# Synthetic haems as mimics for high valent intermediates in haemoprotein catalysed oxidations. Synthesis and oxidation of chloro-7,8,17,18-tetracyano-5,10,15,20-tetraphenylporphyrinatoiron(III), a haem which contains strongly electron-withdrawing groups in the β-pyrrole positions

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**Abstract:** The haem chloro-7,8,17,18-tetracyano-5,10,15,20-tetraphenylporphyrinato- iron(III), which contains strongly electron withdrawing groups in the β-pyrrole positions, has been synthesised as a possible substrate for oxidations which mimic the formation of high valent haems in peroxidase and cytochrome p-450 catalysed reactions. The porphyrin was synthesised by reacting the copper(II) complex of the corresponding tetrabromoporphyrin with CuCN in quinoline. Treatment of the haem with m-chloroperbenzoic acid gave an oxidation product which is stable in solution at room temperature.

## INTRODUCTION

Cytochrome P-450 and the peroxidases are groups of haem containing enzymes which catalyse the oxidation of substrates by molecular oxygen and by peroxides respectively (1, 2). Both groups of enzymes contain iron(III)-protoporphyrin IX as prosthetic group but differ in the nature of the axial ligand (cysteinate in the case of P-450, histidine in the case of many peroxidases e.g. horseradish peroxidase). The intermediates in the catalytic cycles of some of the peroxidases are Compound I which is two oxidation equivalents above the resting enzyme and contains an oxoiron(IV)-porphyrin cation radical and Compound II, resulting from the 1 e⁻ reduction of Compound I and which contains an oxoiron(IV)-porphyrin, Fig. 1. In contrast the nature
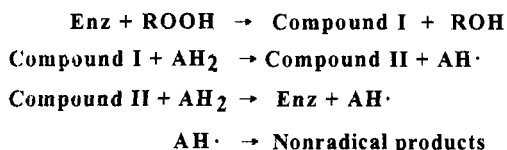
Enz + ROOH → Compound I + ROH
Compound I + AH₂ → Compound II + AH·
Compound II + AH₂ → Enz + AH·
AH· → Nonradical products

Fig. 1 Catalytic cycle of many peroxidase-catalysed reactions.



Fig. 2 Catalytic cycle of cytochrome P-450

of the active oxidant intermediate in the cytochrome P-450 cycle is not well established. This results from the heterolytic breakdown of the haem-bound peroxo ligand with one oxygen atom being displaced as water, Fig. 2. The remaining oxygen remains linked to the iron before transfer to substrate and whether the two oxidising equivalents of the peroxide are transferred to the iron giving an oxoiron(V)-porphyrin as shown in Fig. 2 or one to the iron and one to the porphyrin giving an oxoiron(IV)-porphyrin cation radical is still contentious.

In recent years many model haem systems which give oxoiron(IV)-porphyrin cation radicals or oxoiron(V)-porphyrins have been investigated as mimics for these oxidations. These include the production and characterisation of oxoiron(IV)-porphyrin cation radical species from haem 1, in dichloromethane at -46°C, (3) and from haem 2(a) in THF or DMF at -50 °C (4). The oxidation product from the latter species contains solvent as axial ligand and this is displaceable by 1-methylimidazole. Oxidation of haem 3 with m-chloroperbenzoic acid in dichloromethane gave an oxoiron(IV)-porphyrin cation radical species which is stable in solution at 8°C (5). Addition of methanol to a solution of haem 2(b) in dichloromethane which contained m- chlorobenzoic acid and which had been treated with p-nitroperbenzoic acid gave a species which on the basis of spectroscopic evidence (UV-VIS, $^2$H NMR and ESR) is claimed to be an oxoiron(V)-porphyrin with axially coordinated methanol or methoxide (6). An oxoiron(V) porphyrin with axially coordinated fluoride ligand which is stable in solution at room temperature has been identified as the product of oxidation of haem 2(c) in dichloromethane (7). In these examples the meso-aryl groups of the porphyrin contain bulky or bulky/ electronegative substituents and in the case of haem 3 there are also electronegative substituents (phenyl) in the β-pyrrole positions. Mesotetraphenolichaems have also been investigated as models for formation of high-valent haem intermediates but oxidation of these occurs on the porphyrin giving quinonoid products (8-10). We decided to synthesise porphyrins having very electronegative substituents on the β-pyrrole positions and herein describe the synthesis of 7,8,17,18-tetracyano-5,10,15,20-tetraphenylporphyrin, $H_2$(TPPCN$_4$), its iron(III) complex and its oxidation.



1                                2 (a) X = Cl, Y = OH                                3

(b) X = Cl, Y = m-chlorobenzoate

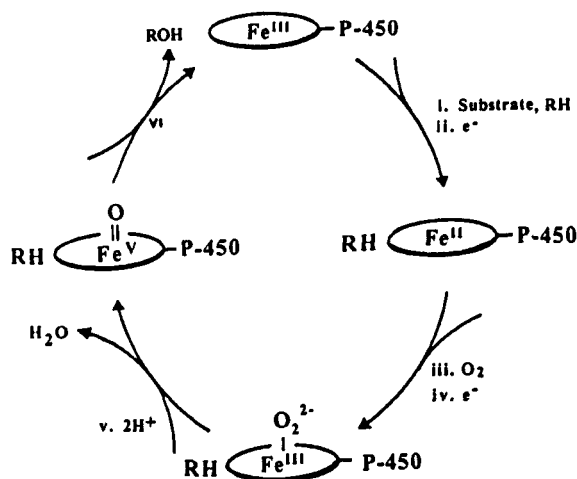(c) X = Y = F

## RESULTS AND DISCUSSION

### Porphyrin and Metalloporphyrin Synthesis

The desired haem containing 7,8,17,18-tetracyano-5,10,15,20-tetraphenylporphyrin, $H_2(TPPCN_4)$, was synthesised from 7,8,17,18-tetrabromo-5,10,15,20-tetraphenylporphyrin, $H_2(TPPBr_4)$, as shown in Fig. 3. The tetrabromotetraphenylporphyrin, **4**, was synthesised in 60% yield from tetraphenylporphyrin using N-bromosuccinimide in chloroform and purified by column chromatography on silica 100. In an earlier synthesis of this compound the structure of the product was misassigned as the 2,7,12,17-tetrabromo isomer, having one Br substituent on each pyrrole/pyrrolenic ring (11). Subsequently the correct structure was assigned on the basis of $^1H$ n.m.r. spectroscopy and nature of reaction products e.g. nucleophilic substitution of Br groups by o-benzenedithiolate to give antipodal ring extended products (12). The regiospecificity of this reaction has been attributed to the intermediacy of a bond-fixed 7,8-dibromochlorin having an aromatic delocalisation pathway which imposes double bond character on the antipodal 17,18 positions. The $^1H$ NMR spectrum of the porphyrin is shown in Fig. 4(a). The iron(III) complex of this porphyrin was synthesised in 95% yield from $FeCl_2 \cdot 6H_2O$ and porphyrin in DMF under reflux.



4            5            6
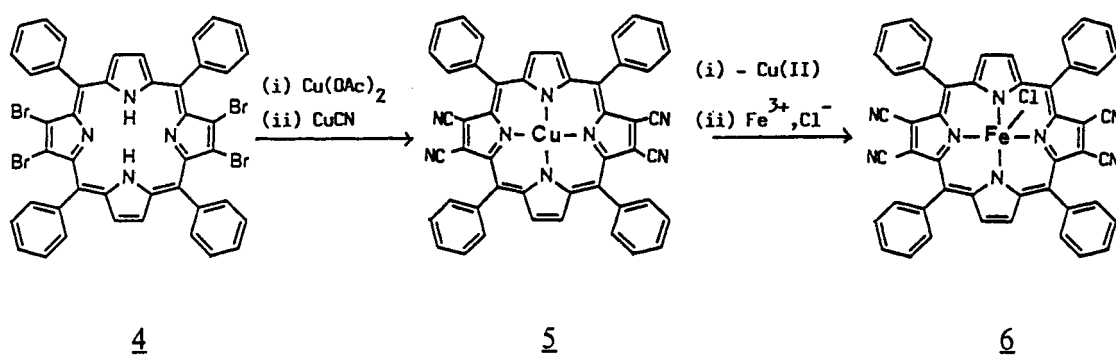
Fig. 3 Synthesis of $Fe(TPPCN_4)Cl$

Several different methods were employed in order to obtain the tetracyanoporphyrin from the tetrabromo derivative. This involved the reaction of the porphyrin alone or several of its metal complexes with various sources of cyanide in different solvents. The method giving the highest yield however involved reaction between the copper(II)-porphyrin and CuCN in quinoline. This reaction may involve a copper(II)-promoted nucleophilic substitution, a reaction type which is well documented in the literature (13). The use of quinoline as solvent permits high reaction temperatures and also stabilises copper(I), the carrier of the CN⁻ nucleophile. The major products of this reaction are the copper(II) complexes of 7,8,17- tricyano-5,10,15,20-tetraphenylporphyrin, and $H_2(TPPCN_4)$, **5**, which were obtained in 7.7% and 23.1% yield respectively, following purification by column chromatography on silica. Demetallation of the complexes proved difficult and required treatment of chloroform solutions with concentrated $H_2SO_4$. The sulphuric acid layer containing protonated porphyrin was diluted, neutralised, extracted into chloroform and purified by column chromatography. The $^1H$ NMR spectrum of $H_2(TPPCN_4)$ is shown in Fig. 4(b). Reaction of $H_2(TPPCN_4)$ with $Fe(ClO_4)_2 \cdot 6H_2O$ in refluxing DMF solution followed by partitioning between $CHCl_3/H_2O$ and washing the organic layer with HCl gave the haem $Fe^{III}(TPPCN_4)Cl$.
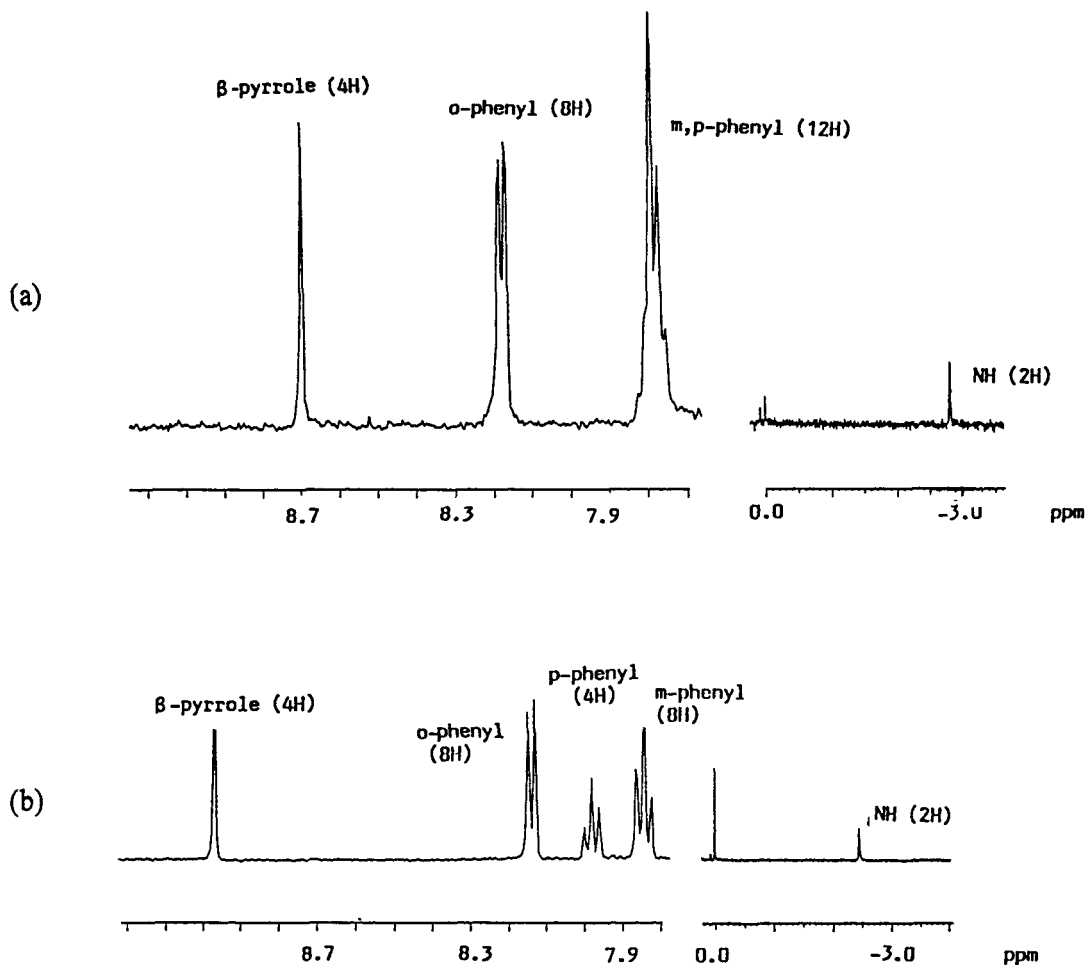
Fig. 4 $^1$H NMR spectra (400 MHz) of (a) H$_2$(TPPBr$_4$) and (b) H$_2$(TPPCN$_4$) in CDCl$_3$

## Oxidation of Fe$^{III}$(TPPBr$_4$)Cl and Fe$^{III}$(TPPCN$_4$)Cl

The attempted oxidation of Fe$^{III}$(TPPBr$_4$)Cl with m-chloroperbenzoic acid (m-CPBA) in dichloromethane resulted in decomposition of the haem and bleaching of the solution. The reaction of Fe$^{III}$(TPPCN$_4$)Cl with m-CPBA caused oxidation and gave a product which is indefinitely stable at room temperature and the UV-VIS spectrum of which is shown in Fig. 5. The oxidation reaction occurs cleanly and the spectral changes are characterised by isosbestic points at ~480, 560 and 650 nm, Fig. 6. A prominent feature of the spectrum of the product is an absorption band at 545 nm. This is similar in position and intensity to a band in the spectrum of the oxidation product (THF) TPP(2,6-Cl) Fe$^{IV}$=O, obtained from haem 2(a) and which is claimed to be characteristic of the ferryl oxidation state (ref. 4). This suggests that the oxidation product we obtained is (TPPCN$_4$) Fe$^{IV}$=O. The product is unlikely to contain a porphyrin cation radical since the formation of these is usually accompanied by a much greater reduction in intensity of the Soret band and in many (5,14) but not all (15,16) cases the Soret band of the product is severely flattened. In support of this it is to be expected that the presence of strongly electron withdrawing groups in the β-pyrrole positions would

strongly destabilise porphyrin cation radicals and mitigate against their formation. The possibility of the product being the $\mu$-oxo dimer [TPPCN4Fe]2O was eliminated since this was synthesised separately and found to have a completely different spectrum. Treatment of the oxidation product with p-cresol in dichloromethane caused slow reduction and following treatment with dilute HCl the absorption spectrum obtained was almost identical to that of Fe$^{III}$(TPPCN4)Cl. In this reaction the oxidation product is reduced by p-cresol back to Fe$^{III}$(TPPCN4), possibly with p-cresolate as an axial ligand, and this was replaced with chloride in the presence of dilute HCl. Attempts to obtain the oxidation product in higher concentration in order to further characterise it and to isolate it as a solid have so far been unsuccessful but work on this is in progress.
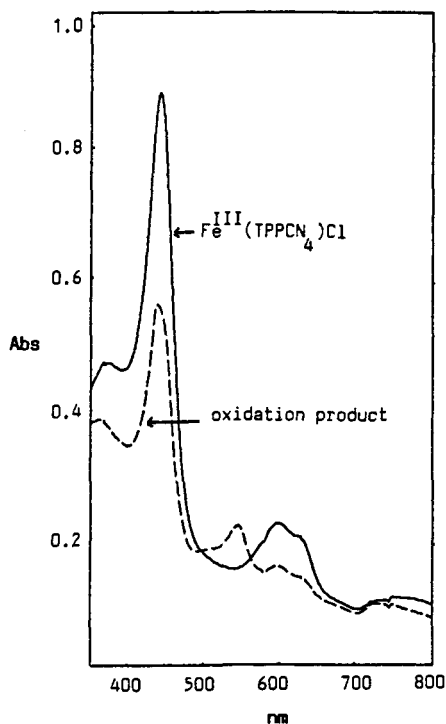


Fig. 5 UV-VIS spectrum of Fe(TPPCN4)Cl
and its oxidation product in CH2Cl2
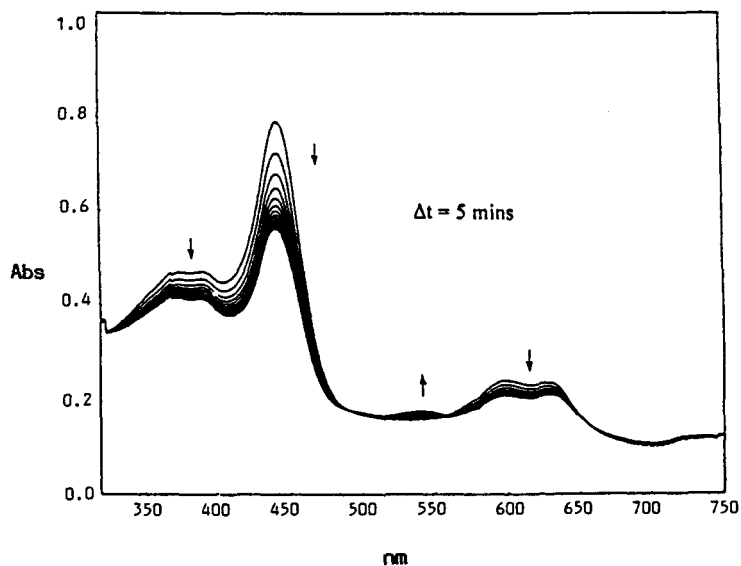
Fig. 6 UV-VIS spectral changes during the
oxidation of Fe(TPPCN4)Cl in CH2Cl2

## EXPERIMENTAL

### Synthesis

The synthesis of the porphyrins and metalloporphyrins are described below and details of their UV-VIS spectra are given in the Table.

*H2(TPPBr4).* To a refluxing solution of meso-5,10,15,20-tetraphenylporphyrin, H2TPP (0.9 g; 1.46 x 10$^{-3}$ mol) in CHCl3 was added N-bromosuccinimide (1.6 g; 9.0 x 10$^{-3}$ mol). The resulting solution was allowed to reflux for 45 minutes then cooled to room temperature and neutralised with an excess of triethylamine. The contents of the reaction vessel were washed three times with deionised water, then dried over anhydrous sodium sulphate and the chloroform removed on a rotary evaporator. The impure porphyrin was purified by chromatography on a silica 100 column using a cyclohexane-chloroform (70:30) eluent.

The first three fractions (green, pink and brown) were impurities and were discarded and the fourth fraction was orange in colour and from its electronic spectrum it was identified as $H_2(TPPBr_3)$. The fifth and major fraction containing $H_2(TPPBr_4)$ was collected. Yield 0.82 g, 60%. Found: C, 56.7; H, 3.3; N, 5.6; Br, 34.6. $C_{44}H_{26}N_4Br_4$, requires C, 56.8; H, 2.8; N, 6.0; Br, 34.4%.

*$Fe^{III}(TPPBr_4)Cl$.* To a refluxing solution of $H_2(TPPBr_4)$ (0.5 g, $5.37 \times 10^{-4}$ mol) in DMF (30 ml) was added $FeCl_2 \cdot 6H_2O$ (0.13 g; $5.53 \times 10^{-4}$ mol) and the resulting solution allowed to reflux for five hours after which time a UV-VIS absorption spectrum showed the reaction was complete. The reaction solution was allowed to cool to room temperature and then partitioned between chloroform-deionised water (50:50). The organic layer was collected and washed 10 times with deionised water to completely remove any traces of DMF, then washed with 4 M HCl and finally dried over sodium sulphate and the solvent removed on a rotary evaporator. Yield 52 mg, 95.0%. Found: C, 51.5; H, 2.9; N, 5.1; Br, 30.5; Cl, 3.2; Fe, 5.4. $FeC_{44}H_{24}N_4Br_4Cl$ requires C, 51.8; H, 2.4; N, 5.5; Br, 31.3; Cl, 3.5; Fe, 5.5 %.

*$Cu^{II}(TPPBr_4)$.* A solution of $Cu(OAc)_2 \cdot H_2O$ (1.5 g, $7.5 \times 10^{-3}$ mol) in methanol (20 ml) was added to a refluxing solution of $H_2(TPPBr_4)$ (0.6 g; $6.44 \times 10^{-4}$ mol) in $CHCl_3$ (200 ml). The resulting solution was allowed to reflux for 30 minutes to ensure complete complex formation. The reaction mixture was cooled to room temperature, washed three times with deionised water then dried over anhydrous sodium sulphate and finally taken to dryness on a rotary evaporator. Yield 0.60 g, 93.9%. Found: C, 53.0; H, 2.5; N, 5.5; Br, 32.1; Cu, 6.1. $CuC_{44}H_{24}N_4Br_4$ requires C, 53.3; H, 2.4; N, 5.5; Br, 32.2; Cu, 6.4%.

*$Cu^{II}(TPPCN_4)$ and $Cu^{II}(TPPCN_3)$.* CuCN (0.6 g; $6.7 \times 10^{-3}$ mol) was added to a warm, stirring solution of $Cu^{II}(TPPBr_4)$ (0.6 g; $6.4 \times 10^{-4}$ mol) in quinoline (110 ml). The temperature was gradually raised to 210°C and the solution refluxed for 30 minutes, then cooled to room temperature. After the addition of chloroform (200 ml) the solution was washed with an equal volume of 4 M HCl to remove quinoline (separation of organic and aqueous layers takes approx. 24 hours). The organic layer was washed a further 10 times with an equal volume of 4M HCl, then dried over anhydrous sodium sulphate and the chloroform removed on a rotary evaporator. The residue (650 mg) was redissolved in chloroform and purified on a silica 100 column using chloroform as eluent. The first three fractions from the column were discarded and the fourth and fifth, both coloured green and containing $Cu^{II}(TPPCN_3)$ and $Cu^{II}(TPPCN_4)$ respectively were collected and taken to dryness. Yield of $Cu^{II}(TPPCN_4) \cdot$quinoline$\cdot^4/_5CHCl_3$ 148 mg, 23.1%. Found: C, 69.5; H, 3.7; N, 11.9; Cu, 6.5. $CuC_{48}H_{24}N_8 \cdot C_9H_7N \cdot ^4/_5CHCl_3$ requires C, 69.3; H, 3.2; N, 12.6; Cu, 6.3. Yield of $Cu^{II}(TPPCN_3) \cdot$quinoline$\cdot CHCl_3$ 49 mg, 7.7%. Found: C, 71.2; H, 4.2; N, 10.6; Cu, 6.0. $CuC_{47}H_{25}N_7 \cdot C_9H_7N \cdot CHCl_3$ requires C, 68.5; H, 3.3; N, 11.2; Cu, 6.4%.

*$H_2(TPPCN_4)$.* $Cu(TPCN_4) \cdot$quinoline$\cdot^4/_5CHCl_3$ (148 mg; $1.5 \times 10^{-4}$ mol) was dissolved in chloroform and shaken with conc. $H_2SO_4$ (50 ml). The $H_2SO_4$ layer was poured onto crushed ice and neutralised with excess $Et_3N$. The porphyrin was extracted into chloroform, the solution washed three times with deionised water, dried over anhydrous sodium sulphate and finally taken to dryness. The porphyrin was purified on a silica 100 column with chloroform as eluent. The main fraction was collected and reduced to dryness. Yield 85 mg, 62.6%. Found: C, 76.9; H, 4.1; N, 14.0. $C_{48}H_{26}N_8 \cdot C_9H_7N \cdot ^1/_2CHCl_3$ requires C, 76.4; H, 3.4; N, 13.5. The quinoline was removed from the porphyrin by washing a chloroform solution several

**Table** UV-VIS spectra of porphyrins and metalloporphyrins in chloroform

| Porphyrin/ metalloporphyrin | $\lambda_{max}$ /nm,(log e) |
|---|---|
| $H_2(TPPBr_4)$ | 437.5 (5.38), 535.7 (4.23), 617.2 (3.58), 683.2 (3.91) |
| $Cu^{II}(TPPBr_4)$ | 427.7 (4.96), 554.4 (3.91), 593.6 (3.68) |
| $Fe^{III}(TPPBr_4)Cl$ | 432.7 (4.54), 520.0 (3.71) |
| $H_2(TPPCN_4)$ | 438.1 (5.23), 449.5 (5.26), 552.9 (4.08), 597.9 (4.29), 667.3 (3.98), 728.8 (4.36) |
| $H_2(TPPCN_3)$ | 441.6 (5.27), 542.9 (4.16), 585.4 (4.10), 645.6 (3.94), |
| $Cu^{II}(TPPCN_4)$ | 437.9 (5.29), 636.3 (4.73) |
| $Cu^{II}(TPPCN_3)$ | 435.8 (5.16), 530.6 (3.76), 571.2 (3.94), 617.9 (4.43) |
| $Fe^{III}(TPPCN_4)Cl$ | 391.0 (4.44), 443.6 (4.68), 600.1 (4.09), 626.4 (4.08) |

times with 0.1 M aqueous HCl. The porphyrin $H_2(TPPCN_3)$ was obtained similarly from its copper(II) complex. Found: C, 75.9; H, 4.0; N, 11.5%. $C_{47}H_{27}N_7 \cdot 2C_9H_7N \cdot CHCl_3$ requires C, 74.3; H, 4.0; N, 11.8%.

***Fe^{III}(TPPCN_4)Cl.*** $Fe(ClO_4)_2 \cdot 6H_2O$ (0.2 g, 5.5 x $10^{-4}$ mol) was added to a refluxing solution of $H_2(TPPCN_4)$ (50 mg, 7.0 x $10^{-5}$ mol) in DMF (30 ml). After 30 minutes complex formation was complete (UV-VIS) and the solution was allowed to cool to room temperature and then partitioned between 50:50 deionised water and chloroform. The organic layer was washed a further 10 times with deionised water, then washed with 4 M HCl, dried over anhydrous $Na_2SO_4$ and taken to dryness on a rotary evaporator. Yield 51 mg, 78.9%. Found: C, 64.0; H, 2.9; N, 12.8; Fe, 5.9; Cl, 14.5. $Fe^{III}C_{48}H_{24}N_8Cl \cdot CHCl_3$ requires C, 63.7; H, 2.7; N, 12.1; Fe, 6.0; Cl, 15.3%.

***[(TPPCN_4)Fe^{III}]_2O.*** This μ-oxo dimer was prepared by literature methods (17) in order to show that it was not the product of oxidation of haem by m-CPBA. A solution of $Fe^{III}TPPCN_4$ (50 mg, 6.5 x $10^{-5}$ mol) in dichloromethane (20 ml) was washed with 20% aqueous KOH (20 ml). The organic layer, which had changed from green to brown, was dried over sodium sulphate and reduced to dryness. UV-VIS in $CHCl_3$: $\lambda_{max}$ 450, 665 nm.

## Oxidation of Fe(TPPCN₄)Cl

A solution of m-CPBA (20 μl, 0.02 M) in $CH_2Cl_2$ was injected into a solution of haem (2 ml, 2.5 x $10^{-5}$ M) in $CH_2Cl_2$ at room temperature and the UV-VIS spectrum was recorded every 5 minutes (Fig.6).

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