Communications to the Editor

Selective Catalytic Hydroxylation of a Steroid by an Artificial Cytochrome P-450 Enzyme

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The ability of oxidizing enzymes, particularly those of the cytochrome P-450 class, to perform selective hydroxylations of unactivated carbons in substrates such as steroids is of great practical importance.[1] It also represents a great challenge for biomimetic chemistry. Indeed the phrase biomimetic chemistry was first coined in 1972 with respect to efforts to achieve selective functionalization of steroids and other hydrocarbon derivatives with use of geometric control to mimic that in enzymes.[2] However, the earliest work involved the functionalization of steroids by reagents or catalysts that were covalently attached to a steroid hydroxyl group.[3] Thus catalytic turnover was not possible. Furthermore, the reactions first examined were directed photolytic insertions, or directed free radical halogenations.

In Nature the relevant enzymatic reactions involve oxidation by metalloporphyrins, with reversible enzyme binding of the substrate in such a geometry that specific substrate positions are within reach of the oxygen atom on the metal. After oxidation the product is released, so catalytic turnover is seen.

To date there is no true mimic of this entire process. Groves has shown that he can use organization in a bilayer to achieve hydroxylation of a steroid,[4] but catalytic turnover was blocked by strong binding of the product. Grieco has shown that a metalloporphyrin can hydroxylate a steroid in an intramolecular reaction if it is covalently attached,[5] but again this is not a catalytic process with turnover. We now wish to describe a system that indeed binds a substrate, performs a hydroxylation catalyzed by a metalloporphyrin, and then releases the product to perform true turnover catalysis. In our best example, the hydroxylation is highly selective for an otherwise unreactive and unremarkable steroid position. Geometry within the complex of substrate with artificial enzyme determines the product formed.

The catalyst builds on our earlier work in which substrates were reversibly bound to metalloporphyrins, and then selectively oxidized. Our first case used auxilliary metal ions to bind coordinating substrates,[6] and our most recent case used a manganese porphyrin that used two (or four) attached cyclodextrins to bind olefins hydrophobically and perform selective epoxidations.[7] We now find that this latter system can perform the directed hydroxylation of an unactivated saturated carbon of a reversibly bound steroid.

The preparation and characterization of catalyst **1** has been described previously.[7] Molecular models suggested that substrate **3** would bind its two *tert*-butylphenyl groups into two trans cyclodextrin rings of **1** and place steroid ring B directly above the metalloporphyrin ring. Thus we carried out the oxidation of 1.0 mM **3** with 10 mol % of **1** and 5 mM iodosobenzene (5 equiv; with 1 equiv the conversion was lower, ca. 10−15%, and less than 5% of product was formed with 0.3 equiv) in water solution in the presence of 10 equiv of pyridine.[8] After 2 h at room temperature the esters were hydrolyzed with 25% KOH and the steroid products examined by thin layer chromatography. Only two significant compounds were present—starting diol **2** and a new product that was identified as triol **4**.

Triol **4** had the expected mass spectrum, and showed three CH−OH groups in the ^{1}H NMR at δ 3.57 (H-17), 3.47 (H-3), and 3.33 (H-6, an axial H with 10.7 Hz coupling to two axial protons and 4.4 Hz coupling to one equatorial proton). The ^{13}C NMR spectrum of **4** showed only three signals between 69 and 83 ppm for monooxygenated carbon, and a DEPT experiment confirmed that all were from CH−OH groups. The H at δ 3.33 showed NOEs with Me-19, H-4, and H-8. Final proof came from spectroscopic comparison with an authentic sample of **4**.[9]

Compound **4** was isolated in ca. 40% conversion, along with recovered **2**, so each catalyst performed at least 4 turnovers.[10] The catalyst was destroyed at that point, but other work makes it clear how to stabilize such metalloporphyrins to oxidation.[11] Controls showed that **3** was not hydroxylated in the absence of oxidant or of catalyst, and that substrate **5**, which lacks the *tert*-butylphenyl binding groups, was not hydroxylated under the same conditions.

We also examined the hydroxylation of substrate **6**, related to a stilbene we had epoxidized with this catalyst previously.[7] We found that **6** was converted quantitatively to **7** with at least 14 turnovers with use of 7% of catalyst **1** and 3 equiv (relative to substrate) of iodosobenzene. An analog of **6** lacking the *tert*-butylphenyl groups was not oxidized under the same conditions. Other controls also exclude a free-radical chain process: the yield of **7** was unaffected in the presence of 12 mol % added potassium nitrosodisulfonate, and with only 0.3 equiv of iodosobenzene the yield of **7** was below 7%. Thus the normal catalytic mechanism[12] is operating.

Our highly selective hydroxylation of **3** depends critically

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(3) Breslow, R. *Acc. Chem. Res.* **1980**, *13*, 170.

(4) (a) Groves, J. T.; Neumann, R. *J. Am. Chem. Soc.* **1989**, *111*, 2900−2909. (b) Groves, J. T.; Neumann, R. *J. Am. Chem. Soc.* **1987**, *109*, 5045−5047. (c) Groves, J. T.; Neumann, R. *J. Org. Chem.* **1988**, *53*, 3891−3893.

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(6) Breslow, R.; Brown, A. B.; McCullough, R. D.; White, P. W. *J. Am. Chem. Soc.* **1989**, *111*, 4517−4518.

(7) Breslow, R.; Zhang, X.; Xu, R.; Maletic, M.; Merger, R. *J. Am. Chem. Soc*, **1996**, *118*, 11678−11679. The preparation of catalyst **1** is described in the Supporting Information of this paper.

(8) (a) Meunier, B.; Guilmet, E.; De Carvalho, M.-E.; Poilblanc, R. *J. Am. Chem. Soc.* **1984**, *106*, 6668−6676. (b) Montanari, F.; Penso, M.; Quici, S.; Viganò, P. *J. Org. Chem.* **1985**, *50*, 4888−4893.

(9) Obtained from Aldrich Chemical Company.

(10) We have also prepared the Fe(III) analog of catalyst **1**, which catalyzes the selective overall conversion of **3** to **4** but in only 5−10% conversion under our standard conditions. Groves and Neumann (ref 4c) report that iron porphyrin catalysts are less effective than are manganese porphyrin analogs.

(11) (a) Traylor, T. G.; Tsuchiya, S. *Inorg. Chem.* **1987**, *26*, 1338−1339. (b) Chang, C. K.; Ebina, F. *J. Chem. Soc., Chem. Commun.* **1981**, 778−779. (c) Nappa, M. J.; Tolman, C. A. *Inorg. Chem.* **1985**, *24*, 4711−4719. (d) Ellis, P. E., Jr.; Lyons, J. E. *J. Chem. Soc., Chem. Commun.* **1989**, 1189−1190. (e) Ellis, P. E., Jr.; Lyons, J. E. *J. Chem. Soc., Chem. Commun.* **1989**, 1315−1316.

(12) (a) Groves, J. T.; Kruper, W. J., Jr.; Haushalter, R. C. *J. Am. Chem. Soc.* **1980**, *102*, 6375−6377. (b) Hill, C. L.; Schardt, B. C. *J. Am. Chem. Soc.* **1980**, *102*, 6374−6375.

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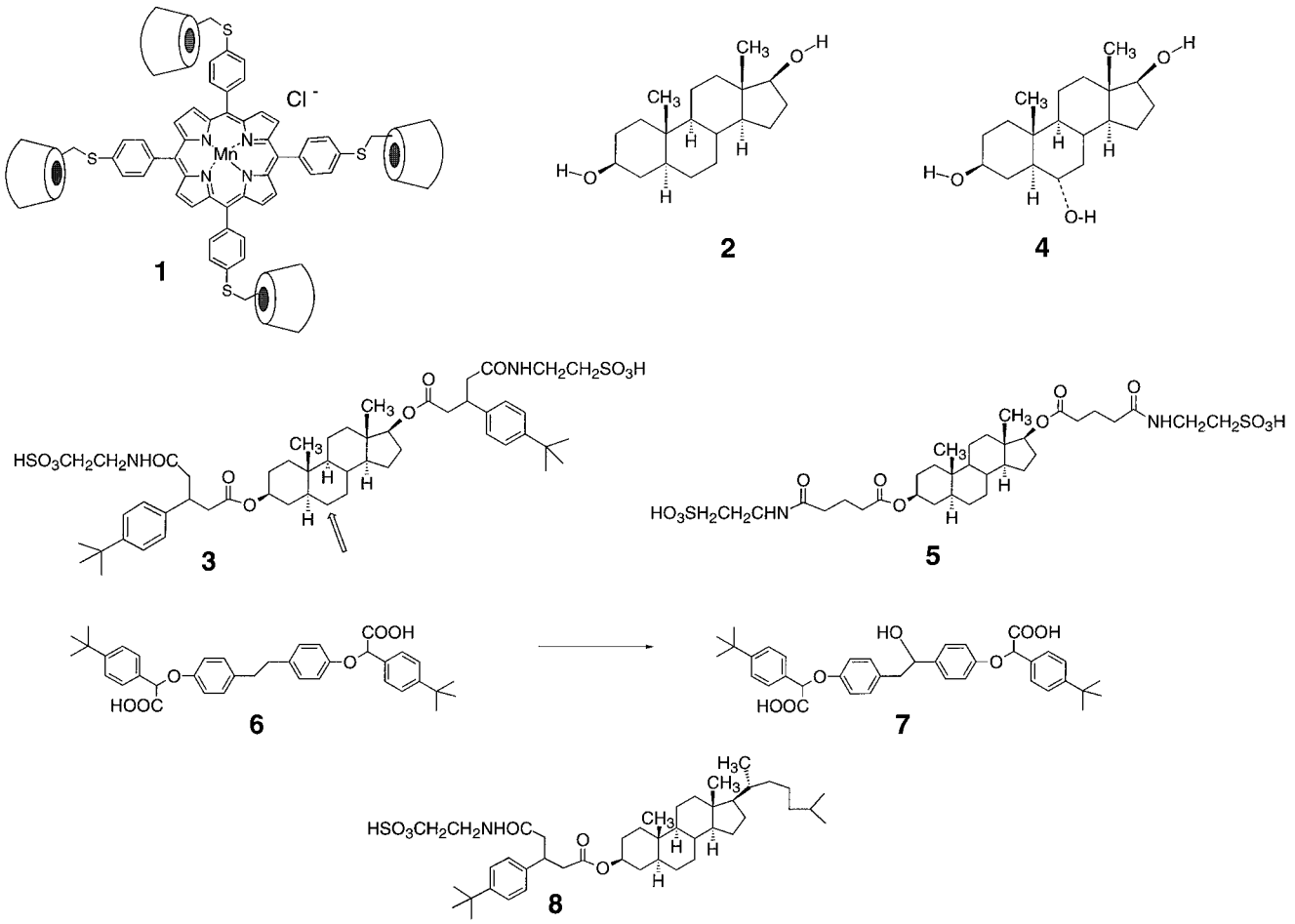
Scheme 1



on its precise fit into the catalyst. Thus compound **8** was also hydroxylated under our conditions, and with at least 10 turnovers, but a mixture of six diols and triols was found after ester hydrolysis.

Of course future developments include working with a more oxidatively stable catalyst, and with alternative binding arrangements that will permit other selective hydroxylations of interest. However, this first success indicates that we can indeed achieve catalytic hydroxylation of substrates by an artificial enzyme system with good regioselectivity, and at carbons less reactive intrinsically (i.e., a CH_2 instead of a CH), because of the geometry of the enzyme−substrate complex.

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