19-nor-10-azasteroids, a new class of steroid 5a-reductase inhibitors. 2. X-ray structure, molecular modeling, conformational analysis of

19-Nor-10-azasteroids, a New Class of Steroid 5α-Reductase Inhibitors. 2.[1] X-ray Structure, Molecular Modeling, Conformational Analysis of 19-Nor-10-azasteroids and Comparison with 4-Azasteroids and 6-Azasteroids

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Received May 7, 1997[⊗]

19-Nor-10-azasteroids are a new class of 5α-reductase inhibitors whose activity depends on the presence of the bridgehead N-10 atom conjugated with the 4-en-3-one moiety in the A ring. The X-ray structure of 19-nor-10-azasteroid **1** has been determined and it is compared with the X-ray structure of testosterone. A complete conformational analysis of these compounds has been performed, determining the number and energy of the possible conformers, as well as the molecular flexibility of the 10-azasteroidal skeleton. Thus, MM2* molecular mechanics calculations and AM1 semiempirical energy refinements revealed that 19-nor-10-azasteroids **1**−**3** have four possible conformations with very small energy differences and that they are very flexible molecules. The conformational analysis has been extended to testosterone (**4**), which also showed conformational flexibility, with three different conformations, and to 6-azasteroid **5** and 4-azasteroid **6**, for which only two thermally accessible conformations have been found. Compared to 19-nor-10-azasteroids **1**−**3**, azasteroids **5** and **6** appear to be more rigid structures. By a best fit analysis of all conformers of **1**−**5** with the global minimum of testosterone (**4-I**) it has been found that the lowest energy conformers of **1**, **3**, and **5** are very close to the structure of **4-I**, and among the conformers of **2**, the best similarity has been observed for the highest energy conformer **2-IV**.

Introduction

The reduction of testosterone (T) to dihydrotestosterone (DHT) in several human tissues is catalyzed by two NADPH-dependent isoenzymes of steroid 5α-reductase (5αR), named type 1 and type 2 (5αR-1 and 5αR-2).[2] The blockade of the formation of DHT by using selective inhibitors of 5αR has recently made possible a new therapeutic approach to the pharmacological treatment of prevalent human diseases such as benign prostatic hyperplasia (BPH), acne, male pattern baldness, and alopecia.[3] Therefore significant research has been carried out to find new potent and selective inhibitors of 5αR-1 and -2.[4]

Recently, we have described the synthesis and biological evaluation of a series of 19-nor-10-azasteroids, a new class of inhibitors for the human steroid-5α-reductases.[1] The inhibitory potency of these compounds, toward 5αR-1 and 5αR-2, was governed by the presence and position of a double bond in the C ring, as well as by the type of substituent at the 17 position. Moreover, the presence in their skeleton of the bridgehead nitrogen atom at position 10, conjugated with the 3-oxo group through the C-4, C-5 double bond, was an essential feature to maintain the inhibitory activity, and in fact, the 19-nor-10-azasteroids lacking in the C-4═C-5 bond were inactive toward the enzyme. Ab initio calculations on tricyclic models[1] indicated that this particular arrangement of atoms could increase the nucleophilic character of the carbonyl group with respect to testosterone, thus causing a stronger interaction with the electrophilic residues present in the 5αR active site.[1,4] However, this electronic effect alone did not explain the difference in the inhibitory potency observed for the $\Delta^{9(11)}$ and $\Delta^{8(9)}$ regioisomers of the most active compounds, for instance, those having a 17*β*-*N*-*tert*-butylcarbamoyl substituent, or the decrease of activity when the double bond in the C ring was absent.[1]

A conformational study on the novel 19-nor-10-azasteroid inhibitors should be the first step in the search of a rationale for the inhibition potency changes mentioned above. Therefore, in this paper we report on the X-ray structure determination of azasteroid **1** and on a molecular modeling study—performed on compounds **1**−**3** (Figure 1)—both aimed at defining the conformational changes, in the steroidal skeleton, determined by the presence of the bridgehead N-10 atom conjugated with two double bonds in the A and C rings (compounds **1** and **2**) or with a single double bond in the A ring (compound **3**). As a reference steroidal structure, we have chosen testosterone (**4**), the natural substrate of 5α-reductase, and thus, the same molecular modeling

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[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1997.

S0022-2623(97)00297-5 CCC: $14.00

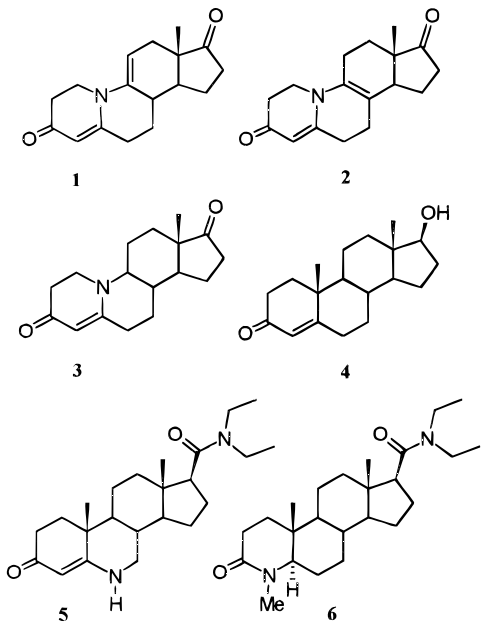


Figure 1.

approach used for compounds **1**−**3** has been extended to **4**. The modeling has consisted of the MM2* energy minimization[5] of each compound, followed by a complete Monte Carlo conformational search, and a final AM1 geometry optimization of the conformers found.

Then, in order to evaluate the backbone conformational differences between 19-nor-10-azasteroids **1**−**3** and other classes of azasteroidal inhibitors, for instance 6- and 4-azasteroids, the same analysis has been applied to compounds **5**[6] and **6**[7] (Figure 1).

The X-ray structure of **1** and the results of the conformational analysis on compounds **1**−**6** are discussed and, finally, the possible bioactive conformations of 19-nor-10-azasteroids are proposed.

Results and Discussion

Synthesis of 19-Nor-10-azasteroids. The strategy used for the synthesis of 19-nor-10-azasteroids always produced these compounds as mixtures of two different isomers having a double bond at the 9(11) or 8(9) position (Scheme 1).[1,8] Usually the 9(11) isomer predominated over the 8(9) isomer, resulting from kinetic control of the reaction. Moreover, the major isomer was also the thermodynamically favored one, since it was predominant after equilibration of the mixtures under basic catalysis. Owing to this equilibration, it was very difficult to obtain a pure single isomer and only in the case of compound **1** this was possible. Thus, the 10:1 reaction mixture of compounds **1** and **2**, after two crystallizations from ethyl acetate, provided pure compound **1**, which, dissolved again in the same solvent, gave crystals suitable for X-ray determination after slow concentration. Compound **1** was also used as a starting material for the synthesis of azasteroid **3**.[1]

Structure Description and Comparison. The description of the steroid conformers is made according to the method reported by Bucourt et al. in their study on the A/B ring fusion of testosterone.[9] With this method, the sign and value of torsion angles are reported inside the cycles, and a quasi-trans and quasi-cis fusion of A and B rings is defined on the basis of the discordance or concordance of the torsional angle signs around the fusion bond (Figure 2).

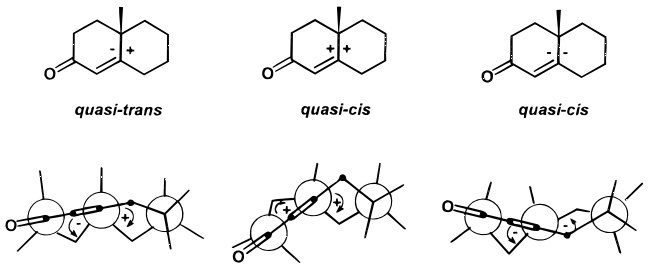


Figure 2. Quasi-cis and quasi-trans A/B ring fusions of steroids.

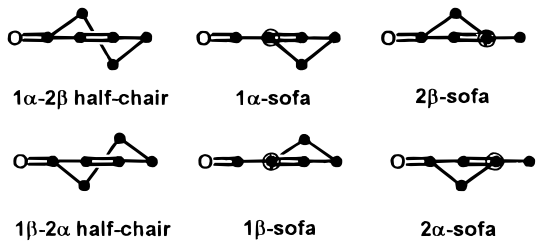
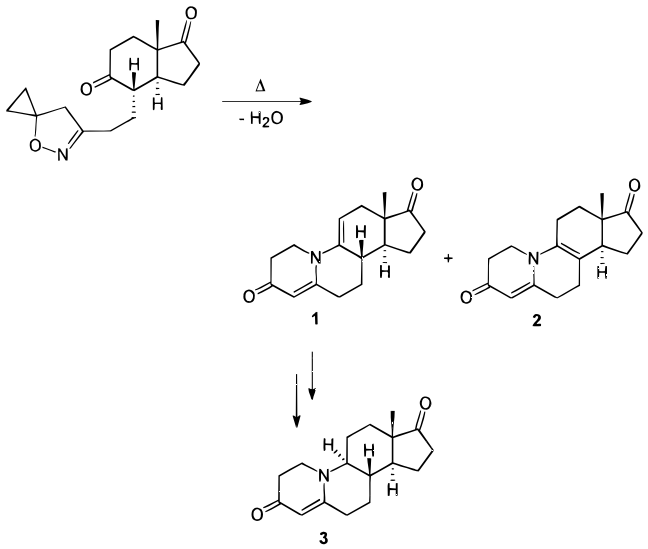


Figure 3. A ring type of steroids defined by the distances of C-1 and C-2 from the C-3, C-4, C-5, and C-10 mean plane.

Scheme 1



Furthermore, because the natural substrates of the enzyme 5α-reductase are 4-en-3-one steroids, we examine the differences in the distorsion of the A ring between the conformers of one substrate (testosterone) and the 10-, 4-, and 6-azasteroid inhibitors with the same approach as Duax et al.[10] They used the distance of C-2 from the C-3, C-4, C-5, and C-10 mean plane as a sensitive measure of the A ring conformation: C-2 is above this plane (positive value of the distance) when the A ring has the 1α−2*β* or 2*β*−sofa conformation, in the plane (distance very close to 0 Å) when the A ring has the 1α−sofa or 1*β*−sofa conformation, and below the plane (negative value of the distance) when the A ring has 1*β*−2α or 2α−sofa conformation (Figure 3).

Duax et al. observed that in most of the crystallographic structures of natural steroids having the 4-en-3-one moiety, the A ring has a conformation ranging from the ideal 1α−2*β* half chair to the ideal 1α−sofa.[10] In these "normal" conformations the C-1 atom is in α position with respect to the plane. However, the same authors pointed out that "inverted" conformations (i.e. with the C-1 in *β* position) are often observed in

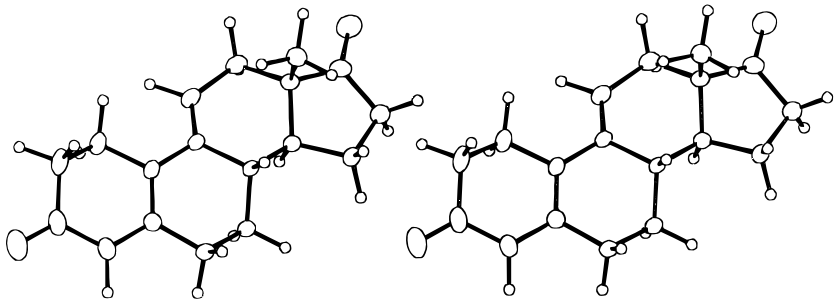


Figure 4. Stereoplot of the crystal structure of **1**.

Table 1. Selected Conformational Data of the Crystallographic Structure of **1** (**1**-X-ray) and the Predicted Conformers of 10-Azasteroids **1**−**3**

entry	structure	A/B ring fusion	A ring type	B ring type	H_f[a] (kcal/mol)	ΔE (kcal/mol)	rms (Å) with X-ray	torsion (deg)[b] O-3=C-3−C-4=C-5		distance from C-3, C-4, C-5, N-10 plane (Å)[c] C-1	C-2	C-6	C-7
					$\Delta^{9(11)}$-19-Nor-10-azasteroid **1**								
1	**1**-X-ray	*q.-trans*	1α−2β	6β−7α	−	−	−	−177.8(4)	(1.87)	−0.319 (4)	0.291 (4)	0.224 (3)	−0.208 (3)
2	**1-I**	*q.-trans*	1α−2β	6β−7α	−53.855	0	0.119	169.66	(10.34)	−0.20	0.33	0.11	−0.47
3	**1-II**	*q.-trans*	2α−sofa	7α−sofa	−52.930	0.925	0.298	−165.32	(14.68)	0.03	−0.44	0.05	−0.36
4	**1-III**	*q.-trans*	1α−2β	7β−sofa	−52.182	1.673	0.291	174.64	(5.36)	−0.39	0.15	0.01	0.89
5	**1-IV**	*q.-trans*	1β−2α	7β−sofa	−51.160	2.695	0.328	−172.36	(7.64)	0.30	−0.23	0.06	0.99
					$\Delta^{8(9)}$-19-Nor-10-azasteroid **2**								
6	**2-I**	*q.-trans*	1β−2α	7β−sofa	−53.406	0		−169.55	(10.45)	0.22	−0.31	0.09	0.74
7	**2-II**	*q.-trans*	1β−2α	7α−sofa	−53.344	0.062		−172.14	(7.86)	0.25	−0.28	0.04	−0.60
8	**2-III**	*q.-trans*	1α−2β	7β−sofa	−53.320	0.086		172.27	(7.73)	−0.29	0.25	−0.01	0.60
9	**2-IV**	*q.-trans*	1α−2β	7α−sofa	−53.307	0.099		169.89	(10.11)	−0.23	0.30	0.08	−0.77
					19-Nor-10-azasteroid **3**								
10	**3-I**	*q.-trans*	1α−2β	6β−7α	−74.280	−		170.00	(10.00)	−0.25	0.28	0.11	−0.58
11	**3-II**	*q.-trans*	1β−2α	7α−sofa	−73.471	0.809		−169.21	(10.79)	0.15	−0.36	0.06	−0.26
12	**3-III**	*q.-trans*	1β−2α	7β−sofa	−73.113	1.167		−173.38	(6.62)	0.38	−0.15	−0.03	1.12
13	**3-IV**	*q.-trans*	1α−2β	7β−sofa	−72.230	2.050		−173.64	(6.36)	−0.36	0.15	−0.05	0.78

[a] Calculated on AM1 energy-minimized structures through Spartan software. [b] The deviation from planarity (180°) is reported in brackets. [c] This is a mean plane.

Table 2. Selected Conformational Data of the Crystallographic Structure of Testosterone (**4**) and the Predicted Conformers of **4**−**6**

entry	structure	A/B ring fusion	A ring type	B ring type	H_f[a] (kcal/mol)	ΔE (kcal/mol)	rms (Å) with X-ray	torsion (deg)[b] O-3=C-3-X-4=C-5[d]		distance from C-3, X-4, C-5, C-10 plane (Å)[c,d] C-1	C-2	Y-6[d]	C-7
					Testosterone (**4**)								
1	**4**-X-ray	*q.-trans*	1α−2β	6β−7α	−	−	−	−179.29	(0.71)	−0.49	0.16	0.19	−0.84
2	**4-I**	*q.-trans*	1α−2β	7α−sofa[e]	−115.584	0	0.098	172.38	(7.62)	−0.42	0.24	0.09	−0.93
3	**4-II**	*q.-cis*	1β−2α	6α−7α	−113.215	2.369	0.384	−167.49	(12.51)	0.26	−0.43	−0.21	−1.54
4	**4-III**	*q.-trans*	1α−2α	6α−7β	−112.14	3.444	0.304	−174.10	(5.9)	−0.72	−0.19	−0.14	0.43
					6-Azasteroid **5**								
5	**5-I**	*q.-trans*	1α−sofa	6β−7α	−96.790	0		179.77	(0.23)	−0.61	0.02	0.12	−0.57
6	**5-II**	*q.-trans*	2α−sofa	7α−sofa	−91.482	5.31		−159.37	(20.63)	0.07	−0.58	0.08	−0.90
					4-Azasteroid **6**								
7	**6-I**	*q.-trans*	1α−2α	6β−sofa	−119.022	0		168.76	(11.24)	−0.77	−0.36	0.61	0.09
8	**6-II**	*q.-trans*	1α−2α	6β−7α	−118.025	0.997		−172.61	(7.39)	−0.65	−1.05	0.38	−0.21

[a] Calculated on AM1 energy-minimized structures through Spartan software. [b] The deviation from planarity (180°) is reported in brackets. [c] This is a mean plane. [d] X = Y = C for compound **4**; X = C, Y = N for compound **5**; X = N, Y = C for compound **6**. [e] The 6β−7α conformation should be more appropriate (see text).

crystallographic structures of semisynthetic steroids, in particular those having additional double bonds in the B or C ring or a 19-nor backbone. Since 19-nor-10-azasteroids have all these features, we have considered useful the application of the same conformational description reported in Figure 3, that is, the distances of C-1 and C-2 from the C-3, C-4, C-5, and N-10 plane, as well as the extension of this approach to the B ring description by considering the sign and value of the distances of C-6 and C-7 from the same mean plane.

X-ray Structure of $\Delta^{9(11)}$-19-Nor-10-azasteroid 1. The crystallographic structure (**1**-X-ray) of $\Delta^{9(11)}$-19-nor-10-azaandrostenedione (**1**) is reported in Figure 4, and a formula with signs and values of the torsional angles is in Figure 6.

A quasi-trans configuration of the A/B and B/C ring fusions is present in **1**-X-ray structure. Other selected structural data are reported in Tables 1 and 3 (entry 1). The most important feature of the crystallographic structure is the presence of a conjugated system extended from O-3 to C-11. Since the N-10 atom is directly connected to the O-3=C-3−C-4=C-5 enone system and to the C-9=C-11 bond, its lone pair should be shared by these two systems. In fact, a strong conjugation of the nitrogen atom is evident, considering the distance of N-10 (in β position) from the C-1, C-5, and C-9 plane, which is only 0.104(2) Å (Table 3, entry 1), and the sum of the bond angles around N-10, which is very close to 360° (358.4°); these values are consistent with the sp^2 hybridization of the N-10 atom. However,

Table 3. Selected Conformational Data of the Crystallographic Structure of **1** and the Predicted Conformers of **1** and **2**

$\Delta^{9(11)}$-19-Nor-10-azasteroid **1**

entry	structure	torsion (deg)[a] C-4═C-5—N-10—C-9		torsion (deg)[a] C-11═C-9—N-10—C-5		distance and position of N-10 from the C-1, C-5, and C-9 plane (Å)
1	**1**-X-ray	−172.6[3]	(7.4)	158.6[3]	(21.4)	0.104[2] (β)
2	**1-I**	−168.36	(11.64)	158.14	(21.86)	0.139 (β)
3	**1-II**	164.67	(15.33)	−175.87	(4.13)	0.109 (α)
4	**1-III**	−172.38	(7.62)	134.84	(45.16)	0.193 (β)
5	**1-IV**	172.31	(7.69)	145.83	(34.17)	0.156 (α)

$\Delta^{8(9)}$-19-Nor-10-azasteroid **2**

entry	structure	torsion (deg)[a] C-4═C-5—N-10—C-9		torsion (deg)[b] C-8═C-9—N-10—C-5		distance and position of N-10 from the C-1, C-5, and C-9 plane (Å)
6	**2-I**	165.97	(14.03)	−1.39	(1.39)	0.170 (α)
7	**2-II**	175.30	(4.70)	19.06	(19.06)	0.121 (α)
8	**2-III**	−174.13	(5.87)	−19.38	(19.38)	0.134 (β)
9	**2-IV**	−165.74	(14.26)	1.9	(1.9)	0.179 (β)

[a] The deviation from planarity (180°) is reported in brackets. [b] The deviation from planarity (0°) is reported in brackets.

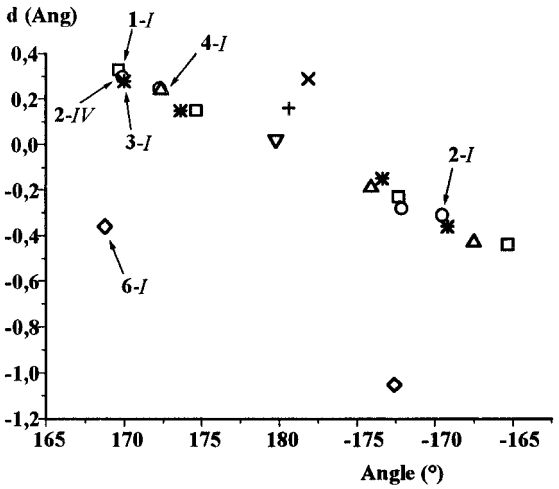


Figure 5. Distance of C-2 from the C-3, C- or N-4, C-5, and C- or N-10 mean plane in the predicted conformers of compounds **1** (□), **2** (○), **3** (*), **4** (△), **5** (▽), and **6** (◇), and in **4**-X-ray (+) and **1**-X-ray (×).

a certain deviation from a complete planarity of the O-3═C-3—C-4═C-5—N-10—C-9═C-11 system exists, as revealed by the angular values of the torsions C-4═C-5—N-10—C-9 [−172.6(3)°] and C-11═C-9—N-10—C-5 [158.6(3)°] lower than 180° (Table 3, entry 1). Moreover, the smaller deviation from planarity of the first torsion angle (≈7° compared to ≈21° of the latter one) and the minor length of the C-5—N-10 bond [1.367(4) Å] with respect to the N-10—C-9 bond [1.417(4) Å] are consistent with a greater conjugation of the N-10 atom with the C-4═C-5 bond than with the C-9═C-11 bond.

Finally, according to the notation of Duax et al.[10] the A ring in **1**-X-ray is in a "normal" 1α−2β conformation, being that the distances of C-1 and C-2 from the C-3, C-4, C-5, and N-10 mean plane are −0.319(4) and 0.291-(4) Å, respectively.

In Figure 5 the deviation of C-2 from this plane is plotted versus the torsion angle O-3═C-3—C-4═C-5, which measures the conjugation of the enone system.

Using this type of analysis, on the basis of a systematic study of the A ring conformation of 119 crystallographic structures of 4-en-3-one steroids, Duax et al.[10] observed that the "normal" conformations are characteristic of 4-en-3-one and 4,9,11-trien-3-one structures, whereas 4,9-dien-3-ones have inverted conformations. Despite $\Delta^{9(11)}$-19-nor-10-azasteroid **1** having only one double bond in the C-ring, it presents a "normal" 1α−2β conformation and thus appears more similar to a 4,9,-11-trien- or 4-en-3-one than a 4,9-dien-3-one steroid. However, being as azasteroid **1** is a competitive inhibitor versus testosterone, a comparison of **1**-X-ray with an X-ray structure of testosterone should be more relevant.

Depending on the crystallization solvent, different conformations in independent crystals of testosterone have been found.[9,11−14] A comparative analysis between the conformations of testosterone obtained by molecular mechanics calculations and crystallographic data has been made by Bucourt et al.,[9] who pointed out that the highest distorsions in the A ring are associated with the structures presenting the strongest hydrogen bond between the O-3 atom and a molecule of the crystallization solvent or another molecule of testosterone. Among the reported crystal structures of testosterone we have chosen for comparison that of the orthorhombic form of hydrated testosterone ($P2_12_12_1$)[11] (**4**-X-ray) which, having a weaker hydrogen bond, has a minor distorsion in the A ring.[9]

The internal torsion angles of **4**-X-ray structure are reported in Figure 6, and some selected structural parameters are reported in Table 2 (entry 1). Looking at the conformation of the A and B rings, the X-ray structure of $\Delta^{9(11)}$-19-nor-10-azasteroid (**1**-X-ray, Table 1, entry 1) shows a considerable similarity to that of testosterone (**4**-X-ray, Table 2, entry 1). In fact both structures have the A ring in the 1α−2β conformation and the B ring in the 6β−7α one, with a quasi-trans A/B ring fusion. Moreover **1**-X-ray and **4**-X-ray structures have very close O-3═C-3—C-4═C-5 torsion angle values [−177.8(4)° for **1**-X-ray and −179.29° for **4**-X-ray], as well as quite similar distances of C-2 from the C-3, C-4, C-5, and C- or N-10 plane (0.291 and 0.16 Å for **1**-X-ray and **4**-X-ray, respectively). Consequently, the two crystallographic structures occupy very close positions in the plot reported in Figure 5.

Conformational Analysis of $\Delta^{9(11)}$-19-Nor-10-azasteroid 1. It is known that the X-ray-derived structure of steroids corresponds in many cases to the global minimum energy conformation or local minimum energy conformations that are less than 2 kcal/mol above the global minimum.[15−17] However, it has been recently pointed out that the flexibility of the skeleton may be an important property of the steroids for best fit to receptors.[18] The absence of the C-19 methyl group has

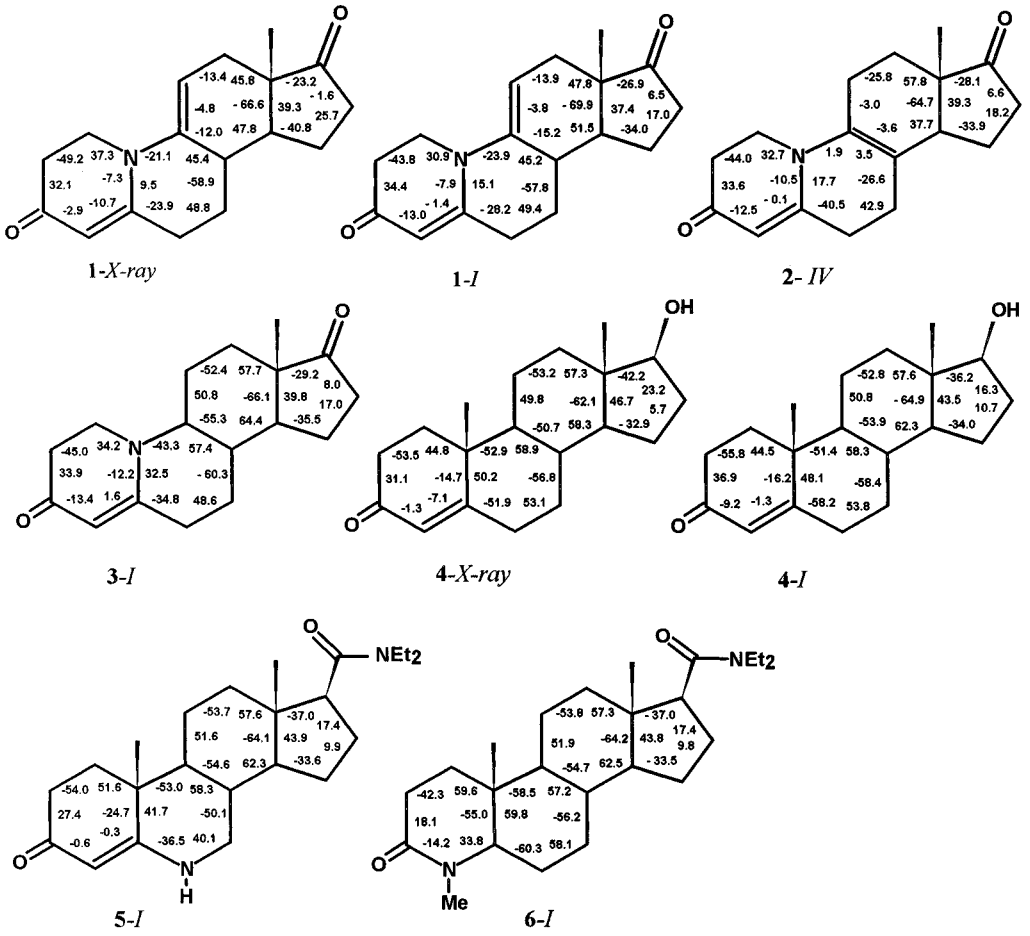


Figure 6. Internal torsion angles of some selected conformations of compounds **1**−**6** and of the X-ray structures of **1** and **4**.

been related to an increase of flexibility of estrogen[18] or 19-norandrogen derivatives as compared to the normal androgens.[9,19] In fact, both 19-nortestosterone and estradiol or estrone can assume different conformations in different crystalline modifications or also in the same crystal. The conformational flexibility of the steroids could also be increased by the presence of endocyclic double bonds which lowers the energy of the transition barriers between different conformers.[20]

Since the most distinctive features of 19-nor-10-azasteroid **1** are the absence of the C-19 methyl group and the presence of two endocyclic C−C double bonds, we have considered the possibility that this compound could have other minimum energy conformations different from that observed in the crystal. Thus a systematic conformational search has been performed, starting from the crystallographic structure, in order to determine the number and the energy of all possible conformers as well as the energy of the transition barriers between them.

The X-ray structure of 19-nor-10-azasteroid **1** was introduced in MacroModel and energy minimized using the MM2* force field, and then an exhaustive conformational analysis was carried out by a Monte Carlo method. This consisted of the systematic breaking and then in the casual recomposition of some selected bonds followed by the minimization process. In particular, three bonds (C-1−C-2, C-6−C-7, and C-11−C-12) of the A, B and C rings were selected and 1000 cycles of conformational analysis were made, generating 1000 structures, each of them minimized until the convergence criterium was reached. These conformations were compared and resulted in nine different conformers. Three conformers having an energy higher than 40 kcal/mol above the global minimum energy were discarded and the remaining six conformers were imported into Spartan and optimized by AM1 semiempirical calculation. Two pairs of the MM2* minimized conformers converged to the same geometry, so that the AM1 calculation produced only four different conformers (named **1-I**−**IV**, entries 2−5 of Tables 1 and 3) having relative energies comprised in a range of only 2.7 kcal/mol with respect to the global minimum.

Comparing the lowest energy conformer **1-I** to **1**-X-ray (Table 1, entries 1 and 2), it is evident that **1-I** closely reproduces the crystallographic structure: the root mean square (rms) fitting on all C and O atoms is 0.119 Å and **1-I** has normal 1α−2β A ring and 6β−7α B ring types, with a distance of C-2 from the C-3, C-4, C-5 and N-10 plane very close to the corresponding distance in **1**-X-ray. In the other conformers (entries 3−5), energy and rms fitting with **1**-X-ray increase with the distortion of the A and B rings, reaching the highest values in conformers **1-IV** (entry 5) which has inverted 1β−2α A ring type and a 7β−sofa B ring. All predicted conformers have a quasi-trans A/B ring fusion.

In Figure 6 the internal torsion angles of the global minimum energy conformation **1-I** are reported. The angle values of **1-I** are in accord with those of **1**-X-ray, with the exception of the torsions related to the 4-en-3-one moiety. In correspondence to this observation, in conformer **1-I** the O-3═C-3−C-4═C-5 dihedral angle, taken as a measure of the 4-en-3-one system conjugation, deviates to some extent (10.34°) from planarity

(Table 1, entry 1). Similar deviations, ranging from 5.36° to 14.68°, are observed for all the other predicted conformers **1-II**−**IV** (entries 3−5) and, as will be shown later, they occur for all the minimized structures of the other 10-azasteroids and testosterone.

Concerning the C-4═C-5−N-10−C-9═C-11 conjugated moiety, also in this case greater deviations from planarity than in **1**-X-ray exist (Table 3, entries 2−5). For example, while the C-11═C-9−N-10−C-5 torsion (158.14°) is substantially unchanged in **1-I**, the other torsion (−168.36°) and the distance of N-10 from the C-1, C-5, and C-9 plane (0.139 Å) differ slightly from the values found by X-ray analysis (Table 3, entry 1). In the same way, in the high-energy conformers **1-II**−**IV** (entries 3−5) either a torsion or the N-10 distance from the plane differ from the corresponding X-ray values. Therefore, on this basis, the extent of the O-3 to C-11 conjugation through the A, B and C rings in the predicted conformations of **1** seems to be underestimated with respect to **1**-X-ray, which instead, especially in the 4-en-3-one moiety, appears more planar. Thus, in order to evaluate the contribution to the energy of the O-3═C-3−C-4═C-5 torsion angle deviation in **1-I** from the value observed in the X-ray structure (−177.8°), we minimized in the usual way a conformer derived from **1-I** in which the 4-en-3-one moiety was constrained at −177.8°. The energy found in such a calculation was only 0.24 kcal/mol different from that of **1-I**, a value which may be taken, therefore, as a measure of the uncertainty on the energy of the conformer **4-I** calculated by AM1 with respect to the X-ray structure. This difference could be related either to packing forces in the crystal of **1**, which constrain the A ring to a greater planarity than in the vacuum, or to the type of calculation performed, which makes lower account of the orbital conjugation with respect to other parameters. In this case, similarly to the calculation carried out on **1-I**, if in conformers **1-II**−**IV** the O-3═C-3−C-4═C-5 torsion angle is constrained to planarity, the energy differences with the unconstrained conformers are only in the 0.3−0.5 kcal/mol range and, therefore, the relative energies of conformers **1-I**−**IV** are only slightly affected.

The conformational analysis of compound **1** requires at this point the evaluation of the flexibility, i.e. the energy determination of the transitional barriers between the predicted conformers **I**−**IV**. This is of crucial importance because, if the barriers are low, during molecular recognition a molecule could be converted, with a low energy cost, to a preferred geometry in the binding site within the enzyme.

The determination of transitional barriers was carried out through a torsion space exploration in MacroModel, optimizing the geometry of each conformation obtained with a combination of sequential rotations in 5° increments from −80° to 80° of both torsion angles N-10−C-1−C-2−C-3 (Φ_1) and C-5−C-6−C-7−C-8 (Φ_2). The 2D conformational MM2* energy map thus obtained for compound **1** is reported in Figure 7 (top). Six distinct local minima (**I**−**VI**, Figure 7, top) have been found, corresponding to the conformers already obtained by the Monte Carlo conformational search. Therefore, the Monte Carlo random approach and the determination of the conformational energy map in terms of Φ_1 and Φ_2 gave the same six conformers. The 2D energy map is approximately centrosymmetric, with the lowest

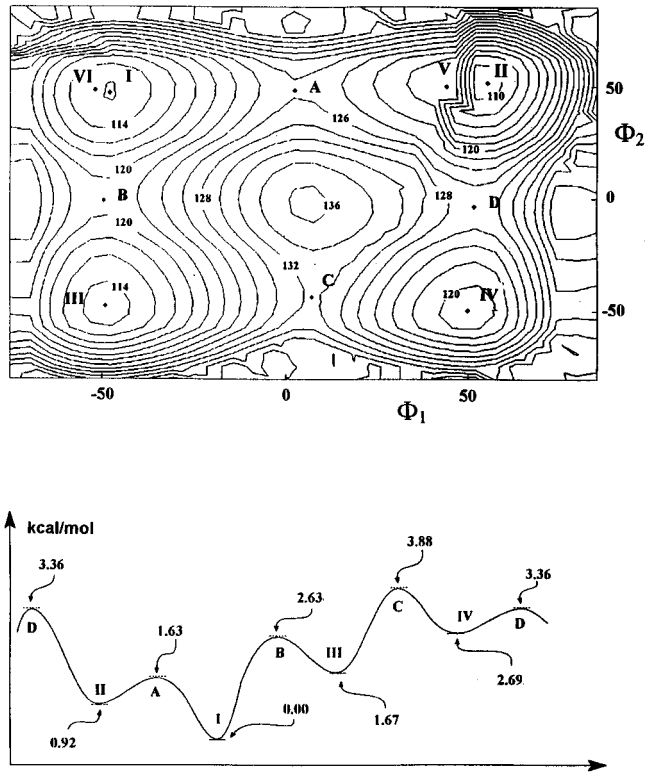
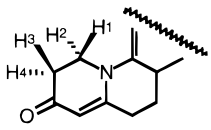


Figure 7. MM2* conformational potential energy map of 19-nor-10-azasteroid **1** (top) and AM1 energy profile of the barriers between the conformers **1-I**−**IV** (bottom). In the 2D map (top), the individual conformers are identified by boldface romanic numbers, the contour level is 2 kJ/mol, and Φ_1 and Φ_2 correspond to the dihedral angles N-10−C-1−C-2−C-3 and C-5−C-6−C-7−C-8, respectively; in the energy profile (bottom) points A, B, C, and D correspond to the barriers between conformers **1-I**−**IV** and energy values are given in kcal/mol.

interconversion barrier values (in the 2.76−5.68 kcal/mol range) found for the conformational changes involving only one of the two torsions. These are the transitions **I** ↔ **II** and **III** ↔ **IV** (Φ_1 torsion involved) and **I** ↔ **III** and **II** ↔ **IV** (Φ_2 torsion involved), and the points A, C, B, and D represent the barriers to these interconversions. On the contrary, the simultaneous rotation of Φ_1 and Φ_2 torsion angles corresponds to transitions (**I** ↔ **IV** and **II** ↔ **III**) involving energy barriers higher than 6 kcal/mol. As already discussed, after AM1 geometry optimization, only four conformers have been found for compound **1**, having two couples of MM2* minima converged to two single conformers (**II** and **V** to **1-II**, and **I** and **VI** to **1-I**). Thus, in order to obtain more consistent transitional barrier values, the energy of the conformations corresponding to the points A−D was refined by rotating the same torsion angles Φ_1 and Φ_2 in 1° increments in a narrower range (−10 ÷ 10°) and optimizing the geometry of the resulting structures by AM1. This process provided the final AM1 energy values (Figure 7, bottom) which, of course, might be affected by the same degree of uncertainty found for the energy of the predicted conformers.

This analysis clearly suggests that compound **1** is a flexible molecule, the highest value of transitional energy being only 3.88 kcal/mol. Therefore, compound **1** should be better described by a fast equilibrium between conformers **1-I**−**IV**, even though, on the basis of their relative energies, the global minimum **1-I** should be the prevailing conformation.

Table 4. Vicinal Coupling Constants for Compound **1**



entry		J_{1-3}	J_{1-4}	J_{2-3}	J_{2-4}
	Observed (Hz)[a]				
1	**1**	7.2	4.0	12.9	5.3
	Calculated (Hz)[b]				
2	**1**-X-ray	4.9	1.5	12.5	4.3
3	**1-I**	6.3	0.9	11.8	5.6
4	(**1-I** + **1-II** + **1-III**)[c]	6.4	2.6	10.9	5.8

[a] ^{1}H NMR (200 MHz) spectrum recorded in $CDCl_3$ at 25 °C. [b] Vicinal coupling constants calculated for all conformers by the NMR option in MacroModel. [c] Calculated on the basis of the Boltzmann distribution of these conformers at 25 °C.

Experimental support to this supposition was found by recording the ^{1}H NMR spectrum of **1** in $CDCl_3$ and measuring the four vicinal coupling constants between the protons on C-1 and C-2 (Table 4, entry 1). The same constants were measured for the X-ray structure and for each predicted AM1 conformer of **1** in MacroModel[21] and calculated on the basis of the relative population of the conformers at room temperature (Table 4, entries 2−4). Even though the conformational analysis of **1** was performed in the vacuum, the conformational property should not be drastically different from that in $CDCl_3$ solution. Thus, comparison of the vicinal constants can be done. The ^3J calculated for **1**-X-ray and **1-I** (which, as expected, are similar; see entries 2 and 3) are quite different from the experimental values (entry 1), especially from those corresponding to the couplings between H_1 and the vicinal H_3 and H_4 (J_{1-3} and J_{1-4}). Instead, a better accord with the observed values (entry 4) can be obtained if the coupling constants are calculated considering the different contribution of the three conformers **I**−**III** (on the basis of their Boltzmann distribution at room temperature), discarding that of the least probable conformer **1-IV**.[22]

Conformational Analysis of Δ$^{8(9)}$-19-Nor-10-azasteroid 2 and Compound 3. The same conformational analysis performed on **1** (Monte Carlo search, followed by AM1 optimization) has been carried out on its Δ$^{8(9)}$ regioisomer **2** and on the C-ring-saturated azasteroid **3**. Four unique conformations have been found for compound **2** (entries 6−9, Table 1). Conformers **2-I**−**IV** have much closer energies than the four minima of **1**, included in the narrow range of 0.1 kcal/mol. The first two conformations (**2-I** and **2-II**) have inverted 1β−2α A ring type and differ by the B ring type (7β− and 7α−sofa). The other two conformers (**2-III** and **2-IV**) have normal 1α−2β A rings and again different B ring conformations. Common features of conformers **2-I**−**IV** are the quasi-trans A/B ring fusion and a deviation from the planarity of the O-3═C-3−C-4═C-5 torsion angle in the 7.7−10.5° range. For this reason, conformers **2-IV** and **1-I** are very close in the plot depicted in Figure 5.

Concerning the π-conjugation through the A, B, and C rings, two conformers (**2-I** and **2-IV**) have the C-8═C-9−N-10−C-5 torsion angle (Table 3, entries 6 and 9) very close to 0° and greater distorsions in the C-4═C-5−N-10−C-9 dihedral angles (≈14°), while the remaining conformers (**2-II** and **2-III**) show inverted deviations (entries 7 and 8). These results, and the fact that the four conformers of **2** have almost the same energy, do not allow assignment of a prevailing conjugation of N-10 with one of the two C−C double bonds present in the molecule.

The presence of a further bridgehead sp^2 C atom (C-8) in compound **2** should provide lower torsional barrier energies than in regioisomer **1**.[20] In fact, the torsional barriers for **2-I**−**IV**, calculated as described for compound **1**, are all in the 1.31−1.34 kcal/mol range, and therefore, a fast equilibrium between four equally probable conformers should be the best description of compound **2**.

With regard to the C-ring-saturated 19-nor-10-azasteroid **3**, four conformations have been found after the usual conformational search. The global minimum energy conformer **3-I** (Table 1, entry 10) has a normal 1α−2β A ring type and a 6β−7α B ring type, similar to **1-I**. The C-2 distance from the C-3, C-4, C-5, and N-10 plane is 0.28 Å, thus very close to the same distance in **1-I** (0.33 Å). As already observed for **1** and **2**, in this case the modeling also gives absolute values for the O-3═C-3−C-4═C-5 torsion angle around 170°, and therefore the lowest energy conformers of **1** and **3** are very close in the plot of Figure 5. The second conformer (**3-II**, entry 11) is 0.81 kcal/mol higher in energy and has inverted A ring type, and conformer **III** (entry 12) also has an inverted A ring type and 7β−sofa B ring, whereas conformer **IV** (entry 13) has a normal A ring type and 7β−sofa B ring. Concerning the determination of the transitional barriers energy map for azasteroid **3**, we should expect that this compound, lacking in the C-19 angular methyl group and, moreover, with a bridgehead N atom instead of a sp^3 C atom, is a molecule at least as flexible as 19-nortestosterone.[9] In fact, the barrier values found are 1.83 (**3-I** ↔ **3-II**), 3.78 (**3-II** ↔ **3-III**), 2.32 (**3-III** ↔ **3-IV**), and 3.92 kcal/mol (**3-I** ↔ **3-IV**), making **3**, similar to azasteroids **1** and **2**, a flexible molecule.

Conformational Analysis of Compounds 4−6. Besides the calculations performed on novel compounds **1**−**3**, we decided to extend our approach to other steroids, in particular testosterone (**4**), taken as a reference structure for the comparative analysis, and two competitive azasteroidal inhibitors **5** and **6** (Figure 1), in order to gain new insights into the conformational features of these molecules.

It has already been shown that testosterone is a quite flexible molecule.[9] It displays different conformations in independent crystals, depending on the presence of cocrystallized solvent molecules or intermolecular H-bonds, all having 1α−2β A ring type and a quasi-trans A/B ring fusion.[9] Bucourt et al. found that testosterone may exist in another form, having an energy 2.7 kcal/mol higher than the form having a conformation corresponding to the X-ray crystal structure. This second conformer has inverted 1β−2α A ring type and a quasi-cis A/B ring fusion.[9]

Starting from the orthorhombic $P2_12_12_1$ crystallographic structure of testosterone,[11] our conformational analysis provided three unique conformers (Table 2, entries 2−4). Among these, the global minimum energy conformation **4-I** closely reproduces the X-ray structure (**4**-X-ray), the rms fitting being 0.098 Å, whereas **4-II** corresponds to the second conformation (relative energy 2.37 kcal/mol) found by Bucourt.[9] Conformer **4-I** has

Table 5. Comparison of Values of Some Selected Torsion Angles of **4-I** with the Corresponding Averaged Values of Four X-ray Structures of Testosterone

	X-ray observations[a]			
	range (deg)	average (deg)	**4-I** (deg)	diff (deg)
	A Ring			
C-10—C-1—C-2—C-3	−53.5 ÷ 57.5	−55.8	−55.8	0.0
C-1—C-2—C-3—C-4	29.2 ÷ 37.6	32.8	36.9	4.1
C-2—C-3—C-4═C-5	0.0 ÷ −8.1	−3.7	−9.2	5.5
C-3—C-4═C-5—C-10	−1.8 ÷ −7.1	−4.3	−1.3	3.0
C-4═C-5—C-10—C-1	−14.7 ÷ −19.6	−17.2	−16.2	1.0
C-2—C-1—C-10—C-5	44.8 ÷ 47.8	46.4	44.5	1.9
	B Ring			
C-10—C-5—C-6—C-7	−48.1 ÷ −51.9	−50.3	−58.2	7.9
C-5—C-6—C-7—C-8	53.1 ÷ 54.4	53.7	53.8	0.1
C-6—C-7—C-8—C-9	−56.5 ÷ −58.7	−57.2	−58.4	1.2
C-7—C-8—C-9—C-10	54.5 ÷ 58.9	56.9	58.3	1.4
C-8—C-9—C-10—C-5	−46.8 ÷ −52.9	−50.0	−51.4	1.4
C-6—C-5—C-10—C-9	44.9 ÷ 50.2	47.2	48.1	1.1
	Other Torsions			
O-3═C-3—C-4═C-5	−179.3 ÷ 179.1	177.8	172.4	5.5

[a] Taken from ref 9.

normal 1α−2*β* A ring type with distances of C-1 and C-2 from the C-3, C-4, C-5, and C-10 plane very close to those of **4**-X-ray (−0.49 and 0.16 Å in **4**-X-ray and −0.42 and 0.24 Å in **4-I**). Conformer **4-II** has 1*β*−2α A ring type and a quasi-cis A/B ring fusion, as was found by Bucourt, and the rms fitting with **4**-X-ray is 0.384 Å. The third conformer found by us (**4-III**, relative energy 3.44 kcal/mol) has a distorted 1α−2α A ring type and a 6α−7*β* B ring type, inverted with respect to that of the global minimum (entry 4) and a quasi-trans A/B ring fusion. The O-3═C-3—C-4═C-5 dihedral angle in the predicted conformers **4-I**−**III** undergoes to some extent a deviation from the planarity, as observed for all predicted conformers of compounds **1**−**3**. In **4**-X-ray the value of this angle is −179.29°, whereas in **4-I** it is 172.38°. This result is consistent with that obtained for azasteroid **1**, and similar reasons can be invoked to explain the difference in the torsion angle value between the minimized and the crystallographic structure of testosterone. However, the difference in the energy due to this distorsion, determined as done in the case of **1-I**, is only 0.1 kcal/mol. Similarly, in conformer **4-II** of testosterone the uncertainty on the energy due to the deviation from planarity of the enone moiety was found to be 0.34 kcal/mol.

The four crystal structures reported for testosterone[9] were determined in different crystalline environments and two are in crystals incorporating solvent. Following a referee's suggestion, it might be more appropriate, for the comparison with the minimized **4-I** conformation, to average the four X-ray structures of testosterone, since such an average should minimize any specific intramolecular interactions present in any of the crystals. In Table 5 some selected torsion angle values of **4-I** and the corresponding averaged values of the four crystal structures are reported. Once again, it is evident how the modeling well reproduces the X-ray structures, with very small differences between calculated and averaged values. Moreover, the differences between the calculated and averaged C-3—C-4═C-5—C-10 and O-3═C-3—C-4═C-5 dihedral angles (3.0° and 5.5°, respectively) are even lower than those obtained considering only the comparison between **4-I** and **4**-X-ray (Table 2, entries 1 and 2, and Figure 6). The most striking difference reported in Table 5 concerns the C-10—C-5—C-6—C-7 torsion angle (7.9°), and this deviation may generate some uncertainty in assigning the B ring conformation in **4-I**. In fact, the C-6 distance found in **4-I** (0.09 Å, Table 2, entry 2) would lead to define the B ring type as 7α−sofa, but likely, this therminology is inappropriate and the 6*β*−7α conformation should be correct.

Bucourt suggested that testosterone might be a flexible molecule and, in particular, that the A ring flexibility is only slightly lowered if the position 10 is occupied by a methyl group or a hydrogen atom as in 19-nortestosterone.[9] A complete search of the conformational space of testosterone performed by us with the procedure above described for **1** gave quite low energy values for the transitional barriers, that is, 4.62 (**4-I** ↔ **4-II**), 4.75 (**4-I** ↔ **4-III**), and 10.31 kcal/mol (**4-II** ↔ **4-III**, this transition involving both the torsions). As expected, transitional barriers of azasteroids **1**−**3** are lower than those of testosterone, confirming that the bridgehead N-10 atom and the further double bond on the C ring enhance the conformational flexibility.

Also for testosterone, the barrier values might be affected by a possible error due to the uncertainty on the energy of **4-I**−**III** (related to the deviation from planarity of the 4-en-3-one moiety). This in any case should not alter the barriers to such an extent to prevent the conformational interconversion between the conformers (barriers higher than 20 kcal/mol are usually required). Therefore, in considering the biological activity of testosterone, the relative populations of the two conformers, where the global minimum **4-I** predominates, should be taken into account, probably, more than the lack of an equilibrium between them.

6-Azasteroidal inhibitor **5** (Figure 1) was synthesized by Glaxo,[6] and like compounds **1**−**4**, it has a 4-en-3-one moiety. Our molecular modeling approach furnished only two conformations after AM1 optimization, **5-I** and **5-II** (see the Experimental Section for the treatment of the 17*β* side chain), the latter having an energy 5.31 kcal/mol higher than the global minimum conformer (Table 2, entries 5 and 6). As evident from the data reported in Table 2 (entry 5) and from the dihedral angle values in Figure 6, **5-I** resembles the X-ray structure of testosterone (**4**-X-ray): the A/B ring fusion is quasi-trans and a 6*β*−7α B ring type is present. The A ring has the normal 1α−sofa conformation,[23] but the distances of C-1 (−0.61 Å) and C-2 (0.02 Å) from the C-3, C-4, C-5, and C-10 plane are quite close to those of **4**-X-ray (−0.49 Å and 0.16 Å, respectively). More important is the very low deviation from the planarity of the O-3═C-3—C-4═C-5 dihedral angle, which is now very close to 180°. Thus, in the plot depicted in Figure 5, conformer **5-I** and **4**-X-ray are very close. The highest energy conformer **5-II** has instead an inverted 2α−sofa A ring type and a 7α−sofa B ring type (Table 2, entry 6) and shows a considerable distorsion of the 4-en-3-one moiety (the corresponding angle being −159.37°).

Recently, the X-ray structure of the 17*β*-[*N*-[1-(4-chlorophenyl)cyclopentyl]carbamoyl]-4-chloro-6-azaandrost-4-en-3-one has been determined.[23] Despite the presence of the bulky substituent at position 17 and the Cl atom on the A ring, which in particular could affect the electronic properties of the enaminone moiety, a comparison of its steroidal skeleton with that of **5-I** is

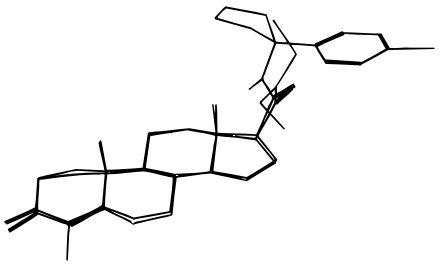


Figure 8. Steroidal backbone superposition of conformer **5-I** and the X-ray structure of 17β-[*N*-[1-(4-chlorophenyl)cyclopentyl]carbamoyl]-4-chloro-6-azaandrost-4-en-3-one.[23]

possible. In fact, the two backbones are well superposable, with a small rms fitting value (0.195 Å). In the X-ray structure, the O-3═C-3—C-4═C-5 torsion angle is very close (−178.2°) to that found in **5-I** and the A ring conformation is 1α−2α, with distances of C-1 and C-2 from the C-3, C-4, C-5, and C-10 plane (−0.64 and −0.15 Å, respectively) well reproduced on the steroidal skeleton of **5-I** (−0.61 and 0.02 Å, Table 2, entry 5). The B ring has a 6β−7α conformation, as found for **5-I**, with N-6 and C-7 distances values of 0.10 and −0.30 Å, respectively. On the whole, the modeling of compound **5** has generated a global minimum conformer whose steroidal skeleton is close to that of the X-ray structure (Figure 8). Interestingly, the C-2 atom lies preferentially in the plane defined by the C-3, C-4, C-5, and C-10 atoms, and as a consequence, the A ring conformation in 6-azasteroids seems to be 1α−sofa (or 1α−2α, but close to the 1α−sofa type).

The internal dihedral angles of the B ring in **5-I** and **5-II** are very close. For example, the torsion usually involved in the barrier energy determination, i.e. C-5—N-6—C-7—C-8, is 40.1° in **5-I** (Figure 6) and 46.5° in **5-II**. On the contrary, the dihedral angles change drastically in the A ring where, for example, the C-2—C-3—C-4═C-5 torsion is −0.6° in **5-I** (Figure 6) and 24.6° in **5-II**. Therefore, two torsions of the A ring have been chosen for the determination of the energy barrier between the two conformers (C-10—C-1—C-2—C-3 and C-2—C-3—C-4═C-5) which was, after the usual procedure, 7.40 kcal/mol. On the basis of this value, the two conformations of **5** are in equilibrium at room temperature; however, owing to the great difference between their energies, 6-azasteroid **5** is practically represented only by the lowest energy conformer **5-I**.

All steroids and azasteroids considered so far have a 4-en-3-one moiety in their A ring, and apart from testosterone, which is a substrate for the enzyme, all the other compounds, according to the transition state analogue theory for the enzyme inhibition, may be thought of as "substrate-like" inhibitors.[1] 4-Azasteroid **6**, developed by Merck,[7] has instead a sp^3 C atom at the position 5, thus making it a "product-like" transition state analogue.[1] After the usual Monte Carlo search and AM1 optimization, two conformers within 1 kcal/mol are obtained (**6-I**,**II**, Table 2, entries 7 and 8) having both a trans A/B ring fusion. Due to the presence of the bridgehead sp^3 C-5 atom, the extension of the comparative analysis performed so far to this compound cannot be done, unless other parameters, different from the distances of C-1 and C-2 from the C-3, N-4, C-5, and C-10 plane, are considered. This is evident from Figure 5. Measuring the distances of C-1 and C-2 from the above mentioned plane, an unusual 1α−2α A ring type is obtained for **6-I**, with values of −0.77 and −0.36 Å, respectively. Similarly, the second conformer (**6-II**) has 1α−2α A ring type with a great distance between C-2 and the plane (−1.05 Å).

For 4-azasteroid **6**, the differences between the two predicted conformers **6-I** and **6-II** are principally due to conformational changes in the A ring and not to distorsions of the B ring, whose internal dihedral angles are almost identical in **6-I** and **6-II**. For this reason, similar to compound **5**, the 2D conformational energy map has been determined using two torsion angles of the A ring (C-10—C-1—C-2—C-3 and C-2—C-3—N-4—C-5), and the resulting barrier between the two conformers was only 1.67 kcal/mol.

On the basis of the results obtained in the determination of the transitional barriers, we may conclude that 4-azasteroids **6** and 6-azasteroid **5** are flexible molecules only in the A ring, and that they should be considered, on the whole, more rigid structures than 10-azasteroids **1**−**3**.

From the analysis performed so far on 19-nor-10-azasteroids **1**−**3**, we have presented evidence that these compounds are very flexible molecules having transitional barriers lower than 4 kcal/mol. For **1** and **3** the global minimum energy conformations are predominant, whereas the four conformations **2-I**−**IV** are almost equally probable.

The flexibility of an inhibitor can be an advantage when the molecule in its lowest energy conformation does not have the right shape for the binding site within an enzyme, because only a little energy is required for a particular conformational change. However, in order to design more rigid analogues, it would be important to find, among all the thermally accessible conformations, the bioactive one, i.e. the most suitable for the fitting with the enzyme cavity. If we take in a simplified approach the global minimum energy conformer of testosterone (**4-I**) as the active conformation of this substrate, we can analyze all conformers of 10-azasteroids **1**−**3** by comparison with that structure.

Thus, all predicted conformers **1-I**−**IV**, **2-I**−**IV**, and **3-I**−**IV** were superimposed with the lowest energy conformation found for testosterone (**4-I**) and the rms fittings were measured. In this superimposition, all C (and N) atoms of the skeleton and the C-18 methyl group were considered, whereas the C-19 methyl group and the C-17 substituent were disregarded. The reason for the choice of **4-I** and not of **4**-X-ray as a reference structure lies in the fact that both the lowest energy conformers of **1** and **4** (**1-I** and **4-I**) have parallel conformational modifications if compared to the corresponding X-ray structures (as evident from Figure 5). Our modeling also has a constant effect on the O-3═C-3—C-4═C-5 dihedral angles, and this suggests that it is more appropriate to compare among minimized structures than among minimized and X-ray structures.

As a second parameter of analysis we chose the distance of the O-3 atom in the superimposed structures from the corresponding atom in **4-I**. In fact, we found that this distance is related to the distortion of the A and B rings with respect to testosterone **4-I** and it can be plotted versus the rms (Figure 9). This helped us to identify the most similar conformations to **4-I**. Indeed, these conformations could be found by the A and B ring type analysis. For example, in Figure 5, two conformers

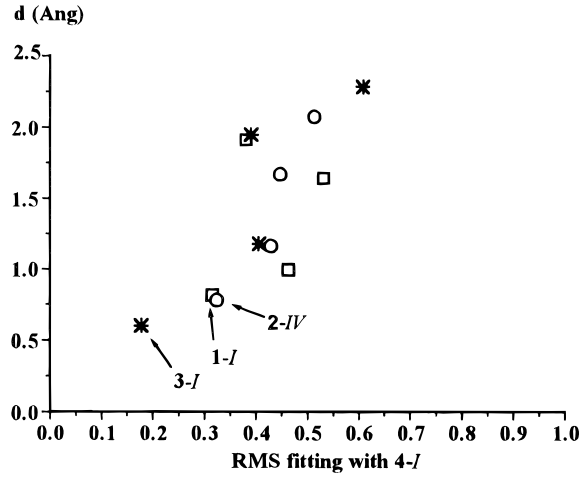


Figure 9. Superposition of conformers **I**−**IV** of compounds **1** (□), **2** (○), and **3** (*) with **4-I**. The O-3 distance of each superimposed conformer from the corresponding atom in **4-I** is plotted versus the rms fitting value.

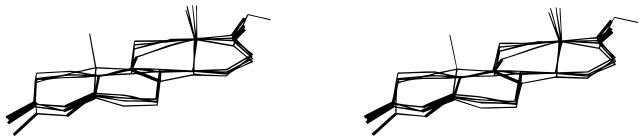


Figure 10. Stereoplot of superposition of conformers **1-I**, **2-IV**, and **3-I** with **4-I**.

of both **1** and **2** are very close to **4-I** (**1-I** and **1-III**, and **2-III** and **2-IV**), each of them having 1α−2β A ring type as in **4-I** and differing by the α or β C-7 atom in the B ring. One should expect that the two conformers of **1** and **2** having also a B ring type similar to that of testosterone would be the most similar structures to **4-I**. This actually occurs; in fact, superimposing all predicted conformers of **1**, **2**, and **3** and plotting as described above (Figure 9) yields only three structures that are close to the lowest energy conformer of **4**, and they are the global minima **1-I** (distance 0.815 Å and rms 0.315 Å) and **3-I** (distance 0.599 Å, rms 0.178 Å) and the conformer **2-IV** (distance 0.782 Å, rms 0.324 Å), all having 1α−2β A ring type and a 7α carbon atom in the B ring (Figure 10). Conformer **3-I** is the closest conformer to **4-I**, and this results also from the comparison of the dihedral angles reported in Figure 6. In fact, excluding those of the D ring, all the other torsions are very similar, although the N-10 atom, conjugated with the 4-en-3-one moiety, causes a decrease of the C-6—C-5—N-10—C-9 torsion angle value in **3-I** (32.5° compared to 48.1° in **4-I**). All the others conformers (Figure 9) have both higher rms (>0.4 Å) and distance (>1 Å), with the A ring distorsion (with respect to **4-I**) contributing to the deviation from the reference structure to a greater extent than the differences in the B ring type. Thus, if with a and A we indicate the normal and inverted A ring types, respectively, and with b and B the normal and inverted B ring types (referring to testosterone), the sequence ab < aB < Ab < AB defines the relative degree of conformational distorsion for the 19-nor-10-azasteroids. This type of analysis is more complete with respect to a simple rms best fit, since it is very useful to bring into consideration the differences between conformers of the same compound. In fact, two or more conformers of an azasteroid, despite having very similar rms fitting values with **4-I**, can occupy distant relative positions in the plot of Figure 9. This can be due to a considerable difference between the distortions of both the A and B rings in the examined conformers not suggested by the analysis of the rms values.

Since 19-nor-10-azasteroids **1**−**3** are competitive inhibitors of 5αR and testosterone (**4**) its natural substrate, the analysis above performed suggests that **1-I**, **2-IV**, and **3-I**, the closest conformers to testosterone, could also reasonably be considered the active conformations of compounds **1**−**3**. We have already shown that the 17β-*N*-*tert*-butylcarbamoyl substituent considerably enhances the inhibitory potency of these compounds.[1] This effect should be related to the interaction of this substituent with the enzyme active site rather than to conformational changes induced by the C-17 substituent. In fact we have observed that the difference in the C-17 hybridization in 19-nor-10-azasteroids has no significant effect on the number (apart from the rotamers due to the presence of a 17β substituent) or type of conformation and skeleton flexibility, which are instead determined by the particular features of the A and B rings.[24] Therefore, the conformers having the 1α−2β A ring type and a 6β−7α or 7α−sofa B ring type can be thought as the bioactive conformations of the 19-nor-10-azasteroid inhibitors.

Finally, applying the same analysis to the other 4-en-3-one azasteroidal inhibitor, i.e. 6-azasteroid **5**, resulted in that the lowest energy conformer **5-I** is very close to **4-I**, having small values of rms (0.104 Å) and distance (0.502 Å). Thus, on the basis of the same considerations done for 19-nor-10-azasteroids, **5-I** could be considered the bioactive conformer for this azasteroid.

Conclusion

Several classes of steroidal and nonsteroidal inhibitors have been synthesized and tested toward 5αR-1 and 5αR-2. However, to our knowledge, few molecular modeling studies on such inhibitors have been reported, and they mainly examine the conformation of the 17β substituent.[23,25,26]

The X-ray structure of steroids is very close in many cases to the global minimum energy conformer.[15−17] When the X-ray structure of a steroidal compound is not available, as in the case of most of azasteroids, calculation may be a useful tool to obtain reliable informations about the conformational features of the molecule, especially if the modeling has been "tested" by reproducing the crystallographic structure of structurally similar compounds and evaluating the possible inaccuracies on particular torsion angles or bond distances.

19-Nor-10-azasteroids are a new class of inhibitors whose activity is dependent on the presence of the bridgehead N-10 atom conjugated with the 4-en-3-one moiety in the A ring.

In the present work, we have performed a molecular modeling study focused on the conformational effects on the steroidal skeleton of modifications to the A, B or C rings, considering in particular three classes of azasteroids in comparison with testosterone.

A complete conformational analysis of these compounds has been performed to determine the number and energy of the possible conformers as well as the molecular flexibility of the 10-azasteroidal skeleton. The same analysis has been then extended to testosterone

(**4**) and to the 6- and 4-azasteroid inhibitors **5** and **6**, respectively. The most important features we have pointed out are as follows:

19-Nor-10-azasteroids **1**−**3**, as well as testosterone (**4**), having low transitional barrier energy values, are flexible molecules.

Four conformations describe azasteroids **1**−**3**, while three conformers are found for testosterone; considering their relative energies, the global minimum energy conformations of **1**, **3**, and **4** are predominant, whereas for azasteroid **2** the four conformations are equally probable.

For compounds **1** and **4**, the conformational analysis indicated that the lowest energy conformers **1-I** and **4-I** well reproduce the corresponding crystallographic structures, although these, concerning the conjugated system in the A rings, appear more planar.

Only two conformations describe 6-azasteroid **5** and 4-azasteroid **6**, which, moreover, are more rigid molecules than 19-nor-10-azasteroids.

From a best fit analysis of all predicted conformers of **1**−**3** with the global minimum of testosterone (**4-I**), taken as the active conformation of testosterone, we have found that the conformers **1-I**, **3-I**, and **2-IV**, having 1α−2β A ring type and a C-7 atom in the position α, are the closest structures to **4-I**. Thus, **1-I**, **2-IV**, and **3-I** could reasonably be thought of as the bioactive conformations of these compounds. Moreover, the conformational properties of the 19-nor-10-azasteroid skeleton being unaffected by the substituent at position 17, the 1α−2β A ring type and the 6β−7α or 7α−sofa B ring types should be the determining conformational features of the bioactive conformers of this class of azasteroid.

This best fit analysis cannot be considered conclusive in explaining the changes in the inhibitory potency determined by the presence and position of the double bond in the C ring of 19-nor-10-azasteroids. Instead it is very useful in identifying the possible active conformation for a particular inhibitor. However, another important finding of this paper is the low number of thermally accessible conformations of 4- and 6-azasteroidal compounds. For this reason, these two classes of inhibitors could be used to develop models of the enzyme active site. Recently, we have proposed a model cavity for 5αR-2 conceived following the "active analogue approach" and based on the 4-azasteroid inhibitors.[27] Similarly, a new model based on the 6-azasteroid inhibitors as mimics of a "substrate-like" transition state[1,4,27] could be now envisaged. In this context, the present results of conformational analysis and determination of the molecular flexibility of 19-nor-10-azasteroids (which can be considered "substrate-like" transition state analogues) should allow the application of such a model with the final goal of explaining the difference in inhibitory activity observed for the $\Delta^{8(9)}$, $\Delta^{9(11)}$, and C-ring-saturated isomers of 19-nor-10-azasteroids.

Experimental Section

Steroids. 19-Nor-10-azasteroids **1**−**3** were synthesized as previously described.[1] A 10:1 mixture of 19-nor-10-azaandrosta-4,9(11)-diene-3,17-dione [or 10-azaestra-4,9(11)-diene-3,17-dione] (**1**) and 19-nor-10-azaandrosta-4,8(9)-diene-3,17-dione [or 10-azaestra-4,8(9)-diene-3,17-dione] (**2**) was crystallized twice from ethyl acetate in order to remove the minor isomer, and suitable crystals for X-ray analysis were obtained by slow concentration of an ethyl acetate solution of pure compound **1**.

X-ray Crystallography of 1. Investigation on a single crystal of 1 of approximate dimensions of 0.15 × 0.30 × 0.45 mm was carried out with an Enraf-Nonius CAD4 X-ray diffractometer by using a $\theta-2\theta$ scan. During data collection three reflections were monitored periodically to check the stability of the diffractometer and the crystal. Intensity data were corrected for Lorentz and polarization effects and an absorption correction was applied when the structure was solved by using the Walker and Stuart method.[28] The structure was solved by direct methods of SIR92[29] and subsequently refined by a full-matrix least square technique on F^2 of SHELXL-93.[30] Atomic scattering factors were taken from the literature.[31] All the non-hydrogen atoms were treated anisotropically, while the hydrogen ones were introduced in calculated positions and refined according to the linked atoms with an overall temperature factor refined to 0.0735 Å^2. The assignment of the absolute configuration was not possible on the basis of the X-ray data, thus the correct enantiomer was set from chemical evidences.

Crystal data were as follows: $C_{17}H_{21}NO_2$, $M_r = 271.35$, $a = 9.829(9)$ Å, $b = 10.380(3)$ Å, $c = 13.988(5)$ Å, $V = 1427(2)$ Å^3, space group = $P2_12_12_1$, orthorhombic, $D_x = 1.263$ g cm^{-3}, λ(Mo Kα) = 0.71069 Å, $T = 298$ K. A total of 1430 reflections were collected ($5° < 2\theta < 50°$). The refinement of the structure used 1419 observed reflections [$I > 2\sigma(I)$]. Refined parameters were 183. Final R indices were $R_1 = 0.0453$ and $wR_2 = 0.1215$. $\Delta\rho$ in the final difference map was within 0.126 and −0.143 e Å^{-3}.

Molecular Modeling. The crystal structure of 19-nor-10-azasteroid **1** was used as input for computer modelization of 19-nor-10-azasteroids **1**−**3** and that of testosterone (T)[11] for the modeling of **4**−**6**.

All models and X-ray structures were displayed on a IBM/RISC 6000 workstation by the MacroModel (version 4.5)[32] molecular modeling software which was also used for the molecular mechanics calculations (MM2* forcefield). Semiempirical (AM1) calculations were carried out through Spartan 3.1.[33] All the described molecular modeling calculations used default values of the specific software unless otherwise indicated. A conformational analysis of the substituent at the position 17 of testosterone (**4**), 6-azasteroid **5**, and 4-azasteroid **6** was carried out. The lowest energy conformation obtained for the C-17 substituent was thus maintained constant during all conformational searchs soon after performed. Conformational analyses were carried out by the Monte Carlo method in MacroModel, and conformational energy surface data of azasteroids and testosterone were generated from torsional space determination using the "Dihedral Driver" option of MacroModel, as described in the Results and Discussion section.

Acknowledgment. The authors thank Ministero dell' Università e della Ricerca Scientifica e Tecnologica (Murst, 60 and 40%), Consiglio Nazionale delle Ricerche (CNR), and "Programma Vigoni" for financial support. E.G.O. thanks University of Florence for a 2-year postdoctoral fellowship.

Supporting Information Available: X-ray data of **1**, including tables of atomic coordinates with equivalent isotropic displacement, bond lengths and angles, crystal data and structure refinement, anisotropic displacement parameters, and hydrogen coordinates, and drawings with the internal torsion angles of conformers **1-I**−**IV**, **2-I**−**IV**, **3-I**−**IV**, **5-II**, and **6-II** (10 pages). Ordering information is given on any current masthead page.

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