Intramolecular γ-Hydroxylations of Nonactivated C–H Bonds with Copper Complexes and Molecular Oxygen

Bruno Schönecker,*[a] Tatjana Zheldakova,[b] Corinna Lange,[a] Wolfgang Günther,[a] Helmar Görls,[c] and Martin Bohl[d]

Abstract: Copper(I) complexes incorporating the isomeric bidentate ligands IMPY (iminomethyl-2-pyridines) or AMPY (aminomethylene-2-pyridines) are quite unusual in their ability to bind and activate molecular oxygen. Using these complexes, hydroxylations of nonactivated CH, CH_2, or CH_3 groups in the γ-position in relation to the imino-nitrogen atom, and with a specific orientation of one H atom with respect to the binuclear Cu–O species, can be achieved in synthetically useful yields. Through mechanistic studies employing conformationally well-defined molecules (for example, cyclic isoprenoids), coupled with solid-state X-ray structure analyses and force-field calculations, we postulate a seven-membered transition state for this reaction in which six atoms lie approximately in a plane. This plane is defined by the positions of the lone pairs on the nitrogen atoms, as well as the copper and the oxygen atoms. For a successful hydroxylation, one hydrogen atom should be located close to this plane. Prediction of the stereochemical course of these reactions is possible based on a simple geometrical criterion. The convenient introduction of IMPY and AMPY groups as auxiliaries into oxo and primary amino compounds and the simple hydrolysis after the hydroxylation procedure has allowed the synthesis of 3-hydroxy-1-oxo and 3-hydroxy-1-amino compounds. If desired, the 3-hydroxy-1-IMPY and -1-AMPY compounds can be reduced with $NaBH_4$ to obtain 3-hydroxy-1-aminomethylpyridines. For a successful hydroxylation procedure, the method employed for the synthesis of the Cu^I complexes is very important. Starting either from Cu^I salts or from Cu^{II} salts with a subsequent reduction with benzoin/triethylamine may turn out to be the better way, depending on the ligand and the molecular structure.

Keywords: C–H activation · copper · hydroxylation · O–O activation · steroids

Introduction

The ability of copper-containing enzymes to regio- and stereoselectively hydroxylate substrates with molecular oxygen[1] has inspired bioinorganic and bioorganic chemists to investigate such reactions employing simple biomimetic copper complexes. Quite some success has been achieved in the past in activating molecular oxygen,[2] the investigation of oxidizing species, and in the hydroxylation of aromatic[3] and benzylic[4] C–H bonds of ligands. It seems to be much more difficult to hydroxylate nonactivated C–H bonds. The work of Thompson (*N*,*N*,*N*′,*N*′-tetraethyl ethylenediamine[5]) and Reglier and co-workers {*N*,*N*-bis[2-(2-pyridyl)ethyl]aminopropane[6] and -cyclopentane[6]}, our own work {17β-*N*-[2-(2-pyridyl)ethyl]amino-, 17β-(2-pyridylmethyl)amino steroids,[7] and a 17a-aza-*N*-[2-(2-pyridyl)ethyl]amino steroid[8]}, and work by Masuda et al. [*cis*,*cis*-1,3,5-tris(isobutylamino)-cyclohexane[9]] is summarized in Figure 1.

Using conformationally more restricted bidentate ligands incorporating an iminomethyl- or iminoethyl-2-pyridine moiety at the 17-position of a steroid molecule, we have observed for the first time a regio- and stereospecific γ-hydroxylation of a nonactivated CH_2 group.

12β-Hydroxylated steroids could be obtained in practically useful yields of 40 to 50%. After the hydroxylation procedure (Scheme 1), hydrolysis or reduction of the imino bond was possible, giving the 12β-hydroxy-17-ketone or 12β-hy-

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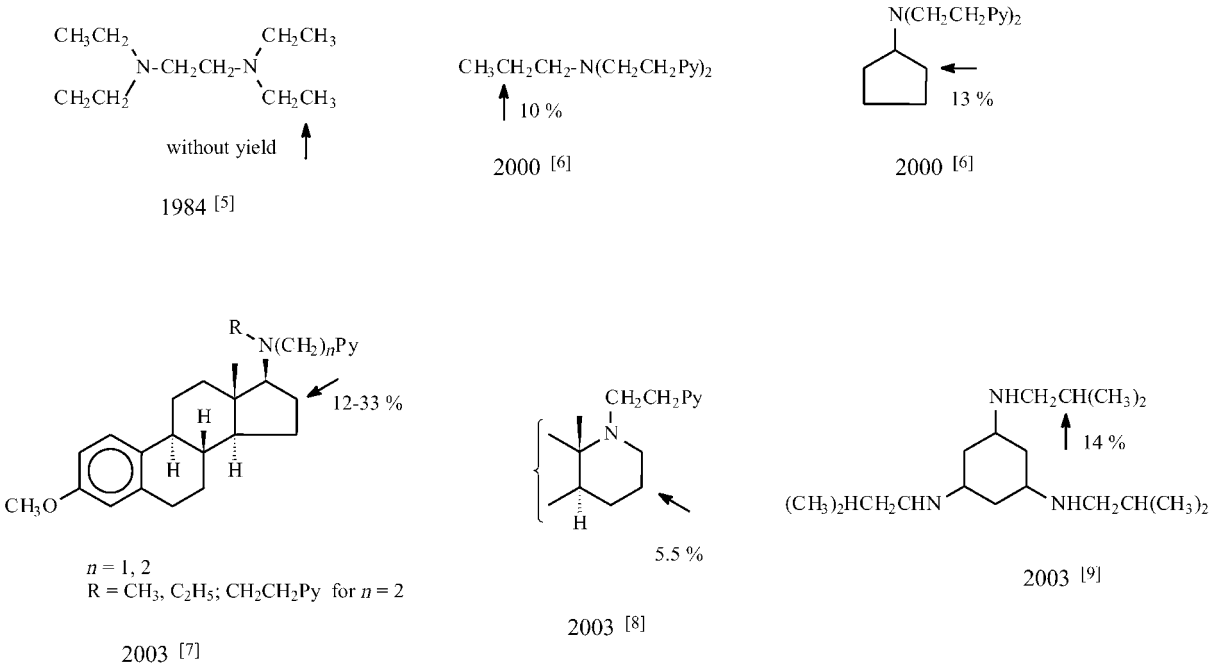
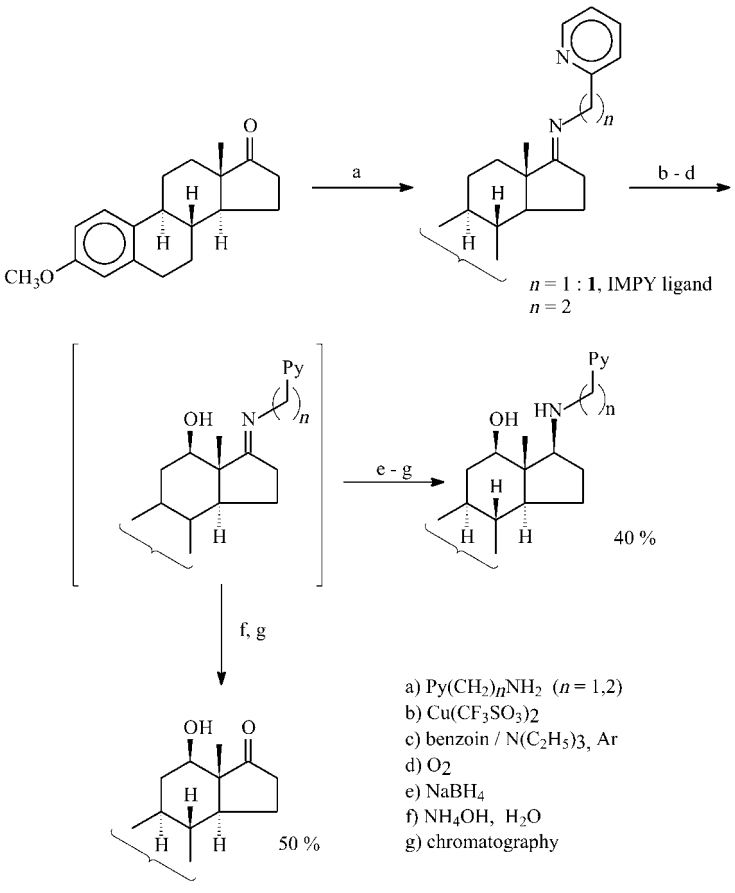


Figure 1. β-Hydroxylation of nonactivated C–H bonds.

Abstract in German: *Kupfer(I)komplexe von zwei Typen isomerer zweizähniger Liganden (IMPY: Iminomethyl-2-pyridine, AMPY: Aminomethylen-2-pyridine) binden und aktivieren molekularen Sauerstoff. Nichtaktivierte CH-, CH_2- oder CH_3-Gruppen in γ-Stellung zum Iminstickstoff, deren H-Atome in die Ebene der Zweikern-Cu-O-Species gelangen können, werden in praktisch verwertbaren Ausbeuten hydroxyliert. An Beispielen konformativ gut definierter Moleküle (cyclische Isoprenoide), Röntgenkristallstrukturanalysen und Kraftfeldberechnungen kann ein siebengliedriger Übergangszustand mit sechs Atomen in nahezu einer Ebene vorgeschlagen werden. Die Ebene ist determiniert durch die freien Elektronenpaare der N-Atome und die Kupfer- und Sauerstoffatome. Vorraussetzung für eine erfolgreiche Hydroxylierung ist, daß ein H-Atom in diese Ebene gelangt. Für eine Voraussage des stereochemischen Verlaufs wird eine einfache geometrische Maßzahl verwendet. Durch die einfache Einführung der IMPY- oder AMPY-Gruppe als Auxiliare in Oxo- bzw. primäre Amino-Verbindungen und die leichte Hydrolyse nach der Hydroxylierung sind auf kurzem Wege und bequem 3-Hydroxy-1-oxo- und 3-Hydroxy-1-amino-Verbindungen zugänglich. Falls gewünscht können jedoch auch die als Primärprodukte entstehenden 3-Hydroxy-1-IMPY- und 3-Hydroxy-1-AMPY-Verbindungen mit $NaBH_4$ zu 3-Hydroxy-1-aminomethyl-pyridinen reduziert werden. Für eine erfolgreiche Hydroxylierung ist die Methode zur Herstellung der Cu^I-Komplexe sehr bedeutsam. In Abhängigkeit von Liganden- und Molekülstruktur ist entweder der Start mit Cu^I-Salzen oder der mit Cu^{II}-Salzen und nachfolgender Reduktion mit Benzoin/Triethylamin der bessere Weg für die folgende Hydroxylierung mit molekularem Sauerstoff.*



Scheme 1. γ-Hydroxylation of a nonactivated CH_2 group.

droxy-17β-*sec*-amine, respectively.[7] For somewhat higher yields, the iminomethylpyridine group (IMPY ligands) proved preferable.

We have presented an initial stereochemical model for H-abstraction from the dinuclear copper–oxygen complex of **1**, which consists of a seven-membered ring with six atoms lying nearly in a plane (Figure 2).

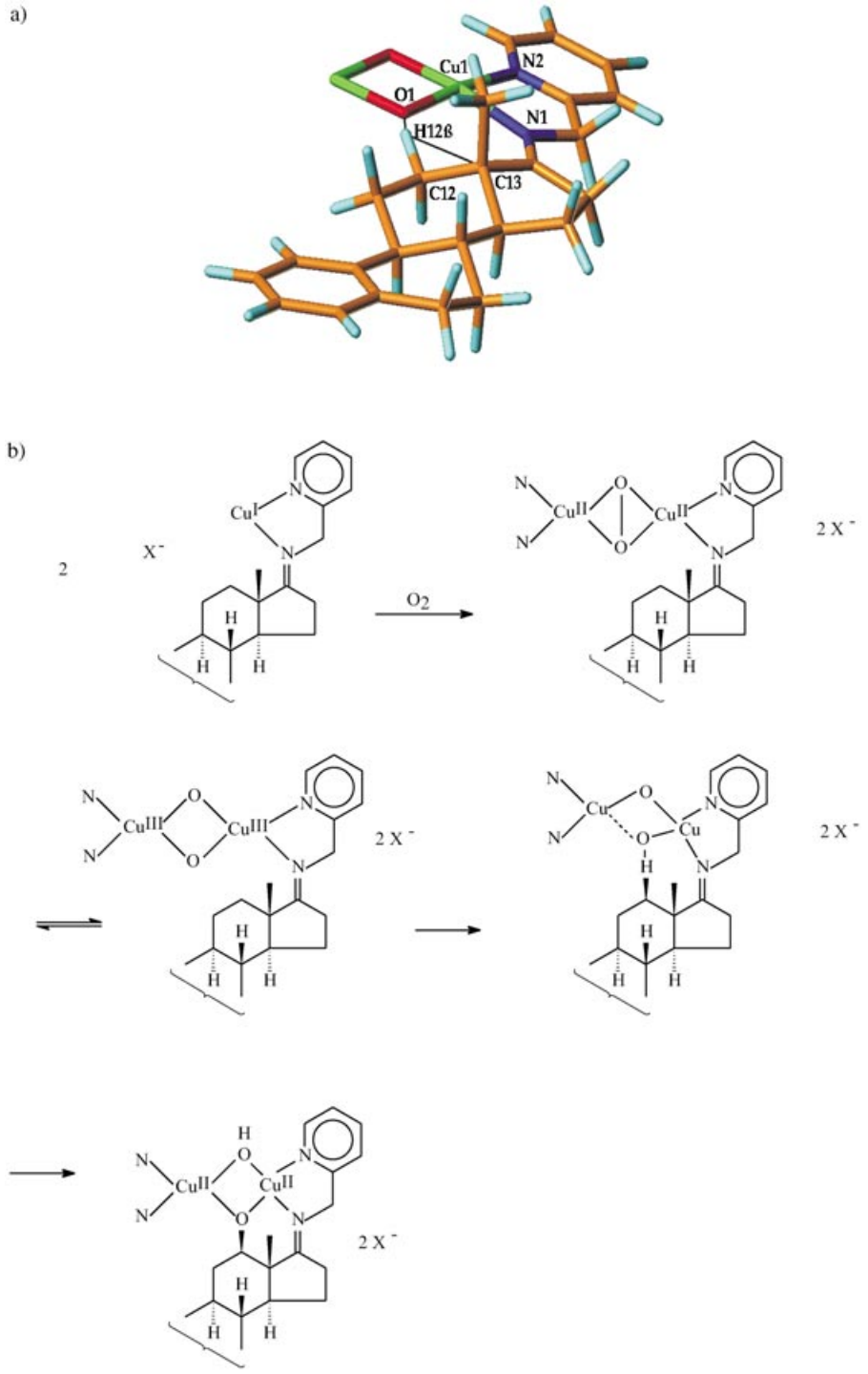


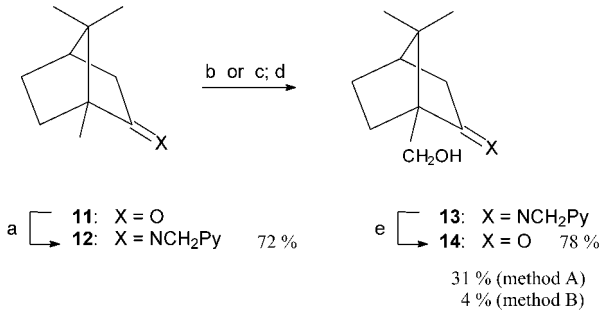
Figure 2. a) Model for the copper–oxygen complex of the 13β-configured IMPY steroid **1** in a conformation forming a seven-membered ring (six atoms N1, Cu1, O1, 12β-H, C13, C17 in a plane; C12 out of this plane) and b) possible mechanism for γ-hydroxylation.

We assume that the orientation of the H atom in this plane is a requirement for a successful hydroxylation procedure. To support this hypothesis, we now report reactions with other IMPY derivatives. To find further suitable ligands, we have investigated isomeric ligands containing an aminomethylene pyridine structure (AMPY ligands) in which the C=N double bond is conjugated with the pyridine ring.[10,11] Finally, we have calculated the conformation of the ligands in the lowest energy state and the conformation of the IMPY and AMPY steroids most appropriate to form the copper–oxygen complex. On the basis of these results, we have modeled the copper–oxygen complexes. We suggest a mechanism similar to that described, and recently also supported by calculations, for the β-hydroxylation of benzylic CH_2 groups with 2-[(2-pyridyl)-ethyl]amino ligands.[4f] Initially generated (μ-η^2:η^2-peroxo)dicopper(II) complexes from copper(I) complexes and molecular oxygen are in equilibrium with bis(μ-oxo)dicopper(III) complexes. These should be the species that are able to attack the C–H bond with subsequent creation of a C–O bond (see Figure 2).

Results and Discussion

Hydroxylations with IMPY ligands: Two methods can be successfully applied for the β-hydroxylation of benzylic groups in the β-position to the central nitrogen of 2-[(2-pyridyl)ethyl]amino ligands. Only 50% of the ligand in the dinuclear complex can be hydroxylated when one starts with copper(I) salts for complexation with subsequent addition of molecular oxygen (method A). To achieve quantitative hydroxyllation, Fukuzumi and co-workers started with copper(II) complexes, which were then reduced with benzoin and triethylamine to copper(I) complexes (method B). After addition of molecular oxygen and completion of hydroxylation turnover, an excess of benzoin/triethylamine was used to reduce the copper(II) complex to the copper(I) complex. This could then react with oxygen once more for further hy-

droxylation of the ligand.[4b,c,d] Interestingly enough, Reglier and co-workers were only able to hydroxylate nonactivated CH_2 groups by way of method B.[6] We also successfully employed method B for β- and γ-hydroxylations of nonactivated CH_2 groups of bidentate ligands.[7] Very recently, however, two examples of β-hydroxylation of nonactivated C–H bonds using method A have been described.[8,9] We were able to show that 17-IMPY-3-methoxy-estra-1,3,5(10)-triene (**1**, Scheme 1) can be stereo- and regioselectively hydroxylated in the 12β-position (γ-hydroxylation) in nearly 50% yield using method B.[7] To investigate the stereochemical implications of this interesting reaction employing IMPY ligands, we describe herein two new 17-IMPY steroidal ligands **3** and **6** (Scheme 2) and the IMPY derivative of (*R*)-camphor **12** (Scheme 3).

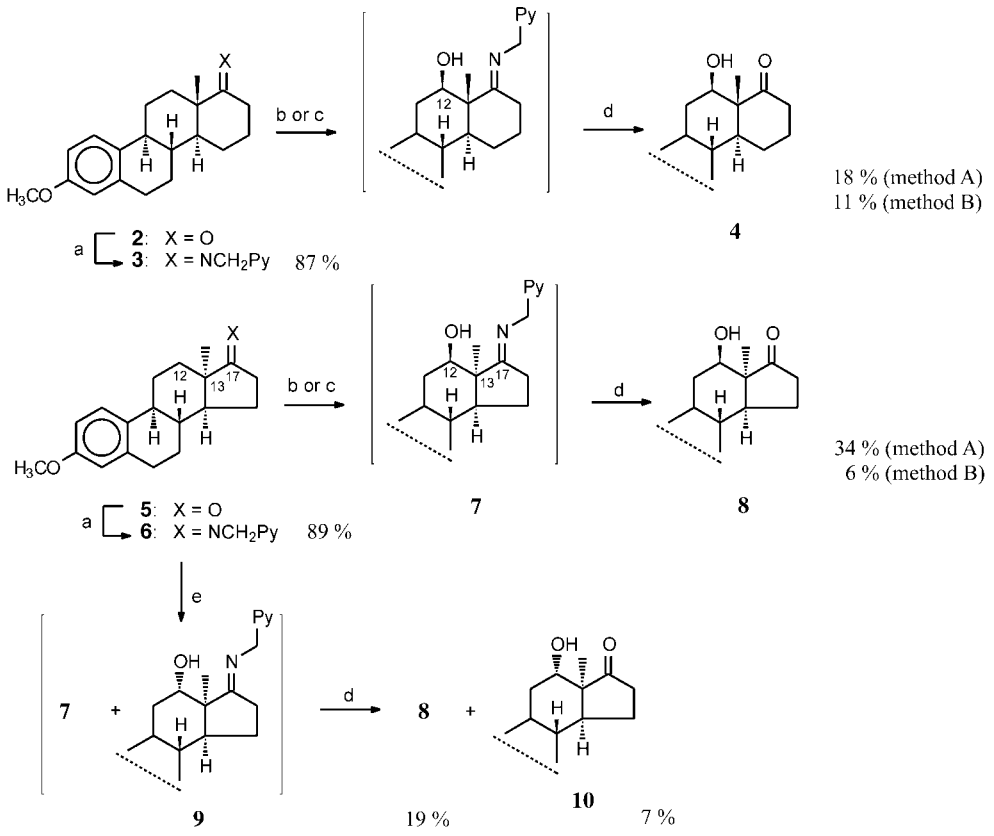


Scheme 3. Hydroxylation of IMPY-(1*R*)-camphor. a) $PyCH_2NH_2$, refluxing xylene, *p*-toluenesulfonic acid; b) 1. $Cu(CH_3CN)_4PF_6$, acetone, Ar; 2. O_2; c) 1. $Cu(CF_3SO_3)_2$, acetone; 2. benzoin, $N(C_2H_5)_3$, Ar; 3. O_2; d) 1. NH_4OH, H_2O; 2. chromatography on silica gel; e) 1. acetic acid, MeOH, 90°C; 2. chromatography on silica gel.

Hydroxylation of the D-homo-17a-IMPY steroid 3: Compounds **2** and **3** (Scheme 2) have a *trans*-perhydro-naphthalene system instead of the *trans*-perhydro-indane system of the IMPY compound **1** (Scheme 1); **3** is quite sensitive to hydrolysis.

As expected, a comparison of the X-ray analyses of both IMPY ligands **1** (Scheme 1) and **3** (Scheme 2) reveals only small geometrical differences.

For comparison between **1** and **3**, the results of a conformational analysis (calculated with the MMFF94 force field) for a model of the 13β-17-IMPY-estratriene steroid **1** are given in Figure 3. Two separated energy minima with nearly the same energy (within 0.01 kcal mol^{-1}) are calculated (blue and orange in Figure 3). The conformation found in the crystal[7] (red in Figure 4) is located in a favorable energy region but was computed to be 1.6 kcal mol^{-1} higher in energy. The energy maps and comparison of the different low-energy conformations indicate that the 17-side chain is flexible enough to adopt quite different orientations with respect to the steroid skeleton. Because of the rigidity imposed by the C17=N1 double bond, there is only one conformation (shown in Figure 2) in which the nitrogen lone pairs are suitably predisposed to bind to the copper ion. The rela-



Scheme 2. γ-Hydroxylation with IMPY ligands of the estra-1,3,5(10)-triene series. a) $PyCH_2NH_2$, refluxing toluene, *p*-toluenesulfonic acid; b) 1. $Cu(CH_3CN)_4PF_6$, acetone, Ar; 2. O_2; c) 1. $Cu(CF_3SO_3)_2$, acetone; 2. benzoin, $N(C_2H_5)_3$, Ar; 3. O_2; d) 1. NH_4OH, H_2O; 2. acetic acid, MeOH, 90°C; 3. chromatography on silica gel; e) 1. $Cu(CF_3SO_3)(CH_3C_6H_5)$, acetone, Ar; 2. O_2.

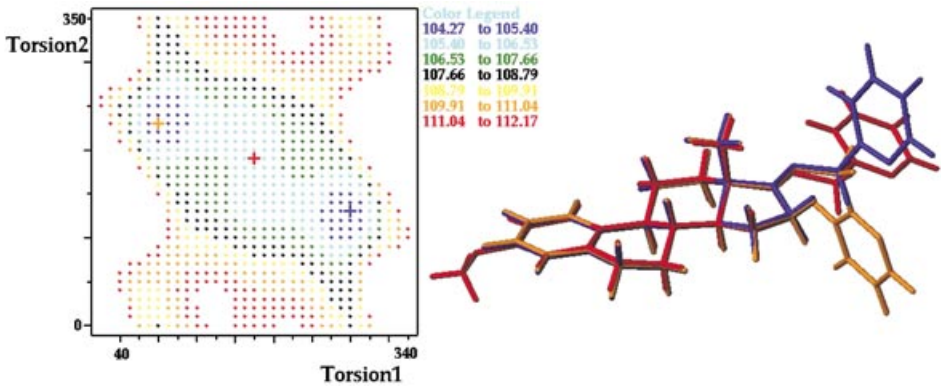


Figure 3. Conformational space of the IMPY steroid model **1** (torsional angle 1: C17=N-C-C_{Py}; angle 2: N-C-C_{Py}-N_{Py}) with color-coded MMFF94 energy in kcal mol^{-1} (left) and superposition (right) of crystal structure[7] (red structure related to red cross left) and two calculated energy-minimum structures (blue and orange structure corresponding to blue and orange cross, respectively).

tive conformational energy required for this steroid (without the four-membered Cu–O ring) to adopt the conformation displayed in Figure 2 is 7.6 kcal mol^{-1}. It is evident that only the 12β-H can be abstracted from the O1 oxygen atom in this conformation. The O1–12βH distance is 0.67 Å. The least-squares plane through the following six atoms has a root-mean-square deviation of 0.09 Å and the following individual displacements (in Å) above/below the plane: N1 (0.06), Cu1 (0.09), O1 (0.02), 12β-H (0.12), C13 (0.13), C17 (0.05). Note that the C12 carbon atom is displaced from this plane by 0.59 Å.

The theoretical results described above and the structural similarity between D-homo compound **3** and compound **1** would lead us to also expect hydroxylation in the 12β-position for the D-homo steroid **3**. We employed method B with acetone as the solvent. After hydroxylation, the reaction mixture was decomplexed and hydrolyzed with aqueous ammonia. We succeeded in isolating 54% of the ketone **2** and only 11% of the expected 12β-hydroxy-17a-ketone **4**. Using method A, 39% of **2** and 18% of **4** were obtained. Despite the possibility of hydrolysis of **3** during the hydroxylation procedure, the higher yield with method A is remarkable and is in contrast to the hydroxylation of **1** (method B: 50%, method A: 29%). These results show a considerable sensitivity to small structural changes.

Hydroxylation of 13α-steroid 6: Another interesting example is the 13α-17-ketone **5** (Scheme 2) which possesses, in contrast to the natural steroids, a non-natural C/D-*cis* hydrindane system. Such non-natural steroids are also interesting because of their conformation and biological activity.[13]

Since 13α-steroids are known to adopt two different C-ring conformations (chair and twist-boat), both of these conformations have been constructed and relaxed by short molecular dynamics simulations and subsequent energy minimizations of representative structures. A model for the C-ring chair steroid **6** with the 17-IMPY side chain was found to be 2.8 kcal mol^{-1} lower in energy than the twist-boat form. This comparison was performed without the Cu–O four-membered ring, but was constrained to conformations of the IMPY group able to bind the copper ion (illustrated in Figure 4 and see Figure 6).

For the energetically favored C-ring chair steroid, the O1 oxygen is located close to the 12β-hydrogen (distance 0.67 Å) and lies near to the least-squares plane formed by the following six atoms, with the displacements from this plane in Å given in parentheses: N1 (0.05), Cu1 (0.09), O1 (0.15), 12β-H (0.31), C13 (0.16), C17 (0.05). The distance from this plane to C12 is 0.54 Å.

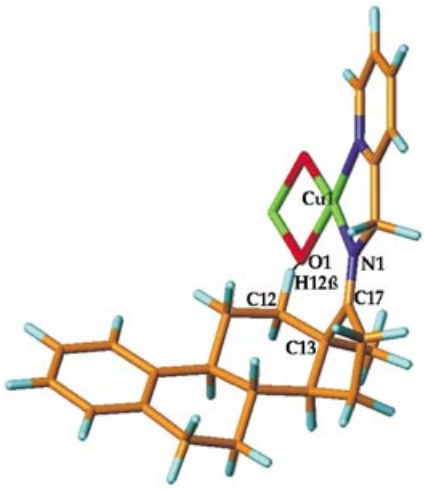


Figure 4. Model for the copper–oxygen complex of the 13α-configured IMPY steroid **6** adopting a C-ring chair conformation (approximate plane through the atoms N1, Cu1, O1, 12β-H, C13, C17; C12 out of plane).

Although the C/D ring junction is different, the orientation of the IMPY group with respect to the equatorial 12β-H is similar to that in the normal 13β-steroid (see Figure 2 and Figure 4). Thus, the 13α-steroid can also be expected to undergo a successful 12β-hydroxylation.

After reaction of the IMPY compound **6** according to method B, followed by decomplexation and hydrolysis, we succeeded in isolating (in addition to the 13α-17-ketone **5**) only a small amount of a hydroxylated product (6%). Using method A, the yield of this product could again be raised to 34%. The structure of this product as the 12β-hydroxy compound **8** was determined by spectroscopic methods and by an X-ray structural analysis (Figure 5).

In one experiment, we investigated the influence of the anion of the copper(I) salt on the hydroxylation process. Reacting **6** with copper(I) triflate instead of the PF_6 salt, we only isolated 19% of the expected 12β-hydroxylated compound **8**. We also obtained a more polar hydroxylation product (7%). The structure of this product was determined by

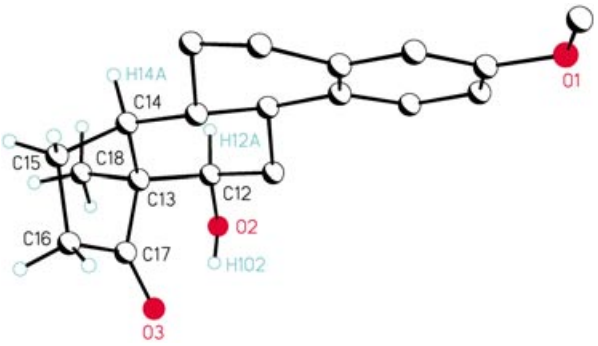


Figure 5. X-ray crystal structure of the 13α-configured steroid **8**.

detailed ^{1}H and ^{13}C NMR spectroscopic analysis to be the epimeric 12α-hydroxy-17-ketone **10**. This rather unexpected result can be rationalized in terms of the two different C-ring conformations[13a] discussed above. Normally, such compounds with an sp^2-hybridized C17 atom possess a C-ring chair conformation (see Figure 4). A 12β-hydroxylation seems to be possible in this conformation. Alternatively, a conformation with a twist-boat C-ring should permit a way for compound **6** to undergo a 12α-hydroxylation (Figure 6).

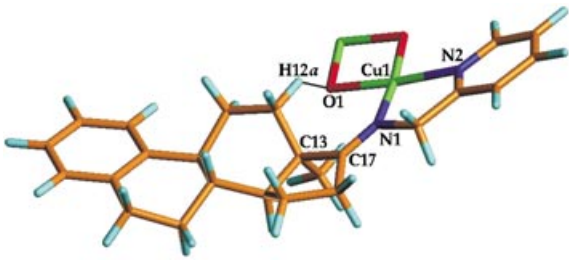


Figure 6. Model for the copper–oxygen complex of the 13α-configured IMPY steroid **6** adopting a C-ring twist-boat conformation and forming a plane through the atoms N1, Cu1, O1, C13, C17.

The model structure in Figure 6 places the O1 oxygen at a distance of 1.23 Å from the 12α-hydrogen. The 12α-H is also displaced by 1.06 Å from the least-squares plane through atoms N1 (0.09), Cu1 (0.12), O1 (0.10), C13 (0.09), and C17 (0.02). Starting from the C-ring twist-boat conformation (Figure 6), the torsion angle C12-C13-C17=N1 was forced by a torsion constraint to adopt distinct values of −20° to −60° in 10° increments. All the remaining internal degrees of freedom in the structure were allowed to relax with the exception of the N1-C-C-N2 planarity. The calculations indicate that the 12α-hydrogen atom can be moved reasonably well into the Cu–O complex plane (distance of 12αH from plane of about 0.2 Å) with an associated energy requirement of approximately 4 kcal mol^{-1}.

If we assume a C-ring chair conformation for the active species with the PF_6^- ion and an equilibrium of chair and twist-boat conformations for the species with the triflate anion, the obtained 12α-hydroxylation results can be understood. The results from this last example clearly underline the importance of the conformation in the hydroxylation process.

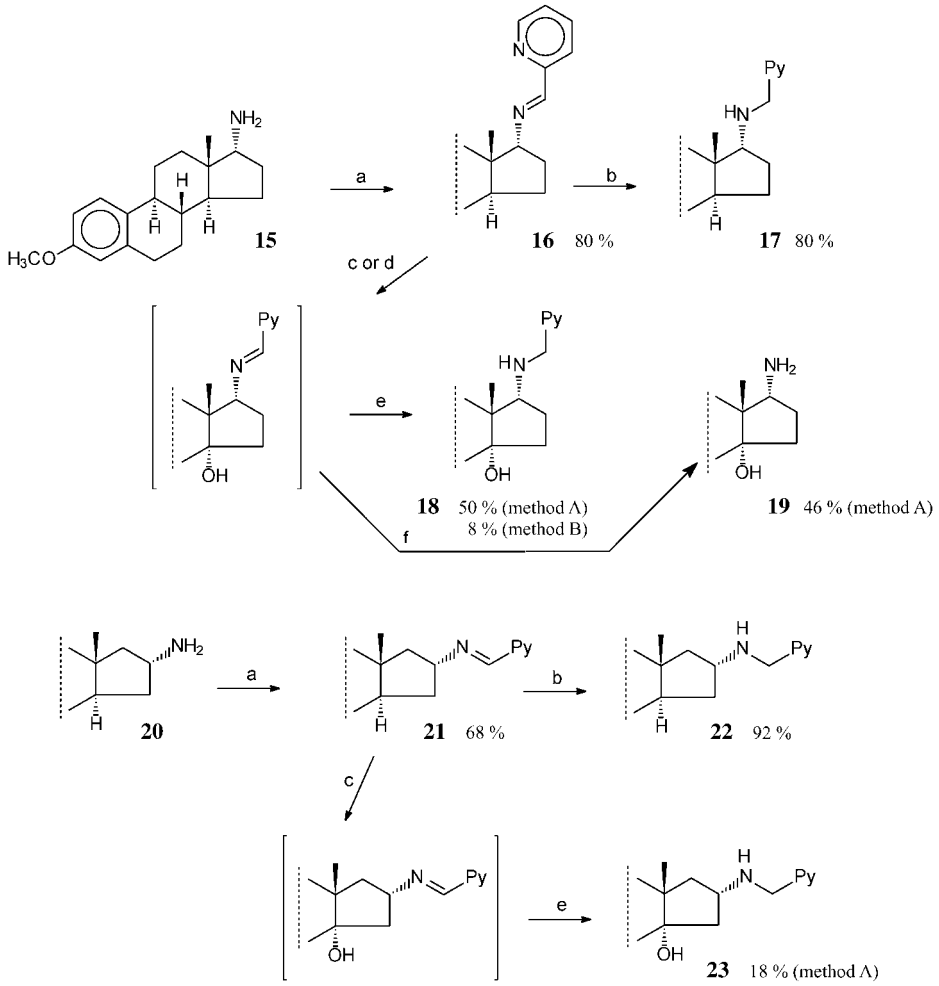
The regio- and stereoselective functionalization of the 12-position in steroid skeletons is not a trivial problem. Related synthetic methods are very scarce, with the application of Breslow's remote oxidation method[14] and the employment of a manganese(III) porphyrin attached through a spacer, whereby a 3α-hydroxyandrostane could be hydroxylated (phenyliodosobenzene as oxidant) in the 12α-position, being one of the few methods having been reported.[15]

Hydroxylation of IMPY-camphor 12: After this successful hydroxylation of nonactivated CH_2 groups, we were interested in uncovering routes to hydroxylate a nonactivated CH_3 group. According to our experience, an IMPY ligand possessing a methyl group in the γ-position with respect to the central nitrogen atom, and with the described six-membered ring plane arrangement of the hydrogen atoms, should be suitable for such a hydroxylation procedure. The camphor molecule should fulfil all such conditions and was thus chosen as a model compound in the form of (1*R*)-camphor **11** (Scheme 3).

The IMPY derivative **12** is a known compound.[16] In contrast to the 17-IMPY steroidal derivative, **12** is stable under chromatographic conditions. Using initially method B, only small amounts (3.7%) of a more polar compound could be detected. In the high-resolution mass spectrum, the *M*+1 peak was consistent with a hydroxylated product. The ^{1}H NMR spectrum showed signals for only two methyl groups, and an additional pair of doublets due to a CH_2 group at $\delta = 3.62$ and 4.01 ppm, indicating that one methyl group had been hydroxylated. Repeating the experiment according to method A, this new compound could be obtained in a yield of 31%. Further detailed NMR experiments confirmed the expected structure **13**, bearing a 10-hydroxy group (Scheme 3). Hydrolysis of the IMPY group of **13** gave the known (1*R*)-10-hydroxy-camphor **14**. The ^{1}H NMR spectrum of **14** was identical to that of the racemic form.[17] Compound **14** is an interesting chiral source,[18] obtained by way of a three-step synthesis starting from (1*R*)-camphor, as has recently been described.[19]

We now possess four IMPY ligands that can be successfully used in hydroxylations. Three of them can be effectively hydroxylated using method A (**3, 6, 12**). Method B is better only in the case of the 17-IMPY-3-methoxy-estra-1,3,5(10)-triene **1** (see Scheme 1). We can only speculate about the reasons for these differences. Possibly, two interdependent oxidizing species, the concentrations of which are method-dependent and, in addition, possess different arrangements relative to the hydrogen atoms that must be abstracted, may be responsible for these results.

Hydroxylations with AMPY ligands: Aminomethylene compounds (AMPY ligands) were chosen for additional experiments because of their similarity to IMPY ligands. In contrast to the IMPY ligands from ring ketones (see Scheme 1), the AMPY ligands obtained from ring amines with pyridine-2-carboxaldehyde possess ring C–N single bonds (see **16** and **21**; Scheme 4). Recently, we were able to show through X-ray analysis that such imines prefer a conformation with a small H_{gem}-C-N=C torsional angle.[20] The stereochemical



Scheme 4. γ-Hydroxylations with 17α- and 16α-AMPY ligands of the estra-1,3,5(10)-triene series. a) Pyridine-2-carbaldehyde, MeOH, 60 °C; b) $NaBH_4$, CH_3OH/THF; c) 1. $Cu(CH_3CN)_4PF_6$, acetone, Ar; 2. O_2; d) 1. $Cu(CF_3SO_3)_2$, acetone; 2. benzoin, $N(C_2H_5)_3$, Ar; 3. O_2; e) 1. $NaBH_4$, CH_3OH; 2. NH_4OH, H_2O; 3. chromatography on silica gel. f) 1. NH_4OH, H_2O; 2. chromatography on silica gel.

outcome of the cyclopropanation reaction of α,β-unsaturated imines also confirmed this conformation in solution.[21]

Hydroxylation of 17α-AMPY compound 16: We started our investigations with the quasi-axial 17α-amine **15**[22] (Scheme 4). The solid-state structure of the corresponding imine **16** exhibits the expected small torsional angle for 17βH-C17-17αN=C (9.8°) and the *E* configuration of the C=N double bond was confirmed.

The results of systematically searching the conformational space of the AMPY structure in steroid **16** by MMFF94 force-field calculations are shown in Figure 7.

The conformation found to have the lowest energy is quite close to the solid-state structure determined experimentally. These two conformations are superimposed on the right-hand-side of Figure 7. Note that both the crystal structure and the calculated minimum-energy structure have two features in common: 1) the lone pairs of the aminomethylenepyridine nitrogens N1 and N2 are oriented in opposite directions (angle 2 being calculated as 180°, and measured as 187° from the crystal structure) and 2) the 17β-hydrogen is almost coplanar with the 17α-AMPY group. A conformational change from the minimum-energy structure (shown in Figure 7) to the appropriate conformation for copper complexation indicated in Figure 8 requires, according to the MMFF94 force field, 8.3 kcal mol^{-1} for the AMPY steroid, without taking into account the energy needed for copper complexation.

In the conformation shown in Figure 8, abstraction of the 14α-H should be possible. The distance between 14α-H and

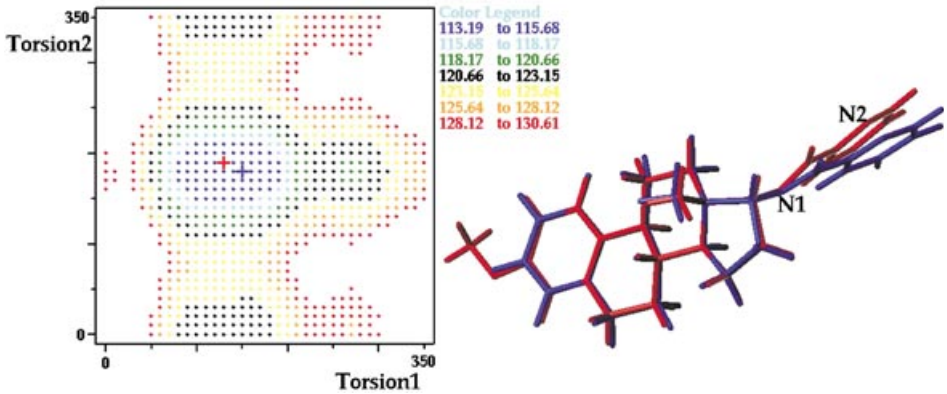


Figure 7. Conformational space of the model for AMPY steroid **16** (torsion angle 1: C13-C17-N=C; angle 2: N=C-C_{Py}-N_{Py}) with color-coded MMFF94 energy in kcal mol^{-1} (left) and superposition (right) of crystal structure (red structure related to red cross left) and calculated energy-minimum structure (blue structure corresponds to blue cross left).

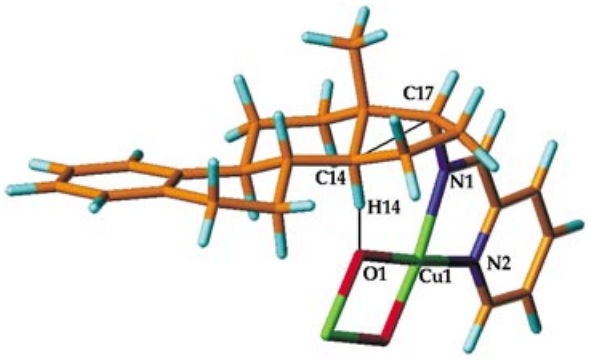


Figure 8. Model for the copper–oxygen complex of the AMPY steroid **16** in a conformation forming a plane through the six atoms N1, Cu1, O1, 14α-H, C14, C17 (C13 out of this plane).

the oxygen atom O1 is 1.16 Å. The least-squares plane through the following six atoms has a root-mean-square deviation of 0.04 Å and the following individual displacements (in Å) above/below the plane: N1 (0.05), Cu1 (0.01), O1 (0.05), 14α-H (0.02), C14 (0.04), C17 (0.07). The carbon C13 is displaced by 0.86 Å from this plane. This model geometry is in excellent agreement with the proposed stereochemical requirement for a successful 14-H abstraction. For the AMPY ligand (Figure 8), the β-C atom (C13) lies out of the six-membered ring plane (for the IMPY ligands the γ-C atom lies out of this plane). For abstraction of the 12α-hydrogen (γ-CH_2 group), a conformational change with an associated energy increase of 10.2 kcal mol^{-1} is required; this would move the O1 oxygen atom to a distance of 0.65 Å from 12α-H (not shown).

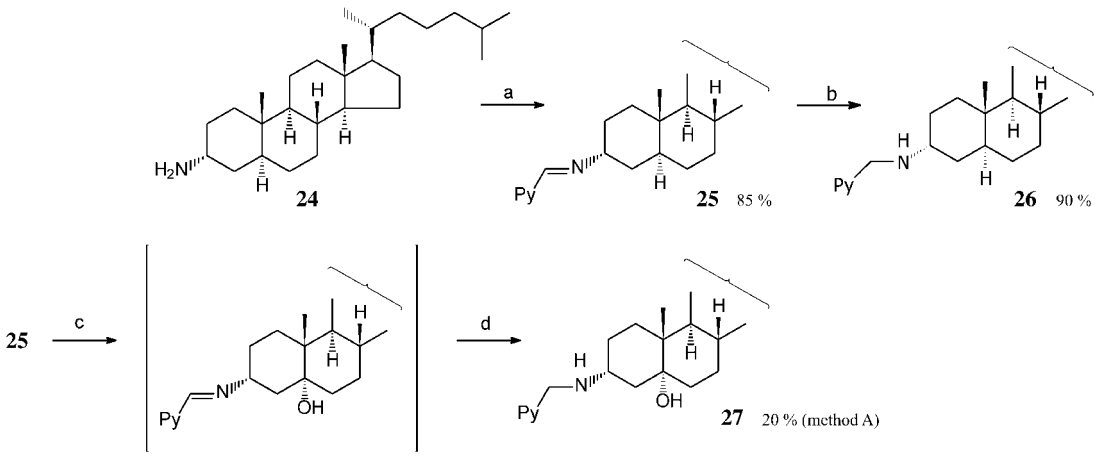
Hydroxylation of the AMPY ligand **16** (Scheme 4) was investigated by means of method B. The reaction mixture was directly reduced with $NaBH_4$ in CH_3OH so that the stable 2-pyridylmethylamino compounds could be isolated by chromatography after decomplexation. In addition to the reduced ligand **17** (main product), a more polar compound could be isolated in 8% yield. This was identified by mass spectrometry and detailed NMR analysis as the expected 14α-hydroxylated 17α-pyridylmethylamino compound **18**. Following method A, **18** was isolated in almost 50% yield. This result clearly confirmed our assumption that AMPY ligands can also be successfully used to hydroxylate nonactivated C–H bonds.

In a second experiment, the reaction mixture for the hydroxylation of **16** was hydrolyzed and separated. In this way, the expected (and hitherto unknown) 1,3-amino alcohol **19** [17α-amino-14α-hydroxy-3-methoxy-estra-1,3,5(10)-triene] could be isolated in a yield of 46%. By using CH_3OH, acetone, or dioxane as the solvent instead of CH_2Cl_2, we obtained comparable yields. For convenience, in subsequent experiments acetone was used as the solvent in both methods A and B.

Hydroxylation of 16α-AMPY and 3α-AMPY steroids 21 and 25: To find further examples of hydroxylations of AMPY ligands with a H-atom at a tertiary γ-C-atom, we started with the 16α-amine **20**[23] (Scheme 4) and with 3α-amino-5α-cholestane **24**[22] (Scheme 5).

The ligands **21** and **25** were obtained as pure crystalline compounds. Assuming the more stable *E* configuration of the C=N double bond and that the discussed imine conformation is preferred, both ligands have a similar orientation of the part needed for complexation with respect to the hydrogens at the tertiary γ-C-atoms (14α-H for **21** and 5α-H for **25**), as in compound **16** (Figure 8). The steric relationships and the six-membered ring planes are shown in Figure 9.

A common steric feature of all three ligands is a nearly axial hydrogen at a tertiary γ-C-atom in a 1,3- disposition relative to the central nitrogen atom. Interestingly, in **16** and **25** further axial hydrogen atoms (belonging to a CH_2 group) are in a 1,3-relationship with respect to the central nitrogen (12α-H of **16** and 1α-H of **25**). Using method A for the hydroxylation of **21** and **25** gave, after reduction with $NaBH_4$, the reduced ligands (**22** and **26**) and the expected hydroxylation products **23** (with a 14α-hydroxy group) and **27** (with a 5α-hydroxy group), in yields of 18 and 20%, respectively, as determined by MS and detailed NMR spectroscopy. The lower yields as compared to the hydroxylation of **16** may



Scheme 5. γ-Hydroxylation of 3α-AMPY-5α-cholestane. a) Pyridine-2-carbaldehyde, MeOH, 60°C; b) $NaBH_4$, CH_3OH; c) 1. $Cu(CH_3CN)_4PF_6$, acetone, Ar; 2. O_2; d) 1. $NaBH_4$, CH_3OH; 2. NH_4OH, H_2O; 3. chromatography on silica gel.

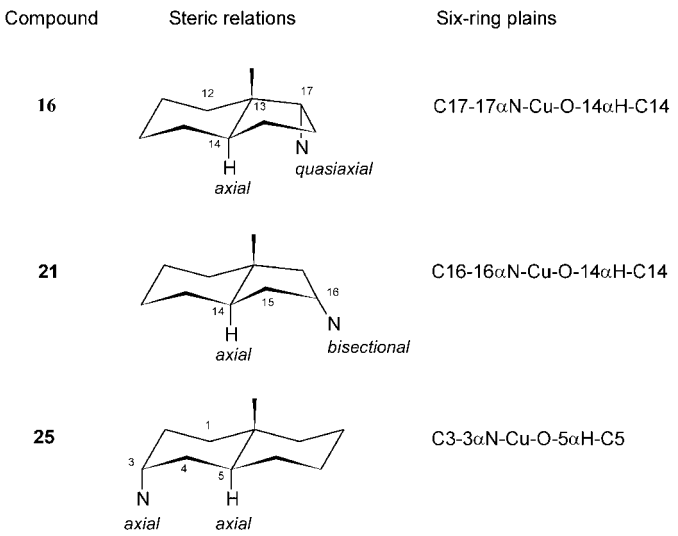


Figure 9. Steric relationships of AMPY groups and H atoms at γ-C atoms.

possibly be accounted for by small structural differences in the active copper–oxygen complexes relating to the hydrogen atom that must be abstracted. Here, method A works best, as demonstrated by the fact that an attempt to hydroxyllate **25** according to method B led only to isolation of the reduced ligand **26**.

These results confirm that AMPY ligands are suitable for copper-mediated hydroxylations of nonactivated C–H bonds at tertiary C-atoms. Prerequisites for a successful reaction are a defined arrangement of the hydrogen atom with respect to the active copper–oxygen species, as well as the carrying out of the reaction with a copper(I) salt (method A). The question as to whether the hydroxylation of nonactivated CH_2 groups is also possible with these AMPY ligands cannot be completely answered with these model compounds. In these few examples, a hydroxylation of the 12-CH_2 group in **16** or the 1-CH_2 group in **25** could not be achieved.

A few inter- and intramolecular methods for the hydroxylation of nonactivated C–H bonds at tertiary C atoms are known, and these have been reviewed by Parish and co-workers.[24] Most of them are not regioselective. External oxidants are ozone,[24] the Gif system,[24] dioxiranes,[24] and chromyl esters.[24] Benzophenones,[14a] Mn^{III} porphyrins,[15,24] and carboxylic acids[10,25] have also been employed as intramolecular groups for oxygen transfer. Recently, Breslow and co-workers have described a catalytic process for hydroxylations involving the use of manganese(III) porphyrins linked to cyclodextrins through spacers.[26]

Conclusion

Using IMPY and AMPY compounds, we have developed a protocol for the hydroxylation of nonactivated CH, CH_2, and CH_3 groups in γ-positions with respect to the central nitrogen. IMPY and AMPY groups can serve as auxiliaries for the starting oxo and *primary* amino compounds. After the hydroxylation procedure and hydrolysis, 3-hydroxy-1-oxo and 3-hydroxy-1-*primary* amino compounds can be obtained. Another possibility is simple reduction of the IMPY and AMPY groups after the hydroxylation procedure, which yields 3-hydroxy-1-(aminomethyl)-α-pyridines.

A clear advantage of this procedure is the simple introduction of IMPY and AMPY groups into complex molecules. The ensuing introduction of a hydroxy group at a nonactivated 3-position is also possible by way of a relatively simple procedure using copper(I) salts and molecular oxygen. Although the influence of the means of preparation of the copper(I) complexes [starting with copper(I), method A; or copper(II) and reduction, method B] needs to be further investigated, conformational analyses based on force-field calculations and X-ray crystal structure results contribute to an understanding of the stereochemical requirements of the oxidizing species in relation to the hydrogen that must be abstracted. On this basis, we suggest a simple procedure to predict if a hydroxylation procedure will be successful and we summarize the yields of our hydroxylation experiments for both methods A and B (Table 1).

Table 1. Collected results of hydroxylations

Compound	Torsion angle [α]	Torsion angle [°] value [α] X-ray (or force field)	Yields [%]	
17-oxo-3-methoxy-estra-1,3,5(10)-triene	O=C17-C13–12βH	8.0		
1[7] 17-IMPY	N1=C17-C13-12βH	6.0 (12.3)	12βOH 17-one	A: 29 B: 50
3 17a-IMPY (D-homo)	N=C17-C13-12βH	9.3	**4**	A: 18 B: 11
5	O=C17-C13-12βH	−14.4		
6 17-IMPY (13α)	N=C17a-C13-12βH	(−18.0)	**8**	A: 34 B: 6
8	O=C17-C13-12βO	0.8		
camphor oxime	N=C2-C1-10H	9.6		
12 IMPY-camphor		n. d.	**13**	A: 31 B: 4
16 17α-AMPY	N1-C17-C14-14αH	7.0 (6.2)	**18**	A: 50 B: 8
			19	A: 46
21 16α-AMPY		n. d.	**23**	A: 18
25 3α-AMPY		n. d.	**27**	A: 20 B: 0

The four atoms defining the torsional angle [α] are part of the seven-membered transition state and should be nearly coplanar for a successful hydroxylation (small [α] values).

The definition, beginning with the central nitrogen, is given in Figure 10. The γ-C atom for the IMPY and the β-C atom for the AMPY compounds, which are not in the plane, are omitted. At the end is the H atom that has to be abstracted. Furthermore, the configuration of the imino bond and the orientation of the lone pairs of the two nitrogen atoms for binding the copper ions have to be taken into account.

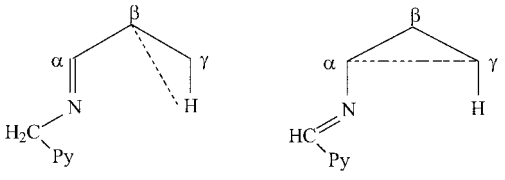


Figure 10. Definition of the torsional angle [α].

The angle [α] can be determined from molecular models or from X-ray data for the IMPY or AMPY compounds. However, the starting carbonyl or amino compounds or derivatives can also be used (see Table 1). In this manner, the likely products of hydroxylations of new compounds can be predicted in a simple manner.

Experimental Section

General methods: Melting points were measured on a Boëtius micro melting point apparatus and are corrected values. Mass spectra were determined on an AMD 402 Intectra instrument with either direct electron impact (EI) or electrospray ionization (ESI) at 70 eV. Optical rotations were measured at room temperature in the solvents given in the individual procedures with a Polamat A (Carl Zeiss Jena) polarimeter and are given in units of $g\,100^{-1}\,mL^{-1}$. Elemental analyses were performed with a CHNS-932 (LECO) instrument. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AC 250 or a DRX-400 spectrometer, in $CDCl_3$ or in CD_2Cl_2 (^1H NMR 250 MHz, 400 MHz; ^{13}C NMR 62.5 MHz, 100 MHz). Signals were assigned with the aid of DEPT, COSY-DQF, TOCSY, NOESY, HMQC, HMBC, and HSQC-TOSCY experiments. All reactions were carried out under an inert atmosphere. The reactions were monitored by TLC on aluminum sheets coated with silica gel 60 F_{254} (Merck), thickness 0.2 mm, detection under UV light (254 nm) or by spraying with a solution of $P_2O_5{\cdot}24\,MoO_3{\cdot}H_2O$ (2.5 g/50 mL; 42% H_3PO_4) and heating at 170 °C. MPLC was performed on Lichroprep Si 60, 15–25 μm (Merck). Solvents were purified, dried, and distilled according to conventional methods.

Crystal structure analysis: Intensity data for compounds **3**, **16**, and **8** were collected on a Nonius KappaCCD diffractometer using graphite-monochromated $Mo_{K\alpha}$ radiation. Data were corrected for Lorentz and polarization effects, but not for absorption effects.[27,28] The structures were solved by direct methods (SHELXS[29]) and refined by full-matrix least-squares techniques against F_2^o (SHELXL-97[30]). For compound **8**, the hydrogen atom of the hydroxy group was located by difference Fourier synthesis and refined isotropically. The other hydrogen atoms were included at calculated positions with fixed thermal parameters. All non-hydrogen atoms were refined anisotropically.[30] XP (Siemens Analytical X-ray Instruments, Inc.) was used for structure representations.[31]

Modeling and conformational analysis: The MMFF94 force field[32] with MMFF94 atomic charges and the grid conformational search option for two torsion angles ranging from 0° to 360° in angular increments of 10° was employed for the conformational analysis of the 13β-configured steroids with AMPY and IMPY groups in the 17-position. In grid searching, a geometry optimization was performed for each grid point, whereby the two selected torsion angles were systematically varied but then kept constrained in the optimization of the remaining internal coordinates. AMPY and IMPY steroid models lacking the 3-methoxy function were used. The starting conformations were built without using crystal structure information and were energy-minimized using the same force-field set-up (maximum number of iterations = 10000; termination with an energy gradient of 0.001). All energy values provided in this article are based on strain energies taken from MMFF94 force-field calculations and are quoted relative to the lowest-energy conformation. To display the energy contour maps, the 900 conformations with the lowest energies were selected from a total of 1369 calculated conformations. For least-squares alignments of steroid structures, all steroid backbone atoms were taken into account.

In the process of modeling the 13α-configured IMPY steroid, unconstrained in vacuo molecular dynamics (MD) computations of 100 ps were employed instead of grid searches in order to better capture the conformational space of the rings (temperature = 300 K; NTV ensemble; time interval step = 1 fs; distance-dependent dielectric function; non-bonded cut-off = 8 Å; nonbonded list update every 25 fs; no periodic boundary conditions). As starting geometries for the two MD simulations, both the C-ring chair and the C-ring twist-boat conformations were constructed and minimized applying the MMFF94 force field. These two basic ring conformations have been found previously in the case of 13α steroids.[33] An average structure from the simulation for each basic C-ring conformation as well as some representative dynamics snapshots were selected; the conformation within the 17-side chain was then adopted so as to allow H abstraction, and these structures were finally energy-minimized once more. The conformations of five- and six-membered rings were judged by using asymmetry parameters ΔC_s and ΔC_2[34] and phase angles of pseudorotation Δ.[35]

To model the binuclear complex with the four-membered ring containing copper and oxygen, the crystal structure of bis[aqua-(μ_2-hydroxo)-(2,2′-bipyrimidinyl-*N*,*N*′)-copper] dinitrate tetrahydrate (CSD refcode:[36] PEMPEC) was selected.[37] This structure provides a Cu–O distance of 1.94 Å.

All molecular modeling calculations were performed on an SGI Octane R14000 computer (500 MHz, main memory 1536 Mbytes) employing the SYBYL® molecular modeling environment[38] in software version 6.9.

Synthesis of the IMPY ligands

17a-(*N*-2-Pyridylmethyl)imino-D-homo-3-methoxy-estra-1,3,5(10)-triene (3): A mixture of 3-methoxy-D-homo-estra-1,3,5(10)-triene-17a-one **2**[12c] (2.0 g, 6.9 mmol), 2-(aminomethyl)pyridine (3.5 mL, 34.7 mmol), and a catalytic amount of *p*-toluenesulfonic acid (30 mg) was dissolved in toluene (30 mL) and refluxed for 9 h in a Dean–Stark apparatus. The reaction mixture was then cooled to room temperature and diluted with ethyl acetate. The organic layer was washed twice with saturated aqueous $NaHCO_3$ solution and water, and dried over Na_2SO_4. The solvent was evaporated and the yellow oily crude product was purified by crystallization from ethyl acetate to give 17-imine **3** as light-yellow crystals (2.28 g, 87%). M.p. 95–98 °C (ethyl acetate); $[\alpha]_D^{24} = 21.9$ ($c = 0.4$ in MeOH); ^1H NMR (250 MHz, CD_2Cl_2): $\delta = 1.10$ (s, 3H; 18-H_3), 2.84 (m, 2H; 6-H_2), 3.75 (s, 3H; CH_3O), 4.63 (m, 2H; CH_2Py), 6.61 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.68 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.23 (d, $^3J = 8.6$ Hz, 1H; 1-H), 7.19, 7.56, 7.68, 8.50 ppm (4m, 4×1H; 4×H_{Py}); MS (EI): *m*/*z* (%): 388 (100) [M^+]; HRMS: calcd. for $C_{26}H_{32}N_2O$: 388.2611; found: 388.2615.

Crystal data for **3**:[31] $C_{26}H_{32}N_2O$, $M_r = 388.54\,g\,mol^{-1}$, colorless prism, size $0.03\times0.03\times0.02\,mm^3$, monoclinic, space group $P2_1$, $a = 6.5906(2)$, $b = 23.1923(9)$, $c = 7.1135(3)$ Å, $\beta = 95.585(2)°$, $V = 1082.15(7)$ Å^3, $T = -90$ °C, $Z = 2$, $\rho_{calcd} = 1.192\,g\,cm^{-3}$, $\mu(Mo_{K\alpha}) = 0.72\,cm^{-1}$, $F(000) = 420$, 4343 reflections in $h(-8/8)$, $k(-29/27)$, $l(-9/9)$, measured in the range $2.88° \le \Theta \le 27.49°$, completeness $\Theta_{max} = 99.6\%$, 4343 independent reflections, 3164 reflections with $F_o > 4\sigma(F_o)$, 262 parameters, 1 restraint, $R1_{obs} = 0.051$, $wR^2_{obs} = 0.111$, $R1_{all} = 0.082$, $wR^2_{all} = 0.126$, GOOF = 1.016, Flack parameter 1.7(18), largest difference peak and hole: 0.151/$-0.152\,e\,Å^{-3}$.

17-(*N*-2-Pyridylmethyl)imino-3-methoxy-13α-estra-1,3,5(10)-triene (6): 2-(Aminomethyl)pyridine (3.6 mL, 35 mmol) and 3-methoxy-13α-estra-

1,3,5(10)-triene-17-one (**5**;[13a] 2.0 g, 7 mmol) were reacted in toluene (30 mL) for 20 h as described for the synthesis of **3**. The sticky brown crude product was crystallized from ethyl acetate to give pure 17-imine **6** (2.35 g, 89%) as a light-yellow amorphous solid. M.p. 115–118 °C (ethyl acetate); $[\alpha]_D^{24} = -48.0$ ($c = 1.5$ in MeOH); ^{1}H NMR (250 MHz, CD_2Cl_2): $\delta = 1.09$ (s, 3H; 18-H_3), 2.80 (m, 2H; 6-H_2), 3.74 (s, 3H; CH_3O), 4.57 (m, 2H; CH_2Py), 6.58 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.68 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.20 (d, $^3J = 8.6$ Hz, 1H; 1-H), 7.09, 7.45, 7.60, 8.46 ppm (4m, 4×1H; 4×H_{Py}); MS (EI): *m*/*z* (%): 374 (100) [M^+]; elemental analysis calcd (%) for $C_{25}H_{30}N_2O$ (374.5): C 80.17, H 8.07, N 7.48; found: C 79.72, H 8.09, N 7.85.

(1*R*)-2-(*N*-2-Pyridylmethyl)iminobornane (12):[16] (1*R*)-Camphor **11** (3.0 g, 19.7 mmol) and 2-(aminomethyl)pyridine (4.0 mL, 39.4 mmol) were dissolved in xylene (100 mL), and a catalytic amount of *p*-toluenesulfonic acid (40 mg) was added. The reaction mixture was refluxed for 36 h in a Dean–Stark apparatus. After the mixture was cooled to room temperature, it was diluted with ethyl acetate, washed twice with saturated aqueous $NaHCO_3$ solution and water, dried, and concentrated. The oily residue was chromatographed on silica gel eluting with CH_2Cl_2/CH_3OH (85:15) to afford the (1*R*)-camphor imine **12** as a yellow oil (3.4 g, 72%). $[\alpha]_D^{24} = -7.3$ ($c = 0.8$ in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.75$ (s, 3H; CH_3), 0.92 (s, 3H; CH_3), 1.02 (s, 3H; CH_3), 4.55 (m, 2H; CH_2Py), 7.09, 7.43, 7.62, 8.48 ppm (4m, 4×1H; 4×H_{Py}); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta = 11.4$ (CH_3), 19.0 (CH_3), 19.6 (CH_3), 27.4 (CH_2), 32.2 (CH_2), 36.1 (CH_2), 43.9 (CH), 47.4 (C_q), 54.2 (C_q), 57.4 (NCH_2Py), 121.6, 121.7, 136.6, 148.9, 160.4 (5×C_{Py}), 185.3 ppm (N=C_q); HRMS (ESI): calcd for $C_{16}H_{23}N_2$ [M^++H]: 243.1861; found: 243.1862.

Synthesis of the AMPY ligands

17α-(*N*-2-Pyridylmethylene)amino-3-methoxy-estra-1,3,5(10)-triene (16): A solution of the 17α-amine **15**[22] (570 mg, 2.0 mmol) in absolute methanol (30 mL) containing pyridine-2-carbaldehyde (0.14 mL, 2.9 mmol) was stirred at 60 °C under an argon atmosphere for 2 h. The solution was then concentrated to half of its original volume and allowed to cool to room temperature. The crystallized product was collected by filtration, washed with cold methanol, dried in vacuo, and recrystallized from methanol to give the 17α-AMPY compound **16** (602 mg, 80%) as colorless crystals. M.p. 118–121 °C (methanol); $[\alpha]_D^{24} = -82.9$ ($c = 0.8$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.85$ (s, 3H; 18-H_3), 2.86 (m, 2H; 6-H_2), 3.42 (m, 1H; 17β-H), 3.76 (s, 3H; CH_3O), 6.62 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.67 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.17 (d, $^3J = 8.6$ Hz, 1H; 1-H), 7.28, 7.70, 8.03, 8.61 (4m, 4×1H; 4×H_{Py}), 8.26 ppm (s, 1H, N=CH); MS (ESI): *m*/*z* (%): 397 (100) [M^++Na]; elemental analysis calcd (%) for $C_{25}H_{30}N_2O$ (374.5): C 80.17, H 8.07, N 7.48; found: C 80.39, H 7.83, N 7.37.

Crystal data for **16**:[31] $C_{25}H_{30}N_2O$, $M_r = 374.51\ g\,mol^{-1}$, colorless prism, size $0.05\times0.04\times0.03\ mm^3$, orthorhombic, space group $P2_12_12_1$, $a = 9.0529(2)$, $b = 13.7690(3)$, $c = 16.6758(4)$ Å, $V = 2078.63(8)$ Å^3, $T = -90$ °C, $Z = 4$, $\rho_{calcd} = 1.197\ g\,cm^{-3}$, $\mu(Mo_{K\alpha}) = 0.73\ cm^{-1}$, $F(000) = 808$, 4739 reflections in $h(-11/11)$, $k(-17/17)$, $l(-21/21)$, measured in the range $1.92° \le \Theta \le 27.49°$, completeness $\Theta_{max} = 99.8\%$, 4739 independent reflections, 3936 reflections with $F_o > 4\sigma(F_o)$, 253 parameters, 0 restraints, $R1_{obs} = 0.044$, $wR^2_{obs} = 0.098$, $R1_{all} = 0.060$, $wR^2_{all} = 0.106$, GOOF = 1.023, Flack parameter −1.7(15), largest difference peak and hole: 0.147/−0.175 e Å^{-3}.

17α-(*N*-2-Pyridylmethyl)amino-3-methoxy-estra-1,3,5(10)-triene (17): $NaBH_4$ (190 mg, 5 mmol) was added to a stirred solution of 17α-imine **16** (375 mg, 1.0 mmol) in absolute MeOH (15 mL) and THF (5 mL) at room temperature. After 90 min, the reaction mixture was poured into an ice/water mixture and extracted with CH_2Cl_2. The organic phase was washed with water, dried, and concentrated. The oily residue was crystallized from *n*-heptane to give **17** as white crystals (300 mg, 80%). M.p. 79–81 °C (*n*-heptane); $[\alpha]_D^{24} = +9.1$ ($c = 0.8$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.74$ (s, 3H; 18-H_3), 2.72 (m, 1H; 17β-H), 2.84 (m, 2H; 6-H_2), 3.75 (s, 3H; CH_3O), 3.86 (m, 2H; CH_2Py), 6.63 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.68 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.10–7.29 (m, 3H; 2×H_{Py} and 1-H), 7.59 and 8.59 ppm (2m, 2×1H; 2×H_{Py}); elemental analysis calcd (%) for $C_{25}H_{32}N_2O$ (376.5): C 79.75, H 8.56, N 7.44; found: C 79.41, H 8.88, N 7.19.

16α-(*N*-2-Pyridylmethylene)amino-3-methoxy-estra-1,3,5(10)-triene (21): 16α-Amine **20**[23] (285 mg, 1.0 mmol) and pyridine-2-carbaldehyde (0.1 mL, 1.5 mmol) were reacted in absolute methanol (20 mL) as described for the synthesis of **16**. The 16α-AMPY compound **21** was obtained as white crystals (255 mg, 68%) after recrystallization from methanol. M.p. 94–97 °C (methanol); $[\alpha]_D^{24} = +112.5$ ($c = 0.8$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.87$ (s, 3H; 18-H_3), 2.83 (m, 2H; 6-H_2), 3.76 (s, 3H; CH_3O), 4.10 (m, 1H; 16β-H), 6.64 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.71 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.21 (d, $^3J = 8.6$ Hz, 1H; 1-H), 7.28, 7.71, 7.98, 8.62 (4m, 4×1H; 4×H_{Py}), 8.26 ppm (s, 1H, N=CH); elemental analysis calcd (%) for $C_{25}H_{30}N_2O$ (374.5): C 80.17, H 8.07, N 7.48; found: C 79.72, H 8.07, N 7.07.

16α-(*N*-2-Pyridylmethyl)amino-3-methoxy-estra-1,3,5(10)-triene (22): Compound **21** (375 mg, 1.0 mmol) and $NaBH_4$ (190 mg, 5.0 mmol) were allowed to react in absolute MeOH and THF as described for the reduction of **16**. Crystallization of the product from *n*-heptane gave **22** as a white solid (347 mg, 92%). M.p. 87–91 °C (*n*-heptane); $[\alpha]_D^{24} = +63.1$ ($c = 0.7$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.76$ (s, 3H; 18-H_3), 2.81 (m, 2H; 6-H_2), 3.36 (m, 1H; 16β-H), 3.75 (s, 3H; CH_3O), 3.85 (m, 2H; CH_2Py), 6.60 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.68 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.11–7.28 (m, 3H; 2×H_{Py} and 1-H), 7.61 and 8.54 ppm (2m, 2×1H; 2×H_{Py}); MS (ESI): *m*/*z* (%): 377 (100) [M^++H]; HRMS (ESI): calcd for $C_{25}H_{33}N_2O$ [M^++H]: 377.2913; found: 377.2607.

3α-(*N*-2-Pyridylmethylene)amino-5α-cholestane (25): 3α-Amino-5α-cholestane (**24**;[22] 1.94 g, 5.0 mmol) and pyridine-2-carbaldehyde (0.5 mL, 7.5 mmol) were reacted in absolute methanol (100 mL) as described for the synthesis of **16**. The solvent was then evaporated to leave a yellow oil. The 3α-AMPY compound **25** was obtained as white crystals (2 g, 85%) after crystallization from methanol. M.p. 40–43 °C (methanol); $[\alpha]_D^{24} = +20.1$ ($c = 1.2$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.65$ (s, 3H; 18-H_3), 0.85–0.97 (m, 12H; 4×CH_3, 19-H_3, 21-H_3, 26-H_3, 27-H_3), 3.60 (m, 1H; 3β-H), 7.26, 7.69, 8.07, 8.60 (4m, 4×1H; 4×H_{Py}), 8.37 ppm (s, 1H, N=CH); MS (EI): *m*/*z* (%): 476 (100) [M^+]; elemental analysis calcd (%) for $C_{33}H_{52}N_2$ (476.4): C 83.12, H 10.99, N 5.88; found: C 83.03, H 10.82, N 5.86.

3α-(*N*-2-Pyridylmethyl)amino-5α-cholestane (26): Imine **25** (476 mg, 1 mmol) and $NaBH_4$ (190 mg, 5 mmol) were allowed to react in absolute CH_3OH as described for the synthesis of **17. 26** was obtained as white crystals after recrystallization from CH_3OH (430 mg, 90%). M.p. 75–77 °C (MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.62$ (s, 3H; CH_3), 0.77 (m, 9H; 3×CH_3), 0.83 (m, 3H; CH_3), 0.85 (m, 3H; CH_3), 2.87 (m, 1H; 3β-H), 3.87 (m, 2H; CH_2Py), 7.11, 7.30, 7.60, 8.52 ppm (4m, 4×1H; 4×H_{Py}); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta = 11.5$ (CH_3), 12.0 (CH_3), 18.6 (CH_3), 20.7 (CH_2), 22.5 (CH_3), 22.7 (CH_3), 23.8 (CH_2), 24.1 (CH_2), 26.1 (CH_2), 28.0 (CH_2), 28.2 (CH_2), 28.8 (CH_2), 32.0 (CH_2), 32.7 (CH_2), 33.4 (CH_2), 35.5 (CH), 35.8 (CH), 36.2 (CH_2), 36.2 (C_q), 39.5 (CH_2), 39.6 (CH), 40.1 (CH_2), 42.6 (C_q), 52.4 (CH), 53.1 (CH_2), 54.3 (CH), 56.3 (CH), 56.6 (CH), 121.7 (CH_{Py}), 121.3 (CH_{Py}), 136.3 (CH_{Py}), 149.1 (CH_{Py}), 160.4 ppm (C_{Py}); MS (ESI): *m*/*z* (%): 479 (100) [M^++H]; HRMS: calcd for $C_{33}H_{55}N_2$ [M^++H]: 479.4365; found: 479.4374; elemental analysis calcd (%) for $C_{33}H_{54}N_2$ (478.4): C 82.85, H 11.30, N 5.86; found: C 82.63, H 10.99, N 5.94.

Hydroxylation procedures with IMPY and AMPY ligands

Method A: Tetrakis(acetonitrile)copper(I) hexafluorophosphate (1.2 equiv) was added to a solution of 1 equivalent of the IMPY (**3**, **6**, **12**) or AMPY ligand (**16**, **21**, **25**) in absolute acetone that had been degassed with argon. The resulting brown solutions were stirred at room temperature. After 1 h, the argon atmosphere was replaced by an O_2 atmosphere. Pure O_2 was then bubbled through the reaction mixtures for approximately 10 min. The solutions were then stirred for about 24 h under O_2. During this time, they turned dark green. The solvent was then distilled off and the oily dark residues were worked-up as described below in the respective syntheses.

Method B: Anhydrous copper(II) triflate (1.2 equiv) was added to a solution of the IMPY or AMPY ligand (**3**, **6**, **12** or **16**, **21**, **25**; 1 equiv.) in absolute acetone. The dark green solutions were stirred at room temperature for about 1 h. Under an argon atmosphere and with constant stirring, benzoin (2 equiv) and triethylamine (2 equiv) were added. After 4 h, pure O_2 was bubbled through the mixtures for 10 min. The yellow-brown solutions were stirred for a further 24 h under O_2, during which they became dark green once more. After removing the solvent, dark, oily

crude products were obtained, which were worked-up as described in detail below.

12β-Hydroxy-D-homo-3-methoxy-estra-1,3,5(10)-triene-17a-one (4): Following method A, compound **3** (388 mg, 1 mmol) was reacted with $Cu^{I}(CH_3CN)_4PF_6$ (450 mg, 1.2 mmol) in acetone (20 mL). The crude product was first dissolved in ethyl acetate and then extracted three times with NH_4OH (25%); the brown organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residual dark brown oil was then redissolved in methanol (20 mL) and treated with glacial acetic acid (20 mL) at 90°C for 6 h. The methanol was then removed, and the mixture was poured into water and extracted with ethyl acetate. The combined organic phases were washed with brine and dried (Na_2SO_4). Evaporation of the volatiles left a dark oil, which was purified and separated by MPLC using Lichroprep Si 60, 15–20 μm, eluting with *n*-hexane/ethyl acetate, 80:20 (column 200×35 mm, rate 25 mL min^{-1}) to yield the D-homo-17a-ketone (116 mg, 39%) and the 12β-hydroxy compound (57 mg, 18%).

Method B: Compound **3** (388 mg, 1 mmol) was allowed to react with $Cu^{II}(CF_3SO_3)_2$ (440 mg, 1.2 mmol), benzoin (425 mg, 2 mmol), and triethylamine (0.3 mL, 2 mmol) in acetone (20 mL). The work-up procedure was performed in the same manner as described above. After MPLC, the 12β-hydroxy compound **4** (35 mg, 11%) was obtained as a white solid in addition to the D-homo-17a-ketone **2** (161 mg, 54%).

4: M.p. 156–159°C (*n*-hexane/ethyl acetate); $[\alpha]_D^{24} = -26.4$ ($c = 1.7$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.16$ (s, 3H; 18-H$_3$), 2.85 (m, 2H; 6-H$_2$), 3.67 (s, 1H; 12β-OH), 3.76 (s, 3H; CH_3O), 4.10 (m, 1H; 12α-H), 6.61 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.70 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.19 ppm (d, $^3J = 8.6$ Hz, 1H; 1-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.4$ (C-18), 22.3 (CH_2), 25.6 (CH_2), 26.6 (CH_2), 29.8 (CH_2), 32.6 (CH_2), 37.4 (CH_2), 38.0 (CH), 40.8 (CH), 48.7 (CH), 53.5 (C-13), 55.2 (H_3CO), 72.5 (C-12), 111.6 (C-2), 113.6 (C-4), 126.1 (C-1), 131.5 (C-10), 137.4 (C-5), 157.7 (C-3), 219.2 ppm (C-17a); MS (ESI): *m*/*z* (%): 337 (100) [M^++Na]; HRMS (ESI): calcd. for $C_{26}H_{32}N_2ONa$ [M^++Na]: 337.1975; found: 337.1780.

12β-Hydroxy-3-methoxy-13α-estra-1,3,5(10)-triene-17-one (8): According to method A, the IMPY ligand **6** (374 mg, 1 mmol) was allowed to react with $Cu^{I}(CH_3CN)_4PF_6$ (450 mg, 1.2 mmol) in acetone (20 mL) as described for the synthesis of **4**. Separation of the oily crude product by column chromatography eluting with *n*-hexane/ethyl acetate (75:25) gave **5**[13a] (74 mg, 26%) and 12β-hydroxy-13α-estrone-3-methyl ether **8** (102 mg, 34%) as white crystals. According to method B, compound **6** (374 mg, 1 mmol) was reacted with $Cu^{II}(CF_3SO_3)_2$ (440 mg, 1.2 mmol), benzoin (425 mg, 2 mmol), and triethylamine (0.3 mL, 2 mmol) in acetone (20 mL). The work-up procedure was performed as described for the synthesis of **4**. After MPLC, **5** (122 mg, 43%) and **8** (19 mg, 6%) were obtained. **8**: M.p. 162–165°C (MeOH); $[\alpha]_D^{24} = +10.0$ ($c = 0.7$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.37$ (s, 3H; 18-H$_3$), 2.82 (m, 2H; 6-H$_2$), 3.69 (m, 1H; 12α-H), 3.75 (s, 3H; CH_3O), 4.27 (d, 1H, $J = 10.8$ Hz; 12β-OH), 6.60 (d, 1H, $J = 2.7$ Hz; 4-H), 6.70 (dd, 1H, $J = 2.7$ Hz, $J = 8.6$ Hz; 2-H), 7.19 ppm (d, 1H, $J = 8.6$ Hz; 1-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 21.0$ (C-18), 21.3 (CH_2), 27.9 (CH_2), 30.1 (CH_2), 34.5 (CH_2), 37.9 (CH_2), 39.7 (CH), 41.4 (CH), 50.2 (CH), 53.2 (C-13), 55.2 (H_3CO), 76.2 (C-12), 111.8 (C-2), 113.7 (C-4), 126.6 (C-1), 131.0 (C-10), 137.8 (C-5), 157.7 (C-3), 225.4 ppm (C-17); MS (ESI): *m*/*z* (%): 323 (100) [M^++Na]; HRMS (ESI): calcd for $C_{19}H_{24}O_3Na$ [M^++Na]: 323.1623; found: 323.1624.

Crystal data for **8**:[31] $C_{19}H_{24}O_3$, $M_r = 300.38$ g mol^{-1}, colorless prism, size $0.03\times0.03\times0.02$ mm^3, orthorhombic, space group $P2_12_12_1$, $a = 7.0319(1)$, $b = 9.3898(2)$, $c = 23.4019(4)$ Å, $V = 1545.18(5)$ Å^3, $T = -90$°C, $Z = 4$, $\rho_{calcd} = 1.291$ g cm^{-3}, $\mu(Mo_{K\alpha}) = 0.86$ cm^{-1}, $F(000) = 648$, 3506 reflections in $h(-9/9)$, $k(-12/12)$, $l(-30/30)$, measured in the range $3.02° \le \Theta \le 27.47°$, completeness $\Theta_{max} = 99.6\%$, 3506 independent reflections, 3023 reflections with $F_o > 4\sigma(F_o)$, 203 parameters, 0 restraints, $R1_{obs} = 0.045$, $wR^2_{obs} = 0.109$, $R1_{all} = 0.057$, $wR^2_{all} = 0.116$, GOOF = 1.066, Flack parameter 1.2(12), largest difference peak and hole: 0.293/−0.363 e Å^{-3}.

12α-Hydroxy-3-methoxy-13α-estra-1,3,5(10)-triene-17-one (10): Following method A, the IMPY ligand **6** (374 mg, 1 mmol) was reacted with $[Cu^{I}(CF_3SO_3)(CH_3C_6H_5)]$ (620 mg, 1.2 mmol) in acetone (20 mL). The reaction mixture was worked-up in a similar manner as described for the synthesis of **4**. Separation of the oily crude product by MPLC eluting with *n*-hexane/ethyl acetate (75:25) gave **5** (68 mg, 24%), **8** (57 mg, 19%), and 12α-hydroxy-13α-estrone-3-methyl ether **10** (21 mg, 7%) as a colorless oil.

10: $[\alpha]_D^{24} = -3.5$ ($c = 1.4$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.16$ (s, 3H; 18-H$_3$), 2.83 (m, 2H; 6-H$_2$), 3.74 (s, 3H; CH_3O), 4.20 (m, 1H; 12β-H), 6.60 (d, 1H, $J = 2.7$ Hz; 4-H), 6.68 (dd, 1H, $J = 2.7$ Hz, $J = 8.6$ Hz; 2-H), 7.15 ppm (d, 1H, $J = 8.6$ Hz; 1-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 20.7$ (C-18), 21.0 (CH_2), 28.2 (CH_2), 30.2 (CH_2), 33.7 (CH_2), 34.0 (CH), 35.9 (CH_2), 41.8 (CH), 46.6 (CH), 53.7 (C-13), 55.2 (H_3CO), 69.3 (C-12), 111.7 (C-2), 113.6 (C-4), 126.7 (C-1), 131.9 (C-10), 138.1 (C-5), 157.5 (C-3), 220.6 ppm (C-17); MS (ESI): *m*/*z* (%): 323 (100) [M^++Na]; HRMS (ESI): calcd for $C_{19}H_{24}O_3Na$ [M^++Na]: 323.1623; found: 323.1625.

(1S)-2-(*N*-2-Pyridylmethyl)imino-10-hydroxybornane (13): According to method A, (1*R*)-IMPY-camphor **12** (1.00 g, 4.1 mmol) was allowed to react with $Cu^{I}(CH_3CN)_4PF_6$ (1.83 g, 4.92 mmol) in acetone (30 mL). The crude product was dissolved in $CHCl_3$ and the resulting solution was extracted with 25% NH_4OH (3×40 mL). The yellow organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel eluting with methyl *tert*-butyl ether (MTBE) and MTBE/$CHCl_3$ (1:1) to afford starting ligand **12** (530 mg, 53%) and **13** (330 mg, 31%) as a light-yellow oil.

According to method B, compound **12** (560 mg, 2.3 mmol) was reacted with $Cu^{II}(CF_3SO_3)_2$ (1.0 g, 2.76 mmol), benzoin (980 mg, 4.6 mmol), and triethylamine (0.7 mL, 4.6 mmol) in acetone (50 mL). The work-up procedure was carried out as described above and yielded an oil. Purification by column chromatography yielded starting ligand **12** (317 mg, 57%) and **13** (22 mg, 4%).

13: $[\alpha]_D^{24} = -13.0$ ($c = 2.0$ in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.89$ (s, 3H; CH_3), 0.99 (s, 3H; CH_3), 3.82 (m, 2H; CH_2OH), 4.52 (m, 2H; CH_2Py), 7.13, 7.39, 7.64, 8.48 ppm (4m, 4×1H; 4×H$_{Py}$); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta = 18.9$ (CH_3), 20.5 (CH_3), 27.0 (CH_2), 28.7 (CH_2), 36.0 (CH_2), 44.9 (CH), 47.1 (C_q), 57.2 (C_q), 57.5 (NCH_2Py), 62.8 (CH_2OH), 121.6, 121.8, 136.7, 148.9, 159.8 (5×C$_{Py}$), 186.1 ppm (N=C$_q$); MS (ESI): *m*/*z* (%): 259 (100) [M^++H]; HRMS (ESI): calcd for $C_{16}H_{23}N_2O$ [M^++H]: 259.1810; found: 259.1808.

(1*R*)-10-Hydroxy-camphor (14):[19] Acetic acid (20 mL) was added to a solution of compound **13** (260 mg, 1.0 mmol) in methanol (20 mL) and the mixture was heated at 90°C for about 6 h. The solution was then concentrated to dryness and the resulting crude product was purified by column chromatography on silica gel eluting with methyl *tert*-butyl ether/*n*-heptane (3:7) to give **14** (131 mg, 78%) as white crystals.

14: M.p. 186–190°C (MeOH); $[\alpha]_D^{24} = +25.9$ ($c = 1.7$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.96$ (s, 3H; CH_3), 0.98 (s, 3H; CH_3), 3.73 ppm (m, 2H; CH_2OH); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta = 19.3$ (CH_3), 20.8 (CH_3), 26.0 (CH_2), 26.7 (CH_2), 43.5 (CH_2), 44.0 (CH), 46.8 (C_q), 60.6 (CH_2OH), 61.6 (C_q), 221.0 ppm (C=O); MS (ESI): *m*/*z* (%): 191 (100) [M^++Na]; HRMS (ESI): calcd for $C_{10}H_{16}O_2Na$ [M^++Na]: 191.1048; found: 191.4200.

14α-Hydroxy-17α-(*N*-2-pyridylmethyl)amino-3-methoxy-estra-1,3,5(10)-triene (18): Following method A, 17α-AMPY ligand **16** (105 mg, 0.28 mmol) was allowed to react with $Cu^{I}(CH_3CN)_4PF_6$ (125 mg, 0.34 mmol) in acetone (30 mL). The crude product was dissolved in MeOH (15 mL) and then $NaBH_4$ (43 mg, 1.12 mmol) was slowly added. The reaction mixture was stirred at room temperature. After 1 h, H_2O (0.5 mL) was added. The dark oily residue that remained after removal of the solvent was redissolved in $CHCl_3$ and the resulting solution was extracted with 25% NH_4OH (4×15 mL). The brown organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The resulting oily product was purified by preparative TLC with methanol as eluent to yield **18** (55 mg, 50%) as a light-yellow oil in addition to the reduced ligand **17** (11 mg, 10%). According to method B, compound **16** (191 mg, 0.51 mmol) was allowed to react with $Cu^{II}(CF_3SO_3)_2$ (222 mg, 0.61 mmol), benzoin (217 mg, 1.02 mmol), and triethylamine (0.15 mL, 1.02 mmol) in acetone (20 mL). The crude product was dissolved in MeOH (15 mL) and reduced with $NaBH_4$ (116 mg, 3.06 mmol) as described above. After column chromatography on silica gel ($CHCl_3$; $CHCl_3$/CH_3OH, 5:1; $CHCl_3$/CH_3OH, 4:1) 15 mg (8%) of **18** and 107 mg (56%) of the reduced ligand **17** were obtained.

18: $[\alpha]_D^{24} = +37.4$ ($c = 1.0$ in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta =$ 0.87 (s, 3H; 18-H_3), 2.84 (m, 2H; 6-H_2), 3.01 (m, 1H; 17β-H), 3.75 (s, 3H; CH_3O), 3.87 (m, 2H; CH_2Py), 6.60 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.67 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.16 (d, $^3J = 8.6$ Hz, 1H; 1-H), 7.25 (m, 2H; 2×H_{Py}), 7.62 and 8.52 ppm (2m, 2×1H; 2×H_{Py}); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta =$ 22.3 (C-18), 23.6 (C-7), 25.8 (C-11), 27.2 (C-16 and C-12), 30.2 (C-6), 33.9 (C-15), 36.5 (C-9), 42.4 (C-8), 48.2 (C-13), 53.0 (CH_2Py), 55.2 (H_3CO), 66.8 (C-17), 83.7 (C-14), 111.4 (C-2), 113.6 (C-4), 122.3 (C_{py}-3), 122.5 (C_{Py}-5), 126.5 (C-1), 133.8 (C-10), 136.7 (C_{Py}-4), 138.0 (C-5), 149.2 (C_{Py}-2), 157.2 (C_{Py}-6), 158.0 ppm (C-3); MS (ESI): *m*/*z* (%): 393 (100) [M^++H]; HRMS: calcd for $C_{25}H_{33}N_2O_2$ [M^++H]: 393.2542; found: 393.2535.

14α-Hydroxy-17α-amino-3-methoxy-estra-1,3,5(10)-triene (19): According to method A, 17α-AMPY ligand **16** (200 mg, 0.53 mmol) was reacted with $Cu^I(CH_3CN)_4PF_6$ (240 mg, 0.64 mmol) in acetone (30 mL). The crude product was dissolved in $CHCl_3$ and treated with NH_4OH (25%) (3×20 mL). The organic layer was then washed with brine, dried over Na_2SO_4, and concentrated. Separation by preparative TLC with CH_2Cl_2/MeOH/NH_4OH (80:20:0.25) as eluent gave **19** (73 mg, 46%) as a colorless oil and 17α-amine **15** (45 mg, 30%).

19: $[\alpha]_D^{24} = +68.4$ ($c = 0.8$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta =$ 0.81 (s, 3H; 18-H_3), 2.84 (m, 2H; 6-H_2), 3.26 (m, 1H; 17β-H), 3.76 (s, 3H; CH_3O), 6.61 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.70 (dd, $^3J = 2.7$ Hz, $^3J =$ 8.6 Hz, 1H; 2-H), 7.20 ppm (d, $^3J = 8.6$ Hz, 1H; 1-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta =$ 23.9 (C-18), 25.7 (C-7), 26.1 (C-11), 26.5 (C-16), 30.3 (C-12), 30.9 (C-6), 34.3 (C-15), 36.5 (C-9), 42.7 (C-8), 47.2 (C-13), 55.1 (H_3CO), 59.9 (C-17), 83.9 (C-14), 111.4 (C-2), 113.6 (C-4), 126.4 (C-1), 134.2 (C-10), 138.1 (C-5), 157.2 ppm (C-3); MS (ESI): *m*/*z* (%): 302 (100) [M^++H]; HRMS: calcd for $C_{19}H_{29}NO_2$ [M^++H]: 302.2120; found: 302.2124.

14α-Hydroxy-16α-(*N*-2-pyridylmethyl)amino-3-methoxy-estra-1,3,5(10)-triene (23): According to method A, 16α-AMPY ligand **21** (105 mg, 0.28 mmol) was allowed to react with $Cu^I(CH_3CN)_4PF_6$ (125 mg, 0.34 mmol) in acetone (30 mL). The crude product was dissolved in MeOH (15 mL) and $NaBH_4$ (98 mg, 2.6 mmol) was added in small portions. The reaction mixture was stirred at room temperature for 1 h, and then H_2O (0.5 mL) was added and the solvent was removed. The residue was redissolved in $CHCl_3$ and extracted with NH_4OH (25%) (4×15 mL). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The resulting oily product was separated by column chromatography on silica gel eluting with MeOH/$CHCl_3$ (1:9) to afford **23** (20 mg, 18%) as a light-yellow oil and the reduced ligand **22** (45 mg, 56%).

23: $[\alpha]_D^{24} = +0.9$ ($c = 0.7$ in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta =$ 0.89 (s, 3H; 18-H_3), 2.84 (m, 2H; 6-H_2), 3.47 (m, 1H; 16β-H), 3.75 (s, 3H; CH_3O), 3.90 (m, 2H; CH_2Py), 6.60 (d, $^3J = 2.7$ Hz, 1H; 4-H), 6.69 (dd, $^3J = 2.7$ Hz, $^3J = 8.6$ Hz, 1H; 2-H), 7.16 (d, $^3J = 8.6$ Hz, 1H; 1-H), 7.27 (m, 2H; 2×H_{Py}), 7.62 and 8.52 ppm (2m, 2×1H; 2×H_{Py}); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta =$ 23.8 (C-18), 24.1 (CH_2), 26.1 (CH_2), 30.1 (CH_2), 32.0 (CH_2), 36.4 (CH), 40.1 (CH_2), 41.0 (CH), 45.5 (CH_2), 46.2 (C-13), 52.9 (CH_2Py), 55.2 (H_3CO), 55.6 (CH), 83.5 (C-14), 111.4 (C-2), 113.6 (C-4), 122.2 (C_{py}-3), 122.5 (C_{Py}-5), 126.6 (C-1), 134.0 (C-10), 136.7 (C_{Py}-4), 138.0 (C-5), 149.2 (C_{Py}-2), 157.2 (C_{Py}-6), 158.5 ppm (C-3); MS (ESI): *m*/*z* (%): 393 (100) [M^++H]; HRMS: calcd for $C_{25}H_{33}N_2O_2$ [M^++H]: 393.2542; found: 393.2544.

3α-(*N*-2-Pyridylmethyl)amino-5α-hydroxy-cholestane (27): Reaction of 3α-AMPY ligand **25** (200 mg, 0.42 mmol) with $Cu^I(CH_3CN)_4PF_6$ (190 mg, 0.5 mmol) in acetone (30 mL) according to method A was followed by reduction with $NaBH_4$ (190 mg, 5.0 mmol) in methanol as described for **23** and resulted in an oily product. Preparative TLC eluting with MeOH/$CHCl_3$ (1:9) gave **27** (41 mg, 20%) as a colorless oil and 110 mg (55%) of 3α-(*N*-2-pyridylmethyl)amino-5α-cholestane **26** (reduced ligand).

27: $[\alpha]_D^{24} = +6.3$ ($c = 1.6$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta =$ 0.63 (s, 3H; CH_3), 0.85 (m, 9H; 3×CH_3), 0.93 (s, 3H; CH_3), 3.05 (m, 1H; 3β-H), 3.88 (m, 2H; CH_2Py), 7.13, 7.23, 7.59, 8.51 ppm (4m, 4×1H; 4×H_{Py}); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta =$ 12.0 (CH_3), 16.0 (CH_3), 18.6 (CH_3), 20.7 (CH_2), 20.9 (CH_2), 22.5 (CH_3), 22.7 (CH_3), 23.9 (CH_2), 24.1 (CH_2), 25.7 (CH_2), 27.3 (CH_2), 27.9 (C_q), 28.3 (CH_2), 34.3 (CH_2), 35.0 (CH_2), 35.1 (CH_2), 35.8 (CH_2), 36.1 (CH), 39.5 (CH_2), 39.9 (C_q), 40.1 (CH_2), 42.7 (C_q), 45.4 (C_q), 52.5 (CH), 52.9 (CH_2), 56.1 (CH), 56.3 (CH_2), 73.9 (C_q), 122.1 (CH_{Py}), 122.6 (CH_{Py}), 136.5 (CH_{Py}), 149.2 (CH_{Py}), 158.6 ppm (C_{Py}); MS (ESI): *m*/*z* (%): 395 (100) [M^++H]; HRMS: calcd for $C_{33}H_{55}N_2O$ [M^++H]: 495.4314; found: 495.4316.

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