Structure-Based Design and Synthesis of Novel Potent Na^+,K^+-ATPase Inhibitors Derived from a 5α,14α-Androstane Scaffold as Positive Inotropic Compounds

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The design, synthesis, and biological properties of novel inhibitors of the Na^+,K^+-ATPase as potential positive inotropic compounds are reported. Following our model of superposition between cassaine and digitoxigenin, digitalis-like activity has been elicited from a non-digitalis steroidal structure by suitable modifications of the 5α,14α-androstane skeleton. The strong hydrophobic interaction of the digitalis or cassaine polycyclic cores can be effectively obtained with the androstane skeleton taken in a reversed orientation. Thus, oxidation of C-6 and introduction in the C-3 position of the potent pharmacophoric group recently introduced by us, in the 17 position of the digitalis skeleton, namely, *O*-(*ω*-aminoalkyl)oxime, led to a series of substituted androstanes able to inhibit the Na^+,K^+-ATPase, most of them with an IC_{50} in the low micromolar level, and to induce a positive inotropic effect in guinea pig. Within this series, androstane-3,6,17-trione (*E,Z*)-3-(2-aminoethyl)oxime (**22b**, PST 2744) induced a strong positive inotropic effect while being less arrhythmogenic than digoxin, when the two compounds were compared at equiinotropic doses.

Introduction

Among inotropic agents, digoxin (Figure 1) is the most prescribed digitalis cardiac glycoside for the treatment of congestive heart failure. Through the inhibition of the Na^+,K^+-ATPase, which promotes the outward transport of Na^+ and the inward transport of K^+, Na^+ concentration inside the cell is increased and, as a consequence, Ca^{2+} is introduced by exchange with Na^+. The final result is a higher concentration of Ca^{2+} available to activate contraction during systole. Digoxin can alleviate symptoms, increase exercise tolerance, and reduce hospitalization. A very well-known drawback of digitalis drugs is their arrhythmogenic side effect. Evidence of digitalis toxicity emerges at 2−3-fold higher serum concentration than the therapeutic dose, such as disturbances of conduction and cardiac arrhythmia which are characteristics of digitalis toxicity.[1]

The selective ability of the natural digitalis compounds to inhibit Na^+,K^+-ATPase is strictly related to their 5*β*,14*β*-androstane three-dimensional structure. Nevertheless, the goal of synthesizing potent Na^+,K^+-ATPase inhibitors structurally not based on the 5*β*,14*β*-androstane steroidal skeleton has been actively pursued, but not yet achieved by medicinal chemists working in the digitalis field. Although some non-digitalis-like compounds are inhibitors of the Na^+,K^+-ATPase, displace ^{3}H-ouabain binding from the Na^+,K^+-ATPase digitalis receptor, and some of them induce positive inotropic effects (cassaine, canrenone, and chlormadinone acetate: Figure 1),[2] their low potency, poor selectivity, and poor therapeutic ratio, have hampered their development as inotropic agents.

In recent years, we proposed a new three-dimensional model for the binding mode of cassaine at the digitalis receptor site, based on the structural and conformational similarities between cassaine and 14,15-secodigitoxigenin analogues.[3] This model was corroborated by the good inhibitory activity on the Na^+,K^+-ATPase of the novel synthesized compounds, thus demonstrating that a suitable functionalization of the lipophilic perhydrophenanthrene (trans, trans, trans) nucleus can result in potent inhibitors of the Na^+,K^+-ATPase that do not possess the structural characteristics peculiar of the digitalis structure.[4]

Following these results, we further explored chemical series structurally unrelated to cardiac glycosides, but still possessing high inhibitory potency and selectivity on the Na^+,K^+-ATPase, with the aim of finding novel inotropic agents endowed with lower proarrhythmogenic effects than classical digitalis compounds.

As recognized by Repke,[5] the perhydrophenanthrene nucleus acts as the minimal pharmacophoric lead structure for bimolecular recognition, a significant contribution to the binding energy being given by the hydrophobic interaction in the *alpha* region of the polycyclic core. Recently, San Feliciano's group and our group reported that inhibition of Na^+,K^+-ATPase and

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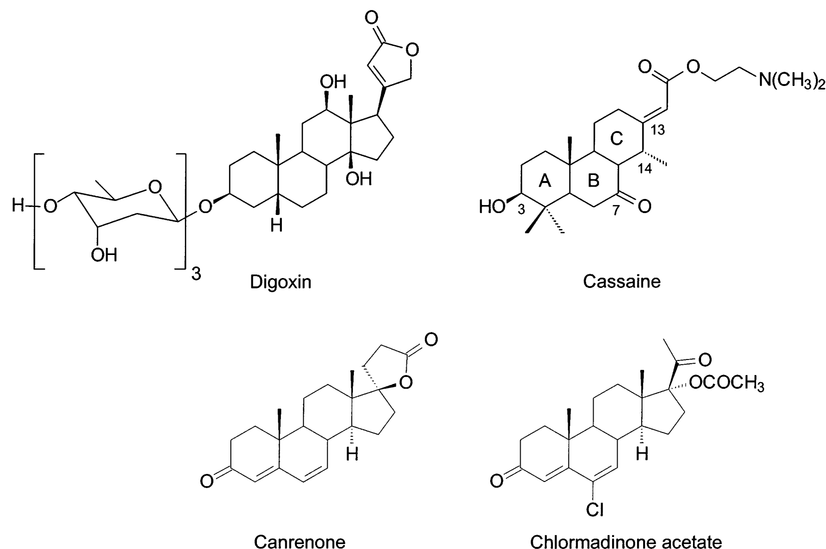


Figure 1. Structures of compounds with digitalis activity.

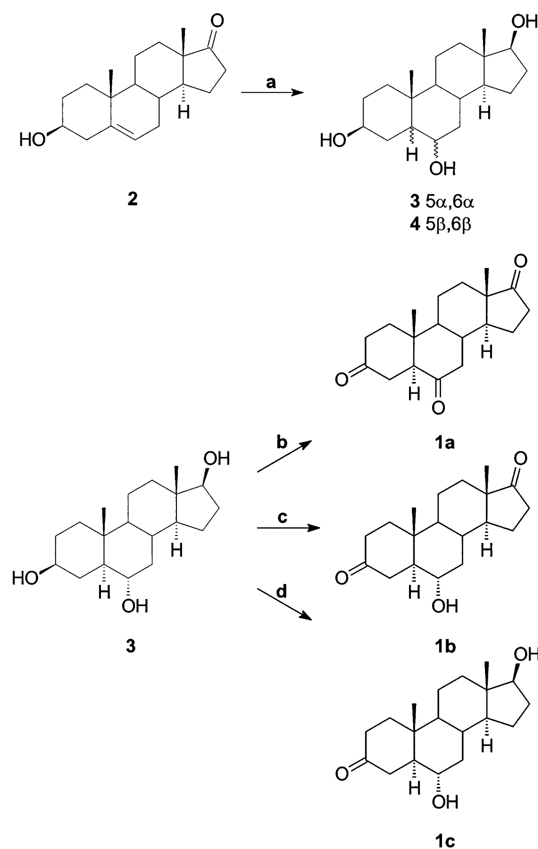
positive inotropic activity, although with weak potency, can be obtained from smaller scaffolds substituted with the appropriate side chains, namely, hydroindene amidinohydrazones and perhydroindene *O*-aminoalkyloximes;[7] these weak potencies could arise from the reduced hydrophobic interaction of the scaffolds.

The result that 3β-(dimethylaminoalkoxy)androst-5-en-17-one derivatives inhibit the Na^+,K^+-ATPase with IC_{50} in the millimolar range (unpublished data) suggested to us the idea that an easily accessible classical steroid skeleton like that of prasterone (3β-hydroxyandrost-5-en-17-one **2**, Scheme 1), beside providing a good support for strong hydrophobic interactions, could serve also as a manageable starting base for chemical elaboration. This would allow the introduction of the very powerful pharmacophoric group *O*-(ω-aminoalkyl)oxime, already investigated for the classical digitalis skeleton,[8] seco-D steroidal derivatives,[4] and the perhydroindene derivatives mentioned above. Moreover, we considered that the 5α,14α-androstane scaffold would be worth exploiting also in a reversed orientation (see Figure 2), facilitating the introduction and matching of some of the functional groups of cassaine, thus extending our model for the binding mode at the digitalis receptor site. In this superposition, rings A, B, and C of androstane are superposed to rings C, B, and A of cassaine, respectively, and the functionalized C-3 and C-6 carbon of androstane coincide with C-13 and C-7 of cassaine, respectively. In this new orientation, the two axial methyl groups of androstane are now oriented toward the hydrophobic *alpha* region, although none of them coincide with the 14α-methyl of cassaine.

We resolved to check the possibility of substituting the cassaine skeleton with the androstane skeleton in the reversed orientation by functionalizing a series of 3-keto androstane derivatives, bearing either oxo or hydroxy groups in positions 6 and 17, and by adding the above-mentioned *O*-(ω-aminoalkyl)oxime groups at position 3.

The best superposition was expected to be obtained with the *E* isomers of 3[$=NO(CH_2)_nNH_2$] (with $n = 2$ or $n = 3$) androstane derivatives, considering that a possible rearrangement in the receptor pocket could require a variable and flexible length of the amino-chain substituent to maximize the binding.

Scheme 1[a]



[a] Reagents and conditions. a: BH_3·THF, THF, −10 °C to room temperature; then $NaBO_3 \cdot 4H_2O$, water, room temperature; b: $NaBrO_3$, $RuO_2 \cdot 2H_2O$, EtOAc/water, room temperature; c: NBS, acetone/water/Py, room temperature; d: act. MnO_2, CH_2Cl_2/acetone/water, 45 °C.

Chemistry. The compounds listed in Table 1 were synthesized from the corresponding ketones of general formula **1** (Figure 3), except for compound **22e** that was prepared by acetylation of **22b**, and the appropriate *O*-(aminoalkyl)hydroxylamines dihydrochlorides in a THF/water solution at room temperature (see Experimental Section).

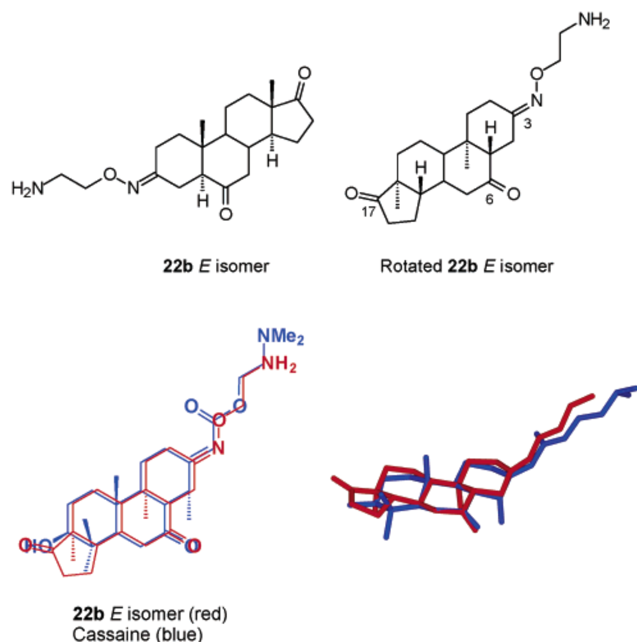


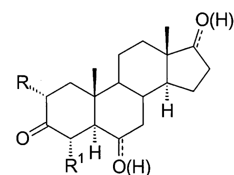
Figure 2. Model of the superposition between cassaine (blue) and *E* isomer of compound **22b** (red).

Table 1. Structure and Na^+,K^+-ATPase Inhibition for Compounds **22a**−**q**

compd	R^2	other[a]	6	17	Na^+,K^+-ATPase inhibition, IC_{50},[b] μM
22a	(*E,Z*) $=NO(CH_2)_2N(CH_3)_2$		oxo	oxo	12.0
22b	(*E,Z*) $=NO(CH_2)_2NH_2$		oxo	oxo	0.2
22c	(*E*) $=NO(CH_2)_2NH_2$	2α-Me	oxo	oxo	25.0
22d	(*E*) $=NO(CH_2)_2NH_2$	4α-Me	oxo	oxo	6.0
22e	(*E,Z*) $=NO(CH_2)_2NHAc$		oxo	oxo	>10.0[c]
22f	(*E,Z*) $=NO(CH_2)_3NH_2$		oxo	oxo	0.8
22g	(*E,Z*) $=NO(CH_2)_2NH_2$		α-OH	oxo	1.2
22h	(*E,Z*) $=NO(CH_2)_2N(CH_3)_2$		α-OH	β-OH	>100.0[d]
22i	(*E,Z*) $=NO(CH_2)_2NH_2$		α-OH	β-OH	1.6
22j	(*E*) $=NO(CH_2)_2NH_2$		α-OH	β-OH	2.0
22k	(*Z*) $=NO(CH_2)_2NH_2$		α-OH	β-OH	12.0
22l	(*E*) $=NO(CH_2)_3NH_2$		α-OH	β-OH	2.0
22m	(*Z*) $=NO(CH_2)_3NH_2$		α-OH	β-OH	3.0
22n	(*E,Z*) $=NO(CH_2)_2NH_2$		β-OH	oxo	2.5
22o	(*E,Z*) $=NO(CH_2)_2NH_2$		β-OH	β-OH	10.0
22p	(*E,Z*) $=NO(CH_2)_2NH_2$		oxo	β-OH	0.6
22q	(*E,Z*) $=NO(CH_2)_2NH_2$		oxo	α-OH	4.5
cassaine					5.0[e]
digitoxigenin					0.5
digoxin					0.4

[a] R and R^1 are hydrogens where not otherwise indicated. [b] Concentrations able to inhibit 50% of enzyme activity; mean of two or three experiments. [c] 35% inhibition at 10 μM. [d] 34% inhibition at 100 μM. [e] Estimated from the reported relative potency of ca. 0.1 in comparison to digitoxigenin (inhibition assays on different enzymes).[5,21] Compounds were isolated as free bases or as salts (see Experimental Section).

The required starting ketones of formula **1a**−**f** and **1i** were obtained as described in the following schemes, while compounds **1g** and **1h** were prepared as described in the literature.[9] In Scheme 1, the syntheses of androstane-3,6,17-trione[10a] **1a**, 6α-hydroxyandrostane-3,17-dione[11] **1b**, and 6α,17β-dihydroxyandrostan-3-one[12] **1c** are reported; these known compounds were obtained by oxidation of the common intermediate **3** at positions 3, 6, and 17 (**1a**) or by regioselective oxidation, at positions 3 and 17 (**1b**) or at position 3 only (**1c**), using the appropriate reagents. Androstane-3β,6α,17β-triol[13] **3** was obtained by hydroboration, and concomitant reduction of the 17 keto group, of the commercially available 3β-hydroxyandrost-5-en-17-one **2**, with BH_3· THF complex in THF solution and $NaBO_3$ in THF/water for the oxidative step. Compound **3** was obtained in 75% yield after crystallization from methanol/ethyl acetate; from the mother liquor the previously unknown 5β-androstane-3β,6β,17β-triol **4** was isolated in 12% yield by flash chromatography on silica gel. Compound **3** was then oxidized with catalytic RuO_2 and $NaBrO_3$[14] in ethyl acetate/water to give androstane-3,6,17-trione **1a** in 95% yield; the same compound could be obtained by reaction with Jones reagent, although in lower yield (74%) and with some amounts of a 5,6-seco-5-oxo-6-carboxylic acid derivative. To obtain 6α-hydroxyandrostane-3,17-dione **1b**, compound **3** was reacted with NBS in acetone in the presence of pyridine; **1b** was isolated in 85% yield. Finally, 6α,17β-dihydroxyandrostan-3-one **1c**, was obtained in low yield (30% after flash chromatography) by reaction with activated MnO_2 in CH_2Cl_2/acetone/water, together with the other monoketones in position 6 or in position 17 and the starting material. However, despite the low yield, this reaction is more favorable when compared, in terms of time and effort, with a more "classical" path with protection and deprotection steps.



1a: R = R^1 = H; 6,17-dioxo
1b: R = R^1 = H; 6α-OH,17-oxo
1c: R = R^1 = H; 6α-OH,17β-OH
1d: R = Me, R^1 = H; 6,17-dioxo
1e: R = H, R^1 = Me; 6,17-dioxo
1f: R = R^1 = H; 6β-OH,17-oxo
1g: R = R^1 = H; 6β-OH,17β-OH
1h: R = R^1 = H; 6-oxo,17β-OH
1i: R = R^1 = H; 6-oxo,17α-OH

Figure 3. Structures of androstane ketones.

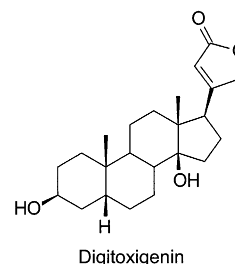
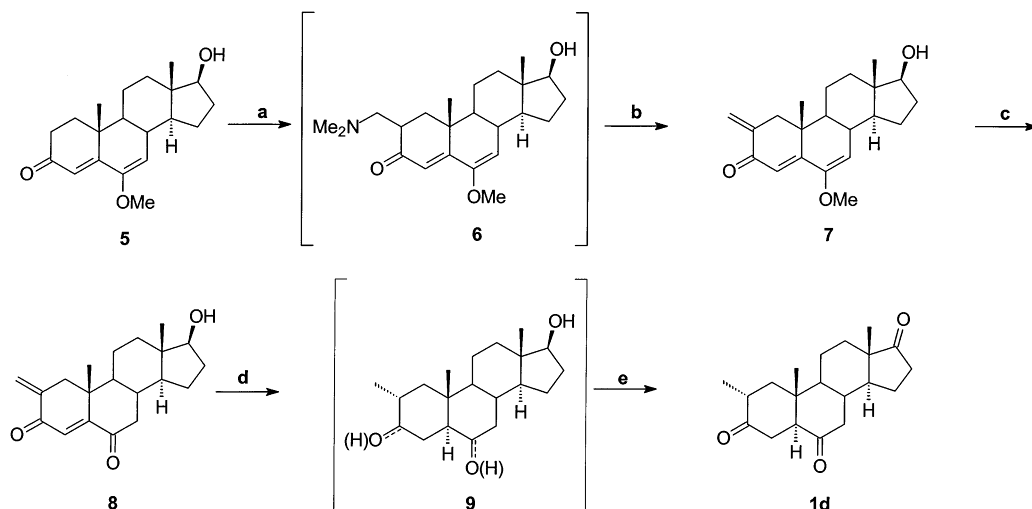


Figure 4. Structure of digitoxigenin.

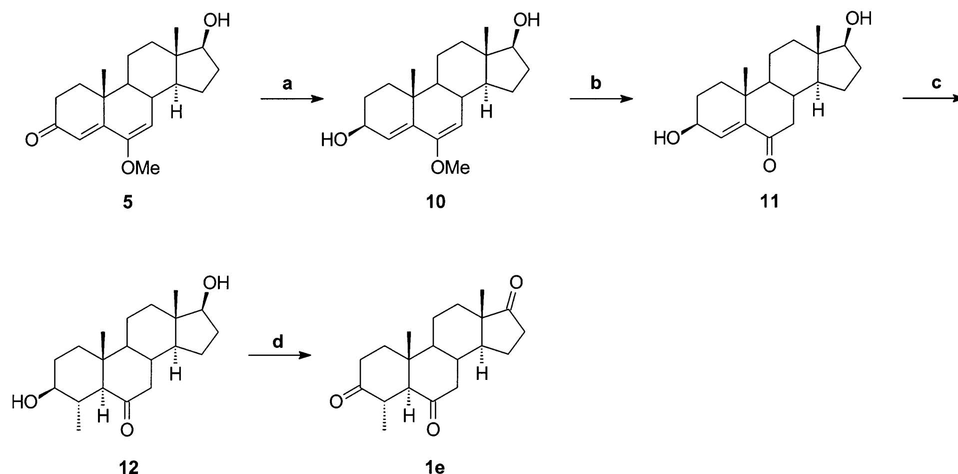
In our model, as stated above and shown in Figure 2, the optimal superposition between the basic chains of cassaine and our compounds would be reached with the *E* isomers of the oxime group. However, the products obtained from the coupling reactions between the keto steroids **1a**−**c** and **1f**−**i** and the appropriate hydroxyl-

Scheme 2[a]



[a] Reagents and conditions. a: $NHMe_2$·HCl, paraformaldehyde, CH_3CN, reflux; b: CH_3I, CH_3CN, room temperature; c: 3 N HCl, dioxane, 80 °C; d: H_2, PtO_2, EtOAc, room temperature; e: IBX, CH_3CN, reflux.

Scheme 3[a]

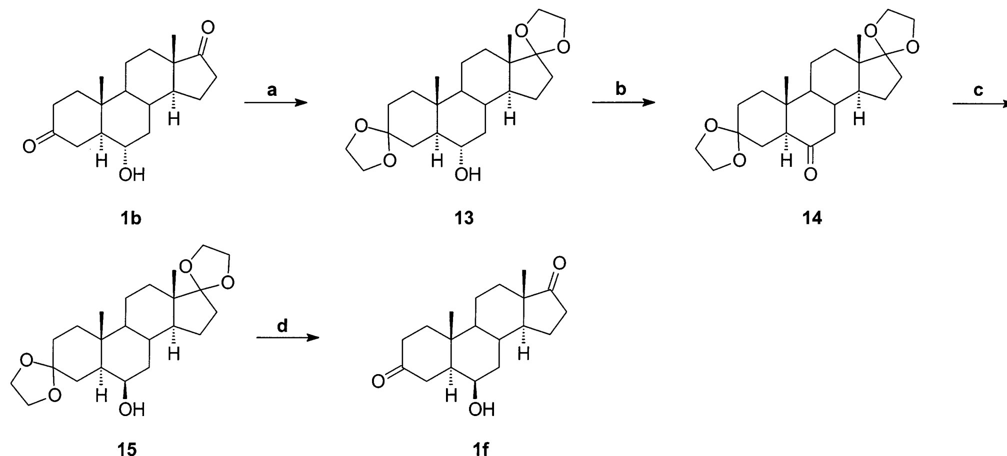


[a] Reagents and conditions. a: $NaBH_4$, $CeCl_3$, MeOH, −5 °C to room temperature; b: 0.5 N HCl, THF, room temperature; c: $LiCuMe_2$, Me_3SiCl, Et_2O/THF, −10 to 0 °C; d: IBX, CH_3CN, reflux.

amines were mixtures of *E* and *Z* isomers in a ratio of about 1:1. For compounds derived from ketone **1a** all efforts to separate the two isomers had poor success and attempts to prepare the pure *E* and *Z* isomers of **22b** and **22f** (see below) by oxidation of the corresponding pure derivatives **22j**−**m** were unsuccessful. In an attempt to bypass this problem, we decided to prepare the analogous ketones **1d** and **1e**, so that the methyl group in position 2α or 4α, respectively, could orient the coupling reaction to give only a single isomer with the oxime chain opposite to the methyl group. The hypothesis was supported by molecular mechanics conformational searching (MacroModel, v. 7.2)[15a] on the *E* and *Z* isomers of **22b**, **22c**, and **22d**.[15b] The preparation of ketone **1d** is reported in Scheme 2. 6-Methoxy-17β-hydroxyandrostane-4,6-diene **5**[16] was reacted with dimethylamine hydrochloride and paraformaldehyde in acetonitrile to give a mixture of **6** and **7** which on treatment with methyl iodide in acetonitrile gave the exomethylene derivative **7** in 83% yield from **5**. Hydrolysis of **7** with 3 N HCl in dioxane gave the diketo derivative **8** in 55% yield. This compound was hydrogenated over PtO_2 in ethyl acetate to give a mixture of keto/hydroxy derivatives **9** that was oxidized with *o*-iodoxybenzoic acid (IBX)[17] in acetonitrile to give the desired 2α-methylandrostane-3,6,17-trione **1d** in 53% yield from **8**. In Scheme 3, compound **5** was reacted with $NaBH_4$ in MeOH in the presence of $CeCl_3$ to give **10** (93% yield) which was in turn treated with 0.5 N HCl in THF to give the α,β-unsaturated ketone **11** in 70% yield. **11** was alkylated in position 4 with $LiCuMe_2$ and Me_3SiCl in Et_2O/THF to give the 4α-Me derivative **12** in 36% yield. Finally, the required 4α-methylandrostane-3,6,17-trione **1e** was obtained in 76% yield by oxidation with IBX in acetonitrile. The reaction between ketones **1d** or **1e** and 2-aminoethoxyamine gave only the pure isomers **22c** and **22d**, respectively, as expected.

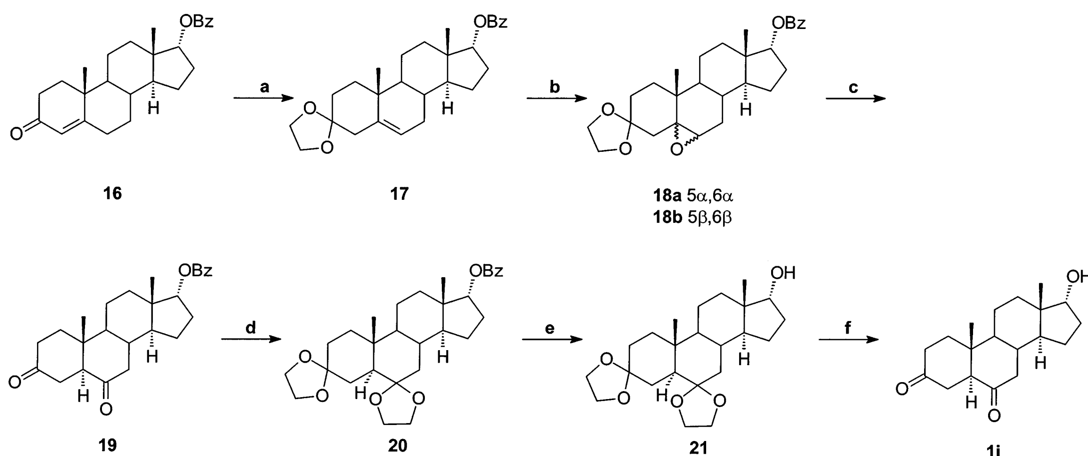
In Scheme 4, **1b** was reacted with ethylene glycol in the presence of *p*TSA in refluxing toluene, to give the protected diketone **13** in 99% yield; oxidation of the 6α-hydroxy group with IBX in DMSO gave **14** in 94% yield. **14** was reduced with $NaBH_4$ in MeOH to obtain **15** in 97% yield; deprotection with 1 N HCl in dioxane

Scheme 4[a]



[a] Reagents and conditions. a: ethylene glycol, *p*TSA, toluene, reflux; b: IBX, DMSO, room temperature; c: $NaBH_4$, MeOH, 0 °C; d: 3 N HCl, dioxane, room temperature.

Scheme 5[a]

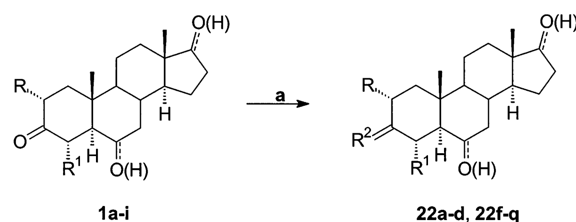


[a] Reagents and conditions. a: ethylene glycol, *p*TSA, toluene, reflux; b: *m*CPBA, CH_2Cl_2, 0 °C to room temperature; c: 4% H_2SO_4, MeOH, reflux; d: ethylene glycol, *p*TSA, toluene, reflux; e: MeLi, Et_2O/THF, −5 °C; f: *p*TSA, acetone, room temperature.

gave the required 6β-hydroxyandrostane-3,17-dione **1f** in 60% yield.

17α-Hydroxyandrostane-3,6-dione **1i** was synthesized as reported in Scheme 5 by using a protection/deprotection approach starting from the known 17α-benzoate **16**[18] (prepared from testosterone in a slightly different manner than reported) because a more straightforward path, such as the Mitsunobu inversion of the 17β-hydroxy group in compound **1h** (Figure 3), was impossible to achieve, even on differently protected derivatives. From **16**, the ethylenedioxy derivative **17** was obtained by reaction with ethylene glycol in the presence of *p*TSA, in toluene at reflux temperature (66% yield); this compound was oxidized with *m*CPBA in CH_2Cl_2 at room temperature to give a mixture of isomeric epoxides **18a,b** in a roughly equal ratio and 97% yield. A small amount of the crude mixture was purified by flash chromatography to give the two pure epoxides **18a** and **18b**. By acidic treatment (4% H_2SO_4 in MeOH, reflux temperature) each isomer gave the diketo derivative **19**; therefore, the reaction was carried out on the crude mixture to give **19** in 60% yield. Attempts to deprotect the 17α-hydroxy group with basic hydrolysis in various conditions caused extensive degradation of the starting compound or isomerization at the 5 position giving mixtures of 5α/5β derivatives; on the other hand, we found the benzoate group resistant to acidic hydrolysis. For these reasons, we were forced to protect again the keto groups as ethylenedioxy derivatives obtaining **20** in quantitative yield. This derivative was then reacted with MeLi in Et_2O/THF at −5 °C to give the 17α-hydroxy derivative **21** in 83% yield. Finally, **21** was deprotected with *p*TSA in acetone to give the desired diketo derivative **1i** in 57% yield.

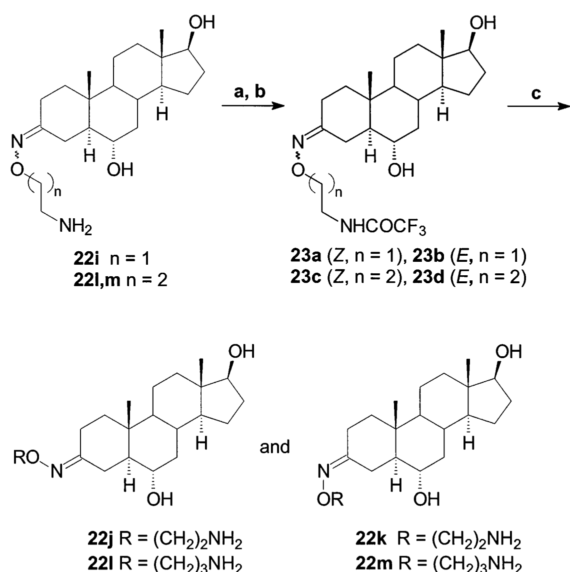
Scheme 6[a]



[a] Reagents and conditions. a: 2-*O*-(dimethylaminoethyl)- or 3-*O*-(aminopropyl)- or 2-*O*-(aminoethyl)hydroxylamine·2HCl, THF/water, room temperature.

Scheme 6 reports the general coupling reaction between the ketosteroids **1a**−**i** and the appropriate (*O*-aminoalkyl)hydroxylamine dihydrochlorides[8] in a THF/water solution at room temperature, to give **22a**−**d**,

Scheme 7[a]



[a] Reagents and conditions. a: trifluoroacetic anhydride, THF, room temperature; then 5% $NaHCO_3$, MeOH, 35 °C; b: flash chromatography; c: K_2CO_3, MeOH/water, room temperature.

22f−**q** (Table 1). It is worth noting the very good regioselectivity of this reaction that, when in the presence of three or two keto groups, led to the derivative at the 3 position with only traces of dioxime side products. Compound **22e** was obtained by acetylation (acetic anhydride/pyridine in methylene chloride) of **22b**. As mentioned above, the oximes were obtained as mixtures of *E* and *Z* isomers that proved to be inseparable by crystallization or flash chromatography. In the case of 16α,17β-diols (**22j**,**k** and **22l**,**m**) the separation was achieved by protection of the amino group as trifluoroacetamide, as described in Scheme 7; the aminoalkyloximes were reacted with trifluoroacetic anhydride in THF and then stirred with 5% aqueous $NaHCO_3$ in MeOH overnight (the hydroxy groups were deprotected by hydrolysis of the trifluoroacetates) to give, after separation by flash chromatography, **23a** (*Z* isomer, 22% yield), **23b** (*E* isomer, 40% yield), and **23c** (*Z* isomer, 25% yield), **23d** (*E* isomer, 41% yield); the separated isomers were deprotected with K_2CO_3 in MeOH/water to give the *E* (**22j** and **22l**) and *Z* (**22k** and **22m**) isomers as pure compounds.

Results and Discussion

All compounds were tested in vitro for their inhibitory activity on purified dog kidney Na^+,K^+-ATPase, measured by ^{32}P-ATP hydrolysis method (see data in Table 1).[19,20] Some compounds showing high inhibitory potency in vitro were investigated in vivo for their inotropic activity and lethal effect by slow intravenous infusion in anesthetized guinea pigs (the results are reported in Table 2). Digoxin was chosen as reference compound because it is the most commonly prescribed cardiac glycoside in the treatment of congestive heart failure (CHF).

The following structure−activity relationships (SAR) are based on the in vitro data shown in Table 1. In the model described above, the keto or hydroxy functions in positions C-6 and C-17 are particularly significant. With regard to the stereochemistry at position C-6, the model predicts that a beta hydroxyl group should have a lower inhibitory potency than an alpha hydroxyl group because the 6β stereochemistry places the hydrophilic hydroxy group in a hydrophobic region of the pharmacophore, while a 6α is located in a similar position to the keto group. In agreement with this prediction the 6β-hydroxy derivative has an inhibitory potency of 1 order of magnitude less than the corresponding 6-keto derivative (**22n** vs **22b**), while the 6α-hydroxy analogue gave a lower decrease (**22g** vs **22b**). The reduction of the keto group in position 17 gave compounds with lower potency: 17β-hydroxy derivatives showed a slightly lower inhibition when compared to the corresponding 17-keto derivatives (**22p** vs **22b**, **22i** vs **22g**, and **22o** vs **22n**), while the reduction to the 17α-hydroxy group resulted in a strikingly lower inhibitory potency (**22q** vs **22b**).

Table 2. Inotropic and Toxic Effects in Anesthetized Guinea Pig

compd	E_{max}[a] % increase in $+dP/dt_{max}$	ED_{max}[b] μmol/kg	ED_{80}[c] μmol/kg	dead/treated	lethal dose/ED_{80}
22b	190	17.90	4.84	7/8	20.2
22f	129	18.20	6.55	1/3	n.c.[d]
22g	207	54.60	5.30	1/5	17.8
22j	158	4.13	0.97	3/3	9.6
22l	99	7.37	1.58	1/3	n.c.
22n	154	111.20	14.80	1/3	9.2
digoxin	127	0.97	0.41	10/10	3.2

[a] Maximal increase in $+dP/dt_{max}$. [b] Dose inducing maximum positive inotropic effect. [c] Inotropic potency: dose increasing $+dP/dt_{max}$ by 80%, calculated from dose−response curves. [d] Not calculated.

On the basis of our model, a higher inhibition was predicted for the *E* isomer of oximes compared with the corresponding *Z* isomer. In the case of 6α,17β-dihydroxy derivatives (**22j**−**m**), it was possible to separate the *E* and *Z* isomers. The compounds with the aminoethoxy chain showed noticeable difference in the inhibitory potency between *E* (**22j**) and *Z* (**22k**) isomers, the *E* being the more potent of the two as predicted by the model. Comparison of the derivatives with the aminopropoxy chain (**22l** and **22m**) shows a smaller difference in the Na^+,K^+-ATPase inhibition than those with the aminoethoxy chain (**22j** and **22k**). Among the aminopropoxy derivatives the *E,Z* isomeric difference may not be relevant because increased conformational freedom in the chain may allow the amino group to reach an appropriate conformation in the *Z* isomer (**22m**) also. As described above, since it was impossible to achieve a separation of the isomers of compounds having 6,17-diketo groups such as **22b**, we introduced a methyl group in the equatorial position 2α to give **22c**, the derivative corresponding to the *Z* isomer of **22b** (**22c** is actually an *E* isomer, because the substituted C-2 has a higher priority than C-4 in this case); the same applies for compound **22d**, where an equatorial 4α-methyl group was introduced to give the *E* derivative. Our prediction proved to be correct because **22d** was more potent than **22c**, even though the presence of the methyl group caused a relative decrease of inhibition when compared with the *E,Z* analogue **22b**. Compound **22f** showed that an augmented chain length brought about a reduced activity (**22f** vs **22b**). By comparing the length of the alkoxyimino chain in position 3, differences were not found in the dihydroxy *E* derivatives (**22j** vs **22l**); on

the contrary, differences were found in the corresponding *Z* derivatives (**22k** vs **22m**); again, the above-mentioned hypothesis can explain these results, i.e., an increased conformational freedom of the chain permits proper recognition of the receptor in the case of less potent compounds. The contrasting results of the different chains in position 3 could be due to subtle adjustments of the ligands at the receptor, depending on the different substitutions in position 6.

As reported in our preceding papers,[4,8] also in this series a stronger (about 2 orders of magnitude) inhibition of Na^+,K^+-ATPase could be obtained with amino chains ending with a primary amino group in comparison with a tertiary one, as demonstrated by **22b** vs **22a** and **22i** vs **22h**. With the aim to demonstrate the importance of a basic group at the end of the chain also in this class of inhibitors, as previously reported for the other classes in our cited papers, we prepared the acetamide derivative **22e**; the expected reduction of inhibition was about 2 orders of magnitude in comparison with the parent compound **22b**.

It should be noted that the most potent compound **22b** (PST 2744) showed a higher inhibitory potency than cassaine, digitoxigenin, and the reference compound digoxin (see Table 1).

Taken as a whole, the high potencies displayed by some compounds and SAR evidence reported here for the inhibition of Na^+,K^+-ATPase are a validation of our model based on superposition of cassaine and (trans, trans, trans) reversed steroids. Furthermore, the introduction in position 3 of the aminoalkyloxime chain together with the oxygenated functions at positions 6 and 17 onto the flat steroid skeleton, generated a new series of potent inhibitors of Na^+,K^+-ATPase. The higher potencies shown by some *E* isomers vs the corresponding *Z* isomers and by the 6-keto and 6α-hydroxy derivatives vs the corresponding 6β-hydroxy compounds further validate our model. Some compounds showing higher inhibitory potency on the isolated enzyme were investigated in vivo in the anesthetized guinea pig (Table 2). Even though none of them showed inotropic potencies (ED_{80}) comparable to digoxin, some displayed safety ratios (LD/ED_{80}) higher than digoxin, namely, compounds **22b** and **22g**. Due to its potency and safety, about six times higher than digoxin, in infused guinea pig, **22b** was chosen to be studied in depth for its inotropic activity and safety in different experimental models; results are summarized here and fully reported elsewhere.[22] Intravenous infusion of **22b** produced a positive inotropic effect at doses substantially lower than those causing cardiac toxicity, without significantly affecting other hemodynamic parameters. Given *iv* in dogs, **22b** combined positive inotropic activity with a favorable hemodynamic profile (no effect on heart rate, reduction of peripheral resistance), absence of alterations of cardiac rhythm and conduction system, fast/high reversibility of action and improved safety index. Preliminary investigations on the pharmacokinetic, electrophysiological, and receptor interaction properties of **22b** indicate that its profile positively differs from that of cardiac glycosides.

Conclusions

On the basis of the new model derived from superposition of cassaine and digitoxigenin, previously proposed by our group, we designed a new series of compounds starting from a planar steroidal skeleton, in contrast to the bent skeleton of digitalis-like compounds, substituted in a reversed orientation. By introducing an aminoalkoxyimino chain in position 3 and oxo or hydroxy groups at position 6 and 17, we obtained compounds having inhibitory activity on the Na^+,K^+-ATPase comparable with that of digoxin, and with positive inotropic activity, thus confirming the value of our model. Most importantly, some of the compounds also displayed a higher safety index than digoxin. From preliminary pharmacological data, PST 2744 (**22b**) emerged as a very promising novel positive inotropic compound that may represent an innovative alternative to digitalis in the treatment of CHF and is a candidate for Phase I clinical trials.

Experimental Section

General Details. General experimental details for the chemistry and pharmacological parts have been reported elsewhere.[8]

Chemistry. Androstane-3β,6α,17β-triol (3) and **5β-androstane-3β,6β,17β-triol (4).** To a stirred solution of dehydroepiandrosterone (**2**) (30.0 g, 104 mmol) in THF (450 mL) at −10 °C, under N_2, was added 1 M BH_3·THF complex in THF (260 mL, 260 mmol). After completing the addition, the mixture was stirred at room temperature for 3 h. H_2O (500 mL) was cautiously added dropwise followed by $NaBO_3$·$4H_2O$ (31.4 g, 204 mmol). After stirring at room temperature overnight, the mixture was filtered. The solid was washed with THF and then discarded. The two liquid phases were separated and the aqueous layer was saturated with NaCl and extracted with THF (3 × 200 mL). The combined organic extracts were dried over NaCl and Na_2SO_4, filtered, and evaporated to dryness. The crude product was crystallized from EtOAc/MeOH (2/1, 10 mL/g) to give a first crop as a white solid (12.5 g). The mother liquors were evaporated and the residue crystallized from EtOAc/MeOH (2/1, 10 mL/g) to give a second crop as a white solid (6.4 g). The procedure was repeated to give a third crop as a white solid (2.1 g; 75% overall yield). **3**: ^{1}H NMR (DMSO-d_6) δ 0.60 (s, 3H, CH_3), 0.72 (s, 3H, CH_3), 3.12 (m, 1H, 6-H), 3.26 (m, 1H, 3-H), 3.42 (dt, 1H, 17-H), 4.24 (d, 1H, OH), 4.42 (m, 1H, OH), 4.44 (m, 1H, OH); mp 232−234 °C. The residue from the final mother liquors was purified by flash chromatography (EtOAc) to give **4** (4.0 g, 12.5%), a white solid. **4** ^{1}H NMR (acetone-d_6) δ 0.61 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 3.40 (m, 2H, 6-H, 17-H), 3.81 (m, 1H, 3-H), 4.21 (d, 1H, OH), 4.30 (m, 1H, OH), 4.42 (m, 1H, OH).

Androstane-3,6,17-trione (1a). Jones oxidation: to a solution of androstane-3β,6α,17β-triol **(3)** (21.0 g, 68 mmol) in acetone (380 mL) was added an excess of Jones reagent (83.5 mL) dropwise, maintaining the temperature below 40 °C. 5 min after completion of the addition, i-PrOH (10 mL) was added and, after further 10 min, the suspension was filtered and the filtrate evaporated to dryness. The residue was treated with H_2O (300 mL) and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with H_2O (100 mL), 5% aqueous $NaHCO_3$ solution (100 mL), H_2O (100 mL), dried over Na_2SO_4 and evaporated to dryness to give **1a** as a white solid (15.3 g, 74%); a sample was crystallized from i-PrOH to give a white solid: mp 195−197 °C. (lit:[10b] 196−196.5 °C).

RuO_2/$NaBrO_3$ oxidation: to a solution of $NaBrO_3$ (22.0 g, 146 mmol) in H_2O (200 mL), RuO_2·$2H_2O$ (0.82 g, 4.87 mmol) and EtOAc (400 mL) were added. After stirring for 10 min, **3** (10.0 g, 32.5 mmol) was added, while maintaining the temperature below 30 °C. After 1 h, i-PrOH (40 mL) was added dropwise. 10 min after the addition, the mixture was filtered through a Celite pad and the filter cake was washed with EtOAc (400 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with brine (100 mL), 5% aqueous

$NaHCO_3$ solution (50 mL), brine (100 mL), dried over Na_2SO_4, and evaporated to dryness to give **1a** as a white solid (9.33 g, 95%); a sample was crystallized from i-PrOH to give a white solid: mp 196−197 °C.

6α-Hydroxyandrostane-3,17-dione (1b). To a stirred solution of **3** (15.0 g, 48.6 mmol) in acetone/H_2O/pyridine (1485/300/8 mL), NBS (freshly crystallized from H_2O; 14.45 g, 81.05 mmol) was added. After 8 h, NBS (freshly crystallized from H_2O; 14.45 g, 81.05 mmol) was added and after stirring at room temperature for 24 h, the solution was quenched with conc HCl (1.5 mL) to pH 3 and, after 10 min, NaOH (small beads, 4.66 g, 0.116 mmol) was added to pH 8. The organic solvent was evaporated and the aqueous phase was extracted with EtOAc (4 × 350 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness to give a brown solid. The crude product was triturated with H_2O (2 × 250 mL) and filtered. After drying under vacuum at 40 °C, **1b** (12.58 g, 85%) was obtained as an off-white solid. ^{1}H NMR (acetone-d_6) δ 0.86 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 3.48 (m, 1H, 6-H), 3.61 (d, 1H, OH); a sample was crystallized from EtOAc to give a white solid: mp 204−206 °C (lit.[11] mp 206−207 °C).

6α,17β-Dihydroxyandrostan-3-one (1c). To a solution of **3** (3.00 g, 9.72 mmol) in CH_2Cl_2/acetone/H_2O (300/150/6 mL), activated MnO_2 (30.0 g, 345 mmol) was added in three portions over 8 h. The mixture was stirred at 45 °C overnight. After cooling the sample to room temperature, the mixture was filtered through Celite. The filtrate was evaporated and the residue was purified by flash chromatography (CH_2Cl_2/*n*-hexane/i-PrOH 10/5/1) to give **1c** (0.89 g, 30%), a white solid. ^{1}H NMR (acetone-d_6) δ 0.75 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 3.43 (m, 1H, H-6), 3.51 (d, 1H, 6-OH), 3.58 (m, 2H, 17H and 17-OH); a sample was crystallized from EtOAc to give a white solid: mp 207−208 °C (lit.[12] mp 208−210 °C).

2-Methylene-6-methoxy-17β-hydroxyandrostane-4,6-dien-3-one (7). A mixture of 6-methoxy-17β-hydroxyandrostane-4,5-dien-3-one[16] (**5**) (4.00 g, 12.6 mmol), paraformaldehyde (0.48 g, 15.98 mmol), and dimethylamine hydrochloride (1.28 g, 15.7 mmol) in acetonitrile (80 mL) was heated to reflux under stirring for 7 h. After cooling, the solution was evaporated and the residue was dissolved in an EtOAc/saturated aqueous Na_2HPO_4 mixture. The layers were separated, and the aqueous phase was extracted with EtOAc (3×). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness. The crude product was dissolved in acetonitrile (100 mL) and CH_3I (0.40 mL, 6.3 mmol) was added. After stirring of the sample at room temperature for 2.5 h, the solution was evaporated to dryness and the yellow crude product was purified by flash chromatography (*n*-hexane/CH_2Cl_2/acetone 6/2/2) to give **7** (3.48 g, 83%), a off-white solid. ^{1}H NMR ($CDCl_3$) δ 0.85 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 3.59 (s, 3H, 5-OCH_3), 3.71 (t, 1H, 17-H), 5.14 (d, 1H, 7-H), 5.29 (t, 1H), 6.08 (dd, 1H), 6.38 (s, 1H, 4-H).

2-Methylene-17β-hydroxyandrostane-4-en-3,6-dione (8). A solution of **7** (3.00 g, 9.09 mmol) and 3 N HCl (3 mL) in dioxane (40 mL) was heated at 80 °C under stirring for 0.5 h. The brown solution was cooled with an ice bath and then quenched by careful addition of a saturated aqueous Na_2HPO_4 solution (50 mL) and brine (20 mL). The mixture was extracted with Et_2O (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and charcoal, filtered, and evaporated to dryness to give **8** (1.60 g, 55%), a off-white solid sufficiently pure to be used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 3.68 (t, 1H, 17-H), 5.38 (m, 1H), 6.11 (m, 1H), 6.42 (bs, 1H, 4-H).

2α-Methylandrostane-3,6,17-trione (1d). A mixture of **8** (1.60 g, 5.0 mmol) and PtO_2 (0.15 g) in EtOAc was stirred under H_2 at atm pressure for 4 h. The mixture was filtered through Celite and the filtrate evaporated to dryness. A mixture of the residue and IBX (2.80 g, 10.0 mmol) in acetonitrile (80 mL) was heated at reflux under stirring for 5 h. The mixture was cooled to room temperature and filtered through Celite. The filtrate was evaporated and the residue was purified by flash chromatography (*n*-hexane/EtOAc 6/4) to give **1d** as a white solid (0.75 g, 53%). ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 3H, CH_3), 1.04 (d, 3H, 2-CH_3), 1.07 (s, 3H, CH_3).

6-Methoxyandrostane-4,6-dien-3β,17β-diol (10). To a solution of $CeCl_3{\cdot}7H_2O$ (3.00 g, 9.4 mmol) and **5** (3.51 g, 9.4 mmol) in MeOH (300 mL) at −5 °C, $NaBH_4$ (0.40 g, 10.6 mmol) was added in five portions over 15 min. The solution was stirred at room temperature for 2 h and quenched with AcOH (2.2 mL, 40 mmol). The mixture was concentrated under reduced pressure, treated with H_2O (100 mL), and extracted with $CHCl_3$ (3 × 100 mL). The combined organic extracts were evaporated to dryness to give **10** as a white solid (2.80 g, 93%). ^{1}H NMR (CD_3OD) δ 0.81 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 3.52 (s, 3H, 6-OCH_3), 3.61 (t, 1H, 17-H), 4.20 (ddd, 1H, 3-H), 4.71 (bs, 1H, 7-H), 5.89 (bs, 1H, 4-H).

3β,17β-Dihydroxyandrostan-4-en-6-one (11). A solution of **10** (2.80 g, 8.8 mmol) and 0.5 N HCl (12 mL) in THF (90 mL) was stirred at room temperature for 45 min and then quenched by addition of saturated Na_2HPO_4 aqueous solution (20 mL). The mixture was extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, and evaporated to dryness to give **11** (2.00 g, 70%), a white solid. ^{1}H NMR (CD_3OD) δ 0.75 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 3.62 (t, 1H, 17-H), 4.19 (m, 1H, 3-H), 6.12 (m, 1H, 4-H).

4α-Methyl-3β,17β-dihydroxyandrostan-6-one (12). To a suspension of CuI (31.8 mg, 167 mmol) in Et_2O (600 mL) at −5 °C under N_2 was added dropwise a 1.6 M MeLi solution in Et_2O (207 mL, 331 mmol). After stirring of the sample at 0 °C for 10 min, the solution was cooled at −10 °C, and Me_3SiCl (106 mL, 860 mmol) was added dropwise, followed by a solution of **11** (3.43 g, 11.1 mmol) in THF (120 mL). After stirring of the sample at 0 °C for 2 h, the solution was poured into cold 1 N HCl. EtOAc (100 mL) was added and the mixture was filtered through Celite. After separation of the organic layer, the aqueous phase was extracted with EtOAc (3×). The combined organic extracts were washed with a saturated aqueous Na_2HPO_4 solution, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2/acetone 90/10). After evaporation of the fractions containing **12**, the residue was crystallized from acetone to give **12** (1.31 g, 36%), a white solid. ^{1}H NMR (CD_3OD) δ 0.73 (s, 3H, CH_3), 0.76 (s, 3H, CH_3), 0.95 (d, 3H, 4-CH_3), 2.25 (d, 1H, 5-H), 3.05 (dt, 1H, 3-H), 3.71 (t, 1H, 17-H).

4α-Methylandrostane-3,6,17-trione (1e). A mixture of **12** (1.36 g, 4.2 mmol) and IBX (2.35 g, 8.4 mmol) in acetonitrile (80 mL) was stirred at reflux for 5 h. After cooling of the sample to room temperature, the mixture was filtered through Celite. The solution was evaporated and the crude product was purified by flash chromatography (*n*-hexane/EtOAc 6/4) to give **1e** as a white solid (1.02 g, 76%). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H, CH_3), 1.02 (d, 3H, 4-CH_3), 1.02 (s, 3H, CH_3), 2.77 (dq, 1H, 4-H).

3,3:17,17-Bis(ethylendioxy)androstan-6α-ol (13). A solution of **1b** (5.00 g, 16.5 mmol), ethylene glycol (37 mL, 610 mmol) and *p*TSA (0.219 g, 11.4 mmol) in toluene (530 mL) was stirred at reflux for 12 h with a Dean−Stark trap. After cooling to room temperature, the mixture was neutralized with 5% aqueous $NaHCO_3$ solution. The organic layer was separated and washed with H_2O (2 × 350 mL), dried over Na_2SO_4 and evaporated to dryness to give **13** as a white solid (6.40 g, 99%). ^{1}H NMR (DMSO-d_6) δ 0.73 (s, 3H, CH_3), 0.74 (s, 3H, CH_3), 3.11 (m, 1H, 6-H), 3.88−3.70 (m, 8H), 4.25 (d, 1H, OH).

3,3:17,17-Bis(ethylendioxy)androstane-6-one (14). A solution of **13** (0.75 g, 1.91 mmol) and IBX (0.88 g, 2.87 mmol) in DMSO (10 mL) was stirred at room temperature for 3 h and then quenched by addition of H_2O (50 mL). After stirring for 15 min, the mixture was filtered and the cake was washed with EtOAc. The layers were separated, and the aqueous phase was extracted with EtOAc (3×). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness to give **14** as a white foam (0.70 g, 94%). ^{1}H NMR (acetone-d_6) δ 0.75 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 2.55 (m, 1H, 5-H), 3.95−3.75 (m, 8H).

3,3:17,17-Bis(ethylendioxy)androstan-6β-ol (15). To a stirred suspension of **14** (1.50 g, 3.82 mmol) in MeOH (20 mL)

at 0 °C and under N_2, $NaBH_4$ (0.22 g, 4.58 mmol) was added. After 2 h at 0 °C, H_2O (50 mL) was added dropwise. The mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated to dryness to give **15** as a white solid (1.46 g, 97%). ^1H NMR (acetone-d_6) δ 0.84 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 3.33 (d, 1H, 6-OH), 3.70 (m, 1H, 6-H), 3.95−3.75 (m, 8H, OCH_2).

6β-Hydroxyandrostane-3,17-dione (1f). 1 N HCl (2.1 mL) was added to a solution of **15** (1.46 g, 3.71 mmol) in dioxane (47.7 mL). After stirring at room temperature for 2 h, the solution was quenched by careful addition of 5% aqueous $NaHCO_3$ solution, and dioxane was evaporated. The aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with H_2O (2 × 100 mL), dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography (cyclohexane/EtOAc 1/1) to give **1f** (0.68 g, 60%), a white solid. ^1H NMR (DMSO-d_6) δ 0.81 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 3.57 (m, 1H, 6-H), 4.47 (d, 1H, OH).

Testosterone 17α-benzoate (16). To a solution of testosterone (6.00 g, 20.8 mmol) in dry toluene (168 mL) under N_2, benzoic acid (3.04 g, 25 mmol), triphenylphosphine (6.54 g, 25 mmol) and DIAD (5.05 g, 25 mmol) were added. The solution was heated at 80 °C under stirring for 1 h. After cooling of the sample, the solution was evaporated to dryness and the crude yellow oil was purified by flash chromatography (*n*-hexane/acetone 95/5) to give **16** (5.88 g, 72%), as a white solid. ^1H NMR (DMSO-d_6) δ 0.82 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 4.94 (d, 1H, 17-H), 5.63 (bs, 1H, 4-H), 8.00−7.45 (m, 5H); mp 133−135 °C (lit.[18] mp 135−137 °C).

3,3-Ethylendioxyandrost-5-en-17α-yl benzoate (17). A solution of **16** (1.71 g, 4.35 mmol), ethylene glycol (8.9 mL, 161 mmol), and *p*TSA (58 mg, 3.0 mmol) in toluene (140 mL) was stirred at reflux for 12 h with a Dean−Stark trap. After cooling to room temperature, the mixture was neutralized with 5% aqueous $NaHCO_3$ solution. The organic layer was separated, washed with H_2O (2 × 100 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was triturated with Et_2O/*n*-hexane 3/7 (10 mL), filtered, and washed with *n*-hexane to give **17** as a white foam (1.23 g, 66%). ^1H NMR (acetone-d_6) δ 0.87 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 3.95−3.80 (m, 4H), 5.03 (dd, 1H, 17-H), 5.29 (m, 1H, 6-H), 8.10−7.45 (m, 5H).

3,3-Ethylendioxy-5α,6α-epoxyandrostan-17α-yl benzoate (18a) and **3,3-ethylendioxy-5β,6β-epoxyandrostan-17α-yl benzoate (18b).** To a stirred solution of **17** (1.22 g, 2.81 mmol) in CH_2Cl_2 (7.4 mL) cooled at 0 °C, a solution of *m*CPBA (0.77 mg, 4.4 mmol) in CH_2Cl_2 (13.6 mL) was added dropwise. After 0.5 h at 0 °C and 0.5 h at room temperature, a 10% aqueous solution of Na_2SO_3 was added. The mixture was neutralized by addition of 5% $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated to dryness to give **18a** and **18b** as a white foam (1/1; 1.24 g, 97%).

Compounds **18a** and **18b** were separated by flash chromatography (cyclohexane/EtOAc 30/70) on a small sample. **18a**, a white solid: ^1H NMR (acetone-d_6) δ 0.81 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 2.72 (d, 1H, 6-H), 3.93−3.77 (m, 4H), 4.99 (dd, 1H, 17-H), 8.1−7.45 (m, 5H). **18b**, a white solid: ^1H NMR (acetone-d_6) δ 0.83 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 3.00 (d, 1H, 6-H), 3.95−3.80 (m, 4H), 5.01 (dd, 1H, 17-H), 8.1−7.4 (m, 5H).

3,6-Dioxoandrostan-17α-yl benzoate (19). To a suspension of **18a** and **18b** (1/1; 1.24 g, 2.73 mmol) in MeOH (290 mL) was added 4% aqueous H_2SO_4 (5 mL) and the mixture was stirred at reflux for 1 h. The solution was concentrated in vacuo and H_2O (100 mL) and CH_2Cl_2 (150 mL) were added. The mixture was stirred for 15 min. The layers were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic extracts were washed with a 5% aqueous $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 7/3) to give **19** as a white solid (0.67 g, 60%). ^1H NMR (acetone-d_6) δ 0.91 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 5.06 (dd, 1H, 17-H), 8.1−7.4 (m, 5H).

3,3:6,6-Bis(ethylendioxy)androstane-17α-yl benzoate (20). A solution of **19** (0.48 g, 1.17 mmol), ethylene glycol (2.42 mL, 43.47 mmol), and *p*TSA (15.3 mg, 0.08 mmol) in toluene (150 mL) was stirred at reflux for 0.5 h a with a Dean−Stark apparatus. After cooling of the sample to room temperature, the mixture was washed with 5% aqueous $NaHCO_3$ solution (50 mL), H_2O (2 × 50 mL), dried over Na_2SO_4 and evaporated to dryness to give **20** as a white foam (0.60 g, 100%). ^1H NMR (acetone-d_6) δ 0.87 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 4.00−3.65 (m, 8H), 5.04 (dd, 1H, 17-H), 8.1−7.45 (m, 5H).

3,3:6,6-Bis(ethylendioxy)androstane-17α-ol (21). To a solution of **20** (0.59 g, 1.18 mmol) in dry THF (40 mL) at −5 °C under N_2, 1.6 M MeLi in Et_2O (1.9 mL, 3.04 mmol) was added dropwise. The solution was stirred at −5 °C for 1.5 h and then quenched by careful addition of brine. The mixture was extracted with THF (3 × 50 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (*n*-hexane/EtOAc 1/1) to give **21** (0.38 mg, 83%), a white foam. ^1H NMR (acetone-d_6) δ 0.67 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 3.38 (d, 1H, OH), 3.95−3.60 (m, 9H, OCH_2 and 17-H).

17α-Hydroxyandrostane-3,6-dione (1i). A solution of **21** (380 mg, 0.97 mmol) and pTSA·H_2O (0.92 g, 4.81 mmol) in acetone (38 mL) was stirred at room temperature for 1.5 h. The solution was neutralized by addition of 5% aqueous $NaHCO_3$, and acetone was evaporated. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (*n*-hexane/EtOAc 1/1) to give **21** as a white solid (167 mg, 57%). ^1H NMR (acetone-d_6) δ 0.71 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 3.53 (d, 1H, OH), 3.71 (dd, 1H, 17-H).

General Procedure for Compounds 22a−d, 22f−i, and **22n−q.** To a stirred solution of the appropriate ketone (1 equiv) in THF (0.15 M), a solution of the appropriate aminoalkoxyamine dihydrochloride (1 equiv) in H_2O (0.30 M) was rapidly added dropwise. After 1.5 h, NaCl (7.5 equiv) was added and the mixture was stirred for 10 min. The phases were separated and the aqueous phase was extracted with THF (2×). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated to give an oily residue. The crude product was dissolved in CH_2Cl_2 (0.15 M) and washed with a saturated aqueous solution of NaCl (3×). The organic layer was dried again over Na_2SO_4 and evaporated to dryness.

(*E,Z*)-3-[2-(*N,N*-Dimethylamino)ethoxyimino]androstane-6,17-dione (22a). Prepared in 65% yield from **1a** and 2-(*N,N*-dimethylamino)ethoxyamine dihydrochloride. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH 90/10/1) and crystallized from MeOH/EtOAc. ^1H NMR (DMSO-d_6) δ 0.88 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 2.30 (s, 6-H, $N(CH_3)_2$), 2.62 (m, 2H, NCH_2), 4.13 (m, 2H, OCH_2); white solid: mp 141−145 °C. Anal. ($C_{23}H_{36}N_2O_2$) C, H, N.

(*E,Z*)-3-(2-Aminoethoxyimino)androstane-6,17-dione hydrochloride (22b). Prepared in 53% yield from **1a** and 2-aminoethoxyamine dihydrochloride. The crude product was crystallized from MeOH/EtOAc. ^1H NMR (DMSO-d_6) δ 0.78 (s, 3H, CH_3), 0.79 (s, 3H, CH_3), 3.00 (t, 2H, NCH_2), 3.08 (m, 1H, 4-H eq. *Z* isomer and 2-H eq. *E* isomer), 4.09 (t, 2H, OCH_2), 8.12 (bb, 3H, NH_3^+); white solid: mp 220−225 °C (dec). Anal. ($C_{21}H_{32}N_2O_3$·HCl) C, H, N, Cl.

(*E*)-2α-Methyl-3-(2-aminoethoxyimino)androstane-6,17-dione fumarate (22c). Prepared in 55% yield from **1d** and 2-aminoethoxyamine dihydrochloride. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH 90/10/1). To the concentrated fractions, a stoichiometric amount of fumaric acid in MeOH was added. After addition of a 1/1 mixture of EtOAc/Et_2O, the precipitate was filtered to give **22c**. ^1H NMR (DMSO-d_6) δ 0.76 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.99 (d, 3H, CH_3 eq), 2.98 (m, 2H, NCH_2), 3.10 (m, 1H, 4-H eq), 4.07 (m, 2H, OCH_2); white solid: mp 140−147 °C dec. Anal. ($C_{22}H_{34}N_2O_3$·$C_4H_4O_4$·1.5 H_2O) C, H, N, H_2O.

(*E*)-4α-Methyl-3-(2-aminoethoxyimino)androstane-6,17-dione hydrochloride (22d). Prepared in 60% yield from **1e** and 2-aminoethoxyamine dihydrochloride. The crude product was crystallized from MeOH/EtOAc. ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H, CH_3), 0.78 (s, 3H, CH_3), 0.98 (d, 3H, CH_3 eq), 2.60 (dq, 1H, 4-H ax), 3.03 (m, 2H, NCH_2), 3.12 (m, 1H, 2-H eq), 4.12 (m, 2H, OCH_2), 8.12 (bb, 3H, NH_3^+); white solid: mp 226–229 °C dec. Anal. ($C_{22}H_{34}N_2O_3$·HCl) C, H, N, Cl.

(*E,Z*)-3-(2-Acetamidoethoxyimino)androstane-6,17-dione (22e). To a stirred solution of **22b** (1.14 g, 2.9 mmol) in CH_2Cl_2 (11.5 mL) at room temperature, Ac_2O (0.54 mL, 5.7 mmol) and pyridine (0.46 mL, 5.7 mmol) were added. The solution was stirred at room temperature for 4 h and evaporated. The residue was dissolved with EtOAc (100 mL), washed with 1 N HCl (1 × 20 mL) and brine (4 × 20 mL). The organic solution was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (cyclohexane/CH_2Cl_2/acetone 2/4/4) to give **22e** (0.80 g, 69%). ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 1.76 (s, 1.5H, $COCH_3$), 1.77 (s, 1.5H, $COCH_3$) 2.96 (m, 0.5H, 4-H eq *Z* isomer), 3.03 (m, 0.5H, 2-H eq *E* isomer), 3.22 (m, 2H, NCH_2), 3.88 (m, 2H, OCH_2), 7.88 (bb, 1H, NH); white solid: mp 101–103 °C. Anal. ($C_{23}H_{34}N_2O_4$·0.5 H_2O) C, H, N, H_2O.

(*E,Z*)-3-(3-Aminopropoxyimino)androstane-6,17-dione hydrochloride (22f). Prepared in 73% yield from **1a** and 3-aminopropoxyamine dihydrochloride. The crude product was crystallized from MeOH/EtOAc. ^{1}H NMR (DMSO-d_6) δ 0.74 (s, 3H, CH_3), 0.75 (s, 3H, CH_3), 2.81 (m, 2H, NCH_2), 2.95 (m, 0.5H, 4-H eq *Z* isomer), 3.00 (m, 0.5H, 2-H eq *E* isomer), 3.99 (m, 2H, OCH_2), 7.85 (bb, 3H, NH_3^+); white solid: mp 83.5–139.5 °C. Anal. ($C_{22}H_{34}N_2O_3$·HCl) C, H, N, Cl.

(*E,Z*)-3-(2-Aminoethoxyimino)-6α-hydroxyandrostane-17-one hydrochloride (22g). Prepared in 70% yield from **1b** and 2-aminoethoxyamine dihydrochloride. The crude product was crystallized from MeOH/EtOAc. ^{1}H NMR (DMSO-d_6) δ 0.77 (s, 3H, CH_3), 0.86 (s, 1.5H, CH_3), 0.87 (s, 1.5H, CH_3), 3.02 (t, 2H, NCH_2), 3.06 (m, 0.5H, 2-H eq. *E* isomer), 3.25 (m, 1H, 6-H), 3.45 (m, 0.5H, 4-H eq. *Z* isomer), 4.08 (t, 2H, OCH_2), 4.52 (bb, 1H, OH), 7.99 (bb, 3H, NH_3^+); white solid: mp 152–155 °C. Anal. ($C_{21}H_{34}N_2O_3$·HCl·H_2O) C, H, N, Cl, H_2O.

(*E,Z*)-3-[2-(*N,N*-Dimethylamino)ethoxyimino]androstane-6α,17*β*-diol (22h). Prepared in 80% yield from **1c** and 2-(*N,N*-dimethylamino)ethoxyamine dihydrochloride. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH 90/10/1) to give **22h**. ^{1}H NMR (CD_3OD) δ 0.75 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 2.30 (s, 6H, $N(CH_3)_2$), 2.64 (m, 2H, NCH_2), 3.58 (m, 1H, 17-H), 4.11 (m, 2H, OCH_2); white solid: mp 102–106 °C. Anal. ($C_{23}H_{40}N_2O_3$·H_2O) C, H, N, H_2O.

(*E,Z*)-3-(2-Aminoethoxyimino)androstane-6α,17*β*-diol oxalate (22i). Prepared in 85% yield from **1c** and 2-aminoethoxyamine dihydrochloride. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH 90/10/1). To the concentrated fractions a stoichiometric amount of oxalic acid in MeOH was added. After addition of a 1/1 mixture of EtOAc/Et_2O, the precipitate was filtered to give **22i**. ^{1}H NMR (DMSO-d_6) δ 0.61 (s, 3H, CH_3), 0.83 (s, 1.5H, CH_3), 0.84 (s, 1.5H, CH_3), 3.04 (t, 2H, NCH_2), 3.07 (m, 0.5H, 2-H eq. *E* isomer), 3.20 (m, 1H, 6-H), 3.42 (m, 1.5H, 4-H eq. *Z* isomer and 17-H), 4.06 (t, 2H, OCH_2), 7.71 (bb, 3H, NH_3^+); white solid: mp 151–156 °C. Anal. ($C_{21}H_{36}N_2O_3$·$C_2H_2O_4$·H_2O) C, H, N, H_2O.

(*Z*)-3-(2-Trifluoroacetamidoethoxyimino)androstane-6α,17*β*-diol (23a) and **(*E*)-3-(2-trifluoroacetamidoethoxyimino)androstane-6α,17*β*-diol (23b).** To a suspension of (*E,Z*) 3-(2-aminoethoxyimino)androstane-6α,17*β*-diol hydrochloride **(22i)** (1.10 g, 2.77 mmol) in THF (40 mL) at 0 °C, $(CF_3CO)_2O$ (0.86 mL, 6.18 mmol) was added dropwise. After stirring at room temperature overnight, the mixture was diluted with EtOAc (100 mL) and washed with a 5% aqueous $NaHCO_3$ solution (2 × 120 mL), H_2O (100 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. To the crude product, MeOH (150 mL) and 5% aqueous $NaHCO_3$ solution (40 mL) were added. After stirring at 35 °C overnight, MeOH was evaporated and the residue was extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with H_2O (2 × 100 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2/EtOAc/acetone 7/2/1) to give **23a** as a white foam (0.28 g, 22%) and **23b** as a white foam (0.50 g, 40%). **23a**: ^{1}H NMR ($CDCl_3$) δ 0.73 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 3.48 (m, 1H, 6-H), 3.55 (m, 1H, 4-H eq.), 3.61 (t, 2H, NCH_2), 3.64 (m, 1H, 17-H), 4.14 (m, 2H, OCH_2), 7.40 (bb, 1H, CONH). **23b**: ^{1}H NMR ($CDCl_3$) δ 0.73 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 3.12 (m, 1H, 2-H eq), 3.48 (m, 1H, 6-H), 3.61 (m, 2H, NCH_2), 3.64 (m, 1H, 17-H), 4.14 (m, 2H, OCH_2), 7.30 (bb, 1H, CONH).

(*E*)-3-(2-Aminoethoxyimino)androstane-6α,17*β*-diol hemifumarate (22j). To a solution of **23b** (0.50 g, 1.08 mmol) in MeOH (6.6 mL) and H_2O (4.0 mL), K_2CO_3 (0.75 g, 5.42 mmol) was added. After stirring of the sample at room temperature overnight, the solution was diluted with CH_2Cl_2 (50 mL) and 1 N NaOH (5 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was dissolved in MeOH (4 mL) and fumaric acid (126 mg, 1.08 mmol) and Et_2O (2 mL) were added. The precipitate was filtered to give **22j** (0.415 g, 80%). ^{1}H NMR (DMSO-d_6) δ 0.61 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 3.08 (m, 3H, NCH_2 and 2-H eq.), 3.21 (m, 1H, 6-H), 3.42 (m, 1H, 17-H), 4.08 (t, 2H, OCH_2), 7.78 (bb, 3H, NH_3^+); white solid: mp 110–180 °C. Anal. ($C_{21}H_{36}N_2O_3$·0.5 $C_4H_4O_4$·1.5 H_2O) C, H, N, H_2O.

(*Z*)-3-(2-Aminoethoxyimino)androstane-6α,17*β*-diol hemifumarate (22k). Prepared in 75% yield from **23a** by the procedure described above for the preparation of **22j**. ^{1}H NMR (DMSO-d_6) δ 0.61 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 2.89 (m, 2H, NCH_2), 3.21 (m, 1H, 6-H), 3.44 (m, 2H, 17-H and 4-H eq), 3.99 (t, 2H, OCH_2); white solid: mp 180–183 °C. Anal. ($C_{21}H_{36}N_2O_3$·0.5 $C_4H_4O_4$·H_2O) C, H, N, H_2O.

(*Z*)-3-(3-Trifluoroacetamidopropoxyimino)androstane-6α,17*β*-diol (23c) and **(*E*)-3-(3-trifluoroacetamidopropoxyimino)androstane-6α,17*β*-diol (23d).** Prepared from **1c** and 3-aminopropoxyamine dihydrochloride by the general procedure described above to give (*E,Z*)-3-(3-aminopropoxyimino)androstane-6α,17*β*-diol. Compounds **23c** and **23d** were prepared from (*E,Z*)-3-(3-aminopropoxyimino)androstane-6α,17*β*-diol by the general procedure described above for the preparation of **23a** and **23b**. **23c** (0.40 g, 25%), a white foam: ^{1}H NMR (MeOD) δ 0.74 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 3.35 (t, 2H, NCH_2), 3.37 (m, 1H, 6-H), 3.57 (m, 1 H, 4-H eq.), 3.58 (m, 1H, 17-H), 4.02 (m, 2H, OCH_2). **23d** (0.66 g, 41%), a white foam: ^{1}H NMR (MeOD) δ 0.74 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 3.18 (m, 1H, 2-H eq), 3.35 (t, 2H, NCH_2), 3.37 (m, 1H, 6-H), 3.58 (m, 1H, 17-H), 4.02 (m, 2H, OCH_2).

(*E*)-3-(2-Aminopropoxyimino)androstane-6α,17*β*-diol hemifumarate (22l). Prepared in 82% yield from **23d** by the procedure described above for the preparation of **22j**. ^{1}H NMR (DMSO-d_6) δ 0.61 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 2.75 (m, 2H, NCH_2), 2.98 (m, 1H, 2-H eq), 3.21 (m, 1H, 6-H), 3.41 (m, 1H, 17-H), 3.98 (m, 2H, OCH_2), 7.78 (bb, 3H, NH_3^+); white solid: mp 147–181 °C. Anal. ($C_{22}H_{38}N_2O_3$·0.5 $C_4H_4O_4$·0.5 H_2O) C, H, N, H_2O.

(*Z*)-3-(2-Aminopropoxyimino)androstane-6α,17*β*-diol hemifumarate (22m). Prepared in 80% yield from **23c** by the procedure described above for the preparation of **22j**. ^{1}H NMR (DMSO-d_6) δ 0.61 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 2.75 (m, 2H, NCH_2), 3.21 (m, 1H, 6-H), 3.40 (m, 2H, 17-H and 4-H eq), 3.98 (m, 2H, OCH_2), 7.80 (bb, 3H, NH_3^+); white solid: mp 125–174 °C. Anal. ($C_{22}H_{38}N_2O_3$·0.5 $C_4H_4O_4$·H_2O) C, H, N, H_2O.

(*E,Z*)-3-(2-Aminoethoxyimino)-6*β*-hydroxyandrostan-17-one hydrochloride (22n). Prepared in 86% yield from **1f** and 2-aminoethoxyamine dihydrochloride. The product was crystallized from MeOH/EtOAc. ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 1.02 (s, 1.5H, CH_3), 1.04 (s, 1.5H, CH_3), 2.82 (dd, 0.5H, 4-H eq. *Z* isomer), 3.03 (t, 2H, NCH_2), 3.06 (m, 0.5H, 2-H eq. *E* isomer), 3.65 (m, 1H, 6-H), 4.07 (t, 2H, OCH_2), 4.45 (bb, 1H, OH), 7.95 (bb, 3H, NH_3^+); white solid: mp 160–170 °C dec. Anal. ($C_{21}H_{34}N_2O_3$·HCl·H_2O) C, H, N, H_2O.

(*E*,*Z*)-3-(2-Aminoethoxyimino)androstane-6β,17β-diol fumarate (22o). Prepared in 75% yield from **1g** and 2-aminoethoxyamine dihydrochloride. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH 90/10/1). To the concentrated fractions, the stoichiometric amount of fumaric acid in MeOH was added, followed by a 1/1 mixture of EtOAc/Et_2O. The precipitate formed was filtered to give **22o**. ^1H NMR (DMSO-d_6) δ 0.64 (s, 3H, CH_3), 1.00 (s, 1.5H, CH_3), 1.01 (s, 1.5H, CH_3), 2.79 (dd, 0.5H, 4-H eq. *Z* isomer), 3.03 (t, 2H, NCH_2), 3.06 (m, 0.5H, 2-H eq. *E* isomer), 3.42 (t, 1H, 17-H), 3.59 (m, 1H, 6-H), 4.05 (t, 2H, OCH_2), 4.40 (bb, 2H, OH), 6.58 (s, 2H fumaric ac.), 8.21 (bb, 3H, NH_3^+); white solid: mp 148−156 °C dec. Anal. ($C_{21}H_{36}N_2O_3\cdot C_4H_4O_4\cdot 2\ H_2O$) C, H, N, H_2O.

(*E*,*Z*)-3-(2-Aminoethoxyimino)-17β-hydroxyandrostan-6-one fumarate (22p). Prepared in 55% yield from **1h** and 2-aminoethoxyamine dihydrochloride. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH 90/10/1). To the concentrated fractions, the stoichiometric amount of fumaric acid in MeOH was added, followed by a 1/1 mixture of EtOAc/Et_2O. The precipitate formed was filtered to give **22p**. ^1H NMR (DMSO-d_6) δ 0.62 (s, 3H, CH_3), 0.74 (s, 3H, CH_3), 2.42 (dd, 0.5H, 5-H, *Z* isomer), 2.47 (dd, 0.5H, 5-H, *E* isomer), 2.99 (m, 2H, NCH_2), 3.02 (m, 0.5H, 4-H eq. *Z* isomer), 3.08 (m, 0.5H, 2-H eq. *E* isomer), 3.46 (t, 1H, 17-H), 4.06 (t, 2H, OCH_2); white solid: mp 110−170 °C. Anal. ($C_{21}H_{34}N_2O_3\cdot C_4H_4O_4\cdot H_2O$) C, H, N, H_2O.

(*E*,*Z*)-3-(2-Aminoethoxyimino)-17α-hydroxyandrostane-6-one hydrochloride (22q). Prepared in 62% yield from **1i** and 2-aminoethoxyamine dihydrochloride. ^1H NMR (DMSO-d_6) δ 0.58 (s, 3H, CH_3), 0.75 (s, 3H, CH_3), 3.01 (t, 2H, NCH_2), 3.53 (dd, 1H, 17-H), 4.09 (t, 2H, OCH_2), 4.35 (d, 1H, OH), 8.01 (bb, 3H, NH_3^+); off-white solid: mp 170−172 °C dec. Anal. ($C_{21}H_{34}N_2O_3\cdot HCl\cdot H_2O$) C, H, N, H_2O.

Conformational Energy Calculations of *E* and *Z* isomers. The calculations of *E* and *Z* isomers of **22b**, **22c**, and **22d** were performed using the AMBER* all atom force field as implemented in the program MacroModel 7.2; PR conjugate gradient was used in all the minimization steps, with the derivative convergence set to 0.05 (kJ/mol)/Å, with a maximum of 5000 iterations. Monte Carlo multiple minimum method was used in the conformational search (AMBER*, 5000 steps, torsion rotations allowed on the amino chain). All conformations within 50 kJ/mol of the identified lowest energy conformer were minimized again (Multiple Minimization routine in the program) using AMBER* in conjunction with the GB/SA continuum model to simulate water solvation.

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