The Synthesis of a Cytochrome c Analogue

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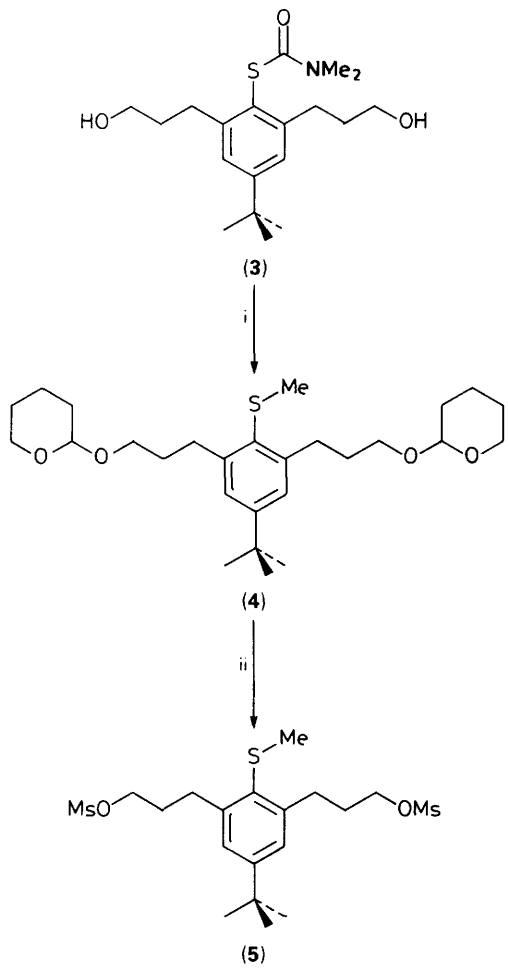
The doubly-bridged iron porphyrins (**1**) and (**8**) have been synthesized carrying a covalently bound, proximal S–Me ligand and an imidazole co-ordinating to the iron; UV and ^{1}H NMR spectroscopy and electrochemistry show that these compounds are close analogues of cytochrome c.

The class I cytochromes c are redox proteins acting in the periplasma as one-electron donors to a terminal oxidizing species, which may be a photo-oxidized chlorophyll or a respiratory enzyme. The centre of redox-chemistry is a modified iron protoporphyrin IX covalently bound to the protein *via* thioether linkages.[1] It is believed that the presence, distance, and orientation of two axial ligands, Met (80) and His (18), to iron triggers the high redox potential in the range 0—500 mV.

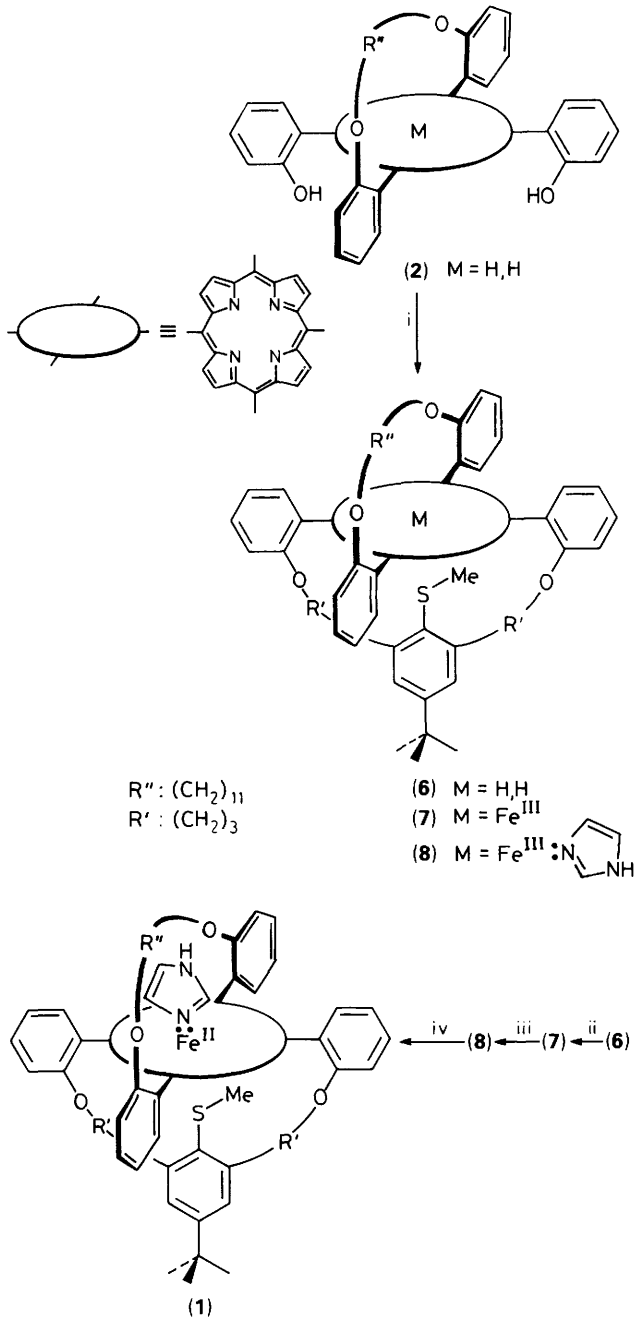
In order to understand the fine-tuning displayed by these



Scheme 1. *Reagents*: i, 3,4-dihydro-2*H*-pyran(DHP)H$^+$, then NaOEt, then MeI; ii, pyridinium toluene-*p*-sulphonate, then MsCl–NEt_3 (Ms = mesyl).

ligands several attempts have been made (i) to prepare variants of cytochrome c by site-directed mutagenesis[2] and (ii) to synthesize porphyrins as models of the active site.[3—8] In contrast to the former the latter approach seems attractive since different ligands can be attached, and their orientation to iron systematically manipulated by synthesis and easily computed. However there is a general synthetic problem owing to the poor ligand binding of thioethers to iron as compared to *e.g.*, imidazoles, which has hampered all the previous attempts.[3—9] In order to circumvent this difficulty (**1**) was envisaged to be an appropriate target since the S–Me group is 'forced' into co-ordination to iron by covalent attachment to the porphyrin and the sixth ligand site is free to accept an even stronger ligand.

Using our experience in the synthesis of an active-site model of cytochrome P-450,[10] the doubly-bridged iron porphyrin (**1**) was prepared starting with the readily available monobridged tetraphenylporphyrin (**2**) and the thiocarbamate (**3**).[10] The OH groups of (**3**) were first protected, the thiocarbamate hydrolysed, and the sulphur methylated to give (**4**) in 75% overall yield from (**3**), Scheme 1. Removal of the THP groups and subsequent mesylation furnished (**5**), which proved to be less prone to intramolecular cyclization then the corresponding dibromide. Condensation of the monobridged dihydroxytetraphenylporphyrin (**2**) with (**5**) in the presence of Cs_2CO_3 under diluted conditions in dimethylformamide (DMF)



Scheme 2. *Reagents*: i, (**5**), $CsCO_3$, DMF; ii, $FeBr_2$; iii, imidazole; iv, $Na_2S_2O_4$.

yielded the doubly-bridged porphyrin (**6**) in 17% yield after HPLC purification, Scheme 2.

Compound (**6**) displays spectroscopical data which are characteristic of a metal-free porphyrin ligand with a Soret band at 422 nm and satellites at 516, 550, 592, and 654 nm, and an ^1H NMR spectrum dominated by the aromatic ring current $\delta_H(CDCl_3)$ −2.20 (S–C*H*$_3$). Iron insertion [$FeBr_2$, 4-*N*,*N*-dimethylaminopyridine (DMAP)–DMF, glove-box] yielded the Fe^{III} porphyrin (**7**) in 95% yield, which turned out to be resistent to dithionite reduction. After addition of a slight excess of imidazole the six-co-ordinate Fe^{III} complex (**8**) was formed quantitatively displaying characteristic UV absorptions at 424 and 558 nm. Reduction of (**8**) with $Na_2S_2O_4$ in a two-phase system (toluene–water) proceeded quantitatively

(5 min, 25 °C) to yield the desired Fe^{II} porphyrin (**1**). The change observed in the UV spectrum during this transformation is very similar to that of native cytochrome c: the long-wave absorption of (**8**) at 558 nm is split into an α- and a β-band at 538 and 570 nm (α : β 0.33), and the Soret-band is shifted by 6 nm from 424 to 430 nm. The ^1H NMR spectrum of (**1**), recorded in [$^2H_8$] toluene/D_2O sat. $Na_2S_2O_4$, displays resonances between δ −5.14 and 22 in agreement with a low-spin Fe^{II} porphyrin. Most remarkable is the singlet for the S–CH_3 group at δ −3.56, which appears very close to the position reported by Wüthrich[11] for the corresponding protons of Met(80) of ferrocytochrome c-551 from *Pseudomonas aeruginosa*. A broad resonance at δ 22 is assigned to the protons of the imidazole co-ordinating to the iron.

In native cytochrome c the corresponding protons of His(18) appear at δ 37,[11] the difference of 15 ppm may be caused by a slightly different orientation of the imidazole in our model system owing to steric interactions with the alkane bridge of (**1**).

Cyclic voltammetry of the redox pair (**8**)/(**1**) in DMF–0.1 M $LiClO_4$ revealed E° =75 mV *vs.* sodium saturated calomel electrode (NaCl-SCE). The difference between E_{pox} and E_{pred}, ΔE = 78 mV, is in the normal range measured for iron porphyrins and accounts for the exchange of one electron.[12] The electron transfer rate, calculated from ΔE, $k_s = 3.5 \times 10^{-3}$ cm s^{-1}, is about five times the value for native cytochrome c.[2] This is no surprise, since it is known that electron transfer rates are dependent on the environment of the active site. *E.g.*, Sorell and Martin have shown[2] that exchanging His(80) by arginine in the wild-type iso-2-cytochrome c from yeast slows down k_s by a factor of 2—10 but leaves E° =40 mV unchanged.

In most respects (**1**) and (**8**), although lacking the protein, mimic the spectroscopy and electrochemistry of native cytochrome c; we believe that this is due to the fact that the S–Me group is 'forced' into co-ordination with the heme iron and the binding of imidazole occurs in a 'hydrophobic' environment provided by the alkane bridge.

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