41. O-Insertion into Nonactivated C–H Bonds: The First Observation of O_2 Cleavage by a P-450 Enzyme Model in the Presence of a Thiolate Ligand[1])

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(30.I.92)

On reaction with 'O'-donors or O_2, the synthetic P-450 analogue **2** undergoes O-insertion at a nonactivated C–H bond of the covalently bound substrate. The mechanism of O-insertion with O_2 most likely involves homolytic cleavage of the O–O bond followed by O-insertion *via* radical recombination.

Introduction. – The cytochrome P-450 enzymes are heme proteins which play an important role in the metabolism of endogenous compounds and xenobiotics in both procaryotic and eukaryotic organisms [1] [2]. The geometry of the active site of one of these proteins is precisely known from a recent series of X-ray studies of the non-membrane-bound, camphor-hydroxylating $P\text{-}450_{cam}$ [3]. These investigations confirmed earlier model studies concerning the coordination of a thiolate ligand to the Fe-atom on the face of the porphyrin opposite to the binding sites of an O_2 and the substrate.

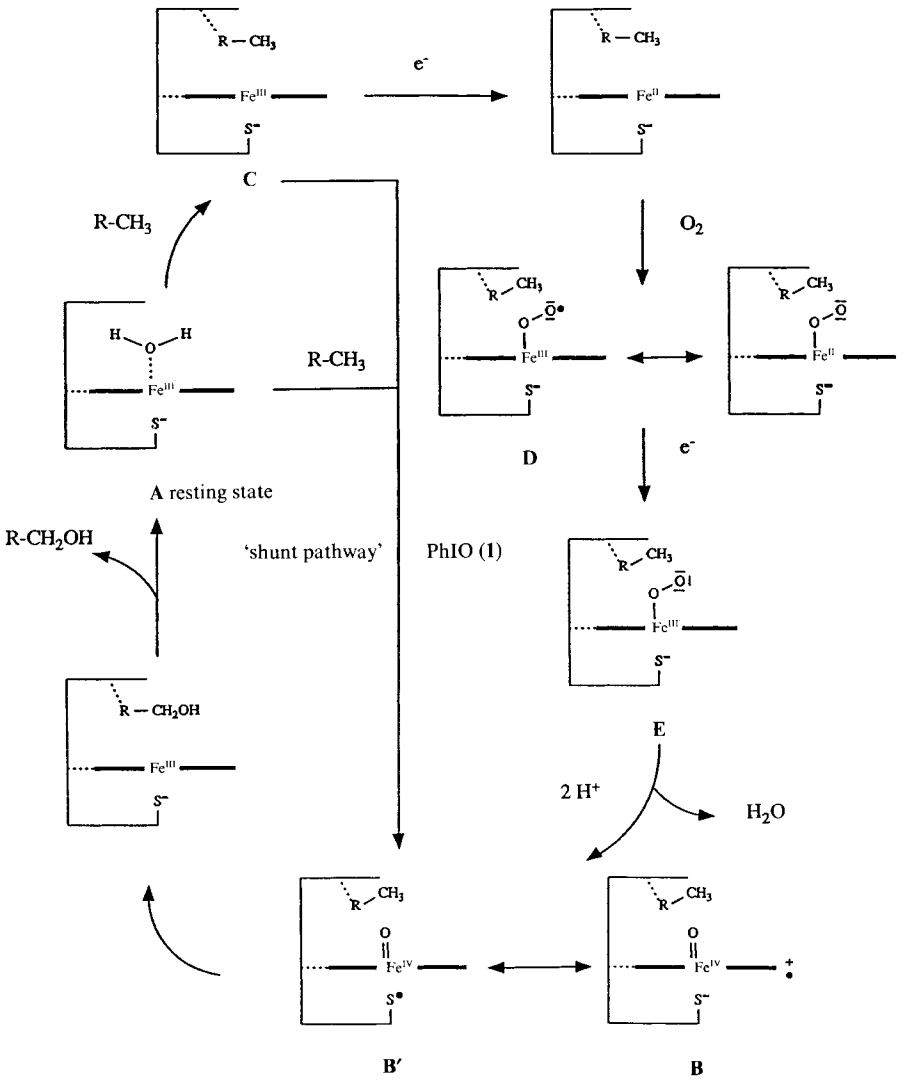
Since the discovery of cytochrome P-450 catalyzed reactions some 30 years ago [4], the unique ability of these enzymes to insert an O-atom regio- and stereospecifically into nonactivated C–H bonds has been a serious challenge to the organic chemist. To simulate these and other P-450 reactions, considerable progress was achieved by circumventing the problematic reductive cleavage of molecular O_2 (see catalytic cycle, *Scheme 1*), and employing synthetic face-protected [5] or perhalogenated iron(III) porphyrinates [6] as analogues of the resting state **A** and, *e.g.*, iodosobenzene (**1**) as the O-source. According to experiments by *Groves* and *Watanabe* [7] using peracids and iron(III)tetramesityl porphyrinate, this so-called 'shunt pathway' leads to an oxoiron(IV) porphyrinate radical cation **B**, which mimics the reactivity characteristic of P-450 enzymes, and, therefore, is believed to be an equivalent of the corresponding transient intermediate of the catalytic cycle in living cells (see reviews [8] [9]). However, in almost all studies using model porphyrins, the significance of the thiolate ligand to the reactivity of the iron porphyrinate was ignored[2]).

We recently showed that the doubly-bridged iron porphyrinates **2** and **3** carrying thiolate ligands are active-site analogues of E·S complexes **C** of cytochrome P-450 with respect to spin states, CO binding, and UV spectroscopy [13] [14]. To investigate whether these compounds are capable of P-450-like reactions, namely the O-insertion into nonac-

[1]) Presented in part at Vth SIMBIC, Parma, Italy, 19.9.90, and at the VIIth International Conference on Biochemistry and Biophysics of Cytochrome P-450, Moscow, Russia, 28.7.91.

[2]) For other approaches, involving non-porphinoid systems, of O-insertion into nonactivated C–H bonds, see [10–12].

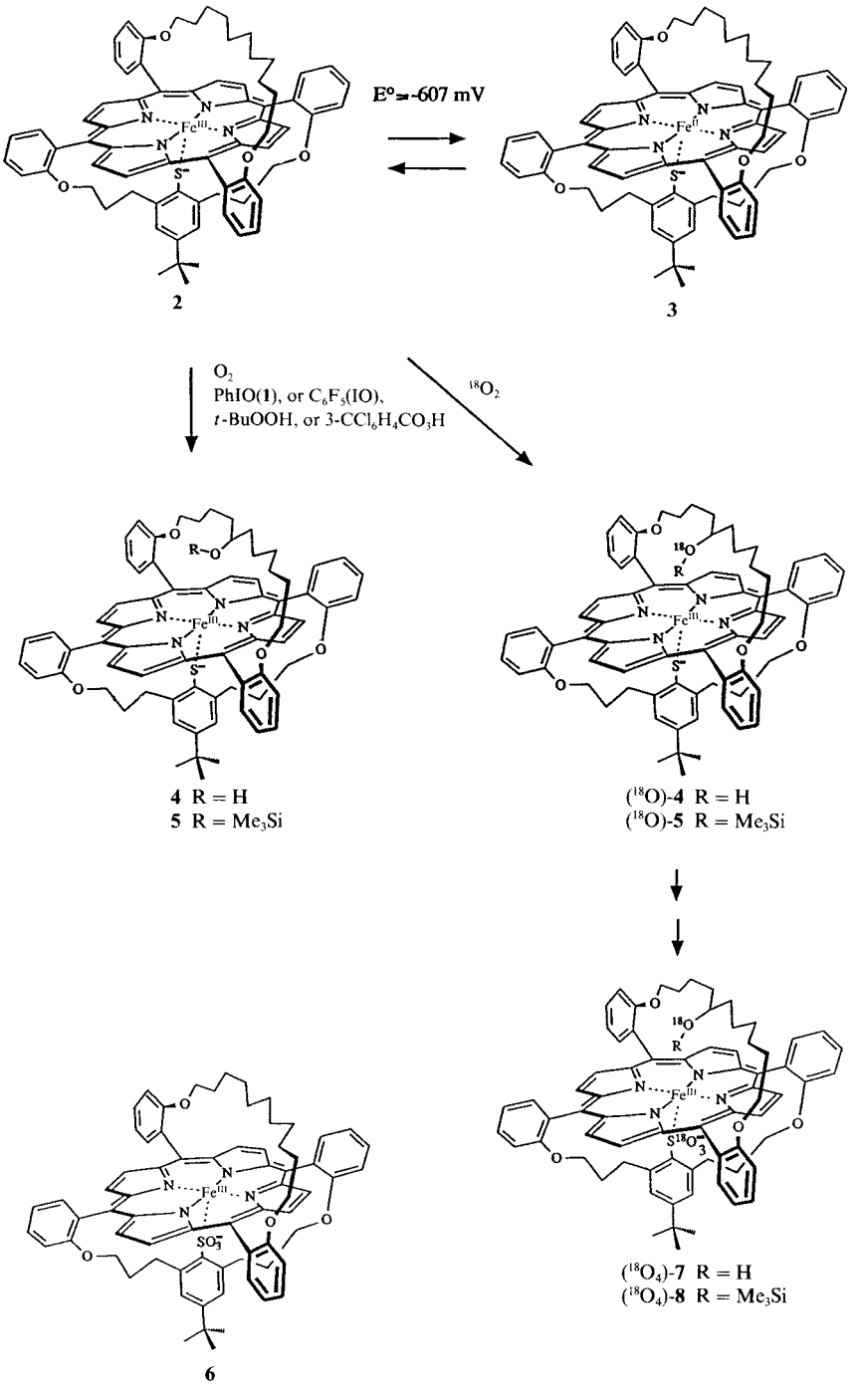
Scheme 1



tivated positions at the covalently bound substrate, a series of reactions of **2** with 'O'-donors and molecular O_2 was carried out.

Results and Discussion. – To a solution of the active-site analogue **2** in abs. toluene the oxidant iodosobenzene (PhIO; **1**), pentafluoroiodosobenzene ($C_6F_5(IO)$), *tert*-butyl hydroperoxide (*t*-BuOOH), or 3-chloroperbenzoic acid (3-$ClC_6H_4CO_3H$) was added under Ar either at −60 or at 25°. In all cases, a significant bathochromic shift of the *Soret* band of **2** from 412 to 418 nm was observed within 2 h, indicating the quantitative formation of a new compound. After chromatography in the glove box, complex **4** was

Scheme 2


E°=-607 mV
2
3
O2
PhIO(1), or C6F5(IO),
t-BuOOH, or 3-CCl6H4CO3H
18O2
4 R = H
5 R = Me3Si
(18O)-4 R = H
(18O)-5 R = Me3Si
(18O4)-7 R = H
(18O4)-8 R = Me3Si
6

isolated in 77% yield (*Scheme 2*). The ^{1}H-NMR spectrum of the sample revealed that **4** is a high-spin iron(III) porphyrinate (very broad signals without fine-splitting). No information could be obtained on the position of O-insertion (H–C(β)'s of pyrrole moieties as 2 broad signals between 80 and 100 ppm), which, therefore, is only tentatively given in *Scheme 2*. However, the MS of **4** (M^+ 1145), and of its trimethylsilyl derivative **5** (M^+ 1217) clearly demonstrate mono-O-insertion at the alkane-bridge spanning the porphyrin face opposite to the thiolate ligand.

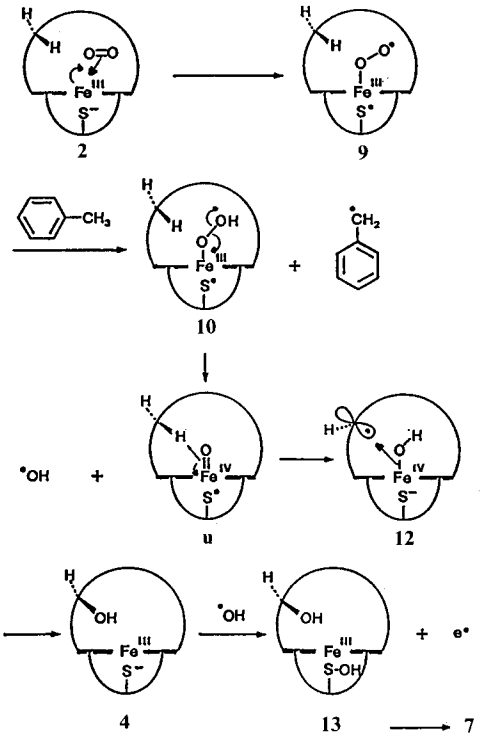
When O_2 was introduced *via* syringe into a 24 μM solution of **2** in abs. toluene at −60°, no reaction was observed during 2 d. However, at 10°, complete conversion to **4** occurred within 3 h. To prepare enough material for MS analysis, the reaction was repeated at higher concentrations of **2** (630 μM in abs. toluene). Surprisingly, a different complex, **6** (416 nm), was obtained, which was isolated as pure compound (HPLC) in 90% yield, after chromatography in the glove box. According to the MS (M^+ 1177), **6** is a sulfonate. At a concentration of 44 μM the addition of O_2 to **2** in abs. toluene yielded an inseparable mixture of **4** and the corresponding sulfonate **7** (418 nm). Silylation of **4**/**7** afforded the derivatives **5** and **8**, displaying both convincing MS in agreement with the proposed structures.

From these experiments, it is evident that depending on the concentrations of O_2 and **2** in toluene, the oxygenated products **4**, **6**, and **7** are produced. To ascertain the origin of the OH group from molecular O_2, the experiments were repeated with $^{18}O_2$. (^{18}O)-**4** and ($^{18}O_4$)-**7** were generated and characterized in the same manner as described for the unlabelled compounds; silylation of ($^{18}O_4$)-**7** gave ($^{18}O_4$)-**8**. Thus, the O-source for both the OH group and the SO_3^- ligand is O_2.

The identification of **6** and **7** confirms our earlier suggestion [13] that P-450 model compounds with thiolate ligands not rigidly attached to the porphyrin moiety will be oxidized at the S-atom when O_2 is used, but not when the oxidant is PhIO or a peroxo compound [15]. Since it was impossible to induce O-insertion with sulfonate **6**, it is evident that **7** originates from **4**.

Most significantly, however, is the formation of **4**. Indeed, for the first time, a synthetic P-450 model carrying a thiolate ligand is shown to cleave O_2 and to induce O-insertion into a nonactivated C–H bond. The rather unusual situation that this reaction occurs with the iron(III) porphyrinate **2** in the absence of a strong reducing agent accounts for the inherent reactivity of the system and can be understood as a mimic for events happening in the absence of electrons or on slow delivery of electrons from reducing proteins like putidaredoxin (see *Scheme 1*). In this context, it is interesting to note that kinetic data from investigations of P-450$_{cam}$/putidaredoxin indicate that the transfer of the second electron is rate limiting [16]. In fact, an intermediate, isoelectronic with **10** (*Scheme 3*) can be derived in the native catalytic cycle simply by protonation of the O_2 adduct **D** (*Scheme 1*). The formation of **4** and **7** involves H$^\cdot$ removal from the solvent toluene by **9** to generate **10**, followed by homolytic cleavage of the O–O bond to yield **11** and OH$^\cdot$. The oxoiron(IV) porphyrinate **11**, having isolated spin density on the S-ligand, is an attractive candidate for removing H$^\cdot$ from nonactivated positions to form the C-radical **12**, which through radical recombination gives the observed alcohol **4**. Intermediate **11** is isoelectronic with the oxoiron(IV) porphyrinate radical cation **B** (*Scheme 1*) which was postulated on the basis of model studies with porphyrinates lacking the thiolate ligand [7].

Scheme 3



Concerning the O–O bond cleavage, it is generally believed that the peroxoiron(III) intermediate **E** is protonated and H_2O is subsequently removed by heterolytic fission of the O–O bond to yield **B** ↔ **B′** (*Scheme 1*). However, as revealed by the X-ray structures of cytochrome P-450$_{cam}$ [3], there is no amino-acid residue near the O_2-binding site which could protonate or acylate the end-on bound O-atom of **E**. Thus, our present understanding of this process rests entirely on experiments with model porphyrinates. In two respects, recent investigations by *Balch et al.* are of significance to our work; first, it was also shown that iron(III) porphyrinates, in particular those alkylated at the Fe-atom ([$Fe^{III}(CH_2R)$(porph)]), react with O_2 [17], and second, it was suggested, in analogy to reactions with hydroperoxides in apolar solvents like toluene [18], that after insertion of O_2 into the Fe–C bond the O–O bond was cleaved homolytically to release RCHO and [Fe^{III}(OH)(porph)]. The identification of the oxoiron(IV) complex [Fe^{IV}(O)(porph)] as the first product of bond fission adds further support to a non-ionic process [19]. However, it was argued that bond cleavage is dependent on base added as a fifth ligand to the Fe-atom and in particular dependent on solvent polarity (see [18] and ref. cit. therein).

To what extent these results and interpretations are relevant to the active site of cytochrome P-450 rests on at least two factors: the polarity of the oxygen/substrate-binding cavity and the significance of the thiolate ligand. The first aspect is rather unexplored, since it is not known how many of the H_2O molecules originally present in the resting-state cavity of P-450$_{cam}$ are forced to leave on binding of the substrate and O_2. However,

recent experiments revealed that the decomposition of hydroperoxides and peracids by chelated protohemin chloride in protic solvents (CH_2Cl_2/ROH) is less dependent on solvent polarity then on alcohol acidity [20]. Solvent isotope effects in the range of 2.0 also account for a reaction involving proton transfer. Thus, *Traylor* and *Xu* [20] argued in favor of a heterolytic O–O bond cleavage catalyzed by H_2O present in the active site.

Our own results indicate the principal assistance of the thiolate ligand in homolytic O–O bond-cleavage and O-insertion in an apolar aprotic environment. Enzyme models with substrate-recognition sites are currently under investigation in order to evaluate the significance of polar groups at the O_2-binding site with respect to O–O bond breaking and in order to perform catalytic reactions [21].

We thank the *Swiss National Science Foundation* and *F. Hoffmann-La Roche AG*, Basel, for generous support.

Experimental Part

1. *General.* Unless otherwise stated, all reactions with porphyrins were carried out in a *Mecaplex-G-B-2201* glove box under dry N_2 free of O_2 (< 10 ppm; *Teldyne Analytical Instruments*). All solvents were purified and degassed by repeated freezing under vacuum as described earlier [13]. Pentafluoroiodobenzene (*Aldrich*) was distilled from activated molecular sieves 3 Å. The 3-chloroperbenzoic acid (*Fluka*) was washed to pH 7.5 with an aq. phosphate buffer $^{18}O_2$ (*MSD Isotopes*) was condensed into a steel vial and dosed *via* a pressure regulator. Natural O_2 (*AGA 4.8*; ≥ 99.998%) was used without purification. TLC: 0.2-mm precoated silica-gel plates *Merck 60 F254*. M.p.: *Mettler FP-52*; uncorrected. UV experiments: low-temperature UV cell (*Helma*) connected to a *Lauda-K-120-W* ultracryostat on a *Hewlett-Packard-8452A* diode-array spectrophotometer; λ_{max} (rel.%) in nm. IR: *Perkin Elmer 297*; in cm^{-1}. ^{1}H-NMR: *Bruker AM-400* (MHz); δ in ppm, J in Hz. ^{13}C-NMR: *Varian XL-200* (50 MHz). MS: *Finnigan MAT 90*, in m/z (rel. %).

2. *Oxidizing Agents ('O'-Donors). Iodosobenzene* (**1**) was prepared according to [22]: (Diacetoxyiodo)benzene (5.00 g, 15.5 mmol; *Fluka*) was vigorously stirred in aq. 3M NaOH (20 ml) for 30 min at r.t. The soln. was then diluted with H_2O (30 ml) and the precipitate filtered off. After emulgation with H_2O (30 ml), the solid was filtered again and washed with cold CH_2Cl_2 (10 ml). After drying for 12 h at 30° *in vacuo*, **1** (2.78 g, 81%) was obtained. Slightly yellow powder. M.p. 234–236°. IR (nujol): 3025*m*, 1575*w*, 1440*m*, 1170*w*, 1050*m*, 1015*m*, 1000*m*, 765*s*, 760*s*, 730*s*, 720*s*, 680*m*, 655*w*. EI-MS: 204 (63, $[M - O]^+$), 145 (9), 77 (100), 51 (43), 50 (27), 44 (27). Anal. calc. for C_6H_5IO (219.95): C 32.75, H 2.29; found: C 30.87, H 2.40.

1,2,3,4,5-Pentafluoro-6-(iodoso)benzene ($C_6F_5(IO)$) was prepared according to [23]: In an intensively ventilated hood, HNO_3 (1.8 ml, $d = 1{,}55$; *Merck*) was slowly added at −30° to a soln. of pentafluoro(iodo)benzene (5.90 g, 20.0 mmol) in $(CF_3CO)_2O$ (10 ml). The soln. was vigorously stirred while slowly warming up to r.t. After 3 h, the evolution of nitrogen oxides stopped, and the soln. was evaporated. The yellow solid (10.58 g) was sublimed (110°/0.1 Torr) to give *1-[bis(trifluoroacetoxy)iodo]-2,3,4,5,6-pentafluorobenzene* (7.52 g, 72%). Clear needles. M.p. 96°. UV/VIS (MeCN): 298, 290 (sh). IR (nujol): 1600*m*, 1330*s*, 1190*s*, 1160*s*, 1115*m*, 885*m*, 785*m*. ^{13}C-NMR ((D$_6$)DMSO): 158.6 (*q*, ^2J(C,F) = 39, CF_3CO); 146.9 (*md*, ^1J(C,F) = 244, C(2)); 140.8 (*md*, ^1J(C,F) = 250, C(4)); 136.9 (*md*, ^1J(C,F) = 253, C(3)); 115,3 (*d*, ^1J(C,F) = 288, CF_3CO); 70.0 (*mt*, ^2J(C,F) = 23, C(1)). CI-MS: 407 (62, $[M - CF_3CO) + H]^+$), 294 (100, $[M - (CF_3CO_2)_2 + H]^+$), 167 (46, $[C_6F_5]^+$). Anal. calc. for $C_{10}F_{11}IO_4$ (519.99): C 23.09; found: C 22.97.

A suspension of 1-[bis(trifluoroacetoxy)iodo]-2,3,4,5,6-pentafluorobenzene (10.0 g, 19.2 mmol) in aq. sat. $NaHCO_3$ soln. (80 ml) was intensively stirred for 25 h at r.t. The solid was filtered off and washed with H_2O, cold $CHCl_3$, and H_2O. The obtained slightly yellow, micro-crystalline powder was dried at 20° *in vacuo*: pure $C_6F_5(IO)$ (4.3 g, 72%). M.p. 146–147° (dec.). UV/VIS (MeCN): 296, 334 (sh). IR (nujol): 1650*m*, 1630*m*, 1515*s*, 1495*s*, 1395*m*, 1290*w*, 1195*m*, 1150*w*, 1135*m*, 1090*s*, 1000*m*, 980*s*, 805*m*, 725*m*. ^{13}C-NMR ((D$_6$)DMSO): 145.6 (*dddd*, ^1J(C,F) = 245, ^2J(C,F) = 16, ^3J(C,F) = 8, ^4J(C,F) = 4, C(1)); 144.0 (*dt*, ^1J(C,F) = 255, C(3)); 137.1 (*dt*, ^1J(C,F) = 230, C(2)); 96.5 (*t*, ^2J(C,F) = 28, C(6)). EI-MS: 294 (7), 293 (100, $[M - O]^+$), 167 (37, $[M - I - O]^+$), 117 (32). Anal. calc. for C_6F_5IO (309.96): C 23.25; found: C 23.43.

3. *Reactions of {5,15-{{[4-(tert-Butyl)-2-mercaptophen-1,3-ylene]bis(trimethyleneoxy)}di(phen-2,1-ylene)}-10,20-[(undecamethylenedioxy)di(phen-2,1-ylene)]porphyrinato}iron(III)* (**2**) *with 'O'-Donors. UV Experiments.*

A low-temperature UV cell was flooded with Ar and filled with 24 μM **2** in toluene (1 ml). The cell was cooled to −60° and the UV/VIS recorded (soln. *A*). Then 1.6 mM 'O'-donor **1**, $C_6F_5(IO)$, *t*-BuOOH, or 3-$ClC_6H_4CO_3H$ in toluene (16 μl) was injected *via* syringe. Every 30 s, an UV/VIS was recorded. When the spectra staid unchanged for 30 min (soln. *B*), a 50-fold excess of the oxidizing agent was added. However, no further change in the absorption could be detected. UV/VIS (toluene, soln. *A*): 606 (9), 570 (6), 512 (14), 412 (100), 332 (sh, 31). UV/VIS (toluene, soln. *B*): 758 (1), 684 (3), 580 (5), 510 (11), 418 (100), 344 (sh, 32).

*{5,15-{{[4-(*tert*-Butyl)-2-mercaptophen-1,3-ylene]bis(trimethyleneoxy)}di(phen-2,1-ylene)}-10,20-{[(hydroxy)undecamethylenedioxy]di(phen-2,1-ylene)}porphyrinato}iron(III)* (**4**). In a flame-dried flask, flooded with Ar, **2** (7.2 mg, 6.4 μmol) in toluene (2.0 ml) was cooled to −78° (CO_2/acetone) before 16.0 mM $C_6F_5(IO)$ in toluene (0.4 ml) was injected *via* syringe. The mixture was kept at −78° for further 3 h and then slowly warmed up to r.t. Evaporation and prep. TLC (toluene/THF 1:1, R_f *ca.* 0.3) in the glove box gave **4** (5.2 mg, 71%). Deeply violet glass. UV/VIS (toluene): 578 (3), 510 (8), 418 (100), 344 (sh, 31). ^{1}H-NMR (CD_2Cl_2, 20°): 81, 77, 71 (3 br. *s*, H–C(*β*)'s of pyrrols); 15.2, 14.3, 13.2, 12.1, 11.9, 11.2, 10.3, 9.9, 8.9, 8.0, 6.1 (11 br. *s*, Ph and bridge protons, not assigned); 2 to −3.5 (several br. *s* aliph. bridge protons). EI-MS[3]: 1145.5 (8), 1144.5 (12, $[M - H]^+$), 1133.5 (7), 1132.5 (25), 1131.5 (57), 1130.5 (100, $[M - O + H]^+$), 1129.4 (74), 1128.4 (34), 1127.5 (16), 1126.5 (15), 1100.4 (7), 1099.6 (16), 1098.5 (22, $[M - S - O + H]^+$), 1097.5 (5), 1096.5 (4), 1073.5 (5), 1072.5 (6, $[M - O - (t\text{-Bu})]^+$), 976.4 (4, $[M$ – alkane bridge + $H]^+$), 885.4 (8), 884.4 (13, $[M - O$ – S-cont. bridge + $H]^+$), 882.3 (4), 731.2 (4), 730.2 (7, $[M - O$ – alkane bridge – S-cont. bridge + $H]^+$).

*{5,15-{{[4-(*tert*-Butyl)-2-mercaptophen-1,3-ylene]bis(trimethyleneoxy)}di(phen-2,1-ylene)}-10,20-{[(trimethylsiloxy)undecamethylenedioxy]di(phen-2,1-ylene)}porphyrinato}iron(III)* (**5**). In the glove box, **4** (1.0 mg, 0.9 μmol) dissolved in DMF (1.0 ml) was stirred with 4-(trimethylsilyloxy)pent-3-en-2-one (0.5 ml, 2.7 mmol; *Fluka*) for 15 h at r.t. Evaporation gave **5** (*ca.* 1.0 mg), still slightly contaminated with **4**. The mixture could not be chromatographed on SiO_2 without decomposition. EI-MS: 1217.3 (4, M^+), 1147.1 (5), 1146.1 (9), 1145.1 (15, $[M - Me_3Si + H]^+$), 1144.1 (12), 1143.1 (5), 1135.1 (6), 1134.1 (11), 1133.1 (34), 1132.1 (70), 1131.1 (100, $[M - Me_3SiO + H]^+$), 1130.1 (40), 1129.1 (28), 1128.1 (6), 1102.1 (5), 1101.1 (15), 1100.1 (31), 1099.1 (40, $[M - S - Me_3SiO + H]^+$), 1098.1 (9), 887.0 (12), 886.0 (37), 885.0 (59, $[M$ – S-cont. bridge – $Me_3SiO + H]^+$), 884.0 (16), 883.0 (9), 732.7 (9), 731.7 (10), 730.7 (12, $[M$ – S-cont. bridge – alkane bridge + $H]^+$), 729.7 (5).

4. *Reactions of* **2** *with Molecular Oxygen. UV Experiments.* As described in *Chapt. 3*, the soln. *A* (**2**) was prepared. Then a balloon with dry O_2 (*AGA*) was attached *via* a cannula. A UV/VIS was recorded every 30 min. When no reaction was detected after 2 d, the setup was slowly warmed up (30°/d). At +10°, a bathochromic shift of the *Soret* band from 412 to 418 nm was observed (soln. *B*). After 3 h, the reaction was complete, and no further change could be detected when warming up to +40° or cooling to −60° again. UV/VIS (toluene, soln. *A*): 606 (9), 570 (6), 512 (14), 412 (100), 332 (sh, 31). UV/VIS (toluene, soln. *B*): 684 (1), 642 (1), 586 (2), 508 (10), 418 (100), 344 (sh, 31).

*{5,15-{{[4-(*tert*-Butyl)-2-sulfonatophen-1,3-ylene]bis(trimethyleneoxy)}di(phen-2,1-ylene)}-10,20-[(undecamethylenedioxy)di(phen-2,1-ylene)]porphyrinato}iron(III)* (**6**). A soln. of **2** (2.1 mg, 1.9 μmol) in toluene (3 ml) under Ar was cooled to 8° and connected *via* cannula to a balloon filled with dry O_2. After 4 h, the reaction had reached completion (UV), and the solvent was evaporated: **6** (2.2 mg, quant.), pure by TLC (toluene/THF 1:1) and HPLC (*Bischoff Nucleosorb 100*, 5 μm; MeOH/CH_2Cl_2/hexane 0.5:20:80). UV/VIS (toluene): 650 (3), 581 (4), 510 (12), 416 (100), 380 (sh, 44). EI-MS: 1181 (5), 1180 (16), 1179 (43), 1178 (86), 1177 (100, M^+), 1176 (15), 1175 (15), 1098 (6, $[M - SO_3 + H]^+$), 1025 (6), 1024 (9, $[M$ – alkane bridge + $H]^+$), 884 (7, $[M$ – S-cont. bridge + $H]^+$).

4 *and* *{5,15-{{[4-(*tert*-Butyl)-2-sulfonatophen-1,3-ylene]bis(trimethyleneoxy)}di(phen-2,1-ylene)}-10,20-{[(hydroxy)undecamethylenedioxy]di(phen-2,1-ylene)}porphyrinato}iron(III)* (**7**). A soln. of **2** (2.5 mg, 2.2 μmol) in toluene (50 ml) under Ar was cooled to 8° and was connected *via* cannula to a balloon filled with dry O_2. After 4 h, the reaction had reached completion, and the solvent was evaporated. A mixture **7**/**4** (2.5 mg) was obtained which could not be separated. UV/VIS (toluene): 646 (4), 578 (6), 510 (14), 418 (100), 342 (sh, 35). ^{1}H-NMR (CD_2Cl_2, 12°): 78.2, 72.6, 71.8 (3 br. *s*, H–C(*β*)'s of pyrrols); 16.2, 15.3, 14.1, 13.2, 13.1, 12.5, 11.9, 11.2, 10.8, 7.8, 7.2, 4.4 (12 br. *s*, Ph and bridge protons, not assigned); 0 to −9 (several br. *s*, aliph. bridge protons). EI-MS (**4**/**7**): 1196.2 (4), 1195.2 (7, $[M(7) + H]^+$), 1194.2 (6), 1180.2 (20), 1179.1 (44), 1178.2 (50, $[M(7) - O]^+$), 1177.2 (8), 1147.1 (8), 1146.1 (10), 1145.1 (12, $[M(4) - H]^+$), 1144.1 (5), 1135.1 (5), 1134.1 (11), 1133.1 (34), 1132.1 (70), 1131.1 (100, $[M(4) - O + H]^+$), 1130.1 (40), 1129.1 (28), 1128.1 (7), 1102.1 (5), 1101.1 (15), 1100.1 (31), 1099.1 (40, $[M(7) - SO_3 + H]^+$, $[M(4) - S + H]^+$), 1098.1 (7), 1073.1 (5, $[M(4) - O - (t\text{-Bu})]^+$), 1025.0 (5, $[M(7)$ – alkane

[3]) Isotope distributions are in agreement with calculated values (*Finnigan SSQ700*, ICIS software).

bridge + H]$^+$), 887.0 (13), 886.0 (37), 885.0 (59, [*M*(**4**, **7**) – S-cont. bridge – O + H]$^+$), 883.0 (10), 732.7 (8), 730.7 (12, [*M*(**4**, **7**) – alkane bridge – S-cont. bridge + H]$^+$).

The reaction was repeated using a 100-fold molar excess of $^{18}O_2$: (^{18}O)-**4**/($^{18}O_4$)-**7**. EI-MS: 1203.8 (9), 1202.8 (26), 1201.8 (61), 1200.8 (82, *M*((^{18}O)-**7**)$^{+\cdot}$), 1199.8 (21), 1198.8 (14), 1185.8 (16), 1184.8 (39), 1183.8 (83), 1182.8 (100, [*M*(($^{18}O_4$)-**7**) – ^{18}O]$^+$), 1181.8 (19), 1180.8 (19), 1151.9 (6), 1150.9 (7), 1145.9 (6, [*M*((^{18}O)-**4**) – H]$^+$), 1144.9 (7), 1143.9 (11), 1133.9 (6), 1132.9 (7), 1131.9 (12), 1130.9 (25), 1129.9 (35, [*M*((^{18}O)-**4**) – ^{18}O + H]$^+$), 1128.9 (31), 1127.9 (6), 1116.9 (5), 1115.9 (9), 1114.9 (7), 1100.0 (9), 1098.9 (20), 1097.9 (28, [*M*((^{18}O)-**4**) – ^{18}O – S + H$^+$, *M* (($^{18}O_4$)-**7**) – ^{18}O – S$^{18}O_3$ + H]$^+$), 1096.9 (5), 1095.9 (5), 1090.0 (9), 1047.8 (7), 1029.8 (9, [*M*((^{18}O)-**7**) – alkane bridge + H]$^+$), 885.9 (5), 884.9 (19), 883.9 (31, [*M*((^{18}O)-**4**, (^{18}O)-**7**) – S-cont. bridge – O]$^+$), 882.9 (5), 732.9 (9), 731.9 (13), 730.9 (10), 729.9 (16, [*M*((^{18}O)-**4**,($^{18}O_4$)-**7**) – alkane bridge – S-cont. bridge]$^+$), 727.9 (6).

Trimethylsilylation of (^{18}O)-**4**/($^{18}O_4$)-**7**. A 1:4 mixture (^{18}O)-**4**/($^{18}O_4$)-**7** (*ca.* 1 mg, 0.9 µmol) in DMF (1 ml) was stirred with 4-(trimethylsilyloxy)pent-3-ene-2-one (0.5 ml, 2.7 mmol; *Fluka*) in the glove box for 12 h at r.t. Evaporation afforded a 1:4 mixture (^{18}O)-**5**/($^{18}O_4$)-**8** (*ca.* 1 mg) which could not be separated without decomposition. EI-MS: 1274.3 (5, *M*(($^{18}O_4$)-**8**)$^{+\cdot}$), 1220.4 (4, *M*((^{18}O)-**5**)$^{+\cdot}$), 1204.4 (10), 1203.4 (37), 1202.4 (55), 1201.4 (60, [*M*(($^{18}O_4$)-**8**) – Me_3Si]$^+$), 1200.1 (15), 1199.4 (13), 1198.4 (6), 1187.4 (7), 1186.5 (14), 1185.5 (39), 1184.5 (72), 1183.5 (100, [*M*(($^{18}O_4$)-**8**) – Me_3Si – ^{18}O]$^+$), 1182.5 (15), 1181.5 (13), 1146.5 (19), 1145.5 (33), 1144.5 (43), 1135.5 (17), 1132.5 (32), 1131.5 (62), 1130.5 (79, [*M*((^{18}O)-**5**) – Mc_3Si – ^{18}O + H]$^+$), 1129.5 (25), 1116.5 (20, [*M*(($^{18}O_4$)-**8**) – Me_3Si – S$^{18}O_3$ + H], [*M*((^{18}O)-**5**) – Me_3Si – S + H]$^+$), 1099.5 (21), 1098.5 (25, [*M*(($^{18}O_4$)-**8**) – Me_3Si – ^{18}O – S$^{18}O_3$ + H]$^+$, [*M*((^{18}O)-**5**) – Me_3Si – ^{18}O – S + H]$^+$), 1031.5 (7, [*M*(($^{18}O_4$)-**8**) – Me_3Si – ^{18}O – alkane bridge + H]$^+$), 886.5 (23), 885.4 (56), 884.5 (93, [*M*((^{18}O)-**5**,($^{18}O_4$)-**8**) – Me_3Si – ^{18}O – S-cont. bridge + H]$^+$), 732.3 (21), 730.3 (23, [*M*((^{18}O)-**5**,($^{18}O_4$)-**8**) – alkane bridge-S-cont. bridge + H]$^+$), 729.3 (10).

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