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Catalytic Oxidation of Alkenes with a Surface-Bound Metalloporphyrin-Peptide Conjugate

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Abstract

A novel surface-bound metalloporphyrin-peptide conjugate was prepared and used to catalytically oxidize alkenes in the presence of iodosylbenzene. The catalyst was found to oxidize a number of alkene substrates in good yield under a variety of reaction conditions. Comparison to control experiments using surface-bound Mn(III)tetraphenylporphyrin showed differences in oxidation yields and ratios of oxidized products. Substrate competition experiments demonstrated the ability of the conjugate catalyst to discriminate between substrates on the basis of size. Both results suggest oxidative catalysis occurred between the porphyrin ring and the peptide chain with the peptide influencing the outcome of the reaction in accord with the catalyst design.

Keywords: catalysis; epoxidation; biomimetic reactions; porphyrins

Introduction

Metalloporphyrin-based catalysts provide a mild method for the oxidation of organic substrates [1]. The development of metalloporphyrin catalysts is inspired by biological systems such as the cytochrome P450 family of monooxygenases [2]. From a biological perspective, P450 catalyzed oxidations play important roles in biosynthesis and metabolism. From a chemical perspective, P450s mediate a rich variety of oxidative transformations. Not only do P450s facilitate challenging reactions such as the hydroxylation of unactivated alkanes and the epoxidation of alkenes, they do so in a regioselective and stereoselective fashion under mild reaction conditions.

The heart of the native enzyme is an iron protoporphyrin IX macrocycle. The iron porphyrin serves as the site of oxygen activation and transfer to the substrate. Thus, interest in mimicking P450 chemistry with abiotic systems is focused on the construction of novel metalloporphyrin systems. Since the publication of the initial work by Groves in this area [3], a significant number of metalloporphyrin-based oxidative catalysts have been reported [1]. As the result of those efforts, the goal of attaining competent catalysts for application in organic synthesis is becoming reasonable. Artificial catalysts have been prepared which oxidize even recalcitrant substrates in good yield with turnovers of more than 10,000 [4]. Other systems are reported to oxidize prochiral substrates with enantioselectivities above 70% [5].

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The major feature distinguishing one catalyst from another is the choice of the auxiliary groups attached to the porphyrin ring. Those auxiliary groups determine which molecules may act as substrates. They also orient the substrate so the oxidation may occur in a regioselective and stereoselective fashion in much the same way the protein of the native enzyme positions the substrate in the binding pocket. Unfortunately, the addition of the auxiliary groups complicates the synthesis, and the effect those groups have on the outcome of a specific oxidation reaction is usually not predictable. Thus, the encouraging reports of good catalytic activity, good stereoselectivity, and good stability are not general to all catalysts acting on all substrates. Furthermore, the synthetic approaches used to construct the modified metalloporphyrin catalysts typically offer limited flexibility for systematic alteration [6][1].

After examining the strengths and weaknesses of reported metalloporphyrin oxidative catalysts, we undertook the development of a novel metalloporphyrin-peptide conjugate (Figure 1). The conjugate system utilizes nature's solution to positioning the substrate—amino acids. By employing only a short peptide chain, we are freed from many of the problems associated with the size and fragile tertiary structure of the native enzyme. In addition, use of a peptide provides a tremendous degree of synthetic flexibility. Simply by varying the amino acid sequence, a large number of catalysts could be generated each with different substrate preferences. Generation of such diversity would not require continuous re-design and re-synthesis of most aspects of the overall catalyst system.

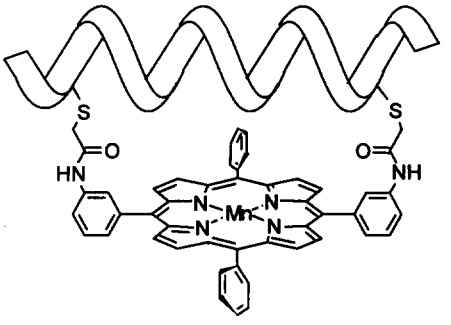


Figure 1. Mn(III)porphyrin-peptide conjugate.

The conjugate was prepared by crosslinking a fourteen residue peptide with a modified tetraphenylporphyrin ring. The peptide was designed to adopt a helical conformation projecting hydrophobic side chains toward the porphyrin ring and hydrophilic residues away from the ring. Such amphiphilicity was chosen in order to form a chiral, hydrophobic substrate binding site between the peptide chain and the porphyrin ring. The design, synthesis and characterization of a free base porphyrin-peptide conjugate has been described by us elsewhere [7]. In this paper we report the final steps in the construction of this initial catalytic system and the results of oxidation experiments under a variety of conditions [8]. This first metalloporphyrin-peptide conjugate catalyst oxidized a number of alkene substrates, and showed substrate discrimination and perturbation of oxidation product ratio consistent with the catalyst design. In the second paper in this series [9], we report and discuss the surprising absence of stereoselectivity in the oxidation reactions.

Results and Discussion

Preparation of the Metalloporphyrin-Peptide Conjugate Catalyst System

After the successful design, synthesis, and characterization of the free base conjugate described in our earlier paper [7], metal insertion and axial ligand preparation were required

[1] Work in Collman's group [6] provides a possible exception, but even their threitol-strapped catalyst lacks the tremendous variability provided by a peptide.

before the system could perform catalysis. Although iron is the choice of the native P450 enzyme, manganese was employed in this study as it has been shown by us[2] and by others [10] to be more suitable for studies with abiotic porphyrin systems. The free base conjugate was stirred in acetic acid with manganese(II)acetate at room temperature [11,12][3]. Incorporation of manganese was complete in 20 hours as monitored by UV-vis spectroscopy and RP-HPLC. No degradation of the conjugate was observed. The purified Mn(III)conjugate was examined by circular dichroism spectroscopy showing it highly helical under a variety of solvent conditions as indicated by the double minima at 208 nm and 222 nm (Figure 2). The molar ellipticity per residue at 222 nm corresponded to about 70-80% helicity [13].

In order to block the unhindered face of the Mn(III)conjugate and force catalysis to occur between the peptide chain and the porphyrin, axial ligation was required. Ligation also helps prevent reduction of the Mn(V)oxo group formed during catalysis [14]. This is important as the Mn(IV)oxo group oxidizes substrates less specifically [14,15]. In model systems, nitrogenous ligands are often used as they are less prone to oxidation than the thiolate ligand of the native P450 [16,17]. In this study a surface-bound imidazole ligand was selected [16e,18]. A surface-bound ligand promotes ligation to the unhindered face, site isolation of metalloporphyrin groups on the surface prevents inter-porphyrin reactions such as the formation of catalytically inactive μ-oxo dimers, and a surface-bound catalyst allows for facile post-oxidation recovery and evaluation of catalyst degradation. Silica gel was selected as the solid support as it is inert to the oxidation conditions and its dimensions are independent of solvent. Imidazole propyl modification of the silica gel was performed using a method previously reported [19]. The propyl groups provide conformational flexibility for the ligand, but not so much as to allow the ligand to bind to the conjugate's hindered face. Before deposition of the metalloconjugate, the imidazole propyl silica gel (IPS) was rinsed with dilute ammonium hydroxide to ensure the imidazole surface was not protonated.

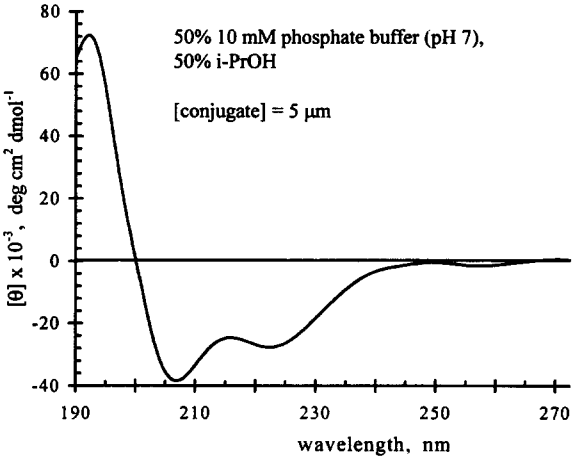


Figure 2. CD Spectrum of the Mn(III)conjugate.

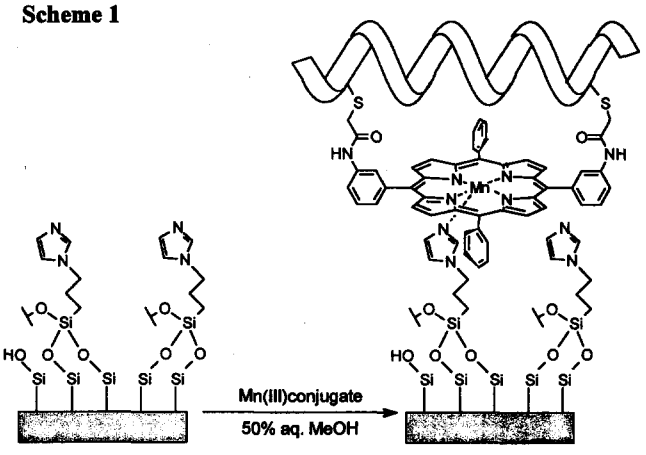
Due to the poor solubility of the Mn(III)conjugate in non-ligating solvents like methylene chloride, the standard approach for adsorbing metalloporphyrins on modified surfaces (mixing and filtration) could not be employed [18a-d,19]. Attempts to deposit the Mn(III)conjugate from ligating solvents such as DMF were not successful. A less common route of deposition by solvent evaporation was examined. Although this method has precedence [18g], there is a risk that the porphyrin may non-specifically precipitate onto the surface rather than properly ligate. In our approach (Scheme 1), Mn(III)conjugate was dissolved in 50% aqueous methanol

[2] Preliminary experiments with Fe(III)TPP/IPS and Fe(III)conjugate/IPS provided much lower oxidation yields than the corresponding manganese systems. The yields were especially lower in the aqueous solvents where the best substrates were oxidized with yields of less than 10%.

[3] Incorporation of manganese could not be performed using manganese(II)acetate in refluxing DMF or acetic acid [12] due to decomposition of the conjugate even with reaction times as short as five minutes.

and IPS was added. Slow evaporation of methanol under vacuum led to adsorption. After 50% of the solvent was removed, the supernatant was colorless.

Scheme 1



Proper ligation of the metalloporphyrin by the modified silica gel was confirmed indirectly. Control experiments were performed with Mn(III)tetraphenylporphyrin (TPP). Mn(III)TPP was deposited onto IPS by evaporation of methylene chloride, DMF, and 50% aqueous methanol solutions, as well as using methylene chloride in the standard approach. In addition, Mn(III)TPP was deposited onto unmodified silica gel using the same four sets of conditions. The behavior of the four Mn(III)TPP/IPS samples were found to be identical to each other and different from the Mn(III)TPP/silica samples. All of the IPS samples were an identical shade of bright green, whereas all of the unmodified silica samples were an identical shade of dull, brown-green. The difference in color was consistent with the expected differences in axial ligation between the IPS samples and the non-IPS silica samples. The identical appearance of the IPS samples regardless of solvent indicated proper adsorption was not solvent dependent. Oxidation experiments were also performed, as the oxidation yield is sensitive to the axial ligation [16a-e]. All of the Mn(III)TPP/IPS samples produced identical oxidation yields for the oxidation of styrene, using both iodosylbenzene (PhIO) and hypochlorite as the oxygen atom donors. Mn(III)TPP/silica samples also produced identical, lower oxidation yields. Using PhIO, the yield of oxidized styrene was 85% with the Mn(III)TPP/IPS and 20% with the Mn(III)TPP/silica samples. Using hypochlorite, yields between 11% and 13% were obtained for the Mn(III)TPP/IPS and yields of between 2% and 5% were obtained for the Mn(III)TPP/silica samples.

Preliminary Single Substrate Oxidation Experiments

A number of preliminary experiments were performed with Mn(III)TPP/IPS in order to develop suitable reaction conditions, decrease the scale of the reaction to minimize catalyst requirements, and to screen substrates for suitability. All experiments were performed using Mn(III)TPP/IPS prepared in an identical fashion to the Mn(III)conjugate/IPS at a substitution level of 5×10^{-6} mmol/mg in keeping with other literature reports [18a,d,f,g]. All experiments used PhIO as the oxygen atom donor.[4] A catalyst scale of 15 nmol was found to be appropriate with a catalyst to substrate to PhIO ratio of 1 : 2000 : 200. That ratio is similar to others reported in the literature [5d,6b,20] and is an attempt to balance loss of catalyst activity from catalyst degradation with the number of experiments possible from a given amount of Mn(III)conjugate. The oxidation reaction results were assessed by ^{1}H NMR spectroscopy. Experiments were performed using three sets of solvents—$CDCl_3$, 50% isopropanol-d_8 in 10

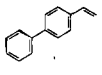
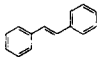
[4] Preliminary experiments were performed with hypochlorite, but the conjugate catalyst suffered rapid degradation under those conditions.

mM phosphate buffered D_2O (pH 7), and 10 mM phosphate buffered D_2O (pH 7). Chloroform was used due to substrate solubility. Buffered D_2O was used in preparation for experiments with the Mn(III)conjugate, to support peptide helicity and to encourage close packing between the hydrophobic residues of the peptide and the porphyrin ring. The 50% aqueous isopropanol was used to provide a balance between substrate solubility and peptide helicity and packing.[5] Initially, eighteen substrates were screened with the Mn(III)TPP/IPS in all three solvents. Only substrates oxidized to quantifiable extents were considered for study with the Mn(III)conjugate.

Mn(III)conjugate/IPS Single Substrate Oxidation Experiments

Table 1 summarizes the results of the Mn(III)conjugate/IPS single substrate oxidation experiments. The experiments were performed side-by-side with Mn(III)TPP/IPS controls.

Table 1
Single substrate Mn(III)conjugate/IPS oxidation experiments with PhIO.

Substrate	Solvent	% Yield [a,b,c]	Relative % Yield [b,d]	Ald : Oxide [c,e] (trans : cis) [c,f]	Relative Ald : Oxide [g] (trans : cis)
	$CDCl_3$	100 *75*	1.3	(0.1 : 1.0) *(0.9 : 1.0)*	(0.11)
	$CDCl_3$	100 (120) *100 (133)*	1.0 (0.90)	0.3 : 1.0 *0.4 : 1.0*	0.75
	$CDCl_3$	84 (109) *100 (120)*	0.84 (0.91)	0.3 : 1.0 *0.2 : 1.0*	1.5
	$CDCl_3$	55 *72*	0.76	(1.0 : 0.0) (1.0 : 0.0)	1.0
	$CDCl_3$	52 (66) *59 (73)*	0.88 (0.90)	0.4 : 1.0 *0.3 : 1.0*	1.3
	$CDCl_3$	29 (47) *24 (38)*	1.2 (1.2)	0.7 : 1.0 *0.6 : 1.0*	1.2
	$CDCl_3$	15 (28) *26 (34)*	0.58 (0.82)	1.0 : 1.0 *0.3 : 1.0*	3.3
	50% iPrOH	77 *92*	0.84	(1.0 : 0.0) *(1.0 : 0.0)*	(1.0)
	50% iPrOH	65 *99*	0.66	0.0 : 1.0 *0.0 : 1.0*	0.0
	50% iPrOH	50 *68*	0.74	(0.25 : 1.0) *(0.11 : 1.0)*	(2.3)

[5] Trifluroethanol (TFE) was also examined, but the chemical shift of the solvent peaks interfered with ^1H NMR analysis of many of the oxide products.

Table 1 (cont.)

	50% iPrOH	0 (41) *0 (14)*	(2.9)	1.0 : 0.0 *1.0 : 0.0*	1.0
	50% iPrOH	0 (35) *15 (37)*	0 (0.95)	1.0 : 0.0 *0.5 : 1.0*	2.0
	50% iPrOH	0 (23) *23 (34)*	0 (0.68)	1.0 : 0.0 *0.5 : 1.0*	2.0
	50% iPrOH	trace *19*	0	NA *0.0 : 1.0*	NA
	50% iPrOH	0 *16 (22)*	0 (0)	NA *0.2 : 1.0*	NA
	D_2O buffer	49 *91*	0.53	0.0 : 1.0 *0.0 : 1.0*	NA
	D_2O buffer	18 *75*	0.24	0.20 : 1.0 *0.40 : 1.0*	0.50

[a] Yields are based upon PhIO.
[b] The values in parentheses include the contribution of aldehyde to the overall yield.
[c] For comparison, results of the side-by-side Mn(III)TPP/IPS control oxidation experiments are provided in italics, beneath each Mn(III)conjugate/IPS result.
[d] The relative yield is the ratio of Mn(III)conjugate/IPS yield to Mn(III)TPP/IPS yield.
[e] The ratio of aldehyde and oxide products determined by ^1H NMR analysis for oxidation of terminal alkenes.
[f] The ratio of trans oxide to cis oxide products for the oxidation of internal alkenes.
[g] The relative aldehyde to oxide ratio is the Mn(III)conjugate/IPS ratio divided by the Mn(III)TPP/IPS ratio.

The substrates were oxidized in good yield under the three sets of conditions. Significantly, the oxidation yields from the Mn(III)conjugate mediated reactions were generally lower than the corresponding Mn(III)TPP reactions. The difference was greatest in the more polar solvents. The observations are consistent with oxidation occurring between the peptide chain and the porphyrin ring, with the peptide regulating substrate approach. In the more polar solvents, the peptide chain and porphyrin were likely packed more intimately due to hydrophobic interactions giving rise to increased differences in oxidation yield between the Mn(III)conjugate and Mn(III)TPP control.

Additionally, the two catalysts provided different ratios of the major oxidation products. Figure 3 highlights the major products detected by ^1H NMR for the oxidation of terminal and internal alkenes. The mechanism by which the various oxidized products are derived is still a source of debate [21], but it is clear that catalyst structure can alter the distribution. Other metalloporphyrin catalysts with a strapping auxiliary group have been reported to produce an increased amount of aldehyde relative to oxide in the oxidation of terminal alkenes [22]. One explanation for

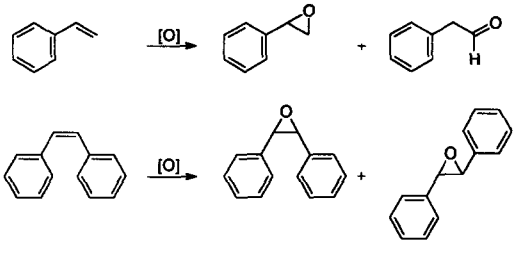


Figure 3. Major oxidation products from terminal and internal alkenes.

the perturbation is inhibition of substrate approach to the Mn(V)-oxo group by the strap, thereby providing greater opportunity for adventitious reductants to reduce the reactive high valent oxo group [14]. The Mn(IV)-oxo group is thought to react by a radical mechanism giving rise to increased amounts of rearranged products, like the aldehyde, relative to the oxide [15]. Alternatively, the presence of the strapping group may alter the transition state geometry of the oxidation leading to increased amounts of aldehyde [23]. The top-on transition state geometry enforced by strapping auxiliary groups may lead to increased formation of rearranged products where as the side-on transition state geometry allowed by the unhindered or picketed catalysts may lead to less rearrangement (Figure 4). Both explanations for the increased amount of aldehyde detected with the Mn(III)conjugate catalyst are consistent with the oxidation occurring from the hindered face of the porphyrin ring, with the outcome of the oxidation being influenced by the peptide chain.

Figure 4. Possible transition state geometries for oxidation.

Differences in the isomerization of cis stilbene during oxidation were also observed between the Mn(III)conjugate and Mn(III)TPP in all three solvents. The perturbations may be explained by physical blocking of isomerization by the peptide chain, and by shielding the substrate from solvent in the hydrophobic binding site. Again, these results are supportive of the general catalyst design.

The behavior of the Mn(III)conjugate/IPS catalyst system in the single substrate oxidation experiments was largely as expected. The catalyst oxidized substrates in the presence of PhIO under a variety of conditions. The observed yields were generally lower for the Mn(III)conjugate relative to the Mn(III)TPP catalyst, and the ratios of oxidized products were perturbed indicating the peptide was regulating some aspects of the oxidation reactions. The differences between the Mn(III)conjugate and Mn(III)TPP reactions were modulated by solvent in a manner consistent with anticipated peptide-porphyrin packing.

Substrate Competition Experiments

The conclusions from the previous section were extended by substrate competition experiments. Both catalyst systems were examined using an equal molar mixture of styrene and cis stilbene. Those substrates were selected as they are of very different size, both were oxidized in all three solvents, and the proton resonances do not overlap in the ^{1}H NMR analysis. The reaction scale and reactant ratios were identical to the earlier studies and all three solvents were examined. Table 2 summarizes the results expressed as a ratio of styrene oxidation products to cis stilbene oxidation products.

A clear preference for the smaller styrene substrate over the larger cis stilbene substrate was observed in the Mn(III)conjugate experiments. The preference became more dramatic as the solvent polarity increased, in agreement with enhanced peptide structure and packing with the porphyrin ring. When compared to the Mn(III)TPP experiments, the discrimination displayed by the Mn(III)conjugate clearly exceeded that explained by inherent differences in substrate reactivity and solubility.[6] The Mn(III)conjugate was 1.1 times more discriminating in

[6] In chloroform the two substrates were equally reactive with the Mn(III)TPP catalyst. In 50% aqueous isopropanol there was a nearly four fold preference for styrene as that substrate was more soluble. In buffer alone, neither substrate was tremendously soluble so the preference displayed by the Mn(III)TPP was lower.

chloroform, 2.8 times more in 50% aqueous isopropanol, and 16 times more in buffer. These results support the single substrate oxidation experiments and the conclusions drawn from those studies.

Table 2
Summary of substrate competition experiments.

Catalyst	Solvent	Relative Oxidation Yields[a]
Conjugate	$CDCl_3$	1.1
TPP	$CDCl_3$	1.0
Conjugate	50% iPrOH	10
TPP	50% iPrOH	3.6
Conjugate	D_2O buffer	30
TPP	D_2O buffer	1.9

[a] The yield of styrene oxidation products (aldehyde and oxide) divided by the yield of cis stilbene oxidation products (cis and trans oxide).

Conclusions

The preparation of a surface-bound metalloporphyrin-peptide conjugate was completed and catalysis experiments were performed. The catalyst mediated the oxidation of a variety of substrates in the presence of PhIO in a trio of solvents. The results matched many expectations such as the substrate discrimination, and perturbation of yield and ratio of oxidation products relative to control experiments performed with Mn(III)TPP. Those differences are consistent with oxidation occurring between the peptide chain and the porphyrin ring with the peptide influencing the outcome of the catalysis in accord with catalyst design.

Experimental

General Methods

All reagents and solvents were obtained from the indicated sources, were of the highest available purity unless otherwise noted, and were used as received unless otherwise noted. Iodosylbenzene was prepared from iodobenzenediacetate (Aldrich) following literature procedure [24]. The iodosylbenzene was periodically subjected to iodometric analysis [25] and the percentage of active oxidant was always greater than 99%. All reaction products were stored in the dark, in dessicators, in a -4°C freezer. RP-HPLC was performed using a Waters 660 solvent programmer with a 6000A pump system and a Perkin-Elmer LC-75 UV-vis spectrophotometric detector, or using a Waters 600E system controller with a Perkin-Elmer LC-95 UV-vis detector. A Kipp and Zonen BD-40 chart recorder was used, and peak integrals were obtained using a Hewlett-Packard HP3394A integrator. Column and solvent conditions will be described where appropriate. Solvent A was 20% acetonitrile (Baker, HPLC grade) in milli-Q water with 0.1% TFA (Advanced Chemtech). Solvent B was 80% aqueous acetonitrile with 0.1% TFA. Unless otherwise indicated, a flow rate of 1 ml/min was used for analytical HPLC and 3 ml/min was used for semi-preparative HPLC. Amino acid analysis was done

using a Waters Pico-Tag workstation and the Waters 600E system controlled HPLC, with the Waters Pico-Tag column. A one hour HCl hydrolysis at 150°C was used. ^{1}H NMR spectra were recorded with Bruker af300 and ac200 spectrometers using TMS as an internal standard. Mass spectra were obtained from a Kratos Profile HV4 ion spray mass spectrometer using a 50% aqueous methanol solvent system with gramicidin as the internal standard. UV-vis spectra were recorded with a Perkin-Elmer Lambda 3B double beam spectrophotometer using 1.0 cm quartz cells. Circular dichroism spectra were recorded using a Jasco J-720 spectropolarimeter. A quartz sample cell with a volume of 300 μL and a pathlength of 1.0 mm was used.

Preparation of Mn(III)Porphyrin-Peptide Conjugate

About 3 mg of free base conjugate [7] was dissolved in 4 ml glacial acetic acid under argon. To the mixture, 50 mg of Mn(II)acetate (Aldrich) was added. The solution was stirred shielded from the light under argon for about 20 hours. The reaction was monitored by UV-vis spectroscopy and by RP-HPLC (C4, Rainin, Microsorb-MV, 30%B to 100%B, 15 minutes) with the Mn(III)conjugate eluting at 15 minutes and the conjugate eluting at 19 minutes. After free base conjugate could not be detected, the solution was filtered to remove undissolved Mn(II)acetate. The solution was directly purified by RP-HPLC. A semi-preparative C4 (Vydac) column was used with a gradient of 40%B to 50%B over 15 minutes. The Mn(III)conjugate eluted as a broad peak at 14 minutes. The homogeneity of the pure Mn(III)conjugate sample was confirmed by performing HPLC in a variety of solvent systems where solvent B was always 100% MeCN, and solvent A was variable (water with 0.1% TFA, 20 mM KPi pH 2, 4.4, and 6.5, and 20 mM HCl). The yield for the metallation was about 80%. ESI-MS: m/z = 1204.2 (M^{+2}), 803.3 (M^{+3}) . UV-vis (DMF): 387 nm (70%), 410 nm (67%), 470 nm (B, 100%), 561 nm (Q1, 7%), 600 nm (Q2, 3%). ε 470 nm (50% aq. MeOH) = 1.03 x 10^5 $M^{-1}cm^{-1}$. Amino acid analysis: ala(2) = 2.0, leu(4) = 4.1, glx(4) = 4.2, lys(2) = 1.9, s-carboxymethylcysteine(2) = 1.7.

Preparation of Imidazole Propyl Modified Silica Gel (IPS)

IPS was prepared following a published procedure [19]. Davisil silica gel (5.0 g), 300Å pore size, 30-40 micron mesh, surface area 250 m^2/g (Alltech) was refluxed for 6-7 hours with 30 ml of 5% HCl. The silica was then rinsed 6 times with 30 ml portions of milli-Q water. The final rinse was of neutral pH. The silica was recovered after each rinse by centrifugation. The silica was dried in an vacuum oven at 100°C overnight. The activated silica gel (4.0 g) was refluxed for 8 hours under argon with 60 ml m-xylene (Aldrich) and 3 ml of freshly distilled 3-chloropropyltrimethoxy silane (Lancaster). The modified silica was rinsed with toluene 5 times, followed by overnight drying in a vacuum oven at 50°C. The dried silica produced a strongly positive Beilstein test. The chloropropyl modified silica gel (4.0 g) was refluxed for 5 hours under argon with 40 ml m-xylene and 2.0 g imidazole (Aldrich). The IPS was rinsed two times with toluene, 3 times with acetone, 2 times with 5% acetic acid, 3 times with water, 1 time with 5% ammonium hydroxide, 2 times with water (pH of second rinse was about 8), and 2 times with acetone. The IPS was dried overnight in a vacuum oven at 50°C. The dried IPS produced a negative Beilstein test.

Preparation of Mn(III)TPP/IPS

Method 1: About 10 mg of Mn(III)TPP (Aldrich) was dissolved in 10 ml methylene chloride. That solution was added to 500 mg of IPS. The mixture was shaken, but not stirred

for 1 hour. The sample was then spun, and the solvent was poured off. The silica was rinsed with methylene chloride until the rinses were nearly colorless (usually 3 to 4 rinses). The darkly colored Mn(III)TPP/IPS was dried overnight in a vacuum oven at room temperature.

Method 2: IPS (200 mg) and 5 ml of solvent were mixed. The solvent was either methylene chloride, DMF, or 50% aqueous methanol. Separately, 0.2 mg Mn(III)TPP was dissolved in 2.0 ml of the same solvent. To the IPS slurry, 700 μl of the Mn(III)TPP solution was added. The slurry was stirred for 30 minutes, then the solvent was evaporated under vacuum. The Mn(III)TPP/IPS was rinsed two times with 25 ml of a 5% aqueous $NaHCO_3$, 0.1 M EDTA solution, 4 times with 5% aqueous $NaHCO_3$, and 5 times with milli-Q water. All rinses were colorless. The Mn(III)TPP/IPS was dried overnight in the vacuum oven at room temperature. In all samples, the expected substitution level of 5×10^{-6} mmol/mg was obtained. For control samples, Mn(III)TPP was deposited on unmodified silica following the exact same procedure. The appearance of the Mn(III)TPP/silica was a brown-green, clearly different from the bright-green appearance of the Mn(III)TPP/IPS samples. The substitution level of the Mn(III)TPP/IPS was determined by rinsing 10.0 mg of Fe(III)TPP/IPS with 1-ml portions of acetic acid. The acetic acid rinses were combined in a 10-ml volumetric flask. The absorbance of the solution was recorded at the Soret band (λ_{max} = 412 nm) and the quantity of porphyrin could be determined ($\varepsilon = 1.03 \times 10^5$ $M^{-1}cm^{-1}$). Three individual trials were performed and the agreement was very good.

Preparation of Mn(III)Conjugate/IPS

Mn(III)conjugate (about 1 mg, 416 nmol) was dissolved in 50% aqueous methanol. The concentration of the solution was determined based on the absorbance of the solution. The solution was added to an appropriate amount of IPS (83 mg for 1 mg Mn(III)conjugate) needed to obtain a substitution level of 5×10^{-6} mmol conjugate/mg IPS. The solvent was evaporated under vacuum to about half of the original volume. The silica was rinsed and dried as described for the preparation of the Mn(III)TPP/IPS. All rinses were colorless. A small sample of the Mn(III)conjugate/IPS was treated with a few drops of acetic acid. The solution was analyzed by RP-HPLC (C4, 40%B to 60%B, 15 minutes, 470 nm) and a single peak corresponding to the Mn(III)conjugate was detected. Amino acid analysis was also performed on the IPS bound sample and the expected residue ratios were obtained.

Single Substrate PhIO Oxidation Procedure

To a 5-ml pear shaped flask, 3.00 mg Mn(III)conjugate/IPS (15 nmol Mn(III)conjugate) was added. Then, 60 μL of solvent was added, followed by 30 μmol of substrate. The reaction was initiated by addition of 0.66 mg ground PhIO (3 μmol). The reaction was allowed to proceed for 90 minutes at room temperature under argon shielded from light. After the reaction, work-up depended on the solvent. For $CDCl_3$ experiments, 550 μL of $CDCl_3$ was added to the reaction flask. The solution was filtered with a Pasteur filter tip pipette into a NMR tube. For aqueous experiments, 600 μL of $CDCl_3$ was added to the reaction flask and the contents were mixed well. The organic layer was transferred to a microfuge tube and dried for 20 minutes over sodium sulfate. The solution was filtered through a Pasteur filter tip pipette into a NMR tube. The sodium sulfate was rinsed with 150 μL of $CDCl_3$. Control experiments with 1:1 mixtures of cis stilbene and cis stilbene oxide, and styrene and styrene oxide indicated the ratio of the two materials was not grossly changed by the work-up. For cis stilbene, a 1:1 ratio was observed in the ^1H NMR spectrum after the work-up. For styrene, the ^1H NMR spectrum was about 5-10% low in styrene. Finally, the ^1H NMR spectrum was recorded. Alkene peaks were

assigned by comparison to starting material. Oxide peaks were found between about 4.5 and 2.7 ppm depending on the substrate. Most of the styrene substrates produced a quartet at 3.85, and doublets at 3.15 and 2.80 ppm. Aldehyde product was most conveniently quantified by integration of the peak from the neighboring methylene protons. These appeared as a doublet at about 3.70 ppm.

Hypochlorite Oxidation Experiments

To a 5-ml pear shaped flask, 3.5 mg Mn(III)TPP/IPS (40 nmol) was added. To the flask, 50 μL of solvent and 2.2 μL styrene (19 μmol) were added. Finally, 41.2 μL of NaOCl solution (Aldrich, 22 μmol) was added. The reaction was continued and work-up was done as already described.

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