First Synthesis of a Binuclear [Mn^{II}(bipy)−Fe^{III}(porphyrin)] Complex: Spectroscopic Characterization and First Evidence of Reversible Formation of Manganese(III) as Manganese Peroxidase

Clotilde Policar, Isabelle Artaud,* and Daniel Mansuy

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA 400, Universite Rene Descartes, 45 rue des St-Peres, 75270 Paris Cedex 06, France

Received June 1, 1995[⊗]

A [(P)Fe^{III}−Mn^{II}] bimetallic complex, mimicking the active site of manganese peroxidase, has been synthesized. A modified highly fluorinated porphyrin, 5,10,15-tris(pentafluorophenyl)-20-(*o*-aminophenyl)porphyrin, has been used to introduce, through a short spacer linked to the amino function, a manganese auxiliary ligand, 6-aminomethyl-2,2′-bipyridine. Two successive metalations by $FeCl_2$ and $MnCl_2$ afforded the [(P)Fe^{III}−Mn^{II}] bimetallic complex that has been characterized by elemental analysis and FAB^+ mass spectrometry. X-band EPR spectroscopy and magnetic susceptibility measurements were in agreement with two high spin Fe(III) and Mn(II) centers without magnetic exchange interaction. Moreover, there is no higher intermolecular association through μ-chloro bridging as observed by EPR with a simpler chloromanganese complex, $Mn(bipy)_2Cl_2$, at high concentration. Addition of pentafluoroiodosobenzene in methanol at 0 °C led to the progressive and complete disappearance of the EPR Mn(II) signals, that were recovered after addition of a phenol. This result is consistent with Mn(III) formation. This production of Mn(III) requires the presence of the iron porphyrin and is proposed to occur through the intermediate formation of a Fe(IV) dimethoxide species which can be related to the oxidation of Mn(II) catalyzed by manganese peroxidase compound II.

Introduction

Manganese peroxidase (MnP) secreted by the white rot fungus *Phanerochaete chrysosporium* is unique among plant and fungal peroxidases since it catalyzes the H_2O_2-dependent oxidation of Mn^{2+} to Mn^{3+}, which in turn oxidizes a wide variety of organic substrates including phenols,[1] phenolic lignin dimer models,[2] and lignin itself.[3] In fact this enzymatically generated Mn^{3+} acts as a diffusible oxidant able to depolymerize lignin. Both from ^{1}H NMR experiments on the native enzyme in the presence of Mn^{2+} [4] and from enzymatic experiments with hydrazines and sodium azide,[5] the Mn^{2+} binding site was first located at about 9 Å from the δ meso heme edge. Very recently, the crystal structure of MnP has been solved, and Mn^{2+} seems more likely to interact directly with one of the heme propionates.[6] Unlike other hemeproteins, the synthesis of model systems based on MnP activity has received little attention.[7] This lack of biomimetic approach arises probably from the following difficulties. Using a heme and a Mn(II) complex free in solution results after addition of an oxene donor in a slow oxidation of Mn^{2+} to Mn^{3+},[7] and thus the further oxidation of a substrate can be catalyzed either by the iron−oxo species or by the Mn^{3+} complex. This leads to an unselective reaction as a result of the competition between oxo transfer vs electron transfer.[8] To overcome these limitations, we have synthesized a [(P)Fe^{III}−Mn^{II}] bimetallic complex in which the manganese site is covalently linked to an iron porphyrin, to mimic the proximity of the manganese binding site to the heme active site of MnP with the aim to favor the electron transfer between Mn(II) and the iron−oxo species and to eliminate as much as possible any direct oxo transfer to the substrate.

In this paper we describe the synthesis and the spectroscopic characterization of the first [(P)Fe^{III}−Mn^{II}] heterobinuclear complex. Then, we give the first evidence showing, in the presence of an iodosylarene, the efficiency of the intramolecular electron transfer between Mn(II) and the ferryl species, leading to Mn(III) formation that reverts back to Mn(II) upon addition of a substrate.

Results and Discussion

Synthesis of the [(P)Fe^{III}−Mn^{II}] Binuclear Complex. The ditopic porphyrin **4** was synthesized in three steps as depicted in Scheme 1, starting from a modified *meso*-tetrakis(pentafluorophenyl)porphyrin **1**, in which one of the pentafluorophenyl group has been replaced by an *o*-nitrophenyl one. Fluorinated porphyrins were chosen for their stability toward oxidants[9] and their high reactivity as catalysts in oxidation reactions.[8,10]

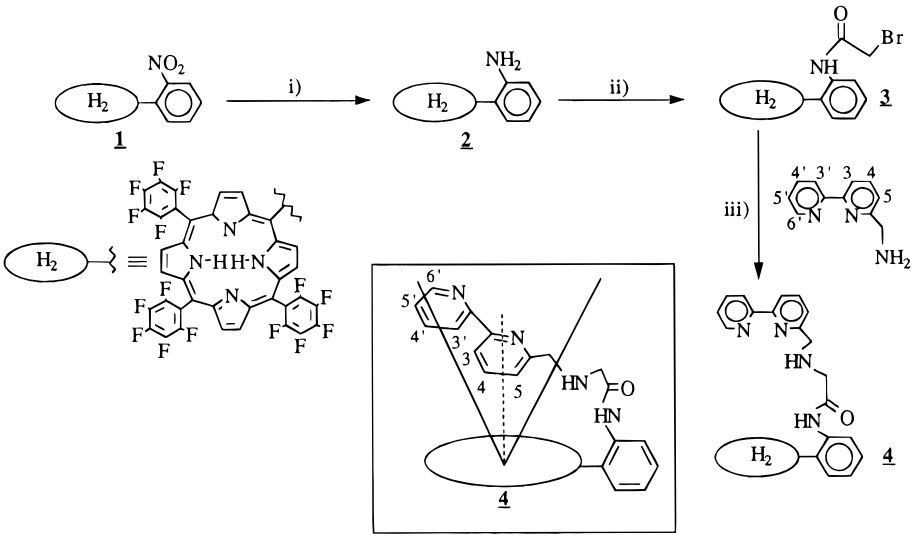
The reducing agent, $SnCl_2$ in concentrated HCl, commonly used for the reduction of nitroporphyrins[11] induces in this case a competitive decomposition of the macrocycle and affords the amino derivative in too low a yield (≤30%) for such a multistep synthesis. A new procedure, more suitable for the reduction

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1995.

(1) Glenn, J. K.; Gold, M. H. *Arch. Biochem. Biophys.* **1985**, *242*, 329−341.
(2) Wariishi, H.; Valli, K.; Gold, M. H. *Biochemistry* **1989**, *28*, 6017−6023.
(3) Wariishi, H.; Valli, K.; Gold, M. H. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 269−275.
(4) Banci, L.; Bertini, I.; Bini, T.; Tien, M.; Turano, P. *Biochemistry* **1993**, *32*, 5825−5831.
(5) Harris, R. Z.: Wariishi, H.; Gold, M. H.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1991**, *266*, 8751−8758.
(6) Sundaramoorthy, M.; Kishi, K.; Gold, M. H.; Poulos, T. L. *J. Biol. Chem.* **1994**, *269*, 32759−32767.
(7) Defrance, S.; Meunier, B. *New J. Chem.* **1992**, *16*, 1015−1016.
(8) Artaud,I.; Ben-Aziza, K.; Mansuy, D. *J. Org. Chem.* **1993**, *58*, 3373−3380.
(9) Traylor, P. S.; Dolphin, D.; Traylor, T. G. *J. Chem. Soc., Chem. Commun.* **1984**, 279−280.
(10) Traylor, T. G.; Tsuchiya, S.; Byun, Y. S.; Kim, C. *J. Am. Chem. Soc.* **1993**, *115*, 2775−2780.
(11) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Bunnenberg, E.; Linder, R. E.; LaMar, G. N.; Gaudio, J. D.; Lang, G.; Spartalian, K. *J. Am. Chem. Soc.* **1980**, *102*, 4182−4192.

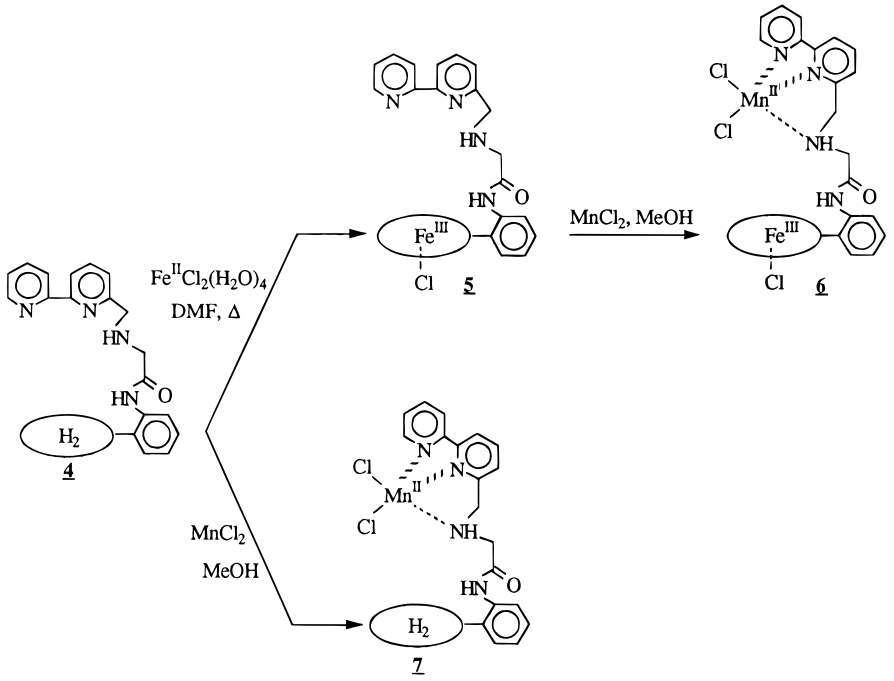
0020-1669/96/1335-0210$12.00/0

Scheme 1. Synthesis of the Ditopic Porphyrin[a]



[a] Key: (i) (1) $CuCl_2$, CH_2Cl_2/MeOH, (2) $Cu(acac)_2$/$NaBH_4$, CH_2Cl_2/EtOH, and (3) H_2SO_4; (ii) $ClCOCH_2Br$, CH_2Cl_2, 0 °C; (iii) $ClCH_2CH_2Cl$, K_2CO_3, 4 °C.

Scheme 2. Metalation of the Free Base **4** by Iron and Manganese: Preparation of the [(P)Fe^{III}−Mn^{II}] Complex



of highly fluorinated porphyrins has been developed, using $NaBH_4$ in the presence of a catalytic amount of copper acetylacetonate. This procedure, which has been previously applied to the reduction of some nitroaromatic compounds,[12] provided the amino derivative **2** in high yield (≥85%). After linkage of the spacer, upon reaction of the amino compound with bromoacetyl chloride leading to the formation of **3**, the 6-(methylamino)-2,2′-bipyridine manganese auxiliary ligand was then introduced by nucleophilic substitution yielding **4**. Such neutral nitrogeneous ligands are known to stabilize the lower oxidation states of metal ions as Mn^{2+}.[13] So, in our model, the Mn ligands are pyridine nitrogens while in manganese peroxidase, the Mn ligands are all oxygens from carboxylates or waters. However, the carboxylate ligands, except in biomolecules, are seldom involved in the formation of monomers, but rather in the formation of dimers or more highly associated complexes, in which two manganese centers are linked through the oxygens of a bridging carboxylate. The free base **4** was characterized by UV−visible and ^{1}H NMR spectroscopies and by elemental analysis. From ^{1}H NMR analysis, the protons of the bipyridine ligand were 0.3−3 ppm upfield shifted, relative to those of the free 6-(methylamino)-2,2′-bipyridine, suggesting that this ligand lies above the porphyrinic macrocycle in a non symmetrical position relative to the anisotropic shielding cone as shown in Scheme 1. The heterobimetallic complex was prepared by two successive metalations (Scheme 2). Iron was first inserted into the porphyrin according to a classical technique using $FeCl_2$ in DMF, yielding complex **5**. The selective incorporation of iron inside the porphyrin has been proved by mass spectrometry and UV−visible spectroscopy. Manganese was then introduced upon treatment with $MnCl_2$ in methanol at

(12) Hanaya, K.; Muramatsu, T.; Kudo, H. *J. Chem. Soc., Perkin Trans 1* **1979**, 2409−2410.
(13) Wieghardt, K. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1153−1172.

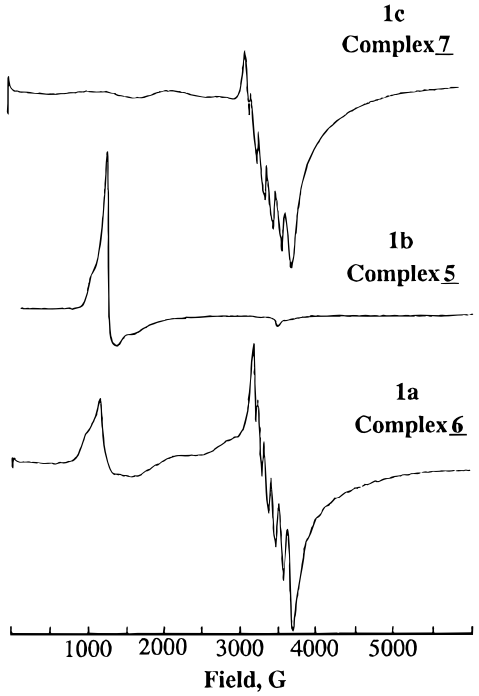


Figure 1. X-Band EPR spectra of complexes **6** (a), **5** (b) and **7** (c) in methanol at 4 K. Instrumental parameters: microwave frequency 9.5 GHz, microwave power 20 mW, and modulation amplitude of 1 G.

room temperature, affording the [(P)Fe^{III}−Mn^{II}] complex **6**, which was further purified by precipitation into a CH_2Cl_2/pentane 2/1 v/v mixture. This binuclear system is air stable, but water sensitive since manganese is readily removed in the presence of water. The structure proposed for complex **6** is consistent with microanalysis, mass spectrometry, and UV−visible spectroscopy results. The electronic spectrum of **6** in CH_2Cl_2, with α bands at 500 and 586 nm indicates the presence of an iron chloroporphyrin. Moreover, the presence of two kinds of chloro ligands is supported by FAB^+ mass spectrometry. This spectrum exhibits a peak at 1303 (38%) whose isotopic pattern is consistent with the presence of two chloro atoms and which corresponds to the mass of complex **6** with the loss of the axial chloro ligand of iron. The peaks at 1268 (100%), 1232 (38%), and 1179 (27%) correspond to the successive loss of the two chloro ligands bound to manganese and finally to the loss of manganese itself. Consequently, as proposed in Scheme 2, manganese seems likely to be either tetracoordinated to the two nitrogen atoms of the bipyridine and to the two exogeneous chloro ligands or pentacoordinated if a loose coordination to the aminomethyl moiety is considered. It is noteworthy that we failed to introduce another exogeneous bipyridine ligand to complete the coordination sphere of manganese. The difficult access to the manganese site could be related to the close proximity of the porphyrinic macrocycle.

EPR Characterization and Magnetic Susceptibility. An X-band EPR spectrum of the [Fe^{III}−Mn^{II}] complex **6** (10^{-3} M in a frozen methanolic solution) shown in Figure 1a has been recorded at 4 K. It can be described as the superimposition of the spectra of the mononuclear iron(III) complex **5** (Figure 1b) and the mononuclear Mn(II) complex **7** (Figure 1c), prepared by direct metalation of the free base **4** with $MnCl_2$ in methanol (Scheme 2). Complexes **5** and **6** exhibit a broad signal at $g =$ 6 which is typical of high spin Fe(III) porphyrins in an axial symmetry, the low component at $g = 2$ observed in the spectrum of complex **5**, being hindered in the case of complex **6** by the Mn(II) signal at $g = 2$. The signal at $g = 6$ is broadened from 5.98 to 7.04 as the consequence of the axial ligand exchange

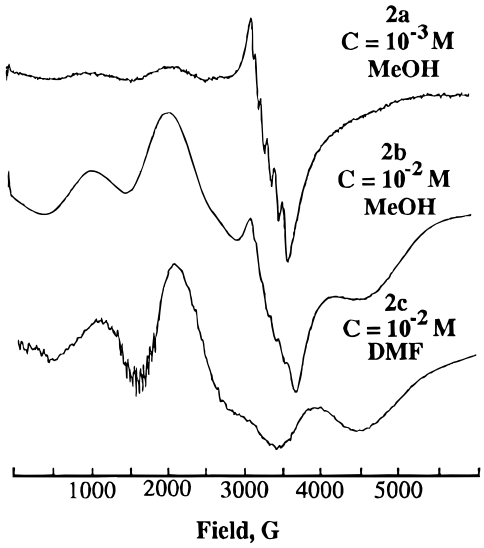


Figure 2. X-Band EPR specra of $Mn(bipy)_2Cl_2$ at 4 K: (a) $c = 10^{-3}$ M in methanol; (b) $c = 10^{-2}$ M in methanol; (c) c $= 10^{-2}$ M in DMF. Mvirowave power was 20 mW for parts a and b and 30 mW for part c.

between chloride and methoxide which occurs upon dissolution of chloro iron highly fluorinated porphyrins in methanol. Indeed, the UV−visible maximum of the α bands is shifted from 500 nm (in CH_2Cl_2) to 580 nm upon dissolution of the chloro complexes **5** and **6** in methanol, suggesting the formation of methoxo complexes.

For the manganese characteristics, the EPR spectrum of complex **6** displays, as that of complex **7,** an intense resonance at $g = 2$ showing a six line hyperfine structure with an average spacing of 85−90 G, typical of monomeric Mn(II) complexes. This main signal is flanked at lower field between 1000 and 3000 G by unresolved broad bands around 2300 and 2900 G for complex **6** and around 1400, 2200, and 2870 G for complex **7**, respectively. No other band has been observed at higher field between 5000 and 10000 G. Such spectra with broad bands at low field have been previously reported for dimeric manganese(II) structures[14] or distorted monomeric Mn(II) compounds.[15]

To choose between these two possibilities, a comparative study has been performed with a simpler Mn(II) complex, $Mn(bipy)_2Cl_2$, whose structure has been previously described.[16] The EPR spectra of this compound are shown in Figure 2 for two concentrations in methanol. The spectrum at 10^{-3} M (Figure 2a) is quite similar to that of complex **7**, but a tenfold more concentrated solution leads to a significant increase of the two bands at low field around 1000 and 2000 G (Figure 2b). At the same concentration in DMF (10^{-2} M) there is a complete disappearance of the resonance at $g = 2$ and a new ^{55}Mn hyperfine structure appears at very low field as a set of 11 lines with a splitting of ca. 45 G (Figure 2c), approximatively half

(14) (a) Mabad, B.; Cassoux, P.; Tuchagues, J. P.; Hendrickson, D. N. *Inorg. Chem.* **1986**, *25*, 1420. (b) Mathur, P.; Crowder, M.; Dismukes, G. C. *J. Am. Chem. Soc.* **1987**, *109*, 5227−5233. (c) Pessiki, P. J.; Khangulov, S. V.; Ho, D. M.; Dismukes, G. C. *J. Am. Chem. Soc.* **1994**, *116*, 891−897.

(15) (a) Dowsing, R. D.; Gibson, J. F.; Goodgame, D. M. L.; Goodgame, M.; Hayward, P. J. *Nature* **1968**, *219*, 1037−1038. (b) Dowsing, R. D.; Gibson, J. F.; Goodgame, M.; Hayward, P. J. *J. Chem. Soc. A* **1969**, 187−193. (c) Dowsing, R. D.; Gibson, J. F.; Goodgame, D. M. L.; Goodgame, M.; Hayward, P. J. *J. Chem. Soc. A* **1969,** 1242−1249. (d) Goodgame, D. M. L.; Goodgame, M.; Hayward, P. J. *J. Chem. Soc. A* **1970**, 1352−1356. (e) Laskowski, E. J.; Hendrickson, D. N. *Inorg. Chem.* **1978**, *17*, 457−470.

(16) Lumme, P. O.; Lindel, E. *Acta Crystallogr.* **1988**, *C44*, 463−465.

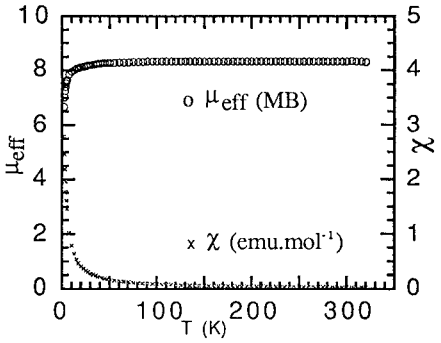
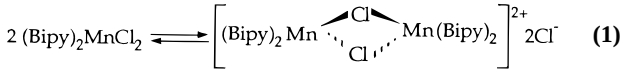


Figure 3. Temperature dependence of the magnetic susceptibility and of the effective magnetic moment of the [Fe^{III}−Mn^{II}] complex **6**.

the hyperfine coupling constant of the monomeric species. This last spectrum is consistent with a dimeric structure,[14] in which the two manganese centers are in magnetic exchange interaction through chloro bridging (eq 1). In methanol, this dimer should



(1)

be in equilibrium with the monomer. More generally, an increase in the concentration or the use of an aprotic solvent, unable to interact through hydrogen bonds with the chloro bridges, favors the dimeric structure.

The EPR spectra of both complexes **6** and **7**, in contrast with that of $Mn(bipy)_2Cl_2$, did not display any concentration effect or solvent effect. This rules out the involvement of such manganese association through μ-chloro bridging, either in the binuclear complex **6** or in the mononuclear complex **7**. Therefore EPR spectra of complexes **6** and **7** with a main line at $g = 2$ and broad resonances of less intensity at lower field could be explained by a slightly distorted monomeric structure. Such spectra with more or less intense components at lower and higher fields have been previously observed for distorted octahedral,[15a,b] tetrahedral,[15a,c] or trigonal pyramidal[15d,e] Mn(II) complexes. From crystallographic data, irregular tetrahedral structures are described when two types of bulky ligands are coordinated to manganese.[17] In the case of complexes **6** and **7**, the close proximity of the porphyrinic macrocycle contributes to increase the steric hindrance of the manganese environment and to decrease the access to the manganese site.

Finally, the lack of magnetic interaction between iron(III) and manganese(II) in the binuclear complex **6**, suggested by EPR, has been confirmed by the magnetic susceptibility measurements. The variation of the magnetic susceptibility has been recorded over the range 2−300 K. Above 20 K, as shown in Figure 3, the effective magnetic moment is almost constant and equal to 8.30 μ_B, a value which corresponds, within experimental error, to the spin-only value expected for an uncoupled $S = {}^5/_2$, $S = {}^5/_2$ Fe(III)/Mn(II) system. Below 20 K, the moment decreases rapidly to 6.61 μ_B at 2 K, as the result of the large zero-field splitting effect common to pentacoordinated iron(III) porphyrins.[18]

Reaction of the Binuclear Complex with Oxidant. Upon addition of pentafluoroiodosylbenzene, C_6F_5IO, to a methanolic solution of complex **6** at 0 °C, we observed by EPR spectroscopy the progressive disappearance of the Mn(II) signal at $g =$ 2 as shown in Figure 4. The complete extinction of Mn(II) resonances occurred after addition of 10 (Figure 4c) or 20 equiv

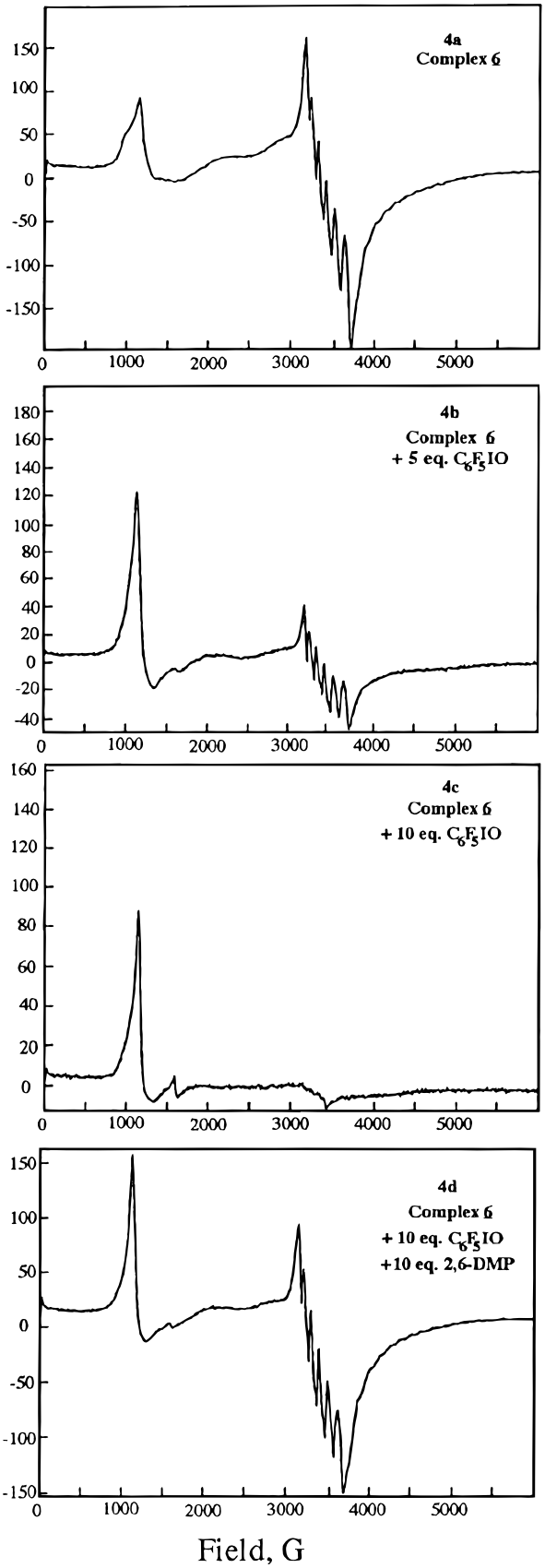
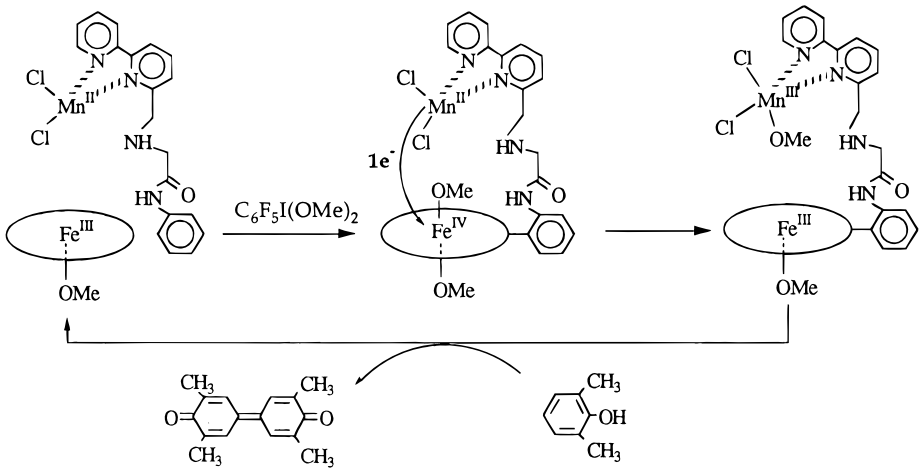


Figure 4. Evolution of the X-band EPR spectra of complex **6** in methanol upon addition of C_6F_5IO: without any oxidant (a); after addition of 5 equiv of C_6F_5IO (b); after addition of 10 equiv of C_6F_5IO (c); after addition of 10 equiv of C_6F_5IO and 10 equiv of 2,6-dimethylphenol (2,6-DMP) (d).

(17) (a) Ahuja, I. S.; Yadava, C. L. *Ind. J. Chem.* **1988**, *27A*, 169−170. (b) Andruh, M.; Roesky, H. W.; Noltemeyer, M.; Schmidt, H. G. Z. *Naturforsch.* **1994**, *49B*, 31−35. (c) Masood, A.; Hodgson, D. J. *Inorg. Chim. Acta* **1994**, *221*, 99−108.

(18) Gunter, M. J.; Mender, N. L.; McLaughlin, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 1470−1473.

Scheme 3. Proposed Mechanism for the Reversible Formation of Mn(III) upon Reaction of Complex **6** with C_6F_5IO



of C_6F_5IO to a solution of complex **6** at 10^{-3} or 10^{-4} M, respectively. This extinction of Mn(II) signals is consistent with Mn(III) formation since Mn(III) is EPR silent. After addition of 2,6-dimethylphenol as a classical substrate of MnP,[1] the characteristic Mn(II) signal at $g = 2$ with its hyperfine structure was fully recovered (Figure 4d). Preliminary catalytic experiments indicated that 2,2′,6,6′-tetramethyldiphenoquinone was the main oxidized product derived from 2,6-dimethylphenol as identified by ^{1}H NMR analysis.

For the sake of comparison, we tried to perform such experiments with mononuclear Mn(II) complexes such as Mn(bipy)$_2$Cl$_2$ or complex **7** and with a 1/1 mixture of chloroiron tetrakis(pentafluorophenyl)porphyrin, Fe(TF$_5$PP)Cl, and Mn(bipy)$_2$Cl$_2$ free in solution. First, neither Mn(bipy)$_2$Cl$_2$ nor complex **7** (10^{-3} M) were oxidized even in the presence of a large excess of C_6F_5IO (30 equiv.). Then, Mn(bipy)$_2$Cl$_2$ in the presence of Fe(TF$_5$PP)Cl at the same concentration of 10^{-3} M was slightly oxidized by 30 equiv of C_6F_5IO, as shown by the partial decrease of the Mn(II) signal at $g = 2$ (data not shown). Such a decrease was not observed at Fe(TF$_5$PP)Cl and Mn(bipy)$_2$Cl$_2$ 10^{-4} M.

The aforementioned results indicate that (i) C_6F_5IO in methanol is unable to oxidize directly a Mn(II) complex (ii) there is an absolute requirement for an iron porphyrin, and (iii) at least at 0 °C, the Mn(III) formation is reversible since Mn(II) is fully recovered upon addition of a phenolic substrate. Moreover, the [(P)Fe^{III}−Mn^{II}] binuclear complex **6** is the most efficient system to catalyze the C_6F_5IO-dependent oxidation of Mn(II) to Mn(III), indicating that, in the dimer complex **6**, the intramolecular electron transfer is highly favored vs the intermolecular one.

In fact, the high solubility of C_6F_5IO in methanol is due to a solvolysis reaction that leads to pentafluoroiodobenzene dimethoxide $C_6F_5I(OMe)_2$.[19] This species is known to react with an iron porphyrin to produce an iron(IV) dimethoxide derivative.[20] The formation of Fe(IV)(OCH$_3$)$_2$(TF$_5$PP) upon reaction of C_6F_5IO with Fe(TF$_5$PP)Cl in methanol has been confirmed by ^{1}H NMR analysis at −40 °C. Its spectrum exhibits a characteristic pyrrole resonance at −22 ppm, as previously described for such a porphyrinic complex.[20] The efficient oxidation of Mn(II) to Mn(III) in complex **6** could be the result of a rapid electron transfer from Mn(II) toward the Fe(IV)(OCH$_3$)$_2$ center providing a [(P)Fe^{III}−Mn^{III}] intermediate which was effectively observed by EPR (Figure 4c). As proposed in Scheme 3, this electron transfer from manganese to iron could be associated with a concomitant methoxide ligand transfer from iron to manganese, that should stabilize the Mn(III) state at low temperature.

This formation of Mn(III) in complex **6**, relayed by the formation of an iron oxo porphyrin, is clearly relevant to manganese peroxidase activity. The [(P)Fe^{III}−Mn^{II}] binuclear system mimics quite well the second step of the catalytic cycle of MnP (eq 2) corresponding to the oxidation of Mn(II) to Mn-

$$\mathrm{MnPII} + \mathrm{Mn^{II}(L)} \rightleftharpoons \left[\mathrm{MnPII}\text{- - -}\mathrm{Mn^{II}(L)}\right] \longrightarrow \mathrm{MnP} + \mathrm{Mn^{III}(L)} \quad (2)$$

(III) catalyzed by the MnP compound II, which is described as a Fe^{IV}═O ferryl species.[21]

Conclusion

This paper describes the synthesis of the first [(P)Fe^{III}−Mn^{II}] heterobimetallic dimer, in which a manganese bipyridine auxillary ligand has been linked to a very reactive iron fluorinated porphyrin. This dimer has been characterized by elemental analysis, mass spectrometry, and UV−visible spectroscopy. As shown by EPR spectroscopy and magnetic susceptibility measurements, there is no magnetic exchange interaction between the Fe(III) and Mn(II) centers and, in contrast with Mn(bipy)$_2$Cl$_2$, no higher intermolecular association through μ-chloro bridging between two manganese sites. Thanks to a rapid intramolecular electron transfer, this dimer is particularly efficient for the reversible formation of Mn(III) in the presence in methanol of C_6F_5IO and a substrate, mimicking quite well the second step of the manganese peroxidase catalytic cycle. In that sense it could be considered as a first promising approach toward the building of a biomimetic system of manganese peroxidase that could be efficient for lignin degradation.

Experimental Section

Spectroscopic Measurements. ^{1}H NMR spectra were recorded at 300 K on a Bruker ARX-250 spectrometer driven by an uxnmr program on an Aspect Station 1. UV−visible spectra were recorded at room temperature on a Varian Cary-210 spectrophotometer. Chemical shifts

(19) Schardt, B. C.; Hill, C. L. *Inorg. Chem.* **1983**, *22*, 1563−1565.
(20) (a) Groves, J. T.; Quinn, R.; McMurry, T. J.; Lang, G.; Boso, B. *J. Chem. Soc., Chem. Commun.* **1984**, 1455−1456. (b) Groves, J. T.; Gross, Z.; Stern, M. K. *Inorg. Chem.* **1994**, *33*, 5065−5072.
(21) (a) Wariishi, H.; Dunford, B. H.: MacDonald, D. I.; Gold,M. H. *J. Biol. Chem.* **1989**, *264*, 3335−3340. (b) Kuan, C. I.; Johnson, K. A.; Tien, M. *J. Biol. Chem.* **1993**, *268*, 20064−20070. (c) Kishi, K.; Wariishi, H.; Marquez, L.; Dunford, B. H.; Gold, M. H. *Biochemistry* **1994**, *33*, 8694−8707.

are reported in ppm downfield from Me_4Si. EPR spectra were performed on a Bruker ESP300 spectrometer operating at a 9.5 GHz microwave frequency, at 4 K, with a 100 kHz modulation frequency and a 1 G modulation amplitude. The microwave power was 20 mW, unless otherwise noted. Variable magnetic susceptibility measurements were made with a Quantum Design MPMS SQUID susceptometer operating with a 1 T applied field.

Mass analyses were achieved at the Ecole Normale Supérieure in Paris for chemical ionization and at the Laboratoire de Spectrométrie de Masse Bioinorganique in Strasbourg, France, for fast atom bombardement. Elemental analyses were done by the microanalysis service at Paris VI University.

Reagents and Solvents. All solvents and reagents were of reagent grade quality and were purchased from Aldrich and Janssen. They were used without further purification. For porphyrins synthesis, anhydrous dichloromethane was purchased from SDS.

Syntheses. Tetrakis(pentafluorophenyl)porphyrin (TF_5PPH_2) and iodosopentafluorobenzene (C_6F_5IO) were synthesized as described previously.[22,23]

6-Methyl-2,2′-bipyridine was synthesized according to the procedure described by Kauffmann et al.[24] and Garber et al.[25] The distilled product was kept at 4 °C under an inert atmosphere, as it darkens in the presence of dioxygen.

6-(Bromomethyl)-2,2′-bipyridine. The preparation was performed according to the procedure described by Newkome et al.[26] 6-Methyl-2,2′-bipyridine was brominated in refluxing CCl_4 with *N*-bromosuccinimide, using azobis(isobutyronitrile) as an initiator. The reaction was monitored by ^{1}H NMR spectroscopy and stopped when the dibromomethyl derivative significantly appeared (after 4−6 h). The mixture was then purified by column chromatography (SiO_2 matrix, 35−70 mesh, $CHCl_3/Et_2O$ 90/10): 6-bromomethyl-2,2′-bipyridine (R_f = 0.35, 35%); 6-dibromomethyl-2,2′-bipyridine (R_f = 0.5, 20%).

^{1}H NMR (CD_2Cl_2), δ: CH_2, 4.65 (s, 2H); $H_{5'}$, 7.34 (dd, J = 3.6, 7.3 Hz, 1H); H_5, 7.47 (d, J = 8.1 Hz, 1H); H_4 and $H_{4'}$, 7.84 (t, J = 8.1 Hz, 2H); H_3 and $H_{3'}$, 8.36 (d, J = 8.1 Hz, 1H), 8.46 (d, J = 8.1 Hz, 1H); $H_{6'}$, 8.66 (d, J = 3.6 Hz, 1H).

6-(Aminomethyl)-2,2′-bipyridine. The product was prepared as previously described by Ziessel et al.[27] The amine was collected as the chlorhydrate derivative (yield 75%) by precipitation in a Et_2O/EtOH (100/2) mixture saturated with HCl gas.

^{1}H NMR of $C_{11}H_{11}N_3$, HCl (D_2O, with presaturation of the solvent signal, based on a WEFT pulse sequence), δ: CH_2, 4.52 (s, 2H); H_{bipy}, 7.69 (d, J = 8.1 Hz, 1H); 8.1 (bm, 2H); 8.32 (d, J = 7.3, 1H); 8.71 (m, 2H); 8.87 (dd, J = 5.8, 1.5 Hz, 1H).

^{1}H NMR of $C_{11}H_{11}N_3$ (CD_2Cl_2: the amine was deprotected in D_2O with NaOD and extracted in CD_2Cl_2), δ: CH_2, 3.98 (s, 2H); H_5 and $H_{5'}$, 7.31 (m, 2H); H_4 and $H_{4'}$, 7.8 (b m, 2H); H_3 and $H_{3'}$, 8.27 (d, J = 7.8 Hz, 1H); 8.47 (d, J = 7.8 Hz, 1H); $H_{6'}$, 8.65 (d, J = 3.9 Hz, 1H).

5,10,15-Tris(pentafluorophenyl)-20-(*o*-nitrophenyl)porphyrin [$F_{15}NO_2$], 1. This macrocycle synthesis was adapted from Lindsey's method.[28,29] A 2.5 L reaction of pentafluorobenzaldehyde (1.87×10^{-2} mol, 3.68 g) and *o*-nitrobenzaldehyde (0.94×10^{-2} mol, 1.41 g) with $BF_3(Et_2O)$ (8.2×10^{-3} mol, 1 mL) in anhydrous and deaerated dichloromethane was performed. The porphyrinogen formation was monitored by UV−visible spectroscopy at 500 nm. Then, 24−48 h later, the reaction mixture was oxidized with a solution of 2,3-dichloro-5,6-dicyanobenzoquinone (4.97 g, 2.2×10^{-2} mol) in toluene (250 mL) at 40 °C. The formation of the porphyrin ring was checked by the increase of the absorbance at 410 nm. The volume was reduced under vacuum to approximately 500 mL and the reaction mixture further purified, first by flash chromatography over basic Al_2O_3 eluted with CH_2Cl_2 and then by column chromatography (SiO_2, CH_2Cl_2/cyclohexane, 1/1) to separate the various porphyrins: 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin ([F_{20}], 10%); 5,10,15-tris(pentafluorophenyl)-20-(*o*-nitrophenyl)-porphyrin ([$F_{15}NO_2$], 25%); *cis*- and *trans*-bis(pentafluorophenyl)bis(*o*-nitrophenyl)porphyrin ([$F_{10}(NO_2)_2$], 10%).

^{1}H NMR ($CDCl_3$), δ: NH, −2.82 (s, 2H); H_{phenyl}, 8.03 (m, 2H), 8.24 (dd, J = 6.5, 2.6 Hz, 1H); 8.51 (dd, J = 7.15, 1.9 Hz, 1H); $H_{\beta pyr}$, 8.74 (d, J = 4.5 Hz, 2H); 8.79 (d, J = 4.5 Hz, 2H); 8.87 (s, 4H).

MS (CI + NH_3): *m*/*e* 930, $[M + 1]^+$, 100%.

Anal. Calcd for $C_{44}H_{14}F_{15}N_5O_2$: C, 56.85; H, 1.52; N, 7.53. Found: C, 57.03; H, 1.87; N, 7.36.

UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 414 (3.2×10^5); 508 (2×10^4); 540 (3×10^3); 584 (7×10^3); 640 (1.2×10^3); 655 (2×10^3).

5,10,15-Tris(pentafluorophenyl)-20-(*o*-aminophenyl)porphyrin [$F_{15}NH_2$], 2: Reduction of the nitro Function. Metalation by Cu(II). To a solution of [$F_{15}NO_2$], **1** (500 mg, 5.4×10^{-4} mol), in CH_2Cl_2 (20 mL), a solution of $CuCl_2$ (1 g, 5.4×10^{-3} mol) in EtOH (10 mL) was added and the mixture refluxed for 20 min. The insertion of copper was monitored by TLC (SiO_2-60F_{254}, Merck, 0.2 mm; CH_2Cl_2/cyclohexane, 1/1) and by UV−visible spectroscopy. After cooling, the excess of cupric salt was removed by washing with water. The organic layer was then dried over Na_2SO_4. [$F_{15}NO_2$]Cu (95% yield: UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 410 (4.7×10^5); 595 (sh); 536 (2×10^4); 570 (7×10^3); 620 (vs).

Reduction of [$F_{15}NO_2$]Cu. This reaction has to be carried out under strictly anaerobic conditions provided by an argon stream. To a stirred solution of copper(II) acetylacetonate (70.5 mg, 2.7×10^{-4} mol) in carefully deaerated ethanol (50 mL) kept at 0 °C, was added dropwise one-third of a solution of $NaBH_4$ (160 mg, 4.2×10^{-3} mol) in 70 mL of deaerated ethanol: a brown complex was formed within a few seconds. A solution of [$F_{15}NO_2$]Cu (536 mg, 5.41×10^{-4} mol) in deaerated CH_2Cl_2 (120 mL) was added dropwise and then the remaining $NaBH_4$ solution. After 1 h at 0 °C, the mixture was heated at 30 °C for 6 h. The reduction was followed by TLC (SiO_2; CH_2Cl_2/cyclohexane, 1/1; $R_{f[F_{15}NO_2]Cu} = 0.55$; $R_{f[F_{15}NH_2]Cu} = 0.35$). Some more $NaBH_4$ (50 mg) might be added if the reaction is not complete. If the solution turns green, the reaction has to be stopped at once by adding H_2O. The reaction mixture was washed four times with H_2O and the organic layer evaporated to dryness.

[$F_{15}NH_2$]Cu, UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 408 (2.5×10^5); 497 (sh); 536 (10^4); 570 (5×10^3); 620 nm (9×10^2).

Demetalation of [$F_{15}NH_2$]Cu. [$F_{15}NH_2$]Cu thus obtained was demetalated within 3 h in concentrated H_2SO_4 (20 mL). The acid solution was poured onto ice and extracted with CH_2Cl_2. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution, with H_2O (up to pH 7), finally dried over Na_2SO_4, and evaporated to dryness. The overall yield of the reduction was 85%.

^{1}H NMR (CD_2Cl_2), δ: NH_{pyr}, −2.87 (s, 2H); NH_{phen}, 3.63 (bs, 2H); H_{phen}, 7.18 (m, 2H); 7.64 (dd, J = 7.9, 7.3 Hz, 1H); 7.85 (d, J = 7.3 Hz, 1H); $H_{\beta pyr}$, 8.87 (d, J = 4.6 Hz, 2H); 8.95 (s, 4H); 9.06 (d, J = 4.6 Hz, 2H).

MS (CI + NH_3): *m*/*e* 900, $[M + 1]^+$, 100%.

Anal. Calcd for $C_{44}H_{16}F_{15}N_5 \cdot 1H_2O$: C, 57.59; H, 1.98; N, 7.63. Found: C, 57.51; H, 1.84; N, 7.75.

UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 412 (2×10^5); 508 (1.5×10^4); 540 (sh); 583 (5×10^3); 637 nm (9×10^2).

5,10,15-Tris(pentafluorophenyl)-20-(*o*-(*N*-(bromoacetyl)amino)-phenyl)porphyrin, 3. Freshly distilled bromoacetylchloride (42 μL, 4.9×10^{-3} mol) was added to a solution of [$F_{15}NH_2$], **2** (300 mg, 3.3 10^{-3} mol), in CH_2Cl_2 (60 mL), cooled at 0 °C. The reaction is complete within 15 min. The reaction mixture was poured onto ice and washed with H_2O (4×200 mL). After the mixture was dried over Na_2SO_4, the solvent was evaporated and the product purified by flash chromatography (SiO_2, CH_2Cl_2). During this reaction, a Br/Cl exchange occurs as shown by ^{1}H NMR and mass spectrometry analysis.

^{1}H NMR (CD_2Cl_2), δ: NH_{pyr}, −2.87 (s, 2H); CH_2, 3.19 (CH_2Br) (s, 75%), 3.40 (CH_2Cl) (s, 25%); NH_{phen}, 7.72 (bs, 1H); H_{phen}, 7.64 (dd, J

(22) Van der Made, A. W.; Hoppenbrouwer, E. J. H.; Nolte, R. J. M.; Drenth, W. *Recl. Trav. Chim. Pays-Bas* **1988**, 15−16.
(23) Schmeisser, M.; Dahmen, K.; Sartori, P. *Chem. Ber.* **1967**, 1633−1637.
(24) Kauffmann, T.; König, J.; Woltermann, A. *Chem. Ber.* **1976**, *109*, 3864−3868.
(25) Garber, T.; Van Wallendael, S.; Rillema, D. P.; Kirk, M.; Hatfield, W. E.; Welch, J. H.; Singh, P. *Inorg. Chem.* **1990**, *29*, 2863−2868.
(26) Newkome, G. R.; Gupta, V. K.; Fronczek, F. R. *Inorg. Chem.* **1983**, *22* 171−174.
(27) Ziessel, R.; Lehn, J. M. *Helv. Chim. Acta* **1990**, 1149−1162.
(28) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827−836.
(29) Lindsey, J. S.; Wagner, R. W. *J. Org. Chem.* **1989**, *54*, 828−836.

= 7.0, 7.6 Hz, 1H); 7.92 (dd, J = 8.0, 7.0 Hz, 1H); 8.16 (d, J = 7.6 Hz, 1H); 8.62 (d, J = 8.0 Hz, 1H); $H_{\beta pyr}$, 8.94 (m, 6H); 8.89 (d, J = 4.6 Hz, 2H).

MS (CI + NH_3): *m*/*e* 1022, $[M + 1]^+$, 100%; *m*/*e* 976, $[M - Br + Cl]^+$, 100%; *m*/*e* 940, $[M - Br]^+$, 6%; *m*/*e* 926, $[M - CH_2Br]^+$, 30%; *m*/*e* 899, $[M - COCH_2Br]^+$, 13%.

UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 414 (2.8×10^5); 471 (sh); 508 (1.9×10^4); 536 (sh); 585 (5.9×10^3); 653 (2×10^3); 634 nm (vs).

5,10,15-Tris(pentafluorophenyl)-20-(*o*-(*N*-(((2,2′-bipyridin-6-yl-methyl)amino)acetyl)amino)phenyl)porphyrin, 4. 6-(Aminomethyl)-2,2′-bipyridine was deprotected by stirring the chlorhydrate derivative (150 mg, 6.8×10^{-4} mol) for 3 h in methanol with a Dowex-Cl^- resin (1×2-200 ion exchange resin, strongly basic anion, 2% cross-linking, 100−200 mesh) (4 g) previously exchanged with OH^-. After filtration and methanol evaporation, 6-(aminomethyl)-2,2′-bipyridine was dissolved in 1 mL of $ClCH_2CH_2Cl$ and added to a solution of **3** (150 mg, 1.47×10^{-4} mol) in 4 mL of $ClCH_2CH_2Cl$ in the presence of K_2CO_3 (500 mg). The mixture was allowed to stand 10 h at 4 °C under magnetic stirring. K_2CO_3 was then filtered off and the solution extracted with HCl (10%). The organic layer was washed with H_2O up to pH 7 and dried over Na_2SO_4 and the solvent evaporated without any heating. Compounds **3** and **4** were easily separated by column chromatography (SiO_2). Two successive elutions with CH_2Cl_2 and CH_2Cl_2/EtOH (80/20) afforded the porphyrins **3** and **4** (80% yield) respectively.

^{1}H NMR (CD_2Cl_2), δ: NH_{pyr}, −3.06 (s, 2H); (bipy)CH_2COCH_2, 1.63 (s, 2H); 2.81 (s, 2H); H_{bipy}, H_5 or H_3, 3.75 (d, J = 7.6 Hz, 1H); H_4, 4.81 (t, J = 7.6 Hz, 1H); H_3 or H_5, 6.59 (d, J = 7.6 Hz, 1H); $H_{5'}$, 7.04 (dd, J = 7.6, 4.2 Hz, 1H); $H_{3'}$, 7.30 (d, J = 7.6 Hz, 1H); $H_{4'}$, 7.38 (t, J = 7.6 Hz, 1H); $H_{6'}$, 8.31 (d, J = 4.2 Hz, 1H); H_{phen}, 7.57 (dd, J = 6.9, 7.6 Hz, 1H); 7.89 (dd, J = 8.3, 6.9 Hz, 1H); 8.07 (d, J = 7.6 Hz, 1H); 8.80 (d, J = 8.3 Hz, 1H); $H_{\beta pyr}$, 8.89 (m, 6H); 8,98 (d, J = 4.1 Hz, 2H); NH, 9.31 (s, 1H), 8.89 (m, 1H).

Chemical shifts were assigned through a 2D-COSY45 HH correlation and double irradiation experiments.

MS (CI + NH_3): *m*/*e* 1125, $[M]^+$, 100%; *m*/*e* 926, $[M - CH_2NHCH_2 - bipy]^+$, 9%; *m*/*e* 900, $[M - COCH_2NHCH_2 - bipy + 1]^+$, 21%.

Anal. Calcd for $C_{57}H_{27}ON_8F_{15}$: C, 60.86; H, 2.42; N, 9.96. Found C, 60.78; H, 2.55; N, 10.00.

UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 287 (2.6×10^4); 415 (2.7×10^5); 477 (sh); 508 (2×10^4); 538 (3×10^3); 585 (6×10^3); 639 (vs); 652 (2×10^3).

Porphyrin Metalation by $FeCl_2$. Complexes **5** and Fe(TF_5PP)Cl were prepared according to the method of Fleischer et al.[30]

Complex 5. MS (CI − NH_3): *m*/*e* 1178, $[M - Cl]^+$, 56%; *m*/*e* 979, $[M - CH_2NHCH_2 - bipy]^+$, 97%; *m*/*e* 953, $[M - COCH_2NHCH_2$-bipy + 1$]^+$, 65%.

UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 287 (3.2×10^4); 355 (4.6×10^4); 414 (8.1×10^4); 500 (2×10^4); 586 (6×10^3); 626 (5.7×10^3); 713 (2.5×10^3).

UV−visible (MeOH) (λ, nm (ϵ, M^{-1} cm^{-1})): 285 (2.7×10^4); 343 (sh); 408 (8.5×10^4); 583 (8.6×10^3).

Preparation of Complex 6. A solution of $MnCl_2$ (176 mg, 1.4×10^{-3} mol) in MeOH (5 mL) was added to a solution of complex **5** (170 mg, 1.4×10^{-4} mol) in MeOH (2 mL). The mixture was stirred for 2 h. After evaporation of the solvent, the porphyrinic derivative was dissolved in anhydrous CH_2Cl_2, in which $MnCl_2$ was insoluble: the salt in excess was thus removed by filtration and the solvent evaporated to dryness. The porphyrinic derivative dissolved in 4 mL of anhydrous CH_2Cl_2 was precipitated upon addition of anhydrous pentane (2 mL), affording 110 mg of complex **6** (60% yield).

MS (FAB^+): *m*/*e* 1302.9, $[M - Cl - 1]^+$, 38%; *m*/*e* 1268, $[M - 2Cl - 1]^+$, 100%; *m*/*e* 1232, $[M - 3Cl - 1]^+$, 38%; *m*/*e* 1179, $[M - 3Cl - Mn]^+$, 27%; *m*/*e* 979, $[M - 3Cl - Mn - CH_2NHCH_2 - bipy]^+$, 38%; *m*/*e* 953, $[M - 3Cl - Mn - COCH_2NHCH_2bipy + 1]^+$, 30%. The isotopic ratio of the peaks at 1302.9 and 1268 were consistent with the presence of two chlorine atoms and one chlorine atom, respectively.

Anal. Calcd for $C_{57}H_{25}F_{15}N_8OMnFeCl_3 \cdot 0.5\ H_2O$: C, 50.75; H, 1.94; N, 8.31; Cl, 7.88. Found: C, 50.72; H, 2.40; N, 8.38; Cl, 7.74.

UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 301 (2.1×10^4); 311 (2.1×10^4); 356 (5×10^4); 413 (9.5×10^4); 502 (1.3×10^4); 590 (6×10^3); 635 (6×10^3).

UV−visible (MeOH) (λ, nm (ϵ, M^{-1} cm^{-1})): 298 (2.7×10^4); 311 (2.8×10^4); 350 (sh); 408 (9.3×10^4); 586 (8.6×10^3); 750 (vs).

Preparation of Complex 7. The aforementioned procedure was applied to the synthesis of complex **7**.

UV−vis (CH_2Cl_2) (λ, nm (ϵ, M^{-1} cm^{-1})): 302 (2.3×10^4); 309 (2.3×10^4); 414 (2×10^5); 508 (1.5×10^4); 537 (3.2×10^3); 584 (5×10^3); 638 (vs); 653 (vs).

EPR Experiments. Reactions with C_6F_5IO. The following solutions were prepared and kept at 0 °C:

$\mathbf{A_1}$, complex **6**, 2.01 mg, 1.5×10^{-6} mol in 1.5 mL of MeOH ($c = 10^{-3}$ M); $\mathbf{A_2}$, Fe(TF_5PP)Cl, 1.59 mg, 1.5×10^{-6} mol in 1.5 mL of MeOH; $\mathbf{A_3}$, Fe(TF_5PP)Cl, 1.59 mg, 1.5×10^{-6} mol, and Mn(bipy)$_2$-Cl_2, 0.657 mg, 1.5×10^{-6} mol in 1.5 mL of MeOH; $\mathbf{A_4}$, Mn(bipy)$_2Cl_2$, 0.657 mg, 1.5×10^{-6} mol in 1.5 mL of MeOH; **B**, C_6F_5IO, 31.1 mg, 10^{-4} mol in 1 mL of MeOH ($c = 0.1$ M); **C**, 2,6-dimethylphenol, 18.3 mg, 1.5×10^{-4} mol in 100 μL of MeOH ($c = 1.5$ M).

All the oxidation reactions were run using the same procedure and thus a typical experiment is described: 5 equiv of C_6F_5IO (**B**, 75 μL) were added twice to $\mathbf{A_1}$ and then 10 equiv of 2,6-dimethylphenol (**C**, 10 μL). After each addition, the mixtures were quickly shaken; 100 μL was taken off and rapidly introduced in a EPR tube which was immediately frozen in liquid nitrogen. For reactions performed at 10^{-4} M, the mother solutions of the complexes and the oxidant were previously diluted to 10^{-4} and 10^{-2} M, respectively.

Acknowledgment. This reseach was supported by the European Union (CEE Contract BIO2-CT94-2052). We thank Prof. P. Monod and R. Chiarelli for helpful assistance during the magnetism and EPR experiments.

IC9506771

(30) Fleischer, E. B.; Palmer, J. M.; Srivastava, T. S.; Chatterjee, A. *J. Am. Chem. Soc.* **1971**, *93*, 3162−3167.