BIOORGANIC CHEMISTRY 18, 440–456 (1990)

REVIEW

Mechanistic Studies and Selective Catalysis with Cytochrome P-450 Model Systems

PETER W. WHITE[1]

Chemistry Department, Columbia University, New York, New York 10027

Received April 4, 1990

Two related uses of cytochrome P-450 model systems are reviewed and evaluated. After a brief summary of the various types of models which have been created, the use of models to study the mechanisms of hydroxylation and especially epoxidation is discussed. The evidence for hydroxylation by initial hydrogen abstraction followed by recombination is clear and convincing. The evidence for and against each proposed epoxidation intermediate is presented. At this time results seem to favor initial charge transfer from the olefin, followed by electrophilic attack. The degree of charge transfer may vary with different substrates and catalysts. Knowledge of hydroxylation and epoxidation mechanisms allows transition-state geometries to be predicted and thus allows one to design a substrate–catalyst or a regioselective oxygenation catalyst. In the final section of the review, several systems are discussed which achieve selectivity by using steric interactions or substrate–catalyst binding.

INTRODUCTION

The cytochrome P-450 monooxygenases carry out an amazing variety of reactions, including hydroxylation of carbon, epoxidation of double bonds, heteroatom release, and oxygenation of nitrogen, phosphorus, sulfur, and halogens. These reactions are important in the metabolism of steroids, fatty acids, and prostaglandins as well as the catabolism of foreign compounds and waste products (*1*). The ability of these enzymes to hydroxylate unactivated carbon atoms and to regiospecifically epoxidize double bonds is of particular interest to many chemists.

This interest has led to an enormous body of work, both in characterizing the enzymes themselves and in constructing model systems which help clarify the reaction mechanism or provide potentially useful catalysts. This review will focus on the mechanism of oxygen transfer to form alcohols and especially epoxides and on the development of P-450 enzyme models as selective hydroxylation and

[1] Present address: Department of Molecular and Cellular Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720.

0045-2068/90 $3.00

epoxidation catalysts. Research on the enzyme itself (*1–3*), and the use of models to study the mechanism (*4, 5*), have been reviewed previously.

The cytochrome P-450 enzymes have been thoroughly studied. The primary sequences of over 60 isozymes have been determined (*6*). The identities of the active site residues have been established with the help of spectroscopic and labeling studies (*3*), and the crystal structure has been determined for the enzyme from the bacterium *Pseudomonas putida* (*7*). A protoporphyrin IX heme ring is buried in the interior of the enzyme. The ring chelates an iron atom and is held in place by hydrophobic contacts and hydrogen bonds between the heme side chain propionates and arginine and histidine residues. The thiolate of a cysteine serves as an axial ligand.

The oxygenation reaction takes place on the heme surface opposite the axial thiolate. The mechanism of all cytochrome P-450 oxidations involves the binding of substrate, followed by the sequential donation of two electrons to the iron by associated flavin- and NADPH-containing enzymes. After the first electron is donated, molecular oxygen binds to the iron and addition of the second electron results in the cleavage of the oxygen–oxygen bond to give a highly activated iron–oxo complex and water (*3*). The precise mechanism of oxygen–oxygen cleavage has not been established and remains an active area of research. Spectroscopic evidence from model systems has established that the intermediate consists of an Fe^{IV}–oxo species and the radical cation of the porphyrin (*8*). The axial thiolate ligand is instrumental in stabilizing this highly activated species. The iron–oxo species then transfers oxygen to the substrate (*3*). Extensive work with model systems, discussed below, has led to an increasingly clear picture of how the oxygen transfer proceeds.

Research on this enzyme was greatly accelerated by two developments. First was the finding that many species besides molecular oxygen itself can donate oxygen to the iron heme. Donors such as hydroperoxides (*9*), sodium chlorite (*10*), sodium periodate (*10*), and iodosobenzene (*11*) (PhIO) are all effective to varying degrees. These oxygen sources facilitate the study of the enzyme because the high-valent iron–oxo species is formed directly on oxygen donation to iron(III); no reducing equivalents from associated enzymes or other added agents are necessary (*1*). Second was the finding that, since no amino acid side chains are directly involved in catalysis, the heme coenzyme or similar metalloporphyrins can be used as enzyme models, especially when the alternative oxygen sources are employed. With these two developments, studies could be conducted with small molecule catalysts, further simplifying the system.

THE RANGE OF SYSTEMS STUDIED

The number of different P-450 model systems is enormous. Early studies showed that iron heme itself was capable of carrying out P-450 chemistry (*12*). However, without the protective enzyme environment, the heme ring is oxidized and destroyed under the reaction conditions. The use of porphyrin rings substituted with phenyl rings at the *meso* carbons (tetraphenylporphyrin (TPP)) produced more

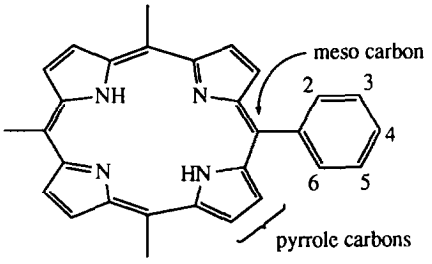


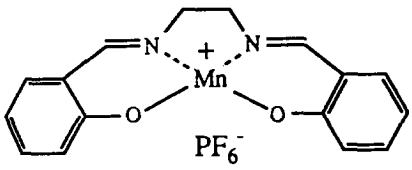
FIG. 1. Nomenclature for tetraaryl porphyrins.

stable systems (*12*), and substituting the phenyl rings with halogens (perfluoro-TPP (*13*), 2,6-dichloro-TPP (*14*)) or alkyl groups (especially tetramesitylporphyrin (*15*)) produced systems which showed very high turnover without significant decomposition. Figure 1 gives the basic geometry and position labels for tetraphenylporphyrin derivatives. It has been possible to produce a wide variety of porphyrin catalysts because the synthesis of these fourfold symmetric porphyrin systems is extremely simple. They are produced in one step by the condensation of 1 eq of pyrrole with 1 eq of an aldehyde (*16*). The aldehyde carbon becomes the *meso* carbon of the porphyrin. For example, the condensation of pyrrole with benzaldehyde gives tetraphenylporphyrin.

Several different oxygen sources have been used in model systems. Molecular oxygen itself is active in model systems in the presence of reducing agents such as ascorbate (*17*) or platinum and hydrogen (*18*). The most popular source, however, has been PhIO or its perfluoro derivative (*19*). Both are insoluble in most organic solvents, though they do dissolve in alcohols. The perfluoro derivative reacts much faster than PhIO, mainly because of its greater solubility (*19*). Sodium (*20*) and lithium (*21*) hypochlorite have also proven to be effective in a number of cases. Amine oxides, peroxides and peracids, potassium persulfate, and sodium periodate have also been employed (*22*). In nearly all cases, the transfer of oxygen to metal is the rate-limiting step in the overall sequence (*23*). With simple oxygen sources such as PhIO, hypochlorite, or persulfate, the transfer can be seen as nucleophilic attack on oxygen by iron, with iodobenzene, chloride, or sulfate serving as the leaving group.

Though iron is the metal present in biological systems, manganese has also proven to be very effective. In fact, manganese porphyrins have been shown to be better catalysts for hydroxylation than iron porphyrins (*24*). Chromium systems also perform this chemistry (*25*), and nickel (*26*), copper (*27*), and ruthenium (*28*) systems have been reported, though the mechanisms of oxygen transfer may be somewhat different in these cases.

Axial ligands similar to the cysteine present in the enzyme are not necessary for oxygenation chemistry, but their use has often resulted in more efficient catalysts (*24*). Little work has been published with thiolate ligands, since these tend to be oxidized in model systems. Woggon has reported a porphyrin system with a thiolate tightly held to the porphyrin by covalent bonds. It mimics the spectral



1

characteristics of P-450 quite well, but its synthesis was discouragingly complex (*29*). Simply adding pyridine and imidazole, on the other hand, has been shown to be very effective. In particular, manganese systems react more cleanly in the presence of imidazoles (*24*).

The model systems do not appear to be sensitive to the solvent used. Work has been done in many organic solvents, including methylene chloride, chloroform, acetonitrile, and benzene (*13*), but the use of periodate or hypochlorite and certain other oxygen sources requires the use of aqueous/organic mixed systems (*20*). With some water-soluble porphyrins, water alone is acceptable (*30*). Alcohols have also been used in some cases to help dissolve oxygen sources or homogenize mixed phase systems (*20*).

Even the special nature of the porphyrin ligand does not seem to be necessary for successful catalysis. Kochi has demonstrated that various substituted salen ligands chelating chromium (*31*), and especially manganese (**1**) (*32*), are as effective as porphyrins. In fact, their reactions probably proceed through a similar mechanism. In particular, their selectivity for *cis* versus *trans* olefins and electron-rich over electron-poor substrates is the same as observed for porphyrins (*vide infra*). Axial ligands were shown to improve catalyst performance in these systems as well. Kochi leaves open the question of whether the active species should be formulated as $O{=}Mn^{V}(salen)^{+}$ or $O{=}Mn^{IV}(salen^{\cdot+})$. Salen ligands are useful model systems because they are easily synthesized by condensing ethylenediamine with 2 eq of a salicylaldehyde derivative (*32*). Several other macrocyclic and multidentate ligands have been shown to be capable of catalyzing oxygenations (*33*). Valentine has even shown that simple nitro and triflate salts of iron, manganese, cobalt, and copper are capable of catalyzing oxygen insertion with PhIO (*34*).

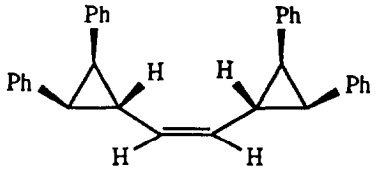
MECHANISMS OF OXYGEN TRANSFER

Despite the variety of metals, ligands, oxygen sources, and solvents employed in these studies, most of these systems are believed to react through the same mechanisms. A mechanism for alkane hydroxylation was proposed by Groves several years ago, both for the enzyme itself (*35*) and for model systems (*36*); it has been accepted without controversy. He proposed that the iron-bound oxygen, which has partial radical character, first abstracts a hydrogen from carbon; the

resulting iron-bound hydroxyl is then transferred to the carbon radical within the solvent cage. Evidence for the intermediacy of a short-lived carbon radical includes the partial, but not complete, retention of stereochemistry at carbon, a preference for tertiary over secondary over primary hydroxylation which parallels the stability carbon radicals, and the formation of side products (such as bromides in the presence of bromotrichloromethane) which are indicative of occasional escape of the radical from the cage. Furthermore, an intermolecular competition between hydrogen- and deuterium-containing substrates gave an isotope effect of 13 (*36*), consistent with carbon–hydrogen bond breaking in the transition state.

The mechanism of hydroxylation has been agreed upon for some time, but the mechanism of olefin epoxidation is still somewhat controversial. Epoxidation is generally much faster with *cis* olefins than with *trans* olefins and is highly stereospecific. Electron-rich olefins are better substrates; terminal olefins are particularly poor substrates (*37*). Side products, most commonly N-alkylated porphyrins (*38*) and carbonyl compounds (*37*), are usually found in small amounts, but it is not clear whether these are produced through an intermediate common with epoxides, or by a distinct but parallel pathway. It has been demonstrated that these side products do not result from secondary reactions of the product epoxides (*38*). With manganese porphyrins (except in the presence of axial ligands) and several non-porphyrin systems, a different stereoselectivity is observed. The *trans* olefins are better substrates and considerable *cis–trans* isomerization is seen. These results imply that a different mechanism takes place with these systems, or at least that a second mechanism competes with that observed in iron porphyrin systems (*39*).

Five structures have been considered as possible intermediates for epoxidation by iron porphyrins and other models, as well as in the enzyme itself (Fig. 2) (*40*). The direct insertion of oxygen illustrated in **I** is generally not considered as a likely possibility, largely because of the small but usually detectable amounts of *trans* epoxide and other side products found with *cis* olefin substrates (*39*). Additional evidence against direct insertion is found in calculations which show that the iron–oxo intermediate has triplet oxenoid character (*41*), so that a spin flip is required at some point before both carbon–oxygen bonds can be formed (*40*). Bruice has presented results of a study with the cyclopropyl olefin **2** which seem to rule out the radical species **II** as a discrete intermediate (*42*). This substrate undergoes a cyclopropylcarbinyl to homallyl radical rearrangement with a rate constant $\geq 2 \times 10^{10}\ s^{-1}$ upon formation of a radical species, such as **II** or **V**. None of the oxygen-containing rearrangement products expected from intermediate **II**



2

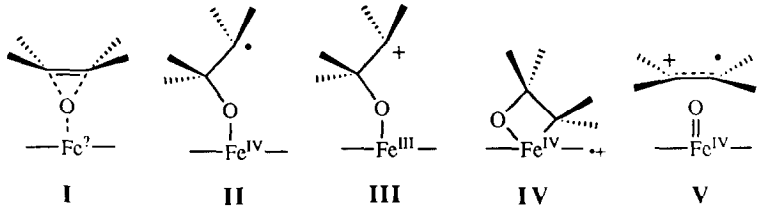


FIG. 2. Proposed intermediates in cytochrome P-450 epoxidations (Ref. (40)).

were detected when this olefin was subjected to epoxidation, so if this structure were an intermediate, it would have to close to product at a rate greater than $2 \times 10^{12}\ s^{-1}$. Rearrangement products not containing oxygen, however, were detected, implying that a species with radical character, presumably **V**, was present. The amounts of the products suggested that the rate constant for collapse to epoxide is fast but $<10^{12}\ s^{-1}$.

The possibility that the intermediate is cation **III** is largely ruled out by Bruice's observation that the ρ^+ for epoxidation of a series of *para*-substituted styrenes with chromium porphyrins is -1.9 (*43*). While this implies the development of some positive charge in the transition state, the value is too low for a fully cationic intermediate. Furthermore, Traylor has found that both *exo* and *endo* epoxides are produced by the reaction of a range of iron tetraarylporphyrins with norbornene. Direct electrophilic, radical, or molecular attack on norbornene is known to proceed exclusively from the *exo* side. Thus this result is evidence against intermediates **I** and **II**, as well as **III** (*44*). In a recent comparison of several chromium, manganese, and iron porphyrins, Traylor found an increase in the amount of *endo* epoxide on proceeding from the relatively electron-rich chromium to electron-poor iron systems. He interpreted this as evidence for some degree of direct attack (via **III**) with chromium but not iron porphyrins (*45*).

Collman proposed the intermediacy of metallacycle **IV** to explain several observations (*21*). He observed in his manganese porphyrin system that the rate of epoxidation was independent of olefin concentration, but that different epoxides were formed at different rates. This was interpreted as meaning that an oxo–olefin complex was formed reversibly and that its breakdown to epoxide was the rate-limiting step. Competition experiments were employed between terminal, disubstituted, and tetrasubstituted olefins to demonstrate that the differences in epoxidation rate for different olefins were largely a consequence of different binding constants of olefin to metal–oxo species (*46*). Groves also reported spectral evidence from studies of FeTMP indicating the reversible formation of an intermediate, consistent with this finding (*47*). The relative rates of epoxidation were more sensitive to steric effects than to the presence of radical or cation stabilizing substituents on the olefin. Also, no isomerization of *cis* to *trans* olefins was observed. These findings argue against the existence of a species with radical or cation character as the reversible intermediate in epoxidation, and the sensitivity to steric effects suggests that the olefin must approach quite closely to the porphyrin ring. Thus he proposed the formation of structure **IV**, a species with some

precedent in the Sharpless epoxidation (*48*), olefin metathesis (*49*), and other transition-metal-catalyzed reactions (*50*).

A great deal of work, however, seems to contradict the possibility that **IV** is the intermediate. Traylor demonstrated that the uv-visible spectrum of an authentic sample of N-alkylated porphyrin was identical with that of the spectra recorded by Groves; this sample also had the same characteristic color observed by Collman. The formation of *N*-alkylporphyrin is reversible, and both its formation and decomposition are slower than epoxidation, so this species is not on the main reaction pathway. The metallacycle proposed by Collman would need to be formed nearly quantitatively under his reaction conditions, but no ESR or uv-visible evidence was found for accumulation of any species besides *N*-alkylporphyrin (*51*).

Bruice studied the kinetics of epoxidations with a computer-generated kinetic model. He ruled out the possibility that a reversible intermediate accumulated on the reaction pathway and also demonstrated, under experimental conditions very similar to Collman's, that the rate of epoxidation was independent of the olefin used (*23*). Collman's finding that different epoxides were produced at different rates presumably reflected the differing efficiencies with which olefins attacked the oxo species to form epoxide, in competition either with other olefins or with side reactions. Bruice's finding means that the small rate differences observed by Collman for reaction with olefins of differing electronic properties do not rule out cationic or radical intermediates since the olefins are not involved in the rate-limiting step. Also, Bruice has recently presented modeling studies and experimental work with iron(III) *meso*-tetrakis(2,6-dibromophenyl)porphyrin, which has a very hindered active site; these indicate that formation of a metallacycle is sterically impossible. Many olefins react very efficiently with this catalyst, even though modeling of this active site indicates that formation of a metallacycle would pose extremely severe steric interactions (*40*). Finally, Traylor has shown that an olefin substituted by two very bulky adamantyl moieties is efficiently epoxidized by a variety of metalloporphyrins (*45*).

Bruice finds these results most consistent with a charge-transfer intermediate (**V**) (*40*). Such a species would require the olefin to interact only with the metal-bound oxygen, rather than with the metal directly, thus relieving the steric requirements significantly. There is some controversy concerning just how close to the metal an olefin needs to approach in order to achieve orbital overlap with the oxygen. Groves has postulated that the oxygen orbitals involved in attack have predominately p_x and p_y character, and thus sideways approach, parallel to the plane of the porphyrin, is necessary (Fig. 3a) (*36*). He uses this postulate to explain the steric selectivity of epoxidation, particularly the preference for *cis* over *trans* olefins. Bruice points out, however, that the oxo intermediate orbitals should be the product of oxygen p and iron d orbital mixing (*52*), and thus have considerable electron density distal to the metal (Figure 3b). This arrangement favors approach at a greater angle relative to the porphyrin plane, placing smaller steric restrictions on olefin attack (*53*). Consistent with Bruice's reasoning are the observations of efficient catalysis by the bromoporphyrin (*40*) and efficient epoxidation of the diadamantyl olefin (*45*), which seem to preclude attack parallel to the porphyrin plane. The finding with the cyclopropyl probe **2**, discussed above, that some

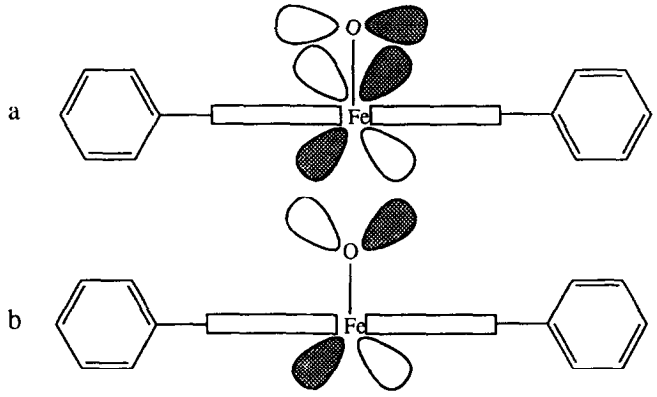


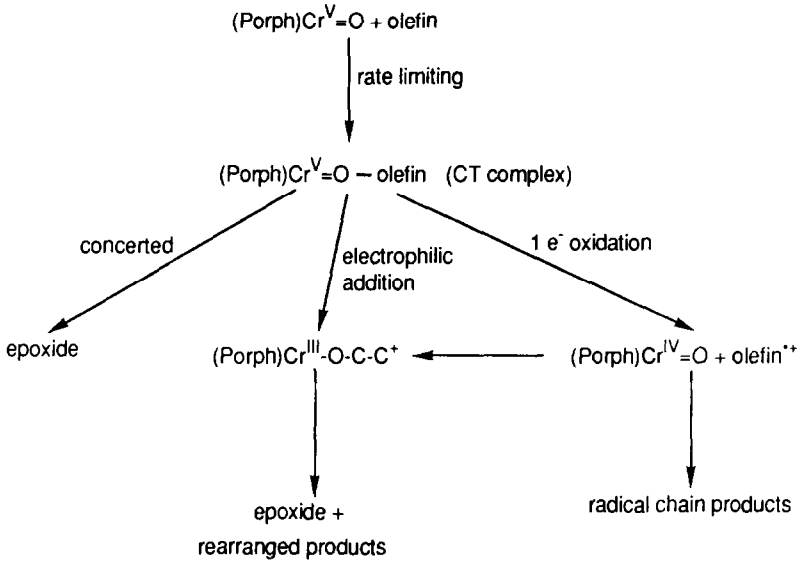
FIG. 3. Orbital arrangements proposed by Groves (a) and Bruice (b) to predict reactivity in cytochrome P-450 oxidations (Refs. (36) and (53)).

rearranged products are produced in the course of catalysis is also strong evidence for the formation of a radical cation by initial charge transfer, although these side products could result from competing pathways (*42*).

The most elegant evidence for a charge-transfer intermediate is a linear free-energy relationship worked out by Bruice using chromium porphyrins and a series of olefins, including a range of substituted styrenes. While this relationship strictly applies only to chromium porphyrins, it is believed to hold for the faster iron and (in most cases) manganese-catalyzed reactions as well. The mechanism is believed to be the same because all the catalysts give similar product distributions and the reactions give similar Hammett ρ^+ values. Bruice was able to generate the stable chromium–oxo species through electrolysis and to use it for kinetic epoxidation studies. The rate of epoxidation was directly proportional to the ionization potential of the olefin. This correlation strongly suggests that initial charge transfer is involved in the reaction (*43*).

A problem with this result is that the linear relationship extends even to substrates for which the ionization potential is greater than the activation energy for epoxidation. Thus full charge transfer cannot occur en route to epoxidation, at least for some substrates. The fact that there is no break in the plot for log (rate) versus ionization potential, however, argues that the same mechanism applies to all substrates. Bruice has explained this apparent contradiction by suggesting that the transition state is achieved by partial charge transfer, followed by direct attack of oxygen on the olefin. Occasionally, full charge transfer may give a radical cation which undergoes side reactions. The finding that certain more easily oxidized olefins give more side products, but that the rates of both epoxidation and side-product formation have the same dependence on ionization potential, is good evidence that partial charge transfer is rate-limiting for all reactions. Subsequent to charge transfer the pathways diverge and the relative importance of each depends on the olefin (Scheme 1) (*43*).

Another possible interpretation of these results is based on Traylor's finding that the amount of *endo* norbornene epoxide produced increases continuously as



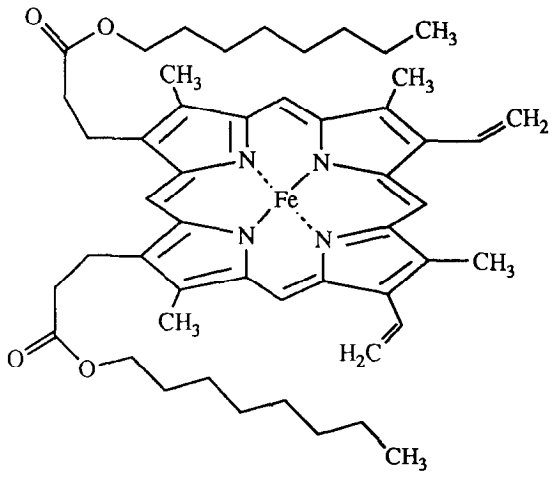
SCHEME I

one proceeds from chromium, manganese, and iron tetraarylporphyrins, ranging from chromium porphyrins with electron-donating substituents to iron porphyrins with electron-withdrawing substituents (*45*). There may be a continuous change in mechanism from direct attack to full electron transfer, or in other words, a continuous change in the degree of electron transfer at the transition state.

The investigations described above have significantly advanced our understanding of the epoxidation mechanism by Mn^{IV}=O and Fe^{IV}=O porphyrin radical cations, but there is evidence that in some cases a different oxo intermediate is involved in epoxidation. As mentioned above, with certain non-porphyrin catalysts and, in some cases, with manganese porphyrins (particularly when no axial ligands are present), a preference for *trans* over *cis* olefin epoxidation is seen, and considerable isomerization of *cis* olefins is observed. Groves was able to generate both the Mn^{IV}=O(porphyrin$^{\cdot+}$) and Mn^{IV}=O(porphyrin) intermediates at low temperatures. He showed that while the selectivity of the first species is similar to that observed with the enzyme or iron porphyrins, the second displayed the product distributions typical of the less selective manganese porphyrins without axial ligands. Noting that the *cis/trans* selectivity of manganese porphyrins could be improved by increasing concentrations of pyridine or imidazole, he postulated that electron donation by an axial ligand stabilizes the more highly oxidized, more selective oxo species (*39*).

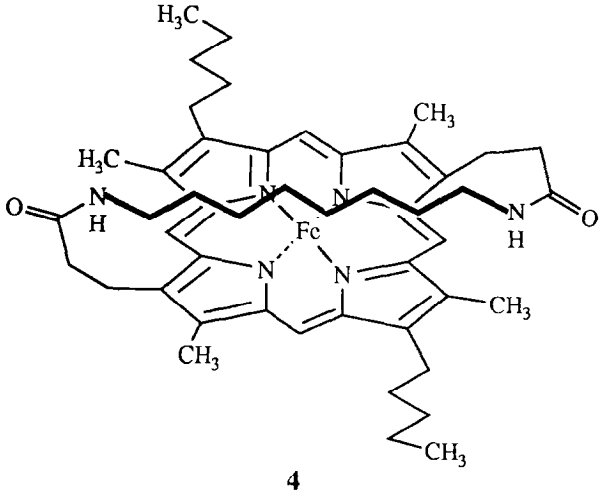
THE USE OF MODEL SYSTEMS AS SELECTIVE CATALYSTS

The sensitivity of these porphyrin reactions to the bulk of substrates indicates that, if the environment around the catalytic center can be carefully controlled, the catalysts have the potential to be quite regiospecific. In addition, the availability

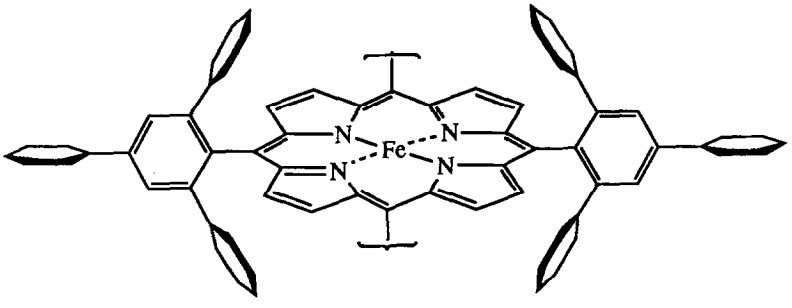


3

of procedures to modify the substituents at both the *meso* and pyrrole carbons should allow one to construct a variety of catalysts with different selectivities (*54*). The first systems in which selectivity was achieved by geometric control, rather than electronic effects (e.g., tertiary over secondary carbon), were published by Groves (*12*) and Change (*55*) nearly 20 years ago. Both systems used a naturally occurring iron porphyrin as the catalyst. The substrate was covalently attached by ester or amide linkages to heme propionates. In Groves's system, **3**, the substrate was octanol; in Chang's, **4**, it was diaminooctane, with two linkages to the porphyrin. In the singly linked case, a pronounced preference was observed for hydroxylation of the middle carbons. In the doubly linked case, the only alcohol obtained was the product of hydroxylation at the middle carbons. Unfortunately,



4



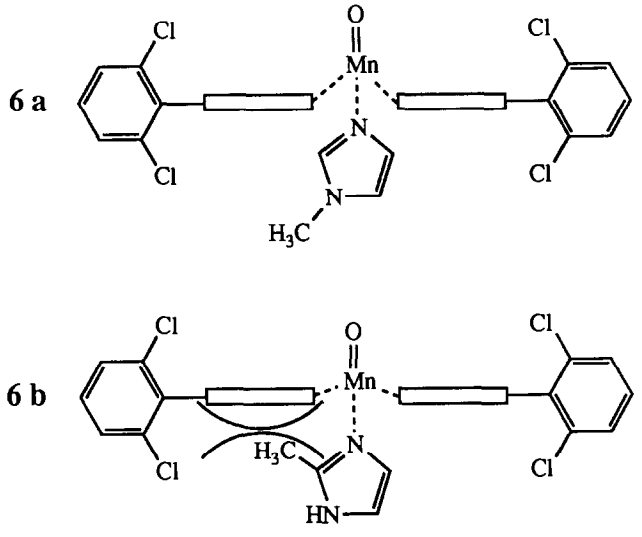
5

both of these catalysts were subject to the degradative side reactions seen with heme rings, and overall yields were very low. No competition reactions were carried out to determined the selectivity for bound versus unbound substrate.

Another way to achieve regioselective oxygenation has been to magnify the steric effects of the catalyst by using extremely bulky substituents on the *meso* phenyl rings of TPP. Suslick has reported a system, **5**, with phenyl substituents *ortho* to the *meso* carbons (*56*). This catalyst exhibits marked selectivity for terminal versus internal double bonds. For example, using 1,4-octadiene as a substrate, $Fe^{III}TPP$ produces 20 times more internal than terminal epoxide, whereas Suslick's bulky catalyst actually produces a slight excess of terminal epoxide (*57*). He has also observed a preference for hydroxylation of unhindered versus hindered (e.g., *tert*-butyl) primary carbons (*56*).

Mansuy has reported another interesting strategy to achieve regiospecificity in olefin epoxidation. A study was carried out using the relatively unhindered manganese(III)–*meso*-tetrakis(2,6-dichlorophenyl)porphyrin to epoxidize limonene. By changing the axial ligand from 1-methyl (**6a**) to 2-methylimidazole (**6b**), he was able to obtain a modest twofold increase in the reactivity of the external double bond relative to that of the more hindered, but normally much more reactive, internal double bond. He postulated that a steric interaction between the 2-methyl group on the imidazole and the porphyrin ring forces the manganese to change its position relative to the plane of the porphyrin. This shift could enhance the steric effects of the dichlorophenyl groups enough for a change in reactivity to be observed (*58*).

Recently, Groves has reported system **7** which hydroxylates and epoxidizes substrates in a highly regiospecific manner. The system consists of iron(III)–tetrakis(*o*-aminophenyl)porphyrin with four steroid derivatives appended to the amines by amide linkages. The porphyrin intercalates into a phospholipid bilayer, forming a well-defined cavity (*59*). With steroid substrates the system reacts only at the side-chain termini. Long-chain unsaturated fatty acids were also substrates for epoxidations, but with much lower selectivity. This was shown, however, to be partly due to a decrease in the rigidity of the bilayer resulting from incorporation of these substrates. Selectivity was improved by adding cholesterol, which rigidifies the bilayer (*59*).

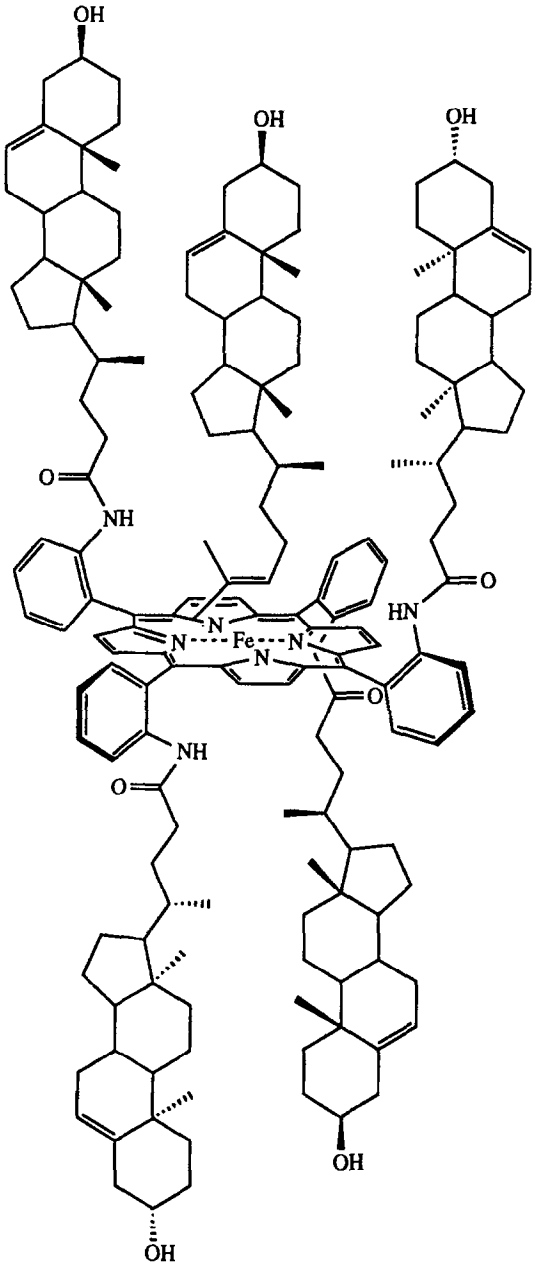


Groves has also used chiral porphyrin **8** to carry out asymmetric epoxidation (*60*). He observed a 50% ee with *p*-chlorostyrene as the substrate. Recently, Kodadek has presented a similar chiral porphyrin in which the binaphthoic moieties are directly linked to the porphyrin *meso* carbons. This system gives slightly lower ee's, with a maximum of 40% for *cis*-β-methylstyrene, but it was significantly more stable to the oxidation conditions, allowing much greater turnover (*61*). Groves has recently reported hydroxylation studies using another chiral porphyrin (**9**) and deuterated ethylbenzene. The measurement of deuterium distribution in the products demonstrated that selectivity depends on a combination of effects on two different reaction rates, hydrogen abstraction and carbon–oxygen bond formation. Best results are obtained for the substrate which is complementary to the catalyst, as expected (*62*). These findings demonstrate that porphyrin systems can serve as stereospecific catalysts, but more work will be necessary to achieve truly useful chiral catalysis.

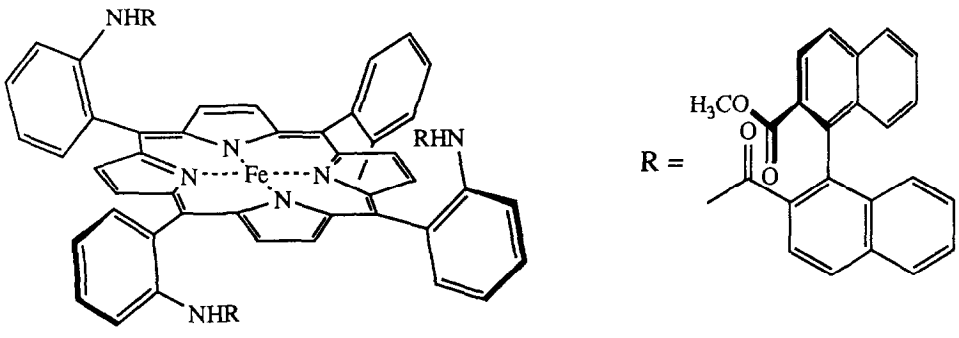
Another potentially selective porphyrin system was synthesized by Kaiser. In this case, a hydrophobic cavity was created by appending four peptides to the porphyrin *meso* carbons. These peptides were known to have a high potential for the formation of amphiphilic α-helices; the helices would have hydrophilic side chains projecting out from the porphyrin and hydrophobic residues on the interior. The peptides were found by circular dichroism to be 70% helical in solution, and the system was shown to be a competent catalyst for aniline hydroxylation (*63*). More detailed studies were not performed, but the system clearly has potential to achieve selective intra- or intermolecular oxidations. The use of amino acid α-helices to create an "active site" makes this an impressively biomimetic catalyst.

In the systems discussed above, substrate selectivity was achieved by steric interactions. Breslow *et al.* reported recently that selectivity can also be obtained

7



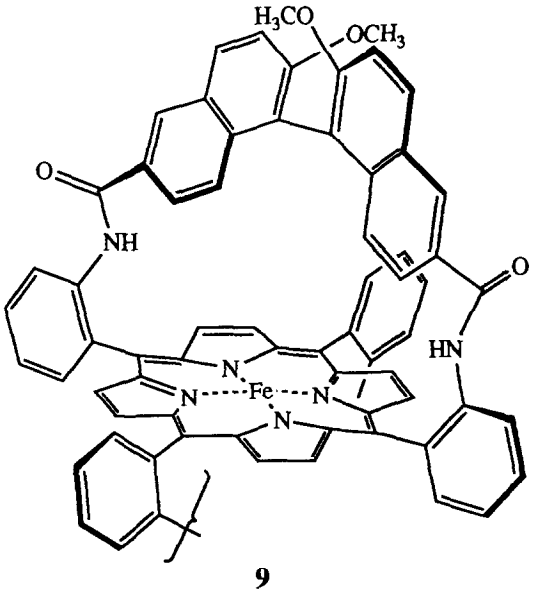
via metal–ligand interactions (*64*). This work used catalyst **10** and another system based on the salen ligand (**1**), which have, under the oxidation conditions, two or more copper ions chelated by bidentate ligands remote from the catalytic center. Substrates with ligands could bind to the catalyst. In dilute solution, this catalyst–metal–substrate binding was found to enhance the reactivity of ligand-con-



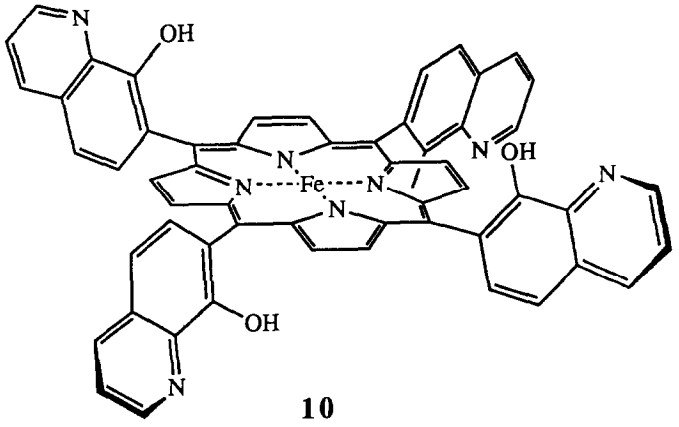
8

taining substrates 40-fold in competition with substrates which could not bind. If this work can be extended to hydroxylation as well as to epoxidation, and regiospecificity can be obtained as well as substrate specificity, metal binding should provide another powerful approach to selective catalysis.

To an organic chemist, the reactions catalyzed by various cytochrome P-450 isozymes, especially the highly specific oxygenation of carbon–hydrogen and carbon–carbon double bonds, are some of the most impressive in enzymology and are clearly of practical interest. The use of P-450 model systems has greatly aided enzymologists in determining the enzyme reaction mechanism and may soon provide selective catalysts of great interest to synthetic chemists.



9



10

ACKNOWLEDGMENTS

I want to thank Ronald Breslow for the encouragement and intellectual stimulation he provided during my graduate career. I also wish to acknowledge the National Science Foundation for a predoctoral fellowship.

REFERENCES

1. GUENGERICH, F. P., AND MACDONALD, T. L. (1984) *Acc. Chem. Res.* **17,** 9–16.
2. ORTIZ DE MONTELLANO, P. R. (1986) "Cytochrome P-450," Plenum, New York.
3. WHITE, R. E., AND COON, M. J. (1980) *Annu. Rev. Biochem.* **49,** 315–356.
4. BRUICE, T. C. (1988) "Mechanistic Principles of Enzyme Activity" (Liebman, J. F., and Greenberg, A., Eds.), pp. 227–278, VCH, New York.
5. MANSUY, D., BATTIONI, P., AND BATTIONI, J.-P. (1989) *Eur. J. Biochem.* **184,** 267–285.
6. NORBERT, D. W., ADESNIK, M., COON, M. J., ESTABROOK, R. W., GONZALEZ, F. J., GUENGERICH, F. P., GUNSALUS, I. C., JOHNSON, E. F., KAMPER, B., LEVIN, W., PHILIPS, I. R., SATO, R., AND WATERMAN, M. R. (1987) *DNA* **6,** 1–11.
7. POULOS, T. L., FINZEL, B. C., AND HOWARD, A. J. (1987) *J. Mol. Biol.* **195,** 687–700.
8. GROVES, J. T., HAUSHALTER, R. C., NAKAMURA, M., NEMO, T. E., AND EVANS, B. J. (1981) *J. Amer. Chem. Soc.* **103,** 2884–2886.
9. KADLUBAR, F. F., MORTON, K. C., AND ZIEGLER, D. M. (1973) *Biochem. Biophys. Res. Commun.* **54,** 1255–1261.
10. HRYCAY, E. G., GUSTAFSSON, J.-Å., INGELMAN-SUNDBERG, M., AND ERNSTER, L. (1975) *Biochem. Biophys. Res. Commun.* **66,** 209–216.
11. LICHTENBERGER, F., NASTAINCZYK, W., AND ULLRICH, V. (1976) *Biochem. Biophys. Res. Commun.* **70,** 939–946.
12. GROVES, J. T., NEMO, T. W., AND MYERS, R. S. (1979) *J. Amer. Chem. Soc.* **101,** 1032–1033.
13. MANSUY, D., LECLAIRE, J., FONTECAVE, M., AND DANSETTE, P. (1984) *Tetrahedron* **40,** 2847–2857.
14. TRAYLOR, P. S., DOLPHIN, D., AND TRAYLOR, T. G. (1984) *J. Chem. Soc., Chem. Commun.* 279–280.
15. BORTOLINI, O., AND MEUNIER, B. (1983) *J. Chem. Soc., Chem. Commun.* 1364–1366.
16. ADLER, A. D., LONGO, F. R., FINARELLI, J. D., GOLDMACHER, J., ASSOUR, J., AND KORSAKOFF, L. (1967) *J. Org. Chem.* **32,** 476.

17. MANSUY, D., FONTECAVE, M., AND BARTOLI, J. F. (1983) *J. Chem. Soc., Chem. Commun.* 253–254.
18. TABUSHI, I., AND KOGA, N. (1979) *J. Amer. Chem. Soc.* **101,** 6456–6458.
19. TRAYLOR, T. G., MARSTERS, J. C., JR., NAKANO, T., AND DUNLAP, B. E. (1985) *J. Amer. Chem. Soc.* **107,** 5537–5539.
20. GUILMET, E., AND MEUNIER, B. (1982) *Nouv. J. Chim.* **6,** 511–513.
21. COLLMAN, J. P., BRAUMAN, J. I., MEUNIER, B., RAYBUCK, S. A., AND KODADEK, T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3245–3248.
22. MEUNIER, B. (1986) *Bull. Soc. Chim. Fr.* 578–594.
23. LEE, R. W., NAKAGOKI, P. C., BALASUBRAMANIAN, P. N., AND BRUICE, T. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 641–644.
24. COLLMAN, J. P., KODADEK, T., RAYBUCK, S. A., AND MEUNIER, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7039–7041.
25. GROVES, J. T., AND KRUPER, W. J., JR. (1979) *J. Am. Chem. Soc.* **101,** 7613–7615.
26. (a) KOOLU, J. D., AND KOCHI, J. K. (1987) *Inorg. Chem.* **26,** 908–916. (b) KINNEARY, J. F., WAGLER, T. R., AND BURROWS, C. J. (1988) *Tetrahedron Lett.* **29,** 877–880.
27. TAI, A. F., MARGERUM, L. D., AND VALENTINE, J. S. (1986) *J. Amer. Chem. Soc.* **108,** 5006–5008.
28. LEUNG, T., JAMES, B. R., AND DOLPHIN, D. (1983) *Inorg. Chim. Acta* **79,** 180–181.
29. STÄUBLI, B., FRETZ, H., PIANTINI, U., AND WOGGON, W.-D. (1987) *Helv. Chim. Acta* **70,** 1173–1193.
30. SHIMIAZU, T., IYODA, T., AND KANDA, N. (1981) *J. Chem. Soc., Chem. Commun.* 1206–1207.
31. SAMSEL, E. G., SRINIVASAN, K., AND KOCHI, J. K. (1985) *J. Amer. Chem. Soc.* **107,** 7606–7617.
32. SRINIVASAN, K., MICHAUD, P., AND KOCHI, J. K. (1986) *J. Amer. Chem. Soc.* **108,** 2309–2320.
33. CHE, C.-M., AND CHENG, W.-K. (1986) *J. Chem. Soc., Chem. Commun.* 1443–1444. (See also Refs. (26) and (27).)
34. VANATTA, R. B., FRANKLIN, C. C., AND VALENTINE, J. S. (1984) *Inorg. Chem.* **23,** 4121–4123.
35. GROVES, J. T., MCCLUSKY, G. A., WHITE, R. E., AND COON, M. J. (1978) *Biochem. Biophys. Res. Commun.* **81,** 154–160.
36. GROVES, J. T., AND NEMO, T. E. (1983) *J. Amer. Chem. Soc.* **105,** 6243–6248.
37. GROVES, J. T., AND NEMO, T. E. (1983) *J. Amer. Chem. Soc.* **105,** 5786–5791.
38. ORTIZ DE MONTELLANO, P. R., MANGOLD, B. L. K., WHEELER, C., KUNZE, K. L., AND REICH, N. O. (1983) *J. Biol. Chem.* **258,** 4208–4213 and references therein.
39. GROVES, J. T., AND STERN, M. K. (1988) *J. Amer. Chem. Soc.* **110,** 8628–8638.
40. OSTOVIĆ, D., AND BRUICE, T. C. (1988) *J. Amer. Chem. Soc.* **111,** 6511–6517.
41. LOEW, G. H., KERT, C. J., HJELMELAND, L. M., AND KIRCHNER, R. F. (1977) *J. Amer. Chem. Soc.* **99,** 3534–3536.
42. BRUICE, T. C., AND CASTELLINO, A. J. (1988) *J. Amer. Chem. Soc.* **110,** 7512–7519.
43. GARRISON, J. M., OSTOVIĆ, D., AND BRUICE, T. C. (1989) *J. Amer. Chem. Soc.* **111,** 4960–4966.
44. TRAYLOR, T. G., NAKANO, T., DUNLAP, B. E., TRAYLOR, P. S., AND DOLPHIN, D. (1986) *J. Amer. Chem. Soc.* **108,** 2782–2784.
45. TRAYLOR, T. G., AND MIKSZTAL, A. R. (1989) *J. Amer. Chem. Soc.* **111,** 7443–7448.
46. COLLMAN, J. P., BRAUMAN, J. I., MEUNIER, B., HAYASHI, T., KODADEK, T., AND RAYBUCK, S. A. (1985) *J. Amer. Chem. Soc.* **107,** 2000–2005.
47. GROVES, J. T., AND WANTANABE, Y. (1986) *J. Amer. Chem. Soc.* **108,** 507–508.
48. SHARPLESS, B., TERANISHI, A. Y., AND BACKVALL, J. E. (1977) *J. Amer. Chem. Soc.* **99,** 3120–3128.
49. GRUBBS, R. H. (1978) *Prog. Inorg. Chem.* **24,** 1–50.
50. (a) IVIN, K. J., ROONEY, J. J., STEWART, C. D., GREEN, M. L. H., AND MAHTAB, R. (1978) *J. Chem. Soc., Chem. Commun.* 604–606. (b) BROOKHART, M. H., TIMMERS, D., TUCKER, J. R., WILLIAMS, G. D., HUSK, G. R., BRUNNER, H., AND HAMMER, B. (1983) *J. Amer. Chem. Soc.* **105,** 6721–6723. (c) YANG, G. K., AND BERGMAN, R. G. (1983) *J. Amer. Chem. Soc.* **105,** 6500–6501.
51. TRAYLOR, T. G., NAKANO, T., MIKSZTAL, A. R., AND DUNLAP, B. E. (1987) *J. Amer. Chem. Soc.* **109,** 3625–3632.
52. STREITWEISER, A., JR., AND OWENS, P. H. (1973) "Orbital and Electron Density Diagrams: An Application of Computer Graphics," pp. 33–47, Macmillan, New York.

53. OSTOVIĆ, D., AND BRUICE, T. C. (1988) *J. Amer. Chem. Soc.* **110,** 6906–6908.
54. KIM, J. B., ADLER, A. D., AND LONGO, F. R. (1978) "The Porphyrins" (Dolphin, D., Ed.), Vol. 1, pp. 85–100, Academic Press, New York.
55. CHANG, C. K., AND KUO, M.-S. (1979) *J. Amer. Chem. Soc.* **101,** 3413–3415.
56. COOK, B. R., REINERT, T. J., AND SUSLICK, K. S. (1986) *J. Amer. Chem. Soc.* **108,** 7281–7286.
57. SUSLICK, K. S., AND COOK, B. R. (1987) *J. Chem. Soc., Chem. Commun.* 200–202.
58. BATTIONI, P., RENAUD, J. P., BARTOLI, J. F., REINA-ARTILES, M., FORT, M., AND MANSUY, D. (1988) *J. Amer. Chem. Soc.* **110,** 8462–8470.
59. GROVES, J. T., AND NEUMANN, R. (1989) *J. Amer. Chem. Soc.* **111,** 2900–2909.
60. GROVES, J. T., AND MYERS, R. S. (1983) *J. Amer. Chem. Soc.* **105,** 5791–5796.
61. O'MALLEY, S., AND KODADEK, T. (1989) *J. Amer. Chem. Soc.* **111,** 9116–9117.
62. GROVES, J. T., AND VISKI, P. (1989) *J. Amer. Chem. Soc.* **111,** 8537–8538.
63. SASAKI, T., AND KAISER, E. T. (1989) *J. Amer. Chem. Soc.* **111,** 380–381.
64. BRESLOW, R., BROWN, A. B., MCCULLOUGH, R. D., AND WHITE, P. W. (1989) *J. Amer. Chem. Soc.* **111,** 4517–4518.