17β-*O*-Aminoalkyloximes of 5β-Androstane-3β,14β-diol with Digitalis-like Activity: Synthesis, Cardiotonic Activity, Structure−Activity Relationships, and Molecular Modeling of the Na^+,K^+-ATPase Receptor

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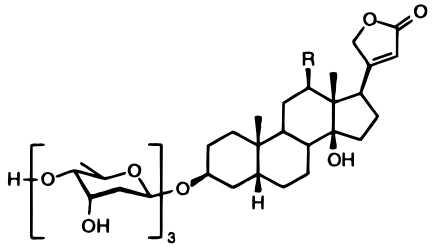
A series of digitalis-like compounds with a 17-aminoalkoxyiminoalkyl or -alkenyl substituent was synthesized and evaluated for inhibition of Na^+,K^+-ATPase and for inotropic activity. The highest inhibition was found with compounds having the substituent in configuration 17β and the amino group at a distance of 6 or 7 bonds from C(17) of the digitoxigenin skeleton. The presence of the oxime function strengthens the interaction with the receptor, more if α,β-unsaturated, thus mimicking the electronic situation of the unsaturated lactone in natural digitalis compounds. The most active compounds showed Na^+,K^+-ATPase inhibitory potencies (IC_{50}) 17−25 times higher than the standards digitoxigenin and digoxin and 3−11 times higher inotropic potencies (EC_{50}) in isolated guinea pig left atria. These features are supported by a molecular model suggesting the possible interactions of the groups described above with particular amino acid residues in the H1−H2 domains of Na^+,K^+-ATPase. Some interactions are the classical ones already described in the literature; a new, very strong interaction of the basic group with the Cys138 was found and adds new possibilities to design compounds interacting with this region of the receptor. The most interesting compounds were also studied in vivo in the anesthetized guinea pig for evaluating their inotropic effect versus the lethal dose. Compounds **9** and **12** showed a slightly higher safety ratio than digoxin and deserve further evaluation.

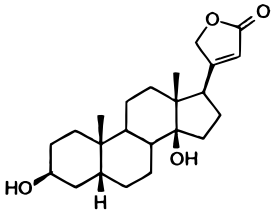
Introduction

Digitalis cardiac glycosides, such as digoxin and digitoxin (Chart 1), are drugs clinically used for the treatment of congestive heart failure (CHF).[1] Although these compounds give an unquestionable improvement in the quality of life of patients suffering from CHF, a major problem is their low therapeutic index due to cardiac proarrhythmogenic activity. A recent NIH-sponsored trial[2] showed a neutral effect for digoxin on mortality and renewed interest in the search for a safer positive inotropic agent acting through the inhibition of Na^+,K^+-ATPase.

In recent articles,[3,4] our group reported a series of 17β-guanylhydrazone derivatives of digitoxigenin skeleton (Chart 1) acting as positive inotropic compounds through the inhibition of Na^+,K^+-ATPase. Structure−activity studies on these compounds indicated that the presence of a basic (guanidino) group at a proper distance, of a 1,2-polarized iminic double bond, or of a 1,4-polarized conjugate system, which could mimic that of the α,β-unsaturated lactone of digitoxigenin, was essential for the above-mentioned activity. The importance of a basic center and of a dipole was confirmed by the high binding activity for the Na^+,K^+-ATPase receptor of a β-dimethylaminoethyl α,β-unsaturated ester of non-digitalis tricyclic perhydrophenanthrene structures, such as cassaine and seco-D 5β-androstane derivatives.[5]

Chart 1





Digitoxigenin

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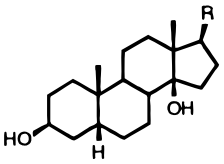
Table 1. Structure, Physical Data, and Na^+,K^+-ATPase Inhibition for Compounds **1**−**42**



compd	R[a]	method of prepn[b]	mol formula[c]	mp, °C	% yield	Na^+,K^+-ATPase inhibition, IC_{50},[d] μM
1	(*E*) CH=N−OH	A	$C_{20}H_{33}NO_3 \cdot H_2O$	229−231	46	100
2	(*Z*) CH=N−OH	A	$C_{20}H_{33}NO_3$	248−250	18	>100[e]
3	(*E*) CH=N−OCH_3	A	$C_{21}H_{35}NO_3 \cdot 0.25H_2O$	172−177	40	32
4	(*E*) CH=N−$O(CH_2)_2N(CH_3)_2$	A	$C_{24}H_{42}N_2O_3 \cdot C_4H_6O_6 \cdot H_2O$	125−135	72	1.0
5	(*E*) CH=N−$O(CH_2)_3N(CH_3)_2$	A	$C_{25}H_{44}N_2O_3 \cdot C_2H_2O_4$[g]	82−90	78	0.25
6	(*E*) CH=N−$O(CH_2)_4N(CH_3)_2$	A	$C_{26}H_{46}N_2O_3 \cdot C_2H_2O_4$[g]$\cdot 0.5H_2O$	48−58	57	2.0
7	(*E*) CH=N−$O(CH_2)_2NH_2$	A	$C_{22}H_{38}N_2O_3 \cdot C_4H_4O_4$[h]$\cdot$EtOH$\cdot 0.5H_2O$	95−135 dec	57	0.16
8	(*E*) CH=N−$O(CH_2)_3NH_2$	A	$C_{23}H_{40}N_2O_3 \cdot C_2H_2O_4$[g]	110−120	60	0.02
9	(*E*) CH=N−$O(CH_2)_4NH_2$	A	$C_{24}H_{42}N_2O_3 \cdot C_2H_2O_4$[g]	161−170	64	0.50
10	(*E*) CH=N−OCH_2COOH	A	$C_{22}H_{35}NO_5$	215−220 dec	79	>100[i]
11	(*E*) CH_2CH=N−$O(CH_2)_2N(CH_3)_2$	B	$C_{25}H_{44}N_2O_3 \cdot 0.5H_2O$	89−91	18	0.63
12	(*E,Z*) $(CH_2)_2CH$=N−$O(CH_2)_2N(CH_3)_2$	B	$C_{26}H_{46}N_2O_3 \cdot C_2H_2O_4$[g]$\cdot H_2O$	125−130	33	0.10
13	(*E,Z*) $(CH_2)_2CH$=N−$O(CH_2)_2NH_2$	B	$C_{24}H_{42}N_2O_3$	165−170	67	0.08
14	(*E,E*) CH=CHCH=N−OCH_3	B	$C_{23}H_{37}NO_3$	176	59	4.0
15	(*E,E*) CH=CHCH=N−$O(CH_2)_2CH_3$	B	$C_{25}H_{41}NO_3 \cdot 0.75H_2O$	127−128	34	10
16	(*E,E*) CH=CHCH=N−$O(CH_2)_2OH$	B	$C_{24}H_{39}NO_4$	189−191	48	6.3
17	(*E,EZ*) CH=CHCH=N−OCH_2COOH		$C_{24}H_{37}NO_5 \cdot 0.25H_2O$	185−188	60	>100[j]
18	(*E,E*) CH=CHCH=N−$O(CH_2)_2N(CH_3)_2$	B	$C_{26}H_{44}N_2O_3.C_2H_2O_4$[g]$\cdot 1.25H_2O$	130−133	48	0.03
19	(*E,E*) CH=CHCH=N−$O(CH_2)_2NH_2$	B	$C_{24}H_{40}N_2O_3 \cdot 0.25H_2O$	162−164	21	0.02
20	(*E,E*) $CH=C(CH_3)CH$=N−OCH_3	A	$C_{24}H_{39}NO_3 \cdot 0.75H_2O$	145−146	59	10
21	(*E,E*) $CH=C(CH_3)CH$=N−$O(CH_2)_2CH_3$	A	$C_{26}H_{43}NO_3$	154−155	66	>10[k]
22	(*E,E*) $CH=C(CH_3)CH$=N−$O(CH_2)_2OH$	A	$C_{25}H_{41}NO_4 \cdot 0.25H_2O$	182−185	65	7.9
23	(*E,E*) $CH=C(CH_3)CH$=N−$O(CH_2)_2N(CH_3)_2$	A	$C_{27}H_{46}N_2O_3 \cdot C_4H_4O_4$[h]$\cdot 0.25H_2O$	198−203	73	0.06
24	(*E,E*) $CH=C(CH_3)CH$=N−$O(CH_2)_2NH_2$	A	$C_{25}H_{42}N_2O_3 \cdot C_4H_4O_4$[h]$\cdot 0.75H_2O$	140−150	70	0.03
25	(*E,E*) $CH=C(CH_3)CH$=N−$O(CH_2)_3NH_2$	A	$C_{26}H_{44}N_2O_3 \cdot C_2H_2O_4$[g]$\cdot 0.5H_2O$	128−136	72	0.20
26	(*E,E*) $CH=C(CH_3)CH$=N−$O(CH_2)_4NH_2$	A	$C_{27}H_{46}N_2O_3.C_2H_2O_4$[g]$\cdot 0.25H_2O$	199−204	48	1.26
27	(*E,E,E*) $(CH=CH)_2CH$=N−$O(CH_2)_2N(CH_3)_2$	B	$C_{28}H_{46}N_2O_3 \cdot C_2H_2O_4$[g]$\cdot 1.25H_2O$	137−151	61	6.30
28	CH_2NHOCH_3	C	$C_{21}H_{37}NO_3 \cdot C_2H_2O_4$[g]$\cdot H_2O$	143−145	32	79
29	$CH_2NHO(CH_2)_2N(CH_3)_2$	C	$C_{24}H_{44}N_2O_3 \cdot 2HCl \cdot H_2O$	182−185	58	50
30	$CH_2NHO(CH_2)_2NH_2$	C	$C_{22}H_{40}N_2O_3 \cdot 2HCl$	202−205	63	1.58
31	$CH_2NHO(CH_2)_3NH_2$	C	$C_{23}H_{42}N_2O_3 \cdot 2HCl \cdot 0.25H_2O$	193−198	64	0.40
32	$CH_2N(CH_3)O(CH_2)_2N(CH_3)_2$		$C_{25}H_{46}N_2O_3 \cdot C_2H_2O_4$[g]$\cdot 1.5H_2O$	160−170 dec	30	>100[l]
33	$(CH_2)_3NHOCH_3$	C	$C_{23}H_{41}NO_3 \cdot HCl \cdot H_2O$	149−151	60	>100[j]
34	$(CH_2)_3NHO(CH_2)_2NH_2$	C	$C_{24}H_{44}N_2O_3 \cdot 0.25H_2O$	145−146	58	1.0
35	(*E*) $CH=CHCH_2NHOCH_3$	C	$C_{23}H_{39}NO_3 \cdot HCl \cdot 0.25H_2O$	172−179	35	40
36	(*E,E*) $(CH=CH)_2(CH_2)_2NH_2$		$C_{25}H_{41}NO_2 \cdot 0.5H_2O$	171−198		0.08
37	(*E,E*) $(CH=CH)_2(CH_2)_3NH_2$		$C_{26}H_{43}NO_2 \cdot 0.25H_2O$	167−173		0.32
38	$(CH_2)_7NH_2$		$C_{26}H_{47}NO_2 \cdot 0.5H_2O$	58(68)−127		1.25
39	CHO		$C_{20}H_{32}O_3 \cdot H_2O$[m]			25
40	$(CH_2)_2CHO$		$C_{22}H_{36}O_3$	135−137		40
41	(*E*) CH=CHCHO		$C_{22}H_{34}O_3$[n]			0.25
42	(*E*) $CH=C(CH_3)CHO$		$C_{23}H_{36}O_3 \cdot 0.25H_2O$	184−192		1.58
digitoxigenin	2,5-dihydro-5-oxo-3-furyl					0.50
digoxin						0.50

[a] The *EZ* mixture is indicated where the amount of *Z* oxime is more than 10%. [b] General methods of preparation: (A) at pH 4.5; (B) in the presence of NaOH; (C) $NaBH_3CN$; where no indications, see experimental. [c] Analyses for C, H, N, Cl, and H_2O are within 0.4% of the theoretical values. [d] Concentrations able to inhibit 50% of enzyme activity; mean of two or three experiments. [e] 30% inhibition at 100 μM. [f] L-(+)-tartrate. [g] Oxalate. [h] Fumarate. [i] 0% inhibition at 100 μM. [j] 40% inhibition at 100 μM. [k] 40% inhibition at 10 μM. [l] 20% inhibition at 100 μM. [m] Reference 7. [n] Reference 4.

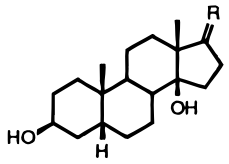
Intrigued by these observations and with the aim to further explore the requirements for a strong interaction with the Na^+,K^+-ATPase receptor and find novel inotropic agents more active than digoxin, we synthesized a new series of digitalis-like steroidal derivatives in which the guanylhydrazone group was replaced by an aminoalkyloxime group; the polarized iminic bond was thus retained together with the basic group of cassaine and seco-D 5β-androstane derivatives reported above.

The aminoalkyloxime substituent hit the target, as some of the compounds of this new series showed the highest inhibitory activities on Na^+,K^+-ATPase ever found with semisynthetic analogues, higher than those of the potent, natural digitalis compounds.

The importance of the iminic double bond was evaluated both through its reduction to the corresponding hydroxylamine function and substitution with a nonpolarized olefinic double bond. The contribution to the activity of the amine group was also assessed either by replacing it with other groups or by leaving it as the unique functional group connected to the steroidal skeleton by a saturated carbon chain.

From these new findings and the data reported in the previous papers,[3−5] we reasoned that this extraordinary activity could be ascribed to an interaction of the protonated amine substituent with a negatively charged site in the receptor. With the aim of finding if this putative "anionic site" really existed, we started a

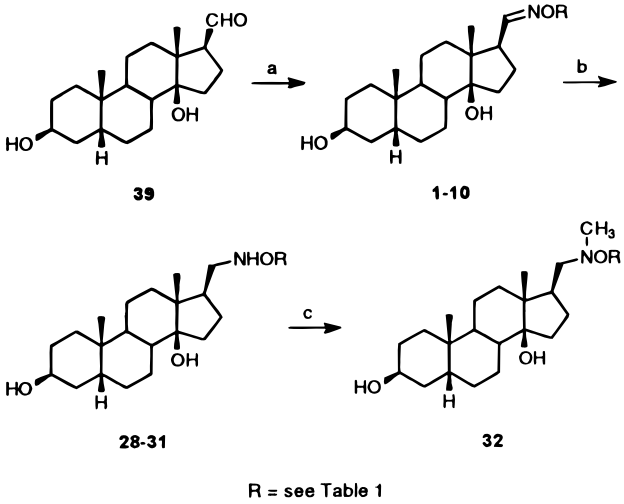
Table 2. Structure, Physical Data, and Na^+,K^+-ATPase Inhibition for Compounds **43**−**60**



compd	R[a]	method of prepn[b]	mol formula[c]	mp, °C	% yield	Na^+,K^+-ATPase inhibition, IC_{50},[d] μM
43	(*E*) $NO(CH_2)_2N(CH_3)_2$	D	$C_{23}H_{40}N_2O_3$	157−159	54	>100[e]
44	(*E*) $NO(CH_2)_3N(CH_3)_2$	D	$C_{24}H_{42}N_2O_3$	169−170	60	>100[e]
45	(*E*) $NO(CH_2)_4N(CH_3)_2$	D	$C_{25}H_{44}N_2O_3$	67−68	45	>100[e]
46	(*E*) $NO(CH_2)_2NH_2$	D	$C_{21}H_{36}N_2O_3 \cdot 0.1EtOH$	161−162	40	>100[e]
47	(*E*,*EZ*) $CHCH{=}N{-}O(CH_2)_2N(CH_3)_2$	A	$C_{25}H_{42}N_2O_3$	157−162	61	12.5
48	(*E*,*E*) $CHCH{=}N{-}O(CH_2)_3N(CH_3)_2$	A	$C_{26}H_{44}N_2O_3$	168−170	42	160
49	(*E*,*EZ*) $CHCH{=}N{-}O(CH_2)_2NH_2$	A	$C_{23}H_{38}N_2O_3 \cdot 0.5EtOH$	208−212 dec	40	2.0
50	(*E*,*EZ*) $CHCH{=}N{-}O(CH_2)_3NH_2$	A	$C_{24}H_{40}N_2O_3 \cdot 0.25H_2O$	150−153	55	>100[f]

[a] The *EZ* mixture is indicated where the amount of *Z* oxime is more than 10%. [b] General methods of preparation: (A) at pH 4.5; (D) at pH 2.0. [c] Analyses for C, H, N, and H_2O are within 0.4% of the theoretical values. [d] Concentrations able to inhibit 50% of enzyme activity; mean of two or three experiments. [e] 0% inhibition at 100 μM. [f] 40% inhibition at 10 μM.

Scheme 1[a]



[a] Reagents and conditions: (a) $H_2NOR \cdot xHCl$, NaOAc, HCl, dioxane, H_2O, pH 4.5; (b) $NaBH_3CN$, MeOH, H_2O, HCl, pH 3; (c) CH_2O, $NaBH_3CN$, MeCN, H_2O.

modeling program of docking the most active compounds with a 3D-model of Na^+,K^+-ATPase receptor, built from the peptidic sequence that many authors indicate as the "receptor sequence".[6]

Chemistry

The synthetic pathways for the compounds listed in Tables 1 and 2 are reported in Schemes 1−7.

The oximes **1**−**27** (Table 1) and **47**−**50** (Table 2) were synthesized from the corresponding aldehydes and appropriate (*O*-substituted)hydroxylamines hydrochlorides or dihydrochlorides in a dioxane/water solution; oximes **1**−**10** (Scheme 1), **17**, **20**−**26** (Scheme 3), and **47**−**50** (Scheme 5) were prepared at pH 4.5 (method A), while oximes **11** (Scheme 2), **12**−**16**, **18** and **19** (Scheme 3), and **27** (Scheme 4) were prepared at pH >9 (method B), in the presence of NaOH (see Experimental Section). In the case of compounds **1**−**10** pH 4.5 was the best compromise to limit the amount of *Z* isomer, relevant at a lower pH, and to prevent the isomerization of the starting 17*β* aldehyde **39**[7] to the 17α isomer, occurring at a higher pH. In the case of compounds **20**−**26** the *E* isomers of the oximes were stable in any case, due to the presence of the 21-CH_3 group, and also the starting aldehyde was more resistant to the epimerization at C(17). Compounds **11**−**16**, **18**, **19**, and **27** were synthesized under basic conditions to limit the amount of *Z* oxime; in these cases no epimerization at C(17) of the corresponding starting aldehydes **54** (Scheme 2, in the form of lactol, as shown by NMR), **40** and **41**[4] (Scheme 3), and **58**[4] (Scheme 4) occurred.

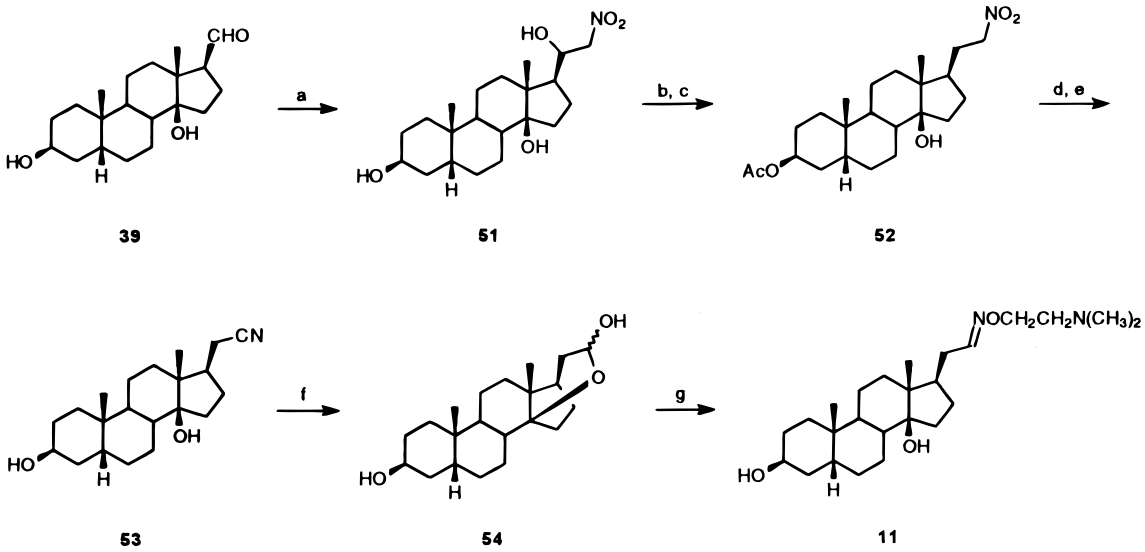
The oximes **43**−**46** (Table 2) were synthesized from ketone **59**[8] (Scheme 5) and the appropriate (*O*-substituted)hydroxylamines dihydrochlorides in ethanol at pH 2.

The oximes reported in Tables 1 and 2 were obtained as almost pure *E* isomers (*Z* isomer ⩽ 10%) except for compounds **12** (40% of *Z* oxime), **17** (25% of *Z* oxime), **47** (30% of *Z* oxime), and **49** and **50** (20% of *Z* oxime). In D_2O/DMSO-d_6 solution at pH 7.4 (phosphate buffer) and 37 °C, oximes **1** and **3**−**9** showed no *E*/*Z* isomerization up to 24 h (^1H NMR analysis), while oximes **11**−**16** and **18**−**19** gave a 6/4 *E*/*Z* equilibrium mixture, after 24 h. At pH 1, oximes **1** and **3**−**9** gave an 8/2 *E*/*Z* mixture after 6 h, while oximes **11**−**19** gave a 6/4 *E*/*Z* equilibrium mixture after 3 h. *Z* oxime **2** reached the equilibrium in a shorter time already at pH 7.4. Compounds **20**−**26**, with a methyl group at position 21, were synthesized to stabilize the *E* isomer and no isomerization was indeed observed for these compounds at any pH.

Hydroxylamines **28**−**31** (Scheme 1) and **33**−**35** (Scheme 3) were prepared from the corresponding oximes by reduction with $NaBH_3CN$ at pH 3, while *N*-methylhydroxylamine **32** (Scheme 1) was prepared by reductive alkylation of **29** with $CH_2O/NaBH_3CN$.

All the attempts to prepare aldehyde **54** from **39** by Wittig reactions with methoxymethylenetriphenylphosphorane or methylenetriphenylphosphorane gave mixtures of 17α- and 17*β*-vinyl epimers in very low yields, the 17α being the undesired, prevalent, and inseparable component. Thus, the homologation was carried out, as shown in Scheme 2, by KF-catalyzed condensation of **39** with nitromethane to give **51** as exclusive isomer (see Templeton et al. for the corresponding 3*β*-*O*-(α-L-rhamnopyranosyl) derivative).[9] Diacetylation of 3*β*- and 20-

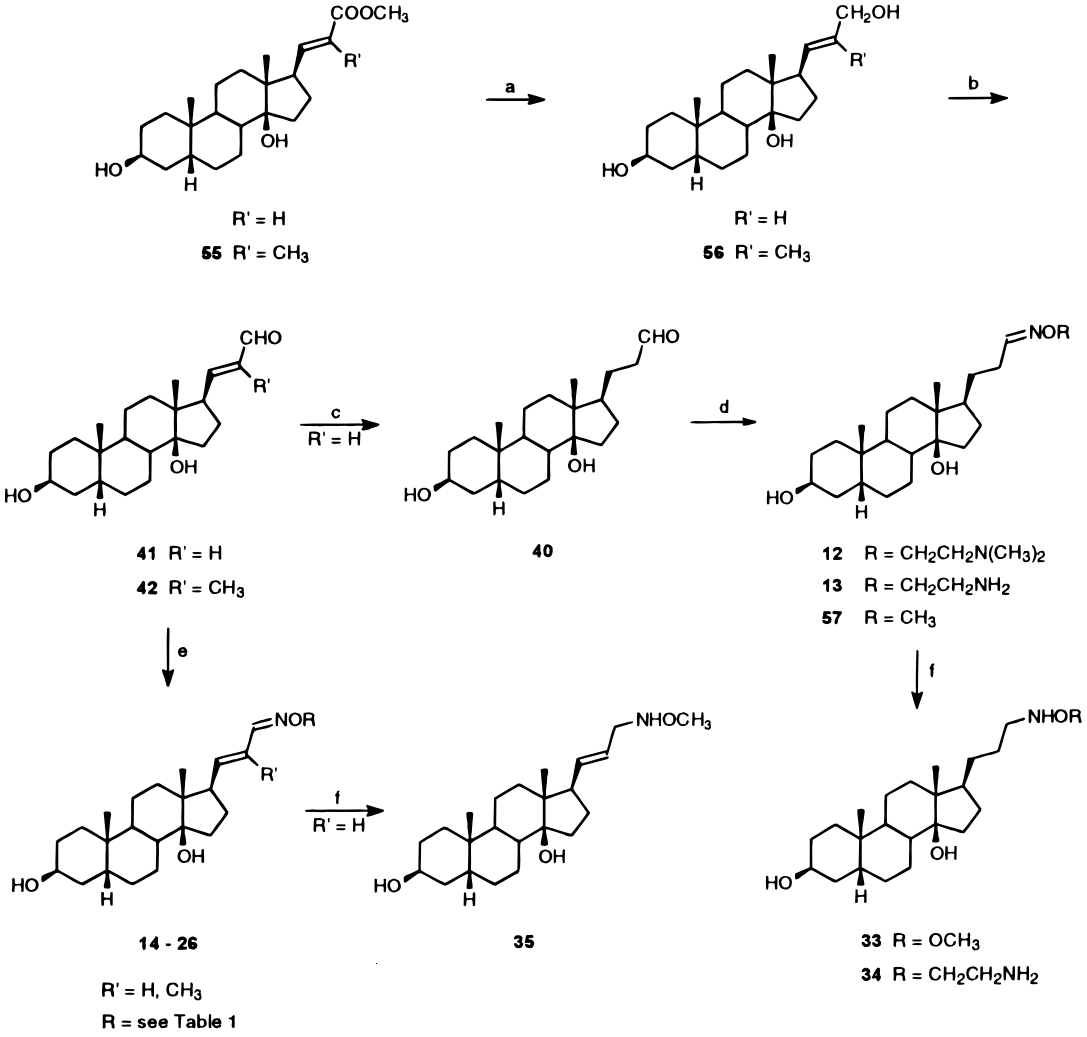
Scheme 2[a]



39 **51** **52** **53** **54** **11**

[a] Reagents and conditions: (a) CH_3NO_2, KF, *i*PrOH; (b) Ac_2O, DMAP, THF; (c) $NaBH_4$, EtOH, Et_2O; (d) DEAD, Ph_3P, CH_2Cl_2; (e) 1 N NaOH, MeOH, THF; (f) DIBAH, THF, 0 °C; (g) $H_2NO(CH_2)_2N(CH_3)_2$·2HCl, NaOH, dioxane, H_2O.

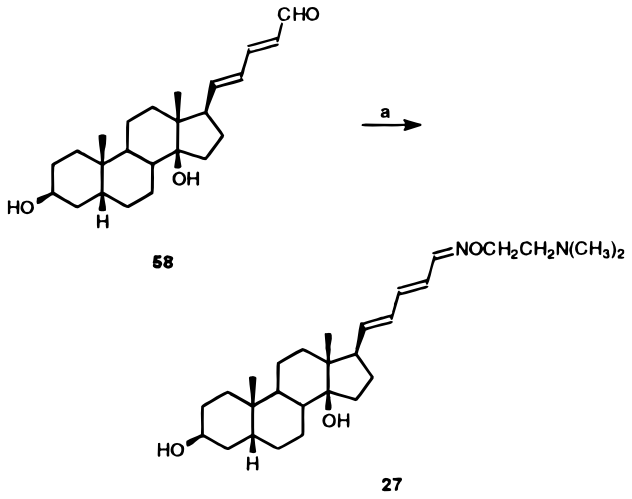
Scheme 3[a]



[a] Reagents and conditions: (a) DIBAH, THF, −60 °C, 2 h; (b) MnO_2, dioxane; (c) H_2, Pd/C, EtOAc; (d) H_2NOR·*x*HCl, NaOH, dioxane, H_2O; (e) H_2NOR·*x*HCl, dioxane, H_2O, pH 4.5 or >9; (f) $NaBH_3CN$, MeOH, H_2O, HCl, pH 3.

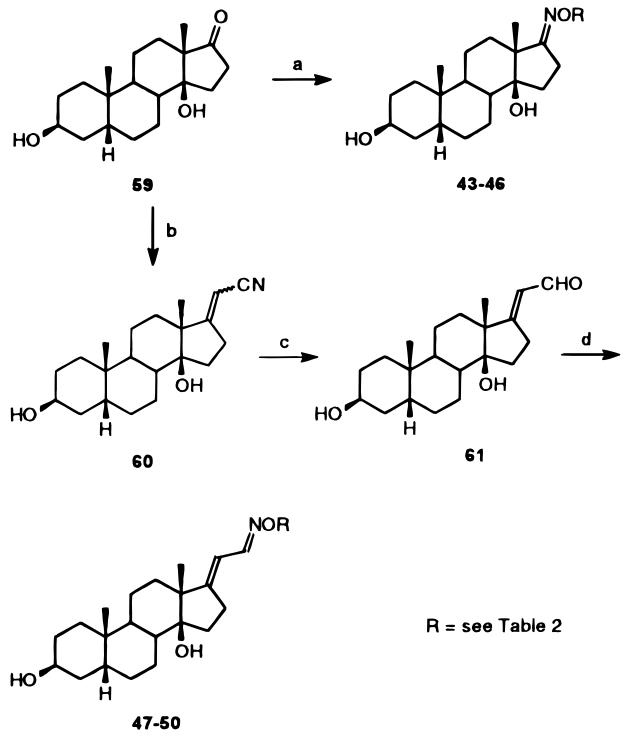
hydroxy groups of **51**, followed by $NaBH_4$ reduction, gave the nitroalkyl derivative **52**,[10] which proved resistant to the direct transformation into the corresponding aldehyde **54** through Nef reaction. The desired aldehyde, in the form of lactol **54**, could be prepared in 38% overall yield from **39**, by transformation of the nitromethylene

Scheme 4[a]



[a] Reagents and conditions: (a) $H_2NOCH_2CH_2N(CH_3)_2 \cdot 2HCl$, NaOH, dioxane, H_2O.

Scheme 5[a]



[a] Reagents and conditions: (a) $H_2NOR \cdot 2HCl$, NaOH, EtOH, pH 2; (b) $NCCH_2PO(OEt)_2$, *t*BuOK, THF; (c) Raney Ni, $NaH_2PO_2 \cdot H_2O$, H_2O/AcOH/pyridine, 1:1:2, 60 °C, 24 h; (d) $H_2NOR \cdot 2HCl$, NaOAc, HCl, dioxane, H_2O, pH 4.5.

group of compound **52** into a nitrile group by treatment with DEAD/Ph_3P,[11] hydrolysis of this derivative with NaOH to compound **53**, and reduction of **53** with DIBAH. Lactol **54** gave the oxime **11** in low yield (18%) because it was stable and reacted poorly under the alkaline reaction conditions we had to choose, to limit the amount of *Z* isomer.

Aldehyde **42** (Scheme 3) was prepared in two steps by reduction with DIBAH of the known ester **55**[12] to the allylic alcohol **56** and subsequent oxidation with MnO_2, following the method already described for **41**.[4] The corresponding saturated aldehyde **40** was prepared by catalytic hydrogenation of **41**.

The *E* aldehyde **61** (Scheme 5) could be obtained by reduction of the *E*/*Z* isomeric mixture of nitriles **60**[13] with Raney Ni and NaH_2PO_2,[14] as the *Z* isomer did not react in the reaction conditions used.

Aminodiene **36** (Scheme 6) was synthesized by $LiAlH_4$ reduction of the azido derivative **63** obtained by palladium-catalyzed cross-coupling of iodoalkene **62** with the stannylalkene **68** of Scheme 8. In turn, iodoalkene **62** was prepared smoothly from aldehyde **39** by Takai reaction.

Aminodiene **37** and aminoalkane **38** were synthesized as shown in Scheme 7. Stille coupling of iodoalkene **62** with the stannylalkene **69** of Scheme 8 gave the cyanodiene **64**, which was reduced to the aminodiene **37** with $LiAlH_4$. Catalytic hydrogenation of **37** afforded the corresponding aminoalkane **38** together with an unseparable unidentified aminoalkene; when we forced the hydrogenation conditions we met with extensive degradation. The same behavior was observed in the catalytic hydrogenation of the cyanodiene **64**, where the desired saturated derivative was obtained together with an unidentified cyanoalkene. Compound **64** was then converted to the corresponding 3β-*O*-acetyl derivative before being hydrogenated; the mixture of **65** and cyanoalkene was treated with MCPBA to obtain a new mixture of the unreacted **65** and the epoxide of the alkene, which could be easily separated by flash chromatography. $LiAlH_4$ reduction of **65** gave the desired aminoalkane **38**.

The intermediate hydroxylamines **76**−**81** were prepared as shown in Scheme 9. Compounds **76**−**80** were obtained by alkylation of benzophenone oxime with the corresponding ω-haloalkylamines in the presence of KOH, followed by acid hydrolysis of the *O*-aminoalkyl derivatives **70**−**74**; compound **81** was obtained by alkylation of *N*-hydroxyphthalimide with commercially available *N*-(4-bromobutyl)phthalimide, followed by hydrazine hydrolysis of the resulting diphthalimido derivative **75**. Propoxyamine,[15] 2-hydroxyethoxyamine,[16] and 2-aminooxyacetic acid hydrochloride,[17] intermediates for compounds, **10**, **15**−**17**, **21**, and **22**, were prepared as described in the literature.

Biological Activity

All compounds were evaluated in vitro for inhibition of dog kidney Na^+,K^+-ATPase activity,[18] measured as percent hydrolysis of ^{32}P-ATP;[19] data are shown in Tables 1 and 2. Compounds showing an inhibitory potency of the enzyme (IC_{50}) at least in the micromolar range were further investigated in vitro for the inotropic activity by measuring their effects on the contractile force of an electrically driven guinea pig left atrium; data are shown in Table 3.

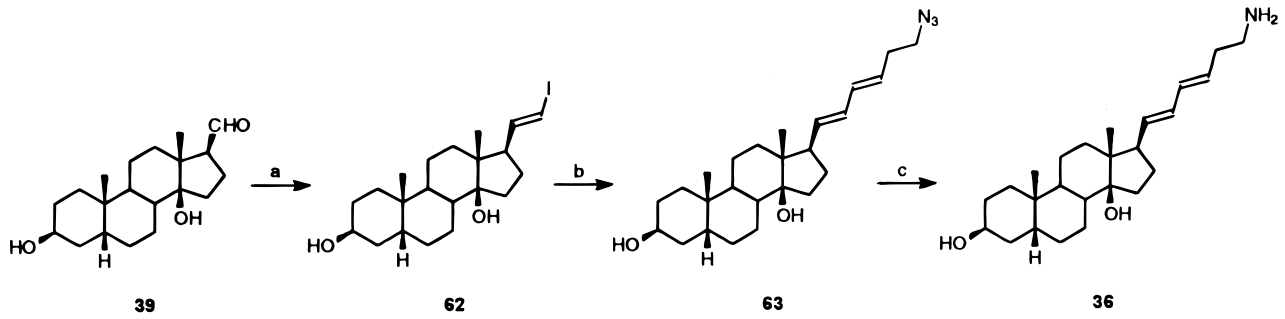
Some compounds showing the highest potency in vitro were investigated in vivo for inotropic activity and lethal effect in anesthetized guinea pigs during slow intravenous infusion; results are reported in Table 4.

Digoxin and digitoxigenin were chosen as reference compounds, as the former is the most commonly prescribed cardiac glycoside in CHF and the latter has the same steroidal aglyconic skeleton of our compounds.

Results and Discussion

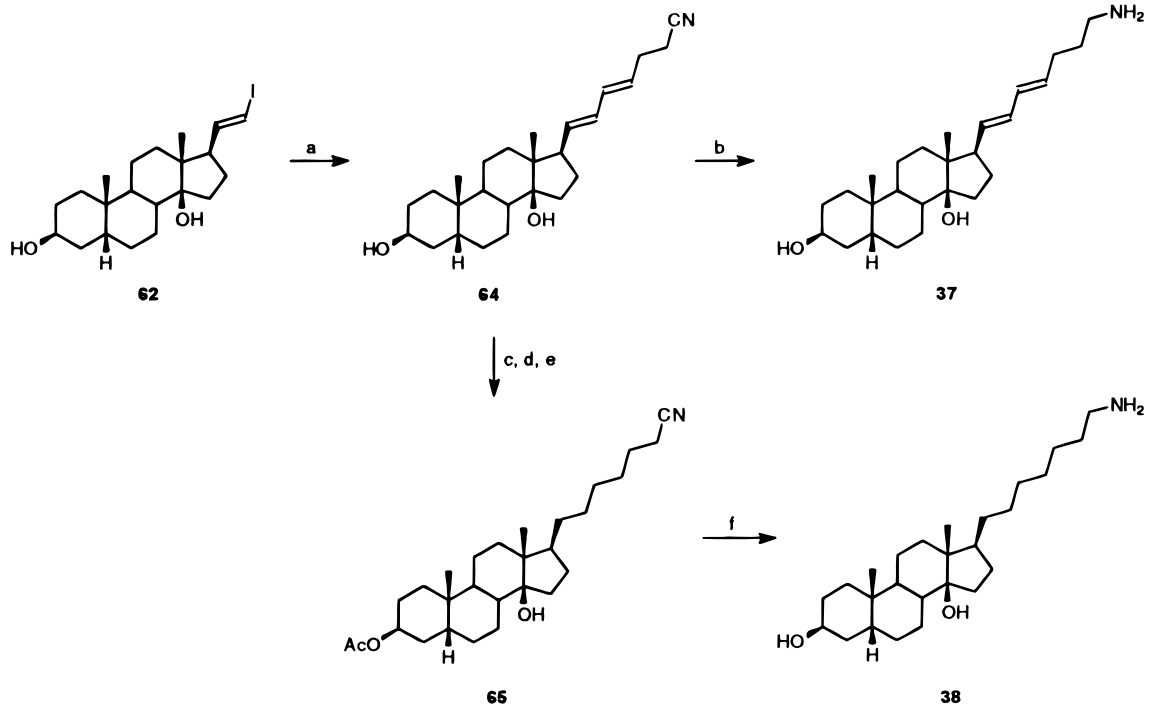
The compounds were tested as isomeric mixtures as obtained from the synthesis. Taking into consideration

Scheme 6[a]



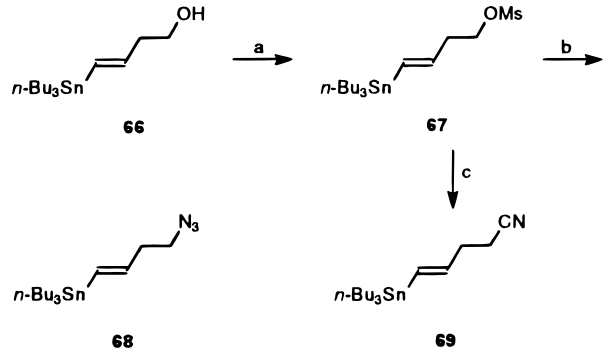
[a] Reagents and conditions: (a) $CrCl_2$, CHI_3, THF, 0 °C, 2 h; (b) **68**, $PdCl_2(CH_3CN)_2$, DMF; (c) $LiAlH_4$, THF.

Scheme 7[a]



[a] Reagents and conditions: (a) **69**, $PdCl_2(CH_3CN)_2$, DMF; (b) $LiAlH_4$, THF, reflux, 24 h; (c) Ac_2O, DMAP, pyridine, CH_2Cl_2; (d) H_2, PtO_2, EtOAc; (e) MCPBA, $CHCl_3$; (f) $LiAlH_4$, THF, reflux, 24 h.

Scheme 8[a]



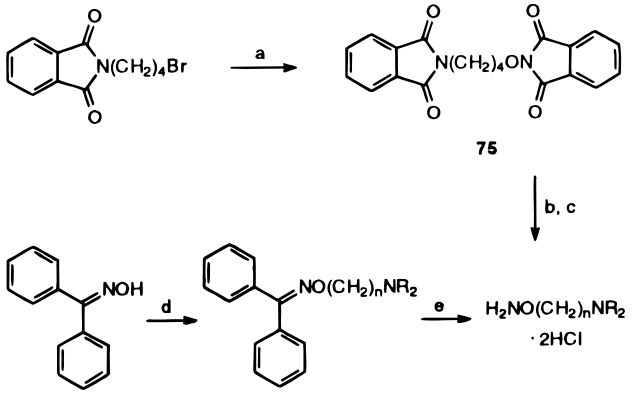
[a] Reagents and conditions: (a) CH_3SO_2Cl, TEA, CH_2Cl_2, 0 °C; (b) NaN_3, DMF, 50 °C, 4h; (c) KCN, KI, DMF, 80 °C, 9 h.

that the biological assays were performed within 1−4 h from the dissolution of the compounds and the long equilibration time at pH 7.4 reported above, we take for granted that no important isomerization of the tested compounds occurred in the biological systems used for the assays.

It should be noted that performing the tests on mixtures of isomers could give defective results in the case of different binding and intrinsic activity of the components. In our hands it was impossible to separate *E*/*Z* isomers, with the exception of compounds **1** and **2**. All the following reasoning is based on the mixtures of components.

Enzyme Inhibition. The oximes **1**−**3**, "parent compounds" of derivatives listed in Table 1, showed IC_{50}s comparable to that of the "parent" aldehyde **39**, suggesting that the same type of polarization of the double bond and of interaction with the receptor occurs. The introduction of the basic side chain in compounds **4**−**9** resulted in a dramatic increase of the inhibitory potencies, from 15- to 5000-fold; the chain length influenced the activities in the following order: $(CH_2)_3 > (CH_2)_2 > (CH_2)_4$, suggesting that six atoms between the basic group and the C17 position of digitoxigenin skeleton could be the optimal distance for a strong interaction with the receptor. As evidence of the importance of the basic group, the acid **10** did not inhibit the enzyme.

Scheme 9[a]



[a] Reagents and conditions: (a) *N*-hydroxyphthalimide, K_2CO_3, DMSO, 80 0C, 0.5 h; (b) hydrazine, EtOH, reflux, 3 h; (c) HCl; (d) $R_2N(CH_2)_nCl$, KOH, DMSO; (e) 6 N HCl, reflux, 2 h.

Table 3. Inotropic Activity on Electrically Driven Guinea Pig Left Atrium

compd	E_{max},[a] % increase from basal force	concn to obtain E_{max}, μM	EC_{50},[b] μM
4	39	3	nd
5	235	10	1.3
6	214	30	4.7
7	145	3	0.62
8	126	1	0.18
9	92	10	2.9
11	73	10	1.8
12	150	3	0.65
13	70	3	1.1
16	179	300	39
18	191	0.3	0.057
19	155	0.3	0.05
22	175	300	23
23	146	0.3	0.07
24	115	1	0.07
25	90	1	0.17
26	106	30	4.0
27	145	100	20
29	18	300	nd
30	135	100	8
31	237	30	3.9
34	71	30	12
36	98	3	0.38
37	73	0.1	0.035
38	43	100	nd
39	79	300	11.0
41	45	10	4
49	136	100	18
digitoxigenin	200	3	0.57
digoxin	184	1	0.38

[a] Maximal increase in force of contraction. [b] Concentrations producing 50% of the maximal increase in force of contraction were calculated from concentration−response curves; nd: not determined.

The 1,4-polarization of aldehyde **41**, mimicking the same electronic situation of the lactone in natural digitalis compounds, gave a more efficient interaction with the receptor, inducing a 100 times increase of potency compared to the 1,2-polarized aldehyde **39**. The same behavior occurred in the α,*β*-unsaturated oximes, although to a lesser extent; in fact, the *O*-methyl α,*β*-unsaturated oxime **14** was only 8 times more potent than the corresponding nonconjugated oxime **3**. The introduction of a basic chain in the 1,4-polarized, α,*β*-unsaturated aldehyde **41** gave the most active compounds, **18** and **19**, with an average inhibitory potency greater than that of the more active basic substituted oximes **5** and **8**, derived from the 1,2-polarized aldehyde **39**. Indeed compound **8** showed the same high activity as **18** and **19**, probably because the amine group in **8** is at the right distance of six atoms (see above) for the best interaction with the complementary site in the receptor, a distance impossible to achieve in the 1,4-polarized series (seven atoms as a minimum) for reasons of chemical stability. The replacement of the amine with a carboxylate gave the inactive compound **17**. The much lower inhibitory potencies of compounds **15** and **16**, where a basic amino group was replaced by a methyl and a hydroxy group, respectively, confirm the importance of the basicity of an amino group when compared with the lipophilicity of a methyl group or the hydrogen-bonding capacity of a hydroxy group.

The 1,4-polarized 21-methyl oximes **20**−**24** had the same general behavior of the corresponding desmethyl derivatives, although with at a bit lower level of Na^+,K^+-ATPase inhibitory activity. That was probably due to the steric hindrance of the methyl substituent, preventing a closer interaction with the receptor (see also the parent aldehyde **42**, 6 times less active than the corresponding desmethyl **41**). The best interaction occurred, as expected, with the dimethylaminoethyl and aminoethyl substituents (compounds **23** and **24**); any further outdistancing of the basic group, as in compounds **25**−**27**, decreased the inhibitory activity.

Although good potencies were retained in the aminoalkyloximes **11**−**13**, where one (**11**) and two (**12**, **13**) methylenes have been placed between the oxime function and the steroidal skeleton, the potencies were lower than those of the most potent compounds, where the aminoalkyloxime function is either direcly linked to the steroidal skeleton (compound **8**) or separated by an ethylene group (compound **19**). Strangely enough, compound **12**, with the basic group at a seven atom distance from C17, was more active than compound **11**, at a six atom distance. The lower potency of these compounds could be easily forecast from the great difference in activity (160 times) of the α,*β*-unsaturated aldehyde **41** vs the saturated one **40**, while the "distance anomaly" can be explained with the presence, in compounds **12** and **13**, but not in compound **11**, of an iminic bond at the same distance from C17 as that of the carbonyl group of the lactone in the natural digitalis derivatives. It is worth noting that the difference in activity between the α,*β*-unsaturated basic oxime **19** vs the corresponding saturated oxime **13** is much less than that of the corresponding α,*β*-unsaturated and saturated parent aldehydes **41** and **40** (4 times vs 160 times). Evidently the strength of the interaction of the basic amino groups with the receptor tends to lower the importance and weight of the other interacting groups.

A further demonstration of the above-mentioned strength of interaction of the basic terminal with the Na^+,K^+-ATPase receptor is the potency of compound **36**, where the oxime group of compound **19** was replaced by a nonpolarized ethylene spacer and the amino group remained the only interacting group present in the side

Table 4. Inotropic and Toxic Effects in Anesthetized Guinea Pig

compd	concn of infused solution, μM	E_{max},[a] % increase in +dP/dt	dose to obtain E_{max}, μmol/kg	ED_{80},[b] μmol/kg	lethal dose, μmol/kg	deaths/ treated	lethal dose/ ED_{80}
8	41.4	163 ± 24	0.435 ± 0.083	0.240 ± 0.041	0.663 ± 0.435	2/4	2.8
9	1000	148 ± 44	5.03 ± 0.221	1.41 ± 0.161	14.8 ± 1.03	3/3	10.5
12	220	132 ± 28	3.65 ± 0.940	0.814 ± 0.295	7.83	1/4	9.6
18	11.0	110 ± 24	0.275 ± 0.018	0.185 ± 0.018	0.385	1/4	2.1
24	12.8	152 ± 29	0.201 ± 0.018	0.128 ± 0.018	0.292 ± 0.018	4/4	2.3
25	375	99 ± 8	0.884 ± 0.075	0.447 ± 0.038	1.67 ± 0.207	4/4	3.7
digoxin	51.2	167 ± 30	0.576 ± 0.064	0.205 ± 0.026	1.25 ± 0.102	11/11	6.1

[a] Maximal increase in +dP/dt. [b] Doses producing 80% of increase in +dP/dt, from basal force, were calculated from dose−response curves; nd: not determined. Data are means ± SEM when possible.

chain: compound **36** is only 4 times less potent than compound **19**. Also in this case the optimal length of the chain between the C17 carbon atom and the amino group was confirmed to be six atoms (see **36** vs **37**). Reduction of the olefinic bonds of **37** to give the saturated compound **38** gave a further 4 times decrease of activity.

Reduction of the iminic bond to give hydroxylamines **28**−**35** caused the activity to drop; when the most active oximes (**4**, **7**, and **8**) were reduced to the corresponding hydroxylamines (**29**−**31**), the decrease was on the order of 10−50 times. The lack of polarization of the iminic bond cannot explain these data completely, since the same situation should also apply to olefinic or aliphatic amines **36**−**38**. A tentative explanation is that an intramolecular hydrogen bond between the protonated amine and the hydroxylamine nitrogen atom could fold the chain, thus making the interaction with the anionic site of the enzyme energetically less favorable.

Oximes **43**−**46** of Table 2 were inactive. The steric situation of these compounds and/or the shortness of the aliphatic chain can be an explanation for their inactivity. In fact, in these compounds, the double bond, directly attached to C17, projects the oxime chain in the same plane of the D ring, while in the case of derivatives **1**−**42** the chain is in position 17β, that is, over the plane of the D ring. The longer chain oximes **47** and **49**, permitting the amine group to reach the proper position for a good interaction with the receptor, showed better activities, being 25 and 4 times less potent than digoxin and digitoxigenin, respectively. Further increase of the chain length in **48** and **50** caused the activity to drop again.

In all cases, primary amines showed inhibitory activities higher than the corresponding tertiary amines, probably due to a limited availability of space around the protonated amine interacting with the anionic site of the receptor.

Inotropic Activity. A highly significant correlation was found between the inhibitory potency on Na^+,K^+-ATPase (IC_{50}) and inotropic potency (EC_{50}) in isolated atria in the set of tested oximes ($r = 0.918$, $r^2 = 0.842$, $n = 17$, $p < 0.0001$). The correlation data between IC_{50} and EC_{50} dropped dramatically on the entire set of compounds (oximes, hydroxylamines, alkenes, alkane, and aldehydes; $r = 0.460$, $r^2 = 0.210$, $n = 25$, $p = 0.0199$). The correlation was even lower ($r = 0.316$, $r^2 = 0.100$, $n = 8$, $p = 0.445$) when all the compounds but the oximes were considered. In particular, alkenes **36** and **37** seem to be more active (EC_{50}) on the atrium than on the isolated enzyme, while the opposite happens for the oximes.

The lack of correlation when the entire set of compounds but the oximes was considered may depend on the different distribution and biochemical characteristics of the Na^+,K^+-ATPase isoforms in different tissues and species. In fact, the IC_{50} was measured on isolated and purified Na^+,K^+-ATPase from dog kidney, which contains the α1 isoform, whereas EC_{50} was measured on the whole guinea pig atria which contains both α1 and α3 isoforms. In this context, it must be remembered that the sensitivity of the Na^+,K^+-ATPase for cardiac glycosides is isoform- and species-dependent (the affinity of the glycosides is higher for the α3 than for the α1 and α2 isoforms, and the Na^+,K^+-ATPase of the dog is more sensitive than that of the rat and the guinea pig). It has also been hypothesized that the inotropic or arrhythmogenic activities may be specifically sustained by one or the other isoform.[20] Therefore, it cannot be excluded that different chemical classes of compounds may differently interact with the Na^+,K^+-ATPase isoforms (α1 and α3) underlying specific functional activities at cardiac level. This hypothesis is currently under evaluation in our laboratory.

The compounds tested in vivo showed good maximum inotropic effects (E_{max}), comparable in some cases to the value of digoxin. Compounds **8**, **18**, and **24**, the most potent both in the inhibition test and in the guinea pig atrium, revealed the same inotropic effect (ED_{80}) at doses comparable to, or even lower than, digoxin. Unfortunately, a parallelism seems to occur between potency (ED_{80}) and toxicity, though for the weakest compounds, **9** and **12**, a safety ratio slightly higher than that of digoxin was found. These compounds deserve further investigation because other factors, such as pharmacokinetics, can be taken into account.

Molecular Modeling of the Ouabain Binding Site. To provide a further insight on the observed activities, a molecular model was created for the receptor site on the basis of the information currently available in the literature. Comprehensive random mutagenesis studies and photoaffinity labeling showed that most of the amino acid substitutions conferring ouabain-resistance relate to the H1−H2 region of the Na^+,K^+-ATPase α1-subunit. In particular, the greatest ouabain resistance is given by substitutions in the extracellular loop, such as Gln111Arg, Asp121(Gly, Glu), or Asn122Asp,[21,22] or Gln118Arg and Asp129Asn in *Torpedo electroplax*,[23] as well as those involving the hydrophobic core of the transmembrane region, such as Cys104(Ala, Phe) or Tyr108Ala,[24] or Tyr124Cys or Ile135Val.[25] A specific role for Cys104 was established, with a putative hydrogen-bond formation with the lactone ring. On this basis, Repke et al.[6,26,27] proposed

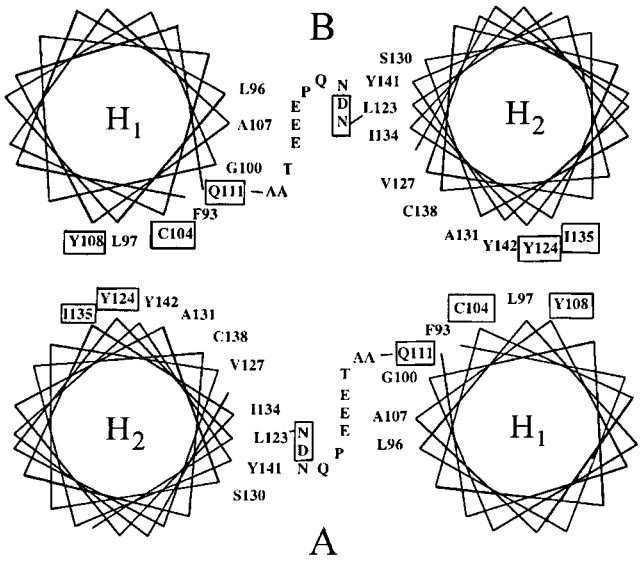


Figure 1. Pictorial representation of the interprotodimeric cleft constituted by two interacting subunits (A and B) of the Na^+,K^+-ATPase. Residues known to be important for digitalis binding are boxed.

a model, formed by two interacting H1−H2 domains arranged in a four-helix bundle to form a cleft, where digitalis sinks and specifically binds.

Although there is substantial agreement in the literature about the length and position of the transmembrane domains (Phe93−Ile110 and Leu123−Ser140 for H1 and H2, respectively, with the connecting loop mainly composed of hydrophilic residues), no information is at present available on the molecular structure of any ATPase α1-subunit at the atomic level nor on the relative orientation of H1 with respect to H2.

Following the work of Repke,[6] we made use of the available information on the topology of the transmembrane segments and the above-mentioned amino acids to build a molecular model of the H1−H2 dimer, where all residues known to be involved in digitalis binding are situated in the inner groove (Figure 1). The PHD-predicted transmembrane segments[28] were arranged in a helical loop geometry, according to the X-ray structure of the purple membrane bacteriorhodopsin,[29,30] with the external loop constituted by the stretch of negatively charged residues (Glu115−Glu117) and by Pro118 situated just in the middle to give an energetically favorable turn conformation for the correct peptide backbone folding.

At first, a suitable orientation of digoxin was found by manually docking the molecule within the groove of a single α1-subunit on the basis of the established interactions, i.e. with the sugar ring positioned at the level of the external loop and the Cys104 SH group forming a H-bond with the lactone ring. The complex was allowed to relax by a cycle of combined energy minimization and molecular dynamics (see experimental for details). A dimer was subsequently built by arranging another α1-subunit in a symmetrical orientation and by energy minimizing the whole complex.

The model thus obtained revealed some interesting features (Figure 2). The steroid moiety is kept in place by a large number of favorable interactions, contributing to build up a negative (attractive) interaction energy between the various functional groups and the amino

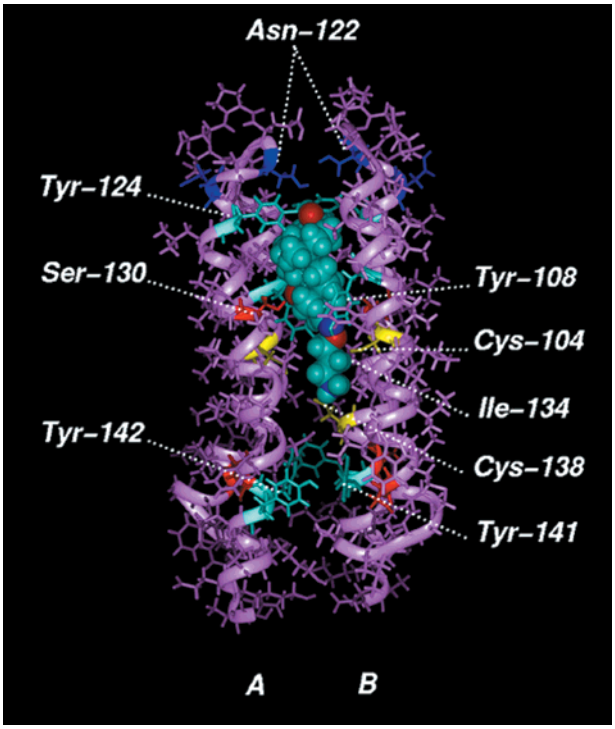


Figure 2. Model of the interprotodimeric cleft constituted by two subunits (A and B) of the Na^+,K^+-ATPase interacting with compound **8**.

acid side chains. In particular, the hydroxyl group at position 3 is held at the beginning of the cleft by hydrogen bonding formed with the Tyr124-OH of both subunits and with the amide group of H2 Asn122. H1 Cys104 interacts with the oxime heteroatoms of many compounds, with Tyr108 giving a further stabilization by means of a hydrogen-bond with the oxime moiety and, in the case of the unsaturated analogues, with attractive van der Waals interactions. Thus, the steroid moiety is held in place by a large network of hydrogen bonds and hydrophobic interactions, involving, for instance, Cys104, Tyr108, Asn122, Tyr124, Ser130, and Ile134. This latter residue is adjacent to Ile135, a residue considered important for digitalis binding. A superimposition of our derivatives with digoxigenin shows that the oxime group held a position analogous to the digoxigenin lactone; thus, the same types of interactions for the steroid moiety and the side chain are created up to the oxime group itself. However, the newly synthesized analogues build up novel interactions within the cleft with other amino acids not previously shown to be involved in the binding. The amino groups of the side chain, whether methylated or not, are found to interact in our model with Cys138. Given the relative acidity of the cysteine SH groups ($pK_a = 8.33$),[31] it may well be that an acid−base interaction occurs between this latter group and the terminal NH_2 of the analogues, giving rise to a salt-bridge of the $-CH_2S^- NH_3^+-$ type. The existence of an ion pair between the thiolate and protonated amine groups has already been demonstrated in the case of glutathione-*S*-transferase P1-1 (GST) in comparison with its K54A mutant.[32] For the wild-type GST a pK_a value of 4.2 for Cys47 was measured and the existence of the thiolate anion, possibly in ion pair with the Lys54 side chain, was detected by the characteristic UV absorbance at 229 nm.

In the Na^+,K^+-ATPase model, along with the cysteine SH groups, also the OH groups of Tyr141 and Tyr142, situated one complete helix-turn further apart, are found in the bottom of the cleft and provide other hydrogen-bonding possibilities for compounds having longer side chains.

Although the model was built on a limited number of molecules and needs further refinement (work in progress) for a quantitative evaluation, it does provide some insight into the structure−activity relationship of many of our newly synthesized compounds. Some general rules may be set on the basis of the model structure: (1) Compounds terminating with a basic group (NH_2 or NMe_2) show higher activity than those terminating with an acidic (COOH) or neutral (OH or OMe) group. (2) Within the class with the highest activity, a further modulation arises from the geometry and the chemical structure of the side chain, the optimum substituent linear chain being constituted by a six or seven atom spacer between the C(17) carbon atom of the steroidal ring and the basic amino group. Compounds **26** and **27**, with a maximum chain length of 10 and 11 atoms, are less active, with an IC_{50} equal to 1.26 and 6.3 μM, respectively.

Our model nicely fits with these general observations. The binding energy is given by a sum of attractive forces, with the terminal basic amino group playing a primary role if positioned at a correct distance from the only acidic groups available within the cleft, identified with Cys138 of both subunits. Thus, compounds **36**−**38** have IC_{50} values (0.08−1.25 μM) comparable to that of digoxin itself (IC_{50} = 0.50 μM), despite lacking any heteroatom capable of hydrogen-bonding with Cys104. Rather bulky amino groups, such as the terminal NMe_2, can be accommodated in the rather large bottom of the cleft, but a longer chain decreases the potency, as in the case of compound **26** (IC_{50} =1.26 μM). This may be explained by the building up of unfavorable repulsive interactions, between the amino group and the bottom of the cleft, constituted in our model by Tyr141 and Tyr142 of both subunits. Cys138 and Tyr142 are localized respectively 8 and 12 residues downstream the primary sequence from Ser130 and reside exactly two and three helix turns further from this residue. Thus, our findings suggest the existence of new side chains with hydrogen-bonding capabilities, apart from the previously ascertained ones, such as Cys104 or Asn122. Such new hydrogen-donor groups are localized about 12 and 16 Å from Ser130 and are buried very close to the bottom of the interprotodimeric cleft.

Conclusions

Among the digitalis-like compounds presented in this paper, some 17β-aminoalkyloxime derivatives showed extraordinary high inhibitory activity on Na^+,K^+-ATPase and inotropic potency on guinea pig atrium. The most active of them (**8**, **18**, **19**, and **24**) were 17−25 times more potent on the isolated dog kidney Na^+,K^+-ATPase (IC_{50}) than the reference compounds digoxin and digitoxigenin and showed inotropic potencies (EC_{50}) 3−11 times higher in isolated guinea pig left atria.

The in vivo evaluation, in the guinea pig, showed that the most potent compounds (**8**, **18**, and **24**) had inotropic effect comparable to or even higher than digoxin. The same happens with toxicity, so that the safety ratio results are slightly lower than that of digoxin. On the contrary, some weaker compounds (**9** and **12**) showed safety ratios slightly higher than digoxin. This behavior deserves further investigation.

The presence of an amino function in the 17β-substituent is of utmost importance for a strong interaction with the receptor. The contemporary presence of an oxime group further strengthens the interaction with the receptor, more if the oxime is an α,β-unsaturated one, thus mimicking the electronic situation of the natural 17β-unsaturated lactone in digitalis derivatives. A proper distance of the amine group from the C(17) of the steroidal skeleton is also of great importance, a spacer of six atoms being the most suitable one. Within the basic group, a primary amine is always more active than a tertiary amine.

These features are supported by a molecular model suggesting the possible interactions of the above-described groups with specific amino acid residues in the H1−H2 domains of Na^+,K^+-ATPase; some of them confirm the classical interactions already described in previous papers and confirmed by mutagenesis studies. On the contrary, the strong interaction of a basic group with the Cys138 is a new one and opens new possibilities to design compounds interacting with this region of the receptor.

In particular, this led to new classes of non-digitalis derivatives, now under study, with strong activities and, more important, a therapeutic index better than the classical digitalis derivatives. This gives us a chance to overcome the failures of the past 50 years of research in finding a valid alternative to the more than a century old digoxin.[6]

Experimental Section

Chemistry. Elemental analyses were performed by Redox, Cologno Monzese, Italy. ^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer at 300.13 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard and coupling constants (J values) are in hertz. ^{1}H NMR assignments were drawn from classical arguments on chemical shift and coupling constant behavior. Mass spectral data were obtained with the electron impact ionization technique at 70 eV from a Finnigan INCOS-50 mass spectrometer using the direct exposure probe (DEP). Chromatographies were carried out on silica gel (Baker 7024-02) in all instances. Solvents and reagents were used as purchased from Aldrich.

Preparation of Oximes. Method A. A solution of NaOAc (4 equiv) and the appropriate hydroxylamine (1.05 equiv) in dioxane/water 3:2 (0.1 M) was adjusted to pH 4.5 with 6 N HCl. A solution of the appropriate aldehyde (1 equiv) in dioxane/water 2:1 (0.2 M) was added dropwise at room temperature. After 1−2 h for compounds **1**−**10** or 4−6 h for compounds **20**−**26**, dioxane was evaporated in vacuo. The residue was diluted with water and 1 N NaOH added until pH 9.5 was reached. The mixture was extracted with EtOAc (3×), and the organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by flash chromatography and, eventually, transformed into the salt by adding the stoichiometric amount of the corresponding acid.

Preparation of Oximes. Method B. To a solution of the appropriate hydroxylamine (1.2 equiv) in 1 N NaOH/dioxane 5:2 (1.7 M) was added dropwise a solution of the appropriate aldehyde (1 equiv) in dioxane (0.5 M) at room temperature. After 1 h the solution was diluted with water and extracted with $CHCl_3$ (3×). The organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by flash

chromatography and, eventually, transformed into the salt by adding the stoichiometric amount of the corresponding acid.

Preparation of Hydroxylamines. Method C. A solution of crude oxime as a base (1 equiv) in MeOH (0.2 M) was adjusted to pH 3.0 with 1 N HCl under stirring at room temperature. $NaBH_3CN$ (1.5 equiv) was added followed by water (0.1 mL/equiv). The reaction was continuously kept at pH 3 by a pH-stat controlling the addition of 1 N HCl. After 6 h $NaBH_3CN$ (0.75 equiv) was added followed by water (0.1 mL/equiv). Additions of $NaBH_3CN$ (0.4 equiv) and water (0.1 mL/equiv) were repeated after 24 and 30 h. After 48 h the solution was brought to pH 1.8 with 1 N HCl. After stirring for 1 h the solution was brought to pH 9.5 with 4 N NaOH and methanol was evaporated in vacuo. The mixture was diluted with water and extracted with EtOAc (3×). The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by flash chromatography and, eventually, transformed into the salt by adding the stoichiometric amount of the corresponding acid.

Preparation of Oximes. Method D. A solution of ketone **59** (1 equiv) and the appropriate hydroxylamine (4 equiv) in ethanol (0.08 M) was adjusted to pH 2 with 0.1 N NaOH and stirred at room temperature for several days. Ethanol was evaporated in vacuo. The residue was diluted with water, 1 N NaOH added, and the mixture extracted with CH_2Cl_2 (3×). The combined organic layers were washed with 1 N NaOH, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by flash chromatography.

(*E*)-17*β*-[(Oximino)methyl]-5*β*-androstane-3*β*,14*β*-diol (1) was prepared following method A. The crude product on chromatography with *n*-hexane/EtOAc, (1:1), R_f 0.34, gave a white solid. ^{1}H NMR ($CDCl_3$): δ 0.94 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.42 (m, 1H, 17-H), 4.13 (m, 1H, 3-H), 6.68 (bb, 1H, NOH), 7.62 (d, 1H, J = 9.5, CHN). MS: *m*/*z* 335 (8, M^+), 203 (42).

(*Z*)-17*β*-[(Oximino)methyl]-5*β*-androstane-3*β*,14*β*-diol (2) was prepared following method A. The crude residue was crystallized twice from EtOAc to give a white solid; R_f 0.26 (*n*-hexane/EtOAc, 1:1). ^{1}H NMR (CD_3OD): δ 0.96 (s, 6H, 2 CH_3), 3.28 (m, 1H, 17-H), 4.04 (m, 1H, 3-H), 6.81 (d, 1H, J = 7.7, CHN). MS: *m*/*z* 335 (1, M^+), 203 (48).

(*E*)-17*β*-[(Methoxyimino)methyl]-5*β*-androstane-3*β*,14*β*-diol (3) was prepared following method A. The crude product on chromatography with cyclohexane/EtOAc, (6:4), R_f 0.33, followed by trituration with *i*-Pr_2O/EtOH, gave a white solid. ^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 2.41 (m, 1H, 17-H), 3.81 (s, 3H, OCH_3), 4.13 (m, 1H, 3-H), 7.56 (d, 1H, J = 9.3, CHN). MS: *m*/*z* 349 (22, M^+), 318 (100).

***(E)*-17*β*-[[(2-Dimethylaminoethoxy)imino]methyl]-5*β*-androstane-3*β*,14*β*-diol L-tartrate (4)** was prepared following method A. EtOAc (55 mL) was added to the solution of the crude product (3.80 g) and the stoichiometric amount of L-tartaric acid in EtOH (15 mL). After standing at 0 °C overnight, the solid was collected to give **4** (4.15 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.89 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.37 (m, 1H, 17-H), 2.89 (s, 6H, $N(CH_3)_2$), 3.42 (m, 2H, NCH_2), 4.04 (m, 1H, 3-H), 4.33 (m, 2H, OCH_2), 4.89 (s, 2H, tartrate), 6.96 (d, 0.1H, J = 7.2, CH═N (*Z*) isomer), 7.56 (d, 0.9H, J = 9.3, CH═N (*E*) isomer). MS: *m*/*z* 406 (5, M^+ base), 58 (100).

(*E*)-17*β*-[[(3-Dimethylaminopropoxy)imino]methyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (5) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1 followed by salt formation with the stoichiometric amount of oxalic acid in EtOH/EtOAc. After evaporation in vacuo of the resulting solution, the residue was triturated with Et_2O/EtOH to give **5**, a white solid. ^{1}H NMR (CD_3OD): δ 0.88 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.33 (m, 1H, 17-H), 2.88 (s, 6H, $N(CH_3)_2$), 3.21 (m, 2H, NCH_2), 4.05 (m, 3H, 3-H and OCH_2), 6.87 (d, 0.1H, J = 8.1, CH═N (*Z*) isomer), 7.56 (d, 0.9H, J = 9.3, CH═N (*E*) isomer). MS: *m*/*z* 420 (3, M^+ base), 302 (70), 71 (100).

(*E*)-17*β*-[[(4-Dimethylaminobutoxy)imino]methyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (6) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1 followed by salt formation with the stoichiometric amount of oxalic acid in EtOH/EtOAc. After evaporation in vacuo of the resulting solution, the residue was triturated with Et_2O/EtOH to give **6**, a white solid. ^{1}H NMR ($CDCl_3$): δ 0.92 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 2.38 (m, 0.9H, 17-H (*E*) isomer), 2.80 (s, 6H, $N(CH_3)_2$), 3.01 (m, 2H, NCH_2), 3.11 (m, 0.1H, 17-H (*Z*) isomer), 4.02 (m, 2H, OCH_2), 4.13 (m, 1H, 3-H), 6.86 (d, 0.1H, J = 8.2, CH═N (*Z*) isomer), 7.60 (d, 0.9H, J = 9.0, CH═N (*E*) isomer). MS: *m*/*z* 434 (0.1, M^+ base), 58 (100).

(*E*)-17*β*-[[(2-Aminoethoxy)imino]methyl]-5*β*-androstane-3*β*,14*β*-diol fumarate (7) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (1.85 g) was dissolved in EtOH (15 mL) at 40 °C and added to a boiling solution of the stoichiometric amount of fumaric acid in EtOH (8 mL). EtOAc (10 mL) was added to the resulting solution. After stirring at room temperature overnight, the solid was collected to give **7** (1.90 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.89 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.37 (m, 1H, 17-H), 3.19 (m, 2H, NCH_2), 4.04 (m, 1H, 3-H), 4.19 (m, 2H, OCH_2), 6.68 (s, 2H, fumarate), 7.64 (d, 1H, J = 9.4, CH═N). MS: *m*/*z* 378 (5, M^+ base), 318 (100).

(*E*)-17*β*-[[(3-Aminopropoxy)imino]methyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (8) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (0.40 g) was dissolved in EtOAc (5 mL) and EtOH (5 mL) and the stoichiometric amount of oxalic acid was added. After evaporation in vacuo, the residue was triturated with EtOAc to give **8** (0.29 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.88 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.32 (m, 1H, 17-H), 3.00 (m, 2H, NCH_2), 4.05 (m, 3H, 3-H and OCH_2), 6.86 (d, 0.1H, J = 8.7, CH═N (*Z*) isomer), 7.56 (d, 0.9H, J = 10.0, CH═N (*E*) isomer). MS: *m*/*z* 392 (2, M^+ base), 302 (100), 250 (90), 203 (32).

(*E*)-17*β*-[[(4-Aminobutoxy)imino]methyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (9) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (2.88 g) was dissolved in EtOH (25 mL) and the stoichiometric amount of oxalic acid was added. After evaporation in vacuo, the residue was triturated with EtOAc to give **9** (2.90 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.87 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.32 (m, 1H, 17-H), 2.95 (m, 2H, NCH_2), 3.99 (m, 2H, OCH_2), 4.04 (m, 3H, 3-H), 6.82 (d, 0.1H, J = 7.5, CH═N (*Z*) isomer), 7.53 (d, 0.9H, J = 9.4, CH═N (*E*) isomer). MS: *m*/*z* 406 (1, M^+ base), 250 (23), 88 (100).

(*E*)-17*β*-[(Carboxymethoxyimino)methyl]-5*β*-androstane-3*β*,14*β*-diol (10) was prepared following method A. The extraction from the aqueous phase was carried out at pH 3 with EtOAc/THF, 9:1, and the crude product was triturated with EtOAc to give a white solid. ^{1}H NMR (CD_3OD): δ 0.90 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.33 (m, 1H, 17-H), 4.04 (m, 1H, 3-H), 4.46 (s, 0.26H, OCH_2 (*Z*) isomer), 4.51 (s, 1.74H, OCH_2 (*E*) isomer), 6.99 (d, 0.1H, J = 7.5, CH═N (*Z*) isomer), 7.63 (d, 0.9H, J = 10.0, CH═N (*E*) isomer). MS: *m*/*z* 250 (66), 203 (6).

(*E*)-17*β*-[2-[(2-Dimethylaminoethoxy)imino]ethyl]-5*β*-androstane-3*β*,14*β*-diol (11) was prepared following method B. The crude product on chromatography with $CHCl_3$/MeOH (8:2) gave a white foam. ^{1}H NMR (CD_3OD): δ 0.97 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.16 (t, 0.9H, NCH_2 (*E*) isomer), 2.18 (t, 0.1H, NCH_2 (*Z*) isomer), 2.31 (s, 6H, $N(CH_3)_2$), 2.30−2.50 (m, 2H, CH_2C═N), 4.04 (m, 1H, 3-H), 4.11 (t, 0.9H, OCH_2 (*E*) isomer), 4.18 (t, 0.1H, OCH_2 (*Z*) isomer), 6.69 (d, 0.1H, J = 5.9, CH═N (*Z*) isomer), 7.36 (d, 0.9H, J = 7.5, CH═N (*E*) isomer). MS: *m*/*z* 420 (4.4, M^+), 332 (33), 58 (100).

(*EZ*)-17*β*-[3-[(2-Dimethylaminoethoxy)imino]propyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (12) was prepared following method B. The crude product was purified by chromatography with $CHCl_3$/MeOH, 8:2. The purified product as a base (0.30 g) was dissolved in Et_2O (25 mL) and EtOAc (5 mL) and the stoichiometric amount of oxalic acid was added. The

precipitate was collected to give **12** (0.27 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.94 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.93 (s, 3.6H, $N(CH_3)_2$ (*E*) isomer), 2.94 (s, 2.4H, $N(CH_3)_2$ (*Z*) isomer), 3.40−3.50 (m, 2H, CH_2N), 4.04 (m, 1H, 3-H), 4.25−4.40 (m, 2H, OCH_2), 6.80 (d, 0.4H, J = 5.6, CH=N (*Z*) isomer), 7.50 (d, 0.6H, J = 6.1, CH=N (*E*) isomer). MS: *m*/*z* 434 (2.8, M^+ base), 203 (45), 58 (100).

(*E*)-17*β*-[3-[(2-Aminoethoxy)imino]propyl]-5*β*-androstane-3*β*,14*β*-diol (13) was prepared following method B. The crude product on chromatography with $CHCl_3$/MeOH/26% NH_4OH (89:10:1) gave **13** as a white solid (1.05 g). ^{1}H NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.69 (m, 2H, NCH_2), 3.63 (m, 1H, 3-H), 3.84 (t, J = 7.5, 1.8H, OCH_2 (*E*) isomer), 3.90 (t, J = 7.5, 0.2H, OCH_2 (*Z*) isomer), 6.68 (d, 0.1H, J = 7.5, CH=N (*Z*) isomer), 7.38 (d, 0.9H, J = 10.0, CH=N (*E*) isomer). MS: *m*/*z* 389 (20, M^+-17), 326 (100).

(*E*,*E*)-17*β*-[3-(Methoxyimino)-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol (14) was prepared following method B. The crude product was purified by chromatography with *n*-hexane/EtOAc (8:2) followed by crystallization from EtOAc to give **14** (1.15 g) as a white solid.^{1}H NMR (DMSO-d_6): δ 0.72 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 2.19 (m, 1H, 17-H), 3.72 (s, 3H, OCH_3), 3.87 (m, 1H, 3-H), 3.97 (s, 1H, 14-OH), 4.17 (d, 3-OH), 5.81 (dd, 1H, J = 9.8, 15.5, H-21), 6.27 (dd, 1H, J = 10.2, 15.5, H-20), 7.72 (d, 1H, J = 9.8, H-22). MS: *m*/*z* 375 (11.7, M^+), 94 (100).

(*E*,*E*)-17*β*-[3-(Propoxyimino)-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol (15) was prepared following method B. The crude product on chromatography with *n*-hexane/EtOAc (8:2) gave **15** as a white foam (0.2 g). ^{1}H NMR (CD_3OD): δ 0.84 (s, 3H, CH_3), 0.94 (t, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.31 (m, 1H, 17-H), 3.92 (t, 2H, OCH_2), 4.04 (m, 1H, 3-H), 5.90 (dd, 1H, J = 10.0, 15.6, CHC=N), 6.37 (dd, 1H, J = 10.3, 15.6, CHC=C=N), 7.68 (d, 1H, J = 10.0, CH=N). MS: *m*/*z* 403 (15, M^+), 203 (83), 94 (100).

(*E*,*E*)-17*β*-[3-[(2-Hydroxyethoxy)imino]-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol (16) was prepared following method B. The crude product on crystallization from EtOAc gave **16** as a white solid (280 mg). ^{1}H NMR (DMSO-d_6): δ 0.73 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 3.54 (q, J = 5.0, 2H, CH_2OH), 3.94 (t, J = 5.0, 2H, OCH_2), 3.88 (m, 1H, 3-H), 5.81 (dd, 1H, J = 15.6, 9.8, 21-H), 6.26 (dd, 1H, J = 15.6, 10.2, 20-H), 7.73 (d, 1H, J = 9.8, 22-H). MS: *m*/*z* 405 (3, M^+), 203 (51), 79 (100).

(*E*,*EZ*)-17*β*-[3-[(Carboxymethoxy)imino]-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol Oxalate (17). To a solution of (*E*)-3*β*,14*β*-dihydroxy-5*β*-pregn-20-ene-21-carboxaldehyde (**41**) (0.25 g, 0.72 mmol) in dioxane (3 mL) was added a solution of 2-aminooxyacetic acid hydrochloride (0.11 g, 0.86 mmol) in water (1 mL). After 1 h the solution was diluted with water and extracted with $CHCl_3$ (3 × 20 mL). The organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was crystallized from EtOAc to give **17** (0.18 g), a white solid. ^{1}H NMR (DMSO-d_6): δ 0.74 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.23 (m, 1H, 17-H), 3.88 (bb, 1H, 14-OH), 3.88 (m, 1H, 3-H), 4.16 (bb, 1H, 3-OH), 4.47 (s, 1.5H, OCH_2 (*E*) isomer), 4.49 (s, 0.5H, OCH_2 (*Z*) isomer), 5.80 (dd, 0.75H, J = 9.9, 15.5, CHC=N (*E*) isomer), 6.33 (dd, 0.75H, J = 10.0, 15.5, CH=CC=N (*E*) isomer), 6.3−6.5 (m, 0.5H, CH=CHC=N (*Z*) isomer), 7.13 (d, 0.25H, J = 8.7, CH=N (*Z*) isomer), 7.81 (d, 0.75H, J = 9.9, CH=N (*E*) isomer), 12.7 (bb, 1H, COOH).

(*E*,*E*)-17*β*-[3-[(2-Dimethylaminoethoxy)imino]-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (18) was prepared following method B. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (2.75 g) was dissolved in EtOAc (30 mL) and EtOH (2 mL) and the stoichiometric amount of oxalic acid was added. The precipitate was collected to give **18** (3.15 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.84 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.31 (m, 1H, 17-H), 2.92 (s, 6H, $N(CH_3)_2$), 3.45 (m, 2H, NCH_2), 4.04 (m, 1H, 3-H), 4.34 (m, 2H, OCH_2), 5.90 (dd, 1H, J = 10.0, 15.6, CHC=N), 6.37 (dd, 1H, J = 10.3, 15.6, CH=CC=N), 7.11 (d, 0.05H, J = 7.5, CH=N (*Z*) isomer), 7.79 (d, 0.95H, J = 10.0, CH=N (*E*) isomer). MS: *m*/*z* 432 (2, M^+ base), 58 (100).

(*E*,*E*)-17*β*-[3-[(2-Aminoethoxy)imino]-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (19) was prepared following method B. The crude product on chromatography with $CHCl_3$/MeOH/26% NH_4OH (89:10:1) gave **19** (0.58 g) as a white foam. ^{1}H NMR (CD_3OD): δ 0.84 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.29 (m, 1H, 17-H), 2.86 (t, 2H, NCH_2), 4.03 (t, 2H, OCH_2), 4.04 (m, 1H, 3-H), 5.87 (dd, 1H, J = 10.0, 15.6, CHC=N), 6.30 (dd, 1H, J = 10.0, 15.6, CH=CC=N), 6.99 (d, 0.04H, J = 7.5, CH=N (*Z*) isomer), 7.74 (d, 0.96H, J = 10.0, CH=N (*E*) isomer). MS: *m*/*z* 404 (99, M^+ base), 326 (100).

(*E*,*E*)-17*β*-(3-Methoxyimino-2-methyl-1-propenyl)-5*β*-androstane-3*β*,14*β*-diol (20) was prepared following method A. The crude product on chromatography with *n*-hexane/EtOAc (8:2) gave **20** (0.17 g) as a white foam. ^{1}H NMR (CD_3OD): δ 0.84 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.75 (s, 3H, $=CCH_3$), 2.71 (m, 1H, 17-H),) 4.04 (m, 1H, 3-H), 5.90 (d, 1H, J = 10, 20-H), 7.61 (s, 1H, CH=N). MS: *m*/*z* 389 (4, M^+), 94 (100).

(*E*,*E*)-17*β*-(3-Propoxyimino-2-methyl-1-propenyl)-5*β*-androstane-3*β*,14*β*-diol (21) was prepared following method A. The crude product on chromatography with *n*-hexane/EtOAc (8:2) gave **21** (0.23 g) as a white foam. ^{1}H NMR (CD_3OD): δ 0.84 (s, 3H, CH_3), 0.94 (t, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 2.71 (m, 1H, 17-H), 3.92 (t. 2H, OCH_2), 4.04 (m, 1H, 3-H), 5.90 (d, 1H, J = 10 20-H), 7.61 (s, 1H, CH=N). MS: *m*/*z* 417 (9, M^+), 143 (100).

(*E*,*E*)-17*β*-[3-(2-Hydroxyethoxy)imino-2-methyl-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol (22) was prepared following method A. The crude product was purified twice by chromatography with hexane/$CHCl_3$/acetone, 36:32:32 and *n*-hexane/$CHCl_3$/acetone, 46:27:27 to give the pure **22** (0.38 g). ^{1}H NMR ($CDCl_3$): δ 0.86 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.09 (s, 1H, 14*β*-OH), 1.67 (d, J = 0.9, CH_3−C=), 2.71 (m, 1H, 17-H), 3.56 (t, 2H, CH_2OH), 3.97 (t, 2H, CH_2ON), 5.96 (d, 1H, J = 5.5, H-20), 7.67 (s, 1H, CH=N). MS: *m*/*z* 419 (1.5, M^+), 94 (100).

(*E*,*E*)-17*β*-[3-(2-Dimethylaminoethoxy)imino-2-methyl-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol fumarate (23) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (0.36 g) was dissolved in EtOAc (8 mL) and added to a solution of the stoichiometric amount of fumaric acid in EtOH (2 mL). After standing for 4 h, the crystals were collected to give **23** (0.41 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.83 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.77 (d, 3H, J = 0.9, $=CCH_3$), 2.70 (m, 1H, 17-H), 2.90 (s, 6H, $N(CH_3)_2$), 3.42 (m, 2H, NCH_2), 4.05 (m, 1H, 3-H), 4.34 (m, 2H, OCH_2), 6.05 (bd, 1H, J = 10.9, CH=CC=N), 6.68 (s, 2H, fumarate), 7.77 (s, 1H, CH=N). MS: *m*/*z* 446 (4, M^+ base), 203 (40), 58 (100).

(*E*,*E*)-17*β*-[3-(2-Aminoethoxy)imino-2-methyl-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol fumarate (24) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (0.27 g) was dissolved in EtOH (5 mL) and the stoichiometric amount of fumaric acid was added. After evaporation in vacuo, the residue was triturated with EtOAc and the solid collected to give **24** (0.32 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.84 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.77 (d, 3H, J = 1.2, $=CCH_3$), 2.71 (m, 1H, 17-H), 3.24 (m, 2H, NCH_2), 4.04 (m, 1H, 3-H), 4.24 (m, 2H, OCH_2), 6.05 (bd, 1H, J = 10.9, CH=CC=N), 6.68 (s, 2H, fumarate), 7.77 (s, 1H, CH=N). MS: *m*/*z* 418 (19, M^+ base), 203 (100).

(*E*,*E*)-17*β*-[3-[(3-Aminopropoxy)imino]-2-methyl-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (25) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (0.31 g) was dissolved in EtOH (5 mL) and the stoichiometric amount of oxalic acid was added. After evaporation in vacuo, the residue was triturated with EtOAc and the solid collected to give **25** (0.37 g), a white solid. ^{1}H NMR (DMSO-d_6): δ 0.72 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 1.68 (bs, 3H, $=CCH_3$), 2.55 (m, 1H, 17-H), 2.81 (m, 2H, NCH_2), 3.87 (m, 1H, 3-H), 4.03 (m, 2H, OCH_2), 5.98 (bd, 1H, J = 10.4,

CH═CC═N), 7.69 (s, 1H, CH═N). MS: *m*/*z* 432 (5, M^+ base), 230 (60), 94 (100).

(*E*,*E*)-17*β*-[3-(4-Aminobutoxy)imino-2-methyl-1-propenyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (26) was prepared following method A. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1. The purified product as a base (0.35 g) was dissolved in EtOH (5 mL) and the stoichiometric amount of oxalic acid was added. After evaporation in vacuo, the residue was triturated with EtOAc and the solid collected to give **26** (0.26 g), a white solid. ^{1}H NMR (DMSO-d_6): δ 0.71 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 1.68 (bs, 3H, ═CCH_3), 2.55 (m, 1H, 17-H), 2.78 (m, 2H, NCH_2), 3.87 (m, 1H, 3-H), 3.96 (m, 2H, OCH_2), 5.96 (bd, 1H, J = 10.4, CH═CC═N), 7.66 (s, 1H, CH═N). MS: *m*/*z* 446 (2, M^+ base), 203 (35), 88 (100).

(*E*,*E*,*E*)-17*β*-[5-(2-Dimethylaminoethoxy)imino-1,3-pentadienyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (27) was prepared following method B. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 95/5/0.5. The purified product as a base (0.31 g) was dissolved in EtOAc (8 mL) and added to a solution of the stoichiometric amount of oxalic acid in EtOAc. After dilution with Et_2O, the solid was collected to give **27** (0.33 g), a white solid. ^{1}H NMR (DMSO-d_6): δ 0.73 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 2.14 (m, 1H, 17-H), 2.71 (s, 6H, $N(CH_3)_2$), 3.25 (m, 2H, NCH_2), 3.88 (m, 1H, 3-H), 4.28 (m, 2H, OCH_2), 5.90−6.70 (m, 4H, $(CH═CH)_2$), 7.26 (d, 0.1H, J = 8.0, CH═N (*Z*) isomer), 7.88 (d, 0.9H, J = 9.9, CH═N (*E*) isomer). MS: *m*/*z* 458 (3, M^+ base), 58 (100).

17*β*-[(Methoxyamino)methyl]-5*β*-androstane-3*β*,14*β*-diol oxalate (28) was prepared following method C. The crude product was purified by chromatography with cyclohexane/EtOAc, 65:35. The purified product as a base (100 mg) was dissolved in EtOAc (8 mL) and added to a solution of the stoichiometric amount of oxalic acid in EtOAc; the precipitate was collected to give **28** (52 mg), a white solid. ^{1}H NMR (CD_3-OD): δ 0.96 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 3.20−3.40 (m, 2H, NCH_2), 3.73 (s, 3H, OCH_3), 4.04 (m, 1H, 3-H). MS: *m*/*z* 351 (3, M^+ base), 302 (80), 94 (100).

17*β*-[(2-Dimethylaminoethoxy)aminomethyl]-5*β*-androstane-3*β*,14*β*-diol dihydrochloride (29) was prepared following method C. The solution of the crude product (2.30 g) in EtOH (13.5 mL) was added with 36% HCl (0.82 mL). After standing at 0 °C overnight, the precipitate was collected. After drying, the solid was crystallized by dissolution in MeOH (10 mL) followed by slow addition of EtOAc (10 mL). After standing at room temperature for 1 h and at 0 °C overnight, the solid was collected to give **29** (1.57 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.98 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 2.98 (s, 6H, $N(CH_3)_2$), 3.40−3.60 (m, 2H, $ONCH_2$), 3.58 (m, 2H, NCH_2), 4.05 (m, 1H, 3-H), 4.53 (t, 2H, OCH_2). MS: *m*/*z* 58 (100).

17*β*-[(2-Aminoethoxy)aminomethyl]-5*β*-androstane-3*β*,-14*β*-diol dihydrochloride (30) was prepared following method C. 36% HCl (4.50 mL) was added to a solution of the crude product (12.20 g) in EtOH (73 mL) at 45 °C. After standing at room temperature for 1 h and at 0 °C overnight, the solid was collected to give **30** (8.90 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.97 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 3.33 (t, 2H, CH_2NH_2), 3.40−3.60 (m, 2H, CH_2NO), 4.05 (m, 1H, 3-H), 4.42 (t, 2H, OCH_2). MS: *m*/*z* 380 (0.5, M^+ base), 250 (100).

17*β*-[(3-Aminopropoxy)aminomethyl]-5*β*-androstane-3*β*,14*β*-diol dihydrochloride (31) was prepared following method C. The crude product was purified by chromatography with $CHCl_3$/MeOH/26% NH_4OH, 84:15:1.5. HCl (36%, 0.20 mL) was added to a solution of the base (0.52 g) in EtOH/MeOH, 8:2 (6 mL). The solution was evaporated in vacuo and the solid was triturated with Et_2O to give **31** (0.57 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.98 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 3.08 (t, 2H, CH_2NH_2), 3.35−3.55 (m, 2H, CH_2NO), 4.04 (m, 1H, 3-H), 4.29 (t, 2H, OCH_2). MS: *m*/*z* 394 (0.4, M^+ base), 286 (100).

17*β*-[*N*-(2-Dimethylaminoethoxy)-*N*-methylaminomethyl]-5*β*-androstane-3*β*,14*β*-diol Oxalate (32). Aqueous CH_2O (36%, 2.2 mL, 29.0 mmol) was added to a mixture of **29** as a base (2.76 g, 5.7 mmol) in MeCN (16.5 mL). After addition of $NaBH_3CN$ (0.68 g, 9.2 mmol) the mixture was stirred for 5 h. HCl (1 N, 15 mL) was added and the solution stirred for 30 min; 1 N NaOH was added until pH 11 was reached and the mixture extracted with EtOAc (5 × 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by two flash chromatographies ($CHCl_3$/MeOH/26% NH_4OH, 95:5:0.5, then $CHCl_3$/MeOH, 9:1). After evaporation to dryness of the fractions containing the pure compound, the residue was dissolved in EtOAc (8 mL) and the stoichiometric amount of oxalic acid added. The waxy solid was triturated with EtOAc to give **32** (0.86 g), a white solid. ^{1}H NMR (CD_3OD): δ 0.95 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.60 (s, 3H, $ONCH_3$), 2.60−3.20 (m, 4H, $ONCH_2CH_2N$), 2.90 (s, 6H, $N(CH_3)_2$), 3.85 (m, 2H, OCH_2), 4.04 (m, 1H, 3-H). MS: *m*/*z* 42 (18, M^+ base), 88 (100).

17*β*-[3-Methoxyaminopropyl]-5*β*-androstane-3*β*,14*β*-diol Hydrochloride (33). 17*β*-(3-Methoxyiminopropyl)-5*β*-androstane-3*β*,14*β*-diol hydrochloride (**57**) was prepared following method A. The crude product was purified by column chromatography (*n*-hexane/EtOAc, 1:1) to give **57** (520 mg, 80%). ^{1}H NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.98 (m, 1H, 3-H), 7.38 (d, 1H, J = 10.0, CH═N). Reduction of **57**, following method C, and crystallization of the crude from EtOAc, followed by salt formation with the stoichiometric amount of HCl (37% solution) in EtOH/Et_2O 1:2, gave **33** as a white solid (342 mg). ^{1}H NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 3.08 (m, 2H, CH_2N), 3.81 (s, 3H, OCH_3), 3.87 (m, 1H, 3-H). MS: *m*/*z* 379 (0.07, M^+ base), 361 (1), 330 (88).

17*β*-[3-(2-Aminoethoxy)aminopropyl]-5*β*-androstane-3*β*,14*β*-diol (34) was prepared following method C. The crude product on chromatography with $CHCl_3$/MeOH/26% NH_4OH, 89:10:1 gave **34** as a white solid (233 mg). ^{1}H NMR (DMSO-d_6): δ 0.81 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.65 (m, 4H, 22-H and CH_2N), 3.48 (t, J = 7.5, 2H, OCH_2), 3.87 (m, 1H, 3-H). MS: *m*/*z* 391 (10, M^+ − 17), 331 (18), 89 (100).

(*E*)-17*β*-(3-Methoxyamino-1-propenyl)-5*β*-androstane-3*β*,14*β*-diol hydrochloride (35) was prepared following method C. The crude product on chromatography with CH_2Cl_2/EtOAc followed by salt formation with the stoichiometric amount of HCl (37% solution) in EtOH/Et_2O, 1:10 gave **35** as a white solid (200 mg). ^{1}H NMR (DMSO-d_6): δ 0.76 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 3.69 (d, J = 10.0, 1H, 22-H), 3.78 (s, 3H, OCH_3), 3.98 (m, 1H, 3-H), 5.21 (dt, 1H, J = 15.3, 6.9, 20-H), 6.07 (dt, 1H, J = 15.3, 10.0, 21-H). MS: *m*/*z* 359 (12, M^+ − H_2O base), 328 (37), 230 (8), 67 (100).

(*E*,*E*)-17*β*-(6-Aminohexa-1,3-dienyl)-5*β*-androstane-3*β*,-14*β*-diol (36). To a solution of (*E*)-21-iodo-5*β*-pregn-20-ene-3*β*,14*β*-diol (**62**) (310 mg, 0.70 mmol) and (*E*)-1-tributyltin-4-azidobutene (**68**) (660 mg, 1.71 mmol) in degassed DMF (6 mL) was added $PdCl_2(CH_3CN)_2$ (17 mg, 0.07 mmol). After 16 h the dark solution was diluted with a 10% KF solution (10 mL), stirred for 15 min, filtered, and extracted with Et_2O (2 × 30 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 7:3) to give (*E*,*E*)-17*β*-(6-azidohexa-1,3-dienyl)-5*β*-androstane-3*β*,14*β*-diol (**63**, 254 mg, 88%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 0.82 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 3.45 (t, 2H, J = 7.0, CH_2N_3), 4.13 (m, 1H, 3-H), 5.5−6.1 (m, 4H, H-20, H-21, H-22, H-23).

A solution of **63** (254 mg, 0.62 mmol) in THF (20 mL) was treated with $LiAlH_4$ (130 mg, 3.42 mmol) and the resulting mixture was stirred at room temperature for 16 h. After this time NaOH (4 N, 1 mL) was added followed by Na_2SO_4 (2 g). After stirring for 1 h, the white precipitate was filtered off and the organic solvent evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH, 89:10:1) to give **36** (72 mg, 30%) as a white foam. ^{1}H NMR (DMSO-d_6): δ 0.71 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.54 (m, 2H, CH_2NH_2), 3.86 (m, 1H, 3-H), 5.4−6.0 (m, 4H, H-20, H-21, H-22, H-23). MS: *m*/*z* 387 (33, M^+), 369 (22), 340 (100).

(*E*,*E*)-17*β*-(7-Aminohepta-1,3-dienyl)-5*β*-androstane-3*β*,14*β*-diol Oxalate (37). To a solution of (*E*)-21-iodo-5*β*-pregn-20-ene-3*β*,14*β*-diol (**62**) (1.12 g, 2.52 mmol) and (*E*)-5-tributyltin-4-pentenenitrile (**69**) (1.40 g, 3.78 mmol) in degassed DMF (20 mL) was added $PdCl_2(CH_3CN)_2$ (60 mg, 0.25 mmol). After 2 h the dark solution is diluted with a 10% KF solution (50 mL), stirred for 15 min, filtered, and extracted with Et_2O (3 × 50 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 7:3) to give (*E*,*E*)-17*β*-(6-cyanohexa-1,3-dienyl)-5*β*-androstane-3*β*,14*β*-diol (**64**, 0.74 g, 74%) as a white foam. ^{1}H NMR ($CDCl_3$): *δ* 0.77 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 2.40 (m, 2H, CH_2CN), 4.15 (m, 1H, 3-H), 5.53 (m, 1H, CH=C*H*CH$_2$), 5.88 (m, 2H, =C*H*C*H*=), 6.14 (dd, 1H, *J* = 14.8, 9.5, C*H*=CH).

A solution of (*E*,*E*)-17*β*-(6-cyanohexa-1,3-dienyl)-5*β*-androstane-3*β*,14*β*-diol (**64**) (445 mg, 1.12 mmol) in THF (30 mL) was treated with $LiAlH_4$ (260 mg, 6.84 mmol) and the resulting mixture was heated under reflux for 24 h. After cooling to room temperature, NaOH (4 N, 2 mL) was added followed by Na_2SO_4 (4 g). After stirring for 1 h, the white precipitate was filtered off and the organic solvent evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH, 89:10:1) to give **37** (200 mg, 44%) as a white foam. ^{1}H NMR (CD_3OD): *δ* 0.82 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.92 (t, 2H, CH_2N), 4.04 (m, 1H, 3-H), 5.4−6.1 (m, 4H, H-20, H-21, H-22, H-23). MS: *m*/*z* 401 (26, M^+ base), 354 (55), 203 (18).

17*β*-(7-Aminoheptyl)-5*β*-androstane-3*β*,14*β*-diol (38). Acetic anhydride (0.19 mL, 2.00 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (50 mg, 0.40 mmol) were added to a solution of (*E*,*E*)-17*β*-(6-cyanohexa-1,3-dienyl)-5*β*-androstane-3*β*,14*β*-diol (**64**) (566 mg, 1.12 mmol) in CH_2Cl_2 (5 mL) and pyridine (0.2 mL), and the mixture was stirred at room temperature for 16 h. Ethanol (1.0 mL) was added and after 2 h the solution was diluted with EtOAc (50 mL) and washed with a saturated solution of NaH_2PO_4 (15 mL). After drying over Na_2SO_4, the organic phase was evaporated to dryness to afford 600 mg of (*E*,*E*)-3*β*-acetoxy-17*β*-(6-cyanohexa-1,3-dienyl)-5*β*-androstane-14*β*-ol as a white foam. The crude product was dissolved in EtOAc (25 mL) and hydrogenated for 6 h over PtO_2 (60 mg, 0.26 mmol). After filtering and evaporating the solvent, the residue was passed through a short silica gel column, eluting with *n*-hexane/EtOAc 4:1, yielding 500 mg of a 4:1 mixture of **65** and an unidentified cyanoalkene. The mixture was dissolved in $CHCl_3$ (25 mL) and treated with MCPBA (520 mg, 3.01 mmol). After stirring for 1 h at room temperature the solvent was removed in vacuo and the residue partitioned between Et_2O (70 mL) and 10% aqueous Na_2CO_3 (20 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated to dryness. Flash chromatography (*n*-hexane/EtOAc, 75:25) afforded pure 3*β*-acetoxy-17*β*-(6-cyanohexyl)-5*β*-androstane-14*β*-ol (**65**) (301 mg, 61% over three steps) as a white foam. ^{1}H NMR ($CDCl_3$): *δ* 0.93 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.08 (s, 3H, *C*H$_3$COO), 2.35 (t, 2H, *J* = 7.2, C*H*$_2$CN), 5.04 (m, 1H, 3-H).

A solution of **65** (290 mg, 0.66 mmol) in THF (25 mL) was treated with $LiAlH_4$ (170 mg, 4.47 mmol) and the resulting mixture was heated under reflux for 24 h. After cooling to room temperature, NaOH (4 N, 1.5 mL) was added followed by Na_2SO_4 (3 g) and the mixture was stirred for 1 h. The white precipitate was filtered off and the organic solvent evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/26% NH_4OH, 89:10:1) to give **38** (165 mg, 63%) as a white foam. ^{1}H NMR (DMSO-d_6): *δ* 0.79 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.48 (m, 2H, C*H*$_2$NH$_2$), 3.87 (m, 1H, 3-H). MS: *m*/*z* 405 (11, M^+), 210 (60).

3*β*,14*β*-Dihydroxy-5*β*-pregnane-21-carboxaldehyde (40). A mixture of (*E*)-3*β*,14*β*-dihydroxy-5*β*-pregn-20-ene-21-carboxaldehyde[4] (**41**) (1.5 g) and 5% Pd/C (0.3 g) in EtOAc (200 mL) was hydrogenated at room temperature and pressure in 1 h. The mixture was filtered and evaporated to dryness. The residue was chromatographed with *n*-hexane/EtOAc, 1:1 to give pure **40** (1.35 g, 90%), a white solid. ^{1}H NMR (DMSO-d_6): *δ* 0.84 (s, 3H, CH_3), 0.86) s, 3H, CH_3), 3.32 (m, 1H, 14*β*-OH), 3.86 (m, 1H, 3-H), 4.17 (d, 1H, 3*β*-OH), 9.64 (t, 1H, *J* = 1.7, CHO).

(*E*)-3*β*,14*β*-Dihydroxy-5*β*-pregn-20-ene-21-methyl-21-carboxaldehyde (42). To a solution of (*E*)-methyl 3*β*,14*β*-dihydroxy-5*β*-pregn-20-ene-21-methyl-21-carboxylate[12] (**55**) (2.20 g, 5.4 mmol) in dry THF (100 mL) at −60 °C was added neat DIBAH (10.0 mL, 56 mmol) dropwise in 30 min. After 2 h at −60 °C the cooling bath was removed and a solution of citric acid (50 g) in water (400 mL) was added dropwise, the temperature being kept below 40 °C. After stirring at room temperature until dissolution of the gelly suspension, the solution was extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with a 5% aqueous solution of Na_2HPO_4 (200 mL), dried over Na_2SO_4, and evaporated to dryness to afford (*E*)-17*β*-(3-hydroxyprop-1-en-1-yl)-21-methyl-5*β*-androstane-3*β*,14*β*-diol (**56**) (1.96 g, 100%).^{1}H NMR ($CDCl_3$): *δ* 0.85 (s, 3H, CH_3), 0.95 (s, 3H, CH_3),1.62 (s, 3H, CH_3C=), 4.01 (2H, C*H*$_2$OH), 4.12 (m, 1H, 3-H), 5.59 (d, 1H, C*H*HCH$_2$OH). This material showed a single spot on TLC and was used in the next step without further purification.

A mixture of crude **56** (1.90 g, 5.2 mmol) and MnO_2 (12.0 g) in dioxane (70 mL) was stirred at room temperature for 2.5 h. The mixture was filtered through a Celite pad by washing with acetone and the solution evaporated to dryness to afford **42** (1.66 g, 88%) as a pale yellow solid showing a single spot on TLC and used in the next step without further purification. A sample was crystallized from EtOAc/Me_2CO to give a white solid. ^{1}H NMR (CD_3OD): *δ* 0.88 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.68 (d, 3H, *J* = 1.2, CH_3C=), 2.84 (m, 1H, 3-H), 4.06 (m, 1H, 3-H), 6.87 (dq, 1H, *J* = 10.6, 1.2, 20-H), 9.34 (s, 1H, CHO).

(*E*)-17-[(2-Dimethylaminoethoxy)imino]-5*β*-androstane-3*β*,14*β*-diol (43) was prepared following method D. The crude product on chromatography with $CHCl_3$/MeOH/NH_3, 9:1:0.5 gave **43** as a white solid (200 mg). ^{1}H NMR ($CDCl_3$): *δ* 0.98 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 2.30 (s, 6H, CH_3N), 2.48−2.71 (m, 4H, CH_2N, 16-CH_2), 4.14 (t, 2H, CH_2O), 4.12 (br s, 1H, 3-H). MS: *m*/*z* 392 (0.5, M^+), 320 (4), 257 (6), 58 (100).

(*E*)-17-[(3-Dimethylaminopropoxy)imino]-5*β*-androstane-3*β*,14*β*-diol (44) was prepared following method D. The crude product on chromatography with CH_2Cl_2/MeOH, 9:1 gave **44** as a white solid (280 mg). ^{1}H NMR ($CDCl_3$): *δ* 0.98 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 2.22 (s, 6H, CH_3N), 2.34 (t, 2H, CH_2N), 2.48−2.71 (m, 2H, 16-CH_2), 4.06 (t, 2H, CH_2O), 4.12 (br s, 1H, 3-H). MS: *m*/*z* 407 (1, $M^+ + 1$), 306 (100).

(*E*)-17-[(2-Dimethylaminobutoxy)imino]-5*β*-androstane-3*β*,14*β*-diol (45) was prepared following method D. The crude product on chromatography with CH_2Cl_2/MeOH/NH_3, 95:5:1 gave **45** as a white solid (300 mg). ^{1}H NMR ($CDCl_3$): *δ* 0.98 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 2.22 (s, 6H, CH_3N), 2.28 (t, 2H, CH_2N), 2.41−2.71 (m, 2H, 16-H), 4.08 (t, 2H, CH_2O), 4.16 (br s, 1H, 3-H).

(*E*)-17-[(2-Aminoethoxy)imino]-5*β*-androstane-3*β*,14*β*-diol (46) was prepared following method D. The crude product on chromatography with CH_2Cl_2/MeOH/NH_3, 9:1:0.1 gave **46** as a white solid (130 mg). ^{1}H NMR ($CDCl_3$): *δ* 0.98 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 2.48−2.71 (m, 2H, 16-H), 2.94 (t, 2H, CH_2N), 4.08 (t, 2H, CH_2O), 4.12 (br s, 1H, 3-H).

17(*E*)-21[(*EZ*)-(2-Dimethylaminoethoxy)imino]-5*β*-pregn-17-ene-3*β*,14*β*-diol (47) was prepared following method A. The crude product on chromatography with CH_2Cl_2/MeOH, 95:5 gave **47** as a white solid (120 mg). ^{1}H NMR ($CDCl_3$): *δ* 0.98 (s, 3H, CH_3), 1.12 (s, 2.1H, CH_3 (*E*) isomer), 1.15 (s, 0.9H, CH_3 (*Z*) isomer), 2.3 (s, 4.2H, CH_3N (*E*) isomer), 2.32 (s, 1.8H, CH_3N (*Z*) isomer), 2.55−2.75 (m, 4H, 16-CH_2 and CH_2N), 4.12 (br s, 1H, 3-H), 4.13−4.23 (m, 2H, CH_2O), 5.91 (m, 0.7H, C=CH (*E*) isomer), 6.4 (m, 0.3H, C=CH (*Z*) isomer), 7.19 (d, 0.3H, *J* = 11.2, CH=N (*Z*) isomer), 7.98 (d, 0.7H, *J* = 11.2, CH=N (*E*) isomer). MS: *m*/*z* 419 (18, $M^+ + 1$), 71 (100).

17(*E*)-21[(*E*)-3-(Dimethylaminopropoxy)imino]-5*β*-pregn-17-ene-3*β*,14*β*-diol (48) was prepared following method A. The crude product on chromatography with CH_2Cl_2/MeOH,

9:1 gave **48** as a white solid (85 mg). ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 3H, CH_3), 1.15 (s, 2.7H, CH_3 (*E*) isomer), 1.18 (s, 0.3H, CH_3 (*Z*) isomer), 2.24 (s, 6H, CH_3N), 2.38 (t, 2H, CH_2N), 4.11 (t, 2H, CH_2O), 4.13 (br s, 1H, 3-H), 5.91 (m, 0.9H, C=CH (*E*) isomer), 6.4 (m, 0.1H, C=CH (*Z*) isomer), 7.19 (d, 0.1H, J = 11.2, CH=N (*Z*) isomer), 7.93 (d, 0.9H, J = 11.2, CH=N (*E*) isomer). MS: *m/z* 433 (2, $M^+ + 1$), 71 (100).

17(*E*)-21[(*EZ*)-(2-Aminoethoxy)imino]-5β-pregn-17-ene-3β,14β-diol (49) was prepared following method A. The crude product on chromatography with $CHCl_3$/MeOH/NH_3, 9:1:0.1 gave **49** as a white solid (60 mg). ^{1}H NMR ($CDCl_3$): δ 0.99 (s, 3H, CH_3), 1.13 (s, 2.4H, CH_3 (*E*) isomer), 1.18 (s, 0.6H, CH_3 (*Z*) isomer), 2.97 (t, 2H, CH_2N), 4.09 (t, 2H, CH_2O), 4.13 (br s, 1H, 3-H), 5.91 (m, 0.8H, C=CH (*E*) isomer), 6.4 (m, 0.2H, C=CH (*Z*) isomer), 7.19 (d, 0.2H, J = 11.2, CH=N (*Z*) isomer), 7.95 (d, 0.8H, J = 11.2, CH=N (*E*) isomer). MS: *m/z* 390 (21, M^+), 82 (100).

17(*E*)-21[(*EZ*)-(3-Aminopropoxy)imino]-5β-pregn-17-ene-3β,14β-diol (50) was prepared following method A. The crude product on chromatography with $CHCl_3$/MeOH/NH_3, 9:1:0.1 gave **50** as a white solid (80 mg). ^{1}H NMR ($CDCl_3$): δ 0.99 (s, 3H, CH_3), 1.12 (s, 2.4H, CH_3 (*E*) isomer), 1.13 (s, 0.6H, CH_3 (*Z*) isomer), 2.84 (t, 2H, CH_2N), 4.08−4.21 (m, 3H, CH_2O and 3-H), 5.93 (m, 0.8H, C=CH (*E*) isomer), 6.39 (m, 0.2H, C=CH (*Z*) isomer), 7.20 (d, 0.2H, J = 11.4, CH=N (*Z*) isomer), 7.95 (d, 0.8H, J = 11.4, CH=N (*E*) isomer). MS: *m/z* 404 (6, M^+), 332 (100).

21-Nitro-5β-pregnane-3β,14β,20(*R*)-triol (51). A mixture of 3β,14β-dihydroxy-5β-androstane-17β-carboxaldehyde[7] (**39**) (3.00 g, 9.3 mmol), nitromethane (1.0 mL, 18.7 mmol), and KF·$2H_2O$ (0.44 g, 4.7 mmol) in *i*-PrOH (30 mL) was stirred for 16 h. The solvent was removed in vacuo and the residue diluted with CH_2Cl_2 (100 mL) and washed with water (30 mL). After drying over Na_2SO_4, the organic phase was evaporated to dryness, affording **51** (3.50 g, 99%) as a white solid, mp 91−92 °C, pure by TLC (cyclohexane/EtOAc, 1:1) and ^{1}H NMR. ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 4.13 (m, 1H, 3-H), 4.26 (dd, 1H, J = 4.3, 11.4), 4.43 (dd, 1H, J = 8.6, 11.4), 4.52 (ddd, 1H, J = 1.0, 4.3, 8.6). Anal. ($C_{21}H_{35}NO_5$·$0.5H_2O$) C, H, N, H_2O.

3β,14β-Dihydroxy-5β-pregnane-21-nitrile (53). To a solution of **51** (3.45 g, 9.0 mmol) in THF (70 mL) were added Ac_2O (3.1 mL, 32.8 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (20 mg). After stirring for 24 h, the solvent was evaporated. The residue was dissolved in EtOH/Et_2O, 1:1 (200 mL), and $NaBH_4$ (0.70 g, 18.5 mmol) was added. After 1 h, the solution was poured into 5% aqueous NaH_2PO_4 (150 mL) and the mixture extracted with CH_2Cl_2 (3 × 100 mL). After drying over Na_2SO_4, the organic phase was evaporated to dryness to afford 3β-acetoxy-21-nitro-5β-pregnan-14β-ol (**52**) (3.55 g, 97%) as a white foam sufficiently pure by TLC (*n*-hexane/EtOAc, 6:4) for use in the following reaction. ^{1}H NMR ($CDCl_3$): δ 0.96 (s, 6H, CH_3), 2.05 (s, 3H, OCH_3), 2.35 (m, 1H, 17-H), 4.35 (m, 2H, CH_2NO_2), 5.09 (m, 1H, 3-H).

To a solution of **52** (3.45 g, 8.5 mmol) in dry CH_2Cl_2 at 0 °C were added DEAD (2.4 mL, 15 mmol) and Ph_3P (7.30 g, 28 mmol). After stirring at room temperature for 40 h, the mixture was evaporated to dryness and the residue triturated with Et_2O (2 × 100 mL). The combined Et_2O layers were evaporated and the residue purified by flash chromatography (CH_2Cl_2/Et_2O, 9:1) to give 3β-acetoxy-14β-hydroxy-5β-pregnane-20-nitrile (3.0 g, 95%) as a white foam. The residue (2.95 g, 7.9 mmol) was dissolved in a mixture of THF (50 mL) and MeOH (100 mL), and aqueous 1 N NaOH (42 mL) was added. After stirring for 18 h, 5% aqueous NaH_2PO_4 (150 mL) was added and the mixture extracted with EtOAc (3 × 100 mL). After drying over Na_2SO_4, the organic phase was evaporated to dryness and the residue purified by flash chromatography ($CHCl_3$/Et_2O, 6:4) to give **53** (1.35 g, 52%) as a white foam. A sample was crystallized from *i*-Pr_2O to give a white solid, mp 160−162 °C. ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 6H, CH_3), 2.21 (m, 1H, 17-H), 2.57 (dd, 1H, J = 12, 5, CH*H*CN), 2.64 (dd, 1H, J = 12, 8, C*H*HCN), 4.13 (m, 1H, 3-H). Anal. ($C_{21}H_{33}NO_2$·$0.75H_2O$) C, H, N, H_2O.

3β,14β-Dihydroxy-5β-pregnane-21-aldehyde (54). To a solution of **53** (1.10 g, 3.3 mmol) in dry THF (65 mL) at 0 °C was added a 1 M solution of DIBAH/THF (13 mL) dropwise. After 3 h, a solution of citric acid (0.90 g) in water (5 mL) was added dropwise. The mixture was allowed to warm to room temperature, stirred for 15 min, and filtered through a Celite pad with thorough washing with EtOAc. The solution was evaporated to dryness to give **54** (0.90 g, 81%) as a mixture of diasteroisomeric lactols, a glassy solid, which was sufficiently pure by TLC and ^{1}H NMR for use in the oxime reaction. ^{1}H NMR ($CDCl_3$): δ 4.13 (m, 1H + 1H, 3-H), 4.62 (m, 1H, CHOH), 5.15 (1H, CHOH).

3β,14β-Dihydroxy-5β-pregn-17(20)-ene-21-aldehyde (61). To a mixture of nitriles **60**[13] (0.750 g, 2.28 mmol) and NaH_2PO_2·H_2O (1.5 g, 17 mmol) in water/acetic acid/pyridine, 1:1:2 (2.5 mL), was added Raney Ni (230 mg). After stirring at 60 °C for 24 h, the suspension was filtered on a Celite pad and washed with warm EtOH. The organic solvent was evapored under reduced pressure. The residue was dissolved with CH_2Cl_2, and the organic phase was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc, 5:2) to give **61** (0.330 g, 45%) as a white solid; some starting material was recovered (0.18 g). ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 3.01−3.12 (1H, 17-H), 4.12 (br s, 1H, 3-H), 5.86 (dt, 1H, J = 7.7, C=CH), 9.91 (d, 1H, J = 7.7, CHO).

(*E*)-21-Iodo-5β-pregn-20-ene-3β,14β-diol (62). To a suspension of $CrCl_2$ (5.00 g, 40.68 mmol) in degassed THF (50 mL) maintained at 0 °C under argon was added a solution of iodoform (5.25 g, 13.33 mmol) and 5β-androstane-3β,14β-diol-17β-carboxaldehyde (**39**) (1.50 g, 4.69 mmol) in THF (50 mL), over 0.5 h. The reaction mixture was stirred for 2 h at 0 °C and filtered through a Celite pad, washing with EtOAc/1% Et_3N (100 mL). The filtrate was stirred with a saturated solution of Na_2HPO_4 (300 mL) and the inorganic precipitate was filtered off. The organic layer was separated, and the aqueous one was extracted with EtOAc/1% Et_3N (2 × 50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 7:3) to give **62** (1.30 g, 66%) as white crystals, mp 167−169 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 4.13 (m, 1H, 3-H), 5.78 (d, 1H, J = 14.8, CH=C*H*I), 6.75 (dd, 1H, J = 14.8, 9.7, C*H*=CHI). Anal. ($C_{21}H_{33}IO_2$) C, H, I.

(*E*)-1-(Tri-*n*-butylstannyl)-4-azidobutene (68). Methanesulfonyl chloride (0.60 mL, 6.12 mmol) was slowly added at 0 °C to a solution of (*E*)-4-(tributylstannyl)-3-butenol (**66**)[33] (2.00 g, 5.54 mmol) and Et_3N (0.98 mL, 7.03 mmol) in CH_2Cl_2 (20 mL). After 1 h the solvent was evaporated in vacuo and the residue treated with water (50 mL) and extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with brine. Evaporation of the solvent gave 2.61 g of crude mesylate **67**. ^{1}H NMR ($CDCl_3$): δ 0.90 (m, 9H, CH_3(CH_2)$_3$Sn), 1.20−1.65 (m, 18H, CH_3(CH_2)$_3$Sn), 2.60 (brq, 2H, J = 7.0, =CH−CH_2), 3.00 (s, 3H, SO_2CH_3), 4.29 (t, 2H, J = 7.0, CH_2-OSO_2CH_3), 5.90 (dt, 1H, J = 18.7, 5.6, =C*H*CH_2), 6.13 (d, 1H, J = 18.7, =C*H*Sn). NaN_3 (1.78 g, 27.4 mmol) was added to a DMF (10 mL) solution of crude **67** and the mixture stirred for 4 h at 50 °C. After this time, water (70 mL) was added and the product was extracted with Et_2O/hexane, 1:1 (4 × 25 mL). The combined organic layers were washed with brine and evaporated under reduced pressure to give 850 mg of crude **68** (72%, over two steps) which was used without purification in the next cross-coupling step. ^{1}H NMR ($CDCl_3$): δ 0.90 (m, 9H, CH_3(CH_2)$_3$Sn), 1.20−1.65 (m, 18H, CH_3(CH_2)$_3$Sn), 2.55 (brq, 2H, J = 7.0, =CHCH_2), 3.49 (t, 2H, J = 7.0, CH_2N_3), 5.92 (dt, 1H, J = 18.7, 5.6, =C*H*CH_2), 6.13 (d, 1H, J = 18.7, =C*H*Sn).

(*E*)-5-(Tri-*n*-butylstannyl)-4-pentenenitrile (69). (*E*)-4-(Tributylstannyl)-3-butenol (**66**) (1.00 g, 2.77 mmol) was converted to crude mesylate **67** (1.40 g) as described above. This was dissolved in DMSO (50 mL) and treated with KCN

(510 mg, 7.83 mmol) and KI (210 mg, 1.26 mmol). After 9 h at 80 °C, the reaction mixture was diluted with water (300 mL) and brine (100 mL) and extracted with Et_2O (3 × 75 mL). The combined organic layers were washed with brine and evaporated under reduced pressure, and the residue was purified on silica gel (pentane/EtOAc, 95:5) to afford 1.48 g of nitrile **69** (72%, over two steps) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9H, $CH_3(CH_2)_3Sn$), 1.20−1.65 (m, 18H, $CH_3(CH_2)_3Sn$), 2.45 (m, 4H, $(CH_2)_2CN$), 5.95 (dt, 1H, J = 18.7, 5.6, =$CHCH_2$), 6.10 (d, 1H, J = 18.7, =$CHSn$).

Preparation of *O*-(*ω*-aminoalkyl)benzophenone Oximes (70−74). *ω*-Chloroalkylamine hydrochloride (1.1 equiv) was added, in portions, to a suspension of KOH powder (1.5 equiv) and benzophenone oxime[34] (1.0 equiv) in DMSO (0.45 M), while the temperature was kept below 20 °C. After 3 h, the mixture was poured into ice/water, the suspension was brought to pH 2 with 1 N HCl, and the mixture was extracted with Et_2O (4×). The aqueous layer was brought to pH 10 with KOH powder and extracted with Et_2O (4×). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to give the desired compounds, sufficiently pure for use in the next step.

***O*-(2-Dimethylaminoethyl)benzophenone oxime (70)** was produced in 98% yield (91.0 g). ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 6H, CH_3), 2.68 (t, 2H, CH_2N), 4.32 (t, 2H, CH_2O), 7.30−7.53 (m, 10H, ArH).

***O*-(2-Aminoethyl)benzophenone oxime (71)** was produced in 82% yield (50.6 g). ^{1}H NMR (DMSO-d_6) δ 2.78 (t, 2H, CH_2N), 4.03 (t, 2H, CH_2O), 7.25−7.50 (m, 10H, ArH).

***O*-(3-Dimethylaminopropyl)benzophenone oxime (72)** was produced in 67% yield (25.0 g). ^{1}H NMR (DMSO-d_6) δ 1.70−1.82 (m, 2H, CH_2CH_2N), 2.17 (s, 6H, CH_3), 2.30 (t, 2H, CH_2N), 4.12 (t, 2H, CH_2O), 7.25−7.50 (m, 10H, ArH).

***O*-(3-Aminopropyl)benzophenone oxime (73)** was produced in 96% yield (13.0 g). ^{1}H NMR ($CDCl_3$): δ 1.82 (q, 2H, CH_2CH_2N), 2.78 (t, 2H, CH_2N), 4.28 (t, 2H, CH_2O), 7.30−7.50 (m, 10H, ArH).

***O*-(4-Dimethylaminobutyl)benzophenone oxime (74)** was produced in 84% yield (10.4 g). ^{1}H NMR ($CDCl_3$): δ 1.50−1.60 (m, 2H, CH_2CH_2N), 1.70−1.80 (m, 2H, CH_2CH_2O), 2.23 (s, 6H, CH_3), 2.28 (t, 2H, CH_2N), 4.22 (t, 2H, CH_2O), 7.25−7.55 (m, 10H, ArH).

***N*-(4-Phthalimidobutoxy)phthalimide (75).** K_2CO_3 (2.76 g, 0.02 mol) was added to a stirred solution of *N*-hydroxyphthalimide (3.26 g, 0.02 mol) and *N*-(4-bromobutyl)phthalimide (5.64 g, 0.02 mol) in DMSO (32 mL) at room temperature. The mixture was heated at 80 °C for 30 min. After cooling at room temperature, water (50 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were washed with water (3 × 25 mL), dried over Na_2SO_4, and evaporated to give **75** (5.77 g, 73%) as an off-white solid sufficiently pure for use in the following reaction. ^{1}H NMR ($CDCl_3$): δ 1.80−1.90 (m, 2H, $OCH_2CH_2CH_2CH_2N$), 1.90−2.00 (m, 2H, $OCH_2CH_2CH_2CH_2N$), 3.70 (t, 2H, CH_2N), 4.27 (t, 2H, CH_2O), 7.70−7.80 (m, 4H), 7.80−7.90 (m, 4H).

Preparation of Aminooxyalkanamines (76−80). A suspension of *O*-(*ω*-aminoalkyl)benzophenone oxime (1.0 equiv) in 6 N HCl (10 equiv) was heated at reflux under stirring for 2 h. After cooling at room temperature, the mixture was extracted with Et_2O (4×). The aqueous phase was evaporated to dryness.

***N*,*N*-Dimethyl-2-(aminooxy)ethanamine Dihydrochloride (76).** The crude material was crystallized from EtOH/H_2O, 9:1 to give **76** (38.2 g, 72%), mp 180−182 °C (lit.[35] mp 180−182 °C). ^{1}H NMR (DMSO-d_6) δ 2.80 (s, 6H, CH_3), 3.42 (t, 2H, CH_2N), 4.43 (t, 2H, CH_2O).

2-(Aminooxy)ethanamine Dihydrochloride (77). The crude material was crystallized from EtOH/H_2O, 9:1 to give **77** (12.9 g, 72%), mp 203−206 °C (lit.[36] mp 181−183 °C). ^{1}H NMR (DMSO-d_6) δ 3.12 (t, 2H, CH_2N), 3.28 (t, 2H, CH_2O). Anal. ($C_2H_8N_2O$·2HCl) C, H, N, Cl.

***N*,*N*-Dimethyl-3-(aminooxy)propanamine Dihydrochloride (78).** The crude material was crystallized from EtOH to give **78** (13.6 g, 95%), mp 123−128 °C. ^{1}H NMR (DMSO-d_6): δ 2.00−2.10 (m, 2H, CH_2CH_2N), 2.72 (s, 6H, CH_3), 3.08 (t, 2H, CH_2N), 4.11 (t, 2H, CH_2O). Anal. ($C_5H_{14}N_2O$·2HCl) C, H, N, Cl.

3-(Aminooxy)propanamine Dihydrochloride (79). The crude material was crystallized from MeOH/Et_2O to give **79** (6.85 g, 82%), mp 200−203 °C (lit.[36] mp 182−185 °C). ^{1}H NMR (CD_3OD): δ 2.02−2.12 (m, 2H, CH_2CH_2N), 3.08 (t, 2H, CH_2N), 4.18 (t, 2H, CH_2O). Anal. ($C_3H_{10}N_2O$·2HCl) C, H, N, Cl.

***N*,*N*-Dimethyl-4-(aminooxy)butanamine Dihydrochloride (80).** The crude material was crystallized from EtOH to give **80** (6.80 g, 94%), mp 119−124 °C. ^{1}H NMR (DMSO-d_6) δ 1.60−1.80 (m, 4H, $CH_2CH_2CH_2N$), 2.70 (s, 6H, CH_3), 3.06 (t, 2H, CH_2N), 4.08 (t, 2H, CH_2O). Anal. ($C_6H_{16}N_2O$·2HCl) C, H, N, Cl.

4-(Aminooxy)butanamine Dihydrochloride (81). Hydrazine hydrate (2.12 mL, 0.044 mol) was added to a stirred suspension of **75** (3.26 g, 0.02 mol) in EtOH (35 mL) at room temperature. The mixture was heated to reflux for 3 h. After cooling at room temperature the precipitate was filtered off and the filtrate evaporated to dryness. The residue was stirred with 37% HCl (2.7 mL) and water (10 mL). The solution was evaporated to dryness to give **81** (2.76 g, 98%), as an off-white solid sufficiently pure for use in the reactions of oxime preparation. ^{1}H NMR (CD_3OD): δ 1.80 (m, 4H, $OCH_2CH_2CH_2CH_2N$), 3.00 (m, 2H, CH_2N), 4.10 (m, 2H, CH_2O). A sample was crystallized from MeOH/EtOAc, mp 135−140 °C (lit.[36] mp 140−142 °C).

Biology. Na^+,K^+-ATPase Inhibition. Na^+,K^+-ATPase was isolated and purified from dog kidney according to the method of Jørghensen.[18] The inhibition of the enzyme activity was measured as the percent of hydrolysis of ^{32}P-ATP in the presence and in the absence of the tested compound.[19] The concentrations able to inhibit 50% (IC_{50}) of enzyme activity were calculated by a nonlinear least-squares curve fitting computer program.

Guinea Pig Atria. Isolated guinea pig left atria (from 300−500 g male animals) were placed in 20 mL organ baths containing a solution of the following composition (mM), NaCl, 131.6; KCl, 5.6; $CaCl_2$, 1.8; NaH_2PO_4, 1.036; $NaHCO_3$, 24.99; glucose, 11; sucrose, 13, under 500 mg resting tension, at 32 °C. The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The preparations were stimulated by platinum electrodes by square-wave pulses at a frequency of 1 Hz (1 ms duration, voltage twice the treshold). After a 60 min equilibration period, cumulative concentrations of the compounds were added, each concentration being left in contact until the maximal response or arrhythmias were observed.

Inotropic and Toxic Effects in Anesthetized Guinea Pigs. Male guinea pigs weighing 400−450 g were anesthetized with urethane (1.5 g/kg ip). Body temperature was mantained at 37 °C by a homeothermic blanket system (Harward Apparatus). A microtip pressure transducer (Millar SPR-407, Houston TX) was introduced into the left ventricle through the right carotid artery to measure ventricular pressure (LVP). Recordings were fed to a Gould (RS 3800) polygraph and to an AST 486 computer and analyzed by IDAS software (Mangoni, Pisa, Italy). A polythene cannula (PE_{50}) was inserted into a jugular vein for drug infusion and the trachea was intubated to facilitate spontaneous respiration. After a stabilization period, the test substance was injected at the rate of 0.16 mL/min by means of a Harvard P22 pump (Harward Apparatus) until the animal died or up to a maximum of 90 min. The doses inducing the maximum inotropic effect and death were determined. The compounds were dissolved in DMSO and the resulting solution diluted with physiological solution to obtain final solutions containing 1% DMSO. The starting concentration of the infused solution was based on the EC_{50} found in the electrically driven guinea pig atria. If no effect was found, the concentration was raised until satisfactory inotropic effect was found or death occurred.

Molecular Modeling. All molecular modeling, including energy minimization and molecular dynamics calculations, were performed on a Silicon Graphics 35GT workstation

running Insight97&Discover (Biosym/Molecular Simulations, San Diego, CA) software package. Molecular models of the digitalis analogues were constructed starting from the residue library included in the software and energy minimized, using a conjugate gradient algorithm.

The coiled structure for the transmembrane H1 and H2 domains of the human cardiac Na^+,K^+-ATPase α1-subunit was modeled on the basis of the available X-ray coordinate set of bacteriorhodopsin protein deposited in the Protein Brookhaven Databank (pdb code 2BRD). The extend and position of the transmembrane helices was checked by means of the PHD prediction[28] available on the Web at the European Molecular Biological Laboratory (EMBL, Heidelberg, Germany) Biocomputing Unit.

The results were found consistent with the reported secondary structure found in Repke.[6] Side-chain/side-chain interactions between the two helical domains were initially energy minimized by molecular mechanics keeping constrained all the backbone atoms coordinates.

All calculations were performed using CVFF[37] as force field with a dielectric constant value equal to 4, to take into account the hydrophobic environment of the membrane. Atomic charges were taken from the fragment library of Insight&Discover.

Initially, to find the best orientation of the digitalis derivatives within the H1−H2, a digoxin molecule was manually docked into the groove by interactive computer graphics taking into account the currently established intermolecular interactions: (a) Cys104−C17 lactone ring; and (b) rhamnose hydroxylic groups pointing toward the extracellular loop. These interactions were used as loosened restraints (d < 3.0 A) for the subsequent energy minimization and a 10 ps run of molecular dynamics simulation at 300 K. Backbone atom coordinates of the peptide chain were always kept constrained. After the correct orientation of digoxin was established, the ligand was replaced by compound **8** and subjected to further energy minimization. Finally, another model of the H1−H2 helical hairpin was added to the complex, keeping preserved the C_2 symmetry of the dimer along its long axis. The whole complex was energy minimized by another run of energy minimization, 6 ps molecular dynamics at 300 K, final energy minimization, keeping constrained the atomic coordinates of the first H1−H2 helical hairpin and allowing the second H1−H2 subunit to move freely and to arrange its orientation in order to optimize intermolecular interactions. The model structures involving the other analogues were analyzed by making the appropriate changes in the C17 side-chain structure, keeping initially fixed the orientation of the steroid moiety, and by a subsequent energy minimization.

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