# Models for Haemoglobin–Myoglobin: Studies with Loosely and Tightly Strapped Imidazole Ligands

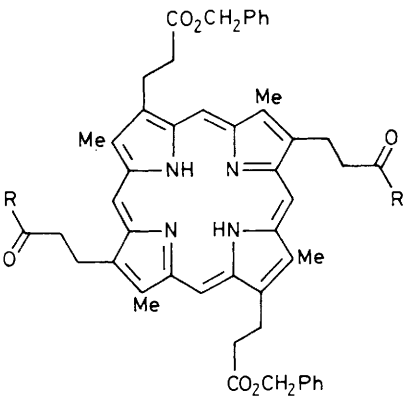
**Alan R. Battersby,* Simon A. J. Bartholomew, and Toyohiko Nitta**
*University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.*

Three doubly bridged $Fe^{II}$-porphyrins are synthesised having a cavity formed on one face by an anthracene-containing bridge and an imidazole ligand strapped across the other face; all three models bind oxygen reversibly at ambient temperature, the oxygenated form of the best system being stable for almost 2 days.

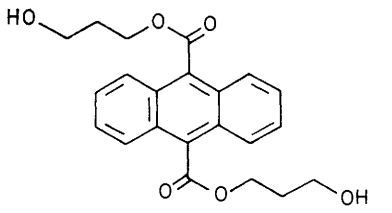
Earlier work in this Laboratory[1] led to the synthesis of a doubly bridged $Fe^{II}$-porphyrin having a cavity on one face (formed by a strapped anthracene unit) and on the other, a pyridine ligand held in place for co-ordination to the $Fe^{II}$ by two covalent attachments at the periphery of the macrocycle. This system bound oxygen reversibly at ambient temperatures but the half-life of the oxygenated species (2—2.5 h) was too low for this model to be suitable for future research. We now outline the synthesis of three doubly bridged porphyrins each carrying an imidazole ligand; one of these models possesses the required stability as an oxygen carrier.

The differentially protected porphyrins (**1a**) and (**1b**) are now available in multi-gram quantities by a new synthesis.[2] Removal of the trichloroethyl or tribromoethyl groups from either (**1a**) or (**1b**) using zinc and acetic acid[3] gave the diacid (**2**), and the corresponding bis-acid chloride reacted under high dilution conditions with the anthracene diol[1] (**3**) to yield the strapped porphyrin† [(**4**), 57%], m.p. 229—230 °C. This substance is also illustrated in diagramatic form as (**4a**) and subsequent complex structures are also shown in this simpli-

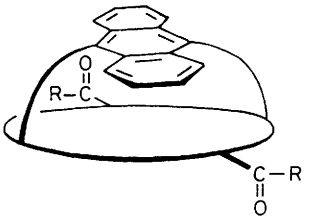
† All the compounds described are new and have been fully characterised spectroscopically (u.v.–visible, mass, and n.m.r.) and by elemental analysis and/or accurate mass determination.



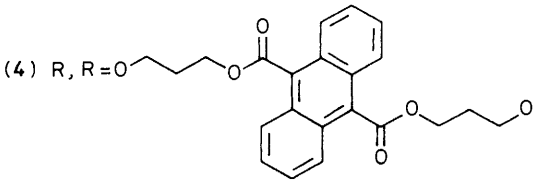
(**1a**) R = $OCH_2CCl_3$
(**1b**) R = $OCH_2CBr_3$
(**2**) R = OH



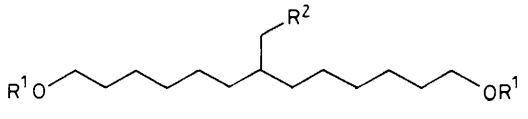
(**3**)



(**4a**) R = $OCH_2Ph$
(**5**) R = OH
(**6**) R = Cl



(**4**) R, R = O

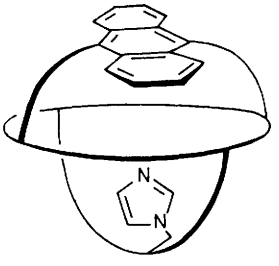


(**7**) $R^1 = CH_2Ph$, $R^2 = OSO_2C_6H_4Me$-*p*

(**8**) $R^1 = CH_2Ph$, $R^2$ =

(**9**) $R^1 = H$, $R^2$ =



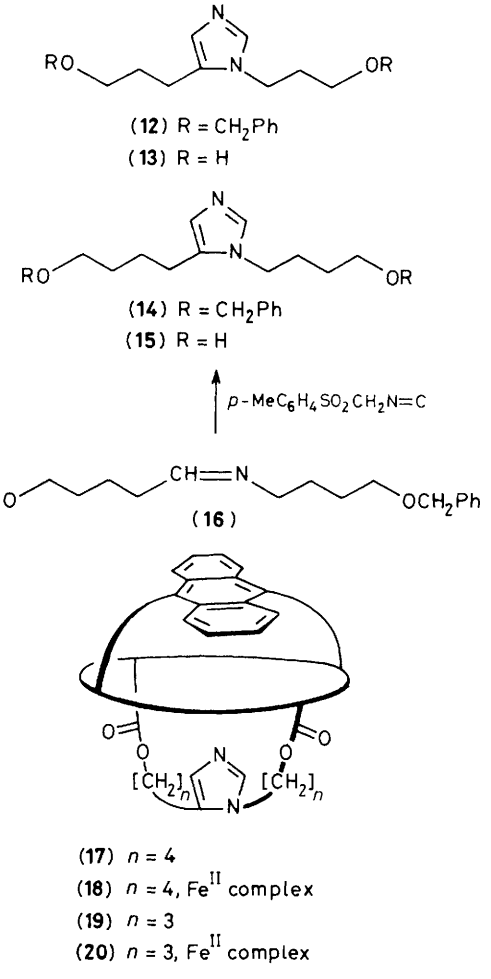


(**10**)
(**11**) $Fe^{II}$ complex

**Table 1.** Spectroscopic data for synthetic and natural systems.

| Structure | U.v.–visible[a] (λ/nm) $Fe^{II}$ | $Fe^{II}$–$O_2$ | $Fe^{II}$–CO | I.r.[c] (ν/cm$^{-1}$) CO-Complex | $^{13}$C N.m.r.[d] (δ/p.p.m.) for CO, CO-Complex |
|---|---|---|---|---|---|
| **(11)** | 413, 546 | 403, 532, 564 | 408, 526, 555 | 1962 | 204.16 |
| **(18)** | 416, 547 | 405, 532, 564 | 410, 528, 558 | 1970 | 205.02 |
| **(20)** | 412, 551 | 404, 535, 567(sh) | 410, 528, 560(sh) | 1970 | 205.22 |
| Deuteromyoglobin[f] | 421, 544 | [b], 532, 565 | 409, 528, 554 | [b] | [b] |
| Haemoglobin[e] | 430, 555[g] | 415, 541, 577[g] | 419, 540, 569[g] | 1951 (α-chain)[h]<br>1952 (β-chain)[h] | 206.7 (α-chain)[i]<br>206.2 (β-chain)[i] |

[a] In DMF for models, in $H_2O$ for proteins. [b] Not quoted. [c] In $CH_2Cl_2$ for models, in $H_2O$ for protein. [d] In wet $Me_2SO$ for models, in aqueous 0.1 M-NaCl for protein. [e] The two vinyl groups attached to this chromophore shift the u.v.–visible absorptions *ca.* 10 nm to longer wavelength. [f] Ref. 7. [g] Ref. 8. [h] Ref. 9. [i] Ref. 10.




fied way. Trimethylsilyl iodide cleaved the benzyl groups[4] from (**4a**) and the diacid (**5**) was converted into the corresponding chloride (**6**) using oxalyl chloride.

Initially, the basic diol (**9**) was selected for attachment to (**6**); this diol was prepared by treating imidazole with the tosylate (**7**) which was synthesised as for the analogue having two methylene groups less.[5] Hydrogenolysis of the benzyl groups in the product (**8**) then gave the diol (**9**), and this was combined as before with the acid chloride (**6**) to afford the doubly bridged porphyrin [(**10**), 22%], m.p. 137—138 °C. Insertion of iron into the macrocycle (**10**) gave the $Fe^{III}$–Cl complex (*m/z* 1314.536, $C_{75}H_{82}FeN_6O_{12}$ requires 1314.534). This was reduced in methylene chloride with aqueous dithionite in a glove box (<5 p.p.m. of $O_2$) and the thoroughly dried $Fe^{II}$-complex (**11**) was transferred to dimethylformamide (DMF). The spectroscopic data for carbon monoxide and oxygen complexes of this model are collected in Table 1, together with the corresponding values for the natural materials where available. The half-life for the oxygenated complex of (**11**) was *ca.* 24 h at room temperature, a considerable improvement over the earlier model based on a pyridine ligand.[1] The oxygenation of (**11**) was reversible (by reducing the pressure) and the system could be taken through four cycles of oxygenation–deoxygenation before irreversible oxidation became significant.

Space-filling models show that the imidazole strap (**9**) is fairly loose so further improvements were sought by synthesis of the tighter straps (**13**) and (**15**). The latter was obtained by cycloaddition[6] of *p*-tolylsulphonylmethyl isocyanide to the imine (**16**) and the resultant ether (**14**) was hydrogenolysed to yield the diol (**15**), picrolonate m.p. 151—152 °C. The diol (**13**), picrolonate m.p. 166—168 °C, was prepared analogously *via* the ether (**12**).

The diol (**15**) and the acid chloride (**6**) reacted together as earlier to yield the doubly strapped porphyrin [(**17**), 23%], m.p. 179—180 °C. This was converted as for (**10**) *via* the $Fe^{III}$-complex (*m/z* 1230.429, $C_{89}H_{70}FeN_6O_{12}$ requires 1230.440) into its $Fe^{II}$-complex (**18**) for binding studies with carbon monoxide and oxygen. Importantly, the oxygenated system was remarkably stable at ambient temperature in DMF, only *ca.* 20% undergoing irreversible oxidation after 2 days; 10 cycles of oxygenation–deoxygenation of (**18**) were carried out as above before significant irreversible oxidation became apparent. Table 1 collects the spectroscopic data on the CO and $O_2$ complexes of model (**18**) which is the one of choice for future work.

As judged from space-filling models, the shorter strap (**13**) can only bridge across the face of porphyrin (**6**) by distorting the porphyrin from planarity. This accounts for the low yield in the bridge-building step [(**13**) + (**6**) → (**19**), 6%, m.p. 174—175 °C]. The corresponding $Fe^{II}$-complex (**20**) bound CO and $O_2$ (see Table 1) and the oxygenated product was less stable than that formed by the model (**18**) having an imidazole bridge which fits tightly but without distortion of the porphyrin.

Our conclusion is that models for natural haem systems having imidazole as the fifth ligand are best constructed using the base (**15**) to form the strap across the porphyrin when the attachment is to two propionic acid units at the periphery of the macrocycle. This is also of importance for our studies on models for several of the cytochromes.

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## References

1 A. R. Battersby and A. D. Hamilton, *J. Chem. Soc., Chem. Commun.*, 1980, 117.
2 A. R. Battersby and T. Mashiko, in preparation.
3 T. W. Green, 'Protective Groups in Organic Synthesis,' Wiley–Interscience, New York, 1981, p. 165.
4 M. E. Jung and M. A. Lyster, *J. Am. Chem. Soc.*, 1977, **99**, 968.
5 A. R. Battersby, W. Howson, and (in part) A. D. Hamilton, *J. Chem. Soc., Chem. Commun.*, 1982, 1266.
6 A. M. van Leusen, J. Wildeman, and O. H. Oldenziel, *J. Org. Chem.*, 1977, **42**, 1153.
7 A. Rossi-Fanelli and E. Antonini, *Arch. Biochem. Biophys.*, 1957, **72**, 243.
8 E. Antonini and M. Brunori, 'Hemoglobin and Myoglobin in their Reactions with Ligands,' American Elsevier, New York, 1971.
9 J. O. Alben and W. S. Caughey, *Biochemistry*, 1968, **7**, 175.
10 R. B. Moon and J. H. Richards, *Biochemistry*, 1974, **13**, 3437; *J. Am. Chem. Soc.*, 1972, **94**, 5093.