Synthesis and Characterization of a New Series of Iron(II) Single-face Hindered Porphyrins. Influence of Central Steric Hindrance upon Carbon Monoxide and Oxygen Binding

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A series of single-face hindered porphyrins has been synthesized from 5,10,15,20-tetrakis-(*o*-aminophenyl)porphyrin having one 'handle' and two pivaloyl 'pickets' as substituents in order both to vary the cavity size and to hold the handle in a central position. They have been characterized from the analysis of the ^{1}H n.m.r. spectra of their four-co-ordinate iron(II) derivatives. The five-co-ordinate complexes obtained by addition of 1-methylimidazole in toluene solution form stable and reversible oxygen adducts. The carbon monoxide and oxygen affinities of these complexes have been determined and are compared with similar data for other model compounds. The systematic decrease of the available space for the iron-bound CO produces a decrease of affinity constants for this ligand which is due to decreased association rates. In contrast, O_2 affinities are only slightly affected. I.r. spectroscopy confirms the presence of a hydrogen bond between O_2 and the NH amide of the handle anchoring groups. No such interaction is observed with the pivaloyl pickets. These compounds display a ratio of CO and O_2 affinity constants from 5000—7. These results are discussed in terms of the distal-side steric effects.

The primary goal in synthesis of iron(II) model compounds of the active site of oxygen-carrier hemoproteins was to define the necessary structural and chemical features involved in the affinity and kinetics of gaseous ligands fixation. These features include (1) resistance to oxidation in the presence of oxygen (both-faces hindrance),[1-2] (2) five-co-ordination of the iron(II) (proximal effect),[3-8] (3) chemical environment of the binding site (distal polar effect),[9-11] (4) steric interference to O_2 and CO ligation (distal-side steric effect).[12-17]

The last factor seems responsible for the greatly reduced affinity for CO of hemoproteins compared with simple model compounds whereas O_2 affinity is not affected. This difference can be explained in structural terms. *X*-Ray analysis of carboxylated hemoproteins revealed that the CO moiety is bent and/or tilted from the axis perpendicular to the porphyrin plane due to interactions with distal residues.[18-20] This structure contrasts with the well known linear iron carbonyl structure in synthetic compounds.[21] Dioxygen adducts of both hemoproteins and model compounds show a bent iron–oxygen configuration.[22-24] It has been proposed that these steric interactions are responsible for the low ratio of the equilibrium constants $M(=K_B^{CO}/K_B^{O_2})$ in hemoproteins compared with most synthetic hemes. Such a low ratio has been correlated with the partial detoxification of carbon monoxide inhalation in respiring organisms.[25] Only few strongly hindered compounds such as 'pocket' porphyrins developed by Collman[26] and 'cyclophane' porphyrins synthesized by Traylor[14,27] show low M values similar to those observed for hemoproteins.

We recently reported the synthesis and ligand binding of ether and amide 'basket handle' porphyrins in which both faces of the porphyrin ring are protected.[4,5] However these compounds presented CO affinities which were as strong as those of flat-open hemes, indicating a weak 'central steric' effect. This may be explained by the fact that the distal handle could be displaced sideways from its average position in the Fe–C (*meso*) plane, preventing the steric effect observed in hemoproteins.

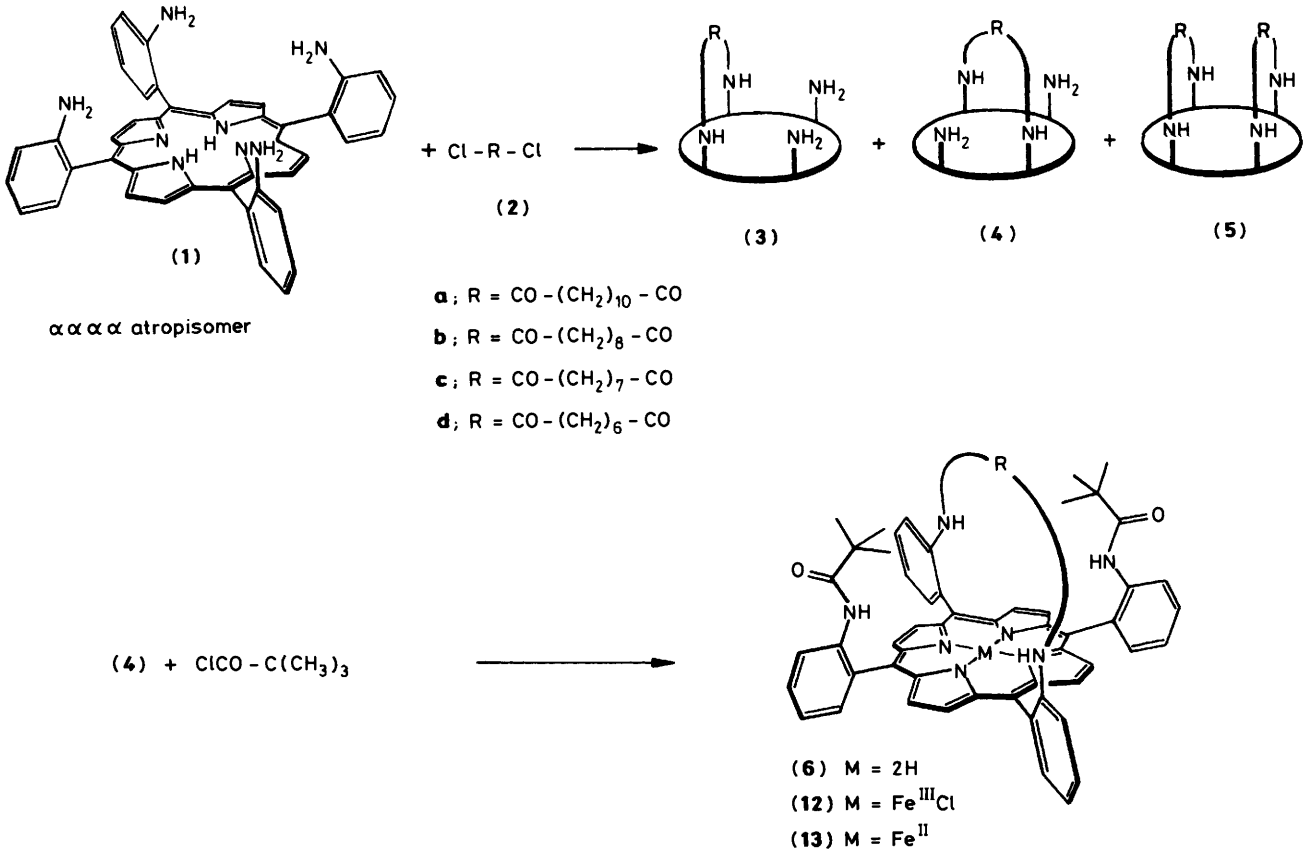
In order to study the influence of this factor in the discrimination of CO ligation relative to O_2 we have developed a new series of four single-face hindered porphyrins designed to hold the distal handle in a central position and thus to increase the central steric hindrance. These so called 'hybrid' models (**6**) have two pivalamido 'pickets' (as 'picket-fence' porphyrins)[9] on each side of an 'amide handle' of variable length in a cross configuration (as amide—'basket handle' porphyrin, a-BHP).[5]

The most striking effect observed in the present series is that K_B^{CO} can be reduced by a factor of 2 500 while $k_B^{O_2}$ changes by less than a factor of 3.5 upon increasing the central steric hindrance.

Results and Discussion

Synthesis.—Compounds (**6a**—**d**) can be easily obtained by a two-step procedure. 5,10,15,20-tetrakis-(αααα-*o*-aminophenyl)-porphyrin (**1**)[9] was treated with an equivalent amount of diacid chloride (**2**) under high-dilution conditions according to Scheme 1. Analytical t.l.c. on silica gel showed two main compounds corresponding to isomeric adjacent and cross mono-handle derivatives (**3**) and (**4**). Assignment of the structure of these products was based on high-field ^{1}H n.m.r. by comparison with the analysis of assignments in the 'basket-handle' porphyrins series.[1] Differences appeared only as very slight changes in chemical-shift values of the methylene protons resonances of the bridge and in the pattern of the resonances of the pyrrole protons at δ 8.5—9. Signals of the methylene protons of the cross isomer (**4**) appear shifted upfield by several p.p.m. compared with the adjacent isomer (**3**) due to a larger effect of the porphyrin diamagnetic ring current. Because of the C_{2v} symmetry of the cross isomer (**4**), the resonances of eight tetrapyrrole protons are expected to appear as an AB spectrum (*J ca.* 5 Hz). In contrast, the adjacent isomer (**3**) has C_s symmetry implying that the pyrrole protons resonance should appear as an AB system (4 H, *J ca.* 5 Hz) and two singlets each corresponding to two protons.

The second step involved the treatment of the cross mono-handle porphyrin (**4**) with an excess of pivaloyl chloride. This reaction routinely proceeded in 70—80% yield after chromatography of compound (**6**) and subsequent crystallization.

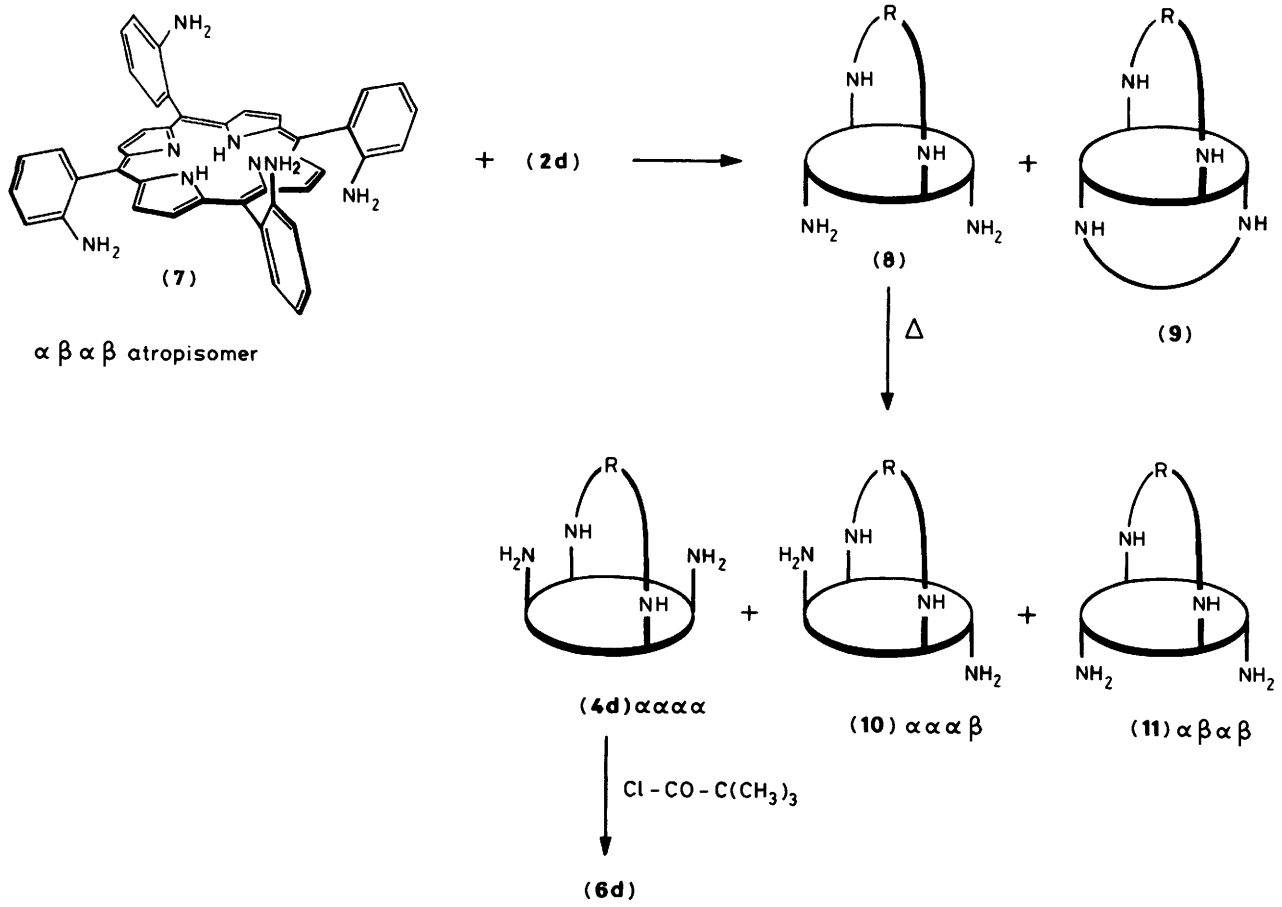


Scheme 1.

Table 1. Absorbance maxima (nm) and molecular extinction coefficients (l mmol^{-1} cm^{-1}) of iron 'hybrid' complexes in toluene

a-$(Piv)_2(C_{12})$	Fe^{III}-Cl^- (**12a**)	417 (104)	507 (13.1)	578 (4)	647 (3.5)
	Fe^{II} (**13a**)	416 (99.2) 441 (88.5)	537 (12.2)		
	Fe^{II}-$(1MeIm)_2$	430 (280)	534 (18.6)	561.5 (4.4)	
	Fe^{II}-(1MeIm)(CO)	423 (280.5)	541 (12.5)		
	Fe^{II}-$(1MeIm)(O_2)$	424.5 (156.5)	543 (16.3)		
a-$(Piv)_2(C_{10})$	Fe^{III}-Cl^- (**12b**)	417 (93)	507 (12)	578 (3.7)	649 (3.2)
	Fe^{II} (**13b**)	416 (97) 442 (86)	538 (11.5)		
	Fe^{II}-(1MeIm)	438.5 (151)	542 (9)	559.5 (8.4)	609 (3)
	Fe^{II}-(1MeIm)(CO)	423 (266)	537 (11.2)		
	Fe^{II}-$(1MeIm)(O_2)$	422 (116)	549.5 (11)		
a-$(Piv)_2(C_9)$	Fe^{III}-Cl^- (**12c**)	418 (98)	508 (12.2)	576 (4.1)	652 (3.3)
	Fe^{II} (**13c**)	415.5 (100.5) 441 (92)	536.5 (12.5)		
	Fe^{II}-(1MeIm)	438 (174)	554 (9.8)	560 (8.5)	609 (3)
	Fe^{II}-(1MeIm)(CO)	422 (257.8)	520 (10.3)		
	Fe^{II}-$(1MeIm)(O_2)$	421.5 (112.8)	553 (11.8)	625 (3.1)	
a-$(Piv)_2(C_8)$	Fe^{III}-Cl^- (**12d**)	418 (98.2)	507 (12.5)	577 (3.9)	650 (3.1)
	Fe^{II} (**13d**)	417 (91) 444 (86.2)	539 (11.5)		
	Fe^{II}-(1MeIm)	438.5 (157.7)	540 (8.4)	561 (7.9)	607 (2.8)
	Fe^{II}-(1MeIm)(CO)	423 (211.9)	514 (9.4)		
	Fe^{II}-$(1MeIm)(O_2)$	422 (96.3)	557.5 (9.8)	595 (3.6)	

The synthesis of the cross mono-handle (**4d**) having a short chain $[(CH_2)_6]$ from αααα-atropisomer (**1**) was not available. The reaction gave exclusively the adjacent isomer (**3d**) in 40% yield. In order to prepare the more sterically hindered compound (**6d**) of the series, we used a modified procedure (Scheme 2). The starting material was 5,10,15,20-tetrakis-(αβαβ-*o*-aminophenyl)porphyrin (**7**).[5] The coupling reaction with suberoxyl chloride (**2d**) led to the formation of the mono-handle derivative (**8**) in 26% yield and of the both-faces hindered compound (**9**). The isolated pure derivative (**8**) was then heated under reflux in



Scheme 2.

Table 2. Binding constants of imidazole derivative for various hemes (Im, imidazole; 1MeIm, 1-methylimidazole; TPP, 5,10,15,20-tetraphenylporphyrin)

	B	K_B/l mol^{-1}	K^B_B/l mol^{-1}	Conditions	Ref.
(13a)	1MeIm	1.9 10^5	510^3	Toluene, 25 °C	This work
(13b)	1MeIm	3.97 10^5	6.8	Toluene, 25 °C	This work
(13c)	1MeIm	2.97 10^5	0	Toluene, 25 °C	This work
(13d)	1MeIm	1.5 10^5	0	Toluene, 25 °C	This work
Fe-(Poc Piv)	1MeIm	2.88 10^4	0	Toluene, 25 °C	13
6,6-Cyclophane heme	1MeIm	3 10^3	5	Benzene, 25 °C	27
7,7-Cyclophane heme	1MeIm	8 10^3	—	Toluene, 25 °C	27
Fe-(TPP)	Im	8.8 10^3	7.9 10^4	Benzene, 25 °C	33*b*
Fe-C_2(Cap)	1MeIm	7.9 10^2	0	Toluene, 23.1 °C	34
Fe-C_3(Cap)	1MeIm	2 10^3	5	Toluene, 23.1 °C	34

xylene–acetone (50:1 v/v) in the presence of silica gel following the procedure described by Lindsey.[28] This thermal conversion of atropisomers and the highest affinity for silica gel of the more polar atropisomer (**4d**) allowed 53% yield to be obtained after chromatography whereas the two other atropisomers [αααβ (**10**) and αβαβ (**11**)] were eluted first (47%). The mono-handle two pickets porphyrin (**6d**) was then easily prepared by treatment of the diamino derivative (**4d**) with pivaloyl chloride and subsequent purification.

Incorporation of iron using iron(II) chloride in dimethylformamide was carried out at low temperature (< 70 °C) to prevent the rotation of pivalamido-substituted phenyl rings. Nearly quantitative yields of chloroiron(III) complexes (**12a—d**) were obtained by these standard procedures.[1] These complexes were reduced in toluene as described in the Experimental section. Their electronic spectral characteristics are listed in Table 1.

^{1}H *N.m.r. Spectroscopy of Iron*(II) *Complexes.*—The structure of these new compounds can be easily analysed by ^{1}H n.m.r. of their paramagnetic iron(II) complexes in [$^2H_6$]benzene. Indeed the square-planar iron(II) complexes of intermediate spin state ($S = 1$) exhibit large paramagnetic shifts of the methylene protons signals by pseudocontact interaction with the central metallic ion (Figure 1A).[29] We have compared the shifts of the methylene protons with those of symmetrical amide-'basket handle' porphyrins (a-BHP) in which the handles on both faces can move away from the symmetry plane passing through the

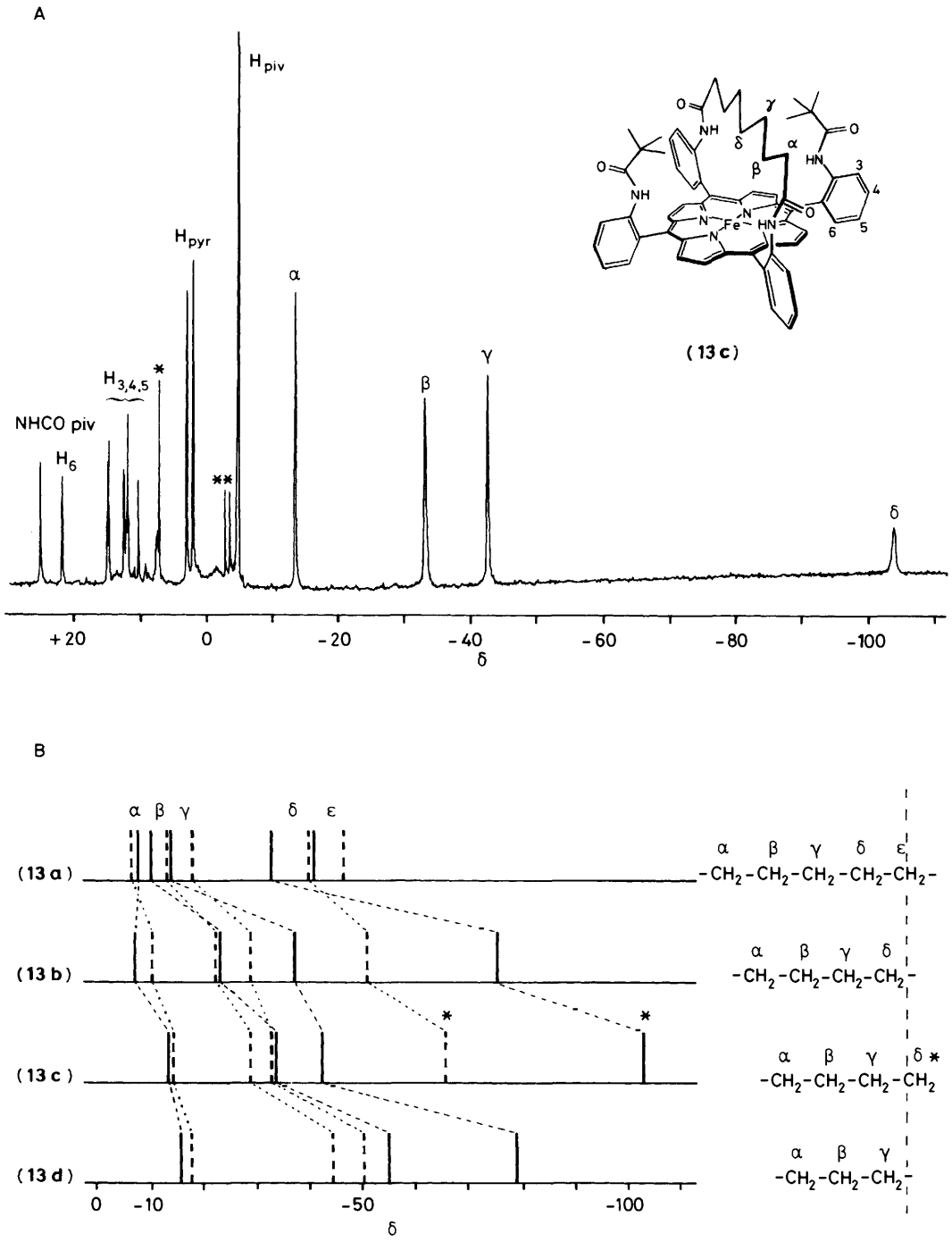


Figure 1. (A) ^{1}H N.m.r. spectrum of compound (**13c**) recorded at 307 K in [$^2H_6$]benzene. * = impurity peaks. (B) Comparison of the high-field part of the ^{1}H n.m.r. spectra of 'hybrid' porphyrins (**13a—d**) (——) and their analogues a-BHP-Fe^{II} (– – –) in [$^2H_6$]benzene

meso carbons of the molecule (Figure 1B). The methylene protons of compounds (**13b—d**) are shifted further upfield than those of a-BHP. This indicates a reduction of the amplitude of the sideways displacement of the handle which appears confined into a better definite axial position due to the presence of the two lateral pickets. On the other hand, in the C_{12} compound (**13a**) the paramagnetic shifts of the methylene protons are lower than those in the corresponding a-BHP, suggesting a much smaller pseudocontact interaction in the former than in the latter due to a greater extension of the handle increasing the distance of the protons from the central ion.

Organic Base Fixation.—The role of proximal histidine on oxygen and carbon monoxide binding to hemoglobin and myoglobin is now well established.[30] In the iron(II) 'hanging base' porphyrins,[3–5,7] or other chelated hemes developed elsewhere by several groups,[31,32] the fifth co-ordination is obtained by covalent attachment of N donor ligands to the heme group. Since such a covalent binding was hardly accessible in the 'hybrid' complexes the effect of steric hindrance on the protected distal face was investigated using external nitrogenous ligands bound to the unprotected face of the porphyrins.

Large changes in the absorption spectra occur upon titration

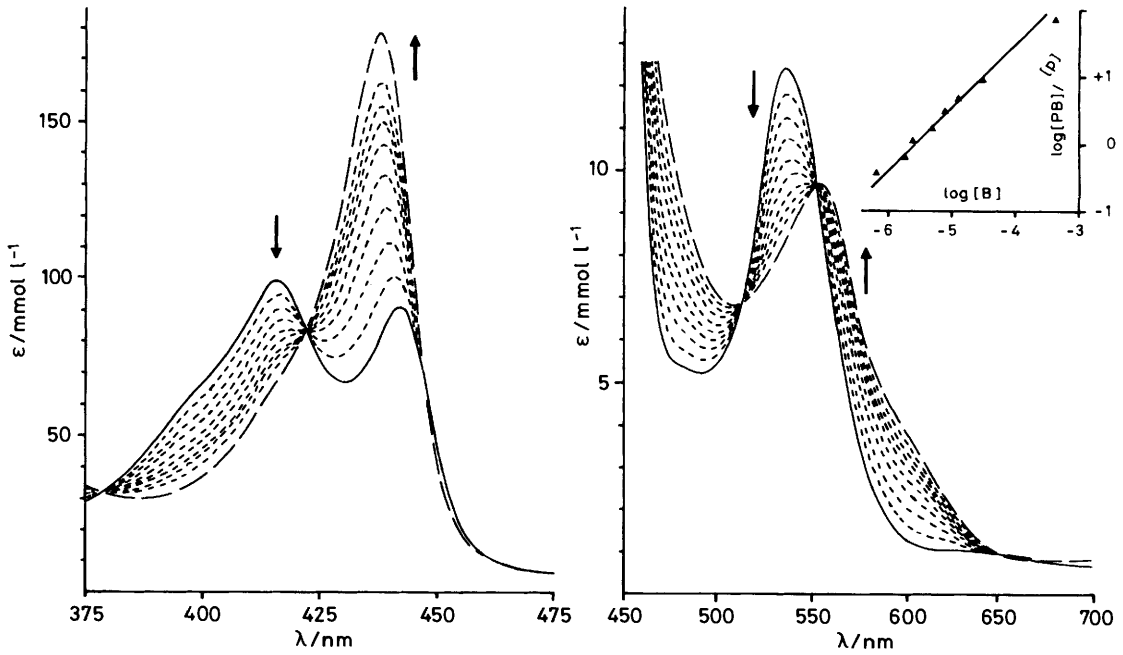


Figure 2. U.v.-visible spectral changes occuring upon titration of a 8.5 10^{-5} mol l^{-1} toluene solution of (**13c**) with 1-methylimidazole under argon. Initial spectrum (——), intermediate spectra (– – – –), final spectrum of (**13c**) (1MeIm) (– – – –). The insert shows the plot of log [PB]/[P] *versus* log [B]

Table 3. Rate and equilibrium constants for CO and O_2 binding to 'hybrid' porphyrins and model hemes, at 20 °C

	$10^{-7}k_B^{+CO}$/ mol^{-1} l s^{-1}	$10^{-3}k_B^{-CO}$/ s^{-1}	$P_{1/2}^{CO}$/ Torr	$10^{-7}k_B^{+O_2}$/ mol^{-1} l s^{-1}	$10^{-3}k_B^{-O_2}$/ s^{-1}	$P_{1/2}^{O_2}$/ Torr	*M*	Ref.
(**13a**)-(1MeIm)[a]	6.3	2.65	4.44 10^{-6}	62.3	0.130	2.98 10^{-2}	4 960	This work
(**13b**)-(1MeIm)[a]	0.18	2.0	1.2 10^{-4}	3.0	0.027	1.3 10^{-1}	814	This work
(**13c**)-(1MeIm)[a]	0.1	2.63	2.8 10^{-4}	2.1	0.005	3.3 10^{-2}	89	This work
(**13d**)-(1MeIm)[a]	0.008	8.16	1.06 10^{-2}	0.22	0.002	1.04 10^{-1}	7	This work
a(BHP)(C_9-Im)(C_{12})[a]	4.0	6.7	1.7 10^{-5}	31.4	0.62	2.9 10^{-1}	12 000	11
Poc Piv-1MeIm[a]	0.058	8.6	1.5 10^{-3}	0.22	0.009	3.6 10^{-1}	270	26
Med Poc Piv-1MeIm[a]	0.15	9.4	6.5 10^{-4}	1.7	0.071	3.6 10^{-1}	550	26
6,6-Anthracene-cyclophane heme-DCIm[b]	0.003	50	1.7 10^{-1}	0.01	0.8	7 10^{2}	4 100	27
7,7-Anthracene-cyclophane heme-DCIm[b]	0.6	50	9 10^{-4}	6.5	1	1.4	1 500	27
6,6-Adamantane-cyclophane heme-DCIm[b]	0.0009	50	57 10^{-2}	0.015	0.69	3 10^{2}	530	14
(C_2Cap)(1MeIm)[a]							4 300	15
Myoglobin[c]	0.05	17	5.4 10^{-3}	2	0.01	23.0	15	25
Hemoglobin[c]	0.65	10	2.6 10^{-2}	5.9	0.012	3 10^{-1}	130	25
			1.1 10^{-3}			1.1 10^{-1}		

[a] Toluene. [b] Benzene. [c] H_2O.

of a toluene solution of iron(II) complexes (**13a**—**d**) with 1-methylimidazole (Figure 2). All compounds exhibit well defined isosbestic points (422, 448, 512, 551, and 650 nm), indicating a simple equilibrium (1) between four- and five-co-ordinated complexes. The association constant K_B determined according to the method of Brault and Rougée[33a] are given in Table 2.

$$PFe^{II} + B \underset{}{\overset{K_B}{\rightleftharpoons}} PFe^{II} - B \qquad (1)$$

A second spectral evolution is observed for compounds (**13a** and **b**) in the presence of an excess of ligand. The final spectrum is characterized by an absorption band at 540 nm very similar to that of bis-(1MeIm)flat-open iron(II)–5,10,15,20-tetraphenylporphyrin (TPP) or iron(II) symmetrical a-BHP. This step could be assigned to the binding of a second base according to equilibrium (2).

$$PFe^{II} - B + B \overset{K'_B}{\rightleftharpoons} B - PFe^{II} - B \qquad (2)$$

Equilibrium constants for the second base fixation are given in Table 2. Except for (**13a**), the compounds of this series remain, therefore, predominantly five-co-ordinated within the range of 1MeIm concentration used for the investigation of the binding of the gaseous ligand.

Equilibrium constants K_B of these compounds were consistently greater than those reported for other single-face hindered hemes[13,14,27,34] or flat-open TPP iron(II).[33b] Thus 'pocket' porphyrins of Collman showed affinity constants for

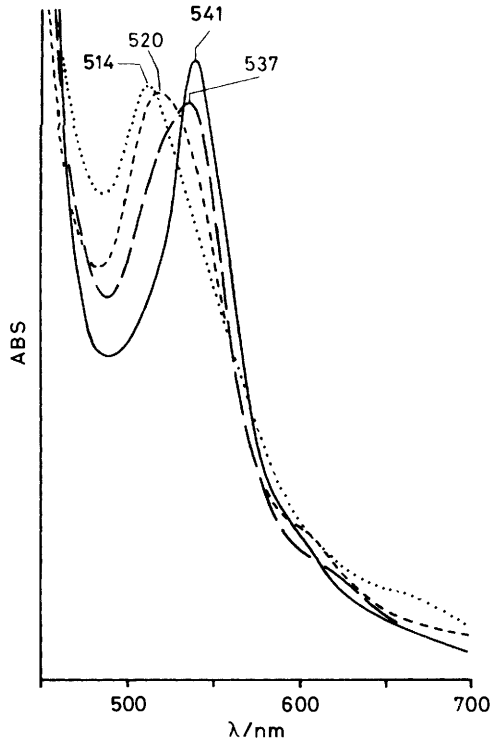


Figure 3. Visible spectra of carbonylated (**13a**—**d**) (1MeIm) in toluene solution: (——) (**13a**); (– –) (**13b**); (– – – –) (**13c**), (· · · ·) (**13d**)

Table 4. CO vibrational frequencies of carbonylated complexes in toluene,[a] benzene,[b] or aqueous solution[c]

Compound	ν_{CO}/cm^{-1}	Ref.
(**13a**) (1MeIm)(CO)[a]	1 958	This work
(**13b**) (1MeIm)(CO)[a]	1 952	This work
(**13c**) (1MeIm)(CO)[a]	1 948	This work
(**13d**) (1MeIm)(CO)[a]	1 948	This work
a-BHP $(C_{12})_2$-(1MeIm)(CO)[a]	1 970	This work
e-BHP $(C_9Im)(C_{12})$(CO)[a]	1 971	This work
a-TpivPP (1MeIm)(CO)[b]	1 969	41
Mb–CO[c]	1 945	38
Hb–CO[c]	1 951	39

the same ligand and in the same solvent which were about one order of magnitude lower.[13] Still more important differences are observed with the 'capped' porphyrins.[34] *X*-Ray crystallographic data of free-base 'capped' porphyrins showed a large doming of the porphyrin ring toward the cap[35] which was suggested to cause the unusual ligation behaviour in this system. However several authors have also emphasized the great sensitivity of ligand binding to the '*cis*' effect of porphyrin.[36,37] It might be expected that electron-withdrawing substituents on the phenyl ring could enhance ligation. This was the case for the chain-anchoring amide in the *ortho*-phenyl position in 'pocket' and our 'hybrid' porphyrins. An opposite situation appears in ether-linked capped porphyrins in which the electron-donating effect of the ether groups decreases ligand affinity.

Interaction with CO *and* O_2.—In the presence of 1MeIm, iron(II) hybrid compounds strongly bind CO to give monocarboxy derivatives displaying characteristic absorption spectra. The variation of the handle length has a remarkable effect on the absorption spectra of the resulting species. The visible band undergoes a hypsochromic shift when the steric hindrance increases without significant shift in the Soret region (Table 1).

Affinity and rate constants are reported in Table 3. Except for compound (**13a**)-(1MeIm), the CO affinities of the model complexes are much lower than those of the 'imidazole' 'hanging base' complex derived from 'basket handle' porphyrins[11] and are similar to those of the strongly hindered 'pocket' and 'cyclophane' hemes. Lowering of CO affinity arises mainly from a decrease in the association rate constant. No major change in the CO dissociation rate is observed.

In carboxy-hemoglobin and -myoglobin CO is known to bind with a bent and/or tilted geometry[18–20] whereas it binds with a normal linear geometry in carboxylated simple heme models.[21] ν(CO) Vibrations are observed respectively at 1 945—1 950[38,39] and 1 970 cm^{-1}[40,41] varying in the same way as the CO affinities. The origin of the difference between natural and synthetic compounds is attributed to a steric interaction of the axial ligand with distal amino acid residues (His-63, Val-67) pushing the ligand off axis in hemoproteins.[41] The frequencies of the CO vibration in our four model compounds are intermediate between those of hemoproteins and simple porphyrins (Table 4). Furthermore, it was found that when the handle length decreases (hence, increasing the steric hindrance or decreasing the CO binding affinity) the CO stretching frequency decreases. This could be correlated to the geometry of the Fe–C–O moiety. However *X*-ray analysis of the carboxy derivative of complex (**13a**)[42] indicates that CO is bound in a linear fashion as in flat-open heme–carbon monoxide complex crystals previously reported.[21] Unfortunately, crystallographic data are not yet available for (**13b**—**d**). Further work is required in order to estimate the relative importance of factors such as electronic distribution within the heme, polarity of the distal cavity, and/or distortion of the porphyrin core upon a particular ligand stretching frequency.

In toluene solution 'hybrid' iron(II) complexes reversibly bind dioxygen at room temperature in the presence of 1MeIm. They show good stability with respect to autoxidation reaction: for example compound (**13d**) ($8.5\ 10^{-5}$ mol l^{-1}) had a lifetime of several days in toluene containing $5\ 10^{-2}$ mol l^{-1} 1MeIm under 1 atmosphere dioxygen. Values of affinity and kinetic constants for O_2 binding are reported in Table 3. These results show that O_2 affinity of the four compounds are slightly higher than those of our synthetic a-BHP analogues[11] and unhindered chelated hemes developed by Traylor[43] or us[44] and similar to the more encumbered model compounds such as 'pocket'[6] or 'cyclophane'[12] porphyrins. The most remarkable fact is that O_2 affinities are changed by less than five-fold within the hybrid series, whereas association *and* dissociation rate constants are simultaneously affected by factors as large as 300—60, respectively. Both electronic and polarity effects are known to play a major role among the factors which stabilize the oxygenated complexes.[10,11,45] The comparison of various porphyrins described elsewhere (a-BHP, picket fence-, pocket-, cyclophane-porphyrins), shows that oxygen affinity is increased by the presence of amide groups as linkage mode between the substituent(s) and the porphyrin ring due to hydrogen bonding between the liganded oxygen molecule and the amide protons.[46] However, since this affects only the dissociation rates[11] H-bonding cannot account for the simultaneous change in the 'on' and 'off' rates observed within the hybrid series. The controversial question as to whether pivalamido pickets could also undergo H-bonding with the bound O_2 molecule was re-investigated using i.r. spectroscopy. I.r. spectra of 'hybrid'-1MeIm complexes in the deoxy form and in the presence of CO and O_2 are illustrated in Figure 4. Spectra of the starting material and their carbonylated adduct in toluene solution are characterized by an intense NH stretching vibration of both pickets and

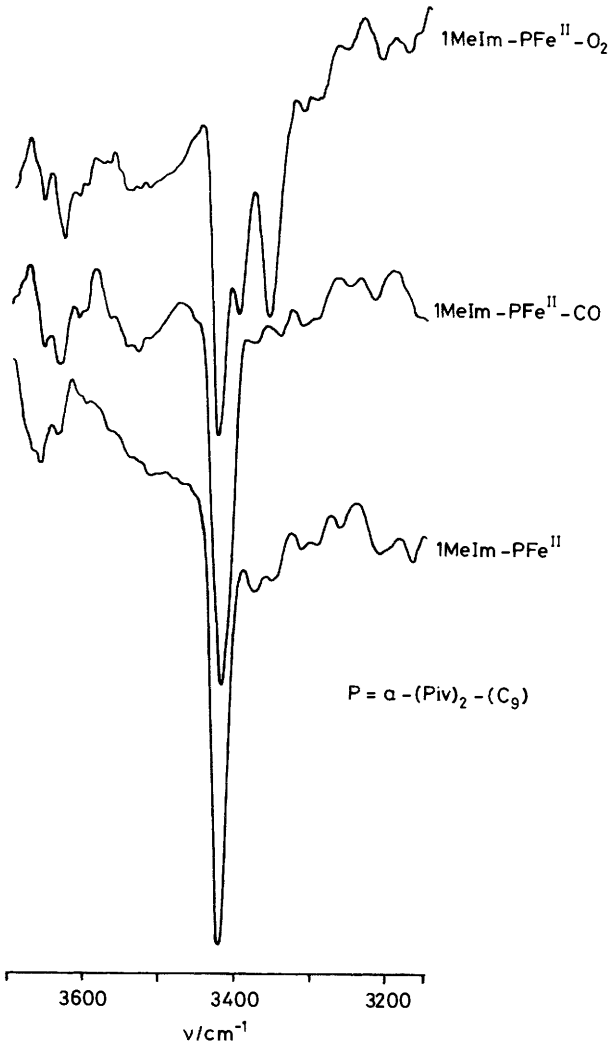


Figure 4. I.r. spectra of (**13c**) (1MeIm) (lower), (**13c**) (1MeIm) (CO) (medium), and (**13c**) (1MeIm) (O_2) (upper) in the NH amide stretching region in toluene solution

Table 5. NH amide vibrational frequencies of oxygenated complexes in toluene solution

Compound	ν_{NHCO}/cm^{-1}	
(**13a**) (1MeIm)(O_2)	3 422	3 372
(**13b**) (1MeIm)(O_2)	3 420	3 366
(**13c**) (1MeIm)(O_2)	3 422	3 364
(**13d**) (1MeIm)(O_2)	3 422	3 358
a-TpivPP (1MeIm)(O_2)	3 418	

handle amide groups at 3 420—3 422 cm^{-1} (Table 5). By contrast, the i.r. spectrum of the oxygenated species reveals two bands at 3 420 and at 3 372—3 358 cm^{-1} (Table 5). The additional band at lower frequency is assigned to the NH bond of one of the amino groups of the handle whose stretching frequency is shifted by intramolecular hydrogen interaction with the co-ordinated oxygen molecule. This shift is slightly dependent upon the length of the distal handle, presumably reflecting variable straight of H-bonding interaction. On the other hand, the unchanged 3 420 cm^{-1} band shows that the amide group of the pivalamido substituents are not involved in H-bonding with oxygen confirming the crystallographic data for the oxygenated iron(II)-'picket-fence' porphyrin in which NH • • • O distances of 3.88 and 4.19 Å are not compatible with hydrogen bonding.[24,47] This band appears always unsymmetrical with a shoulder or a poorly resolved peak on the low-frequency side which should correspond to the second NH group of the distal handle slightly perturbed by the presence of the oxygen molecule.

The degree of steric differentiation between CO and O_2 achieved with the hybrid models is reflected by the *M* values listed in Table 3. *M* decreases from 5 000 to 7 from compound (**13a**) to (**13d**), revealing a direct correlation with increased steric hindrance. This strong decrease is entirely due to a reduction of the CO affinity constants which is controlled only by the association rate. In this connection it is interesting to note that such a decrease in *M* value is observed with other model compounds having both strong steric hindrance and polar groups in the distal cavity[14,15,26,27] (Table 3). A similar situation is observed in hemoglobin and myoglobin in which the imidazole of the distal histidine residue provides both steric hindrance for liganded CO and hydrogen bonding with dioxygen.[22,23,48]

We conclude that the control of affinities for CO and O_2 in hemoproteins and model compounds might be regulated both by steric and by local polar effects.[11] The subtle balance between those two factors should allow one to effectively modify the affinity of gaseous ligands in synthetic compounds designed as models of the active site of oxygen-carrier hemoproteins.

Experimental

All chemicals used were of reagent grade (Aldrich). Reaction solvents (Prolabo) were purified before use. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Dimethylformamide (DMF) was distilled and kept over 4 Å molecular seive. Merck silica gel 60 (40—60 μ) was used for column chromatography. Merck-precoated preparative plates (silica gel 60; 2 mm) were used for t.l.c. Elemental analysis were carried out by the 'Service Central de Microanalyse du C.N.R.S'. Optical spectra in Soret and visible regions were recorded using a Varian DMS 100 spectrometer. ^{1}H N.m.r. spectra of free-base porphyrins and their iron(II) complexes in deuteriochloroform and [$^2H_6$]benzene respectively (C.E.A. France) were measured using a Varian XL 100 spectrometer in the Fourier transform mode using 4 K data points in the frequency domain. Chemicals shifts were referenced to internal tetramethylsilane. I.r. spectra were recorded on a Fourier transform apparatus (Nicolet 5MX) using millimolar solutions.

General Procedure for the Preparation of Mono-handle Porphyrins (**4a**—**c**).—The appropriate diacid chloride (**2**) (1 mmol) obtained according to the usual treatment with oxalyl chloride[5] in THF (50 ml) was added dropwise during 2 h to a mixture of 5,10,15,20-tetrakis-(*o*-aminophenyl)porphyrin (αααα-atropisomer) (**1**) (674 mg, 1 mmol) and triethylamine (280 μl, 2 mmol) in the same solvent (300 ml) at room temperature. Argon was bubbled through the solution during the reaction. After complete addition stirring was continued for 1 h. The solvent was rotary evaporated and the residue dissolved in methylene dichloride. The solution was washed with water (× 3), dried (Na_2SO_4) and concentrated. Subsequent t.l.c. on silica gel, developed with methylene dichloride–acetone gave three main bands. The first band, corresponding to the least polar compound, was assigned by ^{1}H n.m.r. to the 'basket handle' porphyrin, adjacent *cis*-linked isomer (**5**). The second band was the mono-handle porphyrin, adjacent-linked isomer (**3**). The most polar porphyrin was identified as the mono-handle cross-linked isomer (**4**).

Materials.—α-5,15-[2,2′-(*Dodecanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*aminophenyl*)*porphyrin* (**4a**). Decane-1,10-dicarbonyl dichloride was used. The crude material was chromatographed with methylene dichloride–acetone (5:1 v/v). Compound (**4a**) was crystallized from methylene dichloride–hexane (395 mg, 45.5%). α-5,10:α-15,20-Bis-[2,2′-(dodecanediamido)diphenyl]porphyrin (**5a**) (240 mg, 17.4%) and α-5,10-[2,2′-(dodecanediamido)diphenyl]-αα-15,20-bis-(*o*-aminophenyl)-porphyrin (**3a**) (220 mg, 19.5%).

α-5,15-[2,2′-(*Decanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*aminophenyl*)*porphyrin* (**4b**). Sebacoyl chloride was used. The mixture of porphyrins was chromatographed on silica gel, developed with methylene dichloride–acetone (3:1 v/v). Compound (**4b**) was crystallized from methylene dichloride–hexane (311 mg, 37%). α-5,10:α-15,20-Bis-[2,2′-(decanediamido)diphenyl]porphyrin (**5b**). On t.l.c. this yielded 120 mg, 12%. The second band obtained was α-5,10-[2,2′-(decanediamido)diphenyl]αα-15,20-bis(*o*-aminophenyl)porphyrin (**3b**) (176 mg, 21%).

α-5,15-[2,2′-(*Nonanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*aminophenyl*)*porphyrin* (**4c**). Azeloyl chloride was used for the preparation of (**4c**). The mixture of porphyrins was separated on silica gel using methylene dichloride–acetone (2:1 v/v) as developing solvent. Compound (**4c**) was crystallized from methylenedichloride–hexane(220mg,26.6%).α-5,10:α-15,20-Bis-[2,2′-nonanediamido)diphenyl]porphyrin (**5c**) yielded 117 mg, 12%. α-5,10-[2,2′-(Nonanediamido)diphenyl]-αα-15,20-bis-(*o*-aminophenyl)porphyrin (**3c**) yielded 170 mg, 20.5%.

α-5,15-[2,2′-(*Octanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*aminophenyl*)*porphyrin* (**4d**). Suberoyl chloride (316 mg, 1.5 mol) in THF (75 ml) was added dropwise to a mixture of 5,10,15,20-tetrakis-(*o*-aminophenyl)porphyrin (αβαβ atropisomer) (**7**) (1.011 g, 1.5 mmol) and triethylamine (420 μl, 3 mmol) in the same solvent according to the general method used for mono-handle porphyrins (see above). The crude material was chromatographed on a silica gel column (3 × 25 cm). Elution with methylene dichloride–ether (10:5 v/v) gave a first fraction which was the starting TAPP (269 mg, 27%). A second compound, eluted with methylene dichloride–acetone (2:1 v/v), was α-5,15-[2,2′-(octanediamido)diphenyl]-ββ-10,20-bis-(*o*-aminophenyl)porphyrin (**8**) (320 mg, 26%). The third fraction, eluted with methylene dichloride–acetone (1:1 v/v), was identified by n.m.r. spectroscopy as the cross-*trans*-linked isomer of the 'basket-handle' porphyrin α-5,15:β-10,20-bis-[2,2′-(octanediamido)diphenyl]porphyrin (**9**). This compound was crystallized from methylene dichloride–hexane (310 mg, 22%).

Compound (**8**) was dissolved in xylene–acetone (50:1 v/v) followed by the addition of silica gel 60 (7.5 g). The resulting mixture was heated at 130 °C for 18 h under argon, then poured on a silica gel column (3 × 20 cm). Elution with methylene dichloride–acetone (2:1 v/v) gave a first fraction corresponding to a mixture of ααaβ (**10**) and αβαβ (**11**) atropisomers of mono-handle porphyrin (150 mg, 47%). A second fraction was eluted with methylene dichloride–acetone (1:2 v/v). After evaporation of solvent, the residue was crystallized from methylene dichloride–hexane to give 170 mg (53%) of the required αααα-atropisomer (**4d**).

General Procedure for the Preparation of 'Hybrid' Porphyrins (**6**).—Mono-handle porphyrin (**4**) (0.2 mmol) was dissolved in THF (100 ml) and triethylamine (200 μl). The mixture was treated with pivaloyl chloride (200 μl, 0.5 mmol) under argon at room temperature for 2 h. The solvent was evaporated to dryness and the residue was dissolved in methylene dichloride. The organic solution was washed successively with water (× 3), aqueous sodium hydrogencarbonate, and water and dried (Na_2SO_4). The filtrate, on concentration, gave a solid which was purified by t.l.c. on silica gel. The desired compound corresponding to the main band was then crystallized from methylene dichloride–hexane.

α-5,15-[2,2′-(*Dodecanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*pivalamidophenyl*)*porphyrin* (**6a**). This compound was obtained from mono-handle porphyrin (**4a**) (173.6 mg) by the foregoing procedure (168 mg, 81.7%) (Found: C, 74.9; H, 6.9; N, 10.5. $C_{66}H_{68}N_8O_4,H_2O$ requires C, 75.1; H, 6.7; N, 10.6%), δ($CDCl_3$) 8.85 (s, 8 H), 8.79 (2 s, 4 H, *o*-ArH), 7.93—7.42 (6 H, *m,p*-ArH + 2 H, NHCO), 7.14 (2 H, NHCO), 1.58 (t, 2 × α-CH_2), 0.6 (m, 2 × β-CH_2), 0.46 (s, 6 × CH_3), −0.27 (quint, 2 × γ-CH_2), −1.23 (br quint, 2 × δ-CH_2), −1.42 (br quint, 2 × ε-CH_2), and −2.59 (s, 2 × NH).

α-5,15-[2,2′-(*Decanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*pivalamidophenyl*)*porphyrin* (**6b**). An analogous reaction to that described above with mono-handle porphyrin (**4b**) (168 mg) gave the desired compound (146 mg, 73%) (Found: C, 74.3; H, 6.35; N, 10.6. $C_{64}H_{64}N_8O_4,H_2O$ requires C, 74.8; H, 6.5; N, 10.9%), δ($CDCl_3$) 8.89 (2 s, 4 H, *o*-ArH), 8.85 (s, 8 H), 8.35—7.45 (4 H, *m,p*-ArH + 2 H, NHCO), 6.84 (s, 2 H, NHCO), 1.57 (t, 2 × α-CH_2), 0.52 (s, 6 × CH_3), −0.54 (quint, 2 × β-CH_2), −1.78 (br quint, 2 × γ-CH_2), −2.45 (br quint, 2 × δ-CH_2), and −2.56 (s, NH).

α-5,15-[2,2′-(*Nonanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*pivalamidophenyl*)*porphyrin* (**6c**). Compound (**4c**) (165 mg) was used for the preparation of the title compound using the method described above to yield (**6c**) (149 mg, 75.2%) (Found: C, 74.2; H, 6.5; N, 10.8. $C_{63}H_{62}N_8O_4,H_2O$ requires C, 74.7; H, 6.4; N, 11.0%), δ($CDCl_3$) 8.84 (2 s, 4 H, *o*-ArH), 8.84 (s, 8 H), 8.45—7.38 (4 H, *m,p*-ArH + 2 H, NHCO), 6.33 (s, 2 H, NHCO), 1.23 (t, 2 × α-CH_2), −0.92 (quint, 2 × β-CH_2), −1.44 (quint, 2 × γ-CH_2), −2.47 (s, 2 × NH), and −3.27 (quint, δ-CH_2).

α-5,15-[2,2′-(*Octanediamido*)*diphenyl*]-αα-10,20-*bis*-(o-*pivalamidophenyl*)*porphyrin* (**6d**). This compound was prepared from mono-handle porphyrin (**4d**) (162 mg) according to the general method (139 mg, 71%) (Found: C, 75.8; H, 6.9; N, 10.5. $C_{62}H_{60}N_8O_4$ requires C, 75.9; H, 6.2; N, 11.5%), δ($CDCl_3$) 8.99 (2 s, 4 H, *o*-ArH), 8.82 (s, 8 H), 8.6—7.4 (4 H, *m,p*-ArH + 2 H, NHCO), 6 (s, 2 H, NHCO), 0.99 (t, 2 × α-CH_2), 0.48 (s, 6 × CH_3), −1.36 (br quint, 2 × β-CH_2), −2.11 (br quint, 2 × γ-CH_2), and −2.38 (s, 2 H, NH).

Chloroiron(III) *Complexes of 'Hybrid' Porphyrins* (**12a**—**d**).—Insertion of iron into the free base porphyrins was accomplished using $FeCl_2$ in dimethylformamide at 70 °C.[5] The crude iron(III) complex was purified by t.l.c. over silica-gel plates with methylene dichloride–acetone (3:1 v/v). The metalloporphyrin in methylene dichloride was then vigorously shaken with saturated aqueous NaCl solution to generate the chloroiron(III) derivative and dried (Na_2SO_4). Complexes (**12a**—**d**) were crystallized upon addition of hexane.

Preparation of Iron(II) *Complexes* (**13a**—**d**) *for U.v.-visible,* ^{1}H *N.m.r., and I.r. Spectroscopy.*—Chloroiron(III) 'hybrid' porphyrins were dissolved in toluene and shaken with a dilute HCl solution (pH 3—4) to break the possible μ-oxo dimer. After separation of the two phases, the organic layer was transferred under argon into a second vessel containing an aqueous sodium dithionite solution. The reaction was then carried out in a heterogenous two-phase system as previously reported.[1] The reduced compounds in the organic phase were then transferred under argon into the optical or i.r. cell or n.m.r. tube *via* a stainless steel tube.

Determination of the Rate and Equilibrium Constants for CO *and* O_2 *Binding.*—The rate constants $k^{+CO}{}_B$, $k^{+O_2}{}_B$, and $k^{-O_2}{}_B$ were obtained from the kinetics of direct or competitive binding following photodissociation of the carboxyhemochromes or oxyhemochromes by a laser pulse. The apparatus and the techniques used in these experiments have been previously

described.[11] The association rate constants were measured at several 1MeIm concentrations until a convenient range could be found for each 'hybrid'-porphyrin, in which the rate constants were independent of both base and gaseous ligand concentrations. This procedure eliminates kinetic complications due to base elimination or hemochrome formation[11,13]. The following conditions were found to be satisfactory: (**13a**), 10^{-4} mol $l^{-1} \leqslant$ [1MeIm] $\leqslant 10^{-3}$ mol l^{-1}, [CO] 7.2×10^{-3} mol l^{-1}; (**13b**), 3×10^{-3} mol $l^{-1} \leqslant$ [1MeIm] $\leqslant 3 \times 10^{-2}$ mol l^{-1}, [CO] 7.2×10^{-4} mol l^{-1}; (**13c**), 4×10^{-3} mol $l^{-1} \leqslant$ [1MeIm] $\leqslant 8 \times 10^{-2}$ mol l^{-1}, [CO] 7.2×10^{-4} mol l^{-1}; (**13d**), 5×10^{-2} mol $l^{-1} \leqslant$ [1MeIm] $\leqslant 2 \times 10^{-1}$ mol l^{-1}, [CO] 7.2×10^{-3} mol l^{-1}.

The dissociation rates $k^{-O_2}{}_B$ were so low that special precautions were required in the competitive rebinding experiments: attenuation of the monitoring beam to minimize photolysis and choice of $(CO):(O_2)$ ratios such that the exchange relaxation times never exceeded 5 s in order to avoid perturbations by convection movements within the solution.

The great stability of the oxygenated complexes of 'hybrids' towards autoxidation allowed the measurement of the partition coefficient M by photometric titration of the carboxyhemochromes against the oxyhemochromes. The absorbance changes were monitored in the Soret region; five-co-ordination of the porphyrin was ensured by a 1MeIm concentration higher than 10^{-2} mol l^{-1}. The carbon monoxide affinities $K^{CO}{}_B$ were calculated using M and $K^{O_2}{}_B$ determined kinetically. The dissociation rate constants $k^{-CO}{}_B$ were calculated as $k^{+CO}{}_B/K^{CO}{}_B$.

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