Regioselective and Enantioselective Epoxidation Catalyzed by Metalloporphyrins

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Recent progress in regioselective and enantioselective epoxidations catalyzed by metalloporphyrins is discussed here, with an explanation of the biomimetic antecedents of this area and its relevance to synthetic applications. Classification of the catalysts that have been studied allows useful conclusions to be drawn about the development of this field. In particular, both the most promising biomimetic and practical catalysts have arisen from systems that can be systematically modified by convenient synthetic methodology.

Several enzymes tantalize chemists with their remarkable catalytic oxygenation reactions. Heme enzymes such as cytochrome P-450 (*1*) and nonheme enzymes such as methane monooxygenase (*2*) perform intrinsically difficult oxidations (such as hydroxylation of unactivated alkanes), often with high selectivity. The crudest features of these processes are illustrated in Fig. 1A, where an enzymatic cavity capable of discriminating between various potential substrates contains an iron atom at its active site (Fig. 1B). Redox events involving molecular oxygen (or oxygen atom transfer from abiological oxidants) generate an active oxidant that is thought to contain a metal atom in a high oxidation state that is bonded to an oxygen atom. This reactive species then transfers the oxygen atom and, after the product leaves, is ready for another cycle. Improving the selectivity of these challenging oxidations in synthetic systems could affect the development of products ranging from commodity chemicals to specialty pharmaceuticals. Here, we review the recent progress made with regioselective and enantioselective oxidation catalysts. We focus particularly on the selective catalytic epoxidation of unfunctionalized olefins mediated by metalloporphyrin complexes (*3*). The principles that have been elucidated from these studies should facilitate the development of improved catalysts and increase our understanding of enzymatic selectivity.

Epoxide Chemistry and Catalyst Design

Epoxides, which contain a three-membered C–C–O ring, are most often prepared by oxygen atom transfer to olefins. Epoxides are not only found in naturally occurring compounds (*4*) and in the carcinogenic products of biological oxidations of aromatic hydrocarbons (*5*), but are also versatile intermediates in organic synthesis. For example, epoxides can undergo stereospecific ring-opening reactions with nucleophiles to form α-substituted alcohols (*6*).

The crucial difficulty in the selective epoxidation of unfunctionalized alkenes (olefins that have only hydrocarbon substituents) is control of the olefin approach to the active oxidant. With functionalized alkenes (olefins that contain heteroatoms), this problem has been solved with the use of the functional groups to ligate the olefin to the catalyst. The Sharpless epoxidation of allylic alcohols is one outstanding example of the utility of this technique (*7*). With unfunctionalized olefins, however, only low-energy, noncovalent interactions are available. Nevertheless, such nonbonded interactions between a substrate and the elaborate protein superstructure of an enzyme can convey the high degree of selectivity found in many enzymatic reactions. Practical selective epoxidation catalysts for unfunctionalized olefins demand architecturally simpler structures that still have sufficient noncovalent interactions with the substrate to induce high selectivity.

The degree to which trial and error can be avoided in catalyst design is associated with the degree of understanding of the reaction mechanism. Manganese and iron derivatives constitute the most common metalloporphyrin oxidation catalysts. Many studies implicate a high-valent iron-oxo species as the catalytically active intermediate in P-450 dioxygen activation (*1*). Similarly, high-valent metal-oxo species are also implicated when strong oxygen atom transfer reagents such as iodosylbenzene, peracids, or hypochlorite react with biomimetic metalloporphyrin catalysts (*8*). Although several

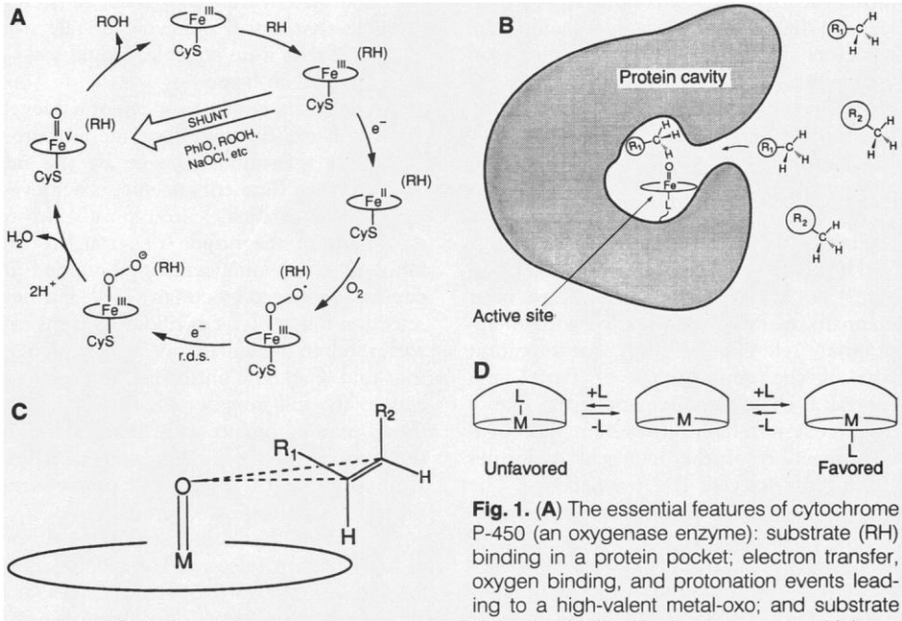

Fig. 1. (**A**) The essential features of cytochrome P-450 (an oxygenase enzyme): substrate (RH) binding in a protein pocket; electron transfer, oxygen binding, and protonation events leading to a high-valent metal-oxo; and substrate oxidation (ROH). The biological pathway may be "short-circuited" with shunt reagents, which are oxygen atom transfer agents; r.d.s., rate-determining step; Ph, phenyl. (**B**) The nature of the protein pocket selects both the substrate (or substrates) that may be used and the bond (or bonds) that may react. (**C**) The side-on approach of an olefin to a metal-oxo believed to precede oxygen atom transfer. (**D**) The use of an excess of a sufficiently bulky ligand (L) favors the desired five-coordinate intermediate in which the ligand binds the exterior face of the metalloporphyrin (M, transition metal).

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variables affect the intimate workings of the epoxidation mechanism, including the *d*-electron count of the metal, the redox potential of the metal-oxo complex, the nature of the oxygen atom source, and the ancillary axial ligand, the details of these mechanistic problems and their associated controversies are beyond the scope of this article (9). Remarkably, a key general feature has arisen from the mechanistic complexity surrounding the metal-catalyzed epoxidations: The initial olefin approach that leads to reaction may be similar for many otherwise dissimilar metal-oxo species. Apparently, whatever the subsequent mechanistic fate may be, a side-on approach of the olefin to the metal-oxo immediately precedes the epoxidation reaction (Fig. 1C). Groves and Nemo first proposed a side-on approach of olefins to porphyrin iron-oxos to account for *cis*-olefins being epoxidized faster than *trans*-olefins (*10*). Such an approach should maximize the interactions between the olefin π orbitals and the Fe=O π antibonding orbitals. This postulate can be used to interpret numerous experimental results and has provided a useful working model in the design of metalloporphyrins and other transition metal–based epoxidation catalysts (*11*).

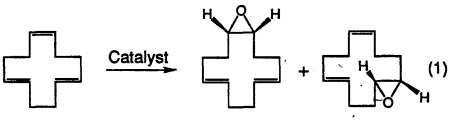
Porphyrins are useful for establishing a catalytic site within a controlled steric environment because their *meso* and β-pyrrolic positions may all be systematically substituted. The central metal atom can bind additional ligands only in the two sites located above and below the porphyrin plane. Although all heme-based oxygenases permit oxygen transfer solely from a single face of the porphyrin, biomimetic systems can be mono-faced or bis-faced, depending on whether one or both faces of the macrocycle have substituents. Mono-faced systems are, in some sense, truer biomodels, but obtaining high selectivity with practical monofaced catalysts will always require that an ancillary ligand be found that will block the unsubstituted face, not the substituted face, and that will be compatible with the catalytic conditions. Preventing "leakage" reactions (oxo transfer from the unsubstituted face) is especially vital with chiral catalysts because enantiomeric products are generally more difficult to separate than regioisomeric products. Control of the reactivity of mono-faced systems is typically accomplished with an excess of an axial blocking ligand that is sterically prevented from binding on the substituted porphyrin face (Fig. 1D). Bis-faced catalysts have the conceptual advantage of not being susceptible to "leakage" reactions because oxo-transfer may occur from either face without loss of selectivity.

Although alkane hydroxylation mediated by metalloporphyrin catalysts is of considerable interest in the general context of hydrocarbon activation (*12*) and particularly the highly selective hydroxylations performed by many P-450 enzymes, it limits the design of epoxidation catalysts. Because many metalloporphyrins that catalyze olefin epoxidation also catalyze alkane hydroxylation, the porphyrin and its superstructure are themselves potential substrates. Natural systems have evolved to cope with this problem. In some systems, the enzyme and its cofactors favor metal-oxo formation only when the substrate molecule is bound within the enzymatic cavity; this is exceedingly difficult to mimic with synthetic catalysts. However, the tertiary protein structure also contributes substantially to enzymatic lifetime by preventing the active species from contacting other potentially oxidizable metalloporphyrins. In addition, the tertiary protein structure conveys rigidity to the pocket above the porphyrin so that the amino acid backbone and side chains do not suffer intramolecular oxidation by contact with the active site. These aspects have been mimicked successfully—the longest lived biomimetic catalysts have bulky, rigid groups distributed about the porphyrin periphery.

Regioselective Epoxidation Catalysts

Although organisms often tailor enzymes for a single substrate, a variety of highly specific reactions can be performed by divergent forms of an enzyme. For example, cytochrome P-450 can catalyze an array of regioselective hydroxylation and epoxidation reactions because more than 150 known P-450 enzymes exist (*13*). Although biological systems respond to environmental pressures through genetic variability, the central challenge to chemists is to develop catalysts that have synthetic variability—that is, catalysts that are readily prepared and readily modified and that promote selective transformations of a variety of substrates.

Investigators initially examined regioselective epoxidations with simple porphyrins such as tetraphenylporphyrin (TPP) and tetramesitylporphyrin (TMP) (Fig. 2, R = H and R = CH_3, respectively). The steric properties of these systems are dictated by varying the size of the ortho substituents on the aryl rings. Even catalysts derived from the relatively unhindered TPP ligand react more readily with *cis*-olefins than with *trans*-olefins. For example, *cis*-stilbene is epoxidized by Fe(TPP)(Cl) with a 77% yield, whereas *trans*-stilbene is unreactive under the same conditions (*10*). This *cis* versus *trans* selectivity can be augmented by increasing the steric bulk of the porphyrin ligand. Thus, in the epoxidization of the *cis* double bond of 1,5,9-*trans*,*cis*,*trans*-cyclododecatriene



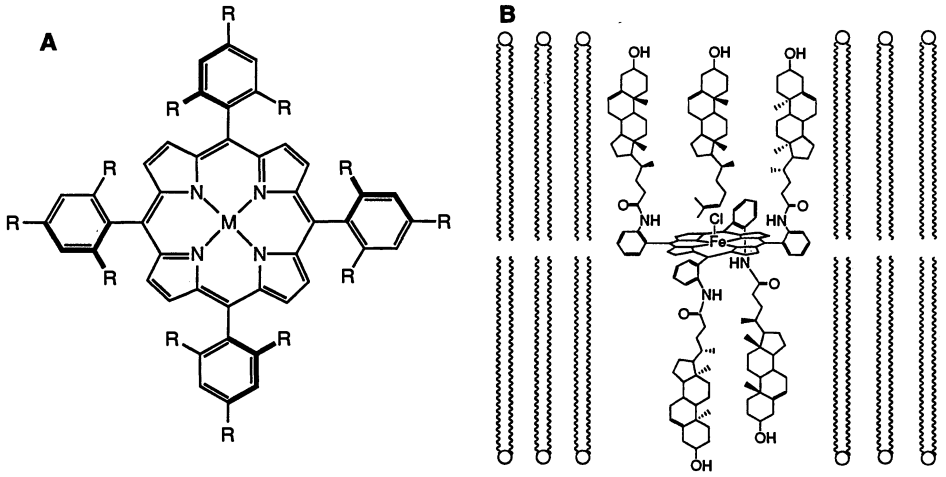
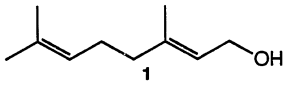


Fig. 2. (**A**) A metalloporphyrin with four *meso* aryl substituents. M, transition metal. (**B**) A steroidal porphyrin intercalated within a lipid bilayer. [Reprinted with permission from (*18*). Copyright 1987 and 1989, American Chemical Society]

Table 1. Epoxidation of 1,5,9-*trans*-*cis*-*trans*-cyclododecatriene (Eq. 1). The *cis*/*trans* selectivity ratio is adjusted to account for the two *trans* double bonds and the one *cis* double bond in the olefin.

Catalyst	Oxidant	*Cis*/*trans* (selectivity ratio)	Reference
Fe(TPP)(Cl)	PhIO	1	(*10*)
Fe(TMP)(Cl)	PhIO	10.4	(*10*)
Mn(TPP)(Cl)	LiOCl	1.4	(*14*)
Mn(TMP)(Cl)	LiOCl	15	(*14*)

TMP-based catalysts yield higher selectivities than do TPP-based catalysts (Table 1) (*10, 14*). Electronic differences between olefins can also be exploited with these simple metalloporphyrin catalysts. For example, Tabushi and Morimitsu (*15*) and De Carvalho and Meunier (*16*) found that the 6,7-tri-substituted olefin of a geraniol derivative (**1**)



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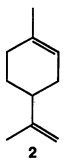
is epoxidized in a 32:1 ratio over the allylic (electron-deficient) 2,3-tri-substituted olefin with a simple metalloporphyrin.

Suslick's group further probed the relation between steric and electronic control by synthesizing an even more hindered catalyst, Mn[5,10,15,20-tetrakis(2′,4′,6′-triphenylphenyl)porphyrin](acetate) [Mn(TTPPP)(OAc)], which has four phenyl groups projecting over each side of the porphyrin plane (Fig. 2A) (R = phenyl) (*17*). Less substituted olefins that react more slowly because of electronic factors can be the preferred substrates in epoxidation reactions with Mn(TTPPP)(OAc) because they are less sterically encumbered. As an illustration, previous epoxidation studies (*16*) showed the internal double bond of limonene (**2**)



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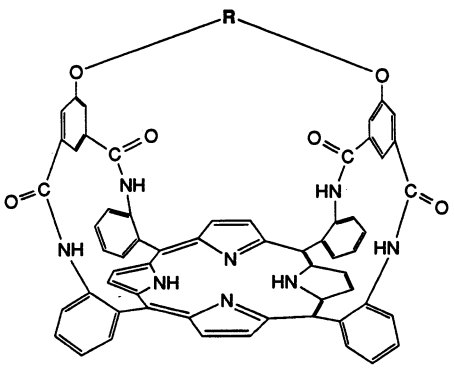
reacts faster than its external double bond, and a similar reactivity pattern is observed with tetrakis(4′-methylphenyl)porphyrin (TTP) and 5,10,15,20-tetrakis(2′,4′,6′-trimethoxyphenyl)porphyrin (TTMPP) (Fig. 2A) (R = OCH_3) systems. However, the highly hindered TTPPP catalysts gave the external epoxide as the major product.

Groves and Newmann addressed the problem of regioselective steroid epoxidation by placing a metalloporphyrin catalyst bearing steroid side chains in a lipid bilayer membrane (Fig. 2B) (*18*). Electron paramagnetic resonance (EPR) experiments indicated that this "pillared" steroidal porphyrin intercalated to the center of the phospholipid bilayer, with the porphyrin ring oriented perpendicular to the phospholipid chains. In this configuration, the manganese and iron derivatives serve as regioselective catalysts for amphiphilic molecules such as hydroxylated steroids or long-chain fatty acids. The alcohol group of a steroidal substrate orients toward the aqueous phase, making the ring olefin inaccessible to the catalyst, although the side chain olefin is well positioned for epoxidation. Thus, although simple metalloporphyrins such as Fe(TTP)(Cl) favor epoxidation of the steroid ring olefin, the pillared steroidal porphyrin catalyst exclusively epoxidizes the side chain olefin. Only moderate regioselectivity was reported with polyunsaturated fatty acids, which was attributed to disruption of the bilayer structure. Rigidifying the phospholipid bilayer by adding cholesterol improved the fatty acid regioselectivity.

The utility of a biomimetic approach for developing regioselective epoxidation catalysts has been demonstrated with "picnic basket porphyrins" (PBPs) developed in Collman's group (*19*). These systems possess a rigid cavity on one face of the macrocycle (Fig. 3) that mimics the protein structure surrounding the active sites of enzymes like cytochrome P-450. The unhindered face of a manganese PBP is blocked by 3,5-di-*tert*-butylphenoxide (OAr), which is prevented sterically from binding inside the cavity. Like enzymatic cavities, the PBP cavities can differentiate olefins by size. However, unlike enzymes the PBPs are easily modified, which allows these synthetic catalysts to be optimized for a variety of substrates. For example, Mn(C_6PBP)(OAr) and Mn(PXYLPBP)-(OAr) (Fig. 3) are 60 to 1000 times more selective in competitive epoxidations of *cis*-2-octene and *cis*-cyclooctene (1:1) than



R	Name
$-(CH_2)_n-$	
$n = 2$	C_2PBP
4	C_4PBP
6	C_6PBP
8	C_8PBP
10	C_{10}PBP
$-CH_2-C_6H_4-CH_2-$	PXYLPBP

Fig. 3. Picnic basket porphyrins. [Reprinted with permission from (*19*). Copyright 1990 and 1992, American Chemical Society]

is either Mn(TPP)(OAr) or Mn(TMP)-(OAr) (Table 2). The smaller PBPs (C_2 and C_4) are too small to accommodate either olefin, whereas the cavities of Mn(C_8PBP)(OAr) and Mn(C_{10}PBP)(OAr) are too large to provide high selectivity. These results demonstrate that steric manipulation and variability are useful in the optimization of selective epoxidation catalysts (*20*).

Asymmetric Epoxidation Catalysts

The increasing demand for enantiomerically pure pharmaceuticals (*21, 22*) has fueled the search for asymmetric epoxidation catalysts that can introduce one or two new stereocenters upon reaction with an olefin of appropriate symmetry. Several studies of abiotic reactions with exogenous olefins have shown that P-450 enzymes can perform asymmetric epoxidations with high enantioselectivities. However, in spite of great strides made by many groups in the preparation of biomimetic systems, the need continues for new catalysts that can epoxidize unfunctionalized olefins enantioselectively (*23*).

Chiral porphyrins (*24*) have been prepared in three ways. (i) The most common approach, first used by Groves and Meyers (*25*), involves attaching chiral units to preformed porphyrins such as amino- or hydroxy-substituted TPP derivatives (Scheme 1). (ii) O'Malley and Kodadek showed that chiral substituents can be introduced at the porphyrin-forming stage by allowing chiral aldehydes to condense with pyrrole (Scheme 2) (*26*). (iii) Chiral porphyrins can also be prepared without the attachment of chiral groups. Inoue and co-workers (*20*) bridged the enantiotopic faces of dihexyletioporphyrin from opposing β-pyrrolic positions and then separated the resulting enantiomers with preparative high-performance liquid

Table 2. Competitive epoxidations of *cis*-2-octene and *cis*-cyclooctene. OAr = 3,5-di-*tert*-butylphenoxide. Catalysts are as in Fig. 3.

Catalyst	 Epoxide ratio
Mn(TPP)(OAr)	1.1
Mn(TMP)(OAr)	0.7
Mn(C_2PBP)(OAr)*	1.1
Mn(C_4PBP)(OAr)*	2.1
Mn(C_6PBP)(OAr)	67
Mn(PXYLPBP)(OAr)	>1000
Mn(C_8PBP)(OAr)	1.6
Mn(C_{10}PBP)(OAr)	0.2

*Slow reaction.

chromatography (HPLC) using a chiral stationary phase (Scheme 3) (*27*).

The catalysts prepared by the above methods can be divided into three structural types (Fig. 4). Type I systems are chiral "picket fence" porphyrins having two (type IA) or four (type IB) chiral pickets above and below the porphyrin unit. Type II systems are chiral basket handle porphyrins in which a strap connects diagonal positions of one (type IIB) or both (type IIA) faces of the macrocycle. In type III systems, adjacent *meso* positions are connected by chiral straps. The unbridged (type IIIC) and bridged (type IIID) single-faced systems are distinguished from the corresponding bis-faced systems, which have eclipsed (type IIIA) or staggered (type IIIB) chiral straps.

As yet, no type IA structures have been especially effective as asymmetric epoxidation catalysts (*25, 26, 28–30*). Only 10 to 33% enantiomeric excess (% ee = the percent excess of one enantiomer over the other = |percent of *R* enantiomer − percent of *S* enantiomer|) is obtained in the epoxidation of styrene with iron or manganese derivatives of **3** to **6** and **8**, whereas the iron derivative of **7** provided up to 51% ee in the epoxidation of 4-chlorostyrene. The flexibility of the chiral substituents in **3** to **6** precludes well-defined catalyst-substrate interactions, and low optical yields result. The flexibility also leaves the chiral substituents vulnerable to hydroxylation by the active species and may also permit the intermolecular oxidation of other porphyrins. Thus, fewer than 100 catalyst turnovers have been realized with these catalysts. Although the more rigid binaphthyl units in **8** do not enhance the optical yields, they do enhance the catalyst stability: 2800 turnovers have been obtained from the manganese derivative of **8**.

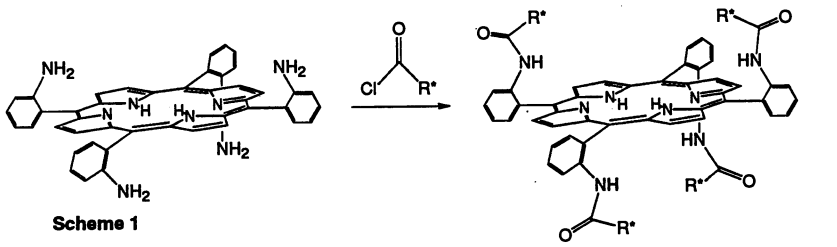
The additional chiral units of the D_4 symmetric type IB system (*31*), **9**, provide more efficient transfer of asymmetry from the catalyst to the substrate. The manganese derivative of **9** gives up to 76% ee in the epoxidation of *cis*-β-methylstyrene. The rigidity of the chiral substituents prevents their intramolecular decomposition, whereas their large size prevents intermolecular decomposition reactions. These factors combined convey the stability needed to obtain up to 2000 turnovers.

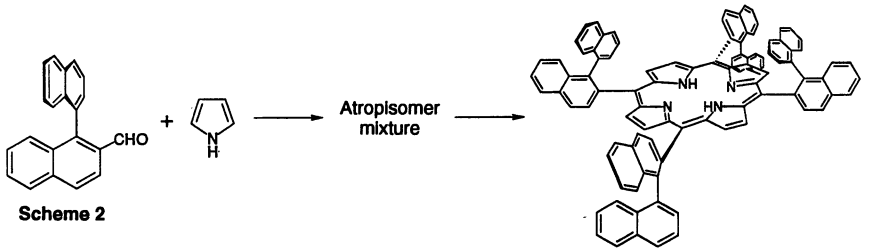
Higher stereoselectivities are also achieved with the type IIA systems **10** to **12** (*28, 32, 33*), the bridged counterparts of the type IA systems. The bridges enhance catalyst effectiveness by positioning the chiral units closer to the active site. For example, the best result obtained in the epoxidation of *cis*-β-methylstyrene with a metal derivative of a type IA system is 40% ee (*25*), whereas that with a similar type IIA system is 72% ee (*33*). However, if the strap is insufficiently rigid and bears readily oxidizable substituents, the proximity of the chiral strap to the metal oxo can limit the lifetime of the catalyst. Thus, **11** (with its rigid binaphthyl strap) gave more than 300 catalyst turnovers (*33*), whereas the dramatic instability of **10** (with its flexible straps) permitted less than two turnovers (*28*).

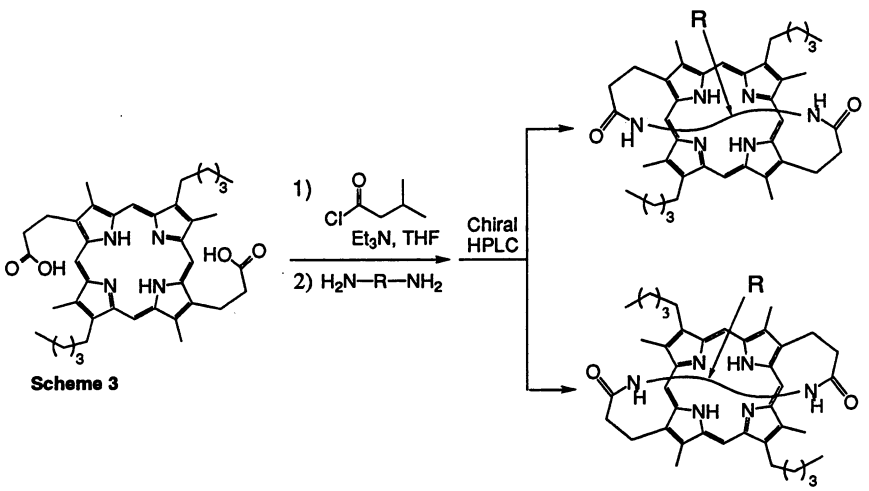
The type IIB systems have revealed additional requirements for effective catalytic asymmetric epoxidation. The manganese derivative of the chiral PBP, **13**, was found to give only 13% ee in the epoxidation of styrene when a bulky phenoxide ligand was used to block the unhindered face of the catalyst (*19*). This low optical yield suggests that the incoming olefin interacts very little with the chiral binaphthyl bridging unit that is more than 6 Å above the porphyrin plane (*34*). Higher optical yields (up to 58% ee for indene) were reported by Inoue and co-workers with manganese derivatives of **14** to **16** when various imidazoles were used as blocking ligands (*20*). The higher optical yields of these systems originate from the closer proximity of the straps to the active site. In addition, because these researchers could easily vary the diamine employed in their porphyrin syntheses, their chiral catalysts were the first to have a steric environment that could be readily and systematically modified. Synthetic variability has been essential to the success of the more effective asymmetric epoxidation catalysts discussed below.

Iron derivatives of the type IIIA and IIIB systems (the "twin coronet" porphyrins of Naruta and co-workers) can be very effective catalysts in the epoxidation of electron-deficient olefins (*35*). For example, up to 96% ee is obtained in the epoxidation of 3,5-dinitrostyrene with an iron derivative of **18**. This high selectivity was attributed to π stacking interactions between the electron-deficient substrate and the electron-rich binaphthyl straps of the catalyst, which are believed to orient the approach of the olefin. Unfortunately, lower stereoselectivities were obtained with other olefins.

The threitol-strapped porphyrins of Collman *et al.* (types IIIC and IIID) are among the most effective asymmetric epoxidation







catalysts derived from metalloporphyrins (36). The chiral frameworks of these systems are easily varied by condensing different aldehydes and ketones with the 2,3-diol of the threitol unit. By using various ketal (type IIIC) and acetal (type IIID) groups, the chiral environment of the catalyst can be optimized. Up to 88% ee can be obtained in the epoxidation of 1,2-dihydronaphthalene

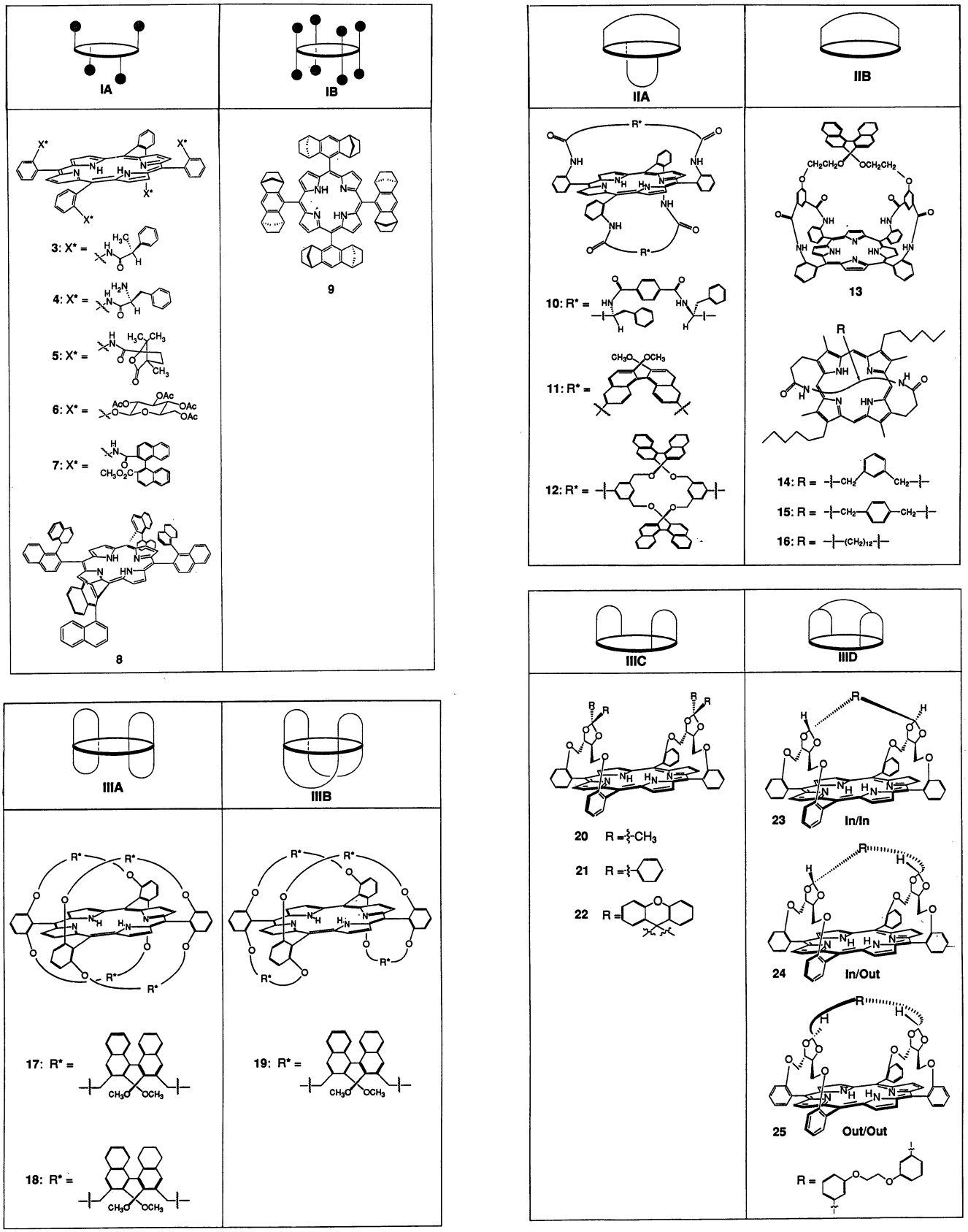


Fig. 4. Structural types of asymmetric epoxidation catalysts. Type I, upper left; type II, upper right; and type III, bottom panels.

with a manganese derivative of **25** when a bulky imidazole ligand is used to block the unhindered face of the catalyst.

The bridge of the type IIID systems pulls the threitol units closer to the center of the macrocycle; this generates higher enantioselectivities but also promotes catalyst degradation. For example, the enantioselectivity obtained with the most effective system (the manganese derivative of **25**) begins decreasing after approximately 100 turnovers. Although the ultraviolet-visible spectrum of the recovered catalyst is unchanged, a mass spectrum of the material indicates that higher molecular weight species are formed that are consistent with the hydroxylation of the catalyst. Apparently, the chiral superstructure is being oxidized because the porphyrinic chromophore is unperturbed. This system remains active, although the alteration of the chiral environment weakens its effectiveness as an asymmetric catalyst.

A recurring theme in the olefin epoxidation studies performed with chiral metalloporphyrin catalysts is that the chiral groups surrounding the macrocycle must be far enough from the central metal to permit facile entry of the substrate and to prevent intramolecular oxidative decomposition, but must be close enough to generate high enantioselectivities. These proximity constraints require careful consideration when chiral porphyrin catalysts are designed, because the chiral substituents attached to the periphery of the macrocycle are at least four bond lengths away from the porphyrin center where the oxo transfer occurs (Fig. 5A). This intrinsic problem of chiral porphyrin systems can be addressed in several ways. First, very large chiral substituents (such as binaphthyl units) can be positioned over the macrocycle, forcing greater interaction with the substrate. Second, a greater number of chiral substituents can be used (as in **9**), which favors a specific orientation of the olefin on its approach to the center of the macrocycle. Third, the chiral substituents can be used to bridge the center of the macrocycle (as in the type II and IIID systems), moving the chirality closer to the reactive species. Fourth, interactions between the chiral unit and the substrate (other than steric repulsion) that favor a specific approach of the olefin can be exploited (such as π stacking interactions with **17** to **19**). The best epoxidation results with chiral metalloporphyrin catalysts are summarized in Table 3.

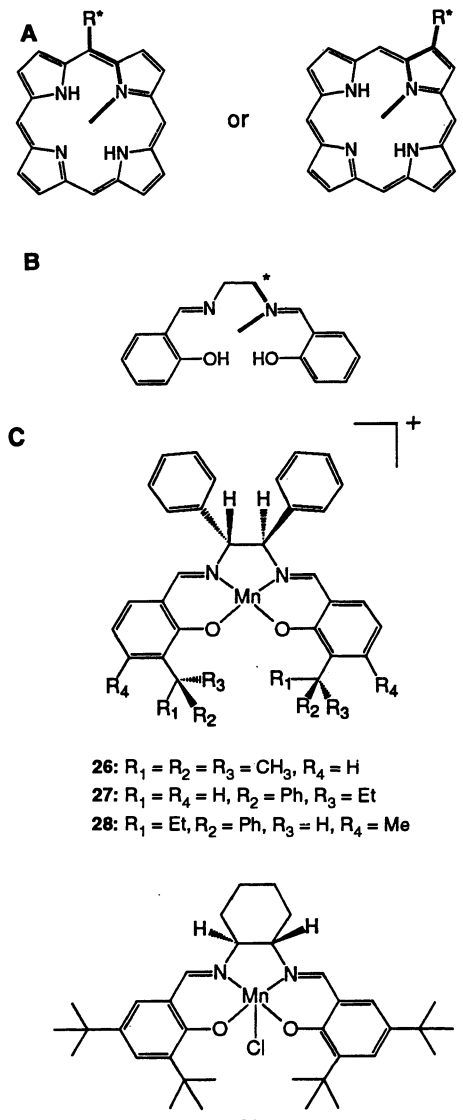


Fig. 5. (**A** and **B**) The difference between porphyrin (A) and salen (B) ligands in the proximity of chiral groups to the active site. (**C**) Chiral Mn(III) salen complexes. Et, ethyl; Me, methyl; and Ph, phenyl.

Salen-Based Catalysts

Although nature has provided an obvious impetus for the creation of effective biomimetic metalloporphyrin catalysts, the vital chemical importance of enantioselective epoxidation has driven the exploration of more abiological systems. Significant catalytic asymmetric epoxidation results have been reported with systems based on chiral salen ligands. Salens, like porphyrins, characteristically bind transition metals with a square-planar configuration. Kochi and coworkers showed that achiral Mn(III)salen complexes catalyze the epoxidation of olefins in the presence of a stoichiometric oxidant (*37*). As in metalloporphyrin systems, the reactive species in metallosalen oxidations is probably a high-valence metal-oxo complex. Asymmetric metallosalen catalysts offer an advantage over chiral porphyrin systems because the salen ligand has two, potentially chiral sp^3-hybridized car-

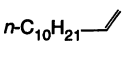
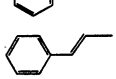
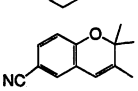
Table 3. Summary of percentages of enantiomeric excess obtained with various olefins and the different metalloporphyrin types.

Substrate	Type I		Type II		Type III			
	IA	IB	IIA	IIB	IIIA	IIIB	IIIC	IIID
	48	52	48	50	54	28	39	69
Cl	51	---	50	42	---	---	44	70
	36	---	63	42	17	---	40	---
NO_2	---	---	---	---	89	54	37	74
O_2N O_2N	---	---	---	---	96	---	---	---
	16	---	---	---	---	---	51	79
	40	76	72	---	63	---	59	80
	---	41	20	58	70	---	---	---
	---	56	42	52	56	---	38	88

bon atoms at its periphery (Fig. 5B). Because these chiral carbon atoms are just two bond lengths away from the metal, their proximity to the reactive site can yield a high degree of stereoselectivity.

Since the first report by Jacobsen and co-workers of asymmetric catalysis with chiral Mn(III)salen complexes (*38*), more than 120 chiral Mn(III)salen derivatives have been studied as catalysts for the epoxidation of a variety of unfunctionalized olefins with iodosylarenes, sodium hypochlorite, or dioxygen as the oxidant (Fig. 5C) (*39–41*). The majority of these systems are derived from chiral 1,2-diamino-1,2-diphenylethane (such as **26** to **28**) or chiral *trans*-1,2-diaminocyclohexane (such as **29**). Greater than 90% ee can be obtained in the epoxidation of various *cis*-disubstituted olefins; Table 4 provides a summary of the best epoxidation results derived from these systems. Facile and relatively inexpensive ligand syntheses, coupled with high enantioselectivities and the ability to use a cheap stoichiometric oxidant, make these the best practical catalysts currently available for epoxidizing unfunctionalized olefins. Merck currently synthesizes antihypertensive chromanol derivatives with one of the Jacobsen catalysts and commercial bleach (hypochlorite) (*22*). Although these Mn(III)salen catalysts are not completely effective with all olefin types, the major limitation facing these systems is the low turnover numbers, which are typically below 40. The instability of the metallosalen catalysts is likely a result of the susceptibility of the imine functional groups to oxidative decomposition, although it is not clear whether the principal decomposition routes involve intra- or intermolecular pathways.

Table 4. Best epoxidation results with chiral Mn(salen)s.

Substrate	Catalyst	% ee
	**26**	57
	**26**	8
	**29**	92
	**28**	83
	**26**	30
	**27**	56
	**26**	59
	**29**	>98

Conclusions

Although great progress has been made in the preparation of catalysts for the regioselective and enantioselective epoxidation of unfunctionalized olefins, these catalysts are only beginning to be useful synthetic tools. Elaborate and expensive catalyst syntheses, low turnover numbers, or both plague each of the systems discussed above to varying degrees. Also, for many of the catalysts discussed here, practical oxidants such as bleach, hydrogen peroxide, *tert*-butylhydroperoxide (TBHP), or dioxygen fail to give the selectivities or turnovers achieved with expensive oxidants such as iodosylbenzene (*42*).

Systematic variability has been the key to the development of the most successful regio- and enantioselective catalysts. Although powerful strides are being made in the areas of molecular mechanics and molecular modeling, computational techniques are still far from supplanting synthetic methods that allow the steric environment of a catalyst to be systematically perturbed and the catalytic competence to be tested. This is particularly true given the relative scarcity of structural and spectroscopic data pertaining to the high-energy metal-oxo species involved in the key catalytic steps. The elucidation of different mechanistic pathways, coupled with a rich variety of stereochemically related biomimetic models, will undoubtedly further our understanding of the intimate workings of oxygenase enzymes and our ability to produce useful, viable catalysts.

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42. Low turnover numbers continue to inhibit the development of practical enantioselective epoxidation catalysts derived from metalloporphyrins or metallosalens. Oxidative decomposition of the porphyrin and oxidative decomposition of the porphyrin superstructure have fundamentally different consequences and can depend on the choice of oxidant and solvent used to generate the active metal-oxo species. Porphyrin oxidation, diagnosed by changes in the ultraviolet-visible spectrum, deactivates the catalyst. However, oxidation of the porphyrin superstructure typically does not perturb this spectrum but may increase the rate of catalysis and seriously diminish the selectivity by opening up the steric environment. It is therefore critical to follow the spectroscopy and product distribution of the reactions as a function of time and catalyst turnover. Mass spectroscopy may be useful for characterizing metalloporphyrin catalysts that have had their superstructures only partially decomposed. For metallosalens, the facile oxidizability of the imine is an inherent problem that conceptually resembles the vulnerability of unsubstituted porphyrins to oxidation at the *meso* position. Metallosalen decomposition may be followed by ultraviolet-visible spectroscopy; fortunately, the decomposition products are catalytically incompetent and selectivity is not compromised.
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RESEARCH ARTICLES

Isolation of New Ribozymes from a Large Pool of Random Sequences

David P. Bartel and Jack W. Szostak

An iterative in vitro selection procedure was used to isolate a new class of catalytic RNAs (ribozymes) from a large pool of random-sequence RNA molecules. These ribozymes ligate two RNA molecules that are aligned on a template by catalyzing the attack of a 3′-hydroxyl on an adjacent 5′-triphosphate—a reaction similar to that employed by the familiar protein enzymes that synthesize RNA. The corresponding uncatalyzed reaction also yields a 3′,5′-phosphodiester bond. In vitro evolution of the population of new ribozymes led to improvement of the average ligation activity and the emergence of ribozymes with reaction rates 7 million times faster than the uncatalyzed reaction rate.

A current view of early evolution is that modern-day life descended from an "RNA world," an era (before proteins) in which all macromolecular catalysts were ribozymes (*1*). One of the most important enzymes of the RNA world would have been an RNA replicase, an RNA molecule capable of autocatalytic replication by virtue of its ability to fulfill two seemingly opposed functions at different times—either folding into an RNA polymerase that uses RNA as a template, or unfolding and acting as a template for another replicase molecule. Previous efforts to design RNA molecules with polymerase and replicase activities have focused on modifying known ribozymes derived from the group I self-splicing introns (*2*, *3*). Although progress has been made on this front, the development of iterative in vitro selection techniques has led to the possibility of isolating such enzymes from completely random-sequence RNA, without bias toward any known sequence or structure. This prospect is appealing in that some versions of the RNA world hypothesis suggest that the first enzyme in the origin of life was a replicase that arose from a prebiotic pool of random RNA or RNA-like sequences (*1*).

Repeated cycles of in vitro selection and in vitro amplification have been widely used to isolate, from large pools of random or degenerate sequences, rare nucleic acid sequences with specified biochemical properties (*4*). Such methods have been used to define protein binding sites on DNA and RNA molecules, to isolate RNAs and DNAs with specific binding sites for a variety of small molecule ligands, and to select for modified catalytic activities from existing ribozymes. We now show that in vitro selection and evolution techniques can be used to generate new classes of ribozymes that catalyze a reaction analogous to a single nucleotide addition during RNA polymerization.

Our selection protocol was designed to effect the enrichment of catalytically active members of a pool of RNA molecules on the basis of their ability to ligate a substrate oligonucleotide to their own 5′ end. The substrate sequence was then used as a tag to separate the rare ligated members of the pool from inactive molecules. We used a substrate oligonucleotide designed to anneal with part of a template region within the 5′ constant region of the pool RNA (Fig. 1A). The pool RNA began with a triphosphate, positioned next to the 3′-hydroxyl of the substrate oligonucleotide by base-pairing of the first few nucleotides of the pool RNA to the remainder of the template region, thus forming an interrupted stem loop. Ligation of the substrate oligonucleotide to the pool RNA was designed to be analogous to chain elongation by one nucleotide during RNA polymerization: in both cases, the growing strand and the triphos-

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