19-Nor-10-azasteroids: A Novel Class of Inhibitors for Human Steroid 5α-Reductases 1 and 2

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Steroid 5α-reductase is a system of two isozymes (5αR-1 and 5αR-2) which catalyzes the NADPH-dependent reduction of testosterone to dihydrotestosterone in many androgen sensitive tissues and which is related to several human endocrine diseases such as benign prostatic hyperplasia (BPH), prostatic cancer, acne, alopecia, pattern baldness in men and hirsutism in women. The discovery of new potent and selective 5αR inhibitors is thus of great interest for pharmaceutical treatment of these diseases. The synthesis of a novel class of inhibitors for human 5αR-1 and 5αR-2, having the 19-nor-10-azasteroid skeleton, is described. The inhibitory potency of the 19-nor-10-azasteroids was determined in homogenates of human hypertrophic prostates toward 5αR-2 and in DU-145 human prostatic adenocarcinoma cells toward 5αR-1, in comparison with finasteride (IC_{50} = 3 nM for 5αR-2 and ∼ 42 nM for 5αR-1), a drug which is currently used for BPH treatment. The inhibition potency was dependent on the type of substituent at position 17 and on the presence and position of the unsaturation in the A and C rings. $\Delta^{9(11)}$-19-Nor-10-azaandrost-4-ene-3,17-dione (or 10-azaestra-4,9(11)-diene-3,17-dione) (**4a**) and 19-nor-10-azaandrost-4-ene-3,17-dione (**5**) were weak inhibitors of 5αR-2 (IC_{50} = 4.6 and 4.4 μM, respectively) but more potent inhibitors of 5αR-1 (IC_{50} = 263 and 299 nM, respectively), whereas 19-nor-10-aza-5α-androstane-3,17-dione (**7**) was inactive for both the isoenzymes. The best result was achieved with the 9:1 mixture of $\Delta^{9(11)}$- and $\Delta^{8(9)}$-17β-(*N-tert*-butylcarbamoyl)-19-nor-10-aza-4-androsten-3-one (**10a,b**) which was a good inhibitor of 5αR-1 and 5αR-2 (IC_{50} = 127 and 122 nM, respectively), with a potency very close to that of finasteride. The results of *ab initio* calculations suggest that the inhibition potency of 19-nor-10-azasteroids could be directly related to the nucleophilicity of the carbonyl group in the 3-position.

Introduction

The enzyme steroid 5α-reductase (E.C. 1.3.99.5) is a system of two NADPH-dependent isozymes (5αR-1 and 5αR-2)[1] which catalyzes the conversion of testosterone (T) to dihydrotestosterone (DHT) in many androgen sensitive cells. The formation of DHT is related to the development of several human endocrine diseases[2] such as benign prostatic hyperplasia (BPH),[3] prostatic carcinoma,[4] male pattern baldness,[5] acne,[6] alopecia in men,[7] and hirsutism in women.[8] The incidence of BPH in the ageing male population is very high, affecting approximately 30% at age 50 and 80% at age 80. A high percentage of sufferers ultimately require costly surgery to alleviate the symptoms.[3] However, pharmacological therapy is now possible due to the development of 5α-reductase inhibitors which can block the formation of DHT without T deprivation.[9] A large number of inhibitors have been identified (for some reviews, see ref 10) and tested *in vitro* toward the human prostatic 5αR (mainly type 2) or toward recombinant human 5αR-1 and 5αR-2.[10] Many of these inhibitors are based on the steroidal structure of testosterone itself; some of the more significant are shown in Figure 1.

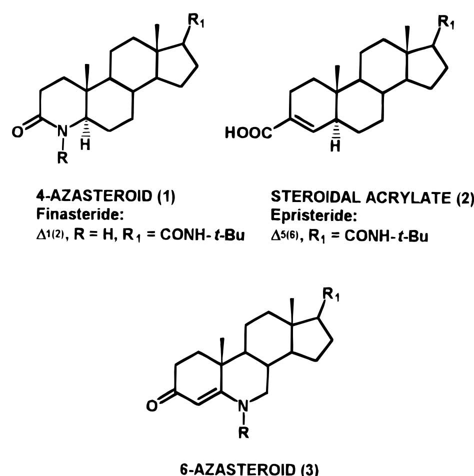

Figure 1.

Finasteride[11] [a member of the 4-azasteroid family (**1**)][12] and epristeride[13] [a member of the steroidal acrylate family (**2**)][14] have been assessed clinically, and finasteride is now on the market as a drug for BPH treatment.[15] 6-Azasteroids **3**, recently synthesized in

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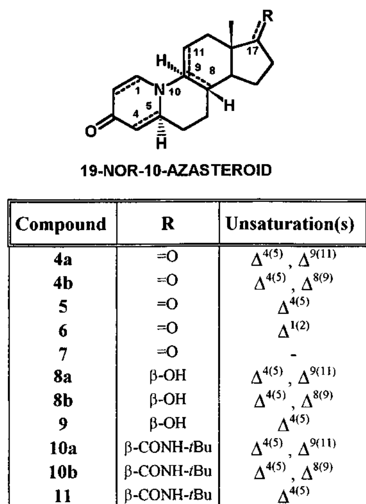
Chart 1



Compound	R	Unsaturation(s)
4a	=O	$\Delta^{4(5)}$, $\Delta^{9(11)}$
4b	=O	$\Delta^{4(5)}$, $\Delta^{8(9)}$
5	=O	$\Delta^{4(5)}$
6	=O	$\Delta^{1(2)}$
7	=O	-
8a	β-OH	$\Delta^{4(5)}$, $\Delta^{9(11)}$
8b	β-OH	$\Delta^{4(5)}$, $\Delta^{8(9)}$
9	β-OH	$\Delta^{4(5)}$
10a	β-CONH-*t*Bu	$\Delta^{4(5)}$, $\Delta^{9(11)}$
10b	β-CONH-*t*Bu	$\Delta^{4(5)}$, $\Delta^{8(9)}$
11	β-CONH-*t*Bu	$\Delta^{4(5)}$

the Glaxo laboratories, appear very promising compounds as dual inhibitors for 5αR-1 and 5αR-2, and the most active of them are now under investigation in *in vivo* experiments.[16] Furthermore it has been recently suggested that the selective inhibition of type 1 isozyme may represent a suitable clinical treatment of androgen-dependent dermatological pathologies such as male pattern baldness, acne, and hirsutism.[5,17] This observation and the fact that finasteride has been clinically proven to be somewhat less effective in treating BPH than originally expected[18] have increased the interest in the synthesis of potent and selective new 5αR inhibitors.

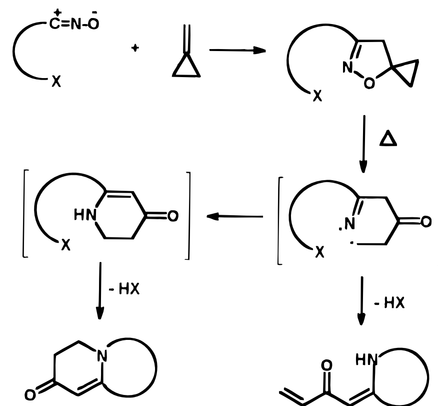
When designing new steroidal inhibitors for this enzyme, two different goals should be attained. Firstly, one should be able to find a suitable structure or modification of the steroid skeleton to have new compounds with a fair inhibition; secondly, one should introduce the suitable substituents on the steroid skeleton useful for increasing the activity or the selectivity toward the enzyme targets (either 5αR-1 or 5αR-2 or both) and decreasing the affinity toward the steroid receptors or the other enzymes involved in the steroid metabolism. This procedure has been applied to the synthesis of 4-azasteroids **1** and 6-azasteroids **3** as well as steroidal acrylates **2**.

Following this rational approach and on the basis of a molecular model of the active site recently developed by us for the type 2 isozyme,[19] we have designed a novel class of inhibitors having as a new feature the nitrogen atom at position 10 of the steroid skeleton. The present paper thus describes the synthesis and the structure–activity relationship of 19-nor-10-azasteroids **4**–**11** which are differentiated by number and position of the double bonds on the A and C rings and by the type of substituent at position 17 (Chart 1). A rational explanation of their mechanism of action, on the basis of the transition state inhibitor paradigm and the results of *ab initio* calculations, is proposed.

Chemistry

The sequential rearrangement–annulation of isoxazoline-5-spirocyclopropane is a well-established procedure for the synthesis of the 4-pyridone moiety embodied in polycyclic compounds.[20] This strategy is illustrated in Scheme 1.

Scheme 1



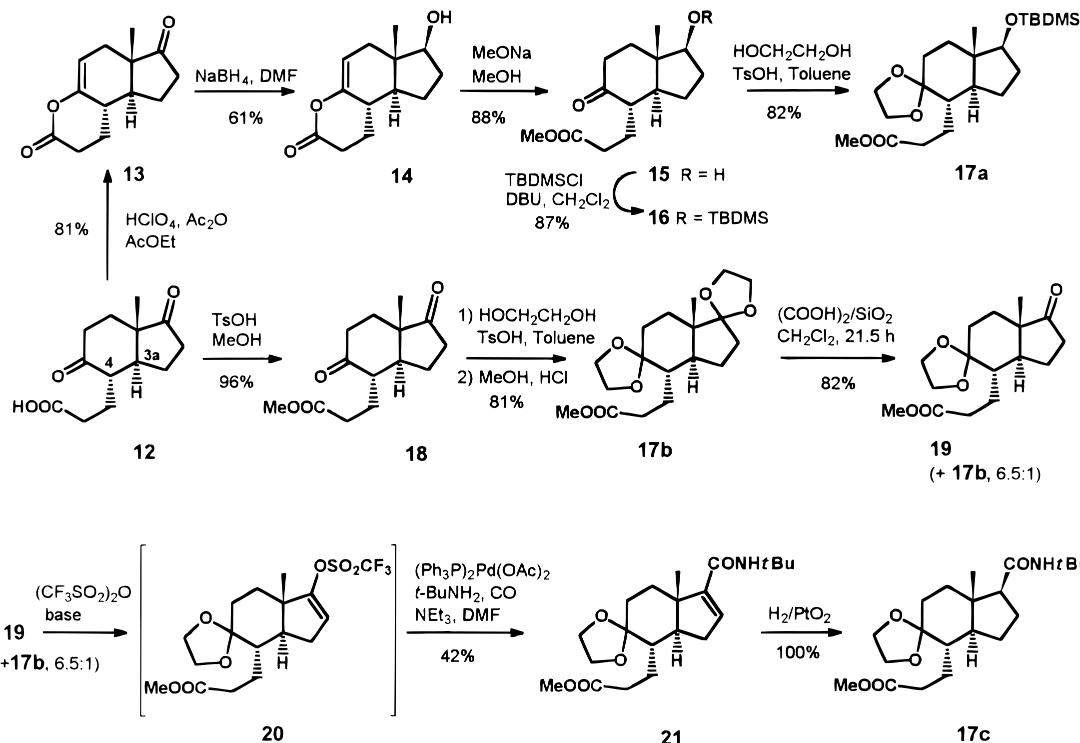
The cycloaddition of nitrile oxides to methylenecyclopropane produces isoxazoline-5-spirocyclopropanes with very high regioselectivity. These compounds undergo a selective sequential rearrangement–annulation when submitted to thermolysis. The driving force of the process results from the strong tendency to relieve the ring strain present in the system.[21] The postulated rearrangement mechanism requires the combined cleavages of the N–O bond of the isoxazoline ring and a C–C bond of the cyclopropane ring to form a highly reactive diradical intermediate which cyclizes to the pyridone moiety or produces an open chain vinyl enamino ketone through a minor side reaction involving a hydrogen transfer.

The presence in the side chain of a reactive X group which can suffer nucleophilic attack by the nitrogen atom of the pyridone nucleus produces directly a polycyclic compound containing a bridgehead nitrogen atom.[22] Thus, according to this strategy, a steroidal skeleton containing a nitrogen atom at position 10 could be produced starting from the suitably functionalized isoxazoline-5-spirocyclopropane. Following this strategy, we previously synthesised (±)-$\Delta^{9(11)}$-19-nor-10-aza-testosterone (**8a**) starting from racemic (1*β*,7a*β*)-2,3,5,6,7,7a-hexahydro-1-hydroxy-7a-methyl-5-oxo-1*H*-indene-4-propionic acid.[23]

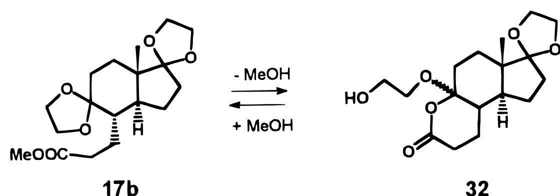
However, the above indene derivative does not appear fit to extend this strategy to the synthesis of a large number of enantiopure azasteroids because of the low stereoselectivity in the C=C bond catalytic hydrogenation[24] and the need for resolution of the starting material itself. This problem has been overcome by using as a starting material (+)-3-[(3a*S*)-(3aα,4α,7a*β*)-1,5-dioxo-7a-methyloctahydro-1*H*-inden-4-yl]propionic acid (**12**), which is commercially available in enantiomerically pure form. This compound is obtained from the microbiological degradation of sterols present in the soya bean; it thus maintains the steroidal configuration of the C and D rings.[25]

The synthetic strategy for the synthesis of 19-nor-10-azasteroids with various substituents in position 17 (shown in Scheme 4) required the modification of the common starting material **12** into the key intermediates **17a**–**c** as shown in Scheme 2. The synthesis of the key intermediate **17a** required the differentiation of the two carbonyl groups of **12**. This was achieved by formation of the enol lactone **13**[26] which was reduced with $NaBH_4$ in DMF in 5–10 min affording with complete stereoselectivity the *β*-alcohol **14**. The short reduction time was essential to avoid the reduction of the C=C bond of the

Scheme 2



Scheme 3



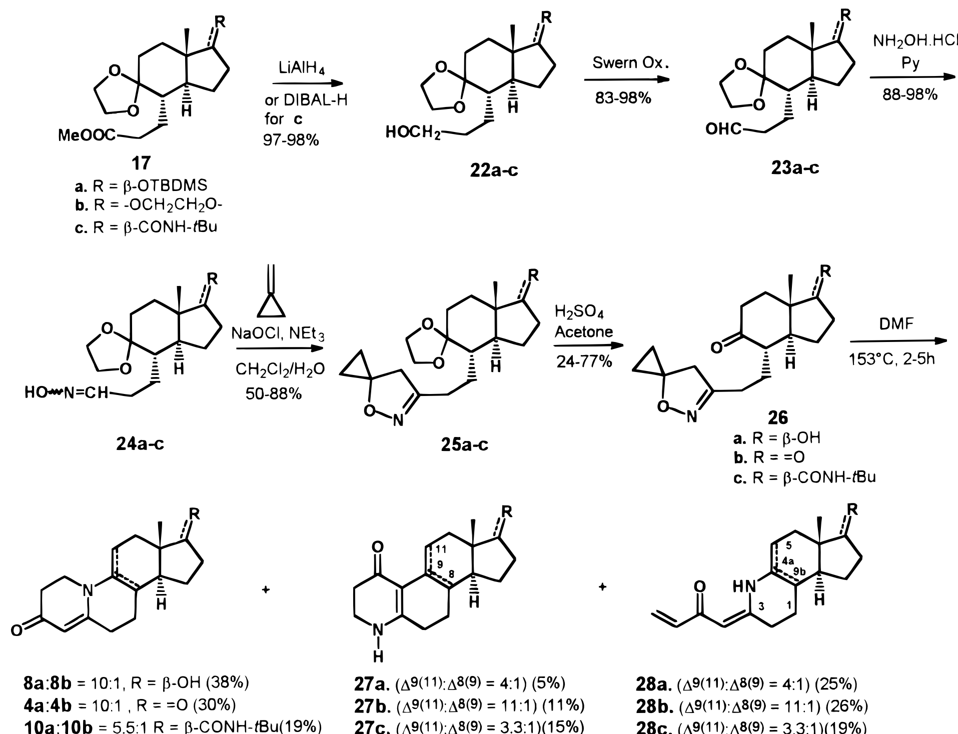
enol moiety; however, a 3:1 mixture of the reduced enol lactone **14** and starting lactone **13** was obtained. After a chromatographic purification, the enol lactone **14** was transformed directly, by methanolysis in the presence of a catalytic amount of MeONa, into the methyl ester **15**. The protection of the hydroxy group as TBDMS ether, to give **16**, followed by the protection of carbonyl group as ketal gave compound **17a**.

The synthesis of the key intermediate **17b** required the esterification of **12** followed by protection of the two carbonyl groups as ketals. However, the acid-catalyzed ketalization of ester **18** afforded **17b**, as well as a large amount of the corresponding hydroxyethyl acetal **32** (shown in Scheme 3). This intramolecular cyclization can occur, to some extent, under acid catalysis, for compounds having the ketal function and a nucleophilic function in the side chain (e.g., the oxygen of the ester group in the above compound **17b**). However, both the methyl ester and ketal functions can be regenerated by treatment of the hydroxyethyl acetal **32** with anhydrous methanol and catalytic *p*-toluenesulfonic acid.[27] This treatment gave **17b** in 81% yield.

The synthesis of the key intermediate **17c** was realized by modification of compound **12** introducing the β-(*N*-*tert*-butylcarbamoyl) function through the enol triflate methodology.[28] The selective hydrolysis of the more hindered ketal group at position 1 was achieved by treatment of the diketal derivative **17b** with oxalic acid and silica gel in dichloromethane,[29] giving the monoketal **19** in a 6:1 ratio with the starting diketal **17b**. However, the remaining diketal **17b** in the mixture was converted into the monoketal during the subsequent enol triflate preparation. This was probably caused by the mild acidity of the reaction medium; in effect, a small amount of the deketalized form of enol triflate **20** was also formed during the reaction. The formation of the enol triflate **20** occurred in good yield, but owing to its instability, the crude product was used directly for the further reactions without purification. The palladium-catalyzed reaction of **20** with CO in the presence of *tert*-butylamine gave in one step the unsaturated amide **21**. However a large quantity of the deketalized form of amide **21** was formed, and a further ketalization reaction was necessary to give only the protected form. The amide **21** was then hydrogenated over PtO_2 affording the saturated β-amide **17c** with complete stereoselectivity. The choice of the catalyst was essential for controlling the stereoselectivity because other catalysts (e.g., Pd/C) gave mixtures of diastereoisomers.

The ester function in the side chain of the key intermediates **17a**–**c** was then transformed into the oxime function through the reactions shown in Scheme 4. The transformation of esters **17a,b** to aldehydes **23a,b** was carried out in two steps by reduction with lithium aluminum hydride followed by Swern oxidation. Alcohols **22a,b** were unstable under storage or to purification by silica gel column chromatography, owing to the intramolecular cyclization between the hydroxy group and the proximal ketal. Oxidation to the aldehydes was effected immediately using crude products without further purification. These two reactions produced the aldehydes **23a,b** in very good yield, better than would otherwise be obtained (as in the racemic synthesis)[23] by direct reduction with DIBAL-H. In fact, the reduction of **17c** with DIBAL-H afforded a 1:2 mixture of the desired aldheyde **23c** and alcohol **22c**,

Scheme 4

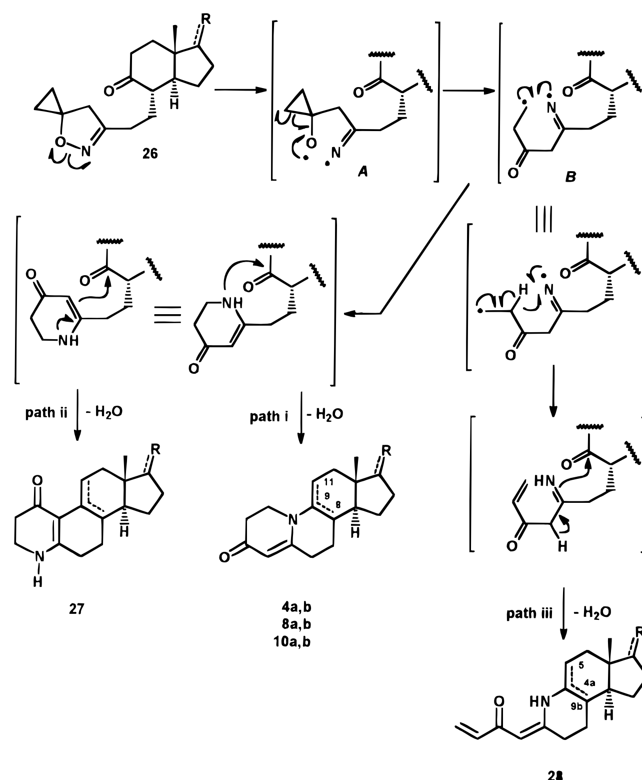


and the Swern oxidation of the mixture was necessary to give only the aldehyde **23c**. The aldehydes **23a**–**c** were converted into a mixture of *Z*- and *E*-oximes **24a**–**c** by reaction with hydroxylamine hydrochloride in pyridine. The next stage of the synthesis, requiring the formation of the isoxazoline-5-spirocyclopropane moiety, was carried out by treatment of the oximes **24a**–**c** in a two-phase dichloromethane/water system with sodium hypochlorite, triethylamine, and methylenecyclopropane.[30] Under these conditions, α-chlorination of the oxime by sodium hypochlorite, followed by elimination of HCl by triethylamine, produced the nitrile oxide function which underwent cycloaddition with methylenecyclopropane present in excess in the reaction mixture. The reactions were regioselective and resulted predominantly in the formation of isoxazoline-5-spirocyclopropanes **25a**–**c** in complete agreement with previous results.[20] Deprotection of isoxazolines **25a**–**c** in refluxing acetone containing an equivalent amount of sulfuric acid (and water for **25a**) afforded deprotected isoxazolines **26a**–**c**.

The thermal rearrangement of isoxazolines **26a**–**c** was carried out in refluxing DMF (2–5 h) affording the desired azasteroids **4, 8,** and **10** in fair yields, as mixture of isomers having the double bond in ring C at the 9(11)- or 8(9)-position, the $\Delta^{9(11)}$ isomer being prevalent in all experiments. In addition to 19-nor-10-azasteroids, 1-oxo-19-nor-4-azasteroids **27a**–**c** (as mixtures of $\Delta^{9(11)}$ and $\Delta^{8(9)}$ isomers) and vinyleneaminones **28a**–**c** (as mixtures of $\Delta^{4a(5)}$ and $\Delta^{4a(9b)}$ isomers) were formed (Scheme 4). The formation of the final products is rationalized on the basis of the rearrangement mechanism (Scheme 5) by analogy with previous studies for similar systems.[20]

The initial step of the reaction is believed to proceed *via* homolytic fission of the nitrogen–oxygen bond of the isoxazoline ring to form the diradical species A which in turn rearranges to form species B. This highly

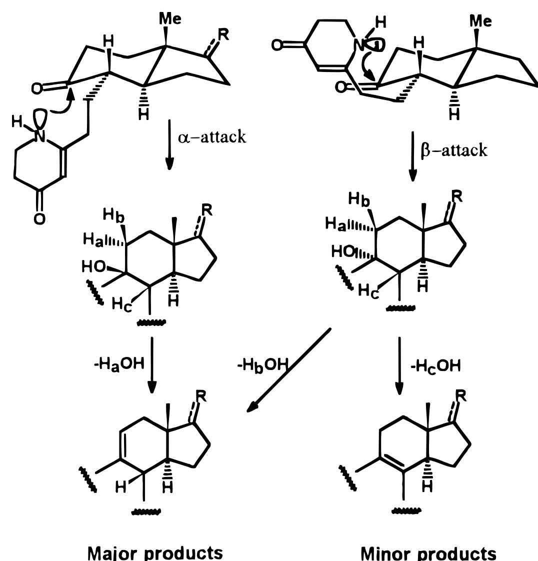
Scheme 5



unstable diradical can produce the rearrangement products through different routes:

(1) Direct coupling of the two radicals giving a pyridone nucleus followed by nucleophilic attack of the nitrogen on the carbonyl group of the hydroindane moiety produces a hydroxy derivative which, after elimination of water, leads to the two isomers of the 19-nor-10-azasteroids (Scheme 5, path i). Alternatively, attack of the nucleophilic carbon of the pyridone nucleus

Scheme 6



on the carbonyl group of the hydroindane moiety affords, after elimination of water, 4-azasteroids **27** (Scheme 5, path ii).

(2) Abstraction of one of the methylene protons adjacent to the acyclic carbonyl group by the nitrogen radical giving an acyclic vinyl ketone moiety, followed by N-attack on the carbonyl group of the hydroindane moiety, leads to vinyleneaminones **28** (Scheme 5, path iii).

The predominant formation of the $\Delta^{9(11)}$ over the $\Delta^{8(9)}$ isomer of the 10-azasteroids could be kinetically or thermodynamically controlled, although a kinetically controlled mechanism seems more consistent with the product distribution obtained, considering the mode of attack of the nitrogen during the ring closure reaction (Scheme 6). The attack on the carbonyl group may occur from either the α or β face of the molecule, producing intermediates with hydroxyl groups on the β or α face, respectively. In the case of β-hydroxyl intermediates, out of the three protons Ha, Hb, and Hc, only Ha is antiperiplanar to the OH group, and this will therefore be lost preferentially for maximum orbital overlap in the transition state, resulting in products with the double bond between C-9 and C-11. In the case of the α-hydroxyl intermediates, however, both Hb and Hc are antiperiplanar to the OH group. Loss of Hb will again lead to products with unsaturation between C-9 and C-11, whereas loss of Hc will lead to products with unsaturation between C-8 and C-9. On a purely statistical basis, if the attacks on the α and β faces of the carbonyl group at the ring closure stage were equally probable, one would expect, assuming only antiperiplanar elimination of water, a 3:1 ratio of products in favor of those with unsaturation between C-9 and C-11. The fact that the observed ratio is generally higher shows that the attack on the two faces does not occur equally readily but that the α (axial) attack is preferred. This can be rationalized by considering that the torsional strain for the axial attack should be much lower than that for the equatorial attack.[22,31]

This kinetically controlled mechanism is thus consistent with the product distribution obtained. A degree of thermodynamic control cannot be completely ruled out, however, since molecular mechanics calculations[32] indicate that the $\Delta^{9(11)}$ isomer **4a** is slightly more stable than the $\Delta^{8(9)}$ isomer **4b**. However, the 10:1 ratio between **4a** and **4b**, obtained from the rearrangement, must be kinetically controlled to a large extent since attempts at equilibration performed on a 3.5:1 mixture of **4a**,**b** with catalytic bases never gave a **4a**:**4b** ratio greater than 5:1.

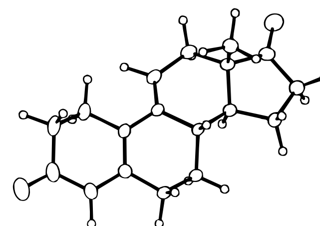


Figure 2. Crystallographic structure of **4a**.

Several attempts to separate completely by chromatography the two isomers of 19-nor-10-azasteroids failed. However, fractions enriched in one of the two isomers can be obtained by repeated chromatography or crystallizations. Only in one case were we able to obtain a pure $\Delta^{9(11)}$ isomer. The 10:1 mixture of **4a**:**4b** was recrystallized from ethyl acetate leading to a 22:1 mixture (by ^{1}H NMR analysis) after the first recrystallization. A second recrystallization yielded pure **4a**, the crystal structure of which was determined by X-ray diffraction and is shown in Figure 2 (full details of the structure will be published elsewhere). ^{1}H NMR analysis of the sample used for the structure determination showed no detectable traces of the isomer **4b**, while analysis of the mother liquor obtained from the first recrystallization, as expected, showed a much larger proportion of the isomer **4b** with a ratio of almost 1:3.5 with respect to **4a**. The structural assignment of the rearrangement products is based on the analytical and spectroscopic data and is in agreement with the results obtained in the synthesis of racemic **8**.[24]

According to the above mechanism, the configuration of the stereocenters 3a and 4 of the starting compound **12** should be retained in the overall synthetic sequence and should remain unchanged in the final products. Thus, the 10-azasteroids having unsaturation at the $\Delta^{9(11)}$ position and the other related compounds **27** and **28** should have the C and D rings *trans*-fused and H-8 in the β position. This was confirmed by the X-ray structure determination of **4a**. As can be seen from the crystal structure (shown in Figure 2), the hydrogens on C-8 and C-14 are *trans* to each other, with the one on C-8 on the β face of the molecule. Additionally, it can be seen that, as expected, the nitrogen atom is sp^2 hybridized and that from C-11 to O-1 the system is totally planar, due to extensive conjugation. Comparison of the methyl signals in ^{1}H NMR spectra of 19-nor-10-azasteroids provided a reliable method of distinguishing between the two stereoisomers **a** and **b** and in assessing their relative abundances.

Solutions enriched in one of the two isomers are, however, unstable and undergo a slow interconversion arriving at an equilibrium ratio which was evaluated as 5:1 for **4a**:**4b**. For this reason, the inhibition assays were made with equilibrated mixtures of the two isomers or with freshly prepared solutions of mixtures enriched in one isomer. The equilibration between the two isomers does not occur under acidic conditions. Instead, the rearrangment of the 10-azasteroid **4** to

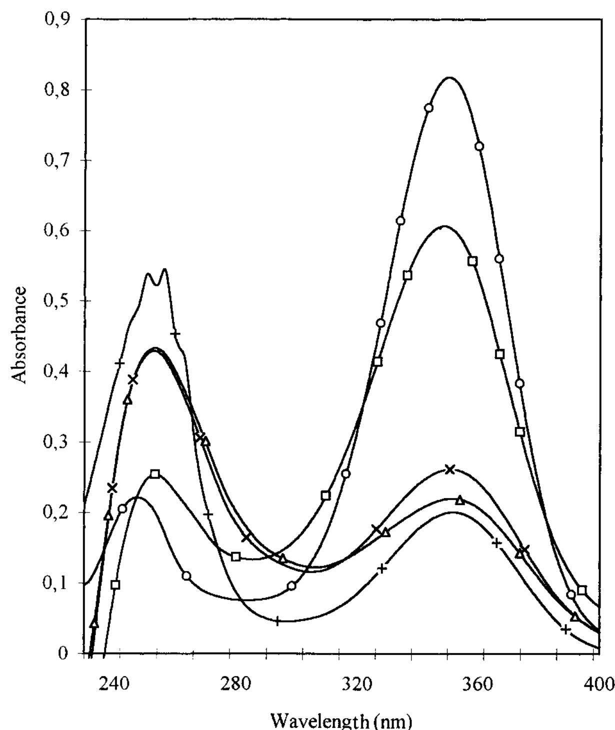
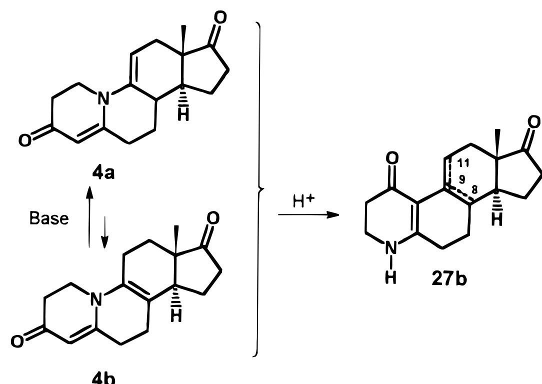


Figure 3. Comparison of absorbance spectra of compound **4** at pH 7 (○), pH 2 after 15 min (□), pH 2 after 3 h (×), and pH 2 after 18 h (△) and compound **27b** at pH 2 (+). UV spectra were recorded in a 2.5% ethanol/97.5% water solution of TRIS-HCl (0.98 mM), EDTA (0.98 mM), and KCl (146 mM) at pH 7 and in a 2.5% ethanol/97.5% water solution of TRIS-citrate (0.98 mM), EDTA (0.98 mM), and KCl (146 mM) at pH 2. Concentration of compounds was 1 ng/μL.

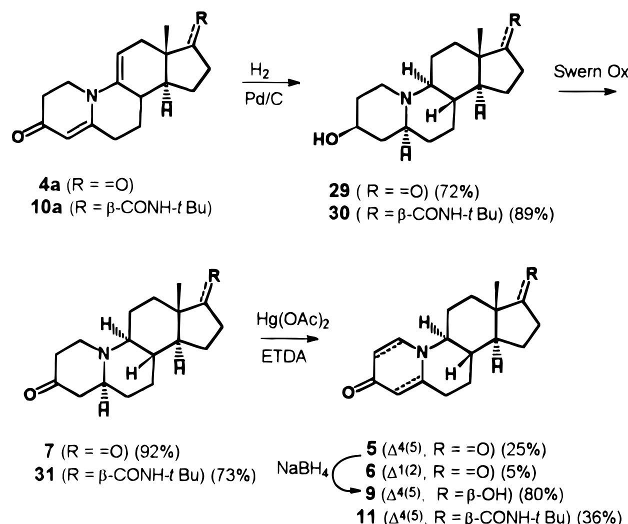
Scheme 7



4-azasteroid **27b** was observed. In fact, the ^{1}H NMR analysis of a $CDCl_3$ solution of **4a,b** (10:1 mixture) revealed, after 24 h, extensive conversion to compound **27b**, although the ratio between **4a** and **4b** was unaffected. The same effect was observed by treating the mixture with *p*-TsOH in toluene for 48 h at room temperature. Moreover by recording the UV spectrum of 10-azasteroid **4** (as a 10:1 mixture of **4a**:**4b**) dissolved in buffered aqueous solution at different pH, no changes were observed at neutral pH. However, the rearrangement became very fast on lowering the pH of the solution, being almost complete in 3 h at pH 2, and the final UV spectrum was very similar to the spectrum of compound **27b** at the same pH (Figure 3). Therefore, in order to avoid such rearrangement, the NMR spectra were recorded using dry and acid-free $CDCl_3$.

This conversion could occur by protonation of the carbonyl at the 3-position and generation of a positive charge on nitrogen at position 10. The cleavage of the N-10–C-9 bond, catalyzed by the presence of water (also in traces), would produce the same pyridone intermediate shown in Scheme 5 as precursor of 4-azasteroid **27b**. This acid lability of the 10-azasteroids having a double bond in the C ring represents a severe limitation to the modifications of the functionalities present in this type of azasteroid. In fact, attempts to transform the carbonyl groups of azasteroid **4** into the corresponding ketals or enol triflates under acid conditions invariably produced 4-azasteroid **27b** as the main product.

Scheme 8



In order to increase the stability of the 19-nor-10-azasteroids and with the aim of determining the influence of the different degree of unsaturation on the inhibition potency, we prepared different azasteroids having only the C=C bond in the A ring (compounds **5**, **6**, **9**, **11**) or without C=C bonds at all (compounds **7**, **31**). Studies were carried out using compound **4**, available in largest amounts, and the strategy developed was then applied to compound **10**. Some attempts to reduce selectively the double bond in position 9(11) of azasteroid **4**, without affecting the carbonyl groups or the double bond in position 4(5), by hydrogenation with different catalysts in heterogeneous or homogeneous phase as well as with other reducing agents, gave invariably the reduction of both the double bonds at positions 4(5) and 9(11) and, to some extent, led to the reduction of both carbonyl groups. Owing to these difficulties the reaction sequence shown in Scheme 8 was selected as the most feasible.

The hydrogenation on Pd/C (5%) of **4a** (as a 10:1 mixture with **4b**) was carried out in 24 h yielding, after chromatographic purification, a product which was identified as the keto alcohol **29** (72% yield). The formation of one main product combined with the above results on reducibility suggested that the hydrogenation of the main isomer **4a** occurs on the α face of the enaminone moiety leading to the hydroxy ketone **29** in a stereospecific manner. The A–B and B–C *trans*-fused structure of **29** was assigned on the basis of the strong Bohlmann bands present at 2856 and 2799 cm^{-1} in the IR spectrum, showing that some hydrogens antiperiplanar to the nitrogen lone pair are present.[33] The ^{1}H NMR spectrum supports this data because in the 3–4 ppm region only one signal at 3.3 ppm is present other than that of the carbinolic proton. This is due to the

effect of the nitrogen lone pair. Protons adjacent to nitrogen atom on the same side of the lone pair experience a strong deshielding effect, whereas the protons on the opposite side of the lone pair experience a strong shielding effect.[34] The signal at 3.3 ppm is thus attribuable to the 1β-H which, being *cis* to the lone pair, is deshielded. The lack of other signals due to protons adjacent to the nitrogen atom in the region above 3 ppm of the spectrum is consistent with the *trans*-fusion of rings A−C. In this case, the protons at the 1α-, 5α-, and 9α-positions are shielded, being on the opposite face with respect to the nitrogen lone pair.

Other diastereoisomers, due to the fact that a mixture of $\Delta^{9(11)}$ and $\Delta^{8(9)}$ isomers was used for these reductions, are obviously present in the reaction mixture with the same abundance as in the starting material. These compounds have presumably a *cis*-fusion of the B and C rings, but owing to their small abundance they were not recovered after chromatographic purification.

The hydrogenation of **10a** (as a 9:1 mixture with **10b**), using the same conditions reported above, gave **30** in 89% yield. The structural assignement was based on the same findings as for compound **29**. The Swern oxidation of **29** and **30** proceeded smoothly giving the diketones **7** and **31**, respectively. The reintroduction of the double bond between C-4 and C-5 was effected using mercuric acetate. This reagent provides a useful means of introducing unsaturation at a carbon atom α to an sp^3-hybridized nitrogen atom. The mechanism of this reaction is believed to involve the initial formation of a mercurated complex through the π-electrons on the nitrogen.[35]

Abstraction of the proton from the tertiary carbon could be a concerted process with cleavage of the nitrogen−mercury bond, which affects the oxidation of the amine moiety. Loss of the proton adjacent to the carbonyl group, i.e., the most acidic proton, results in the formation of the product. This reaction was carried out following the procedure used by Bertin and Perronet[36] in their synthesis of similar substrates, in 5% aqueous acetic acid at 85 °C for 2 h in the presence of the tetrasodium salt of EDTA, which serves as a chelating agent for the Hg(I) species formed in the reaction. The oxidation of **7** using the above condition gave, after flash column chromatography, the desired product **5** as the major fraction (42% isolated yield) together with another minor product (5% yield) identified as the $\Delta^{1(2)}$ isomer **6**. The presence of **5** is clearly shown by a sharp singlet at 4.95 ppm in the ^{1}H NMR spectrum, corresponding to the olefinic proton on C-4. The chemical shift is practically identical with the value found for **4a**. The ^{1}H NMR spectrum of the minor product is similar in many aspects to that of **5** apart from the olefinic region. Here, instead of a singlet at 4.95 ppm, two doublets are present, each of intensity one proton, centered at 4.98 and 7.18 ppm and coupled together with a $J = 7.8$ Hz. The only structure that is consistent with these spectral features is that of **6**. This product obviously results from abstraction of a proton from C-1, after initial formation of the mercurated complex, followed by loss of a proton on C-2 of the iminium salt to produce the $\Delta^{1(2)}$ isomer **6**. The same reaction applied to compound **31** gave **11** as the main product.

Compound **5** was then reduced to 19-nor-10-azatestosterone **9**, with sodium borohydride dispersed in a 1:1 2-propanol/ethanol mixture at 0 °C. Attack occurred only on the α face yielding **9** as the sole diastereomer with respect to the alcohol and methyl functionalities, in 80% yield. 19-Nor-10-azatestosterone **5** has been already prepared by Bertin et al.[36] following a completely different strategy, in 14 steps with a very low yield (less than 0.1%). However, our protocol appears to be a more valuable and efficient strategy because it produced compound **5** in 12 steps with an overall yield of 6.5% starting from the commercially available compound **12**.

Table 1. Inhibition of 19-Nor-10-azasteroids toward 5αR-2 in Human Prostate Homogenate

entry	compd	IC_{50} (nM)	finasteride IC_{50} (nM)	IC_{50} rel (nM)
1[a]	**4a**	4600 ± 2990	3.4 ± 2.2	1389 ± 1295
2[a]	**4a**:**4b** = 5:1	4600 ± 960	4.1 ± 0.7	1123 ± 318
3	**5**	4400 ± 2200	3.5 ± 2.1	1265 ± 1138
4	**6**	46000 ± 18400	2.4 ± 0.8	18843 ± 10053
5	**7**	>>100000	2.9±1.45	
6	**8a**:**8b** = 22:1	2900 ± 1190	3 ± 1.4	981 ± 609
		860[b]	1.4[b]	614[c]
7	**9**	4200 ± 1160	2.7 ± 0.8	1581 ± 664
8[a,d]	**10a**:**10b** = 9.1	37 ± 6.7	2.2 ± 0.4	16.9 ± 4
9[e,d]	**10a**:**10b** = 9.1	122 ± 37[f]	5.7 ± 1.8	21.5 ± 9.4
10[a,g,h]	**10a**:**10b** = 3.5:1	10.2 ± 4.9[i]	3.4 ± 1.3	2.9 ± 1.8
		7.3 ± 3.3[i]	3.1 ± 1.1	2.4 ± 1.4
11[e,d]	**10a**:**10b** = 3.5:1	150 ± 33[f]	4.3 ± 1.5	34 ± 12
12	**11**	460 ± 229	5.5 ± 2.1	83 ± 52
13	**27b**	>>100000	3.8 ± 1.7	
14	**27c**	>>100000	6.1 ± 1.1	
15	**28b**	>>100000	6.1 ± 2.8	

[a] Freshly prepared solution. [b] This value is a K_i. [c] This value is a ratio between K_is. [d] $p < 0.05$, in comparison with IC_{50} curve of finasteride in the same experiment. [e] Solution analyzed 6 months after preparation. [f] $p > 0.05$, in comparison with the corresponding IC_{50} curve of entry 8. [g] $p > 0.05$, in comparison with the IC_{50} curve of finasteride in the same experiment. [h] Values obtained using two different prostate homogenate samples. [i] $p < 0.05$, in comparison with the corresponding IC_{50} curve of sample 8.

Biological Assay

Steroid 5α-Reductase 2. The inhibitory potency of the synthesized azasteroids against 5αR-2 was determined on human prostate homogenates from surgically derived benign hyperplastic tissue according to reported methods.[37] Each inhibitor was tested at 6 different concentrations in the range 10^{-10}−10^{-5} M in duplicate, and finasteride (**1**) ($\Delta^{1(2)}$, R = H, R_1 = CONH-*t*-Bu) was tested for comparison in all inhibition experiments at the same concentrations. The IC_{50} values of all the tested compounds and the IC_{50} values of finasteride determined for each experiment are reported in Table 1.

Owing to the biological variability of the specimens of human prostatic tissue, the redetermination of the IC_{50} values of finasteride for each experiment allows a control of the assay accuracy as well as the calculation of the relative IC_{50} (IC_{50} rel = IC_{50} compound/IC_{50} finasteride). This value can represent a “normalized” index useful to evaluate the changes of the inhibition potency relative to the modification of the inhibitor structures without the influence of biological variability of the sample.

The IC_{50} values were determined by interpolation of the inhibition curves fitted with the program ALLFIT[38] which gave also the SD of the determination. This value is not related to the accuracy of the assay (which is usually in the 10−20% range) but to the imprecision in

fitting the curve. In fact, when the IC_{50} curves are very flat (i.e., for the less potent inhibitors), the IC_{50} evaluation is less accurate (compare the SD for entries 1−6 with those of entries 8−11). Finally, the use of ALLFIT allows the statistical comparison of inhibition curves through the Student *t*-test (*p* value). This enable one to assess if the IC_{50} values obtained in different experiments with the same inhibitor or with different inhibitors are significantly different ($p < 0.05$) or not ($p > 0.05$). For example, the simultaneous comparison of all inhibition curves of finasteride gave an IC_{50} value of 3 ± 0.22 nM, with a $p = 0.87$, indicating that all the determinations are not significantly different.

For the enzymatic conversion assay, the amount of prostate homogenate containing 0.1 mg of protein was incubated with testosterone (30 nM) containing 9% of [1,2,6,7-$^3H_4$]T and 0.3 mM NADPH for 30 min at 37 °C, in TRIS buffer at pH 7.4. In the human prostatic tissue the isozyme 5αR-2 is reported to be prevalent.[39] Both types 1 and 2 are present as active enzymes in human prostatic tissue; however, the activity due to 5αR-1 became measurable only at testosterone concentrations higher than 1 μM at neutral pH.[40] We employed a T concentration of 30 nM which is far too low to activate 5αR-1; thus, the activity measured in this study should be related only to 5αR-2. Moreover, the K_m and V_{max} values [respectively 7.87 nM and 0.84 pmol of DHT/(min·mg of protein)] as well as their ratio [$V_{max}/K_m = 106 \times 10^{-6}$ L/(min·mg of protein)] found for the prostate homogenate with testosterone as substrate agree with the values commonly reported for the human prostate 5αR.[39,40] Finally, the observed IC_{50} value for finasteride (3 ± 0.22 nM) is very close to the values usually found for this inhibitor for 5αR-2.[41−48]

19-Nor-10-azasteroids **4**−**6**, having a carbonyl group at position 17 and one C=C bond in the A ring, are weak inhibitors of 5αR-2, having IC_{50} values in the range 4.4−46 μM. The inhibition potency does not change significantly ($p > 0.5$) when the double bond on the C ring is in the $\Delta^{9(11)}$ or $\Delta^{8(9)}$ position or when it is absent; indeed, the IC_{50} of the pure compound **4a** is identical with the IC_{50} of the 5:1 mixture of **4a**,**b** isomers (compare entries 1 and 2 in Table 1) as well as with the IC_{50} of azasteroid **5** (compare entry 1 with entry 3 in Table 1). However the inhibition seems to be dependent on the presence and position of the double bond on the A ring. In fact, the presence in compound **6** of a double bond at the C-1−C-2 position instead of C-4−C-5 position decreases the inhibition potency (compare entry 3 with entry 4 in Table 1), whereas the absence of a C=C bond in the A ring of compound **7** causes a complete loss of activity (entry 5 of Table 1). The inhibition potency is not modified by changing the carbonyl group at position 17 with a hydroxy group (the comparison between the inhibition curves of entries 1 and 6 gave $p = 0.29$), and also in this case, the absence of the C=C bond on the C ring, as in compound **9**, does not modify significantly the inhibition potency. Thus all the 10-azasteroids of this first series appear to be weaker inhibitors than finasteride; nevertheless, this may be mainly due to the unsuitable functionality present at position 17. In fact, 4-azasteroid **1** ($\Delta^{1(2)}$, R = H, R_1 = β-OH), having a hydroxy group at position 17, has an IC_{50} value of 0.86 μM,[12b] which is very close to the IC_{50} value of $\Delta^{9(11)}$-19-nor-10-azatestosterone (**8**). This observation suggested to us that the introduction of a β-(*N*-*tert*-butylcarbamoyl) group at the 17-position of 10-azasteroids, as in finasteride, may increase significantly the inhibition potency. Indeed, 9:1 or 3:1 mixtures of $\Delta^{9(11)}$- and $\Delta^{8(9)}$-17β-(*N*-*tert*-butylcarbamoyl)-19-nor-10-azasteroid **10a**,**b** were found to have a potency in the nanomolar range, very close to that of finasteride (entries 8−11 of Table 1). In this case, the inhibition potency toward 5αR-2 changes significantly with the position and presence of the C=C bond on the C ring; thus, the decrease in ratio between $\Delta^{9(11)}$ isomer **10a** and $\Delta^{8(9)}$ isomer **10b** increases the inhibition activity [compare entries 8 and 10 of Table 1, their IC_{50} curves were significantly different ($p < 0.05$)]. The 3.5:1 mixture of **10a**,**b** isomers (entry 10 of Table 1) was as potent as finasteride [their IC_{50} curves were not statistically different ($p = 0.4$), IC_{50} rel = 2.9 and 2.4 in two different experiments]. However, owing to the impossibility of complete separation of the two isomers of **10**, we were not able to determine the exact inhibition potency of each isolated isomer. A further limitation in a more precise determination of the inhibition potency of different mixtures of the two isomers derives from the occurring equilibration between the two isomers. Indeed, ethanol solutions of mixtures of the two isomers **10a**,**b**, present in different ratios (9:1 or 3.5:1), analyzed immediately after their preparation gave IC_{50} values significantly different ($p < 0.05$), but reanalyzing them 6 months after their preparation showed very similar IC_{50} values (comparison between curves of entries 9 and 11 gave $p = 0.8$). This may be due to the slow equilibration of the isomer **10b** into **10a**. Probably the 9:1 mixture of **10a**,**b** is very close to the equilibrium because the freshly prepared mixture (entry 8) reanalyzed after 6 months (entry 9) did not gave a significantly different IC_{50} ($p = 0.54$). Moreover, we can exclude that these changes were due to a transformation of compounds **10a**,**b** into the inactive compound **27c** because, by an accurate check of the test solutions by GC−MS, compound **27c** was not detected, although it was impossible to redetermine exactly the ratio between **10a** and **10b** in such dilute solutions.

The problem of the equilibration could be overcome by reducing the C=C bond on the C ring. However 17β-(*N*-*tert*-butylcarbamoyl)-19-nor-10-azasteroid **11** without this double bond was slightly less potent (IC_{50} rel = 83, entry 12 of Table 1). Finally, further modifications of the A ring as in compounds **27b**,**c** and **28** cause the complete loss of activity (see entries 13−15 of Table 1). This may be due to the incorrect disposition of the carbonyl group and the A ring moiety.

Steroid 5α-Reductase 1. The majority of the 19-nor-10-azasteroids were also tested toward the 5αR-1 isoenzyme expressed in the human prostatic carcinoma DU-145 cells. This cell line (ATCC, HTB 81), deriving from brain metastases of an hepithelial human prostate adenocarcinoma, has been demonstrated to express only type 1 isoenzyme[49,50] and has been recently used to test inhibitors toward this 5αR isoform.[51] In our assay, the inhibition potency was determined, according to the reported method,[51] by incubation of 10^5 cells/well with different amounts of inhibitors in the culture medium for 6 h, using tritiated androstenedione (5 nM) as substrate of the enzyme. All the IC_{50} curves were obtained by fitting the data with the ALLFIT program in comparison with the IC_{50} curve of finasteride. The

Scheme 9

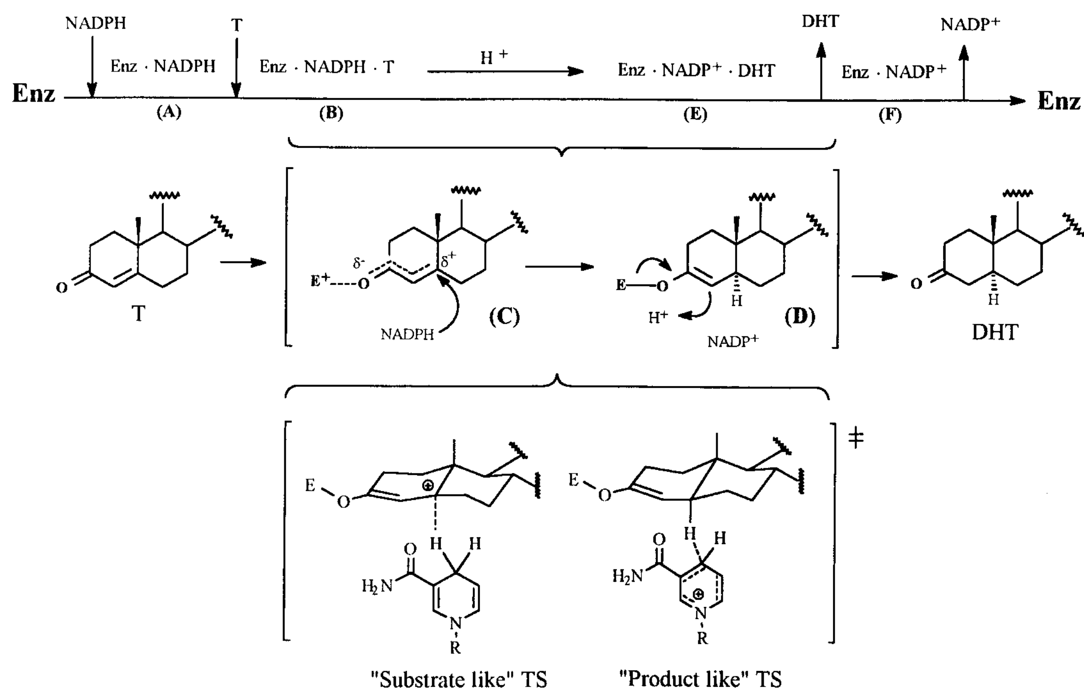


Table 2. Inhibition of 19-Nor-10-azasteroids toward 5αR-1 in DU-145 Cells

entry	compd	IC_{50} (nM)	IC_{50} rel[a] (nM)	selectivity 5αR-2:5αR-1
1[b]	**4a**:**4b** = 5:1	263 ± 63	6.7 ± 2	1:17
2	**5**	299 ± 132	6.4 ± 3	1:15
3	**7**	>>100000		
4	**9**	409 ± 130	10.4 ± 4	1:10
5[c]	**10a**:**10b** = 9.1	127 ± 12	2.8 ± 0.4	1:1
6	**11**	1134 ± 288	24.4 ± 7	2.5:1
7	**27c**	>>100000		

[a] The IC_{50} values of finasteride in this set of experiments ranged from 39.3 ± 8 to 46 ± 5.5 nM. [b] Freshly prepared solution. [c] Solution analyzed 6 months after preparation.

calculated IC_{50} values, the relative IC_{50} values (IC_{50} rel), and the 5αR-2/5αR-1 selectivity (expressed as the ratio between IC_{50} values for 5αR-1 and 5αR-2) are reported in Table 2. All the tested 19-nor-10-azasteroids had a fair inhibition potency toward the isoenzyme 1, with IC_{50} values ranging from 127 nM to 1.1 μM, except for compounds **7** and **27c** which were inactive for this isoform as well as for the isoenzyme 2.

The compounds **4** (as a 5:1 mixture of **4a**,**b**) and **5** having a carbonyl group at position 17 (see entries 1 and 2 of Table 2) have almost the same activity as the 17-hydroxy-10-azasteroid **9** (entry 4 of Table 2) but are less active than the corresponding 10-azasteroid **10** bearing the 17β-(*N*-*tert*-butylcarbamoyl) group. Indeed, the equilibrated 9:1 mixture of **10a**,**b** isomers gave an IC_{50} of 127 nM toward 5αR-1 (entry 5 of Table 2), having a potency very close to that of finasteride which in the 5αR-1 assay gave an IC_{50} of 39.3 nM (their relative IC_{50} is 2.8).

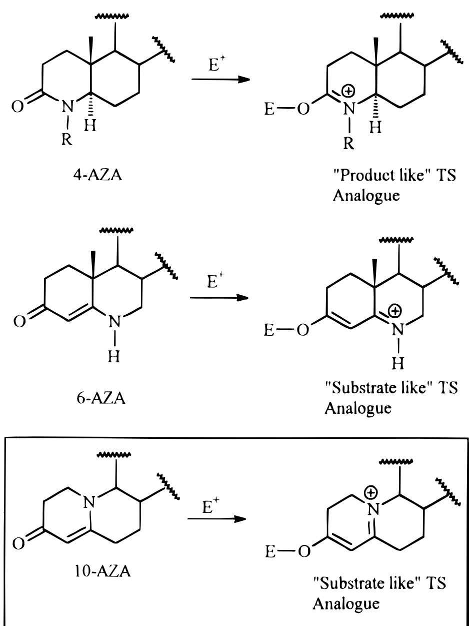
As in the 5αR-2 assay, the absence of the C=C bond in the C ring does not affect the inhibition potency toward 5αR-1 when a carbonyl group is present at position 17 (compare entry 1 with entry 2); however, the potency is sharply decreased when the *N*-*tert*-butylcarbamoyl group is present (compare entries 5 and 6). Concerning the selectivity toward the two isoforms, the 10-azasteroids **4**, **5**, and **9** with a keto or hydroxy group at the 17 position are selective inhibitors for type 1 enzyme (with a selectivity factor of 10−17-fold) having IC_{50} values in the nanomolar range for type 1 and micromolar range for type 2. On the contrary, the 17β-(*N*-*tert*-butylcarbamoyl)-19-nor-10-azasteroid **10** (as a 9:1 mixture of **10a**,**b**) is a dual inhibitor having the same IC_{50} toward 5αR-2 and 5αR-1 isozymes (122 and 127 nM, respectively).

Discussion

We based the design of the new class of 10-azasteroids on the transition state inhibitor paradigm[52] which is common to the three classes of 4-azasteroids **1**, steroidal acrylates **2**, and 6-azasteroids **3**. This concept states that enzyme binding, and so inhibition, should be greater for molecules being mimic of the transition state of the enzymatic process. The proposed mechanism[10] (Scheme 9) of T reduction to DHT by 5αR catalysis, based on the known regio- and stereochemistry of the reduction, involves the formation of a binary complex (A) between the enzyme and NADPH followed by the formation of a ternary complex (B) with the substrate T. The activation of the enone system by a strong interaction with an electrophilic residue (E^+ = proton, positively charged group, proton donor) present in the active site then gives the delocalized carbocation (C) which is reduced selectively at C-5, on the α face, by a direct hydride transfer from NADPH, leading to the formation of the enolate intermediate (D). The enolate, which is presumably coordinated with $NADP^+$ on the α face, is attacked by a proton on the β face at C-4 giving the ternary complex (E). Then, the departure of DHT gives the binary $NADP^+$−enzyme complex (F), and finally the release of $NADP^+$ leaves the enzyme free for further catalytic cycles. The consistency of this mechanism has recently received a further confirmation by the recent isolation of an adduct between finasteride and NADPH.[53]

On the basis of the above mechanism, we have postulated[19] two possible transition states (TS): the

Scheme 10



"substrate-like" TS, in which the C-5 has not yet changed its sp^2 hybridization, where the structures of C-3, C-4, and C-5 atoms are similar to those of carbocation (C), and the "product-like" TS, in which the C-5 has assumed the final sp^3 hybridization and the structures of the C-3, C-4, and C-5 atoms are similar to those of DHT enol (D). Accordingly, 4-azasteroids **1**, similar to the DHT enol,[12d,54] are mimics of the "product-like" TS, while 6-azasteroids **3**,[16] which have an enone structure in the A ring, are mimics of the "substrate-like" TS. Furthermore, the presence of the nitrogen atom, which increases the nucleophilicity of the carbonyl group of the 4- and 6-azasteroids, should favor the interaction with an electrophilic residue in the active site.

The new class of 19-nor-10-azasteroids has structural features which can cause a strong interaction with the active site of 5α-reductase. In fact, the enaminone structure of the A ring of 10-azasteroids is analogous to that of the "substrate-like" TS, and the presence of the nitrogen at position 10 should increase the nucleophilic character of the carbonyl and stabilize the carbocation intermediate by delocalization of the positive charge (Scheme 10). On this basis, the presence of a C=C bond on the A ring is an essential feature in order to maintain the conjugation betwen the carbonyl group and the nitrogen. Thus, 10-azasteroid **7** without this double bond is inactive toward 5αR-2. Although the active sites of the 5αR-1 and 5αR-2 should be different, this stereoelectronic effect seems to be essential for both isoenzymes because compound **7** is also inactive for 5αR-1.

In order to evaluate this effect, in a semiquantitative way, we performed an HF-STO3-21G* *ab initio* calculation[55] on a series of simplified tricyclic models representative of the substrate T, product DHT, and 4-, 6-, and 10-azasteroids (Figure 4). If we consider the partial negative charge on the oxygen as a measure of the nucleophilic character of the C-3 carbonyl in the above compounds, we could relate an increase of this partial charge to a greater interaction with an electrophile in the cavity. Thus, by going from testosterone to DHT, a slight decrease in the negative charge character is

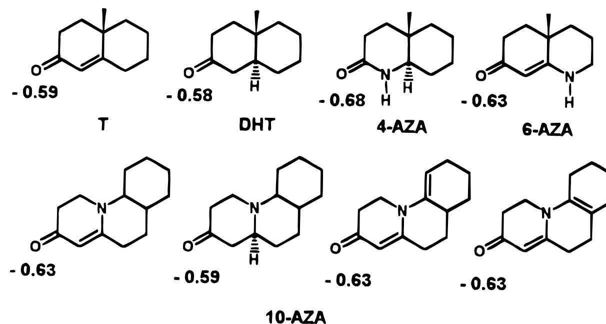


Figure 4. Partial negative charges in some steroid models obtained by HF-STO3-21G* calculations.

observed (from −0.59 to −0.58, respectively); on the contrary, the introduction of the nitrogen at the 4- or 6-position of the azasteroids causes a significant increase of the negative charge (from −0.58 for T to −0.68 for 4-azasteroids or −0.63 for 6-azasteroids).

The presence of the nitrogen at position 10, conjugated with the carbonyl group, confers in 10-azasteroids the same increase in negative charge character (-0.63) as in 6-azasteroids. No effect on the oxygen negative charge was observed in relation to the presence or absence of the $\Delta^{9(11)}$ or $\Delta^{8(9)}$ unsaturation; also, the position of this double bond did not affect the value of the charge (in all these cases the partial charge is −0.63). On the contrary, the reduction of the C-4−C-5 double bond decreases the partial charge to the original value for testosterone (−0.59). These calculations are thus consistent with the complete inactivity of compound **7**, although some steric effect deriving from an incorrect orientation of the carbonyl group in this compound cannot be completely excluded. However, the variation of 5αR-2 inhibition found by changing the ratio between $\Delta^{9(11)}$ and $\Delta^{8(9)}$ isomers in compound **10** does not seem related to an electronic effect because no changes in the partial charge on the oxygen were observed in the models; thus the modification of the inhibition potency may be related to conformational effects.[56]

As in the other classes of azasteroids the inhibition potency of 19-nor-10-azasteroids is strongly dependent on the substituent at position 17. The introduction of the 17*β*-(*N*-*tert*-butylcarbamoyl) group increases significantly the inhibition toward type 1 and 2 isoenzymes, and azasteroid **10** (as a mixture of the two isomers) has a potency very close to that of finasteride.

In addition, the selectivity between the two isoenzymes changes according to the group at position 17. 19-Nor-10-azasteroids **4**, **5**, and **9** bearing a keto or a hydroxy group are selective inhibitors of type 1 enzyme, whereas the compound **10** is an equipotent inhibitor for both isoenzymes. A strong analogy thus exists between the 19-nor-10-azasteroids and 6-azasteroids both having a 17*β*-(*N*-*tert*-butylcarbamoyl) group, because not only are they mimics of the substrate-like transition state, but they are also dual inhibitors of 5α-reductases 1 and 2.

Both 4- and 6-azasteroids are known to display time-dependent inhibition.[16,53] In particular, for finasteride it has been demonstrated that this is due to the addition of NADPH to the C-1−C-2 double bond. The NADP−dihydrofinasteride adduct resulted to be a very potent inhibitor toward 5α-reductase 2 with a potency higher than that of finasteride itself.[53] Although 10-azasteroids

could in principle suffer a similar mechanism, however, preliminar experiments on compound **10** (as a 9:1 mixture of **10a**,**b**) carried out on human prostate homogenates have not revealed significant changes in the IC_{50} curve, with respect to the control, when a preincubation time of 30 min is applied. This result should therefore exclude any time dependence in the inhibitory activity of the tested compound.

Summary and Conclusions

Potent *in vitro* inhibition of human 5α-reductases 1 and 2 has been observed with a series of 19-nor-10-azasteroids. Activity toward both isozymes is enhanced in compounds having both the 17β-(*N-tert*-butylcarbamoyl) group and enaminone mojety and is suppressed when the conjugation between the carbonyl group and nitrogen is interrupted. This observation is consistent with *ab initio* calculations on model compounds which correlate the increase in the nucleophilic character of the carbonyl group of 4-azasteroids, 6-azasteroids, and 19-nor-10-azasteroids with the increase in affinity of these inhibitors toward the enzymes with respect to the natural substrates. This finding should give new insights for a better design of new potent and selective inhibitors of these human enzymes so important for their pathological implications.

Experimental Section

All the reactions were performed under nitrogen, unless otherwise stated. Chromatographic separations were performed under pressure on silica gel using flash column techniques or Chromatospack apparatus; R_f values refer to TLC carried out on 25-mm silica gel plates (Merck F254), with the same eluant indicated for the column chromatography. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer in $CDCl_3$ solution. ^1H NMR (200 MHz) and ^{13}C NMR (50.33 MHz) spectra were recorded on a Varian XL 200 instrument in $CDCl_3$ solution. Mass spectra were carried out in EI at 70 eV on 5790A-5970A Hewlett-Packard and QMD 1000 Carlo Erba instruments. Microanalyses were carried out with a Perkin-Elmer 240C elemental analyzer. Optical activity was measured on a Jasco DIP-370 polarimeter at 25 °C unless otherwise stated. (+)-3-[(3a*S*)-(3aα,4α,7aβ)-1,5-Dioxo-7a-methyloctahydro-1*H*-inden-4-yl]propionic acid (**12**)[25] was purchased from Upjohn Co. (Kalamazoo, MI). Although the nomenclature used in the text describes the synthesized compounds with the trivial name of 19-nor-10-azasteroids, in the Experimental Section the correct nomenclature of estrane derivatives is used.

Enol Lactonization of 12. Compound **13** was prepared as described,[27] starting from pure **12**. Compound **13**: mp 136.5−137.5 °C; R_f = 0.7 (EtOAc); $[\alpha]^{25}_D$ +241 (*c* 1.07, MeOH). Reported[27] mp 136.5−137.5 °C; $[\alpha]^{24}_D$ +263 ± 30.2 (*c* 1.00, EtOH/$CHCl_3$). Elemental anal. for $C_{13}H_{16}O_3$: C, H.

Reduction of Keto Enol Lactone 13. To an ice-cooled solution of **13** (24 g, 109 mmol) in 120 mL of DMF was added dropwise over 10 min a suspension of $NaBH_4$ (8.4 g, 222 mmol) in 90 mL of DMF with stirring. The resulting mixture was stirred for 10 min at 0 °C, and then ice−water (120 mL) was poured quickly into the vessel maintaining the temperature below 10 °C. After 5 min the mixture was extracted with $CHCl_3$ (3 × 80 mL). The separated organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent *in vacuo*, 22.9 g of a 3:1 mixture of compounds **14** and **13** was obtained. Chromatography on Chromatospack apparatus (CH_2Cl_2/EtOAc, 5:1, R_f = 0.39) afforded pure **14** (61% yield): colorless solid; mp 94−95 °C; IR 3613, 1745, 1668 cm^{-1}; ^1H NMR δ 5.27 (m, 1H), 3.79 (t, *J* = 8 Hz 1H), 2.83−2.67 (ddd, *J* = 18, 5.4, 2.8 Hz, 1H), 2.65−2.50 (m, 1H), 2.25−1.35 (m, 11H), 0.83 (s, 3H); ^{13}C NMR δ 168.5 (s), 150.9 (s), 106.0 (d), 81.4 (d), 47.2 (d), 42.8 (s), 35.8 (d), 35.5 (t), 31.4 (t), 31.0 (t), 24.0 (t), 23.7 (t), 11.4 (q); MS *m/z* 222 (M$^+$, 6), 205 (12), 55 (100). Elemental anal. for $C_{13}H_{18}O_3$: C, H.

Methyl (+)-3-[(3a*S*)-(1β,3aα,4α,7aβ)-1-Hydroxy-7a-methyloctahydro-5-oxo-1*H*-inden-4-yl]propionate (15). A solution of the lactone **14** (5.12 g, 23 mmol) in 30 mL of anhydrous MeOH was treated with 1 mL of a MeONa solution (2.3 mmol) prepared from 0.11 g of sodium in 2 mL of methanol. After the mixture stirred at room temperature for 3 h, 132 μL of CH_3COOH (2.3 mmol) was added, and the solvent was removed. The residual oil was dissolved in $CHCl_3$ and filtered to remove the insoluble residue of CH_3COONa and the solution evaporated to give 5.86 g of crude product. Chromatography on Chromatospack apparatus (CH_2Cl_2/EtOAc, 3:1, R_f = 0.25) gave **15** (88% yield): colorless solid; mp 36 °C; $[\alpha]^{25}_D$ +15 (*c* 0.87, $CHCl_3$); IR 3614, 1729, 1704 cm^{-1}; ^1H NMR δ 3.70 (dd, *J* = 15, 8.5 Hz, 1H), 3.63 (s, 3H), 2.52−1.35 (m, 14H), 1.04 (s, 3H); ^{13}C NMR δ 212.6 (s), 174.7 (s), 80.8 (d), 52.0 (d), 50.4 (d), 50.2 (q), 43.7 (s), 38.4 (t), 35.9 (t), 32.2 (t), 31.5 (t), 24.4 (t), 22.3 (t), 11.2 (q); MS *m/z* 254 (M$^+$, 3), 195 (1), 55 (100). Elemental anal. for $C_{14}H_{22}O_4$: C, H.

Methyl (+)-3-[(3a*S*)-(1β,3aα,4α,7aβ)-7a-Methyloctahydro-5-oxo-1-[(*tert*-butyldimethylsilyl)oxy]-1*H*-inden-4-yl]propionate (16). The methyl ester **15** (8.22 g, 32.3 mmol) was added to a stirred solution of TBDMSCl (25.7 g, 165.4 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (15.33 g, 100 mmol) in 100 mL of dry CH_2Cl_2 (freshly distilled from calcium hydride) at room temperature. After 3 days, the reaction mixture was washed with saturated NH_4Cl solution (3 × 60 mL), saturated $NaHCO_3$ solution (3 × 60 mL), and saturated NaCl solution (3 × 60 mL). The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and the residue was chromatographed (hexane/EtOAc, 4:1, R_f = 0.51) to afford **16** (10.36 g, 87%): colorless oil; IR 1729, 1702 cm^{-1}; ^1H NMR δ 3.63 (s, 3H), 3.60 (t, *J* = 7 Hz, 1H), 2.55−2.20 (m, 5H), 2.00−1.30 (m, 9H), 1.00 (s, 3H), 0.86 (s, 9H), −0.01 (s, 6H); ^{13}C NMR δ 212.4 (s), 174.2 (s), 80.3 (d), 51.5 (q), 49.8 (d), 49.4 (d), 43.6 (s), 38.0 (t), 35.8 (t), 31.7 (t), 31.5 (t), 25.8 (q), 24.1 (t), 21.9 (t), 18.0 (s), 10.9 (q), −4.5 (q), −4.9 (q); MS *m/z* 353 (M$^+$ − Me, 1), 338 (3), 311 (100), 237 (9), 203 (93). Elemental anal. for $C_{20}H_{36}O_4Si$: C, H.

Methyl (+)-3-[(3a*S*)-(1β,3aα,4α,7aβ)-5,5-(1,2-Ethylenedioxy)-7a-methyloctahydro-1-[(*tert*-butyldimethylsilyl)oxy]-1*H*-inden-4-yl]propionate (17a). A solution containing **16** (8.90 g, 24.14 mmol), ethylene glycol (24.5 mL, 438 mmol), and *p*-toluenesulfonic acid monohydrate (272 mg, 1.43 mmol) in 190 mL of anhydrous toluene was refluxed for 5.5 h, removing the former water using a Dean−Stark apparatus. The reaction mixture was cooled to room temperature and washed with a saturated Na_2CO_3 solution (3 × 50 mL) and a saturated NaCl solution (3 × 50 mL). After drying (Na_2SO_4) and removal of the solvent, the residue (8.96 g) was chromatographed (hexane/EtOAc, 5:1, R_f = 0.52), affording pure **17a** (8.16 g, 82%): colorless oil; $[\alpha]^{25}_D$ +9 (*c* 1.33, $CHCl_3$); IR 1730 cm^{-1}; ^1H NMR δ 3.98−3.90 (m, 4H), 3.63 (s, 3H), 3.59 (t, *J* = 14 Hz, 1H), 2.45−2.34 (m, 2H), 1.98−1.10 (m, 12H), 0.84 (s, 9H), 0.77 (s, 3H), −0.03 (s, 6H); ^{13}C NMR δ 174.5 (s), 112.2 (s), 80.8 (d), 64.4 (t), 51.3 (q), 46.7 (d), 43.5 (d), 43.4 (s), 33.6 (t), 33.5 (t), 31.1 (t), 31.0 (t), 25.8 (q), 24.1 (t), 21.9 (t), 18.0 (s), 10.6 (q), −4.6 (q), −4.90 (q); MS *m/z* 397 (M$^+$ − Me, 1), 355 (32), 293 (12), 279 (20), 261 (15), 237 (12), 99 (100). Elemental anal. for $C_{22}H_{40}O_5Si$: C, H.

Methyl (+)-3-[(3a*S*)-(3aα,4α,7aβ)-1,5-Dioxo-7a-methyloctahydro-1*H*-inden-4-yl]propionate (18). Acid **12** (100 g, 420 mmol) was dissolved in anhydrous MeOH (460 mL), and *p*-toluenesulfonic acid (7.99 g, 42 mmol) was added. The solution was stirred for 23 h and then concentrated to one-half its original volume. The resulting liquid was added to water (250 mL) and extracted with CH_2Cl_2 (3 × 225 mL). The combined organics were washed with saturated NaCl solution (150 mL) and dried with anhydrous Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure gave **18** as a dark orange oil (101.71 g, 96% yield): $[\alpha]^{25}_D$ +96.5 (*c* 1.00, $CHCl_3$); IR 1729, 1197, 1172 cm^{-1}; ^1H NMR δ 3.64 (s, 3H), 2.62−1.57 (m, 14H), 1.14 (s, 3H); ^{13}C NMR δ 218.2 (s), 210.8 (s), 174.4 (s), 52.9 (q), 49.9 (d), 49.5 (d), 48.0 (s), 37.8 (t), 36.5 (t), 32.1 (t), 30.9 (t), 22.8 (t), 21.9 (t), 13.9 (q); MS *m/z* 252 (M$^+$,

17), 237 (17), 221 (34), 220 (40), 163 (69), 123 (44), 95 (56), 85 (65), 79 (81), 67 (52), 55 (100), 41 (82). Elemental anal. for $C_{14}H_{20}O_4$: C, H.

Methyl (+)-3-[(3a*S*)-(3aα,4α,7a*β*)-1,1-(1,2-Ethylenedioxy)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]propionate (17b). Ester **18** (51.0 g, 202 mmol) was dissolved in toluene (1.30 L), then ethylene glycol (140 mL, excess) and *p*-toluenesulfonic acid (1.95 g, 10.3 mmol) were added, and the resulting solution was refluxed for 8 h removing the water formed using a Dean–Stark apparatus. After cooling, the mixture was washed with saturated Na_2CO_3 (3 × 200 mL) and saturated NaCl (3 × 200 mL) and then dried over Na_2SO_4. Filtration and evaporation of the solvent resulted in an orange/brown oil (76 g). Analysis by GLC revealed a 1:1 mixture of **17b** and **32**. The crude mixture (140 g, combined products of the above reaction and a repeat) was stirred in anhydrous MeOH (400 mL) containing *p*-toluenesulfonic acid (8.18 g). The orange solution was stirred for 4 days, then filtered through a Celite layer, and poured onto ice (200 g). The mixture was extracted with CH_2Cl_2 (3 × 200 mL) and the organic phase washed with saturated NaCl solution (2 × 200 mL). Subsequent drying (Na_2SO_4) and removal of solvent yielded an orange oil (130.51) sufficiently pure for the next reaction. An analytical sample of **17b** was obtained by flash column chromatography (EtOAc/pentane, 1:2, R_f = 0.46): $[\alpha]^{25}_D$ −9.2 (*c* 1.00, $CHCl_3$); IR 2978, 2885, 1728 cm^{-1}; ^1H NMR δ 3.94−3.84 (m, 8H), 3.61 (s, 3H), 2.44−2.34 (m, 2H), 2.05−1.10 (m, 12H), 0.88 (s, 3H); ^{13}C NMR δ 174.6 (s), 118.3 (s), 111.7 (s), 65.2 (t), 64.5 (t), 51.4 (q), 46.4 (s), 45.0 (d), 43.5 (d), 34.4 (t), 33.4 (t), 30.7 (t), 27.2 (t), 23.1 (t), 21.7 (t), 13.6 (q); MS *m/z* 340 (M^+, 2), 309 (4), 279 (3), 241 (3), 152 (6), 100 (28), 99 (100), 86 (36), 55 (14). Elemental anal. for $C_{18}H_{28}O_6$: C, H.

Methyl (+)-3-[(3a*S*)-(3aα,4α,7a*β*)-1-Oxo-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]propionate (19). Silica gel (260 g, ICN-60) was added to CH_2Cl_2 (640 mL) to form a suspension, and a solution of 10% oxalic acid (26.6 g) in water was added dropwise with stirring. When the solution became homogeneous, the crude product from the previous reaction (130.5 g), dissolved in CH_2Cl_2 (100 mL), was added at room temperature. After 21.5 h, dione **18** had started to form, and so the reaction was stopped by filtering the suspension through a sinter funnel. The resulting liquid was concentrated giving an orange oil (112.8 g). GLC analysis indicated a mixture of **19** (87%) and **17b** (13%). An analytical sample of **19** was obtained by chromatography (acetone/pentane, 1:3, R_f = 0.48): $[\alpha]^{25}_D$ +66.7 (*c* 0.35, $CHCl_3$); IR 2954, 2890, 1729, 1436, 1268 cm^{-1}; ^1H NMR δ 3.90−3.83 (m, 4H), 3.58 (s, 3H), 2.50−2.30 (m, 2H), 2.15−1.30 (m, 12H), 0.85 (s, 3H); ^{13}C NMR δ 219.5 (s), 174.3 (s), 111.4 (s), 64.6 (t), 51.5 (d), 47.7 (s), 47.3 (q), 43.0 (d), 35.9 (t), 33.4 (t), 30.5 (t), 28.2 (t), 22.3 (t), 21.3 (t), 13.0 (q); MS *m/z* 296 (M^+, 14), 281 (16), 265 (50), 211 (38), 172 (86), 100 (62), 99 (100), 87 (89), 86 (100), 55 (94). Elemental anal. for $C_{16}H_{24}O_5$: C, H.

Methyl 3-[(3a*S*)-(3aα,4α,7a*β*)-1-[[(Trifluoromethyl)sulfonyl]oxy]-5,5-(1,2-ethylenedioxy)-7a-methylhexahydro-1*H*-inden-4-yl]propionate (20). Crude **19** (38.60 g, 114.0 mmol) was dissolved in freshly distilled anhydrous CH_2Cl_2 (150 mL). 2,6-Di-*tert*-butyl-4-methylpyridine (28.07 g, 137 mmol) was added and the solution cooled to 0 °C. Trifluoromethanesulfonic anhydride (Tf_2O) (21.9 mL, 130.2 mmol) was then added dropwise over a period of 30 min; the solution was stirred at 0 °C for 10 min and then allowed to warm to room temperature. The resulting red solution was stirred for 18 h, checking by GLC. After this time, the mixture was cooled again to 0 °C, further 2,6-di-*tert*-butyl-4-methylpyridine (2.81 g, 13.7 mmol) and Tf_2O (2.2 mL, 13.1 mmol) were added as before, and the solution was stirred for a further 3 days. The solution was filtered and concentrated affording a dark orange oil (67.63 g) containing the triflate **20** and its deketalized form. **20**: ^1H NMR δ 5.60−5.54 (m, 1H), 3.98−3.90 (m, 4H), 3.65 (s, 3H), 2.60−1.60 (m, 12H), 1.03 (s, 3H); MS *m/z* 397 (M^+ − 31, 2), 295 (4), 172 (18), 100 (10), 99 (100), 86 (67), 68 (8), 55 (17). Deketalized triflate: ^1H NMR δ 5.66−5.60 (m, 1H), 3.65 (s, 3H), 2.60−1.60 (m, 12H), 1.20 (s, 3H); MS *m/z* 353 (M^+ − 31, 8), 251 (20), 219 (73), 191 (20), 69 (40), 55 (100).

Methyl (+)-3-[(3a*S*)-(3aα,4α,7a*β*)-1-(*N*-*tert*-Butylcarbamoyl)-5,5-(1,2-ethylenedioxy)-7a-methylhexahydro-1*H*-inden-4-yl]propionate (21). Crude **20** (67.63 g, 158 mmol) and bis(triphenylphosphine)palladium(II) acetate (11.8 g, 15.8 mmol) were dissolved in freshly distilled DMF (280 mL). Et_3N (38 mL, 425 mmol) and *tert*-butylamine (165 mL, 1.57 mmol) were then added, and the solution was left under a CO atmosphere. After 25 h the starting material had all reacted, and water (215 mL) was added. The resulting suspension was filtered, and the liquid was extracted with EtOAc (4 × 250 mL). The combined organics were washed with water (200 mL) and saturated NaCl solution (200 mL), back-extracting the aqueous layers with further EtOAc (200 mL). Finally, all the organics were combined and dried over Na_2SO_4. The liquid was then filtered and concentrated to give a dark orange oil (61.84 g) containing **21** and the deketalized form. Hence, a further reaction was necessary to give only the protected form. The crude mixture (61.84 g, 120 mmol of deketalized form) was dissolved in toluene (500 mL), and *p*-toluenesulfonic acid (1.04 g) and ethylene glycol (100 mL) were added. The solution was then refluxed for 5 h, using a Dean–Stark apparatus to remove the water formed. The resulting liquid was washed with saturated Na_2CO_3 solution (3 × 150 mL), back-extracting the aqueous layers with further toluene (2 × 50 mL). The organics were then washed with saturated NaCl solution (3 × 150 mL), back-extracting once again with toluene (2 × 50 mL). The combined organics were then dried with Na_2SO_4, filtered, and concentrated under vacuum. The resulting orange oil (45.03 g) was divided into three portions and purified by column chromatography using a large sinter funnel (180 g of silica gel) and CH_2Cl_2 followed by petroleum ether/EtOAc, 3:2 (+1% Et_3N), as eluants. All the fractions having R_f = 0.5 with the last eluant were combined and, after removal of solvent under vacuum, yielded **21** as an orange oil (18.20 g, 42% overall yield): $[\alpha]^{25}_D$ +20.79 (*c* 1.04, $CHCl_3$); IR 3438, 2986, 2890, 1727, 1654, 1597 cm^{-1}; ^1H NMR δ 6.12−6.08 (m, 1H), 5.42 (s, 1H), 3.96−3.88 (m, 4H), 3.63 (s, 1H), 2.42−2.32 (m, 2H), 2.20−1.40 (m, 10H), 1.32 (s, 9H), 1.03 (s, 3H); ^{13}C NMR δ 174.4 (s), 165.6 (s), 151.4 (s), 133.2 (d), 111.7 (s), 64.6 (t), 52.1 (d), 51.4 (s), 51.0 (q), 46.7 (d), 42.3 (s), 33.4 (t), 31.9 (t), 31.6 (t), 31.3 (t), 28.9 (q), 22.1 (t), 15.3 (q); MS *m/z* 379 (M^+, 8), 348 (5), 306 (8), 252 (7), 196 (7), 172 (43), 99 (100), 86 (78), 55 (30). Elemental anal. for $C_{21}H_{35}NO_5$: C, H, N.

Methyl 3-[(3a*S*)-(1*β*,3aα,4α,7a*β*)-1-(*N*-*tert*-Butylcarbamoyl)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]propionate (17c). Amide **21** (18.2 g, 48.02 mmol) was dissolved in 480 mL of a EtOAc/EtOH, 3:1, solvent mixture. PtO_2 (4.03 g, 17.74 mmol) was added and the flask sealed under a hydrogen atmosphere. The suspension was left to stir monitoring the reaction's progress by TLC: **21**, R_f = 0.56, UV active; **17c**, R_f = 0.52, non-UV active (pentanol/EtOAc, 1:1). After 4 h the reaction had gone to completion, and the solution was filtered through a bed of Celite to remove the catalyst. Removal of the solvent yielded **17c** as a yellow oil (18.29 g, 100%) used in the next step without further purification. An analytically pure sample was obtained by chromatography (pentanol/EtOAc, 1:1) of a little portion. **17c**: oil; IR 3442, 2966, 1728, 1670 cm^{-1}; ^1H NMR δ 5.03 (s, 1H), 3.94−3.86 (m, 4H), 3.60 (s, 3H), 2.42−1.40 (m, 15H), 1.28 (s, 9H), 0.70 (s, 3H); ^{13}C NMR δ 175.0 (s), 172.0 (s), 112.2 (s), 65.1 (t), 57.2 (d), 52.7 (d), 51.9 (q), 51.5 (s), 44.1 (d), 42.3 (s), 35.7 (t), 33.9 (t), 31.6 (t), 29.5 (q), 25.5 (t), 24.1 (t), 22.8 (t), 12.9 (q); MS *m/z* 381 (M^+, 5), 351 (9), 283 (49), 282 (35), 100 (31), 99 (100), 86 (100), 55 (60). Elemental anal. for $C_{21}H_{35}NO_5$: C, H, N.

3-[(3a*S*)-(1*β*,3aα,4α,7a*β*)-5,5-(1,2-Ethylenedioxy)-7a-methyloctahydro-1-[(*tert*-butyldimethylsilyl)oxy]-1*H*-inden-4-yl]propanol (22a). A suspension of $LiAlH_4$ (0.57 g, 14.41 mmol) in 20 mL of anhydrous Et_2O was added over 20 min to a stirred solution of **17a** (4.73 g, 11.46 mmol) in 24 mL of Et_2O, maintaining the temperature at 15−18 °C. After the addition, the mixture was stirred for 30 min; then 6 mL of a saturated solution of Na_2SO_4 was added dropwise with cooling. The mixture was diluted with 20 mL of Et_2O and dried (Na_2SO_4). Filtration and removal of solvent *in vacuo* afforded **22a** as a crude oil (4.35 g, 98%) which was immediately used in the next

step to avoid degradation: oil; ^{1}H NMR δ 3.93 (m, 4H), 3.60 (m, 3H), 1.95−1.10 (m, 12H), 0.84 (s, 9H), 0.77 (s, 3H), −0.03 (s, 6H); ^{13}C NMR δ 112.4 (s), 80.8 (d), 64.6 (t), 64.4 (t), 47.5 (d), 43.5 (d), 43.4 (s), 33.7 (t), 31.9 (t), 31.1 (t), 30.9 (t), 25.8 (q), 24.2 (t), 22.8 (t), 18.0 (s), 10.6 (q), −4.1 (q), −4.4 (q); MS *m/z* 384 (M$^+$, 0.21), 327 (9), 265 (59), 211 (17), 191 (36), 99 (100), 75 (32).

3-[(3a*S*)-(3aα,4α,7a*β*)-1,1-(1,2-Ethylenedioxy)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]propan-1-ol (22b). Compound **22b** was prepared according to the procedure used for **22a**. Starting from **17b** (20.75 g, 61.0 mmol), 17.88 g of a pale yellow oil was obtained with a GLC purity of 97% and immediately used in the next step. **22b**: ^{1}H NMR δ 3.97−3.80 (m, 8H), 3.58 (q, *J* = 5.0 Hz, 2H), 1.98−1.15 (m, 15H), 0.91 (s, 3H).

(+)-3-[(3a*S*)-(1*β*,3aα,4α,7a*β*)-5,5-(1,2-Ethylenedioxy)-7a-methyloctahydro-1-[(*tert*-butyldimethylsilyl)oxy]-1*H*-inden-4-yl]propanal (23a). To a solution of oxalyl chloride (1.8 g, 14.18 mmol) in CH_2Cl_2 (20 mL, anhydrous and ethanol free) was added dropwise DMSO (1.8 mL, 25.30 mmol) at −60 °C. After 5 min, a solution of **22a** (4.73 g, 12.3 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 30 min maintaining the mixture at −60 °C. The reaction was left at −60 °C under stirring for 25 min; then Et_3N (8 mL) was added over 10 min (−55 °C). After 5 min the cooling bath was removed, and the reaction mixture was left aside to return to room temperature. After addition of 100 mL of water, the mixture was extracted with CH_2Cl_2 (4 × 25 mL), washed with a saturated solution of NaCl, and dried over Na_2SO_4. Removal of the solvent *in vacuo* afforded 4.36 g (100%) of the crude aldehyde which was sufficiently pure to be used in the next step without further purification. Chromatography of a little portion (pentane/EtOAc, 5:1, R_f = 0.42) gave analytically pure **23a**: oil; $[\alpha]^{25}_D$ +14 (*c* 0.286, $CHCl_3$); IR 1722 cm^{-1}; ^{1}H NMR δ 9.69 (t, *J* = 1.6 Hz, 1H), 3.94−3.88 (m, 4H), 3.58 (t, *J* = 7.5 Hz, 1H), 2.58−2.47 (m, 2H), 1.80−1.28 (m, 12H), 0.83 (s, 9H), 0.76 (s, 3H), −0.03 (s, 6H); ^{13}C NMR δ 203.5 (d), 112.6 (s), 81.25 (d), 64.8 (t), 64.7 (t), 47.2 (d), 43.9 (d), 34.0 (t), 31.6 (t), 31.3 (t), 26.3 (q), 19.3 (t), 18.5 (s), 11.1 (q), −4.1 (q), −4.4 (q); MS *m/z* 382 (M$^+$, 0.14), 367 (1), 325 (29), 281 (8), 263 (19), 249 (13), 192 (19), 99 (100). Elemental anal. for $C_{21}H_{38}O_4Si$: C, H.

3-[(3a*S*)-(3aα,4α,7a*β*)-1,1-(1,2-Ethylenedioxy)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]propanal (23b). Compound **23b** was prepared according to the procedure used for **23a**. Starting from **22b** (17.58 g, 56.3 mmol) 17.25 g (95%) of an orange oil (GLC purity 88%) was obtained. An analytically pure sample of **23b** was obtained by chromatography of a little portion of the oil (pentane/EtOAc, 2:1, R_f = 0.33): $[\alpha]^{25}_D$ −16.5 (*c* 2.51, $CHCl_3$); IR 1721 cm^{-1}; ^{1}H NMR δ 9.69 (s, 1H), 3.96−3.80 (m, 8H), 2.55−2.46 (m, 2H), 1.98−1.17 (m, 12H), 0.89 (s, 3H); ^{13}C NMR δ 203.4 (s), 118.7 (s), 112.2 (s), 65.6 (t), 64.9 (t), 64.8 (t), 46.8 (d), 46.5 (s), 43.9 (d), 43.8 (t), 34.7 (t), 31.0 (t), 27.7 (t), 23.6 (t), 18.1 (t), 14.1 (q), 11.0 (t); MS *m/z* 310 (M$^+$, 0.25), 225 (2), 210 (3), 152 (5), 129 (6), 100 (33), 99 (100), 86 (34). Elemental anal. for $C_{17}H_{26}O_5$: C, H.

3-[(3a*S*)-(1*β*,3aα,4α,7a*β*)-1-(*N*-*tert*-Butylcarbamoyl)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]-propanal (23c). Ester **17c** (10.19 g, 26.7 mmol) was dissolved in 145 mL of anhydrous toluene, and the solution was cooled to −75 °C. Then a solution of DIBAL-H was added as follows: (1) 58.3 mL of a 0.56 M DIBAL-H solution (32.5 mmol) at a rate of 18 mL/h (the reaction mixture was worked up at this point, but not all the starting ester had reacted); (2) 14.0 mL of a 1 M DIBAL-H solution (14 mmol) at a rate of 10 mL/h; (3) 14.0 mL of a 1 M DIBAL-H solution (14 mmol) at a rate of 10 mL/h. The suspension was left to stir at −75 °C for 1 h before the cold bath was removed and it was allowed to return to room temperature. After filtering through Celite, the solution was dried over anhydrous Na_2SO_4, filtered again, and concentrated under vacuum. The resulting orange oil (10.25 g) was found to be a mixture of the desired aldehyde **23c** (27%) and the over-reduced alcohol **22c** (49%) by GLC analysis.

A Swern oxidation was carried out to convert the alcohol **22c** back to the aldehyde **23c**. A solution of oxalyl chloride (1.96 mL, 22.8 mmol) in anhydrous CH_2Cl_2 (45 mL) was cooled to −60 °C. DMSO (2.97 mL, 41.8 mmol) was added over 30 min and the solution allowed to stir for a further 15 min. A solution of the crude product from above (10.25 g, ∼19 mmol of alcohol **22c**) in CH_2Cl_2 (60 mL) was then added over 1.5 h. Stirring was continued for 30 min before Et_3N (13.2 mL, 95 mmol) was added dropwise over 45 min. After 15 min the solution was left to return to room temperature, then water (100 mL) was added, and the resulting two layers separated. The aqueous layer was extracted with CH_2Cl_3 (3 × 75 mL), and the combined organics were washed with saturated NaCl solution (200 mL). The organic layer was then dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give **23c** as an orange oil (8.96 g, 95.5% yield), immediately used in the next step without purification to avoid degradation: IR 3441, 2961, 2887, 2727, 1721, 1664 cm^{-1}; ^{1}H NMR δ 9.70 (m, 1H), 5.03 (s, 1H), 3.98−3.86 (m, 4H), 2.85−1.40 (m, 15H), 1.31 (s, 9H), 0.74 (s, 3H); ^{13}C NMR δ 202.9 (d), 171.4 (s), 111.7 (s), 64.4 (t), 52.7 (d), 52.1 (d), 51.1 (s), 43.7 (t), 43.5 (t), 43.3 (d), 35.1 (t), 30.9 (s), 28.9 (q), 24.9 (t), 23.6 (t), 19.1 (t), 12.4 (q); MS *m/z* 252 (13), 178 (7), 99 (100), 86 (45), 69 (38); R_f = 0.30 (petroleum ether/EtOAc, 1:1).

3-[(3a*S*)-(1*β*,3aα,4α,7a*β*)-5,5-(1,2-Ethylenedioxy)-7a-methyloctahydro-1-[(*tert*-butyldimethylsilyl)oxy]-1*H*-inden-4-yl]propanal Oxime (24a). Hydroxylamine hydrochloride (1.05 g, 14.5 mmol) was added to a solution of **23a** (4.34 g, 11.3 mmol) in pyridine (65 mL, freshly distilled from KOH), and the mixture was stirred for 18 h at room temperature. The mixture was concentrated *in vacuo*, and the residue was extracted with Et_2O (90 mL), washed with brine, and dried over Na_2SO_4. Removal of the solvent *in vacuo* afforded 4.69 g of the crude oxime **24a** as 2.5:1 mixture of *E*/*Z* diastereoisomers. Purification by chromatography (CH_2Cl_2/CH_3OH, 9:1, R_f = 0.56) afforded **24a** (3.95 g, 88%) as an *E*/*Z* mixture. One diastereoisomer was isolated in a small amount. **24a**: oil; IR 3591, 1601, 1462, 1388 cm^{-1}; ^{1}H NMR δ 7.37 (t, *J* = 6 Hz, 1H), 6.75 (br s, 1H), 3.98−3.91 (m, 4H), 3.57 (t, *J* = 7 Hz, 1H), 2.45 (m, 1H), 2.23 (m, 1H), 1.9−1.10 (m, 12H), 0.84 (s, 9H), 0.77 (s, 3H), −0.03 (s, 6H); ^{13}C NMR δ 152.5 (d), 112.2 (s), 80.8 (d), 64.5 (t), 64.4 (t), 47.0 (d), 43.7 (d), 43.5 (s), 33.6 (t), 31.1 (t), 30.0 (t), 29.0 (t), 25.8 (q), 24.2 (t), 23.8 (t), 18.0 (s), 10.6 (q), −3.6 (q), −3.9 (q); MS, *m/z* 397 (M$^+$, 0.31), 380 (0.2), 340 (20), 99 (100). Elemental anal. for $C_{21}H_{39}NO_4Si$: C, H, N.

3-[(3a*S*)-(3aα,4α,7a*β*)-1,1-(1,2-Ethylenedioxy)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]propanal Oxime (24b). The same procedure reported above for the preparation of **24a** was used. Starting from **23b** (17.02 g, 55.6 mmol), **24b** (17.31 g, 97%) was obtained as a dense, orange oil (mixture of *E* and *Z* isomers) sufficiently pure for the next step. **24b** (spectroscopic data refer to the *E*/*Z* mixture of diastereoisomers unless otherwise indicated) IR 3588 cm^{-1}; ^{1}H NMR δ 7.37 (m, 1H, *Z*), 7.06 (br s, 1H, *E*), 6.68 (m, 1H, *E*), 7.37−6.68 (m, 2H), 3.97−3.80 (m, 8H), 2.50−2.35 (m, 1H), 2.3−2.15 (m, 1H), 2.1−1.15 (m, 12H), 0.91 (s, 3H); ^{13}C NMR δ 153.4 (s), 152.7 (s), 118.3 (s), 111.7 (s), 65.2 (t), 64.6 (t), 46.8, 46.7, 46.0, 44.0, 43.7, 34.3, 30.7, 30.6, 29.0, 27.3, 24.5, 23.6, 23.3, 23.2, 23.1, 13.6 (q); MS *m/z* 325 (M$^+$, 4), 308 (30), 100 (44), 99 (100), 86 (53), 55 (39); R_f 0.31 (*n*-pentane/EtOAc, 1:1). Elemental anal. for $C_{17}H_{27}NO_5$: C, H, N.

(+)-6-[[(3a*S*)-(1*β*,3aα,4α,7a*β*)-5,5-(1,2-Ethylenedioxy)-7a-methyloctahydro-1-[(*tert*-butyldimethylsilyl)oxy]-1*H*-inden-4-yl]ethyl]-4-oxa-5-azaspiro[2.4]hept-5-ene (25a). Liquid methylenecyclopropane (2.46 g, 45.5 mmol) was transferred by cannula into a cold (−60 °C) solution of **24a** (3.76 g, 9.45 mmol, mixture of *E*/*Z* isomers) and Et_3N (0.232 mL, 1.7 mmol) in CH_2Cl_2 (20 mL), and the reaction flask was sealed. The temperature of the mixture was allowed to rise to 0 °C (ice bath), and a sodium hypochlorite solution (30 mL at 7%) was added dropwise over 1 h. After 3 days of stirring at room temperature, the mixture was extracted with CH_2Cl_2 (4 × 25 mL), and the organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent *in vacuo* afforded 3.76 g of a crude product (88%) which was sufficiently pure to be used directly in the next step. A little amount was purified by FCC (CH_2Cl_2/MeOH, 15:1, R_f = 0.48) affording pure **25a**: oil; $[\alpha]^{25}_D$ +13 (*c* 0.197, $CHCl_3$); IR 1601 cm^{-1}; ^{1}H NMR δ 3.94−3.88 (m, 4H), 3.58 (dd, *J* = 8.2, 6.6 Hz, 1H), 2.97 (m, AB system, 2H),

2.46−2.39 (m, 2H), 2.00−1.15 (m, 14H), 1.09 (m, 2H), 0.84 (s, 9H), 0.78 (s, 3H), 0.68 (m, 2H), −0.03 (s, 6H); ^{13}C NMR δ 160.2 (s), 112.1 (s), 80.7 (d), 64.5 (s), 64.4 (t), 64.3 (t), 46.9 (d), 43.6 (d), 43.4 (s), 42.0 (t), 33.6 (t), 31.0 (t), 30.8 (t), 27.8 (t), 25.7 (q), 24.2 (t), 23.4 (t), 17.9 (s), 11.5 (t), 11.4 (t), 10.6 (q), −4.6 (q), −4.9 (q); MS *m/z* 449 (M^+, 35), 404 (16), 392 (14), 99 (100). Elemental anal. for $C_{25}H_{43}NO_4Si$: C, H, N.

6-[[(3a*S*)-(3aα,4α,7a*β*)-1,1-(1,2-Ethylenedioxy)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]ethyl]-4-oxa-5-azaspiro[2.4]hept-5-ene (25b). The same procedure reported above for the preparation of **25a** was used. Starting from crude **24b** (17.31 g), **25b** (20.35 g, 100%) was obtained as a thick brown oil. This was not purified but used directly in the next step. **25b**: IR 2957, 2886, 1728 cm^{-1}; ^{1}H NMR δ 4.00−3.75 (m, 8H), 2.94 (m, 2H), 2.50−2.35 (m, 2H), 2.10−1.00 (m, 14H), 0.90 (s, 3H), 0.70−0.60 (m, 2H); ^{13}C NMR δ 160.4 (s), 118.2 (s), 111.7 (s), 65.1 (s), 64.6 (t), 64.5 (t), 46.5 (d), 46.0 (d), 43.6 (t), 42.0 (t), 34.3 (t), 30.5 (t), 27.7 (t), 27.3 (t), 23.2 (t), 13.6 (q), 11.6 (t), 11.5 (t); MS *m/z* 377 (M^+, 5), 100 (26), 99 (100), 86 (32), 55 (36).

6-[[(3a*S*)-(1*β*,3aα,4α,7a*β*)-1-(*N*-*tert*-butylcarbamoyl)-5,5-(1,2-ethylenedioxy)-7a-methyloctahydro-1*H*-inden-4-yl]-ethyl]-4-oxa-5-azaspiro[2.4]hept-5-ene (25c). The oxime **24c** was prepared as **24a**: starting from **23c** (8.96 g, 25.5 mmol), **24c** (9.19 g, 98%) was obtained as a green oil. **24c**: ^{1}H NMR δ 7.42−7.30 (m, 2H, *Z*), 6.74−6.60 (m, 1H, *E*), 5.03 (s, 1H), 3.98−3.82 (m, 4H), 2.58−1.35 (m, 15H), 1.31 (s, 9H), 0.74 (s, 3H); R_f = 0.25 (petroleum ether/ethyl acetate, 1:1).

This oil was directly used, with the same procedure reported above for **25a**, for the preparation of **25c**. Starting from crude oxime **24c** (8.76 g, 23.9 mmol), an orange oil (9.17 g) was obtained containing approximately 50% of the desired isoxazoline. This was of difficult purification and therefore used directly in the next step. **25c**: IR 3441, 2971, 2884, 1669, 1503 cm^{-1}; ^{1}H NMR δ 5.05 (s, 1H), 3.96−3.80 (m, 4H), 2.95−2.91 (m, 2H), 2.50−1.35 (m, 15H), 1.28 (s, 9H), 1.10−1.00 (m, 2H), 0.71 (s, 3H), 0.70−0.60 (m, 2H); ^{13}C-NMR δ 171.4 (s), 160.2 (s), 111.6 (s), 111.5 (s), 64.6 (t), 56.5 (d), 52.3 (d), 50.9 (s), 43.6 (t), 43.4 (d), 42.0 (t), 35.1 (t), 30.9 (s), 28.9 (q), 27.8 (t), 25.0 (t), 23.6 (q), 23.5 (q), 12.3 (q), 11.6 (t), 11.4 (t); MS *m/z* 418 (M^+, 7), 373 (4), 218 (3), 99 (100), 86 (23), 58 (11), 55 (19), 41 (30); R_f = 0.52 (EtOAc/petroleum ether, 1:1).

(+)-6-[[(3a*S*)-(1*β*,3aα,4α,7a*β*)-1-Hydroxy-7a-methyloctahydro-5-oxo-1*H*-inden-4-yl]ethyl]-4-oxa-azaspiro[2.4]-hept-5-ene (26a). To 363 mL of a solution prepared from 450 mL of acetone and 450 mg of concentrated H_2SO_4 was added a solution of **25a** (3.66 g, 8.14 mmol) in acetone (10 mL) and water (133 μL, 7.4 mmol). The reaction mixture was refluxed for 3.5 h. Then, after the mixture cooled to room temperature, solid Na_2CO_3 was added and the mixture was concentrated *in vacuo*. The residue was extracted with CH_2Cl_2 (4 × 50 mL), washed with brine, and dried (Na_2SO_4). Removal of solvent and chromatography (EtOAc, R_f = 0.48) afforded **26a** (1.826 g, 77%): oil; $[\alpha]^{25}_D$ +9 (*c* 0.076, $CHCl_3$); IR 3617, 1702, 1616 cm^{-1}; ^{1}H NMR δ 3.70 (t, *J* = 8.2 Hz, 1H), 2.99 (d, 2H), 2.49−1.38 (m, 14H), 1.09 (m, 2H), 1.05 (s, 3H), 0.69 (m, 2H); ^{13}C NMR δ 212.1 (s), 159.9 (s), 80.1 (d), 64.7 (s), 49.8 (d), 49.5 (d), 43.2 (s), 42.2 (t), 37.8 (t), 35.3 (t), 30.0 (t), 26.2 (t), 23.9 (t), 22.9 (t), 11.6 (t), 11.5 (t), 10.6 (q); MS *m/z* 291 (M^+, 100), 272 (22), 124 (39), 111 (78). Elemental anal. for $C_{17}H_{25}NO_3$: C, H, N.

6-[[(3a*S*)-(3aα,4α,7a*β*)-1,5-Dioxo-7a-methyloctahydro-1*H*-inden-4-yl]ethyl]-4-oxa-5-azaspiro[2.5]hept-5-ene (26b). Compound **26b** was prepared according to the procedure used for **26a**. Starting from crude **25b** (21.98 g, 58.2 mmol), after workup, filtration, and evaporation of the solvent, 19.11 g of a thick orange oil was obtained. This crude oil was purified by chromatography (CH_2Cl_2/EtOAc, 1:1, R_f = 0.65), affording **26b** (5.80 g, 40% yield from **24b**) as a white solid: mp 72−73 °C; $[\alpha]^{25}_D$ +85 (*c* 0.09, $CHCl_3$); IR 2968, 1730, 1706 cm^{-1}; ^{1}H NMR δ 2.94 (s, 2H), 2.57−1.50 (m, 14H), 1.09 (s, 3H), 1.02 (m, 2H), 0.64 (m, 2H); ^{13}C NMR δ 217.6 (s), 210.3 (s), 157.4 (s), 64.7 (s), 49.0 (d), 48.9 (d), 47.3 (s), 42.2 (t), 37.2 (t), 35.9 (t), 30.2 (t), 26.1 (t), 22.4 (t), 22.2 (t), 13.3 (q), 11.5 (t), 11.3 (t); MS *m/z* 289 (M^+, 26), 123 (16), 96 (17), 95 (16), 93 (17), 84 (19), 83 (29), 79 (37), 67 (39), 55 (100). Elemental anal. for $C_{17}H_{23}NO_3$: C, H, N.

6-[[(3a*S*)-(1*β*,3aα,4α,7a*β*)-1-(*N*-*tert*-Butylcarbamoyl)-5-oxo-7a-methyloctahydro-1*H*-inden-4-yl]ethyl]-4-oxa-5-azaspiro[2.4]hept-5-ene (26c). The same procedure reported above for the preparation of **26a** was used. Starting from crude ketal **25c** (9.17 g, 21.9 mmol), a dark orange thick oil (7.81 g) was obtained. Column chromatography using a large sinter funnel (150 g of silica gel) and eluting with CH_2Cl_2/EtOAc, 1:1, gave **26c** (R_f = 0.66) as an orange oil with sufficient purity for the next step (1.96 g, 24% yield): IR 3440, 2969, 1703, 1671 cm^{-1}; ^{1}H NMR δ 5.08 (s, 1H), 2.98 (s, 2H), 2.60−1.40 (m, 15H), 1.33 (s, 9H), 1.14−1.02 (m, 2H), 0.98 (s, 3H), 0.72−0.62 (m, 2H); ^{13}C NMR δ 211.6 (s), 170.8 (s), 159.7 (s), 111.6 (s), 111.5 (s), 56.2 (d), 54.1 (d), 51.1 (s), 49.6 (d), 43.6 (t), 42.1 (t), 37.8 (t), 37.0 (s), 28.8 (q), 26.0 (t), 25.1 (t), 23.6 (q), 23.5 (q), 12.3 (q), 11.6 (t), 11.4 (t); MS *m/z* 374 (M^+, 8), 318 (16), 240 (10), 93 (40), 79 (42), 67 (35), 58 (50), 57 (90), 55 (71), 42 (78), 41 (100).

(+)-17*β*-Hydroxy-10-azaestra-4,9(11)-dien-3-one (8). A solution of **26a** (461 mg, 1.49 mmol) in freshly distilled DMF (38 mL) was refluxed for 5 h under N_2. The solvent was removed by distillation *in vacuo* at 50−60 °C affording a brown residue (479 mg). Chromatography by a first elution with EtOAc gave **27a** and **28a** as a 1:4 mixture (both as $\Delta^{9(11)}$:$\Delta^{8(9)}$ isomers in 4:1 mixtures, R_f = 0.65, 101.3 mg, 25%). Then a second elution with EtOAc/MeOH (10:1) afforded **8** (10:1 mixture of $\Delta^{9(11)}$:$\Delta^{8(9)}$ isomers, 153 mg, 38%, R_f = 0.36). Further attempts to separate the two isomers by chromatography failed, but one fraction enriched in $\Delta^{9(11)}$ was obtained (EtOAc/MeOH, 10:1). The following data were obtained by analysis of the fractions enriched in the main isomer.

8 (22:1 mixture of $\Delta^{9(11)}$:$\Delta^{8(9)}$ isomers): yellow solid; mp 83−84 °C; $[\alpha]^{25}_D$ +402 (*c* 0.123, MeOH); IR 3616, 3440, 1618, 1547 cm^{-1}; ^{1}H NMR δ 5.04 (br d, *J* = 6.2 Hz, 1H), 4.93 (s, 1H), 3.93−3.55 (m, 3H), 2.7−1.2 (m, 16H), 0.8 (s, 3H); ^{13}C NMR δ 191.7 (s), 159.6 (s), 139.5 (s), 103.6 (d), 100.5 (d), 81.8 (d), 47.5 (d), 46.3 (t), 41.9 (s), 38.3 (d), 37.5 (t), 35.6 (t), 31.3 (t), 30.7 (t), 24.7 (t), 24.0 (t), 11.2 (q); MS *m/z* 273 (M^+, 100), 258 (15), 214 (49). Elemental anal. for $C_{17}H_{23}NO_2$: C, H, N.

28a (4:1 mixture with **27a**): yellow oil; IR 3615, 1600, 1555 cm^{-1}; ^{1}H NMR δ 11.8 (br s, 1H), 6.3 (dd, *J* = 17, 10 Hz, 1H), 6.10 (dd, *J* = 17, 2.3 Hz, 1H), 5.46 (dd, *J* = 10, 2.3 Hz, 1H), 5.15 (m, 1H), 5.02 (s, 1H), 3.78 (t, *J* = 8 Hz, 1H), 2.55−2.4 (m, 2H), 2.25−1.2 (m, 11H), 0.79 (s, 3H); ^{13}C NMR 186.2 (s), 160.2 (s), 139.5 (d), 135.4 (s), 123.2 (t), 107.3 (d), 95.14 (d), 81.7 (d), 46.1, 36.5, 36.2 (s), 30.6 (t), 28.8 (t), 23.4 (t), 23.2 (t), 11.1 (q).

(+)-10-Azaestra-4,9(11)-diene-3,17-dione (4). Isoxazoline **26b** (5.76 g, 19.9 mmol) was dissolved in freshly distilled DMF (275 mL). The solution was refluxed for 2 h, after which time TLC showed no remaining isoxazoline. The DMF was distilled off under reduced pressure, yielding 5.71 g of a thick oil (**4a**:**4b** ratio of 10:1, by ^{1}H NMR). Purification by Chromatospack apparatus was effected by eluting first with CH_2Cl_2/EtOAc, 1:1, and, when no further products were eluted, with acetone. The fractions containing **4** (R_f = 0.21, acetone, 1.624 g, 30%), **27b** (R_f = 0.69, acetone, 0.568 g, 11%), and **28b** (R_f = 0.67, CH_2Cl_2/EtOAc, 1:1, 1.407 g, 26%) were collected. A first recrystallization from EtOAc gave a **4a**:**4b** mixture in a ratio of 22:1. A second recrystallization afforded pure **4a**: mp 179−180 °C; $[\alpha]^{25}_D$ +536.4 (*c* 0.433, CH_2Cl_2); IR 2969, 1735, 1622, 1547 cm^{-1}; ^{1}H NMR δ 5.07 (t, *J* = 3.9 Hz, 1H), 4.95 (s, 1H), 3.95−3.80 (ddd, *J* = 12.9, 7.1, 4.0 Hz, 1H), 3.7−3.45 (dt, *J* = 12.9, 5.4 Hz, 1H), 2.7−1.9 (m, 11H), 1.75−1.55 (m, 2H), 1.45−1.2 (m, 1H), 0.93 (s, 3H); ^{13}C NMR δ 220.0 (s), 191.2 (s), 158.6 (s), 139.0 (s), 102.1 (d), 100.4 (d), 47.1 (d), 45.9 (t), 45.7 (t), 37.1 (d), 36.1 (t), 35.2 (t), 31.8 (t), 29.9 (t), 23.7 (t), 22.0 (t), 14.1 (q); MS *m/z* 271 (M^+, 100), 256 (38), 254 (74), 214 (35), 91 (22), 77 (20). Elemental anal. for $C_{17}H_{21}NO_2$: C, H, N.

27b: mp 250−252 °C dec; $[\alpha]^{25}_D$ +459.9 (*c* 0.477, CH_2Cl_2), 11:1 ratio of $\Delta^{9(11)}$:$\Delta^{8(9)}$; IR 3437, 1736, 1628, 1548 cm^{-1}; ^{1}H NMR δ 7.02 (t, *J* = 3.3 Hz, 1H), 5.12 (br s, 1H), 3.50−3.40 (m, 3H), 2.70−1.85 (m, 13H), 0.87 (s, 3H); ^{13}C NMR δ 222.3 (s), 191.1 (s), 160.5, 130.4 (s), 117.5 (d), 105.9 (s), 47.2 (d), 45.7 (t), 48.9 (t), 38.2 (d), 38.0 (s), 35.9 (t), 33.6 (t), 29.9 (t), 25.7 (t), 22.5 (t), 14.5 (q); MS *m/z* 271 (M^+, 100), 256 (86), 254 (34),

214 (55), 200 (23), 121 (20), 115 (22), 91 (25). Elemental anal. for $C_{17}H_{21}NO_2$: C, H, N.

28b: $[\alpha]^{25}{}_D$ +163.0 (*c* 0.925, CH_2Cl_2), 11:1 ratio of $\Delta^{9(11)}$:$\Delta^{8(9)}$; IR 2967, 1736, 1588, 1543, 1340, 1149 cm^{-1}; ^{1}H NMR δ 6.4–6.05 (m, 2H), 5.50–5.40 (m, 1H), 5.15 (br t, 1H), 5.01 (s, 1H), 2.60–1.10 (m, 12H), 0.93 (s, 3H); ^{13}C NMR δ 220.0 (s), 185.9 (s), 159.3 (s), 137.6 (d), 135.0 (s), 123.4 (t), 105.6 (d), 94.8 (d), 46.3 (d), 37.3 (s), 35.9 (t), 35.6 (d), 31.5 (t), 28.6 (t), 23.0 (t), 21.7 (t), 14.7 (q); MS *m/z* 271 (M$^+$, 43), 270 (100), 256 (6), 214 (9), 174 (7), 91 (10), 77 (8), 55 (12). Elemental anal. for $C_{17}H_{21}NO_2$: C, H, N.

(+)-17*β*-(*N*-*tert*-Butylcarbamoyl)-10-azaestra-4,9(11)-dien-3-one (10). Isoxazoline **26c** (1.96 g, 5.24 mmol) was dissolved in freshly distilled DMF (150 mL) and the solution refluxed for 3 h. The DMF was then removed by distillation under reduced pressure to give a dark orange oil (2.11 g) containing **10**, **27c**, and **28c** in a 1.3:1:1.3 ratio by ^{1}H NMR analysis of this crude reaction mixture. Purification was carried out by flash column chromatography eluting first with EtOAc, obtaining **27c** (R_f = 0.28, 276 mg, 15%) and **28c** (R_f = 0.55, 360 mg, 19%). A second elution with acetone afforded a mixture of **10a**:**10b** in a 5.5:1 ratio as a pale orange solid (R_f = 0.25, 360 mg, 19%). A recrystallization from EtOAc gave a **10a**:**10b** mixture in a 9:1 ratio, while from the mother liquors a 3.5:1 mixture of **10a**:**10b** was recovered.

The following data refer to the 9:1 mixture of **10a**:**10b**: mp 252 °C; $[\alpha]^{25}{}_D$ +381.08 (*c* 0.569, $CHCl_3$); IR 3440, 2965, 1707, 1662, 1550 cm^{-1}; ^{1}H NMR δ 5.13 (s, 1H), 5.05–4.97 (m, 1H), 4.90 (s, 1H), 3.90–3.76 (m, 1H), 3.70–3.50 (m, 1H), 2.70–1.35 (m, 15H), 1.32 (s, 9H), 0.71 (s, 3H); ^{13}C NMR δ 191.7 (s), 171.9 (s), 159.4 (s), 139.8 (s), 103.0 (d), 100.7 (d), 57.5 (d), 54.3 (t), 52.2 (d), 46.3 (t), 42.6 (s), 39.5 (s), 38.3 (d), 35.7 (t), 30.7 (t), 29.5 (t), 25.3 (q), 25.2 (t), 24.3 (t), 13.5 (q); MS *m/z* 356 (M$^+$, 39), 299 (6), 240 (13), 214 (100), 115 (12), 91 (14), 57 (18). Elemental anal. for $C_{22}H_{32}N_2O_2$: C, H, N.

27c ($\Delta^{9(11)}$ isomer, in a 3:1 mixture with $\Delta^{8(9)}$ isomer): ^{1}H NMR δ 7.22–6.98 (m, 1H), 5.11 (s, 1H), 4.93 (s, 1H), 3.51–3.37 (m, 2H), 2.70–1.40 (m, 17H), 1.32 (s, 9H), 0.66 (s, 3H).

28c ($\Delta^{9(11)}$ isomer, in a 3:1 mixture with $\Delta^{8(9)}$ isomer): ^{1}H NMR δ 6.40–6.06 (m, 2H), 5.48–5.42 (m, 1H), 5.19–5.01 (m, 3H), 2.63–1.61 (m, 16H), 1.34 (s, 9H), 0.72 (s, 3H).

(+)-3*β*-Hydroxy-10-azaestran-17-one (29). Pd/C (5%) (692 mg) was suspended in absolute EtOH (35 mL). A solution of dione **4** (697 mg, 2.57 mmol) in absolute EtOH (50 mL) was added *via* syringe, and under a hydrogen atmosphere, the mixture was left to stir at room temperature until no further traces of starting diketone were observed (26 h). After filtering off the catalyst through a pad of Celite, distillation of the solvent under reduced pressure yielded **29** (555 mg, 72% yield) as a thick oil, which was then purified by chromatography (acetone, 1% Et_3N, R_f = 0.50): $[\alpha]^{25}{}_D$ +96.6 (*c* 1.26, CH_2Cl_2): IR 3609, 2936, 2856, 2799, 1734 cm^{-1}; ^{1}H NMR δ 3.65–3.50 (br m, 1H), 3.33–3.25 (br m, 1H), 2.52–2.38 (m, 1H), 2.2–1.20 (m, 20H), 0.90 (s, 3H); ^{13}C NMR δ 220.4 (s), 69.0 (d), 67.9 (d), 61.7 (d), 49.6 (t), 49.2 (d), 47.4 (s), 42.3 (t), 38.9 (d), 35.9 (t), 34.7 (t), 32.4 (t), 30.3 (t), 28.0 (t), 25.0 (t), 21.2 (t), 13.8 (q); MS *m/z* 277 (M$^+$, 18), 262 (32), 249 (10), 192 (50), 179 (14), 153 (100). Elemental anal. for $C_{17}H_{27}NO_2$: C, H, N.

3*β*-Hydroxy-17*β*-(*N*-*tert*-butylcarbamoyl)-10-azaestrane (30). Pd/C (5%) (100 mg) was suspended in absolute EtOH (10 mL). A solution of the ketone **10** (80 mg, 0.23 mmol) in absolute EtOH (10 mL) was syringed into the flask with stirring. The flask was then placed under hydrogen atmosphere and left to stir at room temperature for 18 h. The suspension was filtered through a pad of Celite and the solvent removed to give **30** as a pale green gummy solid (72 mg, 89%): IR 3440, 3400, 2965, 2935, 2880, 1663 cm^{-1}; ^{1}H NMR δ 5.05 (s, 1H), 3.75–3.50 (m, 2H), 2.70–1.40 (m, 23H), 1.31 (s, 9H), 0.75 (s, 3H); ^{13}C NMR δ 172.0 (s), 69.9 (d), 58.6 (d), 57.4 (d), 54.3 (d), 51.6 (t), 43.7 (d), 41.4 (s), 40.2 (t), 38.9 (s), 37.6 (t), 33.8 (d), 31.8 (t), 31.4 (t), 29.5 (q), 28.9 (t), 25.0 (t), 24.2 (t), 24.0 (t), 13.5 (q); MS *m/z* 362 (M$^+$, 44), 262 (41), 192 (84), 153 (100), 91 (14), 57 (51); R_f = 0.35 (eluant acetone). Elemental anal. for $C_{22}H_{38}N_2O_2$: C, H, N.

10-Azaestrane-3,17-dione (7). Oxalyl chloride (0.22 mL, 2.4 mmol) was added *via* syringe to anhydrous CH_2Cl_2 (3 mL). The temperature was brought to −60 °C, and DMSO (0.32 mL, 4.4 mmol) was added over a period of 5 min, maintaining the temperature at −60 °C. A solution of **29** (555 mg, 2.0 mmol) in CH_2Cl_2 (3 mL) was then added *via* syringe over 20 min. After 15 min, Et_3N (1.5 mL, 10 mmol) was added *via* syringe over a period of 10 min. After a further 5 min the low-temperature bath was removed and the reaction vessel allowed to warm up to room temperature, whereupon water (10 mL) was added. The two phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (4 × 15 mL). The combined organic solution was then washed with saturated NaCl (2 × 50 mL) and dried over anhydrous Na_2SO_4. Removal of the solvent by distillation under reduced pressure yielded **7** as a brown thick oil (515 mg, 92%), which was sufficiently pure for the next reaction: IR 2937, 2860, 2801, 1735, 1712 cm^{-1}; ^{1}H NMR δ 3.62–3.51 (ddd, *J* = 11.4, 6.5, 2.4 Hz, 1H), 2.70–1.20 (m, 20H), 1.15–0.94 (m, 1H), 0.92 (s, 3H); ^{13}C NMR δ 220.2 (s), 208.6 (s), 68.4 (d), 62.9 (d), 50.8 (t), 49.1 (d), 48.5 (t), 47.5 (s), 41.3 (t), 39.3 9d), 35.9 (t), 33.4 (t), 30.4 (t), 27.7 (t), 25.8 (t), 21.2 (t), 13.8 (q); MS *m/z* 275 (M$^+$, 28), 260 (33), 247 (14), 232 (22), 218 (17), 190 (92), 177 (20), 151 (100), 122 (29), 108 (25), 91 (28), 79 (41), 55 (36), 41 (83). Elemental anal. for $C_{17}H_{25}NO_2$: C, H, N.

17*β*-(*N*-*tert*-Butylcarbamoyl)-10-azaestran-3-one (31). Freshly distilled oxalyl chloride (41.2 μL, 0.48 mmol) was dissolved in anhydrous CH_2Cl_2 (1 mL). The solution was then cooled to −65 °C, and freshly distilled DMSO (62.4 μL, 0.88 mmol) was added. Stirring was continued while **30** (65 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (2 mL) was added over a period of 10 min. After 5 min, Et_3N (278 μL, 2.0 mmol) was added. The solution was stirred for 4 min before the cold bath was removed and the solution allowed to return to room temperature. At this point, water (6 mL) was added and the two layers separated, the aqueous one being extracted with CH_2Cl_2 (3 × 8 mL). The combined organics were then washed with saturated NaCl solution before the solvent was removed under vacuum. The resulting oily orange solid (47 mg, 73%) was found to be the desired ketone **31**: IR 3440, 2963, 2930, 2858, 2802, 1840, 1795, 1742, 1663 cm^{-1}; ^{1}H NMR δ 5.06 (s, 1H), 3.64–3.48 (m, 1H), 3.16–2.98 (m, 1H), 2.70–1.40 and 1.25–0.80 (m, 21H), 1.31 (s, 9H), 1.73 (s, 3H); ^{13}C NMR δ 208.3 (s), 172.1 (s), 63.5 (d), 57.7 (d), 54.4 (d), 51.6 (t), 51.0 (t), 48.6 (d), 43.9 (s), 41.4 (t), 39.9 (s), 37.9 (d), 33.6 (t), 30.1 (t), 29.5 (q), 29.2 (t), 26.6 (t), 24.3 (t), 24.0 (t), 13.5 (q); R_f = 0.40 (acetone). Elemental anal. for $C_{22}H_{36}N_2O_2$: C, H, N.

(+)-10-Azaestr-4-ene-3,17-dione (5). Diketone **7** (247 mg, 0.90 mmol) was dissolved in 5% aqueous acetic acid (20 mL). Then, solid mercuric acetate (1.146 g, 3.60 mmol) and tetrasodium ethylenediaminetetraacetate (1.497 g, 3.60 mmol) were added, and the solution was heated to 85 °C and left at this temperature for 2 h. Upon cooling, the solid formed was filtered off and washed with more 5% aqueous acetic acid. The filtrate was extracted with CH_2Cl_2 (3 × 25 mL) and the organic phase washed with water (2 × 75 mL) and dried over anhydrous Na_2SO_4. Removal of the solvent by distillation under reduced pressure yielded 94.3 mg of an orange/brown oil. Purification by flash column chromatography led to the isolation of two fractions: **5** (R_f = 0.14, 103 mg, 42%) and **6** (R_f = 0.54, 9.8 mg, 5%), respectively (eluant EtOAc/MeOH, 19:1, 1% Et_3N).

5: brownish solid; mp 204–205 °C; $[\alpha]^{25}{}_D$ +281.2 (*c* 0.1345, CH_2Cl_2); IR 2945, 1738, 1617, 1545, 1258, 1240, 1153 cm^{-1}; ^{1}H NMR δ 4.95 (s, 1H), 3.77–3.66 (ddd, *J* = 12.2, 5.8, 3.5 Hz, 1H), 3.21–3.04 (ddd, *J* = 13.9, 12.2, 5.2 Hz, 1H), 2.70–1.20 (m, 18H), 0.97 (s, 3H); ^{13}C NMR δ 218.9 (s), 191.6 (s), 163.9 (s), 100.8 (d), 64.4 (d), 48.4 (d), 47.6 (s), 45.8 (t), 38.8 (d), 36.0 (t), 35.8 (t), 30.7 (t), 29.9 (t), 26.7 (t), 23.4 (t), 20.9 (t), 13.9 (q); MS *m/z* 273 (M$^+$, 100), 258 (16), 244 (20), 188 (76), 175 (28), 149 (75), 121 (17). Elemental anal. for $C_{17}H_{23}NO_2$: C, H, N.

6: $[\alpha]^{25}{}_D$ −11.6 (*c* 0.13, $CHCl_3$); IR 2942, 1737, 1627, 1582, 1250 cm^{-1}; ^{1}H NMR δ 7.18 (d, *J* = 7.8 Hz, 1H), 4.98 (d, *J* = 7.8 Hz, 1H), 2.90–1.10 (m, 18H), 0.93 (s, 3H); MS *m/z* 273 (M$^+$, 100), 258 (15), 244 (20), 188 (67), 175 (27), 149 (66).

17*β*-(*N*-*tert*-Butylcarbamoyl)-10-azaestr-4-en-3-one (11). The crude ketone product **31** was carefully dissolved in acetic acid (0.5 mL). Water (2.5 mL) was then slowly dropped into

the stirring solution until the product started to precipitate out of the solution. This gave an orange 20% aqueous acetic acid solution to which were added mercuric acetate (260 mg, 0.8 mmol) and tetrasodium ethylenediaminetetraacetate (330 mg, 0.8 mmol). The suspension was warmed to 85 °C and left to stir at this temperature for 2 h. Upon cooling, the suspension was extracted with CH_2Cl_2 (4 × 10 mL), and the combined organics were washed with water (2 × 15 mL). The resulting orange liquid was filtered through a small quantity of anhydrous Na_2SO_4, washing it thoroughly with further CH_2Cl_2 to ensure no product loss. Removal of solvent under vacuum resulted in an oily orange solid (59.7 mg). Purification by flash column chromatography (EtOAc/MeOH, 19:1, 1% Et_3N, R_f = 0.22) afforded **11** (17 mg, 36%): white solid; mp 271−272 °C; $[\alpha]^{25}_D$ +114 (*c* 0.05, $CHCl_3$); IR ($CDCl_3$) 2982, 2903, 1818, 1794, 1616, 1548, 1468, 1380 cm^{-1}; ^1H NMR δ 5.07 (s, 1H), 4.92 (s, 1H), 3.76−3.62 (m, 1H), 3.20−3.02 (m, 1H), 2.70−1.40 and 1.26−1.18 (m, 18H), 1.33 (s, 9H), 0.77 (s, 3H); ^{13}C NMR δ 228.8 (s), 192.5 (s), 165.3 (s), 101.6 (d), 65.6 (d), 58.1 (d), 54.3 (t), 52.2 (d), 46.7 (s), 44.6 (t), 40.2 (s), 38.0 (d), 37.1 (t), 32.0 (t), 30.0 (q), 28.4 (t), 25.6 (t), 24.7 (t), 24.5 (t), 14.3 (q); MS *m*/*z* 358 (M^+, 100), 329 (14), 258 (24), 188 (44), 149 (87), 121 (22), 57 (34). Elemental anal. for $C_{22}H_{34}N_2O_2$: C, H, N.

17β-Hydroxy-10-azaestr-4-en-3-one (9). Compound **5** (40.9 mg, 0.15 mmol) was dissolved in a 1:1 *i*-PrOH/EtOH mixture (5 mL). The solution was cooled to below 0 °C, and $NaBH_4$ (10.2 mg, 0.27 mmol) dispersed in the solvent mixture (7 mL) was added dropwise over 30 min. The reaction mixture was then left to stir for 15 min, then the ice/salt bath was removed, and when at room temperature, the reaction mixture was left to stir for 10 min. An ice/water mixture (6 mL) was then added, the solution extracted with CH_2Cl_2 (3 × 10 mL), and the organic phase dried overnight over anhydrous Na_2SO_4. Filtration and removal of the solvent by distillation under reduced pressure afforded 33.8 mg (80% yield) of a yellow solid, **9**: $[\alpha]^{21}_D$ +173.0 (*c* 0.80, $CHCl_3$); IR 3617, 2959, 1613, 1542, 1251, 1240 cm^{-1}; ^1H NMR δ 4.89 (s, 1H), 3.80−3.60 (m, 2H), 3.20−3.00 (ddd, 1H, J = 13.9, 12.6, 5.2 Hz, 1H), 2.65−1.05 (m, 17H), 0.81 (s, 3H); ^{13}C NMR δ 191.4 (s), 164.6 (s), 100.1 (d), 80.8 (d), 64.6 (d), 48.3 (d), 45.6 (t), 43.0 (s), 39.2 (d), 35.9 (t), 34.9 (t), 30.8 (t), 30.6 (t), 27.1 (t), 24.0 (t), 22.4 (t), 11.2 (q); MS *m*/*z* 275 (M^+, 33), 246 (10), 188 (85), 149 (60), 121 (69), 119 (79), 88 (100), 82 (100), 46 (100); R_f = 0.12 (EtOAc/MeOH, 9:1). Elemental anal. for $C_{17}H_{25}NO_2$: C, H, N.

Inhibition of Human Prostatic 5α-Reductase 2. Assay for steroid 5α-reductase inhibition was performed on prostatic homogenates from surgically derived benign hyperplastic tissue according to the reported methods.[37] Tissue was stored at −80 °C until used. All procedures were carried out as near as possible to 0 °C. Weighed tissue was cut into small pieces with scissors and homogenized in a Potter homogenizer after addition of 5 vol (w/v) of 50 mM TRIS buffer at pH 7.4 containing NaCl (61 mM), KCl (1.5 mM), $MgCl_2 \cdot 6H_2O$ (1 mM), nicotinamide (15 mM), fumaric acid (1 mM), and sucrose (0.25 mM). The homogenate was centrifuged at 200*g* for 10 min. After separation of the supernatant, the pellet was washed with 1.4 vol (w/v) of the buffer and centrifuged as before. Combined supernatants constituted the "prostate juice"[37b] and were used for the enzymatic assay. Protein content of the various homogenates was determined using the method of Bradford with bovine serum albumin as standard.

For the enzymatic conversion assay the amount of homogenate containing 0.1 mg of protein was added to a preincubated mixture (5 min at 37 °C) of 30 nM testosterone [containing 9% of [1,2,6,7-$^3H_4$]testosterone (Amersham)] and 0.3 mM NADPH in a final volume of 0.4 mL of TRIS buffer at pH 7.4. After 30 min at 37 °C of incubation, the reaction was terminated by addition of 3.5 mL of ethyl acetate. The samples were frozen at −20 °C, and the ethyl acetate layer was separated from the frozen aqueous phase and then dried under nitrogen. Each dry extract was supplemented with 200 μg of testosterone and 200 μg of dihydrotestosterone as carriers, and T and DHT were separated by TLC (20 × 20 cm, 0.25 mm; Merck) using dichloromethane/diethyl ether, 85:15, as eluant. The TLC plates were sprayed with an aqueous solution of TNS (2-*p*-toluidinylnaphthalene-6-sulfonic acid) to identify the DHT lane. Silica gel lanes containing each steroid were scraped and extracted with 2 mL of methanol for 30 min. The methanol extract was added to 5 mL of liquid scintillation cocktail (Instagel, Packard) and counted with a liquid scintillation counter (Betamatic V., Kontron instruments). Percentage conversion (C%) was calculated as follows: C% = DHT counts/(T + DHT) counts × 100. Each determination was performed in duplicate. The CV% in the determination of conversion in different experiments with different homogenates was estimated to be 10−20%.

In the inhibiton experiments the inhibitor was added together with the substrate at the beginning of the assay. For the determinations of the IC_{50} values the concentrations ranged between 10^{-10} and 10^{-5} M for the 10-azasteroids and between 10^{-10} and 10^{-7} M for finasteride. For the K_i determinations the apparent K_m and V_{max} of the enzyme were calculated at different concentrations of inhibitor: 0, 2.5, and 7.5 nM for finasteride and 0, 2.5, and 7.5 μM for compound **6**. Testosterone concentration ranged from 2 to 100 nM for each curve.

Inhibition of Human Prostatic 5α-Reductase 1. The assay was performed according to the reported method[51] with some modifications. DU-145 cells (ATCC, HTB81, human prostatic carcinoma metastasis to brain) between passages 60 and 90 in culture were used. The cells were plated in Falcon 24-well plates at a density of 100 000 cells/well and allowed to become adherent for a period of 24 h. Compounds to be tested were dissolved in ethanol and diluted with MEM without phenol red. 10-Azasteroids and finasteride were tested at five doses ranging from 10^{-9} to 10^{-5} M for measurement of IC_{50} values. The compounds and 5 nM [^{3}H]androstenedione (Amersham) were added to the sample wells in a final volume of 1 mL of medium. Following a 6 h incubation period in 5% CO_2 and 95% air at 37 °C, the media were extracted with 3.5 mL of ethyl acetate and samples processed as described above for the 5αR-2 assay, using androstenedione and androstanedione as carriers. Results are expressed as a percentage of the amount of androstanedione formed with respect to the sum of androstanedione formed and remaining androstenedione. With an incubation time of 6 h only very small amounts of other metabolites were formed.

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