Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 2159–2182

TETRAHEDRON: *ASYMMETRY*

Synthesis of *N*,*N*-bis[2-(2-pyridyl)ethyl]amino steroids and related compounds intended as chiral ligands for copper ions

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Received 28 March 2000; accepted 3 May 2000

Abstract

Compounds with the *N*,*N*-bis[2-(2-pyridyl)ethyl]amino structure (RPY2) are useful tridentate ligands for copper(I) ions, which can bind and activate oxygen from the atmosphere. For diastereoselective and enantioselective oxidation reactions, hitherto unknown chiral ligands possessing tripodal structures have been synthesized starting from homochiral steroids. The double Michael addition of primary steroidal amines and aminoalcohols to 2-vinyl pyridine was not very succesful. However, homochiral bidentate ligands with *N*-[2-(2-pyridyl)ethyl]amino steroid structure could be obtained by this procedure in most cases. New routes (acylation of the bidentate ligands with 2-pyridylacetic acid followed by BH_3·THF reduction, or reductive amination of steroidal ketones, acylation and borane reduction) to the desired tridentate RPY2, also at sterically hindered positions, are described. In the last reaction sequence, 'mixed' tridentate ligands can also be obtained. Copper complexation and oxygen activation with these ligands are briefly discussed.

1. Introduction

The reactivity of copper-dioxygen complexes is an interesting field of research[1] and is inspired from the structure of copper-containing monooxygenases such as dopamine β-hydroxylase.[2] This enzyme is characterized by the ability to hydroxylate dopamine at the benzylic position to give enantioselectively pure (*R*)-noradrenaline. Studies mimicking these enzymes could show that copper(I) complexes with tridentate *N*,*N*-bis[2-(2-pyridyl)ethyl]amino ligands (Fig. 1) are promising compounds for binding and activating dioxygen.[3]

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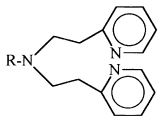
0957-4166/00/$ - see front matter
PII: S0957-4166(00)00168-3



RPY2

Figure 1. *N*,*N*-Bis-[2-(2-pyridyl)ethyl]amino compounds

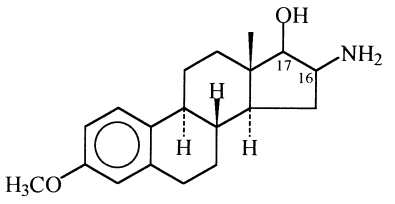
As a result of the activation of dioxygen, hydroxylation of the ligand could be observed in most cases.[3a–e,g] In one case, hydrogen abstraction of exogenous substrates was also described.[3f] More recently, Règlier and co-workers have employed two suitable prochiral ligands (Fig. 1, R = 2-indanyl,[3e] cyclopentyl[3g]) in order to investigate the stereochemistry of the hydroxylation reaction. For both ligands *cis*-hydroxylation to yield racemic *cis*-1-hydroxy-2-amino-indanes[3e] and *cis*-1-hydroxy-2-amino-cyclopentanes[3g] was observed. In spite of recent advances, homochiral RPY2 ligands, which could open the way to homochiral hydroxylation products, have not yet been synthesized. We therefore planned the synthesis of such ligands using a homochiral steroid core with primary amino groups at different positions as starting compounds. In this paper we describe our results for the Michael addition of primary steroidal amines and vicinal aminoalcohols to 2-vinyl pyridine which is currently the standard method to obtain RPY2 ligands.[3d,g,4] New synthetic routes which are also successful for ligand synthesis at sterically hindered positions of steroids have been developed. In addition, we discuss some results of copper ion complexation and dioxygen binding.

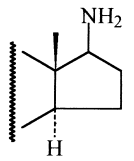
The steroid core has the advantage that substituents can be placed at different positions each having different stereochemical and steric relationships. We chose the D-ring of the 3-methoxy-estra-1,3,5(10)-triene series for substitution since this ring exhibits a relatively restricted, well-defined conformation[5] (Figs. 2 and 3). In particular, we employed derivatives of the four diastereomeric 16-amino-17-hydroxy compounds (**1** to **4**) for the synthesis of copper complexes[5b,6] as well as for the investigation of the diastereoselective formation of $Fe(CO)_3$ complexes possessing a planar chirality.[7] We then included a series of more flexible A-ring-substituted compounds in our investigations (Figs. 2 and 3). Correspondingly, the four diastereomeric 2-amino-3-hydroxy-cholestanes (**7** to **10**) were synthesized from the related azidoalcohols, which we have synthesized by stereoselective synthesis. Furthermore, we employed 3α- and 3β-amino-cholestanes (compounds **11** and **12** with an axial and equatorial amino group, respectively) as well as the sterically more hindered 17-amino-3-methoxy-estra-1,3,5(10)-trienes (**5** and **6**) as model compounds (Fig. 2).

2. Results and discussion

2.1. Synthesis of the steroidal amines and aminoalcohols

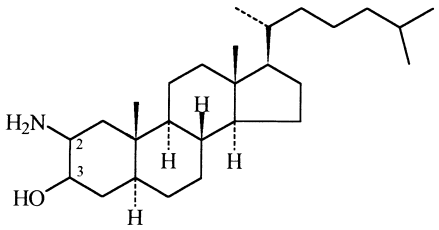
The four diastereomeric 16-amino-17-hydroxy-3-methoxy-estra-1,3,5(10)-trienes (**1** to **4**; Fig. 2) were synthesized as described in the literature.[8] 17β-Amino-3-methoxy-estra-1,3,5(10)-triene **5** (Fig. 2) could be obtained in two steps from 3-methoxy-estra-1,3,5(10)-triene-17-one **13** in an overall yield of 72%. Reduction of the oxime **14**[9] with $MoO_3/NaBH_4$[10a] in methanol gave 86% of the β-amine **5**[10] (Scheme 1). This result is in contrast to a literature report,[10a] which described the

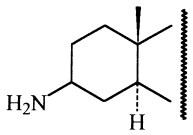




1: 16α, 17α
2: 16α, 17β
3: 16β, 17β
4: 16β, 17α

5: 17β
6: 17α





7 : 2α, 3α
8 : 2α, 3β
9 : 2β, 3β
10 : 2β, 3α

11 : 3α
12 : 3β

Figure 2. Primary steroid amines and aminoalcohols

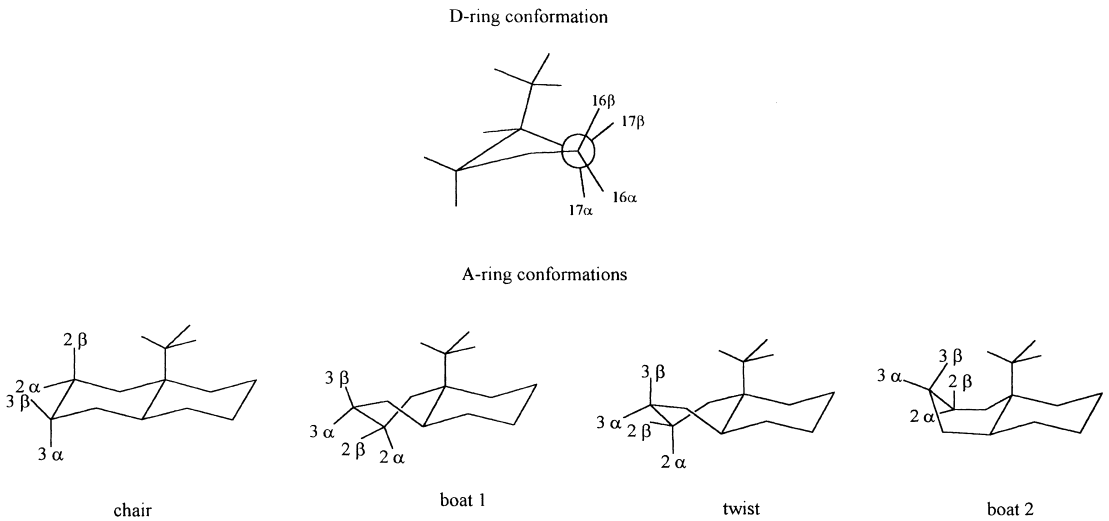
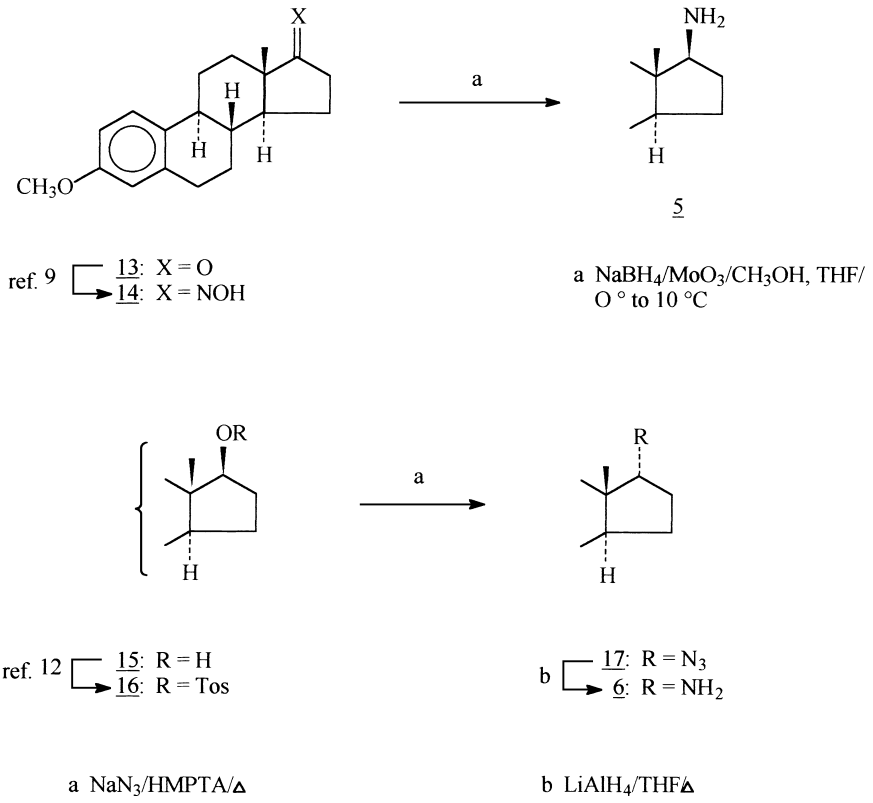


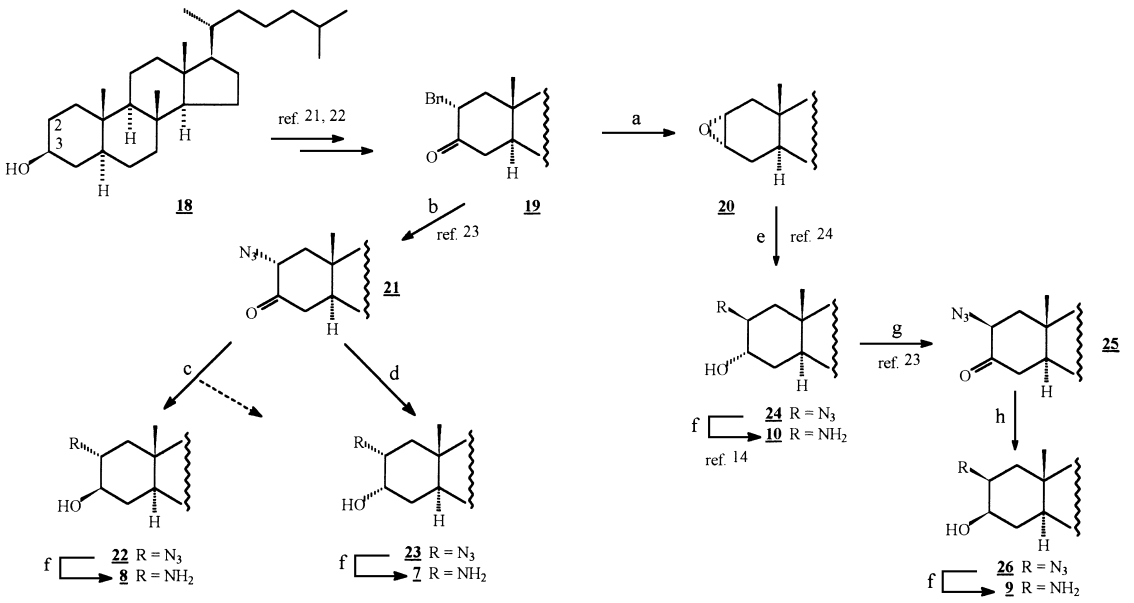
Figure 3. D-Ring and A-ring conformation



Scheme 1. Synthesis of 17-amines

17α-compound as being the main product.[10a] Our result was confirmed by comparing spectral data of the corresponding 17β-acetamide with the literature results.[10] The 17α-amino compound **6** was synthesized, analogously to the androstane and estra-1,3,5(10)-triene series,[11] using the 17β-tosylate **16** [12] for the azide substitution **17** and the subsequent reduction with $LiAlH_4$ (**6**, total yield 57% from **15**, Scheme 1).

The synthetic procedure for obtaining the four diastereomeric aminoalcohols **7** to **10**[13–20] is outlined in Scheme 2. The azidoalcohols **22**, **23**, **24** and **26** are intermediates. Compounds **19**,[21,22] **21**,[23] **24**,[24] **10**,[14] **25**[23] and **26**[23] were synthesized either as described in the literature or with slight variations on the literature procedures. The azidoalcohols **22** and **23** could be obtained by either reduction of the 2α-azido-3-ketone **21**[23] with lithium borohydride (60% of 2α-azido-3β-ol **22**, 33% of 2α-azido-3α-ol **23**, 5% of cholestane-3β-ol **18**) or with LS-Selectride (82% of 2α-azido-3α-ol **23**, 8% of cholestane-3α-ol). Reduction with sodium borohydride in ethanol gave ca. 50% of **22**, 23% of **23** and 15% of cholestane-3β-ol **18**. Reduction of the 2β-azido-3-ketone **25**[23] with sodium borohydride in ethanol/tetrahydrofuran resulted in the 2β-azido-3β-ol **26** as the only product. The aminoalcohols were synthesized from the azidoalcohols by reduction with hydrazine hydrate/Raney-nickel in methanol.[14] 3α-Amino- and 3β-amino-5α-cholestane (**11** and **12**) were synthesized as described in the literature (**11** by azide substitution[11c] of the corresponding 3β-tosyloxy compound and subsequent reduction with $LiAlH_4$,[25,26] **12** by reduction of the corresponding 3-oxime with *n*-amylalcohol/Na[27]).

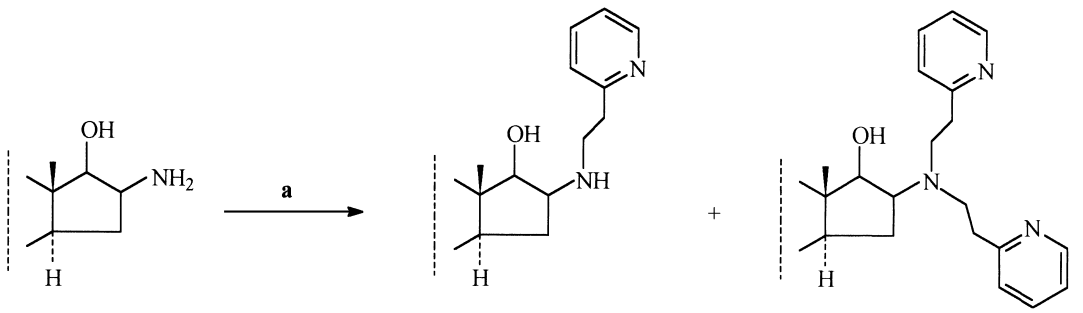


Scheme 2. Synthesis of 2,3-aminoalcohols. (a) (i) L-Selectride; (ii) Zn/AcOH/Δ; (iii) Mg-monoperphthalate; (b) NaN_3/NMP/AcOH/rt; (c) $LiBH_4$; (d) LS-Selectride; (e) NaN_3/DMSO/AcOH/Δ; (f) $N_2H_4{\cdot}H_2O$/Raney-Ni/CH_3OH; (g) Jones ox.; (h) $NaBH_4$

2.2. *Addition of steroidal amines and aminoalcohols to 2-vinyl pyridine*

The Michael addition of primary amines to 2-vinyl pyridine in refluxing methanol and acetic acid is the standard method for the synthesis of RPY2 ligands. A long reaction time (2 to 5 days) is necessary. *m*-Xylylenediamine reacted with 68%,[4] *n*-propylamine with 38%,[3g] cyclopentylamine with 40%[3g] and 2-aminoindane also with 40%.[3d] The results for the four 16,17-aminoalcohols **1** to **4** using this method with a reaction time of 2 days are given in Scheme 3. Only the sterically less hindered 16α-amino compounds **1** and **2** resulted in expected RPY2 compounds (**31**, **32**) in low to moderate yields in addition to the mono(pyridylethyl)amino compounds **27** and **28**. The more hindered 16β-amino compounds formed only the mono(pyridylethyl)amino compounds **29** and **30** in relatively good yields. Variation of the reaction conditions (prolonged reaction times, changing the ratio of 2-vinyl pyridine and acetic acid) did not improve the yields. Due to the restricted conformation of the cyclopentane ring[5] the 16α-substituent has a quasiequatorial or bisectional character. The 16β-substituent also has a quasiaxial or bisectional conformation. However, in contrast to the 16α-substituents, a sterically unfavourable 1,3-interconversion with the 13β-methyl group exists.

The results for the four 2,3-aminoalcohols **7** to **10** are summarized in Scheme 4. The 2α-amino compounds (**7** and **8**) gave the desired RPY2 compounds **37** and **38** only in very low yields. In all cases the mono(pyridylethyl)amino compounds (**33** to **36**) could be obtained in moderate yields. This was an unexpected result since the 2α-substituent is a relatively unhindered equatorial substituent. The 2β-position has an axial character and a strong 1,3-diaxial steric repulsion with the 10β-methyl group. A more flexible twist-boat conformation (Fig. 3) must be considered, which is additionaly favoured for compound **10** of the presence of an intramolecular hydrogen bond in this conformation[13] (Fig. 3), (for IR spectra, see Experimental). Nevertheless, a reaction with two molecules of 2-vinyl pyridine could not be achieved.



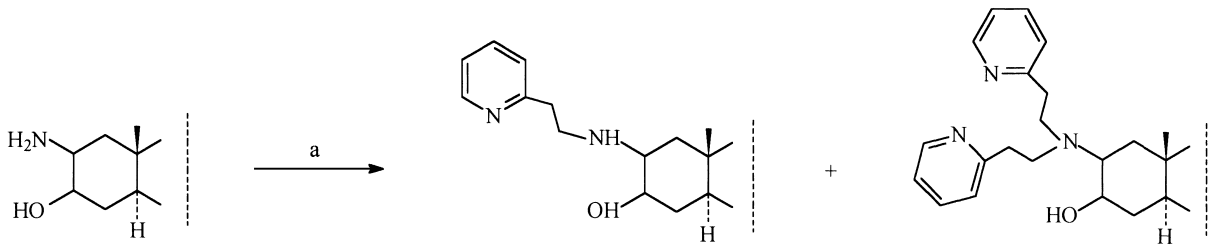
a 2-vinyl pyridine, CH_3OH/AcOH, 75 °C, 48 h

	16-NH_2	17-OH
1	α	α
2	α	β
3	β	β
4	β	α

	yield
27	56%
28	30%
29	75%
30	46%

	yield
31	21%
32	41%

Scheme 3. Hydroamination with 16,17-aminoalcohols



a 2-vinyl pyridine, CH_3OH/AcOH, 75 °C, 48 h

	2-NH_2	3-OH
7	α	α
8	α	β
9	β	β
10	β	α

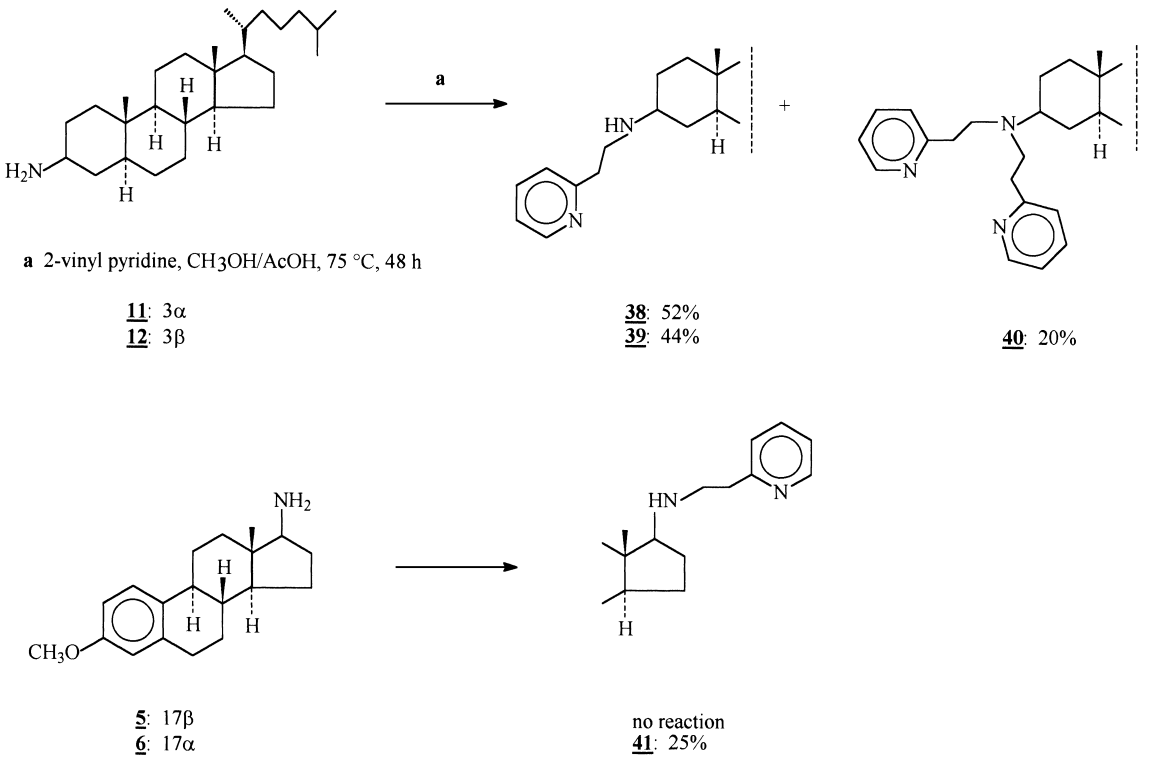
	yield
33	48%
34	54%
35	48%
36	40%

	yield
37	8%
38	11%

Scheme 4. Hydroamination with 2,3-aminoalcohols

To obtain more insight into this addition reaction and also to obtain chiral RPY2 ligands without a neighbouring hydroxy group, we investigated the reaction with the axial 3α-amine **11**, the equatorial 3β-amine **12** (Fig. 2) as well as the 17-amines **5** and **6** (17β-**5**: quasiequatorial, 17α-**6**: quasiaxial).

The last two amines are sterically hindered due to the neighbouring ring junction, which is especially valid for the 17β-amine **5** with a *cis* arrangement to the 13β-methyl group. It can be seen from the results in Scheme 5 that the axial 3α-amine only reacted to form the mono(pyridylethyl)-amine **38**, whereas the equatorial 3β-amine reacted to the desired RPY2 compound **40** in addition to the monadduct **39**. As expected, the hindered amine **6** resulted only in the monoadduct **41** and the most sterically hindered amine **5** did not react at all.



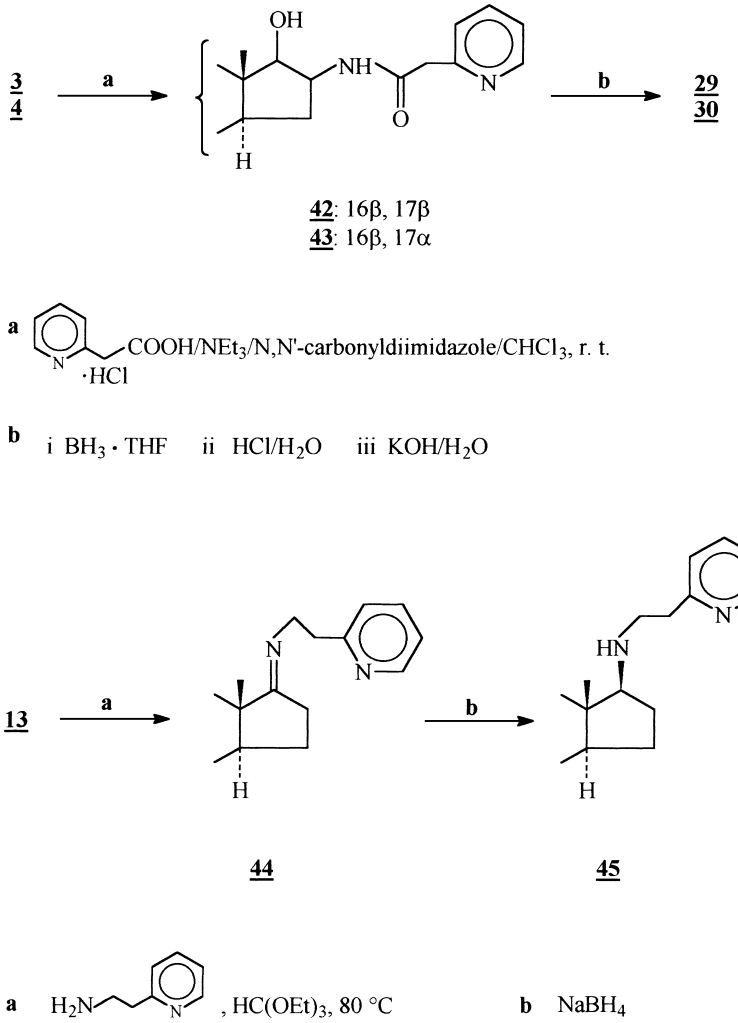
Scheme 5. Hydroamination with 17- and 3-amines

In summary, it is evident that chiral RPY2 ligands with a steroid core generated with low to moderate yields via addition of primary steroid amines to 2-vinyl pyridine are only available when sterically relatively unhindered equatorial or quasiequatorial amines (2α, 3β, 16α) are employed. The corresponding mono(pyridylethyl)amines, however, can generally be synthesized using this method. In addition, axial steroidal amines can be used as starting materials. Only the 17β-amine **5** cannot be employed due to lack of reactivity. When mixtures of mono- and bis-addition products are obtained, chromatographic separation is possible. The RPY2 compounds are in general, less polar than the corresponding monoadducts. The RPY2 steroidal compounds could not be obtained in crystalline form. Our further investigations were then directed to the transformation of mono(pyridylethyl)amines to RPY2 ligands. At this point, we should comment on some very new and important results concerning the bidentate mono(pyridylethyl)amino

ligands. Ítoh, Fukuzumi and co-workers used *N*-ethyl-*N*-[2-(2-pyridyl)ethyl]-2-phenethylamine as a bidentate ligand for Cu(I) ions. The reaction with O_2 at –78°C gave a bis(μ-oxo)dicopper(III) complex. At 25°C the benzylic position of the phenethyl group was hydroxylated.[28] Another bis(μ-oxo)dicopper(III) complex with 2-(diethylaminomethyl)-6-phenylpyridine as a bidentate ligand has the ability to hydroxylate the phenyl ring in *ortho*-position.[29] From these results it is evident that, except for the tridentate RPY2 ligands, bidentate ligands must be considered as ligands for copper ions with the ability to bind, activate and transfer oxygen. Therefore, new routes for the synthesis of *N*-[2-(2-pyridyl)ethyl]amines are also of interest.

2.3. *New routes to* N-*[2-(2-pyridyl)ethyl]amino and* N,N-*bis[2-(2-pyridyl)ethyl]amino steroids*

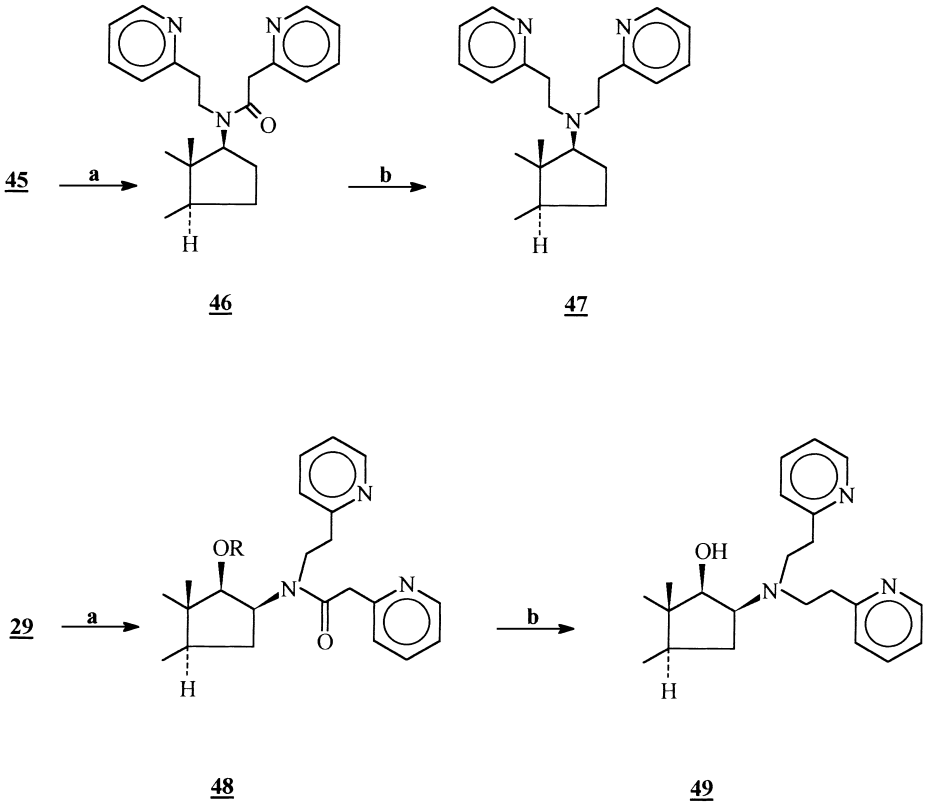
Starting with primary amines of steroids, we employed 2-pyridylacetic acid (commercially available as the hydrochloride) and *N*,*N*′-carbonyldimidazole as acylating agents. The amino-



Scheme 6. Synthesis of mono-(pyridylethyl)amines

alcohols **3** and **4** reacted under mild conditions to give the *N*-pyridylacetamides **42** and **43** in good yields after an aqueous work-up procedure, which is also suitable for saponification of 17-esters. Reduction of the amides with borane in THF[30] under mild conditions also provided the desired *N*-[2-(2-pyridyl)ethyl]amines **29** and **30** in good yields (Scheme 6). Another convenient method is the reductive amination of steroidal ketones with *N*-[2-(2-pyridyl)ethyl]amine and $NaBH_4$ in methanol. In this way we could synthesize from ketone **13** in a one-pot procedure the 17β-*N*-[2-(2-pyridyl)ethyl]amino compound **45**. Compound **45** is not available by addition of the primary amine **5** to 2-vinyl pyridine. It is also possible to isolate the imine **44** in high yields (Scheme 6).

For further transformation of the monopyridylethylamino steroids to bispyridylethylamino steroids we used the acylation/reduction route as previously described. As we could demonstrate with the sterically hindered monopyridylethylamine **45**, the tertiary pyridylacetamide **46** can be obtained in a smooth reaction. Interestingly enough the ^1H NMR spectra of **46** in $CDCl_3$ shows a

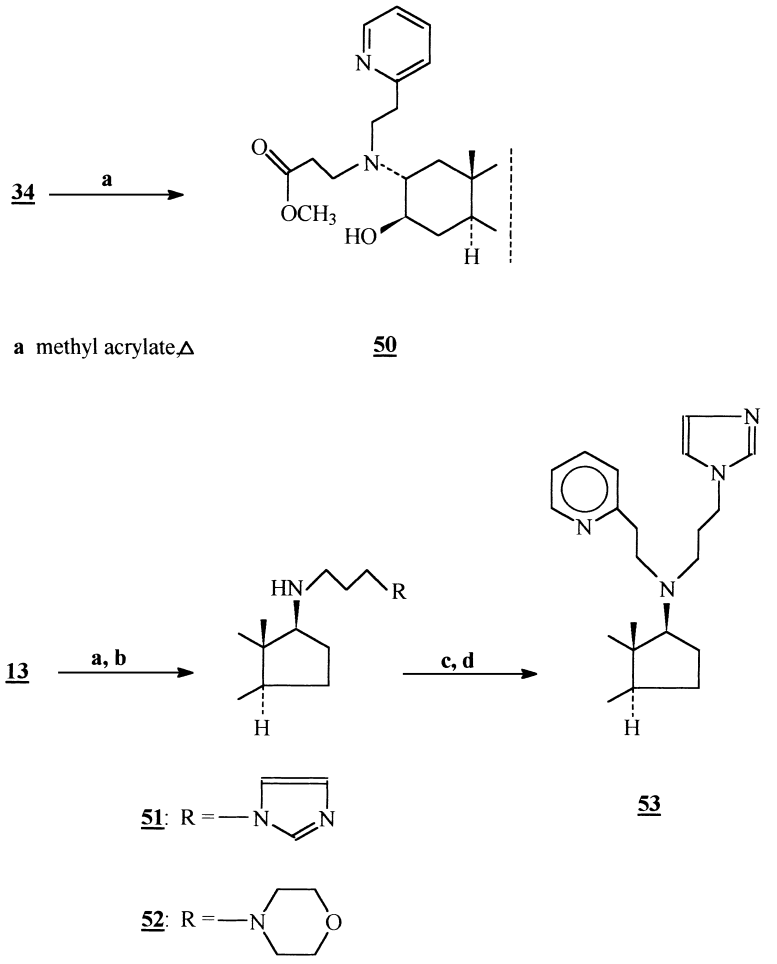


a ·HCl COOH/NEt_3/N,N'-carbonyldiimidazol/$CHCl_3$, r. t.

b i $BH_3 \cdot THF$ ii HCl/H_2O Δ iii KOH/H_2O

Scheme 7. Synthesis of bis-(pyridylethyl)amines

split signal for the 13β-methyl protons at room temperature. At higher temperatures the expected singlet appeared. A slow interconversion of the *E* and *Z* forms of the tertiary amide **46** at room temperature is probably responsible for this observation. The reduction of this amide **46** with borane in THF[30] gave the expected 17β-*N*,*N*-bis[2-(2-pyridyl)ethyl]amino compound **47** in good yield. In addition, the 16β-monopyridylethylamine **29** with an additional 17β-hydroxy group could also be transformed into the 16β-*N*,*N*-bis[2-(2-pyridyl)ethyl]amino-17β-hydroxy compound **49**. However, the yield is lower—especially for the reduction step (Scheme 7).



Scheme 8. Synthesis of mixed ligands

2.4. Synthesis of tridentate 'mixed' ligands

The bidentate monopyridylethylamino ligands described here can be employed in the synthesis of tridentate mixed ligands. One such mixed ligand containing two different arms at the central nitrogen is represented by the amide **46**; the donor activity of the central nitrogen is strongly decreased, however, by the amide structure. A second kind of mixed ligand could be obtained by the Michael addition of **34** to methyl acrylate. Compound **50** represents a tridentate ligand with *N*- and *O*-donor functions (Scheme 8). The reductive amination of ketone **13** with other suitable amines such as 3-(imidazol-1-yl)propylamine or 3-(*N*-morpholino)propylamine furnished the bidentate ligands **51** and **52**, respectively. Acylation of **51** with 2-pyridylacetic acid followed by borane reduction gave the 'mixed' tridentate ligand **53** (Scheme 8).

2.5. Synthesis and reactions of copper complexes (preliminary results)

Copper(II) complexes could be obtained by addition of copper(II) perchlorate dissolved in methanol to a methanolic solution of RPY2 ligands. Reduction of these blue copper(II) complexes with benzoin/triethylamine[3c–e,g] in either tetrahydrofuran, dichloromethane, or acetonitrile, gave yellow solutions of copper(I) complexes. Exposure of these solutions to an oxygen atmosphere caused a colour change to green or blue–green. After 24 h, the isolated copper complexes were investigated by MS spectroscopy and decomplexed with aq. NH_4OH. In the case of the 2α-amino cholestane compound **38** only the unchanged ligand could been isolated after the described procedure. In the case of the 16α-amine **32**, the mass spectra of the complex as well as the decomplexed ligand showed, in addition to the molecular ion peaks of the starting material, peaks of +16 thus indicating the insertion of one oxygen atom. After the described redox procedure with the 3β-amine **40**, only the oxidized ligand could be detected by mass spectroscopy. Further investigations on this remarkable dependence of oxidation behaviour on the position of the steroid ligands, including the structure of the oxidation products and the behaviour of bidentate and mixed ligands, are under way.

3. Conclusions

We have demonstrated that chiral ligands of the type RPY2 with R = steroid core can be synthesized at sterically hindered positions also when new synthetical routes are employed. Furthermore, related bidentate ligands and tridentate 'mixed' ligands were described. These ligands are useful for binding copper ions and dioxygen, thus giving interesting compounds for investigation on diastereoselective and enantioselective oxidations, in dependence on the structure, the position and the configuration of the chiral ligand.

4. Experimental

4.1. General

Melting points were measured on a Boëtius micromelting point apparatus (corrected values). Optical rotations were measured in chloroform with a photoelectronic polarimeter Polamat A

(Carl Zeiss Jena) at 546 and 578 nm and extrapolated to 589 nm (c in g 100^{-1} mL^{-1}). IR spectra were recorded on a Impact 400 spectrometer (NICOLET) in CCl_4. UV/vis spectra were obtained on a Spectrometer Lambda 19 (Perkin–Elmer). Extinction coefficients (ε) are given in l mol^{-1} cm^{-1} in parentheses. ^1H and ^{13}C NMR spectra were recorded on Bruker spectrometers AC 250 or DRX 400 in $CDCl_3$ (^1H NMR 250 or 400 MHz, ^{13}C NMR 62.5 or 100 MHz). Signals were assigned by DEPT, COSY–DQF, TOCSY and NOESY. Mass spectra were recorded on an AMD 402 Intectra instrument with electron impact ionization at 70 eV. Elemental analyses were determined on CHNO-Rapid (HERAEUS) or CHNS-932 (LECO) instruments. MPLC was carried out using a Labomatic equiqment (MD 80 pump, UV detector). All reactions were monitored by TLC aluminium sheets, silica gel 60 F_{254} (Merck), 0.2 mm, detection by UV (254 nm) and spraying with a solution of conc. sulfuric acid (80 mL), EtOH (20 mL) and vanillin (20 mg) and heating at 170°C. Solvents were purified, dried and distilled according to conventional methods. The reactions were carried out using inert conditions. The steroid compounds **13** and **15** were supplied by the Schering AG and the Jenapharm GmbH & Co. KG; the other compounds are commercially available.

4.2. 17β-Amino-3-methoxy-estra-1,3,5(10)-triene **5**

To a solution of 17-hydroximino-3-methoxy-estra-1,3,5(10)-triene[9] **14** (2.42 g, 8.1 mmol) in abs. CH_3OH (200 mL) and abs. THF (40 mL) MoO_3 (2.19 g, 15.2 mmol) were added. After cooling to 0°C $NaBH_4$ (14.8 g, 15.2 mmol) were added in small portions (temperature between 0 and +10°C) under stirring. The reaction mixture was treated after 30 min at room temperature with KOH (5.0 g) in H_2O (20 mL) and filtered off after 12 h at 0°C. The residue was washed twice with methanol (2×30 mL). The filtrate was concentrated in vacuo to 100 mL, poured into ice/water and extracted with $CHCl_3$ (50 mL). The aqueous phase was extracted twice with $CHCl_3$ (2×50 mL). The combined organic phases were washed three times with water (3×70 mL), dried (Na_2SO_4) and evaporated. Yield: 1.7 g (85.8%) of crude **5** as a white powder. M.p. 83–85°C; ^1H NMR ($CDCl_3$): $\delta = 0.65$ (s, 3H, 18-H_3), 2.73 (t, 1H, 17α-H), 2.81 (m, 2H, 6-H_2), 3.75 (s, 3H, OCH_3), 6.61 (s, 1H, 4-H), 6.69 (m, 1H, 2-H), 7.16 (d, $^3J = 8.54$ Hz; 1H, 1-H); after addition of trichloroacetyl isocyanate (TAI): $\delta = 0.80$ (s, 3H, 18-H_3), 3.89 (q, 1H, 17α-H), 7.89 (1H, d, $^3J = 8.57$ Hz, 17β-NHCO), 8.67 (s, 1H, NH). Purification by recrystallization from ether: m.p. 113–115°C, lit.[10b] 119–121°C (CH_2Cl_2–hexane). *N*-Acetylation: 70 mg of **5** were dissolved in acetic anhydride (2.0 mL) under reflux. After 30 min at room temperature the acetic anhydride was removed in vacuo and the crystals of the 17β-acetamide[10] were dried in vacuum. ^1H NMR ($CDCl_3$): $\delta = 0.70$ (s, 3H, 18-H_3), 1.98 (s, 3H, CH_3CO), 3.97 (m, 1H, 17α-H), 5.31 (d, $^3J = 8.55$ Hz, 1H, 17β-NH).

4.3. 17α-Amino-3-methoxy-estra-1,3,5(10)-triene **6**

The 17β-tosyloxy compound **16**[12] (5.16 g, 11.7 mmol) and NaN_3 (5.68 g, 102.8 mmol) were reacted at 100°C in abs. HMPTA (70 mL) and 18-crown-6 (1.5 g) for 20 h. After cooling, the mixture was poured into a ice/water mixture and then extracted with CH_2Cl_2. The organic phase was washed several times with water, dried and evaporated in vacuo. Yield: 3.5 g (96.1%) of 17α-azido compound **17**[11c] as an oily product. ^1H NMR ($CDCl_3$) $\delta = 0.76$ (s, 3H, 18-H_3), 3.57 (d, 1H, 17β-H).

Oily **17** (710 mg, 2.28 mmol), dissolved in abs. THF (10 mL), and $LiAlH_4$ (~1 M in THF, Fluka, 10 mL) were refluxed for 2 h. After cooling (0°C), an ether/H_2O mixture was carefully

added. The mixture was treated with a saturated aqueous NH_4Cl solution and filtered off. The residue was washed with ether and the aqueous solution was extracted twice with ether (2×30 mL). The combined ether solutions were dried and then treated with gaseous HCl. The solid hydrochloride of **6** was filtered off and recrystallized from ethanol. Aqueous KOH solution (1 g KOH, 10 mL H_2O), was then slowly added to a concentrated solution of the hydrochloride in methanol. The mixture was extracted with ether; the ether solution was then washed, dried and evaporated. Yield: 322 mg (49.5%) of amine **6** as a waxy product. ^{1}H NMR ($CDCl_3$): $\delta = 0.71$ (s, 3H, 17β-H), 2.98 (d, 1H, 17β-H); after addition of TAI: $\delta = 0.85$ (s, 3H, 18-H_3), 3.97 (t, 1H, 17β-H), 7.88 (1H, d, $^3J = 8.23$Hz, 17α-NHCO), 8.73 (s, 1H, NH). *N*-Acetylation: acetylation of **6** with acetic anhydride was carried out as described for amine **5**; 17α-acetamide:[10a] m.p. 213–216°C; ^{1}H NMR ($CDCl_3$): $\delta = 0.82$ (s, 3H, 18-H_3), 1.98 (s, 3H, CH_3CO), 4.04 (t, 1H, 17β-H), 5.35 (d, broad, 1H, 17α-NH).

4.4. 2α,3α-Oxido-cholestane **20**

To a stirred solution of the bromo ketone **19** (4.7 g, 1.0 mmol) in abs. THF (60 mL) a solution of L-Selectride (15 mL, 1 M, THF) was added dropwise at –60°C. After 40 min methanol (15 ml, 1 M, –40°C) and aqueous H_2O_2 (30 proz., 10 mL, –20°C) were added. The mixture was poured into ice and water/NaCl. After several hours at 10°C the precipitate was filtered off, washed with water and dried in vacuum. The white solid of 2α-bromo-cholestane-3α-ol[22] (4.5 g, 96%, m.p. 98–105°C) was reduced without further purification by heating with zinc powder (7.5 g, 115 mmol) and acetic acid (110 mL) with stirring under reflux for 30 min. After cooling, the zinc was filtered off and washed with acetic acid. The combined acetic acid solutions were poured into water and extracted with CH_2Cl_2. The organic phase was washed with aqueous $NaHCO_3$ and water, dried and evaporated. Purification of the residue by chromatography (SiO_2, 0.04–0.06 mm, *n*-heptane) gave pure 2-cholestene[31] (1.7 g, 48%, m.p. 72–74°C), lit.[31] m.p. 74–75°C. A solution of 2-cholestene (1.7 g, 4.6 mmol) in CH_2Cl_2 (35 mL) was treated with a solution of magnesium mono-peroxophthalate hexahydrate (~85%, 9.7 g, ~16.7 mmol) in methanol (35 mL) under stirring at room temperature. After 6 h an aqueous sodium hydrosulfite solution was added. After addition of CH_2Cl_2 the organic phase was separated, the aqueous phase was extracted with CH_2Cl_2 and the combined organic solutions were washed with water, dried and evaporated in vacuum. The oily residue was crystallized with methanol giving α-epoxide **20** (1.5 g, 84%, m.p. 100–102°C); lit.[31] m.p. 105–106°C.

4.5. 2β-Azido-cholestane-3α-ol [24] **24**

A mixture of α-epoxide **20** (3.0 g, 7.8 mmol), abs. dimethyl sulfoxide (60 mL), acetic acid (12 mL) and sodium azide (3.6 g, 55.4 mmol) was heated at 110°C under stirring for 4 h. CAUTION! Hydrazoic acid is explosive and poisonous. After cooling, the mixture was poured into ice and water/NaCl. The precipitation was filtered off, washed with water and dried in vacuum giving 3.2 g of azidoalcohol **24** (95%, m.p. 128–138°C), crystallization with methanol gave 2.7 g of **24** (81%, m.p. 139–142°C), lit.[24] m.p. 142°C; $[\alpha]_D^{20} = +45$ ($c = 0.57$, $CHCl_3$), lit.[24] $[\alpha]_D^{20} = +40$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 3.75 (m, 1H, 2α-H), 3.82 (m, 1H, 3β-H); after addition of TAI: 3.93 (m, 1H, 2α-H), 4.89 (m, 1H, 3β-H), 8.32 (s, 1H, NH).

4.6. 2β-Amino-cholestane-3α-ol[14] **10**

To a solution of azidoalcohol **24** (1.0 g, 2.3 mmol) in methanol (30 mL) and THF (5 mL) 80% hydrazine hydrate (2 mL) and a small portion of Raney-nickel were added. After 15 min heating under reflux and stirring the Raney-nickel was filtered off and washed with hot dichloromethane. The solution was concentrated in vacuum for crystallization and after cooling the crystals were filtered off and washed with cold methanol giving 776 mg of **24** (84%); m.p. 180–186°C, lit.[14] m.p. 206–207°C, lit.[13] m.p. 184–187°C,‡ lit.[15] m.p. 177–179°C; $[\alpha]_D^{20} = +29$ ($c = 0.50$, $CHCl_3$), lit.[14]+34 (pyridine), lit.[15]+46.5 (ethanol); IR (CCl_4), ν [cm^{-1}]: 3627 (ms), 3329 (br, ms); ^1H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 0.97 (s, 3H, 19-H_3), 3.00 (m, 1H, 2α-H), 3.66 (m, 1H, 3β-H); after addition of TAI: $\delta = 4.16$ (m, 1H, 2α-H), 5.07 (m, 1H, 3β-H) 8.25 (d, 1H, 2β-NHCO), 8.42 and 8.81 (2×s, 2×1H, 2×NH). $C_{27}H_{49}NO$ HRMS m/z (M^+): 403.3828; calcd: 403.3812.

4.7. Reduction of 2α-azido-cholestane-3-one[23] **21** with $LiBH_4$

To a solution of azido ketone **21** (1.0 g, 2.3 mmol) in methanol (30 mL) and THF (30 mL) lithium borohydride (0.4 g, 18.3 mmol) was successively added under stirring at 0°C. After 30 min acetone (10 mL) was added and the solution was poured into an ice/water mixture. The precipitate was filtered off after cooling for several hours, washed with water and dried in vacuum giving 0.98 g of a white powder.

Chromatography on silica gel (0.04–0.06 mm) with toluene gave 2α-azido-cholestane-3α-ol **23** (0.33 g, 33%), 2α-azido-cholestane-3β-ol **22** (0.60 g, 59%) and cholestane-3β-ol **18** (45 mg, 5%).

Compound **23**: m.p. 105–109°C (methanol); $[\alpha]_D^{20} = -23$ ($c = 0.50$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.63$ (s, 3H, 18-H_3), 0.80 (s, 3H, 19-H_3), 3.53 (m, 1H, 2β-H), 3.95 (m, 1H, 3β-H); after addition of TAI: 3.42 (m, 1H, 2β-H), 5.25 (m, 1H, 3β-H), 8.36 (s, 1H, NH). $C_{27}H_{47}N_3O$ (429.26) calcd: C, 75.54; H, 10.95; N, 9.78; found: C, 75.15; H, 11.24; N, 9.56. HRMS m/z (M^+): 429.3725; calcd: 429.3717.

Compound **22**: m.p. 103–105°C (methanol); $[\alpha]_D^{20} = -42$ ($c = 0.50$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.63$ (s, 1H, 18-H_3), 0.83 (s, 3H, 19-H_3), 3.29–3.47(m, 2H, 2β-H, 3α-H); after addition of TAI: 3.57 (m, 1H, 2β-H), 4.71 (m, 1H, 3α-H), 8.34 (s, 1H, NH). $C_{27}H_{47}N_3O$ (429.26) calcd: C, 75.54; H, 10.95; N, 9.78; found: C, 74.92; H, 10.98; N, 9.32. HRMS m/z (M^+): 429.3724; calcd: 429.3717.

4.8. Reduction of **21** with LS-Selectride

To a solution of **21** (1.0 g, 2.3 mmol) in THF (40 mL) a solution of LS-Selectride in THF (1 M, 10 mL) was added at –60°C under stirring. After 40 min methanol (10 mL, –50°C), sodium hydroxide in methanol (1 M, 10 mL, –30°C) and 30% H_2O_2 (5 mL, –10°C) were added. The mixture was successively added to ice and water/NaCl under stirring. After cooling for several hours the precipitate was filtered off, washed with water, dried in vacuum and purified by chromatography on silica gel (0.04–0.06 mm) with toluene to give **23** (0.82 g, 82%) and cholestane-3α-ol[32] (80 mg, 8%).

4.9. 2β-Azido-cholestane-3β-ol **26**

To a stirred suspension of azido ketone **25** (300 mg, 0.7 mmol) in ethanol (2 mL) $NaBH_4$ (90 mg, 2.4 mmol) was added at 0°C. After 30 min at room temperature ice and water were added.

‡ Lit.[13]: see discussion on m.p. and IR spectra.

The precipitate was filtered off after cooling for several hours, washed with water and dried in vacuum giving 2β,3β-azidoalcohol **26** (269 mg, 89%, m.p. 138–144°C). Crystallization with methanol/ethyl acetate gave white crystals. M.p. 143–147°C; $[\alpha]_D^{20} = +58$ ($c = 1.0$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.63$ (s, 3H, 18-H_3), 0.98 (s, 3H, 19-H_3), 3.59 (m, 1H, 3α-H), 3.99 (m, 1H, 2α-H); after addition of TAI: 4.15 (m, 1H, 2α-H), 4.88 (m, 1H, 3α-H), 8.42 (s, 1H, NH). $C_{27}H_{47}N_3O$ (429.26) calcd: C, 75.54; H, 10.95; N, 9.78; found: C, 75.12; H, 11.04; N, 9.37.

4.10. 2α-Amino-cholestane-3β-ol **8**

A solution of azidoalcohol **22** (1.74 g, 4.0 mmol) in methanol (50 mL) and THF (10 mL) was reacted with 80% hydrazine hydrate (2.5 mL) and Raney-nickel as described for the synthesis of **10**. Crystallization with ethyl acetate and methanol gave **8** as white crystals (1.25 g, 77%, m.p. 158–165°C), lit.[17] m.p. 162–164°C, lit.[18] m.p. 260–265°C;[§] $[\alpha]_D^{20} = +7$ ($c = 0.9$, $CHCl_3$), lit.[17] $[\alpha]_D^{20} = +7$ ($c = 1.06$, $CHCl_3$); IR (CCl_4), ν [cm^{-1}]: 3329 (vbr, s); ^1H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 0.81 (s, 3H, 19-H_3), 2.58 (m, 1H, 2β-H), 3.10 (m, 1H, 3α-H); after addition of TAI: 4.11 (m, 1H, 2β-H), 4.75 (m, 1H, 3α-H), 7.87 (d, 1H, 2α-NHCO), 8.37 and 8.39 (2×s, 2×1H, 2×NH). $C_{27}H_{49}NO$ HRMS m/z (M^+): 403.3834; calcd: 403.3812.

4.11. 2α-Amino-cholestane-3α-ol **7**

A solution of azidoalcohol **23** (1.28 g, 3.0 mmol) in methanol (40 mL) and THF (10 mL) was reacted with 80% hydrazine hydrate (2.3 mL) and Raney-nickel as described for the synthesis of **10**. Crystallization with ethyl acetate/methanol gave **7** as white crystals (1.02 g, 84%, m.p. 205–208°C), lit.[18] m.p. 225–226°C (ethanol).[¶] $[\alpha]_D^{20} = +44$ ($c = 0.64$, $CHCl_3$); IR (CCl_4), ν [cm^{-1}]: 3480 (br, ms), 3329 (br, ms); ^1H NMR ($CDCl_3$), $\delta = 0.62$ (s, 3H, 18-H_3), 0.77 (s, 3H, 19-H_3), 2.97 (m, 1H, 2β-H), 3.68 (m, 1H, 3β-H); after addition of TAI: 4.17 (m, 1H, 2β-H), 5.23 (m, 1H, 3β-H), 7.91 (d, 1H, 2β-NHCO), 8.39 and 8.44 (2×s, 2×1H, 2×NH). $C_{27}H_{49}NO$ (403.70) calcd: C, 80.33; H, 12.23; N, 3.47; found: C, 79.93; H, 12.23; N, 3.43; HRMS m/z (M^+): 403.3829; calcd: 403.3812.

4.12. 2β-Amino-cholestane-3β-ol **9**

A solution of azidoalcohol **26** (300 mg, 0.70 mmol) in methanol (20 mL) was reacted with 80% hydrazine hydrate (1 mL) and Raney-nickel as described for the synthesis of **10** giving after crystallization with ethyl acetate/methanol aminoalcohol **9** (240 mg, 85%, m.p. 155–160°C and 209–211°C), lit.[19] m.p. 166–169°C or 148–149°C,[‖] respectively, lit.[20] m.p. 209–211°C (EtOH); $[\alpha]_D^{20} = +41$ ($c = 0.50$, $CHCl_3$), lit.[20] $[\alpha]_D^{20} = +10$ ($c = 1.0$, $CHCl_3$); IR (CCl_4), ν (cm^{-1}): 3460 (w), 3217 (br, ms); ^1H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 3.13 (m, 1H, 2α-H), 3.51 (m, 1H, 3α-H); after addition of TAI: 4.53 (m, 1H, 2α-H), 4.99 (m, 1H, 3α-H), 8.24 (d, 1H, 2αNHCO), 8.28 and 8.67 (2×s, 2×1H, 2×NH). $C_{27}H_{49}NO \cdot 1/2H_2O$ (412.70) calcd: C, 78.58; H, 12.21; N, 3.39; found: C, 78.08; H, 11.99; N, 3.23. HRMS m/z (M^+): 403.3807; calcd: 403.3812.

[§] Lit.[13]: see discussion on m.p. and IR spectra.
[¶] Lit.[13]: see discussion on m.p. and IR spectra.
[‖] Lit.[13]: see discussion on m.p. and IR spectra.

4.13. General method for the addition of primary amino steroids to 2-vinyl pyridine

4.13.1. Addition of aminoalcohol **1**

Aminoalcohol **1** (500 mg, 1.66 mmol) was dissolved at room temperature in a solution of abs. CH_3OH (10 mL) and 2-vinyl pyridine (10 mL, 9.76 g, 92.8 mmol, purified by passing through a short column with silica gel and ether as solvent, followed by removal of the solvent). After addition of acetic acid (600 mg, 10 mmol), the mixture was stirred for 48 h at 75°C under argon and in the absence of light. The solvents were removed under reduced pressure and the resulting oil was dissolved in CH_3OH (10 mL). Methanolic NaOH (1 M, 5 mL) was added, the solvent was removed and the residue extracted with ether or CH_2Cl_2. After evaporation of the solvent, the reaction mixture was separated with column chromatography on silica gel (50 g) using CH_3OH as solvent or by MPLC on silica gel with ethyl acetate:CH_3OH (7:3) for the chloestane compounds.

Fraction 1: 16α-*N*,*N*-Bis-[2-(2-pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17α-ol **31**, 178 mg (21%), colourless oil; $[\alpha]_D^{20} = +40$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.72$ (s, 3H, 18-H_3), 2.75–3.42 (m, 11H, 6-H_2, 2×CH_2CH_2Py, 16β-H), 3.62 (d, $^3J = 4.1$ Hz, 1H, 17β-H), 3.75 (s, 3H, OCH_3), 6.59 (s, 1H, 4-H), 6.69 (d, $^3J = 8.60$ Hz, 1H, 2-H), 7.05–7.19 (m, 5H, 1-H, 2×3-H_{py} and 5-H_{py}), 7.56 (t, $^3J = 7.6$ Hz, 2H, 2×4-H_{py}), 8.52 (d, $^3J = 5.8$ Hz, 2H, 2×2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 17.15$ (18-C), 25.10, 28.07, 29.86, 31.35, 35.52, 38.83, 43.50, 45.02, 46.91, 51.26, 55.19 (OCH_3), 64.52 (16-C), 78.32 (17-C), 111.40 (C_{ar}), 113.80 (C_{ar}), 121.21 (C_{py}), 123.35 (C_{py}), 126.26 (C_{ar}), 132.98 (C_{ar}), 136.32 (C_{py}), 137.90 (C_{ar}), 149.32 (C_{py}), 157.39 (C_{ar}),160.09 (C_{py}). $C_{33}H_{41}N_3O_2$ (511.72) calcd: C, 77.46; H, 8.07; N, 8.41; found: C, 76.68; H, 8.14; N, 8.26.

Fraction 2: 16α-*N*-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-trien-17α-ol **27**, 380 mg (56%), colourless oil; $[\alpha]_D^{20} = +25$ ($c = 0.50$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.71$ (s, 3H, 18-H_3), 2.75–3.12 (m, 6H, 6-H_2, CH_2CH_2Py), 3.33 (m, 1H, 16β-H), 3.56 (d, $^3J = 4.75$ Hz, 1H, 17β-H), 3.75 (s, 3H, OCH_3), 6.59 (s, 1H, 4-H), 6.68 (d, $^3J = 8.53$ Hz, 1H, 2-H), 7.09–7.18 (m, 3H. 1-H, 3-H_{py} and 5-H_{py}), 7.61 (m, 1H, 4-H_{py}), 8.52 (d, $^3J = 4.0$ Hz, 1H, 2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 16.85$ (18-C), 25.88, 28.06, 29.87, 31.80, 33.72, 37.97, 38.80, 43.51, 45.84, 46.29, 47.91, 55.18 (OCH_3), 59.78 (16-C), 76.36 (17-C), 111.40 (C_{ar}), 113.80 (C_{ar}), 121.41 (C_{py}), 123.35 (C_{py}), 126.31 (C_{ar}), 132.93 (C_{ar}), 136.51 (C_{py}), 137.89, (C_{ar}), 149.27 (C_{py}), 157.38 (C_{ar}), 159.85 (C_{py}). $C_{26}H_{34}N_2O_2$ (406.57) calcd: C, 76.81; H, 8.43; N, 6.89; found: C, 76.19; H, 8.40; N, 6.88.

4.13.2. Addition of aminoalcohol **2**

Aminoalcohol **2** (500 mg, 1.66 mmol) were reacted with 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

Fraction 1: 16α-*N*,*N*-Bis-[2-(2-pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17β-ol **32**, 350 mg (41%), colourless oil; $[\alpha]_D^{20} = +4$ ($c = 0.4$, pyridine); ^{1}H NMR ($CDCl_3$): $\delta = 0.82$ (s, 3H, 18-H_3), 2.75–2.96 (m, 10H, 6-H_2, 2×CH_2CH_2Py), 3.29 (m, 2H, 16β-H, 17α-H), 3.75 (s, 3H, OCH_3), 6.60 (s, 1H, 4-H), 6.68 (d, $^3J = 8.52$ Hz, 1H, 2-H), 6.93–7.19 (m, 5H, 1-H, 2×3-H_{py} and 5-H_{py}), 7.53 (t, $^3J = 7.1$ Hz, 2H, 2×4-H_{py}), 8.49 (d, $^3J = 4.8$ Hz, 2H, 2×2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 12.28$ (18-C), 25.32, 26.07, 27.25, 29.76, 36.20, 37.00, 38.51, 42.41, 43.86, 48.89, 51.44, 55.16 (OCH_3), 66.20 (16-C), 83.81 (17-C), 111.38 (C_{ar}), 113.79 (C_{ar}), 121.08 (C_{py}), 123.48 (C_{py}), 126.21 (C_{ar}), 132.79 (C_{ar}), 136.27 (C_{py}), 137.91 (C_{ar}), 149.00 (C_{py}), 157.40 (C_{ar}), 160.68 (C_{py}). $C_{33}H_{41}N_3O_2$ (511.72) calcd: C, 77.46; H, 8.07; N, 8.41; found: C, 77.19; H, 8.23; N, 6.01.

Fraction 2: 16α-*N*-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17β-ol **28**, 203 mg (30%), colourless oil; $[\alpha]_D^{20} = +30$ ($c = 0.50$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.81$ (s, 3H, 18-H_3),

2.76–3.14 (m, 7H, 6-H_2, 16β-H, CH_2CH_2Py), 3.48 (d, $^3J = 6.5$ Hz, 1H, 17α-H), 3.75 (s, 3H, OCH_3), 6.59 (s, 1H, 4-H), 6.68 (q, 1H, 2-H), 7.10–7.19 (m, 3H, 1-H, 3-H_{py} and 5-H_{py}), 7.60 (m, 1H, 4-H_{py}), 8.50 (m, 1H, 2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 12.26$ (18-C), 26.04, 27.16, 29.74, 31.65, 36.82, 37.87, 38.44, 43.73 (2 C, overlapped), 43.91, 48.06, 55.17 (OCH_3), 63.90 (16-C), 87.94 (17-C), 111.43 (C_{ar}), 113.80 (C_{ar}), 121.33 (C_{py}), 123.39 (C_{py}), 126.20 (C_{ar}), 132.57 (C_{ar}), 136.56 (C_{py}), 137.90 (C_{ar}), 149.16 (C_{py}), 157.42 (C_{ar}), 160.16 (C_{py}). $C_{26}H_{34}N_2O_2$ (406.57) calcd: C, 76.81; H, 8.43; N, 6.89; found: C, 76.50; H, 8.25; N, 6.79.

*4.13.3. Addition of aminoalcohol **3***

Aminoalcohol **3** (400 mg, 1.33 mmol) was reacted with 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

16β-*N*-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17β-ol **29**: 406 mg (75%), colourless oil; ^1H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 2.81 (m, 2H, 6-H_2), 2.92–3.15 (m, 5H, CH_2CH_2Py, 16α-H), 3.37 (d, $^3J = 8.8$ Hz, 1H, 17α-H), 3.75 (s, 3H, OCH_3), 6.60 (s, 1H, 4-H), 6.68 (q, 1H, 2-H), 7.09–7.20 (m, 3H, 1-H, 3-H_{py} and 5- H_{py}), 7.59 (m, 1H, 4-H_{py}), 8.51 (m, 1H, 2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 12.30$ (18-C), 26.23, 27.58, 29.74, 35.16, 37.64, 38.17, 38.63, 43.29, 43.95, 47.18, 49.66, 55.16 (OCH_3), 58.05 (16-C), 79.06 (17-C), 111.42 (C_{ar}), 113.78 (C_{ar}), 121.30 (C_{py}), 123.28 (C_{py}), 126.30 (C_{ar}), 132.69 (C_{ar}), 136.37 (C_{py}), 137.76 (C_{ar}), 149.29 (C_{py}), 157.43 (C_{ar}), 160.00 (C_{py}); $C_{26}H_{34}N_2O_2$ (406.57) calcd: C, 76.81; H, 8.43; N, 6.89; found: C, 77.09; H, 8.73; N, 6.71.

*4.13.4. Addition of aminoalcohol **4***

Aminoalcohol **4** (980 mg, 3.25 mmol) was reacted with 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

16β-*N*-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17α-ol **30**: 610 mg (46%), colourless oil; ^1H NMR ($CDCl_3$): $\delta = 0.81$ (s, 3 H, 18-H_3), 2.81 (m, 2H, 6-H_2), 2.95–3.11 (m, 5H, 16α-H, CH_2CH_2Py), 3.60 (s, 1 H, 17β-H), 3.75 (s, 3 H, OCH_3), 6.61-6.71 (m, 2 H, 4-H, 2-H), 7.04-7.21 (m, 3 H,1-H, 3-H_{py} and 5-H_{py}), 7.57 (m, 1 H, 4-H_{py}), 8.49 (m, 1 H, 2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 17.82$ (18-C), 25.89, 27.94, 29.81, 32.02, 33.51, 37.69, 38.62, 43.35, 44.27, 47.87, 48.03, 55.19 (OCH_3), 68.50 (16-C), 84.63 (17-C), 111.46 (C_{ar}), 113.79 (C_{ar}), 121.34 (C_{py}), 123.37 (C_{py}), 126.25 (C_{ar}), 132.65 (C_{ar}), 136.47 (C_{py}), 137.92 (C_{ar}), 149.14 (C_{py}), 157.45 (C_{ar}), 160.28 (C_{py}). $C_{26}H_{34}N_2O_2$ (406.57) calcd: C, 76.81; H, 8.43; N, 6.89; found: C, 76.74; H, 8.62; N, 6.73.

*4.13.5. Addition of 17α-amine **6***

Compound **6** (400 mg, 1.40 mmol) was reacted with 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

17α-*N*-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene **41**: 140 mg (25%), colourless oil; $[\alpha]_D^{20} = +32.9$ ($c = 0.24$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.71$ (s, 3H, 18-H_3), 2.67 (d, 1H, 17β-H), 2.82 (m, 2H, 6-H_2), 2.90–3.02 (m, 4H, CH_2CH_2Py), 3.75 (s, 3H, OCH_3), 6.60 (s, 1H, 4-H), 6.68 (q, 1H, 2-H), 7.07–7.18 (m, 3H, 1-H, 3-H_{py} and 5-H_{py}), 7.56 (t, 4-H_{py}), 8.52 (m, 1H, 2-H_{py}).

4.13.6. Addition of aminoalcohol 7

Aminoalcohol **7** (800 mg, 1.98 mmol) was reacted with 2-vinyl pyridine as described for the reaction of the aminoalcohol **1**.

Fraction 1: 2α-*N*,*N*-Bis-[2-(2-pyridyl)ethyl]amino-5α-cholestane-3α-ol **37**, 97 mg (8%), colourless oil; $[\alpha]_D^{20} = +13$ ($c = 0.88$, CH_3OH). UV (CH_3OH): λ_{max} (lg ε) = 261 nm (3.6904). ^1H NMR ($CDCl_3$): $\delta = 0.63$ (s, 3H, 18-H_3), 0.74 (s, 3H, 19-H_3), 0.84 (s, 6H, 26-H_3 and 27-H_3), 0.87 (d,

$^3J = 6.6$ Hz, 3H, 21-H_3), 2.55 (m, 1H, 2β-H), 2.81–2.93 (m, 6H, CH_2CH_2Py), 3.18 (m, 2H, CH_2CH_2Py), 3.81 (m, 1H, 3β-H), 6.98–7.06 (m, 4H, 2×3-H_{py} and 5-H_{py}), 7.48 (t, $^3J = 7.6$ Hz, 2H, 2×4-H_{py}), 8.47 (d, 2H, $^3J = 4.2$ Hz, 2×2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 12.0$ (18-C), 12.4 (19-C), 18.6 (21-C), 20.8 (11-C), 22.5 and 22.7 (26-C and 27-C), 23.7 and 24.1 (23-C and 15-C), 27.9 (25-C), 28.1 (6-C), 31.8 (16-C), 34.5 (4-C), 35.0 (7-C and 20-C), 35.7 (8-C and 2×CH_2CH_2Py), 36.1 (22-C), 36.4 (10-C), 38.4 (5-C), 39.4 (24-C and 12-C), 40.0 (1-C), 42.4 (13-C), 49.1 (2×CH_2CH_2Py), 54.4 (9-C), 56.0 and 56.3 (14-C and 17-C), 58.6 (2-C), 67.8 (3-C), 120.9 (2×3-or 5-C_{py}), 123.6 (2×3-or 5-C_{py}), 136.1 (2×4-C_{py}), 148.9 (2×2-C_{py}), 160.4 (2×6-C_{py}). HRMS m/z 595.4864 (M^+–H_2O), calcd: 595.4879 for $C_{41}H_{61}N_3$.

Fraction 2: 2α-*N*-[2-(2-Pyridyl)ethyl]amino-5α-cholestane-3α-ol **33**, 482 mg (48%), white solid. M.p. 73–77°C (CH_3OH); $[\alpha]_D^{20} = +33$ ($c = 1.03$, CH_3OH). UV (CH_3OH): λ_{max} (lg ε) = 260 nm (3.5442). ^{1}H NMR ($CDCl_3$): $\delta = 0.60$ (s, 3H, 18-H_3), 0.76 (s, 3H, 19-H_3), 0.83 (d, 6H, 26- and 27-H_3), 0.86 (d, 3H, 21-H_3), 2.64 (m, 1H, 2β-H), 2.93–3.11 (m, 4H, CH_2CH_2Py), 3.92 (m, 1H, 3β-H), 7.13 (m, 2H, 3- and 5-H_{py}), 7.58 (t, 1H, $^3J = 7.6$ Hz, 4-H_{py}), 8.50 (d, $^3J = 4.1$ Hz, 2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 11.9$ (18-C), 12.4 (19-C), 18.6 (21-C), 20.7 (11-C), 22.5 and 22.7 (26-C and 27-C), 23.7 and 24. 1 (23-C and 15-C), 27.8 (16-C), 27.9 (25-C), 28.1 (6-C), 31.8 (7-C), 33.3 (4-C), 35.0 (20-C), 35.7 and 36.0 (8-C and 22-C), 36.3 (10-C), 38.1 (CH_2CH_2Py), 38.3 (5-C), 39.4 and 39.8 (24-C and 12-C), 39.9 (1-C), 42.4 (13-C), 45.1 (CH_2CH_2Py), 54.1 (9-C), 55.4 (2-C), 56.1 and 56.3 (14-C and 17-C), 64.6 (3-C), 121.3 (3-or 5-C_{py}), 123.4 (3-or 5-C_{py}), 136.4 (4-C_{py}), 149.1 (2-C_{py}), 159.9 (6-C_{py}). HRMS m/z 490.4279 (M^+–H_2O), calcd: 490.4284 for $C_{34}H_{54}N_2$.

*4.13.7. Addition of aminoalcohol **8***

Aminoalcohol **8** (800 mg, 1.98 mmol) was reacted with 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

Fraction 1: 2α-*N*,*N*-Bis-[2-(2-pyridyl)ethyl]amino-5α-cholestane-3β-ol **38**, 134 mg (11%), colourless oil; $[\alpha]_D^{20} = +31$ ($c = 0.83$, CH_3OH). UV (CH_3OH): λ_{max} (lg ε) = 261 (3.8784) and 285 (1.9347); ^{1}H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 0.79 (s, 3H, 19-H_3), 0.83 (d, 6H, 26-H_3 and 27-H_3), 0.85 (d, 3H, $^3J = 6.5$ Hz, 21-H_3), 2.55 (t, 1H, 2β-H), 2.72–3.07 (m, 8H, 2×CH_2CH_2Py), 3.23 (t, 1H, 3β-H), 6.93 and 7.07 (d and m, 2×2H, 2×3-H_{py} and 2×5-H_{py}), 7.50 (t, 2H, 2×4-H_{py}), 8.50 (d, $^3J = 3.9$ Hz, 2H, 2×2-H_{py}). HRMS m/z 595.4872 (M^+-H_2O), calcd: 595.4879 for $C_{41}H_{61}N_3$.

Fraction 2: 2α-*N*-[2-(2-Pyridyl)ethyl]amino-5α-cholestane-3β-ol **34**, 543 mg (54%), white solid. M.p. 126–129°C (CH_3OH); $[\alpha]_D^{20} = +22$ ($c = 0.68$, pyridine). UV (CH_3OH): λ_{max} (lg ε) = 260 nm (3.4867); ^{1}H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 0.79 (s, 3H, 19-H_3), 0.83 (d, 6H, 26-H_3 and 27-H_3), 0.87 (d, 3H, $^3J = 6.5$ Hz, 21-H_3), 2.39 (m, 3H, CH_2CH_2Py), 2.43 (t, 1H, 2β-H), 3.19 (m, 2H, 3α-H and CH_2CH_2Py), 7.11 (m, 2H, 3-H_{py} and 5-H_{py}), 7.58 (t, $^3J = 7.7$ Hz, $^4J = 1.8$ Hz, 1H, 4-H_{py}), 8.50 (d, $^3J = 4.0$ Hz, 1H, 2-H_{py}). HRMS m/z 507.4299 (M^+–1), calcd: 507.4311 for $C_{34}H_{55}N_2O$.

*4.13.8. Addition of aminoalcohol **9***

Aminoalcohol **9** (800 mg, 1.98 mmol) was reacted with 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

2β-*N*-[2-(2-Pyridyl)ethyl]amino-5α-cholestane-3β-ol **35**: 483 mg (48%), white solid. M.p. 137–141°C (CH_3OH); $[\alpha]_D^{20} = +32$ ($c = 0.68$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.60$ (s, 3H, 18-H_3), 0.70 (s, 3H, 19-H_3), 0.83 (d, 6H, 26-H_3 and 27-H_3), 0.87 (d, 3H, $^3J = 6.5$ Hz, 21-H_3), 2.84 (m, 2H, 2α-H and CH_2CH_2Py), 2.94–3.26 (m, 3H, CH_2CH_2Py), 3.48 (m, 1H, 3α-H), 7.14 (m, 2H, 3-H_{py} and 5-H_{py}), 7.58 (t, 1H, $^3J = 6.8$ Hz, $^4J = 1.7$ Hz, 4-H_{py}), 8.50 (d, 1H, $^3J = 4.0$ Hz, 2-H_{py}). HRMS m/z 490.4270 (M^+–H_2O), calcd: 490.4284 for $C_{34}H_{54}N_2$.

*4.13.9. Addition of aminoalcohol **10***

Aminoalcohol **10** (800 mg, 1.98 mmol) was added to 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

2β-*N*-[2-(2-Pyridyl)ethyl]amino-5α-cholestane-3α-ol **36**: 402 mg (40%), white solid. M.p. 101–105°C (CH_3OH); $[\alpha]_D^{20} = +35$ ($c = 0.68$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.61$ (s, 3H, 18-H_3), 0.84 (d, 6H, 26-H_3 and 27-H_3), 0.86 (s, 3H, 19-H_3), 0.89 (d, 3H, $^3J = 6.4$ Hz, 21-H_3), 2.73 (d, 1H, $^3J = 5.2$ Hz, 2α-H), 2.93–3.01 (m, 4H, CH_2CH_2Py), 3.75 (m, 1H, 3β-H), 7.12 (d and t, 2H, 3-H_{py} and 5-H_{py}), 7.57 (t, $^3J = 7.7$ Hz, 1H, 4-H_{py}), 8.50 (d, $^3J = 4.0$ Hz, 1H, 2-H_{py}). HRMS *m*/*z* 507.4316 (M^+–1), calcd: 507.4311 for $C_{34}H_{55}N_2O$.

*4.13.10. Addition of 3α-amine **11***

Compound **11** (718 mg, 1.85 mmol) was reacted with 2-vinyl pyridine as described for the reaction of the aminoalcohol **1**.

3α-*N*-[2-(2-Pyridyl)ethyl]amino-5α-cholestane **38**: 477 mg (52%), white solid. M.p. 115–117°C; $[\alpha]_D^{20} = +19$ ($c = 0.61$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.63$ (s, 3H, 18-H_3), 3.19–3.23 (m, 5H, 3β-H, CH_2CH_2Py), 7.15–7.19 (m, 2H, 3-H_{py} and 5-H_{py}), 7.61 (m, 1H, 4-H_{py}), 8.44 (d, $^3J = 5.6$ Hz, 1H, 2-H_{py}). Bis-picrate (yellow crystals): $C_{46}H_{62}N_8O_{14}$ (951.04) calcd: C, 58.09; H, 6.57; N, 11.78; found: C, 57.55; H, 6.78; N, 11.54

*4.13.11. Addition of 3β-amine **12***

Compound **12** (360 mg, 0.93 mmol) was reacted with 2-vinyl pyridine as described for the reaction of aminoalcohol **1**.

Fraction 1: 3β-*N*,*N*-Bis-[2-(2-pyridyl)ethyl]amino-5α-cholestane **40**, 110 mg (20%), colourless oil; $[\alpha]_D^{20} = +15$ ($c = 0.94$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.61$ (s, 3H, 18-H_3), 0.82 (s, 3H, 19-H_3), 0.86 (d, 6H, 26- and 27-H_3), 0.90 (d, 3H, 21-H_3), 2.56 (m, 1H, 3α-H), 2.89 (m, 6H, 2×CH_2CH_2Py), 7.06 (m, 4H, 2×3-H_{py} and 5-H_{py}), 7.53 (m, 2H, 2×4-H_{py}), 8.48 (m, 2H, 2-H_{py}). Tris-picrate (yellow crystals): $C_{59}H_{72}N_{12}O_{21}$ (1285.29) calcd: C, 55.13; H, 5.65; N, 13.08; found: C, 54.72; H, 5.83; N, 12.44.

Fraction 2: 3β-*N*-Bis-[2-(2-pyridyl)ethyl]amino-5α-cholestane **39**: 200 mg (44%), colourless oil; $[\alpha]_D^{20} = +20$ ($c = 0.96$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.61$ (s, 3H, 18-H_3), 2.46 (m, 1H, 3α-H), 2.94–3.03 (m, 4H, CH_2CH_2Py), 7.07–7.16 (m, 2H, 3-H_{py} and 5-H_{py}), 7.56 (m, 1H, 4-H_{py}), 8.50 (d, 1H, 2-H_{py}). Bis-picrate (yellow crystals): $C_{46}H_{62}N_8O_{14}$ (951.04) calcd: C, 58.09; H, 6.57; N, 11.78; found: C, 57.83; H, 6.71; N, 11.58.

4.14. Acylation procedure with 2-pyridylacetic acid and borane reduction procedure

*4.14.1. 16β-(2-Pyridylacet)amido-3-methoxy-estra-1,3,5(10)-triene-17β-ol **42***

A solution of aminoalcohol **3** (903 mg, 3.0 mmol) in abs. $CHCl_3$ (9 mL) was added to a stirred mixture of 2-pyridylacetic acid hydrochloride (2.08 g, 12 mmol), abs. $CHCl_3$ (15 mL), triethylamine (1.22 g, 12 mmol) and *N*,*N*′-carbonyldiimidazole (1.94 g, 12 mmol). After 18 h at room temperature water was added and the mixture was stirred for 2 h. The organic phase was separated, washed three times with water, dried and evaporated. Crystallization with ethyl acetate gave 650 mg of **42** as white crystals and 450 mg of **42** after column chromatography on silica gel with ethyl acetate and ethyl acetate:CH_3OH (95:5 to 50:50). Yield of **42**: 1.10 g (79%), white crystals (crystals with 0.5 mol ethyl acetate). M.p. 80–2°C, 98–102°C (solidification), 180–185°C; $[\alpha]_D^{20} = +38$ ($c = 1.0$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.73$ (s, 3H, 18H_3), 2.82 (m, 2H, 6-H_2), 3.72–3,75 (m+s, 6H, 17α-H, $COCH_2Py$ and OCH_3), 4.29 (m, 1H, 16α-H), 6.60–6.70 (s+d, 2H, 4-H and 2-H), 7.17–7.26

(m, 2H, 1-H and 3-H_{py} or 5-H_{py}), 7.48 (d, 1H, 3-H_{py} or 5-H_{py}), 7.64 (t, 1H, 4-H_{py}), 8.51 (m, 1H, 2-H_{py}). $C_{26}H_{32}N_2O_3$+$\frac{1}{2}$$CH_3COOC_2H_5$ (464.60) calcd: C, 72.38; H, 7.81; N, 6.01; found: C, 72.58; H, 7.56; N, 6.41. HRMS 420.2380 calcd: 420.2357 for $C_{26}H_{32}N_2O_3$.

*4.14.2. 16β-*N*-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17β-ol **29***

To a stirred solution of borane in THF (1 M, 10 mL, Fluka) a solution of steroid amide **42** (335 mg, 0.72 mmol) in abs. THF (6 mL) was slowly added (45 min). After 3 h at room temperature the solution was heated to 60°C for 4 h. Aqueous hydrochloric acid (6N, 10 mL) was then added and the mixture was heated to 60°C for 1 h under stirring. After cooling to room temperature, aqueous KOH was added and the basic mixture was extracted three times with ether. The combined organic phases were washed with H_2O, dried and evaporated under reduced pressure. The oily residue was crystallized with CH_3OH giving 216 mg (74%) as white crystals. M.p. 132–136°C; $[\alpha]_D^{20}=+76$ ($c=1.0$, $CHCl_3$); ^1H NMR ($CDCl_3$): identical with the spectrum of oily **29**, obtained by reaction of **3** with 2-vinyl pyridine (Section 4.13.3).

*4.14.3. 16β-(2-Pyridylacet)amido-3-methoxy-estra-1,3,5(10)-triene-17α-ol **43***

Aminoalcohol **4** (301 mg, 81.0 mmol) was acylated with 2-pyridylacetic acid hydrochloride as described for the aminoalcohol **3** (Section 4.14.1). Crystallization with ethyl acetate gave 330 mg of **43** (71%, crystallization with 0.5 mol ethyl acetate) as white crystals. M.p. 98–100°C (crystal transformation), 156–160°C (partially) and 166–168°C; $[\alpha]_D^{20}=+23$ ($c=0.88$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta=0.74$ (s, 3H, 18-H_3), 2.82 (m, 2H, 6-H_2), 3.54 (s, 1H, 17β-H), 3.72 (s, 2H, $COCH_2Py$), 3.75 (s, 3H, OCH_3), 3.82 (m, 1H, 16α-H), 6.61 (s, 1H, 4-H), 6.69 (q, 1H, 2-H), 7.17–7.26 (m, 2H, 1-H and 3-H_{py} or 5-H_{py}), 7.65 (m, 2H, 4-H_{py} and 3-or 5-H_{py}), 8.52 (m, 1H, 2-H_{py}). $C_{26}H_{32}N_2O_3$+$\frac{1}{2}$$CH_3COOC_2H_5$ (464.60) calcd: C, 72.38; H, 7.81; N, 6.01; found: C, 71.98; H, 7.64; N, 5.99. HRMS *m/z* 420.2385 (M^+) calcd: 420.2357 for $C_{26}H_{32}N_2O_3$.

*4.14.4. 16β-*N*-[2-(2-Pyridy)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17α-ol **30***

Steroid amide **43** (300 mg, 0.65 mmol) was reduced with borane in THF as described for the reduction of **42**. The oily residue was purified by column chromatography at silica gel with ethyl acetate giving impurities and CH_3OH giving the steroid compound **30** (160 mg, 60%) as a colourless oil; $[\alpha]_D^{20}=+56.6$ ($c=0.9$, pyridine); ^1H NMR ($CDCl_3$): identical with the spectrum of **30**, obtained by reaction of **4** with 2-vinyl pyridine (Section 4.13.4).

*4.14.5. 16β-*N,N*-Bis-[2-(2-pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene-17β-ol **49***

A solution of **29** (307 mg, 0.75 mmol) in abs. $CHCl_3$ (5 mL) was added to a stirred mixture of 2-pyridylacetic acid hydrochloride (520 mg, 3 mmol), abs. $CHCl_3$ (5 mL), triethylamine (304 mg, 3 mmol) and *N*,*N*′-carbonyldiimidazole (486 mg, 3 mmol). After 18 h at room temperature the mixture was worked up as described for the synthesis of **42** (Section 4.14.1). Column chromatography at silica gel with ethyl acetate (yellow nonsteroidal compounds) and ethyl acetate:CH_3OH (95:5 to 50:50) gave 291 mg (74%) of **48** (together with 17β-ester) as a colourless oil; ^1H NMR ($CDCl_3$): $\delta=0.80$ (s, 3H, 18-H_3), 2.76–3.13 (2×m, 6H, 6-H_2, CH_2CH_2Py), 3.72–3.88 (s+m, 6H, OCH_3, $COCH_2Py$, 17α-H), 4.60 (m, 1H, 16α-H), 6.61–6.70 (s+d, 2H, 4-H and 2-H). HRMS *m/z* 507.2860 (M^+-H_2O) calcd: 507.2834 for $C_{33}H_{37}N_3O_2$.

Amide **48** (290 mg, 0.57 mmol) in abs. THF was reacted with borane in THF (1 M, 8 mL) as described for the reduction of **42**. The residue was purified by column chromatography at silica

gel with ethyl acetate giving impurities and ethyl acetate:CH_3OH (95:5 to 50:50) giving compound **49** (100 mg, 43%) as oily product; $[\alpha]_D^{20} = +30.5$ ($c = 0.7$, pyridine); ^1H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 2.82 (m, 2H, 6-H_2), 2.95 and 3.05 (2×m, 8H, 2×CH_2CH_2Py), 3.30 and 3.41 (m+d, AB, 2H, 16α-H and 17α-H), 3.75 (OCH_3), 6.61–6.70 (s+q, 2H, 4-H and 2-H), 7.06–7.20 (m, 5H, 1-H, 2-3-H_{py} and 5-H_{py}), 7.53–7.60 (m, 2H, 4-H_{py}), 8.51 (2H, 2-H_{py}). HRMS m/z 493.3096 (M^+–H_2O) calcd: 493.3045 for $C_{33}H_{39}N_3O$.

4.15. Reductive amination procedure for 3-methoxy-estra-1,3,5(10)-triene-17-one **13**

4.15.1. 17β-N-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene **45**

Compound **13** (2.60 g, 9.1 mmol), 2-(2-pyridyl)ethylamine (8 mL, 7.79 g, 63.8 mmol) and trimethyl orthoformat (25 mL) were heated at 80°C for 20 h under stirring. After cooling and addition of abs. CH_3OH (120 mL) $NaBH_4$ (2.5 g, 66 mmol) were added at 10°C. After 30 min CH_3OH was removed under reduced pressure, $CHCl_3$ was added and the mixture was washed with aqueous NaCl-solution and water. The organic phase was dried and evaporated under reduced pressure. The residue was dissolved in abs. ether, filtered and treated with gaseous HCl at 10°C. The solid amine hydrochloride was isolated by filtration, washed with ether and recrystallized from C_2H_5OH (2.64 g). Methanolic solution (ca. 200 mL) of the hydrochloride was treated with aqueous KOH (2 g KOH, 20 mL H_2O) under cooling and stirring. After 1 h at room temperature ice/H_2O was added and the precipitate isolated by filtration, washed with water and dried in vacuum. Compound **45** (2.1 g, 59%): white solid, m.p. 124–126°C. A sample was recrystallized from isopropanol; m.p. 126–127°C; $[\alpha]_D^{20} = +70$ ($c = 1.1$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.67$ (s, 3H, 18-H_3), 2.65 (t, 1H, 17α-H), 2.81 (m, 2H, 6-H_2), 2.94–3.04 (m, 4H, CH_2CH_2Py), 3.75 (s, 3H, OCH_3), 6.60–6.70 (s and d, 4-H and 2-H), 7.07–7.18 (m, 3H, 1-H, 3-H_{py} and 5-H_{py}), 7.57 (m, 1H, 4-H_{py}), 8.51 (m, 1H, 2-H_{py}); ^{13}C NMR ($CDCl_3$): $\delta = 11.70$ (18-C), 55.17 (19-C), 69.11 (17-C), 111.40 (2-C), 113.76 (4-C), 121.15 (3-C_{py}), 123.26 (5-C_{py}), 126.26 (1-C), 132.82 (10-C), 136.26 (4-C_{py}), 137.99 (5-C), 149.28 (2-C_{py}), 157.38 (3-C), 160.46 (6-C_{py}). $C_{26}H_{34}N_2O$ (390.57) calcd: C, 79.96; H, 8.77; N, 7.17; found: C,79.63; H, 8.93; N, 7.03.

(*E*)-17-*N*-[2-(2-Pyridyl)ethyl]imino-3-methoxy-estra-1,3,5(10)-triene **44**: The imine **44** can be isolated before the reduction procedure by cooling the reaction mixture and filtration of the crystalline imine: 3.29 g (93%). M.p. 158–161°C; $[\alpha]_D^{20} = +66$ ($c = 0.93$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.75$ (s, 3H, 18-H_3), 2.83 (m, 2H, 6-H_2), 3.06–3.66 (2×m, 4H, CH_2CH_2Py), 3.75 (s, 3H, OCH_3), 6.60–6.71 (s, m, 2H, 4-H and 2-H), 7.05–7.21 (m, 3H, 1-H, 3-H_{py} and 5-H_{py}), 7.53 (m, 1H, 4-H_{py}), 8.50 (m, 1H, 2-H_{py}). X-Ray analysis: 17C=N *E*-configuration. $C_{26}H_{32}N_2O$ (388.55) calcd: C, 80.37; H, 8.30; N, 7.21; found: C, 80.14; H, 8.48; N, 7.15.

4.15.2. 17β-N-[3-(1-Imidazolyl)propyl]amino-3-methoxy-estra-1,3,5(10)-triene **51**

Ketone **13** (3.27 g, 11.5 mmol), 1-(3-aminopropyl)imidazol (3.0 mL, 25.1 mmol) and triethyl orthoformate were reacted for 12 h using an ultrasonic apparatus. The clear solution was diluted with abs. ethanol and $NaBH_4$ (1.0 g, 26.4 mmol) was then added under continuous stirring. After 12 h, the ethanol was removed under reduced pressure and ether and water were then added. The organic phase was separated, washed and dried. The etheric solution was treated with gaseous HCl and the hydrochloride isolated by filtration (4.86 g). Aqueous KOH (5.0 g KOH, 30 mL H_2O) was added to a methanolic solution of this hydrochloride (50 mL). The resulting mixture was then poured onto ice/H_2O (250 mL). The precipitate was filtered off, washed with water and dried giving 4.27 g of amine **51** (94%) as a white powder. M.p. 95–97°C; $[\alpha]_D^{20} = +58$ ($c = 0.94$,

$CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.69$ (s, 3H, 18-H_3), 2.53–2.61 (m, 3H, 17α-H and N-CH_2), 2.81 (m, 2H, 6-H_2), 3.75 (s, 3H, OCH_3), 4.03 (m, 2H, CH_2-N), 6.59–6.69 (2×m, 2H, 4-H and 2-H), 6.90 (s, 1H, H_{im}), 7.03 (s, 1H, H_{im}), 7.17 (m, 1H, 1-H), 7.41 (s, 1H, H_{im}). $C_{25}H_{35}N_3O$ (393.57) calcd: C, 76.29; H, 8.96; N, 10.68; found: C, 75.83; H, 8.93; N, 10.59.

*4.15.3. 17β-*N*-[3-(1-Morpholino)propyl]amino-3-methoxy-estra-1,3,5(10)-triene **52***

Compound **13** (2.74 g, 9.6 mmol), 1-(3-aminopropyl)morpholine (2,8 mL, 19.2 mmol) and triethyl orthoformate (10 mL) were reacted as described for the synthesis of **51**. Reduction with $NaBH_4$ (1.0 g, 26.4 mmol) as described (Section 4.15.2) gave the amine **53** (3.65 g, 92%) as a white powder. M.p. 110–112°C; $[\alpha]_D^{20} = +64$ ($c = 0.96$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.71$ (s, 3H, 18-H_3), 2.58 (m, 1H, 17α-H), 2.67 (m, 4H, 2-NCH_2), 2.81 (m, 2H, 6-H_2), 3.68–3.75 (m+s, 7H, 2×OCH_2, OCH_3), 6.59–6.69 (2×m, 4-H and 2-H), 7.18 (m, 1H, 1-H). $C_{26}H_{40}N_2O_2$ (412.61) calcd: C, 75.68; H, 9.77; N, 6.79; found: C, 75.69; H, 10.00; N, 6.66.

*4.15.4. 17β-*N,N*-Bis-[2-(2-Pyridyl)ethyl]amino-3-methoxy-estra-1,3,5(10)-triene **47***

Compound **45** (2.11 g, 5.4 mmol) in abs. $CHCl_3$ (8 mL) was acylated with 2-pyridylacetic acid hydrochloride (1.8 g, 10 mmol), triethylamine (1.1 g, 10 mmol), and *N*,*N*′-carbonyldiimidazole (1.7 g, 10 mmol) in abs. $CHCl_3$ (15 mL) as described for the synthesis of **42** (Section 4.14.1). After 18 h $CHCl_3$ and water were added. The organic phase was separated and washed three times with water, dried and evaporated. The residue was purified by column chromatography at silica gel with ethyl acetate (yellow nonsteroidal compounds) and ethyl acetate:CH_3OH (95:5 to 70:30) giving amide **46** as white foam [2.45 g, 89%, mixture of (*Z*)- and (*E*)-amide]; $[\alpha]_D^{20} = -6$ ($c = 0.9$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.66$ and 0.78 (2×s, 3H, 18-H_3), 2.82 (m, 2H, 6-H_2), 2.8–3.7 (several m, 4H, CH_2CH_2Py), 3.75 (s, 3H, OCH_3), 3.90–4.15 (m, 2.6H, $COCH_2Py$ and 17α-H partially), 4.62 (m, 0.4H, 17α-H partially), 6.59–6.70 (m, 2H, 4-H and 2-H), 7.04–7.20 (m, 4H, 1-H and 3×H_{py}), 7.52 (m, 1H, H_{py}), 7.60–7.66 (m, 2H, 2×H_{py}), 8.50 (m, 2H, 2×2-H_{py}). HRMS *m*/*z* 509.30380 (M^+), calcd: 509.30376 for $C_{33}H_{39}N_3O_2$.

The amide **46** (509 mg, 1 mmol), dissolved in abs. THF (8 mL), was slowly added to a borane solution in THF (1 M, 10 mL). After 3 h at room temperature the solution was heated to 60°C for 7 h. The work-up procedure as described for the reduction of **42** gave a yellow oily product (466 mg), which was purified by column chromatography at silica gel with ethyl acetate (nonsteroidal compounds) and CH_3OH giving the steroid compound **47** as a colourless oil (345 mg, 70%); $[\alpha]_D^{20} = +85$ ($c = 0.2$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.68$ (s, 3H, 18-H_3), 2.71 (t, 1H, 17α-H), 2.81 (m, 2H, 6-H_2), 2.91–3.07 (m, 8H, 2×CH_2CH_2Py), 3.75 (s, 3H, OCH_3), 6.59–6.96 (d+q, 2H, 4-H and 2-H), 7.07–7.17 (m, 5H, 2×3-H_{py} and 5-H_{py}, 1-H), 7.56 (m, 2H, 2×4-H_{py}), 8.50 (2×2-H_{py}). $C_{33}H_{41}N_3O$ (495.70) calcd: C, 79.96; H, 8.34; N, 8.48; found: C, 79.14; H, 8.54; N, 8.03.

*4.16. Procedures for the synthesis of mixed ligands **50** and **53***

*4.16.1. Michael reaction of **34** and methyl acrylate to **50***

N-Pyridylethylamino compound **34** (100 mg, 0.197 mmol), methyl acrylate (20 mg, 0.232 mmol) and abs. ethanol (10 mL) were heated under reflex for 20 h. After evaporation under reduced pressure the residue was purified by MPLC.

2α-*N*-(2-Methoxycarbonylethyl)-*N*-[2-(2-pyridyl)ethyl]amino-5α-cholestane-3β-ol **50**, 96 mg (85%), colourless oil; $[\alpha]_D^{20} = +28$ ($c = 0.67$, $CHCl_3$). UV (CH_3OH): λ_{max} (lg ε) = 261 (3.6180); ^1H NMR ($CDCl_3$): $\delta = 0.62$ (s, 3H, 18-H_3), 0.79 (s, 3H, 19-H_3), 0.84 (d, 6H, 26-H_3 and 27-H_3), 0.88

(d, 3H, $^3J = 6.6$ Hz, 21-H_3), 2.18 (m, 2H, CH_2CH_2), 2.50 (m, 2H, 2β-H and CH_2CH_2), 2.84 (m, 5H, CH_2CH_2), 3.32 (m, 1H, 3α-H), 3.57 (s, 3H, OCH_3), 7.10 (m, 2H, 3-H_{py} and 5-H_{py}), 7.55 (t, 1H, $^3J = 7.7$ Hz, $^4J = 1.7$ Hz, 4-H_{py}), 8.50 (d, 1H, $^3J = 4.0$ Hz, 2-H_{py}). HRMS *m/z* 576.4520 (M^+–H_2O), calcd: 576.4538 for $C_{38}H_{60}N_2O_2$.

*4.16.2. 17β-*N*-[2-(2-Pyridyl)ethyl]-*N*-[3-(1-imidazolyl)propyl]amino-3-methoxy-estra-1,3,5(10)-triene **53***

Steroid **51** (1.97 g, 5.0 mmol) in abs. $CHCl_3$ (3 mL) was acetylated with 2-pyridylacetic acid hydrochloride (1.11 g, 6.4 mmol), triethylamine (1.7 mL, 12.2 mmol) and *N*,*N*′-carbonyldiimidazol (1.04 g, 6.4 mmol) as described for the synthesis of **47**. Purification was accomplished with column chromatography on silica gel with ethyl acetate (600 mL, solution of nonsteroidal compounds) and ethyl acetate:CH_3OH (80:20 to 50:50) giving 1.87 g (73%) of 17β-pyridylacetamide as a colourless oil (^1H NMR: $\delta = 0.55$ and 0.68, 3H, 18-H_3, *Z*/*E*-mixture); 568 mg (1.1 mmol) were directly reduced with borane in THF as described for the synthesis of **47** (Section 4.15.4) giving 413 mg (75%) of **53** as a colourless oil.; $[\alpha]_D^{20} = +53$ ($c = 0.52$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.71$ (s, 3H, 18-H_3), 2.59–2.65 (m, 3H, NCH_2 and 17α-H), 2.81–2.99 (m, 6H, 6-H_2 and CH_2CH_2Py), 3.75 (s, 3H, OCH_3), 3.94 (m, 2H, CH_2N), 6.59–6.70 (2×m, 2H, 4-H and 2-H), 6.90 (s, 1H, H_{im}), 7.04–7.18 (m, 4H, 1-H, 3-H_{py}, 5-H_{py}, H_{im}), 7.45 (s, 1H, H_{im}), 7.56 (m, 1H, 4-H_{py}), 8.50 (d, 1H, 2-H_{py}). Tris-picrate: $C_{50}H_{51}N_{13}O_{22}$ (1186.03) calcd: C, 50.64; H, 4.33; N, 15.35; found: C, 51.20; H, 4.69; N, 14.13. HRMS (ESI in CH_3OH) *m/z* (M+1): 499.3443; calcd: 499.3437 for $C_{32}H_{49}N_4O$.

Financial support from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 436) is gratefully acknowledged. We would also like to thank the Schering AG for their gift of steroidal compounds.

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