroids that potentiate the effects of GABA at $GABA_A$ receptors have therapeutic potential as anticonvulsants,[2] anxiolytics,[3] sedatives, and hypnotics.[4] In addition to the above endogenous steroids, a number of synthetic analogues including alfaxalone (**4**) have been shown to modulate $GABA_A$ receptors allosterically.[5] The in vitro activity of steroids at $GABA_A$ receptors can be evaluated through their ability to inhibit the specific binding of [^{35}S]-*tert*-butyl bicyclophosphorothionate ([^{35}S]TBPS) to rat whole brain membranes.[6] Almost all potent modulators have a 3α-ol and 20-keto function with the natural 17*β*-pregnane side chain. While the conformation of pregnan-20-ones has been extensively studied for optimal binding to progesterone receptors,[7,8] there are few reports regarding the preferred side chain conformation for allosteric modulation of $GABA_A$ receptors. Replacement of the 17-acetyl side chain of allopregnanolone (**1**) with a nitrile is known to retain activity, but inversion of the configuration at C17 reduces activity.[9] The effect of introducing a 16,17-double bond into steroids, including **1**, **3**, and **4**, has also been studied and generally found to be deleterious for in vitro and in vivo activity.[10]

In this paper we report the syntheses of several allosteric $GABA_A$ modulators where the 20-keto group is replaced by an alternative hydrogen bond-accepting moiety and discuss their potency as inhibitors of TBPS binding, proposing from our results a preferred pregnan-20-one side chain conformation for receptor modulation. In addition we report the anesthetic effect of the compounds upon intravenous administration to mice, finding the hypnotic potency to be comparable in certain instances to that of the endogenous steroids.

Chemistry

To try to establish the preferred side chain conformation of pregnan-20-ones for binding to $GABA_A$ receptors, a number of conformationally constrained steroids bearing a hydrogen bond-accepting substituent on the D-ring were synthesized. These included epoxides **5**−**7**, **10**, and **11**, ketones **8** and **9**, oxetanes **12** and **13**, a nitrile **14**, an alcohol **15**, and an ether **16**.

The 16*β*,17*β*-epoxide **5** was prepared by treating the halohydrin **20** with base. The halohydrin was synthesized following the method of Fajkoš and Šorm: i.e., androsterone acetate **17** was converted to the enol ester **18** and brominated to give the 16α-bromo ketone **19** which was reduced to the 17*β*-ol **20** (Scheme 1).[11]

The 16α,17α-epoxide **6**[12] and the 16*β*,17*β*-epoxide **7** were prepared by stereoselective epoxidation of the relevant androstenes **21** and **24** (Scheme 2).[13] The alkene **24** was synthesized from 5*β*-androstan-17-one **22** via the tosylhydrazone **23**.

Androsterone **8**, like the 3α,17*β*-diol **15**, is commercially available. The 16-keto analogue **9**[14] was

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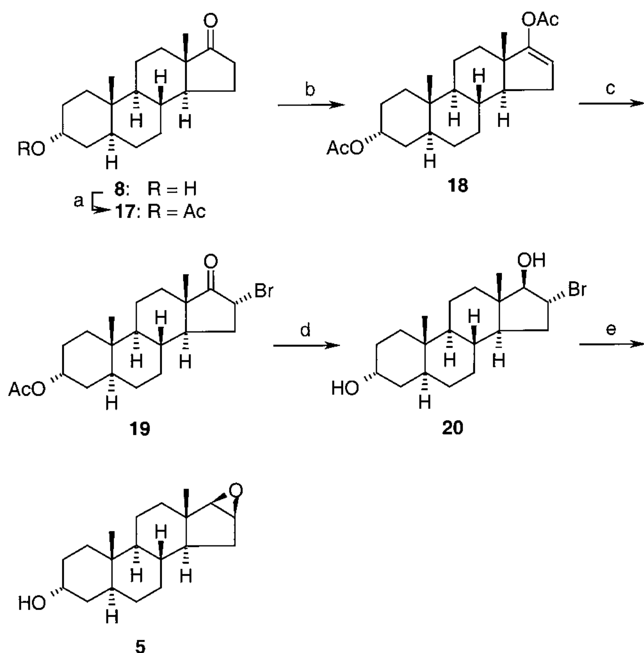
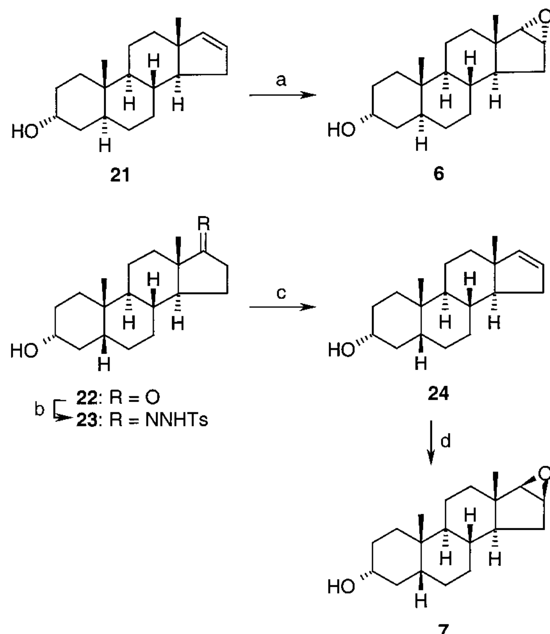
Scheme 1[a]



[a] Reagents: (a) Ac_2O, pyr; (b) $H_2C{=}CMeOAc$, TsOH; (c) Br_2, CCl_4; (d) $LiAlH_4$; (e) KOH, MeOH.

Scheme 2[a]



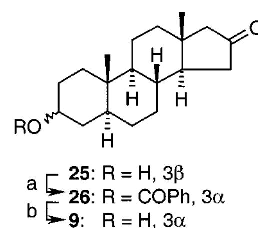
[a] Reagents: (a) AcOOH, NaOAc; (b) $TsNHNH_2$, TsOH; (c) MeLi; (d) NBS, DMSO, H_2O then KOH.

prepared by epimerizing 3β-hydroxy 16-ketone **25** via the benzoate **26** (Scheme 3).

Synthesis of spiro-epoxides **10**[15] and **11** required stereoselective insertion of a methylene unit. This was accomplished by treating ketones **8** and **22** with a sulfonium ylide (Scheme 4). Further reaction of epoxide **10** with a sulfoxonium ylide gave the spiro-oxetane **12**.

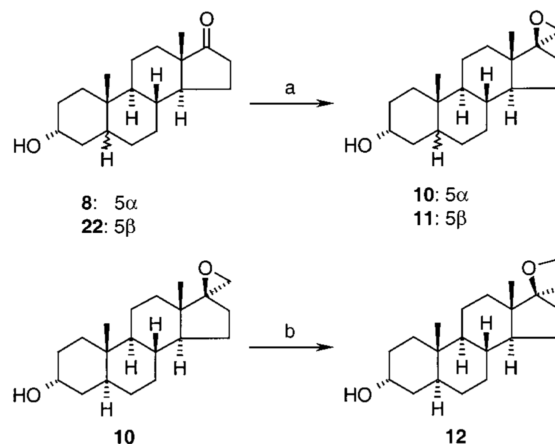
The fused-oxetane **13** (Scheme 5) was prepared by formylation of androsterone **8** followed by stereoselective borohydride reduction to give the triol **28**. Selective tosylation of the primary alcohol, followed by cyclization with sodium methoxide, afforded the 16β,17β-oxetane **13**.

Scheme 3[a]



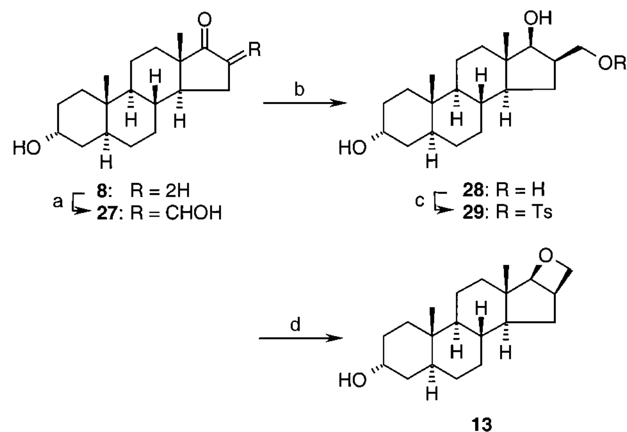
[a] Reagents: (a) BzOH, PPh_3, 1,1′-(azodicarbonyl)dipiperidine; (b) NaOH, MeOH.

Scheme 4[a]



[a] Reagents: (a) Me_3SI, KOBu^t; (b) Me_3SOI, KOBu^t.

Scheme 5[a]

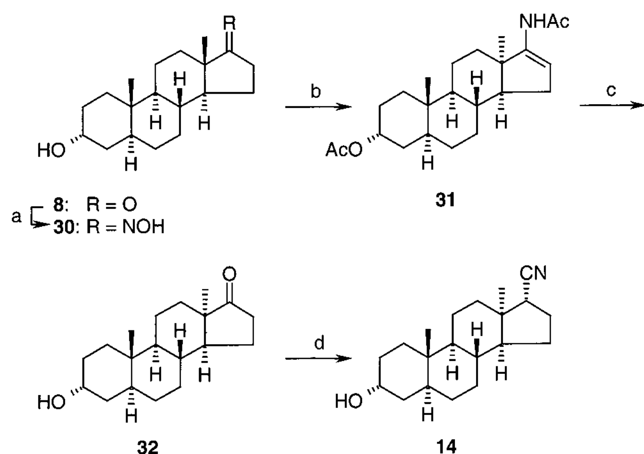


[a] Reagents: (a) NaOMe, HCO_2Et; (b) $NaBH_4$; (c) TsCl, pyr; (d) NaOMe.

For synthesis of the 17α-nitrile **14** (Scheme 6), 13α-androsterone **32** was prepared following the method of Boar et al.[16] Reaction of 13α-androsterone **32** with tosylmethyl isocyanide (TOSMIC) selectively gave the desired 17α-acetonitrile **14** in low yield. The stereochemistry of both structures was confirmed by NMR experiments. For structure **14** NOEs were observed between the 18-Me protons and the 14α-, 15α-, 16α-, and 17β-protons, as well between the 11β-proton and the 11α-, 12α-, and 12β-protons. For structure **32** similar NOEs were observed between the 18-Me protons and the 14α- and 15α-protons.

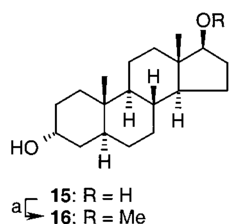
The 17β-ether **16**[17] was prepared by methylation of the diol **15**[18] (Scheme 7).

Scheme 6[a]



[a] Reagents: (a) $NH_2OH·HCl$, pyr; (b) Ac_2O, pyr; (c) HCl, MeOH; (d) $KOBu^t$, TOSMIC.

Scheme 7[a]



[a] Reagents: (a) NaH, MeI.

Pharmacology

The methods used have been previously reported.[5e] The anesthetic potency of the steroids was determined upon their intravenous administration to mice. In each case the dose required to cause a loss of righting reflex for a minimum period of 30 s in 50% of treated mice was determined by probit analysis. This dose is termed the HD_{50} (hypnotic $dose_{50}$). The in vitro effect of the compounds at $GABA_A$ receptors was also assessed, by determination of their ability to inhibit [^{35}S]TBPS binding to rat whole brain membranes. In each case the concentration of drug required to inhibit 50% binding of this radioligand was determined (TBPS IC_{50}). Both in vitro and in vivo results are shown in Table 1.

Discussion

The nature of the binding sites of steroids that allosterically modulate $GABA_A$ receptors has still to be established. Progress has been hindered because $GABA_A$ receptors are membrane-bound proteins which are not conducive to structural determination by methods such as X-ray crystallography and NMR spectroscopy. Moreover all functional mammalian receptors that comprise common subunits (such as α, β, and γ)[19] appear to possess steroid binding sites, although significant differences in response from different brain areas have been noted.[20] A study, which involved constructing chimeras between alfaxalone-sensitive $GABA_A$ receptor α_2 or β_1 subunits and the alfaxalone-insensitive glycine α_1 subunit, suggested the site of action for neurosteroids is on the N-terminal side of the middle of TM2 of $GABA_A$ receptors.[21] Steroids modulate $GABA_A$ receptors at sites that are distinct from other general anesthetics including barbiturates, benzodiazepines, and etomidate.[22] Due

Table 1. Activity of Steroids in the Radioligand ([^{35}S]TBPS) Binding Assay and as Intravenous Hypnotics in the Mouse

compd	TBPS[a] IC_{50} (nM) [*N*][b]	HD_{50}[c] (μmol·kg^{-1})
1	155 ± 6 [4]	10.0 (8.2−12.2)
2	192 ± 28 [3]	18.5
3	145 ± 31 [5]	9.5 (8.1−11.1)
4	423 ± 36 [4]	6.3 (5.6−7.1)
5	255 ± 65 [3]	17.3 (15.6−19.4)
6	5350 ± 450 [2]	34.2−68.4
7	159 ± 29 [3]	38.0 (31.8−45.4)
8	1367 ± 203 [3]	68.9−103.3
9	1767 ± 186 [3]	134.7
10	55 ± 13 [3]	19.8 (16.8−23.5)
11	221 ± 71 [3]	22.6 (20.2−25.4)
12	232 ± 36 [3]	31.4−62.8
13	2533 ± 371 [3]	146.9
14	1236 ± 283 [3]	114.9
15	1867 ± 433 [3]	239.4 (205.2−273.5)
16	210 ± 76 [3]	23.1

[a] IC_{50}: concentration (nM) of steroid required to inhibit 50% of binding of [^{35}S]TBPS from rat whole brain membranes; values are means of experimental determinations ± SEM. [b] Number of experimental determinations. [c] Hypnotic $dose_{50}$ (μmol·kg^{-1}): dose required to cause a loss of righting reflex for a minimum period of 30 s in 50% of treated mice after intravenous administration.

to the problems of studying steroid−receptor interactions, novel steroids that allosterically modulate $GABA_A$ receptors have been identified to date through structural modification of known ligands. Following the discovery that alfaxalone modulates $GABA_A$ receptors, other steroid anesthetics were tested and found to possess similar in vitro activity.[5,9,23] Several papers have appeared in the literature describing such compounds, but all potent derivatives retain a 3α-ol, an androstane nucleus with either 5α- or 5β-configuration, and a hydrogen bond-accepting substituent at the 17β-position. Steroids without an A-ring also have similar potency.[24] So far derivatives with the natural pregnan-20-one side chain (e.g. endogenous neurosteroids) or a 17β-carbonitrile substituent have been shown to produce the greatest potentiation of $GABA_A$ receptor function.[9] The effect of hydrogen bond-donating substituents at the 17β-position has also been examined, but such compounds generally exhibit reduced efficacy at $GABA_A$ receptors.[25] We sought to further broaden the range of modulators by synthesizing conformationally constrained analogues in which the hydrogen bond-accepting substituent is part of a cyclic ether, usually on the β-face of the D-ring.

The analogues were oxiranes such as **5**−**7**, **10**, and **11**, oxetanes such as **12** and **13**, ketones **8** and **9**, an alcohol **15**, and an ether **16**. In addition, the nitrile **14** was prepared to see whether simultaneous inversion of the D-ring stereochemistry at C13 and C17 was tolerated.

The [^{35}S]TBPS assay measures binding to $GABA_A$ receptors in whole rat brains, and the results in Table 1 are therefore an average of binding to a large number of different $GABA_A$ receptor subtypes. It is not known which of these subtypes are most important for anesthesia though a recent report showed the α_1-subunit is crucial for sedation.[26] The affinity of a steroid for $GABA_A$ receptors varies with subunit composition, while anesthetic activity is liable to depend on modulation of specific $GABA_A$ receptor subtypes. For these reasons in vitro and in vivo results are unlikely to correlate

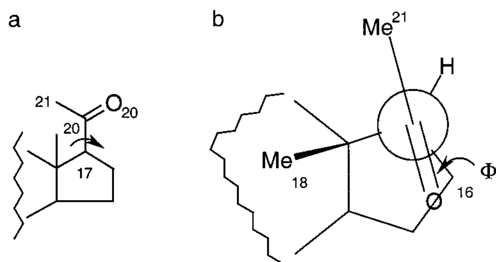


Figure 1. (a) Side chain of pregnan-20-ones indicating rotation about the C17−C20 bond. (b) Newman projection C20 → C17 illustrating the conformation of the side chain in which the C16−C17−C20−O20 torsion angle ϕ is about 25°.

directly. Examination of Table 1 confirms this is the case though some generalizations can be drawn.

Steroids with a [^{35}S]TBPS IC$_{50}$ value in the range 50−500 nM have an HD$_{50}$ ≤ 50 μmol·kg^{-1}, while those with an IC$_{50}$ value > 1000 nM have an HD$_{50}$ ≥ 50 μmol·kg^{-1}. The most active allosteric GABA$_A$ receptor modulators, i.e., steroids **5**, **7**, **10**−**12**, and **16**, have a hydrogen bond-accepting ether function on the β-face. The excellent in vitro and in vivo activity of steroids **10**−**12** is surprising since 17α-substitution is generally deleterious to activity.[9,27,28] Steric constraint within a three- or four-membered ring reduces protrusion of the substituent below the α-face of the steroids. This constraint favors inhibition of [^{35}S]TBPS binding and allosteric modulation of GABA$_A$ receptors. The poorer in vitro activity of the α-epoxide **6** is consistent with the hydrogen bond-accepting substituent being on the less favored α-face. The anesthetic activity of α-epoxide **6** is also lower than that of β-epoxide **5**; this agrees with a previous study which reported 17α-acetyl derivatives to be inferior anesthetics compared with the natural 17β-isomers.[29]

The relationship between the anesthetic activity and the conformation of the A-ring of the steroids has been discussed before, and the results confirm that the configuration at C5 does not markedly affect potency.[5e,29]

Comparison of oxetane **13** with epoxide **5** indicates the additional methylene unit is deleterious for in vitro and in vivo activity. The nitrile **14** retains some in vitro and in vivo activity, but it is apparent that the natural configuration at C13 and C17 is preferred. The alcohol **15** is also a relatively poor GABA$_A$ receptor modulator and has low anesthetic potency. This confirms hydrogen bond-accepting substituents (e.g. the methyl ether **16**) are preferable to hydrogen bond-donating substituents and is consistent with the observation that pregnan-20-ols have 'partial agonist' activity at GABA$_A$ receptors.[9,30]

Computer modeling of steroids **1**−**4** demonstrated there are two conformational minima for the 17β-side chain: one with a C16−C17−C20−O20 torsion angle ϕ varying from −22° to −35° (Figure 1) and the other with C21 eclipsing the C16−C17 bond usually at a slightly higher energy. The normal range in crystal structures for ϕ has been reported as 0° to −41° in pregnan-20-ones, with an average of −22°.[7]

The minimized structures of steroids **1**−**4** were overlaid to align the B- and C-rings as closely as possible, and structures **5** and **10** were then added (Figure 2). The minimum energy position for O20 in pregnan-20-ones **1**−**4** lies between the epoxide oxygen atoms of androstanes **5** and **10**. Other orientations of the 5β-

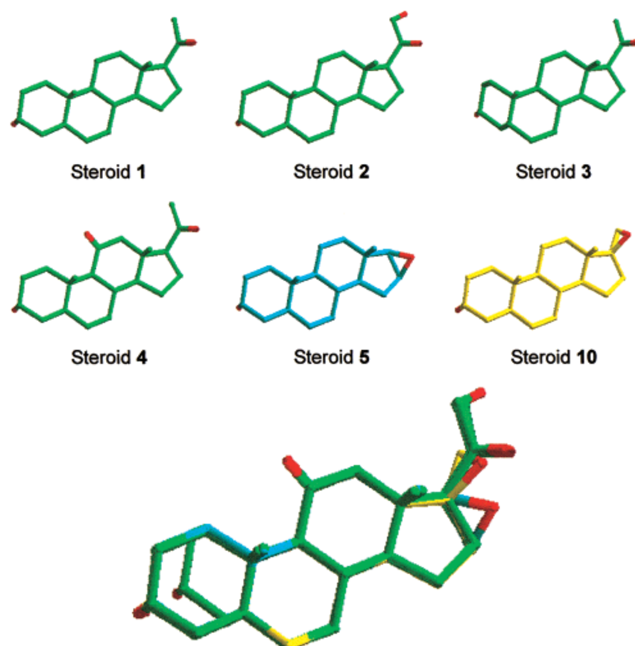


Figure 2. (Top) Structures of steroids **1**−**5** and **10**. (Bottom) Steroids **1**−**5** and **10** superimposed.

steroid **3** that involve improved overlay of the 3α-hydroxyl group with 5α-steroids rather than overlay of the B- and C-rings are also valid.[9] For such orientations it is still beneficial for O20 to lie between the epoxide oxygen atoms of androstanes **5** and **10**.

The in vitro results and the molecular modeling suggest that for optimal GABA$_A$ receptor modulation the hydrogen bond-accepting unit should be near perpendicular to the plane of the D-ring on the β-face of the steroid. This can also account for the poorer activity of the keto androstanes **8** and **9** relative to pregnan-20-one **1** and the good in vitro and in vivo activity of 17β-nitrile-substituted steroids.[9,29]

Conclusion

A number of steroids with a hydrogen bond-accepting substituent attached to the D-ring were prepared and retain good in vitro and in vivo activity relative to the endogenous steroids **1**−**4**. Furthermore, for optimal GABA$_A$ receptor modulation the hydrogen bond-accepting substituent should be near perpendicular to the plane of the D-ring on the β-face of the steroid.

Experimental Section

Pharmacology. In Vitro Experiments ([^{35}S]TBPS assay) and in Vivo Experiments (hypnotic potency). The methods used are identical to those reported previously for water-soluble 2β-morpholinyl steroids.[5e]

Chemistry. General. Melting points were taken with either a Gallenkamp capillary melting point apparatus or a Reichert hot plate apparatus and are uncorrected. Optical rotations were determined at room temperature for solutions in chloroform and c refers to concentration in g/100 mL. ^{1}H NMR (200 or 400 MHz) spectra were obtained using a Bruker AM200 or Bruker DRX400 instrument; chemical shifts (δ) are relative to tetramethylsilane as internal standard. Only discrete or characteristic signals are reported. Coupling constants are given in hertz. IR spectra were obtained with a Perkin-Elmer 16PC FT-IR spectrometer. Elemental analyses were determined on a Perkin-Elmer 2400 CHN elemental analyzer and are within 0.4% of theory unless otherwise noted.

Materials. Reagents were used as supplied from commercial sources. Steroids **1**−**4**, **8** and **15** are commercially available.

Syntheses. Steroid **15** was also isolated as a byproduct from the preparation of steroid **5**. Compounds **5**,[11] **6**,[12] **9**,[14] **10**,[15] and **16**[17] have been previously reported.

(3α,5α,16*β*,17*β*)-16,17-Epoxyandrostan-3-ol (5). Androsterone (**8**) (10 g, 34 mmol) was stirred in pyridine (50 mL) and acetic anhydride (10 mL, 106 mmol) for 3 h then left to stand for 2 days. The product was precipitated with water (600 mL), filtered off, washed with water, taken up in diethyl ether, washed with water, dried over sodium sulfate and the solvent evaporated to give androsterone acetate[31] (**17**) (11.3 g, 34 mmol), which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) *δ* 0.82 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 5.01 (m, 1H).

p-Toluenesulfonic acid (1.13 g, 5.94 mmol) was added to androsterone acetate **17** (11.3 g, 34 mmol) in isopropenyl acetate (20 mL, 182 mmol) and the mixture heated in an oil bath at 130−140 °C for 6 h, collecting the distillate, and replacing each 25 mL of distillate with fresh isopropenyl acetate (7 × 25 mL). After standing overnight, more *p*-toluenesulfonic acid (1.13 g, 5.94 mmol) and isopropenyl acetate were added, and a Vigreux column added to aid fractionation of acetone distillate from isopropenyl acetate. The reaction mixture was heated for a further 8 h. The reaction was cooled to 70 °C, triethylamine (0.85 mL) added, then allowed to cool to room temperature and water (50 mL) added. The solvent was evaporated under reduced pressure until free of isopropenyl acetate and the product was filtered off, washed and dried in vacuo to give crude (3α,5α)-androst-16-ene-3,17-diol diacetate[11] (**18**) (18.3 g), which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) *δ* 0.82 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 5.00 (m, 1H), 5.45 (m, 1H).

A solution of crude diacetate **18** (18.3 g) in carbon tetrachloride (360 mL) was cooled to −5 °C and a solution of bromine (6.4 g, 40 mmol) in carbon tetrachloride (200 mL) added dropwise with stirring over 10 min. The mixture was stirred for a further 20 min and washed with aqueous sodium thiosulfate, aqueous sodium bicarbonate then water, dried over sodium sulfate and the solvent evaporated to give crude (16α)-bromoandrosterone acetate[11] (**19**) (13.6 g, 33.1 mmol): ^{1}H NMR ($CDCl_3$) *δ* 0.82 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 4.53 (m, 1H), 5.01 (m, 1H).

A solution of crude acetate **19** (13.6 g, 33.1 mmol) in dry tetrahydrofuran (186 mL) was added over 15 min to a stirred suspension of lithium aluminum hydride (6.8 g, 179 mmol) in dry diethyl ether (2040 mL). The reaction was stirred at −5 to 0 °C for 1 h, then quenched with ethyl acetate and then water (50 mL). Sodium hydroxide (4 N, 20 mL) was added and the mixture filtered. The filtrate was dried over sodium sulfate and the solvent evaporated to give crude (3α,5α,16α,17*β*)-16-bromoandrostane-3,17-diol[32] (**20**) (11.1 g, 29.9 mmol): ^{1}H NMR ($CDCl_3$) *δ* 0.67 (s, 3H, CH_3), 0.71 (s, 3H, CH_3), 4.00 (m, 1H). NMR also indicated the presence of some (3α,5α,17*β*)-androstane-3,17-diol (**15**).

Crude bromodiol **20** (11.1 g, 29.9 mmol) was refluxed with methanol (150 mL) and potassium hydroxide (10 N, 15 mL) for 2.5 h. The solution was cooled, reduced in volume and the product precipitated by addition of water and filtered off. The solid was taken up in diethyl ether, washed with water, dried over sodium sulfate and the solvent evaporated to give a crude solid. NMR indicated ∼40−50% **5**, ∼25% **15** and ∼25−35% **8**. The crude solid was dissolved in methanol (100 mL), sodium borohydride (500 mg, 13.2 mmol) added and the mixture stirred for 30 min. The mixture was quenched with acetic acid, poured into water and the product extracted with diethyl ether, dried and the solvent evaporated to give a solid (8.3 g). This was taken up in a small amount of methanol and columned on silica, eluting with dichloromethane. Recolumning followed by recrystallization from diethyl ether/heptane gave the title compound **5**[11] (2.33 g, 8.0 mmol, 23% from **8**): mp 196−197 °C (lit. mp 190−191 °C); $[\alpha]_D$ +33.9° (*c* 0.8); ^{1}H NMR ($CDCl_3$) *δ* 0.77 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 3.17 (d, 1H, *J* ∼ 3), 3.47 (d, 1H, *J* ∼ 3), 4.04 (s, 1H); IR (KBr) 3284 (OH), 2931, 2853 (CH) cm^{-1}. Anal. ($C_{19}H_{30}O_2$·0.11H_2O) C, H.

(3α,5α,16α,17α)-16,17-Epoxyandrostan-3-ol (6). To a stirred solution of (3α,5α)-3-hydroxyandrost-16-ene (**21**) (500 mg, 1.82 mmol) in dichloromethane (25 mL) was added a solution of sodium acetate (0.1 g, 1.22 mmol) and peracetic acid (1.0 mL) in water (1.0 mL). After 1 h the solution was washed with aqueous sodium sulfite, water, aqueous sodium carbonate and then water. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure and the residue was crystallized from acetone to give the title compound **6**[12] (337 mg, 1.16 mmol, 64%): mp 144.5−146.5 °C; ^{1}H NMR ($CDCl_3$) *δ* 0.73 (s, 3H, CH_3), 0.79 (s, 3H, CH_3), 1.89 (dd, 1H, *J* ∼ 12, 6), 3.09 (d, 1H, *J* ∼ 3), 3.34 (d, 1H, *J* ∼ 3), 4.04 (m, 1H); IR (KBr) 3490 (OH), 3024, 2979, 2920 (CH) cm^{-1}. Anal. ($C_{19}H_{30}O_2$) C, H.

(3α,5*β*,16*β*,17*β*)-16,17-Epoxyandrostan-3-ol (7). 3α-Hydroxy-5*β*-androstan-17-one (**22**) (2.0 g, 6.89 mmol), *p*-toluenesulfonyl hydrazide (1.42 g, 7.62 mmol) and *p*-toluenesulfonic acid (20 mg, 0.1 mmol) were refluxed in ethanol overnight. Further *p*-toluenesulfonyl hydrazide (700 mg, 3.76 mmol) and *p*-toluenesulfonic acid (20 mg, 0.1 mmol) were added and the mixture refluxed for a further 5 h. The solvent was evaporated under reduced pressure and the residue chromatographed on silica gel (gradient elution, toluene/ethyl acetate) to give crude 3α-hydroxy-5*β*-androstan-17-one *p*-toluenesulfonyl hydrazone (**23**) (4.2 g, 9.2 mmol). To a solution of this steroid in tetrahydrofuran (50 mL) (cooled in an ice−water bath) was added methyllithium (1.4 N in tetrahydrofuran, 21.5 mL, 30 mmol) under nitrogen over 5 min. A precipitate initially formed which redissolved as the addition of methyllithium continued. The dark orange-red solution was stirred for 2 h, then left overnight at room temperature. The reaction was quenched by the slow addition of water, then diethyl ether was added to give two layers and the mixture acidified with 2 N hydrochloric acid. The organic layer was separated, washed with water then brine, dried and the solvent evaporated to give an orange oil (3.1 g), which was chromatographed on silica gel (toluene/ethyl acetate 4/1) to give (3α,5*β*)-3-hydroxyandrost-16-ene[13] (**24**) which solidified on standing (1.14 g, 4.15 mmol, 60% from **22**): ^{1}H NMR ($CDCl_3$) *δ* 0.74 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 3.62 (m, 1H), 5.18 (m, 1H), 5.82 (m, 1H).

To a stirred solution of the androstene **24** (1.1 g, 4.0 mmol) in dimethyl sulfoxide (12 mL) at 5 °C were added water (1.2 mL) and then *N*-bromosuccinimide (1.1 g, 6.2 mmol) portionwise over 5 min. The mixture was stirred for 20 min after which cold water was added and the resulting solid filtered off. This solid was dissolved in ethanol and aqueous potassium hydroxide (10 M, 0.9 mL) added. After heating to 75 °C for 10 min, the ethanol was removed under reduced pressure. After cooling, the residue was diluted with water and the brown gummy precipitate filtered off. This material was dissolved in dichloromethane and the solution washed with water and brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue (1.2 g) was chromatographed on silica gel (gradient elution, toluene/ethyl acetate). Crystallization from diethyl ether gave the title compound **7** (230 mg, 0.79 mmol, 20%): mp 146−150 °C; $[\alpha]_D$ +37.7° (*c* 0.7); ^{1}H NMR ($CDCl_3$) *δ* 0.80 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 3.17 (d, 1H, *J* ∼ 3), 3.47 (d, 1H, *J* ∼ 3), 3.63 (br s, 1H); IR (KBr) 3328 (OH), 2864 (CH) cm^{-1}. Anal. ($C_{19}H_{30}O_2$·0.13H_2O) C, H.

(3α,5α)-3-Hydroxyandrostan-16-one (9). To a stirred mixture of (3*β*,5α)-3-hydroxyandrostan-16-one (**25**) (244 mg, 0.84 mmol), benzoic acid (206 mg, 1.69 mmol) and tri-*n*-butylphosphine (683 mg, 3.38 mmol) in toluene (12.2 mL) was added 1,1′-(azodicarbonyl)dipiperidine (426 mg, 1.69 mmol). After 3.5 h at 60 °C the reaction mixture was diluted with water and extracted with diethyl ether. The combined extracts were washed with water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was chromatographed on silica gel (gradient elution, toluene/diethyl ether) to give (3α,5α)-3-hydroxyandrostan-16-one ben-

zoate (**26**) (318 mg, 0.81 mmol, 96%): ^{1}H NMR (CDCl$_3$) δ 0.89 (s, 6H, 2 $\times$ CH$_3$), 5.30 (m, 1H), 7.50 (m, 3H), 8.07 (m, 2H).

A mixture of the benzoate **26** (307 mg, 0.78 mmol) and aqueous sodium hydroxide (0.9 mL) in methanol (6 mL) was heated under reflux for 4 h, then left to stand overnight. The reaction mixture was diluted with water and extracted with diethyl ether. The combined extracts were washed with water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether) and crystallized from diethyl ether/heptane to give the title compound **9**[14] (91 mg, 0.31 mmol, 40%): mp 173−175 °C (lit. mp 153−154 °C); ^{1}H NMR (CDCl$_3$) δ 0.82 (s, 3H, CH$_3$), 0.88 (s, 3H, CH$_3$), 2.11 (d, 1H, $J \sim 17$), 2.20 (m, 1H), 4.06 (m, 1H); IR (KBr) 3551 (OH), 2924 (CH), 1740 (C=O) cm^{-1}. Anal. ($C_{19}H_{30}O_2 \cdot 0.06H_2O$) C, H.

(3α,5α,17*β*)-Spiro[androstane-17,2′-oxiran]-3-ol (10). To a stirred solution of (3α,5α)-3-hydroxyandrostan-17-one (**8**) (5.0 g, 17.2 mmol) in dimethylformamide (50 mL) were added trimethylsulfonium iodide (5.27 g, 25.8 mmol) and potassium *tert*-butoxide (2.9 g, 25.8 mmol) under nitrogen. After 45 min water was added with stirring and the precipitated pale yellow solid filtered off and washed with water. The crude product was crystallized from aqueous acetone, then flash chromatographed on silica gel (toluene/ethyl acetate 1/1). Crystallization from ethyl acetate gave the title compound **10**[15] (1.58 g, 5.20 mmol, 30%): mp 227−231 °C; [α]$_D$ +7.0° (*c* 0.7); ^{1}H NMR (CDCl$_3$) δ 0.79 (s, 3H, CH$_3$), 0.87 (s, 3H, CH$_3$), 2.00 (m, 1H), 2.60 (d, 1H, $J \sim 5$), 2.90 (d, 1H, $J \sim 5$), 4.05 (br s, 1H); IR (KBr) 3446 (OH), 2881 (CH) cm^{-1}. Anal. ($C_{20}H_{32}O_2$) C, H.

(3α,5*β*,17*β*)-Spiro[androstane-17,2′-oxiran]-3-ol (11). To a stirred solution of (3α,5*β*)-3-hydroxyandrostan-17-one (**22**) (2.0 g, 6.9 mmol) in dimethylformamide (10 mL) and tetrahydrofuran (10 mL) were added trimethylsulfonium iodide (5.27 g, 25.8 mmol) and potassium *tert*-butoxide (2.9 g, 25.8 mmol) under nitrogen. After 2 h more trimethylsulfonium iodide (5.27 g, 25.8 mmol) and potassium *tert*-butoxide (2.9 g, 25.8 mmol) in dimethylformamide (10 mL) was added and stirring maintained for a further 2 h. The reaction mixture was poured into ice/water and after stirring for 15 min the precipitated pale yellow solid was filtered off and washed with water. The solid was flash chromatographed on silica gel (toluene/ethyl acetate 1/1). Crystallization from diethyl ether/heptane gave the title compound **11** (460 mg, 1.51 mmol, 22%): mp 155−158 °C; [α]$_D$ +18.1° (*c* 0.6); ^{1}H NMR (CDCl$_3$) δ 0.87 (s, 3H, CH$_3$), 0.94 (s, 3H, CH$_3$), 2.00 (m, 1H), 2.61 (d, 1H, $J \sim 3$), 2.90 (d, 1H, $J \sim 3$), 3.63 (br s, 1H); IR (KBr) 3381 (OH), 2934 (CH) cm^{-1}. Anal. ($C_{20}H_{32}O_2$) C, H.

(3α,5α,17α)-17,21-Epoxypregnan-3-ol (12). To a stirred suspension of trimethylsulfoxonium iodide (1.45 g, 6.59 mmol) in *tert*-butanol (14.5 mL) at 50 °C was added a solution of potassium *tert*-butoxide (0.74 g, 6.59 mmol) in *tert*-butanol (10.5 mL). After 30 min a solution of (3α,5α,17*β*)-spiro-[androstane-17,2′-oxiran]-ol (**10**) (1.0 g, 3.3 mmol) in *tert*-butanol (20 mL) was added and the mixture heated under reflux for 72 h. The reaction mixture was concentrated to low volume under reduced pressure and diluted with dichloromethane. This solution was washed with water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue (1.0 g) was flash chromatographed on silica gel (toluene/ethyl acetate 3/1). Crystallization from heptane gave the title compound **12** (250 mg, 0.78 mmol, 16%): mp 192−197 °C; [α]$_D$ −29.3° (*c* 1.0); ^{1}H NMR (CDCl$_3$) δ 0.87 (s, 3H, CH$_3$), 0.94 (s, 3H, CH$_3$), 2.02 (m, 2H), 2.27 (m, 1H), 2.78 (m, 1H), 4.05 (br s, 1H), 4.30 (m, 1H), 4.42 (m, 1H); IR (KBr) 3436, 3353 (OH), 2929 (CH) cm^{-1}. Anal. ($C_{21}H_{34}O_2 \cdot 0.25H_2O$) C, H.

(3α,5α,16*β*,17*β*)-16,17-(4*H*)-Dihydro-3-hydroxyandrost-16-eno[17,16-*b*]oxetane (13). A stirred solution of (3α,5α)-3-hydroxyandrostan-17-one (**8**) (4.91 g, 16.9 mmol), sodium methoxide (3.65 g, 67.6 mmol), ethyl formate (41 mL) and toluene (100 mL) was heated under reflux for 7 h, after which a white solid precipitated. The reaction mixture was allowed to cool and water (500 mL) added. After separation the aqueous phase was acidified with dilute hydrochloric acid and the precipitated solid filtered off and washed with water. Evaporation of residual water under reduced pressure afforded (3α,5α)-3-hydroxy-16-hydroxymethyleneandrostan-17-one[33] (**27**) (4.47 g, 14.0 mmol, 83%), which was sufficiently pure for the next stage: ^{1}H NMR (CDCl$_3$) δ 0.76 (s, 6H, 2 $\times$ CH$_3$), 0.97 (m, 1H), 1.78 (m, 1H), 1.88 (m, 1H), 2.48 (m, 1H), 3.80 (m, 1H, C*H*OH), 4.20 (br s, 1H, OH), 7.35 (s, 1H, C=C*H*OH), 10.50 (br s, 1H, OH); IR (KBr) 3373 (OH), 2915 (CH), 1695, 1665, 1629, 1597 (C=O, C=C) cm^{-1}.

To a stirred solution of steroid **27** (4.3 g, 13 mmol) in methanol (150 mL) at ∼5 °C was added sodium borohydride (1.0 g, 26 mmol). After stirring for 5 h at room temperature, acetone was added to decompose any residual sodium borohydride, and the mixture was then concentrated to half volume under reduced pressure. Water was added and the precipitated off-white solid filtered off, washed with water and dried under reduced pressure to give 16-hydroxymethylandrostane-3,17-diol (4.10 g) as a mixture of isomers. This was flash chromatographed on silica gel (toluene/ethyl acetate, gradient elution 1/1 to 1/4) to give (3α,5α,16*β*,17*β*)-16-hydroxymethylandrostane-3,17-diol (**28**) (3.5 g, 11 mmol, 80%), which was sufficiently pure for the next stage: ^{1}H NMR (DMSO) δ 0.64 (s, 3H, CH$_3$), 0.73 (s, 3H, CH$_3$), 3.26 (m, 1H), 3.59 (m, 2H), 3.79 (m, 1H).

To a stirred solution of steroid **28** (3.0 g, 9.3 mmol) in pyridine (30 mL) was added *p*-toluenesulfonyl chloride (1.8 g, 9.4 mmol). After 4 h at room temperature the reaction mixture was poured into ice/water and left to stand overnight. The solid was filtered off, washed with water and dissolved in dichloromethane. This solution was washed with water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue (1.2 g) was triturated with diethyl ether to give [(3α,5α,16*β*,17*β*)-3,17-dihydroxyandrostan-17-yl]methyl *p*-toluenesulfonate (**29**) (1.10 g, 2.31 mmol, 25%), as an off-white solid which was sufficiently pure for the next stage: ^{1}H NMR (CDCl$_3$) δ 0.68 (s, 3H, CH$_3$), 0.77 (s, 3H, CH$_3$), 2.45 (s, 3H, CH$_3$), 3.73 (d, 1H, $J \sim 10$), 4.03 (m, 2H), 4.22 (dd, 1H, $J \sim 10, 7$), 7.34 (d, 2H, $J \sim 8$), 7.79 (d, 2H, $J \sim 8$); IR (KBr) 3521, 3346 (OH), 2928 (CH), 1598 (Ar), 1355, 1173 (SO$_2$) cm^{-1}.

To a stirred suspension of steroid **29** (0.95 g, 2.0 mmol) in methanol (25 mL) was added sodium methoxide (0.47 g, 8.7 mmol) and the mixture heated under reflux for 4 h. The resulting solution was concentrated to low volume under reduced pressure and diluted with water. The precipitated solid was filtered off, washed with water and dried under reduced pressure. The crude product was flash chromatographed on silica gel (toluene/ethyl acetate 2/1) to give the title compound **13** (130 mg, 0.43 mmol, 21%): mp 183−185 °C; [α]$_D$ +4.8° (*c* 0.7); ^{1}H NMR (CDCl$_3$) δ 0.81 (s, 3H, CH$_3$), 1.05 (s, 3H, CH$_3$), 3.15 (m, 1H), 4.05 (m, 1H), 4.22 (t, 1H, $J \sim 6$), 4.56 (d, 1H, $J \sim 8$), 4.74 (dd, 1H, $J \sim 8, 6$); IR (KBr) 3469 (OH), 2910 (CH) cm^{-1}. Anal. ($C_{20}H_{32}O_2$) C, H.

(3α,5α,13α,17α)-3-Hydroxyandrostane-17-carbonitrile (14). To androsterone (**8**) (15 g, 52 mmol) in pyridine (150 mL) and ethanol (150 mL) was added hydroxylamine hydrochloride (15 g, 216 mmol) and the solution heated under reflux for 5 h. The solution was concentrated to approximately 200 mL then cooled and water added to precipitate a colorless solid which was washed with water and dried over anhydrous sodium sulfate, affording androsterone oxime[31] (**30**) (16.18 g, 52 mmol): ^{1}H NMR (CDCl$_3$) δ 0.80 (s, 3H, CH$_3$), 0.90 (s, 3H, CH$_3$), 4.03 (s, 1H); IR (KBr) 3248 (OH), 2926 (CH), 1680 (C=N) cm^{-1}.

The oxime **30** (15 g, 49 mmol) was refluxed gently in acetic anhydride (450 mL) and pyridine (750 mL) with stirring under nitrogen for 12 h. The solution rapidly developed a dark color. It was then allowed to cool, the solvent evaporated and the residue azeotroped with toluene to give a black oily residue. This was shaken for several min with diethyl ether and 5% aqueous sodium carbonate, then filtered through Celite. The granular black residue was washed thoroughly with several portions of diethyl ether, and the combined organic extracts

washed with brine, dried over sodium sulfate and the solvent evaporated. The residue was taken up in diethyl ether, adsorbed on alumina and left for 1 h. Elution with diethyl ether and subsequent evaporation gave a creamy froth, presumed to be crude (13α)-17-acetamidoandrost-16-ene acetate (**31**) (11.5 g, 30.8 mmol).

The crude acetate **31** (11.5 g, 30.8 mmol) was refluxed in methanol (1150 mL) and hydrochloric acid (2 N, 450 mL) for 2 h. The solvent was evaporated to half volume and cooled. Addition of water gave a gum, which was washed by decantation with water. The product was taken up in diethyl ether and washed with water then brine, and the solvent evaporated to give a gum, which upon crystallization from diethyl ether/heptane gave (3α,5α,13α)-3-hydroxyandrostan-17-one[34] (**32**) as creamy prisms (3.74 g, 12.9 mmol, 26% from **31**): ^{1}H NMR ($CDCl_3$) δ 0.62 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.33 (m, 1H), 4.04 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 222.7, 66.3, 51.6, 50.8, 50.1, 38.5, 37.8, 36.1, 35.6, 33.8, 32.9, 32.1, 31.9, 28.8, 28.4, 25.2, 22.2, 21.2, 10.9.

To a stirred solution of potassium *tert*-butoxide (11.2 g, 100 mmol) in *tert*-butyl alcohol (110 mL) was added a solution of steroid **32** (2.90 g, 10 mmol) in diglyme (29 mL) under nitrogen. A solution of *p*-toluenesulfonylmethyl isocyanide (3.91 g, 20 mmol) in diglyme (40 mL) was then added over 1 h. After 4 h more *p*-toluenesulfonylmethyl isocyanide (3.91 g, 20 mmol) in diglyme (40 mL) was added over 1 h, and the mixture left to stand overnight. The mixture was treated with dilute aqueous sodium chloride followed by hydrochloric acid (2 M) until acidic. The aqueous liquors were decanted from the resulting brown gum which was dissolved in dichloromethane. This solution was washed with brine, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The brown residue (2.1 g) was flash chromatographed on silica gel (toluene/ethyl acetate 2/1). Crystallization from acetonitrile gave the title compound **14** (145 mg, 0.48 mmol, 5%): mp 167−170 °C; $[\alpha]_D$ −91.6° (*c* 0.6); ^{1}H NMR ($CDCl_3$) δ 0.70 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 2.88 (t, 1H, $J \sim 9$), 4.05 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 121.6, 66.3, 52.6, 52.2, 45.0, 38.6, 37.8, 36.1, 35.6, 33.5, 33.2, 32.5, 31.8, 28.9, 28.4, 26.4, 26.0, 19.9, 11.1; IR (KBr) 3501 (OH), 2930 (CH), 2236 (CN) cm^{-1}. Anal. ($C_{20}H_{31}NO$) C, H, N.

(3α,5α,17β)-17-Methoxyandrostan-3-ol (16). To a stirred suspension of (3α,5α,17β)-androstane-3,17-diol (**15**) (1.0 g, 3.4 mmol) in 1,2-dimethoxyethane (20 mL) was added sodium hydride (164 mg, 6.8 mmol). After 10 min methyl iodide (0.22 mL, 3.5 mmol) was added and the mixture left to stir under nitrogen for 60 h. The mixture was then poured into dilute aqueous sodium carbonate and the precipitated solid filtered off. This material was dissolved in dichloromethane and the solution washed with water and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue (1.2 g) was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether). Crystallization from acetone gave the title compound **16**[17] (180 mg, 0.59 mmol, 17%): mp 167−168 °C; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3H, CH_3), 0.78 (s, 3H, CH_3), 1.89 (m, 1H), 2.02 (m, 1H), 3.22 (t, 1H, $J \sim 8$), 4.04 (s, 1H); IR (KBr) 3299 cm^{-1} (OH). Anal. ($C_{20}H_{34}O_2$) C, H.

Calculations. All the structures studied were constructed using Chem-X[35] with the Chem-X 2D and 3D building tools. Each structure was then subjected to geometry optimization using the default Gasteiger charges and optimization parameters.

Conformational analyses were performed using Chem-X.[35] For each structure a systematic grid search was carried out by rotating the C17−C20 bond through 360° in 5° increments. Structures were geometry optimized after each step. Global minima were found at C16−C17−C20−O20 torsional values of −34.3°, −34.8° and −21.8° for structures **1**, **3** and **4**, respectively. (The measured angle reported for alfaxalone **4** is −21°.[8]) The analyses showed that torsional angles 10° higher or lower than the minima could be achieved an energy cost of ca. 0.5 kcal in each case. Slightly higher (+0.5−1.5 kcal·mol^{-1}) local minima were also detected at C16−C17−C20−O20 torsional values of 165.6°, 160.0° and 153.2° for structures **1**, **3** and **4**, respectively.

For structure **2** additional rotations were also performed on the C21−O21 bond, which was rotated through 360° in 30° increments, leading to the generation of 864 conformations. In this case four low-energy conformations were detected. Conformations with C16−C17−C20−O20 torsional values of −23.2° and 155.5° had equal lowest energies, while conformations, with C16−C17−C20−O20 torsional values of 84.9° and 44.0° had energy values ca. 0.5−1.5 kcal·mol^{-1} higher.

The alignments shown in Figure 2 were produced by fitting on all carbons in the B- and C-rings of each structure, using the Chem-X rigid fitting procedure. (The various ways of overlaying 5α- and 5β-steroids have been previously discussed.[9]) The structures were transferred via MACCS SD files to Cerius^2,[36] which was used solely to render Figure 2 graphically.

Distance measurements were performed in Chem-X on the alignments shown in Figure 2. The carbonyl oxygens of the minima of structures **1**−**4** in Figure 2 are separated from each other by distances < 0.6 Å. The epoxide oxygen of **10** is 0.8−1.0 Å from the carbonyl oxygens, while the epoxide oxygen of **5** is 1.6−1.8 Å from the carbonyl oxygens.

The default Chem-X parameter files, Gasteiger charges, and molecular mechanics force field were used throughout.

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