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Synthesis and NMR Characterization of a Bis-Strapped Six-coordinate Fe(II) Cytochrome-C Model

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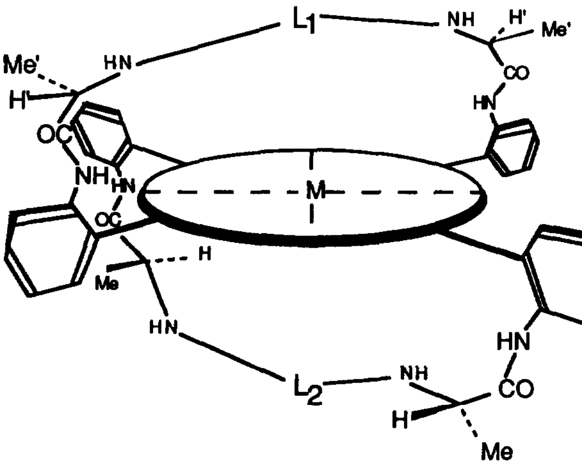
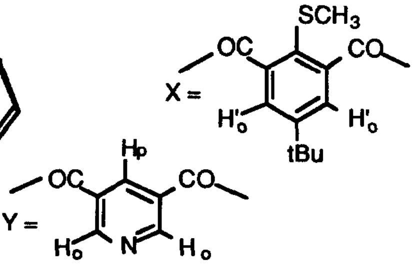
Abstract : The new hexadentate bis-strapped porphyrin 6 has been synthesized with two different ansa, bringing in an intramolecular way, a nitrogen base and a thioether sixth ligand both necessary to mimic the natural cytochrome-C protein. ^1H *NMR spectroscopy indicates clearly the six coordination of the iron atom.*

Cytochromes-C are haemoproteins acting as one electron carriers in the respiratory chain. It is important to note that the iron of the heme prosthetic group is reversibly oxidizable so it can serve as the electron acceptor [1] and that the heme of cytochromes-C (iron-protoporphyrin IX) is covalently attached to the protein by thioether bonds between its vinyl groups and two cysteine residues of the polypeptidic chain (positions 14 and 17). This chain is wrapped around the heme, giving it a very hydrophobic environment and a correspondingly more positive reduction potential than it would have in an aqueous medium. So far, it is difficult to believe that the properties of the cytochrome-C can be explained only by the nature of its two axial ligands (His-18 and Met-80) even on the basis of the few models described. [2-6] In two different studies [7] relative to the semisynthesis of an axial ligand mutant of cytochrome-C via site-directed mutagenesis, by replacement either of Met-80 by Cys, or His-18 by Arg, the coordination number of the iron active site remains unknown and factors which can influence the reduction potential are not really defined.

Figure 1: (The oval represents the 5,10,15,20 tetra-substituted porphyrin)

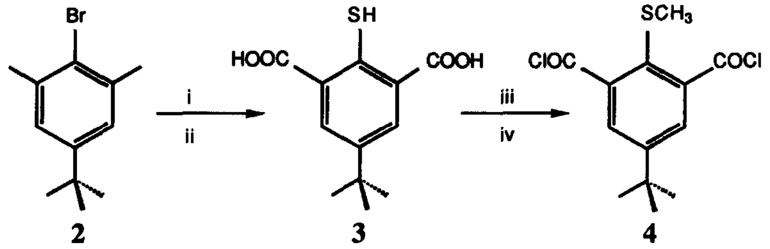


1 L-AlaNH$_2$	M=2H	L_1=L_2= H,H
5* DiSMe	M=2H	L_1=L_2= X
6* SMePy	M=2H	L_1= X L_2= Y
7 DiPy	M=2H	L_1=L_2= Y
8 DiSMeFe(II)	M=Fe(II)	L_1=L_2= X
9 SMePyFe(II)	M=Fe(II)	L_1= X L_2= Y



In this respect, we devised the doubly-bridged porphyrin **6** with the major concept that it should bear both a thioether and a nitrogen base in its own superstructure because of the well-established poor affinity of thioethers in binding heme [8] relative to histidine. We kept in mind the elegant idea of Woggon *et al.*, to avoid the removal of the sulfur atom from the center of the porphyrin for his model [6] in which one of the ligands was supplied intermolecularly, and the fact that a poor electron donating thioether such as a methyl-aryl thioether substituted by two amide bonds at the ortho positions should be a better ligand [9] relative to methyl-phenyl thioether.

Scheme 1: i: $KMnO_4$ ii: NaSH / Diethylene glycol / 170°C iii: Me_2SO_4 iv: $(COCl)_2$ / 50°C/overnight



We applied our synthesis developed for models of dioxygen carriers [10a] to the condensation of the chiral picket porphyrin **1** (fig. 1) with the diacid chloride **4** (scheme 1) and the dichloride of the 3,5-pyridine dicarboxylic acid. Two new chiral porphyrins were obtained : **5** DiSMe (9% yield) and **6** SMePy (24% yield) together with the already described compound **7** DiPy.[10a] Incorporation of iron$^{(II)}$ into **5** and **6** by the usual method ($FeBr_2$ / 2,6-lutidine / refluxing toluene for 1h), yielded quantitatively iron$^{(II)}$ porphyrins **8** DiSMeFe$^{(II)}$ and **9** SMePyFe$^{(II)}$. ^1H NMR and UV-visible spectroscopies show with no ambiguity that **8** is a four-coordinate complex (S=1) while **9** is a low spin (S=0) diamagnetic six-coordinate complex as expected (fig. 3, trace b) [11], indicating clearly the ligation of the two intramolecular ligands. Indeed ^1H NMR of **8** is broadened with a spectrum from *ca* -6 to 16ppm as other intermediate spin state ferrous porphyrins [12] and its UV-visible spectrum exhibits characteristic two bands of roughly equal intensity (424 and 439nm) in the Soret region [13]. It is worthy to note that the resonance of the thiomethyl group at 1.2ppm is unexpected : for such a paramagnetic compound, this shows that the methyl group does not lie at the apical position where it should resonate at a spectacular high field.[10b]

Table 1: ^1H NMR Data of **6** SMePy and **9** SMePyFe$^{(II)}$ in $CDCl_3$ (200 MHz)

	CH	CH'	Me	Me'	tBu	SCH_3	Ho	Hp	Ho'
6 SMePy	3.91	4.07	1.00	0.90	0.66	-0.40	7.17	4.75	6.30
9 SMePyFe$^{(II)}$	4.41	4.37	0.95	0.16	0.74	0.52	2.41	8.38	6.12/6.62

Observing the ^1H NMR spectra of **6** SMePy and **9** SMePyFe$^{(II)}$ (fig.3), one clearly understands that, whereas the nitrogen base has to dive towards the metallic center to ligate it, the sulfur atom ever lies in a good position. This phenomenon is illustrated by the deshielding of the thio-methyl group (SCH_3) in **9** (0.52ppm)

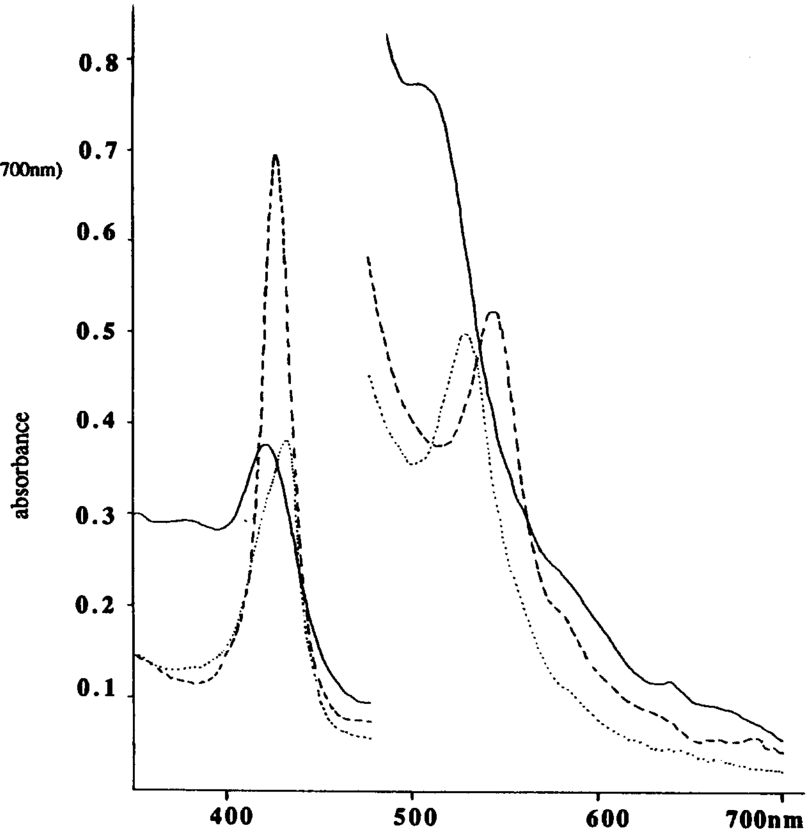


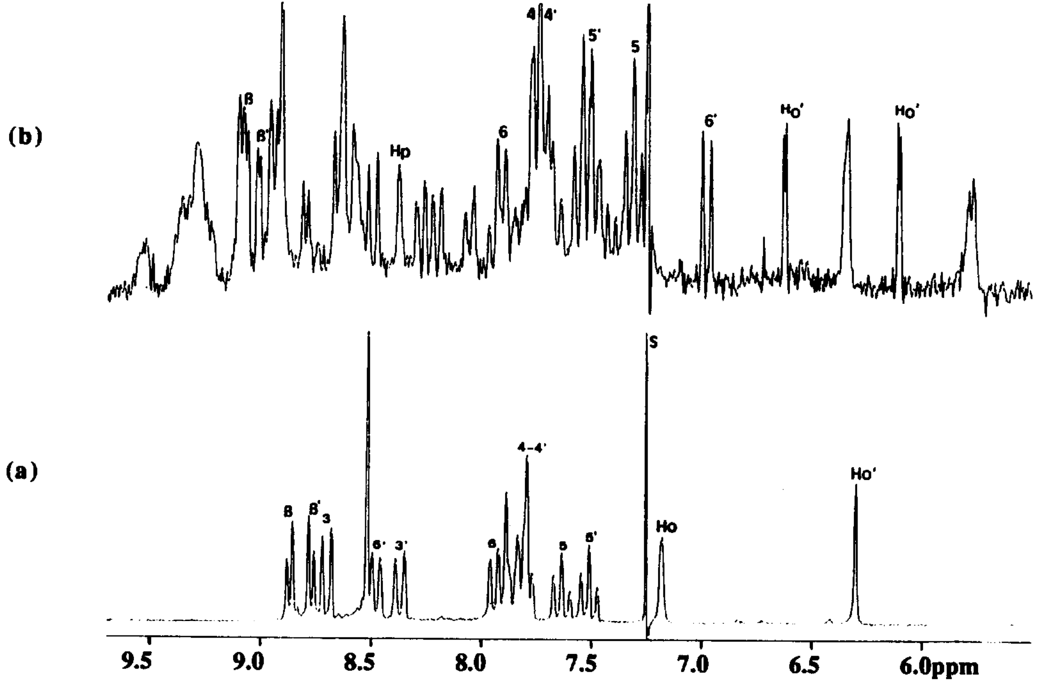
Figure 2: UV-visible spectra (350-700nm) in CHCl$_3$ of :

(——) SMePyFe$^{(III)}$

(....) SMePyFe$^{(II)}$ 9

(----) SMePyFe$^{(II)}$CO

Figure 3 : 200MHz ^{1}H NMR spectra in CDCl$_3$ of (a) **6** SMePy , (b) **9** SMePyFe$^{(II)}$ (same scale ; a gaussian multiplication was performed on both traces using a Bruker spectrometer with LB=-1 and GB=0.1 ; S=solvant, β and β' = β pyrrolic protons).



relative to the free-base **6** (-0.4ppm) and the shielding of the Ho protons of the pyridinic cycle (2.41 versus 7.17ppm) (Table 1). On the other hand, the four-coordinate complex **8** demonstrates again the poor affinity of thio-ligands towards the iron center. Under an atmospheric pressure of CO, **8** becomes a low spin (S=0) six-coordinate porphyrin with a classical NMR spectrum going from 0 to 10 ppm (not shown), in which one of its two potential thioether ligands and the CO coordinate the metal . In other words, the trans influences of CO and of the pyridinic handle (in the case of **9**) induce the coordination of the thioether ligand.

In conclusion, we have shown that the model **9** SMePyFe$^{(II)}$ possesses the right superstructure to force the thioether handle into coordination of the iron center §; this is to our knowledge the first example of a cytochrome-C analogue in which the two axial ligands are so well maintained in an **intramolecular** fashion owing to the *bis-ansa* type of the porphyrin. This represents an improvement to previously reported models in which either the nitrogen base is added [6] or the two ligands are covalently attached by a flexible tailed structure [2].

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§ *According to our results obtained with the model 9, the coordination sphere is easy to reproduce but UV-visible spectroscopy of 9 does not exhibit the well-known α and β absorptions of reduced cytochrome-C at ca 520 and 550nm in aqueous phosphate buffer [14] but absorptions at 592nm and a Soret band at 432nm (fig.2). Slow oxidation of the iron atom gives two new absorptions at 421 and 514nm which probably account for the iron (III) derivative.*

* *All new porphyrins gave satisfactory spectroscopic data (^{1}H NMR, MS) ; potential redox and detailed spectroscopic studies will be described in a full paper.*

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