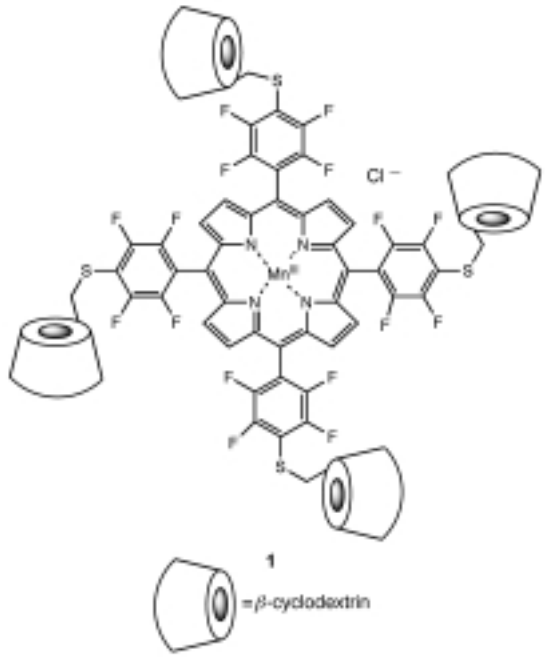
Selective Hydroxylation of a Steroid at C-9 by an Artificial Cytochrome P-450**

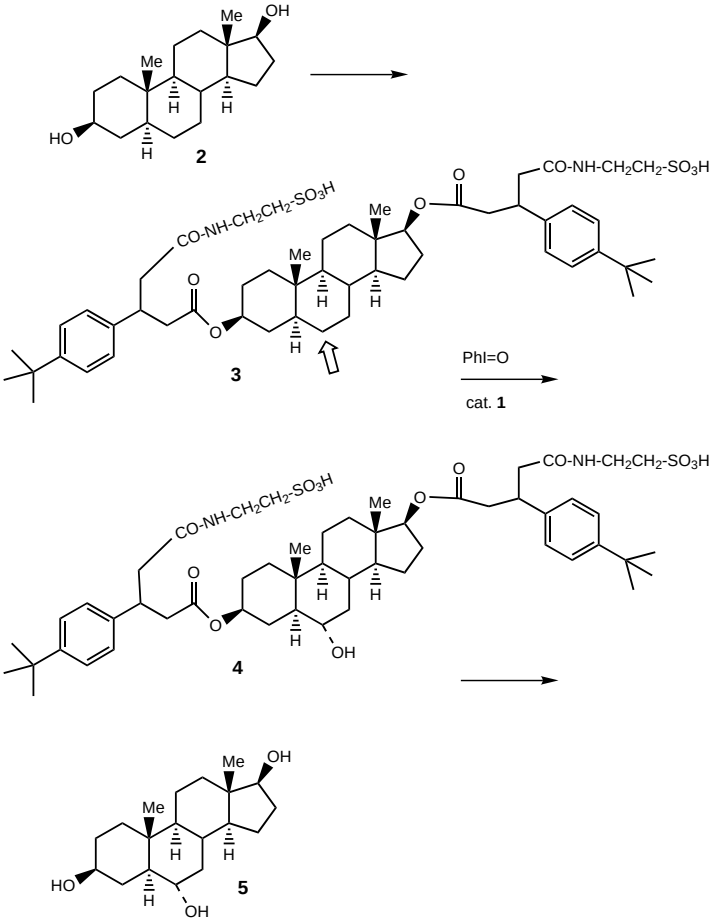
Jerry Yang and Ronald Breslow*

In contrast to the normal dependence of synthetic organic chemistry on the intrinsic reactivity of substrates, enzymatic reactions typically impose selectivity by control of the geometry in the enzyme/substrate complex. Such geometric control can override the reactivity of substrate functional groups. In the oxidation of lanosterol by P-450 enzymes, for instance, methyl groups on saturated carbons are selectively oxidized in the presence of two otherwise much more reactive double bonds and a secondary carbinol group.

We have been pursuing a program for many years to mimic such enzymes, so as to supplement the normal chemistry of functional group manipulation. The process we have termed "remote oxidation" is an example, in which a template is covalently attached to a substrate and guides free radical chlorination, by the "radical relay reaction," to positions in a substrate that are consistent with the geometry imposed by the template.[1] While the templates can then be removed, this is not a true turnover catalytic process.

Recently we reported an example in which **1**, a manganese porphyrin carrying four cyclodextrins attached to fluorinated phenyl groups, was used as a catalyst in the hydroxylation of androstane-3,17-diol (**2**).[2] The steroid **2** was converted to a diester **3** carrying *tert*-butyl groups for binding into the cyclodextrins and sulfonate groups for water solubility (Scheme 1). We saw that substrate **3** was converted to its 6α-hydroxyl derivative **4** in up to 100% yield with 95% conversion using 1 mol% of catalyst **1** and 10 equivalents of PhIO as oxidant. Ester hydrolysis afforded triol **5** (Scheme 1).





Scheme 1. Catalyst **1** converts a doubly bound substrate to the 6α-hydroxy derivative.

The reaction was completely regioselective for the C-6 position of the steroid, stereoselective in forming only the equatorial 6α-hydroxyl derivative, and chemoselective in not oxidizing the hydroxyl group further into the ketone. By contrast, without the *tert*-butylphenyl binding groups no oxidation of the steroid occurred under our conditions. When steroidal alcohols are oxidized with high concentrations of related metalloporphyrin catalysts that do not bind their substrates, we find that the dominant reaction is conversion of the alcohol group into a ketone, not hydroxylation of saturated carbons.

Corey-Pauling-Koltun (CPK) and computer molecular models suggested that our process with the enzyme mimic involves binding of the substrate to present the steroid edge on to the face of the metalloporphyrin (Figure 1). While the selectivity of the results is striking, hydroxylation of a steroid at C-6 is not intrinsically important. However, models indicated (Figure 2) that if we could bind the steroid face to face with the porphyrin we should get attack at the axial C-9α

[*] Prof. Dr. R. Breslow, J. Yang
Department of Chemistry
Columbia University
New York, NY 10027 (USA)
Fax: (+1) 212-854-2755
E-mail: rb33@columbia.edu

[**] We thank the National Institutes of Health, the National Science Foundation, and the Environmental Protection Agency for financial support of this research. J.Y. acknowledges support from an NCER-QA EPA Graduate Fellowship and a Bristol-Myers Squibb Graduate Fellowship.

  0044-8249/00/11215-2804 $ 17.50+.50/0

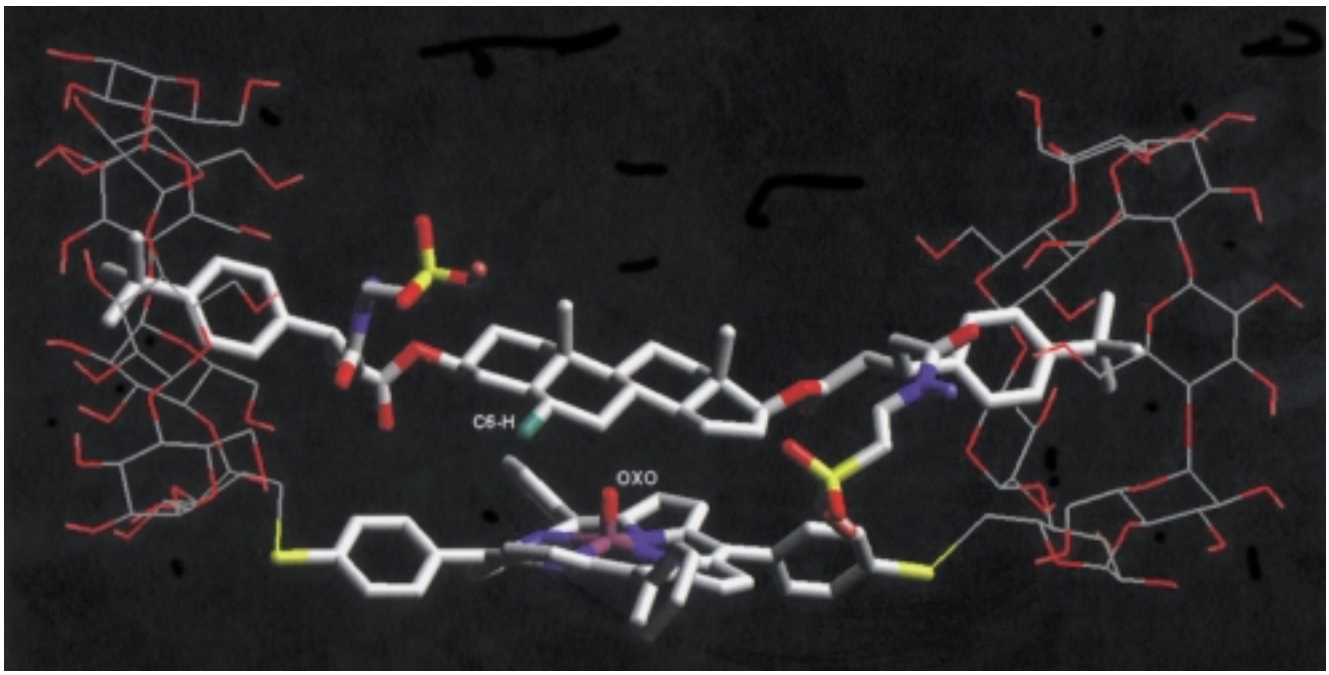


Figure 1. The computed structure of the complex of doubly bound substrate **3** with catalyst **1**, showing that the oxygen atom added to the Mn atom of **1** is in a lateral position to attack the equatorial H atom on C-6 of **3**, as observed. For details of the calculational method, which differs from that in Figure 2, see Experimental Section. For clarity the two cyclodextrins not involved in substrate binding are deleted from the figure, and those binding the substrate are shown with thin lines.

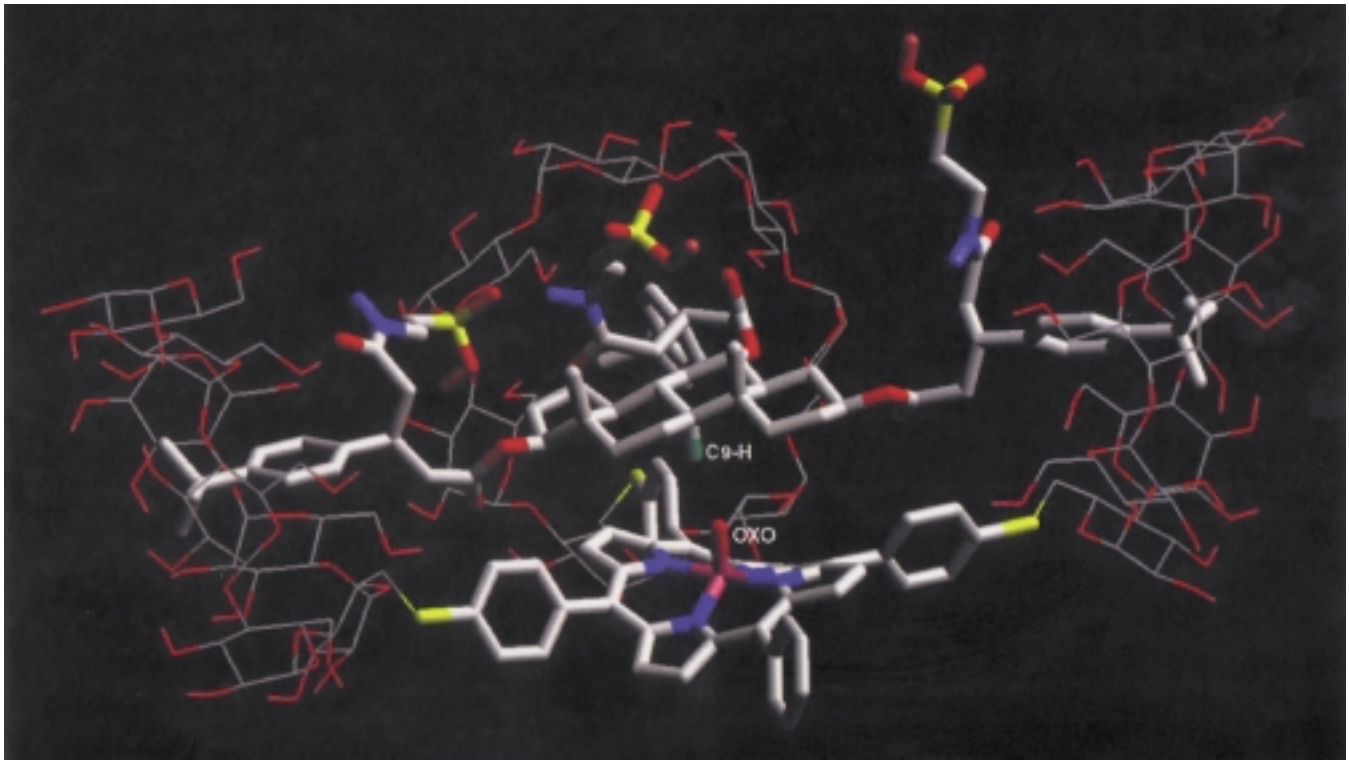


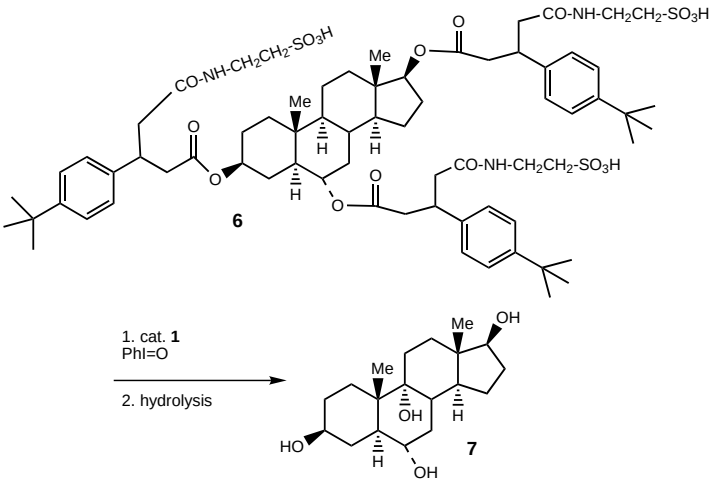
Figure 2. The computed structure of the complex of triply bound substrate **6** with catalyst **1**, showing that the oxygen atom added to the Mn atom of **1** is in a lateral position to attack the axial H atom on C-9 of **6**, as observed. For details of the calculational method, which differs from that in Figure 1, see Experimental Section. For clarity the one cyclodextrin not involved in substrate binding is deleted from the figure, and those binding the substrate are shown with thin lines.

hydrogen, a very desirable process. We have now achieved this, and find that the catalyzed reaction is indeed completely selective for hydroxylation at C-9.

To change the geometry of the complex, we introduced a third binding interaction. The 3β,6α,17β-androstantriol (**5**), synthesized on a preparative scale by our previous hydrox-

  0044-8249/00/11215-2805 $ 17.50+.50/0

ylation reaction,[2] was converted into the triester **6** (Scheme 2), which contains binding and solubilizing groups, and oxidized with 1 equivalent of catalyst **1**, 10 equivalents of PhIO, and 10 equivalents of pyridine in pure deionized water.



Scheme 2. Catalyst **1** converts a triply bound substrate to the 9α-hydroxy derivative.

The oxidant was quenched and the ester groups removed to afford the tetraol **7** as the only detectable product in quantitative yield (Scheme 2). With only 0.1 equivalent of catalyst and excess oxidizing agent the conversion of **6** to **7** was also complete, so there are at least 10 turnovers in this catalytic hydroxylation.

As expected, **7** gave positive ion and negative ion mass spectra indicating one more oxygen than in **5**, and a ^{1}H NMR spectrum showing that there was no new CH–OH group and that the proton at C-9 in **5** was missing in **7**. Relative to **5**, the ^{1}H NMR signal (500 MHz, $CDCl_3$) for the C-18 methyl group was shifted downfield by $\Delta\delta = 0.015$ to $\delta = 0.752$, and that for the C-19 methyl group was shifted downfield by $\Delta\delta = 0.125$ to $\delta = 0.958$. This is consistent with the tabulated effect of a 9-OH group on these methyl NMR signals.[3] The COSY spectrum indicated that the C-5- and C-7α-proton signals moved downfield, as expected from their 1,3-diaxial relationship to the new C-9 hydroxyl group in **7**.

Thus we have achieved selective turnover catalytic hydroxylation of a steroid at C-9, as computer modeling suggested. Such C-9 hydroxysteroids can be dehydrated to introduce the C-9(11) double bond. As previous work shows, such a double bond permits synthesis of corticosteroids with oxygen at C-11 and fluorine at C-9, an important class of medicinal compounds.[4]

These catalytic hydroxylations in the complex of substrates with an artificial P-450 enzyme mimic thus show predictable geometric control that overwhelms intrinsic substrate reactivity, in the sense that the hydroxyl group introduced into compound **5** is not further oxidized in competition with selective hydroxylation of an unactivated methylene group. It remains to be seen how well such enzyme mimics can perform other useful oxidations, and, thus, how generally they succeed in freeing chemistry from the domination of functional group reactivities.

Experimental Section

Catalyst **1** was prepared as described in ref. [2c]. The oxidations and workup were as described in the supplementary material of ref. [2a].

The molecular models shown in Figures 1 and 2 were calculated using version 7.0 of the program MacroModel, with the inclusion of water as a continuous solvent, and with energy minimization as the geometries were varied. In both cases the cyclodextrin rings themselves were fixed in their calculated initial internal geometry before further geometry variations and energy minimizations, to cut down the calculational time required. However, there were some differences in details between the calculations resulting in Figures 1 and 2.

The model in Figure 2 was calculated by substituting a phosphorus atom for the manganese atom in catalyst **1**, since the program has no parameters for manganese. The phosphorus atom was not permitted to move, and the attached oxygen atom was held perpendicular to the porphyrin plane during geometry variation and energy minimization. Essentially the same result was obtained from a calculation using only the porphyrin system and then inserting the included metal (phosphorus atom) and its attached oxygen atom at the end of the calculation. The result was that the oxygen atom in the catalyst/substrate complex of Figure 2 was within contact distance of the hydrogen atom at C-9, consistent with the observed hydroxylation at C-9. The oxygen atom was also calculated to be within the same distance of the axial hydrogen atom on C-1, but no hydroxylation of this position was observed experimentally.

However, the model in Figure 1 was calculated with just the porphyrin system, which lacked metal or oxygen—these were later inserted after energy minimization. This shows the geometry needed to account for our finding that the doubly bound substrate is hydroxylated at C-6. However, when the calculation was done as in Figure 2, with an included phosphorus as a surrogate metal along with the oxygen atom, energy minimization did not bring the edge of the steroid near the oxygen atom, and did not account for the observed hydroxylation at C-6.

Thus, either method of calculation accounts for the observed mode of reaction at C-9 of substrate **6** with catalyst **1**; however when the full catalyst, with bound metal and attached oxygen, is included during energy minimization the calculation does not successfully account for the observed attack on C-6 in the reaction of **3** with **1**. For this reason Figure 1 should be considered simply an illustration of the geometry that explains our observed reaction, not as evidence for the success of the calculation.

Received: March 2, 2000 [Z 14798]

[1] a) R. Breslow, R. J. Corcoran, B. B. Snider, *J. Am. Chem. Soc.* **1974**, *96*, 6791 – 6792; b) R. Breslow, R. J. Corcoran, B. B. Snider, R. J. Doll, P. L. Khanna, R. Kaleya, *J. Am. Chem. Soc.* **1977**, *99*, 905 – 915; c) R. Breslow in *Comprehensive Organic Synthesis*, *Vol. 7* (Ed.: B. M. Trost), Pergamon, Oxford, **1991**, pp. 39 – 52.

[2] a) R. Breslow, X. Zhang, Y. Huang, *J. Am. Chem. Soc.* **1997**, *119*, 4535 – 4536; b) R. Breslow, Y. Huang, X. Zhang, J. Yang, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11156 – 11158; c) R. Breslow, B. Gabriele, J. Yang, *Tetrahedron Lett.* **1998**, *39*, 2887 – 2890.

[3] N. S. Bhacca, D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry: Illustrations from the Steroid Field*, Holden-Day, San Francisco, **1964**, p. 21.

[4] a) C. Bergstrom, R. Nicholson, R. Dodson, *J. Org. Chem.* **1963**, *28*, 2633 – 2640; b) J. Fried, E. Sabo, *J. Am. Chem. Soc.* **1954**, *76*, 1455 – 1456; c) J. Fried, E. Sabo, *J. Am. Chem. Soc.* **1957**, *79*, 1130 – 1141; d) R. Hirschmann, R. Miller, J. Wood, R. Jones, *J. Am. Chem. Soc.* **1956**, *78*, 4956 – 4959; e) R. Dodson, R. Muir, *J. Am. Chem. Soc.* **1961**, *83*, 4631 – 4635; f) R. Dodson, R. Muir, *J. Am. Chem. Soc.* **1958**, *80*, 6148.

  0044-8249/00/11215-2806 $ 17.50+.50/0