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Regioselective Oxidation of Steroids by a Manganese Porphyrin Carrying Metal Coordinating Groups

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A manganese porphyrin having four 2,2′-bipyridyl groups on its meso positions was synthesized. In the presence of Cu^{2+} ions it catalyzes the regioselective oxidation of steroid substrates carrying auxiliary metal coordinating groups.

Key Words: steroid oxidation; metal coordination; cytochrome P450; enzyme mimics.

INTRODUCTION

Enzymes are remarkable catalysts. They are able to transiently bind substrates in their active site in a specific geometric arrangement and then perform reactions whose regio- and stereoselectivity are the result of geometric control rather than of the intrinsic substrate reactivity. Achieving the same level of control using a relatively small synthetic catalyst is still an open challenge for biomimetic chemistry (*1*). A key issue is the incorporation of appropriate recognition elements on substrate and catalyst (*2*). Over the years we have reported the use of covalent binding, ion pairing, metal coordination, and the hydrophobic effect as inducers of geometric control (*1a*, *3*). The possibility of mimicking the reactions catalyzed by cytochrome P-450, in particular the hydroxylation of unreactive C–H bonds, has been particularly studied (*4*). There are several examples from this and other groups about the use of porphyrin-based catalysts that can achieve geometric control via covalent binding (*5*) or hydrophobic binding within vesicles (*6*) or β-cyclodextrins (*7*).

Now we are focusing our attention on the possibility of using metal coordination as the recognition element. Previous work from this laboratory showed that an olefin carrying auxiliary metal ligands is oxidized preferentially over a nonbinding one by an iron porphyrin with appended metal ligand groups in the presence of Cu^{2+} ions (*8*). We decided to extend this approach to the selective hydroxylation of steroids by using a new manganese porphyrin carrying metal coordinating groups. In the designed

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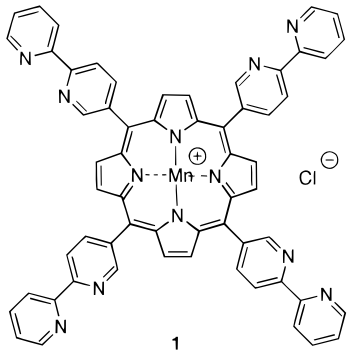
catalytic cycle (Fig. 1), the substrate should coordinate via its auxiliary group to a metal ion on a porphyrin bipyridyl and undergo a geometrically controlled oxidation. Herein we report examples of metal coordination-directed oxidations.

RESULTS AND DISCUSSION

The manganese porphyrin **1** was synthesized by the standard Adler-Longo method (9) from 2,2′-bipyridyl-5-carboxyaldehyde.



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Having obtained the catalyst, we started to evaluate possible metal coordinating groups to be attached to the steroid substrates. In an obvious extension of our previous work on epoxidation (*8*), we tried to oxidize steroid nicotinates. Unfortunately the

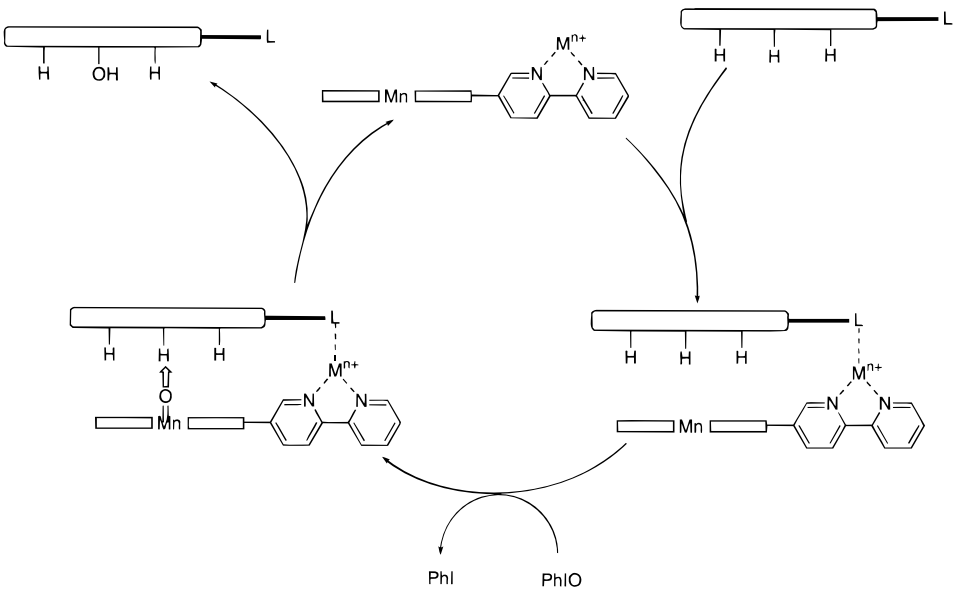
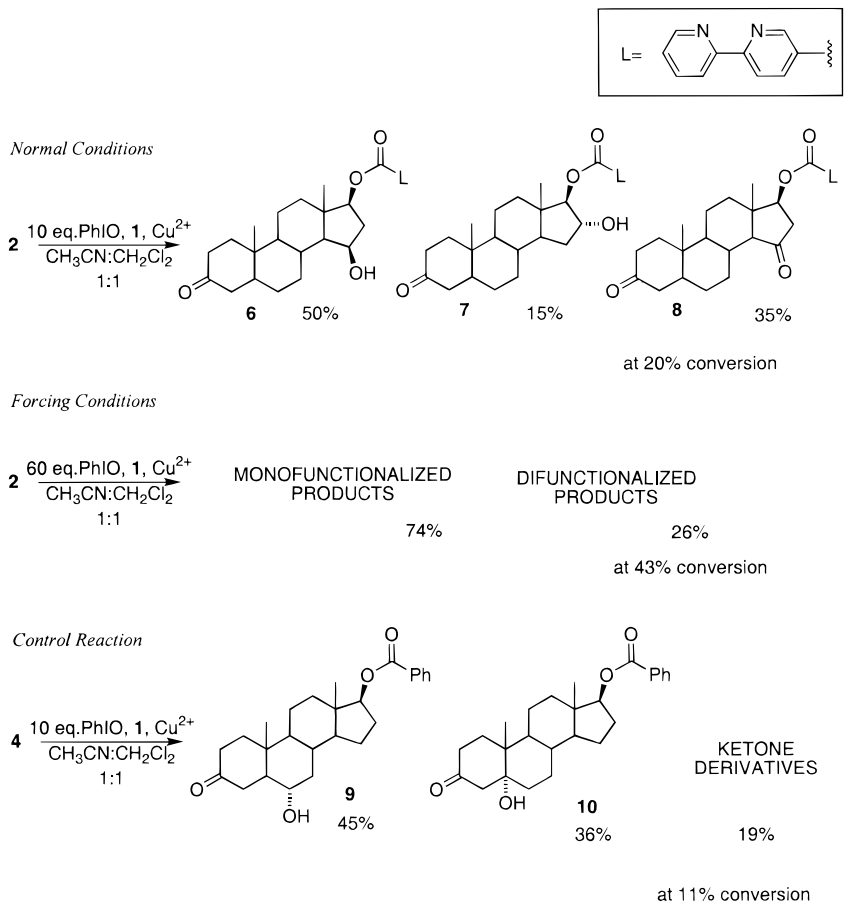


FIG. 1. Selective hydroxylation of a particular carbon on a substrate bound, by metal coordination, to an enzyme mimic with a manganese–porphyrin catalytic group.

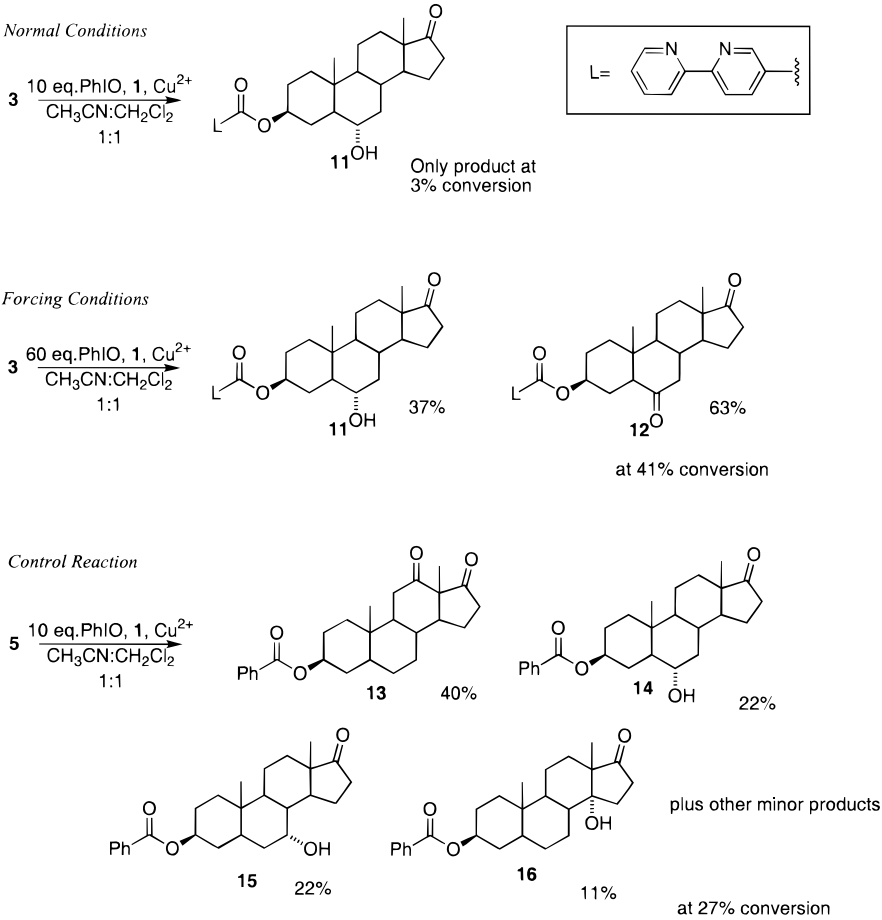
auxiliary group was oxidized to the corresponding N-oxide under the conditions required for C–H activation. Other metal coordinating groups such as picolinate, 2-pyridylhydrazone, and 8-hydroxyquinoline-7-carboxylate were also tested but they all proved to be oxidatively unstable.

We finally settled on the use of 2,2′-bipyridyl-5-carboxylate. The metal chosen for coordination was Cu^{2+}. A bipyridyl group from the substrate and one from the porphyrin should be coordinated around a copper ion in a distorted square planar geometry (*10*). The mutual orientation of steroid and porphyrin within the complex should then determine the regioselectivity of the oxidation.

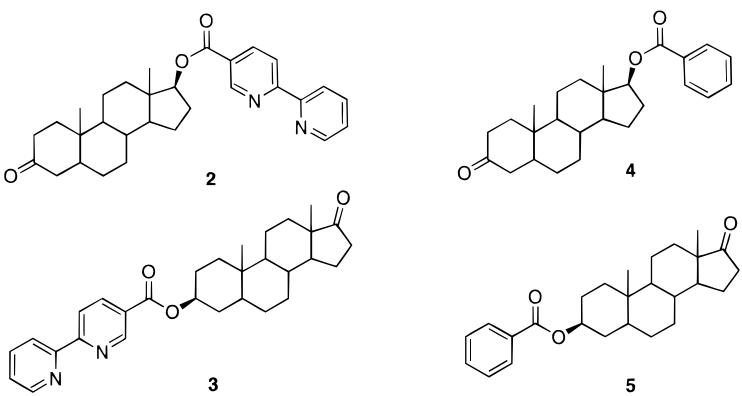
The two androstane derivatives **2** and **3** were prepared and subjected to oxidation by iodosylbenzene (PhIO) catalyzed by **1**. To determine the intrinsic reactivity of these steroid structures in the absence of metal coordination, the corresponding benzoates **4** and **5** were prepared and oxidized in control reactions. The results are reported in Schemes 1 and 2.



SCHEME 1.


Normal Conditions
3
10 eq.PhIO, 1, Cu2+
CH3CN:CH2Cl2
1:1
11
Only product at
3% conversion
L=
Forcing Conditions
3
60 eq.PhIO, 1, Cu2+
CH3CN:CH2Cl2
1:1
11
37%
12
63%
at 41% conversion
Control Reaction
5
10 eq.PhIO, 1, Cu2+
CH3CN:CH2Cl2
1:1
13
40%
14
22%
15
22%
16
11%
plus other minor products
at 27% conversion

SCHEME 2.


2
4
3
5

The androstanolone derivative **2** was first oxidized in a relatively dilute solution (1 mM) in the presence of 0.5 mM (0.5 eq.) catalyst **1** and 2 mM $Cu(OTf)_2$ using 10 eq. of PhIO. The products **6–8**, all derived from oxidation on the D ring of the steroid, were obtained in 50, 15, and 35% yield, respectively, with 20% total conversion.

In a control reaction performed on the corresponding benzoate (**5**), a mixture of products hydroxylated on C5 and C6 was observed together with minor quantities of ketones. It should be noted that in this control reaction higher concentrations were needed for both the substrate and catalyst (5 mM and 1 mM (0.2 eq.), respectively, with 5 mM $Cu(OTf)_2$) in order to obtain detectable amounts of products. Trying to improve the conversion to products, the oxidation of **2** was also attempted at higher substrate and catalyst concentration and adding a larger excess of oxidant (up to 60 eq.) until the reaction did not proceed any further and extensive porphyrin bleaching was observed. Under these forcing conditions 43% conversion to products was obtained, yielding a complex mixture of mono (74%) and difunctionalized (26%) steroids. Although the 15-oxo derivative **6** remained the major product, species derived from the attack on uncomplexed substrate, such as the 5- and 6-hyroxy derivatives, could be detected.

The epiandrosterone derivative **3** appeared to be slightly less reactive than **2**: it was necessary to react a 5 mM solution with 10 eq. of PhIO in the presence of 1 mM (0.2 eq.) catalyst and 5 mM $Cu(OTf)_2$. Under these conditions, it was selectively oxidized to the 6α hydroxy derivative **11** with very low conversion (3%). In the control reaction, run under the same conditions, products of oxidation on C7, C12, and C14 were also observed. In this case we also tried to obtain higher conversion by adding a larger excess of oxidant until no further change was observed. Indeed, higher amounts of products were obtained; at 41% conversion the regioselectivity of oxidation was maintained, and a mixture of 6α hydroxy (37%) and 6-keto (63%) derivatives was obtained.

The results can be rationalized in the context of a slightly distorted square planar Cu^{2+} complex in which both substrate and catalyst contribute a bipyridyl ligand (Fig. 2). The use of CPK models confirms that the observed products are consistent with the proposed model. The orientation effect by metal coordination can be seen at its best in the case of 17-substituted androstanes **2** and **4**. Two completely different sets of products are obtained in the presence or absence of a metal ligand on the substrate.

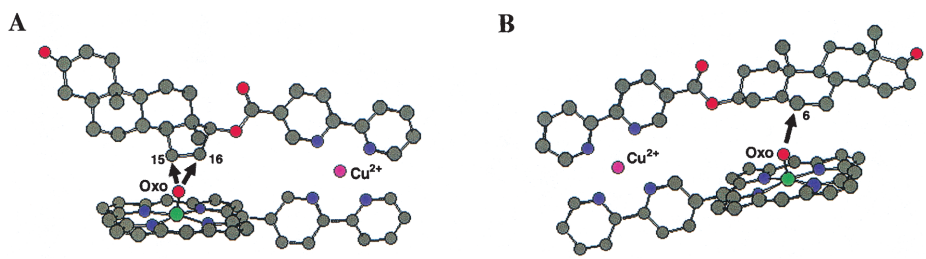


FIG. 2. Molecular models with square planar complexing of catalyst to substrate correctly predict the observed selective hydroxylation products.

In this case intrinsically more reactive positions are completely ignored in favor of the ones that are accessible to the manganese-oxo species within the substrate–catalyst complex. The loss of selectivity under more drastic conditions (higher concentration and larger oxidant excess) is probably due to the oxidation of noncoordinated substrate.

In the case of the 3-substituted steroids **3** and **5**, upon metal coordination the 6 position is selected among the ones that are already intrinsically reactive in the noncoordinating substrate. The observed effect of the metal coordination is to leave only one of those positions accessible for oxidation. Even under forcing conditions the regioselectivity was not lost and catalytic turnover (ca. 2) could be observed.

Taken together, these results indicate that it is possible to induce regiospecific hydroxylation of steroids using metal coordination. The problems encountered in obtaining high conversions, as well as the not perfect selectivity observed in the oxidation of **2**, remain open questions. The low yields and the absence of appreciable catalytic turnovers are probably the results of a combination of low substrate reactivity and the possible prevalence of a "*trans*" configuration around the copper ion, with steroid and porphyrin pointing in opposite directions. Furthermore, the use of the same bipyridyl ligand on substrate and catalyst, motivated by stability considerations, makes possible the abstraction of part of the Cu^{2+} ions from the porphyrin sites. In this situation the formation of unreactive substrate–substrate and porphyrin–porphyrin complexes is likely.

The mixture of products obtained from **2** is comparable to the ones observed in the oxidation of steroids covalently bound to porphyrins (*5*). Furthermore this result is in line with our observation of reduced selectivity and conversion to product whenever a singly binding substrate was used in the epoxidation of alkenes (*8*) or in the hydroxylation of steroids by cyclodextrin based catalysts (*11*).

Attempts to use substrates carrying two bipyridyl moieties resulted in no oxidation, adding further credit to the hypothesis that unreactive homodimers can be formed. Both these problems can probably be solved by using different oxidatively stable and noncompeting ligands. Current work in our laboratory is following this direction.

In conclusion, even though single coordination does not afford complete selectivity, we have shown that it is possible to achieve the geometrically controlled hydroxylation of unactivated C–H bonds using metal coordination as the recognition element in a cytochrome P450 mimic.

MATERIALS AND METHODS

General

Dry CH_2Cl_2 and pyridine were obtained by refluxing over CaH_2. HPLC-grade CH_3CN and all the chemicals used were obtained from Aldrich and used without further purification. PhIO was prepared according to a literature procedure (*12*). The products of hydroxylation were identified based on NMR and MS spectra as indicated below. Calculated chemical shifts were obtained from the substrate methyl shifts, using incremental values from published tables (*13*). Splitting patterns and COSY couplings of new C*H*–OH peaks were consistent with published ones (*14*). UV spectra were recorded with a Cary1E instrument. NMR experiments were run on Bruker 300-, 400-, and 500-MHz instruments. HPLC experiments were run on a Hewlett

Packard 1019 instrument equipped with a Microsorb C18 column. ESI-MS spectra were recorded with a JEOL JMS-LCMate instrument.

Catalyst and Substrate Synthesis

*5,10,15,20-Tetrakis-5-(2,2′-bipyridyl)porphyrin manganese(III)chloride (**1**).* 2,2′-Bipyridine-5-carbaldehyde (*15*) was obtained from the 5-methyl-2,2′-bipyridine (*16*) in a four-step procedure involving oxidation to carboxylic acid, esterification, reduction to alcohol and oxidation under Swern conditions (*17*) in 45% overall yield.

The porphyrin free base was obtained in 19% yield as a purple solid by reaction of 2,2′-bipyridyl-5-carbaldehyde and pyrrole in refluxing propionic acid (9). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 9.54 (br s, 4H), 8.99 (s, 8H), 8.91 (d, J = 8 Hz, 4H), 8.87 (m, 4H), 8.75 (d, J = 8 Hz, 4H), 8.70 (m, 4H), 7.99 (m, 4H), 7.47 (m, 4H), −2.71 (br s, 2H). UV−vis (nm): 424(Soret), 519, 554, 592, 648. ESI-MS: 927 (M + 1).

The title compound was obtained as a green solid in 94% yield by refluxing the free base in a solution of excess Mn_2Cl in DMF under air atmosphere and extracting extensively a CH_2Cl_2 solution of the product with concentrated aqueous EDTA. UV−vis ($CHCl_3$, nm): 245, 284, 378, 404, 423, 480 (Soret), 621.

*3-Oxo-5α-androstan-17β-yl 2,2′-bipyridine-5-carboxylate (**2**).* 2,2′-Bipyridine-5-carboxylic acid (87 mg, 0.44 mmol) was refluxed for 45 min in 5 mL of $SOCl_2$. After solvent removal a solution of androstanolone (160 mg, 0.55 mmol) in 5 mL of dry pyridine was added to the residue and the solution was stirred under Ar at 60°C for 4 h. The product was precipitated by addition of water and purified by preparative TLC (silica gel, CH_2Cl_2:MeOH, 95:5). White solid, 96 mg, 47% yield. ^{1}H NMR (300 MHz, $CDCl_3$) (selected peaks): δ = 9.28 (d, J = 1.8 Hz, 1H), 8.72 (d, J = 4.2 Hz, 1H), 8.50 (m, 2H), 8.39 (dd, $J1$ = 8.4 Hz, $J2$ = 2.4 Hz, 1H), 7.85 (dt, $J1$ = 7.8 Hz, $J2$ = 2.4 Hz, 1H), 7.36 (m, 1H), 4.89 (t, J = 7.8 Hz, 1H, 17α-H), 1.04 (s, 3H, 19-Me), 0.98 (s, 3H, 18-Me). ESI-MS: 473 (M + 1).

*17-Oxo-5α-androstan-3β-yl 2,2′-bipyridine-5-carboxylate (**3**).* The title compound was obtained as a white solid using the procedure described for **2**. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 9.26 (br s, 1H), 8.71 (d, J = 4 Hz, 1H), 8.48 (m, 2H), 8.39 (dd, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.85 (dt, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.36 (m, 1H), 5.00 (m, 1H, 3α-H), 0.925 (s, 3H, 19-Me), 0.88 (s, 3H, 18-Me). ESI-MS: 473 (M + 1).

*3-Oxo-5α-androstan-17β-yl benzoate (androstanolone benzoate) (**4**).* To a solution of androstanolone (46 mg, 0.152 mmol) in 2 mL of dry pyridine was added 1 mL of benzoyl chloride, and the resulting mixture was stirred under inert atmosphere at 60°C for 2 h. The solution was diluted with water and the product extracted with CH_2Cl_2 and washed with water. The crude product was crystallized from ethyl acetate hexanes, yielding the title compound as a white solid (60 mg, 0.158 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) (selected peaks): δ = 8.04 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 4.85 (t, J = 8 Hz, 1H, 17α-H), 1.04 (s, 3H, 19-Me), 0.96 (s, 3H, 18-Me). ESI-MS: 395 (M + 1).

*17-Oxo-5α-androstan-3β-yl benzoate (epiandrosterone benzoate) (**5**).* The compound was obtained in 90% yield from epiandrosterone using the procedure indicated for **4**. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 8.03 (d, J = 7.4 Hz, 2H),

7.54 (t, J = 7.4 Hz, 1H), 7.43 (t, J = 7.4 Hz, 2H), 4.95 (m, 1H, 3α-H), 0.91 (s, 3H, 19-Me), 0.87 (s, 3H, 18-Me). ESI-MS: 395 (M + 1).

Oxidation Procedures

*Oxidation of **2** (normal conditions).* 9.9 mg (20.9 μmol) of substrate **2** were added under Ar to a stirred solution of 9.9 mg of catalyst **1** (9.5 μmol) and 13.9 mg of $Cu(OTf)_2$ (38 μmol) in 20 mL of CH_2Cl_2:CH_3CN, 1:1 (v/v). Then 45 mg (205 μmol, 10 eq. relative to the substrate) of PhIO was added as a solid and the reaction stirred at room temperature under Ar for 16 h. Final reaction conditions: substrate 1 mM, catalyst 0.5 mM, Cu^{2+} 2 mM, PhIO 10 eq. The reaction was quenched by adding an aqueous solution of excess $Na_2S_2O_3$, diluting with CH_2Cl_2 and washing the organic phase with 0.1 M EDTA, pH7, solution (2 $\times$ 10 mL) and water. The organics were collected and dried and the solvent removed. The crude was subjected to a first preparative TLC (silica gel, CH_2Cl_2:MeOH, 95:5) to remove the porphyrin and then analyzed by ^{1}H NMR. The products were isolated by preparative TLC (silica gel, CH_2Cl_2:MeOH, 95:5) and identified by MS and NMR as indicated below.

*Oxidation of **3** (normal conditions).* Substrate **3** (9.3 mg; 19.7 μmol) was added under Ar to a stirred solution of 4.0 mg of catalyst **1** (3.9 μmol), 7.3 mg of $Cu(OTf)_2$ (20 μmol), and 5 μL of pyridine in 4 mL of CH_2Cl_2:CH_3CN, 1:2 (v/v). Then 44 mg (200 μmol, 10 eq. relative to the substrate) of PhIO was added as a solid and the reaction stirred at room temperature under Ar for 16 h. Final reaction conditions: substrate 5 mM, catalyst 1 mM, Cu^{2+} 5 mM, PhIO 10 eq. The reaction was quenched by adding an aqueous solution of excess $Na_2S_2O_3$, diluting with CH_2Cl_2 and washing the organic phase with 0.1 M EDTA, pH 7, solution (2 $\times$ 10 mL) and water. The organics were collected and dried and the solvent removed. The crude was subjected to a first preparative TLC (silica gel, CH_2Cl_2:MeOH, 95:5) to remove the porphyrin and then analyzed by ^{1}H NMR and by HPLC. The products were isolated by preparative TLC (silica gel, CH_2Cl_2:MeOH, 95:5) and identified by MS and NMR as indicated below.

*Oxidation of **2** (forcing conditions).* Substrate, catalyst, $Cu(OTf)_2$, and pyridine were added to 4 mL of CH_2Cl_2:CH_3CN, 1:1 (v/v) in the same quantities indicated above for the normal conditions. PhIO was added every hour in six portions of 44 mg each (6 $\times$ 200 μmol, 60 eq. relative to the substrate total). TLC analysis, performed 1 h after every addition and just before the next one, showed no more conversion to product 1 h after the sixth aliquot had been added. The reaction was stopped at this time. Final reaction conditions: substrate 5 mM, catalyst 1 mM, Cu^{2+} 5 mM, PhIO 60 eq. total. After the usual workup, ^{1}H NMR, MS, and by HPLC analysis revealed the formation of a complex mixture of ketones (mainly the 15-oxo derivative) alcohols and doubly functionalized species. The observed overall conversion (HPLC) was 43%, with 74% of mono- and 26% difunctionalized products.

*Oxidation of **3** (forcing conditions).* The same procedure used for the oxidation of **2** under forcing conditions was used. The solvent was CH_2Cl_2:CH_3CN, 1:2 (v/v). The reaction was quenched and the crude treated as indicated in the previous cases and then analyzed by ^{1}H NMR and by HPLC. The products were isolated by preparative TLC (silica gel, CH_2Cl_2:MeOH, 95:5) and identified by MS and NMR as indicated below.

*Oxidation of **4** and **5** (control reactions).* The benzoates **4** and **5** were oxidized using condition identical to the ones indicated for the oxidation of substrate **2**. Final reaction conditions: substrate 1 mM, catalyst 0.5 mM, Cu^{2+} 2 mM, PhIO 10 eq. The reaction was quenched and worked up in the usual way. The products were quantified in the crude by ^{1}H NMR and HPLC and then isolated by preparative TLC (silica gel, CH_2Cl_2:MeOH, 95:5) and identified by MS and NMR as indicated below.

Identification of the Products

*15β-Hydroxy-3-oxo-5α-androstan-17β-yl 2,2′-bipyridine-5-carboxylate (**6**).* ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 9.29 (br s, 1H), 8.72 (d, J = 4 Hz, 1H), 8.49 (m, 2H), 8.40 (dd, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.86 (dt, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.37 (m, 1H), 4.83 (t, J = 8 Hz, 1H, 17α-H), 4.34 (m, 1H, 15α-H), 1.25 (s, 3H, 18-Me), 1.09 (s, 3H, 19-Me). ESI-MS: 489 (M + 1). COSY: Protons at 4.83 and 4.84 δ are both coupled to a common peak at 2.86 δ (16-H), confirming that the new CH–OH is on C15. The calculated chemical shift values for the methyls are

15α-OH derivative: 18-Me = 1.01δ, 19-Me = 1.05δ

15β-OH derivative: 18-Me = 1.25δ, 19-Me = 1.07δ, so it can be concluded that the product is the 15β-OH derivative.

*16α-Hydroxy-3-oxo-5α-androstan-17β-yl 2,2′-bipyridyl-5-carboxylate (**7**).* ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 9.29 (br s, 1H), 8.72 (d, J = 4 Hz, 1H), 8.49 (m, 2H), 8.40 (dd, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.86 (dt, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.37 (m, 1H), 4.50 (d, J = 4.5 Hz, 1H, 17α-H), 4.30 (m, 1H, 16β-H), 1.05 (s, 3H, 19-Me), 1.03 (s, 3H, 18-Me). ESI-MS: 489 (M + 1). COSY: Protons at 4.50 and 4.30 δ are coupled to each other, confirming that the new CH–OH is on C16. The calculated Me shift values are

16α-OH derivative: 18-Me = 0.99δ, 19-Me = 1.03δ

16β-OH derivative: 18-Me = 1.23δ, 19-Me = 1.06δ, so it can be concluded that the product is the 16α-OH derivative.

*3,15-Dioxo-5α-androstan-17β-yl 2,2′-bipyridyl-5-carboxylate (**8**).* ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 9.29 (br s, 1H), 8.72 (d, J = 4 Hz, 1H), 8.49 (m, 2H), 8.40 (dd, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.86 (dt, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.37 (m, 1H), 5.24 (t, J = 10 Hz, 1H, 17α-H), 2.95 (m, 1H, C16-H), 2.28 (m, 1H, C16-H), 1.05 (s, 3H, 19-Me), 1.06 (s, 3H, 18-Me). ESI-MS: 487 (M + 1). COSY: The two protons at 2.95 and 2.28 δ that are next to the new carbonyl are coupled to each other as well as to 17α-H, indicating the 15-oxo substitution. Calculated chemical shifts: 18-Me = 1.06δ, 19-Me = 1.05δ.

*6α-Hydroxy-3-oxo-5α-androstan-17β-yl benzoate (**9**).* ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 8.03 (d, J = 7.5 Hz, 2H), 7.54 (t, J = 7.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 4.85 (t, J = 8 Hz, 1H, 17α-H), 3.50 (m, 1H, 6β-H), 1.05 (s, 3H, 19-Me), 0.96 (s, 3H, 18-Me). ESI-MS: 411 (M + 1). Calculated chemical shifts: 18-Me = 0.96δ, 19-Me = 1.07δ.

*5α-Hydroxy-3-oxo-androstan-17β-yl benzoate (**10**).* ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 8.04 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 4.85 (t, J = 8 Hz, 1H, 17α-H), 2.70 (d, J = 15 Hz, 1H, 4β-H), 2.12 (d, J = 15 Hz, 1H, 4α-H), 1.20 (s, 3H, 19-Me), 0.96 (s, 3H, 18-Me). COSY:

Protons at 2.70 and 2.12δ are strongly coupled to each other. NOE: Coupling between the 19-Me peak at 1.20δ and the 4β-H peak at 2.70δ. ESI-MS: 411 (M + 1). Calculated chemical shifts: 18-Me = 0.96δ, 19-Me = 1.22δ.

6α-Hydroxy-17-oxo-5α-androstan-3-yl 2,2′-bipyridine-5-carboxylate ***(11)***. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 9.26 (br s, 1H) 8.71 (d, J = 4 Hz, 1H), 8.48 (m, 2H), 8.39 (dd, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.85 (dt, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.36 (m, 1H), 5.00 (m, 1H, 3α-H), 3.50 (m, 1H, 6β-H), 0.94 (s, 3H, 19-Me), 0.88 (s, 3H, 18-Me). ESI-MS: 489 (M + 1). Calculated chemical shifts: 18-Me = 0.88δ, 19-Me = 0.95δ.

6,17-Dioxo-5α-androstan-3-yl 2,2′-bipyridine-5-carboxylate ***(12)***. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 9.26 (br s, 1H), 8.71 (d, J = 4 Hz, 1H), 8.48 (m, 2H), 8.39 (dd, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.85 (dt, $J1$ = 8 Hz, $J2$ = 2 Hz, 1H), 7.36 (m, 1H), 5.00 (m, 1H, 3α-H), 0.925 (s, 3H), 0.88 (s, 3H). ESI-MS: 489 (M + 1).

Upon treatment with 5%KOH in MeOH the ester 12 was converted to the known androstan-3b-ol-6,17-dione. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 3.57 (m, 1H, 3α-H), 0.88 (s, 3H, 19-Me), 0.88 (s, 3H, 18-Me). Literature (18): δ = 0.86 (s, 3H, 19-Me), 0.78 (s, 3H, 18-Me).

12,17-Dioxo-androstan-3β-yl benzoate ***(13)***. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 8.04 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 4.95 (m, 1H, 3α-H), 2.51–2.36 (m, 3H), 2.24–2.14 (m, 1H), 1.21 (s, 3H, 18-Me), 0.98 (s, 3H, 19-Me). ESI-MS: 409 (M + 1). Calculated chemical shifts: 18-Me = 1.25δ, 19-Me = 1.01δ.

6α-Hydroxy-17-oxo-androstan-3β-yl benzoate ***(14)***. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 8.04 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 4.95 (m, 1H, 3α-H), 3.50 (m, 1H, 6β-H), 0.92 (s, 3H, 19-Me), 0.88 (s, 3H, 18-Me). ESI-MS: 411 (M + 1). Calculated chemical shifts: 18-Me = 0.87δ, 19-Me = 0.94δ.

7α-Hydroxy-17-oxo-androstan-3β-yl benzoate ***(15)***. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 8.04 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 4.95 (m, 1H, 3α-H), 4.11 (narrow m, 3H), 0.89 (s, 3H, 19-Me), 0.87 (s, 3H, 18-Me). ESI-MS: 411 (M + 1). Calculated chemical shifts: 18-Me = 0.88δ, 19-Me = 0.90δ.

14α-Hydroxy-17-oxo-androstan-3β-yl benzoate ***(16)***. ^{1}H NMR (500 MHz, $CDCl_3$) (selected peaks): δ = 8.04 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 4.95 (m, 1H, 3α-H), 1.01 (s, 3H, 18-Me), 0.91 (s, 3H, 19-Me). ESI-MS: 411 (M + 1). Calculated chemical shifts: 18-Me = 0.99δ, 19-Me = 0.91δ.

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