However, these spectra were relatively noisy and subject to concerns regarding sample heterogeneity.

Representative normalized XANES spectra[10] for the three galactose oxidase samples are shown in Figure 1. It is clear that the oxidatively activated and reductively inactivated forms have virtually identical edge regions. As expected, the inactive edge is consistent with Cu(II). The nearly identical appearance of the XANES spectra for reductively inactivated and oxidatively activated galactose oxidase suggests that there is at most a minor change in the Cu site on oxidative activation. In particular, the observation that the edge for the oxidatively activated enzyme is, if anything, at a lower energy than the edge for the inactive protein is *inconsistent* with Cu-centered oxidation. Samples were characterized by EPR spectroscopy and enzyme assay before and after XAS analysis and found to be unchanged. Further, a sample of oxidatively activated enzyme containing an oxidant [hexacyanoferrate(III), 7.5 mM] as a trap gave Cu XANES essentially indistinguishable from the oxidant-free sample, removing the possibility of photoreduction of the sample during irradiation. This provides direct evidence that oxidative activation does not involve metal-centered oxidation. In contrast, the edge structure for the anaerobic substrate complex is a classic example of the XANES spectra typical of Cu(I) complexes,[7] demonstrating that substrate reduces the active-site cupric ion.

The present results provide strong support for a catalytic cycle involving [Cu(II) + enzyme radical] ↔ [Cu(I)] as discussed in ref 3. The XANES spectra suggest some structural differences in the Cu sites for the active and inactive enzyme; however, the nature of the difference is unclear. EXAFS studies, which will permit a more detailed structural characterization of each of these species, are in progress.

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First Synthesis of a Pendant-Capped Porphyrin. A Biphenyl Pendant-Capped Porphyrin Model of Catalase

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An ideal chemical model for a specific enzyme would include the following structural characteristics: (i) a domain that holds the catalytic functional groups at the active site in a geometry that mimics the active site structure of the protein enzyme; (ii) a binding domain for the substrate; and (iii) a capping structure that protects the catalytic functional groups from unwanted interaction with species other than the substrate. In principle, the structure of (5,10,15,20-tetraphenylporphinato)metal(III) species ((TPP)M^{III}(X)) offer a unique opportunity to create the desired features of i–iii in the synthesis of catalase, peroxidase, and cytochrome P-450 models. The ligands (phenolic hydroxyl of tyrosine for catalase, imidazole of histidine for peroxidase, and a sulfhydryl of cysteine for cytochrome P-450) that characterize these enzymes are trans to the reactive side of the porphyrin plane such that the substrate-binding domain and the protective ligating cap are located on opposite faces of the porphyrin. The four phenyl rings of TPP would act as the anchors for the binding domain and protective cap. We report here the first synthesis of a TPP structure with a protective pendant ligand cap.[1]

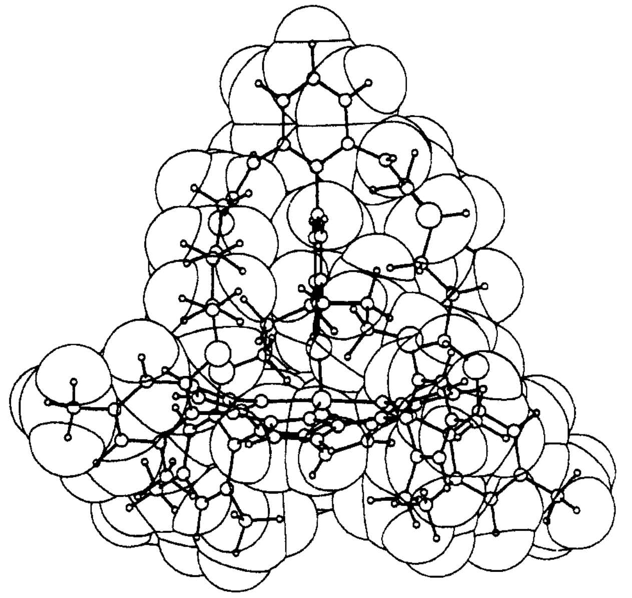
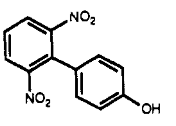
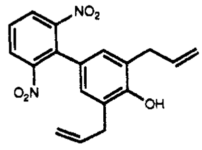


Figure 1. Overlap of "stick and ball" and CPK models of (IX)Fe^{III}. The stick and ball model shows the ligation of iron(III) and phenoxide oxygen as well as the role of the biphenyl substructure in supporting the flying bridges that constitute the capping structure. The CPK model shows how the pendant ligand and iron(III) are protected on the capped face. [The coordinates for (IX)Fe^{III} were created by using $CHARM_m$ (Polygen Corp.). The file PORPHYRINH.RTF was used to generate the iron(III) porphyrin ring, and an RTF generated in the 2-D molecular construction routine of Quanta (Polygen, Corp.) was used to generate the cap. The structure was extensively minimized in $CHARM_m$ with steepest descents and adopted basis Newton–Raphson.]

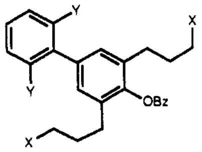
The identities of all intermediates were determined by ^{1}H NMR and mass spectroscopy. 4-Methoxy-2′,6′-dinitrobiphenyl (obtained from reaction of 4-iodoanisole with 2,6-dinitrochlorobenzene in the presence of Cu^0 at 230 °C, 90% yield) on demethylation (BBr_3/CH_2Cl_2 at −70 °C, warming to 25 °C for 17 h) provided 4-hydroxy-2′,6′-dinitrobiphenyl I (90%). Reaction of the sodium salt of I with excess allyl chloride (20 °C, warming to 70 °C for 18 h) and Claisen rearrangement of the resultant allyl ether (bubbling BCl_3 for 1.5 h in chlorobenzene) provided 3-allyl-4-hydroxy-2′,6′-dinitrobiphenyl (II, 91%). Reaction of the sodium salt of II with allyl chloride and Claisen rearrangement (same procedures as used with the sodium salt of I) gave III (88%). Blocking of the phenolic function of III by benzylation (benzyl bromide with K_2CO_3 in DMF, 25 °C) followed by terminal hydroxylation (9-BBN/THF at 25 °C, warming to 65 °C for 2 h, 3 M NaOH at 5 °C followed by reaction with 30% H_2O_2 at ≤25 °C) provided diol IV (76%). Replacement of the OH groups of IV by Br substituents (Ph_3P/CH_2Cl_2 at 23 °C, collidine at −3 °C, and reaction with CBr_4 at −3 °C) and reduction of the NO_2 substituents (NH_4Cl/EtOH–H_2O, Fe^0; 65 °C, 2 h) yielded V



I



III



IV X = OH, Y = NO_2
V X = Br, Y = NH_2

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(1) A tailed (TPP)M^{III}(X) possesses a chain of atoms extending from the 2-position of a single phenyl substituent, which terminates in a ligand species. A strapped (TPP)M^{III}(X) possesses a chain of atoms (which holds a ligand in the vicinity of the M^{III} moiety) extending from the 2-position of a phenyl substituent on the 5-meso carbon of TPP to the 2-position of a phenyl substituent on the 15-meso carbon of TPP. Both tailed and strapped structures allow reaction of reagents with the ligand species. This feature is not shared by a pendant-capped porphyrin because the ligand is hung from the ceiling of a vaulted dome or tepee with protective walls supported by four legs that are anchored to the 2-positions of the four phenyl rings at the 5-, 10-, 15-, and 20-meso carbons of the TPP.

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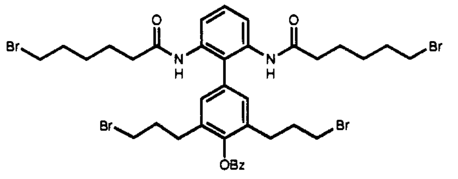
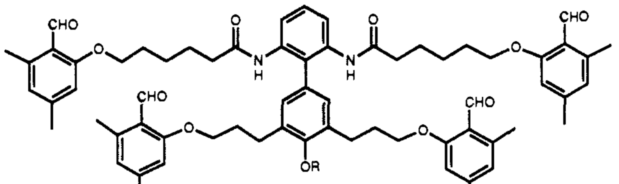
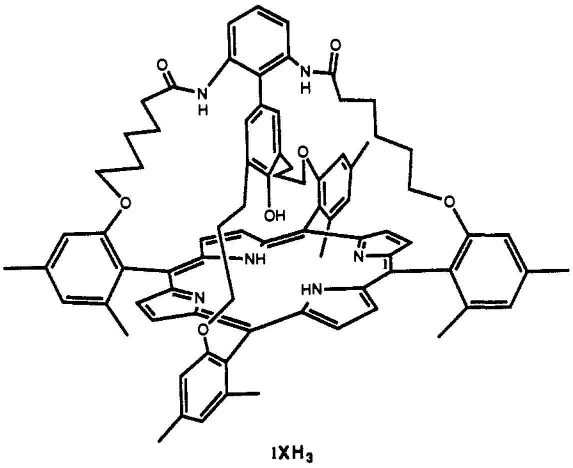
(74%). Reaction of V with 6-bromohexanoyl chloride (in THF/pyridine at −60 °C warming to 10 °C) followed by reaction of the resultant diamide VI with the sodium salt of 2-hydroxy-4,6-dimethylbenzaldehyde[2] (THF/HMPA at 35 °C for 96 h) yielded tetraaldehyde VII (42%). Removal of the *O*-benzyl blocking group with CF_3CO_2H (55 °C for 6 h, 70% yield) and reaction of the resultant phenolic tetraaldehyde VIII with pyrrole, following the method of Lindsey,[3] provided the desired pendant-capped porphyrin IXH_3 in 7% yield (two protons residing on porphyrin pyrrole nitrogens and the third proton on the phenolic substituent). The pendant porphyrin was characterized by ^1H NMR, UV/vis, IR, and mass spectroscopy.[4]



VI



VII R = Bz
VIII R= H



IXH_3

Insertion of iron(III) into IXH_3 to provide (IX)Fe^{III} was carried out after the method of Kobayashi.[5] That the Fe(III) moiety of (IX)Fe^{III} is axially coordinated to the phenoxy substituent of the cap was established from IR, UV/vis, ^1H NMR, and laser desorption mass spectroscopy. In the IR spectra the characteristic phenolic O–H absorption at 3450 cm^{-1} of IXH_3 is lost on conversion to (IX)Fe^{III}. The UV/vis spectrum of (IX)Fe^{III} exhibits absorbance maxima [$CHCl_3$, λ_{max}, nm ($\epsilon \times 10^4$ M^{-1} cm^{-1}), 325 (3.3), 420 (13.5), 490 (1.1), 552 (0.66), 608, 653] comparable to the spectrum of the analogous phenoxy-ligated (tetraphenylporphinato)Fe(III) [(TPP)Fe^{III}(OC_6H_5)].[6] The high-field ^1H NMR spectrum is consistent[6-8] with the above structural assignment [($CDCl_3$, 500 MHz, 23 °C), δ 13.4, 13.1, 11.9, 11.6 (meso-phenyl, meta Hs), 82.6 (β-pyrrolic Hs), 122.4 (Hs meta to Fe^{III}–O bond)]. The spectrum of (IX)Fe^{III} shows the distinctive resonance pattern that is characteristic for a high spin ($S = {}^5/_2$), five-coordinate iron(III) formulation. The line width of the pyrrole resonance (1796 Hz) is consistent with an oxygen donor occupying the fifth coordination site. Moreover, the spectrum of (IX)Fe^{III} shows the distinctly characteristic resonance at 122 ppm corresponding to the axially coordinated phenolate meta resonance. Additionally, the composition of (IX)Fe^{III} was verified by high-resolution laser desorption MS [calcd for $(C_{82}H_{79}N_6OFe + Na)^+$ *m/e* 1338.518, found $(M + Na)^+$ *m/e* 1338.546], which showed no proton on the phenolic oxygen and the absence of any other axial ligand (such as Cl^-).

Chemical properties of (IX)Fe^{III} as well as other metal complexes formed from IXH_3 will be reported at a latter date. The obvious challenge is to synthesize a pendant cap tetraphenylporphyrin that secures in place and protects a S^- ligand. The accomplishment of such a synthesis and the studies that would ensue could be critical to the understanding of the mechanism of cytochrome P-450. Work toward this end is in progress in our laboratory.

Acknowledgment. This investigation was supported by a grant from the National Institutes of Health. High-resolution mass spectrometry was performed by Prof. Charles L. Wilkins, Department of Chemistry, University of California at Riverside.

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(2) 2-Hydroxy-4,6-dimethylbenzaldehyde was prepared as previously described: Ligget, L. M.; Diehl, H. *Proc. Iowa Acad. Sci.* **1945**, *52*, 191.

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(4) 1-D ^1H NMR decoupling and 2-D ^1H NMR COSY experiments were used to assign the chemical shifts. The amide strap carbons are labeled α–ε and the propoxy strap carbons are labeled 1–3. ^1H NMR ($CDCl_3$, 300 MHz, $CHCl_3$ assigned at δ 7.24) δ −2.18 (br s, 2 H, pyrrolic NH), −0.41 to −0.36 (m, 4 H, C-1 Hs), 0.14–0.19 (m, 4 H, δ-CH_2), 0.46–0.53 (m, 4 H, γ-CH_2), 0.55–0.63 (m, 8 H, C-2 Hs and β-CH_2), 1.77 (t, 4 H, J = 7.0 Hz, α-CH_2), 1.85, 2.18, 2.61, 2.64 (s, 6 H each, CH_3s), 3.54 (t, 4 H, J = 7.3 Hz, ε-CH_2), 3.77 (t, 4 H, J = 5.0 Hz, C-3 Hs), 5.73, 6.14, 6.88, 7.03, 7.05, 7.21 (s, 2 H each, amide NH and meso phenyl Hs), 7.06 (t, 1 H, J = 8.4 Hz, H meta to amide), 7.67 (d, 2 H, J = 8.4 Hz, H ortho to amide), 8.67 (d, 4 H, J = 4.8 Hz, β-pyrrolic Hs), 8.70 (d, 4 H, J = 4.8 Hz, β-pyrrolic Hs); UV/vis ($CHCl_3$) λ_{max}, nm ($\epsilon \times 10^4$ M^{-1} cm^{-1}), 402 (sh), 420 (32.2), 512 (12.5), 551 (3.0), 589 (3.0), 643 (0.5); IR ($CHCl_3$) ν, 3450 (br, O–H), 3415 (m, amide NH), 3340 (w, pyrrole NH), 1680 (s, C=O) cm^{-1}; FABMS calcd for $C_{82}H_{82}N_6O_4$ *m/e* 1262.62, found *m/e* 1263.

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$[ECd_8(E'Ph)_{16}]^{2-}$ Cluster Chemistry (E, E′ = S, Se, Te)

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We have established systematic entry to the chemistry of the class of clusters formulated $[ECd_8(E'Ph)_{16}]^{2-}$ (**1**), E, E′ = S, Se, Te. This class adds to the two others we have described, namely, $[E_4M_{10}(E'Ph)_{16}]^{4-}$ (**2**), M = Zn, Cd,[1,2] and $[S_4Cd_{17}(SPh)_{28}]^{2-}$ (**3**).[3]

Complexes $[SCd_8(SPh)_{16}]^{2-}$ (**1a**), $[SCd_8(SePh)_{16}]^{2-}$ (**1b**), $[SeCd_8(SePh)_{16}]^{2-}$ (**1c**), $[TeCd_8(SePh)_{16}]^{2-}$ (**1d**), and $[SCd_8(TePh)_{16}]^{2-}$ (**1e**) can be synthesized by self-assembly of E′Ph$^-$ (E′ = S, Se, Te), CdI_2, and Na_2E or NaEH (E = S, Se, Te) in the composition range 0.2 ≤ E/Cd ≤ 0.3, 1 < E′Ph/Cd < 3, in alcohols/acetonitrile.[4] Crystalline compounds isolated from these reactions and characterized by correct elemental analysis and/or Cd NMR (see below) include the following: **1b** with Me_4N^+,

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(4) $(Et_4N)_2$·**1b**: A deoxygenated solution of NaSePh (15.5 mmol, in ethanol (70 mL)/acetonitrile (70 mL)) was treated with solutions of CdI_2 (6.4 mmol) in acetonitrile (20 mL), Na_2S (1.6 mmol) in methanol (20 mL), and Et_4NCl (2.5 mmol) in acetonitrile (20 mL), yielding a colorless solution. After stripping of all solvent, the colorless solids were extracted with water at 80 °C and ethanol at ambient temperature, then dissolved in acetonitrile at 20 °C, and filtered, and $(Et_4N)_2[SCd_8(SePh)_{16}]$ (ca. 38%, on Cd) crystallized by storage at 0 °C. Anal. $Cd_8SSe_{16}N_2C_{106}H_{120}$, C, H, N, Cd.