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Catalytic Oxidations of Steroid Substrates by Artificial Cytochrome P-450 Enzymes†

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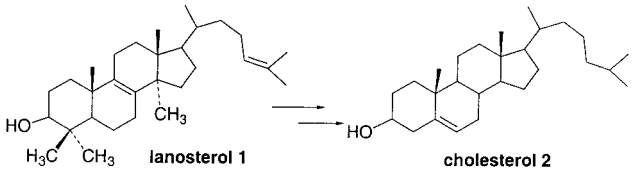
Catalysts comprising manganese−porphyrins carrying cyclodextrin binding groups are able to perform hydroxylations with substrate selectivity and regio- and stereoselectivity and high catalytic turnovers. The geometries of the catalyst/substrate complexes override intrinsic substrate reactivities, permitting attack on geometrically accessible saturated carbons of steroids in the presence of secondary carbinol groups and carbon−carbon double bonds, as in enzymatic reactions. Selective hydroxylations of steroid carbon 9 positions are of particular practical interest.

Introduction

Regioselectivity in organic synthesis is normally achieved by the manipulation of substrate functional groups. Reactions occur at the position of the substrate that has the greatest intrinsic reactivity (sometimes modified by neighboring groups, as in the selective reactivity of the double bonds of allylic alcohols toward coordinating reagents[1a] or the intramolecular attack of a substrate functional group on another position.[1b]) If this is not desired, that most reactive position can be blocked with a protecting group or another position activated by changing the functional group. That is, regioselectivity in chemical synthesis is typically dominated by the competitive reactivity of sections of the substrate toward relatively random reagents.

By contrast, regioselectivity in biochemistry is achieved as the result of the geometry of an enzyme/substrate complex, and that regioselectivity can completely override the normal reactivity pattern of the substrate. A striking example is the selective oxidation of three unactivated methyl groups in lanosterol (**1**) by a cytochrome P-450 enzyme in the biosynthesis of cholesterol (**2**), while two double bonds and a secondary carbinol group are left untouched.[2a] Such dominant geometric control of regioselectivity in enzymatic reactions is so common, and so unlike normal organic synthetic selectivity, that fermentation methods are often used as part of pharmaceutical manufacturing. For example, biological fermentations are used in the synthesis of corticosteroids to achieve the oxygenation of ring C in steroids, replacing much more tedious chemical methods to produce the compounds.[2b,c]



We have been developing chemical methods that imitate such geometric control, to achieve the kinds of regioselectivities heretofore possible only with enzymes. We coined the term biomimetic chemistry to describe such methods.[3] In the earliest approach, we showed that a benzophenone ester of a steroid could undergo intramolecular photochemical hydrogen abstractions, affording regioselective functionalization of the steroid remote from the ester attachment point.[4] We referred to this process as remote functionalization.[5] Benzophenone esters with different geometries afforded different products.

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We then developed methods to achieve remote functionalization by free radical chain reactions. In the first method, we attached a phenyliodine dichloride species to the steroid, again as an ester, and saw that upon irradiation it chlorinated specific carbons remote from the ester attachment point.[6] In a later related scheme, we attached only an iodophenyl group to the substrate as a template and saw that it directed selective chlorination by a process we described as a radical relay reaction.[7] It was also a catalyst for the reaction—without the iodine atom there was no substrate chlorination under the reaction conditions. Other templates and radical reactions were developed as well and their geometries directed the halogenations to other selected spots on the substrates.[8]

These methods are powerful, and indeed, using the radical relay method, we achieved the synthesis of cortisone, in which the ring C functionalization was geometrically directed, as in an enzyme.[9] However, there were drawbacks to these methods. The reagent or template was attached, or in one case coordinated,[10] to the substrate by a single linkage, so there was considerable flexibility. The geometric control was chiefly a control of the *distance* of the attack point from the attachment point. Also, the reagents had some intrinsic chemical selectivity, and functionalizations were partly directed by the greater reactivity of tertiary hydrogens relative to secondary or primary hydrogens.

To achieve completely geometric control of regioselectivity, it would be necessary to have two or even three well-defined interactions between substrate and catalyst, but this becomes unreasonable with covalent attachments. The template catalysts in remote halogenation can be recovered after the process, but there is not true turnover catalysis by such a process (although in one case we attached *three* substrates to such a template and saw that all were selectively halogenated, with thus a turnover of 3).[11] To justify developing a catalyst that could hold a substrate with more than one binding interaction, we need true turnover catalysis so that an expensive catalyst, in terms of the chemical effort needed to make it, can process hundreds or thousands of substrate molecules. Thus, the catalyst/substrate interactions should not use stable covalent bonds.

In one approach, we examined a system in which *two* ion-pairing interactions stretched a flexible substrate above a benzophenone unit.[12] Photolysis did indeed steer reaction into a geometrically well-defined spot, but this is of course not a catalytic reaction. For turnover catalysis, we made a catalyst with an iron porphyrin unit carrying four hydroxyquinoline metal-binding groups. We saw that its Cu^{2+} complex could bind a substrate having two metal coordinating groups and catalyze the epoxidation of its double bond with eight turnovers and good selectivity over noncoordinating substrates.[13] We then turned to hydrophobic binding into cyclodextrins in water solution.

The metalloporphyrin system has been developed extensively to mimic cytochrome P-450 enzymes.[14] We attached cyclodextrin units to it so it could reversibly bind substrates in well-defined positions. The aim was to achieve selective oxidations of the bound substrates directed *entirely* by the geometry imposed by *multipoint binding*. Performing the reactions in water also has environmental advantages, since water is an environmentally benign solvent that can be easily purified. As expected, we saw that performing the reactions in other solvents greatly diminished or erased the yields of products, supporting the importance of hydrophobic binding.

Two related studies on biomimetic regioselectivity should be mentioned here. Groves and Neumann examined the epoxidation and hydroxylation of steroids in a lipid bilayer with a metalloporphyrin carrying hydrophobic side chains.[15] They observed good selectivity, but strong product binding to the catalyst prevented catalytic turnover. Also, Grieco attached a manganese porphyrin to a steroid covalently and saw that the system could perform directed steroid hydroxylation controlled by the distance between the attachment point and the metal oxo species.[16] This covalent system is related to the benzophenone and iodophenyl systems described above, and like them it cannot produce turnover catalysis.

Some of our work has been described in preliminary form elsewhere.[17−20] In this paper, we describe the details of part of the published work and the extension to important new areas.

Results and Discussion

β-Cyclodextrin (β-CD) is a cyclic heptaglucose oligomer that dissolves in water and binds hydrophobic species into its central space. Using the natural cytochrome P-450 enzyme as our model, we synthesized some metalloporphyrin catalysts that retain the natural porphyrin catalytic core and have attached β-CDs to produce an artificial binding pocket (Figure 1). The first example, **3**, with two cyclodextrins and an iron porphyrin core, was poorly water soluble, and the catalyst itself was easily

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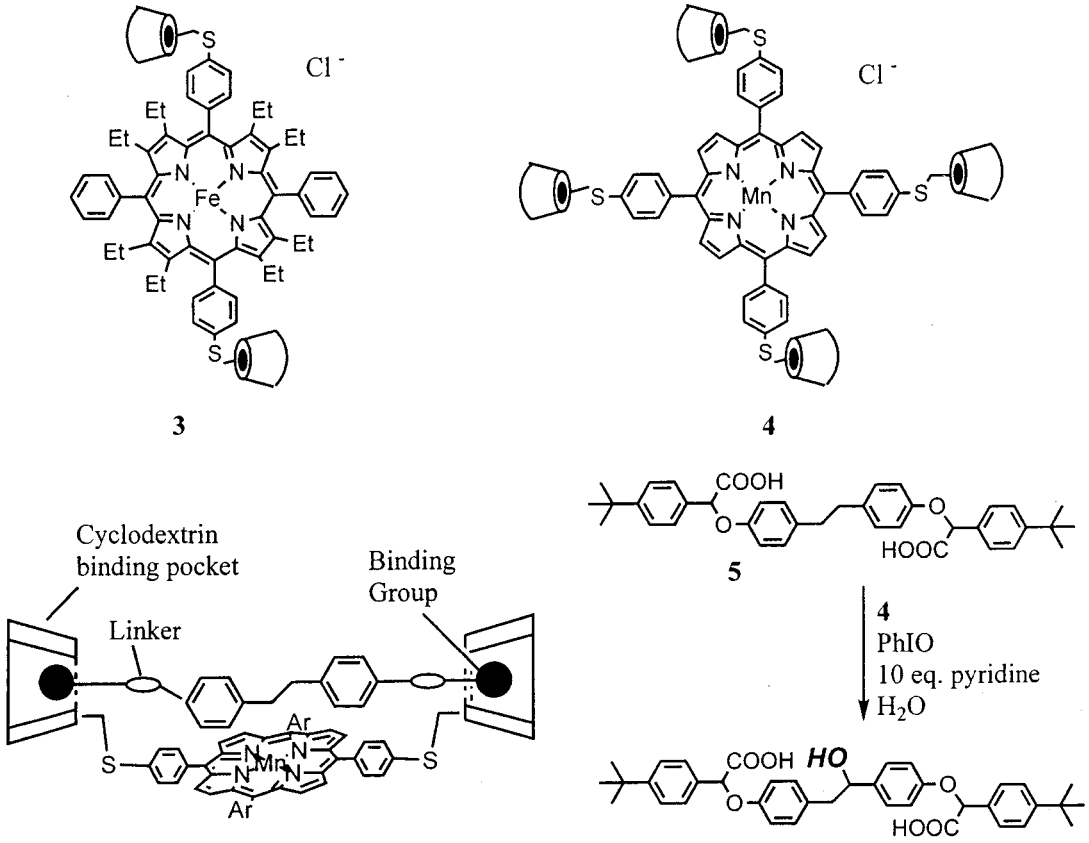

FIGURE 1. Early catalyst **3** (Fe^{III} form) and the better catalyst **4** (Mn^{III} form). Substrate **5** binds to **4** and is hydroxylated with hundreds of turnovers.

destroyed oxidatively. Thus, we turned to catalyst **4**, carrying four cyclodextrin rings to increase water solubility. As metal we used manganese, not the iron used by the enzyme; the iron analogue of **4** was also effective in our system, but less so than the manganese version. Groves has reported such a catalytic preference for manganese in cytochrome P-450 model systems.[21] Catalyst **4** was able to bind a simple dihydrostilbene substrate **5** in water and catalyze the hydroxylation of saturated carbons, using excess iodosobenzene as oxidant. There were up to 650 turnovers and rates somewhat faster than those of natural enzymes for similar benzylic oxidations.[18]

Oxidants other than iodosobenzene have been used in such P-450 model systems,[22] but we find that iodosobenzene is the best reagent in this and the work to be described subsequently. Also, it was important to steer the oxygen atom bonded to the metal, and the bound substrate, to the same face of the catalyst. Thus, in this and the remaining work to be described we added several equivalents of pyridine to bind to one face of the metalloporphyrin and steer substrate and oxygen to the other face; pivalate ion and imidazole were also able to do this, but less effectively than pyridine. At the end of this paper, we will mention a new catalyst in which the pyridine ring is part of the catalyst, and no external pyridine is required.

Such hydroxylations involve the formation of an intermediate porphyrin metal oxo compound, which then oxidizes a C−H bond. There has been some dispute about the hydroxylation mechanism. One choice, proposed by Groves, is the oxygen rebound mechanism—the metal oxo species abstracts hydrogen from the substrate to produce a substrate radical, which then captures the hydroxyl group from the metal.[23] The other choice is a direct oxygen insertion into the C−H bond by the metal oxo species.[24] A theoretical treatment apparently supports the oxygen rebound mechanism.[25]

We examined the epoxidation of some stilbene derivatives that could bind into catalyst **4** and saw sensible substrate selectivity.[17] In line with our modeling, we found that an analogue of **4** carrying only two trans

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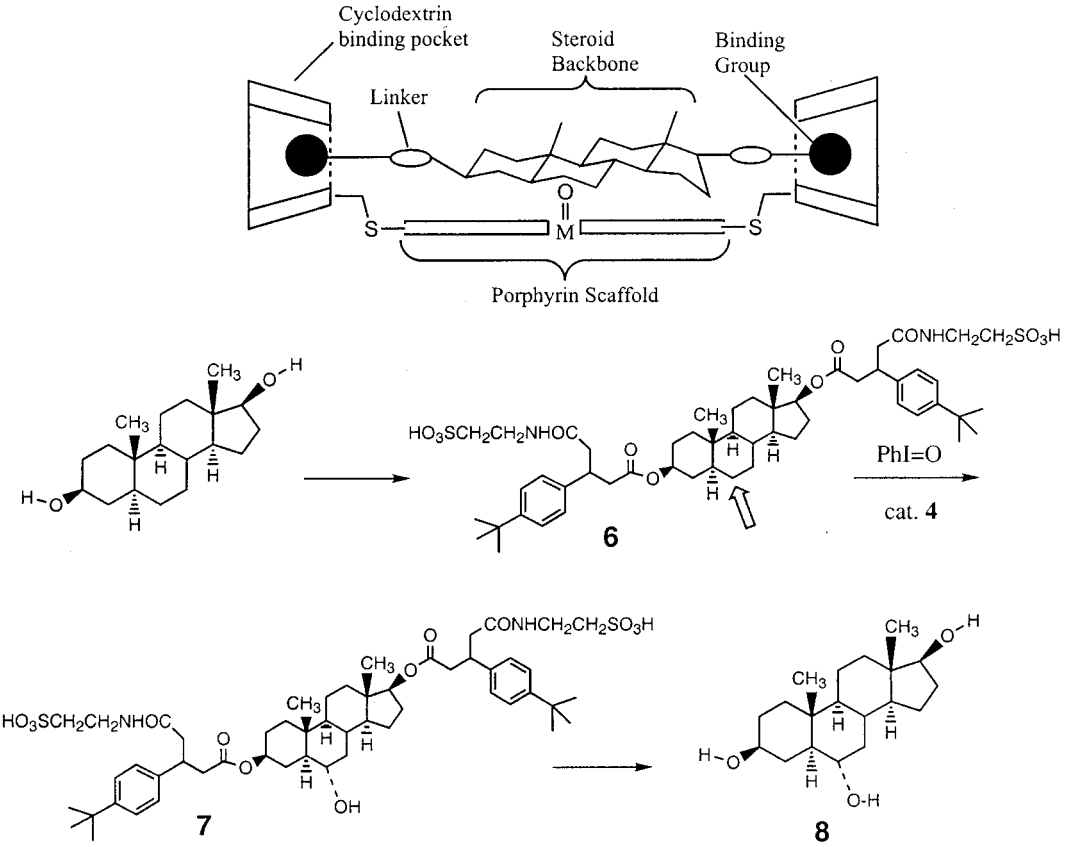

FIGURE 2. Selective hydroxylation of substrate **6** with catalyst **4**, forming only the C-6 alcohol **7**, hydrolyzed to triol **8**.

cyclodextrins and two unsubstituted phenyls was a catalyst comparable to **4**, but the analogous cis isomer was not. For catalysis, the substrates do indeed need to bind *across* the porphyrin ring.

We examined the effect of converting the sulfide links in **4** to sulfone groups, which might occur during oxidation reactions. We synthesized the corresponding tetrasulfone (see the Experimental Section) by oxidation of the porphyrin derivative with magnesium monoperoxyphthalate, followed by manganese incorporation, and found that it was also a catalyst for the hydroxylation of **5**, but with only 18% of the yield seen with **4**. Thus, the sulfide links are better than sulfones in the catalyst.

Of course, there is no unusual positional selectivity in the oxidation of **5**, but we did see that an analogue of **5** lacking the *tert*-butylphenyl groups was not oxidized under these conditions, so there was substrate selectivity. In a more striking and important case, we saw that catalyst **4** was able to bind a steroid **6** carrying two *tert*-butylphenyl binding groups along with water-solubilizing sulfonate groups, but not an analogue without the *tert*-butylphenyl binding group, and perform regioselective hydroxylation at the steroid C-6 position (Figure 2).[18] The reaction was also stereospecific, yielding only the equatorial C-6 alcohol **7**, whose hydrolysis afforded triol **8**. However, this reaction proceeded with only four to five turnovers, in contrast to the 650 turnovers for the more reactive **5**. After these turnovers, UV/vis spectra indicated that the porphyrin system had been oxidatively destroyed.

To obtain higher turnover catalysis in the steroid case, we synthesized catalyst **9** and saw that with iodosobenzene it catalyzed the clean conversion of **6** to **7** in water with the same selectivity as with **4**.[19] With 0.1 mol % catalyst there was 18.7% conversion of **6** to **7**, indicating 187 turnovers. With 1 mol % catalyst there was again 100% yield of **7**, but with only 95 turnovers, so at high conversion there might be some inhibition by product binding to the catalyst. These carefully obtained numbers came from an analysis involving complete conversion of the alcohols to benzoate esters, and then calibrated quantitative hplc analysis of the benzoates.

Fluorination of the phenyl rings has been reported to stabilize tetraarylporphyrins against autoxidative destruction.[26–28] We created a particularly easy synthesis of **9**—reaction of the porphyrin **10** carrying pentafluorophenyl rings with the cyclodextrinthiol (Figure 3). The para fluorines are selectively replaced; ortho and para positions are activated to nucleophilic attack, and the ortho positions are hindered.

The specific hydroxylation of a steroid at C-6 is interesting, and the finding that the product is a secondary alcohol is striking. When steroids are oxidized with high concentrations of unbound porphyrins and iodosobenzene, we find that steroidal secondary alcohols are normally simply oxidized to ketones, before any hydroxylations of saturated carbons occur. This reflects the

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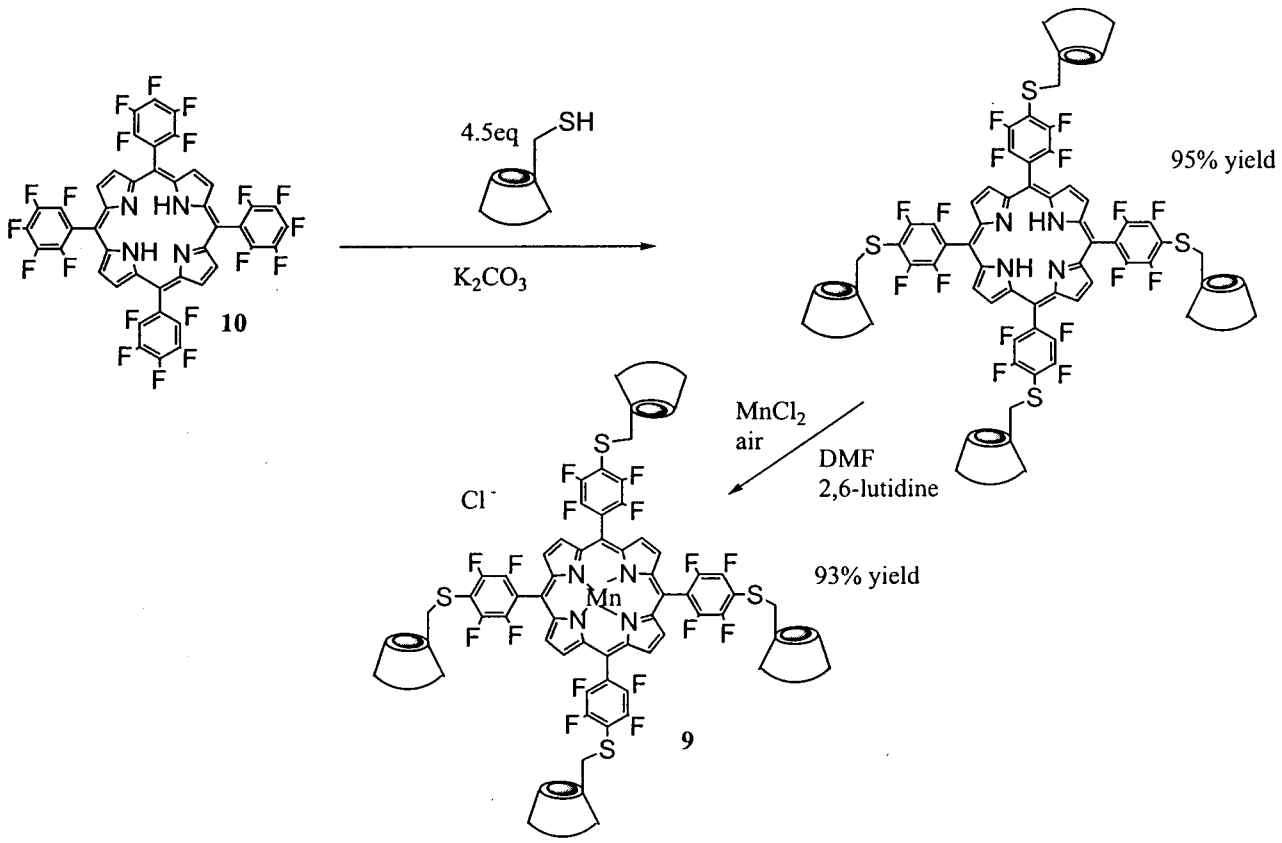


FIGURE 3. Synthesis of the fluorinated catalyst **9** from porphyrin **10**.

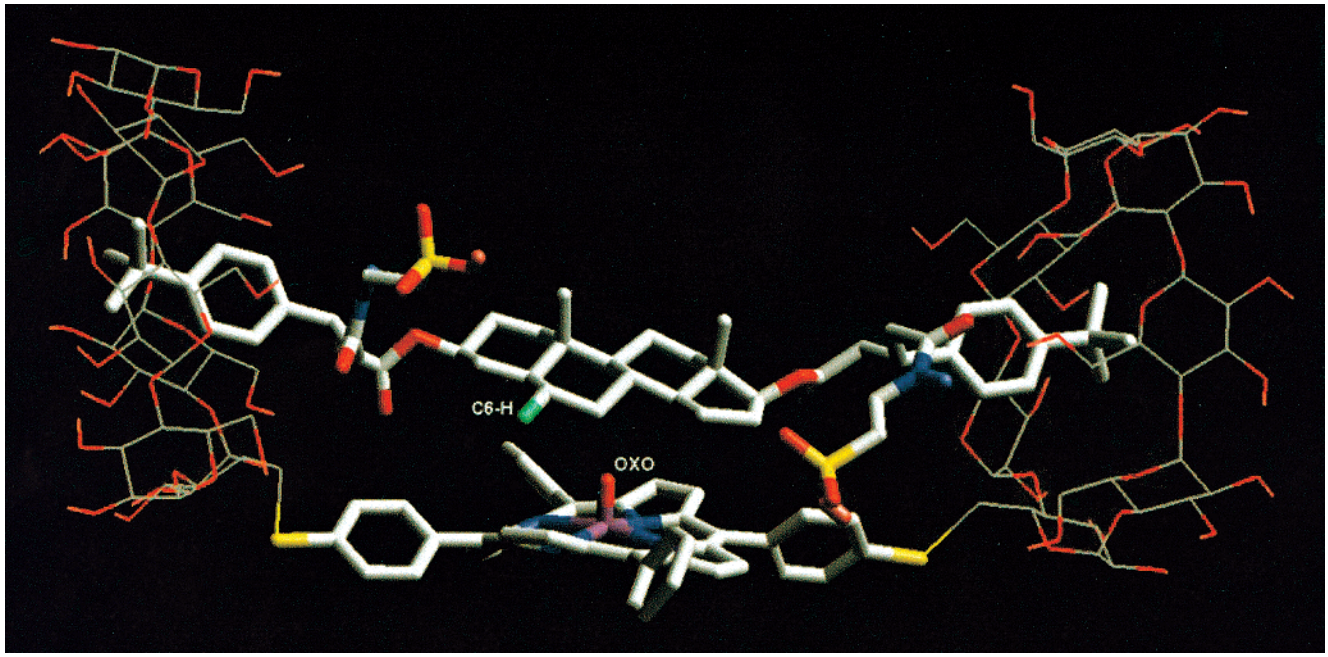


FIGURE 4. Computer model of the complex of substrate **6** with catalyst **9**. For clarity, only the two cyclodextrins involved in binding are shown. The oxygen atom on the manganese is almost in van der Waals contact with the C-6 equatorial hydrogen, but the C-6 axial hydrogen is inaccessible.

higher oxidative reactivity of a secondary carbinol. The fact that we saw *no* ketone in the oxidation of **6** bound to **4** or **9**, even with many turnovers, is an indication that geometric control has overcome normal functional group reactivity. We propose that the oxidation of a carbinol to a ketone by a metal oxo porphyrin intermediate requires that the oxygen atom remove the carbinol hydrogen of the C−H group and that this is not accessible in the

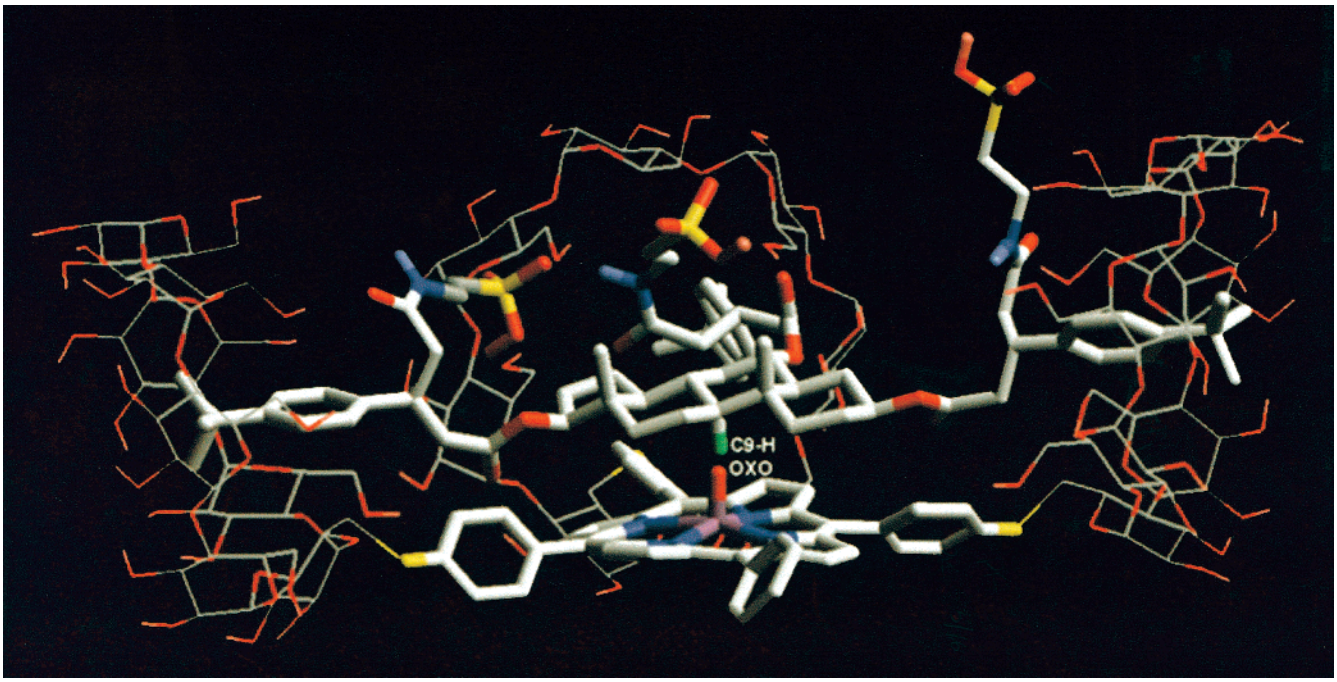


FIGURE 5. Computer model of the complex of substrate **11** with catalyst **9**. For clarity, the cyclodextrin ring not involved in binding is hidden. The oxygen atom on the manganese is in van der Waals contact with the C-9 hydrogen.

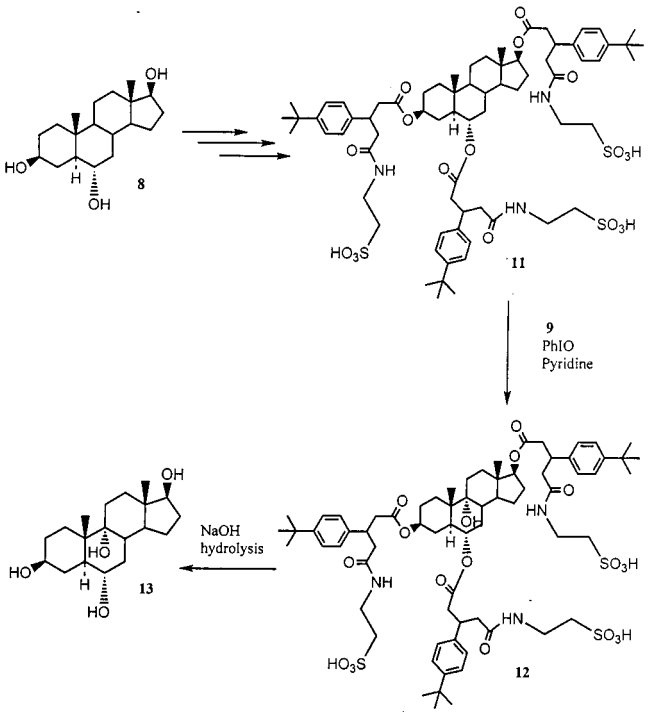


FIGURE 6. Conversion of triol **8** to substrate **11** and its hydroxylation at C-9 to form product **12**, which was hydrolyzed to tetraol **13**.

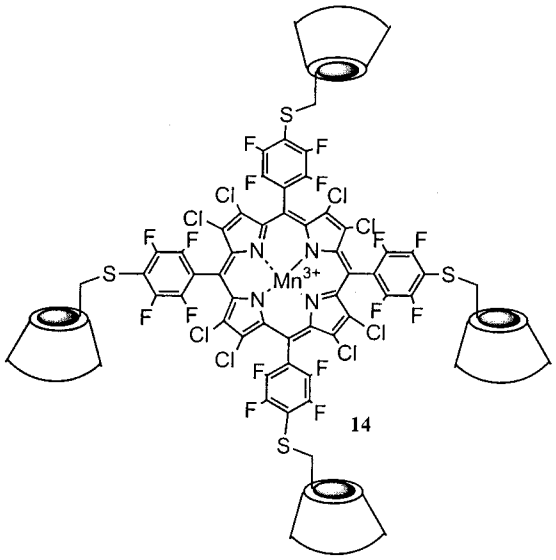
complex of product **7** with the catalysts. The β-hydrogen on C-6 is axial, and models (see Figure 4) show that it is not accessible in the product/catalyst complex.

Hydroxylation of a steroid at C-9 or at C-11 would be more important. Both these positions can be hydroxylated by enzymes in fermentation processes[2] and the resulting carbinols dehydrated to afford a 9(11) double bond. Then epoxidation and HF opening affords 9-fluoro-11-hydroxy steroids that are useful corticosteroid derivatives. Thus, we determined to alter the geometry of our catalyst/substrate complexes so as to direct hydroxylation to C-9 of the steroid.

It was clear from the result and from molecular and computer models (see Figure 4) that the C-6 hydroxylation occurs because the complex of substrate **6** into the catalysts **4** and **9** prefers a geometry in which the steroid edge projects toward the porphyrin unit. To achieve hydroxylation at C-9, it seemed that we simply had to change this geometry so that the steroid α face was above the Mn=O species. Models showed (see Figure 5) that this would occur if we made a *triple* ester of product triol **8** so that *three* of the cyclodextrins in catalyst **9** could bind the substrate. This is what occurred.

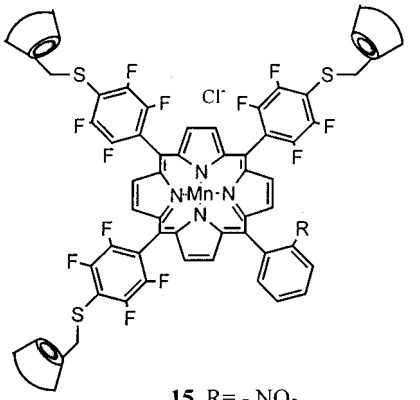
Triol **8** was converted to a triple binding steroid substrate **11**. When this was oxidized by catalyst **9**, the C-9-hydroxylated product **12** was indeed found to be the only significant product of the reaction (Figure 6), consistent with the models. Ester hydrolysis converted the hydroxytriester to the tetraol **13**. With 1 mol % **9**, substrate **11** was converted to **13** with 72% conversion and 100% yield, indicating 72 turnovers. With 0.1 mol % **9**, product **13** was formed in 8% conversion but again 100% yield, so there were 80 turnovers. We never saw any C-9 hydroxylation of the double binding substrate **6** or of its 6-hydroxylated product **7**, so the geometry forced by the triple binding of **11** is apparently not accessed without the extra binding group.

We also synthesized a perhalogenated version **14** of our catalyst, differing from **9** by the incorporation of chlorine atoms at the β-pyrrole porphyrin positions. Catalyst **14** showed a 2-fold increase in efficiency compared with **9,** oxidizing **6** at C-6 with up to 356 turnovers and **11** at C-9 with up to 152 turnovers.



14

In recent work,[29] we have synthesized a manganese porphyrin derivative **15** carrying three cyclodextrin groups and one *o*-nitrophenyl group. Also, compound **16** was derived from it, with a pyridine ring that can coordinate to the manganese atom. The hydroxylation of **6** to **7** again occurred with these catalysts, but with 2000 turnovers for **16** and 3000 turnovers for **15**. The electron-withdrawing group of **15** probably diminishes the rate at which the catalyst itself is oxidatively destroyed, and there may also be steric effects.

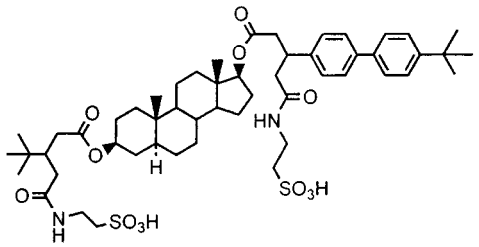


15 R= - NO_2

16 R= - HN

In our earlier studies on template-directed free-radical reactions of steroids, we saw that we could change the position of chlorination by changing the length of the template group. Now we wanted to see whether we could change the position of hydroxylation by using double binding of androstandiol, but with different binding groups at C-3 and C-17. Our first approach was to attach hydrophobic binding groups with short linkers to the C3-OH in 3β,17β-androstanediol and a hydrophobic binding group with a longer linker on the C17-OH. We expected that the relative position of the steroid backbone with respect to the metalloporphyrin would change such as to position steroid carbons in ring D over the reactive metal−oxo species in the catalyst−substrate complex.

In our first rationally designed substrate, we removed the phenyl ring on the linker attached at the steroid 3 position of substrate **6** and inserted it on the phenyl ring of the linker attached at the 17 position of the steroid. The resulting substrate **17**, with a *tert*-butyl binding group on the linker at the 3 position of the steroid and a *tert*-butylbiphenyl binding group on the linker attached to the 17 position of the steroid, would change the position of the steroid in our substrate−catalyst system without changing the length of the substrate. Furthermore, the steroid should be moved by four carbon atoms relative to the porphyrin metal center, so oxidation should occur on the D ring, specifically on C-15, instead of the original C-6 position.



17

Substrate **17** was subjected to our standard oxidizing conditions with catalyst **9** at 0 °C, resulting in a mixture of three steroids after ester hydrolysis. We did see hydroxylation at C-15, but the 7β-hydroxylated product **18** was produced in a 3:1:1 ratio over the 6α-hydroxylated **8** and the 15α-hydroxylated **19** products, as determined by HPLC assay. Our prediction that the major hydroxylated product would be at the steroid 15 position was not correct. This could be due to the ability of the *tert*-butylbiphenyl group in **17** to move within the cyclodextrin cavity such as to bind either phenyl ring in the linker instead of only the desired ring carrying to the *tert*-butyl group.

Therefore, substrate **20**, containing two methyl groups on the *tert*-butylbiphenyl binding group, was synthesized so as to prevent this flexibility of movement of the binding group within the cyclodextrin cavity (Figure 7).

When substrate **20** was subjected to our hydroxylation conditions, the result was a dramatic suppression of the 7-hydroxylated product **18**. However, a 1:1 mixture of the 6α (**8**) and 15α (**19**) product was produced. We rationalize this result by concluding that the *tert*-butyl binding group attached at the steroid 3 position in **20** is not hydrophobic enough to produce tight binding.

Substrate **21** was then synthesized containing an adamantyl binding group linked to the 3 position of the steroid. The adamantylglutaric acid group is a strong binder to β-cyclodextrin, with a measured binding constant (K_a) of approximately 25 000 M^{-1} in pH 10 phosphate buffer. For comparison, 3-*tert*-butylglutaric acid was measured to have a K_a of ~400 M^{-1} to β-cyclodextrin under the same conditions. Hydroxylation of substrate **19** resulted in a 2-fold selectivity for the predicted 15α-hydroxylated product **19** over triols **8** and **18**. Thus, we were indeed able to move the preferred position of hydroxylation to the predicted C-15 position to some

(29) Breslow, R.; Yang, J.; Yan, J. *Tetrahedron* **2002**, *58*, 653−659.

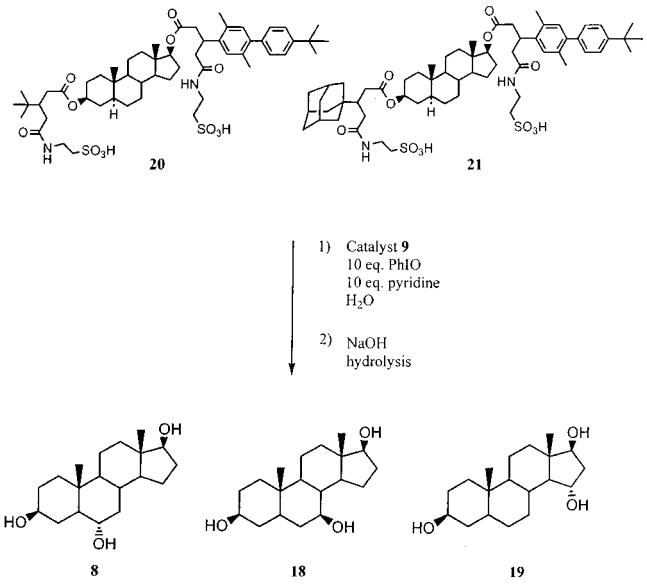


FIGURE 7. Substrates **20** and **21** move the hydroxylation position by catalyst **9** to the right, forming (after hydrolysis) the 7β derivative **18** and the 15α derivative **19**, with a diminished amount of **8**.

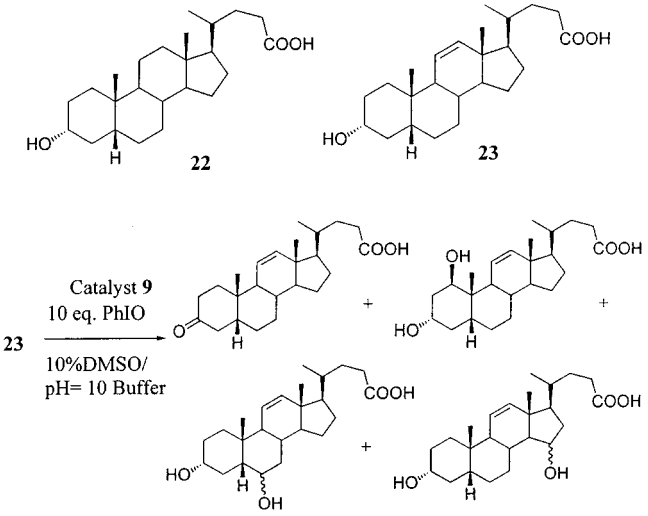


FIGURE 8. Lithocholic acid **22** and lithocholenic acid **23**. Oxidation of the unsaturated **23** with catalyst **9** affords products from hydroxylation in rings A, B, and D, but no product from oxidation of the double bond in ring C, or next to it. In the complex of **23** with **9**, ring C is inside a cyclodextrin and thus inaccessible to the porphyrin catalytic unit.

extent, but there was not such good selectivity as we saw with substrates **6** or **11**. The cyclodextrins are attached to the porphyrin units in our catalysts by relatively flexible links, so the geometries are not well-defined enough for complete selectivity in the hydroxylations. Other work is underway to improve this situation.

Studies on androstane substrates with only one binding group, linked either at the 3 or 17 position of the steroid, resulted in a low yielding mixture of several compounds with no obvious major product. However, with catalyst **9** we were able to hydroxylate lithocholic acid **22** and lithocholenic acid **23**, without any added binding group. These bind well enough[34] into a single cyclodextrin to be functionalized, producing some of the 3 ketone along with 1,3−3,6- and 3,15-diols (α and β at C-15) (Figure 8). The striking finding was that only saturated carbons were hydroxylated in lithocholenic acid. The binding geometry of this compound[34,35] puts the double bond inside a cyclodextrin, out of reach of the catalytic group. Thus, in this case, geometric control in the complex lets us oxidize saturated carbons without attacking the normally more reactive double bond or an allylic position. This finding also indicates that we have enabled geometric control to override intrinsic substrate reactivities, as in natural enzymes.

Conclusions

1. Metalloporphyrins with attached cyclodextrin groups can bind various steroid derivatives and catalyze their selective hydroxylations.
2. The selectivities observed are consistent with molecular and computer models of the complexes.
3. In the best cases, we achieve hundreds and even thousands of catalytic turnovers of the selective reactions.
4. The geometries of the complexes override intrinsic reactivities, preventing oxidation of an otherwise reactive secondary carbinol or of a carbon−carbon double bond. This mimics selectivity effects typically seen only in enzymatic reactions.
5. The catalytic selective hydroxylation at carbon 9 in an androstane derivative provides entry into potential corticosteroid precursors.

Experimental Section

The molecular models shown in Figures 4 and 5 were obtained from Monte Carlo calculations performed using the program Macromodel version 7.0 (Schrödinger, Inc.), using water as continuous solvent and energy minimization as the geometries were varied. Further details of the calculations are described in the references.[20]

Syntheses of the Catalysts. 4-Methylsulfoxybenzaldehyde (**24**) (7.714 g, 46 mmol) was dissolved in 200 mL of propionic acid under air. The solution was heated to 80 °C and allowed to stir for 10 min. Pyrrole (3.18 mL, 46 mmol) was added quickly to the flask, and the solution was heated to reflux at 150 °C for approximately 1 h. The propionic acid was then removed under reduced pressure, and the solid was run through a plug of SiO_2 with 95/5 CH_2Cl_2/MeOH. The crude product was washed several times with pure methanol to remove the white and blue fluorescent impurities. A total of four columns (95/5 CH_2Cl_2/MeOH on SiO_2) were used to purify 2 g of the red/purple product **25** (20% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.06 (s, 12H), 8.05 (d, 8H, J = 8.2 Hz), 8.37 (d, 8H, J = 8.2 Hz), 8.84 ppm (s, 8H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD) δ 43.14, 102.87, 118.66, 122.02, 135.06, 144.21, 144.82. UV/vis (CH_2Cl_2) λ 418, 513, 548, 590, 646 nm.

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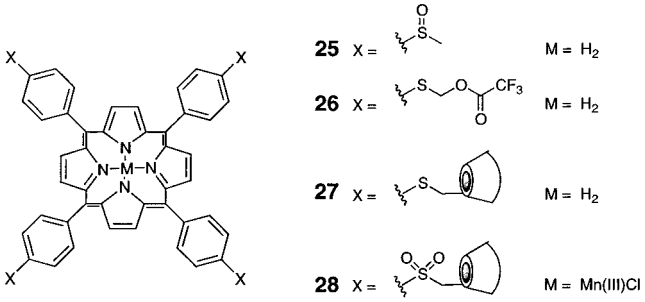
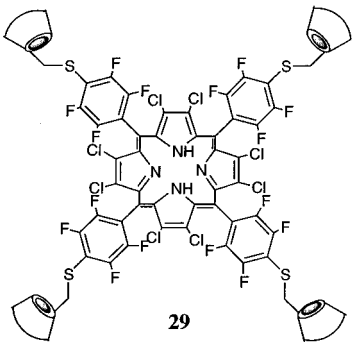
(34) Yang, Z.; Breslow, R. *Tetrahedron Lett.* **1997**, *38*, 6171−6172.
(35) Yang, Z. Ph.D. Thesis, Columbia University, 1999. This thesis contains the X-ray crystal structure of the complex.





29

To the tetrasulfoxide porphyrin **25** (540 mg, 0.626 mmol) in 25 mL of CH_2Cl_2 was added trifluoroacetic anhydride (8 mL, 57 mmol) with stirring. The mixture was gently refluxed at 40 °C for 1.5 h, the solvent was evaporated under reduced pressure, and the product was precipitated with 25 mL of petroleum ether. The blue/green product **26** was collected and dried under vacuum (yield 100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.97 (s, 8H), 8.08 (d, 8H, J = 8.2 Hz), 8.54 (d, 8H, J = 8.2 Hz), 8.63 ppm (s, 8H).

Four equivalents of β-cyclodextrin 6-iodide (2.27 g, 1.83 mmol) was dissolved in 100 mL of anhydrous DMF, NEt_3 (4.2 mL, redistilled from CaH), and anhydrous MeOH (4.2 mL) were added, and the solution was stirred under argon for 20 min. The cyclodextrin solution was then transferred under argon via cannula to a flask containing porphyrin **26**. The transfer was done at a rate such that all solution was added after 20 min. The new solution was stirred at room temperature for 15 min and then heated at 60 °C for 36 h. The DMF was then removed under vacuum, and the product was precipitated with acetone in two portions in a centrifuge tube. The product was then taken up several times in a minimum amount of water and precipitated with mixtures of acetone/MeOH/CH_2Cl_2. The solid was dried in a vacuum, and 2.35 g crude product was obtained. The final product was purified first by a reversed-phase column eluting with pure water, then size-exclusion column (Sephadex G75) eluting with pure water, and again with a reversed-phase column to yield 1.2 g (50%) of pure product **27**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.2−4.2 (m, 168H), 4.3−4.65 (m, 24H), 4.75−5.1 (m, 28H), 5.6−5.9 (m, 56H), 7.83 (d, J = 8 Hz, 8H), 8.12 (d, J = 8 Hz, 8H), 8.92 (s, 8H); ^{13}C NMR (125 MHz, DMF-d_7) δ 35.57, 61.31, 71.65, 73.23, 73.58, 73.78, 74.10, 74.23, 82.84, 86.16, 103.12, 103.30, 103.83, 120.44, 127.44, 132.30, 135.52, 138.58, 139.25; MS (negative ion, electrospray) 1736 $(M - 3H)^{3-}$, 1302 $(M - 4H)^{4-}$; UV/vis (H_2O) 422 (Soret), 523, 559, 590, 650 nm.

Catalyst 7. Porphyrin **27** (0.50 g, 0.0965 mmol) was dissolved in 70 mL of anhydrous DMF and stirred under argon. Then 35 equiv of $MnCl_2$ (425 mg) and 50 equiv of 2,6-lutidine (0.56 mL) was added via syringe, and the solution was heated to 90 °C for 6 h under air and monitored by UV/vis until the reaction was complete. The DMF was removed under reduced pressure, and the green solid was purified on a Sephadex G75 size- exclusion column to isolate and separate the product from $MnCl_2$. The same procedure was repeated with another 500 mg of tetramer porphyrin and a total of 992 mg of catalyst **7** was obtained (>97% yield): UV/vis (H_2O) 383, 405, 428, 470 (Soret band), 569, 607 nm.

28: the Tetrasulfone of Catalyst 7. Porphyrin **27** (20 mg, 3.8 umol) was dissolved in 5 mL of water, magnesium monoperoxyphthalate (23.5 mg, 80% technical grade) was added, and the reaction was stirred overnight at room temperature. TLC showed complete disappearance of starting material. Two drops of concentrated HCl was added and the solution turned blue/green. The solution was extracted with ether, and the aqueous layer was reduced and concentrated. The protonated green porphyrin was lyophilized from water: UV/vis 418 (Soret), 517, 552, 585, 639 nm in pH 7 water; IR 1405, 1282, 1153, 1073, 1030 cm^{-1}.

The sulfone porphyrin (~20 mg) was dissolved in 4 mL of anhydrous DMF and stirred under argon. Thirty-five equivalents of $MnCl_2$ (16.3 mg)) and 50 equiv of 2,6-lutidine were added via syringe, and the solution was heated to 90 °C for 24 h and monitored by UV. The DMF was removed under reduced pressure, and the green solid **28** was purified on a Sephadex G75 size-exclusion column (>97% yield): UV/vis 321, 378, 399, 418, 465 (Soret), 561, 597 nm (in water).

Perfluorinated Catalyst 9. This was first prepared by reaction of the corresponding tetrathiol with β-cyclodextrin 6-iodide, later by reaction of the tetrakispentafluorophenylporphyrin with cyclodextrin thiol. The latter procedure is analogous to the one described below for compound **14**, with eight extra chlorines. For porphyrin **10**: ^{1}H NMR (500 MHz, DMF-d_7) δ −2.73 (s, 2H), 3.5−6.7 (m, 276H), 9.76 (s, 8H); ^{19}F NMR (282.4 MHz, DMF-d_7) δ −138.6, 133.4; UV/vis (H_2O) λ 412, 510, 582 nm. For manganese catalyst **9**: UV/vis (H_2O) λ 369, 458, 555 nm.

Perhalogenated Catalyst 14. The perhalogenated porphyrin was synthesized by reaction of pentafluorobenzaldehyde with 3,4-dichloropyrrole in the standard way (details of the pyrrole and porphyrin syntheses are in the Supporting Information) and had spectroscopic data in line with those reported.[36] This porphyrin (26.9 mg, 0.0215 mmol) and K_2CO_3 (30.1 mg, 0.218 mmol) were dissolved in 1.6 mL of dry DMF. After 5 min, 6-deoxy-6-mercapto-β-cyclodextrin (126 mg, 0.109 mmol) was added under argon, and the solution was stirred at rt for 24 h. The solvent was evaporated and the residue precipitated in acetone to give a dark green solid. The crude product **29** was purified by gel filtration (Sephadex G-75, 20−50 μm) with water as eluent to obtain 95 mg (77% yield) of a brown powder after lyophilization: ^{1}H NMR (300 MHz, DMSO-d_6/D_2O = 95:5 at 353 K) δ 5.0−4.7 (m, 28H), 4.15−4.05 (m, 4H), 3.9−3.1 (m, 244H); ^{19}F NMR (272 MHz, DMSO-d_6/D_2O = 95:5 at 353 K) δ −132.6 to −133.6 (m, 8F), −139.5 to −140.5 (m, 8F); UV/vis (H_2O) λ_{max} 447 (Soret).

Then **29** (25 mg, 0.0043 mmol), $MnCl_2$ (11.6 mg, 0.092 mmol), and 2,6-lutidine (13.8 mg, 0.129) was dissolved in 2.4 mL of dry DMF. The mixture was heated under reflux for 6 h in air. After cooling, the solvent was evaporated and the residue dried under vacuum overnight. The crude product was purified by gel filtration (Sephadex G-75, 20−50 μm) with water as eluent to obtain 23 mg (0.0039) of **14** as a green powder: UV/vis λ_{max} 451 (Soret), 578 nm.

Syntheses of the Substrates. The synthesis of 3-(4-*tert*-butylphenyl)glutaric anhydride **30** will serve as a generic synthesis of the glutaric acid linkers from the corresponding aldehydes.

4-*tert*-Butylbenzaldehyde (24.26 g, 150 mmol) and ethyl cyanoacetate (20.36 g, 180 mmol) in 200 mL of ethanol with 1 mL of pyrrolidine stood for 5 h at room temperature. The ethanol was removed under reduced pressure, and the product was extracted with 200 mL of EtOAc and 50 mL of ammonium chloride. The EtOAc layer was washed with 50 mL of brine and then dried over $MgSO_4$ for several hours. The solvent was

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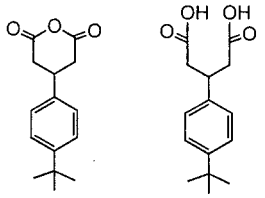

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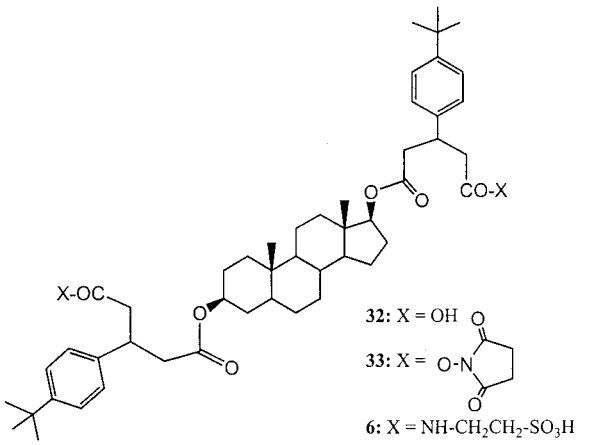

removed under reduced pressure, and the product was taken on to the next step without further purification.

To the above product in 155 mL of EtOH were added Meldrum's acid (23.8 g) and NaOH (6.9 g in 75 mL water). The mixture was stirred at room temperture for 3 h and at 70 °C overnight. Ethanol was removed under reduced pressure, and the product was dissolved in 170 mL of concd HCl and heated under reflux for 10 h. The suspension was filtered, and the HCl solution was extracted with EtOAc. The organic extracts were combined with the filtered solid, the solvent was removed under reduced pressure, the product was then suspended in 150 mL of ethylene glycol with NaOH (35.6 g), and the mixture was heated to 150 °C for 24 h.

After the solution was cooled, 100 mL of ice−water was added, and the solution was then acidified to pH 1−2 with concd HCl and cooled in an ice bath. The solid crude product was filtered and dissolved in 100 mL of 1 N NaOH. The basic solution was extracted with ether to remove most of the impurities, and the aqueous layer was again acidified to pH 1−2 with concd HCl to precipitate the product 3-(4-*tert*-butylphenyl)glutaric acid **31** in an ice bath. This purification scheme was done up to three times to ensure complete removal of impurities. The final product was filtered from the aqueous acid and the washed with 95−98% petroleum ether/ether solution to produce 17 g of **31** as an off-white solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.2 (s, 9H), 2.5 (m, 4H), 3.38 (q, 1H), 7.15 (d, J = 8.4 Hz, 2H), 7.28 ppm (d, J = 8.4 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.3, 34.2, 37.5, 40.3, 125.1, 127.2, 140.4, 148.6, 173.0.

Diacid **31** (5 g) was dissolved in 100 mL of acetic anhydride, the solution was heated at reflux for several hours, and after all starting material disappeared (by TLC) the solvent was removed under reduced pressure. The product 3-(4-*tert*-butylphenyl)glutaric anhydride **30** was washed with a 10:1 petroleum ether/ether solution until the brown color disappeared. An off-white solid was collected (4.6 g) and vacuum-dried: ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.27 (s, 9H), 3.0 (m, 4H), 3.5 (m, 1H), 7.2 (d, J = 8.3 Hz, 2H), 7.39 ppm (d, J = 8.3 Hz, 2H).

5α-Androstane-3,17-diol (200 mg), 3-(4-*tert*-butyl)glutaric anhydride **30** (337 mg), and 20 mg of *p*-toluenesulfonic acid were heated at reflux in 25 mL of toluene under argon overnight. The solvent was removed under reduced pressure, and the product **32** (67% yield) was purified by column chromatography (95/5 CH_2Cl_2/MeOH on SiO_2): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.5−1,8 (m, 45H), 1.98 (m, 1H), 2.5−2.8 (m, 8H), 3.59 (m, 2H), 4.48 (m, 1H), 4.57 (br, 1H), 7.13 (d, J = 8.0 Hz, 4H), 7.30 (d, J = 8.0 Hz, 4H); MS (CI, NH_3) 802 (M + 1 + NH_3).

The diacid derivative **32** (150 mg, 0.19 mmol), 4 equiv of *N*-hydroxysuccinimide (87.5 mg, 0.76 mmol), and 4 equiv of 1-(3-diethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (146 mg, 0.76 mmol) were stirred in 30 mL of methylene chloride overnight. The reaction mixture was quenched with 50 mL of saturated ammonium chloride solution, the organic layer was extracted with water and dried over sodium sulfate, and the solvent was removed under reduced pressure. The product **33** (67% yield) was purified by column chromatography (95/5 CH_2Cl_2/MeOH on SiO_2): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.5−1.7 (m, 45H), 1.97 (m, 1H), 2.5−3.1 (m, 16H), 3.6 (m, 2H), 4.46 (m, 1H), 4.57 (br, 1H), 7.16 (d, J = 8.2 Hz, 4H), 7.31 (d, J = 8.2 Hz, 4H); MS (CI, NH_3) 997 (M + 1 + NH_3).

The activated diester derivative **33** (180 mg, 0.19 mmol), 4 equiv of taurine (95 mg, 0.76 mmol), and 4 equiv of NEt_3 were stirred in 20 mL of anhydrous DMF overnight at room temperature, the DMF was removed under reduced pressure, and the product was precipitated from ∼10 mL of 2 N HCl. The slurry was washed with acetonitrile and 1 N HCl to produce an off-white solid. The product was taken up in water and lyophilized to give 137 mg of substrate **6** (overall 20% yield from androstane-3,17-diol): ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.38−1.51 (m, 45H), 1.82 (m, 1H), 2.2−2.7 (m, 12H), 3.20 (m, 4H), 3.38 (m, 2H), 4.35 (m, 2H), 7.09 (d, J = 8.1 Hz, 4H), 7.24 (d, J = 8.1 Hz, 4H); MS (FAB) 1000 (M + 1).

Triply Linked Androstanetriol Substrate (11) This was prepared from the triol **8** by the sequence described above for substrate **6**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.68 (m, 3H), 7.22 (m, 6H), 7.07 (m, 6H), 4.25 (m, 3H), 3.20 (m, 6H), 0.62 (s, 3H), 0.46 (s, 3H), 3.4−2.28 (21H), 1.85−0.30 (23H).

The syntheses of the unsymmetrically doubly linked androstane derivatives **17**, **18**, and **19** are described in Supporting Information.

Typical Conditions for Hydroxylations of the Steroid Substrates and the HPLC Analysis Methods. A typical experiment: Substrate **6** (20 mg), *x* mol % catalyst **7** or **9**, and 10 equiv (with respect to substrate) of pyridine were dissolved in 20 mL of deionized water and stirred in a closed vessel for 10 min at room temperature in the dark. A solution of 5 equiv of PhIO in MeOH (30 mg/mL) was added to the solution via syringe in 0.2−0.3 mL increments over a period of 20 min. Another 5 equiv of PhIO was added in the same manner over another 20 min. After the solution was stirred at room temperature for ∼2 h, excess $Na_2S_2O_3$ solid was added followed by 10 mL of 3 N NaOH to hydrolyze the esters. The solution was stirred overnight, after which time the solution was extracted with 3 × 20 mL of EtOAc. The combined organic extracts were then washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product mixture (∼0.02 mmol of total steroid) was taken up in 10 mL of distilled pyridine.

Freshly distilled benzoyl chloride (0.5 mL, ∼430 equiv with respect to steroid) was added to the crude pyridine solution along with 1 mg of 4-dimethylaminopyridine (DMAP). The solution was then purged with argon, shaken for 10 min, and put in an 80 °C bath for 20−25 min. The reaction mixture was poured into a separatory funnel containing 50 mL of 0.1 N HCl and 50 mL of 90:10 hexanes/ethyl acetate and then washed once with 20 mL of 0.1 N HCl and once with 20 mL of saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and brought to dryness under reduced pressure to give a brown oil. A small aliquot of this oil was taken up in 1 mL of 2-propanol and analyzed by HPLC.

Authentic samples of the androstanediols and -triols were quantitatively converted to their benzoate esters using the same method described for derivatization of the crude mix-

tures. Further details of the analytical method are described in the Supporting Information.

Typical conditions for hydroxylations of lithocholic **20** and lithocholenic **21** acids and GC analysis methods are described in the Supporting Information.

Spectroscopic Characteristics of Steroid Products. 5α-Androstane-3*β*,17*β*-diol was purchased: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.62 (t, 1H), 3.55 (m, 1H), 0.818 (s, 3H), 0.732 (s, 3H).

5α-Androstane-3*β*,6α,17*β*-triol (8): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.66 (t, 1H), 3.61 (m, 1H), 3.44 (dt, 1H), 0.834 (s, 3H), 0.738 (s, 3H); CI-MS m/z = 326 (M + 1 + NH_3). The NOE spectrum indicates a strong signal between the C6-H to the C19-Me. Triol **8** was compared with an authentic sample obtained from Steraloids, Inc.

5α-Androstane-3*β*,7*β*,17*β*-triol (18): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.62 (t, 1H), 3.58 (m, 1H), 3.38 (m, 1H), 0.849 (s, 3H), 0.762 (s, 3H); CI-MS m/z = 326 (M +1 + NH_3). The new CH-OH and methyl shifts compared to the starting diol are consistent with hydroxylation at C7.[31] The COSY spectrum indicated 3 neighboring protons for the new CH peak. NOESY indicated two cross-peaks with the C7α-H, presumably to the C5-H and C14-H.

5α-Androstane-3*β*,15α,17*β*-triol (19): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.06 (m, 1H), 3.87 (t, 1H), 3.61 (m, 1H), 0.842 (s, 3H), 0.755 (s, 3H); CI-MS m/z = 326 (M + 1 + NH_3). The new CH-OH and methyl shifts compared to the starting diol are consistent with hydroxylation at C15.[31] The COSY spectrum indicated three neighboring protons for the new CH, two of which were the 16 protons as determined by a common COSY to the C17-H. NOESY revealed a strong cross-peak of the C15-H to the C18-Me, indicating that the hydroxyl group is 15α.

5α-Androstane-3*β*,6α,9α,17*β*-tetraol (12): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.73 (t, 1H), 3.58 (m, 1H), 3.43 (dt, 1H), 0.959 (s, 3H), 0.753 (s, 3H); CI-MS m/z = 342 (M + 1 + NH_3), 323 (M − 1, neg). The chemical shifts of the angular methyl groups were consistent with reported values.[31] NOESY indicated that the C5 and C7α protons moved downfield significantly.

3-Keto-5*β*-cholanic Acid. All cholanic and cholenic acid products had the correct masses: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.02 (s, 3H), 0.923 (m, 3H), 0.683 (s, 3H). The product was compared and confirmed against authentic product obtained from Steraloids, Inc.

3-Keto-5*β*-chol-11-enic acid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.02 (d, J = 6 Hz, 3H), 0.98 (s, 3H), 0.768 (s, 3H).

5*β*-Chol-11-enic acid-3α,1*β*-diol: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.05 (br s, 1H), 4.02 (m, 1H), 0.856 (s, 3H), 0.737 (s, 3H). The C1-H and C3-H have a common COSY coupling to the C2-H's.

5*β*-Chol-11-enic acid-3α,6α-diol: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.11 (m, 1H), 3.65 (m, 1H), 0.837 (s, 3H), 0.728 (s, 3H). NOE observed between the C6-H and the C19-Me.

5*β*-Chol-11-enic acid-3α,6*β*-diol: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.83 (br m, 1H), 3.65 (m, 1H), 1.08 (s, 3H), 0.773 (s, 3H).

5*β*-Chol-11-enic acid-3α,15α-diol: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.04 (m, 1H), 3.65 (m, 1H), 0.887 (s, 3H), 0.783 (s, 3H). NOE observed between the C15-H and the C18-Me.

5*β*-Chol-11-enic acid-3α,15*β*-diol: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.26 (m, 1H), 3.65 (m, 1H), 1.05 (s, 3H), 0.922 (s, 3H).

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Supporting Information Available: Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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